Synthesis and biological evaluation of thienopyrimidinone-containing compounds as antitubercular and anticancer agents, novel dicyanoanilines as cell imaging agents and studies towards the total synthesis of quinagolide

by

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Under the supervision of

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Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled, *"Synthesis and biological evaluation of thienopyrimidinone-containing compounds as antitubercular and anticancer agents, novel dicyanoanilines as cell imaging agents and studies towards the total synthesis of quinagolide"*, submitted by *Mr. Pisal Mahesh Madanrao* to the Academy of Scientific and Innovative Research (AcSIR), in partial fulfillment of the requirements for the award of the Degree of *Doctor of Philosophy in Science*, embodies original research work carried-out by the student. We, further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material(s) obtained from other source(s) and used in this research work has/have been duly acknowledged in the thesis. Image(s), illustration(s), figure(s), table(s) *etc.*, used in the thesis from other source(s), have also been duly cited and acknowledged.

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I Mr. Pisal Mahesh Madanrao, a Ph.D. student of the Academy of Scientific and Innovative Research (AcSIR) with Registration No. 10CC17A26006 hereby undertake that, the thesis entitled "Synthesis and biological evaluation of thienopyrimidinone-containing compounds as antitubercular and anticancer agents, novel dicyanoanilines as cell imaging agents and studies towards the total synthesis of quinagolide" has been prepared by me and that the document reports original work carried out by me and is free of any plagiarism in compliance with the UGC Regulations on *"Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions (2018)"* and the CSIR Guidelines for "*Ethics in Research and in Governance (2020)"*.

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Signature of the Supervisor
Name : Dr. Subhash Prataprao Chavan
Date : 22/07/2021
Place : Pune

This dissertation is dedicated to

My sister Archana, parents,

Supriya and Aditya

Whose relentless love, faith and encouragement helped me reach this stage of my life

Contents

Chapter-1

Synthesis and biological evaluation of thienopyrimidinone-containing compounds

Chapter-2

Synthesis and biological evaluation of novel dicyanoanilines and indoles

Chapter-3

Studies directed towards the total synthesis of (±)-quinagolide

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Thank you all…!!!

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Units	
°C	Degree centigrade
g	Gram
mg	Milligram
h	Hour (s)
Hz	Hertz
µg	Microgram
µM	Micromolar
mL	Millilitre
min	Minutes
MHz	Megahertz
mmol	Millimole
nM	Nanometre
ppm	Parts per million
ɗ	Delta
m/z	Mass to charge ratio
cm	Centimetre
Chemical and Biological Notations	
ACP	Acyl carrier protein
AcOH	Acetic acid
AIDS	Acquired immunodeficiency syndrome
$AlCl_3$	Aluminum Trichloride
ATCC	American Type Culture Collection
n-BuLi	*n*-Butyl lithium
t-BuOH	*tert*-Butyl alcohol
$CHCl_3$	Chloroform
CCDC	Cambridge Crystallographic Data Centre
$CDCl_3$	Deuterated Chloroform
$CuCl_2$	Copper(II) chloride
CAN	ceric ammonium nitrate
CNS	Central Nervous System

Conc.	Concentrated
2D	Two Dimensional
3D	Three Dimensional
DAPI	4′,6-diamidino-2-phenylindole
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	N, N'-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d6	Deuterated DMSO
EtOH	Ethanol
EtOAc	Ethyl Acetate
EMA	European Medicines Agency
ESI	Electrospray ionization Mass spectrometry
EC_{50}	Half maximal effective concentration
eq.	Equation
FITC	Fluorescein isothiocyanate
HBr	Hydrogen bromide
FDA	Food and Drug Administration
HIV	Human immunodeficiency virus
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Coherence
HRMS	High Resolution Mass Spectrometry
HCl	Hydrochloric acid
H_2O	Water
IC_{50}	Inhibitory Concentration required for 50% inhibition
IR	Infra-Red
IBX	2-Iodoxybenzoic acid
I_2	Iodine
J	Coupling constant (in NMR)
$KMnO_4$	Potassium permanganate

K_2CO_3	Potassium carbonate
LCMS	Liquid chromatography–mass spectrometry
LiHMDS	Lithium bis(trimethylsilyl)amide
LDA	Lithium diisopropylamide
MDR/RR-TB	Multidrug resistant tuberculosis
MIC	Minimum inhibitory concentration
MnO_2	Manganese dioxide
MeI	Methyl Iodide
MeCN	Acetonitrile
$NaClO_2$	Sodium chlorite
NaH	Sodium hydride
NaH_2PO_4	Sodium dihydrogen phosphate
ND	not done
NMR	Nuclear magnetic Resonance
NR	Nitrate reductase
NTDs	Neglected tropical diseases
$NaIO_4$	Sodium metaperiodate
Na_2SO_4	Sodium sulphate
NH_4Cl	Ammonium chloride
$NaHCO_3$	Sodium bicarbonate
$Na_2S_2O_3$	Sodium thiosulphate
$NaBH_4$	Sodium borohydride
NaOH	Sodium hydroxide
OsO_4	Osmium tetroxide
ORTEP	Oak Ridge Thermal Ellipsoid Plot
PBS	Phosphate-buffered saline
PI	propidium iodide
PPTS	Pyridinium p-toluenesulfonate
PS	Phosphatidylserine
PanC	Pantothenate synthetase
Pd/C	Palladium on charcoal

$Pd(OAc)_2$	Palladium acetate enzyme
i-Pr_2NEt	N,N-Diisopropylethylamine
RCM	Ring-closing metathesis
RIF	Rifamycin
RMSD	Root mean square deviation
rt	Room temperature
R_f	Retention factor
SI	Selectivity index
SiO_2	Silica
SAR	Structure-Activity Relationship
TB	Tuberculosis
TEA (Et_3N)	Triethylamine
$TiCl_4$	Titanium tetrachloride
THF	Tetrahydrofuran
$TMSN_3$	Trimethylsilyl azide
TS	Transition state
TLC	Thin Layer Chromatography
TMS	Trimethyl silyl
p-TSA	*p*-Toluenesulfonic acid
tert	Tertiary
TFA	Trifluoro acetic acid
XDR-TB	Drug resistant TB
XRD	X-Ray Diffraction
$ZnCl_2$	Zinc Cloride

- Independent compound and reference numbering have been used for each chapter as well as for sections of the chapters.
- All reagents and solvents were purchased from commercial suppliers and used as such without any further purification. Starting materials were obtained from commercial suppliers or prepared using known procedures.
- All the known compounds reported in literature were characterized by their NMR spectra.
- Solvents were distilled and dried following standard procedures. Petroleum ether used for column chromatography was of 60-80 °C boiling range.
- Column chromatographic separations were carried out on silica gel (100-200 or 230-400 mesh size).
- All reactions were monitored by TLC with 0.25 mm pre-coated E-Merck silica gel plates (60 F254) and TLC spots were made visible by exposing to UV light, Iodine adsorbed on silica gel or by immersion into an ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde, ninhydrin or $KMnO_4$ followed by heating with a heat gun for ~15sec.
- NMR spectra were recorded on Bruker AV200 (200.13 MHz for ^{1}H NMR and 50.03 MHz for ^{13}C NMR), AV 400 (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR), Jeol-400 (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR), DRX 500 (500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR) and AV 700 (700 MHz for ^{1}H NMR and 176 MHz for ^{13}C NMR)spectrometers.
- Chemical shifts (δ) have been expressed in ppm units relative to tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) were measured in Hertz.
- The following abbreviations were used for ^{1}H NMR: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet and ddd = doublet of doublet of doublet.
- Optical rotations were recorded on a JASCO P-1020 polarimeter at 589 nm (sodium D-line). Specific rotations $[\alpha]^D$ are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent.
- Structures and IUPAC nomenclature were generated using ChemBioDraw Ultra 14.0 software.
- High-resolution mass spectra (HRMS) (ESI) were recorded on an Orbitrap (quadrupole plus ion trap) and TOF mass analyzer.

AcSIR	**Synopsis of the thesis to be submitted to the Academy of Scientific and Innovative Research for award of the Degree of Doctor of Philosophy in Chemistry**
Name of the Candidate	Pisal Mahesh Madanrao
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Title of the Thesis	"Synthesis and biological evaluation of thienopyrimidinone-containing compounds as antitubercular and anticancer agents, novel dicyanoanilines as cell imaging agents and studies towards the total synthesis of quinagolide"
Research Supervisor	Dr. S. P. Chavan

1. Introduction: The synthesis of biologically and medicinally important small synthesized novel molecules as antitubercular and anticancer agents, as well as the synthesis of novel dicyanoanilines and highly substituted indoles as cell imaging agents, are described in this thesis. In addition, study towards the total synthesis of quinagolide is covered. There are three chapters of the thesis. The first chapter covers the synthesis and the antitubercular evaluation of thienopyrimidinone-containing compounds. The second chapter deals with the synthesis, cell imaging properties and photophysical properties of substituted 2,6-dicyanoanilines and derivatives, as well as the synthesis and biological examination of 2,3,5,6,7-pentasubstituted and 2,3,4,5,7-pentasubstituted indoles synthesized from 2,6-dicyanoanilines. The third chapter focuses on advanced synthesis of quinagolide in order to achieve higher yields and shorter synthetic routes, which are needed to keep the drug-costs down.

1. Statement of problem and objectives:

Thieno[2,3-*d*]pyrimidinones: Thieno[2,3-*d*]pyrimidinones compounds have been shown to exhibit a variety of potential biological applications. According to the 2020 global tuberculosis report[1], which includes reports from 202 countries and territories, TB is a major global health problem, with an estimated 10 million people contracting the disease and 2.9 million dying from it in 2019-2020. Also, number of patients suffering from various cancers is increasing every year. With the help of computational prediction and docking study

thieno[2,3-*d*]pyrimidines may be especially useful synthons in the development of highly efficient new antitubercular and anticancer drugs. The search for new scaffolds is necessary

to widen the range of current antitubercularas well as anticancerdrugs.There fore, It was decided to synthesize novel thienopyrimidinones and study whether they could be used as antitubercularor, anticancer drugs.

2,6-Dicyanoanilines and 2,3,5,6,7-pentasubstituted and 2,3,4,5,7 pentasubstitutedindoles: Many uses for 2,6-dicyanoanilines have been found in the literature[2], including biological, dyes, polymers, and textile printing of synthetic or semisynthetic fibers, indicating the importance of these compounds. Synthesizing these target molecules will be significant for the study into different areas of synthetic and applied chemistry. It was decided to study them as possible cell imaging indicators because of their fluorescence properties. Previously 2,3,5,6,7-pentasubstituted and 2,3,4,5,7-pentasubstituted indoles have been synthesized from 2,6-dicyanoanilines. It was thought to see if these indoles could be used for cell imaging or other biological applications.

D2 receptor agonist Quinagolide: Quinagolide is a dopamine D2 receptor selective agonist. It is generally prescribed for the treatment of idiopathic hyperprolactinemia and pituitary tumors that secrete prolactin[3]. It is sold under the brand name NORPROLAC® and is formulated and used as a hydrochloride salt. Quinagolide is a racemate, and Nordmann and co-workers[4] found that the (-) enantiomer is responsible for its clinical action. Banziger*et al*[5]. published a scalablesynthesis of quinagolide intermediate in the year 2000 and this was the same synthetic strategy of Nordmann*et al*. approach. There have been eight synthetic methods published so far out of which two are chiral syntheses. When the overall yield of the product is considered, it is clear that there is a need for more work on this. In this regard, the finding of a new enantioselective synthetic route for (-)-quinagolide, as well as a racemic approach to (±)-quinagolide, is extremely appealing, as it would lead to a shorter synthesis and higher yields. To keep medication costs down, more work on higher yielding and shorter synthetic strategies were needed.

2. <u>Methodology and Results:</u> Each chapter is summarized briefly.

Chapter 1: Synthesis and biological evaluation of thienopyrimidinone-containing compounds

Section 1: Introduction and Literature Reports

This section highlights a brief introduction tothienopyrimidinone. Many studies indicate that thienopyrimidinone is an important example of fused pyrimidines for pharmacological and

biological applications[6].Studies have been focused on the design and synthesis of novel thienopyrimidinones as therapeutic agents for the past four decades, and a significant number of studies have been reported on thienopyrimidinones as they have shown a variety of biological activities such as antiviral, antidiabetic, antioxidant, anticancer, antidepressant, antiplatelet, antihypertensive, anti-inflammatory, antimicrobial, bronchodilatory, and phospodiesterase inhibition. Due to the association with various physiological components, thieno[2,3-*d*]pyrimidines have also been found to exhibit multiple biological activities.

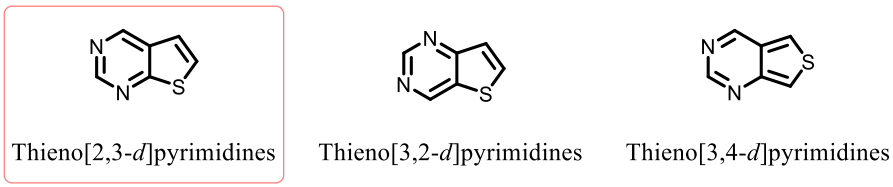


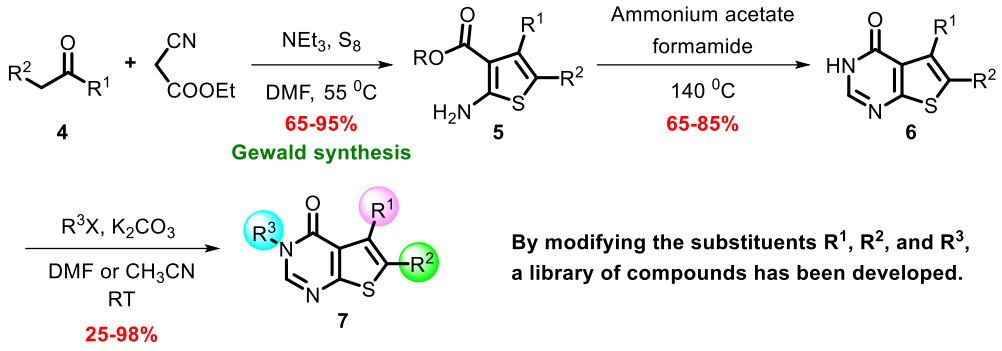
Figure 1:

Synthetic approaches to the development of thieno[2,3-*d*]pyrimidines are well-developed enough. Synthesis of the pyrimidine nucleus on the parent thiophene ring or annulations of the thiophene nucleus on the parent pyrimidine ring have been achieved to build the thienopyrimidine moiety. For the synthesis of thieno[2,3-*d*]pyrimidine, various synthetic strategies have been outlined in three parts 1) Pyrimidine annulations on the thiophene ring 2) Thiophene annulations on the pyrimidine ring 3) Thienopyrimidinones from acyclic compounds

Section 2: Synthesis and evaluation of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents[7]

As a part of the program to introduce new antitubercular agents, a number of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were designed, synthesized, and tested against *Mycobacteria*. Anti-mycobacterial activity was found in some of the compounds against *Mycobacterium*

tuberculosis H37Ra (ATCC25177) and *Mycobacterium bovis* BCG (ATCC 35743). The active compounds were studied for cytotoxicity against four cell lines and were found to be non-cytotoxic. The findings revealed that certain compounds have excellent antimycobacterial activity (MIC in the range of 6–8 μM) and that thienopyrimidinones as a class have the potential to be developed as antitubercular agents.



Scheme 1: Synthesis of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones

Table 1: Antimycobacterial activity data for various thienopyrimidinones

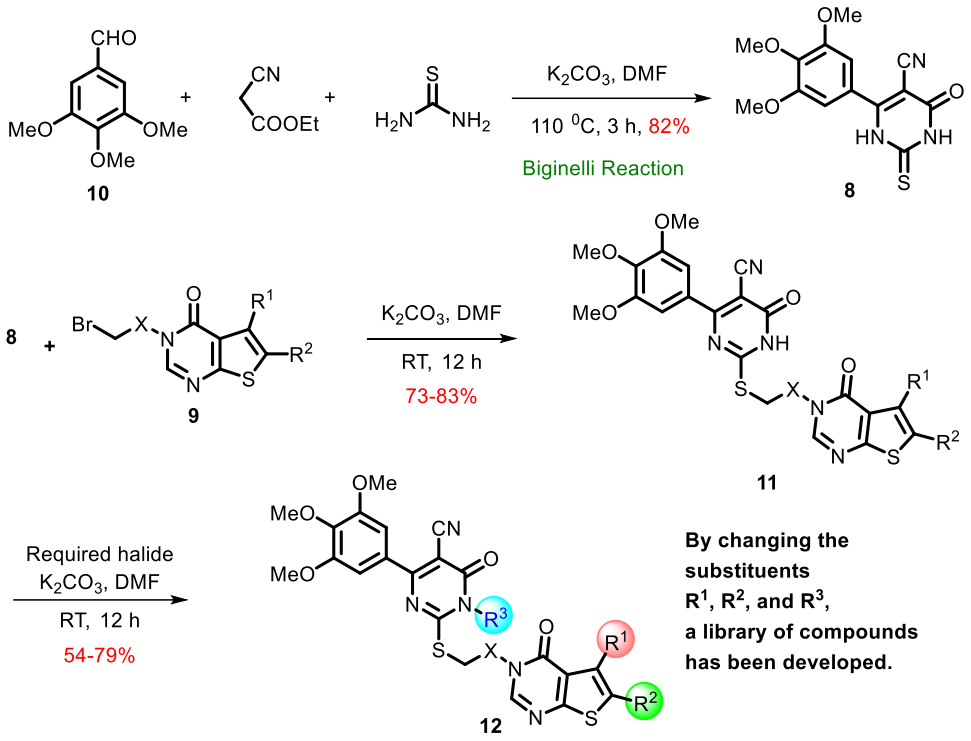
	M. tuberculosis H37 Ra (Active stage) MIC_{90} (µg mL^{-1})	*M. tuberculosis* H37 Ra (Dormant stage) MIC_{90} (µg mL^{-1})	*M. bovis*BCG (Active stage) MIC_{90} (µg mL^{-1})	*M. bovis*BCG (Dormant stage) MIC_{90} (µg mL^{-1})
Selected 11 active compounds, High antimycobacterialactivityhighlitedwith red colour	42.89	36.32	8.51	9.82
	2.51	5.20	1.89	3.02
	38.32	>50	4.95	8.07
	45.86	42.70	1.37	2.34
	49.31	49.13	1.52	2.01
	24.58	35.21	2.29	3.35
	3.50	6.31	2.42	1.40
	17.51	18.49	6.30	9.37
	6.70	15.02	21.43	23.42
	8.42	11.54	4.08	4.51
	2.07	8.33	1.30	2.26
RIF	0.51	0.75	0.45	0.81

Table 2: Cytotoxicity profile of selected compounds against four human cancer cell lines (*In vitro*)

	MCF-7 (Breast) GI_{50} (µg mL^{-1})	A549 (Lung) GI_{50} (µg mL^{-1})	HCT 116 (Colon) GI_{50} (µg mL^{-1})	THP-1 (Leukemia) GI_{50} (µg mL^{-1})
Selected 11 active compounds, High anticancer activityhighlited with red colour	>100	>100	>100	>100
	33.80	27.36	>100	>100
	>100	>100	>100	>100
	>100	>100	>100	>100
	24.83	>100	17.64	>100
	>100	>100	>100	>100
	20.52	35.08	>100	>100
	26.12	45.44	42.41	>100
	19.68	34.15	>100	>100
	>100	>100	>100	>100
	>100	16.17	14.7	>100
	>100	>100	>100	>100
Paclitaxel	0.0048	0.0035	0.0260	0.1374

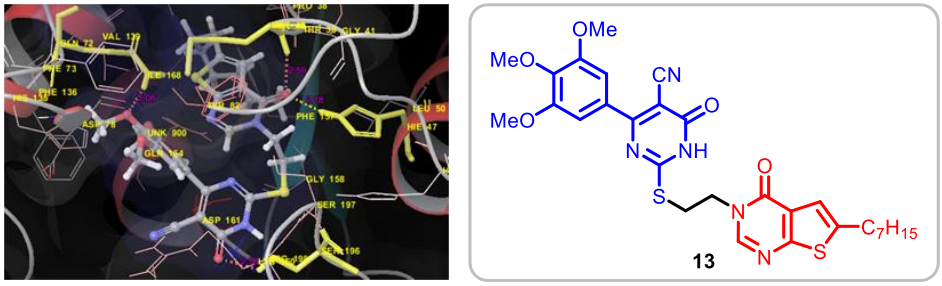
Section 3: Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents[8]

In this portion, a number of hybrid molecules containing thienopyrimidinones and thiouracil moieties were designed, synthesized, and tested against *Mycobacterium tuberculosis H37Ra*, where in antitubercular activity was observed in some of the compounds. Structural modifications of selected compounds were carried out to study the structure-activity relationship. Cytotoxicity studies revealed that these molecules were non-toxic. The docking study of selected compound showed that there is binding with the active site of mycobacterial pantothenatesynthetase.



Scheme 2: Synthesis of hybrid molecules

The compound no. 8 was synthesized using the well-known Biginelli reaction. It involved a three-component base-catalyzed reaction between an aldehyde, ethyl cyanoacetate, and thiourea to yield dihydrothiopyrimidones quickly and easily. A library of hybrid molecules was synthesized by using thienopyrimidinones and thiouracils. The docking study of compounds showed that there was binding with the active site of mycobacterial pantothenatesynthetase which gave valuable information for structural modification in synthesis. Some of the compounds have exhibited cytotoxicity against human cancer cell lines.



Representative example: 3D view of binding of compound with active site of mycobacterial PanC

Figure 2: Docking study of compound **13**

Table 3: Antimycobacterial activity data for selectedthienopyrimidinones

	Intracellular *(Ex Vivo)*		**Extracellular *(In Vitro)***	
	***M. tuberculosis* H37 Ra (Dormant Stage)**	***M. tuberculosis* H37 Ra (Active Stage)**	***M. tuberculosis* H37 Ra (Dormant Stage)**	***M. tuberculosis* H37 Ra (Active Stage)**
	MIC (µg/mL)	**MIC (µg/mL)**	**MIC (µg/mL)**	**MIC (µg/mL)**
Selected 8active compounds, High antimycobacterial activityhighlighted with red colour	**11.9±1.80**	**>30**	**19.1±0.16**	**>30**
	9.8±0.81	**>30**	**12.3±0.27**	**>30**
	7.1±0.91	**>30**	**8.7±1.11**	**>30**
	6.91±0.12	**>30**	**7.6±0.38**	**>30**
	26.1±1.45	**28.8±3.0**	**23.4±1.42**	**25.4±0.65**
	18.8±0.17	**17.1±0.61**	**19.1±0.56**	**15.6±0.59**
	13.6±0.23	**11.1±2.6**	**17.1±0.85**	**11.1±0.15**
	21.5±1.23	**26.9±1.66**	**26.1±1.52**	**27.9±1.82**
Rifampicin	**0.75±0.014**	**0.51±0.012**	**0.48±0.016**	**0.41±0.02**

3. <u>Summary:</u> The literature shows that thieno[2,3-*d*]pyrimidines compounds have a lot of potential for biological applications. Based on these compounds, several biologically active heterocyclic compounds have been discovered. This indicates that thieno[2,3-*d*]pyrimidines may be especially useful synthons in the development of novel highly efficient new drugs with a wide range of bioresponses. In present work Gewald reaction was used for the synthesis of 2-aminothiophene intermediates which were converted into various thienopyrimidinones. Biginelli reaction was used for synthesis of thiouracil intermediate, which was reacted with thienopyrimidinones to obtain hybrid molecules. Some of these molecules exhibited significant antimycobacterial activity. The synthetic strategies used in the present work have potential to prepare a large number of compounds for further refinement of structures and the present results will be very useful in the development of a new class of antimycobacterialas well as anticancer agents.

Chapter 2: Synthesis and biological evaluation of novel dicyanoanilines and indoles

Section 1: Introduction and Literature Reports

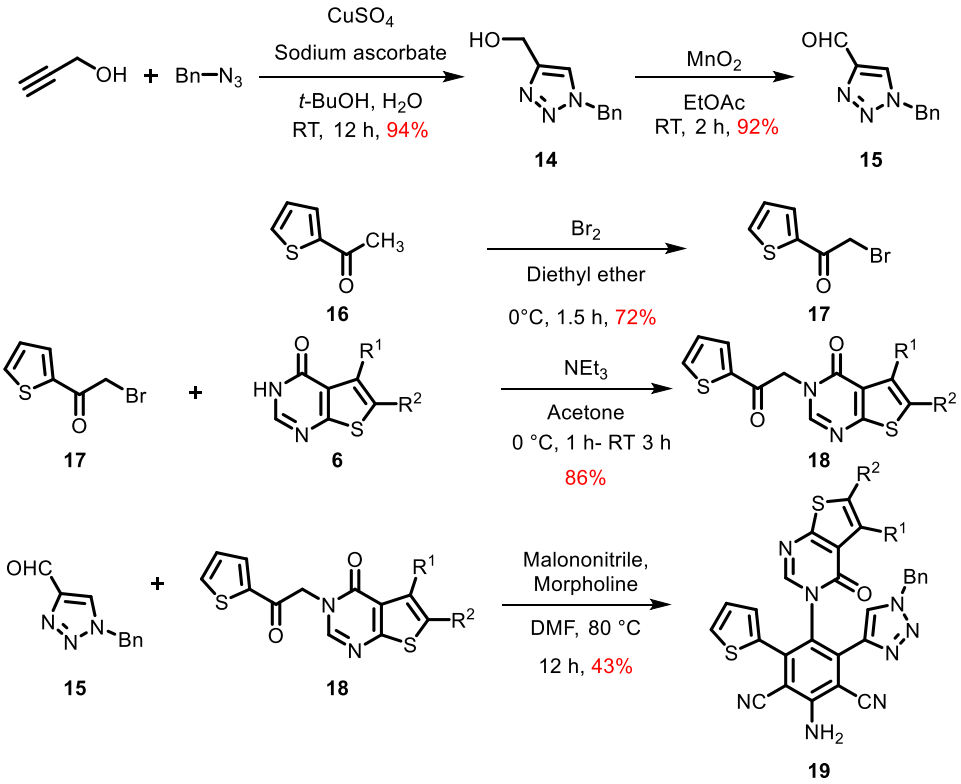
2,6-Dicyanoaniline derivatives, which are acceptor–donor–acceptor systems, are a fascinating class of substituted benzenes whereinbenzene ring contains one donor (NH_2) and two acceptor groups (CN) at 2 and 6 positions. Several compounds with acceptor–donor–acceptor frameworks, including substituted 2,6-dicyanoanilines, exhibit fluorescence. 2,6-Dicyanoaniline exhibits unique properties like organic dyes. Furthermore, different polymers contain 2,6-dicyanoanilines in their structure. 2,6-dicyanoaniline moiety have been used in light-emitting diodes, heat resistant polymers and light absorbing polymers. Various substituted 2,6-dicyanoanilines and associated compounds have also been investigated as chiral phase intermediates in chromatography. The structural features, optical properties, and implementations in liquid crystals of 2,6-dicyanoanilines and associated A-D-A systems have all been investigated as possible photosynthesis mimics. Substituted 2,6-dicyanoaniline derivatives also have biological properties such as anticancer, antimicrobial, antileishmanial, antihyperglycemic, antiamyotrophic lateral sclerosis, and growth-promoting properties due to their structures. Other than this there are various applications reported in literature. Hence the first aim was to investigate these compounds in biological applications.

This group reported synthesis of 2,3,5,6,7-pentasubstituted and 2,3,4,5,7-pentasubstituted indoles from 2,6-dicyanoanilines[9]. As per literature search the indole ring system can be found in a variety of natural products, and various methods for synthesizing the indole framework have been published. The Fischer indolesynthesis, discovered in 1883 by Hermann Emil Fischer and involving the reaction of phenyl hydrazine with an aldehyde or ketone under acidic conditions, is the most widely used method for constructing the indole skeleton. In year 2005 B. Narsaiah and colleagues[10] reported synthesis of substituted indoles by reacting 3,5- substituted- 2,6-dicyanoanilines with ethyl bromoacetate in the presence of potassium carbonate and potassium iodide. This group reported in 2011 synthesis of highly substituted indoles from 4,5-substituted- 2,6-dicyanoanilines. It was decided that the next aim would be to investigate these compounds in biological applications.

Section 2: Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl- 2,6-dicyanoanilines[11]

In this section, synthesis of 3,4,5-triheterocyclyl-2,6-dicyanoanilines, starting from heterocyclic aldehydes and 1,2-diheterocycle-substituted ethanones, is described. 2,6-Dicyanoanilines with one or two heterocyclic substituents have also been synthesized. It was found that some of these molecules have selective cell-staining properties useful for cell imaging applications. Some compounds stained the cytoplasm but not the nucleus of the cells in contact, while one compound hasd an affinity for apoptotic cells, resulting in blue fluorescence. The findings of cell imaging with one compoundis comparable to Annexin V-FITC, a known reagent containing recombinant Annexin V conjugated to green-fluorescent FITC dye, used for detection of apoptotic cells. These compounds were found to be non-cytotoxic and have potential application as cell imaging agents.

Various hitherto unknown 3,4,5-triheterocyclyl-2,6-dicyanoanilines were prepared from 1-benzyl-1,2,3[1*H*]-triazole-4- carboxaldehyde, malononitrile and required 1,2-diheterocyclyl ethanone as exemplified by the synthesis of 3,4,5-triheterocyclyl substituted 2,6-dicyanoaniline **19** shown in Scheme 3.This synthetic route was used to create a compound library for cell imaging study.



Scheme 3: Synthesis of 3,4,5-triheterocyclyl-2,6-dicyanoanilines

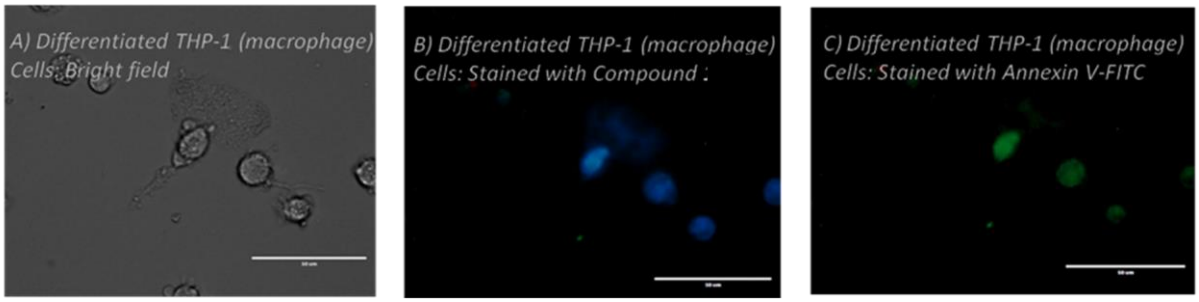
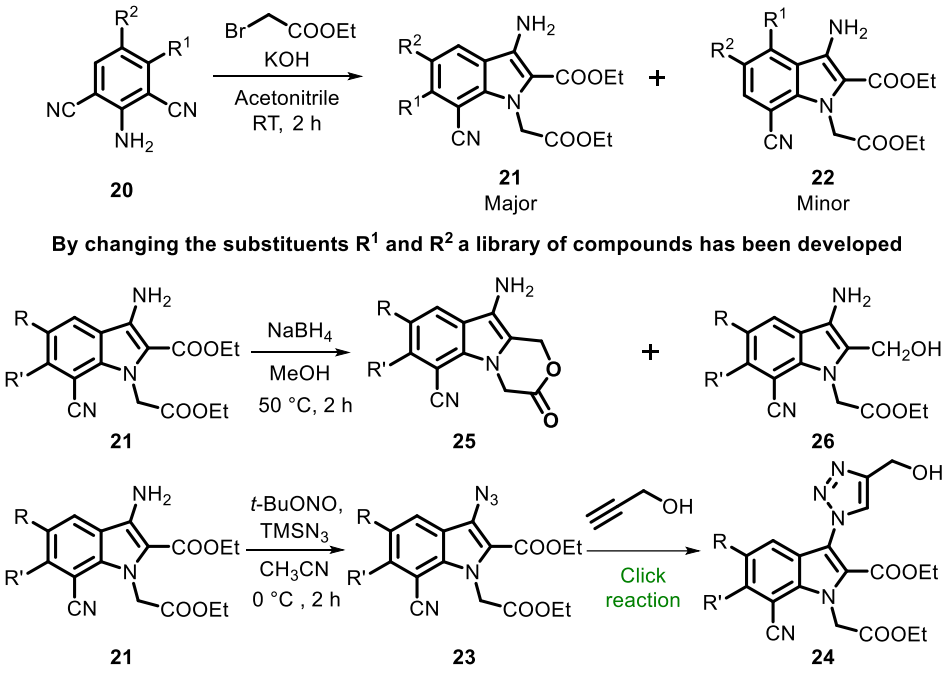


Figure 3: Differentiated THP-1 cells A) In bright field; B) Stained with compound C) Stained with Annexin V-FITC

Section 3: Synthesis and biological evaluation of highly functionalized 2,3,5,6,7- and 2,3,4,5,7-substituted indoles.



Scheme 4: Synthesis of pentasubstitutedindoles and their derivatives

Deal with the synthesis and biological assessment of substituted 2,6-dicyanoanilines in previous work. It was able to more easily synthesis 2,3,5,6,7-and 2,3,4,5,7-substituted indoles using 2,6-dicyanoaniline intermediate molecules. This indole synthesis process is able to produce a large number of new compounds forstructure-property studies to explore their utility as new substrates for different biological applications. Synthetic path of regioisomers 2,3,5,6,7-and 2,3,4,5,7-substituted indoles and their derivatisationis shown in scheme 4.

4.<u>Summary:</u> Synthesized 2,6-dicyanoanilines compounds were found to specifically stain only the cytoplasm of the cells but not the nucleus. This was verified by using known counter stains like SYTO 9 (for nucleus) which gives green fluorescence and Nile red (lipid molecules and cell membrane) which gives red fluorescence. One of the compounds was found to stain selectively some cells out of total cells and the patterns were similar to AnnexinV-FITC which is known to stain apoptotic cells. These results can serve as the basis for further study in the field of detecting apoptotic cells. highly substituted novel dicyanoanilines were converted in to hexasubstituted indoles and screened for anticancer, antibacterial, antioxidant, antitubercular activity. The synthesized indole compounds did not exhibit any activity and futher work is in progress.

Chapter 3: Studies towards the total synthesis of (±)-quinagolide

Section 1: Introduction and Literature Reports[12-14]

This section briefly discusses ergot alkaloids and apomorphine alkaloids. The combined structural characteristics of ergot alkaloids and apomorphineare also shown. Mainly eight synthetic approaches of quinagolide are discussed in brief.

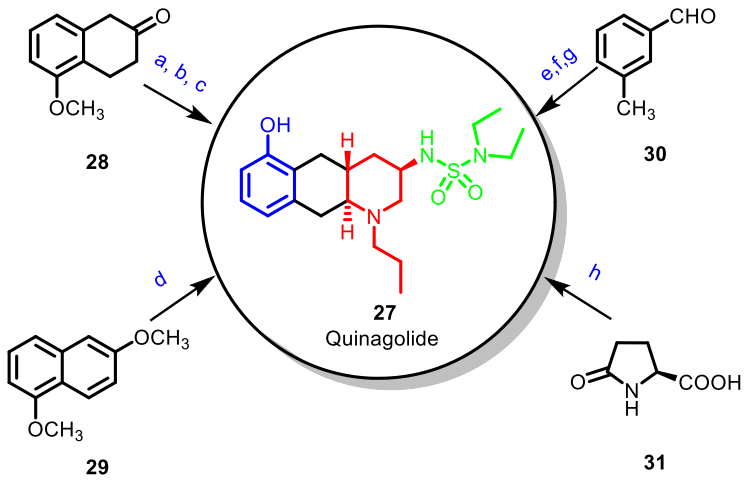
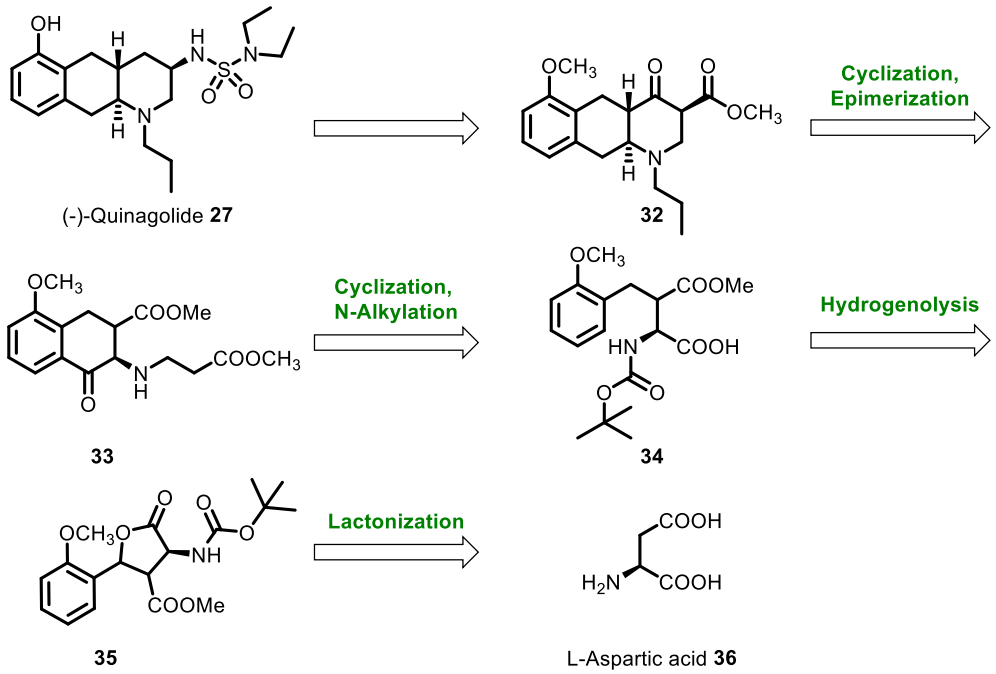


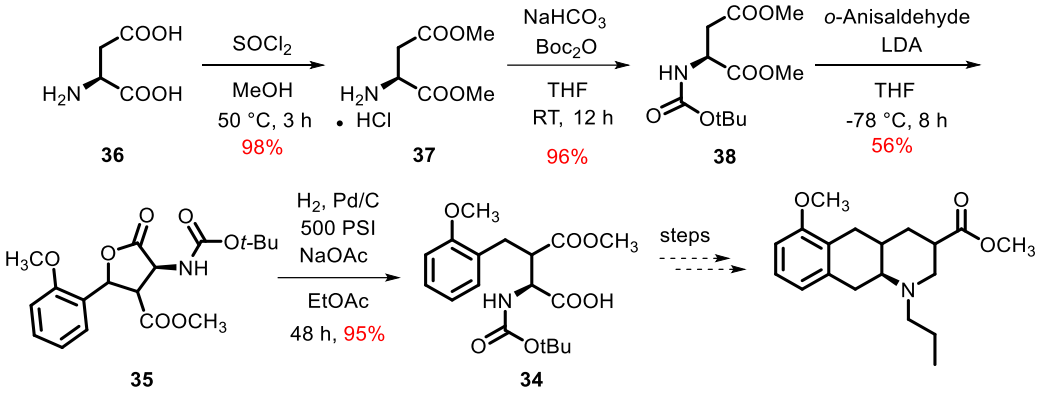
Figure 4: Reported synthesis from starting materials **28, 29, 30** and **31 a)** Nordmann *et al*. *J. Med. Chem.***1985**, *28*, 367-375. **b)** Nordmann *et al*. *J. Med. Chem.***1985**, *28*, 1540-1542. **c)** Morandi *et al*. *J. Am. Chem. Soc.* **2020,** *51*, 21548-21555. **d)** Banziger*et al*. *Org. Process. Res. Dev*,**2000**, *4*, 460-466. **e)** Chavan *et al*. *Org. Lett.* **2018**, *20*, 7011-7014. **f)** Chavan *et al*. *ACS Omega* **2019**, *4*, 8231-8238 **g)** Chavan *et al*. *Org. Lett.***2019**, *21*, 9089-9093. **h)** Chavan *et al*. (communicated)

Section 2, Part A: Studies towards the total synthesis of (-)-quinagolide

Banziger *et al.* published a scalable synthesis of quinagolide intermediate in year 2000[5] and this was in same line of Nordmann *et al.* approach reported earlier[4]. There have been eight synthetic methods published till date, two of which are chiral. Chavan *et al.* group was actively involved in the advanced enantioselective synthesis of (-)-quinagolide because of ongoing research in the synthesis of biologically active compounds. In this regard, finding a new enantioselective synthetic route for (-)-quinagolide, as well as a racemic approach to (±)-quinagolide, was extremely appealing which would lead to a shorter synthesis and higher yield. The current research was aimed at a practical and efficient approach for synthesizing (-)-quinagolide from L-aspartic acid, a low-cost and readily available starting material. In addition, another strategy to synthesize racemic quinagolide was attempted. The key characteristics of (-)-quinagolide synthesis are depicted in Scheme 5.

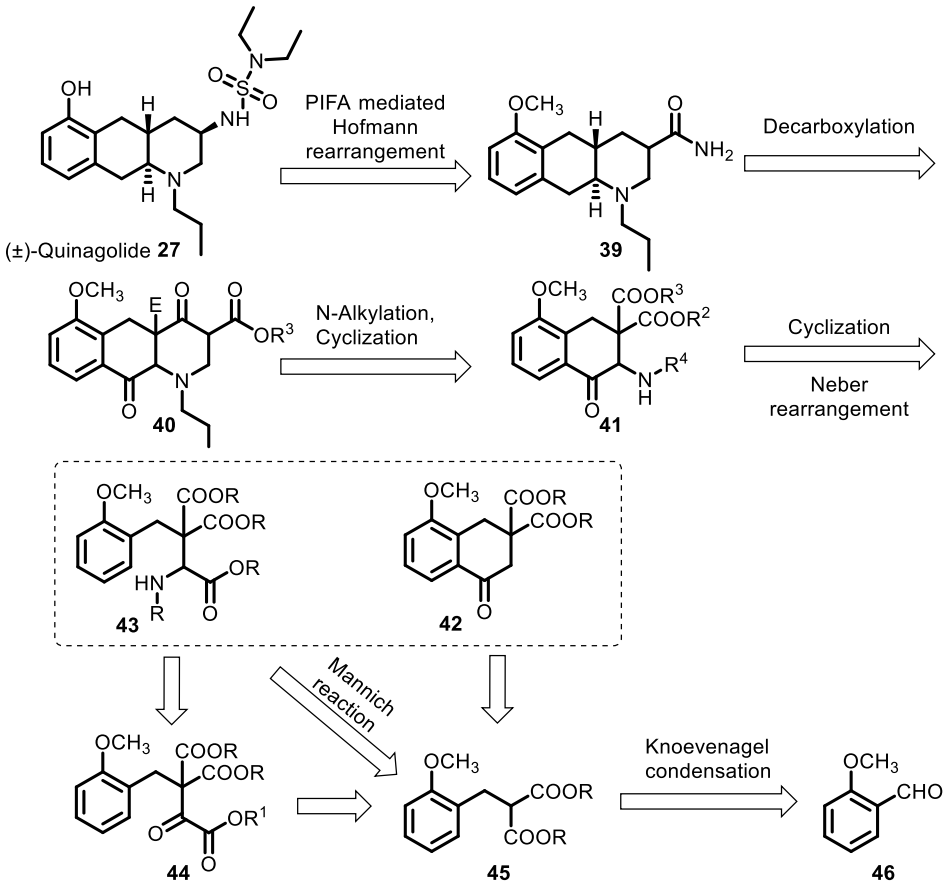


Scheme 5: Retrosynthetic analysis for (-)-quinagolide



Scheme 6: Synthetic studies towards the (-)-quinagolide

Part B: Studies towards the total synthesis of (±)-quinagolide

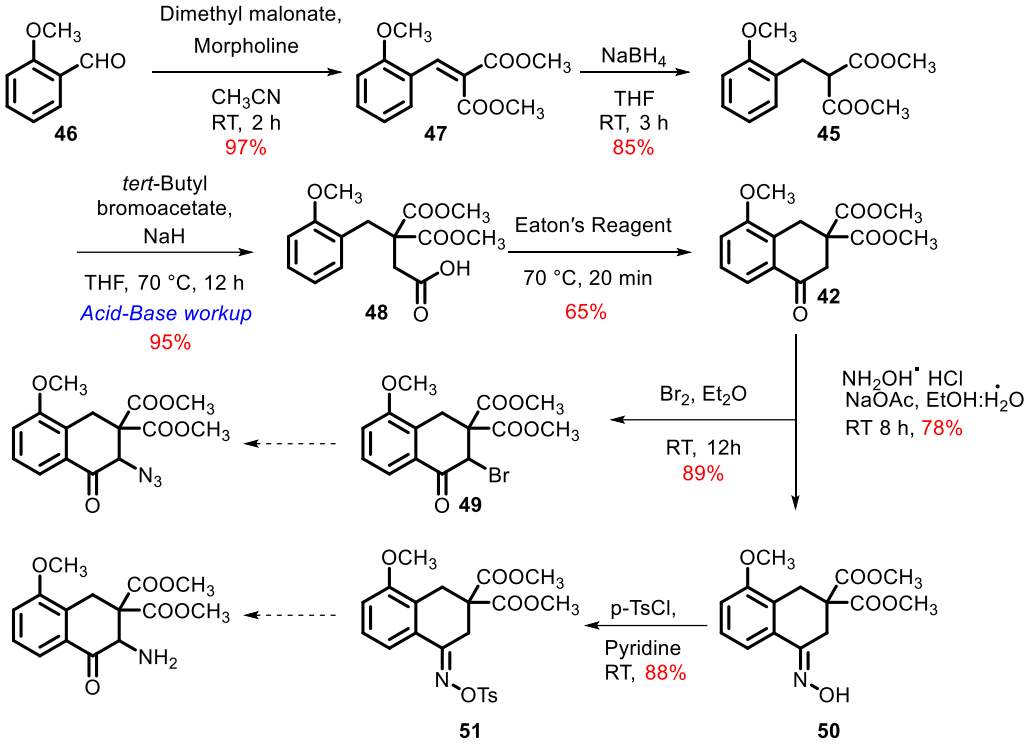


Scheme 7: Retrosynthetic analysis for (±)-quinagolide

Using commercially available and affordable *o*-anisaldehyde, a short and efficient synthetic route for the (±)-quinagolide framework has been intending to use the Mannich reaction and the oxime synthesis reaction. It may be a simple and effective way to make quinagolide. Optimized reactions have given high yield with minimum purification steps up to synthesized

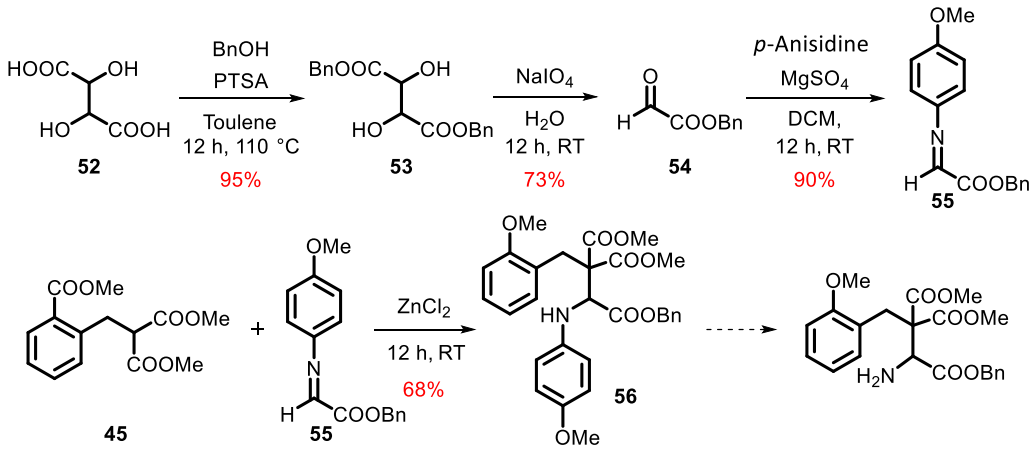
intermediate. Retrosynthetic analysis for the synthesis of (±)-quinagolideis shown in scheme 7. In present work common diester intermediate **45** was synthesized from *o*-anisaldehyde and dimethyl malonate as shown in scheme 8

1. Quinagolide tricyclic intermediate *via*oxime synthesis



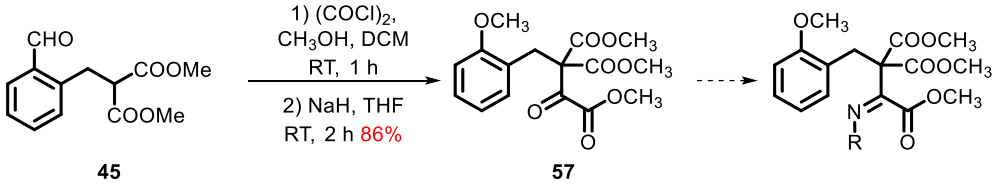
Scheme 8

2. Quinagolide tricyclic intermediate *via*mannich reaction



Scheme 9

3. Synthetic studies towards the (±)-quinagolide *via* imine synthesis



Scheme 10

4.<u>Summary:</u>Various attempts to prepare tricyclic intermediate for racemic as well as chiral quinagolide have been made. Further work is necessary to achieve the goal.

5.<u>Summary (Overall):</u>

- New thienopyrimidinones were synthesized and evaluated for their antimycobacterial potential.
- New thienopyrimidinonecompounds also showed potent anticancer activity against human cancer [MCF-7 (Breast), A549 (Lung) and HCT 116 (Colon)] cell lines.
- New hybrids of thienopyrimidinones and thiouracils were synthesized and evaluated for theirantimycobacterial potential.
- Synthesis of 3,4,5-substituted-2,6-dicyanoanilines, starting from 1-benzyl-1,2,3[1*H*]-triazole-4-carboxaldehyde, malononitrile and required aldehydes/ketones was achieved.
- Synthesized 2,6-dicyanoaniline compounds have considerable potential as valuable tools for cell imaging.
- Highly functionalized 2,3,5,6,7- and 2,3,4,5,7-substituted indoles were synthesized starting from 2,6-dicyanoanilines.
- Preliminary studies realeted to indole compounds have indicated that some of these molecules have selective cellstaining properties useful for cell imaging applications.
- Using commercially available and affordable L-Aspartic acid and o-anisaldehyde, a short and efficient synthetic route for the (-)-quinagolide and (±)-quinagolide framework has been attempted. Optimized reactions have given high yield with minimum purification steps.

6.Future directions:

A number of new molecules containing thienopyrimidinonemoieties have been synthesizedand screened for antitubercular activity as well as cell imaging applications. These molecules can be tested for antifungal activity in order to explore their potential in the treatment of mucormycosis because it is reported that compounds containing thienopyrimidinone moiety exhibit significant antifungal properties. Also, the compounds synthesized in the present work can be structurally modified further to generate new compounds for structure-activity relationship studies.

The methodology used for synthesis of compounds containing indole moiety in the present work, can be extended further to get combretastatin-inspired indoles and screened for anticancer activity.

The synthetic routes for racemic as well as (-)-quinagolide could be pursued further to get the desired molecules as the yields obtained are high in the steps which have been carried out so far in the present work.

7.Publications:

List of Publications:

1) Borate, H. B.; Annadate, R. A.; Vagh, S. S.; **Pisal, M. M.;** Deokate, S. B.; Arkile, M. A.; Jadhav, N. J.; Nawale, L. U.; Sarkar, D.; Synthesis and evaluation of thieno[2,3 *d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents. *MedChemComm* **2015**, *6*, 2209-2215.

2) **Pisal, M. M.;** Nawale, L. U.; Patil, M. D.; Bhansali, S. G.; Gajbhiye, J. M.; Sarkar, D.; Chavan, S. P.; Borate, H. B. Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents: SAR and docking studies. *Eur. J. Med. Chem.* **2017**, *127*, 459-469.

3) **Pisal, M. M.;** Annadate, R. A.; Athalye, M. C.; Kumar, D.; Chavan, S. P.; Sarkar, D.; Borate, H. B. Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl-2,6-dicyanoanilines. *Bioorg. Med. Chem. Lett.* **2017,** *27*, 979-988.

4) Kudale, A. S.; Kamble, S. B.; Gore, A. H.; **Pisal, M. M.**; Salokhe, A. T.; Kolekar, G.B.; Helavi, V.B. One-pot three-component synthesis and photophysical properties of highly fluorescent novel 4-alkyl-3-aryl-2,6-dicyanoanilines by using tris(hydroxymethyl)aminomethane as a catalyst. *CDC.* **2019,** *19*, 100172.

5) Chavan, S.P.; Kawale, S.A.; **Pisal, M. M.;** Kadam, A. L.; Gonnade. Formal Synthesis of (-)-Quinagolide: A Diastereoselective Ring Expansion *via* Bicyclic Aziridiniumion Strategy to Access Octahydrobenzo[*g*]quinoline Architecture. *Manuscript submitted.*

Patents:

1) Borate, H. B.; Chavan, S. P.; **Pisal, M. M.;** Annadate, R. A.; Sarkar, D.; Athalye, M. C. Novel compounds and uses thereof. WO 2016/199172 *Al* ; PCT/IN2016/050179.

8. References:

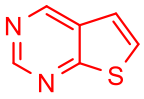
1) World Health Organization, Global tuberculosis report **2020**, ISBN 978-92-4-001314-8.
2) Borate, H. B.; Kudale, A. S.; Agalave, S. G. *Org. Prep. Proc. Int.* **2012**, *44*, 467–521.
3) Barlier, A. & Jaquet, P., *Eur. J. Endocrinol.* **2006,** *154*, 187–195.
4) Nordmann, R. & Widmer, A., *J. Med. Chem.* **1985**, *28*, 1540-1542.
5) Banziger, M.; Cercus, J.; Stampfer, W.; Sunay. U. *Org. Process Res. Dev.* **2000**, *4*, 460−466.
6) Abdel-Megid, M.; Elmahdy, K. M.; Elkazak, A. M.; Seada, M. H; Mohamed, O. F. *J Pharm Appl Chem.***2016**, *2*, 103-127
7) Borate, H.B.; Annadate, R.A.; Vagh, S.S.; Pisal, M.M.; Deokate, S.B.; Arkile, M.A.; Jadhav, N.J.; Nawale, L.U.; Sarkar, D. *MedChemComm* **2015**. *6.* 2209-2215.

8) Pisal, M.M.; Nawale, L.U.; Patil, M.D.; Bhansali, S.G.; Gajbhiye, J.M.; Sarkar, D; Chavan, S.P.; Borate, H.B. *Eur. J. Med. Chem.* **2017**, *127*, 459-469.
9) Sawargave, S. P.; Kudale, A. S.; Deore, J. V.; Bhosale, D. S.; Divse, J. M.; Chavan, S. P.; Borate, H. B. *Tetrahedron Letters*, **2011**, *52*, 5491-5493.
10) Maitraie, D.; Reddy, G. V.; Rama Rao, V. V. V. N. S.; Ravikanth, S.; Narsaiah, B.; Rao, P. S.; Ravikumar K.; Sridhar, B. *Tetrahedron*, **2005**, *61*, 3999-4008.
11) Pisal, M. M.; Annadate, R. A.; Athalye, M. C.; Kumar, D.; Chavan, S.P.; Sarkar, D.; Borate, H.B. *Bioorg. Med. Chem. Lett.* **2017,** *27*, 979-988.
12) Chavan, S. P.; Kadam, A. L.; Lasonkar, P. B.; Gonnade, R. G. *Org. Letters*, **2018**, *20*, 7011-7014.
13) Chavan, S. P.; Kadam, A. L.; Kawale, S. A. *ACS Omega*, **2019**, *4*, 8231-8238.
14) Chavan, S. P. ; Kadam, A. L.; Gonnade, R. G. *Org. Letters*, **2019**, *21*, 9089-9093.

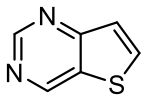
Chapter 1

Section 1

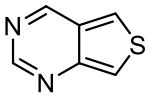
"General Outline of Thienopyrimidinone"



Thieno[2,3-*d*]pyrimidines



Thieno[3,2-*d*]pyrimidines



Thieno[3,4-*d*]pyrimidines

1.1.1. General outline of thienopyrimidinone

For medicinal chemists, pyrimidine has always been a rare heterocyclic moiety of interest. A thorough evaluation on pyrimidines has been carried out, leading to the discovery and marketing of many drugs. Fused heteroaromatic structures are also of much greater concern from the perspective of biological activity compared to the monocyclic molecules that make up the constituents. It seems that development in novel properties of fused heterocyclic molecules, with the introduction of new pharmacophore group to improve the interaction of heterocyclic biologically active compounds with wide range of different receptor are at paramount interest. Moreover, by annealing at various positions of individual heterocyclic fragments, the structure of the molecule can be varied. Due to their versatility and their broad bioactive potential, in the research of medicinal chemistry, fused pyrimidines also attracted significant attention[1-3]. Many studies indicate that thienopyrimidine is a well-known example of fused pyrimidines for pharmacological and biological applications. Design, synthesis of new thienopyrimidines for therapeutic use, are focused since last four decades and a significant number of studies have been reported on thienopyrimidines as they have shown a variety of biological activities such as antiviral, antidiabetic, antioxidant, anticancer, antidepressant, antiplatelet, antihypertensive, anti-inflammatory, antimicrobial, bronchodilatory, and phospodiesterase inhibition. Due to the association with various physiological components, thienopyrimidines shows various biological active properties.[4-6] Purine plays an essential biochemical role as an endogenous scaffold in a number of normal physiological functions such as respiration, inflammation, cell proliferation, *etc*.

1.1.2. Synthetic approaches of thieno[2,3-*d*]pyrimidines

Synthetic approaches of thieno[2,3-*d*]pyrimidines are well-developed. Construction of pyrimidine ring on the thiophene core structure or cyclization of thiophene on the pyrimidine ring has been accomplished to synthesize the thienopyrimidine molecule. For the synthesis of thieno[2,3-*d*]pyrimidine, various synthetic strategies have been outlined.

1) Pyrimidine annulations on the thiophene ring[7-9]

This is the mostly used approach of the synthesis of thieno[2,3-*d*]pyrimidine new In synthesis of new pyrimidine ring, mostly one carbon atom can be introduced in between two adjacent functional group on substituted thiophene to form thienopyrimidine core structure. With respect

to this synthetic strategy substituted thiophene ring with adjacent amine and ester group , thought to be strong building blocks for the synthesis of thienopyrimidines when combined with other reagents. Another example is thiophene compounds with vicinal cyano-amino and vicinal amino carboxamido groups which are thought to be good substrates for synthesizing thienopyrimidines by interacting with other reagents are shown in Figure 1.

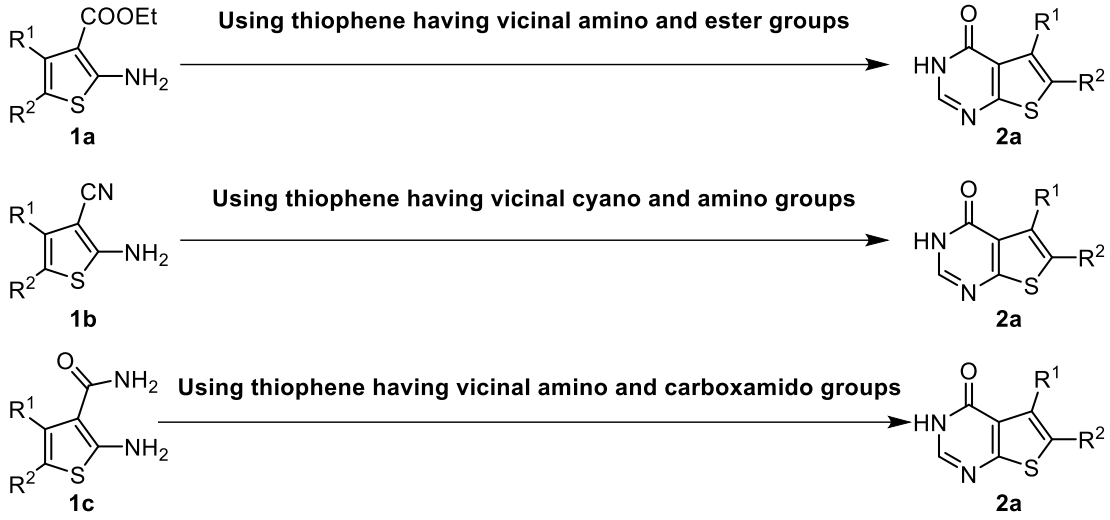


Figure 1. Synthesis of thienopyrimidines

2) Thiophene annulations on the pyrimidine ring[1]

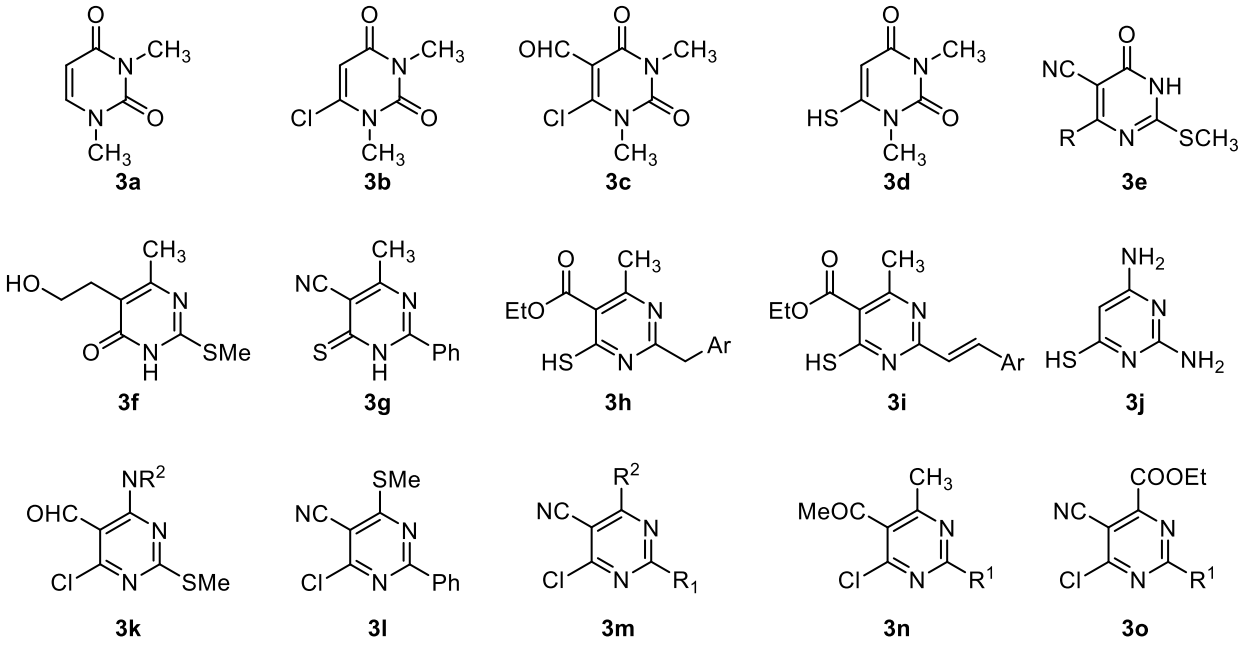
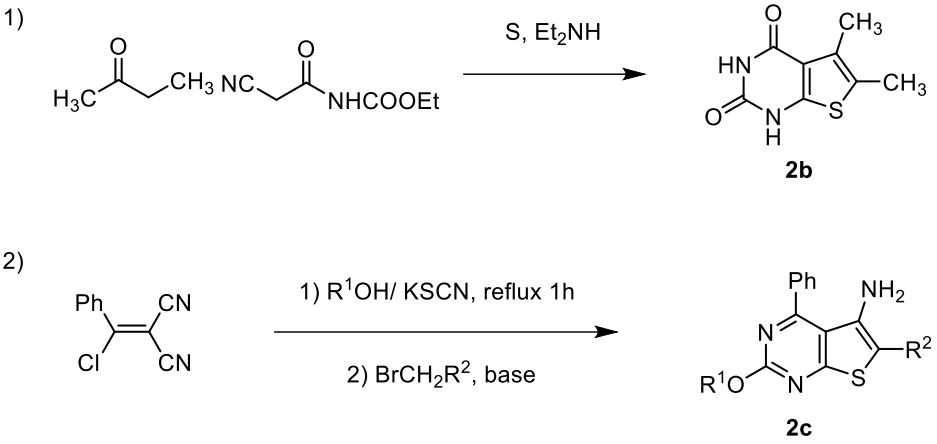


Figure 2. Pyrimidine rings which are used in the synthesis of thieno[2,3-*d*]pyrimidine scaffold

This is another synthetic strategy found in literature. The starting substituted pyrimidines are not easily accessible in thienopyrimidine core synthesis Since the core sulphur in thieno[2,3-*d*]pyrimidines is located at carbon-6, pyrimidine with a sulphur there will be good candidates for forming the thiophene ring. The few reported starting materials which are used in the synthesis of thieno[2,3-*d*]pyrimidine scaffold are shown in Figure 2.

3) Thienopyrimidines from acyclic compounds[1]

In the first approach in this strategy, acyclic compounds are used for one step synthesis of thieno[2,3-*d*]pyrimidines and in the second approach α-cyano-β-chlorocinnamonitrile and active bromomethylene derivatives are treated with potassium thiocyanate in presence of substituted alcohol. These both synthetic paths provide good yields.



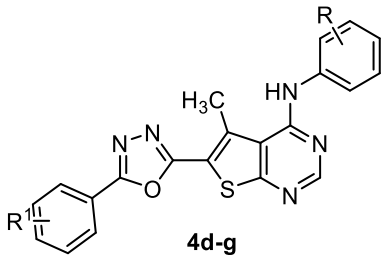
Scheme 1. Acyclic compounds used in the synthesis of thieno[2,3-*d*]pyrimidines

1.1.3. Biological applications

Phosphodiesterase inhibitors, angiotensin receptor, anticancer, anti-proliferative activities, immunosuppressive agents, prophylaxis and therapy of ischemic stroke, malaria, Alzheimer's disease, and Parkinson's disease are just some of the biological and pharmacological activities of thienopyrimidine derivatives that have been covered by a number of patents around the world.

Thienopyrimidinones are used in herbicides, insecticides, and fungicides in agriculture sector as well as they exhibit antiviral, antimicrobial, antiallergic, antibacterial, antihypertensive, anti-inflammatory activities, antiatherosclerotic, antidiabetic, analgetic, antidepressant, antihistaminic, and spasmolytic activities.

Liuyu, D. and coworkers[10] reported the synthesis of 4-substituted aminothieno[2,3-*d*] pyrimidines and the synthesized thienopyrimidinones were evaluated for antioxidant and anticancer biological properties. Compounds **4d-g** and compound **5** exhibited promising antioxidant and anticancer activities.



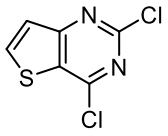
4d-g

4d-R= 2,4-di-F, R^1=4-Cl

4e-R= 2,4-di-F,R^1=H

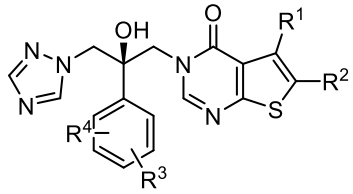
4f-R= 4-F,R^1=H

4g-R= 4-F,R^1=4-Cl

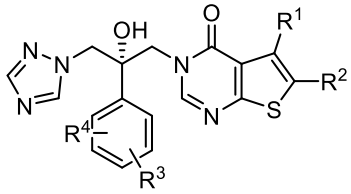


5

H. B. Borate and group reported[11] the synthesis of fluconazole analogues containing thieno[2,3-*d*]-pyrimidinones. Synthesized new molecules were tested as antifungal agents. Synthesized molecules e.g. **6a** and **6b** exhibited promising antifungal activities.



6a



6b

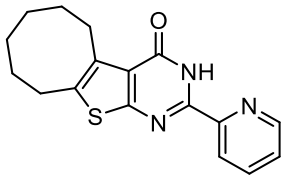
R^1= H, R^2= n-propyl, R^3,R^4= 2F and 4F

R^1= H, R^2= n-pentyl, R^3,R^4= 2F and 4F

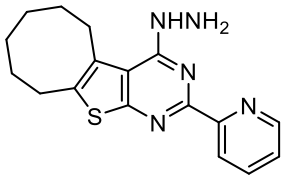
R^1= H, R^2= n-hexyl, R^3,R^4= 2F and 4F

R^1= H, R^2= n-heptyl, R^3,R^4= 2F and 4F

E. M. Gedawy and group[12] reported synthesis and biological activity of thieno[2,3-*d*]-pyrimidines **7a** and **7b** which showed very good activity in lower concentration compared with doxorubicin as potent anticancer drug.

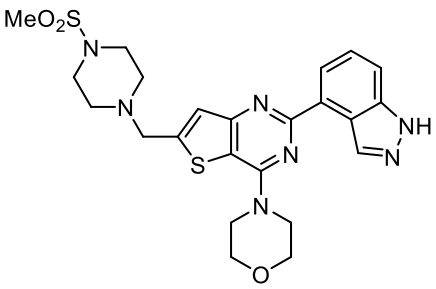


7a

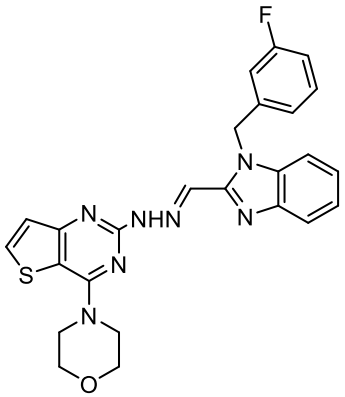


7b

Thienopyrimidine derivative **GDC-0941** and compound **8** showed anticancer activity against human lung cancer (H460), human colon cancer (HT-29), and human breast cancer (MDA-MB-231) cell lines, respectively.[13]

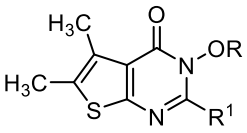


GDC-0941



8

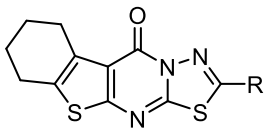
4-Amino thieno[2,3-*d*]pyrimidine derivatives were synthesized by El-Gazzar and coworkers[1]. They reported anti-inflammatory, analgesic and ulcerogenic activity of these compounds.



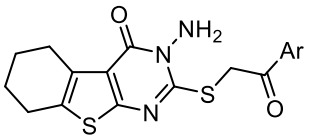
9

R= H, Alkyl, R^1= ethyl

Ashalatha and coworkers reported in year 2006 anti-inflammatory and CNS depressant activities of the synthesized compounds. The pharmacological activities of a few of the compounds **10** and **11** were promising.[14]



10



11

1.1.4. Conclusion and Prospect

The literature shows that thieno[2,3-*d*]-pyrimidines compounds have a lot of potential for biological applications. Based on these compounds, several biologically active heterocyclic compounds have been discovered. This indicates that thieno[2,3-*d*]-pyrimidines may be especially useful synthons in the development of novel highly efficient new drugs with a wide range of bioresponses.

1.1.5. References

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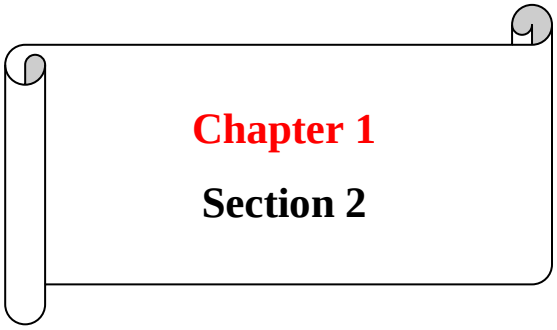
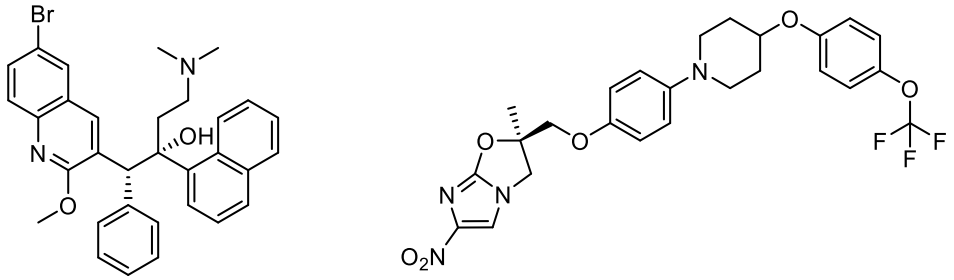


"Synthesis and evaluation of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents"

1.2.1. Introduction

Tuberculosis (TB) is a significant global health epidemic, with an estimated 10 million people contracting the disease and 2.9 million dying from it in 2019-2020, according to the 2020 global tuberculosis report.[1] which contains data from 202 countries and territories. The occurrence of multidrug resistant tuberculosis (MDR/RR-TB) is complicating the situation further because in 2019 globally 3.3% cases reported newly along with previous 18% cases of MDR/RR-TB. These facts indicate that development of new TB drugs is of great importance. In addition, the emergence of extensively drug resistant TB (XDR-TB) also makes the efforts in this direction necessary as generally, about 9.0% of MDR/RR-TB patients are extensive drug resistant TB (XDR-TB) patients. However, the number of new drugs approved for TB treatment is negligible, and in December 2012, U. S. A. Based Food and Drug Administration (FDA) has accepted Bedaquiline **(1)** as shown in Figure 1. For the treatment of pulmonary multi drug resistant tuberculosis, this was the first reported new treatment in last 40 years.



Bedaquiline (1) **Delamanid (2)**

Figure 1. Structures of Bedaquiline and Delamanid.

Further in November 2013 delamanid **(2)** got conditional acceptance by European Medicine Agency (EMA) in the treatment of MDR/RR TB Search for new scaffolds is necessary to overcome the problem of limited choice of current TB drugs. Research in this direction has resulted a few hits e.g. **3**,[2] **4**,[3] **5**,[4,5] **6**[6] and **7**[7] (Fig. 2). We wished to explore the potential of thienopyrimidinones as antitubercular agents. Substituted thienopyrimidinones exhibit various biological activities, as well as constitute molecular backbone with number of other biologically active molecules, but their potential as antitubercular agents is rarely explored.[8] Substituted thieno[2,3-*d*]pyrimidin- 4(3*H*)-ones **10** can be easily prepared from substituted 2-aminothiophene-3-carboxylates **9**, which in turn are prepared in one step by the Gewald synthesis [9,10] from easily available aldehydes or ketones **8** as starting materials (Scheme 1).

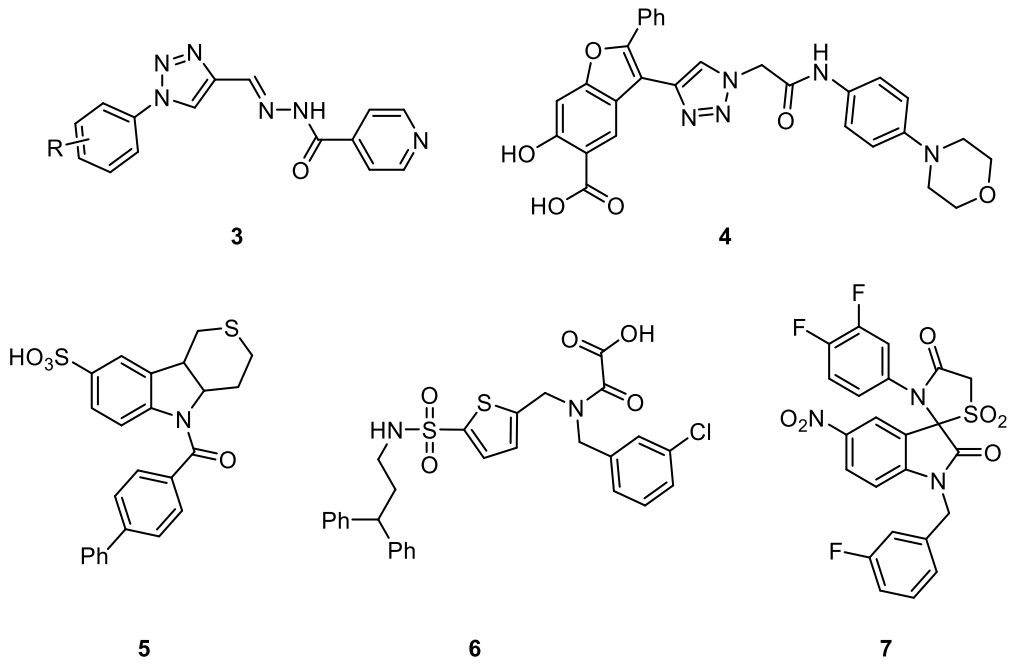


Figure 2. Structures of compounds **3**–**7** exhibiting antimycobacterial activity.

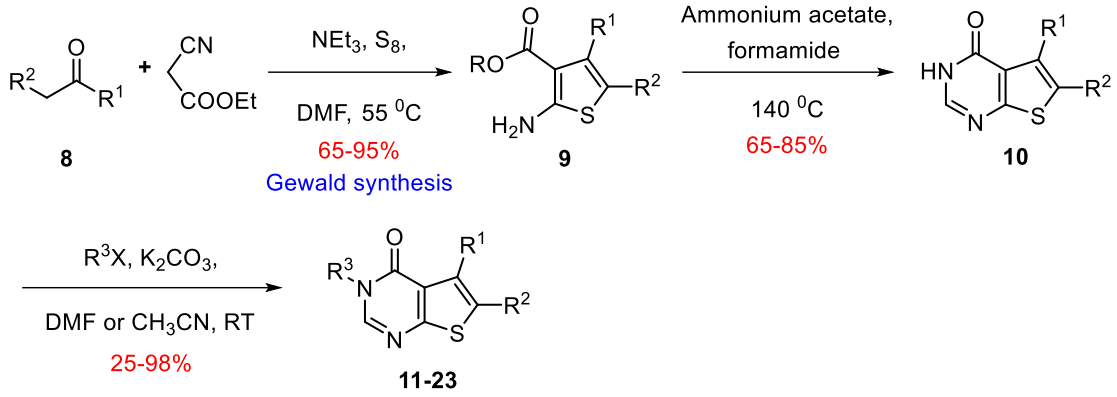
The thienopyrimidinone of type **10** can be functionalized at various positions and thus provide an opportunity to have a number of compounds available for biological activity screening. A library of thienopyrimidinones was designed, synthesized and screened against mycobacteria.[11] Encouragingly, some of the molecules exhibited significant antimycobacterial activity; therefore, the work was continued further and the results are reported herein.

1.2.2. Present work

1.2.2.1. Chemistry

The substituted 2-aminothiophene-3-carboxylates **9** and thienopyrimidinone **10** were synthesized as reported in earlier work.[12–13] Thus, various aldehydes or ketones were subjected to Gewald synthesis using ethyl cyanoacetate and sulphur in the presence of triethyl amine in DMF to obtain the substituted 2-aminothiophene-3-carboxylates **9**[11] which were characterized by ^{1}H NMR, ^{13}C NMR spectroscopies, IR spectroscopy, and HRMS analysis. The spectroscopic analysis was in full agreement with the reported values. The intermediates **9** were reacted further with ammonium acetate and formamide to obtain the corresponding thienopyrimidinones **10**.[11] A number of variously substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones with general structures **11** to **23** were obtained from thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** by reaction with the corresponding

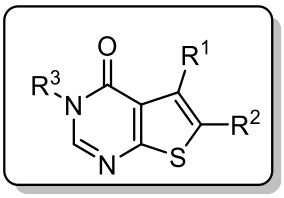
halides in DMF or acetonitrile in the presence of potassium carbonate, and further functional group transformations, as shown in Scheme 1.



Scheme 1. Synthesis of compounds **11-23**.

Thus, reactions of suitable thienopyrimidinones **10** with various alkyl bromides afforded alkylated thienopyrimidinones **11a** to **11m** which were characterized by spectral methods. The compounds **12a** to **12e** were prepared by using benzyl bromide while the compounds **13a** and **13b** were synthesized by using bromoacetonitrile. Using the same reaction strategy, thienopyrimidinones **10** were reacted with (bromomethyl)cyclopropane to obtain compounds **14a** to **14c**. Compound **15** was synthesized by using 1,4-bis(bromomethyl)benzene and thienopyrimidinone **10**. N-Alkylation of thienopyrimidinones **10** with 2-(2-bromoethyl)-1,3-dioxolane and 2-(2-bromoethyl)-1,3-dioxane gave compounds **16a-16c**. The halides selected for N-alkylation where easily available, inexpensive or synthesized easily. For example, 2,4-difluoroacetophenone was reacted with acetic acid and bromine to give 2-bromo-1-(2,4-difluorophenyl)ethanone in quantitative yield. 2-Bromo-1-(2,4-difluorophenyl)ethanone was reacted with the required thienopyrimidinones **10** to give compounds **17a** to **17c**. Compound **18a** was synthesized from required thienopyrimidinone **10** and allyl bromide. Compounds **19a** to **19c** were synthesized by alkylation reaction between required thienopyrimidinones **10** with ethyl 2-bromoacetate and further hydrolysis gave acids **20b** and **20c**. Bromoacetaldehyde diethyl acetal was reacted with the required thienopyrimidinones to get the compounds **21a** and **21b.** Deprotection of **21a** with conc HCl in acetonitrile afforded aldehyde **22a**. The reactions of 2-bromo-1-(thiophen-2-yl)ethan-1-one with various thienopyrimidinones provided corresponding ketones **23a** to **23c**.

Synthesized compounds of 11-23

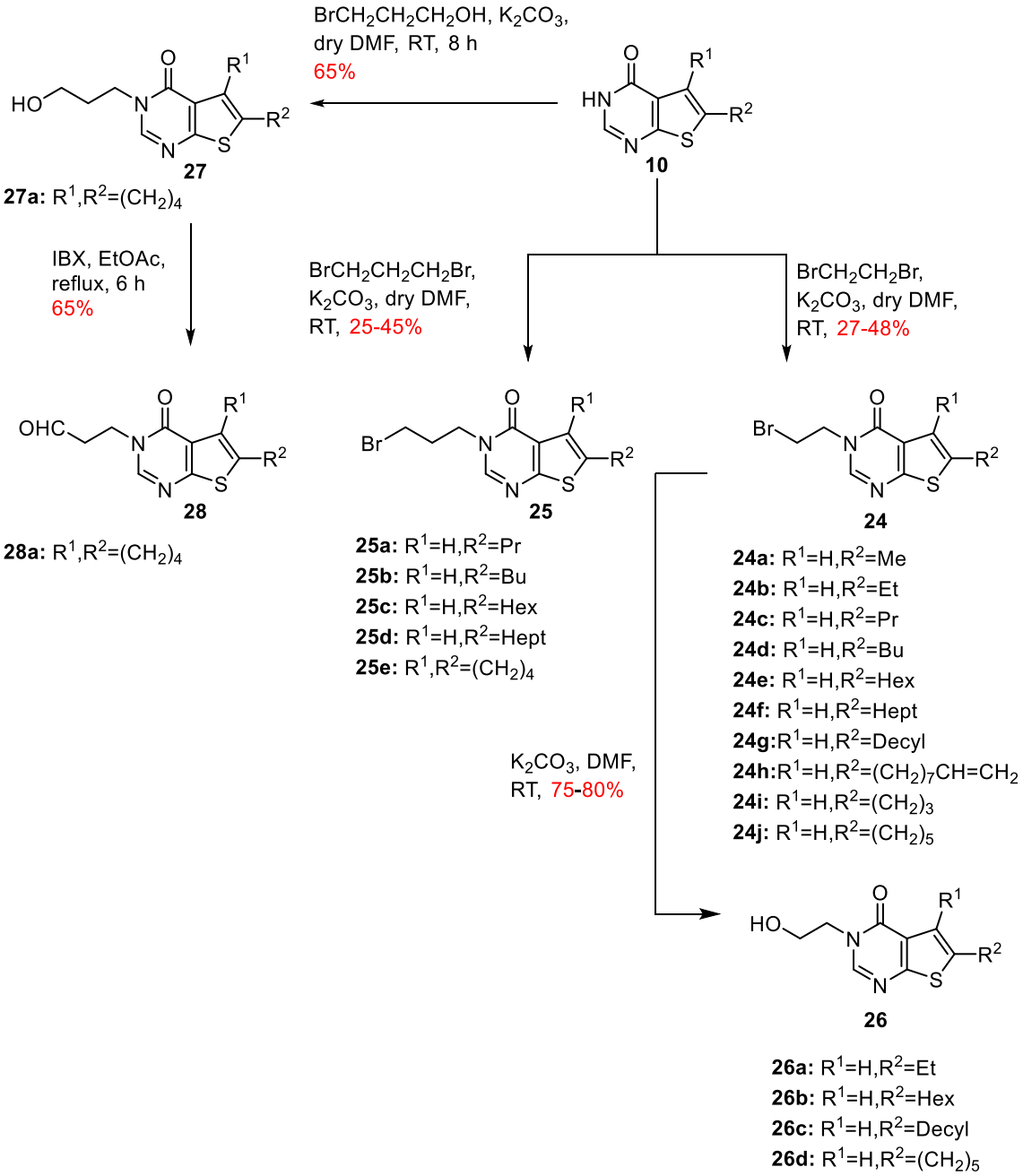


11a: R^1=H, R^2=Me, R^3=Pr
11b: R^1=H, R^2=Me, R^3=i-Pr
11c: R^1=H, R^2=Me, R^3=i-Bu
11d: R^1=H, R^2=Et, R^3=Et
11e: R^1=H, R^2=Et, R^3=Pr
11f: R^1=H, R^2=Et, R^3=i-Pr
11g: R^1=H, R^2=Et, R^3=Pent
11h: R^1=H, R^2=Et, R^3=Oct
11i: R^1=H, R^2=Pr, R^3=Pr
11j: R^1=H, R^2=Pr, R^3=i-Pr
11k: R^1=H, R^2=Pr, R^3=Oct
11l: R^1=H, R^2=Pent, R^3=Me
11m: R^1=H, R^2=Pent, R^3=Pr
11n: R^1,R^2=-$(CH_2)_4$, R^3=Me
12a: R^1=H, R^2=Et, R^3=CH_2Ph
12b: R^1=H, R^2=Pr, R^3=CH_2Ph
12c: R^1=H, R^2=Bu, R^3=CH_2Ph
12d: R^1=H, R^2=Pent, R^3=CH_2Ph
12e: R^1=H, R^2=Hex, R^3=CH_2Ph
13a: R^1=H, R^2=Pent, R^3=CH_2CN
13b: R^1=H, R^2=Hept, R^3=CH_2CN

14a: R^1=H, R^2=Pent, R^3=CH_2-cyclopropyl
14b: R^1=H, R^2=Hex, R^3=CH_2-cyclopropyl
14c: R^1=H, R^2=Hept, R^3=CH_2-cyclopropyl
15: R^1=H, R^2=Pr, R^3=CH_2-(4-bromomethyl)phenyl
16a: R^1=H, R^2=Pent, R^3=2-(1,3-dioxolan-2-yl)ethyl
16b: R^1, R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxolan-2-yl)ethyl
16c: R^1, R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxan-2-yl)ethyl
17a: R^1=H, R^2=Me, R^3=CH_2-CO-2,4-difluorophenyl
17b: R^1=H, R^2=Pr, R^3=CH_2-CO-2,4-difluorophenyl
17c: R^1=H, R^2=Pent, R^3=CH_2-CO-2,4-difluorophenyl
18a: R^1=H, R^2=Bu, R^3=allyl
19a: R^1=H, R^2=Pent, R^3=CH_2COOEt
19b: R^1=H, R^2=Et, R^3=CH_2COOEt
19c: R^1=H, R^2=Me, R^3=CH_2COOEt
20b: R^1=H, R^2=Et, R^3=CH_2COOH
20c: R^1=H, R^2=Me, R^3=CH_2COOH
21a: R^1=H, R^2=Bu, R^3=CH_2-$CH(OEt)_2$
21b: R^1=H, R^2=Me, R^3=CH_2-$CH(OEt)_2$
22a: R^1=H, R^2=Bu, R^3=CH_2-CHO
23a: R^1=H, R^2=Pr, R^3=CH_2-CO-thiophen-2-yl
23b: R^1=H, R^2=Hex, R^3=CH_2-CO-thiophen-2-yl
23c: R^1, R^2=-$(CH_2)_4$, R^3=CH_2-CO-thiophen-2-yl

Figure 3. The synthesized derivatives of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones

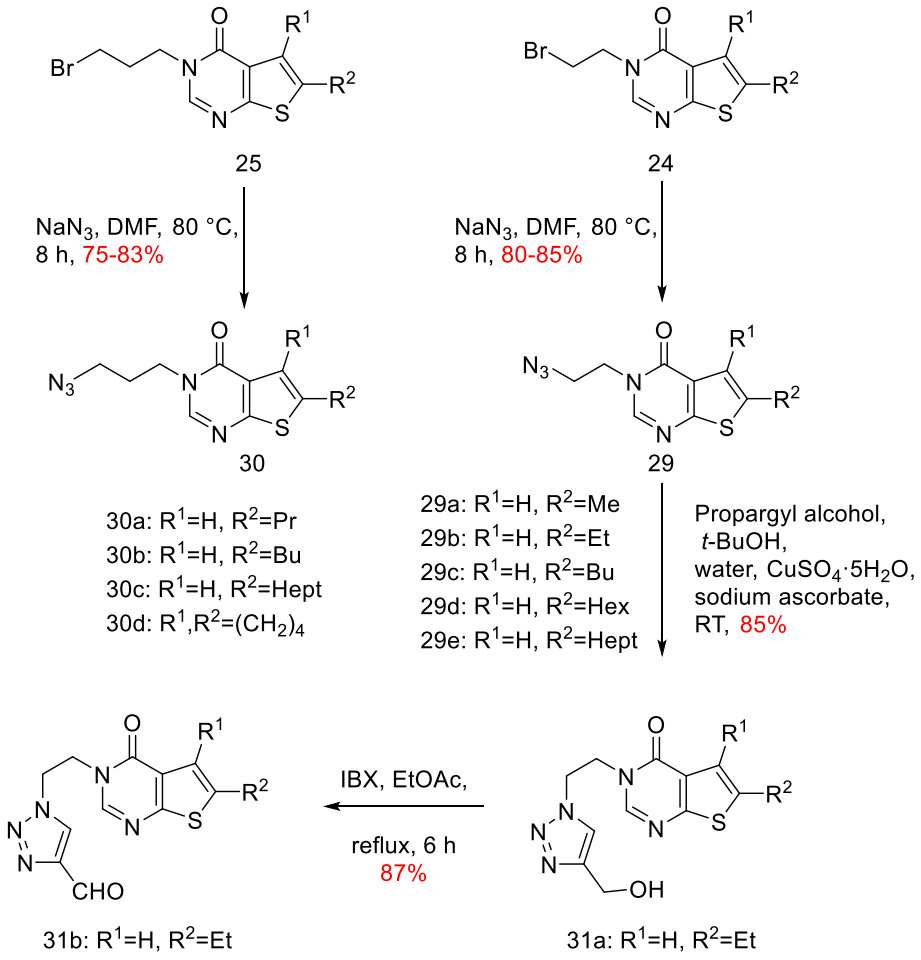
The synthetic scheme for derivatization of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones at nitrogen (N-alkylation):



Scheme 2A.

Compounds **24a** to **24j** were synthesized from required thienopyrimidinones **10** and 1,2-dibromoethane. In this reaction, yield obtained varied in the range of 27 to 48% because it was

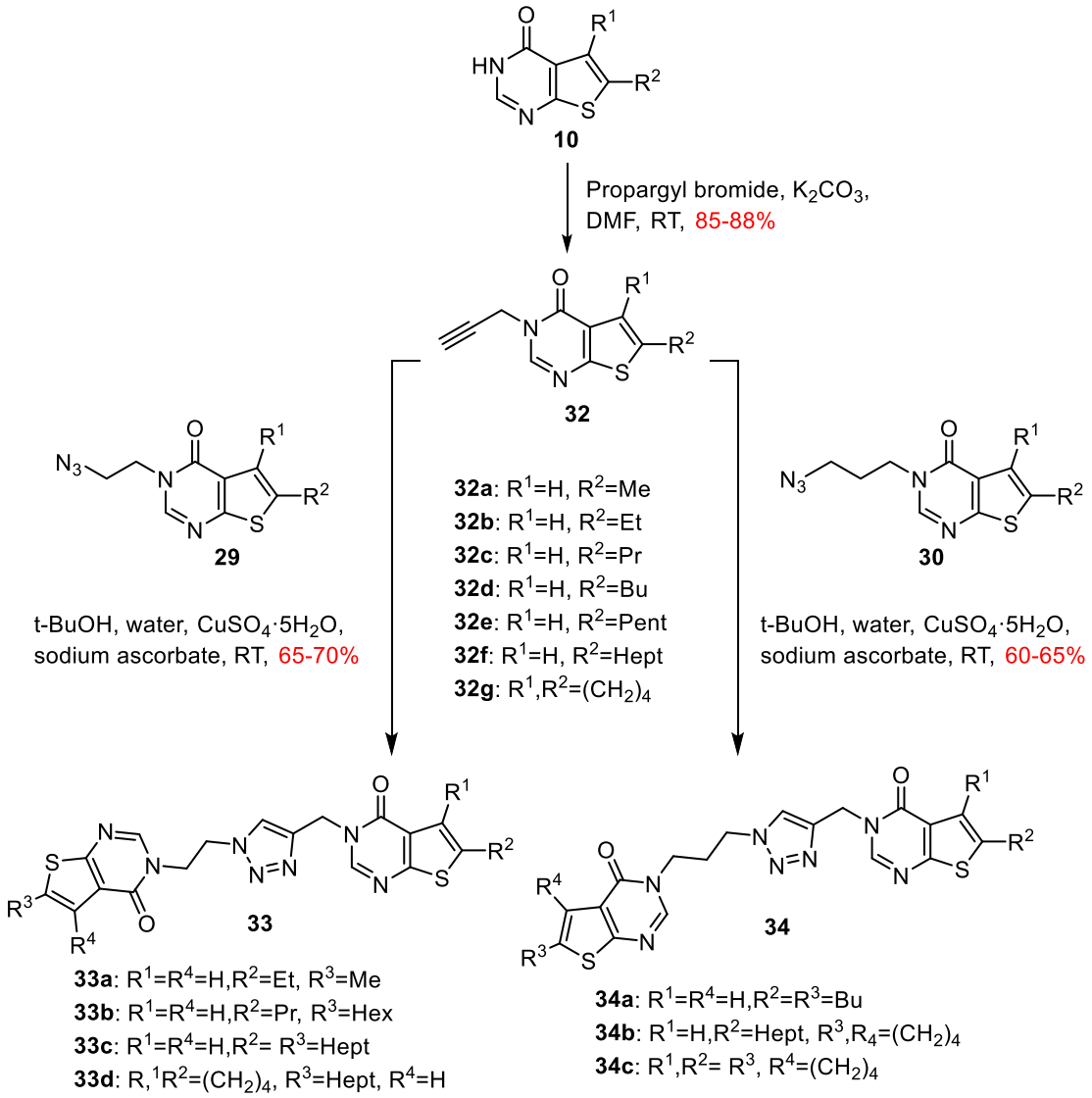
observed that other than bromo compound **24,** side products were formed depending on the base and solvent used, the moisture present in the solvent. The equivalents of the base and 1,2-dibromoethane used for example, when dry DMF was not used in the preparation of **24b**, the compound **26a** was formed predominantly. Also when excess of thienopyrimidinone **10** was used, compound **35** and **36** were formed. Elimination of HBr from compound **24** to give compounds with vinyl side chain was also observed in some cases. Similar reactions took place when dibromoethane was used in the place of dibromopropane.



Scheme 2B.

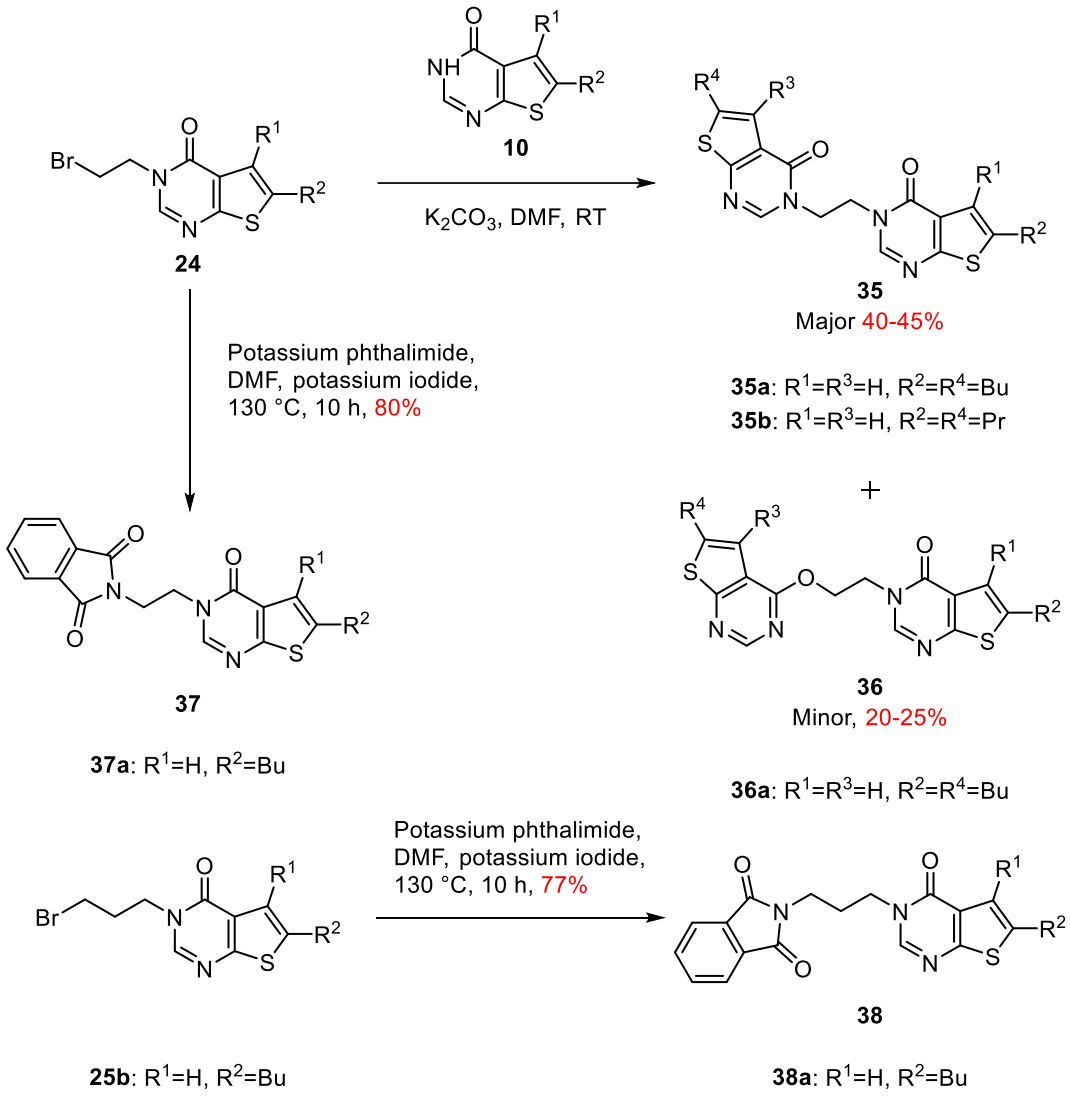
Further conversion of bromo compound **24** to hydroxy compound **26** was accomplished in the range of 75 to 85 % yield using potassium carbonate and DMF with a trace amount of water. Compound **27** was synthesized by *N*-alkylation reaction with required thienopyrimidinone **10** and 3-bromopropan-1-ol in 65% yield. Compound **27** was subjected to oxidation reaction with IBX to give the oxidized product **28** in 65% yields as shown in Scheme 2A.Compound **25** and

compound **24** were converted in azide compounds **29** and **30** respectively by using sodium azide and DMF to give 75 to 85 % yield. Further, azide compound was treated with propargyl alcohol under Cu-catalyzed click reaction condition to give triazole compound **31a** in 85% yield. Compound **31a** was oxidized with IBX to obtain aldehyde **31b** in 87 % of yield as shown in Scheme 2B.



Scheme 3.

The thienopyrimidinone **10** was functionalized *via* N-alkylation reaction by using propargyl bromide to get compound **32** in 85 to 88% yields. The reactions of the acetylene functionality in thienopyrimidinones **32** with the azide functionality thienopyrimidinones **29** and **30** under Cu-catalyzed click reaction resulted in the formation of triazole compounds **33** and **34** in good yield.



Scheme 4.

The reaction of thienopyrimidinone **10** with bromide **24** resulted in the formation of mixture of compounds **35** and **36** which could be separated by column chromatography and characterized by spectral methods. When the bromo compounds **24** and **25** were reacted with potassium phthalimide, the compounds **37** and **38** were obtained in good yields (Scheme 4). The main advantages of the synthetic sequences depicted in schemes 1, 2, 3, and 4, are that all reagents are readily available, low in cost, non toxic, and produce a high yield, thus these things facilitate the maximum derivatization possibilities of the main scaffold thienopyrimidinone **10** and thus suitable for gram scale synthesis. Also these synthetic strategies have potential to generate a large number of compounds for structure-activity relationship studies.

1.2.2.2. Biological evaluation

1.2.2.2.1. Antimycobacterial activity.

All the synthesized compounds were screened for their *in vitro* activity against *M. tuberculosis* H37Ra (MTB) (ATCC 25177) and *M. bovis* BCG (BCG) (ATCC 35743) using a two-fold dilution technique, in order to determine the actual minimum inhibitory concentration (MIC). Activity against MTB was determined through the XTT reduction menadione assay (XRMA), reading absorbance at 470 nm as per the protocol described by Singh *et al*.[14] Briefly, a compound solution (2.5 µl) was added in a total volume of 250 µl of *M. pheli* medium consisting of the MTB and BCG; sealed with plate sealers and allowed to incubate for 8 days (active stage) and 12 days (dormant stage) at 37 °C. The XRMA was then carried out to estimate viable cells present in different wells of the assay plate. To all wells, 200 µM XTT was added and incubated at 37 °C for another 20 min. It was followed by the addition of 60 µM of menadione and incubated at 37 °C for 40 min. The optical density was measured using a microplate reader (SpectraMax Plus 384 plate reader, Molecular Devices Inc.) at 470 nm wavelength against a blank prepared from a well free of cells. Absorbance obtained from the cells treated with 1% DMSO alone was considered as 100% cell growth. The nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG by the compounds.[15] Briefly, in the NR assay, 80 µl of culture from an incubated 96 well plate was taken into another 96 well plate, then 80 µl of 1% sulfanilic acid in 20% of conc. HCl was added, incubated for 10 min at room temperature, and then 80 µl of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution in distilled water was added. Finally, absorbance for the NR assay was measured at 540 nm.

In vitro activity studies against MTB and *M. bovis* BCG at active (8 days) and dormant (12 days) stages were performed using the XRMA and NR assays, respectively, as described above. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(\text{control}–\text{CMP})/(\text{control}–\text{blank})] \times 100$$

where ‘control’ is the activity of mycobacteria without compounds, ‘CMP’ is the activity of mycobacteria in the presence of compounds and ‘blank’ is the activity of the culture medium without mycobacteria.

Table 1: Antimycobacterial activity data for various thienopyrimidinones

Comp no.	*M. tuberculosis* H37 Ra (Active stage) MIC_{90} (μg mL^{-1})	*M. tuberculosis* H37 Ra (Dormant stage) MIC_{90} (μg mL^{-1})	*M. bovis* BCG (Active stage) MIC_{90} (μg mL^{-1})	*M. bovis* BCG (Dormant stage) MIC_{90} (μg mL^{-1})
11g	44.13	43.76	10.09	20.76
11m	42.89	36.32	**8.51**	**9.82**
13b	**2.51**	**5.20**	**1.89**	**3.02**
14a	38.32	>50	**4.95**	**8.07**
14b	45.86	42.70	**1.37**	**2.34**
14c	49.31	49.13	**1.52**	**2.01**
24g	24.58	35.21	**2.29**	**3.35**
24h	**3.50**	**6.31**	**2.42**	**1.40**
25d	17.51	18.49	**6.30**	**9.37**
26c	**6.70**	15.02	21.43	23.42
29d	**8.42**	11.54	**4.08**	**4.51**
29e	**2.07**	**8.33**	**1.30**	**2.26**
RIF	**0.51**	**0.75**	**0.45**	**0.81**

RIF = Rifamycin

1.2.2.2.2. Cytotoxicity

To check the selectivity, selected thienopyrimidinones were assayed for their cytotoxic effects in four different cell lines THP-1, MCF-7, A549 and HCT 116 using the MTT assay[16–18] (Table 2). The cell lines were maintained under standard cell culture conditions under 5% CO_2 at 37 °C in 95% humidified air environment. Each concentration was tested in duplicate in a single experiment. GI_{50} values were calculated using OriginPro Software.[16,17,19]

Table 2: Cytotoxicity profile of selected compounds against four human cancer cell lines (*In vitro*)

Comp. no.	MCF-7 (Breast) GI_{50} (µg mL^{-1})	A549 (Lung) GI_{50} (µg mL^{-1})	HCT 116 (Colon) GI_{50} (µg mL^{-1})	THP-1 (Leukemia) GI_{50} (µg mL^{-1})
11g	>100	>100	>100	>100
11m	**33.80**	**27.36**	>100	>100
13b	>100	>100	>100	>100
14a	>100	>100	>100	>100
14b	**24.83**	>100	**17.64**	>100
14c	>100	>100	>100	>100
24g	**20.52**	**35.08**	>100	>100
24h	**26.12**	**45.44**	**42.41**	>100
25d	**19.68**	**34.15**	>100	>100
26c	>100	>100	>100	>100
29d	>100	**16.17**	**14.7**	>100
29e	>100	>100	>100	>100
Paclitaxel	**0.0048**	**0.0035**	**0.0260**	**0.1374**

1.2.2.2.3. Selectivity index.

The selectivity index (SI) was calculated by dividing the 50% growth inhibition concentration (GI_{50}) for the cell lines (THP-1, A549, MCF-7 and HCT 116) by the MIC for *in vitro* activity against active/dormant MTB and BCG.[20]

Table 3: Selectivity index (SI) of selected thienopyrimidinones on human cell lines against *Mycobacterium tuberculosis* H37Ra and *M. bovis* BCG

	SI on MCF-7		**SI on A549**		**SI on HCT 116**		**SI on THP-1**	
	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG
Comp no.	Active stage of *Mycobacterium tuberculosis* H37Ra and *M. bovis* BCG							
11g	2	10	2	10	2	10	2	10
11m	1	4	1	3	2	12	2	12
13b	**40**	**53**	**40**	**53**	**40**	**53**	**40**	**53**
14a	3	20	3	20	3	20	3	20
14b	1	18	2	**73**	0	13	2	**73**
14c	2	**66**	2	**66**	2	**66**	2	**66**
24g	1	9	1	15	4	**44**	4	**44**
24h	7	11	13	19	12	18	29	**41**
25d	1	3	2	5	6	16	6	16
26c	15	5	15	5	15	5	15	5
29d	12	25	2	4	2	4	12	25
29e	**48**	**77**	**48**	**77**	**48**	**77**	**48**	**77**
RIF	196	222	196	222	196	222	196	222

1.2.2.2.4. Results of antimycobacterial activity.

All the newly synthesized compounds were screened for their *in vitro* activity against *Mycobateria* by using *M. tuberculosis* H37Ra (MTB) and *M. bovis* BCG (BCG) species and the detailed results are given in the publication.[22] In the primary screening, 12 compounds (**11g, 11m, 13b, 14a, 14b, 14c, 24g, 24h, 25d, 26c, 29d and 29e**) were found to be active against both the species (Table 1) which were selected for further dose response screening.

Compounds **13b** and **29e** showed very promising activity against active MTB, with MIC_{90} values of 2.51 μg mL^{-1} (8.68 μM) and 2.07 μg mL^{-1} (6.5 μM) respectively. Compounds **24h, 26c**

and **29d** showed MIC_{90} values in the range of 3 to 8 μg mL^{-1}, while the remaining compounds showed MIC_{90} values in the range of 10–50 μg mL^{-1}.

Similarly, activity was observed against *M. bovis* BCG. Compounds **24g, 29e, 13b, 14b, 14c** and **24h** showed MIC values < 3 μg mL^{-1} while compounds **11m**, **11g**, **29d** and **25d** showed MIC values < 10 μg mL^{-1}.

1.2.2.2.5. Results of cytotoxicity and selectivity index

All the synthesized compounds were further evaluated against four human cancer cell lines (MCF-7, A549, THP-1 and HCT 116) to check the toxicity of these compounds (Table 2). The GI_{50} (>100 μg mL^{-1}) values of compounds **13b** and **29e** indicated that the compounds were potent and specific inhibitors against MTB. Compounds **11m**, **24g**, **24h**, **25d** and **29d** were found to be the most active antiproliferative compounds with IC_{50} values in the range of 14.70–45.44 μg mL^{-1} against the MCF7, A549 and HCT 116 cell lines. Compound **29d** showed the highest cytotoxicity (GI_{50} of 14.70 μg mL^{-1}) against A549. GI_{90} studies also indicated that compound **29d** had the highest cytotoxicity (GI_{90} of 74.66 μg mL^{-1}) against A549. The selectivity of the selected thienopyrimidinones towards the human cell lines against MTB is described in terms of the selectivity index (Table 3). The selectivity index reflects the concentration of the compound at which it is active against *Mycobacteria* but is not toxic towards host cells. A higher selectivity index indicates that the compound can be used as a therapeutic agent. Compounds **13b** (SI: > 40) and **29e** (SI: > 45) showed very high SI index, which are actually good inhibitors of *M. tuberculosis* and *M. bovis* BCG. Although the selectivity index of rifampicin is very high, it is important to consider the significance of this study with respect to the developing resistance of microorganisms against available antibiotics. MDR and XDR mycobacterial strains have been reported to exhibit resistance against known anti-TB drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide.[1] Hence, there is a great need to screen new compounds having therapeutic potential and efforts in the present study were directed towards this. According to a study by Hartkoorn *et al.*[21] on the drug susceptibility of TB, antimycobacterial activity was considered to be specific when the selectivity index was >10. In the current study, both **13b** and **29e** exhibited selectivity index values of >40 indicating their potential as antitubercular agents.

1.2.2.2.6. Structure–activity relationship.

The preliminary studies reported herein indicate that the compounds with a thienopyrimidinone structural unit have potential to be studied further and to be developed as antitubercular agents. Some of the conclusions drawn from the structures of the compounds studied in the present work and antitubercular activity exhibited are as follows: The compounds with a longer alkyl chain at the 6 position of thieno[2,3-*d*]pyrimidin-4(3*H*)-one were observed to exhibit better antitubercular activity than the corresponding compounds with a shorter alkyl chain at the 6 position and the same functional group at the 3 position e.g. antitubercular activity was observed in the order of **29c** < **29d** < **29e**. Similarly, **13a** was observed to be less active than **13b**. The compounds with general structures **24** and **25** with 2-bromoethyl and 3-bromopropyl side chains respectively at the 3 position and alkyl chains at the 6 position exhibited a similar trend in activity with **24a** < **24e** < **24f** < **24g** and **25a** < **25c** < **25d**. Compounds **11a**–**m** in the present work, having alkyl chains both at the 3 and 6 positions of the thienopyrimidinone unit, did not exhibit antitubercular activity. Also, the compounds having a benzyl group at the 3 position of thienopyrimidinone and a 2–6 carbon alkyl chain at position 6 (compounds **12a**–**e**) did not exhibit antitubercular activity. Compounds **14a**–**c** with a cyclopropylmethyl group at position 3 and pentyl, hexyl or heptyl chain at position 6 of thienopyrimidinone exhibited moderate antitubercular activity. Compounds having a propargylic side chain at position 3 of the thienopyrimidinone moiety and an alkyl chain ranging from methyl to heptyl at position 6, with general structure **32**, did not exhibit antitubercular activity. A detailed study with a greater number of compounds would be necessary to refine the structure–activity relationship conclusions drawn from the present preliminary results.

1.2.3. Conclusions

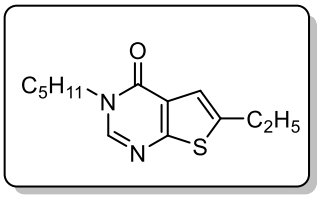
In conclusion, thienopyrimidinones with varying structural features were synthesized for evaluation of antimycobacterial activity. From initial screening studies, it was found that the thienopyrimidinones **11g, 11m, 13b, 14a, 14b, 14c, 24g, 24h, 25d, 26c, 29d** and **29e** exhibited significant activity against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis* BCG. Compounds **13b** and **29e** exhibited greater efficiency in terms of mycobacterial inhibition, specificity and selectivity. Moreover, both the compounds showed satisfactory biocompatibility against human cancer cell lines. These compounds are good candidates for further activity-guided fractionation in the search for new active therapeutic compounds. Our studies point to the

possibility of accessing more active compounds based on the present encouraging findings, as the compounds described in the present work have various functional groups for further structural modifications.

1.2.4. Experimental section

1.2.4.1. Spectral data

Preparation of 6-ethyl-3-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11g)



Potassium carbonate (3.44 g, 0.025 mol) was taken in a 100 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. 6-Ethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (1.5 g, 0.008 mol) was added under nitrogen followed by dry DMF (25 mL) and stirred for 10 min. Pentyl bromide (1.55 ml, 1.88 g, 0.012 mol) was added dropwise and the reaction mixture was stirred at RT for 12 h. It was then diluted with water (100 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 6-ethyl-3-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**11g**) as off-white solid, (1.885 g 90% yield).

Melting Point: 74 °C.

Yield: 90%.

IR ($CHCl_3$): ν_{max} 1672, 2965, 3014 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, J = 7Hz, 3H), 1.28-1.44 (m, 7H), 1.68-1.82 (m, 2H), 2.88 (q, J = 7 Hz, 2H), 3.99 (t, J = 7 Hz, 2 H), 7.17 (s, 1 H), 7.92 (s, 1H).

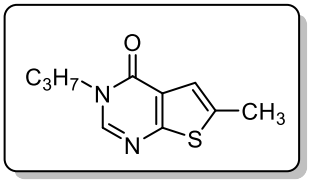
^{13}C NMR (50 MHz, $CDCl_3$): δ 13.80, 15.21, 22.17, 23.91, 28.61, 29.17, 46.80, 117.44, 124.77, 145.62, 145.87, 157.22, 162.24.

HRMS (ESI) *m/z* calcd for $C_{13}H_{19}N_2OS$ $[M + H]^+$: 251.1213, found: 251.1210; $C_{13}H_{18}N_2OS$ [M + Na]: 273.1032 found: 273.1027.

Compounds **11a** to **11f** and **11h** to **11n** were prepared by above method described for **11g** using the corresponding alkyl halides and required thienopyrimidinones. In case of compounds **12a** to **12e**, same procedure was used wherein benzyl bromide was used in place of alkyl halide. Compounds **13a** and **13b** were prepared by using bromoacetonitrile while compounds **14a** to **14c** were prepared by using cyclopropylmethyl bromide. α,α'-dibromo-p-xylene was used for

preparation of compound **15**, (1,3-dioxolan-2-yl)ethyl bromide for compounds **16a** and **16b** whereas (1,3-dioxan-2-yl)ethyl bromide was used for getting compound **16c**. Compounds **17a** to **17c** were obtained when 2-chloro-1-(2,4-difluorophenyl)ethan-1-one was reacted with the corresponding thienopyrimidinones. Allyl bromide was used in the preparation of **18a** while ethyl bromoacetate was employed for getting compounds **19a** to **19c**. Hydrolysis of **19b** and **19c** with ethanolic sodium hydroxide afforded compounds **20b** and **20c** respectively. All the compounds screened in the present study were characterized by spectral methods and the data are given below:

6-Methyl-3-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11a)



Melting Point: 116 °C.

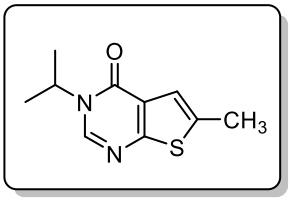
IR (Neat): ν_{max} 1676, 2972, 3055 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, *J*=4 Hz, 3H), 1.75-1.85 (m, 2H), 2.54 (s, 3H), 3.96 (t, *J*= 4 Hz, 2H), 7.13 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 10.95, 15.94, 22.69, 48.30, 119.25, 124.96, 138.45, 145.63, 157.01, 162.38.

HRMS (ESI) *m/z* calculated for $C_{10}H_{13}N_2OS$ $[M + H]^+$: 209.0743; found: 209.0741; $C_{10}H_{12}N_2NaOS$ $[M + Na]^+$: 231.0563, found: 231.0559.

3-Isopropyl-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11b)



Melting Point: 64 °C.

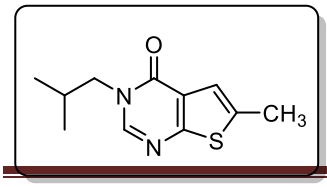
IR (Neat): ν_{max} 1666, 2958, 3055 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.47 (d, *J*=7 Hz, 6H), 2.54 (s, 3H), 5.10-5.31 (m, 1H), 7.13 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.91, 22.13 (2C), 45.58, 119.33, 124.57, 138.28, 142.66, 156.65, 161.91.

HRMS (ESI) *m/z* calculated for $C_{10}H_{13}N_2OS$ $[M + H]^+$: 209.0743, found: 209.0743; $C_{10}H_{12}N_2NaOS$ $[M + Na]^+$: 231.0563, found: 231.0561.

3-Isobutyl-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11c)



Melting Point: 91ºC.

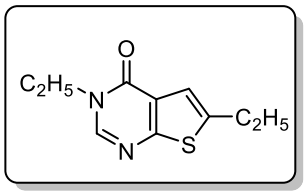
IR (Neat): ν_{max} 1673, 2956, 3061 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (d, *J*=8 Hz, 6H), 2.12-2.25 (m, 1H), 2.54 (s, 3H), 3.80 (d, *J*=7 Hz, 2H), 7.13 (s, 1H), 7.90 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 16.02, 19.81 (2C), 28.14, 53.93, 119.43, 125.07, 138.59, 145.98, 157.24, 162.27.

HRMS (ESI) *m/z* calculated for $C_{11}H_{15}N_2OS$ $[M + H]^+$: 223.0900, found: 223.0898.

3,6-Diethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11d)



Melting Point: 64.1 °C.

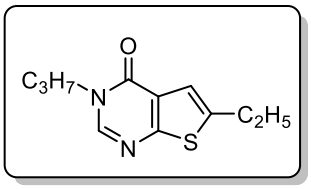
IR (Neat): ν_{max} 1670, 2966, 3051 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, *J*=8 Hz, 3H), 1.41 (t, *J* =7 Hz, 3H), 2.88 (q, *J*= 8 Hz, 2H), 4.07 (q, *J*=7 Hz, 2H), 7.17 (s, 1H), 7.95 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.00, 15.14, 23.84, 41.87, 117.30, 124.67, 145.26, 145.88, 156.96, 162.06.

HRMS (ESI) *m/z* calculated for $C_{10}H_{13}N_2OS$ $[M + H]^+$: 209.0743, found: 209.0741; $[M + Na]^+$: 231.0563, found: 231.0560.

6-Ethyl-3-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11e)



Melting Point: 85 °C.

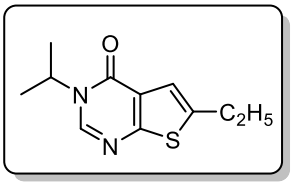
IR (Neat): ν_{max} 1659, 2954, 3059 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.98 (t, *J*=7 Hz, 3H), 1.34 (t, *J*=8 Hz, 3H), 1.70-1.92 (m, 2H), 2.87 (q, *J*=7 Hz, 2H), 3.96 (t, *J*=8 Hz, 2H), 7.15 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 10.85, 15.08, 22.61, 23.78, 48.18, 117.32, 124.63, 145.57, 145.70, 157.09, 162.13.

HRMS (ESI) *m/z* calculated for $C_{11}H_{15}N_2OS$ $[M + H]^+$: 223.0900, found: 223.0899.

6-Ethyl-3-isopropylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11f)



Melting Point: 105 °C.

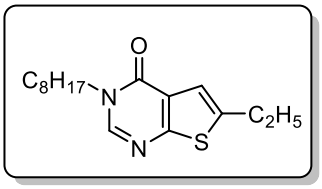
IR (Neat): ν_{max} 1649, 2920, 3047 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (t, *J*=7 Hz, 3H), 1.42 (d, *J*=7 Hz, 6H), 2.82 (q, *J*=7 Hz, 2H), 5.07-5.25 (m, 1H), 7.10 (s, 1H), 7.97 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.20, 22.19 (2C), 23.88, 45.65, 117.54, 124.40, 142.68, 145.81, 156.82, 161.51.

HRMS (ESI) *m/z* calculated for $C_{11}H_{15}N_2OS$ $[M + H]^+$: 223.0900, found: 223.0895; $C_{11}H_{14}N_2NaOS$ $[M + Na]^+$: 245.0719, found: 245.0713.

6-Ethyl-3-octylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11h)



Melting Point: 62 °C.

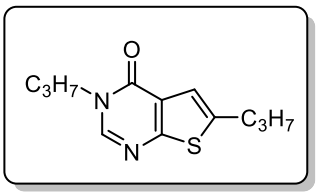
IR ($CHCl_3$): ν_{max} 1676, 2926 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J*=7 Hz, 3H), 1.17-1.42 (m, 13H), 1.72-1.86 (m, 2H), 2.88 (q, *J*=7Hz, 2H), 3.99 (t, *J* =7 Hz, 2H), 7.17 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.00, 15.25, 22.55, 23.97, 26.58, 29.06, 29.09, 29.54, 31.69, 46.89, 117.53, 124.85, 145.64, 145.94, 157.28, 162.29.

HRMS (ESI) *m/z* calculated for $C_{16}H_{25}N_2OS$ $[M + H]^+$: 293.1682, found: 293.1677.

3,6-Dipropylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11i)



Melting Point: 58 °C.

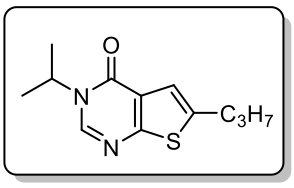
IR ($CHCl_3$): ν_{max} 1674, 2963, 3048 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) : δ 0.92-1.02 (m, 6H), 1.64-1.86 (m, 4H), 2.80 (t, *J*=8 Hz, 2H), 3.95 (t, *J*=8 Hz, 2H), 7.14 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 11.00, 13.74, 22.75, 24.26, 32.57, 48.36, 118.29, 124.78, 144.24, 145.62, 157.26, 162.34.

HRMS (ESI) *m/z* calculated for $C_{12}H_{17}N_2OS$ $[M + H]^+$: 237.1056, found: 237.1055.

3-Isopropyl-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11j)



Melting Point: 95 °C.

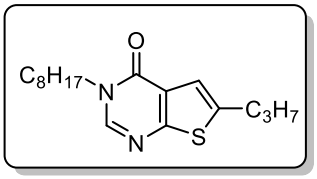
IR ($CHCl_3$): ν_{max} 1657, 2929, 3051 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, *J*=8 Hz, 3H), 1.47 (d, *J*=7 Hz, 6H), 1.65-1.80 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 5.13-5.27 (m, 1H), 7.15 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.49, 22.27 (2C), 24.30, 32.61, 45.69, 118.47, 124.48, 142.68, 144.19, 156.99, 161.92.

HRMS (ESI) *m/z* calculated for $C_{12}H_{17}N_2OS$ $[M + H]^+$: 237.1056, found: 237.1085.

3-Octyl-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11k)



Melting Point: 150 °C.

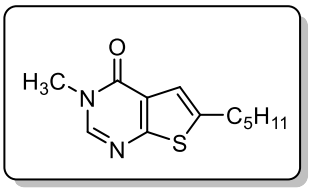
IR ($CHCl_3$): ν_{max} 1662, 2919, 3040 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* =7 Hz, 3H), 1.00 (t, *J* =7 Hz, 3H), 1.17-1.42 (m, 10H), 1.65-1.86 (m, 4H), 2.82 (t, *J*=8 Hz, 2H), 3.99 (t, *J*=8 Hz, 2H), 7.16 (s, 1H), 7.93 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.49, 14.00, 22.56, 24.30, 26.58, 29.06, 29.09, 29.55, 31.70, 32.62, 46.95, 118.37, 124.88, 144.36, 145.62, 157.22, 162.05.

HRMS (ESI) *m/z* calculated for $C_{17}H_{27}N_2OS$ $[M + H]^+$: 307.1839, found: 307.1835; $C_{17}H_{26}N_2NaOS$ $[M + Na]^+$: 329.1658, found: 329.1652.

3-Methyl-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11l)



Melting Point: 88 °C.

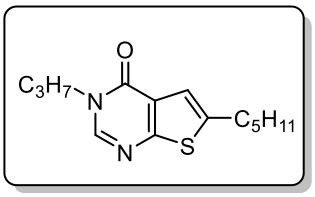
IR ($CHCl_3$): ν_{max} 1664, 3047 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 3H), 1.20-1.45 (m, 4H), 1.60-1.80 (m, 2H), 2.82 (t, *J*=6 Hz, 2H), 3.58 (s, 3H), 7.14 (s, 1H), 7.95 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.91, 22.29, 30.54, 30.65, 31.03, 33.96, 118.01, 124.57, 144.74, 145.75, 157.76, 162.51.

HRMS (ESI) *m/z* calculated for $C_{12}H_{17}N_2OS$ $[M + H]^+$: 237.1056, found: 237.1051; $C_{12}H_{16}N_2NaOS$ $[M + Na]^+$: 259.0876, found: 259.0869.

6-Pentyl-3-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11m)



Melting Point: 141 °C.

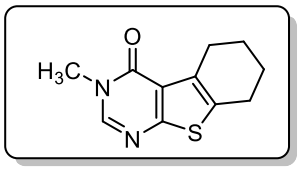
IR ($CHCl_3$): ν_{max} 1682, 2922, 3034 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz , 3H), 1.00 (t, *J*=7 Hz, 3H), 1.27-1.47 (m, 4H), 1.62-1.94 (m, 4H), 2.84 (t, *J*=8 Hz, 2H), 3.98 (t, *J*=7 Hz, 2H), 7.16 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 10.93, 13.83, 22.20, 22.67, 30.44, 30.60, 30.94, 48.28, 118.08, 124.67, 144.44, 145.57, 157.13, 162.11.

HRMS (ESI) *m/z* calculated for $C_{14}H_{21}N_2OS$ $[M + H]^+$: 265.1369, found: 265.1367; $C_{14}H_{20}N_2NaOS$ $[M + Na]^+$: 287.1189, found: 287.1185.

3-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (11n)



Melting Point: 89 °C.

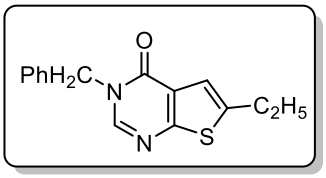
IR ($CHCl_3$): ν_{max} 1658, 2918, 3041 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.75-1.96 (m, 4H), 2.78 (t, *J*=6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.55 (s, 3H), 7.91 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.18, 22.81, 25.16, 25.57, 33.67, 122.58, 131.37, 134.11, 145.61, 158.27, 162.14.

HRMS (ESI) *m/z* calculated for $C_{11}H_{13}N_2OS$ $[M + H]^+$: 221.0743, found: 221.0740 $C_{11}H_{12}N_2NaOS$ $[M + Na]^+$: 243.0563, found: 243.0558.

3-Benzyl-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (12a)



Melting Point: 100 °C.

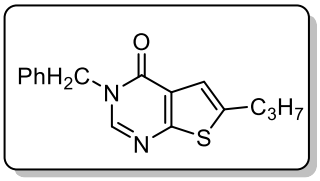
IR (Neat): ν_{max} 1570, 1666, 2968, 3041 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, *J* =7 Hz, 3H), 2.88 (q, *J*=7 Hz, 2H), 5.21 (s, 2H), 7.19 (s, 1H), 7.29-7.39 (m, 5H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.19, 23.88, 49.22, 117.56, 124.74, 127.93 (2C), 128.33, 128.89 (2C), 135.69, 145.48, 146.17, 157.14, 162.05.

HRMS (ESI) *m/z* calculated for $C_{15}H_{15}N_2OS$ $[M + H]^+$: 271.0900, found: 271.0896 $C_{15}H_{14}N_2NaOS$ $[M + Na]^+$: 293.0719, found: 293.0715.

3-Benzyl-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (12b)



Melting Point: 93 °C.

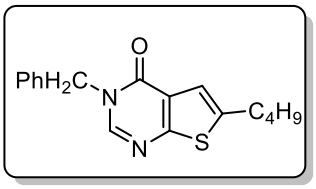
IR (Neat): ν_{max} 1568, 1666, 2959, 3041 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.00 (t, *J*=7 Hz, 3H), 1.60-1.85 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 5.21 (s, 2H), 7.17 (s, 1H), 7.35 (bs, 5H), 8.02 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.54, 24.34, 32.63, 49.31, 118.51, 124.86, 128.06 (2C), 128.37, 129.01 (2C), 135.82, 144.57, 145.54, 157.28, 162.36.

HRMS (ESI) *m/z* calculated for $C_{16}H_{17}N_2OS$ $[M + H]^+$: 285.1056, found: 285.1053 $C_{16}H_{16}N_2NaOS$ $[M + Na]^+$: 307.0876, found: 307.0871.

3-Benzyl-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (12c)



Melting Point: 99 °C.

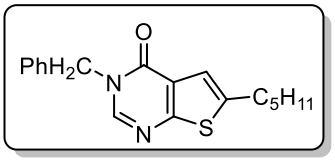
IR ($CHCl_3$): ν_{max} 1560, 1674, 2963, 3048 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J* = 7 Hz, 3H), 1.21-1.53 (m, 2H), 1.60-1.80 (m, 2H), 2.85 (dt, *J* =1, 7 Hz, 2H), 5.21 (s, 2H), 7.17 (t, *J*=1 Hz, 1H), 7.35 (bs, 5H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.61, 21.90, 30.17, 33.00, 49.18, 118.26, 124.72, 127.92 (2C), 128.16, 128.87 (2C), 135.71, 144.68, 145.43, 157.13, 162.19.

HRMS (ESI) *m/z* calculated for $C_{17}H_{19}N_2OS$ $[M + H]^+$: 299.1213, found: 299.1204.

3-Benzyl-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (12d)



Melting Point: 99 °C.

IR ($CHCl_3$): ν_{max} 1571, 1674, 2932, 3014 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, *J*=7 Hz, 3H), 1.30-1.43 (m, 4H), 1.64-1.79 (m, 2H), 2.84 (t, *J*=8 Hz, 2H), 5.21 (s, 2H), 7.17 (s, 1H), 7.35 (bs, 5H), 8.01 (s, 1H).

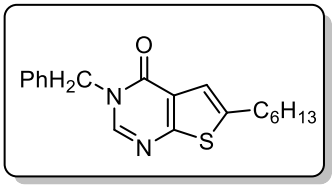
^{13}C NMR (50 MHz, $CDCl_3$): δ 13.86, 22.24, 30.48, 30.63, 30.96, 49.21, 118.27, 124.73, 127.94(2C), 128.18, 128.89 (2C), 135.70, 144.76, 145.43, 157.16, 162.20.

HRMS (ESI) *m/z* calculated for $C_{18}H_{21}N_2OS$ $[M + H]^+$: 313.1369, found: 313.1361; $C_{18}H_{20}N_2NaOS$ $[M + Na]^+$: 335.1189; found: 335.1180.

3-Benzyl-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (12e)

Melting Point: 81 °C.

IR ($CHCl_3$): ν_{max} 1572, 1674, 2929, 3012 cm^{-1}.

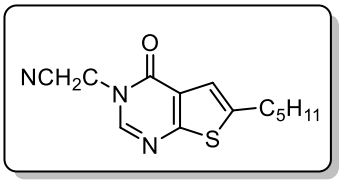


^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 3H), 1.20-1.45 (m, 6H), 1.62-1.74 (m, 2H), 2.83 (t, *J* =8 Hz, 2H), 5.20 (s, 2H), 7.16 (s, 1H), 7.34 (bs, 5H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.39, 28.45, 30.47, 30.88, 31.33, 49.15, 118.21, 124.67, 127.89 (2C), 128.11, 128.83 (2C), 135.68, 144.68, 145.41, 157.10, 162.16.

HRMS (ESI) *m/z* calculated for $C_{19}H_{23}N_2OS$ $[M + H]^+$: 327.1526, found: 327.1519.

2-(4-Oxo-6-pentylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetonitrile (13a)



Melting Point: 88 °C.

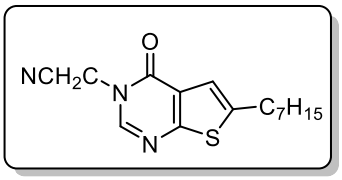
IR ($CHCl_3$): ν_{max} 1638, 2933, 3061 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, *J* =7 Hz, 3H), 1.31-1.41 (m, 4H), 1.65-1.78 (m, 2H), 2.86 (t, *J*=7 Hz, 2H), 4.92 (s, 2H), 7.18 (s, 1H), 8.05 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.85, 22.20, 30.47, 30.59, 30.96, 33.47, 113.77, 118.05, 124.10, 143.59, 146.25, 155.91, 162.18.

HRMS (ESI) *m/z* calculated for $C_{13}H_{16}N_3OS$ $[M + H]^+$: 262.1009, found: 262.1003 $C_{13}H_{15}N_3NaOS$ $[M + Na]^+$: 284.0828, found: 284.0821.

2-(6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetonitrile (13b)



Melting Point: 86 °C.

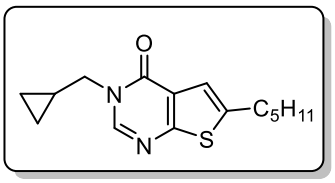
IR ($CHCl_3$): ν_{max} 1686, 2358, 2930, 3021 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 3H), 1.23-1.49 (m, 8H), 1.63-1.78 (m, 2H), 2.86 (t, *J*=8 Hz, 2H), 4.91 (s, 2H), 7.19 (s, 1H), 8.04 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.95, 22.48, 28.79 (2C), 30.51, 30.91, 31.57, 33.47, 113.82, 118.03, 124.09, 143.64, 146.23, 155.93, 162.25.

HRMS (ESI) *m/z* calculated for $C_{15}H_{20}N_3OS$ $[M + H]^+$: 290.1322, found: 290.1318; $C_{15}H_{19}N_3NaOS$ $[M + Na]^+$: 312.1141, found: 312.1136.

3-(Cyclopropylmethyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14a)



Melting Point: 73 °C.

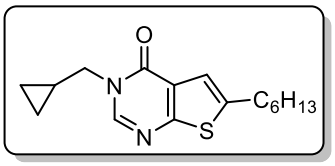
IR ($CHCl_3$): ν_{max} 1671, 2933, 3015 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.32-0.48 (m, 2H), 0.55-0.70 (m, 2H), 0.88 (t, *J*=7 Hz, 3H), 1.17-1.44 (m, 5H), 1.63-1.75 (m, 2H), 2.82 (t, *J* =7 Hz, 2H), 3.86 (d, *J* =7 Hz, 2H), 7.14 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 4.03 (2C), 10.88, 13.83, 22.22, 30.45, 30.60, 30.95, 50.80, 118.14, 124.66, 144.39, 145.26, 157.31, 162.28.

HRMS (ESI) *m/z* calculated for $C_{15}H_{21}N_2OS$ $[M + H]^+$: 277.1369, found: 277.1369; $C_{15}H_{20}N_2NaOS$ $[M + Na]^+$: 299.1189, found: 299.1186.

3-(Cyclopropylmethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14b)



Melting Point: 49 °C.

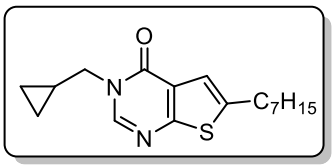
IR ($CHCl_3$): ν_{max} 1683, 2926, 3079 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.36-0.48 (m, 2H), 0.60-0.72 (m, 2H), 0.89 (t, *J* =7 Hz, 3H), 1.17-1.45 (m, 7H), 1.62-1.77 (m, 2H), 2.84 (t, *J* =7 Hz, 2H), 3.88 (d, *J* =7 Hz , 2H), 7.16 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 4.14 (2C), 10.98, 14.03, 22.51, 28.59, 30.62, 31.01, 31.46, 50.93, 118.26, 124.80, 144.56, 145.31, 157.45, 162.39.

HRMS (ESI) *m/z* calculated for $C_{16}H_{23}N_2OS$ $[M + H]^+$: 291.1526, found: 291.1519; $C_{16}H_{22}N_2OS$ $[M + Na]^+$: 313.1345, found: 313.1336.

3-(Cyclopropylmethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14c)



Melting Point: 57 °C.

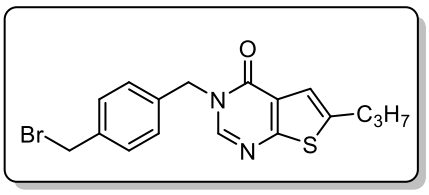
IR ($CHCl_3$): ν_{max} 1678, 2927, 3078 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.35-0.48 (m, 2H), 0.58-0.69 (m, 2H), 0.87 (t, *J* =7 Hz, 3H), 1.14-1.47 (m, 9H), 1.68-1.76 (m, 2H), 2.83 (t, *J* =7 Hz, 2H), 3.86 (d, *J* =7 Hz , 2H), 7.15 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 4.07 (2C), 10.91, 13.98, 22.52, 28.80, 28.86, 30.53, 30.97, 31.62, 50.84, 118.18, 124.71, 144.45, 145.27, 157.35, 162.32.

HRMS (ESI) *m/z* calculated for $C_{17}H_{25}N_2OS$ $[M + H]^+$: 305.1682, found: 305.1683; $C_{17}H_{24}N_2NaOS$ $[M + Na]^+$: 327.1502, found: 327.1500.

3-(4-(Bromomethyl)benzyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (15)



Melting Point: 150 °C.

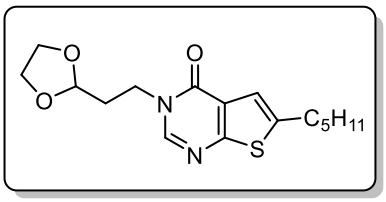
IR ($CHCl_3$): ν_{max} 1572, 1672, 2967, 3039 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, *J*=7 Hz, 3H), 1.68 - 1.80 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 4.46 (s, 2H), 5.19 (s, 2H), 7.17 (s, 1H), 7.30 - 7.35 (m, 2H), 7.35 - 7.41 (m, 2H), 8.01 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.45, 24.24, 32.54, 32.67, 48.95, 118.38, 124.75, 128.36 (2C), 129.58 (2C), 135.96, 137.86, 144.66, 145.33, 157.12, 162.30.

HRMS (ESI) *m/z* calculated for $C_{17}H_{18}{}^{79}Br\,N_2OS$ $[M + H]^+$: 377.0318, found: 377.0311.

3-(2-(1,3-Dioxolan-2-yl)ethyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (16a)



Melting Point: 60 °C.

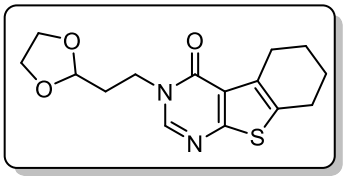
IR ($CHCl_3$): ν_{max} 1290, 1672, 2955, 3040 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz, 3H), 1.20-1.47 (m, 4H), 1.60-1.85 (m, 2H), 2.19 (dt, *J*=5, 7 Hz, 2H), 2.83 (t, *J*=7 Hz, 2H), 3.86 (t, *J*=6 Hz, 2H), 3.99 (t, *J*=6 Hz, 2H), 4.15 (t, *J*=7 Hz, 2H), 4.92 (t, *J*=5 Hz, 1H), 7.15 (s, 1H), 7.97 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.92, 22.31, 30.56, 30.70, 31.06, 32.27, 42.45, 64.95 (2C), 101.97, 118.16, 124.74, 144.55, 146.09, 157.30, 162.41.

HRMS (ESI) *m/z* calculated for $C_{16}H_{23}N_2O_3S$ $[M + H]^+$: 323.1424, found: 323.1424; $C_{16}H_{22}N_2NaO_3S$ $[M + Na]^+$: 345.1243, found: 345.1244.

3-(2-(1,3-Dioxolan-2-yl)ethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (16b)



Melting Point: 77 °C.

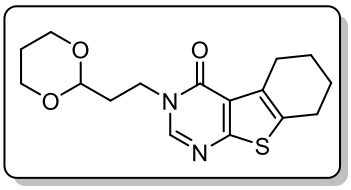
IR ($CHCl_3$): ν_{max} 1292, 1669, 2950, 3044 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68-2.00 (m, 4H), 2.17 (dt, *J*=5 Hz, 2H), 2.77 (t, *J*=6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.86 (t, *J*=6 Hz, 2H), 3.98 (t, *J*=6 Hz, 2H), 4.11 (t, *J*=7 Hz, 2H), 4.92 (t, *J*=5 Hz, 1H), 7.92 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.23, 22.86, 25.21, 25.62, 32.30, 42.19, 64.96 (2C), 102.00, 122.76, 131.56, 133.99, 145.95, 157.81, 162.09.

HRMS (ESI) *m/z* calculated for $C_{15}H_{19}N_2O_3S$ $[M + H]^+$: 307.1111, found: 307.1112; $C_{15}H_{18}N_2NaO_3S$ $[M + Na]^+$: 329.0930, found: 329.0930.

3-(2-(1,3-Dioxan-2-yl)ethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (16c)



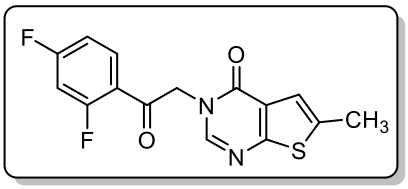
Melting Point: 111 °C.

IR ($CHCl_3$): ν_{max} 1287, 1669, 2947, 3030 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (d, 2H), 1.80-1.92 (m, 4H), 2.04-2.12 (m, 4H), 2.77 (t, *J*=6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.72 (t, *J*=12 Hz, 2H), 4.08-4.22 (m, 2H), 4.59 (t, *J*=6 Hz, 1H), 7.93 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.26, 22.28, 25.21, 25.64 (2C), 33.58 42.12, 66.82 (2C), 99.54, 122.77, 131.59, 133.81, 146.25, 157.86, 162.12.

3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (17a)



Melting Point: 177 °C.

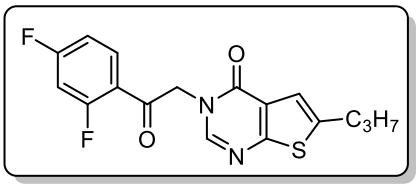
IR ($CHCl_3$): ν_{max} 1570, 1682, 2948, 3018 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.55 (s, 3H), 5.30 (s, 2H), 6.97 (t, *J*=8 Hz, 1H), 7.03 (t, *J*=8 Hz, 1H), 7.12 (s, 1H), 7.87 (s, 1H), 7.97-8.05 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 16.00, 55.01 (d), 104.89 (t), 112.98 (dd), 119.42, 119.52 (d), 124.81, 133.18 (dd), 138.93, 145.78, 156.88, 162.99, 163.26 (dd), 166.69 (dd), 188.28 (d).

HRMS (ESI) *m/z* calculated for $C_{15}H_{11}F_2N_2O_2S$ $[M + H]^+$: 321.0504, found: 321.0507.

3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (17b)



Melting Point: 150 °C.

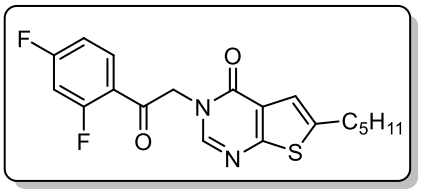
IR ($CHCl_3$): ν_{max} 1572, 1679, 2944, 3020 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, *J*=7 Hz, 3H), 1.68-1.81 (m, 2H), 2.81 (t, *J*=8 Hz, 2H), 5.29 (d, *J*=6 Hz, 2H), 6.91-7.05 (m, 2H), 7.13 (s, 1H), 7.88 (s, 1H), 7.98-8.06 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.47, 24.27, 32.55, 55.00 (d), 104.83 (t), 112.90 (dd), 118.32, 119.39 (dd), 124.47, 133.10 (dd), 144.61, 145.73, 156.95, 162.70, 163.21 (dd), 166.62 (dd), 188.25 (d).

HRMS (ESI) *m/z* calculated for $C_{17}H_{15}F_2N_2O_2S$ $[M + H]^+$: 349.0817, found: 349.0808; $C_{17}H_{14}F_2N_2NaO_2S$ $[M + Na]^+$: 371.0636, found: 371.0627.

3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (17c)



Melting Point: 138 °C.

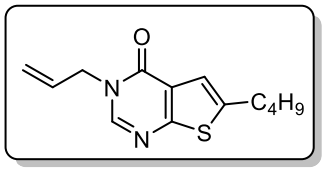
IR ($CHCl_3$): ν_{max} 1569, 1683, 2938, 3038 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, *J*=7 Hz, 3H), 1.26-1.46 (m, 4H), 1.62-1.81 (m, 2H), 2.85 (t, *J*=7 Hz, 2H), 5.31 (d, *J*=5 Hz, 2H), 6.90-7.11 (m, 2H), 7.15 (s, 1H), 7.88 (s, 1H), 7.98-8.14 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.91, 22.30, 30.54, 30.70, 31.03, 55.02 (d), 104.86 (t), 112.95 (dd), 118.25, 119.42 (dd), 124.52, 133.15 (dd), 144.94, 145.69, 156.98, 162.69, 163.24 (dd), 166.66 (dd), 188.26 (d).

MS *m/z* **:** 377.1 $[M+ H]^+$.

3-Allyl-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (18a)



Melting Point: 45 °C.

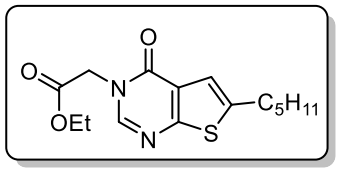
IR ($CHCl_3$): ν_{max} 1676, 2931, 3007 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, *J*=8 Hz, 3H), 1.30-1.45 (m, 2H), 1.60-1.75 (m, 2H), 2.82 (t, *J*=8 Hz, 2H), 4.60 (d, *J*=7 Hz, 2H), 5.16-5.29 (m, 2H), 5.91-6.00 (m, 1H), 7.13 (s, 1H), 7.91 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.62, 21.91, 30.19, 33.01, 48.00, 118.19, 118.73, 124.62, 131.91, 144.62, 145.28, 156.89, 162.28.

HRMS (ESI) *m/z* calculated for $C_{13}H_{17}N_2OS$ $[M + H]^+$: 249.1056, found: 249.1051.

Ethyl 2-(4-oxo-6-pentylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetate (19a)



Melting Point: 80 °C.

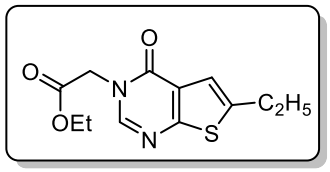
IR ($CHCl_3$): ν_{max} 1670, 1739, 2949, 3041 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz, 3H), 1.25-1.42 (m, 7H), 1.65-1.78 (m, 2H), 2.83 (t, *J*=7 Hz, 2H), 4.27 (q, *J*=7 Hz, 2H), 4.70 (s, 2H), 7.15 (s, 1H), 7.89 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.90, 14.05, 22.29, 30.54, 30.68, 31.02, 47.12, 62.16, 118.24, 124.44, 145.08, 145.42, 156.95, 162.53, 167.21.

HRMS (ESI) *m/z* calculated for $C_{15}H_{21}N_2O_3S$ $[M + H]^+$: 309.1267, found: 309.1263.

Ethyl 2-(6-ethyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetate (19b)



Melting Point: 118 °C.

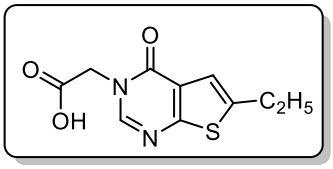
IR ($CHCl_3$): ν_{max} 1667, 1742, 2932, 3041 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (t, *J*=7 Hz, 3H), 1.35 (t, *J*=7 Hz, 3H), 2.89 (q, *J*=7 Hz, 2H), 4.26 (q, *J*=7 Hz, 2H), 4.71 (s, 2H), 7.17 (s, 1H), 7.90 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.09, 15.27, 23.98, 47.21, 62.18, 117.55, 124.47, 145.65, 146.49, 156.97, 162.45, 167.27.

HRMS (ESI) *m/z* calculated for $C_{12}H_{15}N_2O_3S$ $[M + H]^+$: 267.0798, found: 267.0795; $C_{12}H_{14}N_2NaO_3S$ $[M + Na]^+$: 289.0617, found: 289.0613.

2-(6-Ethyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetic acid (20b)



Melting Point: 206 °C.

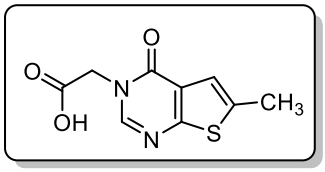
IR ($CHCl_3$): ν_{max} 1213, 1693 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$+ MeOH-d4): δ 1.15 (t, *J*=8 Hz, 3H), 2.68 (q, *J*=8 Hz, 2H), 4.54 (s, 2H), 6.94 (s, 1H), 7.83 (s, 1H).

^{13}C NMR (50 MHz, MeOH-d4): δ 15.88, 24.88, 50.37, 118.31, 125.41, 148.18, 148.53, 159.10, 164.15, 170.82.

HRMS (ESI) *m/z* calculated for $C_{10}H_{11}N_2O_3S$ $[M + H]^+$: 239.0485, found: 239.0481; $C_{10}H_{10}N_2NaO_3S$ $[M + Na]^+$: 261.0304, found: 261.0300.

2-(6-Methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetic acid (20c)



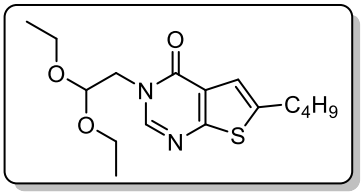
Melting Point: 225 °C.

IR ($CHCl_3$): ν_{max} 1201, 1686 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.28 (s, 3H), 4.47 (s, 2H), 6.84 (s, 1H), 7.79 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 15.34, 46.37, 118.61, 123.94, 137.99, 145.81, 156.23, 162.16, 168.57.

6-Butyl-3-(2,2-diethoxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (21a)



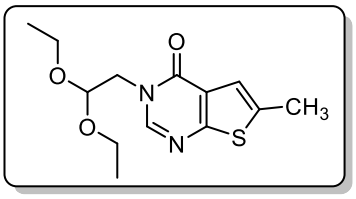
Melting Point: 43 °C.

IR ($CHCl_3$): ν_{max} 1068, 1676 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.07-1.15 (m, 6H), 1.29-1.40 (m, 2H), 1.58-1.70 (m, 2H), 2.78 (t, *J*=7 Hz, 2H), 3.47 (q, *J*=6 Hz, 2H), 3.70 (q, *J*=6 Hz, 2H), 4.01 (d, *J*=6 Hz, 2H), 4.65 (t, *J*=6 Hz, 1H), 7.07 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.45, 14.97 (2C), 21.75, 30.00, 32.84, 48.89, 63.93 (2C), 99.68, 117.81, 123.98, 144.12, 146.63, 157.12, 162.36.

3-(2,2-Diethoxyethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (21b)



Melting Point: 45 °C.

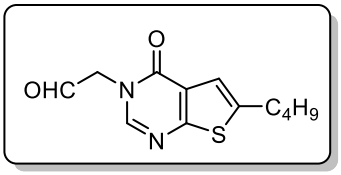
IR ($CHCl_3$): ν_{max} 1062, 1676 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.96-1.22 (m, 6H), 2.44 (s, 3H), 3.31-3.53 (m, 2H), 3.57-3.80 (m, 2H), 3.97 (d, *J*=6 Hz, 2H), 4.62 (t, *J*=6 Hz, 1H), 7.01 (s, 1H), 7.89 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.06 (2C), 15.84, 49.03, 64.09 (2C), 99.75, 119.04, 124.33, 138.31, 146.75, 157.12, 162.73.

HRMS (ESI) *m/z* calculated for $C_{13}H_{19}N_2O_3S$ $[M + H]^+$: 283.1111, found: 283.1104; $C_{13}H_{18}N_2NaO_3S$ $[M + Na]^+$: 305.0930, found: 305.0923.

2-(6-Butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetaldehyde (22)



Melting Point: 90 °C.

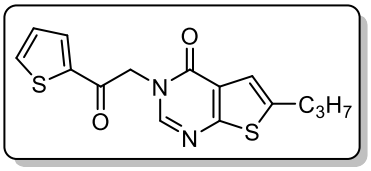
IR ($CHCl_3$): ν_{max} 1670, 1722, 2729 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, *J*=7 Hz, 3H), 1.32-1.43 (m, 2H), 1.61-1.71 (m, 2H), 2.81 (t, *J*=7 Hz, 2H), 4.80 (s, 2H), 7.09 (s, 1H), 7.85 (s, 1H), 9.68 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.63, 21.92, 30.17, 32.98, 54.84, 117.99, 124.23, 145.22, 145.31, 156.86, 162.54, 193.70.

HRMS (ESI) *m/z* calculated for $C_{12}H_{15}N_2O_2S$ $[M + H]^+$: 251.0849, found: 251.0842.

3-(2-Oxo-2-(thiophen-2-yl)ethyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (23a)



Melting Point: 133 °C.

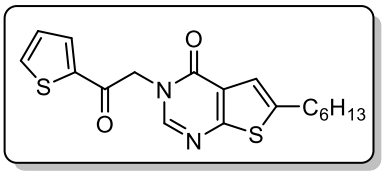
IR ($CHCl_3$): ν_{max} 1676 (b), 2933, 3031 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.99 (t, *J*=7 Hz, 3H), 1.63-1.85 (m, 2H), 2.81 (t, *J*=7 Hz, 2H), 5.34 (s, 2H), 7.13 (s, 1H), 7.16-7.24 (m, 1H), 7.72-7.79 (m, 1H), 7.89-7.97 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.49, 24.29, 32.57, 50.91, 118.33, 124.41, 128.49, 132.92, 135.18, 140.61, 144.65, 145.79, 156.93, 162.67, 184.46.

HRMS (ESI) *m/z* calculated for $C_{15}H_{15}N_2O_2S_2$ $[M + H]^+$: 319.0569, found: 319.0562; $C_{15}H_{14}N_2NaO_2S_2$ $[M + Na]^+$: 341.0389, found: 341.0381.

6-Hexyl-3-(2-oxo-2-(thiophen-2-yl)ethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (23b)



Melting Point: 117 °C.

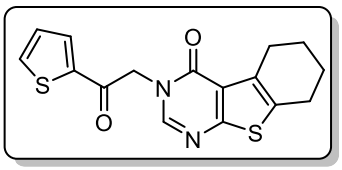
IR ($CHCl_3$): ν_{max} 1676 (b), 2030, 3032 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.25-1.40 (m, 6H), 1.60-1.80 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 5.33 (s, 2H), 7.11 (s, 1H), 7.16-7.20 (m, 1H), 7.72-7.75 (m,1H), 7.89-7.93 (m, 2H).

13C NMR (100 MHz, $CDCl_3$): δ 13.99, 22.45, 28.53, 30.54, 30.95, 31.40, 50.90, 118.16, 124.37, 128.45, 132.90, 135.12, 140.58, 144.90, 145.78, 156.89, 162.60, 184.46.

HRMS (ESI) *m/z* calculated for $C_{18}H_{21}N_2O_2S_2$ $[M + H]^+$: 361.1039, found: 361.1028; $C_{18}H_{20}N_2NaO_2S_2$ $[M + Na]^+$: 383.0858, found: 383.0846.

3-(2-Oxo-2-(thiophen-2-yl)ethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (23c)



Melting Point: 199 °C.

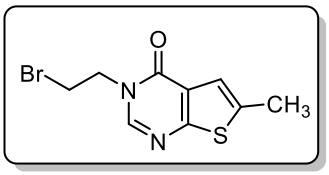
IR ($CHCl_3$): ν_{max} 1674 (b), 2928, 3028 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71-1.94 (m, 4H), 2.79 (t, *J*=6 Hz, 2H), 2.98 (t, *J*=6 Hz, 2H), 5.29 (s, 2H), 7.21 (t, *J*=5 Hz, 1H), 7.76 (d, *J*=5 Hz, 1H), 7.89 (s, 1H), 7.94 (d, *J*=5 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.18, 22.82, 25.20, 25.53, 50.79, 122.55, 128.49, 131.63, 132.86, 134.47, 135.15, 140.69, 145.64, 157.46, 162.35, 184.60.

HRMS (ESI) *m/z* calculated for $C_{16}H_{15}N_2O_2S_2$ $[M + H]^+$: 331.0569, found: 331.0561; $C_{16}H_{14}N_2NaO_2S_2$ $[M + Na]^+$: 353.0389, found: 353.0380.

3-(2-Bromoethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24a)



Melting Point: 137 °C.

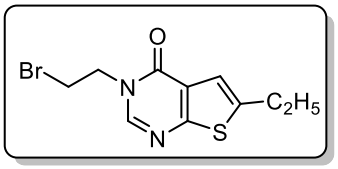
IR (KBr): ν_{max} 1668, 2962, 3014 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.55 (s, 3H), 3.76 (t, *J*=6 Hz, 2H), 4.38 (t, *J* =6 Hz, 2H), 7.13 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.98, 29.70, 48.68, 119.15, 124.75, 138.98, 145.70, 156.86, 162.99.

HRMS (ESI) *m/z* calculated for $C_9H_{10}{}^{79}BrN_2OS$ $[M + H]^+$: 272.9692, found: 272.9690; $C_9H_{10}{}^{81}BrN_2OS$ $[M + H]^+$: 274.9671, found: 274.9659.

3-(2-Bromoethyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24b)



Melting Point: 123 °C.

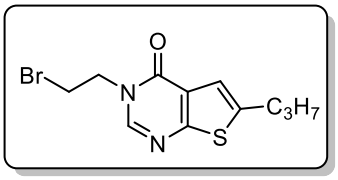
IR ($CHCl_3$): ν_{max} 1676, 3019 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (t, *J*=7Hz, 3H), 2.90 (q, *J*=7 Hz, 2H), 3.77 (t, *J*=6 Hz, 2H), 4.38 (t, *J*=6 Hz, 2H), 7.18 (s, 1H), 7.99 (s 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.29, 24.01, 29.82, 48.75, 117.38, 124.58, 145.80, 146.46, 157.06, 162.74.

HRMS (ESI) *m/z* calculated for $C_{10}H_{12}{}^{79}BrN_2OS$ $[M + H]^+$: 286.9848, found: 286.9847; $C_{10}H_{12}{}^{81}BrN_2OS$ $[M + H]^+$: 288.9828, found: 288.9824.

3-(2-Bromoethyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24c)



Melting Point: 106 °C.

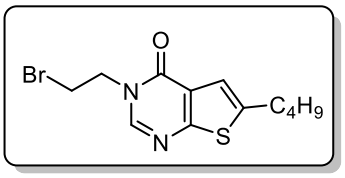
IR ($CHCl_3$): ν_{max} 1668, 2962, 3014 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, *J*=7 Hz, 3H), 1.70-1.81 (m, 2H), 2.83 (t, *J*=8 Hz, 2H), 3.76 (t, *J*=5 Hz, 2H), 4.38 (t, *J*=5 Hz, 2H), 7.16 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.52, 24.30, 29.77, 32.62, 48.76, 118.17, 124.55, 144.81, 145.69, 157.05, 162.810.

HRMS (ESI) *m/z* calculated for $C_{11}H_{14}{}^{81}BrN_2OS$ $[M + H]^+$: 302.9984, found: 302.9976; $C_{11}H_{13}{}^{81}BrN_2NaOS$ $[M + Na]^+$: 359.0450, found: 359.0446.

3-(2-Bromoethyl)-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24d)



Melting Point: 86 °C.

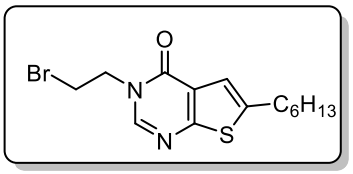
IR ($CHCl_3$): ν_{max} 1672, 2929, 3019 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J*=7 Hz, 3H), 1.30-1.53 (m, 2H), 1.59-1.81 (m, 2H), 2.86 (t, *J*=8 Hz, 2H), 3.77 (t, *J*=6 Hz, 2H), 4.39 (t, *J*=6 Hz, 2H), 7.16 (s, 1H), 8.03 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.67, 21.95, 29.82, 30.25, 31.12, 48.29, 118.15, 124.50, 144.57, 145.70, 157.02, 162.71.

MS *m/z* : 315.0 and 317.0 $[M+H]^+$.

3-(2-Bromoethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24e)



Melting Point: 60 °C.

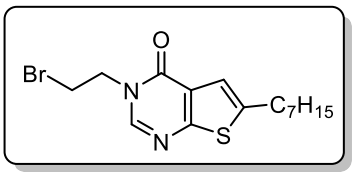
IR ($CHCl_3$): ν_{max} 1676, 2925, 3021 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz, 3H), 1.23-1.47 (m, 6H), 1.66-1.78 (m, 2H), 2.85 (t, *J*=8 Hz, 2H), 3.77 (t, *J*=6 Hz, 2H), 4.39 (t, *J*=6 Hz, 2H), 7.16 (s, 1H), 8.02 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.96, 22.43, 28.51, 29.79, 30.54, 31.13, 31.38, 45.26, 118.00, 124.68, 144.92, 145.57, 157.21, 162.44.

MS *m/z* : 337.1 and 339.1 $[M + H]^+$

3-(2-Bromoethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24f)



Melting Point: 102 °C.

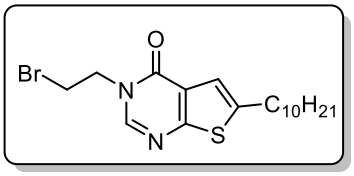
IR ($CHCl_3$): ν_{max} 1674, 2922, 3025 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.20-1.46 (m, 8H), 1.65-1.78 (m, 2H), 2.85 (t, *J*=7 Hz, 2H), 3.76 (t, *J*=6 Hz, 2H), 4.39 (t, *J*=6 Hz, 2H), 7.16 (s, 1H), 8.05 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.98, 22.54, 28.87 (2C), 29.68, 30.57, 30.99, 31.63, 48.71, 118.03, 124.53, 145.06, 145.65, 158.97, 162.63.

HRMS (ESI) *m/z* calculated for $C_{15}H_{22}{}^{79}BrN_2OS$ $[M + H]^+$: 357.0631, found: 357.0628; $C_{15}H_{22}{}^{81}BrN_2OS$ $[M + H]^+$: 359.0450, found: 359.0446.

3-(2-Bromoethyl)-6-decylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24g)



Melting Point: 73 °C.

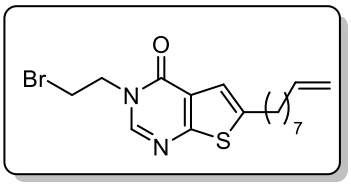
IR ($CHCl_3$): ν_{max} 1676, 2924, 3020 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.19-1.45 (m, 14H), 1.72 (t, *J*=7 Hz, 2H), 2.85 (t, *J*=8 Hz, 2H), 3.77 (t, *J*=6 Hz, 2H), 4.38 (t, *J*=6 Hz, 2H), 7.16 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.07, 22.63, 28.91, 29.25 (2C), 29.46, 29.52, 29.76, 30.60, 31.02, 31.84, 48.73, 118.03, 124.53, 145.08, 145.66, 157.02, 162.75.

HRMS (ESI) *m/z* calculated for $C_{18}H_{28}{}^{79}BrN_2OS$ $[M + H]^+$: 399.1100, found: 399.1102; $C_{18}H_{27}{}^{79}BrN_2NaOS$ $[M + Na]^+$: 421.0920, found: 421.0920.

3-(2-Bromoethyl)-6-(non-8-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (24h)



Melting Point: 150 °C.

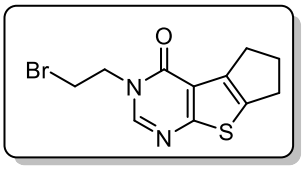
IR ($CHCl_3$): ν_{max} 1674, 2930, 3018 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23-1.49 (m, 8H), 1.65-1.83 (m, 2H), 1.97-2.09 (m, 2H), 2.84 (t, *J* =8 Hz, 2H), 3.76 (t, *J* =6 Hz, 2H), 4.38 (t, *J* =6 Hz, 2H), 4.90-5.07 (m, 2H), 5.73-5.90 (m, 1H), 7.15 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.80, 28.83, 28.90, 29.07, 29.76, 30.58, 30.98, 33.70, 48.74, 114.19, 118.05, 124.53, 139.05, 145.03, 145.67, 157.02, 162.75.

HRMS (ESI) *m/z* calculated for $C_{17}H_{24}{}^{79}BrN_2OS$ $[M + H]^+$: 383.0787, found: 383.0786; $C_{17}H_{23}{}^{79}BrN_2OS$ $[M + Na]^+$: 405.0607, found: 405.0605.

3-(2-Bromoethyl)-3,5,6,7-tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (24i)



Melting Point: 176 °C.

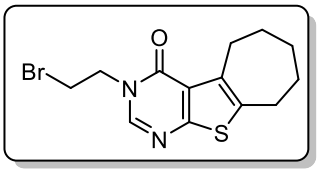
IR ($CHCl_3$): ν_{max} 1667, 2918, 3041 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.40-2.57 (m, 2H), 2.93-3.13 (m, 4H), 3.77 (t, *J*=6 Hz, 2H), 4.37 (t, *J*=6 Hz, 2H), 7.94 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.01, 28.92, 29.59, 29.74, 48.63, 120.17, 140.00, 140.21, 145.28, 157.36, 167.29.

HRMS (ESI) *m/z* calculated for $C_{11}H_{12}{}^{79}BrN_2OS$ $[M + H]^+$: 298.9848, found: 298.9842; $C_{11}H_{12}{}^{81}BrN_2OS$ $[M + H]^+$: 300.9828, found: 300.9820.

3-(2-Bromoethyl)-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (24j)



Melting Point: 142 °C.

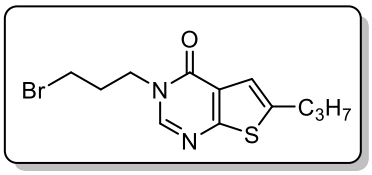
IR (KBr): ν_{max} 1658, 2920, 3040 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.68-1.72 (m, 4H), 1.88-1.91 (m, 2H), 2.81-2.86 (m, 2H), 3.30-3.33 (m, 2H), 3.76 (t, *J*=5 Hz, 2H), 4.34 (t, *J*=5 Hz, 2H), 7.95 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 27.19, 27.66 (2C), 27.93, 30.01, 32.55, 48.69, 123.16, 137.18, 138.93, 145.37, 157.97, 160.48.

HRMS (ESI) *m/z* calculated for $C_{13}H_{16}{}^{79}BrN_2OS$ $[M + H]^+$: 327.0161, found: 327.0153; $C_{13}H_{16}{}^{81}Br\ N_2OS$ $[M + H]^+$: 329.0141, found: 329.0128.

3-(3-Bromopropyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25a)



Melting Point: 107 °C.

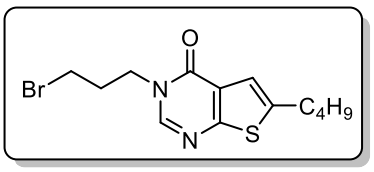
IR (KBr): ν_{max} 1668, 2929, 3020 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.99 (t, *J*=7 Hz, 3H), 1.62-1.85 (m, 2H), 2.27-2.45 (m, 2H), 2.82 (t, *J*=7Hz, 2H), 3.42 (t, *J*=7 Hz, 2H), 4.18 (t, *J*=7 Hz, 2H), 7.14 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.43, 24.22, 29.82, 31.08, 32.51, 45.24, 118.08, 124.64, 144.60, 145.58, 157.19, 162.45.

HRMS (ESI) *m/z* calculated for $C_{12}H_{16}{}^{79}Br\ N_2OS$ $[M + H]^+$: 315.0161, found: 315.0157; $C_{12}H_{15}{}^{79}Br\ N_2NaOS$ $[M + Na]^+$: 336.9981, found: 336.9976.

3-(3-Bromopropyl)-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25b)



Melting Point: 93 °C.

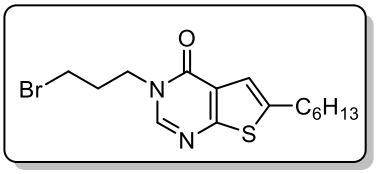
IR ($CHCl_3$): ν_{max} 1672, 2929, 3019 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J*=7 Hz, 3H), 1.28-1.52 (m, 2H), 1.60-1.82 (m, 2H), 2.37 (quint, *J*=6 Hz, 2H), 2.85 (t, *J*=7 Hz, 2 H), 3.43 (t, *J*=6 Hz, 2 H), 4.18 (t, *J*=7 Hz, 2 H), 7.15 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.67, 21.95, 29.82, 30.25, 31.12, 33.06, 45.29, 118.03, 124.70, 144.92, 145.59, 157.25, 162.48.

HRMS (ESI) *m/z* calculated for $C_{13}H_{18}{}^{79}BrN_2OS$ $[M + H]^+$: 329.0318, found: 329.0320; $C_{13}H_{18}{}^{81}BrN_2OS$ $[M + H]^+$: 331.0297, found: 331.0298.

3-(3-Bromopropyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25c)



Melting Point: 70 °C.

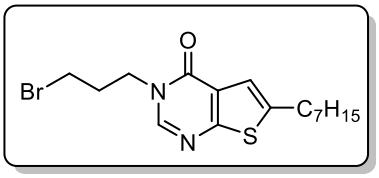
IR ($CHCl_3$): ν_{max} 1672, 2031, 3019 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 3H), 1.16-1.50 (m, 6H), 1.58-1.81 (m, 2H), 2.26-2.46 (m, 2H), 2.84 (t, *J*=8 Hz, 2H), 3.43 (t, *J*=7 Hz, 2H), 4.18 (t, *J*=7 Hz, 2H), 7.15 (s,1H), 8.01 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.99, 22.48, 28.54, 29.82, 30.59, 30.97, 31.16, 31.42, 45.30, 118.04, 124.74, 144.99, 145.59, 157.27, 162.50.

HRMS (ESI) *m/z* calculated for $C_{15}H_{22}{}^{79}BrN_2OS$ $[M + H]^+$: 357.0631, found: 357.0626; $C_{15}H_{22}{}^{81}BrN_2OS$ $[M + H]^+$: 359.0610, found: 359.0601.

3-(3-Bromopropyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25d)



Melting Point: 75 °C.

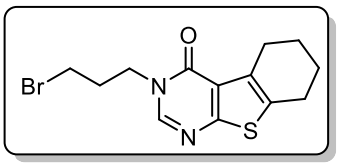
IR ($CHCl_3$): ν_{max} 1670, 2930, 3029 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* =7 Hz, 3H), 1.18-1.40 (m, 8H), 1.58-1.80 (m, 2H), 2.25-2.49 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 3.41 (t, *J*=6 Hz, 2H), 4.17 (t, *J*=6 Hz, 2H), 7.12 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.46, 28.74, 28.79, 29.78, 30.47, 30.90, 31.06, 31.55, 45.19, 117.92, 124.59, 144.82, 145.53, 157.13, 162.36.

HRMS (ESI) *m/z* calculated for $C_{16}H_{24}{}^{79}BrN_2OS$ $[M + H]^+$: 371.0787, found: 371.0785; $C_{16}H_{23}{}^{79}Br\ N_2NaOS$ $[M + Na]^+$: 393.0607, found: 393.0605.

3-(3-Bromopropyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (25e)



Melting Point: 122 °C.

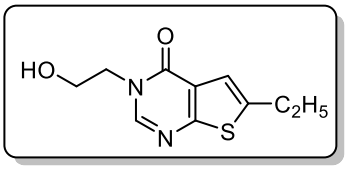
IR ($CHCl_3$): ν_{max} 1669, 2933, 3032 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89-1.94 (m, 4H), 2.31-2.42 (m, 2H), 2.79 (t, *J*=6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.43 (t, *J*=7 Hz, 2H), 4.15 (t, *J*=7 Hz, 2H), 7.79 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.17, 22.79, 25.18, 25.58, 29.91, 31.12, 45.09, 122.72, 131.45 134.39, 145.46, 157.79, 162.13.

HRMS (ESI) *m/z* calculated for $C_{13}H_{16}{}^{79}BrN_2OS$ $[M + H]^+$: 327.0161, found: 327.0156; $C_{13}H_{15}{}^{79}BrN_2NaOS$ $[M + Na]^+$: 348.9981, found: 348.9974.

6-Ethyl-3-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (26a)



Melting Point: 110 °C.

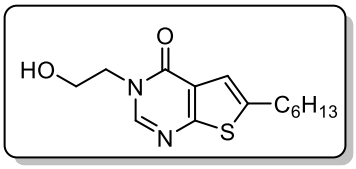
IR ($CHCl_3$): ν_{max} 1672, 3018, 3445 (b) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, *J*=7 Hz, 3H), 2.87 (q, *J*=7 Hz, 2H), 3.15 (bs, 1H), 3.97 (t, *J*=5 Hz, 2H), 4.15 (t, *J*=5 Hz, 2H), 7.07 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d6): δ 15.29, 23.16, 48.33, 58.23, 117.43, 123.84, 144.70, 148.13, 156.47, 161.92.

HRMS (ESI) *m/z* calculated for $C_{10}H_{13}N_2O_2S$ $[M + H]^+$: 225.0692, found: 225.0732; $C_{10}H_{12}N_2NaO_2S$ $[M + Na]^+$: 247.0512, found: 247.0555.

6-Hexyl-3-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (26b)



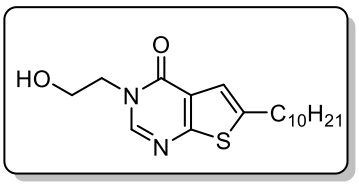
Melting Point: 79 °C.

IR ($CHCl_3$): ν_{max} 1671, 2927, 3016, 3409 (b) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz, 3H), 1.25-1.45 (m, 6H), 1.65-1.76 (m,2H), 2.35 (bs, 1H), 2.83 (t, *J*=8 Hz, 2H), 3.97 (t, *J*=6 Hz, 2H), 4.17 (t, *J*=6 Hz, 2H), 7.10 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.98, 22.48, 28.62, 30.58, 30.95, 31.44, 49.40, 60.48, 117.87, 124.44, 144.84, 146.38, 157.66, 162.48.

6-Decyl-3-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (26c)



Melting Point: 62 °C.

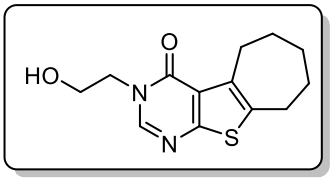
IR ($CHCl_3$): ν_{max} 1675, 2927, 3016, 3408 (b) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.18-1.43 (m, 14H), 1.62-1.78 (m, 2H), 2.82 (t, *J*=8 Hz, 2H), 2.97 (t, *J*=5 Hz, 1H), 3.97 (t, *J*=5 Hz, 2H), 4.16 (t, *J*=5 Hz, 2H), 7.08 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.65, 28.96, 29.27 (2C), 29.49, 29.54, 30.61, 31.03, 31.85, 49.42, 60.70, 117.91, 124.48, 144.92, 146.31, 157.78, 162.56.

HRMS (ESI) *m/z* calculated for $C_{18}H_{29}N_2O_2S$ $[M + H]^+$: 337.1944, found: 337.1937; $C_{18}H_{28}N_2NaO_2S$ $[M + Na]^+$: 359.1764 found: 359.1756.

3-(2-Hydroxyethyl)-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (26d)



Melting Point: 153 °C.

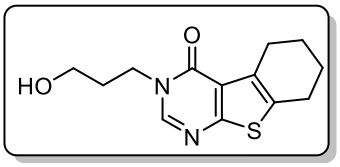
IR ($CHCl_3$): ν_{max} 1670, 2930, 3017, 3402 (b) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65 (m, 4H), 1.86-1.93 (m, 2H), 2.81-2.89 (m, 2H), 3.27-3.35 (m, 2H), 3.97 (t, *J*=5Hz, 2H), 4.17 (t, *J*=5Hz, 2H), 8.05 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 26.58, 27.04, 27.18, 29.21, 31.82, 48.24, 58.76, 122.46, 136.31, 137.06, 146.03, 157.71, 160.06.

HRMS (ESI) *m/z* calculated for $C_{13}H_{17}N_2O_2S$ $[M + H]^+$: 265.1005, found: 265.1013.

3-(3-Hydroxypropyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (27a)



Melting Point: 129 °C.

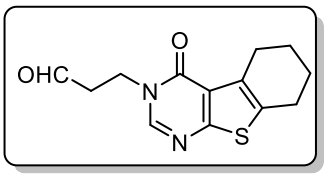
IR ($CHCl_3$): ν_{max} 1662, 2018, 3015, 3416 (b) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.78-1.93 (m, 4H), 1.98 (quint, *J*=6 Hz, 2H), 2.79 (t, *J*=6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.59 (t, *J*=6 Hz, 2H), 4.17 (t, *J*=6 Hz, 2H), 7.94 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.19, 22.81, 25.25, 25.66, 32.41, 42.51, 57.85, 122.51, 131.56, 134.62, 145.43, 158.78, 162.40.

HRMS (ESI) *m/z* calculated for $C_{13}H_{17}N_2O_2S$ $[M + H]^+$: 265.1005, found: 265.1006; $C_{13}H_{16}N_2NaO_2S$ $[M + Na]^+$: 287.0825, found: 287.0822.

3-(4-Oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propanal (28a)



Melting Point: 132 °C.

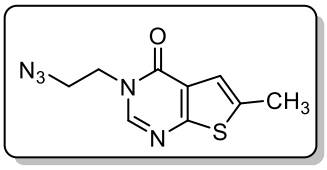
IR ($CHCl_3$): ν_{max} 1667, 1721, 2727 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.77-1.91 (m, 4H), 2.76 (t, *J*=6 Hz, 2H), 2.98 (t, *J*=6 Hz, 2H), 3.08 (t, *J*=6 Hz, 2H), 4.23 (t, *J*=6 Hz, 2H), 8.10 (s, 1H), 9.78 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.18, 22.80, 25.16, 25.55, 40.55, 42.36, 122.58, 131.31, 134.32, 146.22, 157.85, 162.16, 199.37.

HRMS (ESI) *m/z* calculated for $C_{13}H_{15}N_2O_2S$ $[M + H]^+$: 263.0849, found: 263.0844.

3-(2-Azidoethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29a)



Melting Point: 106 °C.

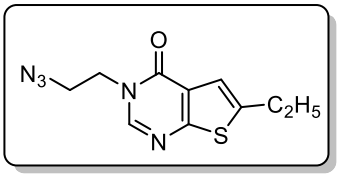
IR (KBr): ν_{max} 1676, 2108, 3012 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.56 (s, 3H), 3.76 (t, *J*=6 Hz, 2H), 4.13 (t, *J*=6 Hz, 2H), 7.14 (s, 1H), 7.99 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 16.04, 46.21, 49.52, 119.23, 124.82, 139.04, 145.85, 157.00, 162.93.

HRMS (ESI) *m/z* calculated for $C_9H_{10}N_5OS$ $[M + H]^+$: 236.0601, found: 236.0598.

3-(2-Azidoethyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29b)



Melting Point: 78 °C.

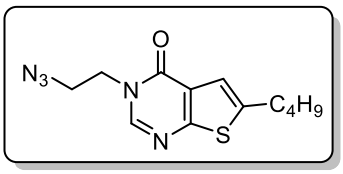
IR ($CHCl_3$): ν_{max} 1670, 2105, 3016 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (t, *J*=8 Hz, 3H), 2.89 (q, *J*=8 Hz, 2H), 3.76 (t, *J*= 6 Hz, 2H), 4.13 (t, *J*=6 Hz, 2H), 7.17 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.25, 23.99, 46.20, 49.50, 117.36, 124.55, 145.76, 146.47, 157.15, 162.67.

MS *m/z*: 250.1 $[M + H]^+$.

3-(2-Azidoethyl)-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29c)



Melting Point: 67 °C.

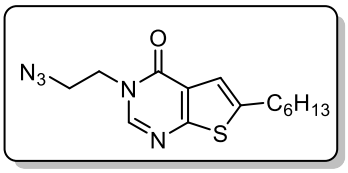
IR ($CHCl_3$): ν_{max} 1682, 2104 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J*=7 Hz, 3H), 1.30-1.52 (m, 2H), 1.61-1.80 (m, 2H), 2.85 (t, *J*=8 Hz, 2H), 3.76 (t, *J*=7 Hz, 2H), 4.12 (t, *J*=7 Hz, 2H), 7.15 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.63, 21.93, 30.21, 33.02, 46.10, 49.43, 117.97, 124.45, 144.93, 145.71, 157.04, 162.62.

HRMS (ESI) *m/z* calculated for $C_{12}H_{16}N_5OS$ $[M + H]^+$: 278.1070, found: 278.1064; $C_{12}H_{15}N_5NaOS$ $[M + Na]^+$: 300.0890, found: 300.0883.

3-(2-Azidoethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29d)



Melting Point: 50 °C.

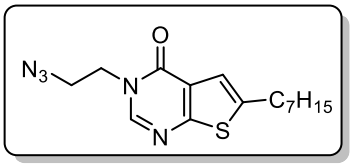
IR ($CHCl_3$): ν_{max} 1674, 2106 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.22-1.45 (m, 6H), 1.65-1.76 (m, 2H), 2.84 (t, *J*=7 Hz, 2H), 3.76 (t, *J*=6 Hz, 2H), 4.12 (t, *J*=6 Hz, 2H), 7.14 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.99, 22.47, 28.55, 30.58, 30.96, 31.42, 46.15, 49.46, 118.01, 124.50, 145.06, 145.71, 157.11, 162.67.

HRMS (ESI) *m/z* calculated for $C_{14}H_{20}N_5OS$ $[M + H]^+$: 306.1383, found: 306.1378; $C_{14}H_{19}N_5NaOS$ $[M + Na]^+$: 328.1203 found: 328.1197.

3-(2-Azidoethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29e)



Melting Point: 50 °C.

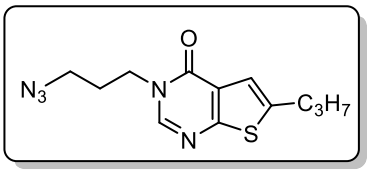
IR ($CHCl_3$): ν_{max} 1676, 2122 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J*=7 Hz, 3H), 1.19-1.45 (m, 8H), 1.62-1.82 (m, 2H), 2.84 (t, *J*=7 Hz, 2H), 3.76 (t, *J*=6 Hz, 2H), 4.12 (t, *J*=6 Hz, 2H), 7.15 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.94, 22.49, 28.79, 28.83, 30.52, 30.94, 31.58, 46.09, 49.43, 117.95, 124.47, 144.99, 145.70, 157.02, 162.55.

HRMS (ESI) *m/z* calculated for $C_{15}H_{22}N_5OS$ $[M + H]^+$: 320.1540, found: 320.1537; $C_{15}H_{21}N_5NaOS$ $[M + Na]^+$: 342. 1359, found : 342.1354.

3-(3-Azidopropyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (30a)



Melting Point: 60 °C.

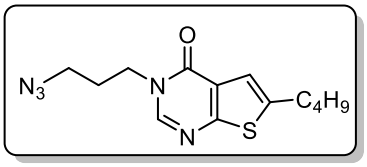
IR ($CHCl_3$): ν_{max} 1669, 2101, 3020 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.96 (t, *J*=7 Hz, 3H), 1.60-1.84 (m, 2H), 1.94-2.15 (m, 2H), 2.79 (t, *J*=7 Hz, 2H), 3.38 (t, *J*=7 Hz, 2H), 4.07 (t, *J*=7 Hz, 2H), 7.11 (s, 1H), 7.93 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.26, 24.05, 28.03, 32.32, 43.98, 48.01, 117.95, 124.45, 144.31, 145.42, 156.96, 162.23.

HRMS (ESI) *m/z* calculated for $C_{12}H_{16}N_5OS$ $[M + H]^+$: 278.1070, found: 278.1066; $C_{12}H_{15}N_5NaOS$ $[M + Na]^+$: 300.0890, found: 300.0884.

3-(3-Azidopropyl)-6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (30b)



Melting Point: 51 °C.

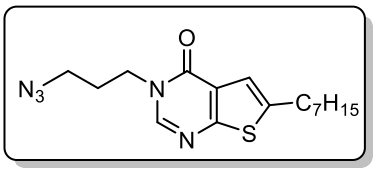
IR ($CHCl_3$): ν_{max} 1673, 2101, 3018 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J* =7 Hz, 3H), 1.32-1.52 (m, 2H), 1.60-1.80 (m, 2H), 1.98-2.16 (m, 2H), 2.85 (t, *J* =7 Hz, 2H), 3.42 (t, *J*=7 Hz, 2H), 4.10 (t, *J*= 7 Hz, 2H), 7.15 (s, 1H), 7.95 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.65, 21.94, 28.20, 30.23, 33.04, 44.19, 48.16, 118.04, 124.68, 144.87, 145.48, 157.19, 162.41.

HRMS (ESI) *m/z* calculated for $C_{13}H_{18}N_5OS$ $[M + H]^+$: 292.1227, found: 292.1224; $C_{13}H_{17}N_5NaOS$ $[M + Na]^+$: 314.1046 found: 314.1042.

3-(3-Azidopropyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (30c)



Melting Point: 52 °C.

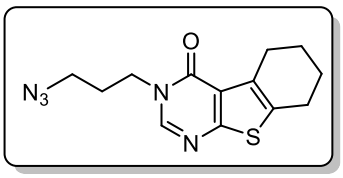
IR ($CHCl_3$): ν_{max} 1674, 2101 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J*=7 Hz, 3H), 1.15-1.38 (m, 8H), 1.60-1.79 (m, 2H), 1.98-2.13 (m, 2H), 2.82 (t, *J*=7 Hz, 2H), 3.40 (t, *J*=7 Hz, 2H), 4.08 (t, *J*=7 Hz, 2H), 7.13 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.00, 22.54, 28.22, 28.83, 28.88, 30.56, 30.98, 31.63, 44.20, 48.18, 118.05, 124.70, 144.95, 145.48, 157.21, 162.43.

HRMS (ESI) *m/z* calculated for $C_{16}H_{24}N_5OS$ $[M + H]^+$: 334.1696, found: 334.1692; $C_{16}H_{23}N_5NaOS$ $[M + Na]^+$: 356.1516, found: 356.1509.

3-(3-Azidopropyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (30d)



Melting Point: 70 °C.

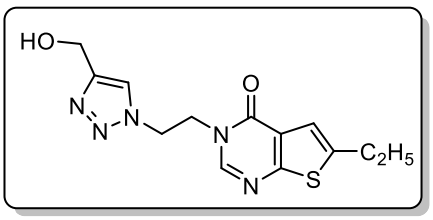
IR ($CHCl_3$): ν_{max} 1668, 2102 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.79-1.95 (m, 4H), 2.02-2.12 (m, 2H), 2.79 (t, *J* =6 Hz, 2H), 3.02 (t, *J*=6 Hz, 2H), 3.41 (t, *J*=6 Hz, 2H), 4.06 (t, *J*=6 Hz, 2H), 7.90 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.14, 22.76, 25.14, 25.54, 28.22, 43.94, 48.26, 122.67, 131.44, 134.29, 145.53, 157.69, 162.06.

HRMS (ESI) *m/z* calculated for $C_{13}H_{16}N_5OS$ $[M + H]^+$: 290.1070, found: 290.1064; $C_{13}H_{15}N_5NaOS$ $[M + Na]^+$: 312.0890, found: 312.0883.

6-Ethyl-3-(2-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)ethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (31a)



Melting Point: 158 °C.

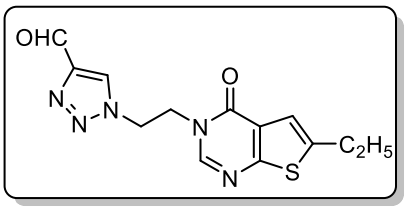
IR (KBr): ν_{max} 1681, 3394 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, *J*=8 Hz, 3H), 2.88 (q, *J*=8 Hz, 2H), 4.55 (t, *J*=6 Hz, 2H), 4.73 (bs, 2H), 4.79 (t, *J*=6 Hz, 2H), 7.16 (s, 1H), 7.42 (s, 1H), 7.50 (s, 1H).

^{13}C NMR (100 MHz, MeOH-d4): δ 15.98, 24.94, 48.15, 49.79, 56.56, 118.39, 124.90, 125.55, 147.81, 148.27, 149.71, 159.13, 164.08.

HRMS (ESI) *m/z* calculated for $C_{13}H_{16}N_5O_2S$ $[M + H]^+$: 306.1019, found: 306.1016; $C_{13}H_{15}N_5NaO_2S$ $[M + Na]^+$: 328.0839, found: 328.0835.

1-(2-(6-Ethyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)-1*H*-1,2,3-triazole-4-carbaldehyde (31b)



Melting Point: 188 °C.

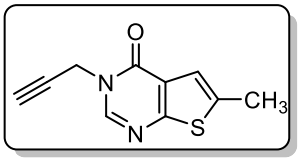
IR ($CHCl_3$): ν_{max} 1673, 1698 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37(t, *J*=7 Hz, 3H), 2.89 (q, *J*=7 Hz, 2H), 4.60 (t, *J*=7 Hz, 2H), 4.90 (t, *J*=7 Hz, 2H), 7.17 (s, 1H), 7.54 (s, 1H), 7.97 (s, 1H), 10.11 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 15.22, 24.01, 46.87, 48.47, 117.22, 124.39, 126.29, 144.70, 147.26, 147.85, 157.17, 162.76, 184.63.

MS *m/z* : 304.1 $[M+ H]^+$.

6-Methyl-3-(prop-2-yn-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32a)



Melting Point: 141 °C.

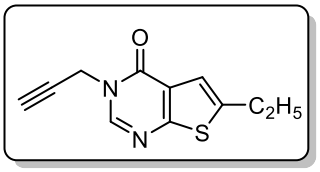
IR (Neat): ν_{max} 1658, 2120, 3195 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.52 (t, *J*=2 Hz, 1H), 2.55 (s, 3H), 4.81 (d, *J*=2 Hz, 2H), 7.14 (s, 1H), 8.25 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.94, 34.94, 75.34, 76.36, 119.22, 124.45, 139.02, 144.31, 156.24, 162.50.

HRMS (ESI) *m/z* calculated for $C_{10}H_9N_2OS$ $[M + H]^+$: 205.0430, found: 205.0428.

6-Ethyl-3-(prop-2-yn-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32b)



Melting Point: 100 °C.

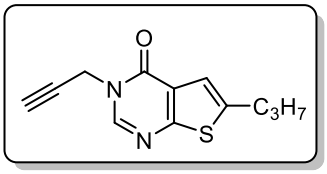
IR (Neat): ν_{max} 1668, 2124, 3306 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, *J*=7 Hz, 3H), 2.51 (t, *J*=3 Hz, 1H), 2.80-2.97 (m, 2H), 4.81 (d, *J*=3 Hz, 2H), 7.17 (s, 1H), 8.23 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 15.10, 23.83, 34.90, 75.24, 76.37, 117.33, 124.16, 144.29, 146.35, 156.34, 162.20.

MS *m/z* : 219.1 $[M+ H]^+$.

3-(Prop-2-yn-1-yl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (32c)



Melting Point: 103 °C.

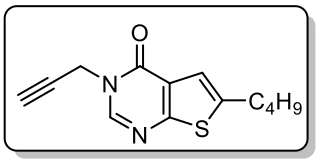
IR (Neat): ν_{max} 1664, 2123, 3310 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.99 (t, *J* =7 Hz, 3H), 1.73-1.75 (m, 2H), 2.51 (t, *J*=2 Hz, 2H), 2.82 (t, *J*=7 Hz, 1H), 4.81 (d, *J*=2 Hz, 2H), 7.17 (s, 1H), 8.25 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.39, 24.16, 32.45, 34.93, 75.26, 76.57, 118.15, 124.16, 144.28, 144.71, 156.35, 162.24.

HRMS (ESI) *m/z* calculated for $C_{12}H_{13}N_2OS$ $[M + H]^+$: 233.0743, found: 233.0740; $C_{12}H_{12}N_2NaOS[M + Na]^+$: 255.0563, found: 255.0558.

6-Butyl-3-(prop-2-yn-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32d)



Melting Point: 89 °C.

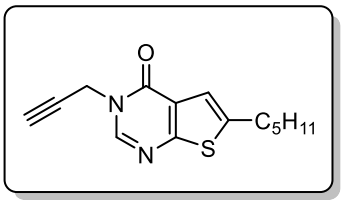
IR (Neat): ν_{max} 1678, 2123, 3307 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J*=7 Hz, 3H), 1.30-1.50 (m, 2H), 1.60-1.80 (m, 2H), 2.51 (t, *J*=3 Hz, 1H), 2.85 (t, *J*=8 Hz, 2H), 4.81(d, *J*=3 Hz, 2H), 7.17 (s, 1H), 8.23 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.63, 21.92, 30.21, 33.01, 34.96, 75.31, 76.37, 118.12, 124.24, 144.27, 145.03, 156.43, 162.33.

HRMS (ESI) *m/z* calculated for $C_{13}H_{15}N_2OS$ $[M + H]^+$: 247.0900, found: 247.0898.

6-Pentyl-3-(prop-2-yn-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32e)



Melting Point: 100 °C.

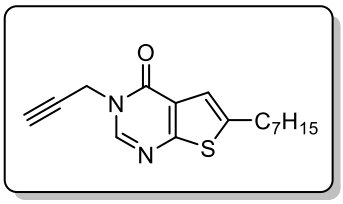
IR (Neat): ν_{max} 1677, 2124, 3311 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, *J*=7 Hz, 3H), 1.28-1.38 (m, 4H), 1.66-1.74 (m, 2H), 2.51 (d, *J*=2 Hz, 1H), 2.84 (t, *J*=7 Hz, 2H), 4.82 (d, *J*=2 Hz, 2H), 7.16 (s, 1H), 8.23 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.93, 22.31, 30.58, 30.70, 31.05, 35.02, 75.40, 76.50, 118.22, 124.33, 144.28, 145.19, 156.53, 162.41.

HRMS (ESI) *m/z* calculated for $C_{14}H_{17}N_2OS$ $[M + H]^+$: 261.1056, found: 261.1053.

6-Heptyl-3-(prop-2-yn-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32f)



Melting Point: 103 °C.

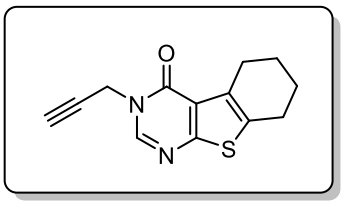
IR (KBr): ν_{max} 1675, 2122, 3320 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 3H), 1.16-1.46 (m, 8H), 1.61-1.81 (m, 2H), 2.52 (t, *J*=3 Hz, 1H), 2.85 (t, *J* =7 Hz, 2H), 4.82 (d, *J* =3 Hz, 2H), 7.17 (s, 1H), 8.28 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.98, 22.53, 28.82, 28.86, 30.56, 30.97, 31.62, 35.01, 75.36, 76.37, 118.16, 124.29, 144.31, 145.14, 156.44, 162.29.

MS *m/z* : 289.2 $[M+ H]^+$.

3-(Prop-2-yn-1-yl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (32g)



Melting Point: 115 °C.

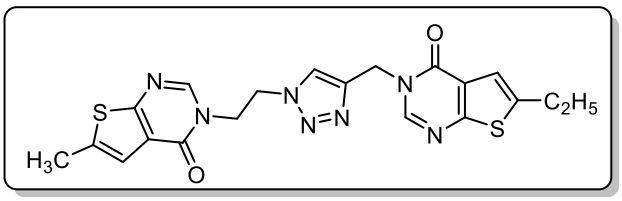
IR (KBr): ν_{max} 1673, 2358, 3315 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.79-2.01 (m, 4H), 2.49 (t, *J*=3 Hz, 1H), 2.78 (t, *J*=6 Hz, 2H) , 3.02 (t, *J*=6 Hz, 2H), 4.78 (d, *J*=3 Hz, 2H), 8.17 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.08, 22.72, 25.11, 25.45, 34.64, 75.08, 76.56, 122.27, 131.50, 134.48, 144.13, 156.89, 161.94.

HRMS (ESI) *m/z* calculated for $C_{13}H_{13}N_2OS$ $[M + H]^+$: 245.0743, found: 245.0739; $C_{13}H_{12}N_2NaOS$ $[M + Na]^+$: 267.0563, found: 267.0556.

6-Ethyl-3-((1-(2-(6-methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (33a)



Melting Point: 162 °C.

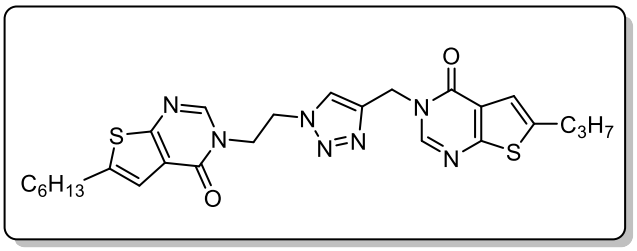
IR (KBr): ν_{max} 1670 (b), 3021 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d6): δ 0.91 (t, *J*=7 Hz, 3H), 2.10 (s, 3H), 2.45 (q, *J*=7 Hz, 2H), 4.06 (t, *J*=5Hz, 2H), 4.33 (t, *J*=5Hz, 2H), 4.82 (s, 2H), 6.63 (s, 1H), 6.65 (s, 1H), 7.26 (s, 1H), 7.51 (s, 1H), 7.86 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$+DMSO-d6): δ 14.23, 14.84, 22.72, 39.91, 45.24, 46.94, 116.23, 117.97, 123.26, 123.31, 123.70, 137.45, 141.33, 141.41, 144.84, 145.00, 155.57, 155.63, 161.24, 161.60.

HRMS (ESI) *m/z* calculated for $C_{20}H_{20}N_7O_2S_2$ $[M + H]^+$: 454.1114, found: 454.1103.

6-Hexyl-3-(2-(4-((4-oxo-6-propylthieno[2,3-d]pyrimidin-3(4*H*)-yl)methyl)-1*H*-1,2,3-triazol-1- yl)ethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (33b)



Melting Point: 141 °C.

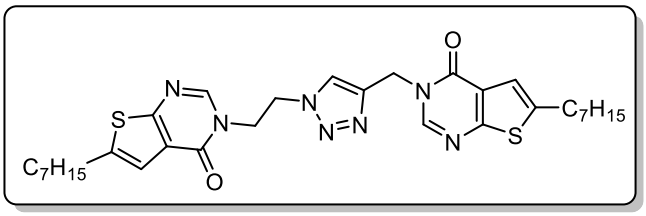
IR (KBr): ν_{max} 1673 (b), 2929, 3019 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84-1.04 (m, 6H), 1.17-1.48 (m, 6H), 1.61-1.87 (m, 4H), 2.81 (t, *J* =7 Hz, 4H), 4.52 (t, *J*=6 Hz, 2H), 4.74 (t, *J*=6 Hz, 2H), 5.21 (s, 2H), 7.09 (s, 1H), 7.14 (s, 1H), 7.48 (s, 1H), 7.63 (s, 1H), 8.22 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.49, 14.00, 22.48, 24.27, 28.62, 30.61, 30.96, 31.44, 32.61, 41.46, 46.77, 48.18, 117.92, 118.08, 124.39, 124.51, 125.08, 142.60, 144.76, 144.91, 145.29, 145.44, 157.03, 157.08,162.67,162.73.

HRMS (ESI) *m/z* calculated for $C_{26}H_{32}N_7O_2S_2$ $[M + H]^+$: 538.2053, found: 538.2043.

6-Heptyl-3-((1-(2-(6-heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (33c)



Melting Point: 159 °C.

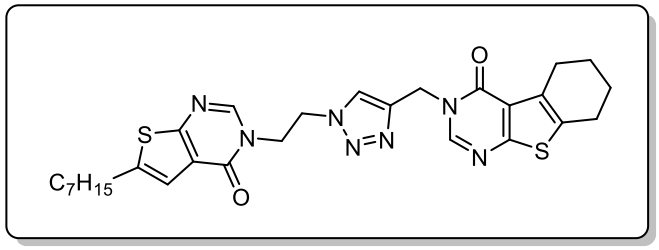
IR (KBr): ν_{max} 1667, 1679, 2930, 3025 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J*=7 Hz, 6H), 1.11-1.50 (m, 16H), 1.52-1.82 (m, 4H), 2.83 (t, *J*=7 Hz, 4H), 4.52 (t, *J*=8 Hz, 2H), 4.74 (t, *J*=8 Hz, 2H), 5.21 (s, 2H), 7.09 (s, 1H), 7.14 (s, 1H), 7.48 (s, 1H), 7.64 (s, 1H) 8.22 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.03 (2C), 22.56 (2C), 28.90 (4C), 30.56 (2C), 30.99 (2C), 31.65 (2C), 41.46, 46.76, 48.15, 117.89 (2C), 124.45, 125.10, 128.37, 131.13, 144.91, 145.07, 145.25, 145.42, 157.05 (2C), 162.64 (2C).

MS *m/z* : 608.3 $[M+H]^+$.

3-((1-(2-(6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (33d)



Melting Point: 150 °C.

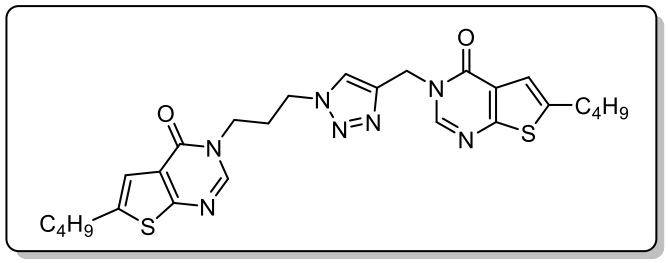
IR (KBr): ν_{max} 1677 (b), 2930, 3028 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J* =7 Hz, 3H), 1.24-1.35 (m, 6H), 1.40-1.85 (m, 8H), 2.77-2.97 (m, 6H), 4.52 (t, *J*=6 Hz, 2H), 4.74 (t, *J*=6 Hz, 2H), 5.17 (s, 2H), 7.14 (s, 1H), 7.47 (s, 1H), 7.62 (s, 1H), 8.17 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.02, 22.16, 22.56, 22.77, 25.16, 25.55, 28.89 (2C), 30.58, 30.98, 31.65, 41.17, 46.79, 48.15, 117.87, 122.51, 124.34, 125.07, 131.34, 134.45, 143.00 (2C), 144.12, 145.43, 157.06, 157.50, 162.30, 162.63.

HRMS (ESI) *m/z* calculated for $C_{28}H_{35}N_7O_2S_2$ $[M + H]^+$: 564.2210, found: 564.2194.

6-Butyl-3-(3-(4-((6-butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (34a)



Melting Point: 173 °C.

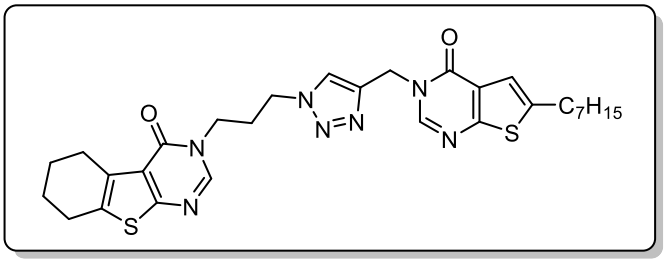
IR ($CHCl_3$): ν_{max} 1668 (b), 2928, 3030 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.91-0.97 (m, 6H), 1.35-1.47 (m, 4H), 1.64-1.75 (m, 4H), 2.37-2.47 (m, 2H), 2.79-2.88 (m, 4H), 4.06 (t, *J* =7 Hz, 2H), 4.39 (t, *J* =7 Hz, 2H), 5.26 (s, 2H), 7.12 (s, 1H), 7.14 (s, 1H), 7.85 (s, 1H), 7.98 (s, 1H), 8.26 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.68 (2C), 22.00 (2C), 29.68, 30.29 (2C), 33.09 (2C), 41.58, 43.96, 47.26, 117.98, 118.07, 124.25, 124.60, 124.69, 128.82, 145.06, 145.14, 145.41, 145.48, 157.11, 157.35, 162.58, 162.71.

HRMS (ESI) *m/z* calculated for $C_{26}H_{32}N_7O_2S_2$ $[M + H]^+$: 538.2053, found: 538.2049.

3-(3-(4-((6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (34b)



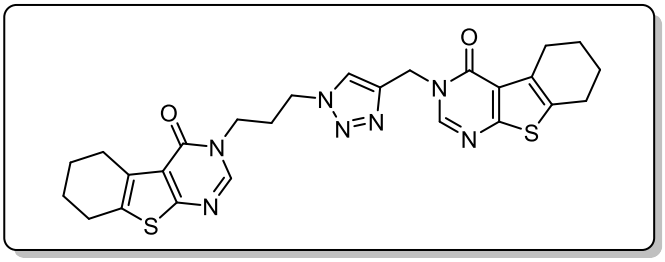
Melting Point: 157 °C.

IR (KBr): ν_{max} 1669 (b), 2925, 3029 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* =7 Hz, 3H), 1.14-1.33 (m, 8H), 1.55-1.95 (m, 6H), 2.40 (quint, *J*=8 Hz, 2H), 2.68-2.86 (m, 4H), 3.00 (t, 2H), 4.03 (t, *J*=7 Hz, 2H), 4.39 (t, *J*=7 Hz, 2H), 5.26 (s, 2H), 7.12 (s, 1H), 7.84 (s, 1H), 7.93 (s, 1H), 8.26 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.01, 22.16, 22.54, 22.78, 25.17, 25.58, 28.87 (2C), 29.63, 30.56, 30.99, 31.63, 41.54, 43.70, 47.29, 117.88 (2C), 124.18, 124.49, 131.43, 134.55, 145.09 (2C), 145.33, 145.37, 157.08, 157.83, 162.17, 162.64.

3-(3-(4-((4-Oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (34c)



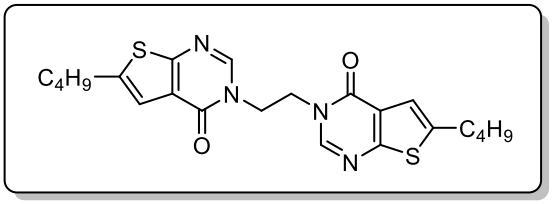
Melting Point: 121 °C.

IR (KBr): ν_{max} 1668 (b), 2931, 3032 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.71-1.95 (m, 8H), 2.25-2.60 (m, 2H), 2.78 (t, *J*=6 Hz, 4H), 3.00 (t, *J*=6 Hz, 4H), 4.03 (t, *J* = 6 Hz, 2H), 4.40 (t, *J*=7 Hz, 2H), 5.23 (s, 2H), 7.83 (s, 1H), 7.94 (s, 1H), 8.82 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.15 (2C), 22.77 (2C), 25.16 (2C), 25.57 (2C), 29.60, 41.22, 43.71, 47.26, 122.62, 122.67, 124.11 (2C), 131.33, 131.40, 134.44, 134.53, 145.30, 145.32, 157.56, 157.82, 162.16, 162.27.

3,3'-(Ethane-1,2-diyl)bis(6-butylthieno[2,3-*d*]pyrimidin-4(3*H*)-one) (35a)



Melting Point: 207 °C.

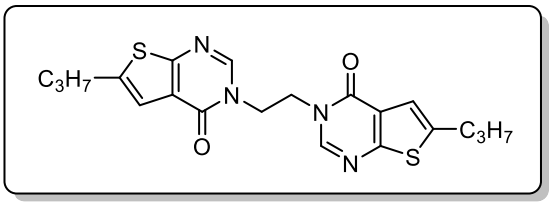
IR (KBr): ν_{max} 1674 (b), 2920, 3047 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.96 (t, *J* =8 Hz, 6H), 1.32-1.50 (m, 4H), 1.64-1.81 (m, 4H), 2.86 (t, *J*=8 Hz, 4H), 4.43 (s, 4H), 7.17 (s, 2H), 7.68 (s, 2H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.69 (2C), 22.03 (2C), 30.32 (2C), 33.07 (2C), 45.51, 45.61, 117.94 (2C), 124.42 (2C), 145.16, 145.30, 145.56, 145.61, 157.30 (2C), 162.43, 162.63.

HRMS (ESI) *m/z* calculated for $C_{22}H_{27}N_4O_2S_2$ $[M + H]^+$: 443.1570, found: 443.1571; $C_{22}H_{26}N_4NaO_2S_2$ $[M + Na]^+$: 465.1389 found: 465.1389.

3,3'-(Ethane-1,2-diyl)bis(6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one) (35b)



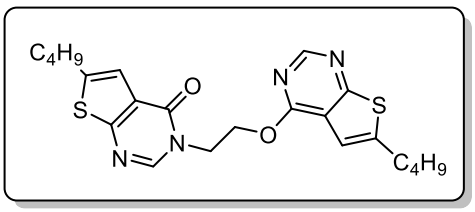
Melting Point: 217 °C.

IR (KBr): ν_{max} 1660 (b), 2922, 3047 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (t, *J*=7 Hz, 6H), 1.65-1.85 (m, 4H), 2.83 (t, *J*=7 Hz, 4H), 4.42 (s, 4H), 7.16 (s, 2H), 7.69 (s, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.54 (2C), 24.27 (2C), 32.64 (2C), 45.48 (2C), 118.03 (2C), 124.40 (2C), 145.12 (2C), 145.29 (2C), 157.35 (2C), 162.86 (2C).

6-Butyl-3-(2-((6-butylthieno[2,3-*d*]pyrimidin-4-yl)oxy)ethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (36a)



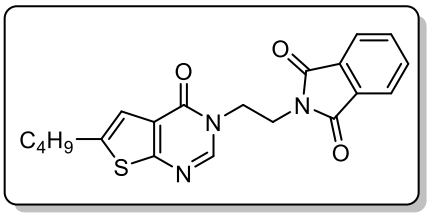
Melting Point: 176 °C.

IR ($CHCl_3$): ν_{max} 1669, 2930,3033 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89-1.00 (m, 6H), 1.34-1.47 (m, 4H), 1.65-1.76 (m, 4H), 2.84 (t, *J*=8 Hz, 2H), 2.90 (t, *J*=8 Hz, 2H), 4.49 (t, *J*=5 Hz, 2H), 4.85 (t, *J*=5 Hz, 2H), 6.94 (s, 1H), 7.17 (s, 1H), 8.00 (s, 1H), 8.51 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.70, 13.74, 21.98, 22.13, 30.27, 30.67, 33.07, 33.09, 45.90, 63.79, 114.04, 118.16, 119.34, 124.70, 144.84, 145.90, 146.32, 152.04, 157.18, 161.72, 162.49, 16 8.27.

2-(2-(6-Butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)isoindoline-1,3-dione (37a)



Melting Point: 139 °C.

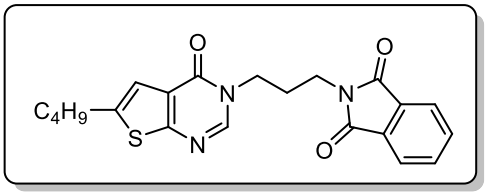
IR ($CHCl_3$): ν_{max} 1674, 1714, 3021 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, *J*=7 Hz, 3H), 1.30-1.52 (m, 2H), 1.58-1.80 (m, 2H), 2.83 (t, *J*=7 Hz, 2H), 4.14 (t, *J*=6 Hz, 2H), 4.33 (t, *J*=6 Hz, 2H), 7.14 (s, 1H), 7.66-7.92 (m, 5H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.71, 22.06, 30.29, 33.02, 36.69, 45.36, 118.22, 123.58 (2C), 124.62, 131.63 (2C), 134.27 (2C), 144.84, 144.98, 157.22, 162.35, 167.98 (2C).

MS *m/z* : 382.1 $[M+ H]^+$.

2-(3-(6-Butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)isoindoline-1,3-dione (38a)



Melting Point: 146 °C.

IR ($CHCl_3$): ν_{max} 1678, 1714, 3022 cm^{-1}.

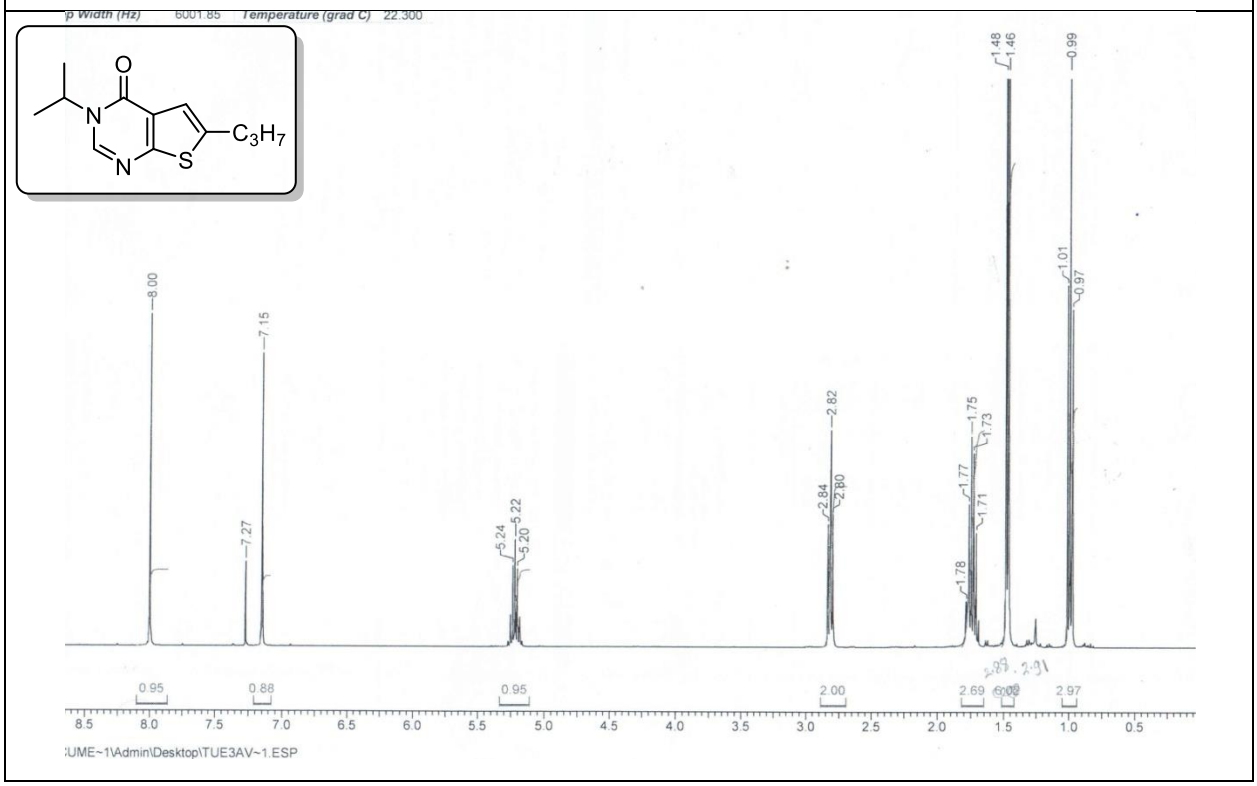
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.94 (t, *J*=7 Hz, 3H), 1.30-1.51 (m, 2H), 1.61-1.79 (m, 2H), 2.11-2.29 (m, 2H), 2.84 (t, *J*=8 Hz, 2H), 3.80 (t, *J*=7 Hz, 2H), 4.06 (t, *J*=7 Hz, 2H), 7.12 (s, 1H), 7.69-7.79 (m, 2H), 7.80-7.91 (m, 2H), 8.07 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.64, 21.92, 28.51, 30.19, 33.01, 35.10, 44.66, 118.05, 123.28 (2C), 124.64, 131.81 (2C), 134.03 (2C), 144.62, 145.63, 157.11, 162.38, 168.25 (2C).

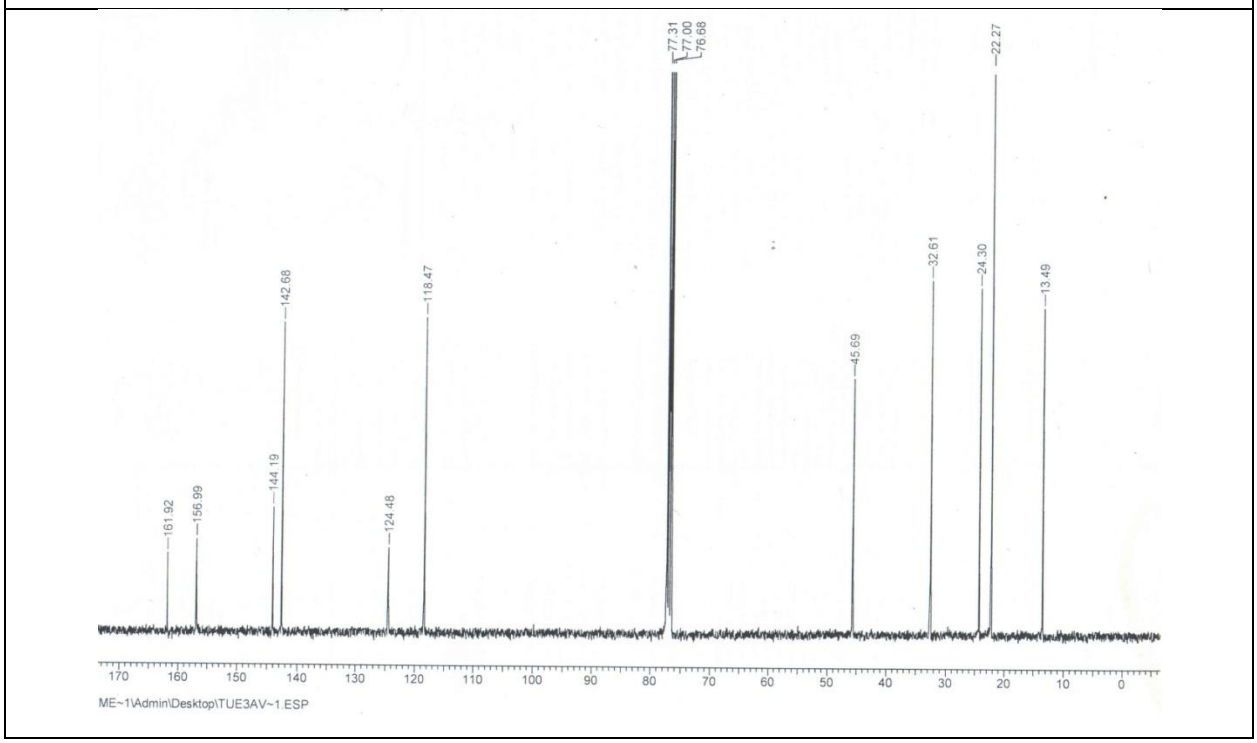
HRMS (ESI) *m/z* calculated for $C_{21}H_{22}N_3O_3S$ $[M + H]^+$: 396.1376, found: 396.1373; $C_{21}H_{21}N_3NaO_3S$ $[M + Na]^+$: 418.1196, found: 418.1190.

1.2.4.2. Representative ^{1}H and ^{13}C NMR

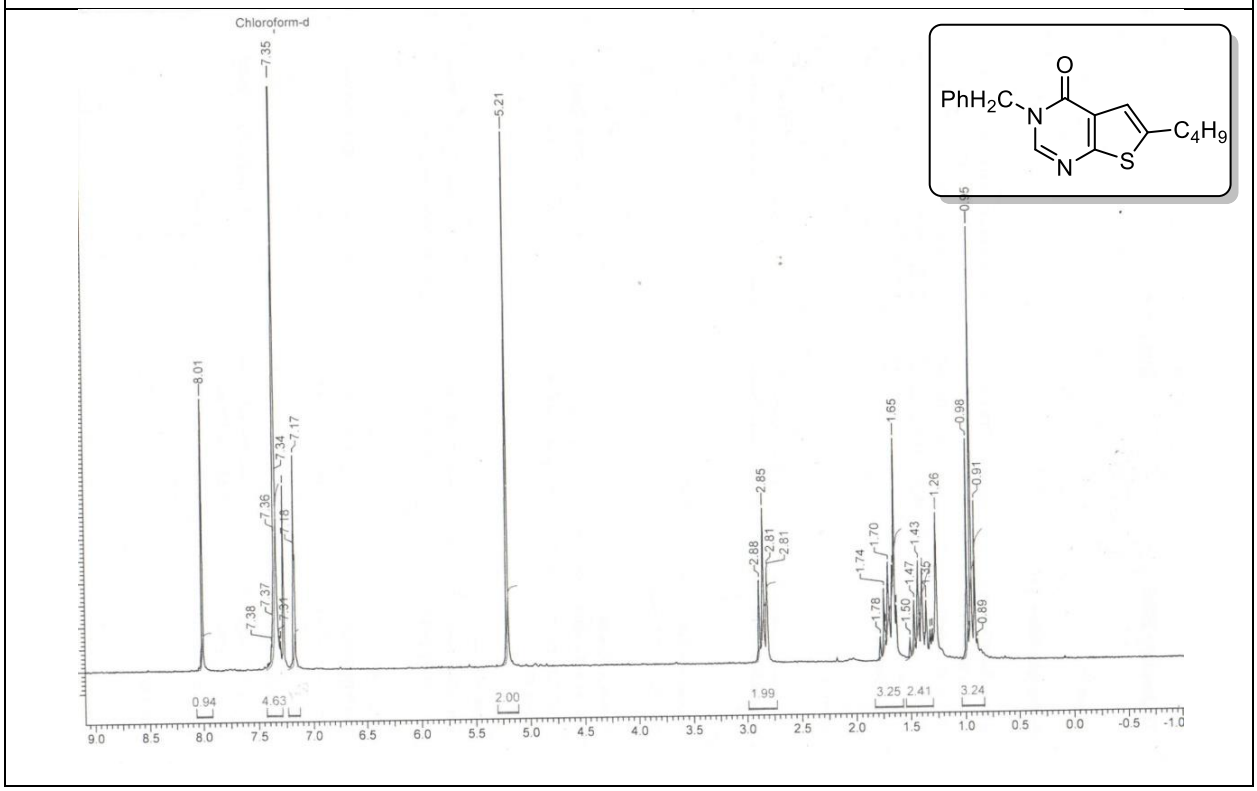
^{1}H NMR (400 MHz, $CDCl_3$) of compound 11j



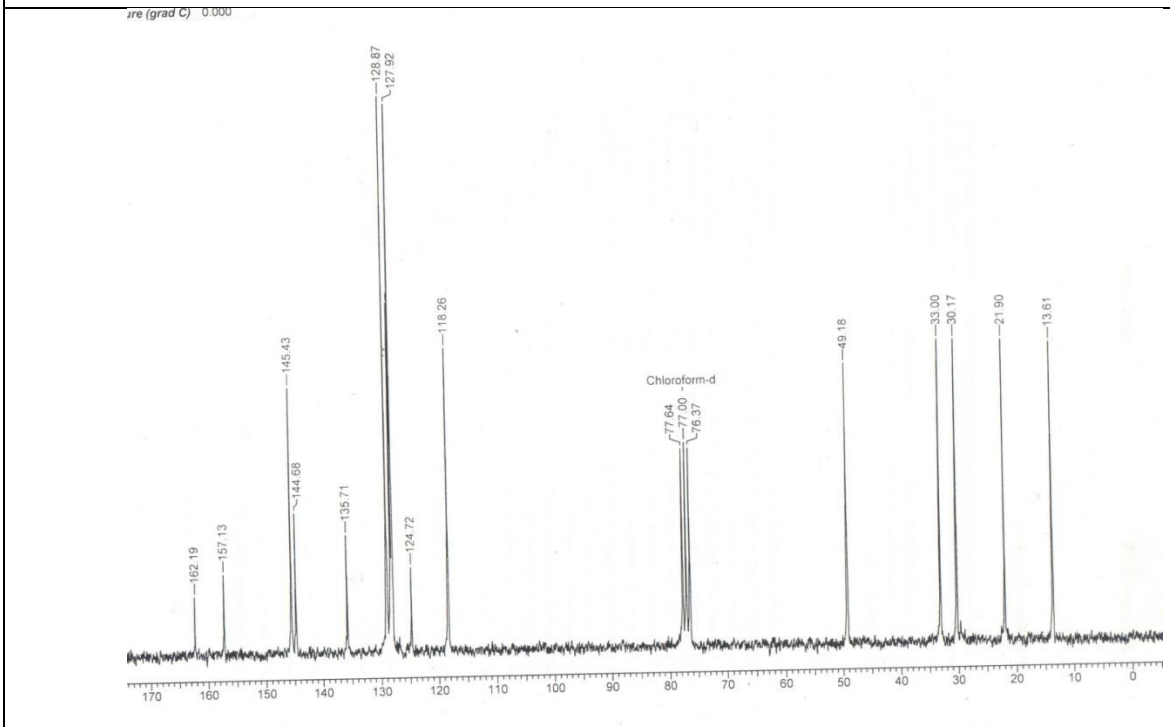
^{13}C NMR (100 MHz, $CDCl_3$) of compound 11j



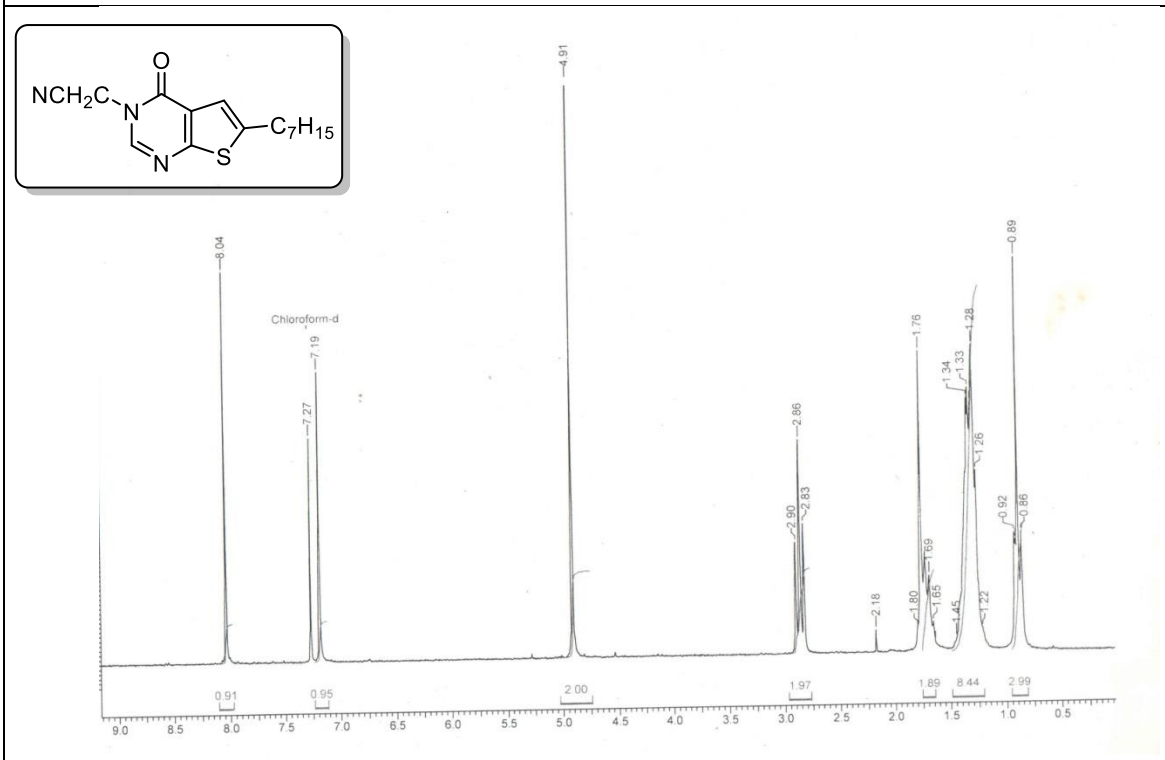
^{1}H NMR (200 MHz, $CDCl_3$) of compound 12c



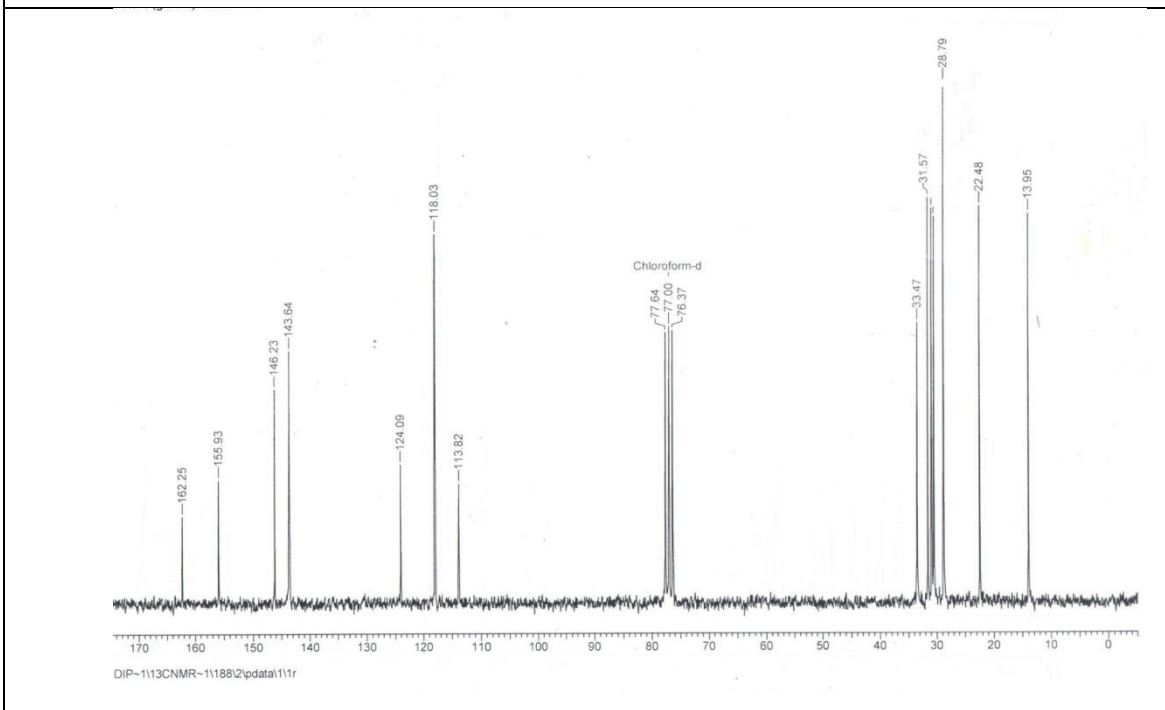
^{13}C NMR (50 MHz, $CDCl_3$) of compound 12c



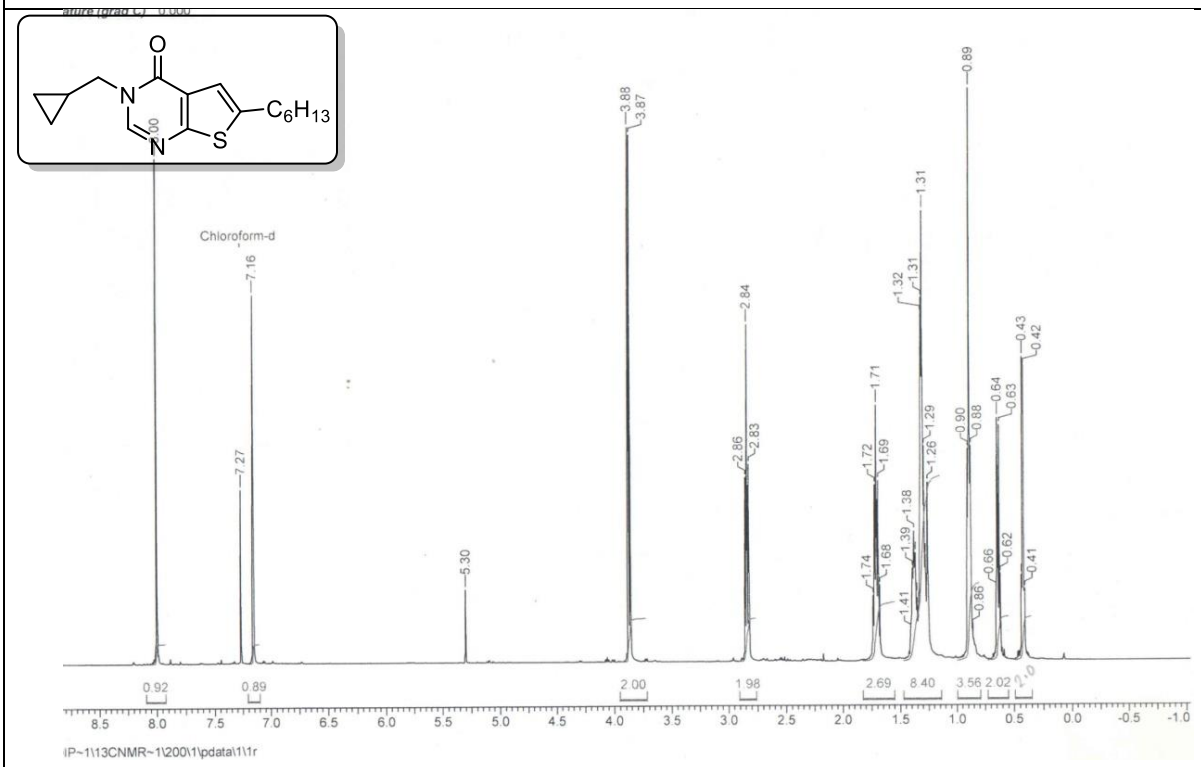
^{1}H NMR (200 MHz, $CDCl_3$) of compound 13b



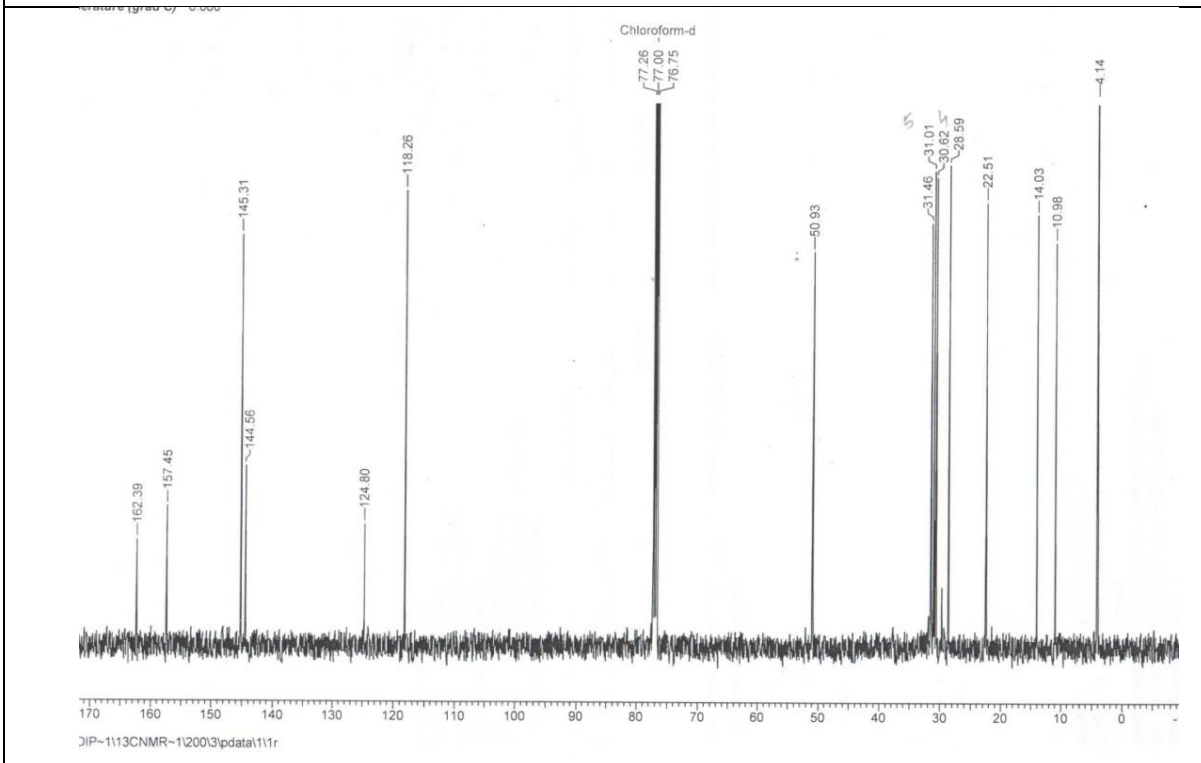
^{13}C NMR (50 MHz, $CDCl_3$) of compound 13b



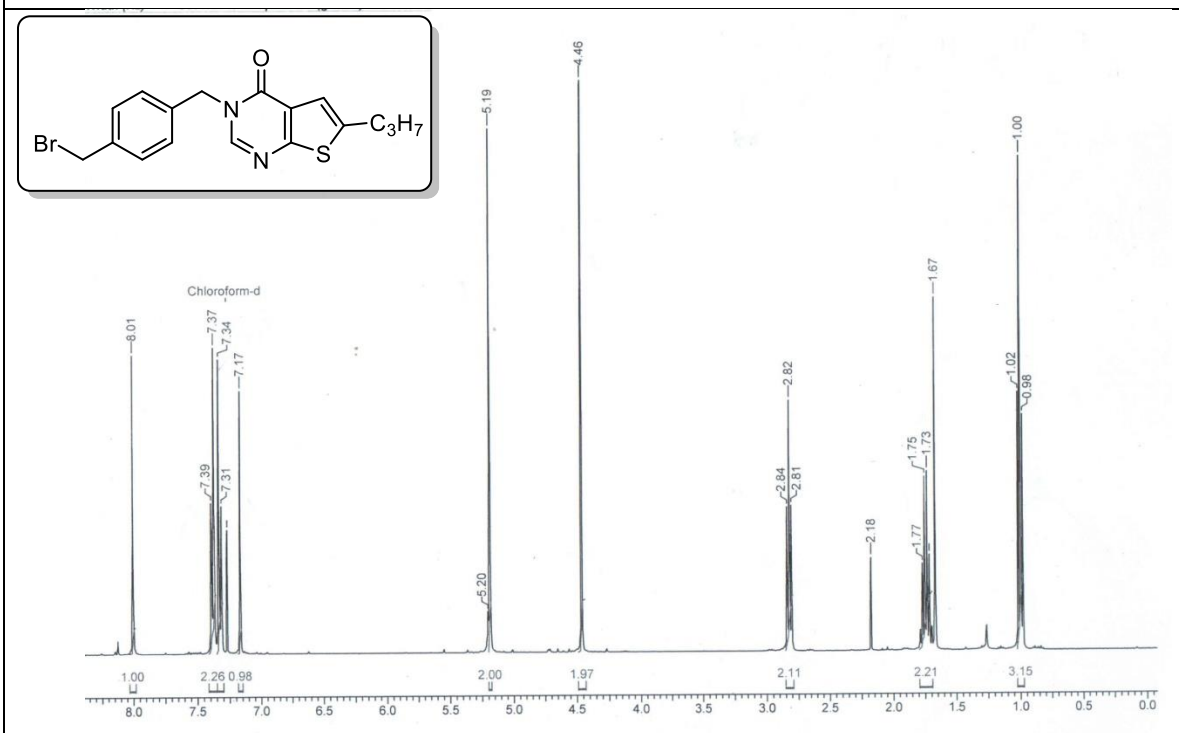
^{1}H NMR (200 MHz, $CDCl_3$) of compound 14b



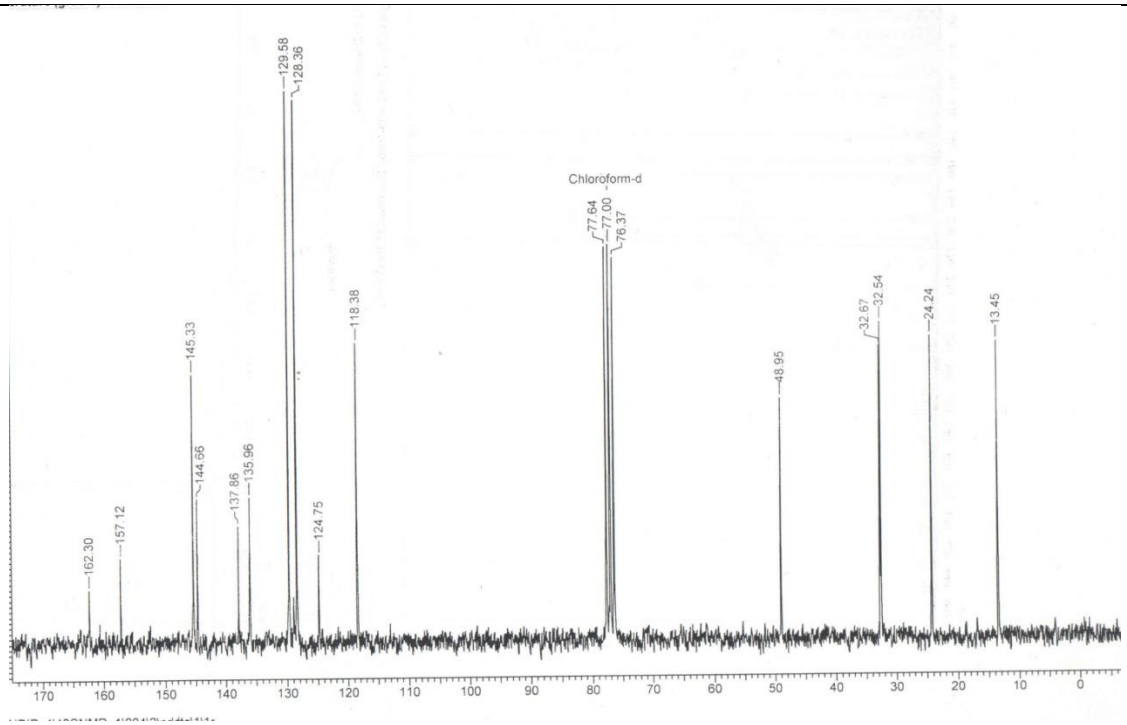
^{13}C NMR (50 MHz, $CDCl_3$) of compound 14b



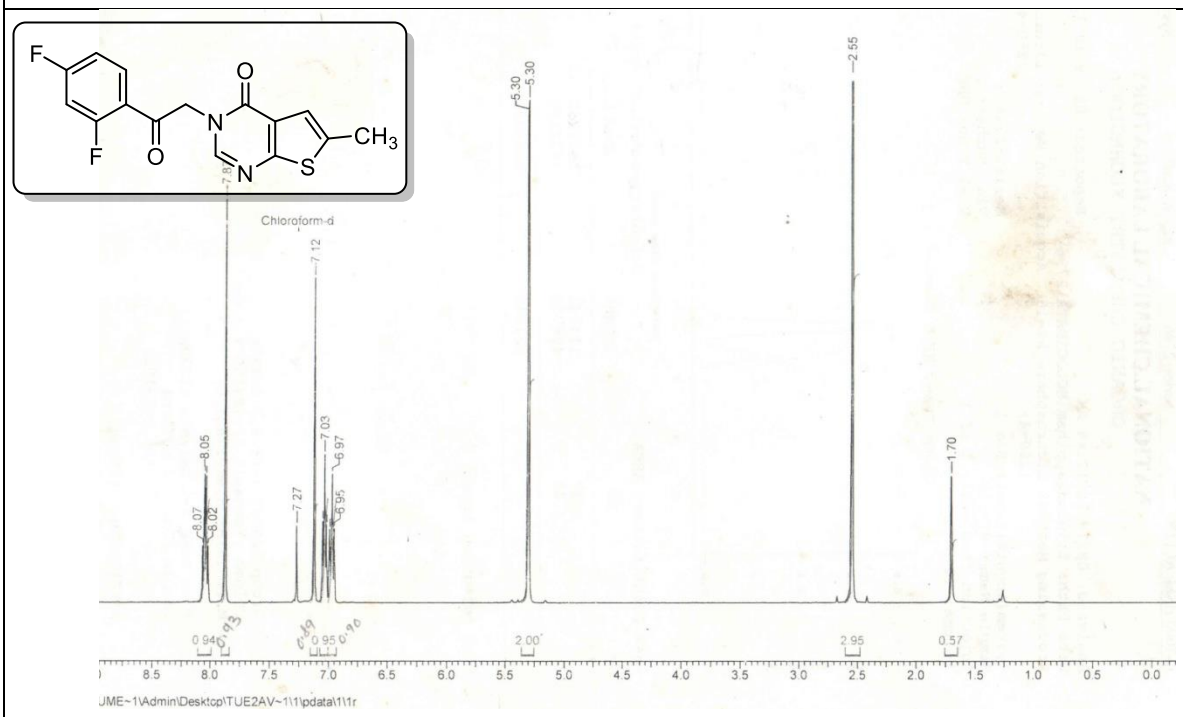
^{1}H NMR (400 MHz, $CDCl_3$) of compound 15



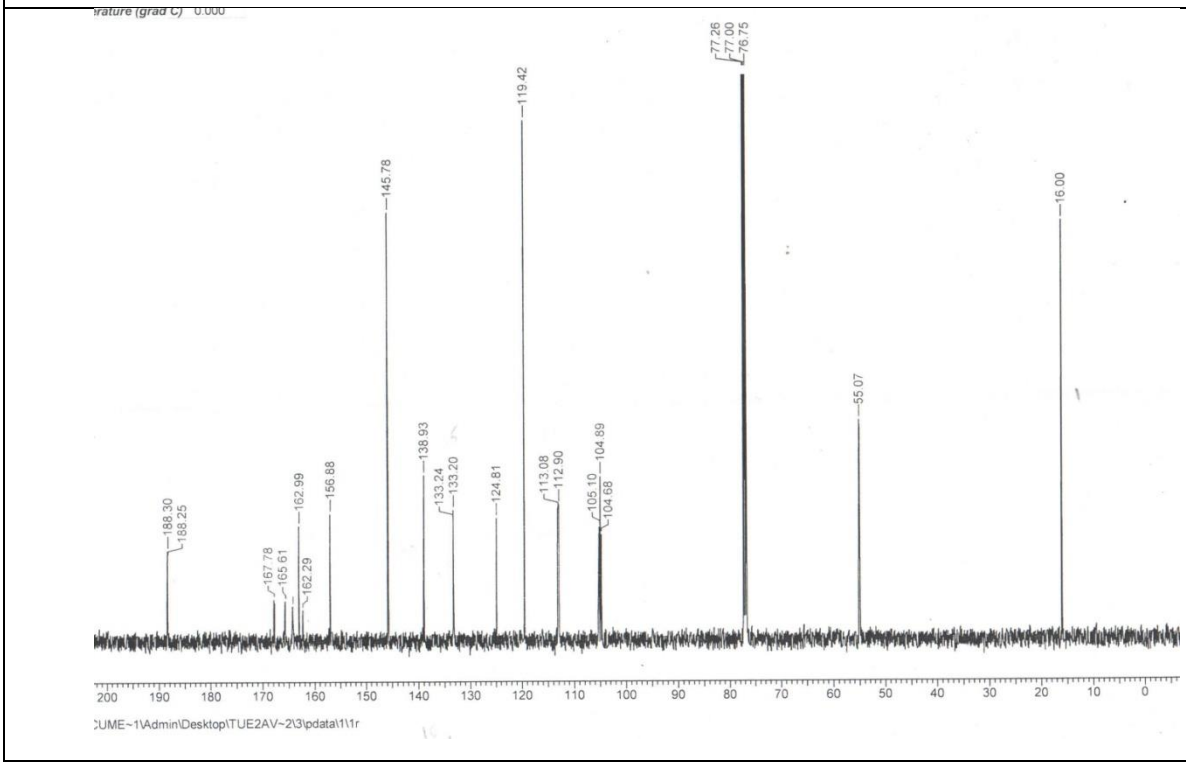
^{13}C NMR (100 MHz, $CDCl_3$) of compound 15



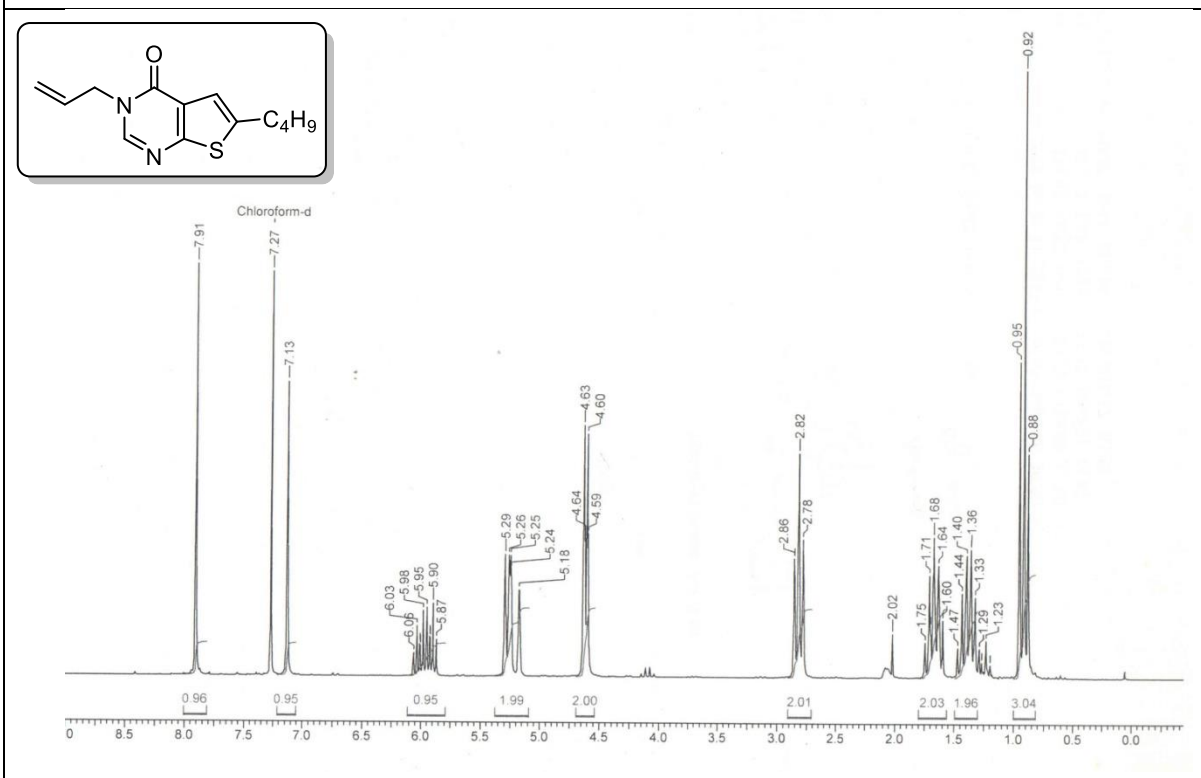
^{1}H NMR (500 MHz, $CDCl_3$) of compound 17a



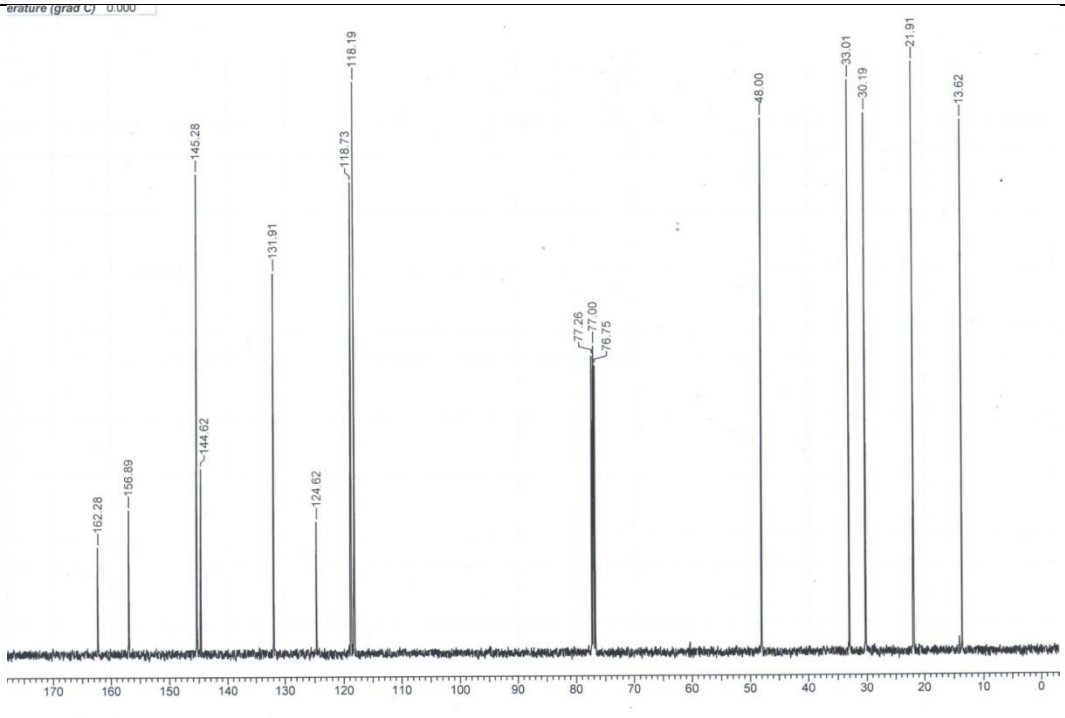
^{13}C NMR (125 MHz, $CDCl_3$) of compound 17a



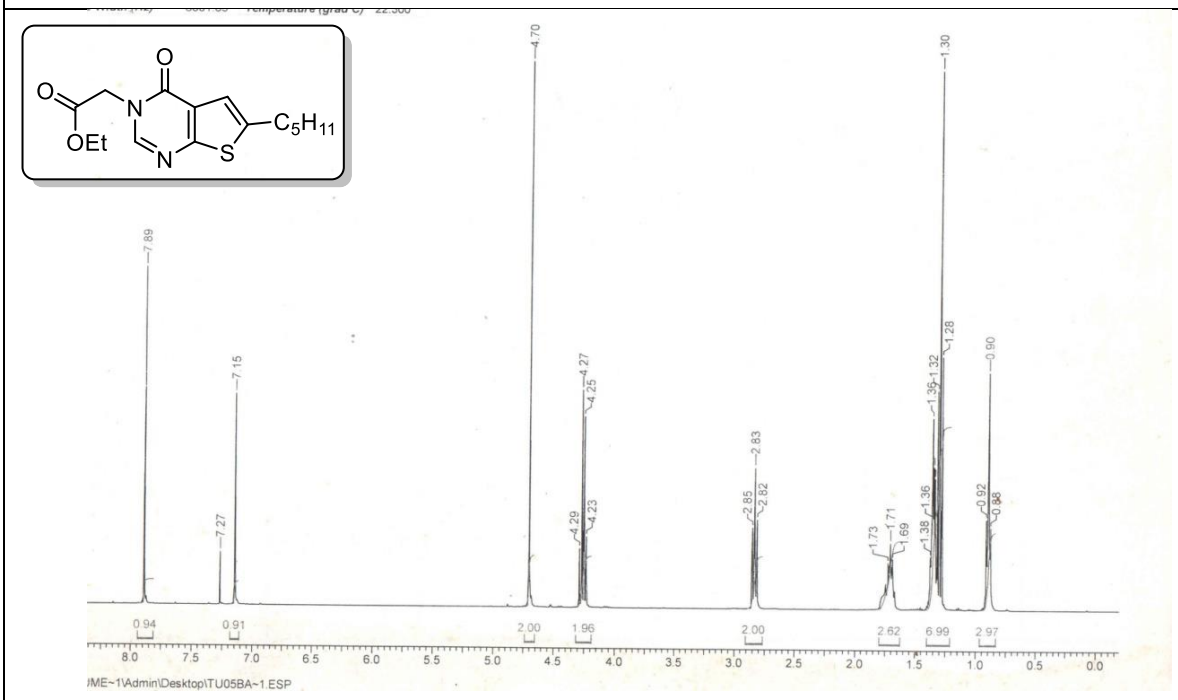
^{1}H NMR (200 MHz, $CDCl_3$) of compound 18a



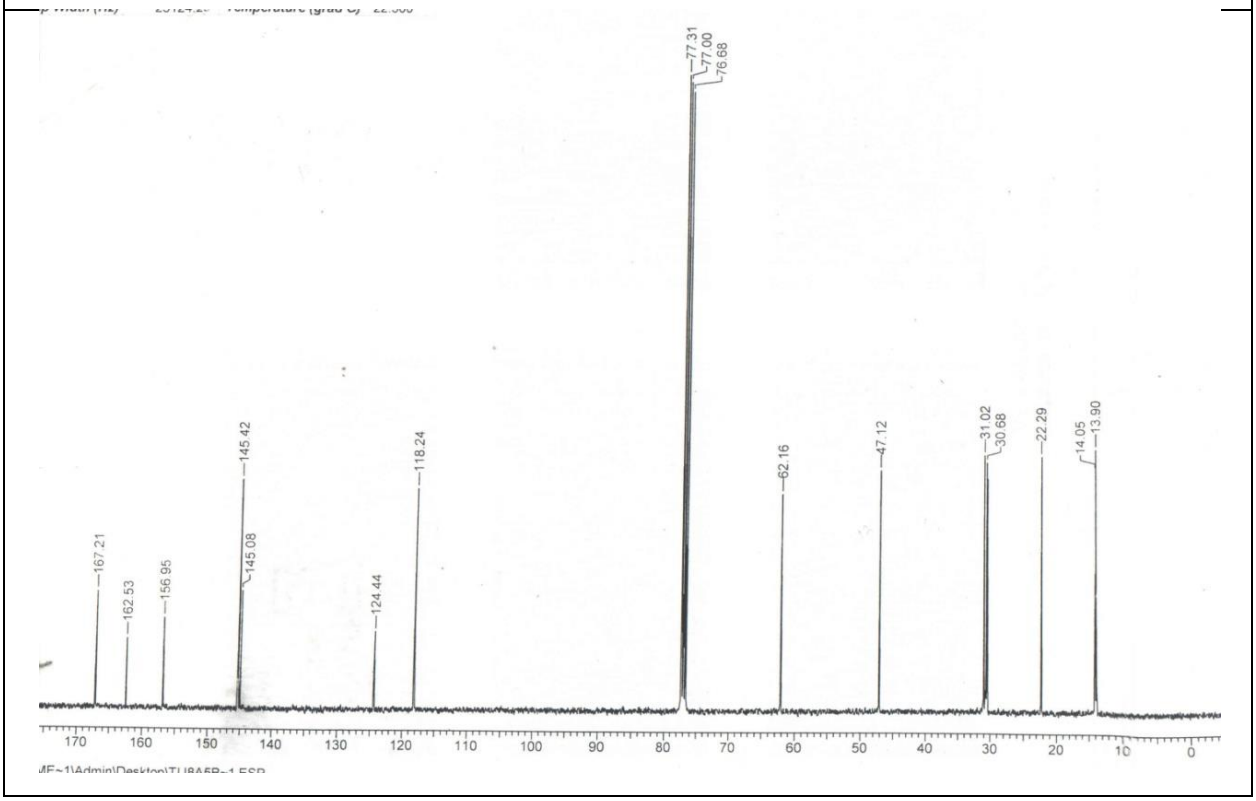
^{13}C NMR (125 MHz, $CDCl_3$) of compound 18a



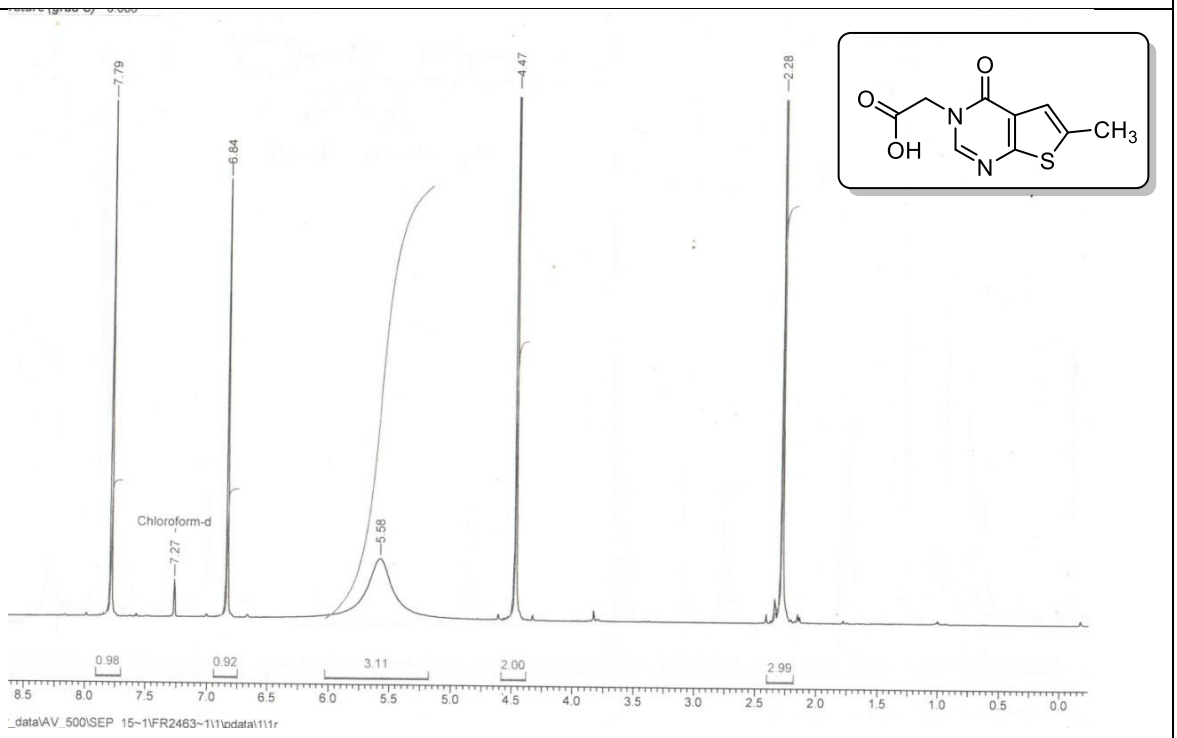
^{1}H NMR (400 MHz, $CDCl_3$) of compound 19a



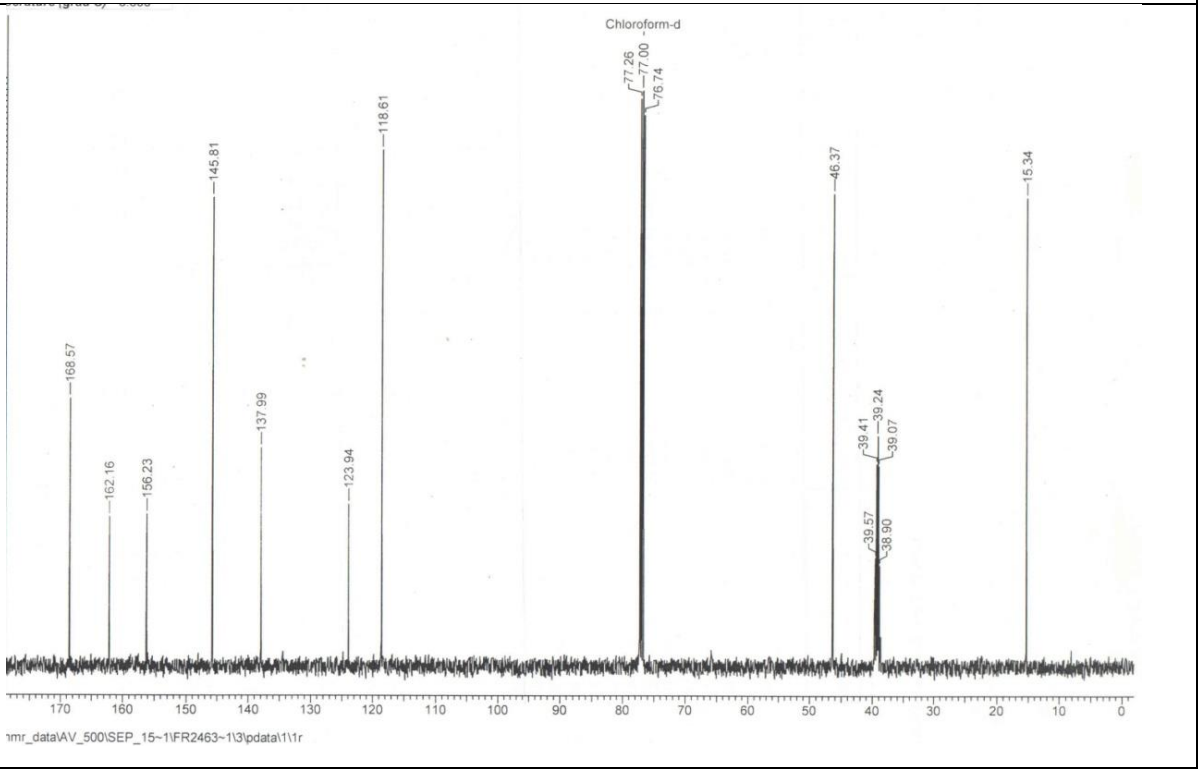
^{13}C NMR (100 MHz, $CDCl_3$) of compound 19a



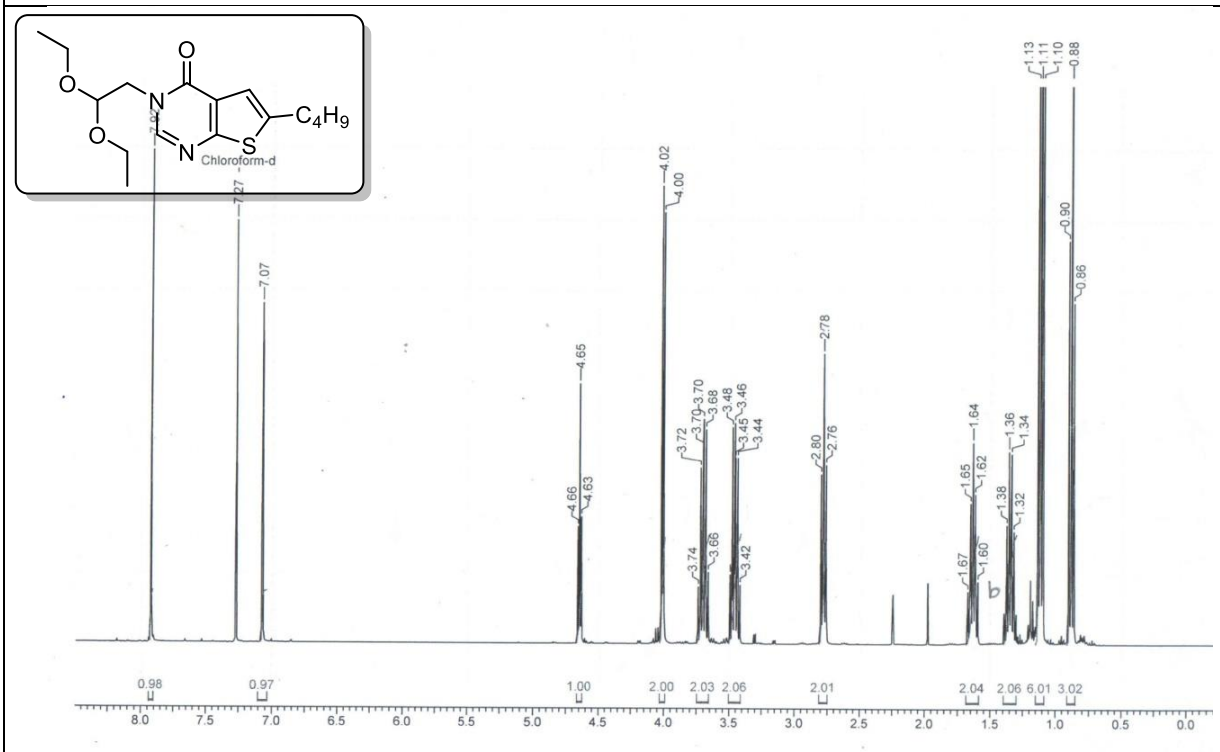
^{1}H NMR (500 MHz, $CDCl_3$) of compound 20c



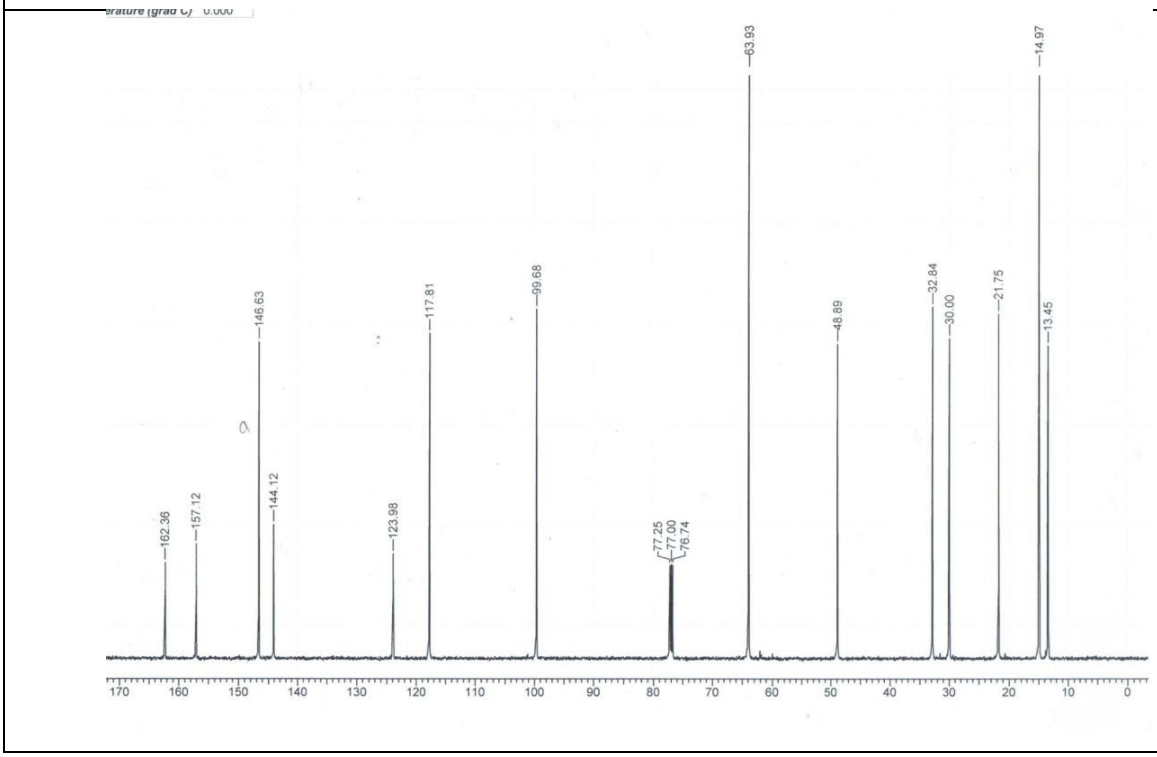
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d6) of compound 20c



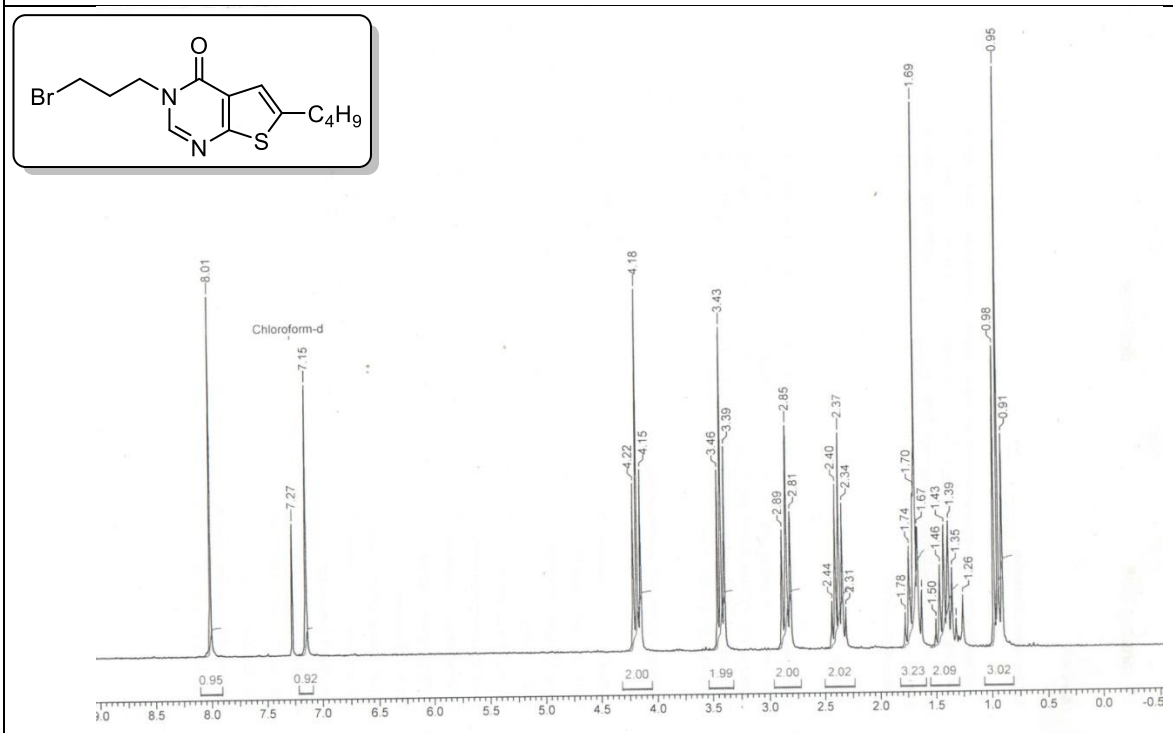
^{1}H NMR (400 MHz, $CDCl_3$) of compound 21a



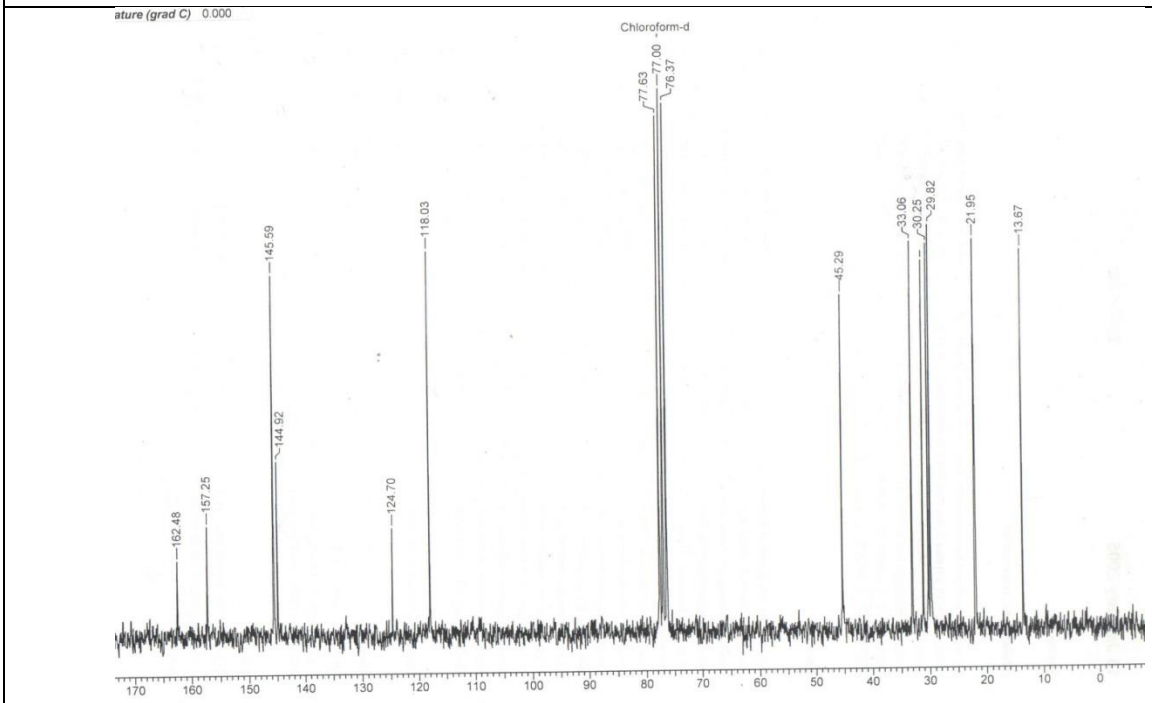
^{13}C NMR (125 MHz, $CDCl_3$) of compound 21a



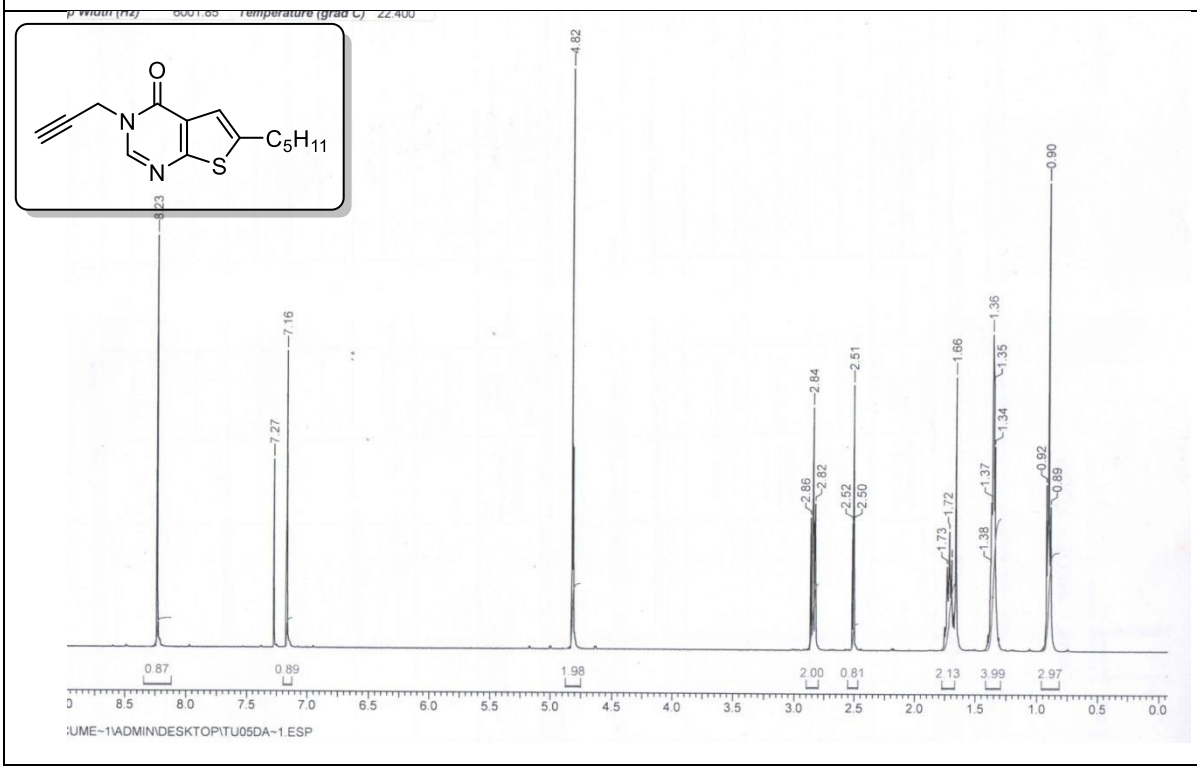
^{1}H NMR (200 MHz, $CDCl_3$) of compound 25b



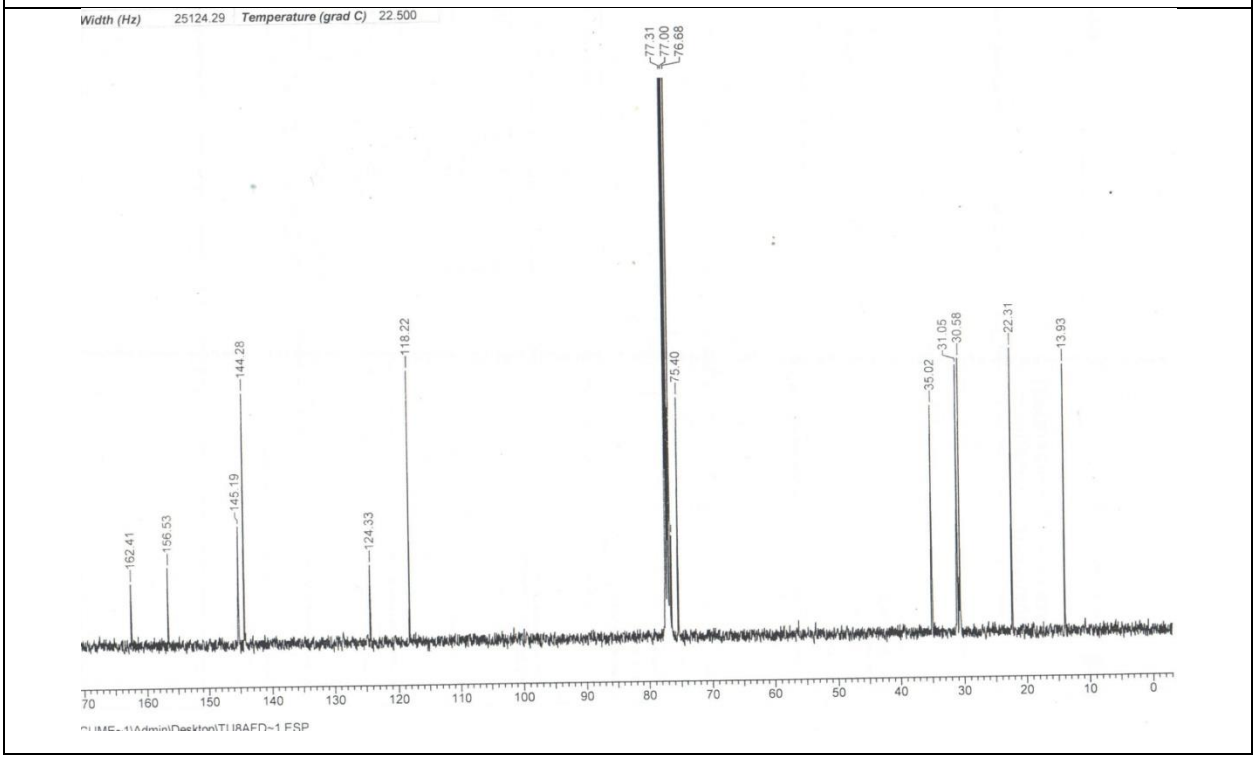
^{13}C NMR (50 MHz, $CDCl_3$) of compound 25b



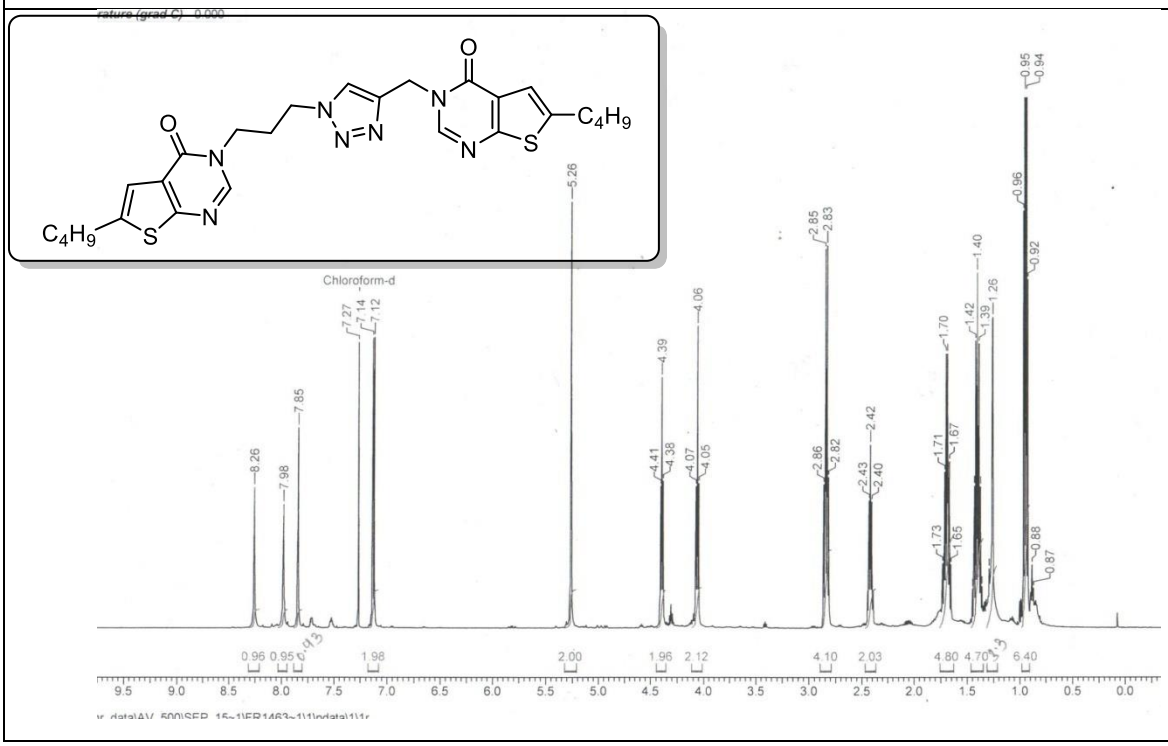
^{1}H NMR (400 MHz, $CDCl_3$) of compound 32e



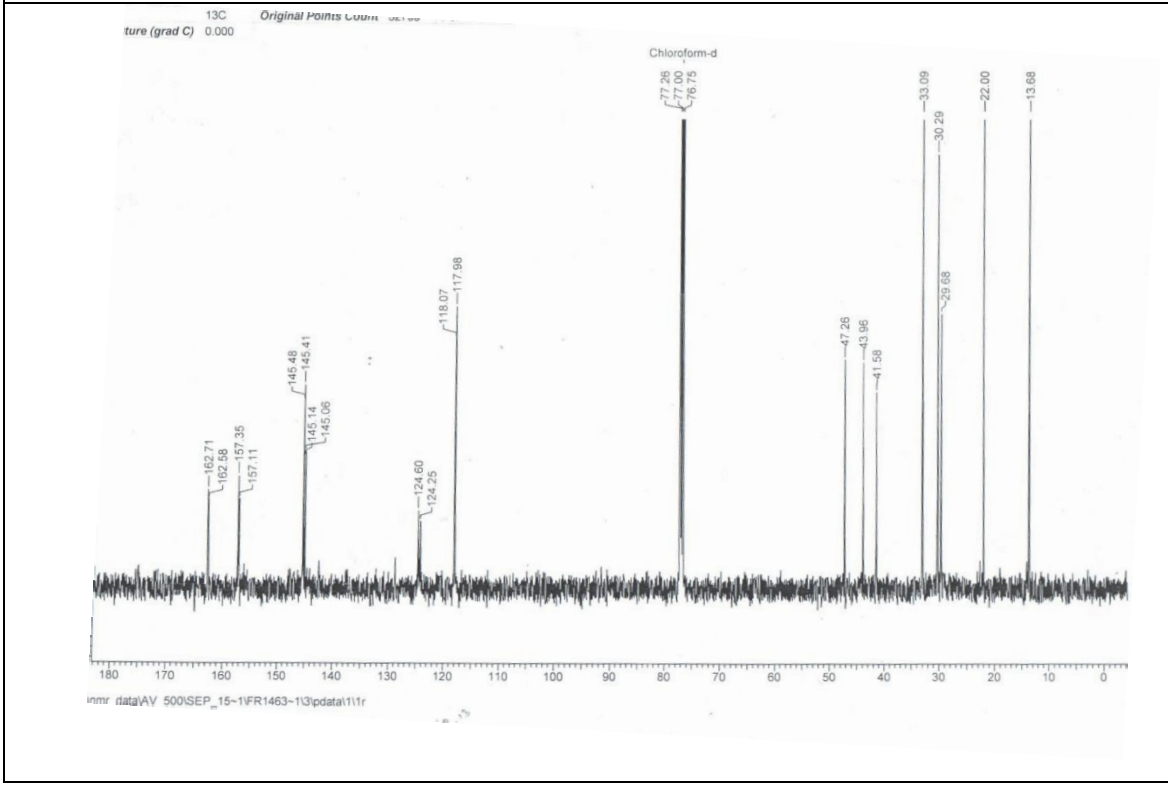
^{13}C NMR (125 MHz, $CDCl_3$) of compound 32e



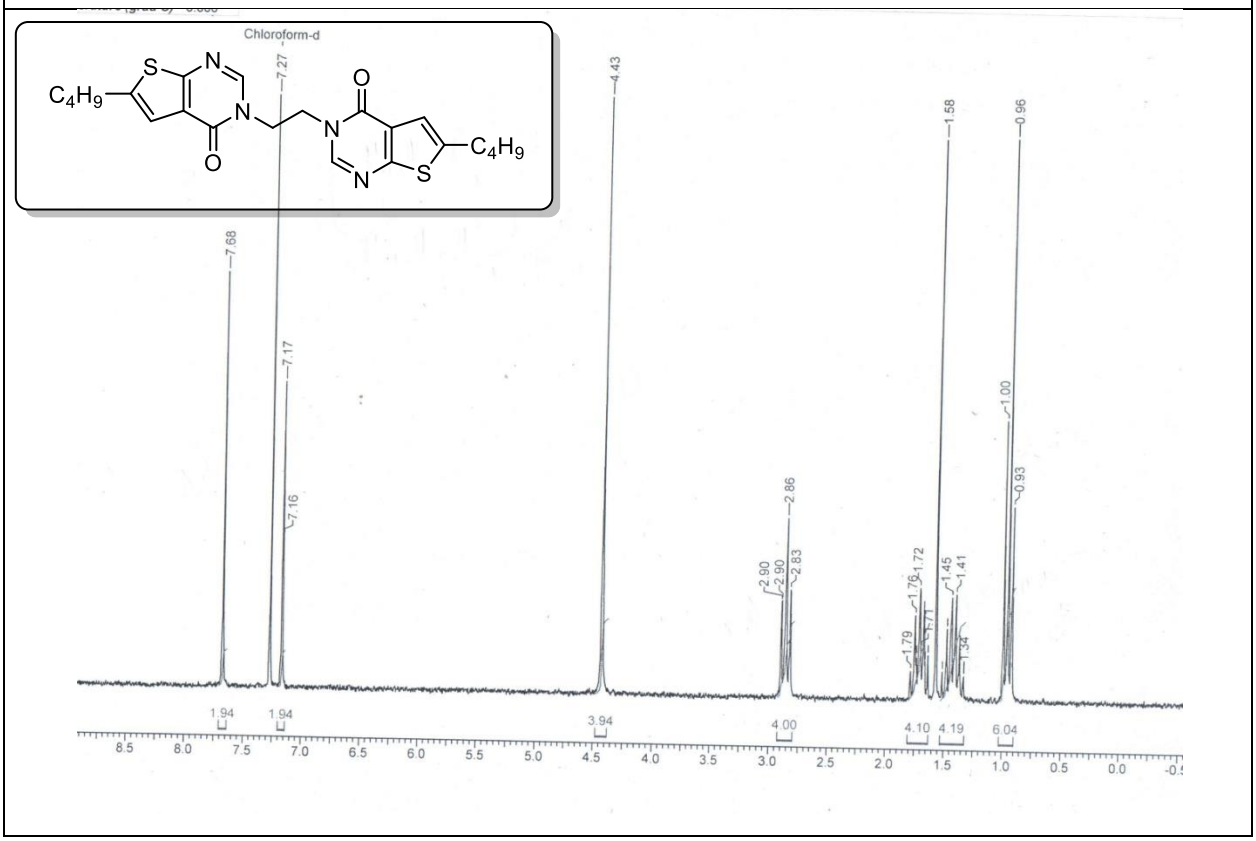
^{1}H NMR (500 MHz, $CDCl_3$) of compound 34a



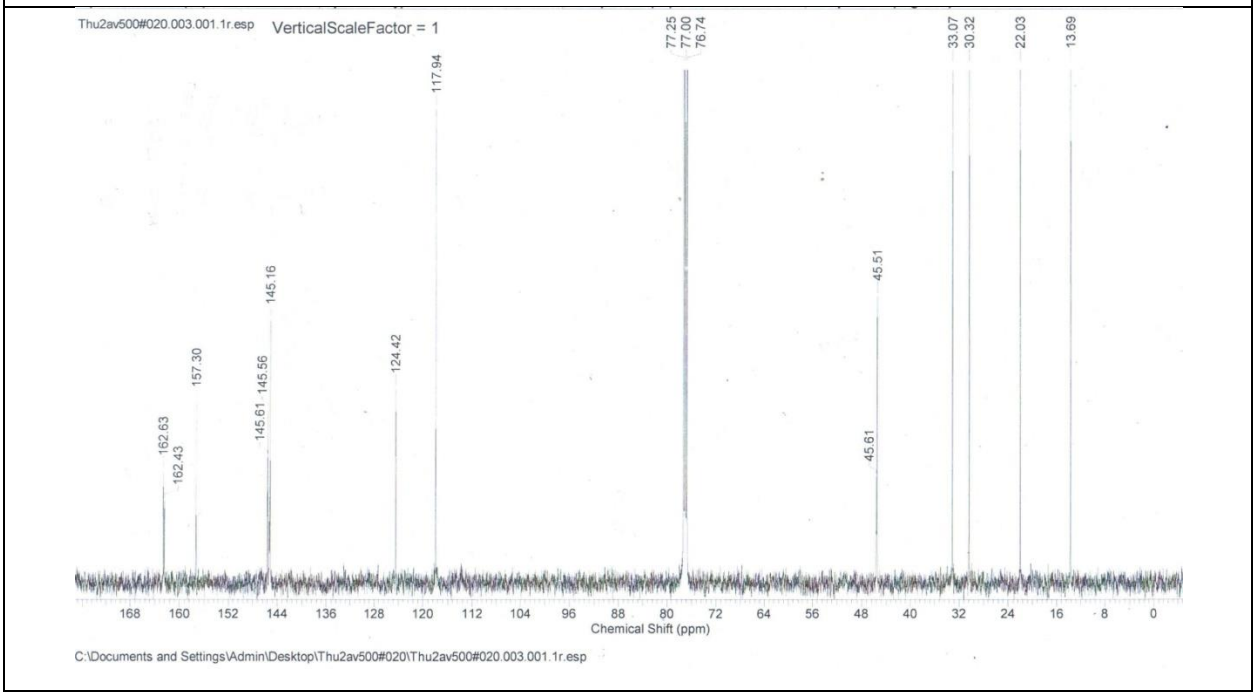
^{13}C NMR (125 MHz, $CDCl_3$) of compound 34a



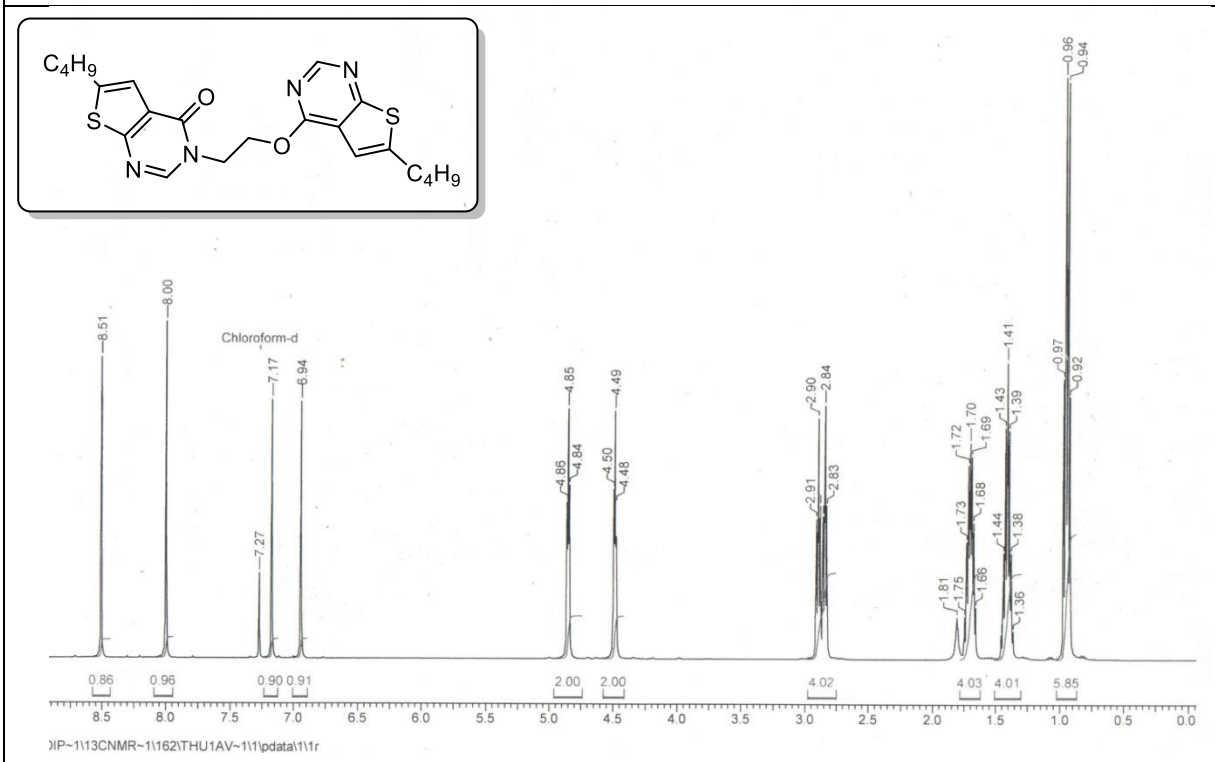
^{1}H NMR (200 MHz, $CDCl_3$) of compound 35a



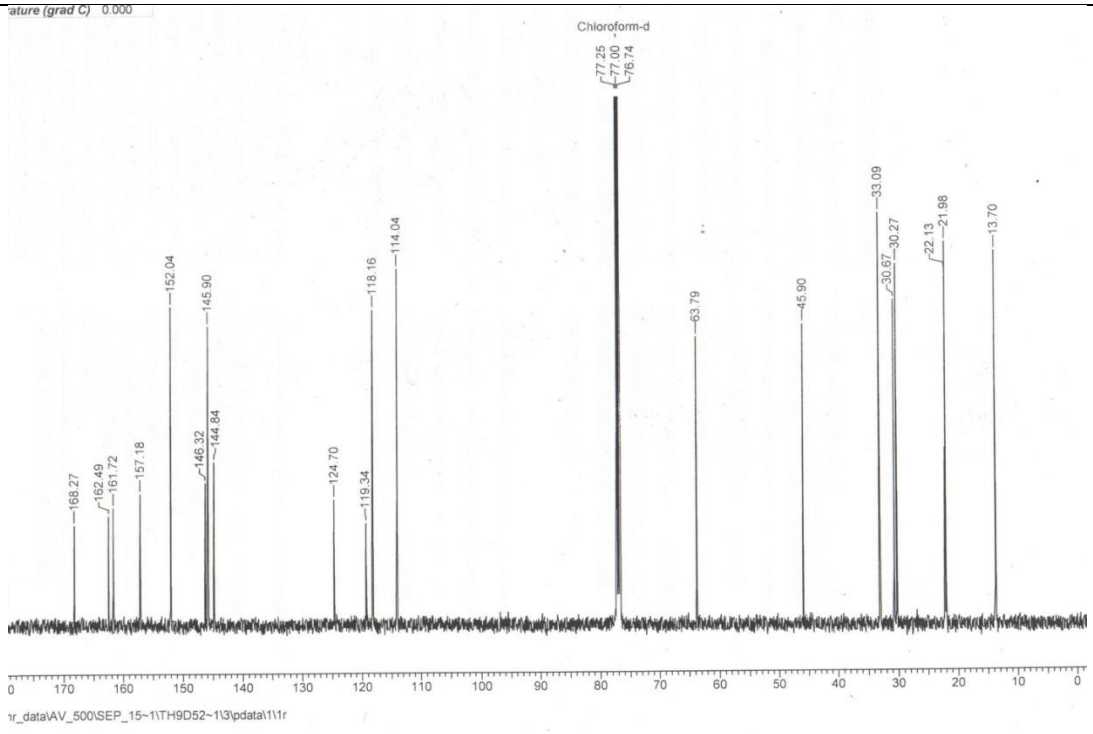
^{13}C NMR (125 MHz, $CDCl_3$) of compound 35a



^{1}H NMR (500 MHz, $CDCl_3$) of compound 36a



^{13}C NMR (125 MHz, $CDCl_3$) of compound 36a



1.1.5. References and notes

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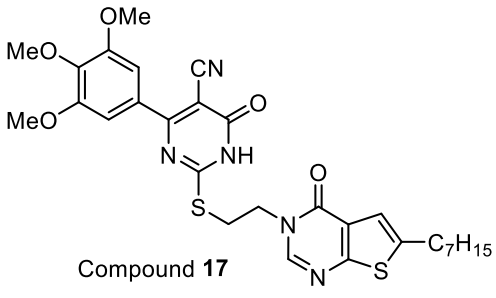
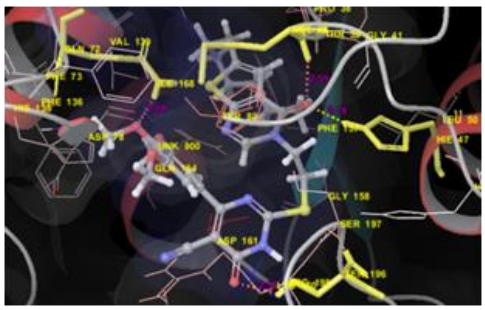
Chapter 1

Section 3

"Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents"



Compound **17**



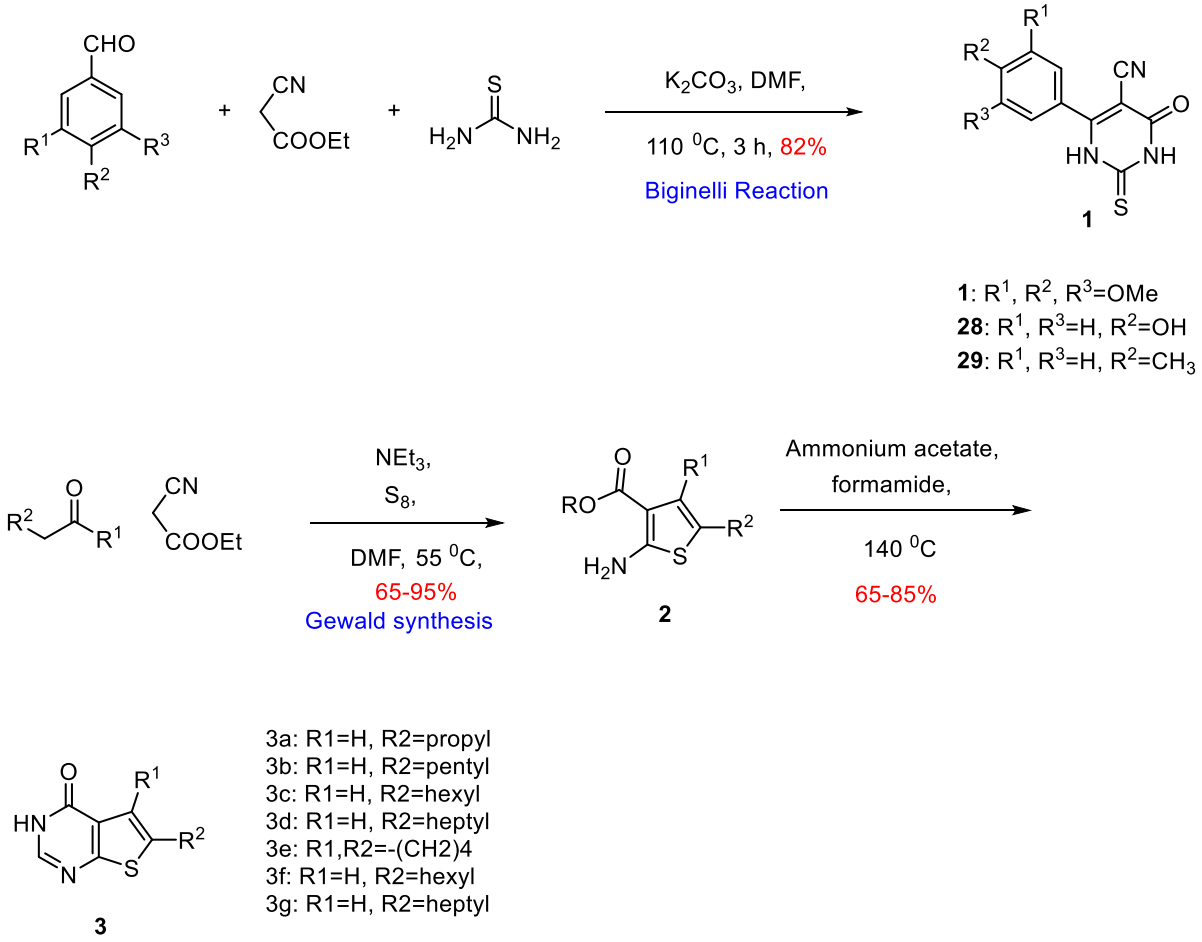
1.3.1. Introduction

Tuberculosis is an infectious disease that claims a number of deaths paralleled only by those from HIV/AIDS.[1] Development of new antitubercular drugs are very important due to occurrence of multidrug resistant tuberculosis (MDR-TB) and emergence of extensively drug-resistant tuberculosis (XDR-TB). However, tuberculosis is one of the neglected tropical diseases (NTDs). NTDs is a diverse group of communicable diseases that induced in tropical and subtropical environment which influence mostly the populations which lives in poverty, where lack of sanitization and close contact with infectious person is the main cause for spread of infection The development of new antitubercular drugs is very slow due to lack of adequate funding. As a result, after a gap of 40 years, the U.S.A. based Food and Drug Administration (FDA) has approved Bedaquiline[2,3] in December 2012 as part of combination therapy in adults to treat pulmonary MDR-TB, followed by the interim guidance on the use of delamanid[4] in 2014. New drug development is a continuous, lengthy process and it is necessary to synthesize and screen a large number of chemical entities as a slight modification in structure can cause dramatic decrease/increase in biological activity. With respect to the program to develop new drugs, several new biologically active molecules has been synthesized and have found that some of them exhibit promising antitubercular activity[5,6] while some exhibit antifungal activity.[7,8] the idea was to explore the potential of hybrid molecules containing thienopyrimidinones and thiouracils (known to posses different biological active properties[9-14] such as, anticancer, antibacterial, anti-inflammatory, and antifungal) because many hybrid compounds reported in literature with better biological activities as compare to their individual counterparts.[15] Accordingly, various new molecules **11-27** were synthesized and characterized with the help of spectral methods. Structure of the representative molecule **18** was confirmed by X-ray crystallography. These molecules were screened against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) thus, it was comes in notice that compound **11-15**, **18**, **22** and **25** exhibited significant antitubercular activity. Based on docking study results for compound **15**, compounds **16** and **17** were prepared and were found to be more active than compound **15** and the results are reported herein.

1.3.2. Present work

1.3.2.1. Chemistry

The synthetic route for various compounds in the present study is shown in Schemes 1a and 1b. The intermediate compound (**1**) was prepared by reaction of 3,4,5-trimethoxybenzaldehyde, ethyl cyanoacetate and thiourea in 82% yield by known method[13] (Scheme 1a). The thienopyrimidinones **3a-e** were prepared by methods described in earlier work[5-8] involving Gewald reaction of required aldehyde/ketone with ethyl cyanoacetate and sulphur in DMF in the



Scheme 1a.

presence of triethylamine to get the corresponding substituted ethyl 2-aminothiophene-3-carboxylate followed by reaction with formamide in the presence of ammonium acetate (Scheme 1a). The bromides **4-10** were obtained from thienopyrimidinones **3a-e** by reacting them with dibromoalkanes in the presence of base. The reactions of 4-oxo-2-thioxo-6-(3,4,5-

trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) with bromides **4-8** in DMF in the presence of potassium carbonate at room temperature afforded the hybrid molecules **11-15**. The compound **15** was reacted with various (un)substituted alkyl halides, propargyl bromide, (un)substituted benzyl bromides or *p*-toluenesulfonyl chloride by using K_2CO_3 as basein DMF at room temperature to obtain novel molecules **18-27** (Scheme 1b).

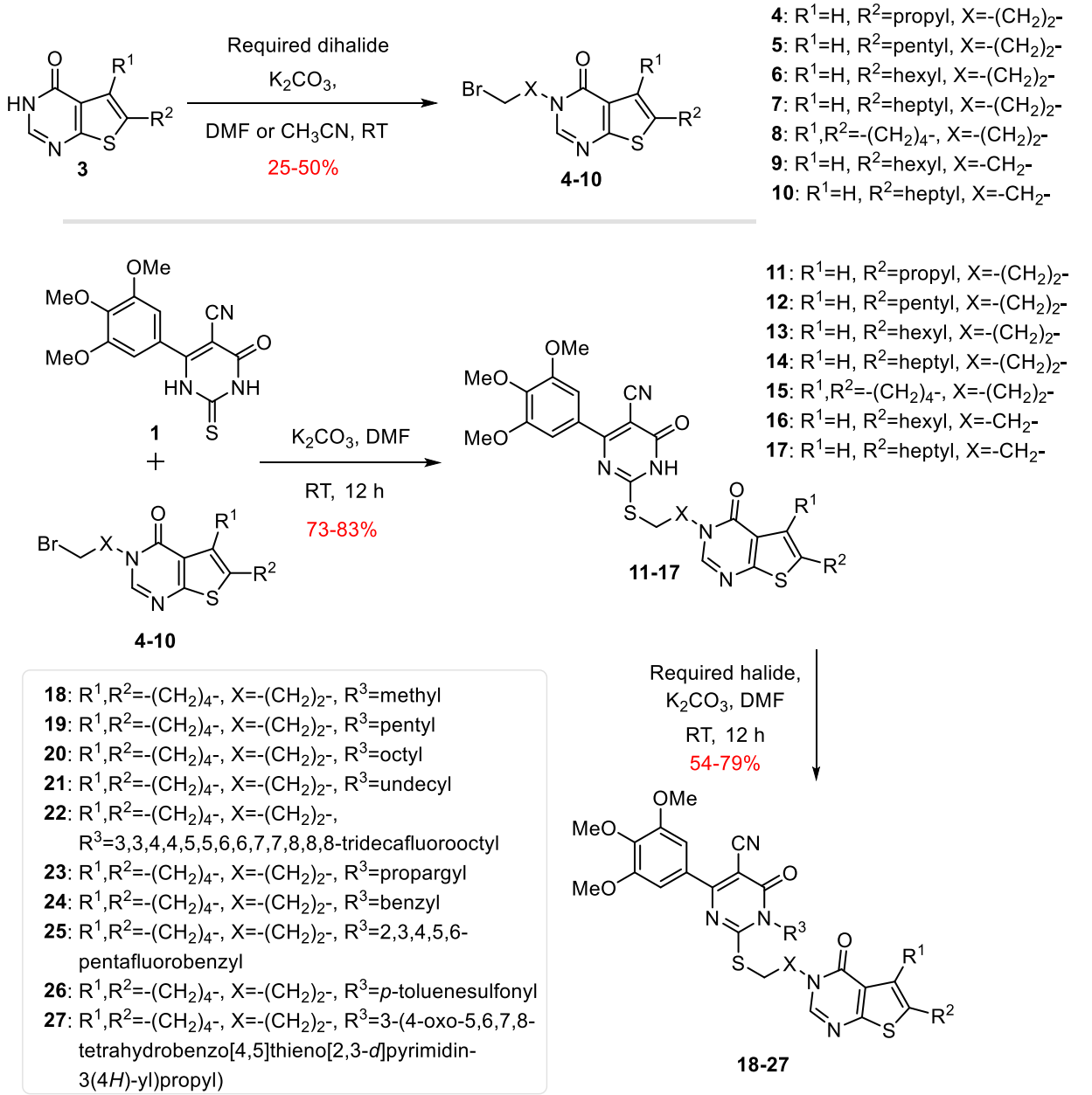
In molecular hybridization two or more different pharmacophore with different structural and biological features combined with each other through covalent linkage. Because of computational and docking assistance, the synthetic efforts are minimized than the prior studies (Chapter 1, Section 2). The docking study of compounds showed that there was binding with the active site of mycobacterial pantothenate synthetase which gave valuable information for structural modification in synthesis. Detailed synthesis is depicted in Schemes 1a and 1b. Obtained novel compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, and Mass spectral analysis and X-ray crystallography unequivocally verified the structure of representative molecule **18**, and the ORTEP diagram is displayed in Figure 4. Compounds **11** to **27,** the thienopyrimidinone-thiouracil hybrids, were synthesized in basic three steps. 1) synthesis of thienopyrimidinone scaffold 2) synthesis of thiouracil scaffold 3) S-alkylation and N-alkylation reactions for thienopyrimidinone and thiouracil molecular hybridization

1) Synthesis of thienopyrimidinone scaffold

To produce substituted 2-aminothiophene-3-carboxylates **2**, different aldehydes or ketones were subjected to Gewald synthesis utilizing ethyl cyanoacetate and sulphur in the presence of triethyl amine in DMF. The thienopyrimidinones **3** were obtained by reacting the intermediates **2** with ammonium acetate and formamide. By reacting thienopyrimidinones **3a-e** with corresponding dibromoalkanes in the presence of base, the bromide compounds **4-10** were synthesized in 25 to 50% yield (Scheme 1a).

2) Synthesis of thiouracil scaffold

The compound **1** was obtained *via* ternary condensation of ethyl cyanoacetate with the 3,4,5-trimethoxybenzaldehyde and thiourea in the presence of anhydrous potassium carbonate in 82% yield (Scheme 1a). Two additional thiouracils were synthesized utilizing the same reaction sequence but using 4-hydroxybenzaldehyde and 4-methylbenzaldehyde, to give compound **28** in 75% yield and compound **29** in 79% yield respectively.



. **Scheme 1b.**

3) S-Alkylation and N-alkylation reactions for thienopyrimidinone and thiouracil molecular hybridization

The hybrid molecules **11-15** were synthesized by reacting compound **1** with bromides **4-8** in DMF in the presence of potassium carbonate at room temperature. Further N-alkylation of compound **15** with required halide gave compounds **18** to **27** (Scheme 1b).

IR spectral analysis confirms that the functional group interpretation as shown in figure 2

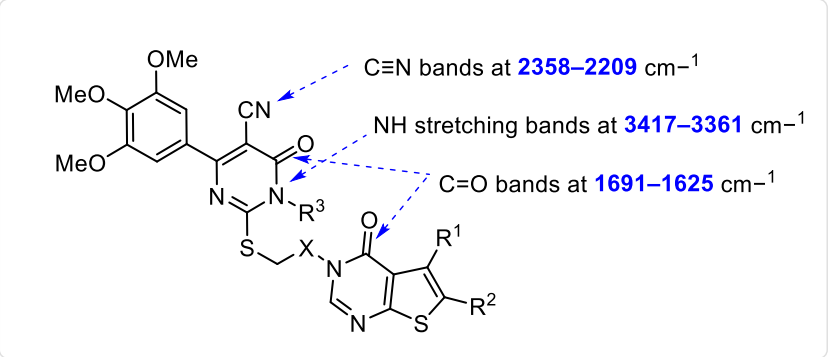


Figure 2. IR spectral analysis

The above compounds showed the existence of bands at 3417–3361 cm^{-1} for NH streaching, bands at 2358–2209 cm^{-1} for CN streaching and bands at 1691–1625 cm^{-1} for C=O were seen in the IR spectra of these compounds.

When compounds **18** to **27** were synthesized, further confirmations of the N-alkylation reactions were required. Because of compounds **11-17** may exist in one of two tautomeric forms. It was thought that N-alkylation reaction goes to nitrogen which is adjacent to carbonyl group because of steric hindrance of substituted trimethoxybenzene group. For final confirmation of N-alkylation the structure of the compound **18** was confirmed by X-ray crystallography and the ORTEP diagram is shown in Figure 4.

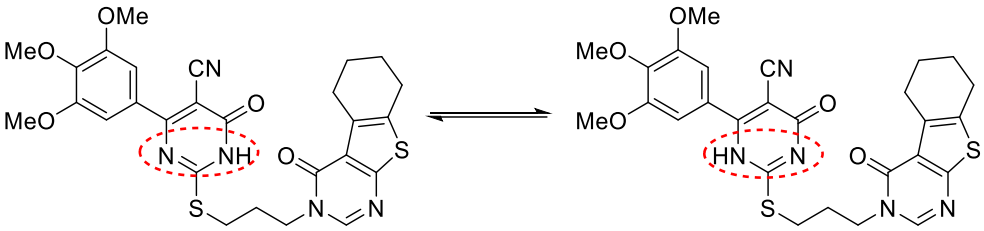


Figure 3. Tautomeric forms of compound **18**

When the thiouracil **1** was reacted with more than one equivalents of the bromide **8**, the N,S-dialkylated compound **27** was obtained in addition to the S-alkylated compound **15**. The N,S-dialkylated compound **27** was also obtained by reacting the S-alkylated compound **15** with the bromide **8**.

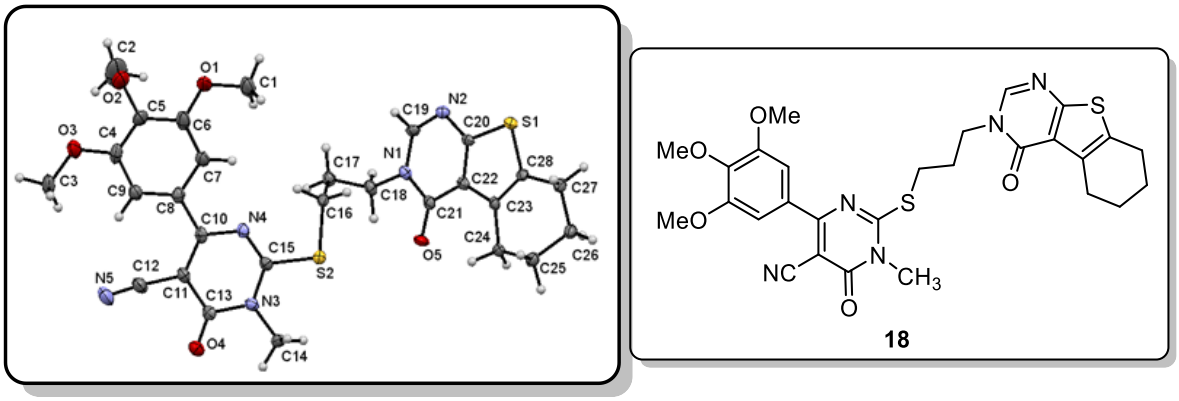


Figure 4. The Oak Ridge Thermal Ellipsoid Plot (ORTEP) of compound **18** showing the atom numbering scheme. The displacement ellipsoids are drawn at the 30% probability level and H-atoms are shown as small spheres with arbitrary radii **(CCDC No. 1504297)**.

Based on the results of antitubercular activity screening and docking study of the molecules **4-15** and **18-27**, compounds **16** and **17** were synthesized by reactions similar to those used for the synthesis of compound **15**.

The compound (**28**) and compound(**29**) were prepared by procedure that was used for compound(**1**) wherein 3,4,5-trimethoxybenzaldehyde was replaced with 4-hydroxybenzaldehyde and 4-methylbenzaldehyde respectively.

1.3.2.2. Evaluation of biological activity

The new molecules synthesized in the present work were checked for antitubercular biological activity (**Table 1**) against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) by *in vitro* and *ex vivo* methods.[16-18] It was found that the compounds **15, 16, 17** and **18** exhibited very good antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra while compounds **1, 11, 12, 13, 14, 22** and **25** exhibited very good antitubercular activity against dormant stage of *M. tuberculosis* H37Ra. The cytotoxicity studies[18-20] against THP-1 monocytes showed that these compounds were non-toxic. The compounds synthesized in the present study were further studied for cytotoxicity against three human cancer cell lines and the results are shown in **Table 2**.

Table 1. Antimycobacterial activity data for various hybrids (**11** to **27**) of thiouracils and thienopyrimidinones and thiouracils **1**, **28** and **29** against H37Ra

Entry no	**Compound no**	**Intracellular *(Ex Vivo)***		**Extracellular *(In Vitro)***		***In vitro***
		1. *M. tuberculosis* H37 Ra (Dormant Stage)	*M. tuberculosis* H37 Ra (Active Stage)	*M. tuberculosis* H37 Ra (Dormant Stage)	*M. tuberculosis* H37 Ra (Active Stage)	Cytotoxicity against THP-1 monocytes
		MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	GI_{50} (µg/mL)
1	**11**	**11.9±1.80**	>30	**19.1±0.16**	>30	>100
2	**12**	**9.8±0.81**	>30	**12.3±0.27**	>30	>100
3	**13**	**7.1±0.91**	>30	**8.7±1.11**	>30	**54.3±4.78**
4	**14**	**6.91±0.12**	>30	**7.6±0.38**	>30	**68.2±0.39**
5	**15**	**26.1±1.45**	**28.8±3.0**	**23.4±1.42**	**25.4±0.65**	**97.6±1.84**
6	**16**	**18.8±0.17**	**17.1±0.61**	**19.1±0.56**	**15.6±0.59**	**96.4±1.55**
7	**17**	**13.6±0.23**	**11.1±2.6**	**17.1±0.85**	**11.1±0.15**	**88.1±3.61**
8	**18**	**21.5±1.23**	**26.9±1.66**	**26.1±1.52**	**27.9±1.82**	**89.7±0.93**
9	**19**	>30	>30	>30	>30	>100
10	**20**	>30	>30	>30	>30	>100
11	**21**	>30	>30	>30	>30	>100
12	**22**	**8.8±0.20**	>30	**11.7±**	>30	>100
13	**23**	>30	>30	>30	>30	>100
14	**24**	>30	>30	>30	>30	>100
15	**25**	**26.5±1.62**	>30	**28.3±**	>30	>100
16	**26**	>30	>30	>30	>30	>100
17	**27**	>30	>30	>30	>30	**81.4±1.11**
18	**1**	**11.3±0.49**	>30	**17.3±0.44**	>30	>100
19	**28**	>30	>30	>30	>30	**93.0±1.35**
20	**29**	>30	>30	>30	>30	**88.9±1.92**
21	**Rifampicin**	**0.75**±0.014	**0.51**±0.012	**0.48**±0.016	**0.41**±0.02	**0.1374**±0.12

***Ex vivo*: Intracellular antitubercular activities of each agent in differentiated THP-1 cells.**

Table 2. Cytotoxicity profile of various hybrids (**11** to **27**) of thiouracils and thienopyrimidinones and thiouracils **1**, **28** and **29** against human cancer cell lines

Entry no	Comp no	A549 (Human lung)	PANC 1 (Human pancreas)	HeLa (Human cervix)
		GI_{50} (µg/mL)	GI_{50} (µg/mL)	GI_{50} (µg/mL)
1	**11**	>100	>100	**52.5±4.91**
2	**12**	>100	**72.8±3.81**	**75.8±4.32**
3	**13**	**90.2±2.73**	**28.1±7.10**	**67.0±2.76**
4	**14**	**90.5±3.54**	>100	**76.1±3.65**
5	**15**	**91.4±3.54**	**52.8±7.04**	**55.7±6.77**
6	**16**	**96.8±1.40**	**36.5±2.60**	**76.3±6.84**
7	**17**	**79.7±7.32**	**72.6±6.48**	**75.4±3.40**
8	**18**	**95.7±4.18**	**24.8±2.53**	**70.7±0.84**
9	**19**	>100	>100	**73.0±3.55**
10	**20**	>100	>100	>100
11	**21**	>100	>100	>100
12	**22**	>100	>100	>100
13	**23**	>100	>100	**66.3±1.83**
14	**24**	>100	**51.9±4.80**	**72.1±2.12**
15	**25**	>100	>100	>100
16	**26**	>100	>100	**64.2±3.91**
17	**27**	**83.1±4.42**	>100	**89.6±2.86**
18	**1**	>100	>100	**63.7±4.71**
19	**28**	**87.4±2.12**	**89.1±6.93**	**90.8±1.43**
20	**29**	86.7±4.33	>100	67.6±3.12
21	**Paclitaxel**	0.0035±0.0014	0.1279±0.022	0.0048±0.0012

It was noticed, the GI_{50} values for all compounds studied were >50 µg/mL indicating that the compounds are non-toxic.

The screening results indicated following points with respect to structure-activity relationship of the compounds investigated in the present work.

1. Synthesized hybrid compounds **11** to **17** with free NH in thiouracil moiety exhibit antitubercular activity against *M. tuberculosis* H37Ra.

The screening results indicated following points with respect to structure-activity relationship of the compounds investigated in the present work.

1. Synthesized hybrid compounds **11** to **17** with free NH in thiouracil moiety exhibit antitubercular activity against *M. tuberculosis* H37Ra.

2. The compounds **11** to **14** with three-carbon linker between thienopyrimidinone and thiouracil moieties and having alkyl substituent on thiophene ring of thienopyrimidinone exhibit antitubercular activity against dormant stage of *M. tuberculosis* H37Ra while corresponding compound **15** with three-carbon linker between thienopyrimidinone and thiouracil moieties and having cycloalkyl substituent on thiophene ring of thienopyrimidinone exhibits antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra.

3. The compounds **16** and **17** with two-carbon linker between thienopyrimidinone and thiouracil moieties and having alkyl substituent on thiophene ring of thienopyrimidinone also exhibit antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra.

4. Methyl group is tolerated on thiouracil moiety of compound **15** but alkylation with longer side chains results in loss of activity (compound **15** v/s compound **18** v/s compounds **19**, **20** and **21**).

5. The reaction of **15** with propargyl bromide, benzyl bromide, p-toluenesulfonyl chloride *etc* results in loss of activity indicating that free NH is preferred (compound **15** v/s compounds **23**, **24** and **26**). The compound **27** is also inactive supporting the above observation.

6. The compounds **20** and **24** are inactive while the corresponding fluorinated compounds **22** and **25** are active against dormant stage of *M. tuberculosis* H37Ra indicating that introduction of fluorine atoms helps to get better antitubercular activity.

The structure-activity relationship studies clearly indicate that it would be possible to get molecules with better antitubercular activity by suitable structural modifications.

1.3.2.3. Docking Studies

In silico based approaches have provided a new perspective in the development of highly efficient chemical leads and have huge potential to impart as starting points in the development of new chemical entities against TB. So, docking studies were performed against mycobacterial pantothenate synthetase due to limited resources availability for carrying out enzyme-based experimental studies to find out the best possible mode of action of the synthesized hybrids of thienopyrimidinones and thiouracil derivatives.

A distinctive characteristic of *M. Tuberculosis* is that its cell wall is enriched with the lipids which are essential for its intracellular endurance, pathogenicity and also it is believed that it makes entry of antimicrobial agents into the cells difficult.[21] In *M. Tuberculosis* genome huge numbers of genes encoding several enzymes are involved in the metabolism of fatty acids,[22] so inhibition of this pathway can be an important target in antitubercular drug discovery.

The enzyme pantothenate synthetase (PS or PanC) is encoded by the gene panC, which is important for the synthesis of pantothenate in bacteria.[21] Pantothenate is required for biosynthesis of coenzyme A (CoA) and acyl carrier protein (ACP) which are the important elements for fatty acid synthesis.[22] In the *in vitro* studies it was found that the gene encoding panC is essential for the optimum growth of bacteria and when it was genetically broken down in *M. tuberculosis*, it made the strain auxotrophic and required supplementation of pantothenate for its growth.[23-25] Also, its pathogenicity is weakened in this strain.[26] In mammals, panC is not present[27,28] so targeting this enzyme will have huge potential for developing drugs which will not have any side effects in the hosts. Thus, it makes pantothenate synthetase an important target for drug discovery against tuberculosis (TB). To continue our ongoing endeavor for discovering new potent antitubercular agents,[6] this study provides valuable guidance for rationally designing more potent inhibitors for treatment of TB. The antitubercular activity exhibited by compounds **11-15** prompted us to carry out docking studies of the compound **15** (**Figure 3**).

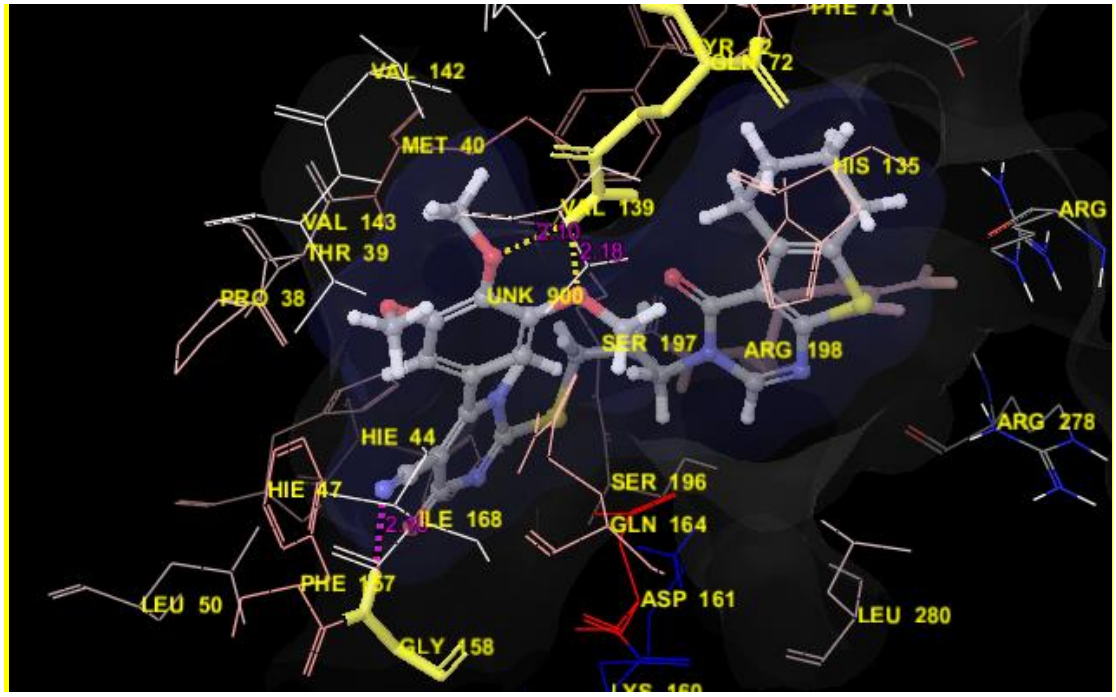


Figure 3a. 3D view of binding of compound **15** with the active site of mycobacterial PanC

From the docking studies, it is clear that the compound **15** is showing hydrogen bonding interactions with Gln72 with the distance of 2.10A° and 2.18A° and with Gly158 with the distance of 2.16A°. Also, it is observed that thiophene ring shows weak π- π stacking interaction with amino acid Arg198 which leads to weak binding of compound **15** in the active site of PanC with the docking score of -5.863.

Encouraged by the antitubercular activity of compounds **11-15** and docking study of compound **15** indicating its binding in the active site of pantothenate synthetase, compounds **16** and **17** were designed and subjected to docking studies. The docking scores of compounds **16** and **17** were -7.949 and -8.666 respectively. Molecular binding interactions (3D and 2D views) of compounds **16** and **17** with the active site of mycobacterial pantothenate synthetase are shown in **Figures 4** and **5** respectively.

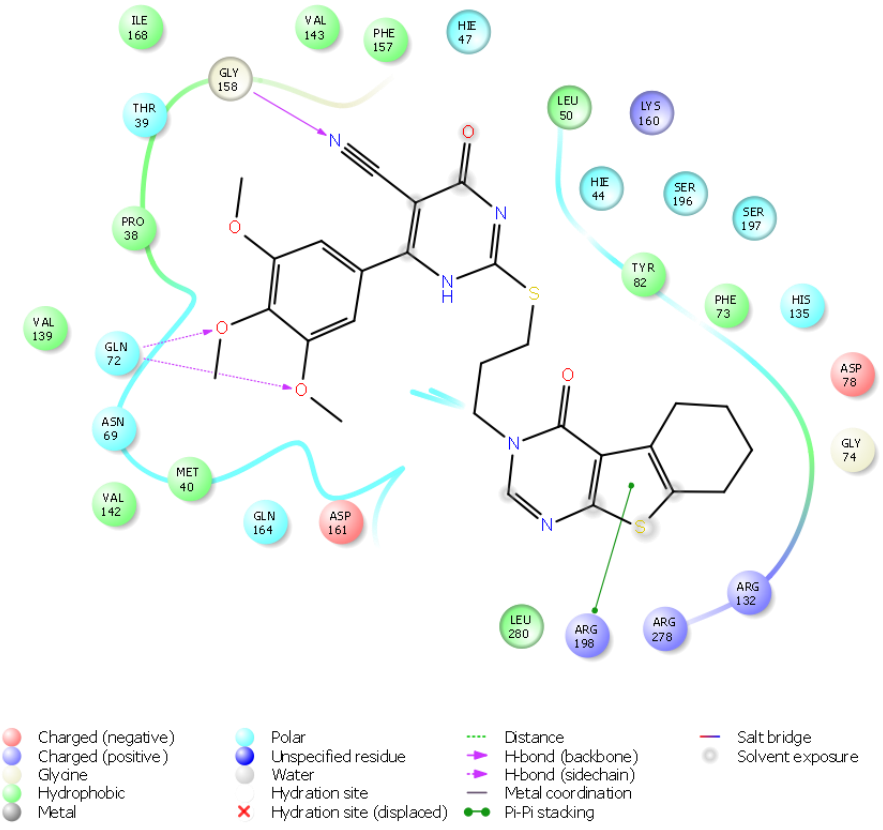


Figure 3b. 2D view of binding of compound **15** with the active site of mycobacterial PanC.

From the lowest energy docking pose of compound **17**, it was noticed that strong hydrogen bonding interactions were observed between methoxy groups present on the phenyl ring and amino acid residue Gln72 with the distance of 2.08A°. Oxo group attached to the pyrimidine ring in compound **17** shows the hydrogen bonding with Ser196 with the distance of 2.53A°. Oxo group attached to thienopyrimidinone shows the hydrogen bonding interactions with the amino acid residue Met40 with the distance of 2.59A°. Further, the heptyl group attached to thienopyrimidinone ring of compound **17** fits into hydrophobic pocket formed by amino acid residues Phe157, Val142, Val143 and Ile168 present within the active site of mycobacterial pantothenate synthetase as seen in **Figure 5**. The strong hydrogen bonding and hydrophobic interaction leads to orientation of ligands within the active site so that they form strong steric and

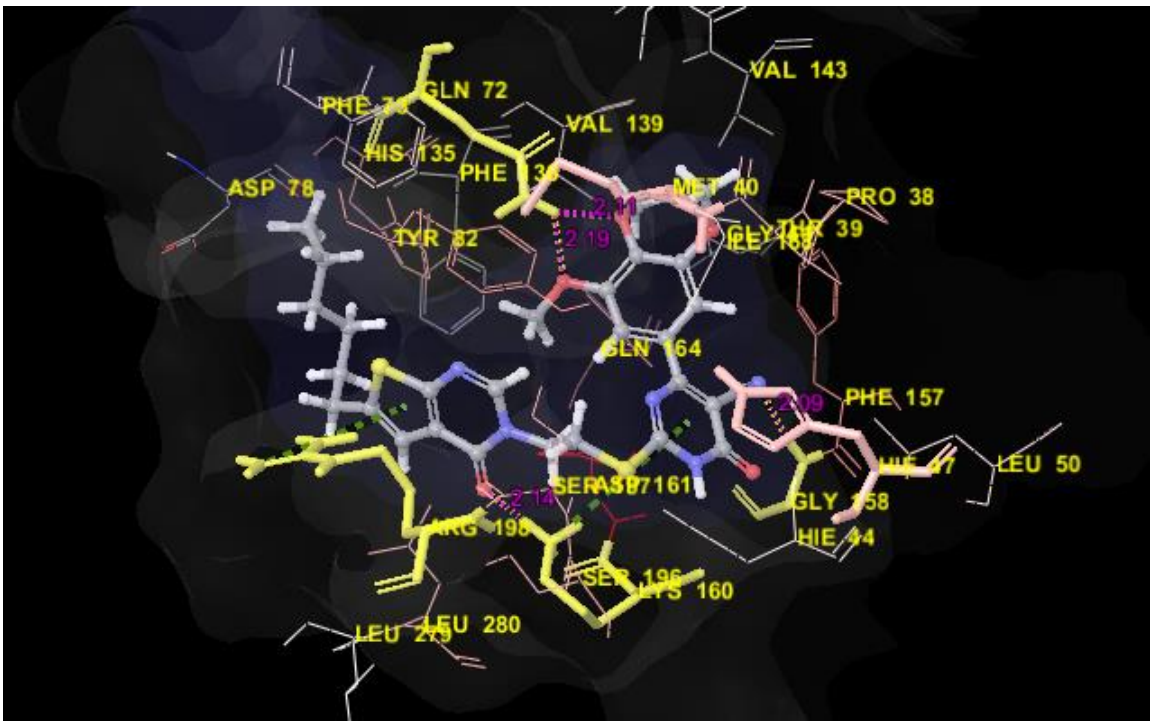


Figure 4a. 3D view of binding of compound **16** with the active site of mycobacterial PanC.

Validation of docking procedure: Co-crystallized ligand FG6 present in the active site of mycobacterial pantothenate synthetase was extracted and was docked again into the active site. Root mean square deviation (RMSD) value was found to be below 1.5Å which validates docking studies.

Accordingly, compounds **16** and **17** were synthesized and screened for antitubercular activity (Table 1, entries 6 and 7) wherein it was found that these compounds were more active than the corresponding compounds **13** and **14** as well as compound **15**. Thus, from the docking studies

and antitubercular activity results, it is clear that hybrids of thienopyrimidinones and thiouracils have significant binding with the active site of mycobacterial pantothenate synthetase. So, the mechanism of action for antitubercular activity of these compounds might be through the inhibition of mycobacterial pantothenate synthetase.

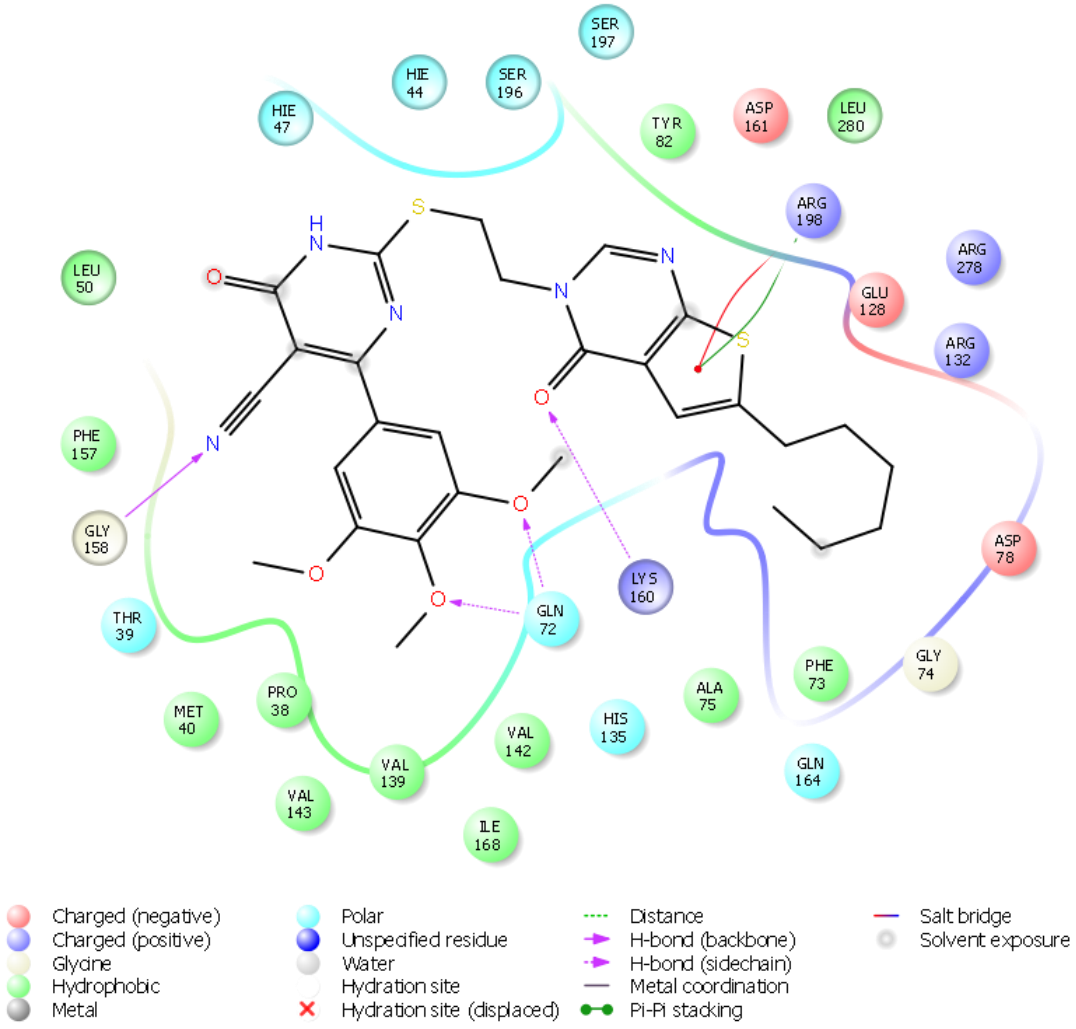


Figure 4b. 2D view of binding of compound **16** with the active site of mycobacterial PanC. electrostatic interactions with the amino acid residues present in the active site of mycobacterial pantothenate synthetase.

The compound **17** shows firm binding with the active site of mycobacterial pantothenate synthetase with the binding energy of -54.413 kcal/mol.

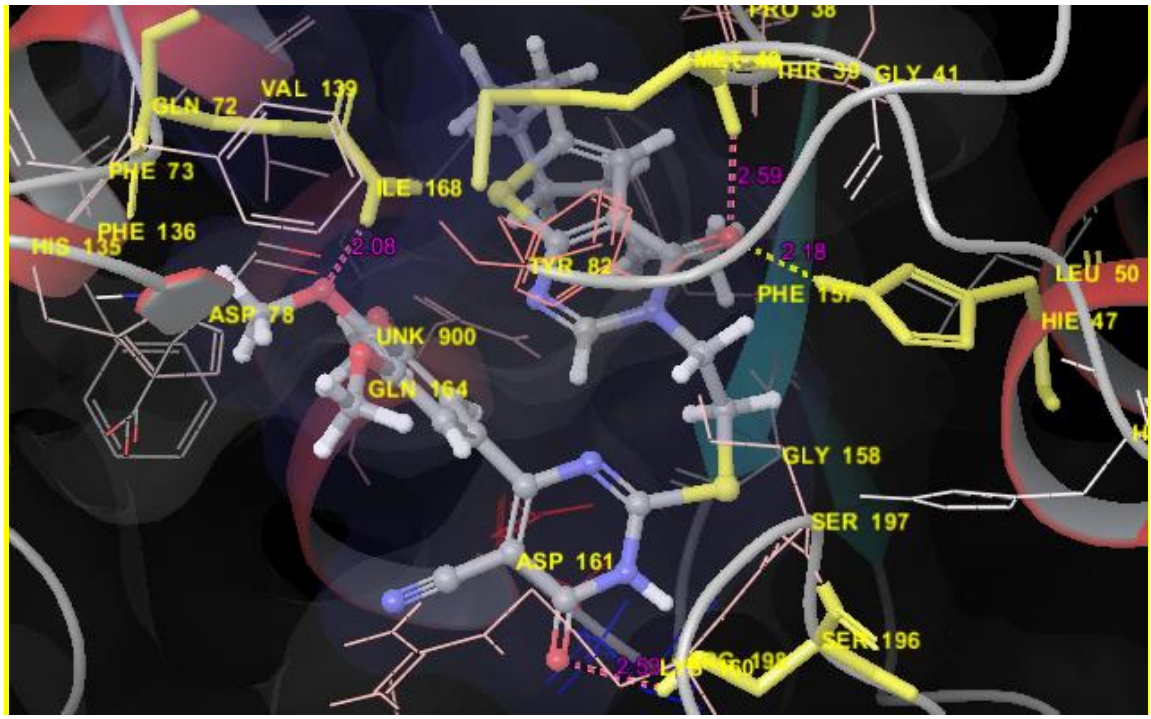


Figure 5a. 3D view of the binding of compound **17** with the active site of mycobacterial pantothenate synthetase

Many favorable Van der Waals interactions were observed with amino acid residues present in the active site. Also, several strong electrostatic interactions were seen with amino acid residues which stabilized the compound **17** in to the active site of mycobacterial pantothenate synthetase.

The docking studies of the compounds **17** and **16** showed the docking score of compounds **17** and **16** to be -8.666 and -7.949 respectively. Molecular binding interactions of compounds **17** and **16** in the active site of mycobacterial PanCare are shown in **Fig. 4a, 4b, 5a** and **5b** respectively. From the lowest energy docking poses of **17** and **16** as observed in **Fig. 4a, 4b, 5a** and **5b** strong hydrogen bonding interactions were observed between methoxy groups present on the phenyl ring and amino acid residue Gln72 with the distance of 2.08A° and 2.19A° respectively. Oxo group attached to the pyrimdine ring in **17** shows the hydrogen bonding with Ser196 with the distance of 2.53A°. Oxo group attached to thienopyrimidinones for both **17** and **16** shows the hydrogen bonding interactions with the amino acid residue Met40 and Lys160 with the distance of 2.59A° and 2.14A° respectively.

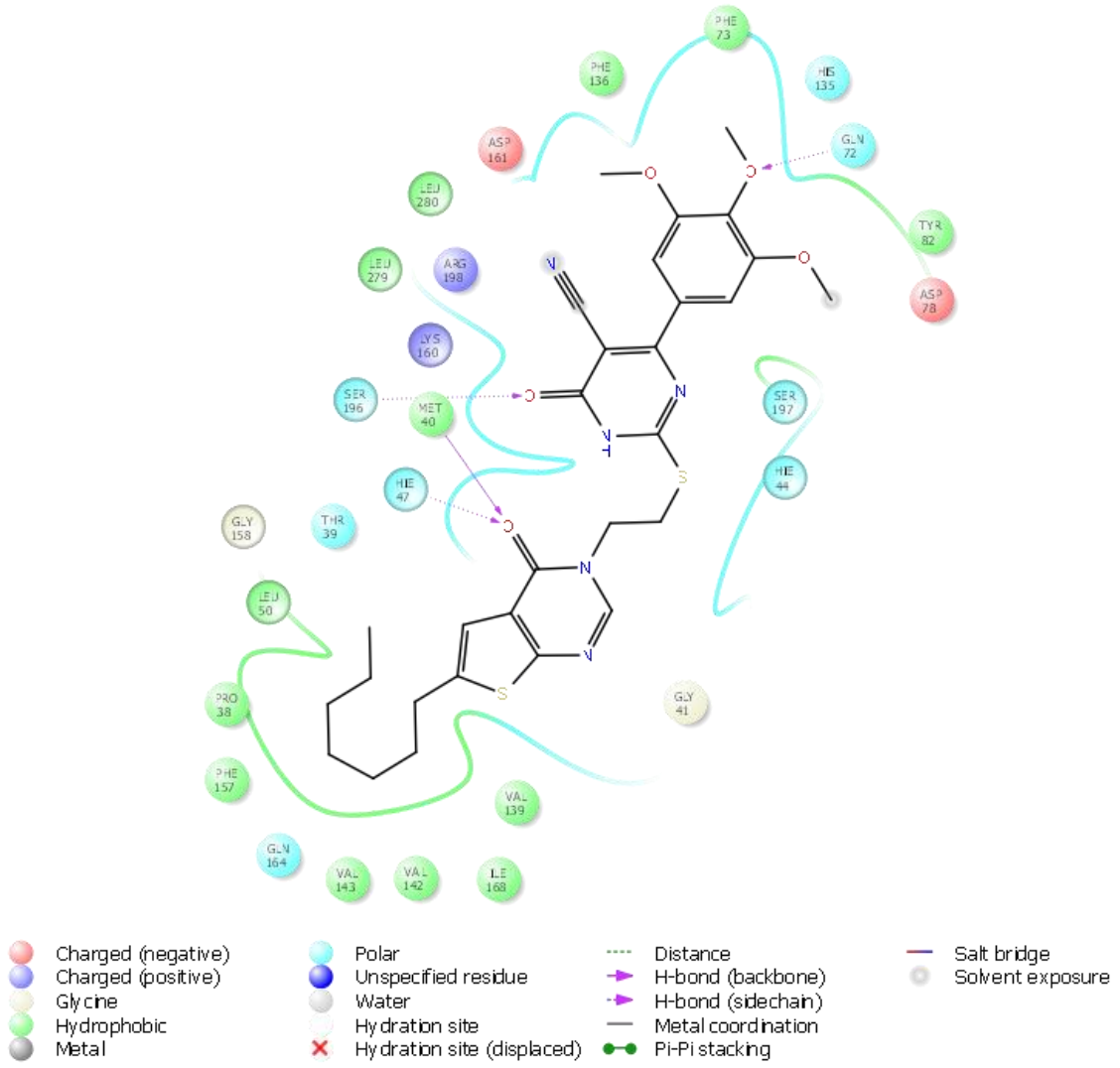


Figure 5b. 2D view of the binding of compound **17** with the active site of mycobacterial pantothenate synthetase.

Also, cyano group attached to the pyrimidine ring shows the hydrogen bonding interactions with the amino acid residue Gly158 with the distance of 2.09A°. Further, the hexyl and heptyl groups attached to thienopyrimidinone ring of **17** and **16** respectively fit into hydrophobic pocket formed by amino acid residues Phe157, Val142, Val143, Ile168 and Phe73 and Gly74 present within the active site of mycobacterial PanC as observed in **Fig. 4a, 4b, 5a** and **5b** respectively. The strong hydrogen bonding and hydrophobic interaction leads to orientation of ligands within the active site so that they form strong steric and electrostatic interactions with the amino acid residues present in the active site of mycobacterial PanC.

The compound **17** shows firm binding with the active site of mycobacterial PanC with the binding energy of -54.413 kcal/mol. Many favorable van der Waals interactions were observed with Leu280 (-2.395 kcal/mole), Arg198 (-4.304 kcal/mole), Ser197 (-2.738 kcal/mol), Ile168 (-0.841 kcal/mol), Gln164 (-4.438 kcal/mol), Asp161 (-3.334 kcal/mole), Lys160 (-1.698 kcal/mole), Phe157 (-2.358 kcal/mole), Val139 (-2.188 kcal/mole), Tyr82 (-4.452 kcal/mol), Phe73 (-2.141 kcal/mol), Gln72 (-2.205 kcal/mol), His47 (-2.507 kcal/mol), Leu44 (-1.198 kcal/mol), Met40 (-7.179 kcal/mol), Thr39 (-2.684 kcal/mol) and Pro38 (-3.397 kcal/mol) residues present in the active site . Also, several strong electrostatic interactions were seen with Leu280 (-2.608 kcal/mole), Arg198 (-2.651 kcal/mole), Ser197 (-2.769 kcal/mol), Ile168 (-0.822 kcal/mol), Gln164 (-3.768 kcal/mol), Asp161 (-10.021 kcal/mole), Lys160 (-3.325 kcal/mole), Phe157 (-2.328 kcal/mole), Val139 (-2.323 kcal/mole), Tyr82 (-4.899 kcal/mol), Lys97 (-7.850 kcal/mole), Phe83 (-4.647 kcal/mole), Phe73 (-1.466 kcal/mol), Gln72 (-8.444 kcal/mol), His47 (-4.657 kcal/mol), Leu44 (-0.832 kcal/mol), Met40 (-7.943 kcal/mol), Thr39 (-3.488 kcal/mol) and Pro38 (-3.186 kcal/mol) residues which stabilized the compound **17** into the active site of mycobacterial PanC.

Compound **16** showed mostly similar interactions with the active site of mycobacterial PanC as seen for **17** (**Fig. 4a, 4b, 5a** and **5b**). The compound **16** showed remarkable Van der Waals interactions with Leu280 (-2.450 kcal/mole), Arg198 (-4.213 kcal/mole), Ser197 (-2.575 kcal/mol), Gln164 (-4.159 kcal/mol), Asp161 (-4.298 kcal/mole), Lys160 (-2.243 kcal/mole), Phe157 (-1.619 kcal/mole), Val139 (-1.300 kcal/mole), Tyr82 (-2.146 kcal/mol), Phe73 (-1.327 kcal/mol), Gln72 (-1.616 kcal/mol), His47 (-3.769 kcal/mol), Leu44 (-0.882 kcal/mol), Met40 (-3.951 kcal/mol), Thr39 (-2.696 kcal/mol) and Pro38 (-2.685 kcal/mol) residues present in the active site . Also, several strong electrostatic interactions were seen with Leu280 (-2.592 kcal/mole), Arg198 (-4.202 kcal/mole), Ser197 (-2.458 kcal/mol), Gln164 (-3.685 kcal/mol), Asp161 (-8.565 kcal/mole), Lys160 (-4.008 kcal/mole), Phe157 (-3.767 kcal/mole), Val139 (-1.302 kcal/mole), Tyr82 (-3.127 kcal/mol), Lys97 (-7.850 kcal/mole), Phe83 (-4.647 kcal/mole), Phe73 (-1.234 kcal/mol), Gln72 (-5.277 kcal/mol), His47 (-3.246 kcal/mol), Leu44 (-0.636 kcal/mol), Met40 (-3.734 kcal/mol), Thr39 (-2.421 kcal/mol) and Pro38 (-2.569 kcal/mol) residues which steadied the compound **16** in to the active site of mycobacterial PanC.

Compound **15** showed similar interactions with the active site of mycobacterial PanC (**Fig. 3a** and **3b**) like the compounds **16** and **17** with weak Van der Waals and electrostatic interactions**.**

Docking studies for compound **15** were performed before synthesis of compounds **16** and **17** and it was used as starting point for further modifications and compounds were designed as per the results of docking studies of modified compounds. Further, these compounds were synthesized and screened against *Mycobacterium tuberculosis* H37Ra (ATCC 25177), wherein as observed in docking studies compounds **16** and **17** were found to be potent against *M. tuberculosis* H37Ra in active as well as dormant stage with lower cytotoxicity.

Thus, from the docking studies it is clear that hybrids of thienopyrimidinones and thiouracil derivatives have significant binding with the active site of mycobacterial PanC. So, the mechanism of action for antitubercular activity of these compounds might be through the inhibition of mycobacterial PanC.

1.3.3. Conclusions

Synthesis of hybrid molecules containing thiouracil and thienopyrimidinone moieties was achieved. The new chemical entities thus synthesized were tested against *Mycobacterium tuberculosis* H37Ra and it was observed that the compounds **11-14** exhibited antitubercular activity against dormant stage while compound **15** exhibited antitubercular activity against dormant as well as active stage. Structural modifications of compound **15** were carried out to study the structure-activity relationship and it was observed that compound **18** exhibited antitubercular activity comparable to compound **15** while compounds **22** and **25** exhibited antitubercular activity against dormant stage. Cytotoxicity studies revealed that these molecules were non-toxic. The docking study of compound **15** showed that there is binding with the active site of mycobacterial pantothenate synthetase with the docking score of -5.863. Further, the compounds **16** and **17** were designed based on docking studies and their docking scores were found to be -7.949 and -8.666 respectively indicating the possibility of these compounds to be more active. The synthesis and antitubercular activity screening of the compounds **16** and **17** was carried out and it was found that these compounds were having potent antitubercular activity supporting the binding of these compounds with mycobacterial pantothenate synthetase. Hence, the compounds **15-18** can be used as starting points for further optimization. In present work,

used synthetic routes have capability to generate wide variety of compounds with further structural refinement. and the present results will be very useful in the development of new class of antimycobacterial agents.

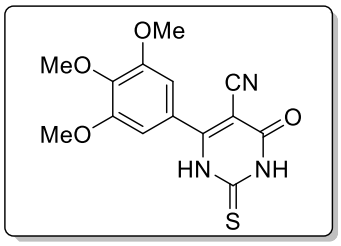
1.3.4. Experimental

1.3.4.1. Synthesis and spectral data

The thiouracils **1**, **28** and **29** were prepared by the reported procedure.[13]

4-Oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1)

Potassium carbonate (21.08 g, 0.15 mol) was taken in a 100 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. A mixture of ethyl cyanoacetate (8.1 mL, 0.07 mol), thiourea (5.81 g, 0.07 mol), and 3,4,5- trimethoxybenzaldehyde (15 g, 0.07 mol) was added under nitrogen followed by dry DMF (30 mL) and the reaction mixture heated at 110 °C for 3 h. The reaction mixture was allowed to cool, then diluted with ice cold water (250 mL) and extracted with ethyl acetate (3 x 150 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 4-oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) as off-white solid, (20 g 82% yield).



Yield: 82%.

Melting point: 283 °C.

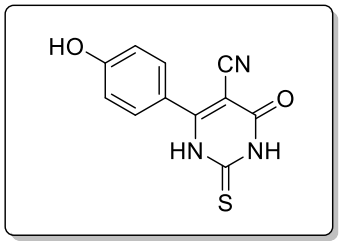
IR ($CHCl_3$): 1241, 1685, 2203, 3417 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 3.47 (s, 3H), 3.51 (s, 6H), 6.60 (s, 2H).

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 55.2 (2C), 59.6, 62.4, 105.4 (2C), 113.6, 122.3, 140.2, 151.7 (2C), 157.6, 158.9, 175.3.

HRMS (ESI) *m/z* calculated for $C_{14}H_{14}N_3O_4S$ $[M+H]^+$: 320.0700, found: 320.0696; $C_{14}H_{13}N_3NaO_4S$ $[M+Na]^+$: 342.0519, found: 342.0516.

6-(4-Hydroxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (28)



Yield: 75%.

Melting point: >295 °C.

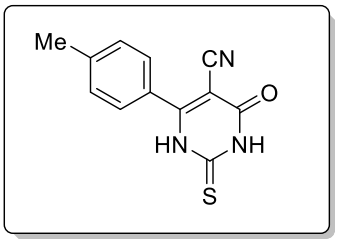
IR ($CHCl_3$): 1238, 1630, 2214, 3313, 3601 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 6.00 (d, J = 8 Hz, 2H), 6.94 (d, J = 8 Hz, 2H), 9.06 (bs, 1H).

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 82.8, 113.0 (2C), 117.4, 126.1, 128.6 (2C), 158.0, 161.6, 165.3, 180.3.

HRMS (ESI) *m/z* calculated for $C_{11}H_8N_3O_2S$ $[M+H]^+$: 246.0332 found: 246.0330.

4-Oxo-2-thioxo-6-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (29)



Yield: 79 %.

Melting point: >295 °C.

IR ($CHCl_3$): 1226, 1665, 2234, 3317 cm^{-1}.

^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (s, 3H), 7.39 (d, J = 8 Hz, 2H), 7.60 (d, J = 8 Hz, 2H), 13.05 (bs, 1H).

^{13}C NMR (50 MHz, DMSO-d_6): δ 21.4, 90.6, 115.1, 126.6, 129.0 (2C), 129.2 (2C), 142.8, 158.8, 161.2, 176.4.

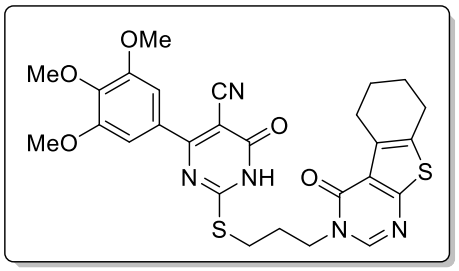
HRMS (ESI) *m/z* calculated for $C_{12}H_{10}N_3OS$ $[M+H]^+$: 244.0538, found: 244.0539; $C_{12}H_9N_3NaOS$ $[M+Na]^+$: 266.0357, found: 266.0359.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (15)

Potassium carbonate (0.50 g, 0.004 mol) was taken in a 100 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. 4-Oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) (0.78 g, 0.002 mol) was added under nitrogen followed by dry DMF (5 mL) and stirred for 10 min. 3-(3-Bromopropyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (0.80 g, 0.002 mol) was added and the reaction mixture was stirred at RT for 12 h. The reaction was monitored with TLC. After completion, it was diluted with ice cold water (100 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(15)** as off-white solid, (0.94 g, 68% yield).

Yield: 68 %;

Melting point: 230 °C.



IR ($CHCl_3$): 1668, 1720, 2210, 3429 cm^{-1}.

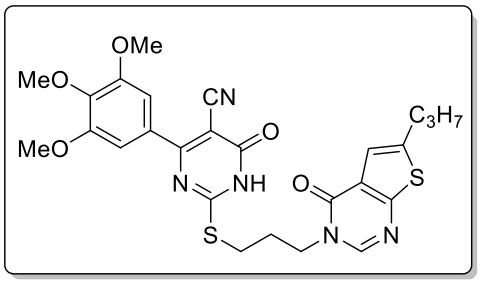
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73-1.91 (m, 4H), 2.33-2.63 (m, 2H), 2.75 (t, J = 6 Hz, 2H), 2.94 (t, J = 6 Hz, 2H), 3.40 (t, J = 7 Hz, 2H), 3.89 (s, 6H), 3.94 (s, 3H), 4.16 (t, J = 7 Hz, 2H), 7.33 (s, 2H), 7.99 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 21.4, 22.0, 24.4, 24.8, 27.2, 27.9, 44.3, 55.6 (2C), 60.1, 92.1, 105.8 (2C), 115.5, 121.8, 129.4, 130.6, 133.5, 140.4, 145.0, 152.2 (2C), 157.0, 161.3, 161.6, 164.7, 166.0.

HRMS (ESI) *m/z* calculated for $C_{27}H_{28}N_5O_5S_2$ $[M+H]^+$: 566.1517, found: 566.1526.

The following compounds (**11, 12, 13, 14, 16** and **17**) were prepared by using procedure described for compound **15**:

6-Oxo-2-((3-(4-oxo-6-propylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (11)



Yield: 73 %.

Melting point: 185 °C.

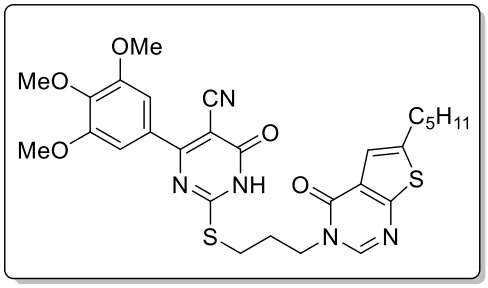
IR ($CHCl_3$): 1683, 1731, 2210, 3450 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, J = 7 Hz, 3H), 1.40-1.70 (m, 2H), 2.19-2.49 (m, 2H), 2.70 (t, J = 6 Hz, 2H), 3.23-3.53 (m, 2H), 3.76 (s, 6H), 3.85 (s, 3H), 4.11-4.37 (m, 2H), 7.04 (s, 1H), 7.15 (s, 2H), 8.01 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 12.7, 23.4, 26.9, 28.1, 31.6, 44.6, 55.4 (2C), 59.9, 90.5, 105.4 (2C), 116.9, 117.3, 123.6, 130.2, 139.6, 143.4, 145.3, 150.2, 152.0 (2C), 156.4, 161.6, 166.1, 167.6.

HRMS (ESI) *m/z* calculated for $C_{26}H_{28}N_5O_5S_2$ $[M+H]^+$: 554.1526, found: 554.1526; $[C_{26}H_{27}N_5NaO_5S_2$ $[M+Na]^+$: 576.1344, found: 576.1346.

6-Oxo-2-((3-(4-oxo-6-pentylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (12)



Yield: 78 %.

Melting point: 183 °C.

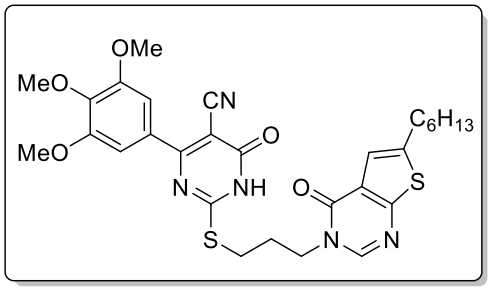
IR ($CHCl_3$): 1651, 1730, 2215, 3415 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, J = 7 Hz, 3H), 1.15-1.40 (m, 4H), 1.60-1.89 (m, 2H), 2.18 (bs, 2H), 2.72 (t, J = 7 Hz, 2H), 3.22 (bs, 2H), 3.77 (s, 6H), 3.86 (s, 3H), 4.11 (t, J = 7 Hz, 2H), 7.05 (s, 1H), 7.14 (s, 2H), 8.00 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9, 22.3, 28.0, 28.7, 30.5, 30.7, 31.1, 45.5, 56.1 (2C), 60.8, 90.3, 106.2 (2C), 117.9, 118.3, 118.4, 124.4, 130.3, 140.8, 145.3, 145.7, 152.7 (2C), 157.4, 162.5, 167.0, 167.1.

HRMS (ESI) *m/z* calculated for $C_{28}H_{32}N_5O_5S_2$ $[M+H]^+$: 582.1838, found: 582.1839.

2-((3-(6-Hexyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (13)



Yield: 81 %.

Melting point: 293 °C.

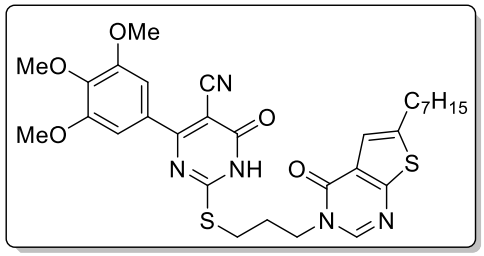
IR ($CHCl_3$):1652, 1726, 2218, 3418 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.20-1.45 (m, 6H), 1.57-1.78 (m, 2H), 2.35 (t, J = 7 Hz, 2H), 2.82 (t, J = 7 Hz, 2H), 3.42 (t, J = 7 Hz, 2H), 3.90 (s, 6H), 3.94 (s, 3H), 4.20 (t, J = 7 Hz, 2H), 7.11 (s, 1H), 7.34 (s, 2H), 8.03 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.5, 28.3, 28.4, 28.6, 30.6, 31.0, 31.4, 45.4, 56.3 (2C), 61.0, 92.5, 106.7 (2C), 115.5, 115.6, 118.0, 124.6, 129.4, 141.8, 145.4, 145.6, 145.9, 153.0 (2C), 157.4, 162.3, 167.5.

HRMS (ESI) *m/z* calculated for $C_{29}H_{34}N_5O_5S_2$ $[M+H]^+$: 596.1995, found: 596.1996; $[C_{29}H_{33}N_5NaO_5S_2$ $[M+Na]^+$: 618.1813, found: 618.1815.

2-((3-(6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (14)



Yield: 83 %.

Melting point: 187 °C.

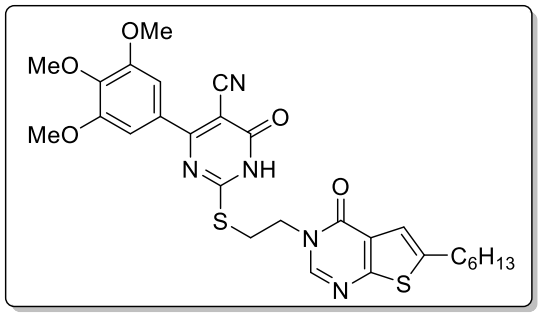
IR ($CHCl_3$): 1655, 1728, 2214, 3418 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (t, J = 6 Hz, 3H), 1.16-1.33 (m, 8H), 1.59 (bs, 2H), 2.17 (bs, 2H), 2.79 (t, J = 7 Hz, 2H), 3.27 (t, J = 7 Hz, 2H), 3.76 (s, 3H), 3.83 (s, 6H), 4.10 (t, J = 7 Hz, 2H), 7.06 (s, 1H), 7.29 (s, 2H), 8.36 (s, 1H).

^{13}C NMR (200 MHz, DMSO-d_6): δ 14.1, 22.2, 27.8, 28.47, 28.50, 28.7, 29.9, 30.8, 31.4, 44.8, 56.2 (2C), 60.4, 92.2, 106.6 (2C), 116.8, 118.4, 124.0, 130.5, 140.6, 143.7, 147.7, 152.7 (2C), 156.7, 162.1, 162.2, 166.3, 166.5.

HRMS (ESI) *m/z* calculated for $C_{30}H_{36}N_5O_5S_2$ $[M+H]^+$: 610.2151, found: 610.2152; $C_{30}H_{35}N_5NaO_5S_2$ $[M+Na]^+$: 632.1968, found: 632.1972.

2-((3-(6-Hexyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (16)



Yield: 80 %.

Melting point: 125 °C.

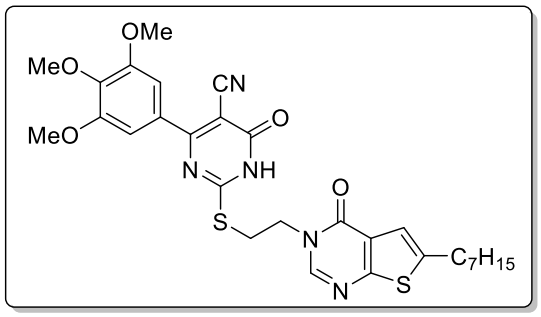
IR ($CHCl_3$): 1672, 1725, 2213, 3403 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, J = 7 Hz, 3H), 1.13-1.40 (m, 6H), 1.41-1.70 (m, 2H), 2.70 (t, J = 7 Hz, 2H), 3.64 (bs, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.39 (bs, 2H), 6.89 (s, 1H), 7.16 (s, 2H), 8.15 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 22.6, 29.0, 29.2, 29.5, 30.4, 30.9, 31.8, 56.1 (2C), 60.9, 106.4 (2C), 116.1, 117.8, 124.3, 129.0, 141.41, 141.42, 145.05, 145.08, 146.02, 146.03, 152.7 (2C), 157.0, 162.1, 167.6.

HRMS (ESI) *m/z* calculated for $C_{28}H_{32}N_5O_5S_2$ $[M+H]^+$: 582.1839, found: 582.1839.

2-((2-(6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)ethyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (17)



Yield: 77 %.

Melting point: 130 °C.

IR ($CHCl_3$): 1666, 1731, 2219, 3412 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, J = 7 Hz, 3H), 1.05-1.35 (m, 8H), 1.42-1.65 (m, 2H), 2.65 (t, J = 6 Hz, 2H), 3.15-3.60 (m, 2H), 3.81 (s, 6H), 3.89 (s, 3H), 4.05-4.50 (m, 2H), 6.95 (s, 1H), 7.08 (s, 2H), 8.03 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0, 22.5, 28.6, 28.9, 29.0, 30.5, 31.1, 31.7, 47.4, 56.0 (2C), 60.8, 88.5, 105.8 (2C), 117.7, 121.1, 124.1, 131.1, 139.9, 145.5, 152.7 (2C), 157.4, 162.8, 167.1, 167.2, 172.4, 174.7.

HRMS (ESI) *m/z* calculated for $C_{29}H_{34}N_5O_5S_2$ $[M+H]^+$: 596.1996, found: 596.1985.

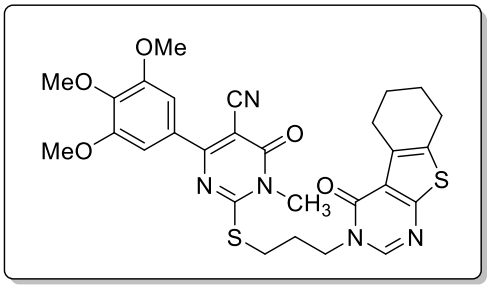
1-Methyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (18)

Potassium carbonate (243 mg, 1.76 mmol) was taken in a 100 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (**15**) (500 mg, 0.88 mmol) was added under nitrogen atmosphere followed by dry DMF (1.5 mL) and stirred for 10 min. Iodomethane (0.08 ml, 188 mg, 1.32 mmol) was added by microlitre syringe and the reaction mixture was stirred at RT for 12 h. It was then diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 1-methyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (**18**) as off-white solid, 435 mg (85%). Melting point: 185 º C.

Yield: 85 %.

Melting point: 185 °C.

IR ($CHCl_3$): 1669, 1724, 2219, 3411 cm^{-1}.



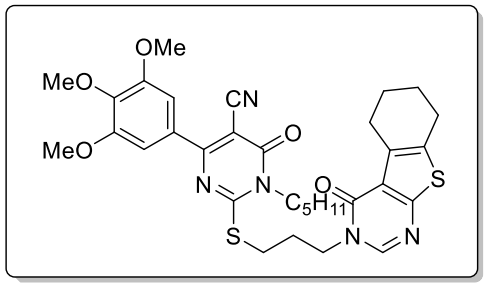
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75-1.91 (m, 4H), 2.22-2.41 (m, 2H), 2.76 (t, J = 6 Hz, 2H), 2.95 (t, J = 6 Hz, 2H), 3.42 (t, J = 7 Hz, 2H), 3.55 (s, 3H), 3.90 (s, 6H), 3.92 (s, 3H), 4.13 (t, J = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 22.7, 25.1, 25.5, 28.3, 29.8, 30.9, 44.9, 56.3 (2C), 60.9, 92.1, 106.5 (2C), 115.7, 122.6, 129.5, 131.4, 134.7, 141.5, 145.0, 153.0 (2C), 157.8, 160.2, 161.9, 164.5, 165.3.

HRMS (ESI) *m/z* calculated for $C_{28}H_{30}N_5O_5S_2$ $[M+H]^+$: 580.1674, found: 580.1683; $C_{28}H_{29}N_5NaO_5S_2$ $[M+Na]^+$: 602.1490, found: 602.1502.

The following compounds **19** to **27** were prepared by using procedure described for compound **18** wherein the required halide was used in place of iodomethane:

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-1-pentyl-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (19)



Yield: 74 %.

Melting point: 86 °C.

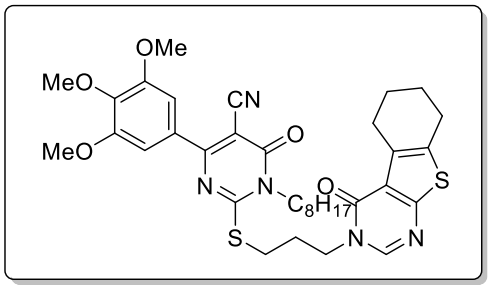
IR ($CHCl_3$): 1669, 1733, 2219, 3418. cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.93 (t, J = 7 Hz, 3H), 1.30-1.52 (m, 4H), 1.70-1.96 (m, 6H), 2.20-2.42 (m, 2H), 2.67-2.85 (m, 2H), 2.91-3.06 (m, 2H), 3.26 (t, J = 7 Hz, 2H), 3.93 (s, 9H), 4.13 (t, J = 7 Hz, 2H), 4.46 (t, J = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.9, 22.1, 22.3, 22.8, 25.2, 25.6, 27.9, 28.1 (2C), 28.5, 45.3, 56.3 (2C), 60.9, 68.8, 87.6, 106.4 (2C), 114.9, 122.7, 129.8, 131.4, 134.4, 141.2, 145.2, 153.1 (2C), 157.7, 162.0, 167.8, 169.8, 174.0.

HRMS (ESI) *m/z* calculated for $C_{32}H_{38}N_5O_5S_2$ $[M+H]^+$: 636.2297, found: 636.2309; $C_{32}H_{37}N_5NaO_5S_2$ $[M+Na]^+$: 658.2114, found: 658.2128.

1-Octyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (20)



Yield: 79 %.

Melting point: 78 °C.

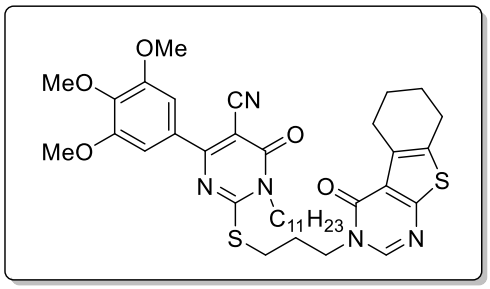
IR ($CHCl_3$): 1677, 1729, 2209, 2911, 3415 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, J = 7 Hz, 3H), 1.21-1.52 (m, 12H), 1.75-1.95 (m, 6H), 2.25-2.38 (m, 2H), 2.79 (t, J = 7 Hz, 2H), 3.00 (t, J = 7 Hz, 2H), 3.27 (t, J = 7 Hz, 2H), 3.94 (s, 9H), 4.14 (t, J = 7 Hz, 2H), 4.47 (t, J = 7 Hz, 2H), 7.34 (s, 2H), 7.89 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.2, 22.6, 22.8, 25.2, 25.6, 25.8, 28.2, 28.5 (2C), 29.16, 29.23, 31.8, 45.3, 56.3 (2C), 61.0, 68.9, 87.7, 106.3 (2C), 115.0, 122.7, 129.9, 131.5, 134.6, 141.1, 145.2, 153.1 (2C), 157.8, 162.0, 167.9, 169.9, 174.0.

HRMS (ESI) *m/z* calculated for $C_{35}H_{44}N_5O_5S_2$ $[M+H]^+$: 678.2765, found: 678.2778.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1-undecyl-1,6-dihydropyrimidine-5-carbonitrile (21)



Yield: 78 %.

Melting point: 74 °C.

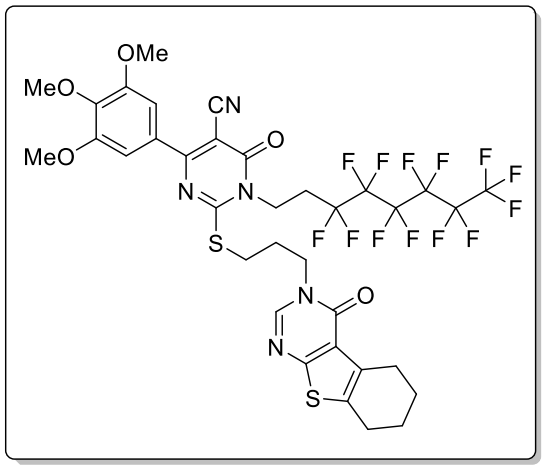
IR ($CHCl_3$): 1638, 1734, 2139, 3412. cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7 Hz, 3H), 1.15-1.55 (m, 16H), 1.70-1.94 (m, 6H), 2.19-2.40 (m, 2H), 2.68-2.85 (m, 2H), 2.89-3.06 (m, 2H), 3.26 (t, J = 7 Hz, 2H), 3.93 (s, 9H), 4.13 (t, J = 7 Hz, 2H), 4.45 (t, J = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0, 22.1, 22.6, 22.8, 25.1, 25.6, 25.8, 28.2, 28.45, 28.53, 29.2 (2C), 29.4, 29.5 (2C), 31.8, 45.3, 56.3 (2C), 60.9, 68.8, 87.6, 106.4 (2C), 114.9, 122.6, 129.8, 131.4, 134.4, 141.2, 145.2, 153.1 (2C), 157.7, 162.0, 167.8, 169.8, 173.9.

HRMS (ESI) *m/z* calculated for $C_{38}H_{50}N_5O_5S_2$ $[M+H]^+$: 720.3248, found: 720.3242; $C_{38}H_{49}N_5NaO_5S_2$ $[M+Na]^+$: 742.3067, found: 742.3060.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-1-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (22)



Yield: 64 %.

Melting point: 140 °C.

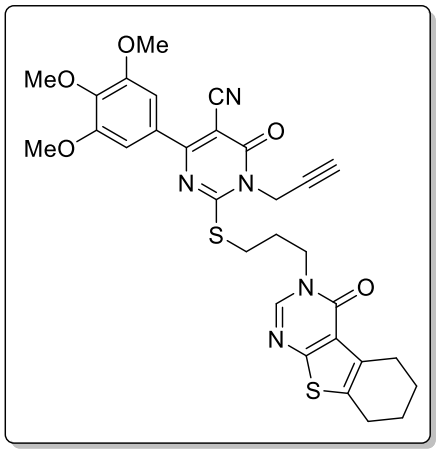
IR ($CHCl_3$): 1608, 1710, 2149, 3420. cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73-1.96 (m, 4H), 2.21-2.42 (m, 2H), 2.54-2.87 (m, 4H), 2.89-3.07 (m, 2H), 3.28 (t, J = 7 Hz, 2H), 3.94 (s, 9H), 4.13 (t, J = 7 Hz, 2H), 4.82 (t, J = 7 Hz, 2H), 7.35 (s, 2H), 7.88 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.2, 22.8, 25.2, 25.6, 28.3, 28.6, 29.7, 30.5 (t), 45.3, 56.3 (2C), 61.0, 87.6, 106.5 (2C), 108.0-119.7 (m, 6C), 114.4, 122.7, 129.5, 131.5, 134.6, 141.5, 145.2, 153.2 (2C), 157.8, 162.0, 168.1, 169.1, 174.1.

HRMS (ESI) *m/z* calculated for $C_{35}H_{31}N_5O_5F_{13}S_2$ $[M+H]^+$: 912.1553, found: 912.1554; $C_{35}H_{30}N_5NaO_5F_{13}S_2$ $[M+Na]^+$: 934.1371, found: 934.1373.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-1-(prop-2-yn-1-yl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (23)



Yield: 71 %.

Melting point: 171 °C.

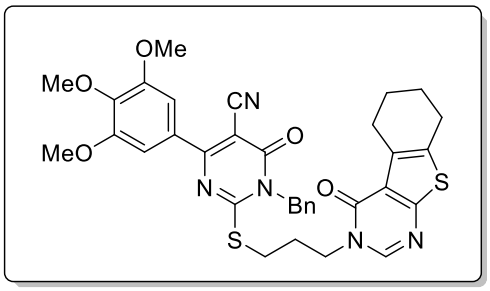
IR ($CHCl_3$): 1655, 1730, 2219, 3415. cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.79-1.91 (m, 4H), 2.28-2.37 (m, 2H), 2.55 (t, J = 2 Hz, 1H), 2.78 (t, J = 6 Hz, 2H), 2.99 (t, J = 6 Hz, 2H), 3.28 (t, J = 7 Hz, 2H), 3.93 (s, 9H), 4.14 (t, J = 7 Hz, 2H), 5.12 (d, J = 2 Hz, 2H), 7.34 (s, 2H), 7.90 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.2, 22.8, 25.2, 25.6, 28.3, 28.7, 45.4, 55.6, 56.3 (2C), 61.0, 76.2, 76.9, 87.6, 106.5 (2C), 114.5, 122.7, 129.5, 131.5, 134.5, 141.4, 145.2, 153.2 (2C), 157.8, 162.0, 168.2, 168.8, 174.2.

HRMS (ESI) *m/z* calculated for $C_{30}H_{30}N_5O_5S_2$ $[M+H]^+$: 604.1675, found: 604.1683; $C_{30}H_{29}N_5NaO_5S_2$ $[M+Na]^+$: 626.1491, found: 626.1502.

1-Benzyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (24)



Yield: 64 %.

Melting point: 110 °C.

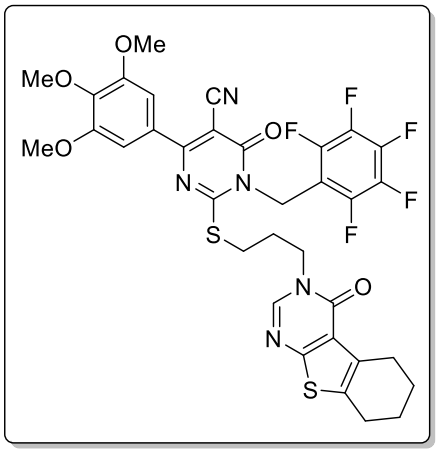
IR ($CHCl_3$): 1624, 1680, 2213, 3417. cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65-2.00 (m, 4H), 2.13-2.44 (m, 2H), 2.59-2.84 (m, 2H), 2.88-3.11 (m, 2H), 3.37 (t, J = 7 Hz, 2H), 3.90 (s, 6H), 3.93 (s, 3H), 4.04 (t, J = 6 Hz, 2H), 5.30 (s, 2H), 7.28-7.56 (m, 7H), 7.75 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.1, 22.7, 25.2, 25.6, 28.1, 30.0, 45.0, 48.0, 56.4 (2C), 61.0, 92.7, 106.6 (2C), 115.6, 122.6, 128.1 (2C), 128.4, 128.8 (2C), 129.4, 131.4, 133.6, 134.7, 141.7, 145.0, 153.0 (2C), 157.7, 160.5, 162.0, 164.5, 165.0.

HRMS (ESI) *m/z* calculated for $C_{34}H_{34}N_5O_5S_2$ $[M+H]^+$: 656.1981, found: 656.1972.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-1-((perfluorophenyl)methyl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (25)



Yield: 76 %.

Melting point: 138 °C.

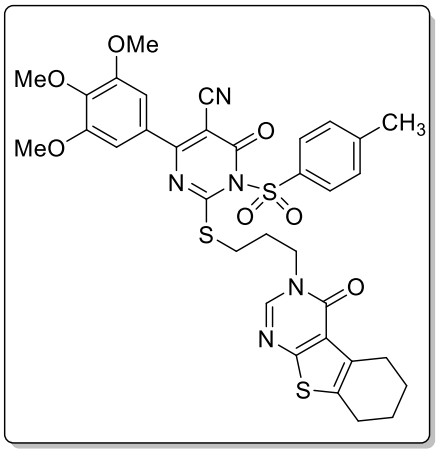
IR ($CHCl_3$): 1628, 1720, 2159, 3411. cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73-2.00 (m, 4H), 2.21-2.45 (m, 2H), 2.75 (t, J = 5 Hz, 2H), 2.94 (t, J = 5 Hz, 2H), 3.31 (t, J = 7 Hz, 2H), 3.93 (s, 9H), 4.14 (t, J = 6 Hz, 2H), 5.60 (s, 2H), 7.32 (s, 2H), 7.90 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.1, 22.7, 25.1, 25.5, 28.3, 28.8, 45.4, 56.3 (2C), 56.8, 60.9, 87.4, 106.5 (2C), 108.4 (dt), 114.3, 122.6, 129.4, 131.4, 134.5, 136.5-146.8 (m, 5C), 141.5, 145.2, 153.1 (2C), 157.7, 162.0, 168.2, 168.8, 174.1.

HRMS (ESI) *m/z* calculated for $C_{34}H_{29}N_5NaO_5F_5S_2$ $[M+Na]^+$: 768.1334, found: 768.1344.

6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (26)



Yield: 58 %.

Melting point: 163 °C.

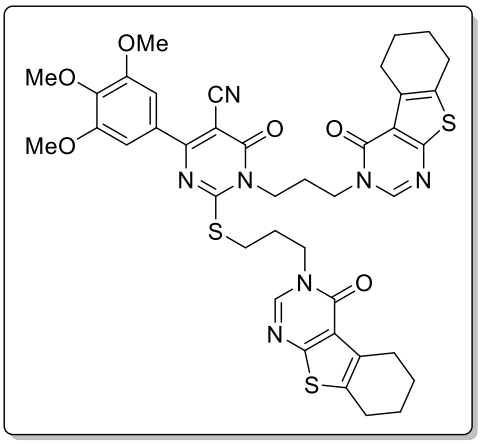
IR ($CHCl_3$): 1668, 1720, 2225, 3414 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.69-2.01 (m, 4H), 2.23-2.44 (m, 2H), 2.51 (s, 3H), 2.78 (t, J = 6 Hz, 2H), 3.02 (t, J = 6 Hz, 2H), 3.26 (t, J = 7 Hz, 2H), 3.93 (s, 9H), 4.10-4.28 (m, 2H), 7.32 (s, 2H), 7.47 (d, J = 8 Hz, 2H), 8.04 (d, J = 8 Hz, 2H), 8.18 (bs, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 21.8, 22.1, 22.8, 25.1, 25.6, 28.6, 29.0, 45.3, 56.3 (2C), 60.9, 88.7, 106.6 (2C), 113.3, 122.7, 128.7 (2C), 128.9, 130.1 (2C), 131.5, 132.5, 134.2, 141.9, 145.6, 146.8, 153.2 (2C), 157.7, 161.8, 165.1, 169.2, 175.1.

HRMS (ESI) *m/z* calculated for $C_{34}H_{34}N_5O_7S_3$ $[M+H]^+$: 720.1609, found: 720.1615; $C_{34}H_{33}N_5NaO_7S_3$ $[M+Na]^+$: 742.1427, found: 742.1434.

6-Oxo-1-(3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (27) (from compound **15** by reaction with bromide **8**)



Yield: 60 %.

Melting point: 129 °C.

IR ($CHCl_3$): 1667, 1722 (b), 2212, 3412 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.75-1.93 (m, 8H), 2.19-2.45 (m, 4H), 2.77 (bs, 4H), 2.97 (bs, 4H), 3.24 (t, J = 7 Hz, 2H), 3.94 (s, 9H), 4.12 (t, J = 7 Hz, 2H), 4.23 (t, J = 7 Hz, 2H), 4.56 (t, J = 7 Hz, 2H), 7.34 (s, 2H), 7.91 (s, 1H), 8.00 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 22.1 (2C), 22.7 (2C), 25.1 (2C), 25.5 (2C), 27.9, 28.2, 28.6, 43.6, 45.3, 56.3 (2C), 60.9, 65.1, 87.3, 106.4 (2C), 114.7,

122.6, 122.7, 129.5, 131.4, 134.3, 134.4, 141.4, 141.5, 145.2, 145.5, 153.1 (2C), 157.7, 157.8, 162.0, 162.1, 167.8, 169.4, 174.2.

HRMS (ESI) *m/z* calculated for $C_{40}H_{42}N_7O_6S_3$ $[M+H]^+$: 812.2347, found: 812.2353; $C_{40}H_{41}N_7NaO_6S_3$ $[M+Na]^+$: 834.2166, found: 834.2173.

1.3.4.2. Biological Screening

1.3.4.2.1. Antitubercular activity

The compounds synthesized in the present work were tested for their *in vitro* and *ex vivo* effects against dormant and active stages MTB using XRMA protocol.[17,18] MTB (ATCC No. 25177) were grown to logarithmic phase (O. D. 1.0) in a M. pheli medium. The stock culture was maintained at -70°C and sub-cultured once in M. pheli medium before inoculation into the experimental culture. All experiments were performed in triplicates and IC_{50} and IC_{90} values were calculated from their dose–response curves[19].

1.3.4.2.2. Cytotoxicity assay

The cytotoxicity of the compounds was determined using MTT assay against three different human cancer cell lines and THP-1 monocytes in duplicate[19, 20, 21]. Leukaemia THP-1, lung A549 adenocarcinoma, pancreatic PANC-1 adenocarcinoma and HeLa cervical carcinoma cell lines were obtained from the European Collection of Cell Cultures (ECCC), Salisbury, UK. Cell lines were maintained under standard cell culture conditions at 37 ^oC and 5% CO_2 in a humidified environment.

1.3.4.2.3. Docking studies

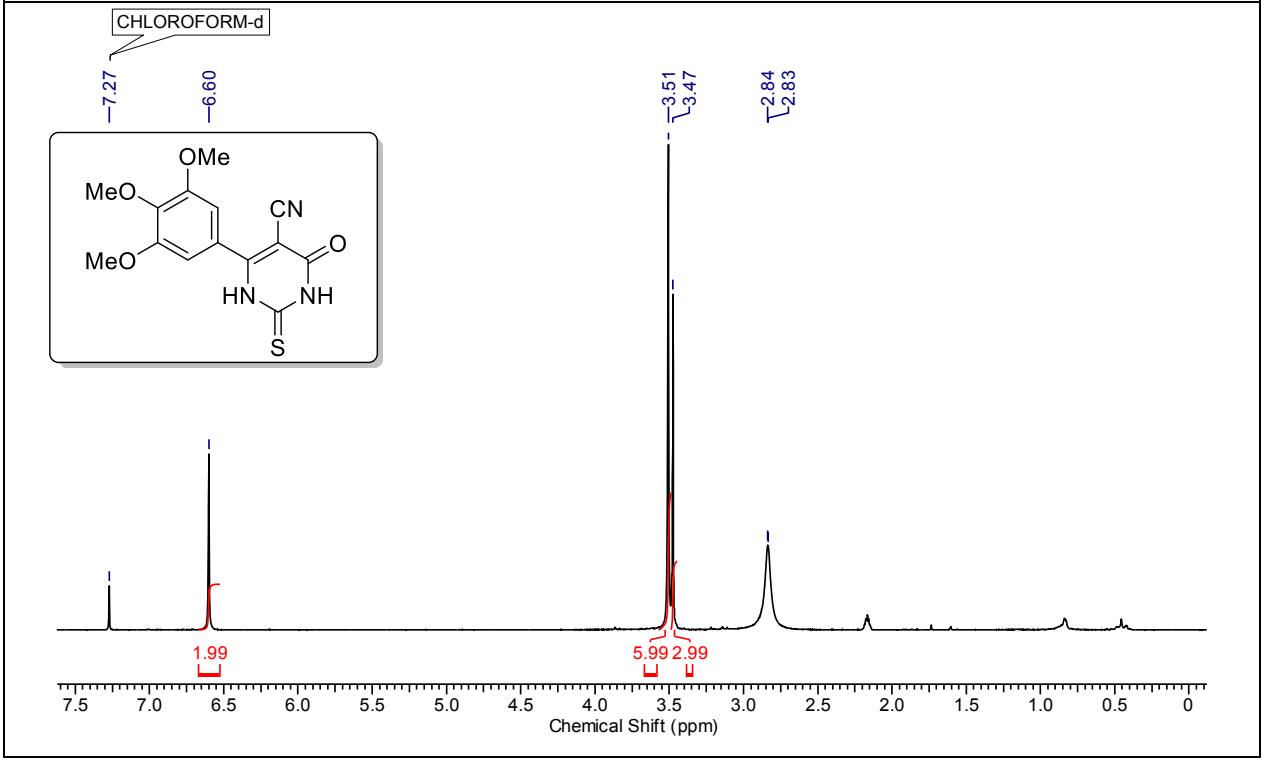
Molecular docking studies were carried out to predict the probable mode of action of antitubercular activity of the synthesized hybrids of thienopyrimidinones and thiouracil derivatives with mycobacterium tuberculosis pantothenate synthetase (PDB id: 3IVX).

To perform docking studies Glide 7.1[29] was used. All chemical structures were drawn using 2D-sketcher incorporated within Maestro 10.6[30]. LigPrep 3.8[31] was used to prepare 3D structures with corrected chiralities and each individual structure was refined with the optimized energy and best possible conformations. Protein obtained was initially purified by adding H-atoms wherever necessary to the amino acid residues and bond order was assigned to find out the correct ionization and tautomeric states of them. All the water molecules present within the active site were removed and missing side chains were added using Prime 4.4[32]. For further

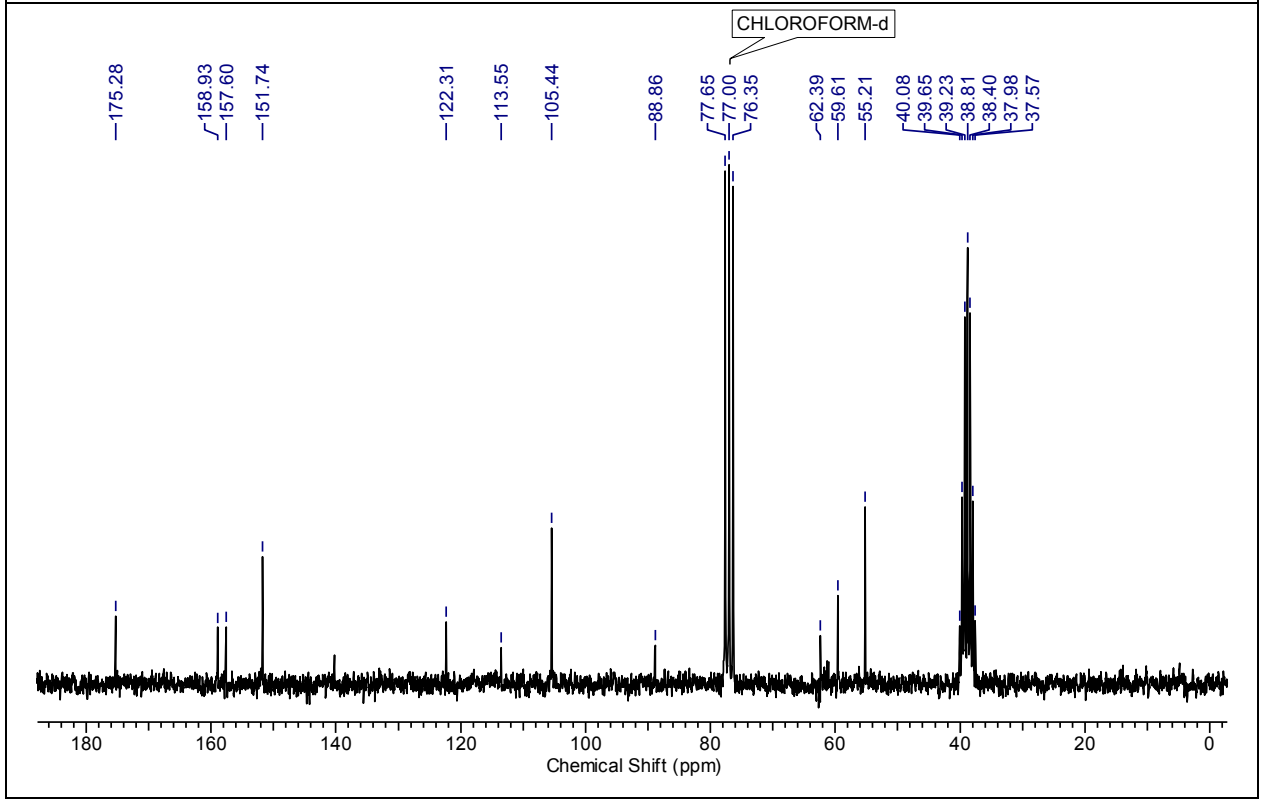
purification protein was refined by checking the protonation state of histidines, terminal acids and of polar hydrogens. The existing steric clashes present within the protein were relaxed using OPLS-2005 force field and was terminated once root mean square deviation reached 0.30 Å[34, 35]. Grid was prepared by selecting the co-crystallized ligand FG6 and the length of enclosing cubic box was set to 20A° to cover the maximum area of active site around the co-crystallized ligand.

1.3.4.3. Spectral Data

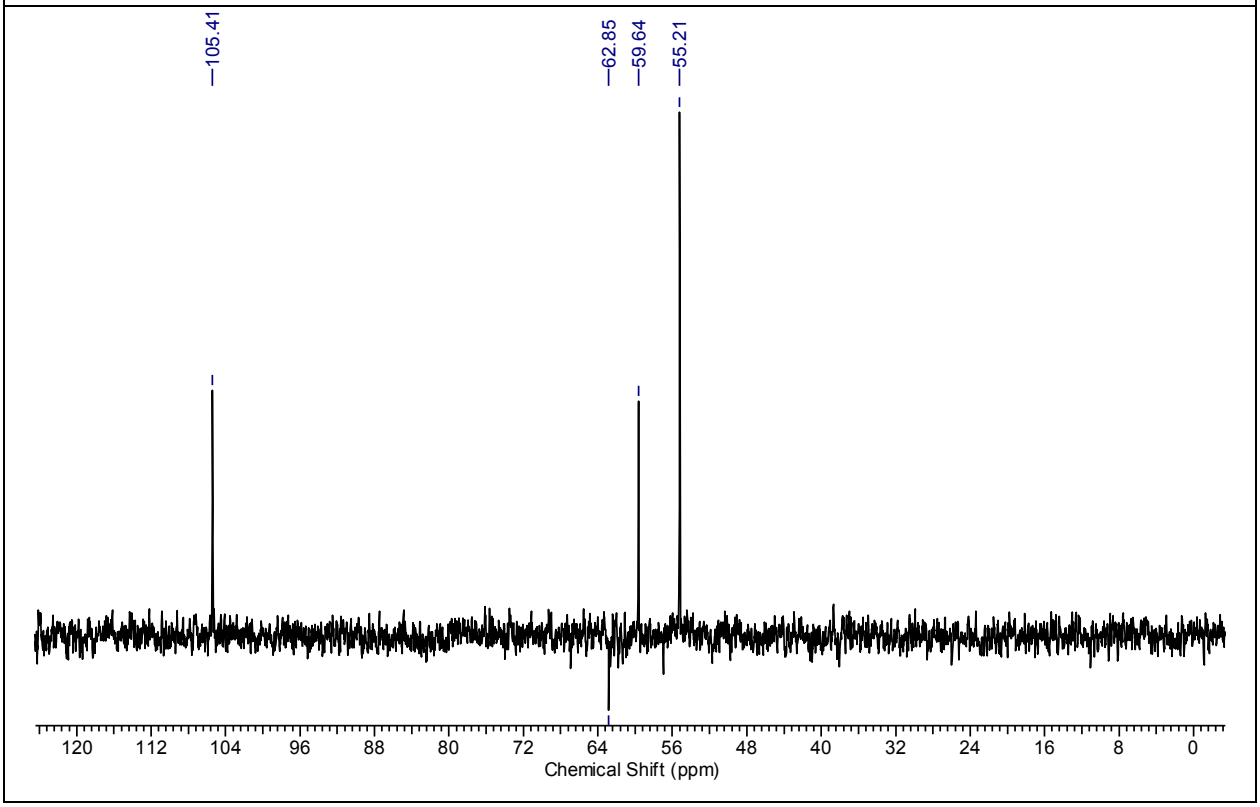
^{1}H NMR (200 MHz, $CDCl_3$ +DMSO-d6) of compound 1



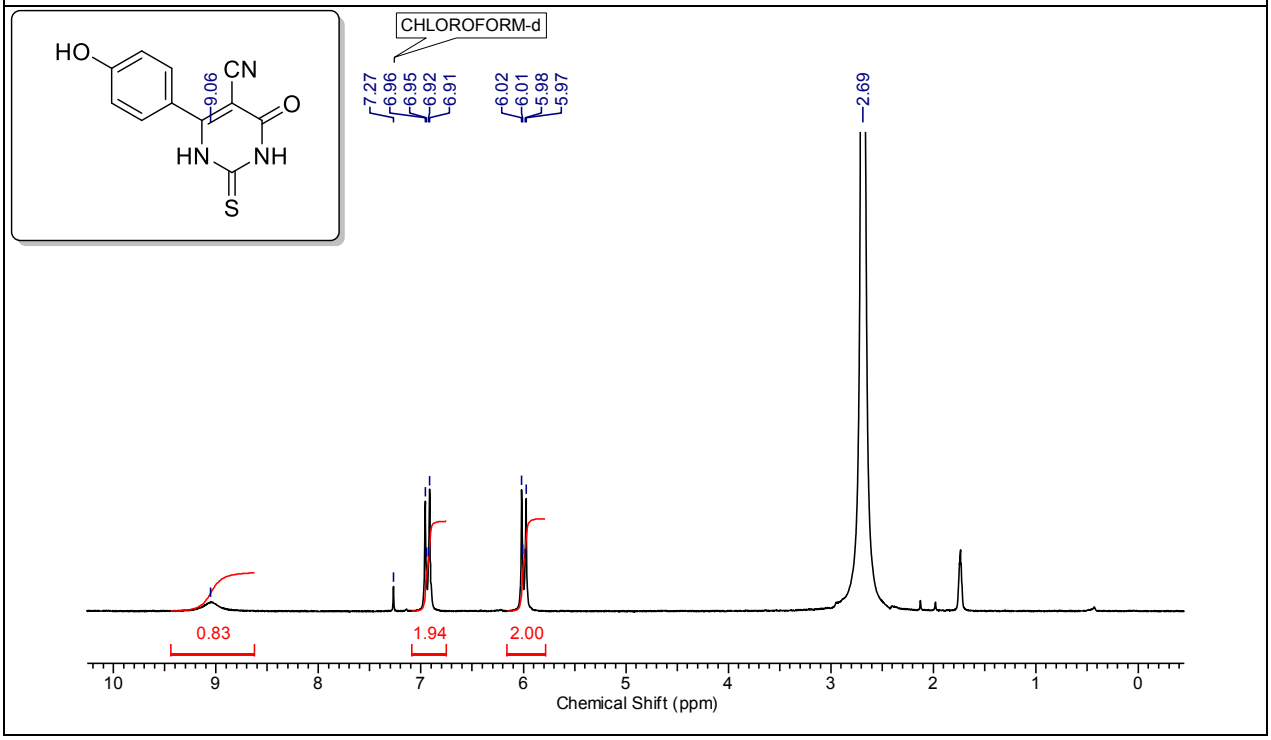
^{13}C NMR (50 MHz, $CDCl_3$ +DMSO-d6) of compound 1



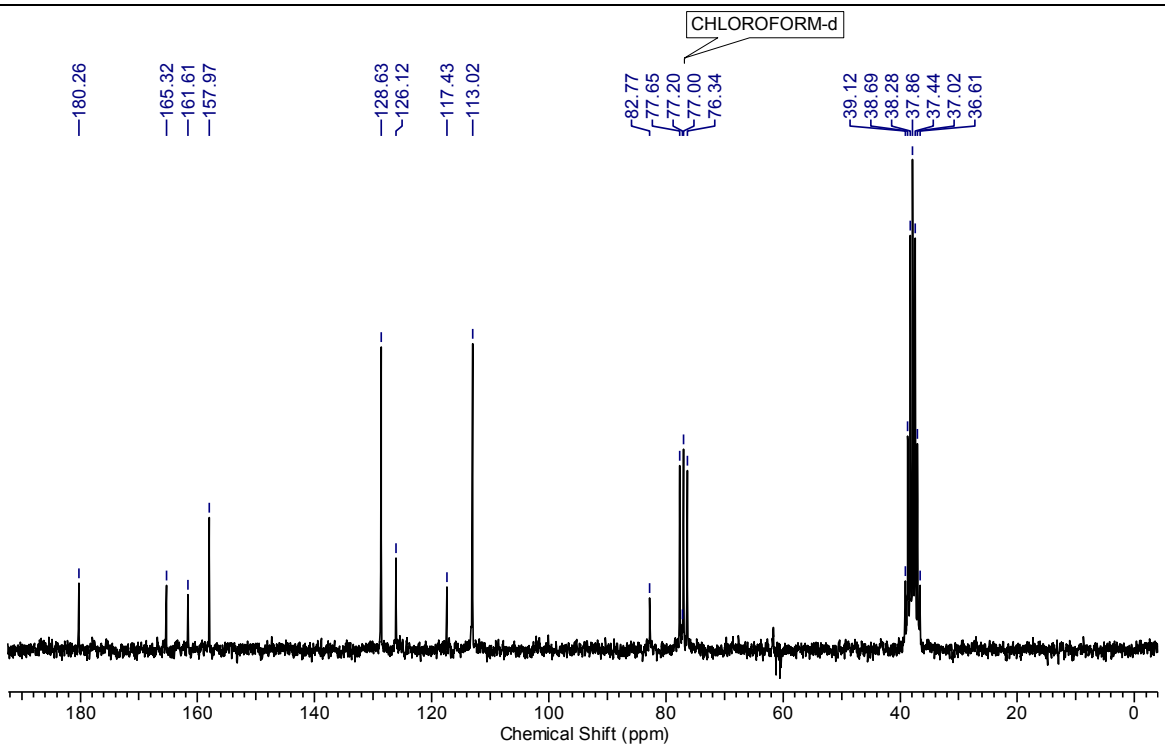
^{13}C NMR DEPT (50 MHz, $CDCl_3$ + DMSO-d6) of compound 1



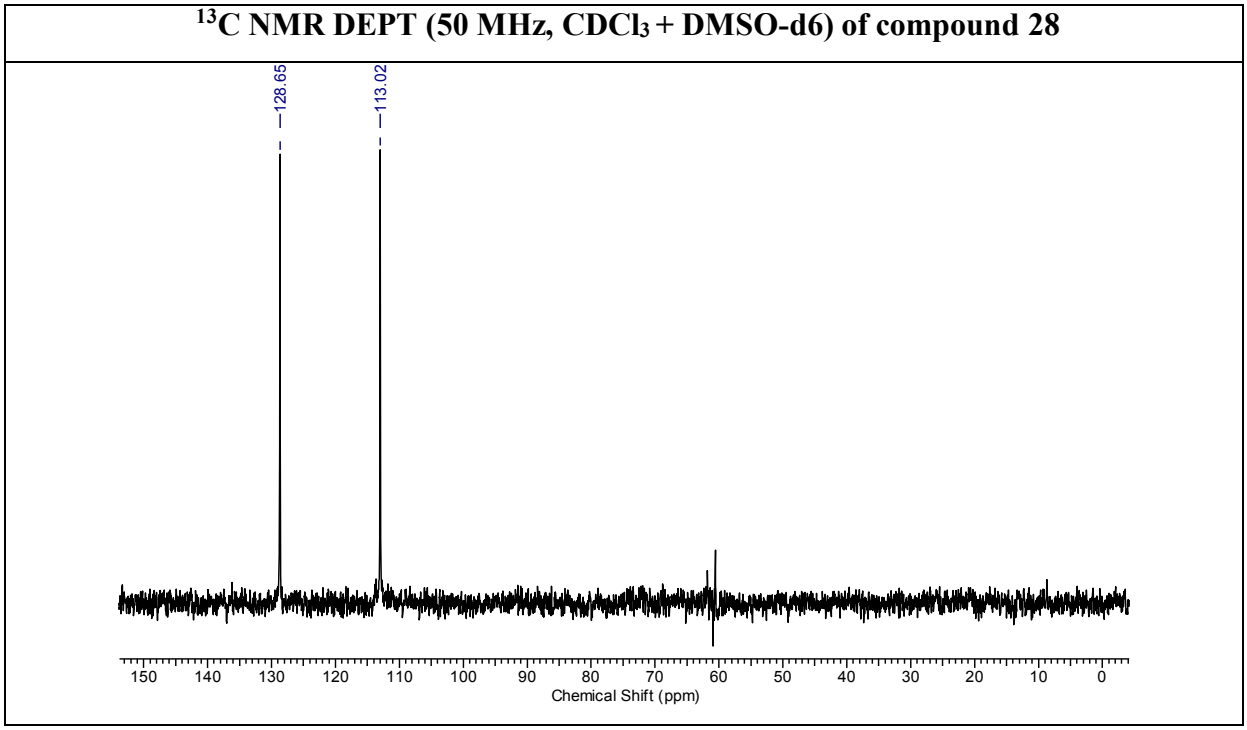
^{1}H NMR (200 MHz, $CDCl_3$ +DMSO-d6) of compound 28



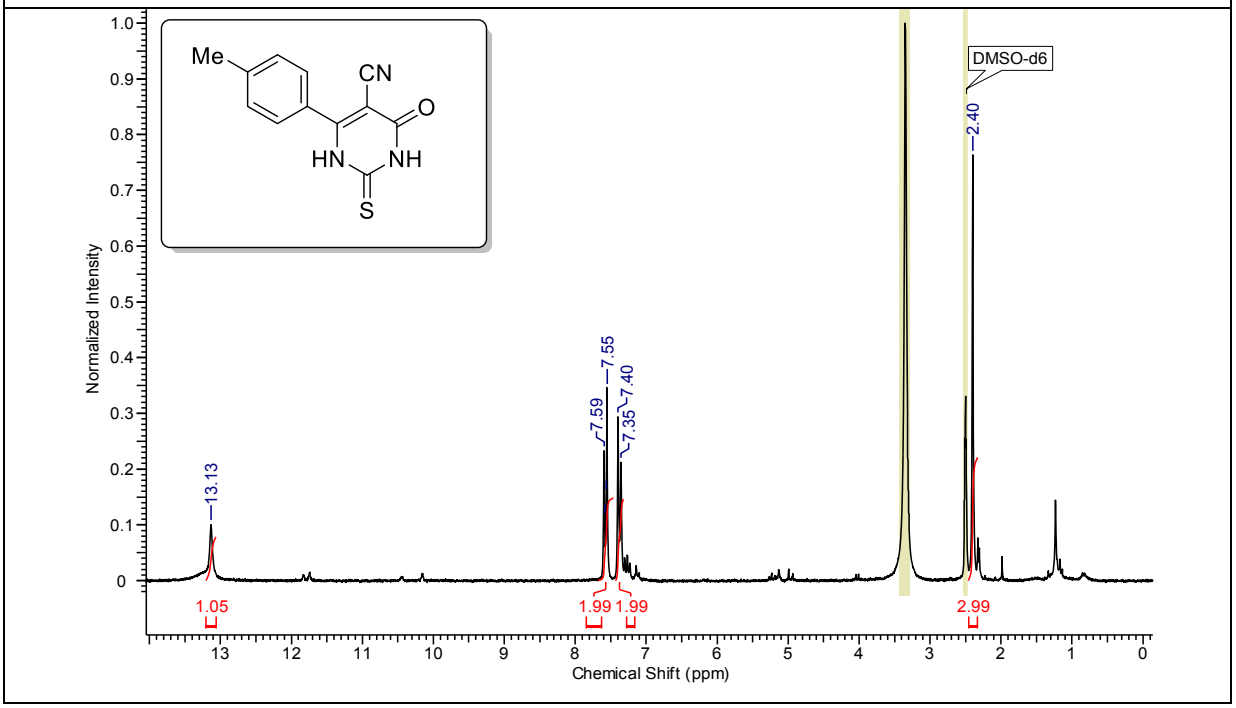
^{13}C NMR (50 MHz, $CDCl_3$ +DMSO-d6) of compound 28



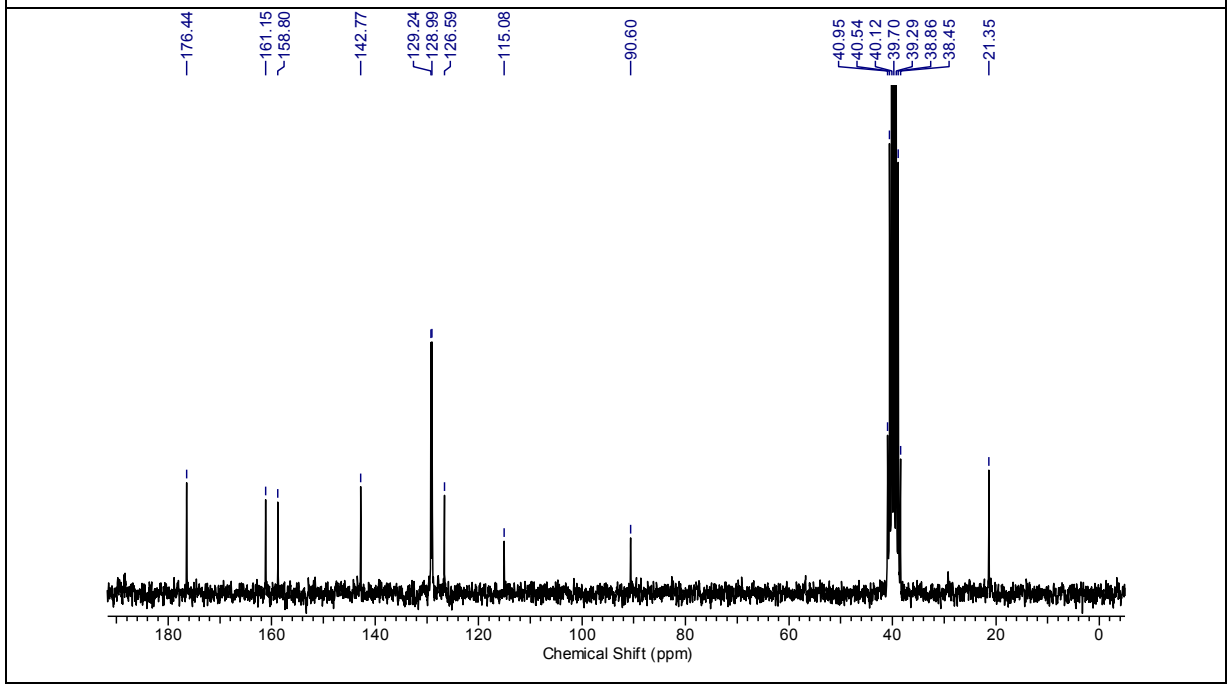
^{13}C NMR DEPT (50 MHz, $CDCl_3$ + DMSO-d6) of compound 28



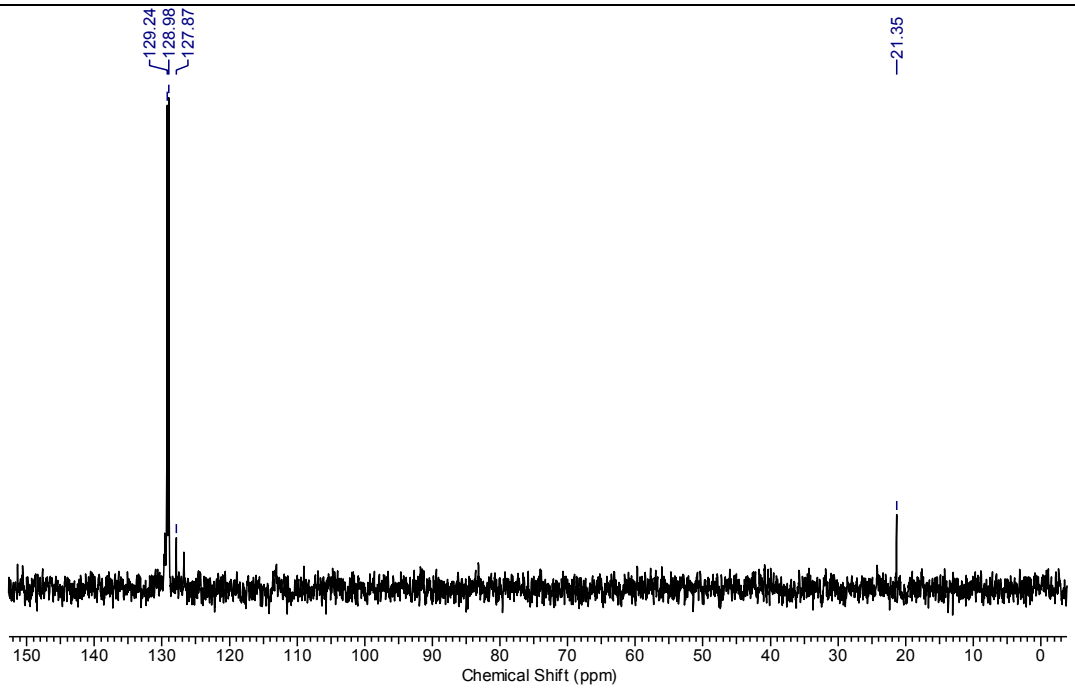
^{1}H NMR (200 MHz, DMSO-d6) of compound 29



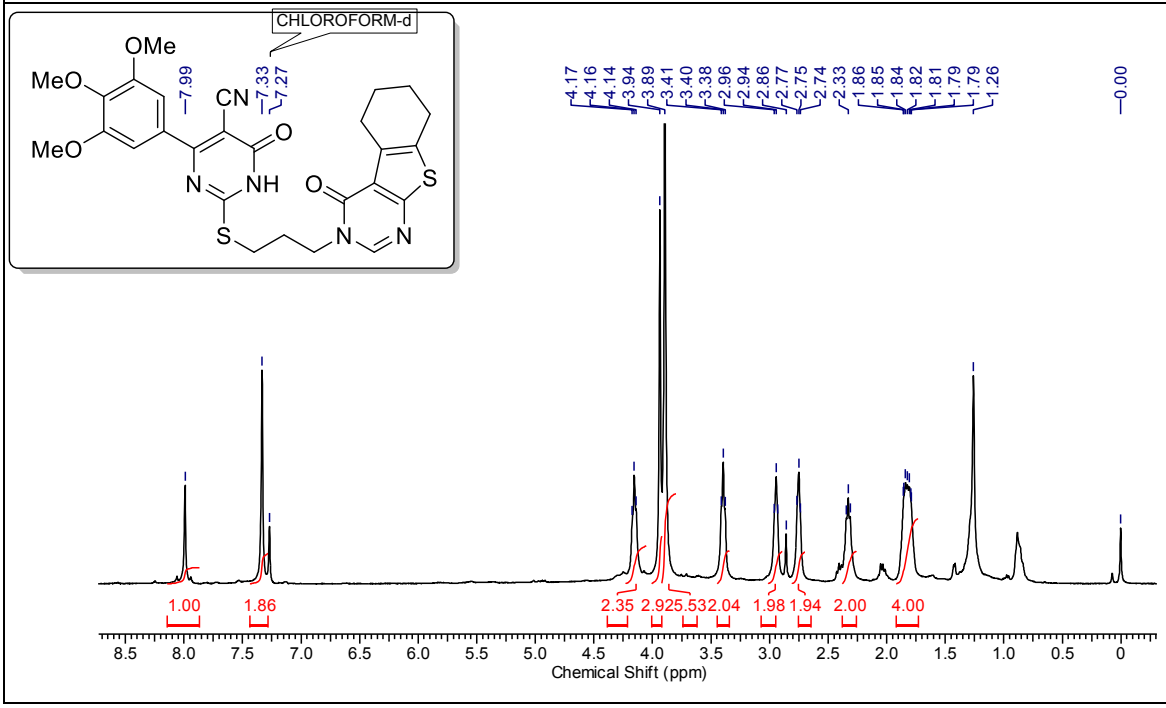
^{13}C NMR (50 MHz, DMSO-d6) of compound 29



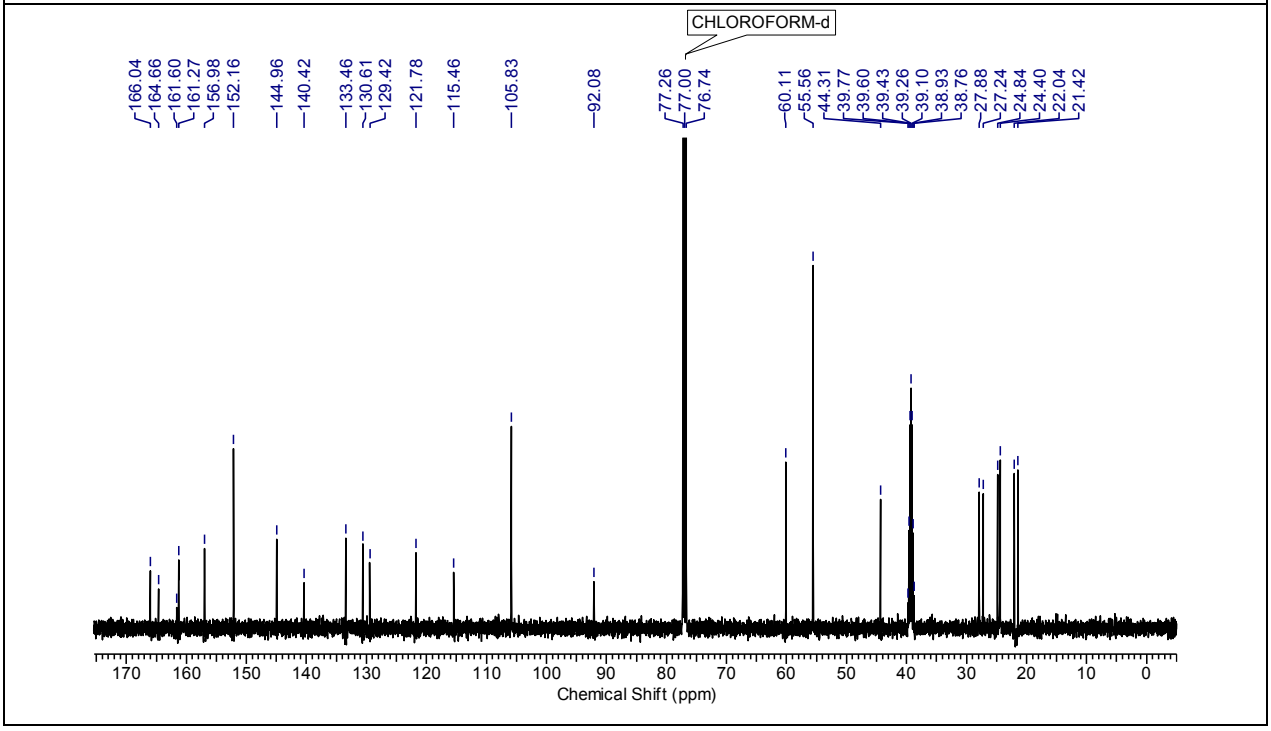
^{13}C NMR (DEPT 50 MHz, DMSO-d6) of compound 29



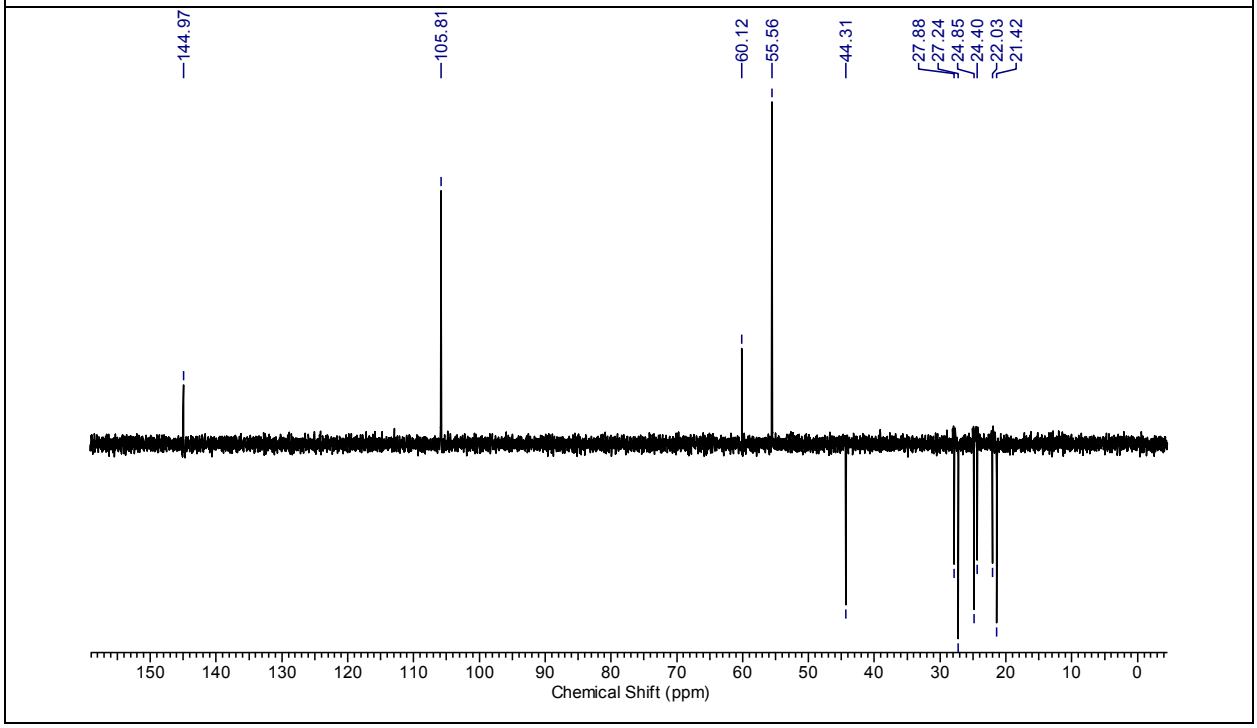
^{1}H NMR (400 MHz, $CDCl_3$) of compound 15



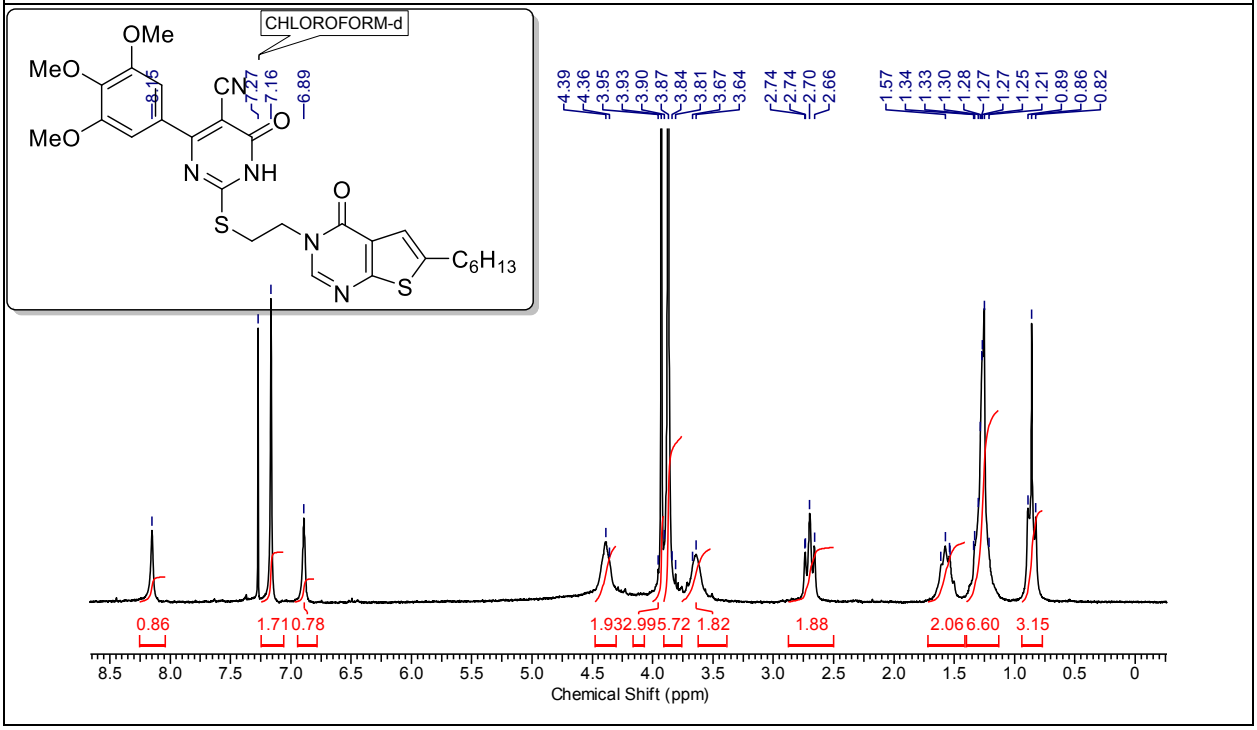
^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d6) of compound 15



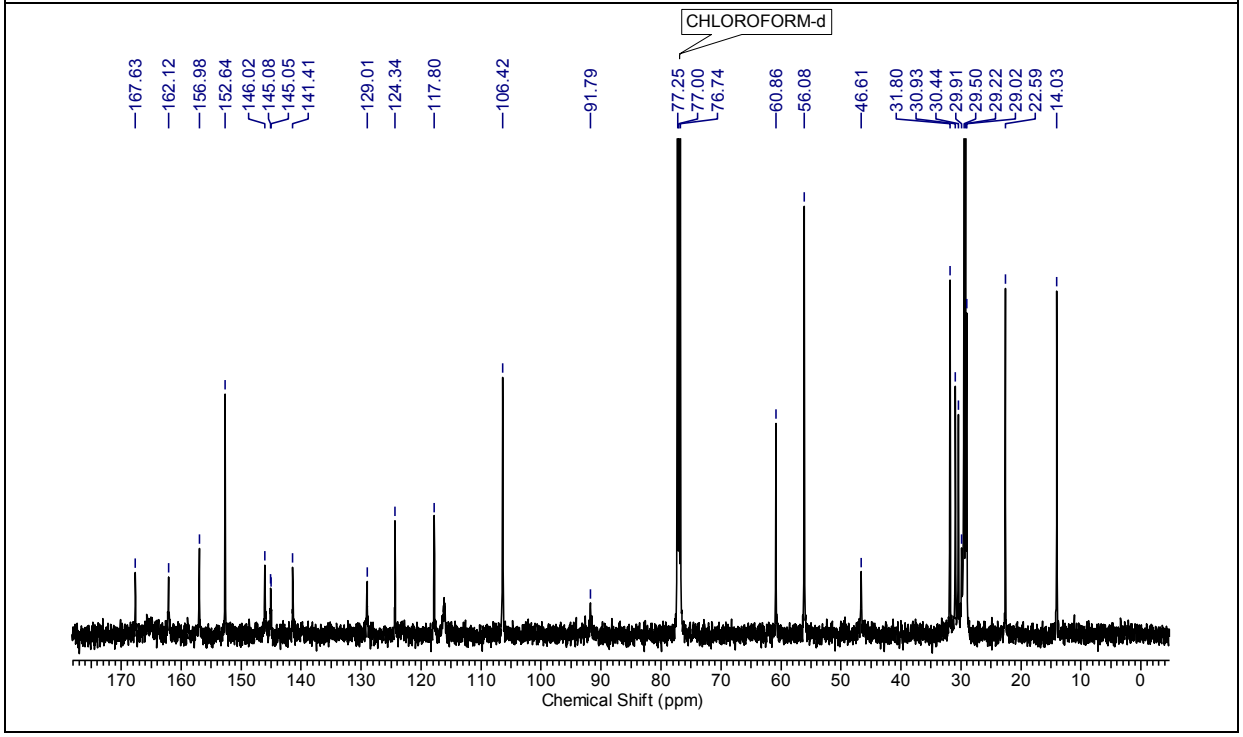
^{13}C NMR DEPT (125 MHz, $CDCl_3$+DMSO-d6) of compound 15



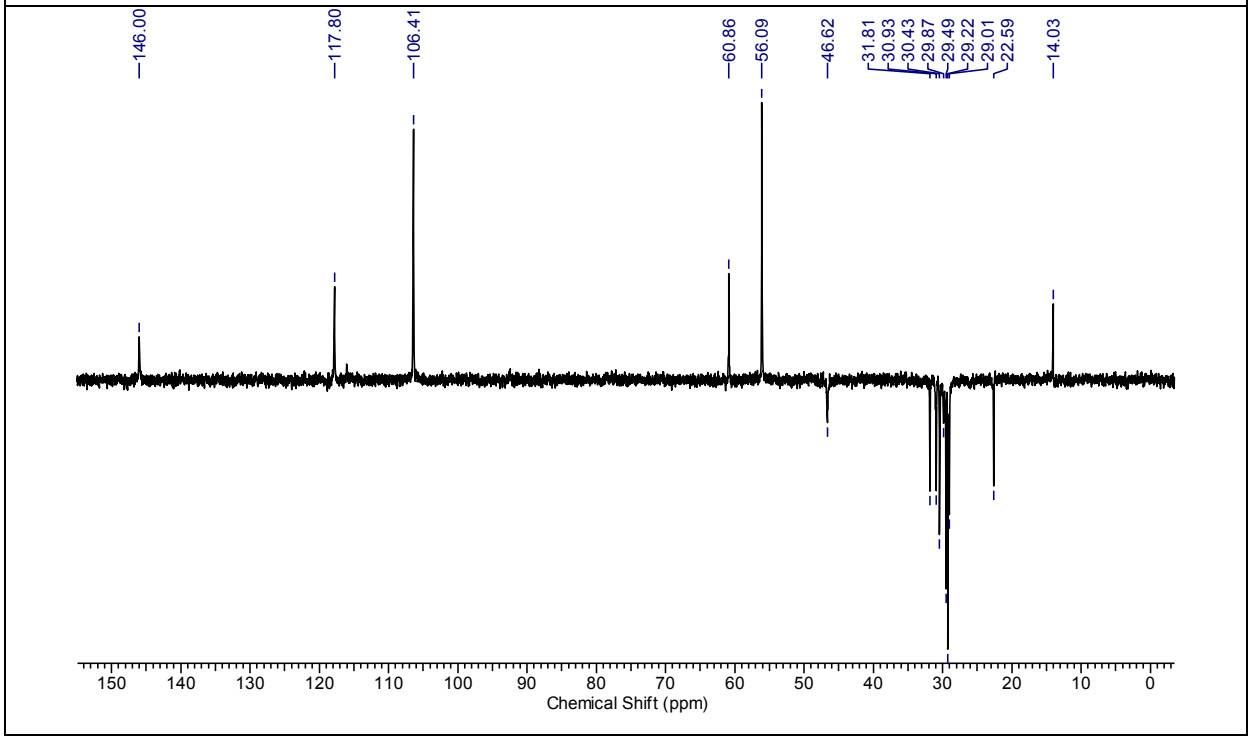
^{1}H NMR (200 MHz, $CDCl_3$) of compound 16



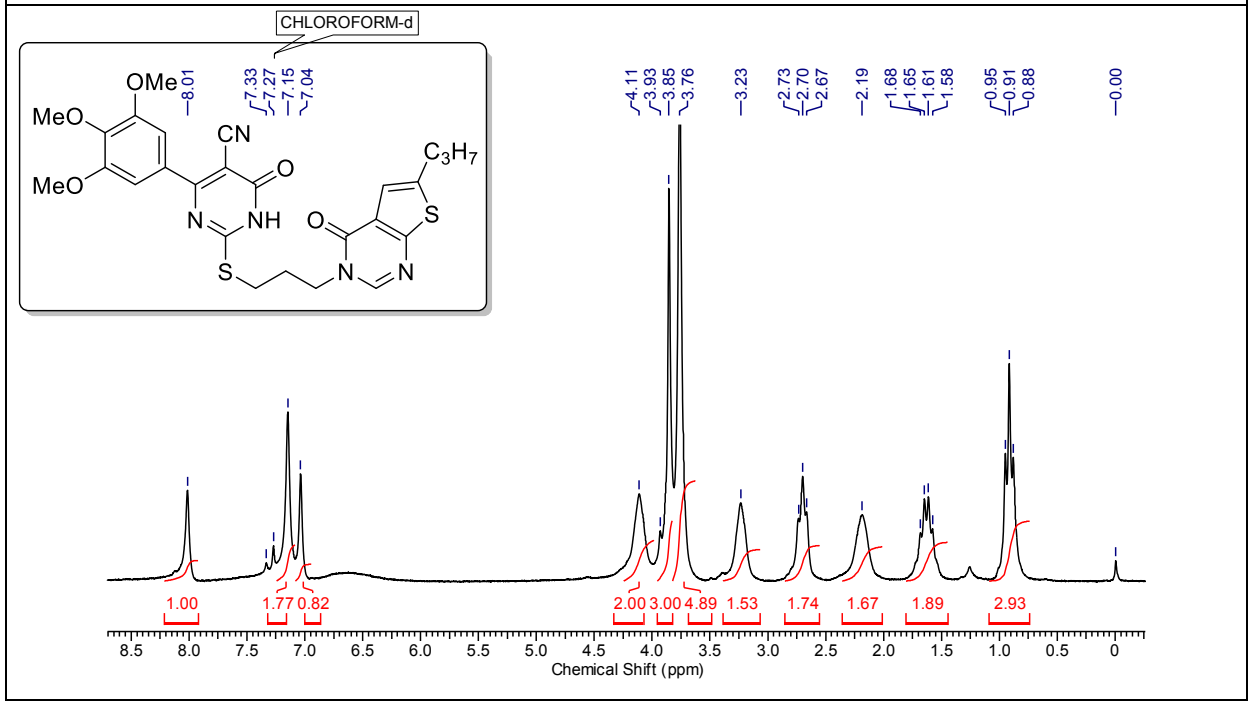
^{13}C NMR (125 MHz, $CDCl_3$) of compound 16



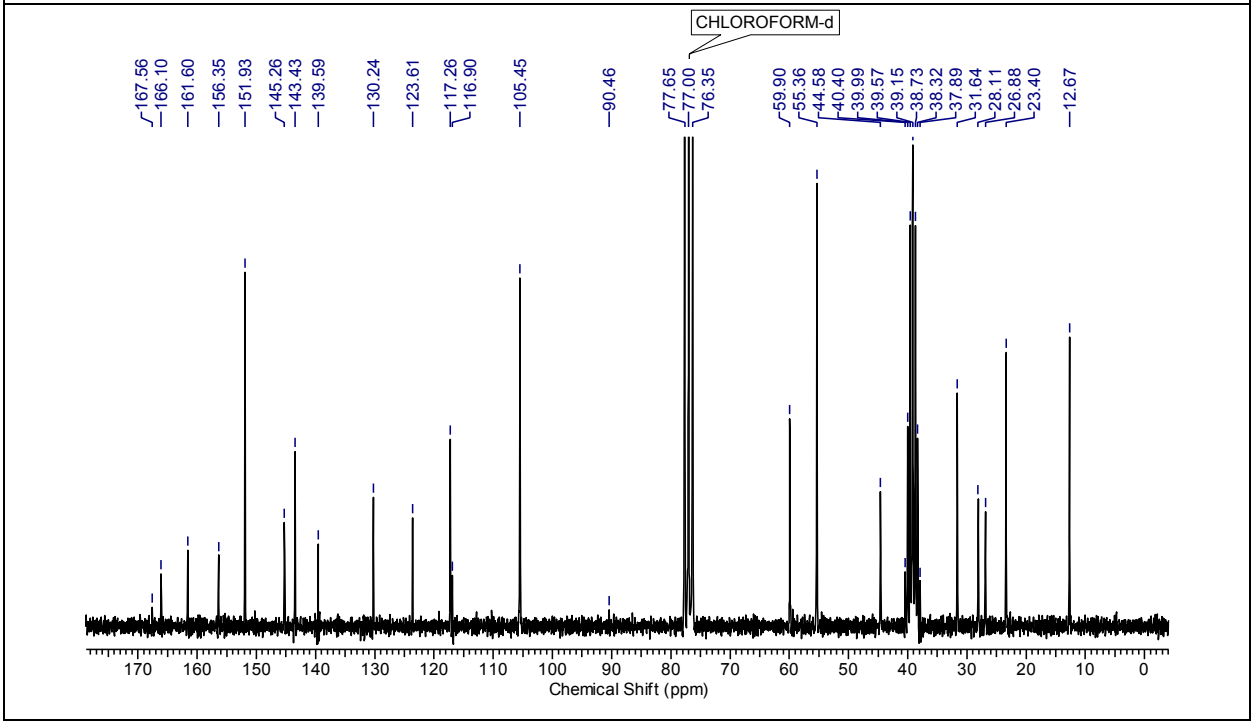
^{13}C NMR (DEPT 125 MHz, $CDCl_3$) of compound 16



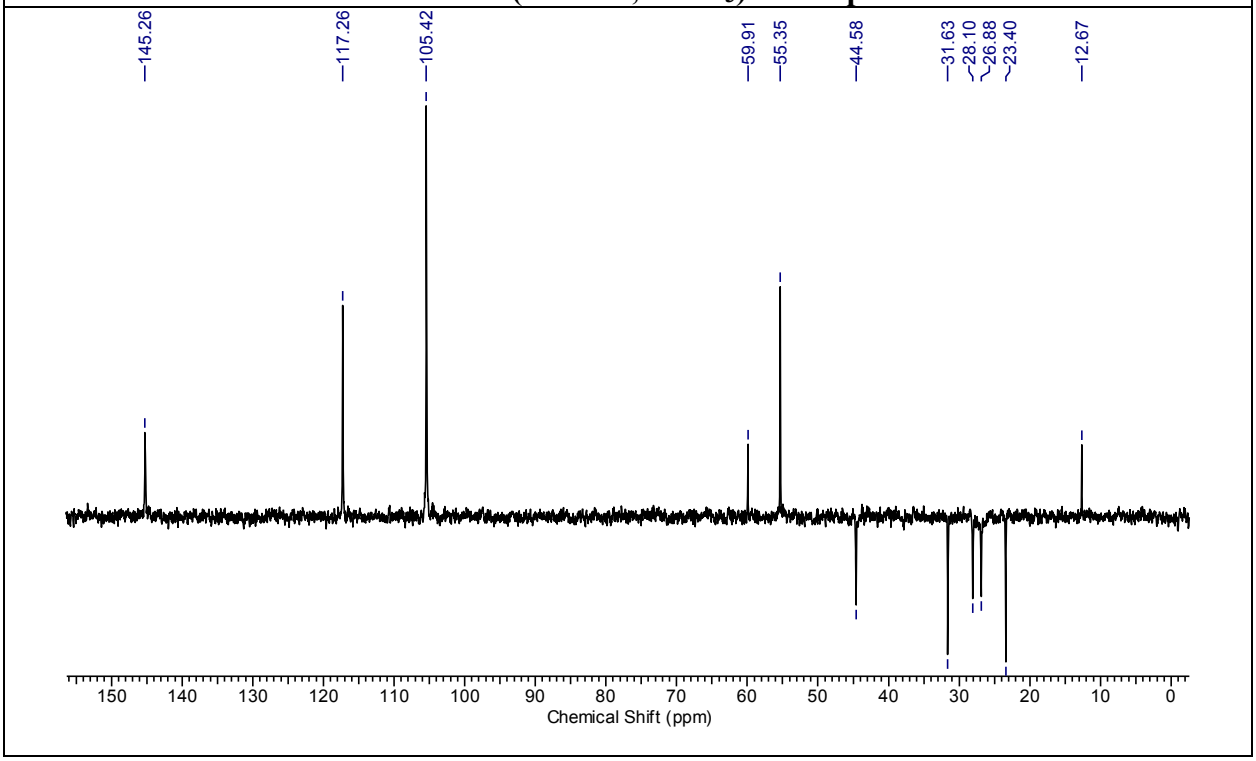
^{1}H NMR (200 MHz, $CDCl_3$) of compound 11



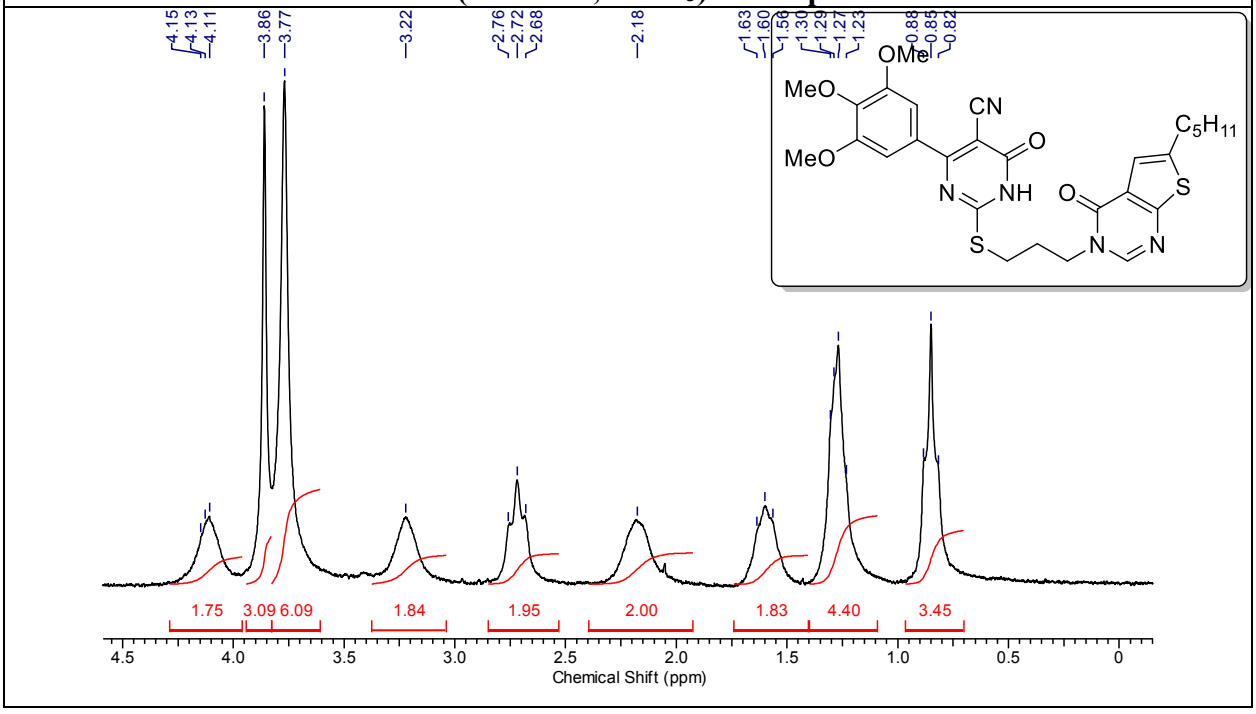
^{13}C NMR (50 MHz, $CDCl_3$) of compound 11



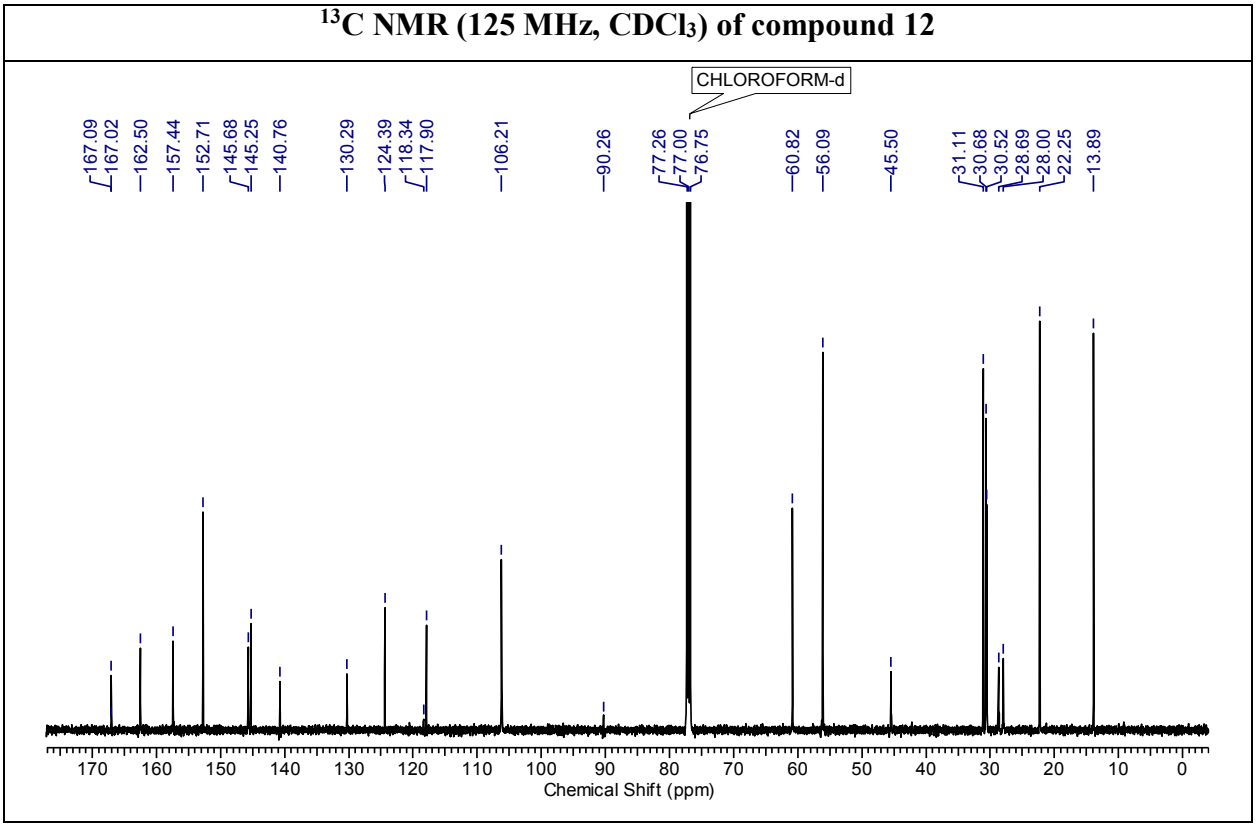
^{13}C NMR DEPT (50 MHz, $CDCl_3$) of compound 11



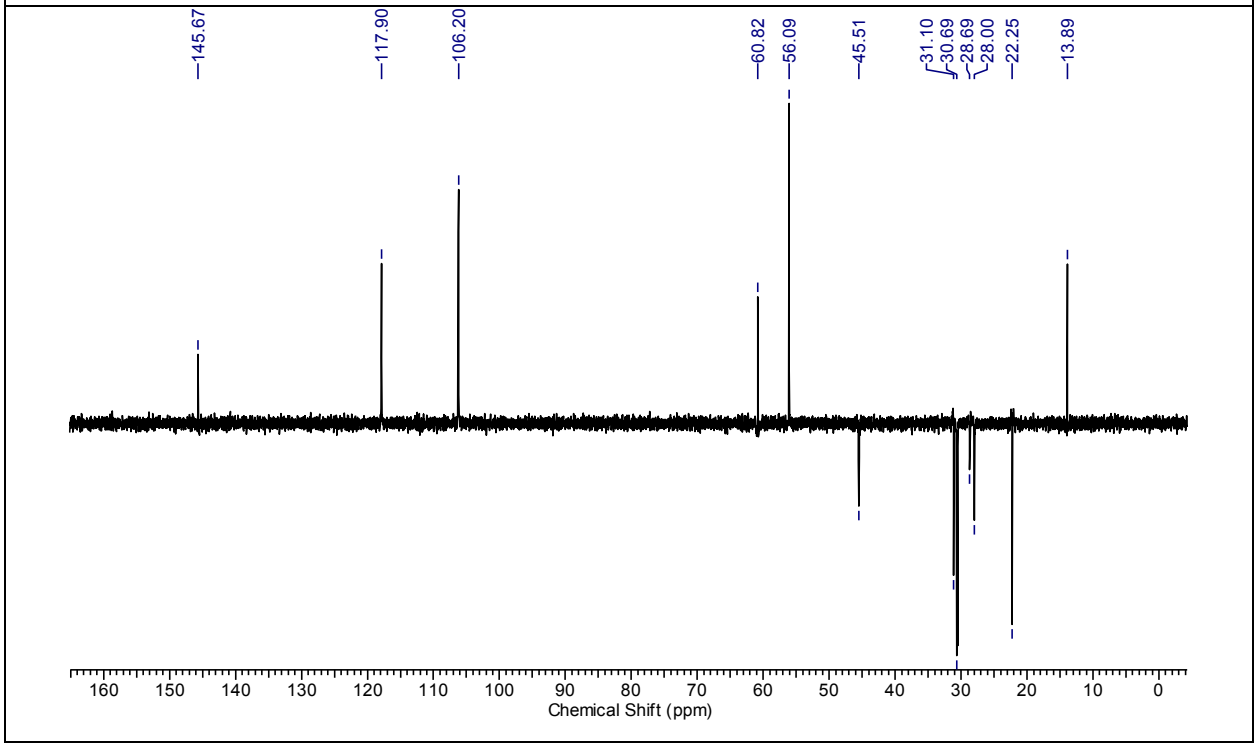
^{1}H NMR (200 MHz, $CDCl_3$) of compound 12



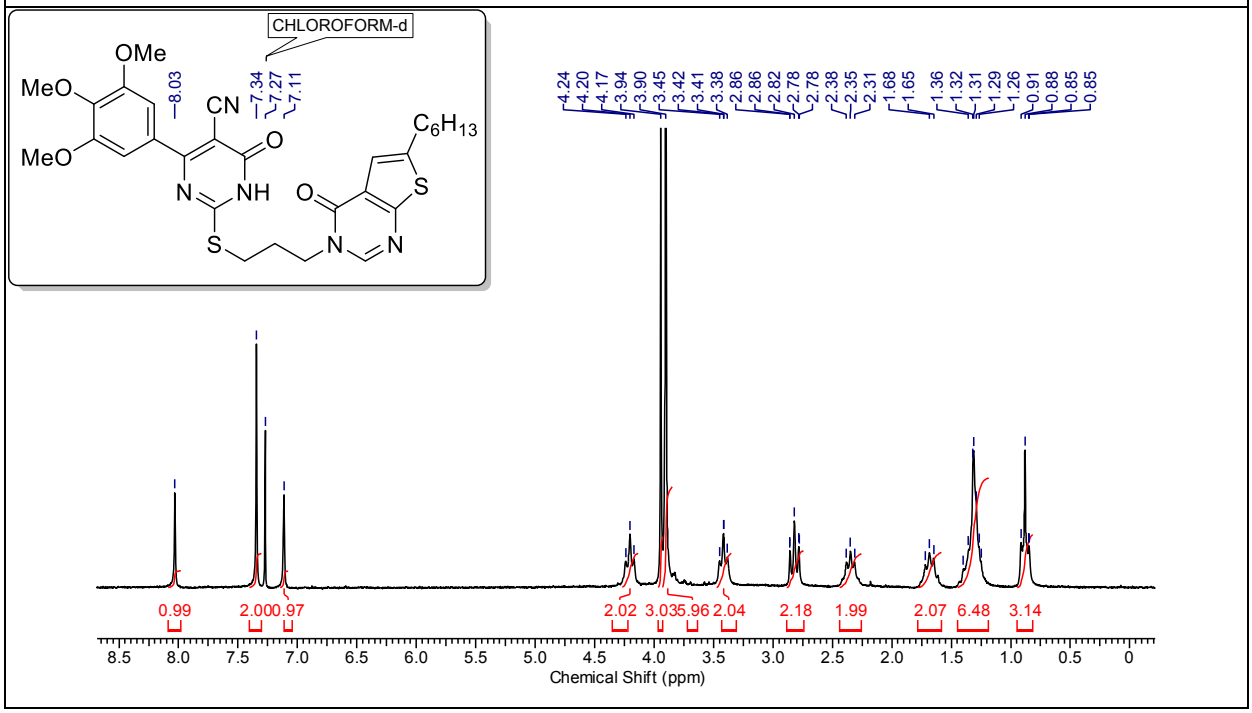
^{13}C NMR (125 MHz, $CDCl_3$) of compound 12



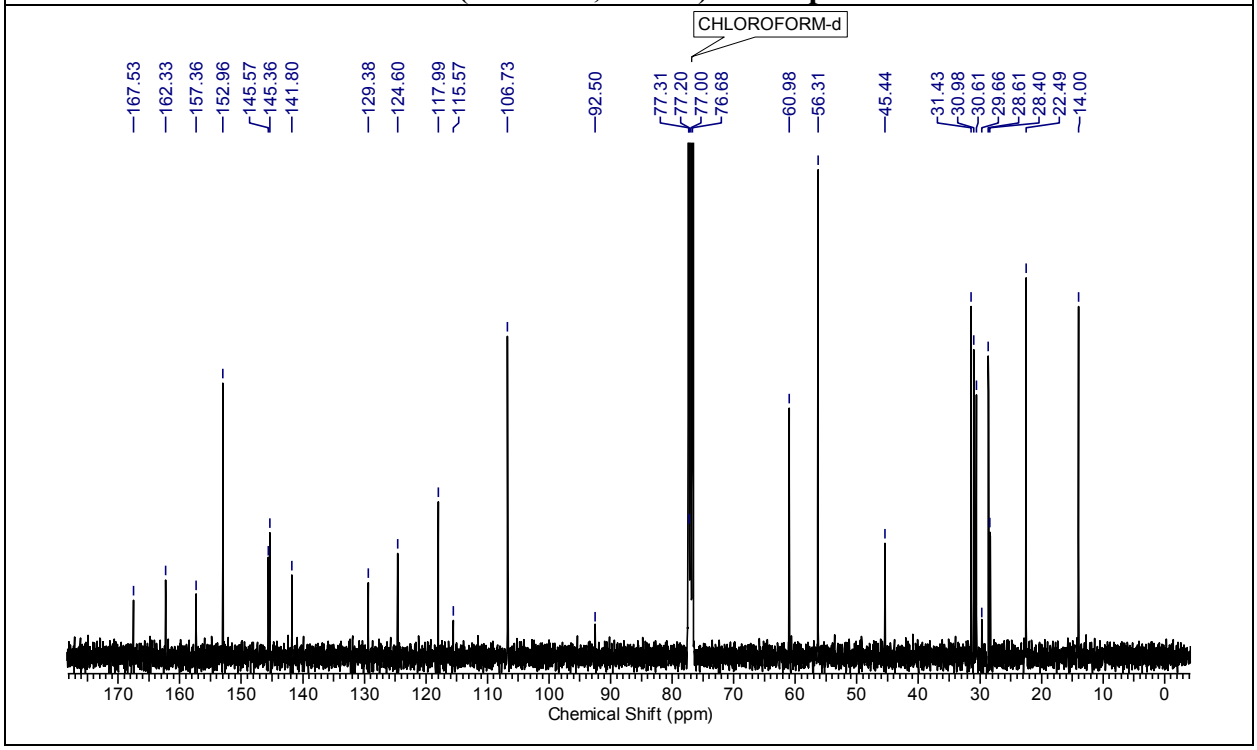
^{13}C NMR DEPT (125 MHz, $CDCl_3$) of compound 12



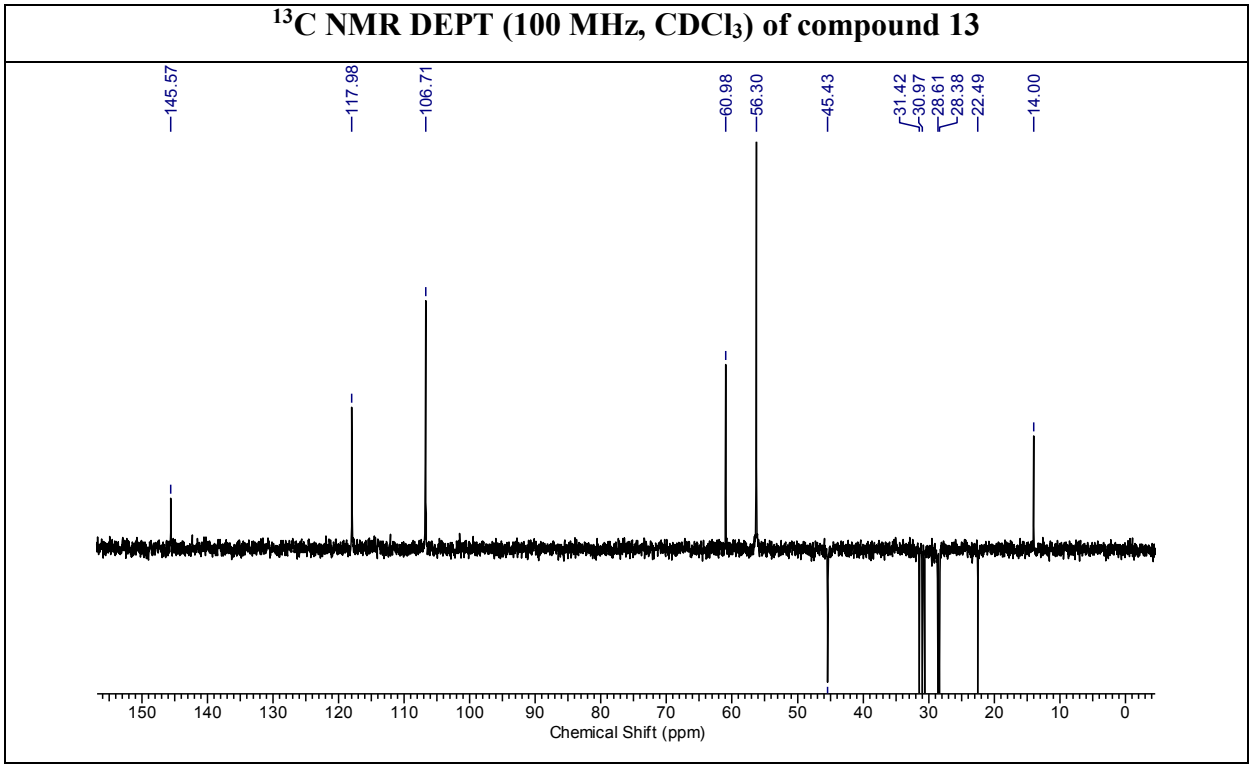
^{1}H NMR (200 MHz, $CDCl_3$) of compound 13



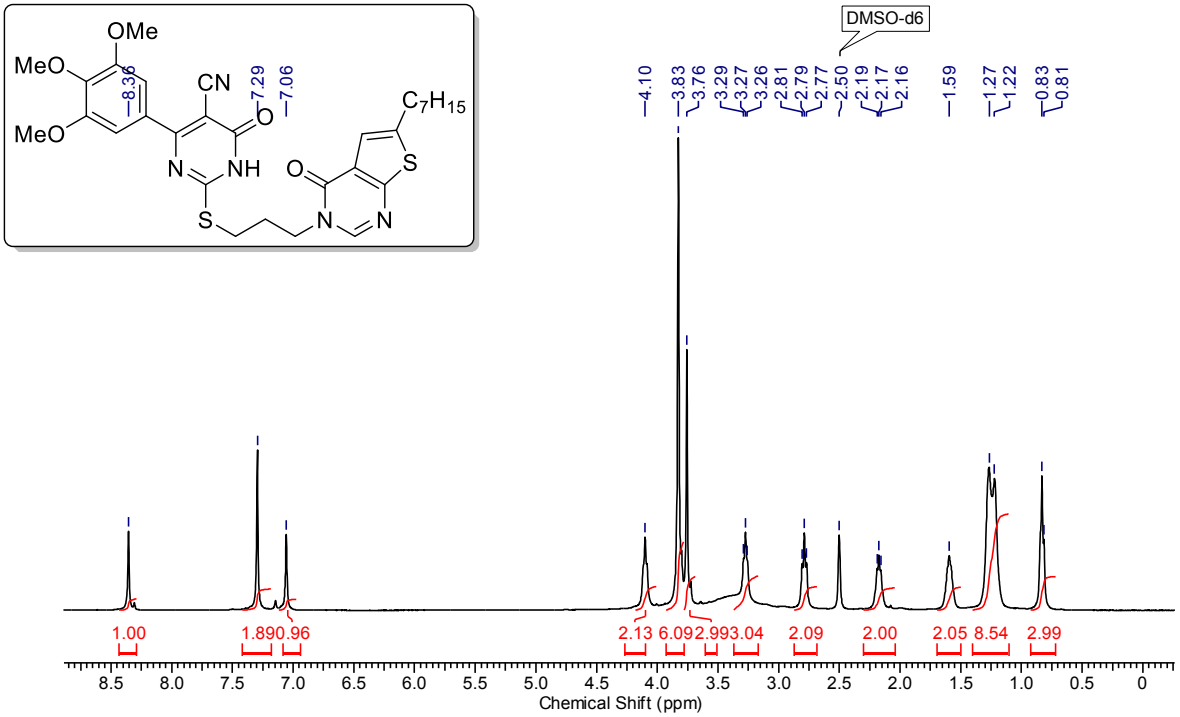
^{13}C NMR (100 MHz, $CDCl_3$) of compound 13



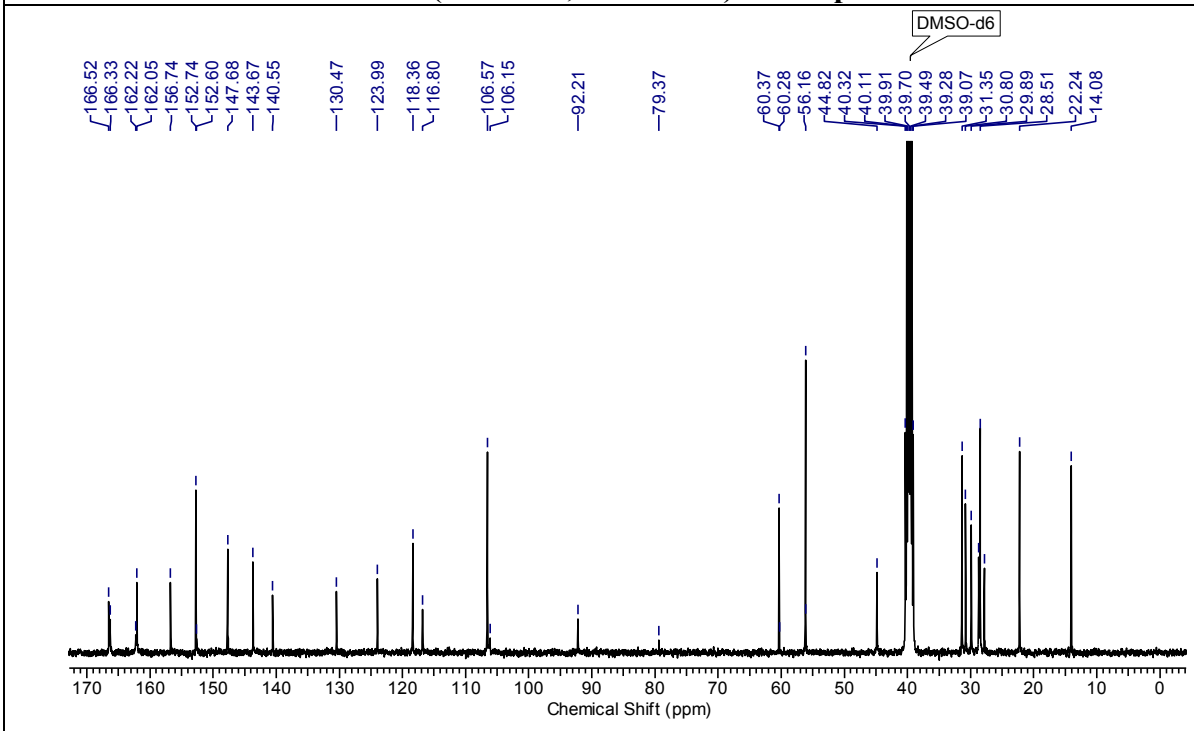
^{13}C NMR DEPT (100 MHz, $CDCl_3$) of compound 13



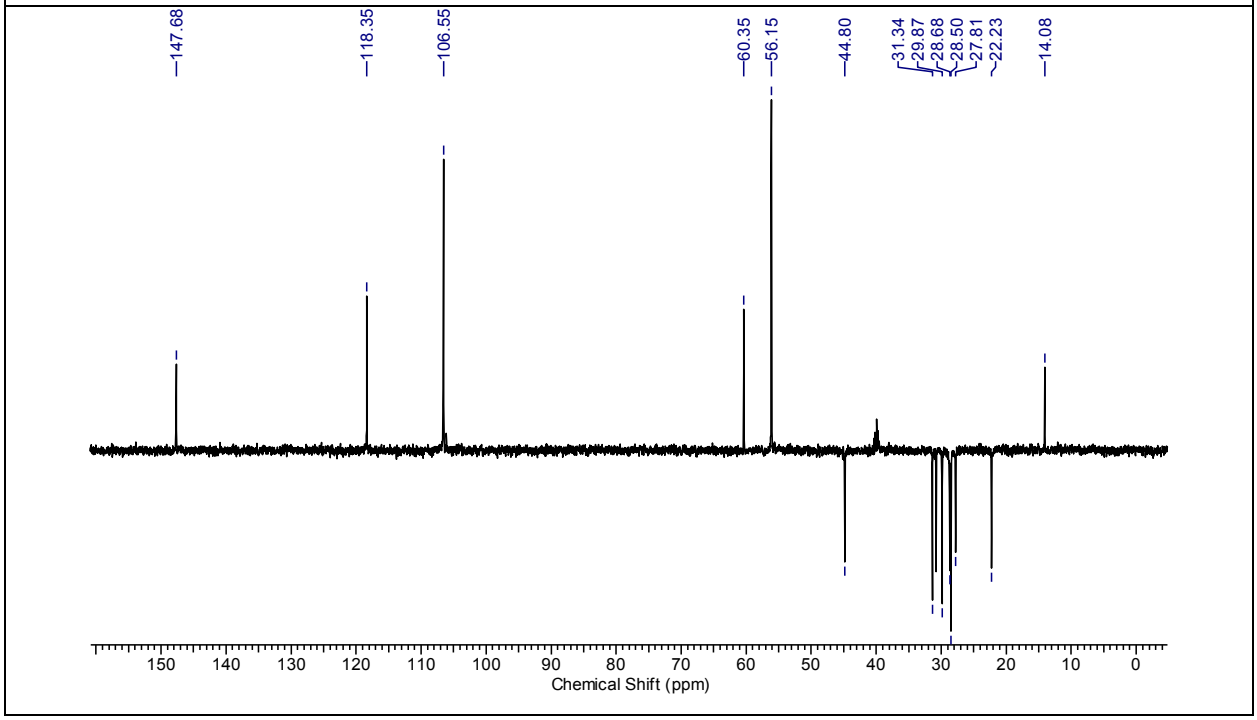
^{1}H NMR (400 MHz, DMSO-d6) of compound 14



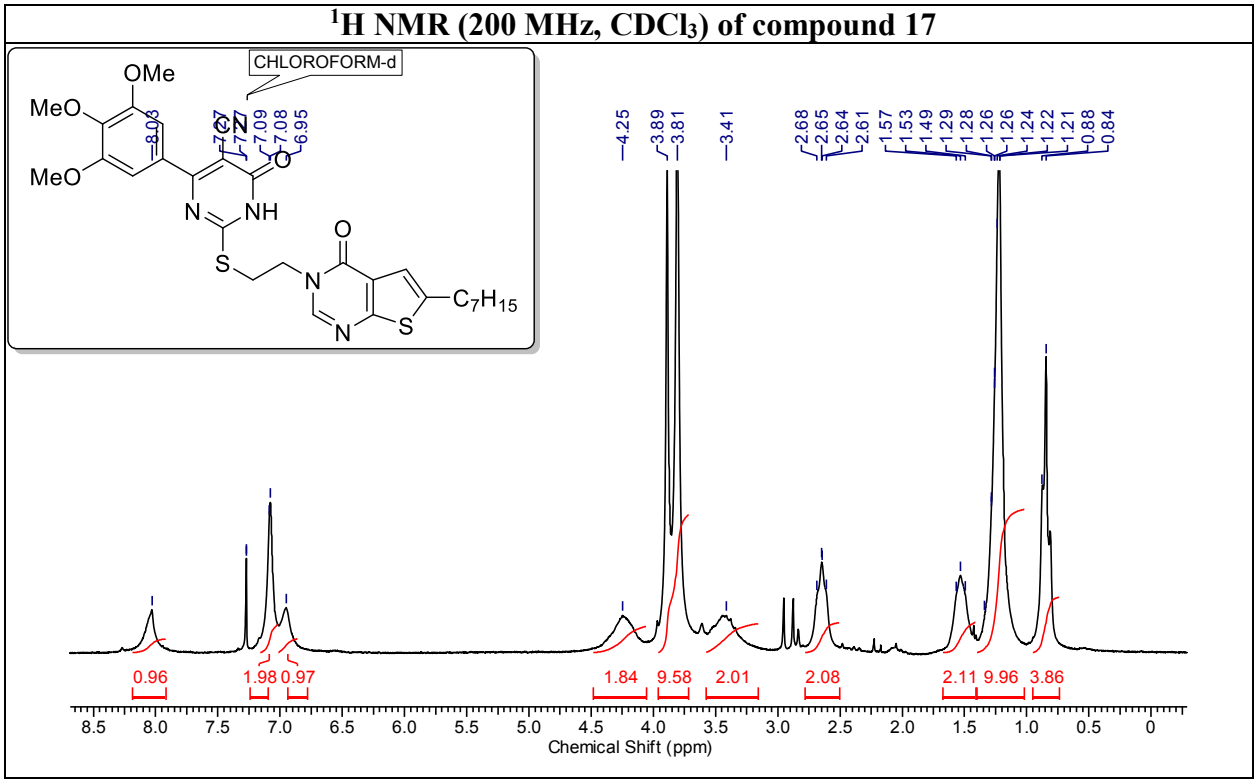
^{13}C NMR (100 MHz, DMSO-d6) of compound 14



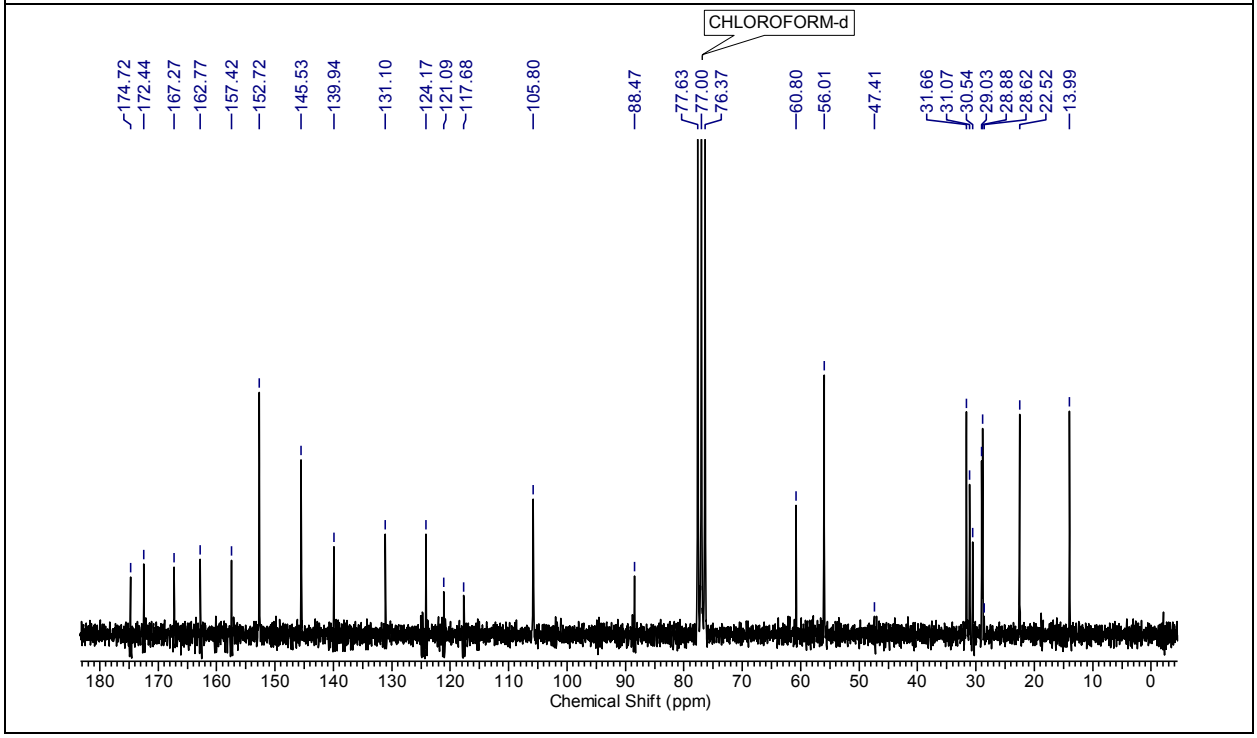
^{13}C NMR DEPT (100 MHz, DMSO-d6) of compound 14



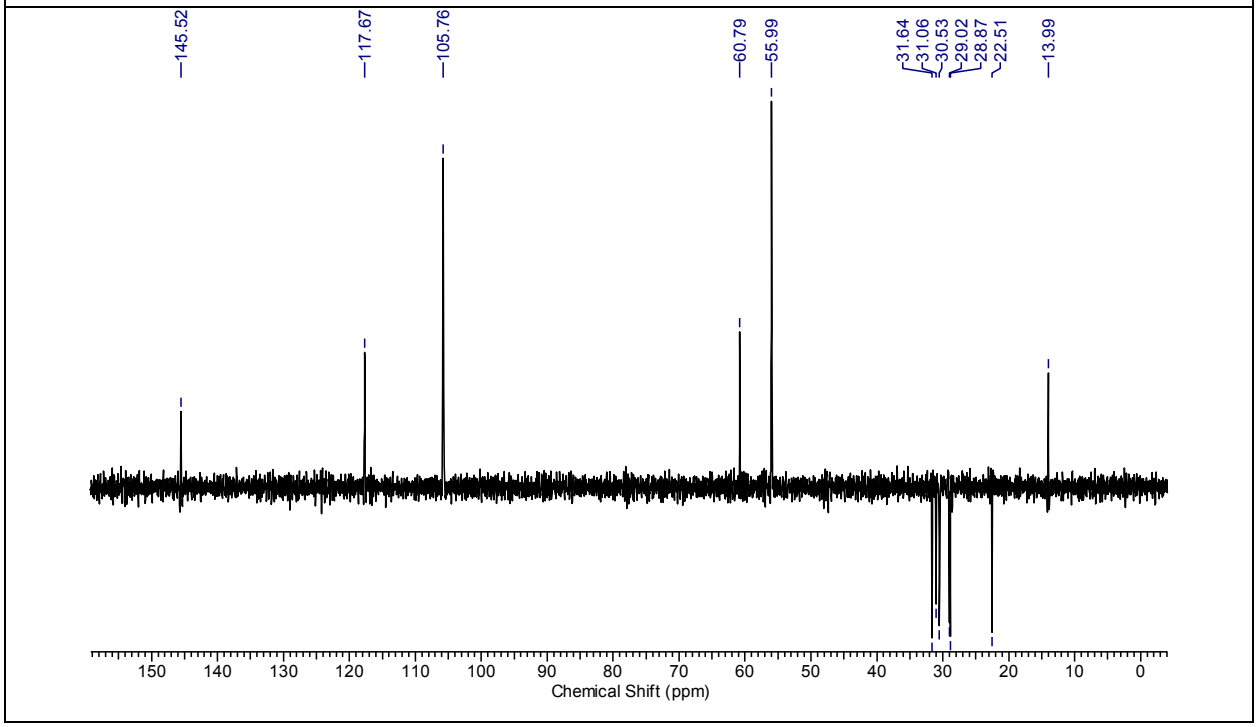
^{1}H NMR (200 MHz, $CDCl_3$) of compound 17



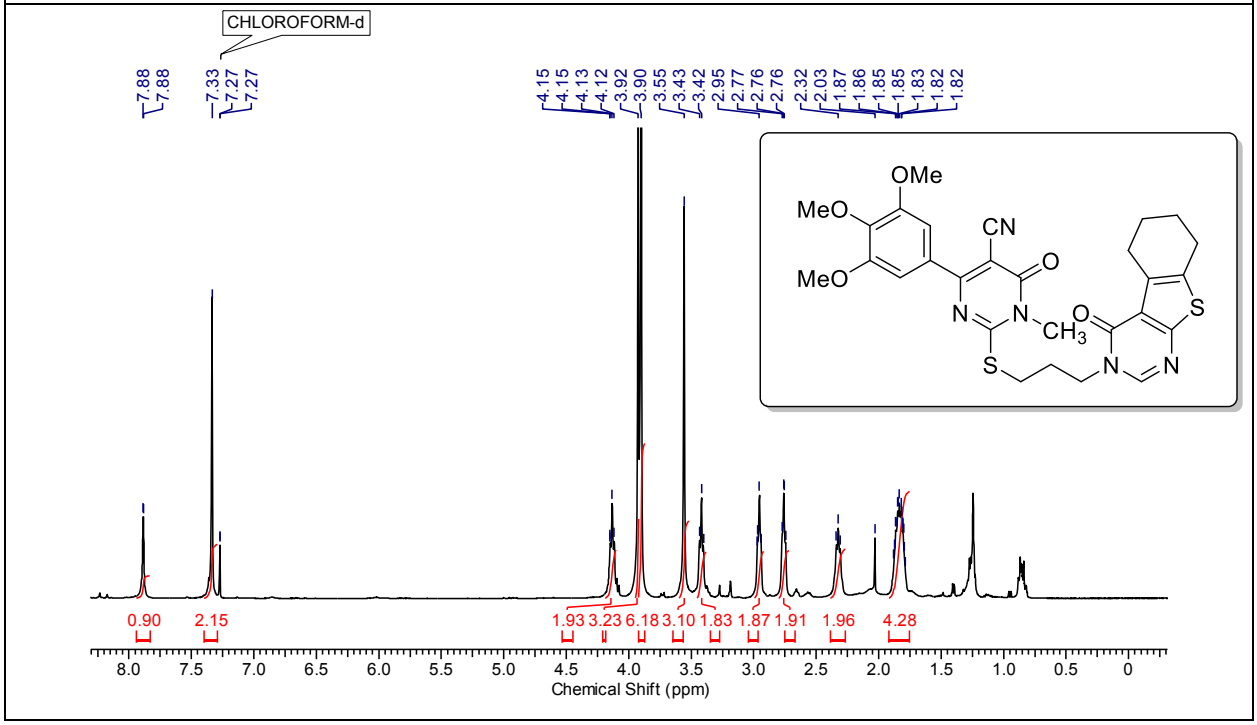
^{13}C NMR (50 MHz, $CDCl_3$) of compound 17



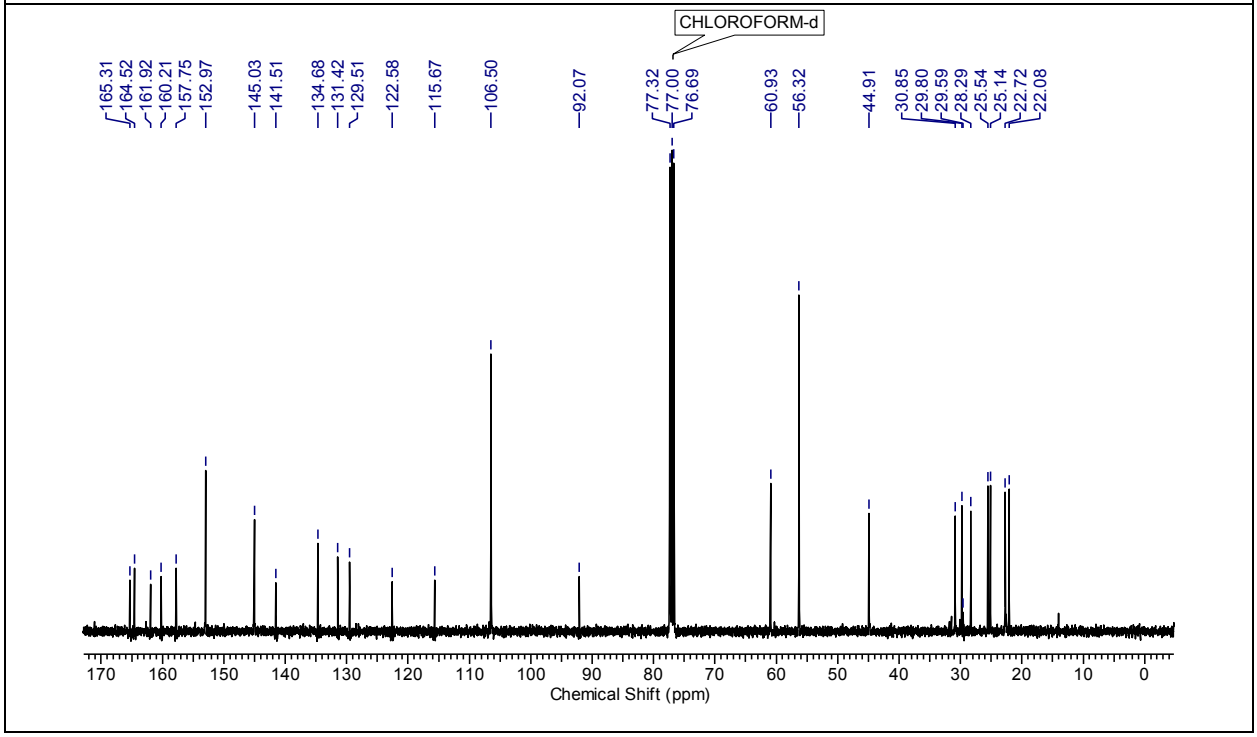
^{13}C NMR (DEPT 50 MHz, $CDCl_3$) of compound 17



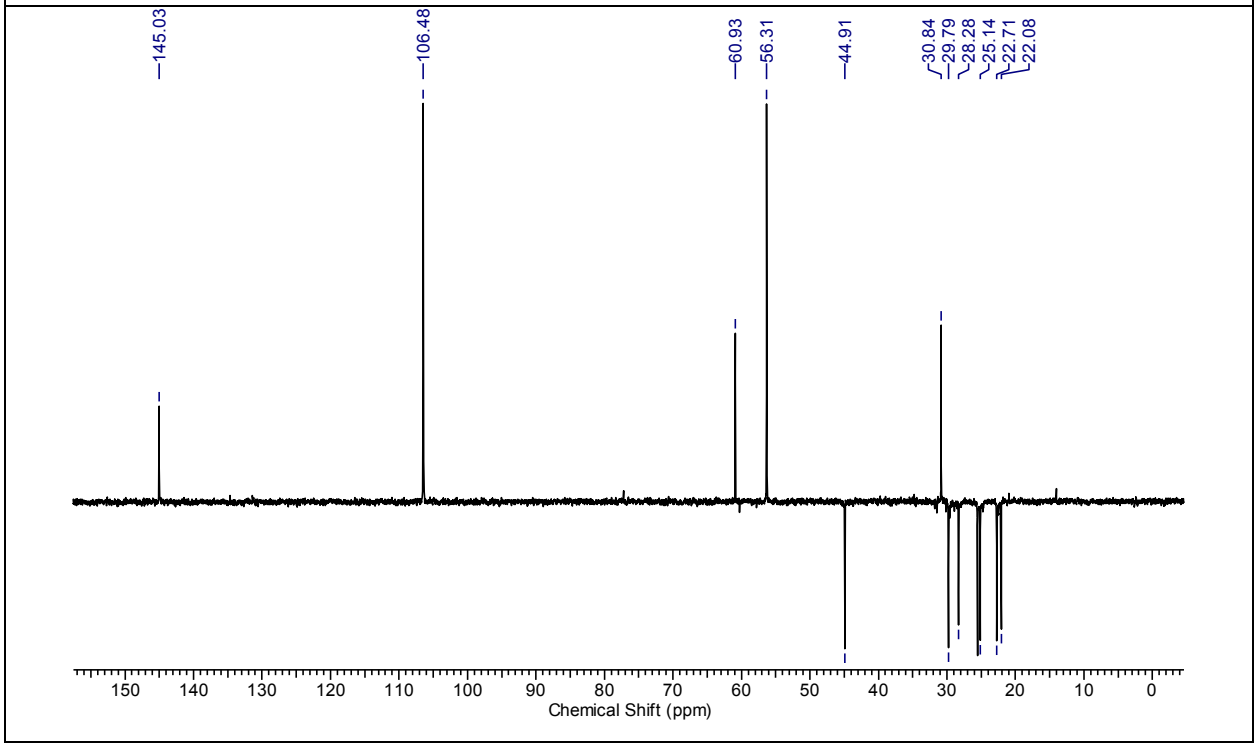
^{1}H NMR (400 MHz, $CDCl_3$) of compound 18



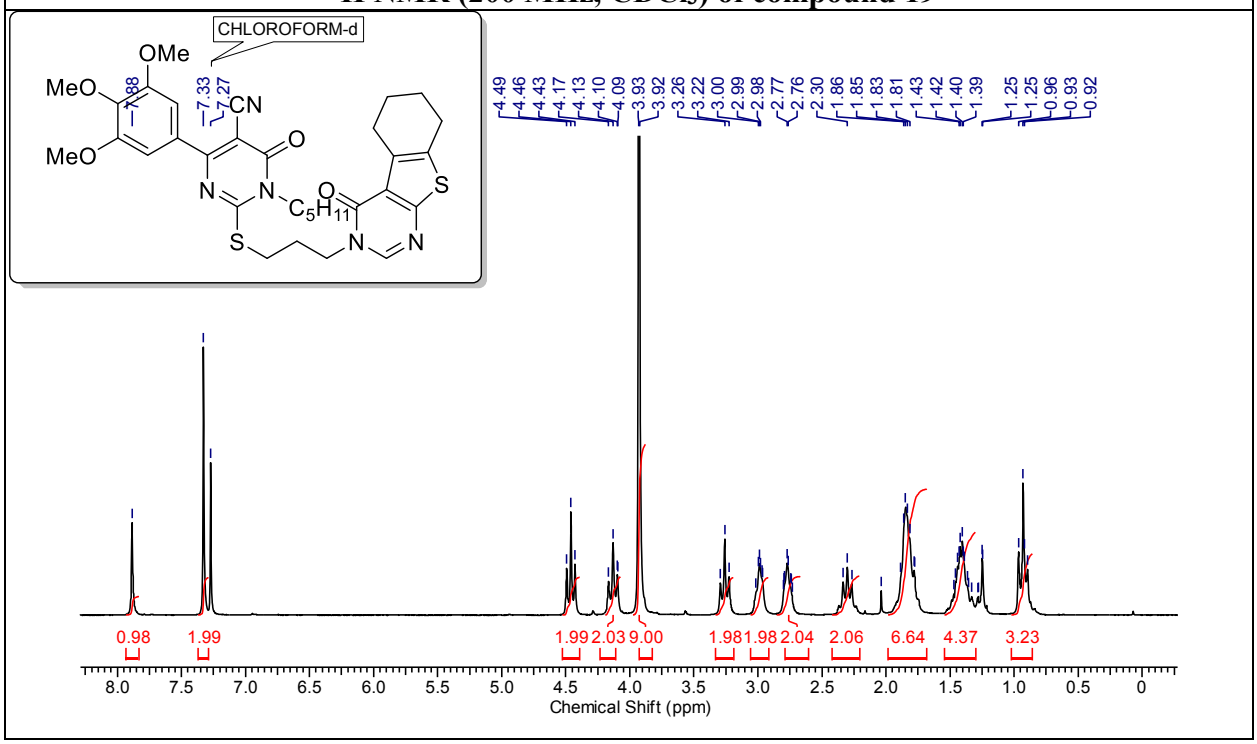
^{13}C NMR (200MHz, $CDCl_3$) of compound 18



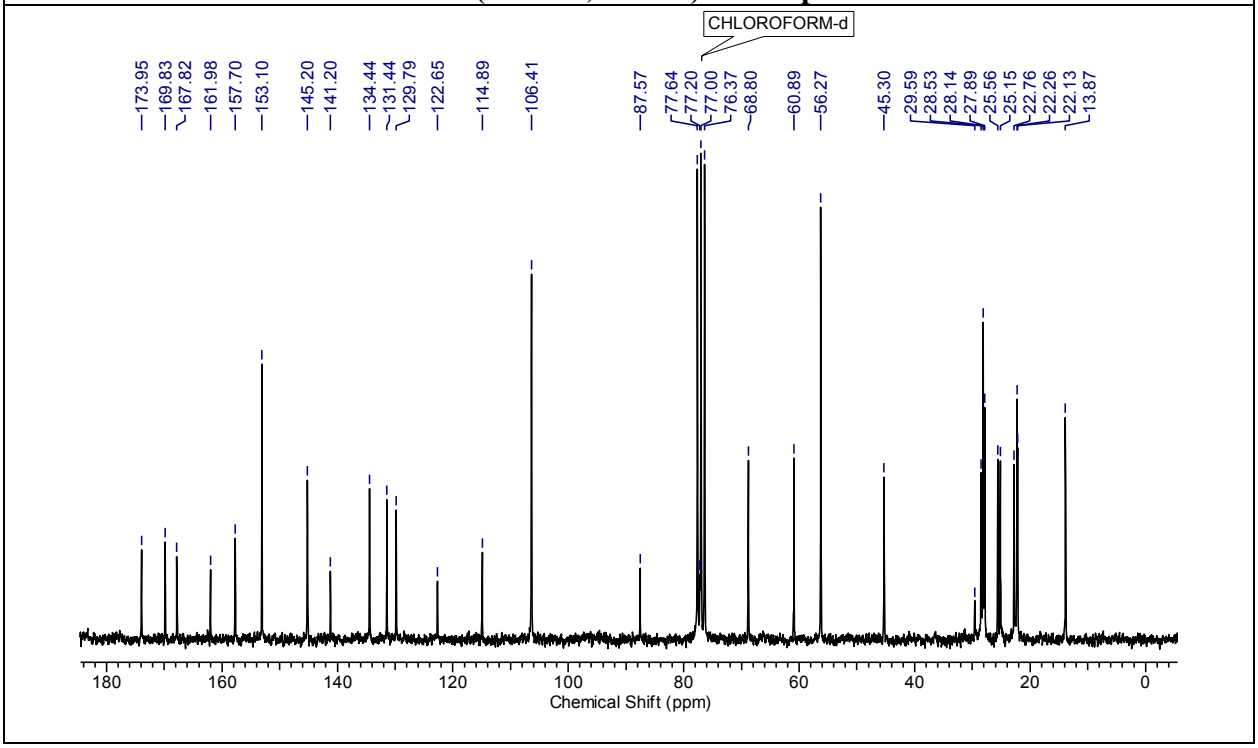
^{13}C NMR DEPT (200MHz, $CDCl_3$) of compound 18



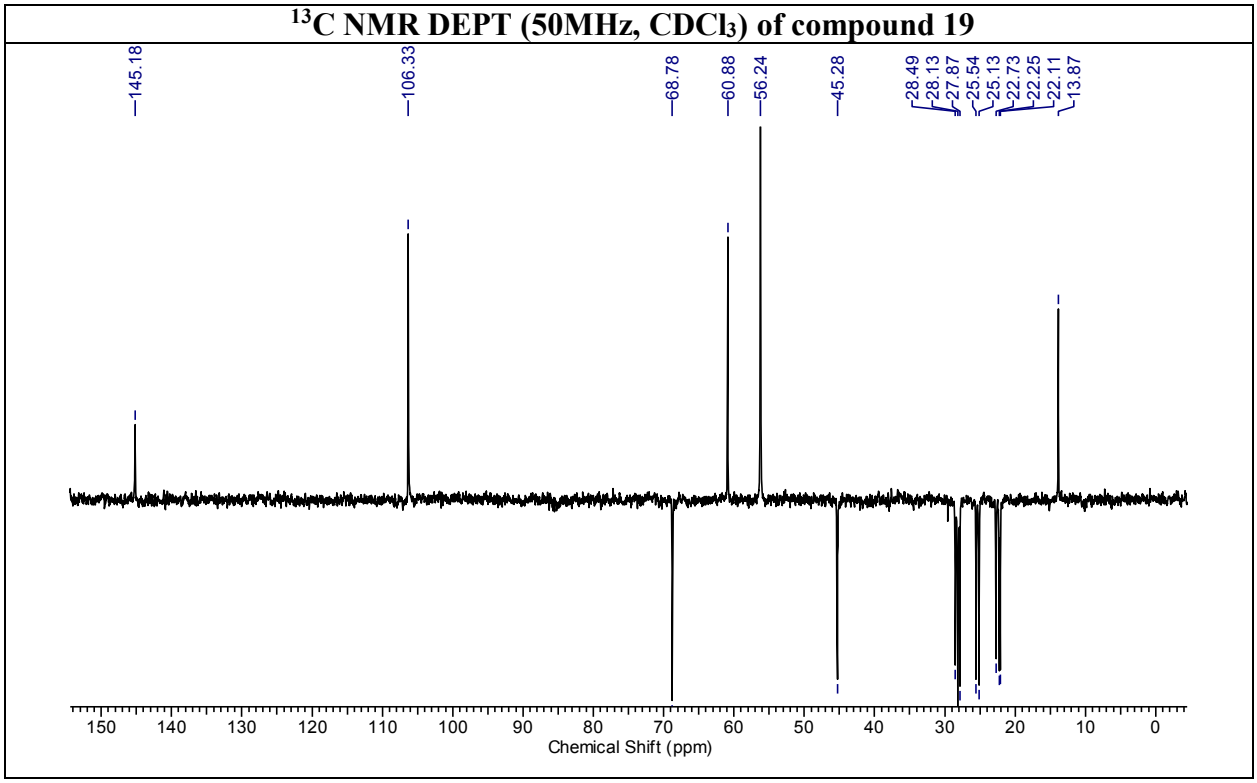
^{1}H NMR (200 MHz, $CDCl_3$) of compound 19



^{13}C NMR (50MHz, $CDCl_3$) of compound 19



^{13}C NMR DEPT (50MHz, $CDCl_3$) of compound 19



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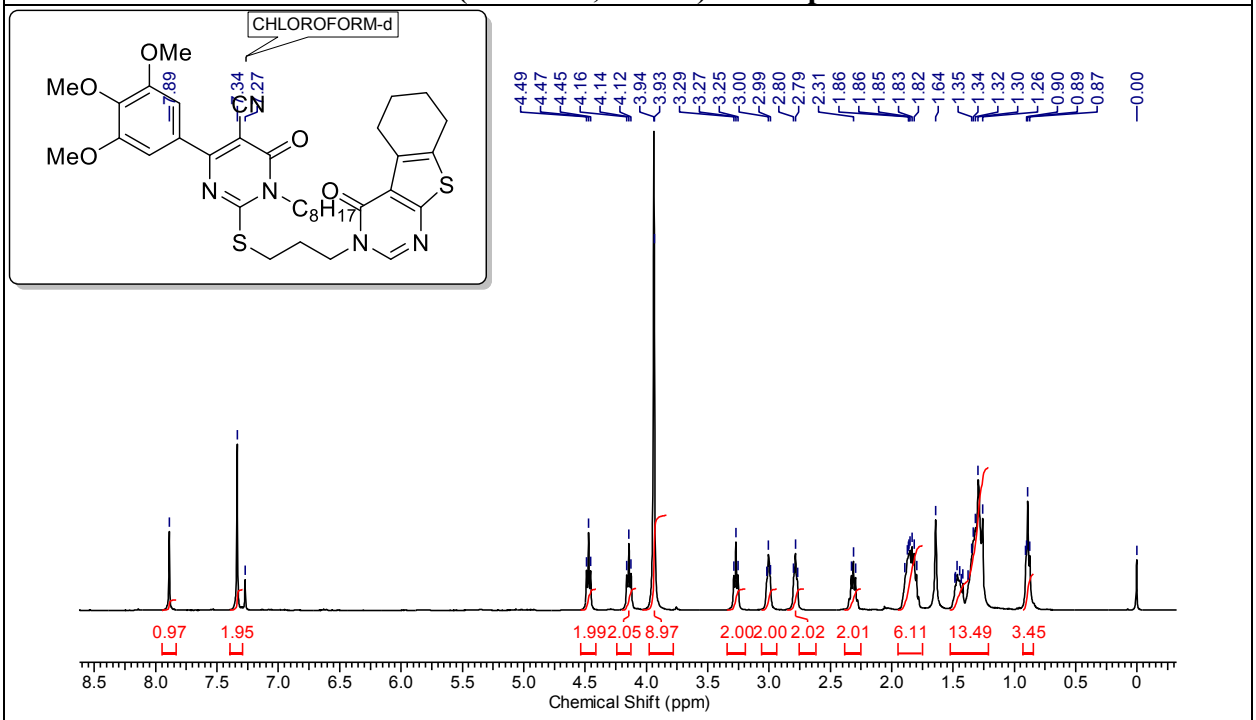

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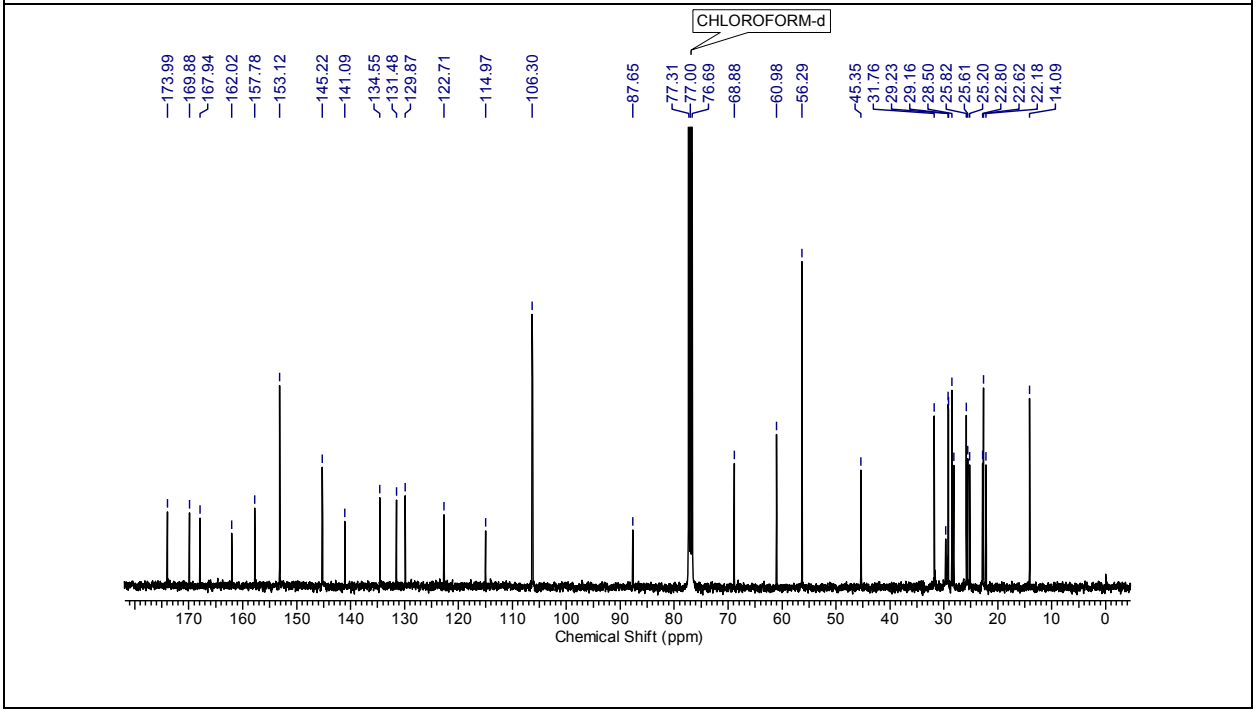

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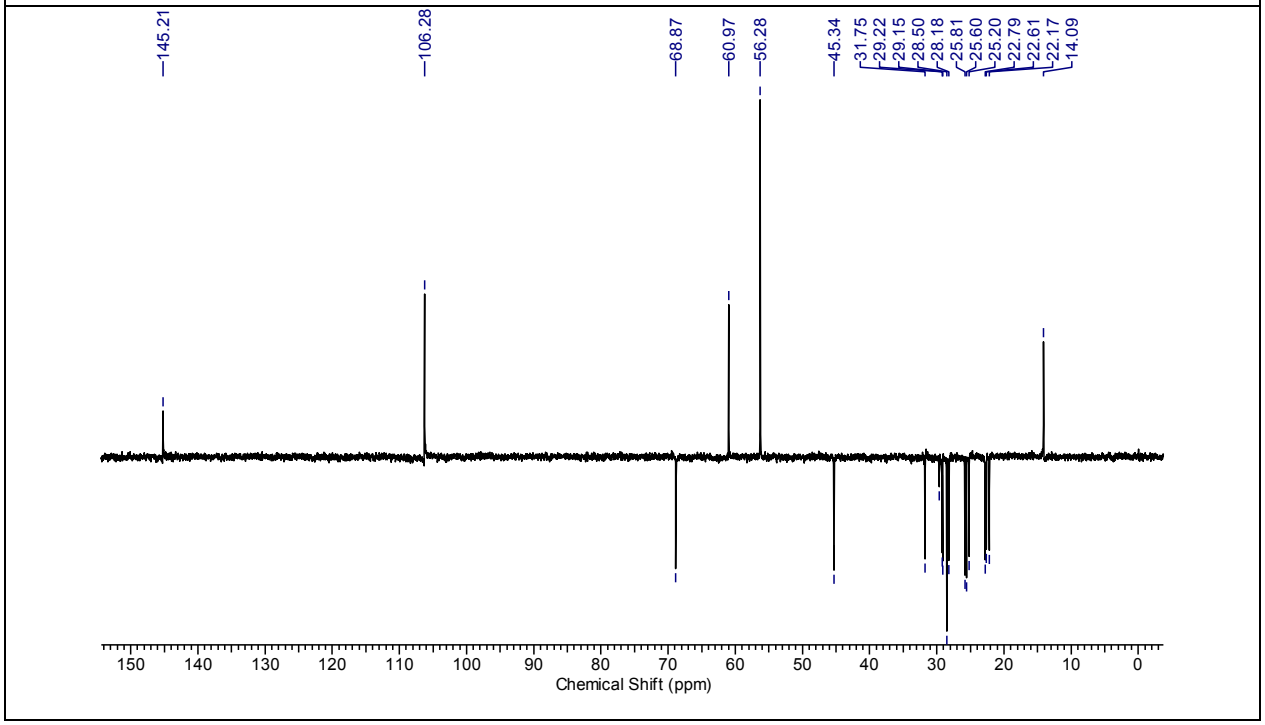

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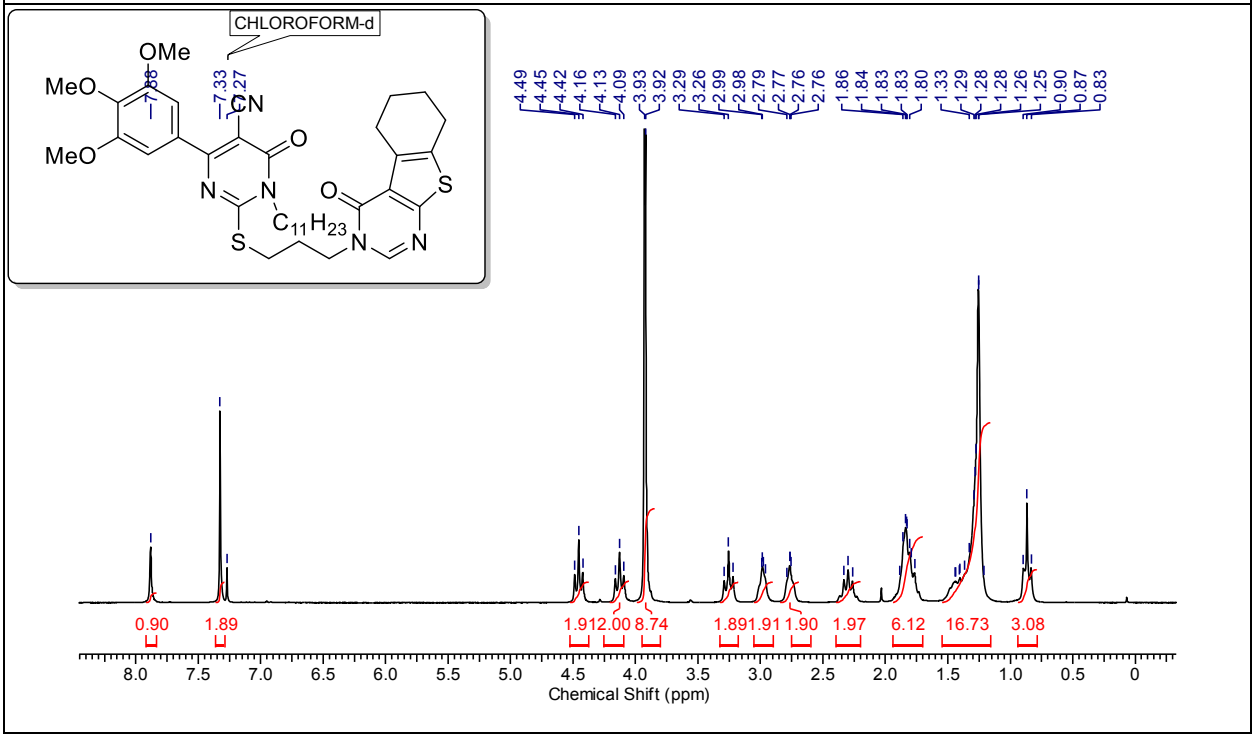

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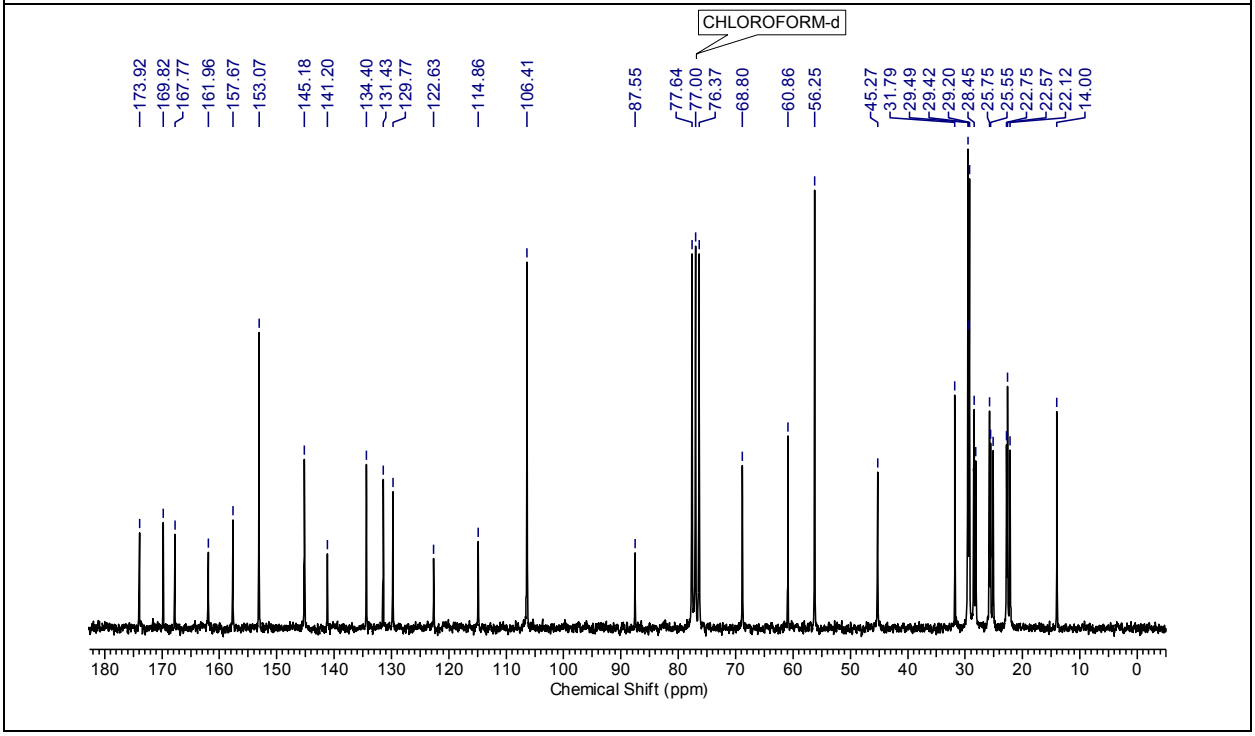

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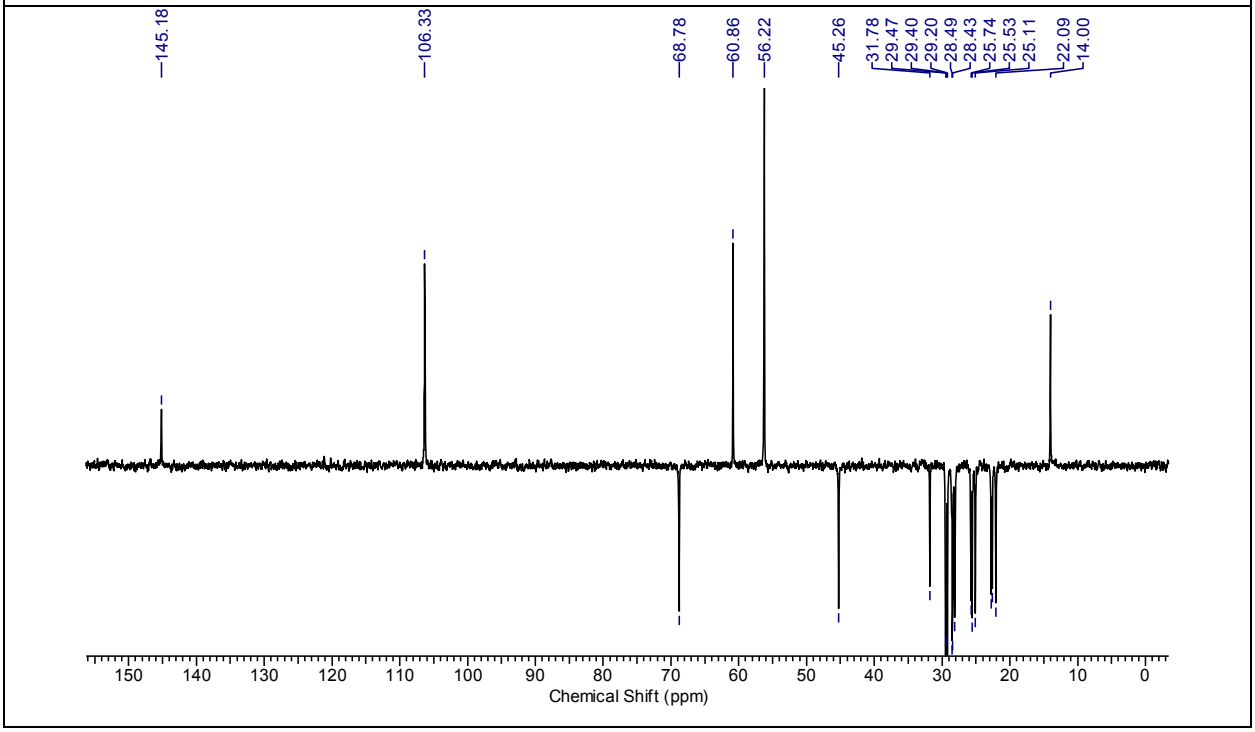

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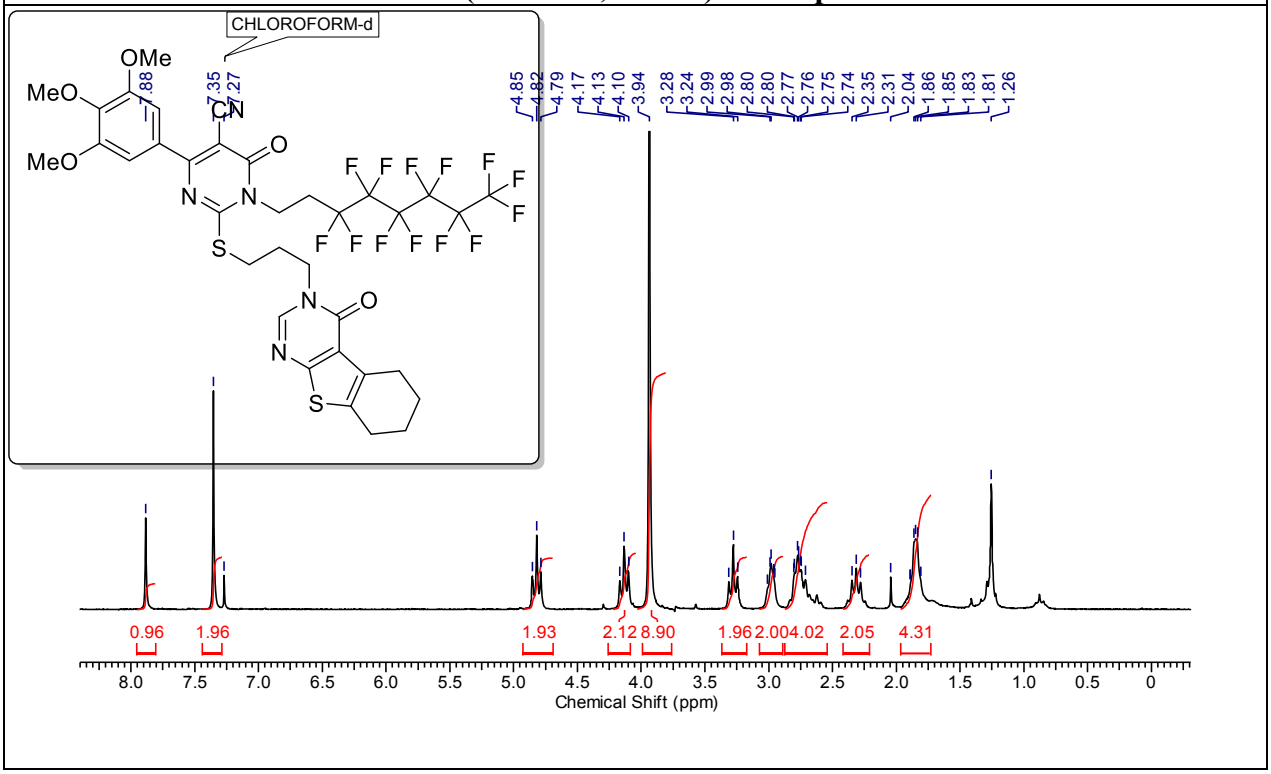

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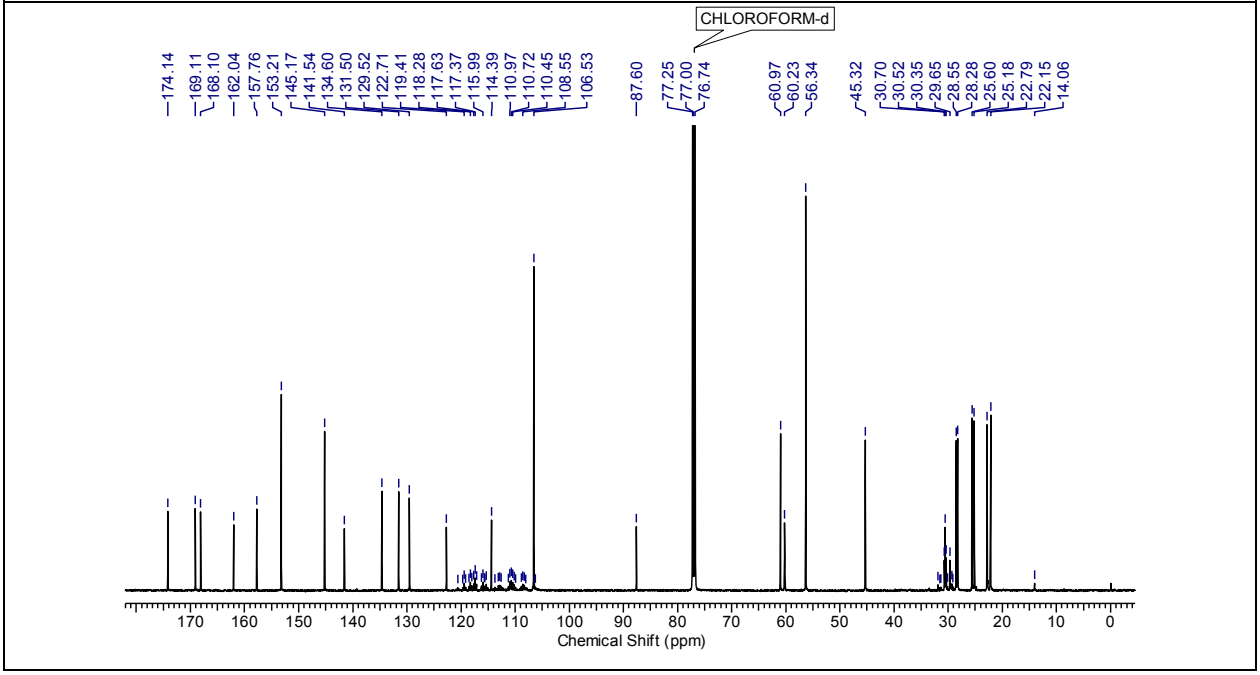

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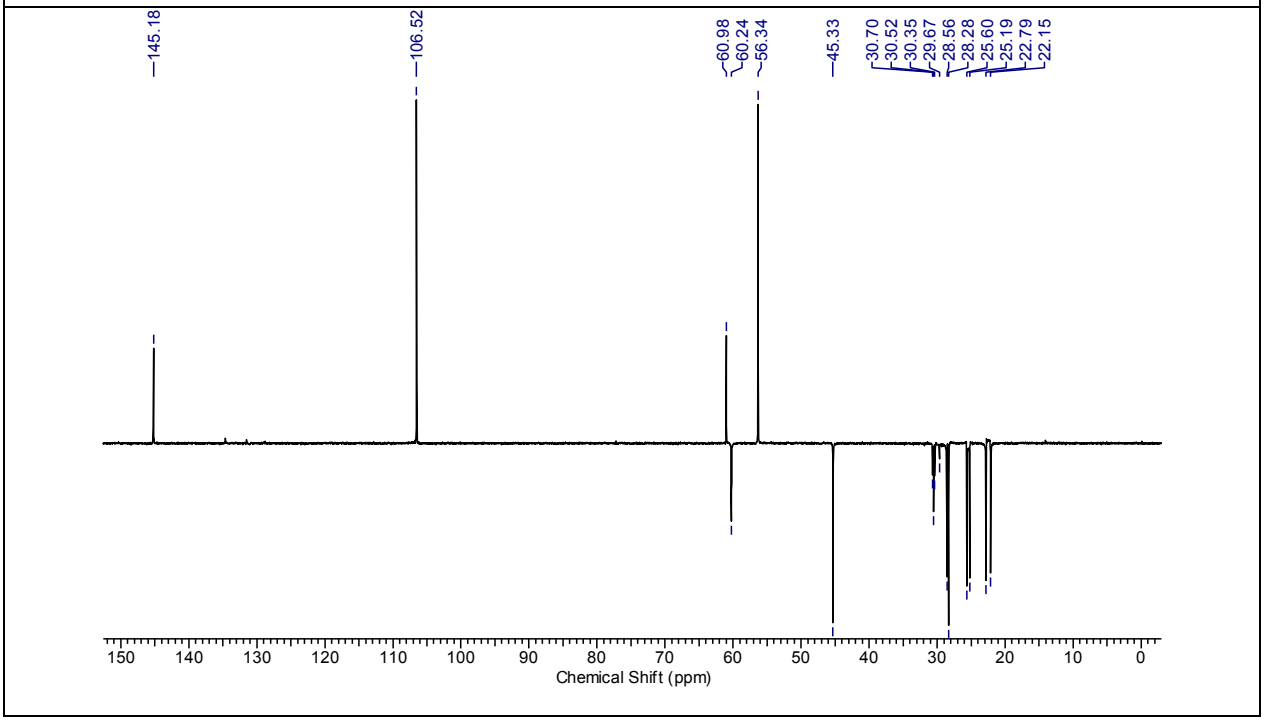

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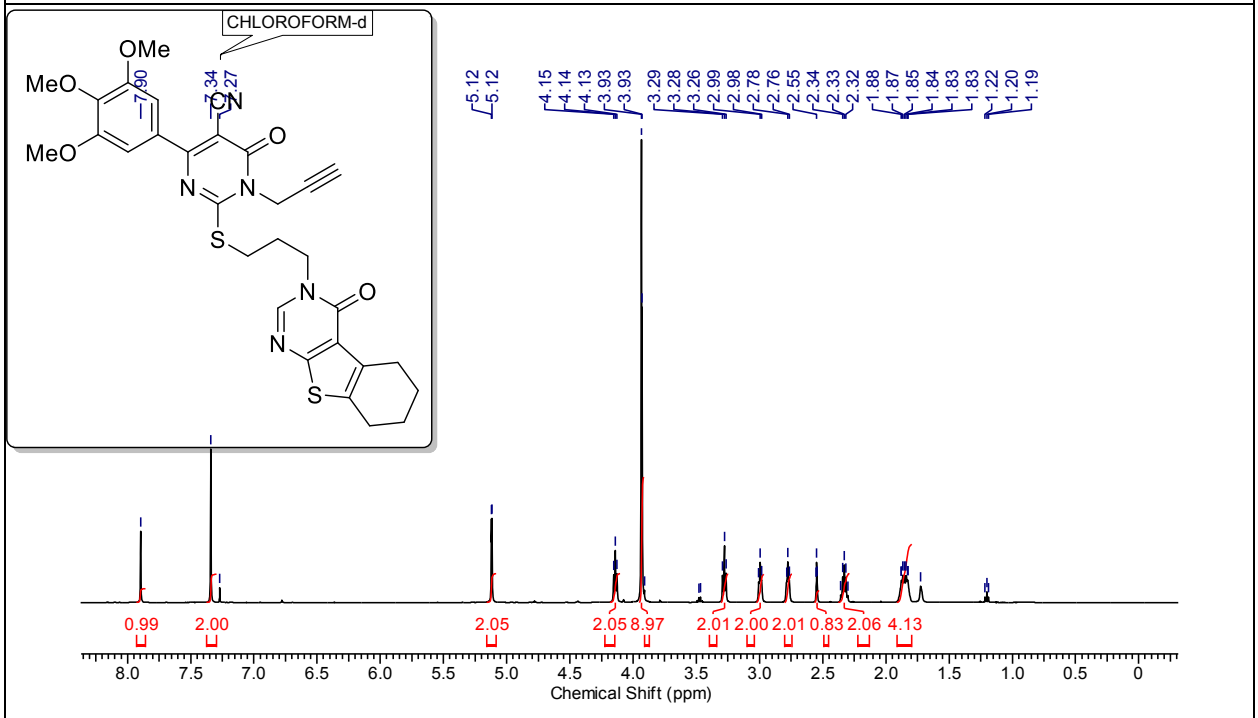

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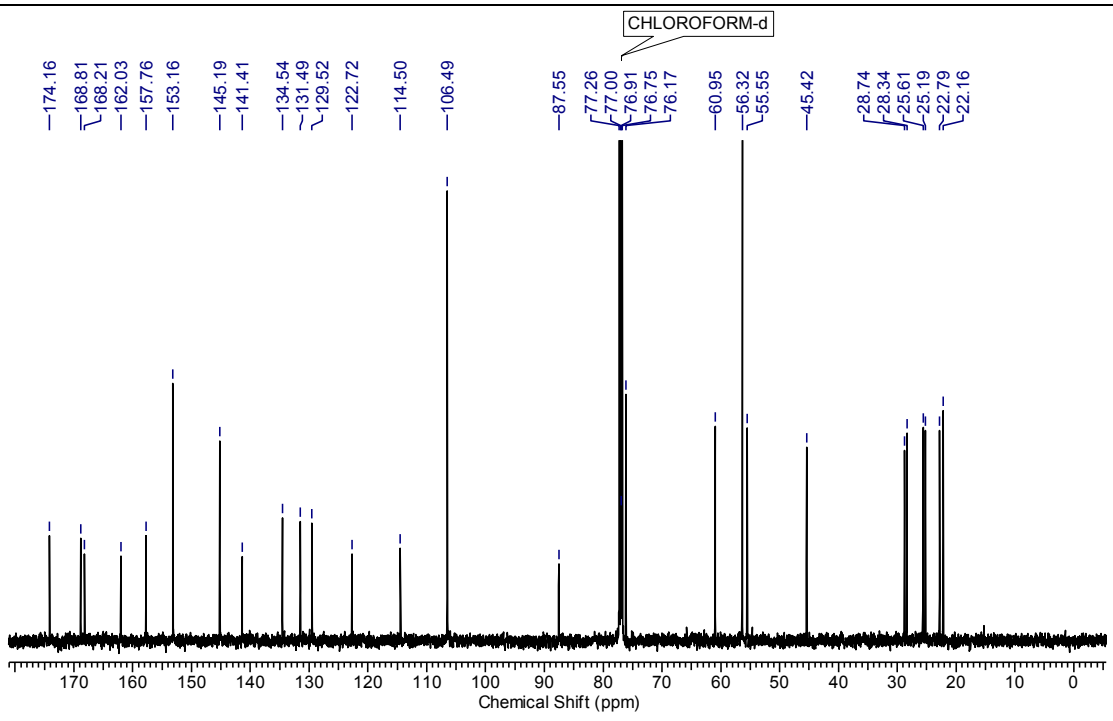
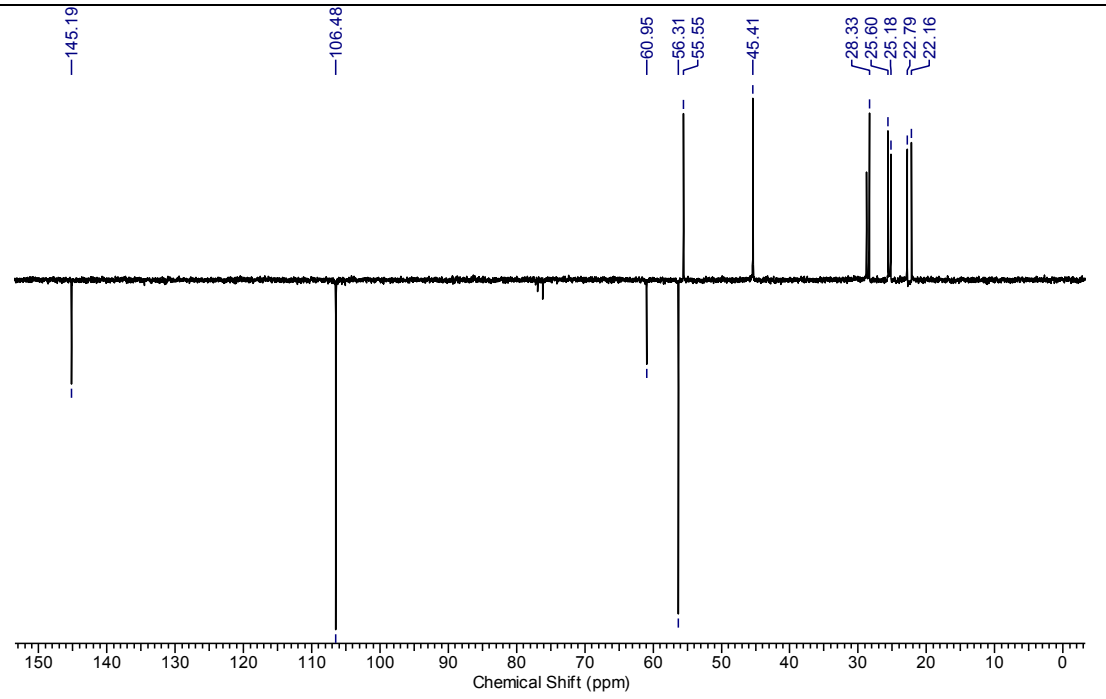
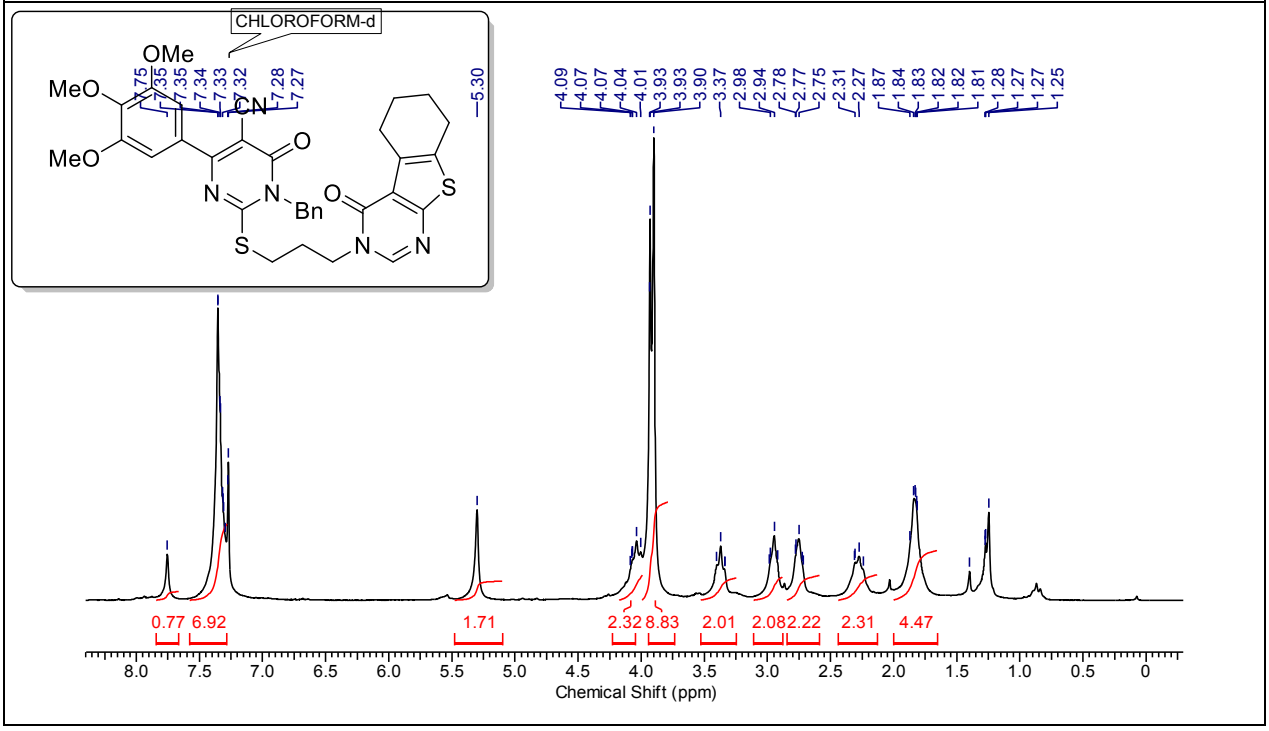


^{13}C NMR DEPT (125 MHz, $CDCl_3$) of compound 23



^{1}H NMR (200 MHz, $CDCl_3$) of compound 24



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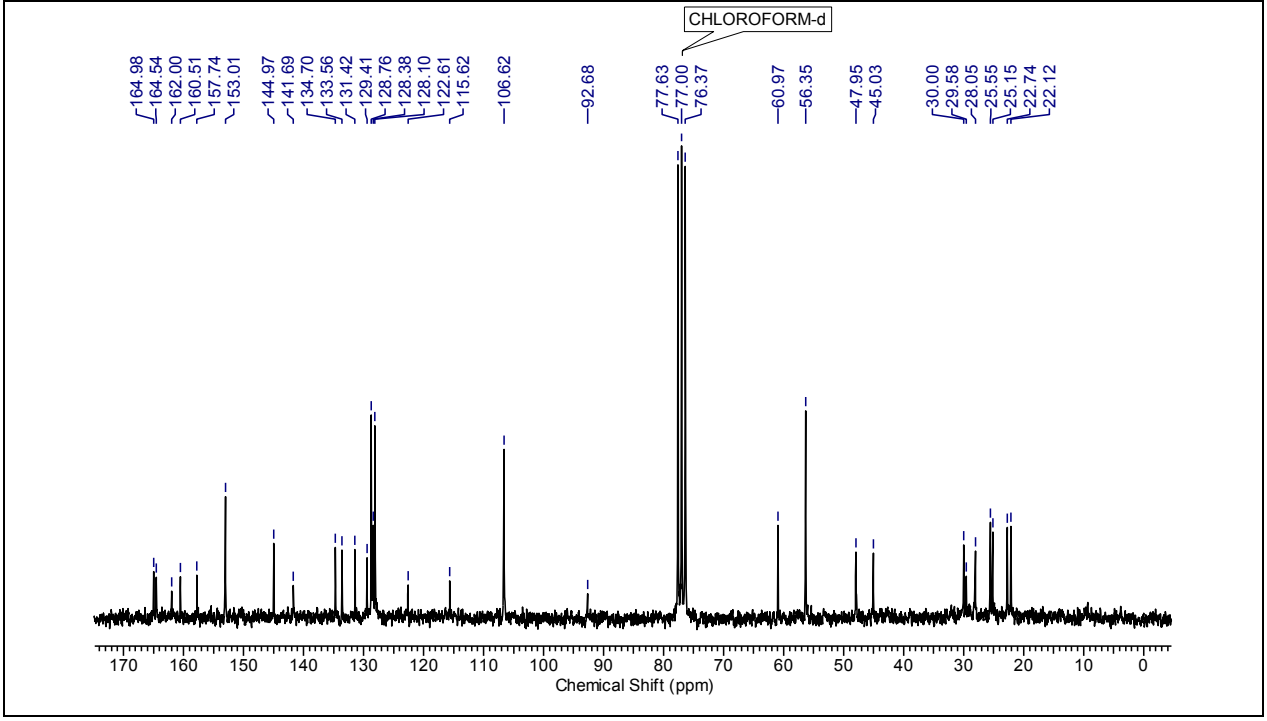

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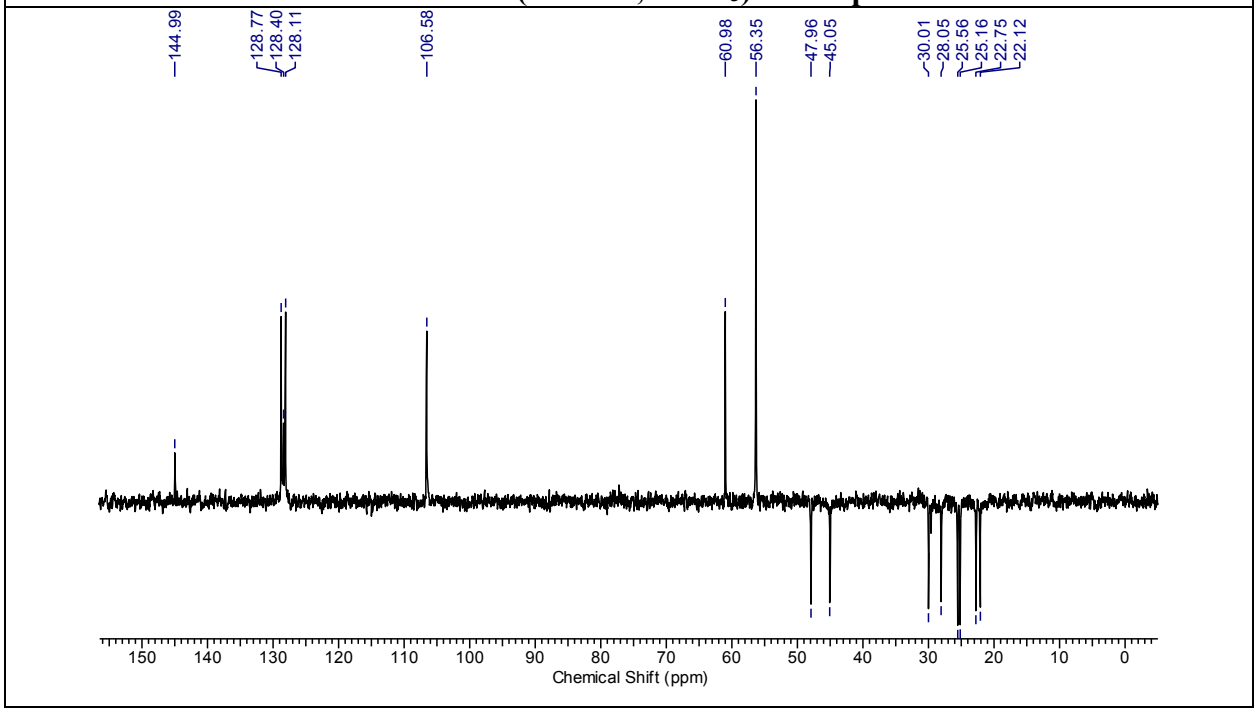

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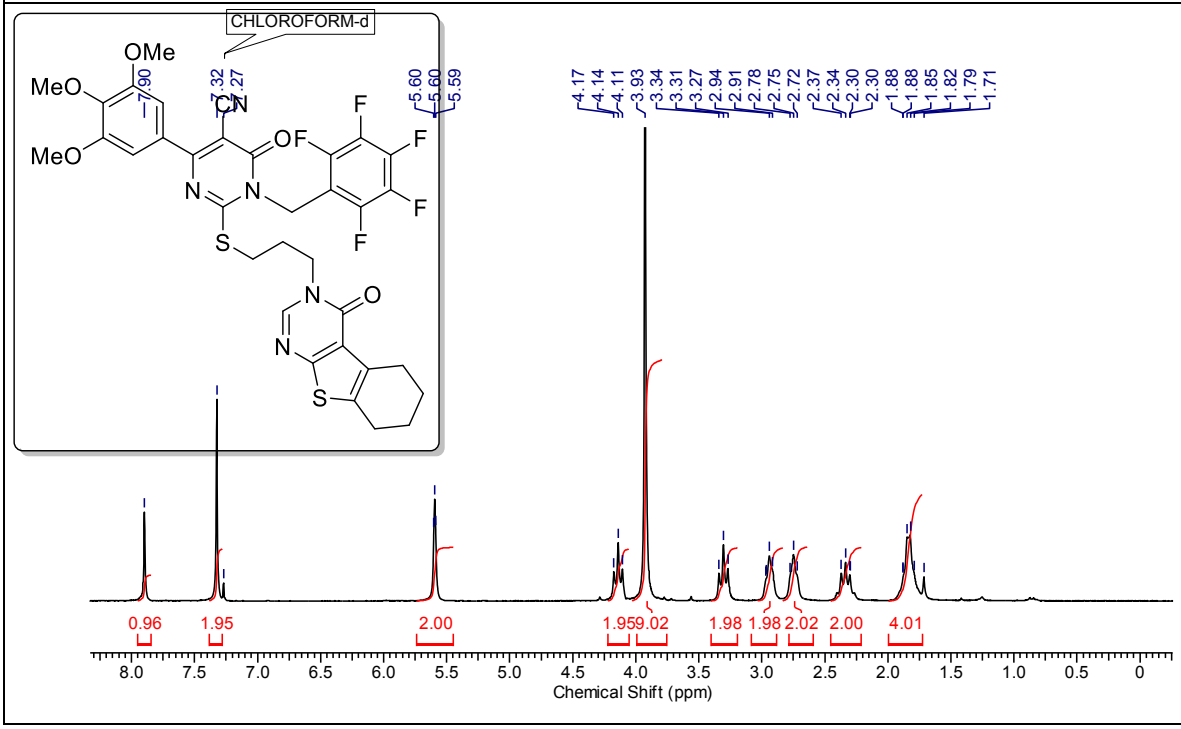
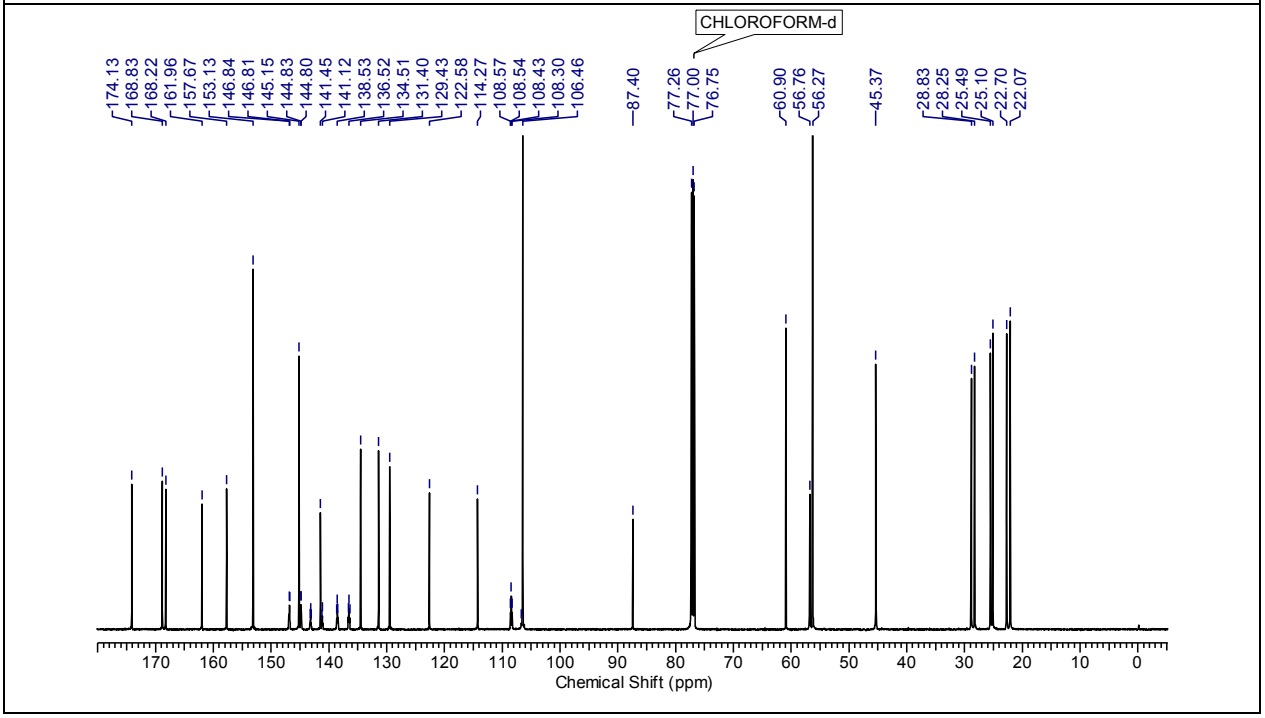


^{13}C NMR (125 MHz, $CDCl_3$) of compound 25



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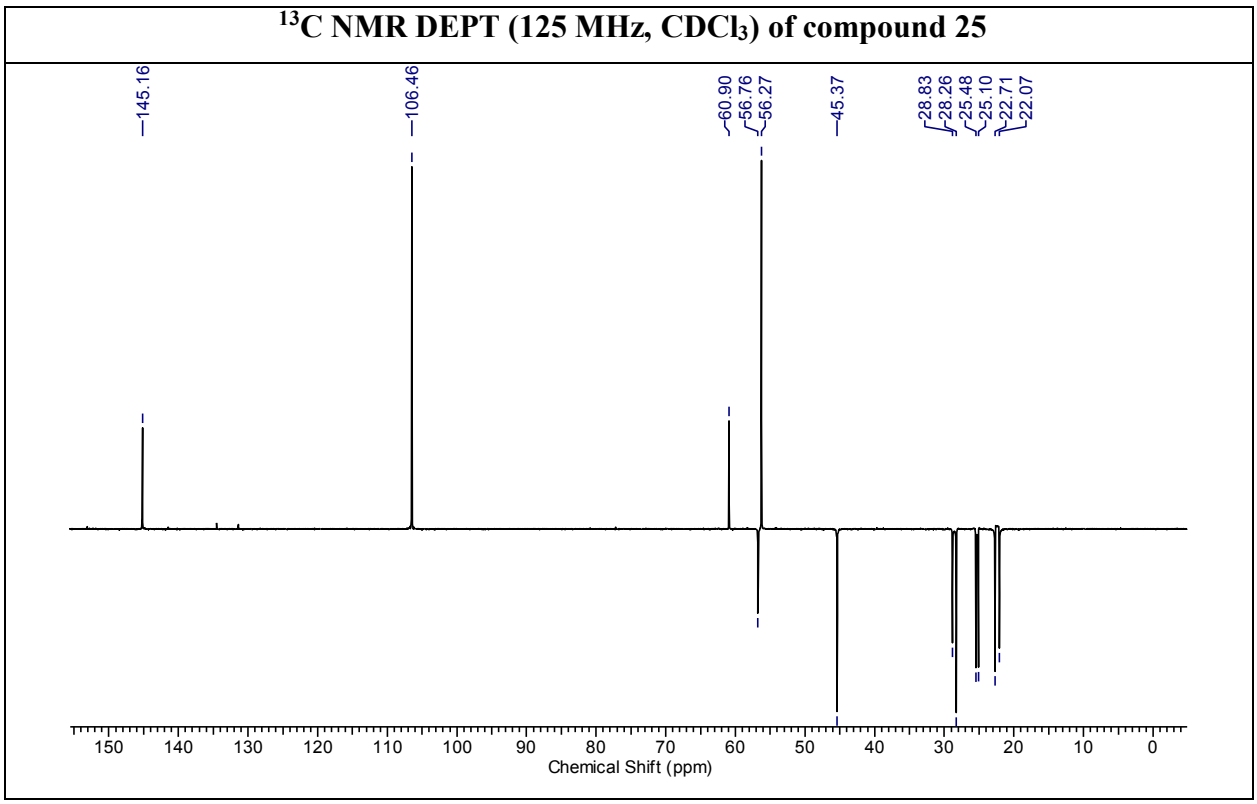
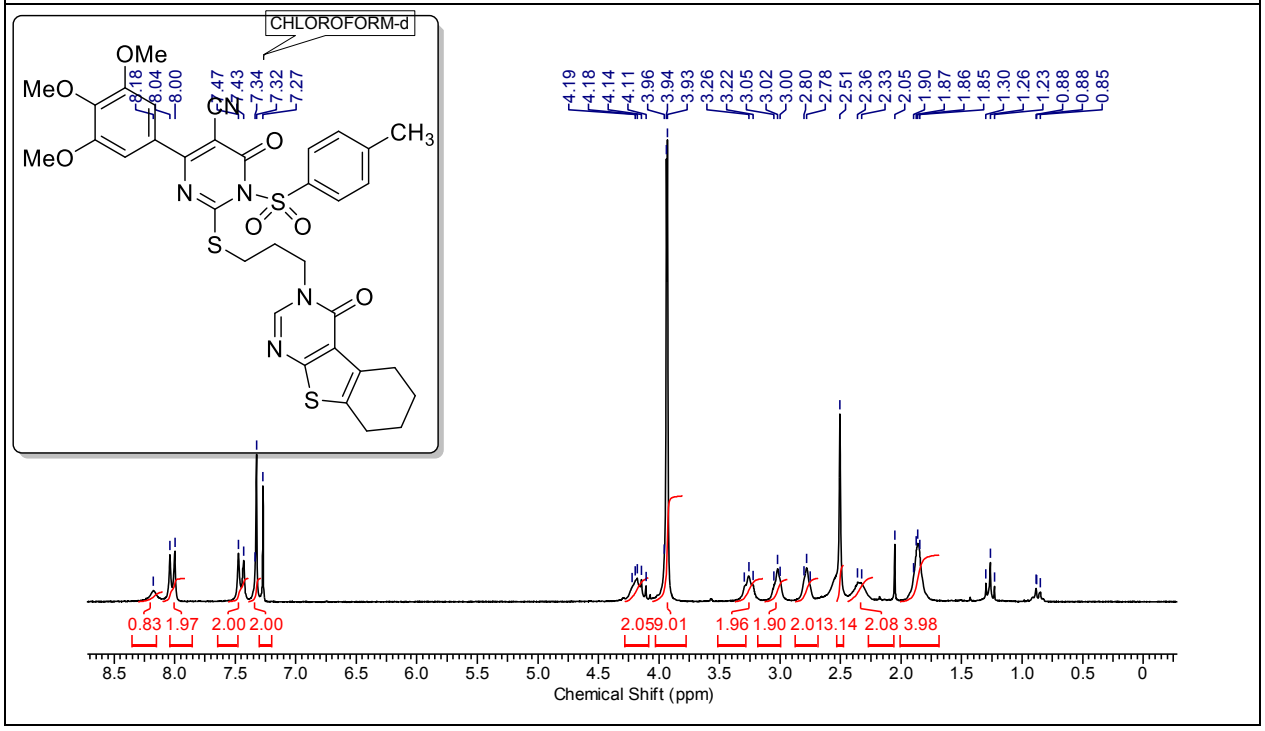
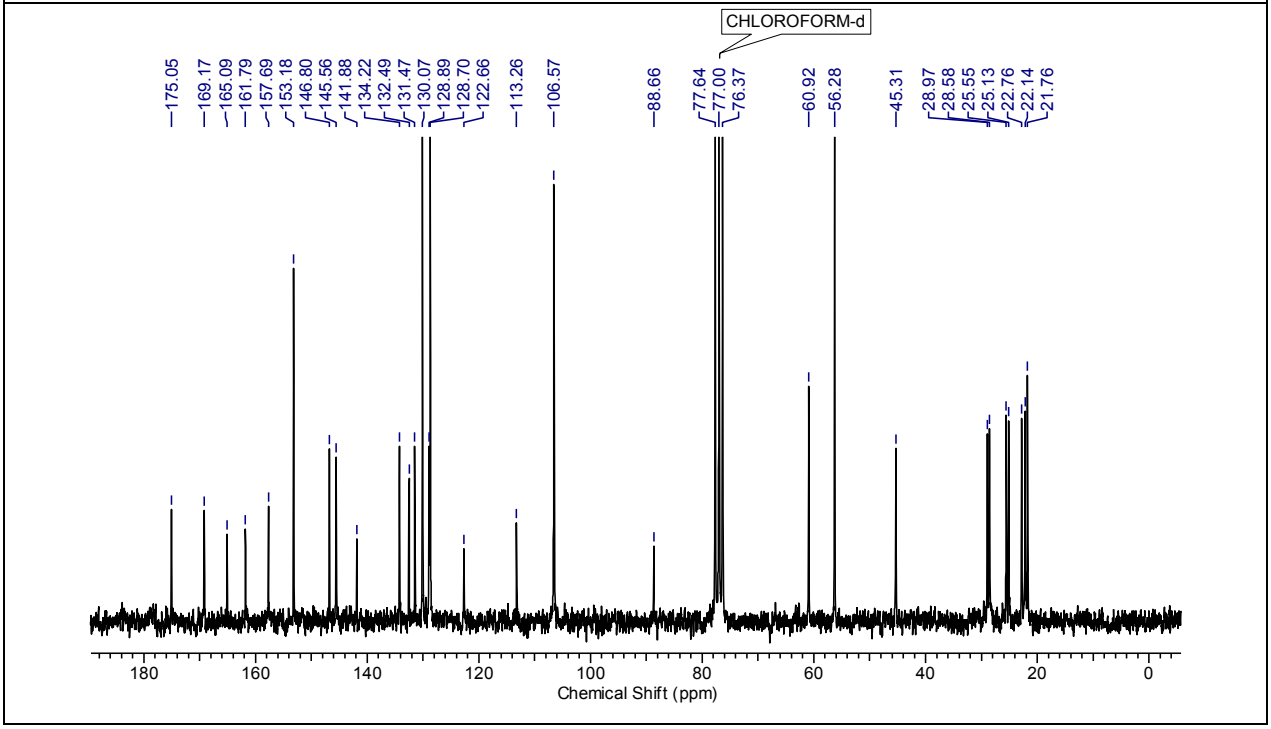


^{1}H NMR (200 MHz, $CDCl_3$) of compound 26



^{13}C NMR (50 MHz, $CDCl_3$) of compound 26



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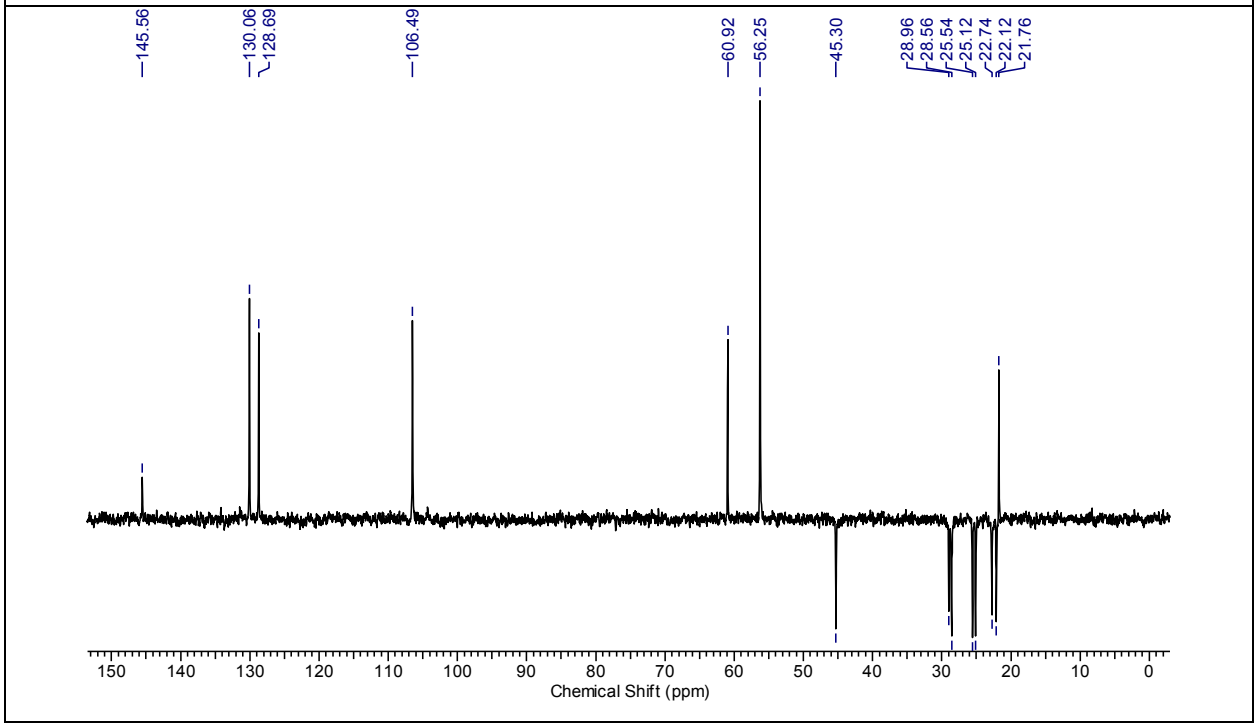

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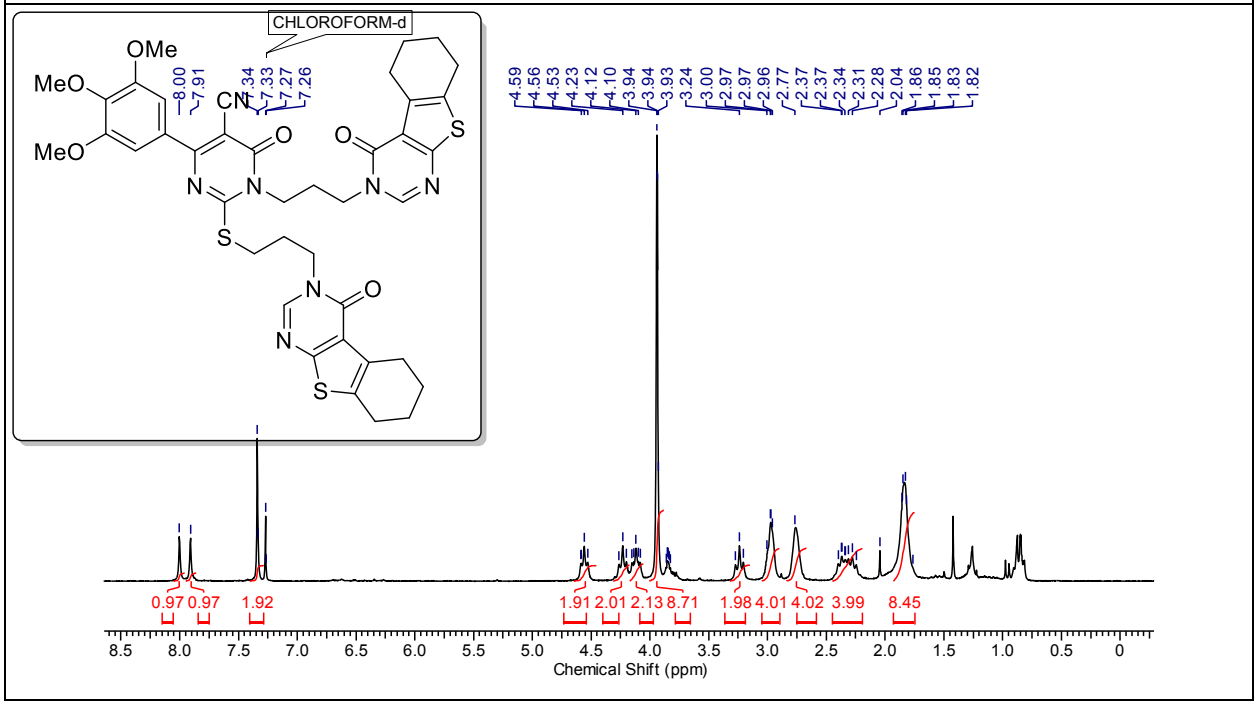
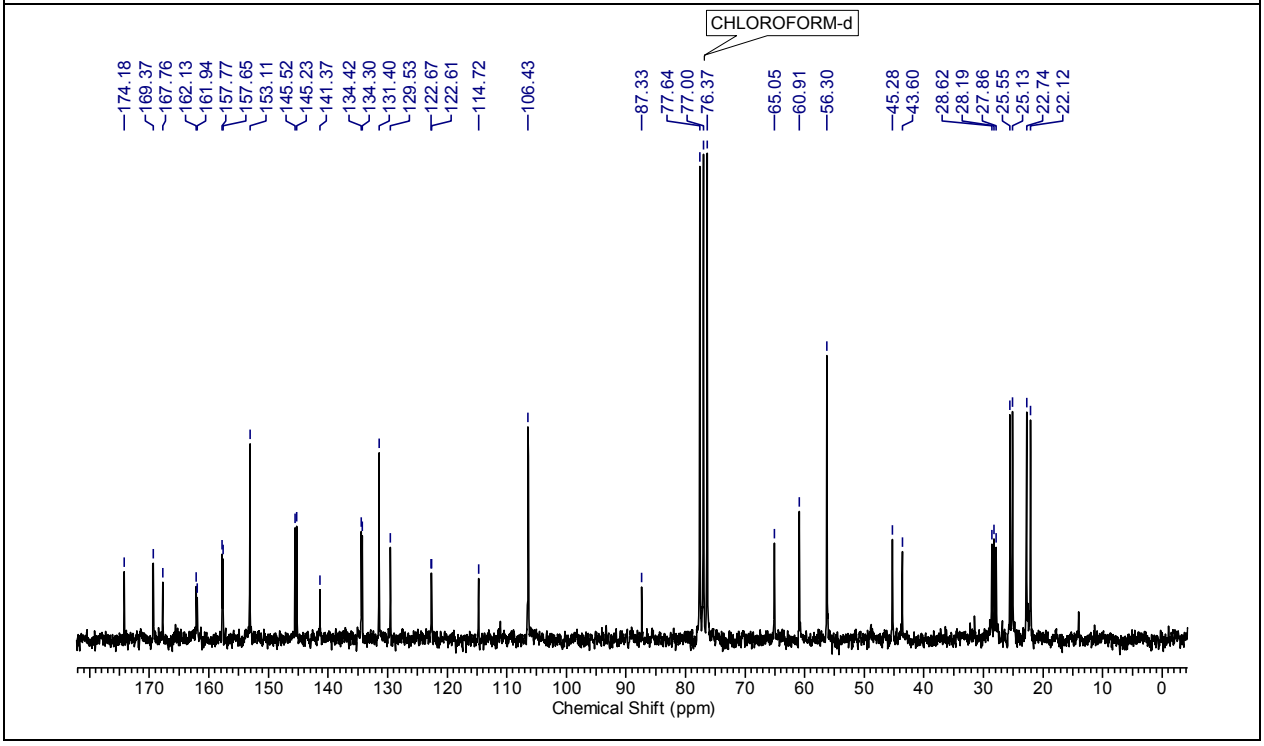


^{13}C NMR (50 MHz, $CDCl_3$) of compound 27



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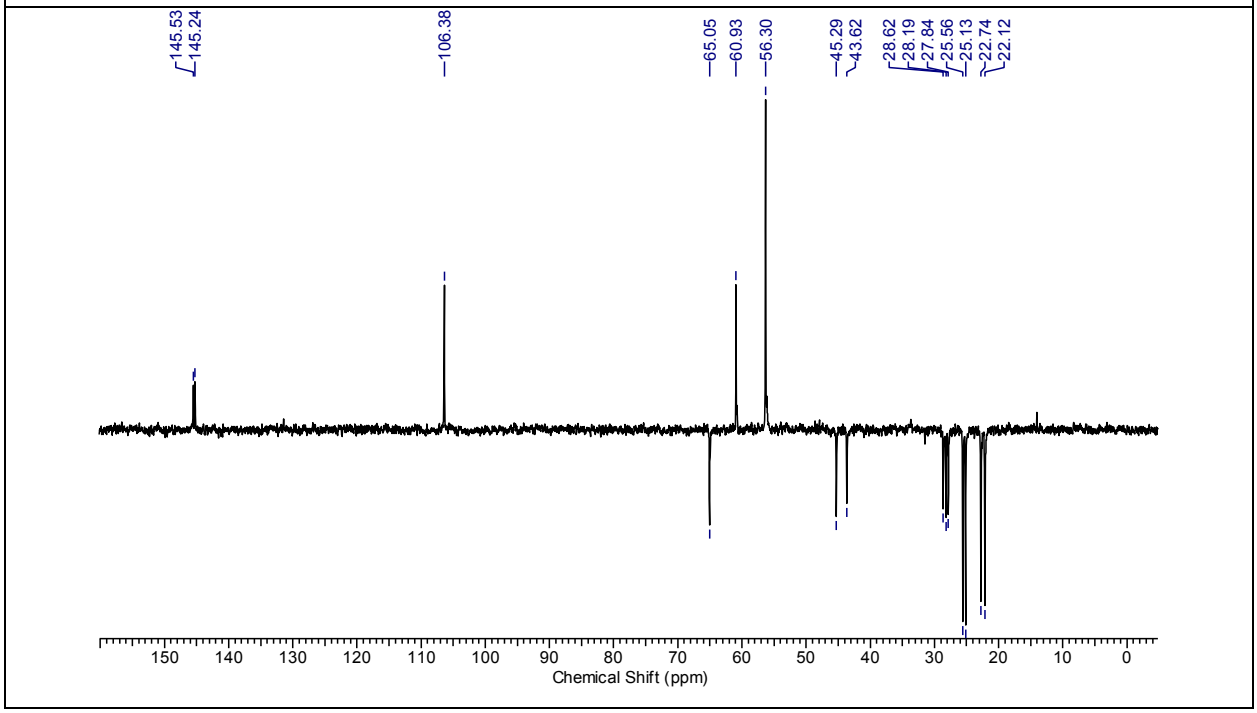

1.3.5. References

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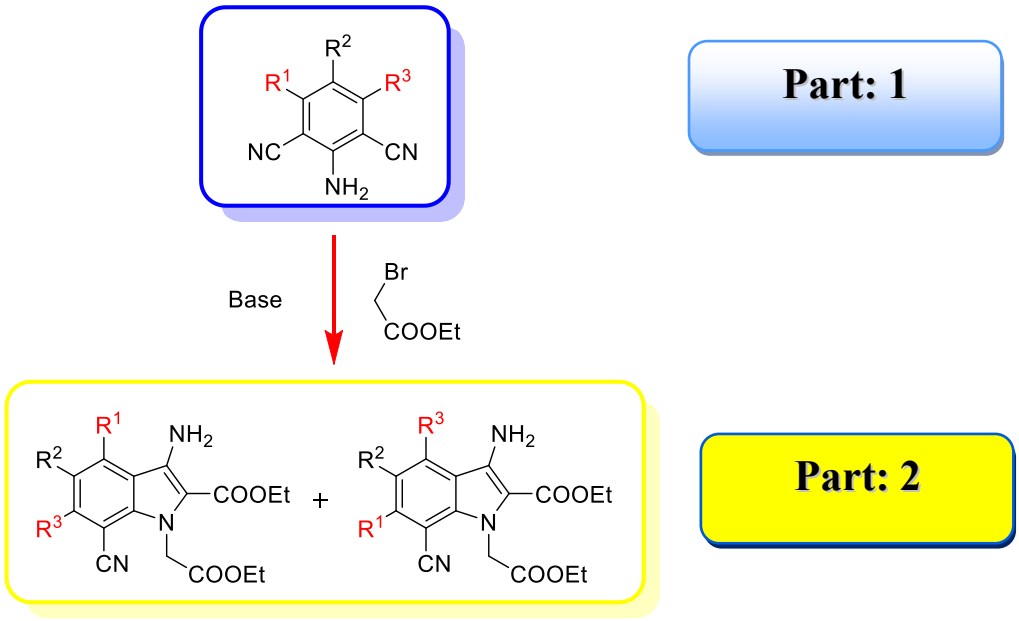
Chapter 2

Section 1

"General Outline of 2,6-Dicyanoanilines and Indoles"



2.1.1. General Outline of 2,6-Dicyanoaniline: Part:1

2,6-Dicyanoaniline derivatives, which are acceptor–donor–acceptor systems, are a fascinating class of substituted benzenes[1] wherein benzene ring contains one donor group (NH_2) and two acceptor groups (CN) at 2 and 6 positions of the donor group. Several compounds with acceptor–donor–acceptor frameworks, including substituted 2,6-dicyanoanilines, exhibit blue fluorescence.[2-5] Because of its unique properties 2,6-dicyanoaniline was used in organic dyes.[6] Furthermore, different polymers[7] and light-emitting diodes,[8] contain 2,6-dicyanoanilines in their structure. As intermediates for warm-safe polymers, magnetic polymers and light absorbing polymers, a variety of compounds containing a 2,6-dicyanoaniline moiety have been used.[9-14] Several 2,6 Dicynoanilines and its derivatives have also been tested as a chiral phase in chiral chromatography.[15-19] The structural features,[20-25] optical properties,[26-28] and implementations in liquid crystals[29] of 2,6-dicyanoanilines and associated derivatives, acceptor-donor-acceptor A-D-A systems are investigated as possible photosynthesis mimics.[30] Substituted 2,6-dicyanoaniline derivatives also have biological properties such as anticancer,[31,32] antimicrobial,[33] antileishmanial,[34] antihyperglycemic,[35] antiamyotrophic lateral sclerosis,[36] and growth-promoting properties due to their structures.[37] They have also been looked at for possible medical applications in the area of cardiovascular activity,[38] anti-inflammatory activity[39] and antifungal activity,[40] Many herbicides[39] and insecticides[40] have also been developed from them. The intermediate 2,6-dicyanoaniline is used in the synthesis of indoles,[41] quinazolines,[42] fluorenones,[43] indazoles,[44] benzoxazines,[45] benzotriazinones,[46] and imines[43] *etc.*

The preparation, properties, and applications of these compounds are described in a large number of articles and patents. There are many synthetic strategies outlined in literature with respect to synthetic route of 2, 6 dicynoanilines, out of this one-pot multi- component reaction is more preferable. Malononitrile and aldehydes or ketones were the key reagents in this one-pot reaction in the presence of bases like NaOH, K_2CO_3, DMAP and piperidine. One-pot multi-component reactions are a fast and environmentally friendly way to make new and useful organic compounds. These reactions are associated with less waste generation, shorter reaction times, lower costs, and the elimination of the intermediate separation step.

2.1.2. Synthetic approaches of 2,6-dicyanoanilines

This group was actively involved in synthesis of 2,6-dicyanoanilines and their biological applications and published a review article in the year 2012.[47] 2,6-Dicyanoaniline synthesis, optical properties, fluorescence study and spectral properties have been reported in many publications and patents.[26, 48-51] Basically in the view of substitution on 2,6-dicyanoaniline ring, these compounds have been divided into three main classes represented in Figure 1. 1) Monosubstituted 2,6-dicyanoanilines subdivided into two types 1a) 3 (5)-Substituted 2,6-dicyanoanilines and 1b) 4-Substituted 2,6-dicyanoanilines. 2) Disubstituted 2,6-dicyanoanilines subdivided into two types 2a) 3,4 (4,5)-disubstituted 2,6-dicyanoanilines 2b) 3,5-disubstituted 2,6-dicyanoanilines and 3) Trisubstituted 2,6-dicyanoanilines.

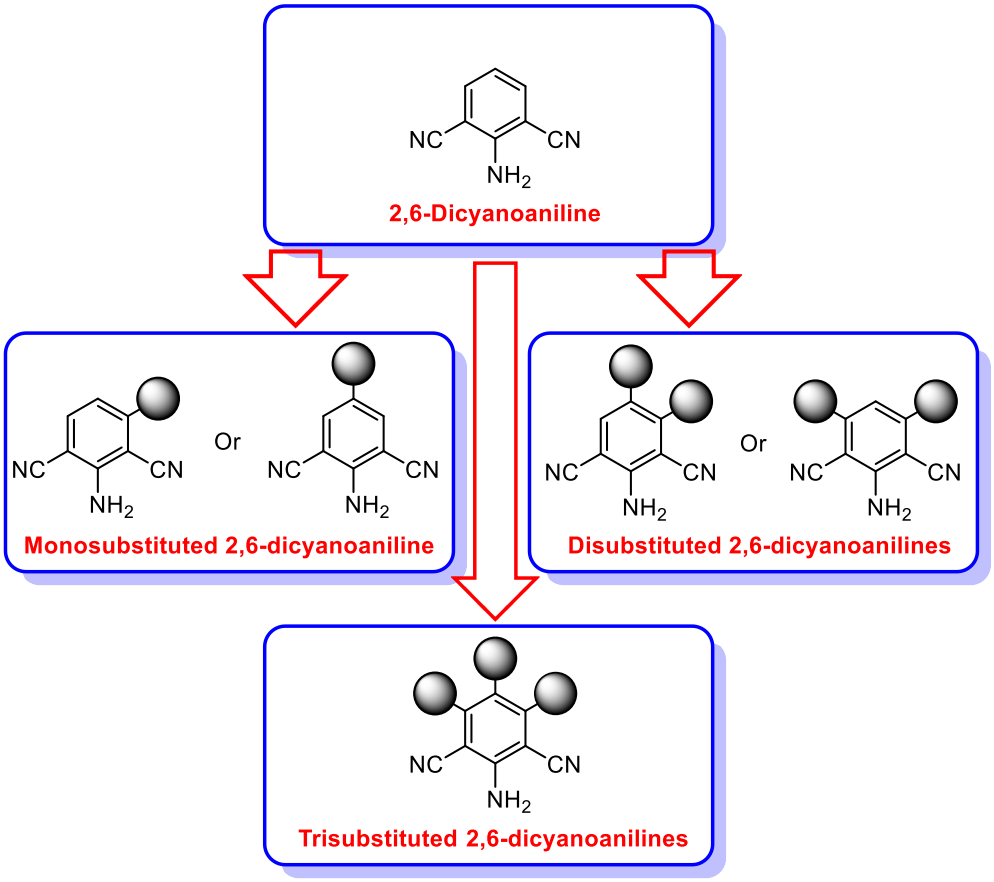


Figure 1. Substitution on 2,6-dicyanoaniline

There are a lot of references for 3-heterocyclyl-2,6-dicyanoanilines, a few for 3,5-diheterocyclyl-2,6-dicyanoanilines, and a few for 4-heterocyclyl-2,6-dicyanoanilines, but there are no references for 3,4-diheterocyclyl-2,6-dicyanoanilines or 3,4,5-triheterocyclyl-2,6- dicyanoanilines.

2.1.3. Conclusion and Prospect

Many applications of 2,6-dicyanoanilines have been reported in the literature, including biological, use in dyes, use in polymers, and textile printing of synthetic or semisynthetic fibers, demonstrating the compound's significance of the compounds. It would be essential to synthesize new molecules of this class, which would aid in the investigation of various aspects of synthetic and applied chemistry. Also, due to the fluorescence properties, it was decided to explore their potential as cell imaging agents.

2.1.4. General Outline of Indole: Part: 2

Heterocyclic chemistry is one of the foremost imperative sources of novel compounds with a range of biological applications, primarily due to the interesting capacity to mimic peptide structures and binding property towards the proteins. Heterocyclic compounds have a wide range of applications. They are enormously utilized as pharmaceuticals, as agrochemicals, and as veterinary products. They moreover have applications as sanitizers, cancer prevention agents, as cytotoxic inhibitors, as copolymers as well as color stuff. They are utilized as vehicles within the blend of other natural compounds. A few of the common items e.g. anti-microbials such as penicillins, cephalosporin; alkaloids such as vinblastine, morphine, reserpine, etc. have heterocyclic moiety and in several of applications, indoles play important role as heterocyclic system.

Indoles are classified as bicyclic heterocyclic compounds containing benzene ring fused to 2 and 3 positions. Indole shares one double bond with a benzene ring and a pyrrole ring. It is a heterocyclic network of 10 electrons from four double bonds and the nitrogen atom in lone pair[52] Indole co-ordinates into proteins within the system of tryptophan amino acid which is the concept of drugs such as indomethacin, thereby giving the structure of indole alkaloids — biologically active compounds from plants such as strychnine and lysergic acid diethylamide (LSD).[53] Indole has become immensely popular in various pharmacological applications as a pharmacophore and Kaushik N. K. *et al*. published a review article in year 2013.[53] The complex molecular architecture draws organic and medicinal chemists to check out its medicinal value and design derivatives.

In synthetic and natural bioactive anticancer compounds indole is very well researched heterocycle in studies. It was highly studied in targeted design and synthesis with respect to achieve the anticancer biological activity,due to its biodiversity and structural versatility. Several researchers reported indole based compounds with distinct pathways involved in the development of possible anticancer activities, suggesting the significance of indole scaffold in the development of anti-cancer drugs.

2.1.5. Synthetic approaches of 2,3,5,6,7-pentasubstituted and 2,3,4,5,7 pentasubstituted indoles from 2,6-dicyanoanilines.

The indole ring system can be found in a variety of natural products, and various methods for synthesizing the indole framework have been published. Hermann Emil Fischer has reported the first synthesis of indole in 1883, where phenyl hydrazine reacted with aldehydes or ketone in acidic condition which was most widely used method for constructing the indole skeleton.[54] In year 2005, B. Narsaiah and colleagues synthesized substituted indoles in presence of K_2CO_3 and KI as a base where 3,5- substituted 2,6-dicyanoanilines reacted with ethyl bromoacetate.

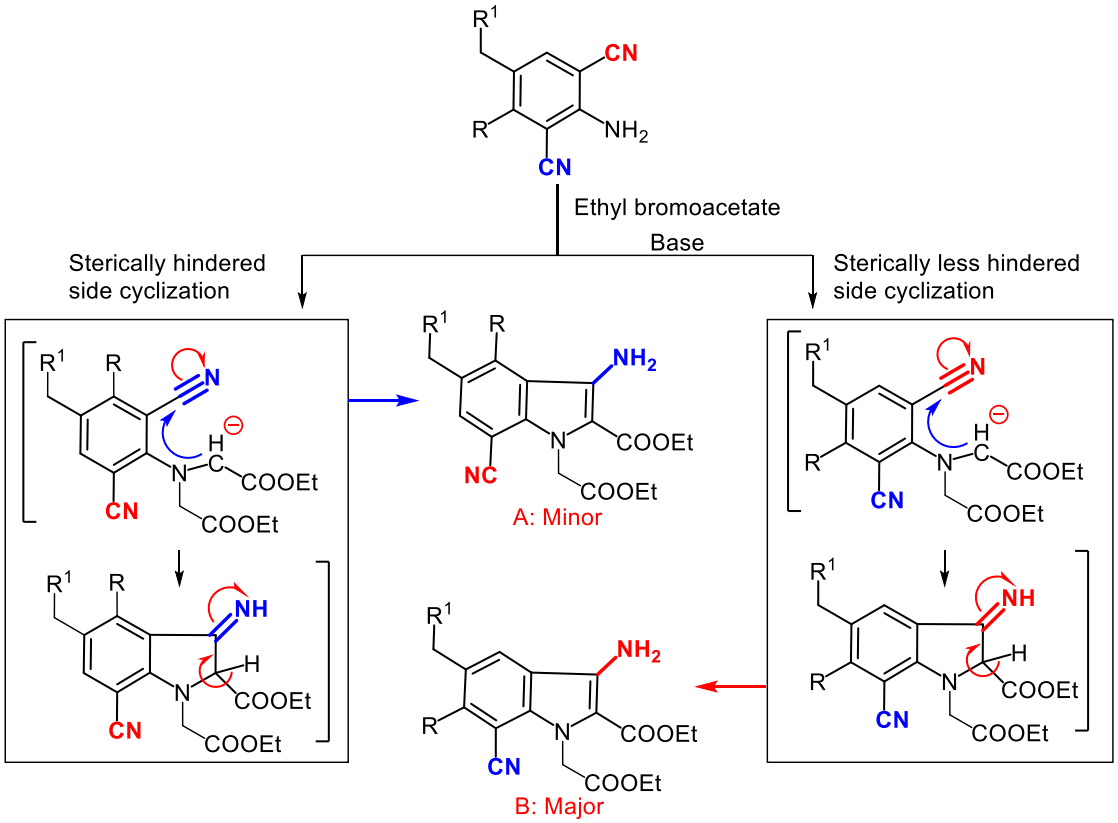


Figure 2. Mechanism of indole synthesis

This group reported synthesis of highly substituted indoles from 4,5-substituted 2,6-dicyanoanilines in 2011.[55] This synthetic strategy decided to use for the synthesis of substituted 2,6-dicyanoanilines precursors of subsequent indole synthesis. Designed substituted indoles planned to screen for biological applications. Figure 2 shows plausible mechanism for the synthesis of substituted indole.

2.1.6. Conclusion

In the literature, many applications of indole-based compounds have been reported, including biological applications. The design and synthesis of these target molecules will be important for research into various aspects of synthetic and applied chemistry.

2.1.7. References

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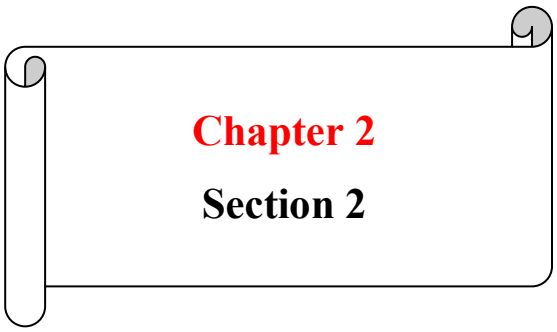


"Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl- 2,6-dicyanoanilines"

2.2.1. Introduction

Substituted 2,6-dicyanoanilines are important compounds studied[1a] as such or as intermediates in diverse fields like optical materials, dyes, textile printing, heat resistant polymers, chiral stationary phases in chromatography *etc*. They also constitute molecular skeleton of a number of compounds with an array of structural variations some of which have been studied for potential medicinal use. The synthetic methods to prepare these moieties and their applications have been reviewed recently[1a] and the continued interest in these compounds has resulted in further publications.[1b,c,2a-c] it was decided to study the synthesis and optical/biological properties of dicyanoanilines with one, two or three heterocyclic substituents. The literature survey revealed that there are many references for 3-heterocyclyl-2,6-dicyanoanilines,[1a] a few references for 3,5-diheterocyclyl-2,6-dicyanoanilines[2] and a very few references for 4-heterocyclyl-2,6-dicyanoanilines[3] but there are no references for 3,4-diheterocyclyl-2,6-dicyanoanilines or 3,4,5-triheterocyclyl-2,6-dicyanoanilines. Herein synthesis of various hitherto unknown 3,4-diheterocyclyl-2,6-dicyanoanilines and 3,4,5-triheterocyclyl-2,6-dicyanoanilines, their fluorescence properties and cell imaging applications is reported. Though there are a large number of reports about cell imaging[4], this is the first report describing cell imaging potential of 2,6-dicyanoanilines and these preliminary results will lead to new applications of dicyanoanilines for cell imaging.

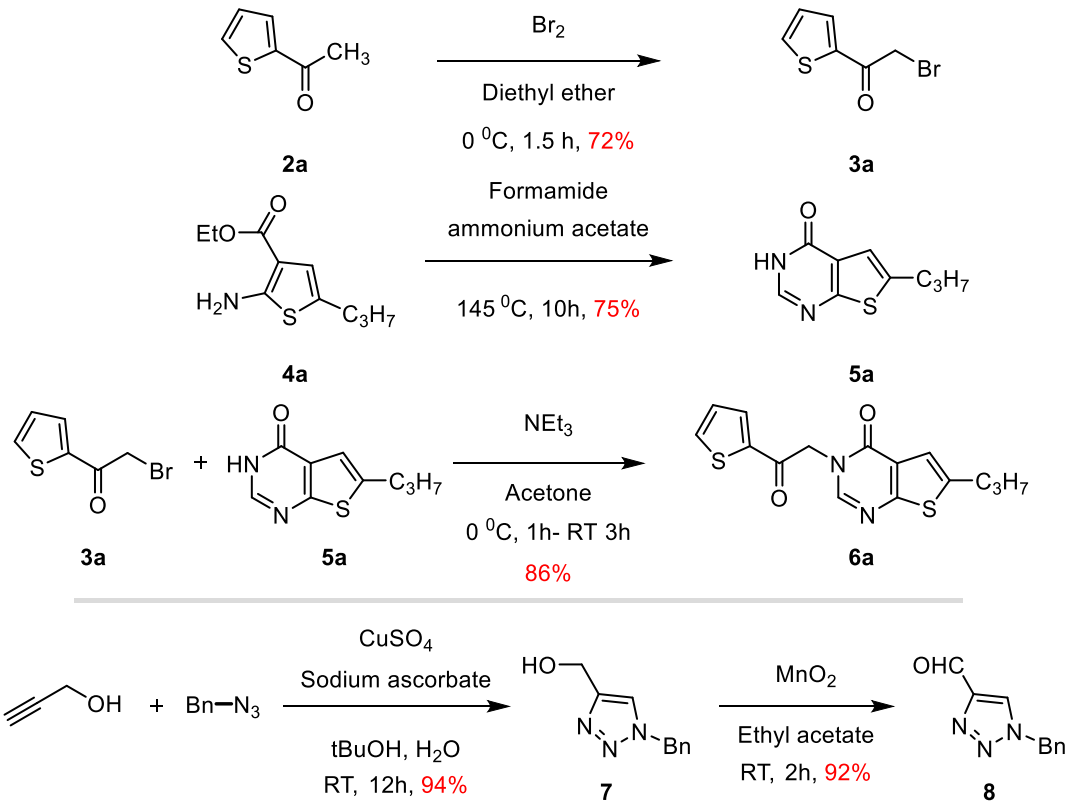
2.2.2. Present work

2.2.2.1. Synthesis of Compounds

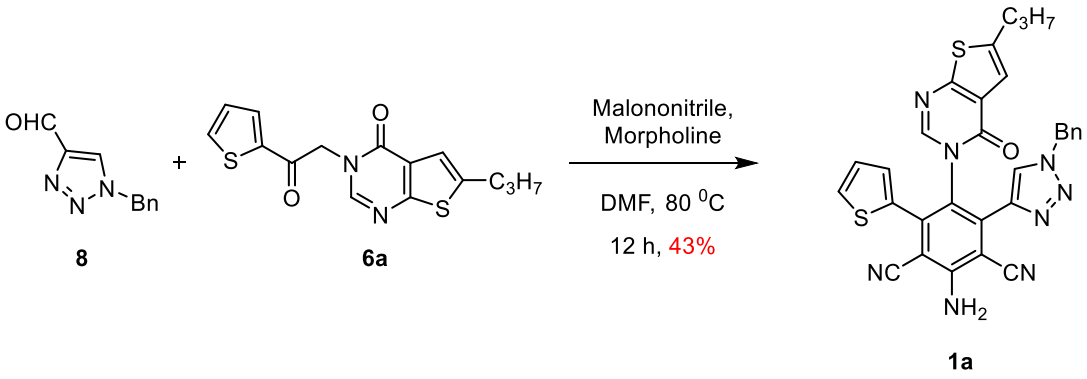
Various hitherto unknown 3,4,5-triheterocyclyl-2,6-dicyanoanilines were prepared from 1-benzyl-1,2,3[1*H*]-triazole-4-carboxaldehyde **8**, malononitrile and required 1,2-diheterocyclyl ethanone as exemplified by the synthesis of compound (**1a**) as shown in Scheme 1b.

Initially, thienopyrimidinone was chosen as one of the heterocycles because thienopyrimidinones[5] with various substituents can be synthesized easily from substituted 2-aminothiophene-3-carboxylates which in turn can be prepared by Gewald synthesis.[6] Thus, ketone **6a** was prepared from 2-bromoacetylthiophene[7] **3a** and compound **5a**[5] in the presence of triethyl amine in ethyl acetate and reacted[8] with 1-benzyl-1,2,3[1*H*]-triazole-4-carboxaldehyde **8**[9] and malononitrile in DMF in the presence of morpholine to obtain the 3,4,5-triheterocyclyl-2,6-

dicyanoaniline **1a** in 43% isolated yield. The 3,4,5-triheterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-5-aryl-2,6-dicyanoanilines thus prepared, using aldehyde **8** and appropriate ketones, are shown in Table 1.



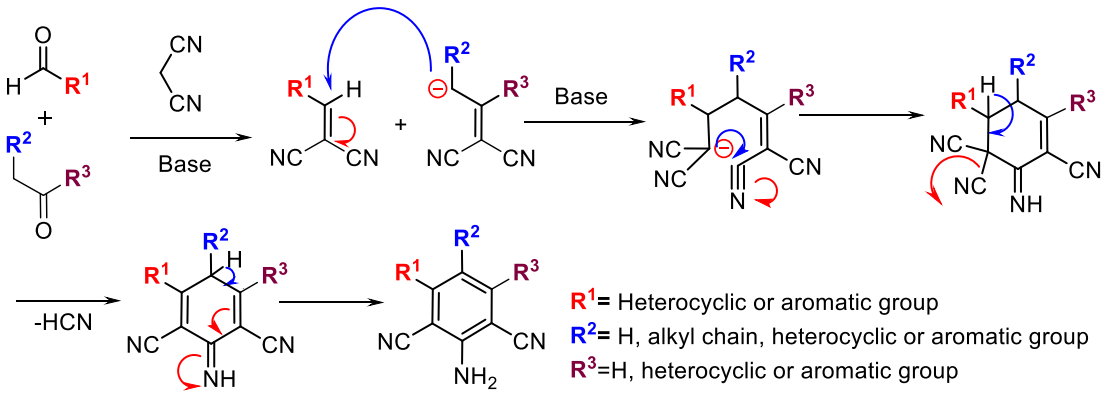
Scheme 1a. Synthesis of intermediate compounds **6a** and **8**



Scheme 1b. Synthesis of compound **1a**

During the course of research on synthesis of various 2,6-dicyanoanilines with one or more heterocyclic substituents, it was noticed that there are a few references[1a,2,3] for preparation of 2,6-dicyanoanilines with heterocyclic substituents at 3,5-positions and 4-position but there are no reports for synthesis of 2,6-dicyanoanilines with heterocyclic substituents at 3,4,5-positions. The preliminary efforts in this direction are presented herein. Synthesis of 3,4,5-triheterocyclyl-2,6-dicyanoanilines was achieved by one step multicomponent reaction. Plausible mechanism for formation of 3,4,5-substituted-2,6-dicyanoanilines is shown in Scheme 2. It was assumed that dehydration reaction after nucleophilic addition of anion of malononitrile to carbonyl carbon of aldehyde and ketone gives Knoevenagel reaction intermediates and in presence of excess base, nucleophilic addition of anion formed by removal of active hydrogen of Knoevenagel product of ketone substrate to that of aldehyde substrate gives condensation product. Further cyclization and aromatization by elimination of hydrogen cyanide result in the formation of substituted-2,6-dicyanoanilines.

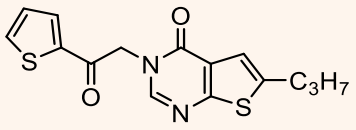
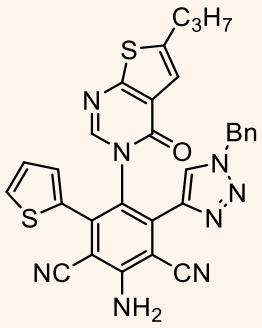
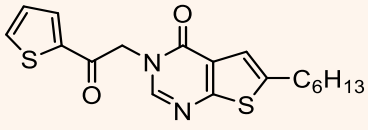
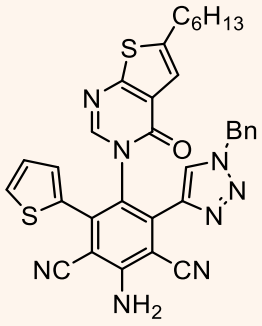
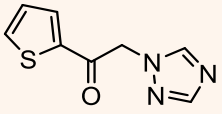
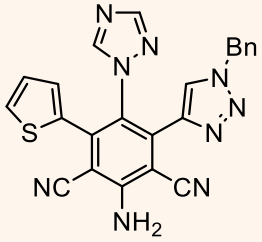
Using this strategy, various hitherto unknown 3-heterocyclyl-2,6-dicyanoanilines **9**, **10a-f**, **11** and **12** were prepared by reacting aldehyde **8** with appropriate aldehyde/ketone (Table 2). Compounds **1a** to **1j** where obtained in lower yield as compared with other compounds because of more bulky substituents on 3,4,5-positions of 2,6-dicyanoanilines and it was fully substituted. In case of compound **11,** yield slightly increased as compared to compounds **1a-1j** as the steric hindrance is less because of heterocyclic and aromatic substituents at 3,5-positions respectively. Also as compared with compound **11**, compounds **10a** to **10f** and compound **12** which are 3,4-substituted-2,6-dicyanoanilines, were obtained in good yields due to less steric hindrance.

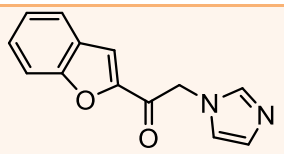
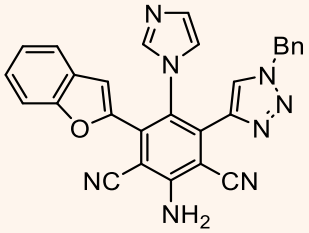
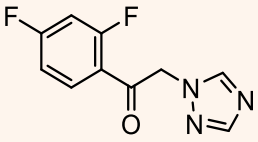
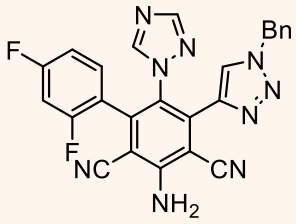
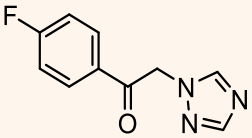
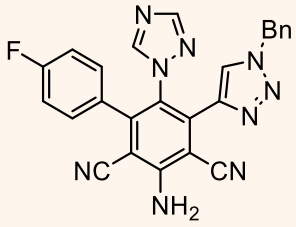
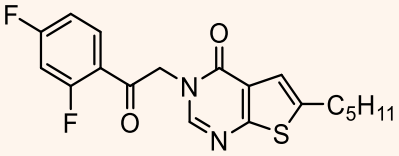
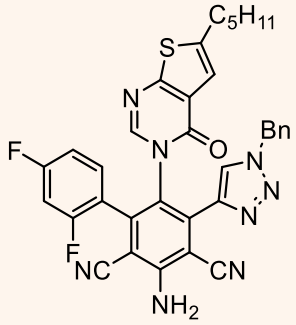
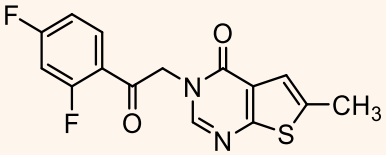
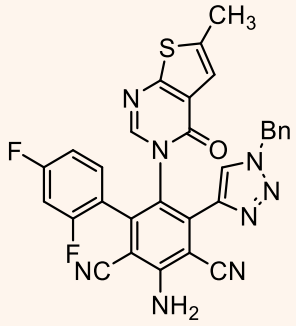


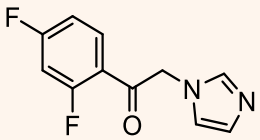
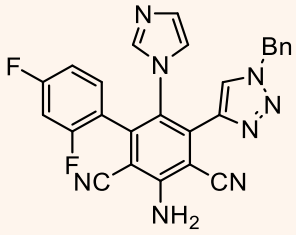
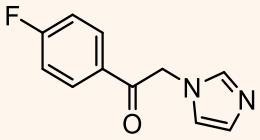
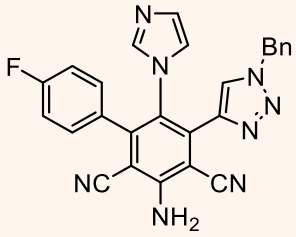
Scheme 2. Plausible mechanism for formation of 3,4,5-trisubstituted-2,6-dicyanoanilines

The dicyanoanilines **1e** and **1f** were methylated[2a] by subjecting them to NaH-MeI in THF to get the corresponding *N,N*-dimethylated compounds **13e** and **13f**.[10] Similarly, compounds **10a-f** were methylated to obtain the corresponding *N,N*-dimethylated compounds **14a-f** (Table 2).

Table 1. The 3,4,5-triheterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-5-aryl-2,6-dicyanoanilines prepared

Entry no	Ketone used	Product obtained	Yield %
1	 **6a**	 **1a**	43
2	 **6b**	 **1b**	45
3	 **6c**	 **1c**	44

4	 **6d**	 **1d**	38
5	 **6e**	 **1e**	49
6	 **6f**	 **1f**	52
7	 **6g**	 **1g**	31
8	 **6h**	 **1h**	37

9	 **6i**	 **1i**	43
10	 **6j**	 **1j**	47

2.2.2.2. X-ray crystallographic characterization

Structures of these compounds were assigned based on ^{1}H NMR, ^{13}C NMR spectroscopies, IR spectroscopy and HRMS data as presented in experimental section. The molecular structures of respective compounds **1c** and **1e** were further confirmed by X-ray crystallography[10] (**Figure 1**).

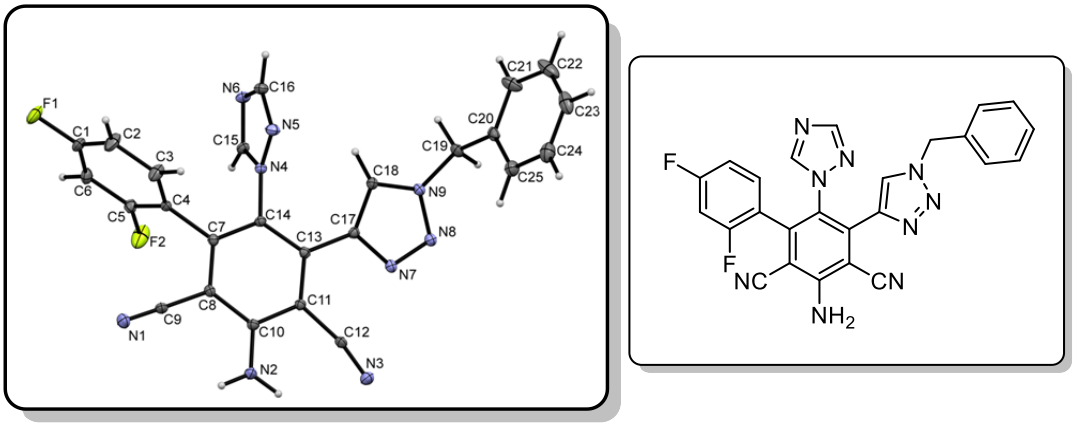


Figure 1a: Compound **1c**

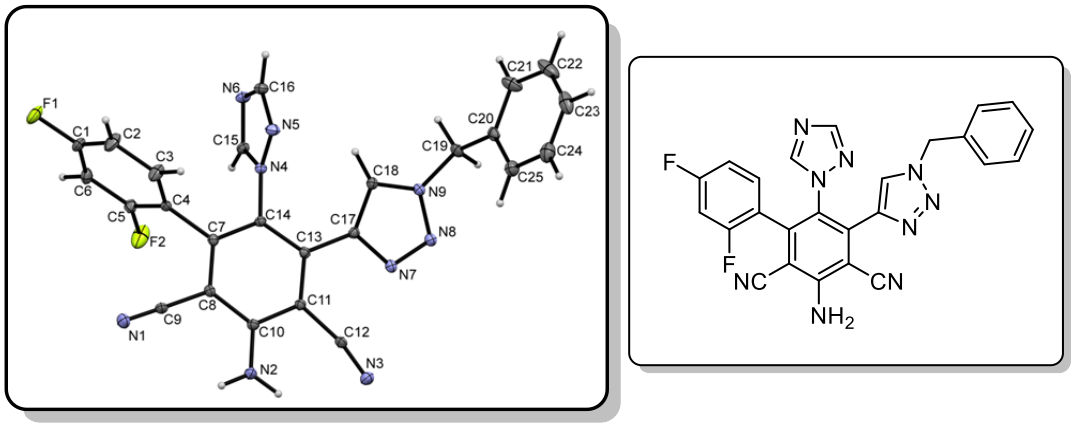
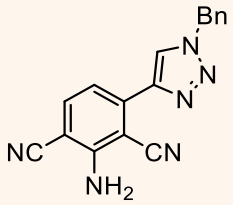
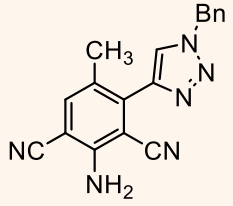
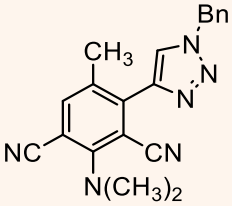
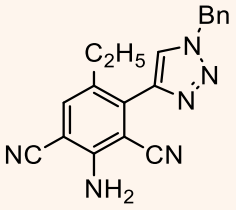
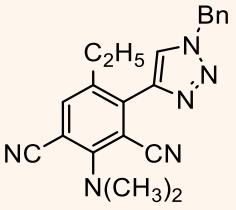
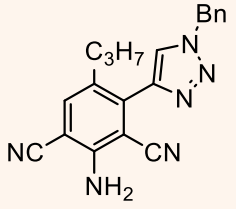
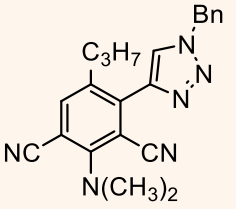
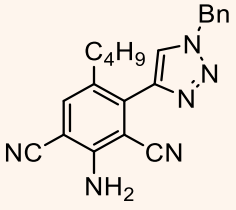
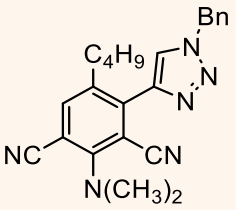
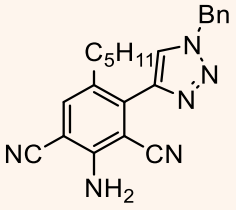
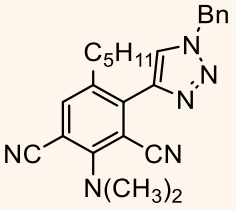
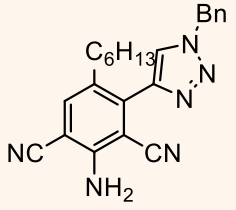
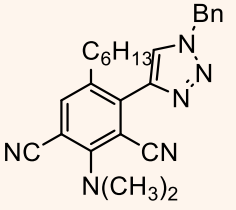


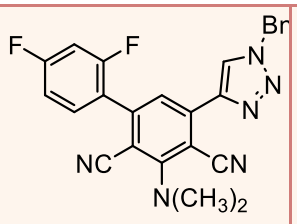
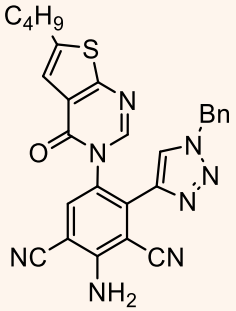
Figure 1b: Compound **1e**

Figure 1. The Oak Ridge Thermal Ellipsoid Plot (ORTEP) of compound **1c** (**Figure 1a**) and compound **1e** (**Figure 1b**) showing the atom numbering scheme. The displacement ellipsoids are drawn at the 30% probability level and H-atoms are shown as small spheres with arbitrary radii (CCDC No. 1437762 and 1437763 for **1c** and **1e** respectively).

Table 2: The novel 3-heterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-2,6-dicyanoanilines prepared

Entry no	Aldehyde/ketone used	Product obtained	Isolated Yield%	Methylated product	Isolated Yield%
1	Acetaldehyde + Compound **8**	 **9**	50	ND	-
2	Propionaldehyde + Compound **8**	 **10a**	61	 **14a**	75

3	Butyraldehyde + Compound **8**	 **10b**	64	 **14b**	73
4	Valeraldehyde + Compound **8**	 **10c**	59	 **14c**	70
5	Hexanal + Compound **8**	 **10d**	60	 **14d**	72
6	Heptanal + Compound **8**	 **10e**	61	 **14e**	69
7	Octanal + Compound **8**	 **10f**	63	 **14f**	77

8	2,4-Difluoro-acetophenone + Compound **8**	 **11**	55	ND	-
9	2-(6-Butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetaldehyde + Compound **8**	 **12**	65	ND	-

* ND: not done

2.2.2.3. Optical properties of compounds

These compounds were studied for their optical properties as they were coloured in solution. The UV-visible absorption spectra, of 2.5 x 10^{-5} M solutions in acetonitrile, for representative 2,6-dicyanoaniline compounds **1e**, **1g**, **10f**, **11** and **12** and *N*,*N*-dimethylated compounds **13e** and **14f** are shown in **Figure 2a**.

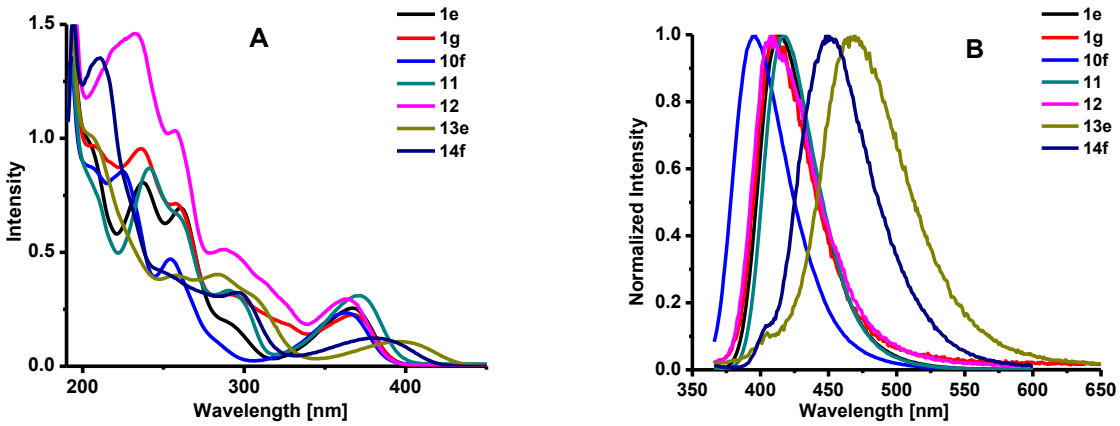


Figure 2a. UV-visible absorption (**A**) and fluorescence (**B**) spectra of compounds **1e**, **1g**, **10f**, **11**, **12**, **13e** and **14f**

It was observed that there was a red shift in absorption maximum of the amino group after methylation due to enhanced electron-donating ability *e.g.* the λ_{max} value for amino compound **1e** was 367 nm while that for its corresponding *N,N*-dimethylated derivative **13e** was 395 nm. It was observed that the Stokes shift for the amino compound **1e** was 47 nm while the Stokes shift for the corresponding *N,N*-dimethylated derivative **13e** was 74 nm indicating that the Stokes shift is increased after *N,N*-dimethylation of amino group. There was change in colour of fluorescence in acetonitrile solutions of same concentration of these compounds.

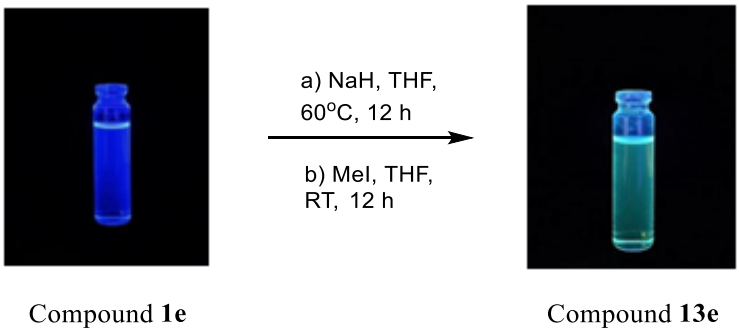


Figure 2. Change in fluorescence after methylation of amino group in compound **1e**

There is a possibility to modify the structures of the compounds in the present work, in order to tune the optical properties, as there is a lot of flexibility in the synthetic schemes described.

2.2.2.4. Biological evaluation

2.2.2.4.1. Cell imaging

The fluorescent molecules have a wide application in biological research where they have been developed as valuable tools to study basic physiological processes within biological systems and there are a number of reports wherein they have been explored for cell imaging studies.[4]

Apoptosis is a process of programmed cell death in which the cell shrinks, nuclear envelope disassembles and DNA breaks into fragments.[12] The cell surface is altered which serves as a signal for the neighboring macrophages to phagocytose it.[13,14] Phosphatidylserine (PS) which is actively held on the interior (cytosolic) side of the lipid bilayer is externalized which serves as a cell surface signal for apoptosis.[15,16] Annexin V has strong affinity toward binding protein phospholipid . It is a conjugated with fluorophore, fluoroscein isothiocynate (FITC) which is widely used to detect this PS externalization which indicates apoptosis.[17] This has a profound application in identifying apoptotic cells in a cell population. In some biological studies it becomes necessary to identify such cell populations in order to interpret cell death inducing mechanisms if necessary. Propidium iodide is another widely used dye which intercalates with the DNA. It is specifically used to detect dead cell population in research[18] and is often used alongwith Annexin-V-FITC.

The fluorescent molecules can be tagged or conjugated to specific moieties and generate fluorescent signals which thus serve as detectable or measurable signals. These have applications in microscopy, fluorimetry, flow cytometry, *etc.* The molecules synthesized in the present work show fluorescent properties which were explored for cell imaging studies. Cell imaging analysis was carried out on fluorescent compounds prepared in the present work and 4 compounds (**1g**, **10f**, **11** and **12**) were found to stain eukaryotic cells. Three cell lines (differentiated THP-1, MCF-7 and MDA-MB-231) were tested for cell staining using known staining agents [SYTO 9 which stains nucleus (green fluorescence), Nile red which stains cell membrane (red fluorescence), Annexin-V-FITC which detects apoptotic cells (green fluorescence) and propidium iodide which stains dead cells (red fluorescence)].

Cell images captured, using High Content Screening System (Array Scan XTI, Thermo Scientific) at 20X magnification, after staining differentiated THP-1 cells with 30 µg/mL solutions of compounds **1g**, **10f**, **11** and **12** are shown in **Figure 3**. Differentiated THP-1 cells were observed to retain these fluorescent compounds.

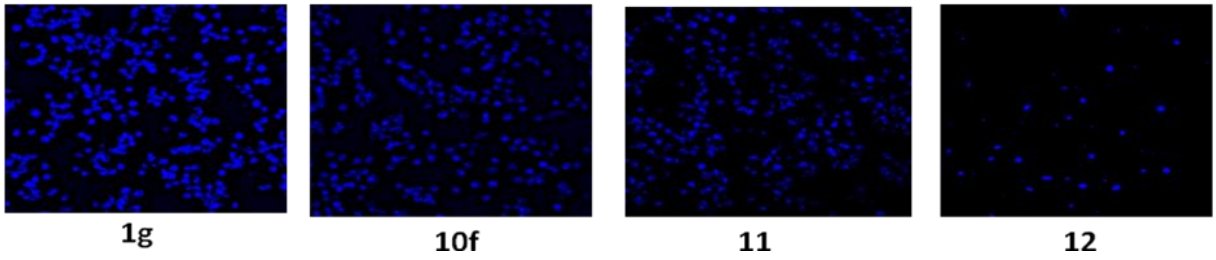


Figure 3. Cell images captured after staining THP-1 cells with compounds **1g**, **10f**, **11** and **12**

Compound 1g, 10f, and 11 revealed to be active in staining the cell which are in contact while compound **12** was found to stain only some cells out of total cells. The compounds **1g**, **10f**, **11** and **12** were studied further for staining MCF-7 and MDA-MB-231. The representative cell-imaging results for the compound **1g** for MCF-7 cells are shown in **Figure 4**.

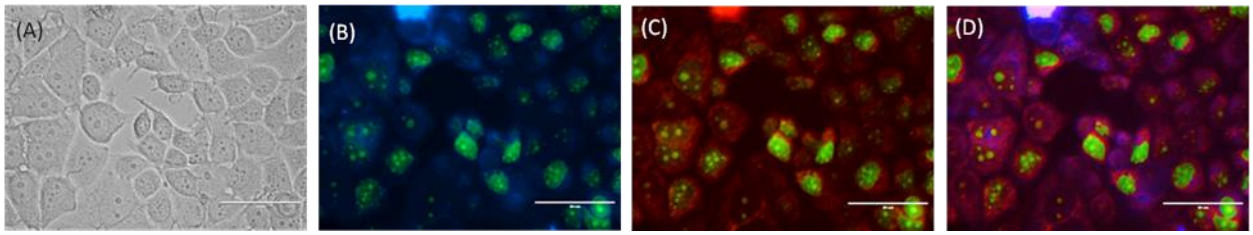


Figure 4. MCF-7 cells stained with the compound **1g**, SYTO 9 and Nile red. The cells were stained with compound **1g** (100 µg/mL, blue fluorescence), SYTO 9 which stains nucleus (green fluorescence) and Nile red which stains cell membrane (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field, (B) Overlay image of blue and green channels, Blue – compound **1g**, Green – SYTO 9 (stains nucleus), (C) Overlay image of green and red channels: Red – Nile red (stains cell membrane), Green – SYTO 9 (stains nucleus), (D) Overlay image of (B) and (C). Scale on image measures 50 µm.

The detailed cell images for staining of differentiated THP-1, MCF-7 and MDA-MB-231 cell lines with the compounds **1g**, **10f, 11** and **12** figure 3 to figure 10. Compounds **1g**, **10f**, **11** showed selective specific staining ability toward cytoplasm and it doesn't stains nuleus. . This

was verified by using known counter stains like SYTO 9 (for nucleus) which gives green fluorescence and Nile red (lipid molecules and cell membrane) which gives red fluorescence.

However, compound **12** was found to stain selectively some cells out of total cells and the patterns were similar to Annexin V-FITC as shown in Figure 5.

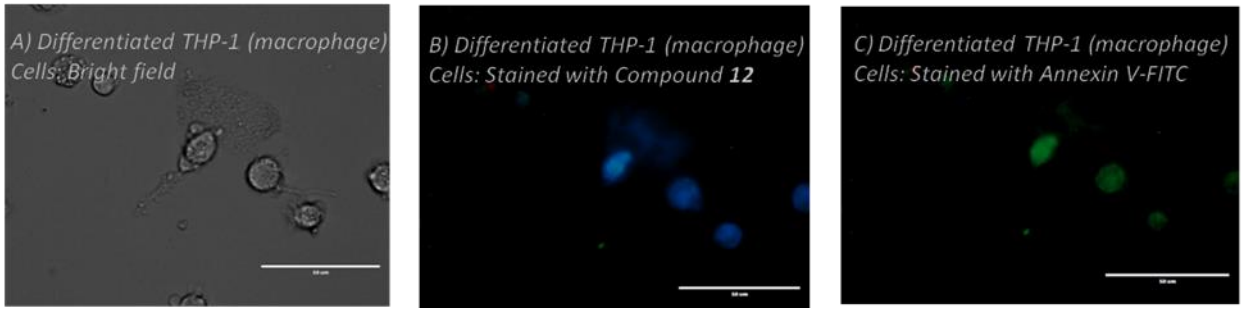


Figure 5. Differentiated THP-1 cells A) In bright field, B) Stained with compound **12**, C) Stained with Annexin V-FITC

This differential staining was studied further using known markers and inducers. In order to study the differential staining property of **12**, the cells were seeded and apoptosis / cell death was induced using known anti-cancer, cytotoxic compounds namely Paclitaxel and Doxorubicin at their IC_{50} and IC_{90} concentrations. Known markers like Annexin-V-FITC for apoptosis and propidium iodide for dead cells were used along with the compound **12**.

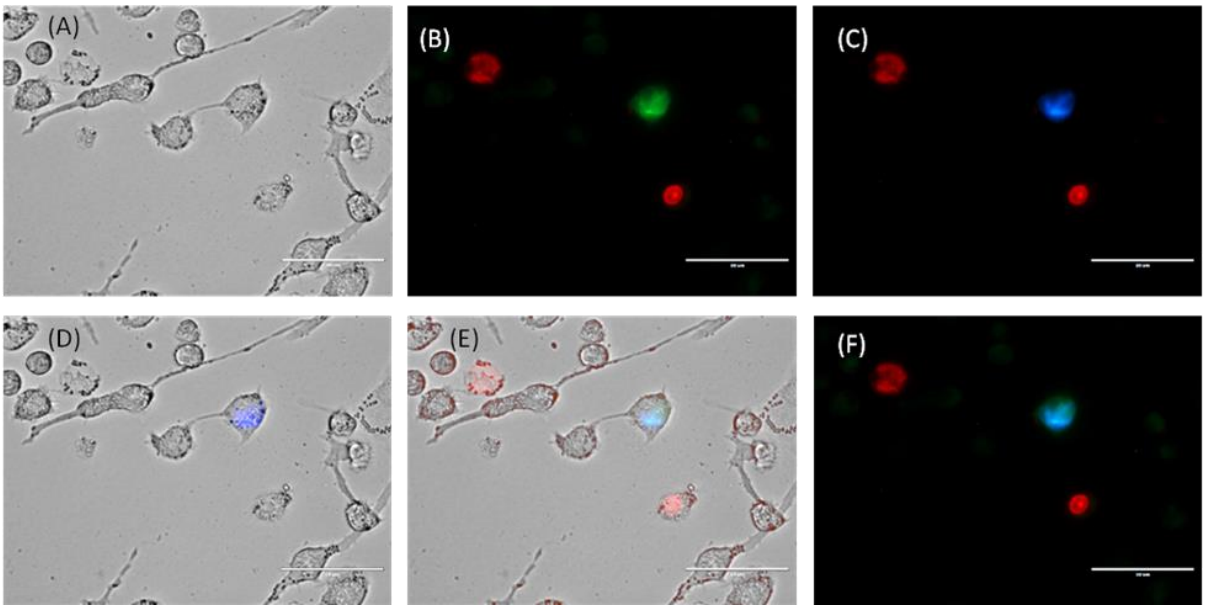


Figure 6. Differentiated THP-1 cells stained with compound **12**, Annexin-V-FITC and propidium iodide. The cells were stained with compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence)which detects apoptotic cells and propidium iodide (PI) (red fluorescence) which stains dead cells Figure 6 It was observed at 60X magnification. (A) Cells seen in bright field, (B) Overlay image of green and red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead), (C) Overlay image of blue and red channels: Blue – compound **12**, Red – PI (dead), (D) Overlay image of bright field with blue channel – Blue – compound **12** (E) - Overlay image of (A), (B) and (C), (F) - Overlay image of (B) and (C).

Standard staining protocol for apoptosis/necrosis using Annexin-V-FITC/PI was carried out with addition of this new compound **12**. Healthy cells were used as negative control. The cell images for differentiated THP-1 (macrophage) cells stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC which detects apoptotic cells (green fluorescence) and propidium iodide (PI) which stains dead cells (red fluorescence) are shown in **Figure 6**.

It was observed that the staining of the differentiated THP-1 (macrophage) cells with the compound **12** corresponds to that with Annexin-V-FITC which is known to stain the apoptotic cells. The compound **12** does not stain dead cells. The results were further confirmed by inducing apoptosis in the THP-1 (macrophage) cells with Paclitaxel and repeating the study (Figure 7).

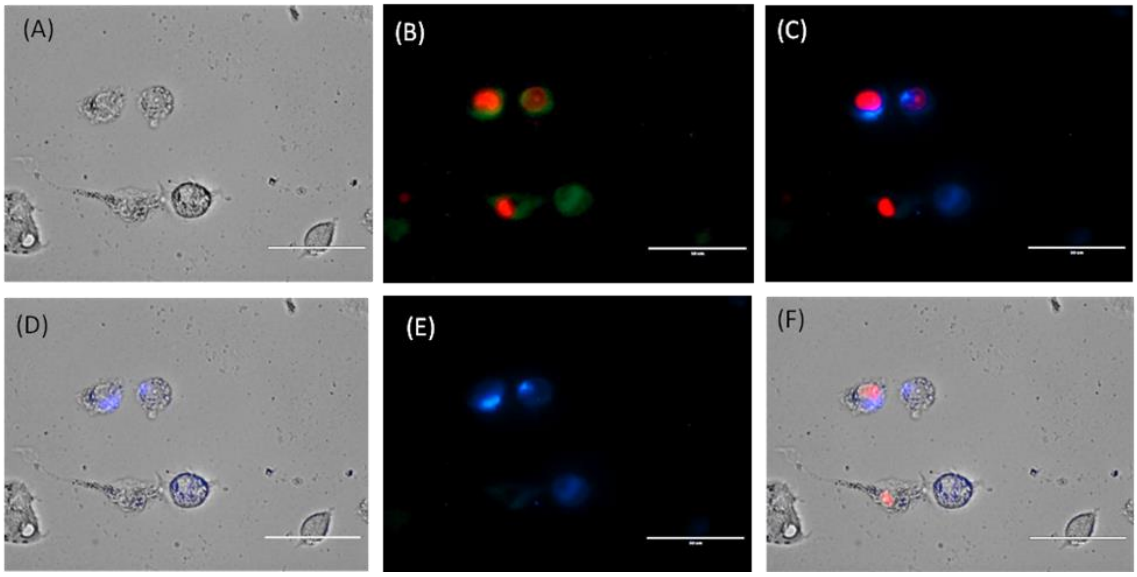


Figure 7. Differentiated THP-1 cells with induced apoptosis using Paclitaxel, stained with the compound **12**, Annexin-V-FITC and propidium iodide. Differentiated THP-1 (macrophage) cells with induced apoptosis using Paclitaxel IC_{90} concentration, were stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence for apoptotic cells) and propidium iodide (PI) (red fluorescence for dead cells) observed at 60X magnification. (A) Cells seen in bright field, (B) Overlay image of green and red channels, Green – Annexin-V-FITC (stains apoptotic cells), Red – PI (stains dead cells), (C) Overlay image of blue and red channels: Blue – compound **12**, Red – PI (stains dead cells), (D) Overlay image of bright field with blue channel– compound **12**, (E) Blue – compound **12**, (F) Overlay image of (A) and (C).

The results of imaging in case of MCF-7 cells stained with the compound **12**, Annexin-V-FITC and propidium iodide are shown in **Figure 8** which also shows that the staining pattern of the compound **12** is similar to Annexin-V-FITC (which shows green fluorescence for apoptotic cells).

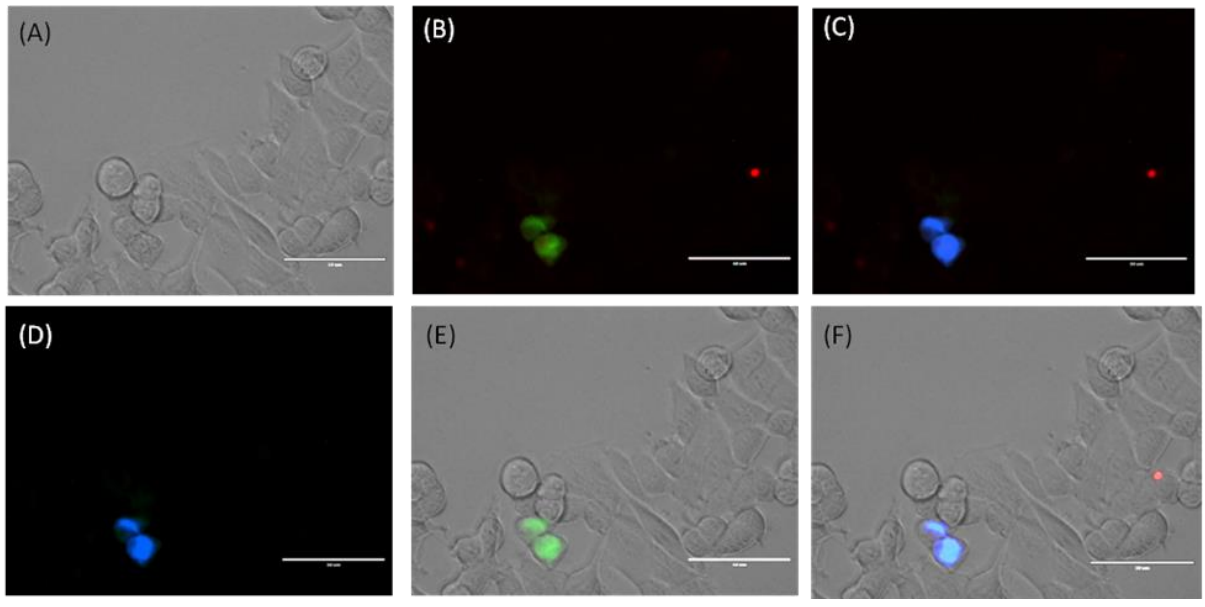


Figure 8. MCF-7 cells stained with the compound **12**, Annexin-V-FITC (green fluorescence) and propidium iodide. The MCF-7 cells were stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field, (B) Overlay image of green and

red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead cells), (C) Overlay image of blue and red channels: Blue – the compound **12**, Red – PI (dead cells), (D) Blue – the compound **12**, (E) - Overlay image of (A) and (B), (F) Overlay image of (A) and (C). Scale on image measures 50 µm.

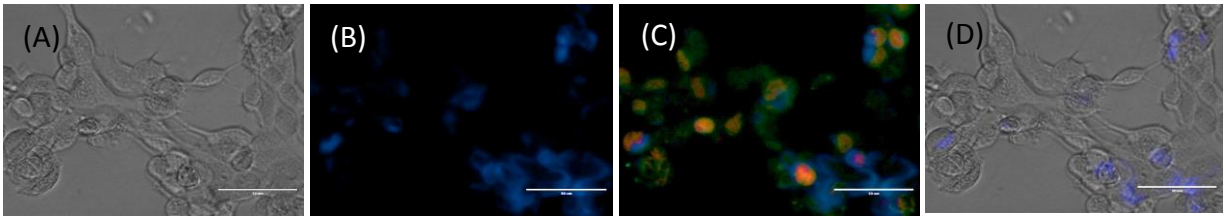


Figure 9. MCF-7 cells with induced apoptosis using Doxorubicin, stained with the compound **12**, Annexin-V-FITC and propidium iodide. MCF-7 cells with induced apoptosis using Doxorubicin IC_{50} concentration were stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field, (B) Blue – compound **12** (C) Overlay image of green, red and blue channels: Green – Annexin-V-FITC (apoptosis), Red – (dead cells) and Blue – compound **12**, (D) - Overlay image of (A) and (B). Scale on image measures 50 µm.

Further, apoptosis was induced in MCF-7 cells by treating them with Doxorubicin and the cells were stained with the compound **12**, Annexin-V-FITC and propidium iodide and the results are shown in Figure 9.

The imaging properties of the compound **12** were further studied with MDA-MB-231 cells. The apoptosis was induced with Doxorubicin and the cells were stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (PI) (red fluorescence) as before (**Figure 10**).

All these results indicated that the staining patterns of the compound **12** (which shows blue fluorescence) are similar to Annexin-V-FITC which shows green fluorescence for apoptotic cells and the compound **12** has potential as staining agent for imaging of apoptotic cells.

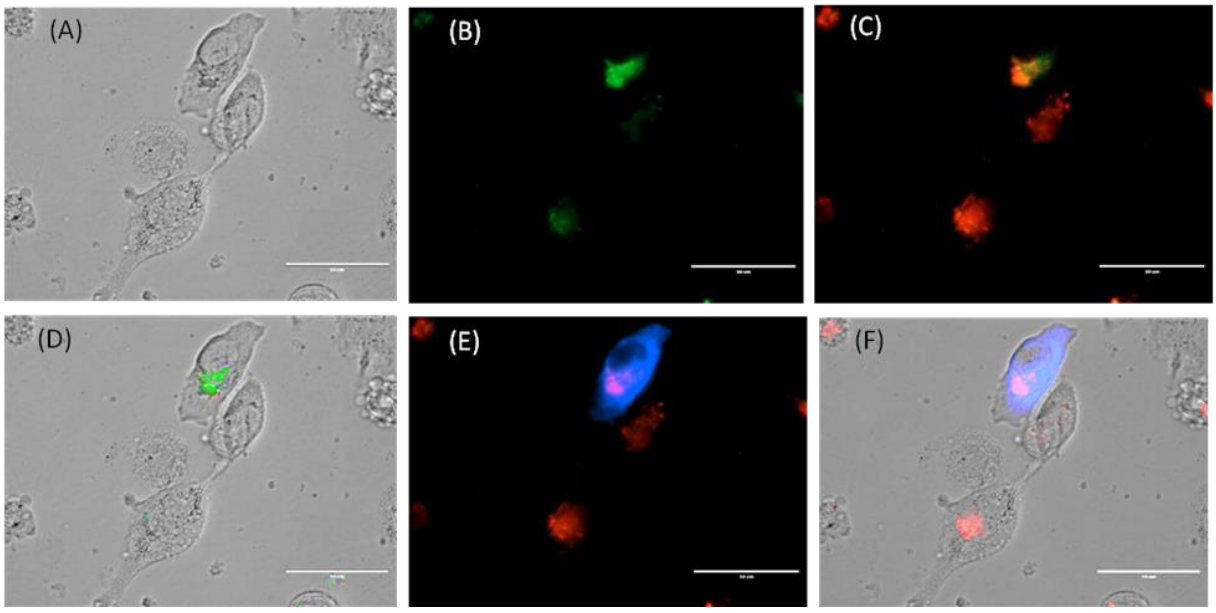


Figure 10. MDA-MB-231 cells with induced apoptosis using Doxorubicin, stained with the compound **12**, Annexin-V-FITC and propidium iodide. MDA-MB-231 cells with induced apoptosis using Doxorubicin IC_{90} concentration, were stained with the compound **12** (100 µg/mL, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field, (B) Green – Annexin-V-FITC (apoptosis), (C) Overlay image of green and red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead cells), (D) - Overlay image of (A) and (B), (E) Bright field with blue – compound **12** and red – PI (dead cells), (F)- Overlay image of (A) and (E). Scale on image measures 50 µm.

2.2.2.4.2. Toxicity

Toxicity of the compounds **1g**, **10f**, **11** and **12** against THP-1 cells was studied[19] and results show that IC_{50} values of compounds are >100 µg/mL so these compounds can be considered as non-cytotoxic. Cytotoxicity of the compounds **1g**, **10f**, **11** and **12** against THP-1 cells (monocytes) was studied by known method[19] MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from SRL and 5 mg/mL stock was made in PBS (pH 7.2). Briefly, 100 µL of growing THP-1 were seeded into 96 well sterile platewith 10^6 cells/mL cell density. which was subsequently incubated at 37 °C for 24 h in 5% CO_2 incubator , compounds at varying concentrations from 100 to 0.7825 µg/mL were added to the wells along with suitable vehicle

controls. Doxorubicin was used as standard inhibitor positive control. 10 µL MTT (5mg/mL in PBS) was added to the 48 h incubated cells Subsequently further incubated for 3,5 h at 37 °C and formazan was dissolved by addition of 100 µL of acidic propanol. Finally absorbance was analyzed at 570 nm (SPECTRA Amax PLUS 384, Molecular Devices) IC_{50} of the compounds was found based on the graph of % inhibition v/s concentration for each compound. The assay was carried out in triplicates and the error bars in graph indicate SD from triplicates. Formula used for the calculation of % cytotoxicity is as follows

$$\% \text{ Cytotoxicity} = [(\text{Control} - \text{Test}) / (\text{Control} - \text{blank})] \times 100$$

Where, **Control** = cells without compounds

Test = cells in presence of compounds

Blank = culture medium without cells

IC_{50} for all four compounds are > 100 µg/mL and hence the compounds can be considered as non-cytotoxic.

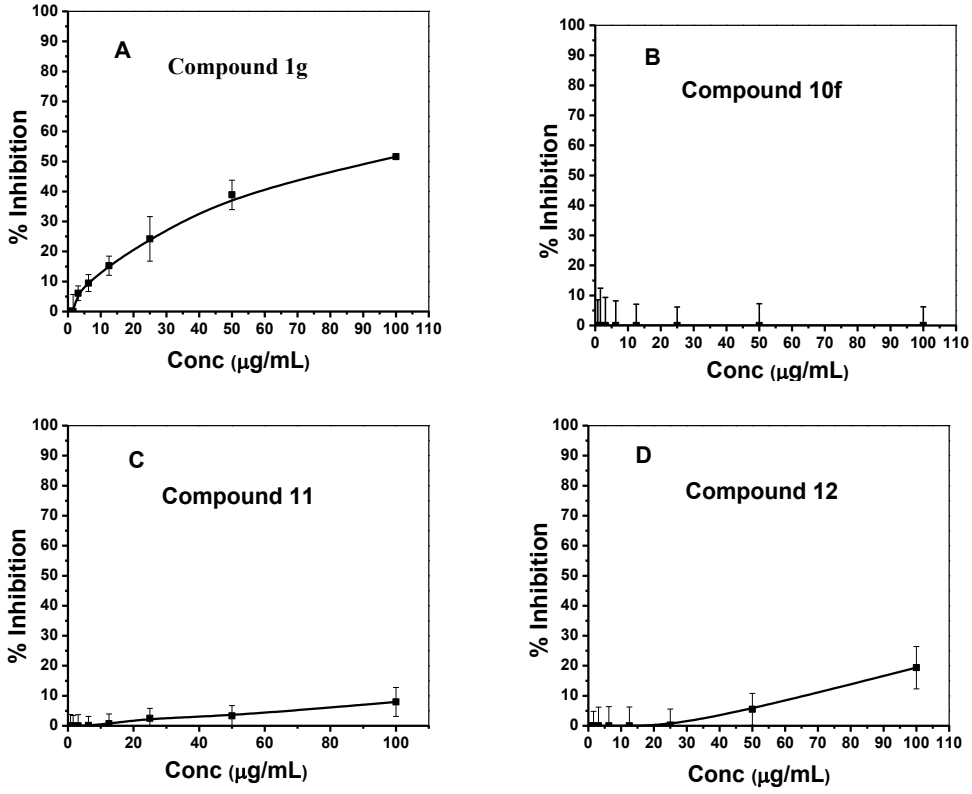


Figure 11. Toxicity of the compounds **1g**, **10f**, **11** and **12** against THP-1 cells

2.2.3. Conclusions

In conclusion, synthesis of 3,4,5-triheterocyclyl/3,4-diheterocyclyl/3-heterocyclyl-2,6-dicyanoanilines, starting from compound **8**, malononitrile and required aldehydes/ketones was achieved. The optical properties of the synthesized compounds were studied which indicated that fluorescence properties of this class of compounds can be modified. The compounds were further studied from the point of their application for cell imaging wherein it was found that compounds1g, 10f and 11 has selectively stains cytoplasm of cell in contact, but not nucleus. Whereas, compound **12** showed specific affinity toward the cells under apoptosis. Stained cells showed blue fluorescence. Compound **12** imaging was compared with a known reagent Annexin V FITC with green fluorescent in imaging of apoptic cells These encouraging results indicate the significant potential of these compounds as valuable tools for cell imaging.

2.2.4. Experimental

2.2.4.1. Synthesis and spectral data

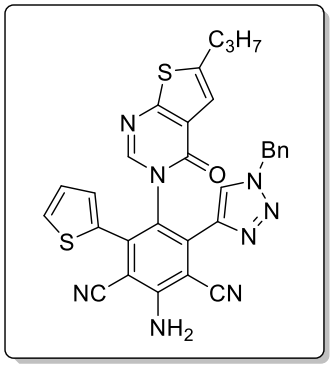
Synthesis of 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(4-oxo-6-propylthieno[3,2-*d*]pyrimidin-3(4*H*)-yl)-6-(thiophen-2-yl)isophthalonitrile (1a)

To a mixture of 3-(2-oxo-2-(thiophen-2-yl)ethyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6a**) (200 mg, 0.62 mmol), 1-benzyl-1*H*-1,2,3-triazole-4-carbaldehyde (**8**) (176 mg, 0.94 mmol) and malononitrile (104 mg, 1.57 mmol) in dry DMF (3 ml) taken in round bottom flask equipped with reflux condenser under argon atmosphere, was added morpholine (0.1 ml, 1 mmol) at 0 ° C. The mixture was allowed to come to RT and then stirred at 80 ° C for 12 h. It was then cooled to room temperature, diluted with ice cold water (30 ml), extracted with ethyl acetate (3 x 20 ml) dried over Na_2SO_4 filtered and concentrated and purified by column chromatography on silica gel (60-120 mesh) using pet. ether- ethyl acetate (30% ethyl acetate in pet. ether) as an eluent to afford 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(4-oxo-6-propylthieno[3,2-*d*]pyrimidin-3(4*H*)-yl)-6-(thiophen-2-yl)isophthalonitrile (**1a**) as a white solid, 156 mg, 43% yield. Melting point: 211 ° C.

Yield: 43%.

Melting point: 211 °C.

IR ($CHCl_3$): 3398, 2218, 1682, 1571 cm^{-1}.



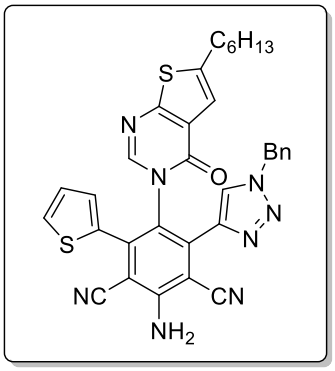
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01 (t, J = 7Hz, 3H), 1.68-1.81 (m, 2H), 2.79 (t, J = 8Hz, 2H), 5.47 (A of ABq, J=16 Hz, 1H), 5.52 (B of ABq, J=16 Hz, 1H), 5.62 (s, 2H, D_2O exchangeable), 6.95-7.10 (m, 4H), 7.26-7.32 (m, 4H), 7.42 (d, J = 5Hz, 1H), 7.66 (s, 1H), 7.78 (d, J = 9Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.70, 24.14, 32.61, 54.31, 97.82, 99.39, 114.48, 114.58, 118.37, 123.89, 124.46, 124.91, 127.57 (2C), 128.79, 129.11 (2C), 129.92, 130.49, 130.68, 132.07, 133.65, 138.61, 139.95, 143.35, 144.87, 145.99, 152.49, 157.52, 162.22.

HRMS (ESI) *m/z* calculated for $C_{30}H_{23}N_8OS_2$ $[M+H]^+$: 575.1431, found: 575.1417.

The following compounds were prepared by using similar procedure

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(6-hexyl-4-oxothieno[3,2-*d*]pyrimidin-3(4*H*)-yl)-6-(thiophen-2-yl)isophthalonitrile (1b)



Yield: 45%.

Melting point: 185 °C.

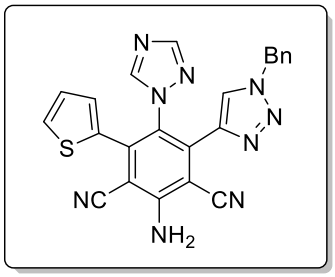
IR ($CHCl_3$): 3395, 2220, 1673, 1584 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 7Hz, 3H), 1.28-1.49 (m, 6H), 1.68-1.80 (m, 2H), 2.81 (t, J = 7Hz, 2H), 5.44 (A of ABq, J=16 Hz, 1H), 5.52 (B of ABq, J=16 Hz, 1H), 5.65 (bs, 2H), 6.93-7.10 (m, 4H), 7.23-7.33 (m, 4H), 7.39 (d, J = 5Hz, 1H) 7.66 (s, 1H), 7.78 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02, 22.49, 28.75, 30.61, 30.84, 31.45, 54.27, 97.82, 99.37, 114.47, 114.57, 118.22, 123.88, 124.47, 124.90 127.55 (2C), 127.72, 128.74, 129.08 (2C), 129.84, 130.46, 132.12, 133.70, 138.63, 139.96, 143.34, 145.10, 145.95, 152.54, 157.55, 162.33.

HRMS (ESI) m/z calculated for $C_{33}H_{29}N_8OS_2$ $[M+H]^+$: 617.1900, found: 617.1894; $C_{33}H_{28}N_8NaOS_2$ $[M+Na]^+$: 639.1720, found: 639.1713.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-6-(thiophen-2-yl)-5-(1*H*-1,2,4-triazol-1-yl)isophthalonitrile (1c):



Yield: 44%.

Melting point: 242 °C.

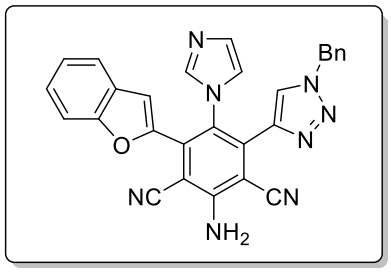
IR ($CHCl_3$): 3356, 2210, 1562 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ 5.42 (s, 2H), 6.69 (s, 2H), 6.92 (t, J = 4Hz, 1H), 7.03 (d, J = 6Hz, 2H), 7.08 (d, J = 3Hz, 1H), 7.25-7.31 (m, 3H), 7.37 (d, J = 5Hz, 1H), 7.39 (s, 1H), 7.64 (s, 1H), 7.85 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 52.74, 96.59, 97.63, 114.62, 114.75, 123.41, 125.16, 127.04, 127.30 (2C), 128.02, 128.64 (2C), 129.90, 130.44, 132.49, 135.47, 138.47, 138.88, 142.41, 147.20, 151.46, 153.68.

MS *m/z* : 450.0 $[M+ H]^+$.

2-Amino-4-(benzofuran-2-yl)-6-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(1*H*-imidazol-1-yl)isophthalonitrile (1d)



Yield: 38%.

Melting point: 227 °C.

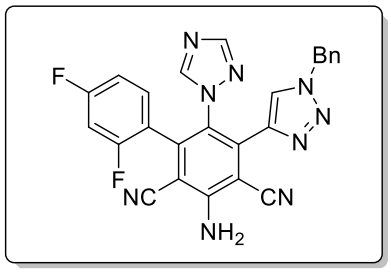
IR ($CHCl_3$): 3350, 2219, 1561 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45 (bs, 2H), 5.83 (bs, 2H), 6.45 (bs, 1H), 6.72 (bs, 2H), 7.02 (bs, 1H), 7.10-7.25 (m, 3H), 7.30-7.60 (m, 7H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 54.19, 95.35, 96.69, 110.53, 111.48, 114.82, 114.91, 120.89, 122.11, 122.48, 123.03, 123.67, 126.81, 127.38, 127.97 (2C), 128.85, 129.10 (2C), 129.96, 133.57, 136.25, 137.91, 137.97, 139.14, 146.18, 153.33, 154.80.

HRMS (ESI) *m/z* calculated for $C_{28}H_{19}N_8O$ $[M+H]^+$: 483.1676, found: 483.1661; $C_{28}H_{18}N_8NaO$ $[M+Na]^+$: 505.1496, found: 505.1473.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1e)



Yield: 49%.

Melting point: 222 °C.

IR ($CHCl_3$): 3397, 2223, 1592 cm^{-1}.

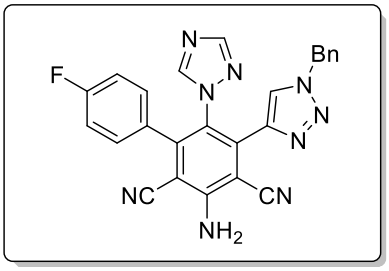
^{1}H NMR (200 MHz, DMSO-d_6): δ 5.60 (s, 2H), 6.98-7.11 (m, 2H), 7.12-7.24 (m, 2H), 7.28-7.49 (m, 6H), 7.78 (s, 1H), 7.99 (s, 1H), 8.38 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 53.89, 97.28, 98.46, 104.22 (t), 111.66 (d), 113.67, 114.24, 116.75, 123.65, 123.77, 127.64 (2C), 128.60, 128.86 (2C), 131.00, 133.33, 137.75, 138.97, 142.86, 145.63, 151.49, 153.05, 158.66 (dd), 163.44 (dd).

HRMS (ESI) *m/z* calculated for $C_{25}H_{16}F_2N_9$ $[M+H]^+$: 480.1491, found: 480.1585; $C_{25}H_{15}F_2N_9Na$ $[M+Na]^+$: 502.1311, found: 502.1409.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-4'-fluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1f)

Yield: 52%.



Melting point: 203 °C.

IR ($CHCl_3$): 3356, 2206, 1560 cm^{-1}.

^{1}H NMR (200 MHz, DMSO-d_6): δ 5.61 (s, 2H), 7-7.16 (m, 3H), 7.22-747 (m, 6H), 7.54 (bs, 2H), 7.79 (s, 1H), 8.02 (s, 1H), 8.44 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 52.54, 95.56, 96.60, 113.43, 113.61, 114.22, 114.39, 122.18, 123.38, 126.52 (2C), 127.36, 127.77 (2C), 129.24, 129.31, 133.32, 136.97, 138.15, 145.31, 147.44, 150.27, 150.30, 152.56, 161.68 (d).

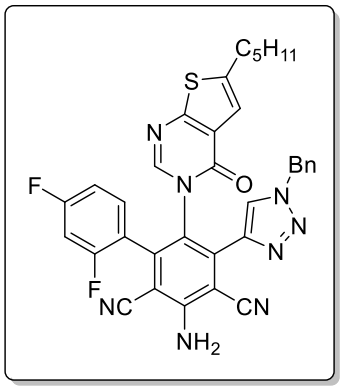
HRMS (ESI) *m/z* calculated for $C_{25}H_{17}FN_9$ $[M+H]^+$: 462.1585, found: 462.1579.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-6-(4-oxo-6-pentylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1g)

Yield: 31%.

Melting point: 196 °C.

IR ($CHCl_3$): 3341, 2220, 1691, 1573 cm^{-1}.

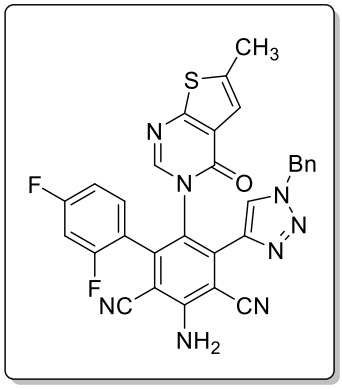


^{1}H NMR (200 MHz, $CDCl_3$): δ 1.37 (t, J= 7Hz, 3H), 1.65-1.92 (m, 4H), 2.11-2.24 (m, 2H), 3.24 (t, J= 8Hz, 2H), 5.91 (A of ABq, J=15 Hz, 1H), 5.99 (B of ABq, J=16 Hz, 1H), 6.0 (bs, 2H), 7.23-7.42 (m, 3H), 7.43-7.60 (m, 2H), 7.66-7.84 (m, 4H), 8.14 (s, 1H), 8.21 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.90, 22.31, 30.51, 30.53, 31.20, 54.32, 98.22, 99.70, 104.64 (t), 112.54 (d), 113.75, 114.53, 118.04, 123.58, 124.43, 125.18, 127.64 (2C), 128.78, 128.83, 129.13 (2C), 131.18, 131.28, 133.59, 138.41, 138.77, 139.75, 144.08, 145.20, 145.41, 152.36, 157.21, 162.35.

HRMS (ESI) *m/z* calculated for $C_{34}H_{27}F_2N_8OS$ $[M+H]^+$: 633.1991, found: 633.1980; $C_{34}H_{26}F_2N_8NaOS$ $[M+Na]^+$: 655.1811, found: 655.1797.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-6-(6-methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1h)



Yield: 37%.

Melting point: 248 °C.

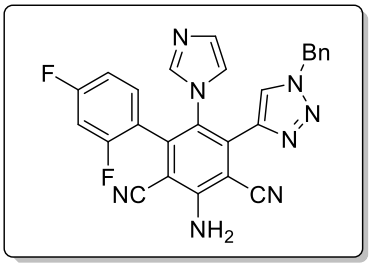
IR ($CHCl_3$): 3314, 2219, 1681, 1574 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.50, (s, 3H), 5.44 (A of ABq, J=16 Hz, 1H), 5.52 (B of ABq, J=16 Hz, 1H), 5.65, (bs, 2H), 6.73-6.97 (m, 3H), 7.01- 7.14 (m, 2H), 7.22-7.36 (m, 4H), 7.68 (d, J = 2Hz, 1H), 7.75 (t, J = 5Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$ +DMSO-d_6): δ 15.97, 54.34, 98.21, 99.68, 104.64 (t), 112.54 (d), 113.75, 114.52, 119.35, 123.84, 124.41, 125.16, 127.63, 127.70 (2C), 128.79, 128.83, 129.12 (2C), 131.19 (dd), 133.55, 138.42, 139.16, 139.73, 144.07, 145.47, 145.97, 152.36, 157.08, 162.65.

HRMS (ESI) *m/z* calculated for $C_{30}H_{19}F_2N_8OS$ $[M+H]^+$: 577.1365, found: 577.1359; $C_{30}H_{18}F_2N_8NaOS$ $[M+Na]^+$: 599.1185, found: 599.1176.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-6-(1*H*-imidazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1i)



Yield: 43%.

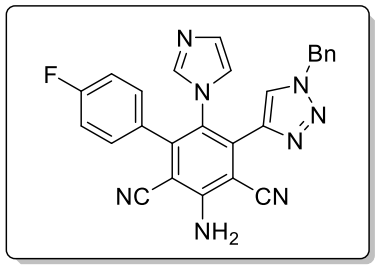
Melting point: 164 °C.

IR ($CHCl_3$): 2217, 1648, 1563 cm^{-1}.

^{1}H NMR (200 MHz, DMSO-d_6): δ 5.60 (s, 2H), 6.66 (bs, 2H), 6.95-7.20 (m, 3H), 7.21-760 (m, 8H), 7.87 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6): δ 53.52, 96.93, 97.98, 104.01 (t), 111.51 (d), 113.64, 114.23, 116.95 (dd), 120.51 (d), 122.81, 123.49, 127.29 (2C), 128.22, 128.53 (2C), 128.93, 130.43 (d), 133.29, 137.02, 137.39, 138.82, 141.97, 152.49, 158.47 (dd), 163.07 (dd).

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-4'-fluoro-6-(1*H*-imidazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (1j)



Yield: 47%.

Melting point: 120 °C.

IR ($CHCl_3$): 2726, 1647, 1560 cm^{-1}.

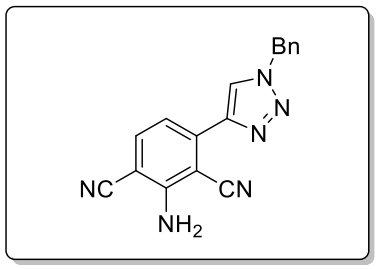
^{1}H NMR (200 MHz, DMSO-d_6): δ 5.59 (s, 2H), 6.63 (s, 1H), 6.88 (s, 1H), 6.95-7.08 (m, 2H), 7.10-7.47 (m, 10H), 7.83 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 53.60, 96.33, 97.54, 114.17, 114.39, 115.25, 115.43, 120.88, 122.80, 123.01, 127.36 (2C), 128.28, 128.59 (2C), 128.99, 129.03, 129.49, 129.56, 133.38, 136.90, 137.69, 139.06, 147.51, 152.59, 162.54 (d).

Synthesis of 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl) isophthalonitrile (9)

To a mixture of 1-benzyl-1*H*-1,2,3-triazole-4-carbaldehyde (100 mg, 0.53 mmol), acetaldehyde (0.05 ml, 0.8 mmol), and malononitrile (0.08 ml, 1.35 mmol) in dry DMF (3 ml) taken in round bottom flask equipped with reflux condenser under argon atmosphere, was added morpholine (0.055 ml, 0.65 mmol) at 0°C. The mixture was allowed to come to RT and then stirred at 80°C for 8 h. It was then cooled to room temperature, diluted with ice cold water (30 ml), extracted with ethyl acetate (3 x 30 ml), dried over anhydrous Na_2SO_4 filtered and concentrated and purified by column chromatography on silica gel (60-120 mesh) using pet ether- ethyl acetate (15

% ethyl acetate in pet ether) as an eluent to give 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)isophthalonitrile as a white solid, 80 mg, 50%. Melting point: 190 °C.



Yield: 50%.

Melting point: 190 °C.

IR ($CHCl_3$): 2213, 1586 cm^{-1}.

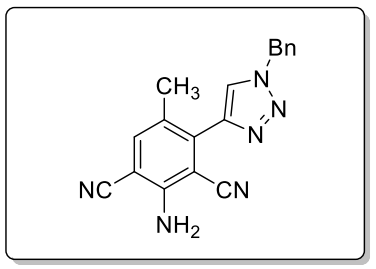
^{1}H NMR (200 MHz, DMSO-d6): δ 5.74 (s, 2H) 6.82 (s, 2H), 7.28 (d, J = 8 Hz, 1H), 7.31-7.45 (m, 5H), 7.85 (d, J = 8 Hz, 1H), 8.84 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 54.52, 93.30, 95.94, 115.81, 116.16, 116.79, 123.09, 128.02 (2C), 129.01, 129.24 (2C), 134.04, 136.98, 138.62, 143.19, 152.12.

HRMS (ESI) *m/z* calculated for $C_{17}H_{13}N_6$ $[M+H]^+$: 301.1196, found: 301.1202; $C_{17}H_{12}N_6Na$ $[M+Na]^+$: 323.1016, found: 323.1022.

The following compounds were prepared by using similar procedure

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-methylisophthalonitrile (10a)



Yield: 61%.

Melting point: 174 °C.

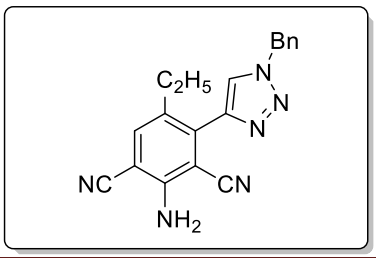
IR ($CHCl_3$): 3344, 2225, 1597 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (s, 3H), 5.08 (bs, 2H), 5.66 (s, 2H), 7.29-7.36 (m, 2H), 7.37-7.44 (m, 3H), 7.51 (s, 1H), 7.78 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 19.13, 53.69, 96.24, 97.47, 115.33, 115.56, 123.80, 126.13, 127.41 (2C), 128.29, 128.66 (2C), 133.95, 137.82, 138.05, 142.29, 150.16.

HRMS (ESI) *m/z* calculated for $C_{18}H_{15}N_6$ $[M+H]^+$: 315.1353, found: 315.1361; $C_{18}H_{14}N_6Na$ $[M+Na]^+$: 337.1172, found: 337.1180.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-ethylisophthalonitrile (10b)



Yield: 64%.

Melting point: 168 °C.

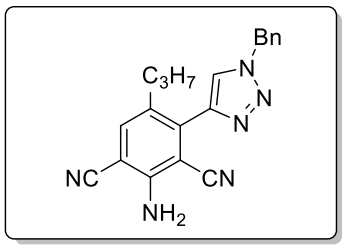
IR ($CHCl_3$): 3313, 2220, 1600 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.08 (t, J = 7Hz, 3H), 2.63 (q, J = 7 Hz, 2H), 5.08 (bs, 2H), 5.66 (s, 2H), 7.28-7.35 (m, 2H), 7.36-7.48 (m, 3H), 7.54 (s, 1H) 7.75 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.07, 25.59, 54.39, 97.38, 98.43, 115.57, 115.78, 123.84, 127.95 (2C), 128.95, 129.26 (2C), 133.93, 134.15, 137.14, 137.92, 142.68, 149.77.

HRMS (ESI) *m/z* calculated for $C_{19}H_{17}N_6$ $[M+H]^+$: 329.1509, found: 329.1502; $C_{19}H_{16}N_6Na$ $[M+Na]^+$: 351.1329, found: 351.1321.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-propylisophthalonitrile (10c)
Yield: 59%.



Melting point: 132 °C.

IR ($CHCl_3$): 3313, 2221 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, J = 7Hz, 3H), 1.09-1.48 (m, 2H), 2.54 (t, J = 7Hz, 3H), 5.10 (bs, 2H), 5.66 (s, 2H), 7.24-7.35 (m, 2H), 7.36-7.46 (m, 3H), 7.51 (s, 1H), 7.73 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 13.12, 23.48, 33.67, 53.62, 96.53, 97.91, 115.21, 115.60, 123.68, 127.21 (2C), 128.21, 128.61 (2C), 131.05, 134.08, 137.39, 137.74, 142.20, 149.99.

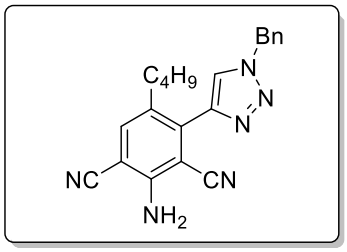
HRMS (ESI) *m/z* calculated for $C_{20}H_{19}N_6$ $[M+H]^+$: 343.1666, found: 343.1661.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-butylisophthalonitrile (10d)

Yield: 60%.

Melting point: 163 °C.

IR ($CHCl_3$): 3401, 2224, 1528 cm^{-1}.

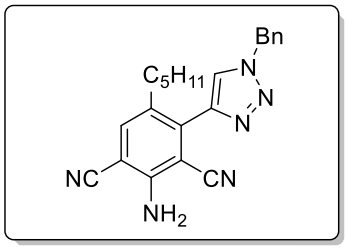


^{1}H NMR (200 MHz, $CDCl_3$): δ 0.78 (t, J= 7Hz, 3H), 1.08-1.45 (m, 4H), 2.56 (t, J= 8Hz, 2H), 5.10 (s, 2H), 5.66 (s, 2H), 7.28-7.35 (m, 2H), 7.36-7.46 (m, 3H), 7.51 (s, 1H), 7.73 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.68, 22.15, 31.98, 32.99, 54.34, 97.24, 98.49, 115.52, 115.77, 123.79, 127.86 (2C), 128.90, 129.21 (2C), 132.66, 134.24, 137.75, 138.04, 142.73, 149.79.

HRMS (ESI) *m/z* calculated for $C_{21}H_{21}N_6$ $[M+H]^+$: 357.1822, found: 357.1819.

2-Amino-4-(1-benzyl-1H-1,2,3-triazol-4-yl)-5-pentylisophthalonitrile (10e)



Yield: 61%.

Melting point: 190 °C.

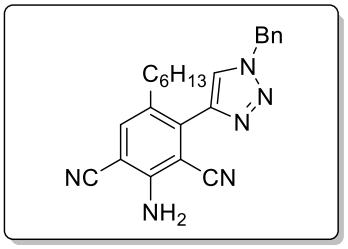
IR ($CHCl_3$): 3395, 2224 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, *J*=7Hz, 3H), 1.05-1.25 (m, 4H), 1.28-1.47 (m, 2H), 2.56 (t, *J*= 8Hz, 2H), 5.07 (s, 2H), 5.66 (s, 2H), 7.26-7.34 (m, 2H), 7.35-7.47 (m, 3H), 7.51 (s, 1H), 7.72 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.80, 22.15, 30.46, 31.14, 32.15, 54.23, 97.11, 98.36, 115.49, 115.77, 123.78, 127.78 (2C), 128.79, 129.11 (2C), 132.46, 134.23, 137.70, 137.97, 142.64, 149.87.

HRMS (ESI) *m/z* calculated for $C_{22}H_{23}N_6$ $[M+H]^+$: 371.1979, found: 371.1973; $C_{22}H_{22}N_6Na$ $[M+Na]^+$: 393.1798, found: 393.1791.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-hexylisophthalonitrile (10f)



Yield: 63%.

Melting point: 133 °C.

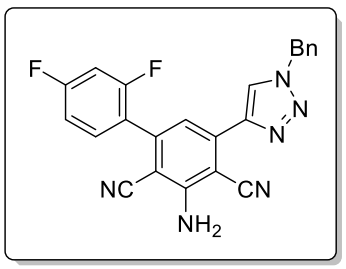
IR ($CHCl_3$): 3398, 2221 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, *J*= 7Hz, 3H), 1.08-1.45 (m, 8H), 2.46-2.65 (m, 2H), 5.08 (s, 2H), 5.66 (s, 2H), 7.28-7.35 (m, 2H), 7.36-7.46 (m, 3H), 7.51 (s, 1H), 7.72 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.37, 28.70, 30.76, 31.32, 32.25, 54.26, 97.13, 98.39, 115.51, 115.78, 123.78, 127.81 (2C), 128.83, 129.14 (2C), 132.52, 134.23, 137.72, 137.99, 142.67, 149.85.

HRMS (ESI) *m/z* calculated for $C_{23}H_{25}N_6$ $[M+H]^+$: 385.2135, found: 385.2130.

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-[1,1'-biphenyl]-2,4-dicarbonitrile (11)



Yield: 55%.

Melting point: 189 °C.

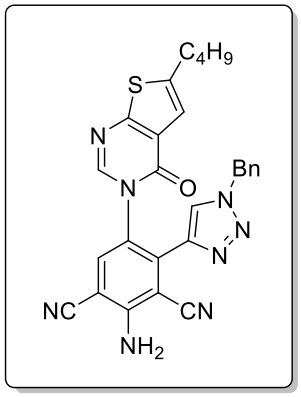
IR ($CHCl_3$): 3274, 2403, 1532 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (s, 2H), 5.64 (s, 2H), 6.90 -7.08 (m, 2H), 7.29-7.36 (m, 2H), 7.37-7.48 (m, 4H), 7.73 (s, 1H), 8.37 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d6): δ 53.18, 92.36, 95.54, 104.64 (t), 112.23 (dd), 115.34, 115.78, 116.96, 121.77, 125.05, 128.06 (2C), 128.31, 128.84 (2C), 132.44 (dd), 135.62, 138.08, 142.59, 143.56, 153.90, 158.92 (dd), 163.03 (dd).

HRMS (ESI) *m/z* calculated for $C_{23}H_{15}F_2N_6$ $[M+H]^+$: 413.1321, found: 413.1319; $C_{23}H_{14}F_2N_6Na$ $[M+Na]^+$: 435.1140, found: 435.1137.

2-Amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(6-butyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)isophthalonitrile (12)



Yield: 65%.

Melting point: 202 °C.

IR ($CHCl_3$): 3398, 2225, 1684, 1569 cm^{-1}.

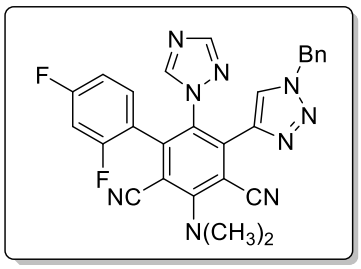
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (t, *J*= 7Hz, 3H), 1.37-1.48 (m, 2H), 1.68-1.75 (m, 2H), δ 2.85 (t, *J*= 7Hz, 2H), 5.47 (A of ABq, J=16 Hz, 1H), 5.52 (B of ABq, J=16 Hz, 1H), 5.64 (s, 2H), 7.04 (s, 1H), 7.05-7.14 (m, 2H), 7.27-7.34 (m, 3H), 7.62 (s, 1H), 7.79 (s, 1H), 7.84 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.71, 22.06, 30.28, 33.05, 54.32, 97.40, 98.13, 114.45, 114.62, 118.29, 124.21, 124.48, 125.37, 127.71 (2C), 128.83, 129.11 (2C), 133.61, 137.54, 138.09, 139.70, 145.38, 145.44, 152.39, 157.11, 162.43.

Synthesis of 5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-3-(dimethylamino)-2',4'-difluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (13e)

3-Amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2',4'-difluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (**1e**) (100 mg, 0.2 mmol) in dry THF (10 ml) was stirred with NaH (19 mg, 0.83 mmol) under argon atmosphere at 60 ºC for 12 h. It was then cooled to RT, methyl iodide (0.14 ml, 340 mg, 2.4 mmol) was added and the reaction mixture was stirred at RT for 12 h. It was then quenched with methanol (2 ml), solvent was removed on rotavapor, water (2 ml) was added and the product was extracted with ethyl acetate (3 x 15 ml). The organic layer was dried with anhydrous Na_2SO_4 filtered and concentrated the residue obtained was purified by

column chromatography to obtain 5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-3-(dimethylamino)-2',4'-difluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (**13e**) as yellow solid (70 mg, 66%). Melting point: 205 °C.



Yield: 66%.

Melting point: 205 °C.

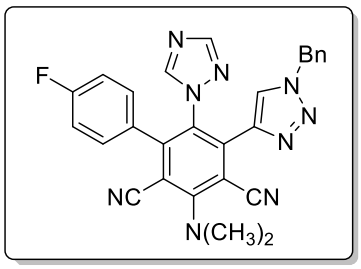
IR ($CHCl_3$): 2229, 1557 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.41 (s, 6H), 5.48 (s, 2H), 6.79-6.88 (m, 2H), 7.05-7.14 (m, 3H), 7.28 (s, 1H), 7.35-7.41 (m, 3H), 7.61 (s, 1H), 7.85 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 44.47 (2C), 54.34, 104.71 (t), 106.91, 107.53, 112.12 (d), 115.25, 115.77, 116.91 (d), 123.99, 127.61, 127.96 (2C), 129.02, 129.25 (2C), 131.08, 133.50, 139.22, 139.99, 144.72, 145.68, 151.92, 159.00, 159.09 (dd), 163.91 (dd).

5-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-3-(dimethylamino)-4'-fluoro-6-(1*H*-1,2,4-triazol-1-yl)-[1,1'-biphenyl]-2,4-dicarbonitrile (13f)

This compound was prepared by procedure described for **13e**.



Yield: 70%.

Melting point: 226 °C.

IR ($CHCl_3$): 2226, 1659, 1558 cm^{-1}.

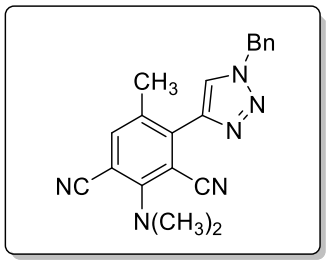
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.39 (s, 6H), 5.47 (s, 2H), 6.93-7.06 (m, 2H), 7.07-7.19 (m, 4H), 7.32-7.42 (m, 4H), 7.61 (s, 1H), 7.73 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 44.39 (2C), 54.22, 106.41, 106.99, 115.67, 115.81 (2C), 116.03, 124.06, 127.17, 127.83 (2C), 128.80 (d), 128.91, 129.16 (2C), 130.20, 130.28, 133.61, 139.33, 139.89, 145.80, 150.20, 151.67, 159.29, 163.21 (d).

HRMS (ESI) *m/z* calculated for $C_{27}H_{21}FN_9$ $[M+H]^+$: 490.1898, found: 490.1899; $C_{27}H_{20}FN_9$ $[M+Na]^+$: 512.1718, found: 512.1721.

Following compounds were prepared by using the procedure similar to **13e** with slight modification. Wherein the compounds were stirred with NaH at room temperature and reacted further with methyl iodide at RT.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(dimethylamino)-5-methylisophthalonitrile (14a)



Yield: 75%.

Melting point: 227 °C.

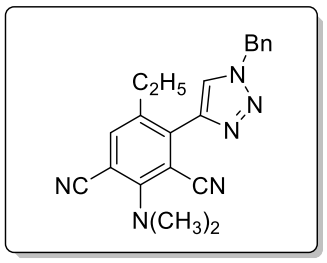
IR ($CHCl_3$): 2228, 1587 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (s, 3H), 3.07 (s, 6H), 5.53 (s, 2H), 7.11-7.22 (m, 2H), 7.23-7.33 (m, 3H), 7.45 (s, 1H), 7.64 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 19.94, 43.86 (2C), 54.32, 106.52, 108.43, 116.87, 117.46, 124.09, 127.92 (2C), 128.88, 129.19 (2C), 131.71, 134.16, 139.86, 140.25, 142.61, 156.76.

HRMS (ESI) *m/z* calculated for $C_{20}H_{19}N_6$ $[M+H]^+$: 343.1666, found: 343.1656; $C_{20}H_{18}N_6Na$ $[M+Na]^+$: 365.1485, found: 365.1475.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(dimethylamino)-5-ethylisophthalonitrile (14b)



Yield: 73%.

Melting point: 121 °C.

IR ($CHCl_3$): 2229, 1584 cm^{-1}.

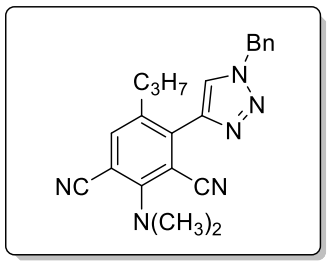
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.08 (t, *J*= 7Hz, 3H), 2.60 (q, *J*= 7Hz, 2H), 3.20 (s, 6H), 5.66 (s, 2H), 7.28-7.33 (m, 2H), 7.34-7.44 (m, 3H), 7.60 (s, 1H), 7.73 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.88, 25.80, 43.86 (2C), 54.32, 106.85, 108.70, 116.79, 117.60, 124.05, 127.85 (2C), 128.86, 129.20 (2C), 134.22, 137.79, 138.97, 139.57, 142.44, 156.54.

HRMS (ESI) *m/z* calculated for $C_{21}H_{21}N_6$ $[M+H]^+$: 357.1822, found: 357.1820.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(dimethylamino)-5-propylisophthalonitrile (14c)

Yield: 70%.



Melting point: 140 °C.

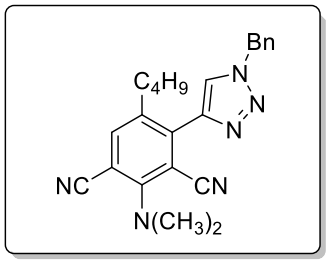
IR ($CHCl_3$): 2229, 1583 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (t, *J*= 7Hz, 3H), 1.38-1.50 (m, 2H), 2.53 (t, *J*= 7Hz, 2H), 3.21 (s, 6H), 5.67 (s, 2H), 7.24-7.33 (m, 2H), 7.33-7.48 (m, 3H), 7.58 (s, 1H), 7.71 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.66, 23.88, 34.56, 43.88 (2C),

54.34, 106.78, 108.85, 116.79, 117.60, 124.03, 127.80 (2C), 128.88, 129.22 (2C), 134.34, 136.43, 139.61, 139.79, 142.58, 156.58.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-5-butyl-2-(dimethylamino)isophthalonitrile (14d)



Yield: 72%.

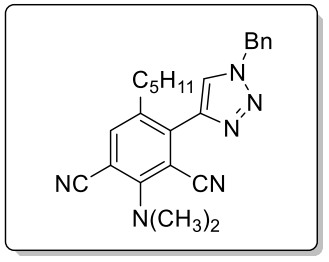
Melting point: 122 °C.

IR ($CHCl_3$): 2228, 1583 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79 (t, *J*= 7Hz, 3H), 1.14-1.24 (m, 2H), 1.32-1.41 (m, 2H), 2.55 (t, *J*= 8Hz, 2H), 3.21 (s, 6H), 5.67 (s, 2H), 7.27-7.31 (m, 2H), 7.35-7.43 (m, 3H), 7.58 (s, 1H), 7.71 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.69, 22.22, 32.24, 32.86, 43.89 (2C), 54.33, 106.71, 108.74, 116.81, 117.63, 123.98, 127.81 (2C), 128.88, 129.21 (2C), 134.32, 136.62, 139.63, 139.76, 142.56, 156.53.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(dimethylamino)-5-pentylisophthalonitrile (14e)



Yield: 69%.

Melting point: 90 °C.

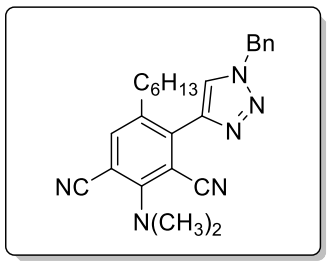
IR ($CHCl_3$): 2227, 1582 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, *J*= 7Hz, 3H), 1.03-1.24 (m, 4H), 1.29-1.49 (m, 2H), 2.54 (t, *J*= 7Hz, 2H), 3.21 (s, 6H), 5.66 (s, 2H), 7.27-7.34 (m, 2H), 7.34-7.46 (m, 3H), 7.57 (s, 1H), 7.70 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.84, 22.21, 30.40, 31.28, 32.52, 43.87 (2C), 54.33, 106.82, 108.85, 116.77, 117.59, 123.97, 127.82 (2C), 128.86, 129.19 (2C), 134.33, 136.73, 139.58, 139.71, 142.56, 156.53.

HRMS (ESI) *m/z* calculated for $C_{24}H_{27}N_6$ $[M+H]^+$: 399.2292, found: 399.2284; $C_{24}H_{26}N_6Na$ $[M+Na]^+$: 421.2111, found: 421. 2102.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(dimethylamino)-5-hexylisophthalonitrile (14f)



Yield: 77%.

Melting point: 83 °C.

IR ($CHCl_3$): 2228, 1582 cm^{-1}.

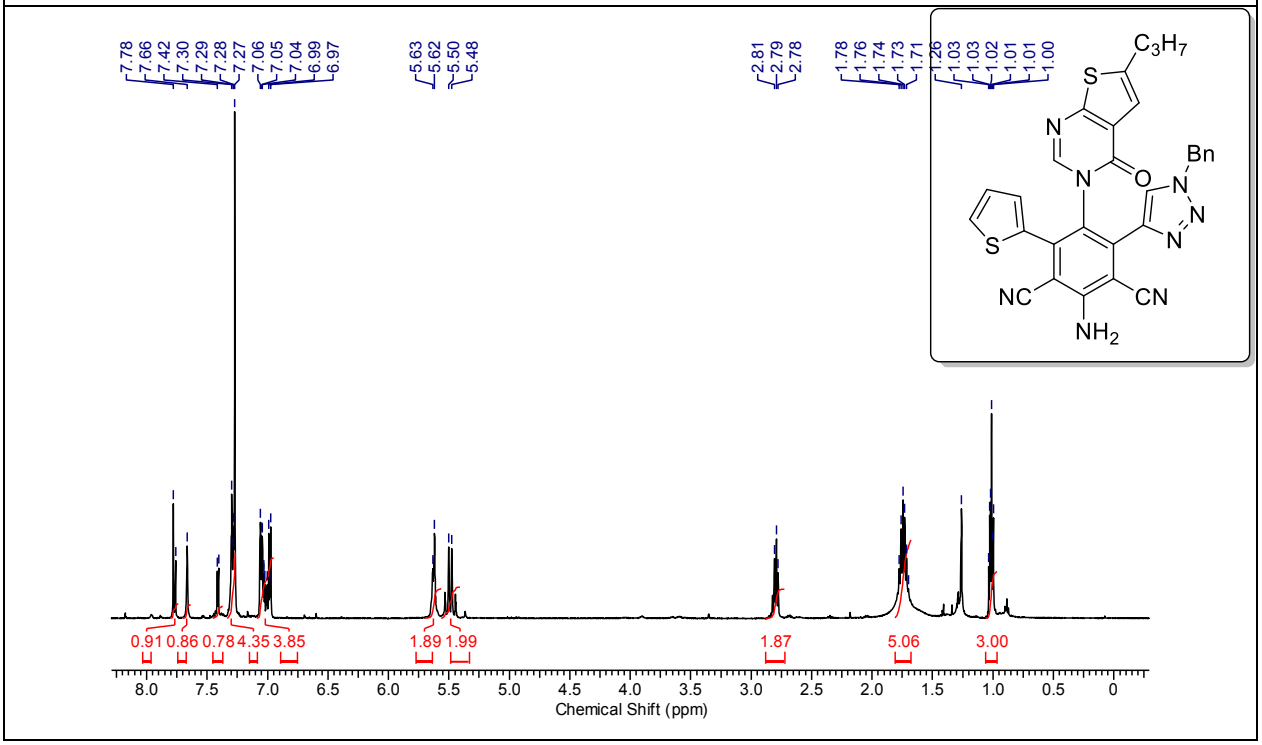
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, *J*= 7Hz, 3H), 1.05-1.28 (m, 6H), 1.28-1.49 (m, 2H), 2.55 (t, *J*= 7Hz, 2H), 3.21 (s, 6H), 5.67 (s, 2H), 7.23-7.33 (m, 2H), 7.33-7.46 (m, 3H), 7.57 (s, 1H), 7.71 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.36, 28.75, 30.62, 31.30, 32.52, 43.81 (2C), 54.24, 106.67, 108.73, 116.73, 117.55, 123.95, 127.74 (2C), 128.79, 129.12 (2C), 134.29, 136.62, 139.53, 139.64, 142.48, 156.45.

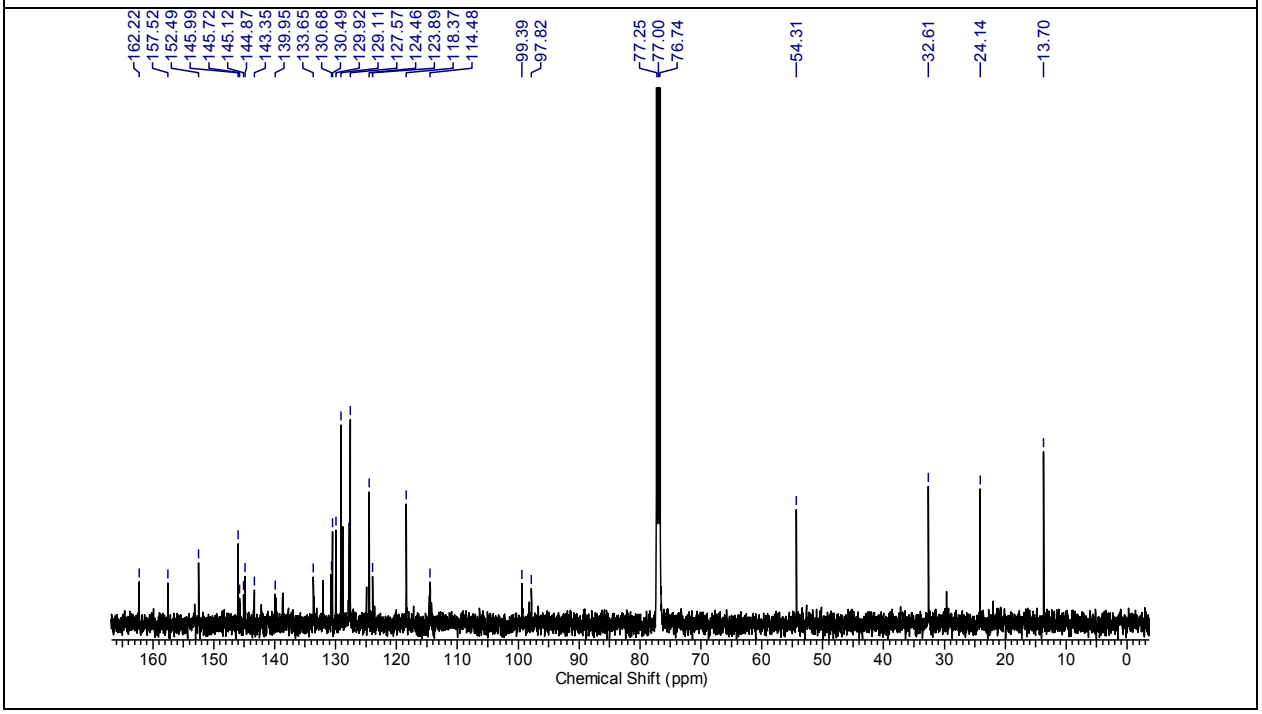
HRMS (ESI) *m/z* calculated for $C_{25}H_{29}N_6$ $[M+H]^+$: 413.2448, found: 413.2439; $C_{25}H_{28}N_6Na$ $[M+Na]^+$: 435.2268, found: 435.2258.

2.2.4.2. Spectral Data

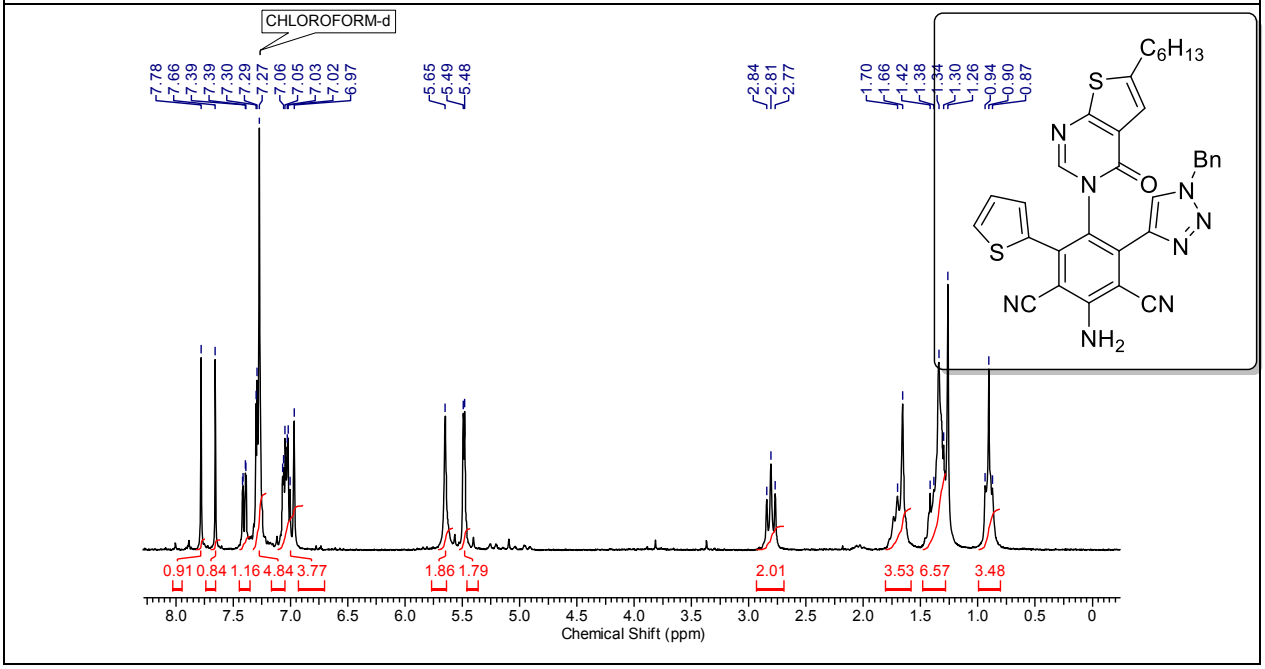
^{1}H NMR (200 MHz, $CDCl_3$) of compound 1a



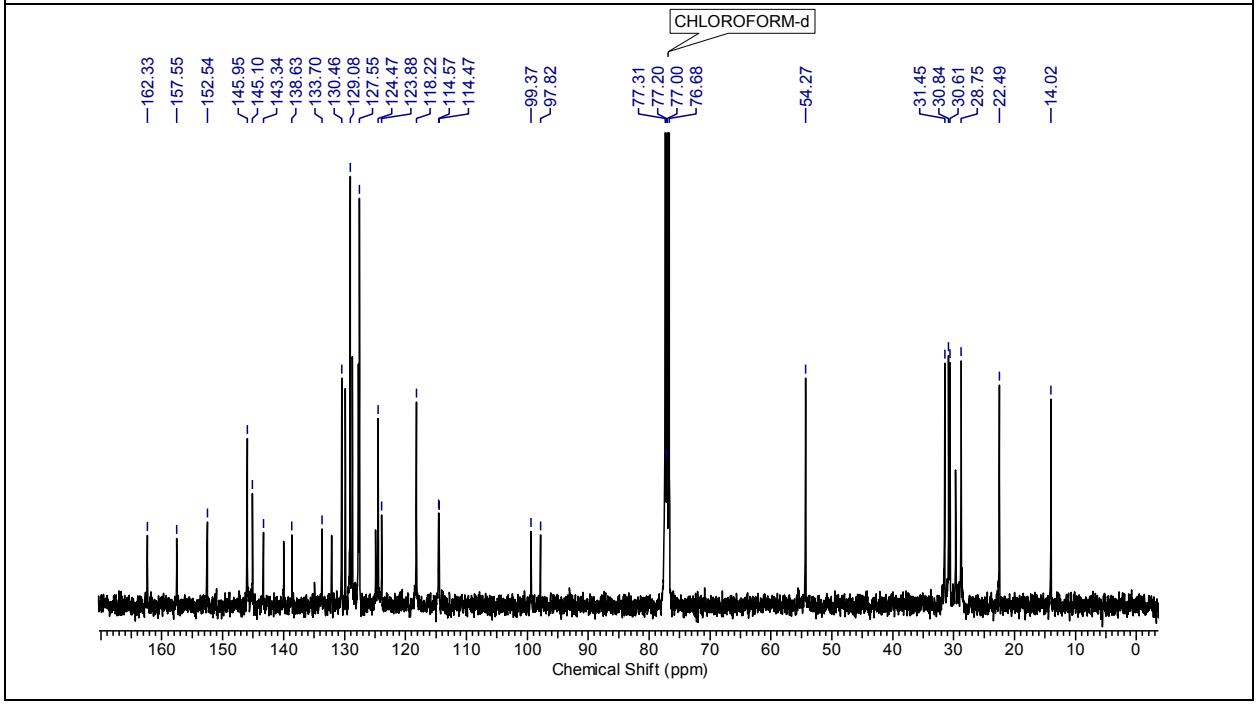
^{13}C NMR (125 MHz, $CDCl_3$) of compound 1a



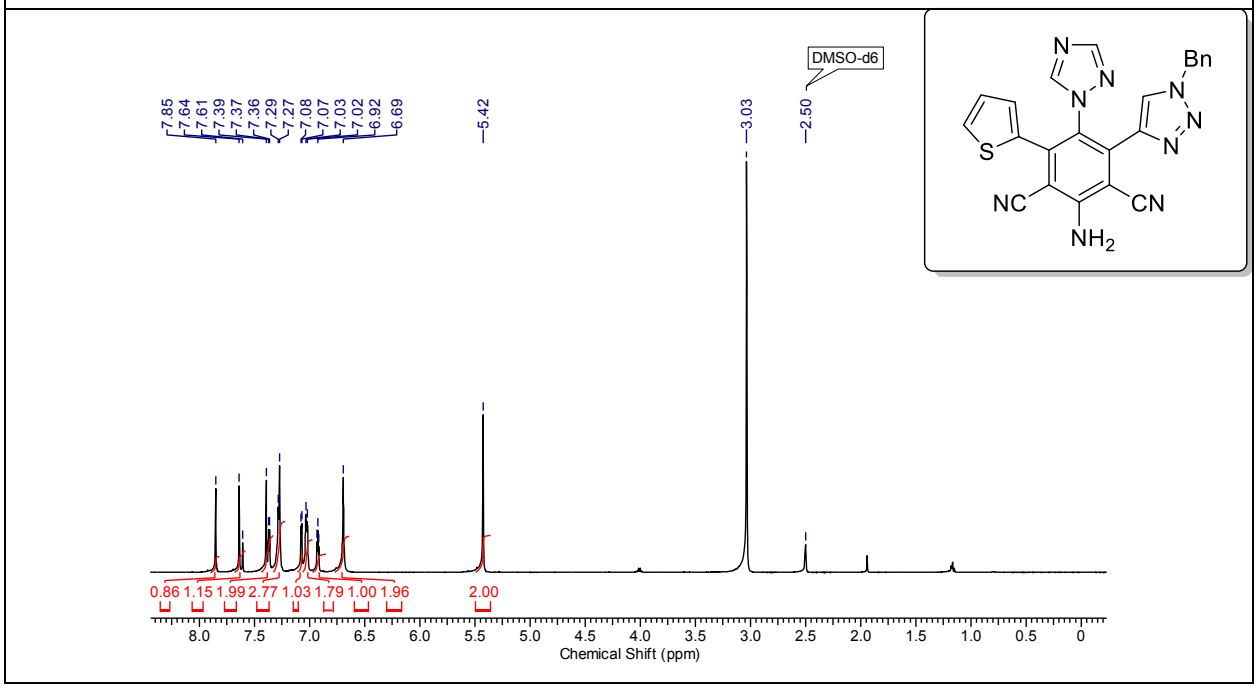
^{1}H NMR (200 MHz, $CDCl_3$) of compound 1b



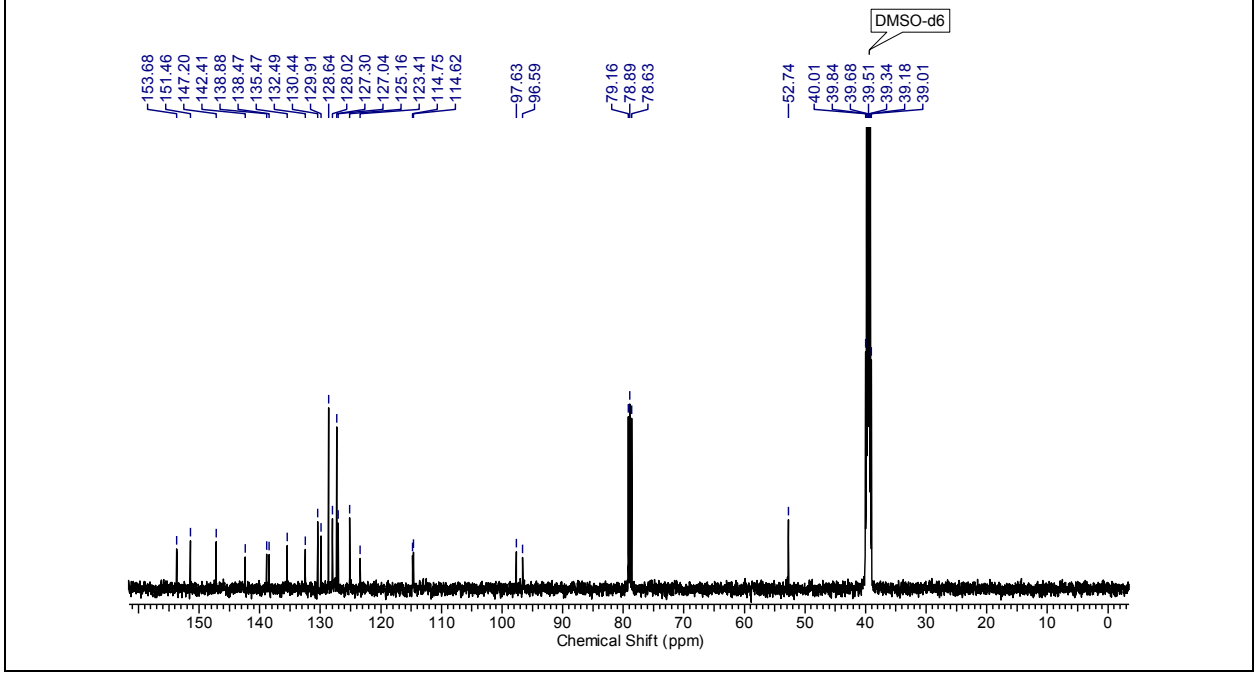
^{13}C NMR (100 MHz, $CDCl_3$) of compound 1b



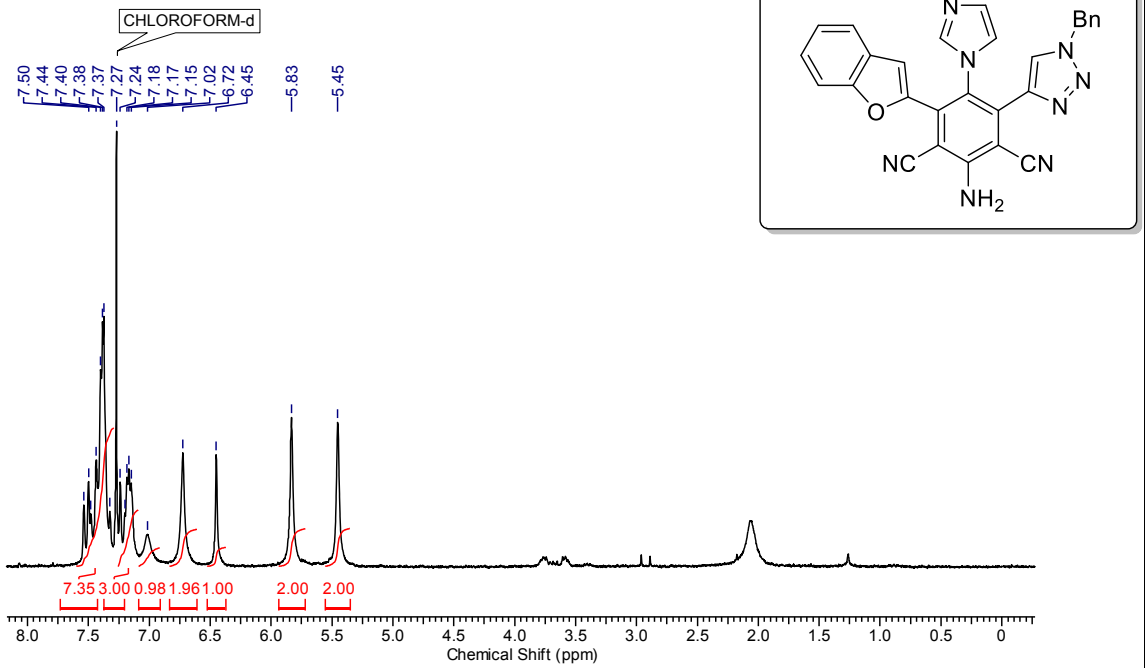
^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) of compound 1c



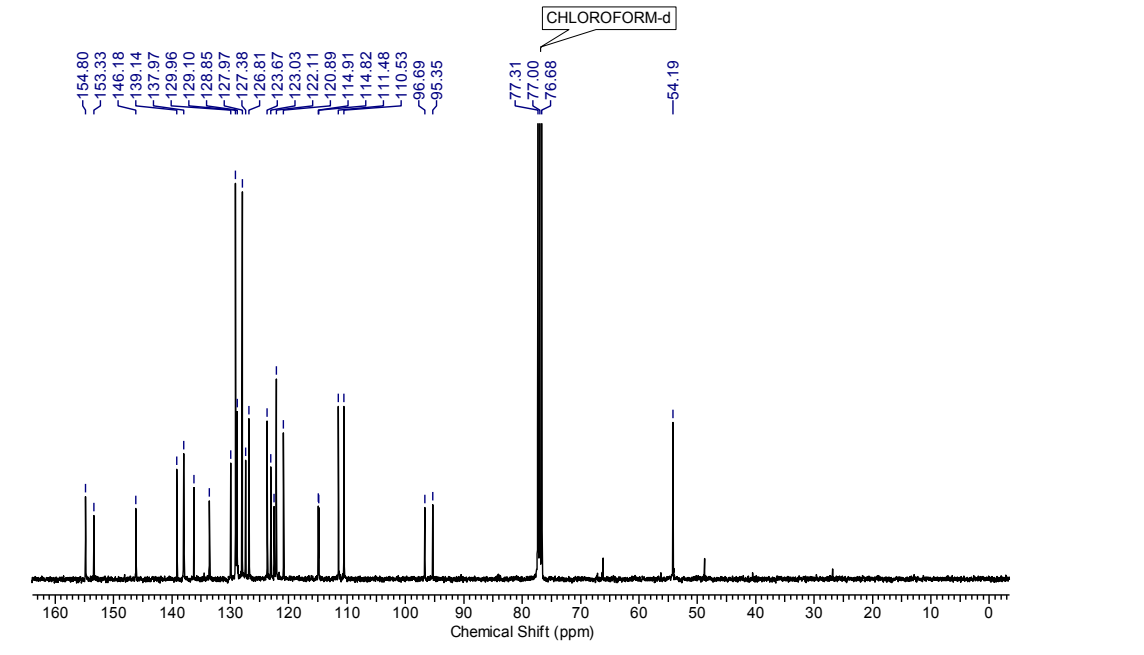
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) of compound 1c



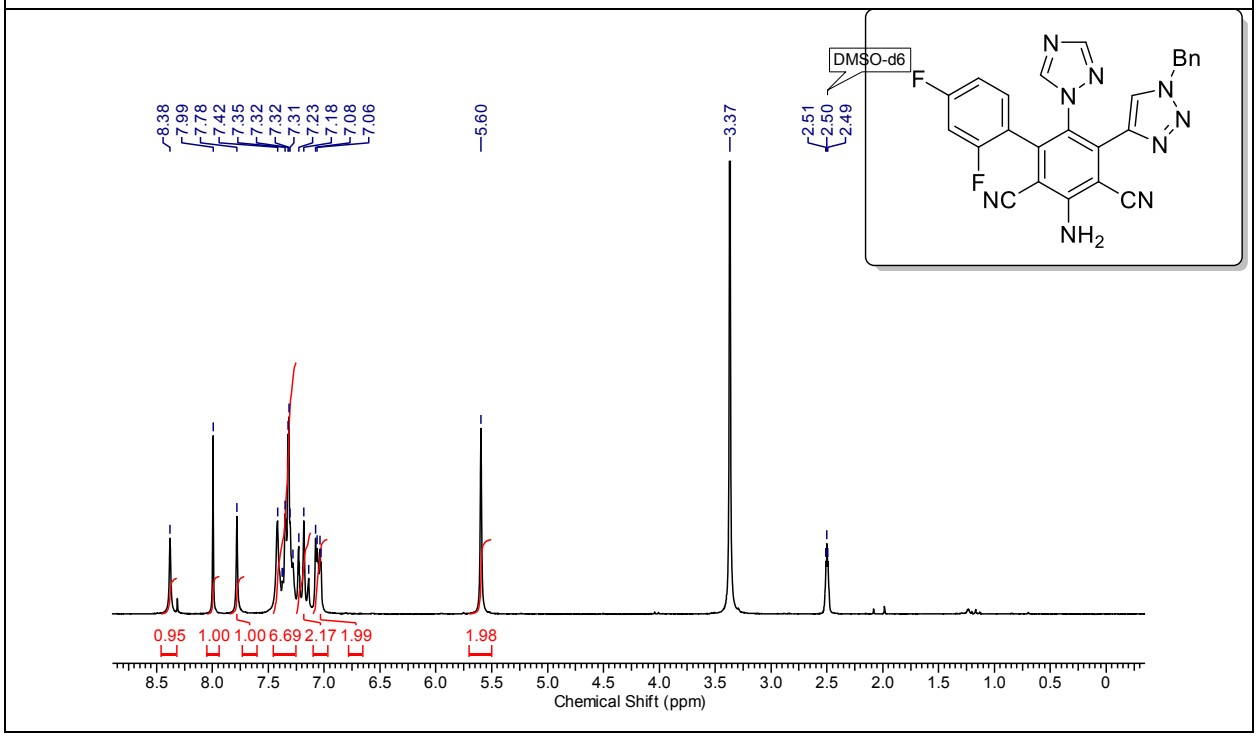
^{1}H NMR (400 MHz, $CDCl_3$) of compound 1d



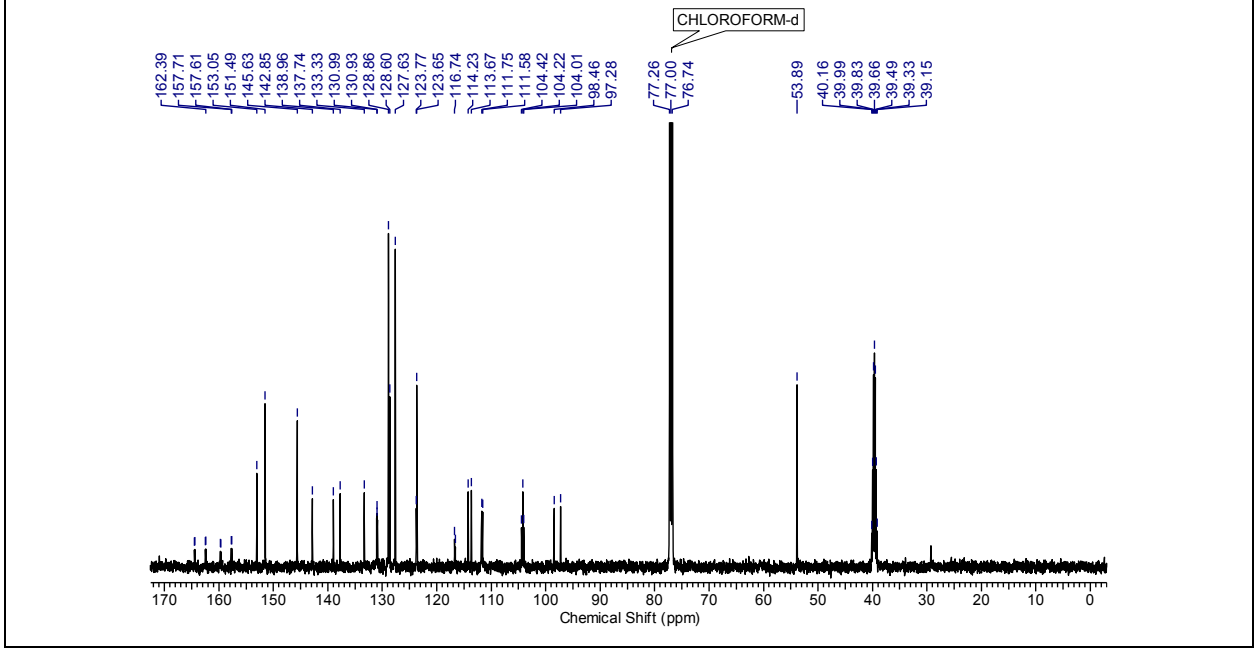
^{13}C NMR (100 MHz, $CDCl_3$) of compound 1d



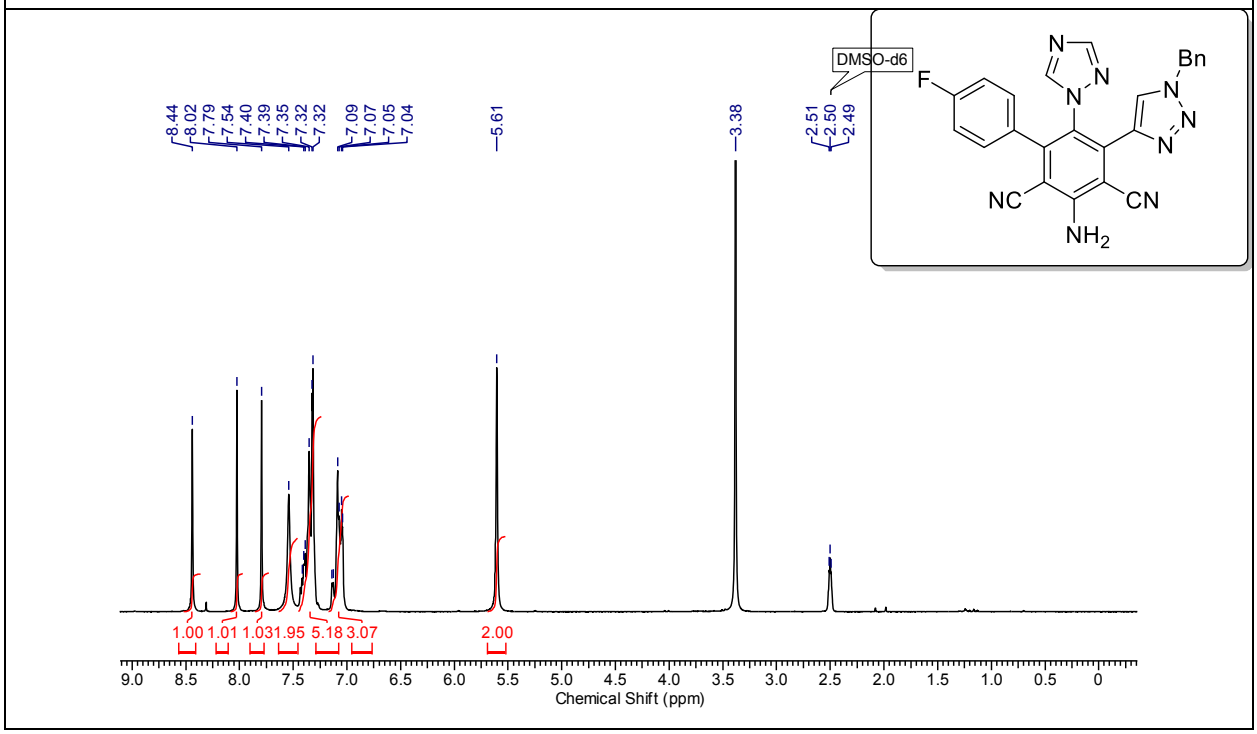
^{1}H NMR (200 MHz, DMSO-d_6) of compound 1e



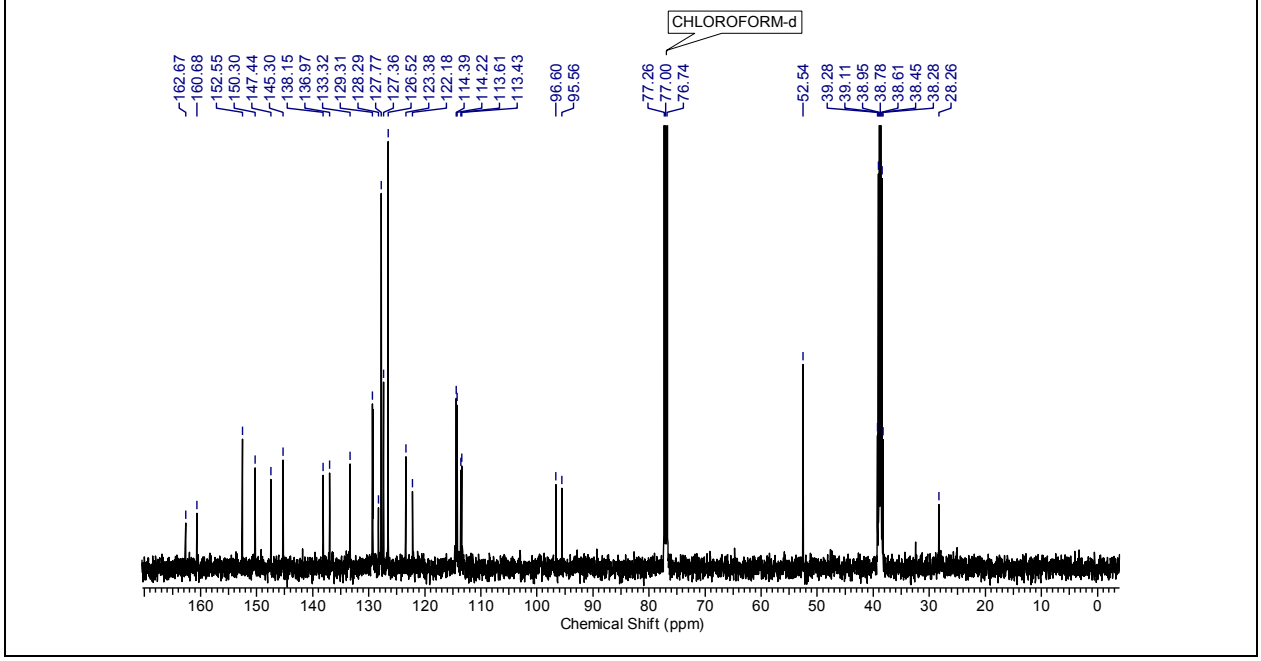
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) of compound 1e



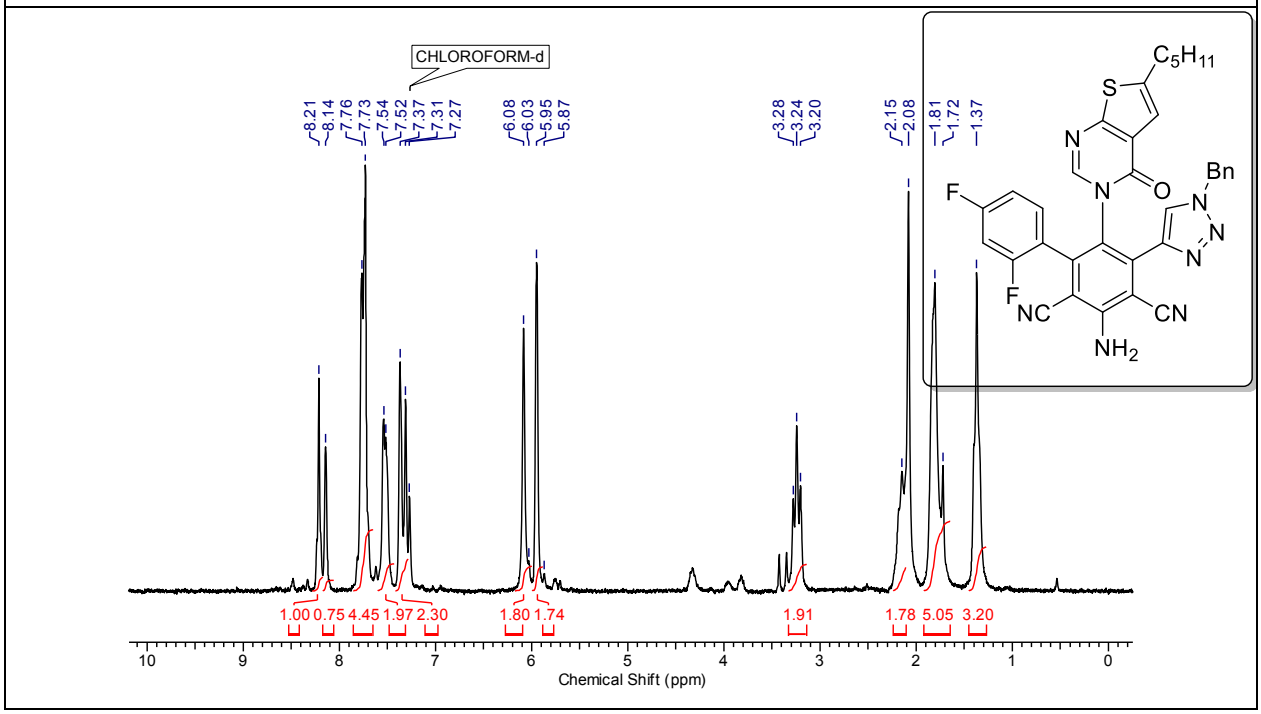
^{1}H NMR (200 MHz, DMSO-d_6) of compound 1f



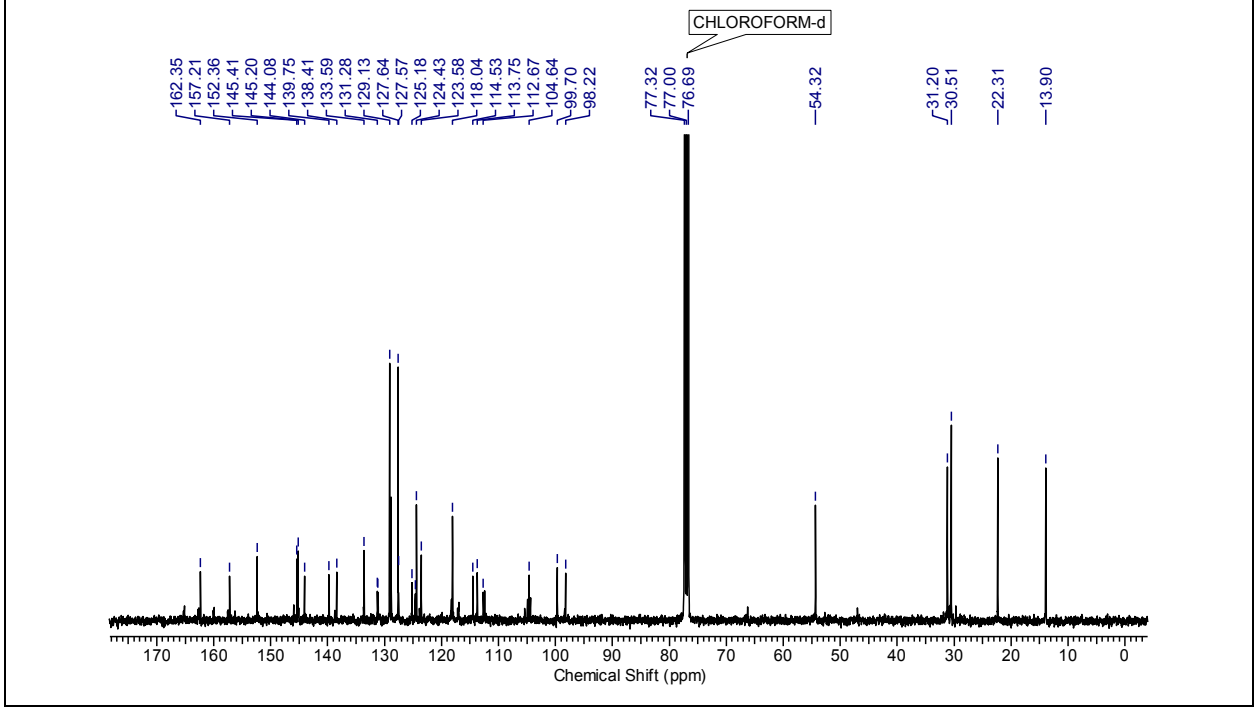
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) of compound 1f



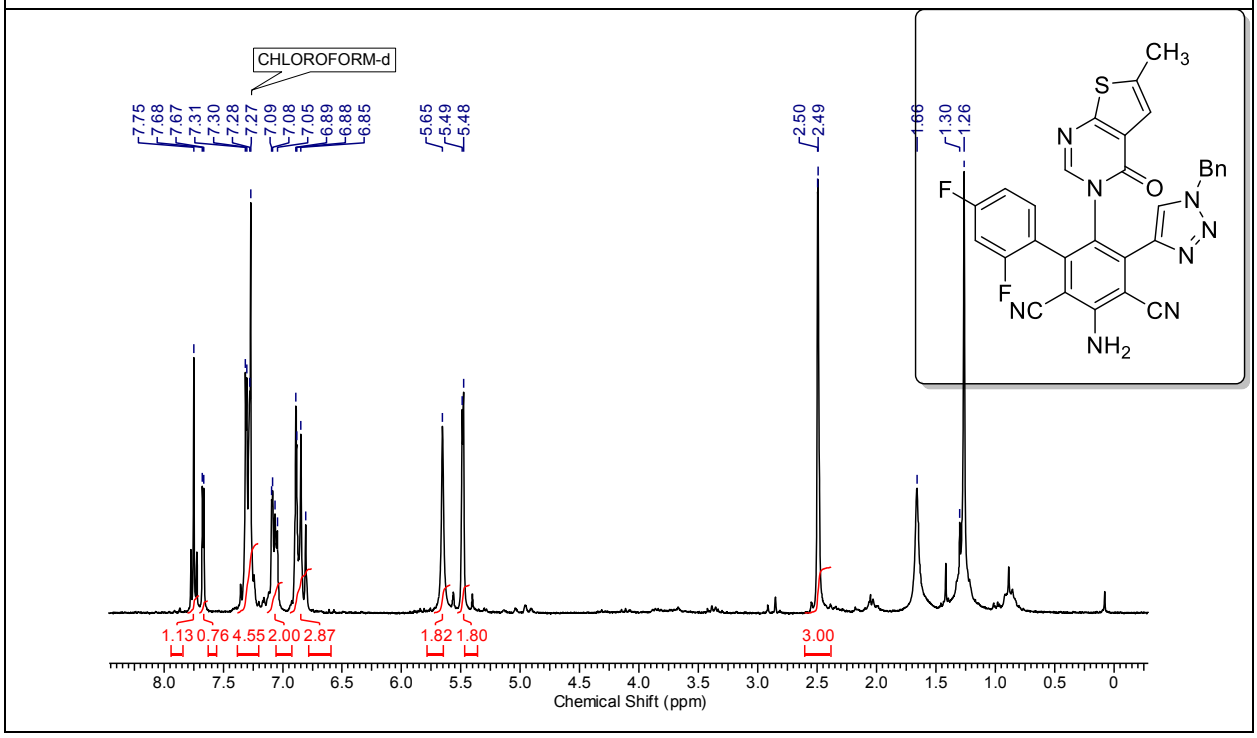
^{1}H NMR (200 MHz, $CDCl_3$) of compound 1g



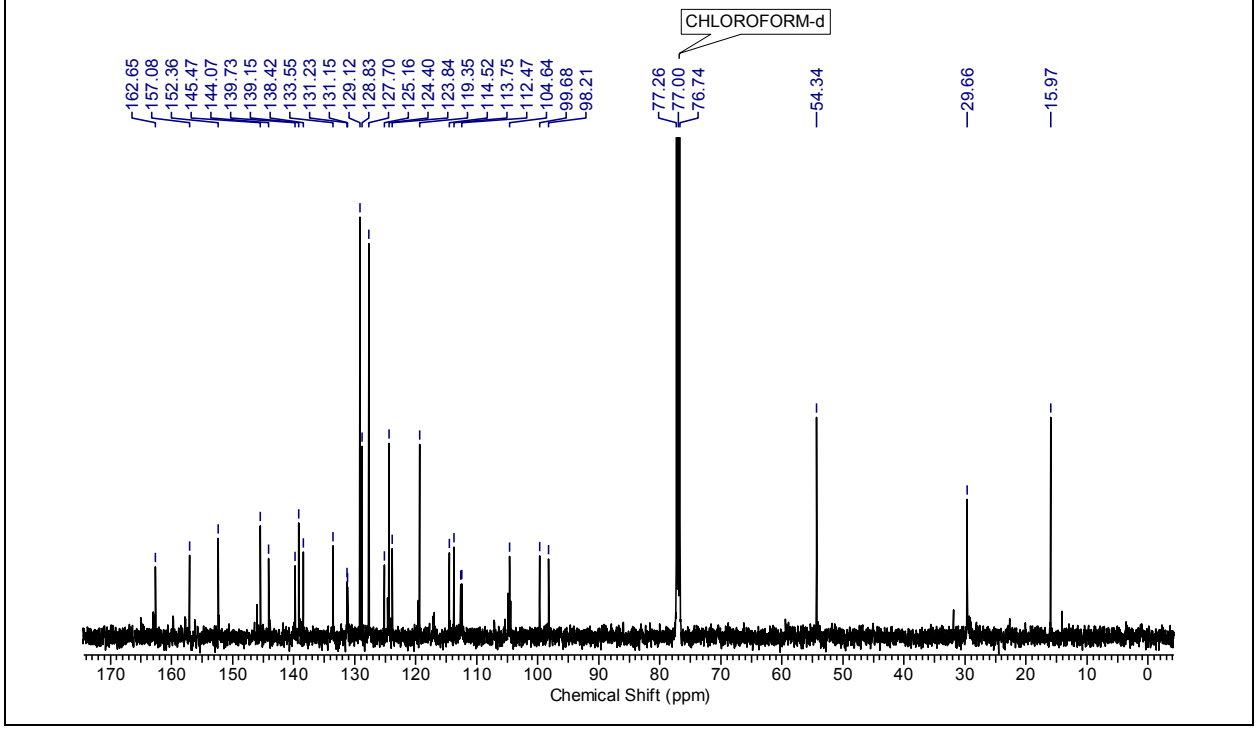
^{13}C NMR (100 MHz, $CDCl_3$) of compound 1g



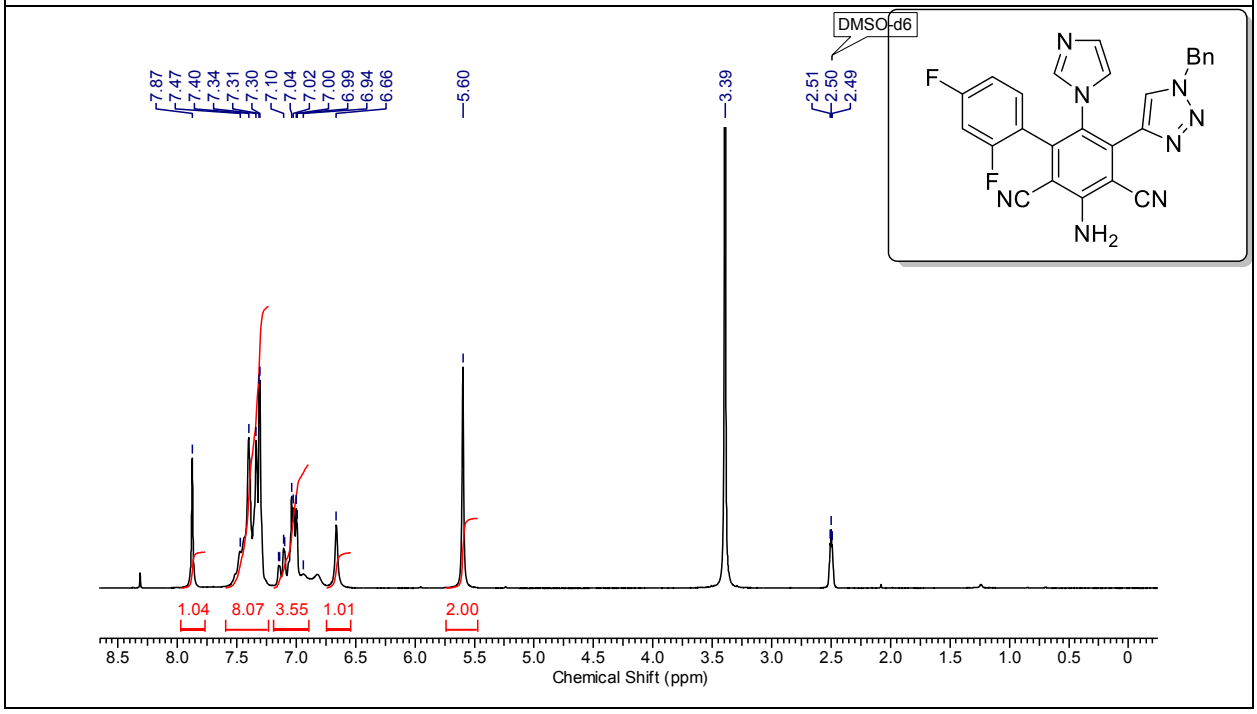
^{1}H NMR (500 MHz, $CDCl_3$) of compound 1h



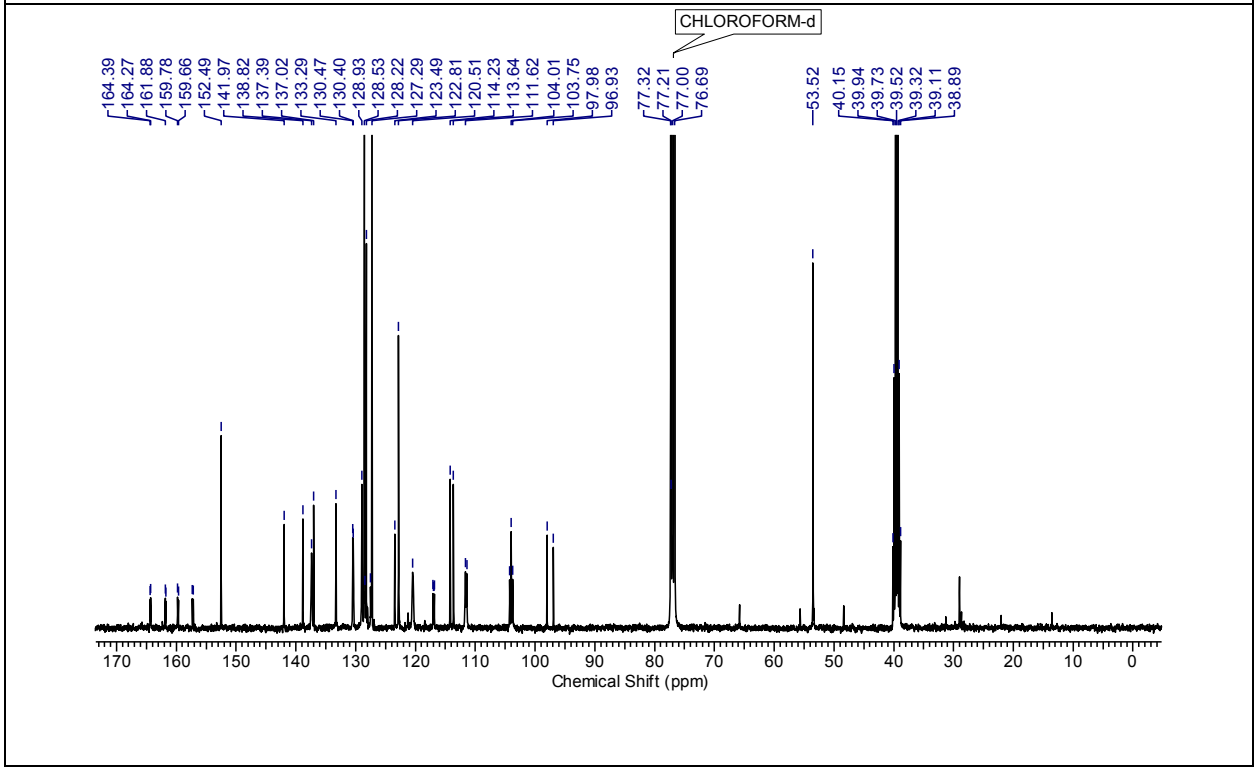
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) of compound 1h



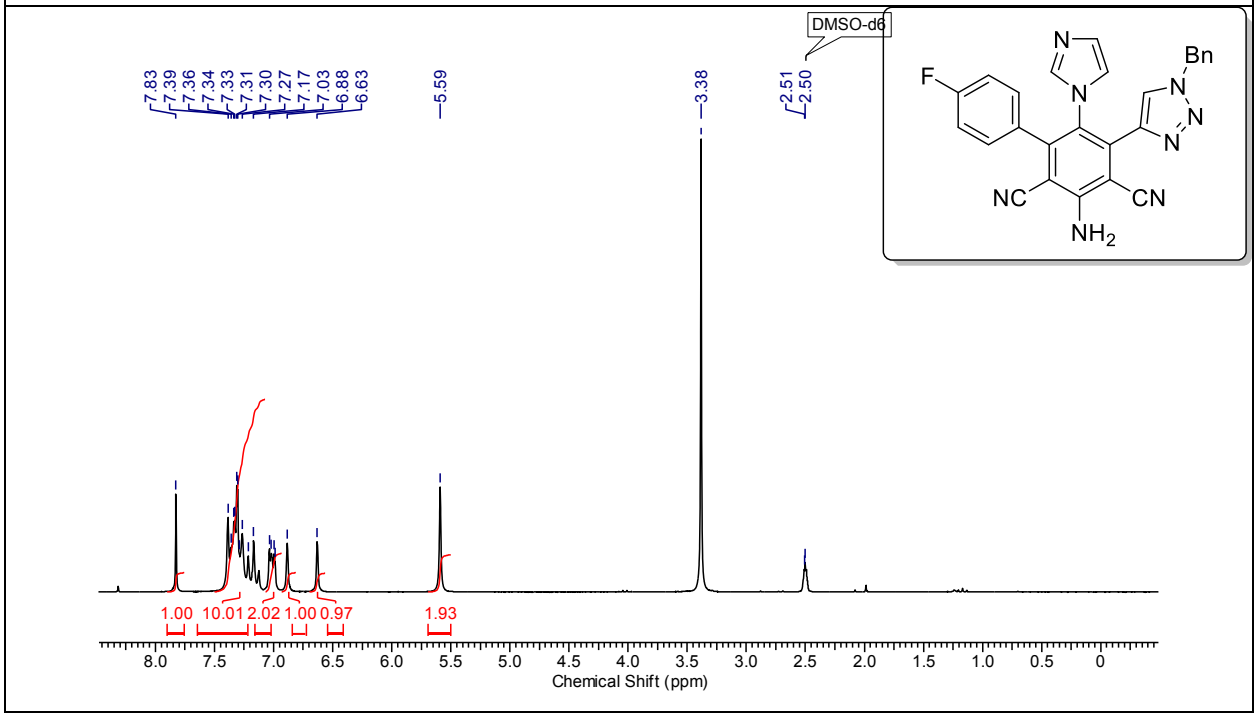
^{1}H NMR (200 MHz, DMSO-d6) of compound 1i



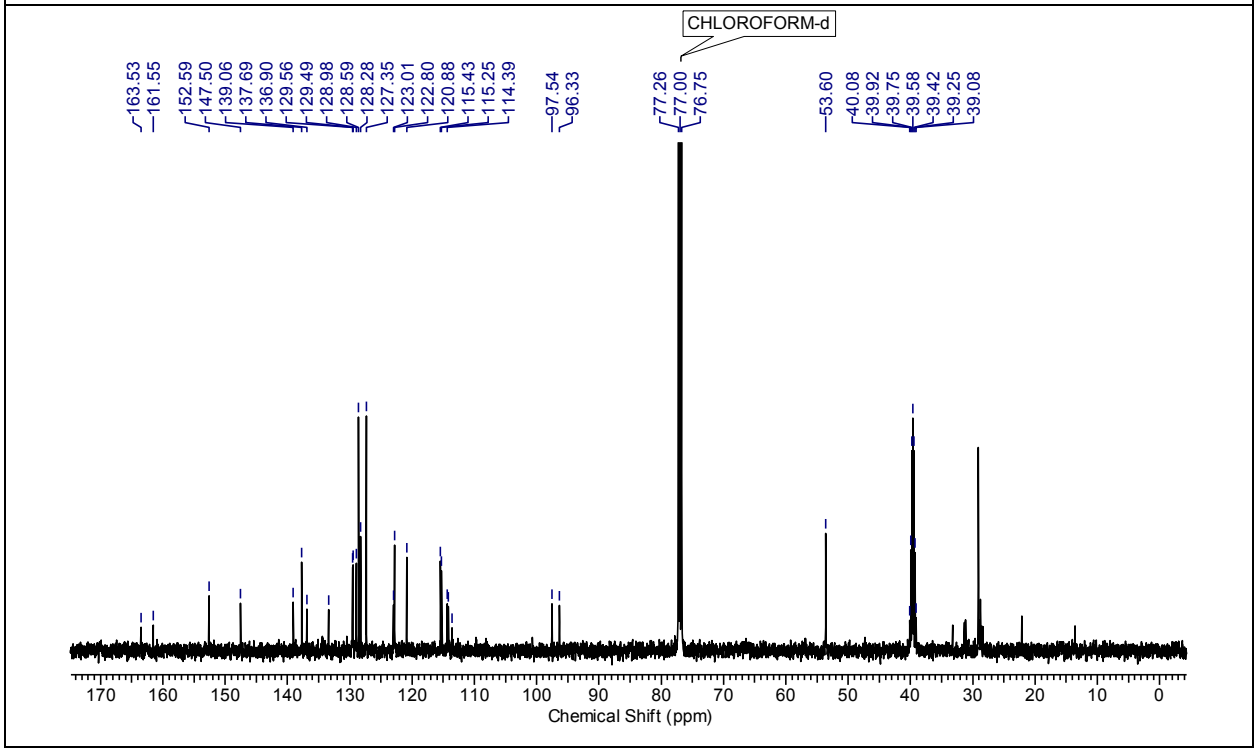
^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) of compound 1i



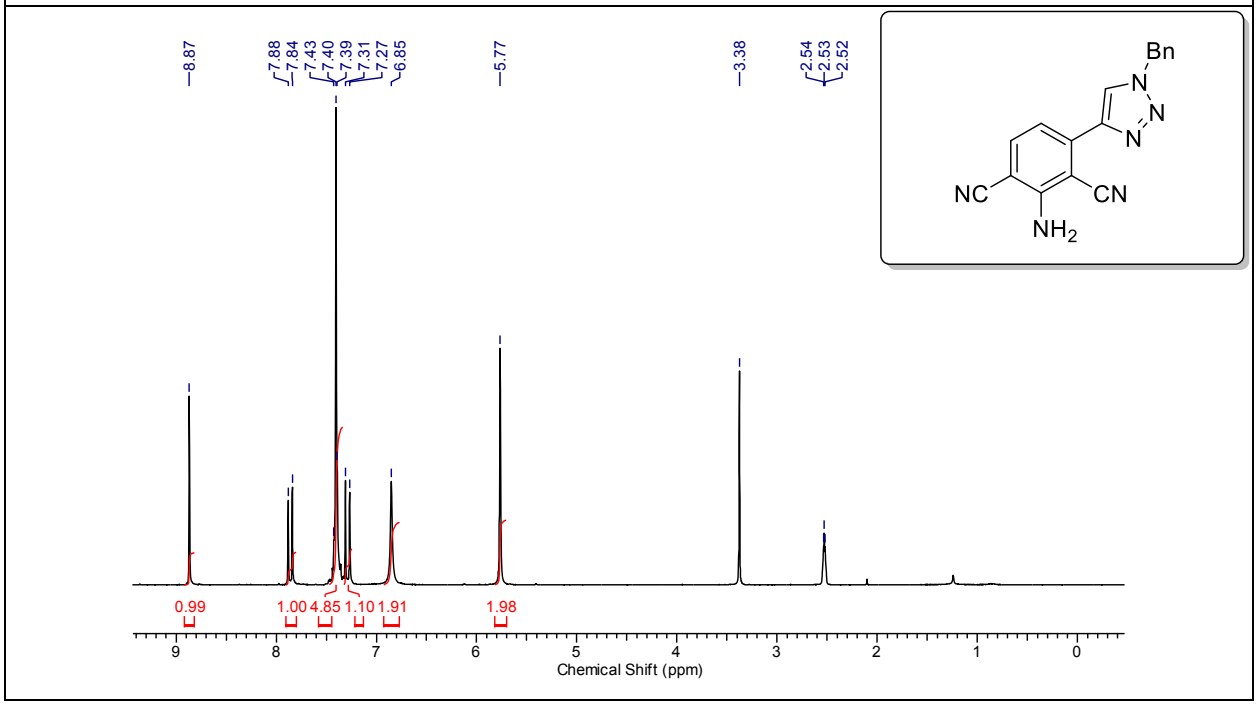
^{1}H NMR (200 MHz, DMSO-d$_6$) of compound 1j



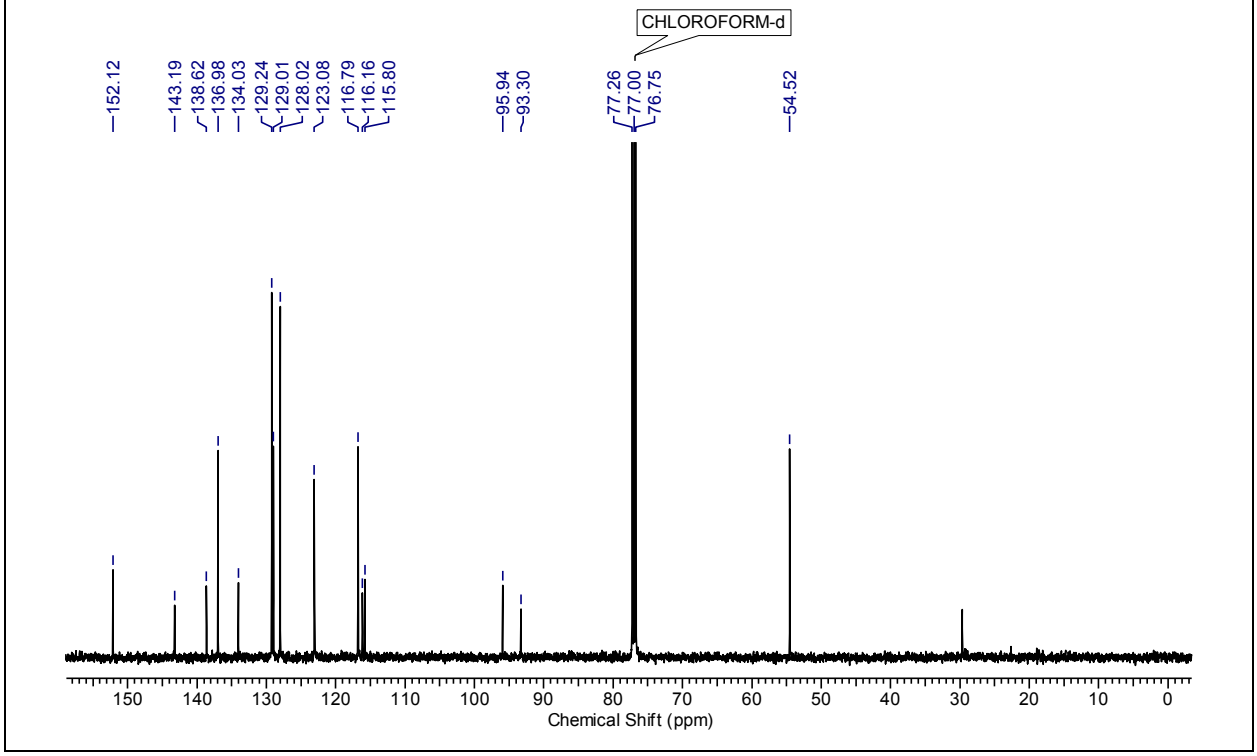
^{13}C NMR (125 MHz, CDCl$_3$ + DMSO-d$_6$) of compound 1j



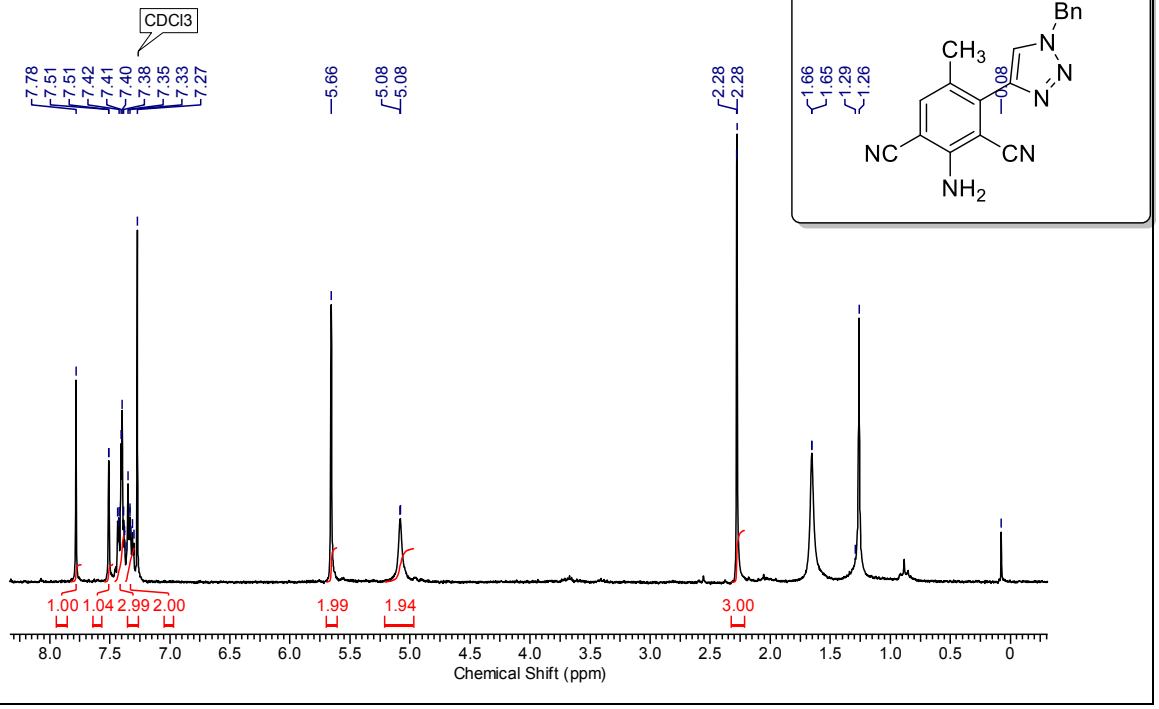
^{1}H NMR (200 MHz, DMSO-d6) of compound 9



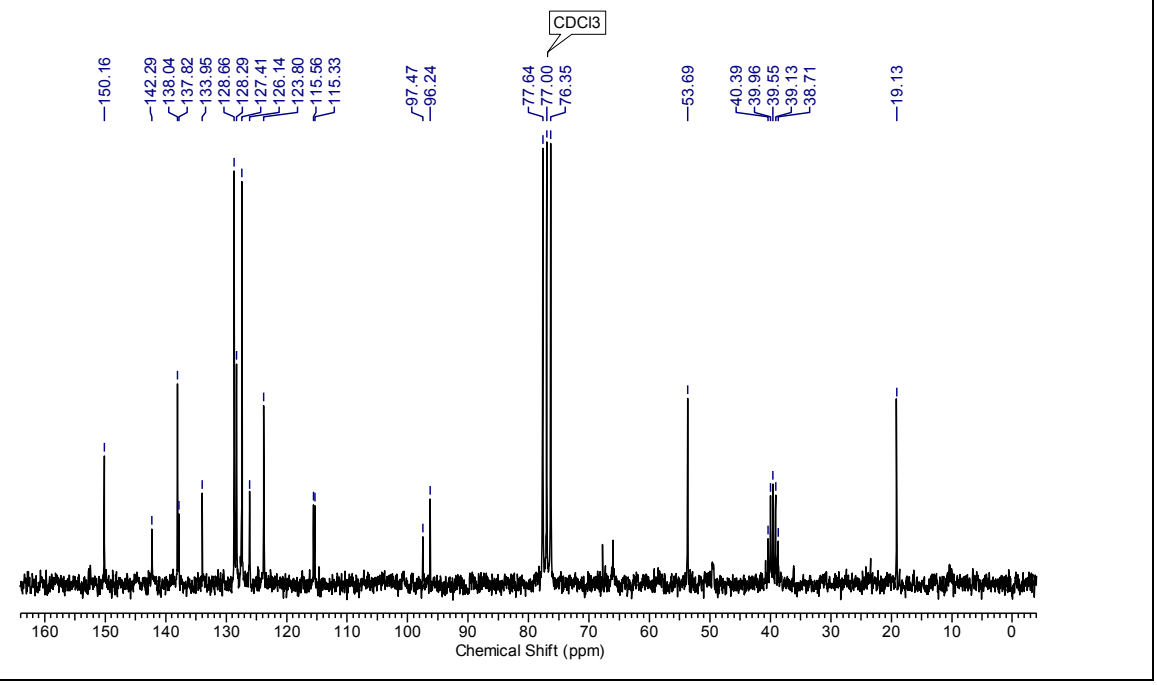
^{13}C NMR (125 MHz, $CDCl_3$) of compound 9



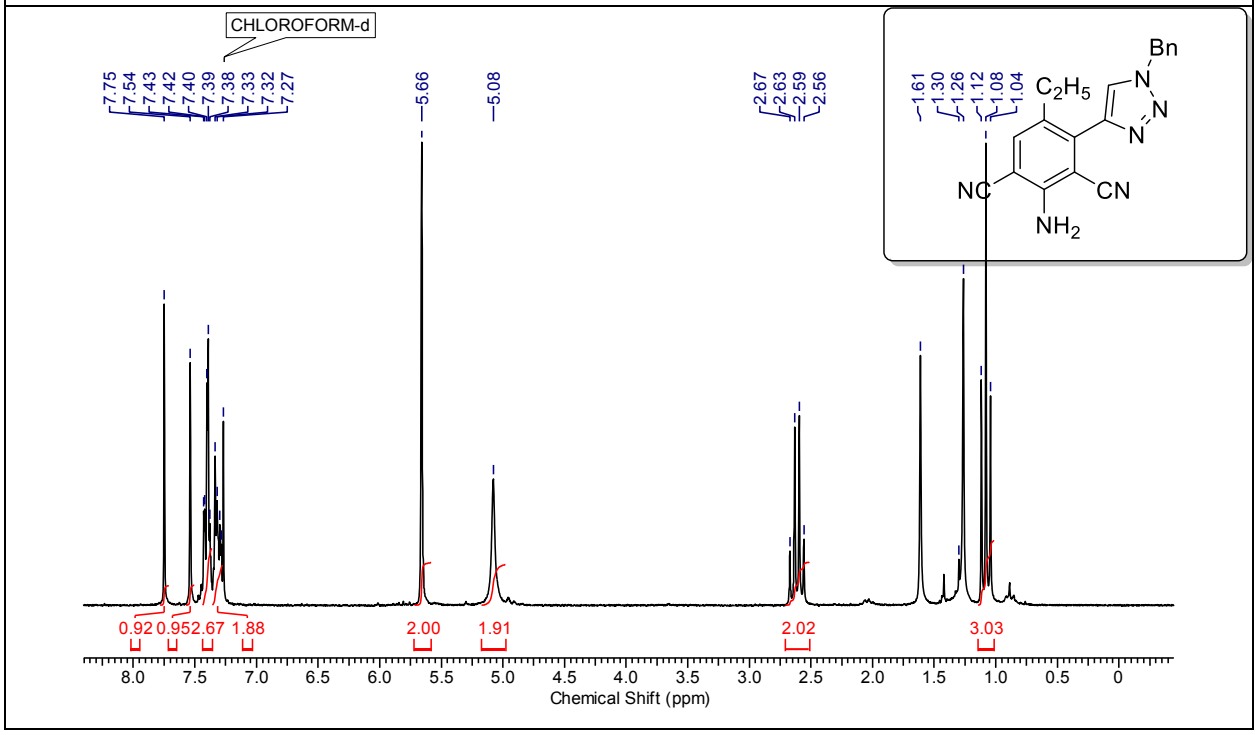
^{1}H NMR (200 MHz, $CDCl_3$) of compound 10a



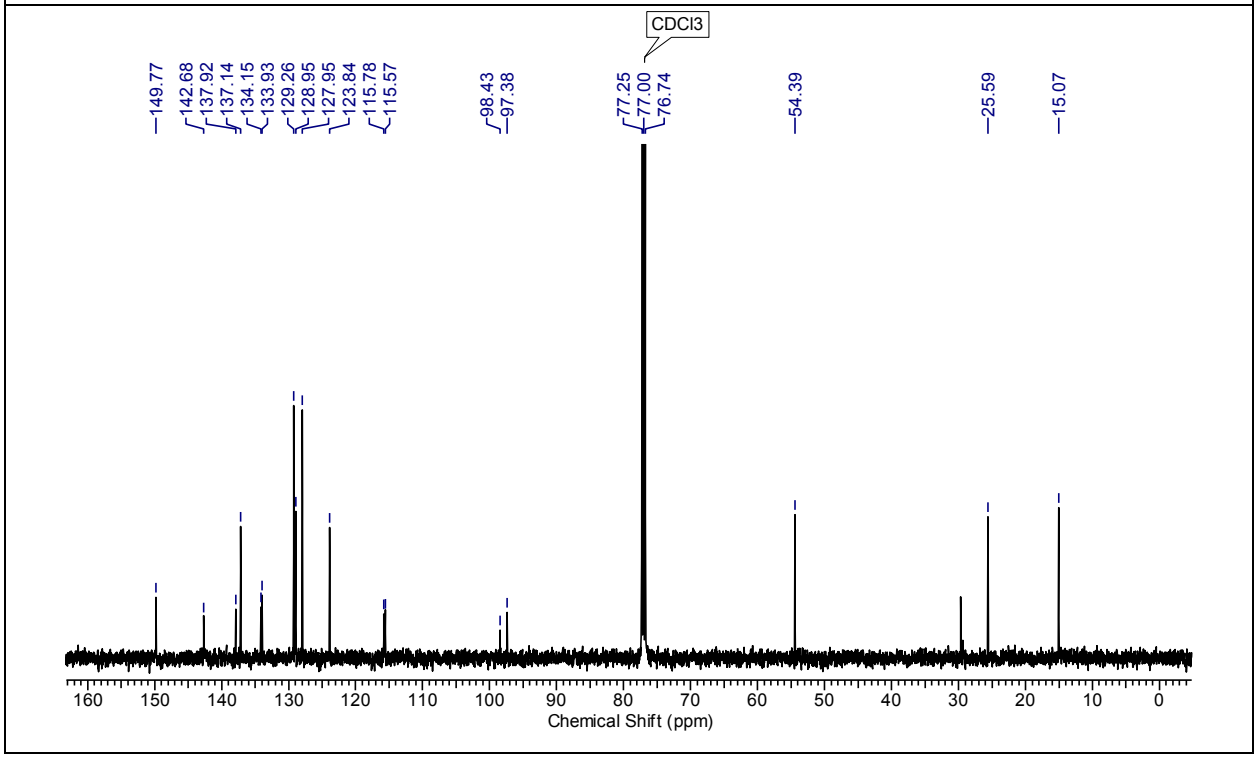
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) of compound 10a



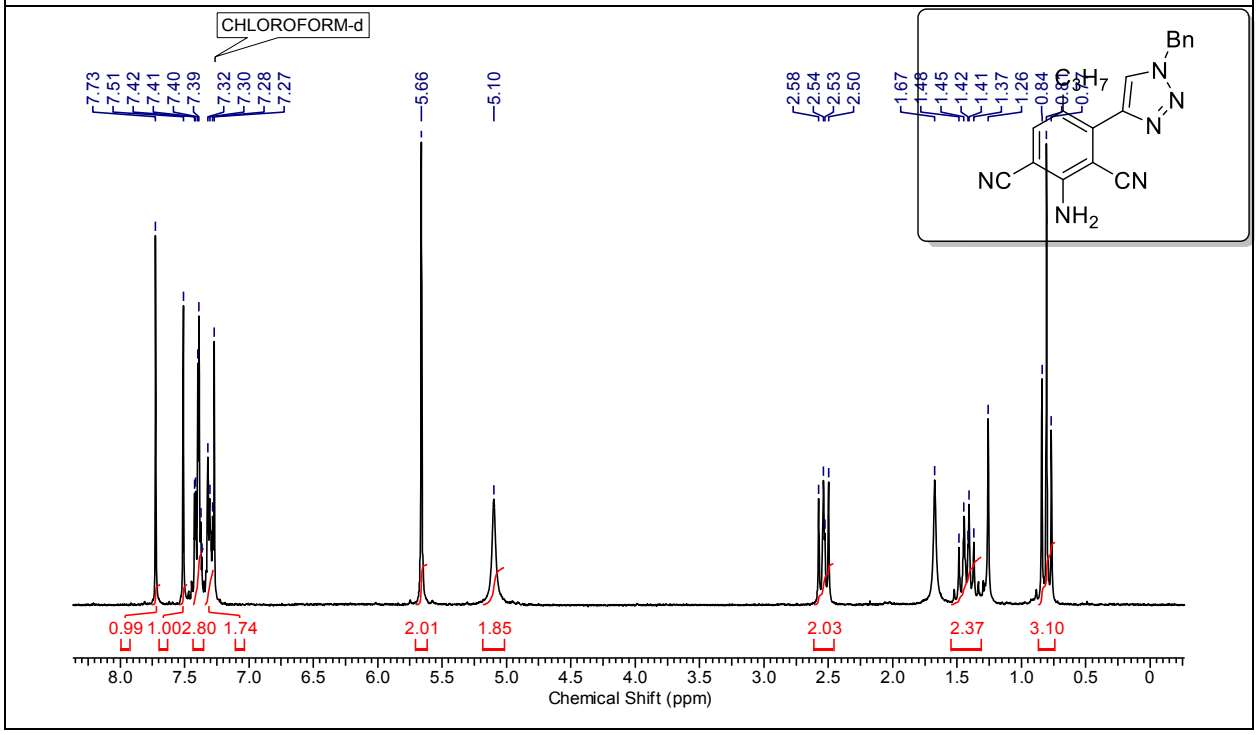
^{1}H NMR (200 MHz, $CDCl_3$) of compound 10b



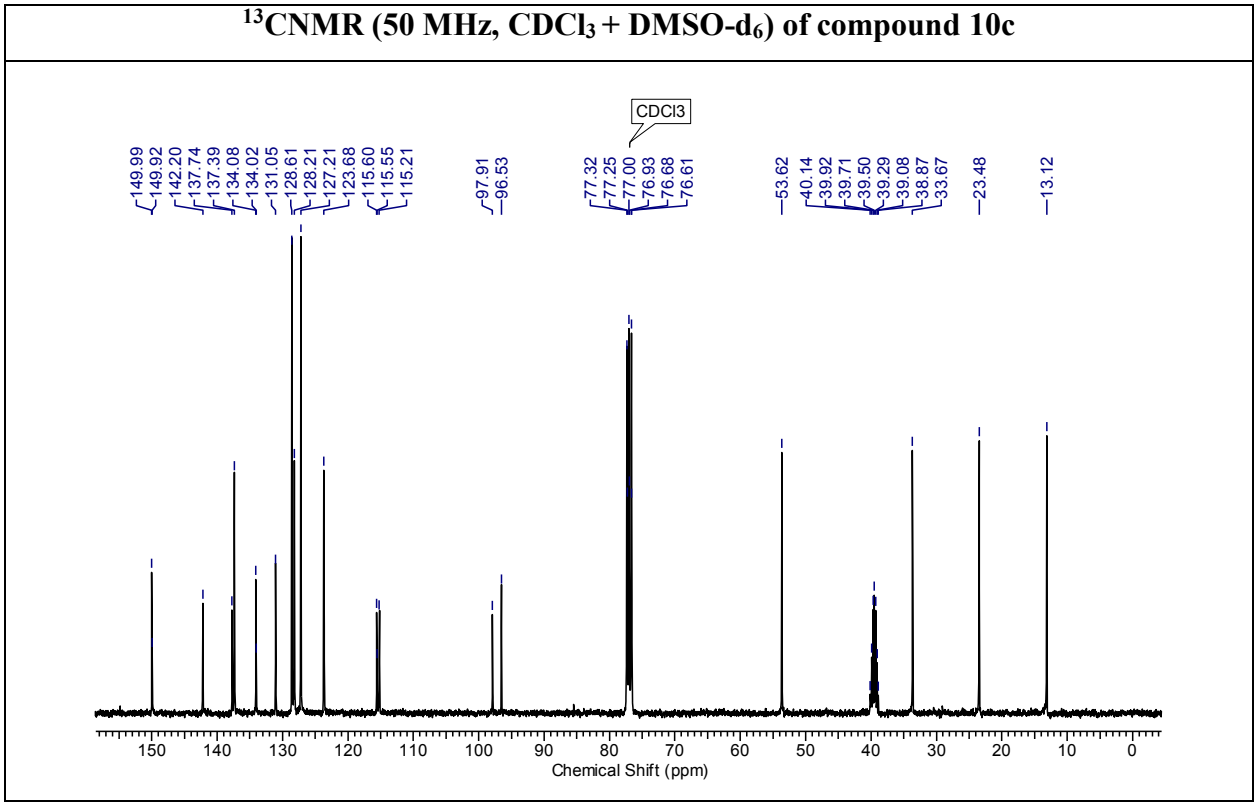
^{13}C NMR (100 MHz, $CDCl_3$) of compound 10b



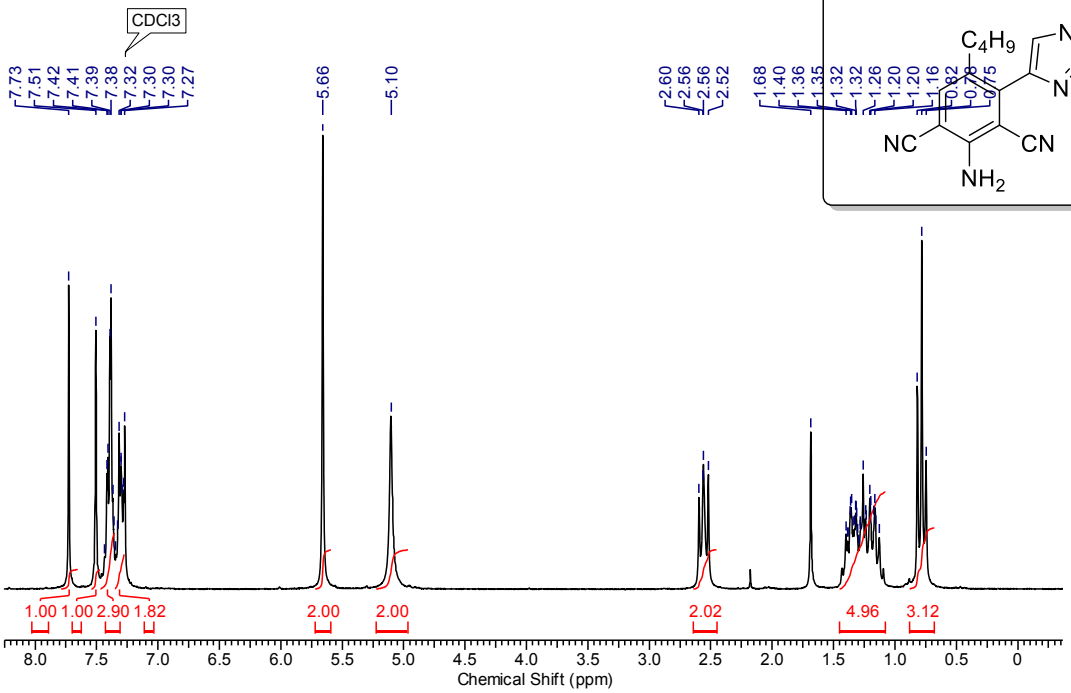
^{1}H NMR (200 MHz, $CDCl_3$) of compound 10c



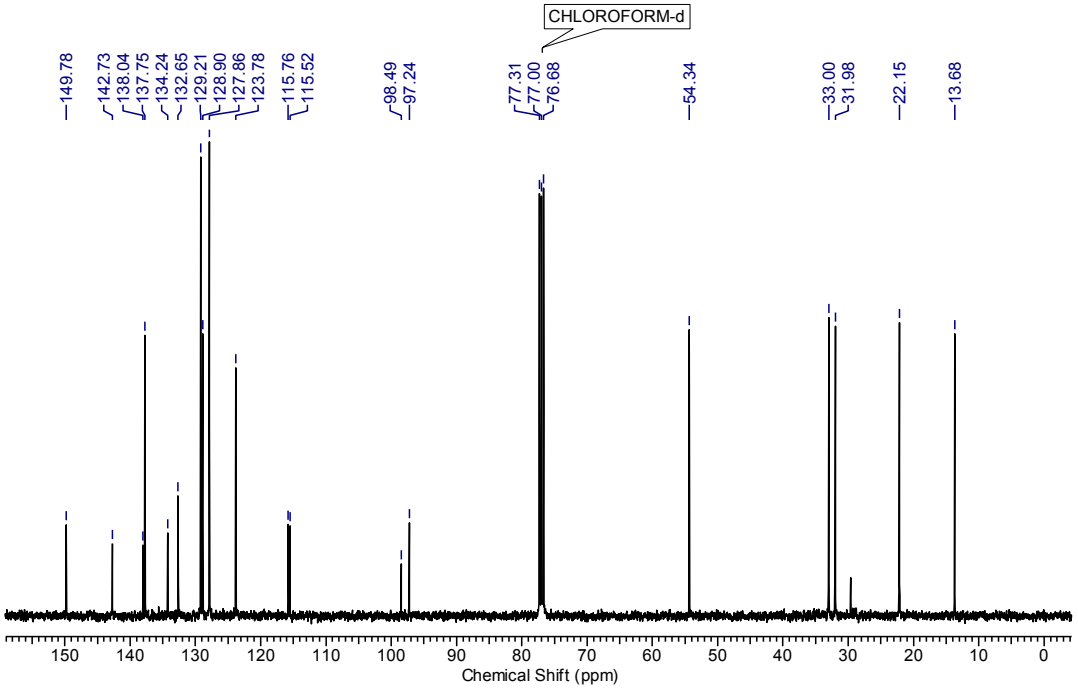
^{13}CNMR (50 MHz, $CDCl_3$ + DMSO-d_6) of compound 10c



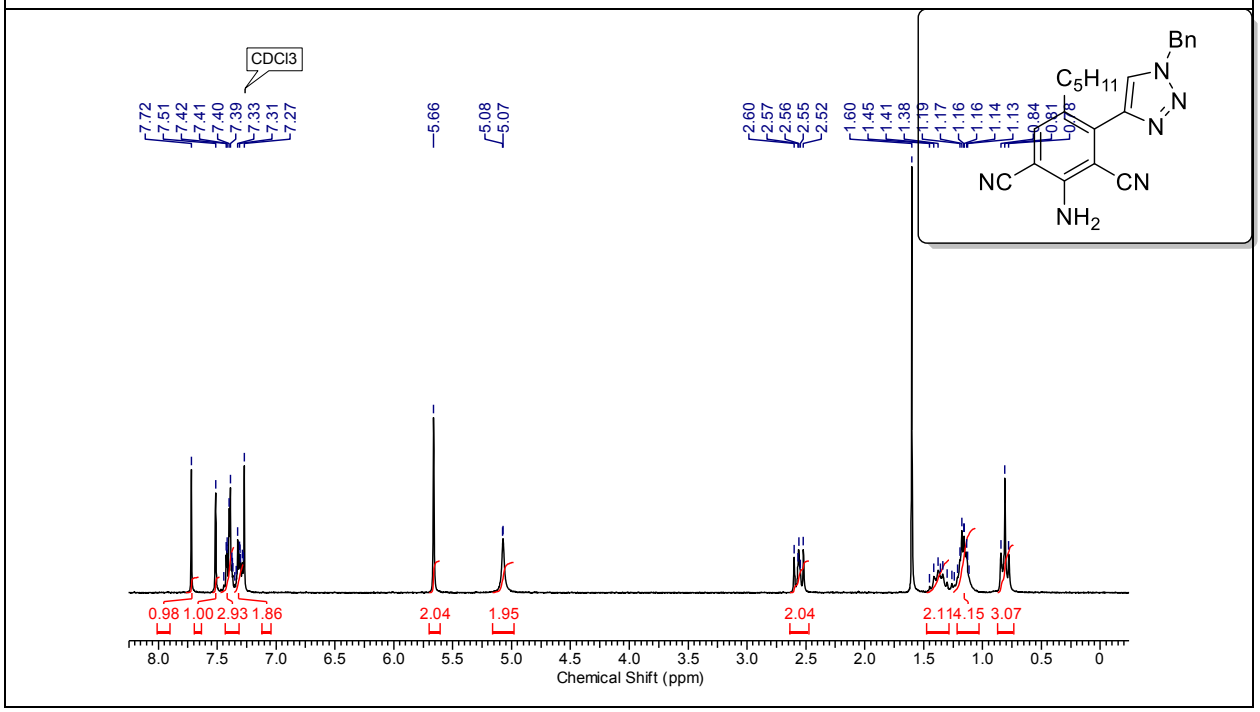
^{1}H NMR (200 MHz, $CDCl_3$) of compound 10d



^{13}C NMR (125 MHz, $CDCl_3$) of compound 10d



^{1}H NMR (200 MHz, $CDCl_3$) of compound 10e



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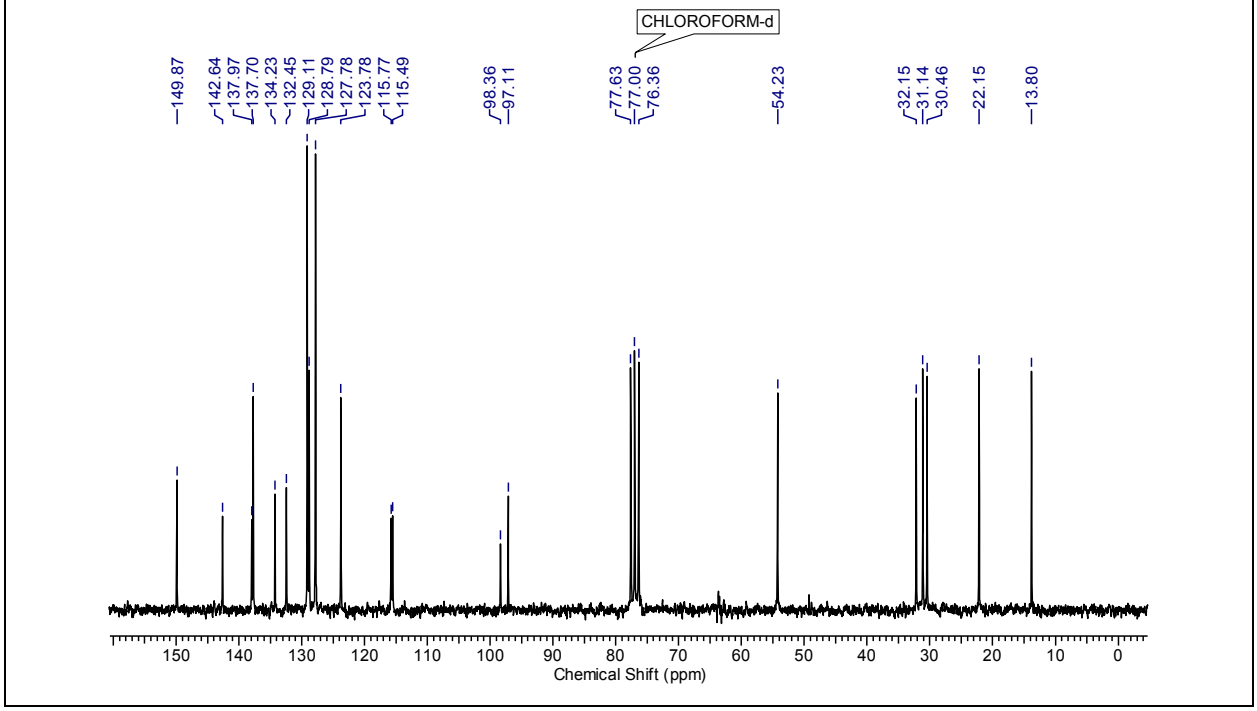
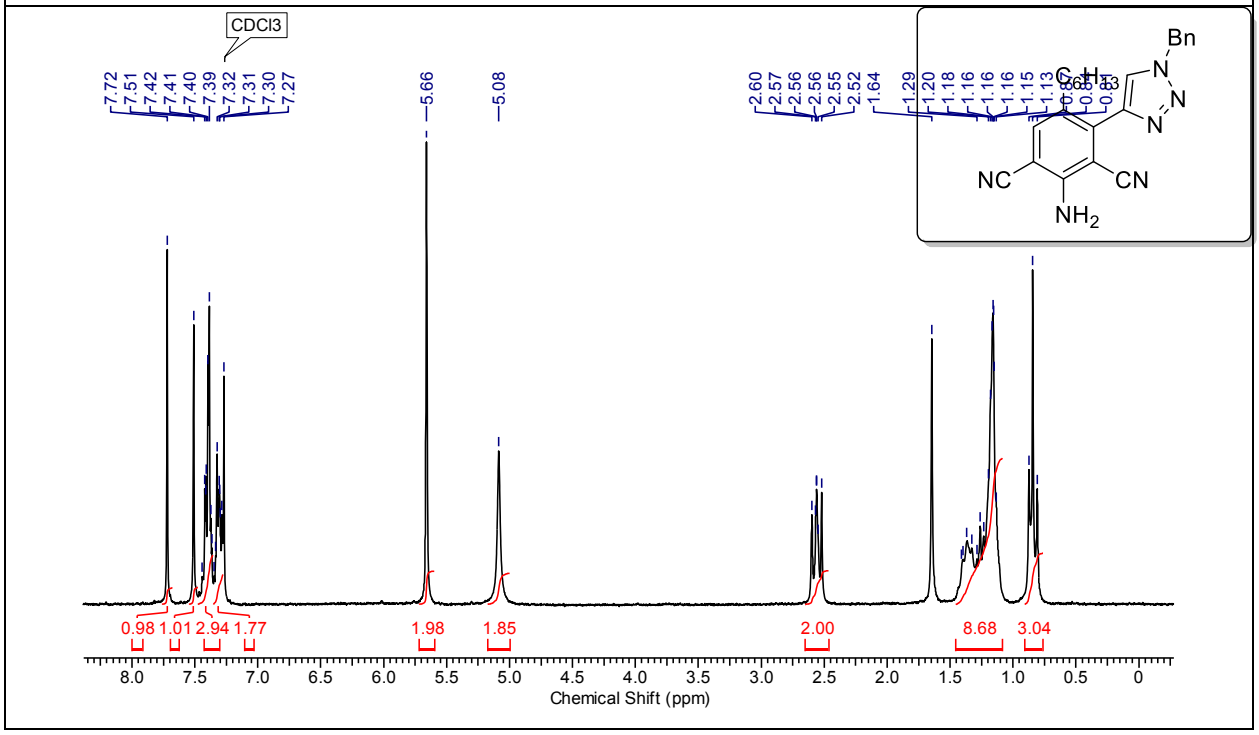


^{1}H NMR (200 MHz, $CDCl_3$) of compound 10f



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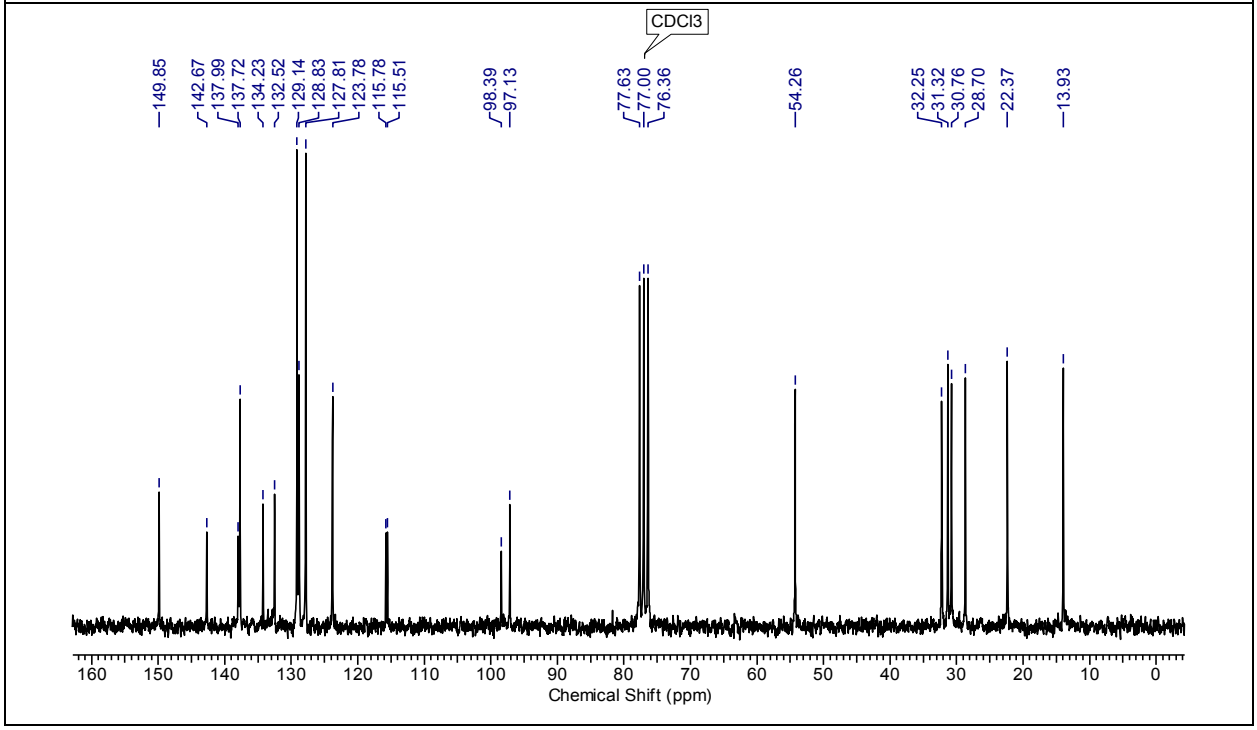
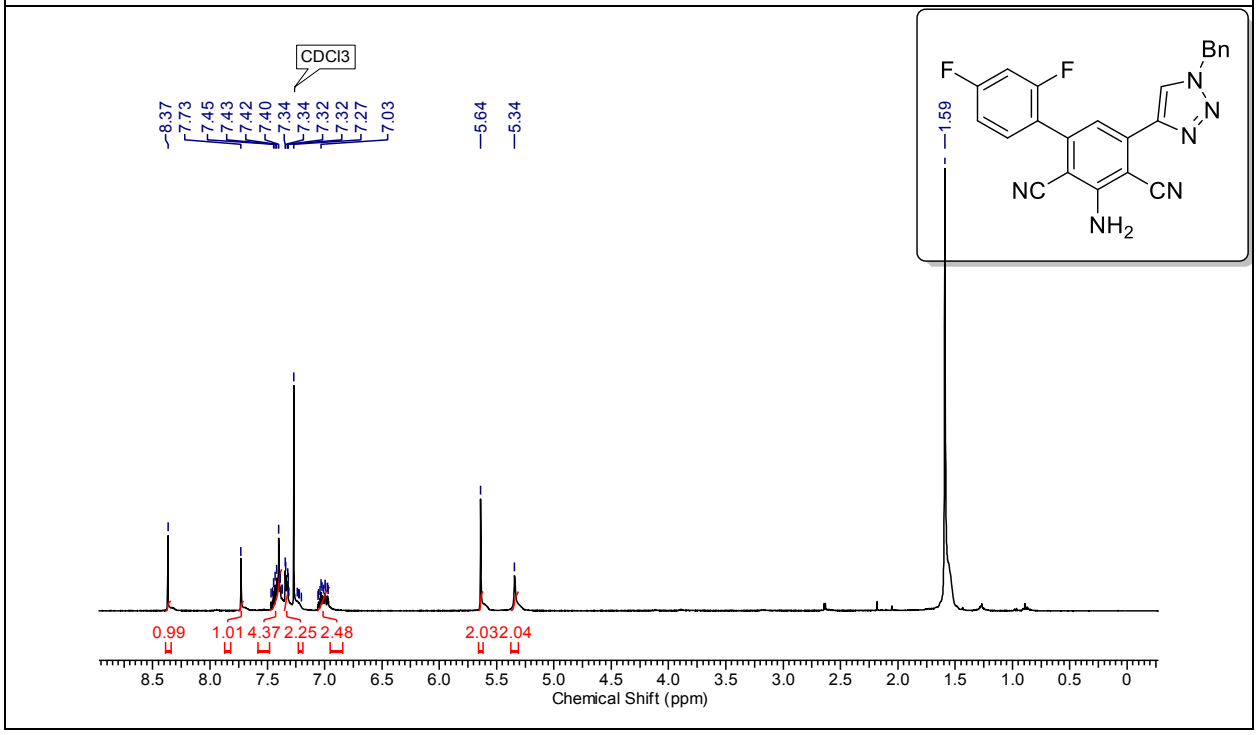
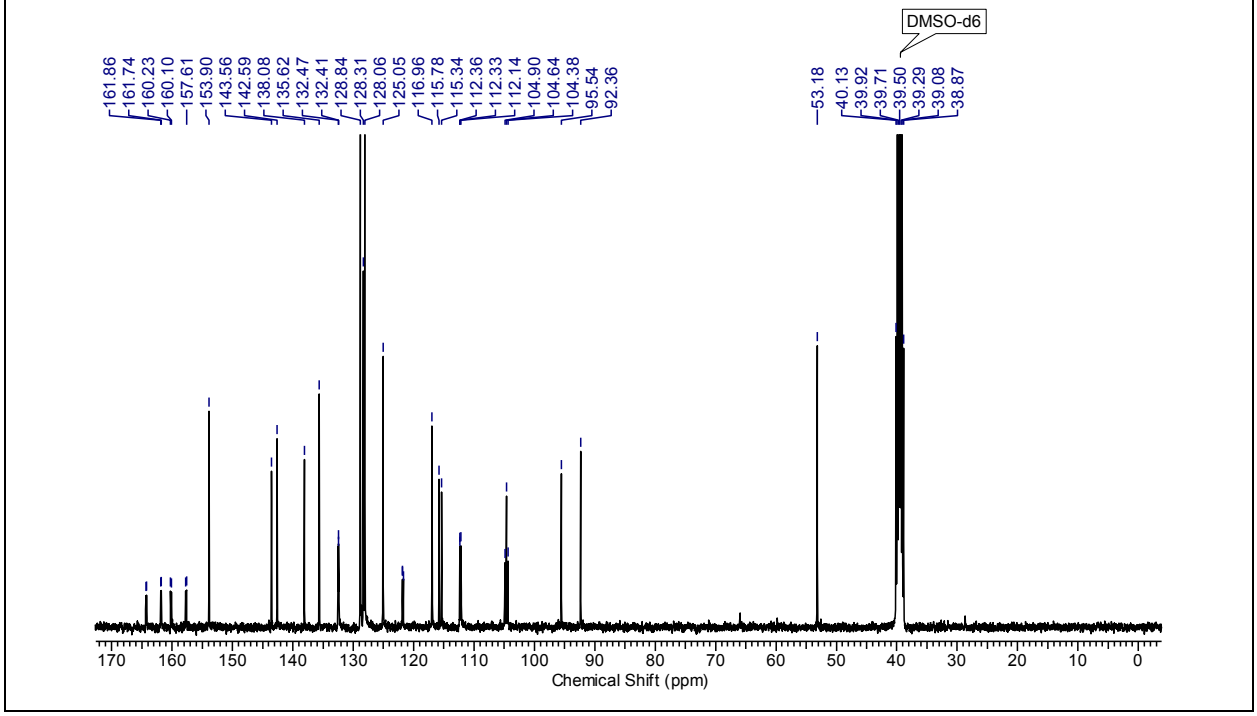


^{1}H NMR (400 MHz, $CDCl_3$) of compound 11



^{13}C NMR (100 MHz, DMSO-d6) of compound 11



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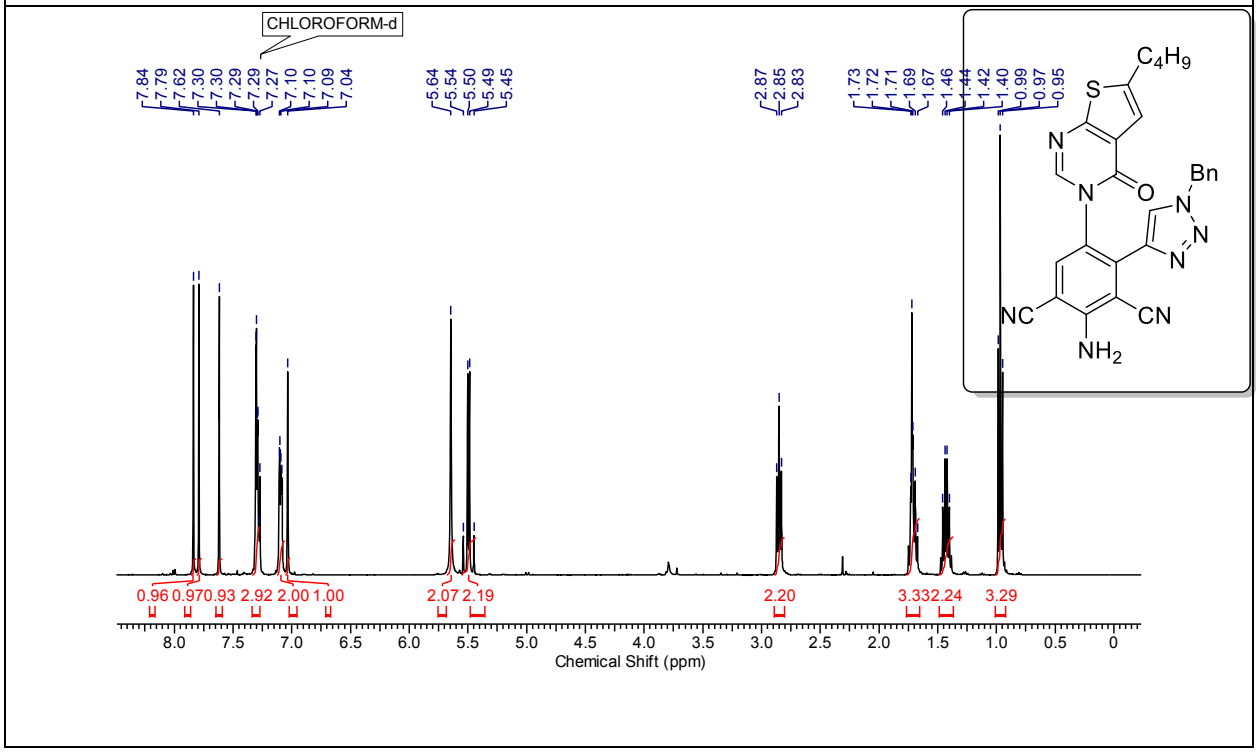

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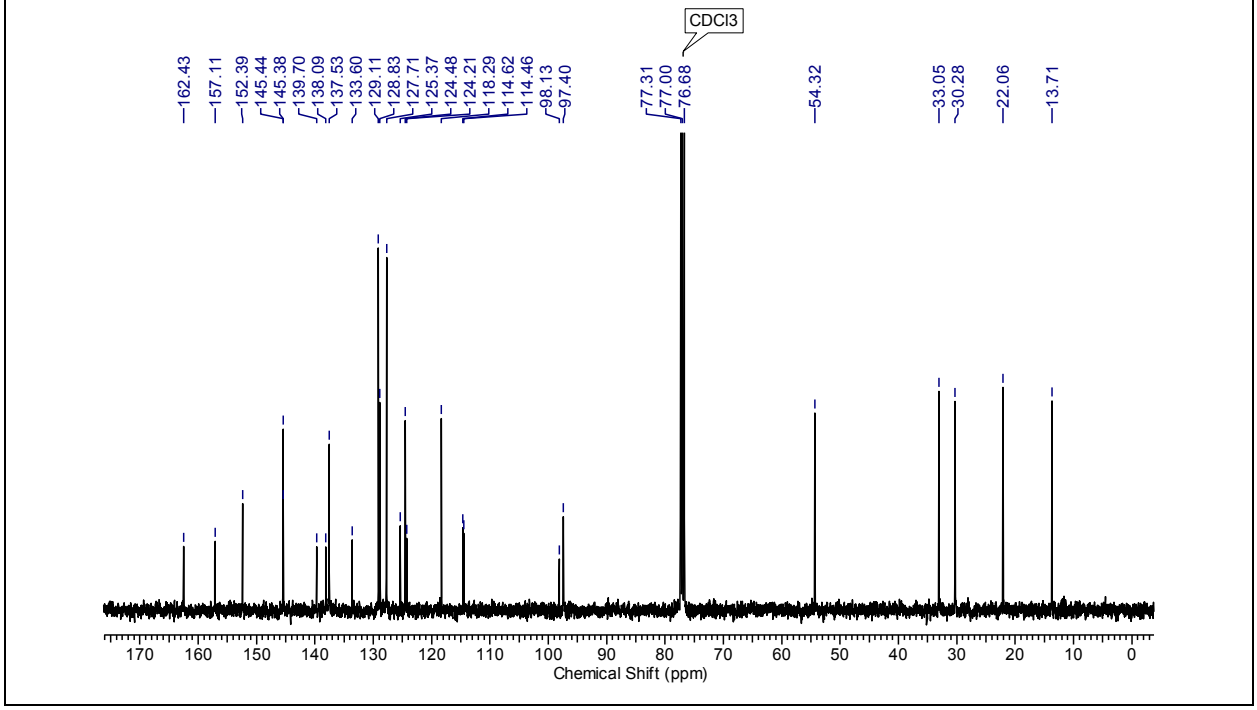
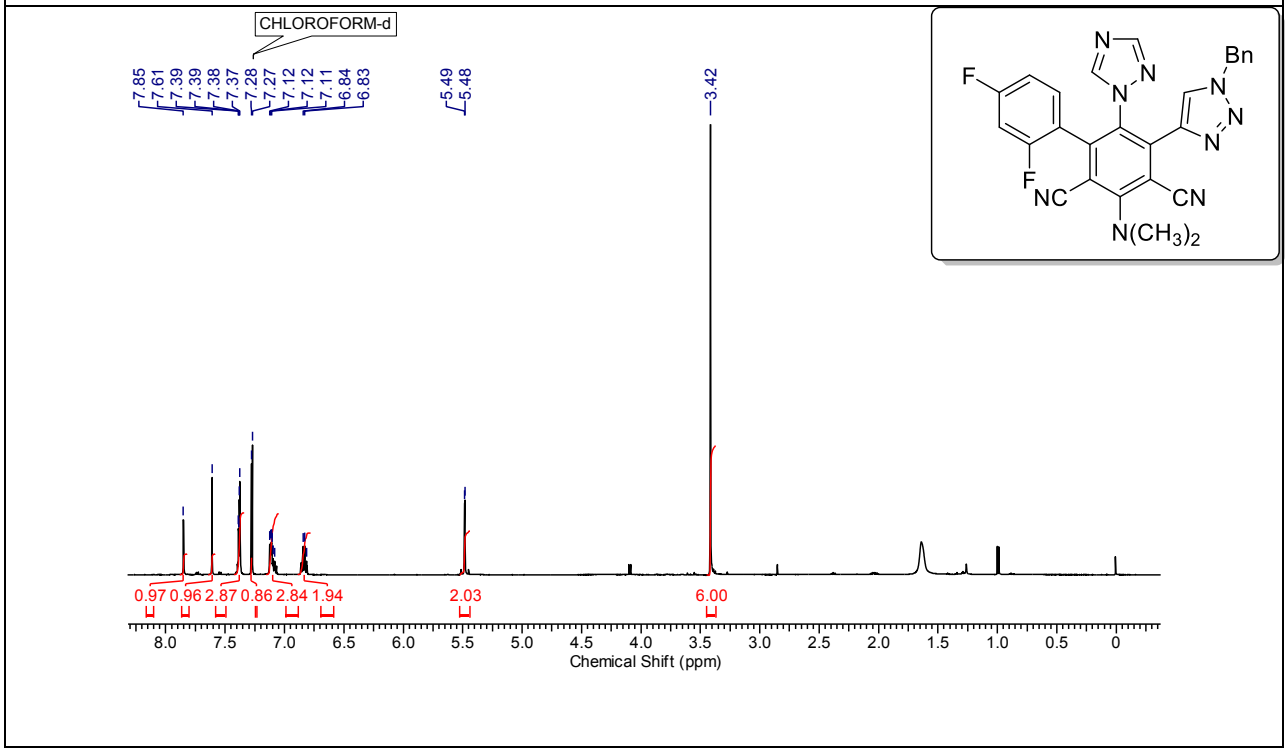
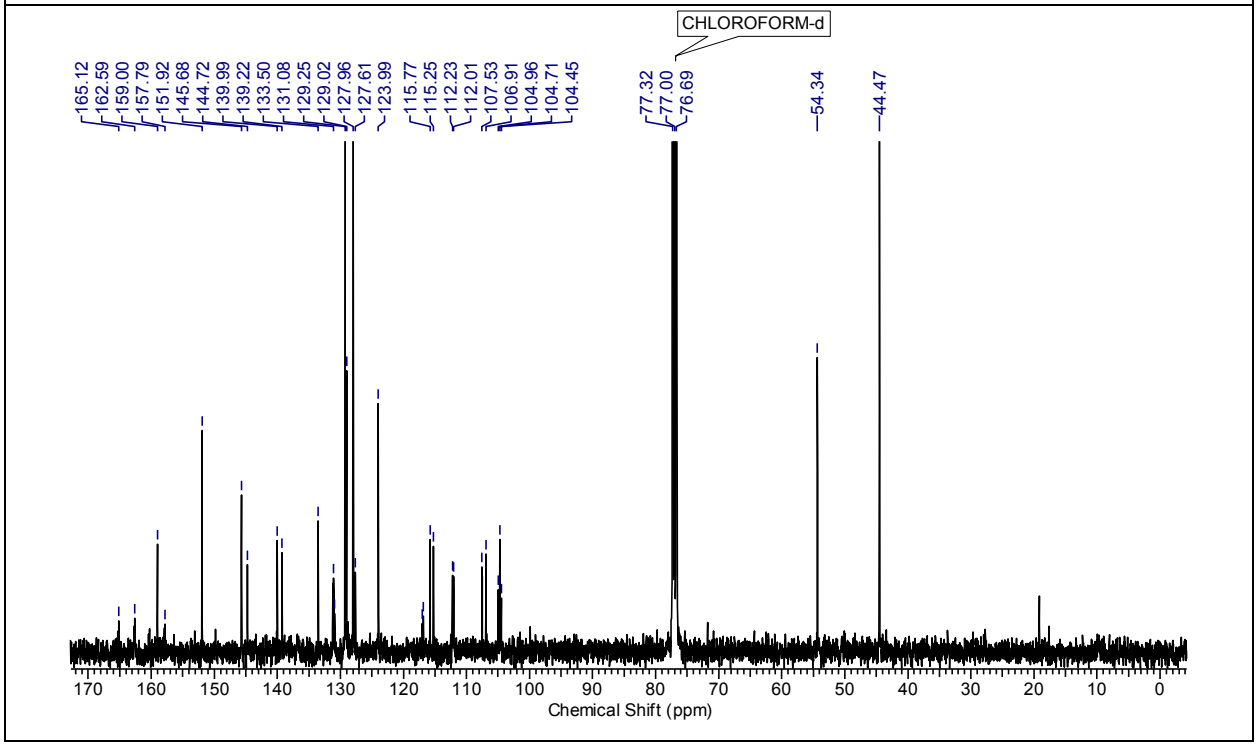


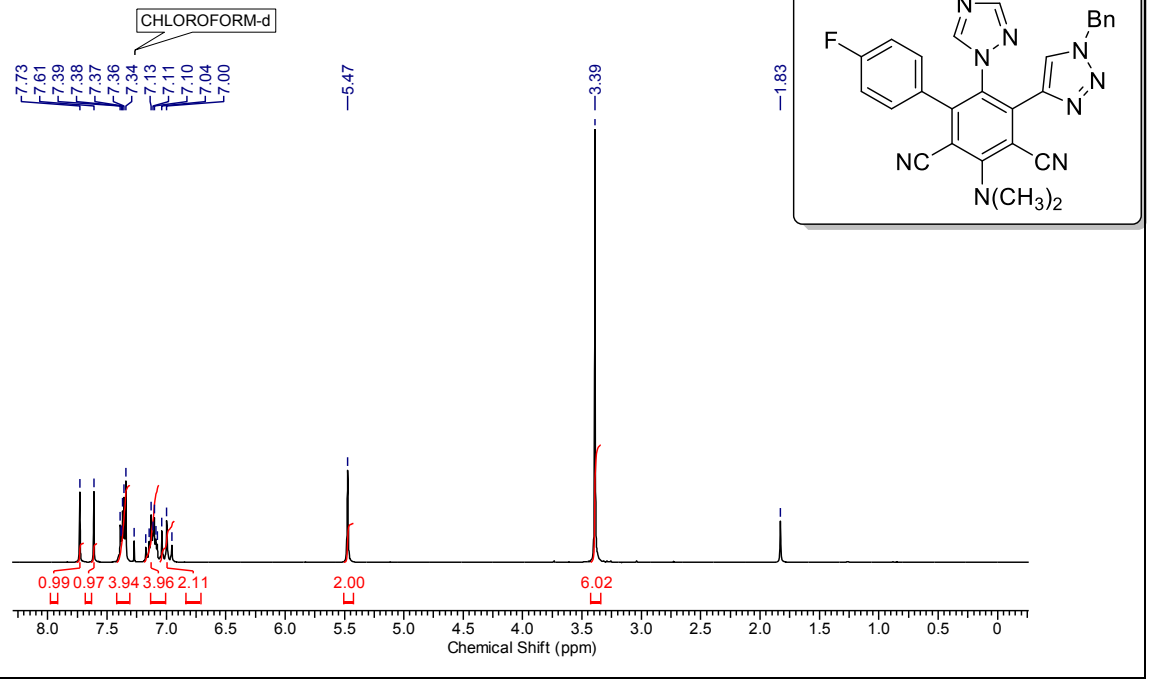
^{1}H NMR (400 MHz, $CDCl_3$) of compound 13e



^{13}C NMR (100 MHz, $CDCl_3$) of compound 13e



^{1}H NMR (400 MHz, $CDCl_3$) of compound 13f



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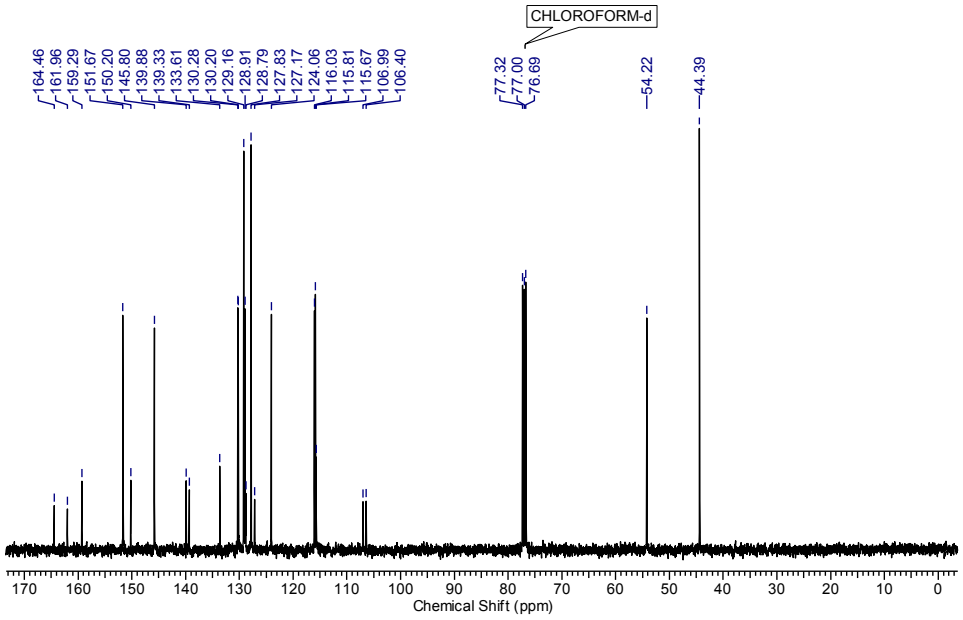
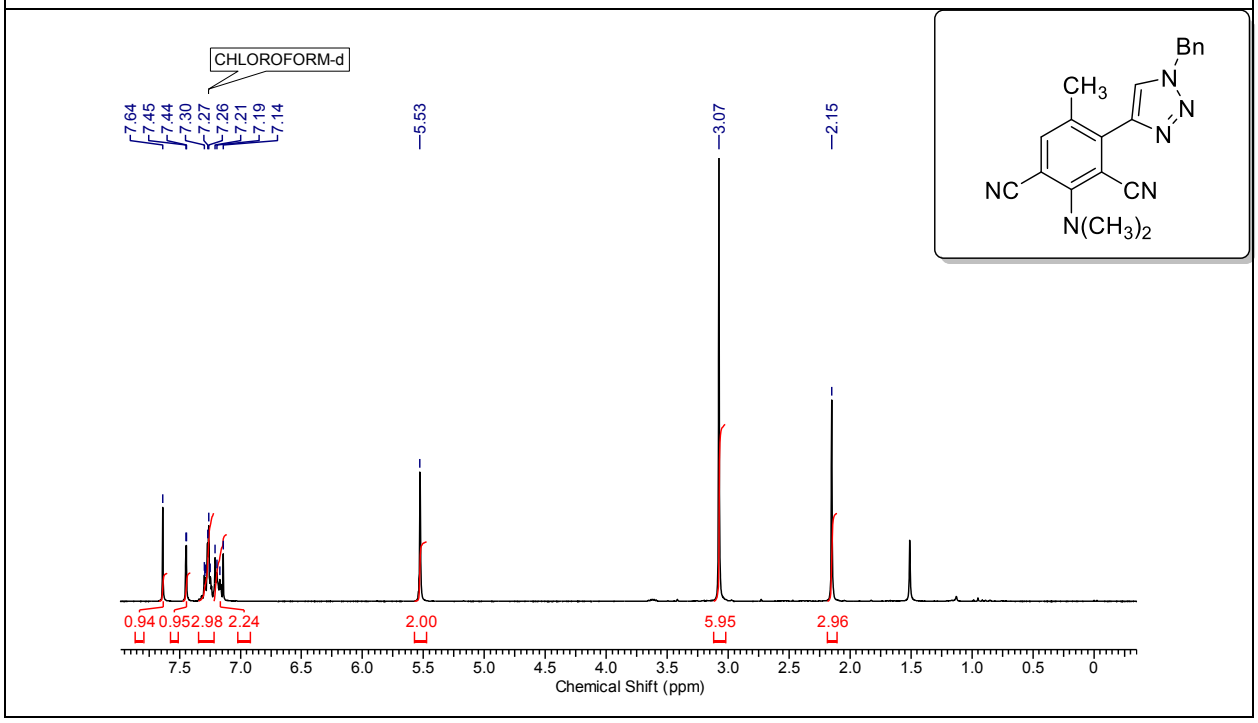
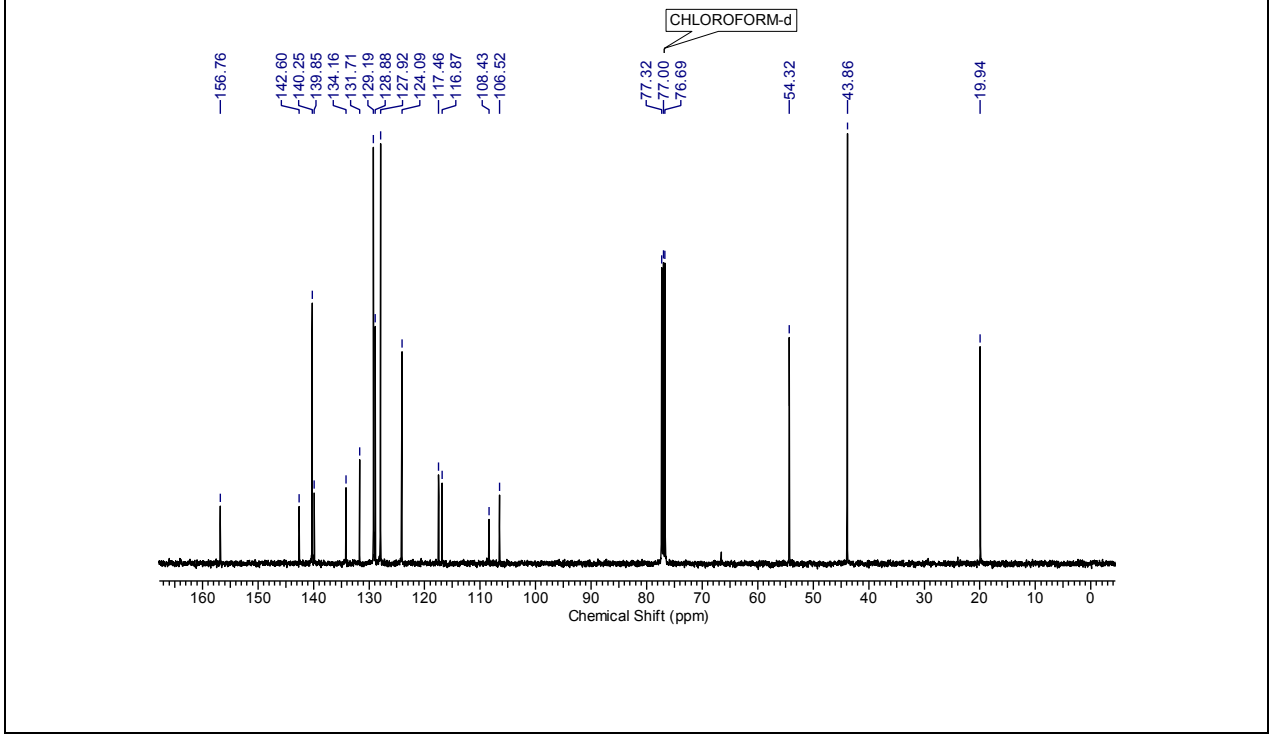


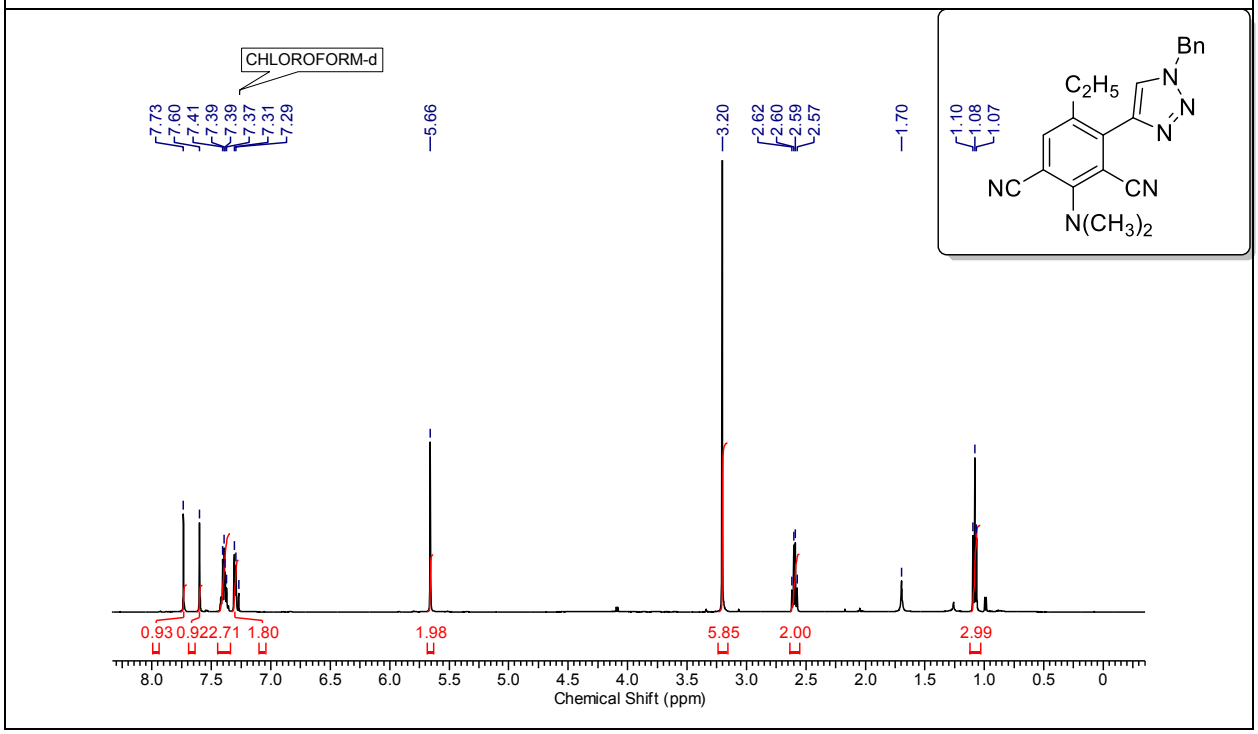
^{1}H NMR (400 MHz, $CDCl_3$) of compound 14a



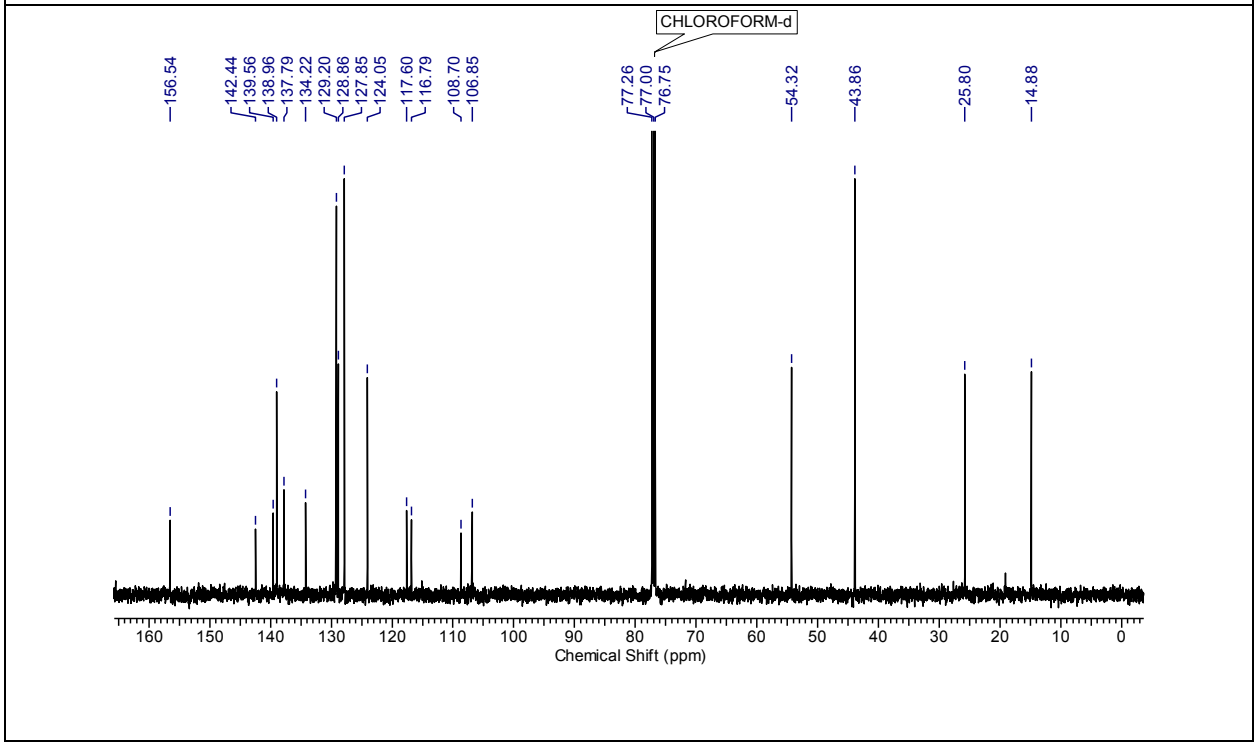
^{13}C NMR (100 MHz, $CDCl_3$) of compound 14a



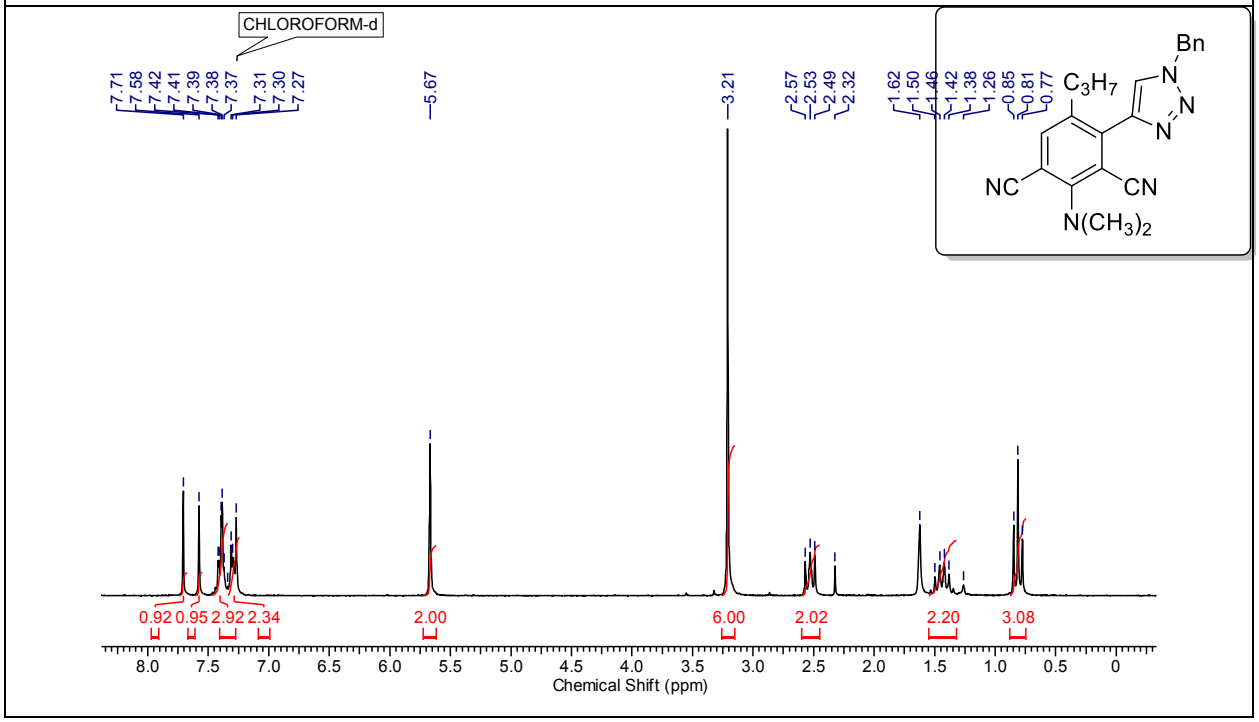
^{1}H NMR (200 MHz, $CDCl_3$) of compound 14b



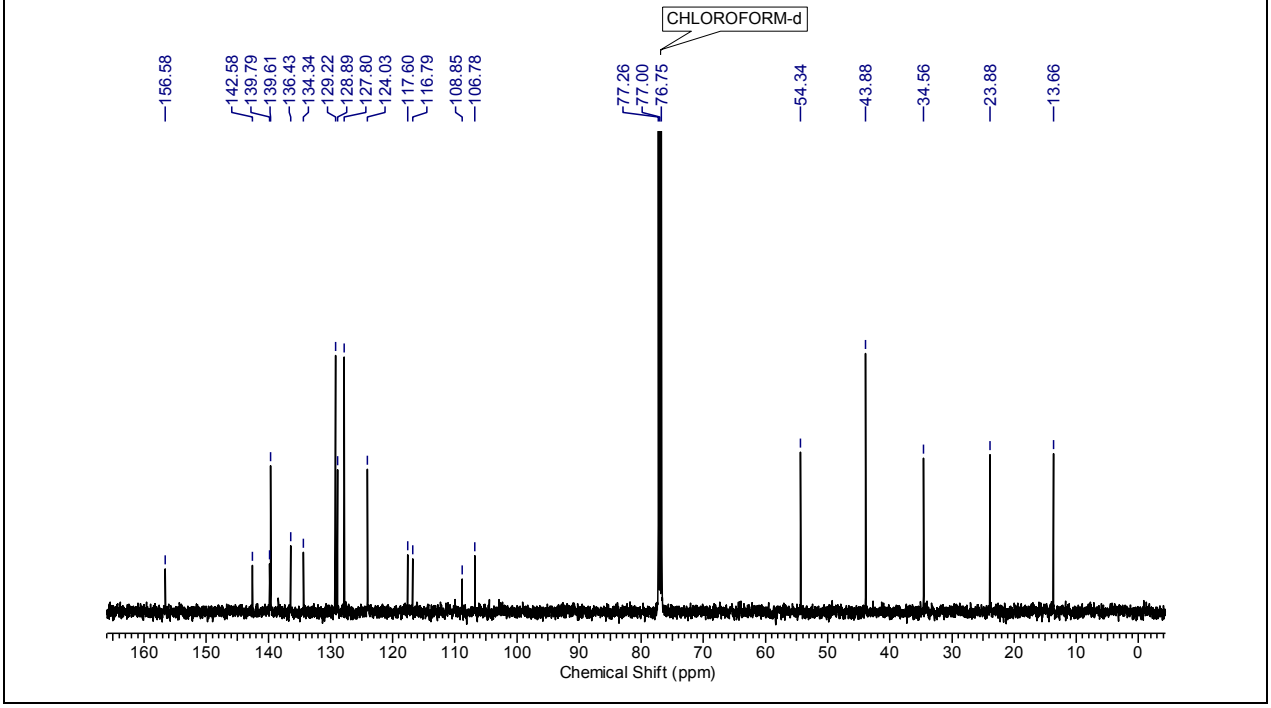
^{13}C NMR (125 MHz, $CDCl_3$) of compound 14b



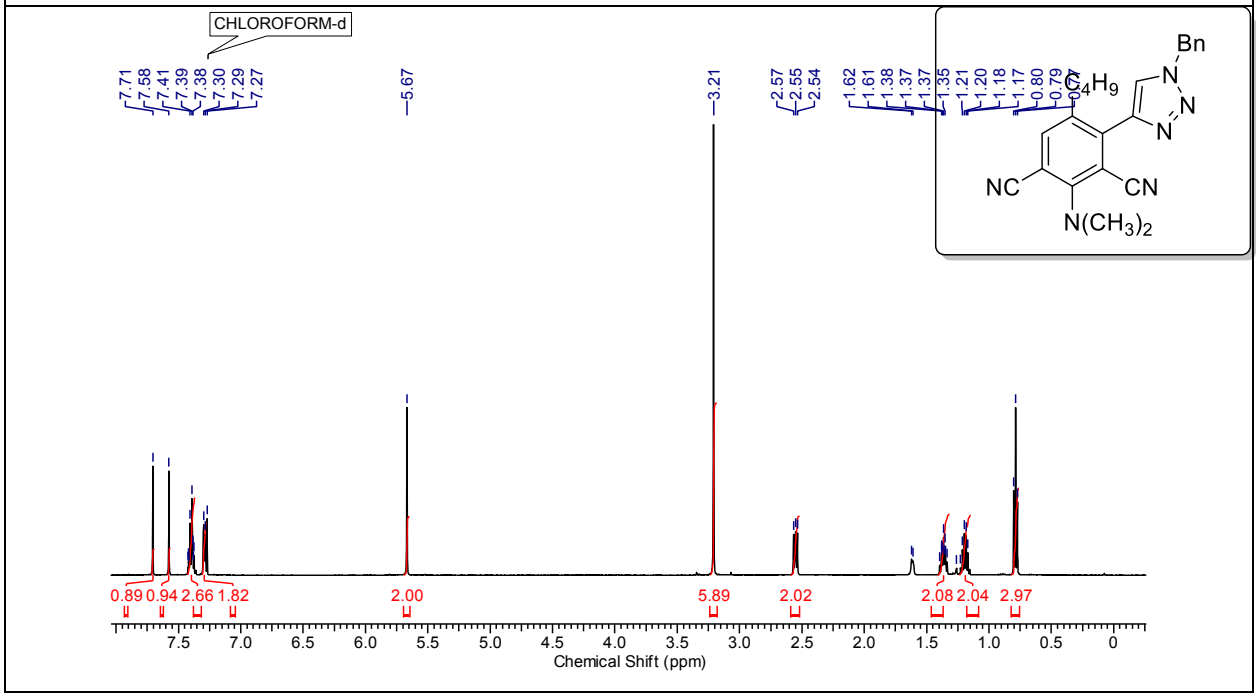
^{1}H NMR (500 MHz, $CDCl_3$) of compound 14c



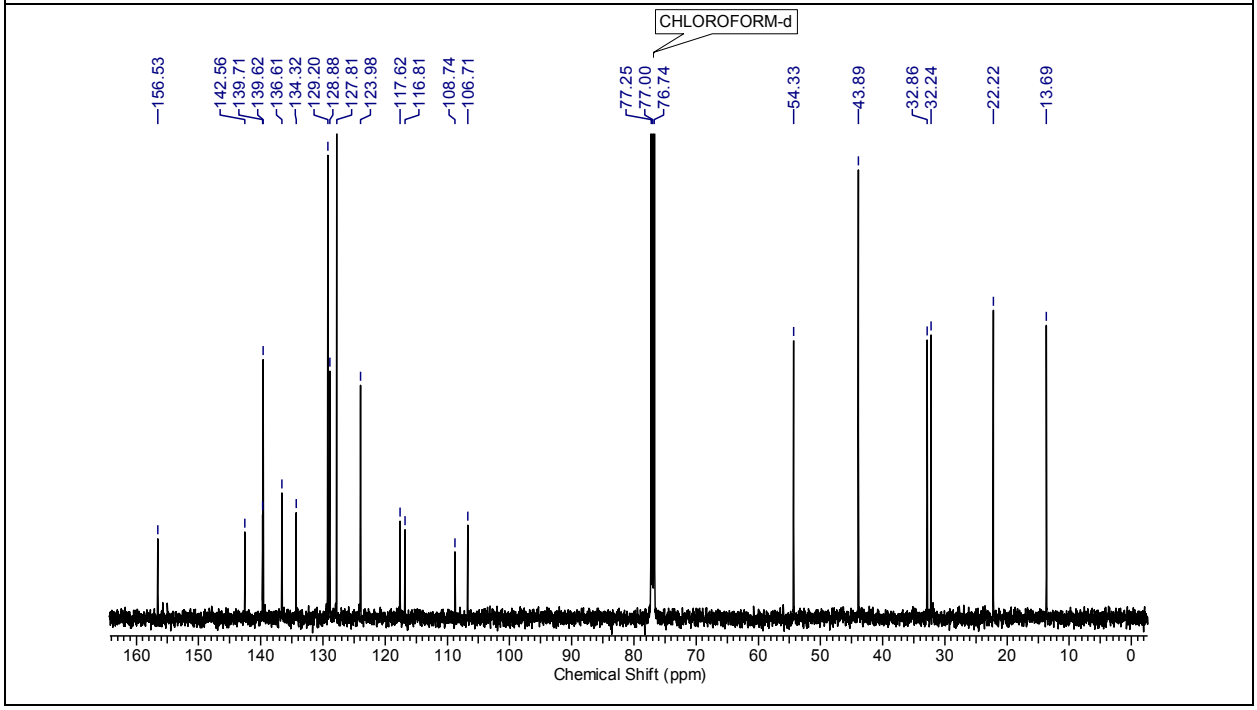
^{13}C NMR (125 MHz, $CDCl_3$) of compound 14c



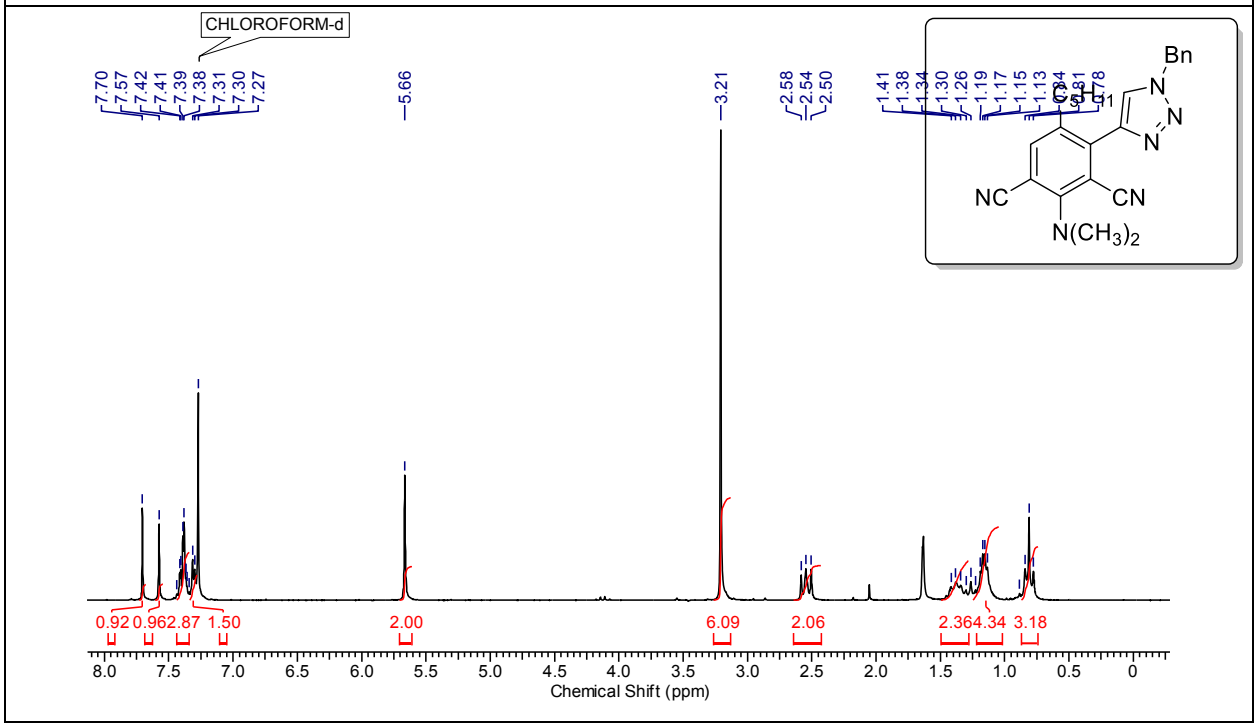
^{1}H NMR (500 MHz, $CDCl_3$) of compound 14d



^{13}C NMR (125 MHz, $CDCl_3$) of compound 14d



^{1}H NMR (200 MHz, $CDCl_3$) of compound 14e



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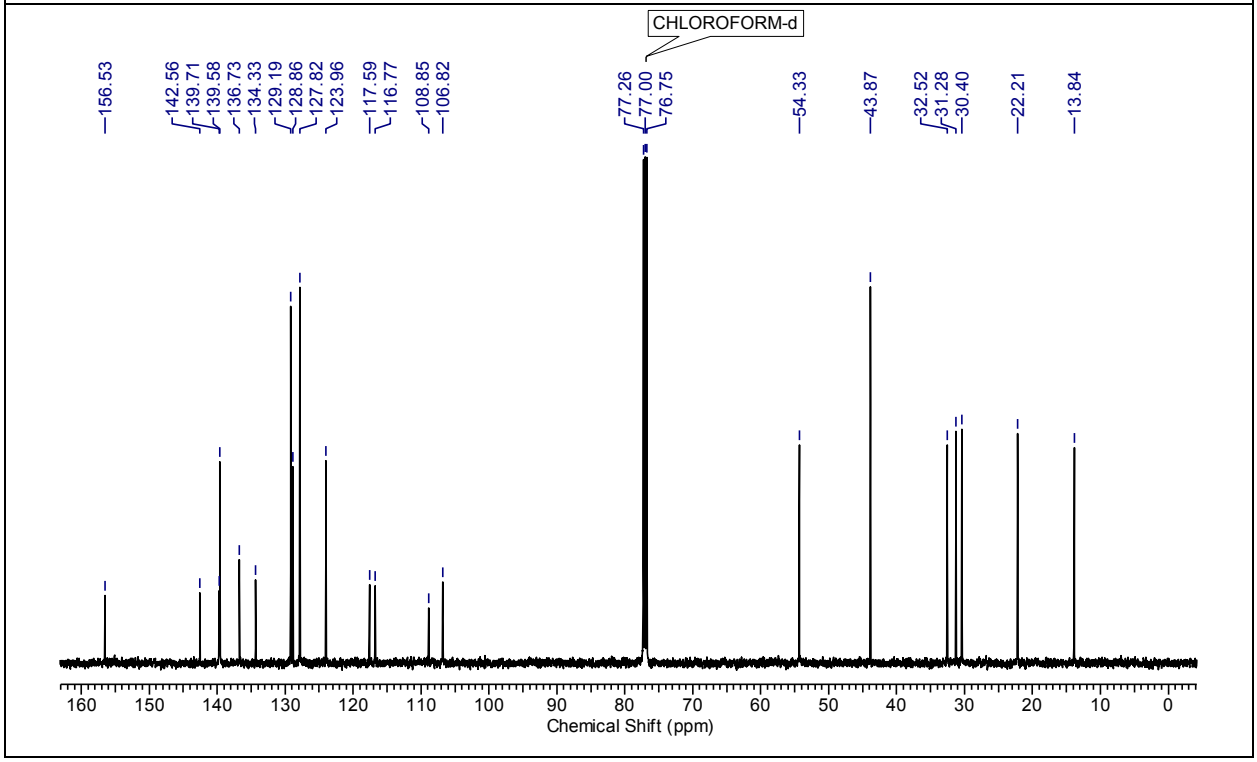

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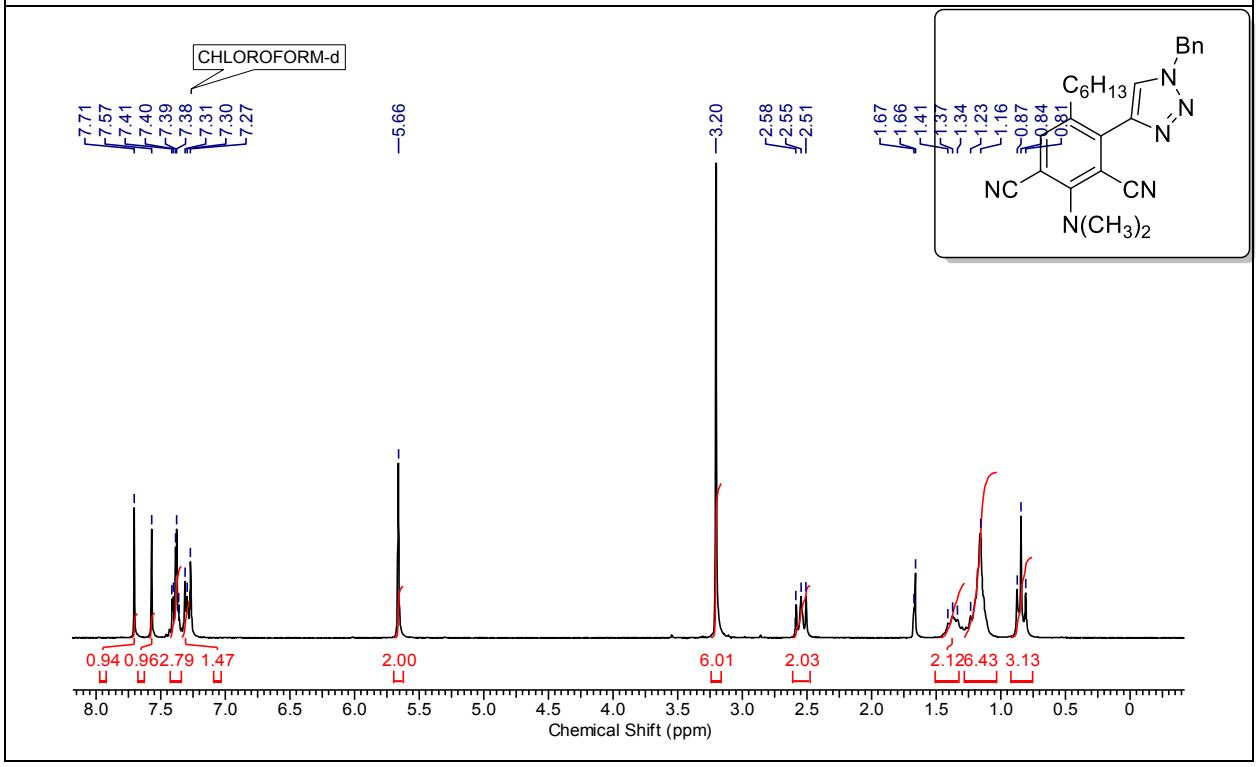

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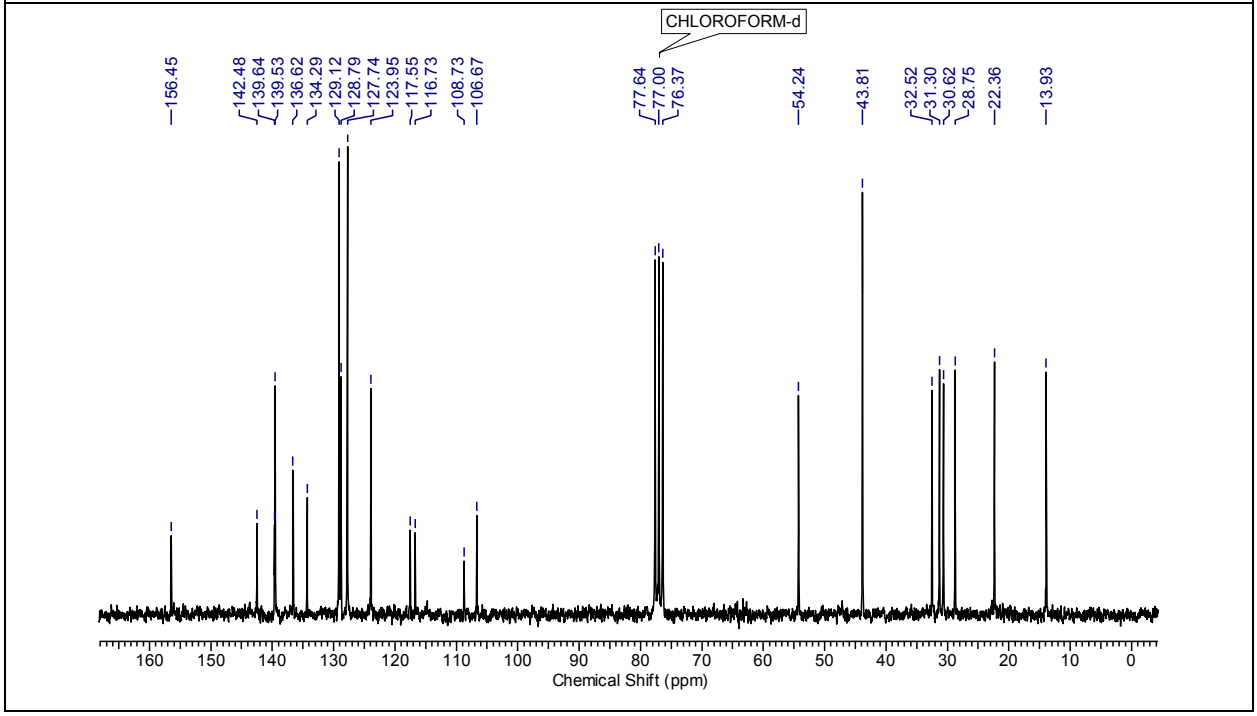

2.2.5. References

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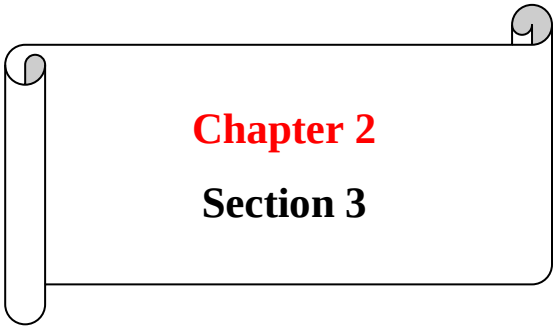


"Synthesis and biological evaluation of highly functionalized 2,3,5,6,7- and 2,3,4,5,7-substituted indoles"

2.3.1. Introduction

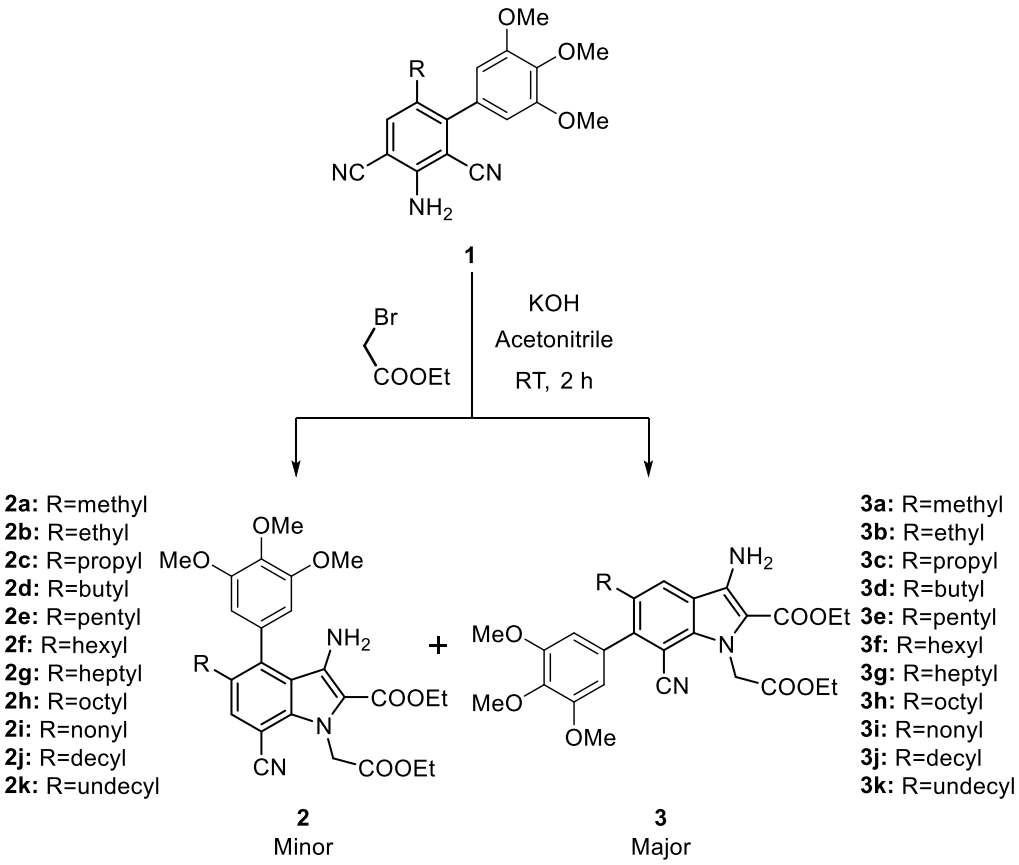
In the year 2005, B. Narsaiah and team reported[1] synthesis of substituted indoles in basic medium of K_2CO_3 and KI by reacting 3,5- substituted 2,6-dicyanoanilines with ethyl bromoacetate. In 2011, Borate and group published a paper[2] on the one-step synthesis of 2,6-dicyanoanilines and their use in the synthesisof highly substituted 2,3,5,6,7- and 2,3,4,5,7-substituted indoles. In an earlier section, the synthesis and biological applications of substituted 2,6-dicyanoanilines[3] were studied and it created a good opportunity to easily synthesize novel 2,3,5,6,7- and 2,3,4,5,7-substituted indoles and study them for further biological evaluation. This indole synthesis method was useful to produce a variety of new indole scaffolds to study the structure activity relationships and investigate their efficiency as new class of substrates for different biological applications.

2.3.2. Present work

2.3.2.1. Synthesis of Compounds

The reaction of aryl aldehyde, alkyl aldehydes and malononitrile in presence of morpholine as a base was employed in the synthesis of 4-alkyl-3-aryl-2,6-dicyanoanilines in one step by using three component at a time. . For structure-activity relationship study, initially, aryl aldehyde (3,4,5-trimethoxy benzaldehyde) was kept constant in all the synthesized compounds, and alkyl aldehydes were chosen selectively for changing the alkyl side-chain. Synthetic path for the regioisomers, 2,3,5,6,7- and 2,3,4,5,7-substituted indoles, is shown in Scheme 1. Minor product, ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-alkyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **(2)** and major product ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-alkyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **(3)** are formed in the regioselective cyclization of 3-amino-3',4',5'-trimethoxy-6-alkyl-[1,1'-biphenyl]-2,4-dicarbonitrile using ethyl bromoacetate, and potassium hydroxide as a base in two different ways known as Thorpe–Ziegler type of cyclization.[4] Substituents present on 2,6-dicyanoaniline ring play major role in the regioisomeric ratio of the product. In case of 3,4-disubstituted 2,6- dicyanoanilines[1] the cyclization involving CN at position 6 is favoured resulting in the formation of major product 3 while cyclization involving CN at position 2 is not favoured due to steric hindrance and the resulting isomer 2 is formed in minor amounts. The size of substituent at position 4 also has some effect on the ratio of the two isomers. As the bulk increases the yield of minor product

decreases e.g. when R sustituent in compound **1** is changed from methyl to undecyl, the yield of **2** decreases from 16% to 5%.. Prepared compounds were analyzed with various spectroscopic techniques such as 1H NMR, 13C NMR (IR) spectroscopy. Where as crystal structure was characterized with X-ray crystallographic analysis

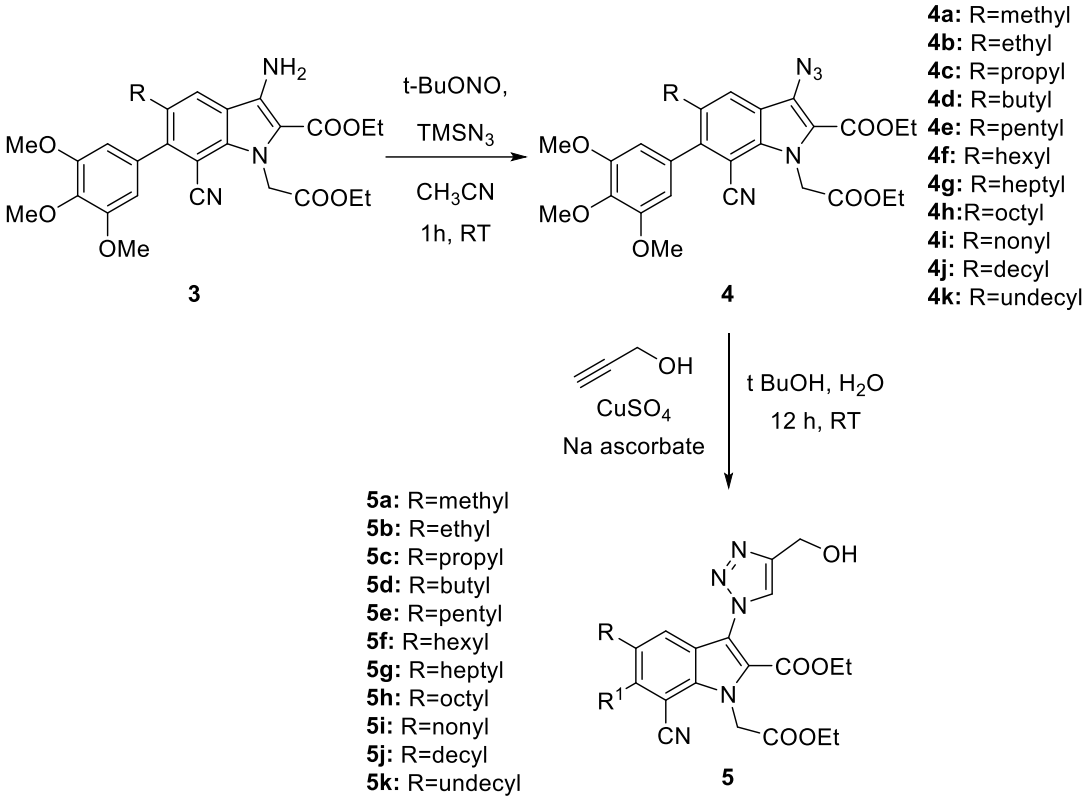


Scheme 1.

After the successful synthesis of compounds **2a**-**2k** and **3a**-**3k**, compounds **3a**-**3k** were functionalized as shown in Scheme 2.

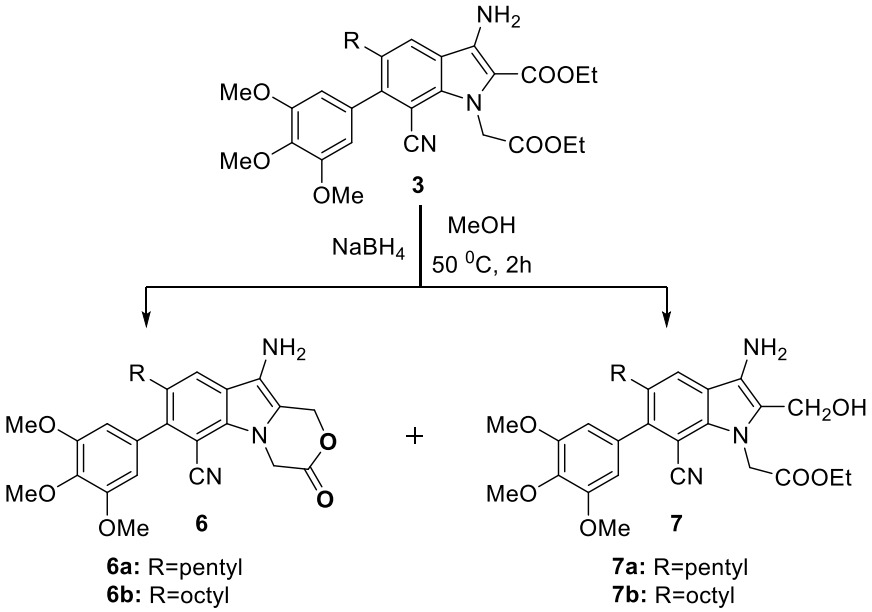
Series of compounds **3** (ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-alkyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate) were converted in azide **4** by reported method[5] compound **4** by reacting with trimethylsilylazide and *tert*-butyl nitrite in 76 to 90 % yield. The synthesized compounds were characterized using IR, ^{1}H NMR, and ^{13}C NMR spectroscopy as given in experimental section. Further, synthesized azide compounds **4a**-**4k were** treated with propargyl alcohol under copper catalyzed click reaction conditions to give the triazole compounds **5a**-**5k** in 81 to 92 % yield and. further these compounds characterized by using ^{1}H

NMR, and ^{13}C NMR spectroscopy, where as functional group analysis was done with IR.



3 4 5

Scheme 2.



Scheme 3.

The primary benefits of this synthetic strategy is that these highly substituted compounds **2** and **3** could be functionalized in a number of ways, resulting in a compound library. For example, compounds **3e** and **3h** were treated with sodium borohydride in presence of methanol as a solvent to give lactone compounds **6a** and **6b** and hydroxy esters **7a** and **7b** respectively. ^{1}H NMR,, ^{13}C NMR spectroscopy and used for structural characterization where as functional group conversion was monitored by IR spectroscopy

2.3.2.2. X-ray crystallographic characterization

Column chromatography (silica gel 60-120) was used to separate the two regioisomers **2** and **3**, it was shown that both isomers were easily separated on basic alumina compared to silica gel. Both the regioisomers were difficult to differentiate using ^{1}H and ^{13}C NMR spectra. Compound **2c** and compound **3c** were characterized using various spectroscopic techniques and the structures were confirmed using X-ray crystallographic characterization as shown in Figures 1a and 1b.

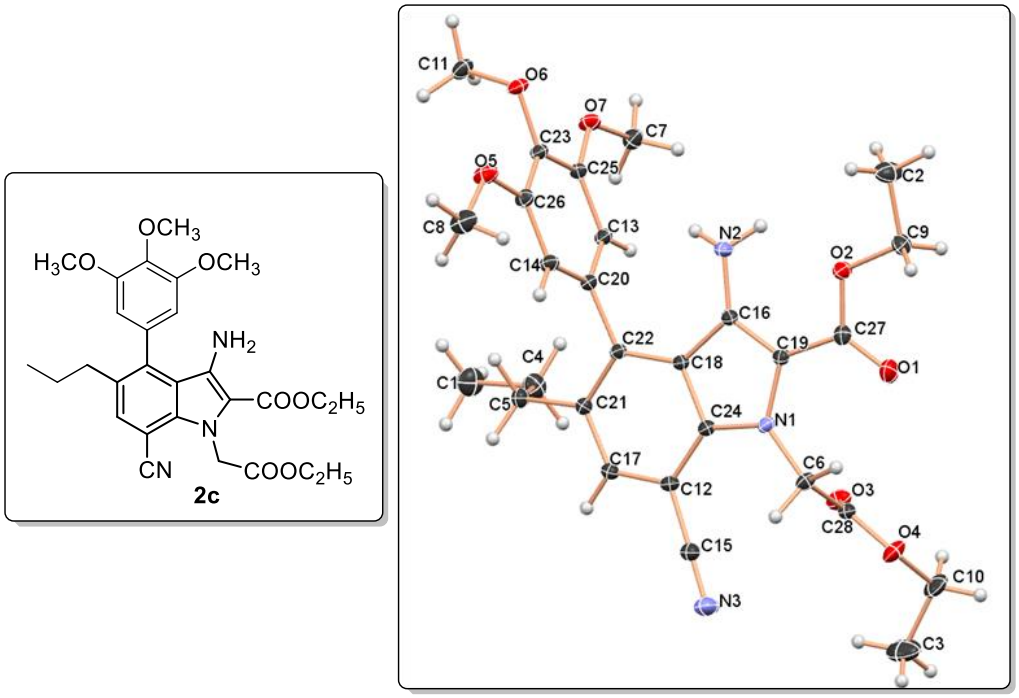


Figure 1a. ORTEP diagram of **2c** (CCDC 1552491)

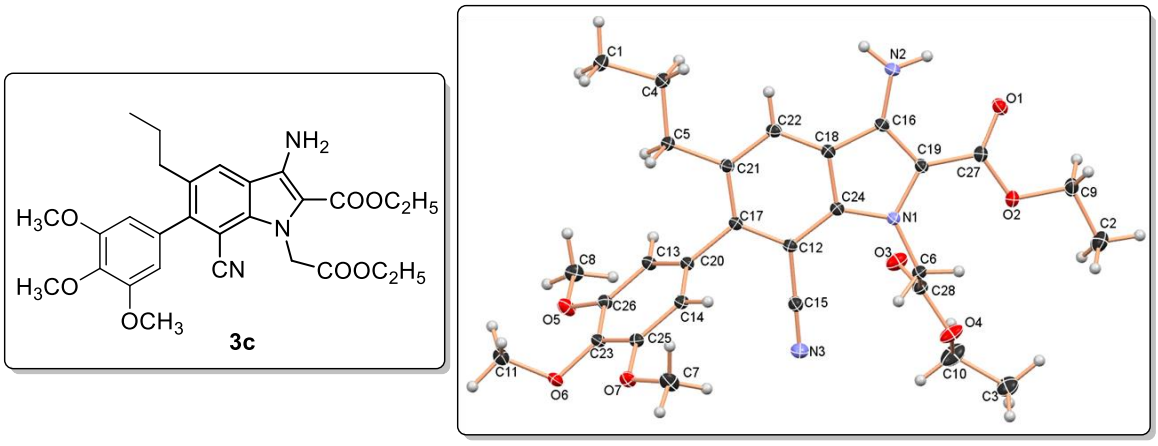


Figure 1b. ORTEP diagram of **3c** (CCDC 1552492)

2.3.2.3. Biological screening

The novel molecules synthesized in this study were subjected to a series of tests given below. It was found that antioxidant, antibacterial, and anticancer activities were not observed in the synthesized compounds. However, some compounds have shown interesting cell imaging properties, as described below.

Anti-oxidant assay: Synthesized compounds antioxidant nature was monitored with DPPH (1,1-diphenyl-2-picrylhydrazyl) assay[6] Ascorbic acid was used as standard control. The compounds in the present study did not have any anti-oxidant properties.

Antimicrobial assay: Antimicrobial activity screening was done against gram-negative bacteria *Escherichia coli* (NCIM 2065; ATCC 8739), gram positive bacteria *Staphylococcus aureus* (NCIM 2901; ATCC 29737) and *Mycobacterium tuberculosis* (ATCC 25177) H37 Ra RFP at the concentrations ranging as 100, 50, 25, 12.5, and 6.25 µg/mL.[7] The results concluded that synthesized compounds did not show any significant inhibition against bacteria at the maximum concentration assessed.

Anticancer activity: The anticancer activity was determined by the MTT dye uptake method[8] against HeLa, DU145, THP-1 and MDA MB cell lines at the concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL. Studies have shown that no significant difference was observed against any cell lines.

Cell imaging: Synthesis with bio-imaging application of mono/di/tri-heterocyclyl-2,6-dicyanoanilines due to their fluroscent propertieds were discussed in the previous section. The optical properties of the substituted 2,6-dicyanoanilines were investigated, and it was observed that the fluorescence properties of this class of compounds can be modified by changing functional groups of 2,6-dicyanoanilines. 2,6-dicyanoanilines were used as precursor for the synthesis of 2,3,5,6,7- and 2,3,4,5,7- substituted indoles and showed fluorescent properties, indicating that they might be useful for cell imaging. According to preliminary findings, the compounds described in the present section have a lot of potential as cell imaging tools.

THP-1 cell lines were seeded ($1X10^6$ cells/mL) on 96 well plates. Culture medium after incubation at 37 °C for 24 h in CO_2 incubator, was taken out and replaced with phosphate-buffered saline (PBS, pH 7.2) containing the synthetic compound at concentrations of 100 µg/mL and was incubated for 20 minutes. Cells washed twice before it examined under fluorescent microscope with PBS (EVOS FL, Invitrogen) at 20X objective magnification.

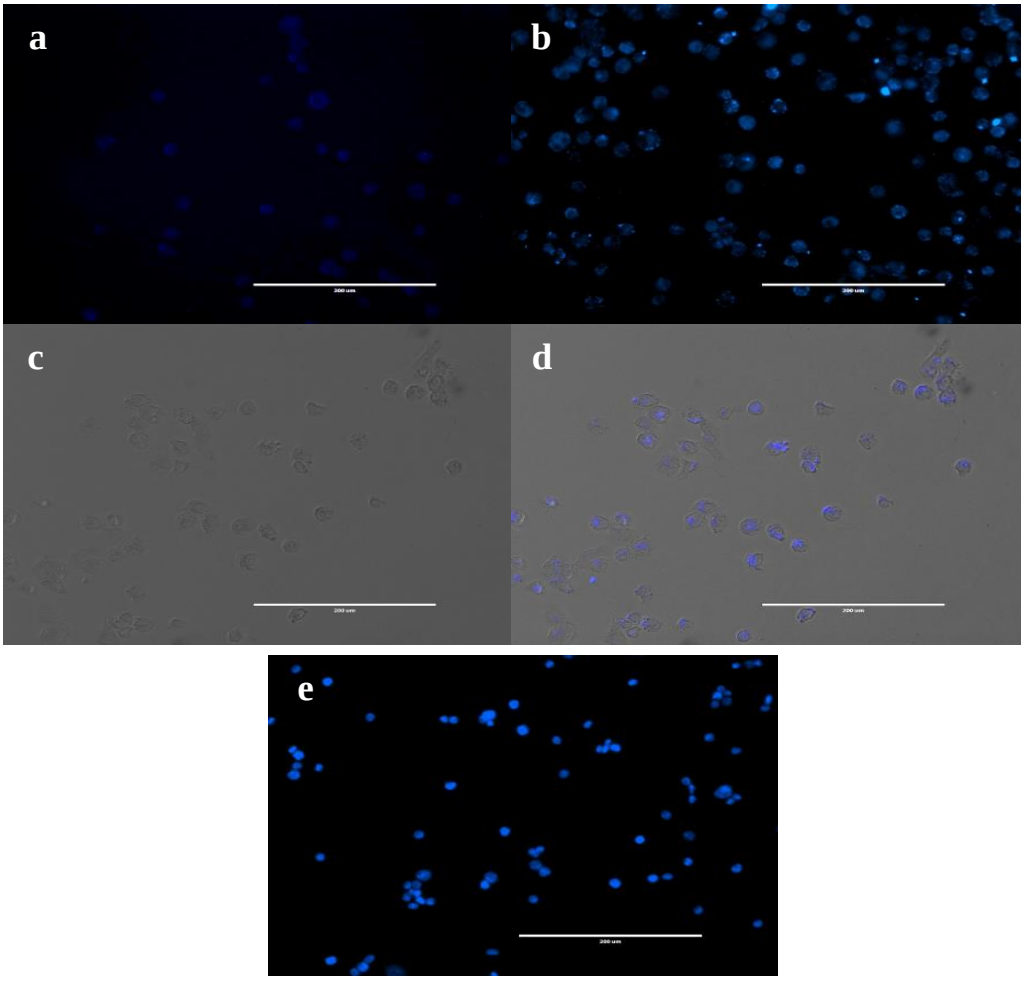


Figure 2. THP-1 cells stained with compounds **2a** and **2e** (100 µg/mL), (A) compound **2a** cells seen in blue channel (B) compound **2e** in the blue channel (C) **2e** in a bright channel (D) **2e** in overlay of the bright and blue channel (E) Control with DAPI in the blue channel

Initial studies on THP-1 cells showed that the compounds **2a** and **2e** stained nuclei as shown in blue (Fig. 1A, B) similar to control with DAPI (4′,6-diamidino-2-phenylindole) (Fig. 1E). Furthermore, THP-1 cells stained with nile red (stained the cell membrane) (Fig. 2A) and overlay of compound showed yellow color as a mixed of red and green (Fig. 2B). The results indicated that synthesized compounds could be used like DAPI which stained nuclei of the cells and also they can be used for cell membrane staining.

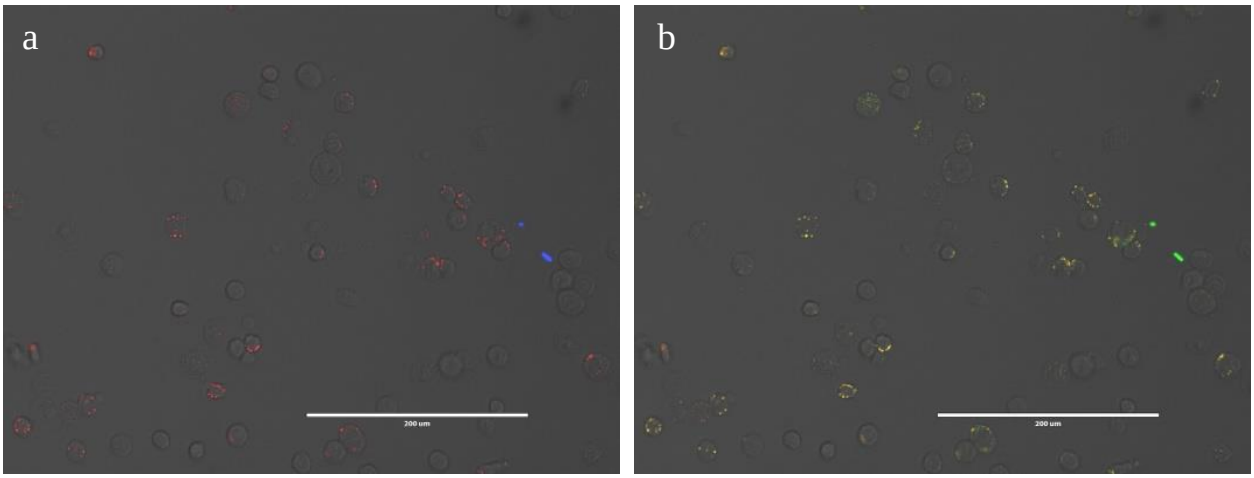


Figure 3. THP-1 cells stained with compound **2a** (100 µg/mL) followed by nile red (A) overlay channel of bright field and red channel (B) overlay image of bright, green (compound) and red (nile red) channel

2.3.3. Conclusions

In conclusion, synthesis of ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-alkyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate and ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-alkyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate, starting from 3-amino-6-alkyl-3',4',5'-trimethoxy-[1,1'-biphenyl]-2,4-dicarbonitrile, ethyl bromoacetate and potassium hydroxide was achieved. Further compound derivatization was achieved by functional group interconversion. The compounds were initially studied from the point of their application for cell imaging wherein it was found that compounds **2a** and **2e** can be used like DAPI which stained nuclei of the cells. These initial results indicate the significant potential of these compounds as valuable tools for cell imaging. Further study is in progress.

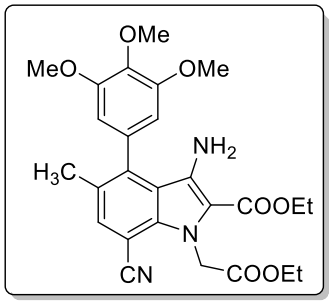
2.3.4. Experimental

2.3.4.1. Synthesis and spectral data

Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate (**2a)** and ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **(3a)**

The 3-amino-3',4',5'-trimethoxy-6-methyl-[1,1'-biphenyl]-2,4-dicarbonitrile (2 gm, 0.006 mol), and ethyl bromoacetate (2 ml, 0.018 mol) were dissolved in acetonitrile (10 ml), in a two necked RB flask under argon atmosphere at RT and the pellets of potassium hydroxide (2 gm, 0.037 mol) were added. The reaction mixture was stirred at room temperature for 2 h. It was then diluted with excess of cold water and extracted with ethyl acetate (3 x 50 ml), dried over anhydrous Na_2SO_4 filtered and concentrated and purified by column chromatography on silica gel (60-120) using pet ether-ethyl acetate as eluent, Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **2a** yellow solid, 0.5 gm, 16%, melting point: 179 °C. Further elution afforded ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **3a** as a yellow solid, 2 gm, 65%, melting point: 178 °C.

2a: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 16 %.

Melting point: 179 ºC.

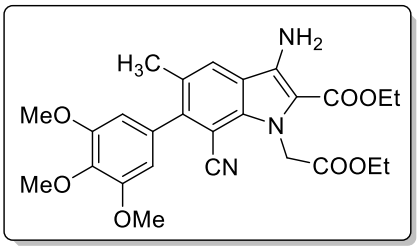
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.27-1.39 (m, 6H), 2.13 (s, 3H), 3.86 (s, 6H), 3.95 (s, 3H), 4.18-4.39 (m, 4H), 5.57 (s, 2H), 6.50 (s, 2H), 7.55 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.07, 14.25, 18.49, 22.57, 29.57, 46.90, 56.14 (2C), 60.17, 60.96, 61.48, 92.89, 105.38 (2C), 109.48, 117.98, 118.10, 126.64, 132.50, 135.42, 137.71, 141.21, 153.39 (2C), 162.35, 169.33.

IR ($CHCl_3$): 3385, 2216, 1745, 1675 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{26}H_{30}N_3O_7$ $[M+H]^+$: 496.2085, found: 496.2078.

3a: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 65 %.

Melting point: 178 ºC.

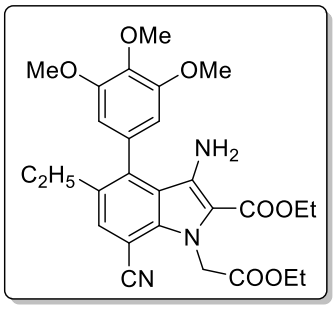
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (t, J = 7Hz, 3H), 1.42 (t, J = 7Hz, 3H), 2.20 (s, 3H), 3.86 (s, 6H), 3.92 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.40 (q, J = 7Hz, 2H), 5.60 (s, 2H), 6.49 (s, 2H), 7.66 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.04, 14.28, 20.42, 46.72, 56.09 (2C), 60.29, 60.82, 61.40, 94.69, 106.27 (2C), 110.30, 116.99, 119.65, 125.06, 127.19, 133.63, 136.04, 136.22, 137.71, 147.31, 153.06 (2C), 162.32, 169.58.

IR ($CHCl_3$): 3365, 2220, 1745, 1677 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{26}H_{30}N_3O_7$ $[M+H]^+$: 496.2079, found: 496.2078; $C_{26}H_{29}N_3NaO_7[M+Na]^+$: 518.1899, found: 518.1898.

2b: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-ethyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 13 %.

Melting point: 168 ºC.

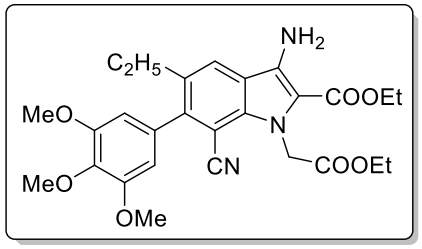
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.11 (t, J = 7Hz, 3H), 1.22-1.43 (m, 6H), 2.44 (q, J = 7Hz, 2H), 3.85 (s, 6H), 3.95 (s, 3H), 4.19-4.41 (m, 4H), 5.58 (s, 2H), 6.52 (s, 2H), 7.59 (s, 1H).

^{13}C NMR(50 MHz, $CDCl_3$): δ 14.07, 14.25, 16.33, 24.79, 46.89, 56.14 (2C), 60.16, 60.99, 61.49, 93.30, 105.67 (2C), 109.43, 117.94, 118.07, 132.10, 132.97, 134.41, 135.54, 137.64, 137.91, 140.63, 153.18 (2C), 162.35, 169.35.

IR ($CHCl_3$): 3384, 2216, 1740, 1679 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{27}H_{32}N_3O_7$ $[M+H]^+$: 510.2232, found: 510.2235; $C_{27}H_{31}N_3NaO_7$ $[M+Na]^+$: 532.2054, found: 532.2054.

3b: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-ethyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 62 %.

Melting point: 176 ºC.

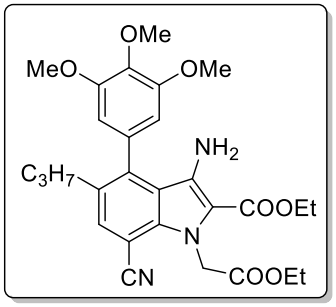
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.11 (t, J = 7Hz, 3H), 1.30 (t, J = 7Hz, 3H), 1.42 (t, J = 7Hz, 3H), 2.52 (q, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.41 (q, J = 7Hz, 2H), 5.61 (s, 2H), 6.50 (s, 2H), 7.70 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.03, 14.28, 15.72, 26.22, 46.73, 56.08 (2C), 60.28, 60.83, 61.38, 94.82, 106.52 (2C), 110.30, 116.96, 119.91, 123.66, 133.37, 133.53, 135.88, 136.36, 137.75, 147.02, 152.94 (2C), 162.34, 169.58.

IR ($CHCl_3$): 3367, 2220, 1773, 1675 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{27}H_{32}N_3O_7$ $[M+H]^+$: 510.2238, found: 510.2235; $C_{27}H_{31}N_3NaO_7$ $[M+Na]^+$: 532.2056, found: 532.2054.

2c: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-propyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 10 %.

Melting point: 162 ºC.

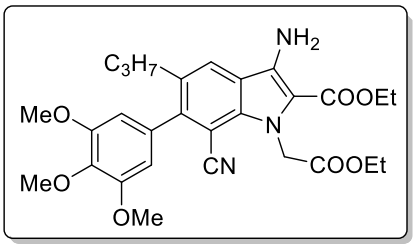
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, J = 7Hz, 3H), 1.26-1.58 (m, 8H), 2.39 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.95 (s, 3H), 4.17-4.41 (m, 4H), 5.57 (s, 2H), 6.51 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.70, 13.98, 14.15, 24.86, 33.36, 46.79, 56.06 (2C), 60.05, 60.90, 61.37, 93.04, 105.72 (2C), 109.29, 117.84, 117.95, 131.31, 132.03, 134.79, 135.36, 137.56, 137.77, 140.78, 153.04 (2C), 162.22, 169.25.

IR ($CHCl_3$): 3385, 2218, 1748, 1674 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{28}H_{34}N_3O_7$ [M+H]: 524.2401, found: 524.2391; $C_{28}H_{33}N_3NaO_7$ [M+Na]: 546.2220, found: 546.2211.

3c: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-propyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 73 %.

Melting point: 173 ºC.

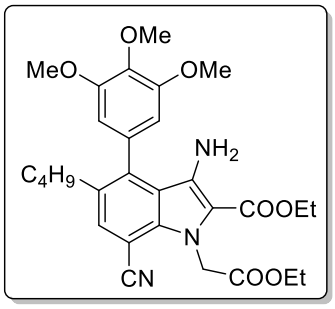
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, J = 7Hz, 3H), 1.29 (t, J = 7Hz, 3H), 1.34-1.57 (m, 5H), 2.47 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.40 (q, J = 7Hz, 2H), 5.60 (s, 2H), 6.50 (s, 2H), 7.67 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.85, 14.12, 14.36, 24.68, 35.13, 46.82, 56.18 (2C), 60.40, 60.94, 61.47, 95.05, 106.67 (2C), 110.55, 117.02, 119.87, 124.27, 132.12, 133.46, 135.99, 136.23, 137.84, 147.26, 152.97 (2C), 162.43, 169.62.

IR ($CHCl_3$): 3366, 2221, 1763, 1680 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{28}H_{34}N_3O_7$ $[M+H]^+$: 524.2397, found: 524.2391; $C_{28}H_{33}N_3NaO_7$ $[M+Na]^+$: 546.2211, found: 546.2211.

2d: Ethyl 3-amino-5-butyl-7-cyano-1-(2-ethoxy-2-oxoethyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 12 %.

Melting point: 156 ºC.

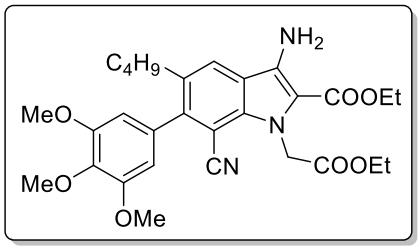
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, J = 7Hz, 3H), 1.13-1.52 (m, 10H), 2.40 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.96 (s, 3H), 4.15-4.43 (m, 4H), 5.58 (s, 2H), 6.52 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.88, 14.18, 14.36, 22.35, 31.45, 31.52, 31.71, 47.03, 56.23 (2C), 60.28, 61.14, 61.63, 93.29, 105.85 (2C), 109.53, 118.04, 118.21, 131.80, 132.24, 135.06, 135.61, 138.01, 140.88, 153.20 (2C), 162.48, 169.49.

IR ($CHCl_3$): 3388, 2220, 1750, 1678 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{29}H_{36}N_3O_7$ $[M+H]^+$: 537.2470, found: 537.2473.

3d: Ethyl 3-amino-5-butyl-7-cyano-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 71 %.

Melting point: 157 ºC.

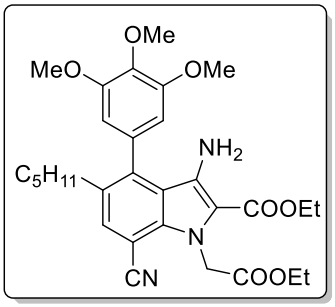
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, J = 7Hz, 3H), 1.10-1.51 (m, 10H), 2.48 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.41 (q, J = 7Hz, 2H), 5.61 (s, 2H), 6.50 (s, 2H), 7.69 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.86, 14.12, 14.37, 22.32, 31.44, 33.11, 46.82, 56.15 (2C), 60.42, 60.96, 61.49, 95.03, 106.59 (2C), 110.56, 117.04, 119.88, 124.25, 132.44, 133.46, 135.95, 136.17, 137.77, 147.23, 152.96 (2C), 162.45, 169.64.

IR ($CHCl_3$): 3377, 2220, 1743, 1675 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{29}H_{36}N_3O_7$ $[M+H]^+$: 538.2549, found: 538.2548.

2e: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-pentyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 10 %.

Melting point: 155 ºC.

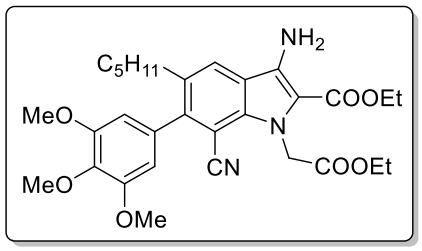
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, J = 7Hz, 3H), 1.13-1.52 (m, 12H), 2.40 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.96 (s, 3H), 4.15-4.43 (m, 4H), 5.58 (s, 2H), 6.52 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.86, 14.16, 14.34, 22.34, 31.43, 31.51, 31.69, 47.00, 56.21 (2C), 60.26, 61.12, 61.60, 93.27, 105.82 (2C), 109.49, 118.02, 118.19, 131.78, 132.22, 135.04, 135.59, 137.69, 137.99, 140.86, 153.19 (2C), 162.47, 169.48.

IR ($CHCl_3$): 3385, 2220, 1751, 1674 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{30}H_{37}N_3NaO_7$ $[M+Na]^+$: 574.2530, found: 574.2524.

3e: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 65 %.

Melting point: 152 ºC.

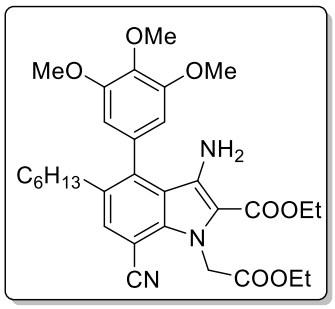
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, J = 7Hz, 3H), 1.10-1.55 (m, 12H), 2.47 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.41 (q, J = 7Hz, 2H), 5.59 (s, 2H), 6.50 (s, 2H), 7.64 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.85, 14.12, 14.36, 22.30, 31.36, 31.43, 33.11, 46.81, 56.15 (2C), 60.41, 60.94, 61.48, 95.02, 106.64 (2C), 110.55, 117.02, 119.88, 124.26, 132.43, 133.46, 135.96, 136.19, 137.81, 147.22, 152.96 (2C), 162.43, 169.63.

IR ($CHCl_3$): 3367, 2218, 1761, 1678 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{30}H_{37}N_3NaO_7$ $[M+Na]^+$: 574.2529, found: 574.2524.

2f: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-hexyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 5 %.

Melting point: 150 ºC.

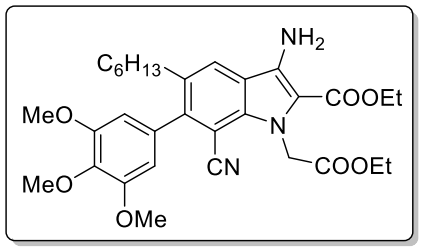
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.83 (t, J = 7Hz, 3H), 1.13-1.54 (m, 14H), 2.39 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.95 (s, 3H), 4.21-4.39 (m, 4H), 5.57 (s, 2H), 6.51 (s, 2H), 7.56 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.97, 14.15, 14.33, 22.40, 28.91, 31.48, 31.54, 31.96, 47.00, 56.20 (2C), 60.23, 61.10, 61.58, 93.27, 105.85 (2C), 109.51, 118.03, 118.17, 131.78, 132.21, 135.01, 135.58, 137.72, 137.98, 140.85, 153.19 (2C), 162.45, 169.45.

IR ($CHCl_3$): 3387, 2221, 1742, 1679 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{31}H_{39}N_3NaO_7$ $[M+Na]^+$: 588.2686, found: 588.2680.

3f: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-hexyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 69 %.

Melting point: 116 ºC.

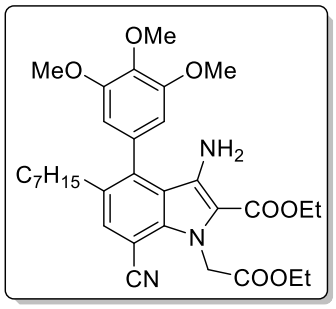
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 (t, *J* = 7Hz, 3H), 1.10-1.50 (m, 14H), 2.47 (t, *J* = 7Hz, 2H), 3.85 (s, 6H), 3.92 (s, 3H), 4.25 (q, *J* = 7Hz, 2H), 4.40 (q, *J* = 7Hz, 2H), 5.58 (s, 2H), 6.50 (s, 2H), 7.65 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.94, 14.10, 14.34, 22.38, 28.89, 31.45, 31.63, 33.12, 46.80, 56.12 (2C), 60.40, 60.92, 61.46, 94.99, 106.61 (2C), 110.59, 117.02, 119.88, 124.28, 132.43, 133.44, 135.92, 136.07, 137.78, 147.20, 152.94 (2C), 162.40, 169.62.

IR ($CHCl_3$): 3370, 2220, 1770, 1660 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{31}H_{39}N_3NaO_7$ $[M+Na]^+$: 588.2682, found: 588.2680.

2g: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-heptyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 9 %.

Melting point: 124 ºC.

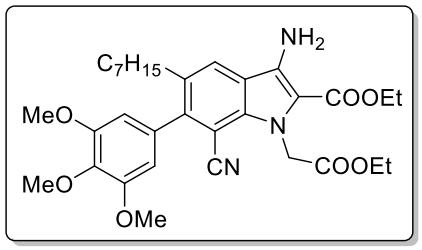
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85(t, *J* = 7Hz, 3H), 1.10-1.55 (m, 16H), 2.39 (t, *J* = 7Hz, 2H), 3.85 (s, 6H), 3.95 (s, 3H), 4.21-4.39 (m, 4H), 5.57 (s, 2H), 6.51 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.99, 14.16, 14.34, 22.54, 28.97, 29.23, 31.56, 31.61, 32.03, 47.01, 56.21 (2C), 60.25, 61.11, 61.59, 93.28, 105.86 (2C), 109.53, 118.03, 118.18, 131.79, 132.21, 135.03, 135.59, 137.73, 137.96, 140.85, 153.20 (2C), 162.46, 169.46.

IR ($CHCl_3$): 3395, 2220, 1680, 1645 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{32}H_{41}N_3NaO_7$ $[M+Na]^+$: 602.2843, found: 602.2837.

3g: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-heptyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 73 %.

Melting point: 110 ºC.

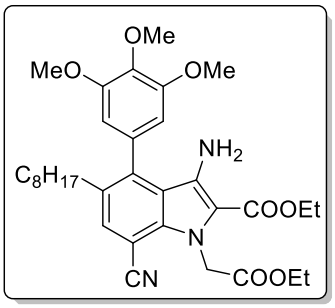
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, J = 7Hz, 3H), 1.05-1.55 (m, 16H), 2.47 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.92 (s, 3H), 4.24 (q, J = 7Hz, 2H), 4.39 (q, J = 7Hz, 2H), 5.58 (s, 2H), 6.50 (s, 2H), 7.65 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.97, 14.10, 14.35, 22.51, 28.95, 29.21, 31.60, 31.70, 33.14, 46.80, 56.13 (2C), 60.42, 60.93, 61.47, 95.00, 106.61 (2C), 110.61, 117.02, 119.88, 124.28, 132.46, 133.44, 135.93, 135.98, 137.79, 147.21, 152.94 (2C), 162.40, 169.62.

IR ($CHCl_3$): 3365, 2220, 1773, 1666 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{32}H_{41}N_3NaO_7$ $[M+Na]^+$: 602.2842, found: 602.2837.

2h: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-octyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 7 %.

Melting point: 118 ºC.

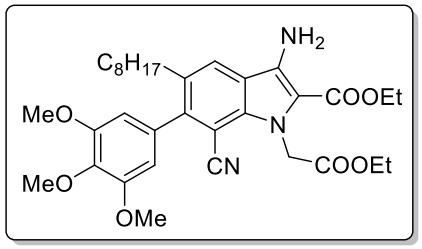
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.14-1.30 (m, 10H), 1.30-1.38 (m, 6H), 1.41-1.52 (m, 2H), 2.40 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.96 (s, 3H), 4.22-4.37 (m, 4H), 4.54 (bs, 2H), 5.58 (s, 2H), 6.51 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.05, 14.18, 14.36, 22.59, 29.13, 29.32 (2C), 31.58, 31.79, 32.08, 47.03, 56.22 (2C), 60.27, 61.13, 61.62, 93.29, 105.85 (2C), 109.52, 118.04, 118.21, 131.80, 132.24, 135.05, 135.61, 137.72, 138.02, 140.88, 153.20 (2C), 162.49, 169.50.

IR ($CHCl_3$): 3385, 2220, 1743, 1670 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{33}H_{44}N_3O_7$ $[M+H]^+$: 594.3181, found: 594.3174.

3h: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-octyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 78 %.

Melting point: 107 ºC.

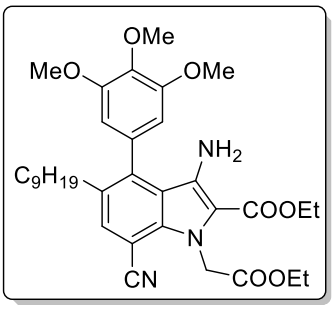
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.13-1.51 (m, 18H), 2.49 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.27 (q, J = 7Hz, 2H), 4.42 (q, J = 7Hz, 2H), 5.60 (s, 2H), 6.50 (s, 2H), 7.64 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02, 14.13, 14.38, 22.57, 29.11, 29.31 (2C), 31.75 (2C), 33.16, 46.82, 56.15 (2C), 60.47, 60.96, 61.50, 95.05, 106.60 (2C), 110.71, 117.03, 119.90, 124.27 (2C), 132.49, 133.44, 135.92, 137.78, 147.25, 152.95 (2C), 162.41, 169.62.

IR ($CHCl_3$): 3367, 2221, 1740, 1687 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{33}H_{44}N_3O_7$ $[M+H]^+$: 594.3176, found: 594.3174; $C_{33}H_{43}N_3NaO_7$ $[M+Na]^+$: 616.2993, found: 616.2993.

2i: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-nonyl-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 8 %.

Melting point: 102 ºC.

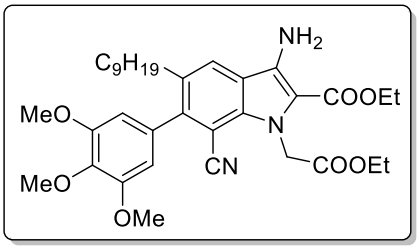
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7Hz, 3H), 1.12-1.52 (m, 20H), 2.40 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.96 (s, 3H), 4.19-4.40 (m, 4H), 4.54 (bs, 2H), 5.58 (s, 2H), 6.51 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.01, 14.12, 14.31, 22.57, 29.18, 29.26, 29.32, 29.37, 31.54, 31.75, 32.02, 46.97, 56.18 (2C), 60.22, 61.07, 61.56, 93.24, 105.85 (2C), 109.50, 118.00, 118.13, 131.75, 132.18, 134.98, 135.56, 137.72, 137.93, 140.82, 153.17 (2C), 162.42, 169.42.

IR ($CHCl_3$): 3386, 2219, 1749, 1678 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{34}H_{46}N_3O_7$ $[M+H]^+$: 608.3336, found: 608.3330; $C_{34}H_{45}N_3NaO_7$ $[M+Na]^+$: 630.3154, found: 630.3150.

3i: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-nonyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 77 %.

Melting point: 102 ºC.

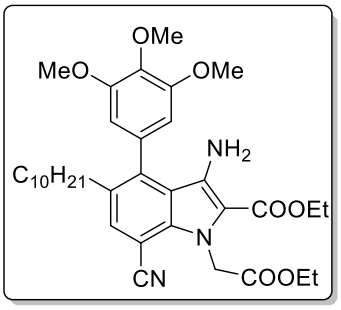
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87(t, J = 7Hz, 3H), 1.10-1.50 (m, 20H), 2.48 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.40 (q, J = 7Hz, 2H), 5.00 (bs, 2H), 5.59 (s, 2H), 6.50 (s, 2H), 7.64 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.04, 14.12, 14.37, 22.60, 29.21, 29.31, 29.36, 29.42, 31.73, 31.79, 33.17, 46.84, 56.16 (2C), 60.51, 60.96, 61.50, 106.64 (2C), 117.00, 119.95, 120.55, 124.29, 132.58, 133.43, 135.90, 137.85, 144.07, 147.27, 152.97 (2C), 154.72, 162.37, 169.58.

IR ($CHCl_3$): 3375, 2220, 1753, 1676 cm^{-1}.

HRMS (ESI) m/z calculated for $C_{34}H_{46}N_3O_7$ $[M+H]^+$: 608.3333, found: 608.3330; $C_{34}H_{45}N_3NaO_7$ $[M+Na]^+$: 630.3153, found: 630.3150.

2j: Ethyl 3-amino-7-cyano-5-decyl-1-(2-ethoxy-2-oxoethyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 7 %.

Melting point: 101 ºC.

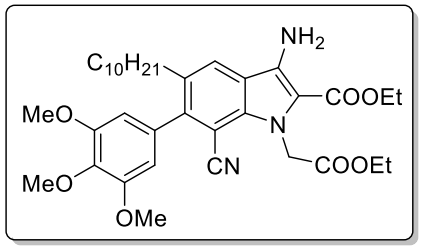
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7Hz, 3H), 1.11-1.40 (m, 22H), 2.40 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.96 (s, 3H), 4.17-4.41 (m, 4H), 5.58 (s, 2H), 6.51 (s, 2H),7.57 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ14.01, 14.11, 14.29, 22.57, 29.19, 29.26, 29.31, 29.41, 29.47, 31.52, 31.78, 32.01, 46.95, 56.16 (2C), 60.20, 61.05, 61.53, 93.23, 105.83 (2C), 109.47, 117.99, 118.13, 131.74, 132.17, 134.97, 135.54, 137.69, 137.93, 140.81, 153.16 (2C), 162.40, 169.41,

IR ($CHCl_3$): 3387, 2220, 1742, 1675 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{35}H_{48}N_3O_7$ $[M+H]^+$: 622.3484, found: 622.3487; $C_{35}H_{47}N_3NaO_7$ $[M+Na]^+$: 644.3306, found: 644.3306.

3j: Ethyl 3-amino-7-cyano-5-decyl-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 86 %.

Melting point: 100 ºC.

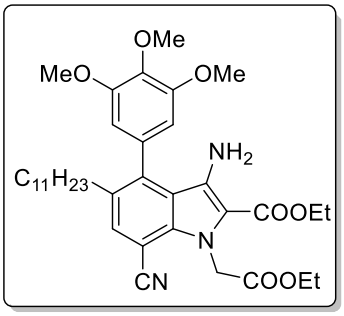
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7Hz, 3H), 1.10-1.50 (m, 22H), 2.48 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.25 (q, J = 7Hz, 2H), 4.40 (q, J = 7Hz, 2H), 5.60 (s, 2H), 6.50 (s, 2H), 7.66 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.98, 14.05, 14.29, 22.54, 24.60, 29.16, 29.28, 29.43, 31.66, 31.74, 33.09, 35.06, 46.75, 56.09 (2C), 60.35, 60.87, 61.42, 94.90, 106.59 (2C), 110.50, 116.99, 119.84, 124.31, 132.08, 132.37, 133.41, 135.89, 137.75, 147.15, 152.89 (2C), 162.34, 169.58.

IR ($CHCl_3$): 3377, 2219, 1766, 1685 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{35}H_{48}N_3O_7$ $[M+H]^+$: 622.3490, found: 622.3487; $C_{35}H_{47}N_3NaO_7$ $[M+Na]^+$: 644.3309, found: 644.3306.

2k: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-4-(3,4,5-trimethoxyphenyl)-5-undecyl-1*H*-indole-2-carboxylate



Yield: 5 %.

Melting point: 98 ºC.

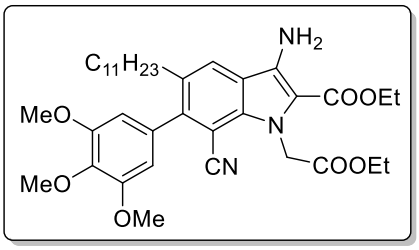
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7Hz, 3H), 1.05-1.47 (m, 24H), 2.40 (t, J = 7Hz, 2H), 3.85 (s, 6H), 3.96 (s, 3H), 4.18-4.40 (m, 4H), 5.58 (s, 2H), 6.52 (s, 2H), 7.57 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 14.01, 14.10, 14.28, 22.57, 29.21, 29.23, 29.25, 29.31, 29.40, 29.49, 31.52, 31.79, 32.00, 46.95, 56.15 (2C), 60.19, 61.03, 61.52, 93.22, 105.83 (2C), 109.50, 117.98, 118.11, 131.74, 132.15, 134.95, 135.51, 137.69, 137.85, 140.79, 153.15 (2C), 162.38, 169.39.

IR ($CHCl_3$): 3388, 2220, 1747, 1675 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{36}H_{50}N_3O_7$ $[M+H]^+$: 636.3644, found: 636.3643; $C_{36}H_{49}N_3NaO_7$ $[M+Na]^+$: 658.3464, found: 658.3463.

3k: Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-5-undecyl-1*H*-indole-2-carboxylate



Yield: 82 %.

Melting point: 98 ºC.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7Hz, 3H), 1.08-1.52 (m, 24H), 2.48 (t, *J* = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.25 (q, *J* = 7Hz, 2H), 4.41 (q, *J* = 7Hz, 2H), 5.60 (s, 2H), 6.49 (s, 2H), 7.68 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 14.01, 14.24, 22.49, 29.14, 29.20, 29.24, 29.34, 29.35, 29.43, 31.60, 31.71, 33.05, 46.70, 56.04 (2C), 60.28, 60.80, 61.36, 94.80, 106.57 (2C), 110.40, 116.95, 119.80, 124.32, 132.30, 133.35, 135.83, 136.04, 137.71, 147.08, 152.84 (2C), 162.26, 169.54.

IR ($CHCl_3$): 3377, 2220, 1762, 1680 cm^{-1}.

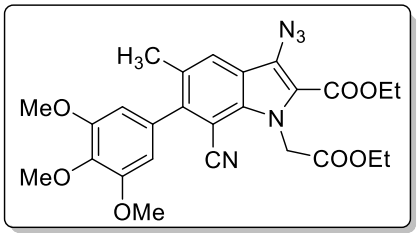
HRMS (ESI) m/z calculated for $C_{36}H_{50}N_3O_7$ $[M+H]^+$: 636.3641, found: 636.3643; $C_{36}H_{49}N_3NaO_7$ $[M+Na]^+$: 658.3463, found: 658.3463.

4a: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate

Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate (**3a**) (500 mg, 1.00 mmol) was dissolved in CH_3CN (5 mL) in a 25 mL round-bottomed flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*-BuONO (156 mg, 180 μL, 1.51 mmol) followed by $TMSN_3$ (140 mg, 160 μL, 1.21 mmol) dropwise. The resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum and the crude product was purified by column chromatography on silica gel (60:120) using pet ether-ethyl acetate as eluent to give the product, ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate **(4a**), as a brown sticky compound (475 mg, 90%).

Yield: 90 %.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (t, *J* = 7Hz, 3H), 1.45 (t, *J* = 7Hz, 3H), 2.21 (s, 3H), 3.85 (s, 6H), 3.90 (s, 3H), 4.27 (q, *J* = 7Hz, 2H), 4.46 (q, *J* = 7Hz, 2H), 5.69 (s, 2H), 6.47 (s, 2H), 7.82 (s, 1H).

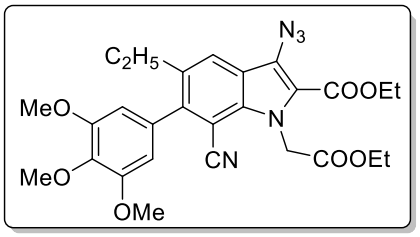


^{13}C NMR (100 MHz, $CDCl_3$): δ 13.99, 14.12, 20.59, 46.85, 56.09 (2C), 60.82, 61.64, 61.78, 95.38, 106.15 (2C), 116.47, 120.06, 122.27, 123.23, 125.34, 129.86, 133.17, 134.34, 137.85, 147.64, 153.14 (2C), 160.49, 168.74.

IR ($CHCl_3$): 2221, 2120, 1747, 1703 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{26}H_{27}N_5NaO_7$ $[M+Na]^+$: 544.1807, found: 544.1803.

4b: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-ethyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 85 %. Brown sticky compound.

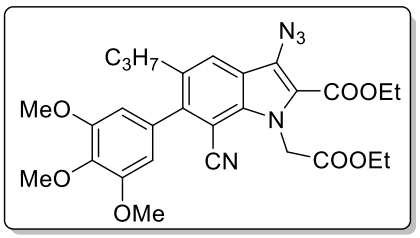
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.12 (t, J = 7Hz, 3H), 1.30 (t, J = 7Hz, 3H), 1.47 (t, J = 7Hz, 3H), 2.56 (q, J = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.29 (q, J = 7Hz, 2H), 4.49 (q, J = 7Hz, 2H), 5.71 (s, 2H), 6.49 (s, 2H), 7.87 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.07, 14.19, 15.59, 26.38, 46.94, 56.17 (2C), 60.93, 61.72, 61.86, 95.61, 106.50 (2C), 116.52, 120.21, 122.63, 123.54, 124.06, 132.99, 134.27, 136.17, 137.99, 147.43, 153.09 (2C), 160.61, 168.86.

IR ($CHCl_3$): 2220, 2120, 1745, 1704 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{27}H_{29}N_5NaO_7$ $[M+Na]^+$: 558.1967, found: 558.1959.

4c: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-propyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 88 %. Brown sticky compound.

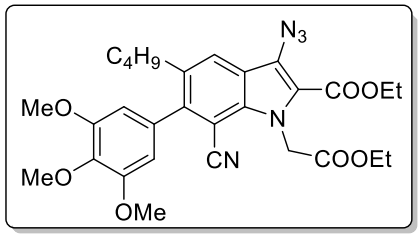
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, J = 7Hz, 3H), 1.31 (t, J = 7Hz, 3H), 1.41-1.57 (m, 5H), 2.49 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.72 (s, 2H), 6.48 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.86, 14.11, 14.24, 24.53, 35.19, 46.98, 56.21 (2C), 61.00, 61.76, 61.92, 95.68, 106.61 (2C), 116.59, 120.24, 122.55, 123.55, 124.81, 133.08, 134.35, 134.67, 137.99, 147.60, 153.07 (2C), 160.66, 168.93.

IR ($CHCl_3$): 2220, 2119, 1744, 1700 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{28}H_{31}N_5NaO_7$ $[M+Na]^+$: 572.2120, found: 572.2116.

4d: Ethyl 3-azido-5-butyl-7-cyano-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 83 %. Brown sticky compound.

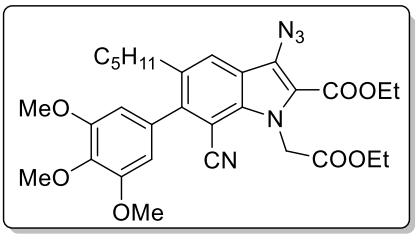
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, *J* = 7Hz, 3H), 1.10-1.55 (m, 10H), 2.50 (t, *J* = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.28 (q, *J* = 7Hz, 2H), 4.48 (q, *J* = 7Hz, 2H), 5.71 (s, 2H), 6.49 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.86, 14.12, 14.37, 22.32, 31.44, 33.11, 46.82, 56.15 (2C), 60.42, 60.96, 61.49, 95.03, 106.59 (2C), 110.56, 117.04, 119.88, 124.25, 132.44, 133.46, 135.95, 136.17, 137.77, 147.23, 152.96 (2C), 162.45, 169.64.

IR ($CHCl_3$): 2221, 2123, 1748, 1701 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{29}H_{33}N_5NaO_7$ $[M+Na]^+$: 586.2285, found: 586.2272.

4e: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



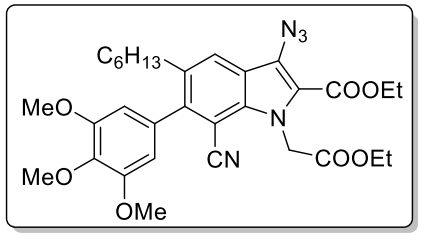
Yield: 85 %. Brown sticky compound.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, *J* = 7Hz, 3H), 1.10-1.57 (m, 12H), 2.50 (t, *J* = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, *J* = 7Hz, 2H), 4.48 (q, *J* = 7Hz, 2H), 5.72 (s, 2H), 6.49 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.86, 14.10, 14.23, 22.30, 31.16, 31.40, 33.15, 46.96, 56.17 (2C), 60.98, 61.75, 61.90, 95.62, 106.54 (2C), 116.58, 120.21, 122.55, 123.52, 124.79, 133.06, 134.31, 134.96, 137.93, 147.53, 153.04 (2C), 160.64, 168.92.

IR ($CHCl_3$): 2220, 2122, 1745, 1703 cm^{-1}.

4f: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-hexyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 76 %. Brown sticky compound.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, *J* = 7Hz, 3H), 1.05-1.55 (m, 14H), 2.50 (t, *J* = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, *J* = 7Hz, 2H), 4.48 (q, *J* = 7Hz, 2H), 5.72 (s,

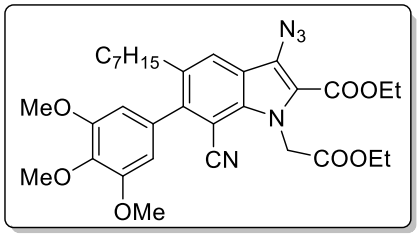
2H), 6.49 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.98, 14.10, 14.24, 22.41, 28.90, 31.46 (2C), 33.20, 46.98, 56.19 (2C), 60.99, 61.76, 61.91, 95.66, 106.61 (2C), 116.59, 120.24, 122.57, 123.53, 124.79, 133.07, 134.34, 134.98, 138.00, 147.56, 153.07 (2C), 160.66, 168.93.

IR ($CHCl_3$): 2220, 2122, 1745, 1703 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{31}H_{38}N_5O_7$ $[M+H]^+$: 592.2772, found: 592.2766.

4g: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-heptyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 90 %. Brown sticky compound.

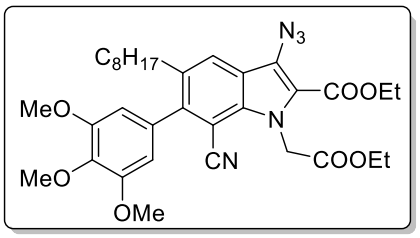
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 7Hz, 3H), 1.10-1.55 (m, 16H), 2.50 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.72 (s, 2H), 6.49 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.01, 14.10, 14.24, 22.55, 28.95, 29.20, 31.51, 31.62, 33.19, 46.97, 56.17 (2C), 60.99, 61.77, 61.92, 95.62, 106.52 (2C), 116.59, 120.21, 122.54, 123.52, 124.80, 133.06, 134.31, 134.96, 137.92, 147.54, 153.03 (2C), 160.65, 168.93.

IR ($CHCl_3$): 2220, 2120, 1747, 1700 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{32}H_{39}N_5NaO_7$ $[M+Na]^+$: 628.2745, found: 628.2742.

4h: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-octyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 86 %. Brown sticky compound.

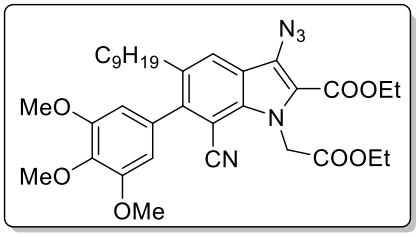
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.10-1.55 (m, 18H), 2.50 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.71 (s, 2H), 6.49 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02, 14.08, 14.21, 22.56, 29.09, 29.26, 29.59, 31.50, 31.75, 33.18, 46.95, 56.16 (2C), 60.96, 61.73, 61.89, 95.62, 106.55 (2C), 116.58, 120.21, 122.54, 123.50, 124.76, 133.04, 134.30, 134.94, 137.95, 147.53, 153.03 (2C), 160.63, 168.90.

IR ($CHCl_3$): 2221, 2123, 1748, 1701 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{33}H_{42}N_5O_7$ $[M+H]^+$: 620.3087, found: 620.3079.

4i: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-nonyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 88 %. Brown sticky compound.

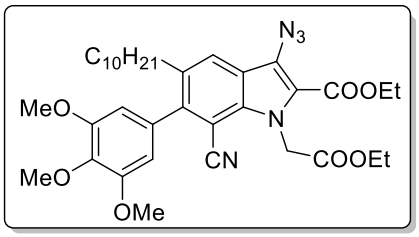
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.10-1.55 (m, 20H), 2.50 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.71 (s, 2H), 6.48 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02, 14.07, 14.21, 22.58, 29.18, 29.23, 29.30, 29.37, 31.49, 31.76, 33.16, 46.94, 56.15 (2C), 60.94, 61.73, 61.87, 95.59, 106.53 (2C), 116.56, 120.18, 122.52, 123.48, 124.75, 133.03, 134.27, 134.92, 137.92, 147.51, 153.01 (2C), 160.61, 168.88.

IR ($CHCl_3$): 2221, 2120, 1747, 1700 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{34}H_{43}N_5NaO_7$ $[M+Na]^+$: 656.3058, found: 655.3055.

4j: Ethyl 3-azido-7-cyano-5-decyl-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



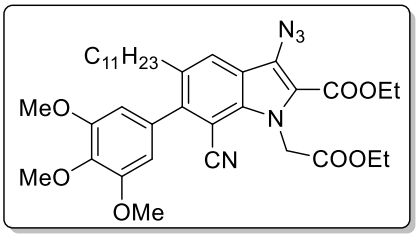
Yield: 79 %. Brown sticky compound.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7Hz, 3H), 1.10-1.55 (m, 22H), 2.50 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.72 (s, 2H), 6.48 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.05, 14.10, 14.22, 22.62, 29.24, 29.27, 29.33, 29.45, 29.51, 31.51, 31.83, 33.19, 46.97, 56.19 (2C), 60.97, 61.73, 61.89, 95.66, 106.63 (2C), 116.58, 120.23, 122.56, 123.52, 124.76, 133.05, 134.33, 134.96, 138.03, 147.55, 153.05 (2C), 160.65, 168.90.

IR ($CHCl_3$): 2222, 2121, 1742, 1703 cm^{-1}.

4k: Ethyl 3-azido-7-cyano-1-(2-ethoxy-2-oxoethyl)-6-(3,4,5-trimethoxyphenyl)-5-undecyl-1*H*-indole-2-carboxylate



Yield: 84 %. Brown sticky compound.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7Hz, 3H), 1.10-1.55 (m, 24H), 2.50 (t, J = 7Hz, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.28 (q, J = 7Hz, 2H), 4.48 (q, J = 7Hz, 2H), 5.72 (s, 2H), 6.48 (s, 2H), 7.85 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 14.13, 14.23, 22.63, 29.27 (2C), 29.34, 29.46, 29.56 (2C), 31.53, 31.85, 33.18, 46.96, 56.16 (2C), 60.98, 61.75, 61.90, 95.60, 106.51 (2C), 116.58, 120.19, 122.54, 123.51, 124.77 (2C), 133.05, 134.29, 134.94, 137.90, 147.54, 153.02 (2C), 160.64, 168.92.

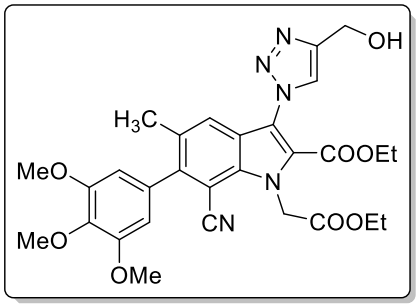
IR ($CHCl_3$): 2220, 2123, 1745, 1702 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{36}H_{48}N_5O_7$ $[M+H]^+$: 662.3548, found: 662.3545.

5a: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate

$CuSO_4.5H_2O$ (6 mg, 0.038 mmol), sodium ascorbate (15 mg, 0.2 mmol), propargyl alcohol (21 mg, 23 μL, 0.383 mmol, 1.0 equiv), water (0.5 mL), ethyl 3-azido-7-cyano 1-(2-ethoxy-2-oxoethyl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate (**4a**) (200 mg, 0.383mmol, 1.0 equiv), and t-BuOH (1 mL) were sequentially added to a 10 mL RB. The mixture was stirred at room temperature for 12 h, diluted with ethyl acetate (60 mL), washed with brine (10 mL), dried over Na_2SO_4 and purified by column chromatography on silica gel (60-120) using pet ether-ethyl acetate as eluent to give ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-methyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate (**5a**) (205 mg, 92%) as a brown sticky compound.

Yield: 92 %.



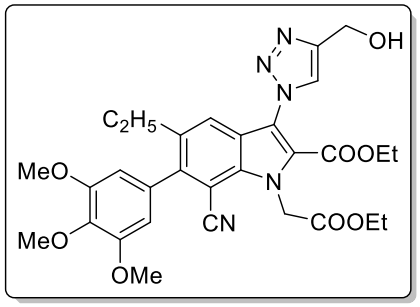
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (t, J = 7Hz, 3H), 1.33 (t, J = 7Hz, 3H), 2.19 (s, 3H), 3.88 (s, 6H), 3.93 (s, 3H), 4.23 (q, J = 7Hz, 2H), 4.32 (q, J = 7Hz, 2H), 4.96 (s, 2H), 5.85 (s, 2H), 6.49 (s, 2H), 7.58 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.61, 14.10, 20.68, 29.67, 47.12, 56.26 (2C), 56.59, 60.98, 62.00, 62.22, 96.20, 106.31 (2C), 116.27, 119.60, 123.62, 123.94, 125.18, 125.57, 132.03, 132.95, 133.90, 138.21, 148.11, 153.37 (2C), 159.64, 168.41.

IR ($CHCl_3$): 3395, 2220, 1750, 1718 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{29}H_{32}N_5O_8$ $[M+H]^+$: 578.2252, found: 578.2245.

5b: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-5-ethyl-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 90 %. Brown sticky compound.

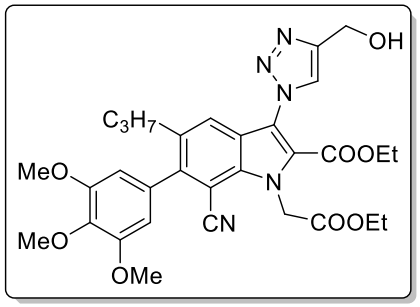
^{1}H NMR (200 MHz, $CDCl_3$): δ 1.03 (t, J = 7Hz, 3H), 1.09 (t, J = 7Hz, 3H), 1.30 (t, J = 7Hz, 3H), 2.50 (q, J = 7Hz, 2H), 3.85 (s, 6H), 3.91 (s, 3H), 4.19 (q, J = 7Hz, 2H), 4.29 (q, J = 7Hz, 2H), 4.94 (s, 2H), 5.82 (s, 2H), 6.49 (s, 2H), 7.58 (s, 1H), 7.93 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.48, 14.00, 15.54, 26.43, 47.02, 56.14 (2C), 60.89, 61.92, 62.13, 96.22, 106.42 (2C), 116.15, 119.59, 123.71, 123.80, 120.90, 123.95, 125.63, 132.62, 133.61, 138.02, 138.14, 146.99, 147.65, 153.08, 159.55 (2C), 168.35.

IR ($CHCl_3$): 3395, 2220, 1751, 1717 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{30}H_{34}N_5O_8$ $[M+H]^+$: 592.2399, found: 592.2402.

5c: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-propyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 81 %. Brown sticky compound.

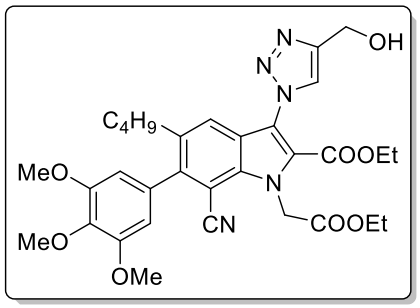
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.79 (t, J = 7Hz, 3H), 1.12 (t, J = 7Hz, 3H), 1.33 (t, J = 7Hz, 3H), 1.38-1.49 (m, 2H), 2.46 (t, J = 7Hz, 2H),3.87 (s, 6H), 3.94 (s, 3H), 4.22 (q, J = 7Hz, 2H), 4.31 (q, J = 7Hz, 2H), 4.97 (s, 2H), 5.58 (s, 2H), 6.50 (s, 2H), 7.58 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.60, 13.87, 14.10, 24.64, 35.28, 47.11, 53.64, 56.24 (2C), 56.59, 61.01, 61.99, 62.22, 96.36, 106.63 (2C), 116.27, 119.73, 123.74, 124.04, 124.06, 124.52, 132.75, 133.76, 136.77, 138.17, 147.87, 153.15 (2C), 159.65, 168.45.

IR ($CHCl_3$): 3395, 2222, 1750, 1719 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{31}H_{36}N_5O_8$ $[M+H]^+$: 606.2565, found: 606.2558.

5d: Ethyl 5-butyl-7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 86 %. Brown sticky compound.

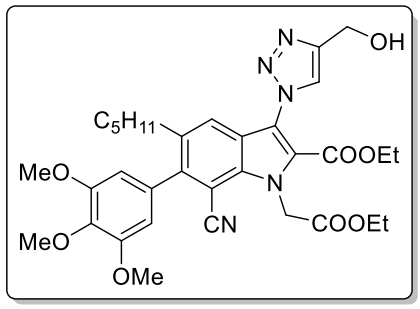
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.78 (t, *J* = 7Hz, 3H), 1.00-1.50 (m, 10H), 2.47 (t, *J* = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.09-4.41 (m, 4H), 4.97 (s, 2H), 5.84 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 7.93 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.54, 13.79, 14.05, 22.17, 31.44, 33.26, 47.06, 56.18 (2C), 56.39, 60.96, 61.96, 62.18, 96.28, 106.57 (2C), 116.24, 119.62, 123.70, 123.99, 124.49, 125.60, 132.71, 133.70, 137.04, 138.09, 146.91, 147.77, 153.08 (2C), 159.61, 168.42.

IR ($CHCl_3$): 3396, 2220, 1750, 1718 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{32}H_{38}N_5O_8$ $[M+H]^+$: 620.2715, found: 620.2715.

5e: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 91 %. Brown sticky compound.

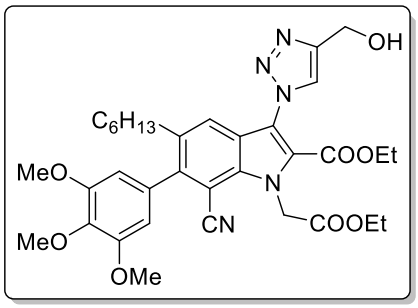
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.77(t, *J* = 7Hz, 3H), 1.00-1.45(m, 12H), 2.47 (t, *J* = 7Hz, 2H), 3.86 (s, 6H), 3.92 (s, 3H), 4.05-4.43 (m, 4H), 4.98 (s, 2H) 5.84 (s, 2H), 6.49 (s, 2H), 7.57 (s, 1H), 7.98 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.55, 13.79, 14.05, 22.17, 29.62, 31.26, 31.44, 33.27, 47.07, 56.19 (2C), 60.96, 61.96, 62.19, 96.30, 106.60 (2C), 114.00, 116.24, 123.70, 124.04, 124.47 (2C), 132.71, 133.71, 137.06, 138.12, 139.20, 147.77, 153.10 (2C), 159.60, 168.42.

IR ($CHCl_3$): 3397, 2221, 1753, 1715 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{33}H_{40}N_5O_8$ $[M+H]^+$: 634.2878, found: 634.2871; $C_{33}H_{39}N_5NaO_8$ $[M+Na]^+$: 656.2695, found: 656.2691.

5f: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-5-hexyl-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



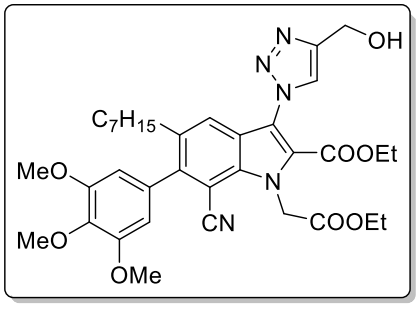
Yield: 87 %. Brown sticky compound.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (t, J = 7Hz, 3H), 1.07-1.44 (m, 14H), 2.48 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.94 (s, 3H), 4.22 (q, J = 7Hz, 2H), 4.32 (q, J = 7Hz, 2H), 4.98 (s, 2H), 5.85 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 7.92 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.61, 13.95, 14.11, 22.38, 28.97, 31.39, 31.59, 33.36, 47.12, 56.24 (2C), 56.69, 61.02, 62.00, 62.24, 96.37, 106.65 (2C), 116.29, 119.70, 119.76, 123.78, 124.04, 124.52, 125.56, 132.75, 133.77, 137.10, 138.21, 147.84, 153.16 (2C), 159.67, 168.46.

IR ($CHCl_3$): 3396, 2220, 1753, 1716 cm^{-1}.

5g: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-5-heptyl-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 83 %. Brown sticky compound.

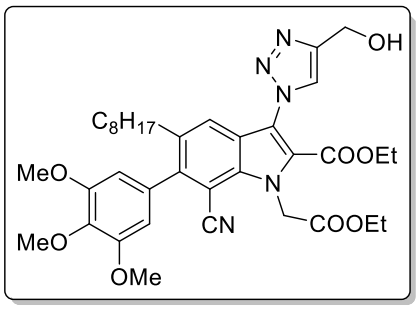
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.08-1.42 (m, 16H), 2.47 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.22 (q, J = 7Hz, 2H), 4.32 (q, J = 7Hz, 2H), 5.00 (s, 2H), 5.85 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.59, 14.06, 14.08, 22.62, 29.26, 29.27, 29.40, 29.44, 29.52, 29.54, 31.73, 31.84, 33.37, 47.09, 56.22 (2C), 60.99, 61.99, 62.21, 96.34, 106.62 (2C), 116.27, 123.73, 124.09, 124.48, 132.72, 133.75, 137.11, 138.17, 147.81, 153.12 (2C), 159.63, 168.46.

IR ($CHCl_3$): 3395, 2220, 1752, 1717 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{35}H_{44}N_5O_8$ $[M+H]^+$: 662.3190, found: 662.3184.

5h: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-octyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 85 %. Brown sticky compound.

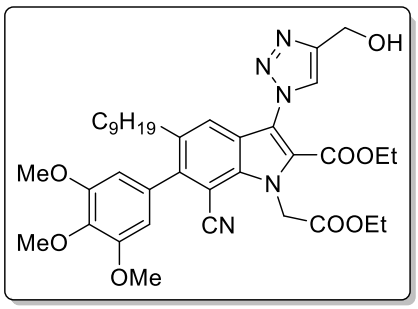
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (t, *J* = 7Hz, 3H), 1.02-1.43 (m, 18H), 2.46 (t, *J* = 7Hz, 2H), 3.85 (s, 6H), 3.91 (s, 3H), 4.19 (q, *J* = 7Hz, 2H), 4.29 (q, *J* = 7Hz, 2H), 4.98 (s, 2H), 5.82 (s, 2H), 6.49 (s, 2H), 7.56 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.50, 13.94, 14.01, 22.46 (2C), 28.99 (2C), 29.11, 29.28, 29.56, 31.63 (2C), 33.27, 47.02 56.12 (2C), 60.91, 61.94, 62.15, 96.23, 106.48 (2C), 116.20, 116.21, 123.63, 124.01, 124.43, 132.67, 133.64, 137.02, 138.00, 147.71, 153.01 (2C), 159.55, 168.39.

IR ($CHCl_3$): 3396, 2222, 1752, 1715 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{36}H_{46}N_5O_8$ $[M+H]^+$: 676..3341, found: 676.3341; $C_{36}H_{45}N_5O_8$ $[M+Na]^+$: 698.3160, found: 698.3160.

5i: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-5-nonyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate



Yield: 88 %. Brown sticky compound.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, *J* = 7Hz, 3H), 0.95-1.50 (m, 20H), 2.47 (t, *J* = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.21 (q, *J* = 7Hz, 2H), 4.31 (q, *J* = 7Hz, 2H), 4.97 (s, 2H), 5.85 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 7.92 (s, 1H).

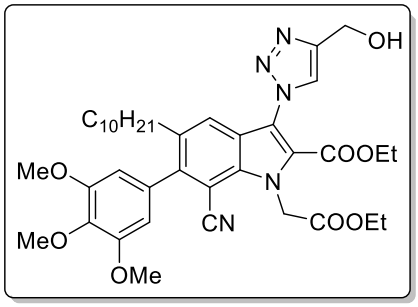
^{13}C NMR (100 MHz, $CDCl_3$): δ 13.57, 14.03, 14.08, 22.58, 29.17, 29.24, 29.37 (2C), 31.70, 31.74, 33.35, 47.07, 56.19 (2C), 56.48, 60.98, 61.97, 62.20, 96.29, 106.53 (2C), 116.27, 119.64, 123.72, 124.00, 124.48, 125.61, 125.68, 132.72, 133.70, 137.07, 138.06, 147.78, 153.08 (2C), 159.63, 168.44.

IR ($CHCl_3$): 3397, 2220, 1755, 1715 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{37}H_{48}N_5O_8$ $[M+H]^+$: 690.3507, found: 690.3497.

5j: Ethyl 7-cyano-5-decyl-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate

Yield: 86 %. Brown sticky compound.



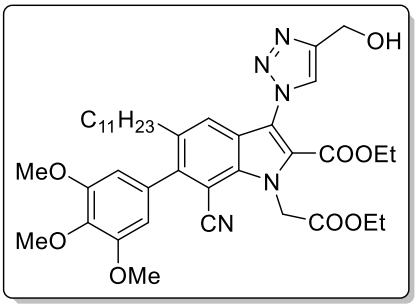
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.04-1.50 (m, 22H), 2.47 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.21 (q, J = 7Hz, 2H), 4.31 (q, J = 7Hz, 2H), 4.97 (s, 2H), 5.84 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 7.94 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.58, 14.04, 14.09, 22.60, 29.21, 29.26, 29.38, 29.43, 29.48, 31.71, 31.81, 33.37, 47.10, 56.22 (2C), 56.52, 60.99, 61.98, 62.21, 96.33, 106.63 (2C), 116.27, 119.65, 123.74, 124.03, 124.49, 125.63, 132.72, 133.74, 137.10, 138.17, 146.87, 147.81, 153.12 (2C), 159.64, 168.44.

IR ($CHCl_3$): 3396, 2220, 1754, 1717 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{38}H_{50}N_5O_8$ $[M+H]^+$: 704.3661, found: 704.3654.

5k: Ethyl 7-cyano-1-(2-ethoxy-2-oxoethyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-5-undecyl-1*H*-indole-2-carboxylate



Yield: 84 %. Brown sticky compound.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.00-1.45 (m, 24H), 2.47 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.21 (q, J = 7Hz, 2H), 4.31 (q, J = 7Hz, 2H), 5.00 (s, 2H), 5.85 (s, 2H), 6.50 (s, 2H), 7.57 (s, 1H), 8.01 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 13.59, 14.06, 14.08, 22.62, 29.26 (2C), 29.27, 29.40, 29.44, 29.52 (2C), 29.54 (2C), 31.73, 31.84, 33.37, 47.09, 56.22 (2C), 56.49, 60.99, 61.99, 62.21, 96.34, 106.62 (2C), 116.27, 123.73, 124.09, 124.48, 132.72, 133.75, 137.11, 138.17, 147.81, 153.12 (2C), 159.63, 168.46.

IR ($CHCl_3$): 3395, 2220, 1751, 1716 cm^{-1}.

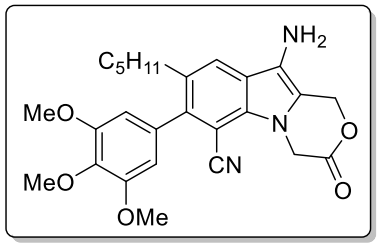
HRMS (ESI) *m/z* calculated for $C_{39}H_{51}N_5NaO_8$ $[M+Na]^+$: 740.3627, found: 740.3630.

10-Amino-3-oxo-8-pentyl-7-(3,4,5-trimethoxyphenyl)-3,4-dihydro-1*H*-[1,4]oxazino[4,3-a]indole-6-carbonitrile (**6a)** and ethyl 2-(3-amino-7-cyano-2-(hydroxymethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indol-1-yl)acetate **(7a)**

Ethyl 3-amino-7-cyano-1-(2-ethoxy-2-oxoethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxylate (**3e**) (500 mg, 1.00 mmol) was dissolved in MeOH (10 mL) in a 25 mL round-bottomed flask and cooled to 0°C in an ice bath. To this stirred mixture was added sodium borohydride (100 mg, 2.71 mmol). The resulting solution was stirred at 50 °C for 2 h under

argon atmosphere and reaction was monitored by TLC. After completion, the reaction mixture was concentrated under vacuum and it was diluted with dropwise addition of cold water and extracted with ethyl acetate (3 x 20 ml), dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (60-120) using pet ether-ethyl acetate as eluent to obtain 10-amino-3-oxo-8-pentyl-7-(3,4,5-trimethoxyphenyl)-3,4-dihydro-1*H*-[1,4]oxazino[4,3-a]indole-6-carbonitrile (**6a**) as a yellow solid (150 mg, 35%) and ethyl 2-(3-amino-7-cyano-2-(hydroxymethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indol-1-yl)acetate (**7a**) (200 mg, 47%).

6a: 10-Amino-3-oxo-8-pentyl-7-(3,4,5-trimethoxyphenyl)-3,4-dihydro-1*H*-[1,4]oxazino[4,3-a]indole-6-carbonitrile



Yield: 35 %.

Melting point: 224 ºC.

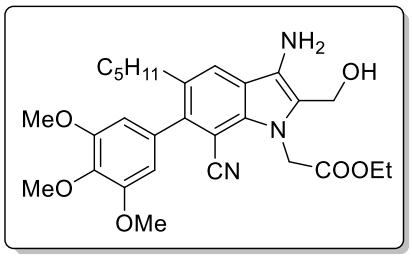
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (t, J = 7Hz, 3H), 1.10-1.27 (m, 4H), 1.38-1.55 (m, 2H),2.51 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.94 (s, 3H), 4.66 (bs, 4H), 5.14 (bs, 2H), 6.51 (s, 2H), 7.69 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.87, 22.33, 31.38, 31.43, 33.14, 40.81, 56.20 (2C), 61.01, 66.54, 95.21, 106.57 (2C), 117.11, 119.05, 124.93 (2C), 132.63, 133.00, 133.32, 136.72, 138.06, 147.39, 153.11 (2C), 161.19.

IR ($CHCl_3$): 3399, 2222, 1673, 1633 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{26}H_{30}N_3O_5$ $[M+H]^+$: 464.2177, found: 464.2180.

7a: Ethyl 2-(3-amino-7-cyano-2-(hydroxymethyl)-5-pentyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indol-1-yl)acetate



Yield: 47 %.

Melting point: 165 ºC.

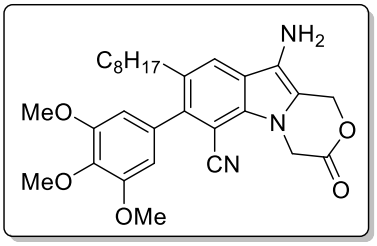
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81 (t, J = 7Hz, 3H), 1.05-1.55 (m, 9H), 2.48 (t, J = 7Hz, 2H), 3.79 (s, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 4.40 (q, J = 7Hz, 2H), 5.00 (bs, 2H), 5.62 (s, 2H), 6.50 (s, 2H), 7.64 (s, 1H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 13.80, 14.31, 22.25, 31.38, 33.06, 46.63, 52.39, 56.12 (2C), 60.37, 60.89, 94.88, 106.61 (2C), 110.38, 117.01, 119.86, 124.32, 132.43, 133.38, 135.88, 136.24, 137.78, 147.18, 152.92 (2C), 162.37, 170.07.

IR ($CHCl_3$): 3447, 2217, 1750, 1676 cm^{-1}.

Compound **3h** was reacted in a similar way to obtain compounds **6b** and **7b**

6b: 10-Amino-8-octyl-3-oxo-7-(3,4,5-trimethoxyphenyl)-3,4-dihydro-1*H*-[1,4]oxazino[4,3-a]indole-6-carbonitrile



Yield: 37 %.

Melting point: 205 ºC.

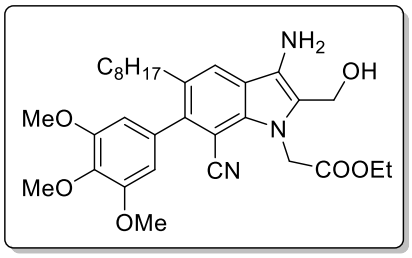
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.10-1.31 (m, 10H), 1.37-155 (m, 2H), 2.51 (t, J = 7Hz, 2H), 3.87 (s, 6H), 3.94 (s, 3H), 4.65 (bs, 4H), 6.50 (s, 2H), 7.69 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.03, 22.57, 29.11, 29.28, 29.30, 31.76 (2C), 33.17, 40.81, 56.19 (2C), 61.00, 66.54, 95.20, 106.55 (2C), 117.10, 119.04, 124.91 (2C), 132.62, 132.98, 133.31, 136.71, 138.05, 147.38, 153.09 (2C), 161.17.

IR ($CHCl_3$): 3395, 2223, 1677, 1635 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{29}H_{35}N_3NaO_5$ $[M+Na]^+$: 528.2469, found: 528.2469.

7b: Ethyl 2-(3-amino-7-cyano-2-(hydroxymethyl)-5-octyl-6-(3,4,5-trimethoxyphenyl)-1*H*-indol-1-yl)acetate



Yield: 47 %.

Melting point: 117 ºC.

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, J = 7Hz, 3H), 1.08-1.50 (m, 15H), 2.49 (t, J = 7Hz, 2H), 3.79 (s, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.40 (q, J = 7Hz, 2H), 5.62 (s, 2H), 6.50 (s, 2H), 7.65 (s, 1H).

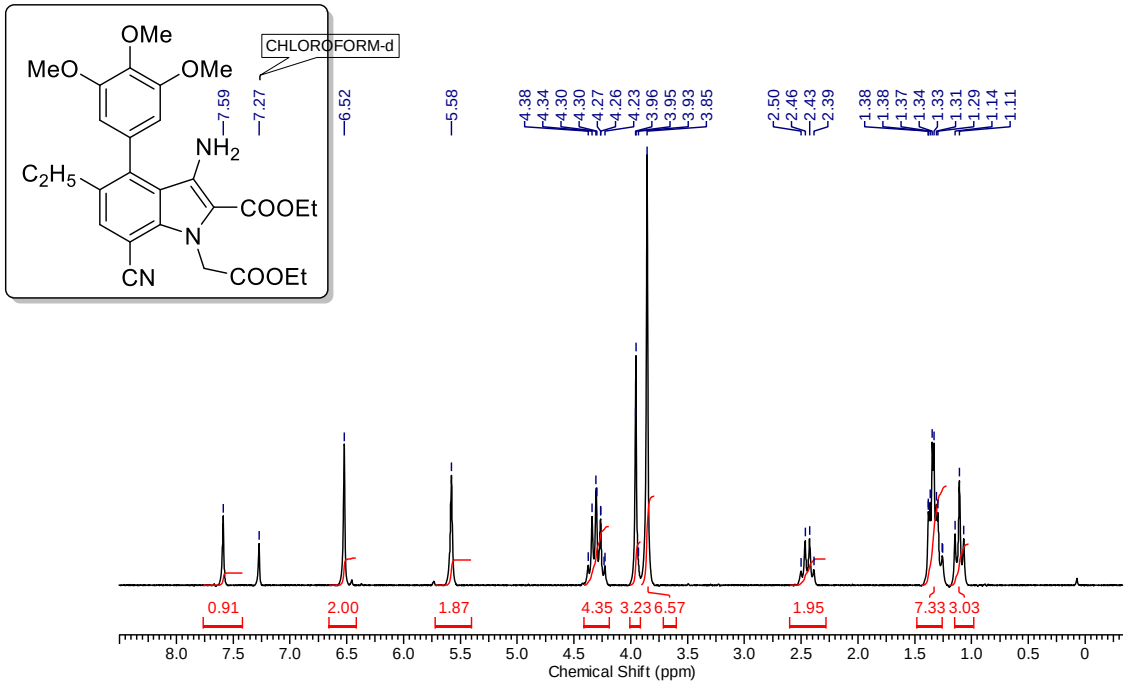
^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02, 14.36, 22.56, 29.10, 29.30 (2C), 31.75, 33.15, 46.68, 52.44, 56.17 (2C), 60.43, 60.95, 95.00, 106.65 (2C), 110.51, 117.04, 119.90, 124.26, 132.50, 133.40, 135.93, 136.21, 137.85, 147.26, 152.97 (2C), 162.43, 170.10.

IR ($CHCl_3$): 3475, 2220, 1753, 1675 cm^{-1}.

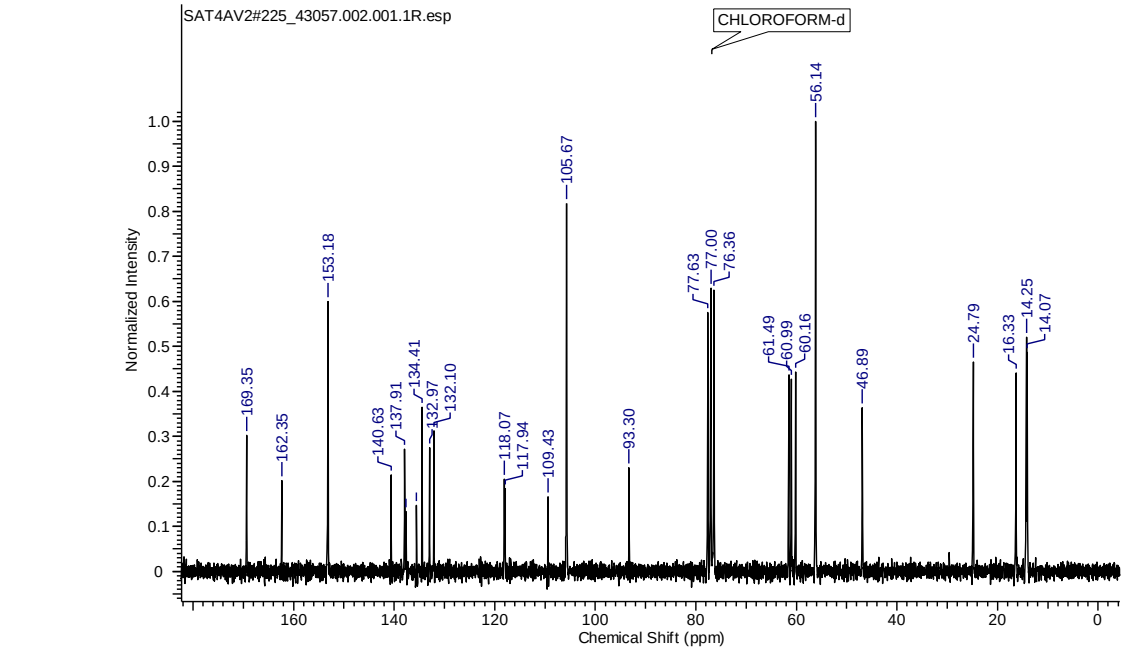
HRMS (ESI) *m/z* calculated for $C_{31}H_{42}N_3O_6$ $[M+H]^+$: 552.2878, found: 552.2871.

2.3.4.2. Spectral Data

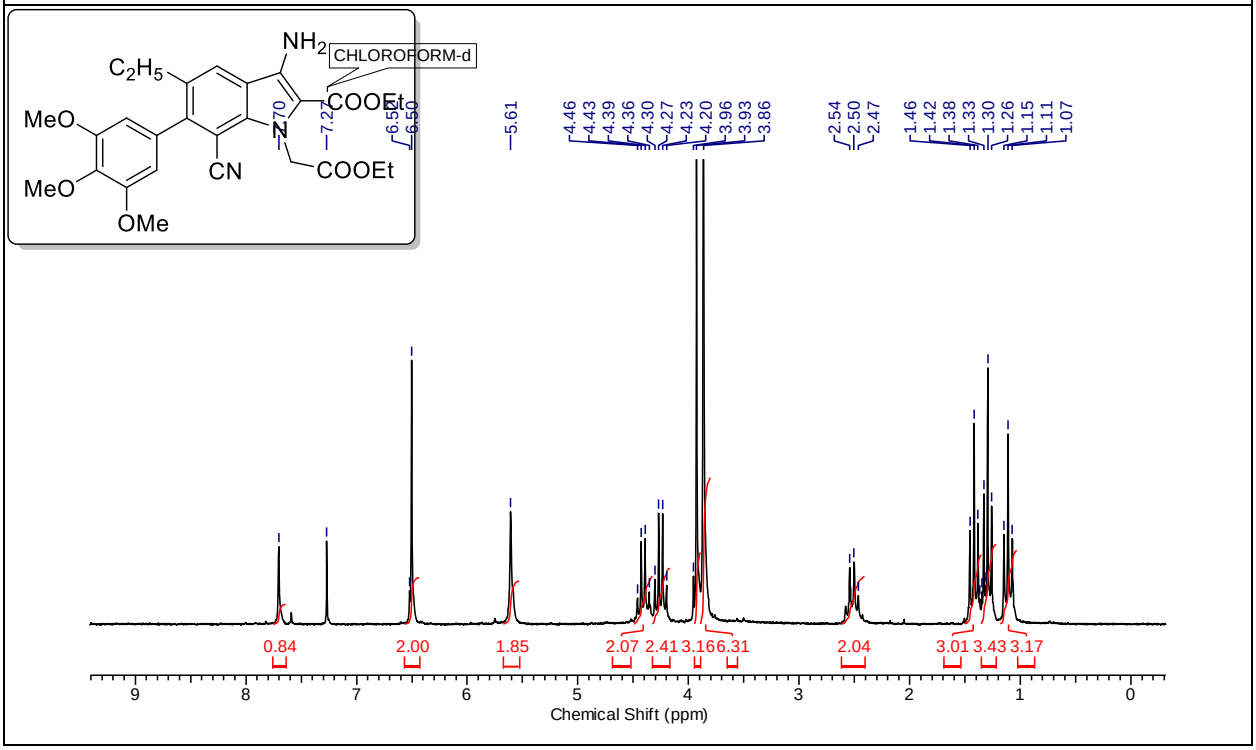
^{1}H NMR (200 MHz, $CDCl_3$) of compound 2b



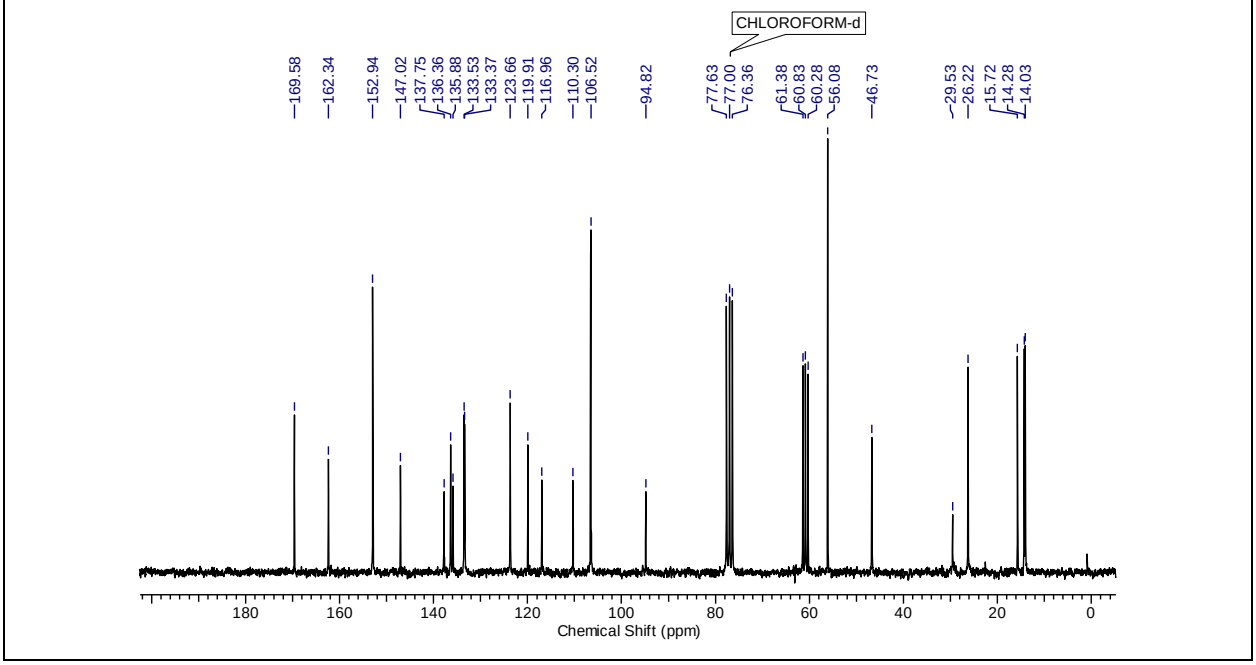
^{13}C NMR (50 MHz, $CDCl_3$) of compound 2b



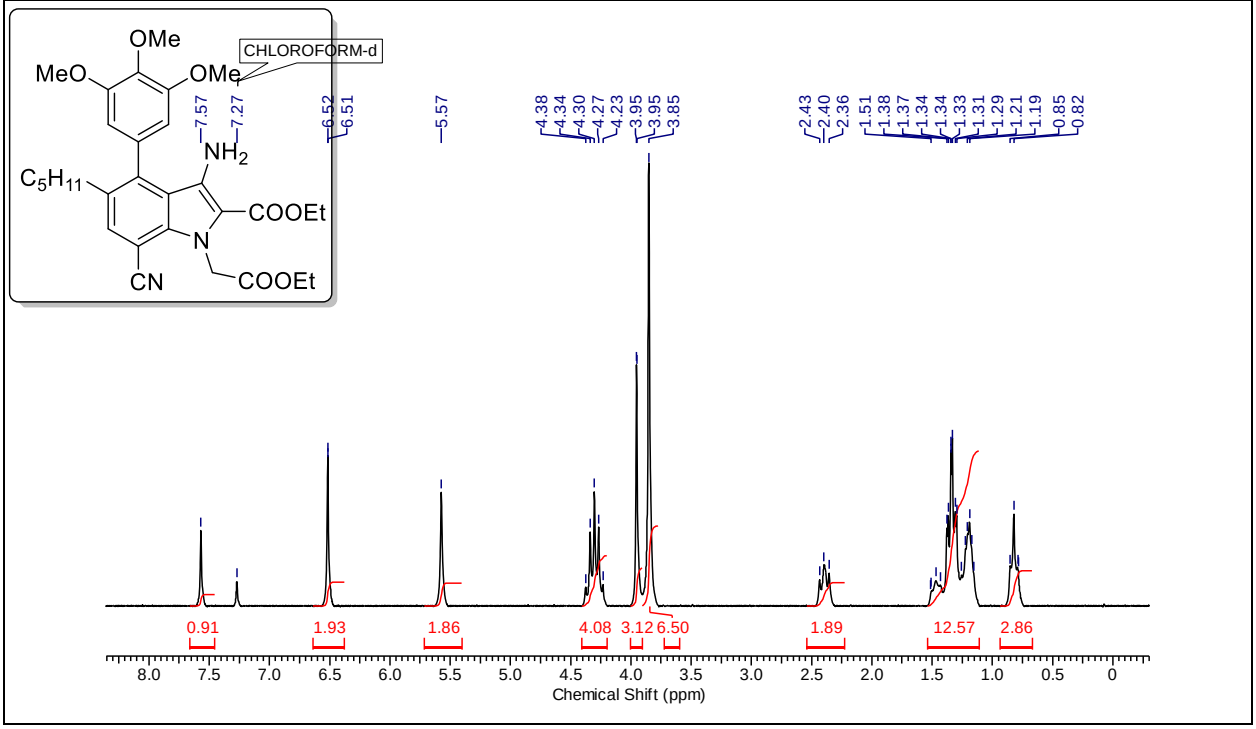
^{1}H NMR (200 MHz, $CDCl_3$) of compound 3b



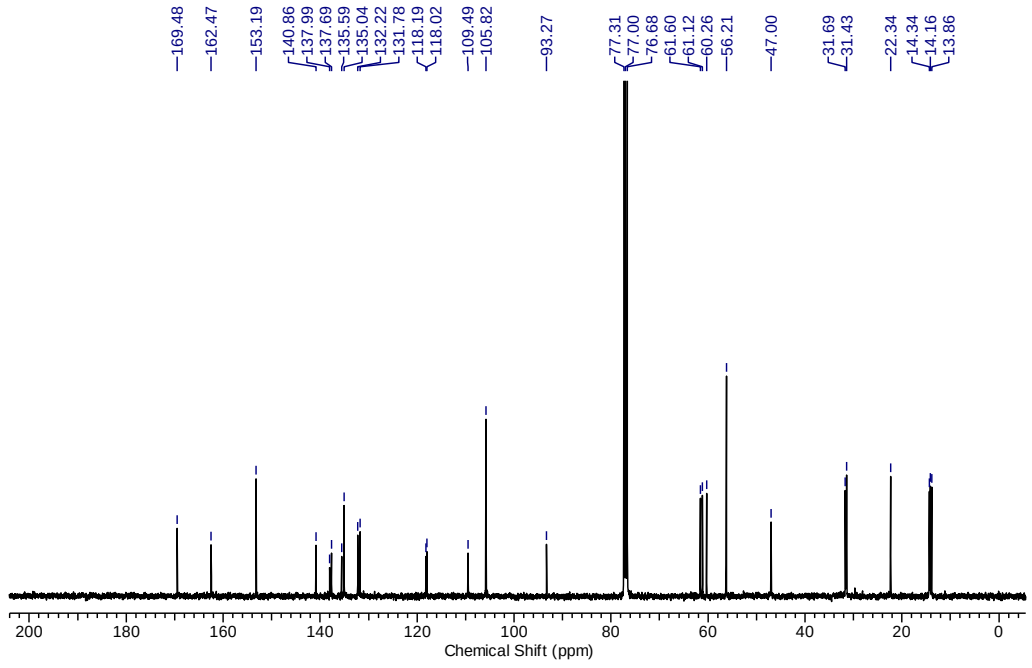
^{13}C NMR (50 MHz, $CDCl_3$) of compound 3b



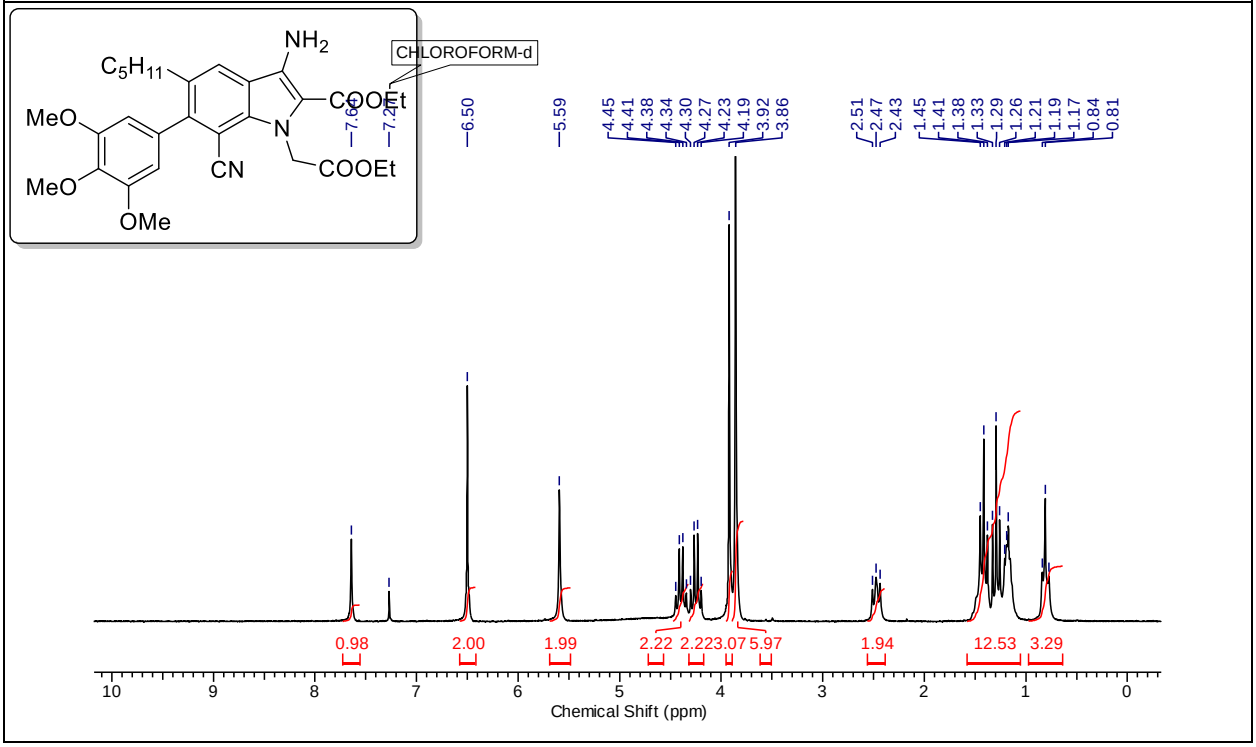
^{1}H NMR (200 MHz, $CDCl_3$) of compound 2e



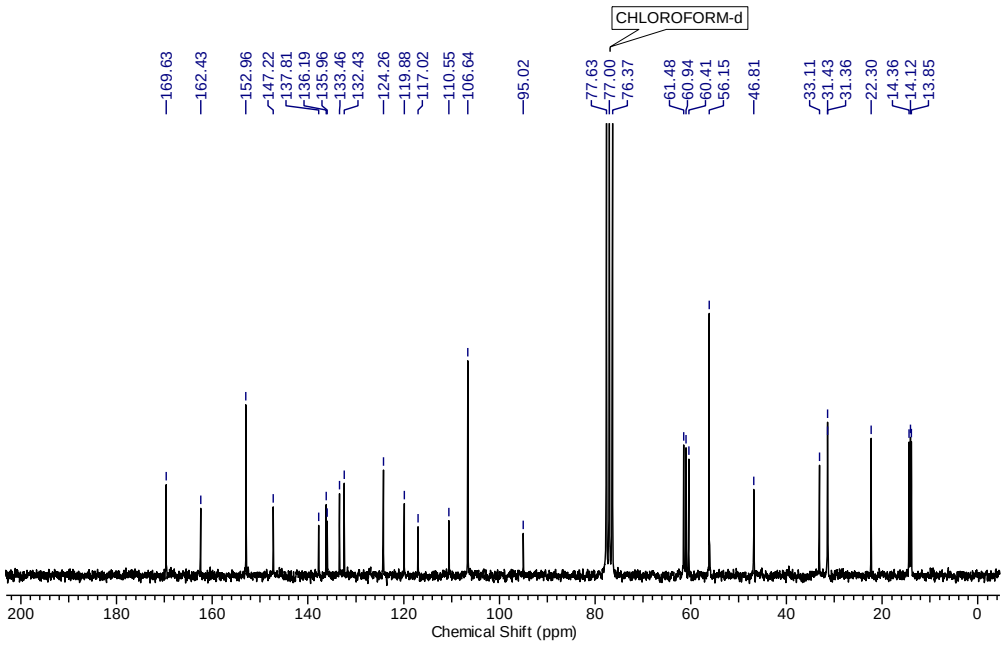
^{13}C NMR (100 MHz, $CDCl_3$) of compound 2e



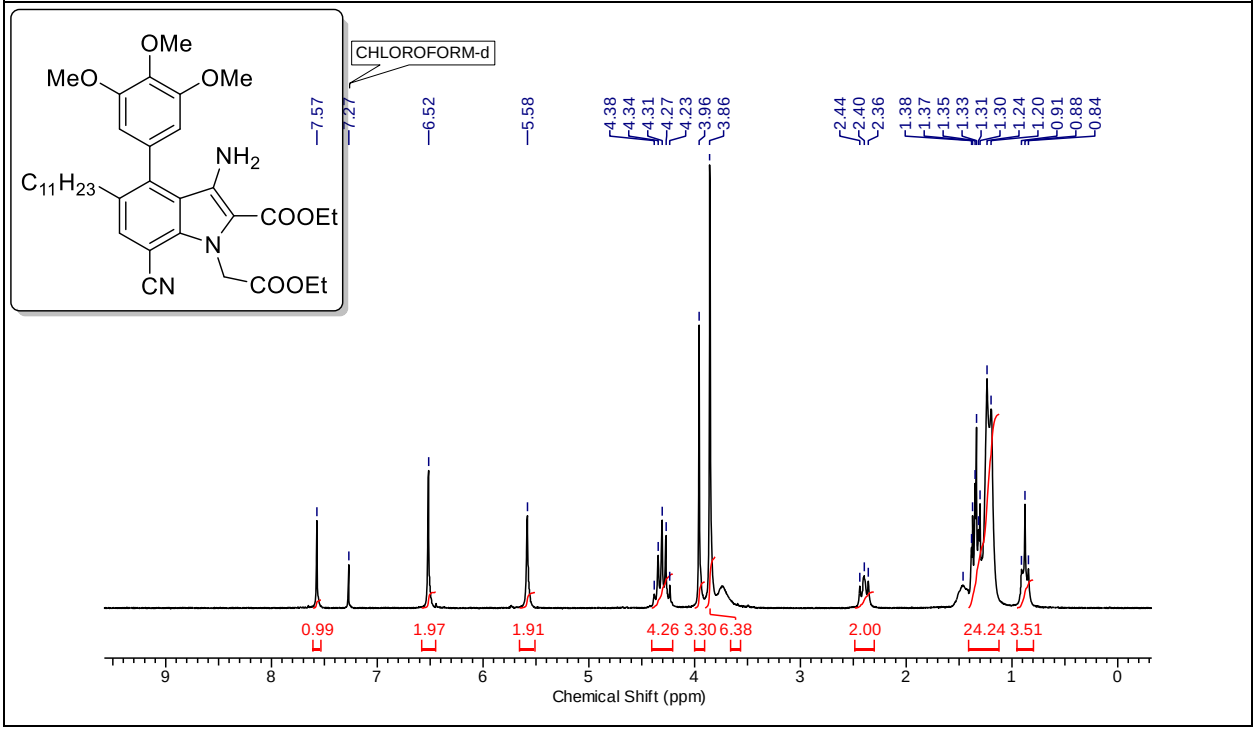
^{1}H NMR (200 MHz, $CDCl_3$) of compound 3e



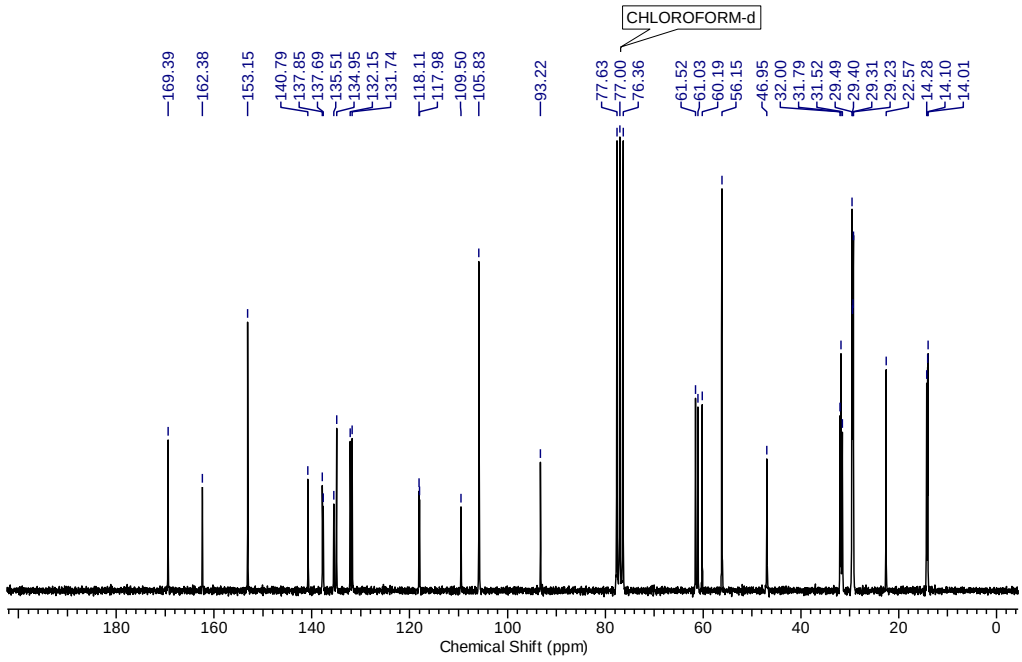
^{13}C NMR (50 MHz, $CDCl_3$) of compound 3e



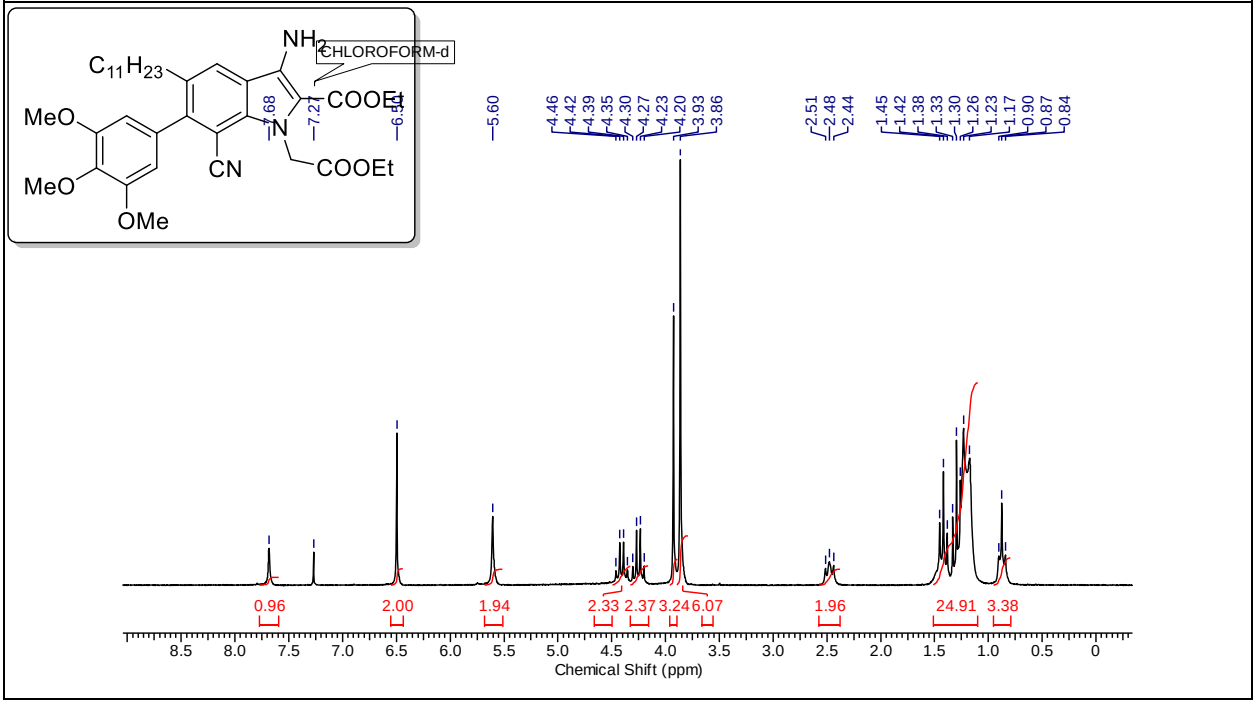
^{1}H NMR (200 MHz, $CDCl_3$) of compound 2k



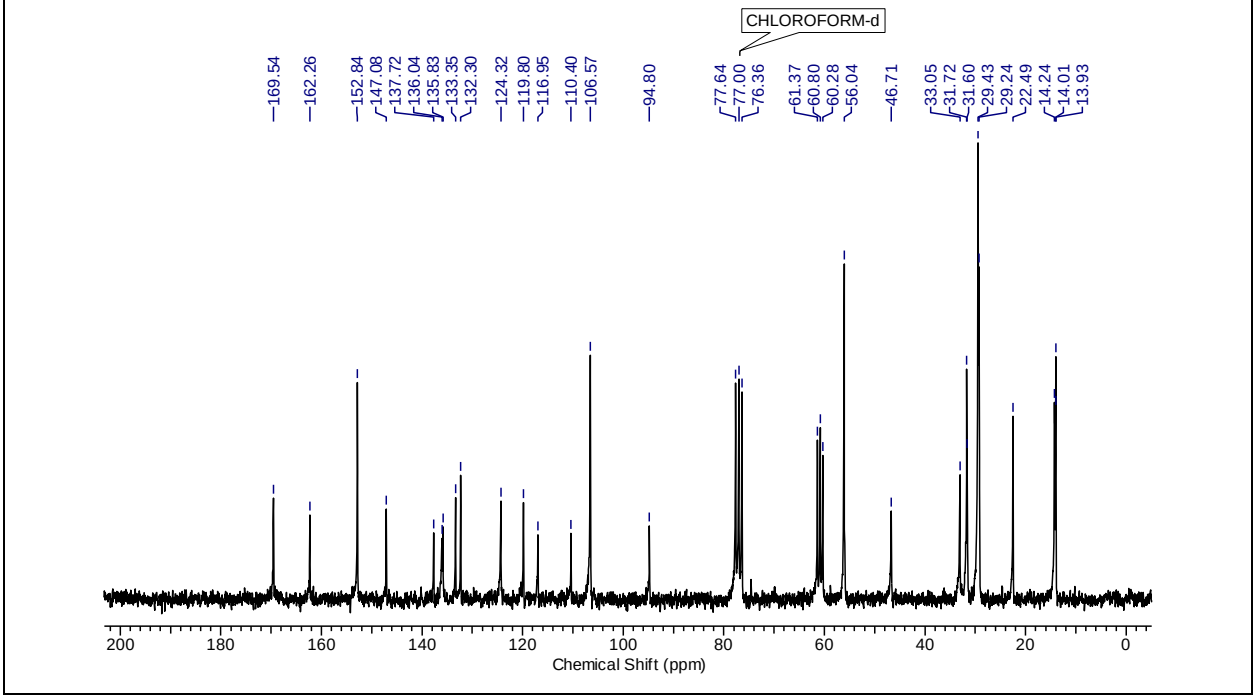
^{13}C NMR (50 MHz, $CDCl_3$) of compound 2k



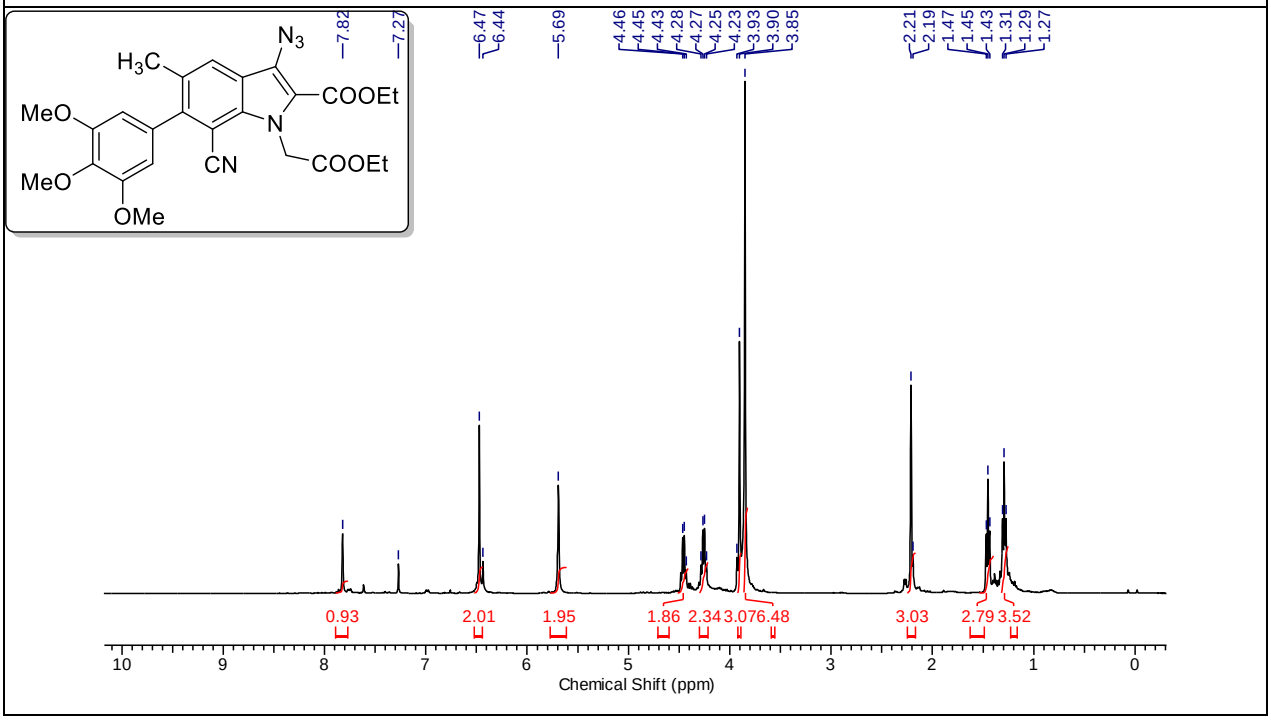
^{1}H NMR (200 MHz, $CDCl_3$) of compound 3k



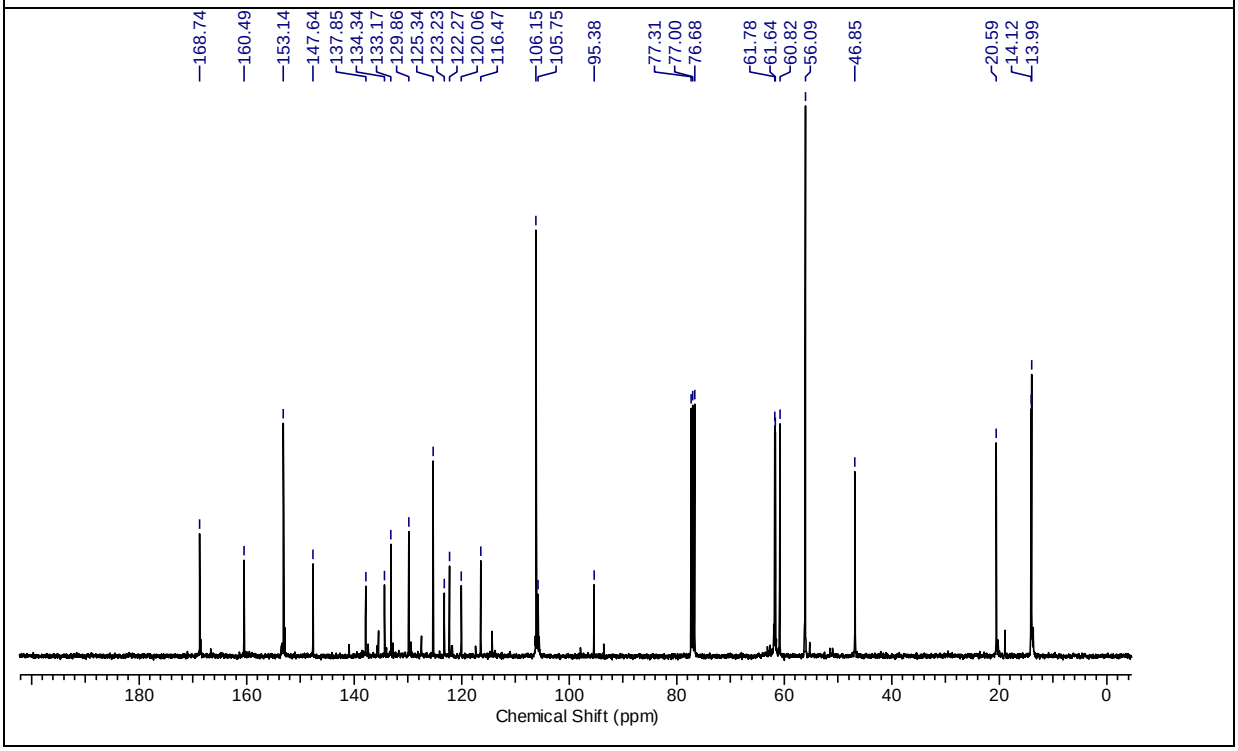
^{13}C NMR (50 MHz, $CDCl_3$) of compound 3k



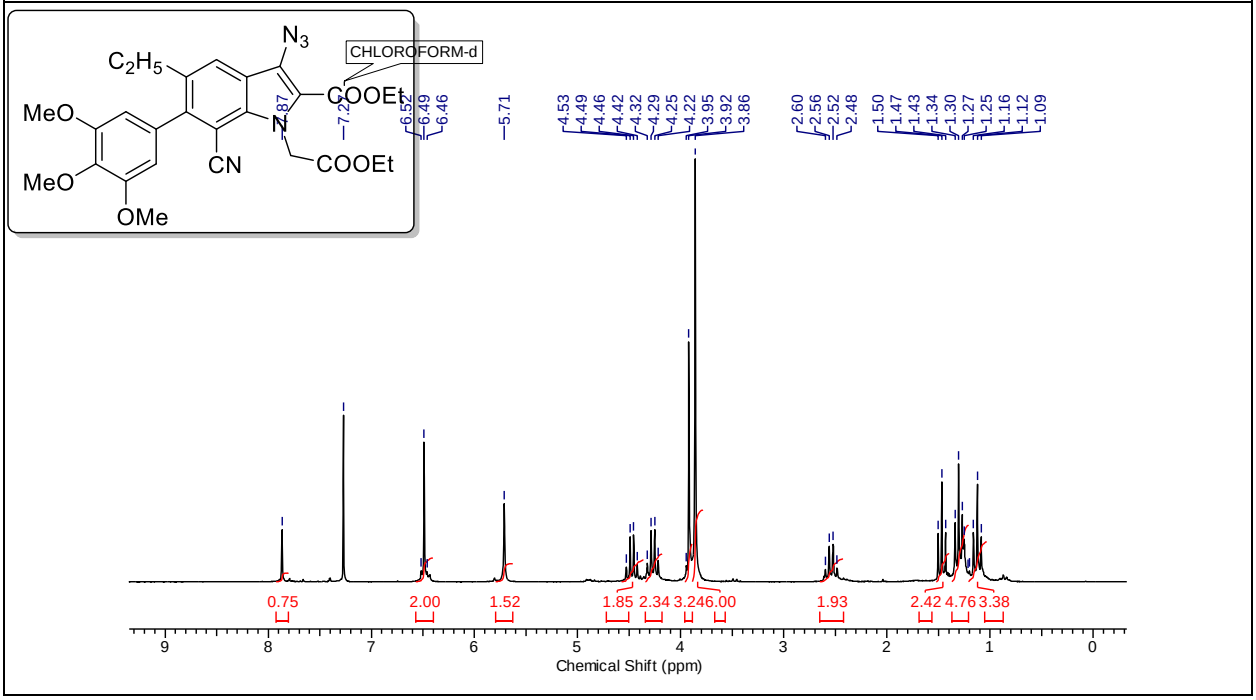
^{1}H NMR (400 MHz, $CDCl_3$) of compound 4a



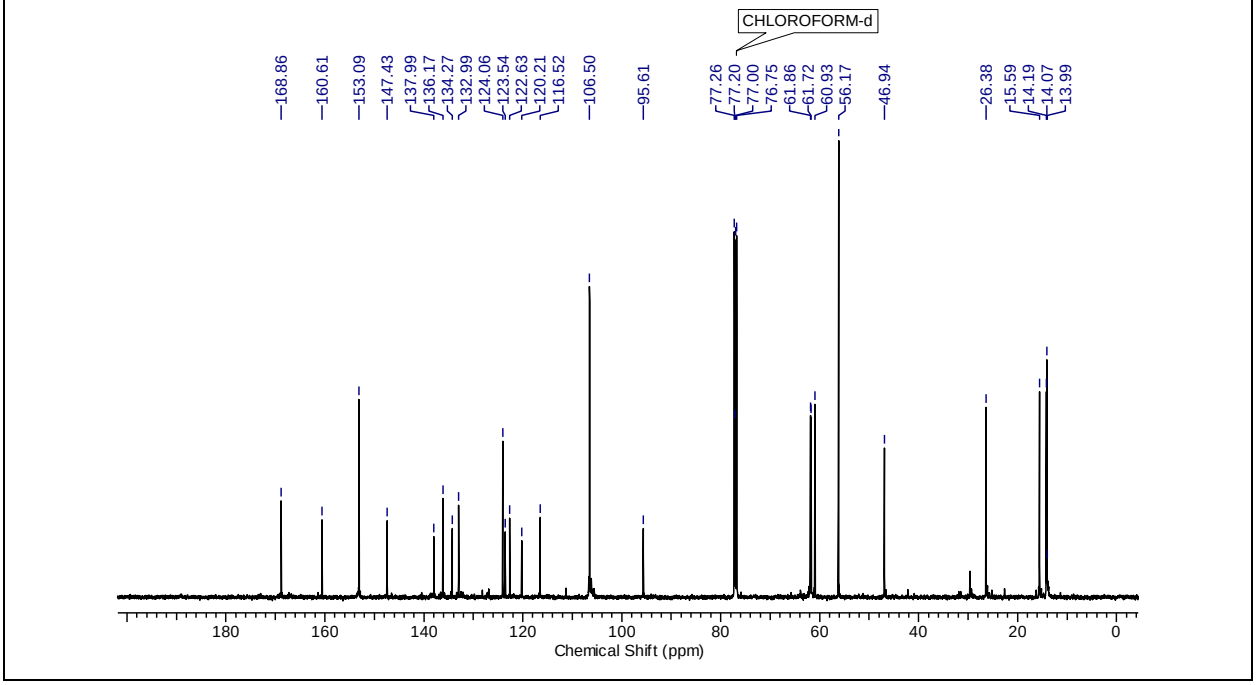
^{13}C NMR (100 MHz, $CDCl_3$) of compound 4a



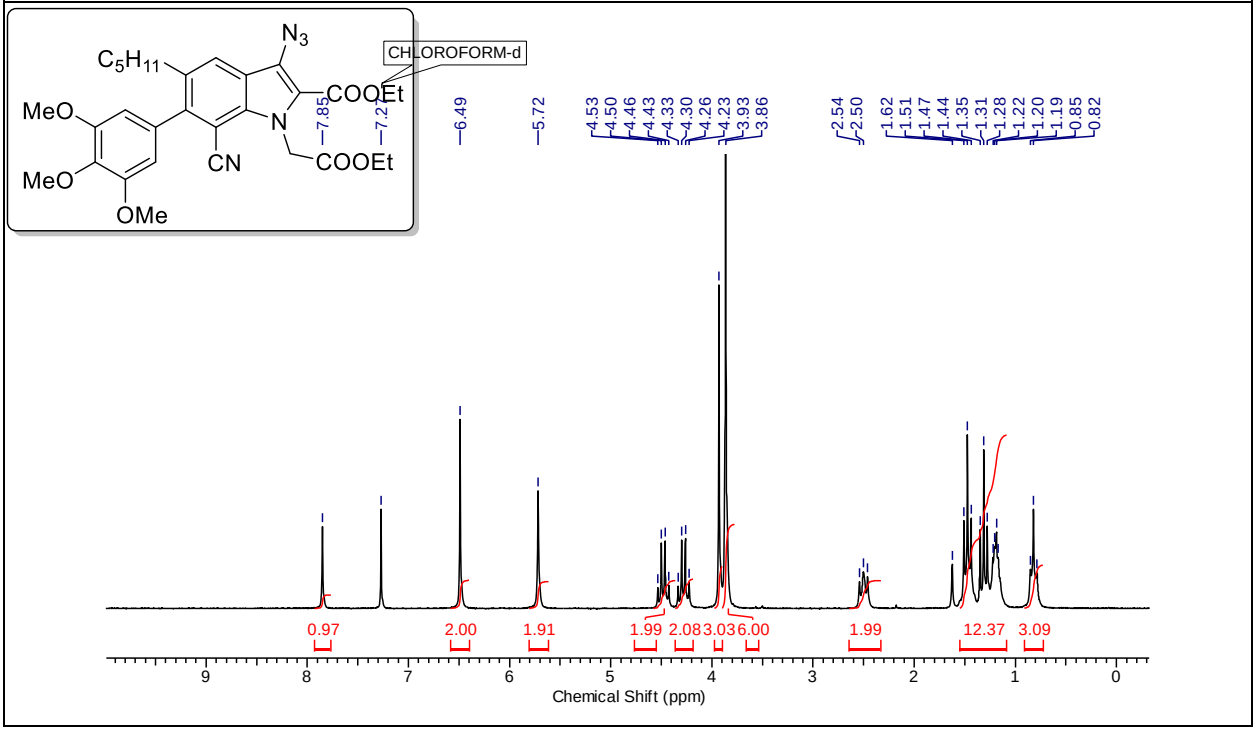
^{1}H NMR (200 MHz, $CDCl_3$) of compound 4b



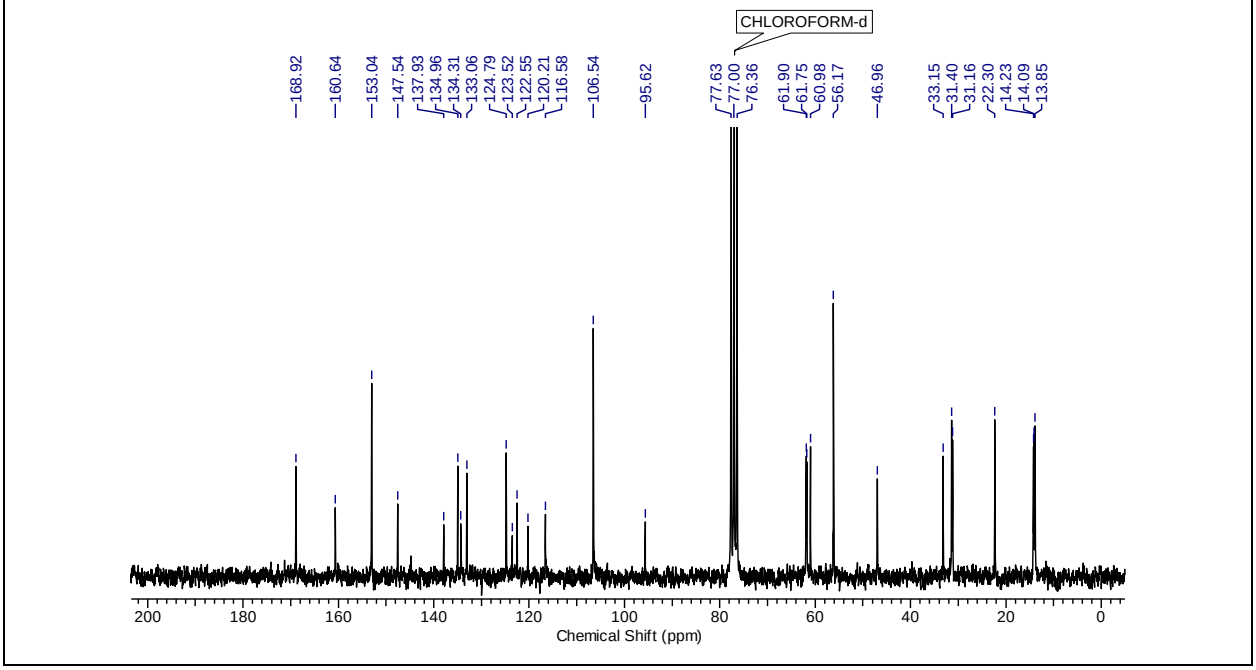
^{13}C NMR (125 MHz, $CDCl_3$) of compound 4b



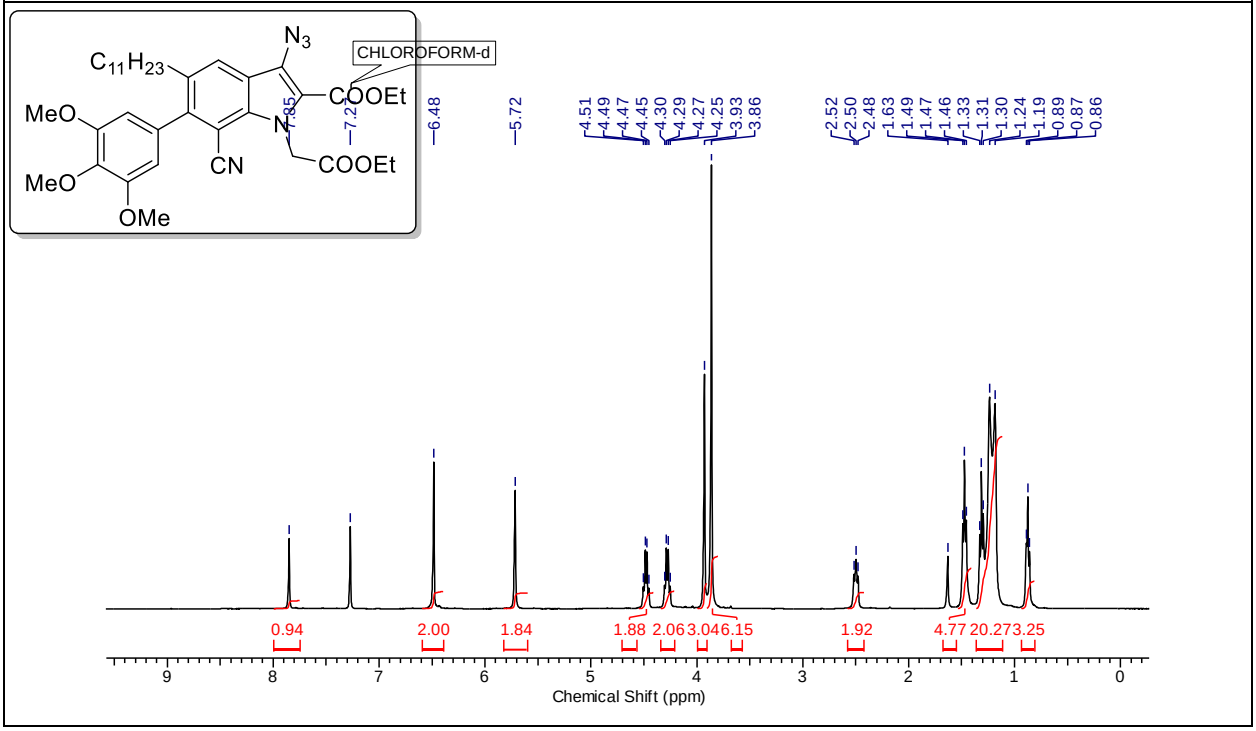
^{1}H NMR (200 MHz, $CDCl_3$) of compound 4e



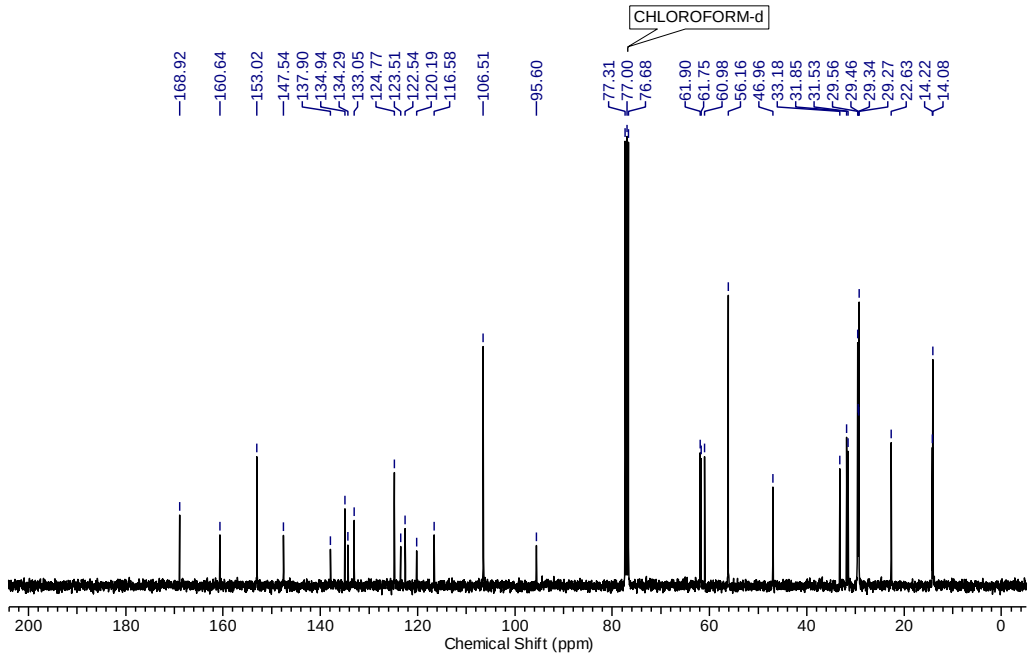
^{13}C NMR (125 MHz, $CDCl_3$) of compound 4e



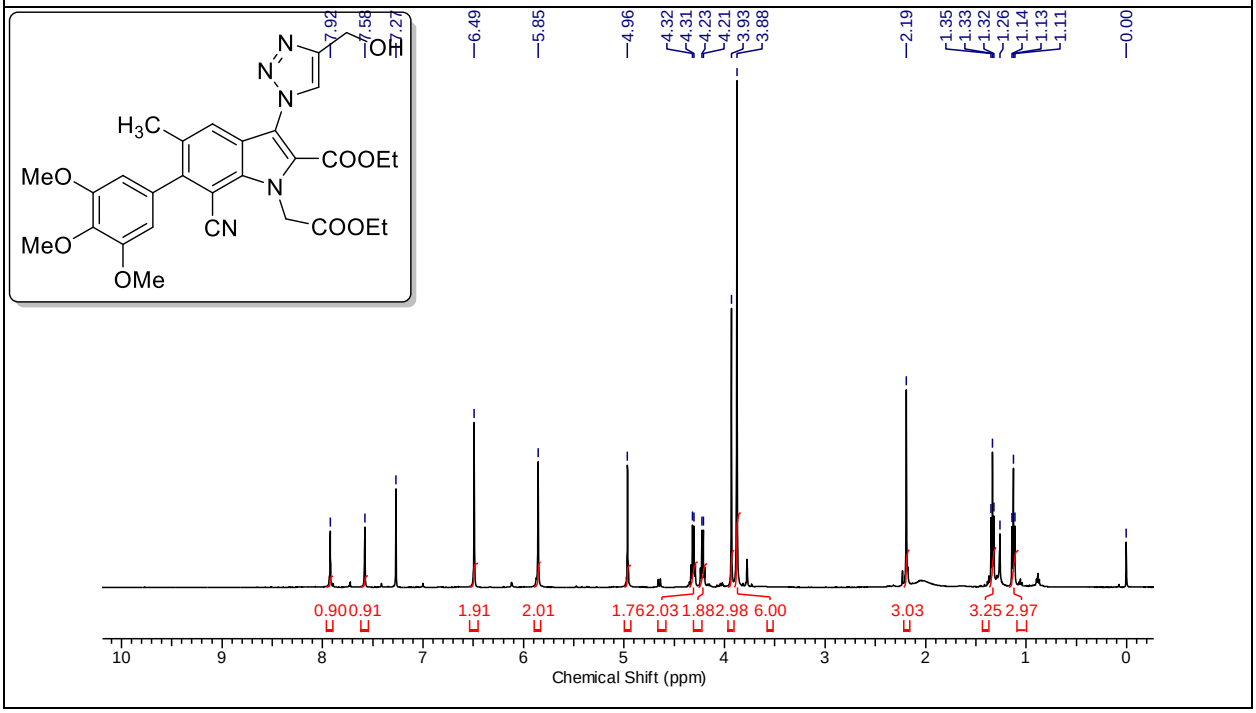
^{1}H NMR (400 MHz, $CDCl_3$) of compound 4k



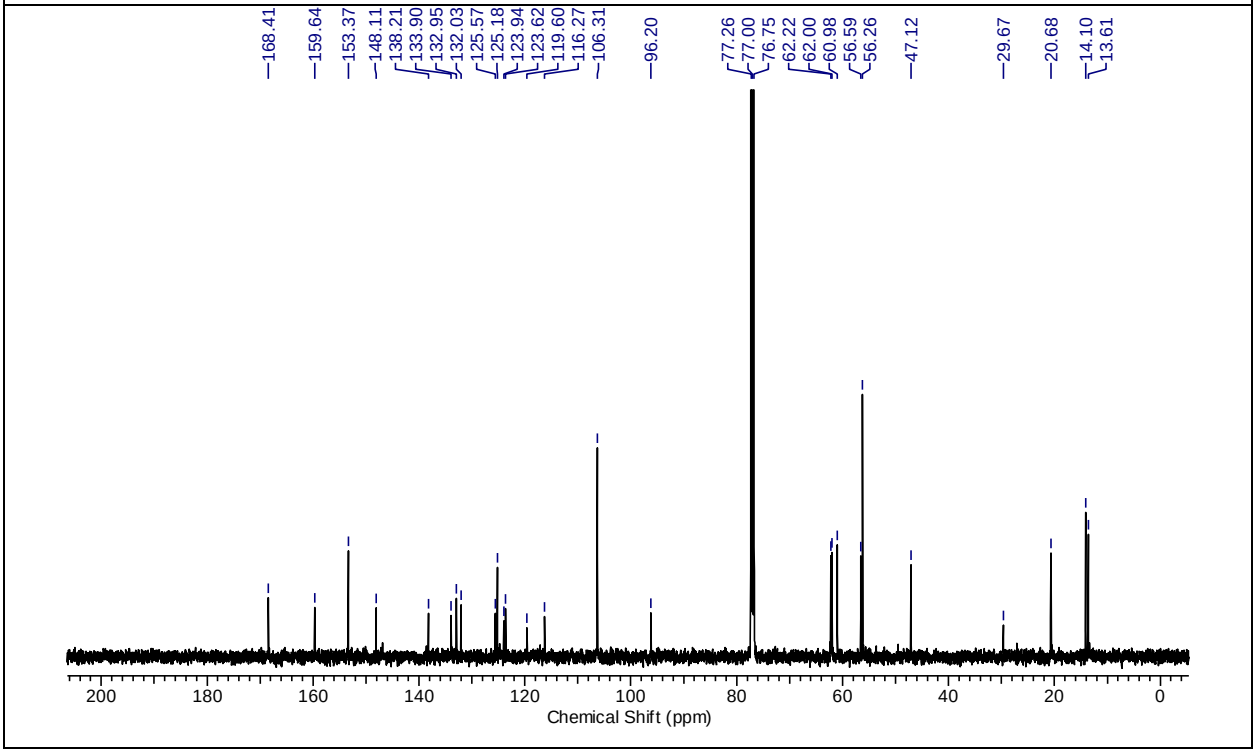
^{13}C NMR (100 MHz, $CDCl_3$) of compound 4k



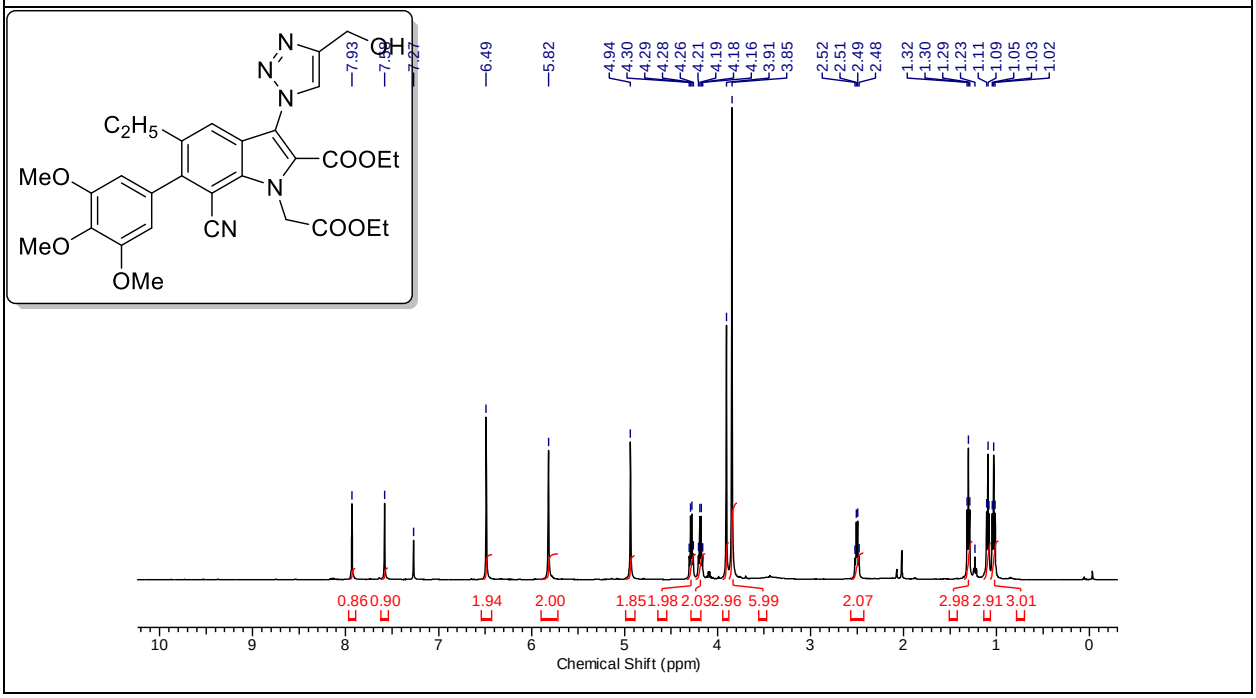
^{1}H NMR (400 MHz, $CDCl_3$) of compound 5a



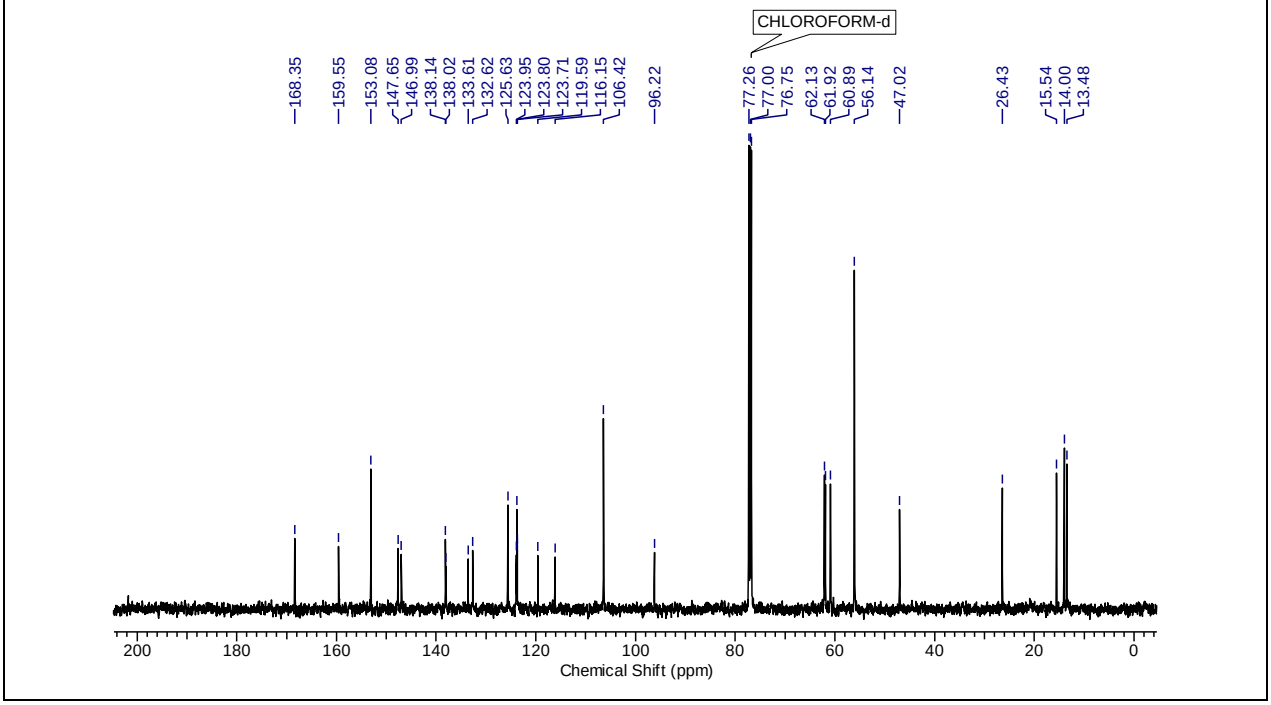
^{13}C NMR (100 MHz, $CDCl_3$) of compound 5a



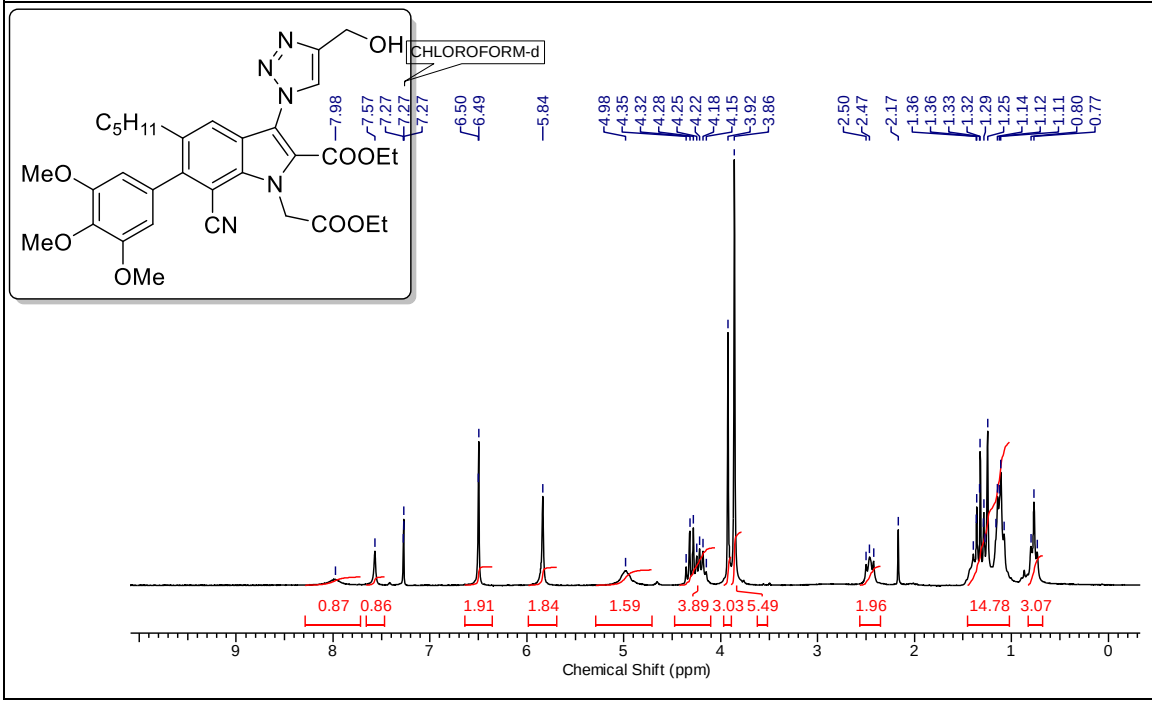
^{1}H NMR (500 MHz, $CDCl_3$) of compound 5b



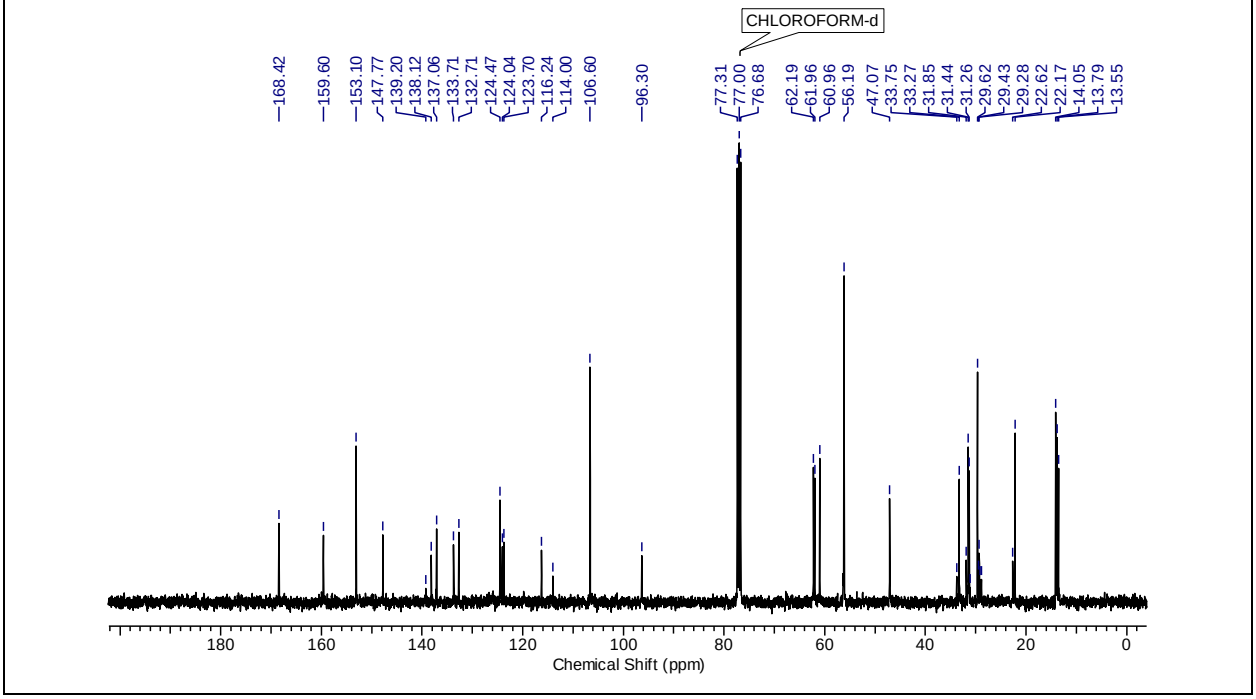
^{13}C NMR (125 MHz, $CDCl_3$) of compound 5b



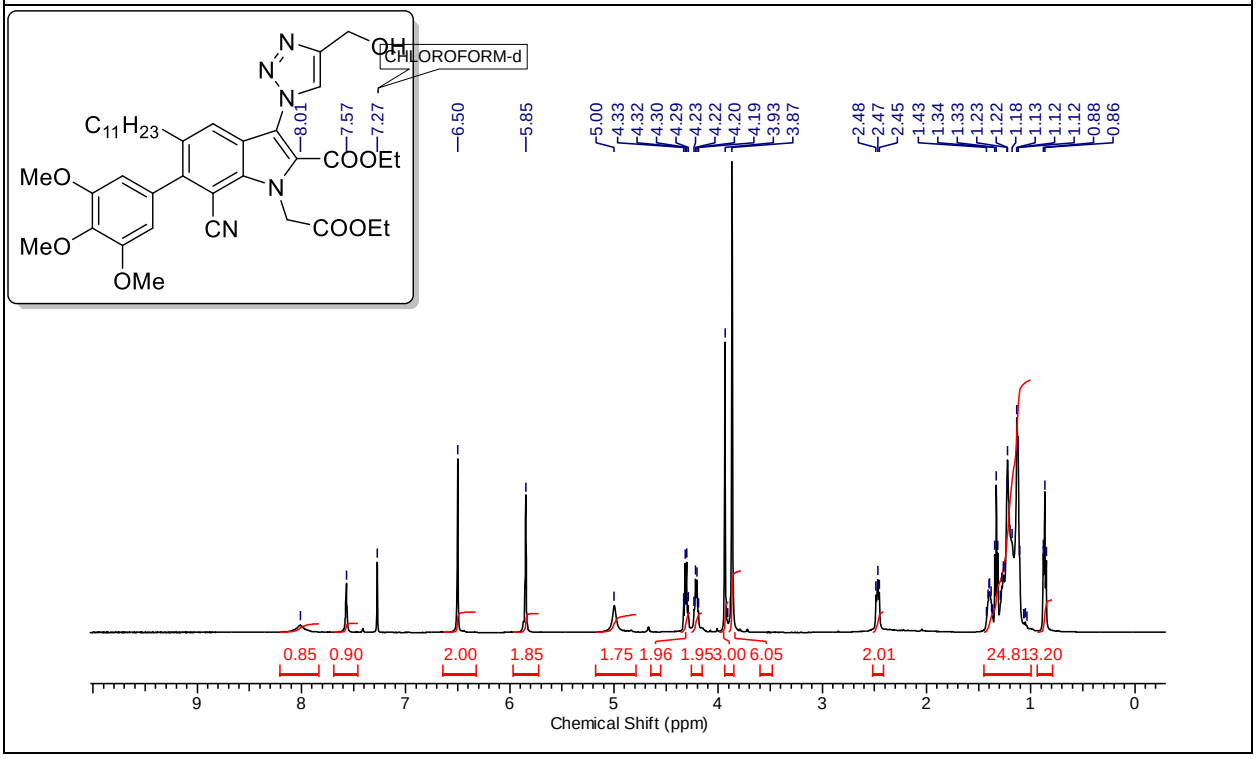
^{1}H NMR (200 MHz, $CDCl_3$) of compound 5e



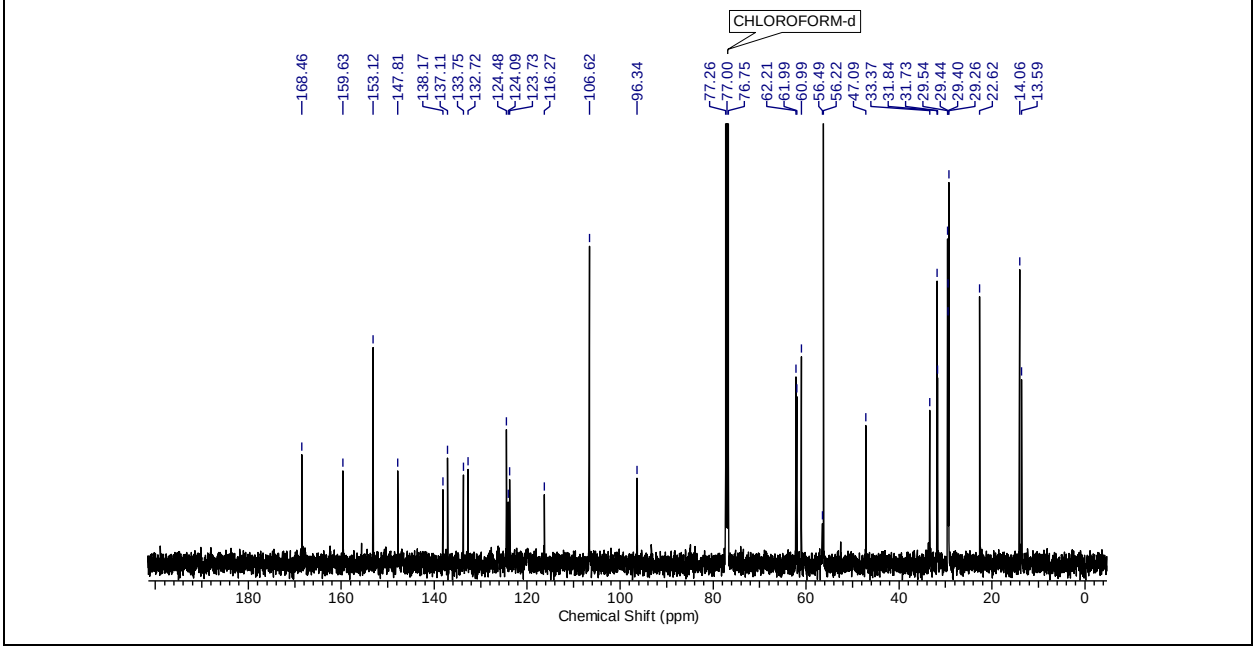
^{13}C NMR (50 MHz, $CDCl_3$) of compound 5e



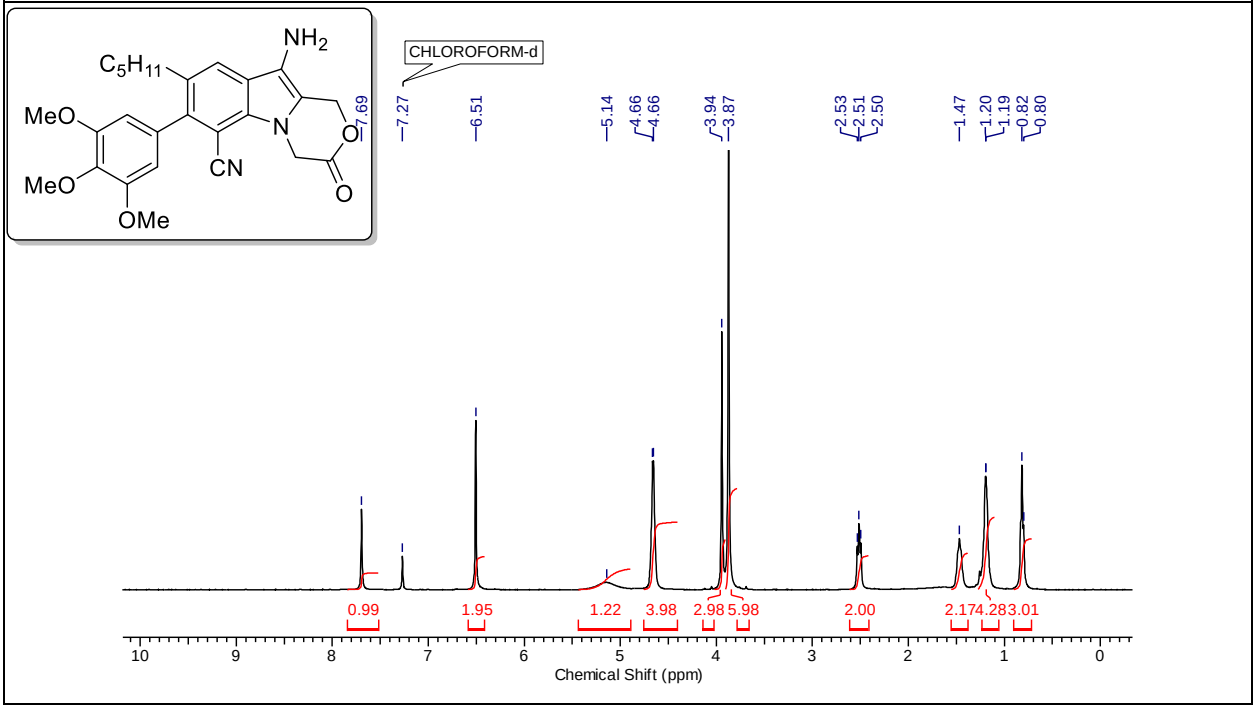
^{1}H NMR (500 MHz, $CDCl_3$) of compound 5k



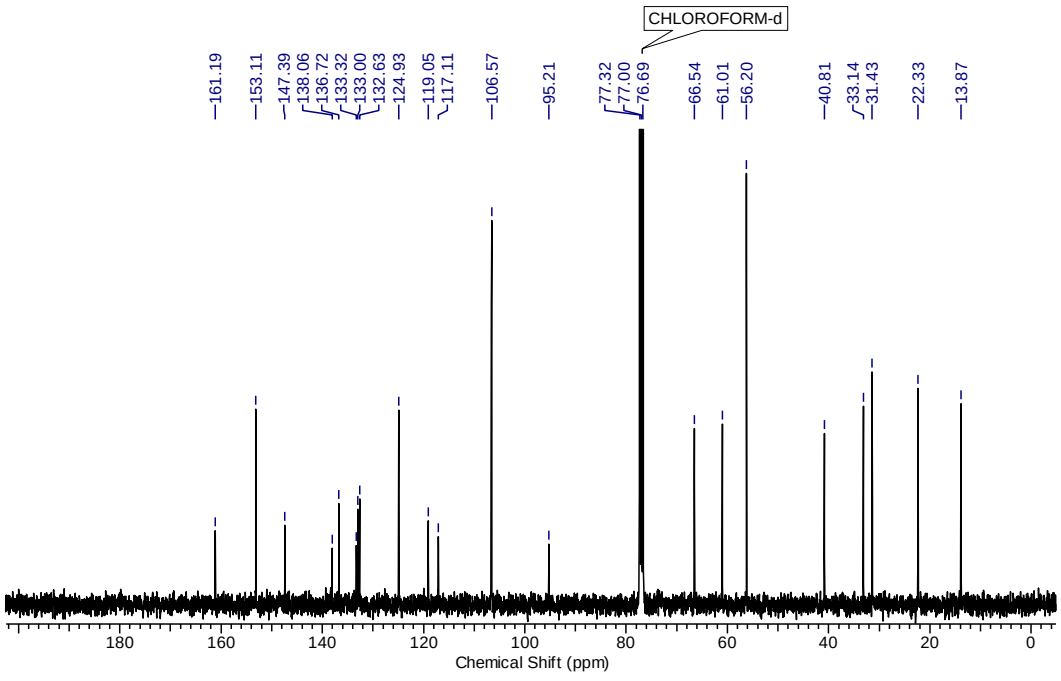
^{13}C NMR (125 MHz, $CDCl_3$) of compound 5k



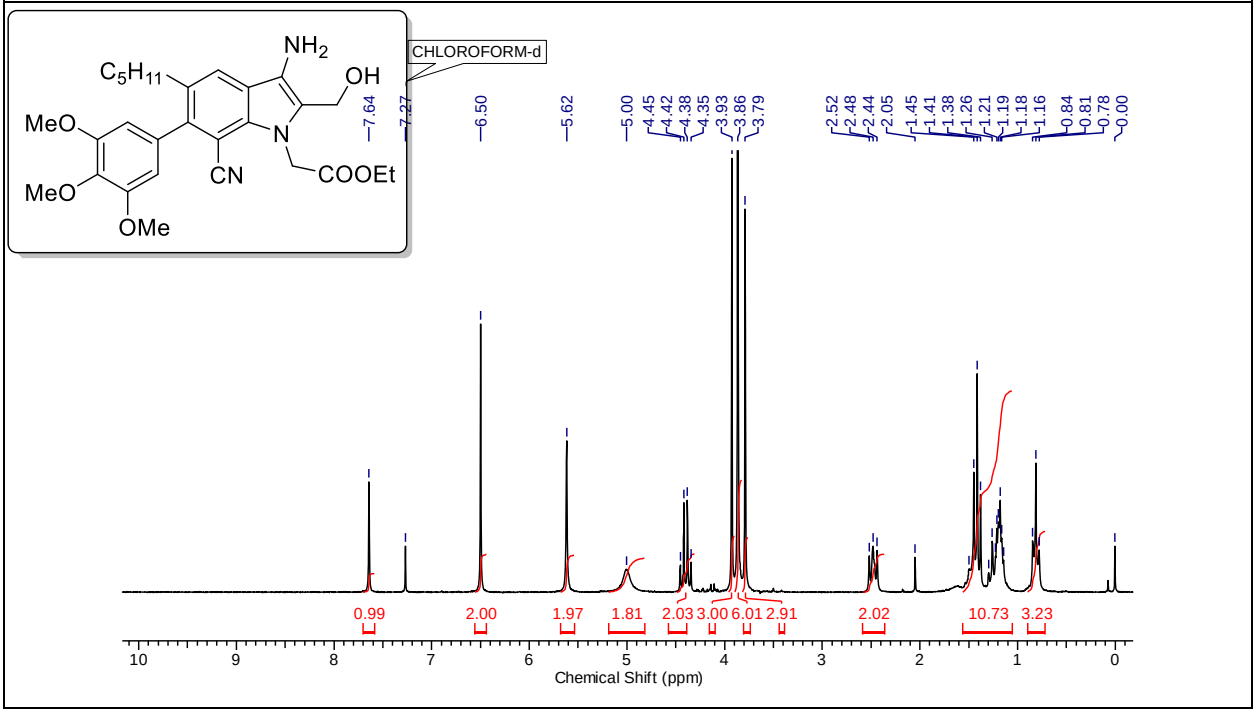
^{1}H NMR (400 MHz, $CDCl_3$) of compound 6a



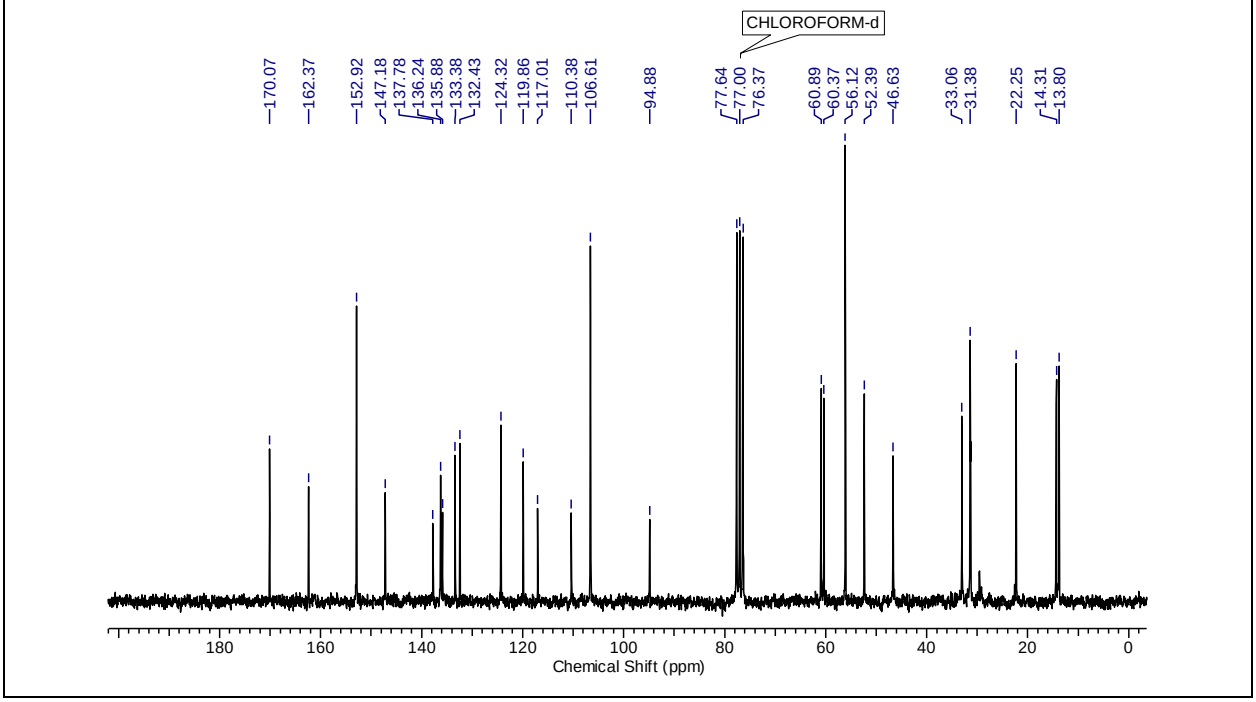
^{13}C NMR (100 MHz, $CDCl_3$) of compound 6a



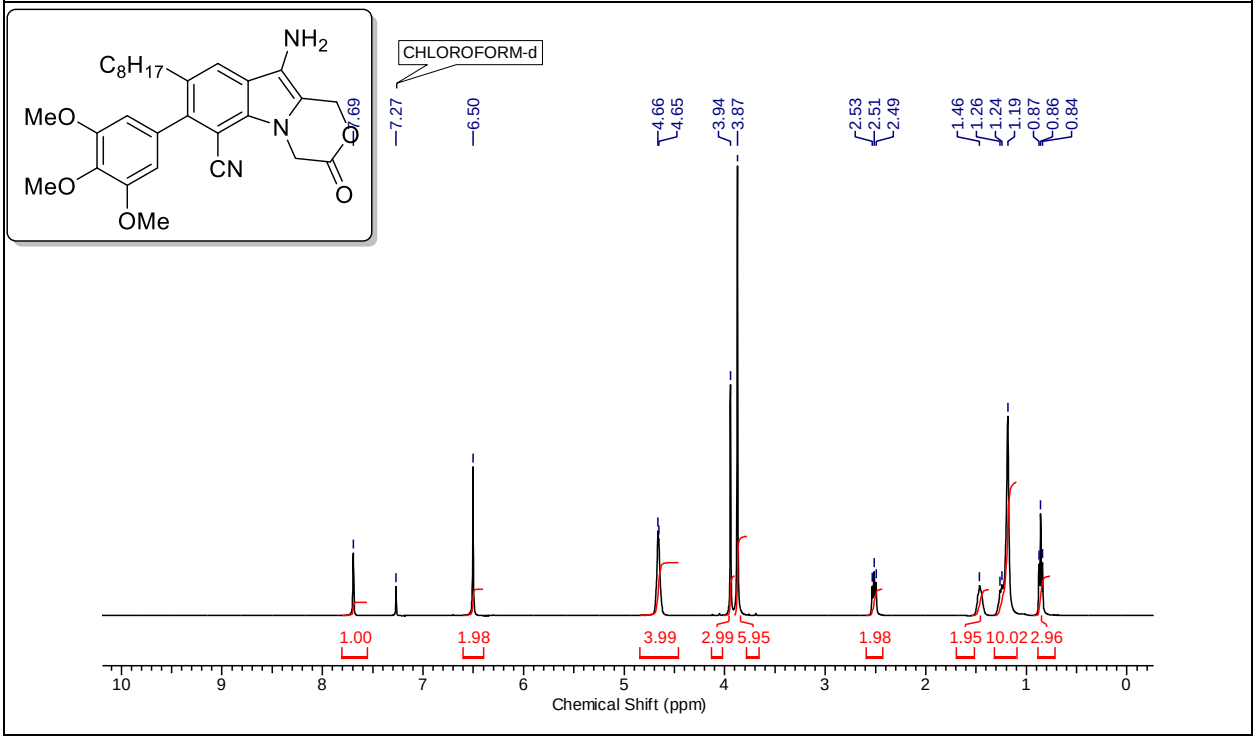
^{1}H NMR (200 MHz, $CDCl_3$) of compound 7a



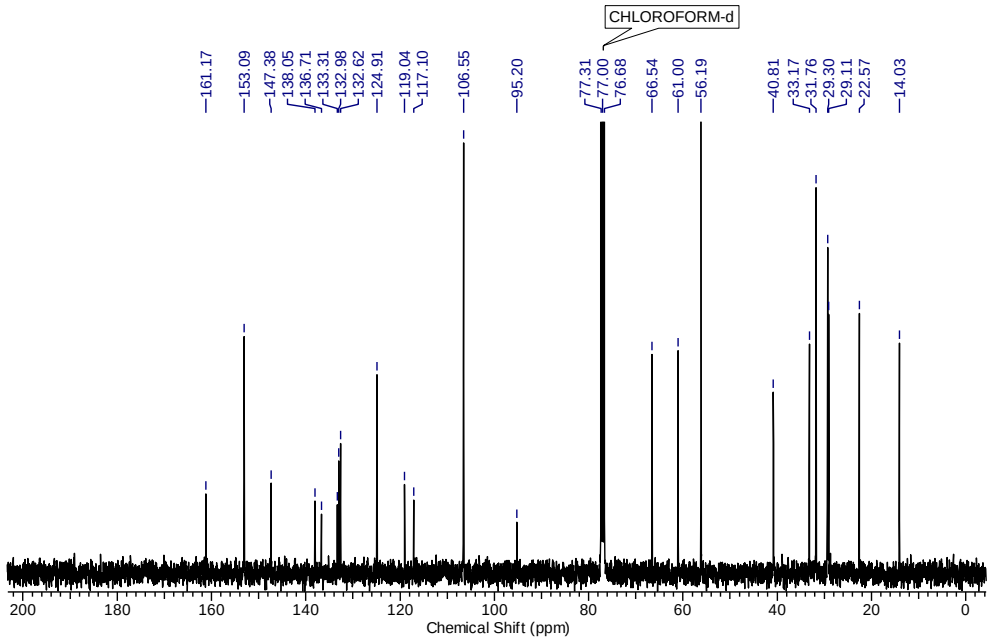
^{13}C NMR (50 MHz, $CDCl_3$) of compound 7a



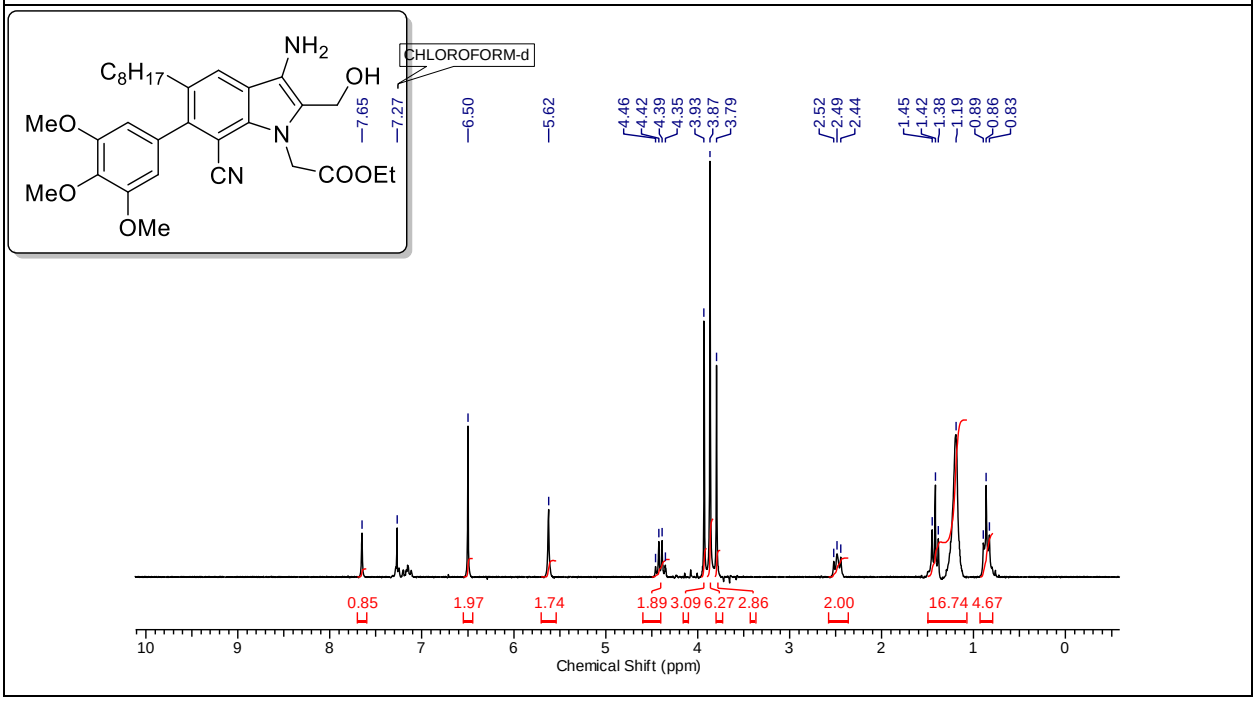
^{1}H NMR (400 MHz, $CDCl_3$) of compound 6b



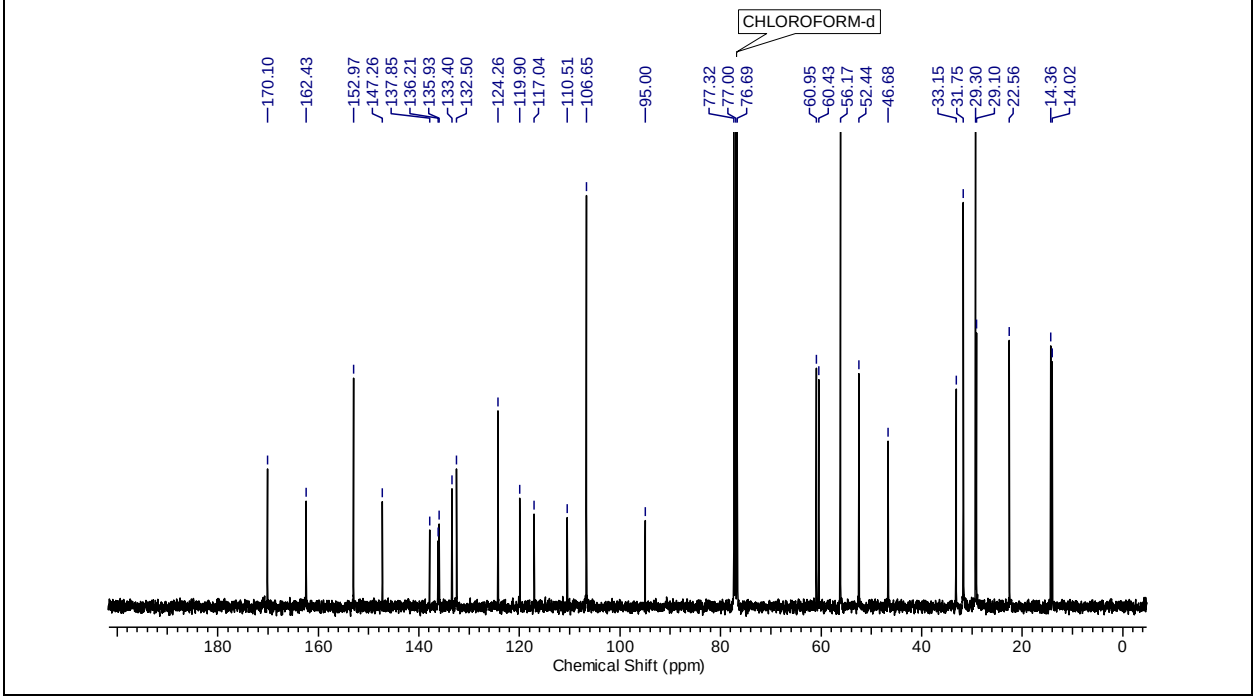
^{13}C NMR (100 MHz, $CDCl_3$) of compound 6b



^{1}H NMR (200 MHz, $CDCl_3$) of compound 7b



^{13}C NMR (100 MHz, $CDCl_3$) of compound 7b



2.3.5. References

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3) Pisal, M. M.; Annadate, R. A.; Athalye, M. C.; Kumar, D.; Chavan, S. P.; Sarkar, D.; Borate, H. B. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 979.
4) Russel, J. S.; Pelkey, E. T.; Greger, J. G.; Gribble, G. W.; Joule, J. A. *Progress in Heterocyclic Chemistry* **2011**, *23*, 155.
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7) Yeware, A., Sarkar, D. *Tuberculosis (Edinb)* **2018**, *110*, 15.
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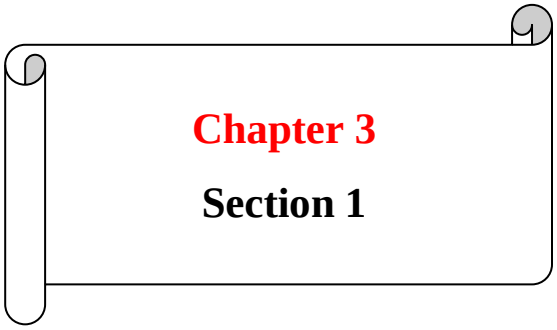


"Introduction and Literature Reports"

3.1.1. Introduction

Ergot alkaloids and synthetic derivatives have long been recognized for their biological activity.[1] Because of their fascinating chemical structures and medicinal potential,[2] they have attracted the interest of many chemists and biologists. To get ergot alkaloids and associated analogues in sufficient amounts, semisynthesis and isolation from natural sources were used. Out of these few are synthesized, well-known dopamine agonists including Bromocriptine, Lisuride, Quinagolide, and Cabergoline, They are all used to treat hyperprolactinemia.[3] Quinagolide and cabergoline, two newer dopamine receptor agonists, have been shown to suppress prolactin secretion more effectively than bromocriptine. Quinagolide is a dopamine agonist that works for a longer time with potent D2 and frail D1 activity.[4] It has been approved to treat idiopathic hyperprolactinemia and prolactin-secreting pituitary tumors. Quinagolide is used to treat high prolactin levels, also known as hyperprolactinemia. Hyperprolactinaemia has been related to gonadal plexus malfunction, such as infertility and reduced libido, in addition to long-term consequences like osteoporosis.

Quinagolide is a racemate, and its therapeutic action is largely due to the (-) enantiomer.[5] It is typically used as a hydrochloride salt in oral tablets marketed by Ferring pharmaceuticals (Lausanne, Switzerland) under the brand name Norprolac®. Nordmann *et al*. published[4] the first synthesis of quinagolide in racemic form using β -tetralone as a starting material and investigated its biological activity. Banziger *et al*. reported[6] a scaled up synthesis of quinagolide in the year 2000 Recently, two strategies for enantioselective synthesis of (-)-quinagolide were published[7,8] by Chavan *et al*. This group out of these two, in first report organocatalyzed Diels-Alder reaction was used to stabilize all three stereocenters on the piperidine group, and the octahydrobenzo[*g*]quinoline was accessed by diastereoselective ring expansion by using bicyclic aziridinium ion strategy in the second article[9]. Along with this, regioselective azidoalkoxylation of enol ether[9] induced by ceric ammonium nitrate (CAN) for the synthesis of 3-azidopiperidine skeleton was reported by Chavan *et al*.[9] Chavan and group developed a method in the lab for the total synthesis of (-)-quinagolide utilizing the ring closure metathesis (RCM) strategy[10] using *meta*-hydroxybenzaldehyde as the precursor material.

3.1.1.1. Ergot alkaloids

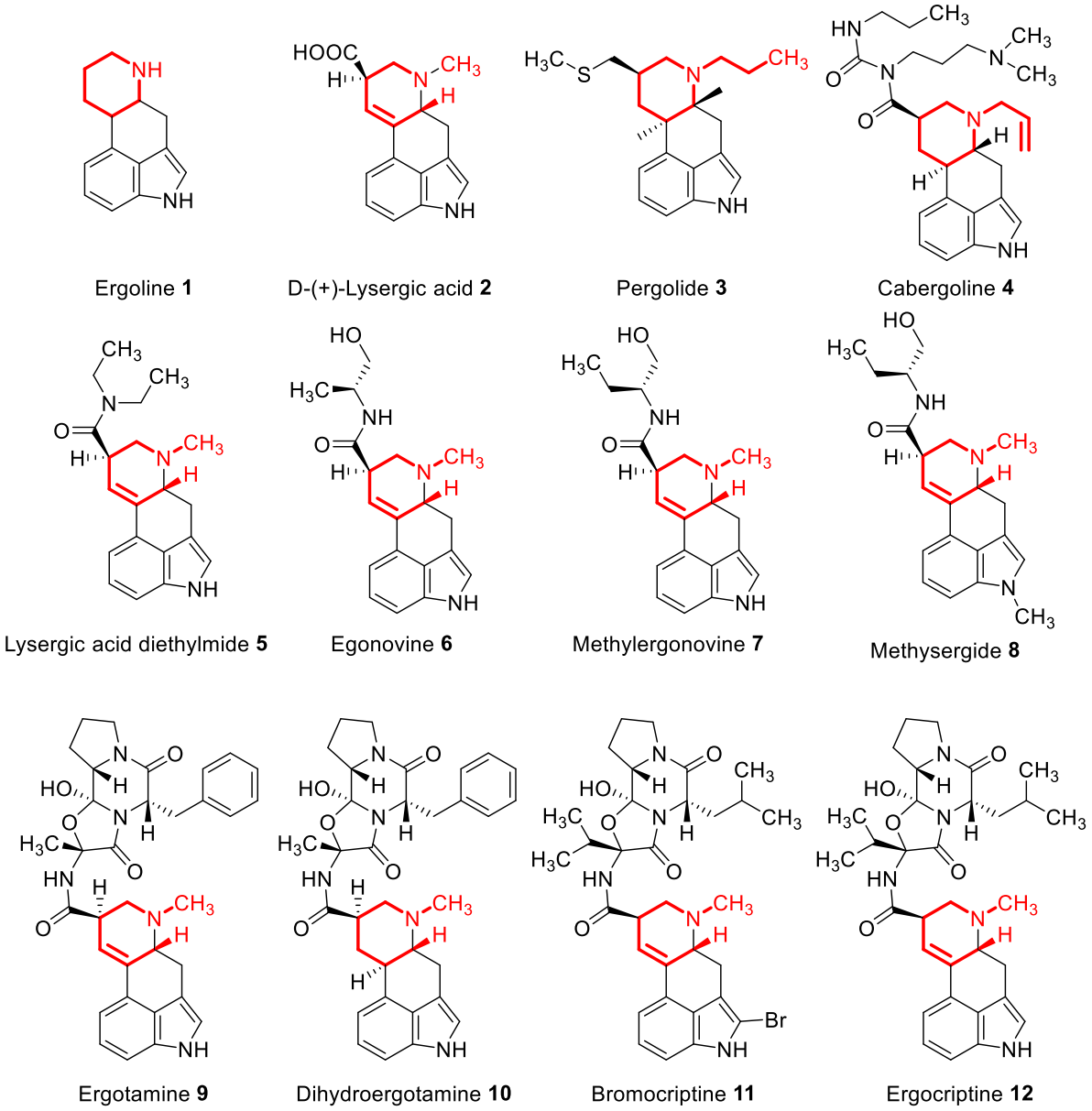


Figure 1. Examples of ergot alkaloids

Alkaloids found in ergot seem to be the most common metabolites of nitrogenous fungi, and they biosynthesize indole core functionality from L-tryptophan.[1] About 80 distinct ergot alkaloids where discovered, mostly in *Claviceps* species, but in various types of fungi and higher plants. The tetracyclic ring system, also known as ergoline, which is a partly hydrogenated indole[4,3-fg]quinoline, is a structural characteristic of ergot alkaloids shown in **Figure 1.** Antiparkinsonian responses are mediated by dopamine agonists, which act specifically on dopamine receptors and

mimic the endogenous neurotransmitter. Ergoline and non-ergoline agonists are indeed the two main types of dopamine agonists. Dopamine D2-type receptors are targeted by each of these subclasses.

The chirality centers in ergot alkaloids are arranged in a variety of ways. The R-chirality at C-5, on the other hand, is stable and nonvariable, indicating that these alkaloids are derived from amino acid L-tryptophan which forms indole ring and fix C-4, C-5 and N-6 atoms contributed from amino acid used accordingly most important center is C-8 because all of the medicinally important ergot alkaloids are C-8 amide or peptide derivatives of (L)-lysergic acid, which has R-chirality at C-8.

3.1.1.2. Aporphine alkaloids

Plants are the most abundant source of aporphine alkaloids.[1] Plants belonging to the genera Beilschmiedia, Nandina (Nandina domestica), Glaucium (horn poppy), and others, for example, also include isoboldine. The synthesis of apomorphine alkaloids is well-documented.[2] Because of its medicinal value and morphine-like similarity, consideration should be given to benzylisoquinoline alkaloids and aporphine alkaloids. The stereocenter of aporphine alkaloids is common, and proaporphin and aporphin alkaloids have structural isomerism. Between aporphine alkaloids the substituents and their positions on the main scaffold differ.[1]

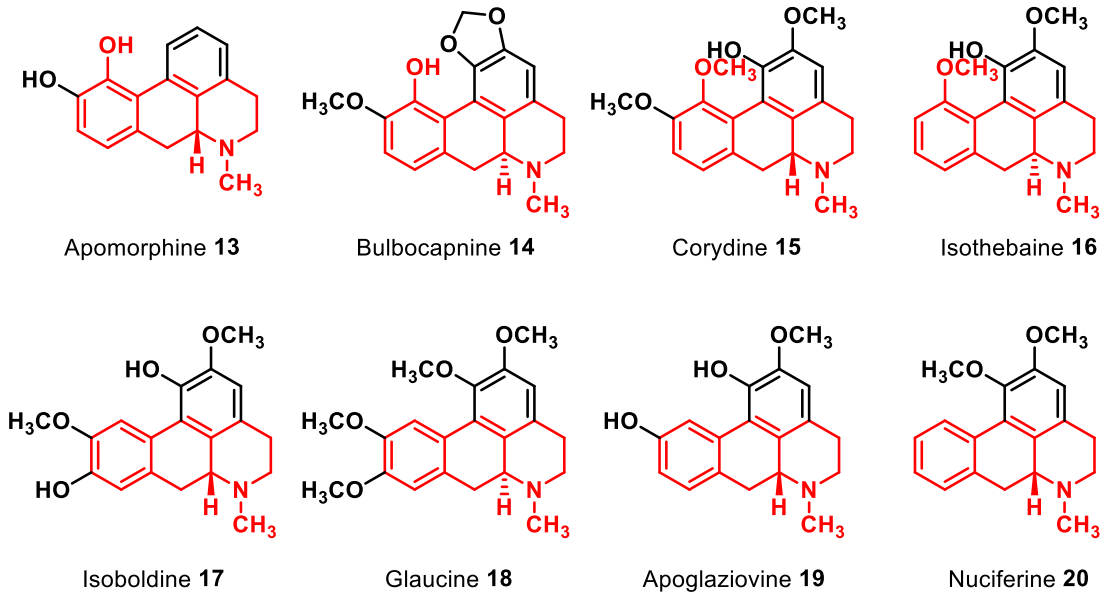
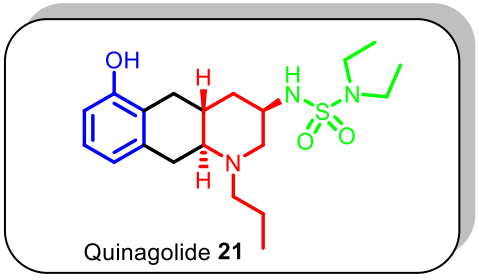


Figure 2. Examples of aporphine alkaloids

Furthermore, their stereochemistry differs, although most are (R)-configured, some, including glaucine, bulbocapnine, and isothebaine, are (S)-configured. Well known examples of aporphine alkaloids and their structural characteristics are shown in **Figure 2.**

3.1.1.3. Combined structural characteristics of ergot alkaloids and apomorphine[4]

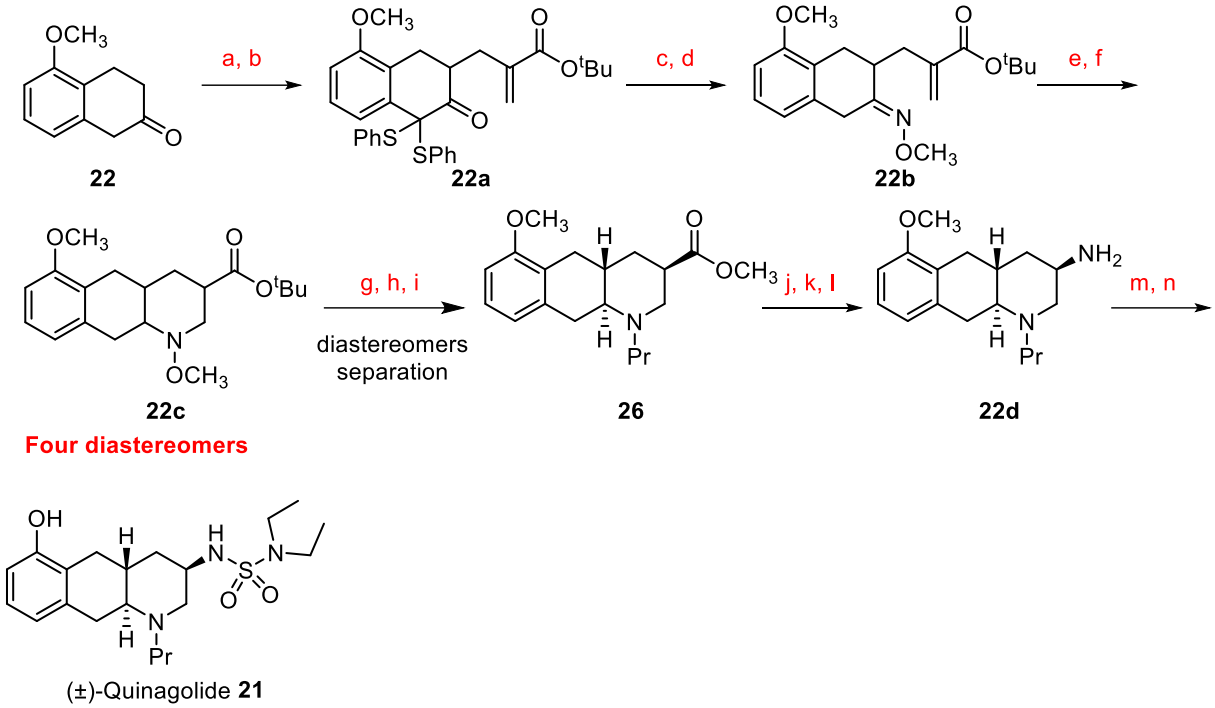
Ergot alkaloids have been shown to have dopaminomimetic activity in several studies.[2] Ergolines CQ32-084, pergolide, and apomorphine are well-known dopamine agonists. The chemical composition of dopaminergic neurons was linked to their behavior. According to Fliickiger *et al.*[1], the pharmacological effects of ergot alkaloids and related synthetic equivalents are diverse depending on minor variations in their chemical structure. In terms of structural features, quinagolide is comparable to dopaminomimetic active ergot alkaloids and aporphine alkaloids. Nordmann *et al.*[4] were the first to use quinagolide in racemic form, and show good dopaminomimetic activity.



Quinagolide **21**

3.1.2. Synthetic approaches

A) 1st Report [(±)-Quinagolide][4]



Scheme 1. Reagents and conditions: **a**: S-phenyl benzenethiosulfonate, NaOAc, MeOH, rt, 82%; **b**: LDA, tert-butyl 2-(bromomethyl)acrylate, Et_2O-THF-HMPT, –78 °C, 66%; **c**: Al(Hg), THF-H_2O, 50 °C, 2 h, 71%; **d**: H_2NOCH_3.HCl, $Na_2HPO_4.2H_2O$, MeOH, rt, 4 h, 72%; **e**: $NaCNBH_3$, MeOH, rt, 12 h; **f**: MeOH, rt, 72 h; **g**: H_2SO_4, MeOH, reflux, overnight, 92%; **h**: Zn, AcOH, H_2O, rt, overnight, 72%; **i**: Propanal, H_2, 10% Pd/C, PrOH, rt, overnight, 74%; **j**: Hydrazine hydrate, MeOH, 50 °C, 20 h, 80%; **k**: NOCl, THF, reflux, 1 h; **l**: HCl, THF, reflux, 1 h, 61% (two steps); **m**: Et_2NSO_2Cl, $CHCl_3$, 50 °C, overnight, 87%; **n**: BBr_3, CH_2Cl_2, –10 °C, 4 h, 89%.

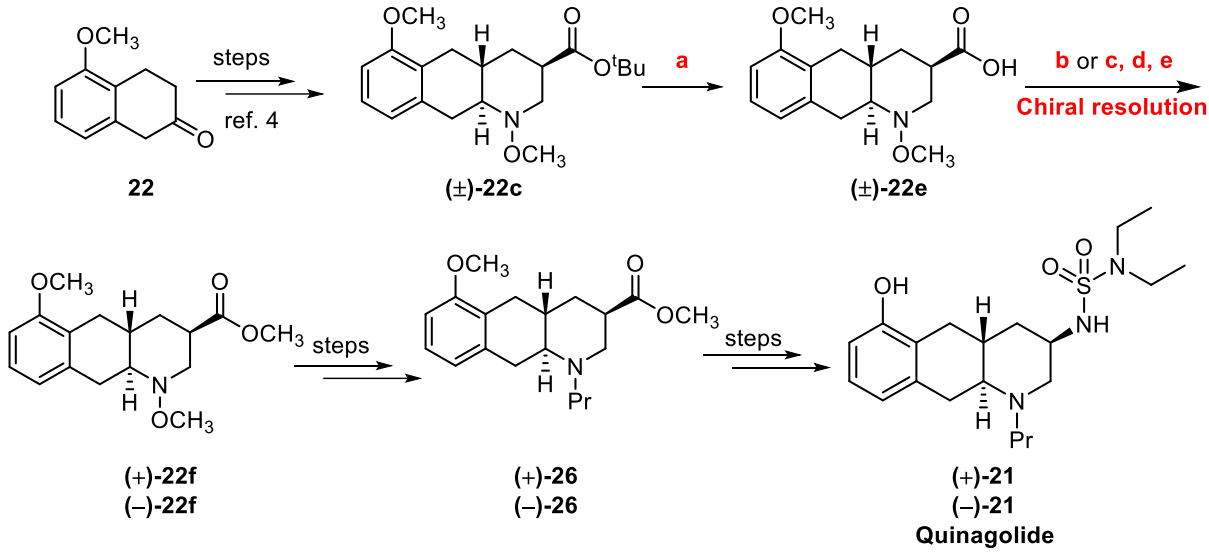
Nordmann *et al.* (Sandoz Ltd.) has reported the total synthesis of a new dopamine agonist quinagolide in 1985 by integrating the structural features of ergot and ampomorphine alkaloids

Key points of synthesis

1) Nordmann and group reported four diastereoisomeric 3-(tert-butoxycarbonyl)-l,6-dimethoxyoctahydrobenzo[g]quinolines.
2) Out of these four isomers, two trans isomers converted into tricyclic analogues which show potent dopaminomimetic activity. The basic apomorphine moiety is combined with the important 8-substituents of ergoline in these two compounds.

3) Silica gel column chromatography was used to isolate the four diastereomers. By alkylation with propanal under hydrogenation conditions, the necessary diastereomer was converted to the methyl ester.
4) To acquire 3-aminopiperidine, the Curtius rearrangement of corresponding azide derived from methyl ester was used.
5) Sulfonation with *N*,*N*-diethyl sulfamoyl chloride and ether cleavage with BBr_3 was used to transform the amine to the final product.

B) 2nd Report [(±)-Quinagolide][5]



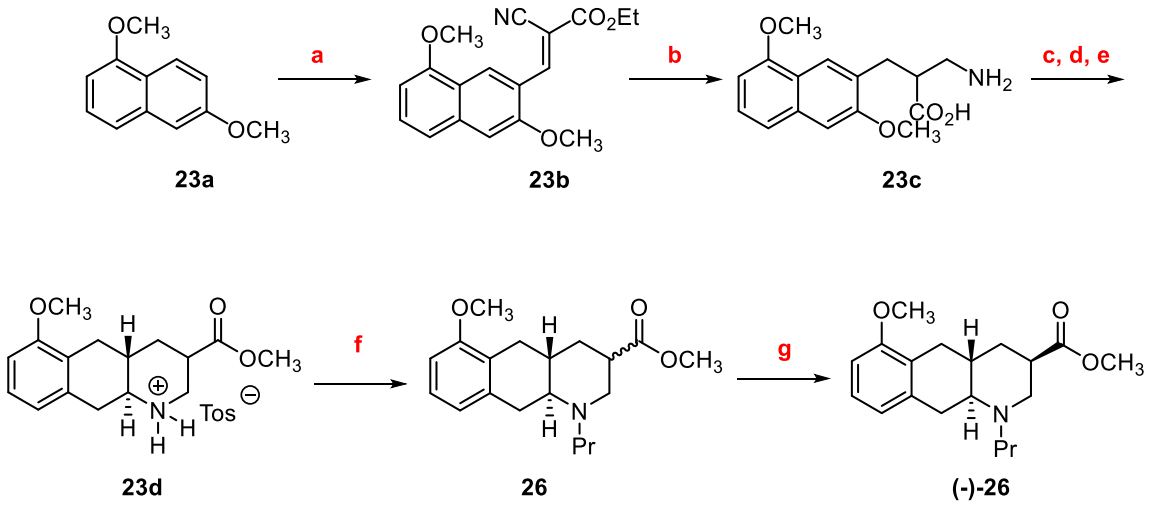
Scheme 2. Reagents and conditions: **a**: TFA, rt, 45 min, 90%; **b**: D-(+)-α-methylbenzylamine CH_2Cl_2-Et_2O, –20 °C, 30%; **c**: L-(–)-α-methylbenzylamine, CH_2Cl_2-Et_2O, –20 °C, 28%; **d**: 1 N HCl; **e**: CH_2N_2, CH_2Cl_2, 98%.

Key points of synthesis

1) *Via* the resolution of racemic intermediates, Nordmann *et al*. revealed the absolute structure and dopaminomimetic activity of quinagolide in the same year (1985).
2) They were unable to overcome the diastereomeric mixture of the parent compound. As a result, D-(+)-methylbenzylamine and L-(-)-methylbenzylamine were used to separate a diasteriomeric mixture of associated acid compounds. The two enantiomers were used to make methyl esters in this reaction sequence.
3) In addition to racemic quinagolide, the (+) enantiomer of quinagolide and (-) enantiomer of quinagolide were tested separately for dopaminomimetic activity *in vivo* and *in vitro*.

4) Biological evaluations indicated that the (-) enantiomer of quinagolide, which has the absolute configuration (3S,4aS,10aR) comparable to the absolute configuration of ergoline CQ32-084, was found to be completely responsible for the dopaminomimetic activity.

C) 3rd Report [(±)-Quinagolide][6]

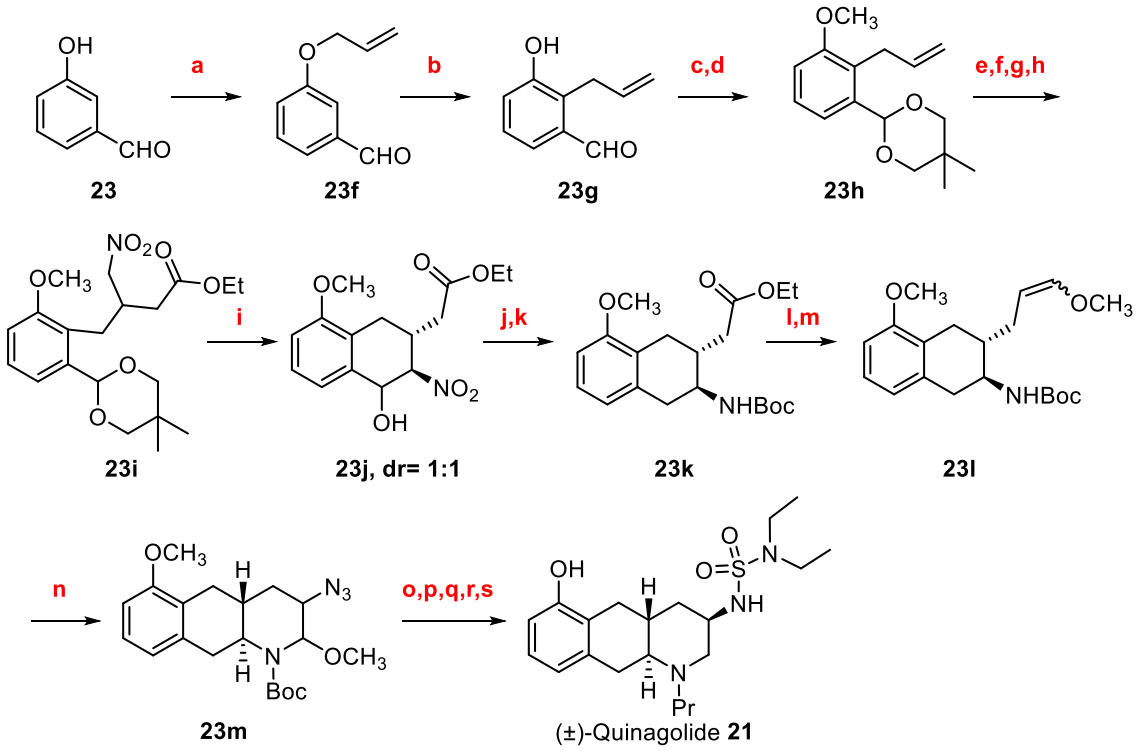


Scheme 3. Reagents and conditions: **a**: Ethoxymethylenecyanoacetate, hexyllithium, THF, –70 °C, 2 h, 55%; **b**: (i) H_2, Pt/C, H_2SO_4, EtOH, 50 °C, 4 h; (ii) NaOH, H_3O^+, 83% (over 2 steps); **c**: Li, NH_3, THF-^{t}BuOH, –70 °C, 1.5 h; **d**: HCl (aq), 95% (over 2 steps); **e**: $NaBH_4$, CH_3OH, –70 °C, 2 h then PTSA, EtOAc, 78%; **f**: n-propyl iodide, K_2CO_3, DMF, 50 °C, 2.5 h; **g**: LDA, TMSCl, THF, –40 °C, 2 h, 85% (over 2 steps).

Key points of synthesis

1) In the year 2000, Novartis Ltd.'s Banziger *et al*. published scalable synthesis of quinagolide using 1,6-dimethoxynaphthalene as a starting material.
2) Centered on 1,6-dimethoxynaphthalene as a starting meterial, a multi-kilogram scale synthesis of quinagolide intermediate was achieved in only 5 isolated steps with a 27–29% overall yield.

D) 4th Report [(±)-Quinagolide][9]

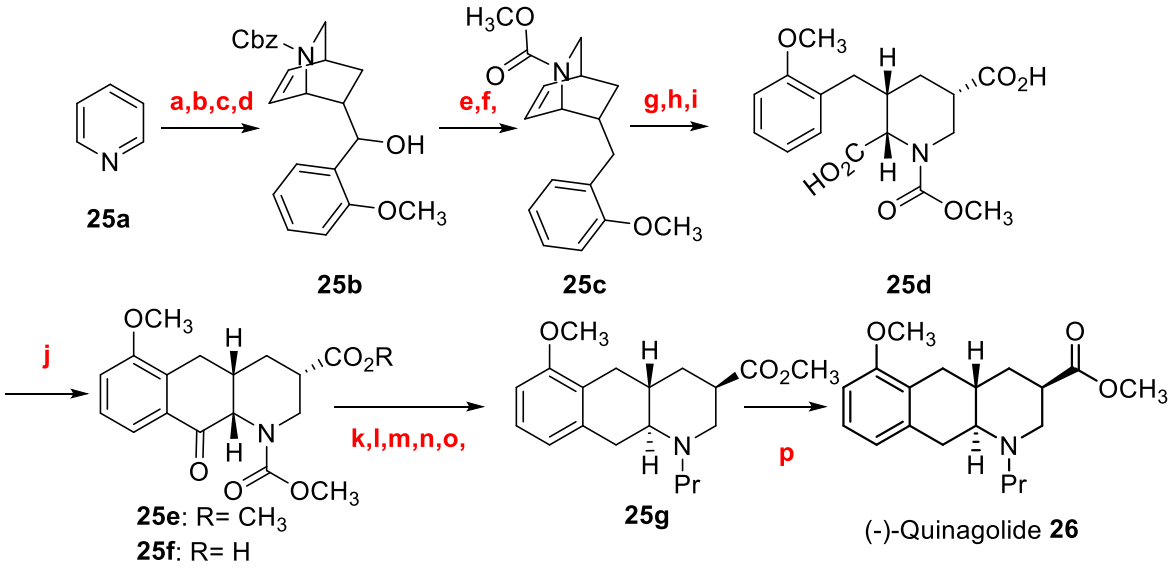


Scheme 4. Reagents and conditions: **a**: Allyl bromide, K_2CO_3, EtOH, reflux, 3 h, 97%; **b**: µw, 800 W, 240 °C, 10 min, 45%; **c**: Dimrthyl sulfate, K_2CO_3, acetone, reflux, 8 h, 95%; **d**: 2,2-Dimethyl-1,3-propanediol, triethyl orthoformate, PTSA, CH_2Cl_2, rt, 24 h, 95%; **e**: OsO_4, NMO, CH_3CN: H_2O (9:1), rt , 24 h, 95%; **f**: $NaIO_4$, acetone: H_2O (3:1), 2 h, rt; **g**: Ph_3PCHCO_2Et, CH_2Cl_2, rt, 3 h, 72% (over 3 steps); **h**: Nitromethane, DBU, reflux, 4 h, 83%; **i**: PPTS, acetone: H_2O (4:5), reflux, 20-30 h, 82%; **j**: H_2 Pd/C, Et_3N, $(Boc)_2O$. EtOAc, 60 psi, 6 h, 90%; **k**: $Pd(OH)_2$/ H_2, cat. HCl, MeOH, 60 psi, 6 h, 83%; **l**: DIBAL-H, CH_2Cl_2, -78 °C, 1.5 h; **m**: $CH_3OCH_2P^+Ph_3Cl^-$, KO^tBu, THF, 0 °C, to rt, 64% (2 steps); **n**: CAN, NaN_3, MeOH, CH_3CN, 0 °C, 20-2 h; **o**: $NaBH_3CN$, TFA-EtOH (1:9), 0 °C, rt, 12 h; **p**: Propyl iodide, K_2CO_3, DMF, 50 °C, 2.5 h, 45% (3 steps), dr: 3:2 **q**: PPh_3, H_2O, THF, reflux, 5 h, 83%; **r**: Diethylsulfamoyl chloride, EtN_3, $CHCl_3$, 50 °C, 12 h, 71%; **s**: $AlCl_3$/EtSH, CH_2Cl_2, rt, 12 h, 66%.

Key points of synthesis

1) This total synthesis was achieved through regioselective azidoalkoxylation of enol ether induced by ceric ammonium nitrate (CAN).
2) Claisen rearrangement, one-pot acetal deprotection catalyzed by PPTS (pyridinium p-toluenesulfonate), and subsequently introduction of trans ring junction and CAN induced regioselective azidoalkoxylation of enol ether which is distereoselective Henry reaction are important features of the synthesis.

E) 5th Report [(-)-Quinagolide][10]

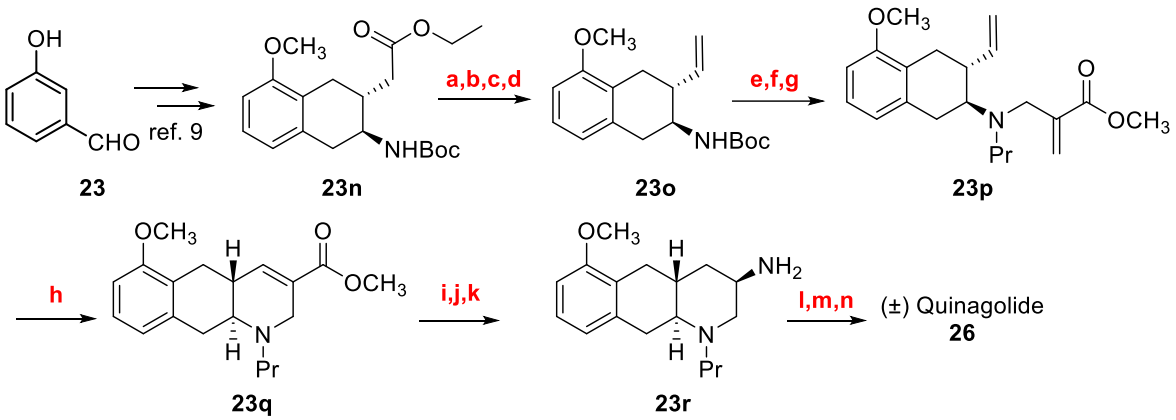


Scheme 5. Reagents and conditions: **a**: Cbz-Cl, $NaBH_4$, MeOH, -50- 0 °C, 2 h, **b**: (S)-(−)-2-Amino-3-methyl-1,1-diphenyl-1-butanol (10 mole%), CH_3CN-H_2O, 0 °C, 24 h; **c**: THF, -30 °C-rt, 2 h, 34% (over 3 steps); **d**: Li, liq NH_3, THF, -78 °C, 15 min; **e**: $ClCO_2CH_3$, Na_2CO_3, CH_2Cl_2, 0- rt °C, 12 h, 64% (over 2 steps); **f**: OsO_4, NMO, THF: H_2O, rt, 2 days; **g**: Silica supported $NaIO_4$, CH_2Cl_2, rt, 1 h; **h**: NaH_2PO_4, $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, 12 h, 95% (over 3 steps), **i**: $(COCl_2)_2$, CH_2Cl_2, 0- rt °C, 1 h, $TiCl_4$, CH_2Cl_2, 0 °C, 2 h, MeOH, -30 °C to rt, 3 h, 62%; **j**: $LiBH_4$, THF, 0 °C to rt, 2 h, 68%; **k**: $NaOCH_3$, MeOH, 0 °C to rt, 12 h, 70%; **l**: NaOH, EtOH, reflux, 1 h, **m**: CH_2N_2 MeOH,-Et_2O, 0 °C to rt, 1 h, 53% (over 2 steps); **n**: PTSA, toluene, reflux, 2 h, $NaBH_3CN$, MeOH; **o**: Propyl iodide, K_2CO_3, DMF, 50 °C, 2.5 h, 61% (over 2 steps); **p**: ref 4

Key points of synthesis

1) This is the first enantioselective formal total synthesis of (−)-Quinagolide.
2) By using following eight consequitive steps, an enantioselective (-)-quinagolide can be Synthesized from pyridine these steps are as, (a) For introducing three stereocenters on the piperidine ring organocatalyzed Diels Alder reaction can be used; (b) protecting group induced deoxygenation in the isoquinuclidine fnctional group can be accomplished by using Birch reduction; (c) Lewis acid ($TiCl_4$) catalyzed Intramolecular Friedel-Crafts induced cyclization of dicarboxylic acid can be catalysed by Lewis acid ($TiCl_4$); and (d) Final trans geometry can be fixed by using diastereoselective ketone reduction and intramolecular cyclization.

F) 6th Report [(±)-Quinagolide][7]

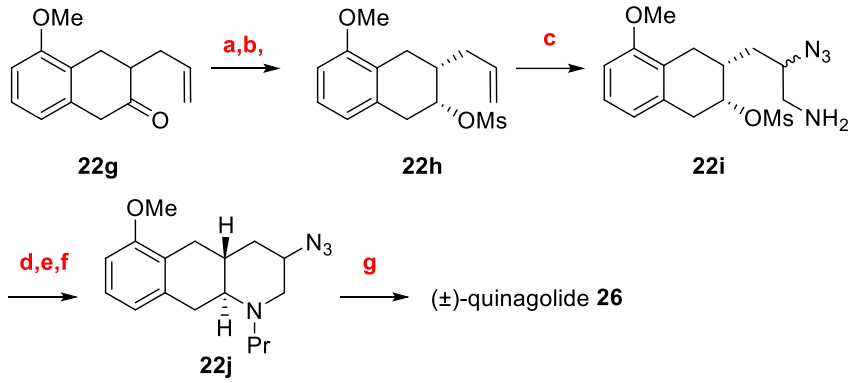


Scheme 6. Reagents and conditions: **a**: $NaBH_4$, LiCl, THF-EtOH, reflux, 16 h, 68%; **b**: $(PhS)_2$ / Bu_3P, THF, rt, 24 h, 95%; **c**: $NaIO_4$, MeOH-H_2O, 0 °C, 12 h, 95%; **d**: $NaHCO_3$/ xylene, reflux, 15 h, 98%; **e**: TFA, CH_2Cl_2, 0 °C- rt, 5 h, **f**: Methyl 2-(acetoxymethyl)acrylate, CH_2Cl_2, rt, 12 h, 80 % (over 2 steps); **g**: Propyl iodide, K_2CO_3, CH_3CN, reflux, 15 h, 70%; **h**: Grubbs' II gen. cat., PTSA, toluene, 80 °C, 14 h, 93%; **i**: H_2, Pd/C, MeOH, 60 psi, 6 h, 87%; **j**: LDA, TMSCl, THF, -40 °C, 2 h, H_3O^+; **k**: $CaCl_2$, NH_3, MeOH, 80 °C, 24 h, 90%; **l**: $PhI(CF_3CO_2)_2$, CH_3CN-H_2O, rt, 12 h, 82%; **m**: Diethylsulfamoyl chloride, EtN_3, $CHCl_3$, 50 °C, 12 h, 71%; **n**: $AlCl_3$/EtSH, CH_2Cl_2, rt, 12 h, 66%.

Key points of synthesis

1) Beginning with *meta*-hydroxybenzaldehyde as the starting material, this synthesis was carried out using the ring closing metathesis (RCM) method.
2) In this report Grubbs II generation catalyst was used for ring closing. Metathesis which shows specific conversion of 23p to the 23q.

G) 7th Report [(±)-Quinagolide][11]



Scheme 7. Reaction conditions: **a**: Reduction; **b**: Mesylation 76%, (over 2 steps); **c**: NaN_3, $PivONH_3OTf$, $Fe(OTf)_2$, MeOH, rt, 16 h, 39%; **d**: Cyclization; **e**: alkylation; **f**: isomer separation 30% (over 2 steps); **g**: ref 9.

Key points of synthesis

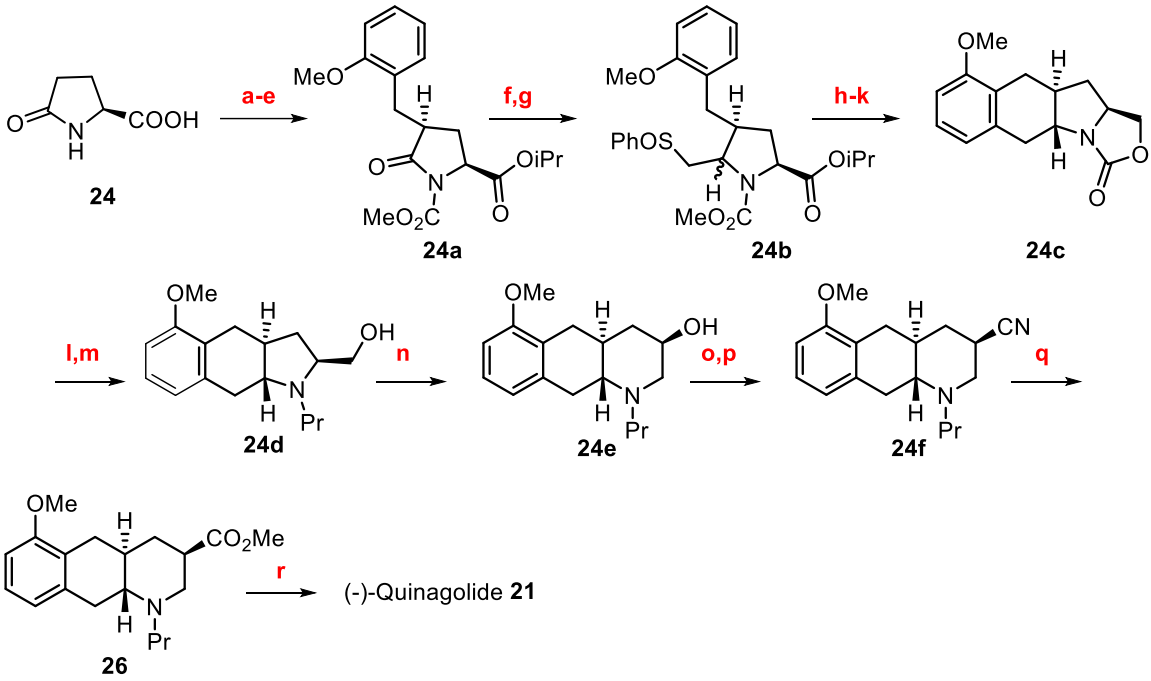
1) Morandi and coworkers recently published an article, on iorn-catalyzed conversion of alkenes to the 2-azidoamines. This approach was used in the formal synthesis of (±)-quinagolide.
2) Using a benign and affordable iron catalyst, offers experimentally easy and stable approach for synthesis of 2-azidoamines selectively in mild resction conditions functionalized trans-fused tetrahydropyridine-3-carboxylates. This strategy yields azido amine intermediate after eight steps only.

H) 8th Report [(-)-Quinagolide][8]

Key points of synthesis

1) This is the second advanced synthesis of (-)-quinagolide using from Chavan's group L-pyroglutamic acid as a starting material.

2) To obtain functionalized prolinate, stereospecific catalytic hydrogenation and diastereoselective Horner-Emmons-Michael cascade were used.
3) Pummerer cyclization with Lewis acid was employed to make a tricyclic fused ring structure.
4) To reach the necessary 3-substituted piperidine scaffold, a thermodynamically preferred diasteoselective ring-expansion through bicyclic aziridinium intermediate was used.



Scheme 8. Reaction conditions: **a**: *p*-TSA, isopropanol, reflux, 6 h, 96%; **b**: methyl chloroformate, Et_3N, Et_2O: DMF (3:1), 0 ^oC, 24 h, 85%; **c**: *o*- Anisaldehyde, Li-HMDS, BF_3:Et_2O, THF, -78 ^oC, 1 h, 75%; **d**: MsCl, Et_3N, CH_2Cl_2, rt, 48 h, 77%; **e**: Pd/C, H_2 (1 atm), EtOAc, 6 h, 82%; **e**: DIBAL-H, THF, -78 ^oC, 1h, 95%; **f**: NaH, DMF, 0 ^oC – rt, 12 h, 85%; **g**: diethyl ((phenylsulfinyl)methyl)phosphonate; **h**: NaOAc, $(Ac)_2O$, 100 ^oC, 1 h or $SOCl_2$, CH_2Cl_2 0 ^oC, 1 h; **i**: $LiBH_4$, THF, rt, 48 h, **j**: Raney-Ni, THF, rt, 30 min; **k**: KOH, methanol, reflux, 1 h, **l**: propyl iodide, K_2CO_3, acetonitrile, 0 ^oC-rt, 3 h, 45 % **m**: TFAA, Et_3N, THF, - 78 ^oC, 1.5 h then 120 ^oC, sealed tube, 24 h, then 5 M NaOH soln., rt, 1 h, 87%; **n**: MsCl, Et_3N, CH_2Cl_2, 0 ^oC, 1h; **o**: NaCN, DMF, 65 ^oC, 1h, 82% (over 2 steps); **p**: HCl, methanol, reflux, 24 h, 90%; **q**: ref 4

3.1.3. Conclusion and Prospect

Because of the *trans*-fused 3-substituted piperidine scaffold, the synthesis of ergot alkaloids is difficult for synthetic chemists. Due to its difficult structural features and therapeutic value, quinagolide is an appealing goal in this sense. Nordmann *et al*. were the first to mention quinagolide in racemic form, and it is prescribed to get good dopaminomimetic activity but biological evaluations found that the (-) enantiomer of quinagolide, which has the absolute configuration (3S,4aS,10aR) comparable to the absolute configuration of ergoline CQ32-084, was found to be completely responsible for the dopaminomimetic activity. this group reported two synthetic routes towards (-)-quinagolide synthesis, but work on higher yielding and shorter synthetic strategy were needed to keep medicine costs down.

3.1.4. References

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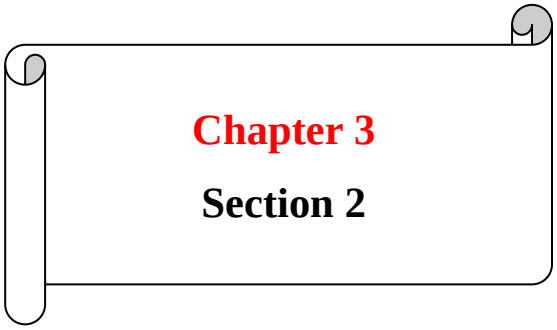


"Advanced synthesis of (±)-quinagolide"

Part A

Studies towards the total synthesis of (-)-quinagolide

3.2.1. Present work

3.2.1.1. Objective

Quinagolide is a dopamine D2 receptor selective agonist.[1] It is prescribed for the treatment of idiopathic hyperprolactinemia and pituitary tumors that secrete prolactin. It is sold under the brand name NORPROLAC® and is formulated and used as a hydrochloride salt. Quinagolide is a racemate, and Nordmann and co-workers[1] found that the (-) enantiomer is responsible for the majority of the drug's clinical action.[2]

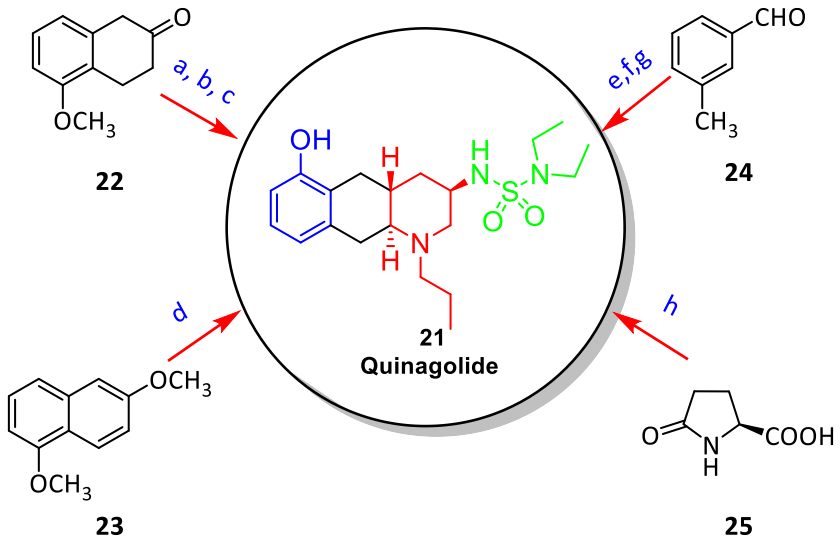


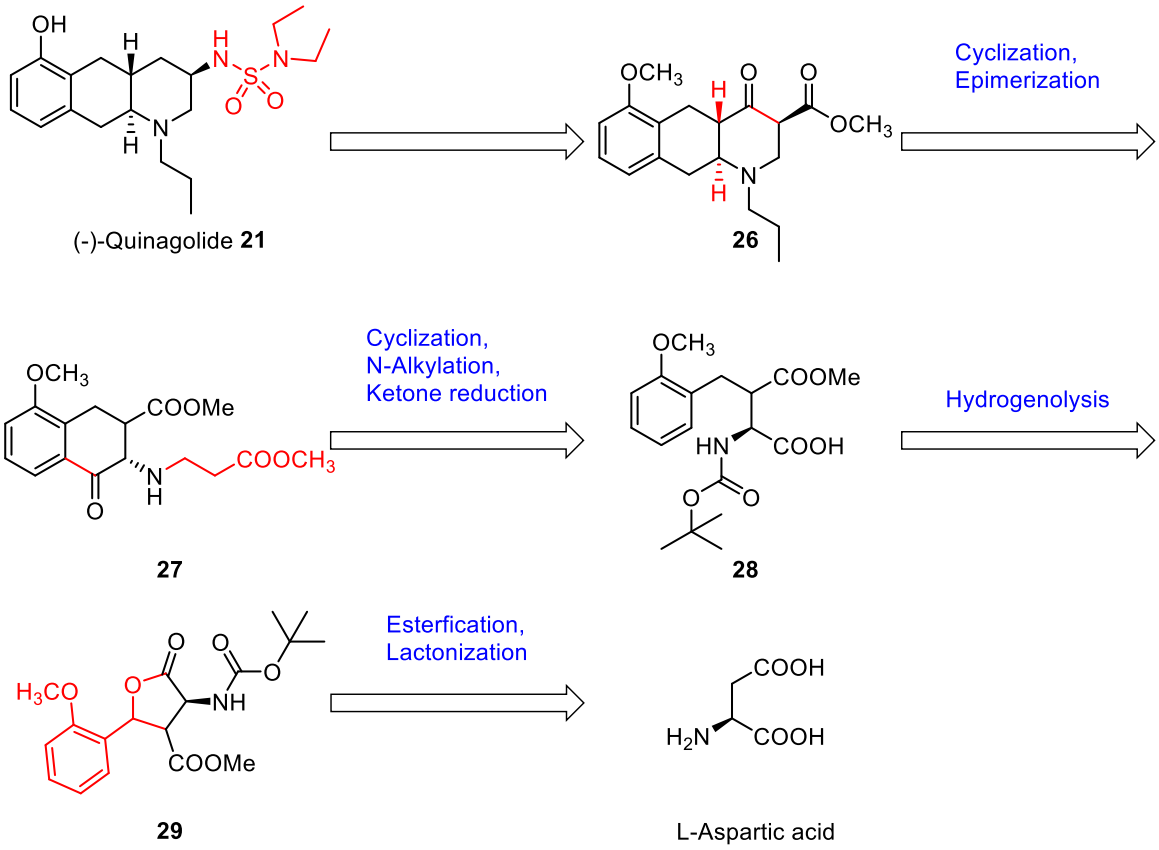
Figure 1. Reported synthesis from starting materials **22, 23, 24** and **25** a) Nordmann *et al.*[1] *J. Med. Chem.* **1985,** *24,* 367. b) Nordmann *et al.*[2] *J. Med. Chem.* **1985,** *28,* 1540. c) Morandi *et al.*[3] *J. Am. Chem. Soc.* **2020,** *51,* 21548. d) Banziger *et al*[4]. *Org. Process. Res. Dev.* **2000,** *4,* 460. e) Chavan *et al.*[5] *Org. Lett.* **2018,** *20,* 7011. f) Chavan *et al.*[6] *ACS Omega* **2019,** *4,* 8231. g) Chavan *et al.*[7] *Org. Lett.* **2019,** *21,* 9089. h) Chavan *et al.*[8] *J. Org. Chem.* 2021, 86, 14, 9344–9352.

Banziger *et al.*[4] published a scalable synthesis of quinagolide intermediate in year 2000 and this was in same line of Nordmann *et al.*[1,2] approach. There have been eight synthetic methods published till date, two of which are chiral. Due to continuous research in the synthesis of biologically active compounds, additional study on improved enantioselective synthesis of (-)-

quinagolide was required. In this regard, the finding of a new enantioselective synthetic route for (-)-quinagolide, as well as a racemic approach to (±)-quinagolide, that leads to a shorter synthesis and higher yield is extremely appealing. The current research outlines a practical and efficient approach for synthesizing (-)-quinagolide from L-aspartic acid, a low-cost and readily available starting material. In addition, it was attempted to synthesize racemic quinagolide by using low-cost and readily available starting material.

3.2.1.2. Retrosynthetic analysis

The key characteristics of (-)-quinagolide synthesis are depicted in Scheme 1.

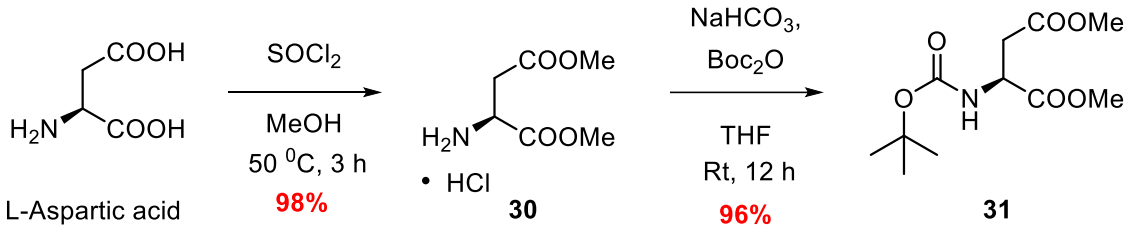


Scheme 1. Retrosynthetic analysis for (–)-quinagolide **21**

(-)-Quinagolide **21** could possibly be synthesized from **26** by functional groups transformations. Compound **26** might possibly be synthesized from compound **27** by cyclization and epimerization. Compound **27** could be achieved from compound **28** by an acid-catalyzed cyclization and N-alkylation and ketone reduction reaction. A one-step N-alkylation and cyclization, which is a condensation reaction catalyzed by an excess base and methyl or ethyl

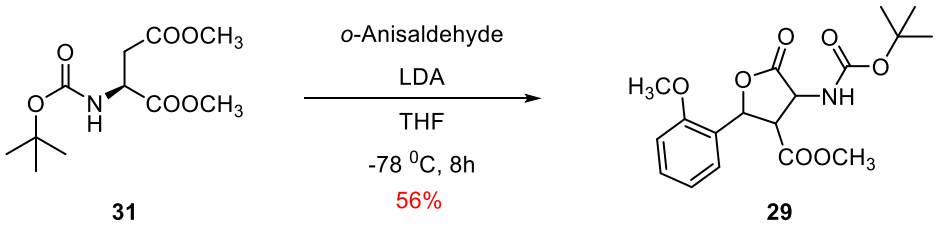
acrylate, is another possibility. To achive compound **28**, the hydrogenolysis reaction could be used to open lactone compound **29**. Compound **29** could be synthesized from commercially available chiral starting materials L-aspartic acid and *o*-anisaldehyde.

3.2.1.3. Results and discussion



Scheme 2. Synthesis of compound **31**

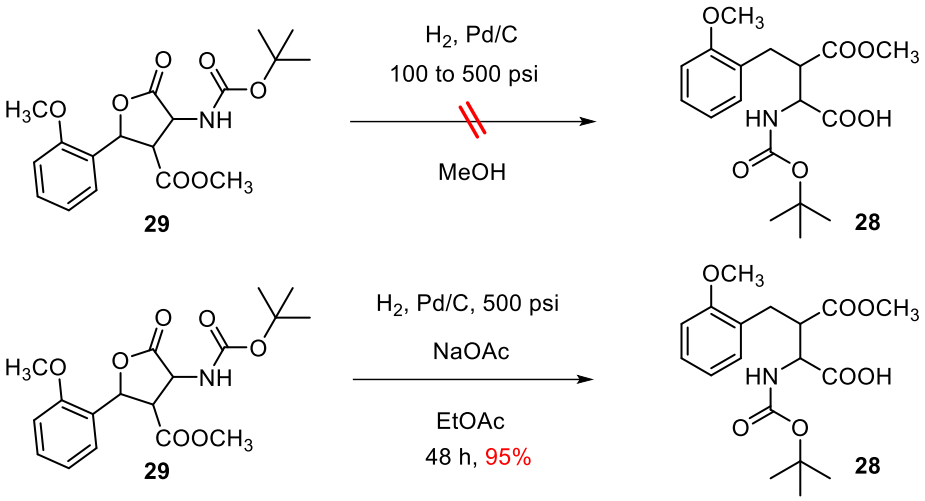
The asymmetric synthesis of quinagolide began with the use of inexpensive and commercially available L-aspartic acid as the starting material, as per the retrosynthetic scheme. In this reaction L-aspartic acid was heated at 50 °C in presence of thionyl chloride and methanol as a solvent to give dimethyl L-aspartate hydrochloride salt **30** in 98% yield. Spectral and optical data results of compound **30** matched well with the reported data.[9] Crude dimethyl L-aspartate hydrochloride salt **30** was the treated with sodium bicarbonate, di-tert-butyl dicarbonate and THF as a solvent to give dimethyl (tert-butoxycarbonyl)-L-aspartate **31** in 95% yield. Synthesized compounds was confirmed with ^{1}H and ^{13}C NMR spectroscopy whereas, functional group conversion was analyzed by IR spectroscopy.



Scheme 3. Synthesis of compound **29**

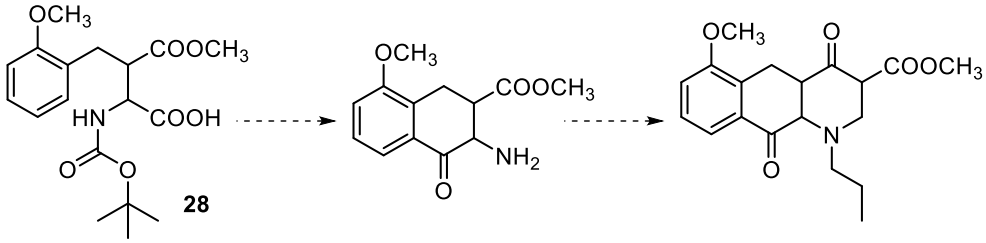
Lactone compound **29** (methyl (4S)-4-((tert-butoxycarbonyl)amino)-2-(2-methoxyphenyl)-5-oxotetrahydrofuran-3-carboxylate) was obtained by reacting compound **31** with commercially available *o*-anisaldehyde at -78 °C for eight hours in the presence of LDA in dry THF in 56% of yield. Compound **29** was separated on column chromatography and first confirmed with ^{1}H NMR, where characteristic peaks for one ester group of cyclized lactone was observed and

protons of cyclized lactone was clearly observed at 2.84 (1H), 3.1 (1H) and 4.49-4.64 (1H), which indicate the successful cyclization of compound **31** to compound **29**. Stereochemistry at 3 three stereo centers of prepared lactone was not confirmed yet.



Scheme 4. Synthesis of compound **28**

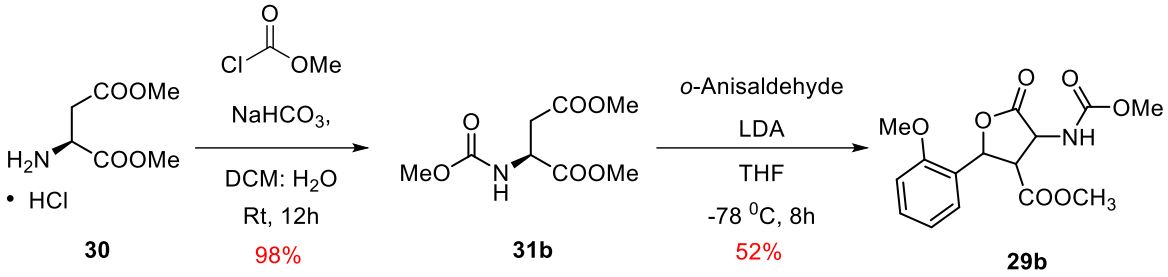
In scheme 4, attempts was made to open the lacone ring using a hydrogenation reaction with H_2, Pd/C, and methanol as the solvent **(Scheme 3)**. H_2 pressure was increased from 100 to 500 psi in Parr reactor but the expected result was not obtained and the starting material was recovered. After that, next attempt was to synthesize compound **28** by reductive cleavage of the benzylic C-O bond in presence of H_2 at 500 psi pressure, in presence of Pd/C, sodium acetate and ethyl acetate as a solvent which successfully afforded acid compound **28** in 95% yield. Then synthesized compound were analyzed by spectroscopic techniques.



Scheme 5. Proposed synthesis of tricyclic core intermediate

The synthesis of tricyclic core intermediate continued further according to retrosynthetic analysis. After successful synthesis of compound **28,** attempts were made to convert into tetralone using acid catalysed cyclization reaction but the cyclized product was not obtained even after several trials. As per literature[7] similar types of cyclization can be achieved in presence of

methyl carbamate as protecting group. The failure in the present work (Scheme 4) compound **28** may be due to Boc protecting group in which is not favorable/stable for Friedel–Crafts cyclization. It was felt that the cyclization can be achieved if Boc protecting group is replaced with methyl carbamate. As a result, a new synthetic route was chosen as shown in Scheme 5.



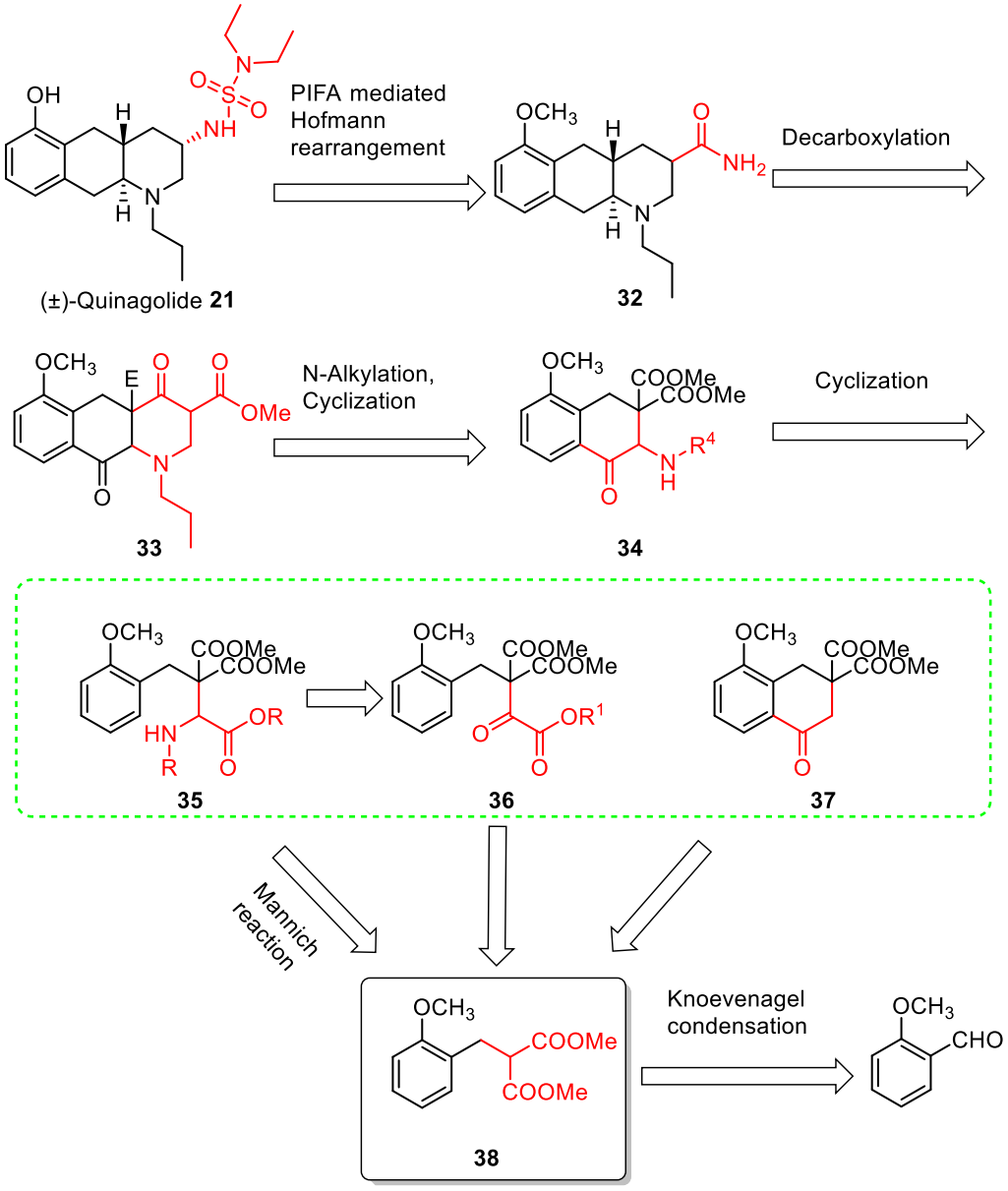
Scheme 6. Synthesis of compound **29b**

Similar to the reaction sequences seen in Scheme 2 and 3, crude dimethyl L-aspartate hydrochloride salt **30** was treated with sodium bicarbonate, methyl chloroformate and DCM, Water (1:1) as a solvent to give dimethyl (methoxycarbonyl)-L-aspartate **31b** in 95% yield. Lactone compound **29b** was obtained by reacting compound **31** with commercially available *o*-anisaldehyde at -78 °C for eight hours in the presence of LDA and solvent dry THF in 52% of yield. Prepared lactone **29b** was further characterized by HRMS, NMR spectroscopy and functional groups characterized by IR spectroscopy. Due to resent COVID-19 pandemic crises and imposed strict lockdown in lab/city which interrupted research activity hence due to paucity of time the meaningful completion of the quinagolide could not be achieved. However by proper choice of reagents and conditions the advanced intermediate could be converted to quinagolide.

Part B

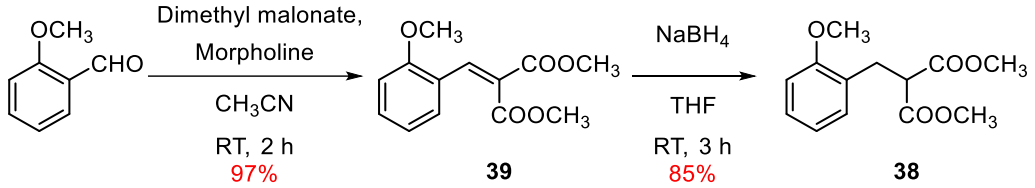
Studies directed towards the total synthesis of (±)-quinagolide

3.2.1.4. Retrosynthetic analysis



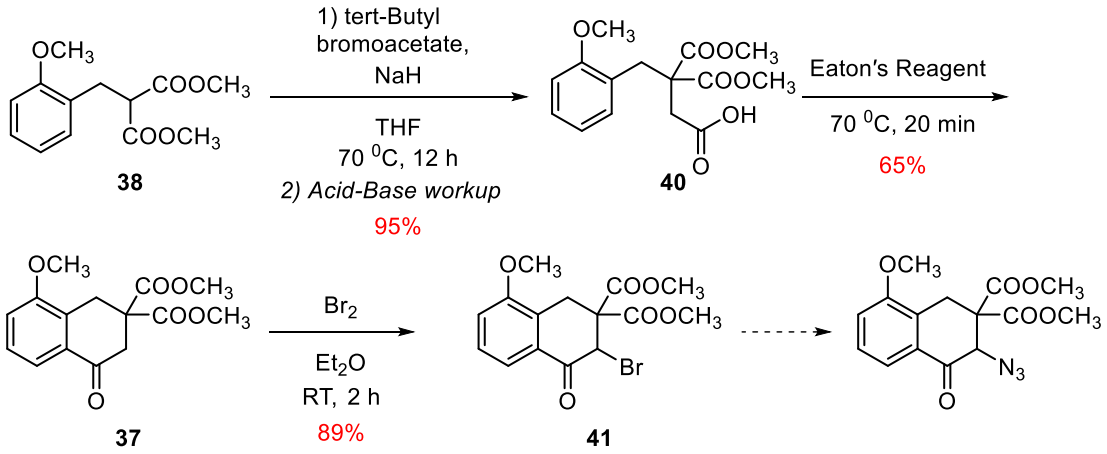
Scheme 7. Retrosynthetic analysis for the synthesis of (±)-quinagolide **21**

This part includes studies directed towards on racemic quinagolide synthesis utilizing commercially accessible, low-cost starting materials. Attempted trials are described below.



Scheme 8. Synthesis of compound **38**

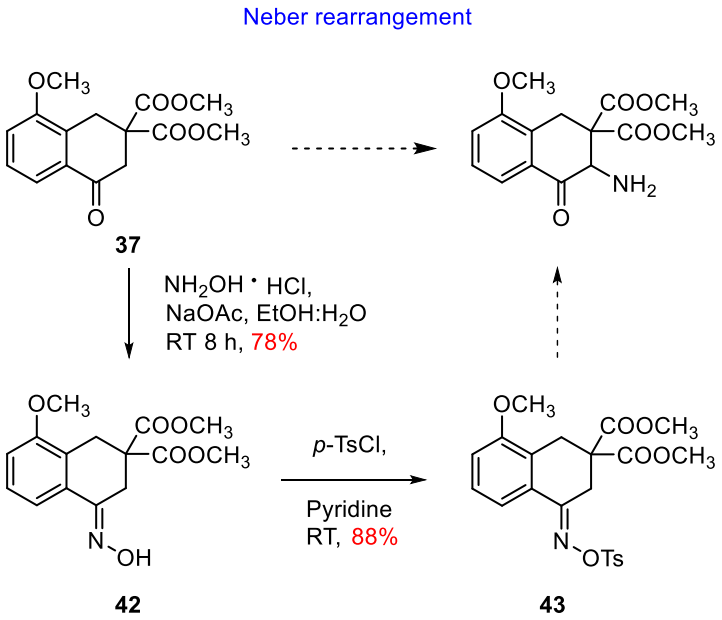
The synthesis of quinagolide began with the use of inexpensive and commercially available *o*-anisaldehyde as the starting material, as proposed in the retrosynthetic scheme. In this reaction *o*-anisaldehyde and dimethyl malonate were heated at 80 °C in presence of morpholine as a base and acetonitrile solvent to give dimethyl 2-(2-methoxybenzylidene)malonate **39** as yellow viscous compound in 97% yield. The spectral data of compound **39** matched well with the reported data. Crude dimethyl 2-(2-methoxybenzylidene)malonate **39** was treated with sodium borohydride in THF to give dimethyl 2-(2-methoxybenzyl)malonate **38** in 85% yield. Finally all compounds molecular structure and functional moieties were confirmed by NMR and IR spectroscopy respectively.



Scheme 9. Synthesis of compound **41**

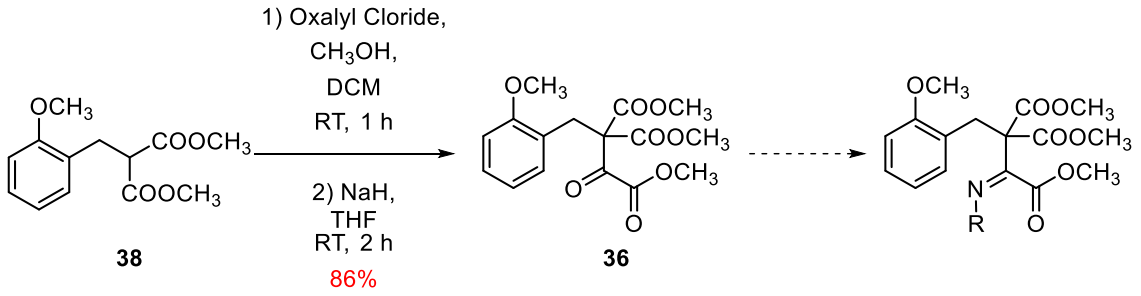
Diester compound **38** was successfully synthesized in high yield, and this compound was utilized to make diester α- tetralone compound **37** on gram scale. Diester compound **38** was treated with sodium hydride in dry THF and t-butyl bromoacetate for C-alkylation of active methlne. After quenching the reaction and work up, the intermediate C-alkylated product was obtained in

quantitative yield and was directly used for deprotection of tert-butyl group in acidic condition, yielding acid **40**. Acid compound **40** was treated with commercially available Eaton's reagent (10 wt% phosphorus pentoxide solution in methanesulfonic acid) to give diester α- tetralone **37** on gram scale. Compound **37** was then brominated by treating it with bromine in diethyl ether to give compound **41.** After separation and purification, prepared molecules were analyzed with ^{1}H and ^{13}C NMR spectroscopy. This compound was attempted to convert the bromo substituents in to azide functionality, however it did not work.



Scheme 10. Oxime synthesis

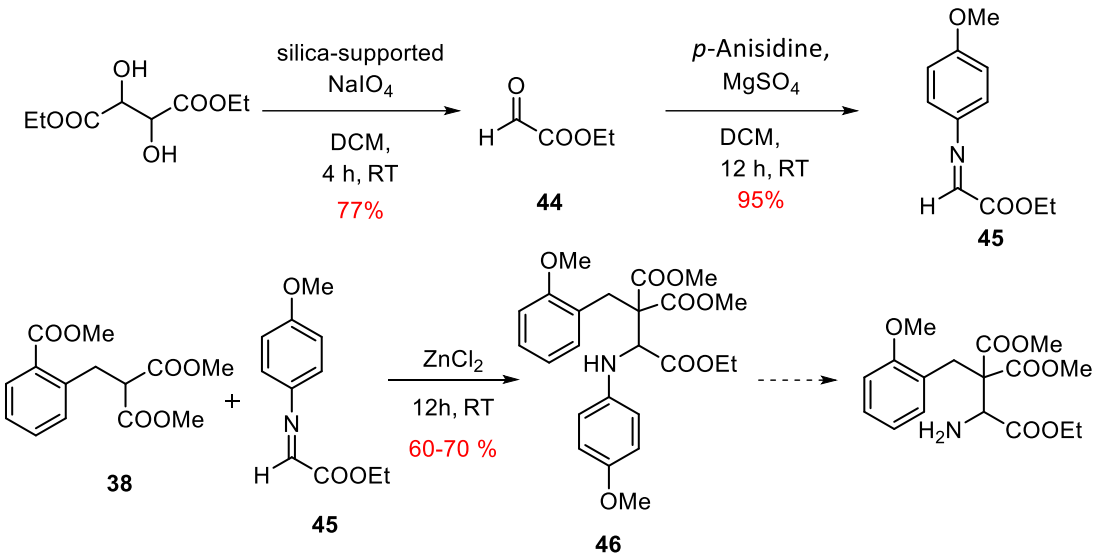
After a series of unsuccessful attempts to synthesize azide, the next choice was Neber rearrangement utilizing the oxime synthesis reaction. Compound **37** was subjected to Neber rearrangement[10] reaction followed by oxime synthesis treating with hydroxylammonium chloride, sodium acetate and as a solvent, a mixture of ethanol and water was used. First one step Neber rearrangement with reported known reaction sequence[10] but it failed to give the target compound. As a result, another synthetic approach was adopted, (**Scheme 8**) where in the oxime compound **42** was obtained successfully. Then the hydroxy group was protected with p-toluenesulfonyl chloride to give intermediate compound **43**. Compound **43** was further treated with different types of bases but did not produce a rearrangement product. Probably the diester group's steric hindrance inhibits the chance of a successful Neber rearrangement.



Scheme 11. Synthesis of compound **36**

Another possibility of constructing a C-N bond in this reactions sequence was the imine synthesis. Diester compound **38** could be converted in to tri-carboxylates compound **36** in a single step. In typical synthesis methyl 2-chloro-2-oxoacetate was made by reacting a stoichiometric ratio of methanol with oxalyl chloride in the presence of dry DCM as a solvent. Subsequently prepared methyl 2-chloro-2-oxoacetate was reacted with diester compound **38** in presence of sodium hydride in dry THF, which gives compound **36** by nucleophilic substitution reaction in one step. Synthesized compound **36** was characterized with ^{1}H NMR and ^{13}C NMR. But compound **36** was not so stable at room temperature and in column chromatography also. Hence, this partially purified compound **36** was reacted with several amines such as p-anisidine, benzyl amine, propylamine, and ammonia in presence of $MgSO_4$ and dry DCM as solvent to get expected imine. But in all trials, the starting material was not stable and amines remained unreacted throughout the reaction time, which was monitored by TLC. Hence this imine synthesis strategy didn't work due to the stability issue of compound **36**.

Hence an alternative approach, was attempted where diethyl tartrate was utilized as the starting material, which was oxidatively cleaved using $NaIO_4$ to give compound **44** following a reported method.[11] Crude aldehyde compound **44** was directly used for imine synthesis with *p*-anisidine to give compound **45** which was directly used for Mannich reaction with diester compound **38** in presence of zinc chloride to give compound **46** which was characterized by LCMS and ^{1}H NMR spectroscopy. Several attempts were made to purify compound **46** but some impurities were observed in it, including p-anisidine. Partially purified compound **46** was subjected to deprotection of the amine nitrogen protecting p-methoxyphenyl (PMP) under several reaction conditions but the reaction did not work.



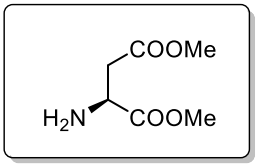
Scheme 12. Synthesis of compound **45**

Due to resent COVID-19 pandemic crises and imposed strict lockdown in lab/city which interrupted research activity hence due to paucity of time the meaningful completion of the quinagolide could not be achieved. However by proper choice of reagents and conditions the advanced intermediate could be converted to quinagolide.

3.2.2. Experimental

3.2.2.1. Synthesis and spectral data

30: Dimethyl L-aspartate



To a suspension of L-aspartic acid (5.0 g, 38 mmol) in methanol (100 mL) at 0 °C was added dropwise thionyl chloride (3.8 mL, 53 mmol) under argon atmosphere. The reaction mixture was allowed to warm to room temperature and then heated under reflux for 3 h. The solution was allowed to cool to room temperature and then concentrated in vacuum, azeotroping with dichloromethane to give dimethyl (2S)-2-aminobutandioate hydrochloride as a white solid (5.9 g, 98%).

Yield: 98 %.

Sp. Rotation: $[\alpha]^D$ +14.8880 (*c* = 1.0, MeOH);

Melting point: 116 ºC.

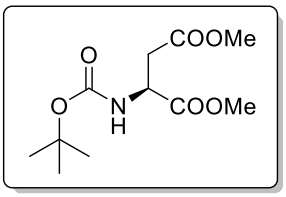
^{1}H NMR (400 MHz, DMSO-d6): δ 2.95-3.09 (m, 2H), 3.64 (s, 3H), 3.72 (s, 3H), 4.31 (t, *J* = 6Hz, 1H), 8.78 (bs, 2H).

^{13}C NMR (100 MHz, DMSO-d6): δ 34.08, 48.53, 52.27, 53.16, 168.75, 169.70.

IR ($CHCl_3$): 1740 (b), 3439 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_6H_{12}NO_4$ $[M+H]^+$: 162.0761, found: 162.0756.

31a: Dimethyl (tert-butoxycarbonyl)-L-aspartate



A flame-dried flask equipped with a magnetic stirrer bar was charged with dry CH_2Cl_2 (30 mL) and the dimethylester (5 g, 0.031 mmol, 1 equiv). Et_3N (8.7 mL, 0.062 mmol, 2 equiv) and di-tert-butyldicarbonate (7.1 mL, 0.031 mmol, 1 equiv) were added over 15 min each with a syringe pump at 0 °C and the reaction was stirred at room temperature for 10 h. The mixture was washed with sat. aq. Na_2CO_3 (100 mL) and the separated aqueous layer was extracted twice with CH_2Cl_2 (2 x 60 mL). The combined organic phases were dried over $NaSO_4$, filtered, and concentrated in vacuo to afford the N-Boc-protected dimethylester white solid which was purified by column chromatography. Yield: (7.7 g, 95%)

Yield: 95 %.

Sp. Rotation: $[\alpha]^D$ +32.7920 (*c* = 1.0, $CHCl_3$);

Melting point: 62 ºC.

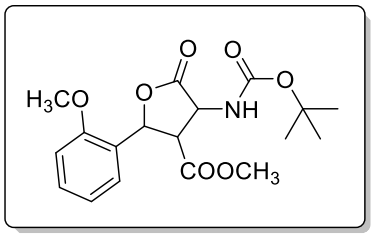
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.84 (dd, *J*= 15Hz, 1H), 3.1 (dd, *J*= 15Hz, 1H), 3.68 (s, 3H), 3.74 (s, 3H), 4.49-4.64 (m, 1H), 5.50 (d, *J*= 8Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.22 (3C), 36.59, 49.85, 51.94, 52.64, 80.08, 155.30, 171.35, 171.48.

IR ($CHCl_3$): 1715 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{11}H_{19}NNaO_6$ $[M+H]^+$: 284.1105, found: 284.1096.

29a: methyl 4-((tert-butoxycarbonyl)amino)-2-(2-methoxyphenyl)-5-oxotetrahydrofuran-3-carboxylate



LDA was prepared by adding BuLi (15 mL, 0.038 mol 2.5 M in hexane) to a solution of diisopropylamine (5.40 mL, 0.038 mol) in 10 mL of dry THF under nitrogen atmosphere, at –78 °C. The solution was stirred 15 min at 0 °C and then cooled again to –78 °C. A solution of Dimethyl (tert-butoxycarbonyl)-L-aspartate (**31**) (5 g, 0.019 mol) in THF (10 mL) was dropwise added to the LDA solution and the mixture was stirred for 30 min at –78 °C. *o*-Anisaldehyde (2.60 g, 0.019 mol) in THF (10 mL) was dropwise added to the reaction mixture. The reaction mixture was stirred for 8 hours, reaction monitored by TLC. After consumed starting material temperature was allowed to rise to 0 °C and quenched with saturated aquous NH4Cl solution. The aqueous phase was extracted with ethyl acetate (3x50 mL), the combined organic phases were dried (NaSO4) and concentrated. By column chromatography, the residue was purified. Yield: (3.9 g, 56%)

Yield: 40 %.

Melting point: 106 ºC.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.84 (dd, *J*= 15Hz, 1H), 3.1 (dd, *J*= 15Hz, 1H), 3.68 (s, 3H), 3.74 (s, 3H), 4.49-4.64 (m, 1H), 5.50 (d, *J*= 8Hz, 1H).

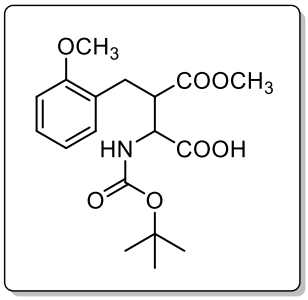
^{13}C NMR (100 MHz, $CDCl_3$): δ 28.20 (3C), 50.06, 51.88, 53.24, 55.26 (2C), 81.00, 110.80, 120.44, 123.70, 128.81, 130.44, 155.04, 156.67, 169.72, 173.78.

IR ($CHCl_3$): 1798, 1715 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{18}H_{24}NO_7$ $[M+H]^+$: 366.1547, found: 366.1535; $C_{18}H_{23}NNaO_7$ $[M+Na]^+$: 388.1367, found: 388.1356.

28: 2-((tert-butoxycarbonyl)amino)-4-methoxy-3-(2-methoxybenzyl)-4-oxobutanoic acid

To a solution of **29** (200 mg, 0.547 mmol) in EtOAc (5.0 mL) were added 10% Pd-C (100 mg) and sodium acetate (100 mg) under H_2 atmosphere (500 psi) in Parr reactor at room temperature. The mixture was stirred for 48 h, filtered through a pad of celite, washed with EtOAc, and concentrated in vacuum to give pure 2-((tert-butoxycarbonyl)amino)-4-methoxy-3-(2-methoxybenzyl)-4-oxobutanoic acid (**28**) (191 mg, 95%) as a colorless sticky compound.



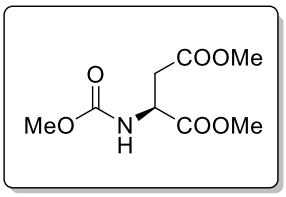
Yield: 98 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H), 3.12 (d, *J*= 16Hz, 1H), 3.27 (d, *J*= 13Hz, 1H), 3.45 (d, *J*= 13Hz, 1H), 3.61 (d, *J*= 16Hz, 1H), 3.70 (s, 3H), 3.78 (s, 3H), 5.82 (bs, 1H), 6.79-6.91 (m, 2H), 7.11 (d, *J*= 7Hz, 1H) 7.19-726 (m, 1H).

IR ($CHCl_3$): 1715 (b), 3582 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{18}H_{26}NO_7$ $[M+H]^+$: 368.1704, found: 368.1694; $C_{18}H_{25}NNaO_7$ $[M+Na]^+$: 390.1523, found: 390.1512.

31b: Dimethyl (methoxycarbonyl)-L-aspartate



A flame-dried flask equipped with a magnetic stirrer bar was charged with DCM (50 mL), H_2O (25 mL) and the dimethylester (9 g, 0.055 mol, 1 equiv). Sodium bicarbonate (23 g, 0.275 mol, 5 equiv) and methyl chloroformate (8.51 mL, 0.110 mol, 2 equiv) were added over 15 min each with a syringe at 0 °C and the reaction was stirred at room temperature for 12 h. The mixture was washed with sat. aq. Na_2CO_3 (100 mL) and the separated aqueous layer was extracted twice with CH_2Cl_2 (2 x 60 mL). The combined organic phases were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuo to afford the *N*-Methylcarbamate dimethylester colorless oily product which was purified by column chromatography. Yield: (12 g, 98%)

Yield: 95 %.

Sp. Rotation: $[\alpha]^D$ +49.1840 (*c* = 1.0, $CHCl_3$);

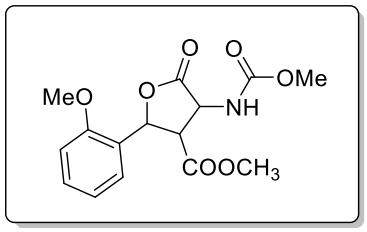
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.83 (dd, *J*= 15Hz, 1H), 2.99 (dd, *J*= 15Hz, 1H), 3.67 (s, 6H), 3.74 (s, 3H), 4.50-4.68 (m, 1H), 5.73 (d, *J*= 8Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 36.38, 50.23, 51.95, 52.35, 52.72, 156.46, 171.13, 171.21.

IR ($CHCl_3$): 1714 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_8H_{14}NO_6$ $[M+H]^+$: 220.0816, found: 220.0810.

29b: Methyl 4-((methoxycarbonyl)amino)-2-(2-methoxyphenyl)-5-oxotetrahydrofuran-3-carboxylate



LDA was prepared by adding BuLi (18 mL, 0.045 mol 2.5 M in hexane) to a solution of diisopropylamine (6.30 mL, 0.045 mol) in 10 mL of dry THF under nitrogen atmosphere, at –78 °C. The solution was stirred 15 min at 0 °C and then cooled again to –78 °C. A solution of dimethyl (methoxycarbonyl)-L-aspartate (**31b**) (5 g, 0.022 mol) in THF (10 mL) was dropwise added to the LDA solution and the mixture was stirred for 30 min at –78 °C. *o*-Anisaldehyde (3.10 g, 0.022 mol) in THF (10 mL) was dropwise added to the reaction mixture. The reaction mixture was stirred for 8 hours, reaction monitored by TLC. After consumption of starting material temperature was allowed to rise to 0 °C and the reaction mixture quenched with saturated aqueous NH_4Cl (10 mL) solution. The aqueous phase was extracted with ethyl acetate (3x50 mL), the combined organic phases were dried anhydrous ($NaSO_4$) and concentrated under vacuum. By column chromatography, the residue was purified. Yield: (3.9 g, 52%)

Yield: 52 %.

Melting point: 157 ºC.

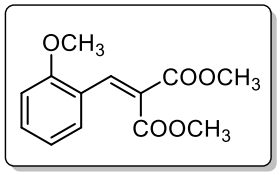
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.21 (s, 3H), 3.72 (s, 3H), 3.88 (s, 3H), 4.10 (t, *J*= 6Hz, 1H), 5.08 (t, *J*= 8Hz, 1H), 5.54 (d, *J*= 8Hz, 1H), 5.90 (d, *J*= 5Hz, 1H), 6.86-6.90 (m, 1H), 6.93-6.98 (m, 1H), 7.28-7.37 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 50.81, 51.80, 52.79, 53.19, 55.46, 74.69, 109.93, 120.53, 122.60, 125.28, 129.71, 155.80, 156.68, 169.06, 172.49.

IR ($CHCl_3$): 1795, 1716 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}NO_7$ $[M+H]^+$: 324.1078, found: 324.1070; $C_{15}H_{17}NNaO_7$ $[M+Na]^+$: 346.0897, found: 346.0887.

39: Dimethyl 2-(2-methoxybenzylidene)malonate



A flame-dried flask equipped with a magnetic stirrer bar was charged with dry acetonitrile (50 ml), dimethyl malonate (11 g, 10 mL, 0.088 mmol, 1.2 equiv) and *o*-anisaldehyde (10 g, 0.073 mmol, 1 equiv).

Morpholine (9.5 mL, 0.110 mmol, 1.5 equiv) were added over 15 min with a syringe at 0 °C and the reaction was stirred at 90 °C for 4 h. The mixture was concentrated in vacuum and EtOAc (100 mL) was added and water (100 mL) and the aqueous layer was extracted twice with EtOAc (2 x 50 mL). The combined organic phases were dried over $NaSO_4$, filtered, and concentrated in vacuo to afford the dimethyl 2-(2-methoxybenzylidene)malonate yellowish semisolid product which was purified by column chromatography. Yield: (17 g, 92%)

Yield: 92 %.

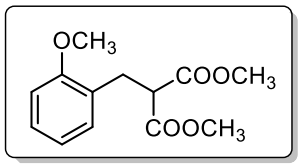
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 6.87-6.96 (m, 2H), 7.31-7.41 (m, 2H), 8.12 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 52.37, 52.47, 55.43, 110.84, 120.52, 122.12, 125.27, 129.00, 132.12, 139.03, 157.99, 164.70, 167.15.

IR ($CHCl_3$): 1735 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{13}H_{15}O_5$ $[M+H]^+$: 251.0914, found: 251.0907; $C_{13}H_{14}NaO_5$ $[M+Na]^+$: 273.0733, found: 273.1725.

38: Dimethyl 2-(2-methoxybenzyl)malonate



To a solution of **39** (10 g, 0.039 mol) in THF (5.0 mL) were added $NaBH_4$ (7.5 g, 0.198 mol) portion wise under nitrogen atmosphere at room temperature. The mixture was stirred for 2.5 h, reaction monitored by TLC and after completion reaction it was quenched by ice, The mixture was concentrated in vacuum and it was added EtOAc (100 mL) and water (100 mL) and the aqueous layer was extracted twice with EtOAc (2 x 50 mL). The combined organic phases were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum to afford the dimethyl 2-(2-methoxybenzyl)malonate as a colorless semisolid product which was purified by column chromatography. Yield: (9.5 g, 94%)

Yield: 94 %.

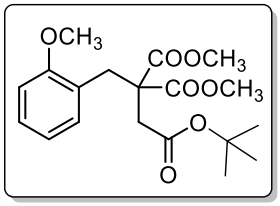
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, *J*= 7Hz, 1H), 3.68 (s, 6H), 3.83 (s, 3H), 3.86 (t, *J*= 7Hz, 1H), 6.80-6.90 (m, 2H), 7.13 (d, *J*= 7Hz, 1H), 7.21 (t, *J*= 7Hz, 1H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 30.23, 51.19, 52.27 (2C), 55.09, 110.06, 120.27, 125.87, 128.16, 130.71, 157.44, 169.51 (2C).

IR ($CHCl_3$): 1735 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{13}H_{17}O_5$ $[M+H]^+$: 253.1071, found: 253.1064; $C_{13}H_{16}NaO_5$ $[M+Na]^+$: 275.0890, found: 275.0881.

40a: 1-(tert-butyl) 2,2-dimethyl 3-(2-methoxyphenyl)propane-1,2,2-tricarboxylate



To a solution of **38** (7 g, 0.027 mmol) in 40 mL dry THF was added NaH (60 % dispersion in mineral oil) (1.33 g, 0.055 mmol) in small amounts and stirred for 2 hr at room temperature. Subsequently, tert-Butyl bromoacetate (6.5 g, 4.90 mL, 0.033 mol) was added and refluxed over night. After cooling, NaH was quenched dropwise addition of saturated solution of ammonium chloride at 0 ^{0}C. Water was added and the mixture extracted three times with EtOAc (50 mL). The combined organic layers were dried over anhydrous $NaSO_4$, filtered and evaporated under reduced pressure. The crude product was obtained as a yellow oil, **40a** (10 g, 98 %), which was used immediately in the next step.

Yield: 98 %.

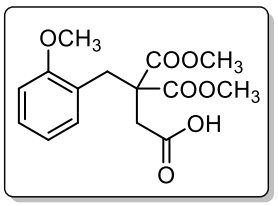
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H), 2.78 (s, 2H), 3.48 (s, 2H), 3.73 (s, 6H), 3.75 (s, 3H), 6.80-6.88 (m, 1H), 6.97-7.03 (m, 1H) 7.18-7.25 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.01 (3C), 32.84, 37.80, 52.45 (2C), 55.09 (2C), 56.09, 80.93, 110.37, 120.35, 124.29, 128.51, 131.72, 157.94, 169.84, 170.84.

IR ($CHCl_3$): 1730 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{19}H_{27}O_7$ $[M+H]^+$: 367.1751, found: 367.1741.

40b: 4-Methoxy-3-(2-methoxybenzyl)-3-(methoxycarbonyl)-4-oxobutanoic acid



To a solution of **40a** (10 g) and 100 mL 2N HCl stirred for 2 hr at room temperature then added EtOAc (100 mL). Then the reaction mixture was basified using $NaHCO_3$ and water. The mixture was extracted three times with EtOAc (50 mL). The combined aqueous layer was acidified with HCl and again the mixture extracted three times with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous $NaSO_4$, filtered and evaporated under reduced pressure. The crude product was obtained as a white solid, **40b** (8.3 g, 98 %)

Yield: 98 %.

Melting point: 145 ºC.

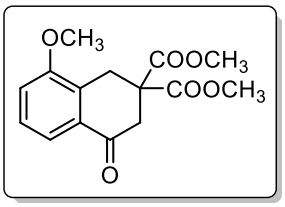
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.93 (s, 2H), 3.51 (s, 2H), 3.74 (s, 3H), 3.75 (s, 6H), 6.82-6.89 (m, 2H), 6.93-6.99 (m, 1H), 7.19-7.26 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 32.30, 36.06, 52.72 (2C), 54.76, 56.11, 110.24, 120.50, 123.81, 128.70, 131.32, 157.76, 170.42 (2C), 177.09.

IR ($CHCl_3$): 1725 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{19}O_7$ $[M+H]^+$: 311.1125, found: 311.1116.

37: Dimethyl 8-methoxy-4-oxo-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate



Eaton's reagent (5.75 g, 0.024 mol) was slowly added to **40b** (5 g, 0.016 mol) and stirred at 80 °C for about 2h under nitrogen atmosphere. Completion of reaction was monitored by TLC. After the completion of reaction, reaction mixture was slowly added to cold saturated solution of sodium bicarbonate and precipitated gummy residue was extracted three times with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous $NaSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography. Yield: (3 g, 63%).

Yield: 63 %.

Melting point: 135 ºC.

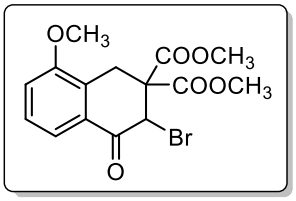
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.09 (s, 2H), 3.50 (s, 2H), 3.71 (s, 6H), 3.87 (s, 3H), 7.05 (d, *J*= 8Hz, 1H), 7.29 (t, *J*= 8Hz, 1H), 7.62 (d, *J*= 8Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.51, 43.13, 53.11 (2C), 54.86, 55.77, 115.08, 118.52, 127.56, 128.28, 132.10, 156.85, 170.40 (2C), 193.85.

IR ($CHCl_3$): 1688, 1733 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{17}O_6$ $[M+H]^+$: 293.1020, found: 293.1016.

41: Dimethyl 3-bromo-8-methoxy-4-oxo-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate



To a solution of the ketone **41** (1 g, 0.003 mol) in Et_2O (20 mL), Br_2 (0.270 g, 87 µL, 0.003 mol) was added dropwise whilst cooling in an ice bath. To complete the reaction, the mixture was stirred for an additional 15 min, then poured onto distilled water (50 mL) and the organic phase was separated, washed with 5% $Na_2S_2O_3$, and once more with distilled water. After drying over anhydrous $NaSO_4$, filtered and evaporation of the solvent, the corresponding bromoketone was obtained almost quantitatively. The resulting products were purified by column chromatography. Yield: (1.1 g, 86 %).

Yield: 86 %.

Melting point: 130 ºC.

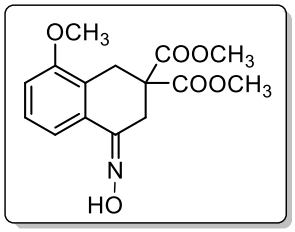
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.46 (d, *J*= 18Hz, 1H), 3.63 (s, 3H), 3.75 (d, *J*= 18Hz, 1H), 3.86 (s, 3H), 3.89 (s, 3H), 5.08 (s, 1H), 7.08 (d, *J*= 8Hz, 1H), 7.34 (t, *J*= 8Hz, 1H), 7.70 (d, *J*= 8Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 25.14, 48.27, 53.50, 53.63, 55.74, 59.49, 115.38, 119.82, 126.55, 128.02, 129.20, 156.76, 167.32, 168.41, 187.93

IR ($CHCl_3$): 1680, 1735 (b) cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{16}BrO_6$ $[M+H]^+$: 371.0125, found: 371.0121.

42: Dimethyl 4-(hydroxyimino)-8-methoxy-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate



A suspension of dimethyl 8-methoxy-4-oxo-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate **37** (1 g, 0.003 mol), hydroxylamine hydrochloride (0.475 g, 0.006 mol), and sodium acetate (0.560 g, 0.006 mol) in ethanol (15 mL) and water (5 mL) was added and the mixture was stirred for 8 h. The progress of the reaction was monitored by TLC. After the reaction was complete, the reaction mixture was concentrated in vacuo. The residue was diluted with EtOAc (50 mL) and water 50 mL. The organic layer was separated and washed with additional water 50 mL. The combined organic layer were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum to afford the crude solid oxime **42** (1.02 g, 97 %). 1. Yeild: 97 %.

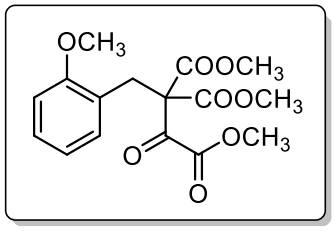
Yield: 97 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.34 (s, 4H), 3.70 (s, 6H), 3.85 (s, 3H), 6.85 (d, *J*= 8Hz, 1H), 7.18 (t, *J*= 8Hz, 1H), 7.47 (d, *J*= 8Hz, 1H), 8.81 (bs, 1H).

IR ($CHCl_3$): 903, 1457, 1717 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}NO_6$ $[M+H]^+$: 308.1129, found: 308.1127.

36: Trimethyl 3-(2-methoxyphenyl)-1-oxopropane-1,2,2-tricarboxylate



Solution of **38** (5 g, 0.019 mmol) in 20 mL dry THF was added NaH (60 % dispersion in mineral oil) (1.42 g, 0.059 mmol) round-bottomed flask and stirred it for 2 hr at 0 ^{0}C under nitrogen atmosphere. Subsequently, in another round-bottomed flask dry

DCM (10 mL), oxalyl chloride (3.77 g, 2.5 mL, 0.029 mol) was added. Reaction temperature was maintained at 0 ^{0}C then added CH_3OH (1.20 mL, 0.029 mol) dropwise. Reaction was stirred for 15 min. The reaction mixture from the second round-bottomed flask was added to the first round-bottomed flask drop wise and stirred it for additional 1 h at 0 ^{0} C. Water was added and the mixture extracted three times with DCM (3 x 100 mL). The combined organic layers were dried over anhydrous $NaSO_4$, filtered and evaporated under reduced pressure. The crude product was obtained as yellow oil, **36** (5.5 g, 82 %).

Yield: 82 %.

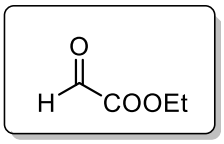
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65 (s, 2H), 3.70 (s, 3H), 3.71 (s, 6H), 3.87 (s, 3H), 6.79 (d, *J*= 7Hz, 1H), 6.85-6.90 (m, 1H), 7.11-7.15 (m, 1H), 7.20-7.26 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 33.37, 52.97 (2C), 67.21, 53.23, 54.79, 109.96, 120.44, 123.12, 128.89, 131.86, 157.55, 160.08, 166.16 (2C), 183.16.

IR ($CHCl_3$): 1733 (b) cm^{-1}.

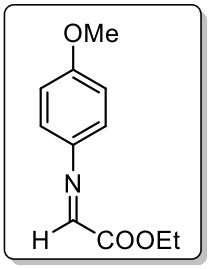
HRMS (ESI) *m/z* calculated for $C_{16}H_{19}O_8$ $[M+H]^+$: 339.1074, found: 339.1062.

44: Ethyl glyoxylate



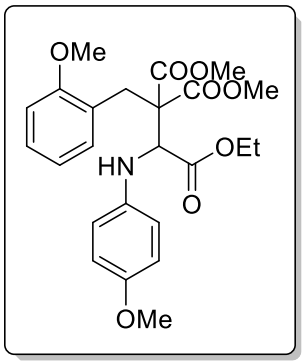
To a solution of diethyl L-tartrate (5 g, 0.024 mol) in DCM (50 mL) at room temperature were sequentially added silica-supported $NaIO_4$ ($NaIO_4$ = 10.3 g, 0.048 mol, 2.0 equiv) and water (5 mL) under vigorous stirring. After 2 h, the cloudy mixture was cooled down to 0 °C. $MgSO_4$ (4 g) was added portion wise. The resulting white suspension was filtered over celite. The solids were washed with DCM (30 mL). The filtrate was evaporated under reduced pressure to give ethyl glyoxylate **44** (2 g, 80 %) as a colorless liquid, which was directly used for the next step without further purification.

45: **Ethyl 2-((4-methoxyphenyl)imino)acetate**



In 20 mL of dichloromethane were added ethyl glyoxylate (2 g, 0.019 mol) and p-methoxyaniline (2.41 g, 0.019 mol) and $MgSO_4$ (5 g). The mixture was stirred at room temperature during 12 h. After filtration, the solution was concentrated to give almost pure imine in quantitative yield.

46: 1-Ethyl 2,2-dimethyl 3-(2-methoxyphenyl)-1-((4-methoxyphenyl)amino)propane-1,2,2-tricarboxylate



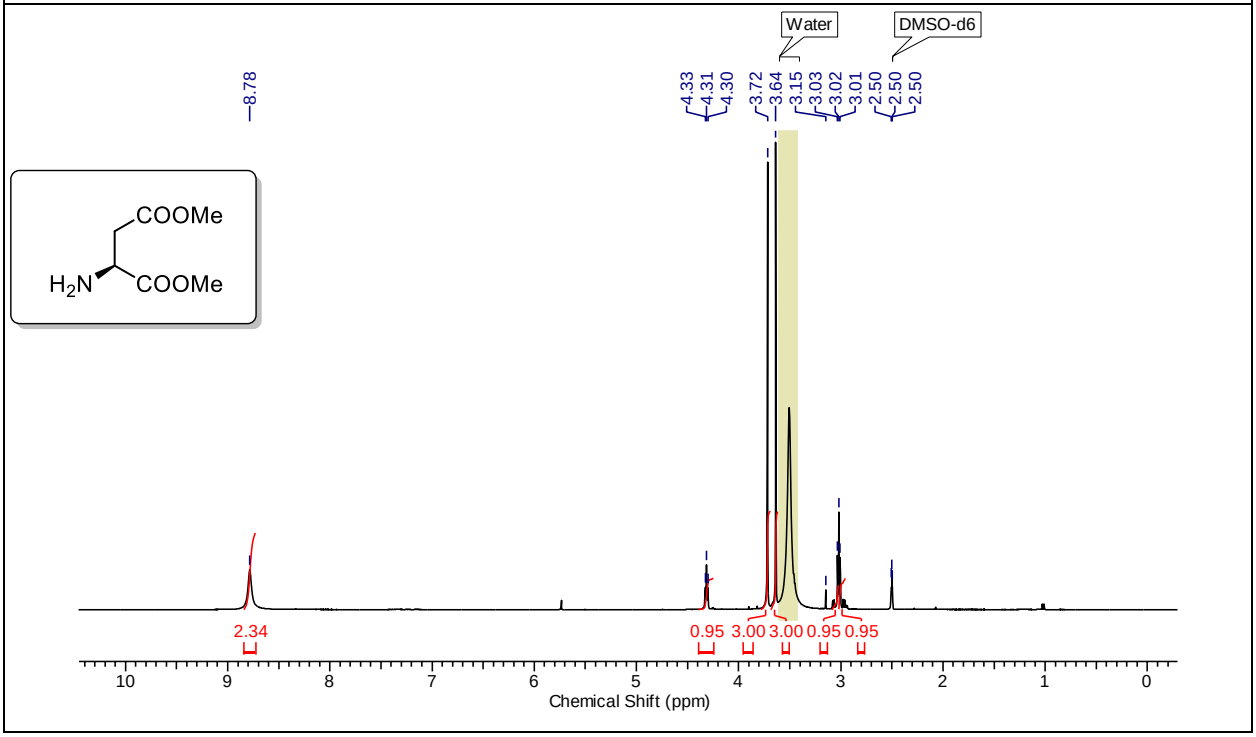
Imine compound **45** (3 g, 0.014 mol) and compound **38** (3.65 g, 0.014 mmol) and $ZnCl_2$ (0.987 g, 0.007 mol). The mixture was stirred at room temperature during 24 h. Water (50 mL) and EtOAc (50 mL) was added and the mixture extracted three times with EtOAc (3 x 50 mL). The combined organic layers were dried over $NaSO_4$ and evaporated under reduced pressure. Resulting products were purified by column chromatography. Yield: (5 g, + Impurities 75 %).

Yield: 75 %.

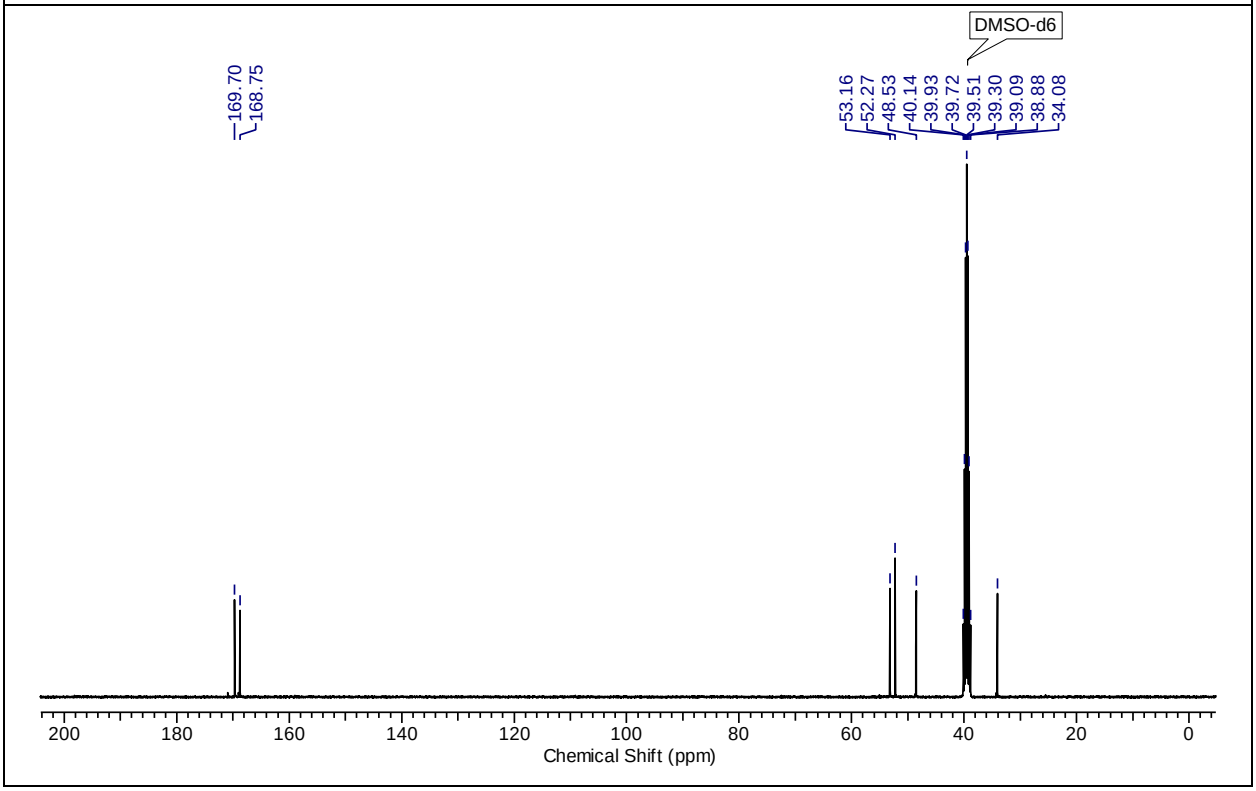
HRMS (ESI) *m/z* calculated for $C_{24}H_{30}NO_8$ $[M+H]^+$: 460.1966, found: 460.1956.

3.2.2.2. Spectral Data

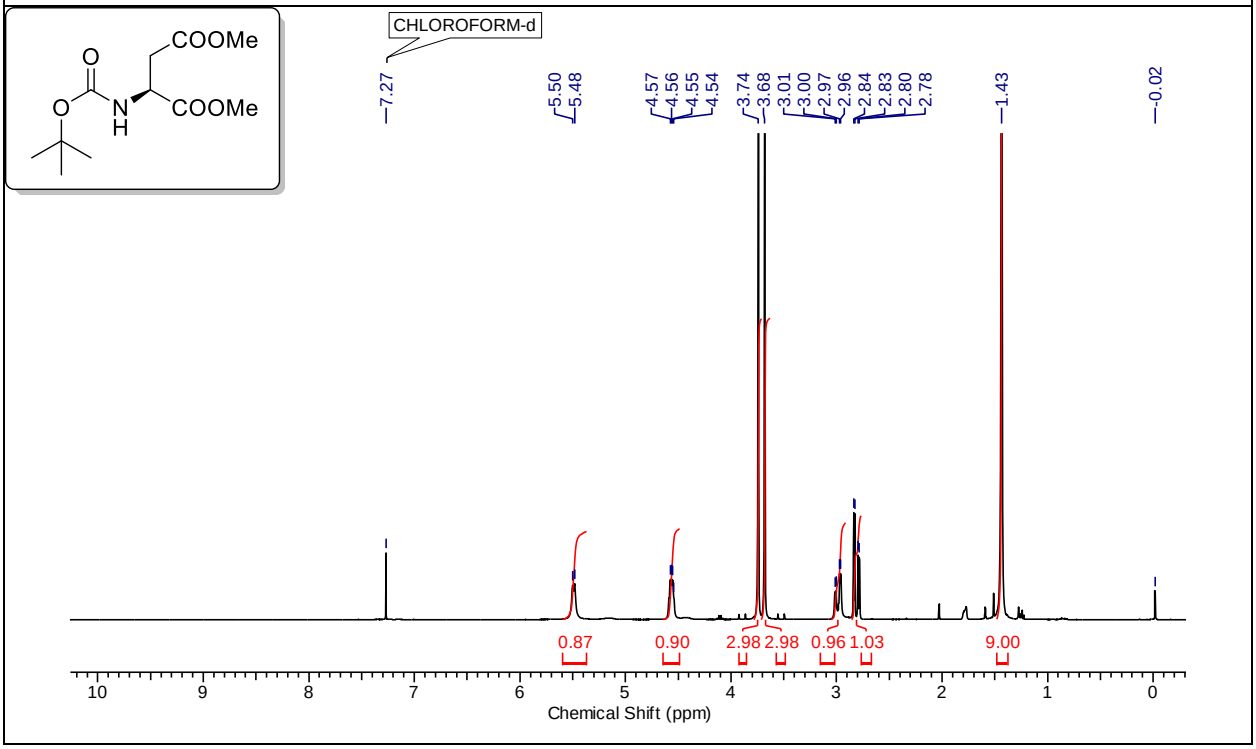
^{1}H NMR (400 MHz, DMSO-d6) of compound 30



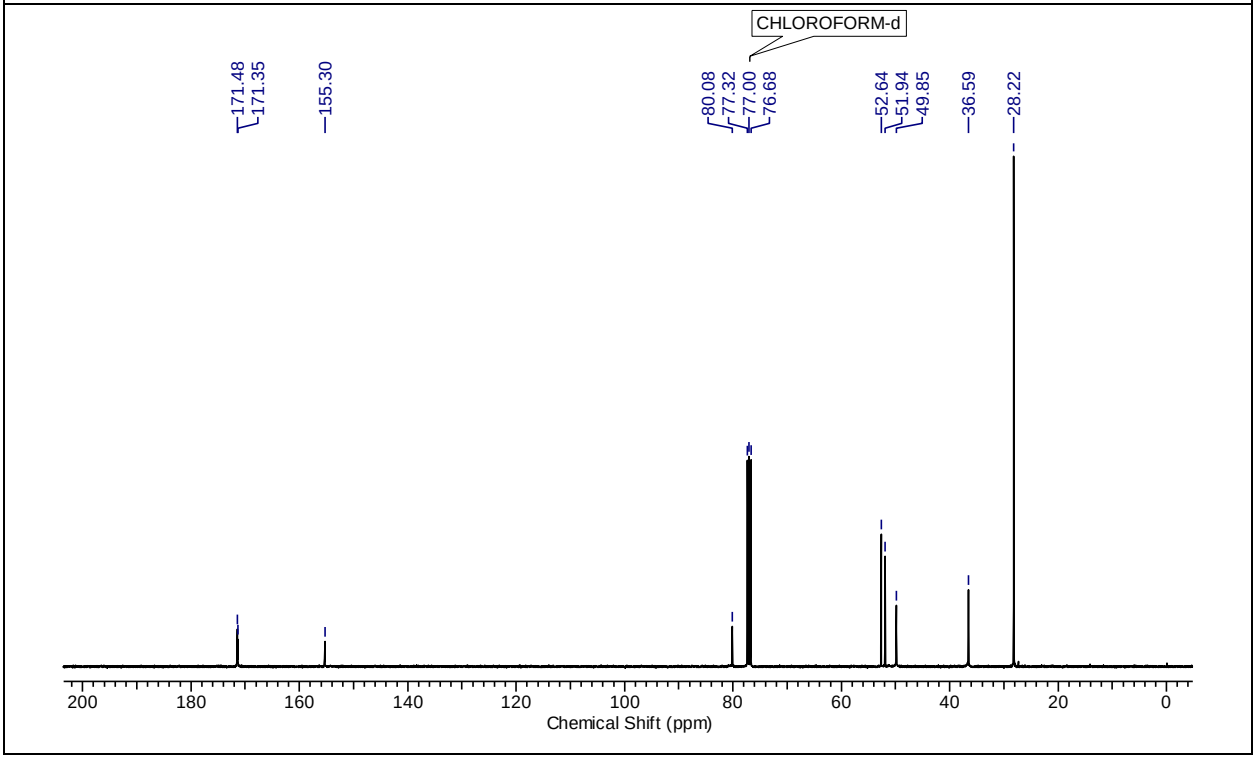
^{13}C NMR (100 MHz, DMSO-d6) of compound 30



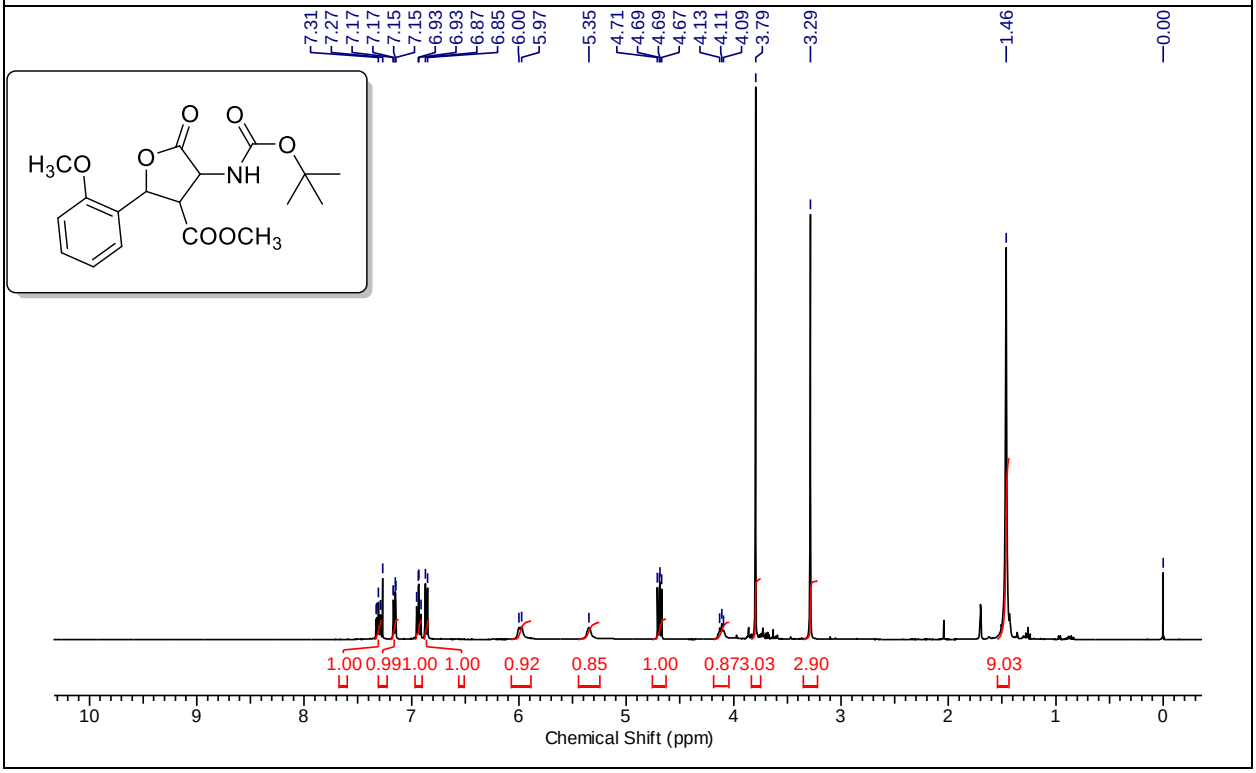
^{1}H NMR (400 MHz, $CDCl_3$) of compound 31a



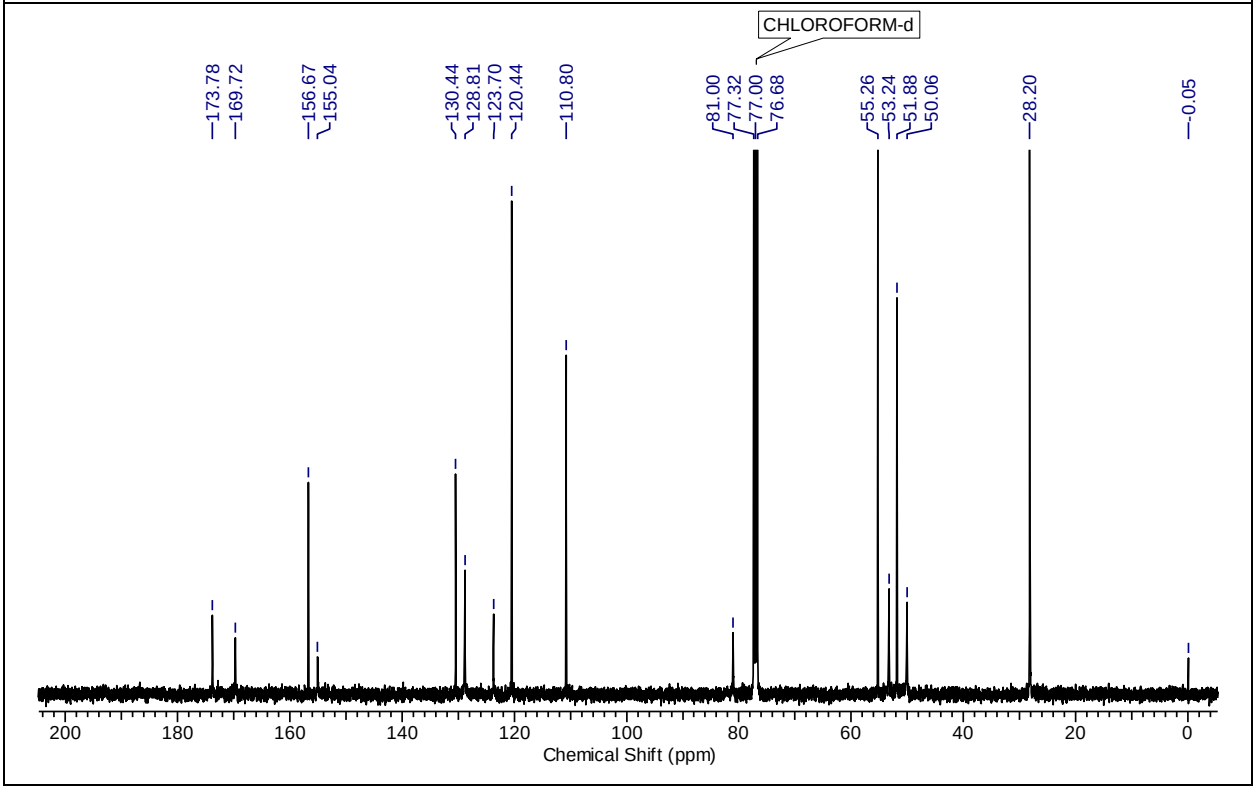
^{13}C NMR (100 MHz, $CDCl_3$) of compound 31a



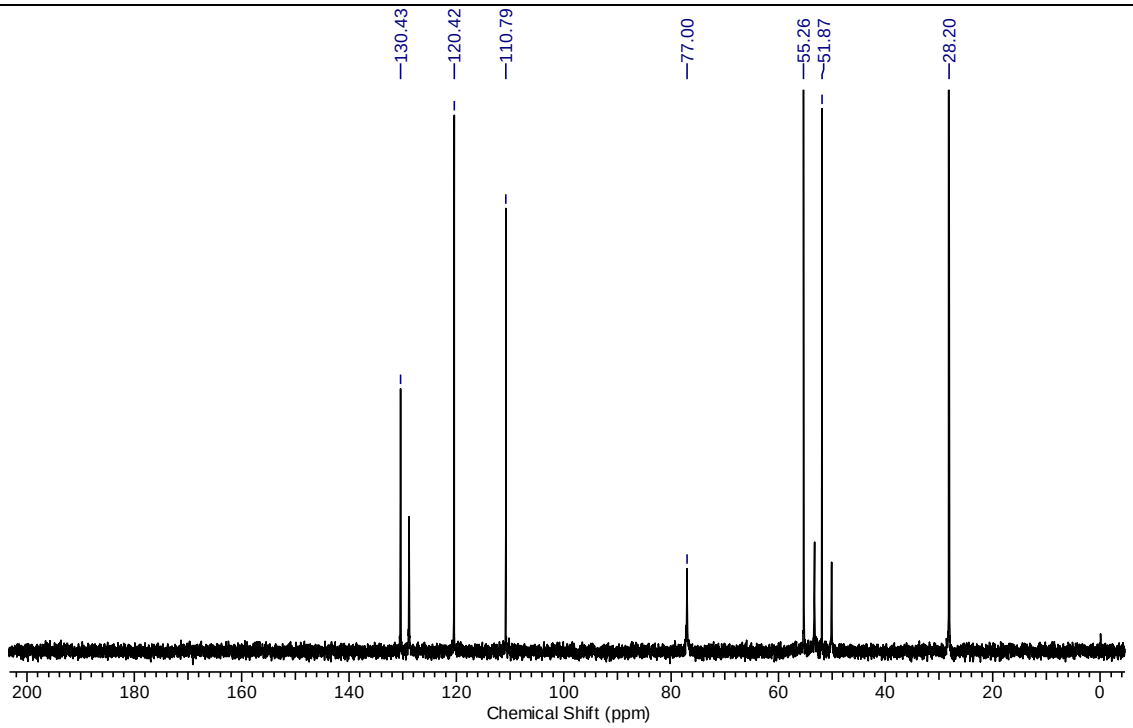
^{1}H NMR (400 MHz, $CDCl_3$) of compound 29a



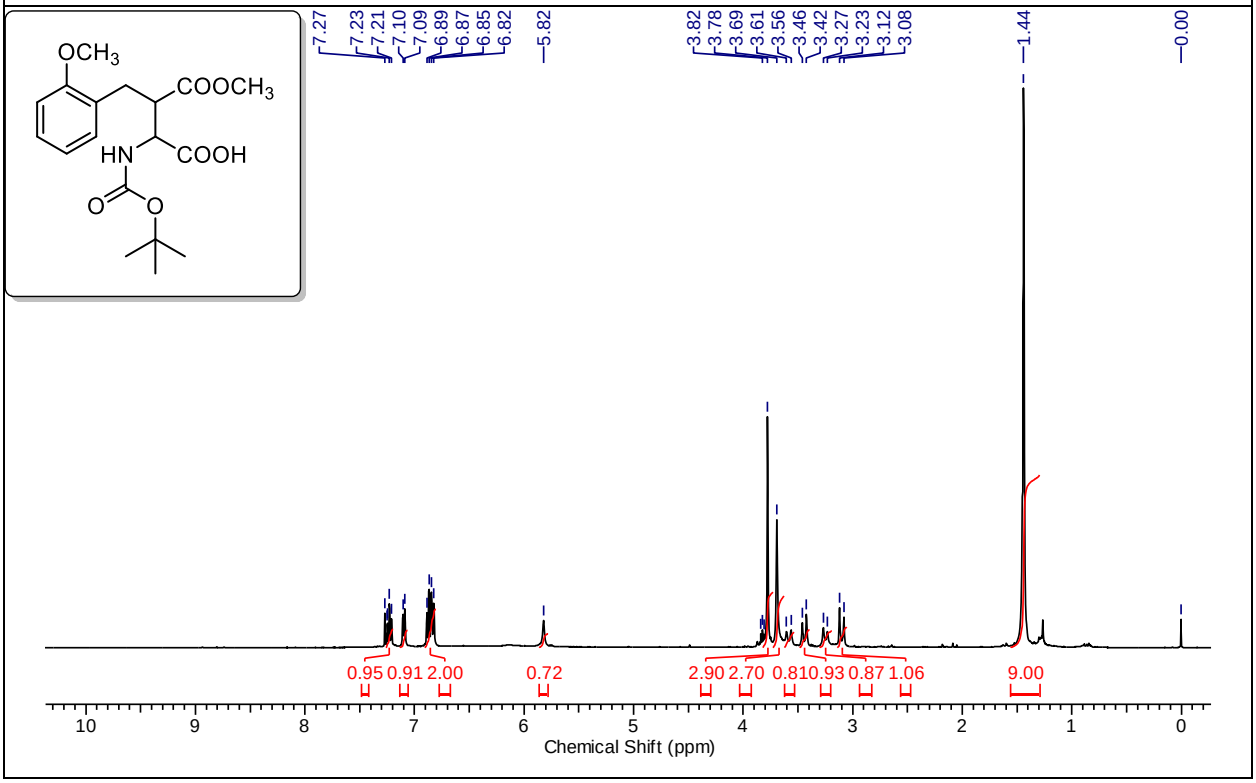
^{13}C NMR (100 MHz, $CDCl_3$) of compound 29a



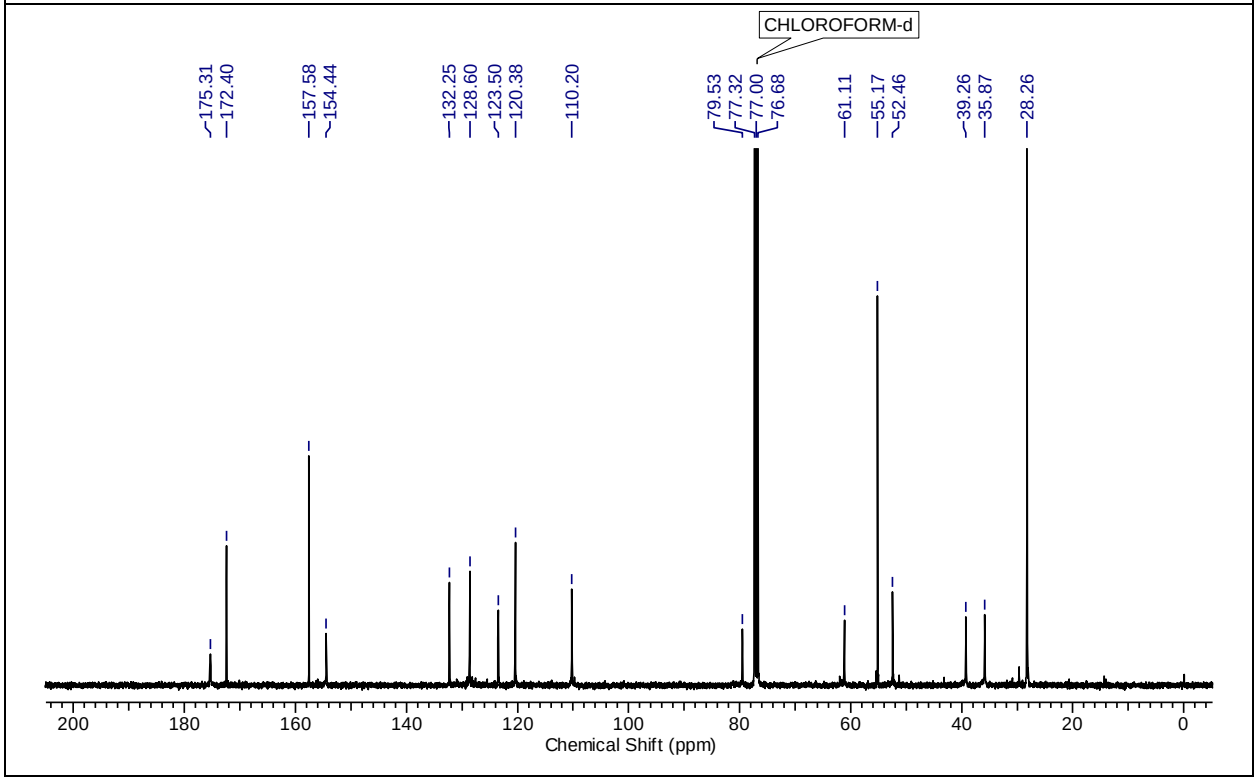
DEPT NMR (100 MHz, $CDCl_3$) of compound 29a



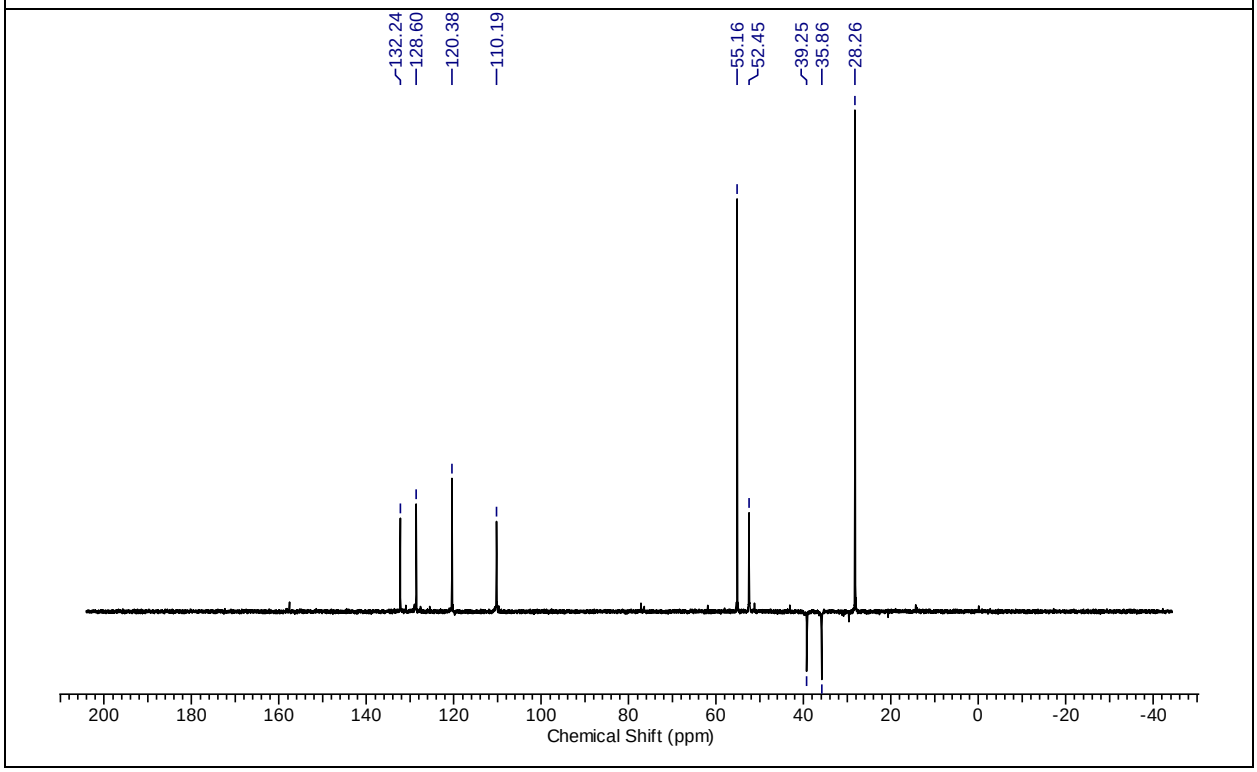
^{1}H NMR (400 MHz, $CDCl_3$) of compound 28



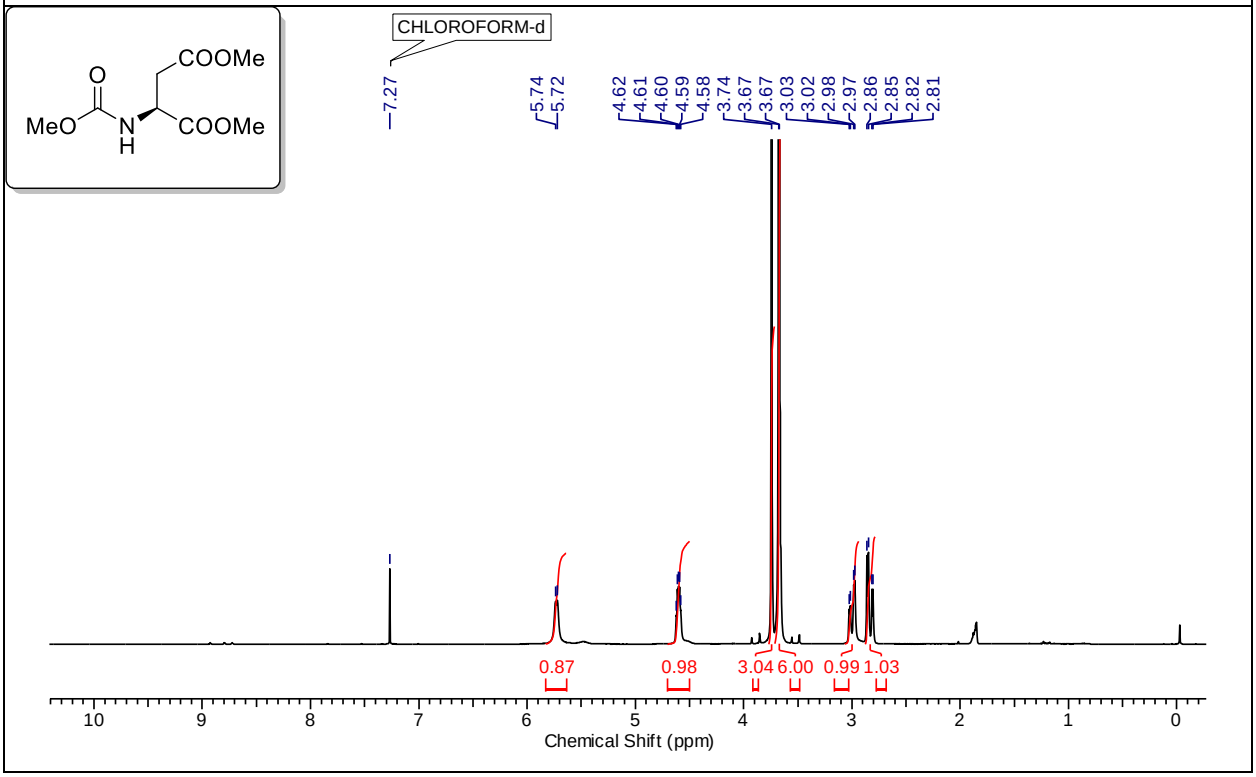
^{13}C NMR (100 MHz, $CDCl_3$) of compound 28



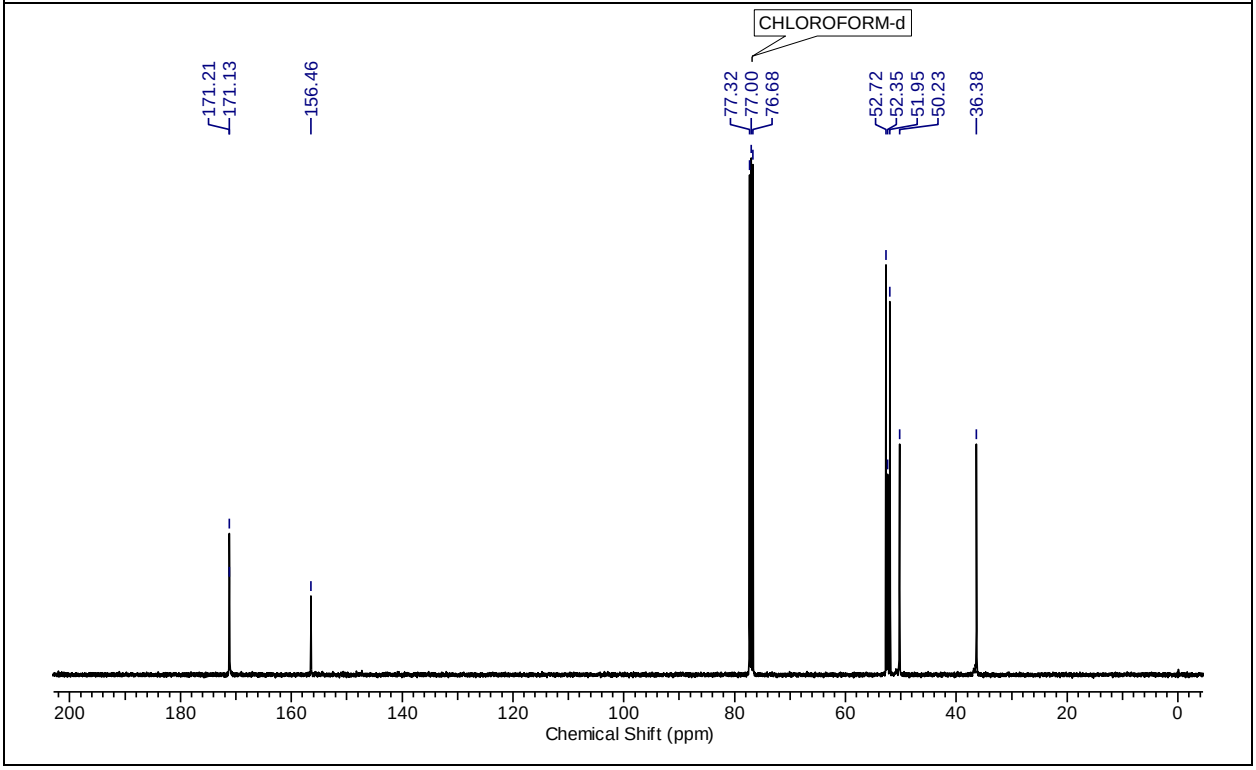
DEPT NMR (100 MHz, $CDCl_3$) of compound 28



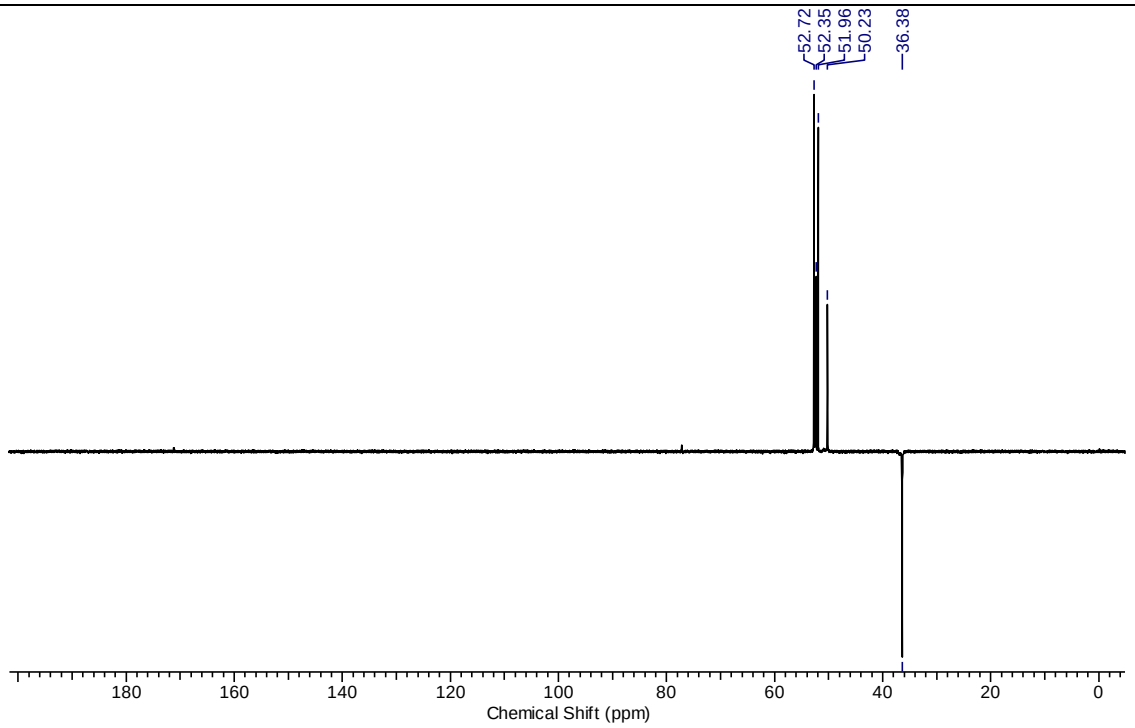
^{1}H NMR (400 MHz, $CDCl_3$) of compound 31b



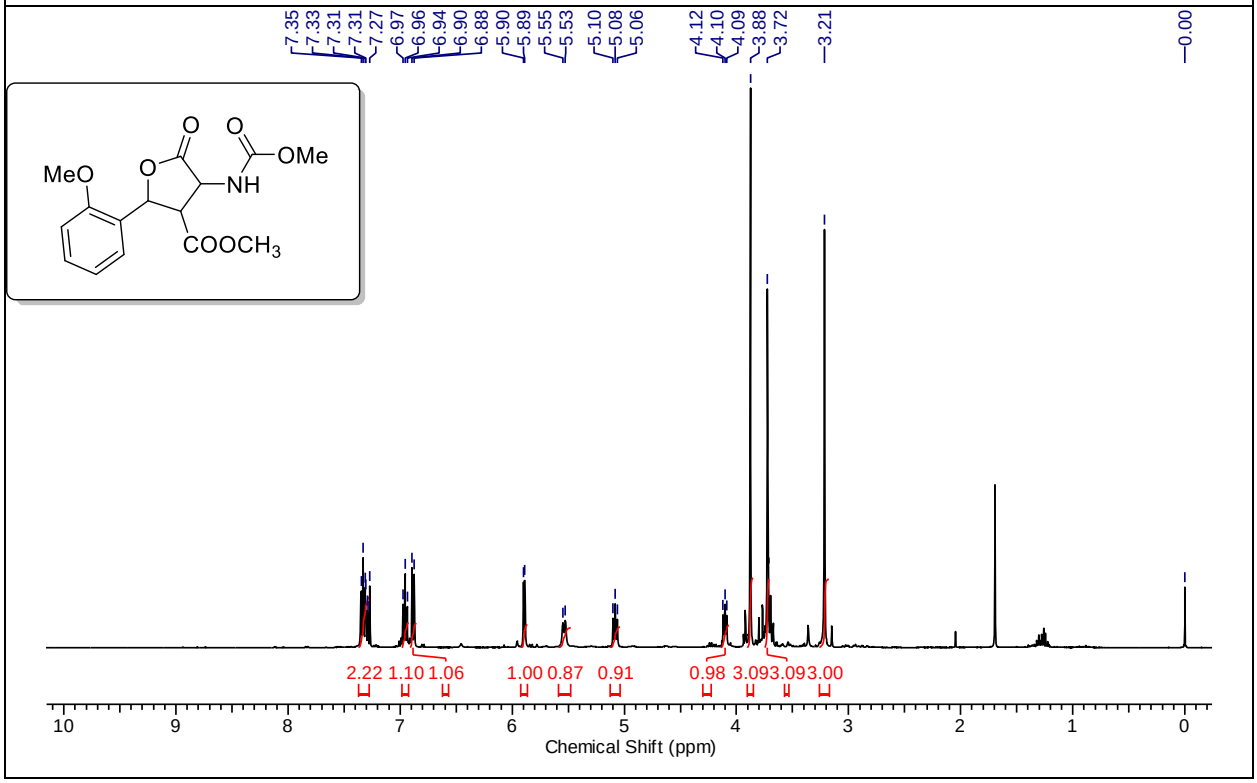
^{13}C NMR (100 MHz, $CDCl_3$) of compound 31b



DEPT NMR (100 MHz, $CDCl_3$) of compound 31b



^1H NMR (400 MHz, $CDCl_3$) of compound 29b



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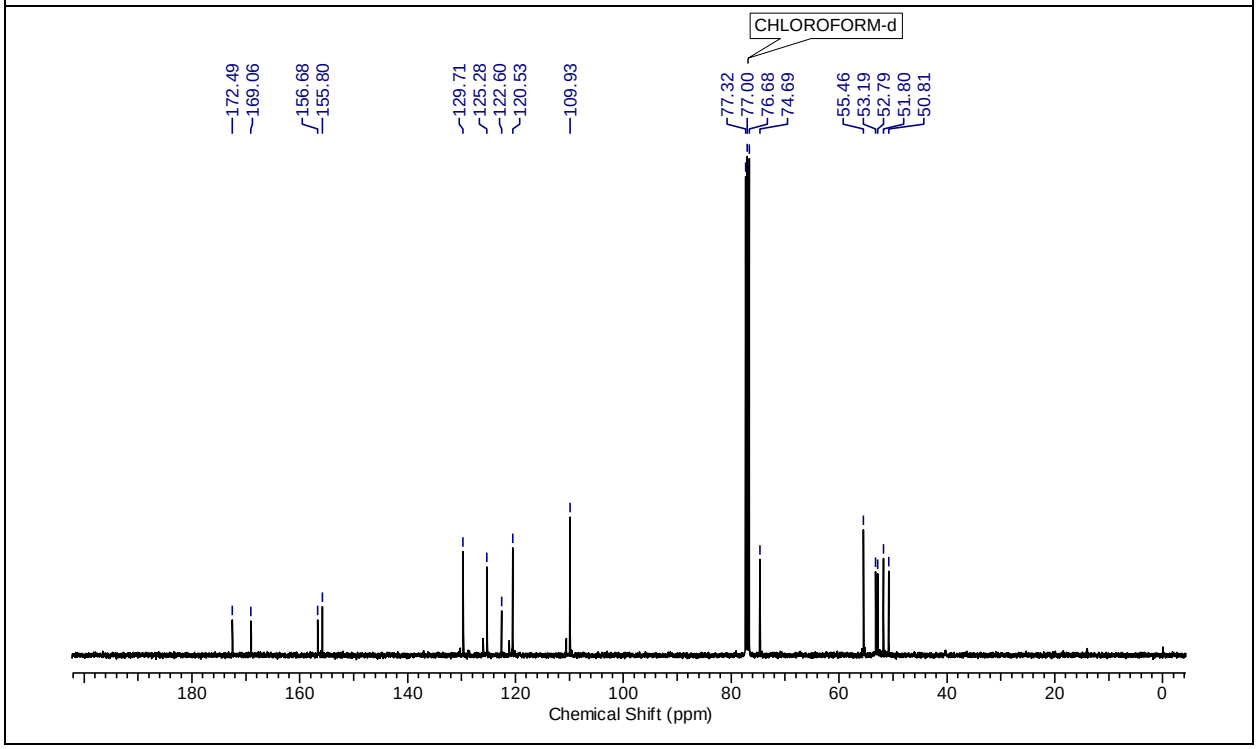
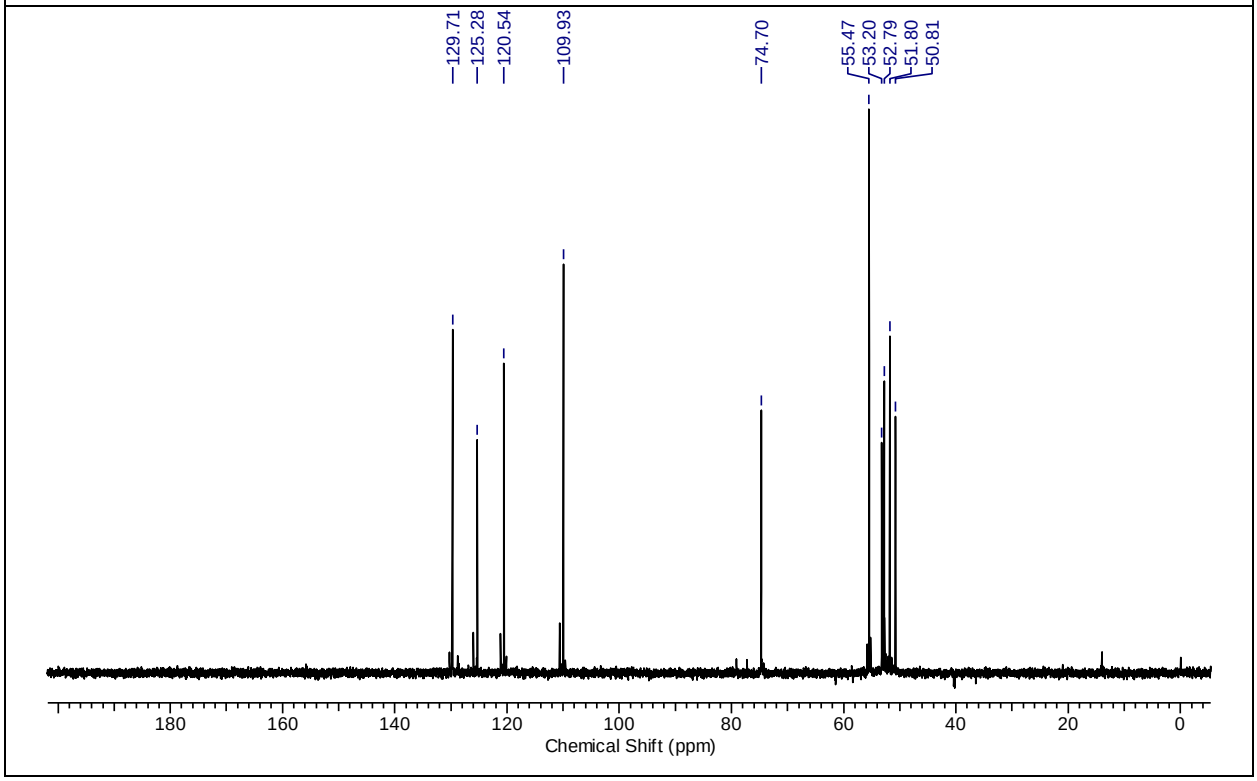
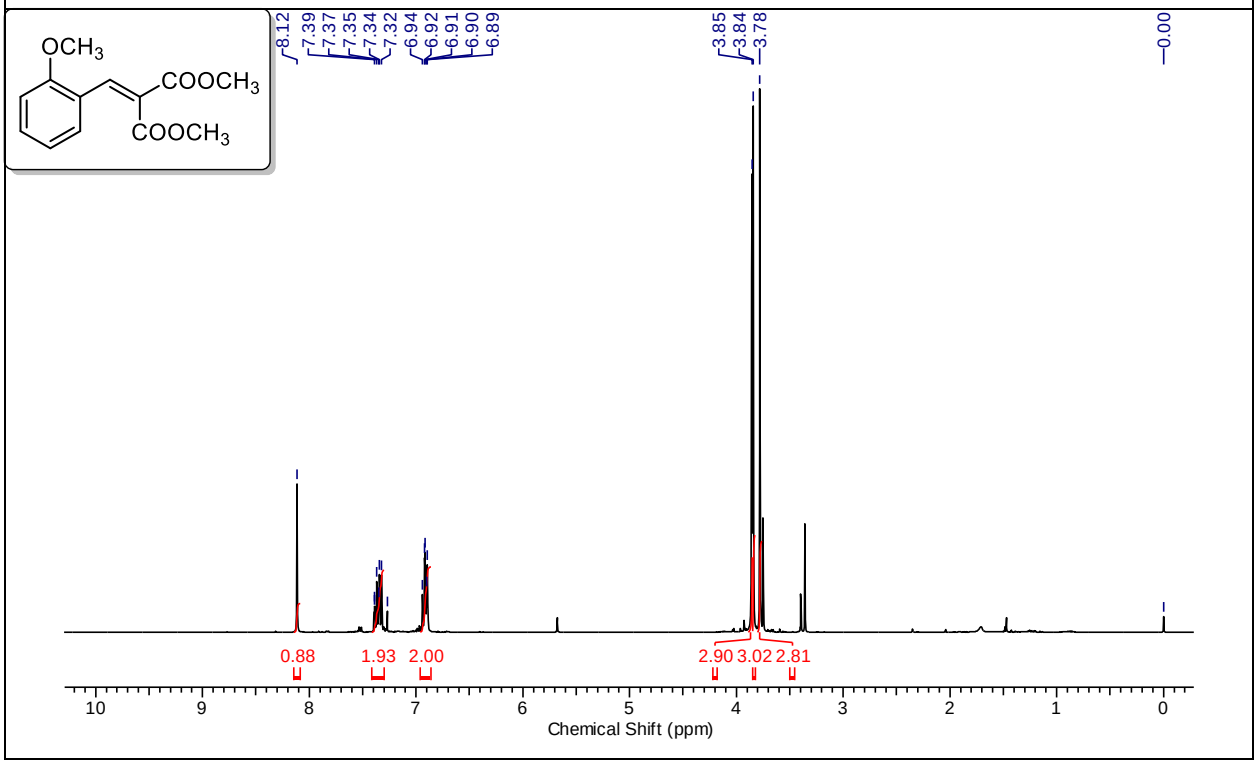


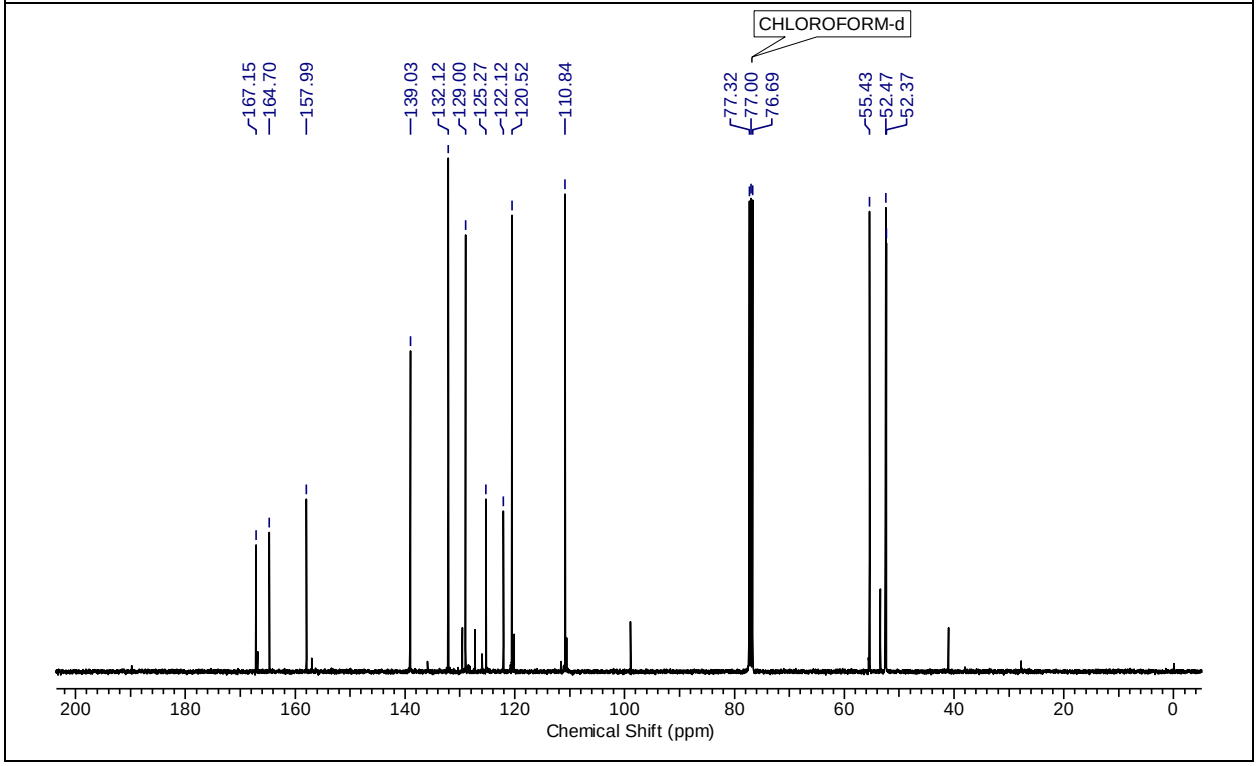
DEPT NMR (100 MHz, $CDCl_3$) of compound 29b



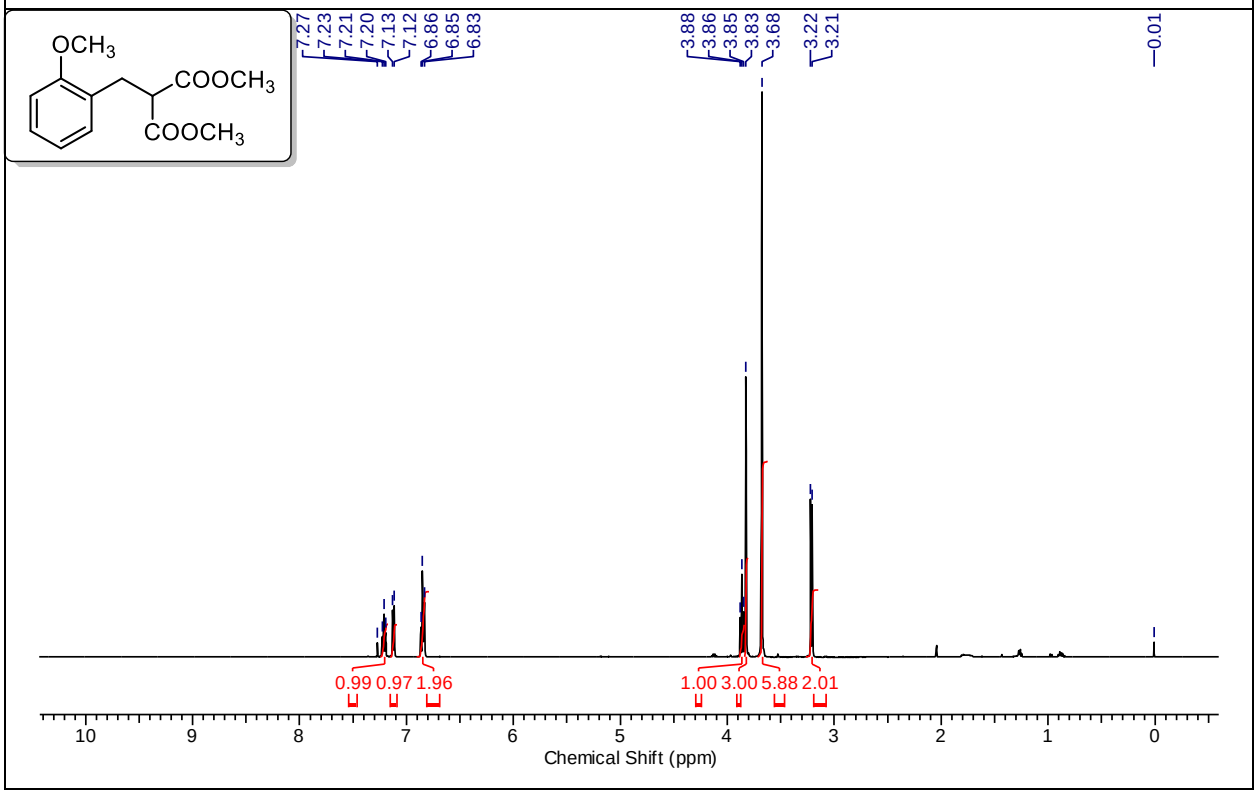
^{1}H NMR (400 MHz, $CDCl_3$) of compound 39



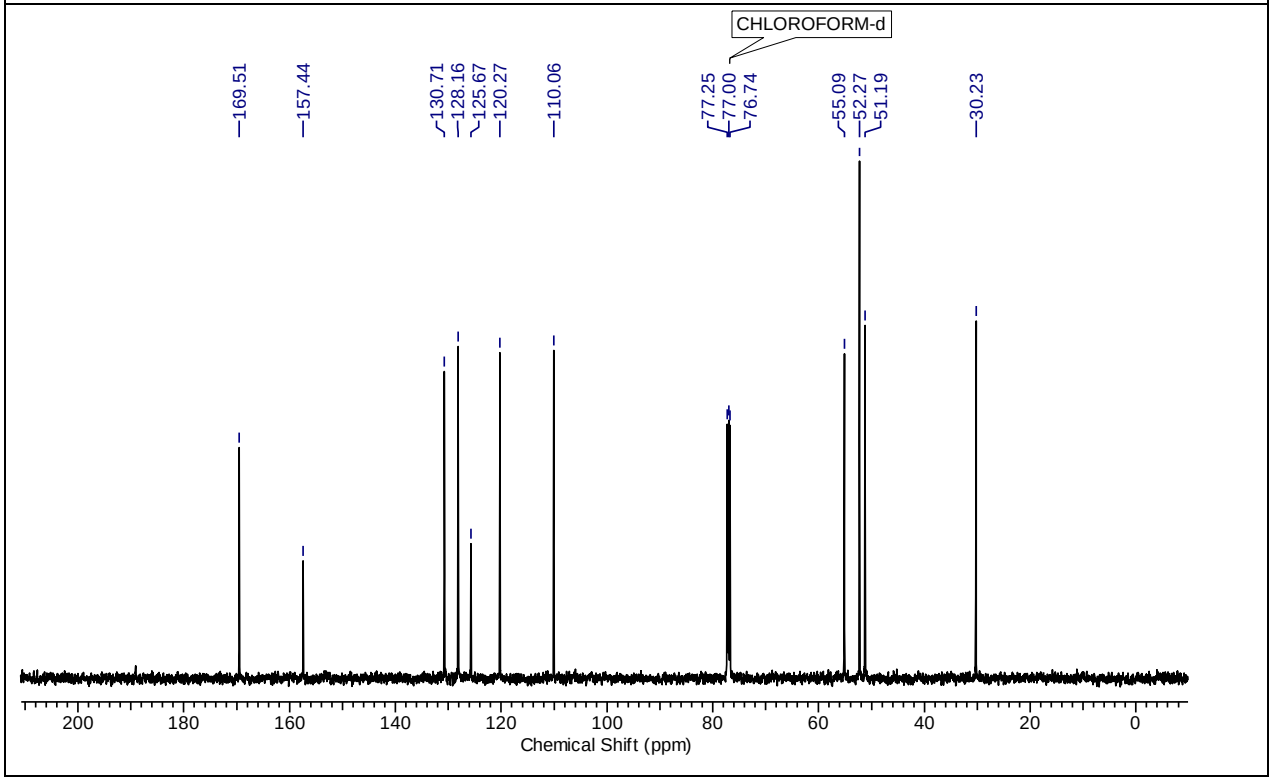
^{13}C NMR (100 MHz, $CDCl_3$) of compound 39



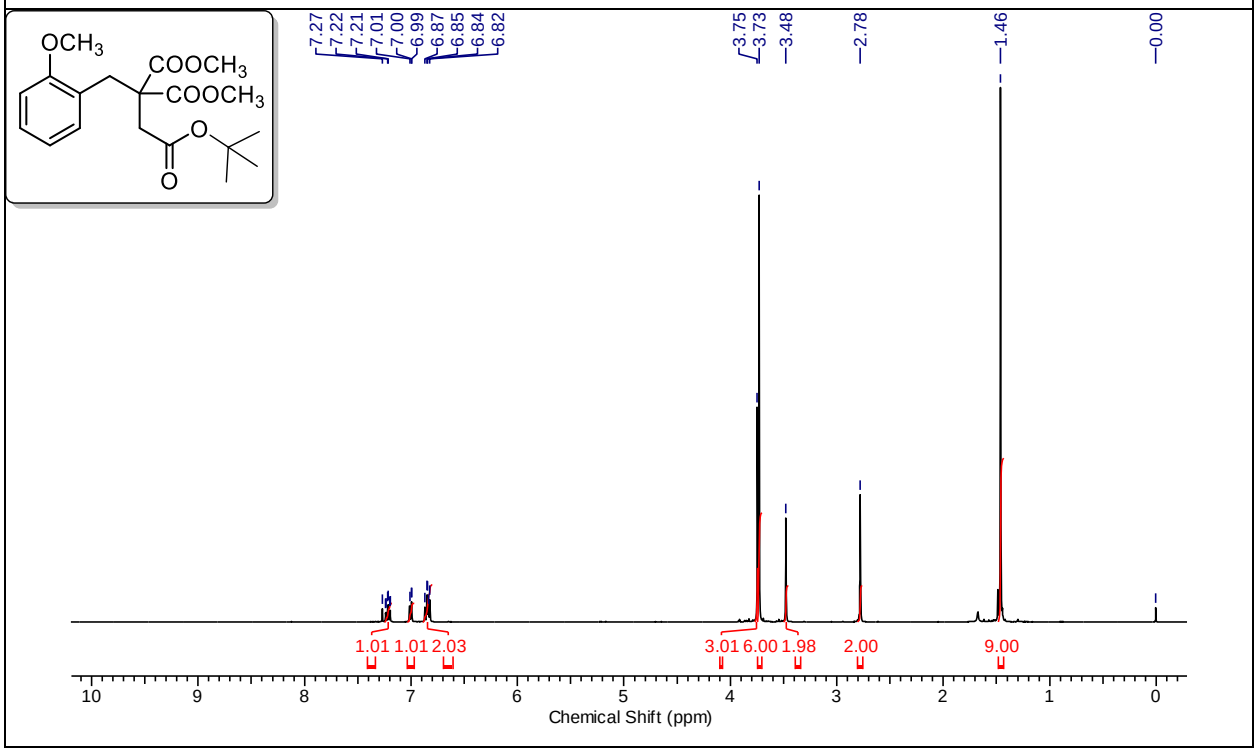
^{1}H NMR (200 MHz, $CDCl_3$) of compound 38



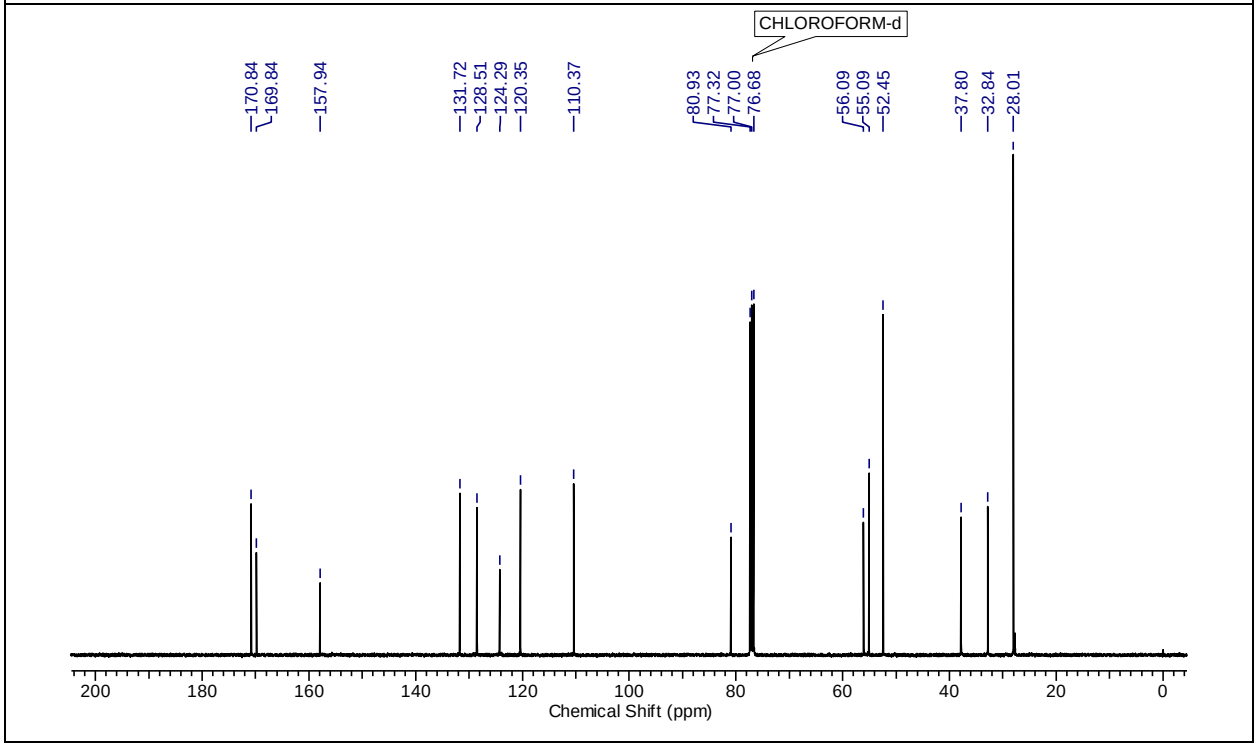
^{13}C NMR (100 MHz, $CDCl_3$) of compound 38



^{1}H NMR (400 MHz, $CDCl_3$) of compound 40a



^{13}C NMR (100 MHz, $CDCl_3$) of compound 40a



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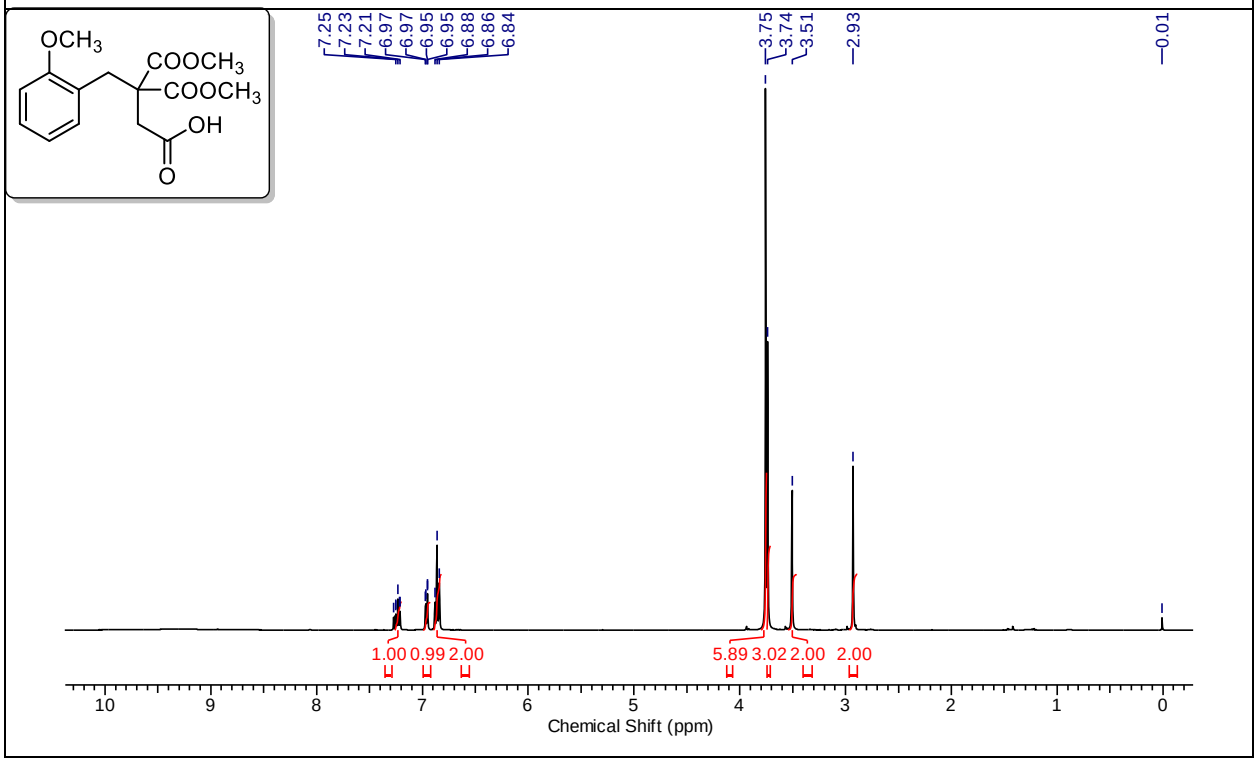

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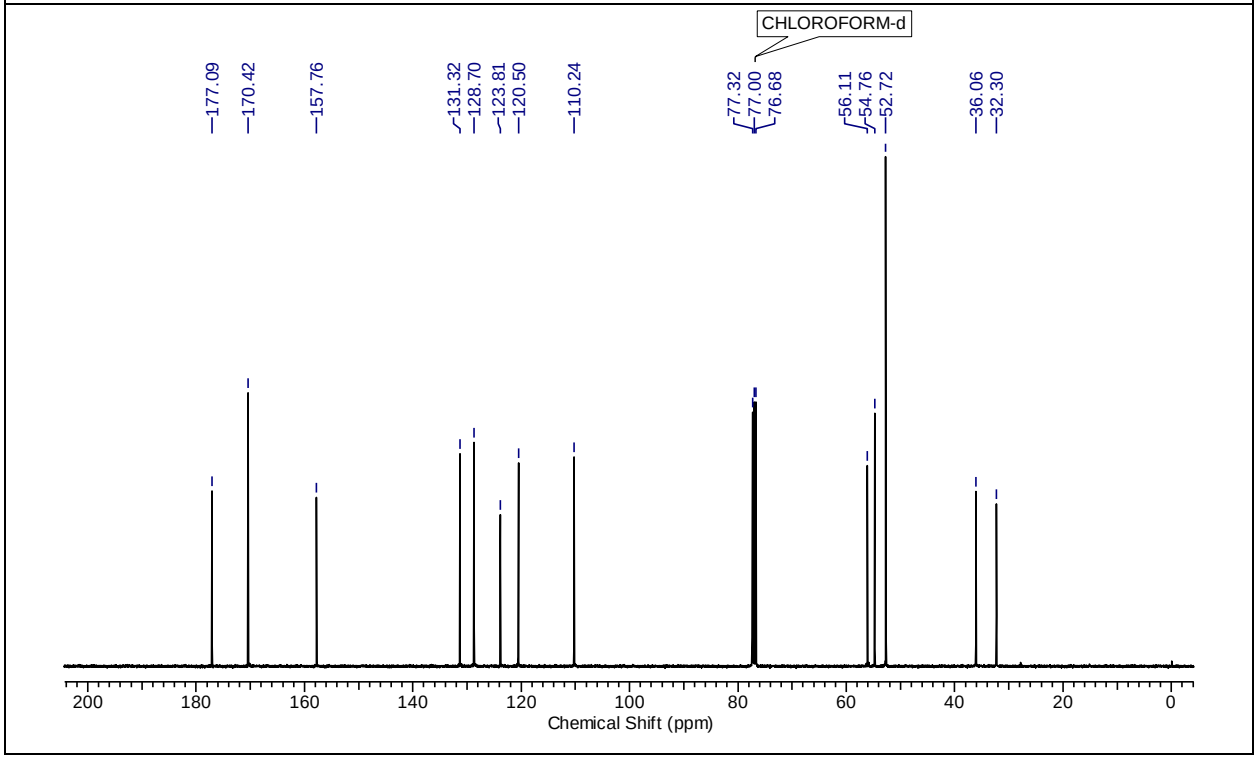
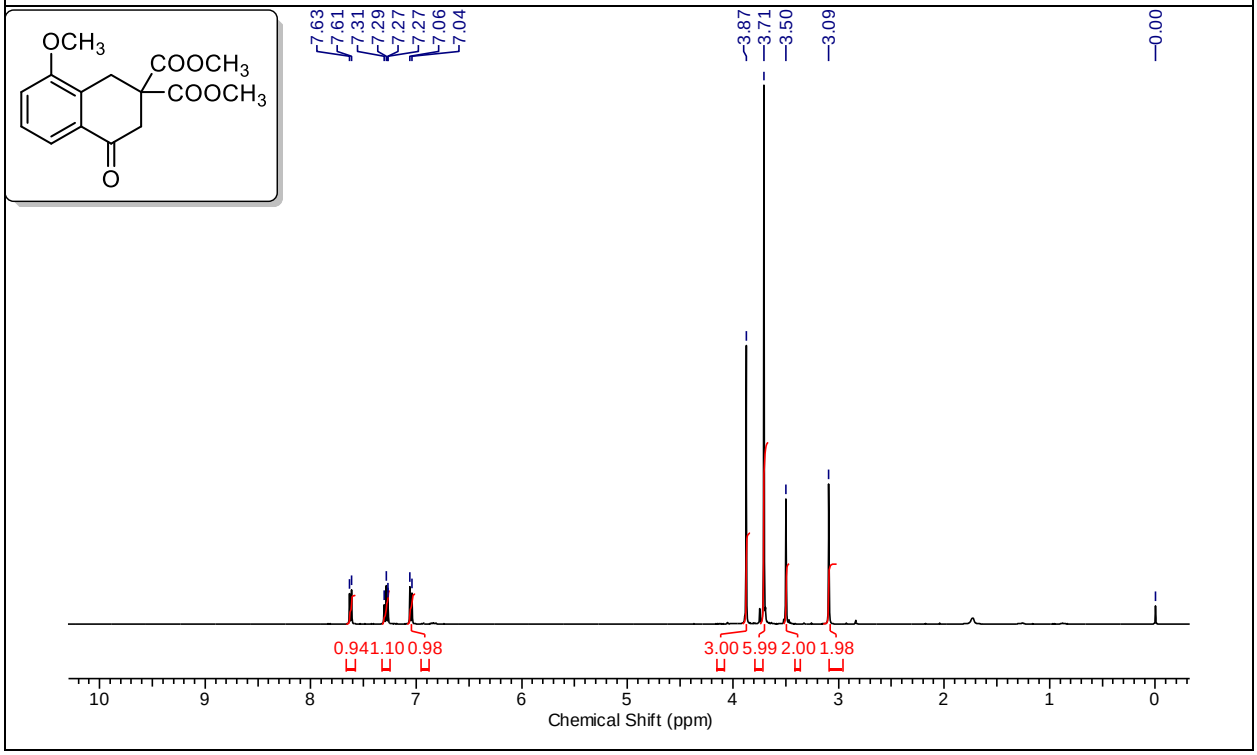
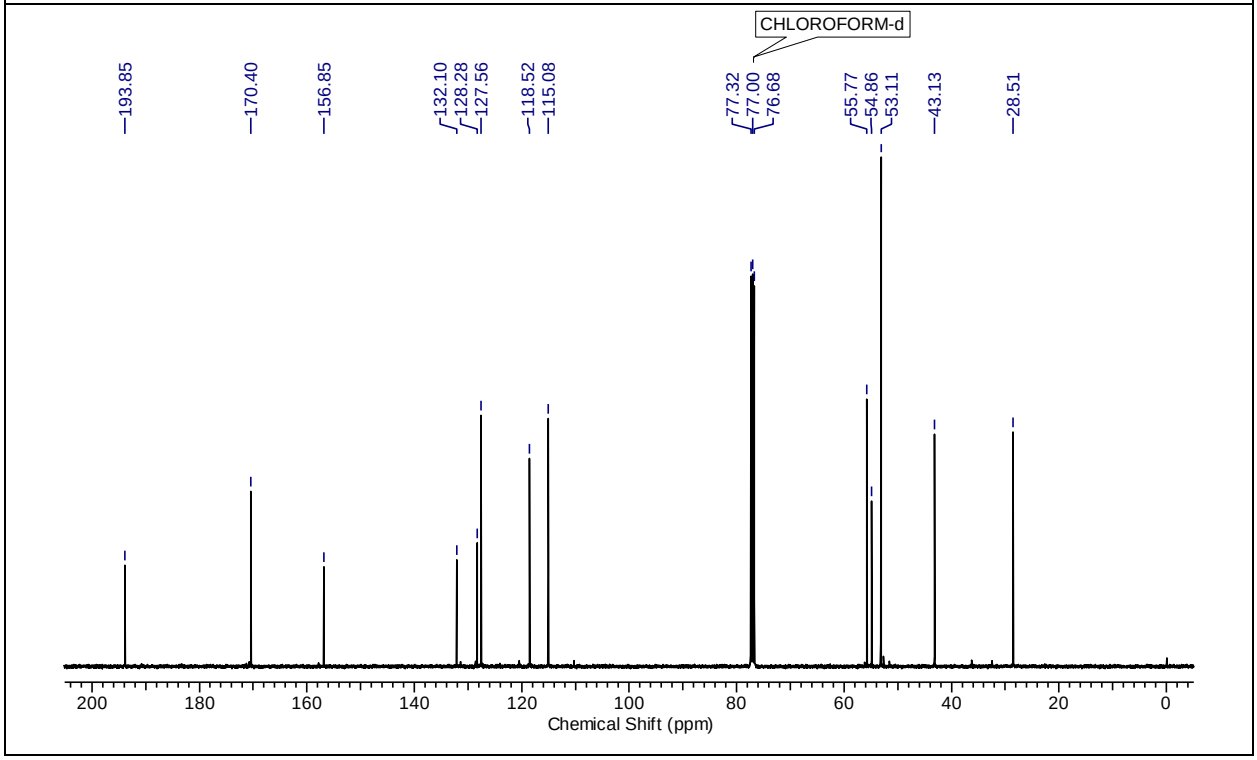


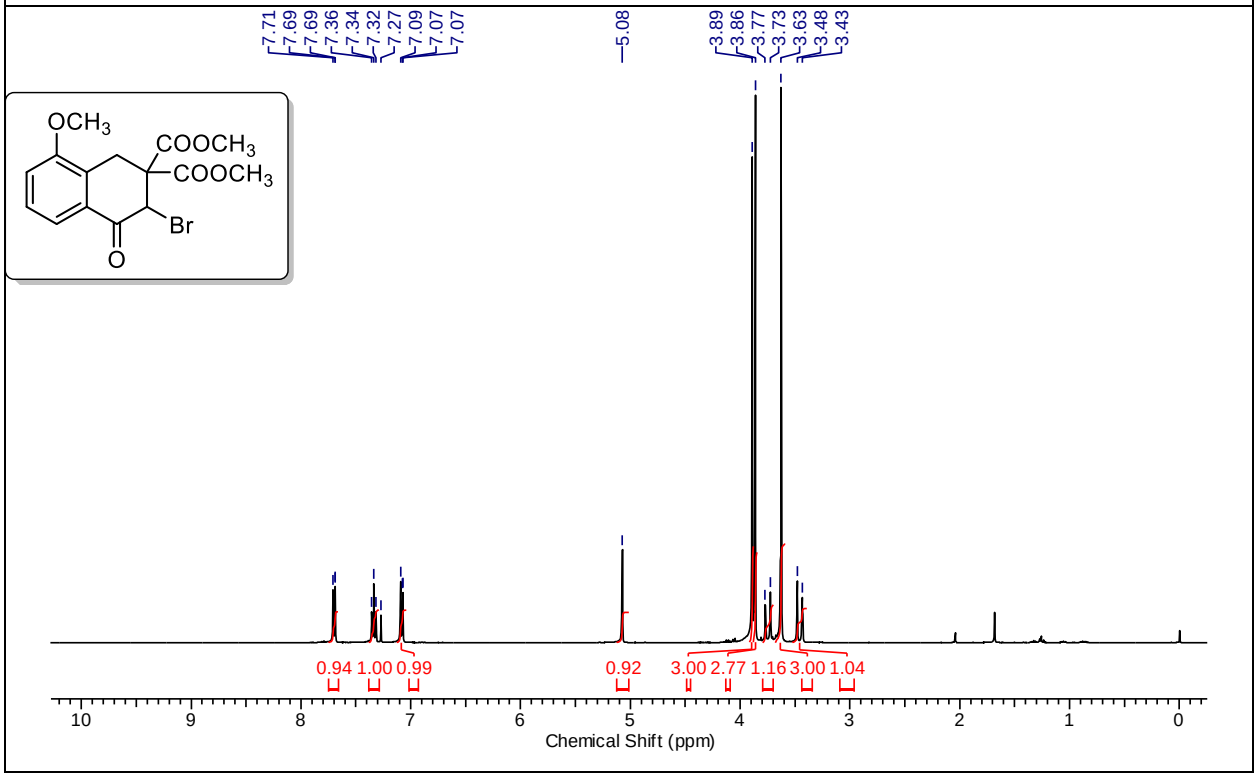
^{1}H NMR (400 MHz, $CDCl_3$) of compound 37



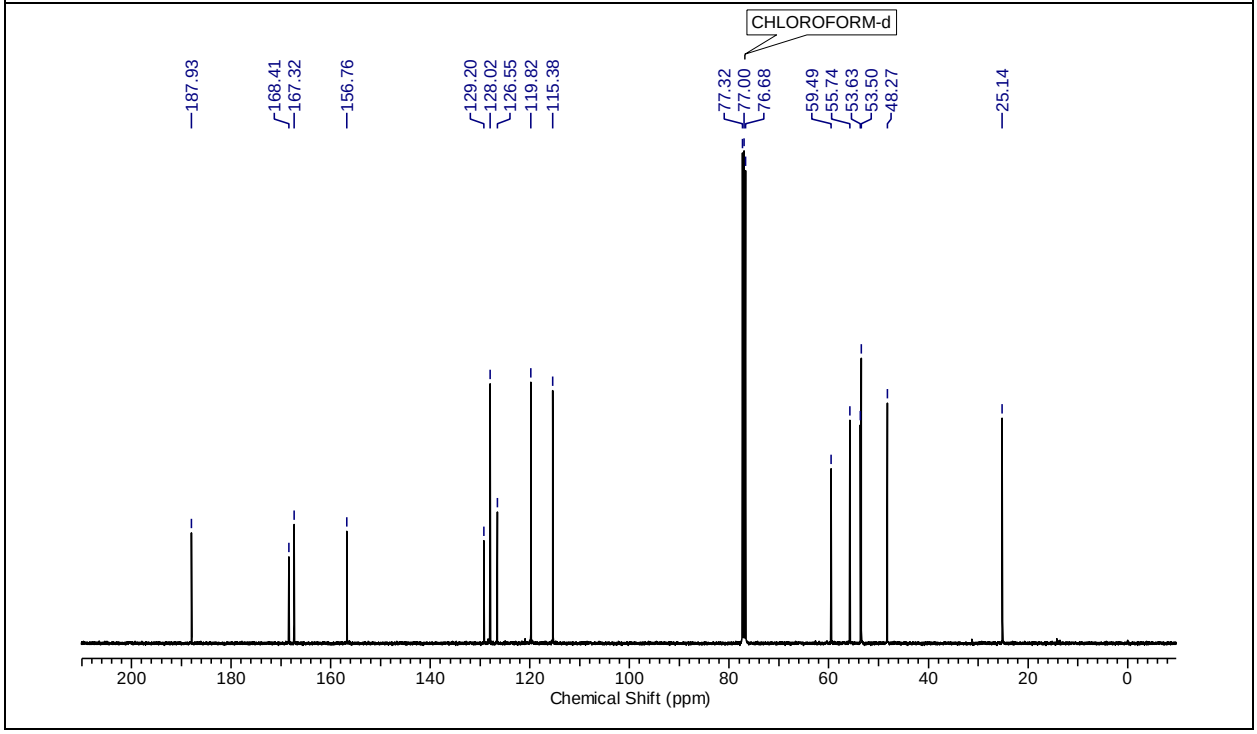
^{13}C NMR (100 MHz, $CDCl_3$) of compound 37



^{1}H NMR (400 MHz, $CDCl_3$) of compound 41



^{13}C NMR (100 MHz, $CDCl_3$) of compound 41



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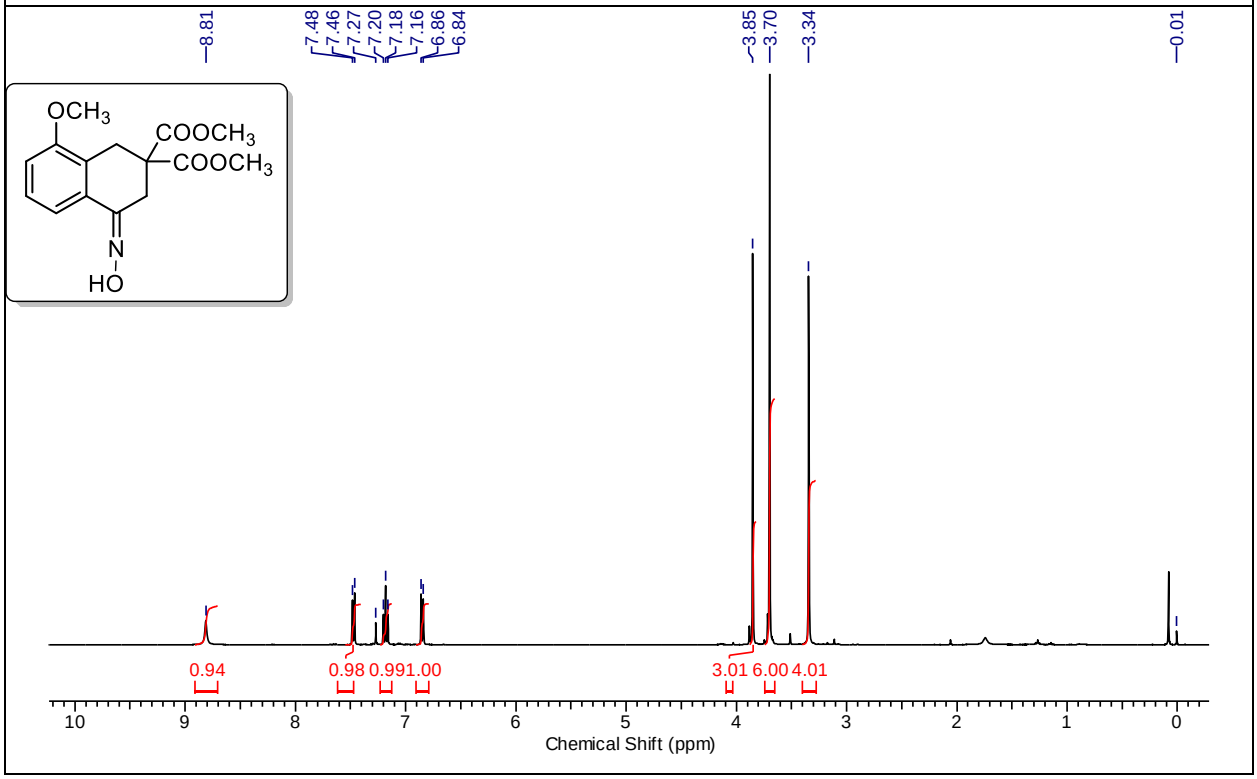
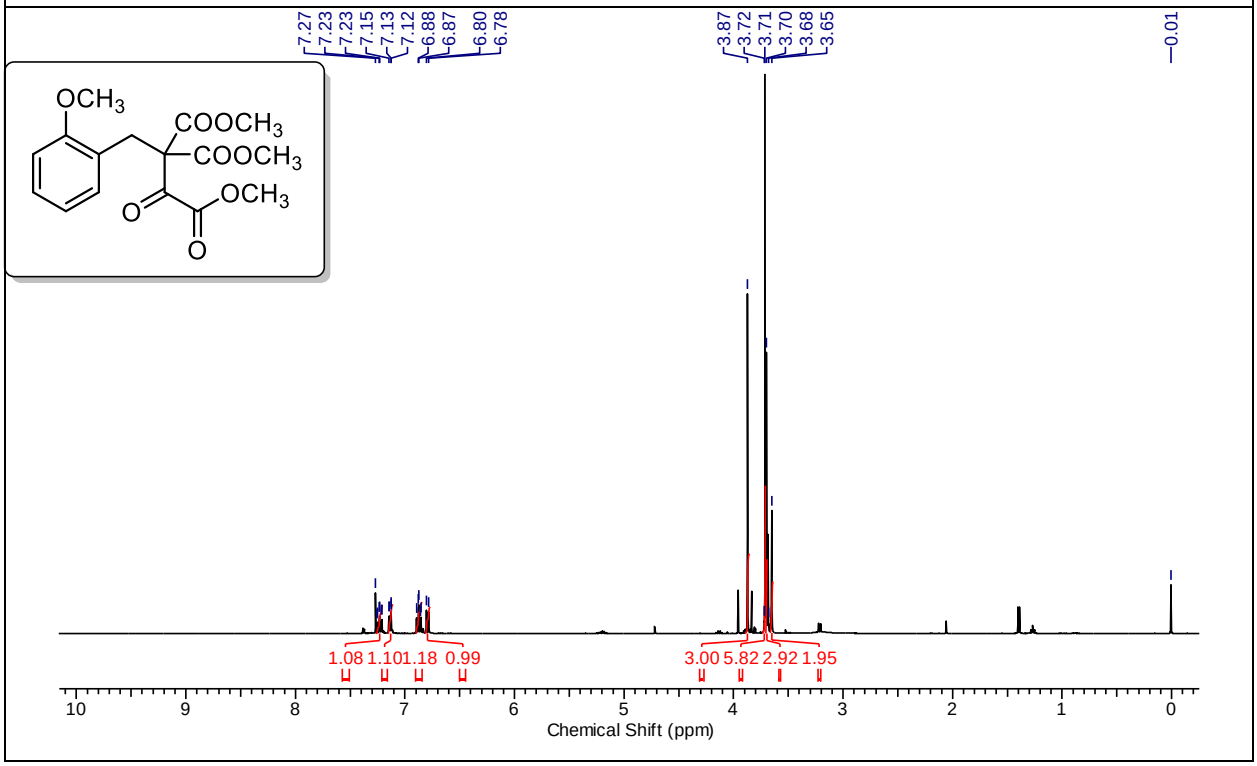
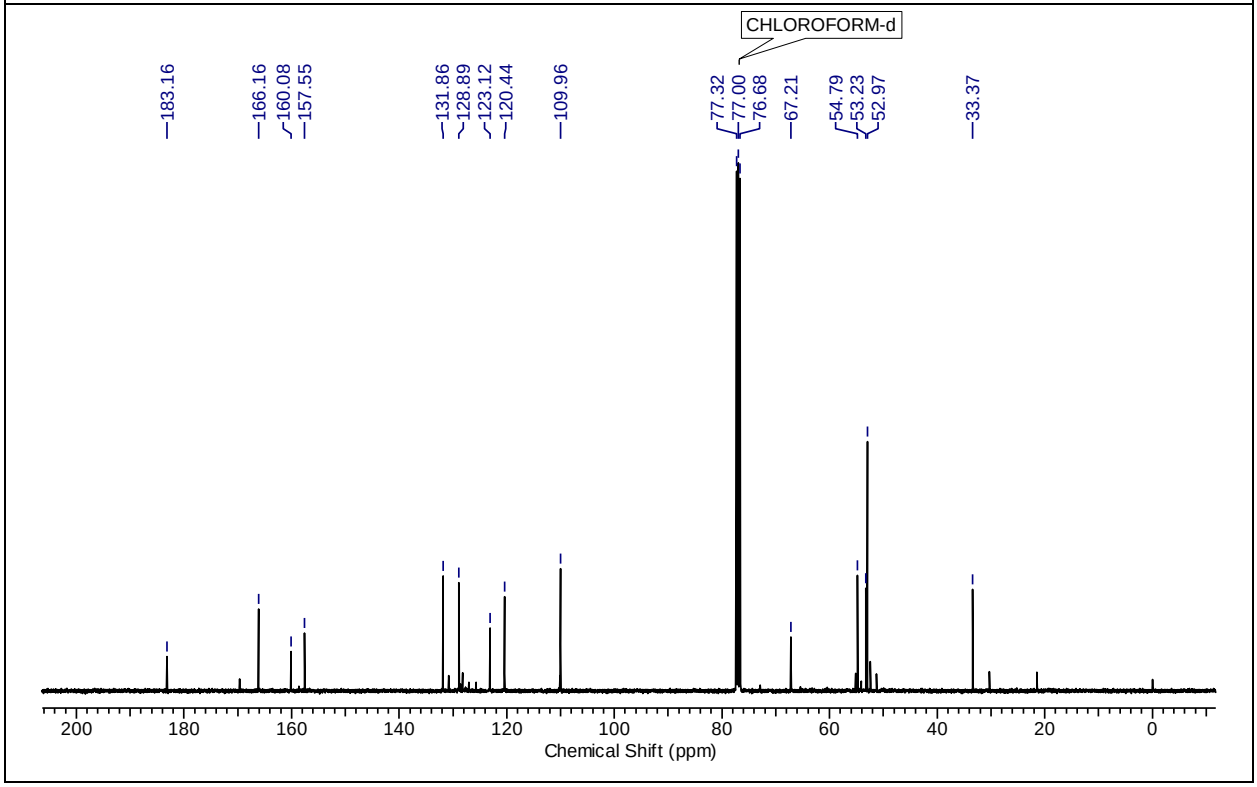


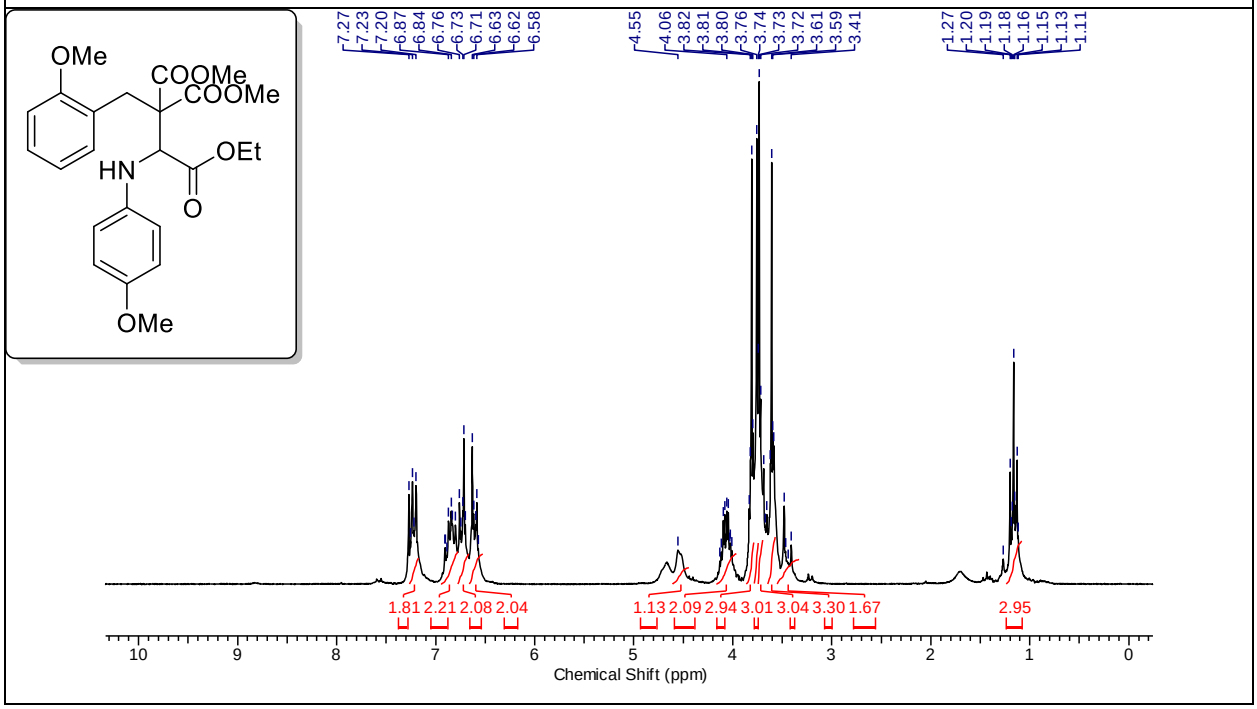
^{1}H NMR (400 MHz, $CDCl_3$) of compound 36



^{13}C NMR (100 MHz, $CDCl_3$) of compound 36



^{1}H NMR (400 MHz, $CDCl_3$) of compound 46



3.2.3. References :

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ABSTRACT

Name of the Student: Pisal Mahesh Madanrao
Registration No.: 10CC17A26006
Faculty of Study: Chemical Science
Year of Submission: 2021
AcSIR academic centre/CSIR Lab:
CSIR-National Chemical Laboratory, Pune
Name of the Supervisor: Dr. Subhash Prataprao Chavan

Title of the thesis: *Synthesis and biological evaluation of thienopyrimidinone-containing compounds as antitubercular and anticancer agents, novel dicyanoanilines as cell imaging agents and studies towards the total synthesis of quinagolide*

The synthesis of biologically and medicinally important small synthesized novel molecules as antitubercular and anticancer agents, as well as the synthesis of novel dicyanoanilines and highly substituted indoles as cell imaging agents, are described in this thesis. In addition, study towards the total synthesis of quinagolide is covered. There are three chapters of the thesis. The first chapter covers the synthesis and the antitubercular evaluation of thienopyrimidinone-containing compounds. The second chapter deals with the synthesis, cell imaging properties and photophysical properties of substituted 2,6-dicyanoanilines and derivatives, as well as the synthesis and biological examination of 2,3,5,6,7-pentasubstituted and 2,3,4,5,7-pentasubstituted indoles synthesized from 2,6-dicyanoanilines. The third chapter focuses on advanced synthesis of quinagolide in order to achieve higher yields and shorter synthetic routes, which are needed to keep the drug-costs down.

List of Publications Emanating from the Thesis Work

1. Borate, H. B.; Annadate, R. A.; Vagh, S. S.; **Pisal, M. M.;** Deokate, S. B.; Arkile, M. A.; Jadhav, N. J.; Nawale, L. U.; Sarkar, D. Synthesis and evaluation of thieno[2,3 *d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents. *MedChemComm*, **2015**, *6*, 2209-2215.
2. **Pisal, M. M.;** Nawale, L. U.; Patil, M. D.; Bhansali, S. G.; Gajbhiye, J. M.; Sarkar, D.; Chavan, S. P.; Borate, H. B. Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents: SAR and docking studies. *Eur. J. Med. Chem.*, **2017**, *127*, 459-469.
3. **Pisal, M. M.;** Annadate, R. A.; Athalye, M. C.; Kumar, D.; Chavan, S. P.; Sarkar, D.; Borate, H. B. Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl-2,6-dicyanoanilines. *Bioorg. Med. Chem. Lett.*, **2017**, *27*, 979-988.

List of Publications Non-Emanating from the Thesis Work

1. Kudale, A. S.; Kamble, S. B.; Gore, A. H.; **Pisal, M. M.**; Salokhe, A. T.; Kolekar, G.B.; Helavi, V.B. One-pot three-component synthesis and photophysical properties of highly fluorescent novel 4-alkyl-3-aryl-2,6-dicyanoanilines by using tris(hydroxymethyl)aminomethane as a catalyst. *CDC.* **2019**, *19*, 100172.
2. Chavan, S.P.; Kawale, S.A.; **Pisal, M. M.;** Kadam, A. L.; Gonnade. Formal Synthesis of (-)-Quinagolide: A Diastereoselective Ring Expansion *via* Bicyclic Aziridiniumion Strategy to Access Octahydrobenzo[*g*]quinoline Architecture. *J. Org. Chem.* 2021, 86, 14, 9344.

Patents

1. Borate, H. B.; Chavan, S. P.; **Pisal, M. M.;** Annadate, R. A.; Sarkar, D.; Athalye, M. C. Novel compounds and uses thereof. *WO 2016/199172 Al; PCT/IN2016/050179.*

List of Posters Presented with Details

1. National Science Day Poster presentation at CSIR-National Chemical Laboratory, Pune (February 26-27, **2020**):

   Title: Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents: SAR and docking studies

   Abstract: A number of hybrid molecules containing thienopyrimidinones and thiouracil moieties were designed, synthesized and tested against Mycobacterium tuberculosis H37Ra wherein it was observed that the compounds 11-14 exhibited antitubercular activity *in vitro* (MIC 7.6-19.1 mg/mL, 12-35 µM) against dormant stage while the compound 15 exhibited antitubercular activity *in vitro* against dormant (MIC 23.4 mg/mL, 41 µM) as well as active (MIC 25.4 mg/mL, 45 µM) stage. Structural modifications of the compound 15 were carried out to study the structure-activity relationship and it was observed that the compound 18 exhibited antitubercular activity comparable to the compound 15. Cytotoxicity studies revealed that these molecules were non-toxic. The docking study of the compound 15 showed that there was binding with the active site of mycobacterial pantothenate synthetase.

MedChemComm

CONCISE ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/c5md00404g

Synthesis and evaluation of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents†

Hanumant B. Borate,*[a] Ritesh A. Annadate,[a] Sandip S. Vagh,[a] Mahesh M. Pisal,[a] Sagar B. Deokate,[a] Manisha A. Arkile,[b] Nandadeep J. Jadhav,[b] Laxman U. Nawale[b] and Dhiman Sarkar[b]

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www.rsc.org/medchemcomm

A number of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were designed, synthesized and screened against *Mycobacteria* as a part of our program to develop new antitubercular agents. It was observed that some of the compounds have significant antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *Mycobacterium bovis* BCG (ATCC 35743). The active compounds were studied for cytotoxicity against four cell lines and were found to be non-cytotoxic. The results showed that compounds **13b** and **29e** were found to exhibit very good antimycobacterial activity (MIC in the range of 6–8 μM) and the thienopyrimidinones as a class have potential to be developed as antitubercular agents.

1. Introduction

Tuberculosis (TB) is a major global health problem and as per the 2014 global tuberculosis report (ref. 1) which includes data compiled from 202 countries and territories, in 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease. The occurrence of multidrug resistant tuberculosis (MDR-TB) is complicating the situation further as globally, 3.5% of new and 20.5% of previously treated TB cases were estimated to have had MDR-TB in 2013. These facts indicate that development of new TB drugs is of great importance. In addition, the emergence of extensively drug-resistant TB (XDR-TB) also makes the efforts in this direction necessary as on average, an estimated 9.0% of patients with MDR-TB had extensively drug resistant TB (XDR-TB). However, the number of new drugs approved for TB treatment is negligible, and on December 28, 2012, the U.S. Food and Drug Administration (FDA) approved bedaquiline (**1**) (Fig. 1) as part of a combination therapy in adults to treat pulmonary multi-drug resistant tuberculosis, the first new treatment in 40 years. Subsequently, delamanid (**2**) received conditional approval by the European Medicines Agency (EMA) for the treatment of MDR-TB in November 2013. The search for new scaffolds is necessary to overcome the problem of limited choice of current TB drugs. Research in this direction has resulted a few hits *e.g.* **3**,[2] **4**,[3] **5**,[4,5] **6** (ref. 6) and **7** (ref. 7) (Fig. 2).

We wished to explore the potential of thienopyrimidinones as antitubercular agents. Substituted thienopyrimidinones exhibit various biological activities, as well as constitute the part of the molecular skeleton of a number of biologically active compounds, but their potential as antitubercular agents is rarely explored.[8] Substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** can be easily prepared from substituted 2-aminothiophene-3-carboxylates **9**, which in turn are prepared in one step by the Gewald synthesis[9,10] from easily available aldehydes or ketones **8** as starting materials (Scheme 1). The thieno[2,3-*d*]pyrimidin-4(3*H*)-ones of type **10** can be functionalized at various positions and thus provide an opportunity to have a number of compounds available for biological activity screening. A library of thienopyrimidinones was designed, synthesized and screened against mycobacteria.[11] Encouragingly, some of the molecules exhibited significant

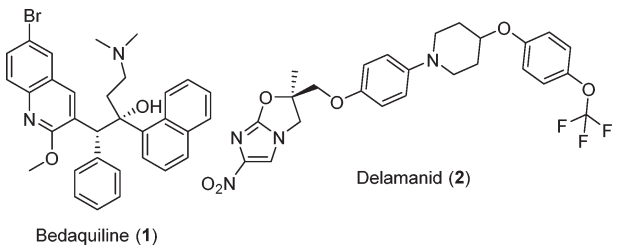

Fig. 1 Structures of bedaquiline and delamanid.

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[b] *Combichem Bio-resource Centre, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India*

† Electronic supplementary information (ESI) available: Details of synthesis, spectral data, and activity data. See DOI: 10.1039/c5md00404g

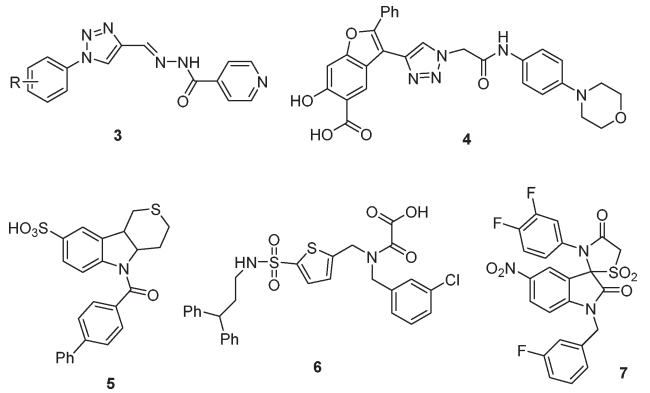
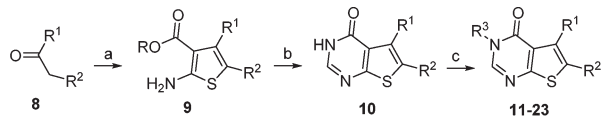


Fig. 2 Structures of compounds **3**–**7** exhibiting antimycobacterial activity.



11a: R^1=H, R^2=Me, R^3=Pr
11b: R^1=H, R^2=Me, R^3=i-Pr
11c: R^1=H, R^2=Me, R^3=i-Bu
11d: R^1=H, R^2=Et, R^3=Et
11e: R^1=H, R^2=Et, R^3=Pr
11f: R^1=H, R^2=Et, R^3=i-Pr
11g: R^1=H, R^2=Et, R^3=Pent
11h: R^1=H, R^2=Et, R^3=Oct
11i: R^1=H, R^2=Pr, R^3=Pr
11j: R^1=H, R^2=Pr, R^3=i-Pr
11k: R^1=H, R^2=Pr, R^3=Oct
11l: R^1=H, R^2=Pent, R^3=Me
11m: R^1=H, R^2=Pent, R^3=Pr
11n: R^1, R^2=-$(CH_2)_4$, R^3=Me
12a: R^1=H, R^2=Et, R^3=CH_2Ph
12b: R^1=H, R^2=Pr, R^3=CH_2Ph
12c: R^1=H, R^2=Bu, R^3=CH_2Ph
12d: R^1=H, R^2=Pent, R^3=CH_2Ph
12e: R^1=H, R^2=Hex, R^3=CH_2Ph
13a: R^1=H, R^2=Pent, R^3=CH_2CN
13b: R^1=H, R^2=Hept, R^3=CH_2CN
14a: R^1=H, R^2=Pent, R^3=CH_2-cyclopropyl
14b: R^1=H, R^2=Hex, R^3=CH_2-cyclopropyl
14c: R^1=H, R^2=Hept, R^3=CH_2-cyclopropyl
15a: R^1=H, R^2=Pr, R^3=CH_2-(4-bromomethyl)phenyl
16a: R^1=H, R^2=Pent, R^3=2-(1,3-dioxolan-2-yl)ethyl
16b: R^1,R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxolan-2-yl)ethyl
16c: R^1,R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxan-2-yl)ethyl
17a: R^1=H, R^2=Me, R^3=CH_2-CO-2,4-difluorophenyl
17b: R^1=H, R^2=Pr, R^3=CH_2-CO-2,4-difluorophenyl
17c: R^1=H, R^2=Pent, R^3=CH_2-CO-2,4-difluorophenyl
18a: R^1=H, R^2=Bu, R^3=allyl
19a: R^1=H, R^2=Pent, R^3=CH_2COOEt
19b: R^1=H, R^2=Et, R^3=CH_2COOEt — d
20b: R^1=H, R^2=Et, R^3=CH_2COOH
19c: R^1=H, R^2=Me, R^3=CH_2COOEt — d
20c: R^1=H, R^2=Me, R^3=CH_2COOH
21a: R^1=H, R^2=Bu, R^3=CH_2-$CH(OEt)_2$ — e
22a: R^1=H, R^2=Bu, R^3=CH_2-CHO
21b: R^1=H, R^2=Me, R^3=CH_2-$CH(OEt)_2$
23a: R^1=H, R^2=Pr, R^3=CH_2-CO-thiophen-2-yl
23b: R^1=H, R^2=Hex, R^3=CH_2-CO-thiophen-2-yl
23c: R^1,R^2=-$(CH_2)_4$, R^3=CH_2-CO-thiophen-2-yl

Scheme 1 Reagents and conditions: (a) Gewald synthesis;[9,10] (b) ref. 12 and 13; (c) R^3X, K_2CO_3, DMF or acetonitrile, RT; (d) NaOH, EtOH, H_2O, RT; (e) conc. HCl, CH_3CN, RT.

antimycobacterial activity; therefore, the work was continued further and the results are reported herein.

2. Results and discussion

2.1 Chemistry

The substituted 2-aminothiophene-3-carboxylates and thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** were prepared as reported in our earlier work.[12–14] A number of variously substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones with general structures **11** to **23** were obtained from thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** by reaction with the corresponding halides in DMF or acetonitrile in the presence of potassium carbonate, and further functional group transformations, as shown in Scheme 1. The structures of these compounds were confirmed by spectral methods.[14] Further functional group conversions provided the compounds with various other substituents.

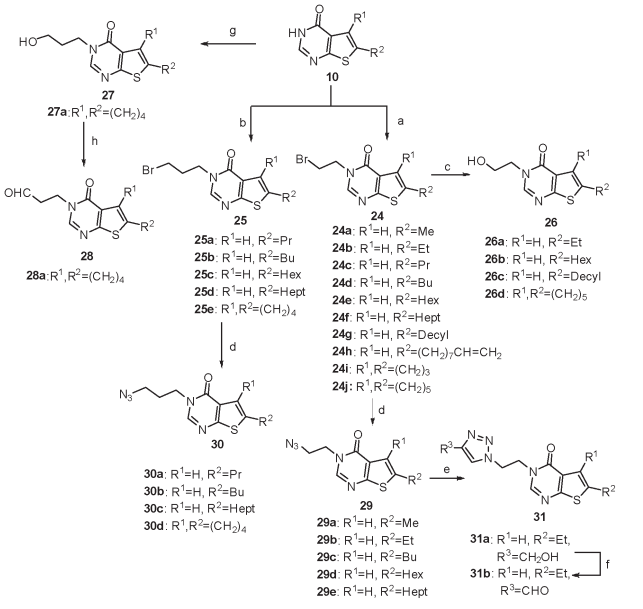
The utility of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** was further explored to obtain various bromides, azides, triazoles, alcohols and aldehydes, as exemplified in Scheme 2. The scheme also shows the flexibility and potential to get a large number of compounds for the biological activity study.

The efforts were continued further (Scheme 3) wherein thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** were reacted with propargyl bromide to obtain the acetylenic compounds **32** which were subjected to a click reaction with azides **29** or **30** to get the triazoles **33** or **34**.

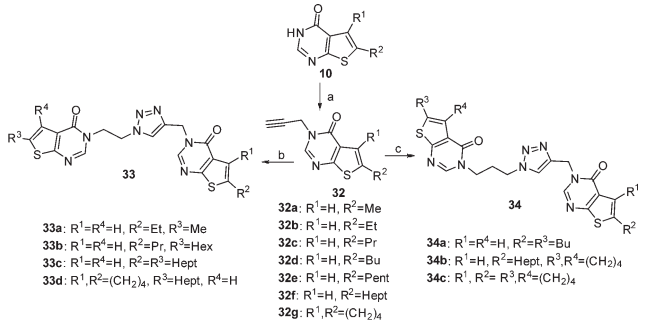
Efforts to prepare the dimeric compounds **35** by the reaction of the bromides **24** with thienopyrimidinones **10** or dibromoethane with excess thienopyrimidinones **10** afforded the desired compounds **35** as the major products and the compounds **36** as the minor products (Scheme 4). The reactions of bromides **24** or **25** with potassium phthalimide afforded the corresponding compounds **37** or **38**.

2.2 Biological evaluation

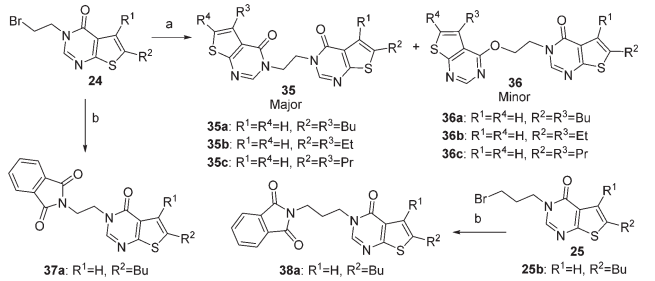
2.2.1 Antimycobacterial activity. All the synthesized compounds were screened for their *in vitro* activity against *M. tuberculosis* H37Ra (MTB) (ATCC 25177) and *M. bovis* BCG (BCG) (ATCC 35743) using a two-fold dilution technique, in order to determine the actual minimum inhibitory concentration (MIC). Activity against MTB was determined through the XTT reduction menadione assay (XRMA), reading absorbance at 470 nm as per the protocol described by Singh *et al.*[15]



Scheme 2 Reagents and conditions: (a) $BrCH_2CH_2Br$, K_2CO_3, dry DMF, RT; (b) $BrCH_2CH_2CH_2Br$, K_2CO_3, dry DMF, RT; (c) K_2CO_3, DMF, RT; (d) NaN_3, DMF, 80 °C; (e) propargyl alcohol, *t*-BuOH, water, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, RT; (f) IBX, DMSO, RT, 3 h; (g) $BrCH_2CH_2OH$, K_2CO_3, dry DMF, RT, 8 h; (h) IBX, EtOAc, reflux, 6 h.



Scheme 3 Reagents and conditions: (a) propargyl bromide, K_2CO_3, DMF, RT; (b) thienopyrimidinone **29**, *t*-BuOH, water, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, RT; (c) thienopyrimidinone **30**, *t*-BuOH, water, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, RT.



Scheme 4 Reagents and conditions: (a) required thienopyrimidinone **10**, K_2CO_3, DMF, RT: (b) potassium phthalimide, DMF, potassium iodide, 130 °C, 10 h.

Briefly, a compound solution (2.5 μl) was added in a total volume of 250 μl of *M. pheli* medium consisting of the MTB and BCG; sealed with plate sealers and allowed to incubate for 8 days (active stage) and 12 days (dormant stage) at 37 °C. The XRMA was then carried out to estimate viable cells present in different wells of the assay plate. To all wells, 200 μM XTT was added and incubated at 37 °C for another 20 min. It was followed by the addition of 60 μM of menadione and incubated at 37 °C for 40 min. The optical density was measured using a microplate reader (SpectraMax Plus 384 plate reader, Molecular Devices Inc.) at 470 nm wavelength against a blank prepared from a well free of cells. Absorbance obtained from the cells treated with 1% DMSO alone was considered as 100% cell growth. The nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG by the compounds.[16] Briefly, in the NR assay, 80 μl of culture from an incubated 96 well plate was taken into another 96 well plate, then 80 μl of 1% sulfanilic acid in 20% of conc. HCl was added, incubated for 10 min at room temperature, and then 80 μl of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution in distilled water was added. Finally, absorbance for the NR assay was measured at 540 nm.

In vitro activity studies against MTB and *M. bovis* BCG at active (8 days) and dormant (12 days) stages were performed using the XRMA and NR assays, respectively, as described above. Percentage inhibition was calculated using the following formula:

$$\%\ \text{inhibition} = [(\text{control}-\text{CMP})/(\text{control}-\text{blank})] \times 100$$

where 'control' is the activity of mycobacteria without compounds, 'CMP' is the activity of mycobacteria in the presence of compounds and 'blank' is the activity of the culture medium without mycobacteria.

2.2.2 Cytotoxicity. To check the selectivity, selected thienopyrimidinones were assayed for their cytotoxic effects in four different cell lines THP-1, MCF-7, A549 and HCT 116 using the MTT assay[17–19] (Table 2). The cell lines were maintained under standard cell culture conditions under 5% CO_2 at 37 °C in 95% humidified air environment. Each concentration was tested in duplicate in a single experiment. GI_{50} values were calculated using OriginPro Software.[17,18,20]

2.2.3 Selectivity index. The selectivity index (SI) was calculated by dividing the 50% growth inhibition concentration (GI50) for the cell lines (THP-1, A549, MCF-7 and HCT 116) by the MIC for *in vitro* activity against active/dormant MTB and BCG.[21]

2.2.4 Results of antimycobacterial activity. All the newly synthesized compounds were screened for their *in vitro* activity against *Mycobateria* by using *M. tuberculosis* H37Ra (MTB) and *M. bovis* BCG (BCG) species and the detailed results are given in the ESI.† In the primary screening, 12 compounds (**11g**, **11m**, **13b**, **14a**, **14b**, **14c**, **24g**, **24h**, **25d**, **26c**, **29d** and **29e**) were found to be active against both the species (Table 1) which were selected for further dose response screening.[14]

Compounds **13b** and **29e** showed very promising activity against active MTB, with MIC_{90} values of 2.51 μg mL^{-1} (8.68 μM) and 2.07 μg mL^{-1} (6.5 μM) respectively. Compounds **24h**, **26c** and **29d** showed MIC_{90} values in the range of 3 to 8 μg mL^{-1}, while the remaining compounds showed MIC_{90} values in the range of 10–50 μg mL^{-1}.

Similarly, activity was observed against *M. bovis* BCG. Compounds **24g**, **29e**, **13b**, **14b**, **14c** and **24h** showed MIC values < 3 μg mL^{-1} while compounds **11m**, **11g**, **29d** and **25d** showed MIC values < 10 μg mL^{-1}.

2.2.5 Results of cytotoxicity and selectivity index. All the synthesized compounds were further evaluated against four human cancer cell lines (MCF-7, A549, THP-1 and HCT 116) to check the toxicity of these compounds (Table 2).[14] The GI_{50} (>100 μg mL^{-1}) values of compounds **13b** and **29e** indicate that the compounds are potent and specific inhibitors against MTB. Compounds **11m**, **24g**, **24h**, **25d** and **29d** were found to be the most active antiproliferative compounds with IC_{50} values in the range of 14.70–45.44 μg mL^{-1} against the MCF7, A549 and HCT 116 cell lines. Compound **29d** showed the highest cytotoxicity (GI_{50} of 14.70 μg mL^{-1}) against A549. GI_{90} studies also indicated that compound **29d** had the highest cytotoxicity (GI_{90} of 74.66 μg mL^{-1}) against A549.[14]

The selectivity of the selected thienopyrimidinones towards the human cell lines against MTB is described in terms of the selectivity index (Table 3). The selectivity index reflects the concentration of the compound at which it is active against *Mycobacteria* but is not toxic towards host cells. A higher selectivity index indicates that the compound can be

Table 1 Antimycobacterial activity data for various thienopyrimidinones

Comp no.	*M. tuberculosis* H37 Ra (Active stage) MIC_{90} ($\mu g\ mL^{-1}$)	*M. tuberculosis* H37 Ra (Dormant stage) MIC_{90} ($\mu g\ mL^{-1}$)	*M. bovis* BCG (Active stage) MIC_{90} ($\mu g\ mL^{-1}$)	*M. bovis* BCG (Dormant stage) MIC_{90} ($\mu g\ mL^{-1}$)
11g	44.13	43.76	10.09	20.76
11m	42.89	36.32	8.51	9.82
13b	**2.51**	**5.20**	**1.89**	**3.02**
14a	38.32	>50	4.95	8.07
14b	45.86	42.70	1.37	2.34
14c	49.31	49.13	1.52	2.01
24g	24.58	35.21	2.29	3.35
24h	3.50	6.31	2.42	1.40
25d	17.51	18.49	6.30	9.37
26c	6.70	15.02	21.43	23.42
29d	8.42	11.54	4.08	4.51
29e	**2.07**	**8.33**	**1.30**	**2.26**
RIF	0.51	0.75	0.45	0.81

RIF indicates rifampicin, and MIC_{90} indicates the minimum inhibitory concentration for 90% (or greater) inhibition ($\mu g\ mL^{-1}$).

Table 2 Cytotoxicity profile of selected compounds against four human cancer cell lines

Comp no.	*In vitro* cytotoxicity of selected thienopyrimidinone compounds: MCF-7 (Breast) GI_{50} ($\mu g\ mL^{-1}$)	A549 (Lung) GI_{50} ($\mu g\ mL^{-1}$)	HCT 116 (Colon) GI_{50} ($\mu g\ mL^{-1}$)	THP-1 (Leukemia) GI_{50} ($\mu g\ mL^{-1}$)
11g	>100	>100	>100	>100
11m	33.80	27.36	>100	>100
13b	>100	>100	>100	>100
14a	>100	>100	>100	>100
14b	24.83	>100	17.64	>100
14c	>100	>100	>100	>100
24g	20.52	35.08	>100	>100
24h	26.12	45.44	42.41	>100
25d	19.68	34.15	>100	>100
26c	>100	>100	>100	>100
29d	>100	16.17	14.7	>100
29e	>100	>100	>100	>100
RIF	>100	>100	>100	>100
Paclitaxel	0.0048	0.0035	0.0260	0.1374

GI_{50} indicates concentration to inhibit 50% growth of cells.

Table 3 Selectivity index (SI) of selected thienopyrimidinones on human cell lines against *Mycobacterium tuberculosis* H37Ra and *M. bovis* BCG

Comp no.	SI on MCF-7		SI on A549		SI on HCT 116		SI on THP-1	
	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG
	Active stage of Mycobacterium tuberculosis H37Ra and *M. bovis* BCG							
11g	2	10	2	10	2	10	2	10
11m	1	4	1	3	2	12	2	12
13b	**40**	**53**	**40**	**53**	**40**	**53**	**40**	**53**
14a	3	20	3	20	3	20	3	20
14b	1	18	2	73	0	13	2	73
14c	2	66	2	66	2	66	2	66
24g	1	9	1	15	4	44	4	44
24h	7	11	13	19	12	18	29	41
25d	1	3	2	5	6	16	6	16
26c	15	5	15	5	15	5	15	5
29d	12	25	2	4	2	4	12	25
29e	**48**	77	**48**	77	**48**	77	**48**	77
RIF	196	222	196	222	196	222	196	222

used as a therapeutic agent. Compounds **13b** (SI: > 40) and **29e** (SI: > 45) showed very high SI index, which are actually good inhibitors of *M. tuberculosis* and *M. bovis* BCG. Although the selectivity index of rifampicin is very high, it is important to consider the significance of this study with respect to the developing resistance of microorganisms against available antibiotics. MDR and XDR mycobacterial strains have been reported to exhibit resistance against known anti-TB drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide.[1] Hence, there is a great need to screen new compounds having therapeutic potential and our efforts in the present study were directed towards this. According to a study by Hartkoorn *et al.*[22] on the drug susceptibility of TB, antimycobacterial activity was considered to be specific when the selectivity index was >10. In the current study, both **13b** and **29e** exhibited selectivity index values of >40 indicating their potential as antitubercular agents.

2.2.6. Structure–activity relationship. The preliminary studies reported herein indicate that the compounds with a thienopyrimidinone structural unit have potential to be studied further and to be developed as antitubercular agents. Some of the conclusions drawn from the structures of the compounds studied in the present work and antitubercular activity exhibited[14] are as follows:

The compounds with a longer alkyl chain at the 6 position of thieno[2,3-*d*]pyrimidin-4(3*H*)-one were observed to exhibit better antitubercular activity than the corresponding compounds with a shorter alkyl chain at the 6 position and the same functional group at the 3 position *e.g.* antitubercular activity was observed in the order of **29c** < **29d** < **29e**. Similarly, **13a** was observed to be less active than **13b**. The compounds with general structures **24** and **25** with 2-bromoethyl and 3-bromopropyl side chains respectively at the 3 position and alkyl chains at the 6 position exhibited a similar trend in activity with **24a** < **24e** < **24f** < **24g** and **25a** < **25c** < **25d**.

Compounds **11a–m** in the present work, having alkyl chains both at the 3 and 6 positions of the thienopyrimidinone unit, did not exhibit antitubercular activity. Also, the compounds having a benzyl group at the 3 position of thienopyrimidinone and a 2–6 carbon alkyl chain at position 6 (compounds **12a–e**) did not exhibit antitubercular activity. Compounds **14a–c** with a cyclopropylmethyl group at position 3 and pentyl, hexyl or heptyl chain at position 6 of thienopyrimidinone exhibited moderate antitubercular activity. Compounds having a propargylic side chain at position 3 of the thienopyrimidinone moiety and an alkyl chain ranging from methyl to heptyl at position 6, with general structure **32**, did not exhibit antitubercular activity. A detailed study with a greater number of compounds would be necessary to refine the structure–activity relationship conclusions drawn from the present preliminary results.

3. Experimental

The general experimental procedures and spectral data for all compounds prepared for antimycobacterial activity testing described in this article and the detailed screening results are given in the ESI.† The spectral data for active compounds are given below.

3.1. Spectral data for active compounds

3.1.1. 6-Ethyl-3-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11g). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, J = 7 Hz, 3H), 1.28–1.44 (m, 7H), 1.68–1.82 (m, 2H), 2.88 (q, J = 7 Hz, 2H), 3.99 (t, J = 7 Hz, 2H), 7.17 (s, 1H), 7.92 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.80, 15.21, 22.17, 23.91, 28.61, 29.17, 46.80, 117.44, 124.77, 145.62, 145.87, 157.22, 162.24. IR ($CHCl_3$): 1672 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{13}H_{18}ON_2S$ + H]: 251.1213, found: 251.1210; [$C_{13}H_{18}ON_2S$ + Na]: 273.1032, found: 273.1027. Melting point: 74 °C.

3.1.2. 6-Pentyl-3-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11m). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 7 Hz , 3H), 1.00 (t, J = 7 Hz, 3H), 1.27–1.47 (m, 4H), 1.62–1.94 (m, 4H), 2.84 (t, J = 8 Hz, 2H), 3.98 (t, J = 7 Hz, 2H), 7.16 (s, 1H), 8.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.93, 13.83, 22.20, 22.67, 30.44, 30.60, 30.94, 48.28, 118.08, 124.67, 144.44, 145.57, 157.13, 162.11. IR ($CHCl_3$): 1672 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{14}H_{20}ON_2S$ + H]: 265.1369, found: 265.1367; [$C_{14}H_{20}ON_2S$ + Na]: 287.1189, found: 287.1185. Melting point: 141 °C.

3.1.3. 2-(6-Heptyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)-acetonitrile (13b). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, J = 7 Hz, 3H), 1.23–1.49 (m, 8H), 1.63–1.78 (m, 2H), 2.86 (t, J = 8 Hz, 2H), 4.91 (s, 2H), 7.19 (s, 1H), 8.04 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.95, 22.48, 28.79 (2C), 30.51, 30.91, 31.57, 33.47, 113.82, 118.03, 124.09, 143.64, 146.23, 155.93, 162.25. IR ($CHCl_3$): 1686, 2358 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{19}ON_3S$ + H]: 290.1322, found: 290.1318; [$C_{15}H_{19}ON_3S$ + Na]: 312.1141, found: 312.1136. Melting point: 86 °C.

3.1.4. 3-(Cyclopropylmethyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14a). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.32–0.48 (m, 2H), 0.55–0.70 (m, 2H), 0.88 (t, J = 7 Hz, 3H), 1.17–1.44 (m, 5H), 1.63–1.75 (m, 2H), 2.82 (t, J = 7 Hz, 2H), 3.86 (d, J = 7 Hz, 2H), 7.14 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 4.03 (2C), 10.88, 13.83, 22.22, 30.45, 30.60, 30.95, 50.80, 118.14, 124.66, 144.39, 145.26, 157.31, 162.28. IR ($CHCl_3$): 1671 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{20}ON_2S$ + H]: 277.1369, found: 277.1369; [$C_{15}H_{20}ON_2S$ + Na]: 299.1189, found: 299.1186. Melting point: 73 °C.

3.1.5. 3-(Cyclopropylmethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14b). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.36–0.48 (m, 2H), 0.60–0.72 (m, 2H), 0.89 (t, J = 7 Hz, 3H), 1.17–1.45 (m, 7H), 1.62–1.77 (m, 2H), 2.84 (t, J = 7 Hz, 2H), 3.88 (d, J = 7 Hz, 2H), 7.16 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 4.14 (2C), 10.98, 14.03, 22.51, 28.59, 30.62, 31.01, 31.46, 50.93, 118.26, 124.80, 144.56, 145.31, 157.45, 162.39. IR ($CHCl_3$): 1683 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{16}H_{22}ON_2S$ + H]: 291.1526, found: 291.1519; [$C_{16}H_{22}ON_2S$ + Na]: 313.1345, found: 313.1336. Melting point: 49 °C.

3.1.6. 3-(Cyclopropylmethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14c). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.35–0.48 (m,

2H), 0.58–0.69 (m, 2H), 0.87 (t, J = 7 Hz, 3H), 1.14–1.47 (m, 9H), 1.68–1.76 (m, 2H), 2.83 (t, J = 7 Hz, 2H), 3.86 (d, J = 7 Hz, 2H), 7.15 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 4.07 (2C), 10.91, 13.98, 22.52, 28.80, 28.86, 30.53, 30.97, 31.62, 50.84, 118.18, 124.71, 144.45, 145.27, 157.35, 162.32. IR ($CHCl_3$): 1678 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{17}H_{24}ON_2S$ + H]: 305.1682, found: 305.1683; [$C_{17}H_{24}ON_2S$ + Na]: 327.1502, found: 327.1500. Melting point: 57 °C.

3.1.7. 3-(2-Bromoethyl)-6-decylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24g). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.19–1.45 (m, 14H), 1.72 (t, J = 7 Hz, 2H), 2.85 (t, J = 8 Hz, 2H), 3.77 (t, J = 6 Hz, 2H), 4.38 (t, J = 6 Hz, 2H), 7.16 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.07, 22.63, 28.91, 29.25 (2C), 29.46, 29.52, 29.76, 30.60, 31.02, 31.84, 48.73, 118.03, 124.53, 145.08, 145.66, 157.02, 162.75. IR ($CHCl_3$): 1676 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{18}H_{27}ON_2S^{79}Br$ + H]: 399.1100, found: 399.1102; [$C_{18}H_{27}ON_2S^{79}Br$ + Na]: 421.0920, found: 421.0920. Melting point: 73 °C.

3.1.8. 3-(2-Bromoethyl)-6-(non-8-enyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (24h). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.49 (m, 8H), 1.65–1.83 (m, 2H), 1.97–2.09 (m, 2H), 2.84 (t, J = 8 Hz, 2H), 3.76 (t, J = 6 Hz, 2H), 4.38 (t, J = 6 Hz, 2H), 4.90–5.07 (m, 2H), 5.73–5.90 (m, 1H), 7.15 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.80, 28.83, 28.90, 29.07, 29.76, 30.58, 30.98, 33.70, 48.74, 114.19, 118.05, 124.53, 139.05, 145.03, 145.67, 157.02, 162.75. IR ($CHCl_3$): 1674 cm^{-1}. HRMS (ESI) m/z calculated for $C_{17}H_{23}ON_2{}^{79}BrS$ + H]: 383.0787, found: 383.0786; [$C_{17}H_{23}ON_2{}^{79}BrS$ + Na]: 405.0607, found: 405.0605. Melting point: 150 °C.

3.1.9. 3-(3-Bromopropyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25d). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, J = 7 Hz, 3H), 1.18–1.40 (m, 8H), 1.58–1.80 (m, 2H), 2.25–2.49 (m, 2H), 2.82 (t, J = 7 Hz, 2H), 3.41 (t, J = 6 Hz, 2H), 4.17 (t, J = 6 Hz, 2H), 7.12 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.46, 28.74, 28.79, 29.78, 30.47, 30.90, 31.06, 31.55, 45.19, 117.92, 124.59, 144.82, 145.53, 157.13, 162.36. IR ($CHCl_3$): 1670 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{16}H_{23}ON_2{}^{79}BrS$ + H]: 371.0787, found: 371.0785; [$C_{16}H_{23}ON_2{}^{79}BrS$ + Na]: 393.0607, found: 393.0605. Melting point: 75 °C.

3.1.10. 6-Decyl-3-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (26c). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.18-1.43 (m, 14H), 1.62–1.78 (m, 2H), 2.82 (t, J = 8 Hz, 2H), 2.97 (t, J = 5 Hz, 1H), 3.97 (q, J = 5 Hz, 2H), 4.16 (t, J = 5 Hz, 2H), 7.08 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.65, 28.96, 29.27 (2C), 29.49, 29.54, 30.61, 31.03, 31.85, 49.42, 60.70, 117.91, 124.48, 144.92, 146.31, 157.78, 162.56. IR ($CHCl_3$): 1675, 3408 (bs) cm^{-1}. HRMS (ESI) m/z calculated for [$C_{18}H_{28}O_2N_2S$ + H]: 337.1944, found: 337.1937; [$C_{18}H_{28}O_2N_2S$ + Na]: 359.1764, found: 359.1756. Melting point: 62 °C.

3.1.11. 3-(2-Azidoethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29d). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.22–1.45 (m, 6H), 1.65–1.76 (m, 2H), 2.84 (t, J = 7 Hz, 2H), 3.76 (t, J = 6 Hz, 2H), 4.12 (t, J = 6 Hz, 2H), 7.14 (s, 1H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.99, 22.47, 28.55, 30.58, 30.96, 31.42, 46.15, 49.46, 118.01, 124.50, 145.06, 145.71, 157.11, 162.67. IR ($CHCl_3$): 1674, 2106 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{14}H_{19}ON_5S$ + H]: 306.1383, found: 306.1378; [$C_{14}H_{19}ON_5S$ + Na]: 328.1203, found: 328.1197. Melting point: 50 °C.

3.1.12. 3-(2-Azidoethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29e). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.19–1.45 (m, 8H), 1.62–1.82 (m, 2H), 2.84 (t, J = 7 Hz, 2H), 3.76 (t, J = 5 Hz, 2H), 4.12 (t, J = 6 Hz, 2H), 7.15 (s, 1H), 7.94 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.94, 22.49, 28.79, 28.83, 30.52, 30.94, 31.58, 46.09, 49.43, 117.95, 124.47, 144.99, 145.70, 157.02, 162.55. IR ($CHCl_3$): 1676, 2122 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{21}ON_5S$ + H]: 320.1540, found: 320.1537; [$C_{15}H_{21}ON_5S$ + Na]: 342.1359, found: 342.1354. Melting point: 50 °C.

4. Conclusions

In conclusion, thienopyrimidinones with varying structural features were synthesized for evaluation of antimycobacterial activity. From our initial screening studies, it was found that the thienopyrimidinones **11g**, **11m**, **13b**, **14a**, **14b**, **14c**, **24g**, **24h**, **25d**, **26c**, **29d** and **29e** exhibited significant activity against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis* BCG. Compounds **13b** and **29e** exhibited greater efficiency in terms of *Mycobacterial* inhibition, specificity and selectivity. Moreover, both compounds showed satisfactory biocompatibility against human cancer cell lines. These compounds are good candidates for further activity-guided fractionation in the search for new active therapeutic compounds. Our studies point to the possibility of accessing more active compounds based on the present encouraging findings, as the compounds described in the present work have various functional groups for further structural modifications.

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Research paper

Hybrids of thienopyrimidinones and thiouracils as anti-tubercular agents: SAR and docking studies

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ABSTRACT

A number of hybrid molecules containing thienopyrimidinones and thiouracil moieties were designed, synthesized and tested against *Mycobacterium tuberculosis* H37Ra wherein it was observed that the compounds **11**–**14** exhibited antitubercular activity *in vitro* (MIC 7.6–19.1 μg/mL, 12–35 μM) against dormant stage while the compound **15** exhibited antitubercular activity *in vitro* against dormant (MIC 23.4 μg/mL, 41 μM) as well as active (MIC 25.4 μg/mL, 45 μM) stage. Structural modifications of the compound **15** were carried out to study the structure-activity relationship and it was observed that the compound **18** exhibited antitubercular activity comparable to the compound **15**. Cytotoxicity studies revealed that these molecules were non-toxic. The docking study of the compound **15** showed that there was binding with the active site of mycobacterial pantothenate synthetase. Further docking studies led to the synthesis of the compounds **16** and **17** and the antitubercular activity screening results showed that these compounds have significant antitubercular activity. The compounds **15**–**18** (MIC 11–29 μg/mL, 19–51 μM) can be used as starting points for further optimization. The synthetic strategies used in the present work have potential to prepare a large number of compounds for further refinement of structures and the present results will be very useful in the development of a new class of antimycobacterial agents.

1. Introduction

Tuberculosis is an infectious disease that claims a number of deaths paralleled only by those from HIV/AIDS [1]. Development of new antitubercular drugs is very important due to occurrence of multidrug resistant tuberculosis (MDR-TB) and emergence of extensively drug-resistant tuberculosis (XDR-TB). However, tuberculosis is one of the neglected tropical diseases (NTDs), a diverse group of communicable diseases that prevail in tropical and subtropical conditions which affect populations living in poverty, without adequate sanitation and in close contact with infectious vectors. The development of new antitubercular drugs is very slow due to lack of adequate funding. As a result, after a gap of 40 years, the U.S. Food and Drug Administration (FDA) approved bedaquiline [2,3] in December 2012 as part of combination therapy in adults to treat pulmonary MDR-TB, followed by the interim guidance on the use of delamanid [4] in 2014. New drug development is a continuous, lengthy process and it is necessary to synthesize and screen a large number of chemical entities as a slight modification in the structure can cause dramatic decrease/increase in the biological activity and the same is applicable to the development of antitubercular agents [5–8]. As a part of a program to develop new drugs, we have synthesized a number of new molecules containing thienopyrimidinone moiety and have found that some of them exhibited promising antitubercular activity [9,10] while some exhibited antifungal activity [11,12]. We wished to explore the potential of hybrid molecules containing thienopyrimidinones and thiouracils (which are known to exhibit various biological activities [13–18] including anticancer, anti-inflammatory, antibacterial, antifungal *etc*) as the hybrid molecules are reported to have better activity in many cases [19]. Accordingly, various new molecules **11**–**15** and **18**–**27** were synthesized and characterized with the help of spectral methods. Structure of the representative molecule **18** was confirmed with X-ray crystallography. These molecules were screened against *Mycobacterium tuberculosis* H37Ra (ATCC

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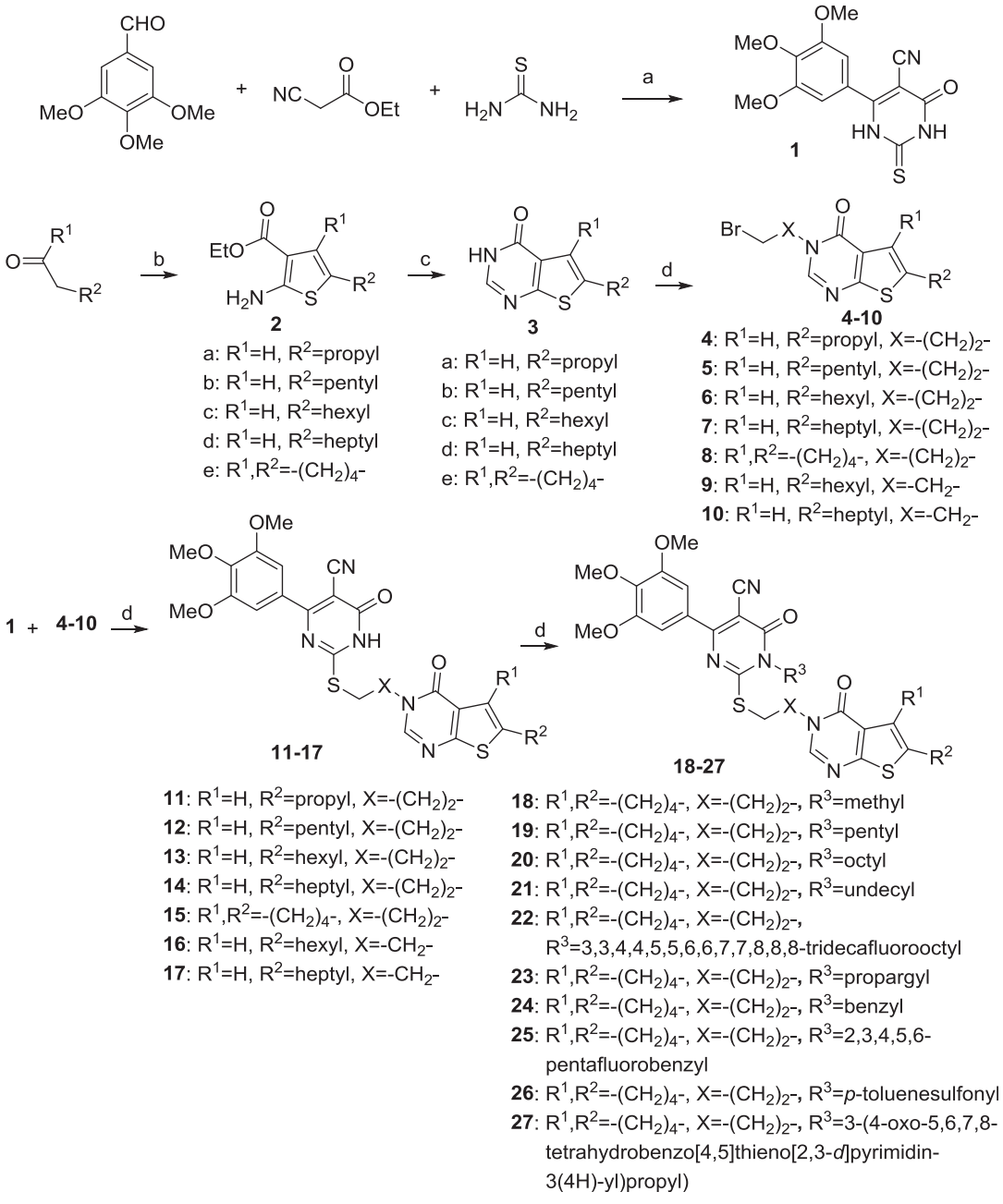
25177) wherein it was observed that the compounds **11**–**15**, **18**, **22** and **25** exhibited significant antitubercular activity. Based on the docking study results for compound **15**, compounds **16** and **17** were prepared and were found to be more active than the compound **15** and the results are reported herein.

2. Results and discussion

2.1. Chemistry

The synthetic route for various compounds in the present study is shown in Scheme 1. The intermediate 4-oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) was prepared by reaction of 3,4,5-trimethoxybenzaldehyde, ethyl cyanoacetate and thiourea by the known method [17]. The thienopyrimidinones **3a**–**e** were prepared by the methods described in our earlier work [9–12] involving Gewald reaction of required aldehyde/ketone with ethyl cyanoacetate and sulphur in DMF in the presence of triethylamine to get the corresponding substituted ethyl 2-aminothiophene-3-carboxylate followed by reaction with formamide in the presence of ammonium acetate. The bromides **4**–**10** were obtained from thienopyrimidinones **3a**–**e** by reacting them with corresponding dibromoalkanes in the presence of base. The reactions of 4-oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) with bromides **4**–**8** in DMF in the presence of potassium carbonate at room temperature afforded the hybrid molecules **11**–**15**. The compound **15** was reacted with various (un)substituted alkyl



Scheme 1. Reagents and conditions: **a**: K_2CO_3, DMF, 110 °C, 3 h; **b**: Ethyl cyanoacetate, sulphur, triethyl amine, DMF, 55 °C, 12 h; **c**: Ammonium acetate, formamide, 145 °C, 12 h; **d**: Required halide, K_2CO_3, DMF, RT, 12 h.

halides, propargyl bromide, (un)substituted benzyl bromides or *p*-toluenesulfonyl chloride in DMF in the presence of potassium carbonate at room temperature to obtain novel molecules **18**–**27**.

The structure of representative molecule **18** was confirmed by X-ray crystallography [20] and the ORTEP diagram is shown in Fig. 1.

When the thiouracil **1** was reacted with more than one equivalents of the bromide **8**, the N,S-dialkylated compound **27** was obtained in addition to the S-alkylated compound **15**. The N,S-dialkylated compound **27** was also obtained by reacting the S-alkylated compound **15** with the bromide **8**.

Based on results of antitubercular activity screening and docking study of the above molecules, compounds **16** and **17** were synthesized by reactions similar to those used for the synthesis of compound **15**.

The 6-(4-hydroxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**28**) and 4-oxo-2-thioxo-6-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**29**) were prepared by procedure [17] that was used for 4-oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) wherein 3,4,5-trimethoxybenzaldehyde was replaced with 4-hydroxybenzaldehyde and 4-methylbenzaldehyde.

2.2. Evaluation of biological activity

The new molecules synthesized in the present work were screened for antitubercular activity (Table 1) against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) by *in vitro* [21] and *ex vivo* methods [22,23].

It was found that the compounds **15, 16, 17** and **18** exhibited very good antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra (MIC 11–29 μg/mL, 19–51 μM) while compounds **1, 11, 12, 13, 14, 22** and **25** exhibited very good antitubercular activity against dormant stage of *M. tuberculosis* H37Ra. The cytotoxicity studies [24,25] against THP-1 monocytes (GI_{50} values > 50 μg/mL) showed that these compounds were non-toxic. The compounds synthesized in the present study were further studied for cytotoxicity against three human cancer cell lines and the results are shown in Table 2.

It was observed that the GI_{50} values for all compounds studied were >50 μg/mL indicating that the compounds were non-toxic.

The results of selectivity of hybrid molecules **11**–**27** towards human cell lines against H37Ra in terms of the selectivity index are shown in Table 3. The selectivity index reflects the concentration of the compound at which it is active against *mycobacteria* but is not toxic towards host cells. According to the study of Hartkoorn *et al.* [26] on the drug susceptibility of TB, antimycobacterial activity was considered to be specific when the selectivity index was >10. Some of the compounds e.g. **14** and **22** showed SI > 10 against dormant *M. tuberculosis* H37Ra, which were found to be good inhibitors of dormant *M. tuberculosis* H37Ra. Although the selectivity index values for other compounds studied in the present work were <10, it is important to consider the significance of this study with respect to the antitubercular activity exhibited by the compounds studied, flexibility of synthetic strategies used in the present work and occurrence of multidrug resistant tuberculosis (MDR-TB) as well as emergence of extensively drug-resistant tuberculosis (XDR-TB). As the synthetic strategies used in the present work have potential to prepare a large number of compounds for further refinement of structures, the present preliminary results will be very useful in the development of a new class of antimycobacterial agents with selectivity index in the desired range by suitable structural modifications.

The antitubercular screening results indicated following points regarding the structure-activity relationship of the compounds studied in the present work.

1.The hybrid molecules **11**–**17** with free NH in thiouracil moiety exhibited antitubercular activity against *M. tuberculosis* H37Ra.
2.The compounds **11**–**14** with three-carbon linker between thienopyrimidinone and thiouracil moieties and having alkyl substituent on thiophene ring of thienopyrimidinone exhibited antitubercular activity against dormant stage of *M. tuberculosis* H37Ra while corresponding compound **15** with three-carbon linker between thienopyrimidinone and thiouracil moieties and having cycloalkyl substituent on thiophene ring of

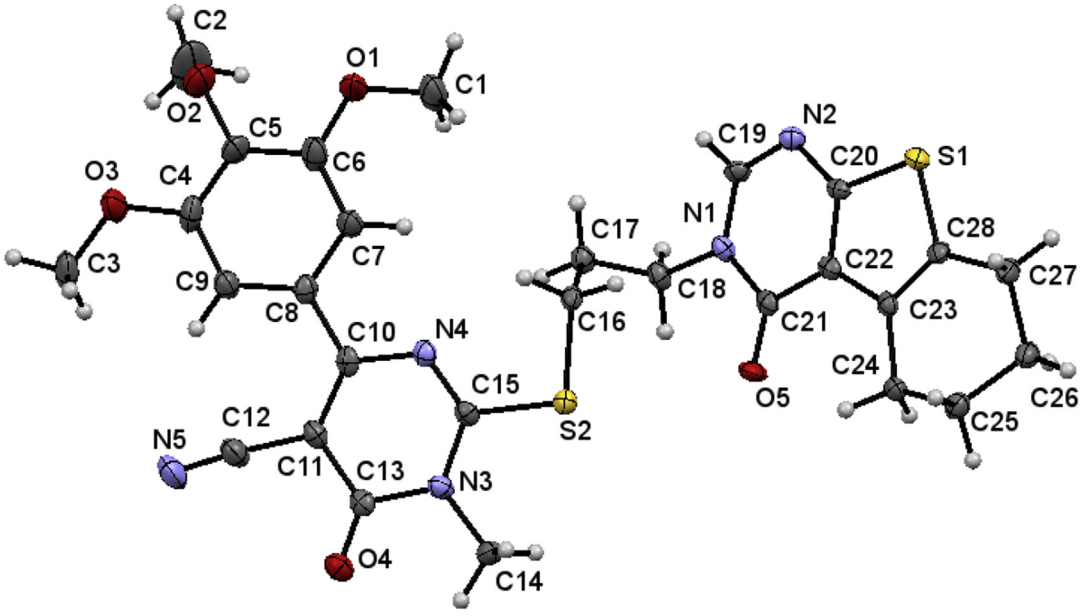


Fig. 1. The Oak Ridge Thermal Ellipsoid Plot (ORTEP) of compound **18** showing the atom numbering scheme. The displacement ellipsoids are drawn at the 30% probability level and H-atoms are shown as small spheres with arbitrary radii (CCDC No. 1504297).

Table 1
Antimycobacterial activity data for various hybrids (**11**–**27**) of thiouracils and thienopyrimidinones and thiouracils **1**, **28** and **29** against H37Ra.

Entry no	Compound no	Intracellular *(Ex Vivo)*		Extracellular *(In Vitro)*		In vitro
		M. tuberculosis H37 Ra (Dormant Stage)	*M. tuberculosis* H37 Ra (Active Stage)	*M. tuberculosis* H37 Ra (Dormant Stage)	*M. tuberculosis* H37 Ra (Active Stage)	Cytotoxicity against THP-1 monocytes
		MIC (μg/mL)		MIC (μg/mL)		GI_{50} (μg/mL)
1	**11**	**11.9** ± 1.80	>30	**19.1** ± 0.16	>30	>100
2	**12**	**9.8** ± 0.81	>30	**12.3** ± 0.27	>30	>100
3	**13**	**7.1** ± 0.91	>30	**8.7** ± 1.11	>30	54.3 ± 4.78
4	**14**	**6.91** ± 0.12	>30	**7.6** ± 0.38	>30	68.2 ± 0.39
5	**15**	**26.1** ± 1.45	**28.8** ± 3.0	**23.4** ± 1.42	**25.4** ± 0.65	97.6 ± 1.84
6	**16**	**18.8** ± 0.17	**17.1** ± 0.61	**19.1** ± 0.56	**15.6** ± 0.59	96.4 ± 1.55
7	**17**	**13.6** ± 0.23	**11.1** ± 2.6	**17.1** ± 0.85	**11.1** ± 0.15	88.1 ± 3.61
8	**18**	**21.5** ± 1.23	**26.9** ± 1.66	**26.1** ± 1.52	**27.9** ± 1.82	89.7 ± 0.93
9	**19**	>30	>30	>30	>30	>100
10	**20**	>30	>30	>30	>30	>100
11	**21**	>30	>30	>30	>30	>100
12	**22**	**8.8** ± 0.20	>30	**11.7**± 2.14	>30	>100
13	**23**	>30	>30	>30	>30	>100
14	**24**	>30	>30	>30	>30	>100
15	**25**	**26.5** ± 1.62	>30	**28.3**± 3.45	>30	>100
16	**26**	>30	>30	>30	>30	>100
17	**27**	>30	>30	>30	>30	81.4 ± 1.11
18	**1**	**11.3** ± 0.49	>30	**17.3** ± 0.44	>30	>100
19	**28**	>30	>30	>30	>30	93.0 ± 1.35
20	**29**	>30	>30	>30	>30	88.9 ± 1.92
21	**Rifampicin**	**0.75** ± 0.014	**0.51** ± 0.012	**0.48** ± 0.016	**0.41** ± 0.02	**0.14** ± 0.12

Ex vivo: Intracellular antitubercular activities of each agent in differentiated THP-1 cells.

Table 2
Cytotoxicity profile of various hybrids (**11**–**27**) of thiouracils and thienopyrimidinones and thiouracils **1**, **28** and **29** against human cancer cell lines.

Entry no	Comp no	A549 (Human lung)	PANC 1 (Human pancreas)	HeLa (Human cervix)
		GI_{50} (μg/mL)	GI_{50} (μg/mL)	GI_{50} (μg/mL)
1	**11**	>100	>100	52.5 ± 4.91
2	**12**	>100	72.8 ± 3.81	75.8 ± 4.32
3	**13**	90.2 ± 2.73	28.1 ± 7.10	67.0 ± 2.76
4	**14**	90.5 ± 3.54	>100	76.1 ± 3.65
5	**15**	91.4 ± 3.54	52.8 ± 7.04	55.7 ± 6.77
6	**16**	96.8 ± 1.40	36.5 ± 2.60	76.3 ± 6.84
7	**17**	79.7 ± 7.32	72.6 ± 6.48	75.4 ± 3.40
8	**18**	95.7 ± 4.18	24.8 ± 2.53	70.7 ± 0.84
9	**19**	>100	>100	73.0 ± 3.55
10	**20**	>100	>100	>100
11	**21**	>100	>100	>100
12	**22**	>100	>100	>100
13	**23**	>100	>100	66.3 ± 1.83
14	**24**	>100	51.9 ± 4.80	72.1 ± 2.12
15	**25**	>100	>100	>100
16	**26**	>100	>100	64.2 ± 3.91
17	**27**	83.1 ± 4.42	>100	89.6 ± 2.86
18	**1**	>100	>100	63.7 ± 4.71
19	**28**	87.4 ± 2.12	89.1 ± 6.93	90.8 ± 1.43
20	**29**	86.7 ± 4.33	>100	67.6 ± 3.12
21	**Paclitaxel**	0.0035 ± 0.0014	0.1279 ± 0.022	0.0048 ± 0.0012

thienopyrimidinone exhibited antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra.

3.The compounds **16** and **17** with two-carbon linker between thienopyrimidinone and thiouracil moieties and having alkyl substituent on thiophene ring of thienopyrimidinone also exhibited antitubercular activity against dormant as well as active stage of *M. tuberculosis* H37Ra.

4.Methyl group was tolerated on thiouracil moiety of compound **15** but alkylation with longer side chains resulted in loss of activity (compound **15** v/s compound **18** v/s compounds **19**, **20** and **21**).

5.The reaction of **15** with propargyl bromide, benzyl bromide, *p*-toluenesulfonyl chloride *etc* resulted in loss of activity indicating that free NH was preferred (compound **15** v/s compounds **23**, **24** and **26**). The compound **27** was also inactive supporting the above indication.

6.The compounds **20** and **24** were inactive while the corresponding fluorinated compounds **22** and **25** were active against dormant stage of *M. tuberculosis* H37Ra indicating that introduction of fluorine atoms helped to get better antitubercular activity.

The structure-activity relationship studies clearly indicated that it would be possible to get molecules with better antitubercular activity/selectivity index by suitable structural modifications.

Table 3
Selectivity index ratio of various hybrids (**11**–**27**) of thiouracils and thienopyrimidinones and thiouracils **1**, **28** and **29** against three human cancer cell lines.

Entry no	Comp no	SI against dormant *M. tuberculosis* H37Ra						SI against active *M. tuberculosis* H37Ra					
		A549		PANC-1		HeLa		A549		PANC-1		HeLa	
		A	B	A	B	A	B	A	B	A	B	A	B
1	**11**	>8	>5	>8	>5	4	3	>3	>3	>3	>3	2	2
2	**12**	>10	>8	>7	>6	8	6	>3	>3	>2	>2	3	3
3	**13**	13	10	4	3	9	8	3	3	1	1	2	2
4	**14**	13	12	14	13	11	10	3	3	3	3	3	3
5	**15**	3	4	2	2	2	2	3	4	2	2	2	2
6	**16**	5	5	2	2	4	4	6	6	2	2	4	5
7	**17**	6	5	5	4	6	4	7	7	6	7	7	7
8	**18**	4	4	1	1	3	3	4	3	1	1	3	3
9	**19**	>3	>3	>3	>3	2	2	>3	>3	>3	>3	2	2
10	**20**	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
11	**21**	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
12	**22**	>11	>8	>11	>8	>11	>8	>3	>3	>3	>3	>3	>3
13	**23**	>3	>3	>3	>3	2	2	>3	>3	>3	>3	2	2
14	**24**	>3	>3	2	2	2	2	>3	>3	2	2	2	2
15	**25**	>4	>4	>4	>4	>4	>4	>3	>3	>3	>3	>3	>3
16	**26**	>3	>3	>3	>3	2	2	>3	>3	>3	>3	2	2
17	**27**	3	3	>3	>3	3	3	3	3	>3	>3	3	3
18	**1**	>9	>6	>9	>6	6	4	>3	>3	>3	>3	2	2
19	**28**	3	3	3	3	3	3	3	3	3	3	3	3
20	**29**	3	3	>3	>3	2	2	3	3	>3	>3	2	2
21	**Rif**	133	208	133	208	133	208	196	244	196	244	196	244

A: *Ex vivo*; B: *In vitro*; Rif: Rifampicin.

2.3. Docking studies

In silico based approaches have provided a new perspective in the development of highly efficient chemical leads and have huge potential to impart as starting points in the development of new chemical entities against TB. So, docking studies were performed against mycobacterial pantothenate synthetase due to availability of limited resources for carrying out enzyme-based experimental studies to find out the best possible mode of action of the synthesized hybrids of thienopyrimidinones and thiouracil derivatives.

A distinctive characteristic of *M. tuberculosis* is that its cell wall is enriched with the lipids which are essential for its intracellular endurance, pathogenicity and also it is believed that it makes entry of antimicrobial agents in to the cells difficult [27]. In *M. tuberculosis* genome, huge numbers of genes encoding several enzymes are involved in the metabolism of fatty acids [28], so inhibition of this pathway can be an important target in antitubercular drug discovery.

The enzyme pantothenate synthetase (PS or PanC) is encoded by the gene panC, which is important for the synthesis of pantothenate in bacteria [27]. Pantothenate is required for biosynthesis of coenzyme A (CoA) and acyl carrier protein (ACP) which are the important elements for fatty acid synthesis [28]. In the *in vitro* studies, it was found that the gene encoding panC is essential for the optimum growth of bacteria and when it was genetically broken down in *M. tuberculosis*, it made the strain auxotrophic and required supplementation of pantothenate for its growth [29–31]. Also, its pathogenicity is weakened in this strain [32]. In mammals, panC is not present [33,34] so targeting this enzyme will have huge potential for developing drugs which will not have any side effects in the hosts. Thus, it makes pantothenate synthetase an important target for drug discovery against tuberculosis (TB). To continue our ongoing endeavor for discovering new potent antitubercular agents [10], this study provided valuable guidance for rationally designing more potent inhibitors for treatment of TB.

The antitubercular activity exhibited by compounds **11**–**15** prompted us to carry out docking studies [35–40] of compound **15** (Fig. 2). From the docking studies (details given in supporting information), it was clear that compound **15** was showing hydrogen bonding interactions with Gln72 with the distance of 2.10A° and 2.18A° and with Gly158 with the distance of 2.16A°. Also, it was observed that thiophene ring showed weak π- π stacking interaction with amino acid Arg198 which led to weak binding of compound **15** in the active site of PanC with the docking score of −5.863.

Encouraged by the antitubercular activity of compounds **11**–**15** and docking study of compound **15** indicating its binding in the active site of pantothenate synthetase, compounds **16** and **17** were designed and subjected to docking studies (details given in supplementary information). The docking scores of compounds **16** and **17** were −7.949 and −8.666 respectively. Molecular binding interactions (3D and 2D views) of compound **17** with the active site of mycobacterial pantothenate synthetase are shown in Figs. 3 and 4.

From the lowest energy docking pose of compound **17**, it was noticed that strong hydrogen bonding interactions were observed between methoxy groups present on the phenyl ring and amino acid residue Gln72 with the distance of 2.08A°. Oxo group attached to the pyrimidine ring in compound **17** showed the hydrogen bonding with Ser196 with the distance of 2.53A°. Oxo group attached to thienopyrimidinone showed the hydrogen bonding interactions with the amino acid residue Met40 with the distance of 2.59A°. Further, the heptyl group attached to thienopyrimidinone ring of compound **17** fitted into hydrophobic pocket formed by amino acid residues Phe157, Val142, Val143 and Ile168 present within the active site of mycobacterial pantothenate synthetase as seen in Fig. 4. The strong hydrogen bonding and hydrophobic interaction led to orientation of ligands within the active site so that they formed strong steric and electrostatic interactions with the amino acid residues present in the active site of mycobacterial pantothenate synthetase.

The compound **17** showed firm binding with the active site of mycobacterial pantothenate synthetase with the binding energy of −54.413 kcal/mol. Many favorable van der Waals interactions (details given in supporting information) were observed with amino acid residues present in the active site. Also, several strong

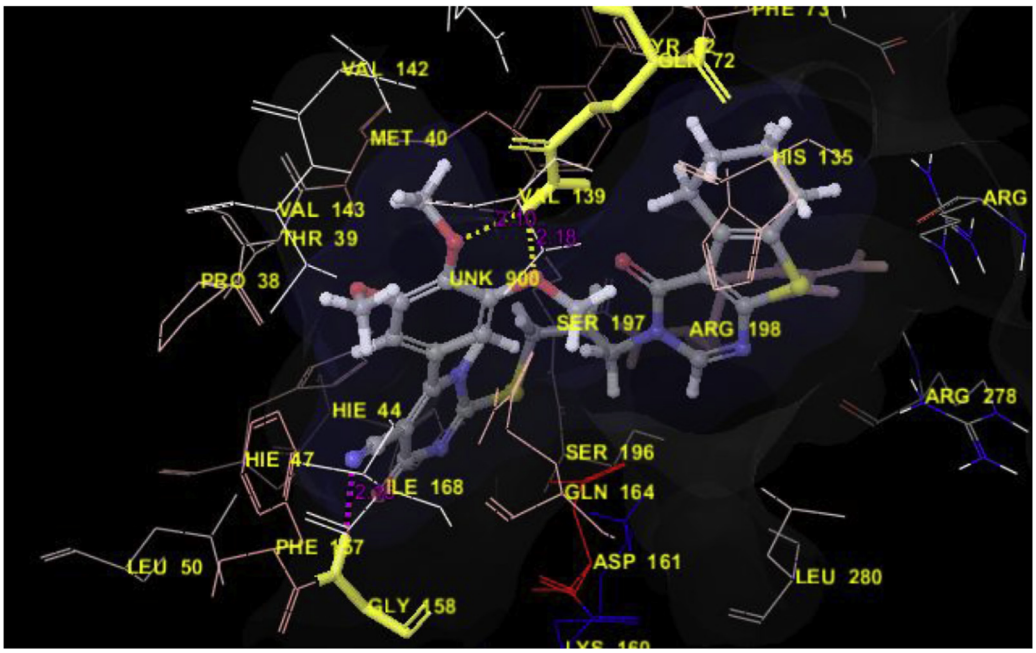


Fig. 2. 3D view of binding of compound **15** with the active site of mycobacterial PanC.

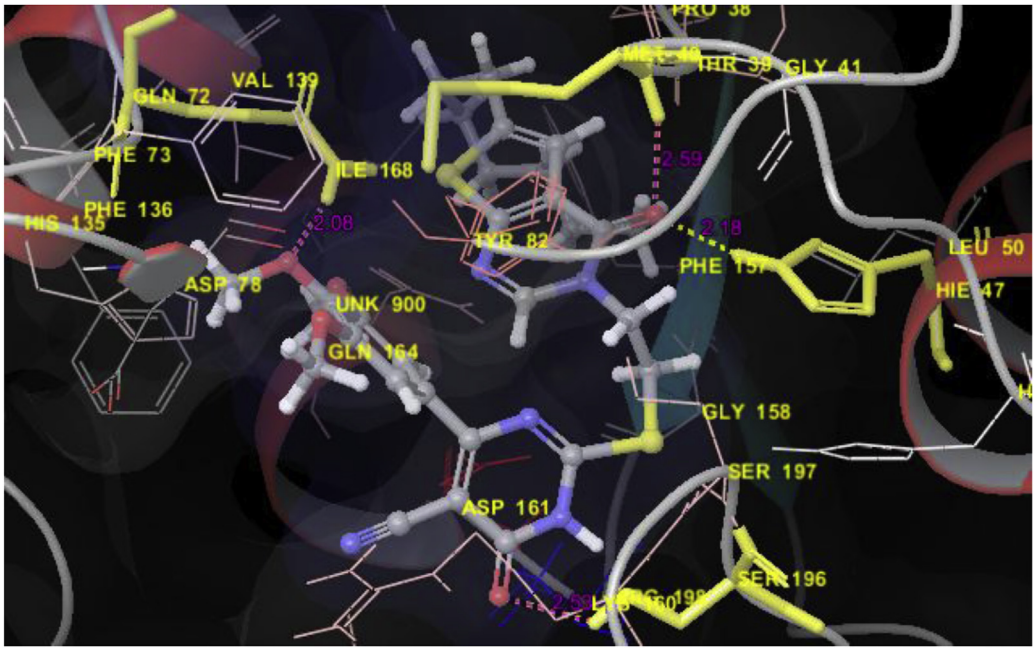


Fig. 3. 3D view of the binding of compound **17** with the active site of mycobacterial pantothenate synthetase.

electrostatic interactions (details given in supporting information) were seen with amino acid residues which stabilized the compound **17** in to the active site of mycobacterial pantothenate synthetase.

Validation of docking procedure: Co-crystallized ligand FG6 present in the active site of mycobacterial pantothenate synthetase was extracted and was docked again into the active site. Root mean square deviation (RMSD) value was found to be below 1.5 Å which validated our docking studies.

Accordingly, compounds **16** and **17** were synthesized and screened for antitubercular activity (Table 1, entries 6 and 7) wherein it was found that these compounds were more active than the corresponding compounds **13** and **14** as well as compound **15**. Thus, from the docking studies and antitubercular activity results, it was clear that hybrids of thienopyrimidinones and thiouracils had significant binding with the active site of mycobacterial pantothenate synthetase. So, the mechanism of action for antitubercular activity of these compounds might be through the inhibition of mycobacterial pantothenate synthetase.

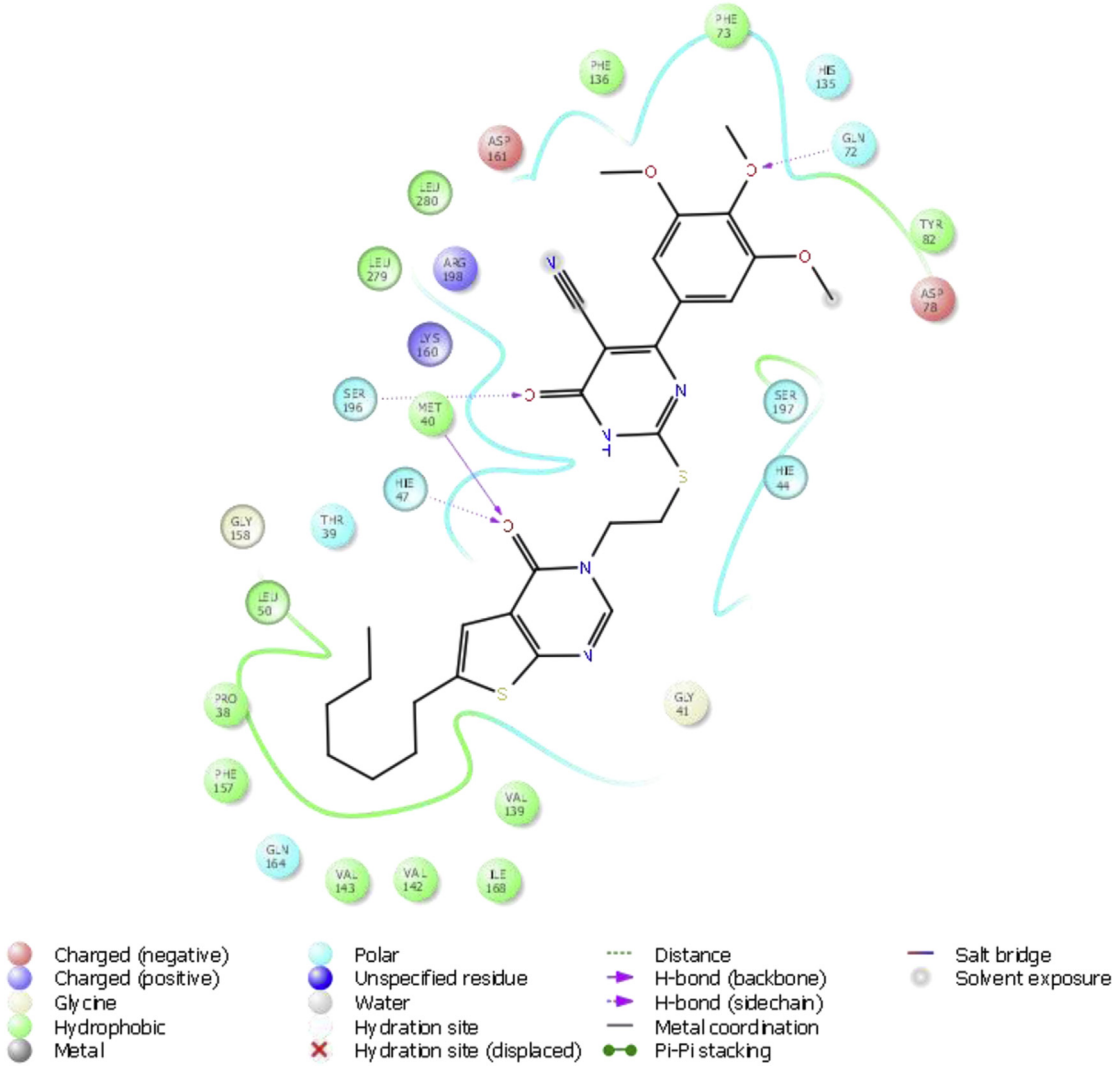


Fig. 4. 2D view of the binding of compound **17** with the active site of mycobacterial pantothenate synthetase.

3. Conclusions

Synthesis of hybrid molecules containing thiouracil and thienopyrimidinone moieties was achieved. The new chemical entities thus synthesized were tested against *Mycobacterium tuberculosis* H37Ra and it was observed that the compounds **11**–**14** exhibited antitubercular activity against dormant stage while compound **15** exhibited antitubercular activity against dormant as well as active stage. Structural modifications of compound **15** were carried out to study the structure-activity relationship and it was observed that compound **18** exhibited antitubercular activity comparable to compound **15** while compounds **22** and **25** exhibited antitubercular activity against dormant stage. Cytotoxicity studies revealed that these molecules were non-toxic. The docking study of compound **15** showed that there was binding with the active site of mycobacterial pantothenate synthetase with the docking score of −5.863. Further, the compounds **16** and **17** were designed based on docking studies and their docking scores were found to be −7.949 and −8.666 respectively indicating the possibility of these compounds to be more active. The synthesis and antitubercular activity screening of the compounds **16** and **17** was carried out and it was found that these compounds were having potent antitubercular activity supporting the binding of these compounds with mycobacterial pantothenate synthetase. Hence, the compounds **15**–**18** can be used as starting points for further optimization. The selectivity index for the compounds studied needs to be improved but the synthetic strategies used in the present work have potential to prepare a large number of compounds for further refinement of structures and the present results will be very useful in the development of new class of antimycobacterial agents.

4. Experimental section

4.1. Chemistry

All reagents and solvents were used as received from the manufacturers. Melting points were recorded in capillary tubes and are uncorrected and the temperatures are in centigrade scale. ^{1}H (200, 400 and 500 MHz) and ^{13}C (50, 100 and 125 MHz) NMR spectra were recorded on AC 200 MHz, AV 400 MHz or AV-500 MHz NMR spectrometers using $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts (δ) and coupling constants (Hz) are reported in the standard fashion with reference to chloroform, δ 7.27 (for ^{1}H) or the central line (77.0 δ) of $CDCl_3$ (for ^{13}C). In the ^{13}C NMR spectra, the natures of the carbons (C, CH, CH_2, or CH_3) were determined by recording the DEPT-135 spectra. The following abbreviations were

used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. FTIR spectra were recorded using KBr plate. The mass spectra were recorded on Orbitrap MS (LC-HRMS). The reaction progress was monitored by the TLC analysis using thin layer plates precoated with silica gel 60 F_{254} and visualized by UV light or iodine or by charring after treatment with *p*-anisaldehyde.

4.1.1. Synthesis of molecules

The thiouracils **1**, **28** and **29** were prepared by the reported procedure [17].

*4.1.1.1. 4-Oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(1)**.* Yield: 82%, White solid, Melting point: 283 °C. ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 3.47 (s, 3H), 3.51 (s, 6H), 6.60 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 55.2 (2C), 59.6, 62.4, 105.4 (2C), 113.6, 122.3, 140.2, 151.7 (2C), 157.6, 158.9, 175.3. IR ($CHCl_3$): 1214, 1689, 2405, 3417 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{14}H_{13}N_3O_4S$ + H]: 320.0700, found: 320.0696; [$C_{14}H_{13}N_3O_4S$ + Na]: 342.0519, found: 342.0516.

*4.1.1.2. 6-(4-Hydroxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(28)**.* Yield: 75%, White solid, Melting point: >295 °C. ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 6.00 (d, *J* = 8 Hz, 2H), 6.94 (d, *J* = 8 Hz, 2H), 9.06 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 82.8, 113.0 (2C), 117.4, 126.1, 128.6 (2C), 158.0, 161.6, 165.3, 180.3. IR ($CHCl_3$): 1225, 1625, 2209 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{11}H_7N_3O_2S$ + H]: 246.0332 found: 246.0330.

*4.1.1.3. 4-Oxo-2-thioxo-6-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(29)**.* Yield: 79%, White solid, Melting point: >295 °C. ^{1}H NMR (200 MHz, DMSO - d_6): δ 2.40 (s, 3H), 7.39 (d, *J* = 8 Hz, 2H), 7.60 (d, *J* = 8 Hz, 2H), 13.05 (bs, 1H). ^{13}C NMR (50 MHz, DMSO- d_6): δ 21.4, 90.6, 115.1, 126.6, 129.0 (2C), 129.2 (2C), 142.8, 158.8, 161.2, 176.4. IR ($CHCl_3$): 1217, 1675, 2232, 3317 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{12}H_9N_3OS$ + H]: 244.0538, found: 244.0539; [$C_{12}H_9N_3OS$ + Na]: 266.0357, found: 266.0359.

*4.1.1.4. Synthesis of 6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(15)**.* Potassium carbonate (6.48 g, 0.047 mol, 1.5 eq) was taken in a 500 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. 4-Oxo-2-thioxo-6-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) (10 g, 0.031 mol, 1.0 eq) was added under nitrogen followed by dry DMF (125 mL) and stirred for 10 min. 3-(3-Bromopropyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (10.20 g, 0.031 mol, 1.0 eq) was added and the reaction mixture was stirred at RT for 12 h. It was then diluted with water (400 mL) and extracted with ethyl acetate (3 × 200 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (**15**) as off-white solid, 12.0 g (68%). Melting point: 230 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73–1.91 (m, 4H), 2.33 (t, *J* = 7 Hz, 2H), 2.75 (t, *J* = 6 Hz, 2H), 2.94 (t, *J* = 6 Hz, 2H), 3.40 (t, *J* = 7 Hz, 2H), 3.89 (s, 6H), 3.94 (s, 3H), 4.16 (t, *J* = 7 Hz, 2H), 7.33 (s, 2H), 7.99 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.4, 22.0, 24.4, 24.8, 27.2, 27.9, 44.3, 55.6 (2C), 60.1, 92.1, 105.8 (2C), 115.5, 121.8, 129.4, 130.6, 133.5, 140.4, 145.0, 152.2 (2C), 157.0, 161.3, 161.6, 164.7, 166.0. IR ($CHCl_3$): 1217, 1243, 1550, 1668, 2210, 2857, 2933, 3429 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{27}H_{27}N_5O_5S_2$+H]: 566.1517, found: 566.1526.

The following compounds were prepared by using procedure described for compound **15**:

*4.1.1.5. 6-Oxo-2-((3-(4-oxo-6-propylthieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(11)**.* Yield: 73%, White solid, Melting point: 185 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7 Hz, 3H), 1.40–1.70 (m, 2H), 2.19 (s, 2H), 2.70 (t, *J* = 6 Hz, 2H), 3.23 (s, 2H), 3.76 (s, 6H), 3.85 (s, 3H), 4.11 (s, 2H), 7.04 (s, 1H), 7.15 (s, 2H), 8.01 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.7, 23.4, 26.9, 28.1, 31.6, 44.6, 55.4 (2C), 59.9, 90.5, 105.4 (2C), 116.9, 117.3, 123.6, 130.2, 139.6, 143.4, 145.3, 150.2, 152.0 (2C), 156.4, 161.6, 166.1, 167.6. IR ($CHCl_3$): 1215, 1683, 2210, 3450 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{26}H_{27}N_5O_5S_2$+H]: 554.1526, found: 554.1526; [$C_{26}H_{27}N_5O_5S_2$ +Na]: 576.1344, found: 576.1346.

*4.1.1.6. 6-Oxo-2-((3-(4-oxo-6-pentylthieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(12)**.* Yield: 78%, White solid, Melting point: 183 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, *J* = 7 Hz, 3H), 1.15–1.40 (m, 4H), 1.60 (t, *J* = 7 Hz, 2H), 2.18 (s, 2H), 2.72 (t, *J* = 7 Hz, 2H), 3.22 (s, 2H), 3.77 (s, 6H), 3.86 (s, 3H), 4.11 (s, 2H), 7.05 (s, 1H), 7.14 (s, 2H), 8.00 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9, 22.3, 28.0, 28.7, 30.5, 30.7, 31.1, 45.5, 56.1 (2C), 60.8, 90.3, 106.2 (2C), 117.9, 118.3, 118.4, 124.4, 130.3, 140.8, 145.3, 145.7, 152.7 (2C), 157.4, 162.5, 167.0, 167.1. IR ($CHCl_3$): 1257, 1651, 2215, 2932, 3415 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{28}H_{31}N_5O_5S_2$+H]: 582.1838, found: 582.1839.

*4.1.1.7. 2-((3-(6-Hexyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(13)**.* Yield: 81%, White solid, Melting point: 293 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J* = 7 Hz, 3H), 1.20–1.45 (m, 6H), 1.57–1.78 (m, 2H), 2.35 (t, *J* = 7 Hz, 2H), 2.82 (t, *J* = 7 Hz, 2H), 3.42 (t, *J* = 7 Hz, 2H), 3.90 (s, 6H), 3.94 (s, 3H), 4.20 (t, *J* = 7 Hz, 2H), 7.11 (s, 1H), 7.34 (s, 2H), 8.03 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.5, 28.3, 28.4, 28.6, 30.6, 31.0, 31.4, 45.4, 56.3 (2C), 61.0, 92.5, 106.7 (2C), 115.5, 115.6, 118.0, 124.6, 129.4, 141.8, 145.4, 145.6, 145.9, 153.0 (2C), 157.4, 162.3, 167.5. IR ($CHCl_3$): 1216, 1258, 1652, 2218, 3418 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{29}H_{33}N_5O_5S_2$+H]: 596.1995, found: 596.1996; [$C_{29}H_{33}N_5O_5S_2$+Na]: 618.1813, found: 618.1815.

*4.1.1.8. 2-((3-(6-Heptyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(14)**.* Yield: 83%, White solid, Melting point: 187 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (t, *J* = 6 Hz, 3H), 1.16–1.33 (m, 8H), 1.59 (t, *J* = 7 Hz, 2H), 2.17 (t, *J* = 7 Hz, 2H), 2.79 (t, *J* = 7 Hz, 2H), 3.27 (t, *J* = 7 Hz, 2H), 3.76 (s, 3H), 3.83 (s, 6H), 4.10 (t, *J* = 7 Hz, 2H), 7.06 (s, 1H), 7.29 (s, 2H), 8.36 (s, 1H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 14.1, 22.2, 27.8, 28.47, 28.50, 28.7, 29.9, 30.8, 31.4, 44.8, 56.2 (2C), 60.4, 92.2, 106.6 (2C), 116.8, 118.4, 124.0, 130.5, 140.6, 143.7, 147.7, 152.7 (2C), 156.7, 162.1, 162.2, 166.3, 166.5. IR ($CHCl_3$): 1255, 1655, 2214, 3418 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{30}H_{35}N_5O_5S_2$+H]: 610.2151, found: 610.2152; [$C_{30}H_{35}N_5O_5S_2$ +Na]: 632.1968, found: 632.1972.

*4.1.1.9. 2-((3-(6-Hexyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)ethyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile **(16)**.* Yield: 80%, White solid, Melting point: 125 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, *J* = 7 Hz, 3H), 1.13–1.40 (m, 6H), 1.41–1.70 (m, 2H), 2.70 (t, *J* = 7 Hz, 2H), 3.64 (s, 2H), 3.87 (s, 6H), 3.93 (s, 3H), 4.39 (s, 2H), 6.89 (s, 1H), 7.16 (s, 2H), 8.15 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 22.6, 29.0, 29.2, 29.5, 30.4, 30.9, 31.8,

56.1 (2C), 60.9, 106.4 (2C), 116.1, 117.8, 124.3, 129.0, 141.41, 141.42, 145.05, 145.08, 146.02, 146.03, 152.7 (2C), 157.0, 162.1, 167.6. IR ($CHCl_3$): 1223, 1672, 2213, 3403 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{28}H_{31}N_5O_5S_2$+H]: 582.1838, found: 582.1838.

4.1.1.10. 2-((2-(6-Heptyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl) ethyl)thio)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(17)***. Yield: 77%, White solid, Melting point: 130 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.84 (s, 3H), 1.22 (s, 8H), 1.53 (s, 2H), 2.65 (t, *J* = 6 Hz, 2H), 3.41 (s, 2H), 3.81 (s, 6H), 3.89 (s, 3H), 4.25 (s, 2H), 6.95 (s, 1H), 7.08 (s, 2H), 8.03 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0, 22.5, 28.6, 28.9, 29.0, 30.5, 31.1, 31.7, 47.4, 56.0 (2C), 60.8, 88.5, 105.8 (2C), 117.7, 121.1, 124.1, 131.1, 139.9, 145.5, 152.7 (2C), 157.4, 162.8, 167.1, 167.2, 172.4, 174.7. IR ($CHCl_3$): 1227, 1666, 2219, 3412 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{29}H_{33}N_5O_5S_2$+H]: 596.1996, found: 596.1985.

4.1.1.11. Synthesis of 1-methyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(18)***. Potassium carbonate (243 mg, 1.76 mmol, 2.0 eq) was taken in a 100 mL two-necked RB flask and heated under vacuum to remove the traces of moisture and flushed with nitrogen. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl) propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (**15**) (500 mg, 0.88 mmol, 1.0 eq) was added under nitrogen followed by dry DMF (1.5 mL) and stirred for 10 min. Iodomethane (0.08 mL, 188 mg, 1.32 mmol, 1.5 eq) was added by microlitre syringe and the reaction mixture was stirred at RT for 12 h. It was then diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 1-methyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)propyl) thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (**18**) as off-white solid, 435 mg (85%). Melting point: 185 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75-1.91 (m, 4H), 2.22–2.41 (m, 2H), 2.76 (t, *J* = 6 Hz, 2H), 2.95 (t, *J* = 6 Hz, 2H), 3.42 (t, *J* = 7 Hz, 2H), 3.55 (s, 3H), 3.90 (s, 6H), 3.92 (s, 3H), 4.13 (t, *J* = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 22.7, 25.1, 25.5, 28.3, 29.8, 30.9, 44.9, 56.3 (2C), 60.9, 92.1, 106.5 (2C), 115.7, 122.6, 129.5, 131.4, 134.7, 141.5, 145.0, 153.0 (2C), 157.8, 160.2, 161.9, 164.5, 165.3. IR ($CHCl_3$): 1218, 1669, 2219, 2358, 3411 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{28}H_{29}N_5O_5S_2$+H]: 580.1674, found: 580.1683; [$C_{28}H_{29}N_5O_5S_2$ +Na]: 602.1490, found: 602.1502.

The following compounds were prepared by using procedure described for compound **18** wherein the required halide was used in place of iodomethane.

4.1.1.12. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-1-pentyl-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(19)***. Yield: 74%, White solid, Melting point: 86 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.93 (t, *J* = 7 Hz, 3H), 1.30–1.52 (m, 4H), 1.70–1.96 (m, 6H), 2.20–2.42 (m, 2H), 2.67–2.85 (m, 2H), 2.91–3.06 (m, 2H), 3.26 (t, *J* = 7 Hz, 2H), 3.93 (s, 9H), 4.13 (t, *J* = 7 Hz, 2H), 4.46 (t, *J* = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.9, 22.1, 22.3, 22.8, 25.2, 25.6, 27.9, 28.1 (2C), 28.5, 45.3, 56.3 (2C), 60.9, 68.8, 87.6, 106.4 (2C), 114.9, 122.7, 129.8, 131.4, 134.4, 141.2, 145.2, 153.1 (2C), 157.7, 162.0, 167.8, 169.8, 174.0. IR ($CHCl_3$): 1217, 1247, 1468, 1536, 1669, 2219, 2358, 2932, 3411 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{32}H_{37}N_5O_5S_2$+H]: 636.2297, found: 636.2309; [$C_{32}H_{37}N_5O_5S_2$ +Na]: 658.2114, found: 658.2128.

4.1.1.13. 1-Octyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(20)***. Yield: 79%, White solid, Melting point: 78 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J* = 7 Hz, 3H), 1.21–1.52 (m, 12H), 1.75–1.95 (m, 6H), 2.25–2.38 (m, 2H), 2.79 (t, *J* = 7 Hz, 2H), 3.00 (t, *J* = 7 Hz, 2H), 3.27 (t, *J* = 7 Hz, 2H), 3.94 (s, 9H), 4.14 (t, *J* = 7 Hz, 2H), 4.47 (t, *J* = 7 Hz, 2H), 7.34 (s, 2H), 7.89 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.2, 22.6, 22.8, 25.2, 25.6, 25.8, 28.2, 28.5 (2C), 29.16, 29.23, 31.8, 45.3, 56.3 (2C), 61.0, 68.9, 87.7, 106.3 (2C), 115.0, 122.7, 129.9, 131.5, 134.6, 141.1, 145.2, 153.1 (2C), 157.8, 162.0, 167.9, 169.9, 174.0. IR ($CHCl_3$): 1197, 1230, 1450, 1530, 1677, 2209, 2911, 3409 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{35}H_{43}N_5O_5S_2$+H]: 678.2765, found: 678.2778.

4.1.1.14. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1-undecyl-1,6-dihydropyrimidine-5-carbonitrile ***(21)***. Yield: 78%, White solid, Melting point: 74 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7 Hz, 3H), 1.15–1.55 (m, 16H), 1.70–1.94 (m, 6H), 2.19–2.40 (m, 2H), 2.68–2.85 (m, 2H), 2.89–3.06 (m, 2H), 3.26 (t, *J* = 7 Hz, 2H), 3.93 (s, 9H), 4.13 (t, *J* = 7 Hz, 2H), 4.45 (t, *J* = 7 Hz, 2H), 7.33 (s, 2H), 7.88 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0, 22.1, 22.6, 22.8, 25.1, 25.6, 25.8, 28.2, 28.45, 28.53, 29.2 (2C), 29.4, 29.5 (2C), 31.8, 45.3, 56.3 (2C), 60.9, 68.8, 87.6, 106.4 (2C), 114.9, 122.6, 129.8, 131.4, 134.4, 141.2, 145.2, 153.1 (2C), 157.7, 162.0, 167.8, 169.8, 173.9. IR ($CHCl_3$):1247, 1280, 1422, 1542, 1638, 2139, 2891, 3401 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{38}H_{49}N_5O_5S_2$+H]: 720.3248, found: 720.3242; [$C_{38}H_{49}N_5O_5S_2$ +Na]:742.3067, found: 742.3060.

4.1.1.15. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-1-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(22)***. Yield: 64%, White solid, Melting point: 140 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73–1.96 (m, 4H), 2.21–2.42 (m, 2H), 1.54–1.87 (m, 4H), 2.89–3.07 (m, 2H), 3.28 (t, *J* = 7 Hz, 2H), 3.94 (s, 9H), 4.13 (t, *J* = 7 Hz, 2H), 4.82 (t, *J* = 7 Hz, 2H), 7.35 (s, 2H), 7.88 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.2, 22.8, 25.2, 25.6, 28.3, 28.6, 29.7, 30.5 (t), 45.3, 56.3 (2C), 61.0, 87.6, 106.5 (2C), 108.0–119.7 (m, 6C), 114.4, 122.7, 129.5, 131.5, 134.6, 141.5, 145.2, 153.2 (2C), 157.8, 162.0, 168.1, 169.1, 174.1. IR ($CHCl_3$): 760, 1250, 1301, 1432, 1492, 1608, 2149, 2931, 3411 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{35}H_{30}N_5O_5F_{13}S_2$+H]: 912.1553, found: 912.1554; [$C_{35}H_{30}N_5O_5F_{13}S_2$+Na]: 934.1371, found: 934.1373.

4.1.1.16. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-1-(prop-2-yn-1-yl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(23)***. Yield: 71%, White solid, Melting point: 171 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.79–1.91 (m, 4H), 2.28–2.37 (m, 2H), 2.55 (t, *J* = 2 Hz, 1H), 2.78 (t, *J* = 6 Hz, 2H), 2.99 (t, *J* = 6 Hz, 2H), 3.28 (t, *J* = 7 Hz, 2H), 3.93 (s, 9H), 4.14 (t, *J* = 7 Hz, 2H), 5.12 (d, *J* = 2 Hz, 2H), 7.34 (s, 2H), 7.90 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.2, 22.8, 25.2, 25.6, 28.3, 28.7, 45.4, 55.6, 56.3 (2C), 61.0, 76.2, 76.9, 87.6, 106.5 (2C), 114.5, 122.7, 129.5, 131.5, 134.5, 141.4, 145.2, 153.2 (2C), 157.8, 162.0, 168.2, 168.8, 174.2. IR ($CHCl_3$): 1220, 1655, 2219, 2230, 2355, 3410 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{30}H_{29}N_5O_5S_2$+H]: 604.1675, found: 604.1683; [$C_{30}H_{29}N_5O_5S_2$+Na]: 626.1491, found: 626.1502.

4.1.1.17. 1-Benzyl-6-oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(24)***. Yield: 64%, White solid, Melting point: 110 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65–2.00 (m, 4H), 2.13–2.44 (m, 2H), 2.59–2.84 (m, 2H), 2.88–3.11 (m, 2H), 3.37 (t, *J* = 7 Hz, 2H), 3.90 (s, 6H), 3.93 (s, 3H),

4.04 (t, $J = 6$ Hz, 2H), 5.30 (s, 2H), 7.28–7.56 (m, 7H), 7.75 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.1, 22.7, 25.2, 25.6, 28.1, 30.0, 45.0, 48.0, 56.4 (2C), 61.0, 92.7, 106.6 (2C), 115.6, 122.6, 128.1 (2C), 128.4, 128.8 (2C), 129.4, 131.4, 133.6, 134.7, 141.7, 145.0, 153.0 (2C), 157.7, 160.5, 162.0, 164.5, 165.0. IR ($CHCl_3$): 1217, 1241, 1455, 1527, 1538, 1624, 1680, 2213, 2901, 3410 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{34}H_{33}N_5O_5S_2$+H]: 656.1981, found: 656.1972.

4.1.1.18. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-1-((perfluorophenyl)methyl)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(25)***. Yield: 76%, White solid, Melting point: 138 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73–2.00 (m, 4H), 2.21–2.45 (m, 2H), 2.75 (t, $J = 5$ Hz, 2H), 2.94 (t, $J = 5$ Hz, 2H), 3.31 (t, $J = 7$ Hz, 2H), 3.93 (s, 9H), 4.14 (t, $J = 6$ Hz, 2H), 5.60 (s, 2H), 7.32 (s, 2H), 7.90 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.1, 22.7, 25.1, 25.5, 28.3, 28.8, 45.4, 56.3 (2C), 56.8, 60.9, 87.4, 106.5 (2C), 108.4 (dt), 114.3, 122.6, 129.4, 131.4, 134.5, 136.5–146.8 (m, 5C), 141.5, 145.2, 153.1 (2C), 157.7, 162.0, 168.2, 168.8, 174.1. IR ($CHCl_3$):1233, 1321, 1442, 1502, 1628, 2159, 2922, 3401 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{34}H_{28}N_5O_5F_5S_2$+Na]: 768.1334, found: 768.1344.

4.1.1.19. 6-Oxo-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(26)***. Yield: 58%, White solid, Melting point: 163 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.69–2.01 (m, 4H), 2.23–2.44 (m, 2H), 2.51 (s, 3H), 2.78 (t, $J = 6$ Hz, 2H), 3.02 (t, $J = 6$ Hz, 2H), 3.26 (t, $J = 7$ Hz, 2H), 3.93 (s, 9H), 4.10–4.28 (m, 2H), 7.32 (s, 2H), 7.47 (d, $J = 8$ Hz, 2H), 8.04 (d, $J = 8$ Hz, 2H), 8.18 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.8, 22.1, 22.8, 25.1, 25.6, 28.6, 29.0, 45.3, 56.3 (2C), 60.9, 88.7, 106.6 (2C), 113.3, 122.7, 128.7 (2C), 128.9, 130.1 (2C), 131.5, 132.5, 134.2, 141.9, 145.6, 146.8, 153.2 (2C), 157.7, 161.8, 165.1, 169.2, 175.1. IR ($CHCl_3$): 1145, 1239, 1284, 1362, 1373, 1396, 1492, 1557, 1668, 2225, 2936 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{34}H_{33}N_5O_7S_3$+H]: 720.1609, found: 720.1615; [$C_{34}H_{33}N_5O_7S_3$+Na]: 742.1427, found: 742.1434.

4.1.1.20. 6-Oxo-1-(3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)-2-((3-(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(4H)-yl)propyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile ***(27)***. Yield: 60% (from compound **15** by reaction with bromide **8**), White solid, Melting point: 129 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.75–1.93 (m, 8H), 2.19–2.45 (m, 4H), 2.77 (s, 4H), 2.97 (s, 4H), 3.24 (t, $J = 7$ Hz, 2H), 3.94 (s, 9H), 4.12 (t, $J = 7$ Hz, 2H), 4.23 (t, $J = 7$ Hz, 2H), 4.56 (t, $J = 7$ Hz, 2H), 7.34 (s, 2H), 7.91 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.1 (2C), 22.7 (2C), 25.1 (2C), 25.5 (2C), 27.9, 28.2, 28.6, 43.6, 45.3, 56.3 (2C), 60.9, 65.1, 87.3, 106.4 (2C), 114.7, 122.6, 122.7, 129.5, 131.4, 134.3, 134.4, 141.4, 141.5, 145.2, 145.5, 153.1 (2C), 157.7, 157.8, 162.0, 162.1, 167.8, 169.4, 174.2. IR ($CHCl_3$): 1127, 1500, 1556, 1667, 2212, 2937 cm^{-1}. HRMS (ESI) *m/z* calculated for [$C_{40}H_{41}N_7O_6S_3$+H]: 812.2347, found: 812.2353; [$C_{40}H_{41}N_7O_6S_3$+Na]: 834.2166, found: 834.2173.

4.2. Biological screening

4.2.1. Antitubercular activity

The compounds synthesized in the present work were tested for their *in vitro* inhibitory activity against dormant (12 days incubation) and active (8 days incubation) *M. tuberculosis* H37Ra using XRMA protocol as described by Singh *et al.* [21]. The absorbance of XRMA was measured at 470 nm. *Ex vivo* activity against dormant and active stages of MTB was estimated through nitrate reductase (NR) assay reading absorbance at 540 nm as per the protocol described by Khan and Sarkar [23]. MTB (ATCC No. 25177) were grown to logarithmic phase (O. D. 1.0) in a M. pheli medium. The stock culture was maintained at −70 °C and sub-cultured once in M. pheli medium before inoculation into the experimental culture. All experiments were performed in triplicates and IC_{50} and IC_{90} values were calculated from their dose–response curves.

4.2.2. Cytotoxicity assay

The cytotoxicity of the compounds was determined using MTT assay against three different human cancer cell lines and THP-1 monocytes in duplicate [24,25]. Leukaemia THP-1, lung A549 adenocarcinoma, pancreatic PANC-1 adenocarcinoma and HeLa cervical carcinoma cell lines were obtained from the European Collection of Cell Cultures (ECCC), Salisbury, UK. Cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humidified environment.

4.2.3. Docking studies

Molecular docking studies were carried out to predict the probable mode of action of antitubercular activity of the synthesized hybrids of thienopyrimidinones and thiouracil derivatives with mycobacterium tuberculosis pantothenate synthetase (PDB id: 3IVX).

To perform docking studies Glide 7.1 [35] was used. All chemical structures were drawn using 2D-sketcher incorporated within Maestro 10.6 [36]. LigPrep 3.8 [37] was used to prepare 3D structures with corrected chiralities and each individual structure is refined with the optimized energy and best possible conformations. Protein obtained was initially purified by adding H-atoms wherever necessary to the amino acid residues and bond order was assigned to find out the correct ionization and tautomeric states of them. All the water molecules present within the active site were removed and missing side chains were added using Prime 4.4 [38]. For further purification, protein was refined by checking the protonation state of histidines, terminal acids and of polar hydrogens. The existing steric clashes present within the protein were relaxed using OPLS-2005 force field and was terminated once root mean square deviation reached 0.30 Å [39,40].

Grid was prepared by selecting the co-crystallized ligand FG6 and the length of enclosing cubic box was set to 20 A° to cover the maximum area of active site around the co-crystallized ligand.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.01.009.

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Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl-2,6-dicyanoanilines

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ABSTRACT

Synthesis of 3,4,5-triheterocyclyl-2,6-dicyanoanilines, starting from heterocyclic aldehydes and 1,2-diheterocycle-substituted ethanones, is described. 2,6-Dicyanoanilines with one or two heterocyclic substituents have also been synthesized. It was found that some of these molecules have selective cell-staining properties useful for cell imaging applications. The compounds **1**g, **10f** and **11** were found to stain cytoplasm of the cells in contact but not the nucleus while the compound **12** showed affinity to apoptotic cells resulting in blue fluorescence. The cell imaging results with compound **12** were similar to Annexin V-FITC, a known reagent containing recombinant Annexin V conjugated to green-fluorescent FITC dye, used for detection of apoptotic cells. These compounds were found to be non-cytotoxic and have potential application as cell imaging agents.

Substituted 2,6-dicyanoanilines are important compounds studied[1a] as such or as intermediates in diverse fields like optical materials, dyes, textile printing, heat resistant polymers, chiral stationary phases in chromatography *etc*. They also constitute molecular skeleton of a number of compounds, with an array of structural variations, some of which have been studied for potential medicinal use. The synthetic methods to prepare these moieties and their applications have been reviewed recently[1a] and the continued interest in these compounds has resulted in further publications.[1b,1c,2a–c] We desired to study the synthesis and optical/biological properties of dicyanoanilines with one, two or three heterocyclic substituents. The literature survey revealed that there are many references for 3-heterocyclyl-2,6-dicyanoanilines,[1a] a few references for 3,5-diheterocyclyl-2,6-dicyanoanilines[2] and a very few references for 4-heterocyclyl-2,6-dicyanoanilines[3] but there are no references for 3,4-diheterocyclyl-2,6-dicyanoanilines or 3,4,5-triheterocyclyl-2,6-dicyanoanilines. We report herein synthesis of various hitherto unknown 3,4-diheterocyclyl-2,6-dicyanoanilines and 3,4,5-triheterocyclyl-2,6-dicyanoanilines, their fluorescence properties and cell imaging applications. Though there are a large number of publications about cell imaging,[4] to our knowledge, this is the first report describing cell imaging potential of 2,6-dicyanoanilines and these preliminary results will lead to new applications of dicyanoanilines for cell imaging.

Various hitherto unknown 3,4,5-triheterocyclyl-2,6-dicyanoanilines were prepared from 1-benzyl-1,2,3[1*H*]-triazole-4- carboxaldehyde **8**, malononitrile and required 1,2-diheterocyclyl ethanone as exemplified by the synthesis of 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(4-oxo-6-propylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)-6-(thiophen-2-yl)isophthalonitrile (**1a**) shown in Scheme 1.

Initially, thienopyrimidinone was chosen as one of the heterocycles because thienopyrimidinones[5] with various substituents can be synthesized easily from substituted 2-aminothiophene-3-carboxylates which in turn can be prepared by Gewald synthesis.[6] Thus, ketone **6a** was prepared from 2-bromoacetylthiophene[7] **3a** and 6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **5a**[5] in the presence of triethyl amine in ethyl acetate and reacted[8] with 1-benzyl-1,2,3[1*H*]-triazole-4-carboxaldehyde **8**[9] and malononitrile in DMF in the presence of morpholine to obtain the 3,4,5-triheterocyclyl-2,6-dicyanoaniline **1a** in 43% isolated yield. The 3,4,5-triheterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-5-aryl-2,6-dicyanoanilines **1a–j** thus prepared, using aldehyde **8** and appropriate ketones, are shown in Table 1.

Structures of these compounds were assigned based on ^{1}H NMR, ^{13}C NMR, IR and HRMS data.[10] The structures of representative

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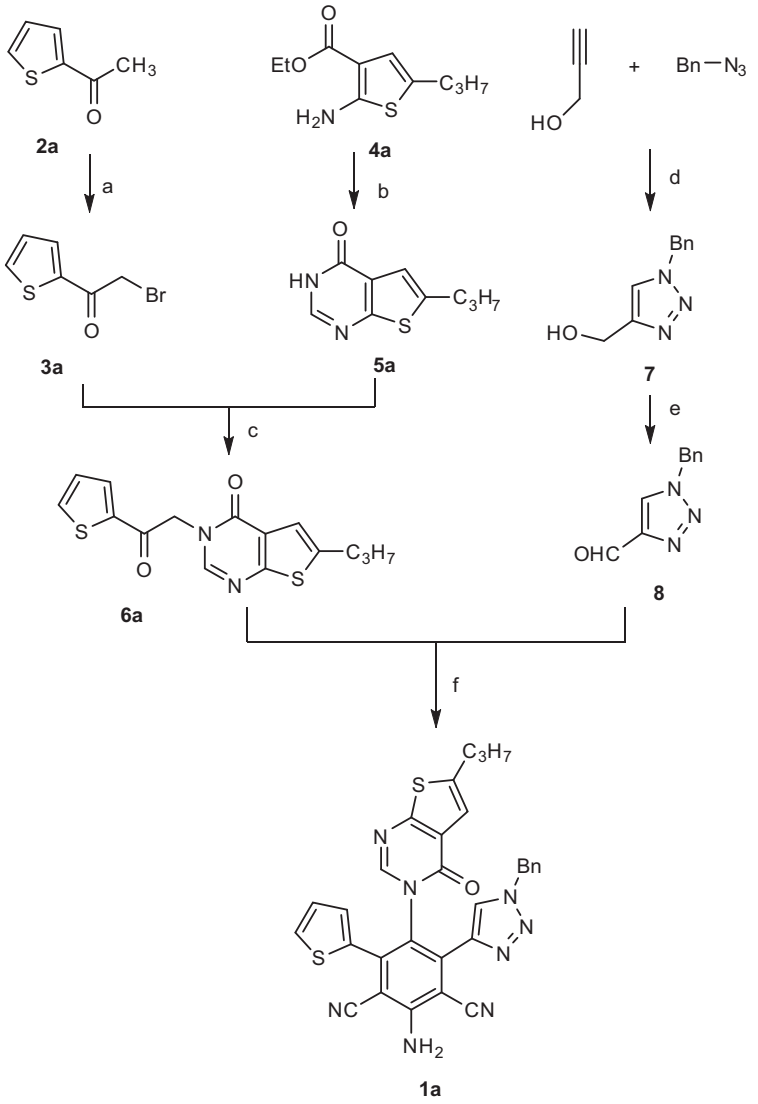


Scheme 1. Reagents and conditions: (a) Br_2, ether, 0 °C, 1.5 h, 72%; (b) Formamide, ammonium acetate, 145 °C, 10 h, 75%; (c) Triethyl amine, acetone, 0 °C, 1 h, RT, 3 h, 86%; (d) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-butanol-water, RT, 12 h, 94%; (e) MnO_2, ethyl acetate, RT, 2 h, 92%; (f) Malononitrile, morpholine, DMF, 80 °C, 12 h, 43%.

compounds **1c** and **1e** were further confirmed by X-ray crystallography.[10]

Using a similar strategy, various hitherto unknown 3-heterocyclyl-2,6-dicyanoanilines **9**, **10a–f**, **11** and **12** were prepared by reacting aldehyde **8** with appropriate aldehyde/ketone (Table 2). The dicyanoanilines **1e** and **1f** were methylated[2a] by subjecting them to NaH-MeI in THF to get the corresponding *N,N*-dimethylated compounds 5-(1-benzyl-1H-1,2,3-triazol-4-yl)-3-(dimethylamino)-2′,4′-difluoro-6-(1H-1,2,4-triazol-1-yl)-[1,1′-biphenyl]-2,4-dicarbonitrile (**13e**) and 5-(1-benzyl-1H-1,2,3-triazol-4-yl)-3-(dimethylamino)-4′-fluoro-6-(1H-1,2,4-triazol-1-yl)-[1,1′-biphenyl]-2,4-dicarbonitrile (**13f**).[10] Similarly, compounds **10a–f** were methylated to obtain the corresponding *N,N*-dimethylated compounds **14a–f** (Table 2).

There is a possibility to modify the structures of the compounds in the present work, in order to tune the optical properties, as there is a lot of flexibility in the synthetic schemes described.

These compounds were studied for their optical properties as they were colored in solution (Please see Supplementary data Table S2).

The UV–visible absorption spectra, of 2.5×10^{-5} M solutions in acetonitrile, for representative 2,6-dicyanoaniline compounds **1e**, **1g**, **10f**, **11** and **12** and N,*N*-dimethylated compounds **13e** and **14f** are shown in Fig. 1**A** while fluorescence spectra for these compounds are shown in Fig. 1**B**.

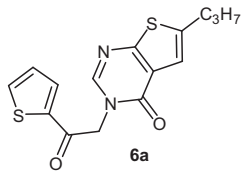
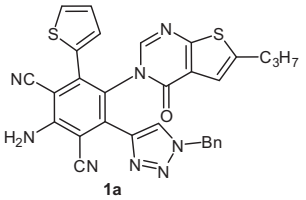
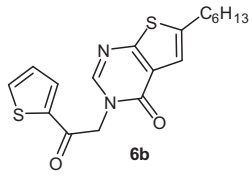
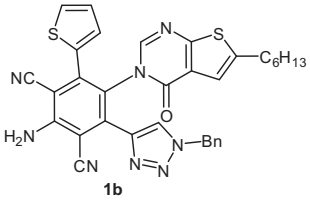
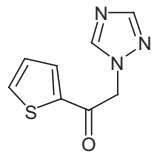
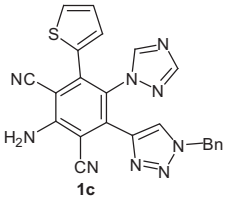
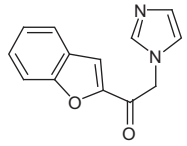
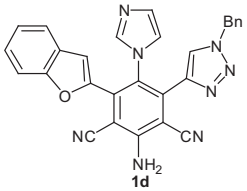
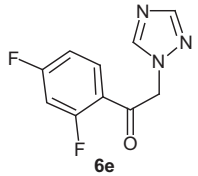
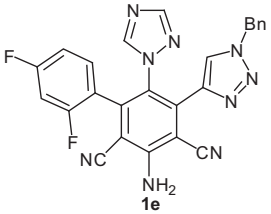
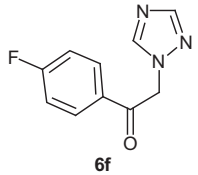
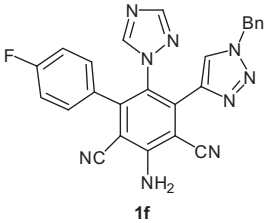
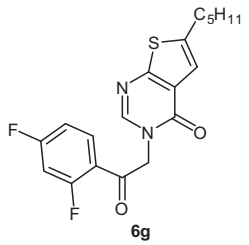
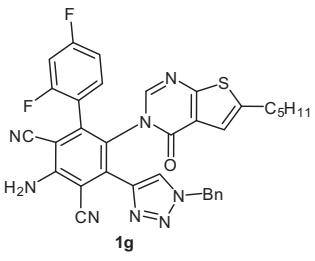
It was observed that there was a red shift in absorption maximum of the amino group after methylation due to enhanced electron-donating ability e.g. the λ_{max} value for amino compound **1e** was 367 nm while that for its corresponding *N,N*-dimethylated derivative **13e** was 395 nm. It was observed that the Stokes shift for the amino compound **1e** was 47 nm while the stokes shift for the corresponding *N,N*-dimethylated derivative **13e** was 74 nm indicating that the Stokes shift is increased after *N,N*-dimethylation of amino group. There was change in color of fluorescence in acetonitrile solutions of same concentration of these compounds. The compounds studied in the present work are soluble in DMSO, DMF, THF, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, methanol and ethanol while they are insoluble in water and pet ether. The HOMO-LUMO gaps estimated from DFT calculations[11] were found to be in good agreement with the experimental values derived from uv-visible spectra (Please see Supplementary data).

The fluorescent molecules have a wide application in biological research where they have been developed as valuable tools to

study basic physiological processes within biological systems and there are a number of reports wherein they have been explored for cell imaging studies.[4]

Apoptosis is a process of programmed cell death in which the cell shrinks, nuclear envelope disassembles and DNA breaks into fragments.[12] The cell surface is altered which serves as a signal for the neighboring macrophages to phagocytose it.[13,14] Phosphatidylserine (PS) which is actively held on the interior (cytosolic) side of the lipid bilayer is externalized which serves as a cell surface signal for apoptosis.[15,16] Annexin V is a Ca^{2+} dependent

Table 1
The 3,4,5-triheterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-5-aryl-2,6-dicyanoanilines prepared.

Entry no	Ketone used	Product obtained	Yield %
1	 **6a**	 **1a**	43
2	 **6b**	 **1b**	45
3	 **6c**	 **1c**	44
4	 **6d**	 **1d**	38
5	 **6e**	 **1e**	49
6	 **6f**	 **1f**	52
7	 **6g**	 **1g**	31

(*continued on next page*)

Table 1 (*continued*)

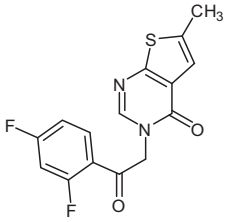
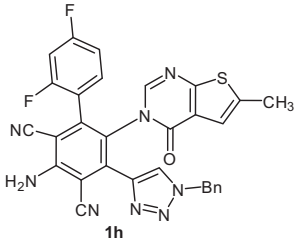
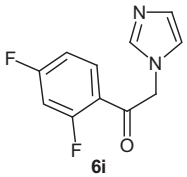
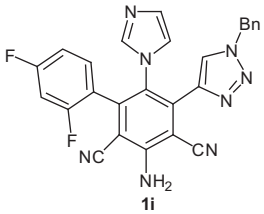
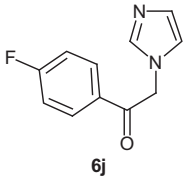
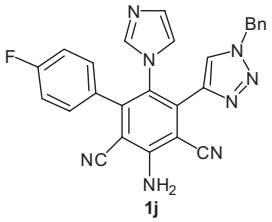
Entry no	Ketone used	Product obtained	Yield %
8	 **6h**	 **1h**	37
9	 **6i**	 **1i**	43
10	 **6j**	 **1j**	47

Table 2
The novel 3-heterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-2,6-dicyanoanilines prepared.

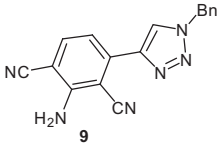
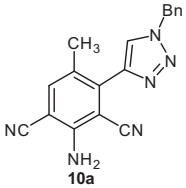
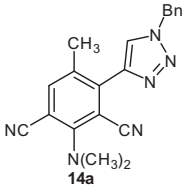
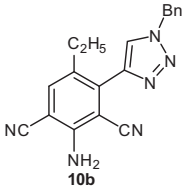
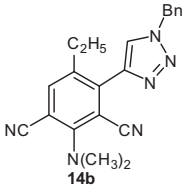
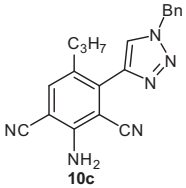
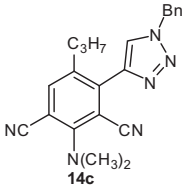
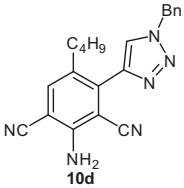
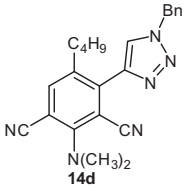
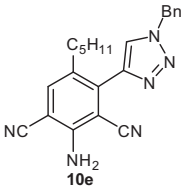
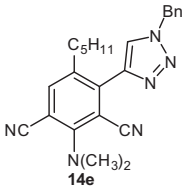
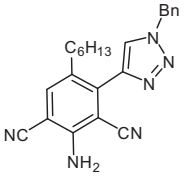
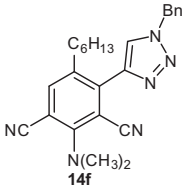
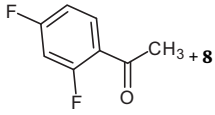
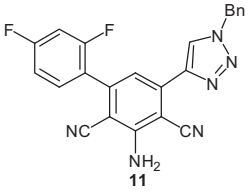
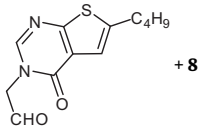
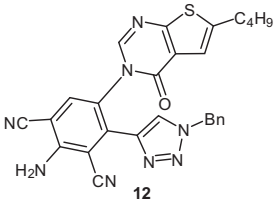
Entry no	Aldehyde/ketone used	Product obtained	Isolated Yield %	Corresponding methylated product prepared	Isolated Yield %
1	Acetaldehyde + **8**	 **9**	50	ND	–
2	Propionaldehyde + **8**	 **10a**	61	 **14a**	75
3	Butyraldehyde + **8**	 **10b**	64	 **14b**	73
4	Valeraldehyde + **8**	 **10c**	59	 **14c**	70

Table 2 (*continued*)

Entry no	Aldehyde/ketone used	Product obtained	Isolated Yield %	Corresponding methylated product prepared	Isolated Yield %
5	Hexanal + **8**	 **10d**	60	 **14d**	72
6	Heptanal + **8**	 **10e**	61	 **14e**	69
7	Octanal + **8**	 **10f**	63	 **14f**	77
8		 **11**	55	ND	–
9		 **12**	65	ND	–

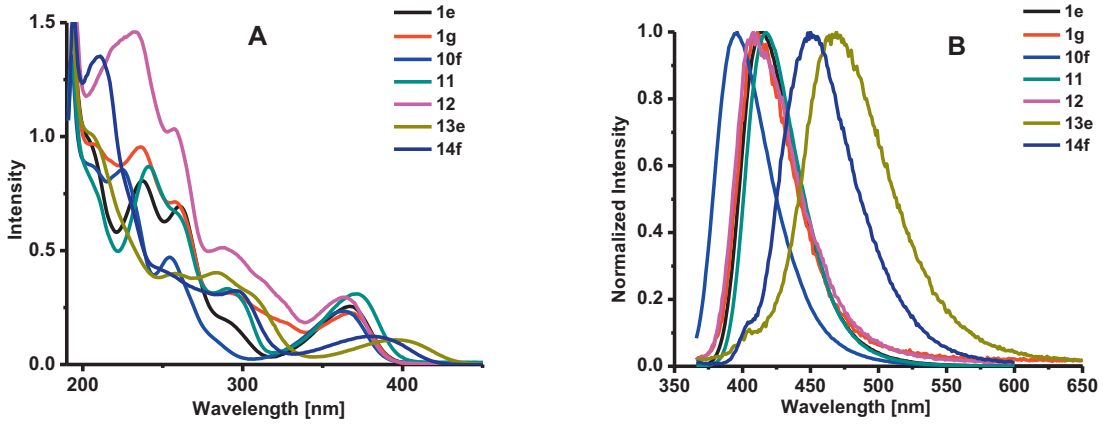


Fig. 1. (A) UV–visible absorption and (B) fluorescence spectra of compounds **1e**, **1g**, **10f**, **11**, **12**, **13e** and **14f**.

phospholipid-binding protein with high affinity for PS. Annexin V conjugated with a fluorophore, fluorescein isothiocyanate (FITC), is widely used to detect this PS externalization which indicates apoptosis.[17] This has a profound application in identifying apoptotic cells in a cell population. In some biological studies it becomes necessary to identify such cell populations in order to interpret cell death inducing mechanisms if necessary. Propidium iodide is another widely used dye which intercalates with the

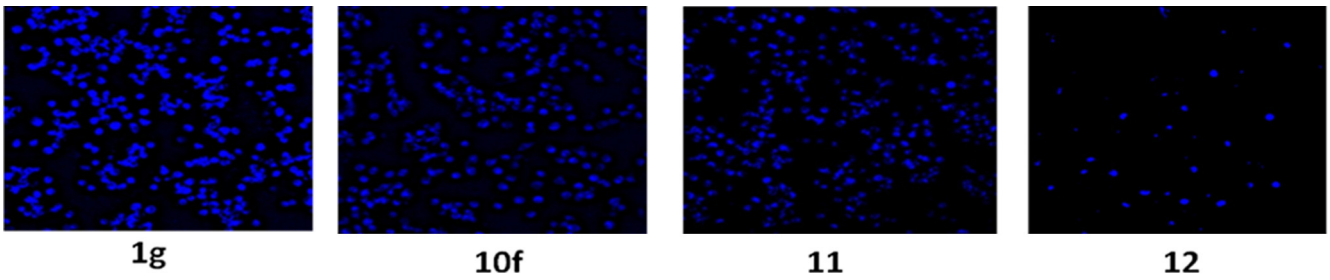


Fig. 2. Cell images captured after staining THP-1 cells with compounds **1g**, **10f**, **11** and **12** (blue fluorescence). Imaging assay buffer: Pre-warmed (37 °C) phosphate-buffered saline (PBS, pH 7.2) containing compounds at 30 μg/mL final concentrations. Incubation time: 20 min, Temperature: 37 °C in CO_2 incubator.

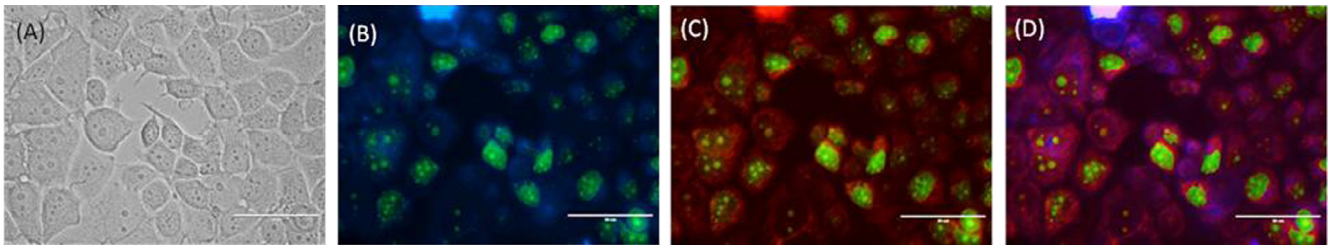


Fig. 3. MCF-7 cells stained with the compound **1g**, SYTO 9 and Nile red. The cells were stained with compound **1g** (100 μg/mL, 0.16 mM, blue fluorescence), SYTO 9 which stains nucleus (green fluorescence) and Nile red which stains cell membrane (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field; (B) Overlay image of blue and green channels, Blue – compound **1g**, Green – SYTO 9 (stains nucleus); (C) Overlay image of green and red channels: Red – Nile red (stains cell membrane), Green – SYTO 9 (stains nucleus); (D) Overlay image of (B) and (C). Scale on image measures 50 μm. Imaging assay buffer: Pre-warmed (37 °C) phosphate-buffered saline (PBS, pH 7.2) containing compound **1g** (100 μg/mL, 0.16 mM), SYTO 9 and Nile red. Incubation time: 20 min, Temperature: 37 °C in CO_2 incubator.

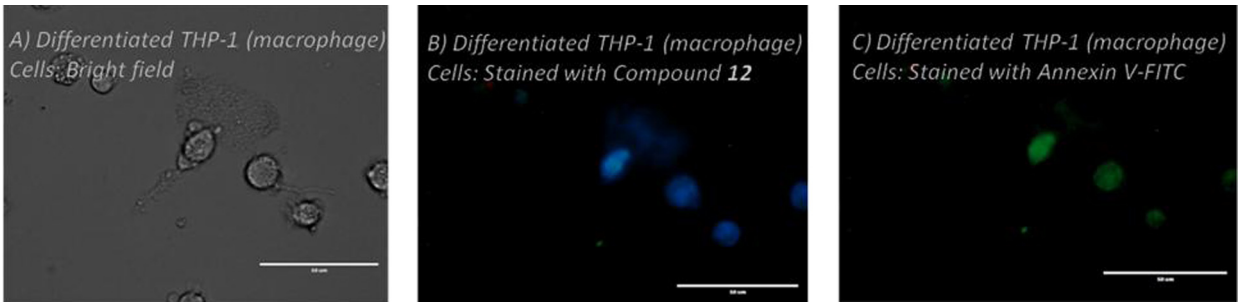


Fig. 4. Differentiated THP-1 cells (A) In bright field; (B) Stained with compound **12**; (C) Stained with Annexin V-FITC. Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, compound **12** (final concentration 100 μg/mL, 0.2 mM), Annexin V-FITC conjugate (5 μL v/v). Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator

DNA. It is specifically used to detect dead cell population in research[18] and is often used along with Annexin-V-FITC.

The fluorescent molecules can be tagged or conjugated to specific moieties and generate fluorescent signals which thus serve as detectable or measurable signals. These have applications in microscopy, fluorimetry, flow cytometry, *etc*. The molecules synthesized in the present work show fluorescent properties which were explored for cell imaging studies. Cell imaging analysis was carried out on fluorescent compounds prepared in the present work and 4 compounds (**1g**, **10f**, **11** and **12**) were found to stain eukaryotic cells. Three cell lines (differentiated THP-1, MCF-7 and MDA-MB-231) were tested for cell staining using known staining agents [SYTO 9 which stains nucleus (green fluorescence), Nile red which stains cell membrane (red fluorescence), Annexin-V-FITC which detects apoptotic cells (green fluorescence) and Propidium iodide which stains dead cells (red fluorescence)].

Cell images captured, using High Content Screening System (ArrayScanXTI, Thermo Scientific) at 20X magnification, after staining differentiated THP-1 cells with 30 μg/mL solutions of compounds **1g**, **10f**, **11** and **12** are shown in Fig. 2. Differentiated THP-1 cells were observed to retain these fluorescent compounds.

The compounds **1g**, **10f** and **11** were found to stain all the cells in contact while compound **12** was found to stain only some cells out of total cells. The compounds **1g**, **10f**, **11** and **12** were studied further for staining MCF-7 and MDA-MB-231 cells. The representative cell-imaging results for the compound **1g** for MCF-7 cells are shown in Fig. 3.

The detailed cell images for staining of differentiated THP-1, MCF-7 and MDA-MB-231 cell lines with the compounds **1g**, **10f** and **11** are given in the Supplementary Information. The compounds **1g**, **10f** and **11** were found to specifically stain only the cytoplasm of the cells but not the nucleus. This was verified by using known counter stains like SYTO 9 (for nucleus) which gives green fluorescence and Nile red (lipid molecules and cell membrane) which gives red fluorescence.

However, compound **12** was found to stain selectively some cells out of total cells and the patterns were similar to Annexin V-FITC as shown in Fig. 4.

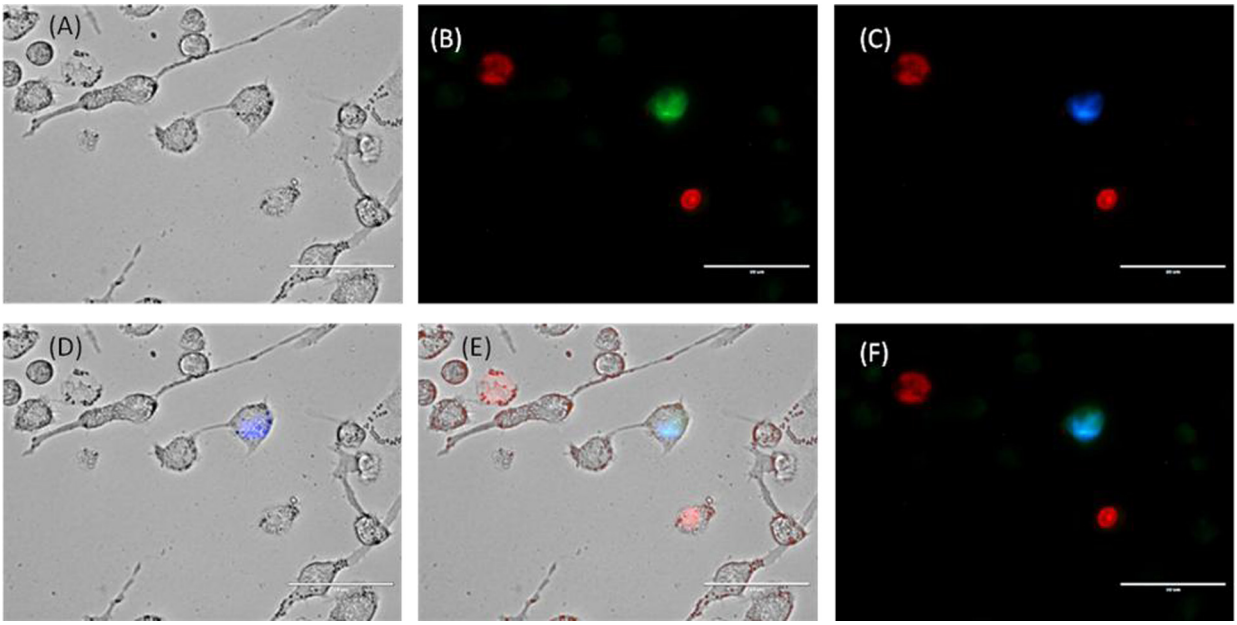


Fig. 5. Differentiated THP-1 cells stained with compound **12**, Annexin-V-FITC and propidium iodide. The cells were stained with compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC which detects apoptotic cells (green fluorescence) and propidium iodide (PI) which stains dead cells (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field; (B) Overlay image of green and red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead); (C) Overlay image of blue and red channels: Blue – compound **12**, Red – PI (dead); (D) Overlay image of bright field with blue channel – Blue - compound **12** (E) - Overlay image of (A), (B) and (C); (F) - Overlay image of (B) and (C). Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, Annexin V-FITC conjugate (5 μL v/v) and PI (10 μg/mL) along with compound **12** at final concentration of 100 μg/mL, 0.2 mM. Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator.

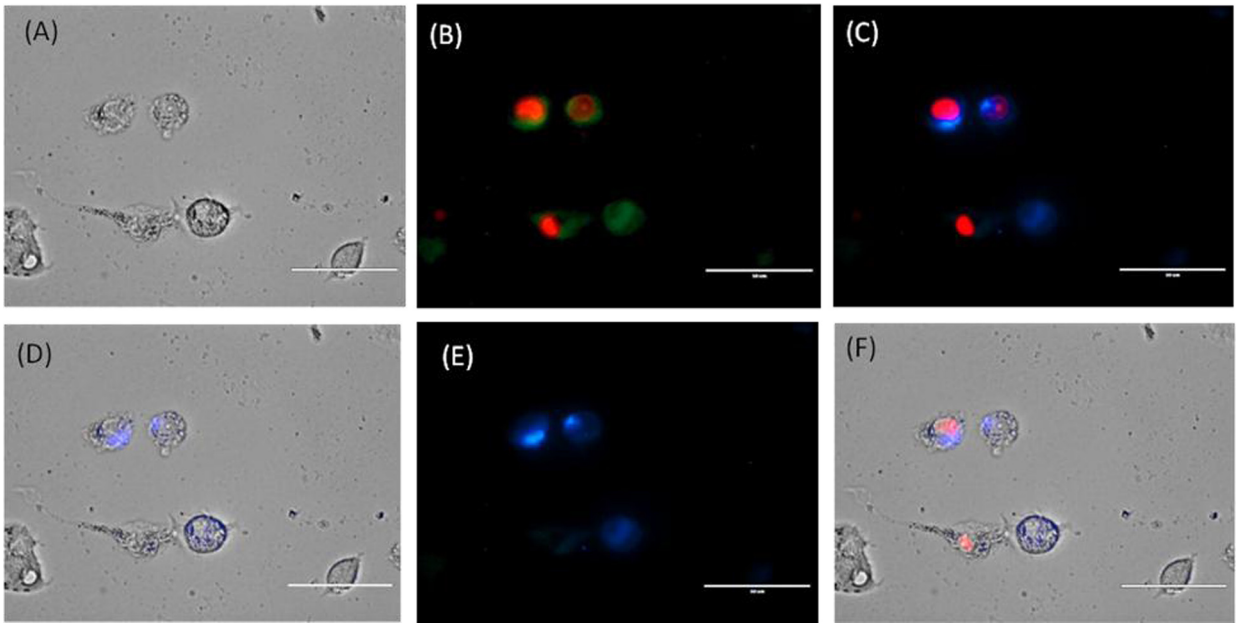


Fig. 6. Differentiated THP-1 cells with induced apoptosis using Paclitaxel, stained with the compound **12**, Annexin-V-FITC and propidium iodide. Differentiated THP-1 (macrophage) cells with induced apoptosis using Paclitaxel IC90 concentration, were stained with the compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC (green fluorescence for apoptotic cells) and propidium iodide (PI) (red fluorescence for dead cells) observed at 60X magnification. (A) Cells seen in bright field; (B) Overlay image of green and red channels, Green – Annexin-V-FITC (stains apoptotic cells), Red – PI (stains dead cells); (C) Overlay image of blue and red channels: Blue – compound **12**, Red – PI (stains dead cells); (D) Overlay image of bright field with blue channel– compound **12**; (E) Blue – compound **12**; (F) Overlay image of (A) and (C). Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, Annexin V-FITC conjugate (5 μL v/v) and PI (10 μg/mL) along with compound **12** at final concentration of 100 μg/mL, 0.2 mM. Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator.

This differential staining was studied further using known markers and inducers. In order to study the differential staining property of **12**, the cells were seeded and apoptosis / cell death was induced using known anti-cancer, cytotoxic compounds namely Paclitaxel and Doxorubicin at their IC50 and IC90 concentrations. Known markers like Annexin V-FITC for apoptosis and

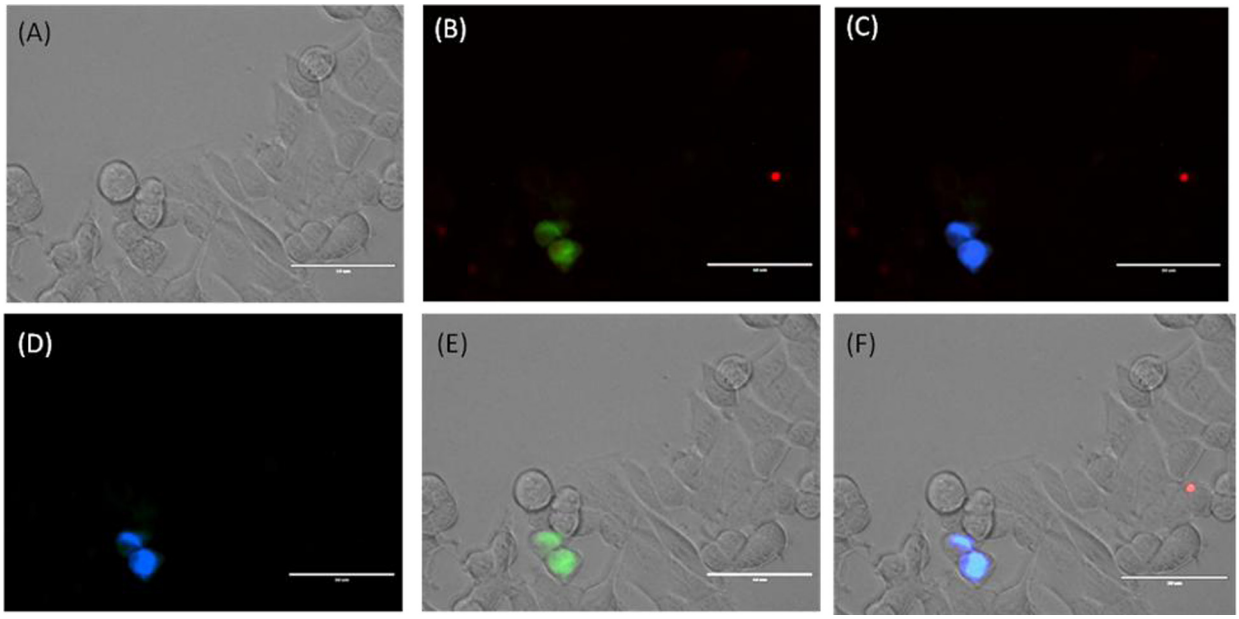


Fig. 7. MCF-7 cells stained with the compound **12**, Annexin-V-FITC (green fluorescence) and propidium iodide. The MCF-7 cells were stained with the compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field; (B) Overlay image of green and red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead cells); (C) Overlay image of blue and red channels: Blue – the compound **12**, Red – PI (dead cells); (D) Blue – the compound **12**; (E) - Overlay image of (A) and (B); (F) Overlay image of (A) and (C). Scale on image measures 50 μm. Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, Annexin V-FITC conjugate (5 μL v/v) and PI (10 μg/mL) along with compound **12** at final concentration of 100 μg/mL, 0.2 mM. Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator.

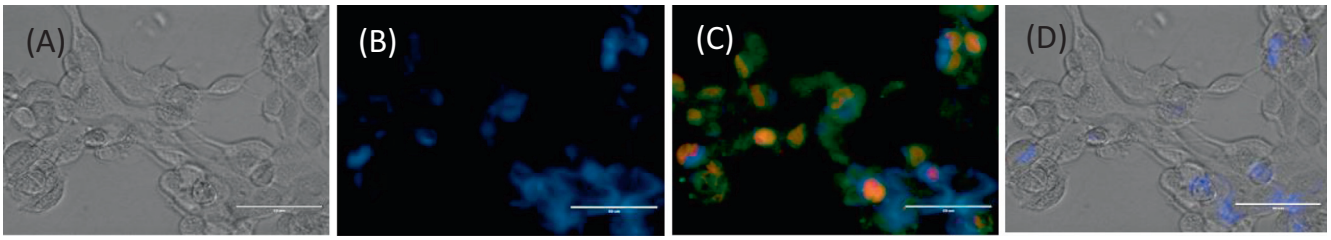


Fig. 8. MCF-7 cells with induced apoptosis using Doxorubicin, stained with the compound **12**, Annexin-V-FITC and propidium iodide. MCF-7 cells with induced apoptosis using Doxorubicin IC50 concentration were stained with the compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field; (B) Blue – compound **12** (C) Overlay image of green, red and blue channels: Green – Annexin-V-FITC (apoptosis), Red – (dead cells) and Blue – compound **12**; (D) - Overlay image of (A) and (B). Scale on image measures 50 μm. Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, Annexin V-FITC conjugate (5 μL v/v) and PI (10 μg/mL) along with compound **12** at final concentration of 100 μg/mL, 0.2 mM. Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator.

propidium iodide for dead cells were used along with the compound **12**. Standard staining protocol for apoptosis/necrosis using Annexin V-FITC/PI was carried out with addition of this new compound **12**. Healthy cells were used as negative control. The cell images for differentiated THP-1 (macrophage) cells stained with the compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC which detects apoptotic cells (green fluorescence) and Propidium iodide (PI) which stains dead cells (red fluorescence) are shown in Fig. 5.

It was observed that the staining of the differentiated THP-1 (macrophage) cells with the compound **12** corresponds to that with Annexin-V-FITC which is known to stain the apoptotic cells. The compound **12** does not stain dead cells. The results were further confirmed by inducing apoptosis in the THP-1 (macrophage) cells with Paclitaxel and repeating the study (Fig. 6).

The results of imaging in case of MCF-7 cells stained with the compound **12**, Annexin-V-FITC and Propidium iodide are shown in Fig. 7 which also shows that the staining pattern of the compound **12** is similar to Annexin-V-FITC (which shows green fluorescence for apoptotic cells).

Further, apoptosis was induced in MCF-7 cells by treating them with Doxorubicin and the cells were stained with the compound **12**, Annexin-V-FITC and Propidium iodide and the results are shown in Fig. 8.

The imaging properties of the compound **12** were further studied with MDA-MB-231 cells. The apoptosis was induced with Doxorubicin and the cells were stained with the compound **12** (100 μg/mL, 0.2 mM, blue fluorescence), Annexin-V-FITC (green fluorescence) and Propidium iodide (PI) (red fluorescence) as before (Fig. 9).

All these results indicated that the staining patterns of the compound **12** (which shows blue fluorescence) are similar to Annexin-V-FITC which shows green fluorescence for apoptotic cells and the compound **12** has potential as staining agent for imaging of apoptotic cells.

Toxicity of the compounds **1g**, **10f**, **11** and **12** against THP-1 cells was studied[19] and the data are given in Supplementary data.

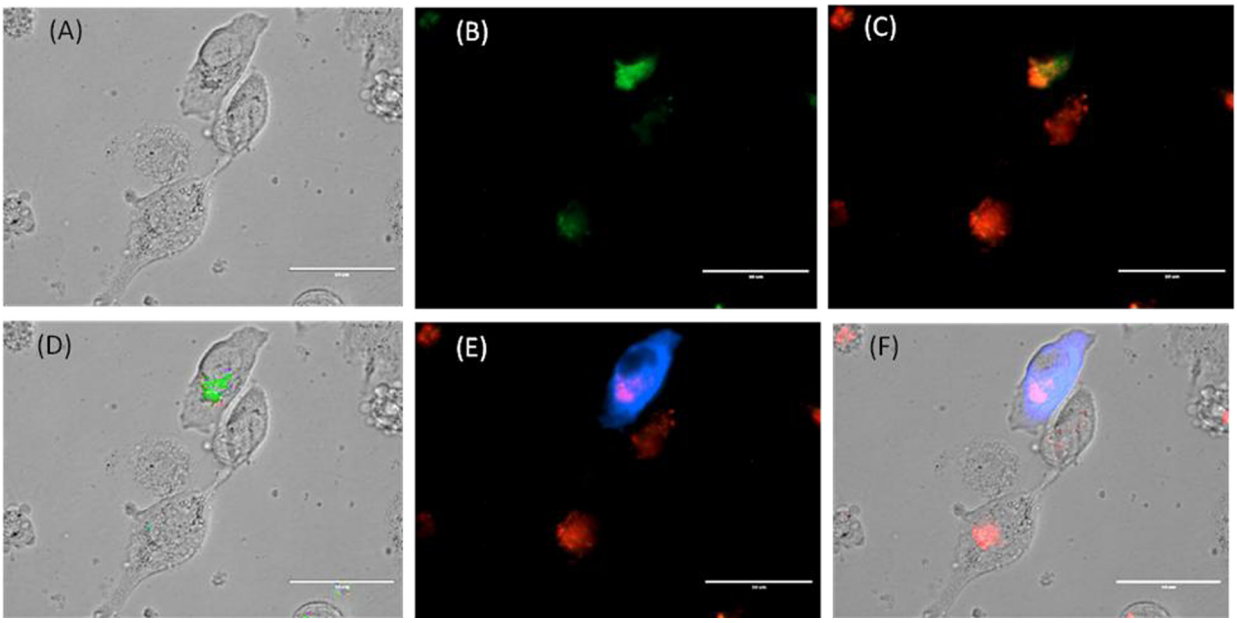


Fig. 9. MDA-MB-231 cells with induced apoptosis using Doxorubicin, stained with the compound **12**, Annexin-V-FITC and propidium iodide. MDA-MB-231 cells with induced apoptosis using Doxorubicin IC90 concentration, were stained with the compound **12** (100 μg/mL, 0.2 mM blue fluorescence), Annexin-V-FITC (green fluorescence) and propidium iodide (red fluorescence) observed at 60X magnification. (A) Cells seen in bright field; (B) Green – Annexin-V-FITC (apoptosis); (C) Overlay image of green and red channels: Green – Annexin-V-FITC (apoptosis), Red – PI (dead cells); (D) - Overlay image of (A) and (B); (E) Bright field with blue – compound **12** and red – PI (dead cells); (F)-Overlay image of (A) and (E). Scale on image measures 50 μm. Imaging assay buffer: 10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4, Annexin V-FITC conjugate (5 μL v/v) and PI (10 μg/mL) along with compound **12** at final concentration of 100 μg/mL, 0.2 mM. Incubation time: 15 min, Temperature: 37 °C in CO_2 incubator.

Since the IC_{50} values of compounds are >100 μg/mL, these compounds can be considered as non-cytotoxic.

In conclusion, synthesis of 3,4,5-triheterocyclyl/3,4-diheterocyclyl/3-heterocyclyl-2,6-dicyanoanilines, starting from 1-benzyl-1,2,3[1*H*]-triazole-4- carboxaldehyde **8**, malononitrile and required aldehydes/ketones was achieved. The optical properties of the synthesized compounds were studied which indicated that fluorescence properties of this class of compounds can be modified. The compounds were further studied from the point of their application for cell imaging wherein it was found that compounds **1g**, **10f** and **11** stain cytoplasm of the cells in contact but not the nucleus while the compound **12** showed selective affinity to apoptotic cells resulting in blue fluorescence. The cell imaging results with compound **12** were compared with Annexin V-FITC, a known reagent containing recombinant Annexin V conjugated to green-fluorescent FITC dye, used for detection of apoptotic cells. These encouraging results indicate the significant potential of these compounds as valuable tools for cell imaging.

Acknowledgements

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A. Supplementary material

Copies of ^{1}H and ^{13}C NMR spectra of all new compounds, X-ray crystallographic data for compounds **1c** and **1e**, optimized geometry and HOMO-LUMO levels of compounds **1g**, **10f**, **11** and **12** and detailed cell imaging data for compounds **1g**, **10f**, **11** and **12**. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.074.

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One-pot three-component synthesis and photophysical properties of highly fluorescent novel 4-alkyl-3-aryl-2,6-dicyanoanilines by using tris(hydroxymethyl)aminomethane as a catalyst

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ABSTRACT

Novel 4-alkyl-3-aryl-2,6-dicyanoanilines were synthesized by a multi-component one-step reaction of aromatic aldehyde, malononitrile and aliphatic aldehyde using tris(hydroxymethyl)aminomethane (THAM) as a catalyst under microwave (MW) irradiation or conventional heating. The optimized reaction condition involved use of 2.5 equivalents of THAM under MW irradiation at 140 W using 20% MW power for 5 min or conventional heating at 80 °C for 8 h in dimethylformamide. The photophysical properties including λ_{max}, quantum yield and Stokes' shifts of newly synthesized molecules were studied. All compounds exhibited quantum yield in the range of 0.04–0.52 with respect to standard quinine sulphate having quantum yield 0.54. The Stokes' shifts of all compounds were found in the range of 41–105 nm. The current strategy provides operationally simple protocol using THAM as a catalyst to synthesize 4-alkyl-3-aryl-2,6-dicyanoanilines with diverse structural features to make them available for exploration of their photophysical as well as biological applications.

Specifications Table

Subject area	Organic chemistry and fluorescence spectroscopy
Compounds	New 4-alkyl-3-aryl-2,6-dicyanoanilines
Data category	Spectral
Data acquisition format	NMR, IR, high resolution-mass spectra, UV–vis and fluorescence
Data type	Analyzed
Procedure	New 4-alkyl-3-aryl-2,6-dicyanoanilines synthesized using THAM catalyst, characterized and studied for their fluorescent properties
Data accessibility	Manuscript and supplementary data enclosed with this article

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2405-8300/

1. Rationale

A number of chemical entities bearing 2,6-dicyanoaniline moiety have been screened for interesting fluorescent properties and various applications. The presence of acceptor–donor–acceptor (A–D–A) system plays a key role in bringing exciting fluorescent properties in resulting compounds. Plenty of research articles reveal that these compounds are well suited in number of fields like nonlinear optical materials, dyes, polymers etc. and have scope for further structural modifications due to easily convertible amino and cyano groups which attracts the attention of various research groups to synthesize and evaluate their potential in various fields [1]. Recently Borate et al. described applications of various mono/di/tri heterocyclyl 2,6-dicyanoanilines as selective cell staining agents in fluorescent cell imaging applications [2]. Further, Yalcin et al. reported the applications of these types of molecules as selective and non-toxic fluorescence probes for double stranded DNA and RNA binders [3]. In view of these recent developments, it is worth to further design, synthesize and explore these categories of compounds for various applications.

A number of useful synthetic methods are available to afford these classes of compounds providing opportunity for screening them for diverse applications. Until now, for the synthesis of 2,6-dicyanoanilines and related compounds, a number of synthetic strategies, diverse bases and intermediates have been utilized [1,4–13]. As a part of our research, we desired to synthesize new 4-alkyl-3-aryl-2,6-dicyanoanilines by use of environment-friendly and benign protocol and study their fluorescent properties. In this context, we came across recent reports by Desai et al. [14] and Kim et al. [15] wherein the catalytic efficiency of tris(hydroxymethyl)aminomethane (THAM) has been evaluated for the synthesis of medicinally important tetrahydrobenzo[*b*]pyrans, pyran-annulated heterocycles and for the synthesis of β-phosphonomalonates. The beauties of THAM catalyst like commercial availability, easy handling, non-toxicity, cost-effectiveness and bio-degradable qualities etc. encouraged us to explore the catalytic utility of THAM in the synthesis of novel 4-alkyl-3-aryl-2,6-dicyanoanilines under microwave irradiation and conventional heating.

Borate et al. [16] reported practical one-step multi-component protocol to afford 4-alkyl-3-aryl-2,6-dicyanoanilines in good yields. We anticipated synthesis of new 4-alkyl-3-aryl-2,6-dicyanoanilines by utilizing various aromatic aldehydes like 1-pyrenecarbaldehyde, 4-biphenylcarbaldehyde, 3-methylbenzo[*b*]thiophene-2-carbaldehyde, 3-(benzyloxy)benzaldehyde and 4-((4-nitrobenzyl)oxy)benzaldehyde. To the best of our knowledge, there is no report of this type of reaction on above mentioned aromatic aldehydes using THAM as a catalyst in microwave and conventional heating methods which can yield novel 4-alkyl-3-aryl-2,6-dicyanoanilines (Table 3) and there will be ample scope to explore the properties and potential of these new chemical entities in multifaceted applications.

2. Experimental

2.1. Materials and methods

Required malononitrile, tris(hydroxymethyl)aminomethane (THAM), dimethyl formamide, silica gel for column chromatography (60–120 mesh), ethyl acetate and petroleum ether (boiling range 60–80 °C) were obtained from Spectrochem while all aromatic/aliphatic aldehydes were procured from Sigma-Aldrich and used without further purification. All the microwave experiments were carried out by using Raga's scientific microwave oven. Thin Layer Chromatography analysis was performed on Merck HPTLC silica gel 60 F_{254} plates. Melting points were determined in an open capillary and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker-AV 200 MHz spectrometer using $CDCl_3$ as solvent and chemical shifts were reported as δ (in ppm). Thermo Fisher FT-IR spectrophotometer in ATR (Attenuated Total Reflection) mode was used to record IR spectra of all compounds in the range of 550–4000 cm^{-1}. The High Resolution Mass Spectra of all compounds were recorded on Thermo Scientific Q-Exactive instrument in ESI mode. UV–vis absorption spectra were recorded on UV–vis double beam spectrophotometer (Analytik Jena Specord Plus) and fluorescence spectra were recorded on spectrofluorometer (FP-8300, JASCO, Japan).

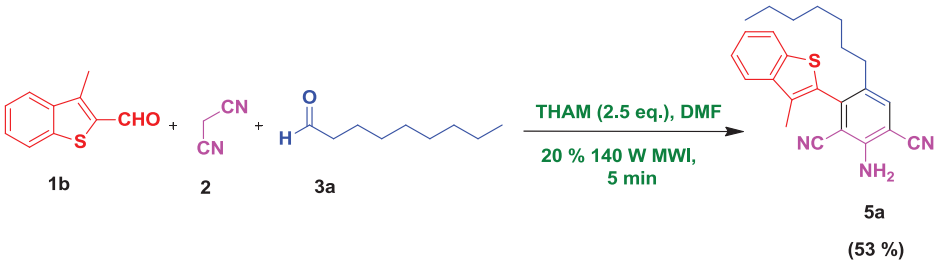
*2.2. Representative procedure for synthesis of 2-amino-5-heptyl-4-(3 methylbenzo[*b*]thiophen-2-yl)isophthalonitrile (**5**)*

2.2.1. Microwave Method A

To a mixture of 3-methylbenzo[*b*]thiophene-2-carbaldehyde (0.1 g, 0.56 mmol), malononitrile (0.094 g, 1.42 mmol) and nonanal (0.104 g, 0.74 mmol) in a round bottom flask, dimethylformamide (1.0 mL) was added. Then THAM catalyst (0.171 g, 1.42 mmol) was added, mixture was mixed thoroughly and irradiated in microwave oven with 20% 140 W for 5 min. The reaction mixture was then allowed to cool at room temperature and ice cold water (20 mL) was added and resultant mixture was extracted with ethyl acetate (3 × 10 mL). Finally, combined organic layers of extract were dried over anhydrous Na_2SO_4, concentrated and purified by using silica gel column chromatography using ethyl acetate and pet ether as mobile phase (4–15% ethyl acetate in pet ether) to get 2-amino-5-heptyl-4-(3-methylbenzo[*b*]thiophen-2-yl)isophthalonitrile (**5**) as white fluffy solid.

2.2.2. Conventional heating Method B

A mixture of 3-methylbenzo[*b*]thiophene-2-carbaldehyde (0.1 g, 0.56 mmol), malononitrile (0.094 g, 1.42 mmol), nonanal (0.104 g, 0.74 mmol), dimethylformamide (3.0 mL) and THAM catalyst (0.171 g, 1.42 mmol) in a round bottom flask equipped



Scheme 1. Model reaction for optimization of MWI condition.

with air condenser, was heated (80 °C) for 8 h. The reaction mixture was then allowed to cool to room temperature and ice cold water (25 mL) was added into it and resultant mixture was extracted with ethyl acetate (3 × 10 mL). Lastly, combined organic layers were dried over anhydrous Na_2SO_4, concentrated and purified by using silica gel column chromatography using ethyl acetate and pet ether (4–15% ethyl acetate in pet ether) as mobile phase to get 2-amino-5-heptyl-4-(3 methylbenzo[*b*]thiophen-2-yl)isophthalonitrile as white fluffy solid. The product (**5**) obtained with this method had similar physical and spectral characteristics with those obtained by microwave Method A.

*2.2.3. Data and values for representative compound (**5**)*

Yield: Method A: 53%, Method B: 50%. Nature and color: White fluffy solid. Melting point: 127 °C. FT-IR (ATR mode, cm^{-1}): ν 3416, 3338 (–NH_2), 2920, 2852 (aliphatic –CH), 2220 (–CN), 1646 (aromatic –C=C). ^{1}H NMR (200 MHz in $CDCl_3$, δ ppm): 0.81 (t, J = 6 Hz, 3H, CH_3), 1.15 (br s, 8H, 4 × CH_2), 1.41 (br s, 2H, CH_2), 2.25 (s, 3H, Ar–CH_3), 2.29–2.61 (m, 2H, Ar–CH_2), 5.11 (s, 2H, NH_2), 7.35–7.53 (m, 2H, Ar–H), 7.56 (s, 1H, Ar–H), 7.78 (dd, J = 6, 2 Hz, 1H, Ar–H), 7.83–7.93 (m, 1H, Ar–H). ^{13}C NMR (50 MHz in $CDCl_3$, δ ppm): 12.3, 13.9, 22.4, 28.8, 28.9, 30.6, 31.5, 31.9, 97.6, 100.3, 114.8, 115.8, 122.2, 122.5, 124.4, 125.1, 130.9, 131.5, 133.4, 137.0, 139.6, 139.8, 142.5, 149.5. HR-MS (ESI) *m/z*: Obtained for M.F. $C_{24}H_{23}N_3S$: 410.1661 as (M+Na); calculated for M.F. $C_{24}H_{23}N_3S$: 410.1667 as (M+Na).

2.3. Photophysical measurement of the synthesized molecules

The UV–vis absorption and fluorescence emission spectra of all the synthesized compounds were recorded in acetonitrile whereas quinine sulphate (QS) in 0.1 M H_2SO_4 as a solvent was used as the standard. The excitation and emission slit width were set at 5–5 nm and with low sensitivity. The quantum yield of the synthesized compounds was evaluated in comparison with standard QS. To measure fluorescence intensity and absorbance, fixed concentration of standard and sample was used (5.0×10^{-6} M). The quantum yield was calculated by using following Eq. (1),

$$QY = QY_{ref} \frac{\eta^2 I\, A_{ref}}{\eta^2_{ref} A\, I_{ref}} \quad (1)$$

where, QY = quantum yield of sample, QY_{ref} = quantum yield of reference quinine sulphate (taken as 0.54), η^2 = square of refractive index of solvent used to dissolve samples (acetonitrile, 1.806605), η^2_{ref} = square of refractive index of solvent used to dissolve standard (0.1 M H_2SO_4, 1.7689), I = fluorescence intensity of sample, A = absorbance of sample, A_{ref} = absorbance of standard, I_{ref} = fluorescence intensity of standard.

3. Results and discussion

3.1. Optimization of reaction conditions

Seferoglu et al. [17], reported solvent-free single-step microwave irradiation synthesis of highly fluorescent coumarins containing 3,5-disubstituted-2,6-dicyanoaniline moiety using piperidine as a catalyst. The microwave irradiation (MWI) synthesis has significant advantages over conventional organic synthesis in terms of reaction time, excellent yields, milder reaction conditions etc. which motivated us to optimize suitable reaction conditions for the synthesis of our targeted novel 4-alkyl-3-aryl-2,6-dicyanoanilines under microwave irradiation and using THAM as a catalyst.

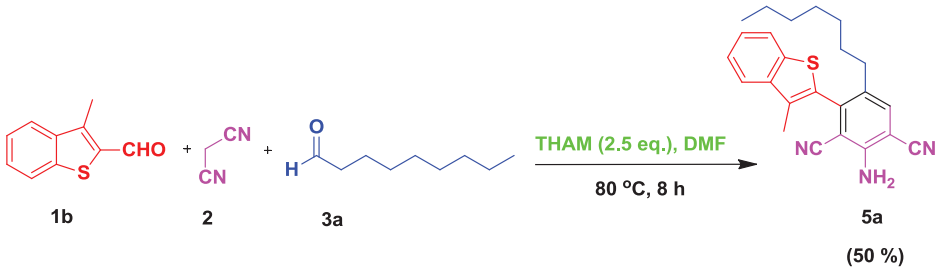
In the beginning, with reference to methodology reported by Borate et al. [16] specifically for synthesis of 4-alkyl-3-aryl-2,6-dicyanoanilines, we replaced the morpholine with THAM and heating with MWI and executed model multi-component reaction of 3-methylbenzo[*b*]thiophene-2-carbaldehyde, nonanal and malononitrile in minimum quantity of dimethylformamide (to make just slurry of all the contents) by using various equivalents of catalyst and MW power for 2–5 min (Scheme 1). The optimization trials are summarized in Table 1.

It is worth to mention that this multi-component reaction yields desired fluorescent product along with its characteristic byproducts viz. product of self cyclization of aliphatic aldehyde (3,4-dialkyl-2,6-dicyanoaniline) and aryl alkyl olefin [16,18]. By checking reaction mixture at different intervals by TLC (20% ethyl acetate in petroleum ether solvent system) we observed

Table 1
Optimization of reaction conditions under microwave irradiation method.

Eq. of THAM and MW power	Time (s)	TLC	Eq. of THAM and MW power	Time (s)	TLC
0.5 and 140 W (20%)	120	NP	1.5 and 140 W (20%)	120	NP
	180	NP		180	TP
	240	TP		240	CRM
	300	TP		300	CRM
2.5 and 140 W (20%)	120	NP	3.5 and 140 W (20%)	120	CRM
	180	CRM		180	CRM
	240	CRM		240	P + MRBP
	300	**P + MRBP**		300	P + MRBP
0.5 and 280 W (40%)	120	NP	1.5 and 280 W (40%)	120	NP
	180	CRM		180	CRM
	240	CRM		240	CRM
	300	CRM		300	CRM
2.5 and 280 W (40%)	120	CRM	3.5 and 280 W (40%)	120	CRM
	180	CRM		180	P + MRBP
	240	P + MRBP		240	P + MRBP
	300	P + MRBP		300	P + MRBP

NP: no product, TP: traces of product, CRM: complex reaction mixture, P: product, MRBP: minor reaction byproducts.



Scheme 2. Model reaction for optimization of conventional heating method.

Table 2
Optimization of reaction conditions by conventional heating method.

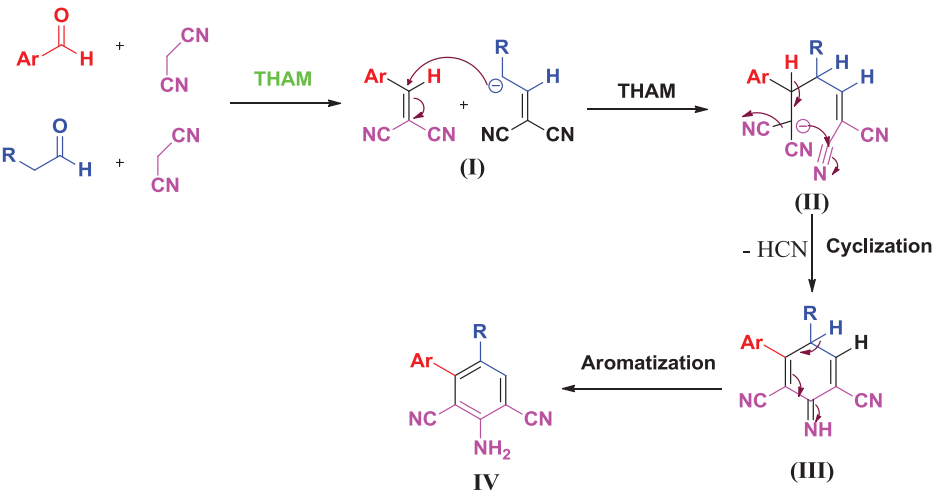
S. no.	Eq. of THAM	Time (h)	Temperature (°C)	TLC analysis
01	2.5	2	R.T.	NP
02		4		NP
03		6		CRM
04		8		CRM
05		12		CRM
06	2.5	2	40	CRM
07		4		CRM
08		6		CRM
09		8		P + CRM
10		12		P + CRM
11	2.5	2	80	P + CRM
12		4		P + CRM
13		6		P + CRM
14		**8**		**P + MRBP**
15		12		P + MRBP

NP: no product, TP: traces of product, CRM: complex reaction mixture, P: product, MRBP: minor reaction byproducts.

that higher MWI (280 W, 40%) power and catalyst amount (3.5 eq.) yielded desired product in less time (3 min) but the most appropriate reaction condition to get desired product in acceptable yield was using 20% 140 W MWI for 5 min and 2.5 eq. of THAM (Table 1).

During standardization of the microwave irradiation reaction condition, it was noted that 2.5 eq. of catalyst was essential to get the product in acceptable yield. It was further decided to check the efficiency of THAM in conventional heating method and same model reaction was performed (Scheme 2) by changing time and temperature conditions and the results are summarized in Table 2.

It was observed that satisfactory results were observed at 80 °C and for 8 h in conventional heating method for the synthesis of 4-alkyl-3-aryl-2,6-dicyanoanilines (Scheme 2, Table 2).



Scheme 3. Plausible mechanism of formation of 4-alkyl-3-aryl-2,6-dicyanoanilines.

As per the optimized reaction conditions, generality of the reaction was checked using different aromatic aldehydes, those previously not used in this type of reaction, with nonanal and hexanal as the aliphatic aldehyde to obtain chain length of seven and four carbons in the product and the results are presented in Table 3, wherein method A involves reaction under microwave irradiation while method B involves heating at 80 °C.

All the newly synthesized compounds (Table 3) have solubility in common polar organic solvents like methanol, ethyl acetate, acetone, acetonitrile, chloroform, dimethylformamide, dimethylsulphoxide etc. while they are insoluble in petroleum ether, *n*-hexane, water etc. The structures of all the compounds were ascertained by spectral methods like ^{1}H NMR, ^{13}C NMR, IR and HR-MS spectroscopy.

The spectral data values and copies of ^{1}H NMR, ^{13}C NMR and IR spectra of all the synthesized compounds are provided in the supporting information.

3.2. Mechanism of reaction

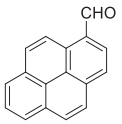
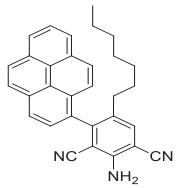
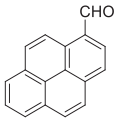
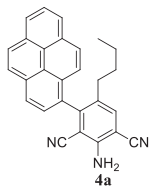
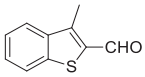
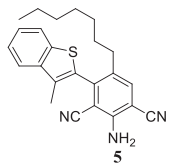
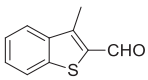
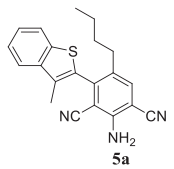
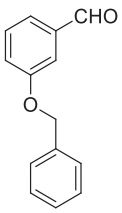
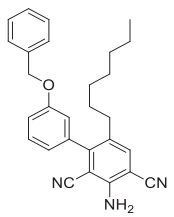
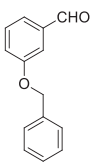
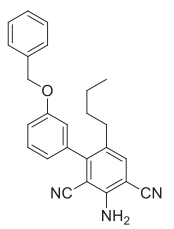
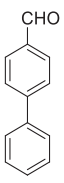
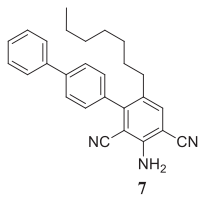
The mechanism of the reaction was supposed to follow steps [16] depicted in Scheme 3. The abstraction of proton from the malononitrile and attack of the corresponding anion on carbonyl carbon of aldehyde followed by dehydration results in the formation of the corresponding aromatic and aliphatic knoevenagel condensation products (I). Further, abstraction of alpha hydrogen from aliphatic knoevenagel product by base creates the cabanion which attacks the aromatic knoevenagel product to form the intermediate (II) which on removal of hydrogen cyanide and subsequent cyclization forms intermediate (III) which on aromatization is converted to the desired product IV.

3.3. Confirmation of positions of 3-aryl and 4-alkyl groups in the resulting 2,6-dicyanoaniline compounds

To confirm the positions of 3-aryl and 4-alkyl groups in the product 5 with respect to the amino group, we studied its ^{1}H NMR and ^{13}C NMR spectra. In the ^{1}H NMR spectrum (Fig. 2) the position of proton on the aromatic ring bearing two cyanide and amino group was assigned to be 7.56 ppm corresponding to single proton (Fig. 1).

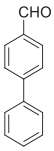
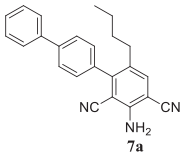
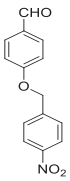
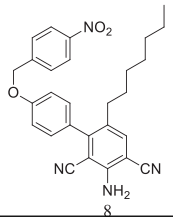
In the ^{13}C NMR spectrum (Fig. 3), two cyanide carbons were assigned the signals at 114.80 and 115.83 ppm. The shielding of cyanide carbon at 114.80 is supposed to be due to the vicinity of benzo[*b*]thiophenyl group and deshielding of cyanide carbon at 115.83 ppm is due to remote electronic cloud of the benzo[*b*]thiophenyl group. Further, the two aromatic carbons bonded to two cyanide groups were assigned to the signals at 97.61 ppm for C-2 (shielded) and 100.36 ppm for C-6 (deshielded) due to the same reason. Further, the deshielded carbon at 137.04 ppm was assigned to the C-5 sandwiched between the C-6 bonded to electron withdrawing cyanide group and C-4 bonded to aliphatic group. We feel that if it is between the aryl and alkyl group, the position of the same will be around 120 ppm due to remote electron withdrawing cyanide group. To confirm the proposed structure of compound **5**, Heteronuclear Multiple Bond Correlation (HMBC) spectra (Fig. 4a and b) of compound **5** were recorded, wherein appearance of strong HMBC correlation between proton at 7.56 ppm and cyanide carbon (115.85 ppm) attached to aromatic C-6 (100.35 ppm) supported the 3-aryl and 4-alkyl positions in the compound **5**.

Table 3
THAM catalyzed synthesis of new 4-alkyl-3-aryl-2,6-dicyanoanilines.

S. no.	Aromatic aldehyde	Aliphatic aldehyde	Product	Yield[a] Method A	Yield[a] Method B
1	 **1a**	Nonanal (3a)	 **4**	50	47
2	 **1a**	Hexanal (3b)	 **4a**	45	42
3	 **1b**	Nonanal (3a)	 **5**	53	50
4	 **1b**	Hexanal (3b)	 **5a**	50	52
5	 **1c**	Nonanal (3a)	 **6**	57	53
6	 **1c**	Hexanal (3b)	 **6a**	55	56
7	 **1d**	Nonanal (3a)	 **7**	64	66

(*continued on next page*)

Table 3 (*continued*)

S. no.	Aromatic aldehyde	Aliphatic aldehyde	Product	Yield[a] Method A	Yield[a] Method B
8	 1d	Hexanal (3b)	 7a	60	57
9	 1e	Nonanal (3a)	 8	55	53

[a] Isolated yields.

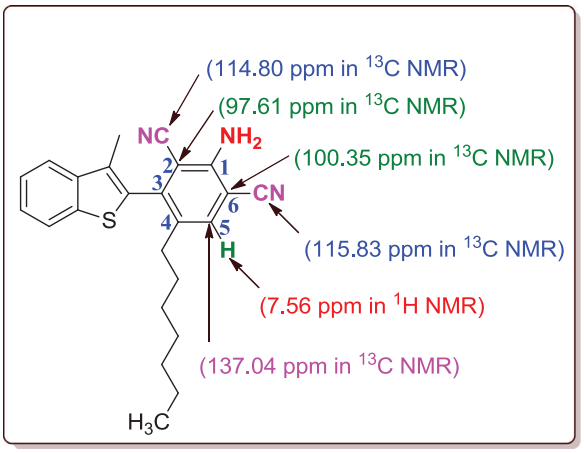


Fig. 1. Structure of compound **5**.

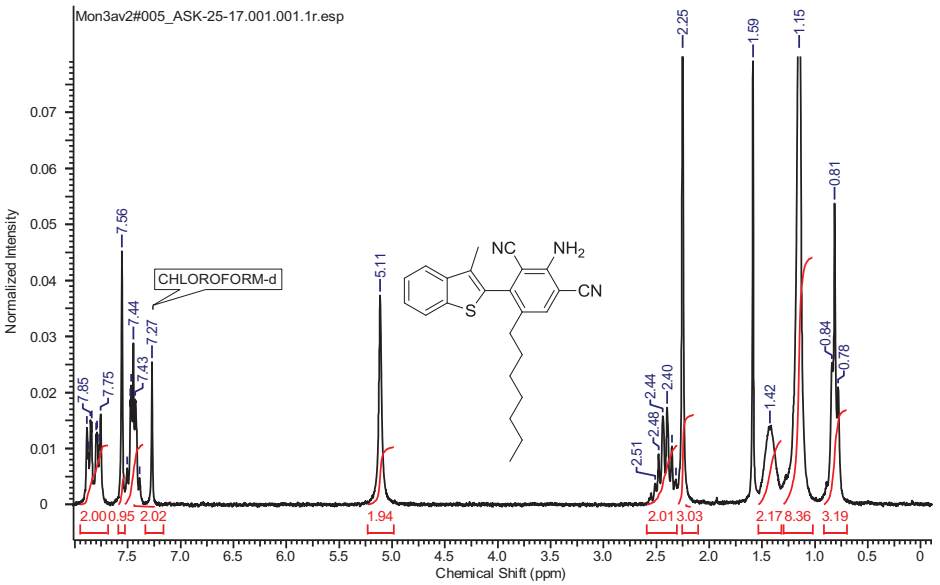


Fig. 2. ^{1}H NMR spectrum of **5**.

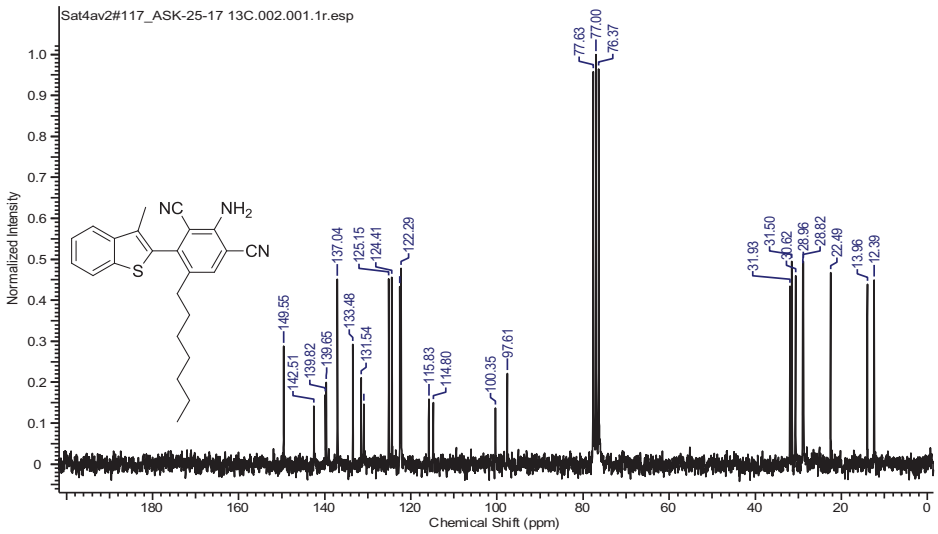


Fig. 3. ^{13}C NMR spectrum of **5**.

Table 4
Optical properties of new 2, 6-dicyanoanilines set as 5×10^{-6} M concentrations.

Sr. no.	Compound no.	UV–vis absorption	Fluorescence emission			Quantum yield ϕ_F	$\lambda_{max.ex}$ (nm)	Stokes' shift
		λ_{max} (nm)	Intensity (a.u.)	$\lambda_{max.emi}$ (nm)	Intensity (a.u.)			
1	**4**	345	0.1726	450	5251.08	0.4041	345	105
2	**4a**	345	0.268	450	6666.39	0.3304	345	105
3	**5**	365	0.0636	407	1053.72	0.2201	365	42
4	**5a**	366	0.0657	407	0925.38	0.1871	363	44
5	**6**	360	0.0482	402	1860.41	0.5127	357	45
6	**6a**	360	0.0456	402	1790.41	0.5216	360	42
7	**7**	360	0.0538	406	1427.16	0.3524	358	48
8	**7a**	360	0.053	406	1255.68	0.3147	358	48
9	**8**	360	0.0473	401	168.33	0.0472	360	41
10	Std QS	350	0.064	449	2656.78	0.5400	349	100

3.4. Photophysical properties of synthesized compounds

The UV–vis absorption and fluorescence emission spectra of all the synthesized compounds are depicted in Figs. 5 and 6 respectively. The absorption and emission maxima of all the synthesized compounds at their respective wavelength are summarized in Table 4.

3.4.1. Effect of varying substitution pattern on quantum yield and Stokes' shift

Fig. 7 provides the quantum yields and Stokes' shifts in the reported molecules with respect to varying substitution pattern of aryl group on the 2,6-dicyanoaniline moiety. The compounds **4, 4a, 5, 5a, 6, 6a, 7** and **7a** exhibit quantum yields in the range of 0.18–0.52 while the compound **8** has quantum yield 0.04. The compound **8** has electron withdrawing nitro group while rest of the compounds do not have the nitro group. This indicates that the presence of electron withdrawing substituent like nitro group drastically reduces the quantum yield.

The compounds **4** and **4a** having pyrenyl group have Stokes' shift 105 nm while rest of the compounds exhibit Stokes' shifts in the range of 41–48 nm. This indicates that the extended conjugation provided by the pyrenyl group increases the Stokes' shift values in the resulting compounds **4** and **4a** higher than the standard quinine sulphate with Stokes' shift 100 nm.

In general, all the newly synthesized compounds prepared in the present work have excitation wavelength in the range of 345–365 nm and fluorescence emission wavelength around 402–450 nm. All these compounds show bright blue luminescence under UV lamp (Fig. 8).

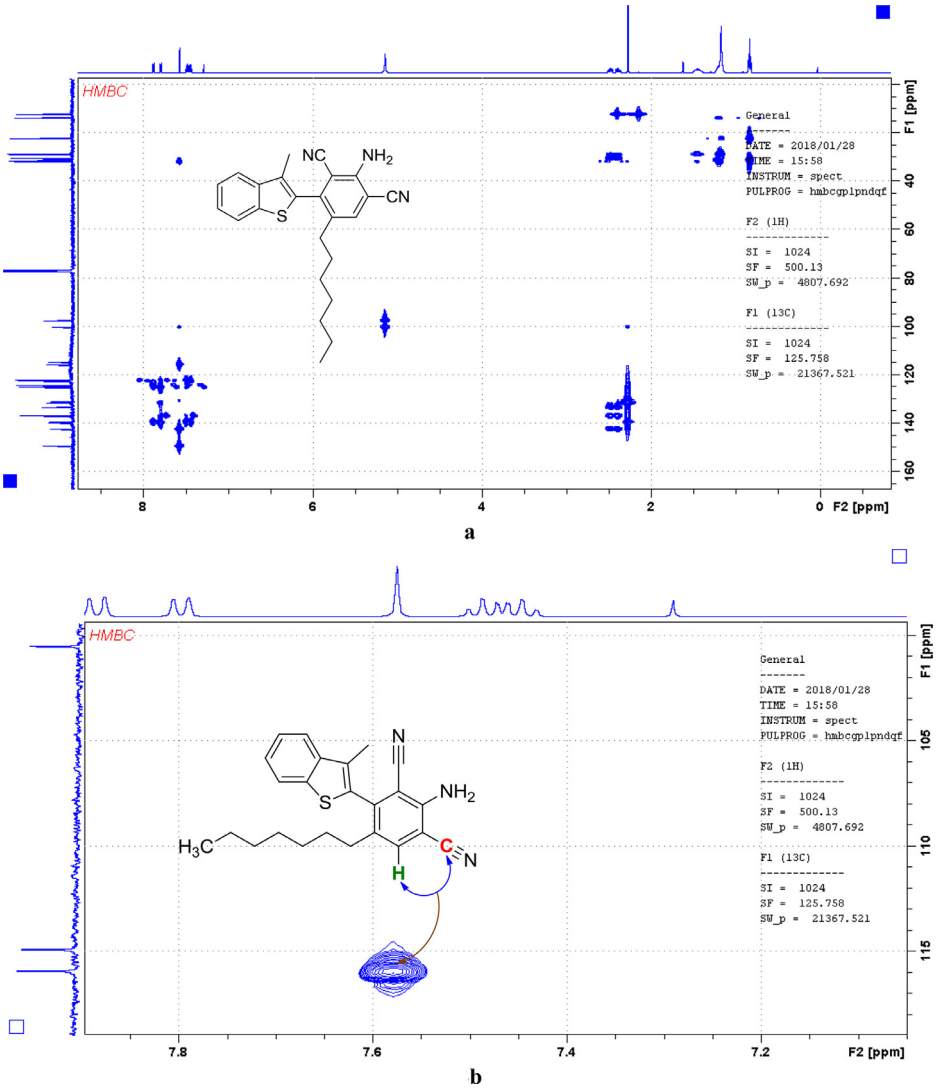


Fig. 4. Heteronuclear multiple bond correlation spectrum of **5**.

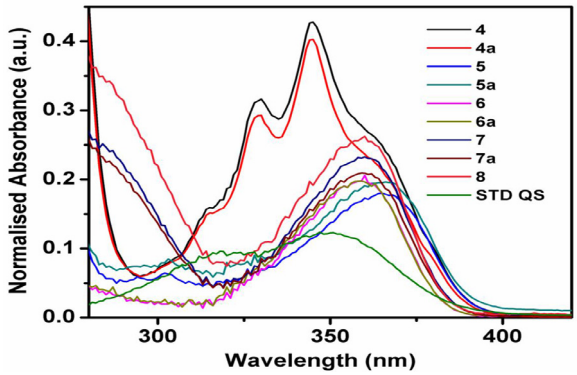


Fig. 5. Normalized UV–vis absorption spectra of all the compounds.

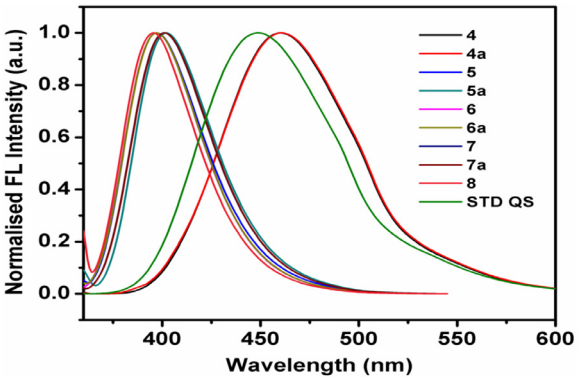


Fig. 6. Normalized fluorescence emission spectra of all the compounds.

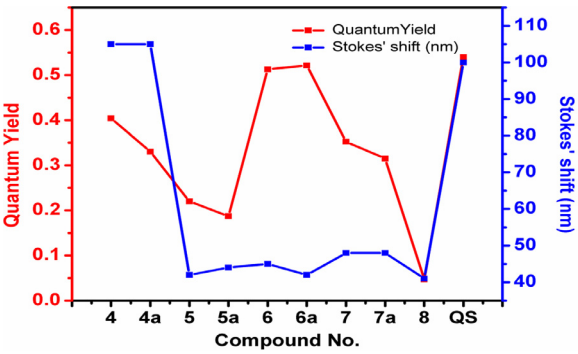


Fig. 7. Effect of varying substitution pattern on quantum yield and Stokes' shift.

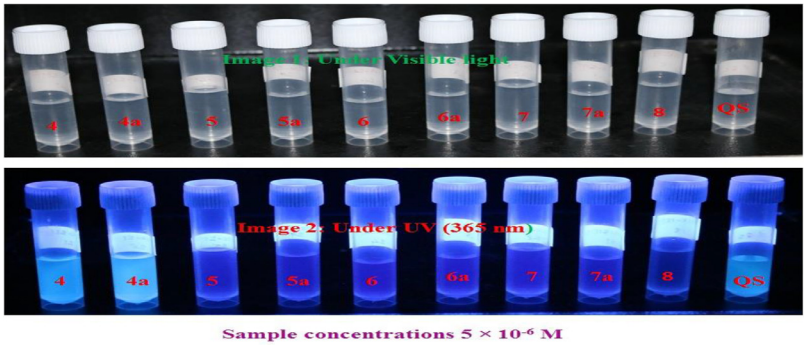


Fig. 8. Photographic images of all newly synthesized compounds under normal (image 1) and UV light (image 2) of wavelength 365 nm.

4. Conclusion

In summary, we have reported a multi-component one-step methodology for the synthesis of novel 4-alkyl-3-aryl-2,6-dicyanoanilines using tris(hydroxymethyl)aminomethane (THAM) as a mild catalyst. Some of the newly synthesized compounds have exhibited good quantum yield and can have potential utility in various biological, photophysical and forensic science applications. We are planning to screen these molecules for various fluorescence bio-imaging applications, trace element detection etc. and the results will be published elsewhere.

Acknowledgments

ASK thanks Government of Maharashtra, Home department for granting the permission for Ph.D. research work and Director, Forensic Science Laboratory, Mumbai for the necessary support. Dr. Anil H. Gore gratefully acknowledges Science and Engineering Research Board (SERB), New Delhi (Government of India) for the partial financial support (File no. YSS/2015/001806). We also thank Dr. Naganath G. Patil for important scientific discussions.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cdc.2018.100172.

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Formal Synthesis of (−)-Quinagolide: Diastereoselective Ring Expansion via a Bicyclic Aziridinium Ion Strategy to Access the Octahydrobenzo[*g*]quinoline Architecture

Subhash P. Chavan,* Sanket A. Kawale, Mahesh M. Pisal, Appasaheb L. Kadam, and Rajesh G. Gonnade

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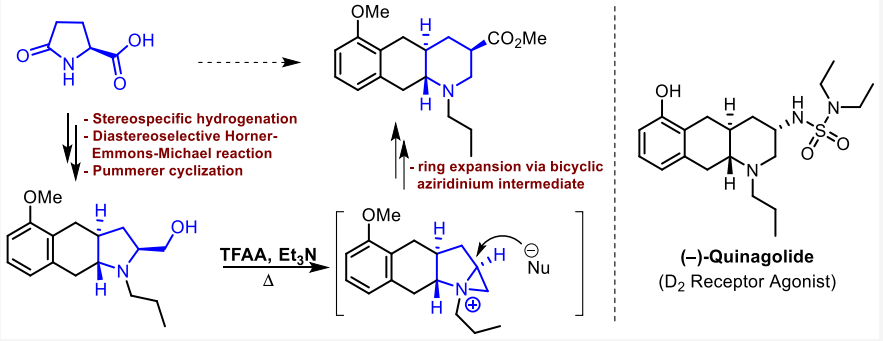

ABSTRACT: The diastereoselective formal synthesis of (−)-quinagolide, a D_2 receptor agonist, has been achieved. The synthesis started from L-pyroglutamic acid and relied on utilization of (a) a stereospecific catalytic hydrogenation and diastereoselective Horner−Emmons−Michael cascade to obtain functionalized prolinate, (b) a Lewis acid mediated Pummerer cyclization to construct a tricyclic fused ring system, and (c) a diastereoselective ring expansion via a bicyclic aziridinium intermediate to access the required 3-substituted piperidine scaffold.

INTRODUCTION

Dopamine agonist agents are used as a first-line treatment to suppress excess prolactin secretion from the pituitary gland.[1] Prolactin is a protein hormone which plays many important roles in the human body, including lactation, but its excess level in the blood is responsible for the disease hyperprolactinemia.[2,3] Dopamine agonist drugs bind to D_2 dopamine receptors on the surface of lactotroph cells in the anterior pituitary gland and inhibit the prolactin secretion.[1,3,4]

Quinagolide is a non-ergot-derived dopamine agonist drug with high affinity for the D_2 receptor, its enantiomer (−)-**1** being the eutomer. Its phenylethylamine framework is a dopamino-mimitic pharmacophore, and the *N*-side chain at C-3 is responsible for its specific dopamine D_2 receptor activity.[5] Due to its more specific nature, it is associated with fewer side effects in comparison to the ergot alkaloids bromocriptine (**2**), carbegoline (**3**), and pergolide (**4**), which are clinically used for hyperprolactinemia (Figure 1).[3a] Its racemic synthesis was targeted five times, wherein all of them followed a stepwise linear approach.[5a,6] Quinagolide (**1**) was first synthesized by Nordmann et al.[5a] (Figure 2). In 2000, Bänziger et al.[6a] reported the scalable synthesis of a quinagolide intermediate. As a continuation of our efforts to explore the new synthetic methods for biologically active compounds, synthetic studies toward quinagolide were initiated in our laboratory. The key task is to install the fused 3-aminopiperidine in a *trans* orientation as per the architectural requirement of quinagolide. Recently, in 2019, we reported the CAN-mediated azidoalkoxylation of enol ethers and ring-closing metathesis reaction for the construction of the 3-aminopiperidine scaffold of quinagolide.[6b,c]

Quinagolide hydrochloride (Norprolac) is available in the market in its racemic form, and there is a need to replace it by its eutomer for optimum treatment and the right therapeutic control for the patient.[7] Accordingly, development of a practical route for the enantioselective synthesis of (−)-quinagolide **1** is highly desirable. Very recently our group reported its first enantioselective synthesis using an organocatalyzed Diels−Alder reaction to fixing all three stereocenters on the piperidine ring (Figure 2).[8] In parallel, an effort was made to develop a chiral pool approach for the synthesis of (−)-quinagolide (**1**), which is described in the present article. This work uses commercially available L-pyroglutamic acid as the starting

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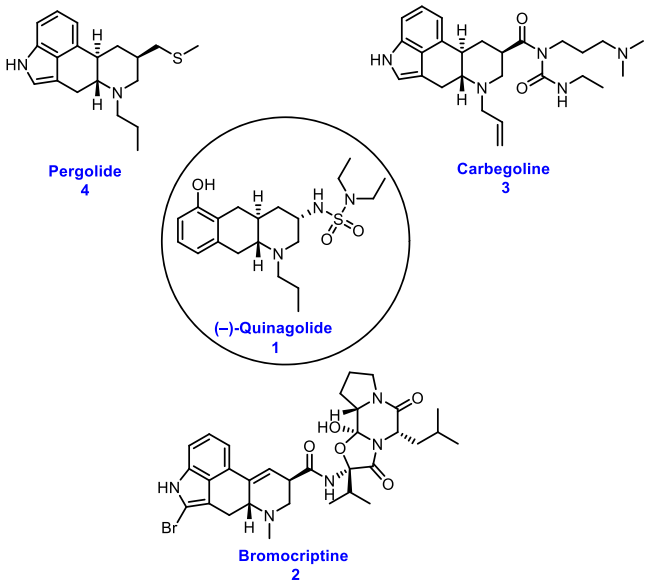

Figure 1. Structures of (−)-quinagolide (**1**), bromocriptine (**2**), carbegoline (**3**) and pergolide (**4**).

material and utilizes the bicyclic aziridinium ion to access the functionalized piperidine subunit of (−)-quinagolide **1**.[9]

The retrosynthetic plan is outlined in Scheme 1. (−)-Quinagolide (**1**) could be obtained from prolinol **5** by a ring expansion rearrangement. A Pummerer cyclization could be utilized to construct a tricyclic fused ring system from the sulfoxide **6**, which could be obtained from protected L-pyroglutamic acid by employing an aldol condensation, hydrogenation, reduction of lactam carbonyl, and Horner−Emmons−Michael reaction sequence.

RESULTS AND DISCUSSION

Accordingly, synthesis of (−)-quinagolide (**1**) was started by the using inexpensive and abundant L-pyroglutamic acid (**7**) as the starting material (Scheme 2).

L-Pyroglutamic acid (**7**) was esterified to its isopropyl ester followed by its *N*-methyl carbamate protection to provide the protected amino acid derivative **8**. A sterically hindered isopropyl group for esterification was chosen to get better diastereoselectivity in the penultimate steps and because of its stability under acidic conditions. An *N*-methyl carbamate protecting group was selected due to its stability under acidic conditions. *cis* adduct **10**, as the structural requirement of **1**, was achieved from **8** and *o*-anisaldehyde by aldol condensation followed by catalytic hydrogenation by following the known literature for a closely related compound.[10] The stereochemistry of the 2-methoxybenzyl group in **9** and **10** was confirmed by two-dimensional (2D) nuclear Overhauser effect (NOE) data (Supporting Information) and was found to be the same as that mentioned in literature for a closely related compound.[10]

A Horner−Emmons−Michael reaction of the hemiaminal with substituted phosphonate is the best way to obtain the 5-substituted prolinate with high 1,4- or 1,2-*trans* diastereoselectivity as our requirement.[11,12] Accordingly, the lactam carbonyl of **10** was reduced using DIBAL-H to obtain hemiaminal **11**, which was treated with diethyl ((phenylsulfinyl)methyl)phosphonate[13] (**12**) to provide sulfoxide **14** in 85% yield as a nonseparable mixture of diastereomers (mixture of four diastereomers; *cis* and *trans* isomers and their two isomers due to sulfoxide). For characterization, sulfoxide **14** was oxidized to the corresponding sulfone, which existed as a mixture of diastereomers with a 4:1 diastereomeric ratio (*trans:cis* ratio ≥4:1), which was separated by using flash silica gel column chromatography (Scheme 3).[14] The *trans* and *cis* stereochemistry of the major and minors isomer of **14** was confirmed by converting both isomers to compounds **18** and **19** as shown in Scheme 4. The preferential intramolecular Michael addition of the nitrogen nucleophile from the sterically less hindered *Si* face of *α,β*-unsaturated sulfoxide **13** possibly occurs in a conformation which minimizes 1,3-allylic strain and leads to the observed diastereoselectivity. To check the possibility of improving the diastereoselectivity by performing a Michael addition at intermediate olefin **13** at lower temperature,[15] an attempt was made to isolate intermediate olefin **13** by performing a Horner−Emmons reaction at 0 °C, which resulted in isolating a mixture of the starting material **11** and cyclized product **14**, which also indicated that the cyclization of **13** to **14** is very fast in comparison to olefination of **11** to **13** and hence the above Horner−Emmons followed by Michael reaction might be a tandem process. On the other hand, the starting material was recovered during a Wittig olefination of hemiaminal **11** with *α*-sulfinylphosphonium ylide or with *α*-sulfidophosphonium ylide to achieve an intermediate olefin.[15,16]

Thus, obtaining the Pummerer cyclized product **17** in good yields from the sulfoxide **14** needed an investigation (Scheme 4).[17] Initially, sulfoxide **14** on treatment with trifluoroacetic anhydride followed by treatment with $BF_3{\cdot}Et_2O$ gave a complex reaction mixture along with the expected cyclized product **17** in 34% yield. The yield of cyclized product **17** was slightly improved to 45% by following a two-step procedure in which sulfoxide **14** was initially heated in Ac_2O in the presence of NaOAc to obtain the corresponding *α*-acetoxy thioether **15**, which in turn underwent Lewis acid induced ($SnCl_4$, CH_2Cl_2, 0 °C) cyclization. The best result was achieved when sulfoxide **14** was treated with $SOCl_2$ to obtain the corresponding *α*-chloro thioether **16**, which gave the Lewis acid induced cyclized product **17** in 67% yield as an inseparable mixture of diastereomers.[18] Reduction of the ester group of **17** followed by reductive desulfurization gave cyclic carbamate **18** as a major product along with alcohol **19**, which was separated by using flash silica gel column chromatography and found to exist in a 3:1 ratio. The structure and absolute stereochemistry of **18**, which contained the required *trans* orientation at the ring junction, was established by its single-crystal X-ray analysis, whereas the structure and *cis* relative stereochemistry of **19** at the ring junction was confirmed on the basis of two-dimensional (2D) nuclear Overhauser effect (NOE) data. The relative stereochemistry at the ring junction in **18** and **19** confirmed the aforementioned *trans:cis* ratio (≥4:1) observed in the Horner−Emmons−Michael reaction. Here, a slight change in dr ratio (4:1 to 3:1) was observed during the Pummerer cyclization and ester reduction followed by desulfurization reaction sequence.

After obtaining the tricyclic product **18** with a *trans* orientation at the ring junction, our next target was to obtain the octahydrobenzo[*g*]quinolone skeleton utilizing an aziridinium ring expansion protocol.[9] For that, cyclic carbamate **18** was deprotected to obtain an amino alcohol, which after *N*-alkylation gave required prolinol **5** in 45% yield (Scheme 5).[19] Treatment of prolinol **5** with trifluoroacetic anhydride (TFAA) in THF followed by addition of triethylamine and heating of the resulting reaction mixture at 120 °C for 24 h led to the ring-expansion product **20** after hydrolytic workup as a single isomer

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J. Org. Chem. 2021, 86, 9344−9352

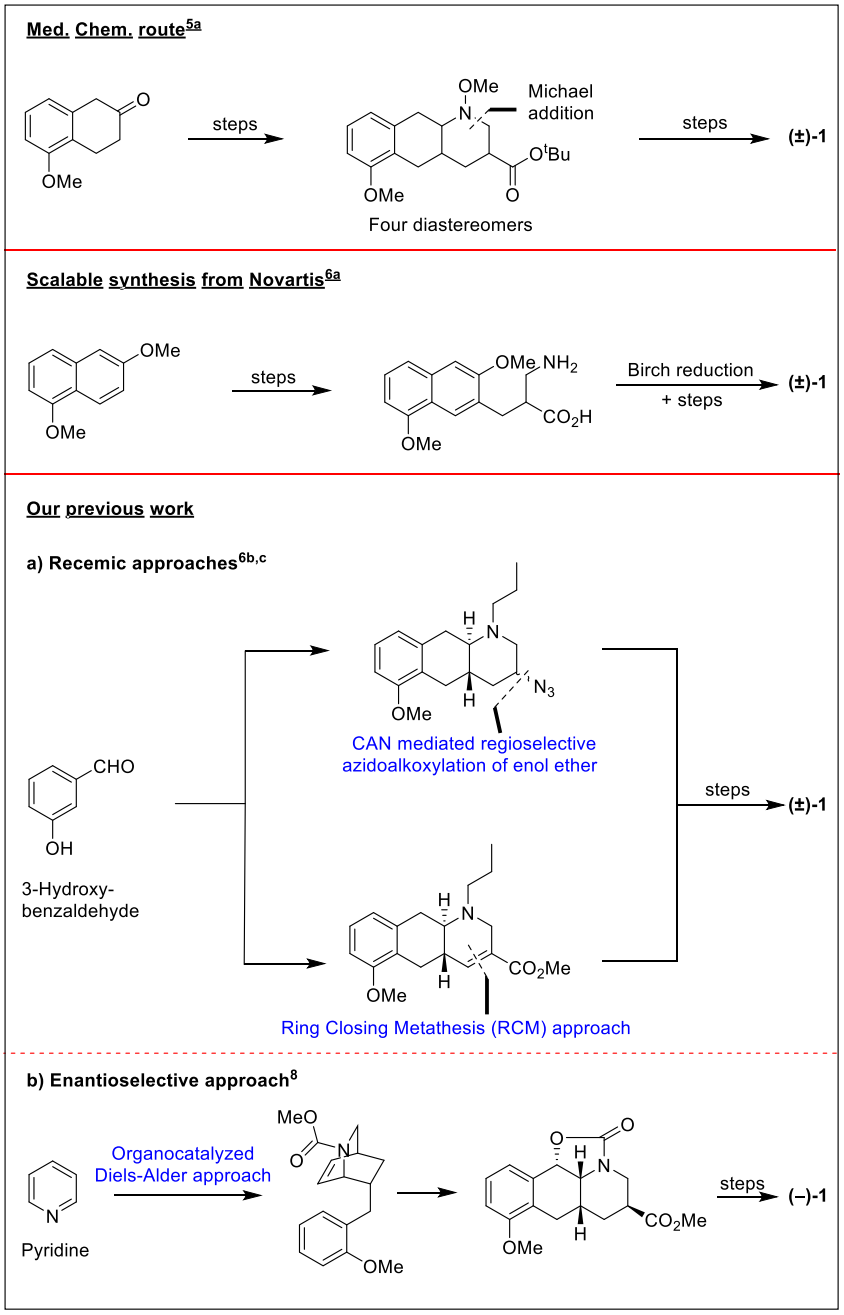

Figure 2. Previous approaches of quinagolide synthesis.

with an *R* configuration at C-3. The observed *R* configuration at C-3 was assigned on the basis of two-dimensional (2D) nuclear Overhauser effect (NOE) data (Supporting Information). It was observed that the ring-expansion product was formed only above 110 °C. Refluxing the reaction mixture in THF for 24 h led to the recovery of starting material **5**, whereas in 1,4-dioxane as a solvent, the formation of product **20** was observed along with the recovery of the starting material **5** when the reaction mixture was refluxed for several hours.

After obtaining 3-hydroxyoctahydrobenzo[*g*]quinoline **20** with the requisite stereochemistry at the ring junction, we decided to introduce an *N*-side chain at the C-3 position with the desired stereochemistry. Accordingly, **20** was converted to its mesylate and treated with sodium azide in order to get the required inversion of configuration product at C-3 (C-3 epimer of **21**) where all three stereocenters will be as exactly same as those of (−)-quinagolide. However, retention of configuration product **21** was observed exclusively instead of the required C-3 epimer of **21**. The structure and relative stereochemistry of **21** was confirmed by a 2D NMR analysis (Scheme 5).

The observed retention of configuration was due to regioselective ring opening of bicyclic aziridinium ion **25** by an azide anion formed by a neighboring-group participation of the lone pair of nitrogen in mesylate **24** to form **21** (Scheme 6).

In an alternate way, the mesylate group in **24** was displaced by cyanide ion to obtain **22** as a single regioisomer again with retention of configuration, which in turn was refluxed in acidic methanol to give ester **23** (Scheme 5). The structure and relative stereochemistry of **23** were confirmed by comparing the spectral data of compound **23** with the spectral details of the same

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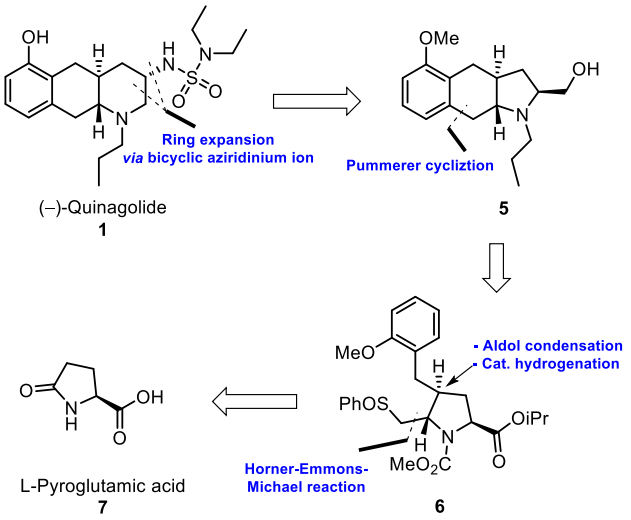
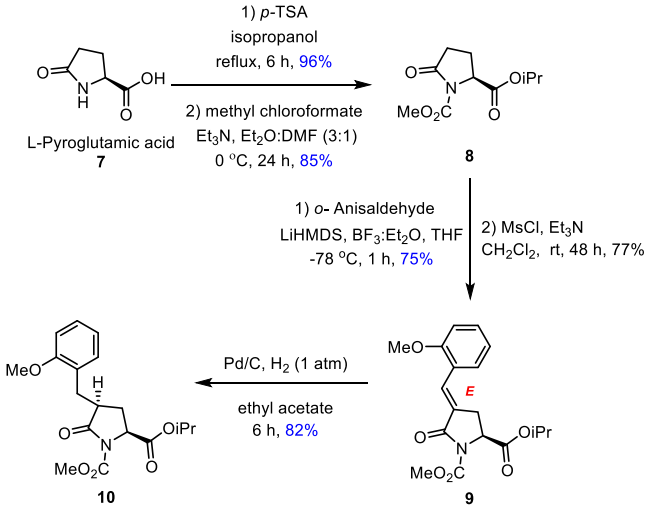
Scheme 1. Retrosynthetic Analysis



Scheme 2. Synthesis of *cis* 4-Substituted Pyroglutamate



compound reported in the literature.[6c] The aforeentioned relative configuration of **22** is assumed to be correct, as it only gave compound **23** after hydrolysis. By following the reaction sequence given in the aforementioned reference, (−)-quinagolide (**1**) could be obtained from (−)-ester **23**; thus, the present work constitutes a formal synthesis of (−)-quinagolide (**1**).

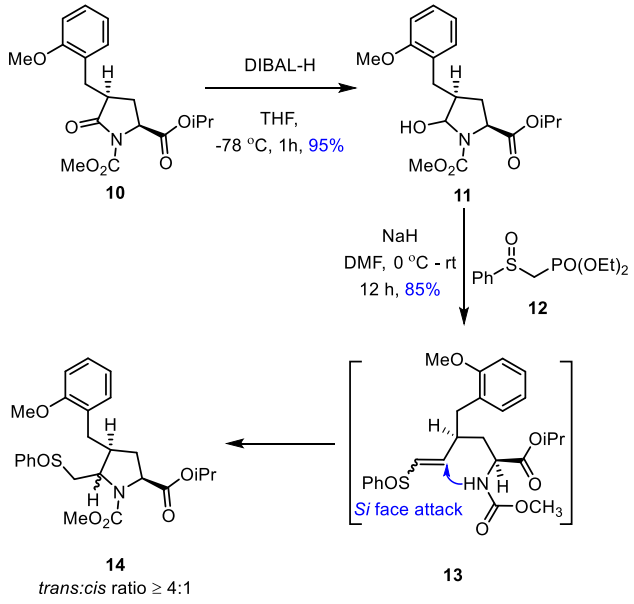
CONCLUSION

In summary, we have achieved a diastereoselective formal synthesis of (−)-quinagolide from commercially available L-pyroglutamic acid. The significant features of this synthesis involve (i) a diastereoselective tandem Horner−Emmons−Michael reaction to obtain a sulfoxide, a substrate for the key Pummerer cyclization, (ii) utilization of Lewis acid catalyzed Pummerer cyclization to construct a tricyclic fused ring system, and (iii) ring expansion via a bicyclic aziridinium ion to access the functionalized piperidine subunit of (−)-quinagolide.

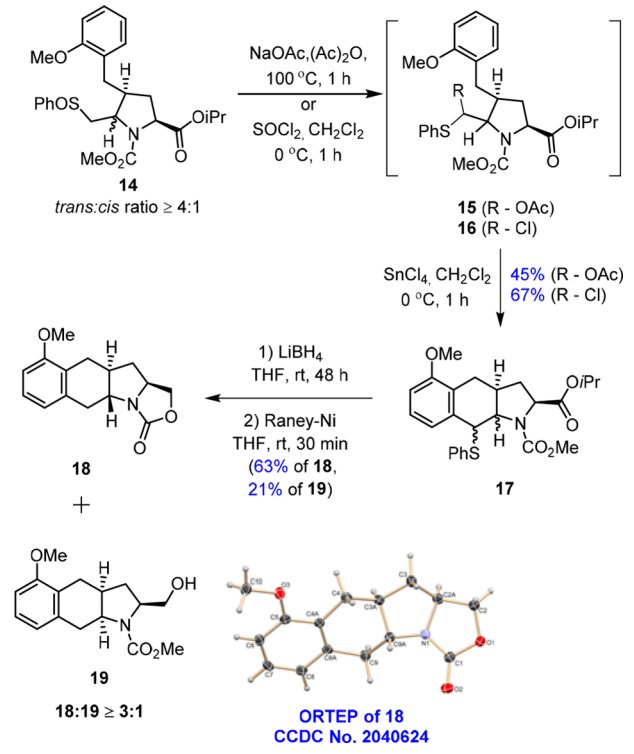
EXPERIMENTAL PROCEDURES

General Considerations. A positive nitrogen or argon pressure was used for all moisture- and air-sensitive reactions by using oven-dried glassware fitted with a rubber septum. All solvents were distilled prior to use. All commercially available reagents and chemicals were used without any purification. All reactions were magnetically stirred, and oil baths were used for reactions that required heating. A syringe or cannula was used to transfer air- and moisture-sensitive solvents and reagents via a rubber septum. Thin-layer chromatography (TLC) with 0.25 mm precoated silica gel plates (60 F254) and mass spectroscopy (MS) were used to monitor all reactions. Phosphomolybdic acid (PMA), *p*-anisaldehyde, 2,4-DNP, $KMnO_4$, or ninhydrin solutions were used to get good TLC images. Anhydrous Na_2SO_4 was used to dry organic layers after extraction, and it was removed by filtration through a cotton plug. The dried organic layer was concentrated and purified as per the requirements. Silica gel (60−120 and 230−400 mesh) and distilled solvents were used for column chromatography separations.

Scheme 3. Synthesis of Prerequisite Sulfoxide for Pummerer Cyclization



Scheme 4. Pummerer Cyclization



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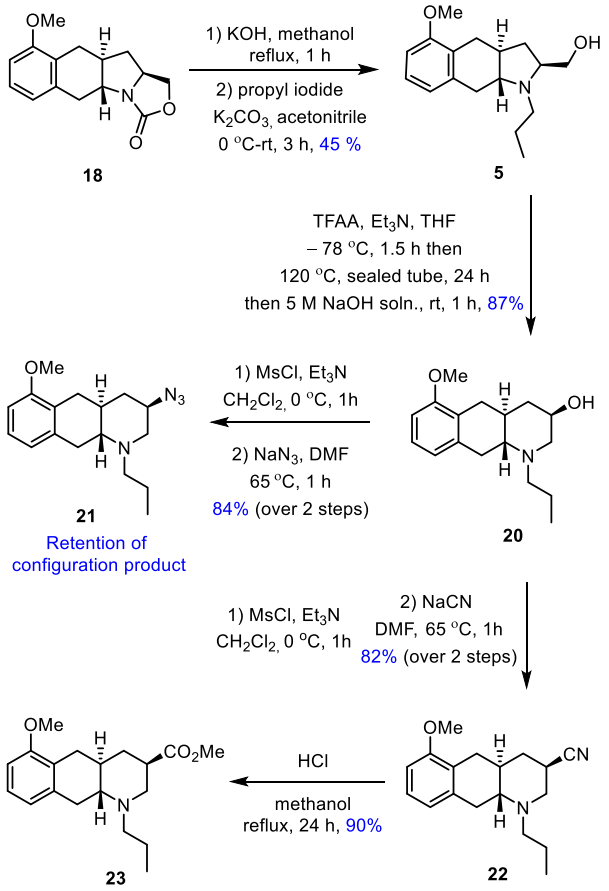
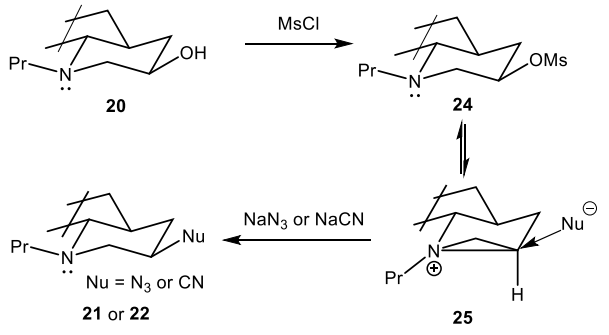
Scheme 5. Formal Synthesis of (−)-Quinagolide



Scheme 6. Possible Mechanism for Observed Retention of Configuration



Melting points are uncorrected. Bruker AV 200, 400, and 500 MHz NMR spectrometers were used to record ^{1}H NMR and ^{13}C NMR spectra. Solvent residue signals were used as an internal standard: ^{1}H NMR, $CDCl_3$ (7.27 ppm), DMSO-d_6 (2.50 ppm); ^{13}C NMR, $CDCl_3$ (77.00 ppm), DMSO-d_6 (39.51 ppm). Orbitrap (quadrupole plus ion trap) and a TOF mass analyzer were used to record HRMS (ESI). The IR spectra were recorded on an FT-IR spectrometer. Structural assignments were made with additional information from gCOSY, gNOESY, gHSQC, and gHMBC experiments.

(−)-2-Isopropyl 1-Methyl (*S*)-5-Oxopyrrolidine-1,2-dicarboxylate (8). A magnetically stirred solution of L-pyroglutamic acid (7; 50.00 g, 387.25 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TSA) (3.68 g, 19.36 mmol, 0.05 equiv) was refluxed in isopropanol (150 mL) under nitrogen for 12 h using a Dean−Stark trap variation (condensate returned through a Soxhlet extractor filled with 4 Å molecular sieves). After it was cooled to room temperature, the reaction mixture was quenched by slow addition of a saturated aqueous $NaHCO_3$ solution (30 mL). The organic solvent was evaporated on a rotary evaporator under reduced pressure, and the obtained residue was washed with ethyl acetate (3 × 250 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain (+)-isopropyl (*S*)-5-oxopyrrolidine-2-carboxylate (63.64 g, 96%) as white prisms. Mp: 68 °C. IR ($CHCl_3$, cm^{-1}): ν_{max} 3020, 1695, 1658, 1215, 766. [α] = +4.00 (*c* 5, MeOH) {lit.[20] [α] = +4.0 (*c* 5, MeOH)}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.00−4.93 (m, 1H), 4.14−4.11 (m, 1H), 2.40−2.32 (m, 1H), 2.30−2.20 (m, 2H), 2.11−2.04 (m, 1H), 1.18 (s, 3H), 1.17 (s, 3H). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ 178.2, 171.5, 68.9, 55.5, 29.1, 24.5, 21.4 (2C).

To a magnetically stirred solution of (+)-isopropyl (*S*)-5-oxopyrrolidine-2-carboxylate (5.00 g, 29.21 mmol) and triethylamine (8.20 mL, 58.41 mmol, 2 equiv) in dry Et_2O/DMF (3/1, 40 mL) at 0 °C under an N_2 atmosphere was slowly added methyl chloroformate (2.94 mL, 37.97 mmol, 1.3 equiv) over a period of 30 min, and the reaction mixture was stirred at the same temperature for 48 h. After complete consumption of the starting material as checked by TLC, the reaction mixture was filtered through a pad of Celite with washing of the residue by ethyl acetate (3 × 50 mL). The organic filtrate was concentrated on a rotary evaporator under reduced pressure, and the residue obtained was purified by using flash silica gel column chromatography with 30% ethyl acetate in petroleum ether as an eluent to obtain the title compound **8** (5.70 g, 85%) as a colorless liquid. R_f = 0.5 (petroleum ether/ethyl acetate, 6/4). IR ($CHCl_3$, cm^{-1}): ν_{max} 3023, 1795, 1736, 1217, 770. [α] = −71.80 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.00−4.94 (m, 1H), 4.52 (d, *J* = 9.2 Hz, 1H), 3.74 (s, 3H), 2.58−2.22 (m, 3H), 1.98−1.93 (m, 1H), 1.18−1.14 (m, 6H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 172.7, 170.2, 151.4, 69.2, 58.6, 53.4, 30.8, 21.5, 21.3, 21.2. HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{10}H_{16}O_5N$ 230.1023, found 230.1021.

(−)-2-Isopropyl 1-Methyl (*S*,*E*)-4-(2-Methoxybenzylidene)-5-oxopyrrolidine-1,2-dicarboxylate (9). To a magnetically stirred solution of **8** (10.00 g, 43.62 mmol) in anhydrous THF (400 mL) at −78 °C was added a 1 M solution of lithium hexamethyldisilazide (48.00 mL, 47.99 mmol, 1.1 equiv) under an N_2 atmosphere. The reaction mixture was stirred for 1 h at −78 °C prior to the addition of a solution of *o*-anisaldehyde (7.13 g, 52.53 mmol, 1.2 equiv) and BF_3·Et_2O (6.46 mL, 52.35 mmol, 1.2 equiv) in anhydrous THF (20 mL). The reaction mixture was stirred for 1 h at −78 °C. The reaction was quenched with saturated NH_4Cl solution (100 mL), the organic layer was separated, and the water layer was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain a residue that was purified by using flash silica gel column chromatography with 35% ethyl acetate in petroleum ether as an eluent to provide the aldol product (11.95 g, 75%) as a colorless liquid.

To a magnetically stirred solution of the aldol adduct (11.95 g, 32.71 mmol) in anhydrous CH_2Cl_2 (100 mL) at 0 °C under an N_2 atmosphere obtained above was added triethylamine (46.00 mL, 327.05 mmol, 10 equiv) followed by methanesulfonyl chloride (3.04 mL, 39.25 mmol, 1.2 equiv), and the mixture was stirred for 2 days at room temperature. After complete consumption of the starting material as checked by TLC (~2 days), water (100 mL) was added to the reaction mixture, which was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to afford crude product **9**, which was purified by using flash silica gel column chromatography with 20% ethyl acetate in petroleum ether as an eluent to obtain compound **9** (8.75 g, 77%) as a colorless crystalline compound. R_f = 0.5 (petroleum ether/ethyl acetate, 7/3) IR ($CHCl_3$, cm^{-1}): ν_{max} 3012, 1785, 1736, 1316, 773. [α] = −86.17 (*c* 1, $CHCl_3$). Mp: 85−90 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (br s, 1H), 7.34−7.23 (m, 2H), 6.92 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 5.03−4.94 (m, 1H), 4.63 (dd, *J* = 3.4, 10.1 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.29 (ddd, *J* = 3.1, 10.4, 17.7 Hz, 1H), 2.88−2.82 (m, 1H), 1.19 (d, *J* = 6.7 Hz, 3H), 1.17 (d, *J* = 6.7 Hz, 3H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 170.2, 166.4, 158.1, 152.2, 131.0, 130.5, 129.0, 126.3, 123.2,

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120.1, 110.8, 69.3, 55.9, 55.3, 53.5, 28.0, 21.4, 21.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{18}H_{22}O_6N$ 348.1442, found 348.1439.

(−)-2-Isopropyl 1-Methyl (2*S*,4*S*)-4-(2-Methoxybenzyl)-5-oxopyrrolidine-1,2-dicarboxylate (10). To a magnetically stirred solution of **9** (2.00 g, 5.72 mmol) in ethyl acetate (20 mL) was added a catalytic quantity of 10% palladium on carbon. The solution was stirred at room temperature under an atmosphere of hydrogen (balloon pressure) for a period of 48 h. After complete consumption of the starting material as checked by TLC, the mixture was filtered over a Celite pad and the Celite rinsed with ethyl acetate (3 × 30 mL). The filterate was evaporated on a rotary evaporator under reduced pressure to provide title compound **10**, which was purified by using flash silica gel column chromatography with 20% ethyl acetate in petroleum ether as an eluent to obtain compound **10** (1.64 g, 82%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 7/3). IR ($CHCl_3$, cm^{-1}): ν_{max} 3023, 1794, 1737, 1216, 764. [α]: −117.15 (c 2, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.21 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.2 Hz, 1H), 6.90−6.82 (m, 2H), 5.13−5.05 (m, 1H), 4.46 (dd, J = 6.5, 8.8 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.29 (dd, J = 4.2, 13.7 Hz, 1H), 3.05−2.93 (m, 1H), 2.68 (dd, J = 10.7, 13.4 Hz, 1H), 2.30 (td, J = 9.2, 13.4 Hz, 1H), 1.78−1.72 (m, 1H), 1.28 (d, J = 6.5 Hz, 3H), 1.24 (d, J = 6.5 Hz, 3H). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 174.5, 170.7, 157.5, 151.9, 130.6, 128.0, 126.6, 120.4, 110.3, 69.2, 57.5, 55.0, 53.6, 42.8, 31.6, 27.0, 21.5 (2C). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{18}H_{24}O_6N$ 350.1598. found 350.1593.

(−)-2-Isopropyl 1-Methyl (2*S*,4*S*)-5-Hydroxy-4-(2-methoxybenzyl)pyrrolidine-1,2-dicarboxylate (11). To a magnetically stirred solution of compound **10** (5.00 g, 14.31 mmol) in anhydrous THF (50 mL) under an atmosphere of argon at −78 °C was added DIBAL-H (18.7 mL, 1 M in toluene, 18.60 mmol, 1.3 equiv) over a period of 1 h. The resulting solution was stirred at −78 °C for fa urther 1 h, before being quenched by the addition of anhydrous MeOH (0.5 mL). After the mixture was warmed to 0 °C, a 1 M aqueous solution of Rochelle's salt (30 mL) and EtOAc (50 mL) was added and the biphasic solution was stirred vigorously for 2 h. The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated on a rotary evaporator under a reduced pressure to afford the crude product, which was purified by using flash silica gel column chromatography with 30% ethyl acetate in petroleum ether as an eluent to obtain a diastereomeric mixture of pure hemiaminal **11** (4.80 g, 95%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 6/4). IR ($CHCl_3$, cm^{-1}): ν_{max} 3022, 1708, 1216, 766. [α] = −82.76 (c 2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$), mixture of diastereomers and rotamers was observed: δ 7.23−7.04 (m, 2H), 6.90−6.80 (m, 2H), 5.42−5.24 (m, 1H), 5.16−4.98 (m, 1H), 4.42−4.12 (m, 1H), 4.06 (br s, 0.3H), 3.82, 3.81, 3.80 (s, 3H), 3.78, 3.71, 3.70, 3.65 (s, 3H), 3.38−3.10 (m, 0.7H), 2.95−2.85 (m, 1H), 2.76−2.44 (m, 2H), 2.42−2.23 (m, 1H), 1.99−1.74 (m, 1H), 1.29−1.22 (m, 6H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$), mixture of diastereomers and rotamers was observed: δ 172.8, 172.3, 172.1, 171.8, 157.6, 157.5, 157.2, 157.1, 155.7, 155.0, 154.7, 154.6, 130.68, 130.66, 130.6, 128.2, 128.1, 127.6, 127.49, 127.47, 120.5, 120.4, 120.23, 120.18, 110.22, 110.16, 87.3, 86.4, 82.6, 82.1, 69.0, 68.7, 68.6, 59.1, 59.0, 58.6, 55.2, 55.1, 55.0, 52.8, 52.8, 52.5, 45.0, 44.7, 44.2, 43.6, 33.6, 32.5, 32.5, 32.3, 32.2, 29.6, 29.1, 28.9, 21.63, 21.59, 21.56. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{18}H_{25}O_6NNa$ 374.1564, found 374.1574.

Diethyl ((Phenylsulfinyl)methyl)phosphonate (12).[13] A solution of sodium metaperiodate (4.50 g, 0.02 mol, 1.05 equiv) in water (75 mL) was added dropwise to a well-stirred and cooled (−5 to 0 °C) solution of diethyl ((phenylthio)methyl)phosphonate (5.21 g, 0.02 mol) in acetone (20 mL) and water (10 mL) during the course of 45 min. After the mixture was stirred further for 4 h at 0 °C, the precipitated sodium iodate was filtered off and the solvent was evaporated under reduced pressure. The residue thus obtained was diluted with water (100 mL) and washed with ethyl acetate (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain the crude product, which was purified by using flash silica gel column chromatography with 60% ethyl acetate in petroleum ether as an eluent to afford pure diethyl ((phenylsulfinyl)methyl)phosphonate **12** (3.59 g, 65%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 20/80). ^1H NMR (400 MHz, $CDCl_3$): δ 7.67−7.65 (m, 2H), 7.45−7.44 (m, 3H), 4.11−3.98 (m, 4H), 3.37−3.18 (m, 2H), 1.25−1.19 (m, 6H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 144.5 and 144.4 (1C), 131.4, 129.1 (2C), 124.0 (2C), 62.7 and 62.6 (1C), 62.5 and 62.5 (1C), 55.23 and 53.9 (1C), 16.1 and 16.0 (1C), 16.0 and 16.0 (1C).

2-Isopropyl 1-Methyl (2*S*,4*S*)-4-(2-methoxybenzyl)-5-((phenylsulfinyl)methyl)pyrrolidine-1,2-dicarboxylate (14). To a magnetically stirred suspension of sodium hydride (0.26 g, 10.93 mmol, 1.2 equiv) in anhydrous DMF (20 mL) under an atmosphere of argon at 0 °C was slowly added diethyl ((phenylsulfinyl)methyl)-phosphonate (**12**; 3.30 g, 11.48 mmol, 1.3 equiv) over a period of 45 min, and then a solution of hemiaminal **11** (3.20 g, 9.11 mmol) in anhydrous DMF (5 mL) was added. The reaction mixture was quickly warmed to room temperature and stirred at the same temperature for 12 h. After complete consumption of the starting material as checked by TLC, the reaction mixture was quenched with saturated aqueous NH_4CI solution (25 mL). The resulting solution was extracted with ethyl acetate (3 × 75 mL). The combined organic extracts were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain a residue which was purified by using flash silica gel column chromatography with 30% ethyl acetate in petroleum ether as an eluent to obtain a diastereomeric mixture of **14** (3.67 g, 85%) as a colorless oil. R_f: 0.5 (petroleum ether/ethyl acetate, 5/5); HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{32}O_6NS$ 474.1945, found 474.1947.

For characterization, compound **14** was oxidized using *m*-CPBA to its sulfone. To a magnetically stirred solution of compound **14** (150.00 mg, 316.73 μmol) in CH_2Cl_2 (3 mL) at 0 °C was added *m*-CPBA (110.00 mg, 633.47 μmol, 2 equiv). After complete consumption of the starting material as checked by TLC (∼2 h), a saturated $NaHCO_3$ solution (3 mL) was added to the reaction mixture. The resulting solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain crude product **14a** as a mixture of diastereomers which was separated by using flash silica gel column chromatography with 15% ethyl acetate in petroleum ether as an eluent to furnish the major diastereomer of sulfone (93.00 mg, 60%) and the minor diastereomer of sulfone (23.00 mg, 15%) as colorless liquids.

Data for the major diastereomer of sulfone of **14** are as follows. R_f = 0.4 (petroleum ether/ethyl acetate, 70/30). IR ($CHCl_3$, cm^{-1}): ν_{max} 1642, 1444, 1217, 770. [α] = −89.59 (c 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$), mixture of rotamers was observed in 6:4 ratio: δ 7.68−7.46 (m, 5H), 7.35−7.24 (m, 1H), 7.05−7.01 (m, 1H), 6.97−6.87 (m, 2H), 5.15−5.06 (m, 1H), 4.25 (d, J = 10.7 Hz, 0.4H), 4.21 (d, J = 10.3 Hz, 0.6H), 3.91 (d, J = 9.5 Hz, 0.6H), 3.86 (s, 3H), 3.80 (d, J = 10.3 Hz, 0.4H), 3.58 (s, 0.4H), 3.55 (s, 2H), 3.40 (s, 1H), 3.34−3.20 (m, 1.6H), 3.19−3.05 (m, 1H), 2.85−2.67 (m, 2H), 2.58−2.50 (m, 0.6H), 2.49−2.41 (m, 0.4H), 1.99−1.90 (m, 1H), 1.30−1.20 (m, 6H). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$), mixture of rotamers was observed: δ 172.0 171.4, 158.1, 157.9, 154.4, 154.2, 139.0, 138.7, 133.5, 131.3, 131.1, 129.1, 129.0, 128.2, 127.9, 127.8, 127.8, 127.6, 120.3, 120.1, 110.5 110.4, 68.8 68.7, 59.2, 57.9, 58.9, 58.7, 58.5, 56.4, 55.1, 52.43 52.36, 41.9 40.3, 34.6, 34.5, 33.0, 31.7, 21.6, 21.5. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{32}O_7NS$ 490.1894, found 490.1890.

Data for the minor diastereomer of sulfone of **14** are as follows. R_f = 0.5 (petroleum ether/ethyl acetate, 70:30). IR ($CHCl_3$, cm^{-1}): ν_{max} 1704, 1639, 1448, 1219, 770. [α] = −7.60 (c 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$), mixture of rotamers was observed: δ 7.72−7.42 (m, 5H), 7.37−7.23 (m, 1H), 7.07 (t, J = 7.3 Hz, 1H), 7.01−6.85 (m, 2H), 5.11−4.94 (m, 1H), 4.34 (t, J = 8.9 Hz, 0.5H), 4.25 (t, J = 8.5 Hz, 0.5H), 3.95−3.76 (m, 4H), 3.71−3.57 (m, 2H), 3.49−3.34 (m, 2H), 3.29−3.13 (m, 1.5H), 3.03 (m, 0.5H), 2.76−2.52 (m, 2H), 2.23−2.13 (m, 1H), 2.10−1.94 (m, 1H), 1.29−1.19 (m, 6H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$), mixture of rotamers was observed: δ 172.3, 172.2, 157.8, 154.4, 154.2, 138.9, 138.7, 133.5, 133.3, 131.1, 131.0, 129.2, 129.0, 128.1, 128.0, 127.93, 127.90, 127.2, 127.1, 120.48, 120.45, 110.5, 110.4, 68.9, 68.7, 58.5, 58.3, 58.2, 57.4, 56.9, 55.1, 55.0, 52.53, 52.49, 42.6,

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41.0, 33.5, 33.4, 32.6, 21.64, 21.59, 21.5. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{32}O_7NS$ 490.1894, found 490.1880.

2-Isopropyl 1-Methyl (2*S*,3a*S*)-5-Methoxy-9-(phenylthio)-2,3,3a,4,9,9a-hexahydro-1*H*-benzo[*f*]indole-1,2-dicarboxylate (17). To a solution of sulfoxide **14** (0.70 g, 1.48 mmol) in anhydrous CH_2Cl_2 (10 mL) under an atmosphere of argon at 0 °C was slowly added thionyl chloride (0.16 mL, 2.22 mmol, 1.5 equiv) diluted in anhydrous CH_2Cl_2. After the resulting solution was stirred at the same temperature for 1 h, the organic solvent and excess thionyl choride were evaporated on a rotary evaporator. The obtained residue was taken up in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 °C before dropwise addition of $SnCl_4$ (0.26 mL, 2.22 mmol, 1.5 equiv). After the mixture was stirred for 15 min at 0 °C, an aqueous saturated $NaHCO_3$ solution was added and the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure. The obtained residue was purified using flash silica gel column chromatography with 10% ethyl acetate in petroleum ether as eluent to afford pure **17** (451.00 mg, 67%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 80/20). IR ($CHCl_3$, cm^{-1}): ν_{max} 1697, 1641, 1434, 1380, 1215, 772. ^1H NMR (400 MHz, $CDCl_3$), mixture of diastereomers and their rotamers was observed: δ 7.62−7.50 (m, 1.4H), 7.37−7.25 (m, 3H), 7.23−7.15 (m, 1H), 7.11−6.95 (m, 1H), 6.87−6.63 (m, 1.7 H), 5.46−4.93 (m, 2H), 4.60−4.24 (m, 1H), 3.97−3.74 (m, 4H), 3.70−3.45 (m, 2.2H), 3.26−3.10 (m, 1.3H), 2.82−2.51 (m, 2H), 2.35 (m, 1H), 1.98−1.87 (m, 0.3H), 1.75−1.63 (m, 0.7H), 1.31−1.21 (m, 6H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$), mixture of diastereomers and their rotamers was observed: δ 172.6, 172.4, 172.1, 157.0, 156.9, 154.8, 154.6, 154.4, 138.3, 138.0, 135.5, 134.8, 134.3, 134.1, 132.1, 131.4, 131.3, 131.0, 128.9, 128.7, 128.6, 127.9, 127.7, 127.6, 127.4, 127.2, 127.0, 126.8, 124.2, 123.5, 123.1, 122.8, 120.1, 110.2, 108.3, 108.2, 68.5, 68.4, 68.4, 64.4, 63.8, 63.5, 61.8, 61.3, 60.5, 59.7, 55.3, 55.22, 55.18, 53.7, 52.3, 52.1, 51.80, 51.78, 39.6, 36.6, 35.9, 35.4, 34.8, 34.7, 33.9, 28.6, 28.5, 21.7, 21.6. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{30}O_5NS$ 456.1839, found 456.1832.

(−)-(4a*R*,10a*S*,11a*S*)-9-Methoxy-4a,5,10,10a,11,11a-hexahydro-1*H*,3*H*-benzo[*f*]oxazolo[3,4-*a*]indol-3-one (18) and (−)-Methyl (2*S*,3a*S*,9a*S*)-2-(Hydroxymethyl)-5-methoxy-2,3,3a,4,9,9a-hexahydro-1*H*-benzo[*f*]indole-1-carboxylate (19). To a magnetically stirred solution of **17** (1.00 g, 2.20 mmol) in anhydrous THF (10 mL) under an atmosphere of argon at 0 °C was added $LiBH_4$ (57.00 mg, 2.63 mmol, 1.2 equiv), and the reaction mixture was stirred for 24 h at room temperature. After complete consumption of the starting material as checked by TLC, excess $LiBH_4$ was destroyed with 2 N HCl solution (10 mL). The organic solvent was evaporated on a rotary evaporator under reduced pressure. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator to obtain a residue. To a magnetically stirred solution of the above obtained residue in THF (10 mL) was added freshly prepared Raney Ni (excess amount) at room temperature. The mixture was stirred at the same temperature for 1 h. After complete consumption of the starting material as checked by TLC, the reaction mixture was filtered through a pad of Celite with ethyl acetate washing (3 × 50 mL). The organic filtrate was evaporated on a rotary evaporator under reduced pressure, and the residue obtained was purified by using flash silica gel column chromatography with 30% ethyl acetate in petroleum ether as the eluent to afford compound **18** (402.00 mg, 63%) as a colorless solid and compound **19** (134.00 mg, 21%) as a colorless liquid in 3:1 ratio.

Data for compound **18** are as follows. R_f = 0.5 (petroleum ether/ethyl acetate, 70/30). IR ($CHCl_3$, cm^{-1}): ν_{max} 1745, 1451, 1217, 766. $[\alpha]$ = −404.13 (c 0.5, $CHCl_3$). Mp: 95−98 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.14 (t, J = 7.9 Hz, 1H), 6.77 (d, J = 7.3 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 4.60−4.58 (m, 1H), 4.32−4.23 (m, 2H), 3.82 (s, 3H), 3.47−3.39 (m, 2H), 3.19 (dd, J = 4.9, 16.5 Hz, 1H), 2.87−2.80 (m, 1H), 2.50−2.43 (m, 1H), 2.29−2.24 (m, 1H), 2.22−2.14 (m, 1H), 1.51 (q, J = 11.0 Hz, 1H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 162.1, 157.2, 136.2, 126.9, 124.6, 122.3, 107.4, 69.1, 62.1, 60.4, 55.2, 45.2, 37.3, 36.5, 28.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{15}H_{18}O_3N$ 260.1281, found 260.1278.

Data for compound **19** are as follows. R_f = 0.4 (petroleum ether/ethyl acetate, 70/30). IR ($CHCl_3$, cm^{-1}): ν_{max} 3017, 1678, 1548, 1535, 1219, 772. $[\alpha]$ = +164.84 (c 1.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.14 (t, J = 7.88 Hz, 1H), 6.77 (d, J = 7.75 Hz, 1H) 6.69 (d, J = 8.13 Hz, 1H), 4.18 (br s, 1H), 3.82 (s, 3H), 3.77 (s, 3H) 3.72 (dd, J = 7.50, 10.38 Hz, 1H), 3.65 (br s, 2H) 3.33 (td, J = 4.63, 10.94 Hz, 1H), 3.15 (dd, J = 4.63, 16.63 Hz, 1H), 2.76−2.70 (m, 1H), 2.30 (dd, J = 12.51, 16.13 Hz, 1H), 2.15−2.04 (m, 1H), 2.00 (br s, 1H), 1.79 (td, J = 9.07, 12.35 Hz, 1H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 157.3 (2C), 136.6, 126.7, 124.7, 122.1, 107.3, 67.7, 62.0, 61.3, 55.2, 52.7, 40.9, 37.3, 32.8, 28.5. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{16}H_{22}O_4N$ 292.1543, found 292.1540.

(−)-((2*S*,3a*S*,9a*R*)-5-Methoxy-1-propyl-2,3,3a,4,9,9a-hexahydro-1*H*-benzo[*f*]indol-2-yl)methanol (5). A mixture of compound **18** (0.10 g, 0.38 mmol) and potassium hydroxide (1.3 g, 23.14 mmol, 60 equiv) in methanol (10 mL) was stirred and refluxed for 1 h. After complete consumption of the starting material as checked by TLC, 3 N aqueous HCl (30 mL) was added to the reaction mixture at 0 °C. Most of the methanol was then evaporated on a rotary evaporator. The pH of the reaction mixture was adjusted to 8−9 with a saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was then extracted with CH_2Cl_2 (5 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure. The obtained residue and K_2CO_3 (69.00 mg, 0.50 mmol, 1.3 equiv) were taken up in anhydrous acetonitrile (3 mL) and cooled to 0 °C before dropwise addition of *n*-propyl iodide (0.04 mL, 0.42 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 12 h, and after complete consumption of starting material as monitored by TLC, the mixture was extracted with CH_2Cl_2 (5 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain the crude product, which was purified by using flash silica gel column chromatography with ethyl acetate as the eluent to afford pure **5** (48. 00 mg, 45%, over 2 steps) as a colorless liquid. R_f = 0.5 (MeOH/ethyl acetate, 05/95). IR ($CHCl_3$, cm^{-1}): ν_{max} 3019, 2400, 1216, 666. $[\alpha]$ = −33.27 (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.12 (t, J = 8.0 Hz, 1H), 6.76 (d, J = 7.6 Hz, 1H), 6.69 (d, J = 8.0 Hz, 1H), 3.82 (s, 3H), 3.68 (dd, J = 4.0, 11.1 Hz, 1H), 3.58 (dd, J = 5.3, 11.4 Hz, 1H), 3.54−3.46 (m, 2H), 3.23 (dd, J = 5.3, 16.8 Hz, 1H), 3.11 (dd, J = 3.8, 14.5 Hz, 1H), 3.04−2.99 (m, 1H), 2.94−2.85 (m, 2H), 2.59−2.54 (m, 1H), 2.35−2.26 (m, 2H), 2.01−1.93 (m, 1H), 1.72−1.64 (m, 2H), 1.53−1.41 (m, 1H), 0.96 (t, J = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 157.3, 136.1, 126.6, 124.8, 122.0, 107.4, 65.3, 64.0, 62.7, 55.2, 51.1, 39.4, 33.7, 33.3, 29.5, 22.0, 11.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{17}H_{26}O_2N$ 276.1958, found 276.1957.

(−)-(3*R*,4a*S*,10a*R*)-6-Methoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinolin-3-ol (20).[3] To a magnetically stirred solution of compound **5** (0.10 g, 0.36 mmol) in anhydrous THF (5 mL) placed in a sealed tube under an atmosphere of argon at −78 °C was added trifluoroacetic anhydride (TFAA) (0.07 mL, 0.47 mmol, 1.3 equiv). After 1 h at −78 °C, triethylamine (0.30 mL, 1.82 mmol, 5 equiv) was added dropwise. The reaction mixture was stirred for 1 h at−78 °C and then heated at 120 °C in the sealed tube. After 24 h, aqueous 2 M NaOH solution (3 mL) was added to the reaction mixture at 0 °C and stirred for 1 h at room temperature. The organic solvent was evaporated on a rotary evaporator, and the water layer was extracted with CH_2Cl_2 (5 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain the crude product **20**, which was purified using flash silica gel column chromatography with ethyl acetate as an elutent to provide **20** (87.00 mg, 87%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 10/90). IR ($CHCl_3$, cm^{-1}): ν_{max} 2942, 1688, 1219, 771. $[\alpha]$ = −49.59 (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.11 (t, J = 7.9 Hz, 1H), 6.74 (d, J = 7.9 Hz, 1H), 6.67 (d, J = 7.9 Hz, 1H), 3.99−3.93 (m, 1H), 3.82 (s, 3H), 3.24 (d, J = 8.5 Hz, 1H), 3.16 (dd, J = 4.6, 16.2 Hz, 1H), 3.00 (dd, J = 4.9, 17.7 Hz, 1H), 2.86−2.60 (m, 4H), 2.30−2.16 (m, 4H), 1.82−1.74 (m, 1H), 1.62−1.52 (m,

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J. Org. Chem. 2021, 86, 9344−9352

2H), 1.16 (dd, J = 11.6, 23.8 Hz, 1H), 0.93 (t, J = 7.0 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 156.7, 136.0, 126.4, 124.2, 121.2, 107.1, 66.5, 60.2, 59.5, 55.2, 54.8, 41.2, 35.8, 34.2, 30.6, 17.1, 11.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{17}H_{26}O_2N$ 276.1958, found 276.1957.

(−)-(3*R*,4a*S*,10a*R*)-3-Azido-6-methoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline (21). To a magnetically stirred solution of alcohol **20** (50.00 mg, 181.56 μmol) and Et_3N (0.050 mL, 363.12 μmol, 2 equiv) in anhydrous CH_2Cl_2 (1 mL) at 0 °C was added dropwise mesyl chloride (0.02 mL, 217.87 μmol, 1.3 equiv). After the reaction mixture was stirred for 1 h at 0 °C, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain the crude mesylate of **20**. To a solution of this mesylate in anhydrous DMF (1 mL) was added NaN_3 (18.00 mg, 272.34 μmol, 1.5 equiv). The reaction mixture was stirred at 65 °C for 1 h under an argon atmosphere. After i was cooled to room temperature, the reaction mixture was diluted with EtOAc (10 mL) and washed with water (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain crude compound **21**. The crude product was purified by using flash silica gel column chromatography with 15% ethyl acetate in petroleum ether as an eluent to give pure azide **21** (46.00 mg, 84%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 75/25). IR ($CHCl_3$, cm^{-1}): ν_{max} 2111, 1523, 1429, 1217, 771. $[\alpha]$ = −44.25 (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.12 (t, J = 8.0 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 3.83 (s, 3H), 3.61−3.54 (m, 1H), 3.20−3.13 (m, 2H), 2.99 (dd, J = 5.1, 17.4 Hz, 1H), 2.79−2.73 (m, 1H), 2.62 (dd, J = 10.7, 15.3 Hz, 1H), 2.59−2.53 (m, 1H), 2.29−2.16 (m, 4H), 1.74−1.68 (m, 1H), 1.57−1.46 (m, 2H), 1.44 (q, J = 12.0 Hz, 1H), 0.92 (t, J = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 156.7, 136.2, 126.4, 124.0, 121.2, 107.0, 59.8, 57.0, 56.7, 55.2, 54.8, 37.3, 36.4, 34.7, 30.4, 17.5, 11.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{17}H_{25}ON_4$ 301.2023, found 301.2023.

(−)-(3*R*,4a*R*,10a*R*)-6-Methoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline-3-carbonitrile (22). To a magnetically stirred solution of alcohol **20** (50.0 mg, 181.56 μmol) and Et_3N (0.050 mL, 363.12 μmol, 2 equiv) in anhydrous CH_2Cl_2 (1 mL) at 0 °C was added dropwise mesyl chloride (0.02 mL, 217.87 μmol, 1.3 equiv). After the reaction mixture was stirred for 1 h at 0 °C, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain the crude mesylate of **20**. To a solution of the this mesylate in anhydrous DMF (1 mL) was added NaCN (13.3 mg, 272.34 μmol, 1.5 equiv). The reaction mixture was stirred at 65 °C for 1 h under an argon atmosphere. After it was cooled to room temperature, the reaction mixture was diluted with EtOAc (10 mL) and washed with water (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain crude compound **22**. The crude product was purified by using flash silica gel column chromatography with 15% ethyl acetate in petroleum ether as an eluent to give compound **22** (42.0 mg, 82%) as a colorless oil. Alternatively, crude compound **22** could be used in the next reaction without further purification. R_f = 0.4 (petroleum ether/ethyl acetate, 75/25). IR ($CHCl_3$, cm^{-1}): ν_{max} 2120, 1529, 1425, 1223, 773. $[\alpha]$ = −30.23 (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.13 (t, J = 7.8 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 3.83 (s, 3H), 3.26 (d, J = 10.7 Hz, 1H), 3.16 (dd, J = 5.0, 16.0 Hz, 1H), 2.97 (dd, J = 5.0, 17.5 Hz, 1H), 2.87 (br s, 1H), 2.80−2.72 (m, 1H), 2.67−2.54 (m, 2H), 2.50 (t, J = 11.4 Hz, 1H), 2.34−2.24 (m, 2H), 2.20 (dd, J = 11.8, 17.2 Hz, 1H), 1.63 (br s, 1H), 1.58−1.48 (m, 2H), 1.44 (q, J = 12.6 Hz, 1H), 0.92 (t, J = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 156.7, 135.9, 126.5, 123.6, 121.2, 120.8, 107.1, 60.0, 55.2, 54.6, 54.4, 36.5, 35.2, 34.5, 30.1, 27.7, 17.7, 11.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{18}H_{25}ON_2$ 285.1961, found 285.1962.

(−)-Methyl (3*R*,4a*R*,10a*R*)-6-Methoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline-3-carboxylate (23). A magnetically stirred solution of compound **22** (15.00 mg, 0.052 mmol) in acidic methanol (methanol saturated by HCl gas) (3 mL) was refluxed for 24 h. The reaction mixture was cooled to room temperature, and 2 M NaOH solution (10 mL) was added to it. After the mixture was stirred for 1 h, the organic solvent was evaporated on a rotary evaporator under reduced pressure and the water layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator under reduced pressure to obtain crude compound **23**. The crude product was purified by using flash silica gel column chromatography with 15% ethyl acetate in petroleum ether as an eluent to give compound **23** (15 mg, 90%) as a colorless oil. R_f = 0.5 (petroleum ether/ethyl acetate, 70/30). IR ($CHCl_3$, cm^{-1}): ν_{max} 1727, 1641, 1464, 1216, 767. $[\alpha]$ = −73.61 (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.12 (t, J = 8.0 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 3.25−3.23 (m, 1H), 3.18 (dd, J = 5.3, 16.0 Hz, 1H), 2.98 (dd, J = 5.3, 17.5 Hz, 1H), 2.82−2.74 (m, 2H), 2.71−2.54 (m, 2H), 2.41 (t, J = 11.4 Hz, 1H), 2.27−2.16 (m, 3H), 1.75−1.65 (m, 1H), 1.59−1.49 (m, 2H), 1.32 (dd, J = 13.0, 25.2 Hz, 1H), 0.91 (t, J = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 174.5, 156.7, 136.3, 126.3, 124.2, 121.2, 106.9, 60.2, 55.2, 55.1, 54.1, 51.7, 41.6, 36.7, 34.7, 34.6, 30.5, 17.4, 12.0. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{19}H_{28}O_3N$ 318.2064, found 318.2063.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00603.

FAIR data, including the primary NMR FID files, for compounds **5**, **8**, **9**−**12**, **14**, and **17**−**23** (ZIP)

^1H and ^{13}C NMR spectra for compounds **5**, **8**, **9**−**12**, **14**, and **17**−**23** and X-ray crystal data and structural refinement details for compound **18** (PDF)

Accession Codes

CCDC 2040624 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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Erratum