Design, Synthesis and Biological Evaluation of Novel Heterocycles and their Encapsulation in Drug Delivery System

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For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMICAL SCIENCES

By

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Under the guidance of

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February 2020

CERTIFICATE

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Design, Synthesis and Biological Evaluation of Novel Heterocycles and their Encapsulation in Drug Delivery System"** submitted by **Ms. Namita Ashok More** to Academy of Scientific and Innovative Research (**AcSIR**) in fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy**, embodies original research work under my supervision. I 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 obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

It is also certified that this work done by the student, under my supervision, is plagiarism free.

Namita Ashok More
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Date: 27th Feb, 2020
Place: CSIR-NCL, Pune.

Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Design, Synthesis and Biological Evaluation of Novel Heterocycles and their Encapsulation in Drug Delivery System"** submitted to Academy of Scientific and Innovative Research (AcSIR) for the award of degree of **Doctor of Philosophy** (Ph.D.) is the outcome of experimental investigations carried out by me under the supervision of **Dr. J. M. Gajbhiye**, Senior Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

February 2020
CSIR-National Chemical Laboratory
Pune 411008

Namita Ashok More
(Research Student)

This dissertation is dedicated to all those people who have always given me the love, trust, and support to come to this stage of my life

~To my Mother and Father~

Acknowledgment

Ph.D. is like a long journey; an experience that takes you through the untraversed path, the green lush meadows and the island of Cyclopes to conquer the final goal fixed in mind. Once you achieve the target and turn back, you realize that all your efforts and the pain were worth going through. The small successes & the serendipitous discoveries, the frustrating failures & unexpected crystallizations, the imparted chemical wisdom & the laboratory camaraderie; they are all important parts of this beautiful voyage. But one can't succeed in this journey without the guidance and support of the research supervisor, friends, and well-wishers. I am taking this opportunity to express my deepest gratitude to everyone who has helped and supported me throughout the course of my research journey.

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I wish to thank the great scientific community whose achievements are a constant source of inspiration for me. Above all, I extend my gratitude to the Almighty God for giving me the wisdom, health, and strength to undertake this research work and enabling me to its completion.

Namita Ashok More

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A Convenient Synthesis of the Enantiomerically Pure (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate using Hydrolytic Kinetic Resolution

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Abbreviations

Abbreviations	What it stand for
Units	
°C	Degree centigrade
ee	Enantiomeric excess
mg	Milligram
h	Hour
Hz	Hertz
µs	Microsecond
µg	Microgram
mL	Millilitre
min.	Minute
MHz	Megahertz
mmol	Millimole
nm	Nanometre
ppm	Parts per million
%	Percentage
Sec	Second
Chemical Notations	
Ac	Acetyl
AcOH	Acetic Acid

MeCN	Acetonitrile
BnBr	Benzyl Bromide
$CDCl_3$	Deuterated chloroform
m-CPBA	*m*-Chloro perbenzoic acid
DMF	*N*, *N'*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EtOH	Ethanol
Et	Ethyl
EtOAc	Ethyl Acetate
EAA	Ethyl Aceto Acetate
MeOH	Methanol
Me	Methyl
py	Pyridine
THF	Tetrahydrofuran
TBAI	Tetrabutylammonium iodide
Other Notations	
CB	Cannabinoid
δ	Chemical shift
J	Coupling constant in NMR
equiv.	Equivalents
GC	Gas Chromatography

HRMS	High Resolution Mass Spectrometry
HKR	Hydrolytic Kinetic Resolution
IR	Infrared
MIC	Minimun Inhibitory Concentration
MT-CY	*Mycobacterium Tuberculosis*-Cytochrome
NMR	Nuclear Magnetic Resonance
rt	Room temperature
TB	Tuberculosis
UV	Ultraviolet

General remarks

- All catalysts were purchased from commercial sources and used as received.
- All reactions were carried out under innert atmosphere following standard procedures using schlenk techniques.
- Deuterated solvents for NMR spectroscopic analyses were used as received. All ^{1}H NMR and ^{13}C NMR analysis were obtained using a Bruker or JEOL 200 MHz, 400 MHz or 500 MHz spectrometers. Coupling constants were measured in Hertz. All chemical shifts are quoted in ppm, relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br =broad.
- HRMS spectra were recorded at UHPLC-MS (Q-exactive-Orbitrap Mass Spectrometer) using electron spray ionization [(ESI^{+}, +/− 5kV), solvent medium: water, acetonitrile, methanol and ammonium acetate] technique and mass values are

expressed as *m/z*. GC-HRMS (EI) was recorded in Agilent 7200 Accurate-mass-Q-TOF.

- All reactions are monitored by Thinlayer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F_{254}). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), p-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec.
- All solvents and reagents were purified and dried by according to procedures given in Vogel's Text Book of Practical Organic Chemistry. All reactions were carried out under nitrogen or argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise specified. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated.
- Column chromatography was performed on silica gel (100-200 or 230-400 mesh size).
- Chemical nomenclature (IUPAC) and structures were generated using ChemDraw Professional 15.1.

Synopsis

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	**Miss Namita Ashok More**
Ac-SIR Enrolment No. & Date	**PhD in Chemical Sciences (10CC17J26023); January 2017**
Title of the Thesis	**Design, Synthesis and Biological Evaluation of Novel Heterocycles and their Encapsulation in Drug Delivery System.**
Research Supervisor	**Dr. J. M. Gajbhiye (CSIR-NCL, Pune)**

Statement of purpose:

This thesis deals with the heterocyclic reactions, in particularly Paal-knorr synthesis, cyclisation, addition-elimination, *etc*. The thesis comprises of four chapters, out of which the first chapter is the introduction where in Heterocyclic chemistry observed in all branches of chemistry. From the second chapter to fourth chapter, all are working chapters which narrates our approaches to the biologically active heterocyclic compounds. The fourth chapter describes importance of drug delivery system in water insoluble heterocyclic compounds.

Chapter 1: Synthesis and biological interpretation of some previous heterocyclic derivatives

Cyclic organic compounds that formulates at least two different elements as a part of ring system. Carbon is essential element in a ring and others are N, O, S, P, *etc*.

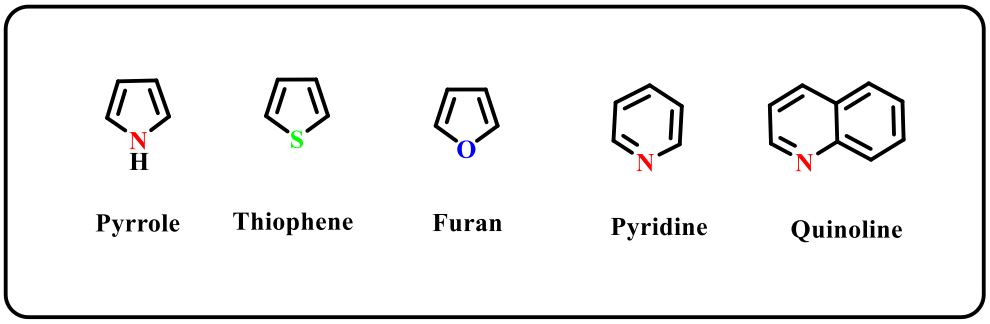


Figure 1: Heterocyclic compounds

Heterocyclic chemistry is the branch of chemistry dealing with the synthesis, properties, and applications of heterocycles. This chapter highlights our traditional methods such as

Paal Knorr synthesis, cyclisation, addition-elimination reactions, biologically important heterocyclic molecules and their applications in drug delivery system.[1]

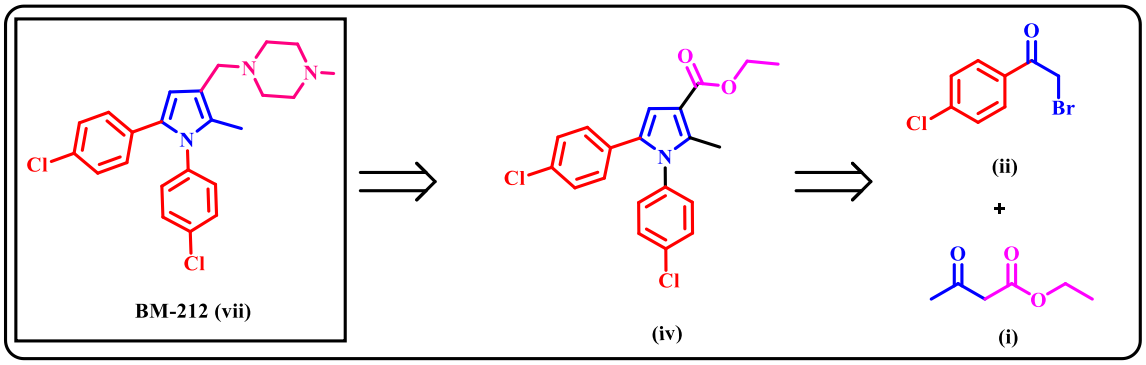
Chapter 2: Anti-tubercular compounds, synthesis and their SAR studies.

Section 2A: Introduction: Tuberculosis

In this chapter introduced Tuberculosis. On March 24, 1882, *M. tuberculosis* a bacteria causes a contagious disease tuberculosis was discovered by Dr. Robert Koch. After some period, 24 March was declared to be the world TB day. This day people were made aware about TB. TB is normally caused by *M. tuberculosis*. TB bacteria are generally found in the lungs when inhaled, but sometimes they can transmit to other parts of the body. Tuberculosis is also known as phthisis, phthisis pulmonalis, consumption and white death.

Section 2B: An efficient synthesis of potent anti-tubercular drug candidate BM-212

A novel five step total synthesis route for an anti-tubercular drug BM-212 starting from 4-chlorophenacyl bromide was developed with 48% overall yield. The synthesis is amiable for the synthesis of biologically active derivatives of BM-212.[2]



Scheme 1: Retrosynthesis of BM-212.

Section 2C: Discovery of Rimonabant and its potential analogues as Anti-TB drug candidates

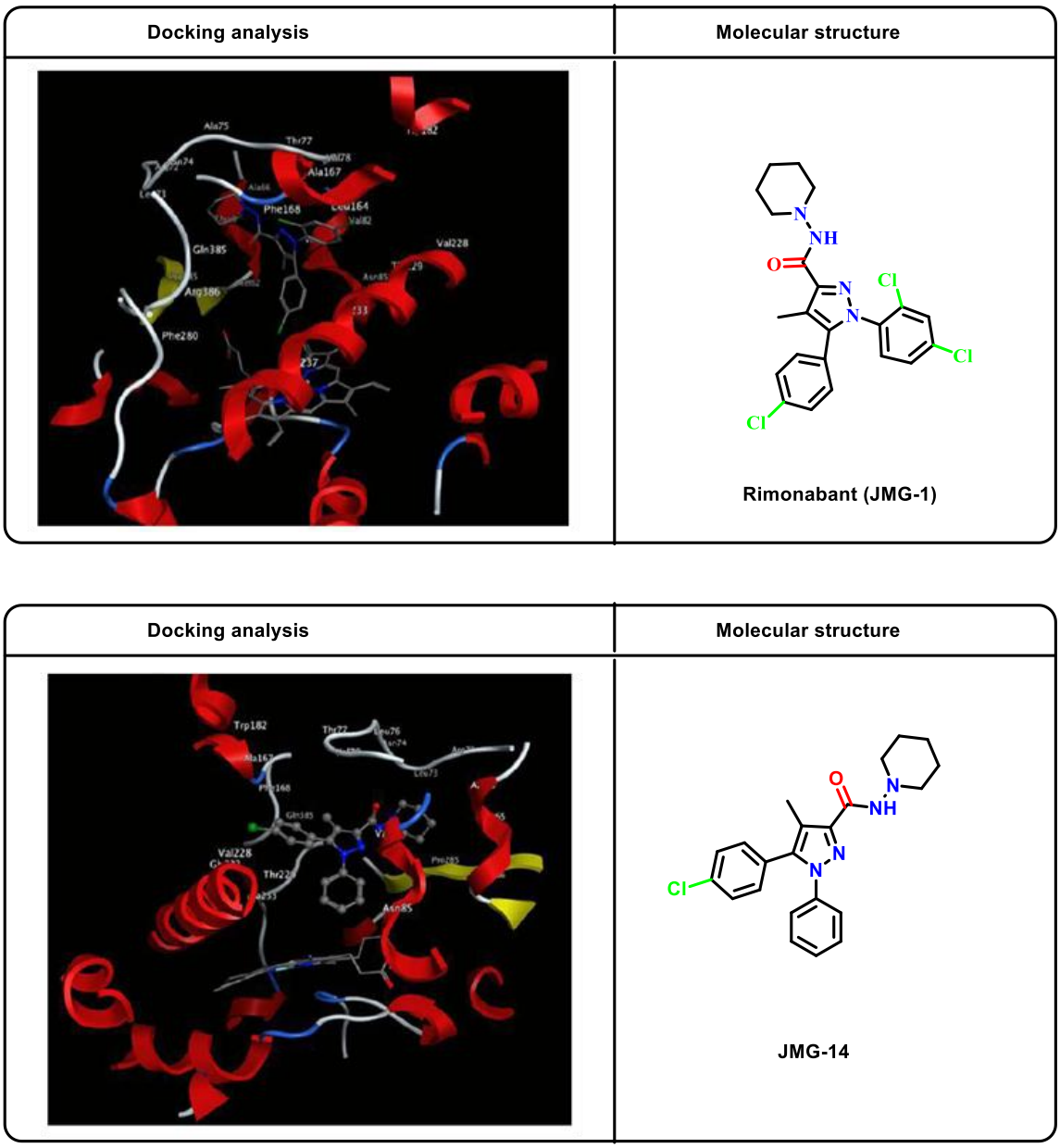
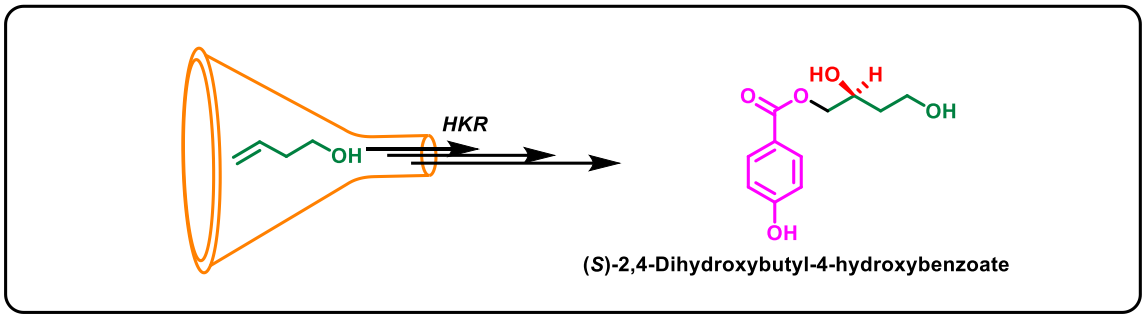


Figure 2 : Docking analysis of JMG-1 and JMG-14

Rimonabant and its analogues have been synthesized in moderate to good yields using a simple synthetic route. All the newly synthesized compounds were subjected to *in vitro* screening against *M. tuberculosis* and *M. smegmatis*. The most potent analogue JMG-14 exhibits MIC value of 3.13 compared to 3.25 and 50 µg/ml for ethambutol and pyrazinamide, respectively. The molecular docking reveals that pyrazole ring, number and position of halogen atoms play crucial role in deciding interactions with MTCYP-121. These findings open up a new avenue in the search of potent anti-TB drugs with Rimonabant and its novel analogue JMG-14 as lead molecule.[3]

Chapter 3: A convenient synthesis of the enantiomerically pure (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate using hydrolytic kinetic resolution

(*S*)-2,4-Dihydroxybutyl-4-hydroxybenzoate was prepared in an extremely simple and practical way with high enantiomeric purity (99% ee) using Jacobsen's Hydrolytic Kinetic Resolution technique as a key step and source of chirality.[4]



Scheme 2. Synthesis of (*S*)-2, 4-dihydroxybutyl-4-hydroxybenzoate

Chapter 4: **Anti-cancer compounds, synthesis and their Drug Delivery System.**

Section 4A: Introduction: Cancer

The aim of this chapter was to develop novel sulfonamides and a combination of chitosan and m-PEG for use in drug delivery system. Controlled drug release systems, is an original technique used to treat cancer cells by targeted delivery.

Section 4B: Sulfonamides encapsulation in drug delivery system

Stuctural diversity of epidermal growth factor receptor (EGFR), single crystal XRD and biological activities of sulfonamides inhibitors containing morpholine has importance in morpholine research. Morpholine compounds are well-known for their anti-cancer activity. Novel compounds obtained from morpholine via nucleophilic addition reactions, provided the desired products in 70 to 90% yield. The *in vitro* antitumor activity of synthesized end products, i.e., **NAM-6** and **G** were tested against MCF-7 and MDA-MB-231 of breast cancer cell line. Amongst the two end-products, sulfonamide group-containing compound **NAM-6** showed significant anti-proliferative activity with IC_{50} 1.811 µM in MCF-7 and 2.143 µM in MDA-MB-231 cells respectively, as compared to 1.883 and 4.688 µM by compound **G**. The results demonstrated that the

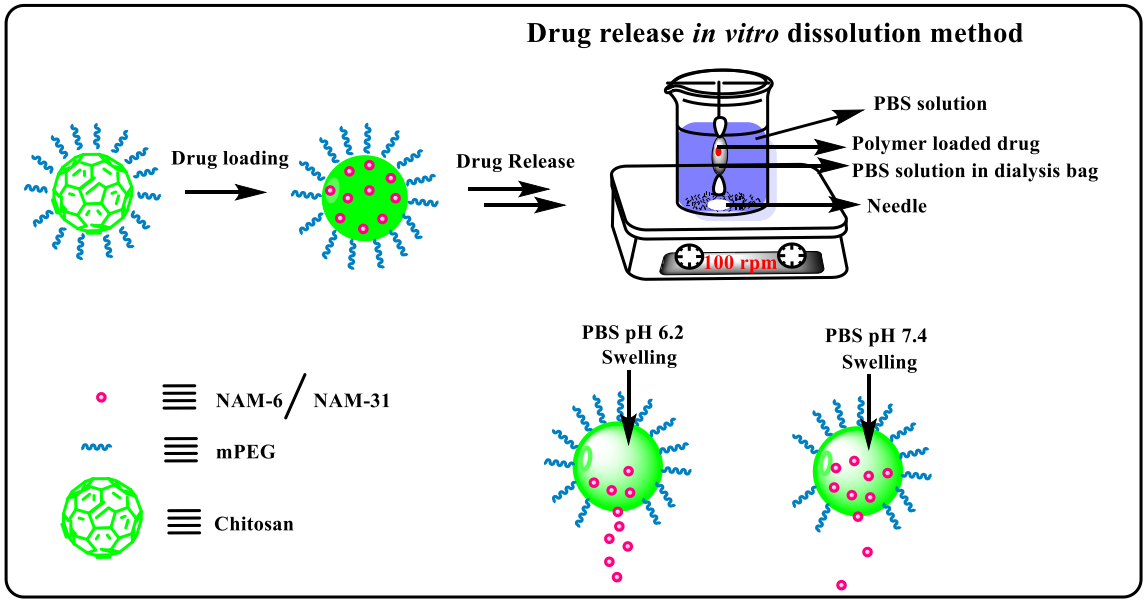


Figure 3: *In vitro* drug release study

synthesized morpholine derivatives have significant potential as anti-cancer agents and have substantial importance in the cancer therapeutics.

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1. Luo, H.; Kang, Y.; Li, Q.; Yang, L. Sulfamic Acid as Efficient and Reusable Catalytic System for the Synthesis of Pyrrole, Furan, and Thiophene Derivatives. *Heteroatom Chem*. **2008**, *19*, 144-148.
2. More, N.; Patil, M.; Garud, D.; Gajbhiye, J. "An efficient synthesis of potent anti-tubercular drug candidate BM212", *RJC*, **2016**, *9*, 806-811.
3. Gajbhiye, J.; More, N.; Patil, M.; Ummanni, R.; Kotapalli, S.; Yogeeswari, P.; Sriram, D.; Masand, V. "Discovery of Rimonabant and its potentialanalogues as anti-TB drug candidates", *Med. Chem. Res.*, **2015**, *24*, 2960-2971.
4. More, N.; Jadhao, N.; Garud, D.; Gajbhiye, J. "A convenient synthesis of the enantiomerically pure (S)-2,4-dihydroxybutyl-4hydroxybenzoate using hydrolytic kinetic resolution", *Syn. Comm.*, **2018**, *48*, 2093-2098.

Chapter 1

Synthesis and biological evaluation of heterocyclic chemistry

1.1 Defination of heterocyclic chemistry

Cyclic organic compounds that contain at least two different elements as a part of ring system are called heterocycles. Carbon is essential element in a ring and others are N, O, S, P *etc*.[1]

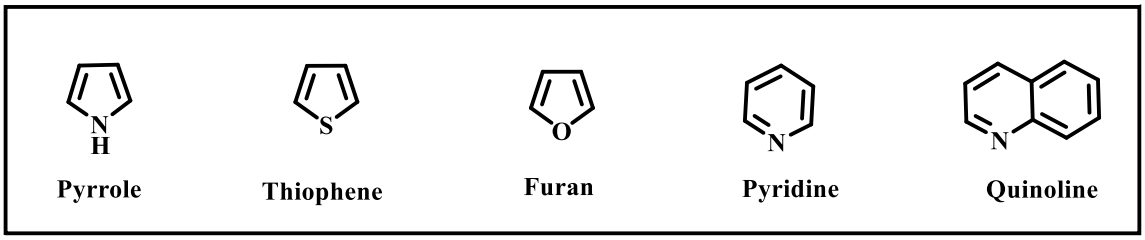


Figure 1.1: Heterocyclic compounds

1.2 Background of heterocyclic chemistry

Heterocyclic chemistry is observed in all branches of chemistry as well as its major role in applications, properties and synthesis of Heterocycles. Development of heterocyclic chemistry was established in the 1800s. Some previous outstanding developments[2] are as below:

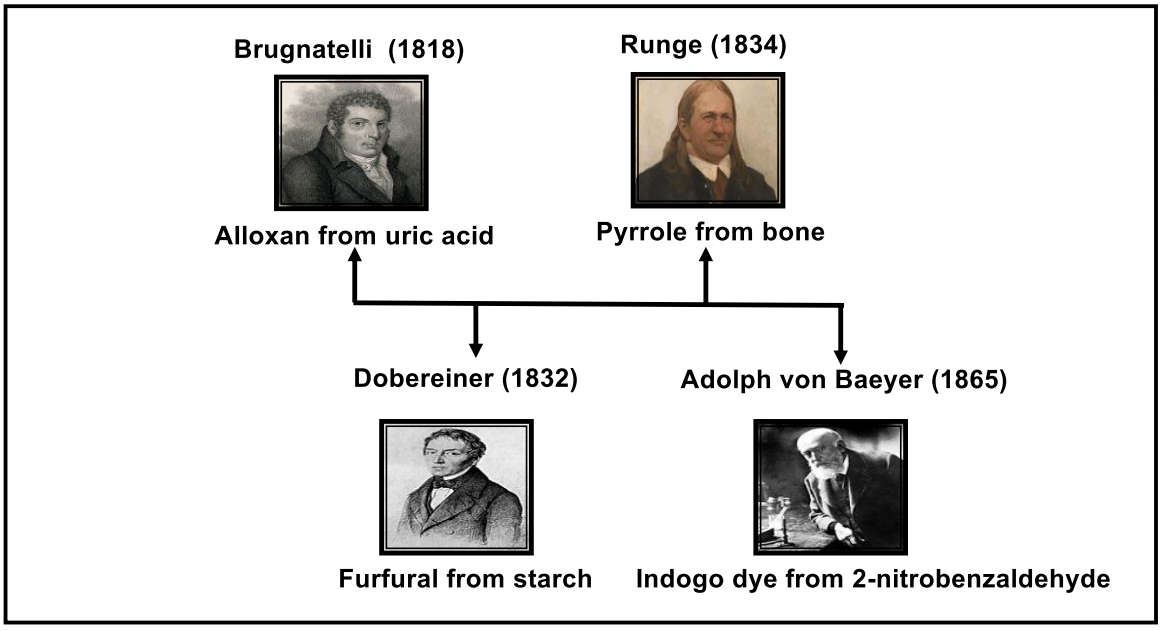
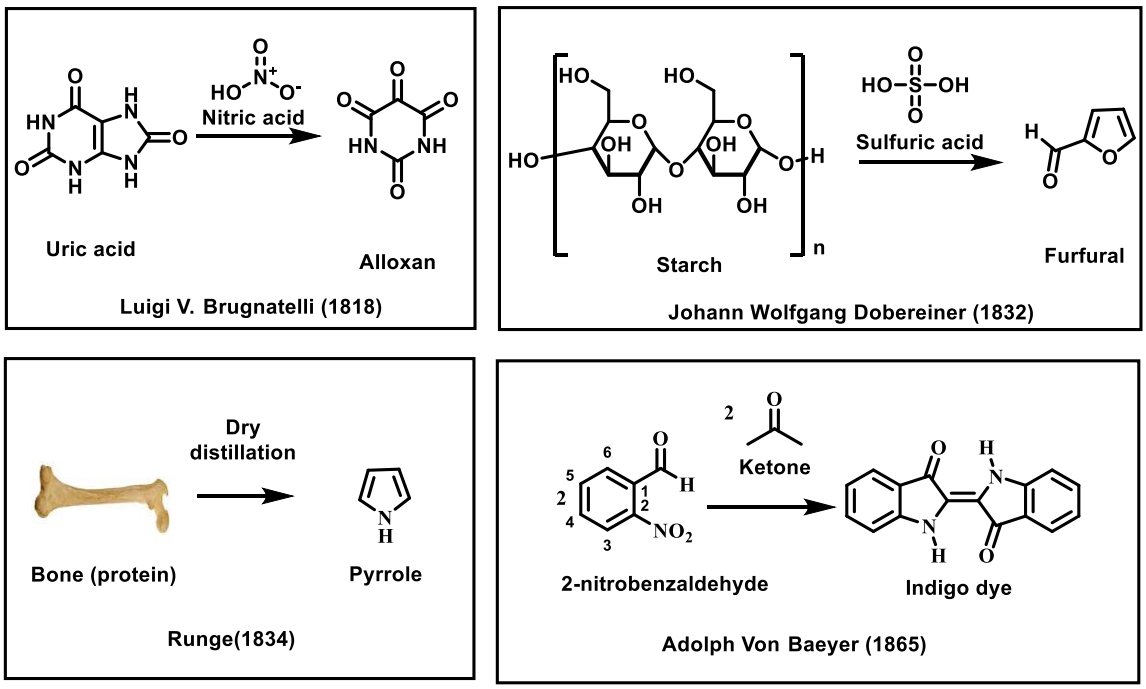


Figure 1.2: History of the heterocyclic chemistry

In 1818 alloxan was first discovered by Luigi V. Brugnatelli. It was isolated from uric acid *via* oxidative degradation of nitric acid.[3] Now a days barbituric acid is used for synthesis of alloxan. Alloxan is used for preparation of dyes. After that Johann Wolfgang Dobereiner had first developed furfural in 1832 as a byproduct of formic acid from starch by treating with sulfuric acid.[4] In 1834 Runge had first discovered pyrrole which was obtained by dry distillation of proteins.[5] Beginning of 1865, Adolph Von Baeyer has synthesized dyes. In 1880 and 1883 indigo was synthesized and determined its structure simultaneously.[6]

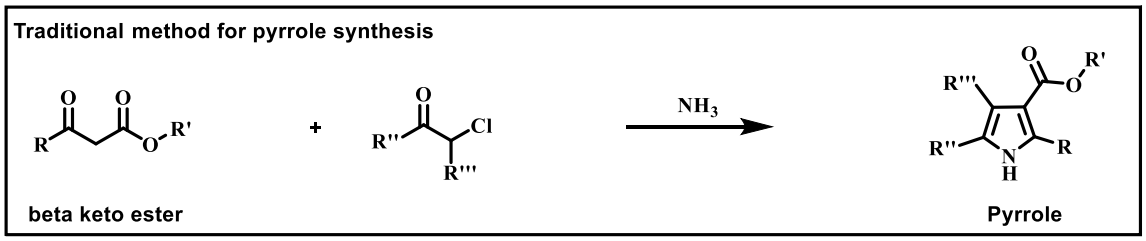


Scheme 1.1 History of the heterocyclic chemistry

1.3 Traditional methods for synthesis of heterocyclic compounds

Pyrroles,[7] furans,[8] pyrazoles[9] and thiophenes[10] are most important moieties in several bioactive natural products as well as pharmaceuticals, having been known for more than 150 years. Pyrrole and furan are most similar in structures to thiophene, just replacement of N, O, S heteroatoms are observed in each structure and essential roles in natural and synthetic chemistry.[11] Pyrroles, furans, pyrazoles and thiophenes have been synthesized by standard named reactions such as Hantzsch reaction.[12] Knorr reaction[13] and Paal–Knorr reaction.[14]

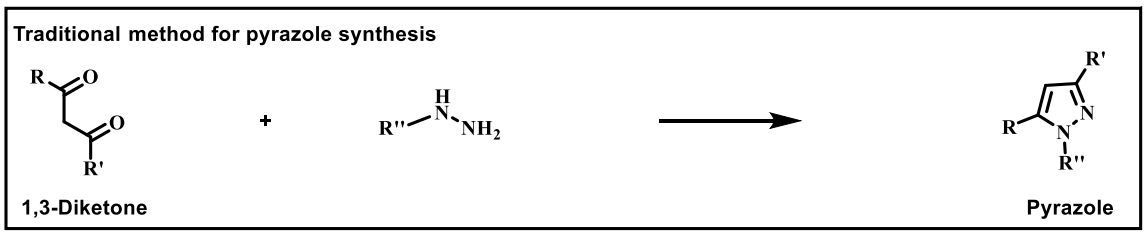
1.3.1 **Hantzsch reaction** (Arthur Rudolf Hantzsch)



Scheme 1.2 Traditional method for pyrrole synthesis

Reaction which provides substituted pyrroles from a α-halo ketones and ammonia (or primary amines) when reacted with β-ketoesters is called as Hantzsch reaction.

1.3.2 **Knorr reaction (**Ludwig Knorr)

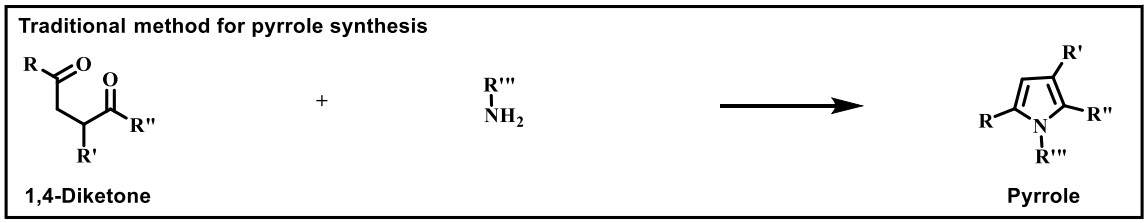


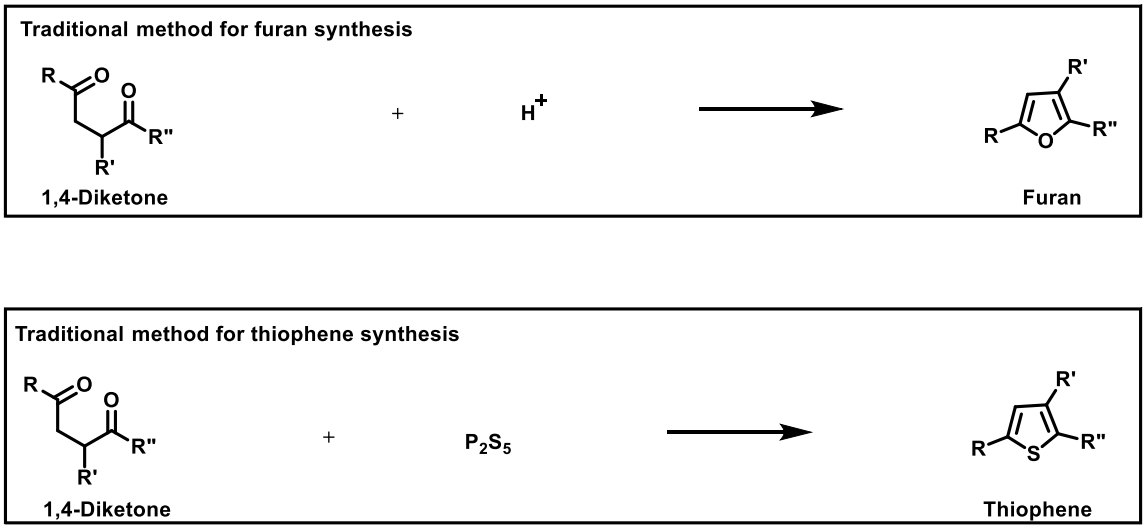
Scheme 1.3 Traditional method for pyrazole synthesis

Synthetic route of Knorr reaction towards pyrazole derivatives started from 1,3-dicarbonyl and hydrazine *via* acid catalyst.

1.3.3 **Paal-Knorr reaction (**Carl Paal and Ludwig Knorr)

One of the most common approaches to pyrrole, furan and thiophene synthesis is from 1,4- dicarbonyl compounds as starting compounds is Paal-Knorr reaction.





Scheme 1.4 Traditional method for pyrrole, furan, thiophene synthesis

Pyrrole is synthesized *via* acid-mediated dehydrative cyclization of 1,4-dicarbonyl compound and primary amine. Synthesis of furan is achieved by similar acid catalyzed dehydrative cyclization. Thiophene and furan were synthesized by similar methods. 1,4-diketone with a sulfurizing agents gave thioketone and 1,4-diketone afforded furan when reacted with acid.

Limitatios of traditional methods:

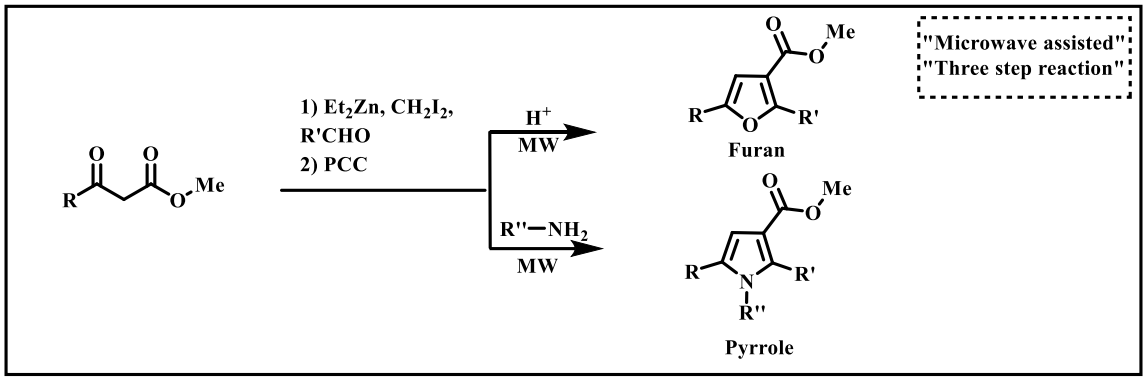
Major limitations of performance in traditional methods are as follows: (i) strong reaction conditions, (ii) prolonged heating (refluxing the reaction mixture), (iii) low atom economy and (iv) high energy consumption.

1.4 Modern green approaches to synthesize heterocyclic compounds

Most of the time heterocyclic compounds are synthesized by traditional methods but reactions require harsh conditions and more time. Heterocyclic compounds are found in natural products, pharmaceutical, molecular recognition, materials science and cosmetics, *etc*. As per requirement, researchers are attracted towards modern organic and bio-organic chemistry reactions which are simple, efficient and eco-friendly leading to the synthesis of different types of five member ring.

In industries, synthesis of drugs is carried out in the presence of organic solvents. These organic solvents are toxic, costly, flammable, and difficult to dispose of. Organic

solvents are having many health issues like cancer. Organic reactions have become demanding in the research area. Due to their limitations in the traditional or reported methods. Therefore researchers choose the modern methods for the synthesis of heterocyclic compounds. They have changed their reaction conditions, such as microwave-assisted, grinding, ultrasound, neat reaction conditions and water-mediated reaction conditions, *etc*. These methods could be less toxic, easy to carry out and compounds easily isolated without column, *etc*.[15]

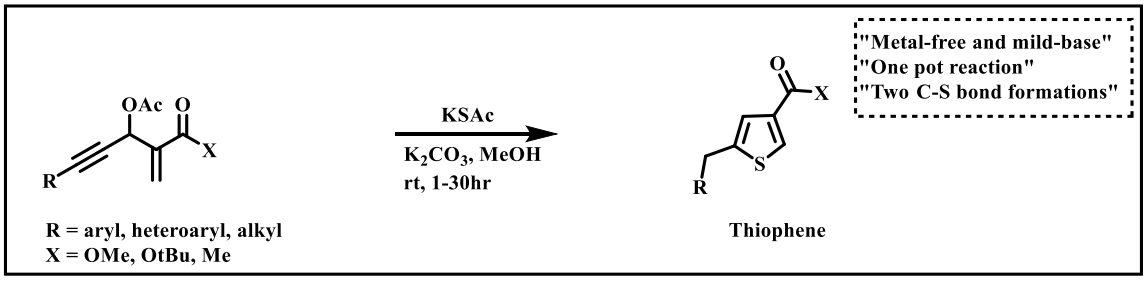


Scheme.1.5 Microwave-assisted furan and pyrrole synthesis

Modification of reported Paal–Knorr synthesis in the presence of different acid catalysts (Brønsted acids or Lewis acids). This is improved by “greener” methodologies, for pyrrole synthesis.

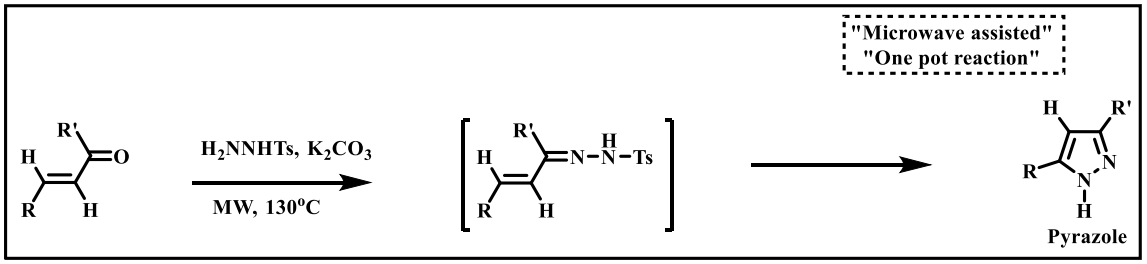
By using microwave irradiation methods reactions are carried out in three steps such as homologation, oxidation and cyclization. Paal-Knorr reactions afforded substituted pyrrole or furans (Scheme 1.5).[16]

A mild and metal-free reaction protocol has been developed for the synthesis of substituted thiophenes. A base-promoted reaction proceed *via* tandem allylic substitution or deacetylative 5-exo-dig-thiocycloisomerization of Morita-Baylis-Hillman acetates with potassium thioacetate (Scheme 1.6).[17]



Scheme.1.6 Thiophene synthesis

Also A. Corradi *et. al.* developed microwave-assisted solvent free reaction protocol for synthesis of pyrazole derivatives from tosylhydrazones of *α,β*-unsaturated carbonyl compounds (Scheme 1.7).[18]



Scheme.1.7 Microwave assisted pyrazole synthesis

1.5 Biological importance of heterocyclic chemistry

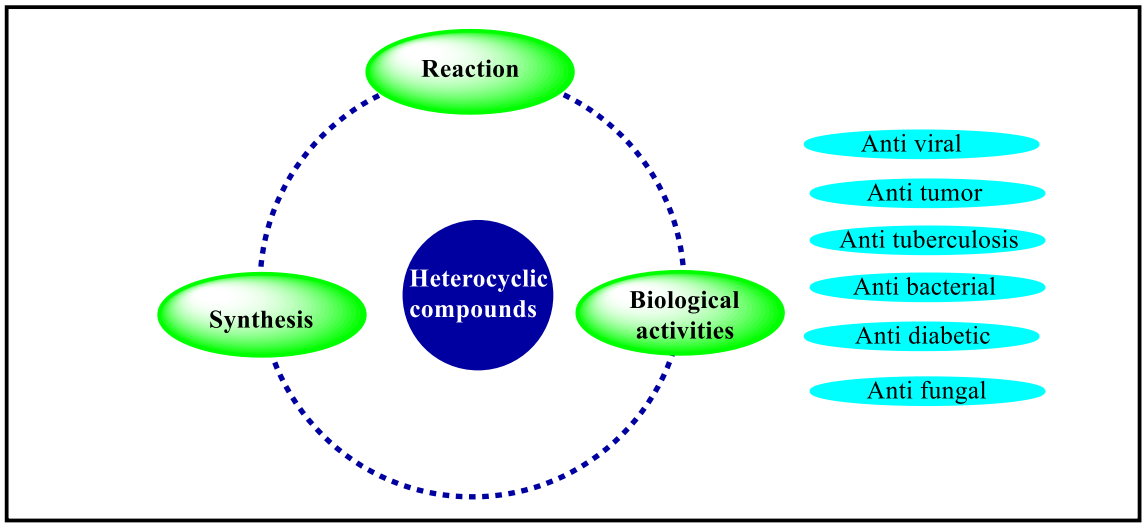


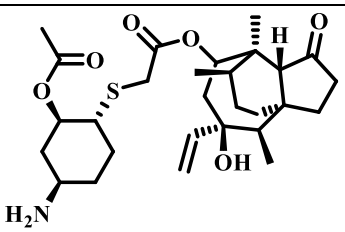
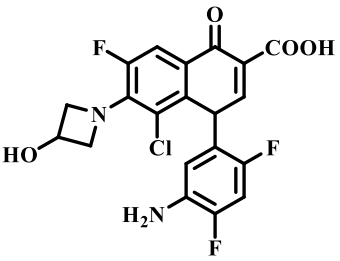
Figure 1.3: Biological importance of heterocyclic chemistry

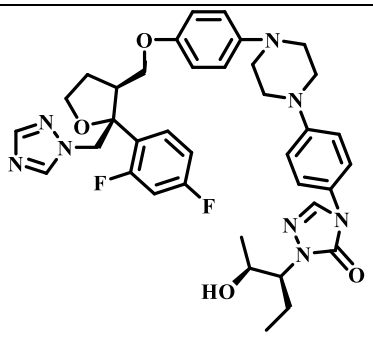
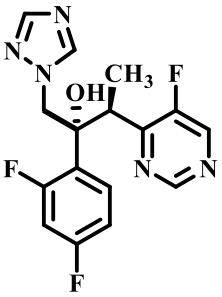
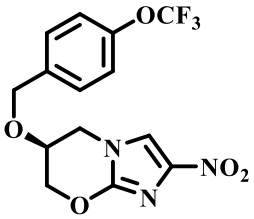
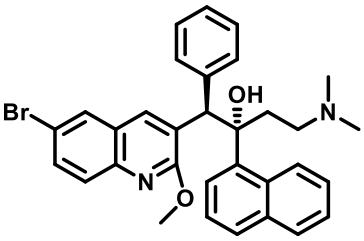
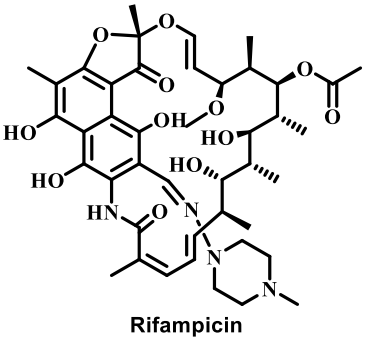
Chemistry is the study of the substances, especially their structure, properties, transformations and the energy changes accompanying the transformation. Organic, inorganic, physical, biochemistry and analytical chemistry are the main branches of chemistry. Organic chemistry is one of the most important branches of chemistry which

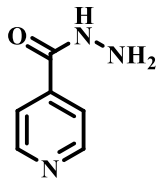
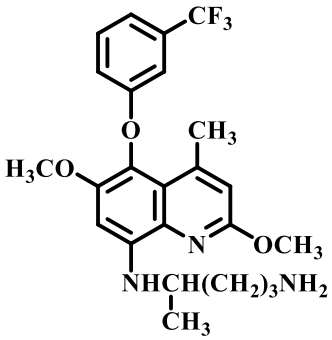
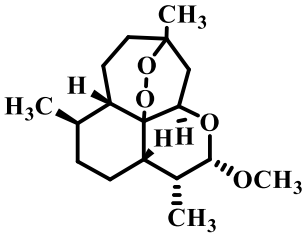
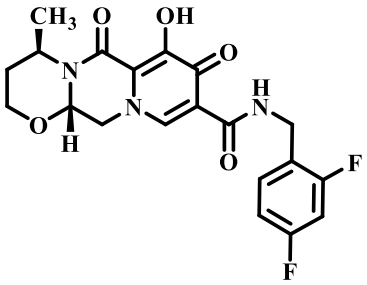
contains carbon-containing compounds and heterocyclic chemistry is the branch of organic chemistry. Heterocyclic chemistry has vast relevance in the industry for synthesis and biological activities.[19]

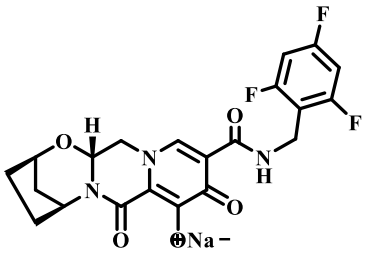
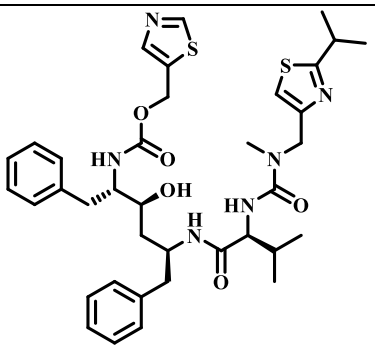
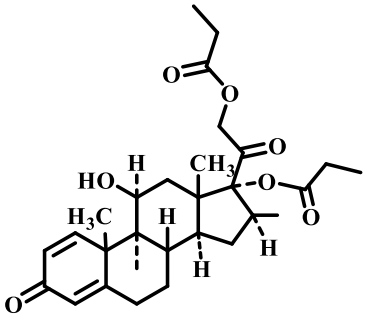
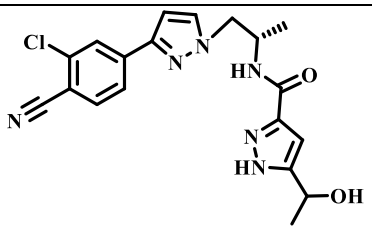
Nature gives us food, plant extracts, ayurvedic medicines *etc* and most of these are made by heterocyclic chemistry and some are synthesized by humans. Many biologically active compounds are heterocyclic compounds. Therefore we chose here heterocyclic compounds for further studies. Synthetic heterocycles exhibit various biological activities, which are shown in Table 1.1.

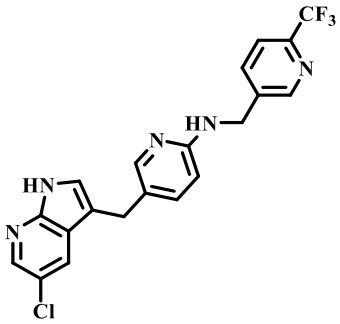
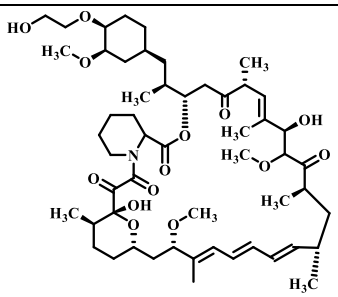
Table.1.1. FDA drug approval and databases

Drug Name	Image	Sructure	Approval year	FDA-approved use
1.5.1 Antibacterial				
Xenleta (Lefamulin)			2019	Bacterial pneumonia
Baxdela (Delafloxacin)			2017	Acute bacterial skin infections
1.5.2 Antifungal				

Noxafil (Posaconazole)			2006	Fungal infections
Vfend (Voriconazole)			2002	Fungal infections
1.5.3 Antituberculosis				
Pretomanid			2019	Drug resistant tuberculosis
Sirturo (Bedaquiline)			2012	Drug resistant tuberculosis
Rifampicin and Isoniazid (RIWELL-IS)			1971	*M. avium* complex, leprosy, and Legionnaies disease.

		 Isoniazid	1952	
1.5.4 Antimalarial				
Krintafel (Tafenoquine)			2018	Prevention of malaria relapse in patients
Coartem (Artemether)			2009	Malaria infections due to *Plasmodium falciparum*
1.5.5 Anti-HIV				
Dovato (Dolutegravir and Lamivudine)			2019	HIV-1 infection in adults

Biktarvy (Bictegravir/ Emtricitabine/ Tenofovir)			2018	HIV-1 infection in adults
Trogarzo (Ibalizumab-uiyk)			2018	Multidrug resistant HIV-1 infection
1.5.6 Antiinflammatory				
Lotrisone (Clotrimazole/ Betamethasone Diproprionate) lotion			2000	Symptomatic inflammatory tinea pedis, tinea cruris, and tinea corporis
1.5.7 Anticancer				
Nubeqa (Darolutamide)			2019	Non-metastatic castration resistant prostate cancer

1.5.8 Antitumor				
Turalio (Pexidartinib)			2019	Symptomatic tenosynovial giant cell tumor
Afinitor (Everolimus)			2011	Advanced pancreatic neuroendocrine tumors

1.6 Applications of biologically active heterocyclic compounds in drug delivery system

Heterocyclic biologically active compounds have their main applications in medicine. Drug delivery and co-drug compounds are two main functions of heterocyclic drug compounds; both are explored for biologically relevant multivalence processes.

Now days researchers efforts are made to design drugs with limited or certain side effects, such as cancer, neurodegenerative diseases and infectious diseases. Therefore they moved towards targeted or controlled drug delivery system and used in controlled rate and location of release of the drug.

1.6.1 Researchers developing drug delivery systems

Current research on drug delivery systems can be described in four broad categories: routes of delivery, delivery vehicles, cargo, and targeting strategies.

1.6.1.1 Routes of delivery

Medicines are introduced into the body by several routes shown in Figure 1.4.

One of the best examples is microneedle arrays, which is a technique drug enters into the body by skin (Figure 1.4). It is a medicine coated very thin needle, easy to use, not to be stored in refrigerator.

Figure 1.5 shows close image of microneedle vaccine patch (1), microneedle array containing influenza vaccine (2) and sequence of microneedle patches which can be administrated to a person (3).

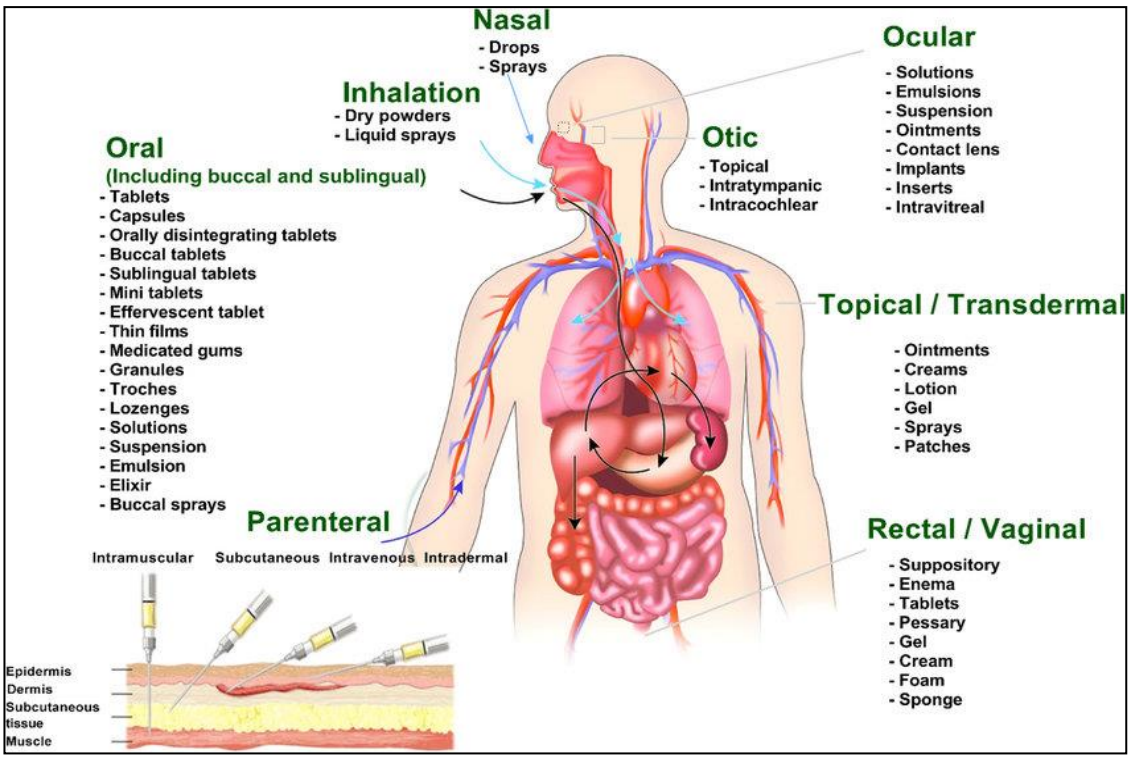


Figure 1.4 Routes of drug administration

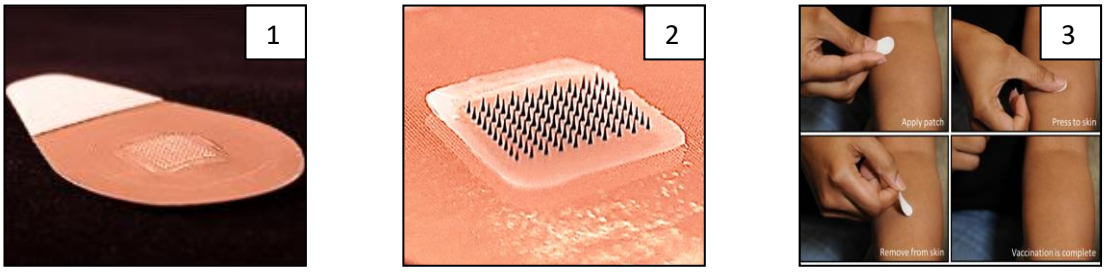


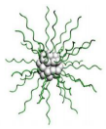
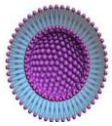
Figure. 1.5 Microneedle vaccine patch

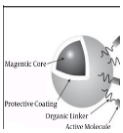
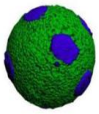
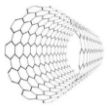
Figure 1.5 shows close image of microneedle vaccine patch (1), microneedle array containing influenza vaccine (2) and sequence of microneedle patches which can be administrated to a person (3).

1.6.1.2 Delivery vehicles

A drug delivery vehicle is helpful in transport of drugs to the particular location in the body. This drug arrives at their destination intact.

Table.2: Different types of carriers in drug delivery system

carriers	carriers	Image	Uses
Polymer	polymeric carriers		
	polymeric micelles		Solubilization of water-insoluble drugs, tumor targeting, and circumvention of MDR
	Dendrimers		*In vitro* diagnostics, contrast agent in MRI, drug delivery, photodynamic therapy, boron neutron capture therapy (BNCT) and vector in gene therapy
Lipids	Liposome (spherical lipid vesicles)		Extended range of morphologies, compositions, ability to envelope and protect many types of therapeutic biomolecules, lack of immunologic response, low cost and their differential release characteristics

Solid Lipid Carrier (lipospheres or solid lipid nanospheres)		delivery of various anticancer drugs, help to solve problems in MDR in cancer therapy
Gold Carrier		Not cytotoxic to human cell lines, PEGylated gold carriers (10 - 30 nm) used to restrict drug delivery to mother while preventing teratogenic effects on the fetus
Magentic Carrier		Contrast agents for magnetic resonance imaging (MRI) and heating mediators (hyperthermia) for cancer therapy
Viral Carrier		Non-toxic, biocompatible, biodegradable and non infectious in humans and other mammals
Carbon Carrier		Construction of biosensors for detection of genetic disorders, molecular abnormalities, substances for cell growth in tissue regeneration, and in drug delivery systems for a broad range of diagnostic and therapeutic agents, no toxic side effects and used in tumor

1.6.1.3 Cargo

Cargo is a carrier which mediates delivery of therapeutics from the site of application to the disease organ/tissues/cells or target area. They are therapeutic or imaging agents and carry drug molecules, chemotherapeutics, antibodies, aptamer, DNA, m-RNA, protein, peptides and vaccines *etc*. The microparticle therapy may be useful in treating autoimmune disorder multiple sclerosis. Oligonucleotide, PEG, protein, *β*-Cyclodextrin, fluorophore, biotin are some cargo's used in drug delivery system.

1.6.1.4 Targeted delivery strategies

In these strategies the delivery systems play a important role, a small amount of drug dose can be delivered to the specific organ and at the same time failing access to the non target site.

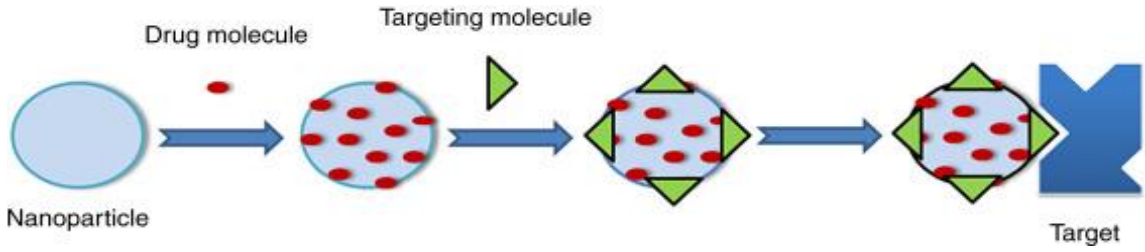


Figure 4: Target strategy of drug delivery system

1.8 References

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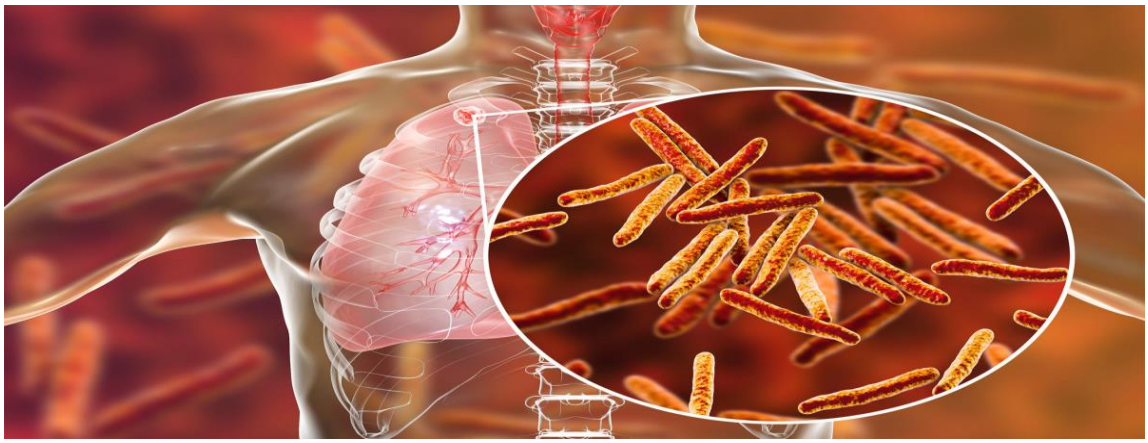
21. Polymer and heterocyclic compounds their utility and application as drug Alajely MS*Frontiers Drug Chemistry Clinical Res, 2019

Chapter 2

Design and Synthesis of Anti-tubercular Compounds and their Structure Active Relationship (SAR) Studies

Chapter 2A

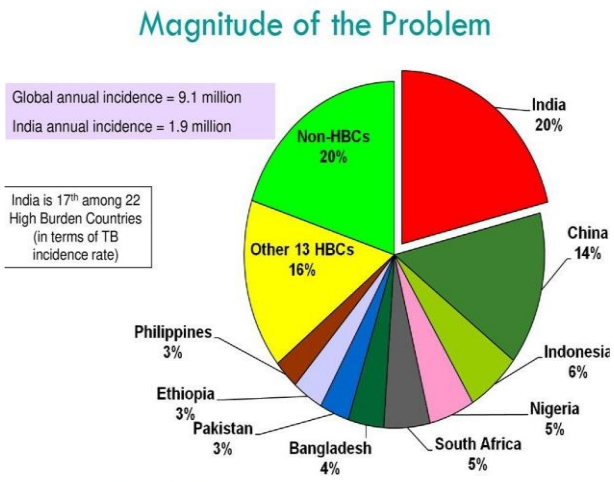
Tuberculosis



2A.1 Introduction

Dr. Robert Koch

On March 24, 1882, *M. tuberculosis* a bacteria causes a contagious disease tuberculosis was discovered by Dr. Robert Koch. After some period, 24 March was declared to be the world TB day. This day people were made aware about TB. TB is normally caused by *M. tuberculosis*. TB bacteria are generally found in the lungs when inhaled, but sometimes they can transmit to other parts of the body. Tuberculosis is also known as phthisis, phthisis pulmonalis, consumption and white death.



In 2018 one-quarter of the world's population was infected by latent TB. In 2017, nearly 10 million people suffered from TB with 1.6 million deaths worldwide. This makes it the number one cause of death from an infectious disease. Every year 0.2 million of 0.7 million HIV patients die due to TB. Therefore, TB is the main killer for HIV infected patients. Nearly 80% of TB patients are from Asian and African countries while 5 - 10% of TB patients were in the United States.

Signs and symptoms of TB

Fever, weight loss, night sweats, nail clubbing, fatigue, chills are the most common signs and symptoms observed in TB disease.[1]

2A.2 Types of mycobacteria that cause TB

2A.2.1 Tubercle bacilli

- *M. tuberculosis* - human
- *M. bovis* - bovine
- *M. microti* - marine

- *M. avium* - avian

2A.2.2 Lepra bacilli

- *M. leprae* - human
- *M. lepraemurium* – rat

2A.2.3 Mycobacterium causing skin cancer

- *M. ulcerans*
- *M. belnei*

2A.2.4 Atypical Mycobacteria – runyon groups

- Photochromogens
- Scotochromogens
- Nanophotochromogens
- Rapid growers

2A.2.5 Johne's bacillus – saprophytic mycobacteria

- *M. butyricum*
- *M. phlei*
- *M. stercoralis*
- *M. smegmatis*

Tuberculosis is generally caused in human and animals by *Mycobacterium* species. *Mycobacterium tuberculosis* is most important bacteria belonging to genus *Mycobacteria*. *M. tuberculosis* has a complex cell wall which consists of mycolic acid layer and a peptidoglycan layer, linked through an arabinogalactan polysaccharide. Cell wall is divided into two layers upper and lower layer.[2]

2A.3 Classification of tuberculosis

2A.3.1 Pulmonary tuberculosis

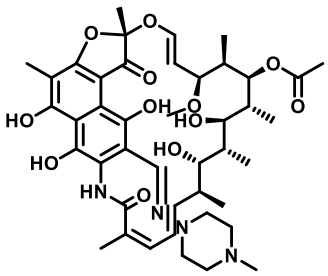
2A.3.2 Extrapulmonary tuberculosis

Tuberculosis is divided into two categories, one is pulmonary tuberculosis and another is extrapulmonary tuberculosis. When the bacteria are present in lungs, it is called as pulmonary tuberculosis and when tuberculosis bacteria are present in another part of the body it is called as extrapulmonary tuberculosis.

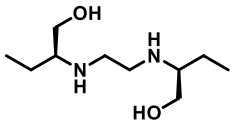
2A.4 Treatment

A number of drugs have been developed for TB treatment. Some are being used from ancient times. The anti-TB drugs are used in different combinations according to different circumstances either of them first line and second line drugs. Rifampicin[1] (**1**), Ethambutol[2] (**2**), Pyrazinamide[3] (**3**) and Isoniazid (**4**) are first line drugs (Figure 2A.1). Rimonabant and isoniazid are used for treating TB for 4 months and after that Rifampicin, Ethambutol, Pyrazinamide and Isoniazid are used in combination for 2 months. If first line drugs are not useful for patients, then second line drugs are used. Ethionamide (**5**), Cycloserine (**6**) and 4-aminosalicylic acid (**7**) the second lines drugs (Figure 2A.2).

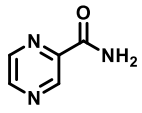
Figure 2A.1. First line drugs



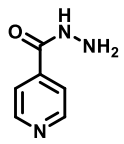
Rifampicin (1)



Ethambutol (2)

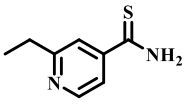


Pyrazinamide (3)

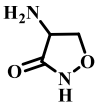


Isoniazid (4)

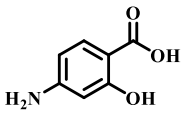
Figure 2A.2. Second line drugs



Ethionamide (5)



Cycloserine (6)



4-Aminosalicylic acid (7)

2A.4.1 New TB drugs

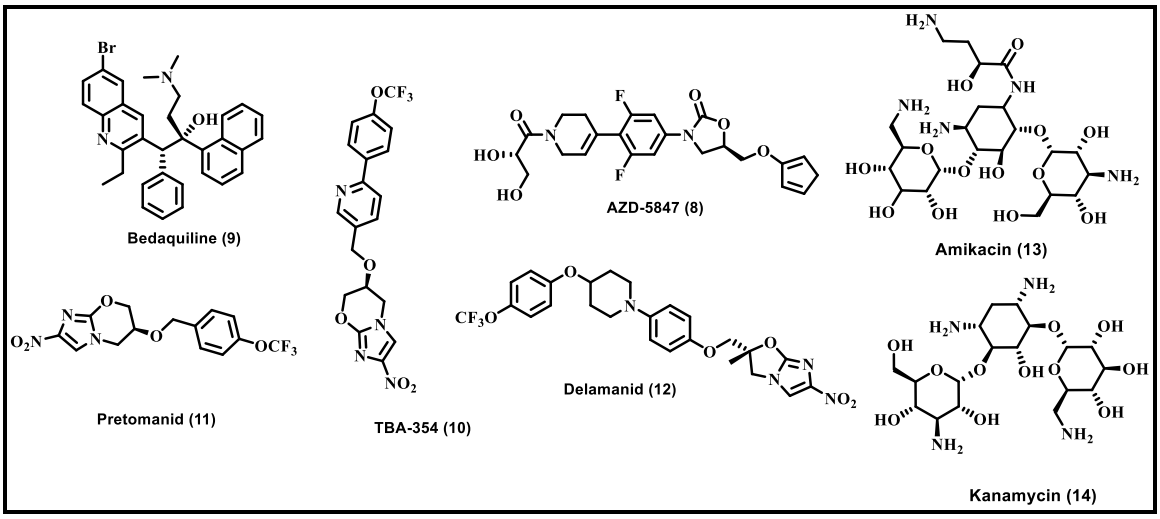
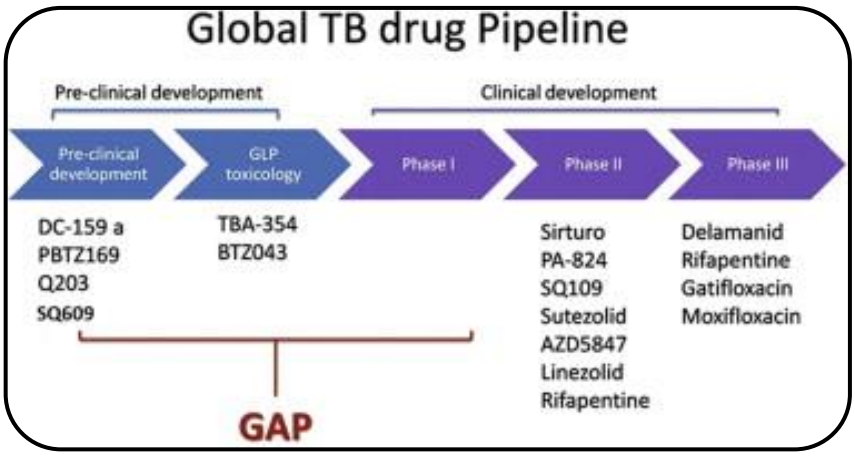


Figure 2A.3. Latest anti tuberculosis drugs available in market

AZD-5847 (**8**), Bedaquiline (**9**), TBA-354 (**10**), Pretomanid (**11**), Delamanid (**12**) are used for drug sensitive and drug resistant TB (Figure.2A.3).

2A.5 Drug resistant tuberculosis

If someone has drug resistant TB, it means TB bacteria in their body do not respond to certain drugs. Mono resistant, poly resistant, MDR and XDR are the types of drug resistant TB. Isoniazid (**4**) and rifampicin (**1**) are examples of drugs used for mono resistant and MDR TB. Amikacin (**13**) and Kanamycin (**14**) are examples of drugs used in the treatment of XDR.



In the market new TB drugs are needed because of current drugs are less effective, more toxic and more expensive. New TB drugs should be simpler, more effective, easily tolerable, less expensive, and safe for drug sensitive, drug resistant and HIV patients with TB. BM-212[8] has less toxic and more active against TB. We modified its synthesis by using simple and easily available starting materials. BM-212 and Rimonabant[9] are more structurally similar to each other. Therefore we planned to investigate Rimonabant activity against TB. For convenience, chapter 2 is divided in two parts. The first part describes total synthesis of BM-212 while second part deals with work related to Rimonabant.

2A.6 References

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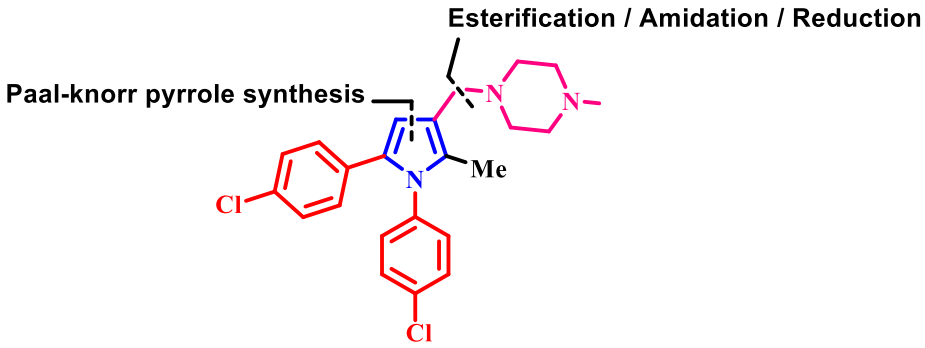
Chapter 2B

An efficient synthesis of potent anti-tubercular drug candidate BM-212



BM-212

In this chapter, total synthesis of anti-tubercular drug BM-212 from commercially available 4-chlorophenacyl bromide was carried out. In 1998, Delia deidda and Giorgio Lampis explored BM-212 molecule, possesing activity against *Mycobacterium tuberculosis* and *nontuberculosis mycobacteria*. The present BM-212 has a broad substrate scope, less toxic and functional group tolerance. It show activity against antifungal and anti TB.

Namita A. More et *al.* *RJC*, 2016, *9*, 806-811

2B.1 Introduction

According to 2013 WHO (World Health Organization) statistics, 9 million people fell ill with TB and 1.5 million died from the disease. Currently, the first-line drugs for TB include Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB) (See chapter 2A, Figure 2A.1) have their own benefits and side effects. However, the emergence of multiple drug resistant (MDR) strains of TB and extreme drug resistant (XDR) strains of TB has created a global epidemic problem. Therefore, development of new drug molecules having novel mode of action is an urgent requirement. In this regard, the quest for a novel anti-mycobacterial drug led to the identification of BM-212 as a promising therapeutic agent against M. Tb.[1-5]

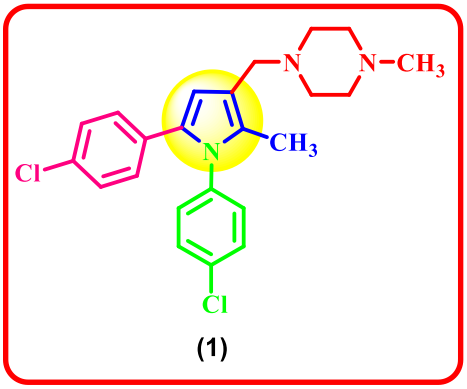


Figure 2B.1 Biologically significant Anti-tubercular BM-212

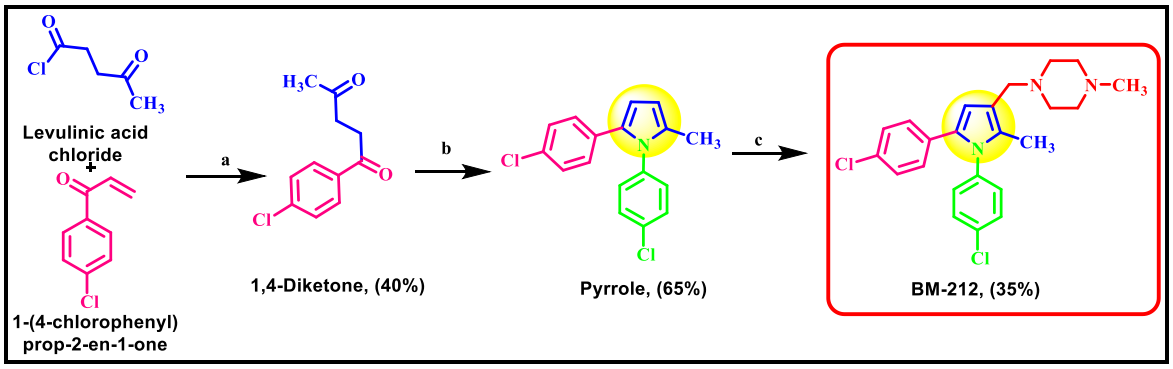
By literature, novel BM-212 has antimycobacterial[6], antifungal[7] and anti TB[8] drug activities with lesser toxicity[9] than the Rifampicin or Isoniazid drugs (See Chapter 2A).[6b]

2B.2 Rationale of the BM-212 molecule

It is surprising to note that only a few literature reports are available that have reported low overall yields and limited scope of pyrrole ring in BM-212.[7-13]

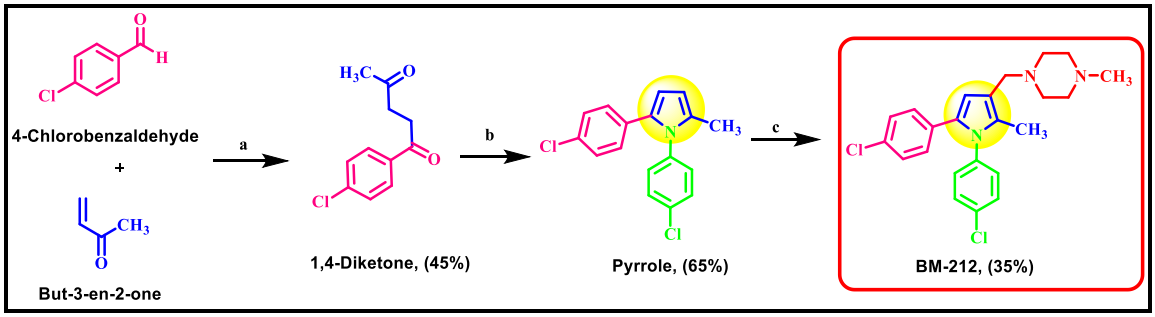
In 1992, F. Cerreto discovered synthetic approaches of BM-212 molecule, which was initiated with the preparation of 1,4-diketone by reacting levulinic acid chloride with 1-(4-chlorophenyl)prop-2-en-1-one following Stetter reaction. After that cyclization with 1,4-diketone and 4-chloro aniline according to Paal-Knorr reaction. Finally, pyrrole mannich reaction using *N*-methylpiperazine and

formaldehyde afforded BM-212 molecule. In this synthetic route, final product



Scheme 2B.1: *Reagents and conditions: (**a**) Thiazolium salt, NEt_3, dry EtOH, 75 - 78 °C, 5 h, 2 N HCl, 40%; (**b**) 4-Chloroaniline, p-TsOH, 100 °C, 5 h, 65%; (**c**) N-Methylpiperazine, CH_2O, AcOH, CAN, rt, 8 h, 20% NaOH, 35%.*

obtained within three steps reactions and afforded 9.10% overall yield. In 2006, Biava and coworkers modified the synthesis of the BM-212 molecule. Biava and coworkers synthesized BM-212 molecule from different starting materials including 4-chlorobenzaldehyde and but-3-en-2-one resulting in marginally increased overall yield from 9.10 to 10.23%.



Scheme 2B.2: *Reagents and conditions: (**a**) Thiazolium salt, NEt_3, 75 - 78 °C, 5 h, 2 N HCl, 40%; (**b**) 4-Chloroaniline, p-TsOH, 100 °C, 5 h, 65%; (**c**) N-Methylpiperazine, CH_2O, AcOH, CAN, rt, 8 h, 20% NaOH, 35%.*

In the above reported two schemes, low overall yields of BM-212 were obtained. These results prompted us to synthesize BM-212 molecule developing can be used new method by using simple starting materials, mild reaction conditions and increase in overall yield. We synthesized BM-212 by using the highly capable and simple synthetic route in five steps and 48% overall yield. Notably many

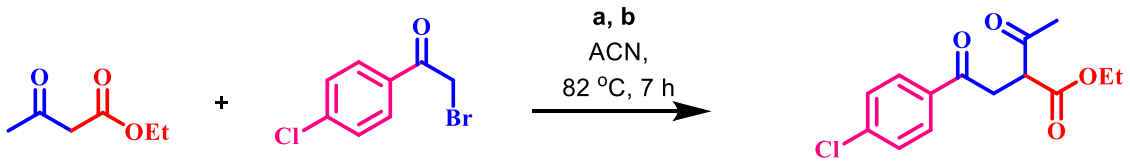
synthetically valuable pyrroles can be obtained by this method using the traditional approach, "Knorr reaction".

2B.3 Results and discussion

2B.3.1 Optimization of reaction condition

An introductory experiment was performed using ethyl acetoacetate (EAA) (**2**) and 4-chlorophenacyl bromide (**3**) in presence of catalyst terabutylammonium iodide (TBAI) at 82 °C in acetonitrile to afford the 1,4-diketone product **4**. In that reaction 1 equiv. of NaOH, KOH, K_2CO_3, $NaHCO_3$, NaH, or KotBu simultaneously were tested for the deprotonation of 1,3 dicarbonyl. With the preliminary results in hand, we were interested to investigate the more appropriate base K_2CO_3 that can potentially improve the yield of the reaction to 73% as shown in Table 2B.1. The same reaction carried was without using TBAI gave the desired 1,4-diketone **4** in 37% yield (Table 2B.1).

Table 2B.1 Optimization of the TBAI catalyst and base to afford 1,4-diketone



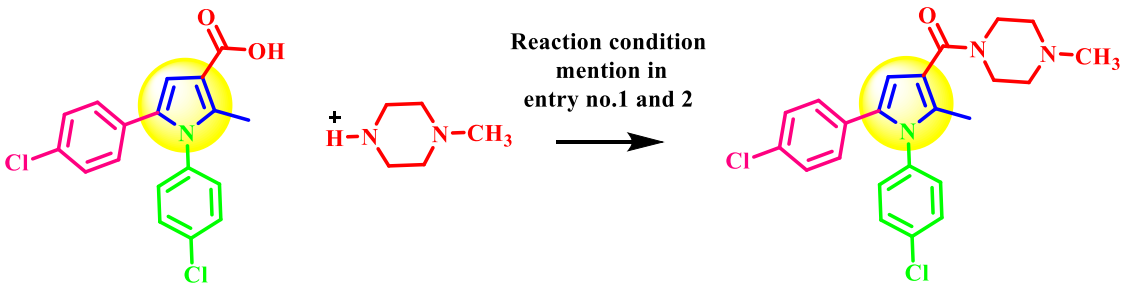
(2) (3) (4)

Entry	Catalyst[a]	Base[b]	Yield 4 (%)
1	TBAI	NaOH	12%
2	TBAI	KOH	14%
3	TBAI	$NaHCO_3$	20%
4	TBAI	NaH	25%
5	TBAI	KOtBu	29%
6	TBAI	K_2CO_3	73%
7	-	K_2CO_3	37%

Carl Paal and Ludwig Knorr observed that in the Paal-Knorr reaction[14-19] (See chapter 1, 1.3.3), using 1,4-diketone (**4**), 4-chloroaniline and 2N H_2SO_4 at 100 °C in toluene, pyrrole was obtained in low yield. To improve the yield 4-chloroaniline was reflux in toluene for

2 h in situ addition of 5 ml 2N H_2SO_4 at 100 ^oC in toluene for 3 h. Followed by addition of 2N NaOH at 100 ^oC for 3 h in hydrolysis in water-ethanol acid **6** in 92% yield.

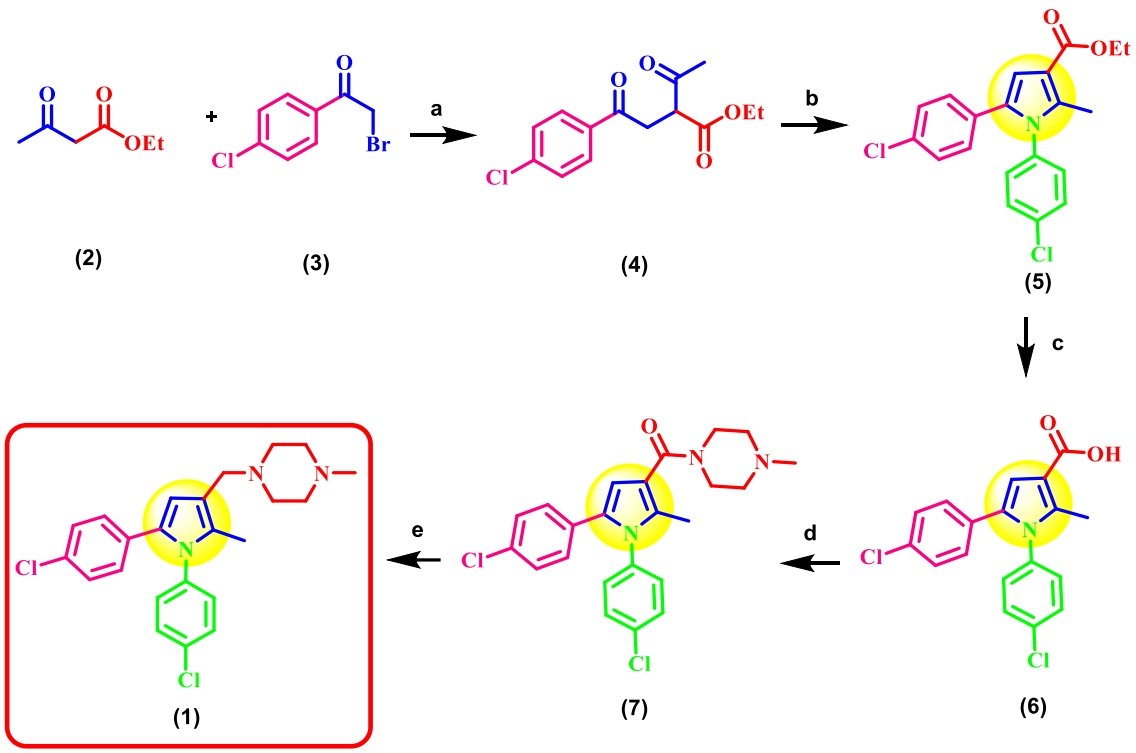
Table 2B.2 Optimization of the microwave-assisted reaction under imidazole catalyst afforded amide



(6) (7)

Entry	Catalyst	Solvent	Temp	Yield 7 (%)
1	DCC, HOBt	DCM	rt	67%
2	Imidazole	Solvent free	100W, MW	100%

Coupling of acid **6** with N-methyl piperazine in the presence of DCC and HOBt yielded amide in 67%. Interestingly, when the same reaction mixture of acid **6** with N-methyl piperazine and imidazole was irradiated at 100W under solvent free conditions for 1 min, the desired amide **7** was formed in quantitative yield. Finally, the reduction of amide **7** using $LiAlH_4$ (3 equiv.) in dry THF under inert conditions readily afforded the target molecule BM212 (**1**) in 85% yield. (Scheme 2B.3). The structure of BM212 (**1**) was confirmed by the studies of ^1H NMR, ^{13}C NMR, and HRMS spectral analyses and is in agreement with the reported data..



Scheme 2B.3: Reagents and conditions: ***(a)*** *K_2CO_3, cat. TBAI, ACN, reflux 7 h, 73%;* ***(b)*** *4-Chloroaniline, 2N H_2SO_4, toluene, reflux 5 h, 84%;* ***(c)*** *NaOH, EtOH-H_2O, reflux 3 h, 92%;* ***(d)*** *N-Methyl piperazine, imidazole, microwave 100W, 1 min, 100%;* ***(e)*** *$LiAlH_4$, dry THF, 0 ^oC – reflux, 4 h, 85%.*

2B.3.2 Scope of BM-212

Pyrrole derivatives appear to show potent and selective antimycobacterial activities. Delia Deidda, Giorgio Lampis in 1998 disclosed pyrrole derivatives of BM-212 molecule. BM-212 is a potent antimycobacterial agent and MmpL3 inhibitor.

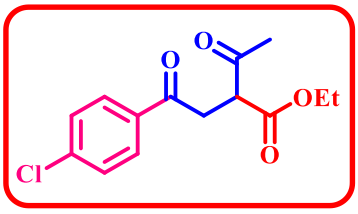
2B.4 Conclusion

In the present chapter 2B, we have reported a simple and highly efficient five step synthesis of BM-212 (**1**) in 48% overall yield from commercially available ethyl acetoacetate and 4-chlorophenacyl bromide. Considering the importance of BM-212 in biological and medicinal chemistry, the present synthesis will allow the incorporation of a variety of functional groups at the 3 position of pyrrole ring. Further exploration of strategy is underway in our laboratory.

2B.5 Experimental section

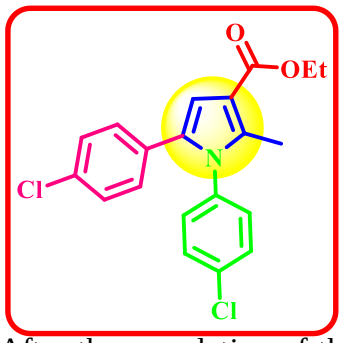
2B.5.1 Experimental procedures and characterization data

Ethyl 2-acetyl-4-(4-chlorophenyl)-4-oxobutanoate (4)



To a 500 mL round bottom flask containing ethyl acetoacetate (**2**) (21.85 mL, 17.10 mmol) in acetonitrile (100 mL) was added by oven dried potassium carbonate (23.675 g, 17.10 mmol) and a catalytic amount of tetrabutylammonium iodide at 0 °C. The reaction mixture was stirred for 30 mins at this temperature and 4-chlorophenacyl bromide (**3**) (40 g, 17.10 mmol) in acetonitrile (80 mL) was added dropwise at 0 °C. The reaction mixture was refluxed for 7 h. After the completion of the reaction, the reaction mixture was filtered and the solvent was evaporated on rota vapor. The residue was extracted with ethyl acetate (3 x 60 mL). The combined organic phase was dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was column chromatograph on silica gel using ethyl acetate: petroleum ether (03:97) as eluent to give the corresponding compound **4**, yield: 35.35 g, 73%; ^{1}H NMR (400 MHz, $CDCl_3$) ᵭ 7.91 (d, *J* = 8.56 Hz, 2H), 7.43 (d, *J* = 8.56 Hz, 2H), 4.17 - 4.26 (m, 3H), 3.66 (dd, *J* = 8.07, 18.34 Hz, 1H), 3.46 (dd, *J* = 5.38, 18.34 Hz, 1H), 2.43 (s, 3H), 1.28 (t, *J* = 7.09 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ᵭ 202.1, 195.9, 168.7, 139.9, 134.4, 129.5, 128.9, 61.8, 53.8, 37.2, 30.2, 14.0; HRMS (ESI): *m/z* Calcd for $C_{14}H_{15}ClNaO_4$ $[M+Na]^+$: 305.0551; Found: 305.0551.

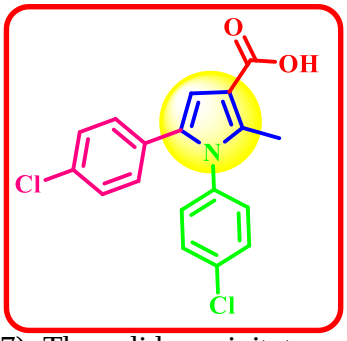
Ethyl 1,5-bis(4-chlorophenyl)-2-methyl-1*H*-pyrrole-3-carboxylate (5)



A round bottom flask containing ethyl 2-(4-chlorophenacyl) acetoacetate (**4**) (18.00 g, 63.66 mmol) and 4-chloro aniline (11.36 g, 89.13 mmol) in toluene (40 mL) was refluxed for 2 h. Then the reaction mixture was cooled to 0 °C. 2N H_2SO_4 (5 mL) was added dropwise to the reaction mixture and stirring was continued under reflux for an additional 3 h. After the completion of the reaction, the reaction mixture was filtered and concentrated on rota vapor. The residue was extracted with ethyl acetate (3 x 40 mL). The combined organic phase was dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was chromatographed on silica gel using ethyl acetate: petroleum ether (04:96)

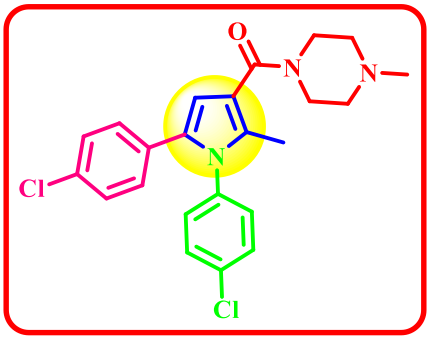
as eluent to give the corresponding product **5**, yield: 20.01 g, 84%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.31 Hz, 2H), 7.12 - 7.18 (m, *J* = 8.56 Hz, 2H), 7.04 - 7.10 (m, *J* = 8.56 Hz, 2H), 6.96 (d, *J* = 8.56 Hz, 2H), 6.79 (s, 1H), 4.33 (q, *J* = 7.09 Hz, 2H), 2.40 (s, 3H), 1.38 (t, *J* = 7.09 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 138.2, 136.3, 134.4, 132.6, 130.5 (2C), 129.6, 129.5 (2C), 129.2 (2C), 128.4 (2C), 113.3, 110.6, 59.6, 14.5, 12.4; HRMS (ESI): *m/z* Calcd for $C_{20}H_{17}Cl_2NNaO_2$ $[M+Na]^+$: 396.0529; Found: 396.0529.

1,5-Bis(4-chlorophenyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid (6)



To a 100 mL round bottom flask containing ester **5** (15.00 g, 40.07 mmol) in ethanol (10 mL) was added a 10 mL aqueous solution of NaOH (4.80 g, 12.24 mmol) at 0 °C and the reaction mixture was refluxed for 3 h. After the completion of the reaction, ethanol was evaporated under *vacuo* and reaction mixture was neutralized with 2N H_2SO_4 (up to pH = 7). The solid precipitate was filtered and washed with cold water and dried to afford acid **6**, yield: 12.766 g, 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (br. s., 1H), 7.53 (d, *J* = 7.58 Hz, 2H), 7.24 - 7.31 (m, 4H), 7.04 (d, *J* = 7.34 Hz, 2H), 6.71 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 137.8, 136.2, 133.2, 131.8, 131.4, 130.7, 130.3 (2C), 129.5 (2C), 129.4 (2C), 128.3 (2C), 113.2, 110.7, 12.1; HRMS (ESI): *m/z* Calcd for $C_{18}H_{14}Cl_2NO_2$ $[M+H]^+$: 346.0395; Found: 346.0395.

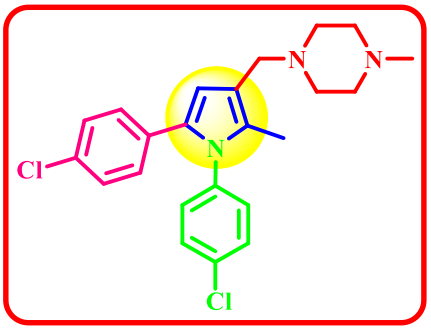
1,5-Bis(4-chlorophenyl)-2-methyl-3-(4-methylpiperazin-1-yl)carbonyl-1*H*-pyrrole (7)



A 25 mL conical flask containing acid **6** (1 g, 2.88 mmol), N-methyl piperazine (0.34 g, 3.46 mmol) and imidazole (0.23 g, 3.46 mmol) was irradiated in a microwave at 100W for 1 min. After the completion of reaction, the reaction mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was chromatographed on silica gel using ethyl acetate: petroleum ether (2:8) as eluent to give the corresponding product **7**, yield: 1.237 g, 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 7.39 (m, 2H), 7.13 (d, *J* = 8.56 Hz, 2H), 7.05 (d, *J* = 8.56 Hz, 2H), 6.94 (d, *J* = 8.56 Hz,

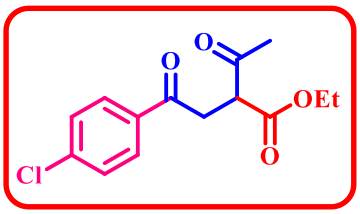
2H), 6.36 (s, 1H), 3.75 (br. s., 4H), 2.45 (br. s., 4H), 2.33 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ɗ 167.1, 136.7, 134.0, 133.0, 132.4, 132.2, 130.7, 129.6 (2C), 129.5 (2C), 129.1 (2C), 128.4 (2C), 116.6, 109.0, 55.2, 46.0, 12.1; HRMS (ESI): *m/z* Calcd for $C_{23}H_{24}Cl_2N_3O$ $[M+H]^+$: 428.1293; Found: 428.1291.

BM-212 (1)

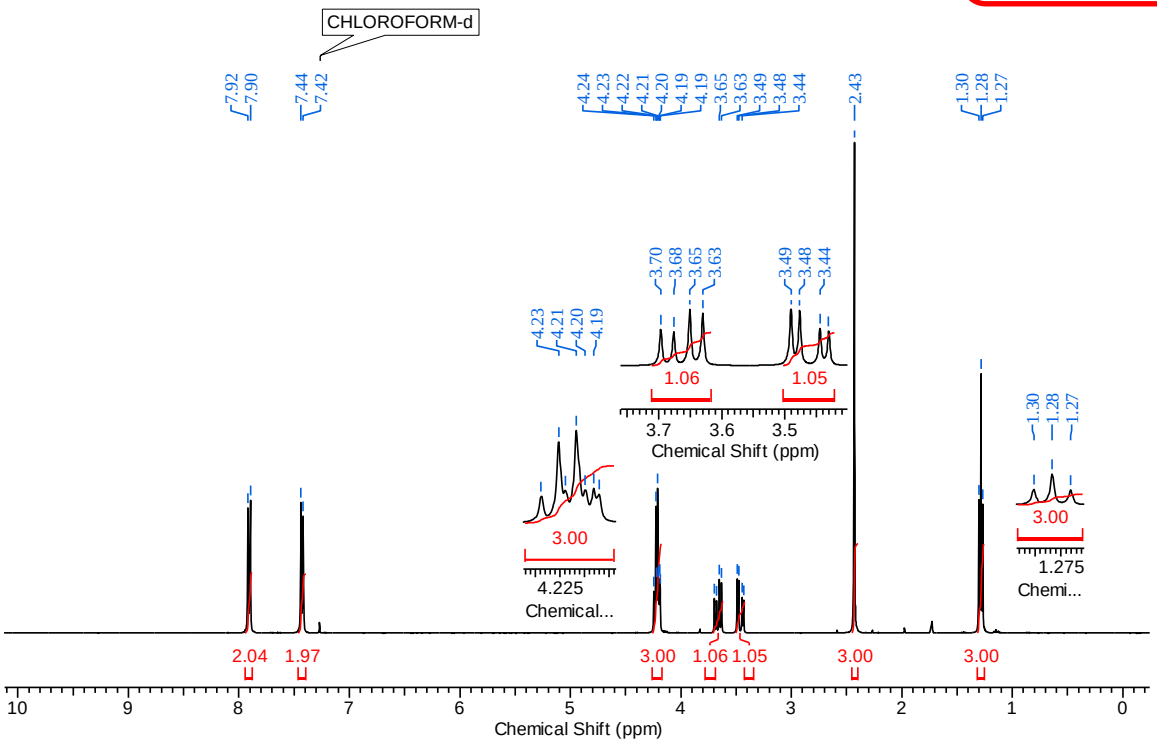


To a two neck round bottom flask containing $LiAlH_4$ (2.12 g, 56.03 mmol) in dry THF (15 mL) at 0 °C was added dropwise a solution of compound **7** (8 g, 18.68 mmol) in dry THF (15 mL). After the complete addition, the reaction mixture was refluxed for 4 h. The excess of $LiAlH_4$ was quenched using methanol, then filtered on celite and washed with methanol. The filtrate was concentrated and the residue was chromatographed on silica gel using ethyl acetate: petroleum ether (9:1) as eluent to give corresponding product BM-212 (**1**), yield: 6.578 g, 85%; ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.30 - 7.38 (m, *J* = 8.56 Hz, 2H), 7.02 - 7.15 (m, 4H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.33 - 6.38 (m, 1H), 3.43 - 3.49 (m, 2H), 2.48 (br. s., 6H), 2.29 (s, 3H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.7, 133.3, 131.8, 131.4, 130.0, 129.6 (2C), 129.2 (2C), 128.7, 128.5, 128.2, 116.9, 111.6, 55.1 (2C), 54.3, 52.7 (2C), 46.0, 11.1; HRMS (ESI): *m/z* Calcd for $C_{23}H_{26}Cl_2N_3$ $[M+H]^+$: 414.1498; Found: 414.1497.

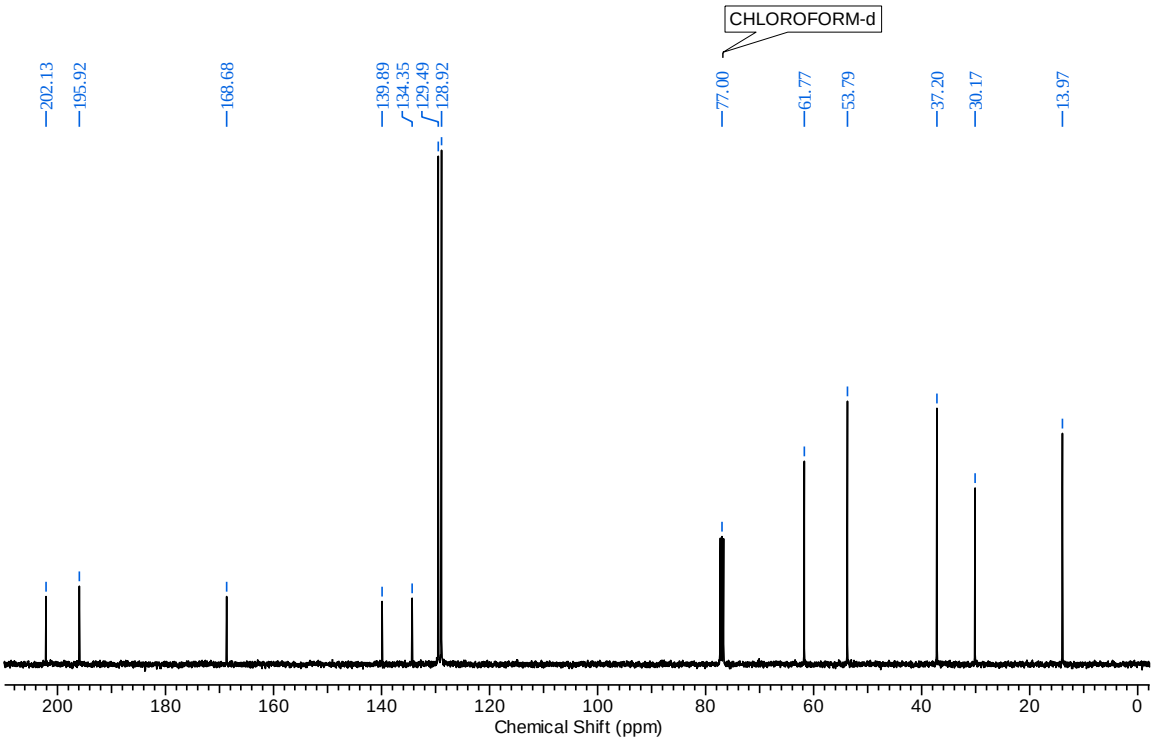
2B.5.2 Spectral data



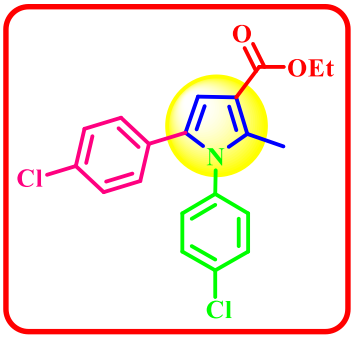
^{1}H NMR (400 MHz, $CDCl_3$)

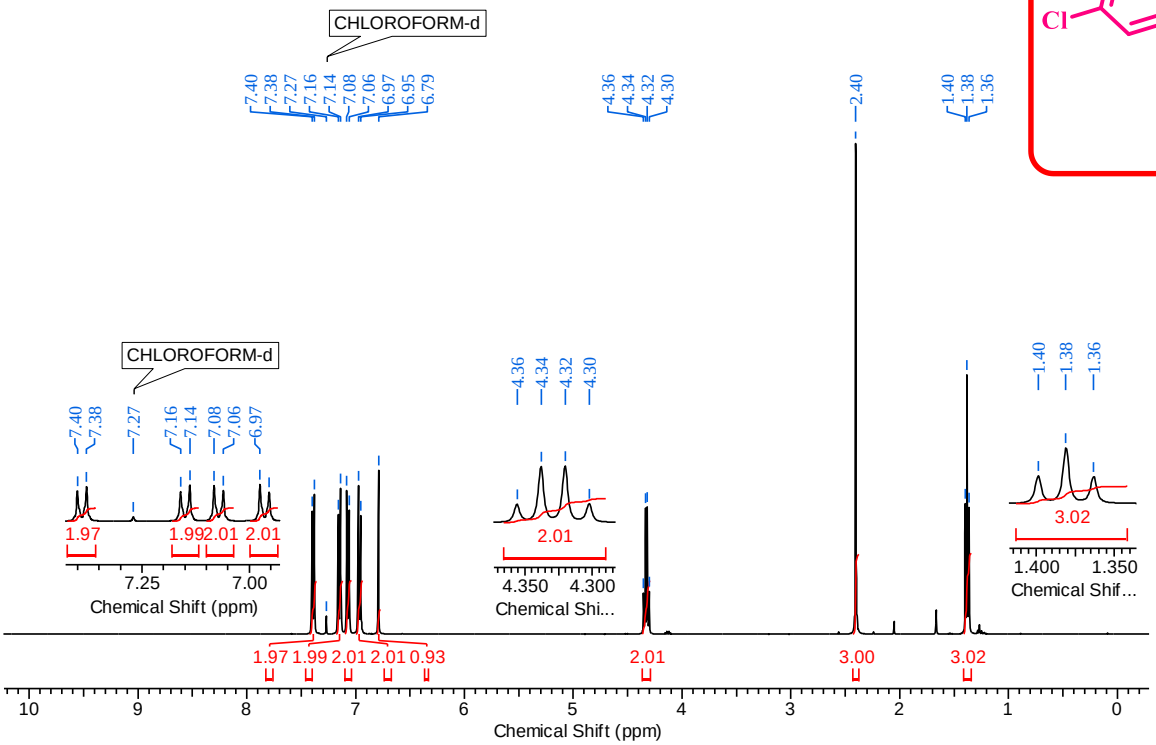


^{13}C NMR (100 MHz, $CDCl_3$)



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





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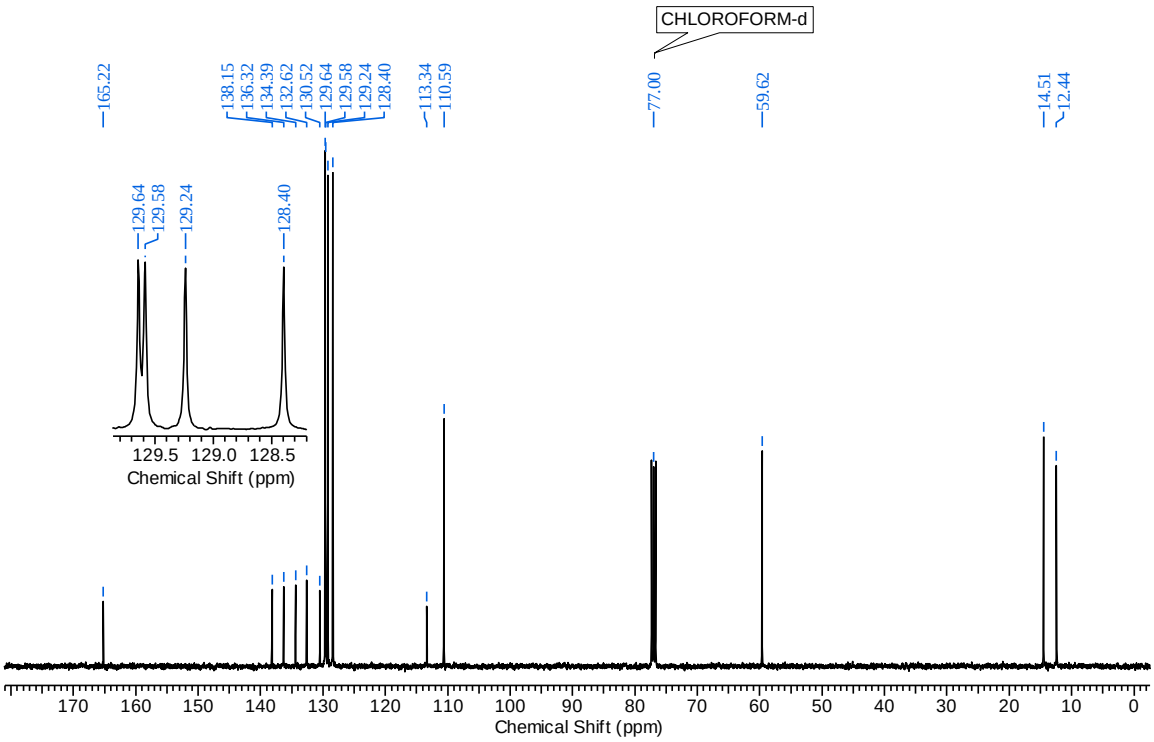
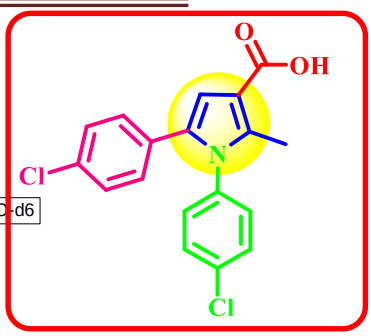
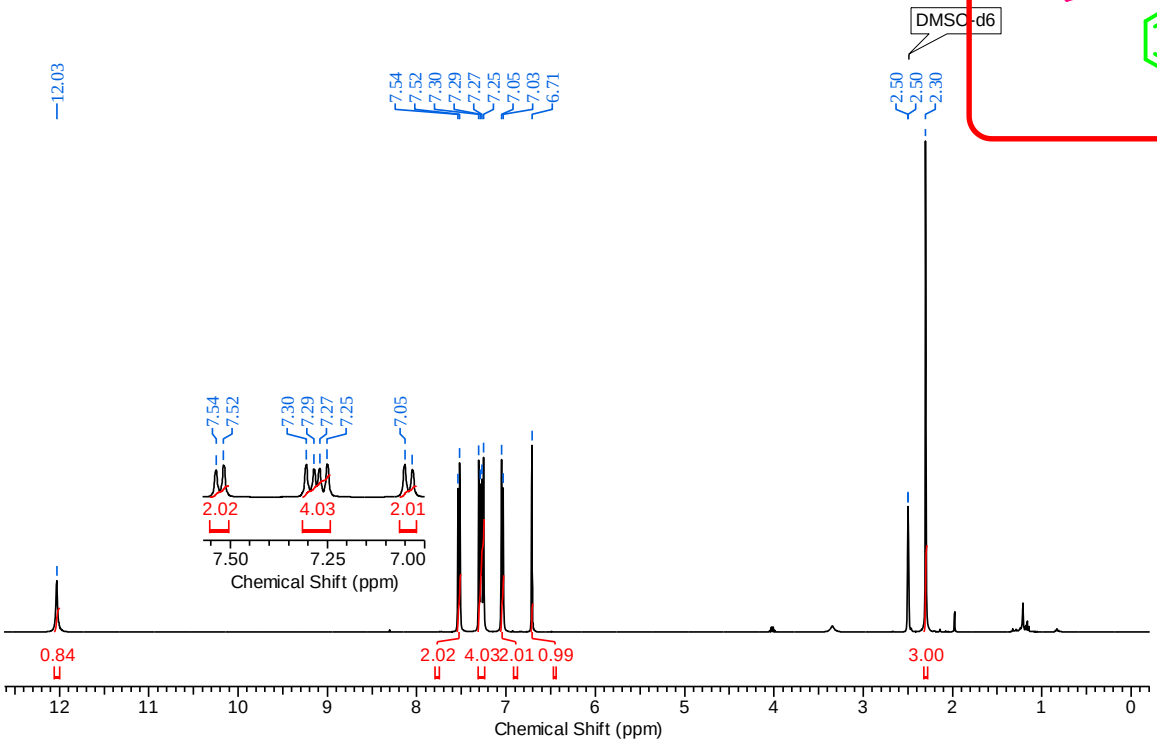


^{1}H NMR (400 MHz, DMSO-d$_6$)





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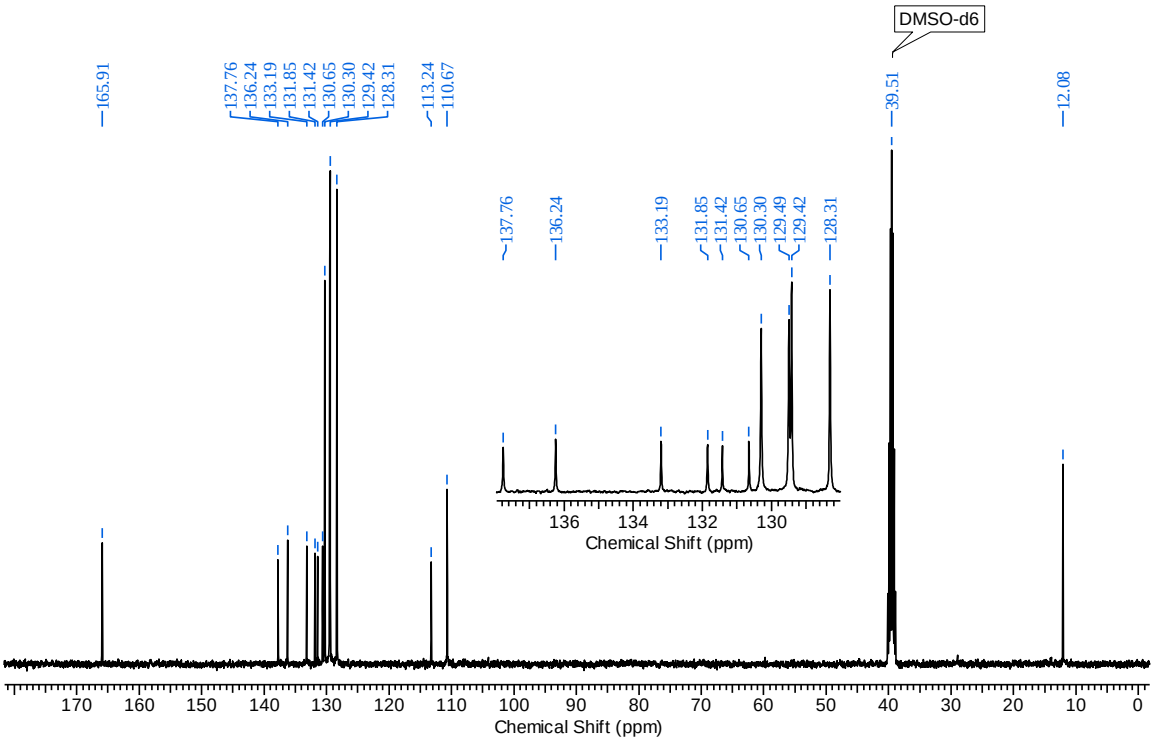
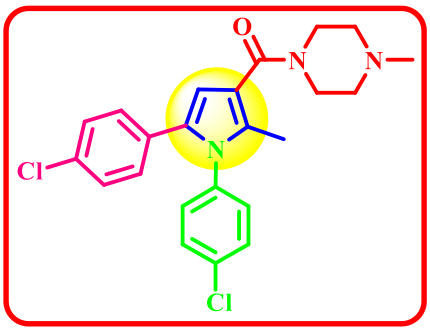
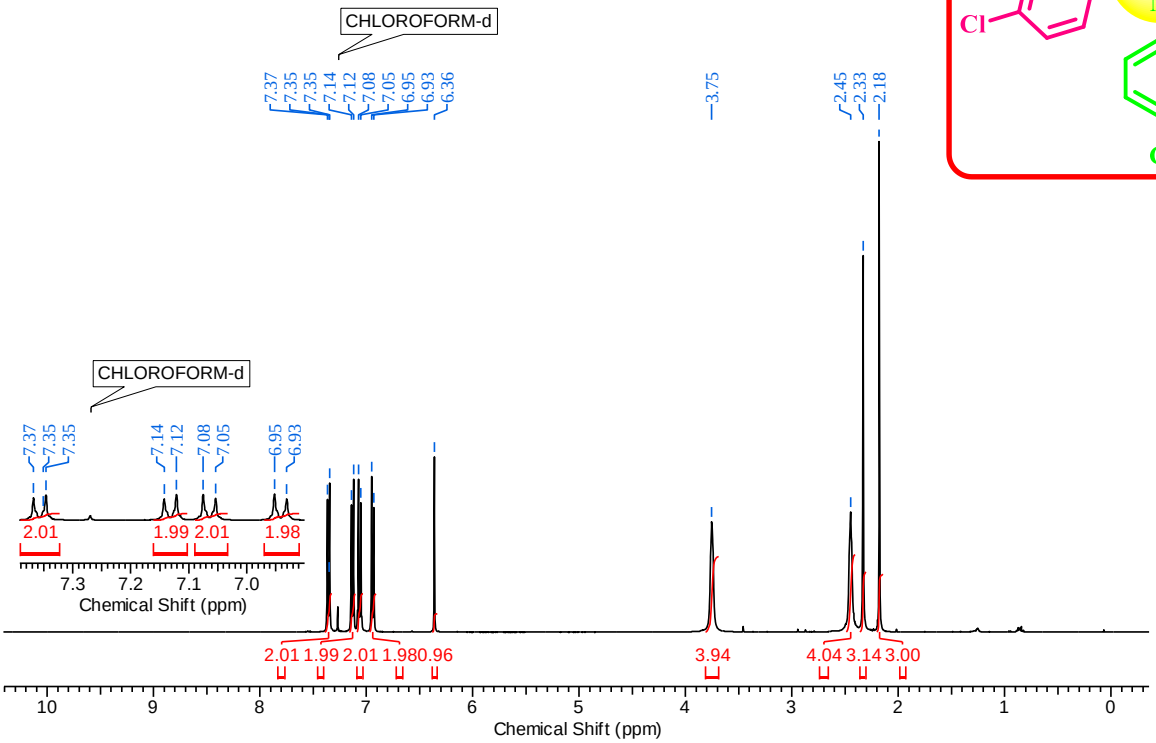
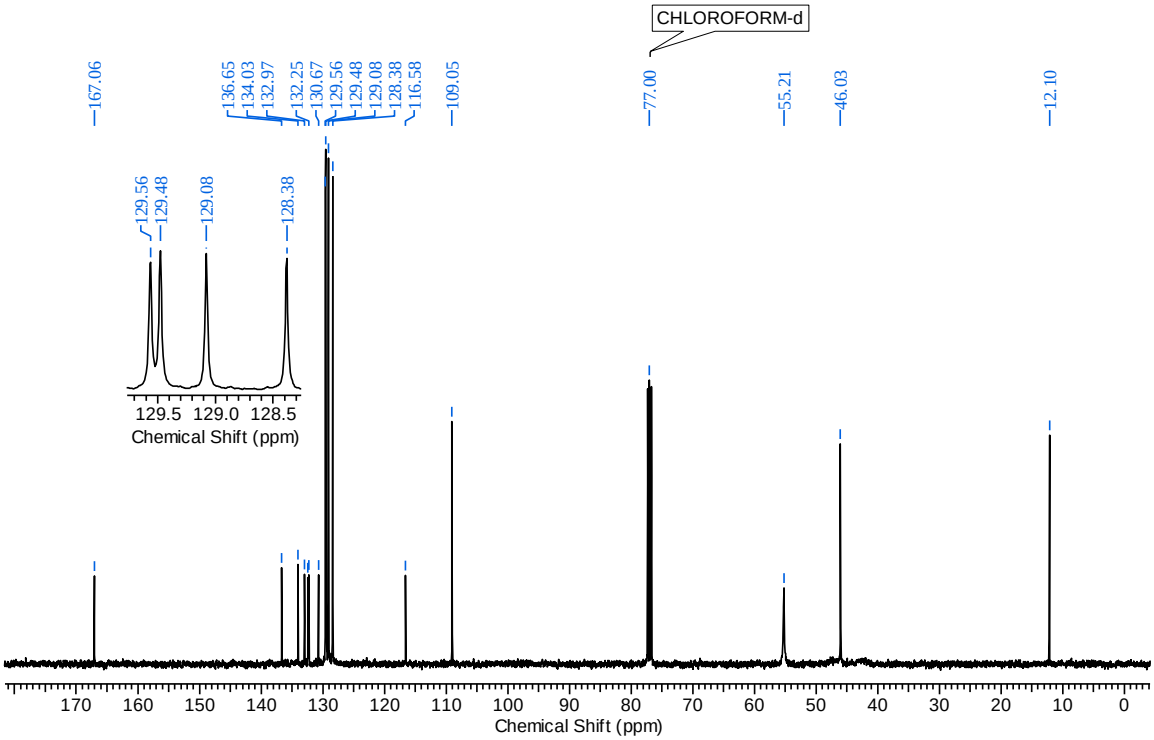


^{1}H NMR (400 MHz, $CDCl_3$)





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



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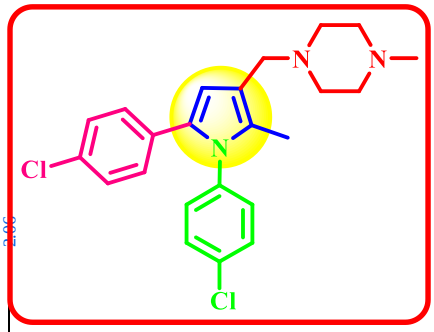

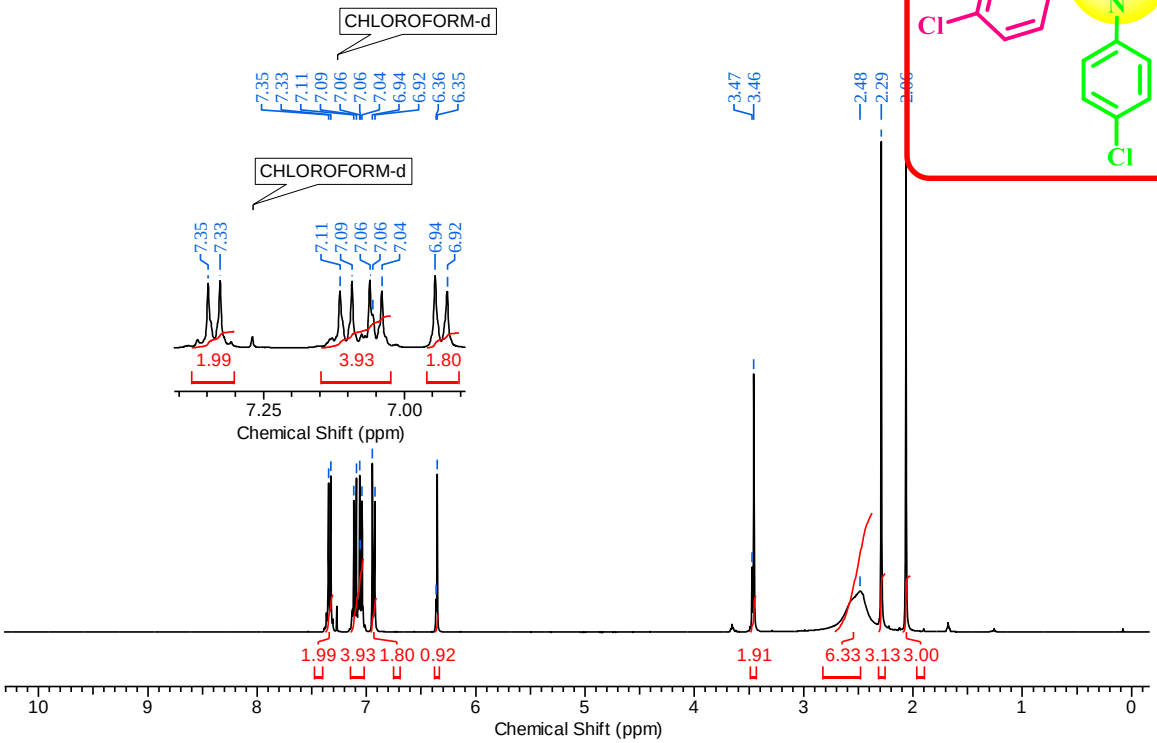

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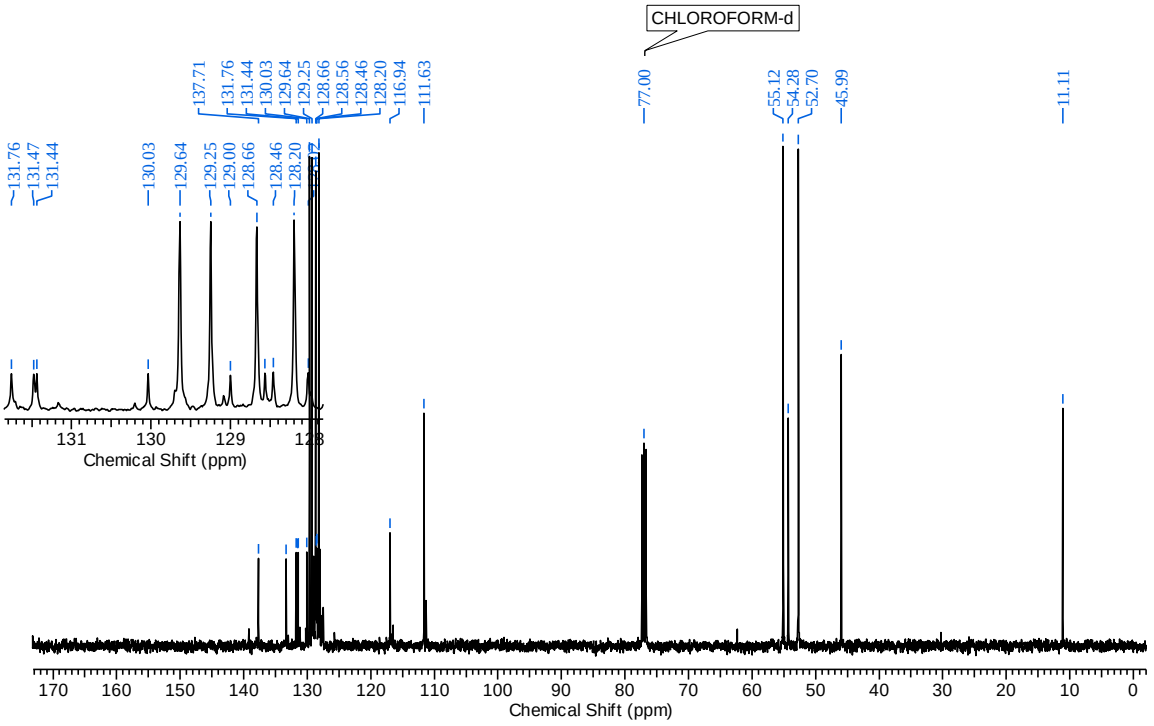

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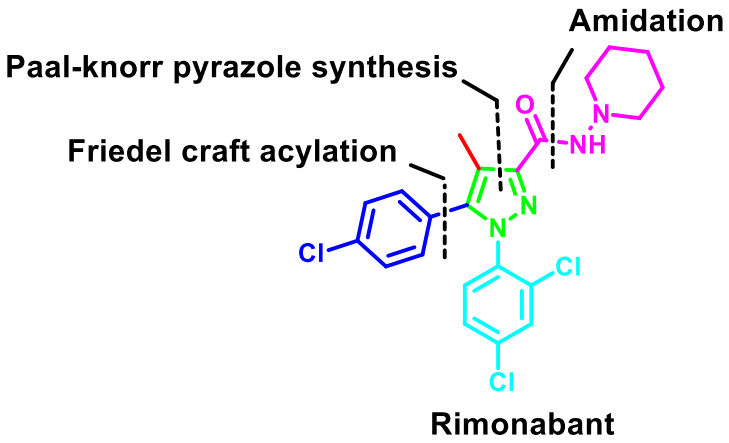
Chapter 2C

Discovery of Rimonabant and its potential analogues as anti-TB drug candidates



Rimonabant

In 2006, Rimonabant was first approved in Europe as anorectic antiobesity drug. It is also known as SR141716, sold under brand name Acomplia and Zimulti retailed by Sanofi-Aventis. It was withdrawn in 2008 due to serious psychiatric side effects. In this chapter we described constructions of Rimonabant and its analogues their screening against *M. tuberculosis* and *M. smegmatis*. The most dynamic analogue JMG-14 exhibits MIC value of 3.13 g/ml compared to ethambutol (3.25 g/ml) and pyrazinamide (50.l g/ml). The molecular docking reveals that pyrazole ring, number and position of halogen atoms play a crucial role in deciding interactions with MTCYP-121. These findings open up a new avenue in the search of potent anti-TB drugs with rimonabant and its novel analogue JMG-14 as lead molecules.

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2C.1 Introduction

Tuberculosis affects a large number of persons and at the current stage, it would be highly beneficial and attractive strategy to identify a drug candidate, which has been approved as a drug by regulatory authorities, effective for the treatment of TB. This would enable a quicker introduction of the drug into the market for TB treatment. In the recent years, azoles *viz*. pyrazoles, imidazoles and triazoles have attracted the attention of researchers. Some clinically used drugs such as clotrimazole, fluconazole and voriconazole, which contain azole moiety, can be used to treat generalized systemic mycoses. Literature survey reveals that selected azole drugs possess potent antimycobacterial activity in the nanomolar range[1] (Figure 2C.1).

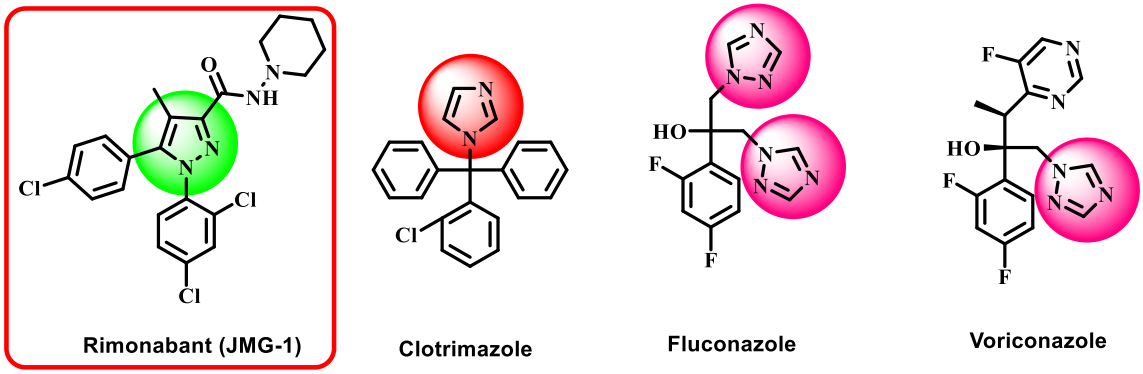


Figure 2C.1 Biologically active azoles moieties

Many azole compounds possessing potent anti-tubercular activity bind to MT-CYP51 and MTCYP121 proteins with crucial role in metabolism of *M. tuberculosis*.[2] In recent years, cytochrome P450 (CYP121) has gained popularity as an attractive target for azole-based drugs against M. tuberculosis. A plausible reason is that MT-CYP121, which has unique catalytic action.[3] Moreover, in many studies, the presence of halogen attached to aromatic moiety has been found to escalate antifungal and anti-mycobacterial activities.[4-6]

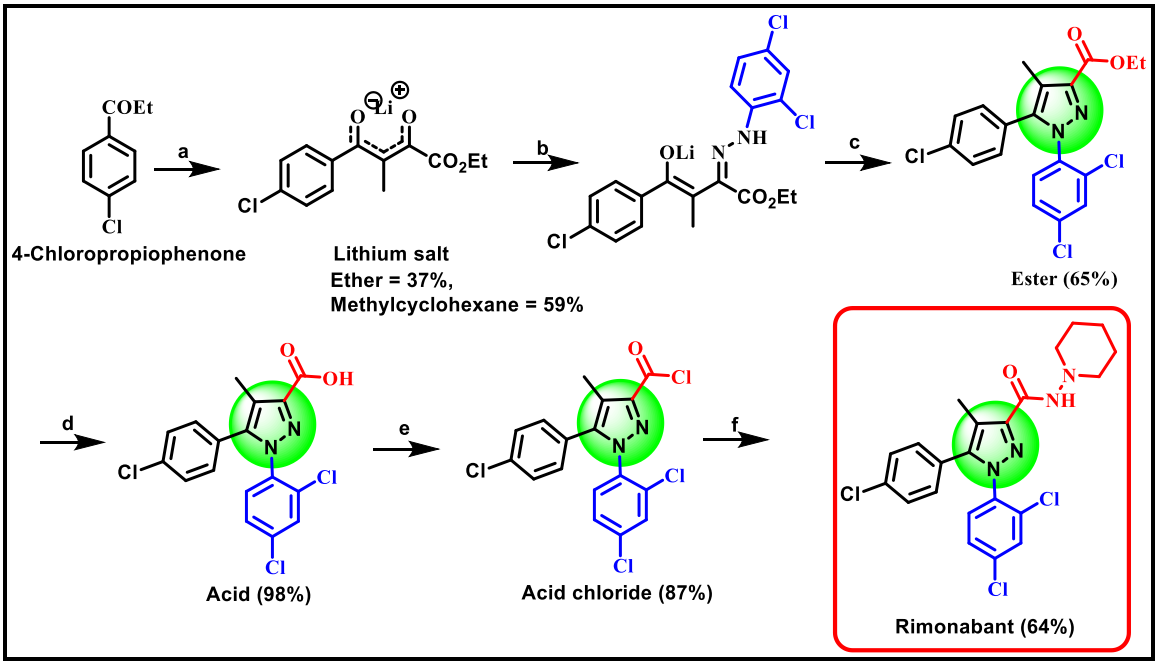
Developing a new drug is a long, expensive and tedious process, often consuming several years and resources. In addition, the regulatory approval process following the drug discovery and design involves several tests and trials. Even after completing the whole process of drug development, certain drugs such as Celecoxib, Sibutramine, Rimonabant, though approved by the regulatory agencies, were withdrawn from the market due to the appearance of side effects.

2C.2 Rationale of the Rimonabant molecule

Composition: 20 mg film coated tablet

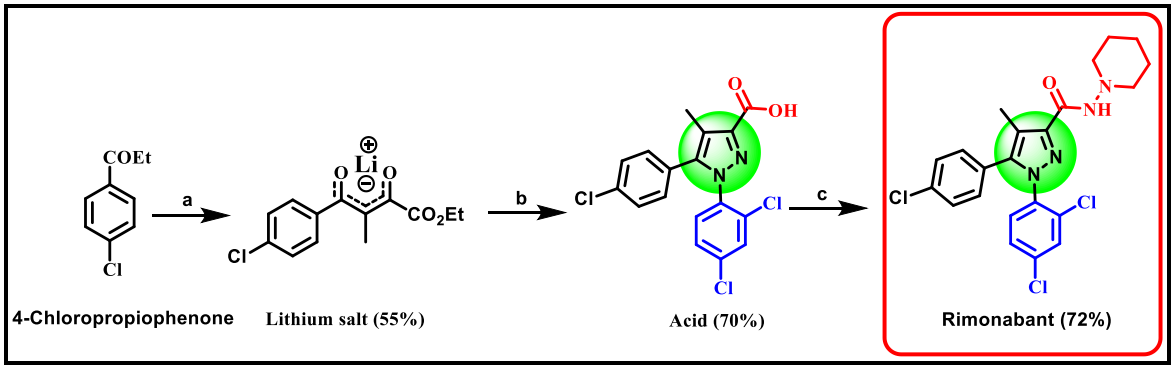
Rimonabant (JMG-1) (Figure 2C.1), is a pyrazole carboxylic acid derivative discovered by Sanofi-Synthelabo in 1994. Rimonabant is a first selective CB1 cannabinoid receptor antagonist. It was sold under trade names of Acomplia or Zimulti, as an anorectic anti-obesity drug, introduced by orally in body. Rimonabant and its analogues were also shown to have good activities and also proved safe to human.[7]

In 1994, Murielle, Rinaldi-Carmona[8] were the first to discover Rimonabant, also known as SR141716A, an antagonist of the cannabinoid receptor. There are many syntheses of Rimonabant reported in the literature with different solvents.



Scheme 2C.1: Reagents and conditions: *(a) LiHMDS, diethyl oxalate, ether, -78 °C, 17 h, 37%; Methylcyclohexane, 59%; (b) 2,4-Dichlorophenyl hydrazine hydrochloride, EtOH, 25 °C, 16 h; (c) AcOH, 118 °C, 24 h, 65%; (d) KOH, MeOH, 118 °C, 24 h, 98%; (e) $SOCl_2$, Toluene, 110 °C, 3 h, 87%; (f) 1-Aminopiperazine, NEt_3, H_2O, 0 °C, 3 h, 64%.*

We discuss them one by one. Starting from F. Barth,[9] design a synthetic methodology of Rimonabant molecule in 1997 from 4-chloropropiophenone. Firstly, chloropropiophenone on condensation with diethyl oxalate in the presence of LiHMDS gave lithium salt. In this reaction he used ether as a solvent and got 37% yield of lithium salt. After that there was an increase in the yield from 37% to 59% when ether was replaced with methylcycohexane. This on condensing with 2,4-dichlorophenyl hydrazine hydrochloride or phenyl hydrazine hydrochloride in ethanol gave hydrazone, which on refluxing in acetic acid for 24 h yielded ester **3** or **8**. Subsequent reaction of ester with potassium hydroxide and methanol at 118 ^{o}C for 24 h yielded 98% acid. These acids on subsequent treatment with thionyl chloride afforded acid chlorides. Finally, the amidation was executed to afford the target compound in 64% yield (Scheme 2C.1). This scheme involves long steps and afforded low overall yield (13.12%). In 2007, from that day forward Kotagiri[10] *et al* (Scheme 2C.1) improved the process of synthesis of Rimonabant. Initiated with 4-chloropropiophenone for the synthesis of Rimonabant and improved preparation of lithium salt by optimising temperature and solvent. By this change he obtained lithium salt in good yield in less time as compared to scheme 2C.1. Subsequent reaction of lithium salt with 2,4-dichlorophenyl hydrazine in 50 % sulfuric acid and ethanol at 79 ^{o}C for 14 h yielded acid in 70%. Finally, the acid was coupled with 1-aminopiperidine in the presence of DCC and HOBt to afford the Rimonabant in 72% yield (scheme 2C.2).



Scheme 2C.2 Reagents and conditions: *(a) LiHMDS, diethyl oxalate, methylcyclohexane, 25 ^{o}C, 6 h, 55%; (b) 2,4-Dichlorophenylhydrazine hydrochloride, 50% aq. H_2SO_4, ethanol, 79 ^{o}C, 14 h, 70%; (c) 1-Aminopiperidine, DCC, HOBt, DCM, 25 ^{o}C, 2.5 h, 72%.*

Thus, in scheme 2C.2 as compared to scheme 2C.1, no cryogenic temperature was required and the number of reaction steps were reduced from six to three with the increase in overall yield from 13.12% to 27.72%.

Rimonabant was recently withdrawn[11] from the market due to depression disorders among users. However, modification due to elimination of the side chain attached to carboxamide[12] (Figure 2C.2) is responsible for crossing the blood–brain barrier which might reduce the side effects. There are several important anti-TB drug candidates such as Pretomanid, Delamanid, TBA-354, AZD-5847 and BM-212 (see in chapter 2A).

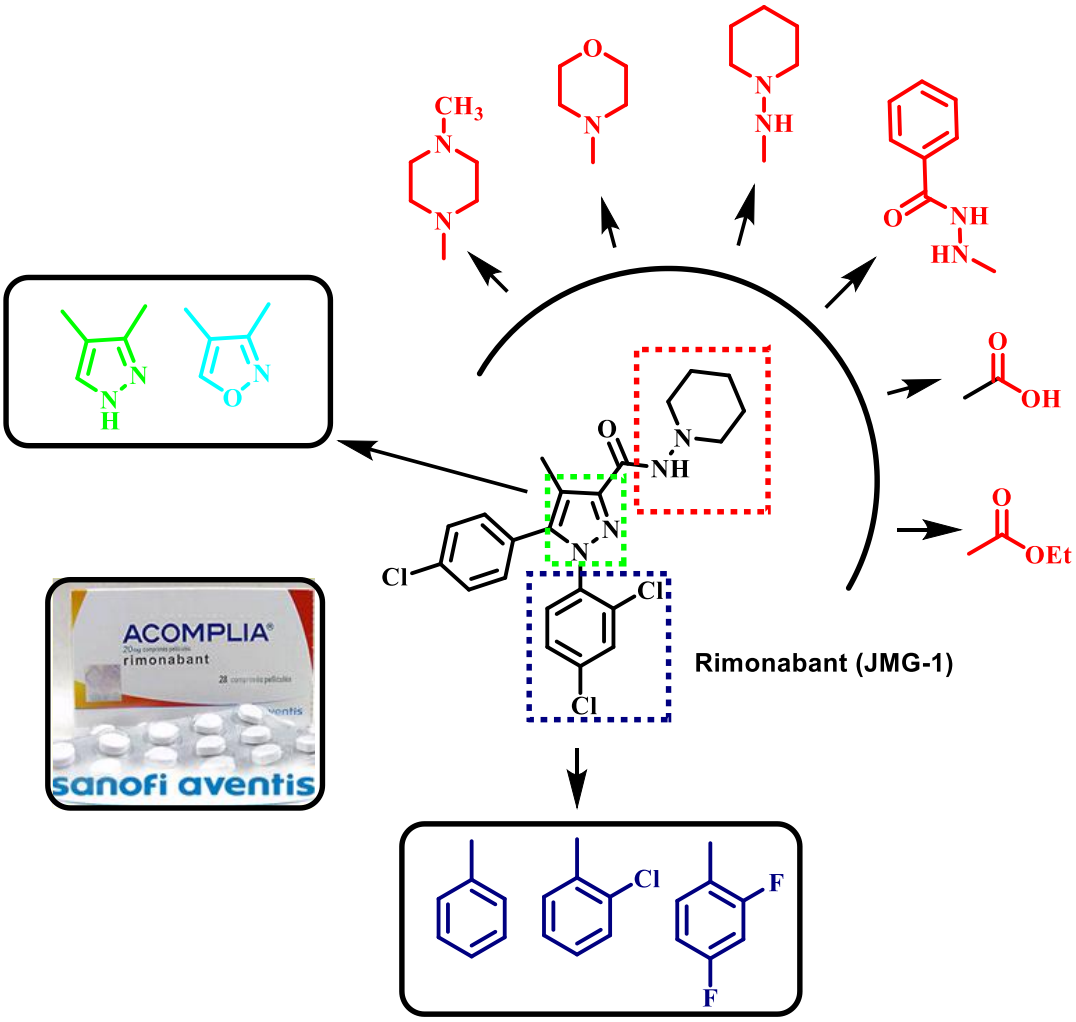


Figure 2C.2 Modification set of Rimonabant (JMG-1)

BM-212, (see in chapter 2B) which are structurally similar to Rimonabant (1), and hence, we were curious to study its anti-TB properties. All these observations prompted us to develop new Rimonabant analogues active against MDR TB.

2C.3 Chemistry

The synthesis of compounds **2**, **3**, **7**, **8**, **9**, **11**, **12**, and **13** was carried out as outlined in the Scheme 2C.3. 4-Chloropropiophenone (A) on condensation with diethyl oxalate in the presence of LiHMDS gave lithium salt (B). This on condensing with 2,4-dichlorophenyl hydrazine hydrochloride or phenyl hydrazine hydrochloride in ethanol gave hydrazones, which on refluxing in acetic acid for 24 h yielded esters **3** or **8**, respectively. Subsequent reaction of B with suitable hydrazines in 50 % sulfuric acid and ethanol at 79 ^{o}C for 14 h yielded desired acids (**2**, **7**, **9**, **11**, **12** and **13**). These acids on subsequent treatment with thionyl chloride afforded acid chlorides. Finally, the amidation was executed to afford the target compounds **1**, **4**, **5**, **6**, **10**, **14**, **15**, **16**, **17**, **18** and **19** in moderate to good yields (Schemes 2C.3-6).

2C.4 Pharmacology

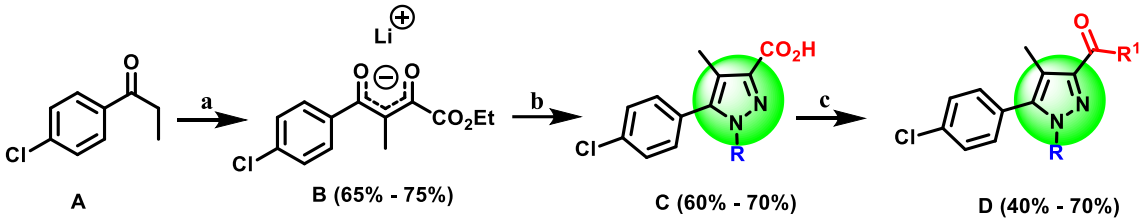
Anti-mycobacterial activity assay: Here M. smegmatis of MC2155 strain used for anti mycobacterial activity by agar dilution of growth inhibition assay followed by turbidimetry method. The assay was semi throughput and conducted in a 96-well plate (sterile). For M. tuberculosis, H37Rv strain was utilized by performing a growth inhibition assay from fresh Middlebrook 7H11 agar slants. This method is used to determine of MIC value in duplicate (NCCLS, 1995).

2C.5 Results and discussion

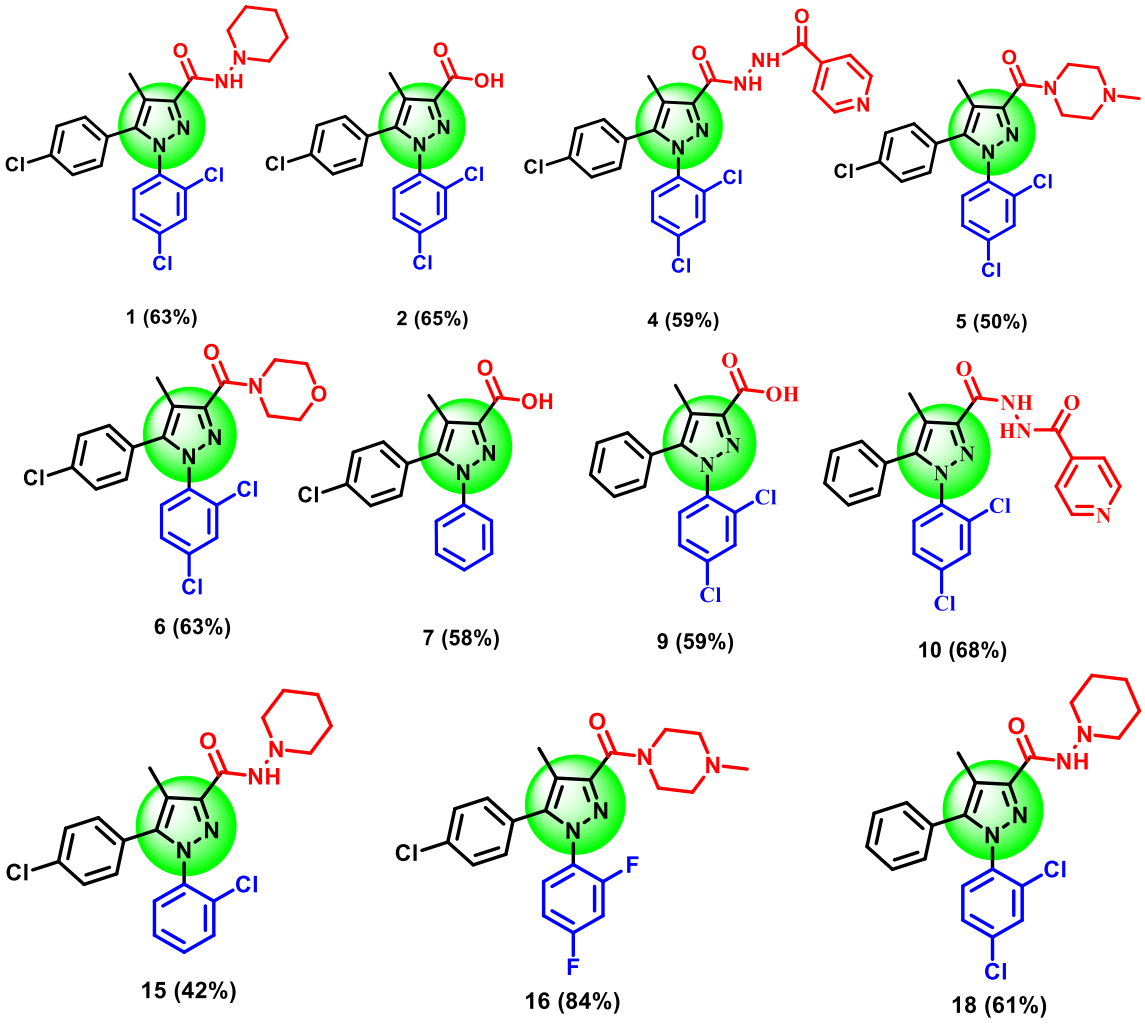
2C.5.1 Optimization of reaction condition

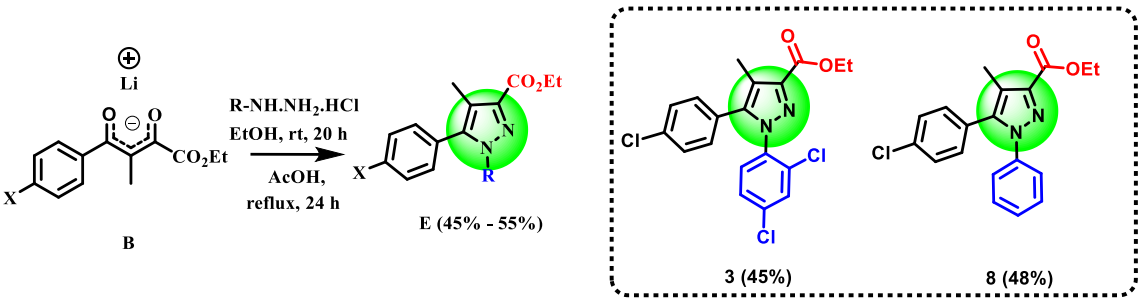
The synthesis of compounds **2**, **3**, **7**, **8**, **9**, **11**, **12**, and **13** was carried out as outlined in the Scheme 2C.3. 4-Chloropropiophenone (**A**) on condensation with diethyl oxalate in the presence of LiHMDS gave lithium salt (**B**). This on condensing with 2, 4-dichlorophenyl hydrazine hydrochloride and phenyl hydrazine hydrochloride in ethanol gave hydrazones, which on refluxing in acetic acid for 24 h yielded esters **3** and **8**, respectively (scheme 2C.4). Subsequent reaction of **B** with suitable hydrazines in 50 % sulfuric acid and ethanol at 79 ^{o}C for 14 h yielded desired acids (**2**, **7**, **9**, **11**, **12** and **13**).

These acids on subsequent treatment with thionyl chloride afforded acid chlorides. Finally, the amidation was executed to afford the target compounds **1**, **4**, **5**, **6**, **10**, **14**, **15**, **16**, **17**, **18** and **19** in moderate to good yields (scheme 2C.3).



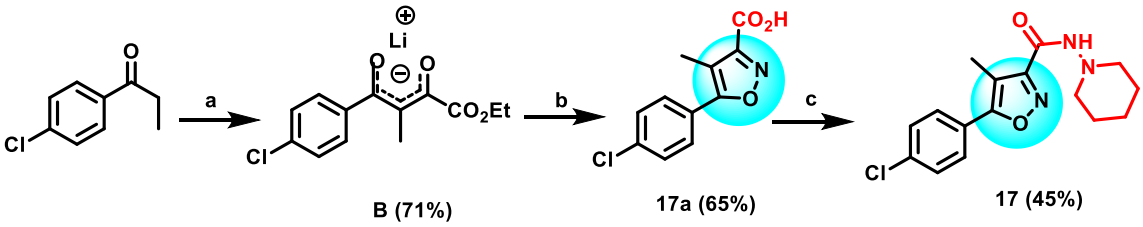
Scheme 2C.3: Reagents and conditions: *(a) LiHMDS, diethyl oxalate, methyl cyclohexane, 25 °C, 6 h, 65 - 75%; (b) Substitutated phenyl hydrazine hydrochloride, 50% aq. H_2SO_4, ethanol, 79 °C, 14 h, 60 - 70%; (c) (i) $SOCl_2$, cat. DMF, toluene, 100 °C, 4 h; (ii) 1-Aminopiperidine, NEt_3, 0 - 25 °C, 12 h, 40 - 70%.*



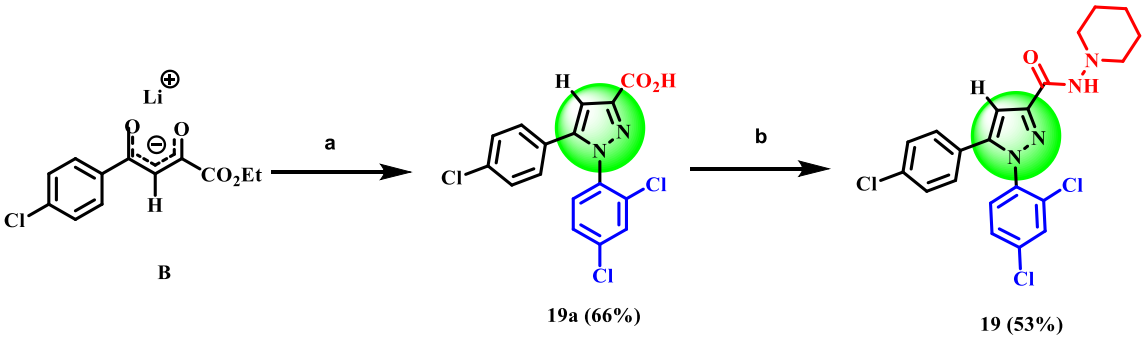


Condensing with R-NH.NH_2.HCl in ethanol gave hydrazones, which on refluxing in acetic acid yielded esters.

Scheme 2C.4: Reagents and conditions: *(a) Substitutated phenyl hydrazine hydrochloride, EtOH, rt, 20 h; (b) AcOH, 80 °C, 24 h, 45 - 55%.*



Scheme 2C.5: Reagents and conditions: *(a) LiHMDS, diethyl oxalate, methyl cyclohexane, 25 °C, 6 h, 71%; (b) $NH_2OH.HCl$, 50% aq. H_2SO_4, EtOH, 79 °C, 14 h, 65%; (c) (i) $SOCl_2$, cat. DMF, toluene, 100 °C, 4 h; (ii) 1-Aminopiperidine, NEt_3, 0 - 25 °C, 12 h, 45%.*



Scheme 2C.3: Reagents and conditions: *(a) 2,4-Dichlorophenyl hydrazine hydrochloride, 50% aq. H_2SO_4, EtOH, 79 °C, 14 h, 66%; (c) (i) $SOCl_2$, cat. DMF, toluene, 100 °C, 4 h; (ii) 1-Aminopiperidine, NEt_3, 0 °C - rt, 12 h, 53%.*

Our initial study involved screening of rimonabant (**1**) against *M. smegmatis*. Interestingly, it exhibited a MIC value of 13.56 compared to 16 µg/ml for isoniazid. Therefore, its 18 analogues were synthesized [13-14] and few of them were screened against M. smegmatis. They showed moderate to high activity (Table 2C.1). Encouraged by the results, rimonabant and all the synthesized analogues were then screened for *Mycobacterium tuberculosis* (virulent strain H37Rv) *in vitro*.[15] The details of the screening data are shown in Table 2C.1

The precursor of rimonabant acid 2 showed improved MTB activity and ester **3** retains the activity as compared to rimonabant. Similarly, the analogues **2**, **4**, **7**, **11** and **15**–**18** exhibited enhanced activity, and the analogues **3**, **5**, **6**, **8**, **10**, **12** and **19** were also found to be active. Most importantly, the analogues **9** and **13** showed very good MTB activity. The analogue JMG-14 came out to be a promising lead with highest activity. Compared

Table 2C.1 Screening of pyrazole based compounds against *M. smegmatis* and *M. tuberculosis*

S. No.	**CLog*P*[c]**	**% inhibition M. Smegmatis**	**MIC (µg/ml) M. TB[b]**	**S. No.**	**CLog*P*[c]**	**% inhibition M. Smegmatis**	**MIC (µg/ml) M. TB**
1	6.471	99.33 MIC 13.56 µg/ml	25	13	4.981	NT	6.25
2	6.121	22.84	12.5	14	5.031	99.70	3.13
3	7.083	14.07	25	15	5.754	NT	12.5
4	5.532	19.21	12.5	16	4.526	NT	12.5
5	5.666	14.50	25	17	3.342	NT	12.5
6	5.335	NT	>25	18	5.756	32.37	12.5
7	4.681	17.70	12.5	19	6.841	NT	25
8	5.234	22.70	25	Isoniazide		16 µg/ml	0.05
9	5.407	15.73	6.25	Rifampicin		2 µg/ml	0.1
10	4.818	1.362	25	Ethambutol		NT	3.25
11	3.035	NT	12.5	Pyrazinamide		NT	50.0
12	5.404	NT	25				

with ethambutol (MIC 3.25 µg/ml), JMG-14 molecule shows good activity. All 18 analogues compared with pyrazinamide, its MIC is 50.0 µg/ml and all molecules

observed moderate activity. Aanalogue **13** also emerged as another lead molecule, which can be explored further. Based on the analogue **9**, a simple replacement of chlorine by hydrogen might provide a more active analogue of **13**.

The main purpose of this study was to introduce modification on the side chain on carboxamide moiety of rimonabant and its analogues to develop new potential anti-TB agents. In the present work, diverse functional groups were introduced on the side chain of the carboxamide moiety of rimonabant to identify structure–activity relationships. It appears that the piperidinyl side chain is not a must for high activity and derivatives with free acids are better candidates for further evaluation. Variation of phenyl ring attached to nitrogen (of pyrazole moiety) shows prominent effect on the activity, and this observation is further vindicated by the docking analysis. The substitution on the ring or its complete removal, as well as replacement of that nitrogen with oxygen shows positive effect. Alteration on the *p*-chlorophenyl ring present on central pyrazole also influences the bioactivity. The methyl group seems to be important for the activity, since the analogue without methyl shows diminished activity.

2C.6 Scope of Rimonabant

Rimonabant (SR141716) was used for treatment of obesity, diabetes, promoting smoking cessation, reducing alcohol consumption and prevents the risk of cardiovascular disease.

2C.7 Molecular docking analysis

Molecular docking analysis was performed to determine the structural features that steer the biological profile of Rimonabant and its novel analogues. As stated earlier, the mechanism of action for anti-TB activity involves interaction of pyrazoles with MTCYP-121; therefore, molecular docking analysis was performed to determine the structural features that govern the anti-TB activity of present series molecules. The docking analysis was performed for all the compounds, but for the sake of convenience, we present the docking poses for compounds 1 and 14 as representatives. The active site of MT-CYP121, a heme-based enzyme that transfers a single oxygen atom to the substrate, comprises of Thr-77, Val-82, Val-83, Asn-85, Met-86, Ala-167, Ph-168, Thr-229, Ala-233, Ser-237, Gln-385 and Arg-386. The catalytic site is located on top of the distal side of the heme, where oxygen binds, near to I-helix.[16-18]

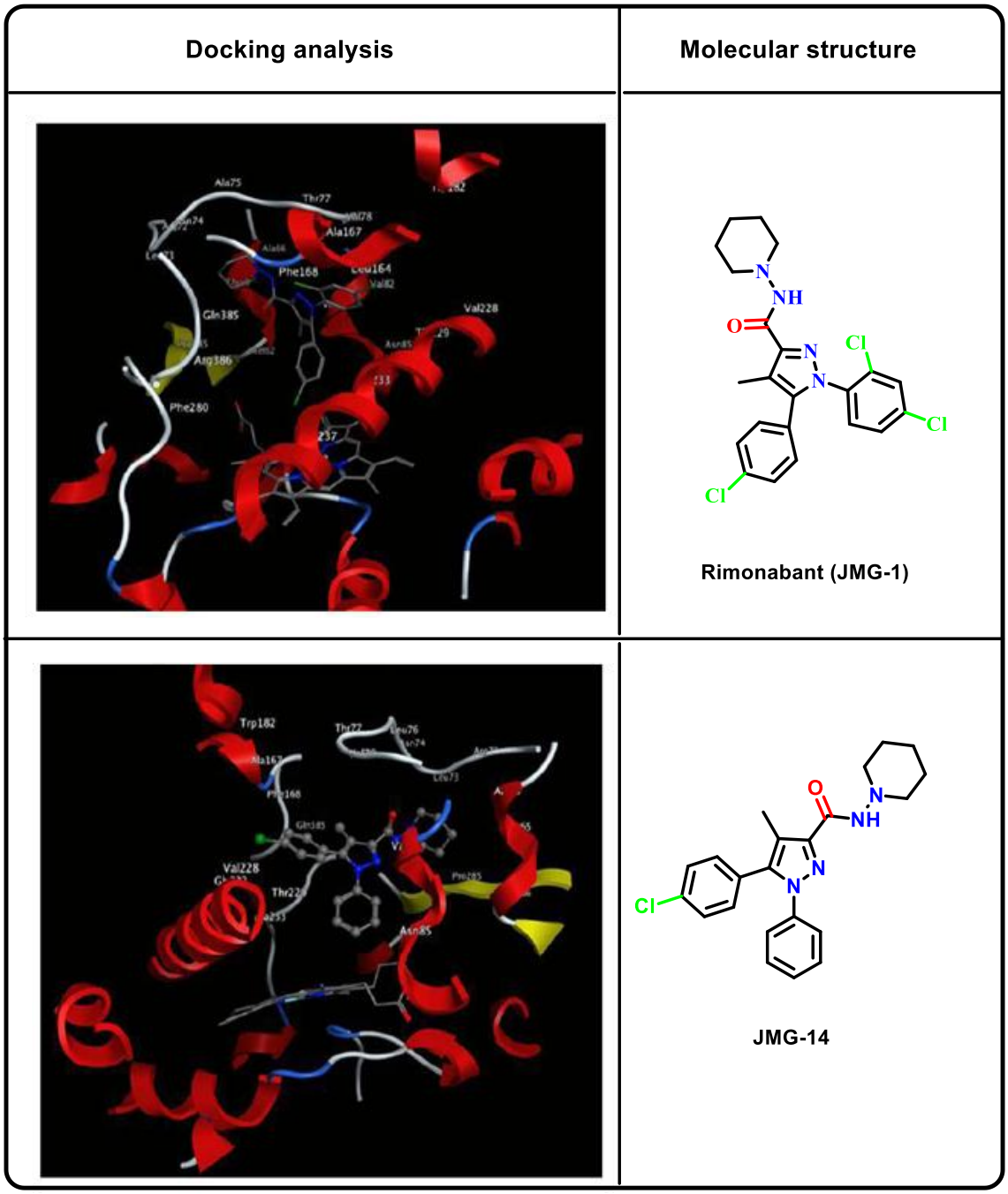


Figure 2C.3 Docking pose for a) rimonabant (JMG-1) and b) most active JMG-14

Furthermore, a strong H-bond between Arg-72 with N of piperidine ring enhances the anchoring of compound 1 with CYP-121. The presence of pyrazole moiety in close proximity to Gln-385 indicates that it plays a crucial role in inhibiting the normal functioning of CYP-121.

According to the results of our docking experiments (see Figure 3), the prominent interactions between compound **1** and the receptor are van der Waals, H-bonding and

hydrophobic in nature. Compound **1** could adopt bent 'T' shape (or weird propeller shape) while interacting with the cytochrome catalytic site (1350 $A^{\circ 3}$ in size) orienting chloro atom attached to benzene ring 1 towards the iron atom of the heme group with the heterocyclic rings (pyrazole and piperidine) pointing toward polar residues. Binding of compound **1** involves hydrophobic side chains of Leu-76, Val-78, Val83, Ala-167 and Phe-168.There are water-mediated H-bond networks with the N (non-substituted) of pyrazole ring and with N of amide group.

Compound **14**, like compound **1**, adopts bent 'T' shape while interacting with CYP-121, but interacts with more number of residues of the active site than the compound **1**. Ring 2 is closer to heme moiety with ring 1 pointing toward hydrophobic residues Val-78, Phe-168, Trp- 182 and Val-228. Another remarkable difference in interaction pattern of compounds **1** and **14** is due to the involvement of one and two water molecules, respectively, in enhancing the polar interactions with the receptor. The binding between the receptor and the substrate is enhanced by the water-mediated H-bonding between Thr-77 and the oxygen atom of amide group as well between Arg-72 and N atom (piperidine moiety).

A plausible reason for bent 'T' shape for compounds **1** and **14** is the presence of bulky Ser-237 in close proximity to phenyl ring attached to N atom of pyrazole moiety. Another reason could be the presence of unusually close other I-helix residues above the heme. Compounds **1** and **14** have adopted exactly opposite conformations while interacting with the receptor, and this could be attributed to the steric repulsion exerted by bulkier -Cl atoms present as substituents on the phenyl ring. Hudson *et al.* recently reported that the azoles do not coordinate with the heme iron, as is typically observed for the azole antifungal CYP inhibitors.[19] Our docking experiment also concurs with this. The N (d^-)/N–H (d^+) with a distance of 2.20 A° (depicted in Figure 2C.3), acting as polar pharmacophore, are responsible for establishing the polar contacts with the receptor.

2C.8 SAR studies

The molecular docking and structure–activity relationships (SAR) have been summarized in Figure 2C.4.

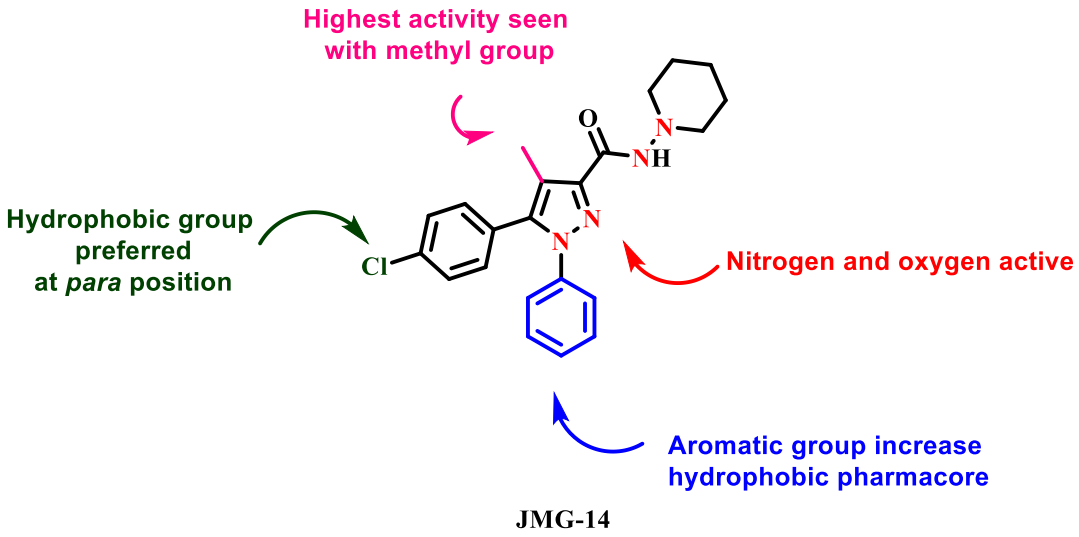


Figure 2C.4 SAR and molecular docking analysis for anti-TB activity of Rimonabant and its analogues

Further studies being carried out in our laboratory to explore the mechanism of action of this scaffold and evaluation of these compounds for anti-bactericidal activity as well as activity under alternate growth conditions such as non-replicating growth conditions.

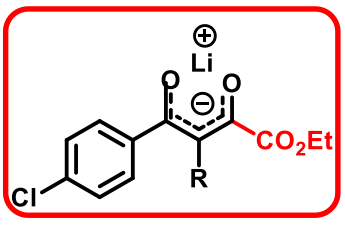
2C.9 Conclusion

For the first time, it has been shown that Rimonabant could be a lead molecule for finding more potent anti-TB drug candidates. In the present work, JMG-14 is the most potent analogue of Rimonabant, emerging as a suitable lead molecule, which can be further optimized using the SAR and molecular docking analyses. Currently, research work is in progress on the development of more rational analogues and their anti-TB activity.

2C.10 Experimental section

2C.10.1 Experimental procedures and characterization data

Lithium salt (B)



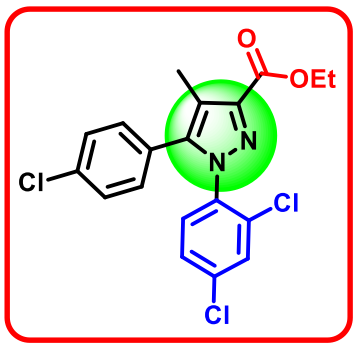
Methylcyclohexane (30 ml) and lithium hexamethyldisilazane (30 ml, 31.7 mmol) were introduced in a round-bottom flask under nitrogen atmosphere and cooled to 15 – 25 °C. A solution of 4-

chloropropiophenone (A, 5.0 g, 29.65 mmol) in methylcyclohexane (12.5 ml) was slowly added with continuous stirring for 2.5 h. To this reaction mixture, diethyl oxalate (4.78 g, 32.6 mmol) was added with stirring. The reaction progress was monitored by TLC; the solid obtained was filtered, washed with methylcyclohexane (30 ml) and dried under vacuum (30 min) to afford the lithium salt; Cream yellow solid; yield: 5.7 g, 71.9 %

General procedure for compounds 3 and 8

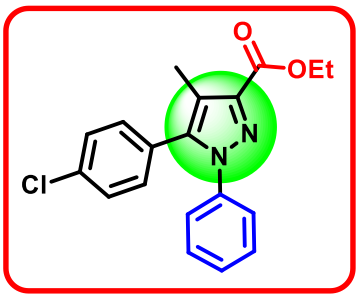
To a continuously stirring solution of lithium salt B (1.0 g, 3.64 mmol) in 15 ml of ethanol was added 2,4-dichlorophenyl hydrazine hydrochloride (0.777 g, 3.64 mmol) at room temperature. The shaking was carried for 20 h till precipitate was obtained. The precipitate so obtained was filtered and washed with ethanol (10 ml) and dried under vacuum to give a pale yellow solid (1.1 g), which was dissolved in acetic acid (10 ml) and refluxed for 24 h. On completion of the reaction (TLC), the reaction mixture was poured into cold water (20 ml) and then extracted with ethyl acetate (3:9:15 ml). The combined extracts were washed with water, saturated sodium bicarbonate solution, brine and then dried under vacuum to give the crude product. It was then purified by column chromatography on silica gel using ethyl acetate/*pet.* ether (1:9) as elution system to give the ester **3** and **8**

Ethyl5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxylate (3)



Yield: 670 mg, 45 %; ^{1}HNMR ($CDCl_3$, 200 MHz): δ 7.39 (d, *J* = 2.02 Hz, 1H), 7.34 (d, *J* = 1.37 Hz, 1H), 7.33 – 7.28 (m, 3H), 7.08 (d, *J* = 8.59 Hz, 2H), 4.51 – 4.41 (q, *J* = 7.08 Hz, 2H), 2.34 (s, 3H), 1.43 (t, *J* = 7.08 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.7, 142.9, 136.0, 135.9, 135.0, 133.0,130.9, 130.7, 130.1, 128.9, 127.7, 127.0, 119.1, 60.9, 14.4, 9.6; HRMS (ESI): *m/z* Calcd for $C_{19}H_1Cl_3N_2O_2$ $[M+H]^+$: 409.0272; Found: 409.0265.

1-(5-(4-Chlorophenyl)-4-methyl-1-phenyl-1-*H*-pyrazol-3-yl)propan-1-one (8)

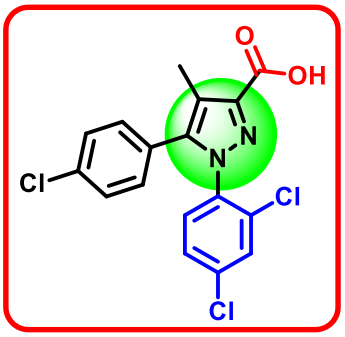


Pale orange solid; yield: 595 mg, 48 %; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31 – 7.36 (m, 5H), 7.24 – 7.27 (m, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 4.48 (q, *J* = 14.14 Hz, 2H), 2.33(s, 3H), 1.46 (t, *J* = 7.07 Hz, 3H); ^{13}C NMR ($CDCl_3$,100 MHz): δ 163.0, 142.3, 140.9, 139.3, 134.7, 131.3, 128.9, 128.0, 127.9, 127.0, 125.4, 120.0, 60.8, 14.4, 9.7; HRMS (ESI): *m/z* Calcd for $C_{19}H_{17}ClN_2NaO_2$ $[M+Na]^+$: 363.0871; Found: 363.0868.

General procedure for compounds 2, 7, 9, 11, 12, 13

A mixture of lithium salt of ethyl 4-(4-chlorophenyl)-3-methyl-4-oxydo2-oxobuten-3-oate (B, 1.0 g, 3.6 mmol), ethanol (25 ml), 2,4-dichlorophenyl hydrazine hydrochloride (0.777 g, 3.6 mmol), and 50 % sulfuric acid (10 ml) was refluxed for 6 h. After the reaction was complete (TLC), ethanol was removed under reduced pressure, and again, a second installment of 50 % sulfuric acid (20 ml) was added, followed by refluxing for 8 h. The reaction mixture was cooled to room temperature (35 ^{o}C) and was poured onto crushed ice, stirred for 15 min, filtered and washed with water (20 ml). The wet solid so obtained was stirred with water (30 ml), and the pH was adjusted to 10 with 20 % dil. NaOH. This aqueous layer was washed with petroleum ether. The aqueous layer was separated, cooled to 0 ^{o}C, and pH was adjusted to 2.0 by concentrated hydrochloric acid. Solid so obtained was filtered, washed with water (100 ml) and dried to afforded desired products.[21]

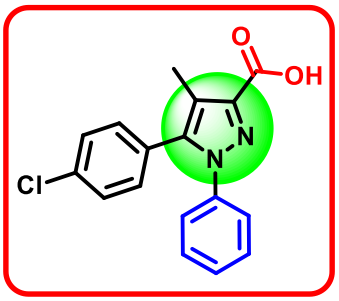
5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxylic acid (2)



Yield: 0.923 mg, 65 %; Off white solid, mp 208 – 209 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44 – 7.41 (m, 1H), 7.35 (d, *J* = 1.89 Hz, 1H), 7.33 – 7.28 (m, 3H), 7.09 (d, *J* = 8.47 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.4, 143.3, 136.2, 135.6, 135.1, 133.5, 130.8, 130.5, 129.8, 128.9, 127.8, 127.7, 126.7, 119.6, 9.6; HRMS (ESI): *m/z* Calcd for $C_{17}H_{11}Cl_3N_2NaO_2$

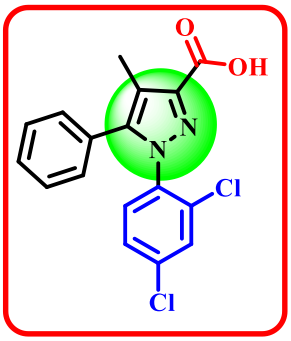
$[M+Na]^+$: 402.9781; Found: 402.9778.

5-(4-Chlorophenyl)-4-methyl-1-phenyl-1-*H*-pyrazole-3-carboxylic acid (7)



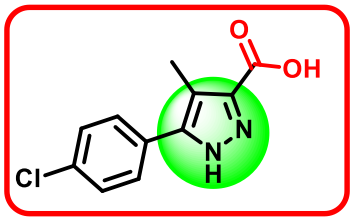
Pale Brown solid: yield: 719 mg, 58 %; mp = 203 – 204 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.46 (s, 1H), 7.32–7.38 (m, 5H), 7.30–7.20 (m, 2H), 7.13 (d, *J* = 6.36 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 165.9, 141.3, 140.9, 139.0, 134.9, 131.3, 129.0, 128.2, 127.6, 125.4, 125.1, 120.5, 9.6; HRMS (ESI): *m/z* Calcd for $C_{17}H_{13}ClN_2NaO_2$ $[M+Na]^+$: 335.0571; Found: 335.0566.

1-(2,4-dichlorophenyl)-4-methyl-5-phenyl-1-*H*-pyrazole-3-carboxylic acid (9)



Cream solid; yield: 865 mg, 59%; mp 188 – 189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46 (s, 1H), 7.16 – 7.27 (m, 5H), 7.08 (s, 2H), 6.02 (s, 1H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.2, 145.6, 136.0, 135.1, 132.2, 131.2, 130.8, 129.6, 129.1, 128.5, 128.3, 128.2, 127.8, 118.0, 9.7; HRMS (ESI): *m/z* Calcd for $C_{17}H_{12}Cl_3N_2NaO_2$ $[M+Na]^+$: 369.0168; Found: 369.0164.

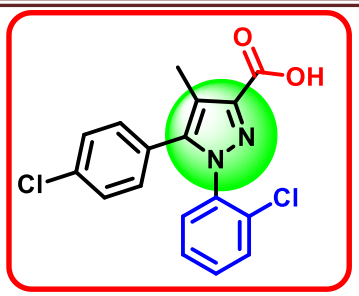
5-(4-chlorophenyl)-4-methyl-1-*H*-pyrazole3-carboxylic acid (11)



Cream solid; yield: 387 mg, 45 %; mp 288 – 290 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.54 (s, 1H), 7.89, (d, *J* = 8.24 Hz, 2H), 7.77 (d, *J* = 8.24 Hz, 2H), 2.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 162.4, 132.7, 130.9, 129.4, 128.9, 128.5, 117.0, 60.5, 9.9; HRMS (ESI): *m/z* Calcd for $C_{11}H_9ClN_2NaO_2$ $[M+Na]^+$: 259.0245; Found: 259.0243.

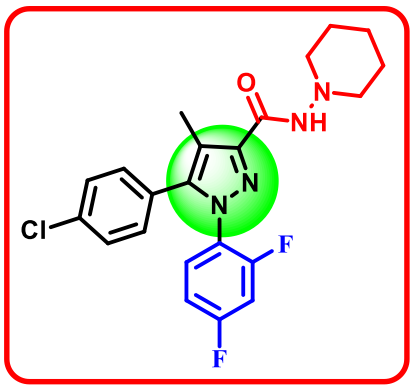
1-(2-chlorophenyl)-5-(4-chlorophenyl)-4methyl-1-*H*-pyrazole-3-carboxylic acid (12)

Pale yellow solid; yield: 656 mg, 52%; mp 204 – 206 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.33 – 7.42 (m, 4H), 7.26 (d, *J* = 6.41 Hz, 2H), 7.04 (d, *J* = 7.79 Hz, 2 H), 2.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.6, 143.3, 141.7, 136.9,



134.9, 131.9, 130.9, 130.3, 130.0, 129.8, 128.8 127.5, 126.9, 119.4, 9.6; HRMS (ESI): *m/z* Calcd for $C_{17}H_{12}Cl_2N_2NaO_2$ $[M+Na]^+$: 369.0169; Found: 369.0168.

5-(4-chlorophenyl)-1-(2,4-difluorophenyl)4-methyl-1-*H*-pyrazole-3-carboxylic acid (13)



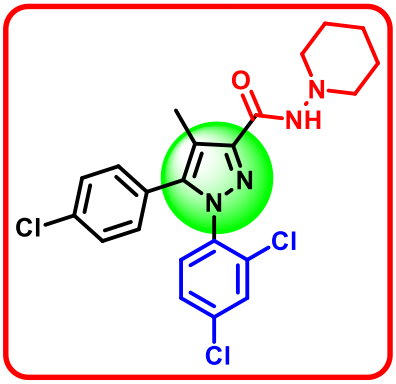
Pale brown solid; yield: 781 mg, 62%; mp 196 – 198 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.31 (bs, 1H), 7.44 (s, 1H), 7.27 (d, *J* = 8.46 Hz, 2H), 7.03 (d, *J* = 8.46 Hz, 2H), 6.87 (s, 1H), 6.72 (s, 1H), 2.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.7, 157.5, 155.5, 143.1, 135.0, 130.7, 130.0, 128.9, 127.0, 123.7, 119.5, 112.1, 111.9, 104.8, 9.6; HRMS (ESI): *m/z* Calcd for $C_{17}H_{11}ClFN_2NaO_2$ $[M+Na]^+$: 371.0370; Found: 371.0369.

General procedure for compounds 1, 4, 5, 6, 10, 14, 15, 16, 17, 18 and 19

To a stirred solution of 2 (382 mg; 1.0 mmol) in toluene (10 ml), one drop of dimethyl formamide was added. The reaction mixture was cooled to 0 °C and thionyl chloride (140 mg: 1.2 mmol) in 2 ml toluene was added dropwise for the period of 2 min at the same temperature. The reaction mixture was allowed to attain room temperature and heated at 100 °C for 4 h. Excess of thionyl chloride and toluene was distilled off under reduced pressure. In another flask under nitrogen atmosphere was introduced substituted amine (100 mg; 1.0 mmol) and triethyl amine (101 mg; 1.0 mmol) in 5.0 ml dichloromethane. The flask was cooled to 0 °C. To this was added a cooled solution of acid chloride dropwise at the same temperature. The resulting reaction mixture was allowed to attain room temperature, and then, it was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with water (10 ml) and organic layer was separated, washed with water (2, 9, 5 ml) and brine solution (5

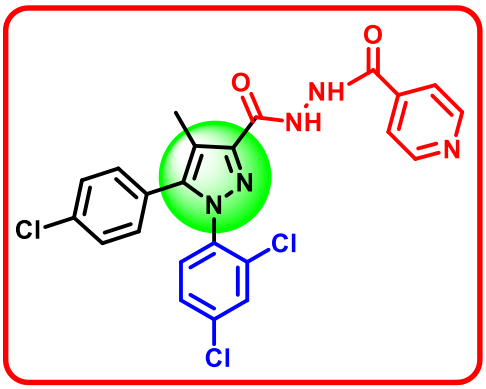
ml), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1-*H*-pyrazole-3-carboxamide (1)



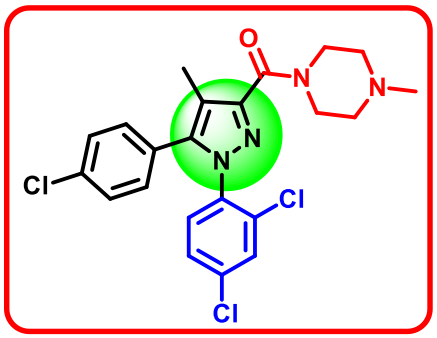
White solid; yield: 292 mg, 63%; mp 182 – 183 ^oC; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.63 (s, 1H), 7.41 – 7.36 (m, 1H), 7.30 – 7.26 (m, 3H), 7.04 (d, *J* = 8.28 Hz, 2H), 2.93 – 2.78 (m, 4H), 2.35 (s, 3H), 1.78 – 1.70 (m, 4H), 1.48 – 1.37 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.9, 144.4, 142.9, 136.0, 135.9, 134.9, 132.9, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 118.2, 57.0, 25.4, 23.3, 9.3; HRMS (ESI): *m/z* Calcd for $C_{22}H_{22}Cl_3N_4O$ $[M+H]^+$: 463.0854; Found: 463.0853

(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carbonyl) isonicotinohydrazide (4)



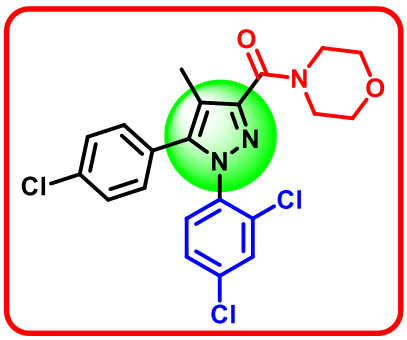
Off white solid; yield: 499 mg, 59%; mp 237 – 239 ^oC; ^1H NMR ($CDCl_3$, 400 MHz): δ 10.33 (s, 1H), 9.44 (s, 1H), 8.71 (s, 1H), 7.74 (s, 2H), 7.41 (s, 1H), 7.30 – 7.32 (m, 5H), 7.05 (d, *J* = 8.53 Hz, 2H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.5, 161.2, 150.2, 143.3, 142.4, 136.2, 135.6, 135.2, 132.7, 130.7, 130.5, 130.3, 128.9, 128.8, 127.9, 126.7, 118.4, 30.9, 9.2; HRMS (ESI): *m/z* Calcd for $C_{23}H_{16}Cl_3N_5O_2$ $[M+H]^+$: 500.0443; Found: 500.0442

(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl)(4-methylpiperazin-1-yl) methanone (5)



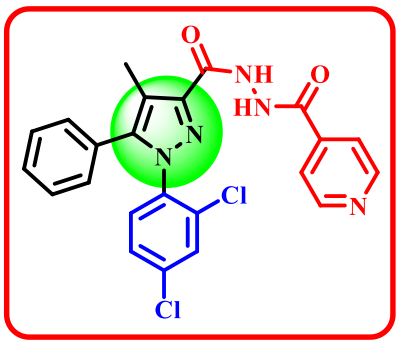
Brown solid; yield: 306 mg, 50%; mp 111 – 113 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (s, 1H), 7.31 – 7.34 (m, 3H), 7.20 (d, *J* = 8.54 Hz, 1H), 7.10 (d, *J* = 8.28 Hz, 2H), 3.99 – 3.80 (m, 4H), 2.61 – 2.45 (m, 4H), 2.37 (s, 3H), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.2, 146.4, 141.9, 135.9, 135.7, 134.8, 133.0, 130.6, 128.9, 127.8, 127.3, 116.8, 55.5, 54.7, 47.1, 45.9, 41.9, 9.0; HRMS (ESI): *m/z* Calcd for $C_{22}H_{21}Cl_3N_4O$ $[M+H]^+$: 463.0862; Found: 463.0854.

(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl) (morpholino) methanone (6)



Cream yellow solid; yield: 590 mg, 63%; mp 170 – 172 ^{o}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.47 (d, *J* = 2 Hz, 1H), 7.33 (d, *J* = 8.54 Hz, 2H), 7.27 – 7.30 (m, 1H), 7.19 (d, *J* = 8.55 Hz, 1H), 7.09 (d, *J* = 8.54 Hz, 2H), 3.85 –3.92 (m, 4H), 3.75 – 3.83 (m, 4H), 2.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.2, 146.0, 142.1, 135.9, 135.8, 134.9, 133.0, 130.6, 130.5, 130.3, 128.9, 127.8, 127.3, 117.2, 67.3, 66.9, 47.8, 42.6, 9.1; HRMS (ESI): *m/z* Calcd for $C_{21}H_{18}Cl_3N_3NaO_2$ $[M+Na]^+$: 472.0357; Found: 472.0355.

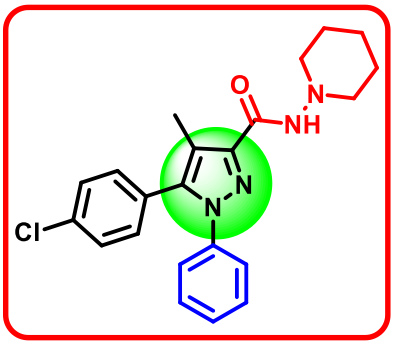
(1-(2,4-dichlorophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carbonyl) isonicotino hydrazide (10)



White solid; yield: 273 mg, 68%; mp 167 – 168 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.28 (bs, 1H), 9.43 (s, 1H), 8.71 (bs, 1H), 7.75 (s, 1H), 7.41 (d, *J* = 1.47, 2H), 7.28 – 7.34 (m, 5H), 7.14 (d, *J* = 4.40, 2H), 7.01 – 7.04 (m, 1H) 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.4, 161.2, 150.3, 144.4, 142.3, 138.8, 135.9, 135.8, 132.8, 130.5, 130.2,

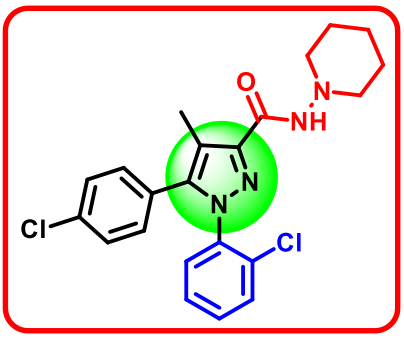
129.5, 128.9, 128.6, 128.2, 127.8, 121.2, 118.3, 9.3; HRMS (ESI): *m/z* Calcd for $C_{23}H_{17}Cl_2N_5O_2$ $[M+H]^+$: 466.0832; Found: 463.0830.

5-(4-chlorophenyl)-4-methyl-1-phenyl-*N*-(piperidin-1-yl)1*H*-pyrazole-3-carboxamide (14)



White solid; yield: 580 mg, 65%; mp 201 – 202 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (bs, 1H), 7.33 (d, *J* = 7.27 Hz, 5H), 7.21 (d, *J* = 7.78 Hz, 2H), 7.08 (d, *J* = 8.28 Hz, 2H), 2.98 – 2.79 (m, 4H), 2.35 (s, 3H), 1.85 – 1.68 (m, 4H), 1.52 – 1.38 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.1, 143.5, 140.9, 139.3, 134.6, 131.2, 129.0, 128.9, 128.0, 127.9, 125.0, 119.1, 57.1, 25.4, 23.3, 9.3; HRMS (ESI): *m/z* Calcd for $C_{22}H_{23}ClN_4O$ $[M+H]^+$: 395.1633; Found: 395.1631.

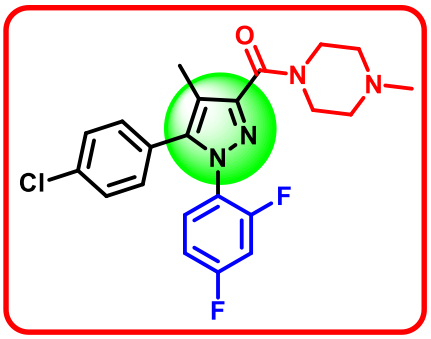
1-(2-chlorophenyl)-5-(4-chlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole -3-carboxamide (15)



Yellow solid; yield: 207 mg, 42%; mp 248 – 250 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.69 (bs, 1H), 7.37 – 7.30 (m, 1H),7.29 – 7.24 (m, 2H), 7.23 – 7.15 (m, 3H), 6.98 (d, *J* = 8.71 Hz, 2H), 2.95 – 2.72 (m, 4H), 2.30 (s, 3H), 1.78 – 1.61 (m, 4H), 1.45 – 1.29 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.3, 143.4, 141.1, 136.5, 135.0, 131.5, 130.9, 130.8, 130.3, 129.8, 128.8, 127.7, 126.6, 118.3, 55.7, 23.8, 21.1, 9.2; HRMS (ESI): *m/z* Calcd for $C_{22}H_{22}Cl_2N_4O$ $[M+H]^+$: 429.1246; Found: 429.1243.

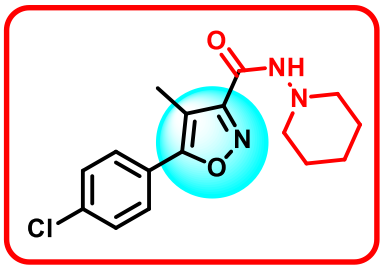
(5-(4-chlorophenyl)-1-(2,4-difluorophenyl)-4-methyl-1*H*-pyrazol-3-yl)(4-methylpiperazin-1-yl) methanone (16)

White solid; yield: 518 mg, 84%; mp 174 - 176 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.30 – 7.24 (m, 1H), 7.24 – 7.20 (m, 2H), 7.06 – 6.96 (m, 2H), 6.91 – 6.69 (m, 2H), 3.91 – 3.63 (m, 4H), 2.56 – 2.34 (m, 4H), 2.29 (s, 3H), 2.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 163.2, 146.6, 141.9, 134.8, 130.5, 129.7, 128.8, 127.4,



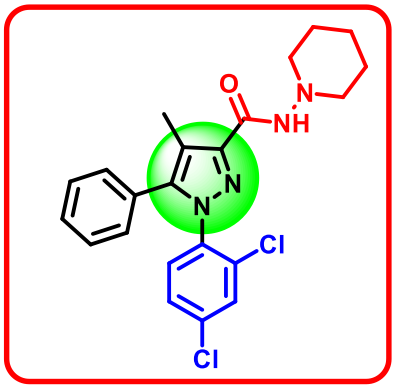
124.1, 116.8, 112.1, 111.6, 105.0, 104.5, 55.5, 54.7, 47.0, 45.9, 41.8, 8.9; HRMS (ESI): *m/z* Calcd for $C_{22}H_{21}ClF_2N_4O$ $[M+H]^+$: 431.1447; Found: 431.1445.

5-(4-chlorophenyl)-4-methyl-N-(piperidin-1-yl) isoxazole3-carboxamide (17)



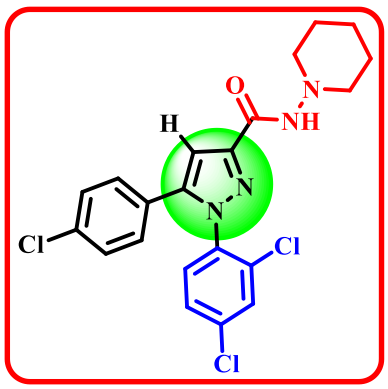
White solid; yield: 240 mg, 45%; mp 103 – 104 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.63 – 7.64 (d, *J* = 8.24 Hz, 1H), 7.57 – 7.58 (d, *J* = 8.24 Hz, 1H), 7.47 – 7.49 (d, *J* = 8.24 Hz, 2H), 2.99 – 2.81 (m, 4H), 2.45 (s, 3H), 1.85 – 1.71 (m, 4H), 1.53 – 1.41 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.8, 163.0, 157.3, 156.4, 136.1, 129.2, 126.0, 111.7, 56.9, 25.2, 23.1, 8.3; HRMS (ESI): *m/z* Calcd for $C_{16}H_{18}ClN_3O_2$ $[M+H]^+$: 320.1161; Found: 320.1160.

1-(2,4-dichlorophenyl)-4-methyl-5-phenyl-*N*-(piperidin-1yl)-1*H*-pyrazole-3-carboxamide (18)



Pale brown solid; yield: 288 mg, 61%; mp 172 – 173 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.72 (s, 1H), 7.41 (s, 1H), 7.27 – 7.32 (m, 5H), 7.11 (s, 2H), 3.05 – 2.75 (m, 4H), 2.38 (s, 3H), 1.91 – 1.65 (m, 4H), 1.54 – 1.34 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.1, 144.2, 144.0, 136.1, 135.7, 133.0, 130.6, 130.2, 129.5, 128.6, 128.6, 128.4, 127.7, 117.9, 57.0, 25.3, 23.2, 9.3; HRMS (ESI): *m/z* Calcd for $C_{22}H_{22}Cl_3N_4O$ $[M+H]^+$: 429.1244; Found: 429.1243.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)1*H*-pyrazole-3-carboxamide (19)



Cream solid; yield: 199 mg, 53%; mp 139 – 140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (d, *J* = 2.29 Hz, 1H), 7.57 (s, 1H), 7.55 (d, *J* = 2.29 Hz, 1H), 7.53 (d, *J* = 1.83 Hz, 1H), 7.42 (d, *J* = 9.15 Hz, 2H), 7.26 (d, 2H), 7.23 (s, 1H), 3.35 – 3.19 (m, 4H), 2.01 – 1.95 (m, 4H), 1.70–1.59 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.8, 146.8, 145.6, 136.3, 135.7, 135.1, 132.8, 130.5, 130.4, 129.1, 129.0, 128.2, 127.3, 107.5, 56.8, 24.9, 22.7; HRMS (ESI): *m/z* Calcd for $C_{21}H_{19}Cl_3N_4O$ $[M+H]^+$: 449.0701; Found: 449.0697.

2C.10.2 *In vitro* study

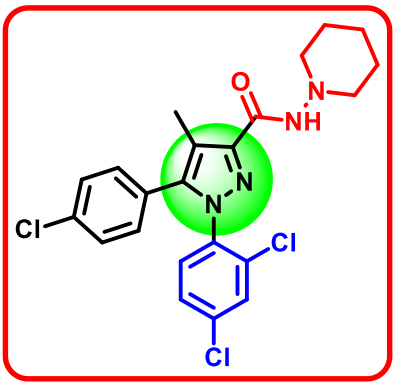
Isolated single colonies of *M. smegmatis* MC2155 (ATCC 14468) grown on 7H10 agar plate were developed overnight in Middlebrook 7H9 medium (0.47% Middlebrook 7H9 broth base, 10% ADS, 0.2% glycerol and 0.1% Tween 80) to mid-exponential phase at 37 °C. When the OD of this culture reached approximately 0.8, a secondary culture was inoculated in 5 ml Middlebrook 7H9 medium. The secondary culture was incubated overnight and allowed to grow at 37 °C to early log phase (OD600 & 0.3). For the anti-mycobacterial assay, 98 µl of 1:1000-folds dilution of the secondary culture was dispensed into 96-well microtiter plate per well along with 2 µl of test compound in triplicate, and 240 µl of sterile water was added to each well of the peripheral rows of 96-well plate to minimize media evaporation during assay incubation. The final concentration of the test compound in each well was 30 µM. Bacterial growth was assessed after 32 h of incubation by measuring turbidity at 600 nm OD600 values using TECAN Infinite 200 PROTM (Tecan Instruments, Switzerland). Depending upon the percentage of growth, the percentage of inhibition was calculated at a standard concentration of 30 µM. Isoniazid and rifampicin were included in every assay plate as positive controls of growth inhibition using stock solutions of INH (10 mg/ml, HiMedia) and rifampicin (10 mg/ml, HiMedia) to achieve the final concentration of 16 µg/ml for INH and 2 µg/ml for rifampicin. Additional controls DMSO (solvent without compound) and medium without inoculums were

included in all the assay plates avoiding intra-assay variability. The results were analyzed as the percentage of growth inhibition.

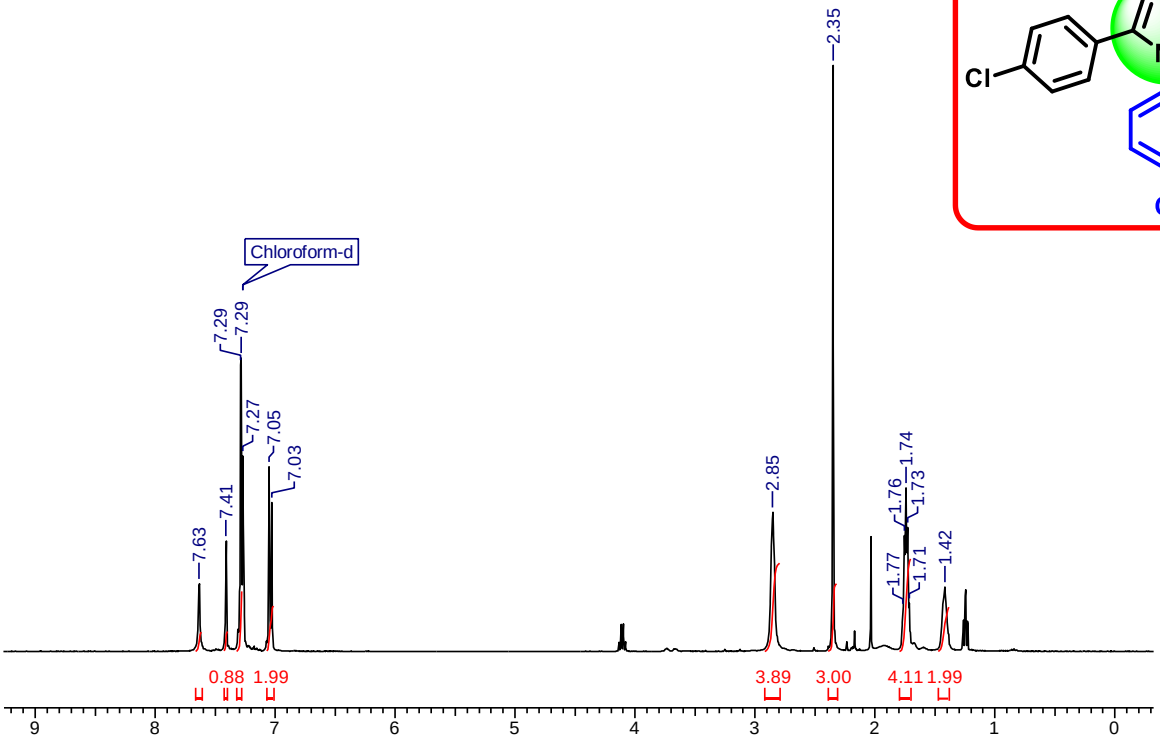
After the compounds were screened for percentage inhibition, the promising compounds were further screened to obtain the MIC values. Minimum inhibitory concentration (MIC) is the concentration of compound which inhibits the 90% growth of bacteria under optimum conditions. The growth inhibition assays were carried out in the same analogy as explained above using various concentrations of the test compounds prepared by serial dilutions 100, 50, 25, 12.5 and 6.25 µM to obtain the final concentrations of 46.37, 23.18, 11.59, 5.79 and 2.89 µg/ml, respectively. From the rate of inhibition bacterial growth, the as curtained MIC of the compound was calculated. The MIC value of the test compound 1 (rimonabant) is 13.56 µg/ml (29.24 lM ± 1.47).

In vitro anti-mycobacterial activity against *M. tuberculosis* H37Rv (MTB).[22-25] MTB H37Rv strain was obtained from National Institute for Research in Tuberculosis (NIRT), Chennai. Tenfold serial dilutions of each test compound were prepared and incorporated into Middlebrook 7H11 agar medium with OADC growth supplement. Inoculation of M. tuberculosis H37Rv ATCC 27294 (MTB) was prepared from fresh Middlebrook 7H11 slants with OADC growth supplement and was adjusted to 1 mg/ml (wet weight) in Tween 80 (0.05 %) saline diluted to 10 – 2 to achieve a concentration of ~107 cfu/ml (culture forming units). Then, a bacterial suspension of 5 µl was spotted in 7H11 agar tubes having tenfold serial dilutions of compounds per ml. Cultures were then incubated at 37 ^{o}C for 4 weeks. The MIC values of the synthesized compounds along with the standard drugs for comparison are tabulated in Table 1.

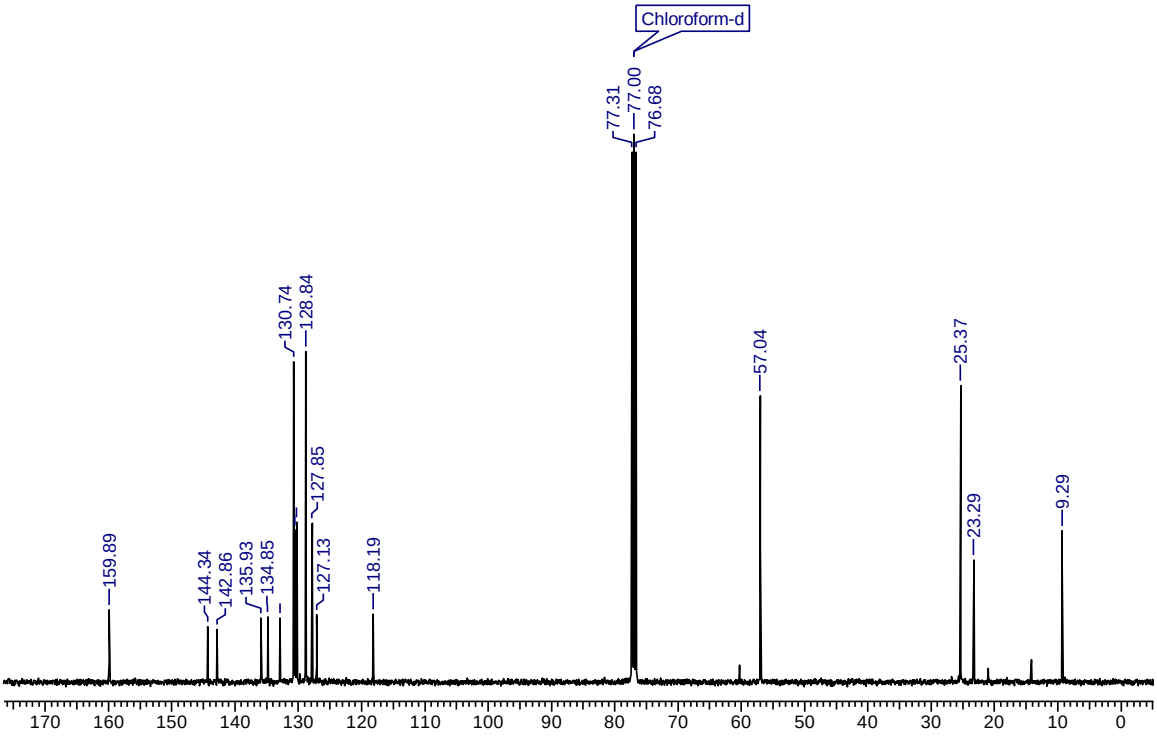
2C.10.3 Spectral data



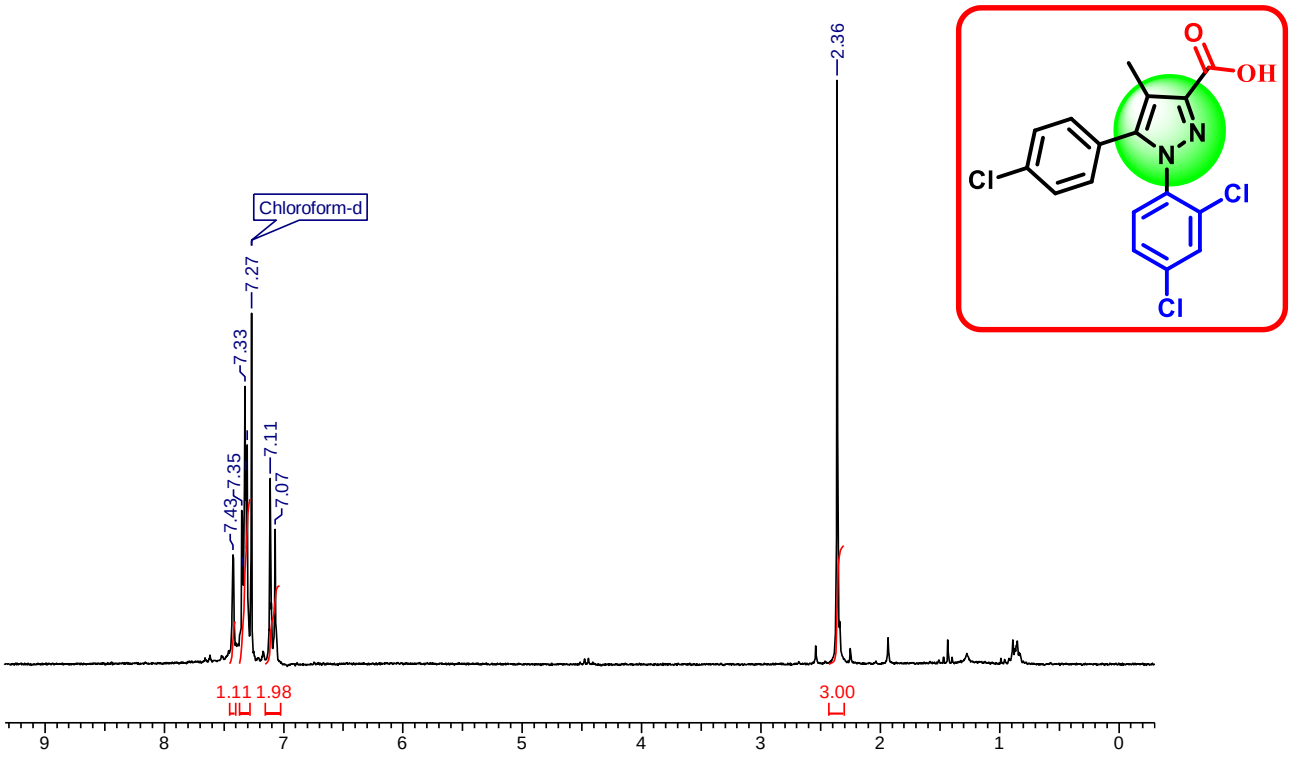
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-1)



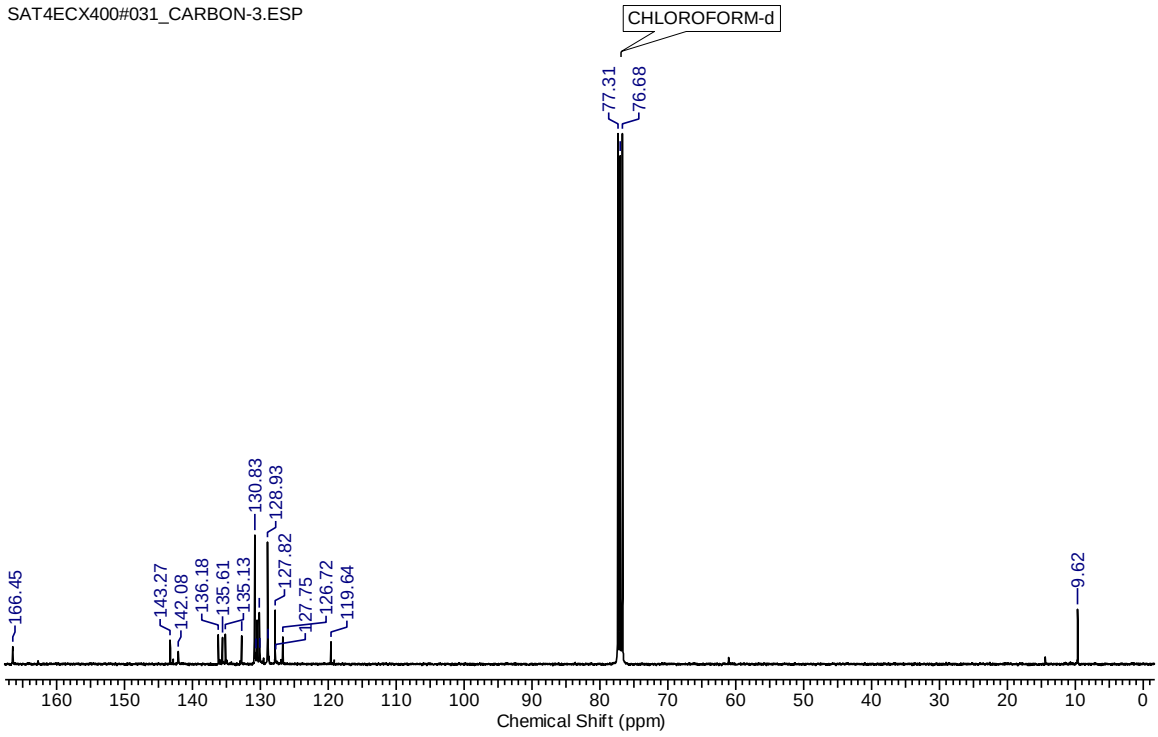
^{13}C NMR (100 MHz, $CDCl_3$)



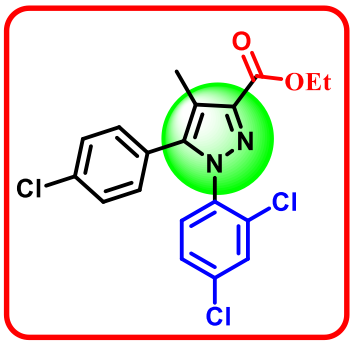
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-2)

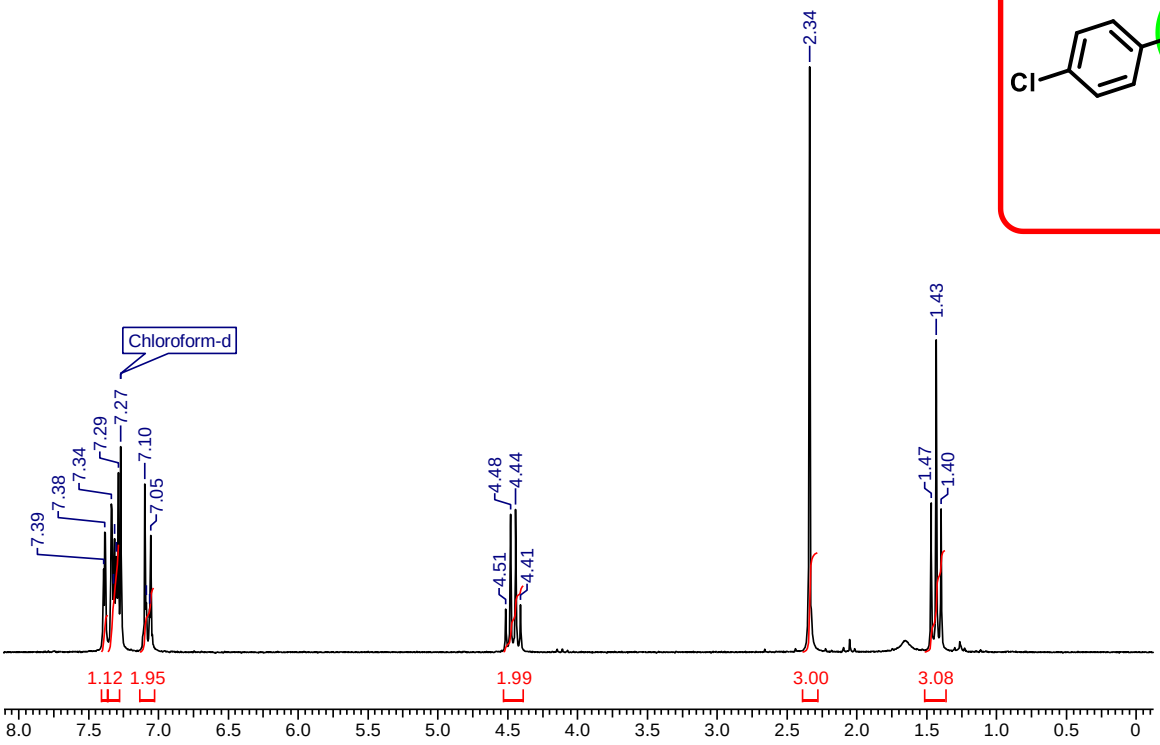


^{13}C NMR (100 MHz, $CDCl_3$)

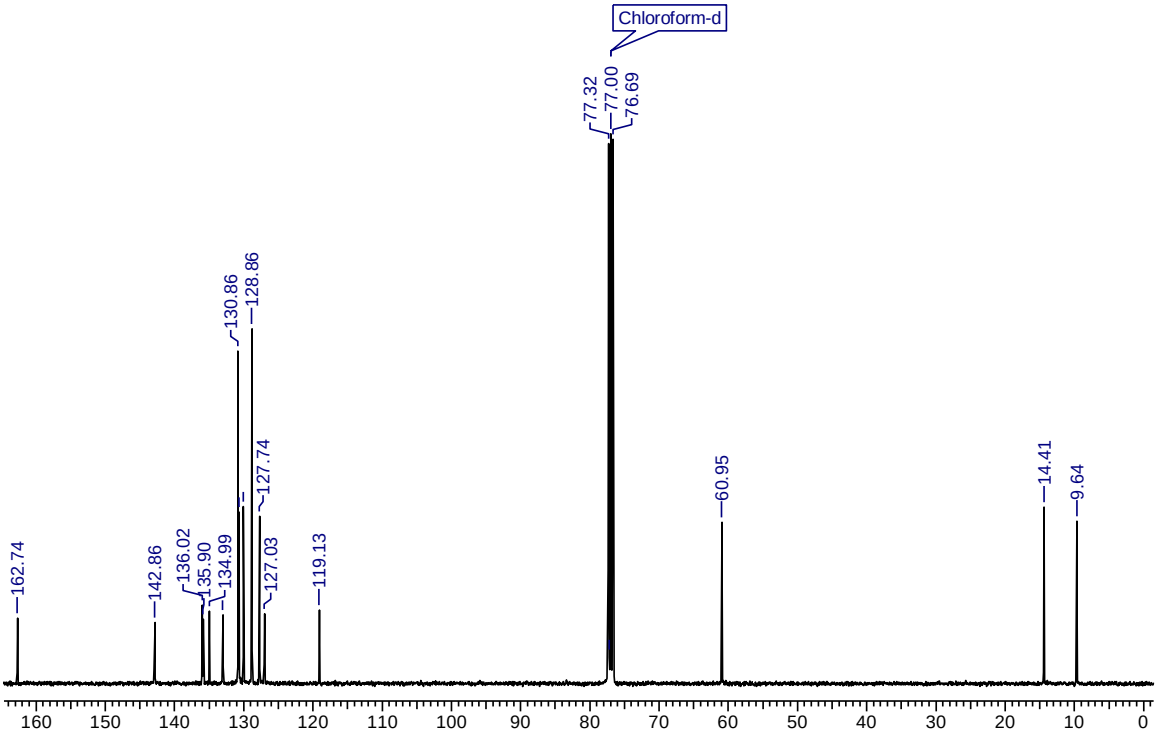


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-3)

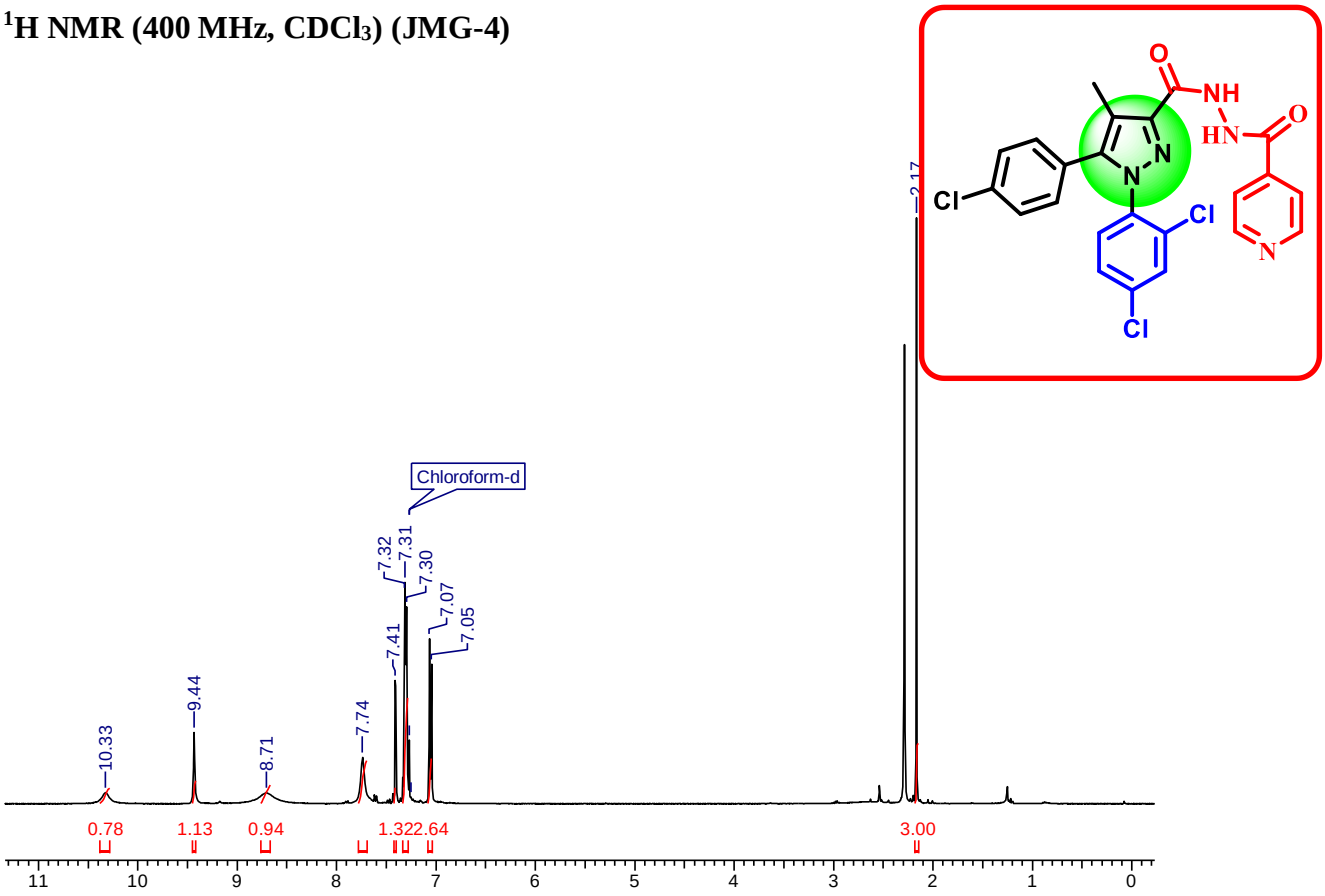




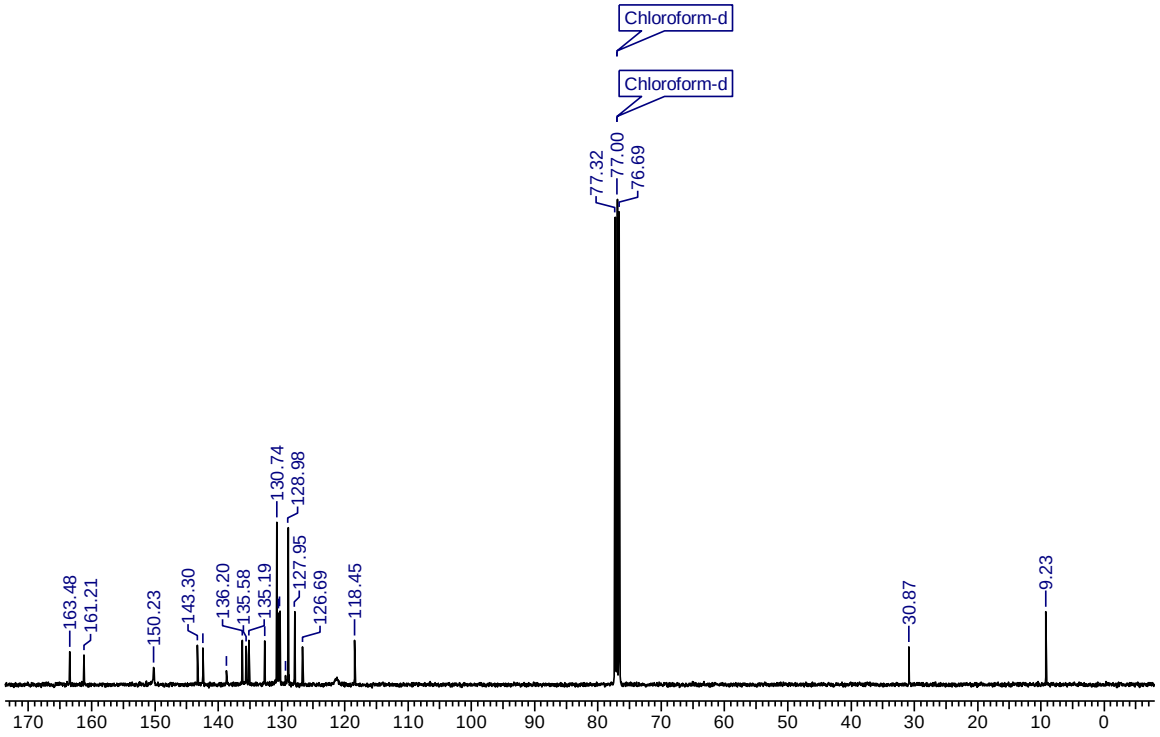
^{13}C NMR (100 MHz, $CDCl_3$)



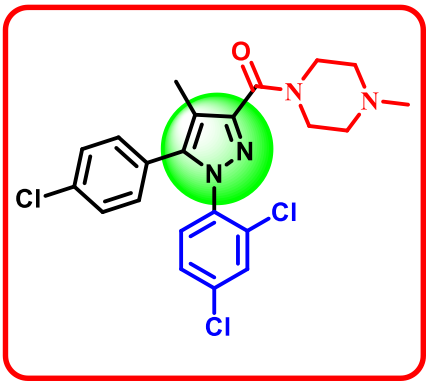
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-4)

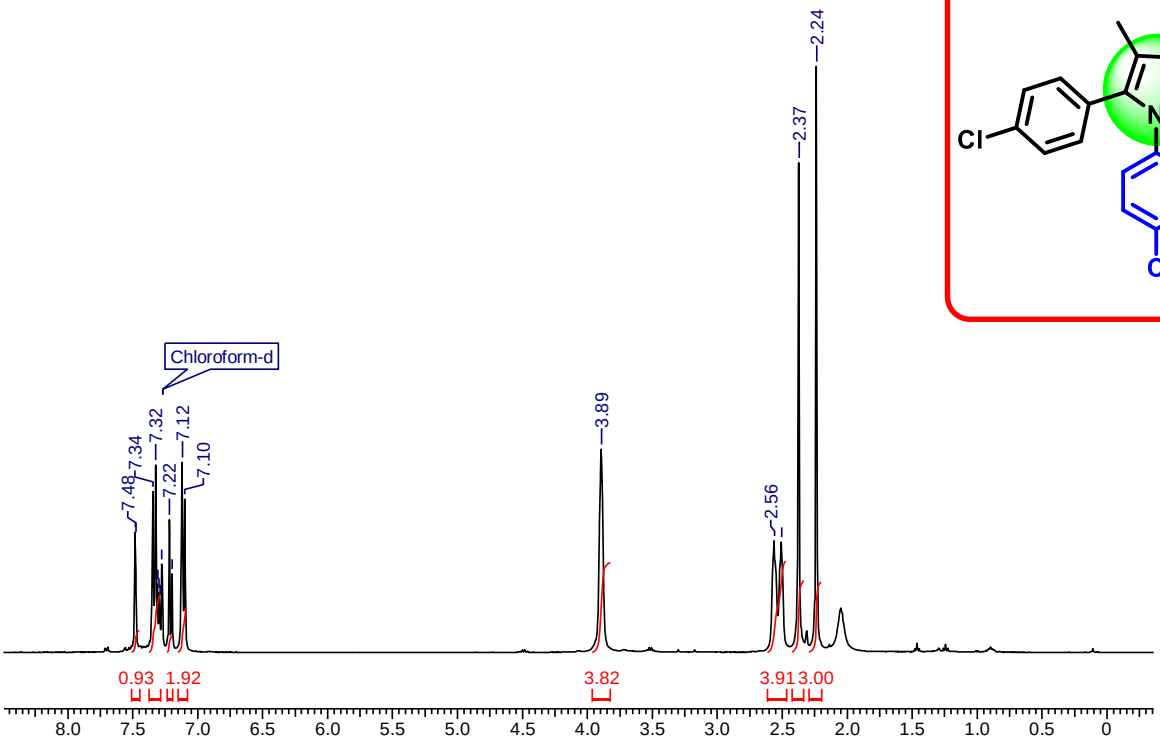


^{13}C NMR (100 MHz, $CDCl_3$)

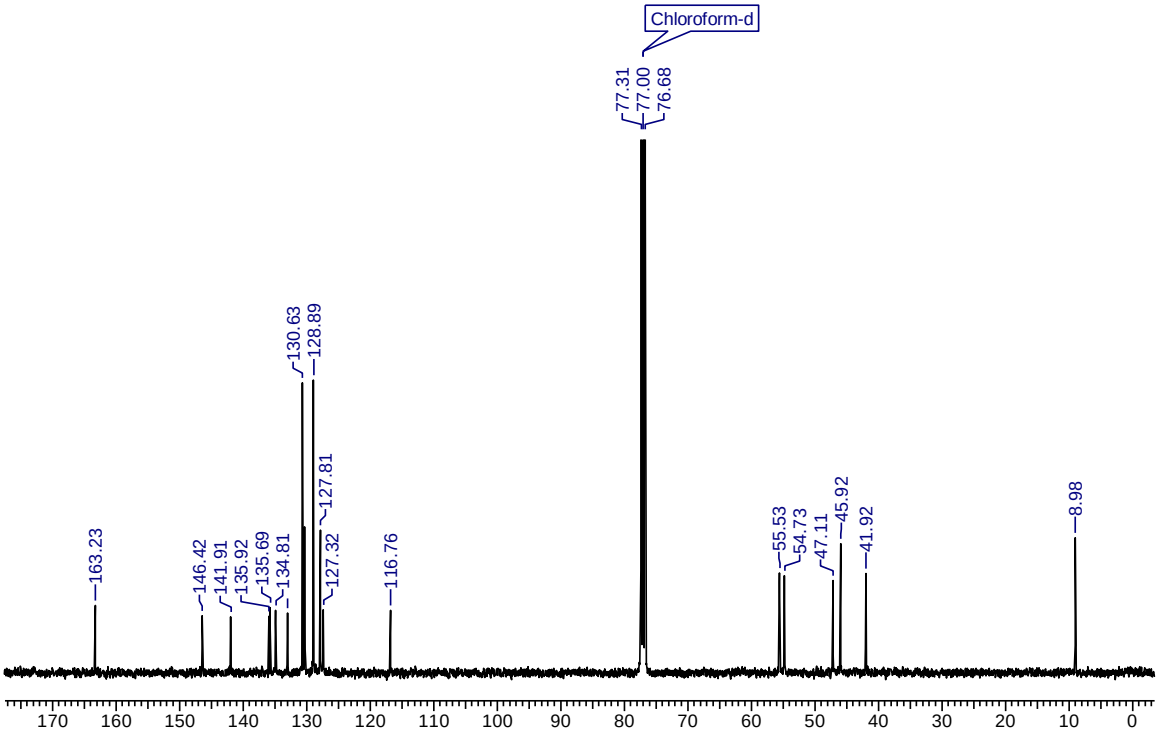


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-5)

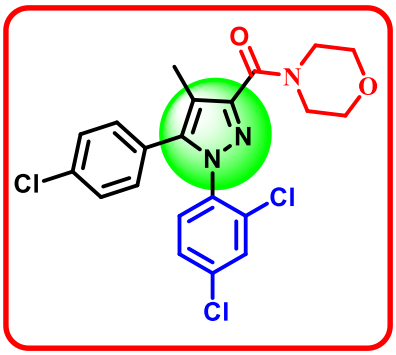


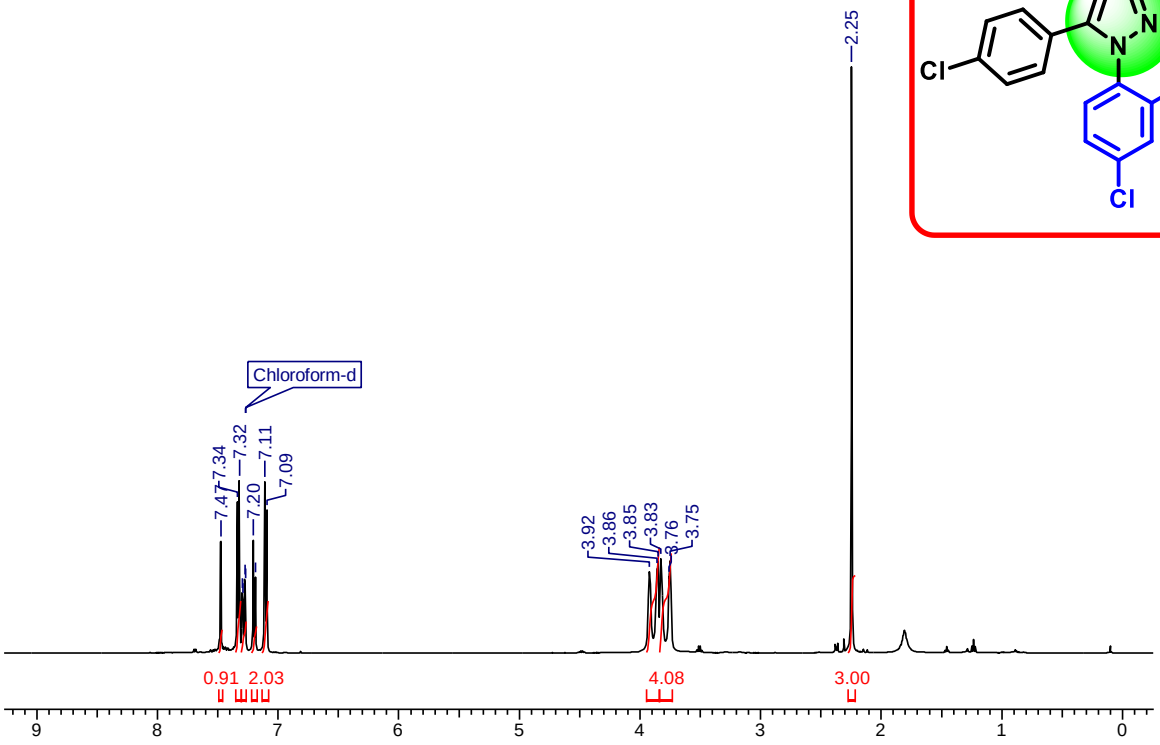


^{13}C NMR (100 MHz, $CDCl_3$)

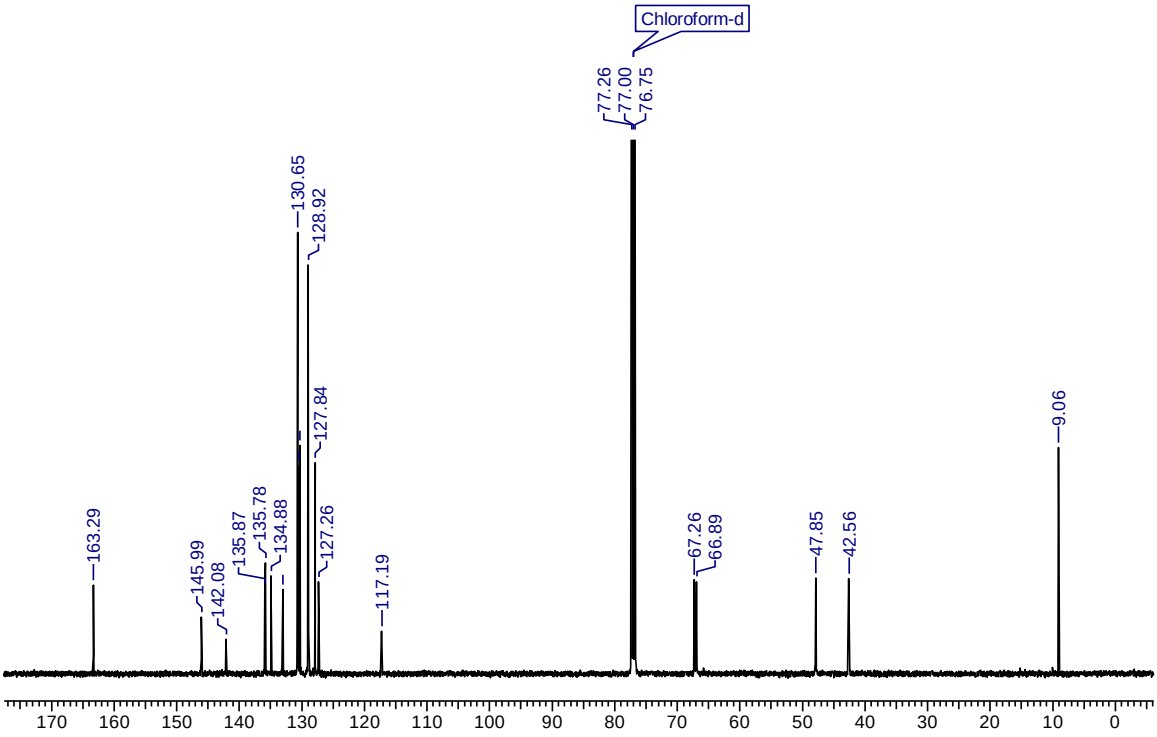


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-6)

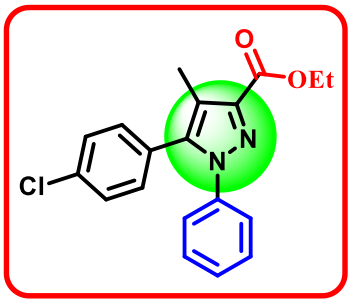


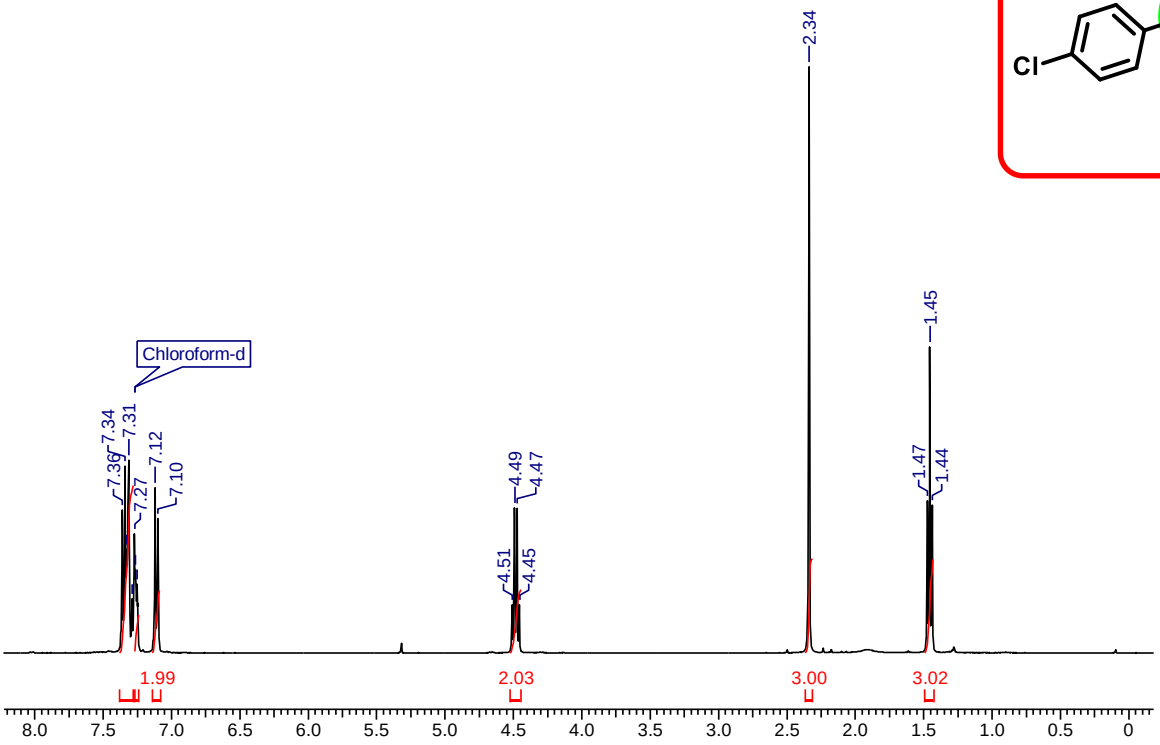


^{13}C NMR (100 MHz, $CDCl_3$)

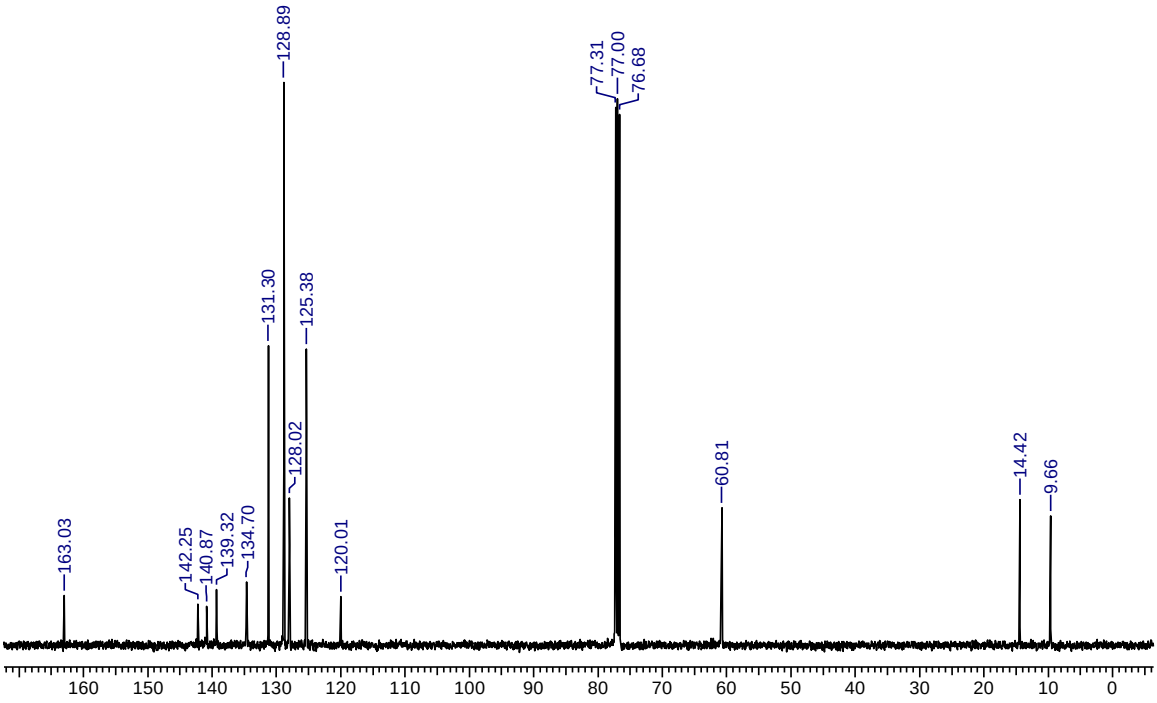


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-8)

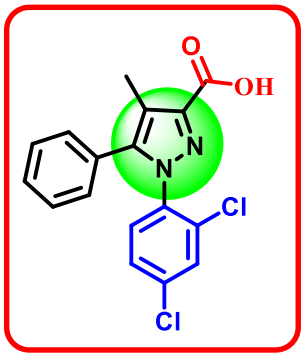




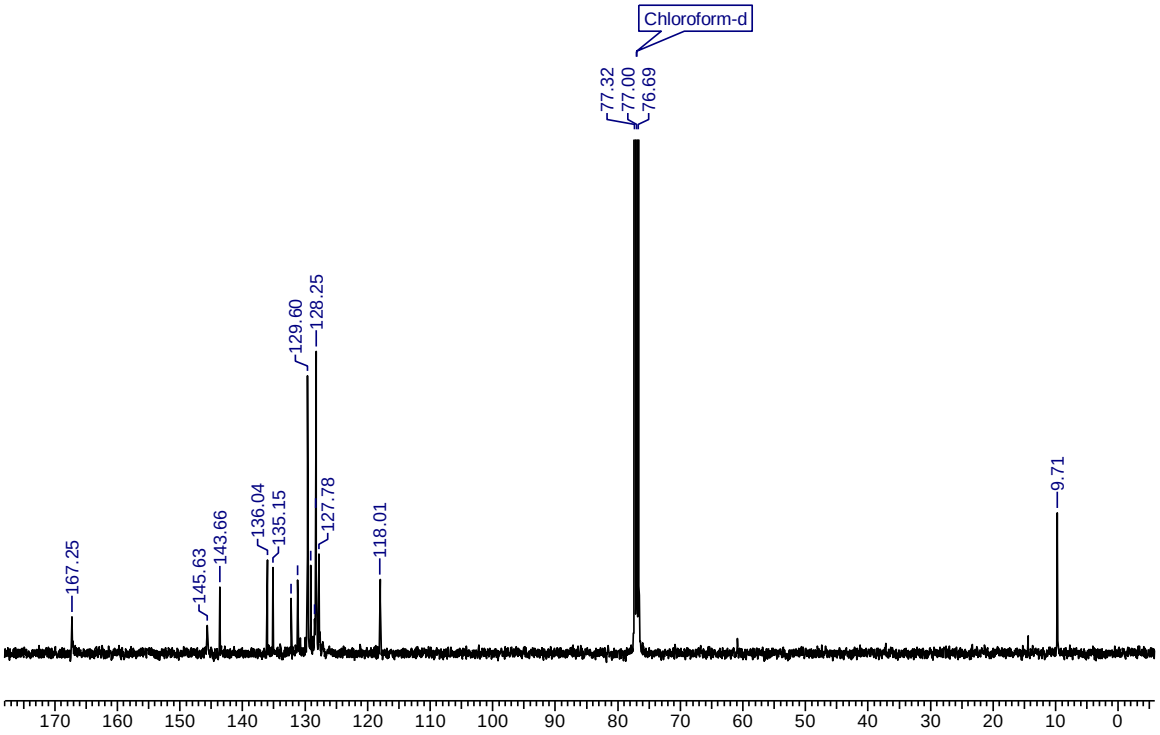
^{13}C NMR (100 MHz, $CDCl_3$)



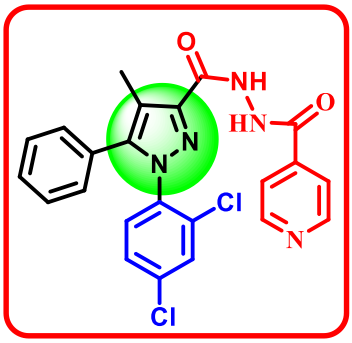
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-9)

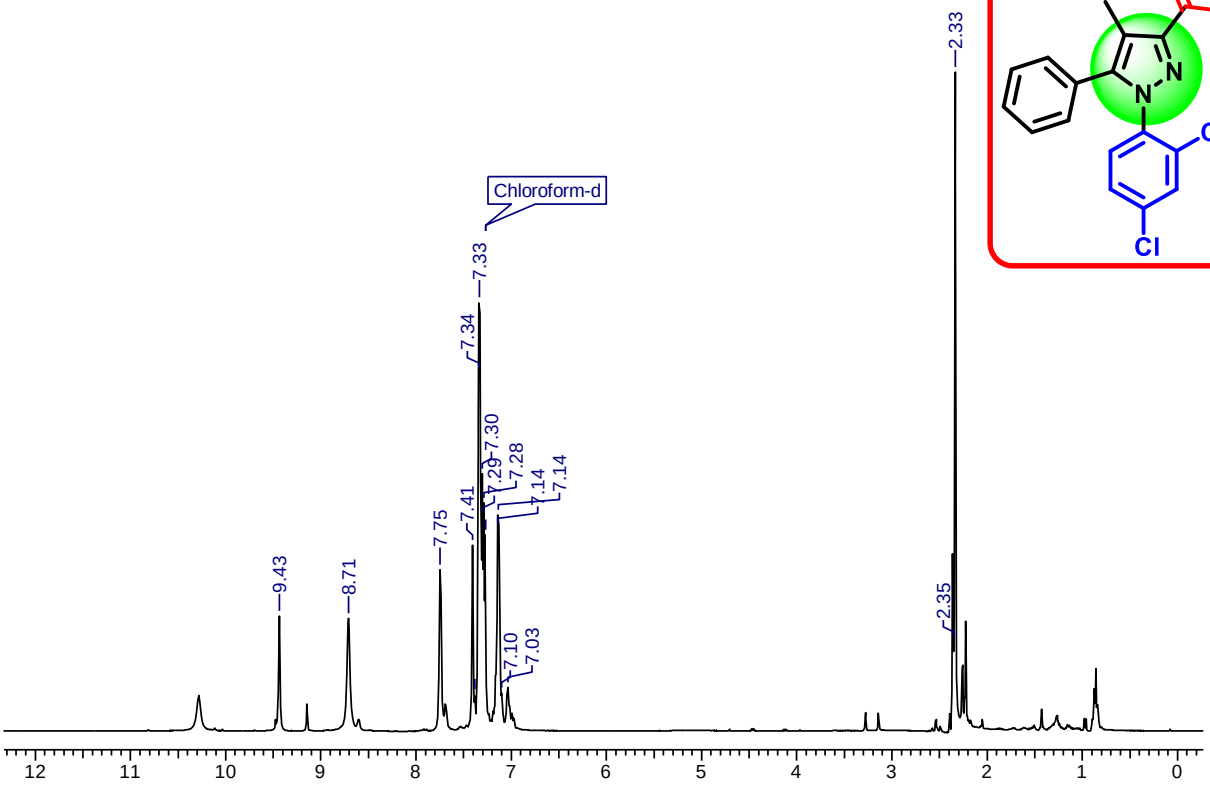


^{13}C NMR (100 MHz, $CDCl_3$)

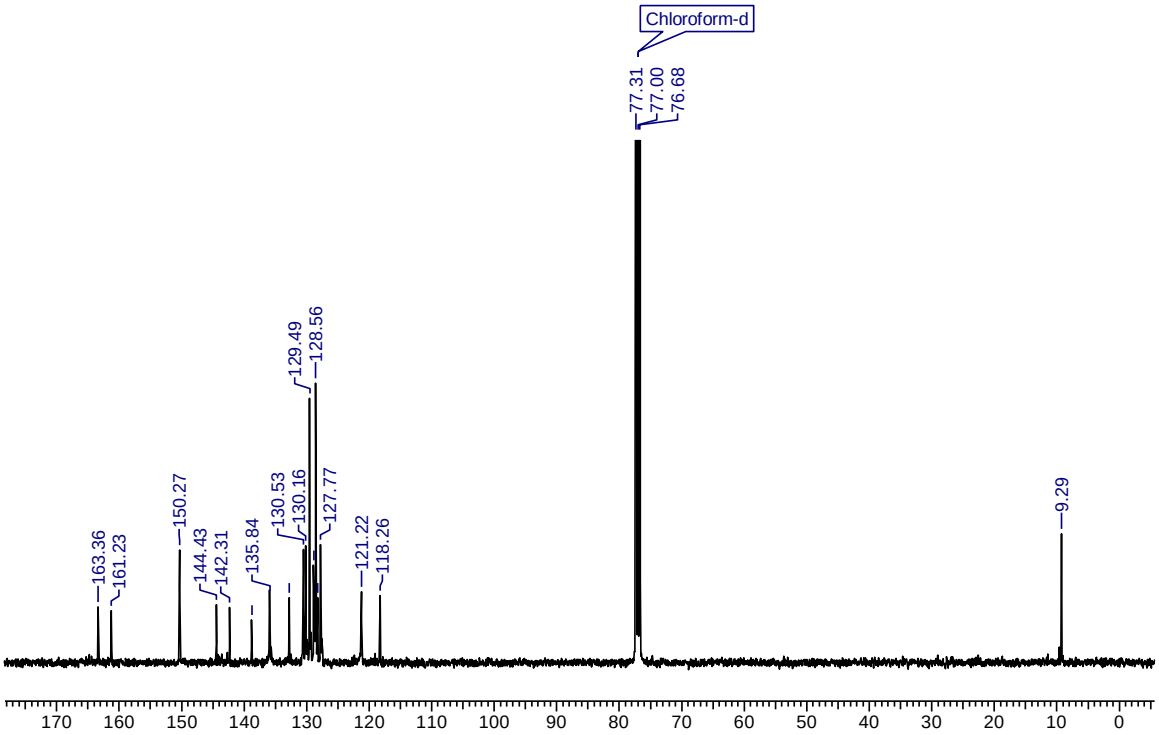


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-10)

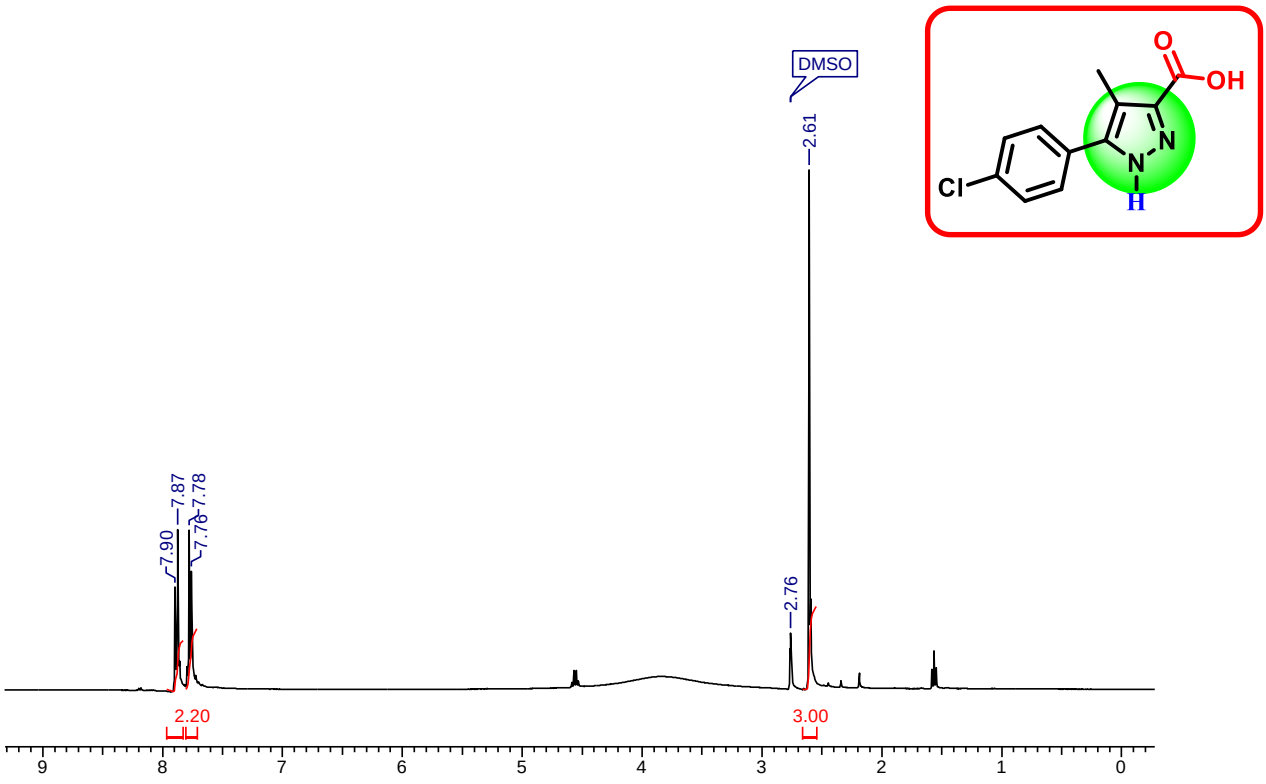




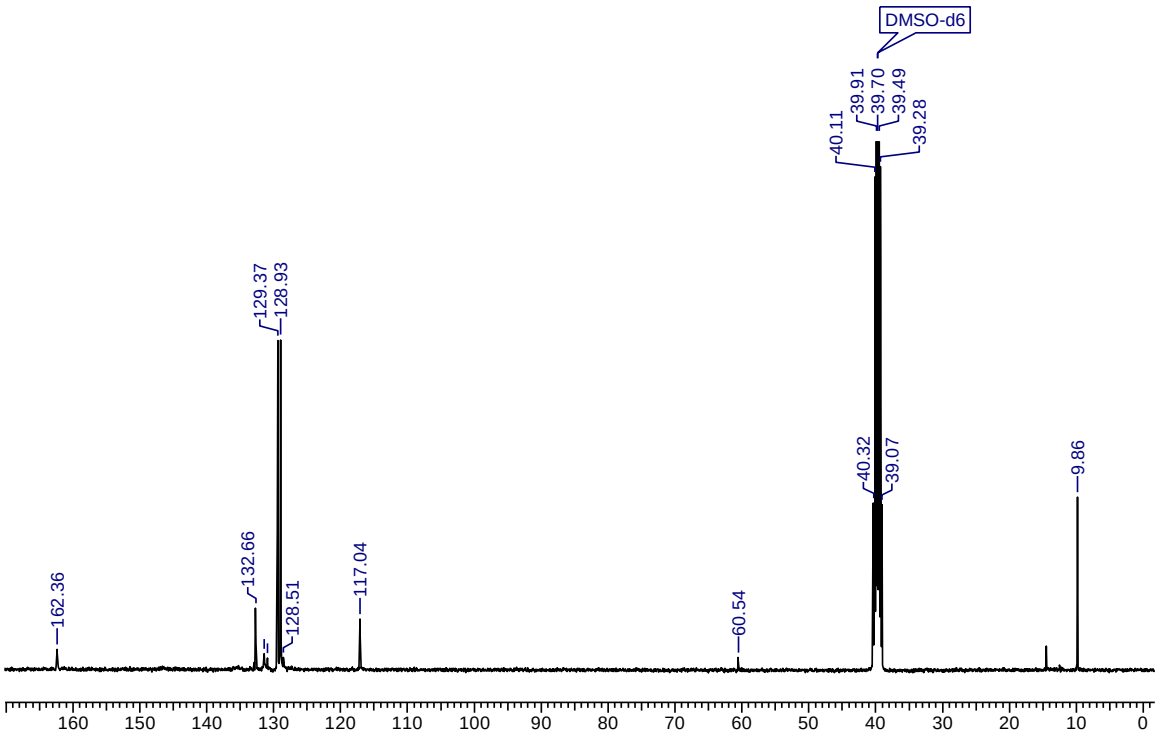
^{13}C NMR (100 MHz, $CDCl_3$)



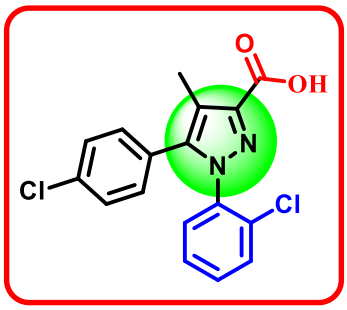
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-11)

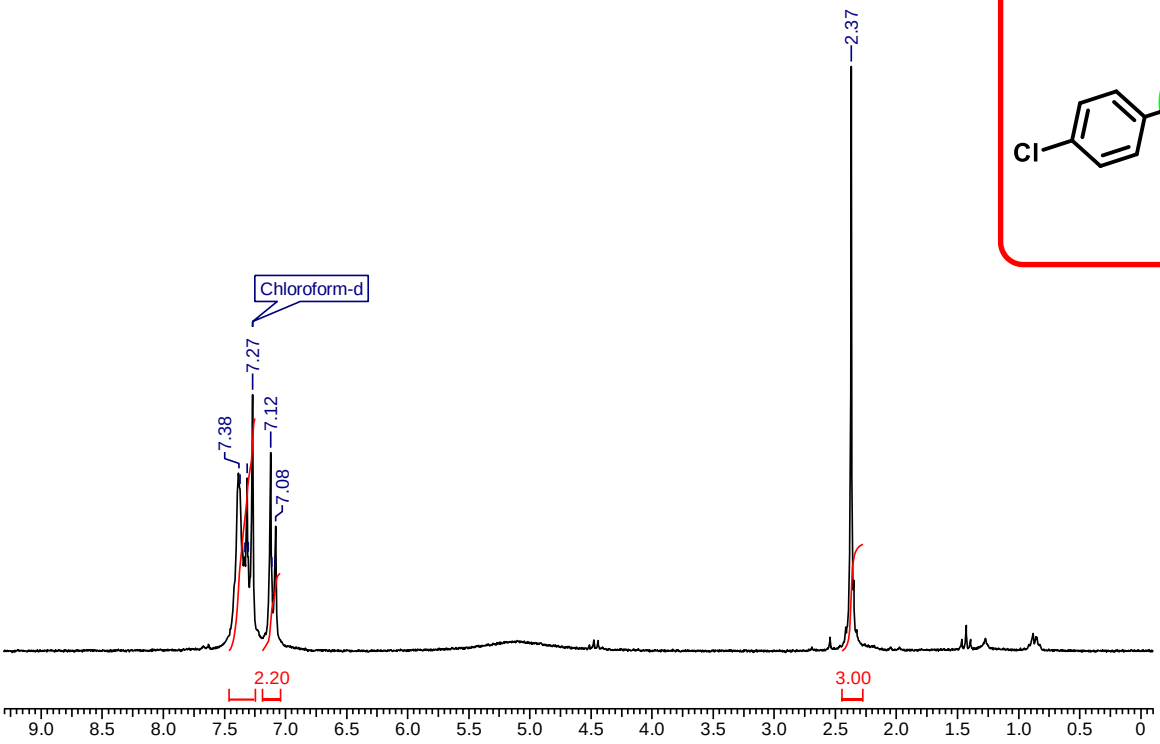


^{13}C NMR (100 MHz, $CDCl_3$)

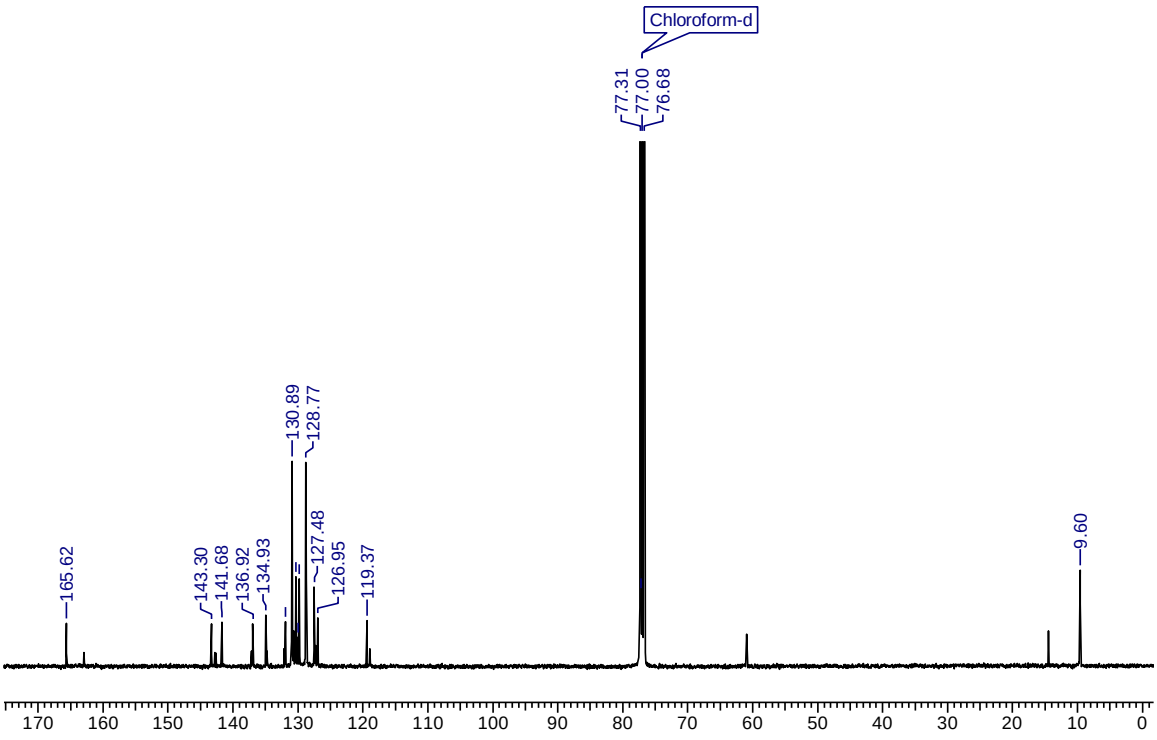


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-12)

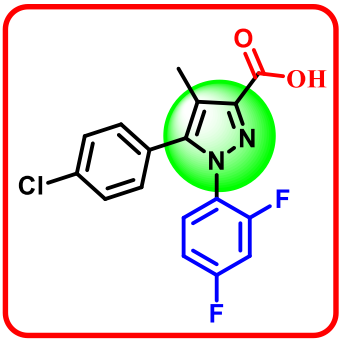


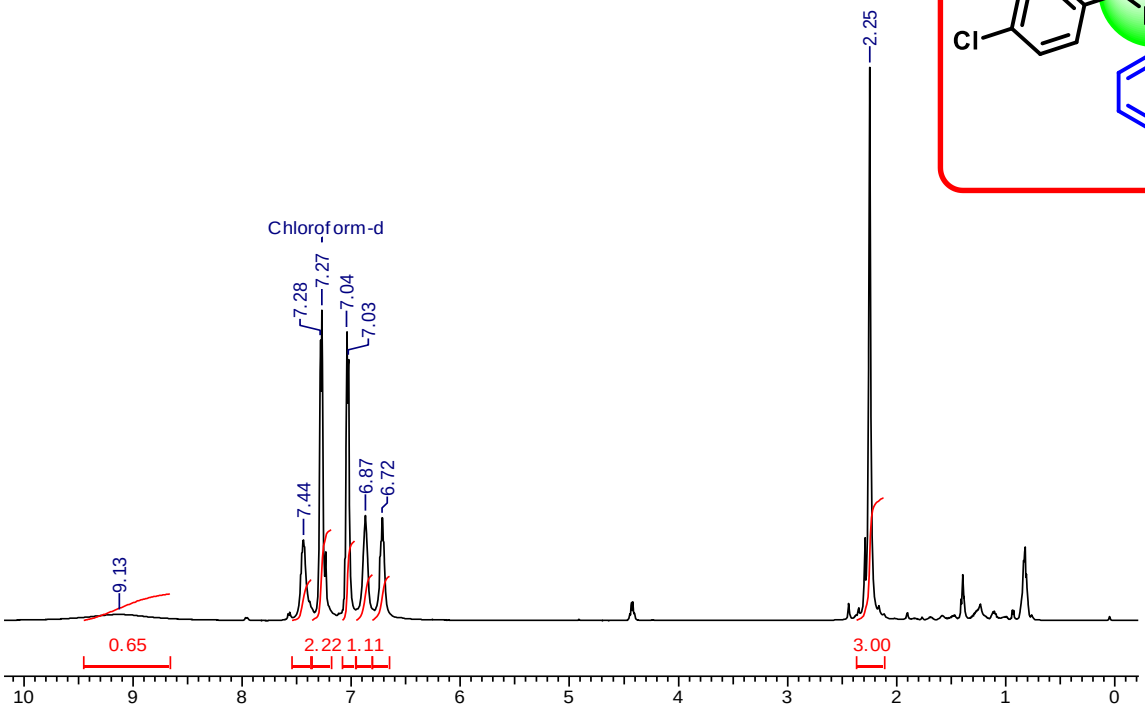


^{13}C NMR (100 MHz, $CDCl_3$)

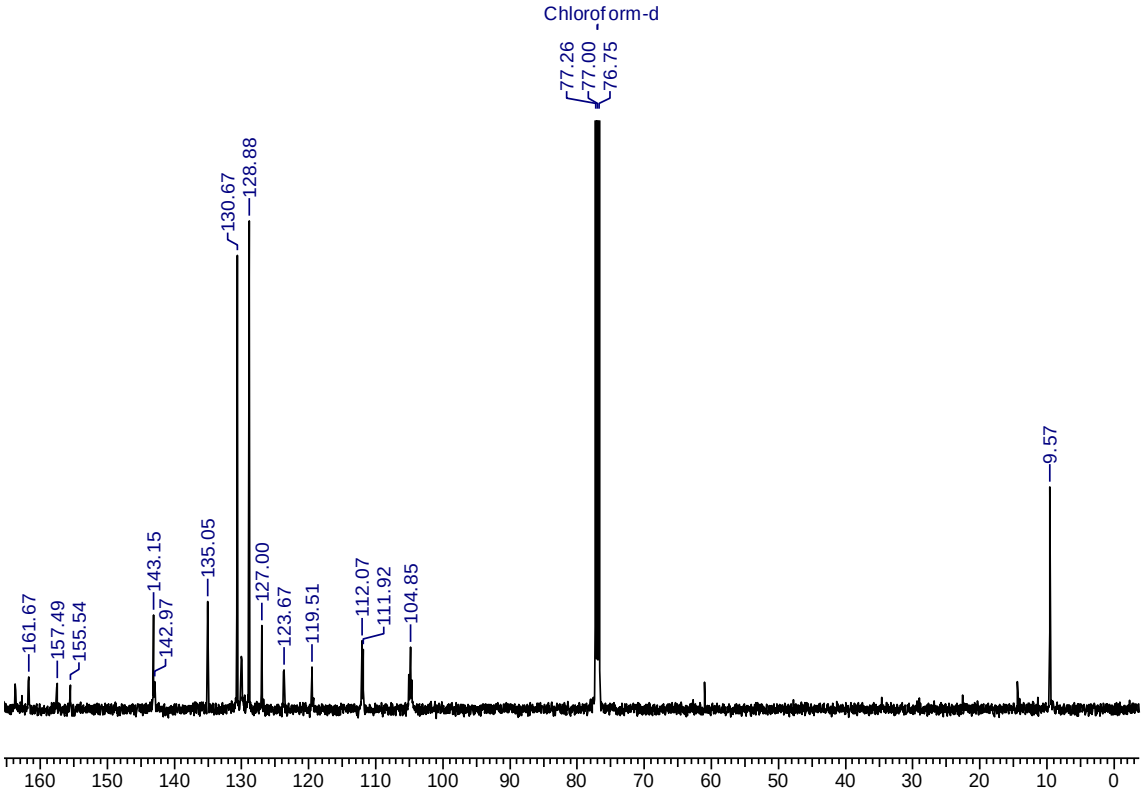


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-13)

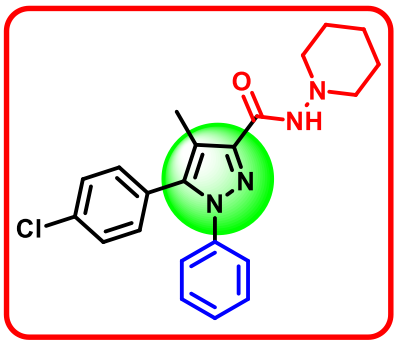


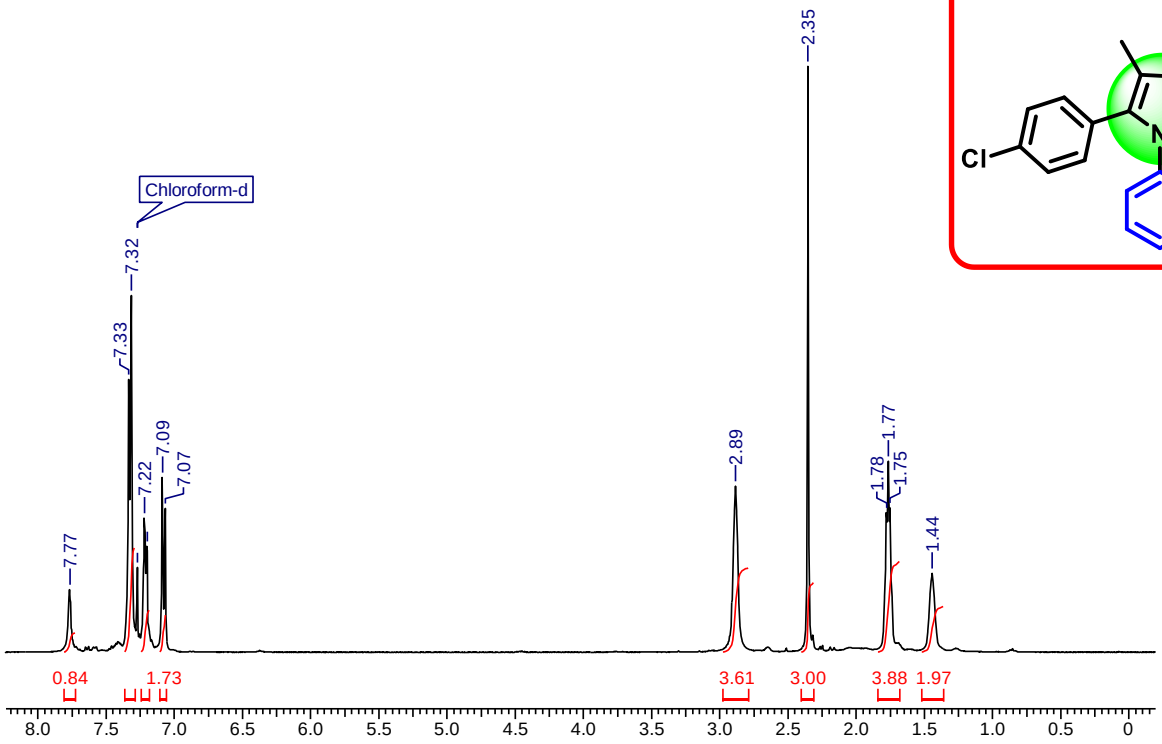


^{13}C NMR (100 MHz, $CDCl_3$)

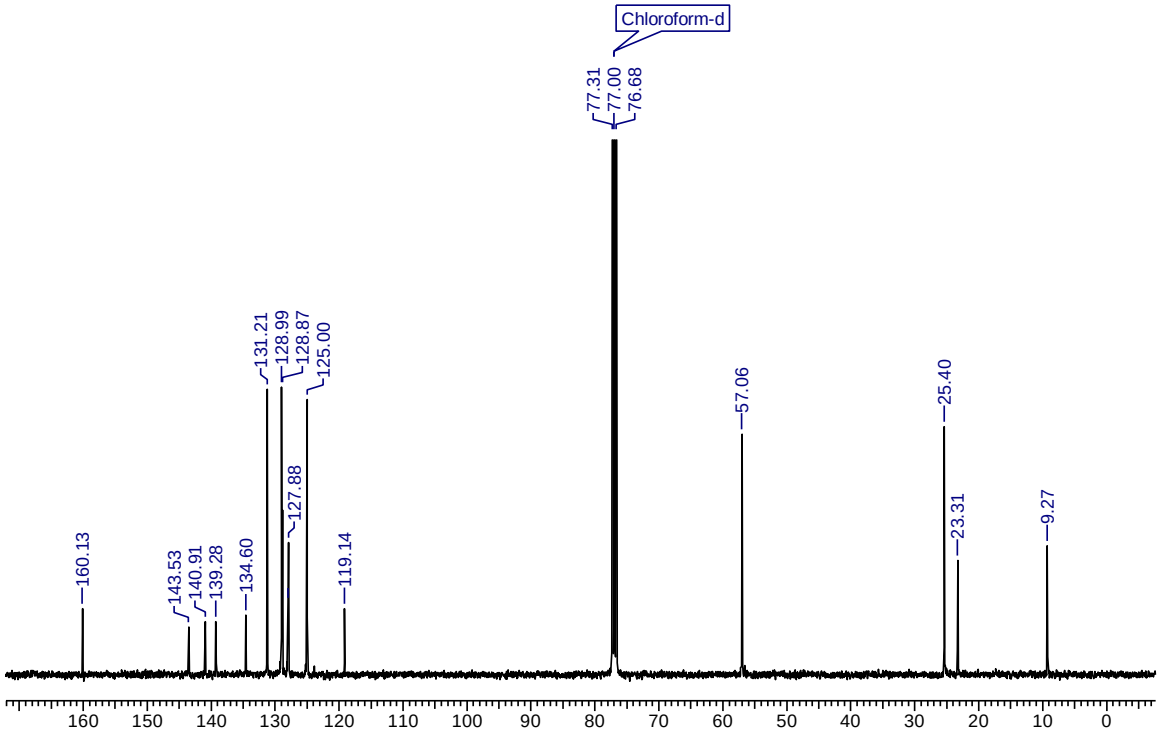


^{1}H NMR (400 MHz, $CDCl_3$) (JMG-14)

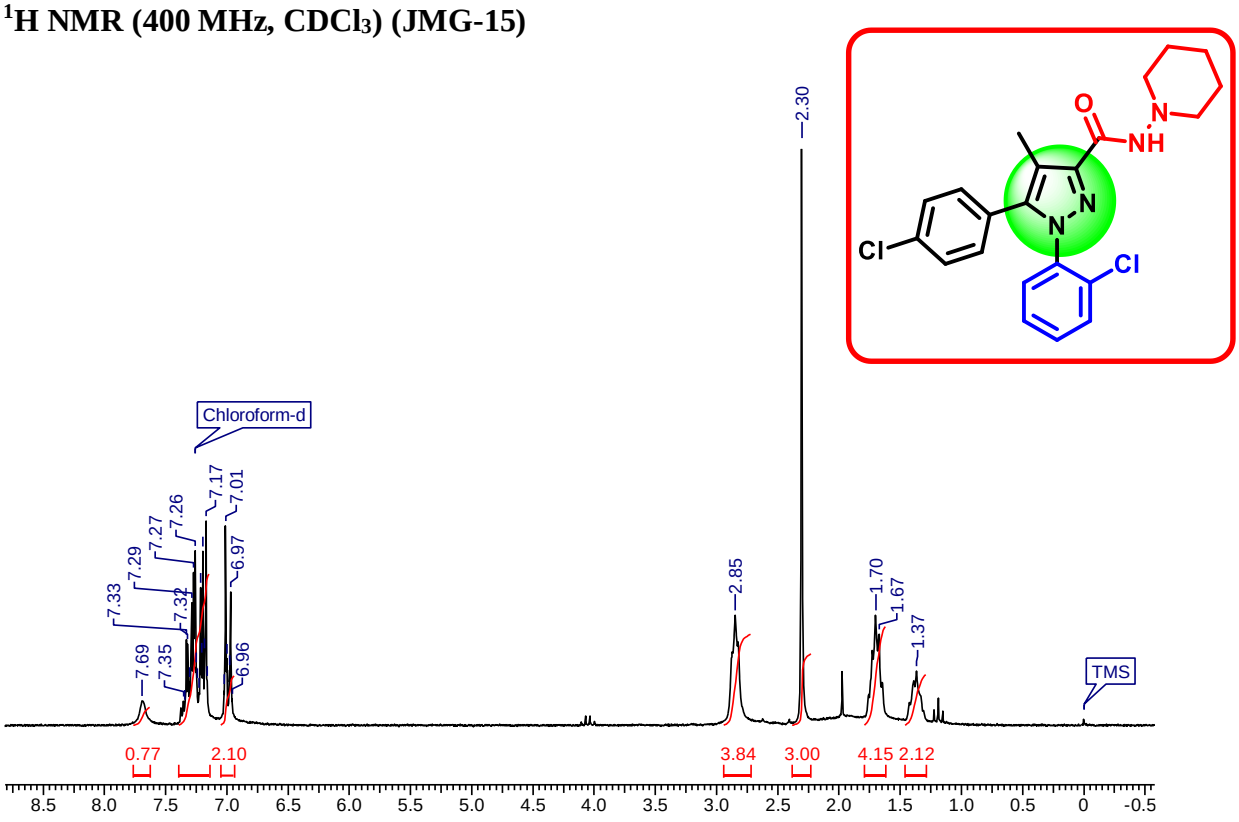




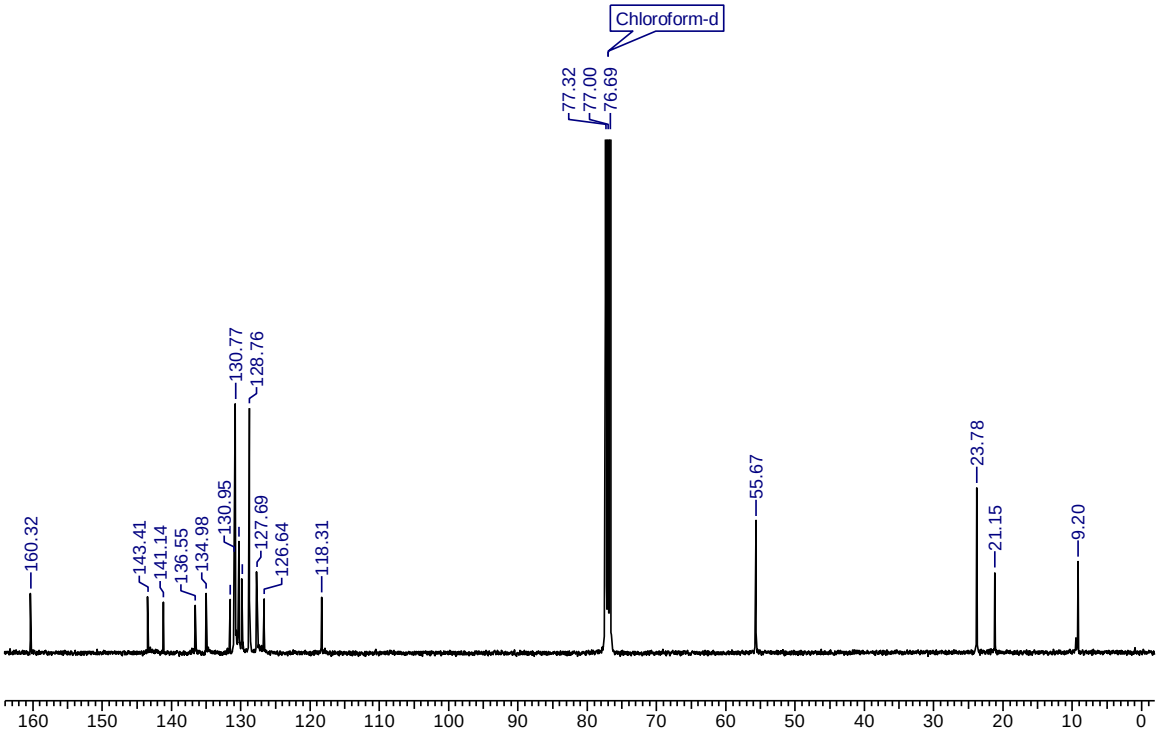
^{13}C NMR (100 MHz, $CDCl_3$)



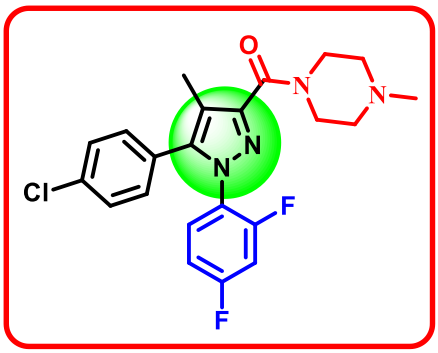
^{1}H NMR (400 MHz, $CDCl_3$) (JMG-15)



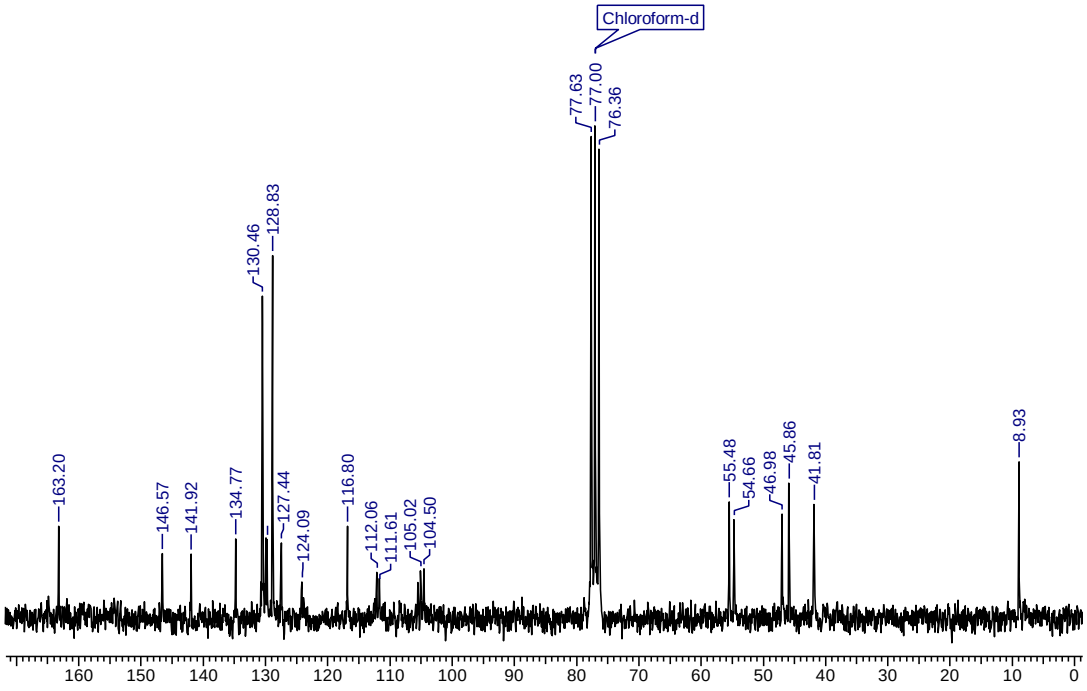
^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$) (JMG-16)



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



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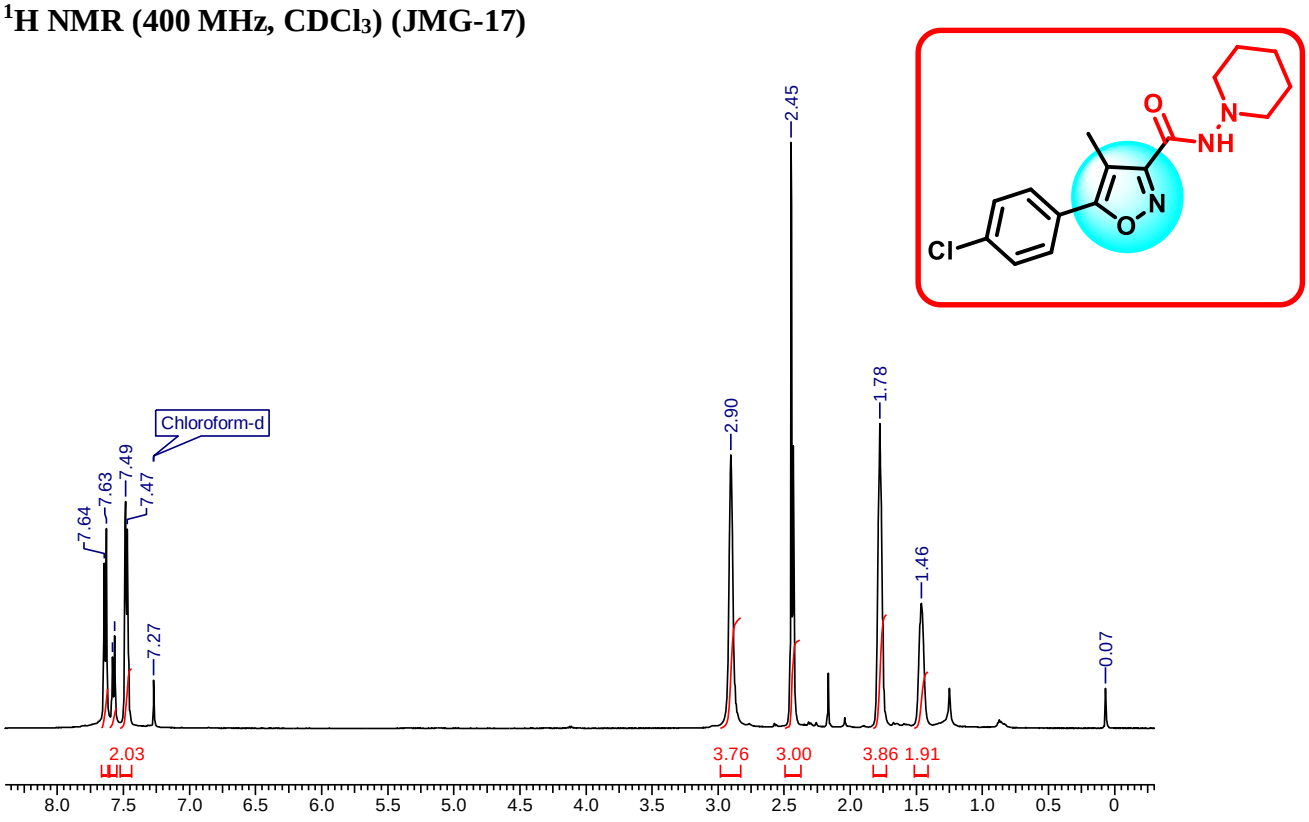
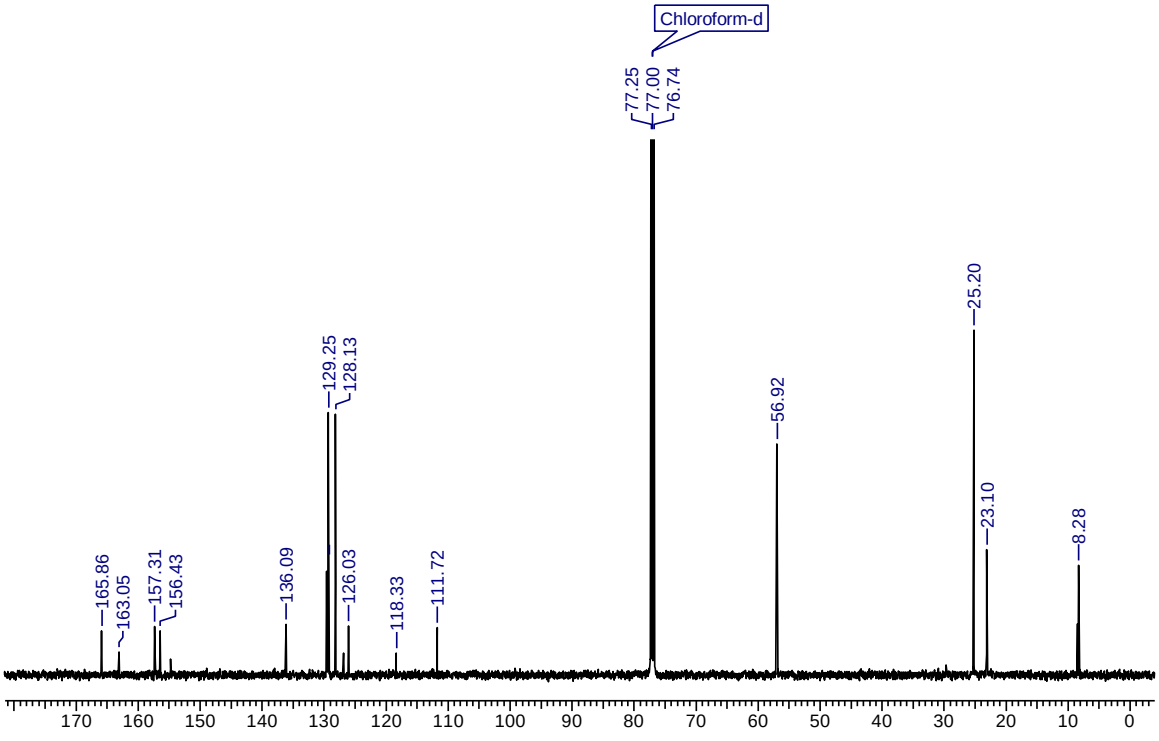


^{13}C NMR (100 MHz, $CDCl_3$)



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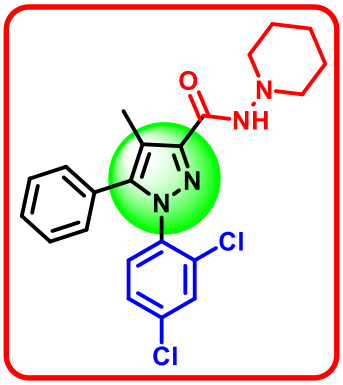
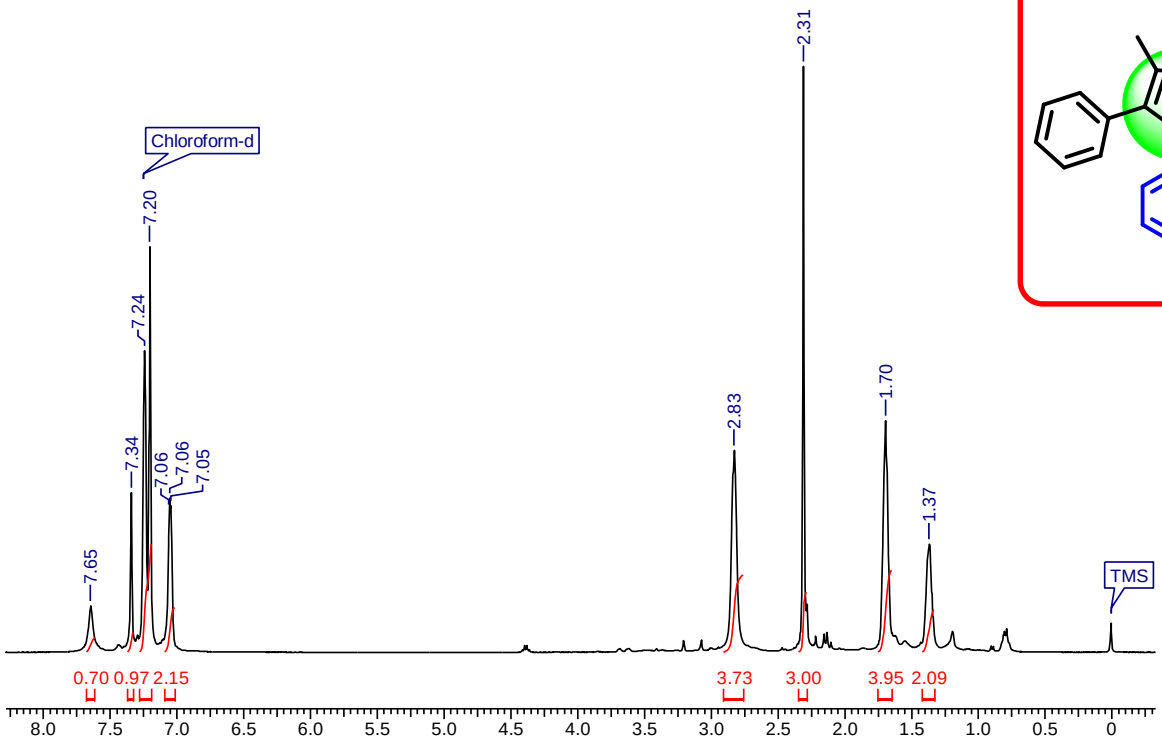
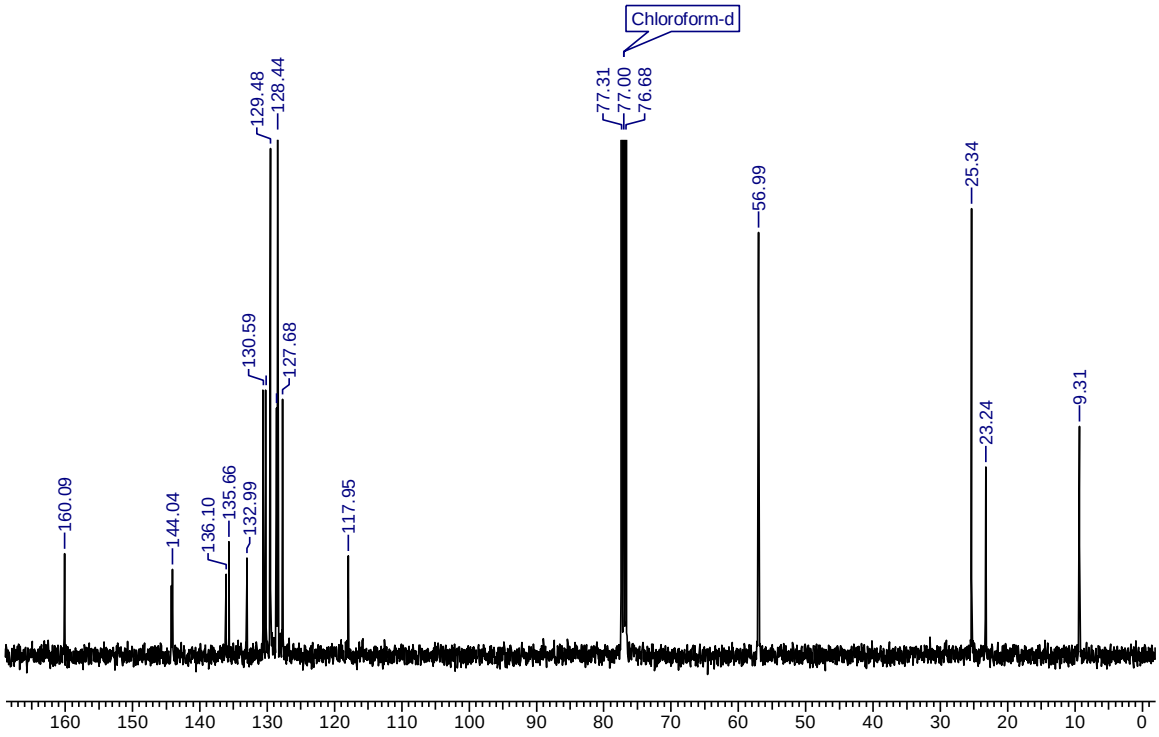
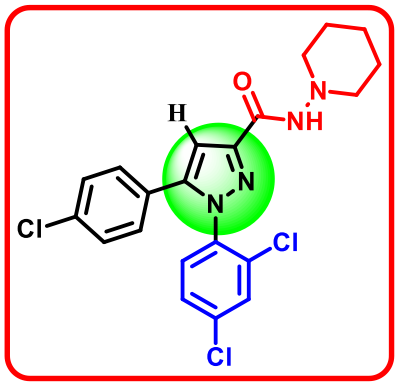




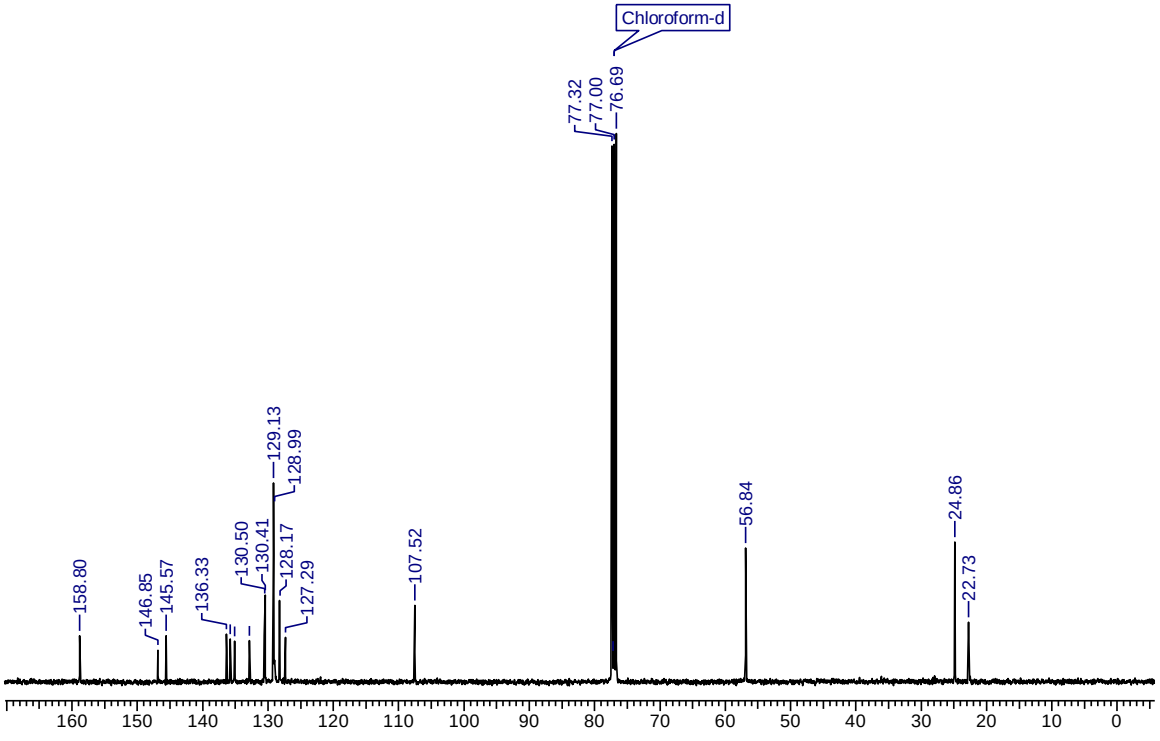
^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$) (JMG-19)



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



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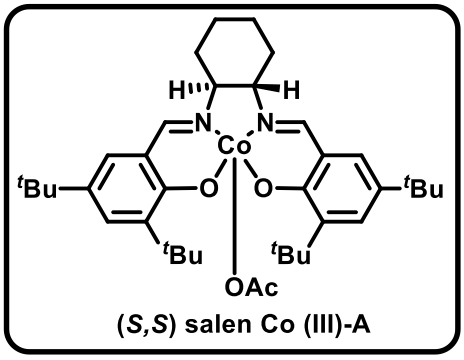
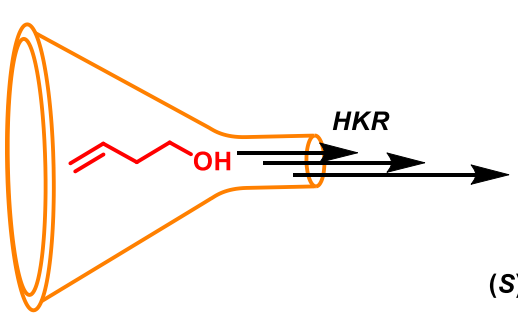
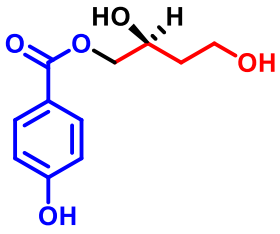
Chapter 3

A convenient synthesis of the enantiomerically pure (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate using hydrolytic kinetic resolution



(*S,S*) salen Co (III)-A





(*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate

(*S*)-2,4-Dihydroxybutyl-4-hydroxybenzoate was prepared in an extremely simple and practical way with high enantiomeric excess (99% ee) using Jacobsen's Hydrolytic Kinetic Resolution technique as a key step and source of chirality.

Namita A. More et *al.* *Synth. Commun.*, 2018, 48, 2093-2098

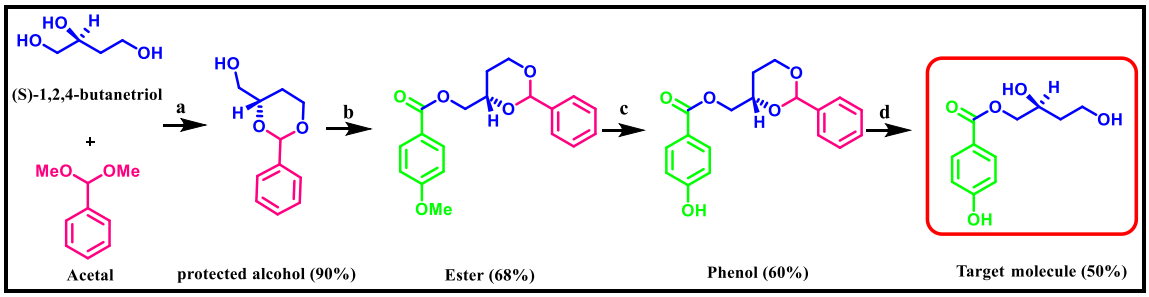
3.1 Introduction

In recent years, marine-microbes have proven to be rich sources of bioactive marine natural products with a unique structure and potent pharmaceutical activity.[1] Biologically active fungi is a natural product as per that reference; marine natural products of *penicillium* fungi show novelties in the structure of compounds and promising biological activities. [2-3] In 2005, the compound (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate (**1**) was isolated from the fungus, *Penicillium aurantiogriseum* (Mycale plumose, Qingdao, China) by Xin *et al.* which showed cytotoxicity against tsFT210 cells at 8.0lμg/ml.[4]

3.2 Rationale of the (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate molecule

It is surprising to note that there is only one report in literature by Seagren et al. for the total synthesis of (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate starting from optically active (*S*)-1,2,4-butanetriol in low yields,[5] (Scheme 3.1) which involves 1,3-diol protected by acetal, followed by Steglich esterification with DCC and DMAP, de-etherification and finally deprotection of acetal like different types of reactions.

Overall yield = 18.36



Scheme 3.1 Reagents and conditions: *(a) p-TsOH, DCM, rt, 24 h, 90%; (b) p-Anisic acid, DCC, DMAP, ACN, rt, 24 h, 68%; (c) t-BuOK / DMF, $Et_2NCH_2CH_2SH.HCl$, rt, 24 h, 60%; (d) TFA, THF : H_2O, 4°C, 50%.*

The simple and clean structural framework, biological properties and limited availability from marine natural sources motivated us to undertake the total synthesis of compound **1**. In recent years, the Jacobsen's hydrolytic kinetic resolution strategy (HKR)[6] has emerged as a powerful tool for the synthesis of various pharmaceutically important

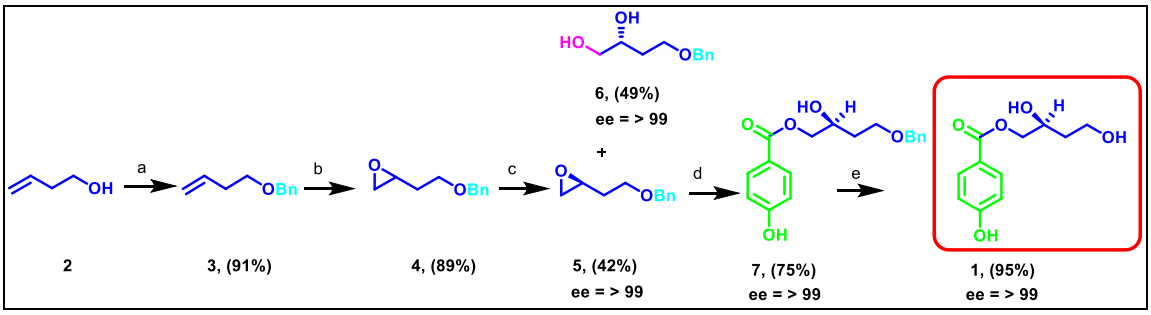
compounds.[7] In continuation of our efforts in the total synthesis of (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate (**1**) *via* Jacobsen's HKR strategy was accomplished.

3.3 Results and discussion

3.3.1 Optimization of reaction conditions

Our synthetic approach to access enantiomerically pure compound **1** began with commercially available 3-buten-1-ol (**2**). Initial attempts to convert the starting compound 3-buten-1-ol into the corresponding epoxide derivative using *m*CPBA resulted in low yields. Therefore, 3-buten-1-ol (**2**) was first protected with benzyl group under basic conditions to afford the compound **3** in 91% yield (Scheme 3.2). The benzyl protected alkene compound **3** was then treated with *m*CPBA to afford the racemic epoxide derivative **4** in 89% yield. Synthesis of enantiomerically pure biologically significant compounds *via* Jacobsen's HKR methodology has emerged as a powerful tool. Therefore, we considered this important methodology to the synthesis of enantiomerically pure epoxide compound **5** from racemic epoxide compound **4** using

Overall yield = 24.23



Scheme 3.2 Reagents and conditions: ***(a)*** *BnBr, NaH, dry THF, 0 °C – rt, 12 h, 91%;* ***(b)*** *m-CPBA, DCM, 0 °C – rt, 4 h 89%;* ***(c)*** *(S,S)-Salen Co$^{(III)}$-OAc, H_2O, 0 °C – rt, 18 h, 42% ((S)-epoxide) and 49% ((R)-1,2-diol)* ***(d)*** *p-Hydroxy benzoic acid, TBAB, dry ACN, 80 °C, 75%;* ***(e)*** *Pd/C, H_2 pressure, dry EA, rt, 4 h, 95%.*

Jacobsen's (S,S)-Salen-Co(III)-OAc catalyst in the presence of water (0.5 equiv.) at 0 °C to room temperature for 18 h resulting in the formation of the desired optically active (*S*)-epoxide **5** in 42% yield ($[\alpha]_D$ = -12.2 (*c*3, $CHCl_3$); ee >99%) along with 49% of (*R*)-diol **6** ($[\alpha]_D$ = +5.5 (*c*1, $CHCl_3$), ee >99%).[9a–f] The enantiomeric excess was determined

by HPLC using chiral column Kromasil 5-Cellucoat for **5** and romasil RP-18 for **6**. Next, the treatment of 4-hydroxybenzoic acid with (*S*)-epoxide **5** and catalytic amount of tetrabutylammonium bromide (TBAB) in anhydrous acetonitrile at reflux conditions resulted in formation of required (*S*)-alcohol **7** in 75% yield (>99% ee) with complete regioselective epoxide ring-opening.[10] Finally, debenzylation of compound **7** was carried out by hydrogenation over 10% Pd/C in ethyl acetate to afford our target molecule (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate (**1**) without loss of optical purity (>99% ee) $[\alpha]_D$ = -8.4, (*c* 0.5, CH_3OH) (Scheme 3.2). The enantiomeric excess of compound **1** was determined by HPLC using chiral column Chiralcel OJ-H. The structure of (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate (**1**) was confirmed by different characterizations.

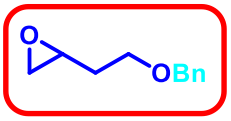
3.4 Conclusion

We have developed a convenient and simple methodology for the synthesis of (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate. Jacobsen's HKR methodology was a source of chirality. The synthetic strategy described here is being explored for the synthesis of biologically significant molecules using structure-activity relationship studies.

3.5 Experimental Section

3.5.1 Experimental procedures and characterization data

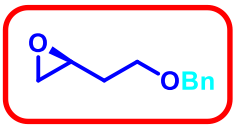
(±)-2-(2-(Benzyloxy) ethyl)oxirane (4)



To an ice-cooled solution of compound 3 (5.0 g, 0.028 mol) in dry DCM (50 ml) *m*CPBA (14.9 g, 0.056 mol) was added in portions over a period of 30 min at 0 °C. After complete addition of *m*CPBA, cooling was remove and stirring was continued at room temperature for 24 h. The reaction mixture was diluted with dichloromethane (50 ml) and washed with an aqueous sodium bicarbonate, followed by water. Organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product which was purified by column chromatography (silica gel; pet. ether/ EtOAc = 90/10) to yield racemic epoxide **4**; yield: 4.91 g, 89%; colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.74-2.02 (m, 2H), 2.52 (dd, *J* = 2.2, 4.8 Hz, 1H), 2.80 (t, *J* = 4.8 Hz, 1H), 3.05-3.14 (m, 1H), 3.64 (t, *J* = 6.8 Hz, 2H), 4.55 (s, 2H), 7.29-7.37 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.2, 128.3 (2C), 127.5 (2C), 126.8, 73.0, 66.5, 50.0, 47.0, 32.9; HRMS (ESI): *m/z*

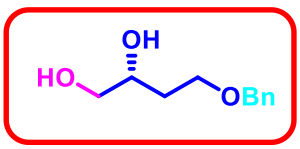
Calcd for $C_{11}H_{14}NaO_2$ $[M+Na]^+$: 201.0887; Found: 201.0886; IR (Neat): 3384, 2926, 2879, 1718, 1492, 1452, 1276, 1113, 1026, 756 cm^{-1}

(*S*)-2-(2-(benzyloxy)ethyl)oxirane (5)



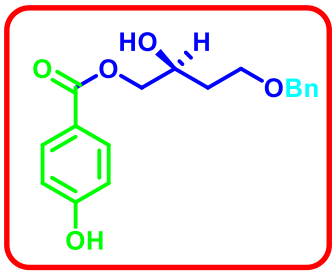
A mixture of racemic epoxide **4** (5.0 g, 0.028 mol) and (S,S) salen Co(III)OAc complex-A (0.10 g, 0.015 mmol) was vigorously stirred for 15 min at 0 °C and water (0.3 ml, 0.017 mol) was added over a period of 1 h, through syringe. The reaction mixture was stirred at rt and monitored by HPLC (kromasil 5-cellucoat column) UV: 220 nm, 3% isopropanol in n-hexane. The reaction mixture was diluted with EtOAc, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel; pet. ether/EtOAc = 93/7). The less polar (*S*)-epoxide **5** eluted first as a colorless liquid; yield: 2.12 g, 42%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.74-2.02 (m, 2H), 2.52 (dd, *J* = 2.2, 4.8 Hz, 1H), 2.80 (t, *J* = 4.8 Hz, 1H), 3.05-3.14 (m, 1H), 3.64 (t, *J* = 6.8 Hz, 2H), 4.55 (s, 2H), 7.29-7.37 (m, 5H); ^{13}C NMR (50MHz, $CDCl_3$): δ 138.2, 128.3 (2C), 127.5 (2C), 126.8, 73.0, 66.5, 50.0, 47.0, 32.9; IR (Neat): 3384, 2926, 2879, 1718, 1492, 1452, 1276, 1113, 1026, 756 cm^{-1}; Specific rotation: $[\alpha]_D$= -12.2° (*c*3, $CHCl_3$) {lit.[9] $[\alpha]_D$= -15.6 (*c* 1.25, $CHCl_3$)}; HPLC: ee >99% [chiral HPLC analysis; Kromasil 5-cellucoat column (250 × 4.6 cm) column; eluent: hexane/isopropanol = 97/3; flow rate: 1.0 ml/min; detector: 220 nm (t_R = 13.47 min), (t_S = 14.08 min)]; HRMS (ESI): *m/z* Calcd for $C_{11}H_{14}NaO_2$ $[M+Na]^+$: 201.0887; Found: 201.0886

Further elution afforded the **(*R*)-diol (6)**



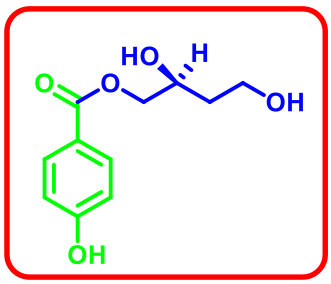
Colorless liquid (2.71g); Yield: 49%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.66-1.85 (m, 2H), 3.46 (dd, *J* = 6.9, 11.4 Hz, 1H), 3.56-3.71 (m, 3H), 3.83-3.94 (m, 1H), 4.52 (s, 2H), 7.24-7.40 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.7, 128.4 (2C), 127.7 (2C), 127.6, 73.2, 70.2, 68.1, 65.5, 34.1; HRMS (ESI): *m/z* Calcd for $C_{11}H_{14}NaO_2$ $[M+Na]^+$: 201.0887; Found: 201.0886; IR (Neat): 3386, 2925, 2867, 1453, 1096, 759, 698cm^{-1}; Specific rotation: $[\alpha]_D$ = +5.5° (*c* 1, $CHCl_3$); HPLC: ee >99% [chiral HPLC analysis; Kromasil RP-18 (150 × 4.6 cm) column; eluent: H_2O/MeOH = 40/60; flow rate: 1.0 ml/min; detector: 254 nm (t_R = 2.98 min)].

(*S*)-4-(benzyloxy)-2-hydroxybutyl 4-hydroxybenzoate (7)



To a solution of (*S*)-epoxide **5** (2.0 g, 0.011 mol) in anhydrous acetonitrile (25 ml) *p*-hydroxybenzoic acid (1.8 g, 0.013 mol) and TBAB (0.9 g, 0.003 mol) was added. The reaction mixture was refluxed for 12 h and concentrated under reduced pressure to afford a residue which was diluted with ethyl acetate and washed with saturated $NaHCO_3$ till the removal of acid. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product which was purified by column chromatography (silica gel; pet. ether/EtOAc = 60/40) to afford the pure compound **7** (2.68 g), Mp = 56 ^oC - 58 ^oC.; Yield: 75%; ^1H NMR (200 MHz, $CDCl_3$): δ 1.82-2.00 (m, 2H), 3.67-3.82 (m, 2H), 4.20-4.38 (m, 3H), 4.56 (s, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 7.27-7.43 (m, 5H), 7.83 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.8, 160.9, 137.4, 131.9 (2C), 128.5 (2C), 127.8 (2C), 121.3, 115.3 (2C), 73.4, 69.6, 68.1, 32.7; HRMS (ESI): *m/z* Calcd for $C_{18}H_{20}NaO_5$ $[M+Na]^+$: 339.1203, Found 339.1200; IR (Neat): 3600-3150, 3020, 2927, 1710, 1273, 1215cm^{-1}; Specific rotation: $[\alpha]_D$ = -8.00 (*c* 0.2, CH_3COCH_3).

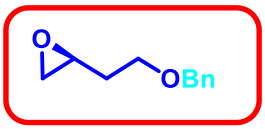
(*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate (1)

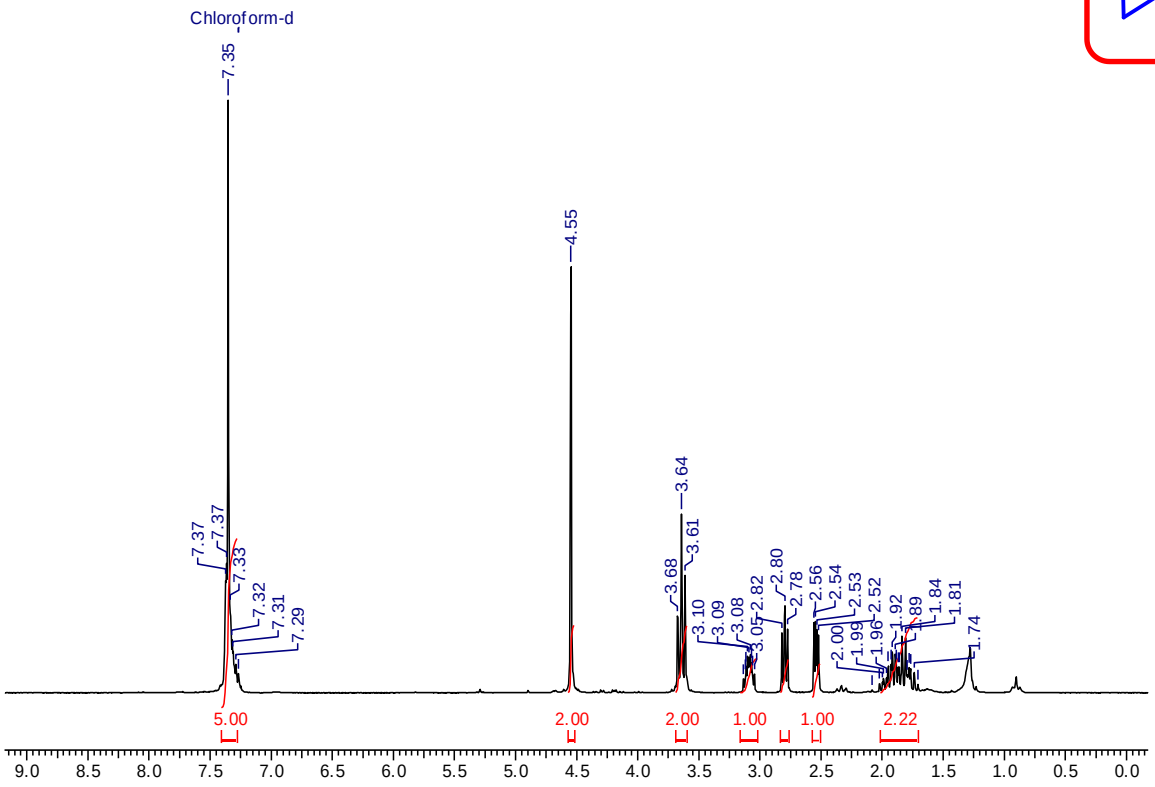


A solution of compound **7** (2.0 g, 0.006 mol) in ethyl acetate (25 ml) was stirred over 10% Pd/C (0.13 g, 0.001 mol) under H_2 pressure (Parr Shaker; 65-psi pressure) for 4 h. After the completion of reaction, the reaction mixture was filtered through a pad of celite, the filtrate was concentrated under reduced pressure to afford the crude product which was purified by column chromatography (silica gel; pet. ether/EtOAc = 50/50) to yield compound 1 (1.36 g), mp = 99 ^oC – 101 ^oC; Yield: 95%; IR (Neat): 3600-3150, 1710, 1608, 1461, 771cm^{-1}; ^1H NMR (200 MHz, CD_3COCD_3): δ 1.68-1.88 (m, 2H), 3.79 (t, *J* = 6.1 Hz, 2H), 4.11-4.26 (m, 3H), 6.93 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (50 MHz, CD_3COCD_3): δ 166.5, 162.6, 132.5, 122.5, 115.9, 69.4, 68.2, 59.8, 37.1; HRMS (ESI): *m/z* Calcd for $C_{11}H_{14}NaO_5$ $[M+Na]^+$: 249.0733, Found 249.0730; Specific rotation: $[\alpha]_D$ = -8.4^o,(*c* 0.5, CH_3OH); HPLC: ee >99% [chiral HPLC analysis; Chiralcel

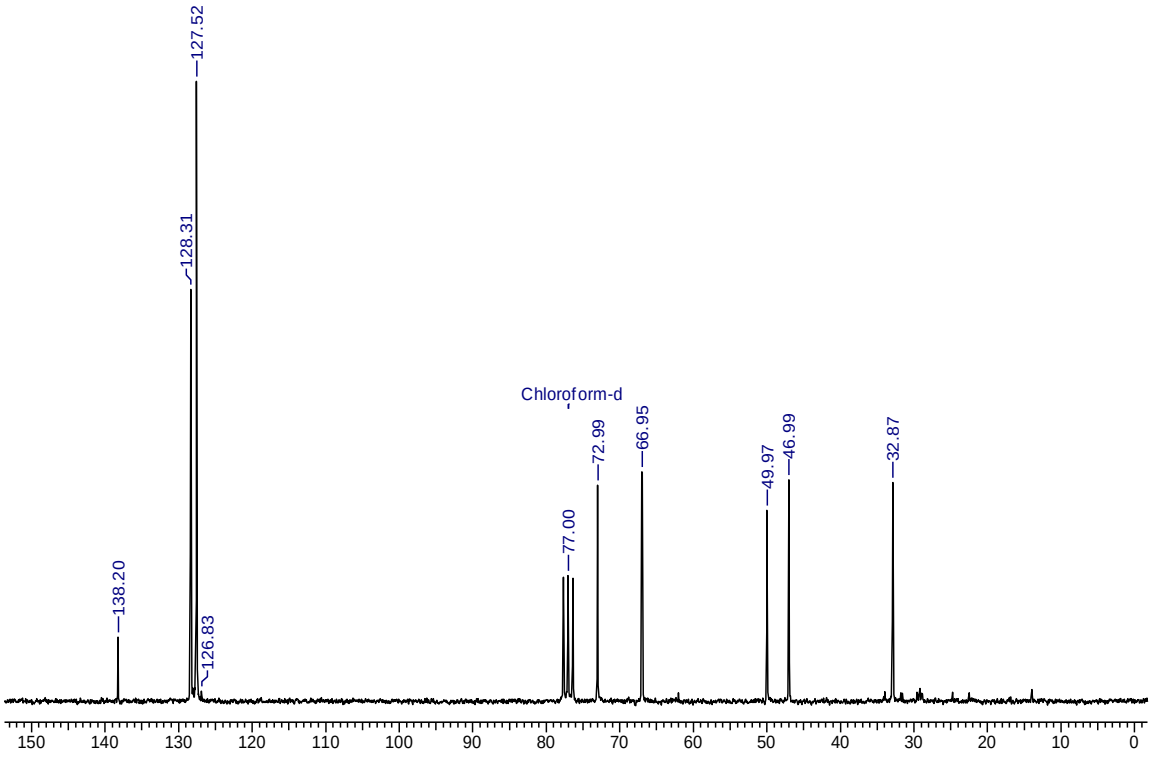
OJ-H column (250 × 4.6cm) column; eluent: hexane/ ethanol = 80/20; flow rate: 1.0 ml/min; detector: 254 nm (t_R = 7.87 min), (t_S = 8.73 min)]

3.5.2 Spectral data: ^{1}H NMR (200 MHz, $CDCl_3$) (5)

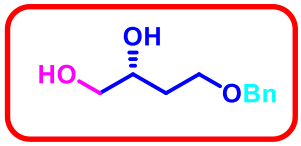


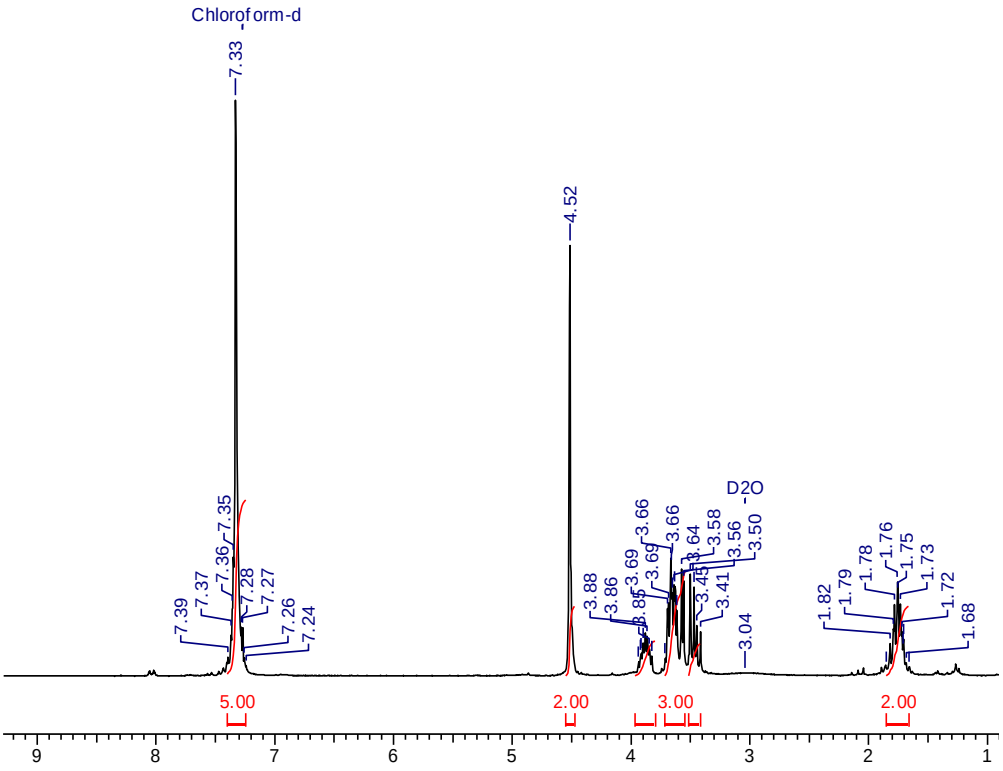


^{13}C NMR (50 MHz, $CDCl_3$)



^{1}H NMR (200 MHz, $CDCl_3$) (6)





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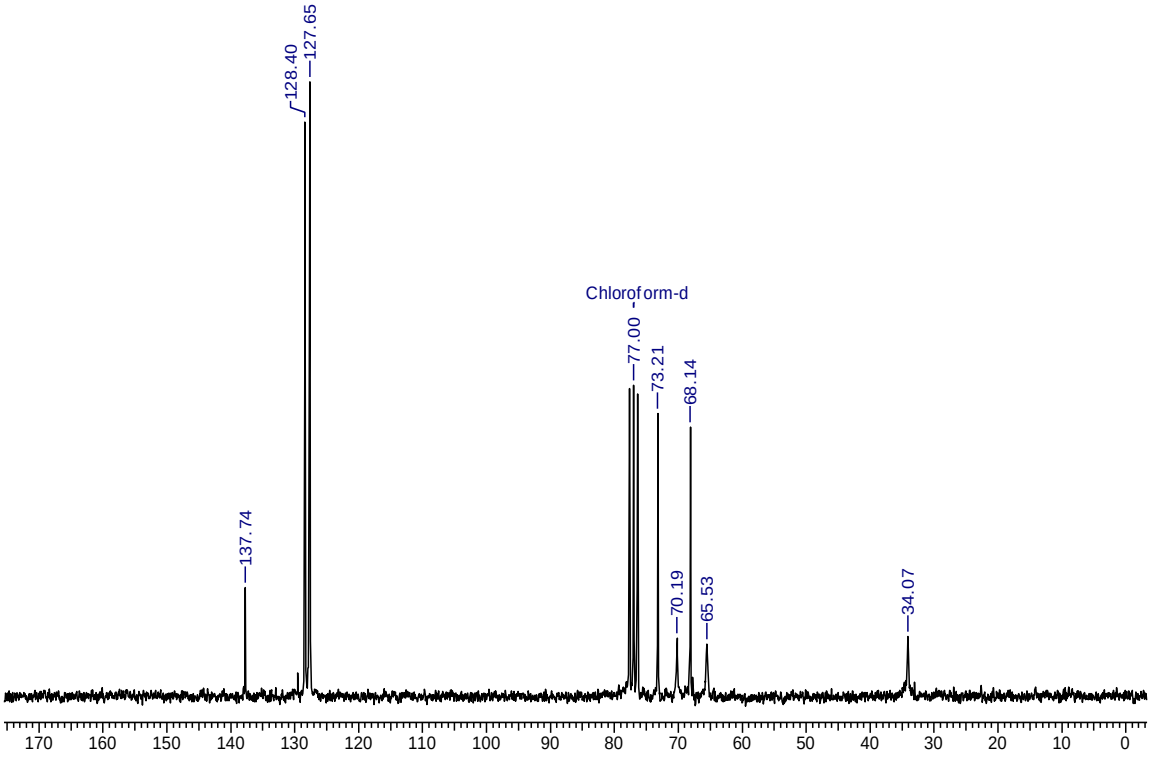
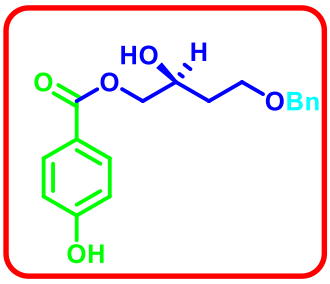
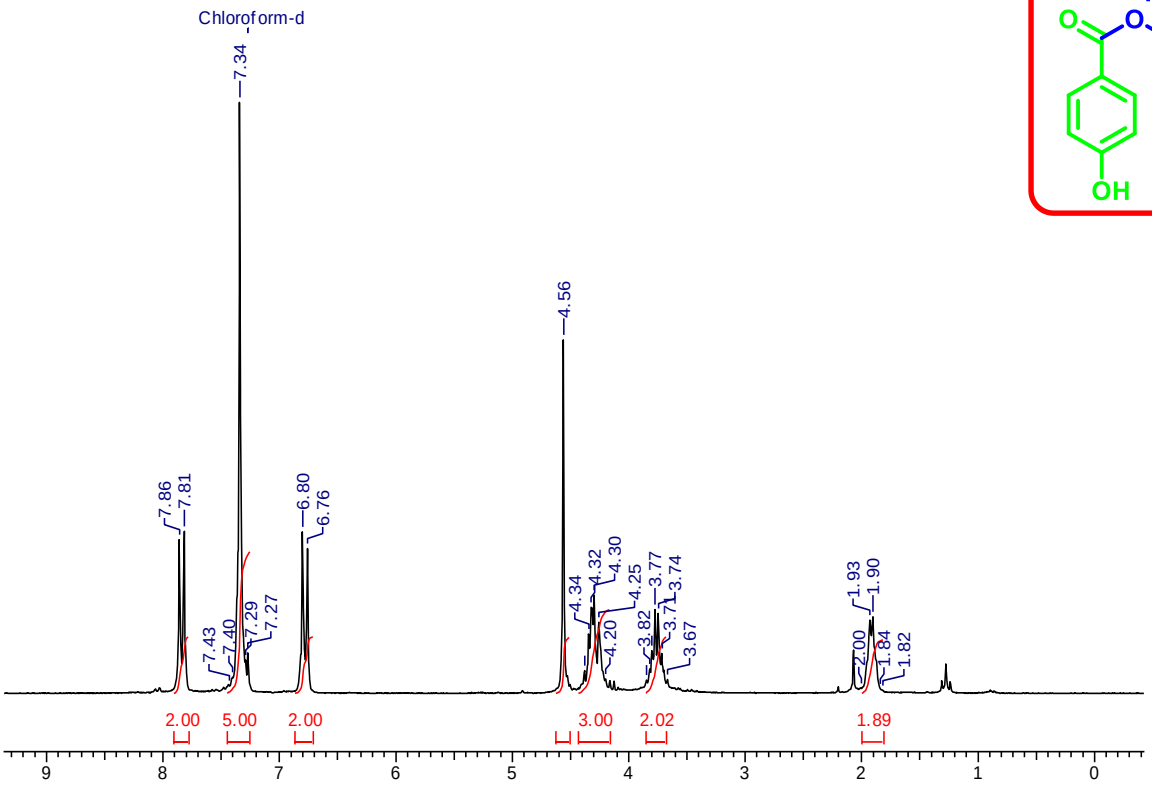
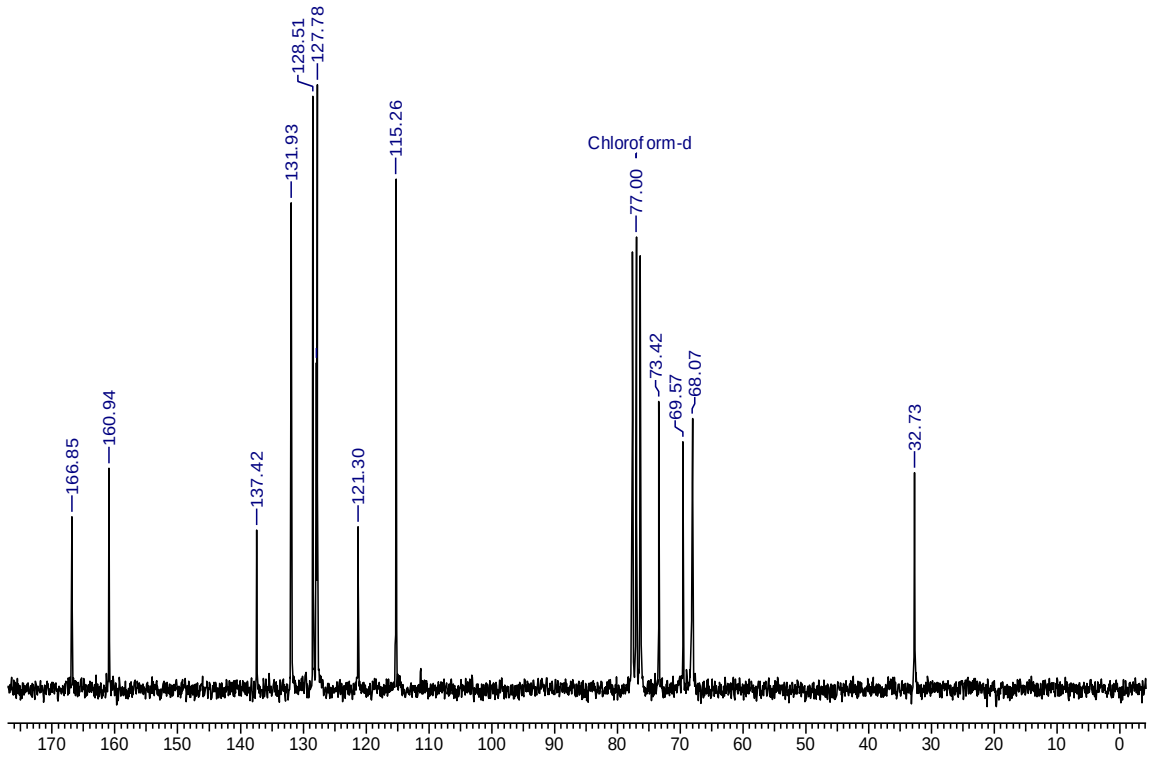


^{1}H NMR (200 MHz, $CDCl_3$) (7)

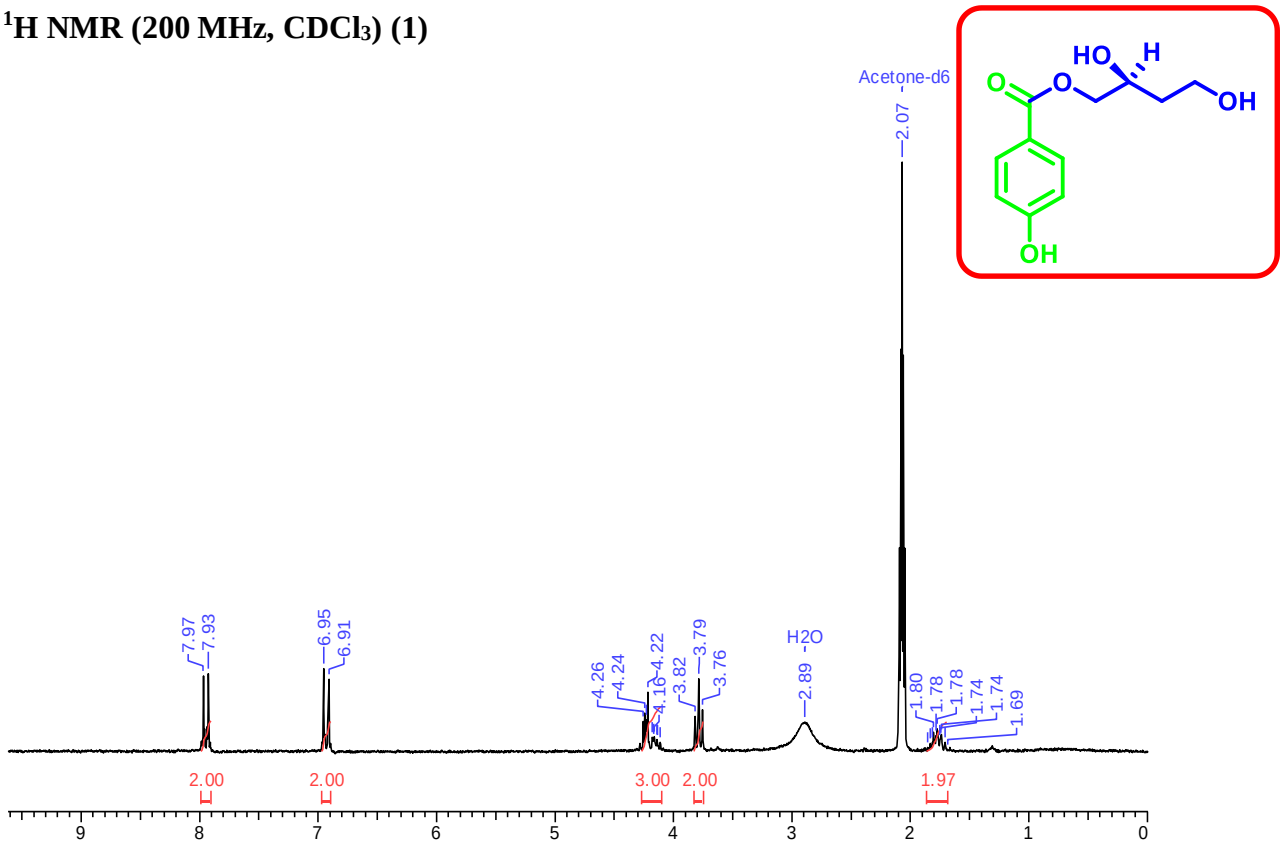




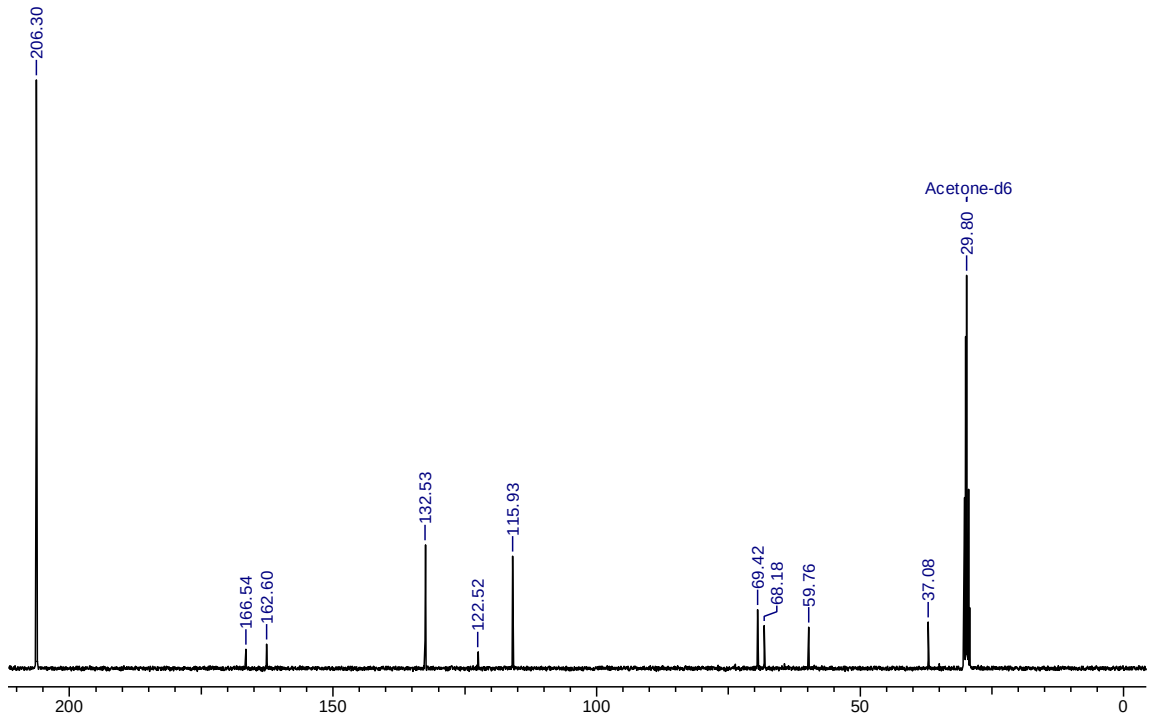
^{13}C NMR (50 MHz, $CDCl_3$)



^{1}H NMR (200 MHz, $CDCl_3$) (1)

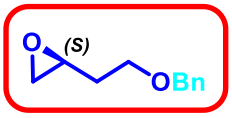


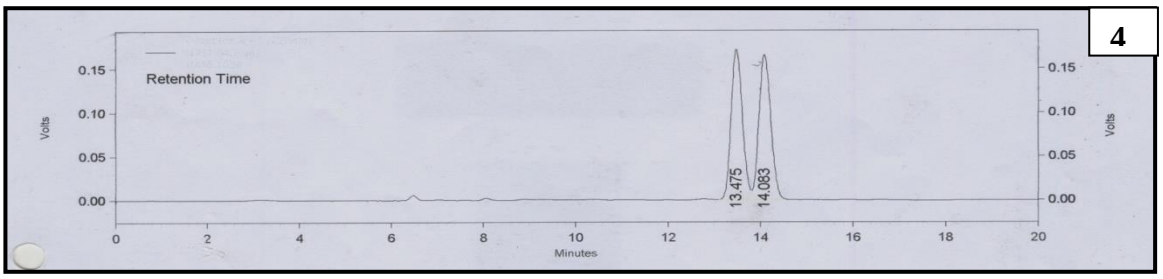
^{13}C NMR (50 MHz, $CDCl_3$)



HPLC:

Racemic standard





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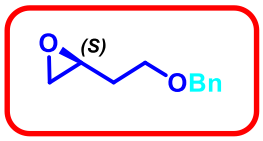
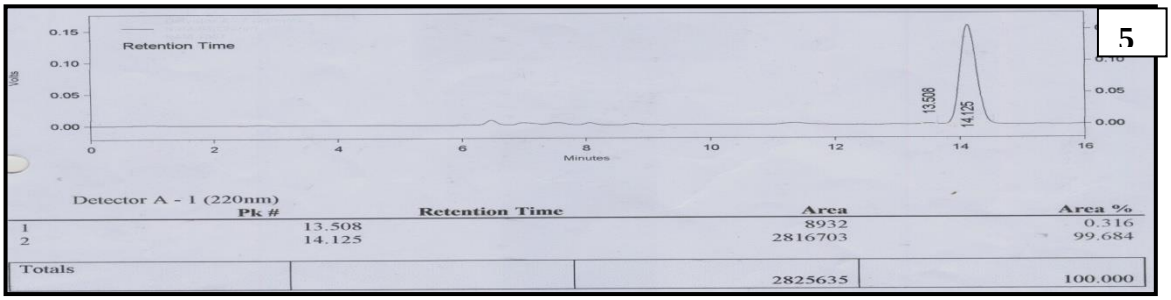
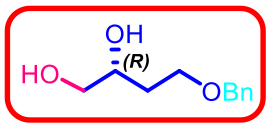


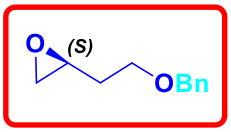


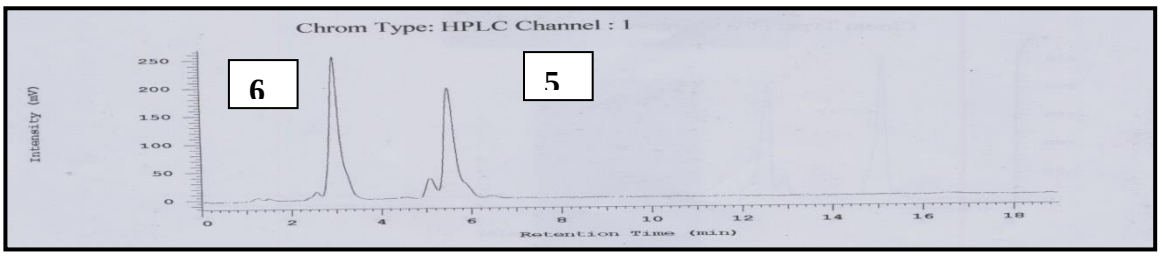
Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	13.508	8932	0.316
2	14.125	2816703	99.684
Totals		2825635	100.000

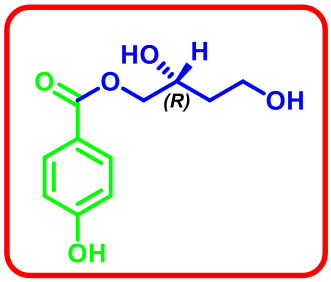
Chiral HPLC

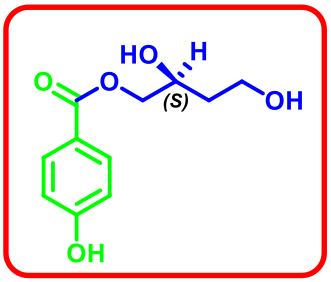


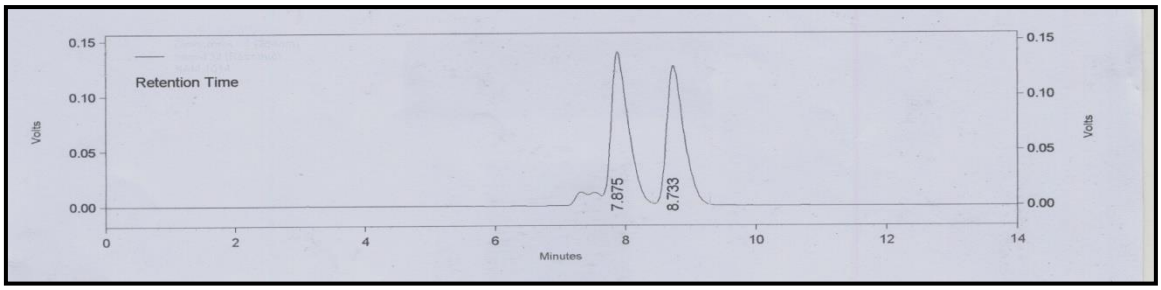




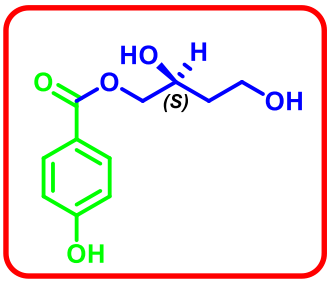
Racemic standard

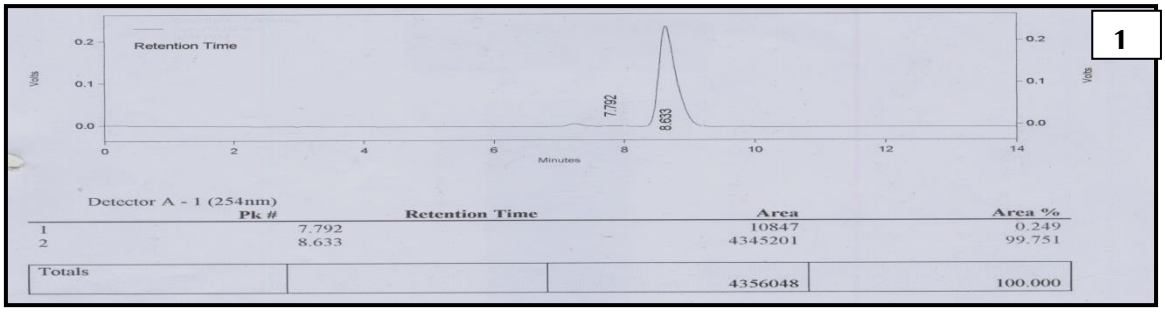






Chiral HPLC





Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %
1	7.792	10847	0.249
2	8.633	4345201	99.751
Totals		4356048	100.000

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Yogeeswari, P.; Sriram, D.; Masand, V. H. *Med. Chem. Res.* **2015**, *24*, 2960–2971. [c] Gajbhiye, J. M.; Chopade, A. U. *Int. J. Chem. Phys. Sci.* **2014**, *3*, 1–4. [d] More, N. A.; Patil, M. D.; Garud, D. R.; Gajbhiye, J. M. *Rasayan J. Chem.* **2016**, *9*, 806–811.

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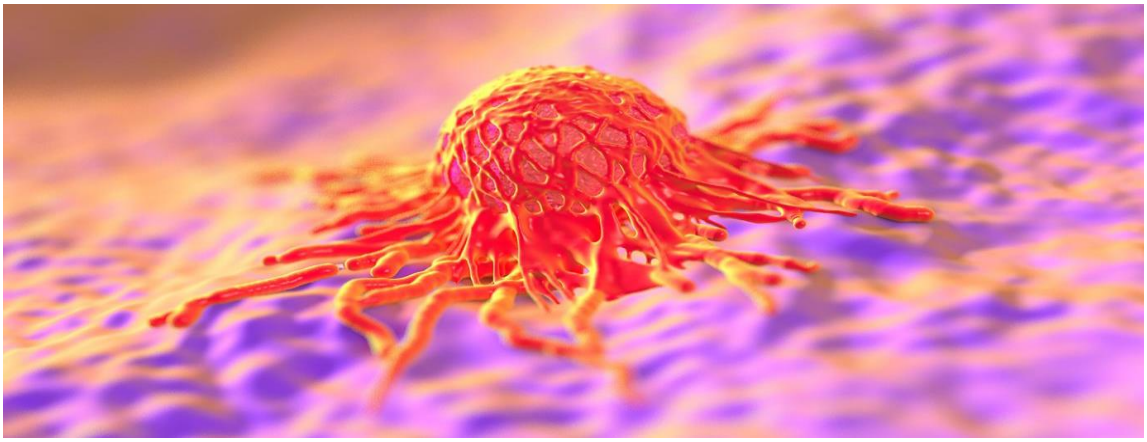
Chapter 4

Design and Synthesis of Anti-tumor Compounds and their Drug Delivery System

Chapter 4A

Cancer



4A.1 Introducion

The aim of this chapter was to develop novel sulfonamides and a combination of chitosan and m-PEG for use in drug delivery system. Controlled drug release systems, is an original technique used to treat cancer cells by targeted delivery.

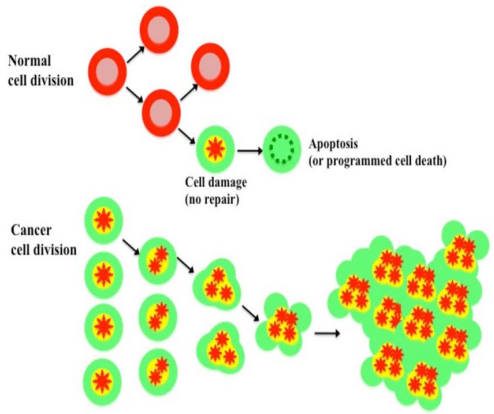


Figure 4A.1 Cell division

Cancer is one of the non-communicable diseases that have been responsible for the increased mortality worldwide and reduced life expectancy in every other country. The growth ability of cancer cells is faster than normal cell present in the body (Figure 4A.1). Therefore, cancer cells easily spread all over the body. Amongst females, high percentage of breast cancer is observed, followed by lung and colorectal cancer.[1] According to the global cancer statistics, 2019, breast cancer alone accounts for 30% of all new cases in women. Statistical estimation shows that in 2019, approximately 62, 930 new breast cancer cases may occur. It has also been observed that the mortality was higher in developing countries as compared to the wealthiest countries.[2] As per the Indian Council of Medical Research (ICMR) reports, the number of cancer cases has almost doubled in the last few years, of which

Table 4A.1 Reason of cancer occurring in different parts

Cancer

Breast cancer	**Oral cancer**	**Cervical cancer**
-Family history 84%	-Tobacco 87%	- STD 80%
-Overweight or obesity 71%	-Smoking 74%	- Unhealthy sanitation 59%
-Dense breast tissue 67%	-Passive smoking 66%	-Age 53%

breast, cervical, oral and lung cancer are regarded as the priority cancers in India. Which is occurring by different reasons shown in table 4A.1.[3] Common anti-cancer therapies to treat cancer include surgery, chemo, radio, immuno, targeted and hormonal therapy,

which are also several times used in combination. The development and application of anticancer drugs started in the 1940s, which included alkylating agents such as nitrogen mustard which was incorporated into the DNA bases causing lesions and cell death.[4] Another class of agents used for cancers involved antimetabolites such as aminopterin and amethopterin which interrupted the DNA replication process, thus causing cell damage. Currently, used chemotherapeutics agents include natural compounds such as anthracyclines, taxanes, doxorubicin, paclitaxel, fluorouracil, cyclophosphamides.[5] Although many scientists have devoted themselves to the discovery of new drugs that would prevent cell proliferation, metastasis and induce apoptosis, considerable hurdles still exist. Nowadays, the treatment of various cancers depends on chemotherapeutic drugs, but their use is limited because of serious side effects, risk of heart problem, low water solubility, and short circulation time. There is no doubt that the identification of novel controlled drug release of targeted therapy, which is different from chemotherapy would optimize the therapeutic outcome in cancer tissues, proteins or specific genes that help to cancer growth. A cancer cell is made by genes and proteins. The main role of controlled drug release study is to inhibits the growth of cancer cell by releasing the high concentration of drug in a specific location of cancer cell without damaging healthy cell. Advantages of the drug delivery system (DDS) as compared to previous therapy is improved stability, reduced side effects, reduced doses, improved bioavailability, reduced cost and also use of hydrophobic drugs with increase biological activity is proved in many literatures. Therefore, the quality of life of the cancer patients and the overall survival would improve by this therapy.[6]

4A.2 Drug delivery system approaches cancer treatment

Gefitinib is a water insoluble compound. In the market different types of anti cancer compounds are available, but most of them are water insoluble. As per this observation researchers move to design and synthesize a drug delivery system.

Drug delivery system is based on organic and inorganic particles. Applications of drug delivery system in organic particles are micelles, polymers, liposomes, nanogels and dendrimers. Inorganic particles are gold nanoparticles, quantum dots. Chemotherapeutic drugs encapsulations by nanoscale device is useful in reduced side effects and enhances the bioavailability of breast cancer drugs. Based on drug delivery system in nanocarrier

platforms, targeting of drug resistant in breast tumor cells is shown in (Figure 4A.2).[7]

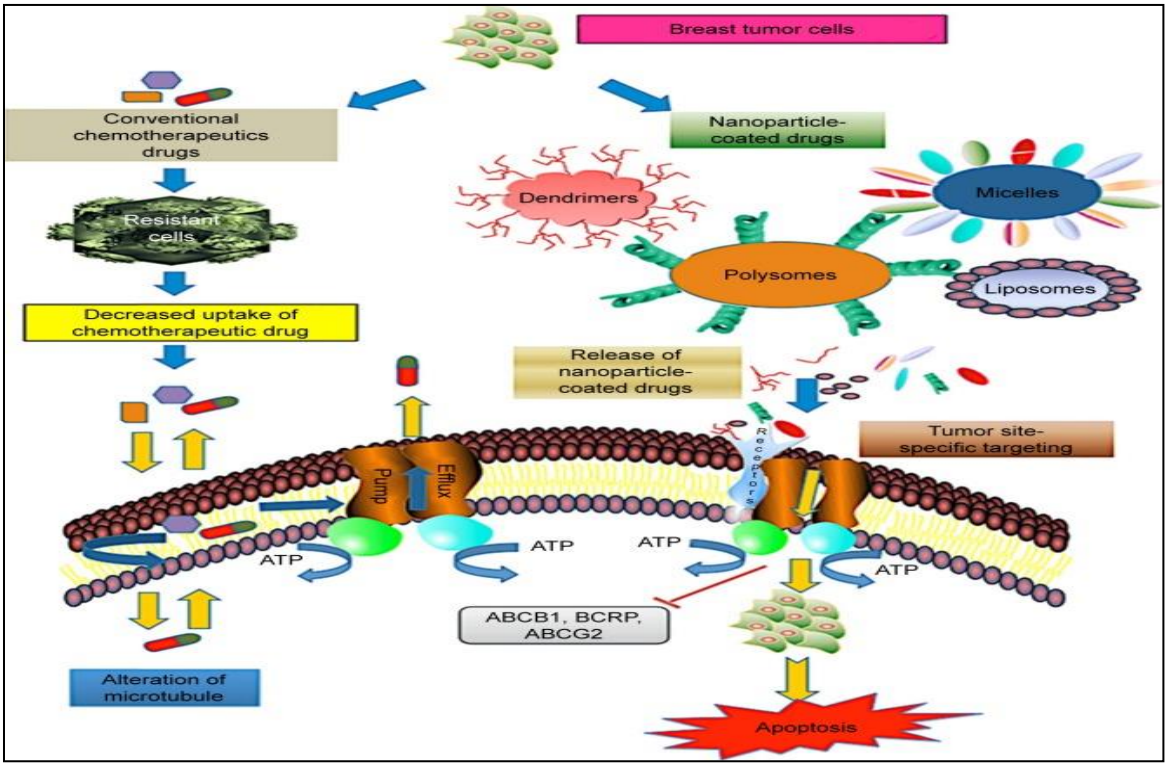


Figure 4A.2 Mechanism of drug resistant of breast cancer cell.

4A.3 Mechanism action of the drug inhibits the EGFR tyrosine kinase

In 1980, Epidermal growth factor receptor (EGFR) was known as an EGFR gene. In 1990, ErbB family discovered EGFR gene with development of inhibitors. ErbB is a protein which consist offour tyrosine kinase receptors such as Erbb-1/ EGFR, Erbb-2/ Her-2, Erbb-3 and Ebbb-4. Her-2 was an excessive expression of a gene in triple-negative breast cancer.[8,9] ErbB is associated with insufficient signal in the human body, developing different types of diseases such as Alzheimer's disease, multiple sclerosis, neurodegenerative diseases, *etc*. At the same time, ErbB is associated with an excessive signal with poor prognosis in the human body, development of different types of tumour cell such as colorectal cancer, lung cancer, endometrial cancer, breast cancer, *etc*.[10]

Schematic diagram representation of Figure 4A.3 (A) is a role aberrant EGFR and, is activated by

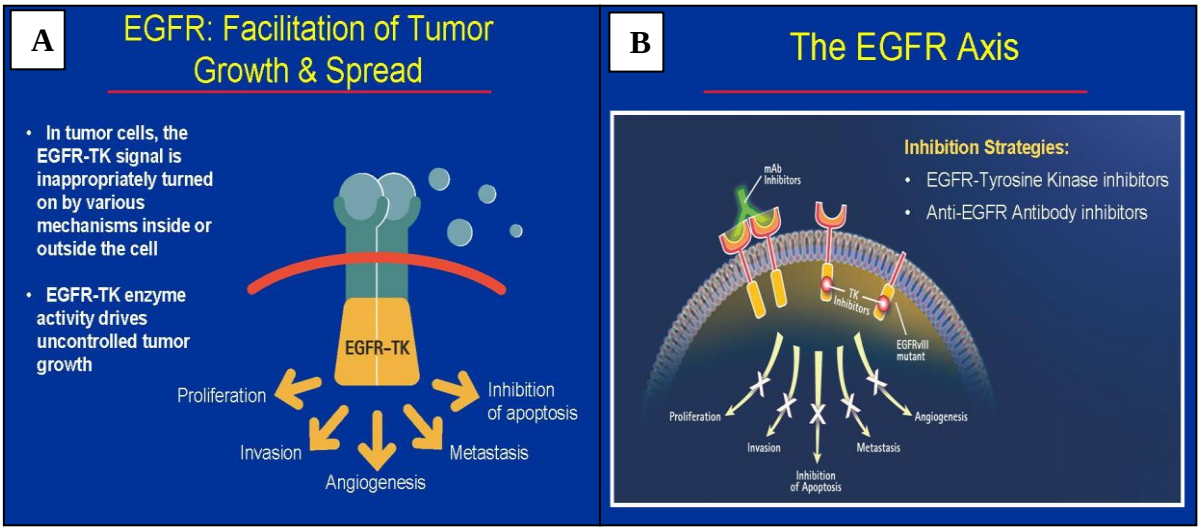


Figure 4A.3 Schematic diagram represention of (A) EGFR: Facilitation of tumor growth and spread and (B) EGFR inhibitors.

oncogenic transformation of cell. EGFR passes excessive signal and formation of uncontrolled tumour cells. In 2003, FDA approved Gefitinib and erlotinib inhibitors of EGFR tyrosine kinase for the formation of tumour cells as shown in Figure 4A.3 (B). Other inhibitors for triple-negative breast cancer are PI3K, mTOR, AKT pathway which are used in the clinical trial phase.[11]

4A.Refferences:

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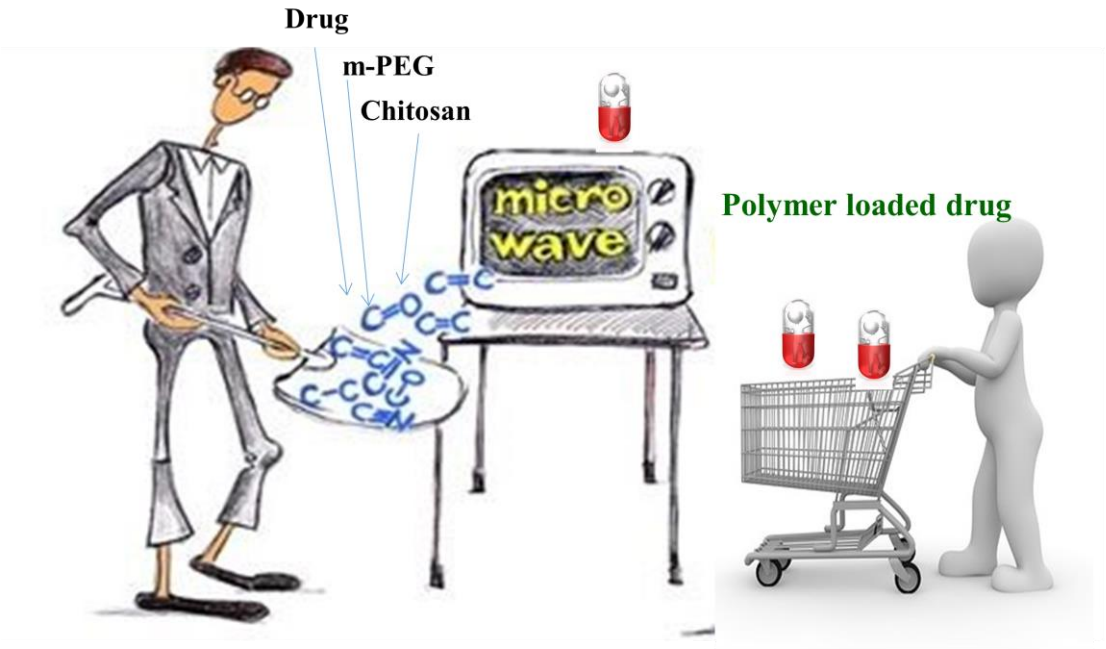
Chapter 4B

Sulfonamides encapsulation in drug delivery system



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Stuctural diversity of epidermal growth factor receptor (EGFR), single crystal XRD and biological activities of sulfonamides inhibitors containing morpholine has importance role in morpholine research. Morpholine compounds are well-known for their anti-cancer activity. Novel compounds obtained from morpholine via nucleophilic addition reactions, provide the desired products in 70 to 90% yield. The *in vitro* antitumor activity of synthesized end products, i.e., **NAM-6** and **G** were tested against MCF-7 and MDA-MB-231 of breast cancer cell line. Amongst the two end-products, sulfonamide group-containing compound **NAM-6** showed significant anti-proliferative activity with IC_{50} 1.811 µM in MCF-7 and 2.143 µM in MDA-MB-231 cells respectively, as compared to 1.883 and 4.688 µM by compound **G**. The results demonstrated that the synthesized morpholine derivatives have significant potential as anti-cancer agents and have substantial importance in the cancer therapeutics.

4B.1 Introduction

Heterocyclic chemistry is known as corner stone in medicinal chemistry.[1] Heterocyclic Compounds have been widely used in clinical applications in making of drugs, having roles in treatments of bacterial, fungal, viral infections as well as different types of tumors.[2] Nitrogen and oxygen-containing heterocyclic compounds such as morpholine are one of the most widely used heterocyclic secondary amines, used as building blocks in biologically active compounds.[3]

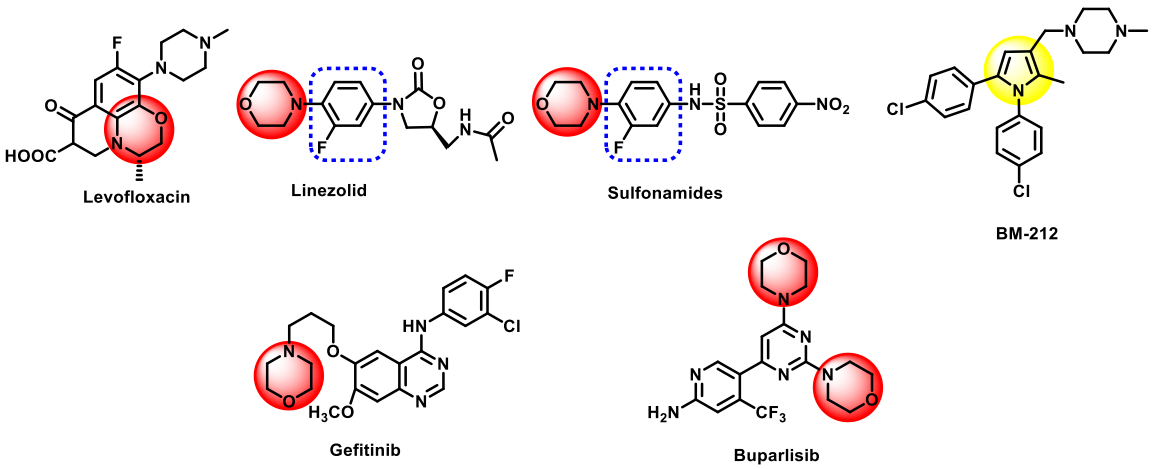


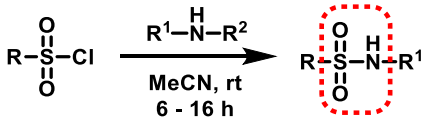
Figure 4B.1 Biologically active heterocyclic molecules.

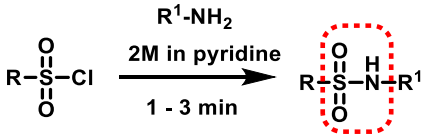
Morpholine is also an important pharmacophoric unit, and its derivatives have received considerable attention and are reported as anti-oxidant,[4] anticancer,[5] antimalarial,[6] anti-bacterial,[7] anti-fungal activities[8] and many more. Drug like Linezolid, levofloxacin, sulphonamides and BM212 containing the morpholine moiety are available in the market (Figure. 4B.1). They have also been used as potent antibacterial agents. Sulfonamides containing sulfur atom attached to nitrogen atom has improved its biological activity such as anti-tumor, diuretics, anti-inflammatory, anti-microbial, *etc*.[9-14] Apart from these compounds, several other drugs such as Gefitinib[15] and Buparlisib[16] (Figure. 4B.1) which are structurally reminiscent to N-(3-fluoro-4-morpholinophenyl)-4-methylbenzenesulfonamide **NAM-6** and **G** play an important role as anti cancer agents. Different synthesis route of gefitinib and buparlisib is previously reported but few of them are reported without substituted phenyl ring. All these observations prompted us to develop new morpholine analogues that may prove effective against breast cancer. In this study, we developed novel morpholine derivatives and studied their anti-tumor activities against MDA-MB-231 and MCF-7 cell line.

In 1977, FDA approved Tamoxifen drug which was used in women breast cancer.[17] As compared to other chemotherapeutics, it has low toxicity and is less harmful. Higher proportion use of Tamoxifen in cancer in different parts of human body made it drug resistant. Drug resistance is a major unfortunate effect of Tamoxifen.[18] Another one is anthracycline therapy, gemcitabine (GEM)[19] can enter in cancer cell as well as healthy tissue also. It means GEM shows deficiency of selectivity of cancer tissues and would damage normal tissue.[20] This drawback is reduced by targeted delivery system.[21]

Another drawbacks of anti cancer drugs is contained high proportion of drugs spreads all over the body as well as poor solubility of drugs in water.[22] After that, various methods such as surgery, chemotherapy, anthracyclic therapy, radio therapy were developed for clinical use.[23] By using these methods, healthy cells are also killed along with cancerous cells and water solubility problem is not reduced. To overcome of this problem, targeted delivery system is the best choice. It can be used to target to kill the cancer cell without any side effects on normal cells. This consists of complexation, lipid-based systems, solid dispersions (SD), micronization, nanonization, and cocrystallization. We came across some literature wherein use of hydrophilic carrier is used for hydrophobic drug. In this process drug properties can be changed. Selection process of drug carrier depends on drug properties and its activity. Whenever any carrier is used for drug delivery process, the drug toxicity might be reduced.

4B.2 Rationale of the sulfonamides molecule





Scheme 4B.1 Synthesis of sulfonamide **Scheme 4B.2** Synthesis of sulfonamides

Introduced in 1935, sulfonamides were discovered by Gerhard Domagk, and used in therapies for bacterial disease commonly known as sulfa drugs. After that a lot of procedures were developed for synthesis of sulfonamides. Out of them here two methods are explained, one discovered by J. Yan in 2007 and another by K. Bahrami in 2009. J. Yan synthesized sulfonamides from substituted sulfonyl chlorides and amine dissolved in acetonitrile solvent within 6 – 16 h.[25] K. Bahrami obtained sulfonamides from substituted sulfonyl chloride and amine in the presence of 2M pyridine within 3 min under microwave irradiation condition.[26] In first method, they got high yield but more

time was required while the second reaction was completed within 3 min but for completion of reaction, pyridine was used as a base, which is not good for human health. Therefore in our synthesis we used simple and green methods for synthesis of sulfonamide compounds which is reported in literature.[27]

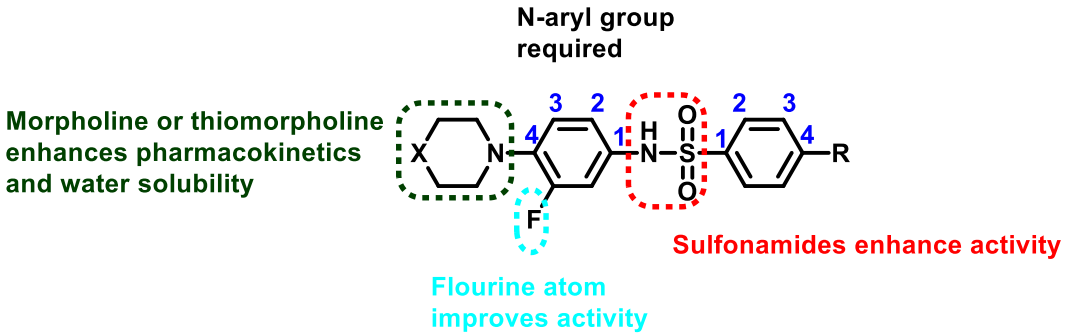


Figure 4B.2 Biologically active heterocyclic molecules

- Sulfa drugs are also used for bacterial infection, urinary tract infection, burns, malaria, pneumonia in HIV/ AIDS patients, leprosy, treatment of different types of cancer
- It is used in anti bacterial, hypoglycemic, anti carbonic anhydrase, antithyroid, diuretic activity
- F atoms improve activity.
- Sulfonyl groups attached to amine (N) enhanced the activity.
- Sulfonyl or sulfonamide group should be attached to 1,4 position on the benzene ring.
- Morpholine, piperazine, substituted piperazine, thiomorpholine, homopiperazine, piperidine all are biologically active compounds against antifungal, antibacterial, antituberculosis, antitumor, *etc*.

4B.1.1 Set of modification

1,4 position of amino group on benzene ring were substituted with- 1) piperidine 2) morpholine, 3) thiomorpholine, 4) piperazine, 5) substituted piperazine, 6) piperidine,

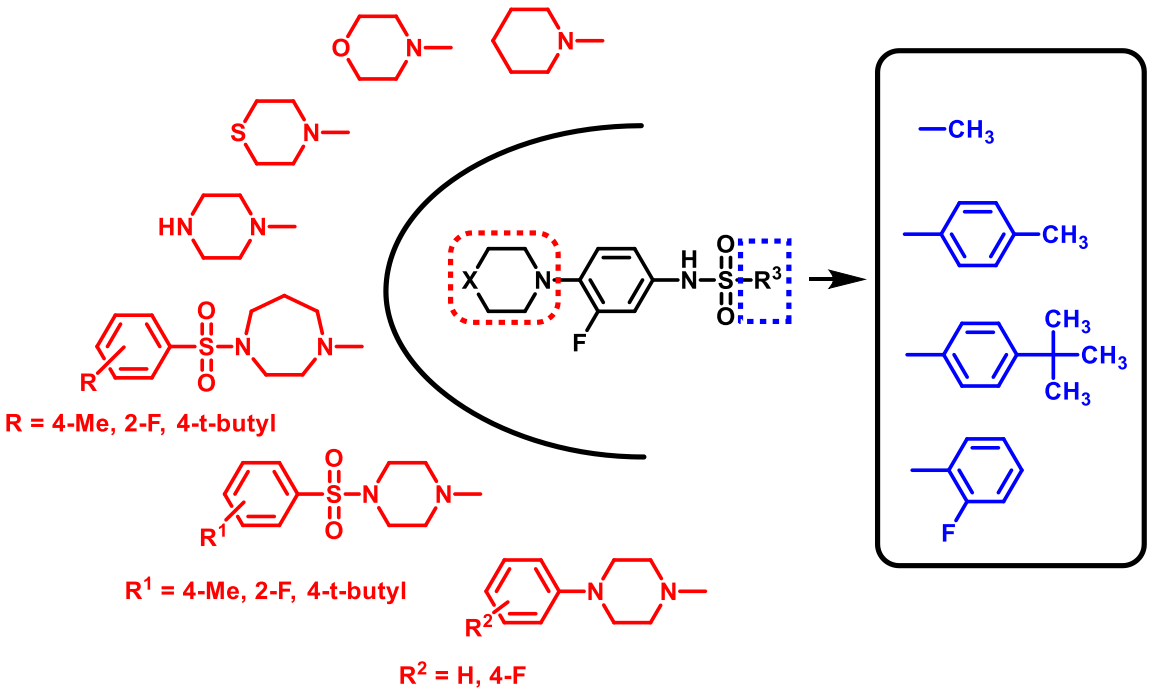


Figure 4B.3 Complete modification set of sulfonamides

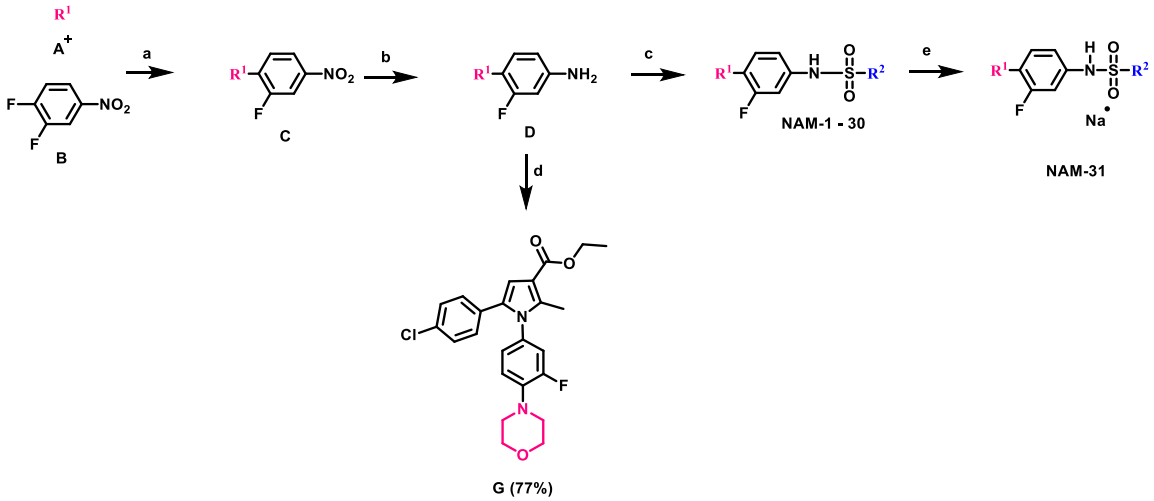
7) homopiperazine to enhance the activity. Finally, last substituent methyl group, 4-methyl benzene group, 4-tert-butyl benzene group, 2-fluoro benzene group attached to benzene ring based on hydrophobic group is a vital key for antitumor activity. Fluorine atoms like halogen atoms are most useful in medicinal chemistry which depends on electronegavity of lewis base and hydrophobic moieties.[28]

4B.3 Results and discussion

4B.3.1 Chemistry

The present study successfully showed the synthesis of novel morpholine derivatives by performing a series of reactions which afforded two novel compounds with significant anti-tumor activity in breast cancer cells. Herein, modifications were implemented to develop these novel anti-cancer compounds whose synthetic strategies have been depicted in **Scheme 4B.1**. In brief, to synthesize compound **D,** firstly intermediate compound **C** was synthesized using commercially available **A** (Spectrochem and sigma-aldrich India) and **B** (Lancaster, India). Compounds **A** and **B** were reacted together, followed by the reduction of the nitro group (NO_2) in the presence of palladium catalyst.[29] Further, structurally related sulfonamides, NAM-1 to NAM-30 were prepared by reacting **D** with *p*-toluenesulfonyl chloride (TsCl) in the presence of 1N aqueous sodium carbonate (Na_2CO_3) and water. The reaction was carried out for 30 min - 12 h at

RT, with 85 - 95% yield.[30] For the synthesis of **G**, condensation of ethyl-2-acetyl-4-(4-chlorophenyl)-4-oxobutanote (**F**) was carried out with **D**. This reaction mixture was refluxed using a catalytic amount of conc. H_2SO_4 using toluene as a solvent for 16 h. The resulting compound was obtained with a good yield (77 %). **F** used in the above reaction was synthesized according to the previously reported literature.[12] All compounds showed more than 99% of purity in HPLC, which was analyzed by C18 column (250×4.6 cm) with eluent MeOH/H_2O.



Scheme 4B.4. *Reagents and conditions: a) DMSO, 50-70°C, 2 h, 92-95%; b) Pd/C, Nitrogen pressure, anhydrous $(CH_3)_2CHOH$, 0-90°C, 3 h, 70 - 80%. c) 1N Na_2CO_3, TsCl, H_2O, RT, 30 min - 12 h, 85 - 95%; d) ethyl 2-acetyl-4-(4-chlorophenyl)-4-oxobutanoate, catalytic conc. H_2SO_4, toluene, 100°C, 16 h, 77%; e) Na metal, THF,*

4B.3.2.1 Preparation of CHS-mPEG (NAM-32-1)

In literature, different concentrations of chitosan-mPEG solution like CHS-0 (100:0), CHS-1 (90:10), CHS-2 (80:20), CHS-3 (70:30), CHS-4 (60:40) have been reported. Among them CHS-2 (80:20) is good for formation of CHS-mPEG polymer, which is proved by different methods of characterization. Here we directly used this concentration for the preparation of polymer. The synthetic route of the CHS-mPEG is shown in Figure 4B.4 Chitosan was dissolved in 2% acetic acid and ethanol. mPEG was added drop by drop in chitosan solution with continuous stirring. Reaction mixture was irradiated in microwave conditions at 60^oC for 15 min. It was then cooled and 1 ml of solution was taken in glass vial and remaining solution used for next step for drug loading. Glass vial solution was dried under lyophilization, which was confirmed by characterization with FTIR, TGA, DSC, SEM and TEM, % of element detection in SEM images.

4B.3.2 Synthesis of polymer–drug conjugates

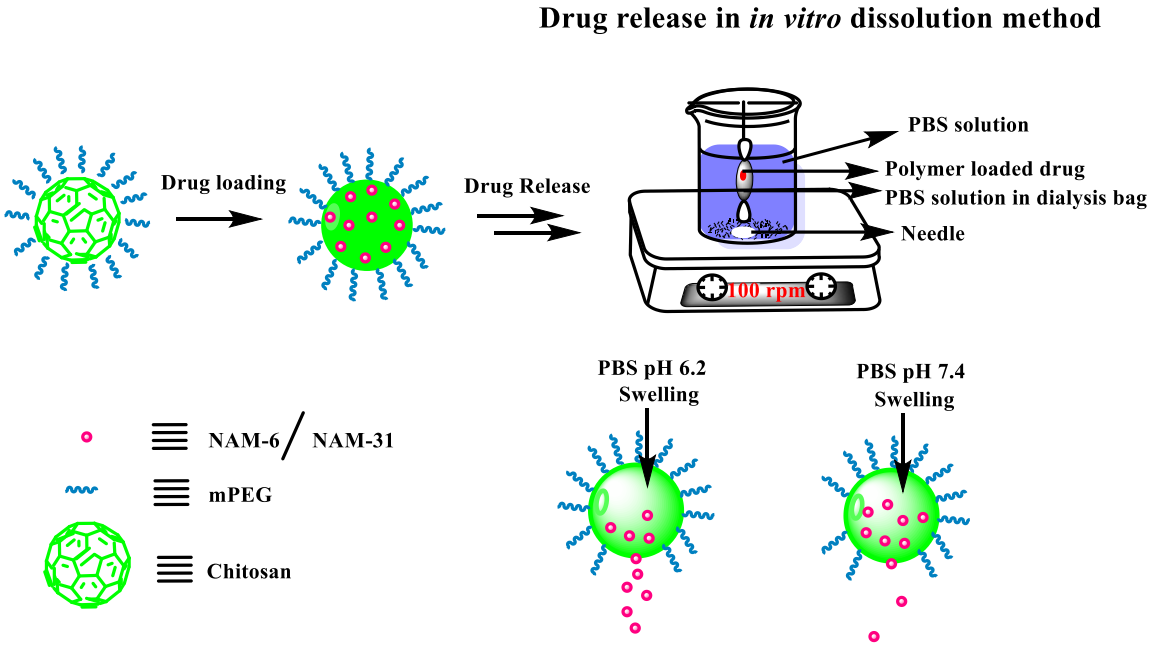


Figure 4B.4 Drug release in *in vitro* dissolution method

4B.3.2.2 Drug loading NAM-32 and NAM-33

Further, to the reaction mixture i.e. NAM-32-1 was added NAM-6 compound and once again irradiated at 60 ^{o}C for 15 min. After completion of 1 h, the reaction mixture was cooled and dried by lyophilization technique. Completion of drug loading process was confirmed by SEM, TEM images, FT-IR, TGA, DSC and % of element detection in SEM images. Same procedure was used for preparation of NAM-33 *via* NAM-31.

SEM images of before and after drug loading

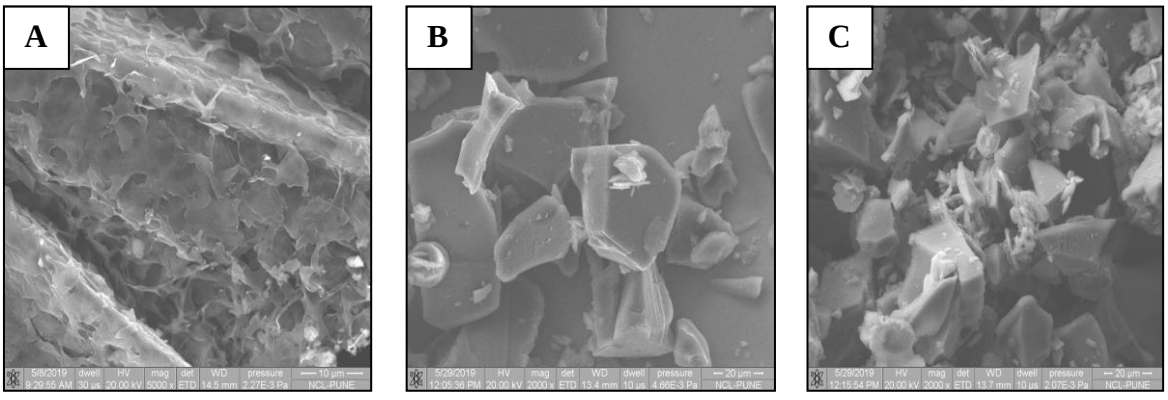


Figure 4B.5 SEM images of (**A**) Polymer before drug loaded, (**B**) NAM-6 (**C**) NAM-31

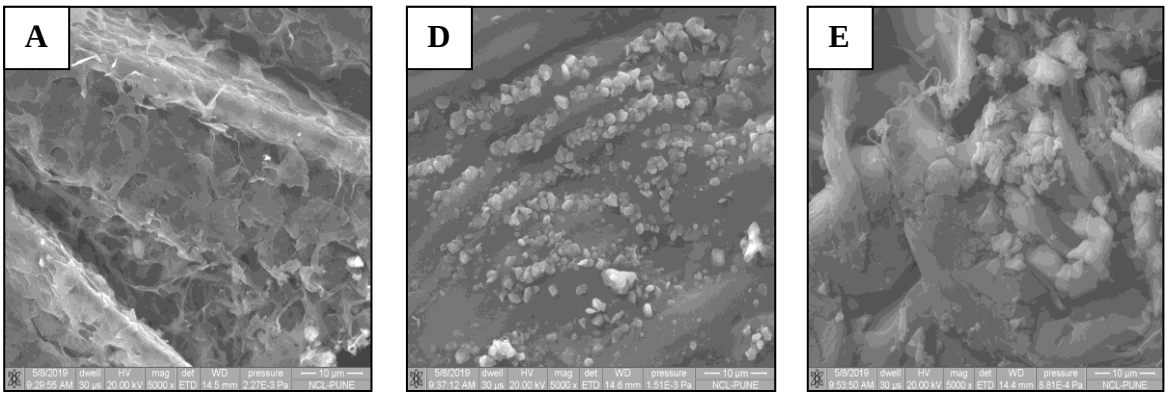


Figure 4B.6 SEM images of (**A**) Polymer before drug loaded, (**D**) Polymer loaded with NAM-6, (**E**) Polymer loaded with NAM-31

Elemental composition

Elemental analysis is used to determine the compositions of element present in compounds. The Table 4B.1 shows the C, O and N elements with its % of weight present in polymer.

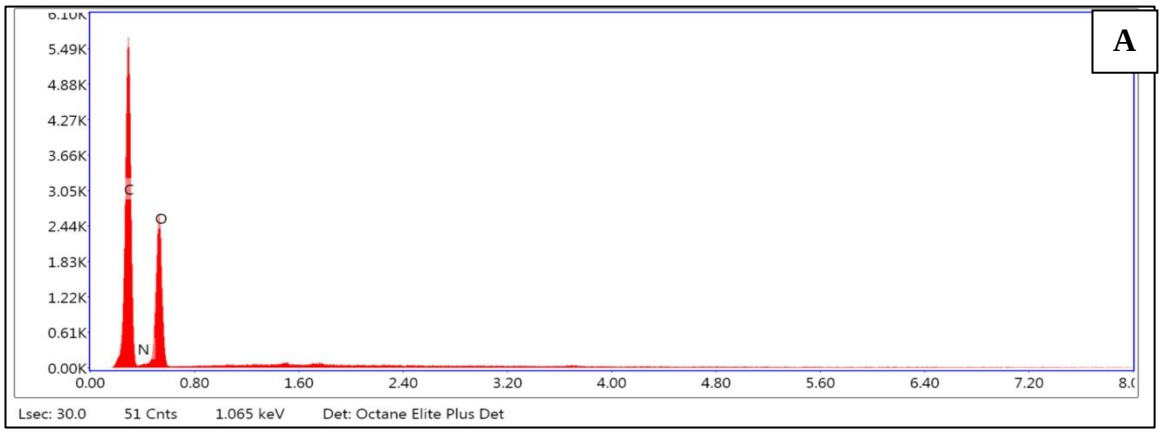


Figure 4B.7 Elemental analysis of Polymer before drug loaded

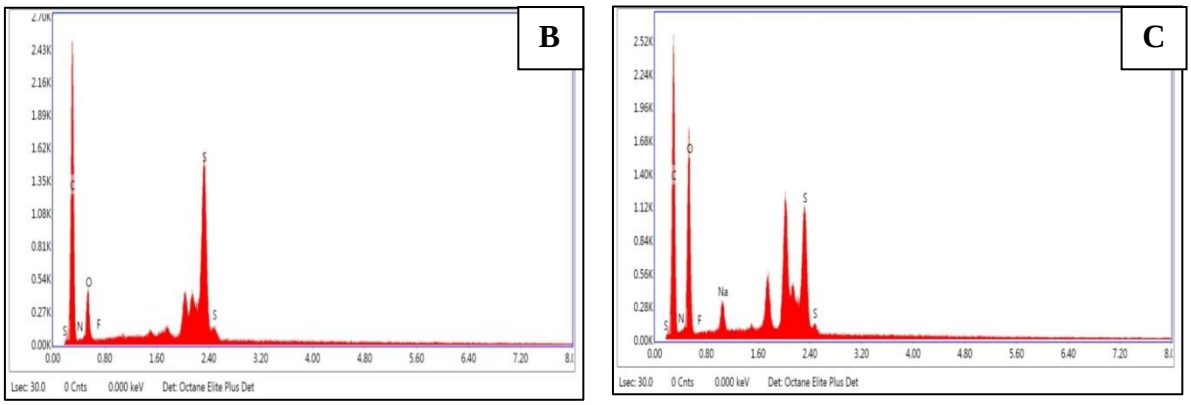


Figure 4B.8 Elemental analysis of NAM-6 and NAM-31

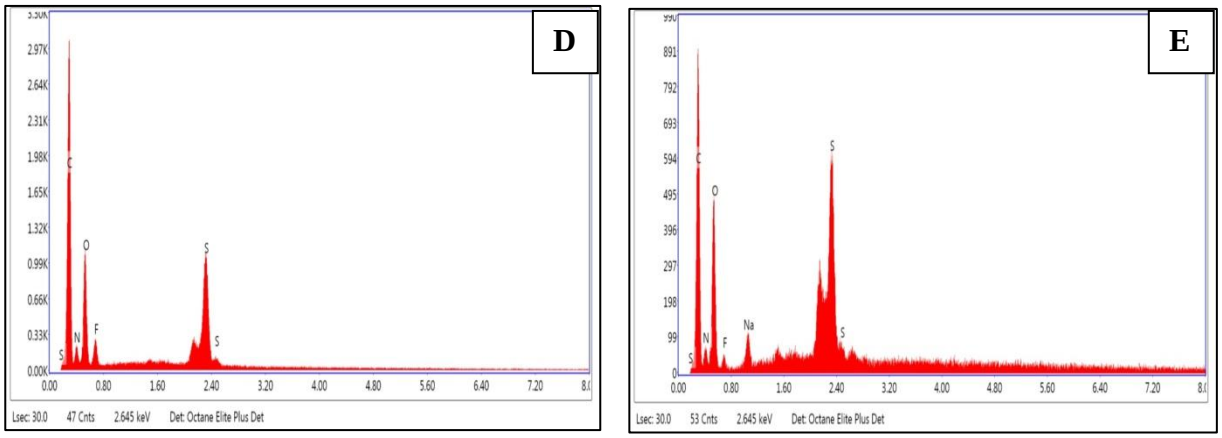


Figure 4B.9 Elemental analysis of polymer loaded with (**D**) NAM-6 and (**E**) NAM-31

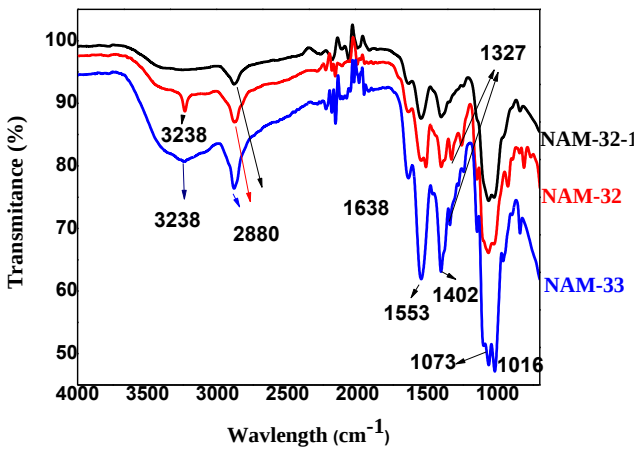
Table 4B.1 Experimental atomic percentage of compounds

No.	Name	% of weight of Elements					
		C	N	O	F	Na	S
1	Polymer	47.13	7.60	45.27	-	-	-
2	NAM-6	66.74	5.61	15.64	1.10	-	10.92
3	NAM-31	49.18	3.81	34.57	1.67	3.28	7.49
4	Polymer+ NAM-6	51.59	11.83	24.50	5.45	-	6.63
5	Polymer+ NAM-31	53.62	4.66	27.97	1.18	1.13	11.43

The elemental composition analysis is used to detect the % of weight of C, H, N, O, Na, S *etc* present in polymer or polymer loaded drugs and the results are shown in table 4B.1.

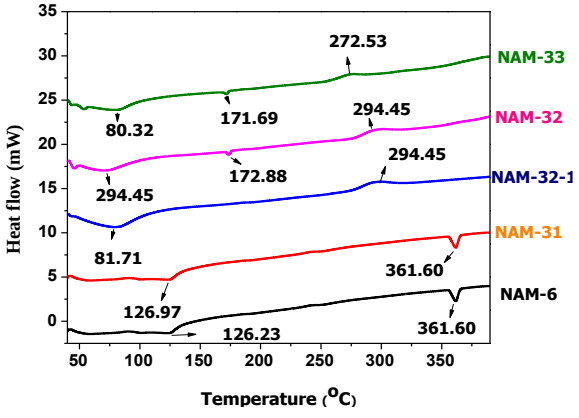
FTIR

FTIR spectroscopy was used to determine interactions between NAM-32-1 and NAM-32 as well as NAM-33. All of the bonds correspond to polymer and polymer are present in NAM-32 and NAM-33 which is occurring from NAM-6 and NAM-31 respectively. A band observed at 2888 cm^{-1} corresponds to C-H asymmetric and symmetric stretching. 1638 cm^{-1} C=O band attached to N atom in N-acetyl group, 1553 cm^{-1} corresponds to secondary N-H bending of amide, asymmetric stretching of S=O observed at 1327 cm^{-1}. A strong band in the region of 3238 cm^{-1} corresponds to N-H group present in NAM-32 and NAM-33 as compared with NAM-32-1. Band at 1073 cm^{-1} corresponds to C-H bending vibration and 1016 cm^{-1} corresponds to C-OH present, both peaks are observed in NAM-32-1 as well as polymer loaded drugs NAM-32 and NAM-33. By observation of FTIR it was found that both the drugs are completely loaded in polymer.



Thermal stability- DSC

DSC is a thermal stability process. DSC is used to measure heat absorbed. The first DSC signal is endothermic. The temperature appears at 79.42 ^{o}C, 76.59 ^{o}C and 80.32 ^{o}C for polymer (NAM-32-1), polymer loaded NAM-6 drug (NAM-32) and polymer loaded NAM-31 drug (NAM-33), respectively. The exothermic temperature appears at 294.45 ^{o}C, 294.45 ^{o}C and 272.53 ^{o}C for polymer (NAM-32-1), polymer loaded NAM-6 drug (NAM-32) and polymer loaded NAM-31 drug (NAM-33). Decomposition of compounds is indicated by endothermic process. The exothermic signals indicate chemical reactions obtained during heating process in DSC studies. NAM-6 and NAM-31 drugs show only endothermic peaks but when drugs are loaded in polymer they show endothermic and exothermic



signals. DSC is used to determine that both drugs are completely encapsulated in polymer because there is no presence of drug signals and only polymer signals are observed.

4B.3.2.3 Drug release

In vitro drug release of sulfonamide drugs loaded in chitosan and m-PEG polymer were studied by dialysis bag method. Polymer loaded drug was placed into dialysis bag and this bag was kept in a beaker containing 100 ml of PBS (phosphate buffer saline) of different pH such as 6.2 and 7.4. This all assembly was kept on magnetic stirrer and stirred it at room temperature and speed maintained at 100 rpm. After each 1 h 2 ml of sample withdrawn from beaker and at a same time added equal amount of fresh PBS solution of same pH in same beaker. After appropriate dilution, samples were analyzed by UV visible spectrometer at 257 nm for NAM-6 and 228 nm for NAM-31. *In vitro* drug release data analyzed by kinetic models showed cumulative % of drug release against time.

In Figure 4B.10 shows a graph of *in vitro* drug release of polymer loaded drug NAM-6 in which 89.77% drug was released at pH 6.2 and 59.87% at pH 7.4 in PBS solution. In case of NAM-31 drug release was 54.13% in pH 6.2 and 63.17% in pH 7.4 in PBS solution. Observation of drug release study at different pH showed that pH 6.2 showed high % of drug release as compared to pH 7.4.

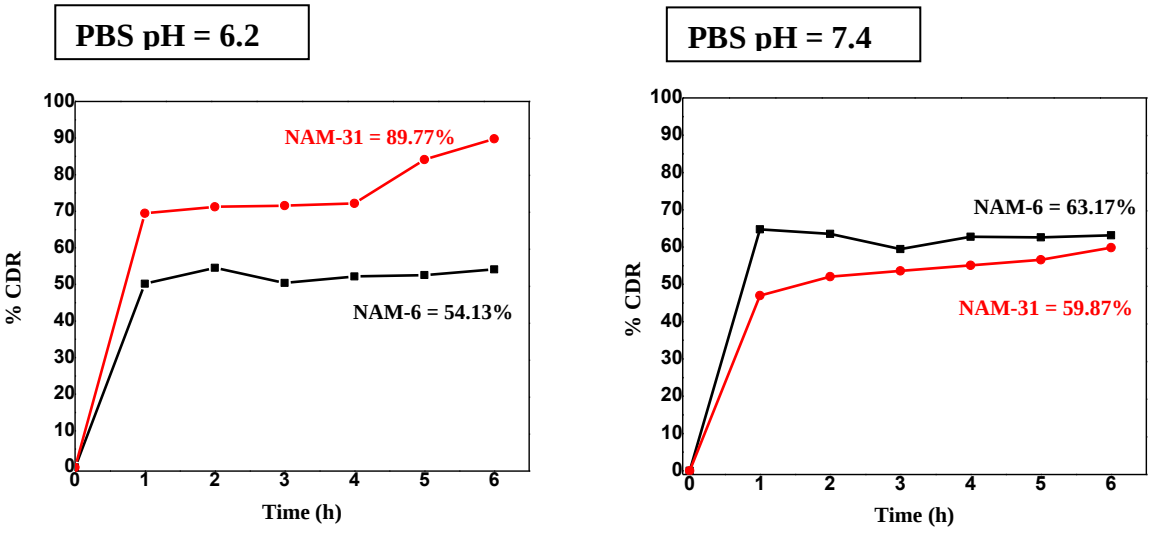
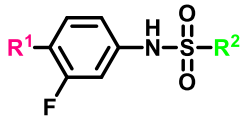
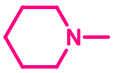
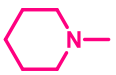
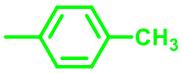
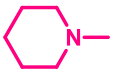
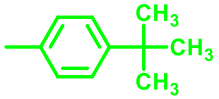
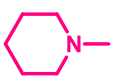
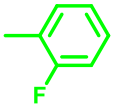
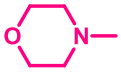
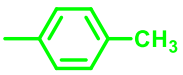
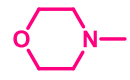
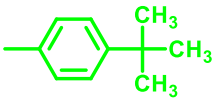
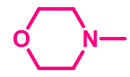
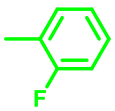
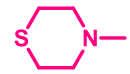
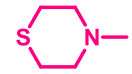
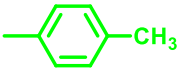
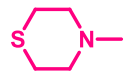
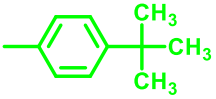
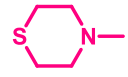
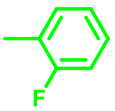


Figure 4B.10. Comparison of in vitro dialysis release methods of sulfonamides encapsulated chitosan-mPEG

4B.2 Table Formation of sulfonamides derivatives



Compound code	R^1	R^2	M.P (°C)
NAM-1			101.2
NAM-2			150.2
NAM-3			153.7
NAM-4			132.0
NAM-5			149.5
NAM-6			166.1
NAM-7			172.1
NAM-8			135.0
NAM-9			162.6
NAM-10			170.6
NAM-11			134.1
NAM-12			146.5
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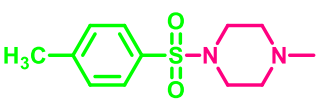
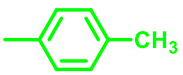
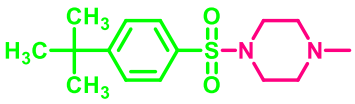
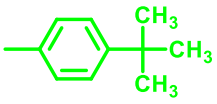
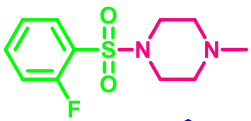
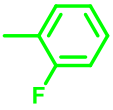
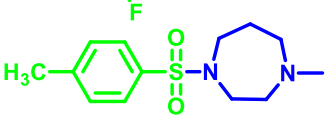
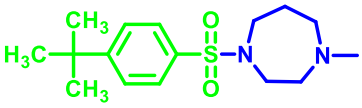
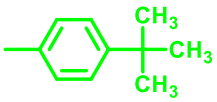
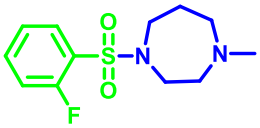
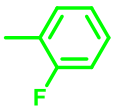
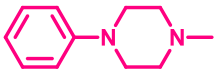
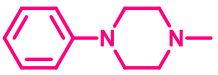
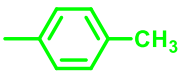
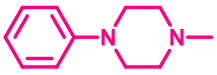
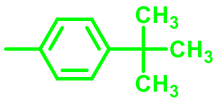
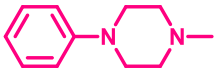
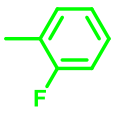
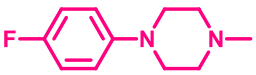
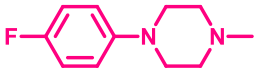
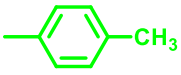
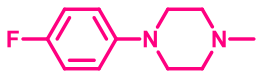
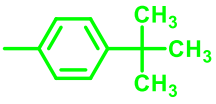
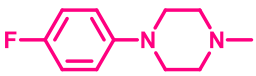
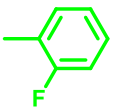
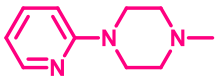
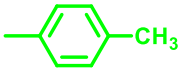
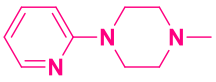
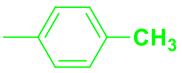
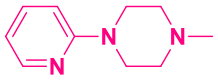
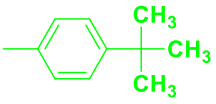
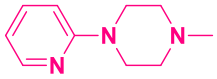
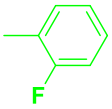
NAM-14			122.9

Compound code	R^1	R^2	M.P (°C)
NAM-15			165.4
NAM-16			156.4
NAM-17			160.2
NAM-18			162.5
NAM-19			139.1
NAM-20			156.9
NAM-21			178.4
NAM-22			167.2
NAM-23			168.2
NAM-24			182.3
NAM-25			185.8
NAM-26			163.7
NAM-27			167.8
NAM-28			145.9

Compound code	R^1	R^2	M.P °C)
NAM-28			175.3
NAM-29			161.2
NAM-30			165.3

4B.3.3 Biological evaluation

The synthesized morpholine derivatives were tested for their anti-cancer activity. Cytotoxic effects of the synthesized compounds on the MTT assay was used for cell viability study. MTT reagent cleaves the living cells and thus dark purple color formazan crystals are formed. These crystals, when dissolved in DMSO, produce purple color, which is indicative of cell viability. After that cell lines of MCF-7 and MDA-MB-231 were incubated at different proportions of compound **NAM-6** and **G** and studied for their anti-cancer activity. The results showed that MCF-7 cells when treated with **NAM-6**, displayed significant toxicity as compared to compound **G** (**Figure 2**). Similar results were obtained when MDA-MB-231 cells were treated with **NAM-6** and **G** (**Figure 2**). The percent cell viability was found to be 37.79 % at the highest concentration (200 µg/mL) when MDA-MB-231 cells were treated with compound **NAM-6,** while 54.19 % viability was found when treated with **G.** Significant cytotoxicity was observed in MCF-7 cells when treated with compound **G**, the percent viability was found to be 35.46 % at the highest concentration (200 µg/mL), while the viability was significantly reduced (20.27 %) when treated with compound **NAM-6**. Compound **NAM-6** reflected higher anti-proliferative activity as compared to **G** in both the cell lines which could be attributed to the presence of sulfonamide group in compound **NAM-6**. The half maximal inhibitory concentration (IC_{50}) values for **G** and **NAM-6** in MCF-7 cells were found to be 1.883 and 1.811, respectively. Moreover, in MDA-MB-231 cells, **G** and **NAM-6** showed IC_{50} values 4.688 and 2.143, respectively.

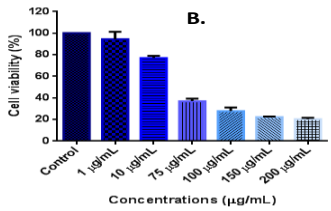
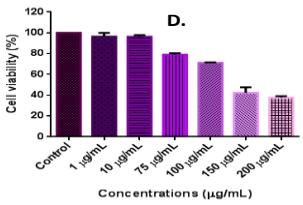
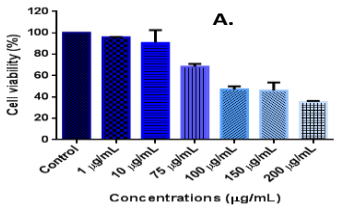
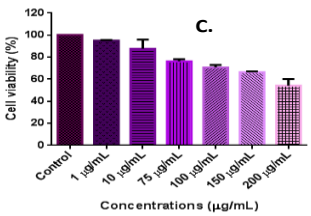
Code	Breast cell lines (anti-proliferative activity) (IC_{50})		Cell viability (200 µg/ml)	
	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231
NAM-6	(IC_{50}) 1.811	(IC_{50}) 2.143	20.27% 	37.79% 
G	(IC_{50}) 1.883	(IC_{50}) 4.688	35.46% 	54.19% 

Table 4B.3 Cell viability of (A, B) compounds **G** and **NAM-6** in MCF-7 cells. (C, D) compounds **G** and **NAM-6** in MDA-MB-231 cells, respectively.

4B.3.4 Molecular docking analysis

Molecular docking analysis was performed to determine the structural features that might steer the biological profile of substituted morpholine and its novel analogues. As stated earlier, the mechanism of action for anti-cancer activity involves interaction of sulfonamides with hydrophobic residues; therefore, molecular docking analysis was performed to determine the structural features that can potentially govern the anti-cancer activity of present series molecules.

In 2005, discovered by Jiang, Gefitinib drug is known to inhibit EGFR kinase to produce anticancer effect against cancer cell like breast cancer and lung cancer. Gefitinib possesses an active morpholine substituent. Interestingly, compounds synthesized in present series also possess morpholine moiety. Therefore, EGFR can be considered as

potential site of action for given series of compounds. As per that result the structure similarity of our compound with Gefitinib, EGFR kinases have been used in docking analysis as targets for sulfonamides. AutoDock was used for molecular docking. In that study we assume ligands might be attached on this receptor and stop growth of cancer cell division.

Table 4B.4 summarizes the binding free energy values of ligands in complex with EGFR. It also features the residue and its corresponding atoms involved in hydrogen bonding along with the name of atom from ligand participating in hydrogen bonding is also tabulated. Hydrogen bonding distance is recorded for each polar contact. Residues involved in nonpolar contacts like hydrophobic contacts or van der waals contact are listed in separate column. Inhibitor-protein complexes are stabilized by force including polar interactions or van der waals contact. The docking analysis was performed for all the compounds, but for the sake of convenience, we present the docking poses for compounds NAM-17, NAM-23, NAM-27 and NAM-30 as representatives. The binding energy of standard compound Gefitinib is -5.61kcal/mol and the binding energy of NAM-17, NAM-23, NAM-27 and NAM-30 is observed to be -7.95, -7.80, -7.58 and -7.69 Kcal/mol respectively. Therefore, these compounds can be considered as potential candidates for further optimization of anticancer activities.

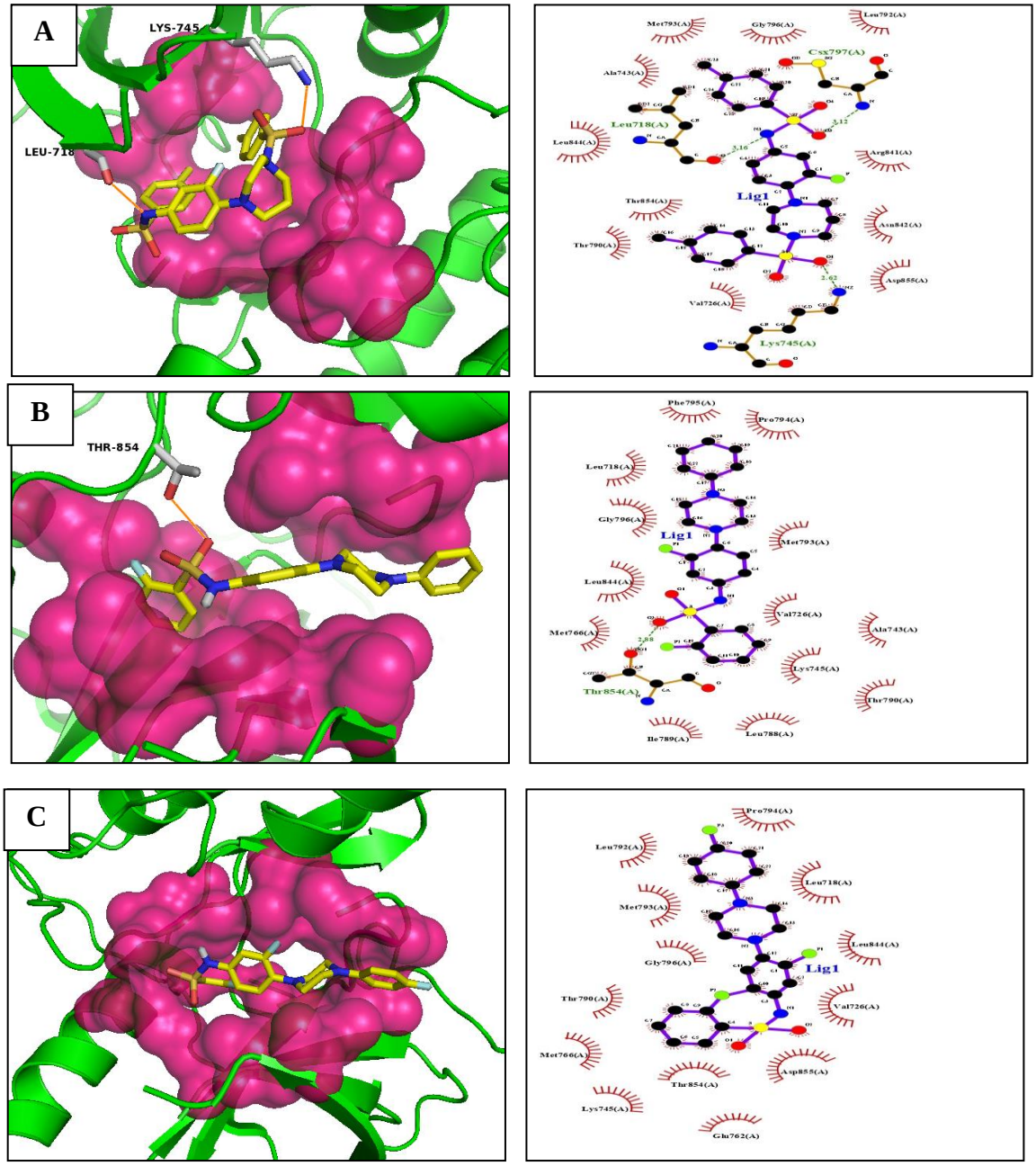
4B.3.4.1 Silico analysis

Tyrosine kinase domain is generally inhibited in epidermal growth factor receptor (EGFR) in cancer cell.

4B.3.4.2 Role of residues in sulfonamides mediated EGFR inhibition

Morpholine is a major structure content of drug Gefitinib. Gefitinib is a EGFR kinase inhibitor, structurally similar to NAM-5 to NAM-8 molecules. Therefore, we hypothesize that morpholine derivatives in present study might inhibit EGFR kinase activity. We performed virtual screening of 30 analogues against tyrosine kinase domain of EGFR and observed that residues like Lys-716, Leu-718, Arg-841, Arg-842, Lys-745, Met-793, Pro-794, Gly-796, Asn-800, Asn-842, Thr-854, Asp-855 form effective hydrogen bonds with present set of compounds (Figure 4B.11). Among them, Met-793, Pro-794, Arg-847 and Thr-854 interact with the hinge region of compounds.

These residues form EGFR kinase contact with N1 lobes of sulfonamides ligand or some inhibitors like gefitinib, TAK-285, WZ-4002 and AFN-941 and it is considered as an important feature in inhibition of EGFR kinase. Leu-718, Pro-794, Asp-800 and Asn-842 interact with N3 lobes of ligand. EGFR is irreversibly inhibited by irreversible inhibitor *via* interaction with residues like Thr-854, Asp-855 and Met-793. The mechanism of action involves an active nucleophilic attack towards these residues by the F electrophilic lobes of the compounds. Fluorine atom is located at meta position to the sulfonamides. Lys-745, Thr-854, Met-793, Lys-716 are observed to provide hydrophobic interaction in sulfonamides as similar in gefitinib.



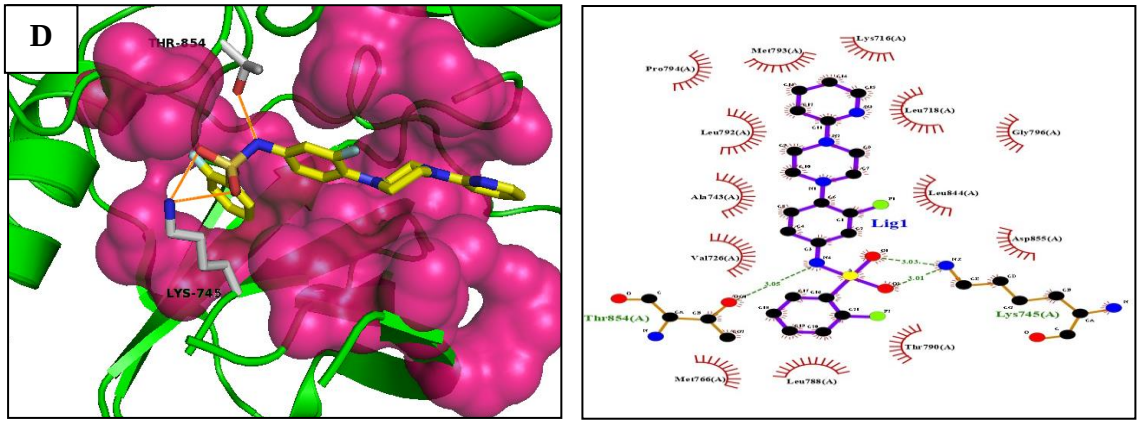


Figure 4B.11 Docked photographs showing polar interaction of (A) NAM- 17, (B) NAM-23, (C) NAM-27, (D) NAM-30 with different residues.

Table 4B.3

Ligand code (Name)	Binding free Energy (Kcal/mol)	Receptor	Ligand	Distance	Residues in hydrophobic interaction or Van der walls contact
Gefitinib	-5.61	-	-	-	
NAM-1	-5.89	Pro-794 : O	N1	2.53	Lys-716, Leu-718, Val-726, Lys-728, Ala-743, Leu-792, Met-793, Gly-796, Leu-844
NAM-2	-5.31	Lys-745 : NZ Thr-854 : OG1	O1 O2	2.73 3.32	Leu-718, Val-726, Glu-762, Met-766, Leu-788, Thr-790, Leu-792, Met-793, Gly-796, Asp-855
NAM-3	-6.46	Thr-854 : OG1 Asp-855 : N	F F	2.59 2.71	Leu-718, Val-726, Lys-745,Glu-762, Met-766, Arg-841, Asn-842, Leu-844, Leu-788, Thr-790, Met-793, Gly-796, Csx-797,

NAM-4	-6.77	Thr-854 : OG1 Lys-745 : NZ	O2 O1	2.64 2.99	Leu-718, Val-726, Ala-743, Met-766, Leu-788, Ile-789, Thr-790, Leu-792, Met-793, Gly-796, Leu-844
NAM-5	-6.61	Met-793 : N	O1	3.08	Leu-718, Val-726, Ala-743, Lys-745, Glu-762, Met-766, Leu-792, Leu-844, Thr-854, Asp-855,
NAM-6	-6.60	Met-793 : N	O1	3.07	Leu-718, Val-726, Ala-743, Lys-745, Glu-762, Met-766, Leu-792, Pro-794, Gly-796, Leu-844, Thr-854, Asp-855
NAM-7	-6.56	Met793 : N Met793 : O	O2 N1	2.83 3.12	Leu-718, Val-726, Ala-743, Lys-745, Thr-790, Leu-792, Gly-796, Csx-797, Asp-800, Leu-844, Thr-854, Asp-855
NAM-8	-5.87	Lys745 : NZ	O1	2.60	Leu-718, Val-726, Ala-743, Ile-744, Leu-788, Thr-790, Gly-796, Csx-797, Leu-844, Asp-855
NAM-9	-5.35	Thr854 : OG1 Met793 : O Gly796 : N	N1 N1 O2	3.03 2.70 2.73	Leu-718, Val-726, Ala-743, Lys-745, Met-766, Leu-792, Pro-794, Phe-795, Leu-844, Thr-854
NAM-10	-6.11	Met793 : N	O1	2.80	Leu-718, Val-726, Ala-743, Thr-790, Leu-792, Gly-796, Leu-844, Thr-854,
NAM-11	-6.52	Met793 : N	O2	2.60	Leu-718, Ala-743, Lys-745, Thr-790, Leu-792,

					Phe-795, Gly-796, Csx-797, Asp-800, Leu-844, Thr-854
NAM-12	-6.88	Lys745 : NZ Lys745 : NZ Thr854 : OG1	O1 O2 N1	3.05 3.05 3.13	Leu-718, Val-726, Ala-743, Met-766, Leu-788, Thr-790, Leu-792, Met-793, Gly-796, Leu-844, Asp-855,
NAM-13	-6.18	Met793 : N Asn842 : OD1	O1 N3	3.15 2.73	Lys-716, Leu-718, Leu-792, Pro-794, Gly-796, Arg-841, Leu-844, Thr-854, Asp-855
NAM-14	-7.05	Leu718 : O Lys745 : NZ	N3 O2	2.62 2.95	Val-726, Glu-762, Met-766, Leu-777, Leu-788, Ile-789, Thr-790, Pro-794, Phe-795, Gly-796, Asp-800, Leu-844, Asp-855
NAM-15	-6.78	Met793 : N Asp800 : OD2	O1 N3	3.09 2.69	Lys-716, Val-717, Leu-718, Val-726, Ala-743, Leu-792, Pro-794, Gly-796, Csx-797, Leu-844
NAM-16	-7.11	Thr854 : OG1	O4	2.87	Leu-718, Val-726, Ala-743, Lys-745, Met-766, Leu-788, Ile-789, Thr-790, Phe-795, Gly-796, Csx-797, Asp-800, Glu-804, Leu-844, Asp-855
NAM-17	**-7.95**	**Leu718 : O Lys745 : NZ Csx797 : N**	**N3 O1 O3**	**3.16 2.62 3.12**	**Val-726, Ala-743, Thr-790, Leu-792, Met-793, Gly-796, Arg-841, Asn-842, Leu-844, Thr-854, Asp-855**
NAM-18	-7.35	Met793 : N	O1	2.98	Leu-718, Gly-719, Ser-

		Asn842 : OD1	N3	2.81	720, Gly-724, Thr-725, Val-726, Ala-743, Thr-790, Leu-792, Gly796,Csx797, Asp800, Arg841, Leu844, Thr854, Asp855
NAM-19	-6.68	Leu718 : O Lys745 : NZ Lys-745 : NZ Csx797 : N	N3 O2 F2 O3	3.06 2.65 3.20 3.14	Gly719, Val726, Ala743, Met793, Gly796, Leu844, Thr854, Asp855
NAM-20	-6.67	Lys716 : NZ Met793 : N Pro794 : O	O2 F N3	3.12 3.27 2.62	Leu718, Val726, Leu792, Ala743,Gly796, Leu844, Thr854
NAM-21	-6.69	Lys716 : NZ Pro794 : O	O2 N3	2.90 2.47	Leu718, Val726, Lys728, Ala743, Lys745, Leu792, Met793, Gly796, Leu844, Thr854
NAM-22	-7.30	Csx797 : OD Arg841 : O	N1 N1	2.91 3.25	Lys716, Leu718, Val726, Lys728, Lys745, Leu792, Met793, Pro794, Gly796, Leu844, Lys745, Thr854
NAM-23	**-7.80**	**Thr854 : OG1**	**O2**	**2.88**	**Leu718, Val726, Ala743, Lys745, Met766, Leu788, Ile789, Thr790, Met793, Pro794, Phe795, Gly796, Leu844**
NAM-24	-6.68	Lys716 : NZ Lys745 : NZ Pro794 : O	O2 F2 N3	3.02 3.00 2.52	Leu718, Val726, Lys728, Ala743, Leu792, Met793, Gly796, Leu844, Thr854
NAM-25	-7.39	Lys745 : NZ	O1	2.81	Leu718, Val726, Ala743, Ile744, Met766, Leu790, Ile789, Thr790, Leu792,

					Met793, Pro794, Gly796, Leu844, Thr854, Asp855
NAM-26	-7.41	Csx797 : OD	N1	2.92	Lys716, Leu718, Val726, Lys728, Lys745, Thr790, Leu792, Met793, Pro794, Gly796, Arg841, Leu844, Thr854
NAM-27	**-7.58**	**-**	**-**	**-**	**Leu718, Val726, Lys745, Glu762, Met766, Thr790, Leu792, Met793, Pro794, Gly796, Leu844, Thr854, Asp855**
NAM-28	-6.91	Leu718 : O Lys745 : NZ Thr854 : OG1	N3 O1 N4	2.92 2.64 3.29	Ala743, Ile744, Leu788, Ile789, Thr790, Gly796, Csx797, Asp800, Leu844, Asp855
NAM-29	-7.15	Csx797 : N	F	2.59	Lys716, Leu718, Val726, Lys745, Glu762, Met766, Thr790, Leu792, Met793, Pro794, Gly796, Leu844, Thr854, Asp855
NAM-30	**-7.69**	**Lys745 : NZ** **Lys745 : NZ** **Thr854 : OG1**	**O1** **O2** **N4**	**3.03** **3.01** **3.05**	**Lys716, Leu718, Val726, Ala743, Met766, Leu788, Thr790, Leu792, Met793, Pro794, Gly796, Leu844, Asp855**

4B.4 Conclusion

We have synthesized number of thirty one of novel sulfonamide derivatives, by employing a convenient and straightforward N-alkylation of the amine with tosyl chloride or nucleophilic addition reaction and cyclization reaction methodology. The successfully produced final compounds, **G** and **NAM-6** were assessed for their anti-

proliferative activity in MCF-7 and MDA-MB-231 cells. Significant anti-proliferative activity was shown by compound **NAM-6** in MCF-7 as compared to **G** in MCF-7 as well as MDA-MB-231 cells. Interestingly, both the compounds did not show significant toxicity in MDA-MB-231 cells as that found in MCF-7 cells. Overall, this data demonstrates that the synthesized novel morpholine derivatives have the potential to be used as anti-cancer agents in breast cancer and can be studied in different types of cancers as well.

4B.4 Experimental section

All reactions were performed by standard syringe septa technique under an inert atmosphere. The solvents were purified and dried by conventional methods before use. Column chromatography was performed on silica gel (60-120) or (230-400) with ethyl acetate (EtOAc) or hexane as eluents. The progress of all reactions was monitored by thin layer chromatography (TLC) on alumina sheets precoated with silica gel 60F254 to a thickness of 0.5 nm (Merck, Germany KGaA) and locating the spots using UV light or iodine vapors as the visualizing agent. Melting points were obtained by an open capillary method and were uncorrected. Percent purity was measured with HPLC using a C-18 column. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on 200 and 50 MHz spectrometers at ambient temperature using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were measured in delta scale, i.e. parts per million (ppm). The splitting pattern abbreviations were designated as singlet (s), doublet (d), double doublet (dd), triplet (t), the quartet (q) and multiplet (m). The HRMS mass spectra were recorded on thermo scientific Q-TOF exactive (YA-105) spectrometer in ESI mode.

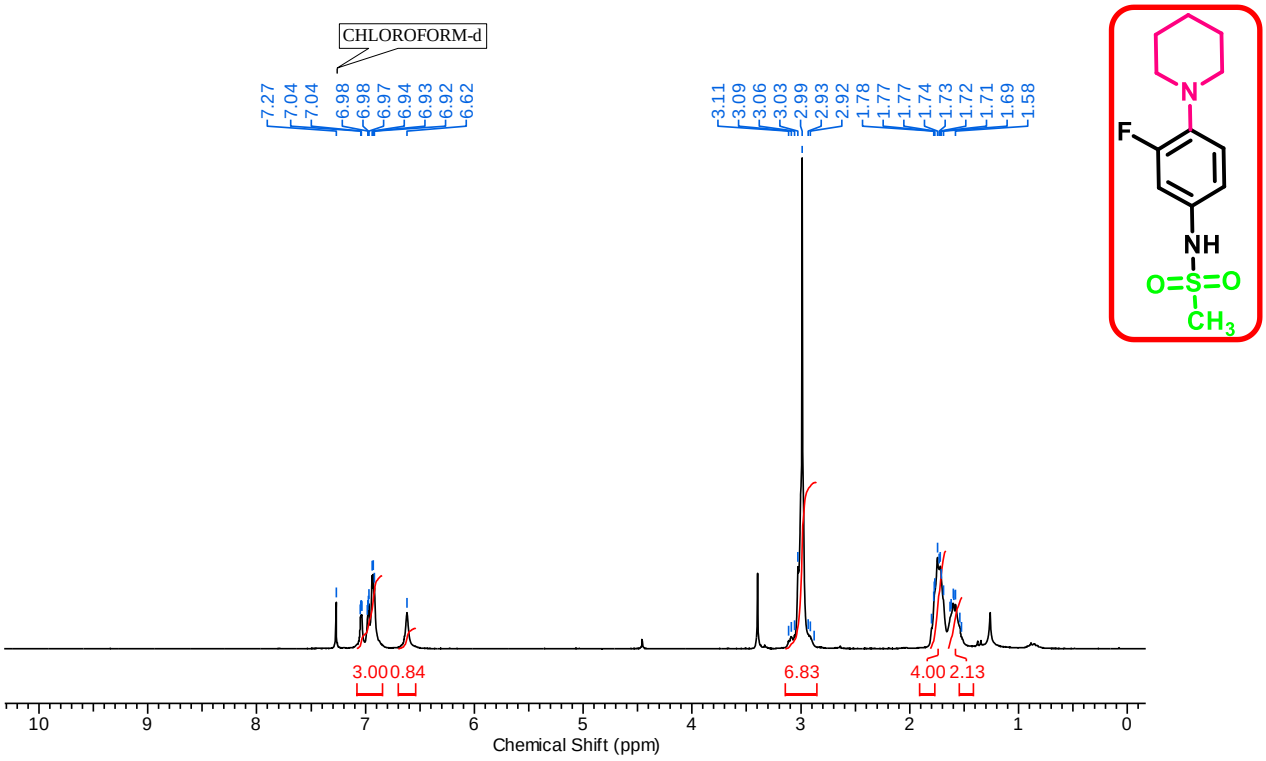
Substituted nitro (3)

To a warm solution of morpholine **(1)** (3.30 mL, 37.70 mmol) in DMSO (40 mL), 1,2-difluoro-4-nitrobenzene **(2)** (2.00 mL, 18.85 mmol) was added dropwise. The solution was stirred at 70 °C for 2 h, cooled to rt, diluted with EtOAc, washed with water, brine, dried over anhydrous sodium sulfate (Na_2SO_4), and filtered. The solvent was extracted *in vacuo* to obtain the title compound.

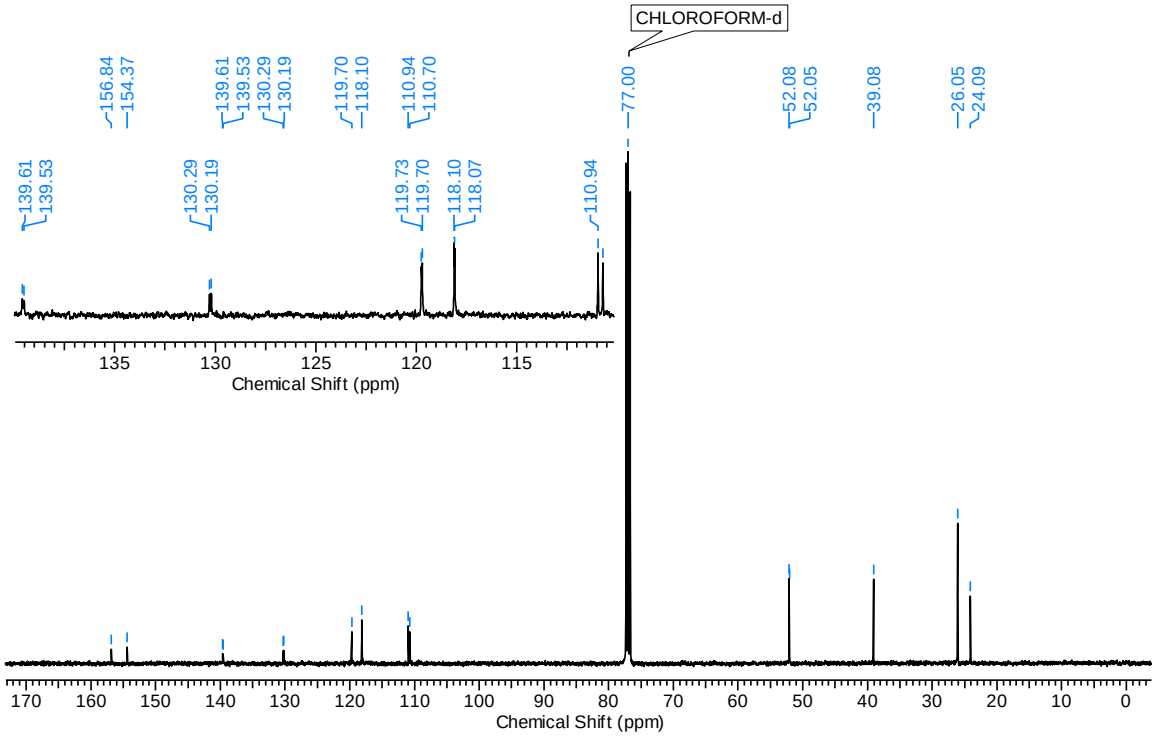
Aniline (4)

Palladium catalyst (Pd/c) (5 mmol) was added to the solution of compound **(3)** (3.00 g, 13.26 mmol) in 2-propanol, and the mixture was refluxed in the presence of 99 %

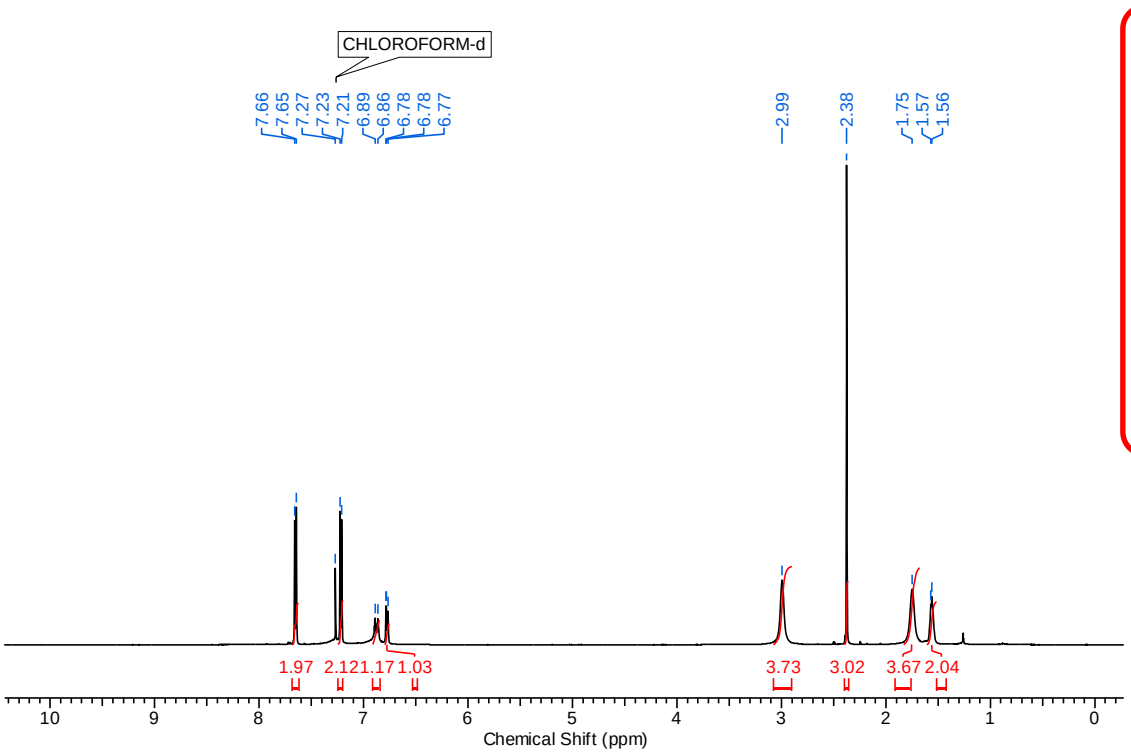
Spectral data: ^{1}H NMR (200 MHz, $CDCl_3$): NAM-1



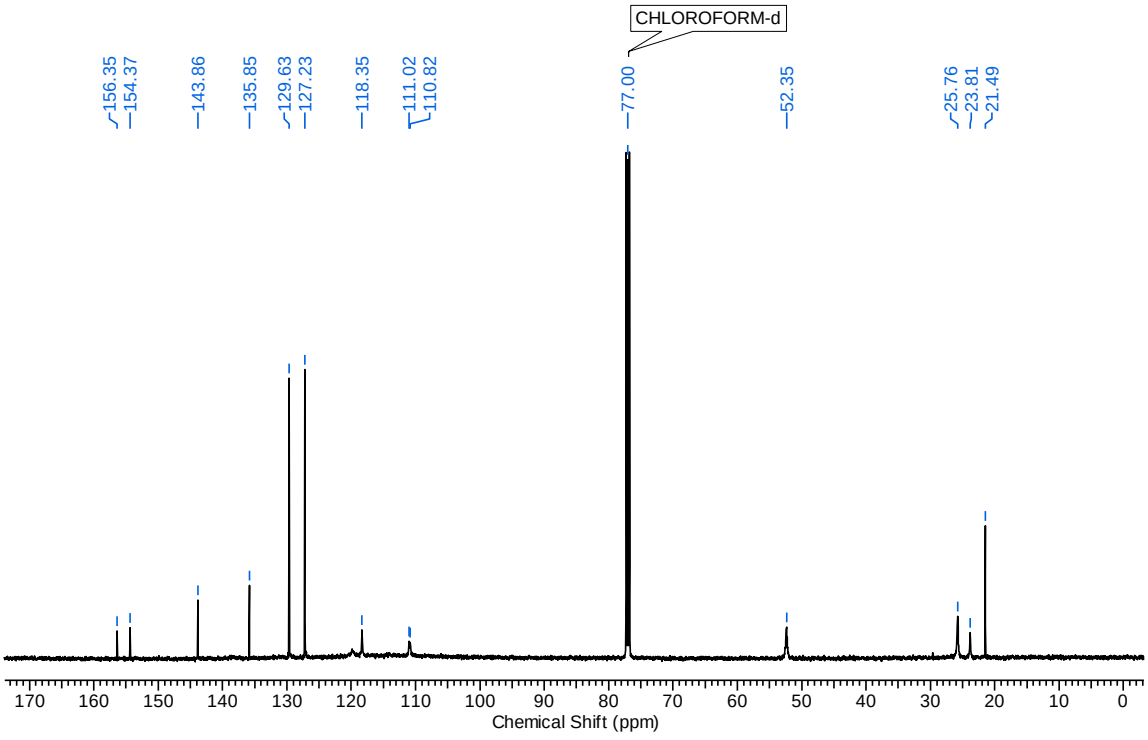
^{13}C NMR (50 MHz, $CDCl_3$)



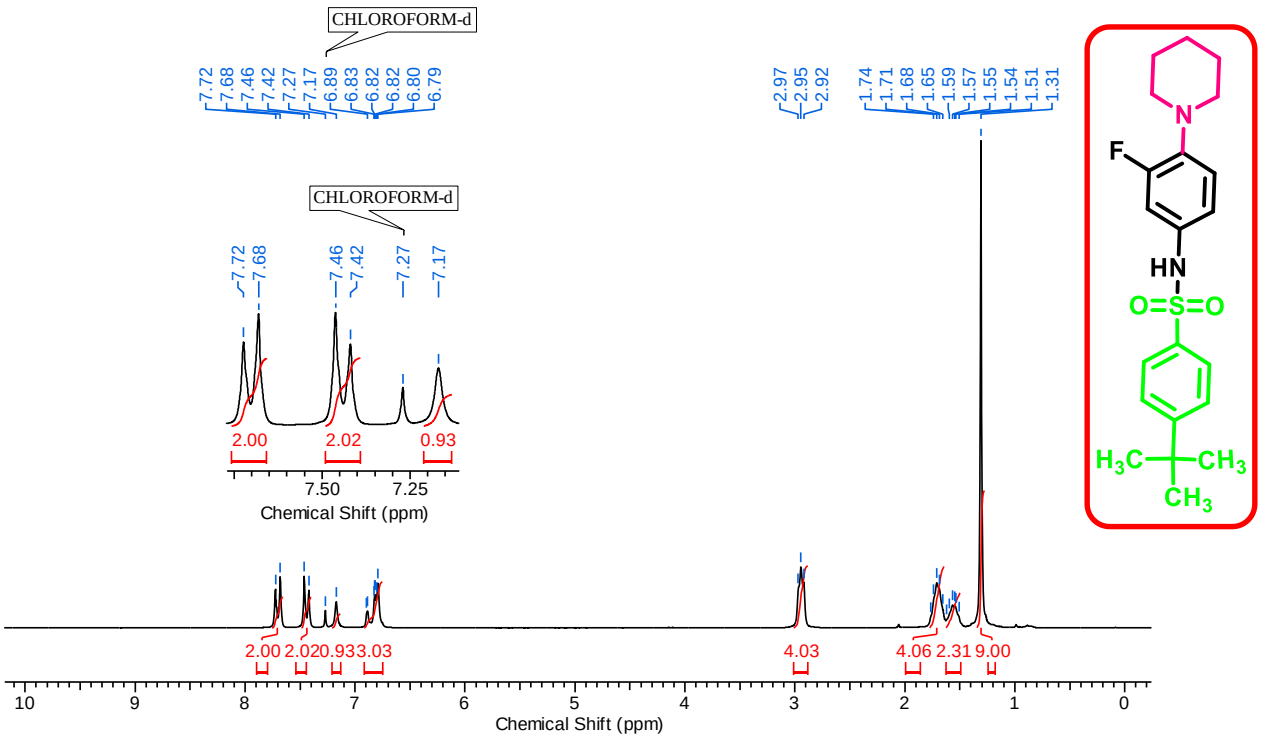
^{1}H NMR (500 MHz, $CDCl_3$): NAM-2



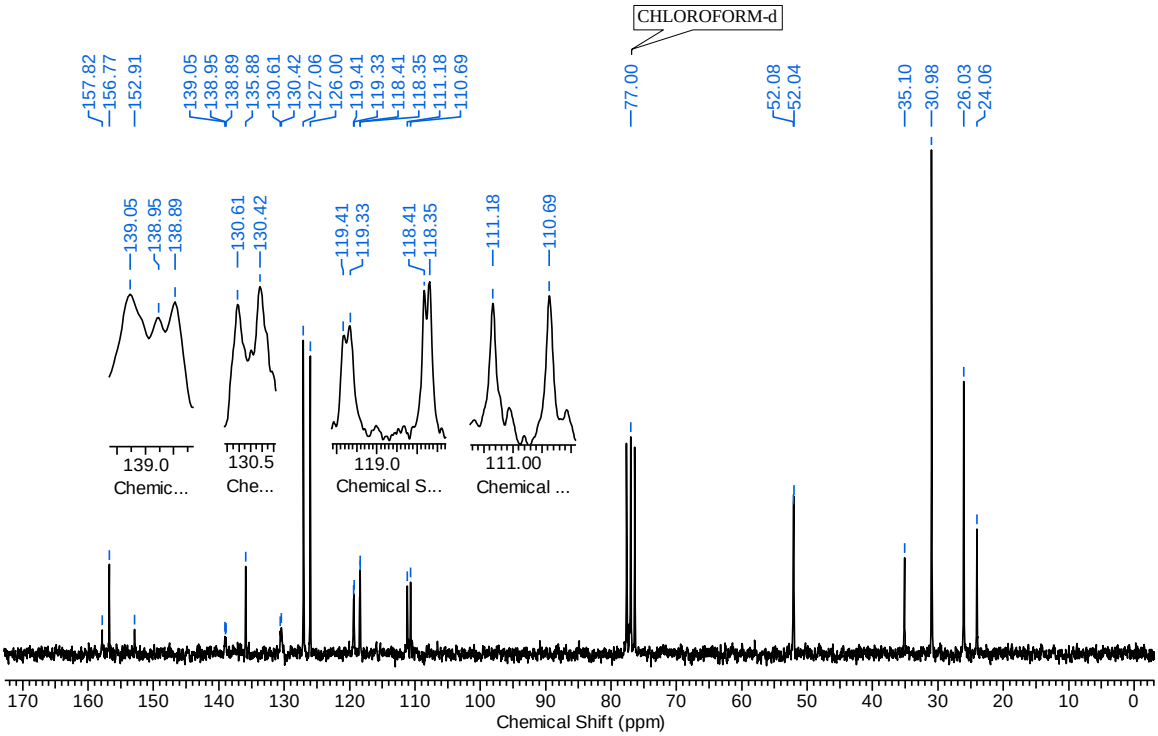
^{13}C NMR (125 MHz, $CDCl_3$)



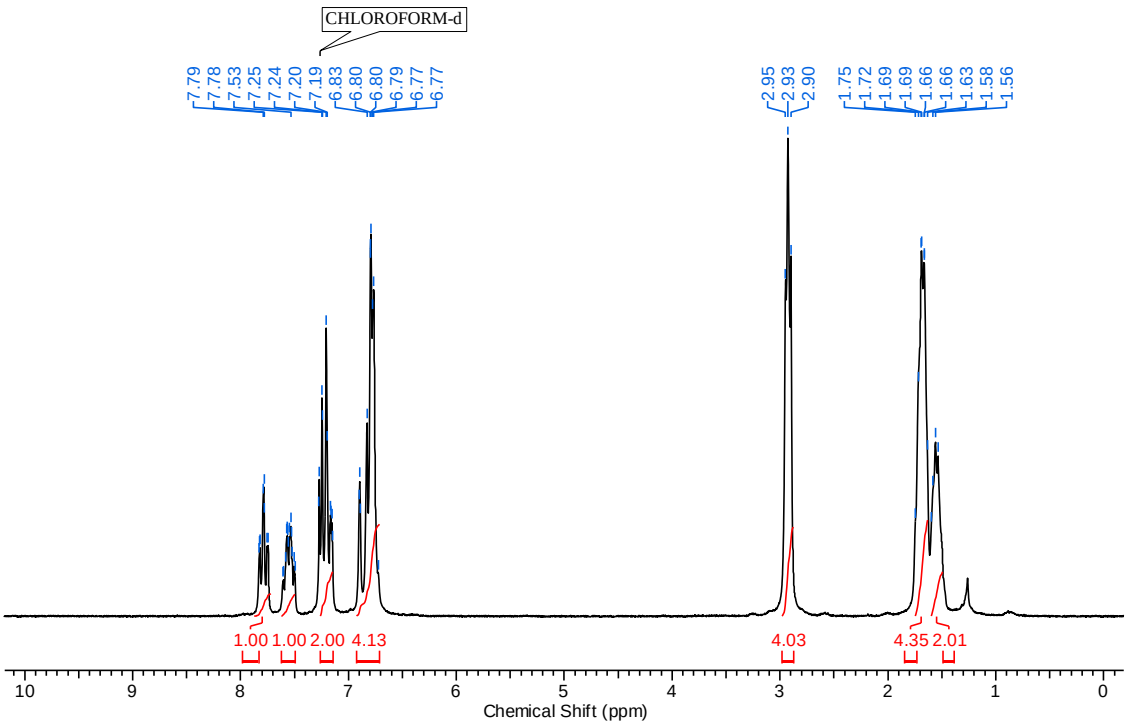
^{1}H NMR (200 MHz, $CDCl_3$): NAM-3

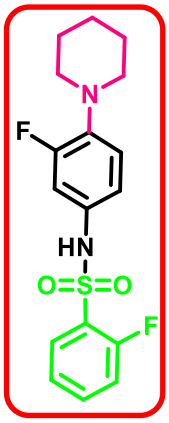


^{13}C NMR (50 MHz, $CDCl_3$)

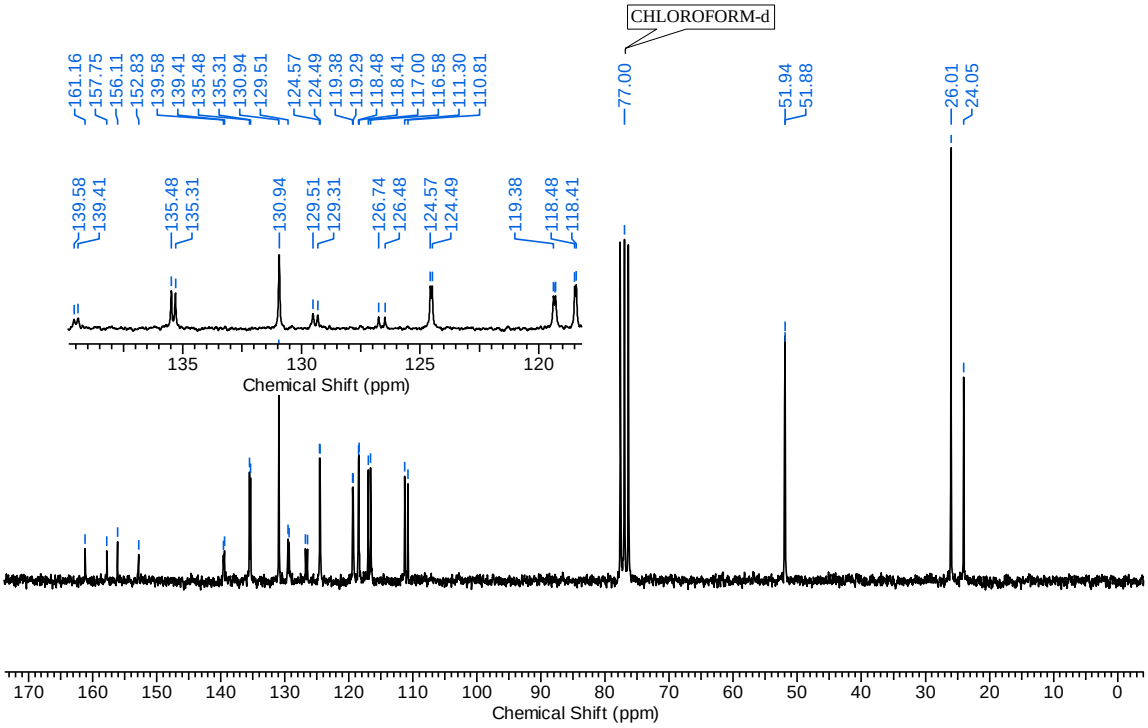


^{1}H NMR (200 MHz, $CDCl_3$): NAM-4

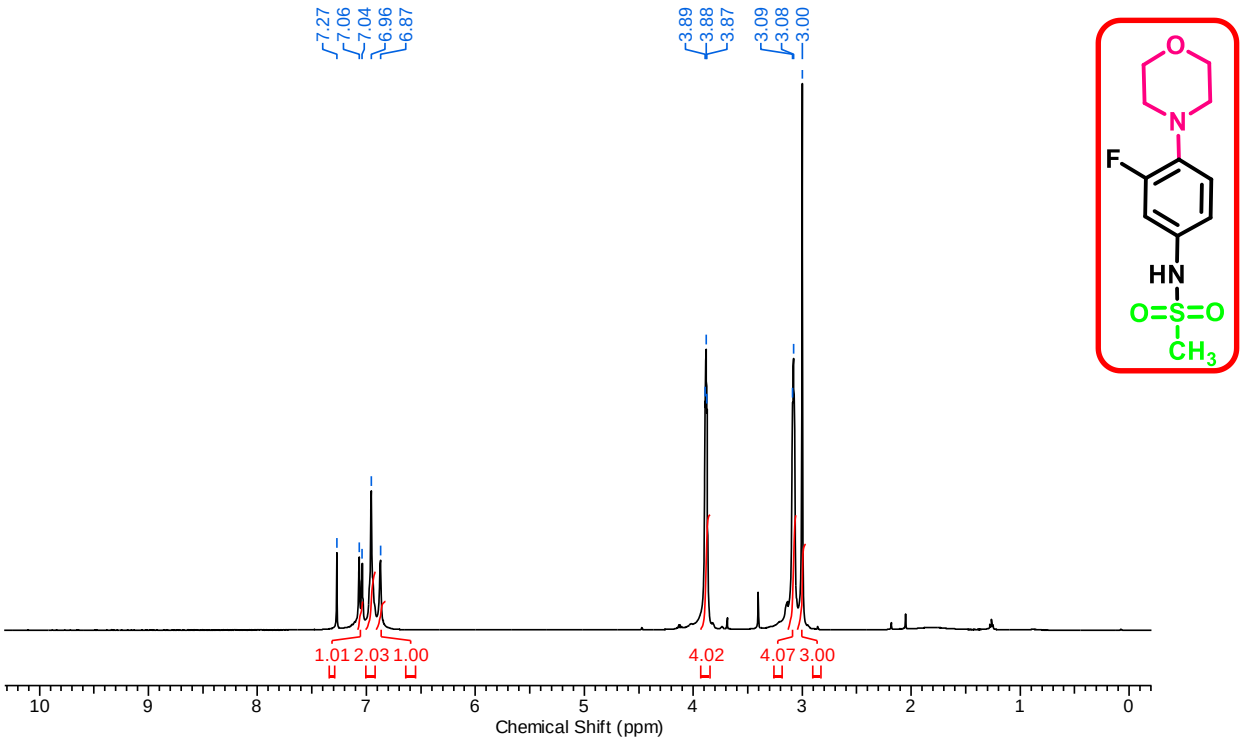




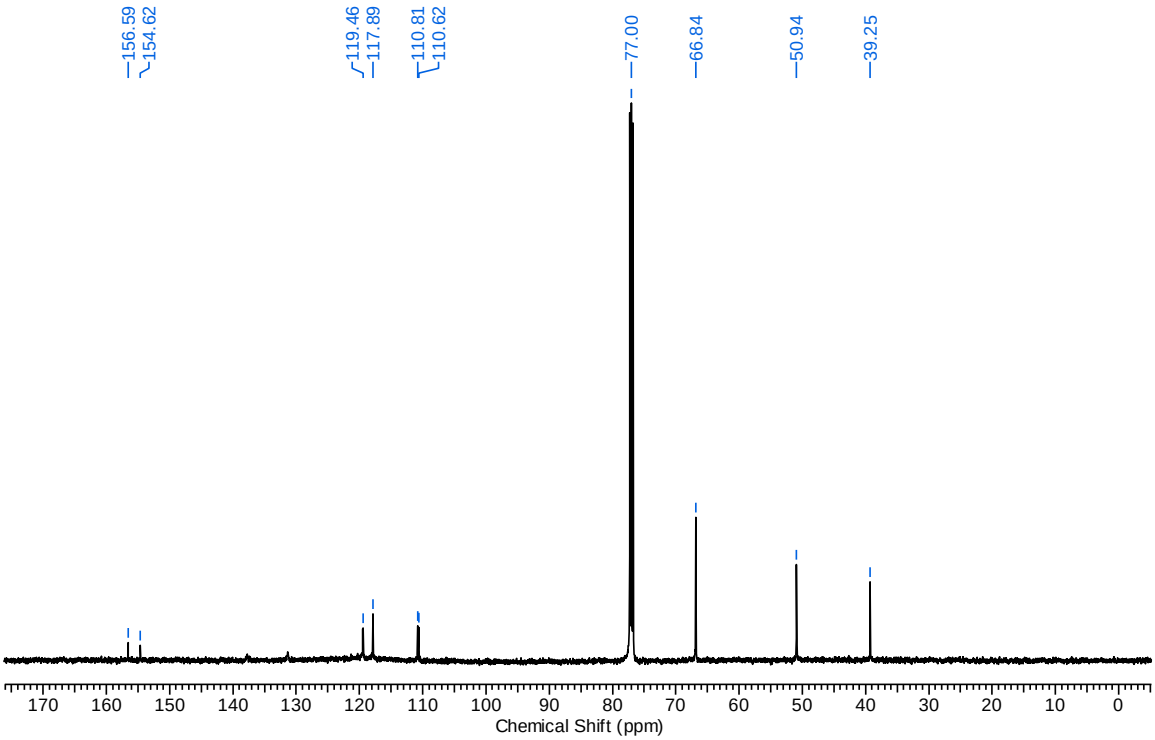
^{13}C NMR (50 MHz, $CDCl_3$)



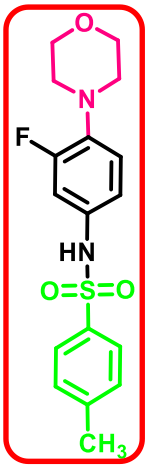
^{1}H NMR (400 MHz, $CDCl_3$): NAM-5

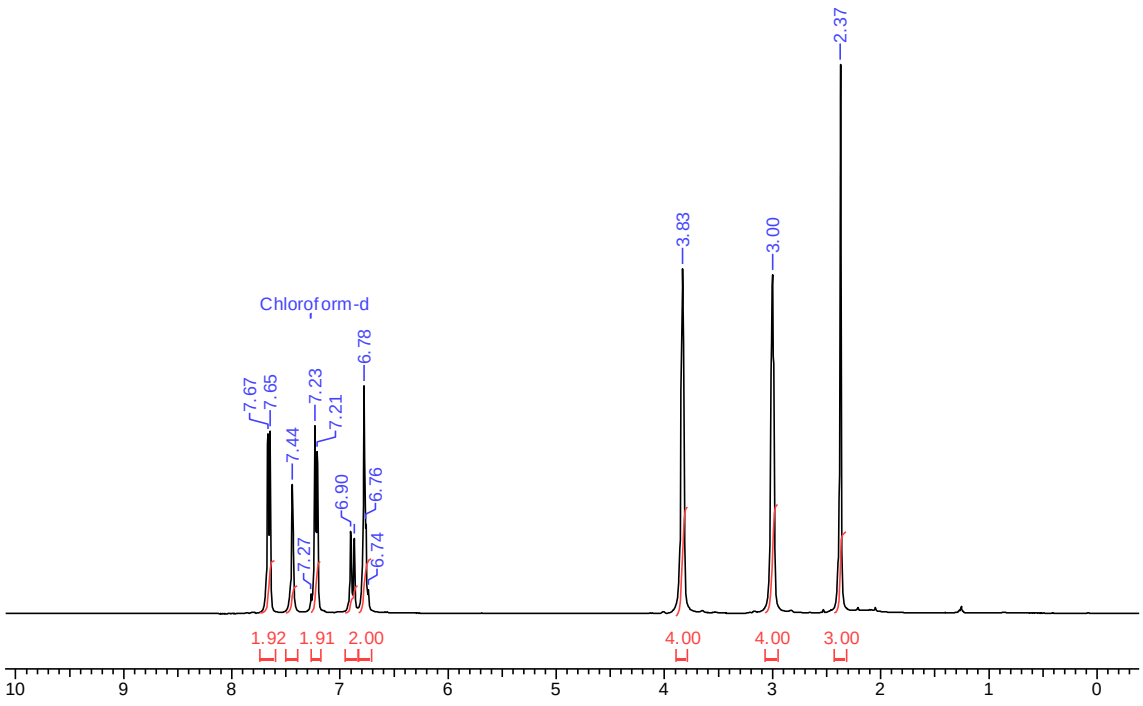


^{13}C NMR (100 MHz, $CDCl_3$)

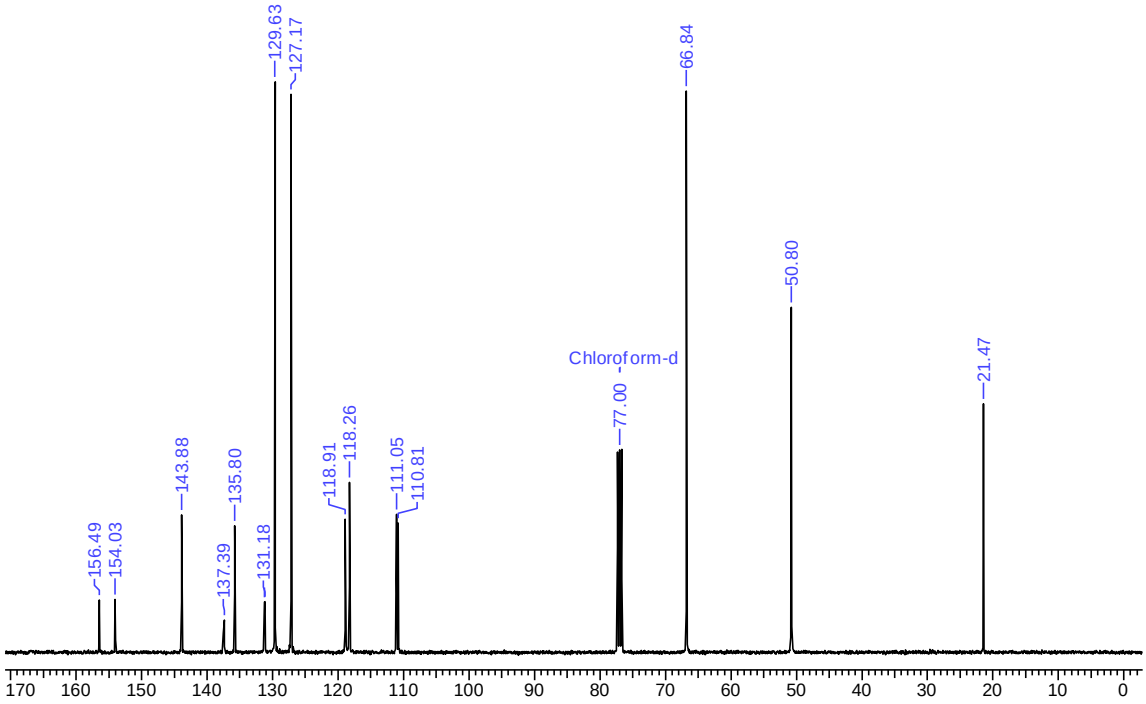


^{1}H NMR (400 MHz, $CDCl_3$): NAM-6

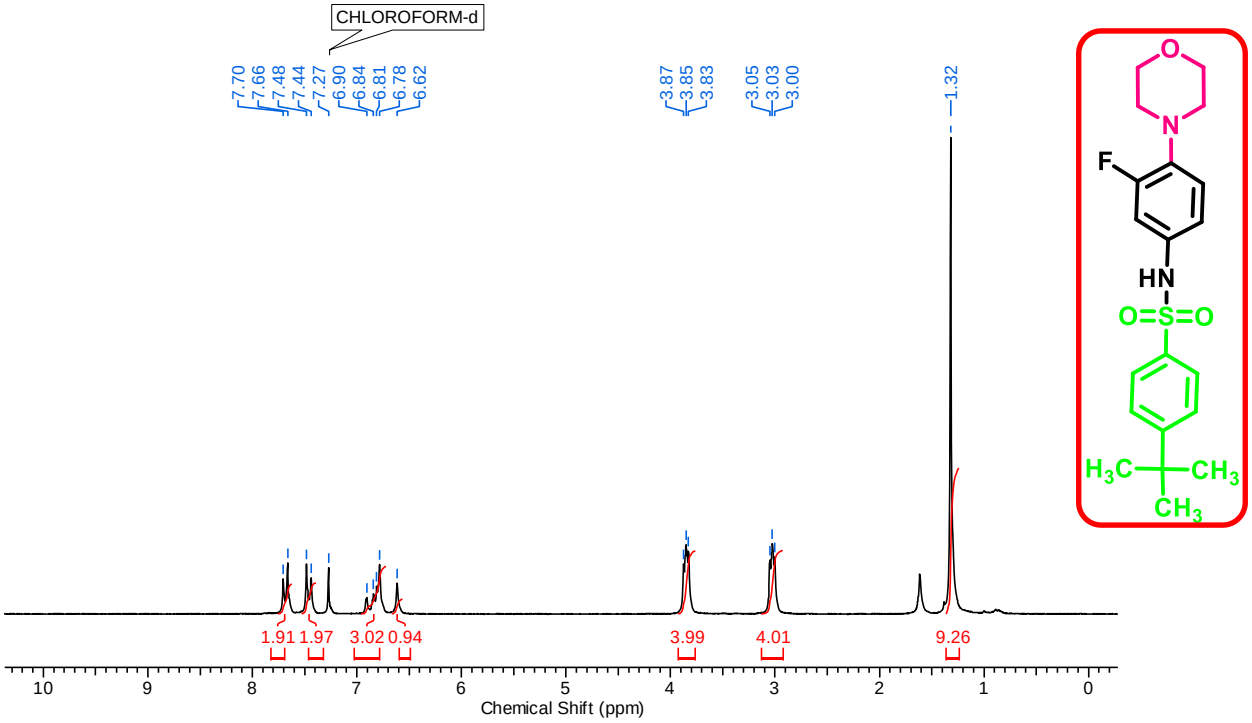




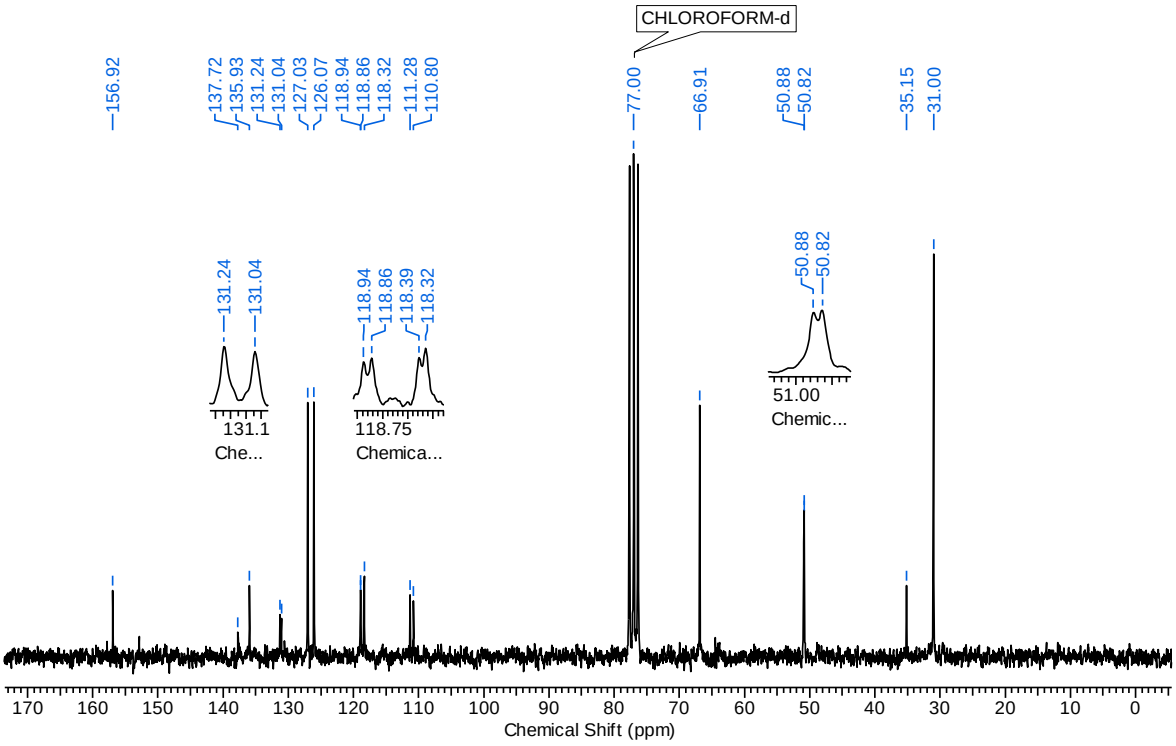
^{13}C NMR (100 MHz, $CDCl_3$)



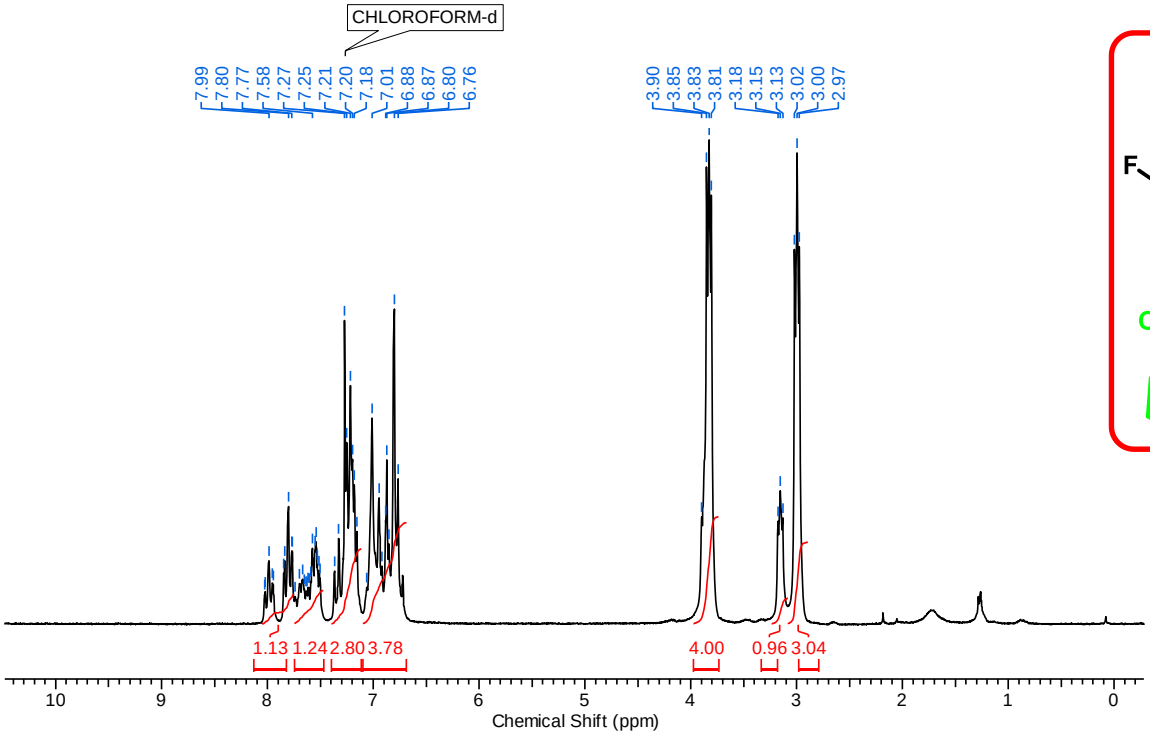
^{1}H NMR (200 MHz, $CDCl_3$): NAM-7

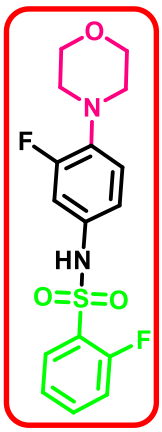


^{13}C NMR (50 MHz, $CDCl_3$)

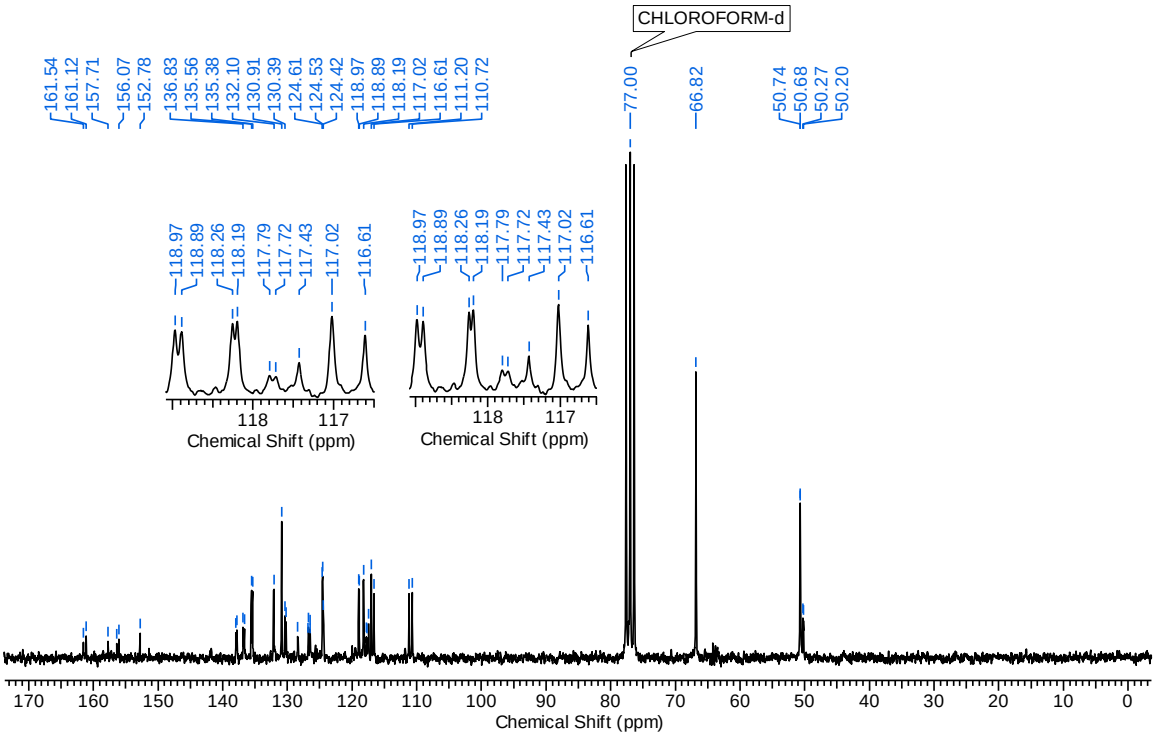


^{1}H NMR (200 MHz, $CDCl_3$): NAM-8

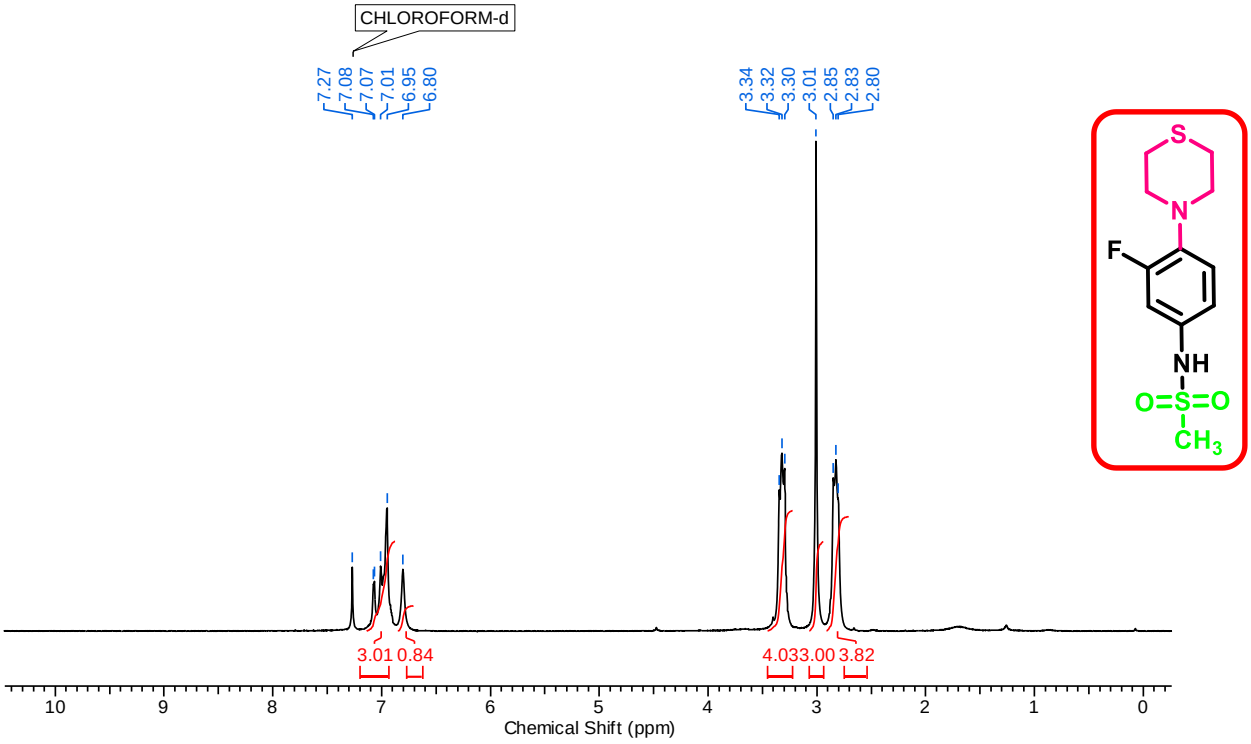




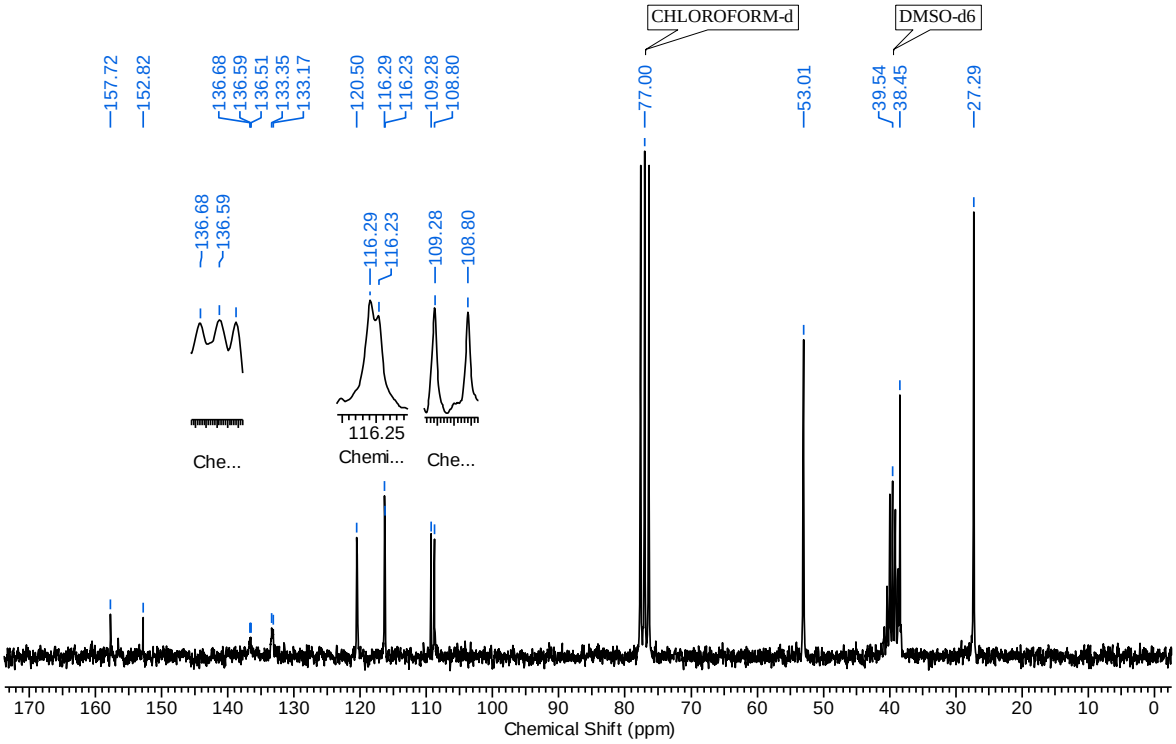
^{13}C NMR (50 MHz, $CDCl_3$)



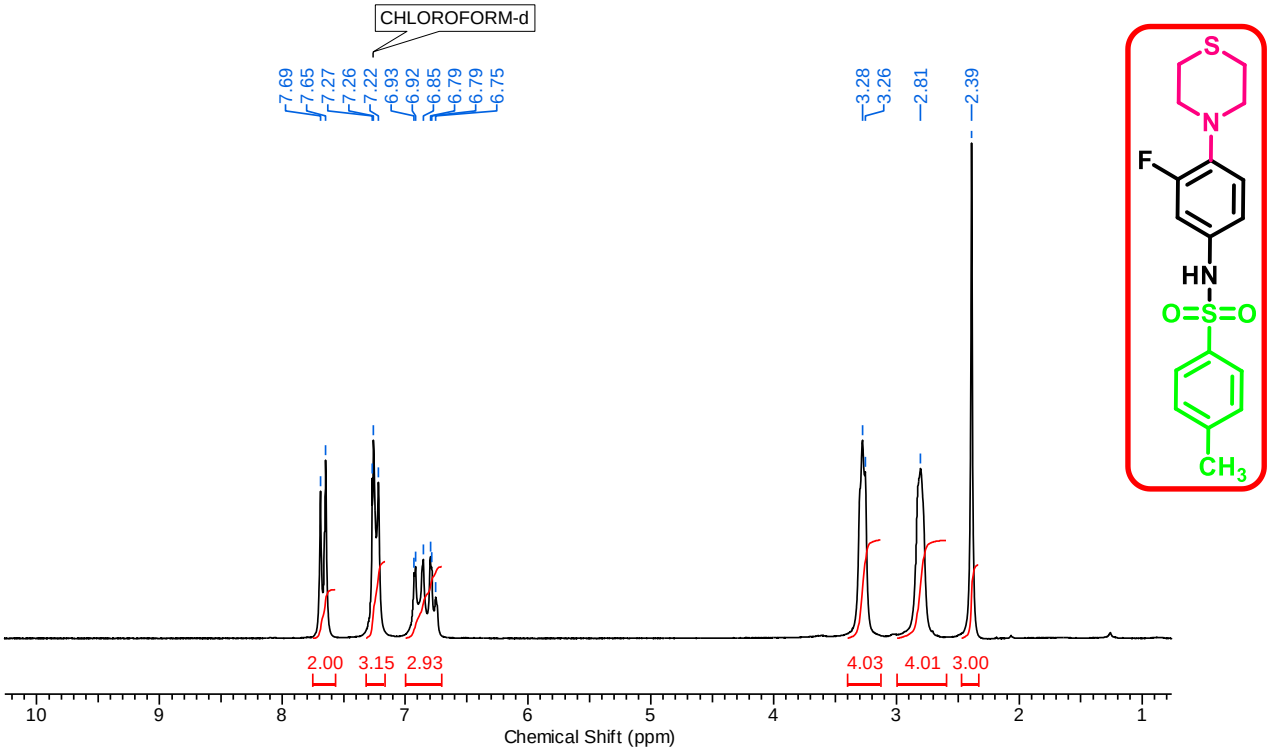
^{1}H NMR (200 MHz, $CDCl_3$): NAM-9



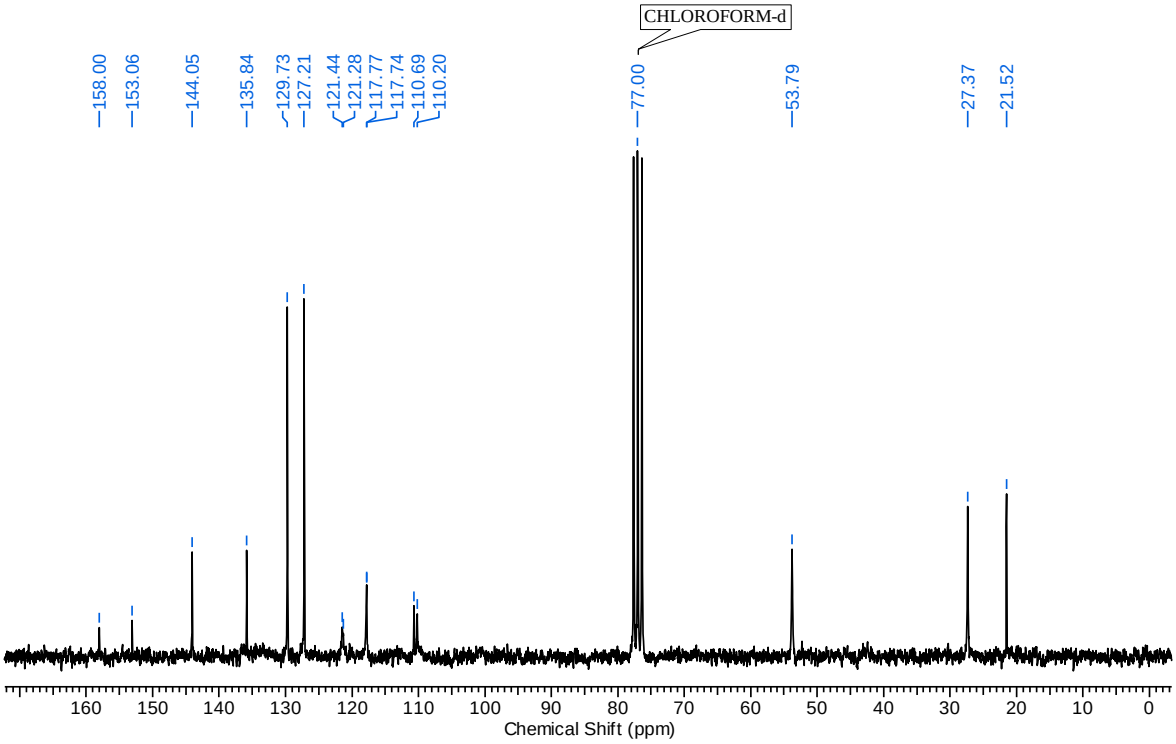
^{13}C NMR (50 MHz, $CDCl_3$): NAM-9



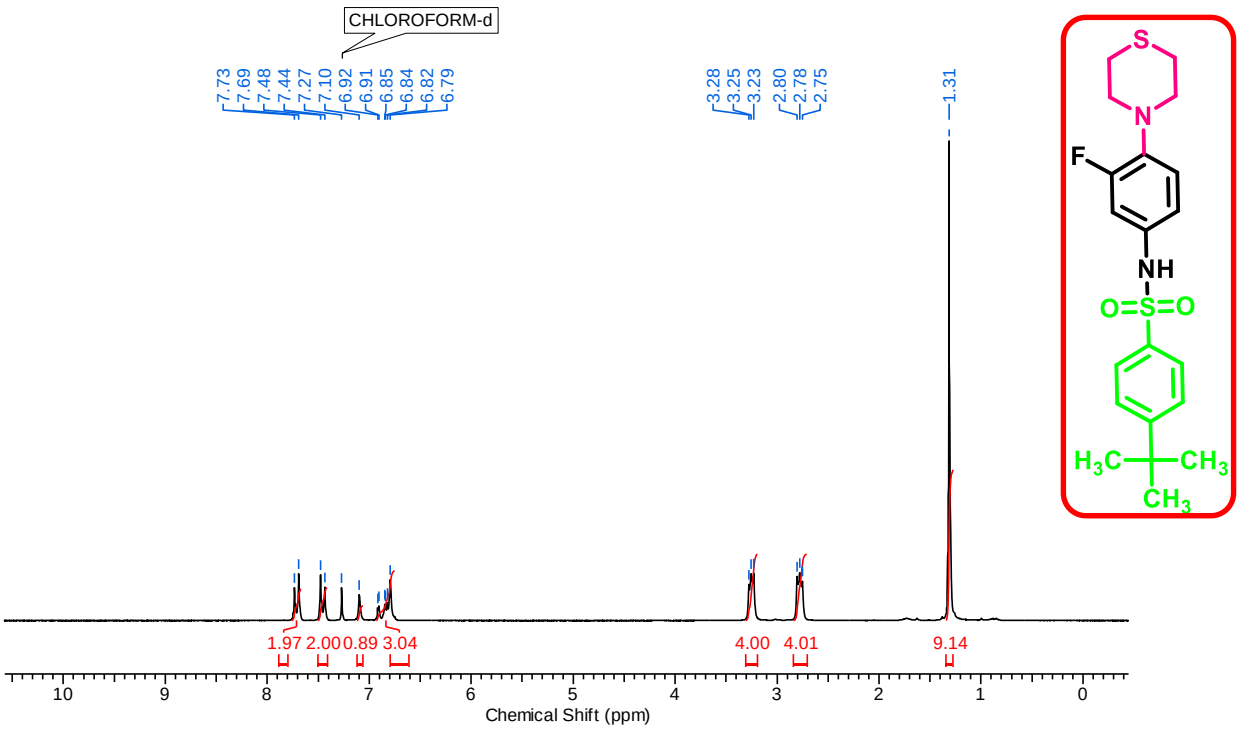
^{1}H NMR (200 MHz, $CDCl_3$): NAM-10



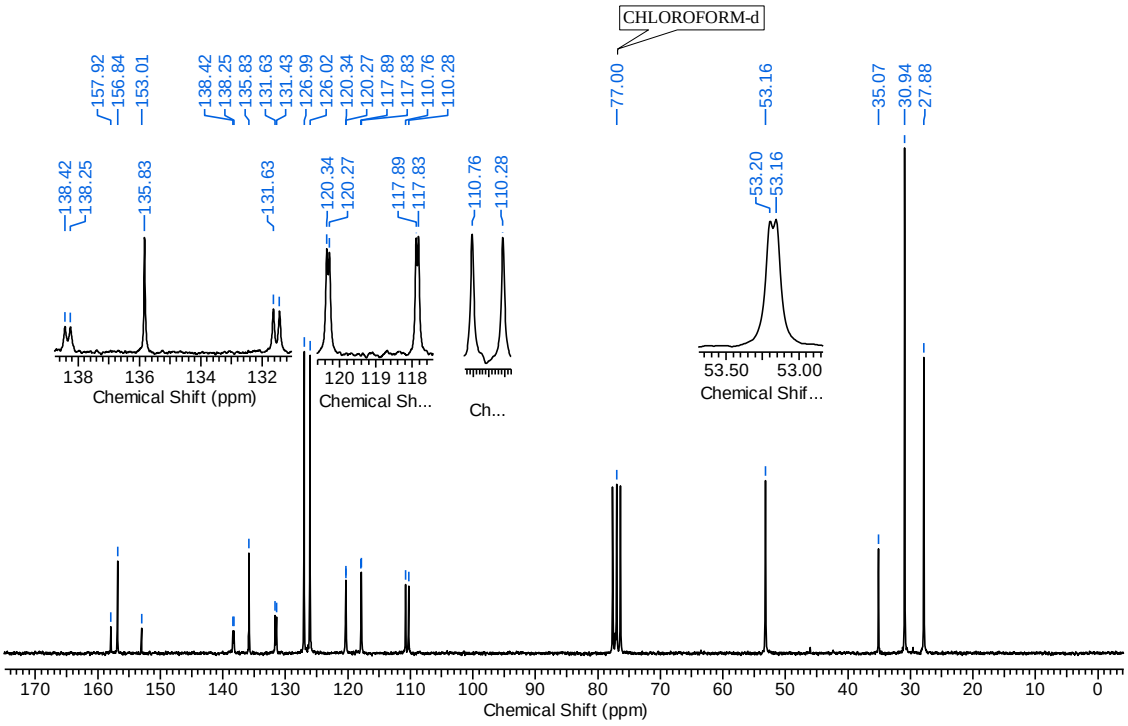
^{13}C NMR (50 MHz, $CDCl_3$): NAM-10



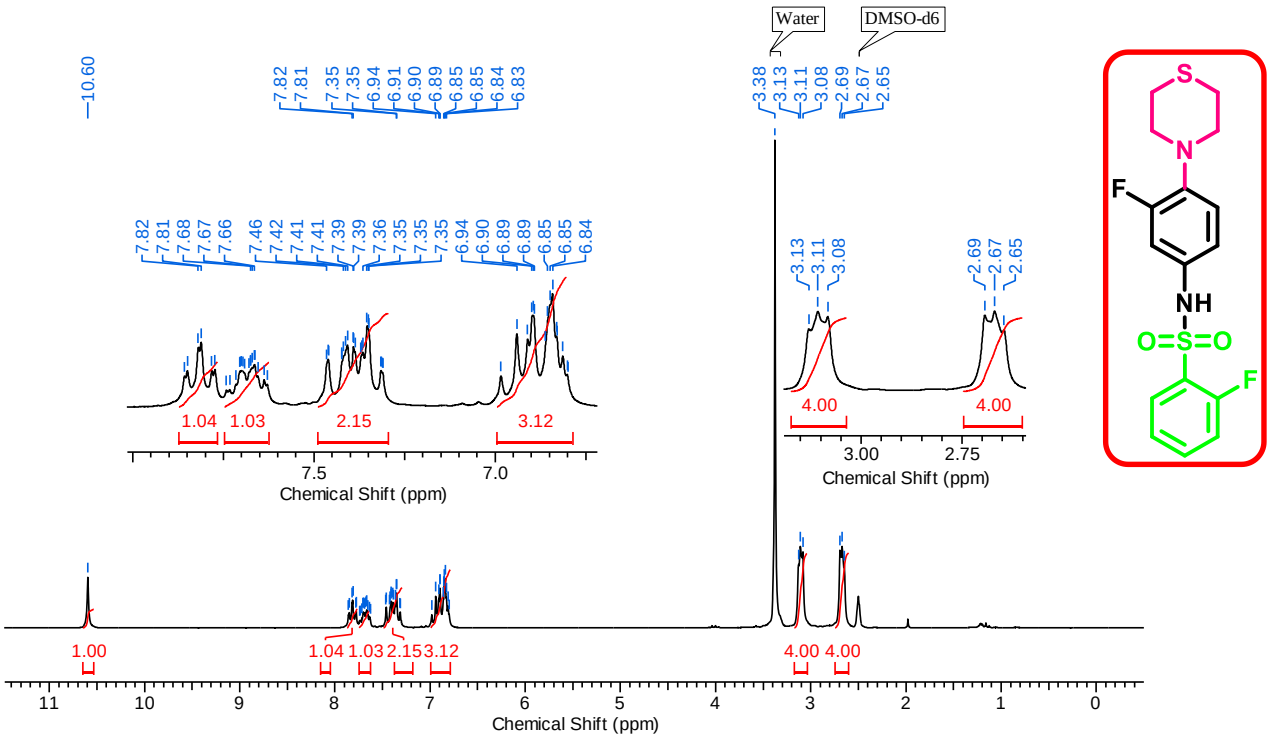
^{1}H NMR (200 MHz, $CDCl_3$): NAM-11



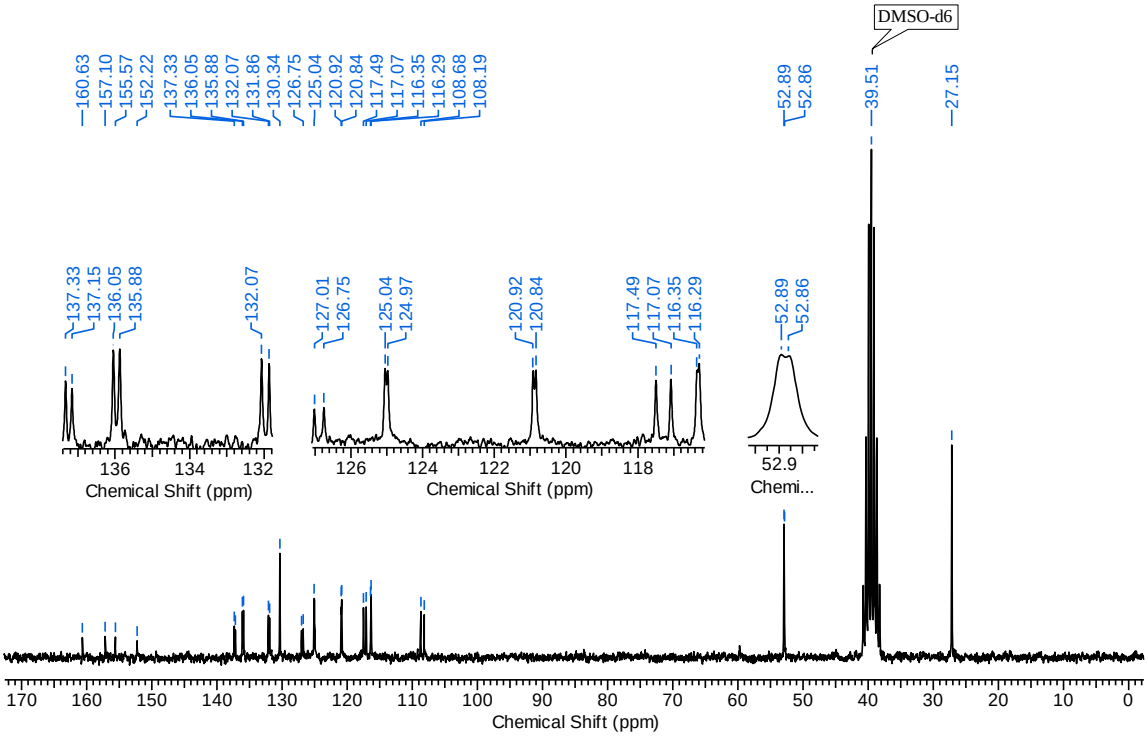
^{13}C NMR (50 MHz, $CDCl_3$): NAM-11



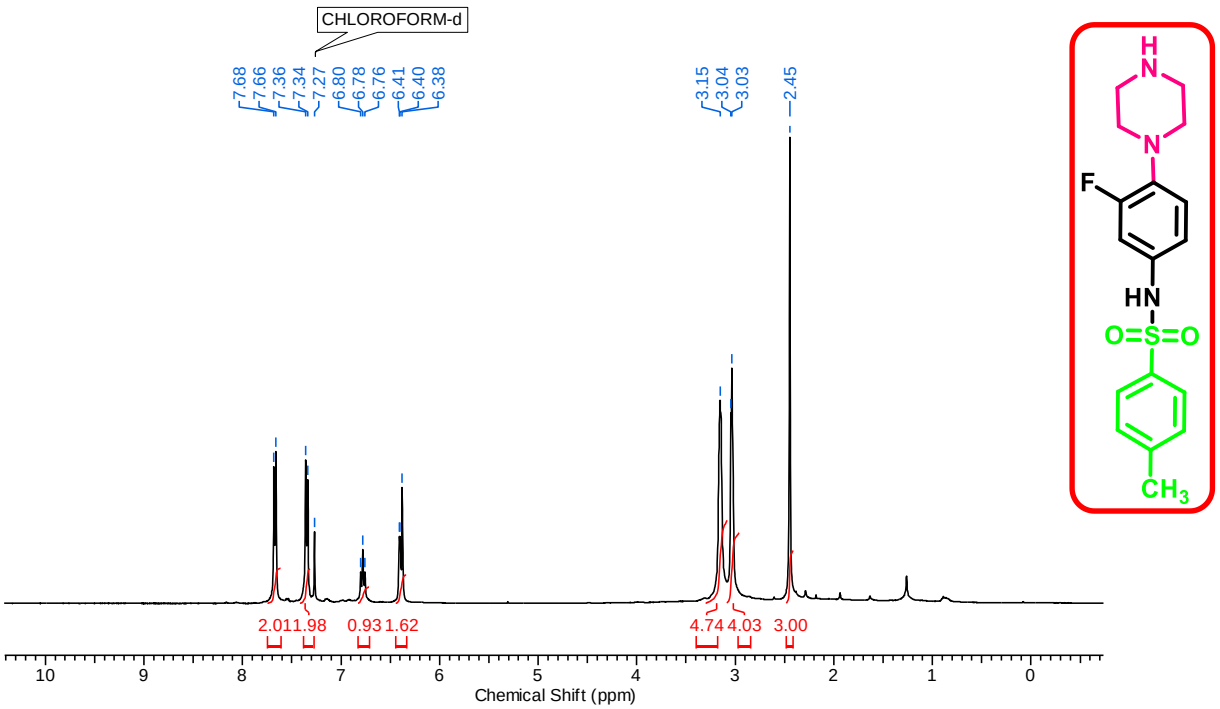
^{1}H NMR (200 MHz, DMSO-d$_6$): NAM-12



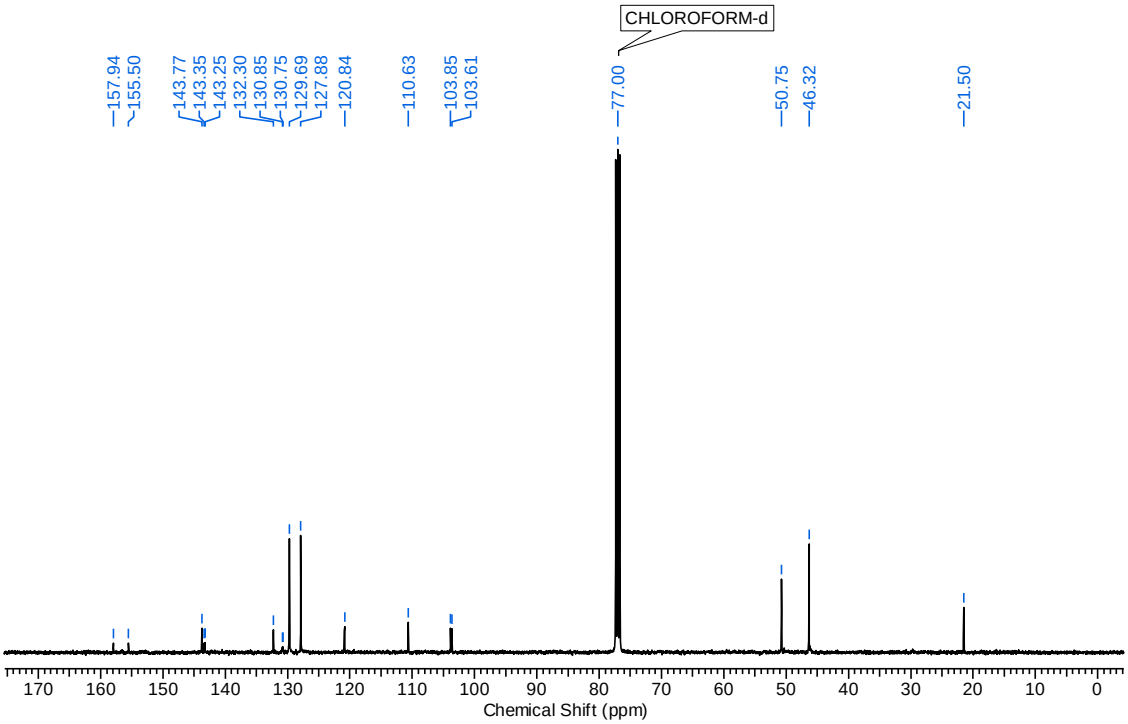
^{13}C NMR (50 MHz, DMSO-d$_6$)



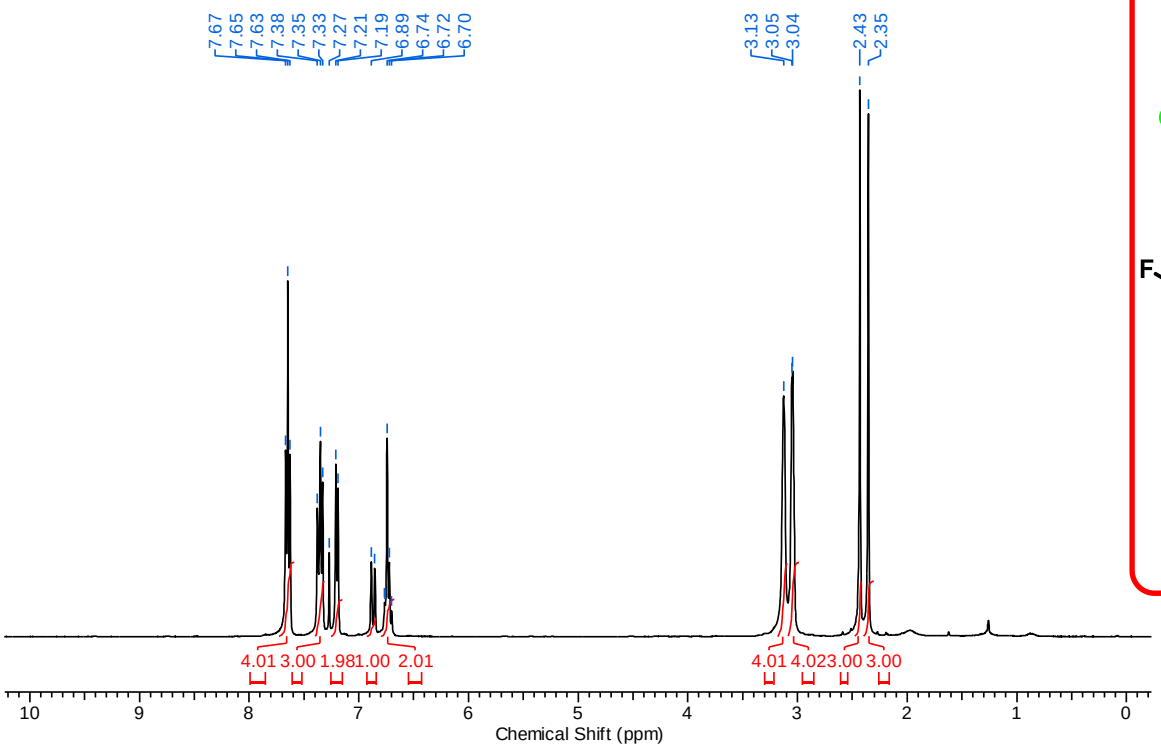
^{1}H NMR (500 MHz, $CDCl_3$): NAM-13

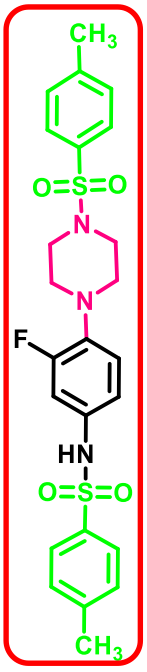


^{13}C NMR (125 MHz, $CDCl_3$)

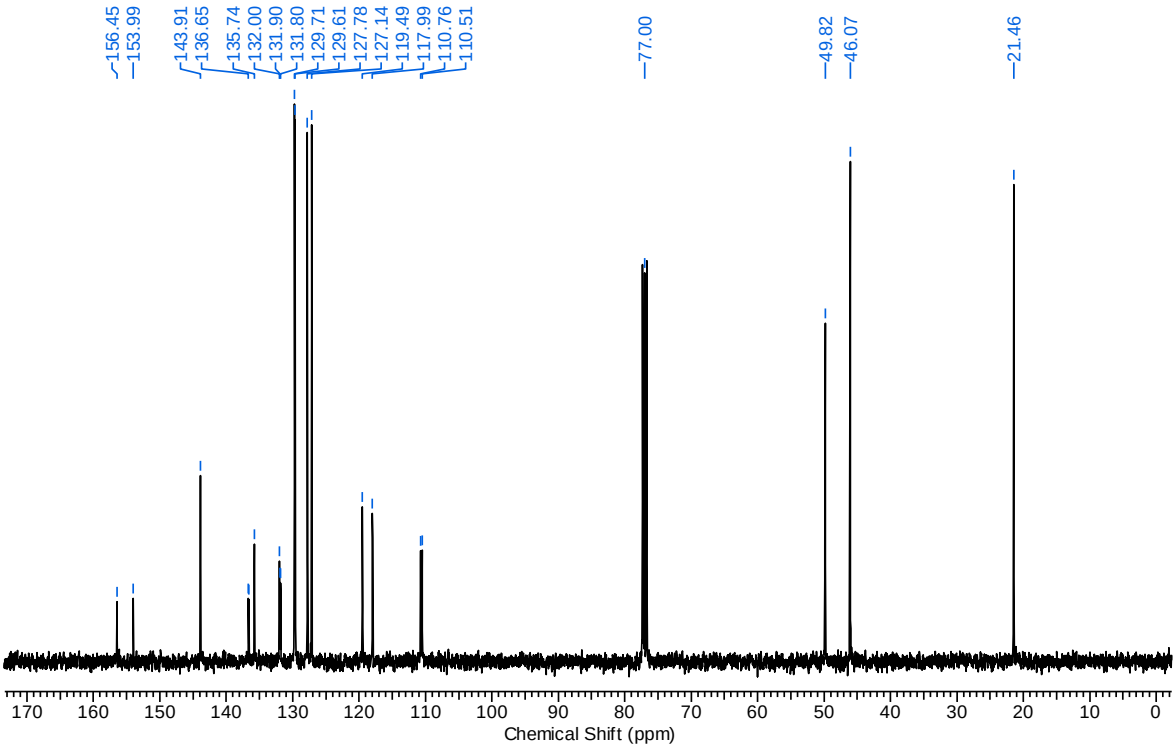


^{1}H NMR (200 MHz, $CDCl_3$): NAM-14





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



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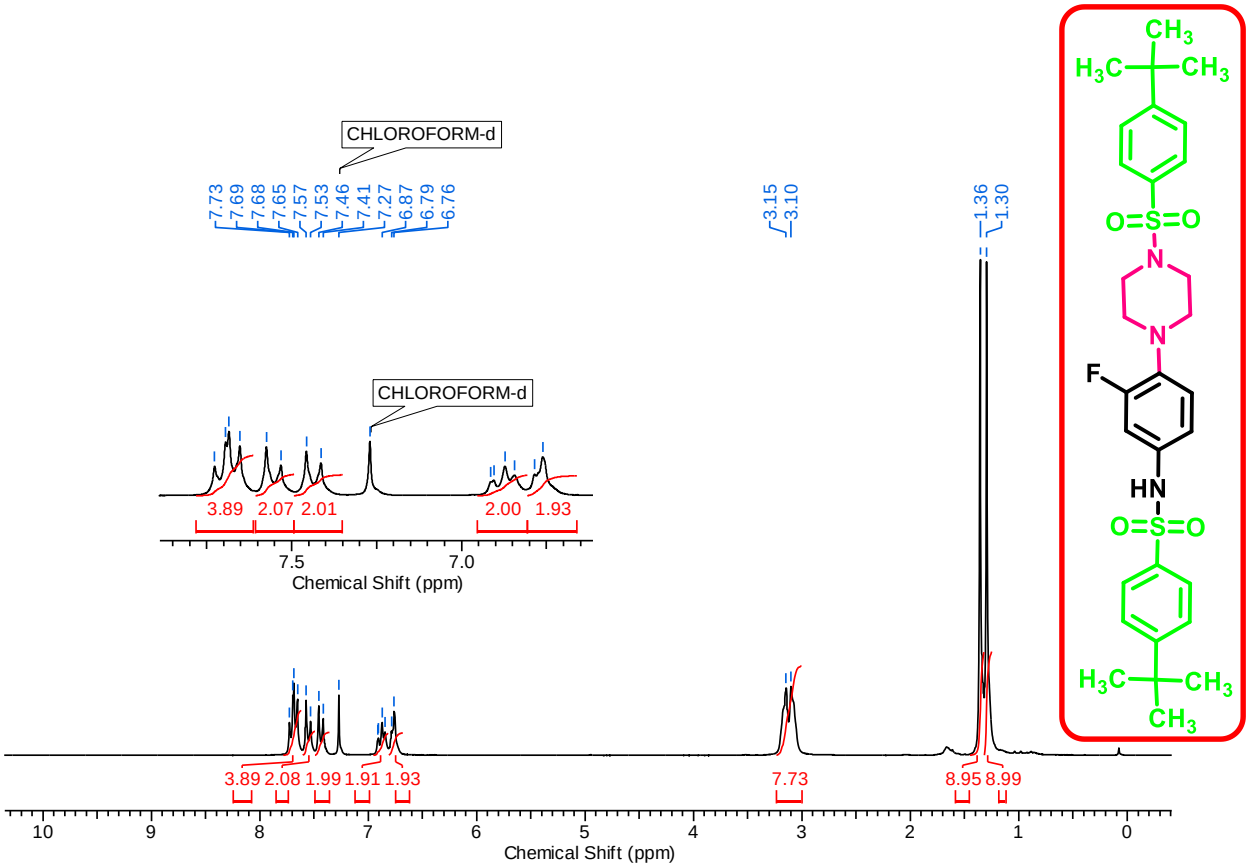
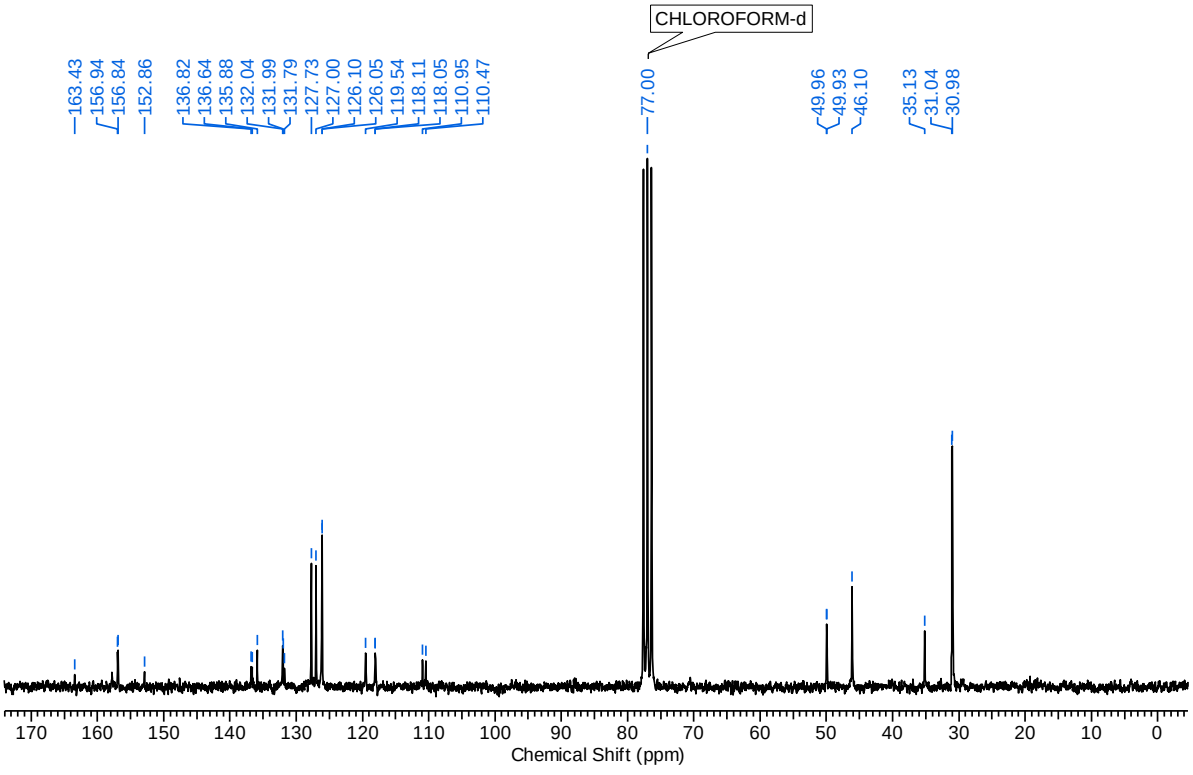
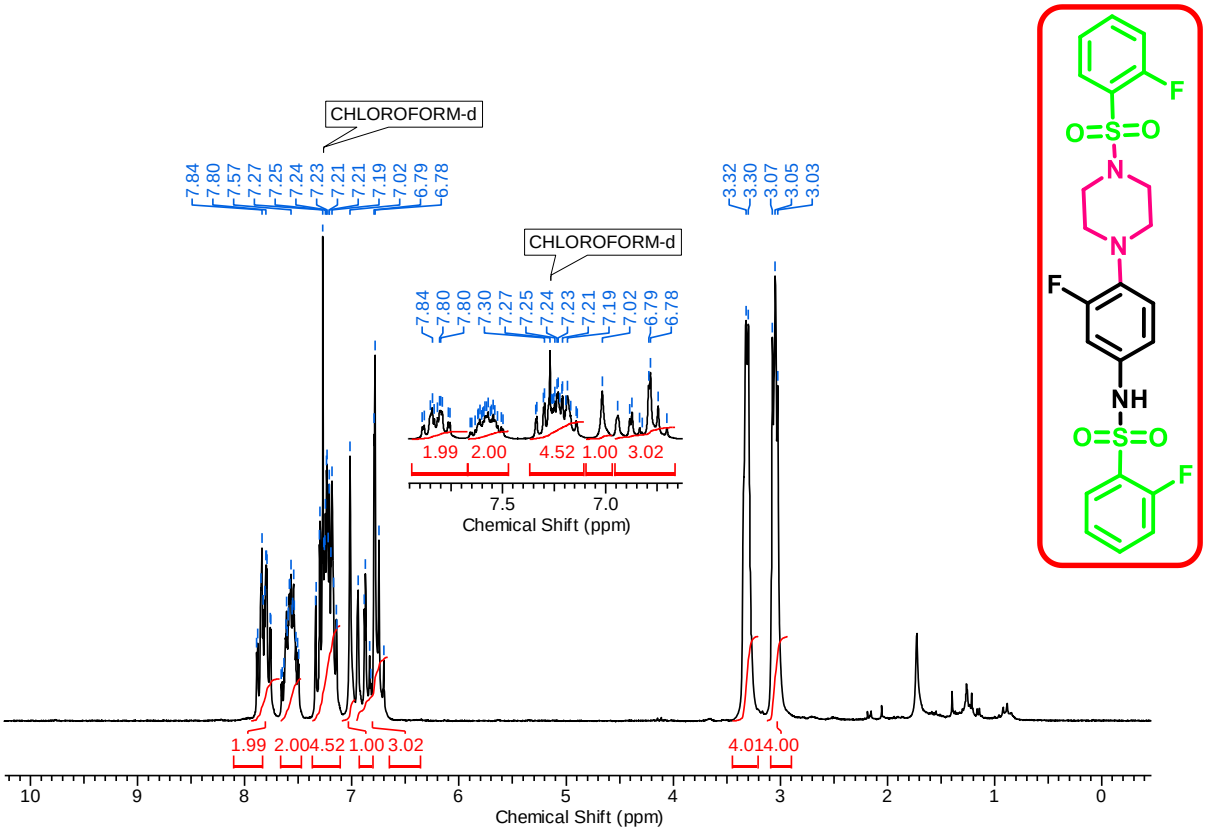


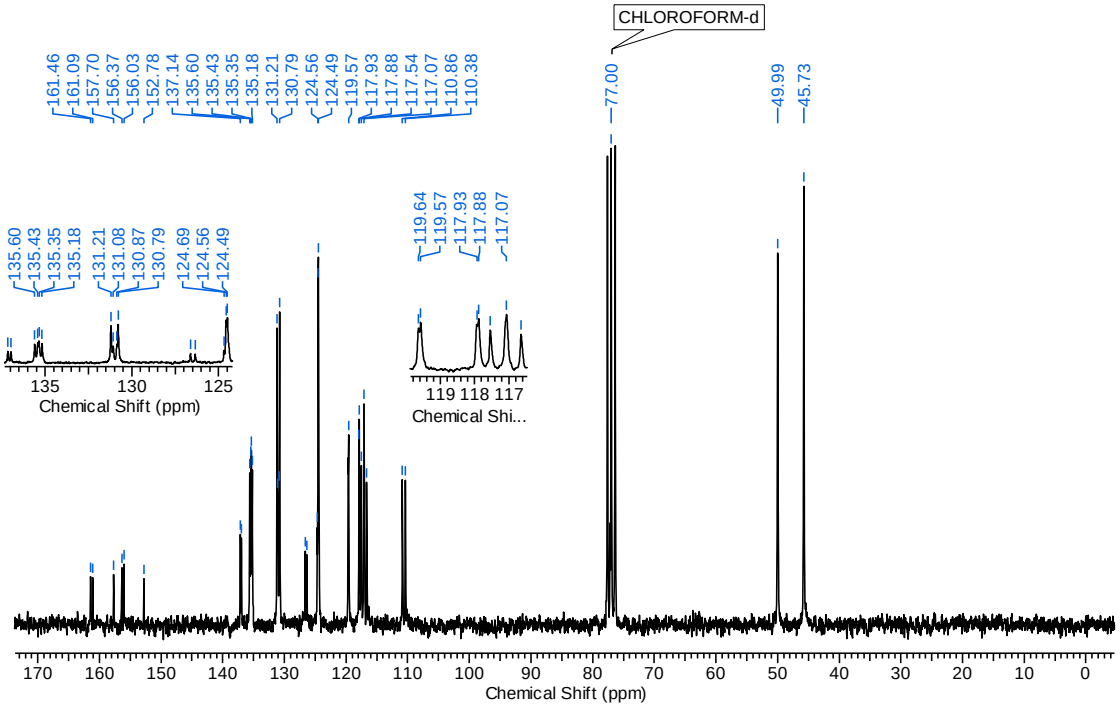
^{13}C NMR (50 MHz, $CDCl_3$)



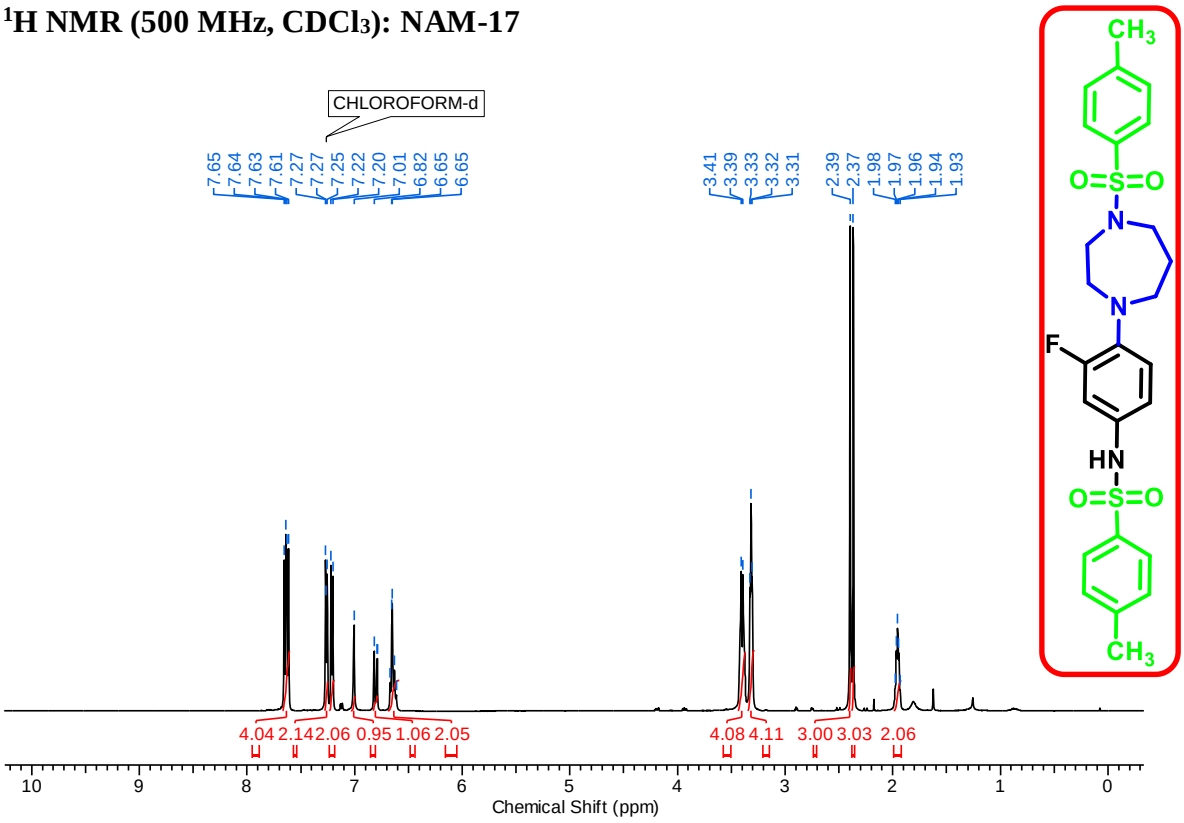
^{1}H NMR (200 MHz, $CDCl_3$): NAM-16



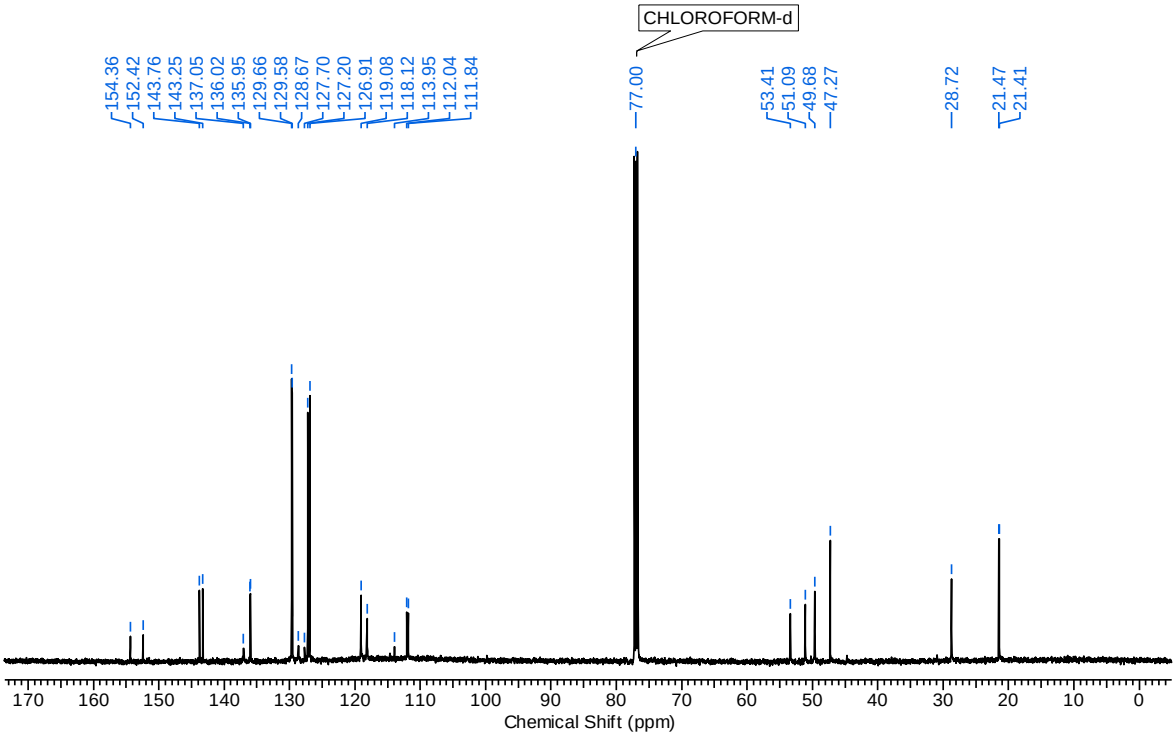
^{13}C NMR (50 MHz, $CDCl_3$)



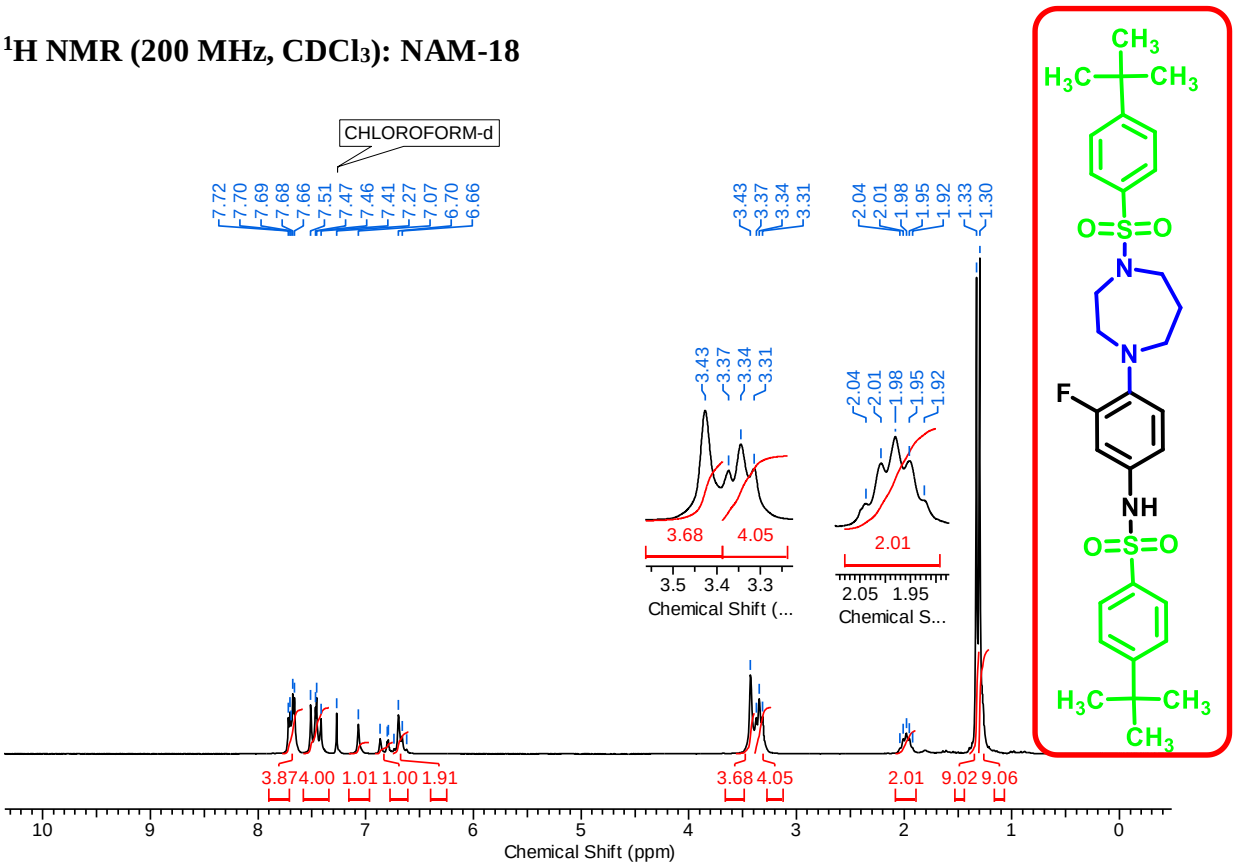
^{1}H NMR (500 MHz, $CDCl_3$): NAM-17



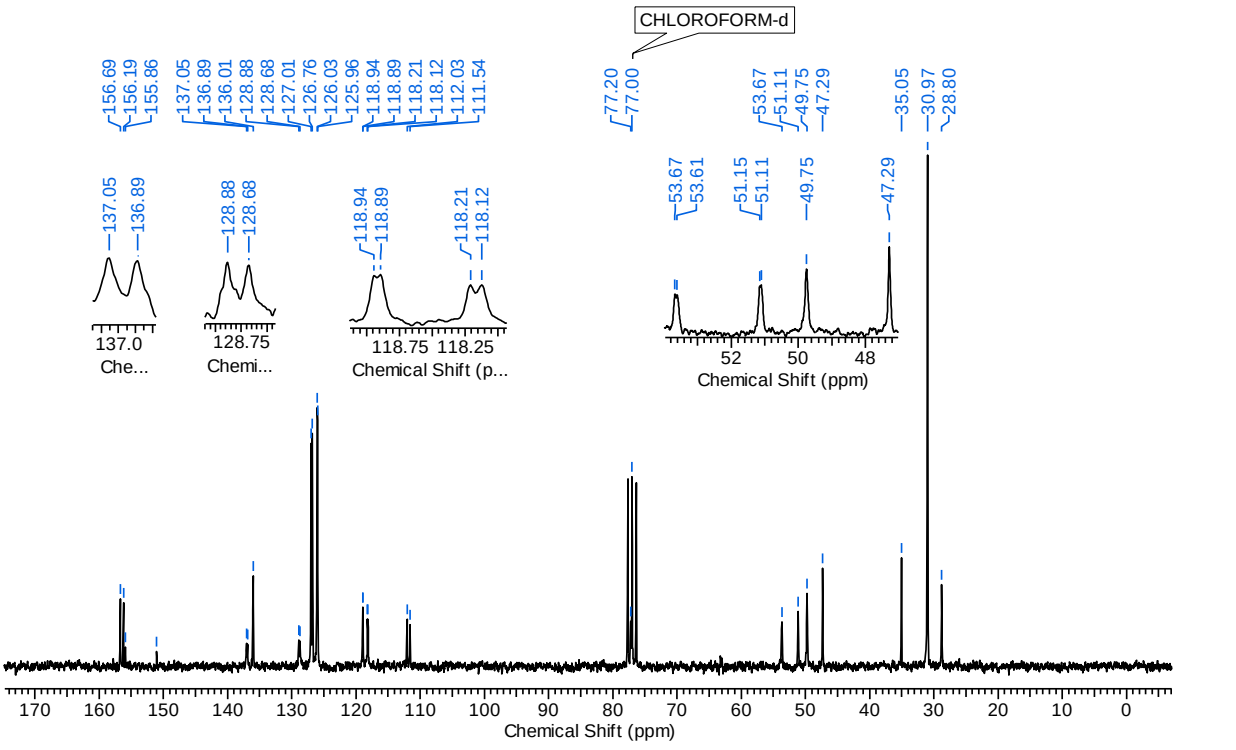
^{13}C NMR (125 MHz, $CDCl_3$)



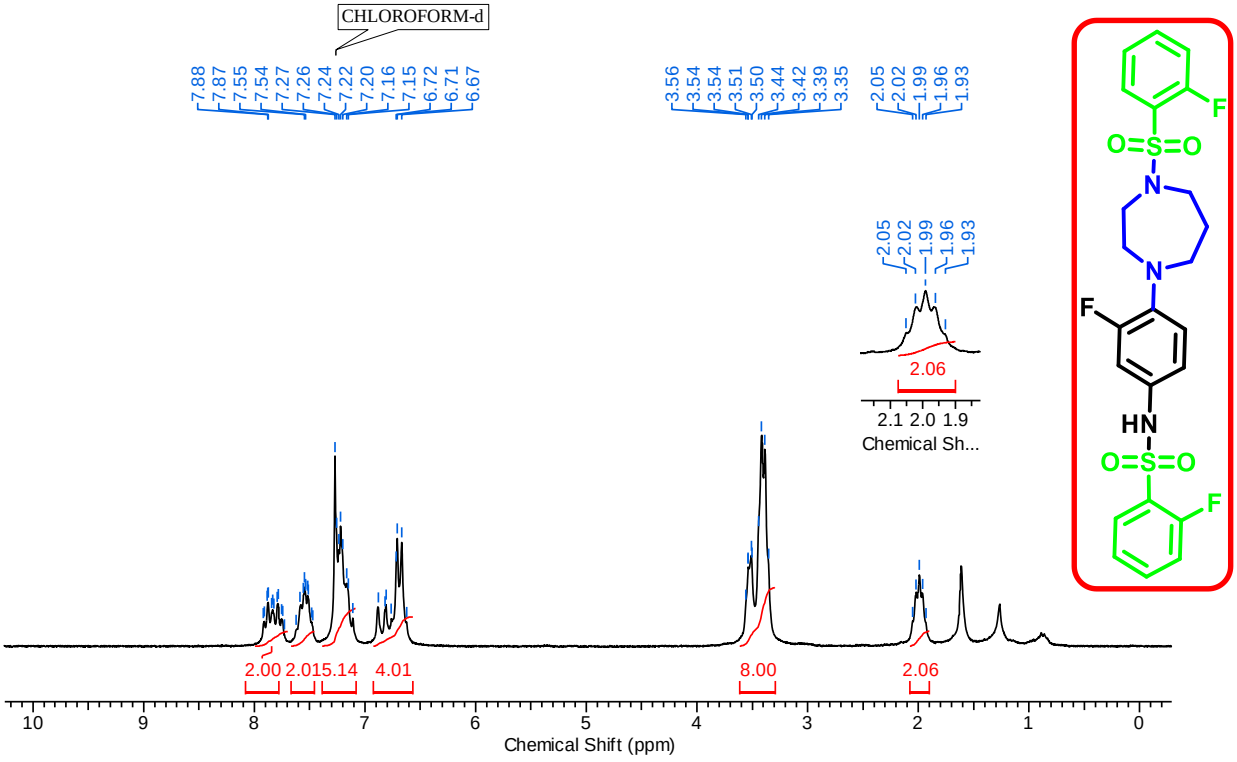
^{1}H NMR (200 MHz, $CDCl_3$): NAM-18



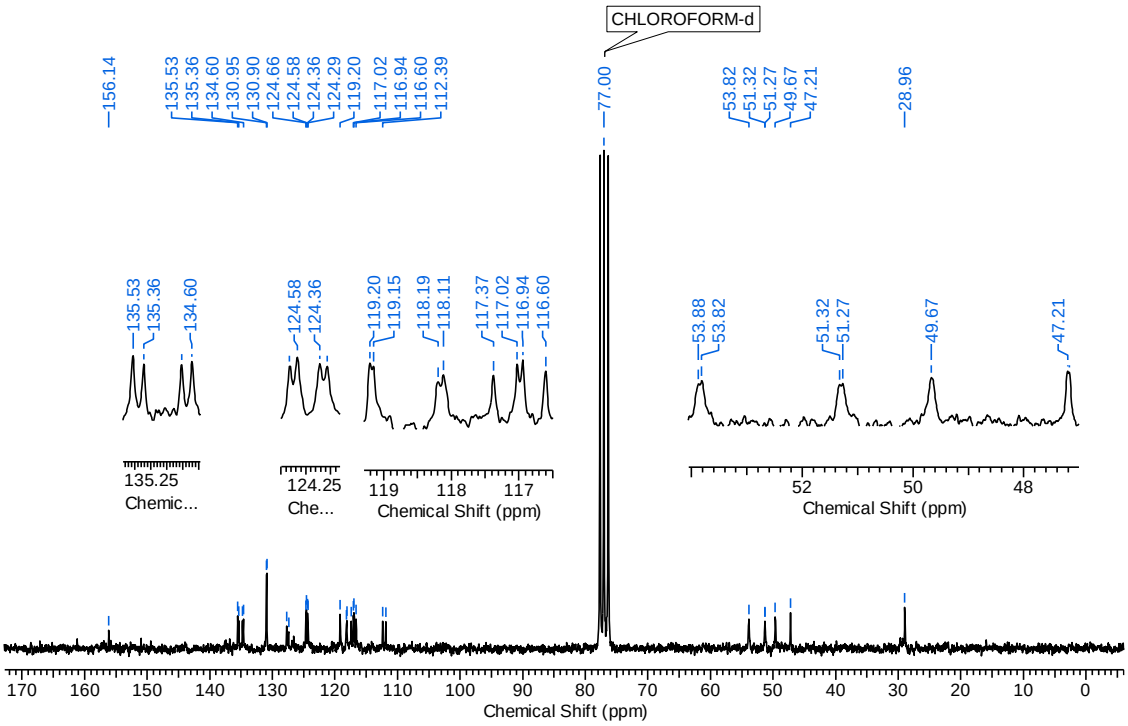
^{13}C NMR (50 MHz, $CDCl_3$)



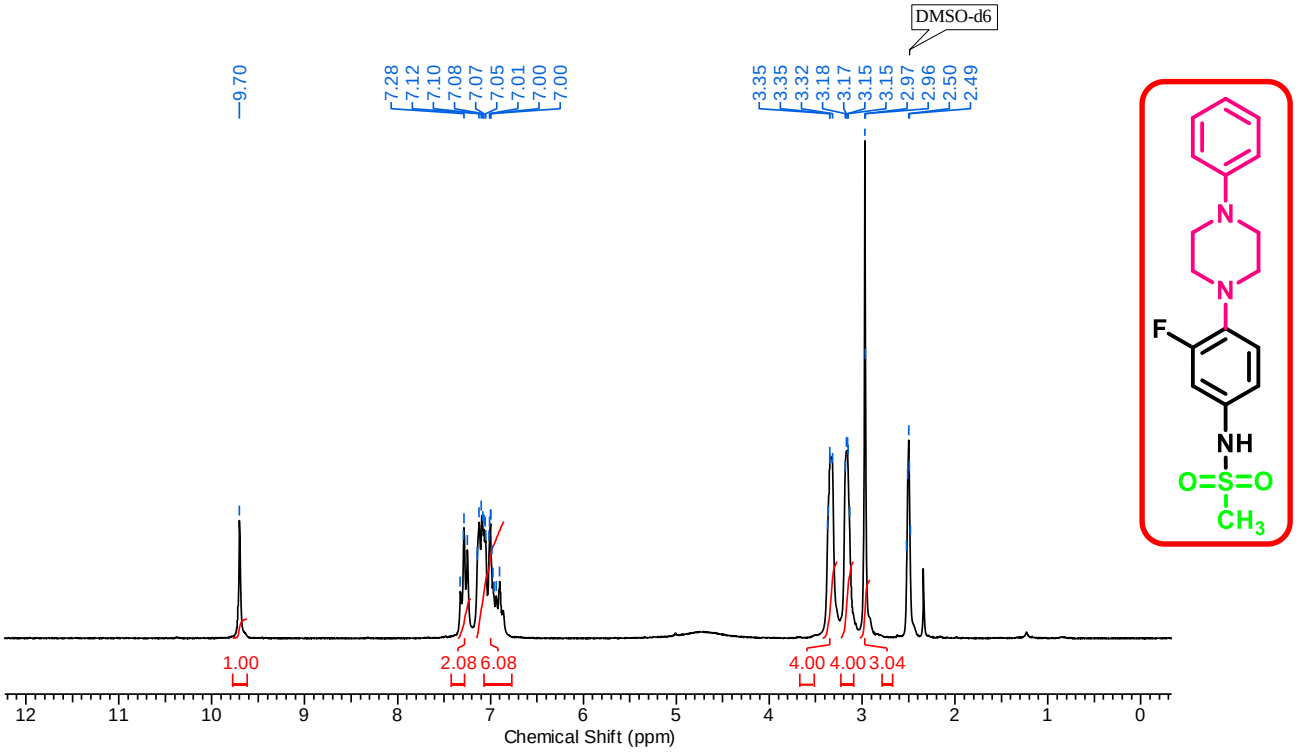
^{1}H NMR (200 MHz, $CDCl_3$): NAM-19



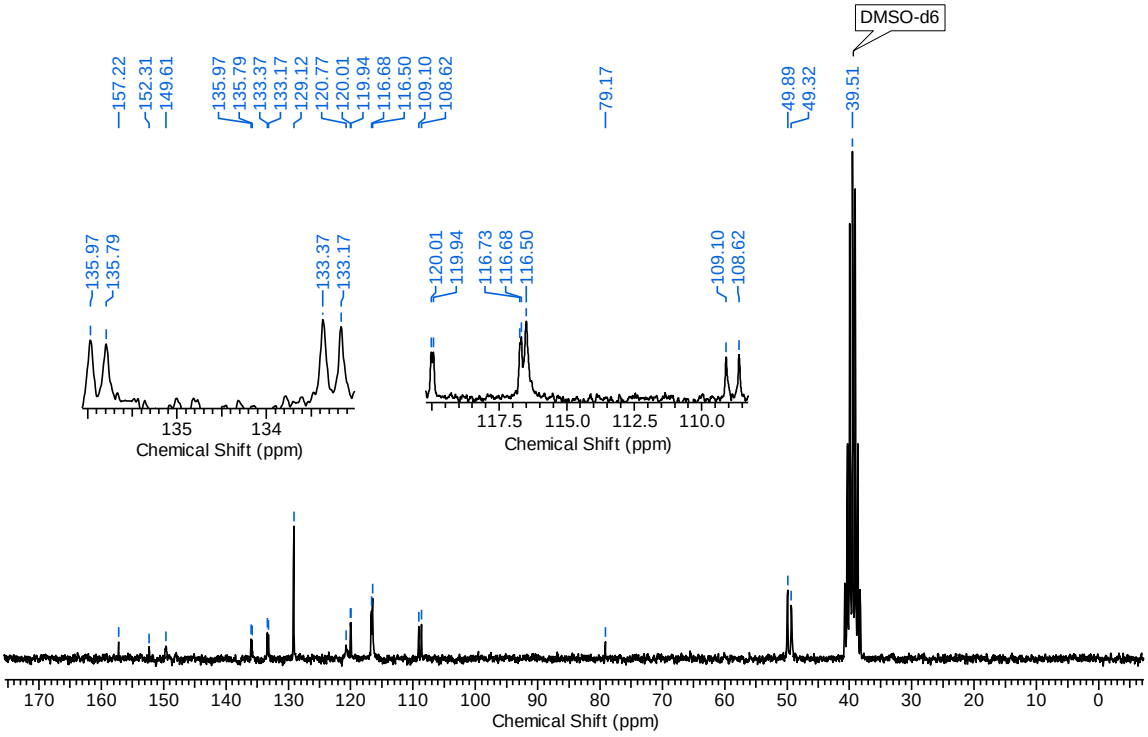
^{13}C NMR (50 MHz, $CDCl_3$)



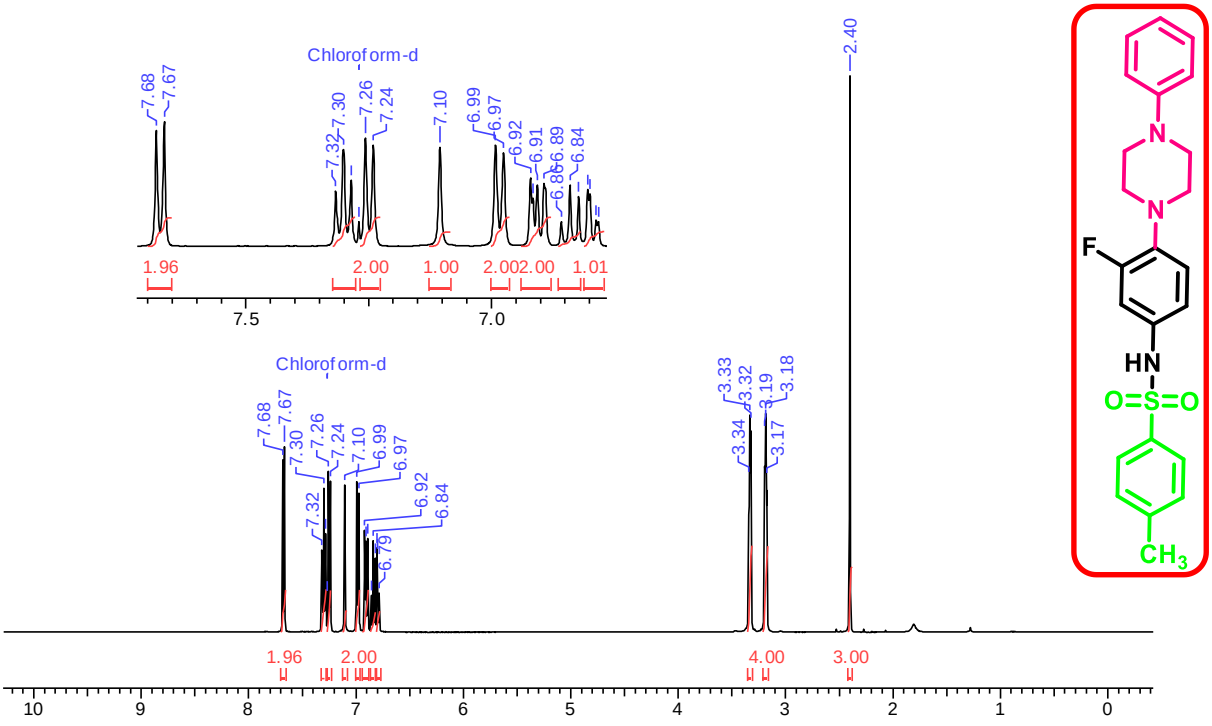
^{1}H NMR (200 MHz, $CDCl_3$): NAM-20



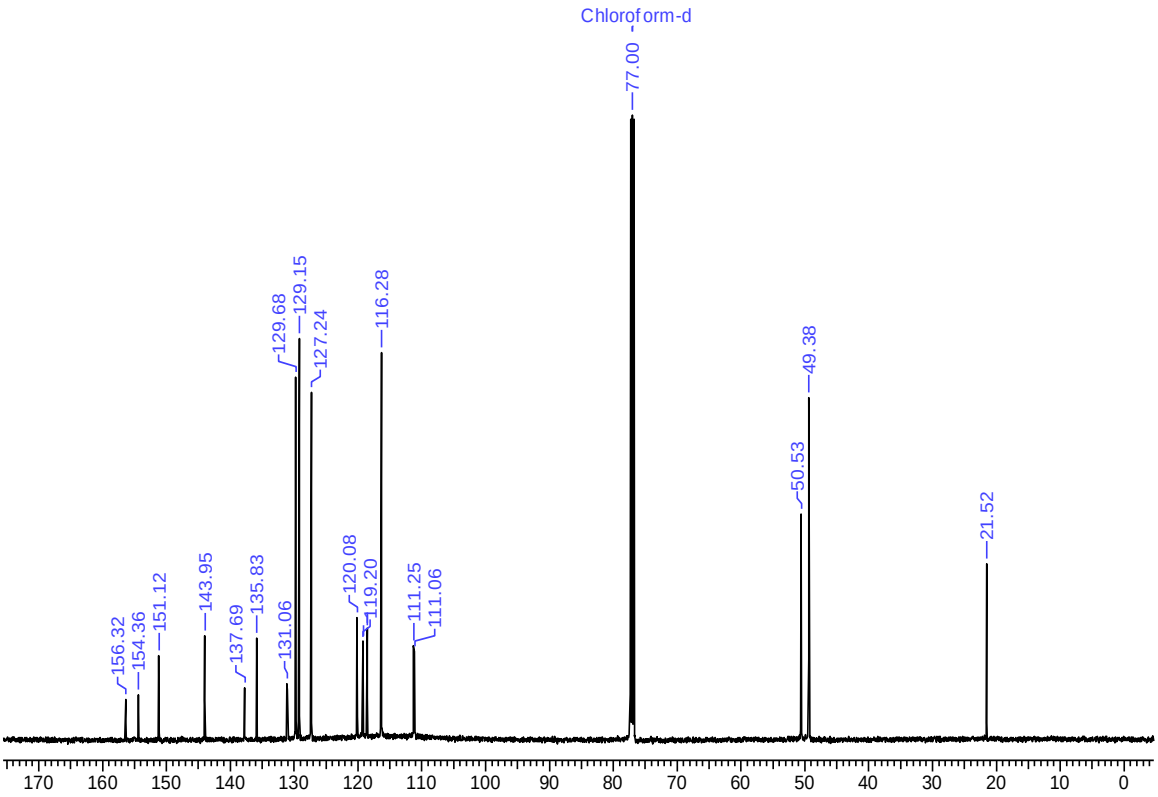
^{13}C NMR (50 MHz, $CDCl_3$)



^{1}H NMR (500 MHz, $CDCl_3$): NAM-21



^{13}C NMR (125 MHz, $CDCl_3$)



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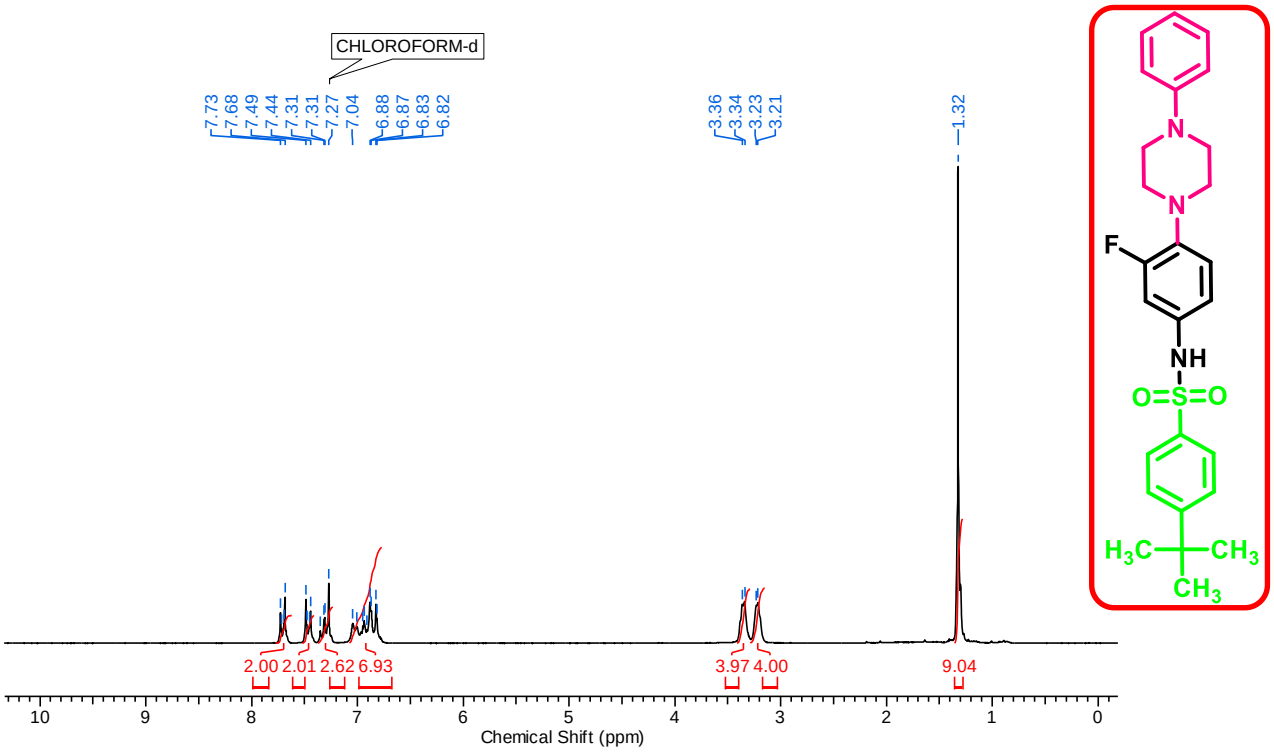

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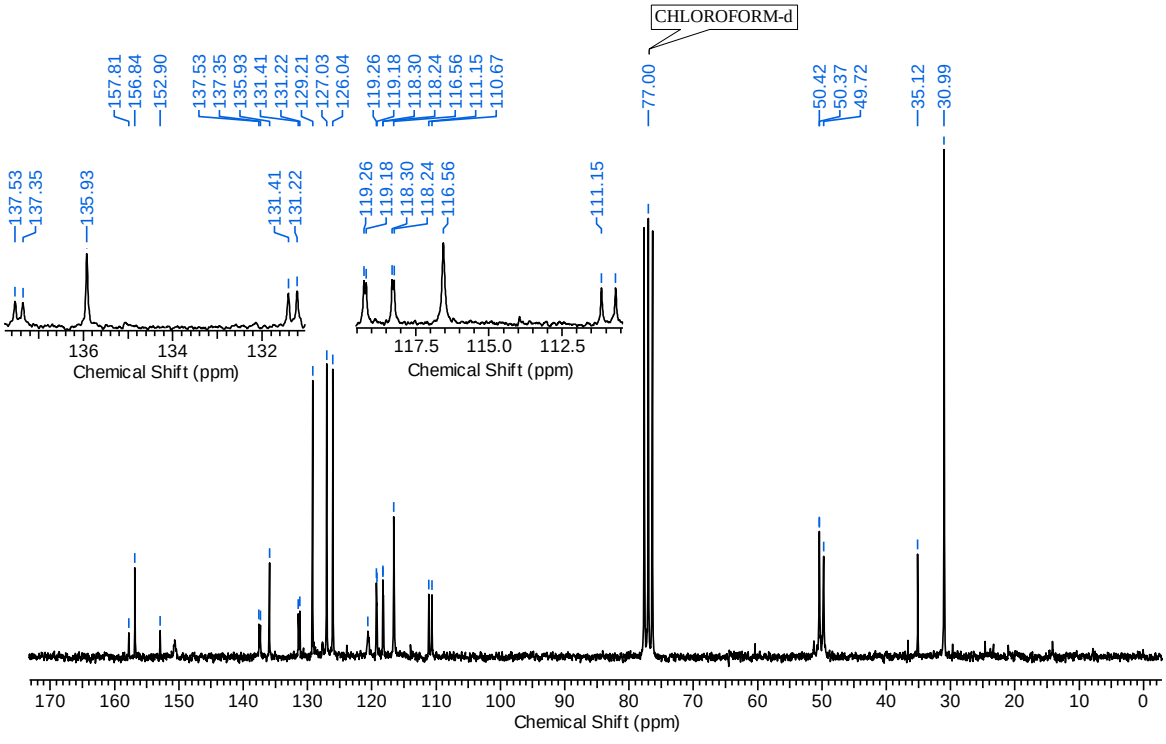
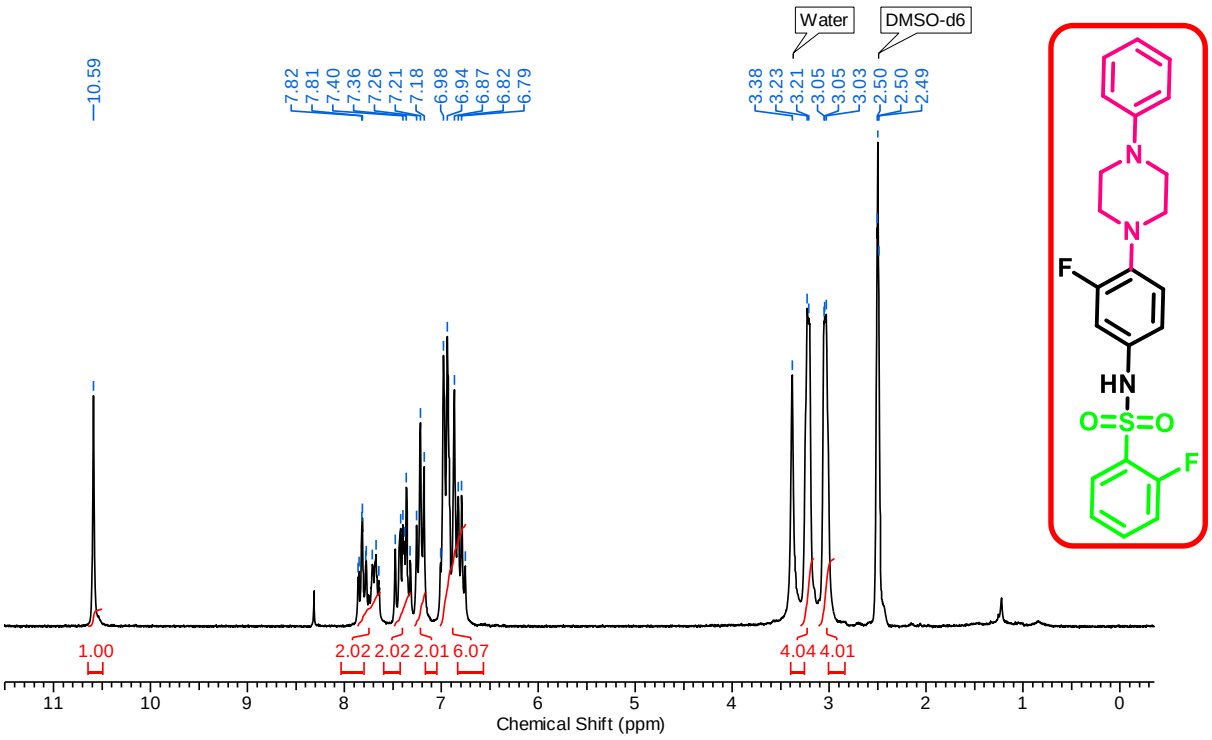
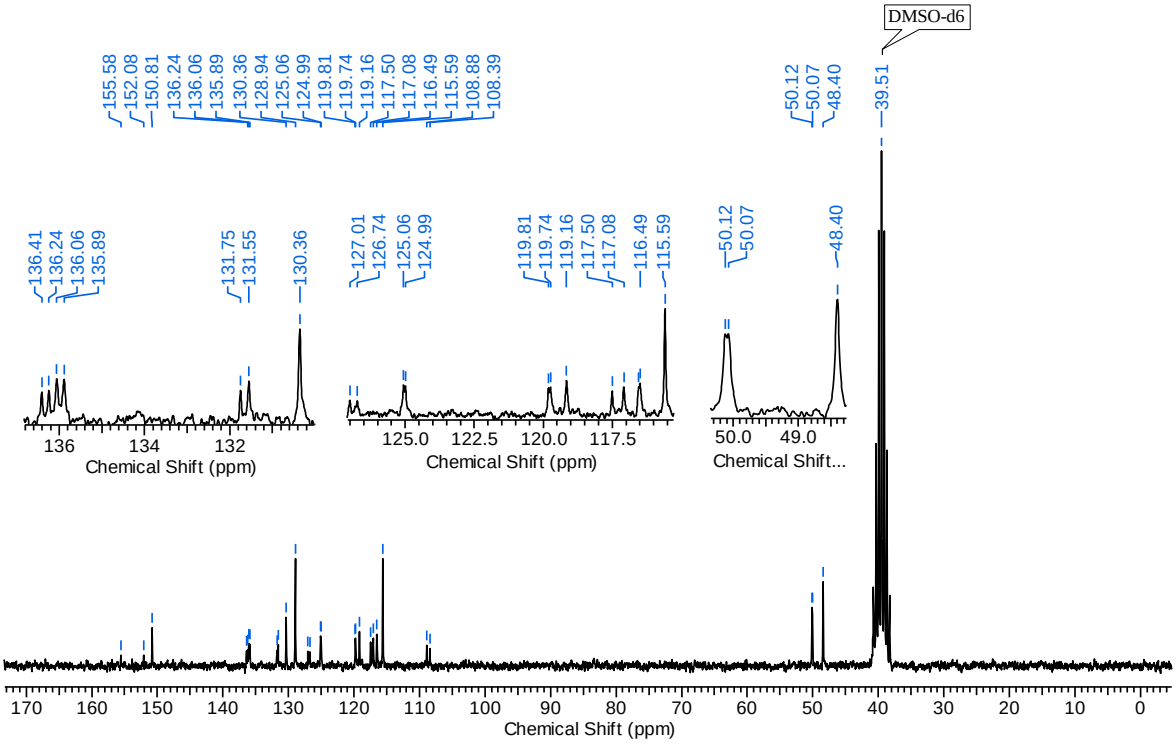


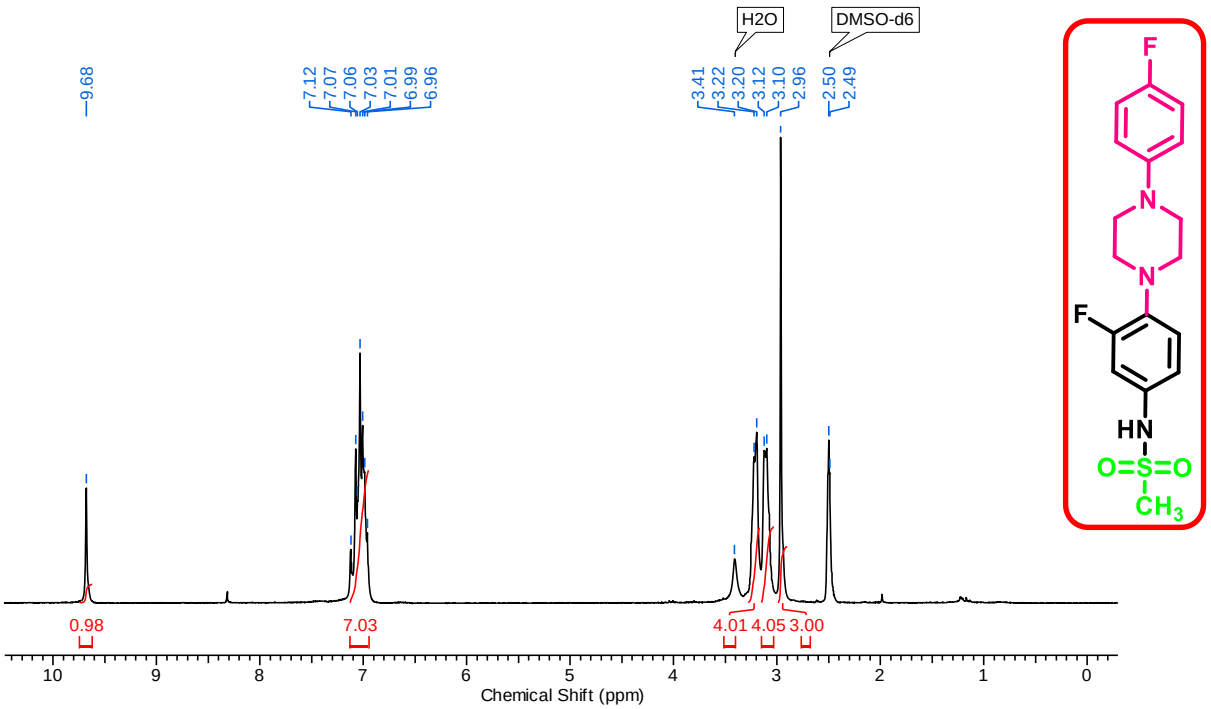
^{1}H NMR (200 MHz, DMSO-d$_6$): NAM-23



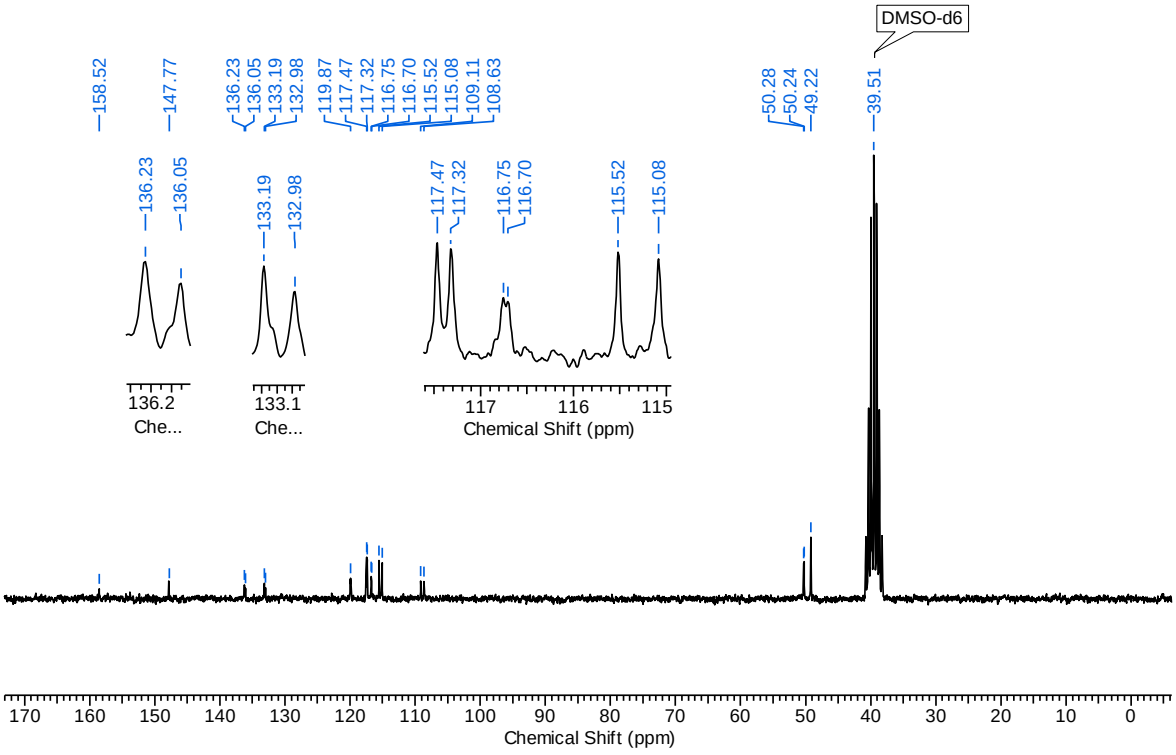
^{13}C NMR (50 MHz, DMSO-d$_6$)



^{1}H NMR (200 MHz, $CDCl_3$): NAM-24



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



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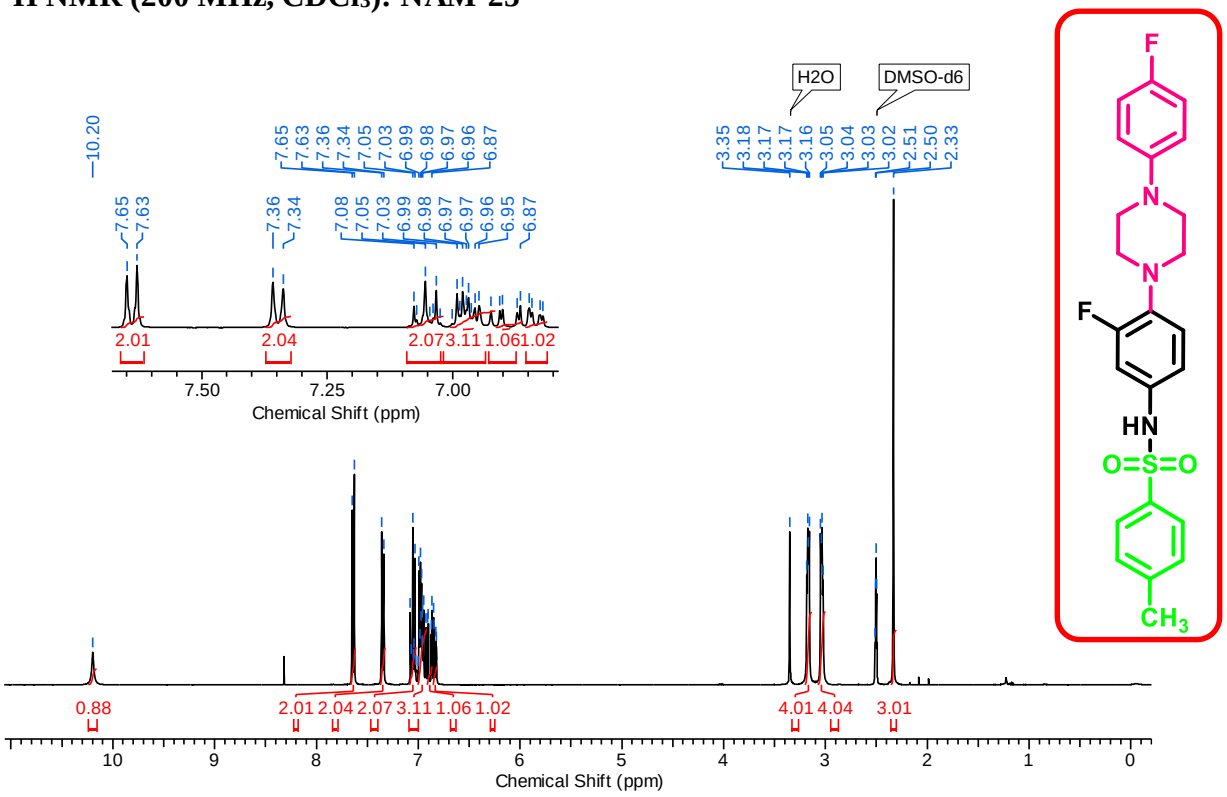
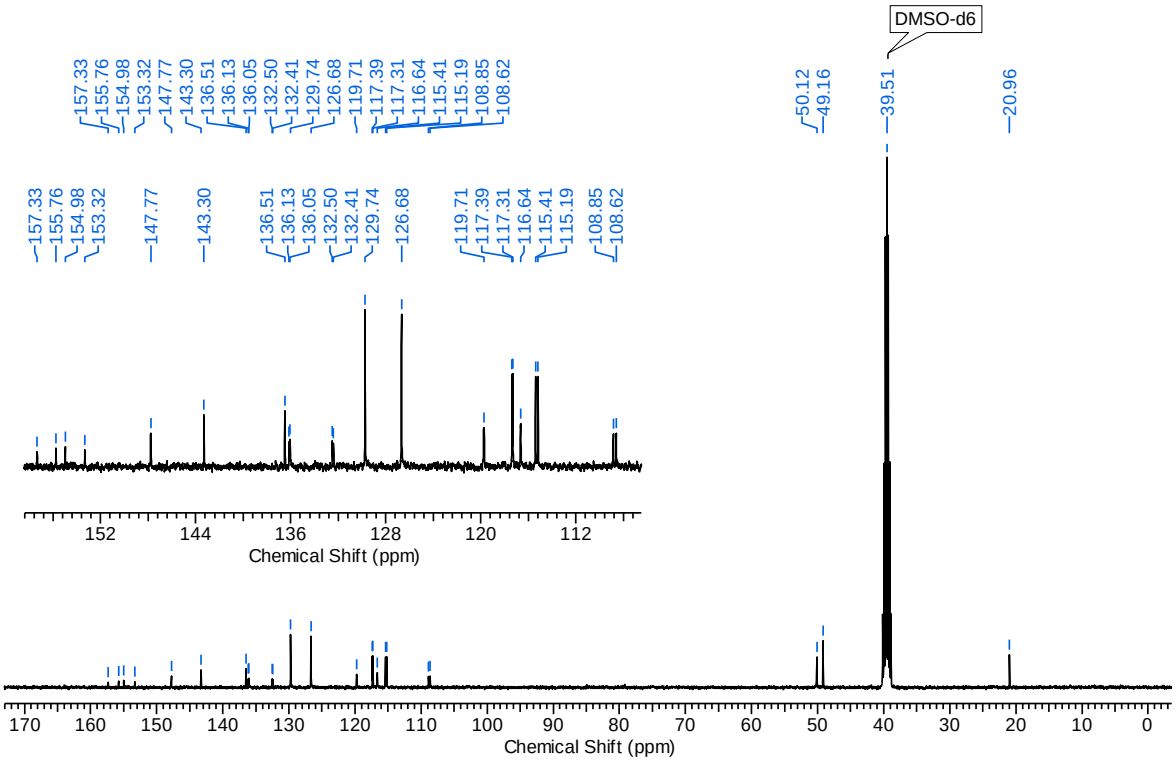


^{13}C NMR (50 MHz, $CDCl_3$)



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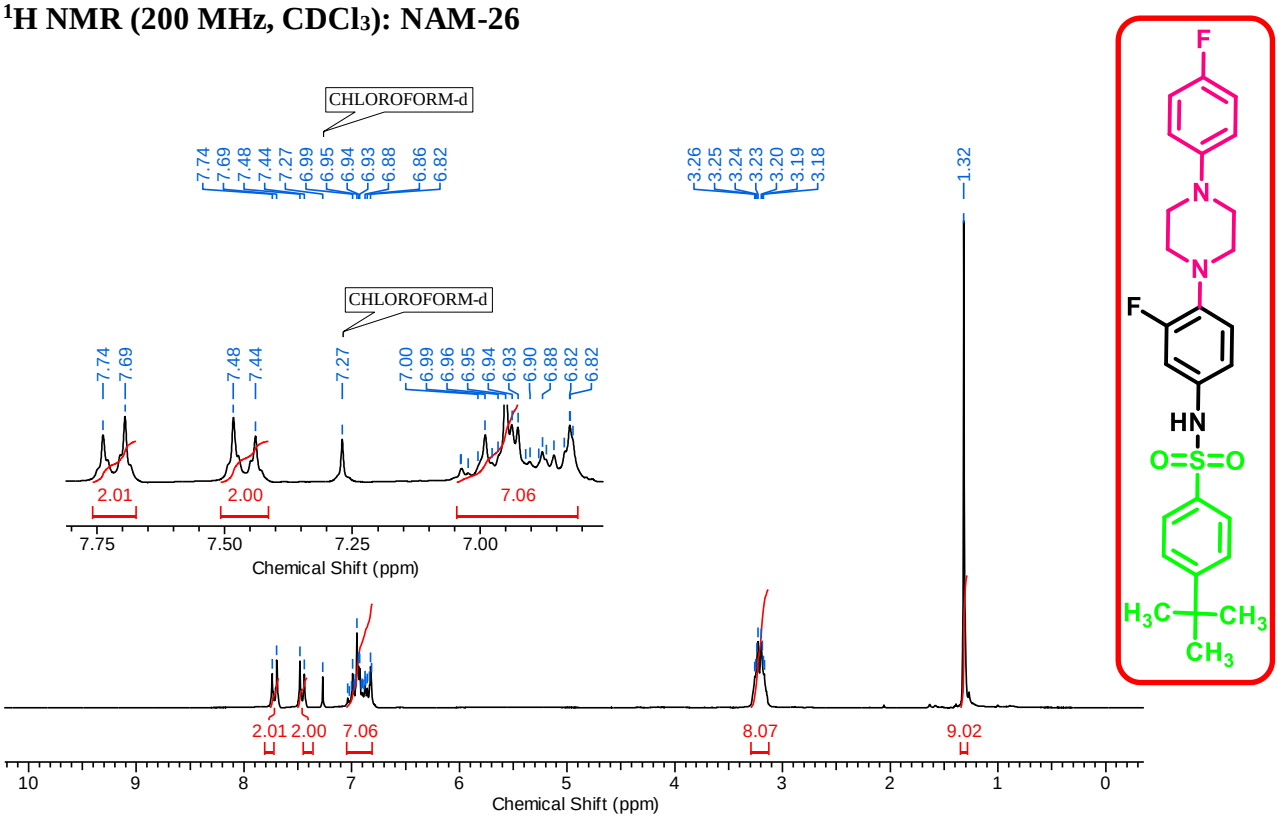
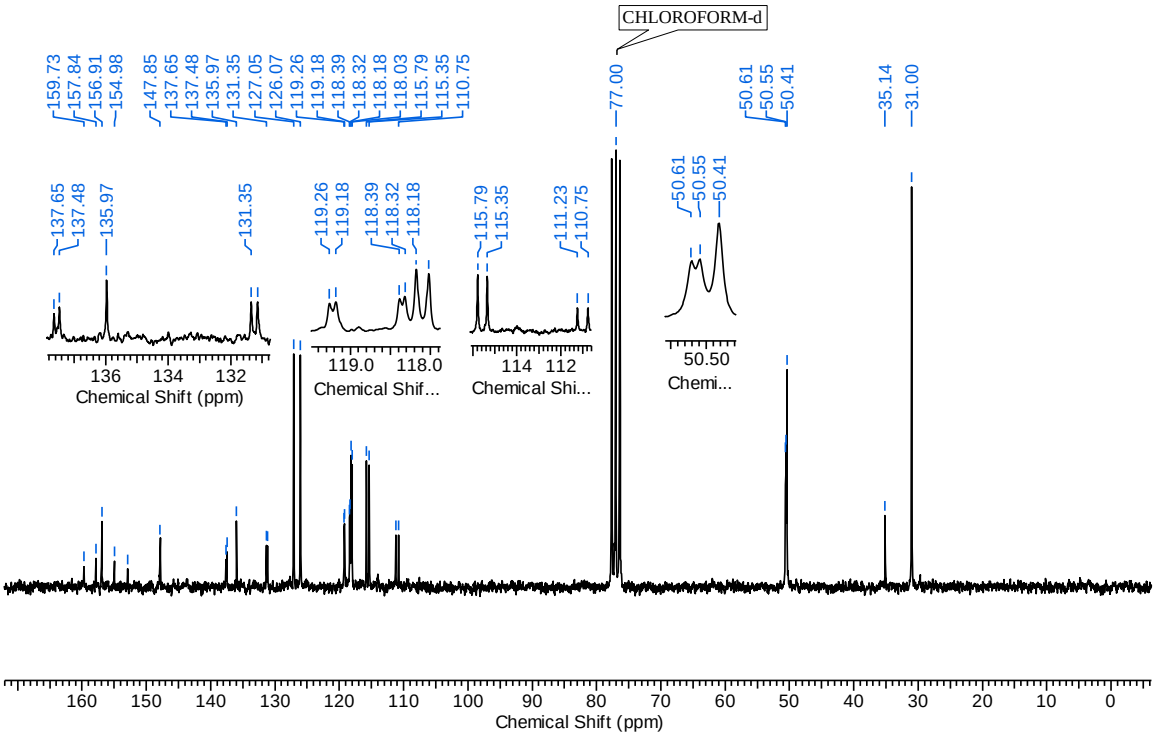


^{13}C NMR (50 MHz, $CDCl_3$)



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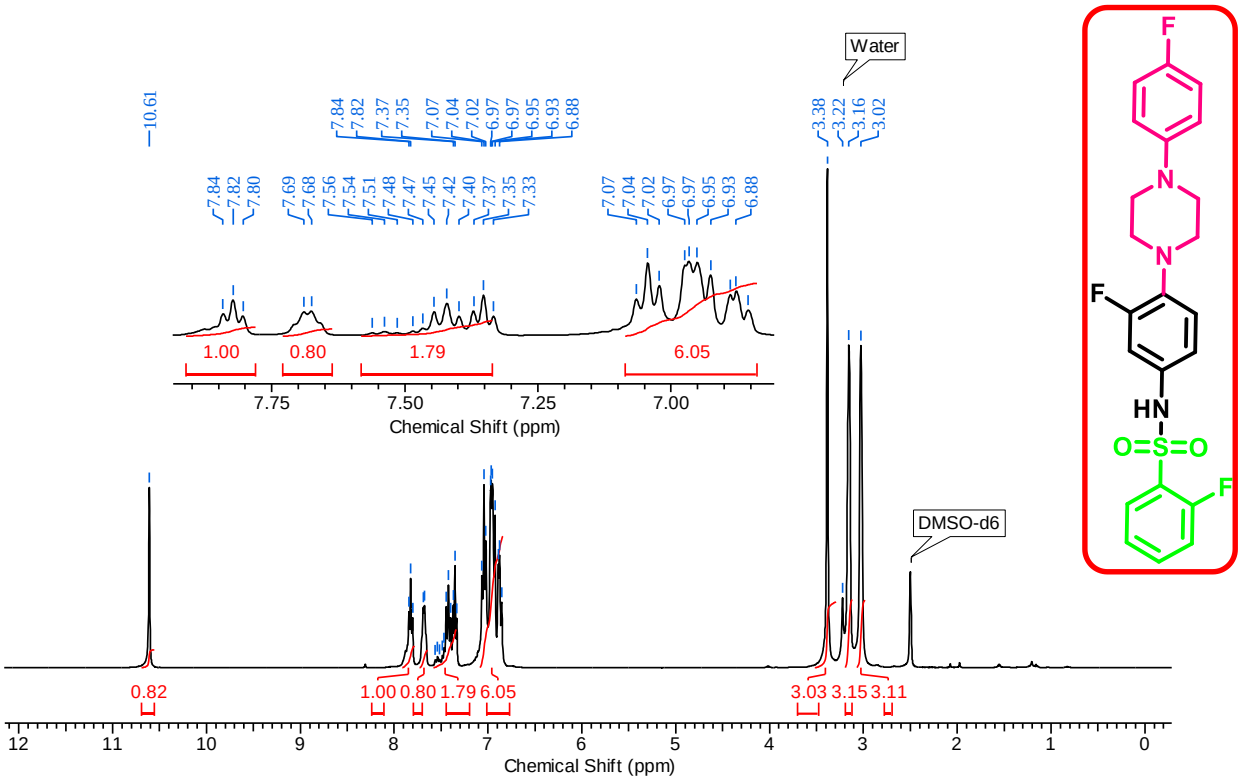
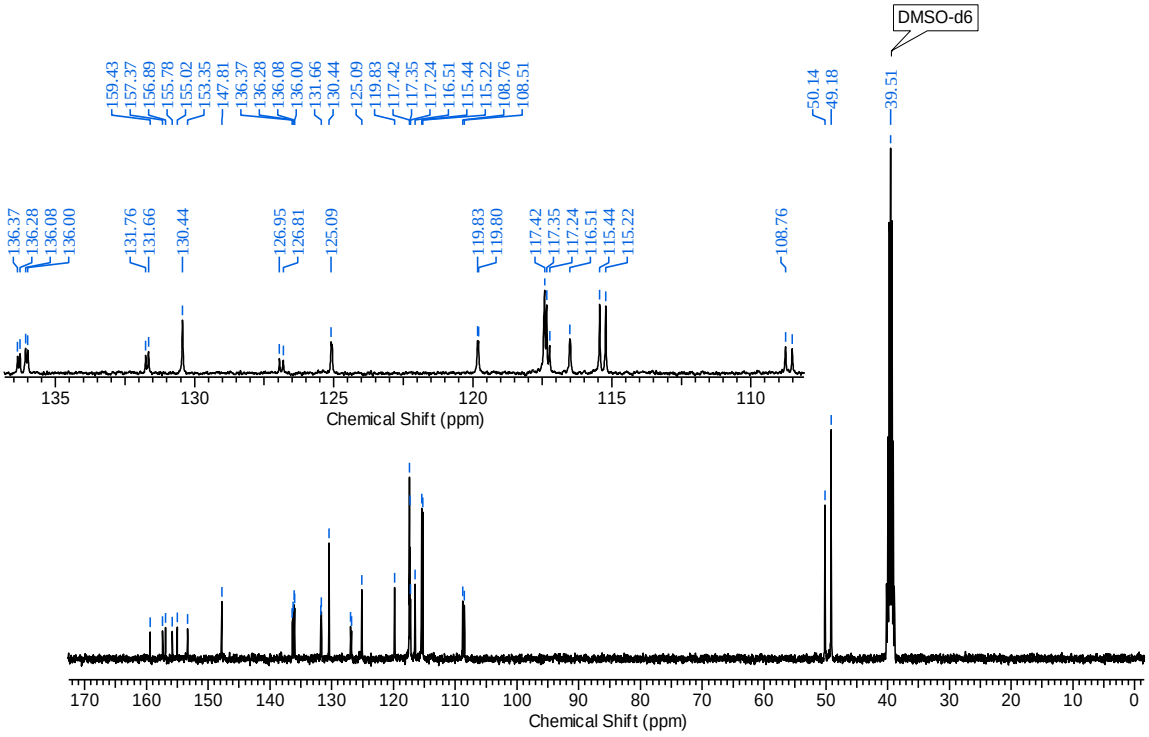
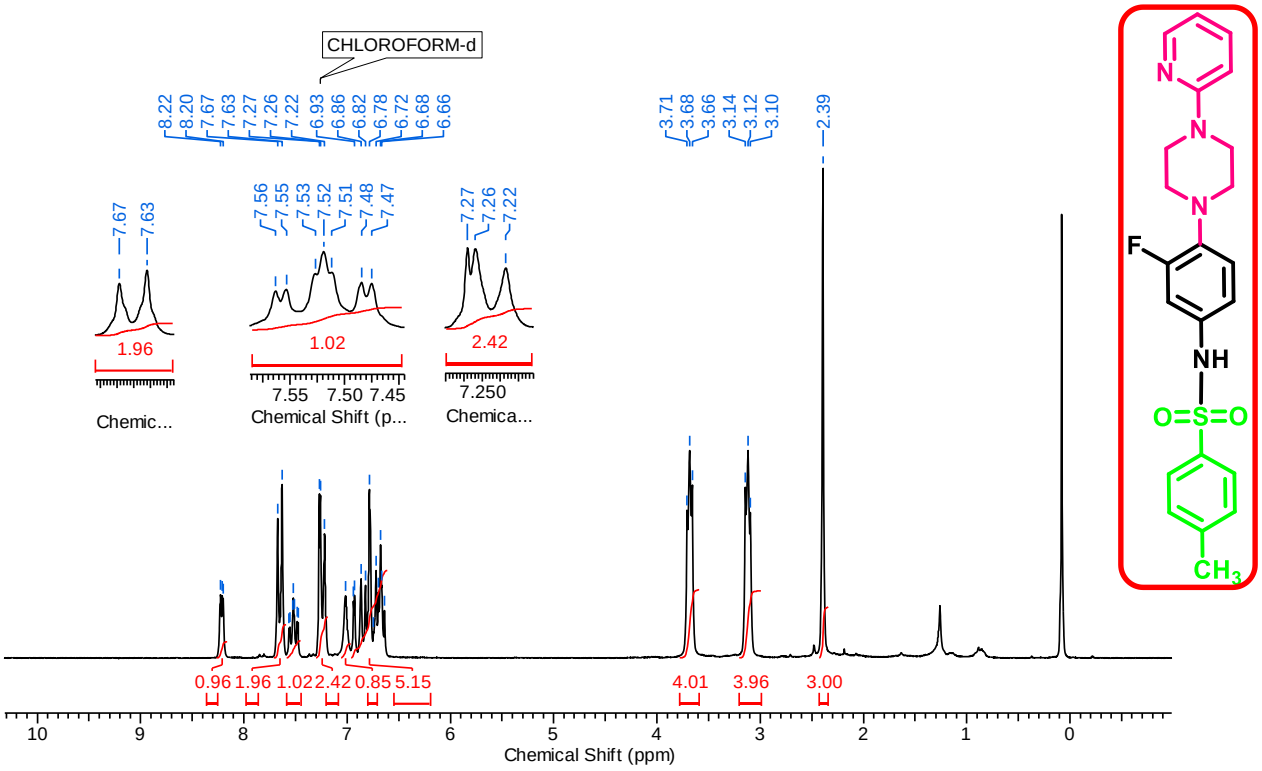


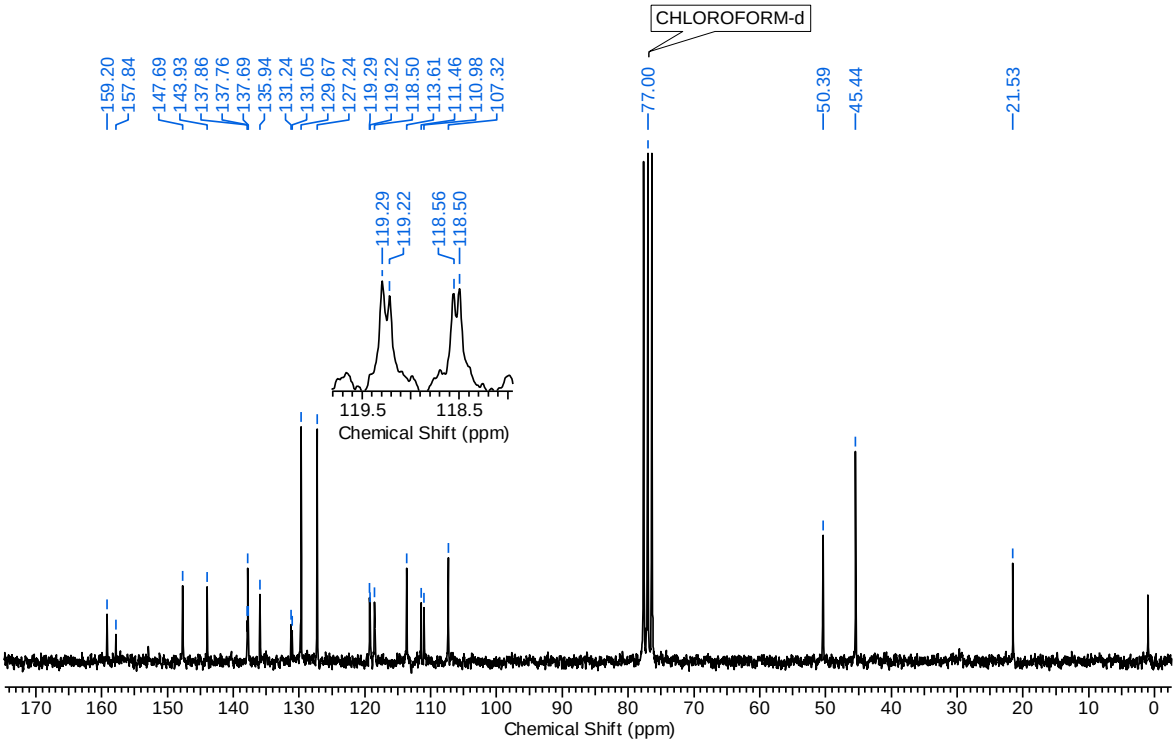
^{13}C NMR (50 MHz, $CDCl_3$)



^{1}H NMR (200 MHz, $CDCl_3$): NAM-28



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



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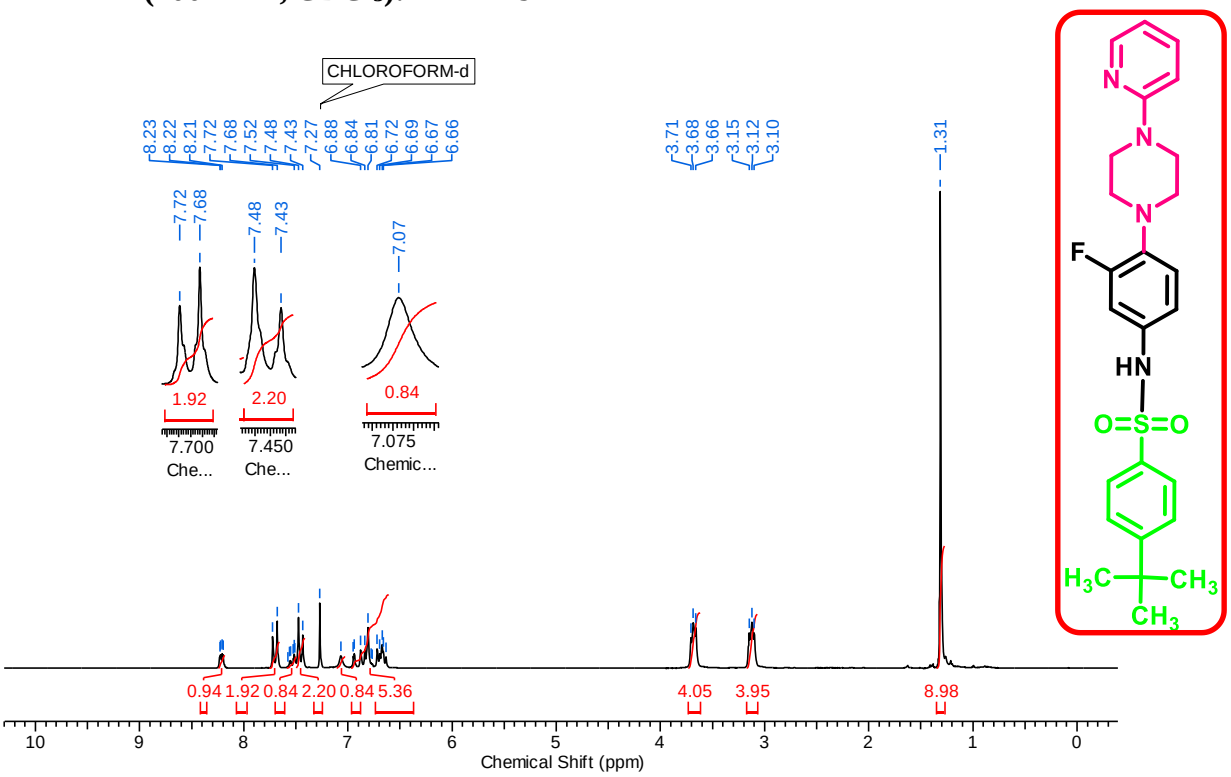
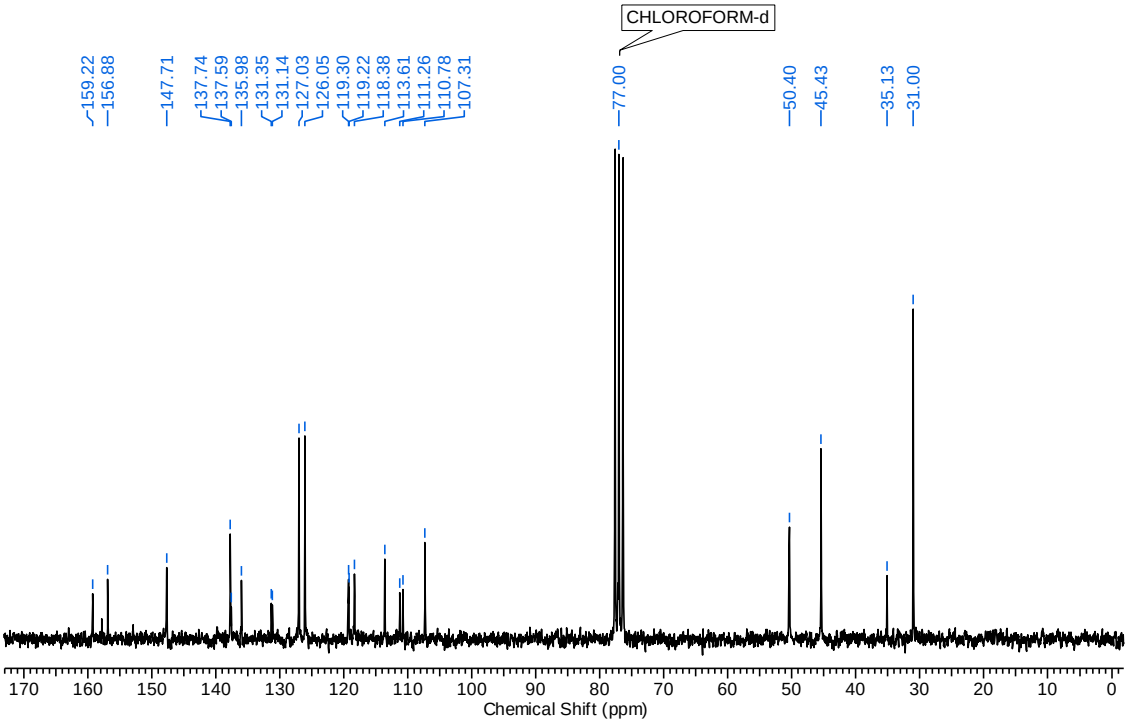
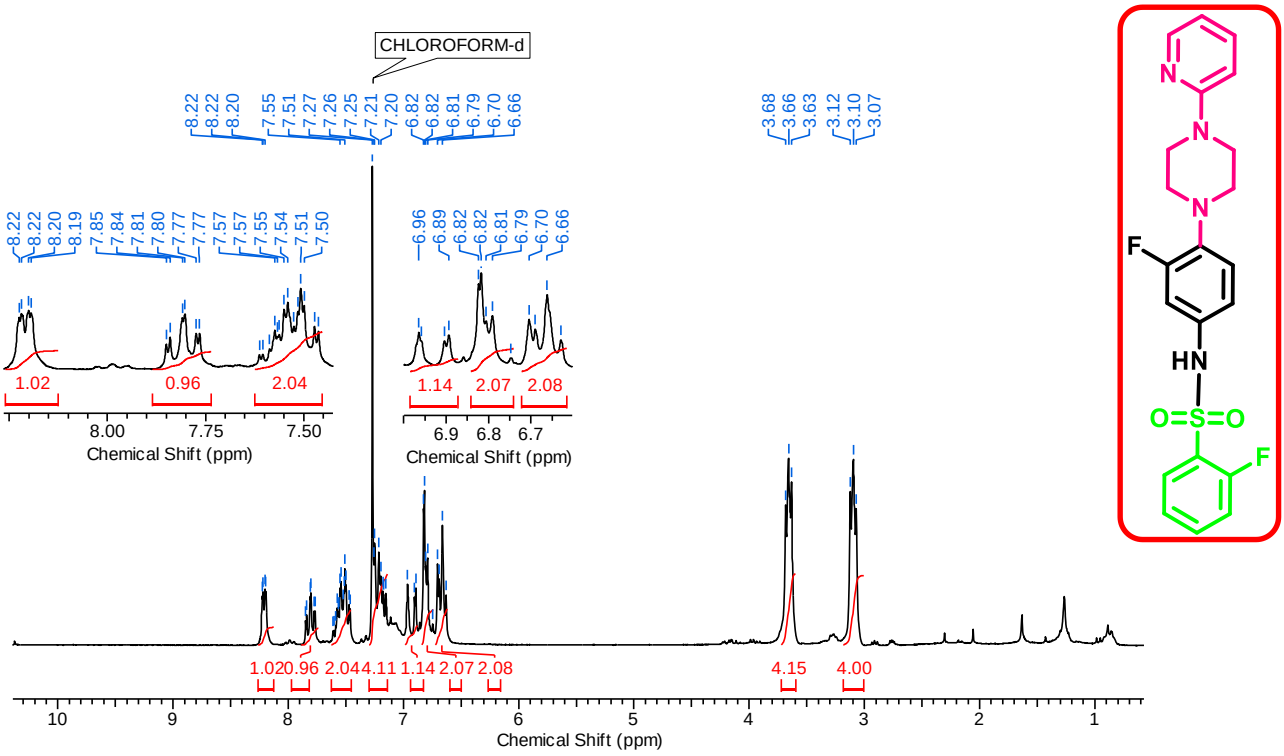


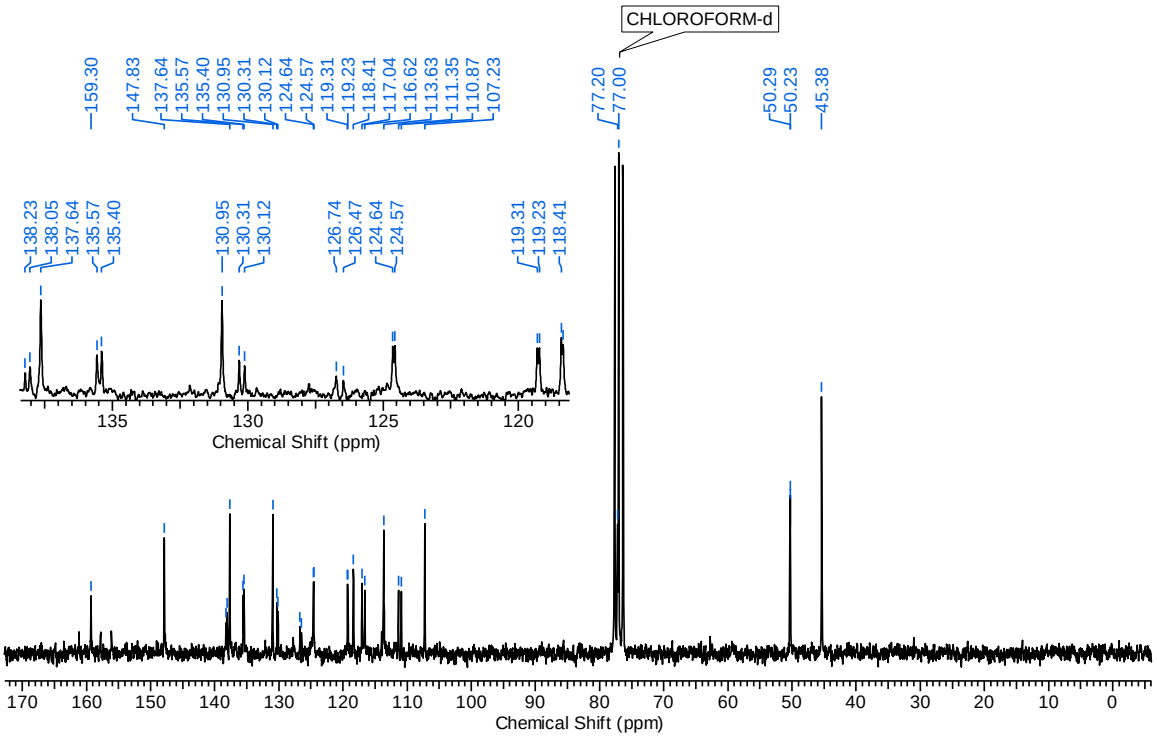
^{13}C NMR (50 MHz, $CDCl_3$)



^{1}H NMR (200 MHz, $CDCl_3$): NAM-30



^{13}C NMR (200 MHz, $CDCl_3$)



Biology

Cell culture.

Cell culture conditions were maintained as per the need of the respective cell lines used. Human mammary adenocarcinoma (MCF-7) and (MDA-MB-231) cells were purchased from National Centre for Cell Science (Pune, India). MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, USA) with 10 % Fetal Bovine Serum (FBS, Invitrogen) and 1 % penicillin/streptomycin (Himedia) at 37°C in a humidified CO_2 (5%) incubator.

***In vitro* cell viability assay.**

To study the cytotoxicity of compounds **(5)** and **(7)** on the cell viability, MCF-7 and MDA-MB-231 cells were seeded at a density of $1x10^4$ in a 96-well plate, respectively and grown for 24 h at 37˚C in a CO_2 incubator. Post 24 h, the old media was discarded and the cells were treated with the compounds at varying concentrations for 24 h in serum-free media. The viability of the cells was then determined by adding 20 µL of MTT reagent (5 mg/mL) and incubating for 4 h. Followed by MTT additions and incubation, 200 µL of DMSO was added to solubilize the formazan crystal. Subsequently, the absorbance was measured at 570 nm on BioTek Synergy Instrument.

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Publications

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AN EFFICIENT SYNTHESIS OF POTENT ANTI-TUBERCULAR DRUG CANDIDATE BM212

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ABSTRACT

A novel five step total synthesis route for an anti-tubercular drug BM212 starting from 4-chlorophenacyl bromide was developed with 48% overall yield. The synthesis is amiable for the synthesis of biologically active derivatives of BM 212.

Keywords: Anti-tubercular, BM212, Paal-Knorr synthesis.

INTRODUCTION

Tuberculosis (TB), a widespread infectious bacterial disease mainly caused by *Mycobacterium tuberculosis*, is the second leading cause of death from a single infectious agent, after the human immuno deficiency virus (HIV). According to 2013 WHO (World Health Organization) statistics, 9 million people fell ill with TB and 1.5 million died from the disease.[1] Currently, the first-line drugs for TB include Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB) have their own benefits and side effects (figure 1).[2] However, the emergence of multiple drug resistant (MDR) strains of TB and extreme drug resistant (XDR) strains of TB has created a global epidemic problem.[3] Therefore, development of new drug molecules having novel mode of action is an urgent requirement.[4-6] In this regards, the quest for a novel anti-mycobacterial drug led to the identification of BM212 as a promising therapeutic agent against M. Tb (figure 1).[7] The molecule BM212 shows potent and selective antimycobacterial[8] and antifungal[9] activities with lesser toxicity[10] than the existing drugs. However, it is surprising to note that only a few reports are available in the literature for the synthesis of BM212.[8b,9-13] The previously reported methods have low overall yields and limited scope for inclusion of substituents at 3 position of pyrrole ring in BM212. Within an ongoing project aimed at synthesis of biologically significant heterocycles, we recently decided to search for a new protocol that would allow rapid access to BM212. Herein, we report a simple and highly efficient synthesis of BM212 in five steps with 48% overall yield.

EXPERIMENITAL

All reactions were performed in round-bottom flask fitted with balloon filled with nitrogen, otherwise specified. Transfer of air- and moisture-sensitive liquids were performed via cannula under a positive pressure of nitrogen. TLC analysis was performed on Merck TLC (silica gel $60F_{254}$ on glass plate). Evaporation and condensation were carried out *in vacuo*. Visualization of TLC was carried out by using Iodine or by charring solutions such as molybdenum, anisaldehyde and ninhydrin. All the starting materials were purchased from the commercial sources (Sigma-Aldrich and Spectrochem) and used without further purification. The reagents and the solvents were dried and purified before use by usual procedures and kept under inert condition. Column chromatographic purifications were carried out on

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Discovery of Rimonabant and its potential analogues as anti-TB drug candidates

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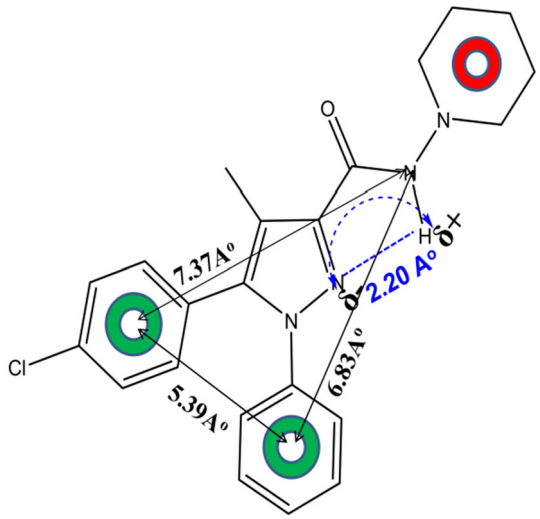
Abstract Rimonabant and its analogues have been synthesized in moderate to good yields using a simple synthetic route. All the newly synthesized compounds were subjected to in vitro screening against *M. tuberculosis* and *M. smegmatis*. The most potent analogue JMG-14 exhibits MIC value of 3.13 compared to 3.25 and 50 μg/ml for ethambutol and pyrazinamide, respectively. The molecular docking reveals that pyrazole ring, number and position of halogen atoms play a crucial role in deciding interactions with MTCYP-121. These findings open up a new avenue in the search of potent anti-TB drugs with rimonabant and its novel analogue JMG-14 as lead molecules.

Graphical Abstract



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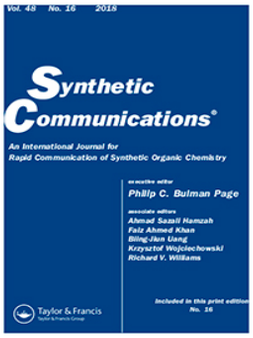
Keywords Rimonabant · Diaryl pyrazoles · Tuberculosis · Mycobacterium tuberculosis · H37Rv · MTCYP-121

Introduction

Tuberculosis (TB) is among the deadly diseases afflicting mankind, and it still remains a major public health burden in many developing countries (Corbett *et al*., 2003). Approximately one-third of the world's population is still under the ill influence of TB. In 2011, nearly 9 million people around the world suffered from TB (WHO 2013), with 1.4 million deaths worldwide. Recent reports reveal that every year 0.2 million from 0.7 million HIV patients die due to TB,



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A convenient synthesis of the enantiomerically pure (*S*)-2,4-dihydroxybutyl-4-hydroxybenzoate using hydrolytic kinetic resolution

Namita A. More, Nitin L. Jadhao, Dinesh R. Garud & Jayant M. Gajbhiye

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A convenient synthesis of the enantiomerically pure (*S*)-2, 4-dihydroxybutyl-4-hydroxybenzoate using hydrolytic kinetic resolution

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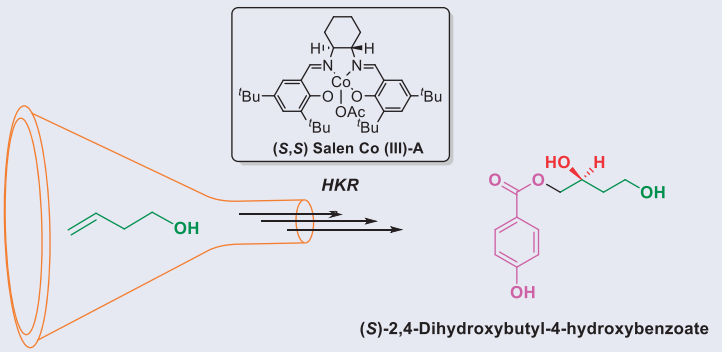
ABSTRACT

(*S*)-2,4-Dihydroxybutyl-4-hydroxybenzoate was prepared in an extremely simple and practical way with high enantiomeric excess (99% ee) using Jacobsen's Hydrolytic Kinetic Resolution technique as a key step and source of chirality.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
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KEYWORDS
Anti-tumor; enantioselective; hydrolytic kinetic resolution

Introduction

In recent years, marine-microbes have proven to be rich sources of bioactive marine natural products with a unique structure and potent pharmaceutical activity.[1] The filamentous fungi are a vital source of bioactive natural products for their large metagenomes and more complex genetic backgrounds and play an important role in the discovery of lead compounds and environmental restoration.[2] In this regard, Penicillium fungi have received great attention among all marine-derived fungi and are becoming a promising reservoir of marine natural products with bioactivities and novel

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This article has been republished with minor changes. These changes do not impact the academic content of the article.
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Supplemental data for this article can be accessed on the publisher's website.

Curriculum vitae

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Objective

My career objective is to work in an environment where my qualifications and expertise are recognized, and in-turn utilized for the progress of the organization

Academic Qualification

Dec 2016 – August 2020	**Ph. D in Organic Chemistry** Research supervisor: Dr. J. M. Gajbhiye Department of Organic Chemistry, CSIR-National Chemical Laboratory, pune.
July 2010 – July 2012	**Master of Science in Organic Chemistry** First class (60.2%) Modern College, Ganeshkhind, Pune, Department of Organic Chemistry University of Pune, India
July 2007 - June 2010	**Bachelor of Science in Chemistry** First class (69.53%) Modern College, Ganeshkhind, Pune, Department of Organic Chemistry University of Pune, India

Research projects done

- At my master level I have undergone the project entitled **"Synthesis of Naftifine"** from Dec-2011 to April 2012 at Modern College, Ganeshkhind, Pune.
- I have completed the project entitled **"Novel of anti-tubercular drug**

candidates and process for preparation thereof" from Feb 2013 to Apr 2017 at CSIR-NCL, Pune.

- I have completed the project entitled **"Innovative processes and technologies for indian pharmaceutical and agrochemical industries"** from March 2018 to Jan 2020 at CSIR-NCL, Pune.
- I have completed my Ph.D in Organic Chemistry, the thesis entitled **"Design, Synthesis and Biologically Evaluation of Heterocycles and their Encapsulation in Drug Delivery System"** from Jan 2017 to August 2020 at CSIR-NCL, Pune.

Skill Set

- Multi-step organic synthesis in organic chemistry.
- Synthesis and performing air, moisture, and microwave assisted and light sensitive reactions.
- Well versed with various chemistry related computer packages viz. Chem Draw, SciFinder, *etc*.
- Knowledge of Techniques and expertise in handling instruments like FT-IR, NMR, HPLC, LCMS, X-ray crystallography, SEM and TEM images, *etc*.
- Skilled in Column Chromatography

Research Interests

- Total synthesis of biologically active natural compounds
- Medicinal chemistry
- Drug release study
- Patent analysis
- Publication editing

Publications

1) J. M. Gajbhiye*, **N. A. More**, Manoj D. Patil, R. Ummanni, S. S. Kotapalli, P. Yogeeswari, D. Sriram, V. H. Masand, "**Discovery of Rimonabant and its potentialanalogues as anti-TB drug candidates**", *Med. Chem. Res.*, **2015,** *24*, 2960-2971.

2) Pankaj S. Mahajan, Subhash D. Tanpure, **Namita A. More**, Jayant M. Gajbhiye and Santosh B. Mhaske*,"**Ammonium persulfate activated DMSO as the one carbon synthon for an efficient access to methylenebisamides and some other applications**", *RSC Adv*., **2015**, *5*, 101641-101646.

3) **Namita A. More**, M. D. Patil, D. R. Garud & J. M. Gajbhiye*, "**An efficient synthesis of potent anti-tubercular drug candidate BM212**", *RJC*, **2016**, *9* **(4)**, 806-811.

4) **Namita A. More**, N. L. Jadhao, D. R. Garud & J. M. Gajbhiye*, "**A convenient synthesis of the enantiomerically pure (S)-2,4-dihydroxybutyl-4- hydroxybenzoate using hydrolytic kinetic resolution**", *Syn. Comm.*, **2018**, *48* **(16)**, 2093-2098.

5) Jaisingh M. Divse, Vandana S. Pore, **Namita More**, Sunita Kunte and Santosh Mhaske*, "**Cholic acid based trans-4-hydroxy-(L)- proline derivative as recyclable organocatalyst for highly diastereo and enantioselective asymmetric direct aldol reactions in water**" (Manuscript to be submitted).

6) **Namita A. More**, K. R. Gajbhiye, Prajakta Tambe, V. Gajbhiye, Jayant M. Gajbhiye*, "**Novel 3-fluoro-4-morpholinoaniline derivatives: Synthesis and its assessment of anti-cancer activity in breast cancer cells"** (Manuscript to be submitted).

Conference papers and presentation

- Participated in the National Seminar On Next Two Decades of Chemical Sciences & Technology at the National Chemical Laboratory, Pune (Sep 2011)
- Participated in the National Seminar on New trends in chemical research and writing of scientific research paper at Modern college, Ganeshkhind, Pune (Feb 2012)
- Presented Poster entitled "Discovery of Rimonabant and its potential analogues as anti-TB drug candidates" in National Chemical Laboratory, Pune (Feb 2017)

- Participated in the National conference on Recent Advances in Chemical Sciences at Sri Shivaji college of arts, commerce and science, Akola (Feb 2018)
- Participation in the conference organized by CSIR-National Chemical Laboratory (Nov 2018).

Personal Details

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Thank you very much for spending your very precious time for having a look at my Curriculum Vitae.

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References

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