SYNTHETIC STUDIES TOWARDS FLUCONAZOLE ANALOGUES

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Dedicated To MY FATHER

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CERTIFICATE

This is to certify that the work presented in this thesis entitled **"SYNTHETIC STUDIES TOWARDS FLUCONAZOLE ANALOGUES"** submitted by Mr. Suleman R. Maujan, has been carried out by the candidate at National Chemical Laboratory, Pune, India, under my supervision. Such materials as obtained from other sources have been duly acknowledged in the thesis. This work is original and has not been submitted for any other degree or diploma of this or any other university.

MAY 2010

Dr. H. B. BORATE

CANDIDATE'S DECLARATION

I hereby declare that the research work presented in the thesis entitled **"SYNTHETIC STUDIES TOWARDS FLUCONAZOLE ANALOGUES"** was carried out by me at the National Chemical Laboratory, Pune, India, under the supervision of **Dr. H. B. BORATE**, Sc. F, Division of Organic Chemistry, National Chemical Laboratory, Pune, India and submitted for the degree of Doctor of Philosophy in Chemistry to the University of Pune. This work is original and has not been submitted in part or full by me for any other degree or diploma of this or any other university.

MAY 2010 **SULEMAN R. MAUJAN**

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*I owe my deepest gratitude and affection to my Father "****ABBAJAN****". He inspired me to become a DOCTOR. No words would suffice to express my gratitude and love to AMMIJAN, my brother (Bhaijan)* ***AEJAZ*** *(The Right Man In The Right Place), adored sisters (Aapa) NAZNEEN and MUMTAJ, uncle Sd. ABDULLAH and brother-in-law KHALED and JAWED and all family members for their continuous showering of boundless affection on me and supporting me in whatever I chose or did. It is my parent's prayer, constant struggle and relentless hard work to overcome the odds of life, which has inspired me to pursue life with a greater optimism. The moral values of my parents guided me throughout my life and I would always look up to them for strength no matter what I have to go through. This thesis is a result of the extraordinary wills, efforts and sacrifices of my parents. My successes are dedicated to them now and always.*

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Suleman R. Maujan

CONTENTS

Chapter 2: Synthesis of dimeric fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one and their bioevaluation

Chapter 3: Synthesis of fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-ones or 2*H*-1,4-benzoxazin-3(4*H*)-ones and one-step preparation of α-chlorostyrenes

Section I: Synthesis of fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-ones or 2*H*-1,4-benzoxazin-3(4*H*)-ones

Section II: One-step preparation of α-chlorostyrenes

GENERAL REMARKS

- ^{1}H NMR spectra were recorded on Bruker AC–200 MHz, Bruker AC–400 MHz and DRX–500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C NMR spectra were recorded on Bruker AC–200, Bruker AC–400 and DRX–500 instruments operating at 50 MHz, 100 MHz and 125 MHz respectively.
- EI Mass spectra were recorded on Finngan MAT–1020 spectrometer at 70 *eV* using a direct inlet system and applied Biosystems API QSTAR Pulsar Mass Spectrometer (Electrospray ionization, direct infusion method, solvents used were acetonitrile/methanol).
- The X-Ray crystal data were collected on *Bruker SMART APEX* CCD diffractometer using Mo K_{α} radiation with fine focus tube with 50 kV and 30 mA.
- Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and measured in cm^{-1}.
- Optical rotations were measured with a JASCO DIP 370 digital polarimeter.
- All reactions were monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F–254) with UV light, I_2 or *p*-anisaldehyde in ethanol as developing agents.
- All dry 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.
- All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 45 °C unless otherwise specified.
- Silica gel (60–120, 100–200 or 230–400 mesh) was used for column chromatography.

ABBREVIATIONS

Ac	Acetyl
Ac_2O	Acetic anhydride
$AlCl_3$	Aluminium chloride
AIBN	2,2'-Azobisisobutyronitrile
aq	Aqueous
b	Broad
$BF_3.OEt_2$	Borontrifluoride diethyl etherate
Bn	Benzyl
BnBr	Benzyl bromide
Boc	*tert* – Butyloxycarbonyl
$CDCl_3$	Deuterated chloroform
cm	Centimeter
d	Doublet
DMAP	*N, N'*-Dimethylaminopyridine
DCM	Dichloromethane
dm	Decimeter
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Duterated dimethyl sulfoxide
EDC	Ethylene dichloride
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et_3N	Triethyl amine
g	Grams
h	Hours
HPLC	High performance liquid chromatography
IR	Infra red
K_2CO_3	Potassium carbonate
KI	Potassium iodide
L	Liter

LDA	Lithium diisopropylamide
m	Multiplet
M^+	Molecular ion
mg	Milligrams
MHz	Megahertz
min	Minutes
ml	Milliliter
mmol	Millimole
MP	Melting point
n-BuLi	*n*-Butyllithium
Na_2SO_4	Sodium sulfate
$NaHCO_3$	Sodium bicarbonate
NaH	Sodium hydride
NaI	Sodium iodide
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
p	Pentet
psi	Per square inch
q	Quartet
rt	Room temperature
RT	Retention time
s	Singlet
t	Triplet
TBAF	Tetrabutylammonium fluoride
TBAB	Tetrabutylammonium bromide
TBDMS	*tert*-Butyldimethylsilyl
TEA	Triethyl amine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography

ABSTRACT

The thesis entitled "**Synthetic Studies Towards Fluconazole Analogues**" consists of three chapters. The first chapter is divided into two sections. First section describes the synthesis of fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one. Second section explains the Structure Activity Relationship (SAR) Study of synthesized compounds. The second chapter deals with the synthesis of dimeric molecules with fluconazole pharmacophores and substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one. The third chapter is divided into two sections wherein the first section deals with the synthesis of fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties, and their SAR studies while the second section deals with one-step preparation of α-chlorostyrenes.

Chapter 1: Section-I

Synthesis of fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one

Introduction

The incidence of life threatening fungal infections has tremendously increased in recent years due to greater use of immunosuppressive drugs, prolonged use of broad-spectrum antibiotics, wide-spread use of indwelling catheters, and also in cancer and AIDS patients. The presently marketed antifungal drugs are either highly toxic (amphotericin B) or becoming ineffective due to appearance of resistant strains (flucytosine and azole antifungals)[1]. Azole antifungals are strong inhibitors of lanosterol 14α-demethylase which is involved in the biosynthesis of ergosterol, a major component of fungal cell membrane[2]. Fluconazole (**1**) is one of the best drugs used as antifungal agents. It is an orally effective, potent, and safe triazole based antifungal drug, with favorable pharmacokinetic characteristics and low toxicity[3]. Also it has excellent oral bioavailability and half-life period of 30 hours so it is used safely for treatment of systemic infections in cancer, leukemia, transplant and AIDS patients.

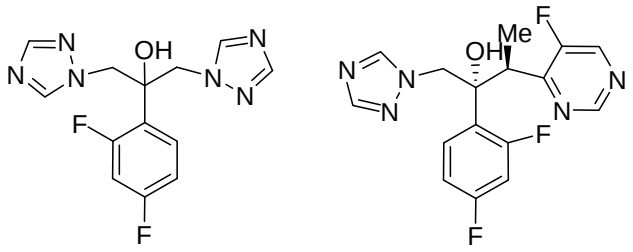


Figure 1. Fluconazole (**1**) and Voriconazole (**2**)

Worldwide efforts to obtain analogues of fluconazole[4] effective against resistant strains have resulted in synthesis of many novel azole antifungals such as Ravuconazole (**3**)[3], Voriconazole (**2**)[2,5], *etc*.

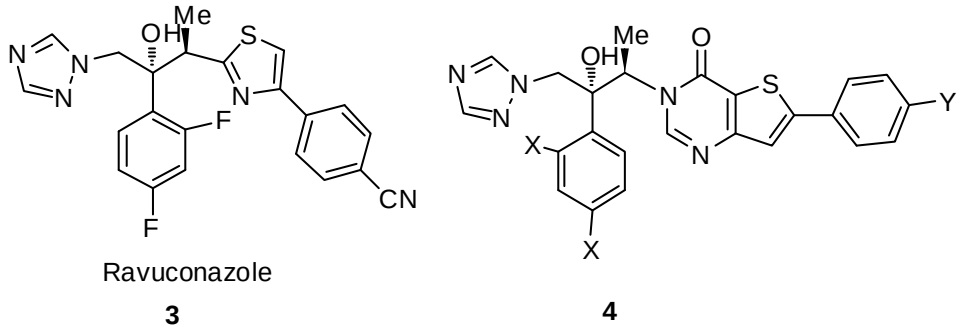
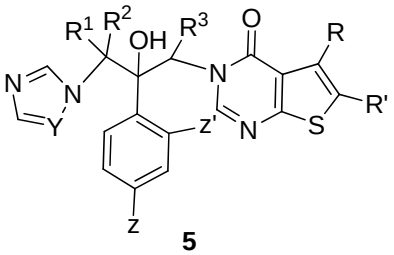


Figure 2. Ravuconazole (**3**) and fluconazole analogues

It is observed that the presence of one triazole ring, halogenated phenyl ring and tertiary oxygen functionality in fluconazole are necessary for activity (concluded from SAR studies). Voriconazole (**2**) is one more example out of many azole antifungals reported in literature containing these structural features.[6] Bartroli *et al.*[7] reported azole antifungals containing heterocycle-carboxamide derivatives of 3-amino-2-aryl-1-azolyl-2-butanol. Some of the analogues having general structure **4** reported by them contained substituted phenylthieno-[3,2-*d*]pyrimidin-4(3*H*)-ones. The synthetic strategy involved amidation of 3-amino-5-arylthiophene-2-carboxylic acid followed by cyclization to build up the pyrimidinone ring. We have synthesized some analogues of fluconazole, where one of the triazole is replaced by various substituted thieno-[2,3-*d*]pyrimidin-4(3*H*)-ones.

Present work

In order to seek new triazole antifungal agents, we designed a series of triazole compounds depicted by general formula **5**.



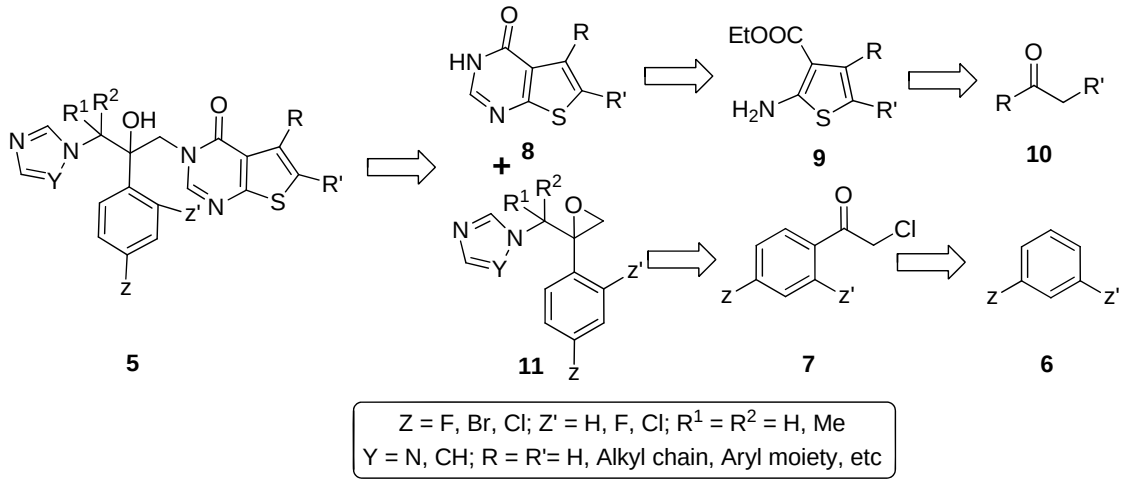
5

Z = F, Br, Cl; Z' = H, F, Cl; $R^1 = R^2 = R^3$= H, Me;
Y = N, CH; R = R'= H, Alkyl chain, Aryl moiety, etc

Figure 3. Fluconazole analogues containing thieno-[2,3-*d*]pyrimidin-4(3*H*)-ones

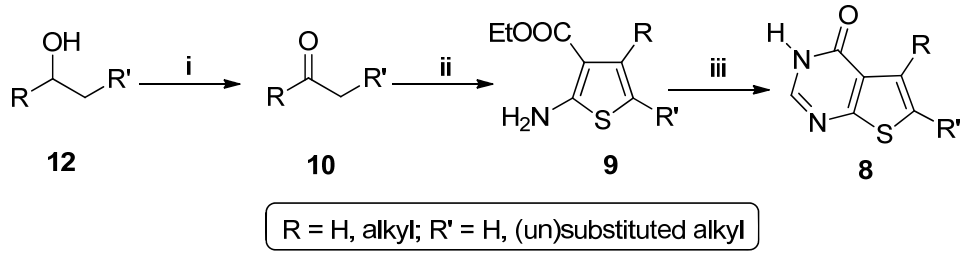
As the structure activity study of fluconazole has revealed that the left half portion of the molecule is essential for the high antifungal activity, a number of analogues have been reported in literature, in attempt to develop better antifungal agents wherein one of the triazole ring of fluconazole is replaced by other groups. This type of research is important to solve the problem of treatment of infections caused by fluconazole-resistant fungal strains. Substituted thienopyrimidinones exhibit anticancer, herbicidal, antibacterial and antifungal activity[8]. In this section efforts directed towards replacement of triazole moiety with variously substituted thienopyrimidinones in order to make a number of compounds available for biological activity have been described. The retrosynthetic analysis for preparation of compounds **5** (when R^3 = H) is shown in Scheme 1.

Compound **5** (when R^3 = H) could be synthesized from the epoxide opening of **11** by the thienopyrimidinone **8**. Thienopyrimidinone **8** could be synthesized from 4 and/or 5-substituted 2-aminothiophene-3-carboxylate **9**, which in turn could be synthesized from respective aldehyde or ketone by Gewald synthesis[9] whereas epoxide **11** could be synthesized from difluorobenzene **6** *via* its acylated intermediate **7** by a known method[10].



Scheme 1. Retrosynthetic analysis for preparation of compounds **5**

The various substituted thienopyrimidinones were prepared by the synthetic route shown in Scheme 2.

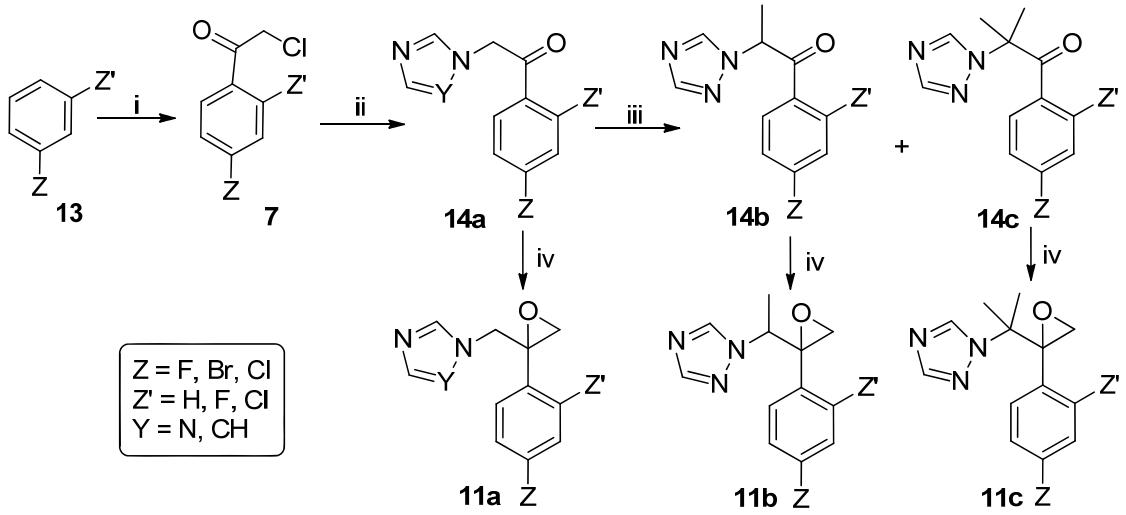


Scheme 2. Reagents and conditions**:** i) DMSO, $(COCl)_2$, Et_3N, DCM, -78 °C, 3-4 h, 60-68 % ii) Ethyl cyanoacetate, sulphur, DMF, TEA, 50 °C, 10-12 h, 84.3-94.6 % iii) NH_4OAc, $HCONH_2$, 140-145 °C, 4-5 h, 74.7-91.8 %.

A substituted alcohol **12** (depending on side chain desired in thienopyrimidinone) was subjected for oxidation to get aldehyde **10**, which was subjected to Gewald synthesis[9] to afford 4 and/or 5-substituted 2-aminothiophene-3-carboxylate **9**. The intermediate **8** was obtained by cyclization of 4 and/or 5-substituted 2-aminothiophene-3-carboxylate **9** in presence of ammonium acetate and formamide.

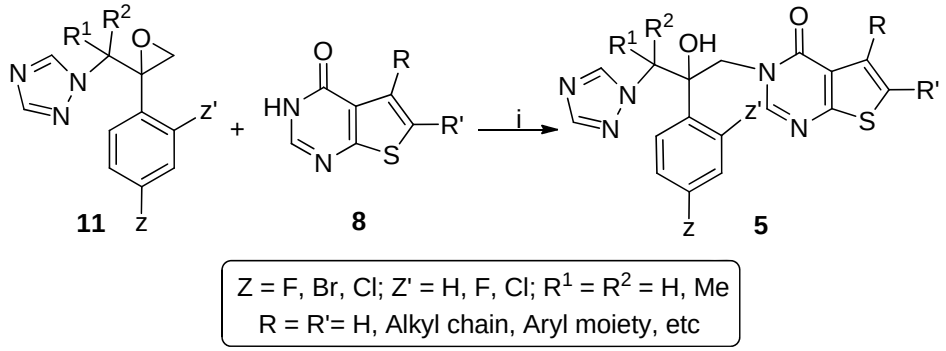
Synthesis of the epoxide **11** was carried out by known method[10] as shown in Scheme 3. 1,3-Dihalobenzene was acylated with chloroacetyl chloride in presence of aluminium chloride. The acylated product **7** thus obtained was treated with triazole in presence of potassium carbonate to get ketone **14a**. This triazole containing ketone **14a** on treatment with methyl iodide in presence of sodium hydride in THF yielded mixture of **14b** and **14c** which were separated by column chromatography. These triazole containing ketones **14a,**

14b and **14c** were converted into respective epoxides **11a, 11b** and **11c** by reaction with trimethylsulphoxonium iodide and aq potassium hydroxide.



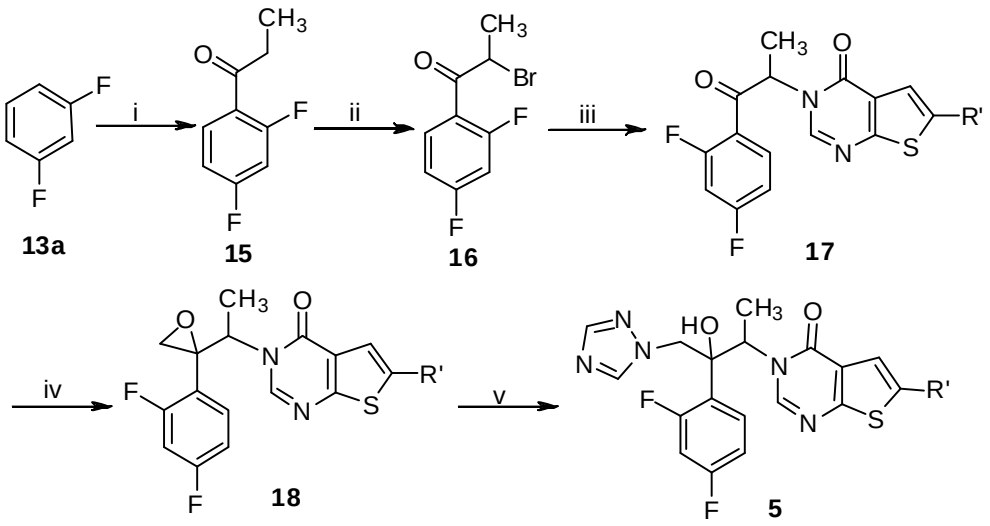
Scheme 3. Reagents and conditions**:** i) Chloroacetyl chloride, $AlCl_3$, DCM, 0 °C, 4 h, 80-90 %. ii) K_2CO_3, 1,2,4-Triazole, EtOAc, 80 °C, 10 h, 69-76 %. iii) Methyl iodide, NaH, THF, 3 h, 63-70 % iv) Trimethylsulphoxonium iodide, aq KOH, DCM, 12 h, 81-91 %.

The thienopyrimidinone **8** was then reacted with the epoxide **11** in presence of potassium carbonate to afford the target molecules (Scheme 4).



Scheme 4. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 61.8-68.9 %.

For the synthesis of compound **5** with $R^1 = R^2 = H$ and $R^3 = Me$, the synthetic strategy used is shown in Scheme 5.



Scheme 5. Reagents and conditions**:** i) Propionyl chloride, $AlCl_3$, DCM, 0 ^oC-rt, 8 h, 90 % ii) NBS, AIBN, carbon tetrachloride, 77 ^oC, 12 h, 78 % iii) Thienopyrimidinone **8** (R=H), NaH, DMF, 0 ^oC-rt, 4 h, 73 % iv) Trimethylsulphoxonium iodide, NaH, DMSO, rt, 17 h 69-73 %. v) 1,2,4-Triazole, K_2CO_3, DMF, 80 °C, 10 h, 63-67 %.

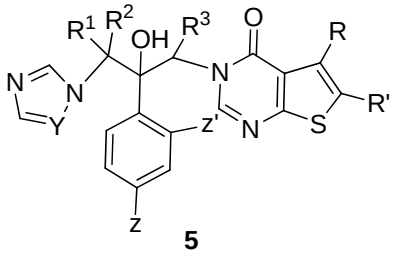
Bromoketone **16** was synthesized[11] from 1,3-difluorobenzene **13a** by its acylation using propionyl chloride in presence of $AlCl_3$ in DCM followed by the bromination of α-carbon using N-bromosuccinimide and AIBN in carbon tetrachloride. Reaction of bromoketone **16** with thienopyrimidinone **8** in presence of sodium hydride in dimethyl formamide gave ketone **17** in good yield. It was then treated with trimethylsulphoxonium iodide and sodium hydride in DMSO at room temperature to get oxirane **18**. Then it was converted into **5** by epoxide opening using 1*H*-1,2,4-triazole in presence of potassium carbonate in DMF at 80 ^oC (Scheme 5).

Using above synthetic strategies (Scheme 4 and 5) a library of compounds was synthesized. These compounds were screened for antifungal activity against various fungi and the results of the structure activity relationship study have been presented.

Chapter 1: Section-II

Bioevaluation of fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one

All the newly synthesized fluconazole analogues depicted by general formula **5** were tested for antifungal activity against various fungi including *Candida albicans* ATCC 24433, *Aspergillus niger* ATCC 16404 and *Fusarium proliferatum* ATCC 10052. *In vitro* evaluation of antifungal activity was performed by determining the minimum inhibitory concentration (MIC) following standard methods[12,13]. Antifungal susceptibility testing of these compounds was done by broth dilution method using RPMI 1640 medium with MOPS (3-(N-morpholino)propanesulfonic acid) buffer. Known antifungal agents like fluconazole and amphotericin B were used as positive control. End points were determined after 48 hours visually and by using spectrophotometer wherever necessary. Different dilutions were tried and various sets of experiments were performed.



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Z = F, Br, Cl; Z' = H, F, Cl; $R^1 = R^2 = R^3$= H, Me;
Y = N, CH; R = R'= H, Alkyl chain, Aryl moiety, etc

Figure 1. Fluconazole analogues containing thieno-[2,3-*d*]pyrimidin-4(3*H*)-ones

The activity data indicated following points regarding the structure-activity relationship of the compounds studied in the present section.

1. In case of the compounds with aliphatic side chain at R', activity gradually increases with increase in the number of carbons. Activity is maximum for R'= $(CH_2)_4CH_3$, $(CH_2)_5CH_3$ or $(CH_2)_7CH_3$ after which the activity decreases rapidly.

2. The compounds with R = Br and R' = alkyl chain show less activity, when compared with the corresponding compounds with R = H.

3. The compounds with polar groups in side chain at R' possess less activity but the activity increases on protection of these polar groups.

4. The compounds with aliphatic rings at R, R' show less activity when compared with the compounds with R = H, R' = alkyl chain and activity vanishes in larger rings.

5. The compounds with R^3 = Me exhibited equivalent activity with respect to unsubstituted molecules.

6. The compounds with methyl and dimethyl substitution at R^1 and R^2 show very less activity when compared with respective unsubstituted molecules.

In continuation of the above work, we resolved the compound **5** to obtain R and S enantiomers. Resolution was done using chiral preparative High Performance Liquid Chromatography (HPLC). Compound chosen for the separation was the most active compound from the list. Primary screening indicated that R-(+) enantiomer is more active than S-(-) enantiomer.

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Chapter 2

Synthesis of dimeric fluconazole analogues containing thieno[2,3-*d*]pyrimidin-4(3*H*)-one moieties

Introduction

It is now well known that many biologically active molecules on dimerization lead not only to increased potency and selectivity, but also long residence time particularly at epithelial surfaces in comparison to the respective monomers. Use of linkers between two monomers results into dimeric compounds. Linkers used are of different kind such as straight chain and branched hydrocarbons including alkyl, alkenyl or alkynyl. The linker may also include one or more of N, O and S and one or more functional groups such as amide, amine, carbonyl and carboxy.

Sulfides and sulphoxides were used as linkers for synthesis of dimeric analogues of fluconazole and its derivatives and a library of compounds with general structures **1** and **2** was synthesized for the structure activity relationship study[1]. It was observed that the activity of dimeric compounds was enhanced in comparison to the respective monomers. During the work presented in this chapter, efforts were made to synthesize dimeric fluconazole analogues containing thieno[2,3-*d*]pyrimidin-4(3*H*)-one moieties depicted by general formula **3** (Figure 1).

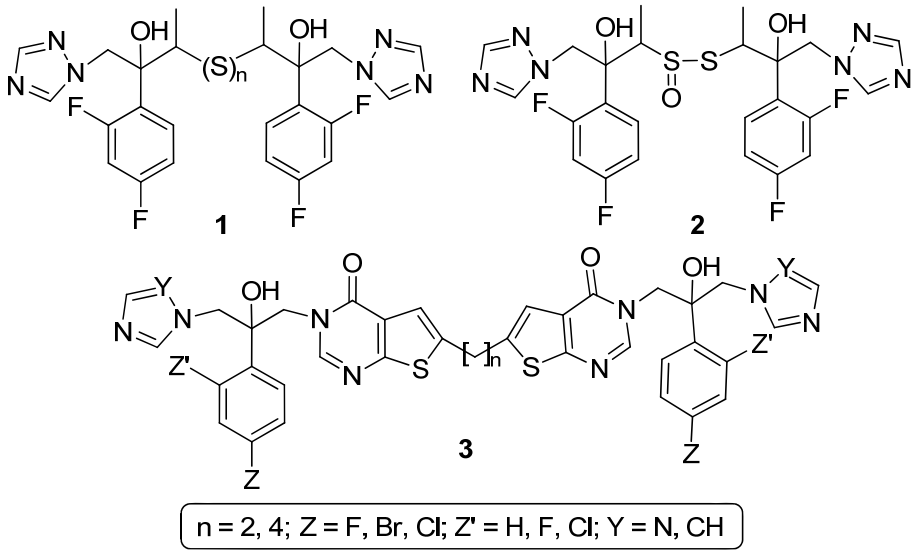


Figure 1. Dimeric fluconazole analogues

Present work

As we had synthesized molecules of general formula **4**, discussed in first chapter, we thought of dimerizing the synthesized molecules in order to get new molecules.

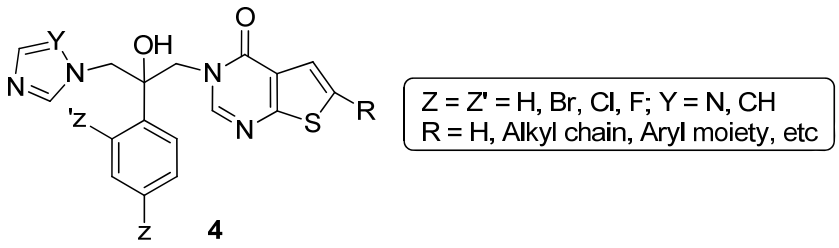
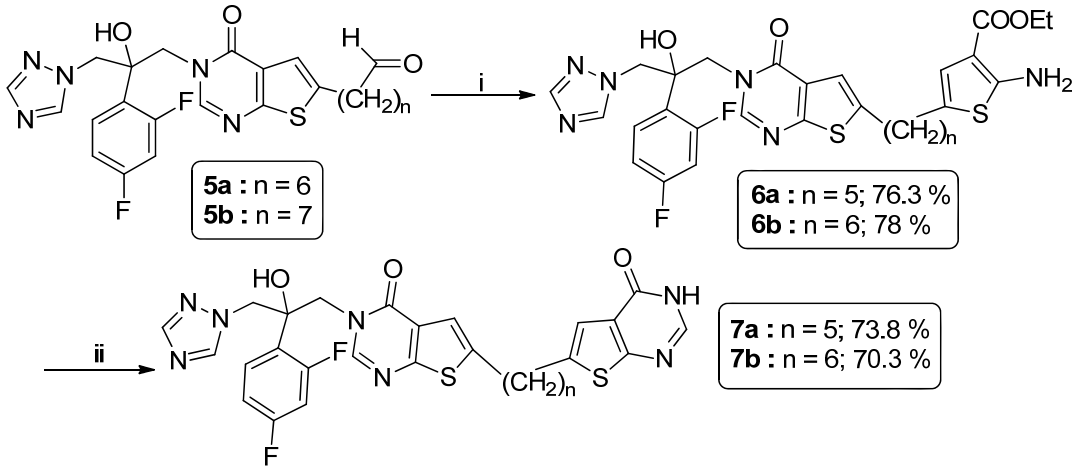


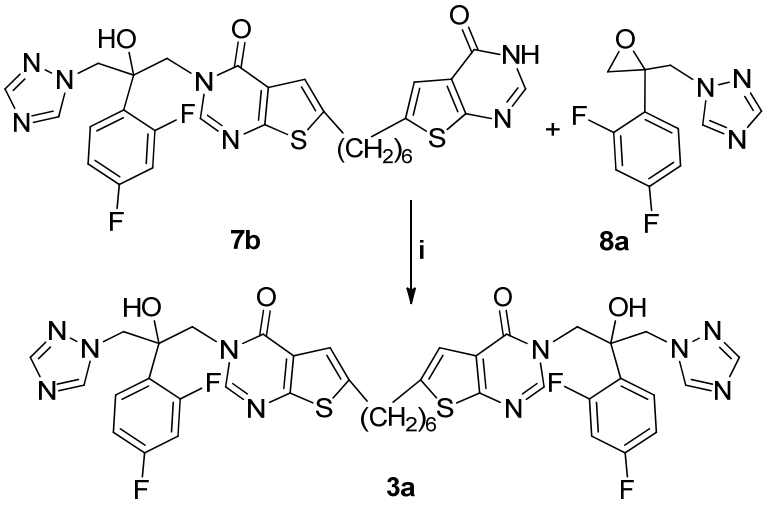
Figure 1. Fluconazole analogues containing thieno-[2,3-*d*]pyrimidin-4(3*H*)-ones

For doing so, alkyl chain (R) with terminal olefin was oxidized to get adehyde **5** (discussed in 1st Chapter) which was subjected to Gewald synthesis[2] to afford 2-aminothiophene-3-carboxylate **6**. Then it was cyclized in presence of ammonium acetate and formamide to get the key intermediate **7**, as shown in Scheme 1.



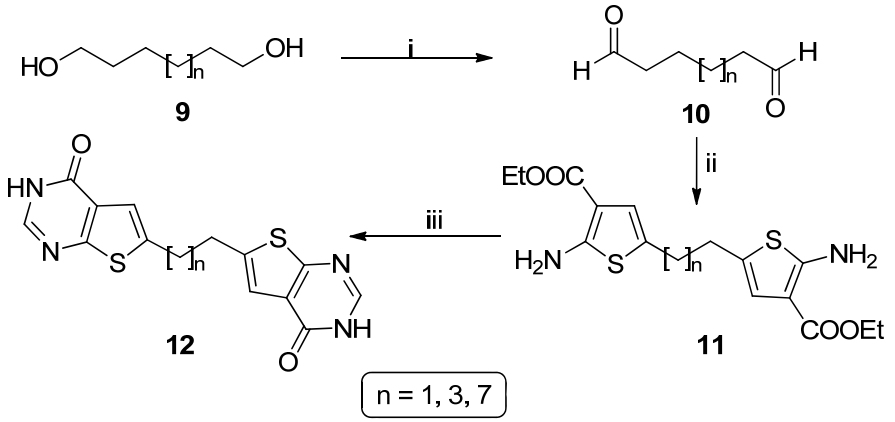
Scheme1. Reagents and conditions: i) Ethyl cyanoacetate, sulphur, DMF, TEA, 50 °C, 10-12 h. ii) NH_4OAc, $HCONH_2$, 140-145 °C, 4-5 h

Having **7b** in hand, it was then treated with 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **8a** (Synthesis of 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **8a** was done using literature procedure[3] described in 1st Section of 1st Chapter, Scheme 3) in presence of potassium carbonate in ethyl acetate at refluxing temperature to afford the target molecule **3a** (Scheme 2).



Scheme 2. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 64.8 %.

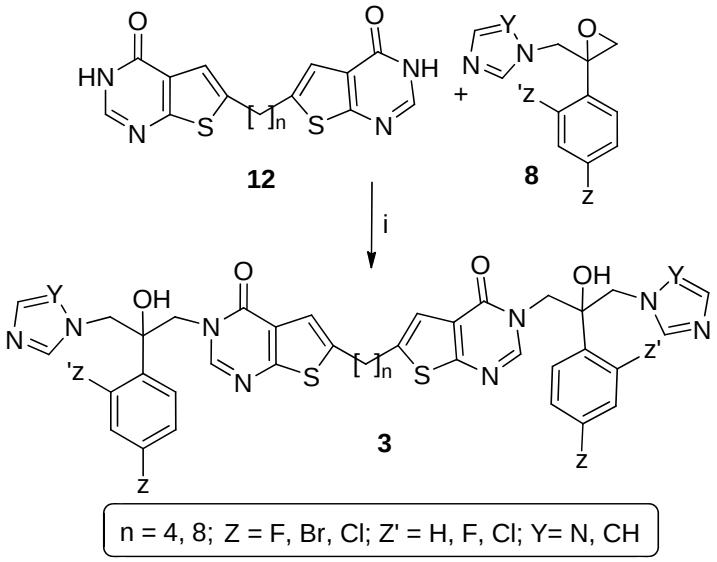
This synthetic route developed for preparation of dimeric fluconazole analogues containing thieno[2,3-*d*]pyrimidin-4(3*H*)-one moieties **3**, was lengthy and time consuming hence to reduce the steps involved in the synthesis, an alternate strategy was started from respective diols (Scheme 3).



Scheme 3. Reagents and conditions: i) DMSO, $(COCl)_2$, Et_3N, DCM, -78 ^oC - rt, 4 h; ii) Ethyl cyanoacetate, S_8, morpholine, EtOH, 80 ^oC, 10-12 h, 77.4-89.8 %; iii) NH_4OAc, $HCONH_2$, 140-145 ^oC, 5 h, 75.4-77.1 %.

A suitable diol **9** (depending on linker chain expected in dimer) was subjected for oxidation to get di-aldehyde **10**, which was subjected to Gewald synthesis[2] to afford 5,5'-(alkane-diyl)bis(2-aminothiophene-3-carboxylic acid) **11** which was then reacted with ammonium acetate and formamide to get 6,6'-(alkane-diyl)dithieno[2,3-*d*]pyrimidin-4(3*H*)-

one **12**. Intermediate **12** was then treated with epoxide **8** (synthesis of epoxide[3] was described in Chapter 1) to get the dimeric molecule **3** (Scheme 4).



Scheme 4. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 57.3-69.8 %.

All the newly synthesized dimeric fluconazole analogues containing thieno[2,3-*d*]pyrimidin-4(3*H*)-one moieties **3** were tested for antifungal activity against various fungi as described in 2nd section of Chapter 1. The activity data indicated following points regarding the structure-activity relationship of the compounds studied in the present chapter.

1. Most of the dimeric compounds exhibit significant antifungal activity.
2. The compounds with difluorophenyl moieties show higher activity than compounds with 4-fluoro, 4-bromo and dichlorophenyl moieties.
3. The compounds with imidazole moieties possess less activity than those with triazole moieties.

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Chapter 3: Section-I

Synthesis of fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties

Introduction

Various fluconazole (**1)** derivatives and analogues have been proposed, synthesized and checked for the antifungal activity against different fungal strains. This included derivatisation at tertiary alcohol of fluconazole as ethers, esters, phosphates and so on or the substitution of one of the triazole unit by different moieties[1]. In some of the cases, difluorophenyl ring in fluconazole was replaced by other moieties such as 1,4-benzothiazinones to obtain compounds **2**[2]. The analogues having general structure **3** were also synthesized and screened against *Candida albicans*[3]. In all the above 1,4-benzothiazinones and 1,4-benzoxazinones, amide nitrogen was protected as alkyl chain such as methyl or ethyl *etc*.

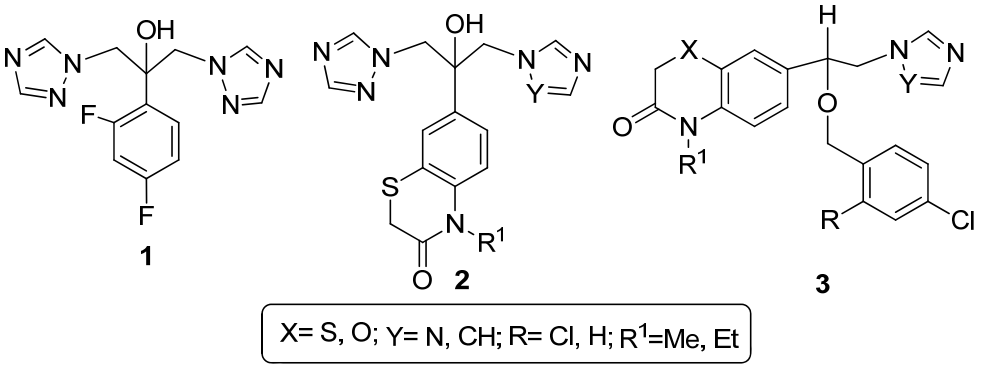
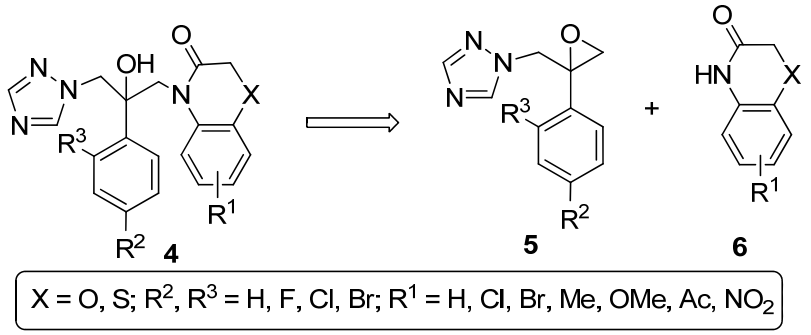


Figure 1. General structures of azole analogues containing benzothiazinone and benzoxazinone moieties

In continuation with previous work[4] to develop new antifungal drugs, in this section we have synthesized a number of fluconazole analogues wherein one of the triazole moieties in fluconazole was replaced with 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moiety and Structure Activity Relationship Study was carried out for these analogues against various fungi.

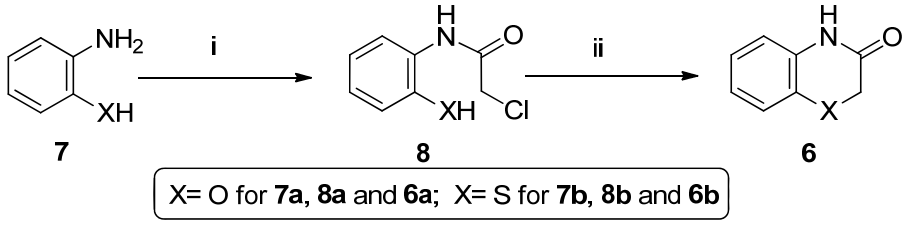
Present Work

The retrosynthetic analysis of compound **4** is shown in Scheme 1. The synthesis of compounds **4** was planned to be achieved from epoxide **5** and (un)substituted benzothiazinone or benzoxazinone moieties.



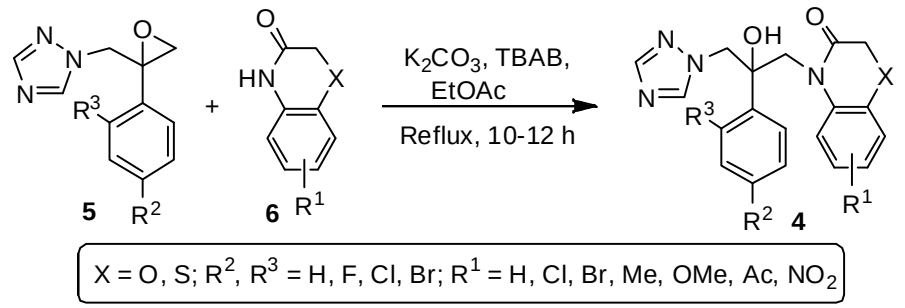
Scheme 1. Retrosynthetic analysis for fluconazole analogues **4** containing benzothiazinone and benzoxazinone moieties

The synthesis of epoxide **5** was described in Scheme 3 of Chapter 1. The synthesis of benzoxazinone **6a** and benzothiazinone **6b** was started from commercially available 2-aminophenol **7a** and 2-aminothiophenol **7b** respectively. Stirring 2-aminophenol **7a** with chloroacetyl chloride in DCM at room temperature for 2 h yielded N-acylated intermediate **8a**, which on treatment with potassium carbonate in ethyl acetate at room temperature yielded benzoxazinone **6a** as shown in scheme 2. The same reaction sequence using 2-aminothiophenol **7b** afforded benzothiazinone **6b**.



Scheme 2. Reagents and conditions: i) Chloroacetyl chloride, DCM, rt, 2 h, 79-81 % ii) K_2CO_3, EtOAc, rt, 12 h, 78-84 %.

Benzoxazinone and benzothiazinone thus prepared were reacted with epoxide **5** in presence of potassium carbonate and tetrabutylammonium bromide (TBAB) in ethyl acetate at refluxing temperature for 10-12 h to yield the desired compounds **4** in good yields as shown in Scheme 3. Rest of the benzoxazinones and benzothiazinones were either prepared by the synthetic sequence given in Scheme 2 or were commercially available and reacted with different epoxides to get the biologically active compounds **4**.



Scheme 3. Synthesis of fluconazole analogues **4** containing benzothiazinone and benzoxazinone moieties

All the newly synthesized compounds **4** were tested for antifungal activity against various fungi using the methods described in Chapter 1. The activity data indicated following points regarding the structure-activity relationship of the compounds studied in the present work.

1. The compounds containing benzothiazinone moiety are more active than those containing benzoxazinone .

2. In the benzothiazinone series, replacement of fluorine by hydrogen reduces the activity, the same is true for benzoxazinone series.

3. In the benzothiazinone series, halogen substituent at C7 position is tolerated with slight decrease in activity, while substituents like methyl and methoxy decrease the activity considerably.

4. In case of benzoxazinone series, no substituent at C6 is tolerated and all compounds having substituents like Cl, Br, NO_2 or Ac lost the activity.

In continuation of the above work, we have obtained the R and S enantiomers from racemic compound **4**. Resolution was done using chiral preparative High Performance Liquid Chromatography (HPLC). Compound chosen for the separation was the most active compound from the list and it was observed that S enantiomer is having higher activity than R.

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Chapter 3: Section-II

One -step preparation of α-chlorostyrenes

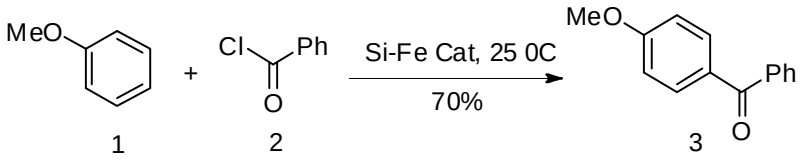
Introduction

In continuation with our previous work directed towards the synthesis of epoxide (compound **11** described in Chapter 1) which included Friedel-Crafts acylation as the first step wherein $AlCl_3$ was used, we wished to overcome the problems associated with this reaction such as use of excess equivalents of $AlCl_3$, tiresome work-up, *etc*. We thought to use some heterogeneous catalysts for the reaction, so that the catalyst could be easily removed from the reaction mixture during work-up and could be reused. As a part of ongoing efforts of our group to explore the utility of heterogeneous catalysts for various organic reactions[1-4], during the course of our work, we studied heterogeneous Si-Fe catalyst and found that it catalyzed Friedel-Crafts acylation of aromatic compounds. It afforded α-chlorostyrenes in one step as the major product in case of acid chlorides having α-methylene groups and the results are presented in this section.

α-Chlorostyrenes are used as intermediates in the preparation of α-halomethyl ketones[5], 1-diarylphosphino-2-arylethylenes[6], 2-arylallyltitanocenes[7], arylacetylenes[8] α-thiocyanatostilbenes[9] *etc*. α-Chlorostyrenes are prepared by number of reactions and reaction sequences reported in literature[10a-10i]. These methods suffer from the disadvantages such as heating at high temperatures[10b], costly or rare reagents[10d-10g], low yields[10h] *etc*. In fact, easy methods for preparation of α-chlorostyrenes will lead to further exploration of utility of these compounds in various fields. We undertook the present work to develop efficient method for preparation of α-chlorostyrenes.

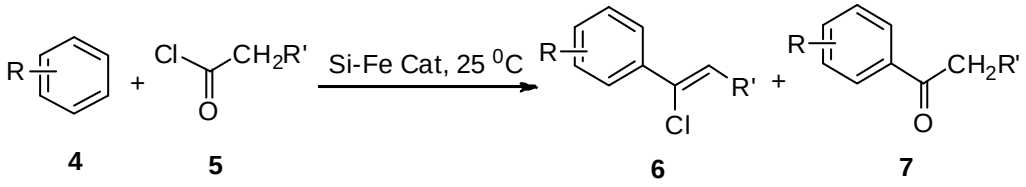
Present work

Initially anisole was reacted with benzoyl chloride in presence of various catalysts. The Si-Fe catalyst, prepared from sodium trisilicate and ferric nitrate in presence of ammonia and ammonium carbonate, afforded the corresponding Friedel-Crafts acylation product by stirring the neat reaction mixture at rt in 70 % isolated yield demonstrating the possibility of exploring its application in organic chemistry as shown in scheme 1.



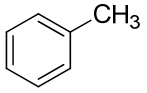
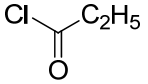
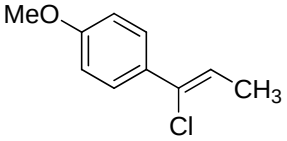
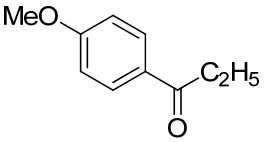
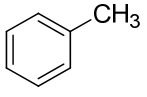
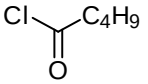
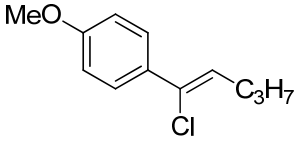
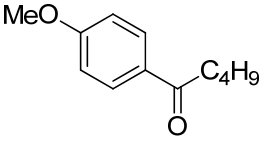
Scheme 1. Friedel-Crafts acylation reaction of anisole and benzoyl chloride

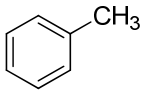
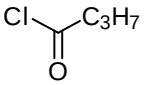
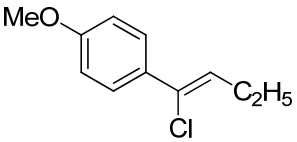
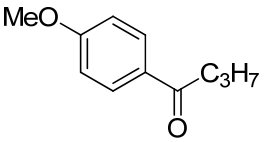
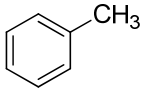
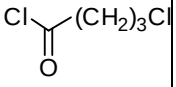
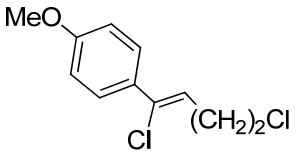
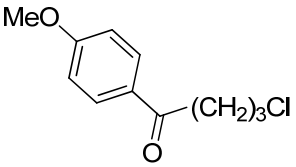
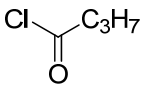
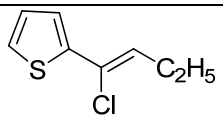
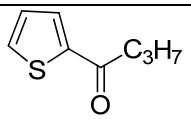
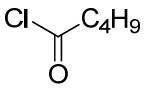
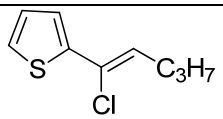
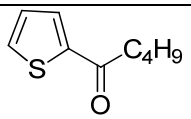
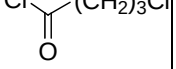
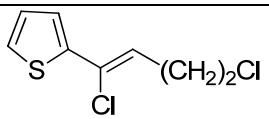
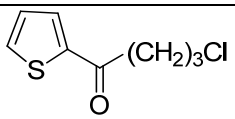
When reaction of anisole with propionyl chloride was carried out, the product was a mixture of two compounds which were easily separated by column chromatography. One of them was found to be the Friedel-Crafts acylation product while other was α-chlorostyrene[10c] (Table 1, Entry 1). Literature survey revealed that the α-chlorostyrenes are obtained as side products in Friedel-Crafts acylation using acid- treated montmorillonite-$FeCl_3$ catalyst[10h] in 1-9 % yields. The yields obtained in our reaction prompted us to explore the opportunity to develop these results into one-step method for preparation of α-chlorostyrenes. Accordingly, reactions of various aliphatic acid chlorides were carried out to obtain the corresponding α-chlorostyrenes in good yields.



Scheme 2. Preparation of α-chlorostyrenes

Table 1. α-Chlorostyrenes and Friedel–Crafts acylated products obtained using Si-Fe catalyst

Sr No	Aromatics	Acid chlorides	Products (% yields) α-chlorostyrenes	Products (% yields)
1	 **1**	 **8**	 **9:** 65 %	 **10:** 28 %
2	 **1**	 **11**	 **12:** 67 %	 **13:** 25 %

3	 **1**	 **14**	 **15:** 43 %	 **16:** 49 %
4	 **1**	 **17**	 **18:** 41 %	 **19:** 48 %
5	 **20**	 **14**	 **21:** 26 %	 **22:** 66 %
6	 **20**	 **11**	 **23:** 17 %	 **24:** 73 %
7	 **20**	 **17**	 **25:** 19 %	 **26:** 68 %

In conclusion, α-chlorostyrenes were prepared by a one-step method from aromatics and acid chlorides using heterogeneous Si-Fe catalyst at room temperature.

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Chapter 1

Synthesis of fluconazole analogues containing substituted thieno[2,3-d]pyrimidin-4(3H)-one and their bioevaluation

Chapter 1: Section I

Synthesis of fluconazole analogues containing substituted thieno[2,3-d]pyrimidin-4(3H)-one

Synthesis of fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one

1.1.1 Abstract

A new class of antifungal agents (Figure 1) was synthesized based on structural features of fluconazole wherein one of the triazole moiety in fluconazole (**1**) was replaced with various substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones. The strategy developed can be used for the synthesis of number of such derivatives with very good to excellent yields even on large scale. Efforts to vary substituents on thiophene ring of thienopyrimidinone have been carried out to generate a library of compounds. This was achieved by varying the starting materials in the synthesis. Methyl and dimethyl substitution has also been done on the carbon flanked between triazole nitrogen and quaternary carbon bearing hydroxyl group in the molecule. Attempts were also made to put methyl substituent on the carbon connecting to thienopyrimidinone moiety. All the synthesized compounds containing triazole moiety were screened against different fungal strains. It was observed that some of the newly synthesized compounds exhibited better antifungal activity when compared with parent molecule fluconazole and/or potent antifungal agent amphotericin B.

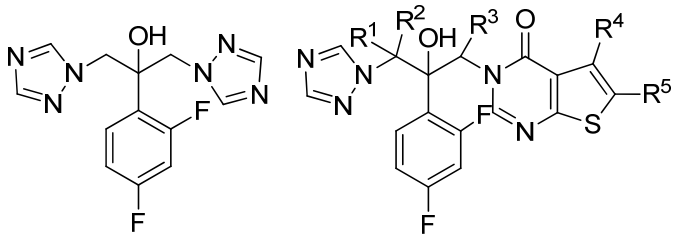


Figure 1. Structues of fluconazole (**1**) and compounds synthesized in present work

1.1.2 Introduction

Not only the development of new synthetic strategies for synthesis of known antifungal agents and their derivatives, and synthesis of new antifungal agents has attracted many organic chemists in the medicinal chemistry, but also the life threatening fungal infections and the drug resistant fungal strains invited the synthetic chemists to come up with new antifungal agents. In human beings infections due to fungi range from the very common to critical diseases, such as dermatophytoses and onycomycoses to deeply invasive and disseminated, such as candidiasis and aspergillosis. In the last two decades the

frequency of systemic fungal infections has increased dramatically along with the number of invasive, mostly opportunistic species. Recently used antifungal agents are associated with severe side effects mainly due to the lower degree of specificity towards the desired target. Due to longer use of these drugs, resistance gets developed, especially in the opportunistic fungi active during the immunosuppressive stage. This has attained greater importance especially because of the HIV infected patients, where the resistance goes down increasing the chances of the entry of the active fungi. The main factor for the increase is the increased population of severely immunocompromised patients either with AIDS, undergoing cancer chemotherapy or immunosuppressive therapy for organ transplantation. The additional factors include treatment with broad-spectrum antibacterial drugs or glucocorticosteroids, invasive procedures such as surgery, in-dwelling catheters or prosthetic devices and parenteral nutrition or dialysis. So as to understand the antifungal agents and their synthesis, it is important to know about the pathogens and their infections.

1.1.3 Review of literature

Antifungal agents

Fungi are plant-like organisms that lack chlorophyll and are one of the five kingdoms of life. According to Hawksworth, there are over 1,500,000 species of fungi known.[1] Fungal infections have emerged as a significant clinical problem in recent years. Both the number of fungal infections and the number of fungal species causing them are increasing, as a result of the exponential increasing number of immunosuppressed and immunocompromised patients.[2] Clinically, candidosis, aspergillosis and cryptococcosis are the three major infections in the immunocompromised individuals.[3] Due to the development of resistance to the current treatment,[4] and increasing frequency of fungal infections, mycology is today undergoing a true renaissance. Invasive fungal infections are nowadays a major cause of morbidity and mortality in patients such as with Acquired Immune Deficiency Syndrome (AIDS), organ transplantation, neutropenic, *etc*. Infections can be superficial, i. e. situated at or close to the surface of the skin, or systemic, which means they can affect the body as a whole rather than individual parts or organs. Mycoses are classified into five classes according to the tissue levels initially colonized:[5-8] 1) Superficial mycoses, 2) Cutaneous mycoses, 3) Subcutaneous mycoses, 4) Systemic mycoses due to primary pathogens and 5) Systemic mycoses due to opportunistic pathogens.

The responsible factors for the development of fungal infections are as follows[7c]:

• Use of drugs that suppress the immune system, e. g. anticancer drugs, corticosteroids.

• Diseases and conditions, such as AIDS, kidney failure, diabetes, lung diseases, leukemia, organ transplantation *etc*.

• Fungal infections are extremely difficult to diagnose and therefore delays in initiation of treatment.

The fungal cell

Knowledge of fungal cell structure and function is essential for understanding the pharmacology of antifungal agents. Like mammalian cells and unlike bacteria, fungi are eukaryotes with chromosomes within the cell nucleus and have distinct cytoplasmic organelles including endoplasmic reticulum, golgi apparatus, mitochondria, and storage vacuoles.[7b] This homology to mammalian cells also extends to biosynthetic pathways, where fungi share similar mechanisms for DNA replication and protein synthesis. The similarity of fungal and mammalian cells creates a number of problems for designing drugs that are selectively toxic to fungal cells but not to the human host. Thus the issue of selectivity predominates in the search for safe and effective chemotherapeutic remedies for mycoses.

Fungal and mammalian cells both contain a cell membrane that plays vital role in cell structure, division and metabolism. It is composed of complex lipids such as sterols, which account for approximately 25 % of the weight of the cell membrane. In all pathogenic fungi, the principle sterol is ergosterol, whereas the mammalian cell membranes contain primarily cholesterol. Although the sterols are different, the main route for the biosynthesis of ergosterol parallels with the biosynthesis of cholesterol, therefore selectivity plays a crucial role in the quest for safe and effective chemotherapeutic drugs.

The current therapeutic treatment of mycoses

Antifungal agents currently used for the treatment of mycoses and their targets in fungal pathogens and toxicities are as follows:

Polyene antifungals: Amphotericin B (**2**) and Nystatin (**3**) are currently used polyene antifungal drugs. The polyenes act by binding to ergosterol in the fungal cell membrane.

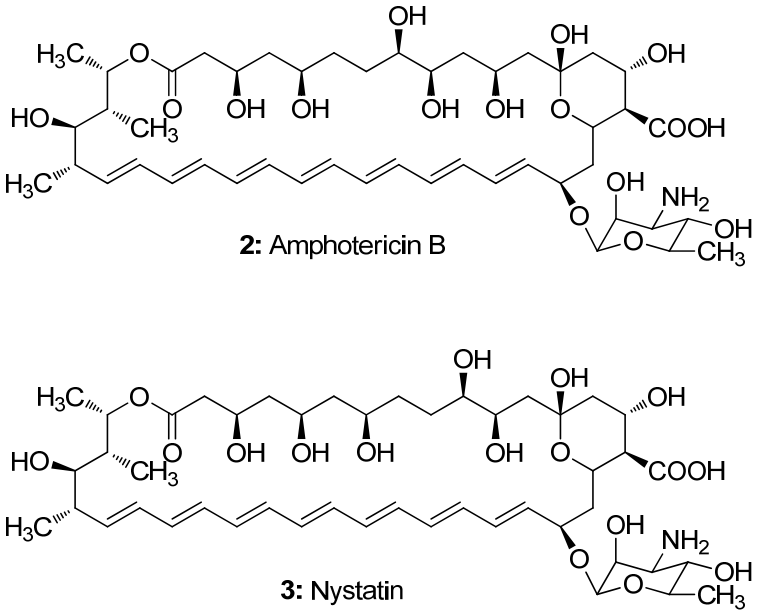


Figure 2. Polyene antifungals

The binding results in depolarization of the membrane and formation of pores that causes leakage of cell contents, eventually leading to cell death. Although, amphotericin B binds approximately 10 times more strongly to fungal cell membrane components than mammalian cell membrane cholesterol, it definitely disrupts mammalian cell giving rise to adverse side effects. Therefore polyenes have greater toxicities for mammalian cells and cause nephrotoxicity that limits the clinical use of polyenes. Resistant strains have also been isolated under laboratory conditions with alteration in the nature and amount of sterols present in the membrane.

Azole antifungals: Azole antifungals are the major class of drugs which are widely used clinically.[6] Ketoconazole (**4**), miconazole (**5**), clotrimazole (**6**) are the topical agents and fluconazole (**1**) and itraconazole (**7**) are useful in the treatment of systemic mycoses (Figure 3).

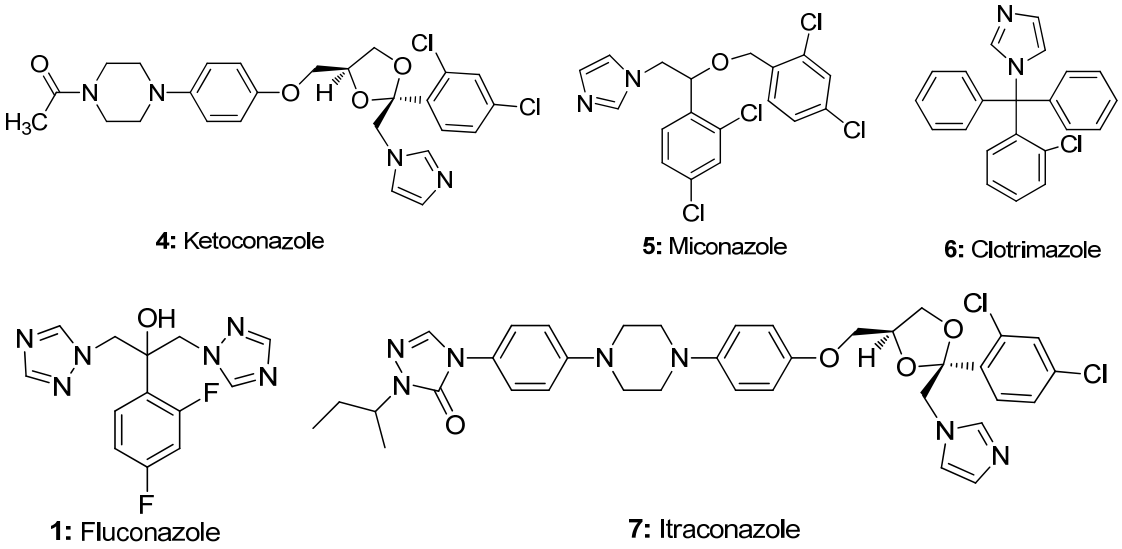


Figure 3. Imidazole and triazole antifungals

The mode of action of azole antifungals is inhibition of ergosterol biosynthesis by inhibiting the fungal cytochrome P450 3-A dependent enzyme, lanosterol 14-α-demethylase, thereby interrupting the synthesis of ergosterol (**11**) as shown in Figure 4.

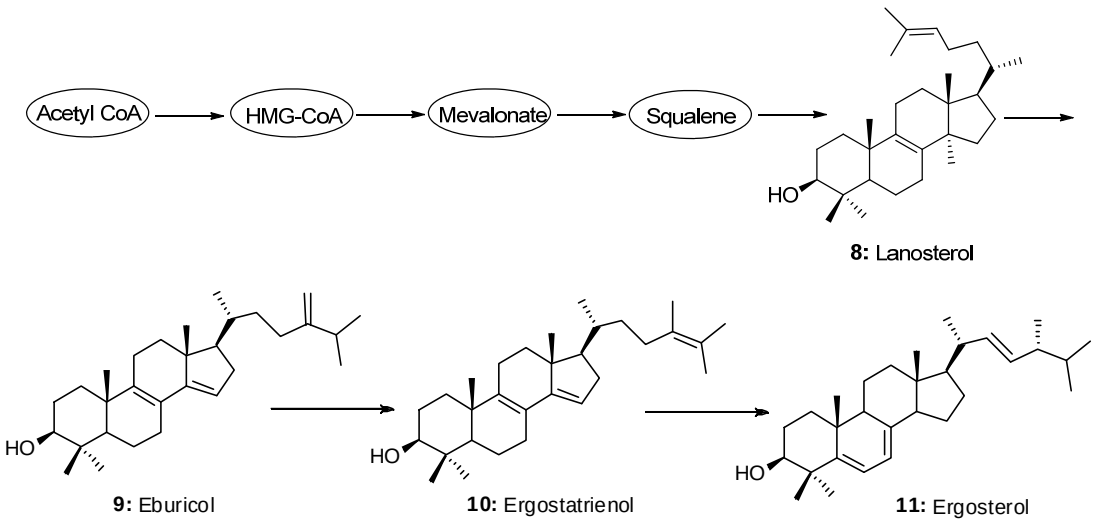
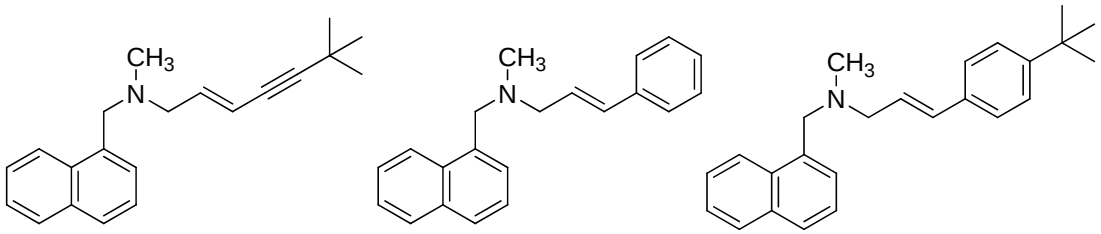


Figure 4. The steps illustrated for ergosterol synthesis at the endoplasmic reticulum: target for azoles, allylamines, phenyl-morpholines. These are the major steps found in all fungi.

Inhibition of the enzyme leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth. Azole antifungals can also inhibit mammalian cytochrome P450-dependent enzymes involved in hormone synthesis or drug metabolism. Therefore, azole antifungals cause hepatotoxicity. Azoles are only fungistatic[6b] and due to the increased administration of azole antifungals for the treatment of systemic fungal infections, pathogenic yeasts are developing resistance to these drugs. Target modification is a common factor contributing to clinical resistance to azole therapy. However, azole moiety itself has been proved to be effective pharmacophore.

Allylamines as antifungals: Allylamines are the other class of antifungals that also work in a similar fashion i.e. by inhibiting the synthesis of ergosterol. However, allylamines act at an earlier step in the ergosterol synthesis pathway by inhibiting the enzyme squalene epoxidase leading to the accumulation of intracellular squalene that causes fungicidal effect upon exposure to the drug. Like the azoles, terbinafine (**12**) causes hepatic toxicity and has the potential for drug interaction with other medications metabolized through the mammalian cytochrome P-450 pathway. Naftifine (**13**) and butenafine (**14**) have been used as antifungal drugs with the same mode of action.



12: Terbinafine **13:** Naftifine **14:** Butenafine

Figure 5. Allylamine antifungals

Other antifungal agents of medicinal interest: Besides above classes of antifungals, there are a few other classes of antifungal agents.

Flucytosine: Flucytosine or 5-fluorocytosine (**15**) was originally developed in 1957 as a potential antineoplastic agent. It was found to have antifungal activity in 1968 to treat candida and cryptococcal infections in human. Flucytosine inhibits DNA synthesis by

blocking the functions of a key enzyme thymidylate synthetase in the DNA replication. Flucytosine is also incorporated in fungal RNA, thereby disrupting transcription and translation. Selectivity is achieved because mammalian cells are unable to convert flucytosine to 5-fluorouracil. But flucytosine can be converted to 5-fluorouracil by bacteria residing in the gastrointestinal tract.

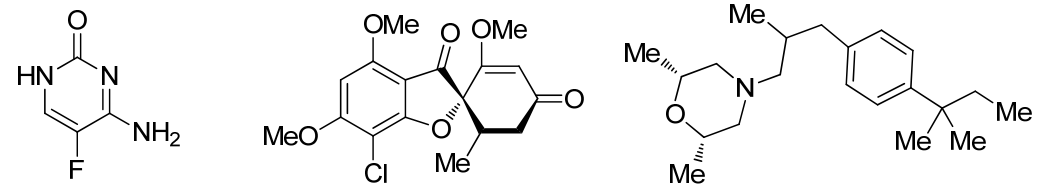


Figure 6. Antifungal agents: Flucytosine, Griseofulvin and Amorolfine

The most common adverse effects seen with flucytosine are similar to 5-fluorouracil chemotherapy (diarrhea, nausea and vomiting, bone marrow suppression) however with reduced intensity. The serious side effects associated with flucytosine are hematological, manifested as leucopenia and thrombocytopenia.

Griseofulvin: Griseofulvin (**16**) is a natural product first isolated in 1939 from *Penicillium griseofulvum*. It inhibits fungal cell mitosis by disrupting mitotic spindle formation, a critical step in cellular division. Griseofulvin served as first line drug for treatment of dermatophytosis for many years. Because of its limited efficacy and untoward side effects, it is recently being replaced by itraconazole (**7**) and terbinafine (**12**).

Amorolfine: Morpholine antifungal amorolfine (**17**) is known to inhibit sterol synthesis. There are also cell wall antagonists like echinocandins e.g. cilofungin or nikkomycins. The echinocandins are fungal secondary metabolites comprising a cyclic hexapeptide core with a lipid sidechain responsible for antifungal activity. Antifungal activity in the prototypes, echinocandin B and aculeacin A, was discovered by random screening in the 1970s. The target for the echinocandins is the complex of proteins responsible for synthesis of cell wall β-1,3 glucan polysaccharides. The sordarin antifungal class, although not developed for

clinical use, merits mention among the new mechanisms of action. Sordarins inhibit protein synthesis by blocking the function of Fungal Translation Elongation Factor 2.

Need for further research in antifungal agents

There are mainly three challenging problems for antifungal researchers in development of an effective drug in combating severely invasive mycosis.

1. Resistance of yeasts to clinically useful antifungal agents: There are three different resistance mechanisms known in pathogenic yeasts. (i) The reduced access of the agents to the target cytochrome P450 enzyme because of increased efflux of antifungals, caused by the action of resistance gene products. (ii) Overproduction of cytochrome P450 enzyme, possibly by gene amplification. (iii) Resistance mechanism due to a structural alteration in cytochrome P450 enzyme which results in lower susceptibility to azole antifungals.

2. Emergence of newer strains by mutation: The treatment of immunosuppressed and immunocompromised patients such as cancer and AIDS patients needs long term administration of antifungal drugs to treat the invasive infection caused by opportunistic pathogenic fungi. The consequence leads to the development of resistance of fungi to these drugs by mutation in the genes leading to newer resistant strains.

3. Toxicity of currently used antifungal agents: Most of the currently administered drugs are only fungistatic and cause several side effects such as nephrotoxicity (polyenes) and hepatotoxicity (azoles), as the fungi share similar cellular components and mechanism as that of mammalian cells.

A unique target: The fungal cell wall

The fungal cell wall is critical for the cell viability and pathogenicity. Beyond serving as a protective shell and providing cell morphology, the fungal cell wall is a critical site for exchange and filtration of ions and proteins, as well as metabolism and catabolism of complex nutrients. The fungal cell wall is a unique target because mammalian cells lack a cell wall; it represents an ideal, safe and specific target for antifungal therapy. Cell wall is

present in all fungi, therefore cell wall biosynthesis inhibitors would exhibit broad spectrum of antifungal activity.[4,7]

In spite of significant research on antifungal agents, the azoles remain the mainstay of therapy for systemic life threatening fungal infections as they have fungistatic, orally active and broad-spectrum activities against most yeasts and filamentous fungi. Azole antifungals are strong inhibitors of lanosterol 14α-demethylase, which is essential for conversion of lanosterol into ergosterol and the latter is the major component of fungal cell membrane.[6] Fluconazole (**1**) is a 1,2,4-triazole based drug that has established an exceptional therapeutic record for *Candida* infections, including oropharyngeal and esophageal candidiasis, vulvovaginal candidiasis, candidemia and disseminated candidiasis. It is an antifungal agent of choice for the treatment of infections by *Candida albicans* and *Cryptococcus neoformans* due to its potent activity, excellent safety profile and favorable pharmacokinetic characteristics. However, fluconazole is not effective against invasive aspergillosis and is not fungicidal. In addition, extensive use of fluconazole has increased the number of fluconazole-resistant *C. albicans* isolates.[8] Itraconazole (**7**) is an improvement of fluconazole in its broad-spectrum activity and better toleration but shows low bioavailability and oral absorption. Therefore, great efforts have been made to modify the chemical structure of fluconazole, in order to broaden its antifungal spectrum of activity and to increase its potency.[9]

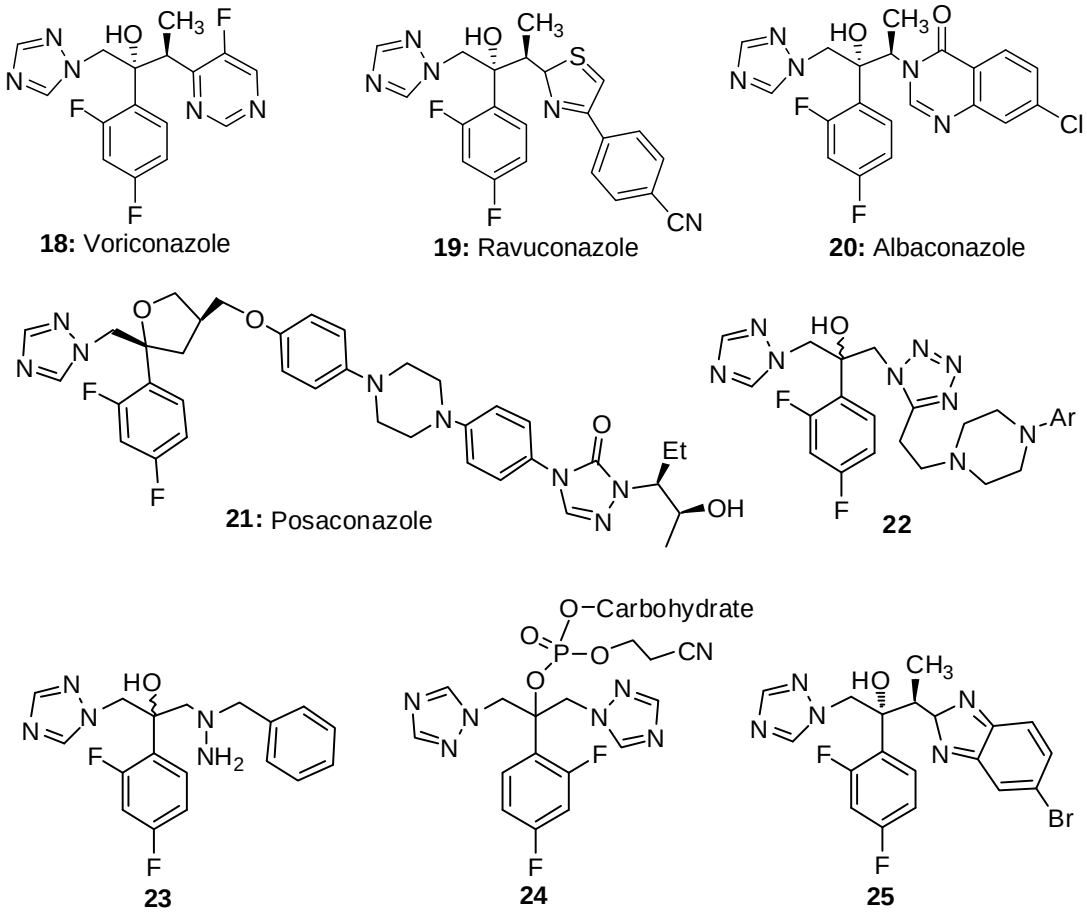


Figure 7. Azole antifungals containing 1,2,4-triazole

Several new azoles, containing 1,2,4-triazole and 1,3-difluorobenzene moieties, such as voriconazole (**18**), ravuconazole (**19**), albaconazole (**20**), posaconazole (**21**), *etc*. are marketed or in the late stages of clinical trials. Other modified fluconazole analogues **22-25** are reported in literature (Figure 7). Azoles exert antifungal activity through inhibition of CYP51 by a mechanism in which the heterocyclic nitrogen (N-3 of imidazole or N-4 of 1,2,4-tiazole) binds to the sixth coordination of heme iron atom of the porphyrin in the substrate binding site of the enzyme.[10] Based on the structure of the active site of CYP51 and the extensive investigation of the structure-activity relationships of azole antifungals, it was found that 1,2,4-triazole ring and 2,4-difluorophenyl group are essential for the high antifungal activity. Several reports on the synthesis and biological activity of structurally modified new analogues of fluconazole are known in the literature.[11-14]

Thienopyrimidinones: biologically important leads

Thienopyrimidinone derivatives represent important building blocks in organic and medicinal chemistry due to their pharmacological properties. H. M. Hosni *et al.* reported[15] thienopyrimidinone derivatives **26, 27, 28** and **29** showing molluscicidal and larvicidal activity.

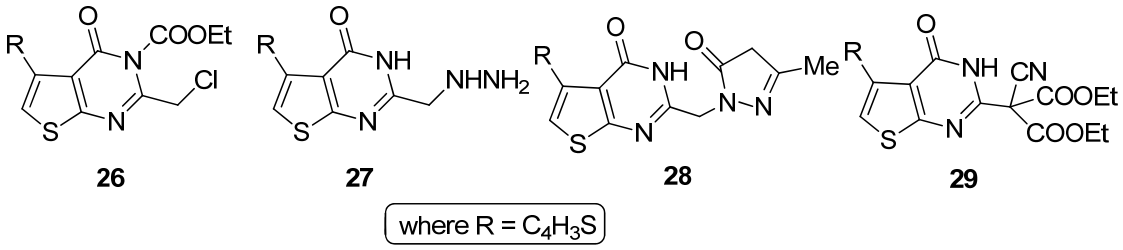


Figure 8. Thienopyrimidinones exhibiting molluscicidal and larvicidal activity

Compounds **26** and **27** were prepared from same intermediate 2-chloromethyl-5-(2 thienyl)thieno[*2,3-d*]-pyrimidin-4(3*H*)-one by treating it with ethyl chloroformate and hydrazine hydrate respectively. Reaction of 2-hydrazinomethylthieno[*2,3-d*]pyrimidinone **27** with ethyl acetoacetate gave compound **28** and so on. They have prepared some more derivatives of thienopyrimidinone, out of which above represented examples showed excellent activity values. Yong Sun *et al.* reported[16] thienopyrimidinone derivatives having triazole moiety in the molecule (representative example **30**) exhibiting fungicidal activity. These compounds were synthesized from tetrasubstituted thiophene, which in turn was synthesized from 1-(1*H*-1,2,4-triazol-1-yl)acetone, ethyl cyanoacetate and sulfur using Gewald method. Compounds of this type possess inhibitory activity against six fungal strains *Fusarium oxysporium*, *Rhizoctonia solani*, *Botrytis cinereapers*, *Gibberella zeae*, *Dothiorella gregaria* and *Colletotrichum gossypii.* Modica *et al.* reported[17] that various thienopyrimidinone derivatives, such as compound **31**, possess higher affinity and selectivity towards serotonin receptor sub type 5-HT_{1A} which is involved in physiological processes and psychiatric disorders. Hafez *et al.* reported[18] compounds **32**, **33** and **34** containing thienopyrimidinone exhibiting anti-inflammatory, antifungal and analgesic activity (Figure 9).

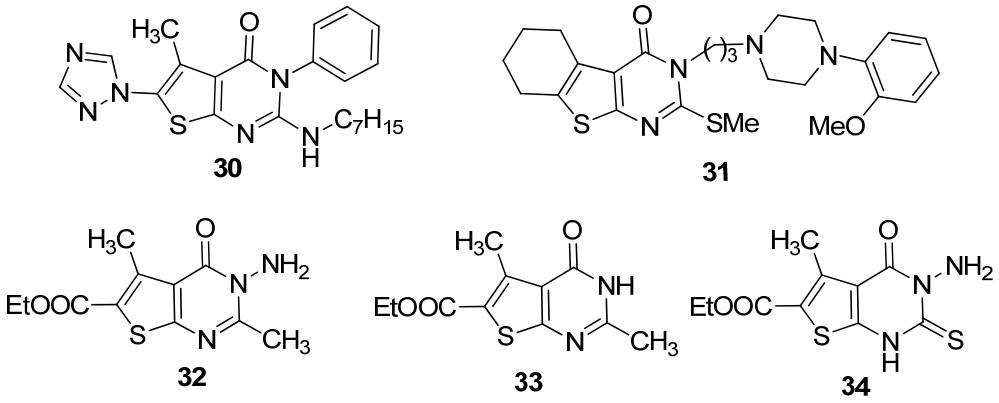


Figure 9. Thienopyrimidinones exhibiting biological activities

It is observed that rigid isomeric thienopyrimidinone derivatives[19] **35** and **36** possess good affinity for human melanin concentrating hormone receptor 1 (MCH R1). It is conjectured that brain penetrant antagonists of this receptor may have applications in the treatment of human obesity. The syntheses of these isomeric thienopyrimidinones were started from 3-amino-5-phenylthiophene-2-carboxylic acid methyl ester and 2-amino-5-phenyl-thiophene-3-carboxamide respectively. E. Duval *et al.* reported[20] thienopyrimidinone derivatives **37** and **38** as transglutaminase (TGase) inhibitors. TGases are the enzymes in many different tissues which play a vital role in blood clotting, epithelia formation *etc*. However, it is becoming increasingly evident that some TGase isozymes are involved in diverse pathological conditions such as celiac disease, neurodegenerative disorders, *etc*. Furthermore, some reports[21] demonstrate that thienopyrimidinone derivatives (representative example **39**) are potential inhibitors of tumor cell proliferation.

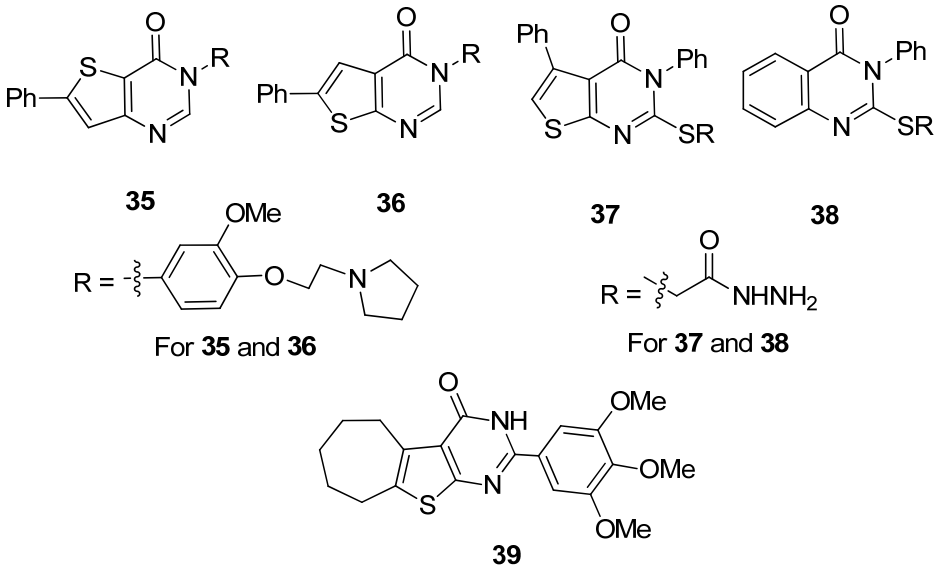


Figure 10. Thienopyrimidinones exhibiting biological activities

Fluconazole and its analoues

Amongst all azole antifungal agents, fluconazole (Trade name Diflucan) is the most important antifungal agent used in day to day life. This is because of the following reasons: i) it is having less toxicity compared with other azole antifungals ii) it has antifungal activity equivalent to amphotericin B and iii) fluconazole is having activity against broad range of fungi. Due to such properties fluconazole is in focus for its extensive use and hence for the synthesis. It is known in literature that azole derivatives including fluconazole act by inhibiting lanosterol P450 14α–demethylase enzyme (CYP51) in fungi through a mechanism in which the heterocyclic nitrogen atom (N-4 of triazole) binds to the heme iron atom[22a]. The crystal structure of CYP51 of fungi has not been obtained. Ji *et al.* [22b] reported a 3D model of lanosterol P450 14α-demethylase from *Candida albicans* and its interaction with azole antifungal compounds. In their study, the triazole ring was positioned perpendicular to the porphyrin plane, with a ring N-atom coordinated to the heme iron, which was found to be of key importance for antifungal activity. The halogenated phenyl group of azole inhibitors was deeply buried in the same hydrophobic binding cleft at the active site of the enzyme, and long chains of some inhibitors, such as itraconazole or ketoconazole, surpassed the active site, interacting with residues in the substrate access channel.

The binding mode of fluconazole with CYP51 is shown in Figure 11. In general, the active site of CYP51 for ligand binding can be divided into four subsites: an area in contact with heme, a hydrophilic H-bonding region, a hydrophobic region, and a narrow hydrophobic cleft formed by the residues in the helix B'-meander 1-loop and the N-terminus of helix I.

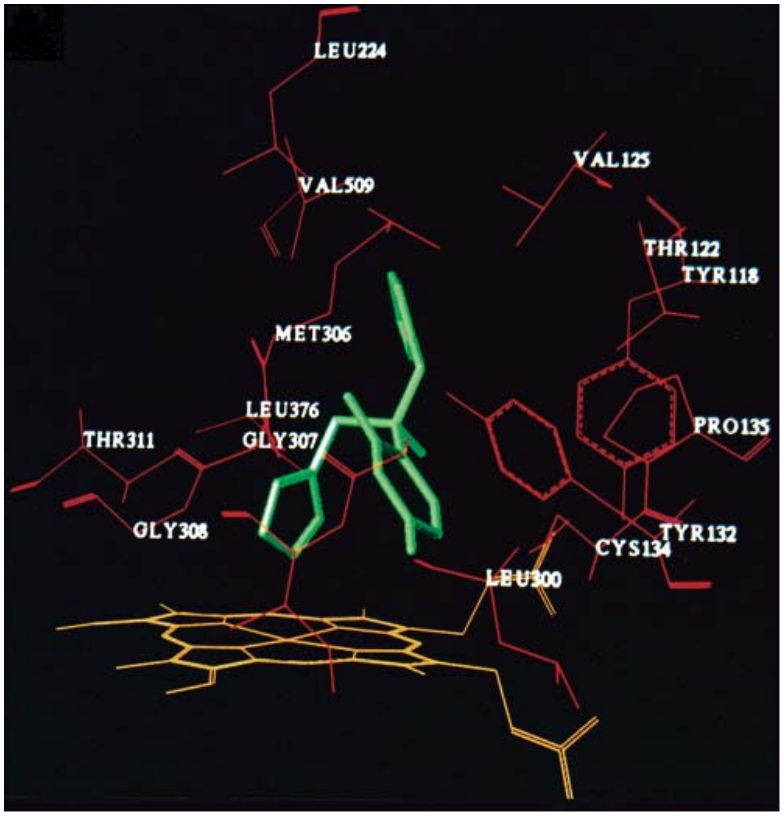
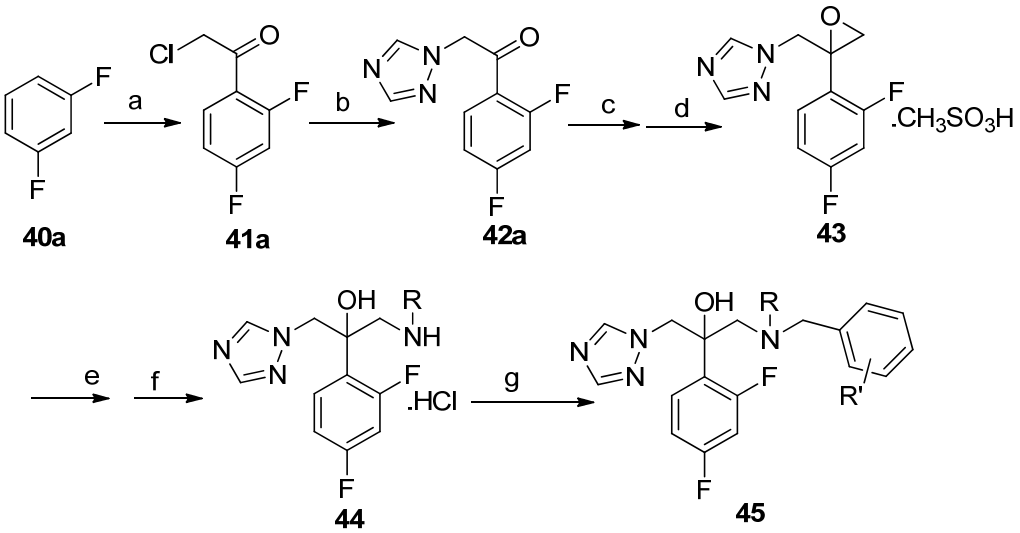


Figure 11. Stereoview of active site of lanosterol 14α-demethylase of *C. albicans* with bound fluconazole

Based on above study X. Chai *et al.*[23] constructed 3D model of CYP51 from *C. albicans* and analyzed the binding between fluconazole and CYP51. Depending upon the results of computational docking to the active site of the cytochrome P450 14α-demethylase (CYP51), a series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-substituted benzylamino-2-propanols **45** as analogues of fluconazole were designed, synthesized, and evaluated as antifungal agents[23] (Scheme 1).



Scheme 1. Reagents and conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50 ^oC, 5 h, 87 % (b) $C_6H_5CH_3$, $NaHCO_3$, 1*H*-1,2,4-triazole, reflux, 5 h, 87 % (c) $C_6H_5CH_3$, $(CH_3)_3SOI$, NaOH, cetylmethylammonium bromide, 60 ^oC, 3 h, 86 % (d) CH_3SO_3H, 0 ^oC, 1 h, 89 % (e) CH_3CH_2OH, Et_3N, primary amine, reflux, 6 h (f) $HCl_{(g)}$, 80–90 % (g) CH_3CN, KI, K_2CO_3, substituted benzyl bromide, rt, 5–6 h, 50–70 %.

Synthesis of the compound **45** was accomplished using chemistry illustrated in Scheme 1. The intermediate oxirane **43** was synthesized by known procedure and was allowed to react with n-propylamine in the presence of triethylamine in ethanol and then HCl gas was introduced to form the hydrochloride **44**. The reaction of compound **44** and substituted benzyl bromide in the presence of KI and K_2CO_3 in acetonitrile afforded compounds **45**. Results of preliminary antifungal tests against eight human pathogenic fungi *in vitro* showed that these analogues exhibited excellent activities with broad spectrum.

Pore and co-workers reported[24] fluconazole analogues (showed by general formulae **46** and **47**) with (non)substituted 1,2,3-triazole ring which are isosteres of fluconazole. They also reported bile acid conjugates of fluconazole isosteres as shown in Figure 12.

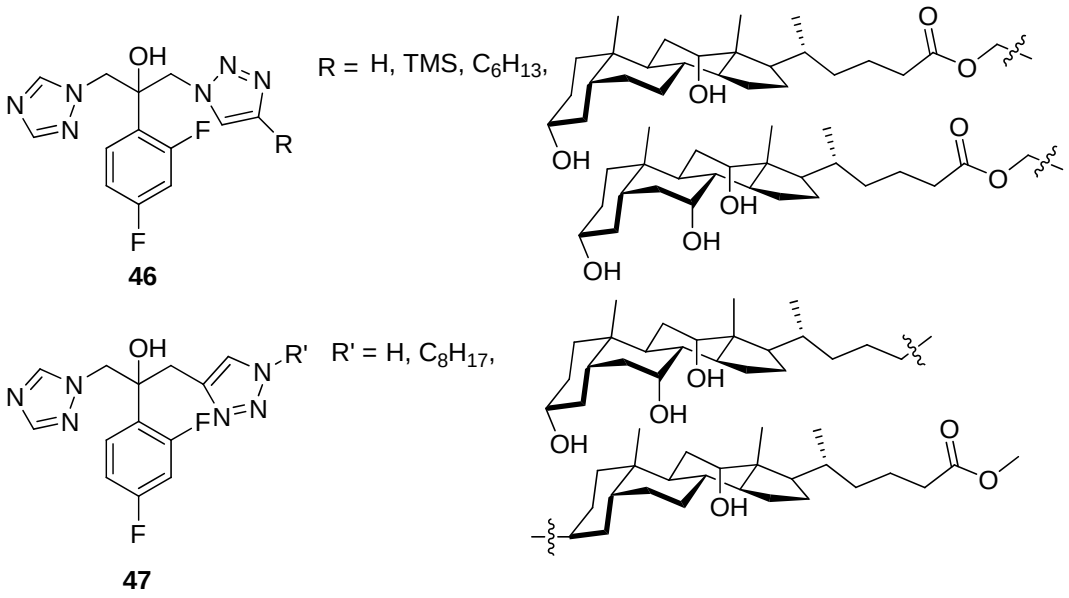
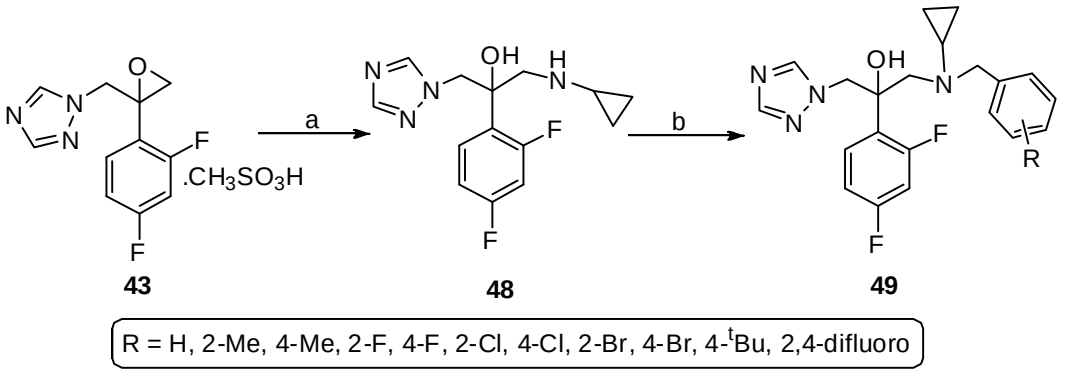


Figure 12. Fluconazole analogues and their bile acid conjugates

These molecules were evaluated *in vivo* against *Candida albicans* in Swiss mice model and antiproliferative activities were tested against human hepatocellular carcinoma Hep3B and human epithelial carcinoma A431. It was observed that one of the compounds resulted in 97.4 % reduction in fungal load in mice and did not show any profound proliferative effect at lower dose. Some of them were observed to be more toxic than fluconazole but less toxic than ketoconazole.

Depending upon the model study carried out by Ji *et al.*, Qui-Ye Wu and coworkers[25] designed fluconazole analogues **49** by computational docking experiments to the active site of the cytochrome P450 demethylase (CYP51). They synthesized the compounds **49** shown in Scheme 2 as follows. The triazole **48** was obtained by nucleophilic epoxide-ring opening of **43** (a known intermediate) with cyclopropylamine in EtOH in the presence of Et_3N as base. Finally, the target compounds **49** were obtained by reaction of **48** with differently substituted benzyl bromides ($ArCH_2Br$) in MeCN in the presence of K_2CO_3, in yields of 40 –60 %.



Scheme 2. Reagents and conditions**:** a) Cyclopropylamine, Et_3N, EtOH, 80 ^oC, 6–8 h; 60 %; b) $ArCH_2Br$, MeCN, K_2CO_3, 70–90 ^oC, 6–10 h; 40 –60 %.

Preliminary biological tests showed that most of the target compounds exhibit significant activities against the eight most-common pathogenic fungi. The most potent derivative, 1-[(4-*tert*-butylbenzyl)(cyclopropyl)amino]-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**49** wherein R = 4-*t*Bu), was found to exhibit a broad antifungal spectrum, being more active against *Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans*, *Microsporum canis*, and *Trichophyton rubrum* than the standard clinical drug itraconazole. The observed affinities of the lead molecules towards CYP51 indicated that a cyclopropyl residue enhances binding to the target enzyme.

N. Lebouvier *et al.* reported[26] that fluconazole analogues with azaheterocycle moiety showed by general formula **50** exhibited antifungal activity against *Candida albicans* and *Aspergillus fumigatus*. Four analogues out of 15 compounds synthesized exerted high antifungal activity against *C. albicans* with MIC values 3 to 28 fold lower than that of fluconazole.

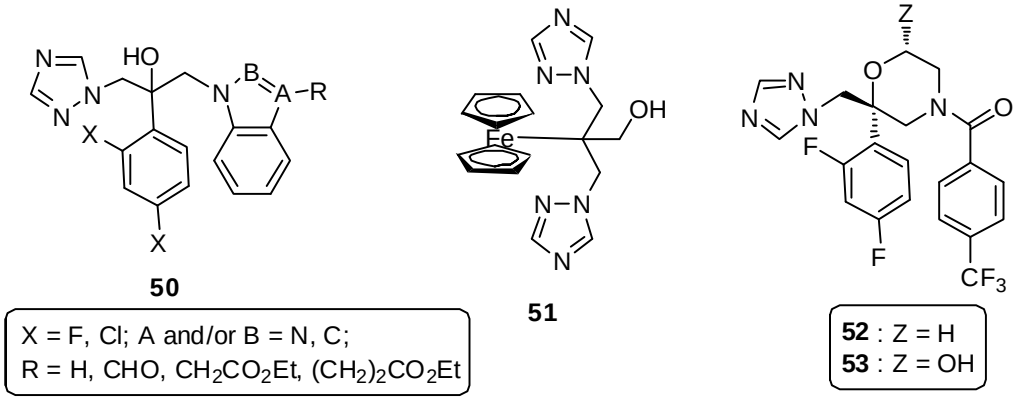


Figure 13. The structures of the fluconazole analogues **50**, **51**, **52** and **53**

C. Biot *et al.* reported[27] ferrocene-fluconazole analogue **51** and investigated its activity against different yeast strains. Surprisingly, *in vitro* tests revealed a slight increase in fungal growth and a reversal of the effect of fluconazole at minimal inhibitory concentrations. Bartroli *et al.* reported[28] encouraging results in their synthesized fluconazole analogues containing morpholine ring. Out of 25 compounds synthesized and tested for antifungal activity against 17 different strains of fungi, all of them exhibited good to excellent activities and two of them, **52** and **53**, showed approximately 10 times more potency than fluconazole in murine coccidioidomycosis and superior to itraconazole in murine histoplasmosis. Preliminary toxicity data for these compounds in the rat showed excellent tolerance. Microscopic observations of these samples did not show any alteration in internal organs of the rat models. However, these compounds failed to show any particular activity in murine models of aspergillosis.

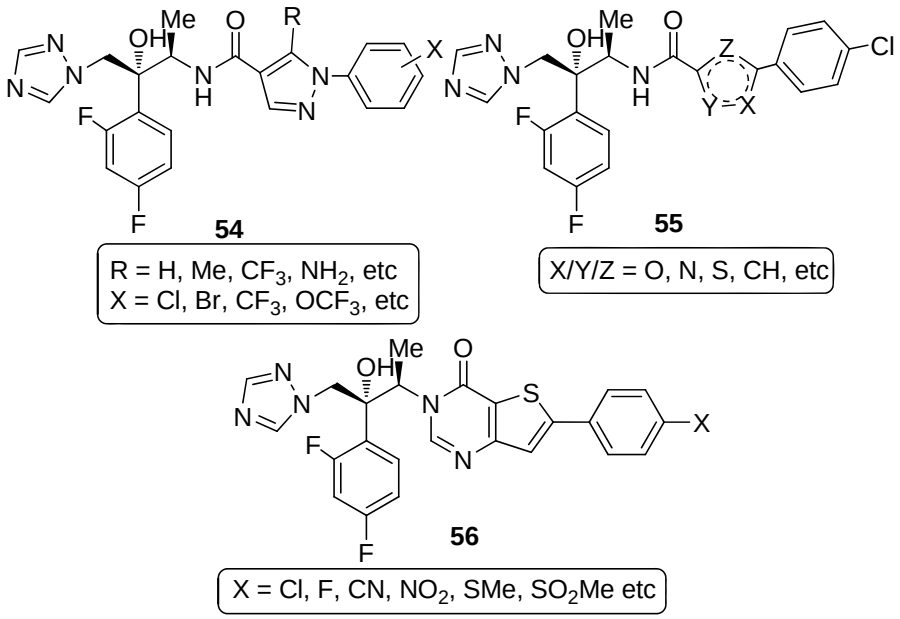


Figure 14. Fluconazole analogues containing heterocyclic rings

Furthermore, Bartroli *et al.* reported[29] the synthesis and antifungal activity of fluconazole analogues **54**, **55** and **56** containing various heterocycles such as thiazole, pyrazole, oxazole, imidazole, thienopyrimidinone and so on.

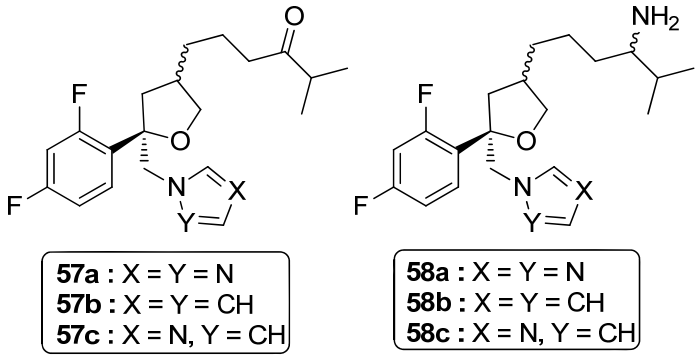


Figure 15. Fluconazole analogues containing tetrahydrofuran ring

S.-K. Chung *et al.* reported[30] the synthesis of various azole containing compounds **57** and **58**, tested their antifungal activity and observed that imidazole and triazole groups play a major role in the antifungal activity. R. S. Upadhayaya *et al.* reported[31] the synthesis of compounds containing tetrazole moiety depicted by general structure **59** in Figure 16.

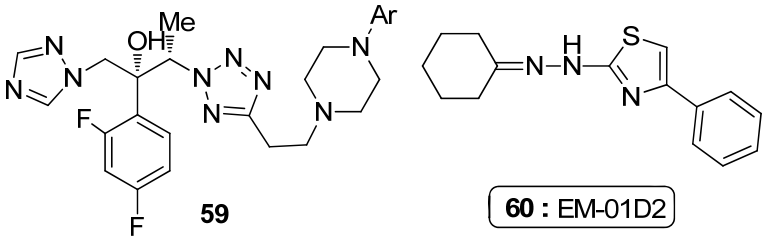
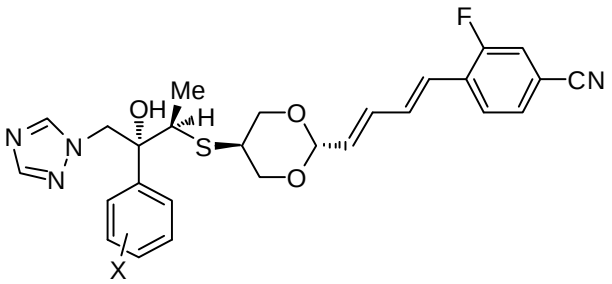


Figure 16. Fluconazole analogues containing tetrazole ring **59** and EM-01D2 (**60**)

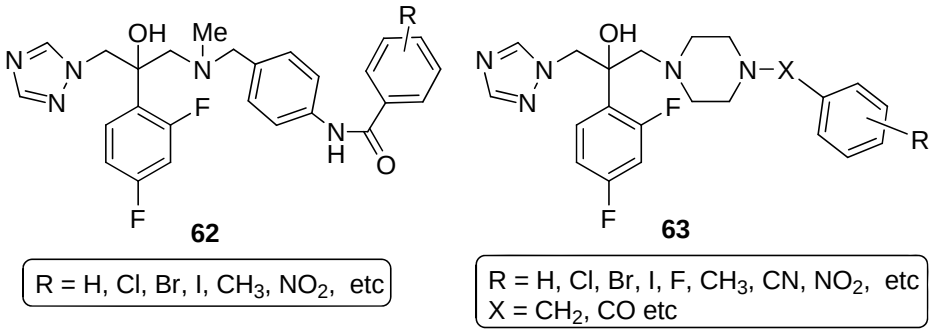
They observed that the antifungal activity of some of the synthesized compounds was better than fluconazole and was equivalent to itraconazole, voriconazole, posaconazole and ravuconazole. De Logu *et al.* reported[32] the 2-cyclohexylidenehydrazo-4-phenyl-thiazole (EM-01D2) (**60**) as potent antifungal agent. Furthermore, it has low toxicity, broad spectrum and activity against *C. albicans* and *C. krusei* at concentrations lower than those shown by amphotericin B and fluconazole, and it maintained potent *in vitro* activity against fluconazole-resistant *C. albicans* isolates.



61	a	b	c	d	e
X	2-F	4-F	2,3-F_2	2,4-F_2	2,5-F_2

Figure 17. Fluconazole analogues containing dioxane ring

Y. Kogoshima *et al.* reported[33] the synthesis of fluconazole analogues containing dioxane ring **61** including the most active member CS-758 (**61d**) as shown in Figure 17 and studied the effect of fluorine atoms on aromatic ring.



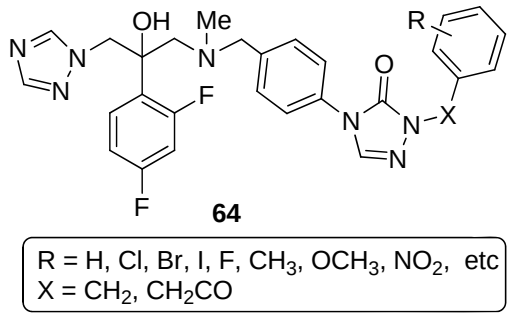


Figure 18. Fluconazole analogues

W. Zang and coworkers reported[34a] the synthesis of a series of azole molecules depicted by general formulae **62**, **63** and **64** and studied the antifungal activity and

calculated the interaction energies for the complexes of compounds **62**, **63** and **64** with the active site of CYP51. Pharmacological and toxicological evaluation of these compounds may help in discovery and optimization of the lead compounds. Compounds depicted by general formula **62** with free primary amine were also studied and reported[34b] by F. Giraud *et al.*

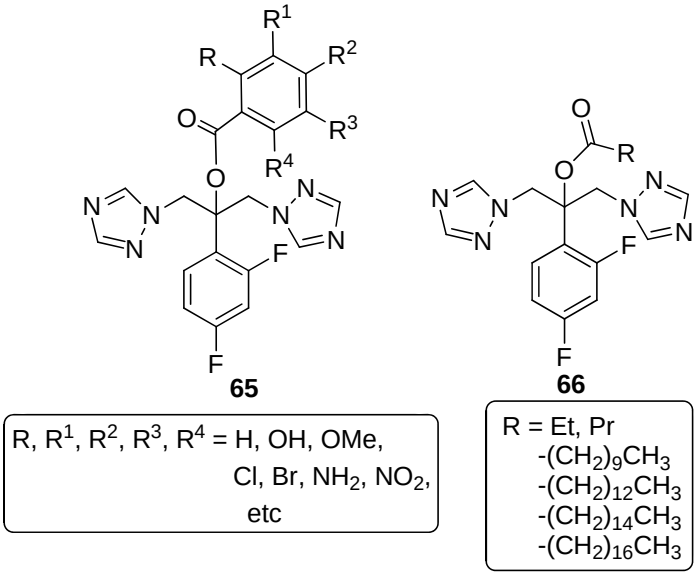


Figure 19. Ester derivatives of fluconazole

R. Ohlan *et al.* reported[35] the synthesis of various ester derivatives of fluconazole **65** and **66**, wherein fluconazole was treated with various aliphatic and aromatic acid chlorides. These esters were screened for the antifungal activity and it was found that the long chain esters exhibited better activity than respective short chain esters and aromatic esters. Furthermore, a series of such ester analogues of fluconazole were also reported[36] by A. R. Katritzky *et al.* The synthesized compounds were screened against various fungal strains and evaluated for antifungal activity.

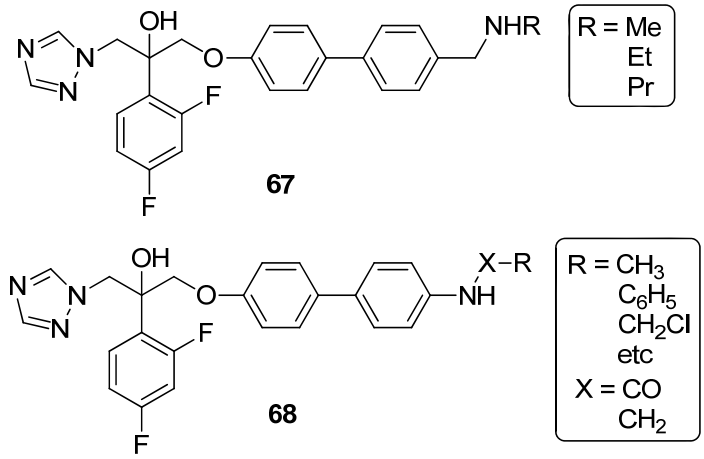


Figure 20. Biphenyloxyl analogues of fluconazole

P. Liu *et al.* reported[37] the synthesis of ether analogues of fluconazole **67** and **68**. These biphenyloxyl analogues were screened for antifungal activity against yeast and mold fungi species.

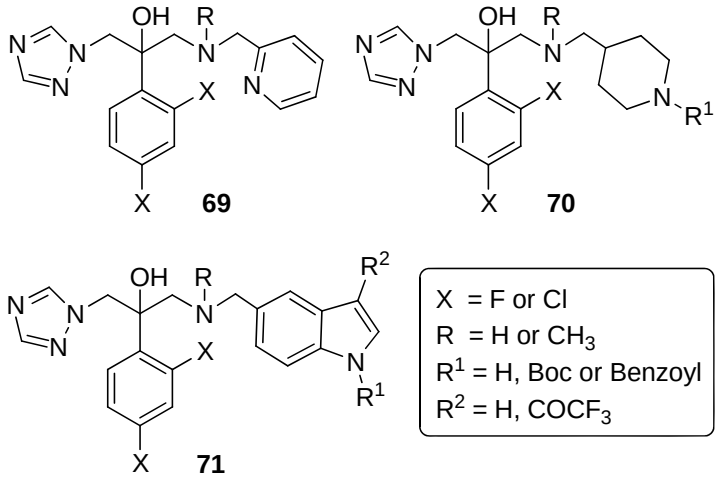


Figure 21. Pyridine, piperidine and indole analogues of fluconazole

C. Loge and coworkers reported[38,39] the synthesis of fluconazole analogues containing substituted pyridine, substituted piperidine and substituted indole depicted by general formulae **69**, **70** and **71** respectively (Figure 21) and studied the π- π stacking and hydrogen bonding interaction in the active site of CYP51 in *Candida albicans*.

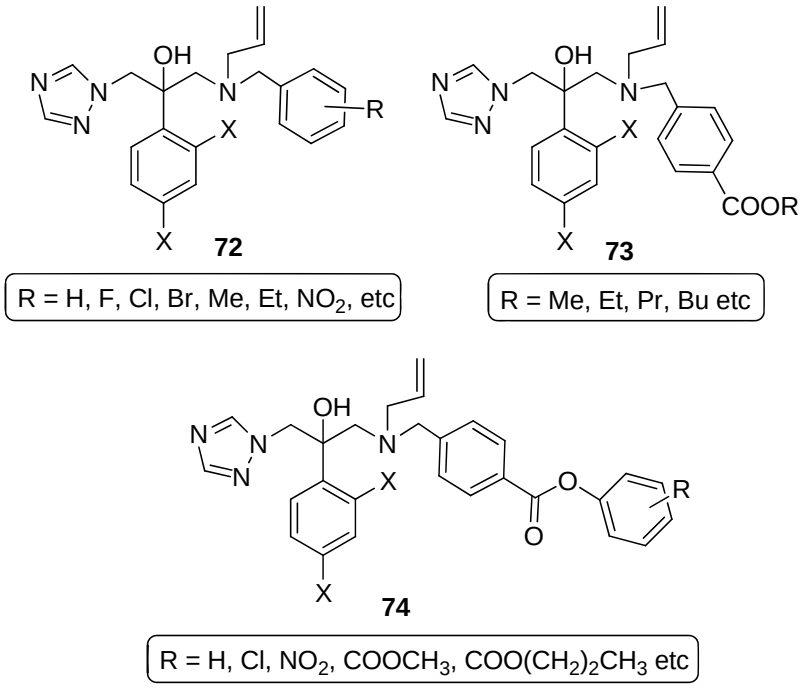


Figure 22. Fluconazole analogues containing substituted aryl moieties

X. Chai *et al.* reported[40] the synthesis of fluconazole analogues **72**, **73** and **74** containing substituted aryl moieties and screened against various fungal strains. They observed that some of the compounds exhibited better antifungal activity than fluconazole and amphotericin B.

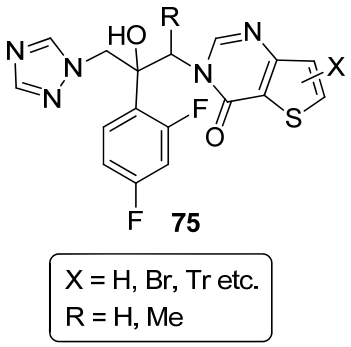


Figure 23. Fluconazole analogues containing thieno-[3,2-*d*]pyrimidinone moieties

Apart from this, in our group efforts have been made for the synthesis of fluconazole analogues[41] containing thieno[3,2-*d*]pyrimidinone moieties

shown by general formula **75**. Synthesized compounds exhibited antifungal activity but the route involved in the synthesis was lengthy and the starting materials were costly. This made us to design and synthesize novel fluconazole analogues described in this chapter.

1.1.4 Present work

1.1.4.1 Objective

Researches indicated that the triazole ring, the difluorophenyl group and the hydroxyl group were the pharmacophores of fluconazole. The side chain located in the narrow hydrophobic cleft was also important. The optimization of the side chain attached to the pharmacophore remains an attractive subject of research for the organic chemist. We intended to alter the side chains to find potent systemic antifungal agents with a broad antifungal spectrum and less potential to develop resistance. We aimed at the designing of compounds, where we systematically altered the structure of fluconazole (Figure 24) based on the literature results of computational docking to the active site of the CYP51, and the modification was focused on one triazole moiety of fluconazole.

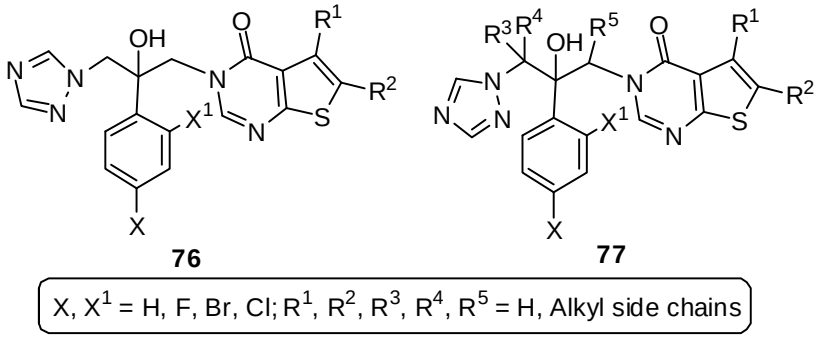


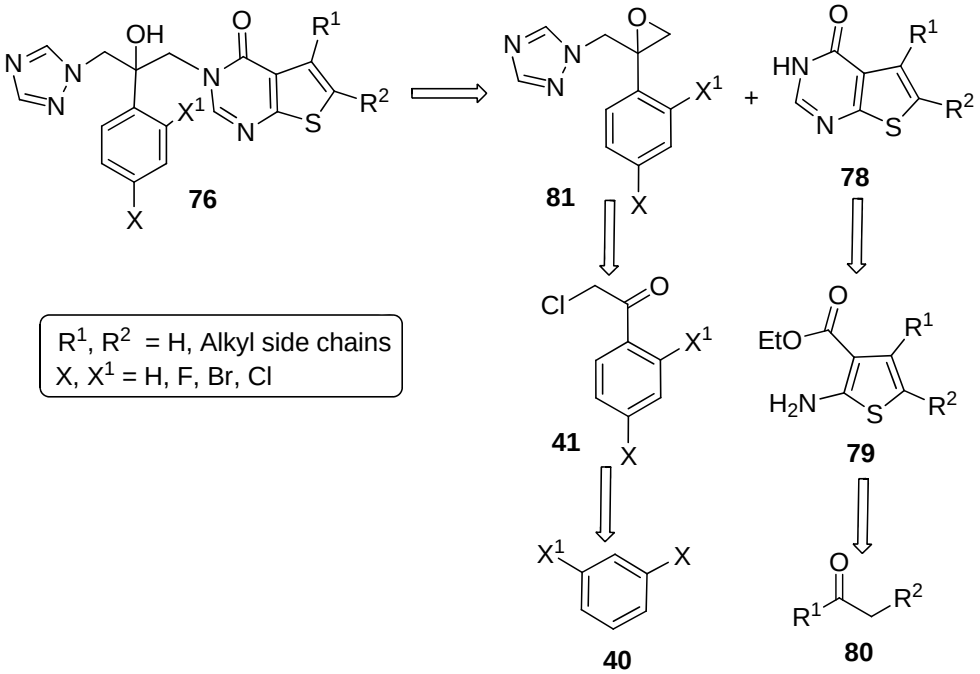
Figure 24. Proposed fluconazole analogues with thienopyrimidinone moieties

In order to seek new triazole antifungal agents, we designed a series of triazole compounds depicted by general formulae **76** and **77**. As the structure activity study of fluconazole has revealed that the left half portion of the molecule is essential for the high antifungal activity, a number of analogues have been reported in literature, in attempt to develop better antifungal agents wherein one of the triazole rings of fluconazole is replaced by other groups. This type of research is important to solve the problem of treatment of fluconazole-resistant fungal strains. Furthermore, substituted thienopyrimidinones exhibited

biological activity hence these moieties were chosen to be introduced in fluconazole molecule. In the work described in this section, efforts were directed towards replacement of triazole moiety with variously substituted thienopyrimidinones in order to make a number of compounds available for biological activity. So we targeted to synthesize a molecule where pharmacophore of fluconazole was attached to the biologically active theinopyrimidinone moiety so that we could get a modified fluconazole with enhanced antifungal activity. First of all we focused on the synthesis of target molecule **76** where one of the triazoles from the fluconazole molecule was replaced with thienopyrimidinone. The significant results obtained in case of molecules represented by general structure **76** made us to undertake synthesis of compounds with general structure **77** wherein structures of the molecules **76** were modified further. The structure-activity relationship study for these compounds is presented in second section of this chapter.

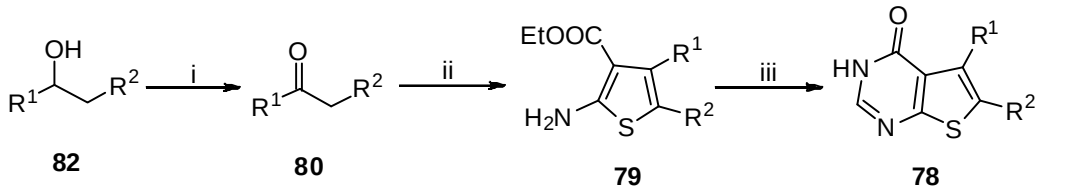
1.1.4.2 Results and discussion

The retrosynthetic analysis for preparation of compounds depicted by general formula **76** is shown in Scheme 3.



Scheme 3. Retrosynthetic analysis for preparation of compounds **76**

Compound **76** could be synthesized from the epoxide opening of **81** by the thienopyrimidinone **78**. Thienopyrimidinone **78** could be synthesized from 4 and/or 5-substituted 2-aminothiophene-3-carboxylate **79**, which in turn could be synthesized from respective aldehyde or ketone **80** by Gewald reaction.[42] The epoxide **81** could be synthesized from (di)halobenzene **40** *via* its acylated intermediate **41** by a known method[43]. The various substituted thienopyrimidinones **78a-r** were prepared by the synthetic route shown in Scheme 4.



	R^1	R^2
a	H	H
b	H	CH_3
c	H	CH_2CH_3
d	H	$(CH_2)_2CH_3$

	R^1	R^2
e	H	$(CH_2)_4CH_3$
f	H	$(CH_2)_5CH_3$
g	H	$(CH_2)_6CH_3$
h	H	$(CH_2)_8CH_3$

	R^1	R^2
i	H	$(CH_2)_9CH_3$
j	H	$(CH_2)_6CH=CH_2$
k	H	$(CH_2)_7CH=CH_2$
l	H	$(CH_2)_3OCH_2Ph$

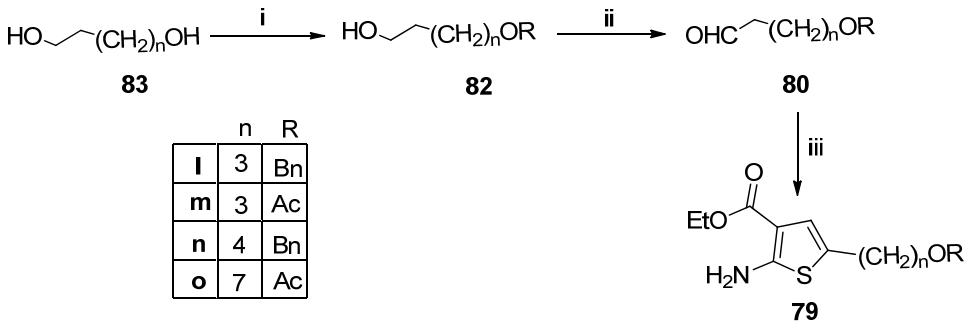
	R^1	R^2
m	H	$(CH_2)_3OAc$
n	H	$(CH_2)_4OCH_2Ph$
o	H	$(CH_2)_7OAc$

	p	**q**	**r**
R^1, R^2	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_{10}$

Scheme 4. Reagents and conditions: i) DMSO, $(COCl)_2$, Et_3N, DCM, -78 °C, 3-4 h, 60-68 %; ii) Ethyl cyanoacetate, sulphur, DMF, TEA, 50 °C, 10-12 h, 84.3-94.6 %; iii) NH_4OAc, $HCONH_2$, 140-145 °C, 4-5 h, 74.7-91.8 %.

A substituted alcohol **82** (depending on side chain desired in thienopyrimidinone) was subjected for oxidation to get aldehyde or ketone **80**, which was subjected to Gewald synthesis[42] to afford 4 and/or 5-substituted 2-aminothiophene-3-carboxylate **79**, which was then cyclized in presence of ammonium acetate and formamide to get the key intermediate, a substituted thienopyrimidinone **78**. Thienopyrimidinone **78a** was synthesized from readily available ethyl 2-aminothiophene-3-carboxylate **79a**. Evidences for the formation of thienopyrimidinone **78a** were shown by the PMR value of aromatic proton at δ 8.15 and disappearance of protons for ethyl ester indicating cyclisation. Furthermore, C=O frequency in IR shifted from 1745 to 1659 cm^{-1}. Thienopyrimidinones **78b** to **78i** were synthesized from respective aldehydes by Gewald synthesis and were characterized by PMR, CMR, Mass and IR spectroscopy. For the synthesis of **79j** and **79k**, starting materials used were 9-

decen-1-ol and 10-undecen-1-ol respectively. These two alcohols were oxidized using Swern oxidation conditions and the crude aldehydes obtained in the reaction were used without purification for the Gewald reaction to obtain ethyl 2-amino-5-(oct-7-en-1-yl)thiophene-3-carboxylate (**79j**) and ethyl 2-amino-5-(non-8-en-1-yl)thiophene-3-carboxylate (**79k**) respectively. ^{1}H NMR spectra showed singlets in aromatic region at δ 6.68 and 6.66 respectively for one proton, which indicated the formation of compounds **79j** and **79k** respectively. Synthesis of **79l** to **79o** was started using respective diols as shown in Scheme 5.

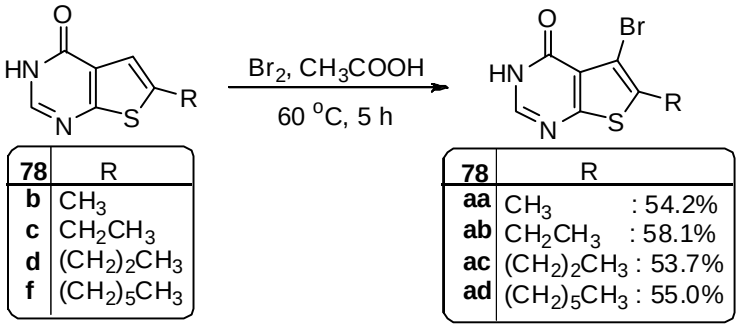


	n	R
l	3	Bn
m	3	Ac
n	4	Bn
o	7	Ac

Scheme 5. Reagents and conditions: i) For R = Bn: BnBr, NaH, THF, 0 ^{o}C-rt, 6 h; for R = Ac: Acetic anhydride, NaH, THF, rt, 12 h, 65.5-69.4 %; ii) DMSO, $(COCl)_2$, Et_3N, DCM, -78 °C, 3-4 h; iii) Ethyl cyanoacetate, sulphur, DMF, TEA, 50 °C, 10-12 h, 68.5-71 % (over two steps).

Monoprotection of pentanediol and hexanediol was carried out using benzyl bromide in presence of sodium hydride in THF to get **82l** and **82n** respectively. Characteristic singlet for two protons at δ 4.21 for **82l** and δ 4.50 for **82n** in PMR and absorption at 3400 cm^{-1} in IR spectrum indicated monoprotection of diol. For the synthesis of **82m** and **82o**, acetic anhydride was used for the monoprotection of pentanediol and nonanediol respectively as shown in Scheme 5. Synthesis of **79p, 79q** and **79r** was carried out by subjecting cyclopentanone, cyclohexanone and cyclododecanone respectively to Gewald synthesis. Thus synthesized 4 and/or 5-substituted 2-aminothiophene-3-carboxylates **79**, were cyclized to get respective substituted thienopyrimidinones **78** except **78aa** to **78ad**. Formations of thienopyrimidinones **78a**-**78r** were confirmed by the appearance of aromatic singlet in the range δ 7.94-8.15 for one proton. Bromination of **78b**, **78c**, **78d** and **78f** in presence of bromine and acetic acid gave **78aa**, **78ab**, **78ac** and **78ad** respectively as shown in Scheme 6. One of the peak in the aromatic region disappeared due to the substitution of the bromine

atom in the thiophene ring of thienopyrimidinone moiety indicating the formation of brominated thienopyrimidinones, which was again confirmed by mass spectroscopy.

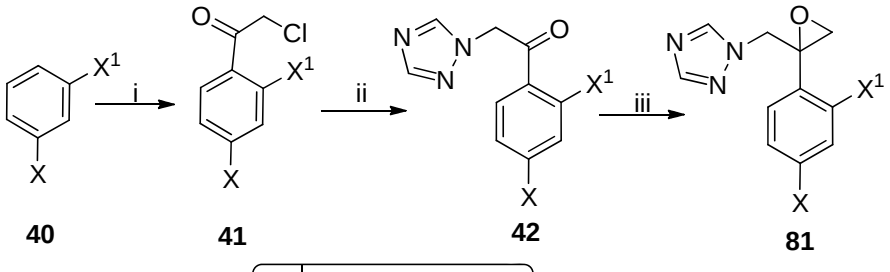


78	R
b	CH_3
c	CH_2CH_3
d	$(CH_2)_2CH_3$
f	$(CH_2)_5CH_3$

78	R	
aa	CH_3	: 54.2%
ab	CH_2CH_3	: 58.1%
ac	$(CH_2)_2CH_3$	: 53.7%
ad	$(CH_2)_5CH_3$	: 55.0%

Scheme 6. Synthesis of thienopyrimidinones **78aa** to **78ad**

Synthesis of the epoxides **81** was carried out by known methods[11,13,43a-c,47] shown in Scheme 7 as follows.



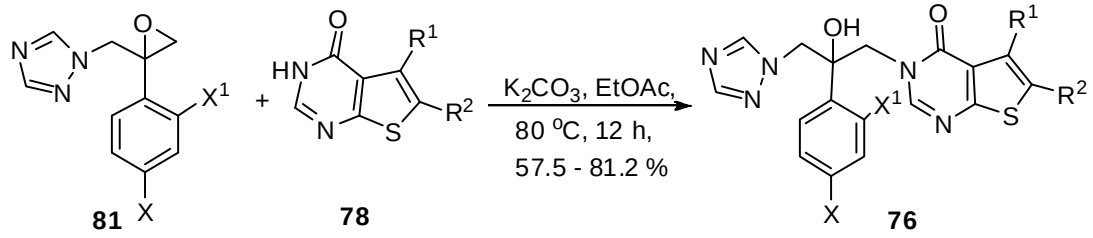
40 **41** **42** **81**

	a	b	c	d
X	F	F	Br	Cl
X^1	F	H	H	Cl

Scheme 7. Reagents and conditions: i) Chloroacetyl chloride, $AlCl_3$, DCM, 0 °C-rt, 8 h, 80-90 %; ii) K_2CO_3, 1,2,4-triazole, EtOAc, 80 °C, 10 h, 69-76 %; iii) Trimethylsulphoxonium iodide, aq. KOH, DCM, 45 °C, 12 h, 81-91 %.

Halobenzenes **40a-d** were acylated with chloroacetyl chloride in presence of aluminium chloride. The acylated products **41a-d** thus obtained were treated with 1,2,4-triazole in presence of potassium carbonate to get ketones **42a-d** respectively. These triazole containing ketones **42a-d** were converted into respective epoxides **81a-d** under Corey-Chaykovsky reaction conditions[43e-f] using trimethylsulphoxonium iodide and aq potassium hydroxide. The comparison of the spectroscopic data of epoxides **81a-d** with the literature values confirmed the product formation in the reaction.

The thienopyrimidinones **78a-r** were then reacted with the epoxides **81a-d** in presence of potassium carbonate in ethyl acetate at refluxing temperature to afford the target molecules **76a-t** and **76aa-ad** as shown in Scheme 8. Generally four doublets, each for one proton in the range of δ 4.04-4.98 with coupling constant in the range of J = 14 Hz in PMR spectra were observed which confirmed the formation of product. These protons are diastereotopic in nature and hence showed doublet in PMR spectra of these molecules.



For **76s** and **76t** X = Br, X^1 = H and for rest of the compounds X = X^1 = F

	R^1	R^2
a	H	H
b	H	CH_3
c	H	CH_2CH_3
d	H	$(CH_2)_2CH_3$

	R^1	R^2
e	H	$(CH_2)_4CH_3$
f	H	$(CH_2)_5CH_3$
g	H	$(CH_2)_6CH_3$
h	H	$(CH_2)_8CH_3$

	R^1	R^2
i	H	$(CH_2)_9CH_3$
j	H	$(CH_2)_6CH=CH_2$
k	H	$(CH_2)_7CH=CH_2$
l	H	$(CH_2)_3OCH_2Ph$
m	H	$(CH_2)_3OAc$

	R^1	R^2
n	H	$(CH_2)_4OCH_2Ph$
o	H	$(CH_2)_7OAc$
s	H	CH_3
t	H	CH_2CH_3

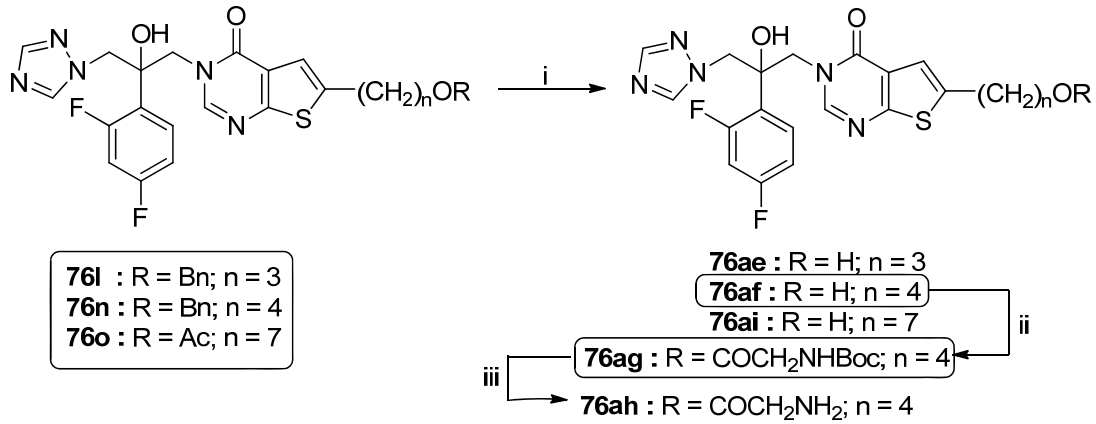
	p	**q**	**r**
R^1, R^2	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_{10}$

	R^1	R^2
aa	Br	CH_3
ab	Br	CH_2CH_3

	R^1	R^2
ac	Br	$(CH_2)_2CH_3$
ad	Br	$(CH_2)_5CH_3$

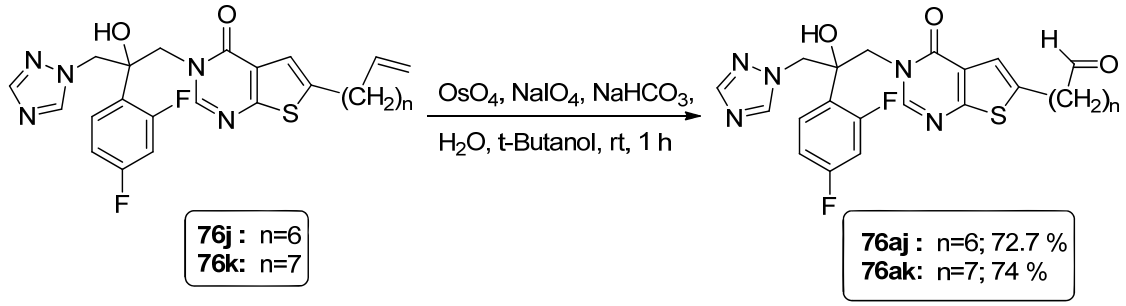
Scheme 8. Synthesis of fluconazole analogues **76**

Debenzylation[44] of **76l** and **76n** was done with boron trifluoride-etherate in acetonitrile to afford the corresponding diols **76ae** and **76af** respectively. Synthesis of compound **76ai** was achieved by deprotection of **76o** using potassium carbonate in methanol/water system at room temperature. Reaction of **76af** with N-Boc-alanine in dichloromethane in presence of EDCI and DMAP at room temperature[45] resulted in the formation of ester **76ag**. Treatment of **76ag** with trifluoroacetic acid in dichloromethane[46] afforded the amine **76ah** as shown in Scheme 9. In all above deprotection reactions, functional groups in the side chain were unmasked hence disappearance of peaks due to protecting group in PMR and CMR spectra indicated the product formation.



Scheme 9. Reagents and conditions i) a. $BF_3.Et_2O$, NaI, acetonitrile, 0 ^oC-rt, 2 h, 81-88 % (For **76l** and **76n**) or b. K_2CO_3, MeOH:H_2O (1:1), rt, 3 h, 96.7 % (For **76o**); ii) N-Boc alanine, EDCI, DMAP, DCM, rt, 12 h, 46.8 %; iii) TFA, DCM, rt, 4 h, 56.8 %.

For the synthesis of **76aj** and **76ak** oxidation of **76j** and **76k** was done using osmium tetraoxide, sodium periodate and sodium bicarbonate in water *t*-butanol at room temperature as shown in Scheme 10. Aldehydic proton in the range of δ 9.7-9.8 in PMR spectrum of **76aj** and **76ak** indicated the formation of product in oxidation reaction.



Scheme 10. Synthesis of **76aj** and **76ak**

Apart from the above compounds (**76a-t** and **76aa-ak**) synthesized, which were available for the structure activity relationship (SAR) study (explained in second section of this chapter), we targeted towards the synthesis of **84** where we thought to put a methyl substituent on the carbon flanked between nitrogen of thienopyrimidinone and carbon bearing hydroxyl group as shown in Figure 25. Such type of methyl substituent was observed in some potent antifungal molecules such as voriconazole, ravuconazole *etc*.

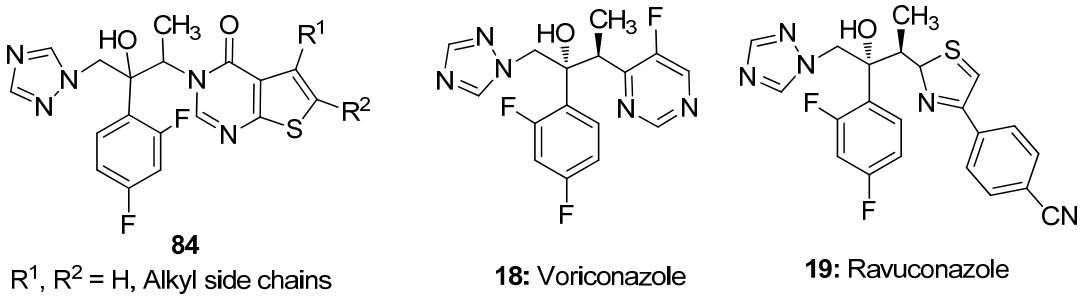
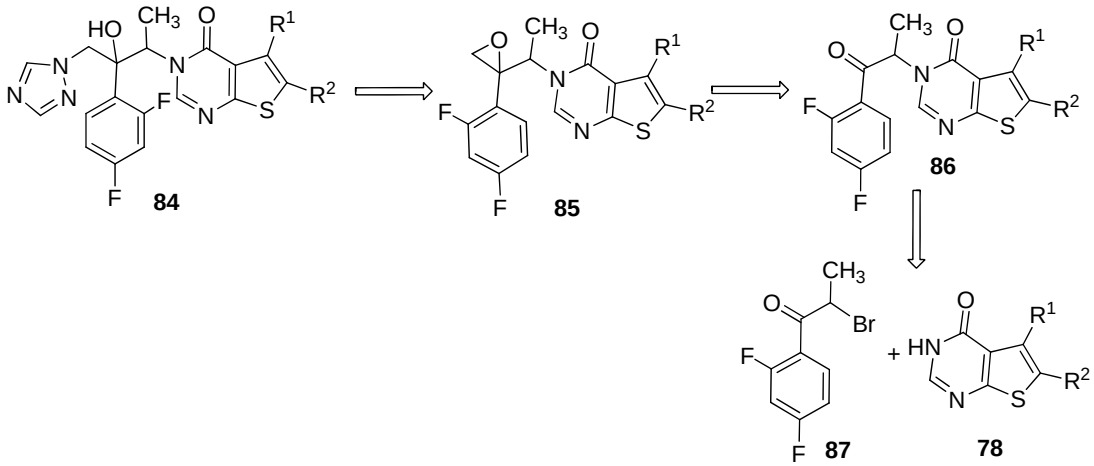


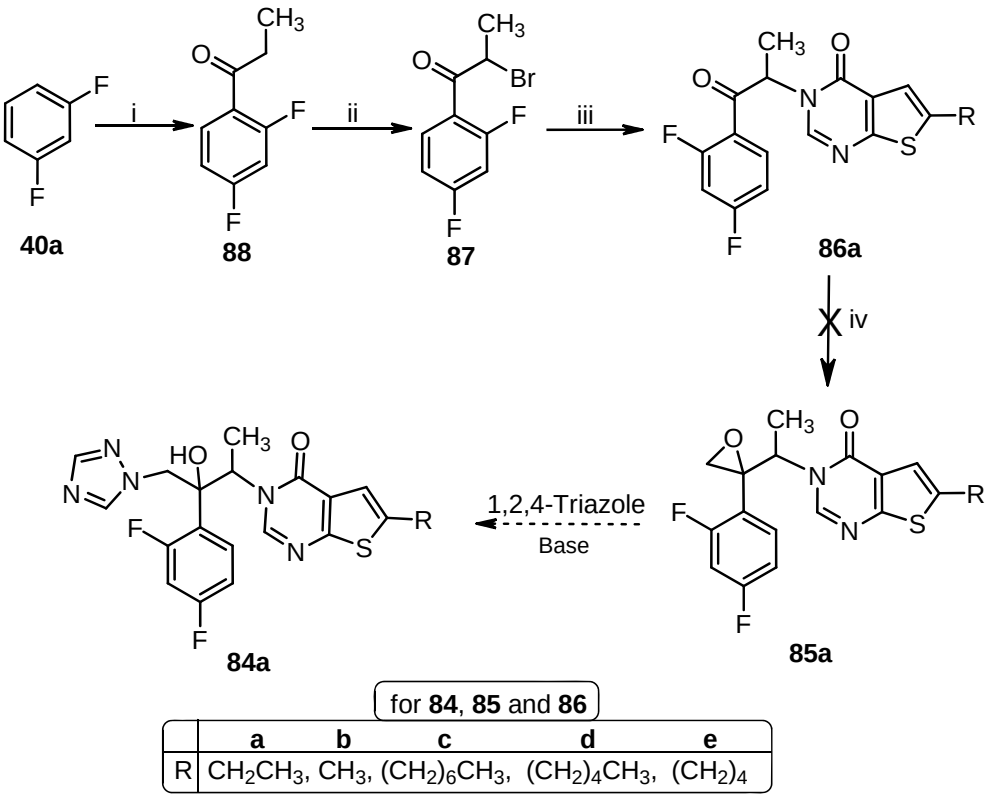
Figure 25. Antifungal agents

Retrosynthetic analysis (Scheme 11) suggested that, the target molecule **84** could be synthesized from epoxide **85** by reaction with 1,2,4-triazole in basic media. Epoxide **85** could be prepared from the corresponding ketone **86** which in turn could be obtained from alkylation of thienopyrimidinone **78** with the bromoketone **87**.



Scheme 11. Retrosynthetic analysis for preparation of compounds **84**

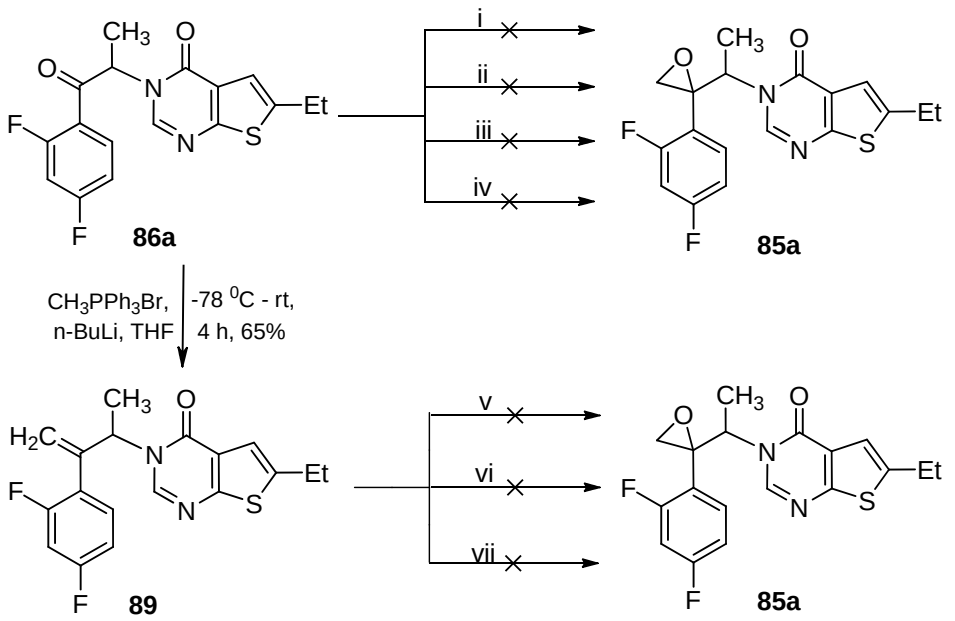
Accordingly, bromoketone **87** was synthesized[43d] from 1,3-difluorobenzene **40a** by its acylation using propionyl chloride in presence of $AlCl_3$ in DCM followed by the bromination of α-carbon using N-bromosuccinimide and AIBN in carbon tetrachloride as shown in Scheme 12.



Scheme 12. Reagents and conditions: i) Propionyl chloride, $AlCl_3$, DCM, 0 ^oC-rt, 8 h, 90 %; ii) NBS, AIBN, carbon tetrachloride, 77 ^oC, 12 h, 78 %; iii) Thienopyrimidinone, NaH, DMF, 0 ^oC-rt, 4 h, 73 %; iv) Trimethylsulphoxonium iodide, aq. KOH, cetrimide, DCM, 45 ^oC, 12 h.

Reaction of bromoketone **87** with thienopyrimidinone **78c** (synthesis of compound **78c** is shown in Scheme 4) in presence of sodium hydride in dimethyl formamide gave ketone **86a** in good yield (compounds **86b**, **86c**, **86d** and **86e** were synthesized using respective thienopyrimidinones under similar conditions). The ketone **86a** was subjected to Corey-Chaykovsky reaction[43e-f] conditions *i.e.* reaction with trimethylsulphoxonium iodide and aq potassium hydroxide but we failed to get oxirane **85a** under these conditions.

So as to achieve the synthesis of target molecule **84a**, synthesis of intermediate oxirane **85a** from ketone **86a** was important. Hence we tried a number of reaction conditions and reagents shown in Scheme 13 as follows.

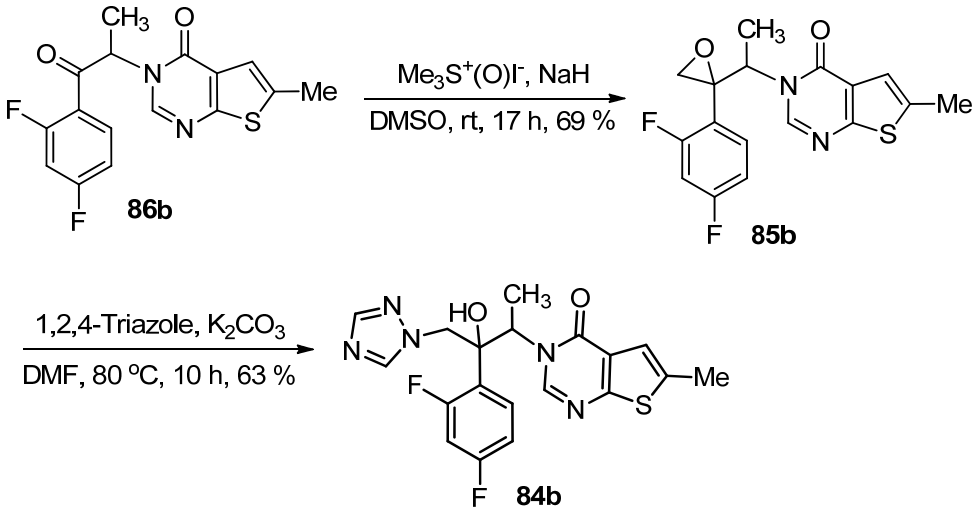


Scheme 13. Reagents and conditions: i) $Me_3S^+(O)I^-$, aq. KOH, cetrimide, DCM, 40 ^oC, 12 h; ii) $Me_3S^+(O)I^-$, NaH, DMF, 85 ^oC, 12 h; iii) $Me_3S^+I^-$, NaH, DMF, 85 ^oC, 12 h; iv) $Me_3S^+I^-$, aq. KOH, cetrimide, DCM, 40 ^oC, 12 h; v) mCPBA, DCM, rt, 12 h; vi) H_2O_2, NaOH, MeOH, H_2O, rt, 6 h; vii) Ag_2O, I_2, dioxane, rt, 7 h.

For the synthesis of oxirane **85a** from ketone **86a**, trimethylsulphoxonium iodide was used along with different bases such as potassium hydroxide, sodium hydride *etc* in different solvents such as dichloromethane, dimethylformamide *etc*. and also at varying temperatures, but none of the above reaction conditions resulted in product formation. Every reaction resulted in either recovery of starting material or a complex reaction mixture. Then we converted compound **86a** into olefin **89** using Wittig reaction. Two doublets at δ 5.59 and 5.68 in PMR spectrum confirmed the formation of olefin **89**. It was then subjected to epoxidation using different reagents like mCPBA, H_2O_2, Ag_2O in presence of I_2 *etc*. but none of these reagents converted olefin **89** into expected oxirane **85a**.

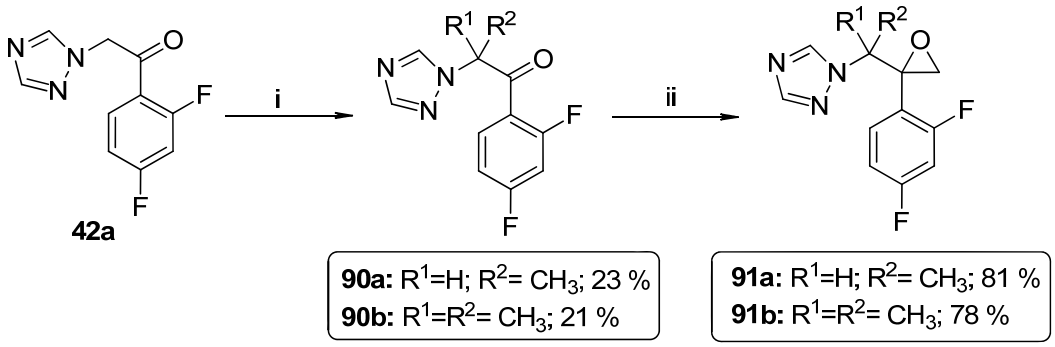
At last use of trimethylsulphoxonium iodide in presence of sodium hydride in DMSO at room temperature converted ketone **86b** into oxirane **85b** and this was confirmed by the disappearance of IR frequency above 1700 cm^{-1} and two protons were observed in the region δ 6.87-7.02 in PMR spectrum. Compound **85b** was then converted into **84b** by epoxide opening using 1,2,4-triazole in presence of potassium carbonate in DMF at 80 ^oC as shown in Scheme 14. In the PMR spectrum two doublets, each for one proton at δ 3.99 and

5.19 with coupling constant J = 14 Hz, indicated the product formation. Compound **84a** was synthesized using the same procedure without purification and characterization of intermediate oxirane **85a**. Intermediates **86c**, **86d** and **86e** under similar reaction conditions after two steps gave complex reaction mixture which on separation and purification with column chromatography gave respective thienopyrimidinone as the only isolated product.



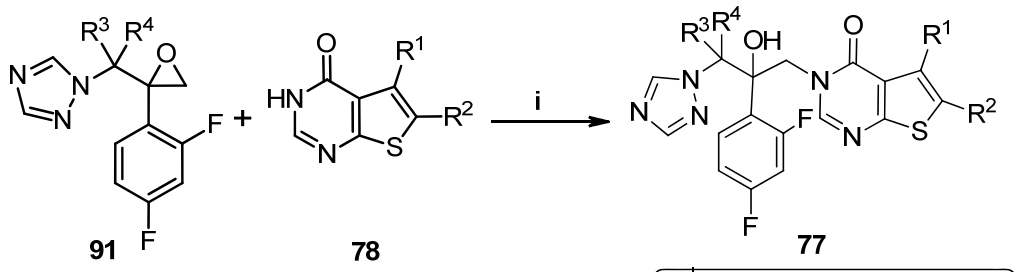
Scheme 14. Synthesis of fluconazole analogue **84b**

We then thought to synthesize target molecules **77** wherein methyl group would be present on carbon adjacent to triazole moiety and SAR studies can be carried out. So as to synthesize molecule **77**, it was necessary to synthesize the intermediate epoxide **91**. This was synthesized by a known literature procedure[43b,g,h] as shown in Scheme 15.



Scheme 15. Reagents and conditions: i) MeI, NaH, THF, rt, 11 h; ii) Trimethylsulphoxonium iodide, aq KOH, DCM, 45 °C, 12 h.

Synthesis of the triazole containing ketone **42a** was described in this chapter in Scheme 7. This ketone **42a** was then treated with me thyl iodide in THF in presence of sodium hydride to yield a mixture of products. The mixture was separated on column chromatography to afford substituted ketones **90a** and **90b** in 23 % and 21 % yields respectively (along with recovered mixture of **90a** and **90b**) and then separately treated with trimethylsulphoxonium iodide in presence of aq potassium hydroxide to yield the epoxides **91a** and **91b**.



77	R^1	R^2	R^3	R^4
a	H	CH_3	H	CH_3
b	H	CH_2CH_3	H	CH_3
c	H	$(CH_2)_2CH_3$	H	CH_3
d	H	$(CH_2)_4CH_3$	H	CH_3
e	H	$(CH_2)_5CH_3$	H	CH_3
f	H	$(CH_2)_8CH_3$	H	CH_3
g	$R^1=R^2=(CH_2)_3$		H	CH_3
h	H	CH_2CH_3	CH_3	CH_3
i	$R^1=R^2=(CH_2)_3$		CH_3	CH_3

Scheme 16. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 61.8-68.9 %.

The various thienopyrimidinones **78** were then reacted with the epoxide **91a** or **91b** in presence of potassium carbonate to afford the target molecules **77a-i** with monomethyl or dimethyl substitutions as shown in Scheme 16. The various compounds obtained as described above were screened for antifungal activity (Section 2 of this Chapter).

Resolution of 76e

Out of 42 compounds synthesized, a group of 5 compounds was chosen for the chiral separation. These five compounds **76d**, **76m**, **76t**, **76n** and **76e** were analysed by HPLC on chiral column to develop the conditions for preparative chiral HPLC in order to isolate the

enantiomers in pure form. Along with these five compounds epoxide **81a** was also resolved under following conditions:

HPLC Column	Chiralcel OD-H (250 X 4.6 mm) (DAICEL)
Mobile Phase	IPA: PE (40:60)
Wavelength	240 nm
Flow rate	0.5 ml/min (430 psi)

It was observed that all six compounds were resolved under these conditions. Since **76e** was observed to be the most active compound within this group (described in second section of this chapter), it was chosen for further study and for the separation of the R and S enantiomers from the racemic compound using preparative HPLC. Apart from compound **76e**, racemic epoxide **81a** was also separated on preparative HPLC to get R and S enantiomers of **81a**. Both of these compounds were separated on preparative HPLC under following conditions:

HPLC Column	Chiralcel-OD (250 X 4.6 mm) (DAICEL)
Mobile Phase	Ethanol: n-Hexane (15:85)
Wavelength	254 nm
Carried out by	Normal Phase

The chiral HPLC chromatograms for racemic **76e** and its (S) and (R) enantiomers are shown in Figure 26.

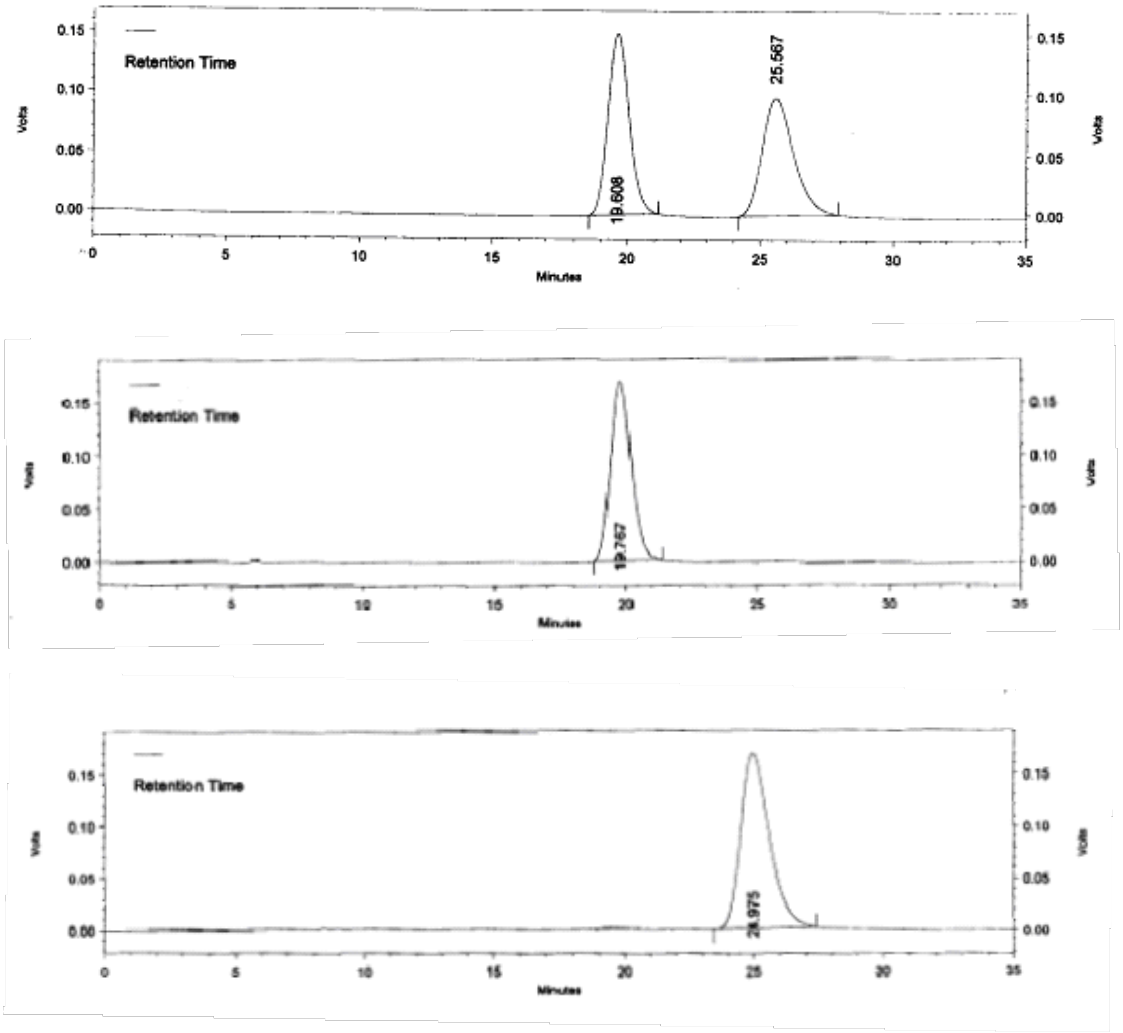


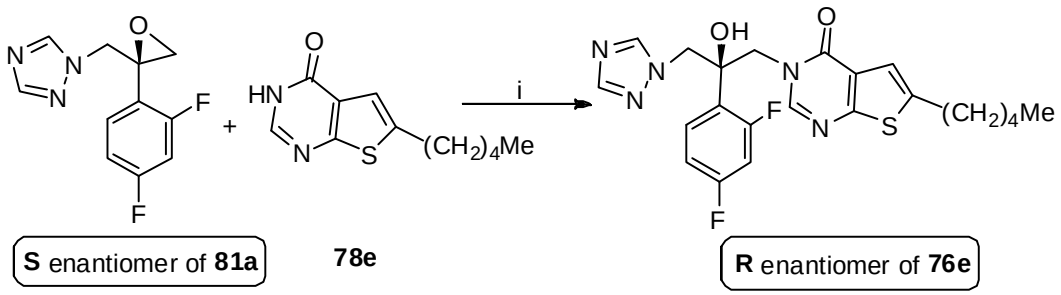
Figure 26. Resolution of **76e** using chiral HPLC

After resolution and separation of enantiomers of **81a** and **76e** was done, specific rotation of every compound was calculated from the observed rotations as shown in Table 1.

Table 1. Specific rotations of enantiomers of **81a** and **76e**

Comp No.	Peaks In Chromatogram	Retention Time (RT) min	Observed Rotation c = 10 mg/ml l = 0.5 dm	Specific Rotation $[\alpha]_D^{24} = \frac{\text{Obs. Rotation X 100}}{\text{Conc. X Length}}$
81a	A	14.518	-0.04	-8 ^{o} (C = 1.0, THF)
	B	15.200	+0.04	+8 ^{o} (C = 1.0, THF)
76e	A	19.608	-0.55	-110 ^{o} (C = 1.0, Chloroform)
	B	25.567	+0.55	+110 ^{o} (C = 1.0, Chloroform)

It is known in literature[47b] that "**S**" enantiomer of **81a** is having $[\alpha]_D$ = -8 ^{o}. When we treated **78e** with "**S**" enantiomer of **81a,** the product obtained had $[\alpha]_D$ = –110 ^{o} and should have R configuration as shown in Scheme 17.



Scheme 17. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 72 %.

Structure confirmation

All the compounds synthesized in reaction sequences in this section were fully characterized by PMR, CMR, IR and Mass spectroscopic methods and showed satisfactory spectral data described in experimental section.

It is possible that the thienopyrimidinones used in the present work may exist in two resonating forms as shown in Figure 27. Hence there was a possibility of epoxide opening by **N3** nitrogen or **N1** nitrogen from thienopyrimidinone, which might result into the formation of regioisomers.

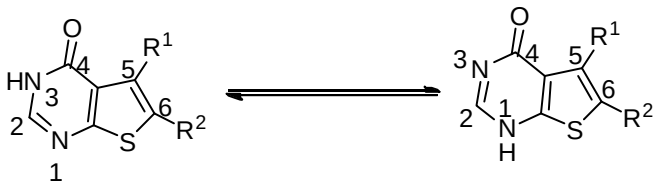


Figure 27. Resonating structures of thienopyrimidinone

So as to confirm the structure of one of the compounds from the series of **76a-t**, compound **76n** was recrystallised from hot ethanol. Using X-ray crystallography, the structure of **76n** was confirmed as shown in the ORTEP diagram in Figure 28.

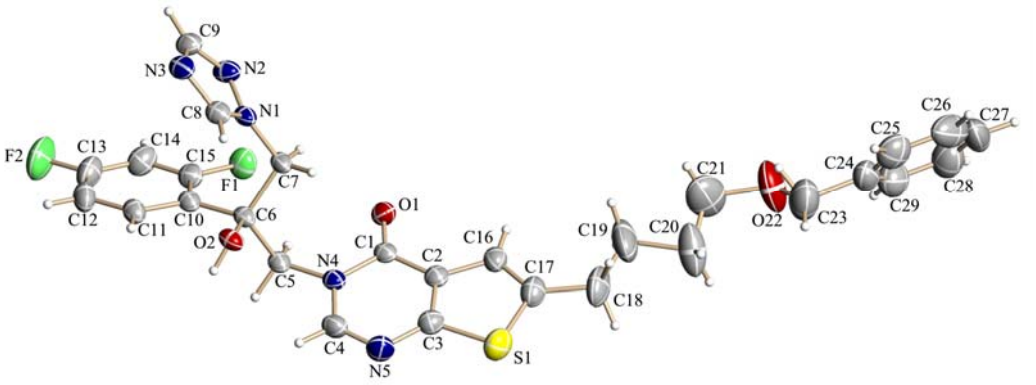


Figure 28. ORTEP diagram of compound **76n**

The data collection and refinement parameters are listed in the tables 2, 3 and 4. Confirmation of the structure was carried out to know the bonding between **C5** carbon and **N4** nitrogen of thienopyrimidinone.

Table 2. Crystal data and structure refinement for **76n**

Identification code	**76n**
Empirical formula	$C_{28}H_{27}F_2N_5O_3S$
Formula weight	551.61
Temperature	297(2) K
Wavelength	0.71073Å
Crystal system, space group	MONOCLINIC, P 21/c
Unit cell dimensions	a = 15.525(8) Å, α = 90º. b = 11.452(6) Å, β = 102.383(8)º. c = 15.658(8) Å, γ = 90º.
Volume	2719(2) $Å^3$
Z, Calculated density	4, 1.347 Mg/m^3
Absorption coefficient	0.172 mm^{-1}
F(000)	1152
Crystal size	0.78 x 0.58 x 0.18 mm
Theta range for data collection	2.22 to 25.00 º.
Limiting indices	-18<=h<=18, -13<=k<=13, -18<=l<=18
Reflections collected / unique	19092 / 4775 [R(int) = 0.0247]
Completeness to theta = 25.00	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9697 and 0.8775
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4775 / 16 / 389
Goodness-of-fit on F^2	1.022
Final R indices [I>2sigma(I)]	R1 = 0.0492, wR2 = 0.1295
R indices (all data)	R1 = 0.0583, wR2 = 0.1383
Largest diff. peak and hole	0.326 and -0.192 $eÅ^{-3}$

Table 3. Bond lengths [Å] and angles [º] for **76n**

S(1)-C(3)	1.729(2)
S(1)-C(17)	1.736(3)
O(1)-C(1)	1.224(2)
O(2)-C(6)	1.414(2)
F(1)-C(15)	1.364(3)
F(2)-C(13)	1.356(3)
N(1)-C(8)	1.324(2)
N(1)-N(2)	1.358(2)
N(1)-C(7)	1.459(2)
N(2)-C(9)	1.316(3)
N(3)-C(8)	1.320(3)

N(3)-C(9)	1.346(3)
N(4)-C(4)	1.367(3)
N(4)-C(1)	1.405(2)
N(4)-C(5)	1.462(3)
N(5)-C(4)	1.293(3)
N(5)-C(3)	1.365(3)
C(1)-C(2)	1.436(3)
C(2)-C(3)	1.368(3)
C(2)-C(16)	1.423(3)
C(5)-C(6)	1.550(3)
C(6)-C(10)	1.529(3)
C(6)-C(7)	1.540(3)
C(10)-C(15)	1.378(3)
C(10)-C(11)	1.394(3)
C(11)-C(12)	1.383(3)
C(12)-C(13)	1.361(4)
C(13)-C(14)	1.367(4)
C(14)-C(15)	1.372(4)
C(16)-C(17)	1.352(3)
C(17)-C(18)	1.507(4)
C(18)-C(19A	1.417(13)
C(18)-C(19)	1.554(10)
C(19)-C(20)	1.594(11)
C(20)-C(21)	1.285(13)
C(21)-O(22)	1.435(12)
O(22)-C(23)	1.313(10)
C(19A)-C(20A)	1.599(13)
C(20A)-C(21A)	1.374(12)
C(21A)-O(22A)	1.549(12)
O(22A)-C(23)	1.299(8)
C(23)-C(24)	1.547(5)
C(24)-C(25)	1.340(5)
C(24)-C(29)	1.346(5)
C(25)-C(26)	1.375(6)
C(26)-C(27)	1.375(7)
C(27)-C(28)	1.355(7)
C(28)-C(29)	1.354(5)
C(3)-S(1)-C(17)	91.39(12)
C(8)-N(1)-N(2)	109.06(16)
C(8)-N(1)-C(7)	129.82(17)
N(2)-N(1)-C(7)	120.19(15)
C(9)-N(2)-N(1)	102.59(17)
C(8)-N(3)-C(9)	102.41(17)
C(4)-N(4)-C(1)	122.01(18)
C(4)-N(4)-C(5)	119.68(17)
C(1)-N(4)-C(5)	118.03(16)
C(4)-N(5)-C(3)	113.62(19)
O(1)-C(1)-N(4)	120.59(18)

O(1)-C(1)-C(2)	126.67(18)
N(4)-C(1)-C(2)	112.74(17)
C(3)-C(2)-C(16)	112.9(2)
C(3)-C(2)-C(1)	119.30(19)
C(16)-C(2)-C(1)	127.76(19)
N(5)-C(3)-C(2)	126.1(2)
N(5)-C(3)-S(1)	122.70(17)
C(2)-C(3)-S(1)	111.19(17)
N(5)-C(4)-N(4)	126.2(2)
N(4)-C(5)-C(6)	114.08(15)
O(2)-C(6)-C(10)	111.45(15)
O(2)-C(6)-C(7)	104.58(15)
C(10)-C(6)-C(7)	111.32(16)
O(2)-C(6)-C(5)	110.06(15)
C(10)-C(6)-C(5)	108.40(15)
C(7)-C(6)-C(5)	111.03(15)
N(1)-C(7)-C(6)	110.54(15)
N(3)-C(8)-N(1)	111.12(19)
N(2)-C(9)-N(3)	114.8(2)
C(15)-C(10)-C(11)	115.9(2)
C(15)-C(10)-C(6)	122.54(18)
C(11)-C(10)-C(6)	121.53(18)
C(12)-C(11)-C(10)	121.7(2)
C(13)-C(12)-C(11)	118.7(2)
F(2)-C(13)-C(12)	119.3(3)
F(2)-C(13)-C(14)	118.1(3)
C(12)-C(13)-C(14)	122.6(2)
C(13)-C(14)-C(15)	116.9(3)
F(1)-C(15)-C(14)	116.5(2)
F(1)-C(15)-C(10)	119.2(2)
C(14)-C(15)-C(10)	124.2(2)
C(17)-C(16)-C(2)	112.8(2)
C(16)-C(17)-C(18)	128.4(3)
C(16)-C(17)-S(1)	111.72(19)
C(18)-C(17)-S(1)	119.9(2)
C(19A)-C(18)-C(17)	117.7(6)
C(17)-C(18)-C(19)	111.9(4)
C(18)-C(19)-C(20)	118.3(7)
C(21)-C(20)-C(19)	121.3(9)
C(20)-C(21)-O(22)	110.1(13)
C(23)-O(22)-C(21)	97.9(9)
C(18)-C(19A)-C(20A)	102.5(9)
C(21A)-C(20A)-C(19A)	91.8(10)
C(20A)-C(21A)-O(22A)	110.5(9)
C(23)-O(22A)-C(21A)	122.4(7)
O(22A)-C(23)-C(24)	108.5(5)
O(22)-C(23)-C(24)	113.0(6)
C(25)-C(24)-C(29)	119.4(3)

C(25)-C(24)-C(23)	118.0(4)
C(29)-C(24)-C(23)	122.5(4)
C(24)-C(25)-C(26)	121.5(4)
C(25)-C(26)-C(27)	118.2(4)
C(28)-C(27)-C(26)	119.9(4)
C(29)-C(28)-C(27)	120.0(4)
C(24)-C(29)-C(28)	120.9(4)

Symmetry transformations used to generate equivalent atoms:

Table 4. Torsion angles [º] for **76n**

C(8)-N(1)-N(2)-C(9)	0.3(2)
C(7)-N(1)-N(2)-C(9)	170.29(17)
C(4)-N(4)-C(1)-O(1)	176.39(18)
C(5)-N(4)-C(1)-O(1)	2.5(2)
C(4)-N(4)-C(1)-C(2)	-2.6(2)
C(5)-N(4)-C(1)-C(2)	-176.45(15)
O(1)-C(1)-C(2)-C(3)	-177.44(19)
N(4)-C(1)-C(2)-C(3)	1.5(3)
O(1)-C(1)-C(2)-C(16)	0.9(3)
N(4)-C(1)-C(2)-C(16)	179.81(18)
C(4)-N(5)-C(3)-C(2)	-0.8(3)
C(4)-N(5)-C(3)-S(1)	-179.33(18)
C(16)-C(2)-C(3)-N(5)	-178.4(2)
C(1)-C(2)-C(3)-N(5)	0.2(3)
C(16)-C(2)-C(3)-S(1)	0.3(2)
C(1)-C(2)-C(3)-S(1)	178.88(14)
C(17)-S(1)-C(3)-N(5)	178.9(2)
C(17)-S(1)-C(3)-C(2)	0.12(18)
C(3)-N(5)-C(4)-N(4)	-0.5(4)
C(1)-N(4)-C(4)-N(5)	2.3(3)
C(5)-N(4)-C(4)-N(5)	176.0(2)
C(4)-N(4)-C(5)-C(6)	88.1(2)
C(1)-N(4)-C(5)-C(6)	-97.86(19)
N(4)-C(5)-C(6)-O(2)	-59.1(2)
N(4)-C(5)-C(6)-C(10)	178.77(15)
N(4)-C(5)-C(6)-C(7)	56.2(2)
C(8)-N(1)-C(7)-C(6)	67.4(3)
N(2)-N(1)-C(7)-C(6)	-100.3(2)
O(2)-C(6)-C(7)-N(1)	-72.5(2)
C(10)-C(6)-C(7)-N(1)	48.0(2)
C(5)-C(6)-C(7)-N(1)	168.87(16)
C(9)-N(3)-C(8)-N(1)	0.2(2)

N(2)-N(1)-C(8)-N(3)	-0.3(2)
C(7)-N(1)-C(8)-N(3)	-169.04(18)
N(1)-N(2)-C(9)-N(3)	-0.2(2)
C(8)-N(3)-C(9)-N(2)	0.0(3)
O(2)-C(6)-C(10)-C(15)	176.75(17)
C(7)-C(6)-C(10)-C(15)	60.4(2)
C(5)-C(6)-C(10)-C(15)	-62.0(2)
O(2)-C(6)-C(10)-C(11)	-1.8(2)
C(7)-C(6)-C(10)-C(11)	-118.19(19)
C(5)-C(6)-C(10)-C(11)	119.42(19)
C(15)-C(10)-C(11)-C(12)	0.8(3)
C(6)-C(10)-C(11)-C(12)	179.52(19)
C(10)-C(11)-C(12)-C(13)	-0.7(4)
C(11)-C(12)-C(13)-F(2)	179.2(2)
C(11)-C(12)-C(13)-C(14)	0.2(4)
F(2)-C(13)-C(14)-C(15)	-178.9(2)
C(12)-C(13)-C(14)-C(15)	0.1(4)
C(13)-C(14)-C(15)-F(1)	-179.0(2)
C(13)-C(14)-C(15)-C(10)	0.1(4)
C(11)-C(10)-C(15)-F(1)	178.56(18)
C(6)-C(10)-C(15)-F(1)	-0.1(3)
C(11)-C(10)-C(15)-C(14)	-0.5(3)
C(6)-C(10)-C(15)-C(14)	-179.2(2)
C(3)-C(2)-C(16)-C(17)	-0.7(3)
C(1)-C(2)-C(16)-C(17)	-179.2(2)
C(2)-C(16)-C(17)-C(18)	-179.8(3)
C(2)-C(16)-C(17)-S(1)	0.8(3)
C(3)-S(1)-C(17)-C(16)	-0.5(2)
C(3)-S(1)-C(17)-C(18)	-179.9(2)
C(16)-C(17)-C(18)-C(19A)	3.8(8)
S(1)-C(17)-C(18)-C(19A)	-176.9(6)
C(16)-C(17)-C(18)-C(19)	-23.0(6)
S(1)-C(17)-C(18)-C(19)	156.3(4)
C(19A)-C(18)-C(19)-C(20)	75.1(19)
C(17)-C(18)-C(19)-C(20)	-176.0(6)
C(18)-C(19)-C(20)-C(21)	-55.4(15)
C(19)-C(20)-C(21)-O(22)	-177.0(9)
C(20)-C(21)-O(22)-C(23)	179.6(12)
C(17)-C(18)-C(19A)-C(20A)	179.9(6)
C(19)-C(18)-C(19A)-C(20A)	-98(2)
C(18)-C(19A)-C(20A)-C(21A)	-165.9(11)
C(19A)-C(20A)-C(21A)-O(22A)	-176.7(11)
C(20A)-C(21A)-O(22A)-C(23)	-44.7(19)
C(21A)-O(22A)-C(23)-O(22)	84.5(13)
C(21A)-O(22A)-C(23)-C(24)	-171.3(9)
C(21)-O(22)-C(23)-O(22A)	-71.7(10)
C(21)-O(22)-C(23)-C(24)	-164.5(8)
O(22A)-C(23)-C(24)-C(25)	139.3(7)

O(22)-C(23)-C(24)-C(25)	-174.8(6)
O(22A)-C(23)-C(24)-C(29)	-41.6(8)
O(22)-C(23)-C(24)-C(29)	4.2(8)
C(29)-C(24)-C(25)-C(26)	-1.5(5)
C(23)-C(24)-C(25)-C(26)	177.6(4)
C(24)-C(25)-C(26)-C(27)	1.0(6)
C(25)-C(26)-C(27)-C(28)	0.5(6)
C(26)-C(27)-C(28)-C(29)	-1.5(7)
C(25)-C(24)-C(29)-C(28)	0.5(6)
C(23)-C(24)-C(29)-C(28)	-178.6(4)
C(27)-C(28)-C(29)-C(24)	1.0(6)

Symmetry transformations used to generate equivalent atoms

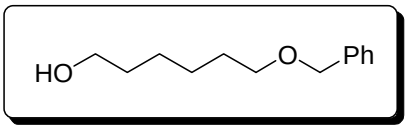
New chemical entities (NCEs) synthesized in this section were subjected for testing their antifungal activity and their structure activity relationship study is discussed in second section of this chapter.

1.1.5 Conclusion

Fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were synthesized by a short route with good to excellent yields. Variations in the side chains can be done by varying the starting materials, most of which are cheap and readily available or functional group modifications of some of the products. These reactions can be carried out conveniently on large scales. Apart from these syntheses, we have resolved the racemic mixture of most active fluconazole analogue **76e** into its R and S enantiomers to make them available for SAR studies. Further work to develop this molecule into an antifungal drug is in progress at FDC Ltd. Mumbai. This investigation is helpful to improve the design and development of more potent antifungal agents with superior antifungal activity and their synthesis by short chemical sequences.

1.1.6 Experimental Section

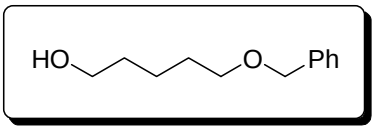
Preparation of 6-(benzyloxy)hexan-1-ol (82n)[48]



Sodium hydride (1.01 g, 0.0423 mol) was taken in two-necked round bottom flask equipped with guard tube; THF (20 ml) was added to it and cooled to -10 ^{o}C. 1,6-Hexanediol (**83n**) (5 g, 0.0423 mol) in THF (30 ml) was added over a period of 10 min and stirred at same temperature for 30 min. Benzyl bromide (7.23 g, 0.0423 mol) in THF (30 ml) was added slowly and whole reaction mixture was stirred at room temperature for 6 h. THF was evaporated under reduced pressure using rotary evaporator, diluted with water (200 ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography yielded pure product as colorless oil (6.12 g, 69.4 %).

Nature: Colorless oil; **Yield:** 69.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.35-1.44 (m, 4H), 1.55-1.68 (m, 4H), 3.47 (t, *J* = 6 Hz, 2H), 3.64 (t, *J* = 6 Hz, 2H), 4.50 (s, 2H), 7.33 (bs, 5H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 25.7 (2C), 27.3, 29.2, 62.7, 69.9, 72.9, 125.8, 127.5 (2C), 128.2 (3C); **MS** (ESI) *m*/*z***:** 231.0517 (M + Na); **Anal. Calcd for** $C_{13}H_{20}O_2$**:** C, 74.96; H, 9.68 %. **Found:** C, 75.21; H, 9.44 %.

5-(Benzyloxy)pentan-1-ol (82l)[49]

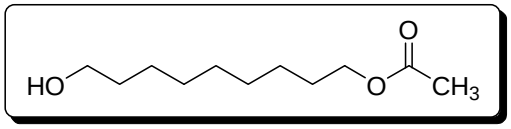


Compound **82l** was prepared from 1,5-pentanediol and benzyl bromide using the procedure given for the compound **82n**.

Nature: Colorless oil; **Yield:** 65.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.43-1.70 (m, 6H), 3.49 (t, *J* = 6 Hz, 2H), 3.65 (t, *J* = 6 Hz, 2H), 4.21 (s, 2H), 7.33 (bs, 5H); **^{13}C NMR** (50

MHz, $CDCl_3$)**:** δ 26.3, 27.1, 31.2, 59.5, 62.1, 71.8, 106.1, 127.3 (2C), 128.3 (3C); **MS** (ESI) *m*/*z***:** 217.1356 (M + Na); **Anal. Calcd for** $C_{12}H_{18}O_2$**:** C, 74.19; H, 9.34 %. **Found:** C, 74.51; H, 9.28 %.

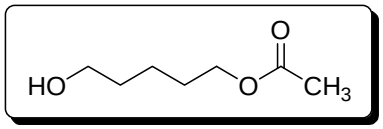
Preparation of 9-hydroxynonyl acetate (82o)[50]



1,9-Nonanediol (**83o**) (10 g, 0.0625 mol) in dichloromethane (100 ml) was taken in two necked round bottom flask equipped with guard tube and triethyl amine (6.31 g, 9 ml, 0.0625 mol) was added slowly at 0 ^oC and stirred for 30 min. Acetyl chloride (4.85 g, 4.4 ml, 0.0625 mol) was added over a period of 5 min and stirred at room temperature for 12 h. The reaction mixture was then diluted with water (200 ml), extracted with dichloromethane (3 X 100 ml), dried over Na_2SO_4 and concentrated under reduced pressure using rotary evaporator. Purification by column chromatography yielded pure product as colorless oil (8.4 g, 66.5 %).

Nature: Colorless oil; **Yield:** 66.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.13-1.35 (m, 6H), 1.43-1.68 (m, 8H), 2.29 (s, 3H), 3.49 (t, *J* = 6 Hz, 2H), 4.09 (t, *J* = 6 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.3, 26.1 (2C), 29.4 (4C), 31.8, 61.4, 64.1, 169.5; **MS** (ESI) *m*/*z***:** 225.0407 (M + Na); **Anal. Calcd for** $C_{11}H_{22}O_3$**:** C, 65.31; H, 10.96 %. **Found:** C, 65.57; H, 10.33 %.

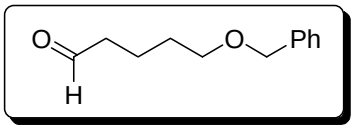
5-Hydroxypentyl acetate (82m)[51]



Compound **82m** was prepared by reacting 1,5-pentanediol with acetyl chloride using the procedure given for **82o**.

Nature: Colorless oil; **Yield:** 66.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.47-1.68 (m, 6H), 2.24 (s, 3H), 3.48 (t, *J* = 6 Hz, 2H), 4.21 (t, *J* = 6 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.5, 27.2 (2C), 31.1, 62.1, 64.4, 170.1; **MS** (ESI) *m*/*z***:** 169.0976 (M + Na); **Anal. Calcd for** $C_7H_{14}O_3$**:** C, 57.51; H, 9.65 %. **Found:** C, 57.73; H, 9.41 %.

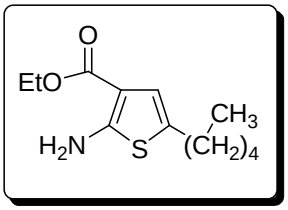
Preparation of 5-(benzyloxy)pentanal (80l)[49]



DMSO (5.62 g, 5.1 ml, 0.072 mol) in dichloromethane (120 ml) was taken in two necked round bottom flask cooled at -78 ^oC and equipped with guard tube; oxalyl chloride (4.54 g, 3 ml, 0.036 mol) was added slowly and stirred for 45 min. 5-(Benzyloxy)pentan-1-ol (**82n**) (5 g, 0.024 mol) in dichloromethane (30 ml) was added at -78 ^oC and stirred further for 45 min. Triethyl amine (14.54 g, 20.7 ml, 0.144 mol) was added to reaction mixture and the reaction mixture was allowed to warm slowly to 25 ^oC with constant stirring. It was then diluted with water (300 ml), extracted with dichloromethane (3 X 100 ml), dried over Na_2SO_4 and concentrated under reduced pressure using rotary evaporator to get colorless oil (8.4 g, 66.5 %) which was used as such for next reaction.

The aldehydes **80m, 80n** and **80o** were prepared from the corresponding alcohols **82m**, **82n** and **82o** using the procedure given for **80l** and all these compounds were used without purification for further reaction.

Preparation of ethyl 2-amino-5-n-pentyl-thiophene-3-carboxylate (79e)

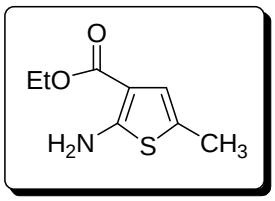


Heptanal (100 g, 122 ml, 0.88 mol) was taken in two necked round bottom flask equipped with reflux condenser and guard tube, DMF (700 ml) was added followed by sulphur powder (28.16 g, 0.88 mol), ethyl cyanoacetate (93.5 ml, 99.11 g, 0.88 mol) and triethyl amine (63 ml, 44.1 g, 0.44 mol). The reaction mixture was stirred at 50-55 ºC for 10 h, cooled, diluted with water (1 L), extracted with ethyl acetate (3 X 500 ml), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography yielded pure product as thick red oil (188.42 g, 89.3 %).

Nature: Thick red oil; **Yield:** 89.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.28-1.37 (m, 7H), 1.51-1.62 (m, 2H), 2.55 (t, *J* = 8 Hz, 2H), 4.24 (q, *J* = 6 Hz, 2H), 5.65 (bs, 2H), 6.61 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.7, 14.3, 22.2, 29.4, 30.5, 30.9, 59.3, 105.7, 121.1, 126.5, 161.3, 165.2; **IR** (Chloroform)**:** 3440, 3334, 1675 cm^{-1}; **MS** (ESI) *m*/*z***:** 280.1105 (M + K); **Anal. Calcd for** $C_{12}H_{19}NO_2S$**:** C, 59.72; H, 7.93; N, 5.80 %. **Found:** C, 60.05; H, 8.17; N, 5.42 %.

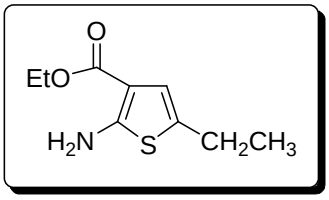
The amino esters **79b-79r** were prepared from the corresponding aldehydes or ketones **80** using the procedure given for the compound **79e**.

Ethyl 2-amino-5-methylthiophene-3-carboxylate (79b)[52]



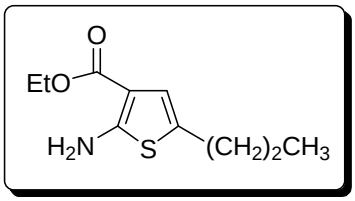
Nature: Dark brown oil; **Yield:** 85.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.28 (t, *J* = 8 Hz, 3H), 2.36 (s, 3H), 4.31 (q, *J* = 8 Hz, 2H), 5.43 (bs, 2H), 6.66 (s, 1H). **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.4, 15.3, 59.4, 124.9, 128.1, 138.2, 158.4, 164.5; **IR** (Chloroform)**:** 3449, 3294, 1672 cm^{-1}; **MS** (ESI) *m*/*z***:** 186.1607 (M +1). **Anal. Calcd for** $C_8H_{11}NO_2S$**:** C, 51.87; H, 5.99; N, 7.56 %. **Found:** C, 52.21; H, 6.14; N, 7.11 %.

Ethyl 2-amino-5-ethyl-thiophene-3-carboxylate (79c)[53,54]



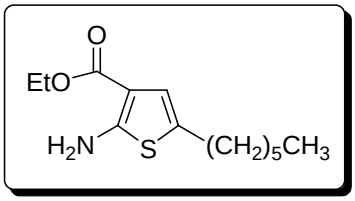
Nature: Reddish brown oil; **Yield:** 94.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.23 (t, *J* = 7 Hz, 3H), 1.34 (t, *J* = 7 Hz, 3H), 2.62 (q, *J* = 7 Hz, 2H), 4.26 (q, *J* = 7 Hz, 2H), 4.62 (bs, 2H), 6.64 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.1, 16.3, 22.8, 60.3, 125.3, 127.6, 136.8, 157.8, 163.7; **IR** (Chloroform)**:** 3483, 3350, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 238.9905 (M + K). **Anal. Calcd for** $C_9H_{13}NO_2S$**:** C, 54.25; H, 6.58; N, 7.03 %. **Found:** C, 54.63; H, 6.41; N, 7.37 %.

Ethyl 2-amino-5-n-propyl-thiophene-3-carboxylate (79d)



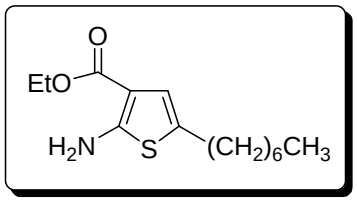
Nature: Reddish brown oil; **Yield:** 93.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.95 (t, *J* = 7 Hz, 3H), 1.34 (t, *J* = 7 Hz, 3H), 1.51-1.71 (m, 2H), 2.56 (t, *J* = 7 Hz, 2H), 4.26 (q, *J* = 7 Hz, 2H), 4.75 (bs, 2H), 6.64 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.4, 13.9, 24.2, 41.0, 58.3, 125.6, 127.6, 143.8, 158.4, 162.8; **IR** (Chloroform)**:** 3436, 3320, 1659 cm^{-1}; **MS** (ESI) *m*/*z***:** 214.2233 (M + 1). **Anal. Calcd for** $C_{10}H_{15}NO_2S$**:** C, 56.31; H, 7.09; N, 6.57 %. **Found:** C, 56.62; H, 7.31; N, 6.19 %.

Ethyl 2-amino-5-n-hexyl-thiophene-3-carboxylate (79f)



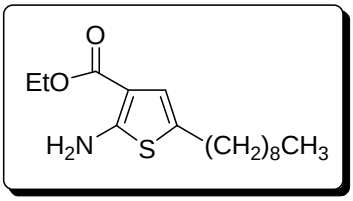
Nature: Reddish brown oil; **Yield:** 92.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.90 (t, *J* = 6 Hz, 3H), 1.22-1.42 (m, 9H), 1.49-1.63 (m, 2H), 2.57 (t, *J* = 7 Hz, 2H), 4.26 (q, *J* = 7 Hz, 2H), 5.79 (bs, 2H), 6.61 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.6, 14.1, 22.5, 28.7, 29.9 (2C), 37.9, 62.4, 125.7, 127.6, 143.2, 162.0, 165.3; **IR** (Chloroform)**:** 3440, 3335, 1677 cm^{-1}; **MS** (ESI) *m*/*z***:** 256.4565 (M + 1); **Anal. Calcd for** $C_{13}H_{21}NO_2S$**:** C, 61.14; H, 8.29; N, 5.48 %. **Found:** C, 61.47; H, 8.13; N, 5.23 %.

Ethyl 2-amino-5-heptyl-thiophene-3-carboxylate (79g)



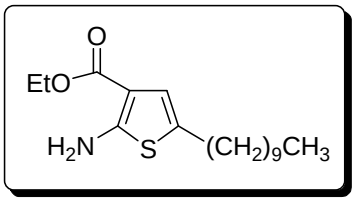
Nature: Red oil; **Yield:** 93.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.89 (t, *J* = 6 Hz, 3H), 1.30-1.38 (m, 11H), 1.51-1.62 (m, 2H), 2.58 (t, *J* = 8 Hz, 2H), 4.26 (q, *J* = 6 Hz, 2H), 5.49 (bs, 1H), 6.63 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.1, 14.5, 22.6, 28.9, 29.0, 29.7, 31.0, 31.7, 59.5, 106.6, 121.3, 127.1, 160.6, 165.2; **IR** (Chloroform)**:** 3440, 3358, 1674 cm^{-1}; **MS** (ESI) *m*/*z***:** 308.2732 (M + K); **Anal. Calcd for** $C_{14}H_{23}NO_2S$**:** C, 62.42; H, 8.61; N, 5.20 %. **Found:** C, 62.11; H, 8.87; N, 5.46 %.

Ethyl 2-amino-5-n-nonyl-thiophene-3-carboxylate (79h)



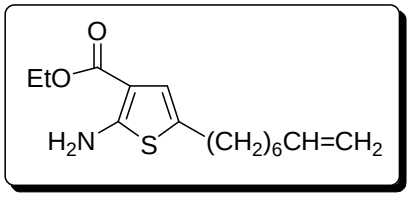
Nature: Brown oil; **Yield:** 90.0 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.26-1.35 (m, 15H), 1.48-1.63 (m, 2H), 2.54 (t, *J* = 6 Hz, 2H), 4.24 (q, *J* = 6 Hz, 2H), 5.78 (bs, 2H), 6.61 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 14.2, 22.4, 28.7, 29.0, 29.1, 29.3, 29.4, 30.8, 31.6, 59.2, 105.6, 121.0, 126.3, 161.3, 165.1; **IR** (Chloroform)**:** 3482, 3349, 1668 cm^{-1}; **MS** (ESI) *m*/*z***:** 298.1825 (M + 1); **Anal. Calcd for** $C_{16}H_{27}NO_2S$**:** C, 64.60; H, 9.15; N, 4.71 %. **Found:** C, 64.91; H, 8.74; N, 4.64 %.

Ethyl 2-amino-5-n-decyl-thiophene-3-carboxylate (79i)



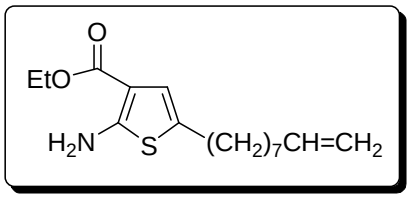
Nature: Brown semisolid; **Yield:** 89.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.25-1.37 (m, 17H), 1.50-1.65 (m, 2H), 2.56 (t, *J* = 6 Hz, 2H), 4.25 (q, *J* = 8 Hz, 2H), 5.40 (bs, 2H), 6.63 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 14.2, 22.4, 28.8, 29.1, 29.2, 29.3, 29.4, 29.5, 30.8, 31.7, 59.2, 105.7, 121.1, 126.4, 161.3, 165.2; **IR** (Chloroform)**:** 3481, 3347, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 350.3279 (M + K); **Anal. Calcd for** $C_{17}H_{29}NO_2S$**:** C, 65.55; H, 9.38; N, 4.50 %. **Found:** C, 65.84; H, 9.11; N, 4.61 %.

Ethyl 2-amino-5-(oct-7-en-1-yl)thiophene-3-carboxylate (79j)



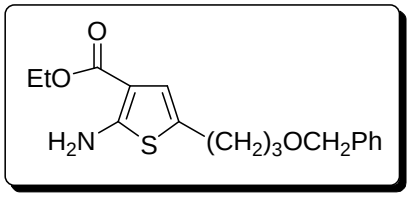
Nature: Red oil; **Yield:** 88.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.27-1.47 (m, 9H), 1.51-1.69 (m, 2H), 2.02-2.12 (m, 2H), 2.61 (t, *J* = 8 Hz, 2H), 4.29 (q, *J* = 8 Hz, 2H), 4.93-5.08 (m, 2H), 5.60 (bs, 2H), 5.74-5.94 (m, 1H), 6.68 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.3, 28.5 (3C), 29.4, 30.8, 33.5, 59.3, 105.9, 114.0, 121.1, 126.6, 138.8, 161.1, 165.2; **IR** (Chloroform)**:** 3461, 3342, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 282.2072 (M + 1), 304.1728 (M + Na); **Anal. Calcd for** $C_{15}H_{23}NO_2S$**:** C, 64.02; H, 8.24; N, 4.98 %. **Found:** C, 63.76; H, 8.48; N, 5.31 %.

Ethyl 2-amino-5-(non-8-en-1-yl)thiophene-3-carboxylate (79k)



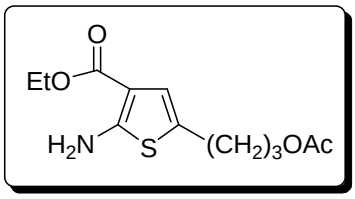
Nature: Red oil; **Yield:** 86.9 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.31-1.38 (m, 11H), 1.51-1.63 (m, 2H), 1.99-2.09 (m, 2H), 2.59 (t, *J* = 6 Hz, 2H), 4.27 (q, *J* = 6 Hz, 2H), 4.90-5.04 (m, 2H), 5.17 (bs, 2H), 5.71-5.91 (m, 1H), 6.66 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.2, 28.7 (3C), 30.1 (2C), 30.6, 33.8, 58.7, 106.4, 115.7, 120.2, 128.1, 140.1, 162.9, 165.6; **IR** (Chloroform)**:** 3438, 3309, 1680 cm^{-1}; **MS** (ESI) *m*/*z***:** 297.2689 (M + 2), 354.4633 (M + K) ; **Anal. Calcd for** $C_{16}H_{25}NO_2S$**:** C, 65.05; H, 8.53; N, 4.74 %. **Found:** C, 65.49; H, 8.27; N, 4.51 %.

Ethyl 2-amino-5-(3-benzyloxypropyl)-thiophene-3-carboxylate (79l)



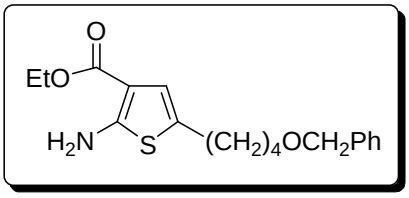
Nature: Dark brown oil; **Yield:** 72.1 % (Over two steps); **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.34 (t, *J* = 8 Hz, 3H), 1.80-1.96 (m, 2H), 2.70 (t, *J* = 7 Hz, 2H), 3.51 (t, *J* = 7 Hz, 2H), 4.25 (q, *J* = 7 Hz, 2H), 4.51 (s, 2H), 5.80 (bs, 2H), 6.64 (s, 1H), 7.34 (bs, 5H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 14.5, 26.2, 31.0, 59.3, 68.7, 72.8, 105.9, 121.8, 125.2, 127.2 (3C), 128.2 (2C), 138.0, 161.4, 165.1; **IR** (Chloroform)**:** 3447, 3343, 1674, 1096 cm^{-1}; **MS** (ESI) *m*/*z***:** 320.13 (M + 1), 342.12 (M + Na), 359.19 (M + K); **Anal. Calcd for** $C_{17}H_{21}NO_3S$**:** C, 63.92; H, 6.63; N, 4.39 %. **Found:** C, 64.26; H, 6.87; N, 4.24 %.

Ethyl 5-(3-acetoxypropyl)-2-aminothiophene-3-carboxylate (79m)



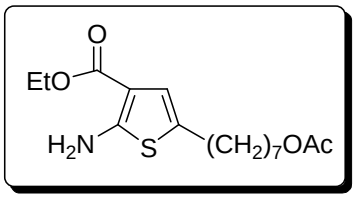
Nature: Brown oil; **Yield:** 68.5 % (Over two steps); **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.28 (t, *J* = 8 Hz, 3H), 1.85-1.97 (m, 2H), 2.09 (s, 3H), 2.77 (t, *J* = 7 Hz, 2H), 3.86 (t, *J* = 7 Hz, 2H), 4.19 (q, *J* = 7 Hz, 2H), 5.60 (bs, 2H), 6.66 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 14.3, 20.9, 22.3, 34.6, 60.7, 63.8, 122.8, 126.3, 142.3, 160.6, 164.8, 169.4; **IR** (Chloroform)**:** 3440, 3335, 1735, 1674 cm^{-1}; **MS** (ESI) *m*/*z***:** 271.64 (M + 1); **Anal. Calcd for** $C_{12}H_{17}NO_4S$**:** C, 53.12; H, 6.32; N, 5.16 %. **Found:** C, 53.46; H, 6.59; N, 5.31 %.

Ethyl 2-amino-5-(4-benzyloxybutyl)-thiophene-3-carboxylate (79n)



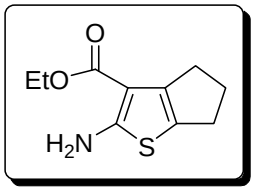
Nature: Dark brown oil; **Yield:** 73.6 % (Over two steps); **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.33 (t, *J* = 8 Hz, 3H), 1.60-1.75 (m, 4H), 2.59 (bt, *J* = 6 Hz, 2H), 3.48 (bt, *J* = 6 Hz, 2H), 4.25 (q, *J* = 8 Hz, 2H), 4.50 (s, 2H), 5.77 (bs, 2H), 6.62 (s, 1H), 7.33 (bs, 5H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.6, 27.7, 29.0, 29.5, 59.4, 69.9, 72.8, 105.8, 121.7, 125.9, 127.6 (3C), 128.3 (2C), 138.5, 161.7, 165.3; **IR** (Chloroform)**:** 3432, 3340, 1678, 1095 cm^{-1}; **MS** (ESI) *m*/*z***:** 334.16 (M + 1), 356.13 (M + Na), 372.12 (M + K); **Anal. Calcd for** $C_{18}H_{23}NO_3S$**:** C, 64.84; H, 6.95; N, 4.20 %. **Found:** C, 65.14; H, 7.13; N, 4.23 %.

Ethyl 2-amino-5(7-acetoxy-n-heptyl)-thiophene-3-carboxylate (79o)



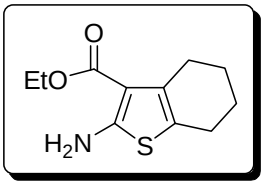
Nature: Brown oil; **Yield:** 71.8 % (Over two steps); **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.22-1.32 (m, 9H), 1.42-1.67 (m, 4H), 2.00 (s, 3H), 2.52 (t, *J* = 8 Hz, 2H), 4.00 (t, *J* = 8 Hz, 2H), 4.20 (q, *J* = 8 Hz, 2H), 5.57 (bs, 2H), 6.57 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 14.2, 20.7, 25.5, 28.3, 28.5, 28.6, 29.3, 30.6, 59.2, 64.3, 105.9, 112.9, 121.2, 126.3, 165.0, 170.9; **IR** (Chloroform)**:** 3444, 3337, 1738, 1674 cm^{-1}; **MS** (ESI) *m*/*z***:** 350.5910 (M + Na), 366.6381 (M + K); **Anal. Calcd for** $C_{16}H_{25}NO_4S$**:** C, 58.69; H, 7.70; N, 4.28 %. **Found:** C, 58.94; H, 7.36; N, 3.88 %.

Ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylate (79p)[54]



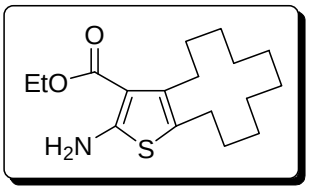
Nature: Dark brown oil; **Yield:** 84.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.32 (t, *J* = 7 Hz, 3H), 2.22-2.38 (m, 2H), 2.65-2.90 (m, 4H), 4.24 (q, *J* = 7 Hz, 2H), 5.85 (bs, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.4, 25.5, 26.7, 29.3, 59.1, 104.8, 127.7, 139.2, 161.3, 165.6; **IR** (Chloroform)**:** 3476, 3341, 1666 cm^{-1}; **MS** (ESI) *m*/*z***:** 250.0978 (M + K); **Anal. Calcd for** $C_{10}H_{13}NO_2S$**:** C, 56.85; H, 6.20; N, 6.63 %. **Found:** C, 57.12; H, 6.45; N, 6.46 %.

Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (79q)[54, 56a]



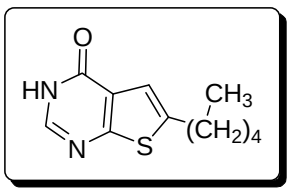
Nature: Brown semisolid; **Yield:** 93.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.27 (t, *J* = 7 Hz, 3H), 1.62-1.78 (m, 4H), 2.35-2.50 (m, 2H), 2.54-2.70 (m, 2H), 4.19 (q, *J* = 7 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 14.4, 22.8, 23.2, 24.5, 26.9, 59.3, 105.7, 117.5, 132.4, 161.7, 166.1; **IR** (Chloroform)**:** 3483, 3345, 1659 cm^{-1}; **MS** (ESI) *m*/*z***:** 226.1385 (M + 1); **Anal. Calcd for** $C_{11}H_{15}NO_2S$**:** C, 58.64; H, 6.71; N, 6.22 %. **Found:** C, 58.79; H, 6.56; N, 6.38 %.

Ethyl 2-amino-4,5,6,7,8,9,10,11,12,13-decahydro-[1]cyclododeca[b]thiophene-3-carboxylate (79r)



Nature: Red semisolid; **Yield:** 88.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.21-1.50 (m, 15H), 1.55-1.71 (m, 4H), 2.54-2.71 (m, 4H), 4.27 (q, *J* = 7 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.2, 22.1, 23.0, 24.2, 24.9 (2C), 25.2, 25.3, 25.4 (2C), 28.4, 29.5, 105.5, 121.9, 134.9, 162.3, 165.9; **IR** (Chloroform)**:** 3417, 3387, 1665 cm^{-1}; **MS** (ESI) *m*/*z***:** 332.0684 (M + Na); **Anal. Calcd for** $C_{17}H_{27}NO_2S$**:** C, 65.98; H, 8.79; N, 4.53 %. **Found:** C, 66.34; H, 8.76; N, 4.19 %.

Preparation of 6-(n-pentyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78e)



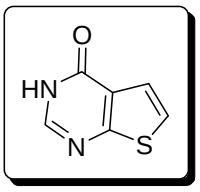
A mixture of ethyl 2-amino-5-n-pentylthiophene-3-carboxylate (100 g, 0.41 mol), ammonium acetate (31.95 g, 0.41 mol) and formamide (330 ml, 8.3 mol) was taken in a two necked round bottom flask equipped with reflux condenser and a guard tube and stirred at 145-150 °C for 10 h. The reaction mixture was then cooled to room temperature, diluted with chilled water (800 ml) and stirred for 30 min to obtain a white solid product which was filtered, washed with water (3 X 300 ml) followed by washing with 20 % ethyl acetate in pet-ether (3 x 20 ml) to obtain 6-(n-pentyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one as off-white solid (74.89 g, 81.3 %).

Nature: Off-white solid; **MP:** 170-173 ^oC; **Yield:** 81.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.89 (t, *J* = 6 Hz, 3H), 1.31-1.38 (m, 4H), 1.65-1.75 (m, 2H), 2.84 (t, *J* = 8 Hz, 2H), 7.13 (s,

1H), 8.07 (s, 1H), 12.9 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 22.2, 30.4, 30.5, 31.0, 117.3, 124.7, 142.9, 144.9, 159.8, 164.2; **IR** (Chloroform)**:** 3434, 1663 cm^{-1}; **MS** (ESI) *m*/*z***:** 223.3916 (M + 1); **Anal. Calcd for** $C_{11}H_{14}N_2OS$**:** C, 59.43; H, 6.35; N, 12.60 %. **Found:** C, 59.21; H, 6.26; N, 12.89 %.

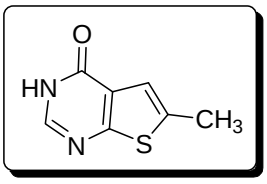
Compounds **78a-78r** were prepared by reacting the corresponding substituted 2-amino-thiophene-3-carboxylates **79a-79r** with formamide in presence of ammonium acetate using the procedure given for the preparation of **78e**.

Thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78a)[55]



Nature: White solid; **MP:** 220-222 ^{o}C; **Yield:** 91.8 %; **^{1}H NMR** (200 MHz, DMSO-d_6)**:** δ 7.41 (d, *J* = 6 Hz, 1H), 7.60 (d, *J* = 6 Hz, 1H), 8.15 (s, 1H), 12.54 (bs, 1H); **^{13}C NMR** (50 MHz, DMSO-d_6)**:** δ 121.9, 124.1, 124.9, 145.8, 157.8, 164.5; **IR** (Chloroform)**:** 3387, 1676 cm^{-1}; **MS** (ESI) *m*/*z***:** 153.2947 (M + 1); **Anal. Calcd for** $C_6H_4N_2OS$**:** C, 47.36; H, 2.65; N, 18.41 %. **Found:** C, 47.82; H, 2.48; N, 18.31 %.

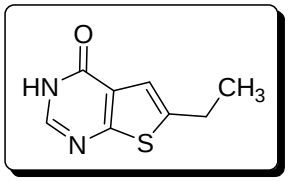
6-Methyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78b)



Nature: Light pink solid; **MP:** 269-271 ^{o}C; **Yield:** 78.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.35 (s, 3H), 7.07 (s, 1H), 7.94 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.6, 117.3, 122.1, 140.1, 144.8, 156.8, 168.5; **IR** (Chloroform)**:** 3391, 1674 cm^{-1}; **MS** (ESI) *m*/*z***:** 167.1805 (M

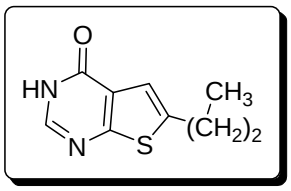
+ 1); **Anal. Calcd for** $C_7H_6N_2OS$**:** C, 50.59; H, 3.64; N, 16.86 %. **Found:** C, 50.74; H, 3.37; N, 17.11 %.

6-Ethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78c)



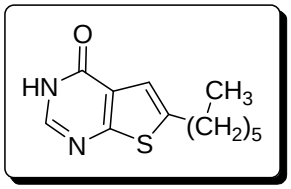
Nature: Off-white solid; **MP:** 193 ^oC; **Yield:** 85.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.34 (t, *J* = 8 Hz, 3H), 2.88 (q, *J* = 8 Hz, 2H), 7.17 (s, 1H), 8.02 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.5, 22.8, 113.8, 116.7, 138.4, 143.9, 157.3, 167.2; **IR** (Chloroform)**:** 3319, 1661 cm^{-1}; **MS** (ESI) *m*/*z***:** 181.05 (M + 1), 203.03 (M + Na), 219.07 (M + K); **Anal. Calcd for** $C_8H_8N_2OS$**:** C, 53.31; H, 4.47; N, 15.54 %. **Found:** C, 53.64; H, 4.11; N, 15.69 %.

6-(n-Propyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78d)



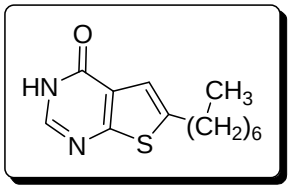
Nature: Pale yellow solid; **MP:** 198-199 ^oC; **Yield:** 88.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.99 (t, *J* = 6 Hz, 3H), 1.67-1.83 (m, 2H), 2.83 (t, *J* = 6 Hz, 2H), 7.15 (s, 1H), 8.05 (s, 1H), 12.87 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 21.4, 31.8, 116.7, 123.5, 144.1, 146.3, 157.9, 163.8; **IR** (Chloroform)**:** 3336, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:** 195.4443 (M + 1); **Anal. Calcd for** $C_9H_{10}N_2OS$**:** C, 55.65; H, 5.19; N, 14.42 %. **Found:** C, 55.78; H, 5.32; N, 14.67 %.

6-(n-Hexyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78f)



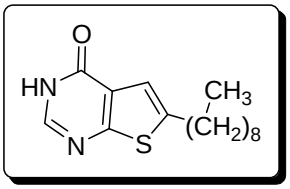
Nature: Dark brown solid; **MP:** 181 ^{o}C; **Yield:** 84.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.90 (t, *J* = 6 Hz, 3H), 1.21-1.50 (m, 6H), 1.61-1.86 (m, 2H), 2.87 (t, *J* = 8 Hz, 2H), 7.15 (s, 1H), 8.03 (s, 1H), 12.80 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 22.5, 28.8, 30.5, 30.9, 31.6, 117.4, 124.8, 143.0, 145.0, 159.6, 163.9; **IR** (Chloroform)**:** 3388, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 237.1471 (M + 1); **Anal. Calcd for** $C_{12}H_{16}N_2OS$**:** C, 60.99; H, 6.82; N, 11.85 %. **Found:** C, 61.36; H, 6.66; N, 12.07 %.

6-(n-Heptyl)- thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78g)



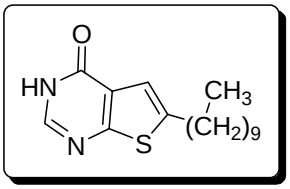
Nature: Off-white solid; **MP:** 170-171 ^{o}C; **Yield:** 91.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.89 (t, *J* = 6 Hz, 3H), 1.26-1.37 (m, 8H), 1.69-1.76 (m, 2H), 2.87 (t, *J* = 6 Hz, 2H), 7.17 (s, 1H), 8.05 (s, 1H), 12.82 (bs, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$)**:** δ 13.7, 22.2, 28.6 (2C), 30.3, 30.6, 31.3, 117.1, 124.5, 142.6, 144.6, 159.6, 164.1; **IR** (Chloroform)**:** 3319, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 273.2424 (M + Na); **Anal. Calcd for** $C_{13}H_{18}N_2OS$**:** C, 62.37; H, 7.25; N, 11.19 %. **Found:** C, 62.76; H, 7.49; N, 10.76 %.

6-(n-Nonyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78h)



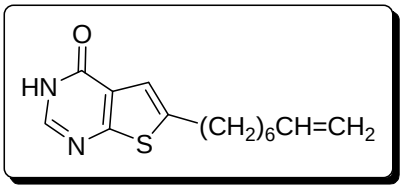
Nature: White solid; **MP:** 167 ^{o}C; **Yield:** 88.0 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.91 (t, *J* = 6 Hz, 3H), 1.14-1.55 (m, 12H), 1.65-1.92 (m, 2H), 2.89 (t, *J* = 6 Hz, 2H), 7.19 (s, 1H), 8.09 (s, 1H), 12.96 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.3, 21.9, 28.2, 28.5 (2C), 28.7, 29.9, 30.3, 31.1, 116.8, 124.2, 142.4, 144.4, 159.0, 163.4; **IR** (Chloroform)**:** 3319, 1663 cm^{-1}; **MS** (ESI) *m*/*z***:** 301.2506 (M + Na); **Anal. Calcd for** $C_{15}H_{22}N_2OS$**:** C, 64.71; H, 7.96; N, 10.06 %. **Found:** C, 64.51; H, 8.21; N, 10.33 %.

6-(n-Decyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78i)



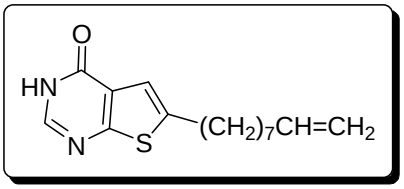
Nature: Off-white solid; **MP:** 158 ^{o}C; **Yield:** 85.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.24-1.35 (m, 14H), 1.66-1.80 (m, 2H), 2.86 (t, *J* = 6 Hz, 2H), 7.17 (s, 1H), 8.15 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.3, 23.9, 30.2, 30.5 (2C), 30.7, 30.8, 31.8, 32.2, 33.1, 118.7, 142.3, 145.7, 146.3, 158.7, 163.7; **IR** (Chloroform)**:** 3386, 1677 cm^{-1}; **MS** (ESI) *m*/*z***:** 315.4655 (M + Na); **Anal. Calcd for** $C_{16}H_{24}N_2OS$**:** C, 65.71; H, 8.27; N, 9.58 %. **Found:** C, 65.36; H, 7.93; N, 9.91 %.

6-(Oct-7-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78j)



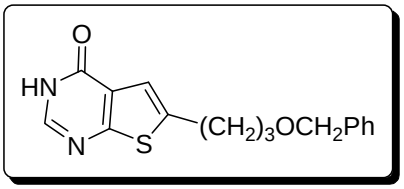
Nature: White solid; **MP:** 156 ^{o}C; **Yield:** 78.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.24-1.45 (m, 6H), 1.65-1.76 (m, 2H), 2.00-2.11 (m, 2H), 2.86 (t, *J* = 6 Hz, 2H), 4.90-5.09 (m, 2H), 5.70-5.90 (m, 1H), 7.15 (s, 1H), 8.05 (s, 1H), 12.79 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 28.5 (3C), 30.4, 30.7, 33.5, 114.1, 117.3, 124.7, 138.7, 143.0, 144.6, 159.4, 164.1; **IR** (Chloroform)**:** 3382, 1666 cm^{-1}; **MS** (ESI) *m*/*z***:** 263.3785 (M + 1), 285.3744 (M + Na), 301.4319 (M + K); **Anal. Calcd for** $C_{14}H_{18}N_2OS$**:** C, 64.09; H, 6.91; N, 10.68 %. **Found:** C, 63.78; H, 7.27; N, 10.34 %.

6-(Non-8-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78k)



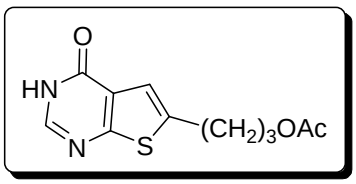
Nature: Grey solid; **MP:** 130-132 ^{o}C; **Yield:** 74.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.24-1.36 (m, 8H), 1.66-1.80 (m, 2H), 1.99-2.10 (m, 2H), 2.87 (t, *J* = 6 Hz, 2H), 4.91-5.04 (m, 2H), 5.71-5.90 (m, 1H), 7.17 (s, 1H), 8.16 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 28.6, 28.7, 28.8, 28.9, 30.4, 30.8, 33.5, 114.0, 117.3, 124.7, 138.9, 142.9, 144.7, 159.5, 164.1; **IR** (Chloroform)**:** 3375, 1665 cm^{-1}; **MS** (ESI) *m*/*z***:** 299.3179 (M + Na), 315.3037 (M + K); **Anal. Calcd for** $C_{15}H_{20}N_2OS$**:** C, 65.18; H, 7.29; N, 10.14 %. **Found:** C, 64.84; H, 7.51; N, 9.78 %.

6-(3-Benzyloxypropyl)- thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78l)



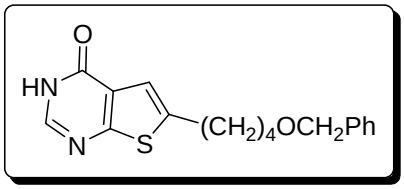
Nature: Pale yellow solid; **MP:** 161-163 ^{o}C; **Yield:** 78.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.91-2.12 (m, 2H), 3.01 (t, *J* = 6 Hz, 2H), 3.53 (t, *J* = 6 Hz, 2H), 4.51 (s, 2H), 7.17 (s, 1H), 7.33 (bs, 5H), 8.03 (s, 1H), 12.84 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$, CCl_4)**:** δ 27.3, 31.0, 68.6, 73.0, 117.9, 124.8, 127.6 (3C), 128.4 (2C), 138.2, 143.0, 143.8, 159.8, 164.6; **IR** (Chloroform)**:** 3318, 1665, 1100 cm^{-1}; **MS** (ESI) *m*/*z***:** 301.11 (M + 1), 323.09 (M + Na), 339.06 (M + K); **Anal. Calcd for** $C_{16}H_{16}N_2O_2S$**:** C, 63.98; H, 5.37; N, 9.33; %. **Found:** C, 64.30; H, 5.49; N, 9.08 %.

6-(3-Acetoxypropyl)- thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78m)



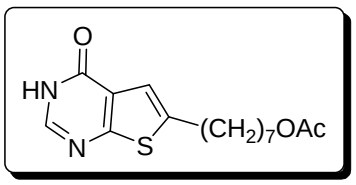
Nature: Off-white solid; **MP:** 158 ^{o}C; **Yield:** 80.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.08 (s, 3H), 2.93-3.03 (m, 2H), 4.14 (t, *J* = 6 Hz, 2H), 4.25 (t, *J* = 6 Hz, 2H), 7.21 (s, 1H), 8.08 (s, 1H), 9.67 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 20.4, 22.1, 32.4, 63.1, 123.1, 134.1, 144.1, 146.4, 157.6, 162.8, 172.2; **IR** (Chloroform)**:** 3319, 1725, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 254.0803 (M + 2); **Anal. Calcd for** $C_{11}H_{12}N_2O_3S$**:** C, 52.37; H, 4.79; N, 11.10 %. **Found:** C, 52.66; H, 5.00; N, 10.74 %.

6-(4-Benzyloxybutyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78n)



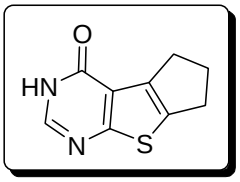
Nature: Grey solid; **MP:** 117-119 ^{o}C; **Yield:** 87.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.61-1.92 (m, 4H), 2.89 (t, *J* = 6 Hz, 2H), 3.51 (t, *J* = 6 Hz, 2H), 4.51 (s, 2H), 7.17 (s, 1H), 7.34 (bs, 5H), 8.02 (s, 1H), 12.54 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 27.7, 29.0, 30.4, 69.7, 72.9, 117.8, 124.9, 127.6 (3C), 128.4 (2C), 138.4, 143.0, 144.4, 159.5, 164.4; **IR** (Chloroform)**:** 3318, 1665, 1101 cm^{-1}; **MS** (ESI) *m*/*z***:** 315.12 (M + 1), 337.10 (M + Na), 353.07 (M + K); **Anal. Calcd for** $C_{17}H_{18}N_2O_2S$**:** C, 64.94; H, 5.77; N, 8.91 %. **Found:** C, 64.79; H, 5.67; N, 9.06 %.

6-(7-Acetoxyheptyl)- thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78o)



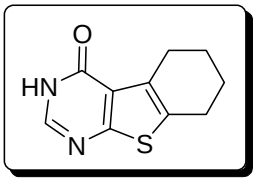
Nature: White solid; **MP:** 135-137 ^{o}C; **Yield:** 84.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.27-1.32 (m, 6H), 1.51-1.65 (m, 4H), 1.97 (s, 3H), 2.79 (t, *J* = 8 Hz, 2H), 3.98 (t, *J* = 7 Hz, 2H), 7.08 (s, 1H), 8.03 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 20.8, 25.6, 28.3, 28.4, 28.6, 30.3, 30.7, 64.3, 117.4, 124.7, 143.0, 144.5, 159.5, 164.1, 171.0; **IR** (Chloroform)**:** 3319, 1727, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 347.4229 (M + K); **Anal. Calcd for** $C_{15}H_{20}N_2O_3S$**:** C, 58.42; H, 6.54; N, 9.08 %. **Found:** C, 58.91; H, 6.44; N, 8.82 %.

3,5,6,7-Tetrahydrocyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78p)



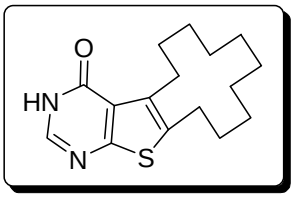
Nature: Grey solid; **MP:** 249 ^{o}C; **Yield:** 81.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.21-1.50 (m, 2H), 2.12-2.68 (m, 2H), 2.73-2.87 (m, 2H), 8.02 (s, 1H), 12.80 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 25.6, 28.9, 31.4, 117.7, 125.1, 139.1, 145.9, 158.6, 165.4; **IR** (Chloroform)**:** 3369, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 215.1919 (M + Na); **Anal. Calcd for** $C_9H_8N_2OS$**:** C, 56.23; H, 4.19; N, 14.57 %. **Found:** C, 56.49; H, 4.55; N, 14.78 %.

5,6,7,8-Tetrahydrobenzothieno[2,3-*d*]pyrimidin-4(3*H*)-one (78q)[56]



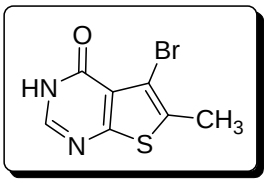
Nature: Yellow solid; **MP:** 253 ^{o}C; **Yield:** 89.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4 + DMSO-d_6)**:** δ 1.36-1.52 (m, 4H), 2.31-2.40 (m, 2H), 2.51-2.62 (m, 2H), 7.45 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4 + DMSO-d_6)**:** δ 22.2, 22.9, 25.0, 25.7, 123.3, 131.3, 132.8, 144.1, 158.5, 163.0; **IR** (Chloroform)**:** 3271, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 229.2190 (M + Na); **Anal. Calcd for** $C_{10}H_{10}N_2OS$**:** C, 58.23; H, 4.89; N, 13.58 %. **Found:** C, 57.88; H, 5.21; N, 13.33 %.

5,6,7,8,9,10,11,12,13,14-Decahydrocyclododeca[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (78r)



Nature: White solid; **MP:** 258-259 ^{o}C; **Yield:** 75.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.21-1.58 (m, 12H), 1.68-2.01 (m, 4H), 2.79-2.99 (m, 4H), 7.98 (s, 1H), 12.08 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 25.8 (2C), 26.3 (2C), 26.4 (2C), 28.3 (2C), 31.1 (2C), 122.4, 135.4, 135.7, 142.2, 157.5, 159.8; **IR** (Chloroform)**:** 3316, 1663 cm^{-1}; **MS** (ESI) *m*/*z***:** 292.1834 (M + 2); **Anal. Calcd for** $C_{16}H_{22}N_2OS$**:** C, 66.17; H, 7.64; N, 9.65 %. **Found:** C, 66.55; H, 7.41; N, 9.81 %.

Preparation of 5-bromo-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (78aa)



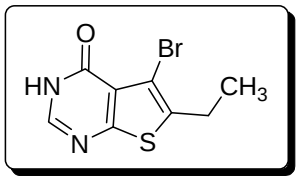
6-Methyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5 g, 0.0301 mol) was taken in two necked round bottom flask with guard tube, acetic acid (25 ml) was added, stirred for 10 min and bromine (1.8 ml, 0.0331 mol) in acetic acid (18 ml) was added drop wise. After complete addition, whole reaction mixture was stirred at 60 ºC for 5 h. Reaction mixture was then cooled to room temperature and diluted with water. The solid obtained was filtered and dried to obtain 5-bromo-6-methyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one as a white solid (4.0 g, 54.2 %).

Nature: White solid; **MP:** 256 ^{o}C; **Yield:** 54.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 2.33 (s, 3H), 7.77 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 13.9, 102.8, 130.8, 143.9, 156.0, 161.0, 178.8; **IR** (Chloroform)**:** 3375, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:**

266.2892 and 268.2869 (M + Na), 281.2457 and 283.2837 (M + K); **Anal. Calcd for** $C_7H_5BrN_2OS$**:** C, 34.30; H, 2.06; Br, 32.60; N, 11.43 %. **Found:** C, 34.55; H, 1.73; Br, 32.31; N, 11.67 %.

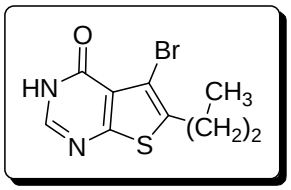
Compounds **78ab, 78ac** and **78ad** were prepared by bromination of the corresponding thienopyrimidinones **78c**, **78d** and **78f** using the procedure given for the compound **78aa**.

5-Bromo-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (78ab)



Nature: Grey solid; **MP:** 242 ^oC; **Yield:** 58.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.20 (t, *J* = 8 Hz, 3H), 2.77 (q, *J* = 8 Hz, 2H), 7.81 (s, 1H), 12.16 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 13.8, 22.1, 101.8, 121.2, 138.3, 144.1, 156.4, 161.2; **IR** (Chloroform)**:** 3393, 1657 cm^{-1}; **MS** (ESI) *m*/*z***:** 280.3729 and 282.3782 (M + Na); **Anal. Calcd for** $C_8H_7BrN_2OS$**:** C, 37.08; H, 2.72; Br, 30.84; N, 10.81 %. **Found:** C, 37.16; H, 2.46; Br, 31.08; N, 10.99 %.

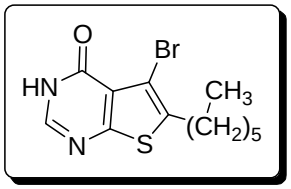
5-Bromo-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (78ac)



Nature: Pale yellow solid; **MP:** 205 ^oC; **Yield:** 53.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.03 (t, *J* = 6 Hz, 3H), 1.71-1.77 (m, 2H), 2.87 (t, *J* = 6 Hz, 2H), 8.13 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.5, 23.5, 31.3, 138.9, 143.9, 148.1, 149.0, 153.0, 158.5; **IR** (Chloroform)**:** 3400, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 272.2528 and 274.2715 (M+); **Anal. Calcd**

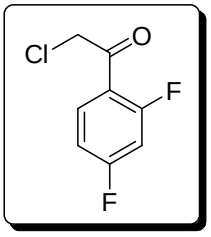
for $C_9H_9BrN_2OS$**:** C, 39.57; H, 3.32; Br, 29.25; N, 10.26 %. **Found:** C, 39.77; H, 3.04; Br, 29.55; N, 9.89 %.

5-Bromo-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (78ad)



Nature: Off-white solid; **MP:** 147-148 ^oC; **Yield:** 55.0 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.90 (t, *J* = 6 Hz, 3H), 1.28-1.45 (m, 6H), 1.63-1.78 (m, 2H), 2.88 (t, *J* = 8 Hz, 2H), 8.12 (s, 1H), 12.47 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 22.4, 28.6, 29.3, 30.0, 31.3, 102.9, 121.6, 139.1, 144.1, 158.5, 162.5; **IR** (Chloroform)**:** 3370, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:** 314.9961 and 316.9981 (M + 1); **Anal. Calcd for** $C_{12}H_{15}BrN_2OS$**:** C, 45.72; H, 4.80; Br, 25.35; N, 8.89 %. **Found:** C, 46.11; H, 5.07; Br, 25.14; N, 8.64 %.

Preparation of 2-chloro-1-(2,4-difluorophenyl)ethanone (41a)[13]



To a solution of 1,3-difluorobenzene (5.7 g, 50 mmol) in dichloromethane (30 ml), anhydrous aluminum chloride (7.98 g, 60 mmol) was added at 25-30 °C and stirred for 30 min. The reaction mixture was then cooled to 0 °C and chloroacetyl chloride (6.21 g, 54 mmol) in DCM (15 ml) was added over a period of 30 min at 0-10 °C. The reaction mixture was stirred at 25-30 °C for 7 h and diluted with the DCM (30 ml) and poured into chilled water (150 ml). The product was extracted with DCM (2 X 50 ml) and the combined organic layer was washed with water (2 X 20 ml), brine (20 ml) and dried over anhydrous Na_2SO_4.

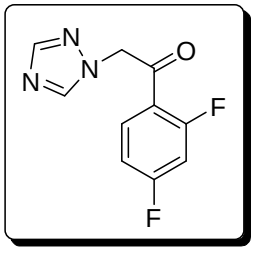
The solvent was concentrated under reduced pressure to yield the product **41a** (7.60 g, 80 %).

Caution: Product **41a** is highly lachrymatory and skin irritating hence reaction must be carried out under vacuum hood and use of hand gloves is essential during reaction work-up.

Nature: Yellow solid; **MP:** 47.5 ºC; **Yield:** 80 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.70 (d, *J* = 2 Hz, 2H), 6.86-7.06 (m, 2H), 7.96-8.08 (m, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 49.7 (d), 104.5 (t), 112.5 (d), 119.2 (d), 133.0 (d), 161.62 (dd), 166.7 (dd), 187.4; **Anal. Calcd for** $C_8H_5F_2N_3O$**:** C, 50.42; H, 2.64; Cl, 18.60; F, 19.94 %. **Found:** C, 50.59; H, 2.46; Cl, 18.29; F, 20.16 %.

Compounds **41b, 41c** and **41d** were prepared from flurobenzene, bromobenzene and dichlorobenzene using the procedure given for the compound **41a**. All these compounds were lachrymatory and skin irritating, hence reactions were monitored using thin layer chromatography (TLC) and without purification they were used for further reactions.

Preparation of 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone (42a)[13, 43c]



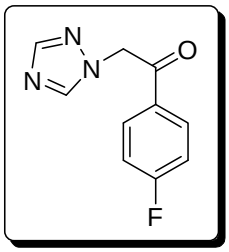
2-Chloro-1-(2,4-difluorophenyl)ethanone **41a** (9.05 g, 47.5 mmol), 1*H*-1,2,4-triazole (3.93 g, 57.01 mmol) and potassium carbonate (7.88 g, 57.00 mmol) were taken in round bottom flask, equipped with a condenser and guard tube, containing ethyl acetate (50 ml) and the reaction mixture was refluxed for 10 h. It was then cooled to room temperature, diluted with water (100 ml) and extracted with ethyl acetate (2 X 50 ml). The combined organic layer was washed with water (2 X 20 ml), brine (20 ml) and dried over anhydrous Na_2SO_4. The

solvent was concentrated under reduced pressure on rotary evaporator. Purification by column chromatography yielded pure product as white solid (7.30 g, 69 %).

Nature: White solid; **MP:** 105 ºC; **Yield:** 69 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 5.59 (d, *J* = 2 Hz, 2H), 6.93-7.10 (m, 2H), 7.99-8.11 (m, 1H), 8.21 (bs, 2H); **^{13}C NMR** ($CDCl_3$, 50 MHz)**:** δ 58.3 (d), 104.8 (t), 112.9 (d), 118.9 (d), 132.9 (d), 144.7, 151.7, 162.1 (dd), 168.7 (dd), 187.4; **IR** (Chloroform)**:** 1688 cm^{-1}; **Anal. Calcd for** $C_{10}H_7F_2N_3O$**:** C, 53.82; H, 3.16; N, 18.83 %; **Found:** C, 54.10; H, 3.02; N, 18.71 %.

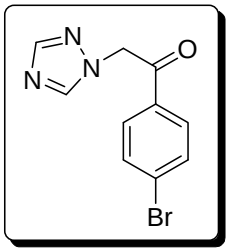
Compounds **42b, 42c** and **42d** were prepared by reacting **41b**, **41c** and **41d** with 1,2,4-triazole using the procedure given for the compound **42a**.

1-(4-Fluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone (42b)[43c]



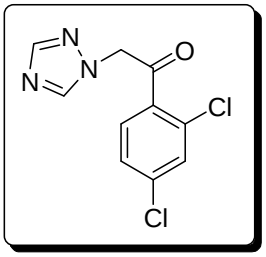
Nature: White solid; **MP:** 76 ºC; **Yield:** 68 % (over two steps); **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 6.10 (s, 2H), 7.48-7.52 (m, 2H), 8.05 (s, 1H), 8.12-8.22 (m, 2H), 8.41 (s, 1H).

1-(4-Bromophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone (42c)[43c]



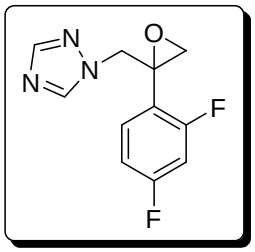
Nature: Off-white solid; **MP:** 172-174 ºC; **Yield:** 63 % (over two steps); **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 5.98 (s, 2H), 7.79 (d, *J* = 8 Hz, 2H), 7.98 (d, *J* = 8 Hz, 2H), 8.10 (s, 1H), 8.48 (s, 1H).

1-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone (42d)[43c]



Nature: White solid; **MP:** 116-117 ºC; **Yield:** 71 % (over two steps); **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 5.85 (s, 2H), 7.59-7.65 (m, 1H), 7.82 (d, *J* = 2 Hz, 1H), 8.02 (d, *J* = 6 Hz, 1H), 7.89 (s, 1H), 8.67 (s, 1H).

Preparation of 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (81a)[43c]



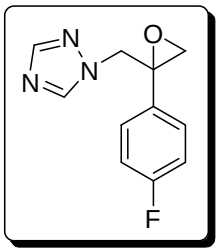
A mixture of 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazolyl)-ethanone **42a** (15.00 g, 0.067 mol), trimethylsulfoxonium iodide (22.20 g, 0.1 mol), and cetrimide (0.245 g, 0.00067 mol) in dichloromethane (150 ml) was stirred at room temperature for 10 min. Then a solution of KOH (9.42 g, 0.168 mol) in water (20 ml) was added to it. This mixture was refluxed at 40-45 ^oC for 12 h, cooled to room temperature and diluted with water (60 ml). The two layers were separated, the aqueous layer was extracted with dichloromethane (3 X 150 ml) and the combined organic extracts were dried over Na_2SO_4. After the solvent was concentrated *in*

vacuo, the residue was subjected to chromatography on silica gel to afford pure 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **81a** (13.55 g, 85 %).

Nature: Colourless thick liquid, **Yield:** 85 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.85 (d, *J* = 6 Hz, 1H), 2.95 (d, *J* = 6 Hz, 1H), 4.50 (d, *J* = 16 Hz, 1H), 4.80 (d, *J* = 16 Hz, 1H), 6.76-6.89 (m, 2H), 7.12-7.26 (m, 1H), 7.83 (s, 1H), 8.07 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 52.1, 53.5, 56.2, 104.0 (t), 111.7 (d), 119.5 (d), 129.54 (t), 144.0, 151.8, 159.3 (dd), 164.3 (dd).

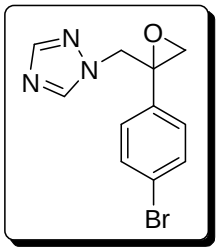
Compounds **81b, 81c** and **81d** were prepared from **42b**, **42c** and **42d** using the procedure given for the epoxide **81a**.

1-[(2-(4-Fluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]-triazole (81b)[43c]



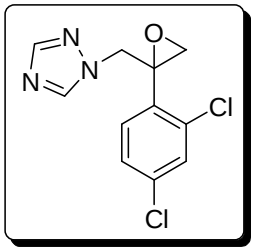
Nature: Red thick liquid, **Yield:** 81 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.87 (d, *J* = 6 Hz, 1H), 3.10 (d, *J* = 6 Hz, 1H), 4.66 (d, *J* = 16 Hz, 1H), 4.97 (d, *J* = 16 Hz, 1H), 7.15-7.24 (m, 2H), 7.39-7.48 (m, 2H), 7.98 (s, 1H), 8.39 (s, 1H).

1-[(2-(4-Bromophenyl)oxiranylmethyl]-1*H*-[1,2,4]-triazole (81c)[43c]



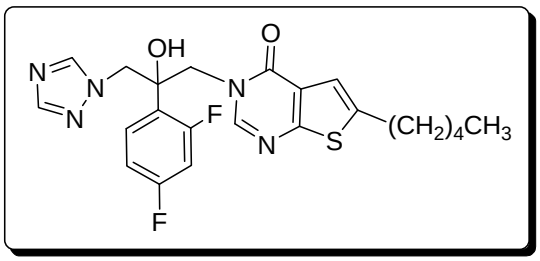
Nature: Red thick liquid, **Yield:** 84 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 2.81 (d, *J* = 6 Hz, 1H), 2.87 (d, *J* = 6 Hz, 1H), 4.59 (d, *J* = 14 Hz, 1H), 4.80 (d, *J* = 14 Hz, 1H), 7.20 (d, *J* = 8 Hz, 2H), 7.47 (d, *J* = 8 Hz, 2H), 7.89 (s, 1H), 8.08 (s, 1H).

1-[2-(2,4-Dichlorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (81d)[43c]



Nature: Red thick liquid, **Yield:** 91 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 2.92 (d, *J* = 4 Hz, 1H), 3.01 (d, *J* = 4 Hz, 1H), 4.53 (d, *J* = 14 Hz, 1H), 4.90 (d, *J* = 14 Hz, 1H), 7.16-7.46 (m, 3H), 7.92 (s, 1H), 8.13 (s, 1H).

Preparation of 3-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-6-n-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76e)



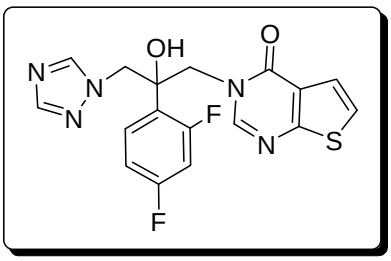
6-(n-Pentyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **78e** (46 g, 0.21 mol) was taken in two necked round bottom flask equipped with reflux condenser and guard tube. Ethyl acetate (250 ml) was added followed by the addition of flame dried potassium carbonate (28.6 g, 0.42 mol) and tetrabutylammonium bromide (66.93 g, 0.21 mol). The mixture was stirred at room temperature for 1 h and 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **81a** (49.1 g, 0.21mol) in ethyl acetate (200 ml) was added. The mixture was stirred under reflux

for 12 h, cooled, diluted with water (500 ml), extracted with ethyl acetate (3 X 300 ml), dried over Na_2SO_4 and concentrated. Purification by recrystallization from ethyl acetate afforded the pure product (71.12 g, 73.8 %).

Nature: Pale yellow solid; **MP:** 152 ^oC; **Yield:** 73.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.90 (t, *J* = 6 Hz, 3H), 1.27-1.42 (m, 4H), 1.64-1.76 (m, 2H), 2.82 (t, *J* = 8 Hz, 2H), 4.21 (d, *J* = 15 Hz, 1H), 4.52 (d, *J* = 15 Hz, 1H), 4.71 (d, *J* = 15 Hz, 1H), 4.78 (d, *J* = 15 Hz, 1H), 6.24 (s, 1H), 6.76-6.88 (m, 2H), 7.06 (s, 1H), 7.50-7.64 (m, 1H), 7.81 (s, 1H), 7.90 (s, 1H), 8.09 (s, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$ + CCl_4)**:** δ 13.8, 22.2, 30.5, 30.6, 31.0, 53.3, 55.4, 75.9, 104.2 (t), 112.1 (d), 117.9, 122.6, 123.8, 130.1, 144.6, 145.3, 146.0, 151.5, 158.7, 159.8 (dd), 161.7 (dd), 162.9; **IR** (Chloroform)**:** 3372, 1698 cm^{-1}; **MS** (ESI) *m*/*z***:** 482.5296 (M + Na); **Anal. Calcd for** $C_{22}H_{23}F_2N_5O_2S$**:** C, 57.50; H, 5.05; F, 8.27; N, 15.24 %. **Found:** C, 57.84; H, 4.77; F, 8.39; N, 15.06 %.

The fluconazole analogues **76a-76r** and **76aa-76ad** were prepared from the corresponding epoxides and thienopyrimidinones using the procedure given for the compound **76e**.

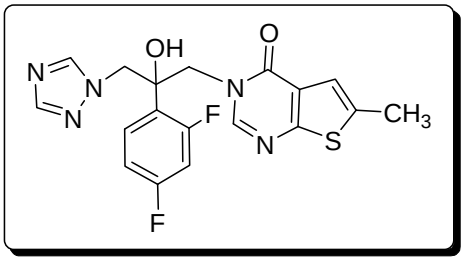
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76a)



Nature: White solid; **MP:** 91 ^oC; **Yield:** 57.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.30 (d, *J* = 14 Hz, 1H), 4.71 (d, *J* = 14 Hz, 1H), 4.80 (d, *J* = 14 Hz, 1H), 4.95 (d, *J* = 14 Hz, 1H), 6.74-6.90 (m, 2H), 7.27 (d, *J* = 6 Hz, 1H), 7.41 (d, *J* = 6 Hz, 1H), 7.44-7.55 (m, 1H), 7.99 (s, 1H), 8.05 (s, 1H), 8.72 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 52.9, 55.2, 76.0, 104.3 (t), 112.1 (d), 121.8, 123.6, 124.4, 129.9 (d), 144.6, 146.9 (2C), 151.6, 155.0 (dd), 159.1, 162.8 (dd), 164.0; **IR** (Chloroform)**:** 3317, 1666 cm^{-1}; **MS** (ESI) *m*/*z***:** 390.0604 (M + 1), 412.0347

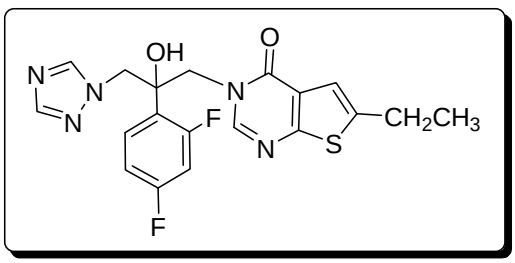
(M + Na); **Anal. Calcd for** $C_{17}H_{13}F_2N_5O_2S$**:** C, 52.44; H, 3.37; F, 9.76; N, 17.99 %. **Found:** C, 52.67; H, 3.12; F, 9.91; N, 17.74 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6 methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76b)



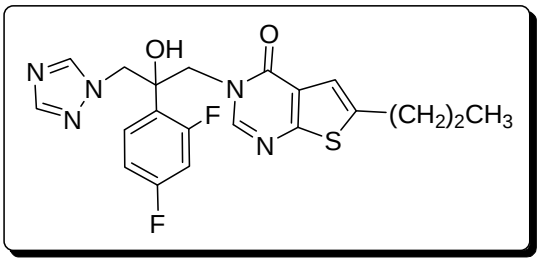
Nature: White solid; **MP:** 172 ^oC; **Yield:** 72 %; **^{1}H NMR** (300 MHz, $CDCl_3$)**:** δ 2.51 (s, 3H), 4.23 (d, *J* = 12 Hz, 1H), 4.53 (d, *J* = 12 Hz, 1H), 4.69 (d, *J* = 12 Hz, 1H), 4.82 (d, *J* = 12 Hz, 1H), 6.22 (bs, 1H), 6.71-6.85 (m, 2H), 7.04 (s, 1H), 7.50-7.67 (m, 1H), 7.83 (s, 1H), 7.90 (s, 1H), 8.10 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.5, 53.2, 55.8, 77.1, 104.3 (t), 112.1 (d), 121.4, 123.7, 124.4, 130.0 (d), 144.3, 146.8 (2C), 152.4, 156.2 (dd), 158.8, 162.9 (dd), 163.8; **IR** (Chloroform)**:** 3408, 1660 cm^{-1}; **MS** (ESI) *m*/*z***:** 442.4805 (M + K); **Anal. Calcd for** $C_{18}H_{15}F_2N_5O_2S$**:** C, 53.59; H, 3.75; F, 9.42; N, 17.36 %. **Found:** C, 53.67; H, 3.81; F, 9.31; N, 17.22 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76c)



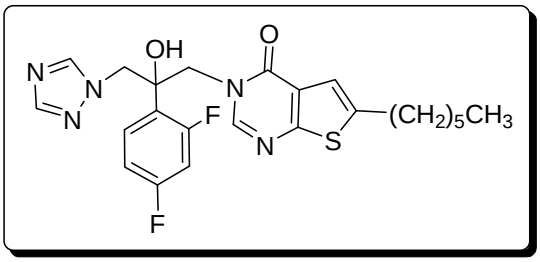
Nature: White solid; **MP:** 108 ^{o}C; **Yield:** 68.9 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.35 (t, *J* = 7 Hz, 3H), 2.87 (q, *J* = 7 Hz, 2H), 4.27 (d, *J* = 14 Hz, 1H), 4.60 (d, *J* = 14Hz, 1H), 4.72 (d, *J* = 14 Hz, 1H), 4.83 (d, *J* = 14Hz, 1H), 6.26 (bs, 1H), 6.77-6.91 (m, 2H), 7.08 (s, 1H), 7.48-7.61 (m, 1H), 7.89 (s, 1H), 7.92 (s, 1H), 8.31 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.0, 23.9, 53.4, 55.5, 75.9, 104.3 (t), 112.1 (d), 117.1, 121.6 (d), 123.8, 130.1 (d), 144.7, 146.1, 146.9, 151.5, 158.4 (dd), 158.9, 162.8, 163.6 (dd); **IR** (Chloroform)**:** 3380, 1657 cm^{-1}; **MS** (ESI) *m*/*z***:** 418.12 (M + 1), 440.10 (M + Na), 456.09 (M + K); **Anal. Calcd for** $C_{19}H_{17}F_2N_5O_2S$**:** C, 54.67; H, 4.10; F, 9.10; N, 16.78 %. **Found:** C, 54.44; H, 4.08; F, 9.20; N, 16.95 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76d)



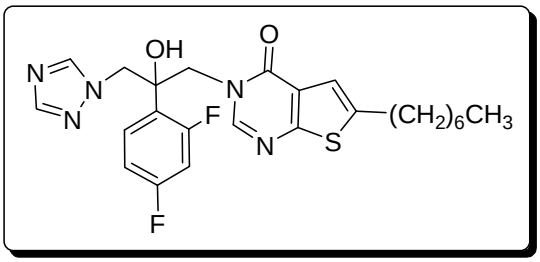
Nature: Off-white solid; **MP:** 94 ^{o}C; **Yield:** 74.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.98 (t, *J* = 8 Hz, 3H), 1.63-1.80 (m, 2H), 2.79 (t, *J* = 8 Hz, 2H), 4.25 (d, *J* = 14 Hz, 1H), 4.58 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.82 (d, *J* = 14 Hz, 1H), 6.30 (bs, 1H), 6.74-6.90 (m, 2H), 7.07 (s, 1H), 7.47-7.60 (m, 1H), 7.87 (s, 1H), 7.92 (s, 1H), 8.24 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.5, 24.2, 32.6, 53.2, 56.0, 75.8, 104.3 (t), 112.2 (d), 118.0, 122.0 (d), 123.9, 130.1(d), 144.3, 145.3, 146.1, 149.7, 158.0 (dd), 158.8, 162.8, 163.3 (dd); **IR** (Chloroform)**:** 3395, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:** 432.3541 (M + 1); **Anal. Calcd for** $C_{20}H_{19}F_2N_5O_2S$**:** C, 55.67; H, 4.44; F, 8.81; N, 16.23 %. **Found:** C, 55.41; H, 4.52; F, 8.93; N, 16.10 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76f)



Nature: Pale brown solid; **MP:** 90 ^{o}C; **Yield:** 75.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.21-1.48 (m, 6H), 1.61-1.76 (m, 2H), 2.82 (t, *J* = 8 Hz, 2H), 4.22 (d, *J* = 14 Hz, 1H), 4.52 (d, *J* = 14 Hz, 1H), 4.72 (d, *J* = 14 Hz, 1H), 4.80 (d, *J* = 14 Hz, 1H), 6.24 (s, 1H), 6.75-6.89 (m, 2H), 7.07 (s, 1H), 7.48-7.62 (m, 1H), 7.83 (s, 1H), 7.91 (s, 1H), 8.10 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 13.9, 22.4, 28.5, 30.5, 30.8, 31.3, 53.3, 55.4, 75.9, 104.2 (t), 112.1 (d), 117.8, 122.4, 123.8, 130.1, 144.6, 145.4, 146.0, 151.5, 158.4 (dd), 158.7, 162.8, 163.1 (dd); **IR** (Chloroform)**:** 3312, 1656 cm^{-1}; **MS** (ESI) *m*/*z***:** 475.3563 (M + 2); **Anal. Calcd for** $C_{23}H_{25}F_2N_5O_2S$**:** C, 58.34; H, 5.32; F, 8.02; N, 14.79 %. **Found:** C, 58.13; H, 5.30; F, 8.19; N, 14.92 %.

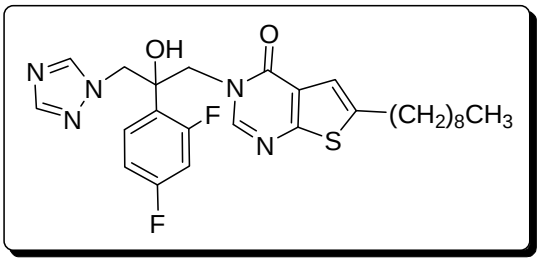
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76g)



Nature: Cream white solid; **MP:** 98 ^{o}C; **Yield:** 81.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.86 (t, *J* = 6 Hz, 3H), 1.21-1.32 (m, 8H), 1.62-1.72 (m, 2H), 2.81 (t, *J* = 8 Hz, 2H), 4.23 (d, *J* = 15 Hz, 1H), 4.53 (d, *J* = 15 Hz, 1H), 4.72 (d, *J* = 15 Hz, 1H), 4.80 (d, *J* = 15 Hz, 1H),

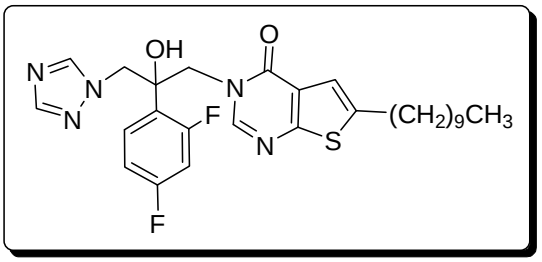
6.24 (bs, 1H), 6.74-6.89 (m, 2H), 7.06 (s, 1H), 7.48-7.60 (m, 1H), 7.84 (s, 1H), 7.91 (s, 1H), 8.13 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.0, 22.6, 28.9 (2C), 30.8, 31.0, 31.6 (2C), 53.3, 55.9, 75.9, 104.4 (t), 112.2 (d), 117.1, 122.3 (d), 123.9, 130.3 (d), 144.5, 145.6, 146.1, 150.6, 158.5 (dd), 158.9, 162.9, 163.1 (dd); **IR** (Chloroform)**:** 3396, 1656 cm^{-1}; **MS** (ESI) *m*/*z***:** 489.3757 (M + 2); **Anal. Calcd for** $C_{24}H_{27}F_2N_5O_2S$**:** C, 59.12; H, 5.58; F, 7.79; N, 14.36 %. **Found:** C, 58.95; H, 5.61; F, 8.03; N, 14.14 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-nonylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76h)



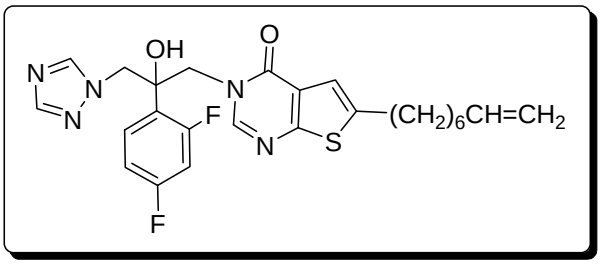
Nature: Off-white solid; **MP:** 132 ^{o}C; **Yield:** 76.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.86 (t, *J* = 6 Hz, 3H), 1.13-1.42 (m, 12H), 1.54-1.80 (m, 2H), 2.80 (t, *J* = 8 Hz, 2H), 4.26 (d, *J* = 14 Hz, 1H), 4.57 (d, *J* = 14 Hz, 1H), 4.72 (d, *J* = 14 Hz, 1H), 4.82 (d, *J* = 14 Hz, 1H), 6.27 (bs, 1H), 6.73-6.92 (m, 2H), 7.06 (s, 1H), 7.44-7.61 (m, 1H), 7.87 (bs, 1H), 7.92 (s, 1H), 8.25 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 22.4, 28.7, 29.0 (2C), 29.4, 30.4, 30.8, 31.6, 53.1, 55.5, 104.1 (t), 111.9 (d), 117.7, 122.5 (d), 123.7, 130.1 (d), 145.4, 146.0 (2C), 151.3, 158.2 (dd), 158.7, 162.7, 163.7 (dd); **IR** (Chloroform)**:** 3380, 1657 cm^{-1}; **MS** (ESI) *m*/*z***:** 516.4371 (M + 1); **Anal. Calcd for** $C_{26}H_{31}F_2N_5O_2S$**:** C, 60.56; H, 6.06; F, 7.37; N, 13.58 %. **Found:** C, 60.21; H, 5.84; F, 7.58; N, 13.76 %.

6-Decyl-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76i)



Nature: White solid; **MP:** 123 ^{o}C; **Yield:** 71.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.87 (t, *J* = 6 Hz, 3H), 1.19-1.38 (m, 14H), 1.62-1.73 (m, 2H), 2.81 (t, *J* = 8 Hz, 2H), 4.27 (d, *J* = 14 Hz, 1H), 4.61 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.85 (d, *J* = 14 Hz, 1H), 6.30 (bs, 1H), 6.74-6.88 (m, 2H), 7.06 (s, 1H), 7.46-7.59 (m, 1H), 7.90 (s, 1H), 7.93 (s, 1H), 8.35 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 22.4, 28.8, 29.0, 29.2, 29.3, 30.4, 30.8, 31.6, 53.1, 55.4, 76.1, 104.2 (t), 112.0 (d), 117.7, 122.2 (d), 123.7, 130.0 (d), 145.4, 146.0 (2C), 151.2, 158.6 (dd), 158.7, 162.6 (dd), 162.7; **IR** (Chloroform)**:** 3345, 1658 cm^{-1}; **MS** (ESI) *m*/*z***:** 530.5315 (M + 1); **Anal. Calcd for** $C_{27}H_{33}F_2N_5O_2S$**:** C, 61.23; H, 6.28; F, 7.17; N, 13.22 %. **Found:** C, 61.23; H, 6.14; F, 7.33; N, 13.21 %.

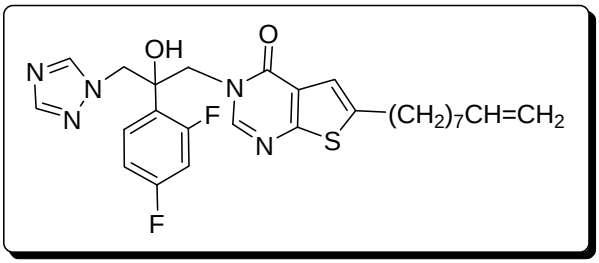
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(oct-7-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76j)



Nature: Off-white solid; **MP:** 126-128 ^{o}C; **Yield:** 68.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.30-1.45 (m, 6H), 1.63-1.71 (m, 2H), 1.98-2.07 (m, 2H), 2.80 (t, *J* = 8 Hz, 2H), 4.38 (d, *J* = 14 Hz, 1H), 4.76 (d, *J* = 14 Hz, 1H), 4.86 -5.03 (m, 4H), 5.69-5.87 (m, 1H), 6.73-6.90 (m,

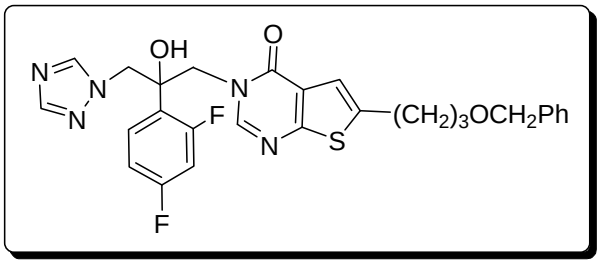
2H), 7.04 (s, 1H), 7.39-7.51 (m, 1H), 8.03 (s, 1H), 8.08 (s, 1H), 9.10 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 28.5 (2C), 30.4, 30.6, 30.7, 33.4, 53.1, 55.5, 75.8, 104.2 (t), 112.0 (d), 114.1, 117.7, 122.2 (d), 123.7, 130.0 (d), 138.7, 145.3, 146.0 (2C), 151.1, 158.3 (dd), 158.7, 162.7, 163.0 (dd); **IR** (Chloroform)**:** 3369, 1660 cm^{-1}; **MS** (ESI) *m*/*z***:** 500.1953 (M + 1); **Anal. Calcd for** $C_{25}H_{27}F_2N_5O_2S$**:** C, 60.10; H, 5.45; F, 7.61; N, 14.02 %. **Found:** C, 60.33; H, 5.56; F, 7.29; N, 13.37 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(non-8-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76k)



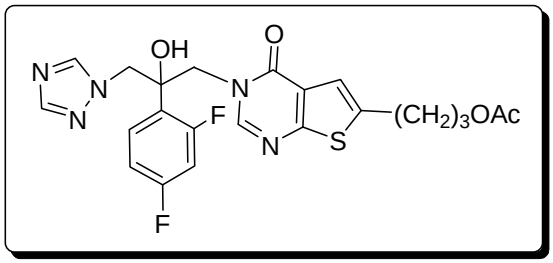
Nature: White solid; **MP:** 108 ^{o}C; **Yield:** 65.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.23-1.34 (m, 8H), 1.61-1.72 (m, 2H), 1.97-2.08 (m, 2H), 2.81 (t, *J* = 6 Hz, 2H), 4.25 (d, *J* = 14 Hz, 1H), 4.57 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.83 (d, *J* = 14 Hz, 1H), 4.89-5.03 (m, 2H), 5.69-5.89 (m, 1H), 6.27 (bs, 1H), 6.74-6.88 (m, 2H), 7.06 (s, 1H), 7.47-7.59 (m, 1H), 7.86 (s, 1H), 7.92 (s, 1H), 8.23 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 26.8, 28.7 (2C), 30.4, 30.7, 33.5, 36.1, 53.2, 55.3, 75.8, 104.2 (t), 112.0 (d), 114.9, 117.7, 122.3 (d), 123.7, 130.1 (d), 138.8, 144.6, 145.3, 146.0, 151.5, 158.1 (dd), 158.7, 162.7, 162.9 (dd); **IR** (Chloroform)**:** 3418, 1671 cm^{-1}; **MS** (ESI) *m*/*z***:** 514.5428 (M + 1); **Anal. Calcd for** $C_{26}H_{29}F_2N_5O_2S$**:** C, 60.80; H, 5.69; F, 7.40; N, 13.64 %. **Found:** C, 60.58; H, 5.91; F, 7.33; N, 13.55 %.

6-(3-(Benzyloxy)propyl)-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76l)



Nature: Pale brown solid; **MP:** 111 ^{o}C; **Yield:** 71.6 %; **^{1}H NMR** (200 MHz, $CDCl_3$+ CCl_4)**:** δ 1.91-2.06 (m, 2H), 2.95 (t, *J* = 6 Hz, 2H), 3.51 (t, *J* = 6 Hz, 2H), 4.21 (d, *J* = 14 Hz, 1H), 4.49 (s, 2H), 4.52 (d, *J* = 14 Hz, 1H), 4.72 (d, *J* = 14 Hz, 1H), 4.79 (d, *J* = 14 Hz, 1H), 6.22 (s, 1H), 6.72-6.88 (m, 2H), 7.08 (s, 1H), 7.31 (bs, 5H), 7.49-7.62 (m, 1H), 7.83 (s, 1H), 7.91 (s, 1H), 8.09 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 27.2, 30.9, 53.3, 55.7, 68.5, 73.0, 75.8, 96.1, 104.3 (t), 112.2 (d), 118.3, 122.4 (d), 123.9, 127.6 (3C), 128.3 (2C), 130.1, 138.1, 144.7, 146.2, 151.1, 158.8, 158.9 (dd), 162.9, 163.1 (dd), 175.2; **IR** (Chloroform)**:** 3303, 1659, 1106 cm^{-1}; **MS** (ESI) *m*/*z***:** 538.19 (M + 1), 560.16 (M + Na), 576.23 (M + K); **Anal. Calcd for** $C_{27}H_{25}F_2N_5O_3S$**:** C, 60.32; H, 4.69; F, 7.07; N, 13.03 %. **Found:** C, 60.11; H, 4.85; F, 7.09; N, 13.15 %.

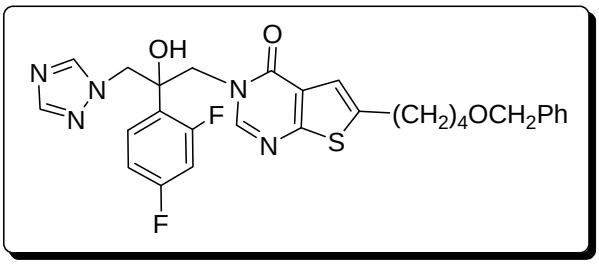
6-(3-Acetoxypropyl)-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one (76m)



Nature: Off-white solid; **MP:** 100-102 ^{o}C; **Yield:** 70.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.96-2.10 (m, 5H), 2.92 (t, *J* = 6 Hz, 2H), 4.11 (t, *J* = 6 Hz, 2H), 4.28 (d, *J* = 14 Hz, 1H),

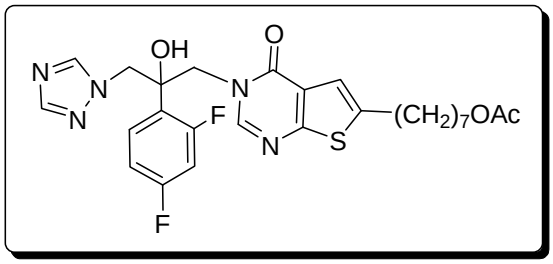
4.61 (d, *J* = 14 Hz, 1H), 4.74 (d, *J* = 14 Hz, 1H), 4.86 (d, *J* = 14 Hz, 1H), 6.75-6.90 (m, 2H), 7.10 (s, 1H), 7.46-7.58 (m, 1H), 7.91 (s, 1H), 7.95 (s, 1H), 8.36 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 20.7, 26.8, 29.5, 53.0, 55.4, 62.8, 75.8, 104.2 (t), 112.0 (d), 118.3, 122.2 (d), 123.7, 130.0 (d), 143.3, 146.3, 151.3, 158.0 (dd), 158.6, 162.9, 163.8 (dd), 170.8; **IR** (Chloroform)**:** 3368, 1735, 1660 cm^{-1}; **MS** (ESI) *m*/*z***:** 491.3670 (M + 2); **Anal. Calcd for** $C_{22}H_{21}F_2N_5O_4S$**:** C, 53.98; H, 4.32; F, 7.76; N, 14.31 %. **Found:** C, 54.23; H, 4.21; F, 7.51; N, 14.41 %.

6-(4-Benzyloxybutyl)-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one (76n)



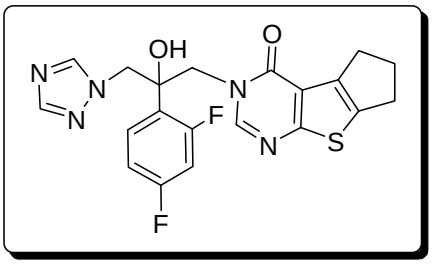
Nature: Off-white solid; **MP:** 80 ^{o}C; **Yield:** 76.8 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 1.61-1.86 (m, 4H), 2.84 (t, *J* = 6 Hz, 2H), 3.49 (t, *J* = 6 Hz, 2H), 4.23 (d, *J* = 16 Hz, 1H), 4.49 (s, 2H), 4.53 (d, *J* = 16 Hz, 1H), 4.72 (d, *J* = 16 Hz, 1H), 4.80 (d, *J* = 16 Hz, 1H), 6.22 (s, 1H), 6.72-6.90 (m, 2H), 7.08 (s, 1H), 7.32 (bs, 5H), 7.50-7.62 (m, 1H), 7.84 (s, 1H), 7.92 (s, 1H), 8.10 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 27.8, 29.1, 30.4, 53.5, 55.5, 69.5, 72.9, 76.0, 104.3 (t), 112.2 (d), 118.2, 122.4, 123.9, 127.5 (3C), 128.3 (2C), 130.2, 138.3, 144.7, 144.9, 146.1, 151.6, 158.1 (dd), 158.7, 163.0, 163.5 (dd); **IR** (Chloroform)**:** 3354, 1656, 1106 cm^{-1}; **MS** (ESI) *m*/*z***:** 552.20 (M + 1), 574.18 (M + Na), 590.16 (M + K); **Anal. Calcd for** $C_{28}H_{27}F_2N_5O_3S$**:** C, 60.97; H, 4.93; F, 6.89; N, 12.70 %. **Found:** C, 61.12; H, 5.11; F, 6.80; N, 12.83 %.

6-(7-Acetoxyheptyl)-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one (76o)



Nature: Pale brown solid; **MP:** 94-96 ^{o}C; **Yield:** 74.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.21-1.36 (m, 6H), 1.51-1.80 (m, 4H), 2.03 (s, 3H), 2.81 (t, *J* = 8 Hz, 2H), 4.03 (t, *J* = 6 Hz, 2H), 4.26 (d, *J* = 14 Hz, 1H), 4.57 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.83 (d, *J* = 14 Hz, 1H), 6.75-6.87 (m, 2H), 7.06 (s, 1H), 7.51-7.59 (m, 1H), 7.87 (s, 1H), 7.92 (s, 1H), 8.23 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 20.8, 22.3 (2C), 23.5, 27.06 (2C), 29.7, 53.2, 55.5, 62.9, 75.9, 104.3 (t), 112.2 (d), 118.5, 122.4 (d), 123.9, 130.2 (2C), 143.5, 146.5, 151.5, 158.3 (dd), 158.7, 163.1, 163.2 (dd), 171.0; **IR** (Chloroform)**:** 3401, 1746, 1659 cm^{-1}; **MS** (ESI) *m*/*z***:** 584.7058 (M + K); **Anal. Calcd for** $C_{26}H_{29}F_2N_5O_4S$**:** C, 57.24; H, 5.36; F, 6.96; N, 12.84 %. **Found:** C, 57.48; H, 5.16; F, 7.17; N, 12.61 %.

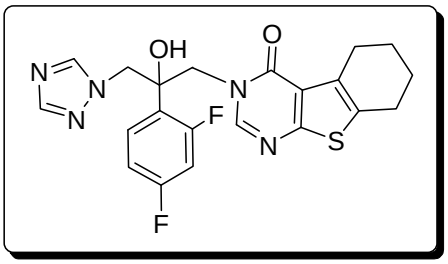
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6,7-dihydro-3*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4(5*H*)-one (76p)



Nature: White solid; **MP:** 200 ^{o}C; **Yield:** 66.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 2.39-2.55 (m, 2H), 2.95 (t, *J* = 7 Hz, 2H), 3.02 (t, *J* = 7 Hz, 2H), 4.18 (d, *J* = 14 Hz, 1H), 4.55 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.80 (d, *J* = 14 Hz, 1H), 6.26 (bs, 1H),

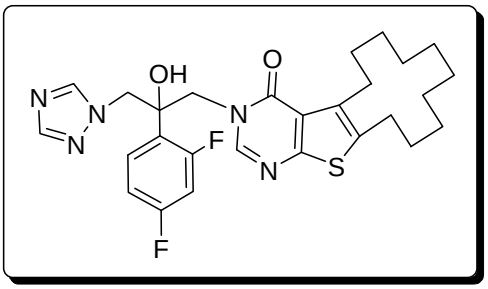
6.74-6.92 (m, 2H), 7.51-7.63 (m, 1H), 7.85 (s, 2H), 8.15 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 25.9, 27.0, 27.6, 49.0, 53.0, 73.0, 102.2 (t), 109.2 (d), 117.7, 121.7 (d), 127.8 (d), 136.8, 138.2, 143.0, 145.8, 148.6, 155.9, 156.7 (dd), 160.7 (dd), 165.1; **IR** (Chloroform)**:** 3313, 1656 cm^{-1}; **MS** (ESI) *m*/*z***:** 430.0470 (M + 1); **Anal. Calcd for** $C_{20}H_{17}F_2N_5O_2S$**:** C, 55.94; H, 3.99; F, 8.85; N, 16.31 %. **Found:** C, 56.37; H, 4.14; F, 8.61; N, 16.06 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76q)



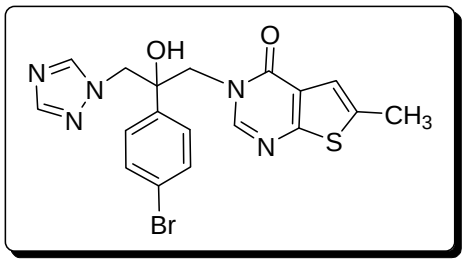
Nature: White solid; **MP:** 170 ^{o}C; **Yield:** 68.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.74-1.96 (m, 4H), 2.71-2.81 (m, 2H), 2.90-3.02 (m, 2H), 4.18 (d, *J* = 14 Hz, 1H), 4.55 (d, *J* = 14 Hz, 1H), 4.70 (d, *J* = 14 Hz, 1H), 4.77 (d, *J* = 14 Hz, 1H), 6.29 (bs, 1H), 6.72-6.92 (m, 2H), 7.51-7.62 (m, 1H), 7.85 (s, 2H), 8.13 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 21.9, 22.6, 25.0, 25.4, 53.0, 55.6, 75.9, 104.2 (t), 112.0 (d), 121.9, 122.5 (d), 130.1, 131.2, 134.9, 144.6, 145.9, 151.3, 156.4 (dd), 159.4, 162.5, 162.9 (dd); **IR** (Chloroform)**:** 3436, 1663 cm^{-1}; **MS** (ESI) *m*/*z***:** 445.2642 (M + 2); **Anal. Calcd for** $C_{21}H_{19}F_2N_5O_2S$**:** C, 56.88; H, 4.32; F, 8.57; N, 15.79 %. **Found:** C, 57.16; H, 4.48; F, 8.31; N, 15.54 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-5,6,7,8,9,10,11, 12,13,14-decahydrocyclododeca[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76r)



Nature: White solid; **MP:** 123 ^{o}C; **Yield:** 67.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.10-1.57 (m, 12H), 1.59-1.83 (m, 4H), 2.66-2.97 (m, 4H), 4.27 (d, *J* = 14 Hz, 1H), 4.58 (d, *J* = 14Hz, 1H), 4.66 (d, *J* = 14 Hz, 1H), 4.79 (d, *J* = 14 Hz, 1H), 6.33 (bs, 1H), 6.72-6.90 (m, 2H), 7.46-7.90 (m, 1H), 7.86 (s, 1H), 7.89 (s, 1H), 8.20 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.9, 23.1, 23.6, 24.6 (2C), 25.2 (2C), 25.3, 28.3, 29.5, 53.1, 55.7, 75.9, 104.2 (t), 112.2 (d), 122.1, 122.3 (d), 130.1 (d), 133.9, 139.4, 144.8, 146.0, 151.5, 158.0 (dd), 159.1, 163.1, 163.3 (dd); **IR** (Chloroform)**:** 3310, 1673 cm^{-1}; **MS** (ESI) *m*/*z***:** 528.1685 (M + 1); **Anal. Calcd for** $C_{27}H_{31}F_2N_5O_2S$**:** C, 61.46; H, 5.92; F, 7.20; N, 13.27 %. **Found:** C, 61.23; H, 6.16; F, 7.04; N, 13.12 %.

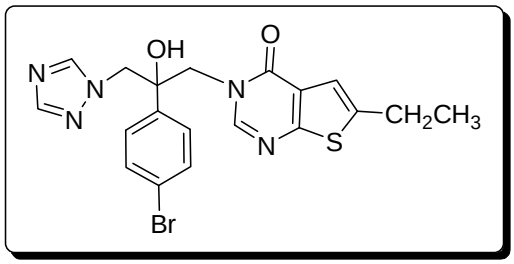
3-(2-(4-Bromophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76s)



Nature: Off-white solid; **MP:** 168 ^{o}C; **Yield:** 65.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.53 (s, 3H), 4.06 (d, *J* = 14 Hz, 1H), 4.48 (d, *J* = 14 Hz, 1H), 4.79 (d, *J* = 14 Hz, 1H), 4.81 (d, *J* = 14 Hz, 1H), 7.31 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8 Hz, 2H), 7.47 (s, 1H), 7.96 (s, 1H), 8.03

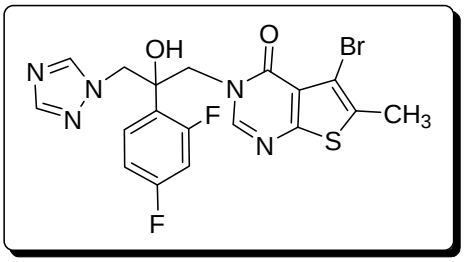
(s, 1H), 8.33 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 16.0, 53.6, 55.9, 76.3, 119.0, 122.5, 124.2, 126.8 (2C), 131.9 (2C), 138.9, 139.4, 144.8, 146.5, 151.8, 158.6, 163.2; **IR** (Chloroform)**:** 3352, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 446.2450 and 448.2449 (M + 1); **Anal. Calcd for** $C_{18}H_{16}BrN_5O_2S$**:** C, 48.44; H, 3.61; Br, 17.90; N, 15.69 %. **Found:** C, 48.21; H, 3.79; Br, 18.13; N, 15.41 %.

3-(2-(4-Bromophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76t)



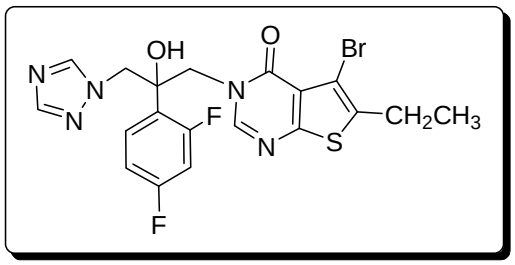
Nature: Brown solid; **MP:** 173 ^{o}C; **Yield:** 67.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.34 (t, *J* = 8 Hz, 3H), 2.87 (q, *J* = 8 Hz, 2H), 3.71 (bs, 1H), 4.12 (d, *J* = 14 Hz, 1H), 4.53 (d, *J* = 14 Hz, 1H), 4.80 (d, *J* = 14 Hz, 2H), 7.10 (s, 1H), 7.31 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8 Hz, 2H), 8.01 (s, 1H), 8.05 (s, 1H), 8.49 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.2, 24.0, 53.7, 56.0, 76.4, 117.3, 122.6, 124.0, 126.9 (2C), 132.0 (2C), 139.0, 144.6, 146.6, 146.9, 151.9, 158.8, 163.0; **IR** (Chloroform)**:** 3418, 1666 cm^{-1}; **MS** (ESI) *m*/*z***:** 499.6406 and 501.6458 (M + K); **Anal. Calcd for** $C_{19}H_{18}BrN_5O_2S$**:** C, 49.57; H, 3.94; Br, 17.36; N, 15.21 %. **Found:** C, 49.74; H, 4.21; Br, 17.13; N, 15.06 %.

5-Bromo-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76aa)



Nature: Off-white solid; **MP:** 142 ^{o}C; **Yield:** 72.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.46 (s, 3H), 4.04 (d, *J* = 14 Hz, 1H), 4.46 (d, *J* = 14 Hz, 1H), 4.89 (d, *J* = 14 Hz, 1H), 4.96 (d, *J* = 14 Hz, 1H), 5.96 (s, 1H), 6.77-6.90 (m, 2H), 7.51-7.62 (m, 1H), 7.83 (s, 1H), 8.04 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.1, 52.6, 55.1, 76.0, 97.8, 104.2 (t), 112.1 (d), 120.8, 122.4 (d), 130.1 (d), 133.7, 144.6, 147.3, 151.7, 157.7, 159.3 (dd), 161.4, 162.5 (dd); **IR** (Chloroform)**:** 3444, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 482.0249 and 484.0200 (M +1); **Anal. Calcd for** $C_{18}H_{14}BrF_2N_5O_2S$**:** C, 44.83; H, 2.93; Br, 16.57; F, 7.88; N, 14.52 %. **Found:** C, 45.08; H, 3.21; Br, 16.32; F, 7.71; N, 14.33 %.

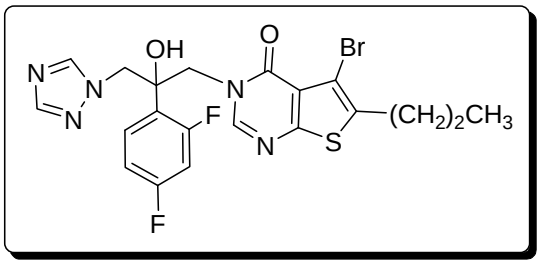
5-Bromo-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ab)



Nature: Pale yellow solid; **MP:** 133 ^{o}C; **Yield:** 78.9 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.31 (t, *J* = 8 Hz, 3H), 2.88 (q, *J* = 8 Hz, 2H), 4.06 (d, *J* = 14 Hz, 1H), 4.47 (d, *J* = 14 Hz, 1H), 4.90 (d, *J* = 14 Hz, 1H), 4.96 (d, *J* = 14 Hz, 1H), 5.95 (bs, 1H), 6.77-6.89 (m, 2H), 7.53-7.62 (m, 1H), 7.82 (s, 1H), 8.03 (s, 1H), 8.05 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ

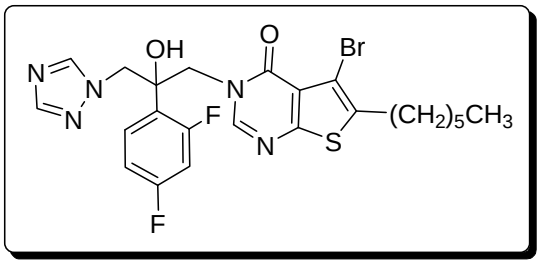
14.4, 23.0, 52.5, 55.1, 76.2, 102.8, 104.3 (t), 112.1 (d), 120.8, 122.3 (d), 130.1, 140.9, 147.2, 151.3, 157.2 (dd), 157.8, 161.3, 161.4 (dd); **IR** (Chloroform)**:** 3468, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 498.7345 and 500.7357 (M + 3); **Anal. Calcd for** $C_{19}H_{16}BrF_2N_5O_2S$**:** C, 45.98; H, 3.25; Br, 16.10; F, 7.66; N, 14.11 %. **Found:** C, 46.25; H, 3.12; Br, 16.27; F, 7.41; N, 14.00 %.

5-Bromo-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ac)



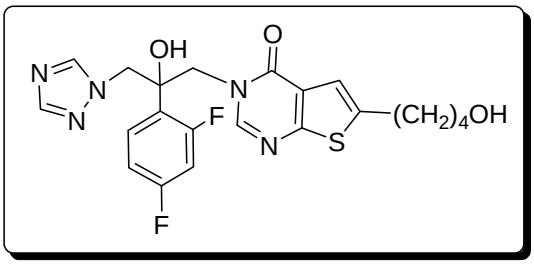
Nature: White solid; **MP:** 163 ^{o}C; **Yield:** 73.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.02 (t, *J* = 8 Hz, 3H), 1.63-1.78 (m, 2H), 2.84 (t, *J* = 6 Hz, 2H), 4.04 (d, *J* = 14 Hz, 1H), 4.46 (d, *J* = 14 Hz, 1H), 4.91 (d, *J* = 14 Hz, 1H), 4.98 (d, *J* = 14 Hz, 1H), 5.95 (bs, 1H), 6.77-6.89 (m, 2H), 7.50-7.62 (m, 1H), 7.82 (s, 1H), 8.03 (s, 1H), 8.04 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.5, 23.4, 31.3, 52.5, 55.0, 76.1, 103.4, 104.3 (t), 112.1 (d), 120.7, 122.3 (d), 130.1 (d), 139.3, 144.6, 147.3, 151.7, 157.8, 158.2 (dd), 161.5, 163.3 (dd); **IR** (Chloroform)**:** 3429, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 532.1171 and 534.1528 (M + Na); **Anal. Calcd for** $C_{20}H_{18}BrF_2N_5O_2S$**:** C, 47.07; H, 3.55; Br, 15.66; F, 7.45; N, 13.72 %. **Found:** C, 46.86; H, 3.61; Br, 15.46; F, 7.31; N, 13.68 %.

5-Bromo-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ad)



Nature: Pale yellow solid; **MP:** 115 ^{o}C; **Yield:** 75.0 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 8 Hz, 3H), 1.20-1.43 (m, 3H), 1.60-1.75 (m, 2H), 2.84 (t, *J* = 6 Hz, 2H), 4.04 (d, *J* = 14 Hz, 1H), 4.46 (d, *J* = 14 Hz, 1H), 4.90 (d, *J* = 14 Hz, 1H), 4.97 (d, *J* = 14 Hz, 1H), 5.94 (bs, 1H), 6.78-6.89 (m, 2H), 7.48-7.61 (m, 1H), 7.81 (s, 1H), 8.02 (s, 1H), 8.04 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.2, 22.5, 27.7, 28.8, 31.0, 31.5, 53.1, 55.7, 76.1, 104.3 (t), 112.2 (d), 122.8, 128.7, 130.1 (d), 138.1, 144.4, 144.5, 145.9, 151.3, 158.4 (dd), 159.8, 162.01, 163.1 (dd); **IR** (Chloroform)**:** 3436, 1673 cm^{-1}; **MS** (ESI) *m*/*z***:** 552.9600 and 554.9618 (M + 1); **Anal. Calcd for** $C_{23}H_{24}BrF_2N_5O_2S$**:** C, 50.01; H, 4.38; Br, 14.46; F, 6.88; N, 12.68 %. **Found:** C, 49.84; H, 4.51; Br, 14.27; F, 7.04; N, 12.32 %.

Preparation of 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(4-hydroxybutyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76af)



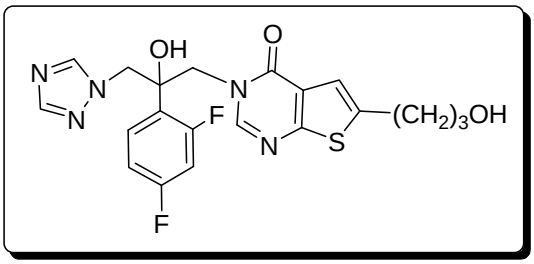
In a two necked round bottom flask, a mixture of 6-(4-benzyloxybutyl)-3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**76n**) (150 mg, 0.27 mmol) and sodium iodide (82 mg, 0.54 mmol) was taken in dry

acetonitrile (10 ml) and cooled to 0 ºC. $BF_3.OEt_2$ (96 mg, 0.11 ml, 0.68 mmol) was added drop wise over a period of 5-6 min. Then the reaction mixture was stirred at room temperature for 2 h, poured into ice-cold water and treated with few drops of aq. sodium thiosulphate. It was then extracted with dichloromethane (3 X 10 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product (110 mg, 87.3 %).

Nature: Off-white solid; **MP:** 148 ^oC; **Yield:** 87.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.50-1.90 (m, 4H), 2.87 (t, *J* = 6 Hz, 2H), 3.59 (t, *J* = 6 Hz, 2H), 4.26 (d, *J* = 14 Hz, 1H), 4.56 (d, *J* = 14 Hz, 1H), 4.77 (d, *J* = 14 Hz, 1H), 4.89 (d, *J* = 14 Hz, 1H), 6.22 (bs, 1H), 6.72-6.92 (m, 2H), 7.07 (s, 1H), 7.35-7.50 (m, 1H), 7.76 (bs, 1H), 8.00 (s, 1H), 8.22 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 26.8, 33.6, 38.9, 52.3, 55.3, 60.5, 75.4, 104.1 (t), 111.6 (d), 118.2, 122.5 (d), 123.9, 129.8 (d), 144.4 (2C), 146.7 (2C), 158.3 (dd), 158.4, 162.6, 163.1 (dd); **IR** (Chloroform)**:** 3382, 1675 cm^{-1}; **MS** (ESI) *m*/*z***:** 461.3047 (M+), 462.3002 (M + 1); **Anal. Calcd for** $C_{21}H_{21}F_2N_5O_3S$**:** C, 54.66; H, 4.59; F, 8.23; N, 15.18 %. **Found:** C, 54.51; H, 4.62; F, 8.31; N, 15.09 %.

Compound **76ae** was prepared using the procedure given for the compound **76af**.

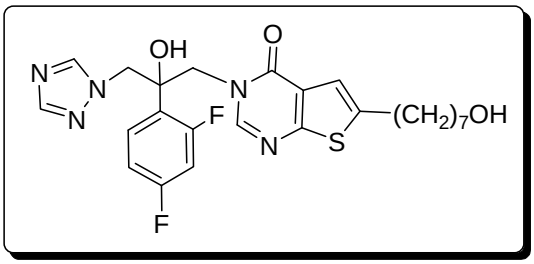
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(3-hydroxypropyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ae)



Nature: White solid; **MP:** 108-110 ^oC; **Yield:** 86.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.69-1.82 (m, 2H), 2.77 (t, *J* = 8 Hz, 2H), 3.47 (t, *J* = 6 Hz, 2H), 4.13 (d, *J* = 14 Hz, 1H), 4.48 (d, *J* = 14 Hz, 1H), 4.61 (d, *J* = 14 Hz, 1H), 4.83 (d, *J* = 14 Hz, 1H), 6.55-6.74 (m, 2H), 6.93 (s, 1H), 7.27 (s, 2H), 7.86 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ

26.4, 33.2, 51.9, 54.9, 60.1, 75.0, 103.7 (t), 111.2 (d), 117.8, 122.2 (d), 123.5, 129.4 (2C), 144.0, 146.3, 157.8 (dd), 157.9, 162.2, 162.9 (dd); **IR** (Chloroform)**:** 3444, 1671 cm^{-1}; **MS** (ESI) *m*/*z***:** 486.5786 (M + K); **Anal. Calcd for** $C_{20}H_{19}F_2N_5O_3S$**:** C, 53.68; H, 4.28; F, 8.49; N, 15.65 %. **Found:** C, 53.52; H, 4.46; F, 8.14; N, 15.47 %.

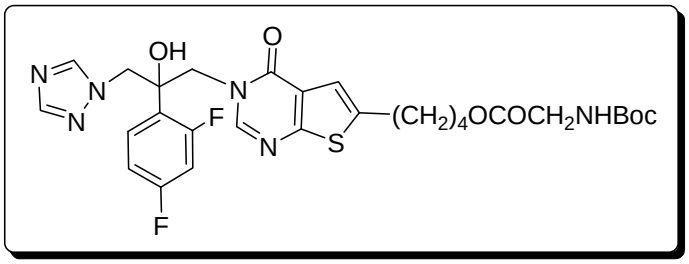
Preparation of 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(7-hydroxyheptyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ai)



7-(3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)heptyl acetate (**76o**) (3.0 g, 5.5 mmol) was taken in a round bottom flask. A mixture of methanol and water (1**:**1, 20 ml) was added to it and stirred for 10 min. Then potassium carbonate (2.27 g, 5.5 mmol) was added to the reaction mixture and stirred for 3 h. Methanol was evaporated under vacuum and diluted with water (50 ml), extracted with ethyl acetate (3 X 20 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product (2.67 g, 96.7 %).

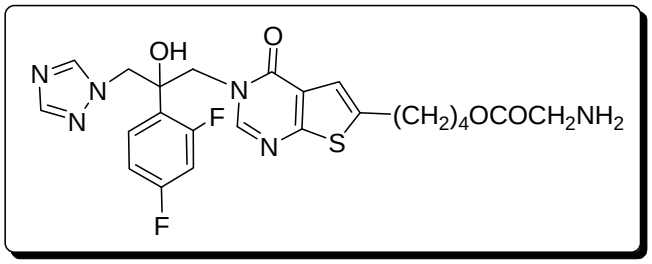
Nature: Off-white solid; **MP:** 109-110 ^oC; **Yield:** 96.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.29-1.37 (m, 6H), 1.49-1.79 (m, 4H), 2.50 (bs, 1H), 2.81 (t, *J* = 8 Hz, 2H), 3.62 (t, *J* = 6 Hz, 2H), 4.26 (d, *J* = 14 Hz, 1H), 4.58 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.83 (d, *J* = 14 Hz, 1H), 6.27 (bs, 1H), 6.75-6.88 (m, 2H), 7.06 (s, 1H), 7.46-7.59 (m, 1H), 7.87 (s, 1H), 7.92 (s, 1H), 8.25 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 25.4, 28.6, 28.8, 30.4, 30.7, 32.4, 53.1, 55.4, 62.4, 75.8, 104.2 (t), 112.0 (d), 117.8, 122.2, 123.8, 130.0, 144.6, 145.3, 146.1, 151.1, 158.7, 159.3 (dd), 162.4 (dd), 162.7; **IR** (Chloroform)**:** 3375, 1675 cm^{-1}; **MS** (ESI) *m*/*z***:** 542.00 (M + K); **Anal. Calcd for** $C_{24}H_{27}F_2N_5O_3S$**:** C, 57.24; H, 5.40; F, 7.55; N, 13.91 %. **Found:** C, 56.97; H, 5.43; F, 7.68; N, 13.83 %.

Preparation of 3-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]-6-(4-N-Boc-aminoacetyloxybutyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ag)



In a two necked round bottom flask equipped with guard tube, a mixture of 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(4-hydroxybutyl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one (**76af**) (500 mg, 0.895 mmol), 2-((tert butoxycarbonyl)amino)acetic acid (188 mg, 1.073 mmol) and DMAP (10 mg) was taken in dry DCM (10 ml) and cooled to 0 ^{o}C. EDCI (257 mg, 1.346 mmol) in DCM (5 ml) was added to reaction mixture and stirred at room temperature for 12 h. The reaction mixture was then diluted with water (50 ml), extracted with DCM (3 X 20 ml), dried over Na_2SO_4 and concentrated on rotary evaporator. Purification by column chromatography yielded pure product (300 mg, 46.8 %). **Nature:** Greenish thick liquid; **Yield:** 46.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.47 (s, 9H), 1.62-1.90 (m, 4H), 2.80-2.96 (m, 2H), 3.78-3.98 (m, 2H), 4.05-4.32 (m, 3H), 4.55-4.91 (m, 3H), 5.05 (bs, 1H), 6.29 (bs, 1H), 6.73-6.89 (m, 2H), 7.12 (s, 1H), 7.50-7.65 (m, 1H), 7.92 (bs, 1H), 7.98(s, 1H), 8.35 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.6, 14.2, 14.6, 23.8, 25.2, 34.0, 38.1, 52.8, 55.1 (2C), 63.5, 75.7, 104.3 (t), 111.9 (d), 113.8, 115.5 (d), 119.4 (d), 130.4, 131.3, 136.4, 142.5, 146.1 (dd), 148.7, 151.0, 152.3 (dd), 155.4, 158.0, 161.3; **IR** (Chloroform)**:** 3315, 1713, 1679 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 618.3011 (M+); **Anal. Calcd for** $C_{28}H_{32}F_2N_6O_6S$**:** C, 54.36; H, 5.21; F, 6.14; N, 13.58 %. **Found:** C, 54.61; H, 5.29; F, 6.01; N, 13.38 %.

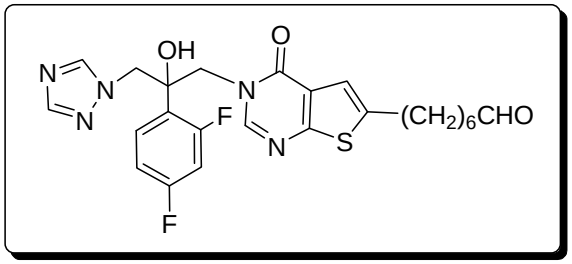
Preparation of 3-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4triazol-1-yl)-propyl]-6-(4-aminoacetyloxybutyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (76ah)



In a two necked round bottom flask equipped with guard tube, 3-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4triazol-1-yl)-propyl]-6-(4-N-Boc-aminoacetyloxybutyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**76ag**) (200 mg, 0.28 mmol) in dry DCM (6 ml) was taken and cooled to 0 ^{o}C. Trifluoroacetic acid (0.2 ml, 1.64 mmol) was added slowly and the reaction mixture was stirred for 4 h at room temperature. It was then diluted with water (30 ml), extracted with DCM (3 X 10 ml), dried over Na_2SO_4 and concentrated on rotary evaporator. Purification by column chromatography yielded pure product (100 mg, 56.8 %).

Nature: Brown semisolid; **Yield:** 56.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.45-1.80 (m, 4H), 2.68-2.90 (m, 2H), 4.05-4.29 (m, 4H), 4.32-4.61 (m, 3H), 4.65-4.90 (m, 3H), 5.81 (bs, 1H), 6.68-6.91 (m, 2H), 7.03 (s, 1H), 7.39-7.58 (m, 1H), 7.74 (s, 1H), 7.91 (s, 1H), 8.08 (s, 1H); **IR** (Chloroform)**:** 3412, 1730, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 541.7506 (M + Na); **Anal. Calcd for** $C_{23}H_{24}F_2N_6O_4S$**:** C, 53.27; H, 4.67; F, 7.33; N, 16.21 %. **Found:** C, 53.04; H, 4.74; F, 7.24; N, 16.19 %.

Preparation of 7-(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)heptanal (76aj)

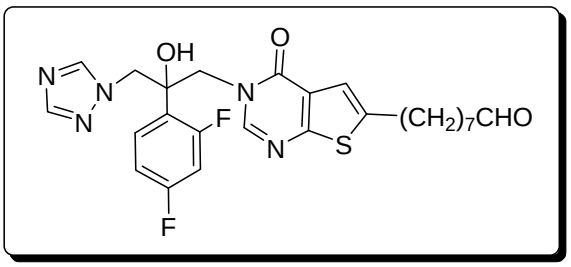


In a round bottom flask, 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(oct-7-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**76j**) (5 g, 10.02 mmol) was dissolved in a mixture of t-butanol (250 ml) and water (50 ml) and then solution of OsO_4 (0.2 ml, 0.01 mmol) in t-butanol was added to it. The reaction mixture was stirred for 5 min, when dark black color appeared, sodium bicarbonate (3.36 g, 4.0 mmol) and sodium periodate (21.4 g, 10.0 mmol) were added and the mixture was stirred for 1 h. It was then diluted with water (300 ml) and extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product (3.65 g, 72.7 %).

Nature: White solid; **MP:** 148 ^oC; **Yield:** 72.7 %; **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 1.36-1.42 (m, 4H), 1.60-1.74 (m, 4H), 2.44 (t, *J* = 8 Hz, 2H), 2.83 (t, *J* = 8 Hz, 2H), 4.30 (d, *J* = 16 Hz, 1H), 4.66 (d, *J* = 16 Hz, 1H), 4.75 (d, *J* = 16 Hz, 1H), 4.79 (d, *J* = 16 Hz, 1H), 6.33 (bs, 1H), 6.77-6.86 (m, 2H), 7.07 (s, 1H), 7.51-7.55 (m, 1H), 7.95 (s, 1H), 7.96 (s, 1H), 8.52 (s, 1H), 9.77 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.6, 28.5, 30.4, 30.5, 43.5 (2C), 53.1, 55.4, 75.5, 104.2 (t), 112.0 (d), 117.8, 122.3 (d), 123.7, 130.0, 144.5, 145.0, 146.1, 151.1, 158.3 (dd), 158.7, 162.7, 163.2 (dd), 202.4; **IR** (Chloroform)**:** 3393, 1721, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 502.4689 (M + 1); **Anal. Calcd for** $C_{24}H_{25}F_2N_5O_3S$**:** C, 57.47; H, 5.02; F, 7.58; N, 13.96 %. **Found:** C, 57.72; H, 5.16; F, 7.25; N, 13.88 %.

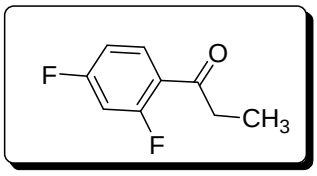
Compound **76ak** was prepared using the procedure given for the compound **76aj**.

8-(3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)octanal (76ak)



Nature: White solid; **MP:** 130 ^{o}C; **Yield:** 74 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.22-1.40 (m, 6H), 1.55-1.71 (m, 4H), 2.43 (dt, *J* = 2, 6 Hz, 2H), 2.82 (t, *J* = 8 Hz, 2H), 4.24 (d, *J* = 14 Hz, 1H), 4.54 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.82 (d, *J* = 14 Hz, 1H), 6.22 (bs, 1H), 6.75-6.89 (m, 2H), 7.08 (s, 1H), 7.49-7.62 (m, 1H), 7.85 (s, 1H), 7.93 (s, 1H), 8.11 (s, 1H), 9.76 (t, *J* = 2 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.7, 28.4, 28.7 (2C), 30.3, 30.6, 43.5 53.0, 55.3, 75.6, 104.1 (t), 111.9 (d), 117.7, 122.0 (d), 123.7, 130.0 (d), 144.5, 145.1, 146.1, 151.0, 158.6, 158.7 (dd), 162.7, 163.5 (dd), 202.5; **IR** (Chloroform)**:** 3443, 1714, 1667 cm^{-1}; **MS** (ESI) *m*/*z***:** 515.4219 (M+); **Anal. Calcd for** $C_{25}H_{27}F_2N_5O_3S$**:** C, 58.24; H, 5.28; F, 7.37; N, 13.58 %. **Found:** C, 57.87; H, 5.45; F, 7.69; N, 13.38 %.

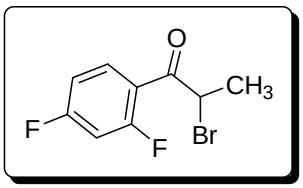
1-(2,4-Difluorophenyl)propan-1-one (88)[43]



Compound **88** was prepared from 1,3-difluorobenzene and propionyl chloride using the procedure given for the chloroketone **41a**.

Nature: Pale yellow oil; **Yield:** 90 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.20 (t, *J* = 8 Hz, 3H), 2.98 (q, *J* = 8 Hz, 2H), 6.82-7.01 (m, 2H), 7.89-8.01 (m, 1H).

Preparation of 2-bromo-1-(2,4-difluorophenyl)propan-1-one (87)[43d]



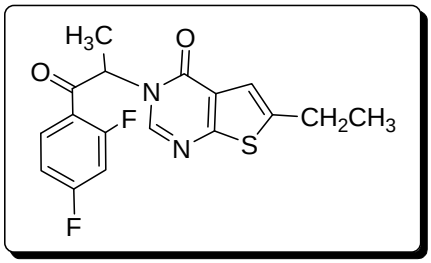
1-(2,4-Difluorophenyl)propan-1-one (**88**) (11 g, 0.064 mol), N-bromosuccinimide (12.48g, 0.16 mol) and AIBN (50 mg) were taken in a round bottom flask containing carbontetrachloride (110 ml) and equipped with reflux condenser and guard tube. Reaction

mixture was stirred at 90 ^{o}C for 6 h, cooled and filtered through a funnel containing celite bed. The filtrate was evaporated under reduced pressure using rotary evaporator to afford crude product. Purification by column chromatography yielded pure product as red oil (12.5 g, 78 %).

Caution: Product **87** is lachrymatory hence reaction must be carried out under vacuum hood and use of hand gloves is essential during reaction work-up.

Nature: Red oil; **Yield:** 78 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.83 (d, *J* = 6 Hz, 3H), 5.16 (q, *J* = 6 Hz, 1H), 6.77-6.99 (m, 2H), 7.85-7.97 (m, 1H).

Preparation of 3-(1-(2,4-difluorophenyl)-1-oxopropan-2-yl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (86a)



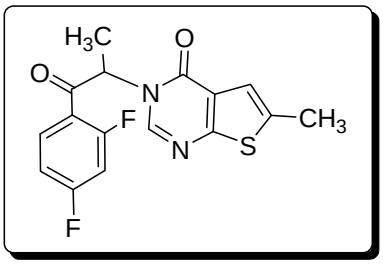
Sodium hydride (1.44 g, 0.06 mol) was taken in two necked round bottom flask equipped with guard tube, DMF (50 ml) was added to it and cooled to 0 ^{o}C. 6-Ethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**78c**) (9 g, 0.05 mmol) in DMF (50 ml) was added over a period of 10 min and stirred at same temperature for 1 h. 2-Bromo-1-(2,4-difluorophenyl)propan-1-one (**87**) (12.45 g, 0.05 mol) was added slowly and the whole reaction mixture was stirred at room temperature for 3 h. It was then diluted with water (200 ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2 SO_4 and concentrated under vacuum on rotary evaporator. Purification by column chromatography yielded pure product as brown semisolid (12.7 g, 73 %).

Nature: Brown semisolid; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.34 (t, *J* = 8 Hz, 3H), 1.77 (d, *J* = 6 Hz, 3H), 2.87 (q, *J* = 8 Hz, 2H), 6.08 (q, *J* = 8 Hz, 1H), 6.83-7.13 (m,

2H), 7.10 (s, 1H), 7.91-8.03 (m, 1H), 8.22 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.1, 15.9, 23.9, 58.4, 104.7 (t), 112.8 (d), 117.4, 120.1 (d), 124.1, 133.5 (d), 144.2, 146.4, 156.5, 161.6, 162.2 (dd), 166.6 (dd), 192.9; **IR** (Chloroform)**:** 1671 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 371.5112 (M + Na), 387.5271 (M + K); **Anal. Calcd for** $C_{17}H_{14}F_2N_2O_2S$**:** C, 58.61; H, 4.05; F, 10.91; N, 8.04 %. **Found:** C, 58.47; H, 3.87; F, 11.06; N, 8.13 %.

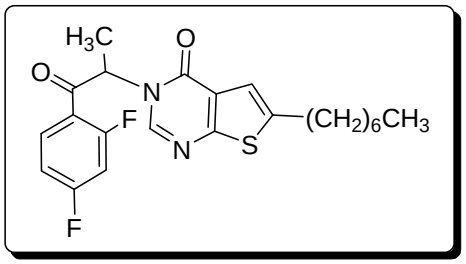
Compounds **86b**, **86c**, **86d** and **86e** were prepared by reaction of bromoketone **87** with the corresponding thienopyrimidinones **78b**, **78g**, **78e** and **78q** using the procedure given for the ketone **86a**.

3-(1-(2,4-Difluorophenyl)-1-oxopropan-2-yl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (86b)



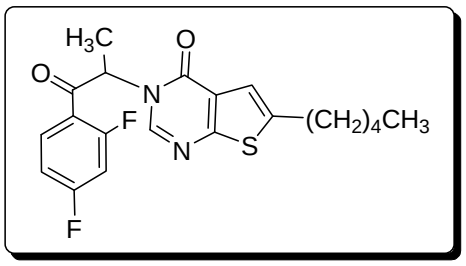
Nature: Yellow solid; **MP:** 130 ^{o}C; **Yield:** 74.5 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.77 (d, *J* = 6 Hz, 3H), 2.64 (s, 3H), 6.09 (q, *J* = 8 Hz, 1H), 6.76-7.04 (m, 2H), 7.11 (s, 1H), 7.87-7.95 (m, 1H), 8.23 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 16.0, 24.1, 58.1, 104.7 (t), 112.9 (d), 117.5, 119.9 (d), 124.2, 133.5 (d), 144.7, 146.6, 156.5, 161.6, 162.1 (dd), 166.8 (dd), 193.0; **IR** (Chloroform)**:** 1671 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 373.0643 (M + K); **Anal. Calcd for** $C_{16}H_{12}F_2N_2O_2S$**:** C, 57.48; H, 3.62; F, 11.36; N, 8.38 %. **Found:** C, 57.32; H, 3.79; F, 11.52; N, 8.14 %.

3-(1-(2,4-Difluorophenyl)-1-oxopropan-2-yl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (86c)



Nature: Off-white semisolid; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.88 (t, *J* = 6 Hz, 3H), 1.24-1.33 (m, 8H), 1.65-1.78 (m, 5H), 2.81 (t, *J* = 6 Hz, 2H), 6.03 (q, *J* = 8 Hz, 1H), 6.81-6.99 (m, 2H), 7.07 (s, 1H), 7.90-7.98 (m, 1H), 8.04 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 15.8, 22.4, 28.8, 30.4 (2C), 30.9, 31.5, 58.4, 104.7 (t), 112.8 (d), 118.0, 120.2 (d), 124.0, 133.3 (d), 144.1, 144.8, 156.6, 162.3, 162.7 (dd), 165.6 (dd), 192.9; **IR** (Chloroform)**:** 1666 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 418.3813 (M+); **Anal. Calcd for** $C_{22}H_{24}F_2N_2O_2S$**:** C, 63.14; H, 5.78; F, 9.08; N, 6.69 %. **Found:** C, 63.11; H, 5.97; F, 9.19; N, 6.32 %.

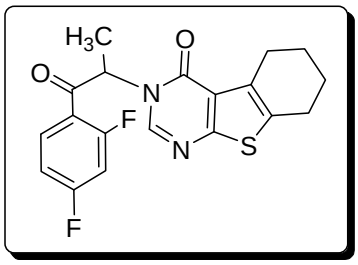
3-(1-(2,4-Difluorophenyl)-1-oxopropan-2-yl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (86d)



Nature: White solid; **MP:** 154-156 ^oC; **Yield:** 65.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.90 (t, *J* = 6 Hz, 3H), 1.30-1.39 (m, 4H), 1.66-1.74 (m, 2H), 1.76 (d, *J* = 8 Hz, 3H), 2.82 (t, *J* = 8 Hz, 2H), 6.05 (q, *J* = 8 Hz, 1H), 6.82-7.01 (m, 2H), 7.08 (s, 1H), 7.91-8.01 (m, 1H), 8.08 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 15.9, 22.2, 30.6 (2C), 31.0, 58.3, 104.7 (t),

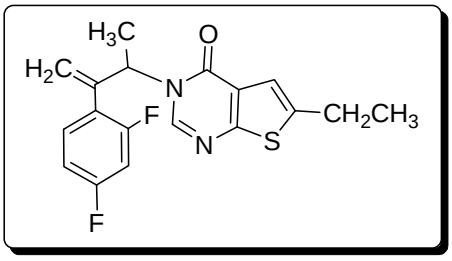
112.7 (d), 118.1, 120.2 (d), 124.1, 133.4 (d), 144.1, 144.8, 156.6, 162.3, 162.8 (dd), 164.8 (dd), 192.0; **IR** (Chloroform)**:** 1665 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 391.1400 (M + 1); **Anal. Calcd for** $C_{20}H_{20}F_2N_2O_2S$**:** C, 61.52; H, 5.16; F, 9.73; N, 7.17 %. **Found:** C, 61.41; H, 5.27; F, 9.64; N, 7.34 %.

3-(1-(2,4-Difluorophenyl)-1-oxopropan-2-yl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (86e)



Nature: Pale yellow solid; **MP:** 178-179 ^oC; **Yield:** 74.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.72 (d, *J* = 6 Hz, 3H), 1.76-1.82 (m, 4H), 2.74 (t, *J* = 6 Hz, 2H), 2.88 (t, *J* = 6 Hz, 2H), 5.93 (q, *J* = 6 Hz, 1H), 6.78-7.01 (m, 2H), 7.87-7.99 (m, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** 15.8, 22.0, 22.7, 25.1, 25.4, 58.2, 104.6 (t), 112.7 (d), 119.9 (d), 122.1, 131.4, 133.5 (d), 134.2, 143.9, 157.0, 161.3 (dd), 161.9, 166.5 (dd), 192.8; **IR** (Chloroform)**:** 1666 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 376.4914 (M + 2); **Anal. Calcd for** $C_{19}H_{16}F_2N_2O_2S$**:** C, 60.95; H, 4.31; F, 10.15; N, 7.48 %. **Found:** C, 60.83; H, 4.64; F, 10.17; N, 7.54 %.

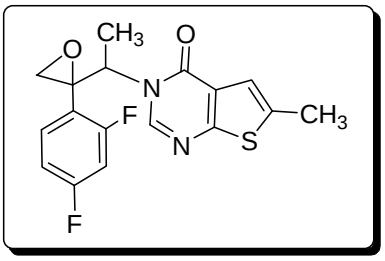
Preparation of 3-(3-(2,4-difluorophenyl)but-3-en-2-yl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (89)



Methyltriphenylphosphonium bromide (5.12 g, 14.36 mmol) in freshly distilled THF (30 ml) was taken in a two necked round bottom flask under inert atmosphere (maintained by using argon gas filled in balloon), cooled to -78 ^{o}C and n-BuLi (8.97 ml, 1.6 M, 14.36 mmol) was added dropwise over a period of 5 min with constant stirring. After 30 min, 3-(1-(2,4-difluorophenyl)-1-oxopropan-2-yl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**86a**) (1 g, 2.87 mmol) in THF (10 ml) was added and stirred for 3 h at room temperature. THF was evaporated under reduced pressure using rotary evaporator, diluted with water (200 ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography yielded pure product as white solid (6.12 g, 69.4 %).

Nature: White solid; **MP:** 162 ^{o}C; **Yield:** 69.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.33 (t, *J* = 6 Hz, 3H), 1.64 (d, *J* = 6 Hz, 3H), 2.85 (q, *J* = 6 Hz, 2H), 5.59 (d, *J* = 2 Hz, 1H), 5.68 (d, *J* = 2 Hz, 1H), 6.20 (q, *J* = 6 Hz, 1H), 6.71-6.82 (m, 2H), 7.10 (s, 1H), 7.17-7.25 (m, 1H), 7.93 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.0, 18.7, 23.7, 51.0, 104.2 (t), 111.3 (d), 117.5, 119.7, 121.7 (d), 130.1 (d), 141.6 (2C), 142.9, 145.8, 156.4, 158.7 (dd), 161.7, 163.3 (dd); **IR** (Chloroform)**:** 1672 cm^{-1}; **MS** (ESI) *m*/*z***:** 347.1721 (M + 1); **Anal. Calcd for** $C_{18}H_{16}F_2N_2OS$**:** C, 62.41; H, 4.66; F, 10.97; N, 8.09 %. **Found:** C, 62.65; H, 4.87; F, 10.48; N, 7.96 %.

Preparation of 3-(1-(2-(2,4-difluorophenyl)oxiran-2-yl)ethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (85b)



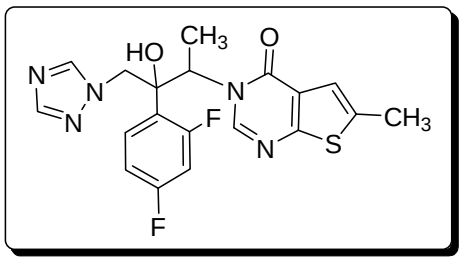
Sodium hydride (0.158 g, 6.89 mmol) in DMSO (30 ml) was taken in two necked round bottom flask equipped with guard tube, trimethylsulfoxonium iodide (0.688 g, 6.89 mmol) was added to it and stirred at room temperature for 15 min. 3-(1-(2,4-Difluorophenyl)-1-oxopropan-2-yl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**86b**) (1.9 g, 5.74 mmol) in

DMSO (20 ml) was added over a period of 5 min and the reaction mixture was stirred at room temperature for 8 h. It was then diluted with water (200 ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated under vacuum on rotary evaporator. Purification by column chromatography yielded pure product as white solid (1.4 g, 69 %).

Nature: White solid; **MP:** 130 ^oC; **Yield:** 69 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.15 (d, *J* = 8 Hz, 3H), 2.57 (s, 3H), 4.07-4.18 (m, 1H), 4.40-4.51 (m, 1H), 5.75 (q, *J* = 8 Hz, 1H), 6.87-7.02 (m, 2H), 7.19 (s, 1H), 7.57-7.69 (m, 1H), 8.52 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.3, 16.0, 30.5, 51.6, 66.3, 104.4 (t), 111.2 (d), 119.7 (d), 124.2, 126.7, 128.1 (d), 138.4, 144.8, 157.5, 161.2 (dd), 162.3, 163.9 (dd); **IR** (Chloroform)**:** 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 349.8457 (M + 1); **Anal. Calcd for** $C_{17}H_{14}F_2N_2O_2S$**:** C, 58.61; H, 4.05; F, 10.91; N, 8.04 %. **Found:** C, 58.50; H, 3.74; F, 11.10; N, 8.19 %.

Epoxide **85a** was prepared from the ketone **86a** using same procedure and used without purification for further reaction.

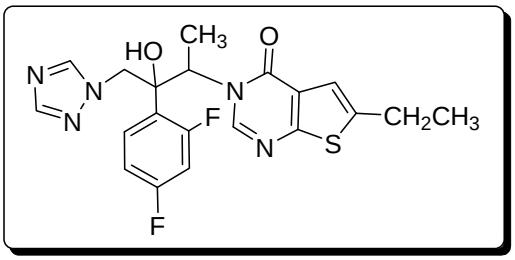
Preparation of 3-(3-(2,4-difluorophenyl)-3-hydroxy-4-(1*H*-1,2,4-triazol-1-yl)butan-2-yl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (84b)



3-(1-(2-(2,4-Difluorophenyl)oxiran-2-yl)ethyl)-6-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**85b**) (1.92 g, 5.54 mmol) in DMF (10 ml) was taken in two necked round bottom flask equipped with guard tube. Flame dried potassium carbonate (2.19 g, 16.56 mmol) and 1*H*-1,2,4-triazole (1.09 g, 16.56 mmol) were added to it. The mixture was stirred at 80 ^oC for 12 h, cooled, diluted with water (100 ml), extracted with ethyl acetate (3 X 50 ml), dried over Na_2SO_4 and concentrated under vacuum on rotary evaporator. Purification by column chromatography yielded pure product as white solid (1.44 g, 63 %).

Nature: White solid; **MP:** 223 ^{o}C; **Yield:** 63 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.28 (d, *J* = 8 Hz, 3H), 2.57 (s, 3H), 3.99 (d, *J* = 14 Hz, 1H), 5.19 (d, *J* = 14 Hz, 1H), 5.50 (bs, 1H), 5.97 (q, *J* = 8 Hz, 1H), 6.76-6.89 (m, 2H), 7.16 (s, 1H), 7.42-7.54 (m, 1H), 7.75 (s, 1H), 7.77 (s, 1H), 8.47 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 15.5, 16.0, 50.8, 54.3, 78.1, 104.4 (t), 111.9 (d), 119.4, 122.7 (d), 123.9, 130.4 (t), 138.9, 144.0, 145.1, 152.0, 157.7, 158.1 (dd), 162.6, 163.0 (dd); **IR** (Chloroform)**:** 3393, 1661 cm^{-1}; **MS** (ESI) *m*/*z***:** 417.5859 (M+); **Anal. Calcd for** $C_{19}H_{17}F_2N_5O_2S$**:** C, 54.67; H, 4.10; F, 9.10; N, 16.78 %. **Found:** C, 54.44; H, 3.95; F, 9.42; N, 16.94 %.

3-(3-(2,4-Difluorophenyl)-3-hydroxy-4-(1*H*-1,2,4-triazol-1-yl)butan-2-yl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (84a)

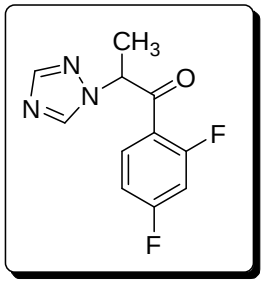


Nature: Off-white solid; **MP:** 214 ^{o}C; **Yield:** 51 % (over 2 steps); **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.28 (d, *J* = 8 Hz, 3H), 1.37 (t, *J* = 8 Hz, 3H), 2.90 (q, *J* = 8 Hz, 2H), 3.97 (d, *J* = 14 Hz, 1H), 5.19 (d, *J* = 14 Hz, 1H), 5.50 (bs, 1H), 5.97 (q, *J* = 8 Hz, 1H), 6.76-6.87 (m, 2H), 7.18 (s, 1H), 7.41-7.54 (m, 1H), 7.74 (s, 1H), 7.77 (s, 1H), 8.48 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 15.2, 15.5, 23.9, 50.7, 54.3, 78.0, 104.4 (t), 111.8 (d), 117.5, 122.8 (d), 123.6, 130.3 (t), 143.9, 145.0, 146.2, 151.9, 154.1 (dd), 157.7, 162.2, 162.9 (dd); **IR** (Chloroform)**:** 3310, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:** 432.1369 (M + 1); **Anal. Calcd for** $C_{20}H_{19}F_2N_5O_2S$**:** C, 55.67; H, 4.44; F, 8.81; N, 16.23 %. **Found:** C, 56.01; H, 4.17; F, 8.59; N, 16.13 %.

Preparation of 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)propan-1-one (90a) and 1-(2,4-difluorophenyl)-2-methyl-2-(1*H*-1,2,4-triazol-1-yl)propan-1-one (90b)

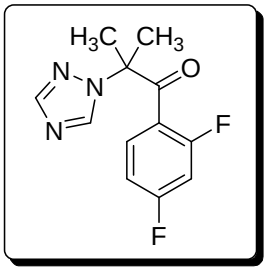
Sodium hydride (0.743 g, 0.0309 mol) and freshly distilled dry THF (20 ml) were taken in two necked round bottom flask equipped with guard tube, 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone (**42a**) (5 g, 0.022 mol) in freshly distilled dry THF (30 ml) was added to it and stirred at room temperature for 1 h. Iodomethane (1.75 ml, 4.01 g, 0.028 mol) was added over a period of 5 min and stirred at room temperature for 10 h. THF was evaporated under reduced pressure using rotary evaporator, reaction mixture was diluted with water (200 ml), extracted with dichloromethane (3 X 50 ml), dried over Na_2SO_4 and concentrated under vacuum. The residue containing a mixture of **90a** and **90b** was separated by column chromatography to obtain pure **90a** (1.22 g, 23 %) and **90b** (1.18 g, 21 %) in addition to 1.85 g mixture of **90a** and **90b**.

1-(2,4-Difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)propan-1-one (90a)[43b,g,h]



Nature: Pale yellow thick liquid; **Yield:** 23 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 1.80 (d, *J* = 8 Hz, 3H), 5.54 (q, *J* = 8 Hz, 1H), 6.83-7.03 (m, 2H), 7.83-7.95 (m, 2H), 8.33 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 17.0, 62.9, 104.7 (t), 112.9 (d), 119.1 (d), 133.5 (d), 143.0, 151.4, 161.4 (dd), 166.5 (dd), 191.4.

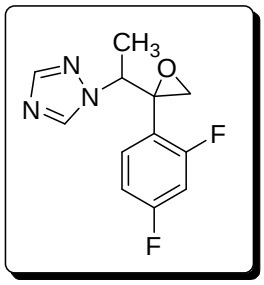
1-(2,4-Difluorophenyl)-2-methyl-2-(1*H*-1,2,4-triazol-1-yl)propan-1-one (90b)[43g,h]



Nature: Pale yellow solid; **MP:** 44-46 ºC; **Yield:** 21 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 2.01 (s, 6H), 6.72-6.92 (m, 2H), 7.16-7.27 (m, 1H), 7.92 (s, 1H), 8.35 (s, 1H).

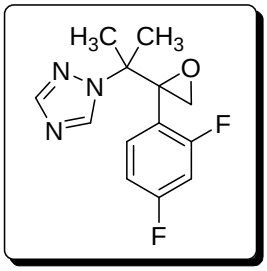
Epoxides **91a** and **91b** were prepared from the ketones **90a** and **90b** using the procedure given for the epoxide **43a**.

1-(1-(2-(2,4-Difluorophenyl)oxiran-2-yl)ethyl)-1*H*-1,2,4-triazole (91a)[43b,g,h]



Nature: White solid; **MP:** 89 ºC**; Yield:** 81 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 1.61 (d, *J* = 8 Hz, 3H), 2.64 (d, *J* = 4 Hz, 1H), 2.80 (d, *J* = 4 Hz, 1H), 4.92 (q, *J* = 8 Hz, 1H), 6.77-6.89 (m, 2H), 6.98-7.13 (m, 1H), 7.94 (s, 1H), 8.16 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.1, 51.7, 58.8, 103.7 (t), 111.6 (d), 119.2 (d), 130.1 (d), 142.5, 151.0, 158.9 (dd), 163.9 (dd); **IR** (Chloroform)**:** 1094 cm^{-1}.

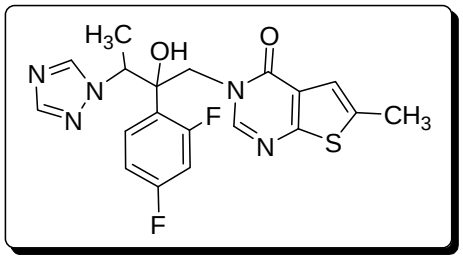
1-(2-(2-(2,4-Difluorophenyl)oxiran-2-yl)propan-2-yl)-1*H*-1,2,4-triazole (91b)[43b,g,h]



Nature: White solid; **MP:** 80 ºC; **Yield:** 78 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 1.92 (s, 6H), 3.36 (bs, 1H), 3.42 (bs, 1H), 6.66-6.84 (m, 2H), 7.09-7.20 (m, 1H), 7.83 (s, 1H), 8.25 (s, 1H); **IR** (Chloroform)**:** 1099 cm^{-1}.

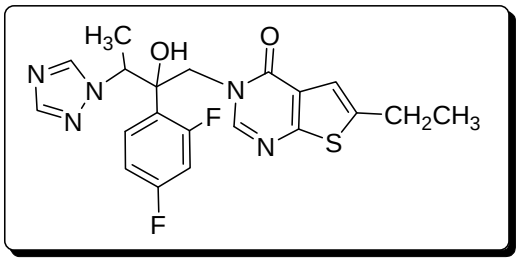
The fluconazole analogues **77a-77i** were prepared by reacting epoxide **91a** or **91b** with suitable thienopyrimidinone using the procedure given for the compound **76e**.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6 methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77a)



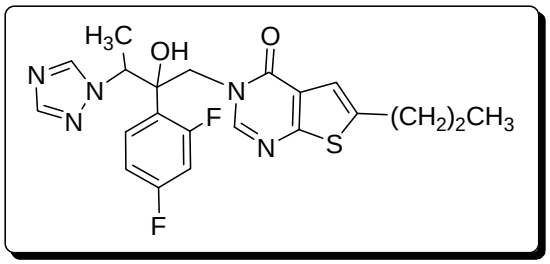
Nature: White solid; **MP:** 176 ºC; **Yield:** 67.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.33 (d, *J* = 8 Hz, 3H), 2.46 (s, 3H), 3.92 (d, *J* = 14 Hz, 1H), 4.49 (d, *J* = 14 Hz, 1H), 5.22 (q, *J* = 8 Hz, 1H), 6.77-6.89 (m, 3H), 6.93 (s, 1H), 7.61-7.74 (m, 2H), 7.95 (s, 1H), 8.54 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 15.7, 15.9, 56.2, 61.4, 77.4, 104.2 (t), 112.2 (d), 118.8, 122.6 (d), 123.7, 130.6 (d), 139.6, 143.2, 145.1, 150.4, 157.8 (dd), 159.2, 162.7, 163.7 (dd); **IR** (Chloroform)**:** 3446, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 418.3419 (M + 1), 440.3413 (M + Na), 456.3327 (M + K); **Anal. Calcd for** $C_{19}H_{17}F_2N_5O_2S$**:** C, 54.67; H, 4.10; F, 9.10; N, 16.78 %. **Found:** C, 54.82; H, 3.93; F, 9.25; N, 16.67 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77b)



Nature: White solid; **MP:** 171 ^{o}C; **Yield:** 64.8 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 1.25-1.34 (m, 6H), 2.81 (q, *J* = 6 Hz, 2H), 3.95 (d, *J* = 14 Hz, 1H), 4.49 (d, *J* = 14 Hz, 1H), 5.24 (q, *J* = 6 Hz, 1H), 6.78-6.84 (m, 3H), 6.99 (s, 1H), 7.62-7.76 (m, 2H), 7.98 (s, 1H), 8.55 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.9, 15.8, 23.8, 56.2, 61.6, 77.5, 104.2 (t), 112.3 (d), 116.9, 122.6 (d), 123.5, 130.6 (d), 145.1 (2C), 147.2, 150.4, 157.8 (dd), 159.3, 162.9, 163.0 (dd); **IR** (Chloroform)**:** 3279, 1667 cm^{-1}; **MS** (ESI) *m*/*z***:** 471.8992 (M + 2); **Anal. Calcd for** $C_{20}H_{19}F_2N_5O_2S$**:** C, 55.67; H, 4.44; F, 8.81; N, 16.23 %. **Found:** C, 55.49; H, 4.62; F, 9.02; N, 16.11 %.

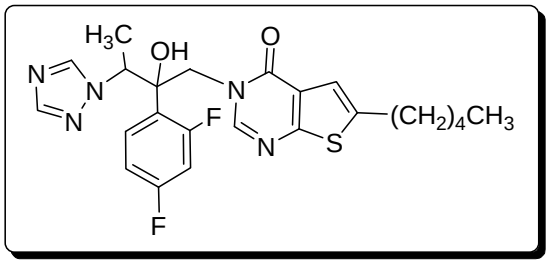
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77c)



Nature: White solid; **MP:** 178 ^{o}C; **Yield:** 66.5 %; **^{1}H NMR** ($CDCl_3$, 200 MHz)**:** δ 0.96 (t, *J* = 8 Hz, 3H), 1.35 (d, *J* = 6 Hz, 3H), 1.60-1.78 (m, 2H), 2.76 (t, *J* = 8 Hz, 2H), 2.92 (bs, 1H), 3.97 (d, *J* = 16 Hz, 1H), 4.50 (d, *J* = 16 Hz, 1H), 5.27 (q, *J* = 6 Hz, 1H), 6.80-6.89 (m,

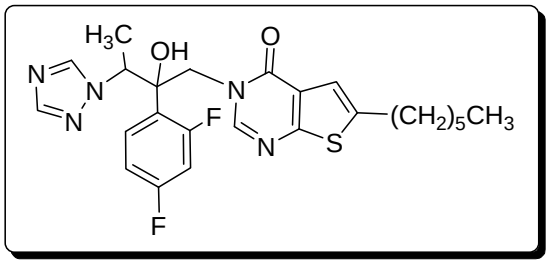
2H), 6.99 (s, 1H), 7.62-7.74 (m, 2H), 8.05 (s, 1H), 8.67 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.4, 15.7, 24.0, 32.4, 56.1, 61.5, 77.6, 104.2 (t), 112.2 (d), 117.6, 122.6 (d), 123.4, 130.6 (d), 143.2, 145.1, 145.5, 150.4, 158.5 (dd), 159.4, 162.4 (dd), 162.9; **IR** (Chloroform)**:** 3389, 1672 cm^{-1}; **MS** (ESI) *m*/*z***:** 446.2289 (M + 1); **Anal. Calcd for** $C_{21}H_{21}F_2N_5O_2S$**:** C, 56.62; H, 4.75; F, 8.53; N, 15.72 %. **Found:** C, 56.89; H, 4.91; F, 8.20; N, 15.58 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77d)



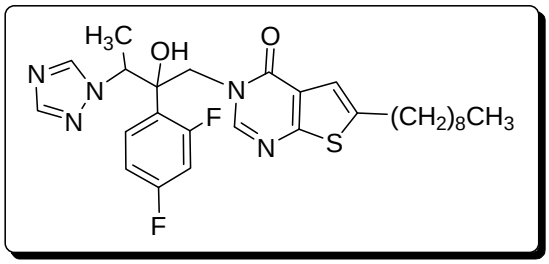
Nature: White solid; **MP:** 118 ^{o}C; **Yield:** 67.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$+ CCl_4)**:** δ 0.88 (t, *J* = 8 Hz, 3H), 1.26-1.39 (m, 7H), 1.53-1.73 (m, 2H), 2.77 (t, *J* = 6 Hz, 2H), 3.68 (bs, 1H), 3.98 (d, *J* = 14 Hz, 1H), 4.51 (d, *J* = 14 Hz, 1H), 5.27 (q, *J* = 8 Hz, 1H), 6.80-6.89 (m, 2H), 6.98 (s, 1H), 7.61-7.73 (m, 2H), 8.08 (s, 1H), 8.74 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 15.8, 22.2, 30.5 (2C), 31.0, 56.2, 61.8, 76.4, 104.3 (t), 112.4 (d), 117.6, 122.5 (d), 123.5, 130.8 (d), 143.2, 145.1, 145.9, 149.8, 157.6 (dd), 159.5, 162.6 (dd), 162.9; **IR** (Chloroform)**:** 3379, 1673 cm^{-1}; **MS** (ESI) *m*/*z***:** 474.3755 (M +1), 496.3517 (M + Na); **Anal. Calcd for** $C_{23}H_{25}F_2N_5O_2S$**:** C, 58.34; H, 5.32; F, 8.02; N, 14.79 %. **Found:** C, 58.67; H, 5.46; F, 7.78; N, 14.82 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77e)



Nature: Colorless glassy solid; **MP:** 105 ^{o}C; **Yield:** 68.9 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.86 (t, *J* = 6 Hz, 3H), 1.22-1.38 (m, 9H), 1.57-1.68 (m, 2H), 2.77 (t, *J* = 8 Hz, 2H), 3.98 (d, *J* = 14 Hz, 1H), 4.51 (d, *J* = 14 Hz, 1H), 5.27 (q, *J* = 8 Hz, 1H), 6.79-6.88 (m, 2H), 6.98 (s, 1H), 7.61-7.73 (m, 2H), 8.08 (s, 1H), 8.75 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 15.7, 22.3, 28.5, 30.5, 30.7, 31.3, 56.1, 61.7, 77.5, 104.2 (t), 112.3 (d), 117.5, 122.4 (d), 123.5, 130.6 (d), 143.2, 145.2, 145.8, 150.2, 157.7 (dd), 159.4, 162.8 (dd), 162.9; **IR** (Chloroform)**:** 3405, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 488.2552 (M + 1), 510.2458 (M + Na); **Anal. Calcd for** $C_{24}H_{27}F_2N_5O_2S$**:** C, 59.12; H, 5.58; F, 7.79; N, 14.36 %. **Found:** C, 59.21; H, 5.74; F, 7.61; N, 14.22 %.

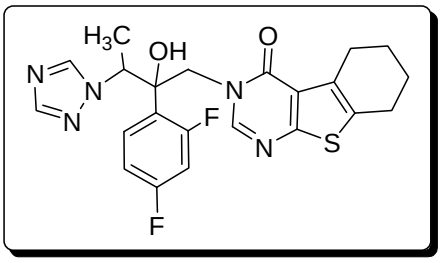
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-nonylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77f)



Nature: White solid; **MP:** 102 ^{o}C; **Yield:** 63.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 0.85 (t, *J* = 6 Hz, 3H), 1.19-1.35 (m, 13H), 1.55-1.73 (m, 4H), 2.83 (t, *J* = 8 Hz, 2H), 3.96 (d, *J* = 14 Hz, 1H), 4.51 (d, *J* = 14 Hz, 1H), 5.25 (q, *J* = 6 Hz, 1H), 6.77-6.86 (m, 2H), 6.96 (s, 1H),

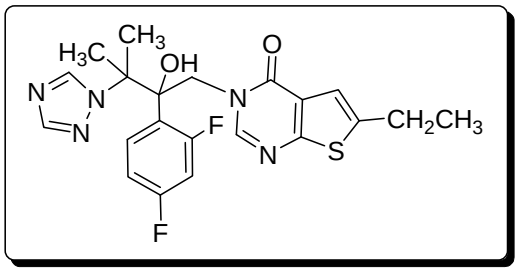
7.13 (s, 1H), 7.59-7.71 (m, 1H), 8.04 (s, 1H), 8.67 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 15.7, 22.5, 28.8, 29.1 (2C), 29.3, 30.5, 30.7, 30.9, 56.1, 61.8, 77.4, 104.2 (t), 112.1 (d), 117.5, 124.8 (d), 130.6 (d), 142.9 (2C), 144.7, 145.8, 159.4, 162.9, 163.4 (dd), 164.1, 166.6 (dd); **IR** (Chloroform)**:** 3344, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 568.92 (M + 1); **Anal. Calcd for** $C_{27}H_{33}F_2N_5O_2S$**:** C, 61.23; H, 6.28; F, 7.17; N, 13.22 %. **Found:** C, 61.07; H, 6.36; F, 7.22; N, 13.17 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)butyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (77g)



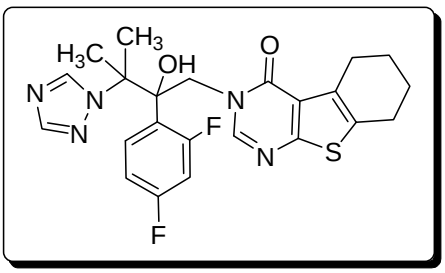
Nature: White solid; **MP:** 212 ^{o}C; **Yield:** 68.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.32 (d, *J* = 6 Hz, 3H), 1.72-1.88 (m, 4H), 2.69-2.89 (m, 4H), 3.97 (d, *J* = 14 Hz, 1H), 4.44 (d, *J* = 14 Hz, 1H), 5.24 (q, *J* = 6 Hz, 1H), 6.80-6.91 (m, 3H), 7.58 (s, 1H), 7.62-7.74 (m, 1H), 7.99 (s, 1H), 8.56 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.8, 21.9, 22.5, 25.1, 25.3, 55.9, 61.6, 76.4, 104.3 (t), 112.4 (d), 121.7, 122.6 (d), 130.7 (d), 131.1, 135.3, 143.1, 144.9, 150.1, 158.6 (dd), 160.1, 162.6, 163.7 (dd); **IR** (Chloroform)**:** 3444, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 458.4910 (M + 1); **Anal. Calcd for** $C_{22}H_{21}F_2N_5O_2S$**:** C, 57.76; H, 4.63; F, 8.31; N, 15.31 %. **Found:** C, 57.53; H, 4.89; F, 8.24; N, 15.39 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-methyl-3-(1*H*-1,2,4-triazol-1-yl)butyl)-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (77h)



Nature: White solid; **MP:** 139-141 ^{o}C; **Yield:** 61.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.40 (t, *J* = 8 Hz, 3H), 1.79 (s, 3H), 1.88 (s, 3H), 2.92 (q, *J* = 8 Hz, 2H), 4.61 (d, *J* = 14 Hz, 1H), 5.11 (d, *J* = 14 Hz, 1H), 6.70-6.79 (m, 1H), 6.81-6.99 (m, 1H), 7.11 (s, 1H), 7.39 (bs, 1H), 7.82-7.95 (m, 2H), 8.14 (s, 1H), 8.30 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 15.0, 23.5 (2C), 23.8, 54.6, 67.1, 79.6, 103.3 (t), 112.2 (d), 116.9, 121.2 (d), 123.5, 132.8 (d), 142.9, 146.0, 147.0, 150.2, 157.8, 160.1 (dd), 162.9, 163.3 (dd); **IR** (Chloroform)**:** 3221, 1667 cm^{-1}; **MS** (ESI) *m*/*z***:** 446.8782 (M + 1); **Anal. Calcd for** $C_{21}H_{21}F_2N_5O_2S$**:** C, 56.62; H, 4.75; F, 8.53; N, 15.72 %. **Found:** C, 56.46; H, 4.90; F, 8.75; N, 15.37 %.

3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-methyl-3-(1*H*-1,2,4-triazol-1-yl)butyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (77i)

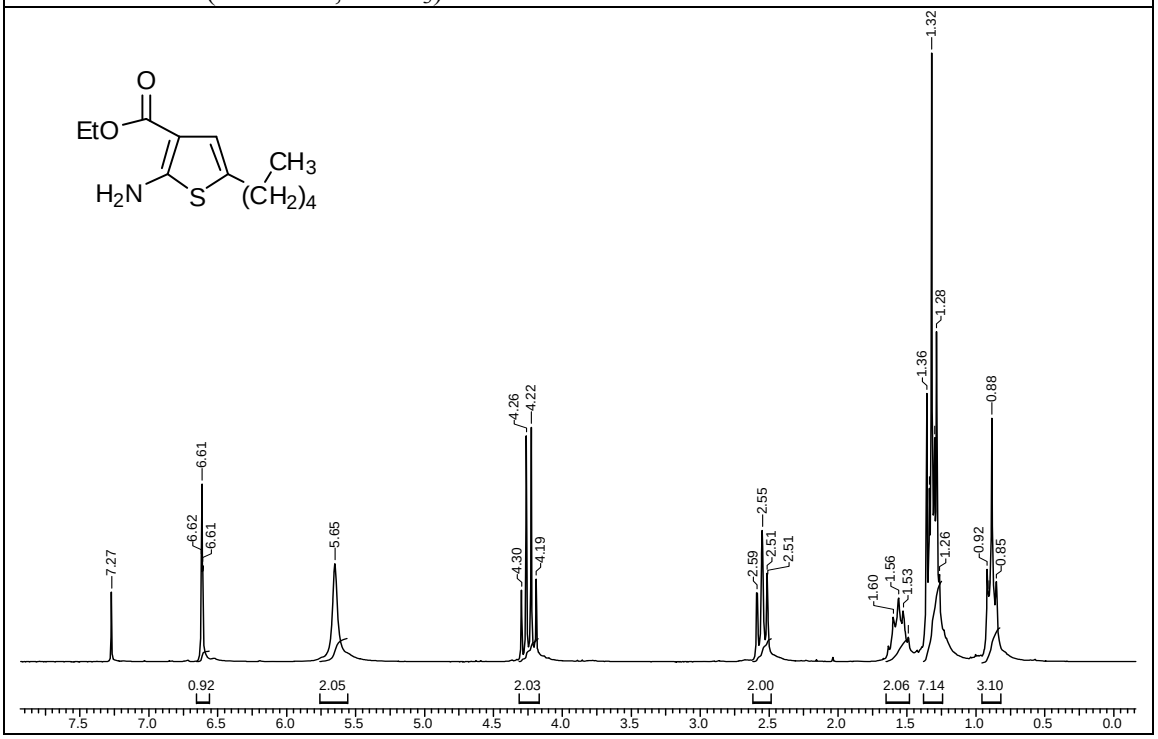


Nature: White solid; **MP:** 199-201 ^{o}C; **Yield:** 65.2 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 1.65-1.78 (m, 10H), 2.68-2.96 (m, 4H), 4.51 (d, *J* = 14 Hz, 1H), 4.97 (d, *J* = 14 Hz, 1H), 6.59-6.70 (m, 1H), 6.82-6.89 (m, 1H), 7.31 (bs, 1H), 7.78-7.89 (m, 2H), 7.96 (s, 1H), 8.12 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3 + CCl_4$)**:** δ 22.0, 22.6, 23.6 (2C), 25.1, 25.4, 54.1, 67.0,

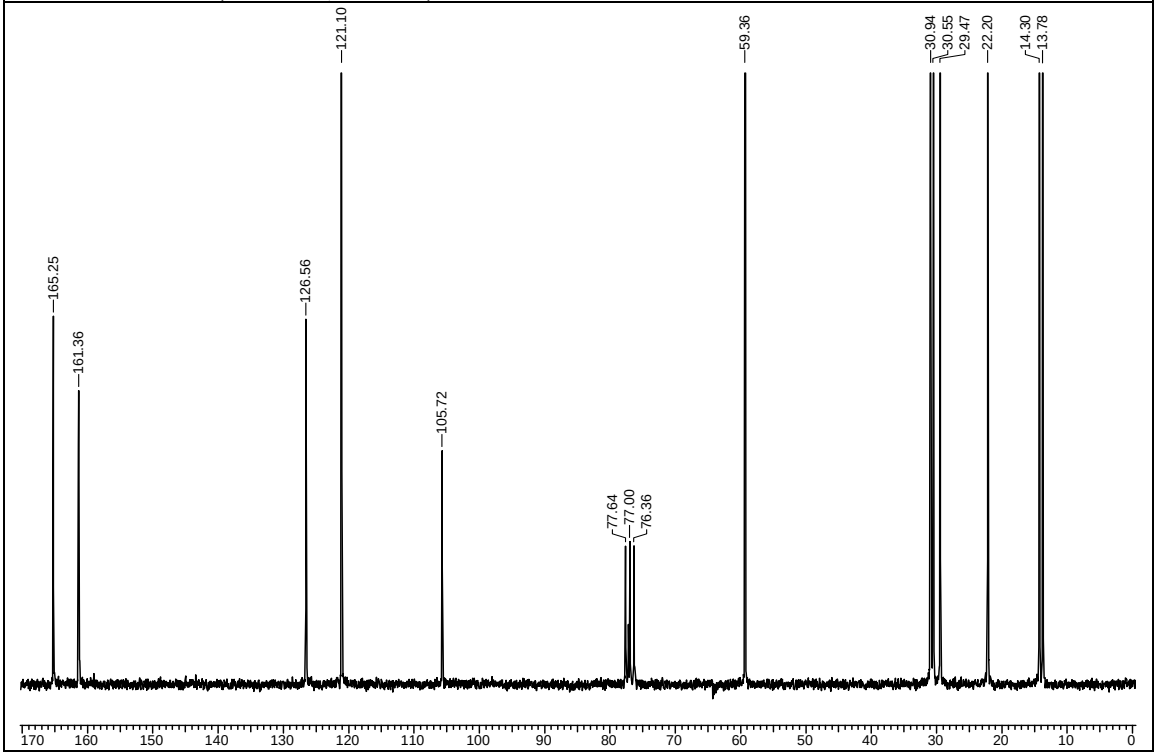
79.8, 103.9 (t), 111.8 (d), 121.3 (d), 121.7, 131.1, 133.0, 135.2, 145.7, 145.9, 150.5, 157.6 (dd), 160.7, 162.5 (dd), 162.7; **IR** (Chloroform)**:** 3445, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 510.3703 (M + 1); **Anal. Calcd for** $C_{23}H_{23}F_2N_5O_2S$**:** C, 58.59; H, 4.92; F, 8.06; N, 14.85 %. **Found:** C, 58.61; H, 5.17; F, 8.25; N, 14.66 %.

1.1.7 Selected spectra

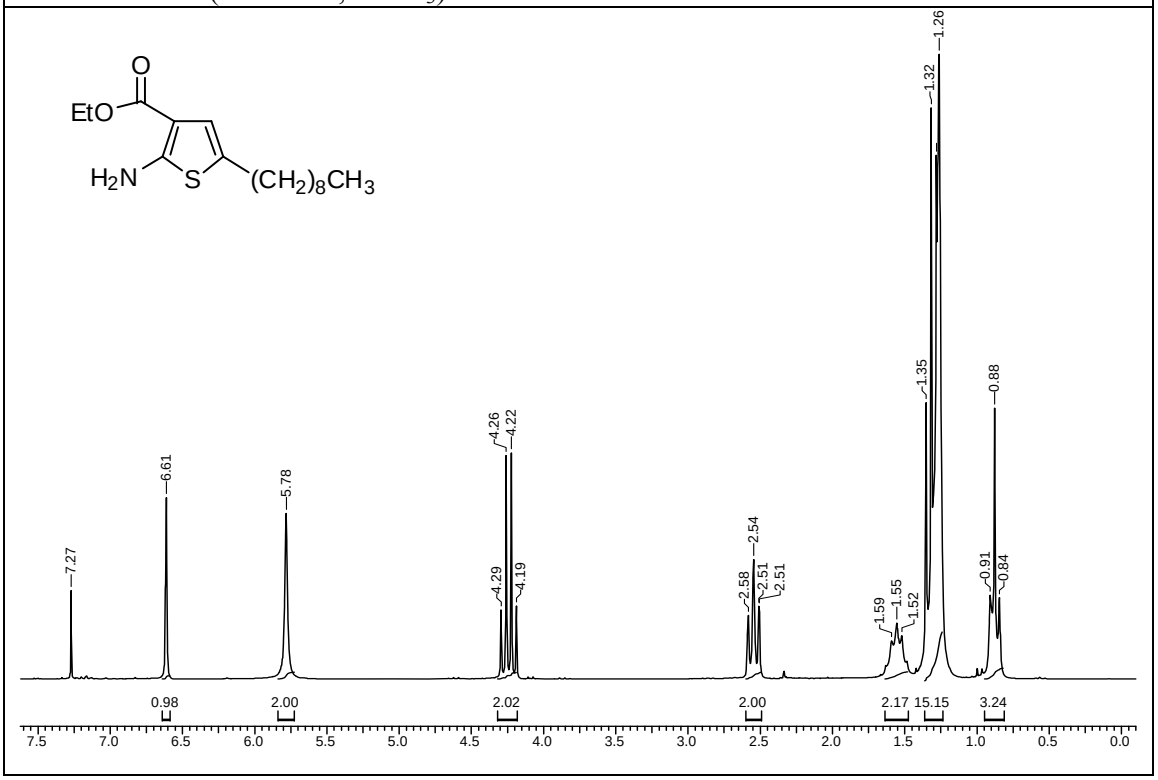
79e: ^{1}H NMR (200 MHz, $CDCl_3$)



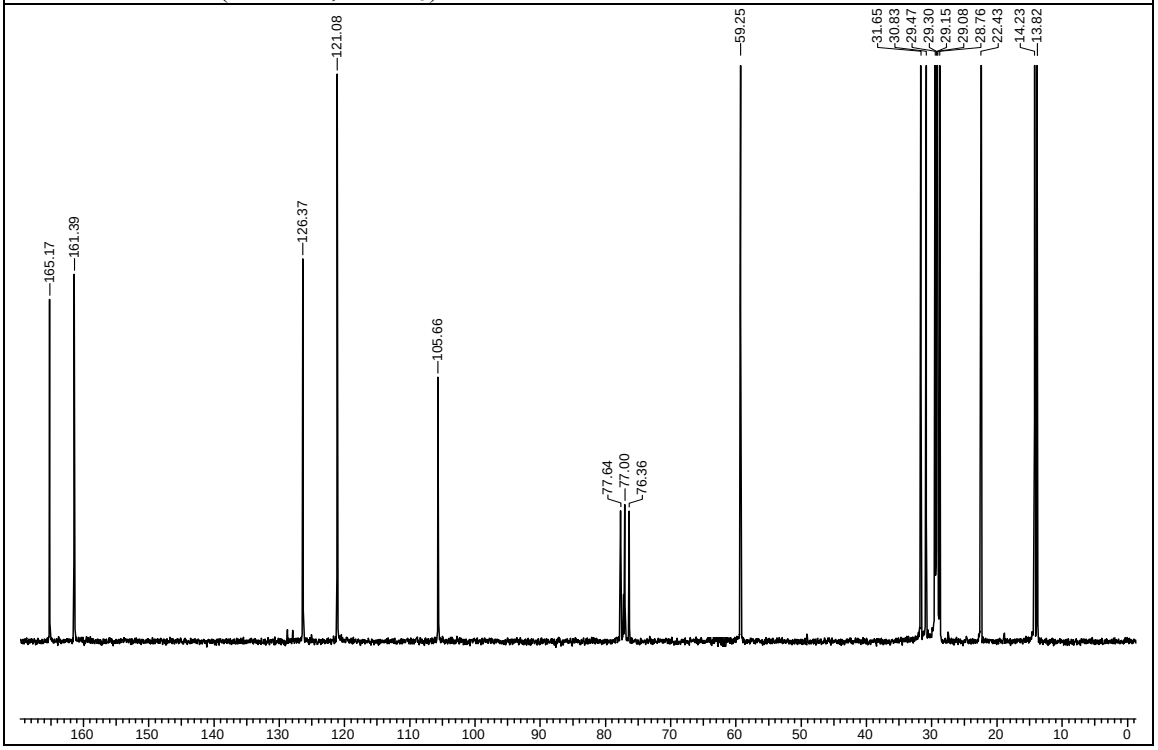
79e: ^{13}C NMR (50 MHz, $CDCl_3$)



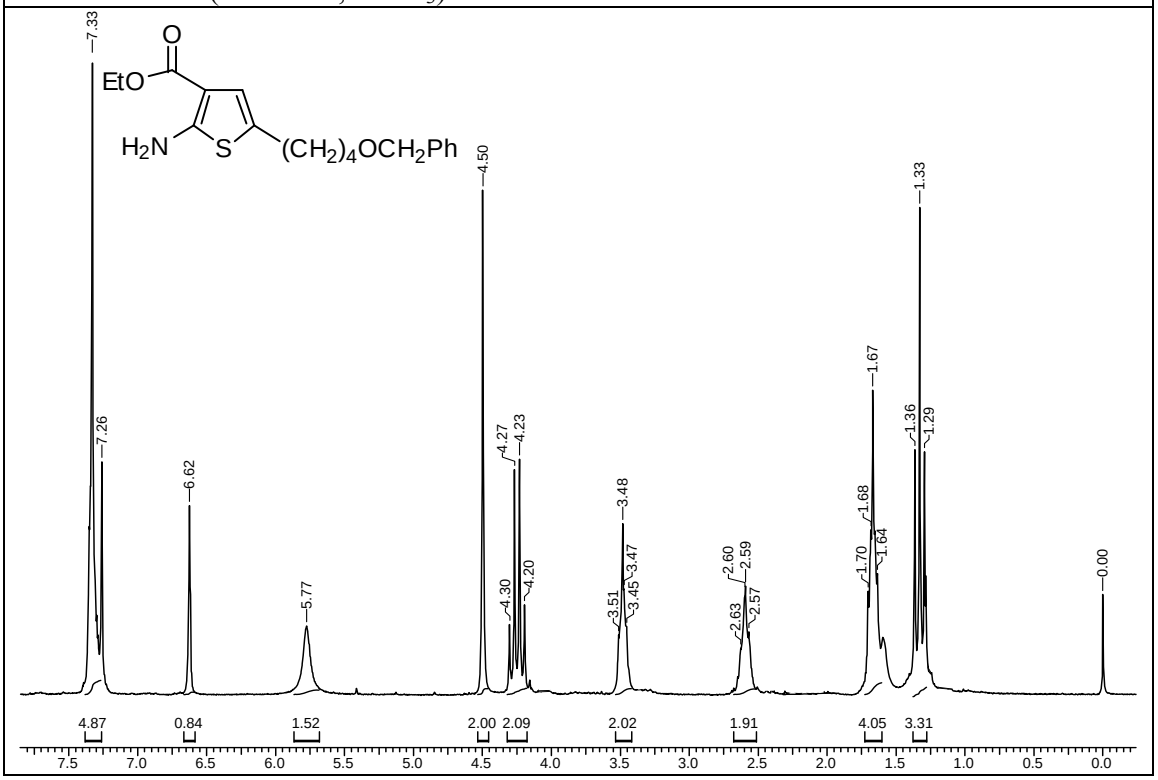
79h: ^{1}H NMR (200 MHz, $CDCl_3$)



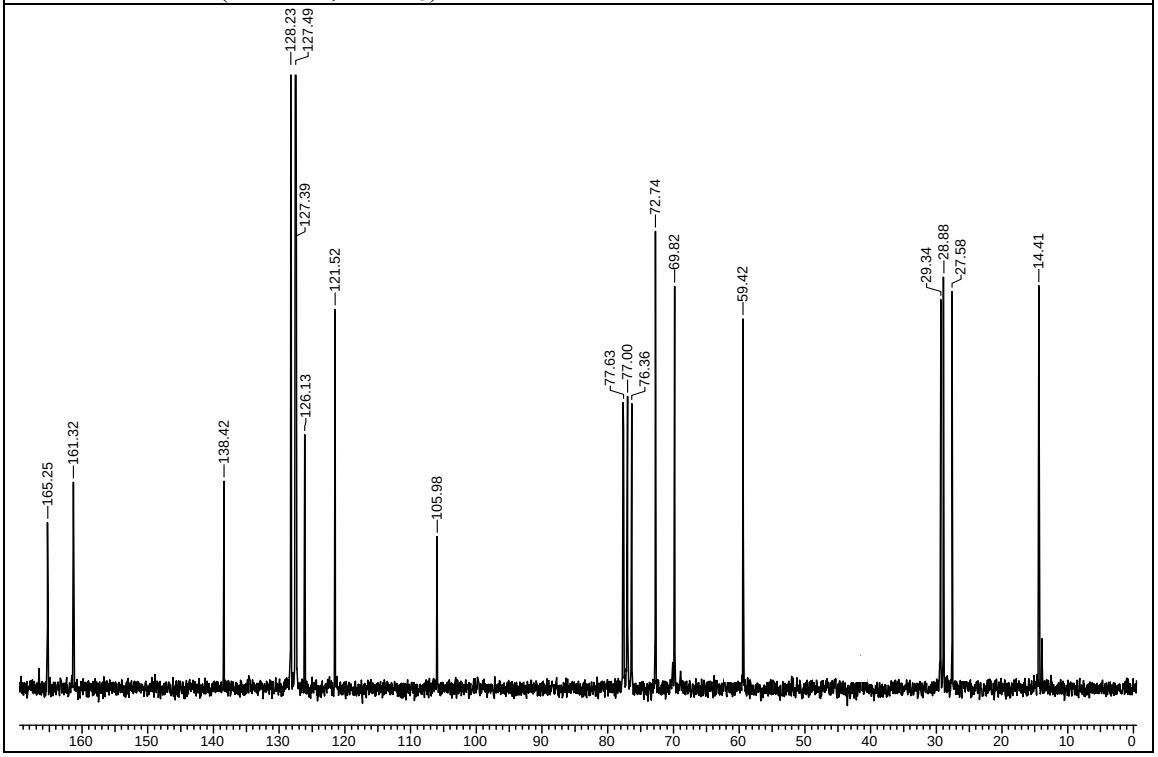
79h: ^{13}C NMR (50 MHz, $CDCl_3$)



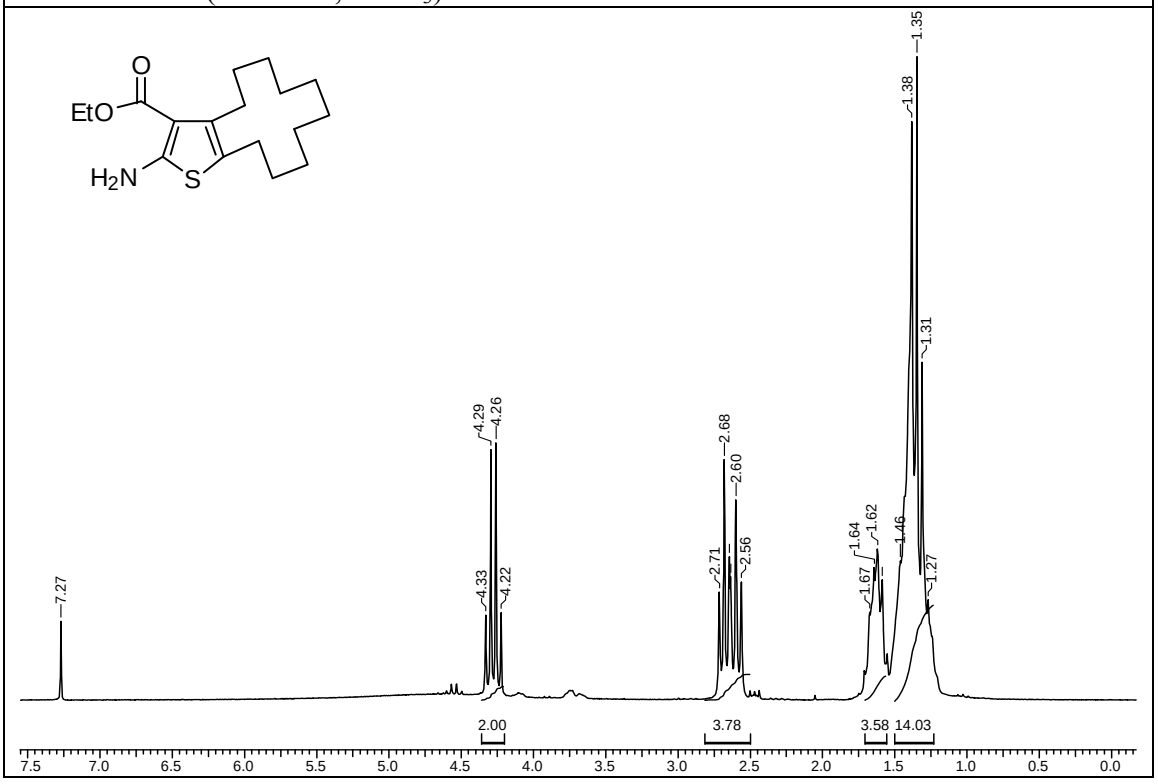
79n: ^{1}H NMR (200 MHz, $CDCl_3$)



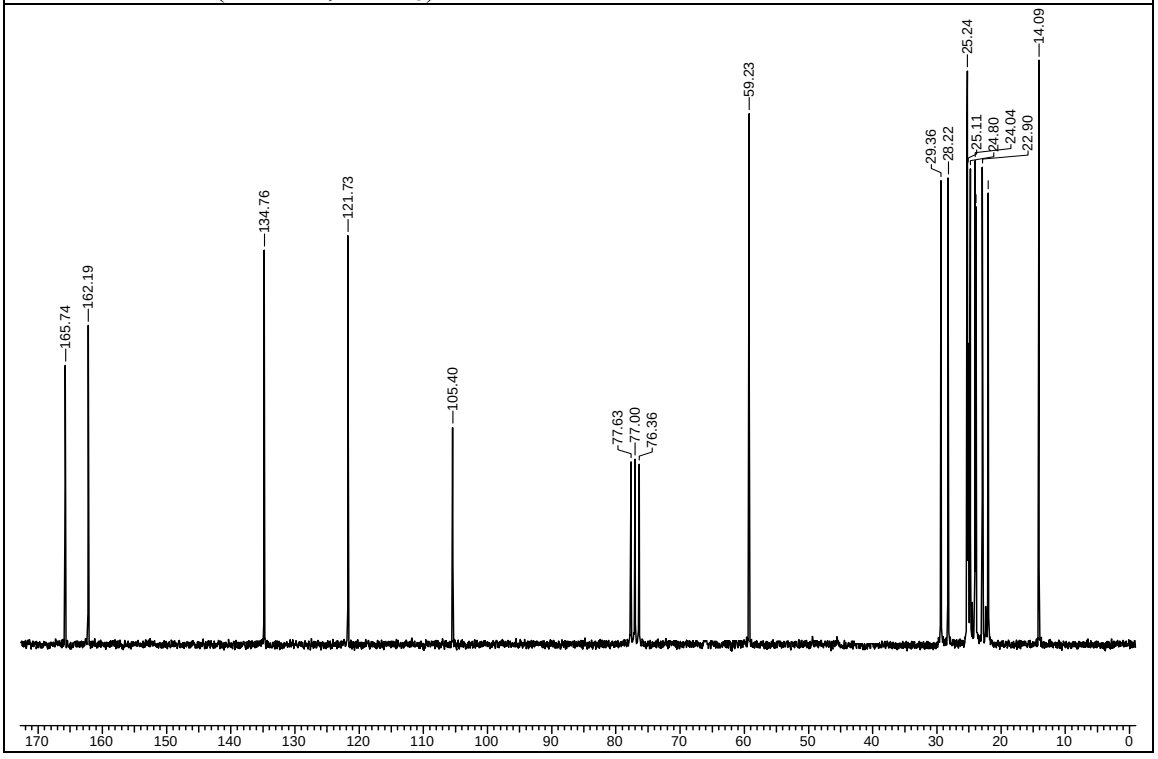
79n: ^{13}C NMR (50 MHz, $CDCl_3$)



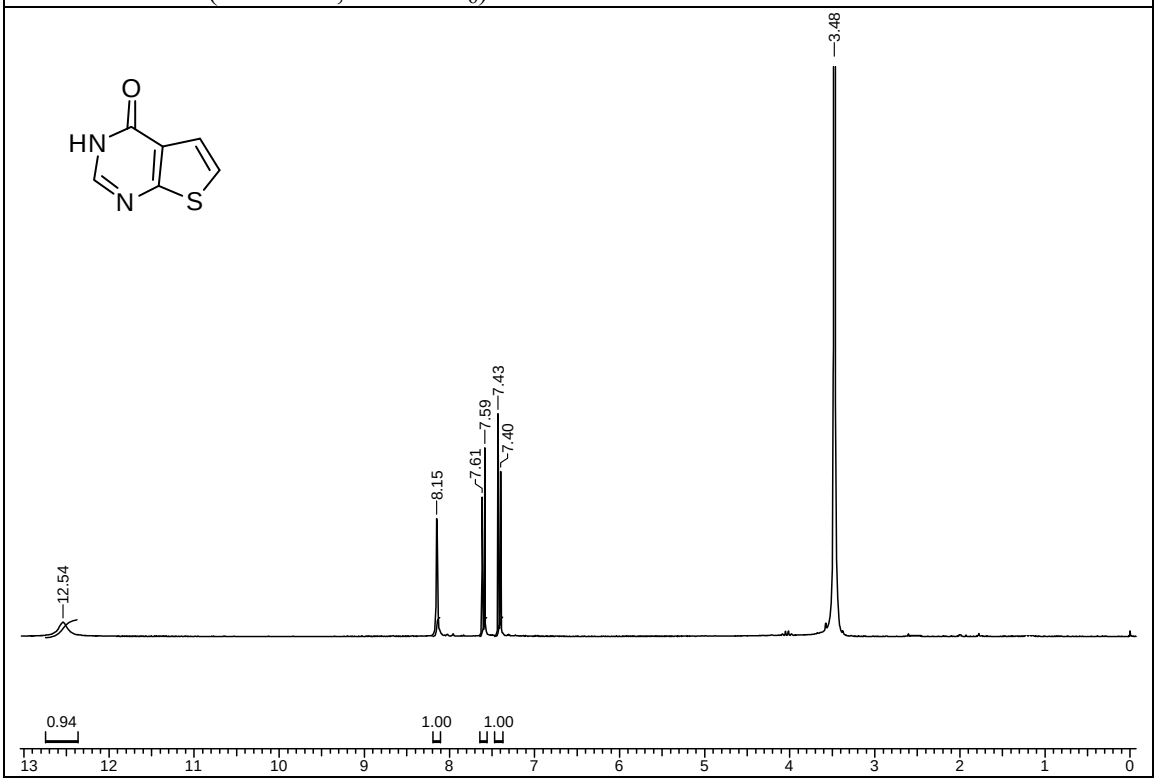
79r: ^{1}H NMR (200 MHz, $CDCl_3$)



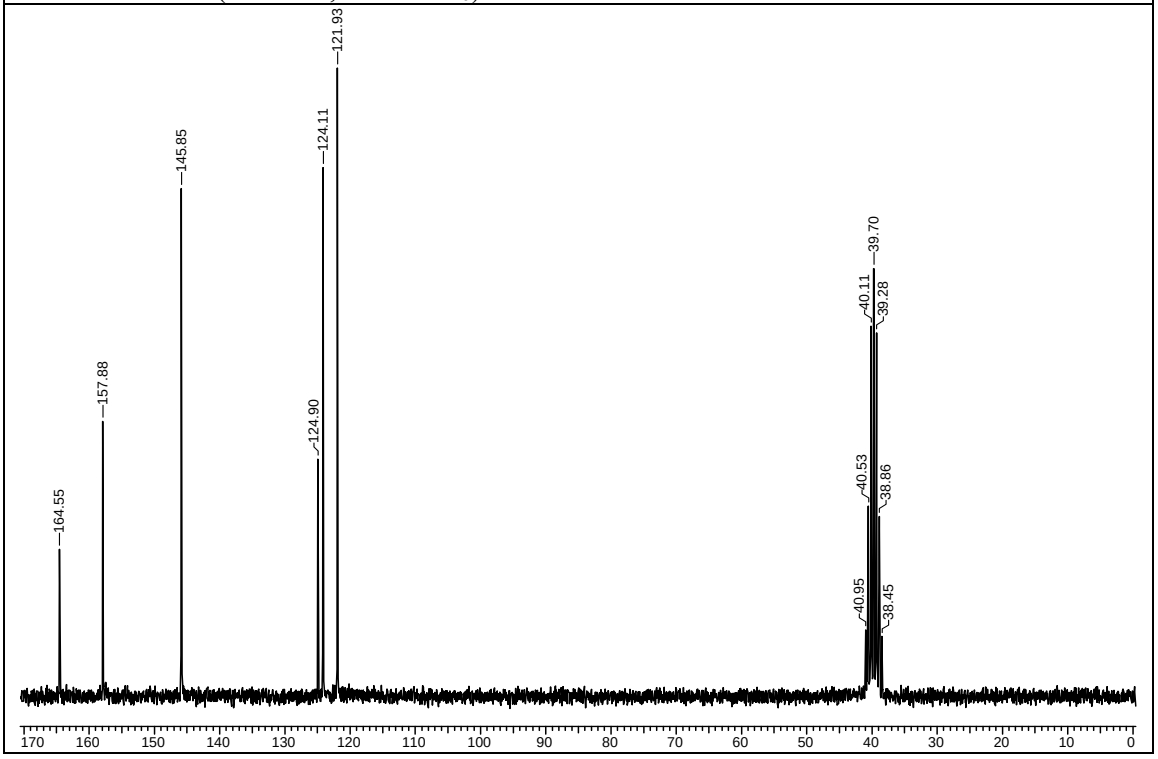
79r: ^{13}C NMR (50 MHz, $CDCl_3$)



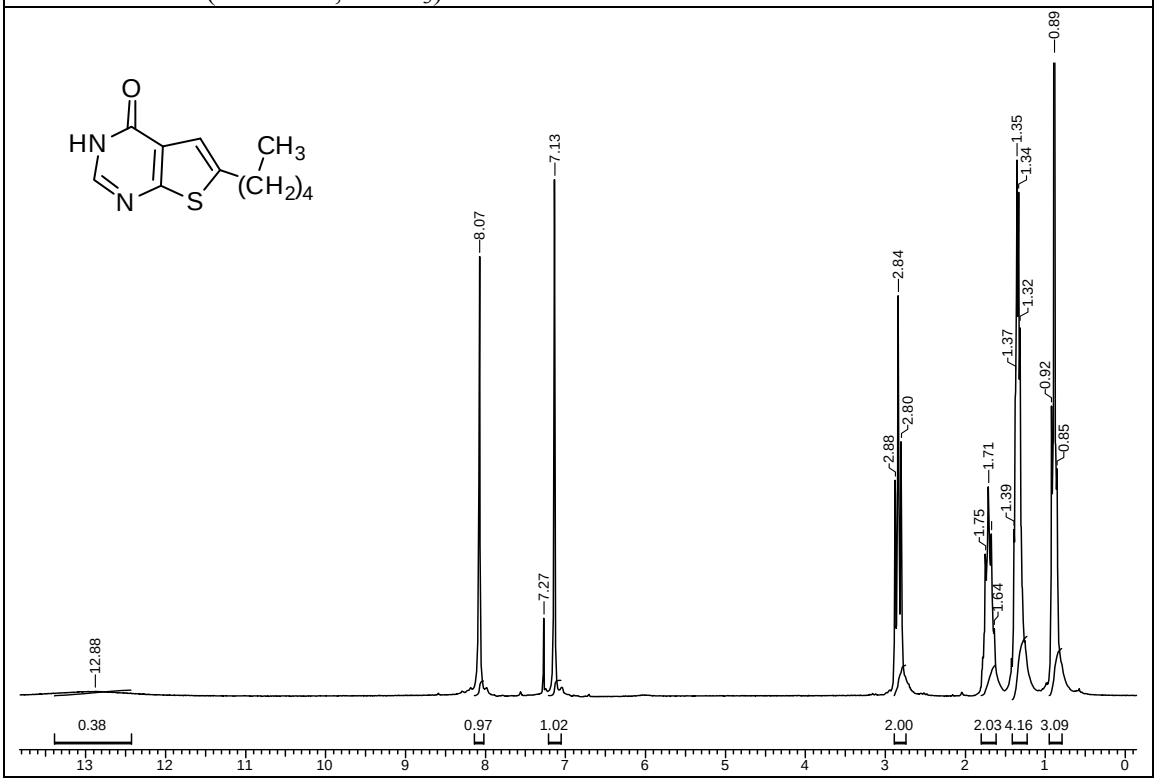
78a: ^{1}H NMR (200 MHz, DMSO-d_6)



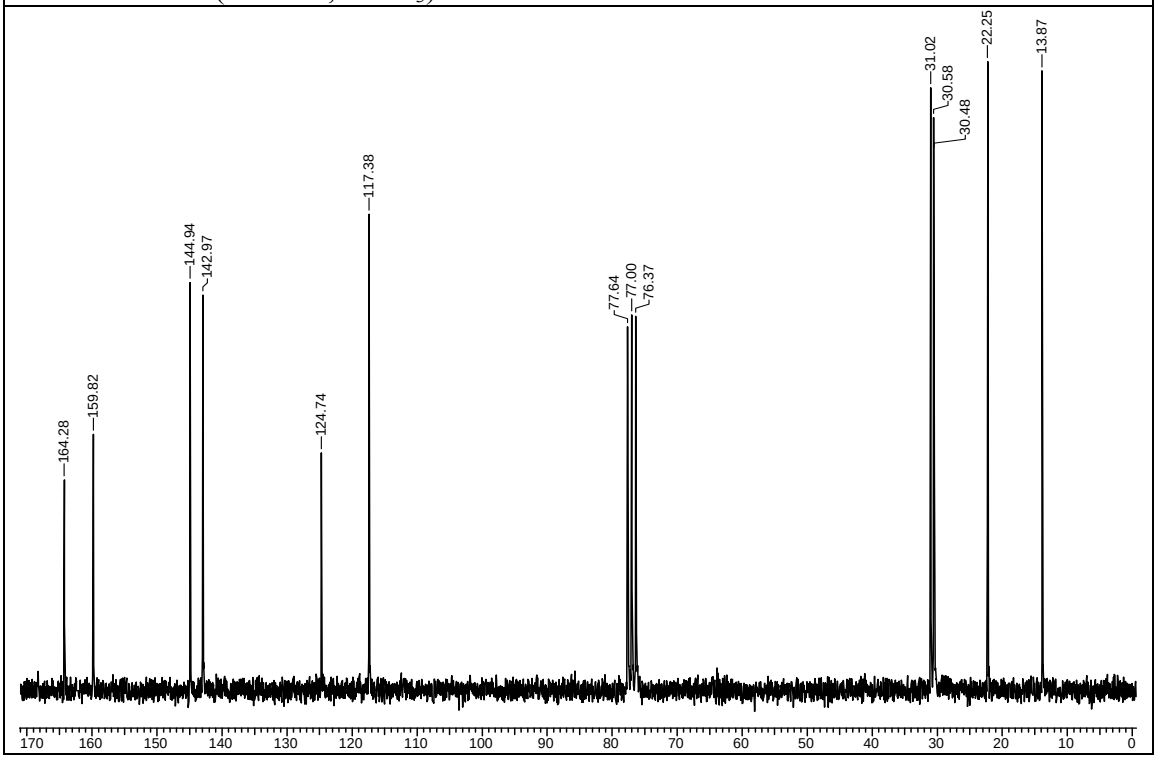
78a: ^{13}C NMR (50 MHz, DMSO-d_6)



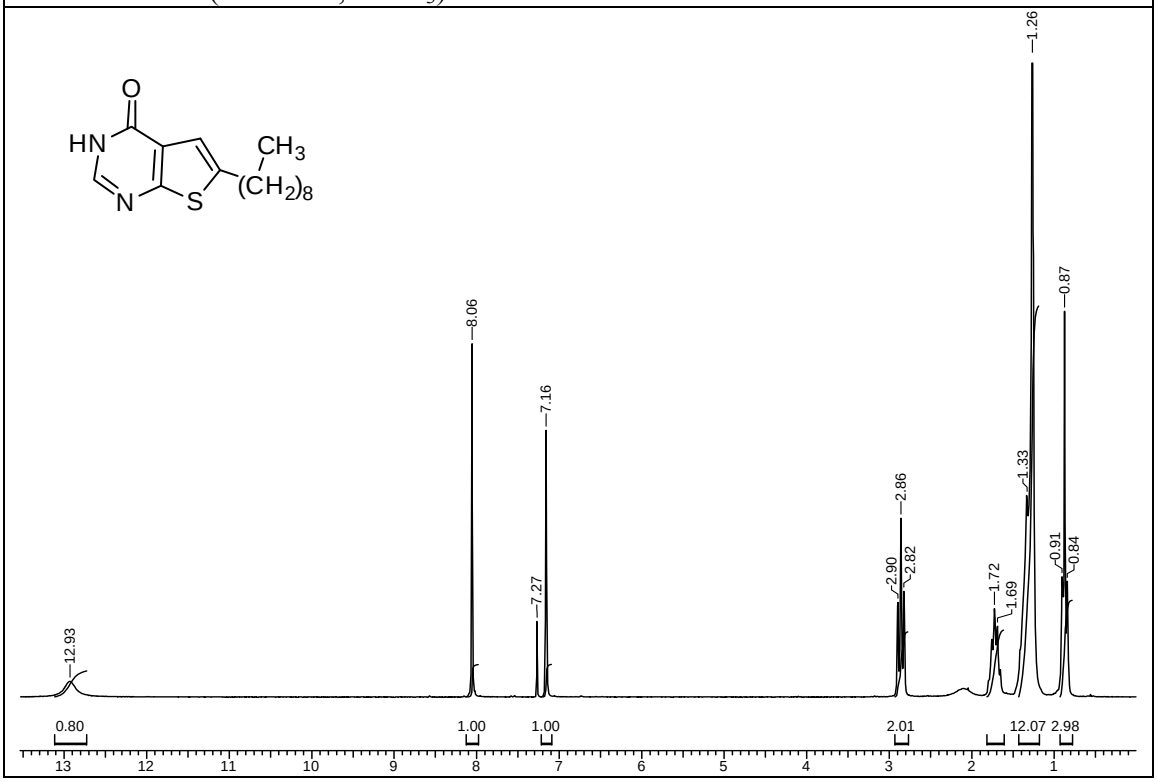
78e: ^{1}H NMR (200 MHz, $CDCl_3$)



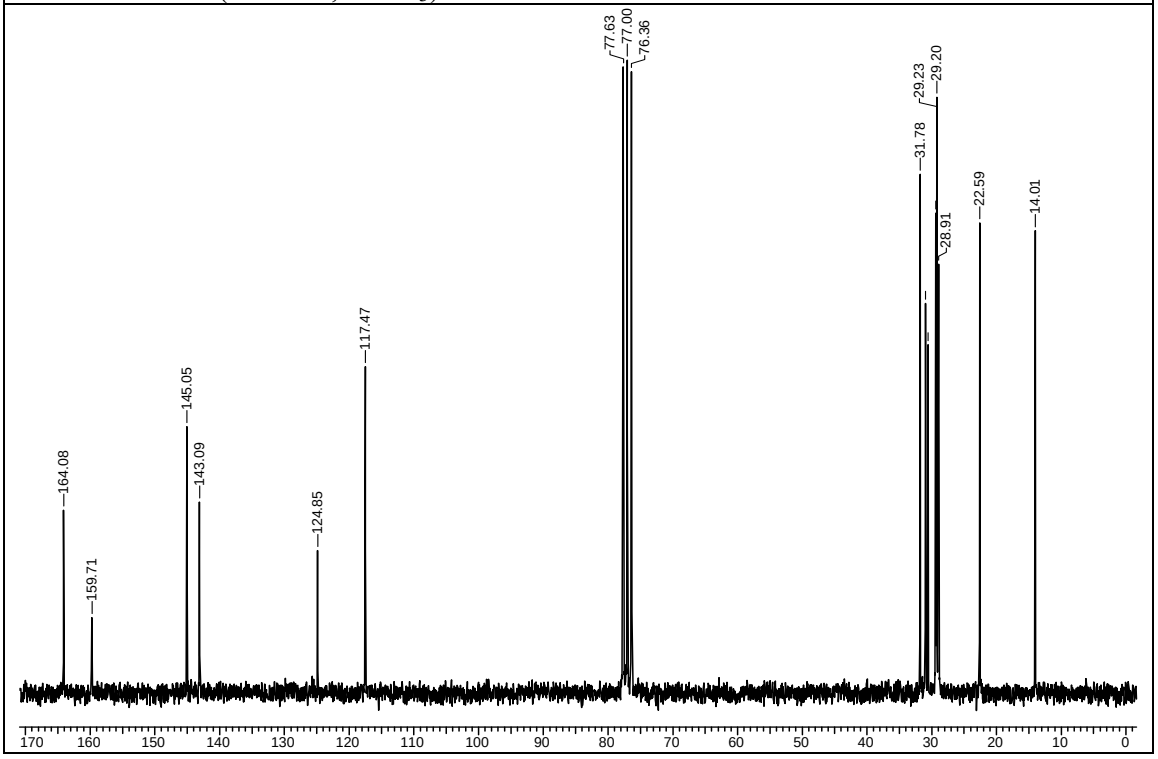
78e: ^{13}C NMR (50 MHz, $CDCl_3$)



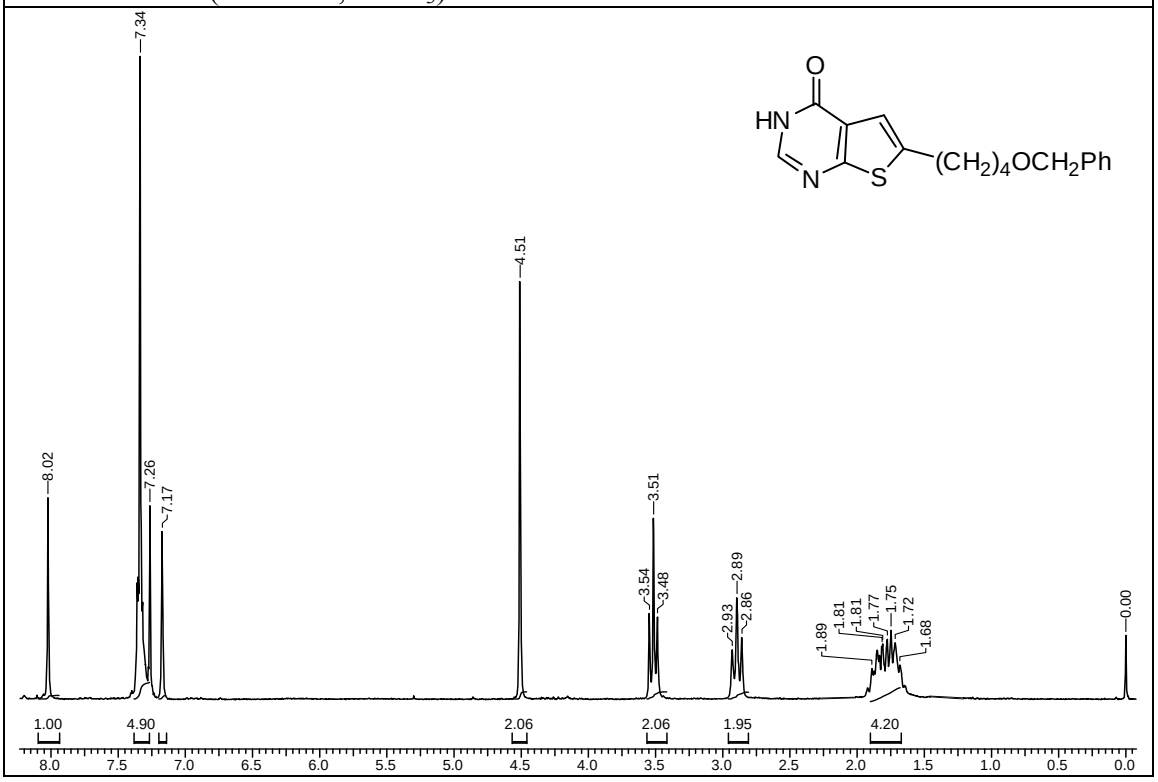
78h: ^{1}H NMR (200 MHz, $CDCl_3$)



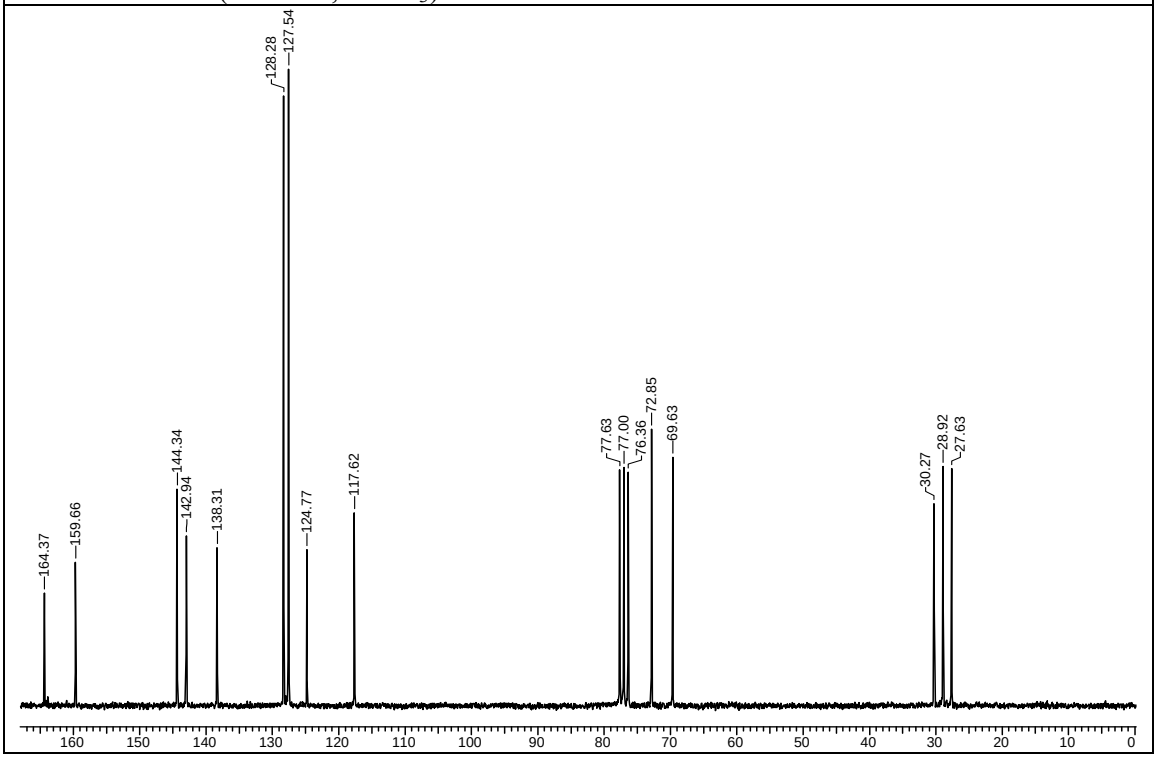
78h: ^{13}C NMR (50 MHz, $CDCl_3$)



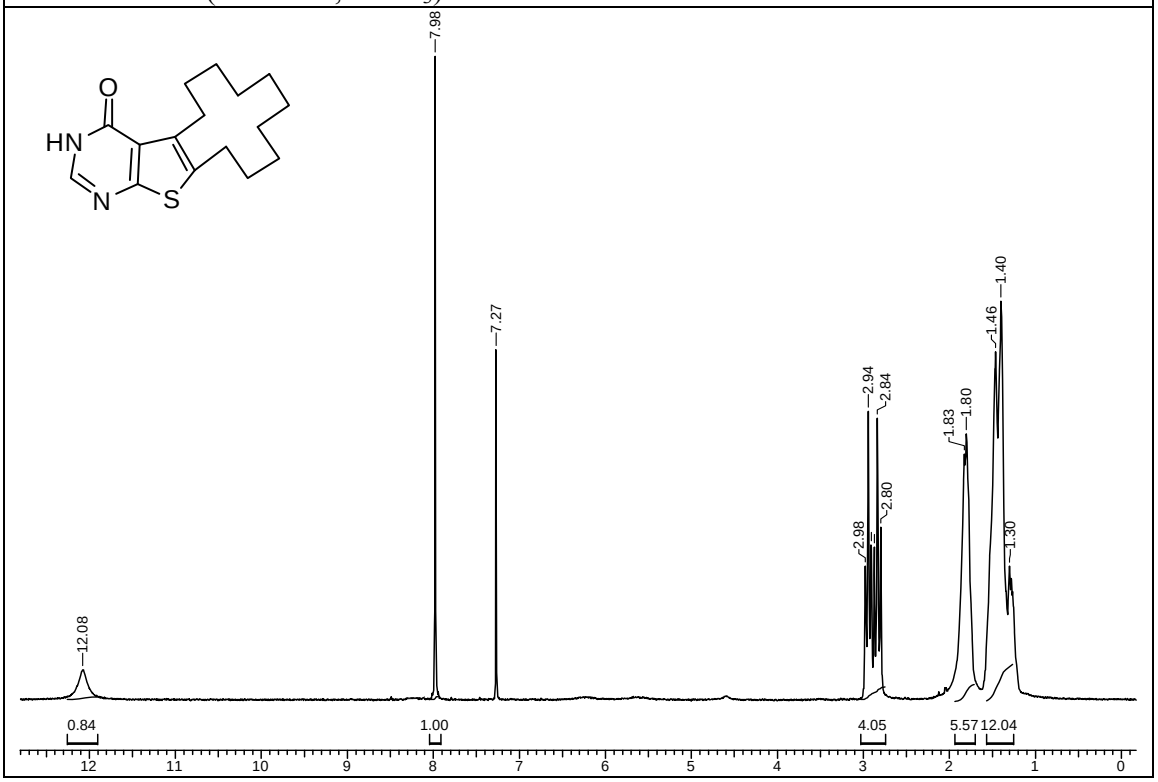
78n: ^{1}H NMR (200 MHz, $CDCl_3$)



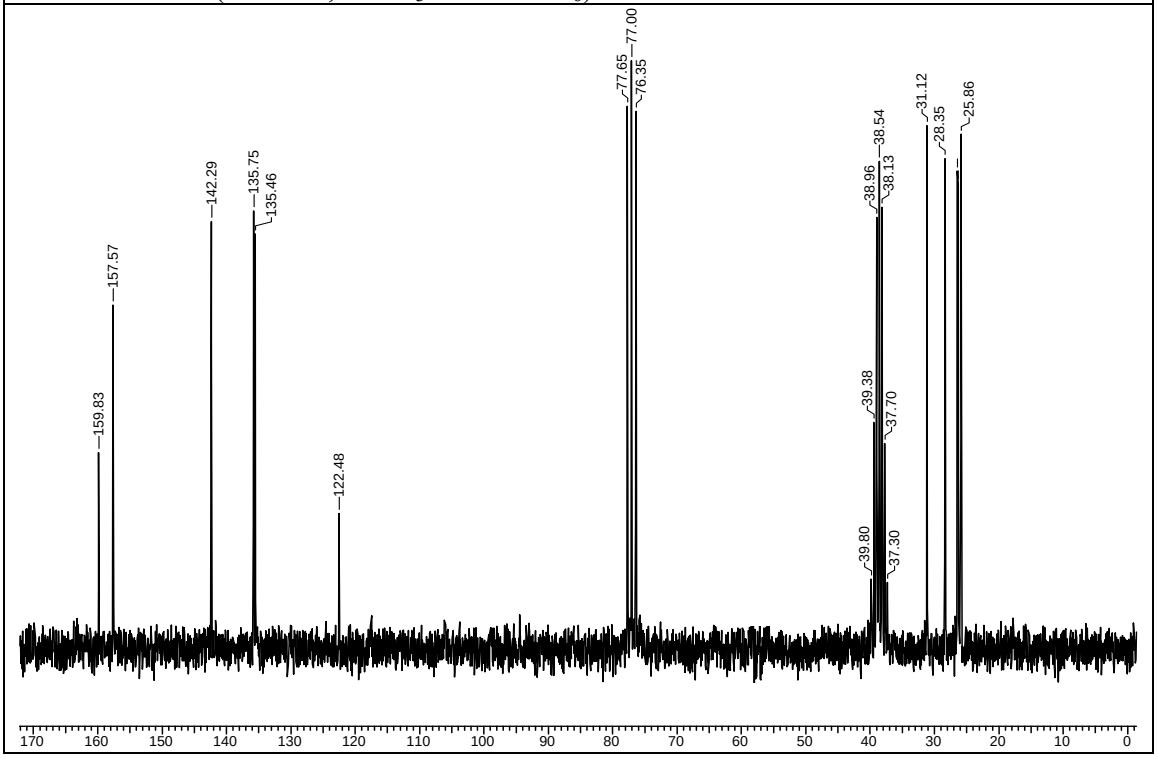
78n: ^{13}C NMR (50 MHz, $CDCl_3$)



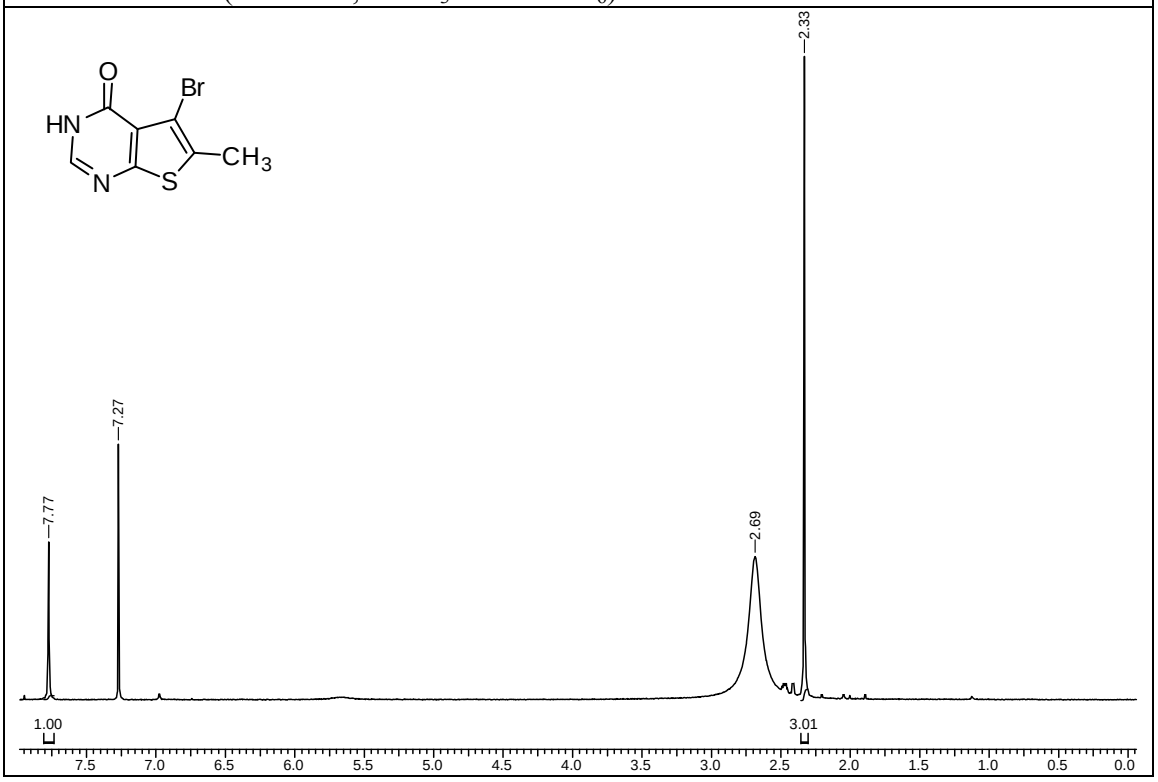
78r: ^{1}H NMR (200 MHz, $CDCl_3$)



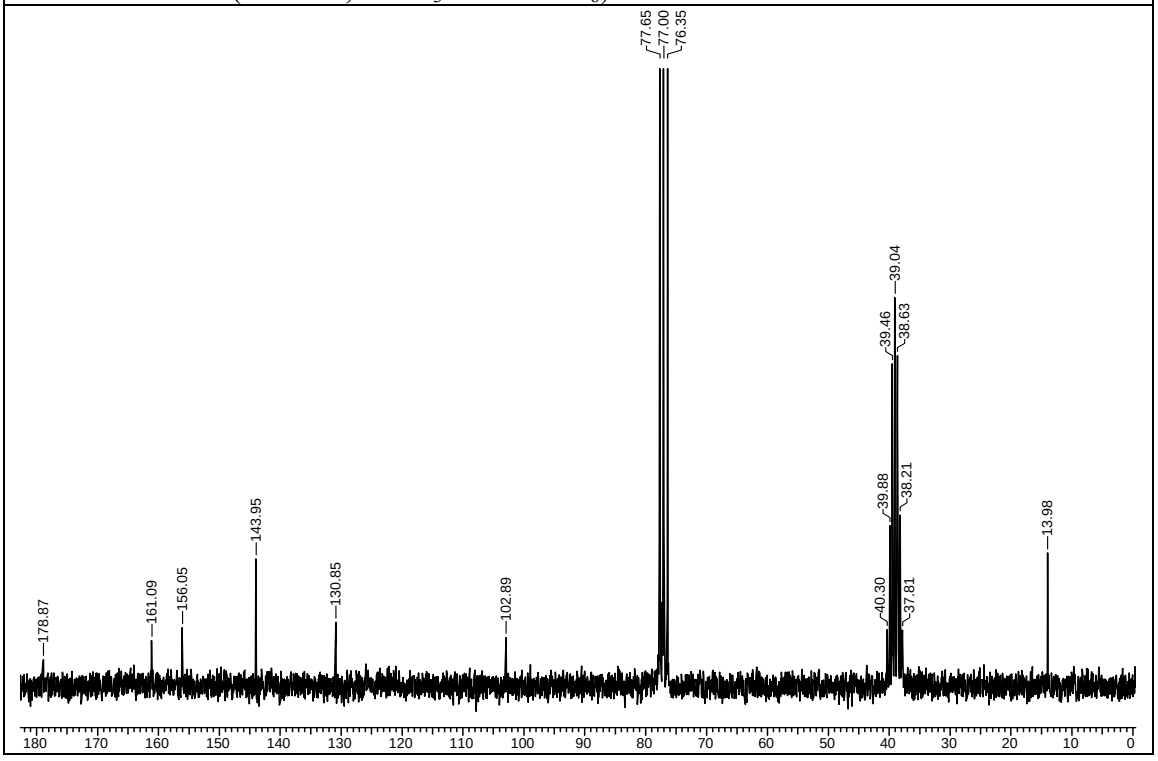
78r: ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)



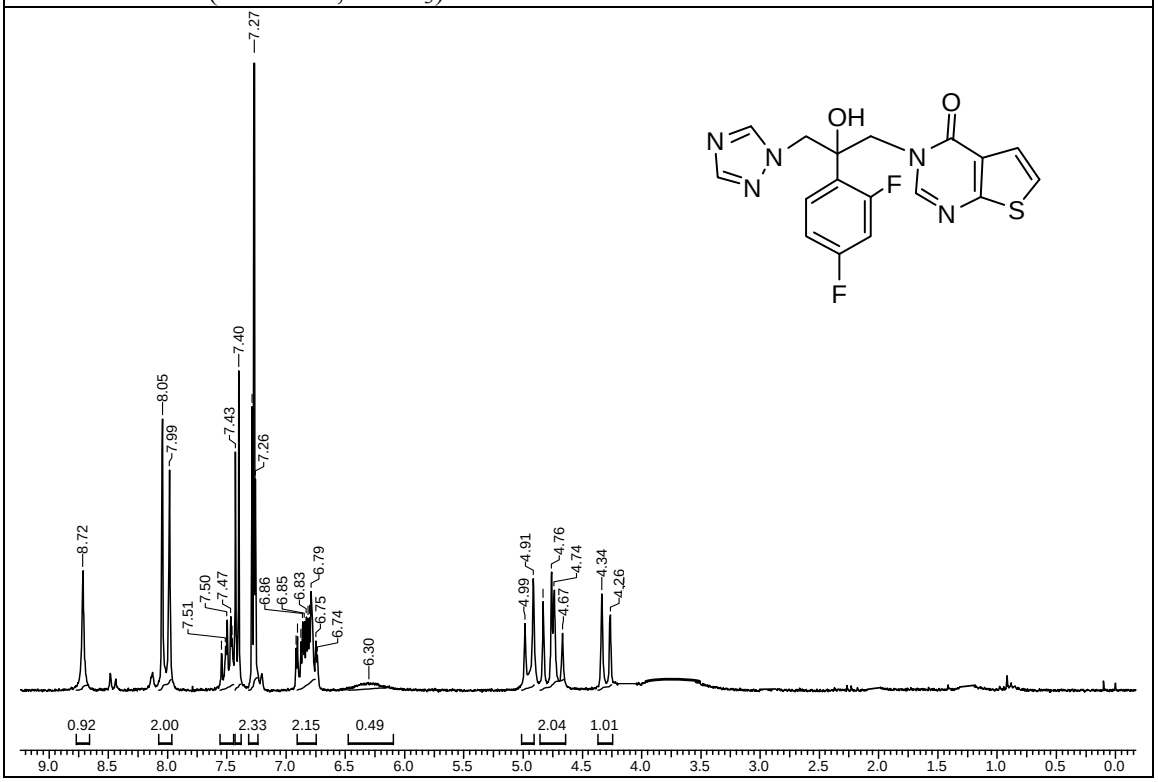
78aa: ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6)



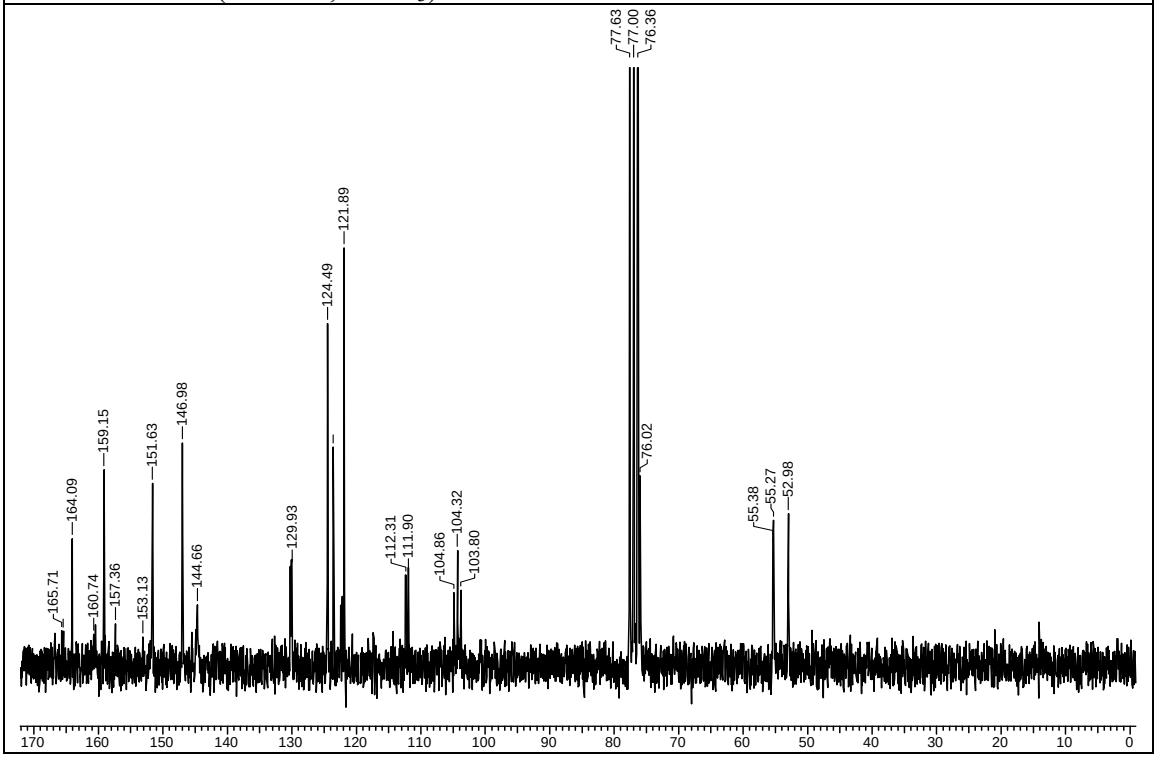
78aa: ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)



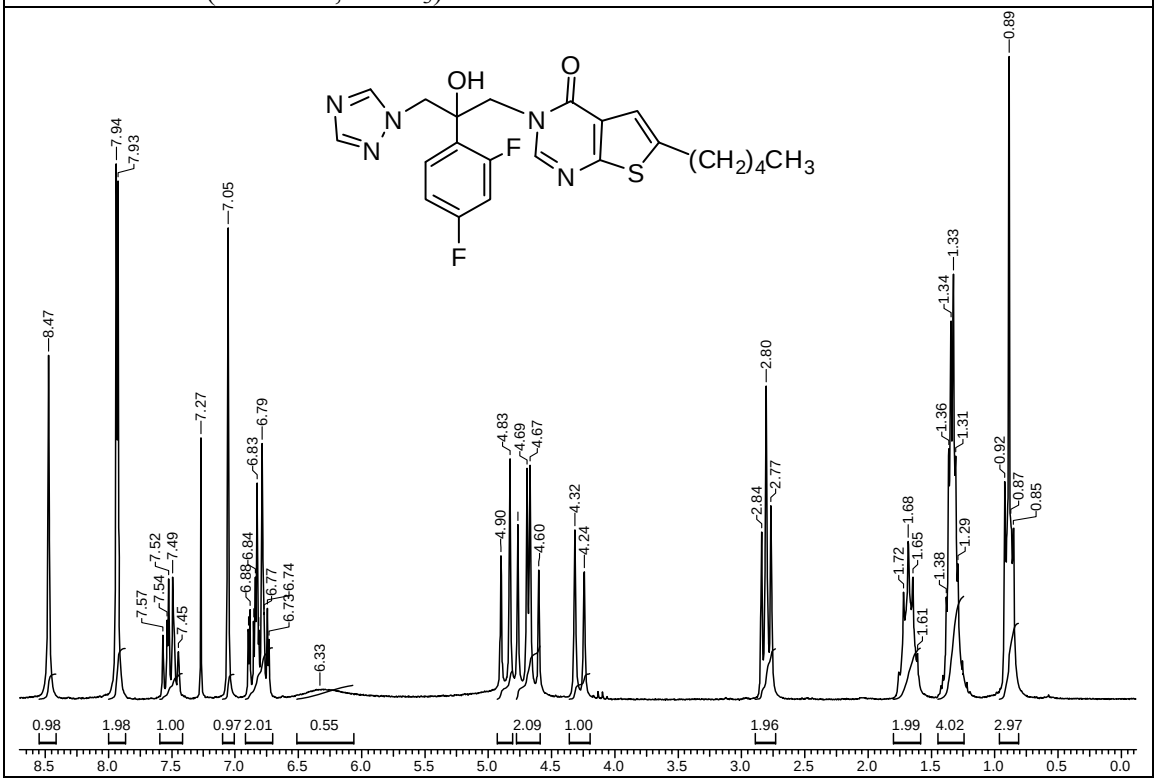
76a: ^{1}H NMR (200 MHz, $CDCl_3$)



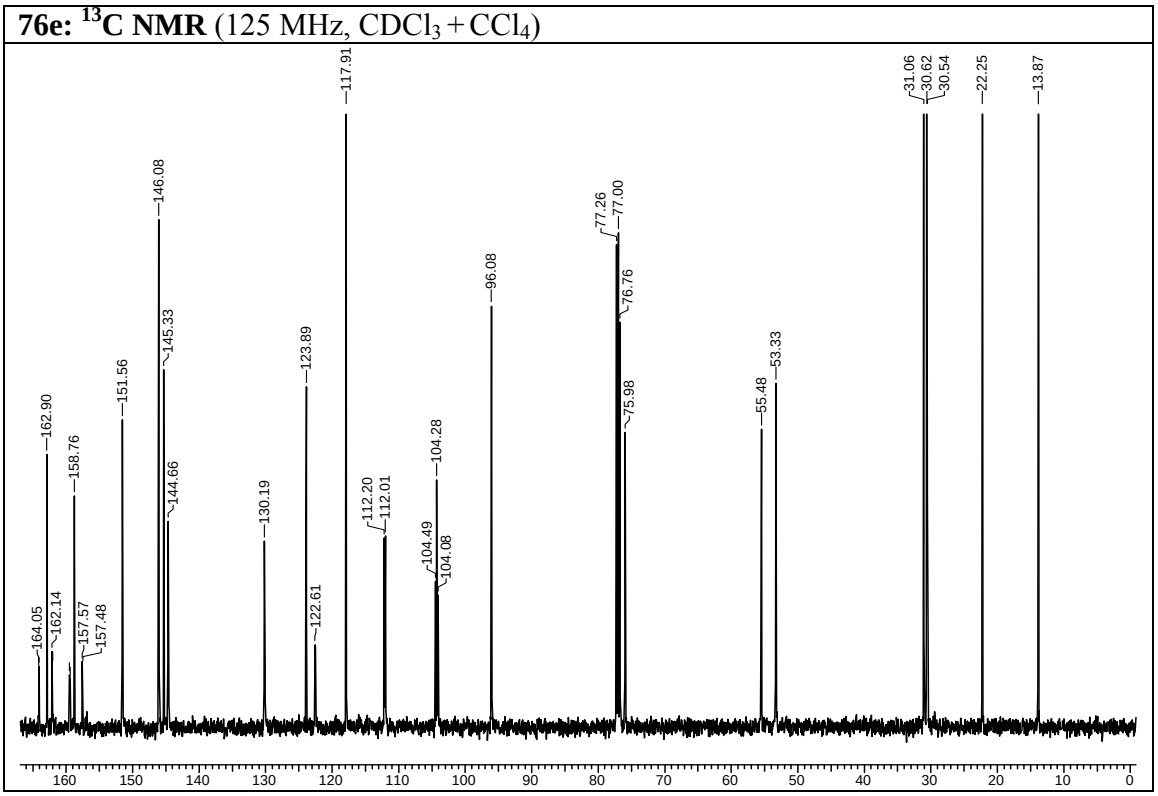
76a: ^{13}C NMR (50 MHz, $CDCl_3$)



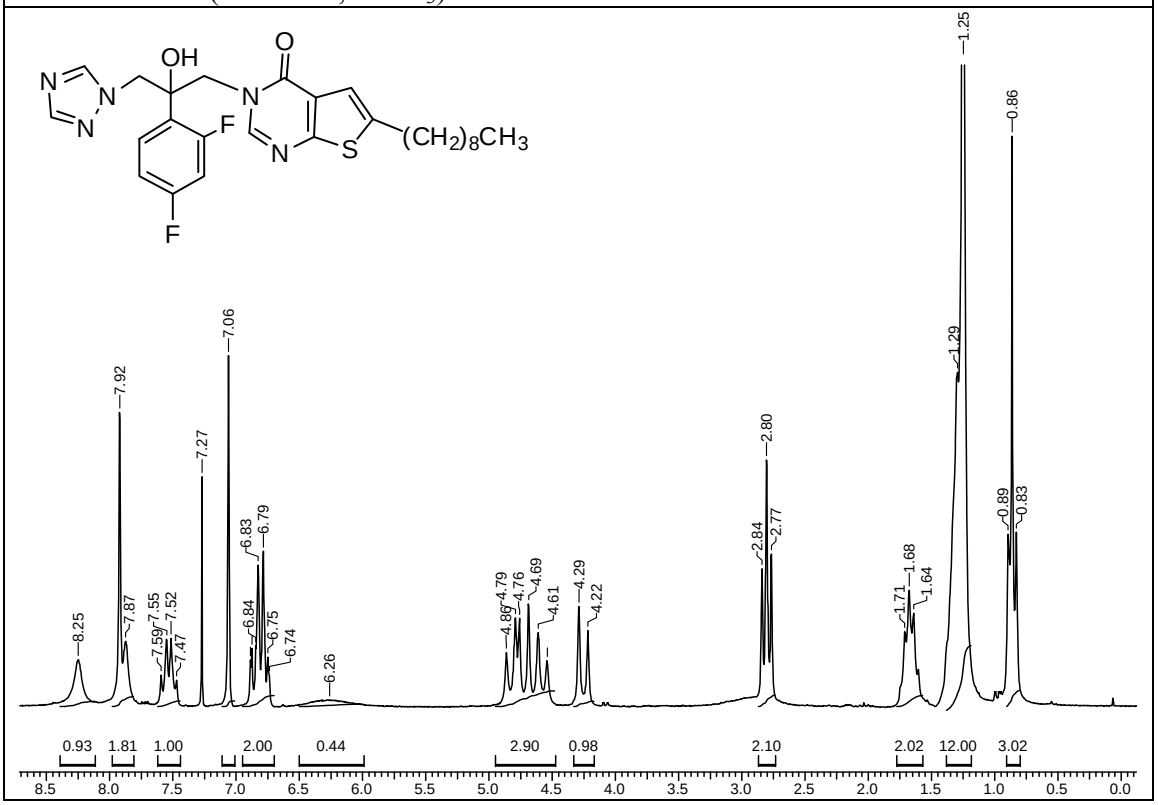
76e: ^{1}H NMR (200 MHz, $CDCl_3$)



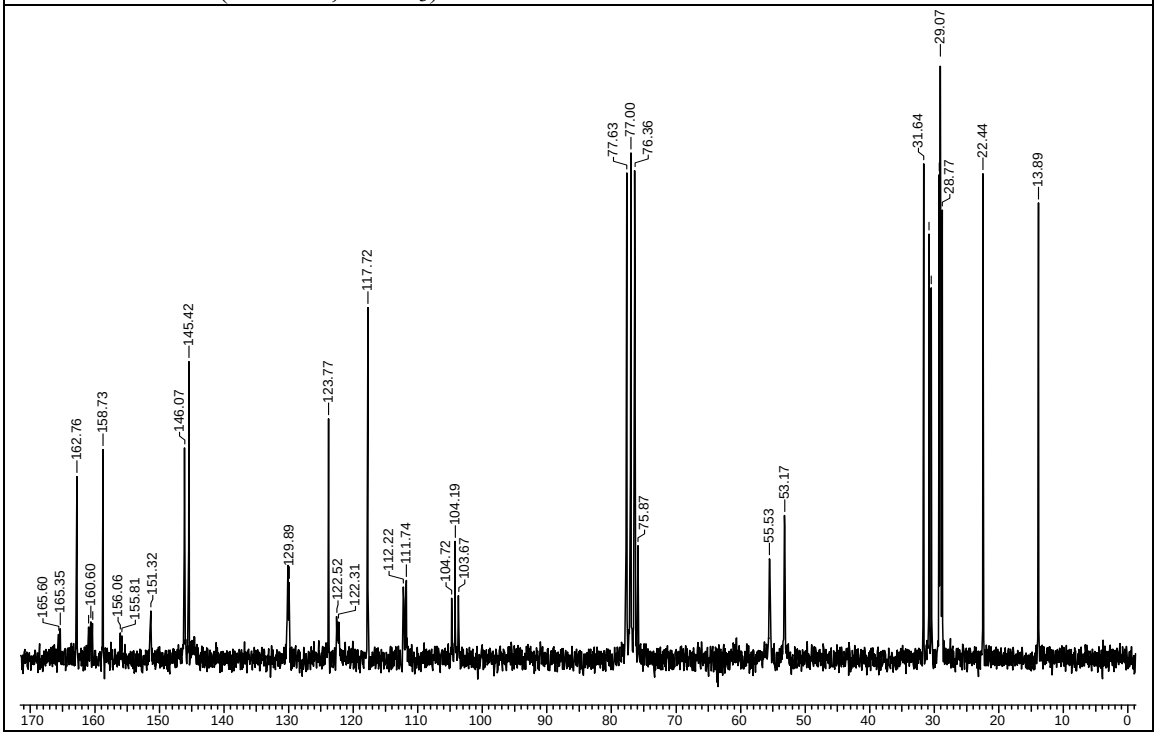
76e: ^{13}C NMR (125 MHz, $CDCl_3 + CCl_4$)



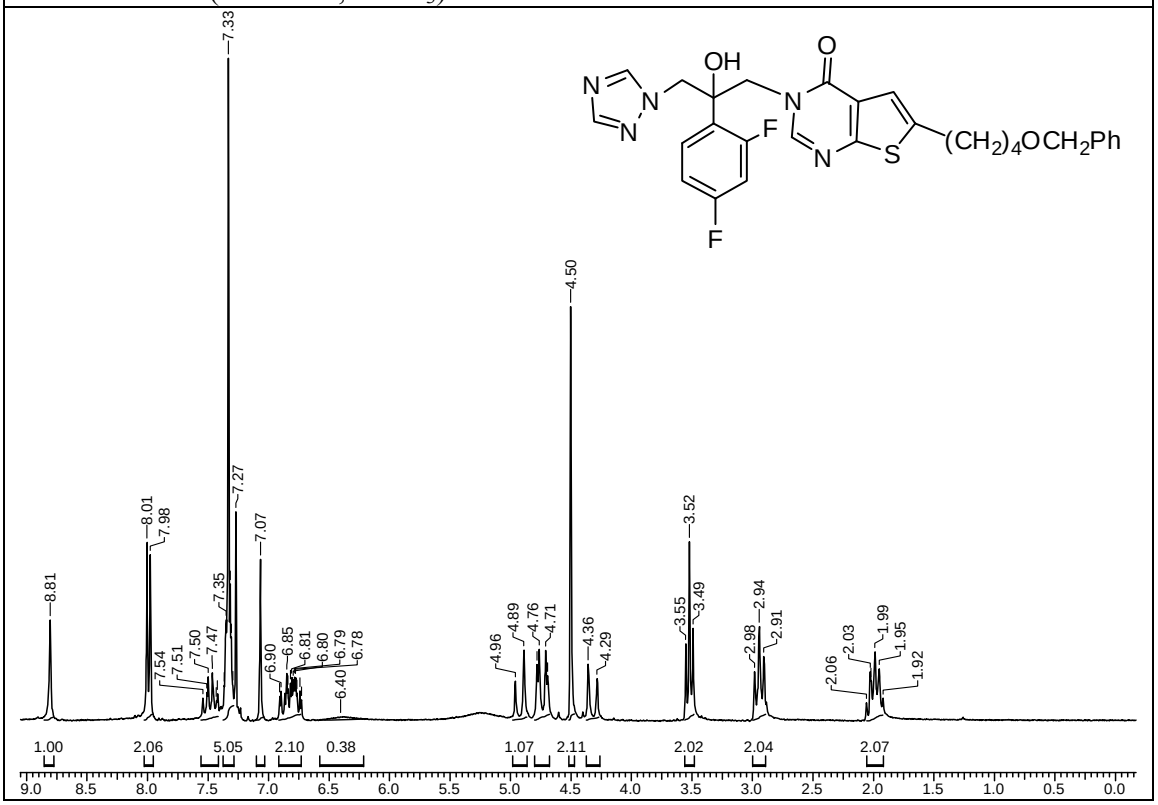
76h: ^{1}H NMR (200 MHz, $CDCl_3$)



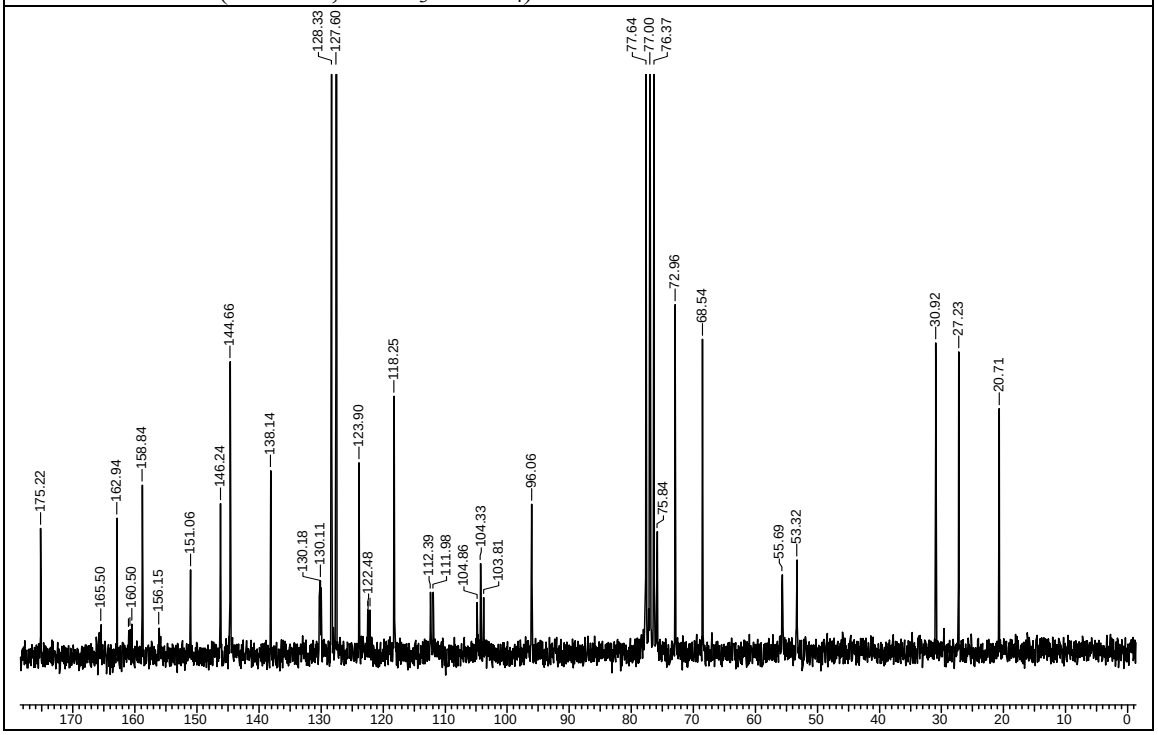
76h: ^{13}C NMR (50 MHz, $CDCl_3$)



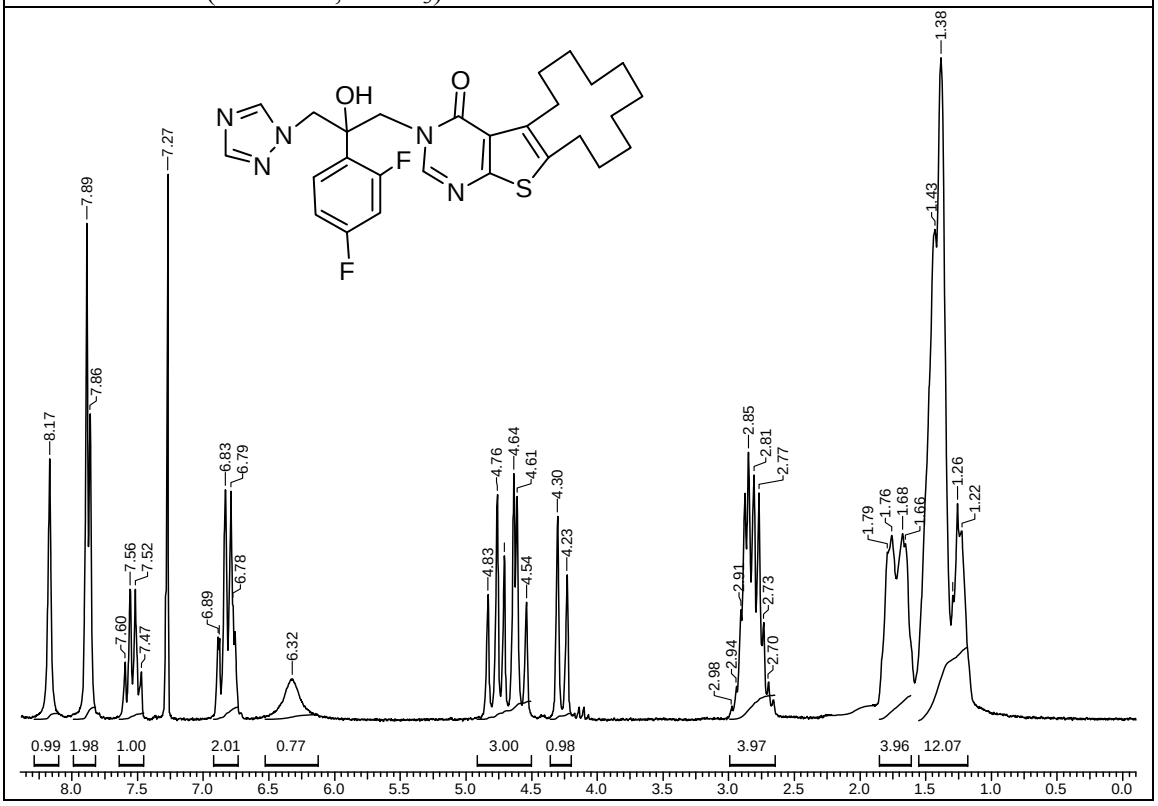
76n: ^{1}H NMR (200 MHz, $CDCl_3$)



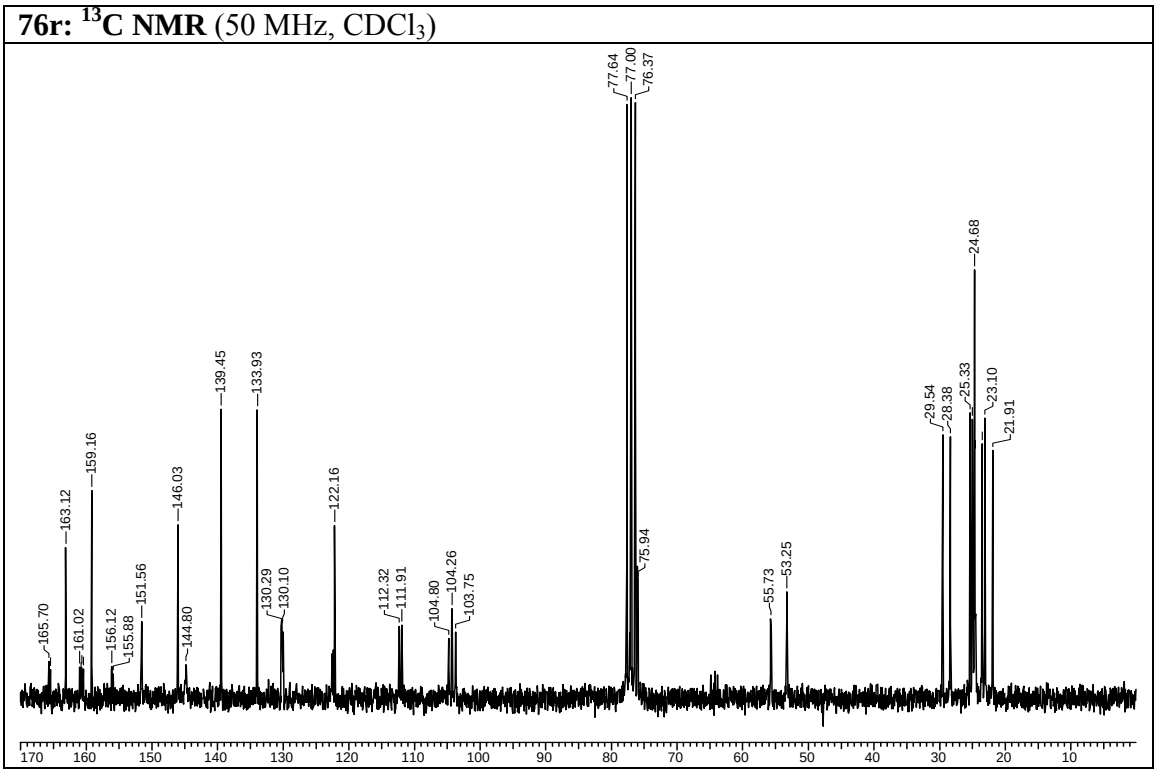
76n: ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4)



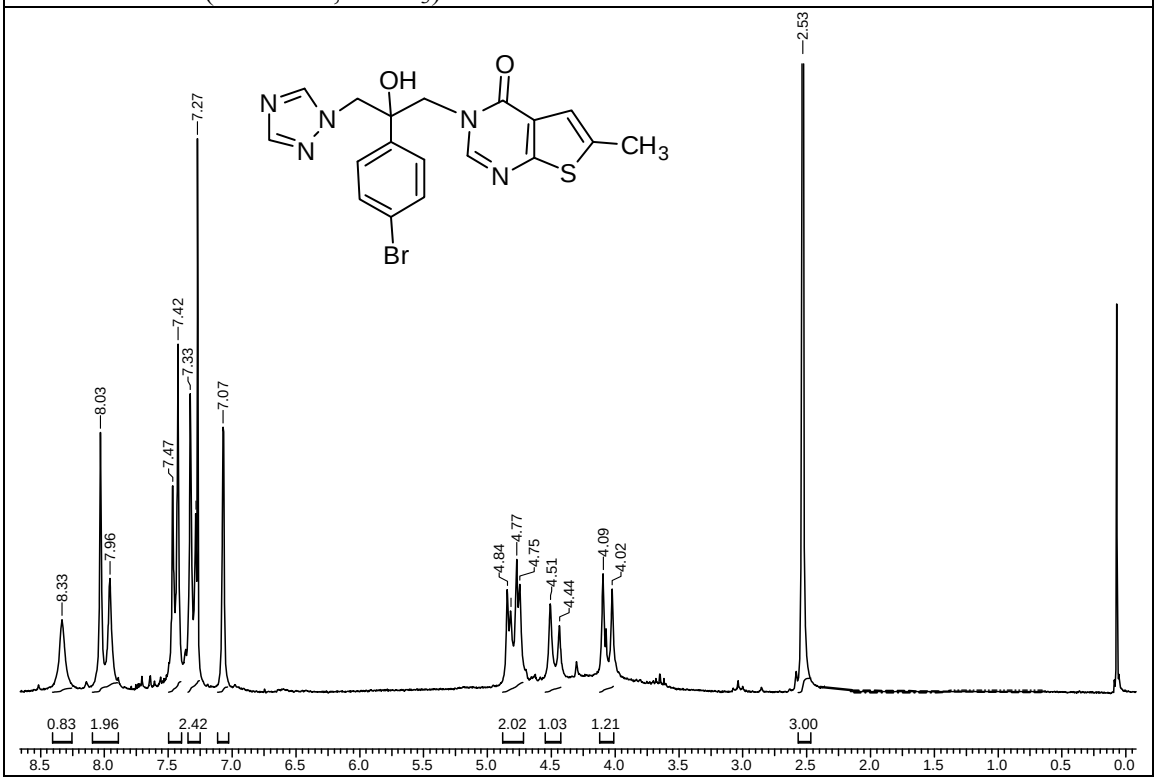
76r: ^{1}H NMR (200 MHz, $CDCl_3$)



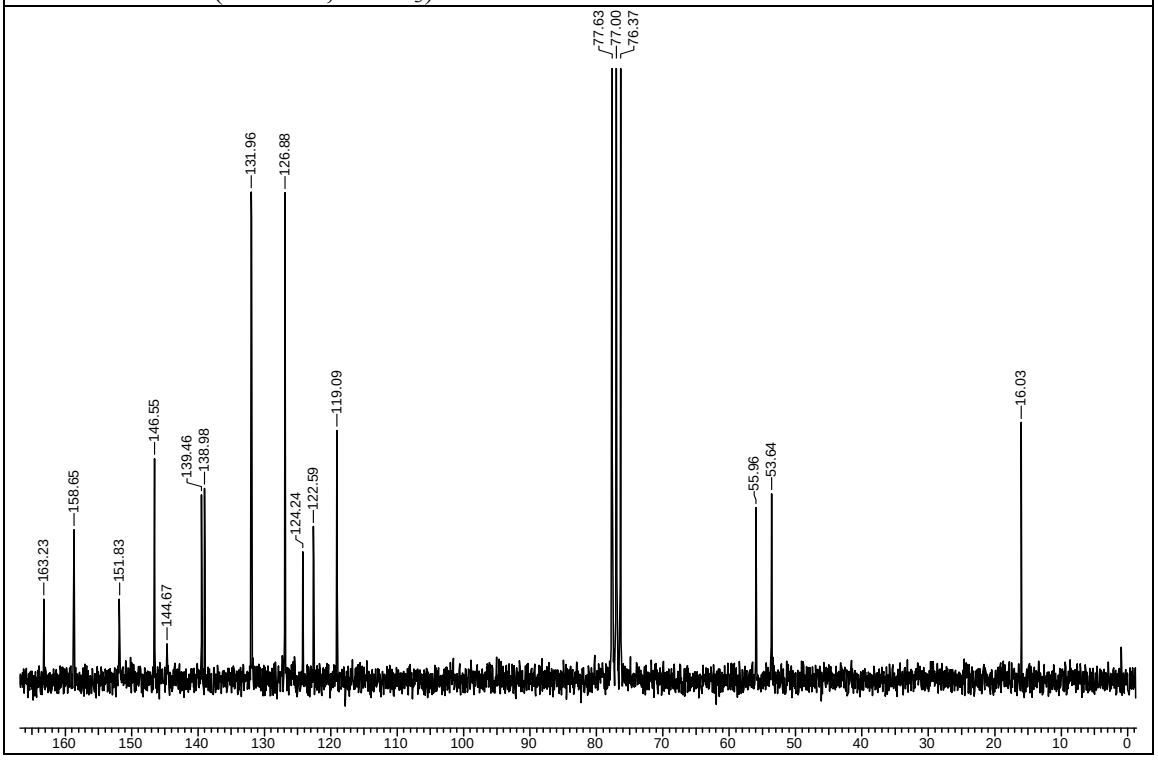
76r: ^{13}C NMR (50 MHz, $CDCl_3$)



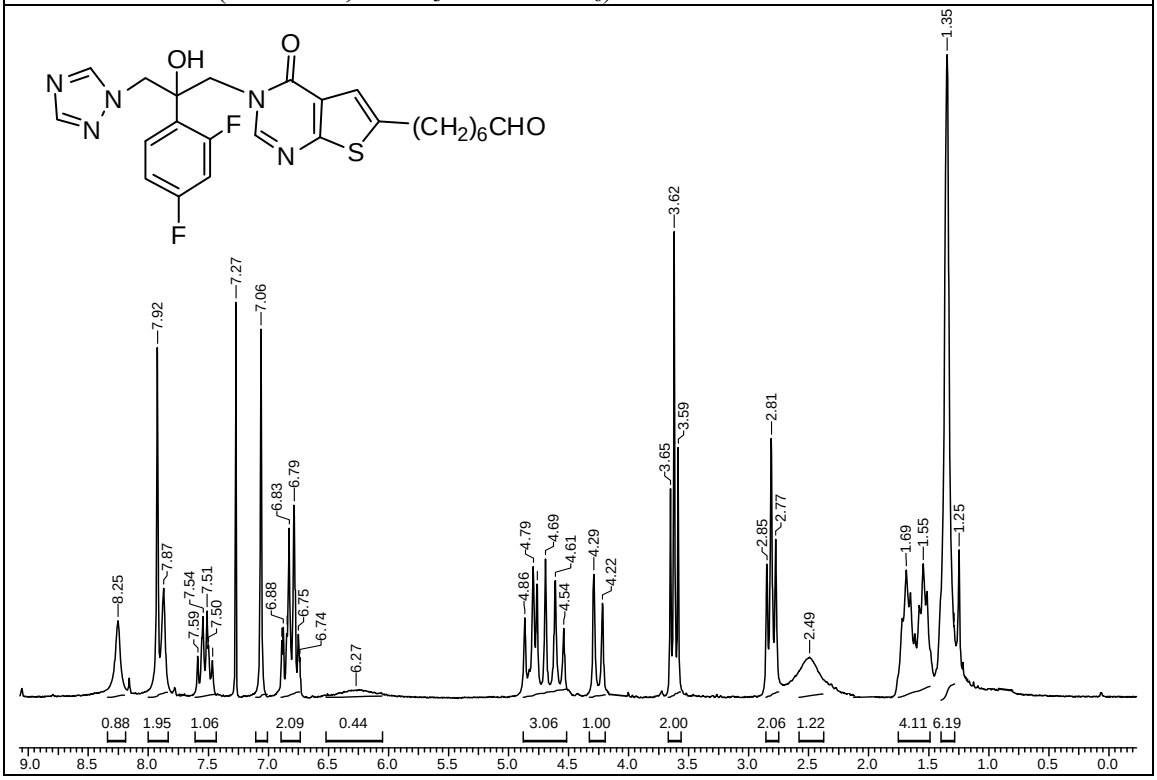
76s: ^{1}H NMR (200 MHz, $CDCl_3$)



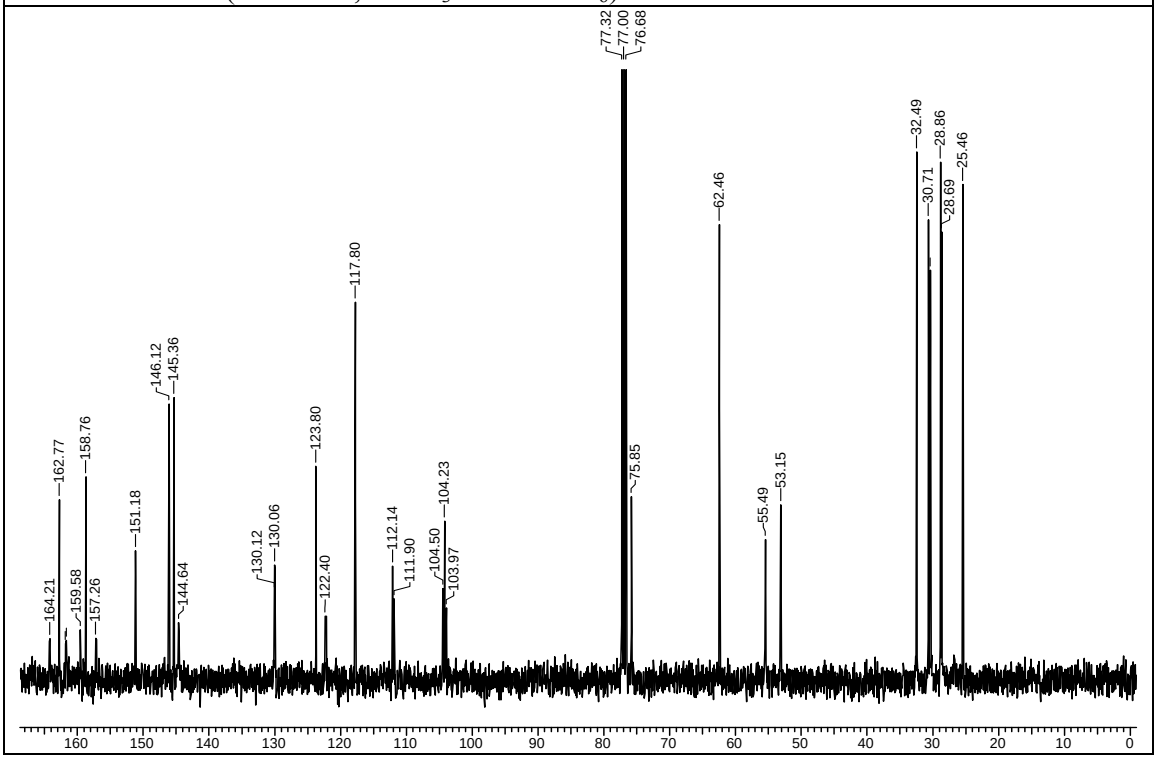
76s: ^{13}C NMR (50 MHz, $CDCl_3$)



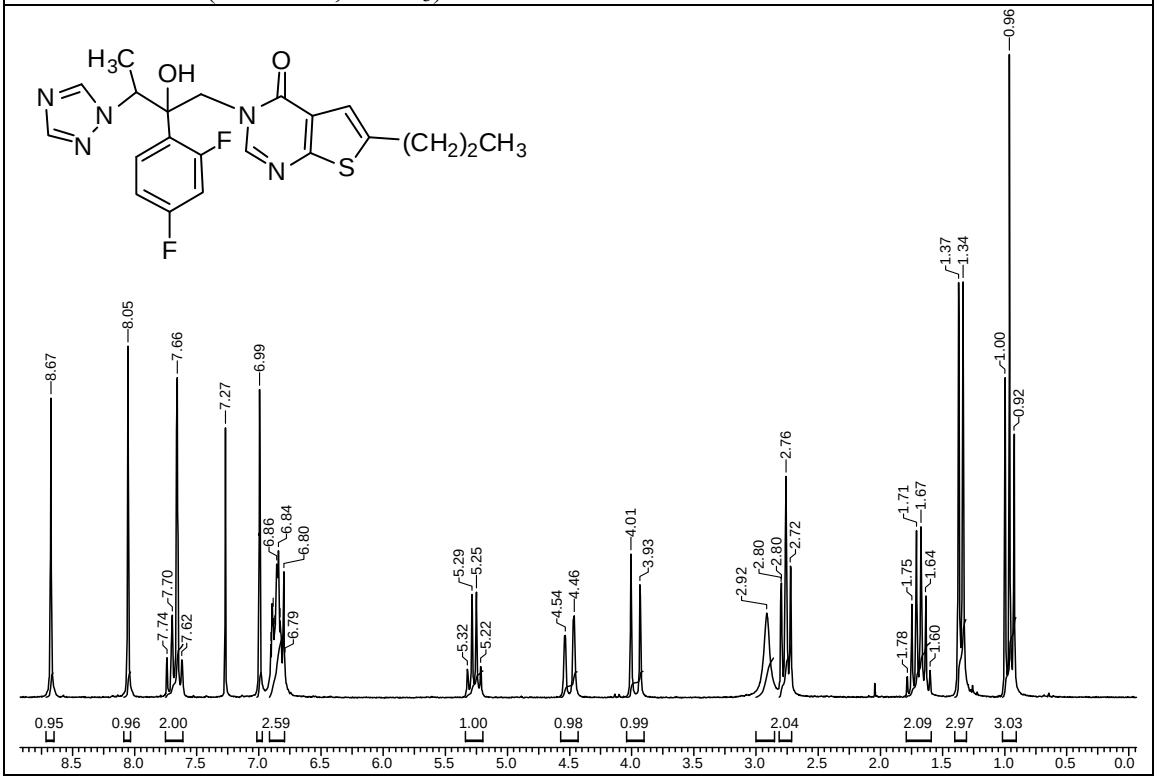
76ai: ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6)



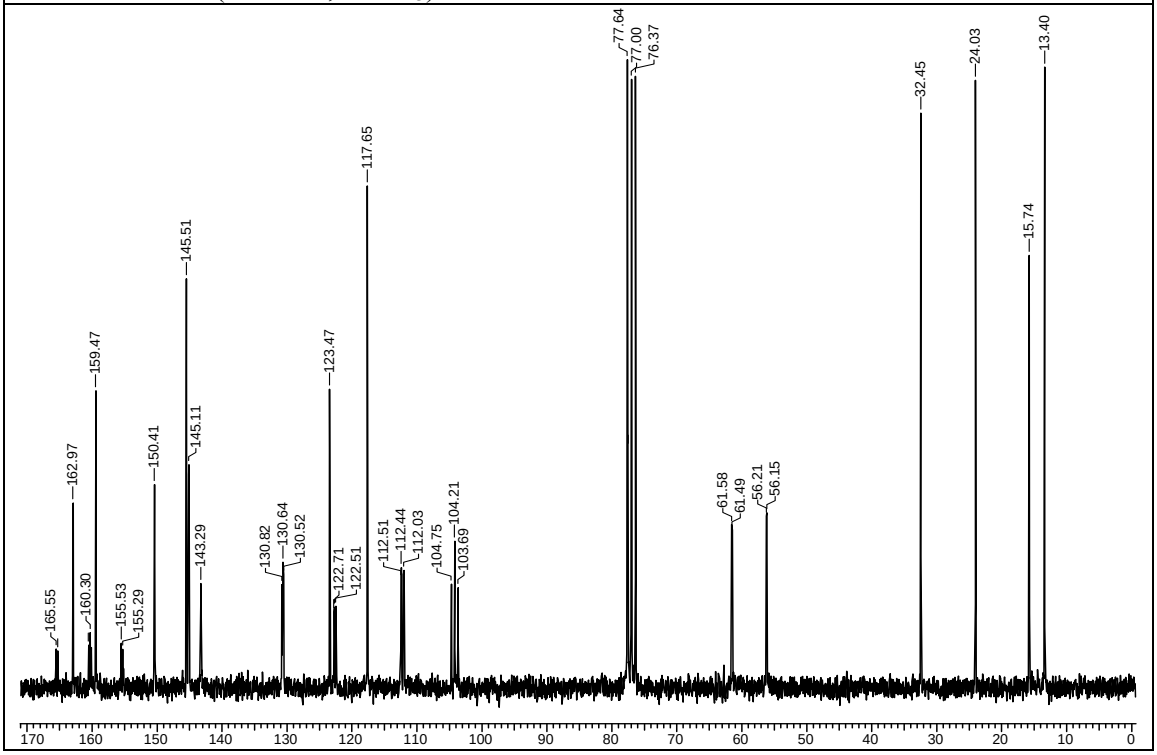
76ai: ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6)



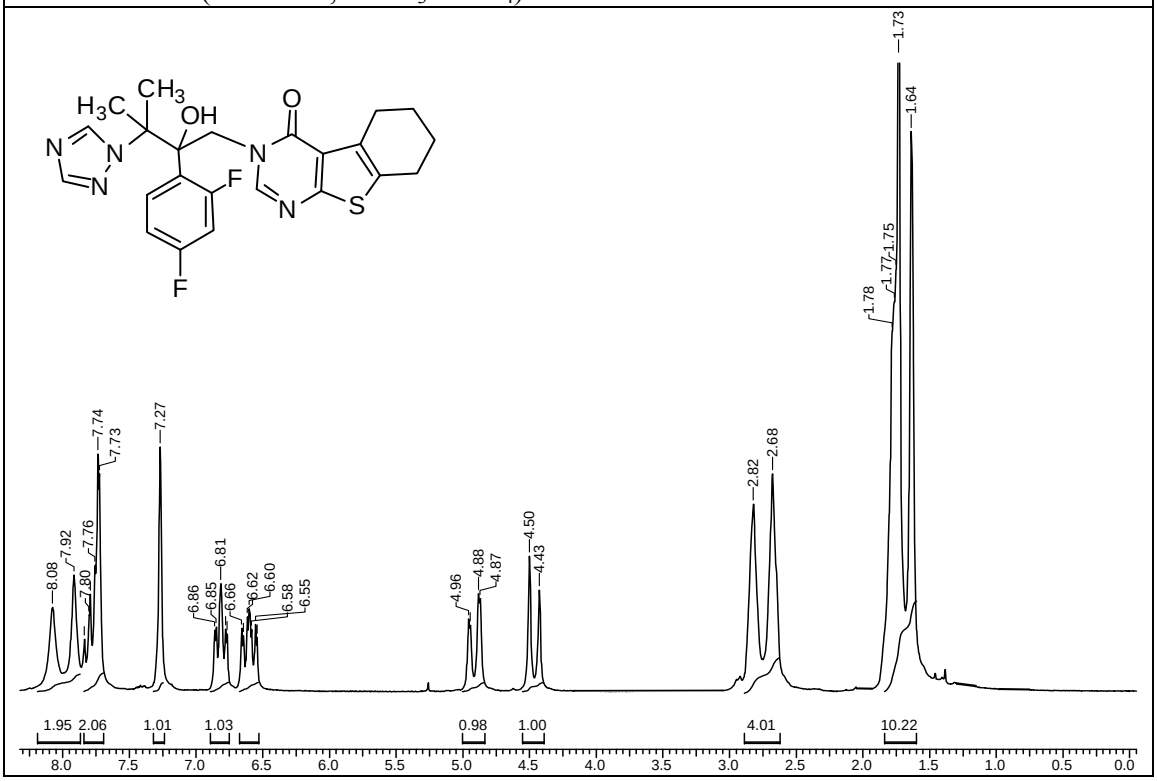
77c: ^{1}H NMR (200 MHz, $CDCl_3$)



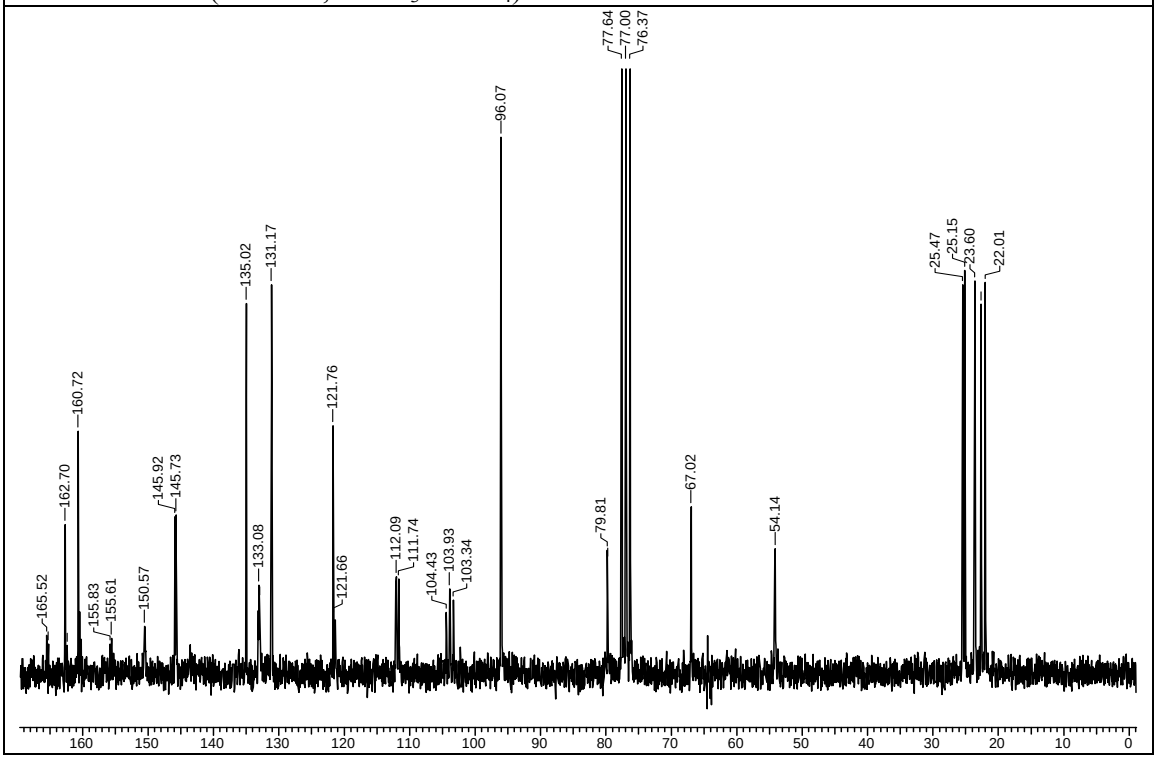
77c: ^{13}C NMR (50 MHz, $CDCl_3$)



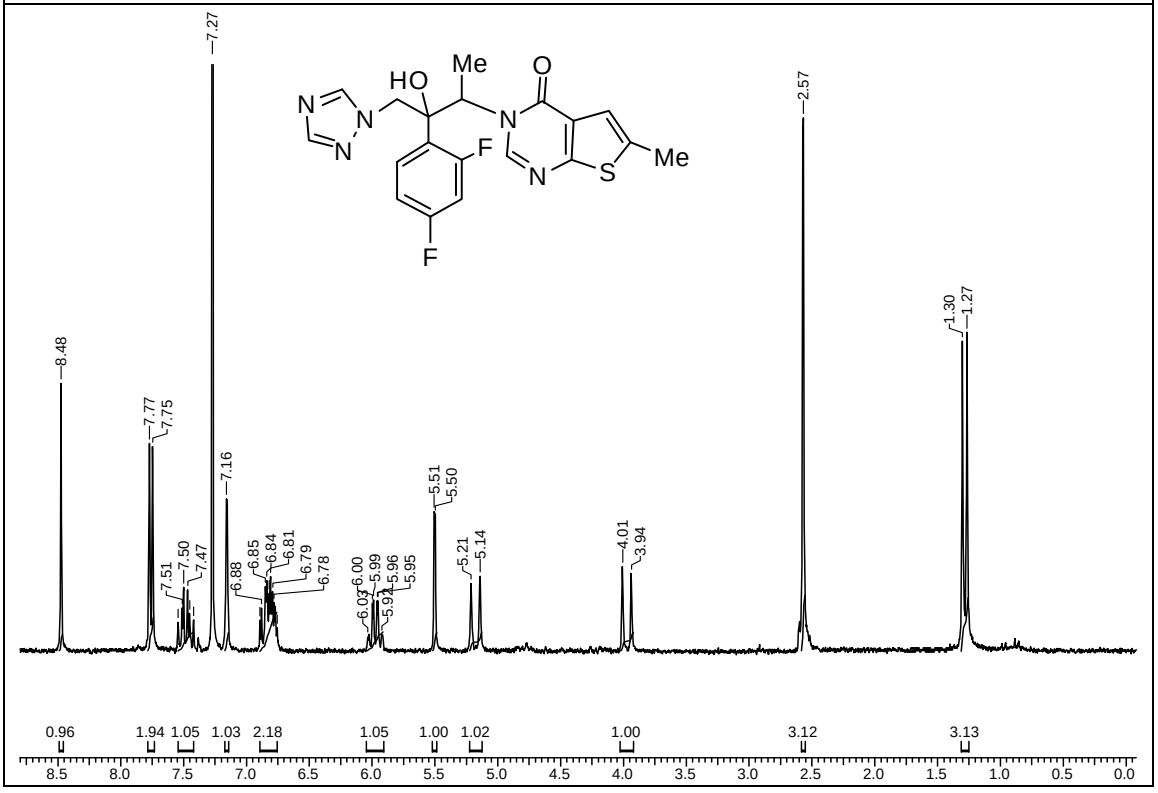
77i: ^{1}H NMR (200 MHz, $CDCl_3$+ CCl_4)



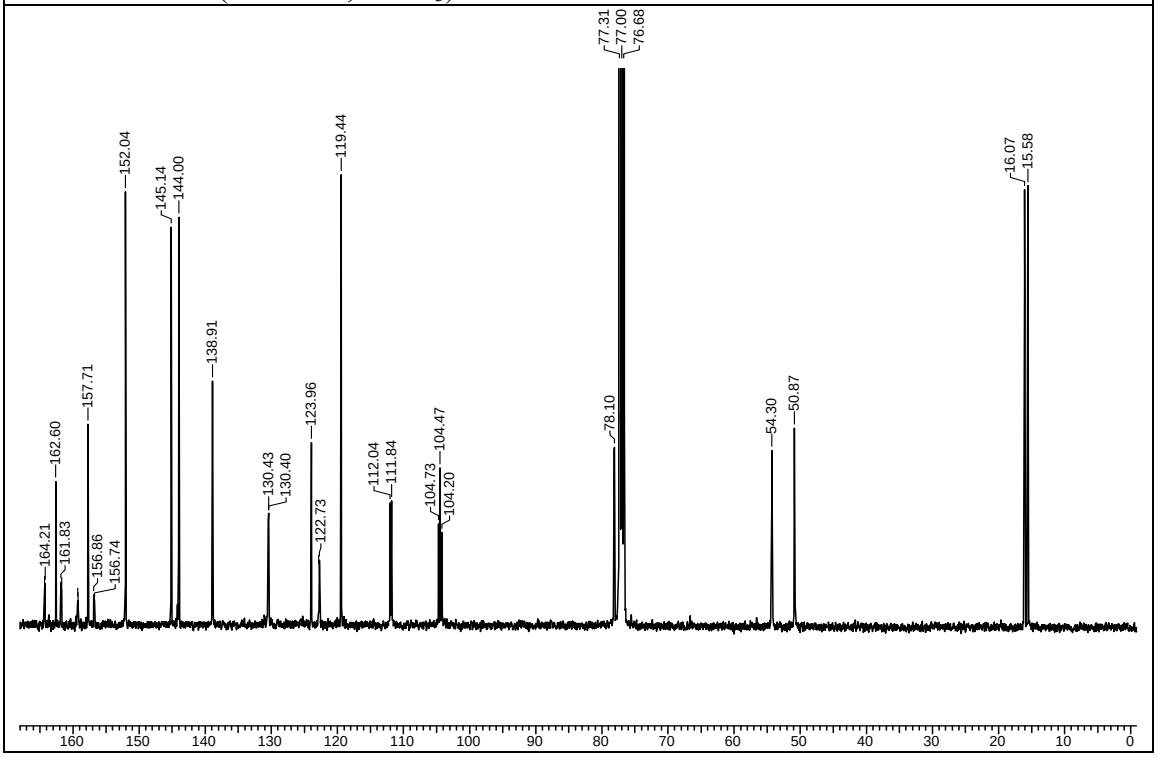
77i: ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4)



84b: ^{1}H NMR (200 MHz, $CDCl_3$)



84b: ^{13}C NMR (100 MHz, $CDCl_3$)



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Chapter 1: Section II

Bioevaluation of fluconazole analogues containing substituted thieno[2,3-d]pyrimidin-4(3H)-one

Bioevaluation of fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one

1.2.1 Introduction

Bioevaluation of new chemical entities is an important exercise in the development of new drugs. The results of the activity of molecules against different bacteria, fungi, cancer cell lines or various diseases give information regarding pharmacophores of particular class of compounds for specific diseases and thereby provides guidelines for designing new molecules with improved activity. The structure-activity relationship study of a number of such molecules is a necessary step in the development of superior drugs. We intended to develop new antifungal agents with more activity against various fungi, especially resistant strains and/or less toxicity, as the incidence of systemic fungal infections such as Candidiasis, Cryptococcosis and Aspergillosis has been increasing recently due to an increase in the number of immunocompromised hosts. Synthesis of a number of new chemical entities was achieved as described in Section 1. The screening of these molecules for antifungal activity was an equally important task for refining the structure of active molecules in order to get the most active compounds. This exercise was undertaken in collaboration with FDC Ltd, Mumbai and the results are described in this section.

1.2.2 Present Work

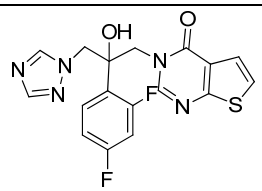
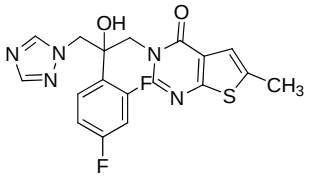
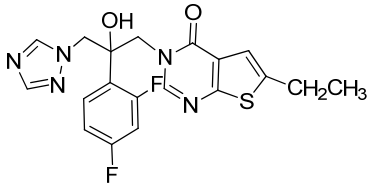
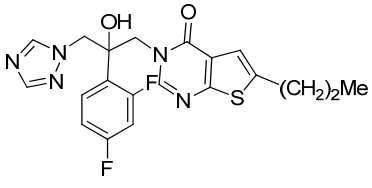
The synthesized fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones described in the first section of this chapter were screened for antifungal activity at FDC Ltd., Mumbai. Three standard strains of fungal cultures were used for the primary screening; *Candida albicans* ATCC 24433, *Aspergillus niger* ATCC 16404 and *Fusarium proliferatum* ATCC 10052. The activity of these compounds was studied by macro broth dilution method[1,2] optimized by using fluconazole and amphotericin B as the standards. The compounds exhibiting significant antifungal activity were studied further by micro broth dilution method against additional fungal strains.

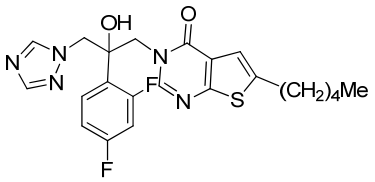
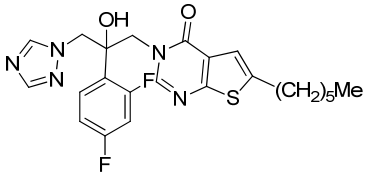
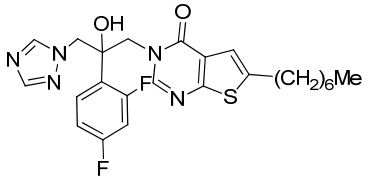
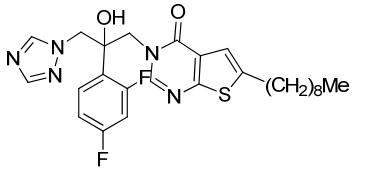
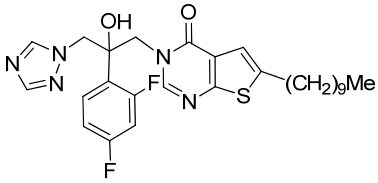
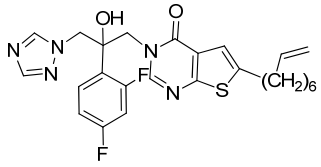
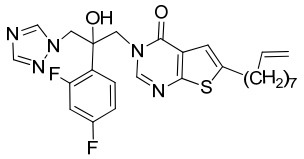
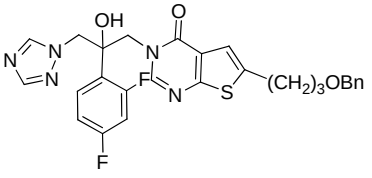
1.2.3 Results and discussion

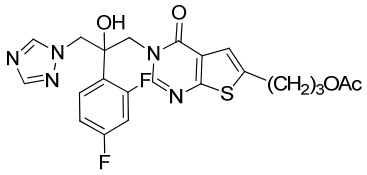
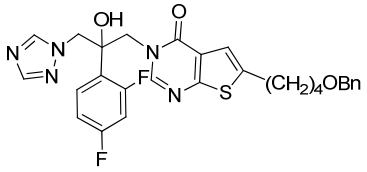
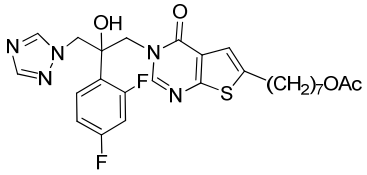
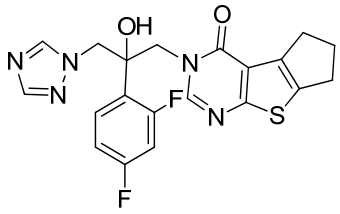
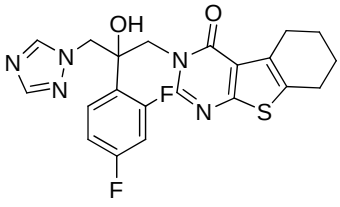
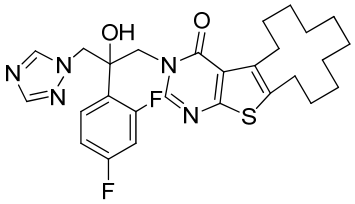
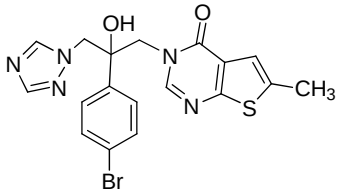
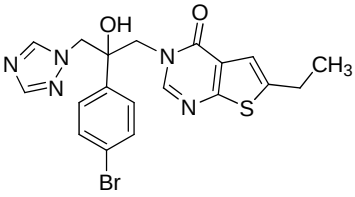
All the newly synthesized compounds having general structures **76**, **77** and **84** were tested for antifungal activity against various fungi including as shown in Table 1. *In vitro* evaluation of antifungal activity was performed by determining the minimum inhibitory concentration (MIC) following standard methods[1,2]. Antifungal susceptibility testing of

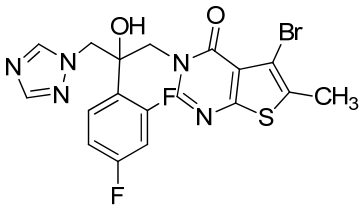
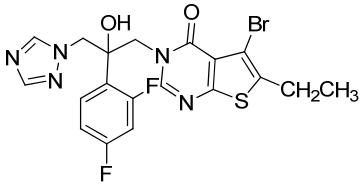
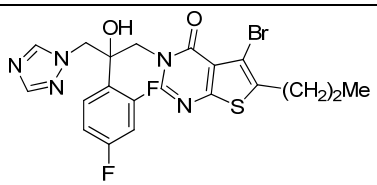
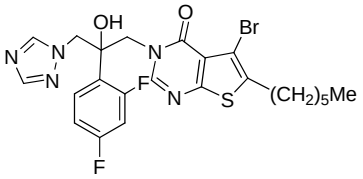
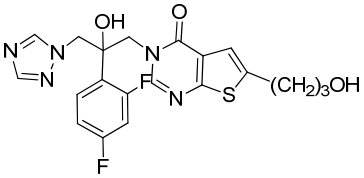
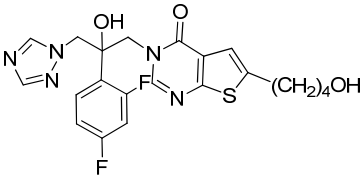
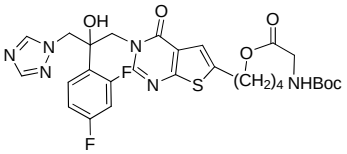
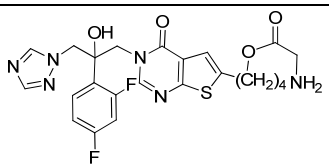
these compounds was done by broth dilution method using RPMI 1640 medium with MOPS (3-(N-morpholino)propanesulfonic acid) buffer. Known antifungal agents like fluconazole and amphotericin-B were used as positive control and end points were determined after 48 hours visually and by using spectrophotometer wherever necessary. Different dilutions were tried and various sets of experiments performed. The activity parameters are enumerated in Table 1.

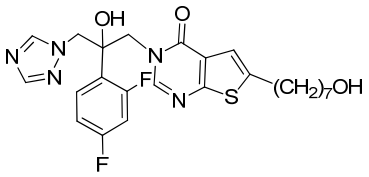
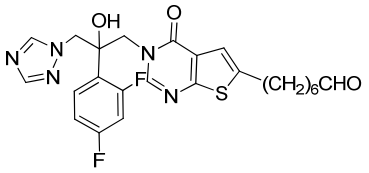
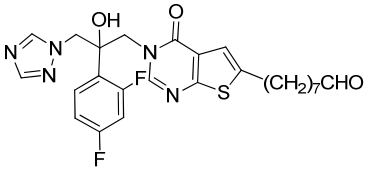
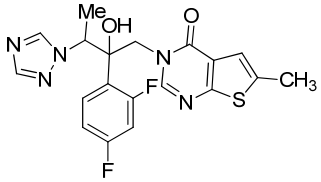
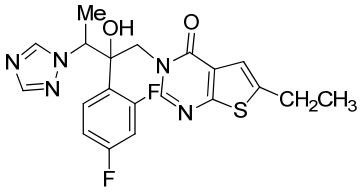
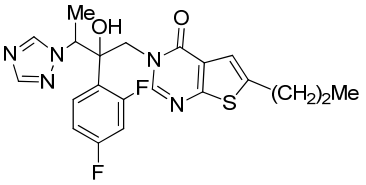
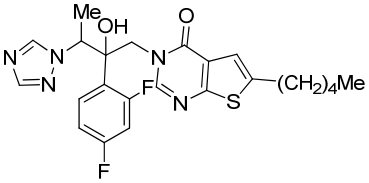
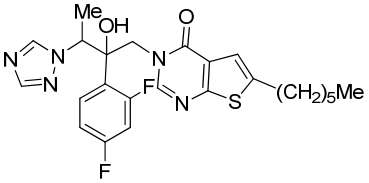
Table1. Antifungal activity data of fluconazole analogues

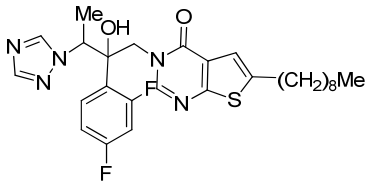
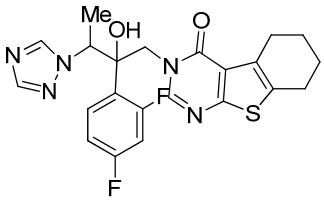
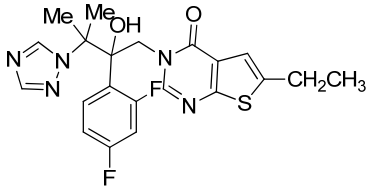
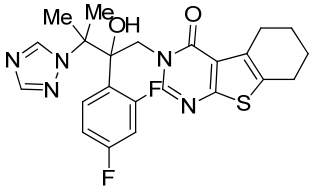
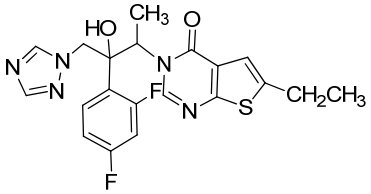
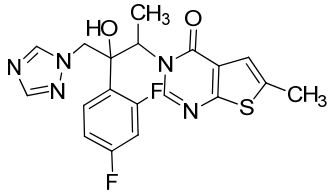
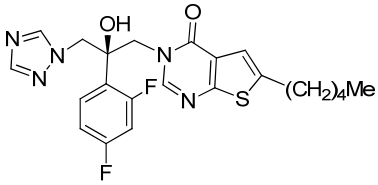
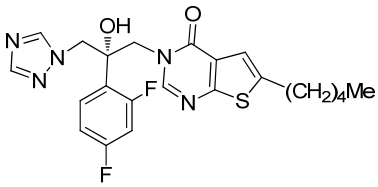
Sr No	Comp. Code	Structures	MIC_{80} in µg/ml		
			C. albicans ATCC 24433	*A. niger* ATCC 16404	*F. proliferatum* ATCC 10052
A		Fluconazole	**0.25-1**	64-128	>128
B		Amphotericin-B	**0.12-0.25**	0.25-1	1-2
1	**76a**		1-2	>128	>128
2	**76b**		0.5-1	NA	NA
3	**76c**		**0.06-0.12**	NI till 16	NI till 16
4	**76d**		**0.06-0.12**	NI till 32	NI till 32

5	**76e**		**0.03-0.06**	NI till 4	NI till 4
6	**76f**		**0.03-0.06**	NI till 2	NI till 2
7	**76g**		**0.06-0.12**	2	2
8	**76h**		**0.25-0.5**	4	4
9	**76i**		1-2	2	2
10	**76j**		**0.25-0.5**	>4	>4
11	**76k**		**0.25-0.5**	>2	>2
12	**76l**		**0.06-0.12**	NI till 4	NI till 4

13	**76m**		1-2	NI till 64	NI till 64
14	**76n**		**0.06-0.12**	NI till 4	NI till 4
15	**76o**		**0.12-0.25**	2	2
16	**76p**		**0.25-0.5**	2	2
17	**76q**		**0.25-0.5**	8	8
18	**76r**		NI till 2	NI till 2	NI till 2
19	**76s**		0.5-1.0	>32	>32
20	**76t**		**0.25-0.5**	>16	>16

21	**76aa**		4-8	>32	>32
22	**76ab**		2-4	>16	>16
23	**76ac**		2-4	>8	>8
24	**76ad**		2-4	>4	>4
25	**76ae**		2-4	128	12
26	**76af**		1-2	NI till 64	NI till 64
27	**76ag**		0.5-1	NI till 16	NI till 16
28	**76ah**		4-8	NI till 8	NI till 8

29	**76ai**		**0.12-0.25**	16	16
30	**76aj**		**0.25-0.5**	>32	>32
31	**76ak**		**0.25-0.5**	>16	>16
32	**77a**		16-32	64	64
33	**77b**		4-8	NI till 32	NI till 32
34	**77c**		8-16	16	16
35	**77d**		4-8	NI till 8	NI till 8
36	**77e**		NI till 1	NI till 1	NI till 1

37	**77f**		NI till 4	NI till 4	NI till 4
38	**77g**		NI till 8	NI till 8	NI till 8
39	**77h**		2-4	NA	NA
40	**77i**		2-4	NA	NA
41	**84a**		**0.06-0.12**	>16	>16
42	**84b**		**0.12-0.25**	>4	>4
43	**76e (R)**		2-4	ND	ND
44	**76e (S)**		**0.015-0.03**	ND	ND

NI: No inhibition, NA: Not active, ND: Not done
For azoles: For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 80% inhibition as compared to the positive control.
For amphotericin B: MIC is recorded as the concentration exhibiting complete inhibition.

From the biological data it was observed that most of these new chemical entities (NCEs) exhibited antifungal activity which means that thienopyrimidinone moiety is tolerated in fluconazole pharmacophore. The insertion of thienopyrimidinone moiety in fluconazole molecule either enhances or retains the antifungal activity of these NCEs. In some of the cases the activity vanishes probably due to steric bulk. All these NCEs showed potent antifungal activity, some of the analogues exhibited activity more than that of the fluconazole. Amongst the three stains of fungi, *Candida albicans*, *Aspergillus niger* and *Fusarium proliferatum*, these NCEs were found to be more active against *Candida albicans* but activity against *A. niger* and *F. proliferatum* was also observed to some extent.

Chain length of substituents on thienopyrimidinone exerts effect on the antifungal activity of these NCEs. Activity enhances with increase in chain length as follows: **76a** < **76b** < **76c** < **76d** < **76e**. The same trend was observed on replacing 2,4-difluorophenyl moiety with 4-bromophenyl moiety (**76s** < **76t**). Further elongation in side chain by one carbon showed steady MIC values (**55e** = **55f**) but activity decreases with further elongation in side chain (**76f** > **76g** > **76h** > **76i**). Thus activity was observed to be optimum up to five carbons in side chain. The NCEs with polar groups in side chains exhibited less activity than that of the nonpolar side chains (e.g. **76d** and **76g** are more active than **76ae** and **76ai** respectively). Furthermore, protected polar groups exhibited better activity than unprotected polar groups in the side chains (e.g. **76l**, **76n** and **76ag** exhibited better activity than **76ae**, **76af** and **76ah** respectively).

Cyclic rings in thienopyrimidinone are tolerated up to some extent, but as ring size increases, the activity decreases (e.g. **76p** ~ **76q** > **76r**). The compounds with brominated thienopyrimidinone were also observed to be not suitable for the activity. They showed less activity compared to the compounds with non-brominated thienopyrimidinones (e.g. **76aa**, **76ab**, **76ac** and **76ad** were less active than **76b**, **76c**, **76d** and **76f** respectively).

Apart from this, we synthesized some compounds wherein we put a methyl substituent on the carbon flanked between nitrogen of thienopyrimidinone and carbon bearing hydroxyl group and also we synthesized some compounds bearing methyl and

dimethyl substituent on the carbon flanked between nitrogen of triazole and carbon bearing hydroxyl group (described in first section of this chapter) so as to study the effect of alkyl group on the antifungal activity of resultant molecules. We observed that compounds **84a** and **84b** exhibited equivalent activity with respect to unsubstituted molecules **76c** and **76b**. Furthermore, methyl and dimethyl substituent on the carbon flanked between nitrogen of triazole and carbon bearing hydroxyl group was not tolerated in the molecule. Activity of all these molecules having general structure **77** dramatically decreased when compared with respective unsubstituted molecules with general structure **76**.

Compound **76e** was chosen for the chiral separation and was resolved using chiral HPLC to get R and S enantiomers out of which S enantiomer exhibited more activity than R enantiomer hence it can be stated that activity exhibited by the racemic mixture of **76e** is mainly due to S enantiomer.

1.2.4 Conclusion

The present work demonstrated that the designed fluconazole analogues containing thieno[2,3-*d*]pyrimidin-4-(3*H*)-ones having general structures **76**, **77** and **84** showed considerable antifungal activity. Some of the NCEs showed very good antifungal activity as compared to amphotericin B and fluconazole. The results have been protected in the form of patents and some of the compounds are being studied further in order to develop them as new antifungal drugs.

1.2.5 References

1. CLSI: Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, second edition M27-A2, **2002**.
2. CLSI: Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard M38-A, **2002**.

Chapter 2

Synthesis of dimeric fluconazole analogues containing substituted thieno[2,3-d]pyrimidin-4(3H)-one and their bioevaluation

Synthesis of dimeric fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one and their bioevaluation

2.1 Abstract

New dimeric molecules containing pharmacophores of fluconazole and thienopyrimidinone moieties with aliphatic carbon chains as linker units were synthesized in good yields. The strategy developed can be used for the synthesis of number of such dimers, with varying number of carbon atoms in the linker units. Two synthetic pathways were used for the synthesis of these dimeric molecules. All of them were tested against different fungal strains for their biological activity and it was observed that these molecules exhibited antifungal activity against *C. albicans* but they were not active against *A. niger* or *F. proliferatum*. Furthermore, these molecules come across solubility problem in routinely used organic solvents.

2.2 Introduction

It is now well known that many biologically active molecules on dimerization lead not only to increased potency and selectivity, but also long residence time particularly at epithelial surfaces in comparison to the respective monomers. Dimers, oligomers and polymers of the biologically active molecules have been prepared to increase the bioavailability of these molecules in the body, so that concentration of these molecules at the active site remains high. So as to maintain the bioavailability, drug molecules were loaded on the different carrying units such as sugars, amino acids, bile acids, biodegradable polymers, *etc*. depending upon the site of action. These carrier units play a very important role in carrying the active molecule to the site of infections. They help in absorption of the drug into the body, increase lipid solubility hence help in crossing of blood-brain barrier, protecting them from the metabolism and helping in slow release of drug molecule in the body. In addition to this, in some of the cases linker units are used between two monomers which results into the formation of dimers. Linkers used are of different kind such as straight chain and branched hydrocarbons including alkyl, alkenyl or alkynyl. The linker may also include one or more heteroatom such as N, O and S. They may also include one or more functional groups such as amide, amine, carbonyl and carboxy. These linker units either remain attached to the active molecule after absorption in the body, or get detached as a result of metabolism. In some of the cases it was observed that an active molecule reaches to

the target area in dimeric form, and exhibits better activity compared to the respective monomer unit depending upon the conditions, topology and reaction site.

2.3 Review of literature

It is known that nature has its own library of millions and billions of biologically active compounds, from many of the sources such as plants, micro-organisms, marine invertebrates, fish, insects and so on. This library contains many examples, wherein dimeric, oligomeric and polymeric molecules exhibit better activity than the respective monomeric units. Furthermore, the activity of a molecule also depends upon many factors like size, solubility, functional groups present, *etc*. and also upon the site of action. These dimeric molecules include two monomeric units joined head to head, head to tail or tail to tail fashion and also joined together by bonds or some linkers. Some of the naturally occurring and/or synthetically prepared dimeric molecules are discussed below.

Dimeric molecules other than antifungal agents

Artemisinin (**1**) is a drug used to treat multi-drug resistant strains of falciparum malaria. This compound is isolated from the plant *Artemisia annua*. In addition to its well-known antimalarial activity, artemisinin derivatives possess potent activity against cancer cells. P. M. O'Neill and co-workers[1,2] have synthesized a series of dimers of artemisinin (Figure 1).

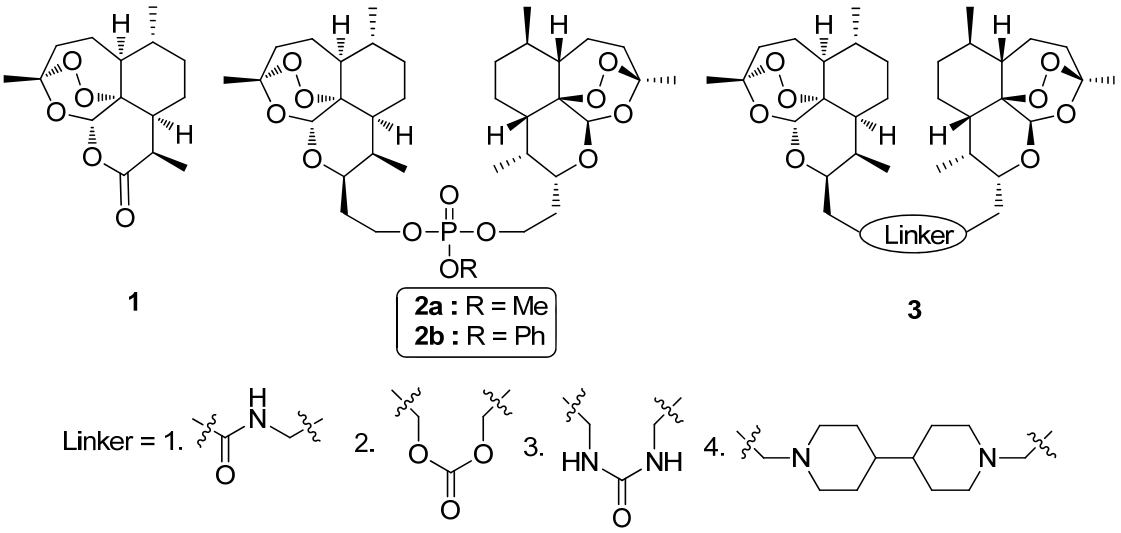


Figure 1. Artemisinin (**1**) and its various dimers having linkers

These dimers were synthesized using various linkers (Figure 1) and it was found that the dimers **2a** and **2b** exhibited excellent antimalarial activity at nanomolar concentrations.

There are three monoamine neurotransmitters dopamine (DA), serotonin (SER) and norepinephrine (NE). The transporter proteins for these neurotransmitters are dopamine transporter proteins (DAT), serotonin transporter proteins (SERT) and norepinephrine transporter proteins (NET), respectively. These transporters play a vital role in many psychiatric diseases such as depression, Parkinson's and Alzheimer's diseases. DAT, SERT and NET binders are used to regulate the function of these transporters and help in the treatment of diseases. Chloro derivative **4** is one such prime example from the class of phenyl tropanes which is a selective DAT binder[3]. S. Nielsen *et al*. reported[4] dimeric phenyl tropanes shown by general formula **5** with various linkers as shown in Figure 2.

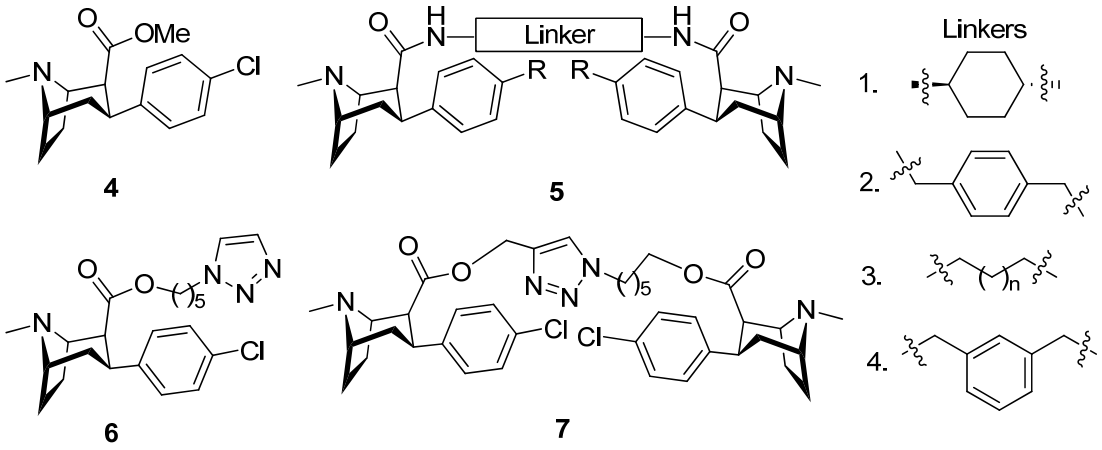
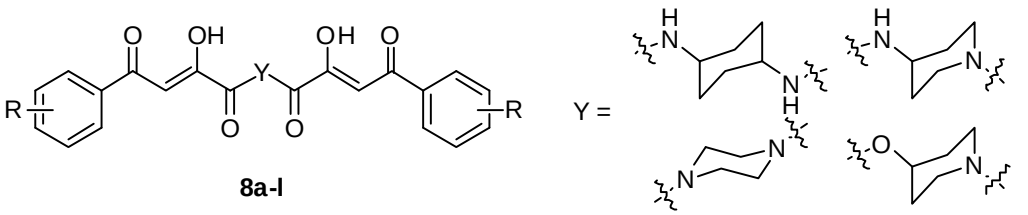


Figure 2. Phenyl tropanes monomers **4** and **6** and their various dimers

The various dimers synthesized were tested for their binding capacity to DAT, SERT and NET and the effect of linkers and functional group present was studied. They observed that the dimeric compound **7** exhibited 5.5 fold tighter binding affinity for DAT compared to monovalent **6**, furthermore, significant increase was found towards SERT (23 fold) and even more pronounced towards NET (45 fold).

Aryldiketo acids (ADK) and their bioisosters are known to be the most promising HIV-1 integrase (IN) inhibitors. L-F. Zeng *et al*. reported[5] the synthesis and activity of dimeric aryldiketo acid derivatives **8a-l** (Figure 3). It was observed that these dimeric derivatives exhibited moderate IN inhibitory activity.



8	a	b	c	d	e	f	g	h	i	j	k	l
R	3-OBn	3-OBn 4-OMe	2-F	4-F	3-NHCbz	3-(2'-fluorobenzyl)	3-NBn_2	3-Me 4-Cl	2-Cl	4-CF_3	3-OMe	4-NO_2

Figure 3. Aryl diketoacid derivatives with varying aromatic rings and the linkers

Pyrrolobenzodiazepine ring systems are known for their DNA interstrand cross-linking property and one of the representative examples is DC-81 (**9**). Thurston and co-workers[6] tethered two DC-81 units at the C8-position by using different alkane spacers to give bisfunctional-alkylating agents (representative example **10**) capable of cross-linking DNA (Figure 4).

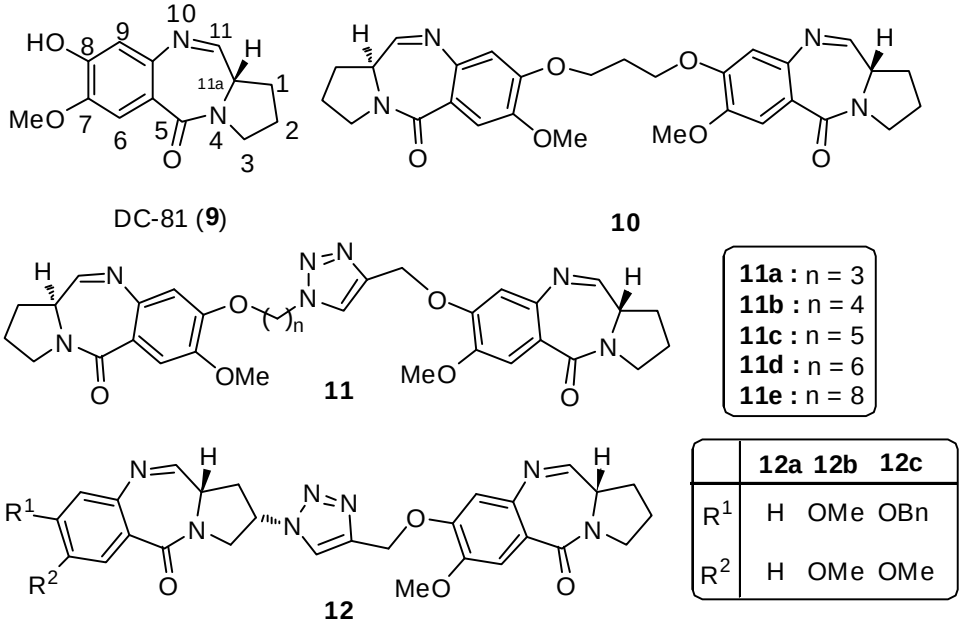


Figure 4. DC-81 (**9**) and its dimeric molecules

Furthermore, A. Kamal *et al.* reported[7] the synthesis of C8-C8 / C2-C8 linked triazolo pyrrolobenzodiazepine dimers with general structures **11** and **12** (Figure 4) by employing click chemistry and studied their DNA binding affinity. Representative dimer **11d** has shown significant DNA-binding ability. B. D. Giacomo *et al.* reported[8] the synthesis

and biological activity of melatonin dimeric derivatives **14** and **15**. Melatonin (**13**) is a hormone secreted by the pineal gland in the brain that helps regulate other hormones and maintains the body's circadian rhythm. The circadian rhythm is an internal 24 hour time-keeping system that plays a critical role in determining when we fall asleep and when we wake up. The melatonin and its dimeric derivatives are shown in Figure 5.

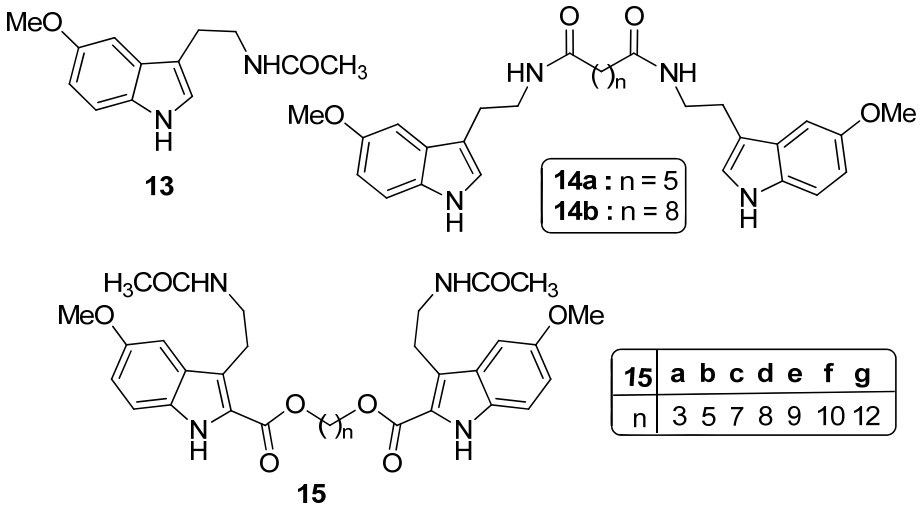


Figure 5. Melatonin (**13**) and its dimeric molecules

Synthesized dimers of melatonin were studied for their affinity towards melatonin receptors, and it was observed that all dimers possess moderate affinity. Amongst all dimers, **15d** showed good affinity for melatonin receptors at nanomolar concentrations.

Dimeric molecules as antifungal agents

J. N. Tabudravu *et al.* reported[9] isolation of psammaplin A (**16a**), psammaplin K (**16b**) and psammaplin L (**16c**) from the marine sponge *Aplysinella rhax*. The compounds isolated were characterized and checked for their biological activity, and it was observed that they exhibited chitinase inhibitory activity. The importance of chitinase in many biological processes made these inhibitors important targets as potential antifungal and insecticidal agents as well as antimalarial agents. Psammaplin A (**16a**) was observed to be the most potent chitinase inhibitor amongst three when compared to allosamidin, the most potent chitinase inhibitor known.

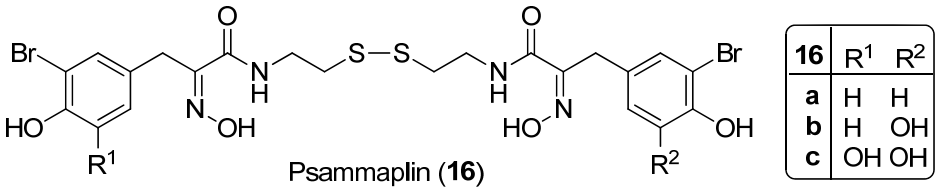


Figure 6. Psammaplin A (**16a**), psammaplin K (**16b**) and psammaplin L (**16c**)

S. Mishra *et al.* reported[10] the curcumin bioconjugates of poperine **19a** and **19b** (Figure 7) as potential antifungal drugs although it was observed that curcumin (**17**) and piperine (**18**) as such were less active than their bioconjugates.

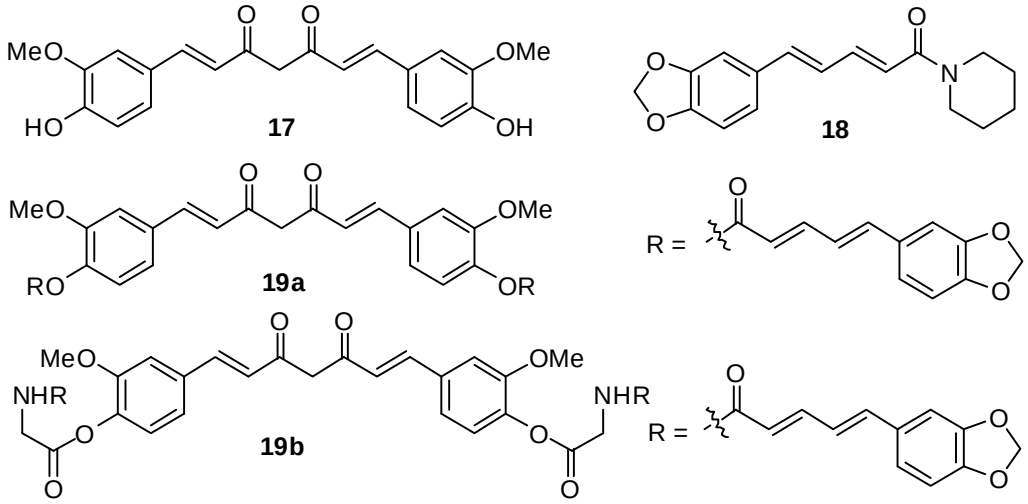


Figure 7. Curcumin (**17**), piperine (**18**) and their bioconjugates **19**

M. Costa *et al.* reported[11] the synthesis of dimeric chromene derivatives **20** and **21** (Figure 8). The activity of compound **20a** on *Aspergillus* spp. growth and on ochratoxin A production was evaluated. The results of the bioassays indicated that compound **20a**, applied at concentrations of 2 mM, totally inhibited the growth of the fungi.

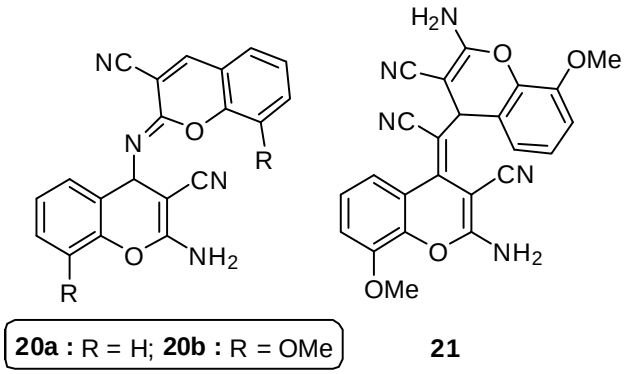
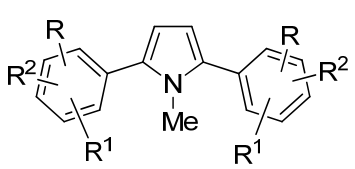


Figure 8. Dimeric chromene derivatives **20** and **21**

G. H. Jana *et al.* reported[12] the synthesis and biological activity of 2,5-bis(guanidine-aryl)-1-methyl-1*H*-pyrroles **22a-h** (Figure 9). The antifungal activities of these compounds were evaluated by *in vitro* agar diffusion and broth dilution assay against *Candida* and *Aspergillus* species.



22a-h

	a	b	c	d	e	f	g	h
R	H	2-OMe	2-Me	2-Cl	3-CF_3	4-F	4-Cl	2-F
R^1	H	H	H	H	H	H	H	4-F
R^2	4-R^2	4-R^2	4-R^2	4-R^2	4-R^2	3-R^2	2-R^2	4-R^2

R^2 = NHC(=NH)NH_2

Figure 9. 2,5-Bis(guanidine-aryl)-1-methyl-1*H*-pyrroles

Compound **22c** from the series was found to be equipotent or more potent than fluconazole, whereas compound **22d** was comparable to fluconazole against most of the tested strains.

Some phenolic dimeric molecules, such as **23a**, **23b** and **23c** (Figure 10) exhibiting anti-Candidial activity were reported[13] by L. Ejim *et al.* These compounds act as inhibitors of yeast homoserine (Hse) dehyrogenase, which is essential for the fungal homoserine biosynthesis.

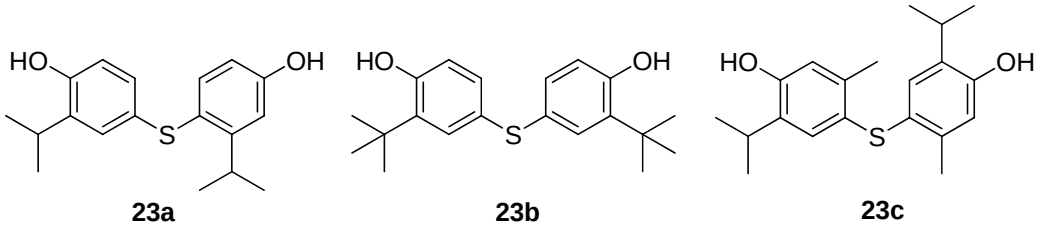


Figure 10. Inhibitors of yeast homoserine biosynthesis

Triazole containing compounds were reported[14] as antifungal agents by S. Emami *et al.*, wherein bisphenacyl triazolium compounds **24a-c** (Figure 11) showed superior activity compared to their respective monomeric molecules.

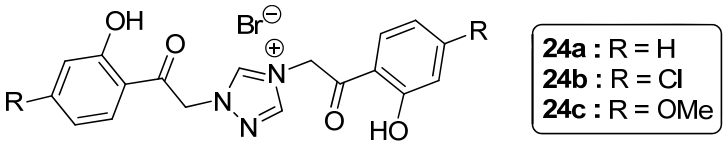


Figure 11. (Un)Substituted 2-hydroxyphenacyl-azolium compounds

Furthermore, compound **24a** with MIC value of 1 μg/mL showed good activity against *Saccharomyces cerevisiae* comparable to miconazole and 32-fold more potent than fluconazole. Oceanaposide (**25a**) was isolated from the temperate marine sponge *Oceanapia phillipensis*, and its algycone **25b** exhibited ~10-fold improved antifungal activity against *C. glabrata* compared to D-Sphingosine (**26**), reported[15] by T. F. Molinski and co-workers.

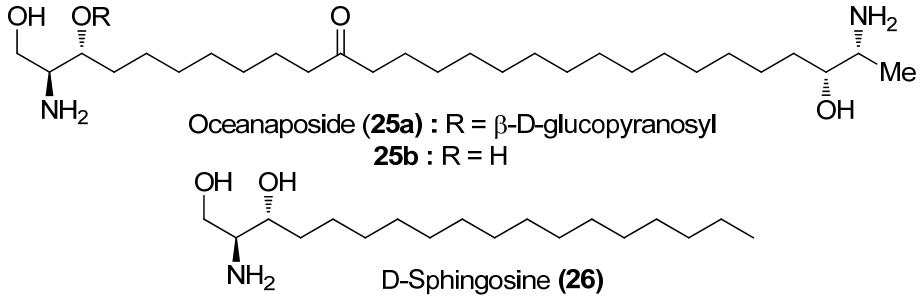
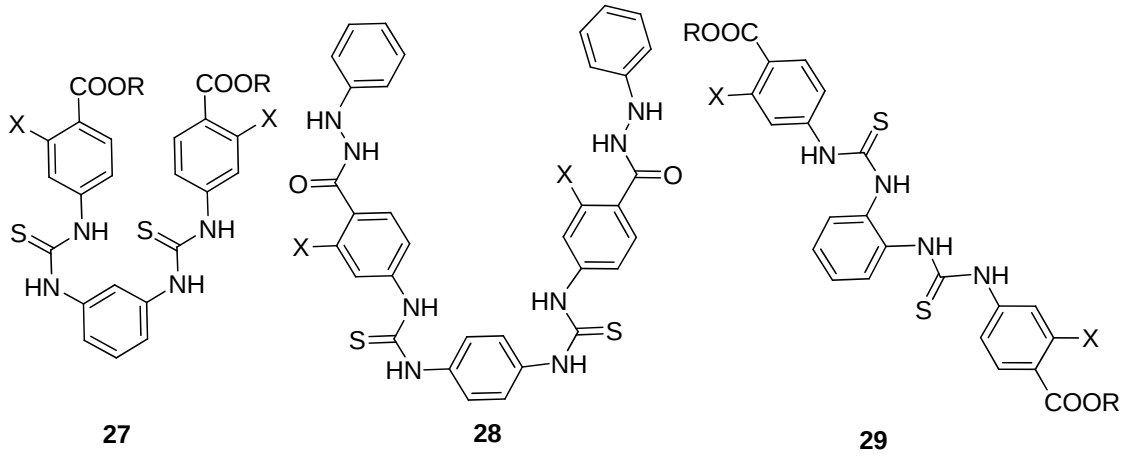


Figure 12. Oceanaposide (**25a**), its algycone **25b** and D-Sphingosine (**26**)

T. Phuong *et al.* reported[16] the synthesis of 1,3-, 1,4- and 1,2-phenylenedithiourea derivatives with general structures **27**, **28** and **29** respectively (Figure 13). The synthesized

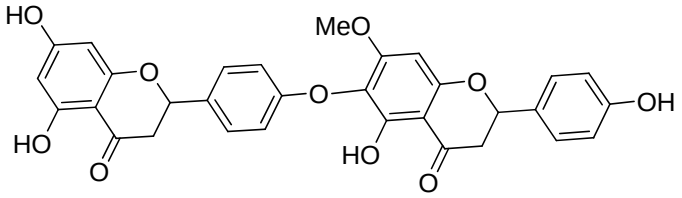
compounds were tested against various fungal strains for their biological activity, and it was observed that some of the compounds showed the antifungal potency comparable to ketoconazole with a broad antifungal spectrum.



27 **28** **29**

Figure 13. Phenylenedithiourea derivatives as antifungal agents

J. Lee *et al.* investigated[17] the antifungal properties of isocryptomerin (**30**), which is a biflavonoid isolated from *selaginella tamariscina* used in traditional medicines. To understand the mode of action(s) of isocryptomerin, author conducted experiments on *Candida albicans*, and concluded that the antifungal activities of isocryptomerin might be due to its membrane-disruption mechanism(s).



30

Figure 14. Isocryptomerin (**30**)

B. G. Hazra and co-workers reported[18] the synthesis and biological activity of the steroidal dimers **31** and **32** (Figure 15). The synthesized dimers were linked with various linkers such as ethylenediamine and diethylenetriamine.

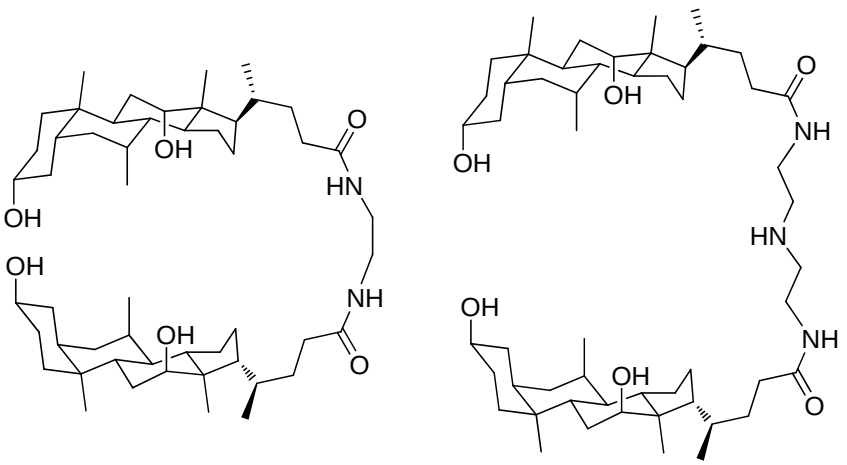


Figure 15. Steroidal dimers **31** and **32** as antifungal agents

The steroidal dimers when tested against both the pathogenic and nonpathogenic fungi, the activity data suggested that compounds **32a** and **32b** have potential as antifungal agents when compared to cycloheximide, a standard inhibitor.

Dimeric molecules of Amphotericin B, Fluconazole and Thienopyrimidinone

Synthesis of dimeric molecules of amphotericin B and their biological activity has been studied by M. Murata and co-workers.[19,20] It was observed that these dimeric molecules **33a** and **33b** (Figure 16) exhibited potent hemolytic activity and dimeric compounds **34c**, **34d** and **34e** exhibited the prominent hemolytic activity, exceeding that of amphotericin B, but these dimers were associated with poor solubility in most of the solvents.

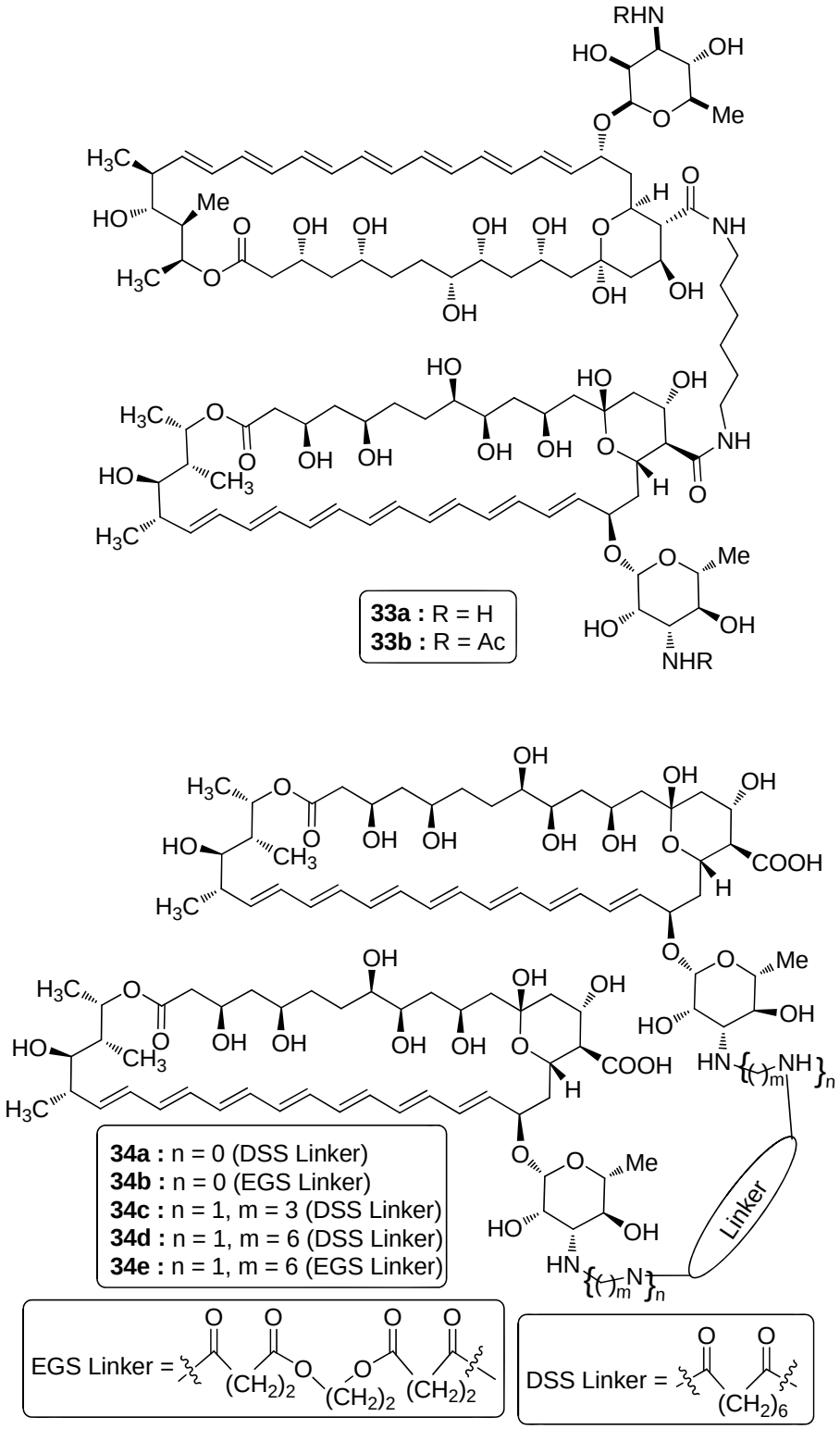


Figure 16. Dimeric molecules of amphotericin B

Sulfides and sulphoxides were used as linkers for fluconazole and its derivatives and a library of compounds with general structures **35** and **36** (Figure 17) was synthesized for the structure activity relationship study[21]. It was observed that the activity of dimeric compounds was enhanced in comparison to the respective monomers.

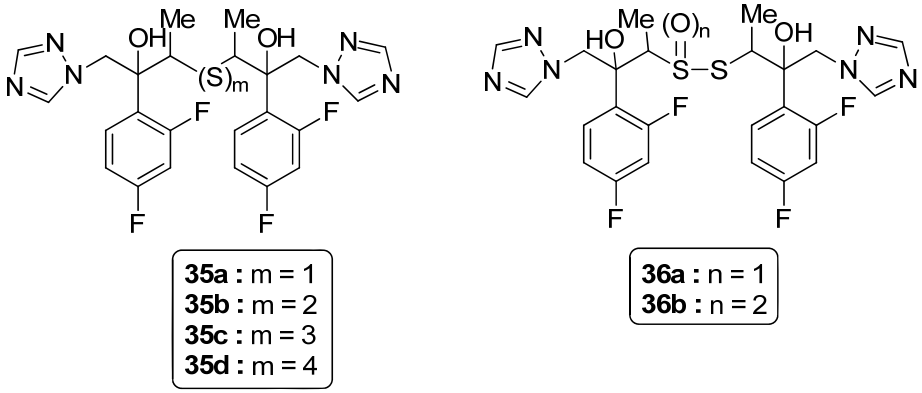


Figure 17. Dimeric fluconazole derivatives

Apart from these dimeric molecules synthesized and studied for their biological activity, synthesis of dimeric thienopyrimidinone molecules **37a** and **37b** was reported by F. E. M. El-Baih and co-workers[22]. Though the biological part has not been studied by the author, the synthesis of such molecules may help in constructing the newer and hybrid lead molecules of biological importance.

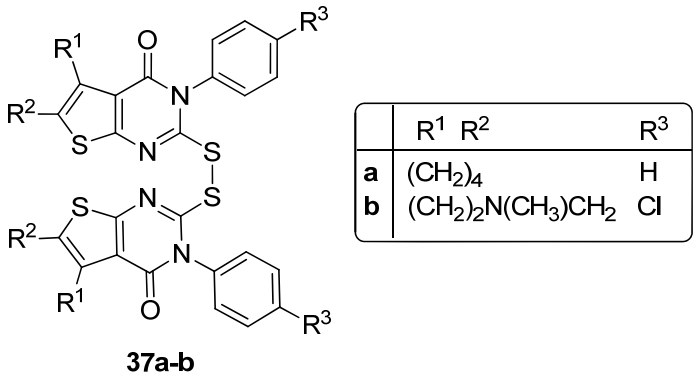


Figure 18. Dimeric thienopyrimidinone molecules **37a** and **37b**

2.4 Present Work

2.4.1 Objective

As discussed in first chapter we planned to develop new antifungal agents, and a series of fluconazole analogues was designed and synthesized, wherein one of the triazole moieties in fluconazole was replaced with various substituted thienopyrimidinone moieties. The new chemical entities thus synthesized were screened against various strains of fungi and it was observed that most of the compounds were the potent inhibitors of *Candida* strains. Exploring the chemistry used for the synthesis, and extending the utilization of the molecules for further development, we thought to synthesize the dimeric fluconazole analogues having thienopyrimidinone moieties. The molecules we planned are depicted with general formula **38** and **39**, wherein the two monomeric molecules are linked with the suitable linkers. The use of monomeric molecules exhibiting antifungal activity could be further developed to get dimeric molecules and this may lead to the synthesis of new fluconazole analogues exhibiting enhanced antifungal activity.

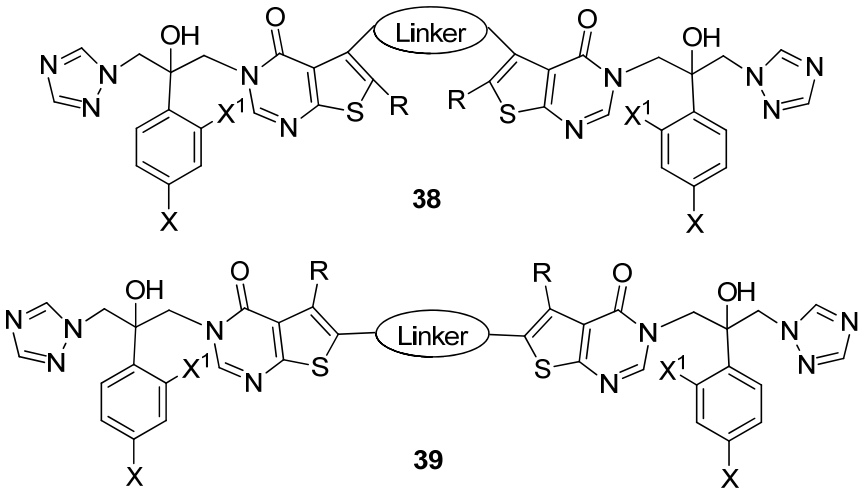


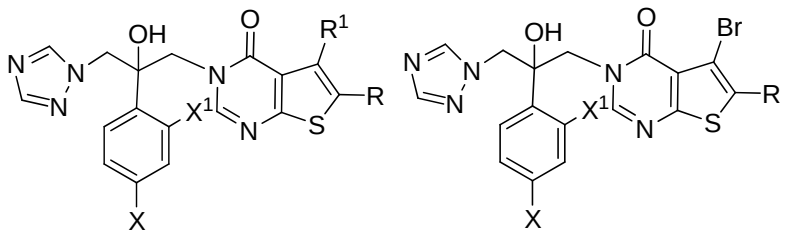
Figure 19. Dimeric fluconazole analogues containing thienopyrimidinone moieties

The structure activity relationship study of fluconazole has revealed that one of the triazole rings, halogenated aromatic ring and *tert.* hydroxyl group are essential for the antifungal activity because this portion of the molecule is involved in the binding to the enzyme at the site of action. Hence, without disturbing this part of the molecule, we thought to put the linker chains in between the thienopyrimidinone moieties of the two monomers. In

order to make a number of compounds available for biological activity and to study the effect of linker chains we planned to vary the linker chain length and halogens on the aromatic rings. So, initially we directed our efforts towards the synthesis of dimeric molecules depicted by general formula **38** followed by synthesis of compounds depicted by general formula **39**. The dimeric molecules synthesized were tested for their antifungal activity against various strains of fungi.

2.4.2 Results and discussion

As we had synthesized molecules of general formula **40**, discussed in first chapter, we thought of dimerizing the synthesized molecules in order to get new molecules. For doing so, we thought to put a handle on the thienopyrimidinone, and hence we brominated the thiophene ring of the thienopyrimidinone moiety. The fluconazole analogues having brominated thienopyrimidinone moiety could be used for the coupling purpose using suitable linker. Keeping this idea of coupling of two monomer units in the mind, we synthesized the compounds **41a-d** from the suitable starting materials (discussed in Chapter 1, Scheme 8).



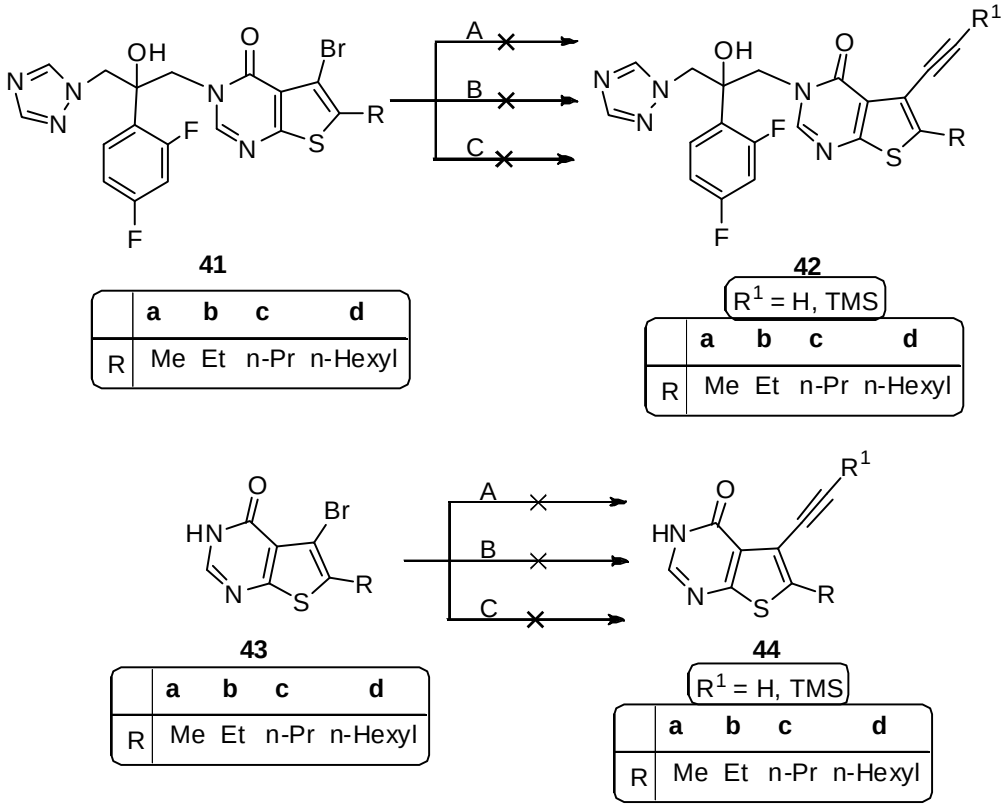
40 **41**

	a	b	c	d
R	Me	Et	*n*-Pr	*n*-Hexyl

Figure 20. Fluconazole molecules containing thienopyrimidinone moieties

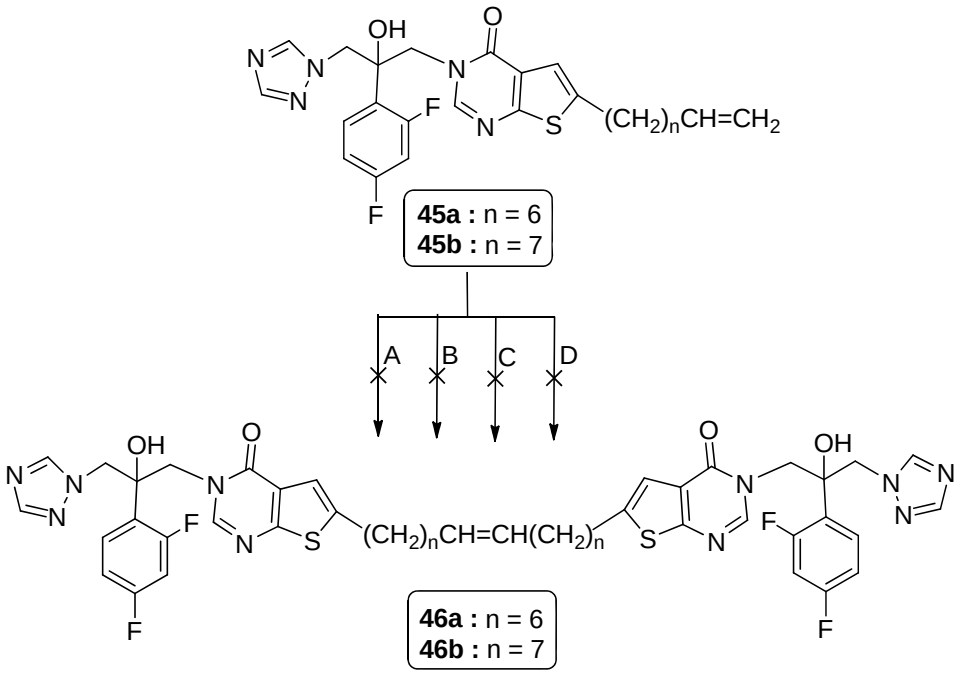
Having compounds **41a-d** in hand, we thought to couple them with the ethynyltrimethylsilane using Sonogashira coupling reaction[23] conditions. Accordingly, compound **41a** was treated with acetylene/ethynyltrimethylsilane in presence of palladium catalyst at room temperature and also at 50 ^{o}C but unfortunately we recovered starting material. Then we varied the reaction conditions by varying temperature and reagent, but in each case we observed the recovery of starting material. We varied starting material from

41a to **41b**, **41c** and **41d** to see the effect of side chain present on the thienopyrimidinone moiety but the reactions failed in all the cases. Furthermore, we thought to couple the thienopyrimidinone molecules **43a-d** first and then to construct the rest of the molecule (Scheme 1), but this route also failed to give the coupled product.



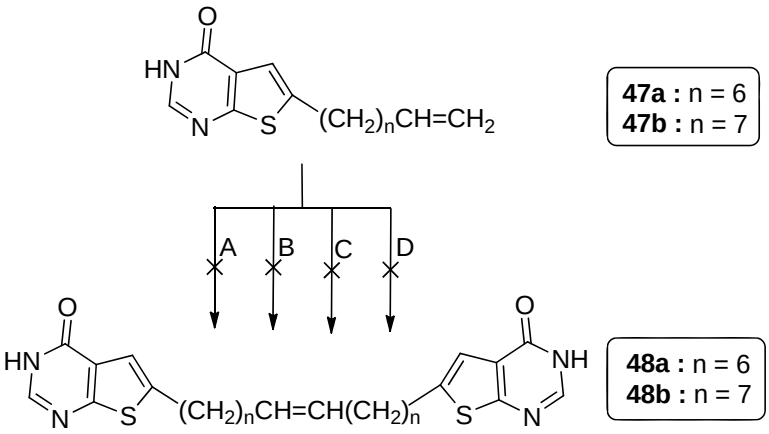
Scheme 1. Reagents and conditions**:** A) Acetylene, TEA, $(PPh_3)_2PdCl_2$, THF, rt, 6-8 h; B) Ethynyltrimethylsilane, TEA, $(PPh_3)_2PdCl_2$, THF, rt, 6-8 h; C) Ethynyltrimethylsilane, TEA, $(PPh_3)_2PdCl_2$, THF, 50 ^oC, 6-8 h

If we could have successfully obtained products **42** with R^1 = TMS and /or **44** with R^1 = TMS, then we could have deprotected the TMS group and further reacted with another monomeric unit under similar reaction conditions to get the dimeric molecule. Failures in case of Sonogashira coupling reaction made us to attempt the synthesis of dimeric molecules, using Grubb's catalyst, from the monomeric units having terminal olefin side chains on the thienopyrimidinone moiety. The molecules having terminal olefinic group such as **45a** and **45b** were synthesized using the strategy discussed in first chapter (Scheme 8 in Chapter 1, Section 1) and used for the self metathesis reaction using Grubb's catalyst as shown in Scheme 2.



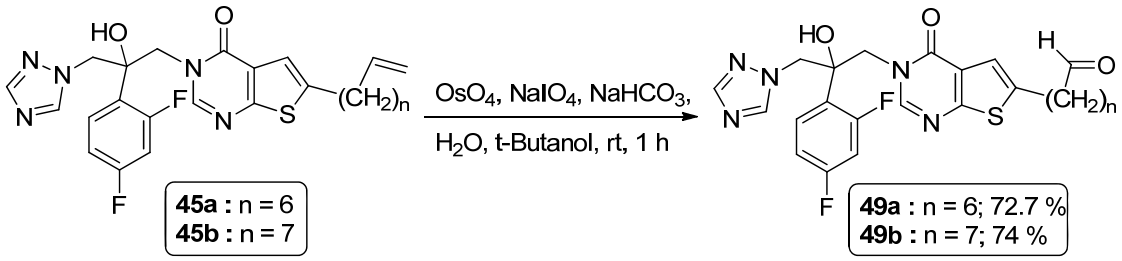
Scheme 2. Reagents and conditions: A) Grubbs catalyst 1st generation, DCM, rt, 6-8 h; B) Grubbs catalyst 2nd generation, DCM, rt, 6-8 h; C) Hoveyda-Grubbs catalyst 1st generation, DCM, rt, 8-10 h; D) Hoveyda-Grubbs catalyst 2nd generation, DCM, rt, 8-10 h

In case of the self metathesis of monomers with terminal olefin, none of the Grubb's catalyst worked to yield the product. The starting material was recovered in every case. Furthermore, our efforts to synthesize the dimeric thienopyrimidinone molecules **48a** or **48b** using Grubb's catalyst also failed when the reaction of self metathesis between the thienopyrimidinone molecules bearing terminal double bond **47a** or **47b** was attempted as shown in Scheme 3.



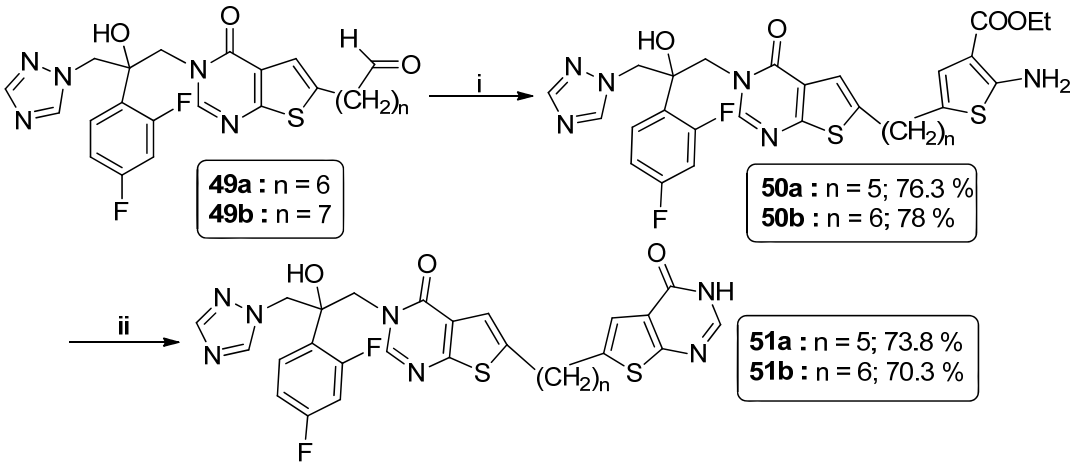
Scheme 3. Reagents and conditions: A) Grubbs catalyst 1st generation, DCM, rt, 6-8 h; B) Grubbs catalyst 2nd generation, DCM, rt, 6-8 h; C) Hoveyda-Grubbs catalyst 1st generation, DCM, rt, 8-10 h; D) Hoveyda-Grubbs catalyst 2nd generation, DCM, rt, 8-10 h

After this failure, we thought of making use of the terminal olefin moiety in the fluconazole analogues **45a** and **45b**, which is nothing but the masked aldehyde. The olefins **45a** and **45b** were subjected to reaction with osmium tetroxide and sodium periodate to yield the aldehydes **49a** and **49b** respectively as shown in Scheme 4.



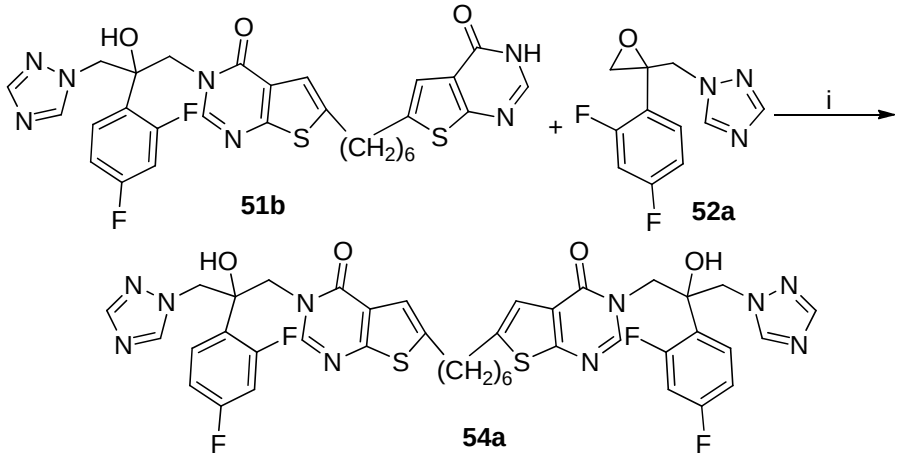
Scheme 4. Synthesis of **49a** and **49b**

Having compound **49** in hand, it was subjected to Gewald synthesis[24] to afford 2-aminothiophene-3-carboxylate **50**. In PMR spectrum aldehydic proton above δ 9.75 disappeared and protons for ethyl ester were observed. Furthermore, in IR spectrum C=O frequency at 1721 cm^{-1} along with 1667 cm^{-1} was observed which confirmed the formation of compound **50**. Then it was cyclized in presence of ammonium acetate and formamide to get the key intermediate **51**, as shown in Scheme 5. Product formation was confirmed by PMR as well as Mass spectroscopy.



Scheme 5. Reagents and conditions**:** i) Ethyl cyanoacetate, sulphur, DMF, TEA, 50 °C, 10-12 h; ii) NH_4OAc, $HCONH_2$, 140-145 °C, 4-5 h

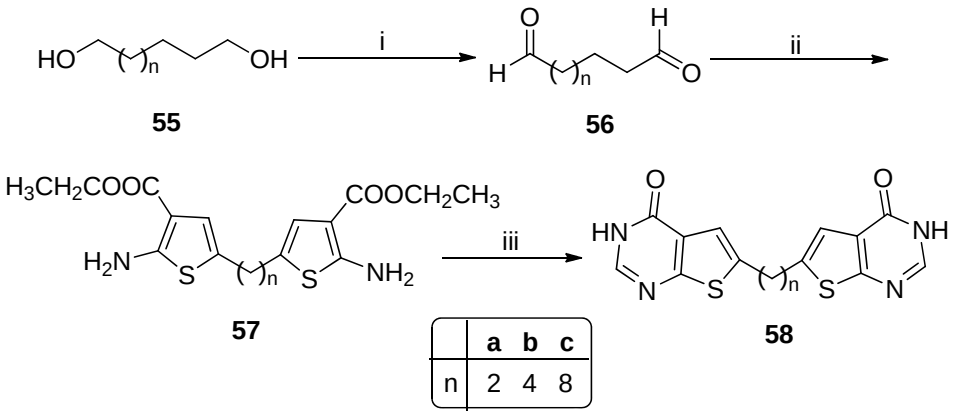
Having **51b** in hand, it was then treated with 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **52a** (Synthesis of 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole **52a** was achieved using literature procedure[25] described in 1st Section of 1st Chapter, Scheme 7) in presence of potassium carbonate in ethyl acetate at refluxing temperature to afford the target molecule **54a** as shown in Scheme 6.



Scheme 6. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 7 h, 64.8 %.

The route developed for the synthesis of the dimeric molecule **54a** was lengthy and time consuming as the steps involved in the synthesis were too many which directly affected the overall yield of the molecule. Hence it was decided to reduce the steps involved in the

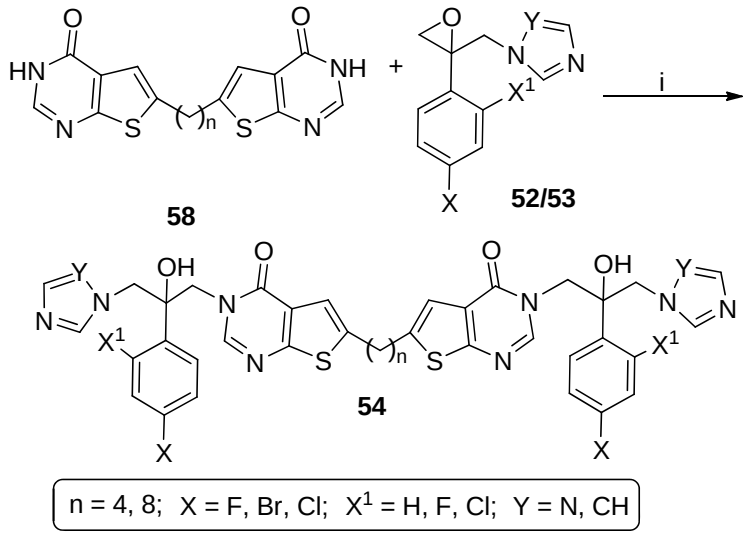
synthesis by modification in the synthetic sequence. Previously, we had mono protected the diols and oxidized the primary alcohol to get the aldehyde, which was followed by the Gewald reaction to get 5-substituted 2-aminothiophene-3-carboxylates (Compounds **79l**, **79m**, **79n** and **79o** described in Scheme 5 in Chapter 1). Based on these results, it was proposed to synthesize dimeric fluconazole analogues **54** containing thieno[2,3-*d*]pyrimidin-4(3*H*)-one moieties from suitable diols as shown in Scheme 7. Thus, a suitable diol **55** (depending on linker chain expected in dimer) was subjected for oxidation to get di-aldehyde **56**, which was subjected to Gewald synthesis[24] to afford 5,5'-(alkane-diyl)bis(2-aminothiophene-3-carboxylic acid) **57**. PMR spectrum showed singlet peak for two protons in aromatic region above δ 6.60 indicating the formation of compound **57**. This was then reacted with ammonium acetate and formamide to get 6,6'-(alkane-diyl)dithieno[2,3-*d*]pyrimidin-4(3*H*)-one **58**. Evidence for the formation of thienopyrimidinone **58** was shown by the PMR value of aromatic proton in the region δ 8.06-8.08 and C=O frequency in IR in the region 1660-1690 cm^{-1}.



Scheme 7. Reagents and conditions: i) DMSO, $(COCl)_2$, Et_3N, DCM, -78 ^oC - rt, 4 h; ii) Ethyl cyanoacetate, S_8, morpholine, EtOH, 80 ^oC, 10-12 h, 77.4-89.8 % over 2 steps; iii) NH_4OAc, $HCONH_2$, 140-145 ^oC, 5 h, 75.4-77.1 %.

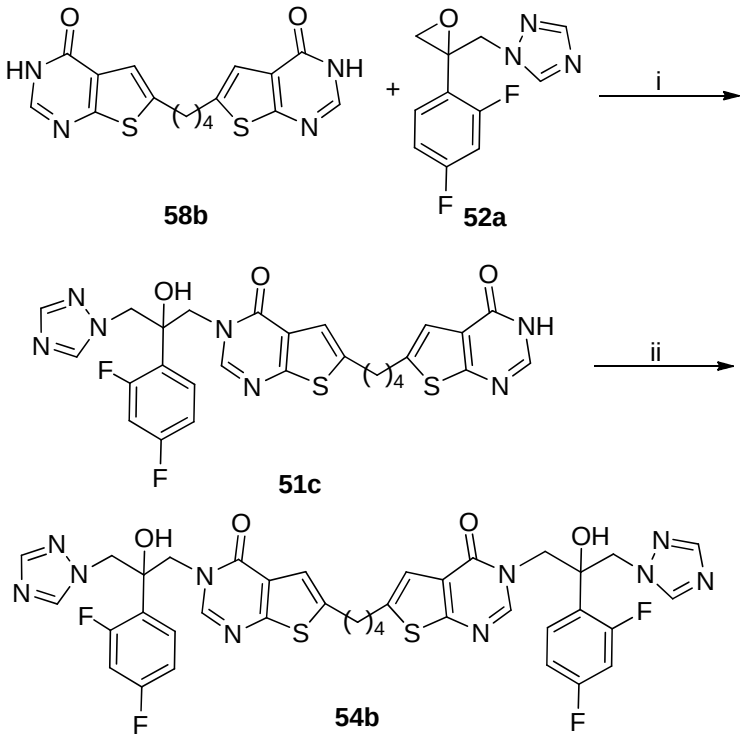
It is noteworthy that in case of 1,6-hexanediol the corresponding diethyl 5,5'-(ethane-1,2-diyl)bis(2-aminothiophene-3-carboxylate) (**57a)** was obtained but the product obtained after its reaction with formamide and ammonium acetate was insoluble in all routinely used organic solvents even at elevated temperatures and hence could not be characterised or reacted further.

After getting compound **58** in hand, it was treated with excess epoxide **52** or **53** (Synthesis of epoxide **52** was done using literature procedure[25] described in 1st Section of 1st Chapter, Scheme 7. For the synthesis of epoxide **53**, same procedure was utilized with imidazole in place of 1,2,4-triazole) to get the dimeric molecule **54** as shown in Scheme 8. Four doublets, each for two protons in the range of δ 3.32-5.30 with coupling constant J = 14 in PMR confirmed the formation of product. These four protons are diastereotopic in nature and hence showed doublets in PMR spectrum.



Scheme 8. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 12 h, 57.3-69.8 %.

When **58b** was treated with one equivalent of **52a** under similar reaction conditions i.e. in presence of potassium carbonate in ethyl acetate at refluxing temperature, we observed the formation of **51c** as shown in Scheme 9. Formation of compound **51c** was confirmed by CMR and Mass spectroscopy.



Scheme 9. Reagents and conditions: i) K_2CO_3, EtOAc, 80 °C, 7 h, 58 %; ii) **52a**, K_2CO_3, EtOAc, 80 °C, 12 h, 57.3 %.

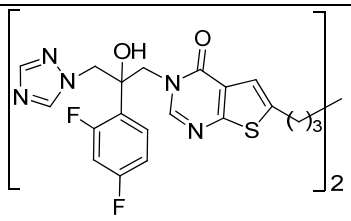
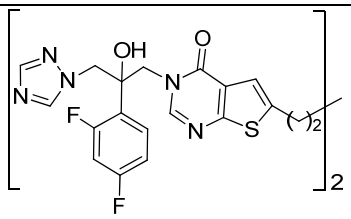
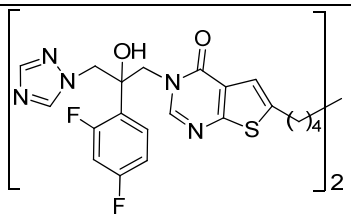
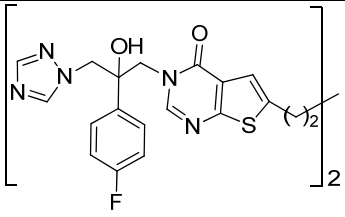
The compound **51c** thus formed was isolated and characterized, and again it was treated with one equivalent of epoxide **52a** under similar reaction conditions to get **54b**. For rest of the reactions of epoxide opening, we used excess equivalents of **52/53**. Some of the compounds synthesized in present chapter such as **50**, **51**, **54**, **57** and **58** showed poor solubility in common solvents such as ethyl acetate, chloroform, dichloromethane, methanol *etc*. at room temperature, but they were found to be fairly soluble in dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylacetamide (DMAC).

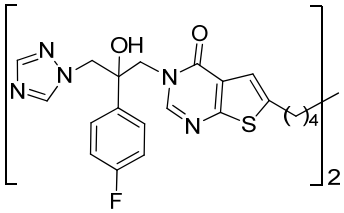
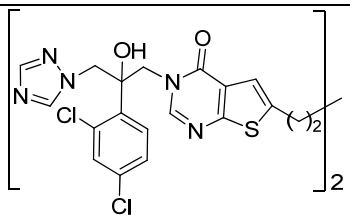
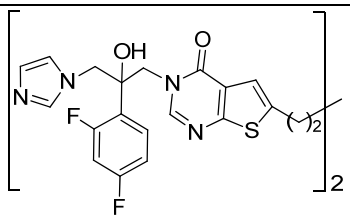
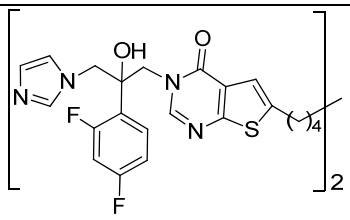
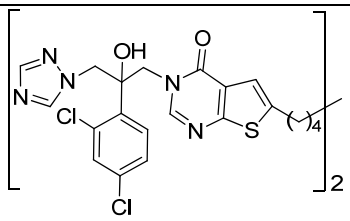
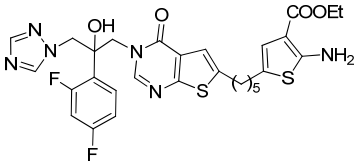
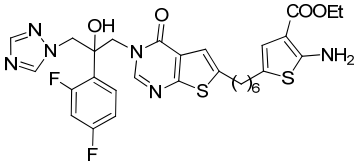
Bioevaluation of dimeric fluconazole analogues

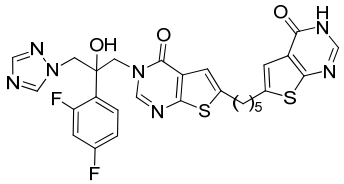
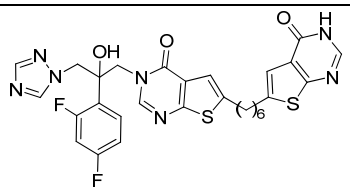
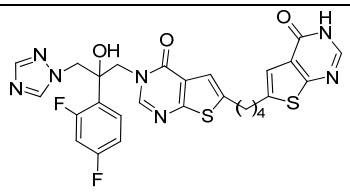
All the newly synthesized compounds **54** as well as **50** and **51** were tested for antifungal activity at FDC Ltd, Mumbai. *In vitro* evaluation of antifungal activity was performed by determining the minimum inhibitory concentration (MIC) following standard methods.[28,29] Antifungal susceptibility testing of these compounds was done by broth dilution method. Known antifungal agents like fluconazole and amphotericin B were used as positive control and end points were determined after 48 hours visually and by using

spectrophotometer wherever necessary. The fungal strains used were *Candida albicans* ATCC 24433, *Aspergillus niger* ATCC 16404 and *Fusarium proliferatum* ATCC 10052 as shown in Table 1. Different dilutions were tried and various sets of experiments performed. The activity parameters are enumerated in Table 1.

Table1. Antifungal activity data of dimeric fluconazole analogues

Sr. No.	Comp. Code	Structures	MIC_{50} in μg/ml		
			C. albicans ATCC 24433	*A. niger* ATCC 16404	*F. proliferatum* ATCC 10052
A		Fluconazole	**0.25-1**	64-128	>128
B		Amphotericin B	**0.12-0.25**	0.25-1	1-2
1	**54a**		1-2	>128	>128
2	**54b**		0.5-1	NA	NA
3	**54c**		**0.06-0.12**	>4	>4
4	**54d**		**0.06-0.12**	NI till 32	NI till 32

5	**54e**		**0.03-0.06**	NI till 4	NI till 4
6	**54f**		**0.03-0.06**	NI till 2	NI till 2
7	**54g**		**0.06-0.12**	2	2
8	**54h**		**0.25-0.5**	4	4
9	**54i**		1-2	2	2
10	**50a**		1-2	>4	>4
11	**50b**		**0.25-0.5**	>2	>2

12	**51a**		**0.06-0.12**	NI till 4	NI till 4
13	**51b**		1-2	NI till 64	NI till 64
14	**51c**		**0.06-0.12**	NI till 4	NI till 4

NI: No inhibition
For azoles: For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 50 % inhibition as compared to the positive control.
For amphotericin B: MIC is recorded as the concentration exhibiting complete inhibition.

The activity data indicated following points regarding the structure-activity relationship of the compounds studied in the present chapter.

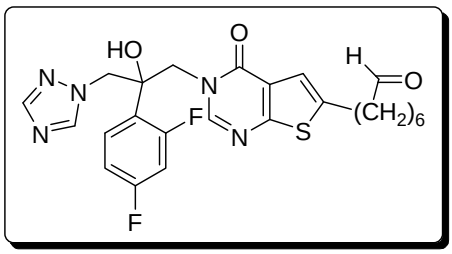
1. Most of the dimeric compounds exhibit significant antifungal activity against *Candida albicans* but they do not show activity against *Aspergillus niger* or *Fusarium proliferatum.*
2. The compounds with 2,4-difluorophenyl moieties show higher activity than compounds with 4-fluorophenyl and 2,4-dichlorophenyl moieties.
3. The compounds with 1,2,4-triazole moieties possess higher activity than those with imidazole moieties.

2.5 Conclusion

Dimeric fluconazole analogues containing substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were synthesized using two routes with good yields. Variation in the linker chains can be done by varying the starting materials, most of which are readily available. These reactions can be carried out on large scales. The designed new chemical entities (NCEs) showed considerable antifungal activity but these NCEs had less solubility than respective monomeric units. This investigation is helpful for the improvement in design and development of new antifungal agents with superior antifungal activity and short chemical sequences.

2.6 Experimental Section

Preparation of 7-(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)heptanal (49a)

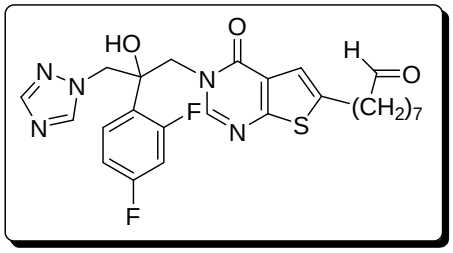


In a round bottom flask, 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(oct-7-en-1-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**45a**) (5 g, 10.02 mmol) was dissolved in a mixture of t-butanol (250 ml) and water (50 ml) and a solution of OsO_4 (0.05 M, 0.2 ml, 0.01 mmol) in t-butanol was added to it. The reaction mixture was stirred for 5 min at room temperature, when dark black color appeared, sodium bicarbonate (3.36 g, 4.0 mmol) and sodium periodate (21.4 g, 10.0 mmol) were added to reaction mixture and further stirred for 1 h. It was then diluted with water (300 ml) and extracted with ethyl acetate (3 X 100 ml), organic layer was combined, dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product (3.65 g, 72.7 %).

Nature: White solid; **MP:** 148 ^oC; **Yield:** 72.7 %; **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 1.36-1.42 (m, 4H), 1.60-1.74 (m, 4H), 2.44 (t, *J* = 8 Hz, 2H), 2.83 (t, *J* = 8 Hz, 2H), 4.30 (d, *J* = 16 Hz, 1H), 4.66 (d, *J* = 16 Hz, 1H), 4.75 (d, *J* = 16 Hz, 1H), 4.79 (d, *J* = 16 Hz, 1H), 6.33 (bs, 1H), 6.77-6.86 (m, 2H), 7.07 (s, 1H), 7.51-7.55 (m, 1H), 7.95 (s, 1H), 7.96 (s, 1H), 8.52 (s, 1H), 9.77 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.6, 28.5, 30.4, 30.5, 43.5 (2C), 53.1, 55.4, 75.5, 104.2 (t), 112.0 (d), 117.8, 122.3 (d), 123.7, 130.0, 144.5, 145.0, 146.1, 151.1, 158.3 (dd), 158.7, 162.7, 163.2 (dd), 202.4; **IR** (Chloroform)**:** 3369, 1720, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 502.4689 (M + 1); **Anal. Calcd for** $C_{24}H_{25}F_2N_5O_3S$**:** C, 57.47; H, 5.02; F, 7.58; N, 13.96 %. **Found:** C, 57.72; H, 5.16; F, 7.25; N, 13.88 %.

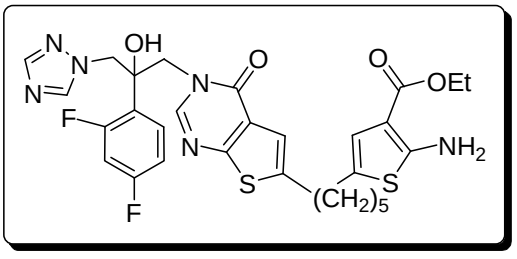
The aldehyde **49b** was prepared from the olefin **45b** using the same procedure given for the compound **49a**.

8-(3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)octanal (49b)



Nature: White solid; **MP:** 130 ^{o}C; **Yield:** 74 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.22-1.40 (m, 6H), 1.55-1.71 (m, 4H), 2.43 (dt, *J* = 2, 6 Hz, 2H), 2.82 (t, *J* = 8 Hz, 2H), 4.24 (d, *J* = 14 Hz, 1H), 4.54 (d, *J* = 14 Hz, 1H), 4.73 (d, *J* = 14 Hz, 1H), 4.82 (d, *J* = 14 Hz, 1H), 6.22 (bs, 1H), 6.75-6.89 (m, 2H), 7.08 (s, 1H), 7.49-7.62 (m, 1H), 7.85 (s, 1H), 7.93 (s, 1H), 8.11 (s, 1H), 9.76 (t, *J* = 2 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 21.7, 28.4, 28.7 (2C), 30.3, 30.6, 43.5 53.0, 55.3, 75.6, 104.1 (t), 111.9 (d), 117.7, 122.0 (d), 123.7, 130.0 (d), 144.5, 145.1, 146.1, 151.0, 158.6, 158.7 (dd), 162.7, 163.5 (dd), 202.5; **IR** (Chloroform)**:** 3403, 1720, 1673 cm^{-1}; **MS** (ESI) *m*/*z***:** 516.3182 (M+1); **Anal. Calcd for** $C_{25}H_{27}F_2N_5O_3S$**:** C, 58.24; H, 5.28; F, 7.37; N, 13.58 %. **Found:** C, 57.87; H, 5.45; F, 7.69; N, 13.38 %.

Preparation of ethyl 2-amino-5-(5-(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)pentyl)thiophene-3-carboxylate (50a)

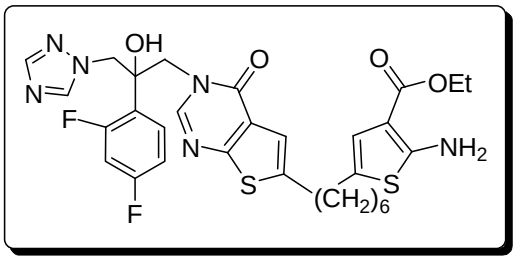


7-(3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)heptanal (**49a**) (17 g, 33 mmol) was taken in two-necked round bottom flask equipped with guard tube, DMF (150 ml) was added followed by

sulphur powder (1.05 g, 33 mmol), ethyl cyanoacetate (3.51 ml, 3.73 g, 33 mmol) and triethyl amine (2.38 ml, 1.6 g, 16.5 mmol). The reaction mixture was stirred at 55 ºC for 10 h, cooled, diluted with water (300 ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated under vacuum to obtain the crude product which on purification by column chromatography yielded pure product as dark green semisolid (16.16 g, 76.3 %).
Nature: Dark green semisolid; **Yield:** 76.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.20-1.36 (m, 5H), 1.64-1.74 (m, 4H), 2.57 (t, *J* = 6 Hz, 2H), 2.81 (t, *J* = 6 Hz, 2H), 4.24 (q, *J* = 8 Hz, 2H), 4.68 (d, *J* = 14 Hz, 1H), 4.75 (d, *J* = 14 Hz, 1H), 4.87-4.94 (m, 2H), 5.09 (bs, 2H), 6.60 (s, 1H), 6.73-6.89 (m, 2H), 7.05 (s, 1H), 7.44-7.54 (m, 1H), 7.97 (s, 1H), 8.02 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.3, 27.8, 29.2, 30.2, 30.3, 30.4, 52.8, 55.3, 59.3, 75.6, 104.1 (t), 105.6 (2C), 111.8 (d), 117.8, 121.3, 122.4 (d), 123.7, 125.8, 129.9 (d), 144.8, 146.1, 150.7, 158.5, 158.6 (dd), 161.3, 162.4, 163.5 (dd), 165.1; **IR** (Chloroform)**:** 3368, 1667 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 628.5713 (M + 1); **Anal. Calcd for** $C_{29}H_{30}F_2N_6O_4S_2$**:** C, 55.40; H, 4.81; F, 6.04; N, 13.37 %. **Found:** C, 55.12; H, 5.15; F, 5.95; N, 13.11 %.

Compound **50b** was prepared by reacting **49b** with ethyl cyanoacetate and sulphur in presence of triethyl amine using the same procedure given for compound **50a**.

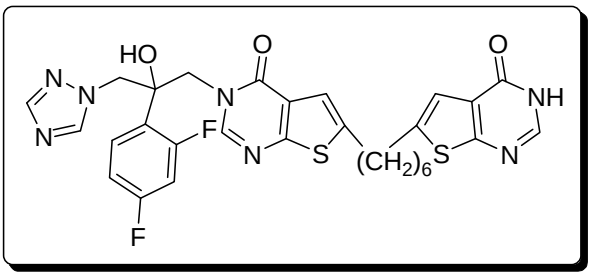
Ethyl 2-amino-5-(6-(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)hexyl)thiophene-3-carboxylate (50b)



Nature: Pale brown solid; **MP:** 77 ^oC; **Yield:** 78 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.23-1.39 (m, 7H), 1.61-1.75 (m, 4H), 2.56 (t, *J* = 6 Hz, 2H), 2.82 (t, *J* = 6 Hz, 2H), 4.25 (q, *J* = 6 Hz, 2H), 4.28 (d, *J* = 14 Hz, 1H), 4.53 (d, *J* = 14 Hz, 1H), 4.74 (d, *J* = 14 Hz, 1H), 4.82 (d, *J* = 14 Hz, 1H), 5.83 (bs, 2H), 6.21 (bs, 1H), 6.61 (s, 1H), 6.76-6.89 (m, 2H), 7.08 (s,

1H), 7.49-7.61 (m, 1H), 7.85 (s, 1H), 7.93 (s, 1H), 8.10 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 14.4, 28.2, 28.4, 29.4, 30.5, 30.7 (2C), 53.2, 55.3, 59.4, 75.8, 104.2 (t), 105.9, 112.0 (d), 117.9, 121.3, 122.2 (d), 123.8, 126.2, 130.0 (d), 144.6, 145.3, 146.1, 151.5, 158.8, 159.2 (dd), 161.2, 162.8, 163.4 (dd), 165.2; **IR** (Chloroform)**:** 3395, 1671 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 643.5668 (M + 1), 665.5656 (M + Na); **Anal. Calcd for** $C_{30}H_{32}F_2N_6O_4S_2$**:** C, 56.06; H, 5.02; F, 5.91; N, 13.08 %. **Found:** C, 55.79; H, 5.37; F, 6.07; N, 13.29 %.

Preparation of 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(6-(4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)hexyl)thieno[2,3-d]pyrimidin-4(3*H*)-one (51b)



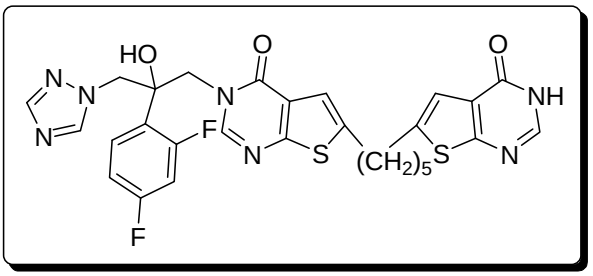
A mixture of ethyl 2-amino-5-(6-(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)hexyl)thiophene-3-carboxylate (**50b**) (4 g, 6.23 mmol), ammonium acetate (0.479 g, 6.23 mmol) and formamide (4.9 ml, 124 mmol) was taken in a two-necked round bottom flask equipped with reflux condenser and a guard tube and stirred at 145-150 °C for 10 h. The reaction mixture was then cooled to room temperature, diluted with ice-cold water (400 ml) and stirred for 30 min to obtain a solid mass which was filtered, washed with water (3 X 50 ml) followed by washing with 20 % ethyl acetate in petroleum ether (3 X 8 ml) to get pure product as off-white solid (2.73 g, 70.36 %).

Nature: Off-white solid; **MP:** 147 ^{o}C; **Yield:** 70.36 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.19-1.27 (m, 4H), 1.48-1.59 (m, 4H), 2.58-2.70 (m, 4H), 4.09 (d, *J* = 14 Hz, 1H), 4.38 (d, *J* = 14 Hz, 1H), 4.62 (d, *J* = 14 Hz, 1H), 4.72 (d, *J* = 14 Hz, 1H), 6.05 (bs, 1H), 6.54-6.74 (m, 2H), 6.90 (s, 1H), 6.93 (s, 1H), 7.21-7.32 (m, 1H), 7.59 (s, 1H), 7.70 (s, 1H), 7.84 (s, 1H), 7.96 (s, 1H), 11.97 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ

26.7 (2C), 28.5 (2C), 29.1 (2C), 49.4, 53.2, 73.3, 102.4 (t), 109.3 (d), 116.3, 116.6, 121.8 (d), 122.3, 123.5, 128.0 (d), 141.2, 142.1, 143.2, 146.2, 149.0, 155.8, 156.0, 157.4 (dd), 160.5 (2C), 161.6, 161.7 (dd); **IR** (Chloroform)**:** 3384, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 624.5758 (M + 1), 646.5770 (M + Na), 662.5542 (M + K); **Anal. Calcd for** $C_{29}H_{27}F_2N_7O_3S_2$**:** C, 55.85; H, 4.36; F, 6.09; N, 15.72 %. **Found:** C, 55.71; H, 4.31; F, 6.14; N, 15.91 %.

Compound **51a** was prepared by reacting **50a** with ammonium acetate and formamide using the procedure given for the compound **51b**.

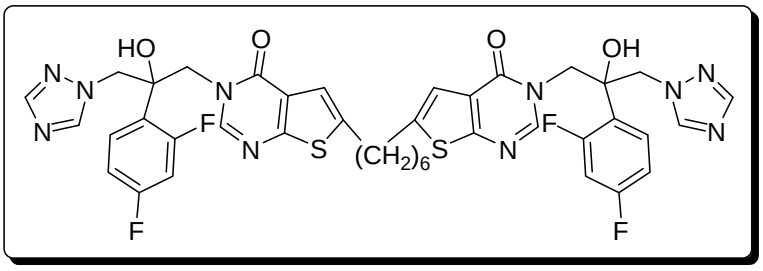
3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(5-(4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)pentyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (51a)



Nature: Brown semisolid; **Yield:** 73.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.38-1.44 (m, 4H), 1.68-1.79 (m, 2H), 2.00-2.15 (m, 2H), 2.81-2.90 (m, 2H), 4.51-4.62 (m, 2H), 4.78-4.90 (m, 2H), 5.85 (bs, 1H), 6.21 (s, 1H), 6.30 (s, 1H), 6.75-6.87 (m, 1H), 7.02-7.07 (m, 1H), 7.47-7.59 (m, 1H), 7.97 (s, 1H), 8.07 (s, 1H), 8.19 (s, 1H), 8.26 (s, 1H), 12.32 (bs, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$)**:** δ 27.4, 30.0 (2C), 30.4 (2C), 53.3, 55.5, 75.9, 104.3 (t), 112.2 (d), 117.9, 118.2, 122.4 (d), 123.9, 124.9, 130.1 (d), 142.9, 143.9, 144.7, 145.1, 151.5, 158.8, 158.9, 160.1 (dd), 163.0, 163.1, 164.1, 164.2 (dd); **IR** (Chloroform)**:** 3439, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 610.5294 (M + 1), 632.5236 (M + Na), 648.4856 (M + K); **Anal. Calcd for** $C_{28}H_{25}F_2N_7O_3S_2$**:** C, 55.16; H, 4.13; F, 6.23; N, 16.08 %. **Found:** C, 54.89; H, 4.27; F, 6.36; N, 15.92 %.

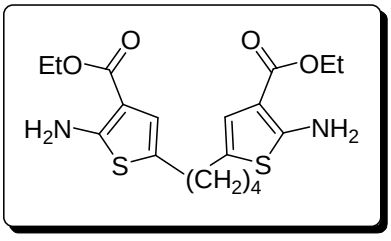
Compound **54a** was prepared by reacting **51b** with epoxide **52a** using the procedure given for the preparation of compound **54b**.

6,6'-(Hexane-1,6-diyl)bis(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54a)



Nature: White solid; **MP:** 162 ^{o}C; **Yield:** 64.8 %; **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 1.34-1.45 (m, 4H), 1.61-1.74 (m, 4H), 2.81 (t, *J* = 6 Hz, 4H), 4.23 (d, *J* = 14 Hz, 2H), 4.54 (d, *J* = 14 Hz, 2H), 4.73 (d, *J* = 14 Hz, 2H), 4.82 (d, *J* = 14 Hz, 2H), 6.21 (bs, 2H), 6.75-6.88 (m, 4H), 7.06 (s, 2H), 7.49-7.61 (m, 2H), 7.84 (s, 2H), 7.93 (s, 2H), 8.11 (s, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 28.4 (2C), 30.4 (2C), 30.7 (2C), 53.4 (2C), 55.5 (2C), 76.0 (2C), 104.3 (t, 2C), 112.2 (d, 2C), 117.9 (2C), 122.3 (d, 2C), 123.9 (2C), 130.2 (d, 2C), 144.7 (2C), 145.2 (2C), 146.1 (2C), 151.7 (2C), 158.4 (dd, 2C), 158.9 (2C), 163.0 (2C), 163.3 (2C); **IR** (Chloroform)**:** 3373, 1698 cm^{-1}; **MS** (ESI) *m*/*z***:** 861.9924 (M + 1), 883.9329 (M + Na), 899.9227 (M + K); **Anal. Calcd for** $C_{40}H_{36}F_4N_{10}O_4S_2$**:** C, 55.81; H, 4.21; F, 8.83; N, 16.27 %. **Found:** C, 56.14; H, 4.12; F, 8.69; N, 16.32 %.

Preparation of diethyl 5,5'-(butane-1,4-diyl)bis(2-aminothiophene-3-carboxylate) (57b)



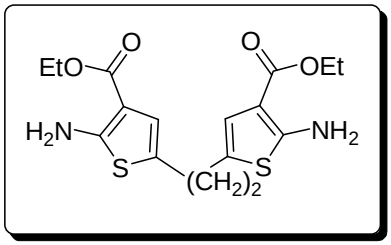
1,8-Octanedial (20 g, 0.14 mol) was taken in two-neck round bottom flask equipped with guard tube, DMF (150 ml) was added followed by sulphur powder (9.01 g, 0.28 mol), ethyl cyanoacetate (30.0 ml, 31.8 g, 0.28 mol) and triethyl amine (20.3 ml, 14.2 g, 0.14 mol) and stirred at 50-55 ºC for 11 h. The reaction mixture was then cooled, diluted with water (300

ml), extracted with ethyl acetate (3 X 100 ml), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography yielded pure product as pale yellow solid (50.08 g, 89.8 %).

Nature: Pale yellow solid; **MP:** 164-165 ^oC; **Yield:** 89.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.22 (t, *J* = 6 Hz, 6H), 1.42-1.65 (m, 4H), 2.38-2.54 (m, 4H), 4.13 (q, *J* = 6 Hz, 4H), 6.51 (s, 2H); **IR** (Chloroform)**:** 3420, 1748 cm^{-1}; **MS** (ESI) *m*/*z***:** 397.1793 (M + 1); **Anal. Calcd for** $C_{18}H_{24}N_2O_4S_2$**:** C, 54.52; H, 6.10; N, 7.06 %. **Found:** C, 54.67; H, 5.96; N, 7.15 %.

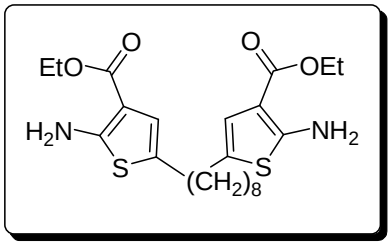
The amino esters **57a** and **57c** were prepared from 1,6-hexanedial and 1,12-dodecanedial respectively under Gewald reaction conditions using the procedure given for the preparation of compound **57b**.

Diethyl 5,5'-(ethane-1,2-diyl)bis(2-aminothiophene-3-carboxylate) (57a)



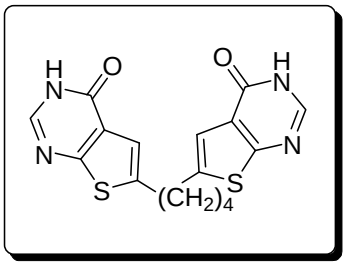
Nature: Grey solid; **MP:** 221-222 ^oC; **Yield:** 77.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.33 (t, *J* = 6 Hz, 6H), 2.85 (s, 4H), 4.26 (q, *J* = 6 Hz, 4H), 5.81 (bs, 4H) 6.65 (s, 2H) ; **^{13}C NMR** (125 MHz, $CDCl_3$)**:** δ 14.3 (2C), 31.2 (2C), 59.5 (2C), 106.0 (2C), 122.3 (2C), 124.4 (2C), 161.5 (2C), 165.2 (2C) ; **IR** (Nujol)**:** 3447, 1747 cm^{-1}; **Anal. Calcd for** $C_{16}H_{20}N_2O_4S_2$**:** C, 52.15; H, 5.47; N, 7.60 %. **Found:** C, 52.41; H, 5.28; N, 7.51 %.

Diethyl 5,5'-(octane-1,8-diyl)bis(2-aminothiophene-3-carboxylate) (57c)



Nature: Brown solid; **MP:** 178 ^{o}C; **Yield:** 86.4%; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.25-1.37 (m, 12H), 1.56 (t, *J* = 6 Hz, 6H), 2.57 (t, *J* = 6 Hz, 4H), 4.26 (q, *J* = 6 Hz, 4H), 5.19 (bs, 4H), 6.64 (s, 2H) ; **MS** (ESI) *m*/*z***:** 453.2407 (M + 1), 475.2279 (M + Na) ; **IR** (Nujol)**:** 3422, 1751 (b) cm^{-1}; **Anal. Calcd for** $C_{22}H_{32}N_2O_4S_2$**:** C, 58.38; H, 7.13; N, 6.19 %. **Found:** C, 58.71; H, 6.87; N, 6.27 %.

Preparation of 6,6'-(butane-1,4-diyl)bis(thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (58b)



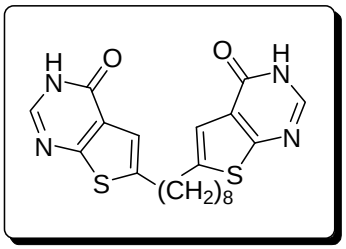
A mixture of diethyl 5,5'-(butane-1,4-diyl)bis(2-aminothiophene-3-carboxylate) (**57b**) (15 g, 37.83 mmol), ammonium acetate (5.82 g, 75.6 mmol) and formamide (29.99 ml, 756 mmol) was taken in a two-necked round bottom flask equipped with reflux condenser and a guard tube and stirred at 145-150 °C for 12 h. The reaction mixture was then cooled to room temperature, diluted with ice-cold water (400 ml) and stirred for 30 min. The solid mass was filtered, washed with water (3 X 100 ml) followed by washing with 20 % ethyl acetate in petroleum ether (3 X 10 ml) to obtain product as yellow solid (10.23 g, 75.4 %).

Nature: Yellow solid; **MP:** The compound decomposes at 389 ^{o}C; **Yield:** 75.4 %; **^{1}H NMR** (500 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 2.48-2.53 (m, 4H), 2.85-2.92 (m, 4H), 7.13 (s, 2H), 8.06

(s, 2H), 12.45 (bs, 2H); **^{13}C NMR** (125 MHz, DMSO-d_6)**:** δ 29.4 (2C), 30.1 (2C), 118.6 (2C); 125.0 (2C), 142.7 (2C), 145.3 (2C), 157.3 (2C), 163.2 (2C); **IR** (Chloroform)**:** 3436, 1667 cm^{-1}; **Anal. Calcd for** $C_{16}H_{14}N_4O_2S_2$**:** C, 53.61; H, 3.94; N, 15.63 %. **Found:** C, 53.74; H, 4.18; N, 15.26 %.

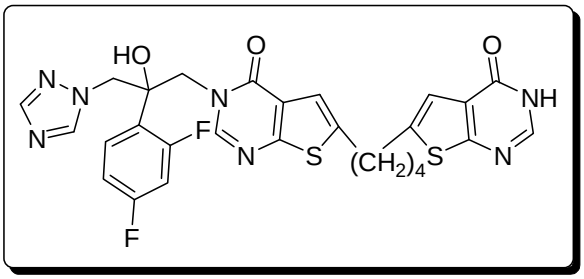
Compound **58c** was prepared by reacting compound **57c** with formamide in presence of ammonium acetate using the procedure given for the preparation of compound **58b**.

6,6'-(Octane-1,8-diyl)bis(thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (58c)



Nature: Grey solid; **MP:** 299 ^{o}C; **Yield:** 77.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.24-1.30 (m, 8H), 1.60-1.74 (m, 4H), 2.83 (t, *J* = 8 Hz, 4H), 7.11 (s, 2H), 8.07 (s, 2H), 12.46 (bs, 2H); **IR** (Chloroform)**:** 3434, 1662 cm^{-1}; **MS** (ESI) *m*/*z***:** 437.7197 (M + Na); **Anal. Calcd for** $C_{20}H_{22}N_4O_2S_2$**:** C, 57.95; H, 5.35; N, 13.52 %. **Found:** C, 58.12; H, 5.49; N, 13.71 %.

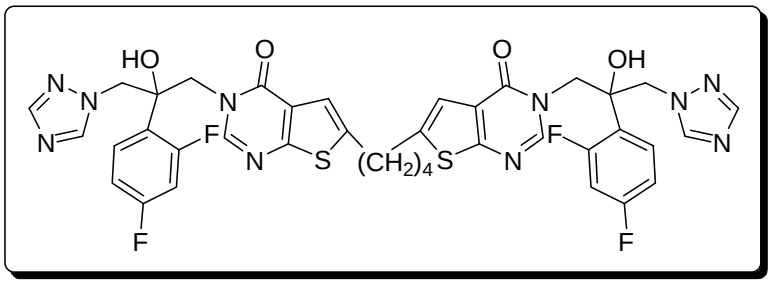
Preparation of 3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(4-(4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)butyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (51c)



6,6'-(Butane-1,4-diyl)bis(thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (**58b**) (500 mg, 1.39 mmol) was taken in two-necked round bottom flask equipped with guard tube. Dimethyl formamide (10 ml) was added followed by flame-dried potassium carbonate (578 mg, 4.19 mmol). The mixture was stirred at room temperature for 1 h and 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (**52a**) (662 mg, 2.79 mmol) in DMF (5 ml) was added. The mixture was stirred at 95 ^{o}C for 7 h. The reaction mixture was then cooled, diluted with water (50 ml), extracted with ethyl acetate (3 X 20 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product as pale brown solid (487 mg, 58 %).

Nature: Pale brown solid; **MP:** 156 ^{o}C; **Yield:** 58 %; **^{1}H NMR** (200 MHz, CD_3OD)**:** δ 1.78-1.83 (m, 4H), 2.90-2.98 (m, 4H), 4.57 (d, *J* = 14 Hz, 1H), 4.67 (d, *J* = 14 Hz, 1H), 4.75 (d, *J* = 14 Hz, 1H), 5.01 (d, *J* = 14 Hz, 1H), 6.78-6.85 (m, 1H), 6.97-7.06 (m, 1H), 7.08 (s, 1H), 7.15 (s, 1H), 7.29-7.41 (m, 1H), 7.86 (s, 1H), 8.05 (s, 1H), 8.16 (s, 1H), 8.43 (s, 1H); **IR** (Chloroform)**:** 3401, 1664 cm^{-1}; **MS** (ESI) *m*/*z***:** 618.1814 (M + Na); **Anal. Calcd for** $C_{27}H_{23}F_2N_7O_3S_2$**:** C, 54.44; H, 3.89; F, 6.38; N, 16.46 %. **Found:** C, 54.61; H, 4.01; F, 6.48; N, 16.21 %.

Preparation of 6,6'-(butane-1,4-diyl)bis(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54b)

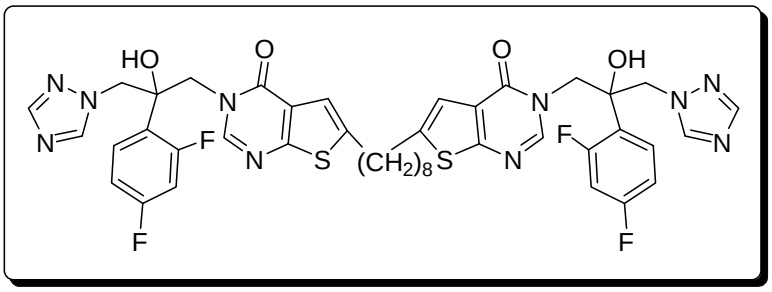


3-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-6-(4-(4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)butyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**51c**) (200 mg, 0.33 mmol) was taken in two-necked round bottom flask equipped with guard tube. Dimethyl formamide (8 ml) was added followed by flame-dried potassium carbonate (231

mg, 1.68 mmol). The mixture was stirred at room temperature for 1 h and 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (**52a**) (159 mg, 0.67 mmol) in DMF (4 ml) was added. The mixture was stirred at 95 ^{o}C for 7 h, cooled, diluted with water (40 ml), extracted with ethyl acetate (3 X 15 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product as off-white solid (160 mg, 57.3 %).

Nature: Off-white solid; **MP:** 147 ^{o}C; **Yield:** 57.3 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.22-1.35 (m, 4H), 1.59-1.71 (m, 4H), 4.26 (d, *J* = 14 Hz, 2H), 4.60 (d, *J* = 14 Hz, 2H), 4.74 (d, *J* = 14 Hz, 2H), 4.85 (d, *J* = 14 Hz, 2H), 6.30 (bs, 2H), 6.76-6.90 (m, 4H), 7.06 (s, 2H), 7.48-7.59 (m, 2H), 7.90 (s, 2H), 7.93 (s, 2H), 8.32 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 29.1, 29.4, 31.2, 31.3, 52.8 (2C), 55.8, 56.0, 75.8 (2C), 104.7 (t, 2C), 112.3 (d, 2C), 118.3 (2C), 122.6 (d, 2C), 124.2 (2C), 130.2 (2C), 145.9 (4C), 147.1 (4C), 158.8 (dd, 2C), 159.0 (2C), 162.9 (2C), 164.1 (2C); **IR** (Chloroform)**:** 3362, 1670 cm^{-1}; **MS** (ESI) *m*/*z***:** 911.2338 {(M + 2K) +1}; **Anal. Calcd for** $C_{38}H_{32}F_4N_{10}O_4S_2$**:** C, 54.80; H, 3.87; F, 9.12; N, 16.82 %. **Found:** C, 55.11; H, 3.74; F, 9.04; N, 16.71 %.

Preparation of 6,6'-(octane-1,8-diyl)bis(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54c)

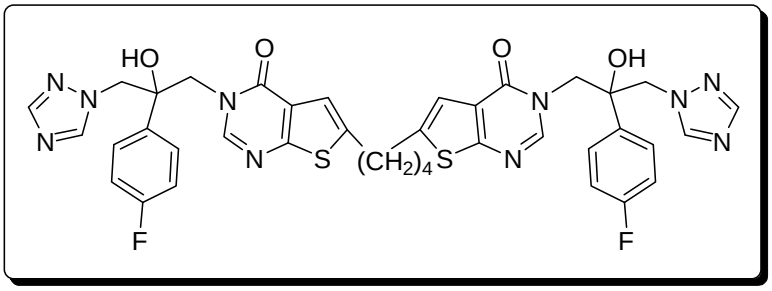


6,6'-(Octane-1,8-diyl)dithieno[2,3-*d*]pyrimidin-4(3*H*)-one (**58c**) (500 mg, 1.2 mmol) was taken in two-necked round bottom flask equipped with guard tube. Dimethyl formamide (10 ml) was added followed by flame-dried potassium carbonate (500 mg, 3.62 mmol). The mixture was stirred at room temperature for 1 h and 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (**52a**) (858 mg, 3.62 mmol) in DMF (5 ml) was added. The reaction mixture was stirred at 95 ^{o}C for 12 h, cooled, diluted with water (50 ml),

extracted with ethyl acetate (3 X 20 ml), dried over Na_2SO_4 and concentrated. Purification by column chromatography yielded pure product as pale brown solid (654 mg, 61 %).
Nature: Pale brown solid; **MP:** 249 ^oC; **Yield:** 61 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.69-1.92 (m, 12H), 2.75-2.83 (m, 4H), 4.23 (d, *J* = 14 Hz, 2H), 4.47 (d, *J* = 14 Hz, 2H), 4.64 (d, *J* = 14 Hz, 2H), 4.78 (d, *J* = 14 Hz, 2H), 6.65-6.83 (m, 4H), 6.98 (s, 2H), 7.31-7.44 (m, 2H), 7.73 (s, 2H), 7.89 (s, 2H), 8.07 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 29.9 (8C), 52.2 (2C), 52.7 (2C), 75.4 (2C), 104.2 (t, 2C), 111.8 (d, 2C), 118.0 (2C), 122.2 (2C), 123.7 (2C), 129.8 (2C), 144.4 (2C), 146.4 (2C), 150.9 (2C), 153.6 (dd, 2C), 158.4 (dd, 2C), 158.6 (2C), 162.6 (2C), 163.0 (2C); **IR** (Chloroform)**:** 3395, 1676 cm^{-1}; **MS** (ESI) *m*/*z***:** 871.2155 {(M + 1)-H_2O}; **Anal. Calcd for** $C_{42}H_{40}F_4N_{10}O_4S_2$**:** C, 56.75; H, 4.54; F, 8.55; N, 15.76 %. **Found:** C, 56.56; H, 4.69; F, 8.75; N, 15.49 %.

Compounds **54d-54i** were prepared by reacting thienopyrimidinones **58a**, **58b** or **58c** with epoxides **52** or **53** using the procedure given for compound **54c**.

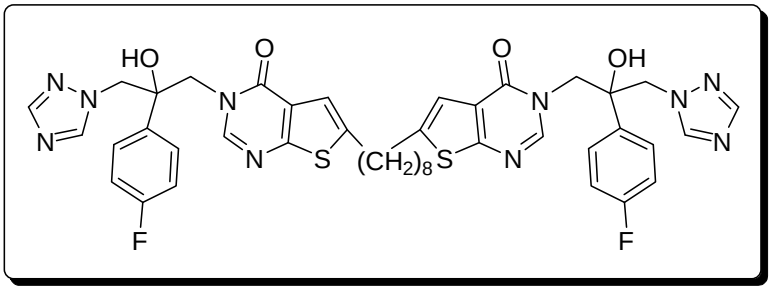
6,6'-(Butane-1,4-diyl)bis(3-(2-(4-fluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54d)



Nature: Pale yellow solid; **MP:** 135 ^oC; **Yield:** 68.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.45-1.56 (m, 4H), 2.54-2.62 (m, 4H), 3.95 (d, *J* = 14 Hz, 2H), 4.18 (d, *J* = 14 Hz, 2H), 4.42 (d, *J* = 14 Hz, 2H), 4.46 (d, *J* = 14 Hz, 2H), 5.77 (bs, 2H), 6.65-6.79 (m, 6H), 7.12-7.19 (m, 4H), 7.44 (s, 2H), 7.80 (s, 2H), 7.83 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 29.5 (2C), 29.6 (2C), 52.7 (2C), 56.2 (2C), 75.9 (2C), 114.6 (2C), 115.0 (2C), 117.9 (2C), 123.5 (2C), 126.8 (2C), 126.9 (2C), 135.3 (2C), 143.3 (2C), 146.7 (2C), 150.2 (d, 2C), 157.7 (2C), 159.2 (2C), 162.2 (4C); **IR** (Chloroform)**:** 3401, 1670 cm^{-1}; **Anal.**

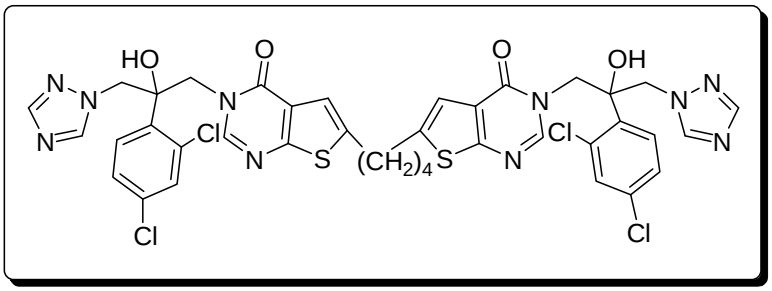
Calcd for $C_{38}H_{34}F_2N_{10}O_4S_2$**:** C, 57.28; H, 4.30; F, 4.77; N, 17.58 %. **Found:** C, 57.34; H, 4.41; F, 4.48; N, 17.60 %.

6,6'-(Octane-1,8-diyl)bis(3-(2-(4-fluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54e)



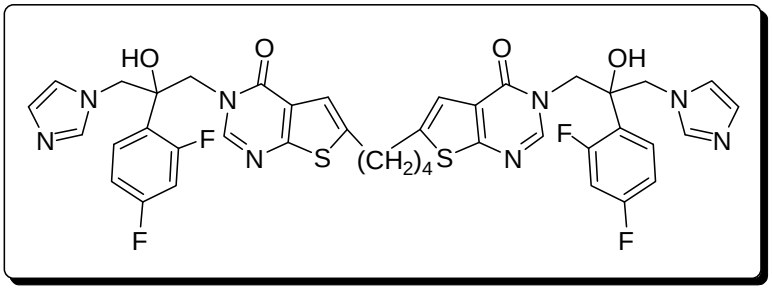
Nature: Yellow solid; **MP:** 90 ^{o}C; **Yield:** 69.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.26-1.38 (m, 8H), 1.66-1.72 (m, 4H), 2.38 (t, *J* = 6 Hz, 4H), 4.00 (d, *J* = 14 Hz, 2H), 4.36 (d, *J* = 14 Hz, 2H), 4.75 (d, *J* = 14 Hz, 2H), 4.86 (d, *J* = 14 Hz, 2H), 5.74 (bs, 2H), 6.97-7.05 (m, 4H), 7.11 (s, 2H), 7.38-7.44 (m, 4H), 7.86 (s, 2H), 7.94 (s, 2H), 8.03 (s, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 28.7 (2C), 29.0 (2C), 30.5 (2C), 30.8 (2C), 53.7 (2C), 56.1 (2C), 76.4 (2C), 115.4 (2C), 115.9 (2C), 117.9 (2C), 123.9 (2C), 127.0 (4C), 135.9 (2C), 144.5 (2C), 144.9 (2C), 146.5 (2C), 151.7 (2C), 158.7 (2C), 162.8 (2C), 163.8 (d, 2C); **IR** (Chloroform)**:** 3395, 1663 cm^{-1}; **MS** (ESI) *m*/*z***:** 853.7123 (M + 1), 875.7145 (M + Na); **Anal. Calcd for** $C_{42}H_{42}F_2N_{10}O_4S_2$**:** C, 59.14; H, 4.96; F, 4.45; N, 16.42 %. **Found:** C, 58.79; H, 5.14; F, 4.31; N, 16.11 %.

6,6'-(Butane-1,4-diyl)bis(3-(2-(2,4-dichlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54f)



Nature: Off-white solid; **MP:** 239 ^{o}C; **Yield:** 67.8 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.41-1.50 (m, 4H), 1.81-1.87 (m, 4H), 3.32 (d, *J* = 14 Hz, 2H), 3.45 (d, *J* = 14 Hz, 2H), 4.01 (d, *J* = 14 Hz, 2H), 4.26 (d, *J* = 14 Hz, 2H), 6.06 (s, 2H), 6.19 (dd, *J* = 8, 2 Hz, 2H), 6.33 (d, *J* = 8 Hz, 2H), 6.53 (d, *J* = 2 Hz, 2H), 6.61 (s, 2H), 7.19 (s, 2H), 7.22 (s, 2H); **IR** (Chloroform)**:** 3393, 1670 cm^{-1}; **Anal. Calcd for** $C_{38}H_{32}Cl_4N_{10}O_4S_2$**:** C, 50.79; H, 3.59; Cl, 15.78; N, 15.59 %. **Found:** C, 50.61; H, 3.74; Cl, 15.94; N, 15.44 %.

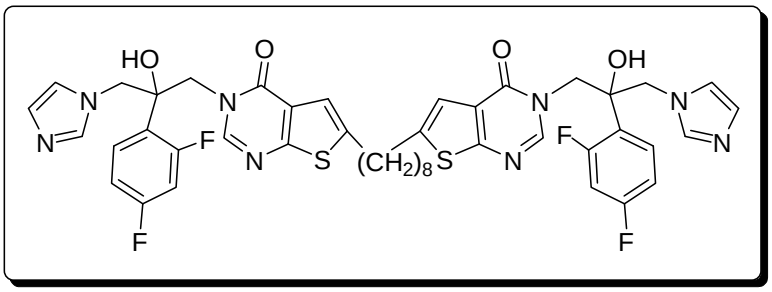
6,6'-(Butane-1,4-diyl)bis(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-imidazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54g)



Nature: Off-white solid; **MP:** 288 ^{o}C; **Yield:** 67.2 %; **^{1}H NMR** (400 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 1.68-1.72 (m, 4H), 2.84-2.90 (m, 4H), 4.21 (d, *J* = 14 Hz, 2H), 4.33 (d, *J* = 14 Hz, 2H), 4.57-4.63 (m, 4H), 6.35 (s, 2H), 6.63 (s, 2H), 6.77 (s, 2H), 6.81-6.86 (m, 2H), 7.05 (s, 2H), 7.09-7.15 (m, 2H), 7.21-7.28 (m, 2H), 8.15 (s, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$ + DMSO-d_6)**:** δ 27.5 (2C), 27.9 (2C), 48.7 (2C), 50.4 (2C), 73.2 (2C), 102.1 (t, 2C), 109.2 (d,

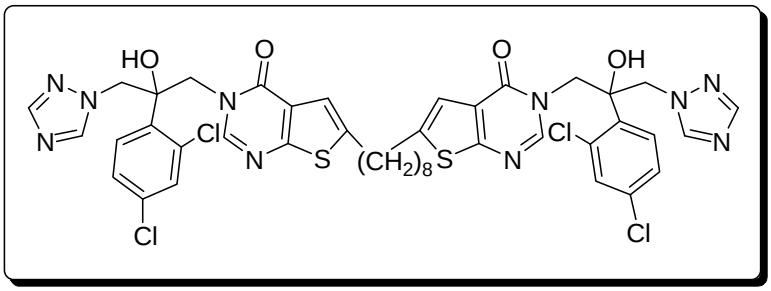
2C), 116.6 (2C), 118.4 (2C), 121.7 (4C), 125.4 (2C), 128.1 (2C), 136.0 (2C), 141.1(2C), 146.4 (2C), 155.0 (2C), 157.3 (dd, 2C), 159.9 (2C), 161.1 (dd, 2C); **IR** (Chloroform)**:** 3376, 1670 cm^{-1}; **Anal. Calcd for** $C_{40}H_{34}F_4N_8O_4S_2$**:** C, 57.82; H, 4.12; F, 9.15; N, 13.49 %. **Found:** C, 58.12; H, 4.29; F, 8.77; N, 13.35 %.

6,6'-(Octane-1,8-diyl)bis(3-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-imidazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54h)



Nature: Off-white solid; **MP:** 235 ^oC; **Yield:** 65.4 %; **^{1}H NMR** (200 MHz, $CDCl_3$, DMSO-d_6)**:** δ 1.41-1.49 (m, 8H), 1.75-1.81 (m, 4H), 2.95 (t, *J* = 8 Hz, 4H), 4.44 (d, *J* = 14 Hz, 2H), 4.62 (d, *J* = 14 Hz, 2H), 4.69-4.80 (m, 4H), 6.85 (s, 2H), 6.89-6.95 (m, 2H), 7.00 (s, 2H), 7.08-7.21 (m, 4H), 7.36-7.46 (m, 2H), 7.57 (s, 2H), 8.21 (s, 2H); **IR** (Chloroform)**:** 3310, 1670 cm^{-1}; **Anal. Calcd for** $C_{44}H_{42}F_4N_8O_4S_2$**:** C, 59.58; H, 4.77; F, 8.57; N, 12.63 %. **Found:** C, 59.64; H, 4.59; F, 8.66; N, 12.69 %.

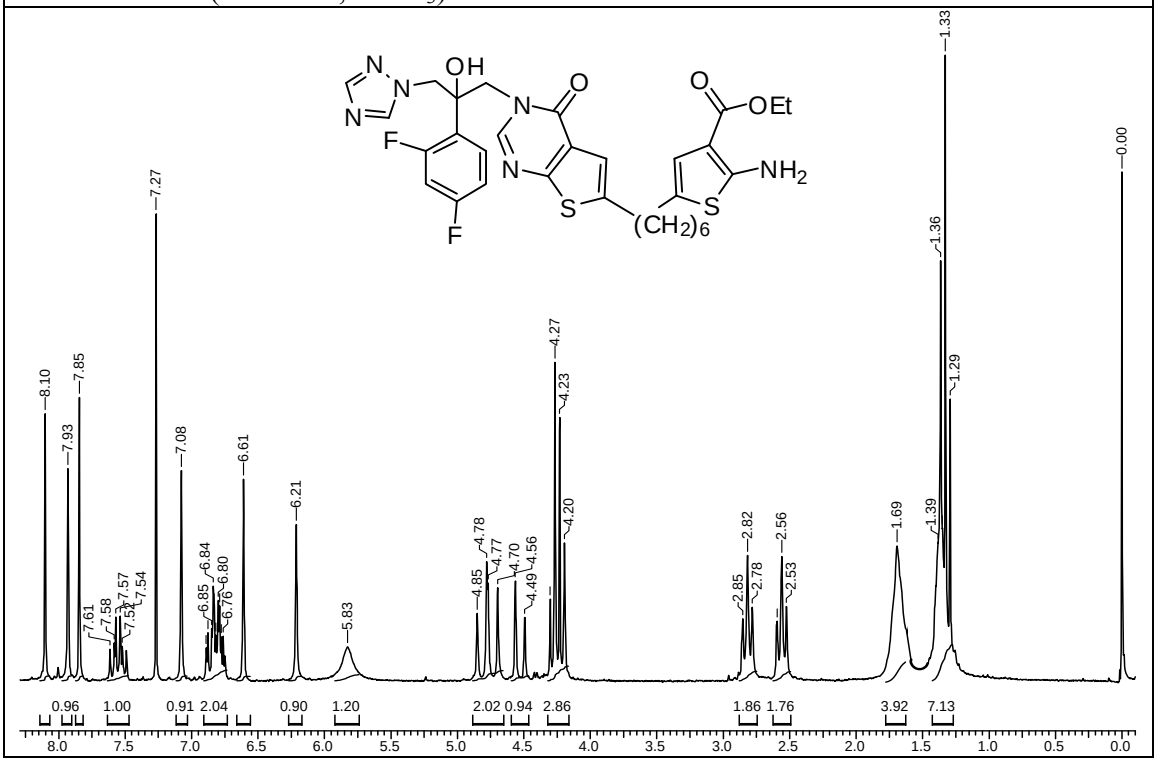
6,6'-(octane-1,8-diyl)bis(3-(2-(2,4-dichlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one) (54i)



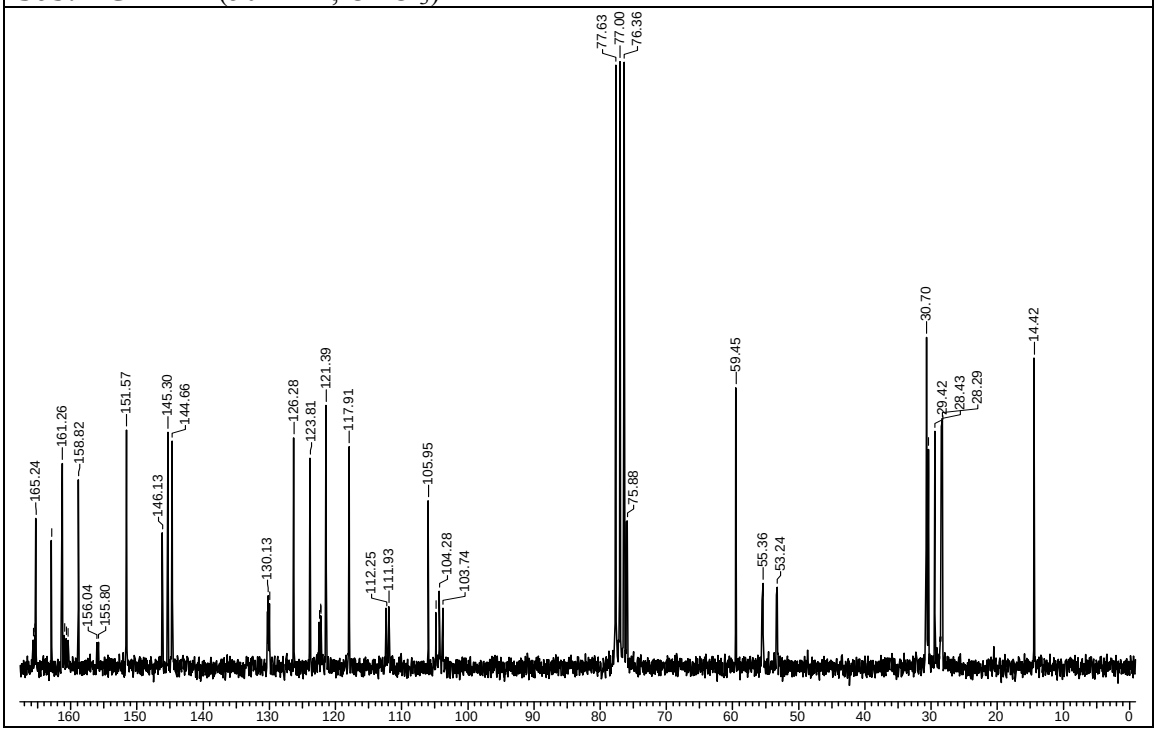
Nature: Pale yellow solid; **MP:** 166 ^{o}C; **Yield:** 67.9 %; **^{1}H NMR** (200 MHz, DMSO-d_6)**:** δ 1.24-1.30 (m, 8H), 1.60-1.69 (m, 4H), 2.51 (t, *J* = 2 Hz, 4H), 4.31 (d, *J* = 14 Hz, 2H), 4.47 (d, *J* = 14 Hz, 2H), 5.09 (d, *J* = 14 Hz, 2H), 5.30 (d, *J* = 14 Hz, 2H), 6.41 (s, 2H), 7.11 (s, 2H), 7.24-7.29 (m, 1H), 7.39 (d, *J* = 8 Hz, 1H), 7.60 (d, *J* = 2 Hz, 2H), 7.68 (s, 2H), 8.18 (s, 2H), 8.24 (s, 2H); **^{13}C NMR** (50 MHz, DMSO-d_6)**:** δ 28.5 (2C), 28.8 (2C), 29.9 (2C), 30.9 (2C), 49.9 (2C), 53.4 (2C), 76.0 (2C), 118.7 (2C), 123.9 (2C), 127.2 (2C), 130.3 (2C), 130.8 (2C), 132.0 (2C), 133.5 (2C), 137.0 (2C), 143.8 (2C), 145.2 (2C), 148.9 (2C), 150.8 (2C), 157.4 (2C), 162.0 (2C); **IR** (Chloroform)**:** 3362, 1661 cm^{-1}; **Anal. Calcd for** $C_{42}H_{40}Cl_4N_{10}O_4S_2$**:** C, 52.83; H, 4.22; Cl, 14.85; N, 14.67 %. **Found:** C, 53.04; H, 4.51; Cl, 14.64; N, 14.91 %.

2.7 Selected spectra

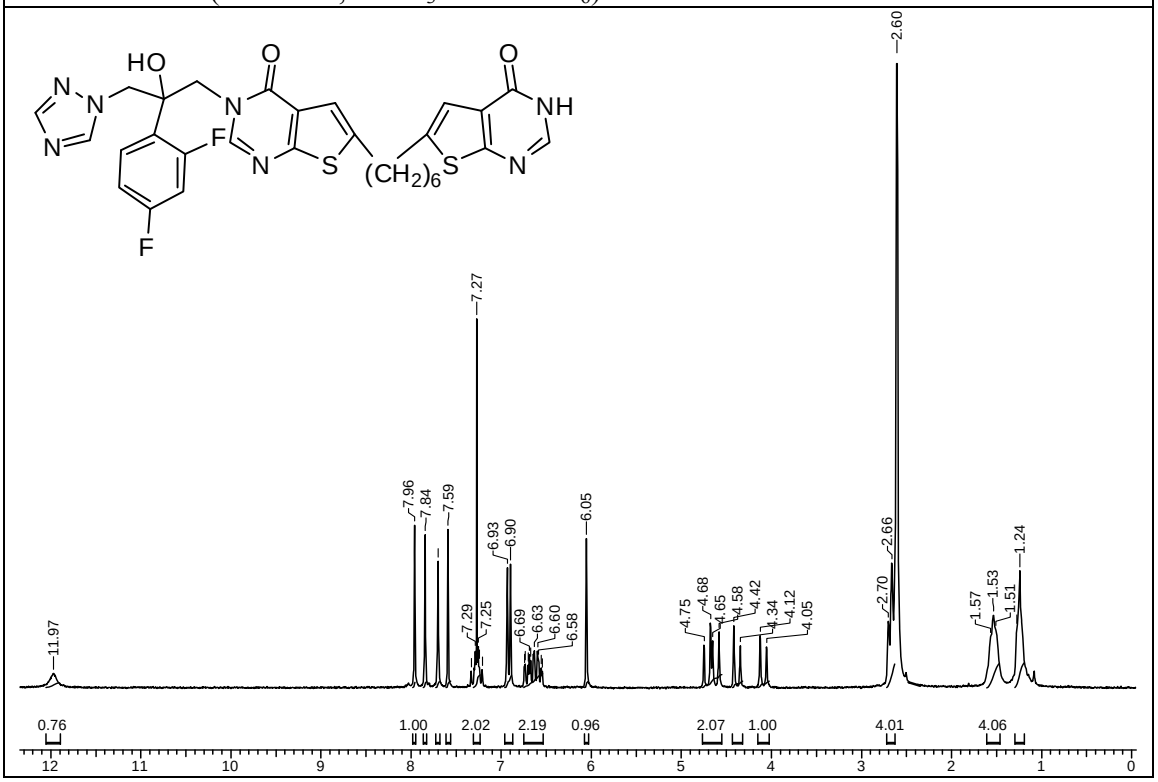
50b: ^{1}H NMR (200 MHz, $CDCl_3$)



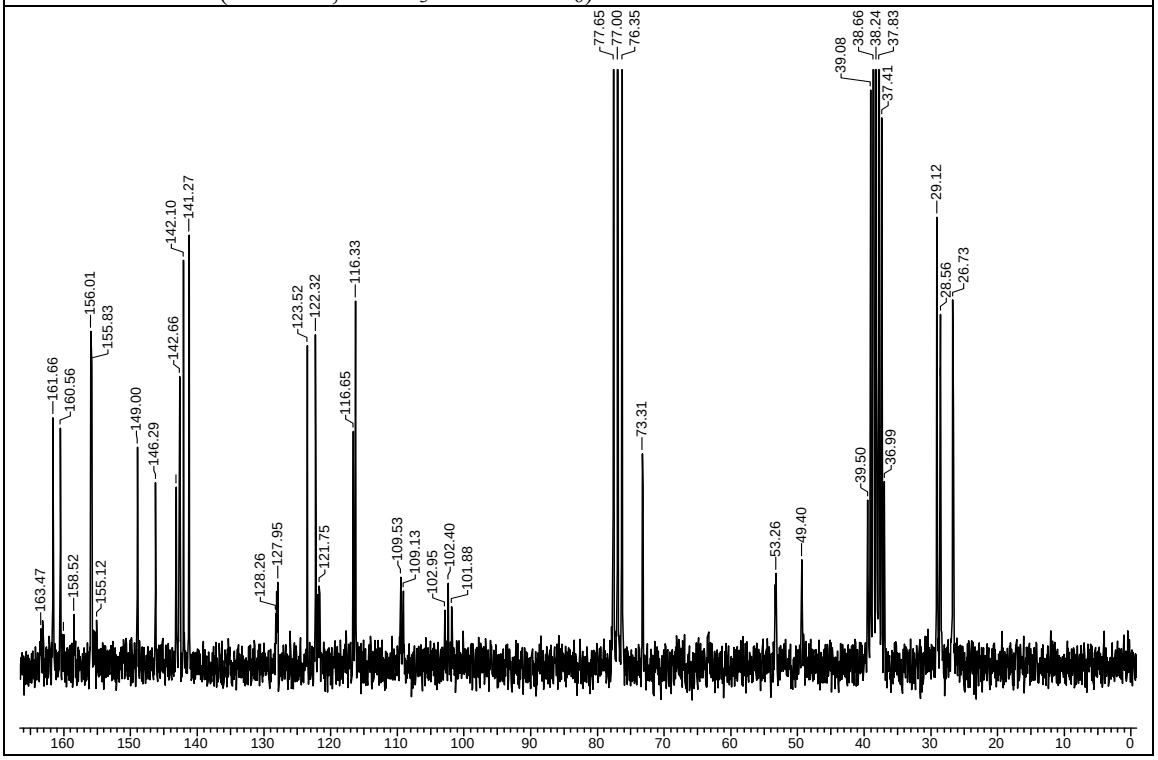
50b: ^{13}C NMR (50 MHz, $CDCl_3$)



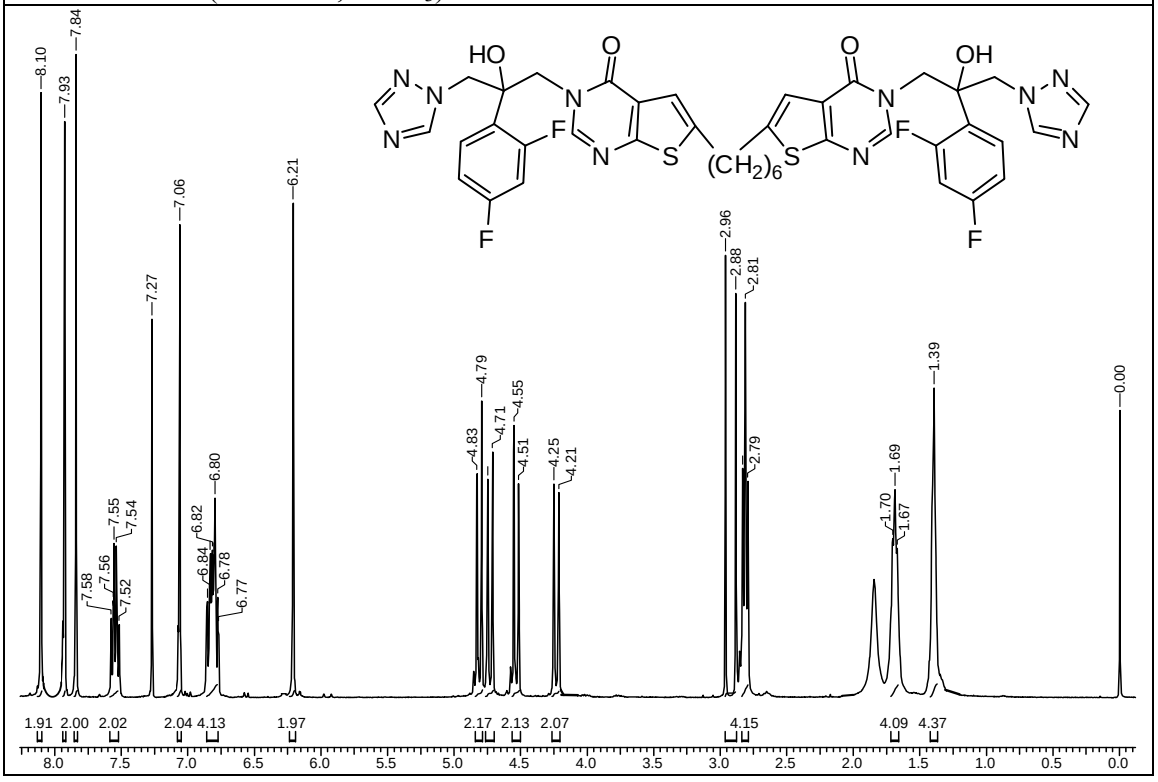
51b: ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6)



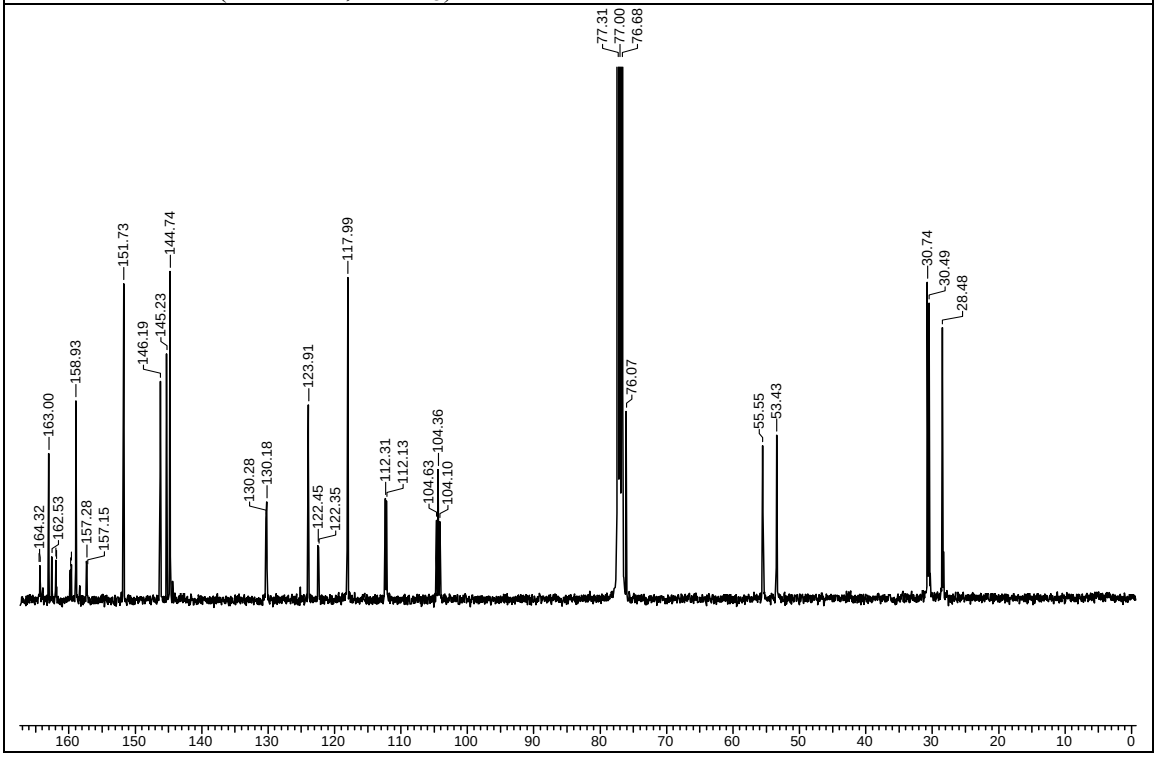
51b: ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)



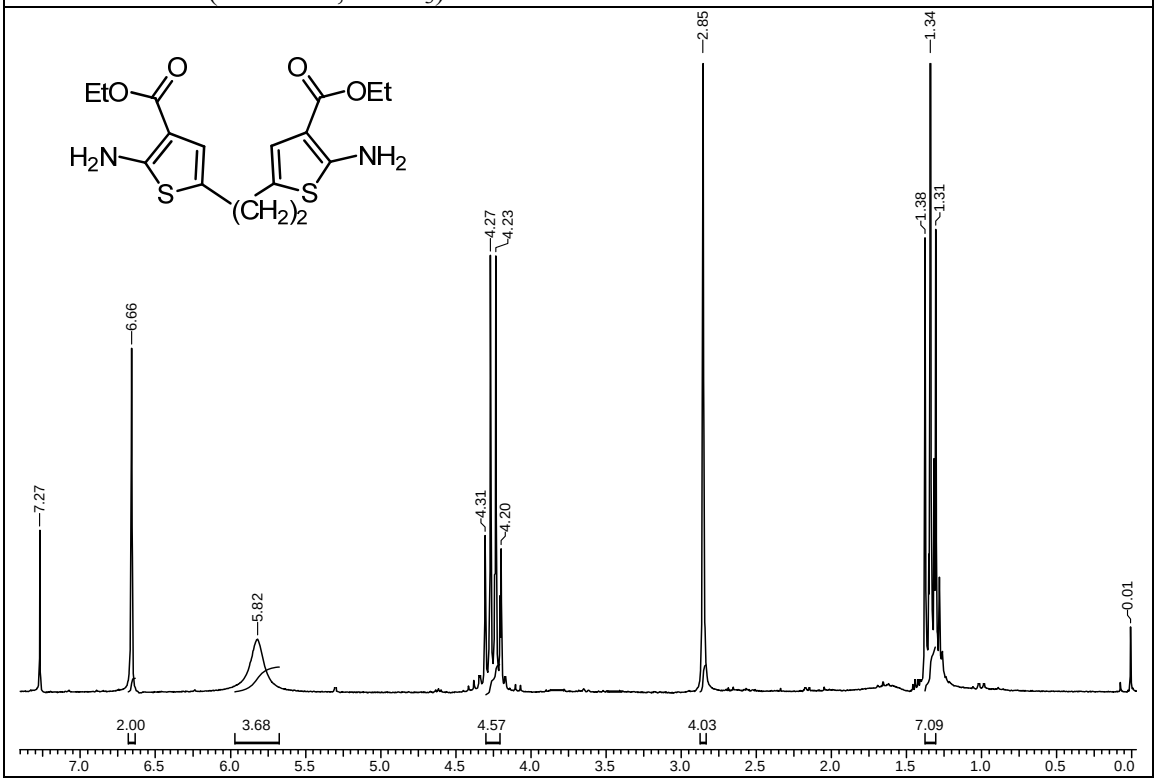
54a: ^{1}H NMR (400 MHz, $CDCl_3$)



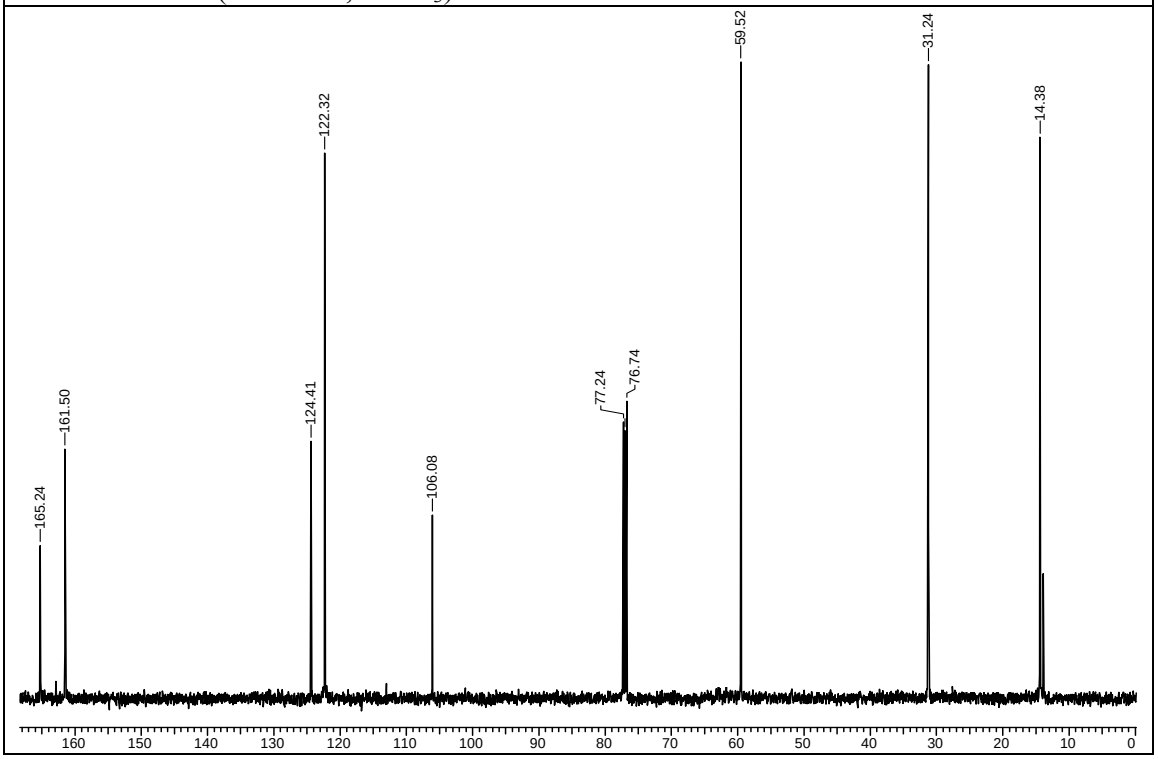
54a: ^{13}C NMR (100 MHz, $CDCl_3$)



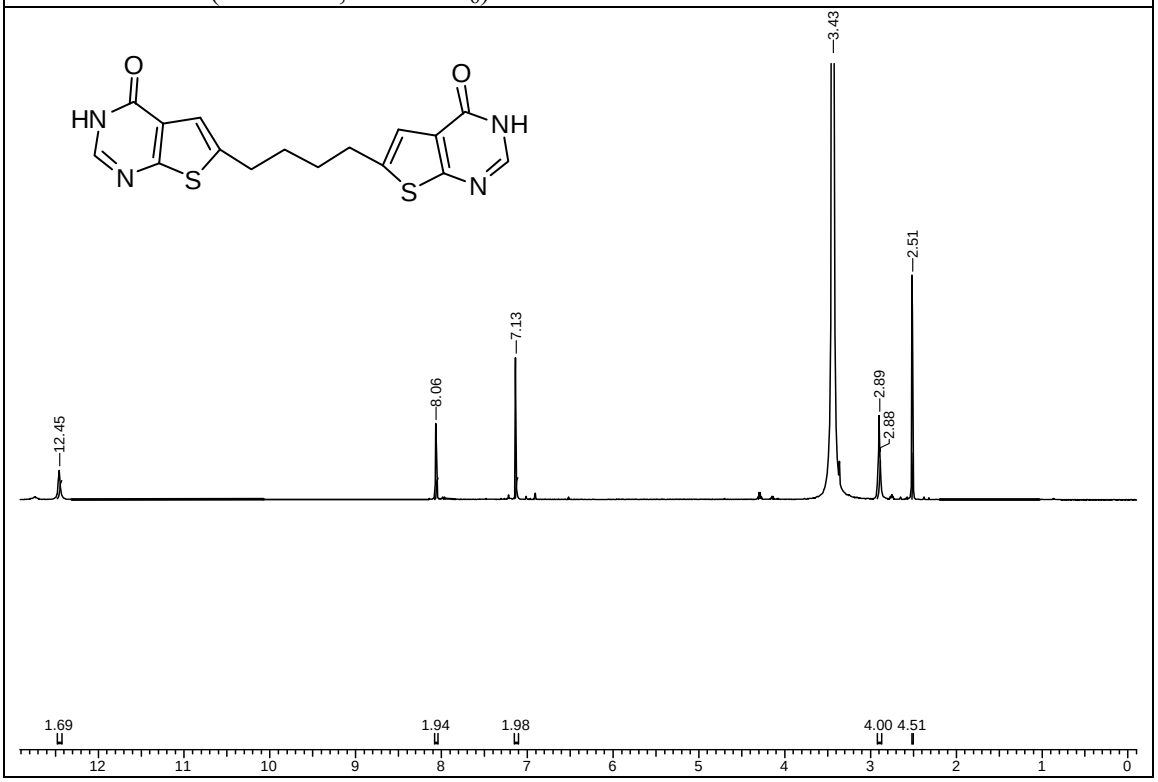
57a: ^{1}H NMR (200 MHz, $CDCl_3$)



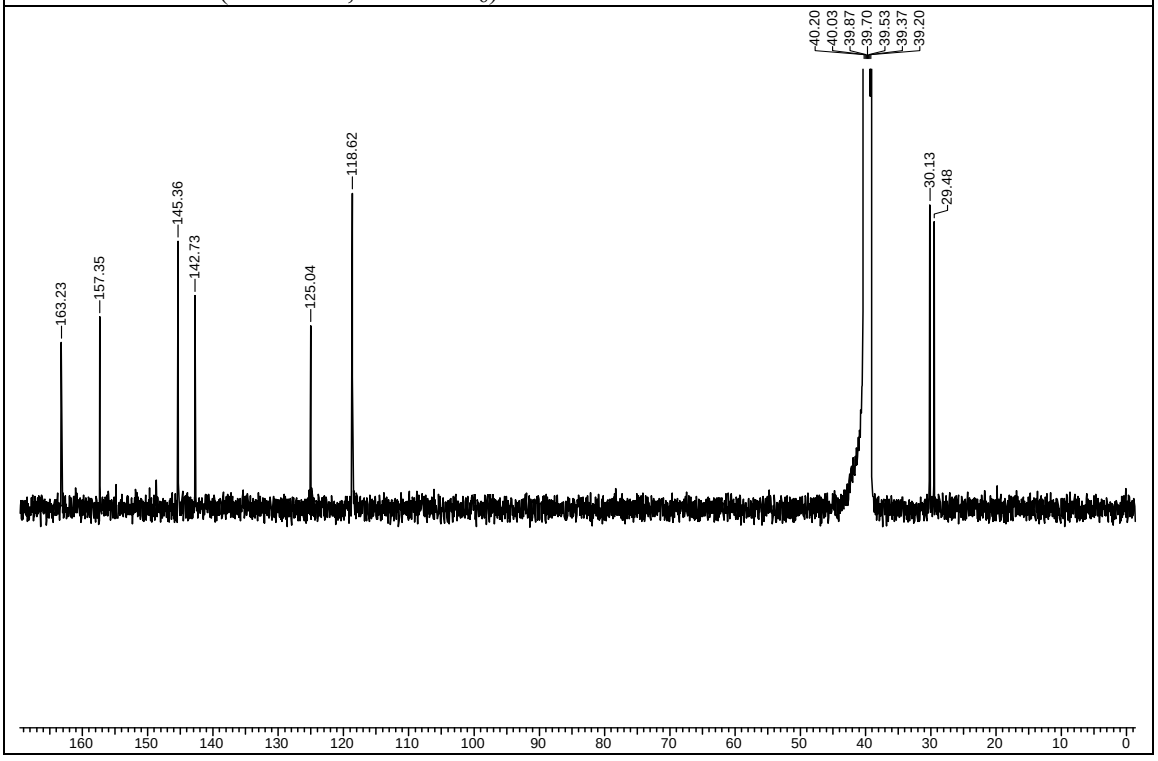
57a: ^{13}C NMR (125 MHz, $CDCl_3$)



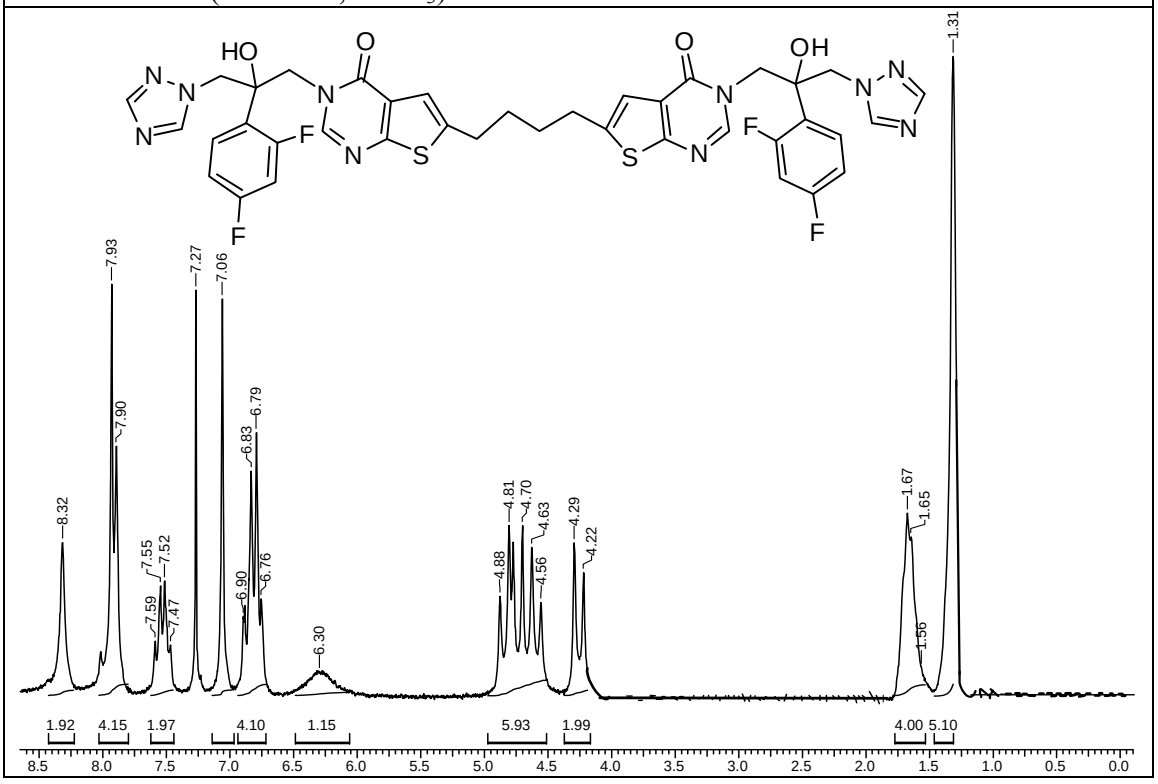
58b: ^{1}H NMR (500 MHz, DMSO-d_6)



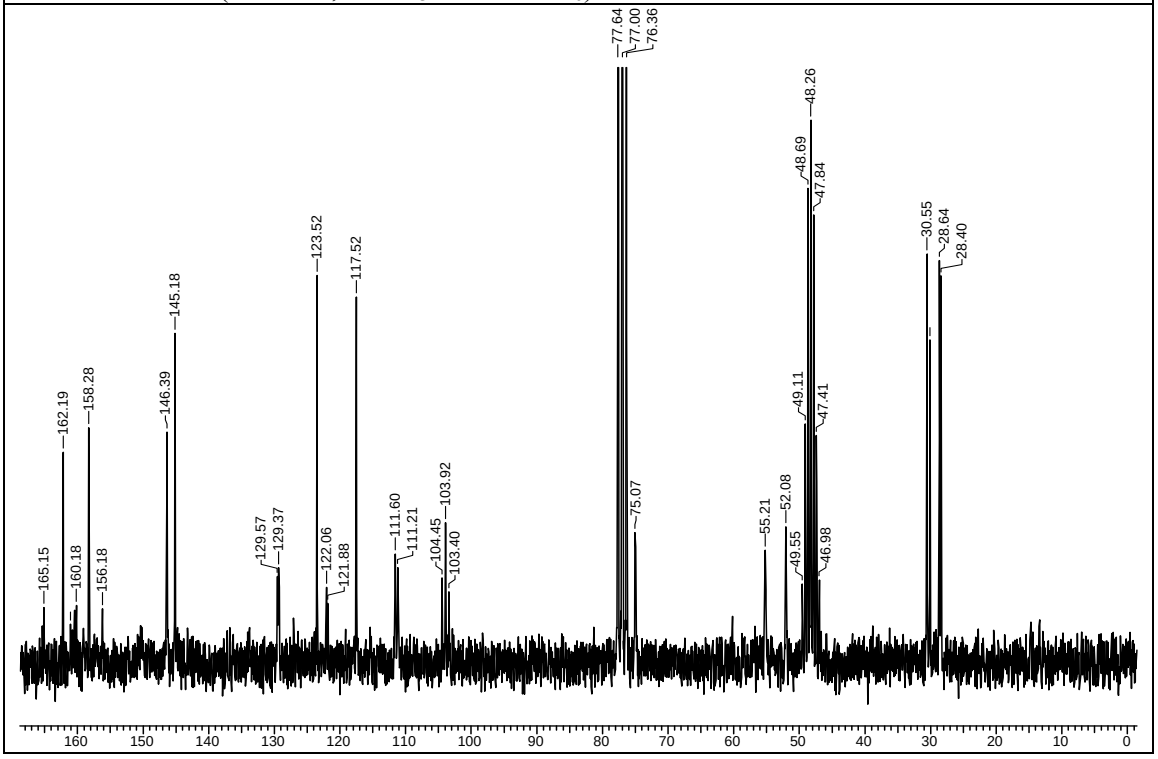
58b: ^{13}C NMR (125 MHz, DMSO-d_6)



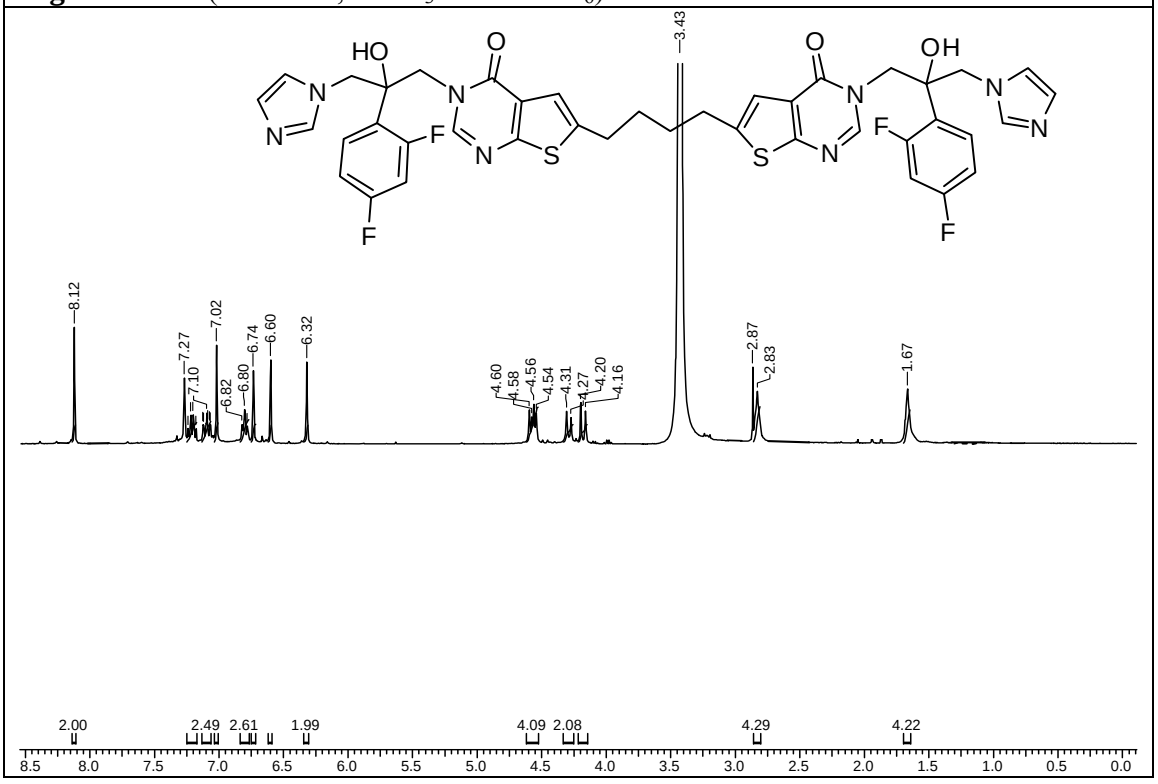
54b: ^{1}H NMR (200 MHz, $CDCl_3$)



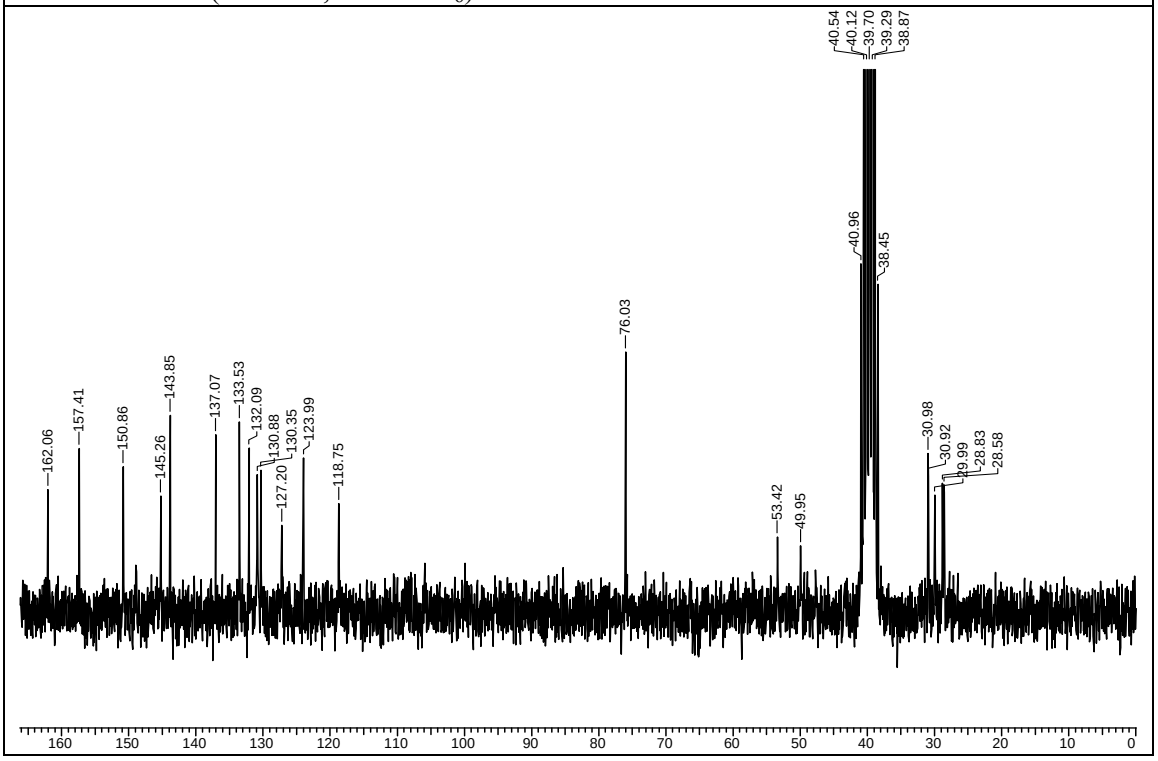
54b: ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)



54g: ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6)



54i: ^{13}C NMR (50 MHz, DMSO-d_6)



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Chapter 3

Synthesis of fluconazole analogues containing 2H-1,4-benzothiazin-3(4H)-ones or 2H-1,4-benzoxazin-3(4H)-ones and one-step preparation of α-chlorostyrenes

Chapter 3: Section I

Synthesis of fluconazole analogues containing 2H-1,4-benzothiazin-3(4H)-ones or 2H-1,4-benzoxazin-3(4H)-ones

Synthesis of fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)- ones or 2*H*-1,4-benzoxazin-3(4*H*)-ones

3.1.1 Abstract

New class of antifungal agents was synthesized with various substituted 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties as a replacing unit in place of one of the triazoles from fluconazole since it is known that benzothiazinones, benzoxazinones and their derivatives exhibit biological activity against various pathogens, pests and micro-organisms. The strategy developed can be used for the synthesis of number of such derivatives with good to excellent yields. All the synthesized fluconazole analogues containing benzothiazinone and benzoxazinone moieties were screened against different fungal strains. It was observed that some of the newly synthesized compounds exhibited better antifungal activity when compared with parent molecule fluconazole (**1**) and potent antifungal agent amphotericin B (**2**).

3.1.2 Introduction

Complications in the fungal infections have increased in the number and severity in recent years as a result of an increasing number of immunocompromised hosts, such as patients suffering from tuberculosis, infected with HIV and undergoing organ transplantations and cancer chemotherapy, due to the use of immunosuppressant agents. Though there are effective antifungal agents available in the market, they have quite a few shortcomings such as toxicity, limited range of activity for the fungal strains, high price and limited penetration through central nervous system. Fluconazole (**1**)[1] is an important antifungal agent used against various fungal strains, but its extensive use has increased the number of fluconazole-resistant fungi due to mutations. Amphotericin B (**2**) is the antifungal agent of choice used in fluconazole-resistant fungal infections but it has a higher degree of toxicity than that of fluconazole.[2]

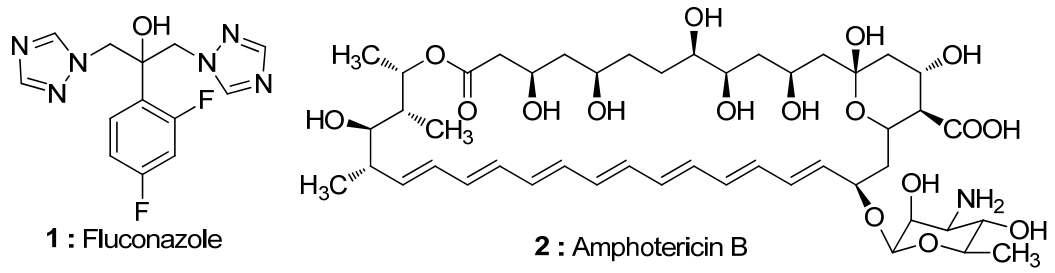


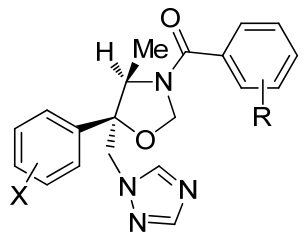
Figure 1. Structures of fluconazole (**1**) and amphotericin B (**2**)

The immunocompromised patients have to take long term antifungal therapy to prevent relapses and this causes the development of resistance in fungal strains. This compels the synthetic chemists to design and synthesize new chemical entities in an effort to come up with new drugs which can be used in place of current drugs like fluconazole (**1**) or amphotericin B (**2**) against the mutated fungal strains. Various fluconazole derivatives and analogues have been synthesized and checked for the antifungal activity against different fungal strains. This included derivatisation at tertiary alcohol of fluconazole as ethers, esters, phosphates and so on or the substitution of one of the triazole unit by different moieties.[3] Some of such fluconazole analogues were discussed in 1st chapter. Some more antifungal agents containing benzothiazinones, benzoxazinones and their derivatives as well as synthesis and antifungal activity of some more fluconazole analogues are described below.

3.1.3 Review of literature

Fluconazole analogues

Triazole compounds **3** (Figure 2) with an oxazolidine ring were reported[4] by S. Oida and co-workers as the potential inhibitors of fungal cytochrome P450 14α-demethylase. Furthermore, some of the methyloxazolidine derivatives exhibited remarkably high efficacy against a mouse systemic *Candida albicans* infection.



3

X = 2,4-F_2, 2,4-Cl_2, 4-Cl etc
R = 4-Cl, 4-CF_3, etc

Figure 2. General structure of oxazolidine derivatives of fluconazole

The potent activity of such compounds was hypothesized to be a consequence of a structural resemblance between **3** and lanosterol (a target molecule of the cytochrome P450 14α-demethylase discussed in 1st chapter).

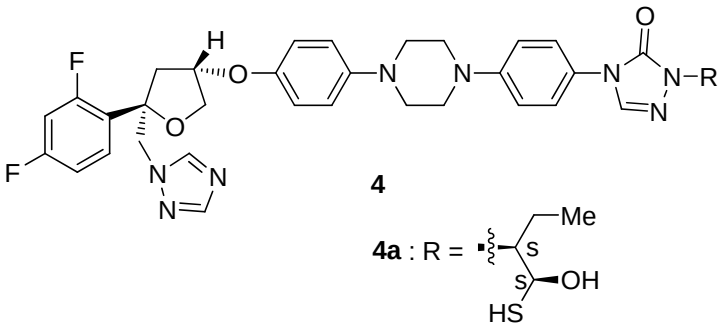


Figure 3. Structure of posaconazole (**4a**)

F. Bennett *et al.* reported[5] the synthesis of hydroxylated potential antifungal agents shown by general formula **4**. Out of a series of compounds synthesized, posaconazole (**4a**) with overall superior profile was selected for clinical studies. T. Konosu and co-workers reported sulfur analogues **5**[6a], carbon analogues **6**[3l] and amide analogues **7**[6b] of antifungal dioxane-triazole derivatives with general formulae shown in Figure 4.

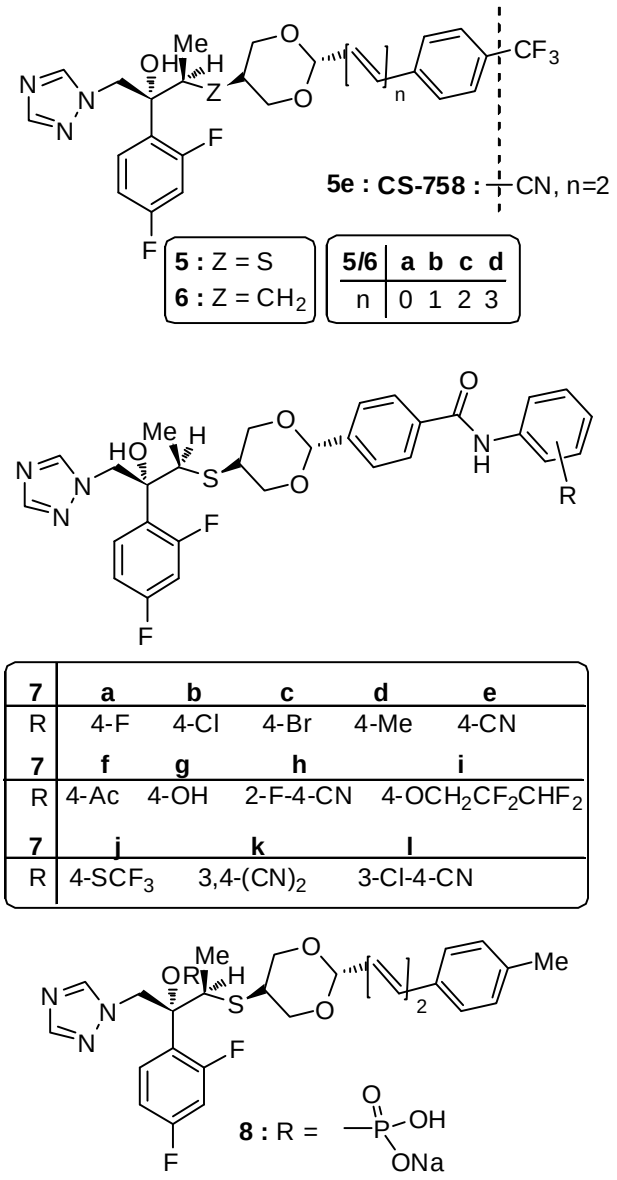
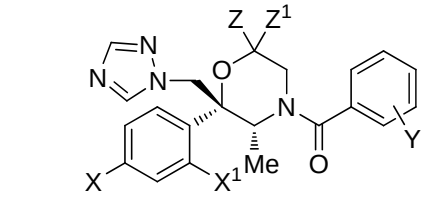


Figure 4. Structures of antifungal dioxane-triazole derivatives

All the synthesized compounds exhibited excellent *in vitro* activity and out of the series of these compounds, CS-758 (**5e**) was chosen as a candidate compound for the further development on the basis of its minimum inhibitory concentration, solubility and chemical/metabolic stability and its overall excellent profile. After choosing **5e**, its number of water soluble ester derivatives and analogues were made[6c], wherein the phosphate ester monosodium salt **8** was observed to be comparable to or slightly superior to CS-758 (**5e**).

Bartroli *et al.* reported[3a] a series of azole derivaties carrying *N*-acylmorpholine ring depicted by general formula **9** which were lactones, lactols and cyclic ethers.



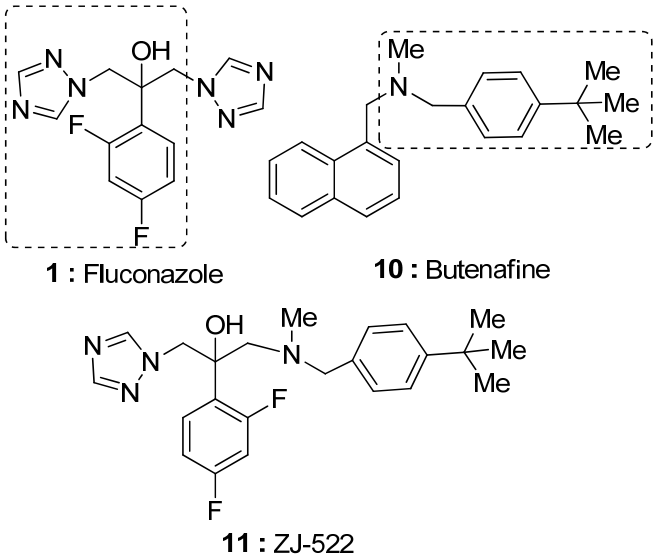
9

X, X^1 = H, F, Cl, CF_3
Z, Z^1 = H, OH, =O
Y = CF_3, OCF_3, OCH_2CF_3, $OCH_2CF_2CF_2H$

Figure 5. Azole derivaties carrying an *N*-acylmorpholine ring

One of the compounds from the series exhibited excellent activity, and in vaginal model of murine systemic candidosis, it was observed to be superior in potency when compared with fluconazole.

P.-H. Gao *et al.* reported[7] a hybrid molecule ZJ-522 (**11**) restructured from fluconazole (**1**) and butenafine (**10**) (Figure 3) exhibiting antifungal activity.



1 : Fluconazole

10 : Butenafine

11 : ZJ-522

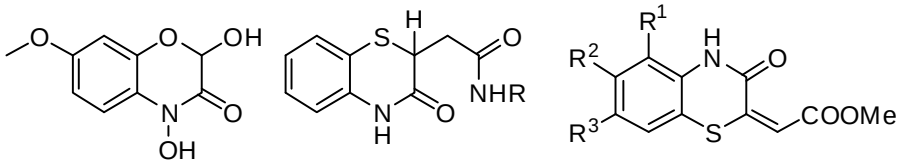
Figure 3. Structures of fluconazole (**1**), butenafine (**10**) and hybrid molecule ZJ-522 (**11**)

ZJ-522 was tested against 43 strains of fungi representing 13 fungal species, and observed to be about 50-fold and 2 to 16-fold more potent than fluconazole against yeasts

and filamentous fungi respectively. This research demonstrated that hybrid molecules having pharmacophores/structural features of two molecules of different classes may have potential to exhibit superior activity than the parent molecules.

Benzothiazinones and benzoxazinones: biologically important leads

Benzothiazinones and benzoxazinones are known to exhibit biological activities and are present as a core structure in many of the naturally occurring biologically active molecules. Benzoxazinone derivatives are the key defense chemicals of cereals which are used to protect the crops from the various lepidopteran pests, aphid species and fungal infections[8]. In number of cereals, benzoxazinones are the major secondary metabolites and have been shown to confer resistance against herbivorous insects and pathogens. Maize contains mainly DIMBOA [2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (**12**)] which is stored in the vacuole as the D-glucoside. Upon tissue disruption the glycoside is hydrolyzed by β-glucosidase yielding the more toxic aglycone DIMBOA. The highest concentrations of DIMBOA can be found in seedlings or in the younger parts of a plant. As the plant matures, the levels of DIMBOA and other benzoxazinone derivatives decline rapidly. However, insect feeding, artificial damage and pathogen infection may induce the accumulation of benzoxazinones which were observed, isolated and studied[9a-f].



12 : DIMBOA **13** **14**

Figure 7. DIMBOA (**12**), *N*-(alkyl/aryl)-2-(3-oxo-1,4-benzothiazin-2-yl)acetamide **13** and benzothiazinone derivatives **14**

A series of *N*-(alkyl/aryl)-2-(3-oxo-1,4-benzothiazin-2-yl)acetamide shown by general formula **13** was synthesized and screened for their antifungal activity against various fungal strains, as reported[10] by G. Gupta *et al.* Furthermore, they reported that in primary screening, some of the compounds exhibited appreciable activity. N. D. Heindel *et al.*

reported[11] the synthesis of benzothiazinone derivatives **14** and tested their biological activity and observed that some of these molecules exhibited sedative-hypnotic activity.

It is known that benzothiazinone derivatives are Ca^{2+}-activated potassium channel openers and also can act as calcium entry blockers. V. Calderone *et al.* reported[12] the synthesis of benzothiazinone derivatives **15** (Figure 8) and elaborated the vasorelaxing potency and efficacy of these molecules. Furthermore, they observed that 1,4-benzothiazinone heterocyclic nucleus is a suitable backbone for designing novel potassium channel openers. Author observed that some of the derivatives of **15** exhibited vasorelaxing potency comparable or superior to that of reference potassium channel activators.

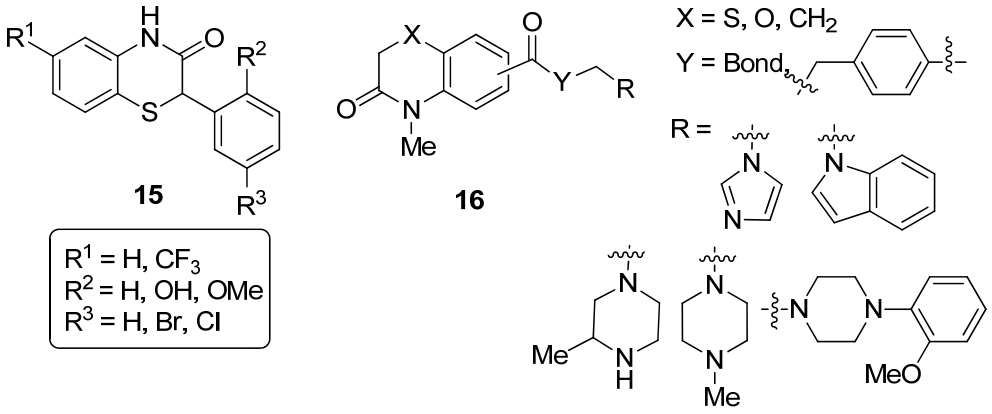


Figure 8. Benzothiazinone derivatives as potassium channel openers

R. Fringuelli and co-workers[13] reported the synthesis of various benzothiazinones and benzoxazinones **16** (Figure 8) and their apoptotic activity. 1,4-Benzothiazine induced neurotoxic effects have been hypothesized to play a role in neurodegenerative diseases. They observed that transformation of 1,4-benzothiazine skeleton into 1,4-benzoxazine reduces activity. D. W. Combs *et al.* reported[14a] the synthesis of long-acting cardiotonic bemoradan (**17a**), a compound from the 1,4-benzothiazinylpyridazinone class exhibiting cardiotonic activity. Furthermore, 1,4-benzothiazinylpyridazinones can act as peripheral vasodilator agents[14b]. The authors also observed that changing the oxygen heteroatom by sulfur gave more potent enzyme inhibitor.

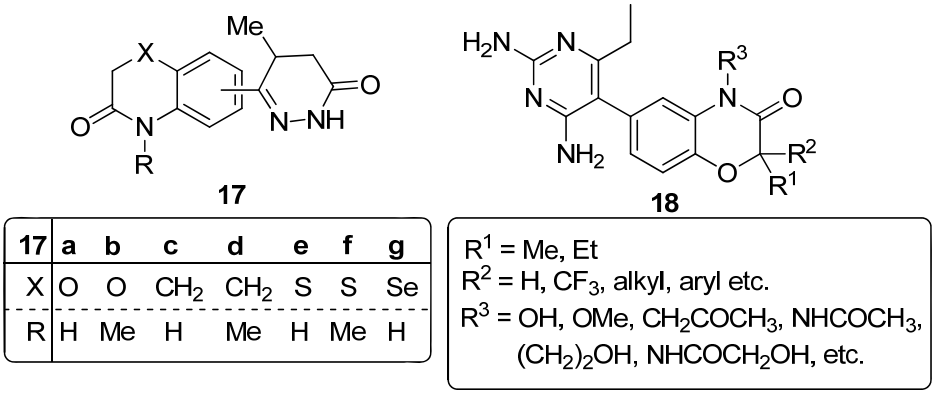


Figure 9. Bemoradan analogues **17** and renin inhibitors **18**

N. A. Powell and co-workers reported[15] the synthesis of a series of 6-(2,4-diaminopyrimidinyl)-1,4-bezoxazin-3-ones **18** as orally bioavailable small molecule inhibitors of renin. The renin angiotensin system (RAS) is well established as an endocrine system involved in blood pressure regulation and fluid electrolyte balance. Some of the compounds synthesized exhibited potent renin inhibition and good permeability, solubility and metabolic stability. Author observed that bioavailability was found to be dependent on metabolic clearance and cellular permeability of compounds. Dudley and co-workers reported[16a] the synthesis of benzoxazinone derivatives **19** and their biological activity as Factor Xa (FXa) inhibitor. FXa is a trypsin-like serine protease that plays a key role in the blood coagulation cascade. Out of a series of compounds synthesized and tested for their activity, compound **19a** was found to be a potent FXa inhibitor with high selectivity for FXa over other related serine proteases and was efficacious when dosed intravenously in rabbit and dog antithrombotic models.

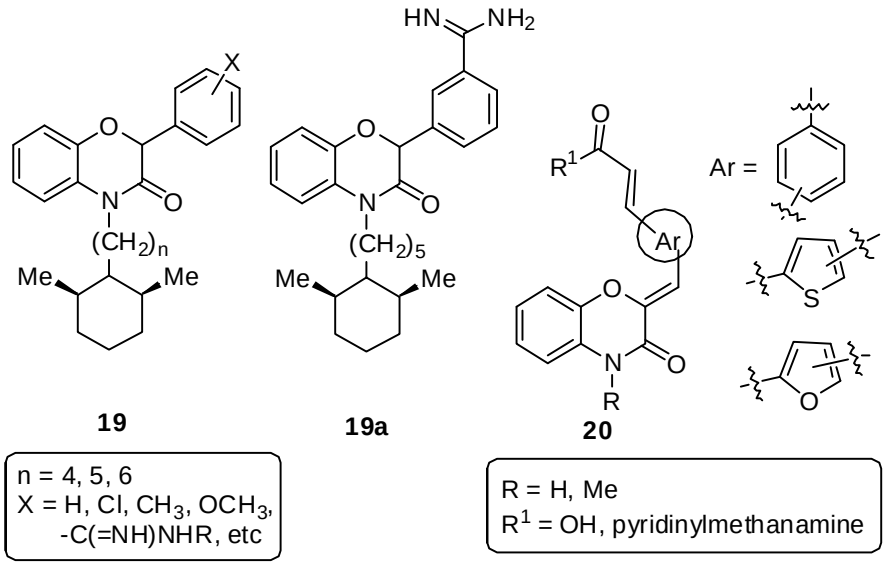


Figure 10. FXa inhibitors (**19** and **19a**) and tyrosine kinase inhibitors (**20**) containing benzoxazinone

Further study of replacement of benzoxazinone derivatives with benzothiazinone and further modification in the molecule was reported from the same group[16b]. Derivatives of 1,4-benzoxazin-3-one **20** were reported[17] as inhibitors of tyrosine kinases. Furthermore, it was concluded that 1,4-benzoxazin-3-one framework was favourable structure for inhibiting tyrosine kinases receptor.

Azole derivatives of benzothiazinones and benzoxazinones

R. Fringuelli *et al.* reported[18] the synthesis of fluconazole analogues containing benzothiazinone and benzoxazinone moieties. They synthesized analogues **21**, **22** and **23** wherein, benzothiazinone was incorporated in the molecule and evaluated for the *in vitro* and *in vivo* activity against *Candida albicans*.

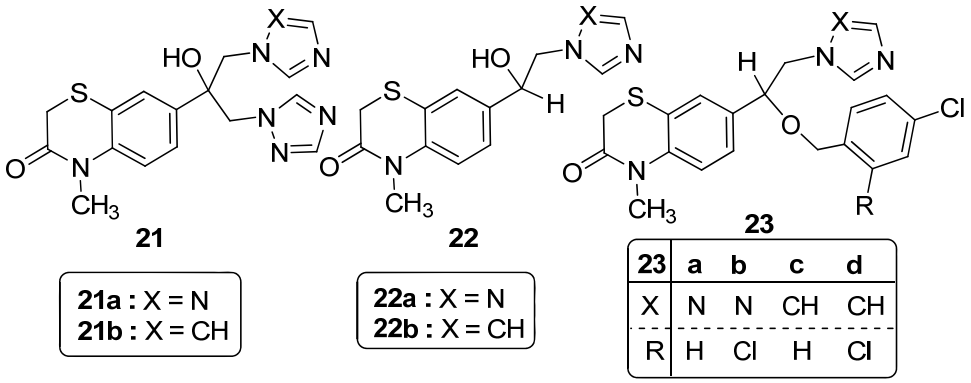


Figure 11. Fluconazole analogues containing benzothiazinone moieties

It was observed that compounds **22b** and **23c** showed appreciable *in vivo* antifungal activity. Compound **23c** was chosen for the further modification[18b] wherein, benzothiazinone was replaced by benzoxazinone and the results demonstrated that replacement of sulfur by oxygen improved immune response against *Candida albicans* infection.

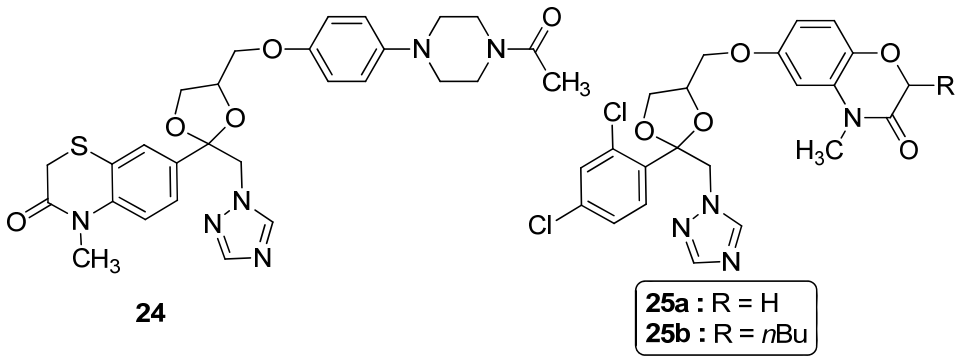


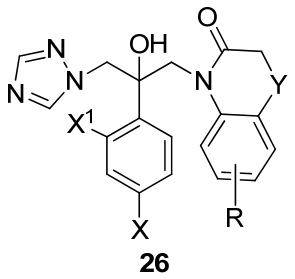
Figure 12. Ketoconazole analogues containing benzothiazinone and benzoxazinone moieties

R. Fringuelli and co-workers reported[19] not only the synthesis of fluconazole derivatives containing benzothiazinone and benzoxazinone moieties as discussed above, but also the synthesis of ketoconazole analogues containing benzothiazinone and benzoxazinone moieties. They evaluated the antifungal activity of synthesized ketoconazole analogues **24** and **25** and also studied the various aspects of the molecules synthesized.

3.1.4 Present work

3.1.4.1 Objective

Keeping in view the potential biological activities and importance of benzothiazinone and benzoxazinone moieties and their various derivatives, we thought to utilize these moieties for incorporation into the fluconazole molecule in such a way that the important part of fluconazole molecule which is responsible for the antifungal activity (that is triazole ring, difluorophenyl group and the hydroxyl group) does not get disturbed and benzothiazinone and benzoxazinone moieties get incorporated with easy means. Since it was perceived that if benzothiazinone or benzoxazinone moiety and the important part of fluconazole molecule responsible for the antifungal activity are synergized in a single nucleus, the new compounds obtained were likely to possess significant biological activities. In this quest, we aimed at the designing of compounds depicted by general formula **26** (Figure 13) wherein, one of the triazole rings in fluconazole was replaced systematically with the benzothiazinone or benzoxazinone moiety.



26

Y = O, S; X, X^1 = H, F, Cl, Br; R = H, Cl, Br, Me, OMe, Ac, NO_2

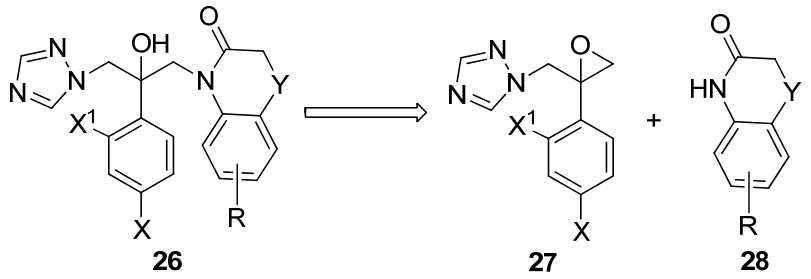
Figure 13. Fluconazole analogues containing benzoxazinone and benzothiazinone moieties

During the work described in this section, efforts were directed towards replacement of triazole moiety with various (un)substituted benzothiazinones or benzoxazinones in order to make a number of compounds available for biological activity. Accordingly we synthesized a number of molecules where pharmacophore of fluconazole was attached to the biologically active benzothiazinone or benzoxazinone moiety to get hybrid molecules. The newly synthesized molecules were tested against various fungi and the structure-activity relationship study was carried out.

3.4.1.2 Results and discussion

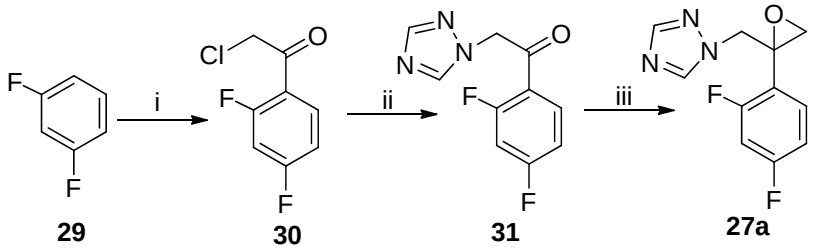
Synthesis of fluconazole analogues 26

The retrosynthetic analysis of compound **26** is shown in Scheme 1. The synthesis of compounds **26** was planned to be achieved from epoxide **27** and (un)substituted benzothiazinone or benzoxazinone moieties.



Scheme 1. Retrosynthetic analysis for preparation of fluconazole analogues **26**

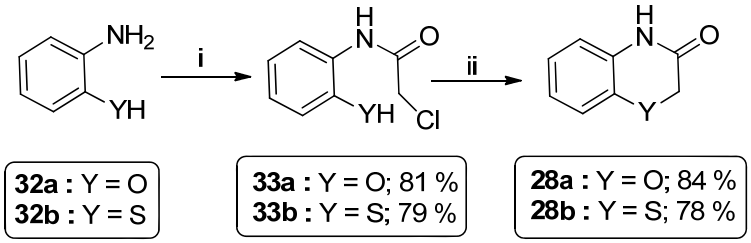
1-[2-(2,4-Difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (**27a**) was prepared from ketone **31** by Corey-Chaykovsky epoxidation method. Ketone **31** was in turn obtained from 1, 2-difluorobenzene (**29**) *via* its acylated intermediate (**30**) by known method[20,21] as shown in Scheme 2. Other epoxides such as 1-[(2-(4-bromophenyl)oxiranylmethyl]-1*H*-[1,2,4]-triazole (**27b**) and 1-[(2-(4-fluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]-triazole (**27c**) were synthesized using same strategy from their respective starting materials.



Scheme 2. Reagents and conditions i) Chloroacetyl chloride, $AlCl_3$, 0 ^oC to rt, 10 h, 80 %; ii) 1,2,4-Triazole, K_2CO_3, DMF, 85 ^oC, 8 h, 69 %; iii) Trimethylsulfoxonium iodide, cetrimide, aq KOH, DCM, 45 ^oC, 12 h, 85 %.

The synthesis of benzoxazinone **28a**[22] and benzothiazinone **28b**[22] was started from commercially available 2-aminophenol **32a** and 2-aminothiophenol **32b** respectively. Stirring 2-aminophenol **32a** with chloroacetyl chloride in DCM at room temperature for 2 h yielded N-acylated intermediate **33a** which on treatment with potassium carbonate in ethyl

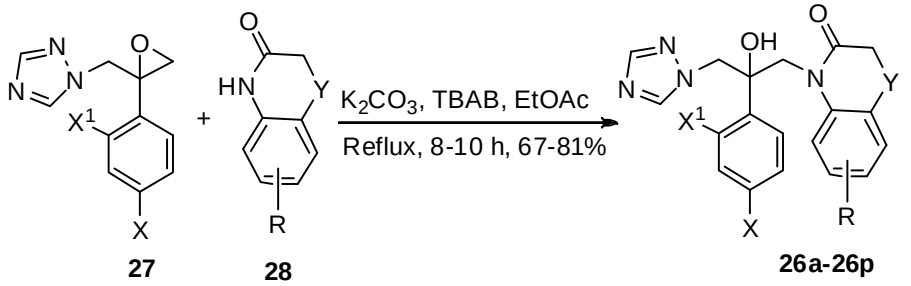
acetate at refluxing temperature yielded benzoxazinone **28a** as shown in Scheme 3. The same reaction sequence using 2-aminothiophenol **32b** afforded benzothiazinone **28b**.



Scheme 3. Reagents and conditions i) $ClCOCH_2Cl$, DCM, rt, 2 h; ii) K_2CO_3, EtOAc, rt, 12 h.

Benzoxazinone and benzothiazinone so prepared were reacted with epoxide **27** in presence of potassium carbonate and tetrabutylammonium bromide (TBAB) in ethyl acetate at refluxing temperature for 8-10 h to yield **26** in good yields as shown in Scheme 4. Rest of the benzoxazinones **28c-28f** and benzothiazinones **28g-28j** were commercially available and reacted with different epoxides **27** to get the biologically active compounds **26**.

All the compounds synthesized in these reaction sequences in this section were fully characterized by **PMR**, **CMR**, **IR** and **Mass** spectroscopic methods and showed satisfactory spectral data described in experimental section.



27a : X = X^1 = F
27b : X = Br, X^1 = H
27c : X = F, X^1 = H

28	a	c	d	e	f
Y	O	O	O	O	O
R(6)	H	Cl	Br	NO_2	Ac

28	b	g	h	i	j
Y	S	S	S	S	S
R(7)	H	Cl	Br	Me	OMe

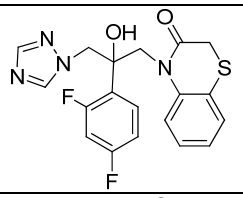
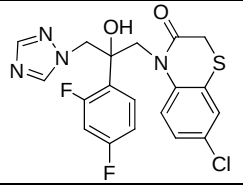
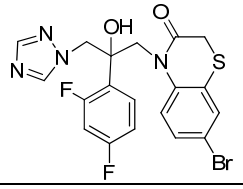
Structures of compounds **26a-26p** are shown in Table 1

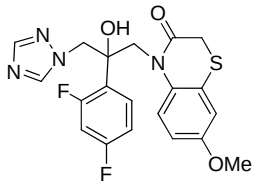
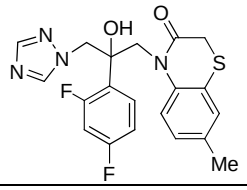
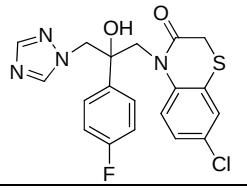
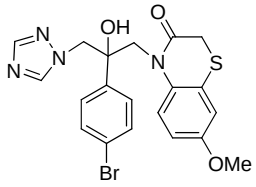
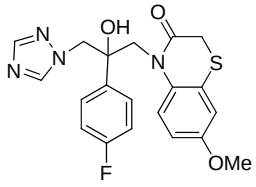
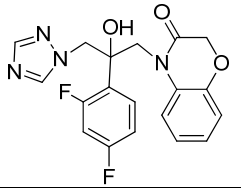
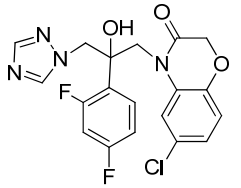
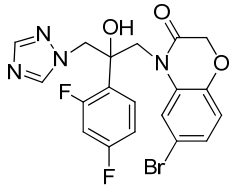
Scheme 4. Synthesis of fluconazole analogues **26**

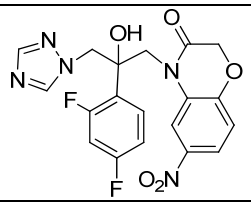
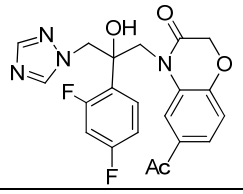
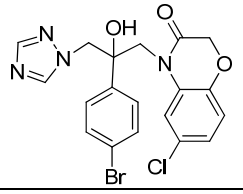
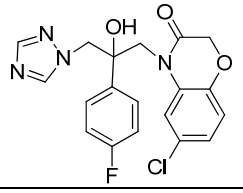
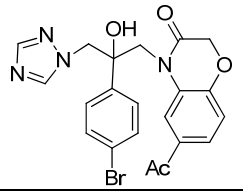
Bioevaluation of fluconazole analogues 26

All the newly synthesized compounds **26** were tested for antifungal activity against various fungi including *Candida albicans* ATCC 24433, *Aspergillus niger* ATCC 16404 and *Fusarium proliferatum* ATCC 10052 (Table 1, Sr. no. 3-18). *In vitro* evaluation of antifungal activity was performed by determining the minimum inhibitory concentration (MIC) following standard methods.[23,24] Antifungal susceptibility testing of these compounds was done by broth dilution method using RPMI 1640 medium with MOPS (3-(N-morpholino)propanesulfonic acid) buffer. Known antifungal agents like fluconazole and amphotericin B were used as positive control. End points were determined after 48 hours visually and by using spectrophotometer wherever necessary. Different dilutions were tried and various sets of experiments performed. The activity parameters are enumerated in Table 1.

Table 1: MIC obtained by broth macro-dilution method

Sr no	Comp no	Structure 26	Activity against organisms MIC In µg/ml		
			***C. albicans* ATCC 24433**	***A. niger* ATCC 16404**	***F. proliferatum* ATCC 10052**
1		Fluconazole	**0.25-1**	64-128	>128
2		Amphotericin B	**0.12-0.25**	0.25-1	1-2
3	**26a**		**0.12-0.25**	NI	NI
4	**26b**		**0.5-1**	NI	NI
5	**26c**		**0.5-1**	NI	NI

6	**26d**		2-4	NI	NI
7	**26e**		**1-2**	NI	NI
8	**26f**		**1-2**	NI	NI
9	**26g**		**1-2**	NI	NI
10	**26h**		4-8	NI	NI
11	**26i**		**0.25-0.5**	NI	NI
12	**26j**		4-8	NI	NI
13	**26k**		8-16	NI	NI

14	**26l**		16-32	NI	NI
15	**26m**		NI	NI	NI
16	**26n**		2-4	NI	NI
17	**26o**		8-16	NI	NI
18	**26p**		16-32	NI	NI

NI**:** No inhibition
For azoles**:** For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 80% inhibition as compared to the positive control.
For amphotericin B**:** MIC is recorded as the concentration exhibiting complete inhibition.

The antifungal activity exhibited by the compounds in the present work was confirmed by secondary screening of **26a** and **26i** against various strains of *Candida* and it was observed that activity of **26a** is excellent against the *C. albicans* ATCC 24433, *C. albicans* ATCC 90028 and *C. albicans* ATCC 90030 when compared with amphotericin B and fluconazole as shown in Table 2.

Table 2: MIC obtained by broth micro-dilution method

Fungus	MIC in µg/ml			
	Amphotericin B	**Fluconazole**	**26a**	**26i**
C. albicans ATCC 24433	**0.25**	**0.25**	**0.03**	**0.12**
C. albicans ATCC 10231	**0.5**	**1.0**	**0.12**	**0.5**
C. albicans ATCC 2091	**0.5**	4	2	4
C. albicans ATCC 90028	**0.5**	**0.5**	**0.03**	**0.12**
C. glabrata ATCC 90030	**0.25**	4	**0.06**	**0.25**
C. krusei ATCC 6258	**0.5**	64	4	16
C. tropicalis ATCC 750	**0.5**	2	**0.5**	2

For azoles**:** For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 50% inhibition as compared to the positive control.
For amphotericin B**:** MIC is recorded as the concentration exhibiting complete inhibition.

The above results indicated following points regarding the structure-activity relationship of the compounds studied in the present work.

1. The compounds containing benzothiazinone moiety are more active than those containing benzoxazinone (compound no **26a** v/s **26i**).

2. In the benzothiazinone series, replacement of fluorine by hydrogen reduces the activity (compound nos **26b** v/s **26f** and **26e** v/s **26h**). The same is true for benzoxazinone series (compound no **26i** v/s **26o**).

3. In the benzothiazinone series, halogen substituent at C7 position is tolerated with slight decrease in activity (compound no **26a** v/s **26b** or **26c**) while substituents like methyl and methoxy decrease the activity considerably (compound no **26a** v/s **26d** or **26e**).

4. In case of benzoxazinone series, no substituent at C6 is tolerated and all compounds having substituents like Cl, Br, NO_2 or Ac lost the activity (compound no **26i** v/s **26j**, **26k**, **26l** or **26m**).

Resolution of 26a

Compound **26a** was observed to be the most active compound within this group, hence it was chosen for the separation of the R and S enantiomers from the racemic compound using preparative HPLC. Apart from compound **26a**, racemic epoxide **27a** was

also separated on preparative HPLC to get R and S enantiomers (HPLC method was described in chapter 1). Compound **26a** was separated on preparative HPLC under following conditions**:**

HPLC Column	Chiralcel-OD (250 X 4.6mm) (DAICEL)
Mobile Phase	Ethanol**:** n-Hexane (25**:**75)
Wavelength	254 nm
Carried out by	Normal Phase

The HPLC chromatograms of **26a** are shown in Figure 14.

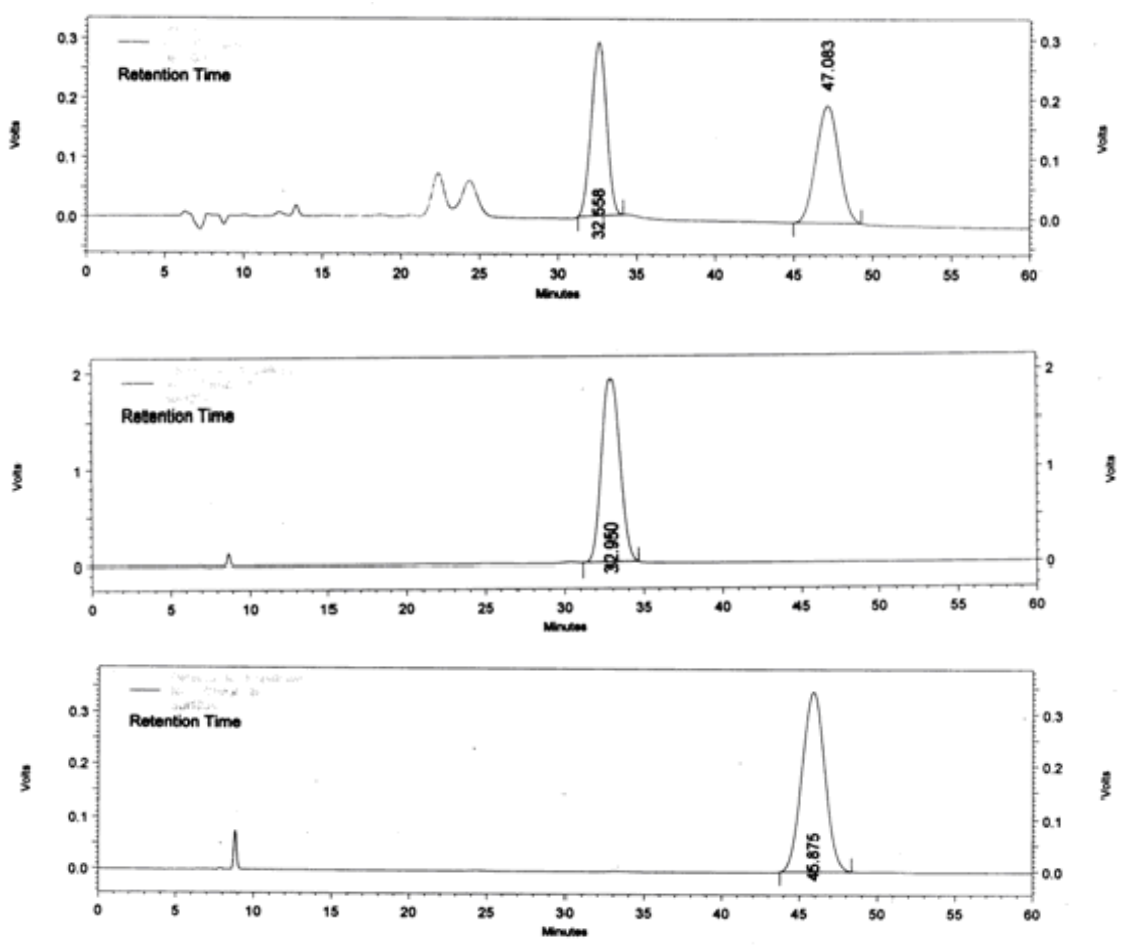


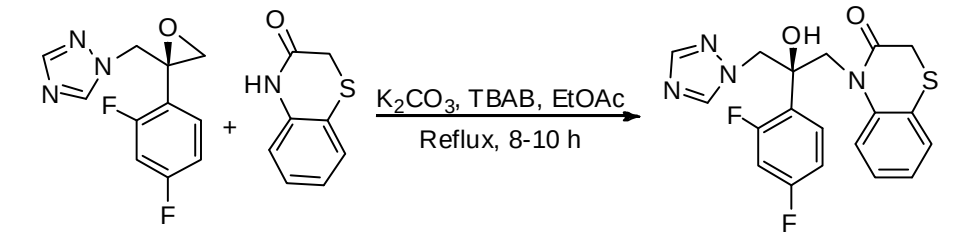
Figure 14. HPLC chromatograms of **26a**

After resolution and separation of enantiomers of **26a** was done, specific rotation of every compound was calculated from the observed rotations as shown in Table 3.

Table 3. Specific rotations of enantiomers of **27a** and **26a**

Comp No.	Peaks In Chromatogram	Retention Time (RT)	Observed Rotation	Specific Rotation $[\alpha]_D$ C =10 mg/ml and l =0.5 dm
27a	A	14.518	-0.04	-8 ^{o} (C = 1.0, THF)
	B	15.200	+0.04	+8 ^{o} (C = 1.0, THF)
26a	A	32.558	-0.285	-57 ^{o} (C = 1.0, Chloroform)
	B	45.875	+0.285	+57 ^{o} (C = 1.0, Chloroform)

Specific rotations of **R** and **S** enantiomers of epoxide **27a** were known (described in chapter 1). Hence we treated **28b** with **S** enantiomer of **27a** as shown in Scheme 5.



S enantiomer of **27a** **28b** **R** enantiomer of **26a**

Scheme 5. Synthesis of R enantiomer of fluconazole analogue **26a**

The product obtained had $[\alpha]_D = -57$ ^{o}, and should have **R** configuration. These two enanteriomers were tested for their antifungal activity against various strains of *Candida* and it was observed that **S** enantiomer is having higher activity than **R** as shown in Table 4.

Table 4: MIC obtained by broth macro-dilution method

Fungus	MIC in µg/ml				
	Amphotericin B	**Fluconazole**	**26a Racemic**	**26a R enantiomer**	**26a S enantiomer**
C. albicans ATCC 24433	0.25	1	**0.25**	16	**0.12**
C. albicans ATCC 10231	0.5	1	**0.12**	16	**0.06**
C. albicans ATCC 2091	0.5	0.5	**0.12**	8	**0.06**
C. albicans ATCC 90028	0.5	0.5	**0.03**	4	**0.015**
C. glabrata ATCC 90030	0.25	4	**0.06**	4	**0.03**
C. krusei ATCC 6258	0.5	64	**4**	>128	**2**
C. tropicalis ATCC 750	0.5	2	**0.5**	>128	**0.25**

For azoles**:** For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 80% inhibition as compared to the positive control.
For amphotericin B**:** MIC is recorded as the concentration exhibiting complete inhibition.

3.1.5 Conclusion

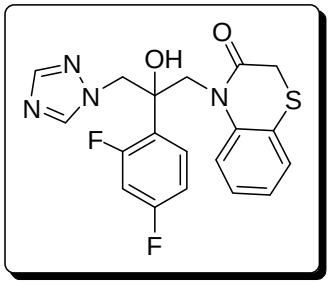
Fluconazole analogues containing various substituted 2*H*-1,4-benzothiazin-3(4*H*)-one and/or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties were synthesized by a short route with good to excellent yields. Antifungal activity studies revealed that the designed new chemical entities showed considerable antifungal activity. Furthermore, we have resolved the racemic mixture of most active fluconazozole analogue **26a** into its **R** and **S** enantiomers to make them available for SAR studies. It was observed that the S enantiomer is far more active than the R enantiomer. This investigation will be helpful to improve the design and development of more potent antifungal agents with superior antifungal activity and short chemical sequences.

3.1.6 Experimental section

General procedure for the synthesis of fluconazole analogues 26a-26p

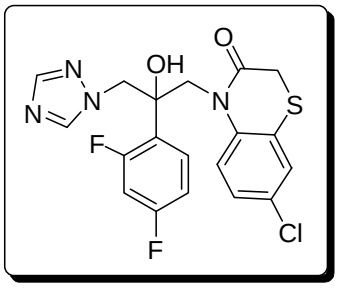
To a flame dried K_2CO_3 (0.18 mol), tetra-butylammonium bromide (TBAB, 0.09 mol) was added followed by the addition of compound **28** (0.09 mol) in dry ethyl acetate (200 ml). Reaction mixture was stirred at reflux for 30 min. Then 1-[2-(halophenyl)-oxiranylmethyl]-1*H*-[1,2,4]triazole (**27**) (0.09 mol) dissolved in dry ethyl acetate (200 ml) was added to the refluxing mixture dropwise over a period of 10 min and stirring was continued for further 12 h at the same temperature. It was then cooled to room temperature, diluted with water (800 ml), extracted with ethyl acetate (3 X 400 ml), dried over Na_2SO_4, concentrated and purified by column chromatography to give pure compound.

4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]thiazin-3-one (26a)



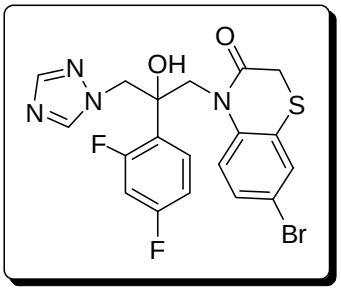
Nature: Pale yellow solid; **MP:** 123 ^{o}C; **Yield:** 78 %; **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 3.17 (d, *J* = 14 Hz, 1H), 3.24 (d, *J* = 14 Hz, 1H), 4.43 (d, *J* = 14 Hz, 1H), 4.48 (d, *J* = 14 Hz, 1H), 4.60 (s, 2H), 5.52 (bs, 1H), 6.41-6.47 (m, 1H), 6.68-6.73 (m, 1H), 6.92-6.98 (m, 1H), 7.13-7.26 (m, 3H), 7.50-7.58 (m, 1H), 7.76 (s, 1H), 8.15 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ 31.9, 52.3, 56.1, 76.4, 103.3 (t), 111.3 (d), 119.1, 122.8, 123.9, 125.2, 126.9, 128.2, 130.1, 139.4, 144.6, 151.0, 157.4 (d), 162.8 (d), 168.4; **IR** (Chloroform)**:** 3398, 1651 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 403.3074 (M + 1), 425.2999 (M + Na); **Anal. Calcd for** $C_{19}H_{16}F_2N_4O_2S$**:** C, 56.71; H, 4.01; F, 9.44; N, 13.92 %. **Found:** C, 56.64; H, 3.82; F, 9.37; N, 14.08 %.

7-Chloro-4-[2-(2,4-difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]thiazin-3-one (26b)



Nature: Reddish brown solid; **MP:** 183 ^{o}C; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.14 (d, *J* = 14 Hz, 1H), 3.26 (d, *J* = 14 Hz, 1H), 4.29 (d, *J* = 14 Hz, 1H), 4.49 (d, *J* = 14 Hz, 1H), 4.56 (d, *J* = 14 Hz, 1H), 4.66 (d, *J* = 14 Hz, 1H), 5.71 (bs, 1H), 6.42-6.56 (m, 1H), 6.63-6.78 (m, 1H), 7.06-7.21 (m, 3H), 7.40-7.52 (m, 1H), 7.80 (s, 1H), 8.25 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 31.8, 52.3, 56.0, 76.4, 103.5 (t), 111.5 (d), 120.3 122.8, 126.9, 127.0, 127.7, 129.2, 130.0 (d), 138.1, 144.7, 151.2, 155.2 (dd), 163.1 (dd), 168.0; **IR** (Chloroform)**:** 3355, 1652 (b) cm^{-1}; **MS** (ESI) *m*/z**:** 433.4146 (M + 1), 455.4147 (M + Na), 471.3987 (M + K); **Anal. Calcd for** $C_{19}H_{15}ClF_2N_4O_2S$**:** C, 52.24; H, 3.46; Cl, 8.12; F, 8.70; N, 12.82 %. **Found:** C, 52.15; H, 3.54; Cl, 8.03; F, 8.84; N, 12.76 %.

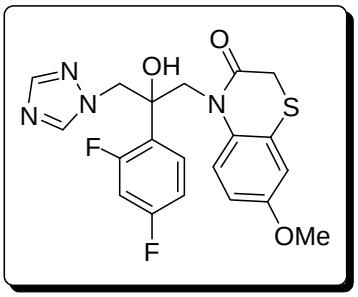
7-Bromo-4-[2-(2,4-difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]thiazin-3-one (26c)



Nature: Pale brown solid; **MP:** 115 ^{o}C; **Yield:** 72 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.22 (d, *J* = 14 Hz, 1H), 3.36 (d, *J* = 14 Hz, 1H), 4.28 (d, *J* = 14 Hz, 1H), 4.67 (d, *J* = 14 Hz, 1H),

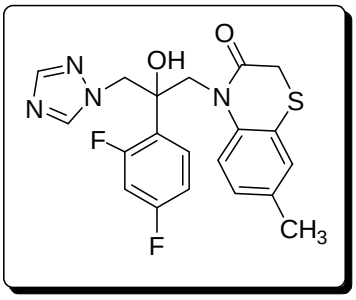
4.73 (d, *J* = 14 Hz, 1H), 4.85 (d, *J* = 14 Hz, 1H), 5.72 (bs, 1H), 6.56-6.67 (m, 1H), 6.70-6.85 (m, 1H), 7.18 (d, *J* = 8 Hz, 1H), 7.34 (dd, *J* = 8, 2Hz, 1H), 7.41 (d, *J* = 2 Hz, 1H), 7.43-7.55 (m, 1H), 7.97 (bs, 1H), 8.90 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 31.6, 52.0, 55.7, 76.4, 103.4 (t), 111.4 (d), 116.5, 120.5, 122.8, 127.1, 129.7 (2C), 129.9 (d), 130.4, 138.5, 151.1, 158.2 (dd), 163.2 (dd), 167.8; **IR** (Chloroform)**:** 3337, 1654 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 481.8374 and 483.8357 (M + 1), 503.8335 and 505.8369 (M + Na); **Anal. Calcd for** $C_{19}H_{15}BrF_2N_4O_2S$**:** C, 47.41; H, 3.14; Br, 16.60; F, 7.89; N, 11.64 %. **Found:** C, 47.51; H, 3.07; Br, 16.69; F, 7.96; N, 11.43 %.

4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-7-methoxy-4*H*-benzo[1,4]thiazin-3-one (26d)



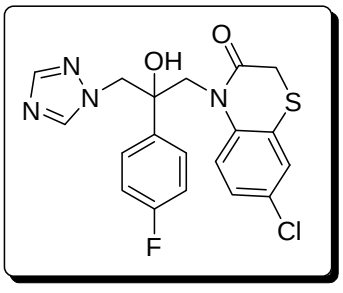
Nature: Pale yellow solid; **MP:** 127 ^{o}C; **Yield:** 77 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.15 (d, *J* = 10 Hz, 1H), 3.22 (d, *J* = 10 Hz, 1H), 3.70 (s, 3H), 4.38 (s, 2H), 4.54 (s, 2H), 5.89 (bs, 1H), 6.35-6.49 (m, 1H), 6.61-6.76 (m, 3H), 7.10 (d, *J* = 10 Hz, 1H), 7.42-7.58 (m, 1H), 7.76 (s, 1H), 8.13 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 32.4, 52.8, 55.6, 56.3, 76.6, 103.5 (t), 111.4 (d), 113.0, 113.3, 120.2, 126.8 (2C), 130.2 (d), 133.0, 144.6, 151.0, 155.8, 159.1 (dd), 163.0 (dd), 168.6; **IR** (Chloroform)**:** 3335, 1644 cm^{-1}; **MS** (ESI) *m*/*z***:** 455.7493 (M + K); **Anal. Calcd for** $C_{20}H_{18}F_2N_4O_3S$**:** C, 55.55; H, 4.20; F, 8.79; N, 12.96 %. **Found:** C, 55.74; H, 4.10; F, 8.61; N, 13.12 %.

4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-7-methyl-4*H*-benzo[1,4]thiazin-3-one (26e)



Nature: Pale brown semisolid; **Yield:** 79 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.28 (s, 3H), 3.20 (d, *J* = 12 Hz, 1H), 3.28 (d, *J* = 12 Hz, 1H), 4.48 (s, 2H), 4.61 (s, 2H), 5.92 (bs, 1H), 6.42-6.56 (m, 1H), 6.68-6.88 (m, 2H), 6.99-7.16 (m, 2H), 7.49-7.64 (m, 1H), 7.82 (s, 1H), 8.20 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 20.2, 32.0 52.3, 55.9, 74.7, 103.3 (t), 111.4 (d), 118.7, 125.0, 127.7, 128.5, 130.1 (d), 134.0, 136.9, 144.6, 151.0, 158.3 (dd) 163.0 (dd), 168.7, 170.9; **IR** (Chloroform)**:** 3326, 1646 (b) cm^{-1}; **MS** (ESI) *m*/*z***:** 417.9047 (M + 1), 439.9010 (M + Na), 455.9399 (M + K); **Anal. Calcd for** $C_{20}H_{18}F_2N_4O_2S$**:** C, 57.68; H, 4.36; F, 9.12; N, 13.45 %. **Found:** C, 57.81; H, 4.21; F, 9.02; N, 13.67 %.

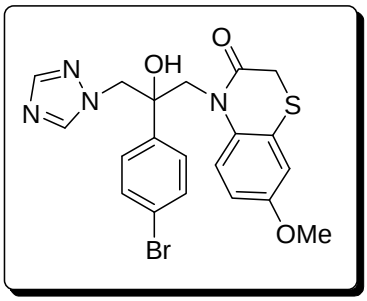
7-Chloro-4-[2-(4-fluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]thiazin-3-one (26f)



Nature: Pale yellow semisolid; **Yield:** 74 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.27 (d, *J* = 14 Hz, 1H), 3.40 (d, *J* = 14 Hz, 1H), 4.11 (d, *J* = 14 Hz, 1H), 4.66 (d, *J* = 14 Hz, 1H), 4.69-4.83 (m, 2H), 6.87-6.95 (m, 2H), 7.15 (dd, *J* = 8, 2 Hz, 1H), 7.30-7.40 (m, 4H), 7.94 (s, 1H), 8.67

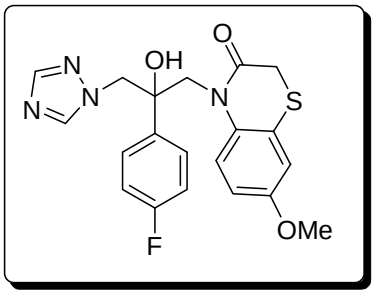
(s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 31.6, 54.2, 56.9, 77.6, 115.1 (d), 120.7, 125.6, 126.4, 127.0 (d, 3C), 127.1, 127.7, 129.1, 135.9, 138.6, 151.0, 160.0 (d), 167.1; **IR** (Chloroform)**:** 3382, 1667 cm^{-1}; **MS** (ESI) *m*/*z***:** 419.0630 (M + 1), 441.0702 (M + Na), 457.1034 (M + K); **Anal. Calcd for** $C_{19}H_{16}ClFN_4O_2S$**:** C, 54.48; H, 3.85; Cl, 8.46; F, 4.54; N, 13.38 %. **Found:** C, 54.64; H, 3.91; Cl, 8.27; F, 4.61; N, 13.21 %.

4-[2-(4-Bromophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-7-methoxy-4*H*-benzo[1,4]thiazin-3-one (26g)



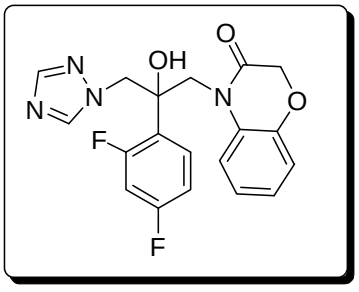
Nature: Brown solid; **MP:** 136-138 ^{o}C; **Yield:** 77 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.26 (d, *J* = 14 Hz, 1H), 3.37 (d, *J* = 14 Hz, 1H), 3.78 (s, 3H), 4.22 (d, *J* = 14 Hz, 1H), 4.52 (d, *J* = 14 Hz, 1H), 4.60-4.75 (m, 2H), 6.72 (dd, *J* = 10, 4 Hz, 1H), 6.80 (d, *J* = 4 Hz, 1H), 7.17-7.31 (m, 5H), 7.94 (s, 1H), 8.53 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 32.2, 54.4, 55.5, 56.9, 77.6, 112.8, 113.2, 120.4, 121.8, 126.4, 127.2 (2C), 131.1 (2C), 131.6, 133.4, 139.2, 150.9, 155.6, 167.7; **IR** (Chloroform)**:** 3367, 1655 cm^{-1}; **MS** (ESI) *m*/*z***:** 475.0584 and 477.0539 (M + 1); **Anal. Calcd for** $C_{20}H_{19}BrN_4O_3S$**:** C, 50.53; H, 4.03; Br, 16.81; N, 11.79 %. **Found:** C, 50.71; H, 3.79; Br, 17.04; N, 11.54 %.

4-[2-(4-Fluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-7-methoxy-4*H*-benzo[1,4]thiazin-3-one (26h)



Nature: Pale yellow semisolid; **Yield:** 78 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.26 (d, *J* = 14 Hz, 1H), 3.37 (d, *J* = 14 Hz, 1H), 3.78 (s, 3H), 4.22 (d, *J* = 14 Hz, 1H), 4.54 (d, *J* = 14 Hz, 1H), 4.61 (d, *J* = 14 Hz, 1H), 4.70 (d, *J* = 14 Hz, 1H), 6.71-6.91 (m, 4H), 7.23-7.33 (m, 3H), 7.91 (s, 1H), 8.41 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 32.0, 54.5, 55.4, 57.1, 71.6, 113.0 (d), 114.8 (d), 120.3, 126.2, 127.0, 127.2, 128.6, 130.7, 133.4, 135.9, 144.5, 150.9, 155.5, 161.9 (d), 165.5; **IR** (Chloroform)**:** 3393, 1657 cm^{-1}; **MS** (ESI) *m*/*z***:** 414.3148 (M+), 436.2464 (M + Na), 452.2196 (M + K); **Anal. Calcd for** $C_{20}H_{19}FN_4O_3S$**:** C, 57.96; H, 4.62; F, 4.58; N, 13.52 %. **Found:** C, 58.12; H, 4.71; F, 4.41; N, 13.61 %.

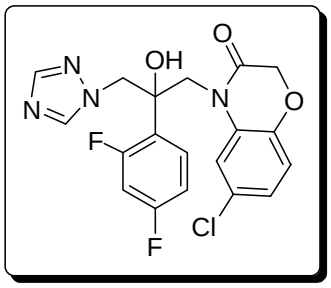
4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]oxazin-3-one (26i)



Nature: White solid; **MP:** 159-160 ^oC; **Yield:** 79 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + DMSO-d6)**:** δ 4.14 (d, *J* = 14 Hz, 1H), 4.49-4.70 (m, 4H), 4.94 (d, *J* = 14 Hz, 1H), 5.98 (s, 1H), 6.70-6.80 (m, 2H), 6.82-7.00 (m, 3H), 7.10-7.22 (m, 1H), 7.35-7.49 (m, 1H), 7.62 (s, 1H),

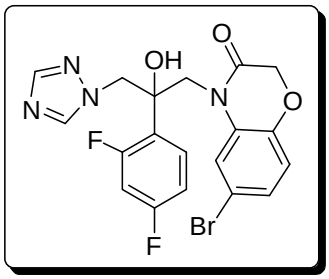
8.22 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + DMSO-d6)**:** δ 48.7, 55.3, 67.3, 76.7, 104.0 (t), 111.6 (d), 116.8, 117.1, 118.7, 118.8, 127.6, 129.9 (d), 130.1, 143.6, 144.4, 151.5, 154.4 (dd), 157.0 (dd), 166.0; **IR** (Chloroform)**:** 3368, 1682 cm^{-1}; **MS** (ESI) *m*/*z***:** 386.5672 (M+) **Anal. Calcd for** $C_{19}H_{16}F_2N_4O_3$**:** C, 59.07; H, 4.17; F, 9.83; N, 14.50 %. **Found:** C, 58.84; H, 4.47; F, 9.75; N, 14.29 %.

6-Chloro-4-[2-(2,4-difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]oxazin-3-one (26j)



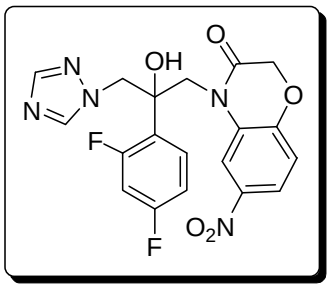
Nature: Pale brown semisolid; **Yield:** 74 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.13 (d, *J* = 15 Hz, 1H), 4.35-4.62 (m, 4H), 5.03 (d, *J* = 15 Hz, 1H), 5.53 (bs, 1H), 6.66-6.98 (m, 4H), 7.23 (d, *J* = 2 Hz, 1H), 7.49-7.64 (m, 1H), 7.82 (bs, 1H), 8.09 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 48.6, 55.5, 67.3, 76.6, 104.0 (t), 111.8 (d), 116.8, 117.8, 123.0, 124.0, 127.5, 129.9 (d), 130.1, 143.8, 144.5, 151.5, 154.3 (dd), 156.9 (dd), 166.0; **IR** (Chloroform)**:** 3383, 1693 cm^{-1}; **MS** (ESI) *m*/*z***:** 421.8764 (M + 1), 459.9421 (M + K); **Anal. Calcd for** $C_{19}H_{15}ClF_2N_4O_3$**:** C, 54.23; H, 3.59; Cl, 8.43; F, 9.03; N, 13.31 %. **Found:** C, 54.47; H, 3.68; Cl, 8.31; F, 8.89 N, 13.27 %.

6-Bromo-4-[2-(2,4-difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]oxazin-3-one (26k)



Nature: Off-white solid; **MP:** 119 ^{o}C; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.11 (d, *J* = 14 Hz, 1H), 4.49-4.74 (m, 4H), 5.06 (d, *J* = 14 Hz, 1H), 5.55 (bs, 1H), 6.68-6.85 (m, 3H), 7.08 (dd, *J* = 10, 2 Hz, 1H), 7.32 (d, *J* = 2 Hz, 1H), 7.46-7.60 (m, 1H), 7.87 (s, 1H), 8.51 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 48.4, 55.2, 67.2, 76.4, 103.9 (t), 111.7 (d), 114.5, 118.1, 119.5, 123.0, 126.8, 129.9 (d), 130.2, 144.2, 144.3, 151.1, 158.2 (dd), 163.2 (dd), 165.8; **IR** (Chloroform)**:** 3383, 1693 cm^{-1}; **MS** (ESI) *m*/*z***:** 465.8742 and 467.8794 (M + 1), 503.9364 and 505.9311 (M + K); **Anal. Calcd for** $C_{19}H_{15}BrF_2N_4O_3$**:** C, 49.05; H, 3.25; Br, 17.17; F, 8.17; N, 12.04 %. **Found:** C, 48.86; H, 3.51; Br, 17.01; F, 8.29; N, 11.85 %.

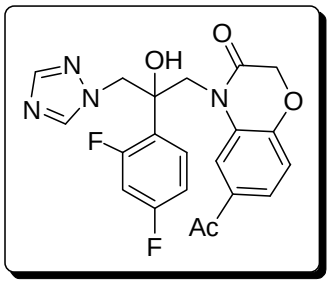
4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-6-nitro-4*H*-benzo[1,4]oxazin-3-one (26l)



Nature: Pale yellow solid; **MP:** 175 ^{o}C; **Yield:** 67 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.16 (d, *J* = 14 Hz, 1H), 4.54 (d, *J* = 14 Hz, 1H), 4.61-4.82 (m, 3H), 5.12 (d, *J* = 16 Hz, 1H), 5.57 (bs, 1H), 6.68-6.86 (m, 2H), 7.05 (d, *J* = 10 Hz, 1H), 7.52-7.66 (m, 1H), 7.82 (s, 1H), 7.93

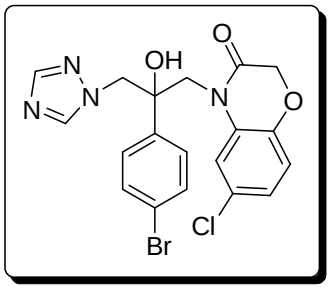
(dd, *J* = 10, 2 Hz, 1H), 8.02 (s, 1H), 8.28 (d, *J* = 2 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 48.6, 55.0, 67.2, 76.4, 104.2 (t), 111.1 (d), 113.1, 117.1, 120.1, 122.6, 129.5 (2C), 130.0 (d), 142.8, 149.9, 151.9, 153.9 (dd), 158.8 (dd), 164.6; **IR** (Chloroform)**:** 1689 cm^{-1}; **MS** (ESI) *m*/*z***:** 432.8953 (M + 1), 454.9330 (M + Na); **Anal. Calcd for** $C_{19}H_{15}F_2N_5O_5$**:** C, 52.90; H, 3.51; F, 8.81; N, 16.24 %. **Found:** C, 53.01; H, 3.38; F, 8.94; N, 16.11%.

6-Acetyl-4-[2-(2,4-difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]oxazin-3-one (26m)



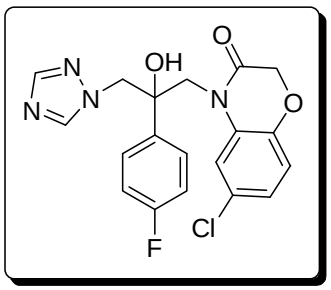
Nature: White solid; **MP:** 139-141 ^{o}C; **Yield:** 81 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.59 (s, 3H), 4.30 (d, *J* = 14 Hz, 1H), 4.60-4.79 (m, 4H), 5.09 (d, *J* = 14 Hz, 1H), 6.68-6.84 (m, 2H), 7.03 (d, *J* = 8 Hz, 1H), 7.51-7.67 (m, 2H), 7.87 (bs, 1H), 7.91 (d, *J* = 2 Hz, 1H), 8.31 (bs, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 26.2, 48.4, 55.4, 67.2, 76.4, 104.0 (t), 111.8 (d), 116.7 (2C), 122.8, 125.3, 129.0 (2C), 129.8, 131.8, 149.0, 151.1, 155.2 (dd), 161.1 (dd), 165.6, 196.0; **IR** (Chloroform)**:** 3258, 1694, 1669 cm^{-1}; **MS** (ESI) *m*/*z***:** 429.1651 (M + 1), 451.1171 (M + Na), 467.0826 (M + K); **Anal. Calcd for** $C_{21}H_{18}F_2N_4O_4$**:** C, 58.88; H, 4.24; F, 8.87; N, 13.08 %. **Found:** C, 58.56; H, 4.40; F, 8.74; N, 13.27 %.

4-[2-(4-Bromophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-6-chloro-4*H*-benzo[1,4]oxazin-3-one (26n)



Nature: Pale brown solid; **MP:** 73-75 ^{o}C; **Yield:** 74 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.93 (d, *J* = 14Hz, 1H), 4.53-4.69 (m, 4H), 4.85 (d, *J* = 14 Hz, 1H), 6.86-6.99 (m, 2H), 7.30-7.45 (m, 5H), 7.88 (s, 1H), 8.35 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 50.2, 55.6, 66.8, 76.0, 116.8, 117.7, 121.7, 123.5, 126.5 (3C), 127.0, 129.6, 131.0 (2C), 139.0, 143.2, 150.7, 165.3; **IR** (Chloroform)**:** 3401, 1690 cm^{-1}; **MS** (ESI) *m/z***:** 485.2167 and 487.2116 (M + Na), 501.2784 and 503.2754 (M + K); **Anal. Calcd for** $C_{19}H_{16}BrClN_4O_3$**:** C, 49.21; H, 3.48; Br, 17.23; Cl, 7.65; N, 12.08 %. **Found:** C, 49.37; H, 3.54; Br, 17.07; Cl, 7.80; N, 12.11 %.

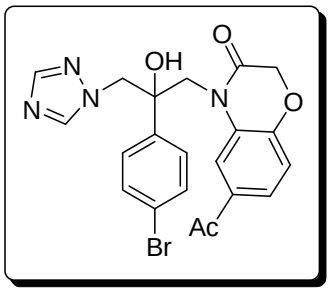
4-[2-(4-Fluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-6-chloro-4*H*-benzo[1,4]oxazin-3-one (26o)



Nature: Pale yellow solid; **MP:** 103-105 ^{o}C; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.95 (d, *J* = 14 Hz, 1H), 4.52-4.68 (m, 4H), 4.85 (d, *J* = 14 Hz, 1H), 6.86-7.03 (m, 4H), 7.34 (d, *J* = 2 Hz, 1H), 7.41-7.47 (m, 2H), 7.87 (s, 1H), 8.28 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 50.8, 56.3, 67.2, 76.3, 115.3 (d), 117.4 (d), 123.9, 126.9 (2C), 127.0 (2C), 127.4,

130.0, 136.0, 142.6, 144.3, 151.2, 162.6 (d), 165.7; **IR** (Chloroform)**:** 3362, 1685 cm^{-1}; **MS** (ESI) *m*/*z***:** 403.4927 (M + 1), 425.4877 (M + Na), 441.4519 (M + K); **Anal. Calcd for** $C_{19}H_{16}ClFN_4O_3$**:** C, 56.65; H, 4.00; Cl, 8.80; F, 4.72; N, 13.91 %. **Found:** C, 56.54; H, 4.15; Cl, 8.94; F, 4.58; N, 13.84 %.

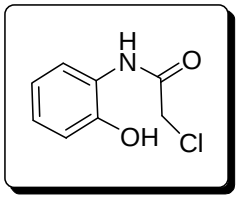
6-Acetyl-4-[2-(4-bromophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]oxazin-3-one (26p)



Nature: Pale brown semisolid; **Yield:** 80 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.49 (s, 3H), 4.06 (d, *J* = 14 Hz, 1H), 4.50-4.64 (m, 4H), 4.79 (d, *J* = 8 Hz, 1H), 6.92 (d, *J* = 8 Hz, 1H), 7.27-7.35 (m, 4H), 7.53 (dd, *J* = 8, 2 Hz, 1H), 7.84-7.86 (m, 2H), 8.38 (s, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 26.3, 50.5, 56.3, 67.2, 71.7, 116.7, 117.3, 122.2, 125.4, 127.0 (2C), 128.8, 129.9, 130.8, 131.6 (2C), 131.8, 139.4, 148.9, 165.5, 196.2; **IR** (Chloroform)**:** 3393, 1677 cm^{-1}; **MS** (ESI) *m*/*z***:** 470.2361 and 472.2327 (M+); **Anal. Calcd for** $C_{21}H_{19}BrN_4O_4$**:** C, 53.52; H, 4.06; Br, 16.95; N, 11.89 %. **Found:** C, 53.67; H, 3.87; Br, 17.13; N, 11.79 %.

Preparation and spectral data of compounds **27a, 27b** and **27c** was described in the experimental part of 1st Chapter.

Preparation of 2-chloro-N-(2-hydroxyphenyl)acetamide (33a)[24]

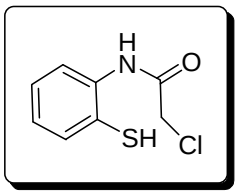


Chloroacetyl chloride (20 g, 14.6 ml, 0.183 mol) was added dropwise to a stirred solution of 2-aminophenol (30 g, 0.183 mol) in dry dichloromethane (300 ml) at room temperature in a two necked round bottom flask equipped with calcium chloride guard tube. After stirring for 2 h, the mixture was added to saturated solution of sodium bicarbonate and stirred for 10 min, diluted with water, extracted with dichloromethane (3 X 100 ml) and dried over sodium sulphate. Evaporation of solvent left the lachrymatory residue of 2-chloro-N-(2-hydroxyphenyl)acetamide which was purified by column chromatography to give pure compound **33a** as white solid (41.29 g, 81.1 %).

Nature: White solid; **Yield:** 81.1 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.16 (s, 2H), 6.77-6.99 (m, 3H), 7.95 (d, *J* = 6 Hz, 1H), 9.03 (bs, 1H).

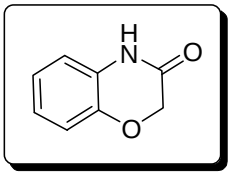
For the synthesis of 2-chloro-N-(2-mercaptophenyl)acetamide **33b** same experimental procedure was repeated starting with 2-aminothiophenol.

2-Chloro-N-(2-mercaptophenyl)acetamide (33b)[24]



Nature: White solid; **Yield:** 79.2 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.70 (bs, 1H), 4.11 (s, 2H), 6.70-6.92 (m, 3H), 7.93 (d, *J* = 8 Hz, 1H), 8.99 (bs, 1H).

Preparation of 2*H*-benzo[b][1,4]oxazin-3(4*H*)-one (28a)[24]

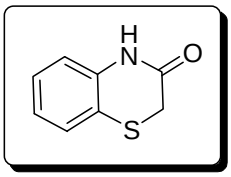


2-Chloro-N-(2-hydroxyphenyl)acetamide (**33a**) (40 g, 0.269 mol) was dissolved in dry ethyl acetate (500 ml) in two necked round bottom flask equipped with calcium chloride guard tube. The solution was charged with anhydrous potassium carbonate (74.39 g, 0.539 mol) and was allowed to stir at room temperature for 8-10 h. After complete conversion, water was added to reaction mixture. The mixture was extracted with ethyl acetate (3 X 100 ml), dried over sodium sulphate and concentrated to get crude product. After purification by silica gel column chromatography, pure 2*H*-benzo[b][1,4]oxazin-3(4*H*)-one **28a** was obtained.

Nature: Off-white solid; **MP:** 174-176 ^{o}C; **Yield:** 84 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 4.64 (s, 2H), 6.86-6.99 (m, 4H), 9.76 (bs, 1H).

For the synthesis of 2*H*-benzo[b][1,4]thiazin-3(4*H*)-one **28b**, same experimental procedure was repeated starting with 2-chloro-N-(2-mercaptophenyl)acetamide **33b**.

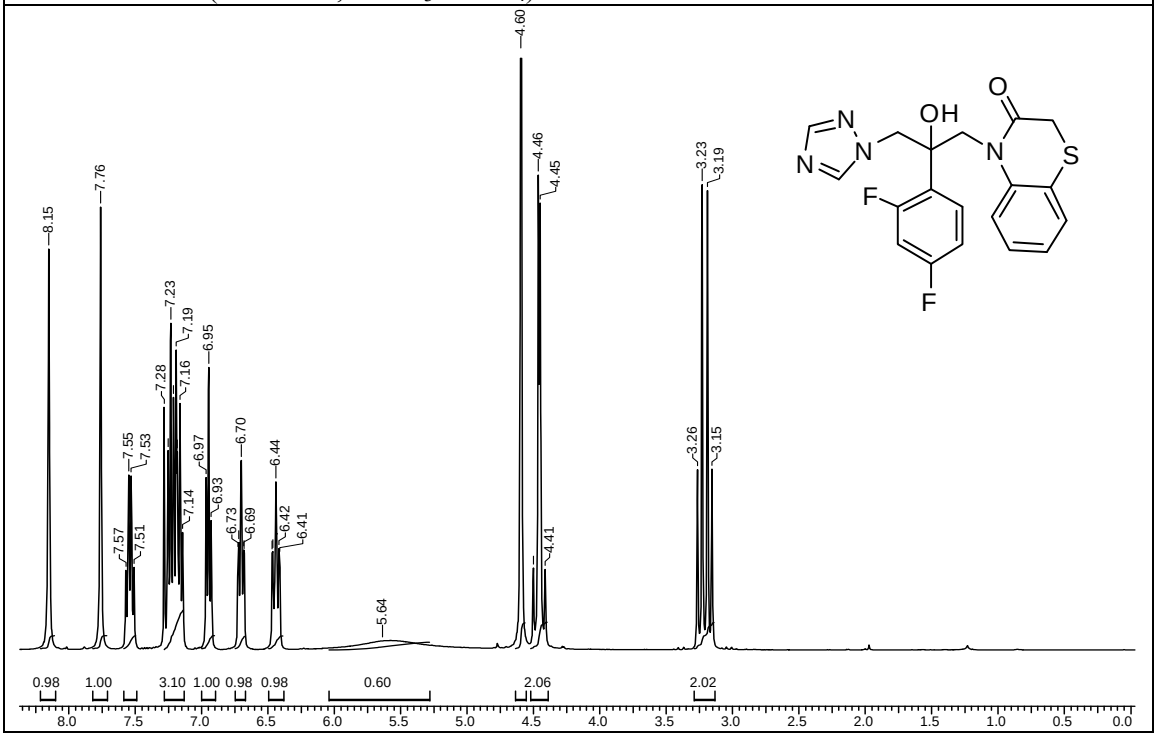
2*H*-Benzo[b][1,4]thiazin-3(4H)-one (28b)[24]



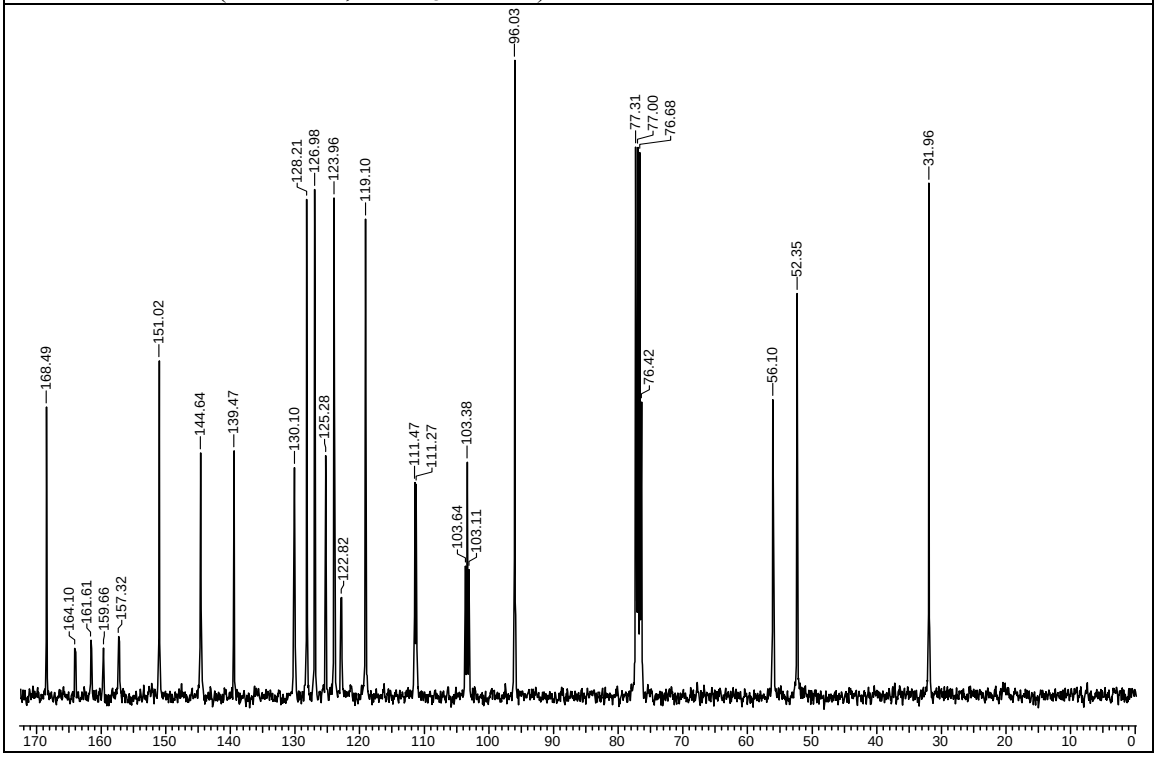
Nature: Brown solid; **MP:** 180-182 ^{o}C; **Yield:** 78.7 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.36 (s, 2H), 6.84-6.97 (m, 2H), 7.06-7.24 (m, 2H), 9.46 (bs, 1H).

3.1.7 Selected spectra

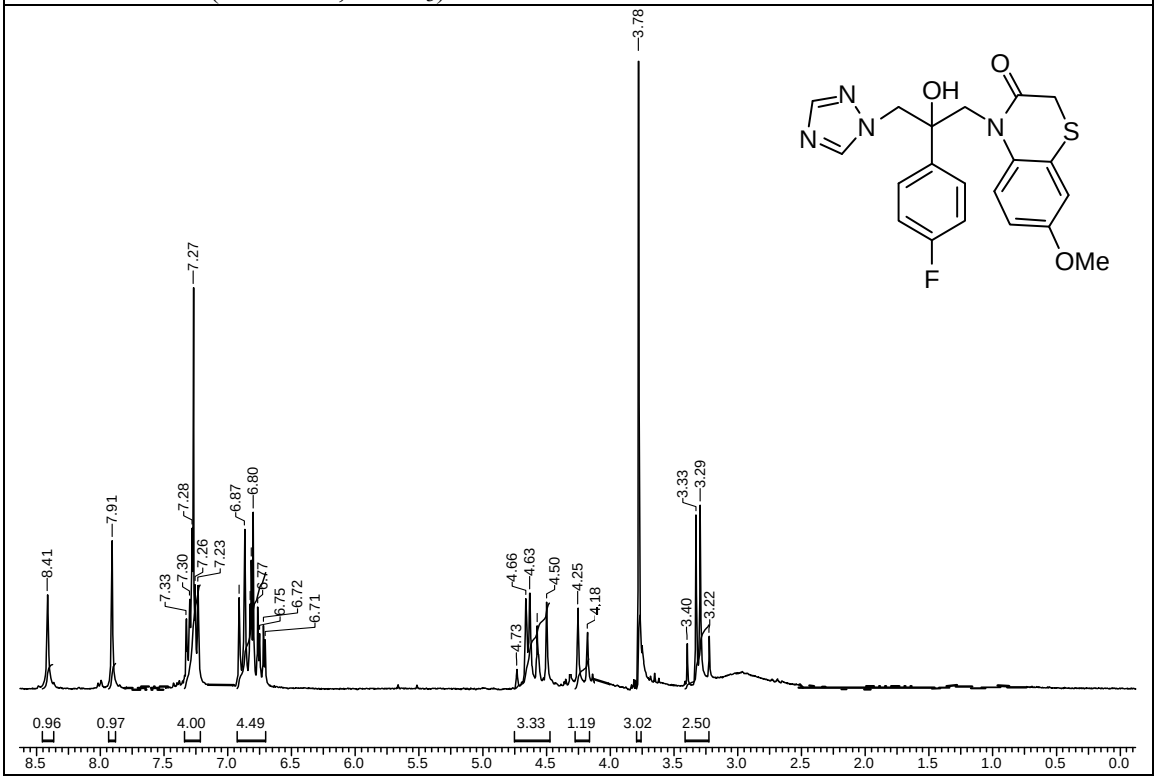
26a: ^{1}H NMR (400 MHz, $CDCl_3$ + CCl_4)



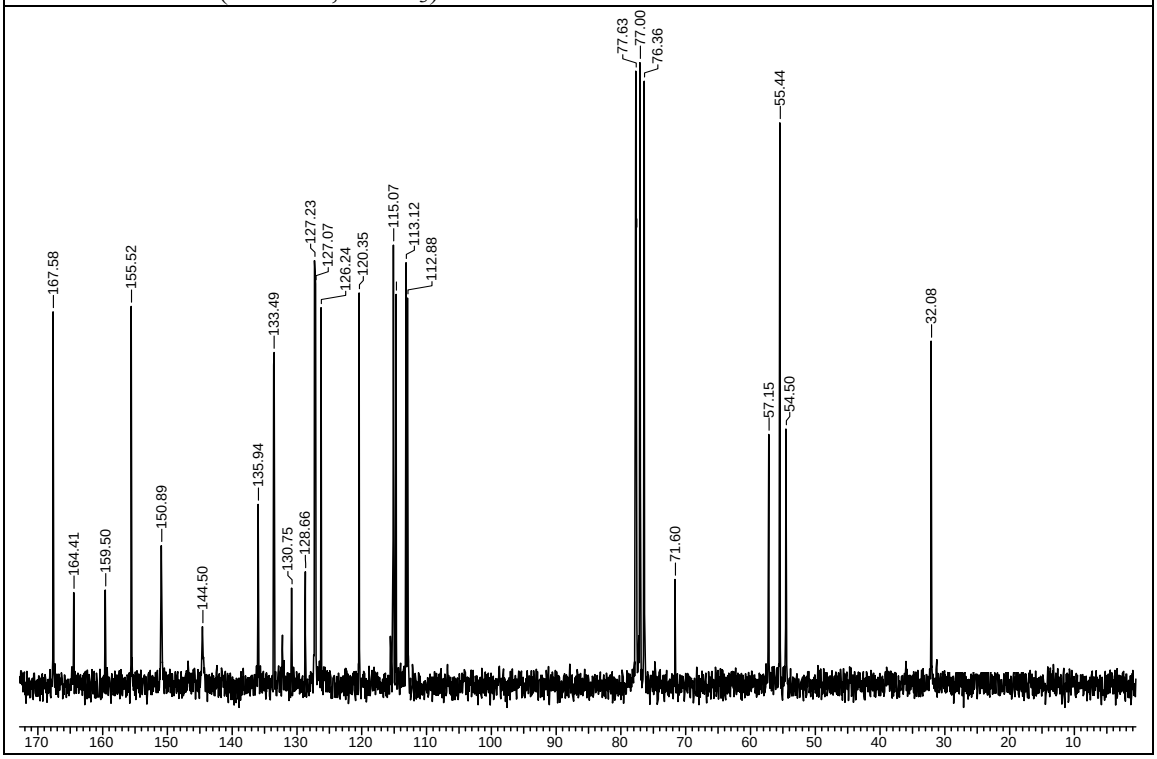
26a: ^{13}C NMR (100 MHz, $CDCl_3$ + CCl_4)



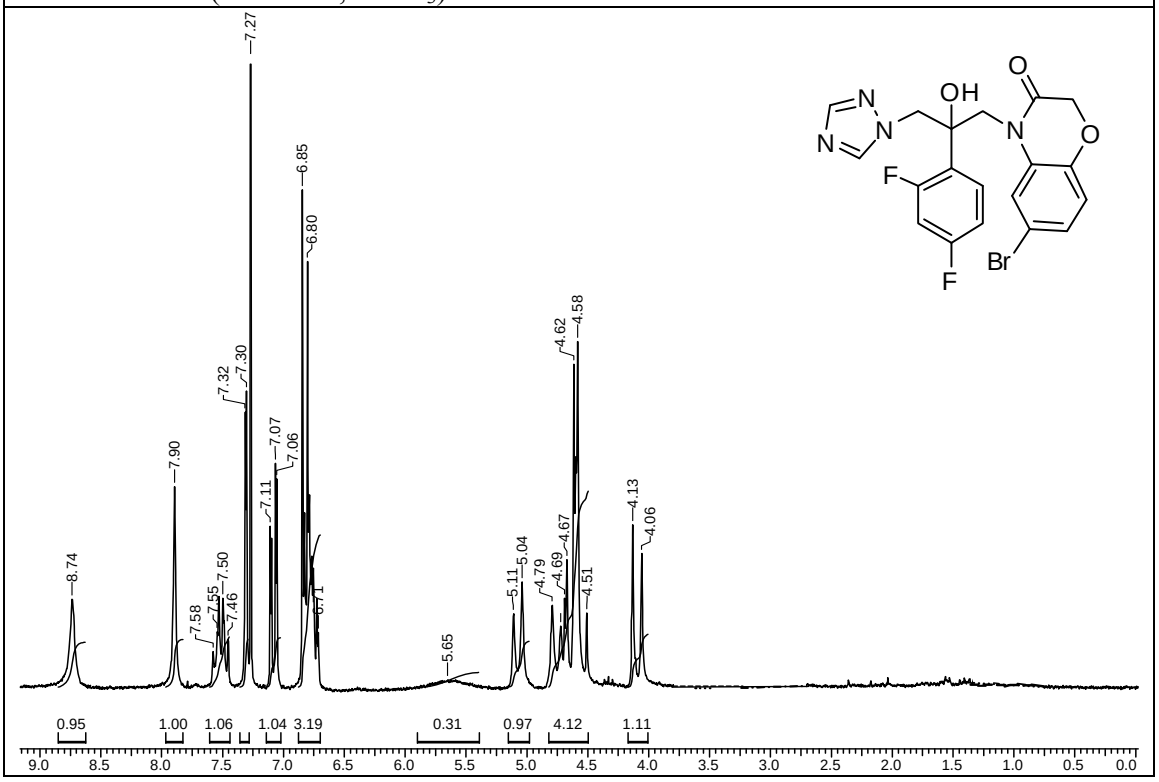
26h: ^{1}H NMR (200 MHz, $CDCl_3$)



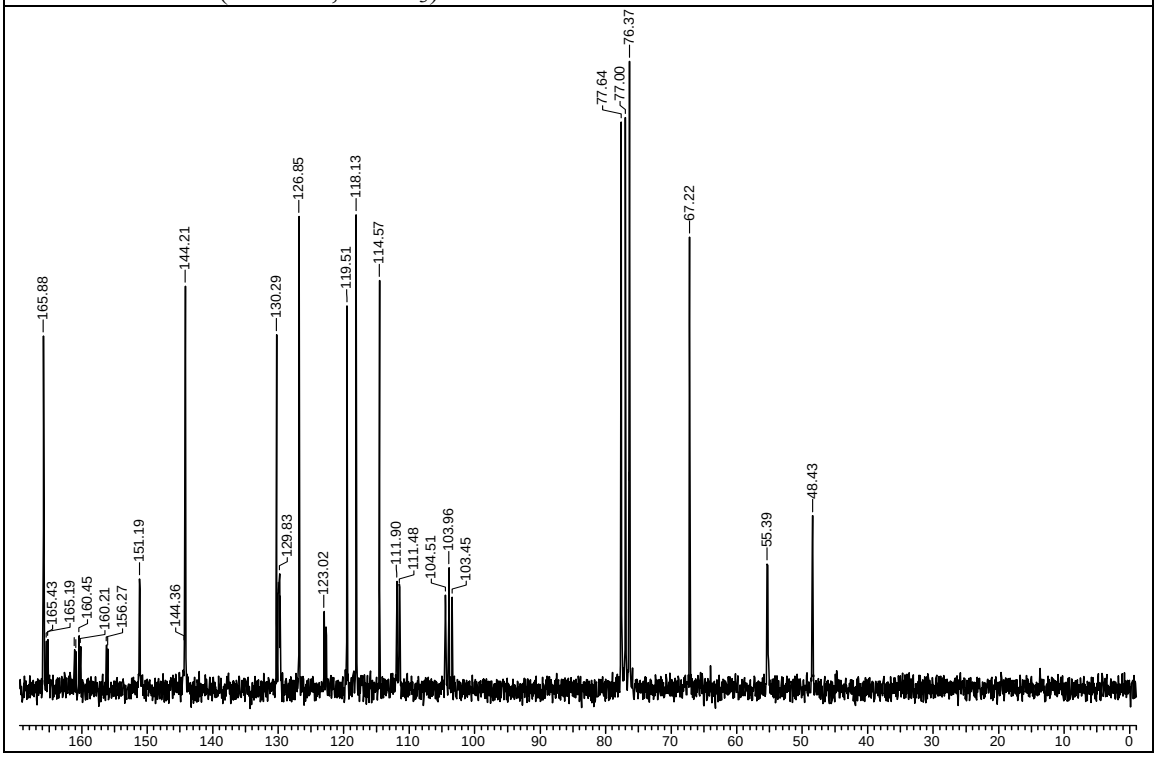
26h: ^{13}C NMR (50 MHz, $CDCl_3$)



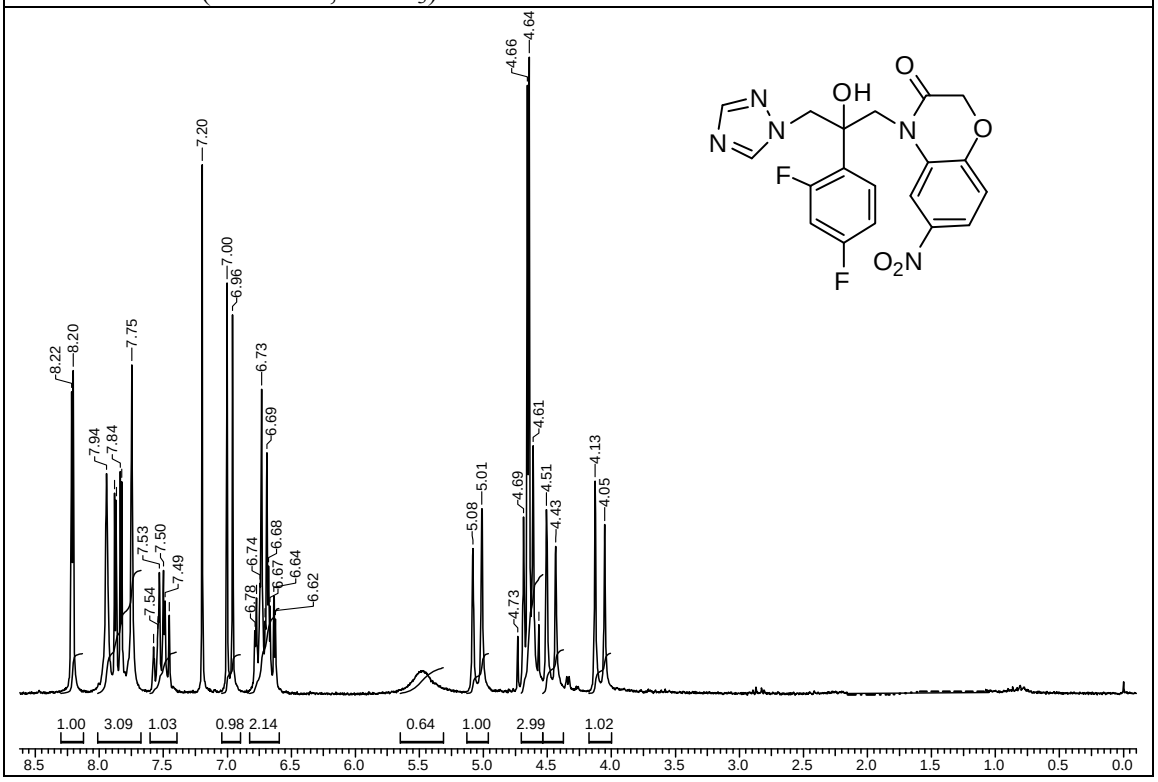
26k: ^{1}H NMR (200 MHz, $CDCl_3$)



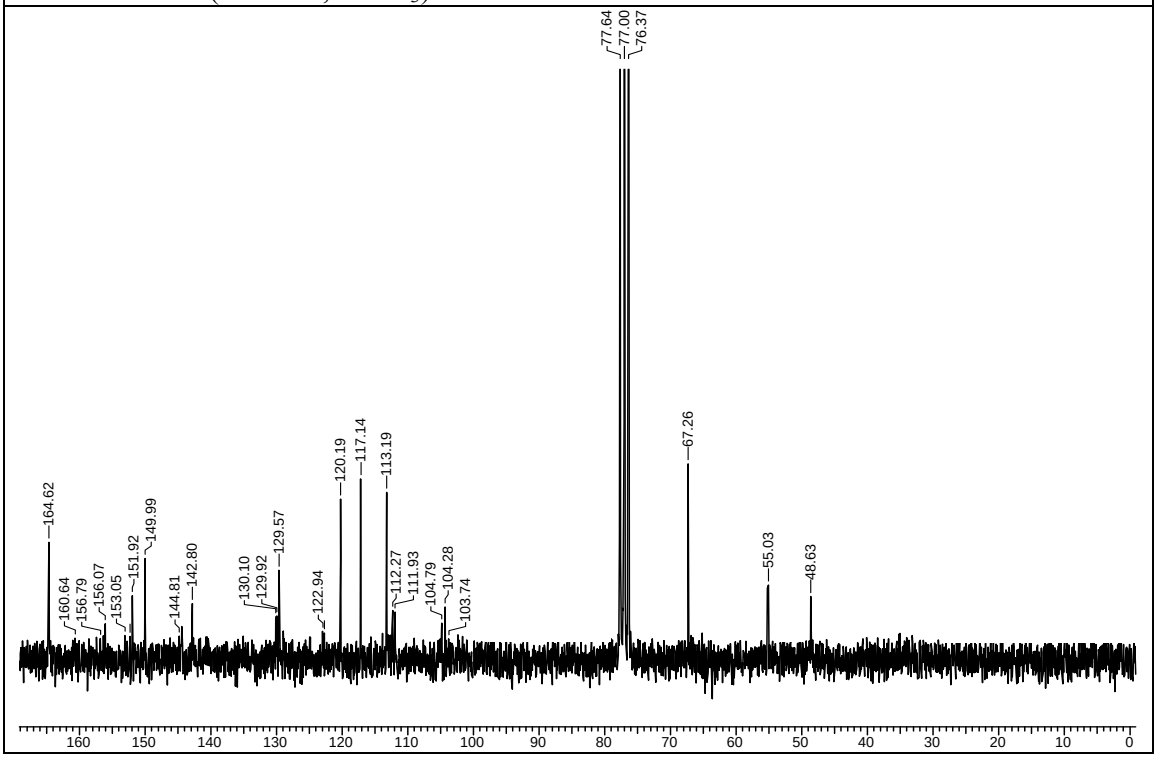
26k: ^{13}C NMR (50 MHz, $CDCl_3$)



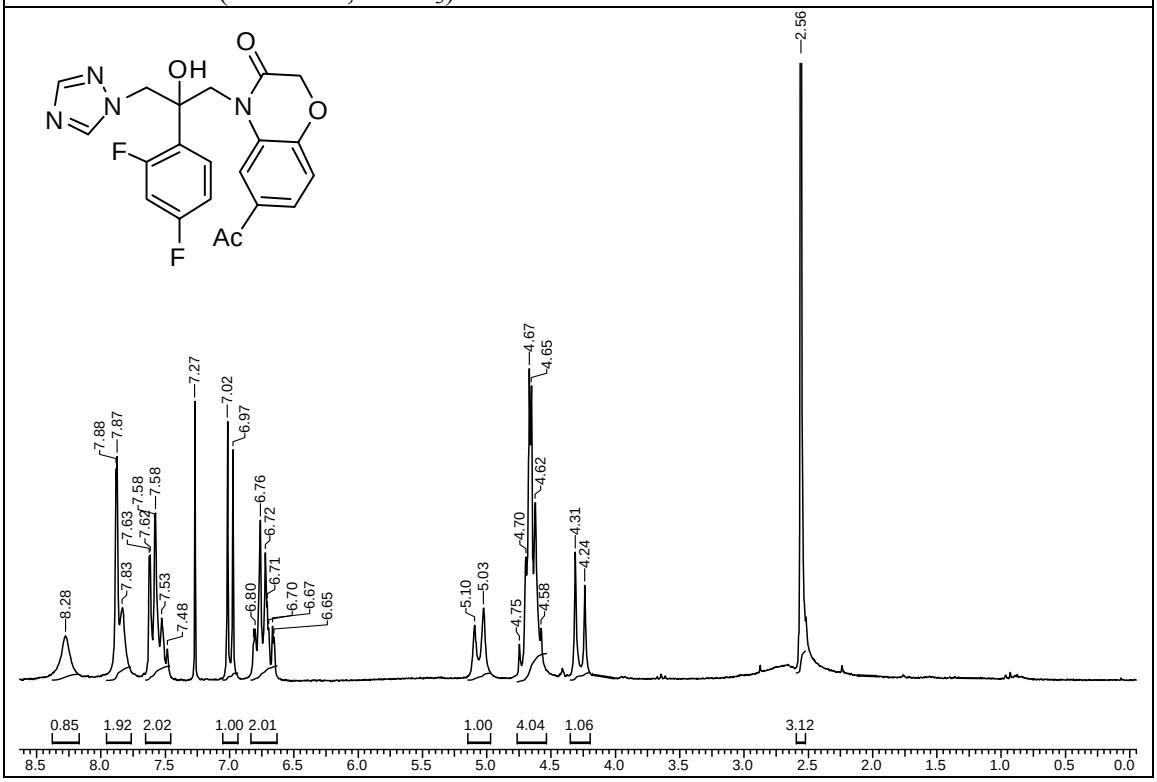
26l: ^{1}H NMR (200 MHz, $CDCl_3$)



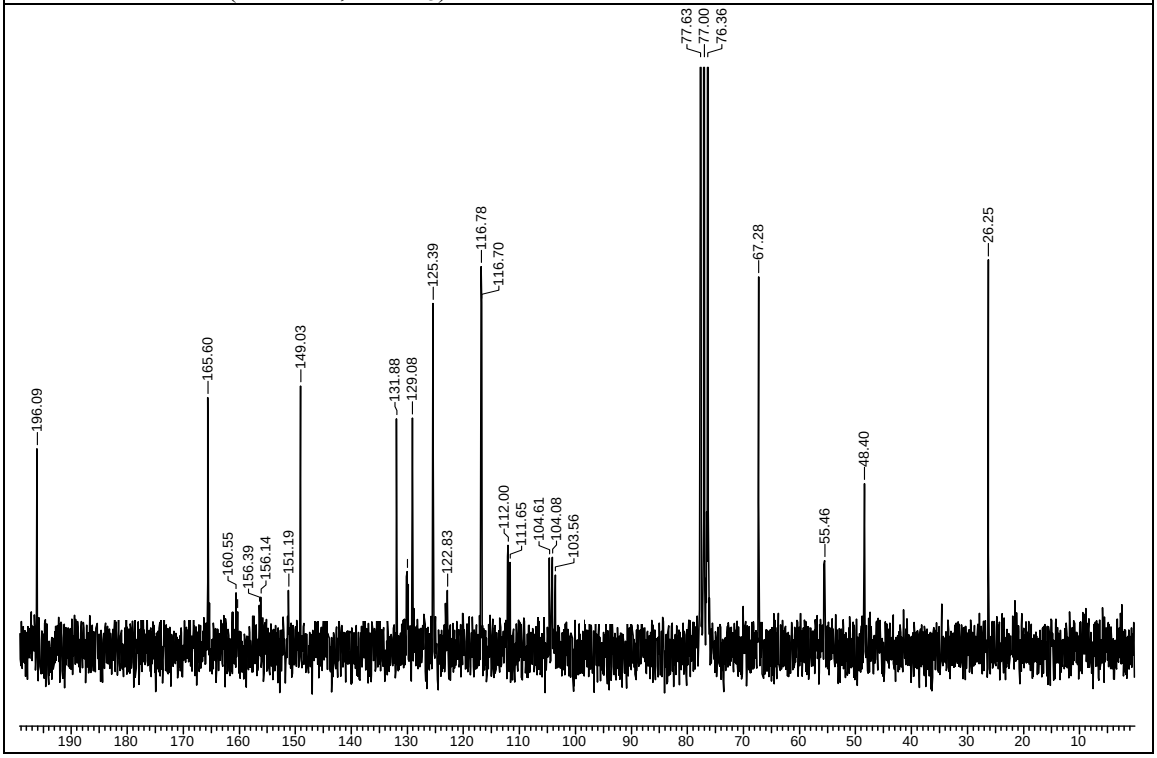
26l: ^{13}C NMR (50 MHz, $CDCl_3$)



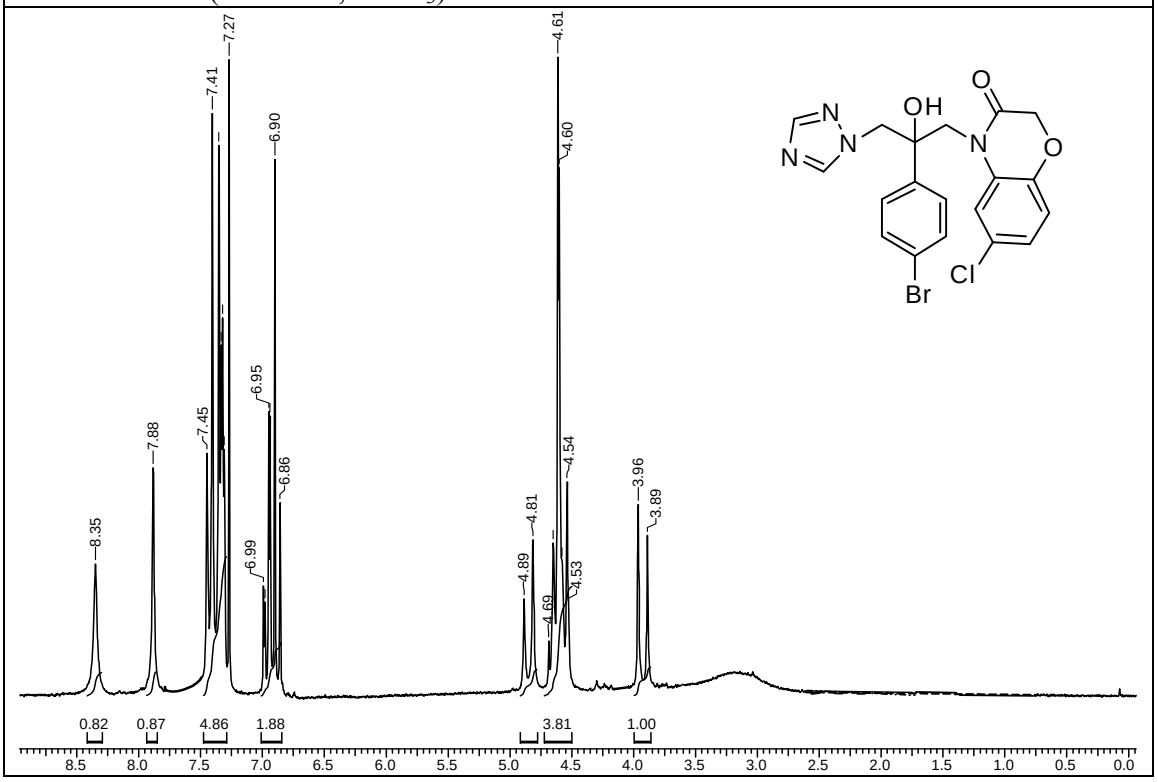
26m: ^{1}H NMR (200 MHz, $CDCl_3$)



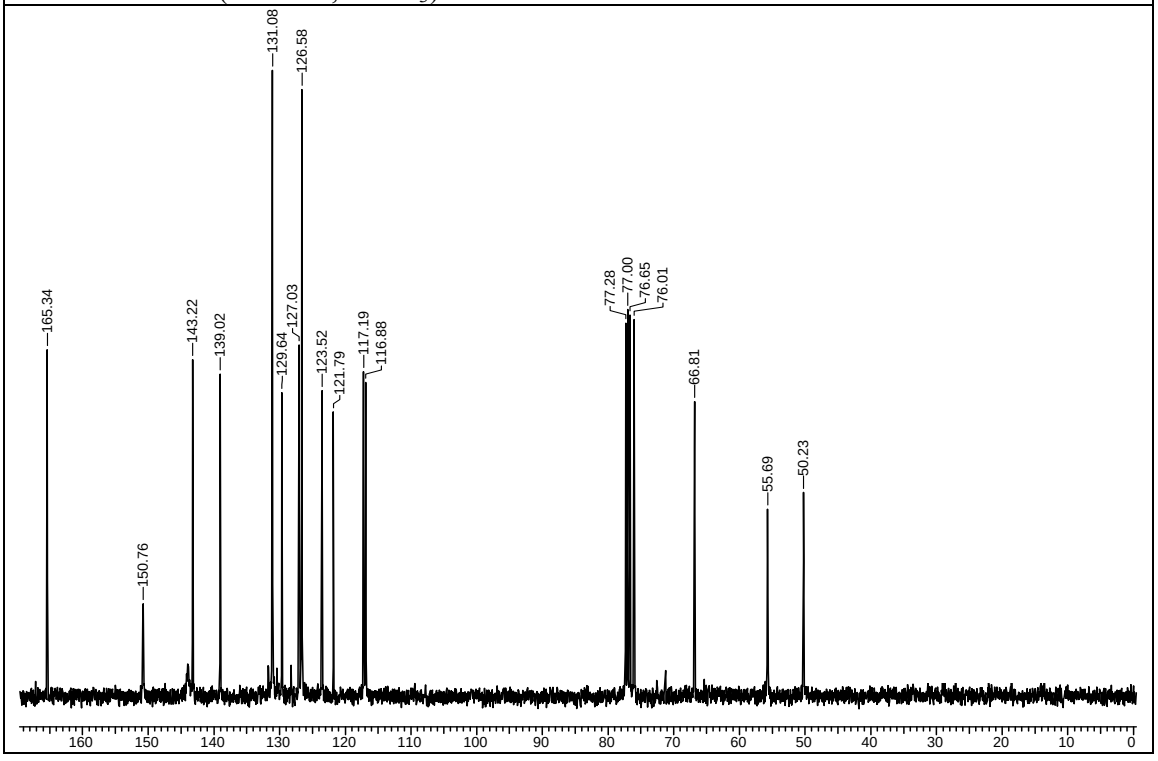
26m: ^{13}C NMR (50 MHz, $CDCl_3$)



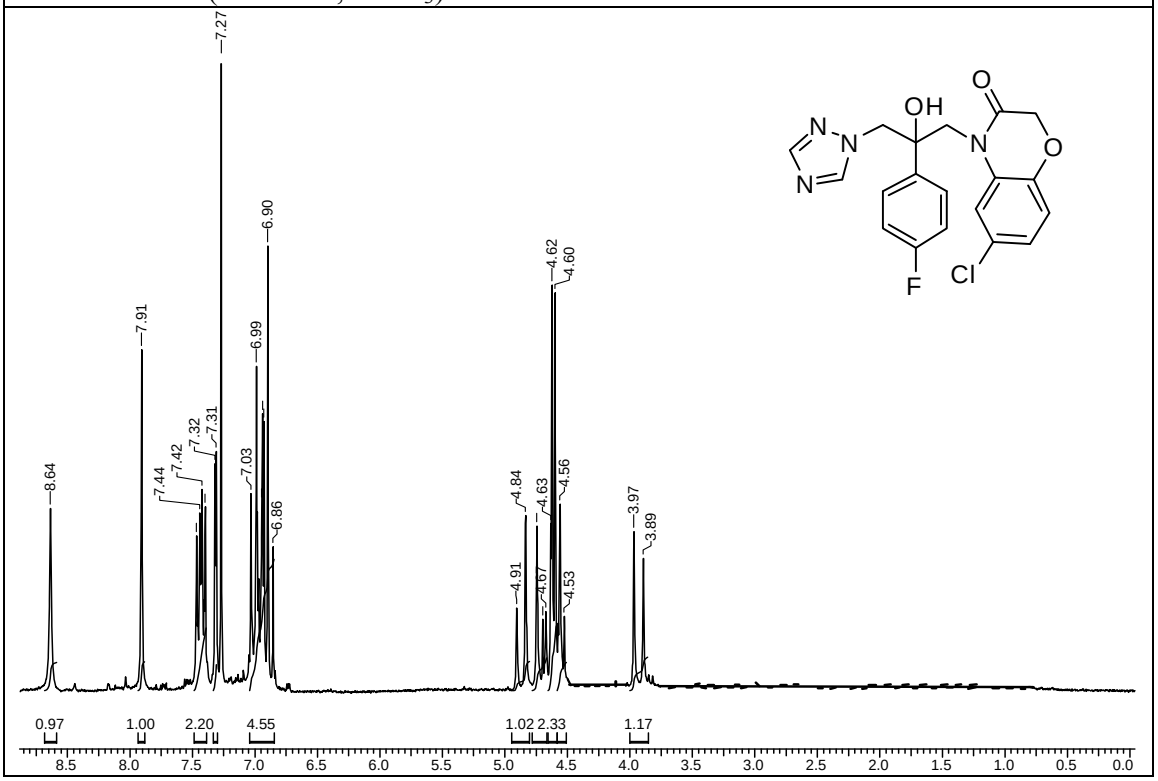
26n: ^{1}H NMR (200 MHz, $CDCl_3$)



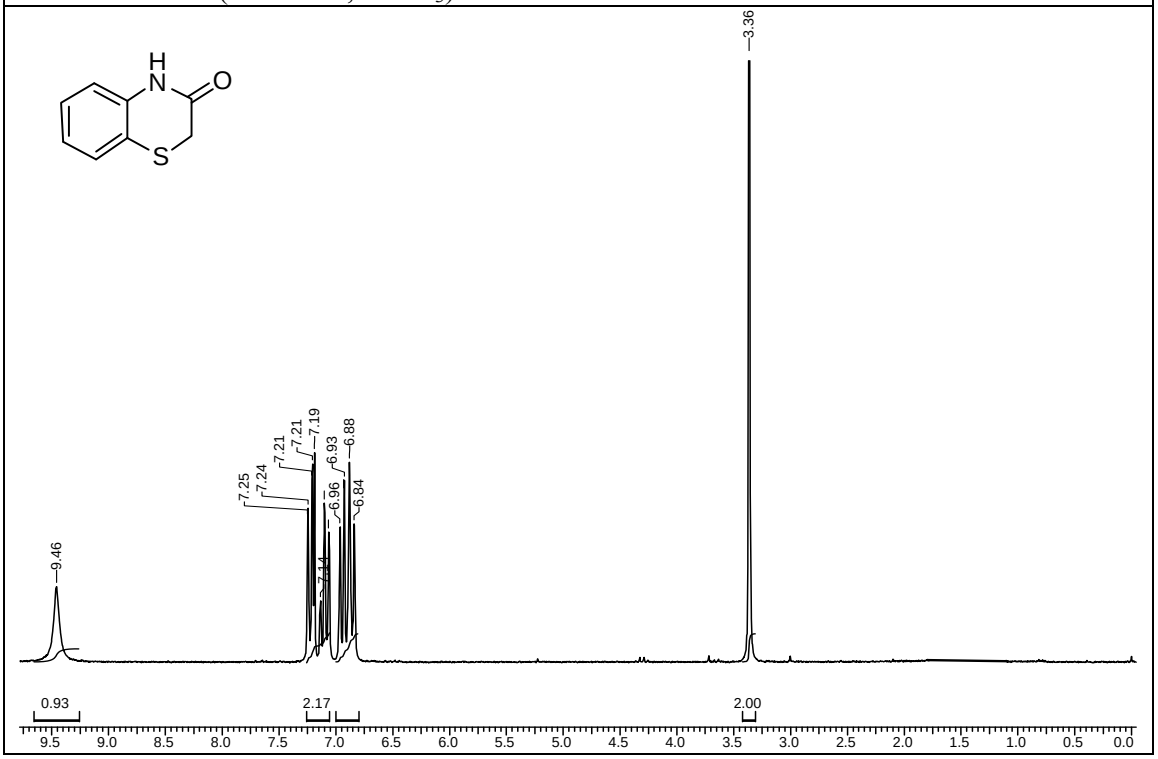
26n: ^{13}C NMR (50 MHz, $CDCl_3$)



26o: ^{1}H NMR (200 MHz, $CDCl_3$)



28b: ^{13}C NMR (200 MHz, $CDCl_3$)



3.1.8 References

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24. CLSI**:** Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard M38-A, 2002.

Chapter 3: Section II

One-step preparation of α-chlorostyrenes

3.2.1 Abstract

α-Chlorostyrenes were prepared *via* a one-step method involving Friedel–Crafts reaction of various aromatic substrates with acid chlorides in the presence of a heterogeneous Si–Fe catalyst. The catalyst was synthesized and screened for various organic transformations, and it was observed that it catalyzed the Friedel–Crafts acylation of aromatic compounds. Furthermore, it was observed that along with the corresponding Friedel-Crafts acylation product, α-chlorostyrenes were obtained as the major product in the case of acid chlorides having α-methylene groups. The higher yields of α-chlorostyrenes obtained in the reaction prompted us to develop this method for the one-step preparation of α-chlorostyrenes.

3.2.2 Introduction

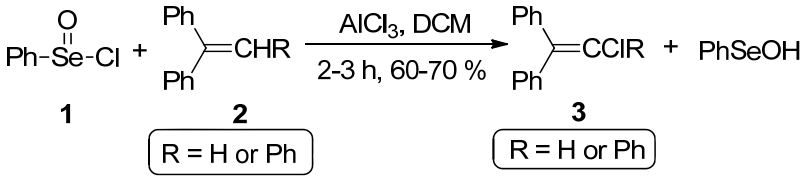
During our work directed towards synthesis of fluconazole analogues, 1-((2-(2,4-dihalophenyl)oxiran-2-yl)methyl)-1*H*-1,2,4-triazole was used as an intermediate (Compound **81** in Chapter 1). The synthetic sequence for the preparation of this epoxide involved Friedel-Crafts acylation as the first step, wherein $AlCl_3$ was used as Lewis acid as depicted in Scheme 7 in 1st Section of Chapter 1. We wished to overcome the problems associated with this reaction such as requirement of excess equivalents of $AlCl_3$, quenching of unused $AlCl_3$ while work-up which results in the formation of $Al(OH)_3$ and makes the work-up lengthy, *etc*. We thought to use some heterogeneous catalysts for the reaction, so that the catalyst could be easily removed from the reaction mixture during work-up and could be reused as well. Heterogeneous catalysts offer a number of advantages over homogeneous catalysts from the point of ease of work-up, recyclability of catalyst, effluent treatment *etc*. As a part of ongoing efforts of our group to explore the utility of heterogeneous catalysts for various organic reactions[1-4], we prepared heterogeneous Si-Fe catalyst and studied its activity for various reactions, and found that it catalyzed Friedel-Crafts acylation of aromatic compounds. When anisole was reacted with various aromatic acid chlorides the corresponding Friedel-Crafts acylation products were obtained showing the utility of the catalyst. Interestingly, α-chlorostyrenes were obtained in one step as the major product in case of acid chlorides having α-methylene groups. When we glance through the literature, we comprehend the importance of preparation as well as the use of the α-chlorostyrenes at

various places. Hence we developed this method for the one-step preparation of α-chlorostyrenes.

3.2.3 Review of literature

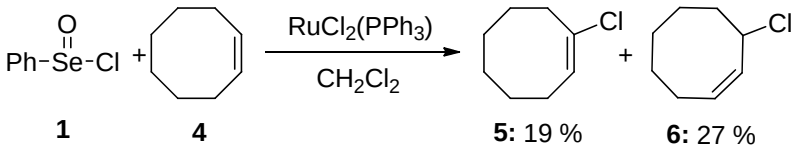
Preparation of α-halostyrenes

There are many methods known for the preparation of α-chlorostyrenes in the literature such as Wittig reaction of ketones with Ph_3P=CHCl or addition of HCl to acetylenes in the presence of zinc chloride reported[5] by M. Hanack *et al.* Use of selenium reagent in presence of aluminum chloride or ruthenium(II) phosphine complex for the preparation of α-chloro-olefins was reported[6] by N. Kamigata *et al.* They observed that the reaction of benzeneseleninyl chloride (**1**) with olefins **2** in the presence of aluminum chloride in DCM afforded chloro-olefins **3** as shown in Scheme 1.



Scheme 1. Preparation of α-chloro-olefins **3** in presence of aluminum chloride

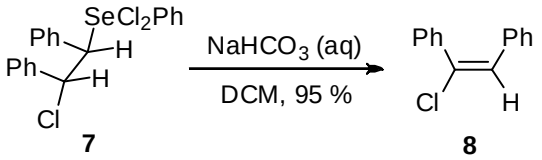
Furthermore, reaction of benzeneseleninyl chloride (**1**) with cyclooctene (**4**) in the presence of dichlorotris (triphenylphosphine) ruthenium(II) gave 1-chlorocyclooctene (**5**) and 3-chlorocyclooctene (**6**) as shown in Scheme 2.



Scheme 2. Preparation of chloro-olefins **5** and **6** in presence of ruthenium catalyst

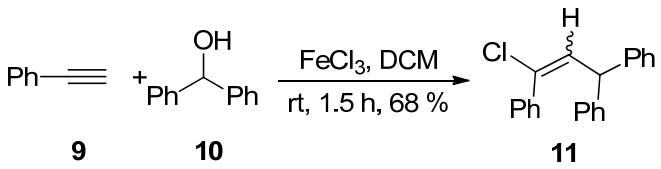
L. Engman *et al.* reported[7] the addition of phenylselenium trichloride to number of olefinic compounds to produce (β-chloroalkyl)phenylselenium dichlorides, which on treatment with aq sodium hydrogen carbonate in DCM, readily hydrolyzed to selenoxide,

which underwent the usual selenoxide elimination reaction to produce an allylic or a vinylic chloride. One such example of preparation of α-chloro-stilbene is shown in Scheme 3.



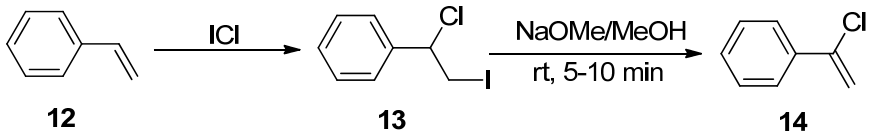
Scheme 3. Preparation of α-chloro-stilbene **8**

S. Biswas *et al.* reported[8] the reaction of phenylacetylene (**9**) with benzhydrol (**10**) in presence of $FeCl_3$ in DCM to afford the (3-chloroprop-2-ene-1,1,3-triyl)tribenzene (**11**) in 68 % yield of *E* and *Z* mixture as shown in Scheme 4. This methodology was generalized with some more examples and was studied with various Lewis acids in various reaction conditions by the author.



Scheme 4. Preparation of (3-chloroprop-2-ene-1,1,3-triyl)tribenzene (**11**)

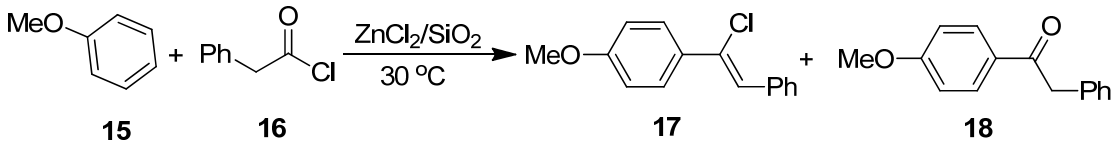
R. G. Amiet *et al.* reported[9] the synthesis of α-chlorostyrene **14** from styrene **12** by the addition of iodine monochloride, followed by the sodium methoxide-initiated dehydrohalogenation of the intermediate **13** as shown in Scheme 5. Furthermore, if reaction was not stopped immediately and kept on refluxing for an hour, it was observed that chloro group was replaced by methoxy group.



Scheme 5. Preparation of α-chlorostyrene **14**

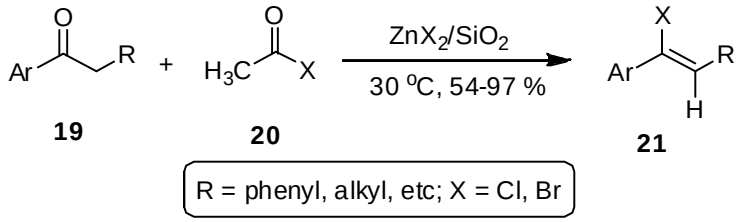
M. Kodomari *et al.* reported[10] the use of silica gel-supported zinc chloride for the preparation of α-chlorostyrenes and α-chlorostilbenes as shown in Schemes 6 and 7. Author

observed that when anisole (**15**) was treated with phenylacetyl chloride (**16**) using silica gel-supported zinc chloride as a catalyst, α-chlorostilbene **17** as well as the expected acylated product **18** were obtained as shown in Scheme 6.



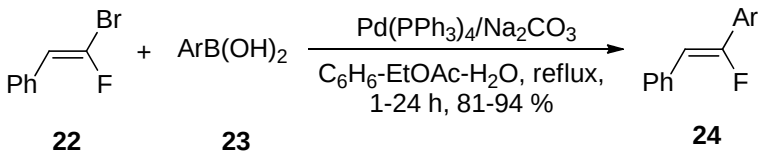
Scheme 6. Preparation of α-chlorostilbene **17**

Furthermore, when aryl ketones were treated with acetyl halides in presence of silica gel-supported zinc halide, the products formed were aryl-substituted halo olefins as shown in Scheme 7. Probably reaction proceeds through the addition of acetyl halides to the carbonyl, resulting in the formation of α-haloacetate, followed by elimination of acetic acid.[11]



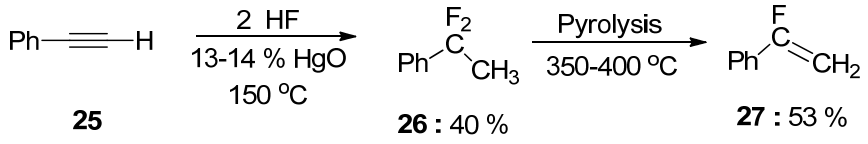
Scheme 7. Preparation of α-chlorostyrenes/α-chlorostilbenes **21**

C. Chen *et al.* reported[12] the synthesis of α-fluorostilbenes using Suzuki coupling reaction. When 1-fluoro-2-phenylvinyl bromide (**22**) was coupled with arylboronic acid **23** in the presence of $Pd(PPh_3)_4$ under Suzuki conditions, α-fluorostilbenes **24** were obtained in 81-94 % yields as shown in Scheme 8.



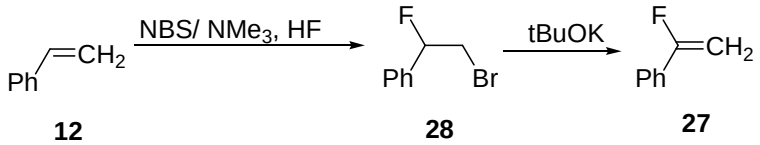
Scheme 8. Preparation of α-fluorostilbenes **24**

α-Fluorostyrenes are the monomer units for the synthesis of poly(α-fluorostyrenes), hence their syntheses were carried out using various methods. Matsuda *et al.* reported[13] the synthesis of α-fluorostyrenes (**27**) starting from the ethynylbenzene (**25**) as shown in Scheme 9.



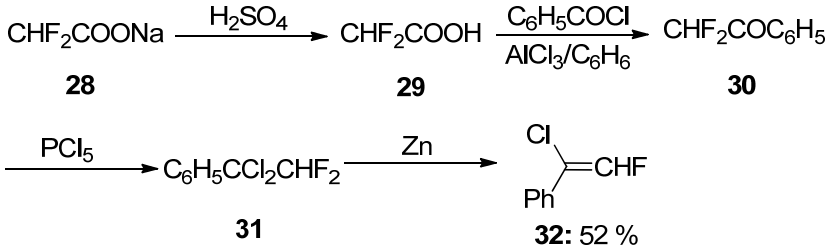
Scheme 9. Preparation of α-fluorostyrenes **27**

Meyer *et* al. reported[14] the synthesis of α-fluorostyrenes (**27**) in overall 68 % yield by bromofluorination of styrene (**12**) and subsequent dehydrobromination of thus-formed vicinal bromofluoride **28** as shown in Scheme 10.



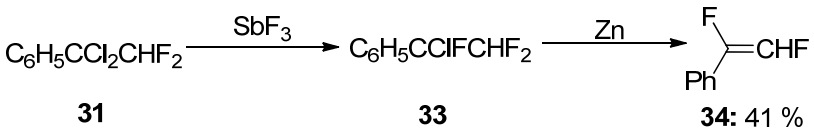
Scheme 10. Preparation of α-fluorostyrenes **27**

Synthesis of another monomer unit α-chloro-β-fluorostyrene (**32**) used for the polymerization was reported[15] by M. Prober *et al.* This multi-step synthetic route resulted in α-chloro-β-fluorostyrene formation in overall 52 % yield as shown in Scheme 11.



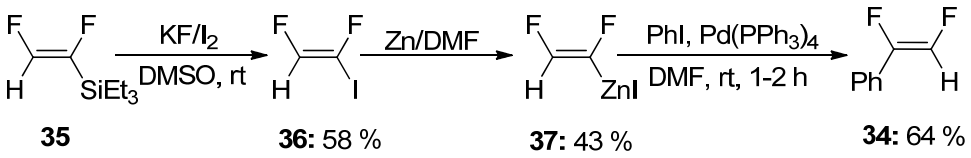
Scheme 11. Synthesis of α-chloro-β-fluorostyrene (**32**)

Furthermore, synthesis of α,β-difluorostyrene (**34**) was reported[15] by M. Prober *et al.* using intermediate **31** by treating it with SbF_3 followed by Zn or NaOH as shown in Scheme 12.



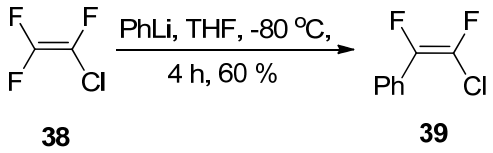
Scheme 12. Synthesis of α,β-difluorostyrene (**34**)

For the synthesis of α,β-difluorostyrene (**34**), Burton and co-workers reported[16] some more methods with varying reagents and conditions and one of them is shown in Scheme 13. For the synthesis of α,β,β-trifluorostyrene same synthetic strategy was utilized using appropriate starting materials.[16g]



Scheme 13. Synthesis of α,β-difluorostyrene (**34**)

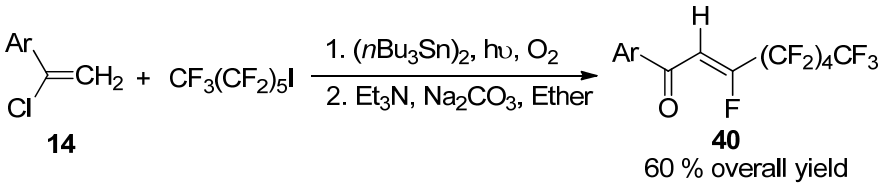
S. Dixon reported[17] the synthesis of β-chloro-α,β-difluorostyrene (**39**) wherein it was observed that chlorotrifluoroethylene (**38**) reacted with phenyl lithium to eliminate lithium fluoride and gave β-chloro-α,β-difluorostyrene (**39**) in 60 % yield instead of the stilbene derivative as shown in Scheme 14.



Scheme 14. Synthesis of β-chloro-α,β-difluorostyrene (**39**)

Synthesis and uses of **27**, **32**, **34**, **39** and other α-halostyrenes in polymerization were reported[18] by R. Souzy *et al.* Furthermore, α-chlorostyrenes were known to be used as

intermediates in the preparation of α-halomethyl ketones,[19] 1-diarylphosphino-2-arylethylenes,[20] 2-arylallyltitanocenes, [21] α-thiocyanatostilbenes, [22] arylacetylenes, [23] *etc*.



Scheme 15. Synthesis of fluoroalkylated α,β-unsaturated ketones **40**

α-Chlorostyrenes **14** were used for the synthesis of fluoroalkylated α,β-unsaturated ketones **40** by oxygenative perfluoroalkylation method in presence of perfluoroalkyl radicals reported[24] by M. Yoshida *et al.* as shown in Scheme 15. These fluoroalkylated α,β-unsaturated ketones **40** are the important building blocks for various fluoroalkylated heterocyclic compounds[25].

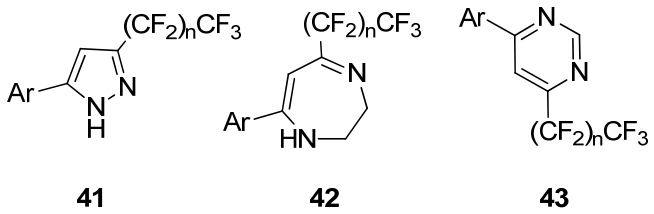


Figure 1. Fluoroalkylated heterocyclic compounds **41**, **42** and **43**

Furthermore, synthesis of **41**-**43** (Figure 1) was achieved in one pot from α-chlorostyrenes *via* fluoroalkylated α,β-unsaturated ketone intermediates as reported[26] by Yoshida and co-workers.

Thus it is seen that α-halostyrenes are industrially important compounds and newer methods for their synthesis are desirable to make them available by simple reactions/short reaction sequences.

3.2.4 Present work

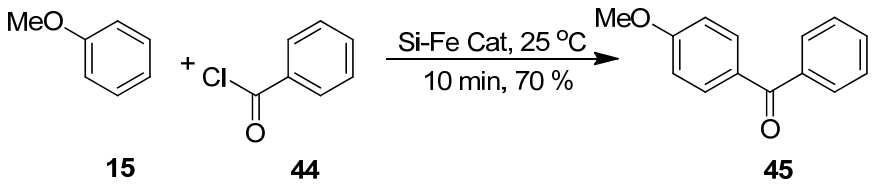
Preparation of Si-Fe catalyst

Heterogeneous Si-Fe catalyst was synthesized based on the procedure used for the synthesis of hydrotalcites[27]. Hydrotalcites are nothing but layered double hydroxides (LDH) and comprise an unusual class of layered materials with positively charged layers and charge balancing anions located in the interlayer region.

The Si–Fe catalyst was prepared from sodium trisilicate as a source of silica and ferric nitrate as source of iron in the presence of ammonia and ammonium carbonate at room temperature. The solid mass formed in the aqueous solution was stirred for 12 h, filtered, dried at 120 ^{o}C for 12 h, calcined at 500 ^{o}C for 3 h, powdered and used for reactions. The detailed procedure of preparation is described in experimental section. The acidity of this iron silicate type catalyst was determined by the ammonia method[28], and it was observed to be 0.4 mmol/g. Surface area was measured using BET method[29] and was found to be 271 m^2/g while the atomic weight ratio was calculated using EDAX (Energy Dispersive X-ray analysis) and was observed to be as follows: C = 4.14 %, O = 64.6 %, Si = 18.11% and Fe = 13.15 %. During synthesis of Si-Fe catalyst, we assumed that the product of the reaction will result into the formation of hydrotalcites, but we observed that nature of the catalyst synthesized was amorphous and not crystalline. This indicated that the catalyst synthesized falls in the category of a hydrotalcite-type anionic clays.[27b]

Preparation of α-chlorostyrenes

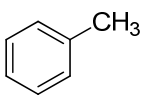
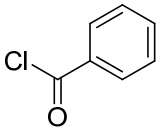
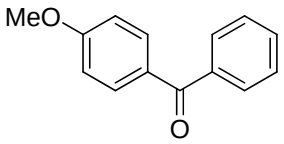
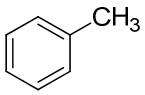
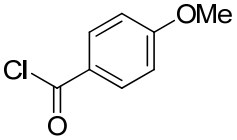
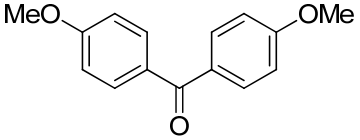
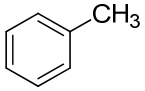
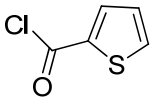
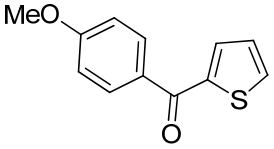
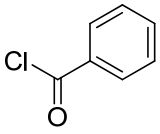
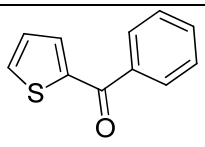
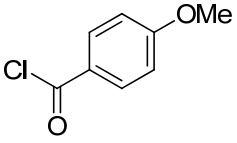
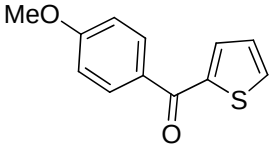
Initially anisole (**15**) was reacted with benzoyl chloride (**44**) in presence of activated Si-Fe catalyst. This afforded the corresponding Friedel-Crafts acylation product (4-methoxyphenyl)(phenyl)methanone (**45**) by stirring the neat reaction mixture at rt in 70 % isolated yield as shown in Scheme 16.



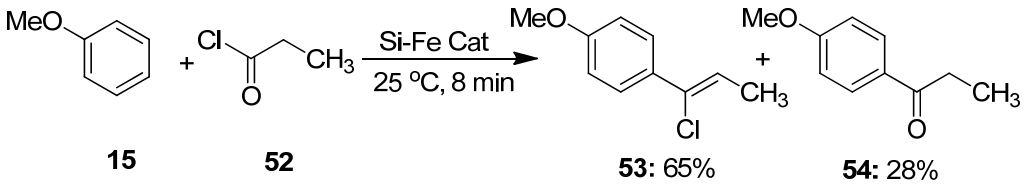
Scheme 16. Friedel–Crafts acylation reaction

Then to generalize the reaction, anisole was treated with 4-methoxybenzoyl chloride (**46**) and thiophene-2-carbonyl chloride (**47**) to obtain bis(4-methoxyphenyl)methanone (**48**) and (4-methoxyphenyl)(thiophen-2-yl)methanone (**49**) respectively. Furthermore, when thiophene (**50**) was treated with benzoyl chloride (**44**) and with 4-methoxybenzoyl chloride (**46**), the formation of phenyl(thiophen-2-yl)methanone (**51**) and (4-methoxyphenyl)(thiophen-2-yl)methanone (**49**) respectively was observed in excellent yields as shown in Table 1.

Table 1. Friedel–Crafts acylation with aromatic acid chlorides

Entry	Substrate	Acid chloride	Product	%Yield
1	 **15**	 **44**	 **45**	70
2	 **15**	 **46**	 **48**	76
3	 **15**	 **47**	 **49**	75
4	 **50**	 **44**	 **51**	79
5	 **50**	 **46**	 **49**	77

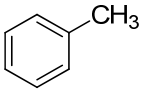
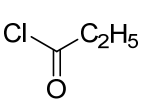
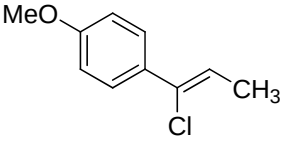
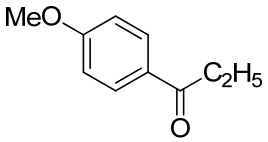
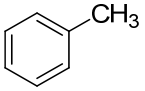
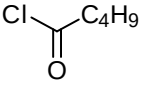
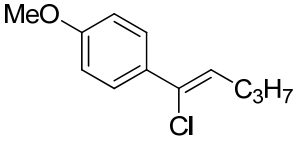
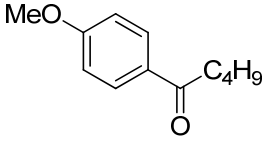
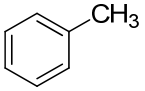
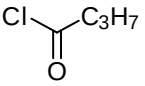
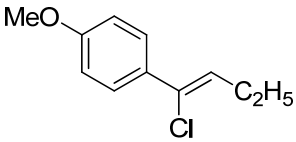
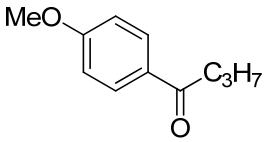
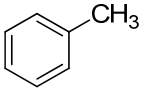
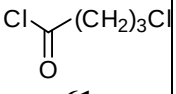
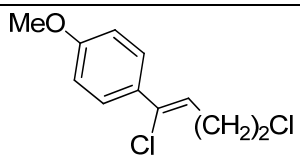
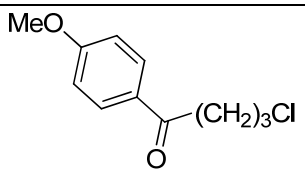
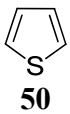
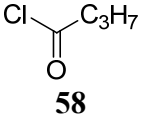
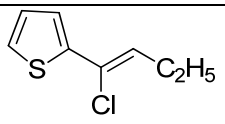
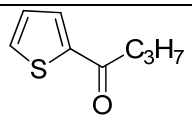
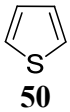
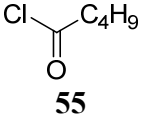
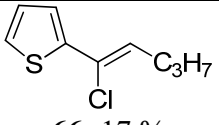
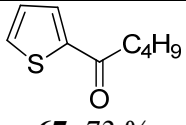
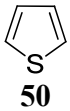
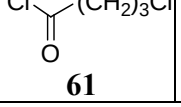
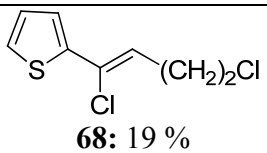
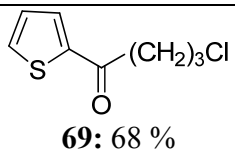
When anisole (**15**) was reacted with propionyl chloride (**52**) as shown in Scheme 17, a mixture of two products was obtained which was easily separated by column chromatography. The slower moving product obtained in 28 % yield was found to be the Friedel–Crafts acylation product 1-(4-methoxyphenyl)propan-1-one (**54**) while the faster moving product, isolated in 65 % yield, turned out to be the corresponding α-chlorostyrene 1-(1-chloroprop-1-en-1-yl)-4-methoxybenzene (**53**).



Scheme 17. Preparation of α-chlorostyrene **53**

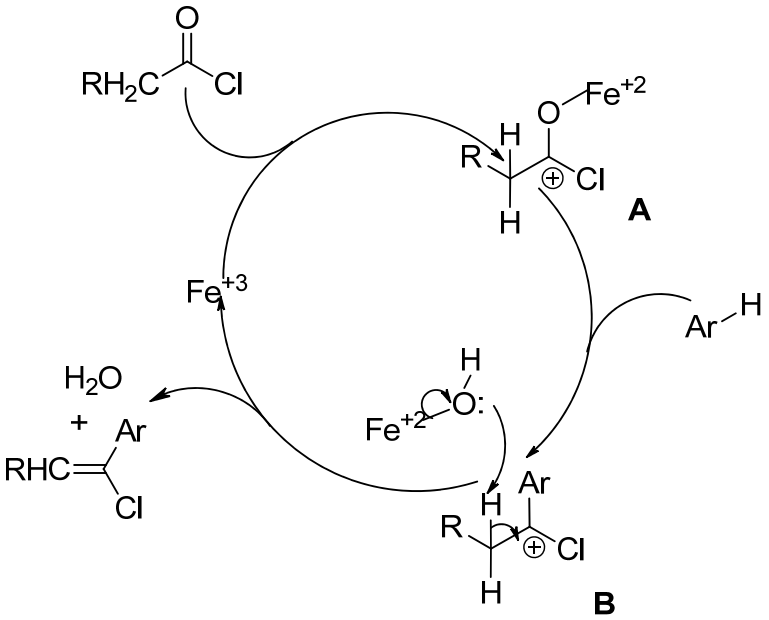
Accordingly, reactions of various aliphatic acid chlorides such as propionyl chloride (**52**), pentanoyl chloride (**55**), butyryl chloride (**58**) and 4-chlorobutanoyl chloride (**61**) with aromatic substrates such as anisole (**15**) and thiophene (**50**) were carried out to afford the corresponding α-chlorostyrenes in good yields along with Friedel–Crafts acylation product as shown in Table 2.

Table 2. α-Chlorostyrenes and Friedel–Crafts acylated products obtained using Si-Fe catalyst

Sr No	Aromatics	Acid chlorides	Products (% yields) α-chlorostyrenes	Products (% yields) ketones
1	 **15**	 **52**	 **53:** 65 %	 **54:** 28 %
2	 **15**	 **55**	 **56:** 67 %	 **57:** 25 %
3	 **15**	 **58**	 **59:** 43 %	 **60:** 49 %
4	 **15**	 **61**	 **62:** 41 %	 **63:** 48 %
5	 **50**	 **58**	 **64:** 26 %	 **65:** 66 %
6	 **50**	 **55**	 **66:** 17 %	 **67:** 73 %
7	 **50**	 **61**	 **68:** 19 %	 **69:** 68 %

The α-chlorostyrenes were easily separated from the corresponding Friedel–Crafts acylation products by column chromatography. These compounds had variable stability ranging from 1–2 days to 1–2 weeks after which they either decomposed or got converted into the corresponding Friedel–Crafts acylation products. Clark *et al.* have proposed a

mechanism for the formation of α-chlorostyrenes from aromatic substrates[30] involving a chlorovinyl cation or chlorohydroxy cation. We envisage that the Fe^{3+} present in Si–Fe catalyst helps the formation of cation **A** from the acid chloride, which on reaction with the substrate leads to the formation of cation **B** and a Fe^{2+} species. Cation **B** is transformed into the α-chlorostyrene and Fe^{3+} is regenerated as shown in Scheme 18.



Scheme 18. Proposed mechanism for the formation of α-chlorostyrenes

3.2.5 Conclusion

α-Chlorostyrenes were prepared by a one-step method from aromatics and acid chlorides using a heterogeneous, Si–Fe catalyst at room temperature. The catalyst used in the present work was prepared from easily available starting materials by a simple procedure. The reactions are over in a short time and the catalyst can be recycled. The desired α-chlorostyrenes were easily separated from the corresponding Friedel–Crafts acylation products by column chromatography.

3.2.6 Experimental section

Preparation of Si-Fe catalyst

In a round bottom flask a solution of sodium trisilicate (24.21 g, 0.3 mol) in water (250 ml) was taken and a solution of ferric nitrate (40.4 g, 0.1 mol) in water (75 ml) and a solution of ammonium carbonate containing ammonia [ammonium carbonate (2.34 g, 0.05 mol), 25 % ammonia solution (34 ml, 0.5 mol) diluted to 75 ml] were added simultaneously at room temperature with stirring over a period of 30 min. The solid mass formed in the aqueous solution was stirred for 12 h, filtered, dried at 120 ^{o}C for 12 h, calcined at 500 ^{o}C for 3 h and powdered.

Atomic weight ratio : C = 4.14 %, O = 64.6 %, Si = 18.11 % and Fe = 13.15 %
[by Energy Dispersive X-ray analysis (EDAX)]

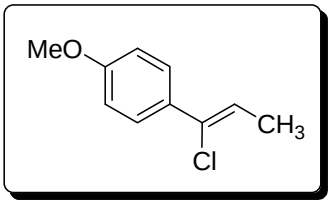
Surface area **:** 271 m^2/g (by BET method)[29]

Acidity **:** 0.4 mmol/g (by the ammonia method)[28]

Preparation of 1-chloro-1-(4-methoxyphenyl)-1- propene (53) and 1-(4-methoxyphenyl)-propan-1-one (54)

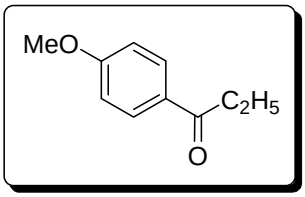
To a two-neck flask equipped with a guard tube ($CaCl_2$), activated (150 ^{o}C, 2 h) catalyst (10 % by weight, 20 mg) was added. To this was added anisole (0.2 ml, 1.85 mmol) followed by the addition of propionyl chloride (0.16 ml, 1.85 mmol). The reaction mixture was allowed to stir at 25 ^{o}C for 10 min. The reaction mixture was then filtered to remove the catalyst and washed with ethyl acetate (5 ml). The filtrate and washings were combined, washed with water, dried over sodium sulfate and concentrated to give a crude product. Purification by column chromatography over silica gel afforded 1-chloro-1-(4-methoxyphenyl)-1- propene (**53**) (220 mg, 65 %) as the faster moving spot and further elution afforded 1-(4-methoxyphenyl)-propan-1-one (**54**) (85 mg, 28 %).

1-(1-Chloro-propenyl)-4-methoxy-benzene (53)[5]



Nature: Red oil; **Yield:** 65 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.94 (d, *J* = 8 Hz, 3H), 3.83 (s, 3H), 6.09 (q, *J* = 8 Hz, 1H), 6.88 (d, *J* = 10 Hz, 2H), 7.50 (d, *J* = 10 Hz, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$ + CCl_4)**:** δ 15.1, 55.2, 113.5 (2C), 120.3, 127.6 (2C), 131.2, 133.6, 160.6; **IR** (Chloroform)**:** 1509, 1178, 1036 cm^{-1}; **GCMS:** *m/z* 184, 182 (M+), 147, 131, 115, 103, 91, 71, 63, 51, 41, 40; **Anal. Calcd for** $C_{10}H_{11}ClO$**:** C, 65.76; H, 6.07; Cl, 19.41 %. **Found:** C, 65.43; H, 6.23; Cl, 19.32 %.

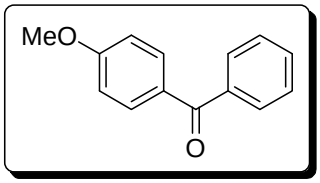
1-(4-Methoxy-phenyl)-propan-1-one (54)[31]



Nature: Yellow liquid; **Yield:** 28 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.21 (t, *J* = 8 Hz, 3H), 2.96 (q, *J* = 8 Hz, 2H), 3.87 (s, 3H), 6.93 (d, *J* = 10 Hz, 2H), 7.95 (d, *J* = 10 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 8.3, 31.3, 55.4, 113.6 (2C), 129.9, 130.1 (2C), 163.2, 199.4; **IR** (Chloroform)**:** 1677, 1074, 844 cm^{-1}; **GCMS:** *m/z* 164 (M+), 135, 107, 92, 77, 64, 50, 40; **Anal. Calcd for** $C_{10}H_{12}O_2$**:** C, 75.00; H, 7.31 %. **Found:** C, 75.25; H, 6.95 %.

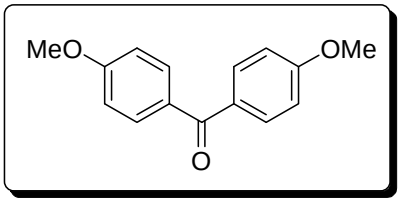
Preparation of rest of all compounds was done using procedure given for compounds **53** and **54.**

(4-Methoxyphenyl)-phenyl-methanone (45)[32,37]



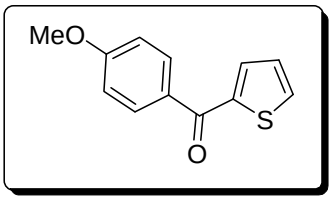
Nature: White solid; **MP:** 59-61 ^{o}C; **Yield:** 70 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.89 (s, 3H), 6.97 (d, *J* = 8 Hz, 2H), 7.43-7.62 (m, 3H), 7.77 (d, *J* = 8 Hz, 2H), 7.84 (d, *J* = 8 Hz, 2H).

Bis-(4-methoxyphenyl)-methanone (48)[33,37]



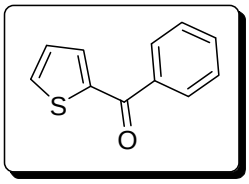
Nature: Colorless oil; **Yield:** 76 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 3.90 (s, 6H), 6.97 (d, *J* = 8 Hz, 4H), 7.80 (d, J = 8 Hz, 4H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 55.4 (2C), 112.4 (4C), 130.8 (4C), 128.6 (2C), 163.7 (2C), 186.2.

(4-Methoxyphenyl)(thiophen-2-yl)methanone (49)[34,37]



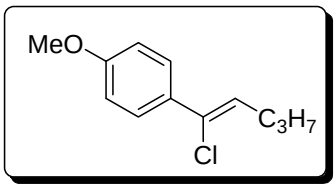
Nature: Pale yellow solid; **MP:** 74 ^{o}C; **Yield:** 75 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 3.90 (s, 3H), 6.99 (d, *J* = 8 Hz, 2H), 7.14-7.18 (m, 1H), 7.64-7.70 (m, 2H), 7.91 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 55.4, 113.6 (2C), 127.7, 130.6, 131.5 (2C), 133.3, 133.9, 143.7, 163.0, 186.8.

Phenyl-thiophen-2-yl-methanone (51)[35, 37]



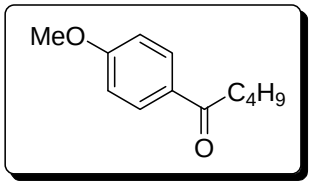
Nature: Grey solid; **MP:** 57 ^{o}C; **Yield:** 79 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 7.13-7.17 (m, 1H), 7.48-7.65 (m, 4H), 7.69-7.72 (m, 1H), 7.83-7.88 (m, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 127.8, 128.3 (2C), 129.1 (2C), 132.1, 134.0, 134.6, 138.1, 143.6, 187.7.

1-(1-Chloro-pent-1-enyl)-4-methoxy-benzene (56)



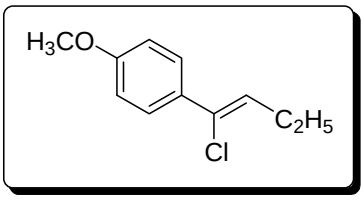
Nature: Yellow semisolid; **Yield:** 67 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.00 (t, *J* = 8 Hz, 3H), 1.44-1.63 (m, 2H), 2.36 (q, *J* = 8 Hz, 2H), 3.83 (s, 3H), 6.04 (t, *J* = 8 Hz, 1H), 6.88 (d, *J* = 8 Hz, 2H), 7.51 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.9, 22.0, 31.6, 55.3, 113.5 (2C), 126.2, 127.6 (2C), 131.1, 132.4, 159.6; **IR** (Chloroform)**:** 1509, 1178, 1034 cm^{-1}; **GCMS:** *m/z* 212, 210 (M+), 209, 183, 181, 145, 135, 115, 103, 83, 77, 63, 51, 41; **Anal. Calcd for** $C_{12}H_{15}ClO$**:** C, 68.40; H, 7.18; Cl, 16.83 %. **Found:** C, 68.79; H, 6.84; Cl, 16.47 %.

1-(4-Methoxyphenyl)-pentan-1-one (57)[36]



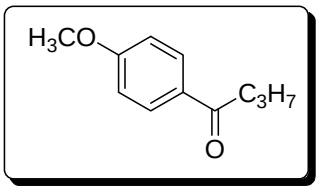
Nature: Colorless thick oil; **Yield:** 25 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 0.95 (t, *J* = 6 Hz, 3H), 1.35-1.46 (m, 2H), 1.63-1.78 (m, 2H), 2.91 (t, *J* = 6 Hz, 2H), 3.86 (s, 3H), 6.91 (d, *J* = 8 Hz, 2H), 7.93 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 14.0, 22.5, 26.7, 37.9, 55.3, 113.6 (2C), 130.2 (2C), 163.2 (2C), 198.8; **Anal. Calcd for** $C_{12}H_{16}O_2$**:** C, 74.97; H, 8.39 %. **Found:** C, 75.24; H, 8.24 %.

1-(1-Chlorobut-1-enyl)-4-methoxy-benzene (59)



Nature: Brown semisolid; **Yield:** 43 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 1.02 (t, *J* = 8 Hz, 3H), 2.33-2.48 (m, 2H), 3.72 (s, 3H), 5.90 (t, *J* = 8 Hz, 1H), 6.74 (d, *J* = 8 Hz, 2H), 7.38 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 13.3, 23.0, 55.1, 113.5 (2C), 127.4, 127.6 (2C), 131.0, 132.1, 159.6; **IR** (Chloroform)**:** 1504, 1176, 1027 cm^{-1}; **GCMS:** *m/z* 198, 196 (M+), 181, 161, 145, 131, 115, 103, 91, 77, 63, 51, 40; **Anal. Calcd for** $C_{11}H_{13}ClO$**:** C, 67.18; H, 6.66; Cl, 18.03 %. **Found:** C, 67.41; H, 6.38; Cl, 18.19 %.

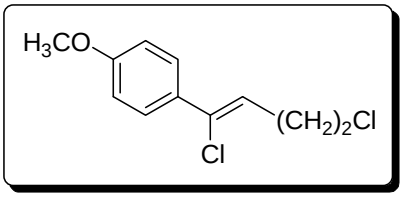
1-(4-Methoxyphenyl)-butan-1-one (60)[31,36]



Nature: Colorless oil; **Yield:** 49 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.00 (t, *J* = 8 Hz, 3H), 1.71-1.82 (m, 2H), 2.90 (t, *J* = 8 Hz, 2H), 3.87 (s, 3H), 6.92 (d, *J* = 8 Hz, 2H), 7.95 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 17.9, 40.1, 55.3, 113.5 (2C), 130.2 (2C),

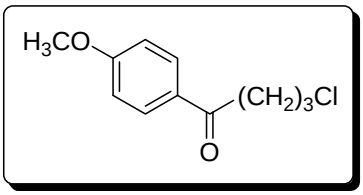
163.2 (2C), 199.0; **Anal. Calcd for** $C_{11}H_{14}O_2$**:** C, 74.13; H, 7.92 %. **Found:** C, 74.48; H, 8.30 %.

1-(1,4-Dichlorobut-1-enyl)-4-methoxy-benzene (62)



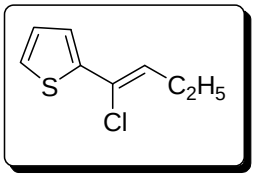
Nature: White solid; **MP:** 162-163 ^{o}C; **Yield:** 41 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 2.85 (q, *J* = 6 Hz, 2H), 3.67 (t, *J* = 6 Hz, 2H), 3.84 (s, 3H), 6.11 (t, *J* = 6 Hz, 1H), 6.89 (d, *J* = 10 Hz, 2H), 7.53 (d, *J* = 10 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 26.6, 35.7, 44.6, 114.2 (2C), 120.2, 127.4 (2C), 129.7, 134.4, 159.8; **IR** (Chloroform)**:** 1504, 1027 cm^{-1}; **Anal. Calcd for** $C_{11}H_{12}Cl_2O$**:** C, 57.16; H, 5.23; Cl, 30.68 %. **Found:** C, 56.74; H, 5.51; Cl, 30.81 %.

4-Chloro-1-(4-methoxyphenyl)-butan-1-one (63)[37]



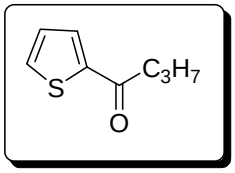
Nature: Brown semisolid; **Yield:** 48 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 2.15-2.29 (m, 2H), 3.13 (t, *J* = 6 Hz, 2H), 3.67 (t, *J* = 6 Hz, 2H), 3.88 (s, 3H), 6.94 (d, *J* = 8 Hz, 2H), 7.96 (d, *J* = 8 Hz, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 26.9, 34.8, 44.6, 55.3, 113.7 (2C), 129.9, 130.2 (2C), 163.5, 197.1; **Anal. Calcd for** $C_{11}H_{13}ClO_2$**:** C, 62.12; H, 6.16; Cl, 16.67 %. **Found:** C, 62.35; H, 6.02; Cl, 16.83 %.

2-(1-Chloro-but-1-enyl)-thiophene (64)



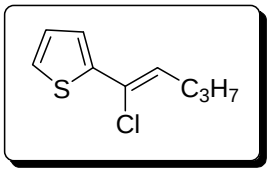
Nature: White solid; **MP:** 121-123 ^{o}C; **Yield:** 26 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 1.30 (t, *J* = 8 Hz, 3H), 2.51-2.66 (m, 2H), 6.34 (t, *J* = 8 Hz, 1H), 7.12-7.17 (m, 1H), 7.35-7.43 (m, 2H); **^{13}C NMR** (50 MHz, $CDCl_3 + CCl_4$)**:** δ 13.0, 22.6, 124.8, 125.0, 125.8, 127.0, 127.9, 142.0; **IR** (Chloroform)**:** 1519, 1141 cm^{-1}; **GCMS:** *m/z* 174, 172 (M+), 159, 157, 137, 121, 109, 97, 77, 67, 45; **Anal. Calcd for** C_8H_9ClS**:** C, 55.65; H, 5.25; Cl, 20.53 %. **Found:** C, 55.26; H, 5.12; Cl, 20.71 %.

1-Thiophen-2-yl-butan-1-one (65)[38,39]



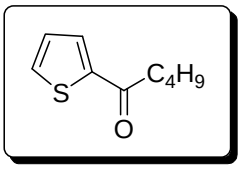
Nature: Dark brown oil; **Yield:** 66 %; **^{1}H NMR** (200 MHz, $CDCl_3 + CCl_4$)**:** δ 1.01 (t, *J* = 8 Hz, 3H), 1.70-1.88 (m, 2H), 2.88 (t, *J* = 8 Hz, 2H), 7.10-7.14 (m, 1H), 7.59 (d, *J* = 2 Hz, 1H), 7.69 (d, *J* = 2 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3 + CCl_4$)**:** δ 13.9, 18.1, 41.2, 127.9, 131.4, 133.1, 144.6, 192.9; **Anal. Calcd for** $C_8H_{10}OS$**:** C, 62.30; H, 6.54 %. **Found:** C, 62.13; H, 6.71 %.

2-(1-Chloropent-1-enyl)thiophene (66)



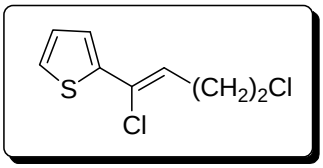
Nature: Dark brown semisolid; **Yield:** 17 %; **^{1}H NMR** (200 MHz, $CDCl_3$)**:** δ 1.05 (t, *J* = 8 Hz, 3H), 1.50-1.68 (m, 2H), 2.41 (q, *J* = 8 Hz, 2H), 6.22 (t, *J* = 8 Hz, 1H), 7.01-7.07 (m, 1H), 7.23-7.31 (m, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$)**:** δ 13.8, 21.8, 31.2, 124.8, 125.0, 126.2, 126.5, 127.1, 142.1; **IR** (Chloroform)**:** 1504, 1123, 1027 cm^{-1}; **Anal. Calcd for** $C_9H_{11}ClS$**:** C, 57.90; H, 5.94; Cl, 18.99 %. **Found:** C, 58.25; H, 6.16; Cl, 18.67 %.

1-Thiophen-2-yl-pentan-1-one (67)[39]



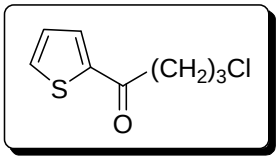
Nature: Black oil; **Yield:** 73 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 0.95 (t, *J* = 8 Hz, 3H), 1.35-1.46 (m, 2H), 1.66-1.81 (m, 2H), 2.90 (t, *J* = 8 Hz, 2H), 7.10-7.14 (m, 1H), 7.62 (d, *J* = 4 Hz, 1H), 7.71 (d, *J* = 4 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 13.9, 22.5, 26.8, 39.1, 127.9, 131.4, 133.1, 144.6, 193.0; **IR** (Chloroform)**:** 1664 cm^{-1}; **Anal. Calcd for** $C_9H_{12}OS$**:** C, 64.25; H, 7.19 %. **Found:** C, 64.61; H, 7.03 %.

2-(1,4-Dichlorobut-1-enyl)thiophene (68)



Nature: Dark brown semisolid; **Yield:** 19 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 2.89 (q, *J* = 8 Hz, 2H), 3.69 (t, *J* = 8 Hz, 2H), 6.25 (t, *J* = 8 Hz, 1H), 7.00-7.06 (m, 1H), 7.27-7.32 (m, 2H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 32.4, 42.8, 121.5, 125.6, 125.9, 127.3, 128.7, 141.3; **IR** (Chloroform)**:** 1609, 818, 757 cm^{-1}; **Anal. Calcd for** $C_8H_8Cl_2S$**:** C, 46.39; H, 3.89; Cl, 34.23 %. **Found:** C, 46.51; H, 4.11; Cl, 34.67 %.

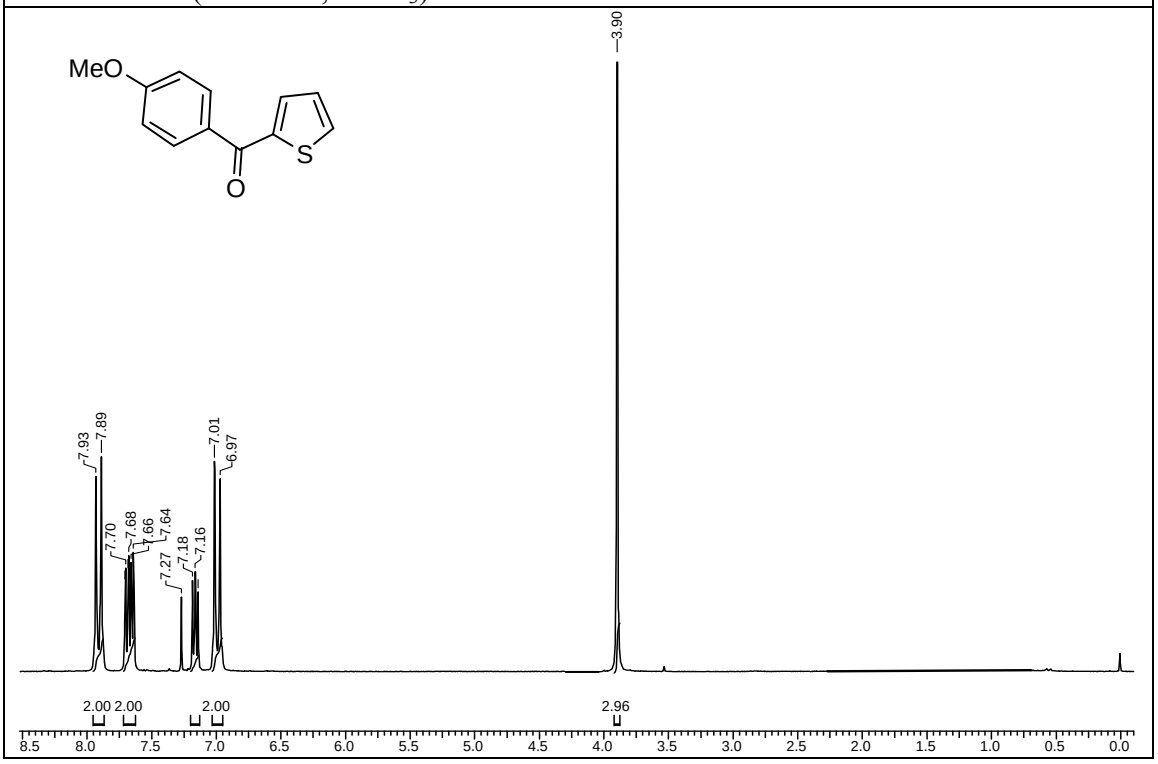
4-Chloro-1-thiophen-2-yl-butan-1-one (69)



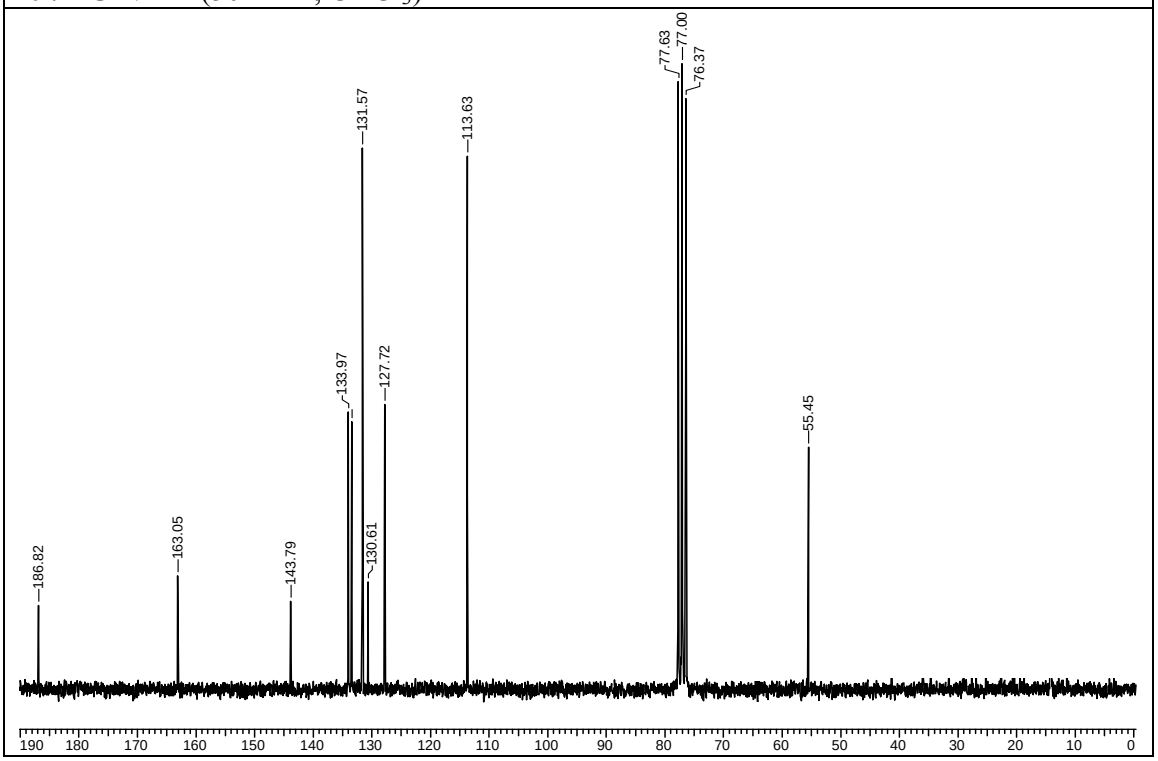
Nature: Brown semisolid; **Yield:** 68 %; **^{1}H NMR** (200 MHz, $CDCl_3$ + CCl_4)**:** δ 2.23 (t, *J* = 6 Hz, 2H), 3.10-3.17 (m, 2H), 3.67 (t, *J* = 6 Hz, 2H), 7.12-7.17 (m, 1H), 7.65 (d, *J* = 4 Hz, 1H), 7.76 (d, *J* = 4 Hz, 1H); **^{13}C NMR** (50 MHz, $CDCl_3$ + CCl_4)**:** δ 26.9, 35.8, 44.3, 128.0, 131.8, 133.5, 144.0, 191.5; **Anal. Calcd for** C_8H_9ClOS**:** C, 50.93; H, 4.81; Cl, 18.79 %. **Found:** C, 51.31; H, 4.58; Cl, 18.84 %.

3.2.7 Selected Spectra

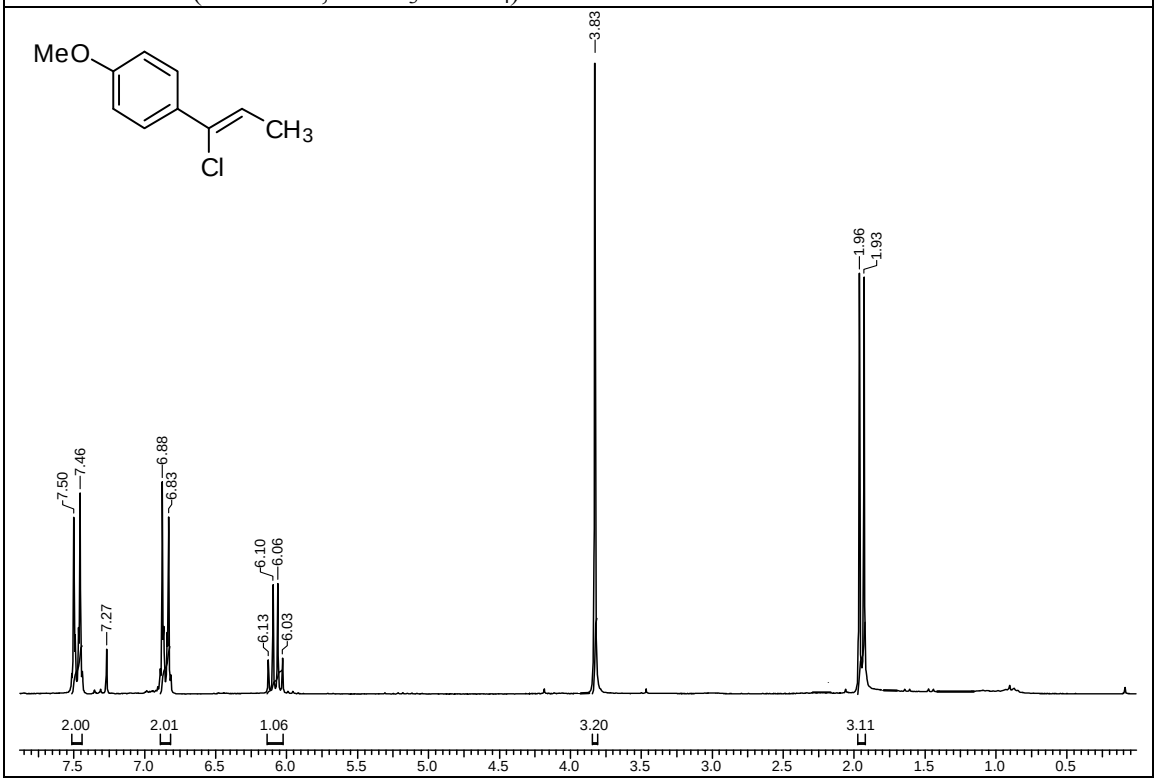
49: ^{1}H NMR (200 MHz, $CDCl_3$)



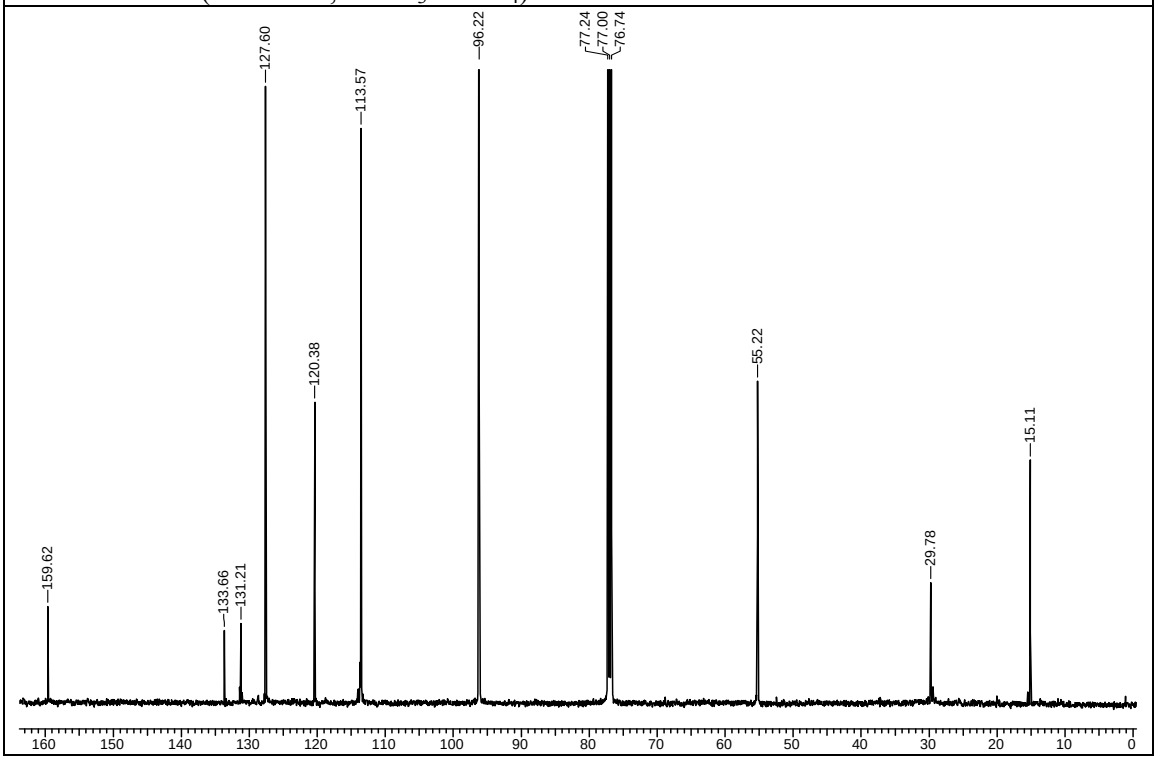
49: ^{13}C NMR (50 MHz, $CDCl_3$)



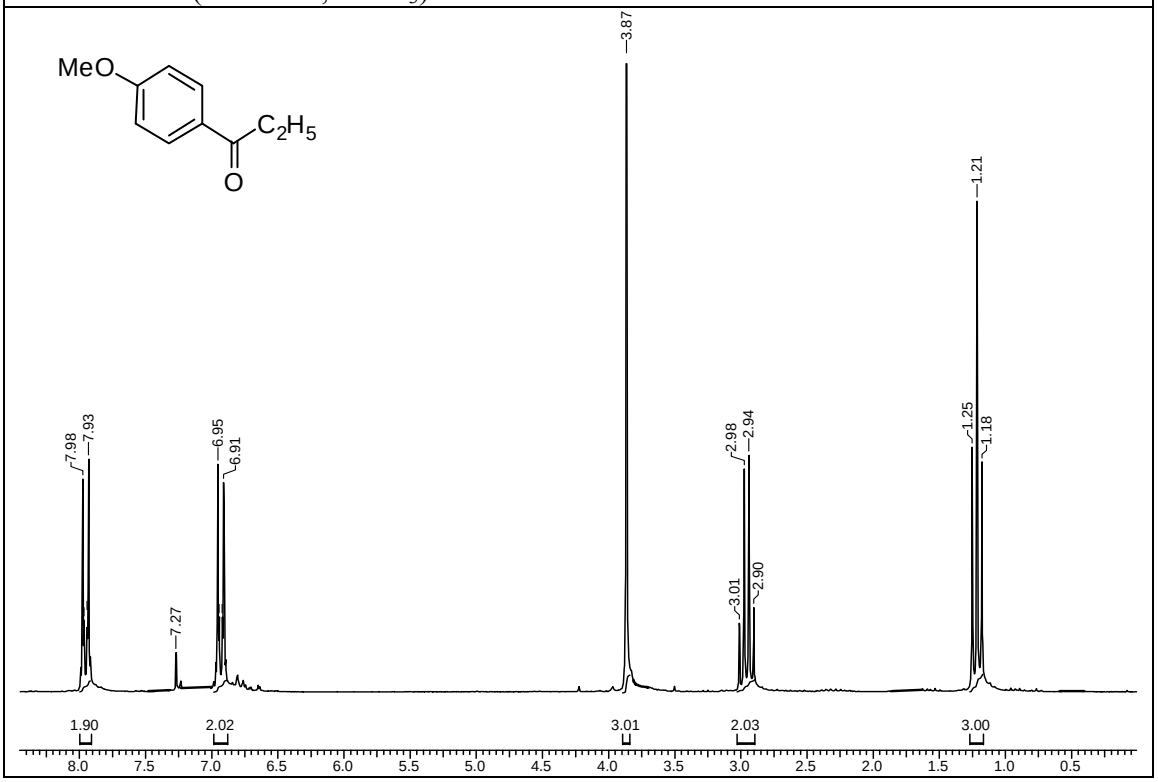
53: ^{1}H NMR (200 MHz, $CDCl_3 + CCl_4$)



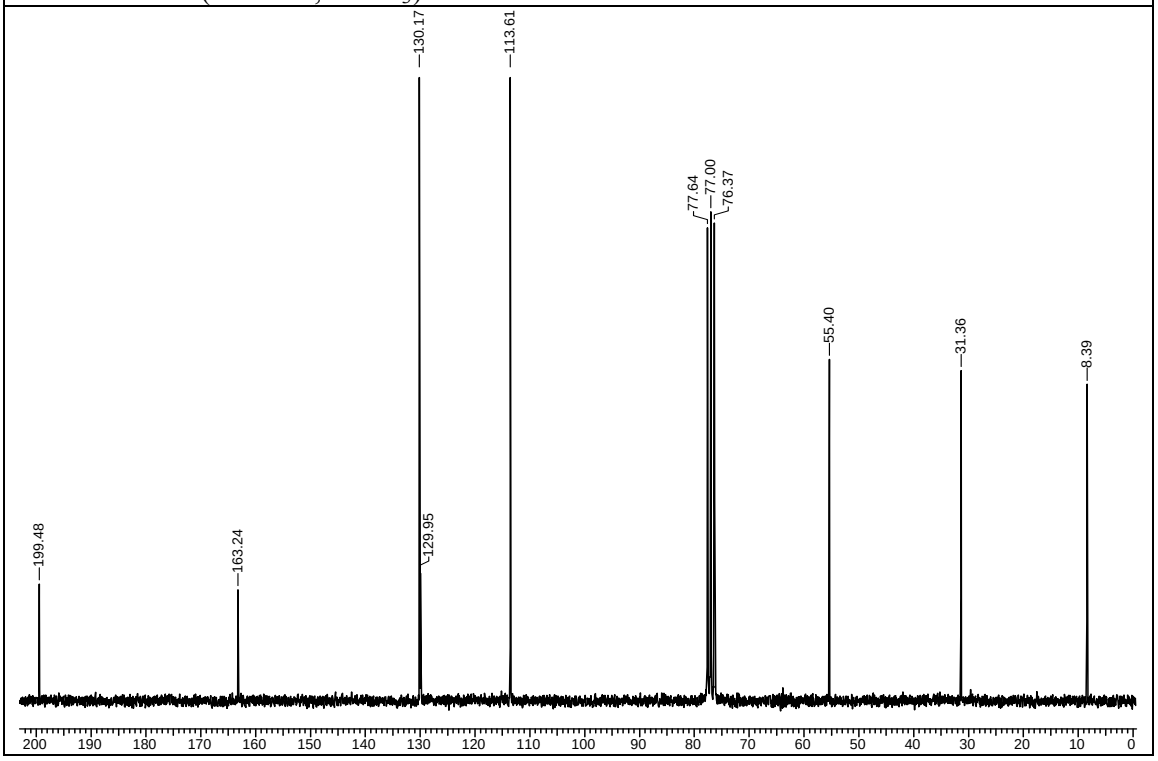
53: ^{13}C NMR (125 MHz, $CDCl_3 + CCl_4$)



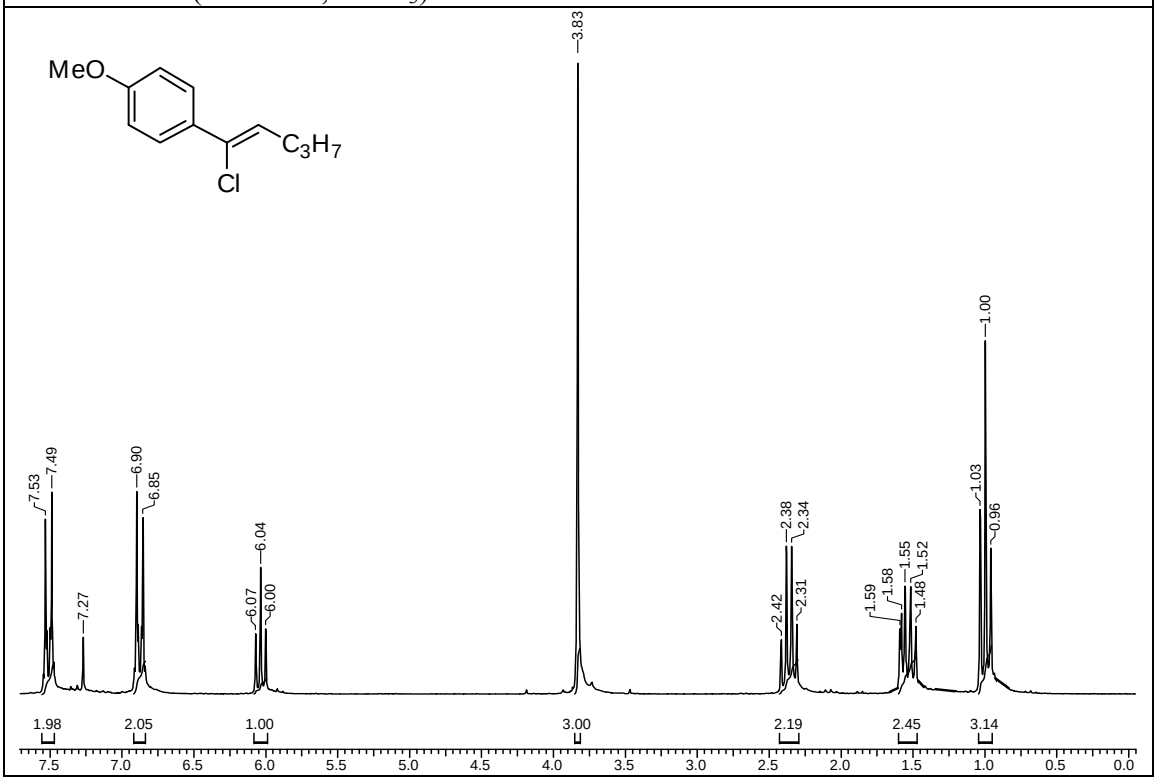
54: ^{1}H NMR (200 MHz, $CDCl_3$)



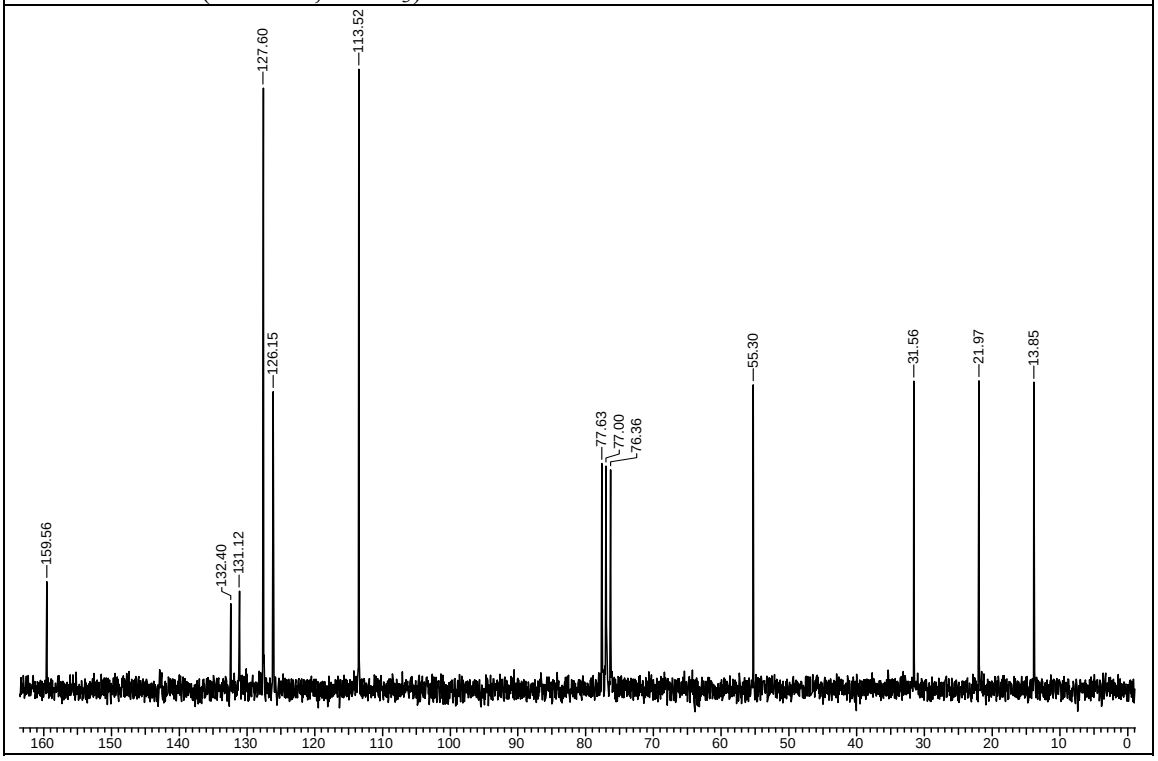
54: ^{13}C NMR (50 MHz, $CDCl_3$)



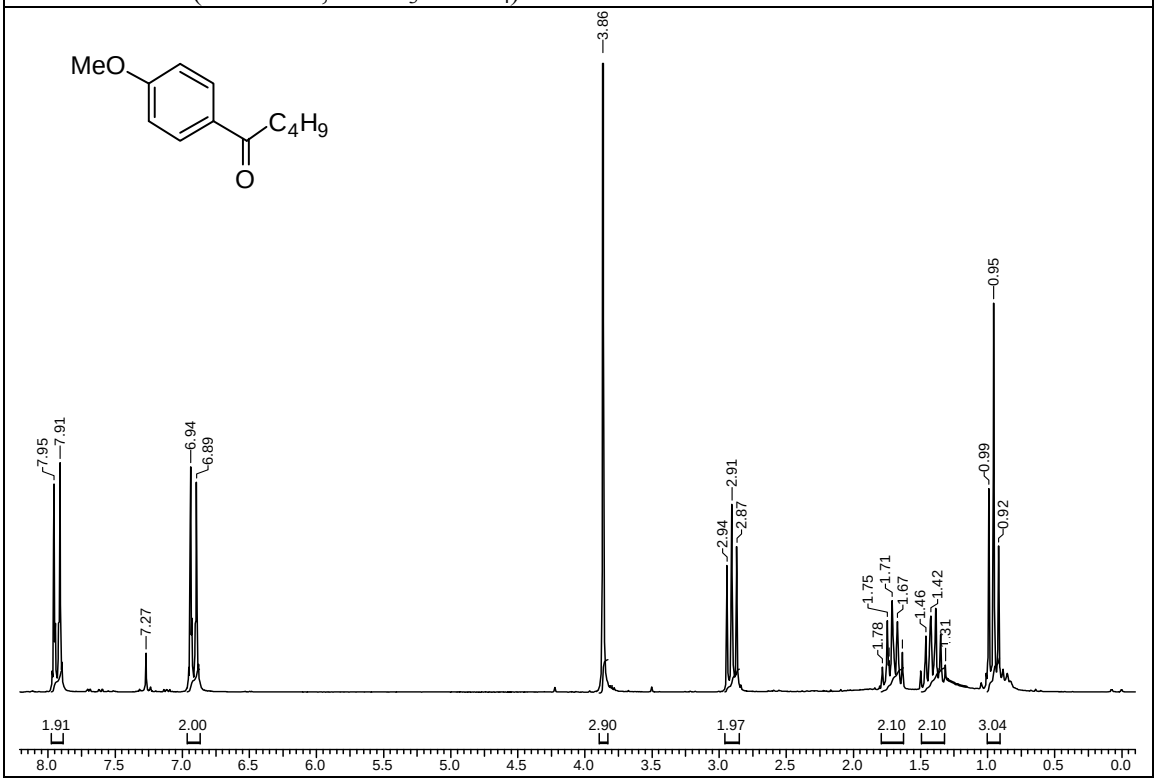
56: ^{1}H NMR (200 MHz, $CDCl_3$)



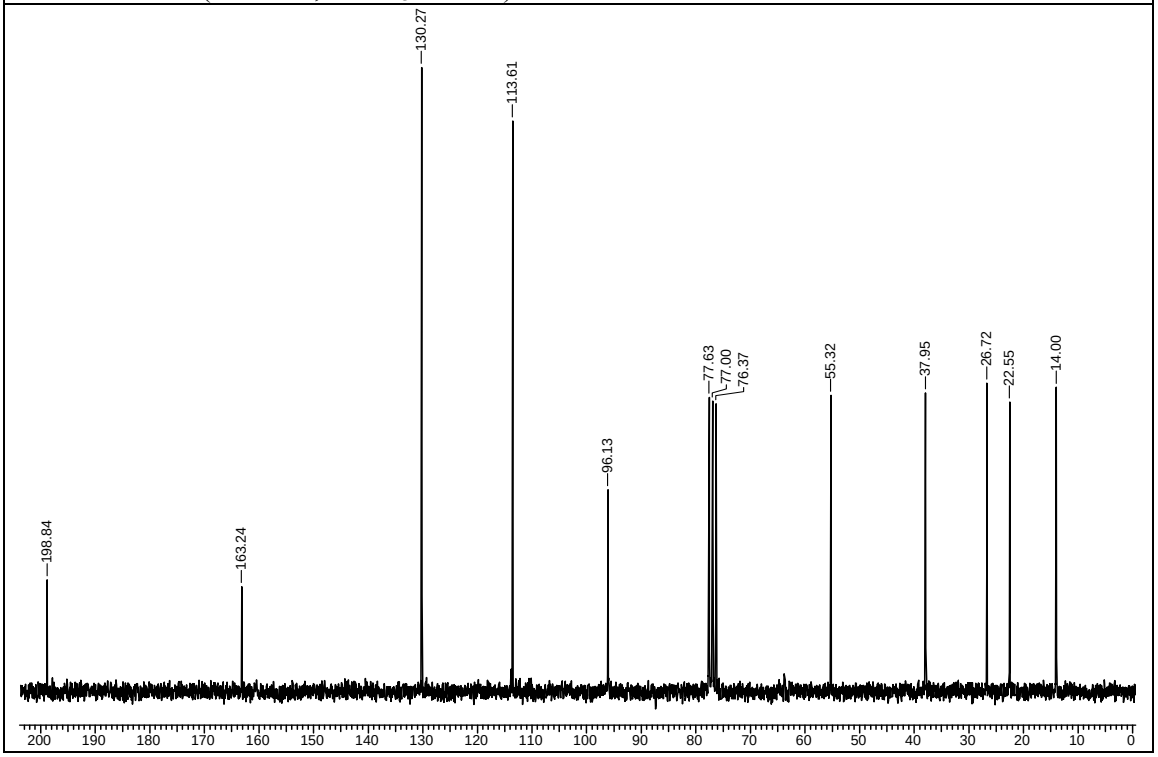
56: ^{13}C NMR (50 MHz, $CDCl_3$)



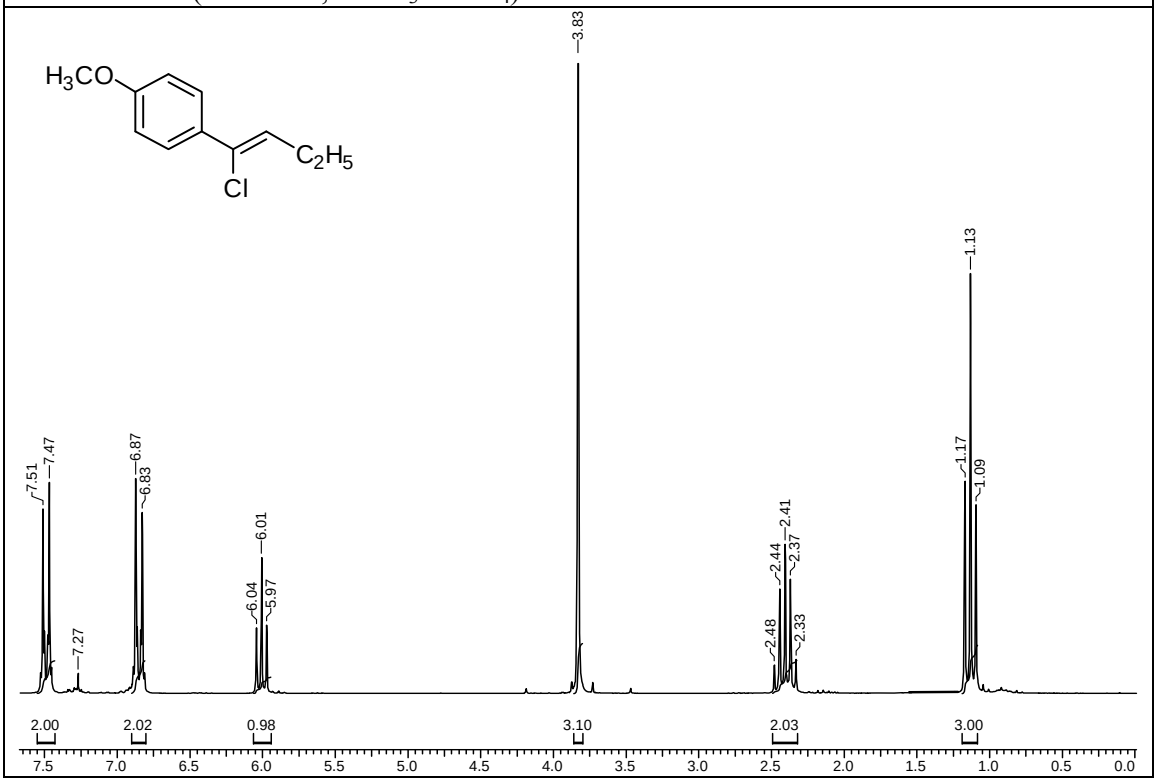
57: ^{1}H NMR (200 MHz, $CDCl_3 + CCl_4$)



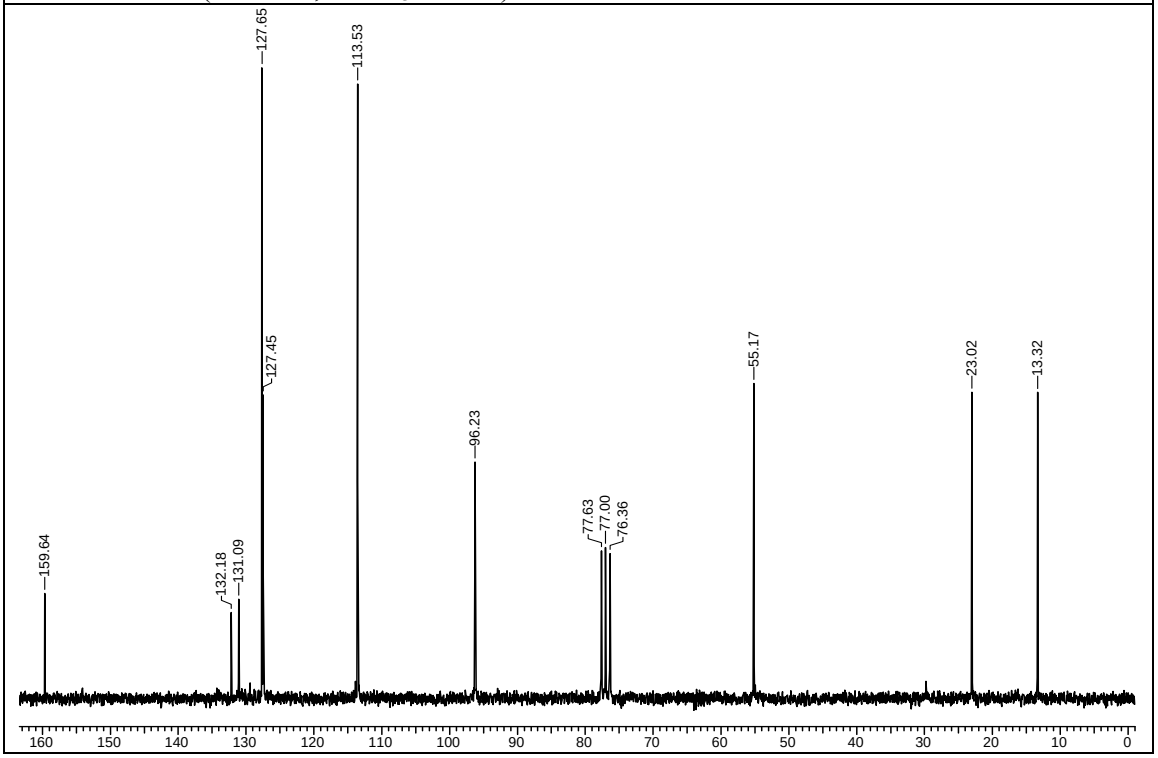
57: ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$)



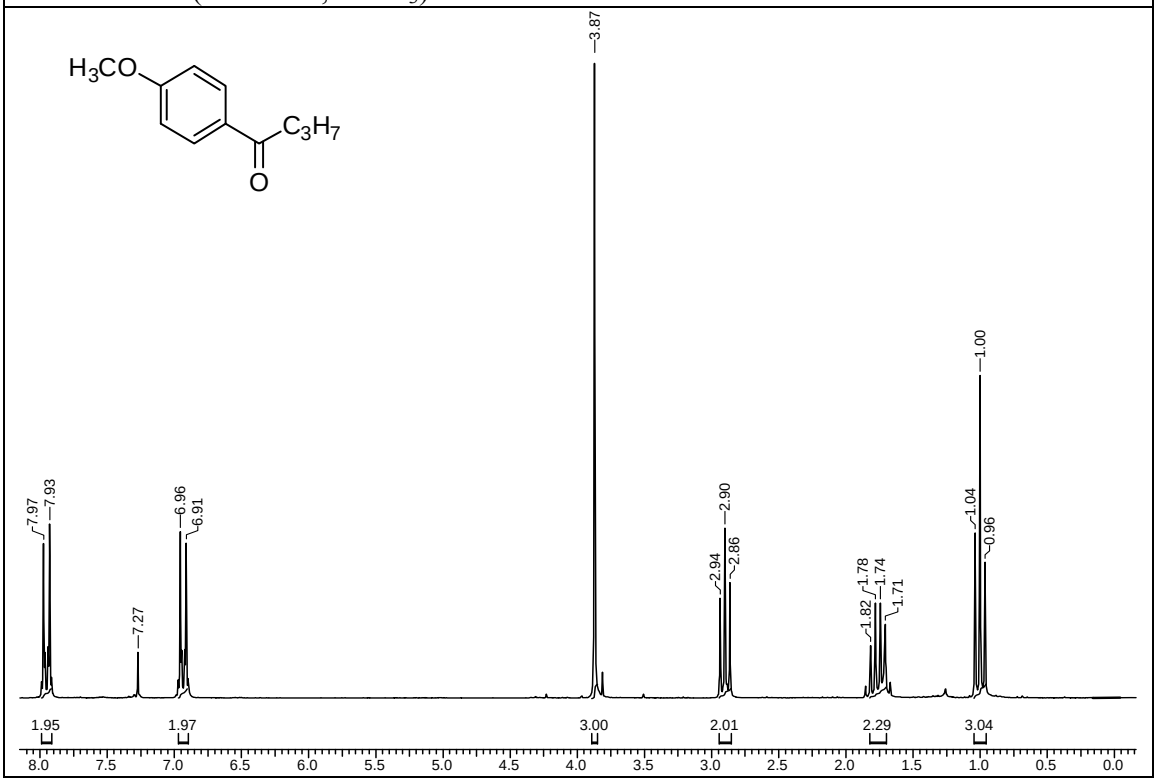
59: ^{1}H NMR (200 MHz, $CDCl_3 + CCl_4$)



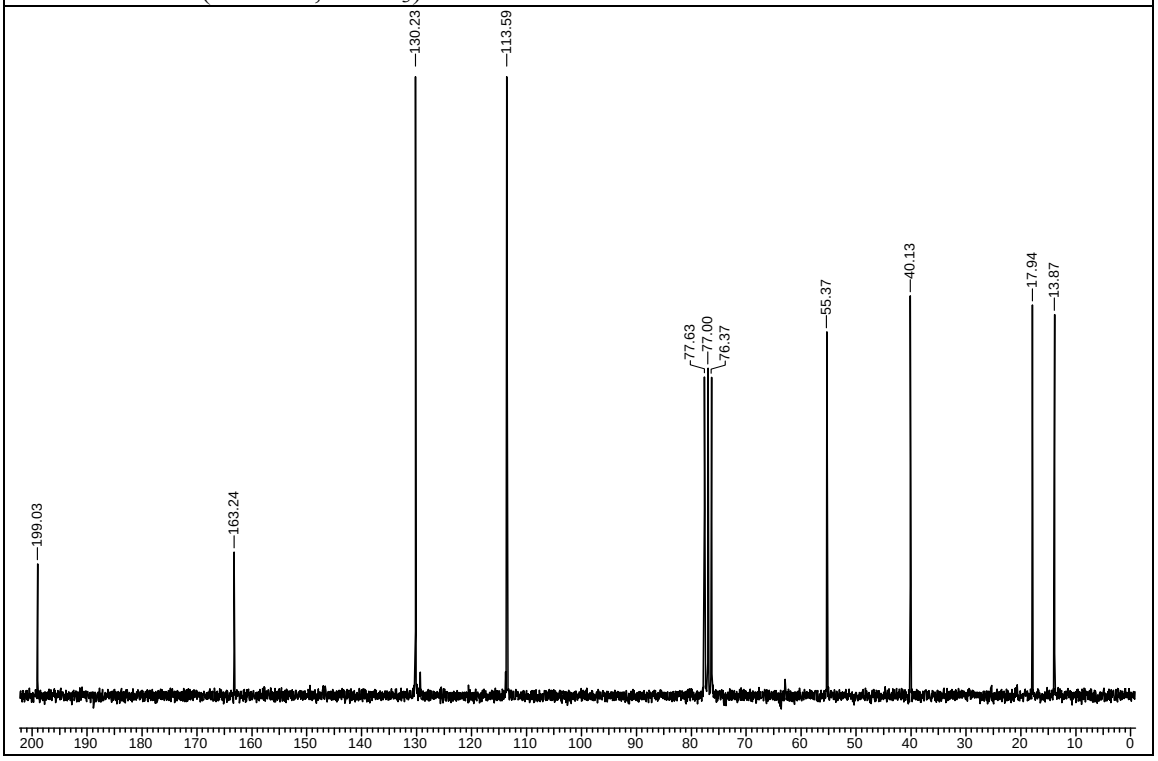
59: ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$)



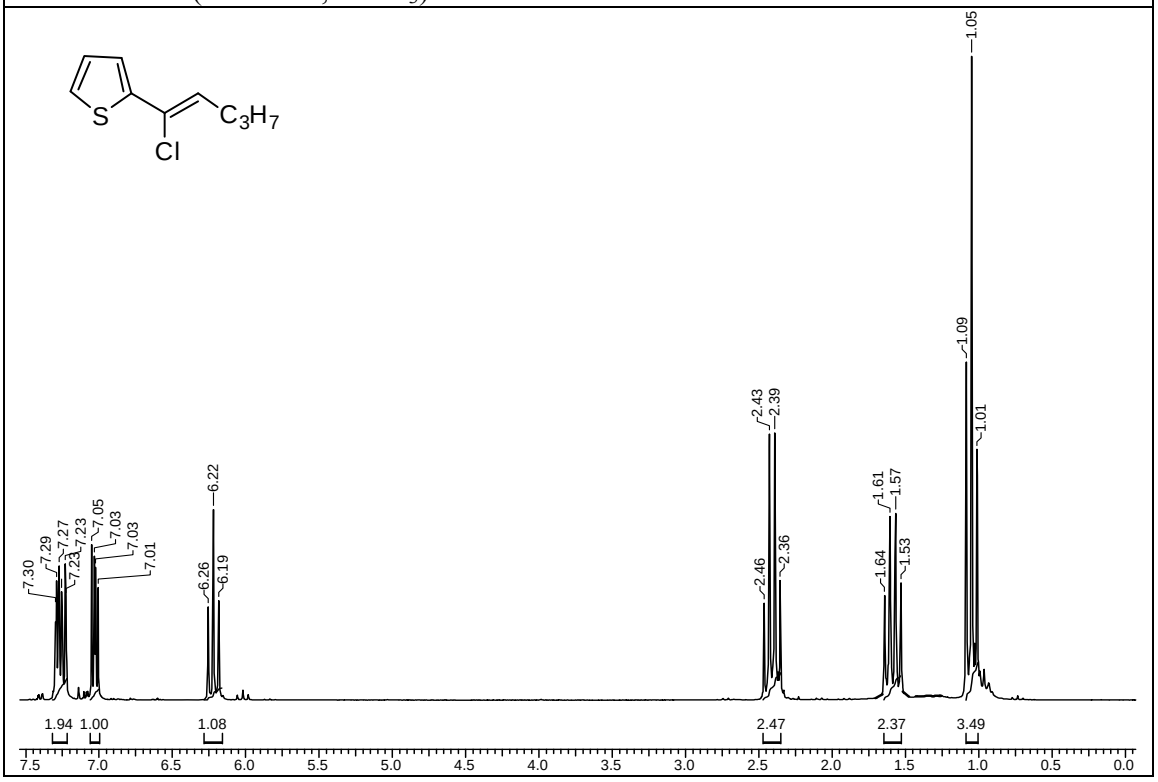
60: ^{1}H NMR (200 MHz, $CDCl_3$)



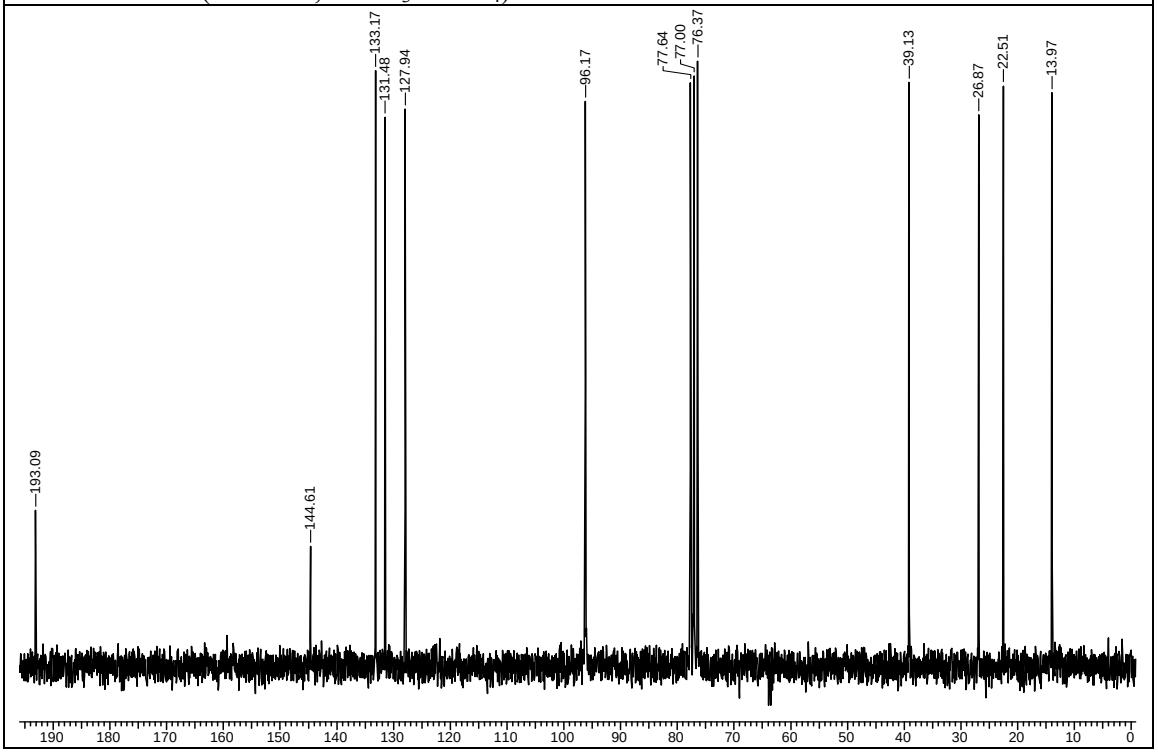
60: ^{13}C NMR (50 MHz, $CDCl_3$)



66: ^{1}H NMR (200 MHz, $CDCl_3$)



67: ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$)



3.2.8 References

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List of Publications

PAPERS

1. "Oxidation of benzylic alcohols and sulfides over LaCoO3 and TBHP" Mahesh L. Patil, **Suleman R. Maujan**, Hanumant B. Borate, Balu S. Uphade *ARKIVOC,* **2007**, *(i)*, 70-75.

2. "One-step preparation of α-chlorostyrenes" Hanumant B. Borate, Abaji G. Gaikwad, **Suleman R. Maujan**, Sangmeshwer P. Sawargave, Kamalakar M. Kalal *Tetrahedron Lett.,* **2007**, *48*, 4869-4872.

3. "A short synthesis of 3,6-disubstituted N-2-thienyl/aryl-indoles" Hanumant B. Borate, Sangmeshwer P. Sawargave, **Suleman R. Maujan** *Tetrahedron Lett.,* **2009**, *50* 6562-6566.

4. "Fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties, a novel class of anti-Candida agents" Hanumant B. Borate, **Suleman R. Maujan**, Sangmeshwer P. Sawargave, Mohan A. Chandavarkar, Sharangi R. Vaiude, Vinay A. Joshi, Radhika D. Wakharkar, Ramki Iyer, Ramesh G. Kelkar, Subhash P. Chavan, Sunita S. Kunte *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 722–725.

PATENTS

1. "Thieno-[2,3-*d*]pyrimidin-4(3*H*)-one compounds with antifungal properties and process thereof" Hanumant B. Borate, **Suleman R. Maujan**, Sangmeshwer P. Sawargave, Mohan A. Chandavarkar, Shreerang V. Joshi, Sharangi R. Vaiude Ind. Patent Appl. No. 438/MUM/2008 (3-3-2008); PCT International Application No. PCT/IN08/000571 dated 05/09/2008; WO 2009/109983 A1.

2. "Thiophene containing analogues of fluconazole as antifungal agents and process thereof" Hanumant B. Borate, Sangmeshwer P. Sawargave, **Suleman R. Maujan**, Mohan A. Chandavarkar, Sharangi R. Vaiude, Vinay A. Joshi Ind. Patent Appl. No. 1595/MUM/2007 (27-8-2007); PCT/IN2009/000543; WO 2010/046912 (published on 29-4-2010).

3. "Antifungal compounds containing benzothiazinone, benzoxazinone or benzoxazolinone and process thereof" Hanumant B. Borate, **Suleman R. Maujan**, Sangmeshwer P. Sawargave, Ramesh G. Kelkar, Radhika D. Wakharkar, Mohan A. Chandavarkar, Sharangi R. Vaiude, Vinay A. Joshi Ind. Patent Appl. No. 2306/MUM/2008 (24-10-2008); WO 2010/046931 (published on 29-4-2010).