SYNTHESIS, SPECTRAL ANALYSIS AND BIOLOGICAL EVALUATION OF SOME NOVEL FLUOROBENZOTHIAZOLE INCORPORATED 1, 3, 4 -THIADIAZOLE

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai – 600 032.

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY
(PHARMACEUTICAL CHEMISTRY)

Submitted by

R. HEMACHANDER

Register No. 26106032

Under the Guidance of

Mr. M. SENTHIL @ PALANIAPPAN, M. Pharm.,
Professor.
(Department of Pharmaceutical Chemistry)

&

Mr. M. SUGUMARAN, M. Pharm., (Ph.D.)
Associate Professor.
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ADHIPARASAKTHI COLLEGE OF PHARMACY
(Accredited By "NAAC" with CGPA of 2.74 on a Four Point Scale at "B" Grade)
MELMARUVATHUR – 603 319.

MAY – 2012

CERTIFICATE

This is to certify that the research work entitled **"SYNTHESIS, SPECTRAL ANALYSIS AND BIOLOGICAL EVALUATION OF SOME NOVEL FLUOROBENZOTHIAZOLE INCORPORATED 1,3,4 THIADIAZOLE"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Chemistry) was carried out by **R. HEMACHANDER (Register No. 26106032)** in the Department of Pharmaceutical Chemistry under our direct guidance and supervision during the academic year 2011-2012.

Mr. M. SENTHIL@PALANIAPPAN, M. Pharm.,
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Place: Melmaruvathur
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Date:

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Today what I am is all due to my most beloved and revered parents my father **Mr. E. Rajaraman**, my mother **Mrs. R. Vimalarani** who have blessed, carved, and encouraged me with moral support. I am immensely pleased in expressing my deepest sense of gratitude and regards to them, the real sources of inspiration at each and every front of my life to transform my dreams into reality.

Above all I dedicate myself and my work to **Almighty**, who is the source of knowledge and for showering all his blessings and grace upon me.

Mere words and acknowledgement are not enough to express the valuable help and encouragement rendered by all people. I finally conclude with a saying that “thanking may just be a formality, but if done inwardly, it surely reflects your noblest thoughts within”.

R. HEMACHANDER

DEDICATED

TO MY

BELOVED PARENTS

CONTENTS

SYMBOLS AND ABBREVIATIONS

$^{\circ}C$	:	Degree Centigrade
δ	:	Chemical shift
µg	:	Microgram
µm	:	Micrometer
µM	:	Micromolar
%	:	Percentage
ANOVA	:	Analysis of Variance
Ar	:	Aromatic
Chem.	:	Chemicals
CFU	:	Colony Forming Unit
cm	:	Centimeter
CMC	:	Carboxy methyl cellulose
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
DMF	:	Dimethyl formamide
DMSO-d_6	:	Deuterated Dimethyl sulphoxide
d	:	Doublet
ED_{50}	:	Effective Dose
Eg.	:	Example
gm	:	Gram
^{1}H-NMR	:	Proton Nuclear Magnetic Resonance
h	:	Hour
IR	:	Infra Red
KBr	:	Potassium bromide

kg	:	Kilogram
LD_{50}	:	Lethal Dose
Ltd.	:	Limited
m	:	Multiplet
M^+	:	Molecular ion
MeOH	:	Methanol
MIC	:	Minimum Inhibitory Concentration
min	:	Minutes
mg	:	Milligram
ml	:	Milliliter
m/z	:	Mass / charge
nm	:	Nanometer
OECD	:	Organization for Economic Corporation and Development
ppm	:	Parts per million
pH	:	Hydrogen ion concentration
Pvt.	:	Private
QSAR	:	Quantitative Structure Activity Relationship
R_f	:	Retention factor
SEM	:	Standard Error Mean
s	:	Singlet
TLC	:	Thin Layer Chromatography
t	:	Triplet
w/v	:	weight/volume
UV	:	Ultra Violet

INTRODUCTION

1. INTRODUCTION

Medicinal chemistry concerns with the discovery, development, identification and interpretation of the mode of biologically active compounds at the molecular level.

Medicinal chemistry also concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds. The primary function of medicinal chemists is to discover new drugs, but knowledge of the underlying principles of biochemical action should be immense value for the design of new drug molecules. The development of drug therapy could not progress until knowledge of anatomy and physiology had reached the status of science.

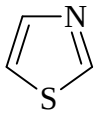
The current trend in the drug design is to develop new clinically effective agents through the structural modification of a lead moiety. The lead moiety is a new active compound which is typically found by screening many compounds for the desired biological properties. These lead can come from natural sources, such as plants, animals etc., more often it can come from synthetic sources such as historical compound collections and combinational chemistry.

Once a lead compound has been discovered for a particular therapeutic use, the next step is to optimize the lead compound. An important method of lead optimization is optimization by bioisosterism. Bioisosters are substituted group that have similar physical and chemical properties and hence similar biological activity patterns. Bioisosteric replacement may help to decrease toxicity or to change the pharmacokinetic profile.

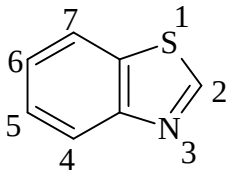
The final step involves the rendering of lead compounds suitable for use in clinical trials. This involves the optimization of synthetic route for bulk production and the preparation of compounds suitable for drug formulation.

1.1) 1, 3 – Benzothiazoles:

A number of heterocyclic derivatives containing nitrogen and sulphur atom serve as a unique and versatile scaffolds for experimental drug design. Benzothiazole is one of the most important heterocycle that has received overwhelming response owing to its diversified molecular design and remarkable optical, liquid and electronic properties. Benzothiazole consists of thiazole ring fused with benzene ring and possess multiple applications.



Thiazole



1,3 Benzothiazole

Thiazole is structurally related to thiophene and pyridine, but in most of its properties it resembles the latter. Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. The numbering in thiazole starts from the sulphur atom. The basic structure of 1, 3 benzothiazole consist of benzene ring fused with 4, 5-position of thiazole. The two rings together constitute the basic nucleus 1, 3-benzothiazole.

Molecular Formula : C_7H_5NS

Molecular Weight : 135.19

Physical and Chemical Properties of 1, 3 - Benzothiazole:

(Anonymous http:// www.wikipedia.org)

Description : Yellow liquid with unpleasant odour

Boiling Point : 227 - 228° C

Melting Point : 36° F (or) 2.00° C @ 760.00mm Hg

Flash Point : >230.00° F

Density	:	1.238 g/ml at 25° C
Refractive Index	:	n20/D 1.642(lit)
Vapor Pressure	:	34 mm Hg at 131° C
Vapor Density	:	4.66 (vs air)
Solubility	:	Slightly soluble in water; very soluble in ethanol, diethyl ether and carbon disulfide; soluble in acetone
Reactivity	:	Reacts with aldehydes or ketones to generate ahydroxy carbonyl compounds
Storage	:	Stable (Regarded as highly persistent in the environment. Incompatible with strong oxidizing agent)

The compounds encompassing benzothiazole moiety are of great interest and have been extensively used in pharmaceutical chemistry and agriculture division. Heterocycles bearing a benzothiazole ring residue are reported to show antimicrobial, anti-cancer, anti-inflammatory, analgesic, muscle-relaxant, sedative, anti-tubercular, diuretic, anticonvulsant, anti-allergic, anti-malarial, antiviral, anti-HIV, antioxidant, CNS depressant, anti-psychotic, schictosomicidal, anti-diabetic and plant growth regulatory activity etc. In addition, benzothiazole forms an important pharmacaphore in fungicidal, herbicidal and insecticidal, agents. (Priyanka *et al.*, 2010)

Different substitutions on benzothiazole moiety on different position are found to posses different activity, for example if substitution on 2 position on benzothiazole by any phenyl ring are used as an anticancer drug, if substitution on 2,5,6 position is used as anti-inflammatory agent. Substitution of phenyl imidazole ring used as an anthelmintic activity. Substitution of 4-acetamidophenyl sulphonamide on 2 position used as anti-tubercular activity. Substitution of 4-aminophenylsulphonamide on 2 position and halo compounds on 6 position are used as anticonvulsant activity.

Substitution on 2 position by 4-aminophenyl and on 6 position by methoxy group used in Alzheimer's disease.

General Methods For The Synthesis of 1,3-benzothiazole:

(Sukhbir L Khokra *et al.*, 2011)

1. **Condensation of o-aminothiophenol with aldehydes:**

Treatment of o-aminothiophenols with substituted aldehydes affords the synthesis of 2-substituted benzothiazoles using different catalysts and reaction conditions.

Catalysts (a-f):

a. Montmorillonite, SiO_2/Graphite; Microwave, p-TsOH

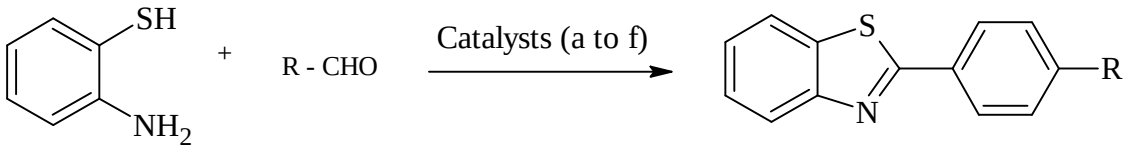
b. Diethyl bromophosphonate/tert-Butyl hypochlorite; acetonitrile

c. Cerium (IV) ammonium nitrate

d. H_2O_2/HCl system in ethanol

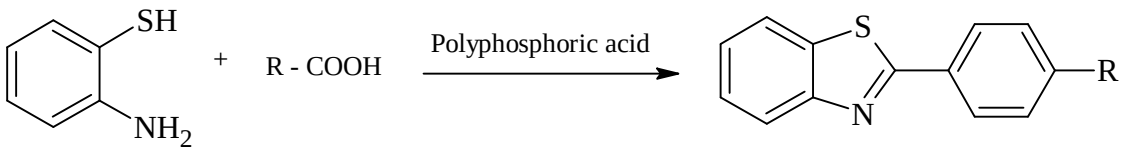
e. AcOH/Air; Microwave/ Thermal Heating

f. Baker's yeast, Dichloro methane



2. **Condensation of o-aminothiophenol with acids:**

Treatment of o-aminothiophenols and substituted aromatic acids in the presence of polyphosphoric acid provides a good method to synthesis of 2-substituted benzothiazoles and gives good yield.



3. Cyclization of Thioformanilides Using Different Reagent:

Substituted thioformanilides can be converted to 2-aminobenzothiazoles via intramolecular C-S bond formation/C-H functionalization utilizing various reagents and catalysts.

Catalysts (a-e):

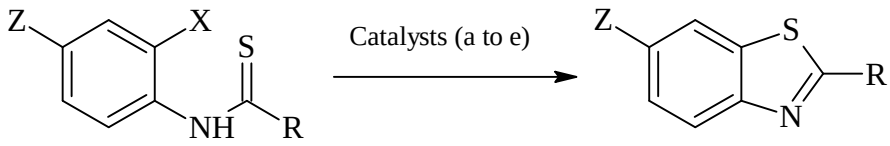
a. CuI; 1, 10-Phenanthroline, CS_2CO_3, reflux

b. Manganese triacetate

c. CS_2CO_3, Dioxane

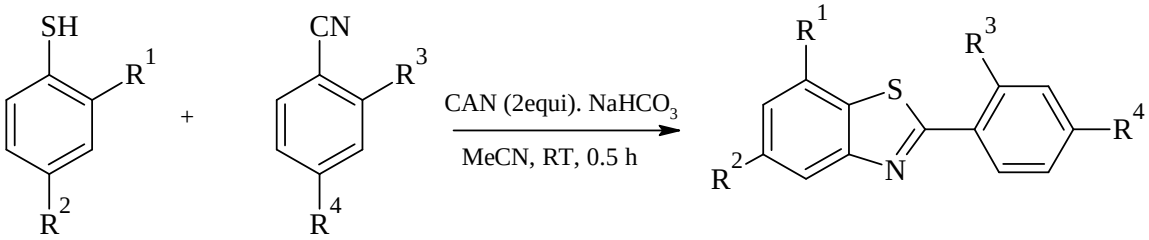
d. Photochemical cyclization induced by chloranil

e. Pd $(PPh_3)_4/MnO_2$ system under an oxygen atmosphere



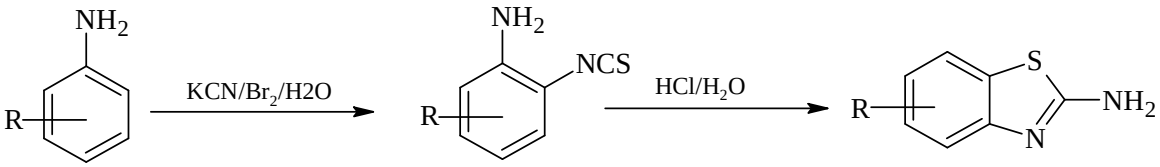
4. Coupling Between Thiophenols and Aromatic Nitriles:

Thiophenols when treated with aromatic nitriles to affords a smooth reaction mediated by ceric ammonium nitrate to give corresponding 2-arylbenzothiazoles in excellent yield.



5. Synthesis Using Anilines:

Different substituted anilines when treated with potassium thiocynate in presence of glacial acetic acid to synthesize 2-substituted benzothiazoles.



Fluorine in Bioactive Molecules:

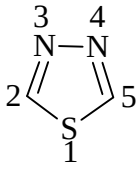
The incorporation of fluorine in drug molecule as a mean of increasing therapeutic efficacy is based on several considerations,

1. Fluorine, the second smallest substituent, closely mimics hydrogen with respect to steric requirements at enzyme receptor sites (Vander waal's radii F=1.35A°; H=1.2A°)
2. The strong electron withdrawing inductive effect of fluorine can significantly influence reactivity and stability of functional groups and the reactivity of neighboring reaction centers.
3. The substitution of hydrogen by fluorine at or near a reactive site frequently causes inhibition of metabolism because of the high C-F bond energy.
4. The replacement of hydrogen by fluorine usually increases lipid solubility, thereby enhancing the rate of absorption and transport of drug *in vivo.*
5. Some time the presence of fluorine instead of hydrogen actually blocks an essential biochemical reaction: the fluorine behaves as a receptor group Eg. 5- fluorouracil.

1.2) 1, 3, 4-Thiadiazole

1, 3, 4-Thiadiazole is a isomer of thiadiazole series. It is a versatile moiety that exhibits a wide variety of biological activities. They act as “hydrogen binding domain” and “two-electron donor system”. It also acts as a constrained pharmacophore.

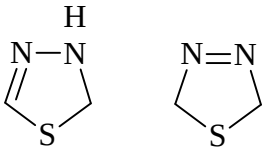
1, 3, 4-thiadiazole are sulfur containing aromatic heterocycle with nitrogen atoms at the 3 and 4 positions and are numbered as shown below.



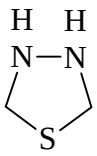
1,3,4 Thiadiazole

The C-N bond length in 1,3,4-thiadiazole is very close to that in thiazole and the C-S bond length is nearly similar to that in thiophene. The N-N bond in 1,3,4-thiadiazole also acquires some double bond character. The bond lengths in 1,3,4-thiadiazole reflect that the single bonds acquire double bond character while double bonds acquire some single bond character and therefore suggest larger delocalization of π-electrons in 1,3,4-thiadiazole.

1,3,4-Thiadiazole exists in two partially reduced (dihydro) forms named as 1,3,4-thiadiazolines depending on the position of the double bond. The completely reduced (tetrahydro) 1,3,4-thiadiazole is known as 1,3,4-thiazolidine.



1,3,4 - thiadiazolines
(dihydroforms)



1,3,4 - thiazolidine
(tetrahydroform)

Molecular Formula : $C_2H_2N_2S$

Molecular Weight : 86.11

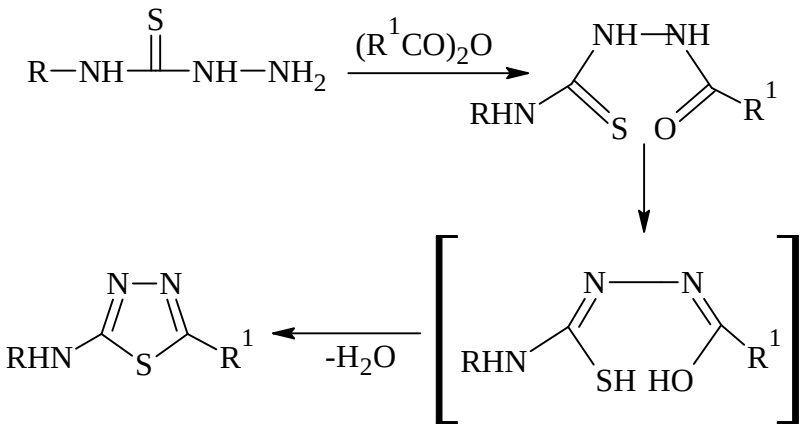
1,3,4-Thiadiazole is a versatile pharmacophore which exhibits a wide variety of biological activities like anti-microbial, anti-tubercular, anti-tumor, anti-inflammatory, anti-hypertensive, anti-convulsant, anti-diabetic, anti-viral, anthelmintic, diuretic, anti-depressants, antioxidants, analgesic, oxidative inhibitors, anti H-pylori, hepatoprotective, anti-mycotic, cardiotonic etc.

(Barve Ashutosh *et al.,* 2009)

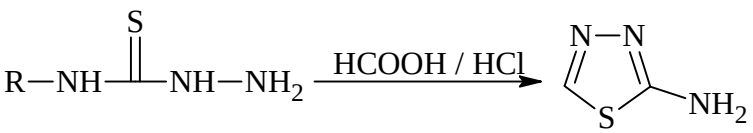
General Methods For The Synthesis of 1,3,4-Thiadiazole:

1. From Thiosemicarbazides:

This is the most common method to synthesize 5-substituted 2-amino-1,3,4-thiadiazoles which involves acyclation of thiosemicarbazide followed by dehydrative cyclization using any of the below mentioned agents sulfuric acid, phosphorus oxychloride, polyphosphoric acid, phosphorus halides or more recently methane sulfonic acid.

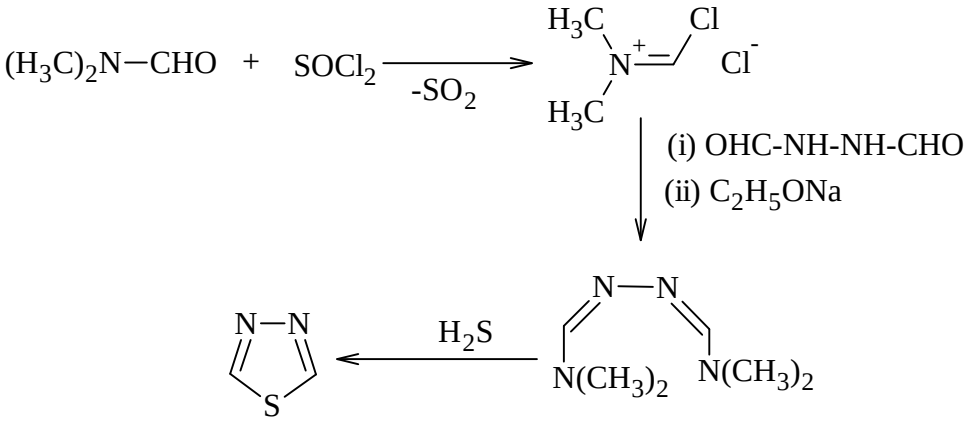


However, 2-amino-1,3,4-thiadiazole is obtained by heating thiosemicarbazide with a mixture of formic acid and hydrochloric acid.



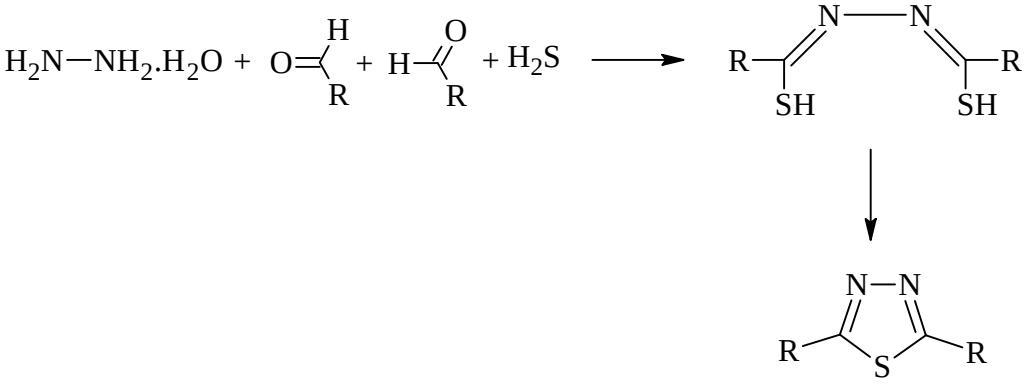
2. From Dimethylformamaide:

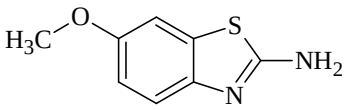
The reaction of N,N-dimethylformamide with thionyl chloride produces formamidoyl chloride which on treatment with N,N-diformylhydrazine and with sodium ethoxide gives a free base. The free base obtained undergoes cyclization in the presence of hydrogen sulfide with the formation of parent 1,3,4-thiadiazole.



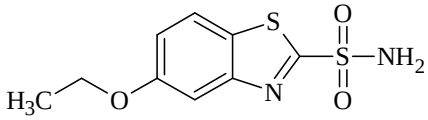
3. From Hydrazine:

This is the one pot-synthesis of 2,5-dialkyl-1,3,4-thiadiazoles and involves the reaction of hydrazine with aldehyde and elemental sulfur. The reaction proceeds via an intermediate which is subsequently cyclized to 2,5-dialkyl-1,3,4-thiadiazole with the evolution of hydrogen sulfide involving the formation of C-S bond.

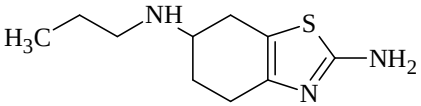




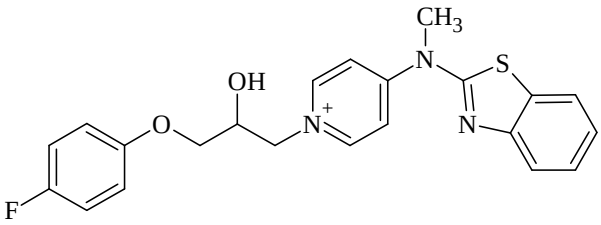
Riluzole (Anti-Epileptic drug)



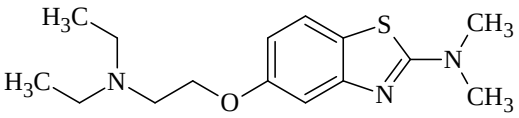
Ethoxzolamide (Diuretic)



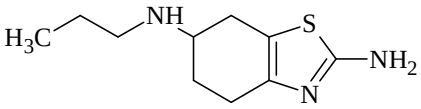
Pramipexole (Anti-Depressants)



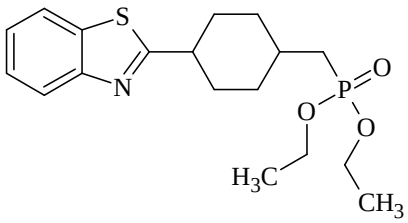
Sabeluzole (Anti-Fungal)



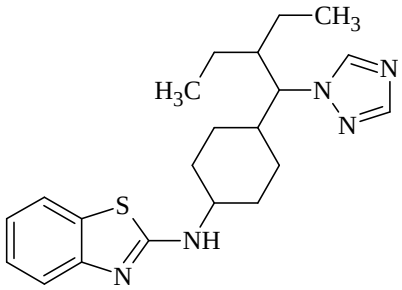
Dimazole (Anti-Fungal)



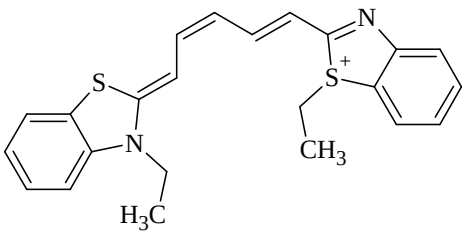
Dexpramipexole (Anti-Epileptic)



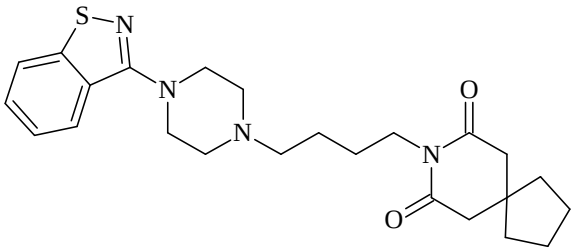
Fostedil (Anti-Hypertensive agent)



Talarozole (To treat acne & psoriasis)

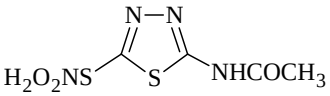


Dithiazanine Iodide (Anthelmintic)

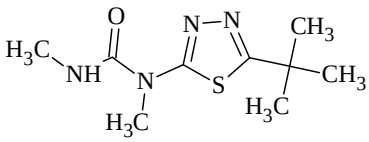


Tiospirone (Anti-Psychotic drug)

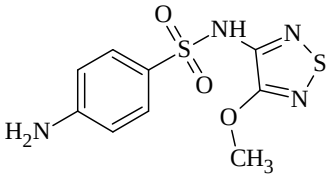
Fig-1: Compounds Containing 1, 3 - Benzothiazole Nucleus



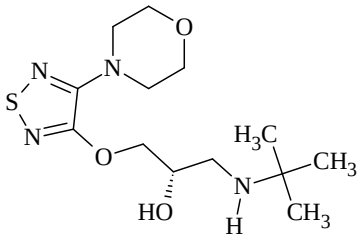
Acetazolamide (Diuretic)



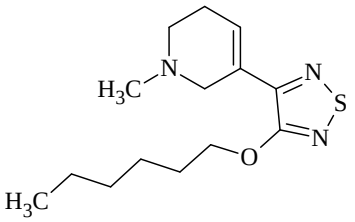
Tebuthiuron (Herbicides)



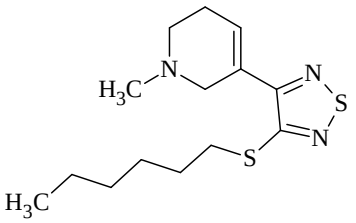
Sulfametrole (Anti-bacterial)



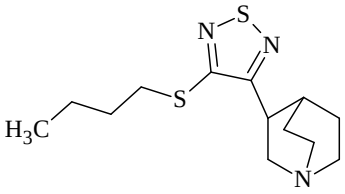
Timolol (b-adrenergic blocker)



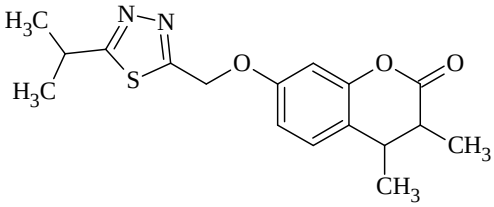
Xanomeline (M5 receptor antagonist)



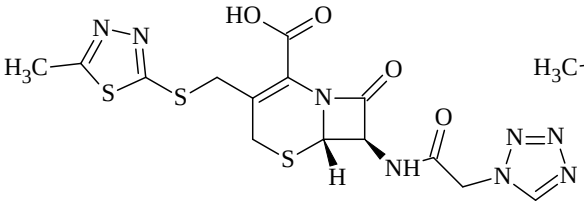
Tazomeline (Muscarnic ACH receptor agonist)



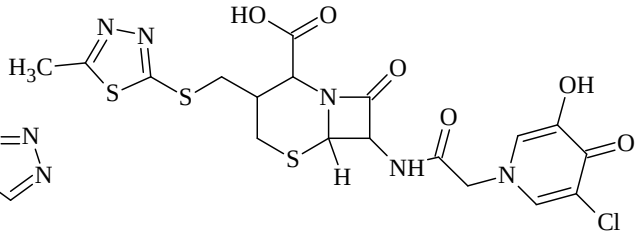
Vedaclidine (Analgesic)



Atibeprone (Anti-depressant)



Cefazolin (Antibiotic)



Cefazedone (Antibiotic)

Fig-2: Compounds Containing 1, 3, 4 - Thiadiazole Nucleus

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Literature Review For 1, 3 Benzothiazole

2.1.1) Bobade AS *et al.,* (2010) synthesized some phenyl thioureido sulfonamide benzothiazoles (Fig-3). These compounds were evaluated for their anti-bacterial and anti-fungal activity against gram positive *Staphylococcus aureus* (ATTCC 3750), gram negative *Salmonella typhi* (NCTC 786) and fungal strain *Candida albicans.* It was observed that the electron donating moieties attached to the benzothiazole nucleus proved to be more potent than the electron withdrawing moieties. Among all the compounds tested methyl substituted benzothiazole (V_a) showed highest potency against all three pathogenic strains. Although comparatively electron withdrawing moieties did not show good activity one of the derivatives (V_i) was relatively potent against all three pathogenic strains. This may be due to the presence of two fluorine moieties present on the benzothiazole ring.

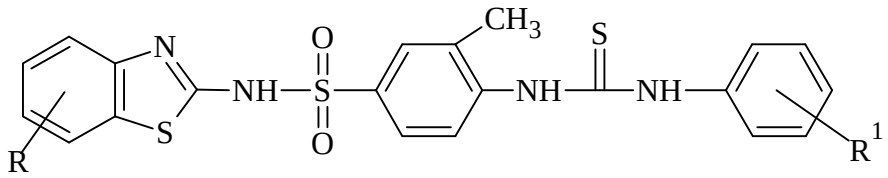
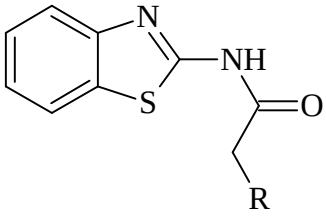


Fig-3

Code	R	R^1
V_a	6 – CH_3	H
V_i	4F, 6F	H

2.1.2) Velingkari VS *et al.,* (2010) synthesized series of substituted 2-amino benzothiazoles (Fig-4). The compounds were confirmed by Thin Layer Chromatography and spectroscopic methods like UV, IR, ^{1}H NMR and Mass. In addition the elemental analysis (Thermo Finnigan) was carried out manually by combustion method. The synthesized compounds were screened for their *in vitro* anti-inflammatory activity.



S.No.	R
1	p – toluidino
2	Ethanol amino
3	p – Hydroxy phenyl
4	Dibutyl amino
5	Diethyl amino

Fig-4

2.1.3) Bhanushali MD *et al.*, (2010) synthesized a series of various substituted benzothiazole derivatives containing 7-chloro-6-fluro-N (substituted hydrozones) – benzothiazole (Fig-5). All the compounds were tested for their purity by TLC and melting point. The structures of these compounds were confirmed by IR, NMR, and CNH analysis. All these were found to be satisfactory. The selected synthesized compounds $MIII_C$, $MIII_F$ and $MIII_J$ were evaluated for anti-inflammatory activity by paw edema method using carragennan. The inflammation was measured by plethysmometer and synthesized compounds were given orally. There was significant reduction in the inflammation and compound $MIII_J$ has shown more significant anti-inflammatory activity.

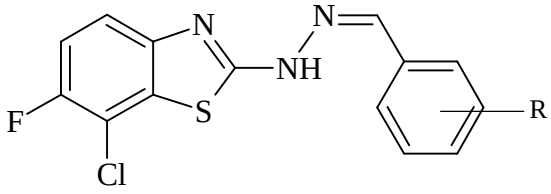


Fig-5

Code	Ortho	Meta	Para
$MIII_C$	H	OCH_3	OH
$MIII_F$	H	NO_2	H
$MIII_J$	OCH_3	OCH_3	OCH_3

2.1.4) Nitendra K Sahu *et al.*, (2011) synthesized a series of 1,3-benzothiazole-2-yl-hydrazones and evaluated for antibacterial activity against four different bacterial species and antifungal activity against two different fungal species by disk diffusion method displaying different degree of antimicrobial activity. All the synthesized

compounds were in good agreement with elemental and spectral data (FT-IR, ^{1}H NMR and Mass spectroscopy). *In vitro* antimicrobial activity was evaluated against the four pathogenic bacterial strains *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas alkaligenes* and three fungal strains *Aspergillus niger, Rhizopus oryzae* and *Candida albicans*. All the compounds have shown moderate activity. Compounds 3a (Fig-6) and 3b (Fig-7) were found to be most active.

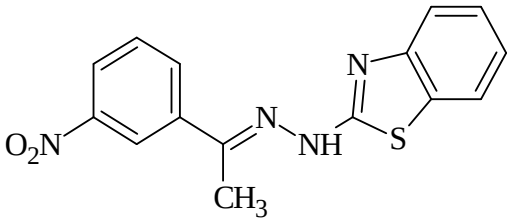


Fig-6

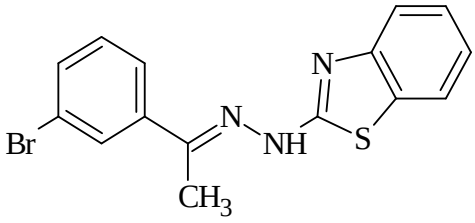


Fig-7

2.1.5) Balram Soni *et al.*, (2011) synthesized a novel series of N-(1, 3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide in order to determine their antimicrobial activities. The synthesized compounds were tested *in vitro* against two gram positive, one gram negative bacteria and two fungal strains in comparison with control drugs. Microbiological results showed that the synthesized compounds possessed a broad spectrum of antibacterial activity against the tested microorganisms. The compounds with a 4-amino group (Fig-8) and 4-dimethylamino group (Fig-9) on the aromatic ring possessing azomethine linkage showed better antimicrobial activity; almost similar to that of standard drugs thus they could be promising candidates for novel drugs.

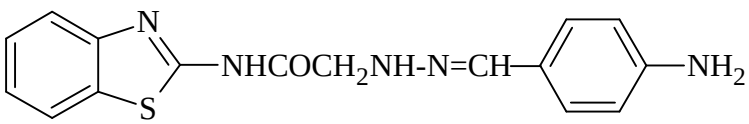


Fig-8

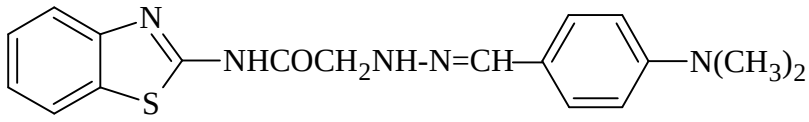


Fig-9

2.1.6) Padmavathi P Prabhu *et al.*, (2011) synthesized a series of Schiff's base of several benzothiazole derivatives (Fig-10). The structure of synthesized compounds was characterized by IR, ^{1}H NMR and Mass spectral data. Purity of the individual compound was confirmed by TLC. Then, each product was evaluated for their *in vitro* growth inhibiting activity against several microbes. All the compounds have shown significant antibacterial activity with the reference standard ampicillin and ketoconazole.

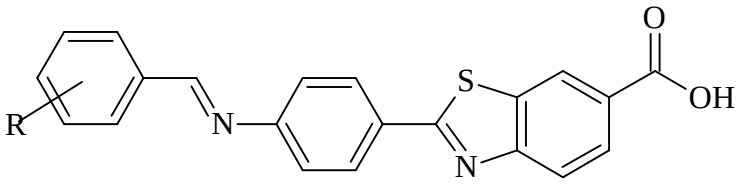


Fig-10

Code	R
P5a	H
P5b	para- Cl
P5c	meta-F
P5d	para-NO_2
P5e	para- OCH_3
P5f	4-F, 3-OCH_3
P5g	para-CH_3
P5h	para-OH

2.1.7) Priyanka Yadav *et al.*, (2010) synthesized a series of novel 2-substituted hydrazino-6-fluoro-1,3-benzothiazole derivatives (Fig-11). The structures of few compounds were elucidated by IR and NMR. The efficacy of compounds was determined against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, *Pseudomonas aeruginosa* for their microbiological activities. All the newly synthesized compounds showed good antimicrobial activity against *Staphylococcus epidermidis* (D_1-D_4), *S.epidermidis* (D_2-D_4), *P.aeruginosa* (D_1-D_3) and *E.coli* (D_1 & D_4). The compound D_3 has better antimicrobial activity against *S. aureus* than other compounds. Compound D_4 showed better activity against *S.epidermidis* and

compound D_2 showed better activity against *P.aeruginosa* whereas compound D_1 shows potent activity against *E.coli.*

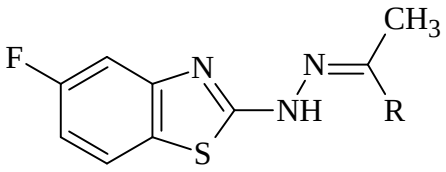
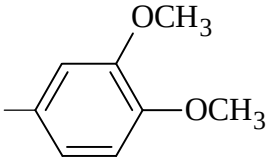
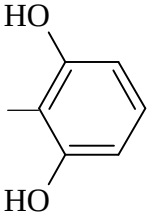
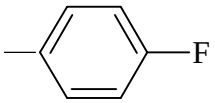
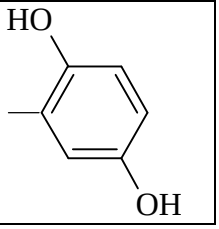
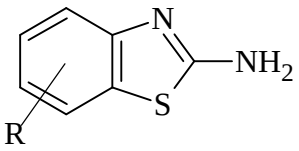


Fig-11

Code	D_1	D_2	D_3	D_4
R				

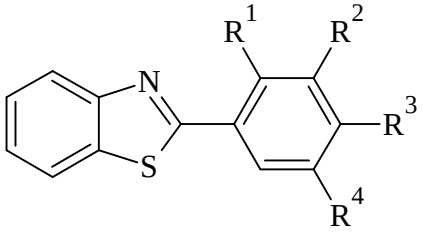
2.1.8) Venkatesh P *et al.*, (2009) synthesized a series of some novel 2-amino benzothiazole derivatives and evaluated for anti-inflammatory activity (Fig-12). The compounds were synthesized from the substituted aromatic amines through the intermediate substituted 1-phenylthiourea oxidation by bromine water in acidic medium. The purity of the synthesized compounds was judged by their C, H and N analysis and the structure was analyzed on the basis of IR, ^{1}H NMR and Mass spectral data. The anti-inflammatory activities of new compounds were determined by carrageenan-induced mice paw edema method using diclofenac sodium as a standard. Among the compounds tested three compounds Bt_2 (5-chloro-1, 3-benzothiazole-2-amine), Bt_1 (6- ethoxy-1, 3-benzothiazole-2-amine) and Bt_7 (6-methoxy-1, 3-benzothiazole-2-amine) were the most active compounds in these series when compared with diclofenac sodium. In the SAR study, the phenyl ring substituted with chloro at 5 position, methoxy substitution at 4 and 6-position in benzothiazole ring system showed better anti-inflammatory activity.



Code	R
Bt_1	H
Bt_2	5-Cl
Bt_7	4-OCH_3

Fig-12

2.1.9) Devmurari VP *et al.*, (2010) synthesized a series of seven substituted 2-phenyl-benzothiazole and substituted 1,3-benzothiazole-2-yl-4-carbothiaote derivatives. Substituted 2-phenyl-benzothiazole were synthesized by condensing substituted benzoic acid with 2-amino thiophenol in the presence of phosphoric acid and 3-benzothiazole-2-yl-4-substituted carbothiaote derivatives were prepared by condensing 2-mercaptobenzothiazole with substituted acid chloride. Structures of all the compounds were characterized by spectral and elemental analysis. All the synthesized novel compounds were screened for anticancer activity. It was also found that compounds 1, 2 (Fig-13) 6 (Fig-14) and 7 (Fig-15) showed very good anticancer activity whereas all the other compounds have showed mild to moderate anticancer activity when compared to standard drug.



Compound	R^1	R^2	R^3	R^4
1	H	H	NH_2	H
2	F	Cl	Cl	H

Fig-13

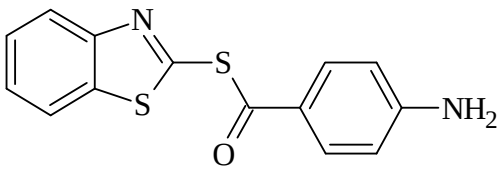


Fig-14

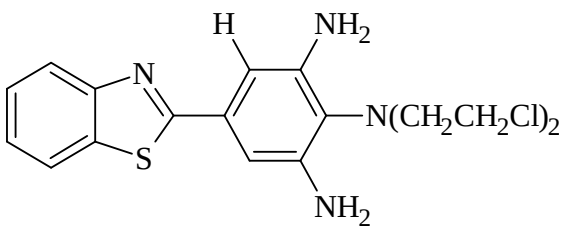


Fig-15

2.1.10) Sambhaji P Vartale *et al.*, (2011) reported convenient and practical procedure for the synthesis of 10-methyl 14, 15-di-imino benzothiazolo[2,3-b] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole derivatives by using milder reaction conditions, simple workup with good yield. The structure of these newly synthesized compounds was established on the basis of elemental analysis, IR, NMR and Mass spectral data. Spectral studies of all compounds shows that compounds are stable & do not exhibit any tautomerism. All the newly synthesized compounds were tested for their antimicrobial activity using disc diffusion technique against *S. aureus, B. Substilis, E. Coli and S. Typhi.* The standard antibiotics norflaxacin showed zones of inhibition 14-24 mm, against bacterial strains. Among, all the newly synthesized compound 5h (Fig-16) show very good activity against *B. Substilis* whereas moderately active against *S. Aureus, E. Coli* and *S. Typhi*.

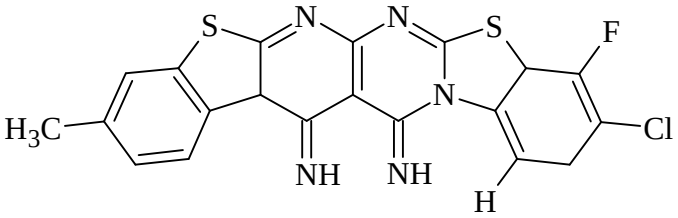


Fig-16

2.1.11) Andrew D Westwell *et al.*, (2006) synthesized a series of new 2-phenylbenzothiazoles on the basis of the discovery of the potent and selective *in vitro* anti-tumor properties of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole. Synthesis of analogues substituted in the benzothiazole ring was achieved via the reaction of o-aminothiophenol disulfides with substituted benzaldehydes under reducing conditions. Compounds were evaluated *in vitro* in lung, colon, and breast cancer cell lines, compound 8n (Fig-17) was found to possess potent anti-proliferative activity. Structure-activity relationships established that the compound 8n stands on a pinnacle of potent activity, with most structural variations having a deactivating *in vitro* effect.

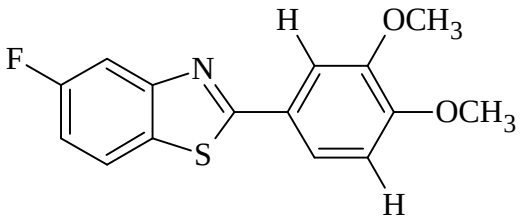


Fig-17

2.1.12) Yaseen A Al-Soud *et al.*, (2008) synthesized a series of benzothiazole bearing piperazino-arylsulfonamides (Fig-18) and arylthiol analogues (Fig-19) as well as substituted benzothiazoles having sulfonamides. All compounds were evaluated in vitro for their anti-proliferative activity against a large panel of human tumor-derived cell lines. Compounds 5c, 5d, 5j, 6b, 6c and 6j were the most potent analogues in this series, showing activity against both cell lines derived from haematological and solid tumors (CC 50range = 8-24 μM), only 5d was found to be selective and not cytotoxic to normal human tissues.

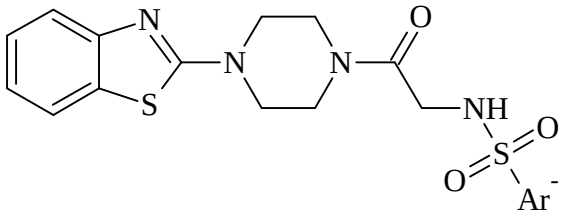
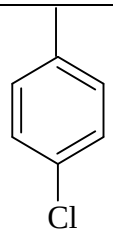
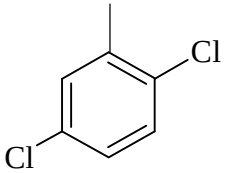
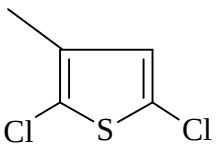


Fig-18

Code	5c	5d	5j
Ar=			

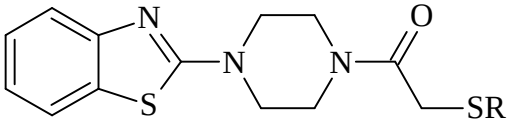
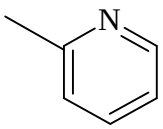
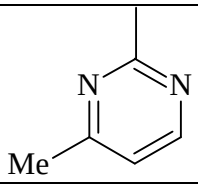
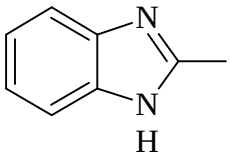


Fig-19

Code	6b	6c	6j
R			

2.1.13) Bhusari KP *et al.*, (2009) synthesized some 4-amino-N-(1,3- benzothiazol-2-yl) benzenesulphonamide derivatives and evaluated for *in vitro* anti-mycobacterial activity against *H37Rv* strain of M*ycobacterium tuberculosis.* Out of which the below mentioned compound (Fig-20) was found to have good anti-mycobacterial activity.

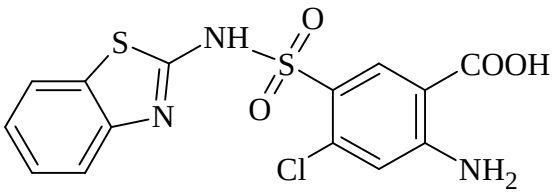


Fig-20

2.1.14) Barot HK *et al.*, (2010) synthesized nitrogen mustards of fluoro benzothiazoles and evaluated for their antibacterial and antifungal activity of against *S. aureus, B. subtilis, C. tropicans, A. niger* and *F. heterosporium*. The nitrogen mustards (Fig-21) and (Fig-22) showed excellent inhibition at a concentration of 50 µg/ 0.1ml.

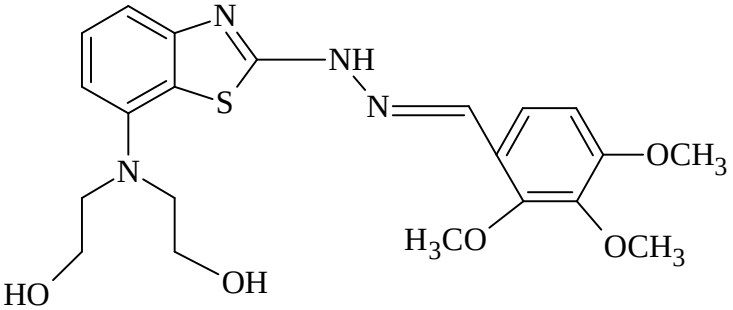


Fig-21

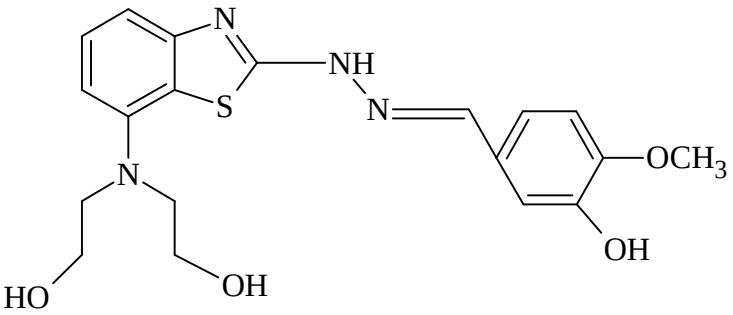


Fig-22

2.1.15) Patel NB *et al.*, (2010) synthesized a new series of compounds 2-[(6-methyl-1, 3- benzothiazol- 2- yl) amino]- N- [2- (substituted phenyl/ furan- 2- yl)- 4- oxo- 1, 3- thiazolidin- 3-yl] nicotinamides (Fig 23 and 24) and examined to possess good *in vitro* antimicrobial activity against two gram positive (*S. aureus* and *S. pyrogens*), two gram negative (*E. coli* and *P. aeruginosa*) bacteria and three fungal species (*C. albicans, A. niger, A. clvatus*).

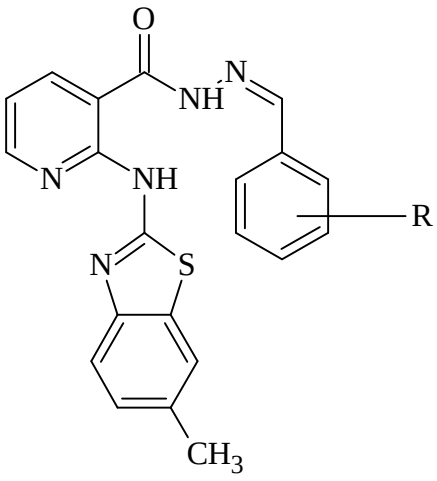


Fig-23

R=H, 2-Cl, 2-NO_2, 3-No_2, 4-OH, 4-OMe, 3-OMe-4-OH 3- OMe-4OH-5-NO_2 and 2-furyl

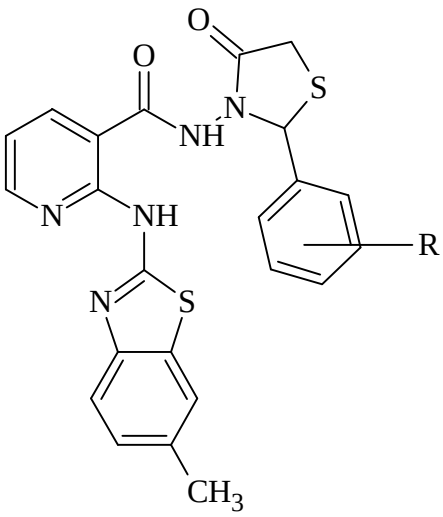


Fig-24

R=H, 2-Cl, 2-NO_2, 3-No_2, 4-OH, 4-OMe, 3-OMe-4-OH, 3- OMe-4OH-5-NO_2 and 2-furyl

2.1.16) Huang W *et al.*, (2006) synthesized 2-(3, 4-difluoro-benzylsulfanyl)-4-fluoro benzothiazole (Fig-25) exhibited most interesting antifungal activities against *R.*

solani, *B. cinereapers* and *D. gregaria* among a series of polyfuorinated 2-benzylthiobenzothiazoles.

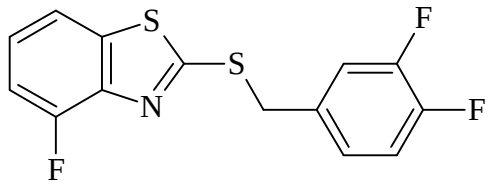


Fig-25

2.1.17) Arun Pareek *et al.*, (2010) synthesized some N-(4, 5-dihydro-1*H*-imidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines and *N*-(1*H* benzimidazol- 2-yl)-6-substituted-1,3-benzothiazol-2-amines. All the synthesized compounds were characterized by elemental analysis, IR spectra, ^{1}H NMR and Mass spectral studies. They were screened for their anti-inflammatory, anti-ulcer, anti-tumor, entomological (anti-feedant, acaricidal, contact toxicity and stomach toxicity) and antibacterial activities. Among the synthesized compounds 3a (Fig-26) and 4a (Fig-27) compounds were found to possess good activity. The compounds also showed potent antiulcer, anti-inflammatory and antitumor. Antifeedant activity, contact toxicity and stomach toxicity tested against *Spodoptera litura* and acaricidal activity tested against *Tetranychus urticae.*

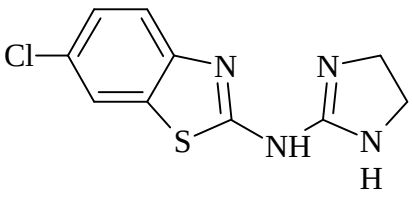


Fig-26

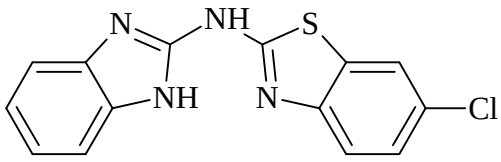
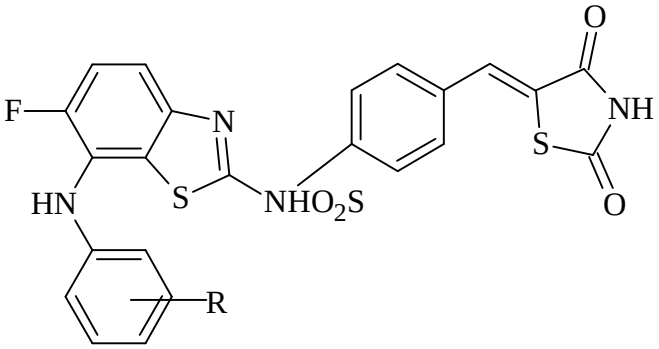


Fig-27

2.1.18) Pattan SR *et al.*, (2005) synthesized a series of 2-amino [5'(4-sulphonnyl benzylidine)-2-4- thiazolidinedione]-7-chloro-6-flurobenzothiazole and screened for their antidiabetic activity by alloxan induced tail tipping method on albino rats. All

the compounds showed good anti-diabetic activity and the below mentioned compound (Fig-28) showed maximum anti-diabetic activity.



Code	R
1	H
2	meta- NO_2
3	para-COOH

Fig-28

2.1.19) Pankaj Arora *et al.*, (2010) synthesized various chromene -2- one derivatives and evaluated for antipsychotic activity. All the synthesized compounds showed antipsychotic activity with muscle relaxant property. The compound N-(fluoro benzothiazol-2-yl)-(4-Methyl-2-oxo-chromene-7-yloxy) (Fig-29) acetamide has been found to have significant atypical behavior.

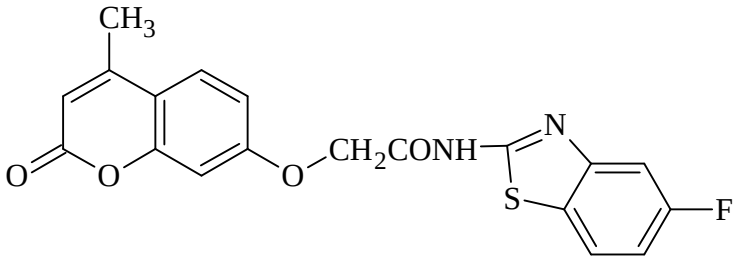
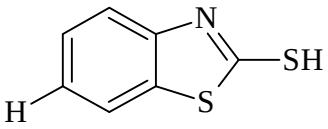


Fig-29

2.1.20) Ghassoub Rima *et al.*, (2009) reported the synthesis and characterization of new compounds derived from benzothiazoles and thiadiazoles. They observed that structural modifications on these skeletons affected the antioxidant activity. Thiol and aminothiol compounds derived from thiadiazoles and benzothiazoles showed an interesting antioxidant property. The radioprotective activity has also been evaluated in mice. *In vivo* tests showed an efficient radioprotective effect at $LD_{99.9/30days}$-IRR for

the compounds 4a (Fig-30) and 5a (Fig-31) and particularly for 2-mercapto-6-methylbenzothiazole 4b (Fig-32) compared to WR-272.



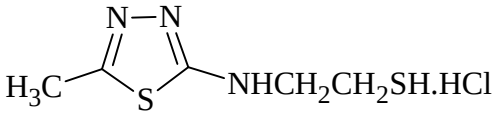


Fig-30 **Fig-31**

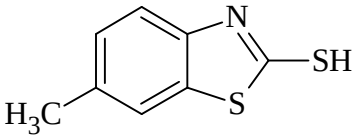


Fig-32

2.2 Literature Review For 1,3,4-Thiadiazole

2.2.1) Weerasak Samee *et al.*, (2011) synthesized a series of 2,5-dimercapto-1,3,4-thiadiazole (Fig-33) derivatives by nucleophilic substitution reaction. The cytotoxic activity was determined by green fluorescent protein (GFP)-based assay and anti-candida activity was determined by Resazurin Microplate Assay (REMA). Compounds 1, 2 and 3 showed *in vitro* cytotoxic activities against Vero cells (African green monkey kidney). Compounds 1 and 4 exhibited anti-candida activities against *Candida albicans* (ATCC 90028) with IC50 values of 1.94 and 19.10 μg/ml, respectively. Docking studies on the catalytic site of cytochrome P450 14a-demethylase were used to identify the chemical structures in the molecule responsible for cytotoxic and anti-candida activities of the synthesized compounds.

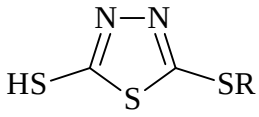
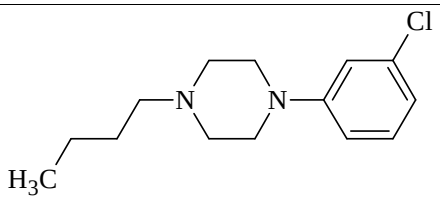
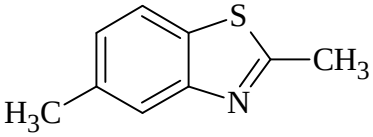
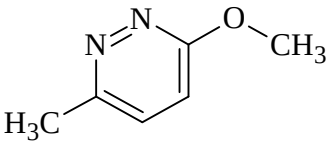
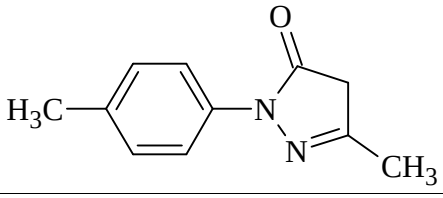


Fig-33

S.No.	R	S.No.	R
1		3	
2		4	

2.2.2) Dhanya Sunil *et al.*, (2009) synthesized a series of 3,6-disubstituted 1,2,4–triazolo[3,4-b]-1,3,4-thiadiazoles were synthesized from 3-substituted-4-amino-5-mercapto-1,2,4-triazoles and 3-substituted 4-carboxypyrazoles. Elemental analyses, IR, ^{1}H NMR and Mass spectral data confirmed the structures of all newly synthesized compounds. Most of the newly synthesized compounds were screened for their anticancer activity in hepatic cell lines. Many of the compounds were found to be potent. The perturbations brought by the substituent can affect various parameters of the molecule like its electron density, its steric environment, its bioavailability etc. The thiadiazole with naphthyloxymethyl and fluorophenyl groups (Fig-34) as substituent's showed (Fig-34) excellent anti-proliferative effect.

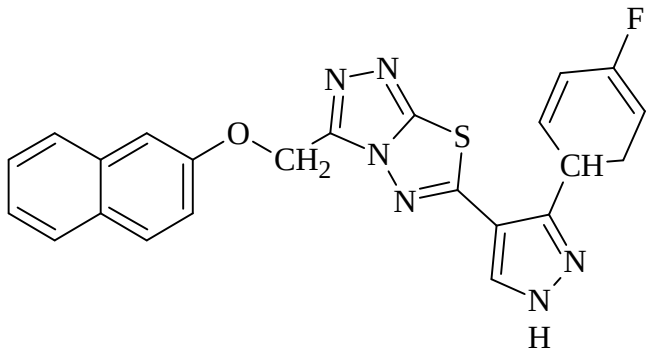


Fig-34

2.2.3) Arvind Kumar Singh *et al.*, (2011) synthesized some 2, 5 substituted 1,3,4-thiadiazoles (Fig-35). The structures of the synthesized compounds were confirmed

by IR, NMR, and Mass spectra data. The compounds were evaluated for antibacterial and anti-inflammatory activity. The anti-inflammatory activity was carried out by carrageenean induced paw oedema method using indomethacine as standard drug. Among all synthesized compounds 2 and 5 shown good anti-inflammatory activity. Whereas all the synthesized compounds shown moderate antimicrobial activity against *S. aureus, E. coli,* and *A. niger.*

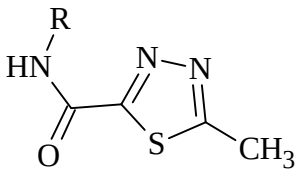


Fig-35

S.No.	R
1	C_6H_5
2	2-C_6H_4Cl
3	3-C_6H_4Cl
4	4-C_6H_4Cl
5	2-$C_6H_4NO_2$
6	3-$C_6H_4NO_2$
7	4-$C_6H_4NO_2$

2.2.4) Himaja M *et al.*, (2011) synthesized some 2-amino-5-aryl-5H-thiazole [4, 3-b]-1,3,4-thiadiazoles (Fig-36) by using aromatic aldehydes, thioglycolic acid and thiosemicarbazide. Structures of all synthesized compounds were confirmed by FT-IR, ^{1}H NMR and Mass spectral data. Their anti-tubercular activity was studied. All the synthesized compounds have shown good anti-tubercular activity.

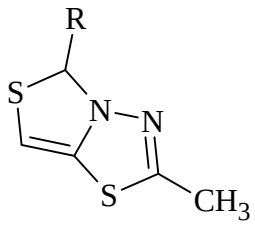
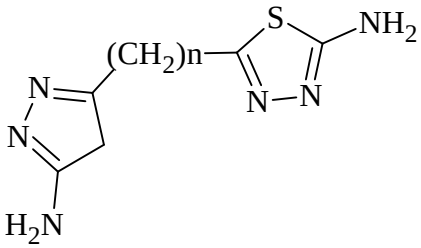


Fig-36

S.No.	R
1	Ph
2	4-MeC_6H_4
3	4-HOC_6H_4
4	4-HOC_6H_4
5	4-$O_2C_6H_4$
6	4-$Me_2NC_6H_4$
7	2-ClC_6H_4
8	2,4-$Cl_2C_6H_3$

2.2.5) Barve Ashutosh *et al.*, (2009) synthesized a series of 1,3,4-thiadiazol-2-amine (Fig-37). The structures of the compounds were confirmed by IR, NMR and Mass

spectral analysis. The newly synthesized compounds were evaluated for the antibacterial and antifungal activity. The results show that compound a, e, f, and h exhibit moderate to good antibacterial and antifungal activity at 5-100mcg/ ml.



Compound
a=n=1 Malonic acid
e=n=5 Pimelic acid
f=n=6 Suberic acid
h=n=8 sebacic acid

Fig-37

2.2.6) Shiv K Gupta *et al.*, (2011) synthesized a new series of 5-(*o*-hydroxy phenyl)-2-[4'aryl-3'chloro-2'azetidinon-1-yl]-1,3,4-thiadiazoleand the structures of the new compounds were established on the basis of IR, ^{1}H NMR spectral data. *In vitro* antifungal activity (MIC activity) was evaluated and compared with standard drug of ketoconazole. The below mentioned compound (Fig-38) has shown interesting antifungal activity against both *C. albicans* and *A. niger* fungus. In the gratifying result, most of the compounds were found to have moderate antifungal activity.

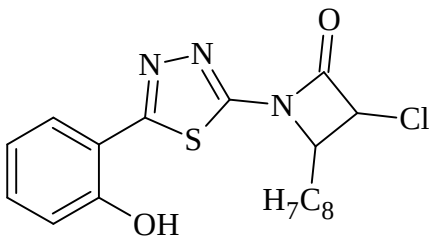


Fig-38

2.2.7) Nandini R Pai *et al.*, (2010) synthesized some novel aryloxy propanoyl thiadiazoles (Fig-39) as potential anti-hypertensive agents based on structure activity relationship with propanalol. The structure of the compounds was confirmed on the basis of both analytical and spectroscopic data. The synthesized compounds are mentioned below.

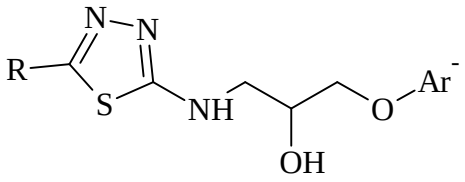
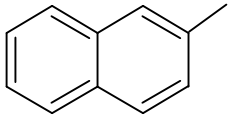
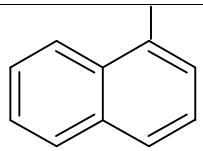
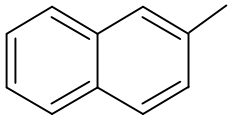
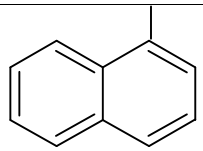
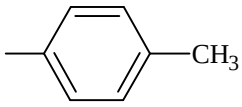
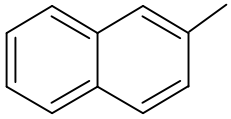


Fig-39

S.No.	R	Ar^-
1	$-CH_3$	
2	$-CH_3$	
3	$-CH_2CH_3$	
4	$-CH_2CH_3$	
5		

2.2.8) Foroumadi A *et al.*, (2007) synthesized several novel 2-amino-5-[4-chloro-2-(2-chlorophenoxy)phenyl]-1,3,4-thiadiazole derivatives and the anticonvulsant activity was determined by evaluation of the ability of these compounds to protect mice against convulsion induced by a lethal doses of pentylentetrazole (PTZ) and maximal electroshock (MES). The result of anticonvulsant data shows that among the synthesized compounds, 5-[4-chloro-2-(2-chlorophenoxy) phenyl]-N-ethyl-1,3,4-thiadiazol-2-amine (Fig-40) was the most active compound in both MES and PTZ tests with an ED50 of 20.11 and 35.33 mg/kg, respectively.

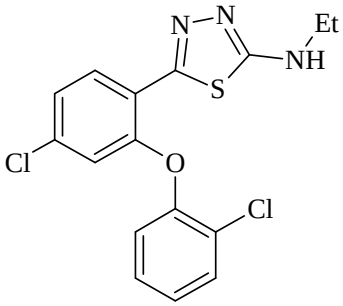


Fig-40

2.2.9) Sudhir Bharadwaj *et al.*, (2011) synthesized some 4-(substituted benzylidene)-1-(5-mercapto-1,3,4-thiadiazol-2-yl) -2-phenyl-1H-imidazol-5(4H)-one (Fig-41) by using microwave irradiation method and confirmed the structure of all the compounds by elemental analysis and spectral analysis (^{1}H NMR, IR and Mass).

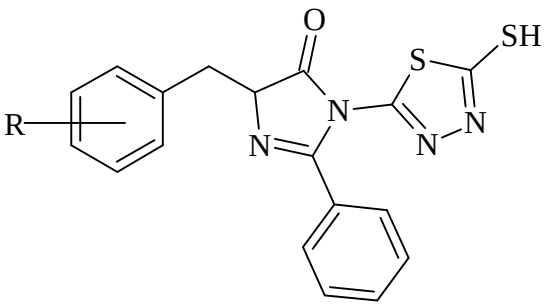


Fig-41

S.No.	R
1	H
2	4-F
3	4-Cl
4	2-Cl
5	4-NO_2
6	3-NO_2
7	4-OH
8	4-OH
9	4-OCH_3
10	4-$N(CH_3)_2$

2.2.10) Bijo Mathew *et al.*, (2010) synthesized some Schiff bases of 1, 3, 4 thiadiazole derivatives and evaluated for anthelmintic activity. The structures of the compounds were confirmed by IR, ^{1}H NMR and elemental analysis. The physicochemical properties involve determination of drug-like property of the synthesized compounds. It is based on the Lipinski's rule of 5 and can be determined by using molinspiration cheminformatics software. All the synthesized compounds showed zero violation of Lipinski's rule of five, which indicates good bioactivity and

bioavailability. The anthelmintic activity of those compounds was investigated by method described in details by Kuppast and Nayak. Parameter under study was mean paralysis and mean lethal time in Pheretima posthuma. The below mentioned compounds (Fig-42) produce good anthelmintic activity.

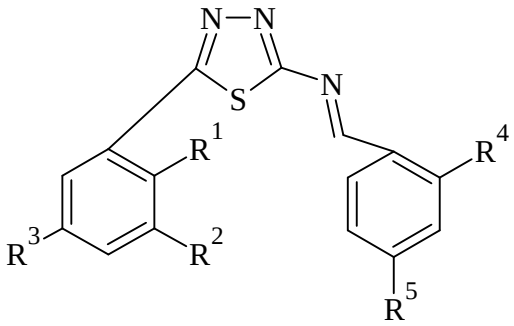


Fig-42

S.No.	R^1	R^2	R^3	R^4	R^5
1	OH	H	H	H	Cl
2	OH	NO_2	NO_2	H	Cl
3	$OCOCH_3$	NO_2	NO_2	H	Cl
4	H	H	H	H	H
5	H	NO_2	H	H	Cl
6	H	NO_2	H	OH	H
7	H	NO_2	H	H	H

2.2.11) Byran Gowramma *et al.*, (2011) synthesized a series of 1, 3, 4-thiadiazole derivatives (Fig-43). The structures of the compounds were established by means of IR, ^{1}H NMR and Mass spectrum analysis. All the compounds were evaluated for antibacterial and antifungal activities. Most of the compounds have shown significant antibacterial and antifungal activity when compared with the standard drugs.

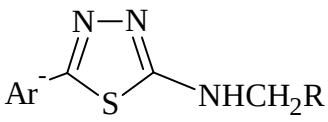


Fig-43

R = C_6H_6N & C_6H_5NCl

Ar = C_6H_5, C_6H_4OH, $C_6H_4N(CH_3)_2$, C_6H_4OH, C_6H_4Cl, $C_6H_4NO_2$ & C_4H_3S

2.2.12) Foroumadi A *et al.*, (2007) synthesised some novel 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole derivatives and evaluated for their anti-tuberculosis activity. QSAR studies were subsequently used to find the structural requirements for activity of this series of compounds. It was concluded that the below mentioned compound (Fig-44) was the anti-mycobacterial agents showing MIC value of 6.25 μg.ml-1. The results of QSAR study demonstrated that electronic distribution is among the most important determining factors for activity in this series of compounds.

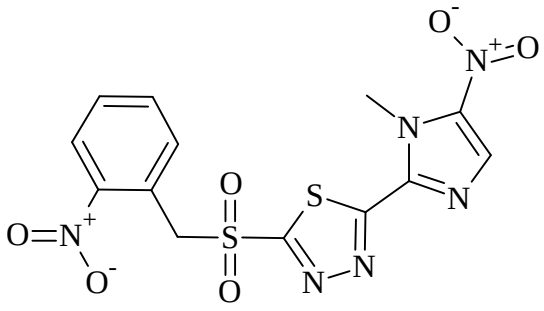


Fig-44

2.2.13) Mohammad Asif *et al.*, (2009) designed, synthesized and investigated the *in vivo* anti-inflammatory activity of some 2,4-disubstituted-5-imino1, 3, 4-thiadiazole derivatives. The below mentioned compound IIIg (Fig-45), (2-p-aminophenyl-4-phenyl-5-imino-Δ2-1, 3, 4-thiadiazole) exhibited highest anti-inflammatory activity (P <0.0001) with a percentage inhibition of 35.5. It was concluded that the thiadiazole compounds can potentially be developed into useful anti-inflammatory agents.

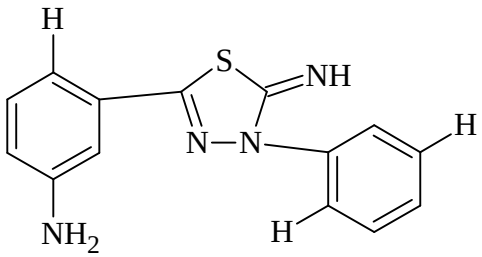


Fig-45

2.2.14) Mahmoud M M Ramiz *et al.*, (2011) synthesized a number of new 2,5-disubstituted 1,3,4-thiadiazole and their S- or N-substituted derivatives as well as the corresponding sugar hydrazone and tested them for their antimicrobial activity against *Bacillus subtilis* (gram positive), *Pseudomonas aeruginosa* (gram negative), and Streptomyces species (Actinomycetes). The results revealed that compound 9 (Fig-46) showed the highest inhibition activity against *Pseudomonas aeruginosa*, whereas compound 6 (Fig-47) were the most active among the series of tested compounds against Streptomyces species with MIC values of 75 µg/mL.

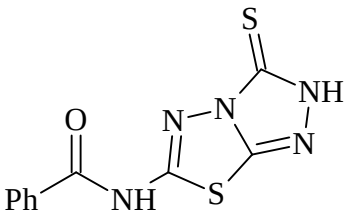


Fig-46

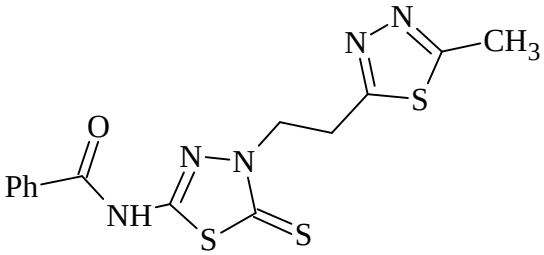


Fig-47

2.2.15) Brijendra Kumar Soni *et al.*, (2011) synthesized 1,3,4-thiadiazole derivatives (Fig-48) and were investigated for their *in vitro* antioxidant activity. The results revealed that, some of tested compounds showed potent antioxidant activity. Among all the compounds, compound 1 has shown good activity. While the compound 2 has shown moderate activity.

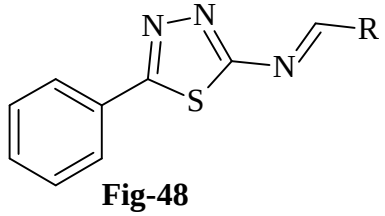


Fig-48

S.No.	R
1	4-OCH_3-C_6H_4-
2	2-Furyl

2.2.16) Parmar Kokila *et al.*, (2011) synthesized some new and biologically active [1,2,4] triazolo [3,4-b][1,3,4] thiadiazole-2-aryl-thiazolidinone-4-ones by reaction of

Schiff bases with mercapto acetic acid in presence of THF with adding anhydrous $ZnCl_2$. The structures of the synthesized compounds have been established on the bases of IR, PMR, CMR and elemental analysis. The compounds have been evaluated for antibacterial activity against *B. subtilis, S. aureus, P. aeruginosa and E. coli*. The compound 1, 2, 3 and 4 (Fig-49) were shown significant activities.

S.No.	Ar
1	4-OCH_3-C_6H_5
2	4-OH-C_6H_5
3	2-OH-C_6H_5
4	3-OC_2H_5-4-OC_2H_5-C_6H_4

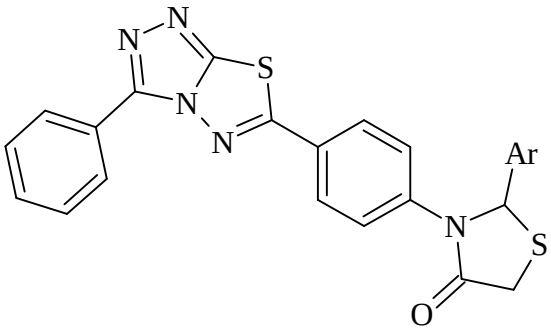


Fig-49

2.2.17) Mohammed N Al - Ahdal *et al.*, (2009) synthesized some new 1,3,4 thiadiazole analogs for the inhibition of growth of *Leishmania donovani*, the causative agent of visceral leishmaniasis, which is transmitted by sand flies and replicates intracellularly in their mammalian host cells. Fourty four 1,3,4-thiadiazole derivatives and related compounds were tested *in vitro* for possible anti-leishmanial activity against the promastigotes of *L. donovani,* out of which seven compounds were identified with potential antigrowth agents of the parasite. The below mention compound (Fig-50) was the most active compound at 50µM among all.

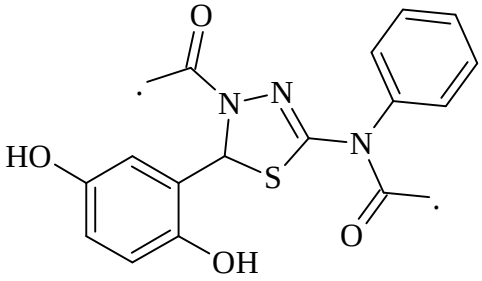


Fig-50

2.2.18) Bahar Ahmed *et al.*, (2008) synthesized a series of new imine derivatives of 5–amino-1,3,4-thiadiazole and their anti-depressant activity was tested by using imipramine as reference drug. In present study two compounds (Fig-51 & 52) have shown significant anti-depressant activity, which decreased immobility time compared to the standard imipramine. All the compounds in the series have passed neurotoxicity tests.

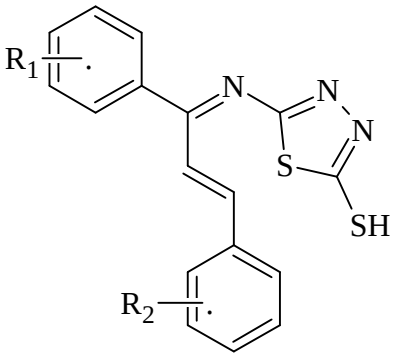


Fig-51

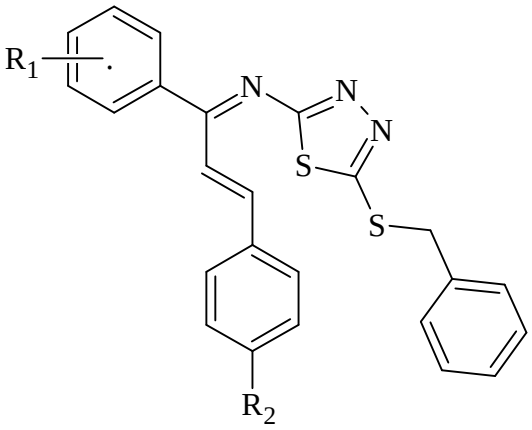


Fig-52

R_1 --- H; OCH_3; $(CH_3)_2N$; Cl; OH. **R_2** --- H; Cl.

2.2.19) Alireza Foroumadi *et al.*, (2005) synthesized a novel series of 1,3,4–thiadiazole derivatives and evaluated for *in vitro* leishmanicidal activity against *Leishmania major* promastigotes. The leishmanicidal data discovered that compounds had strong and much better leishmanicidal activity than the reference drug pentostam. The compound piperizene (Fig-53) analog was most active compound.

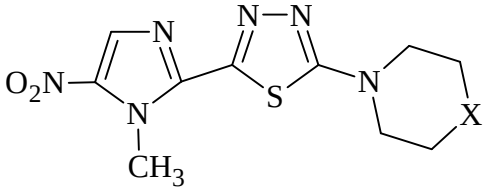


Fig-53

X --- CH_2; O; NH; NMe; NPh; NCOMe; NCOPh.

2.2.20) Mohammed K Abdel-Hamid *et al.*, (2007) synthesized a new series of 1,3,4-thiadiazole derivatives (Fig-54 and 55) and assayed for the inhibition of three physiologically relevant carbonic anhydrase isozymes, the cytosolic human isozymes I and II, and the transmembrane. In addition, docking of the tested compounds into carbonic anhydrase II active site was performed in order to expect the affinity and orientation of these compounds at the isozyme active site. The results showed similar orientation of the target compounds at CA II active site compared with reported sulfonamide type CA I with the thione group acting as a zinc-binding moiety.

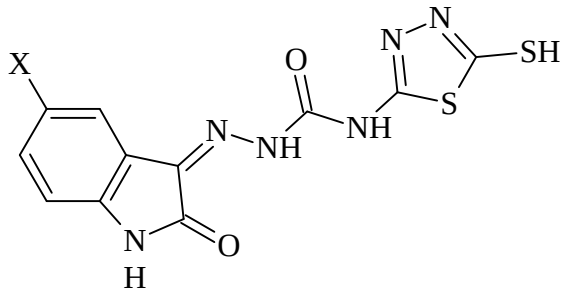


Fig-54

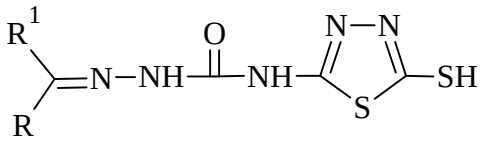


Fig-55

X --- H; Br; NO_2; **R** --- H; CH_3; C_6H_5.

R^1 --- C_6H_5;4-(OH)C_6H_4; 3-(OCH_3)C_6H_4; 4-(OCH_3)C_6H_4; 2-(Cl)C_6H_4; 4-(Cl)C_6H_4; 3-(OCH_3)-4-(OH)C_6H_3; 3-(Br)C_6H_4; 4-(Br)C_6H_4; 4-(F)C_6H_4; 2-(NO_2)C_6H_4; 4-(NO_2)C_6H_4; 2-[$N(CH_3)_2$]C_6H_4; C_6H_5; 4-(CH_3)C_6H_5; 3-Pyridyl; 2-Furyl.

AIM
&
PLAN OF WORK

3. AIM AND PLAN OF WORK

AIM

Research for the development of new therapeutic agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to discover newer, more potent molecules, with higher specificity and reduced toxicity than the existing ones. In addition, the various types of resistant microorganisms that are discovered now-a-days are becoming a great challenge for the scientists.

The existing drugs that are available are either very expensive or are prone to microbial resistance. Most of the drugs that are marketed today are modified derivatives of existing pharmacophores. No new pharmacophore having a novel mechanism of action has been identified in the recent past. To overcome these problems, it becomes necessary for further investigating newer molecules to treat infections at affordable costs.

Fluorine incorporated benzothiazole and 1,3,4 - thiadiazole derivatives are known to possess a variety of physiological properties like anti-microbial, anti-tubercular, anti-tumor, anti-inflammatory, anti-hypertensive, anti-convulsant, anti-diabetic, anti-viral, anthelmintic, diuretic, anti-depressants, antioxidants, analgesic etc., It was felt interesting to bring these two biologically active moieties within a molecular frame work with a view to study their additive effect on biological properties.

With the dual aim of developing potential therapeutic agents and studying their chemistry we undertook the synthesis of biologically active fluorobenzothiazole incorporated with 1,3,4 - thiadiazole compounds for anti-bacterial and anti-inflammatory activity with minimum toxic levels.

PLAN OF WORK

1. Literature was collected on related benzothiazole and 1, 3, 4 - thiadiazole compounds.
2. Synthesis of fluorobenzothiazole incorporated 1, 3, 4 - thiadiazole compounds by appropriate method as per literature.
3. Characterization of the newly synthesized compounds by physical data such as
    - ★ Colour and nature
    - ★ Melting point
    - ★ Solubility
    - ★ Thin layer chromatography (R_f value)
    - ★ UV – Visible Spectroscopy
4. Conformation of the structures of the synthesized compounds by
    - ★ IR Spectroscopy
    - ★ NMR Spectroscopy
    - ★ Mass Spectroscopy
5. Evaluation of antimicrobial activities for synthesized compounds (SH_6 and SH_{11}) by disc diffusion method.
6. Evaluation of anti-inflammatory activity for the synthesized compounds (SH_8 and SH_{11}) by carrageenan induced paw oedema method.

EXPERIMENTAL

4. EXPERIMENTAL

4.1. Materials

4.1.1. List of Chemicals and Drugs Used:

S. No	Name of Chemicals / Drugs	Manufacture
1	3-chloro-4-fluoro aniline	Sisco Research Lab. Pvt. Ltd., Mumbai.
2	2 – Furoic acid	Central Drug House Pvt. Ltd., Chennai.
3	2 – Nitro aniline	Loba Chem. Pvt. Ltd., Mumbai.
4	4 – Nitro aniline	Loba Chem. Pvt. Ltd, Mumbai.
5	Anthranilic acid	Spectro Chem. Pvt. Ltd., Mumbai.
6	Ammonia solution	Loba Chem. Pvt. Ltd., Mumbai.
7	Benzene	Qualigens Fine Chem. Pvt. Ltd., Mumbai.
8	Bromine	Loba Chem. Pvt Ltd, Mumbai.
9	Carbon disulphide	Loba Chem. Pvt. Ltd., Mumbai.
10	Chloroform	Spectro Chem. Pvt. Ltd., Mumbai.
11	Carrageenan sodium	Sigma Chemical Co. Ltd., USA
12	Diclofenac sodium	German Remedies Ltd., Mumbai.
13	Dimethyl formamide	Loba Chem. Pvt. Ltd., Mumbai.
14	Ethanol	Changshu Yangyuan Chemicals, China.
15	Glacial acetic acid	Qualigens Fine Chem. Pvt. Ltd., Mumbai.
16	Hydrazine hydrate	Loba Chem. Pvt. Ltd., Mumbai.
17	Methanol	Qualigens Fine Chem. Pvt. Ltd., Mumbai.
18	Nicotinic acid	Loba Chem. Pvt. Ltd., Mumbai.
19	Phosphrous oxychloride	Loba Chem. Pvt. Ltd., Mumbai.
20	Potassium thiocyanate	Loba Chem. Pvt. Ltd., Mumbai.
21	Polyethylene glycol	Spectro Chem. Pvt. Ltd., Mumbai.
22	Sodium chloro acetate	Himedia Laboratories Pvt. Ltd., Mumbai.

4.1.2. List of Instruments Used:

S. No	Name of Instrument	Manufacture
1	^{1}H-NMR spectrophotometer	JEOL JNM-α 400 spectrometer
2	Digital electronic balance	Shimadzu Ax - 200
3	Double beam UV-spectrophotometer	Shimadzu 1601 UV-Spectrophotometer, Japan.
4	FT-IR spectrophotometer	Perkin-Elmer and Fischer Nicolet Spectroscopy,
5	Heating mantle	Ajay, Thermo Electrics, Chennai
6	Hot air oven	Precision Scientific Co., Chennai.
7	Mass spectrophotometer	Jeol SX102/DA-6000 Mass Spectrometer
8	Magnetic Stirrer	Remi Equipments, Chennai.
9	Melting point apparatus	Elico Ltd., Hyderabad.
10	Plethysmometer	Inco Rat Paw Plethysmograph Mercury Model, Ambala, India.

Analytical grade solvents and reagents were used throughout the experiment. All the glasswares of borosil A grade and were as oven or flame dried for moisture sensitive reactions. When necessary, solvents and reagents were dried prior to use. Solution or extracts in organic solvents were dried over anhydrous sodium sulphate or fused calcium chloride before evaporation to under vacuum using rotary evaporator. Analytical samples were dried in vaccum and were free of significant impurities on TLC.

4.1.3. Microorganisms:

The gram positive bacteria (*Micrococcus luteus* NCTC 4635), gram negative bacteria (*Proteus vulgaris* NL98) and fungal strain *(Aspergillus flavus* ATCC 46646) were procured from Microbial Resources Division, Kings Institute of Preventive

Medicine, Guindy, Chennai. The agar medium was purchased from HI Media Laboratories Ltd., Mumbai, India.

4.1.4. Animals:

The animals (Wistar albino rats of either gender) were procured from the Kings Institute of Preventive Medicine, Guindy, Chennai. Animals were housed in animal house in Adhiparasakthi College of Pharmacy in standard environmental conditions of temperature (25 ± 20º C), humidity (55 ± 10%) and light (12 : 12 hour light: dark cycle). The animals were fasted prior to dosing but water was given *ad-libitum*. The anti-inflammatory activity was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines after obtaining the approval from the Institutional Animal Ethical Committee.

4.1.5. Analytical Techniques

4.1.5.1. Physical Data:

Melting points of the synthesized compounds were taken in open capillaries on Guna Enterprises melting point apparatus and are uncorrected.

4.1.5.2. Thin Layer Chromatography (TLC):

Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and various combinations of ethyl acetate and chloroform (2:1) mobile phase. The spots resolved were visualized in UV chamber or Iodine vapour.

4.1.5.3. Instrumentation:

The techniques employed for the characterization of the synthesized compounds were UV, IR spectra, ^{1}H NMR spectra, Mass spectra.

UV spectra:

The Ultra Violet Spectroscopy of the synthesized compounds was recorded on double beam UV spectrophotometer (SHIMADZU -1700) at Adhiparasakthi College of Pharmacy.

Infrared Spectra:

The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (Perkin-Elmer and Fischer Nicolet) in the range of 4000 – 450 cm^{-1} by KBr pellets technique. It was recorded at Indian Institute of Technology Madras, Chennai and Ideal Analytical and Research Institution, Puducherry.

Nuclear Magnetic Resonance Spectra (^{1}H NMR):

^{1}H NMR spectra were recorded at Sophisticated Analytical Instrumentation Facility, IITM, Chennai on (Bruker-NMR 400mHZ) spectrometer using DMSO-d_6 as solvent and chemical shifts were reported in δ values using tetramethylsilane as internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, m-multiplet).

Mass Spectra:

The mass spectrum of the synthesized compounds was recorded on Jeol SX102/DA-6000 apparatus at Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology Madras, Chennai.

4.2 Methodology:

Monitoring of Synthetic Reaction Procedures:

Established synthetic procedures were employed for synthesis of compounds SH_1 to SH_{11} and the reactions were monitored by Thin Layer Chromatography (TLC) employing 6'' X 2'' plates coated with 0.25 mm thick layer of silica gel (pre-activation by heating at 110° C for one hour). Solvent systems of varying combinations of ethyl acetate and chloroform (2:1) mobile phase was used to monitor the reactions. The plates were visualized in UV chamber or Iodine vapour.

Purification Techniques:

Recrystallization: The crude products were recrystallized by charcoal treatment in appropriate solvent. Wherever possible combinations of solutions were used.

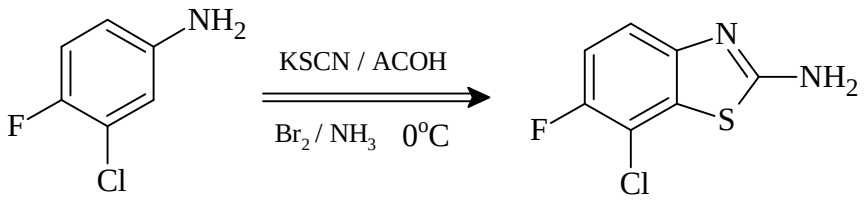
Authentication of Chemical Structures and Purity of Compounds:

Chemical structure of products and their purity were ascertained by thin layer chromatography, UV-Visible spectrometer, melting point and various spectral techniques including Ultra Violet Spectrophotometry, Fourier Transform Infra Red Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, and Mass Spectroscopy.

4.3. Synthesis of Compounds:

4.3.1 Synthesis of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole: (SH_1)

Reaction:



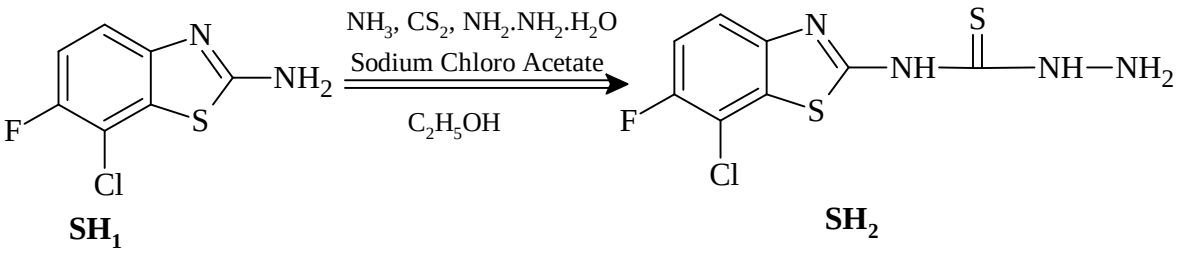
3-chloro-4-fluoro aniline **SH_1**

Procedure:

To the glacial acetic acid (20 ml) which was cooled below room temperature, added 8 gm (0.08 mole) of potassium thiocyanate and 1.44 gm (0.01 mole) of 4-flouro-3-chloro aniline. The mixture was placed in cold mixture of ice and salt and mechanically stirred. 1.6 ml of bromine in 6 ml of glacial acetic acid was added from a dropping funnel at such a rate that temperature never rose beyond 0º C. After all the bromine was added (105 minutes) the solution was stirred for 2 hours in ice cold condition and at room temperature for 10 hours. It was then allowed to stand overnight, during the period orange precipitate settled at the bottom, water (6 ml) was added quickly in it and slurry was heated to 85º C on a steam bath and filtered while hot. The orange residue was placed in a reaction flask and treated with 10 ml of glacial acetic acid heated again to 85º C on a steam bath and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution up to pH-6. The precipitate was collected and recrystallized from benzene and ethanol (1:1) after treatment with charcoal gave yellow crystal of 2-amino-6-fluoro-7-chloro benzothiazole. (Bhushankumar S Sathe *et al.*, 2011)

4.3.2 Synthesis of 6-fluoro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide: (SH_2)

Reaction:



SH_1 **SH_2**

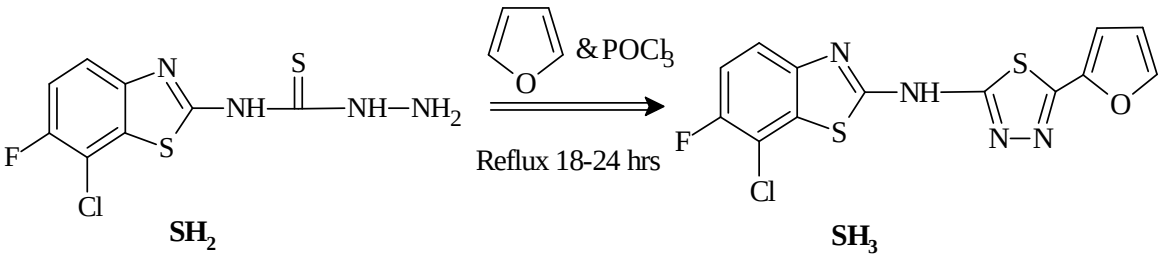
Procedure:

2-amino-6-fluoro-7-chloro benzothiazole (SH_1) 20.1 gm (0.1mole) was dissolved in 50 ml of ethanol (95%) and 8 ml of ammonia solution was added to it. The reaction mixture was cooled below 30º C and 8 ml of carbon disulphide was added slowly within 15 minutes with continuous shaking. After complete addition of disulphide the solution was cooled to stand for 1 hour. Then 9.4 gm (0.1 mole) sodium chloro acetate was added to it. The reaction was exothermic. To it 20 ml of 50% hydrazine hydrate was added. The mixture was warmed gently, filtered and boiled to half of its volume and kept overnight. Next day, the product thiosemicarbazide was filtered and recrystalised from ethanol.

(Vedavathi M *et al.*, 2010)

4.3.3 Synthesis of 7-chloro-6-fluoro-N-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine: (SH_3)

Reaction:



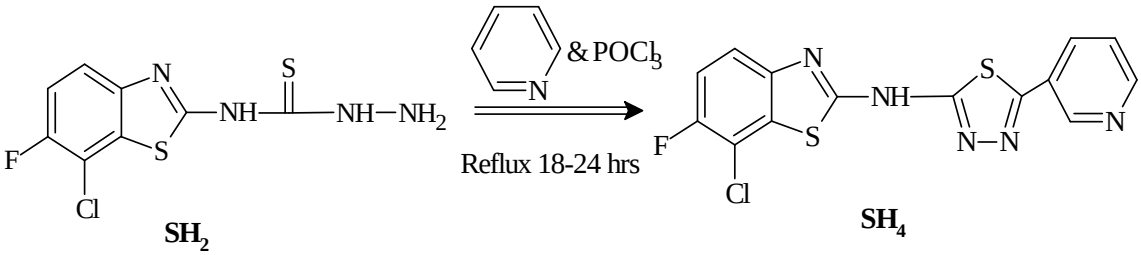
SH_2 **SH_3**

Procedure:

A mixture of 6-fluro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide (0.01 mole), 2 – furoic acid (0.1mole) and phosphrous oxychloride 25ml was refluxed for 18 – 24 hours. The reaction mixture was cooled to the room temperature and was slowly poured in to crushed ice and kept overnight. The solid separated was filtered, dried and recrystallized from methanol. (Senthil Kumar G P *et al.,* 2011)

4.3.4 Synthesis of 7-chloro-6-fluoro-N-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine: (SH_4)

Reaction:



SH_2 **SH_4**

Procedure:

A mixture of 6-fluro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide (0.01 mole), nicotinic acid (0.1mole) and phosphrous oxychloride 25ml was refluxed for 18 – 24 hours. The reaction mixture was cooled to the room temperature and was slowly poured in to crushed ice and kept overnight. The solid separated was filtered, dried and recrystallized from methanol. (Senthil Kumar G P *et al.,* 2011)

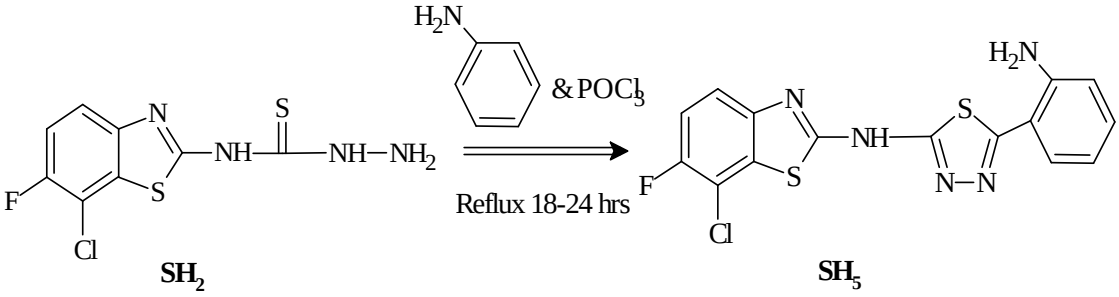
4.3.5 Synthesis of 7-chloro-6-fluoro-N-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-1-3-benzothiazol-2-amine: (SH_5)

Procedure:

A mixture of 6-fluro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide (0.01 mole), anthranilic acid (0.1 mole) and phosphrous oxychloride 25 ml was refluxed for 18 – 24 hours. The reaction mixture was cooled to the room temperature and was

slowly poured into crushed ice and kept overnight. The solid separated was filtered, dried and recrystallized from methanol. (Senthil Kumar G P *et al.*, 2011)

Reaction:



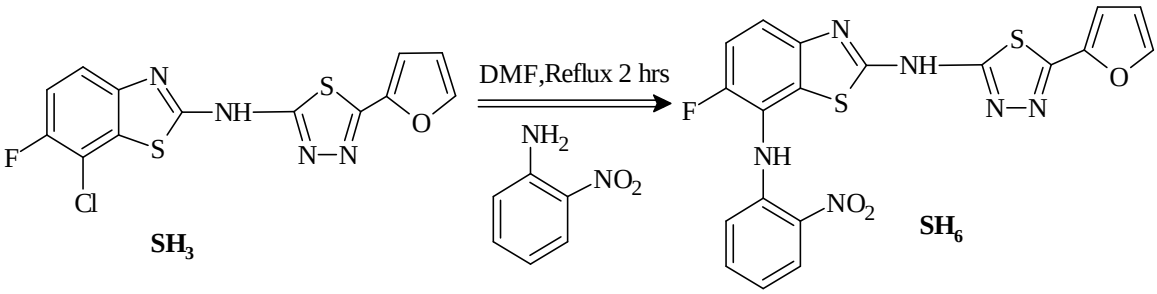
4.3.6 Synthesis of 6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-N^7-(2-nitrophenyl)-1,3-benzothiazole-2,7-diamine: (SH_6)

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 2 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.*, 2010)

Reaction:

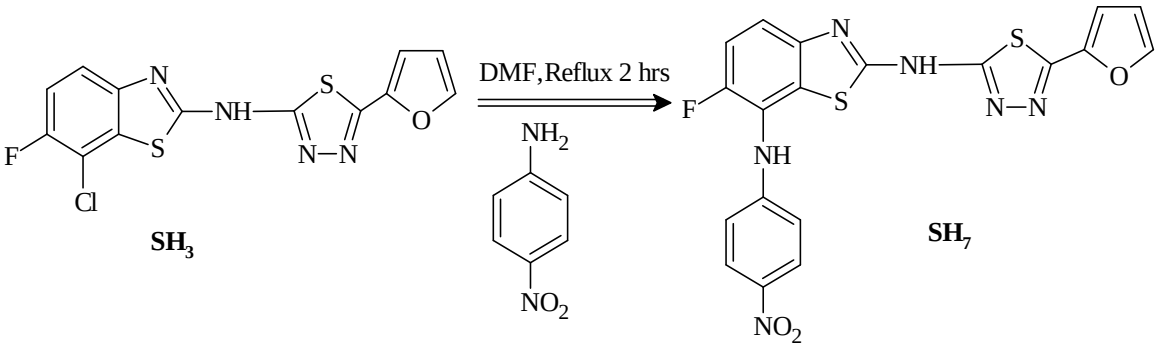


4.3.7 Synthesis of N^7-(2,4-dinitrophenyl)-6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine: (SH_7)

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 4 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.,* 2010)



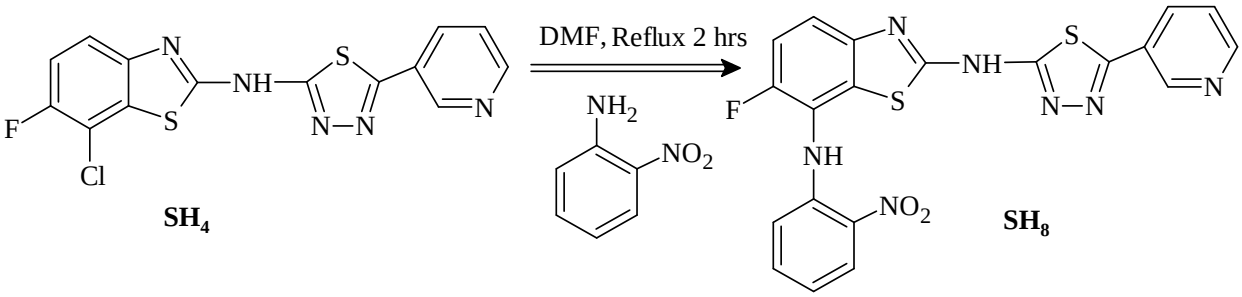
4.3.8 Synthesis of 6-fluoro-N^7-(2-nitrophenyl)-N^2-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine:(SH_8)

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 2 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.,* 2010)

Reaction:



SH_4 **SH_8**

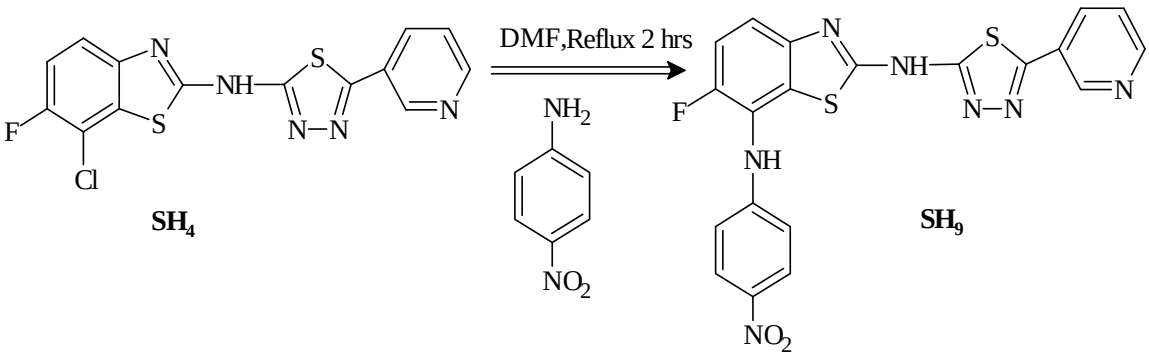
4.3.9 Synthesis of N^7-(2,4-dinitrophenyl)-6-fluoro-N^2-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine: (SH_9)

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 4 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.*, 2010)

Reaction:



SH_4 **SH_9**

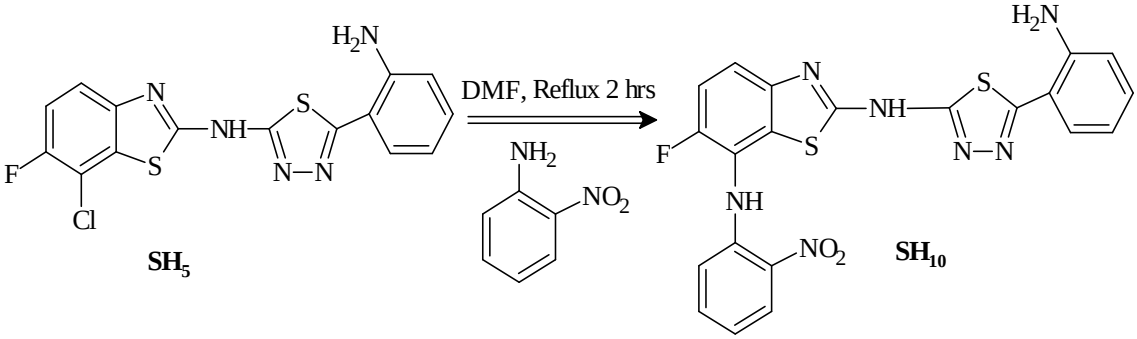
4.3.10 Synthesis of 6-fluoro-7 N^2-[5-(2-aminophenyl)-1, 3, 4-thiadiazol-2-yl] -N^7-(2-nitrophenyl)-1, 3-benzothiazole-2, 7-diamine:(SH_{10})

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-1-3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 2 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.*, 2010)

Reaction:



SH_5 **SH_{10}**

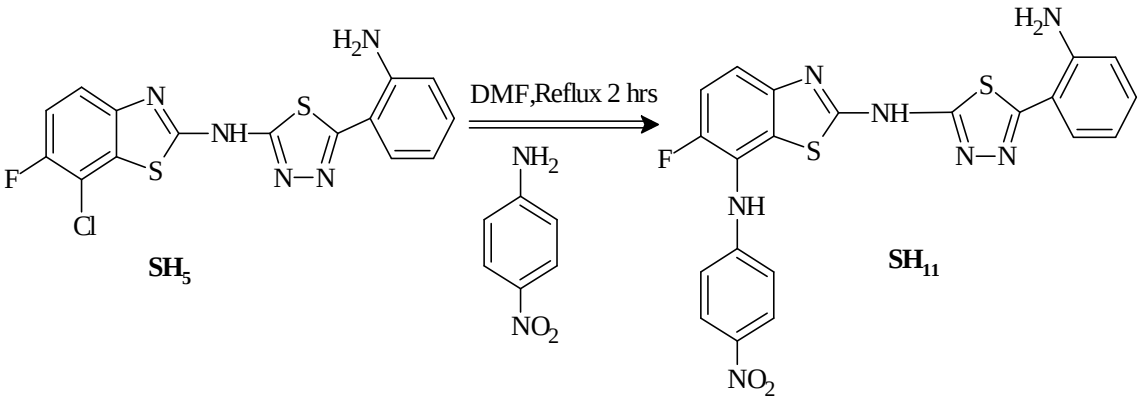
4.3.11 Synthesis of 6-fluoro-N^2-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl] -N^7-(4-nitrophenyl)-1,3-benzothiazole-2,7-diamine: (SH_{11})

Procedure:

0.0025 mole of 7-chloro-6-fluoro-N-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-1-3-benzothiazol-2-amine was treated with equimolar quantity (0.0025 mole) of 4 – nitro aniline was substituted and refluxed for 2 hours in the presence of DMF. The mixture was then cooled and poured in to crushed ice. The solid separated was filtered, dried and recrystallized from benzene and absolute alcohol (1:1).

(Gupta Akhilesh *et al.*, 2010)

Reaction:



SH_5 **SH_{11}**

BIOLOGICAL EVALUATION

5. BIOLOGICAL EVALUATION

5.1. Evaluation of Antimicrobial Activity:

The antimicrobial screening of the synthesized compounds (SH_6-SH_{11}) were carried out by determining the zone of inhibition using disc diffusion method. The synthesized compounds were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Final inoculums of 100 µl suspension containing 10^8 CFU/ ml of each bacterium and fungus used. Nutrient agar (anti-bacterial activity) and sabouraud's dextrose agar medium (anti-fungal activity) was prepared and sterilized by an autoclave (121° C and 15 lbs for 20 minutes) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45° C, and fungal organism in sterile sabouraud's dextrose agar medium at 45° C in aseptic condition. Sterile Whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25 and 100 µg/disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 minutes to allow the diffusion of compounds at room temperature. Antibiotic discs of ciprofloxacin (100 µg/disc) and ketaconazole (100 µg/disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 hours at 37 ± 1° C for antibacterial activity and 48 hours at 37 ± 1° C for anti-fungal activity. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the disc.

5.2. Acute Oral Toxicity Studies:

The oral acute toxicity of synthesized compounds SH_8 and SH_{11} was

determined by using albino mice of either sex (20 – 25 gms) maintained under standard husbandry conditions. This involves the estimation of median lethal dose (LD_{50}), which is the dose that kills 50% of the animal population within 24 hours of the post treatment of the test substance. The acute oral toxic class method (OECD 423) was adopted. The animals used for the studies where in accordance with principles of laboratory animal care and were approved by institutional animal ethical committee. The animals were fasted overnight prior to the experiment and were then grouped (3 mice per group). They were treated intra-peritoneally with different doses of the test compounds (5, 50, 300 mg/kg). The animals were then observed for 24 hours for any behavioral effects such as nervousness, excitement, dullness, incoordination or even death. Acute toxic class (OECD guidelines No. 423) method of CPCSEA was adopted for toxicity studies. $1/5^{th}$ of the lethal dose was taken as effective dose ED_{50} (Therapeutic dose).

5.2. Evaluation of Anti-Inflammatory Activity:

The anti-inflammatory activity of the synthesized derivative SH_8 and SH_{11} was evaluated by carrageenan-induced paw oedema method. Wistar albino rats of either sex (150 – 200 g) were randomly selected and the animals were divided into control, standard and test groups, each consisting of three animals. The first group was treated with 1% polyethylene glycol suspension which served as control, second group was administered with a dose of 20 mg/kg suspension of diclofenac sodium intra-peritoneally which served as standard and other groups were treated with 50 mg/kg of suspension of test compounds in 1% polyethylene glycol. After 30 minutes, the rats were injected with 0.1 ml of carrageenan (1% w/v) to the sub plantar region of left paw of the rats. The volume of paw was measured using mercury displacement

technique with the help of plethysmograph in control and animals treated with standard and test compounds at 0, 1, 2 and 3 hour after injection of carrageenan.

The percentage inhibition of oedema was calculated by using formula,

$$\text{Percentage Reduction} = \frac{V_c - V_t}{V_c} \text{ X } 100$$

Where, V_t = mean paw volume of the test drug,

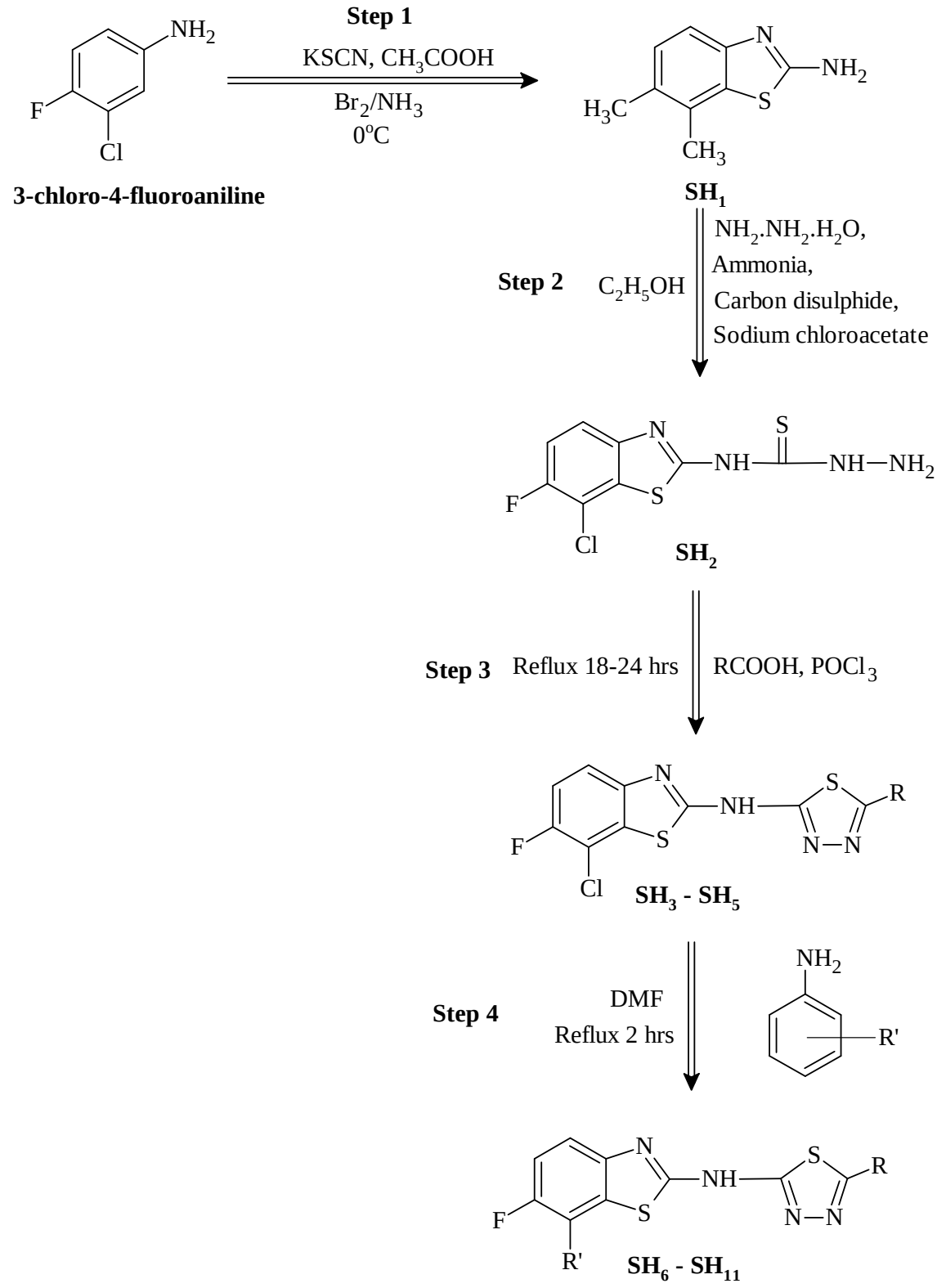
V_c = mean paw volume of the control

From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated.

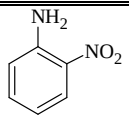
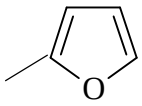
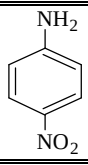
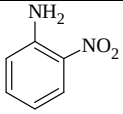
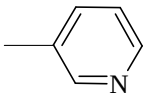
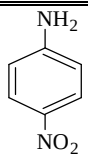
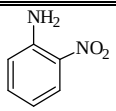
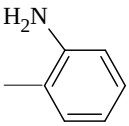
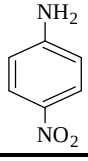
RESULTS
&
DISCUSSION

6. RESULTS AND DISCUSSION

6.1. Synthetic Scheme



Different substitutions in the compounds $SH_6 - SH_{11}$

S. No	Compound Code	R'	R
1	SH_6		
2	SH_7		
3	SH_8		
4	SH_9		
5	SH_{10}		
6	SH_{11}		

1,3-benzothiazole and 1,3,4-thiadiazole are the most common and important groups among the nitrogen and sulphur containing heterocyclic compounds. Many methods had been reported in the literature for the synthesis of 1,3 benzothiazole such as condensation of o-aminothiophenol with aldehydes, cyclization of thioformanilides using different reagent, coupling between thiophenols and aromatic nitriles, synthesis using anilines and also by using microwave irradiation method. Among this synthesis of benzothiazole by using anilines is the most common method which involves the reaction of different substituted aniline with potassium thiocynate in the presence of glacial acetic acid.

The substitutions of fluorine in the benzothiazole moiety increases lipid solubility which in turn increases the transport and absorption of drug *in vivo* and a

strong electron withdrawing inductive effect of fluorine can significantly influence reactivity and stability of functional groups and the reactivity of neighboring reaction centers. (Kuntal Hazra *et al.,* 2011) Hence to fluorinate the benzothiazole 4-fluoro 3-chloro aniline was chosen.

1,3,4-thiadiazole can be synthesized from thiosemicarbazides, dimethyl formamaide and hydrazine. The most common method to synthesize 5-substituted 2-amino-1,3,4-thiadiazoles is form thiosemicarbazides which involves acyclation of thiosemicarbazide followed by dehydrative cyclization using any of the below mentioned agents sulfuric acid, polyphosphoric acid, phosphorus oxychloride, phosphorus halides or more recently methane sulfonic acid.

In this present study, a series of fluorobenzothiazole incorporated 1,3,4-thiadiazole compounds were synthesized by the following strategy: initially 3-chloro 4-fluoro-aniline reacted with potassium thiocynate in the presence of glacial acetic acid and bromine at 0° C to give the corresponding 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole, which further reacts with hydrazine hydrade, ammonia, carbon disulphide, sodium chloroacetate in the presence of ethanol to form 2-thiosemicarbazide substituted 6-fluoro-7-chloro-(1,3)-benzothiazole. The third step involves acyclation of 2-thiosemicarbazide followed by dehydrative cyclization using phosphorous oxy chloride to form 1,3,4 thiadiazole moiety, in this step different aromatic acids were also substituted. Then the 1,3,4 thiadiazole substituted compound was treated with ortho and para nitro anilines, in the presence of DMF to obtain different fluorobenzothiazole incorporated 1,3,4 thiadiazole compounds.

6.2. Interpretation of spectral data of synthesized compounds ($SH_1 – SH_{11}$)

6.2.1. 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole (SH_1)

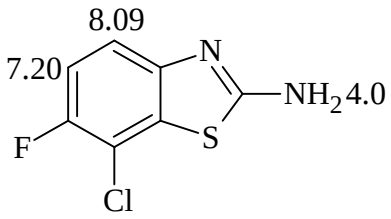
UV: (Fig-56)

λ_{max} (MeOH) 240.0 **(**ε_{max} 1.0140)

IR (Kbr): (Fig-57)

S. No	Wave Number (cm^{-1})	Assignment
1	3288	N – H stretching
2	3083	Aromatic C – H stretching
3	1637	C = N stretching
4	1543	Aromatic C = C ring stretching
5	1346	Aromatic C = N stretching
6	1193	C – F stretching
7	683	C – Cl stretching
8	617	C – S stretching

NMR (DMSO – d_6): (Fig-58)



(2 aromatic protons, 2 protons on Nitrogen)

S. No	δ	Assignment
1	8.09	1H, d, Ar – H of benzothiazole
2	7.20	1H, d, Ar – H of benzothiazole
3	4.0	2H, s, NH

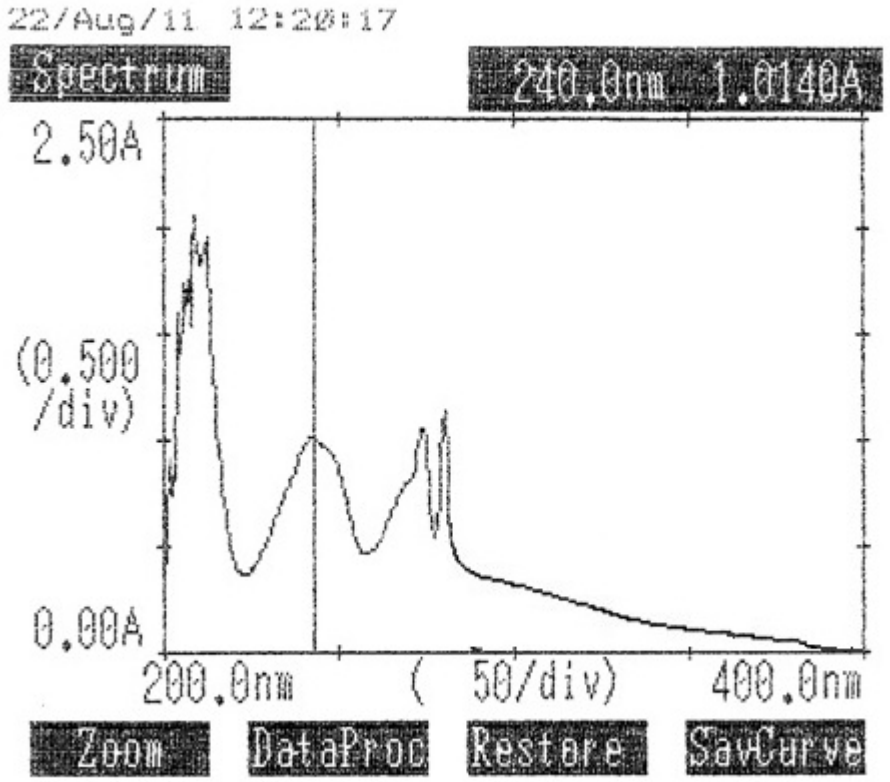


Fig.56: UV Spectrum of compound SH_1

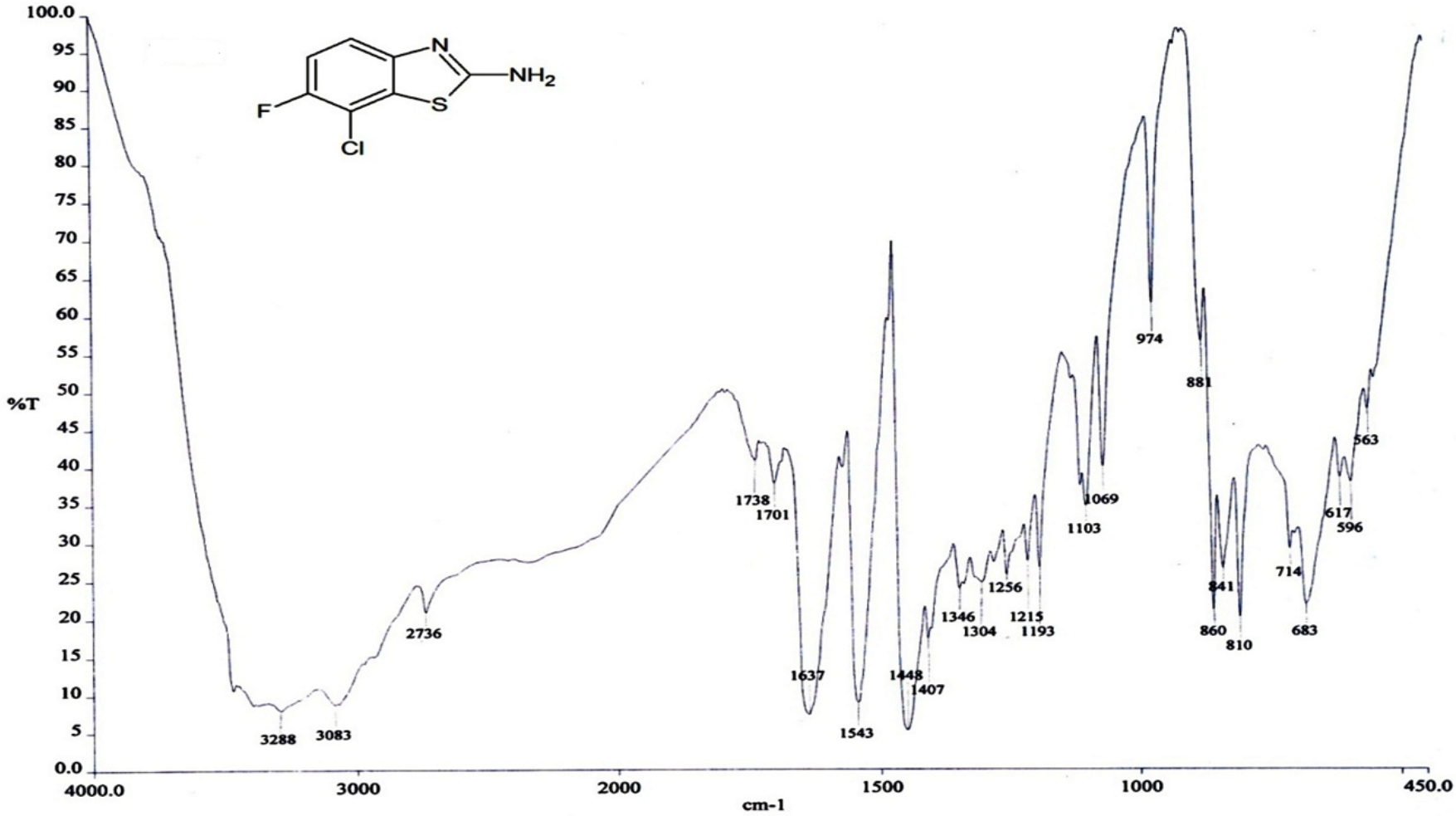


Fig.57: IR Spectrum of compound SH_1

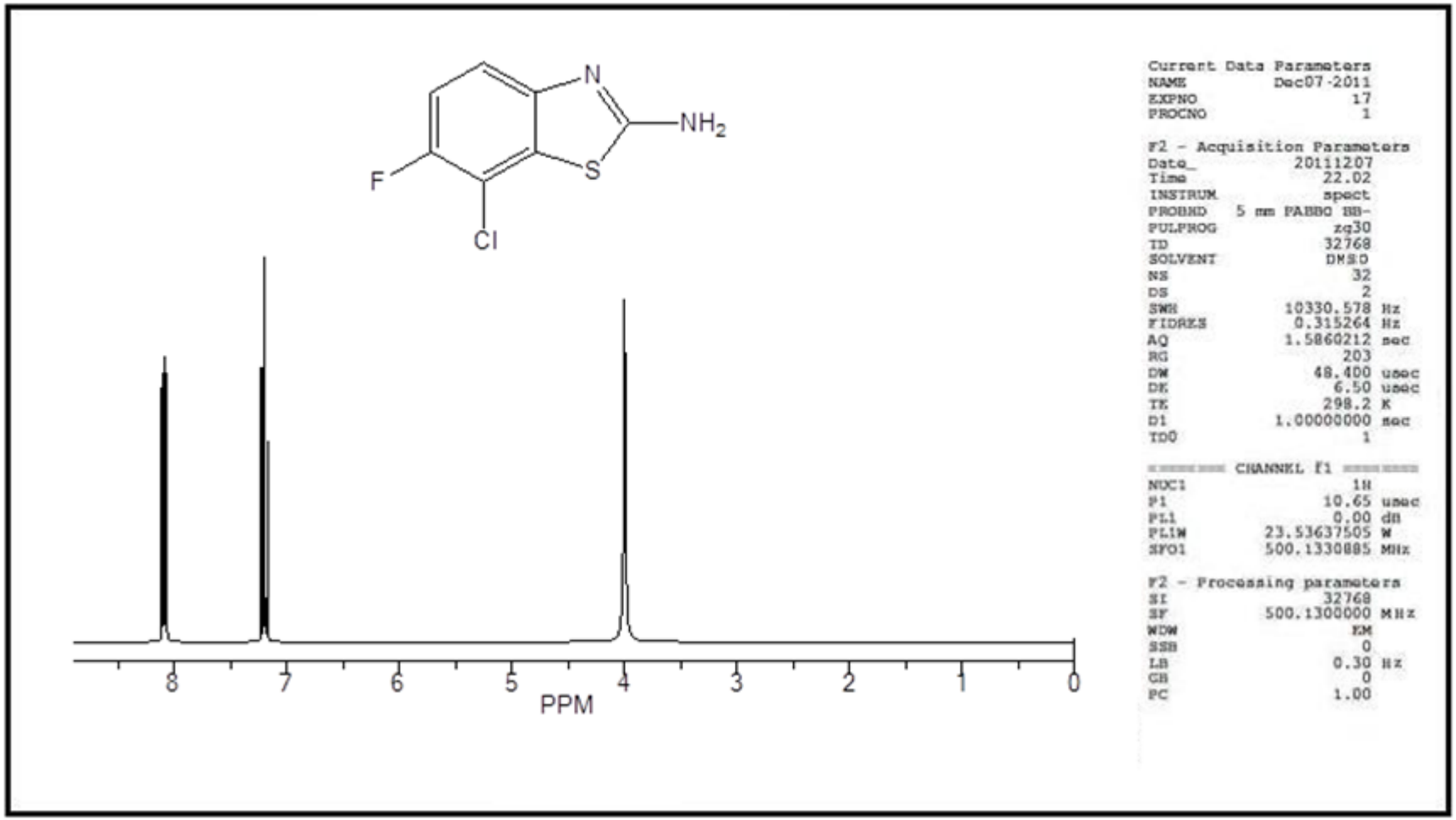


Fig-58: ^{1}H NMR Spectra of the compound SH_1

6.2.2. N-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)hydrazine carbothioamide (SH_2)

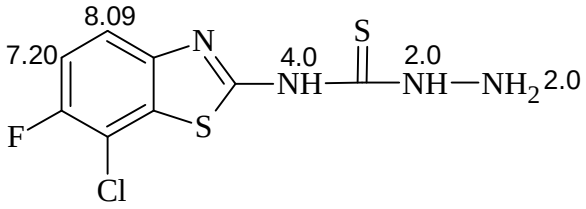
UV: (Fig-59)

λ_{max} (MeOH) 265.5 (ε_{max} 1.6844)

IR (KBr): (Fig-60)

S. No	Wave Number (cm^{-1})	Assignment
1	3473	N – H stretching
2	3077	Aromatic C – H stretching
3	1645	C = N stretching
4	1452	Aromatic C = C ring stretching
5	1337	Aromatic C – N secondary vibrations
6	1216	Aliphatic C – N vibrations
7	1194	C – F stretching
8	1071	Aliphatic C = S stretching
9	715	C – Cl stretching
10	686	C – S stretching

NMR (DMSO – d_6): (Fig-61)



(2 aromatic protons, 4 protons on nitrogen)

S. No	δ	Assignment
1	8.09	1H, d, Ar – H of benzothiazole
2	7.20	1H, d, Ar – H of benzothiazole
3	4.0	1H, s, NH
4	2.0	3H, s, NH

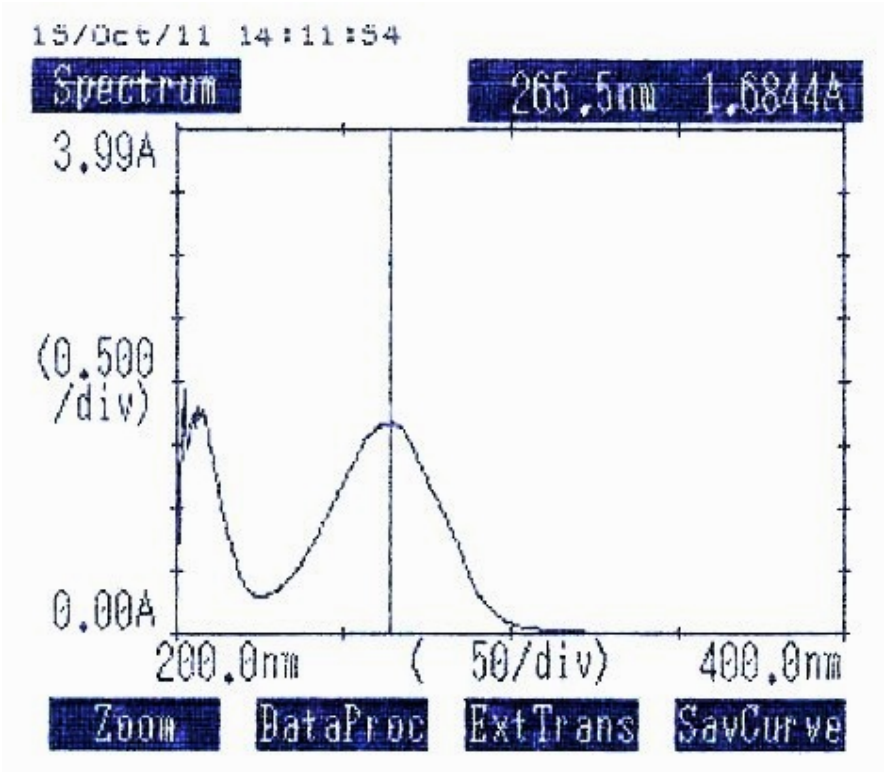


Fig.59: UV Spectrum of compound SH_2

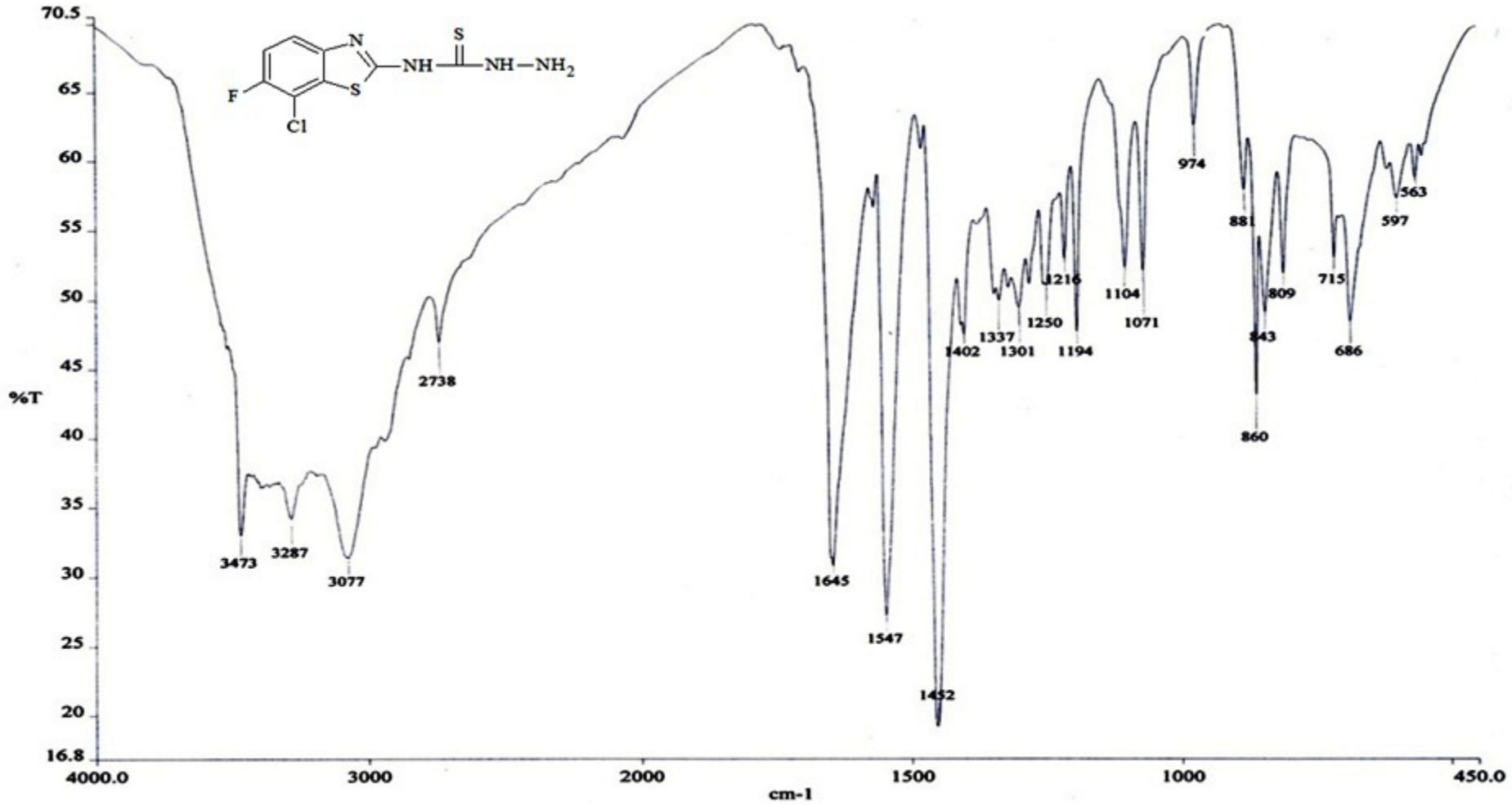


Fig.60: IR Spectrum of compound SH_2

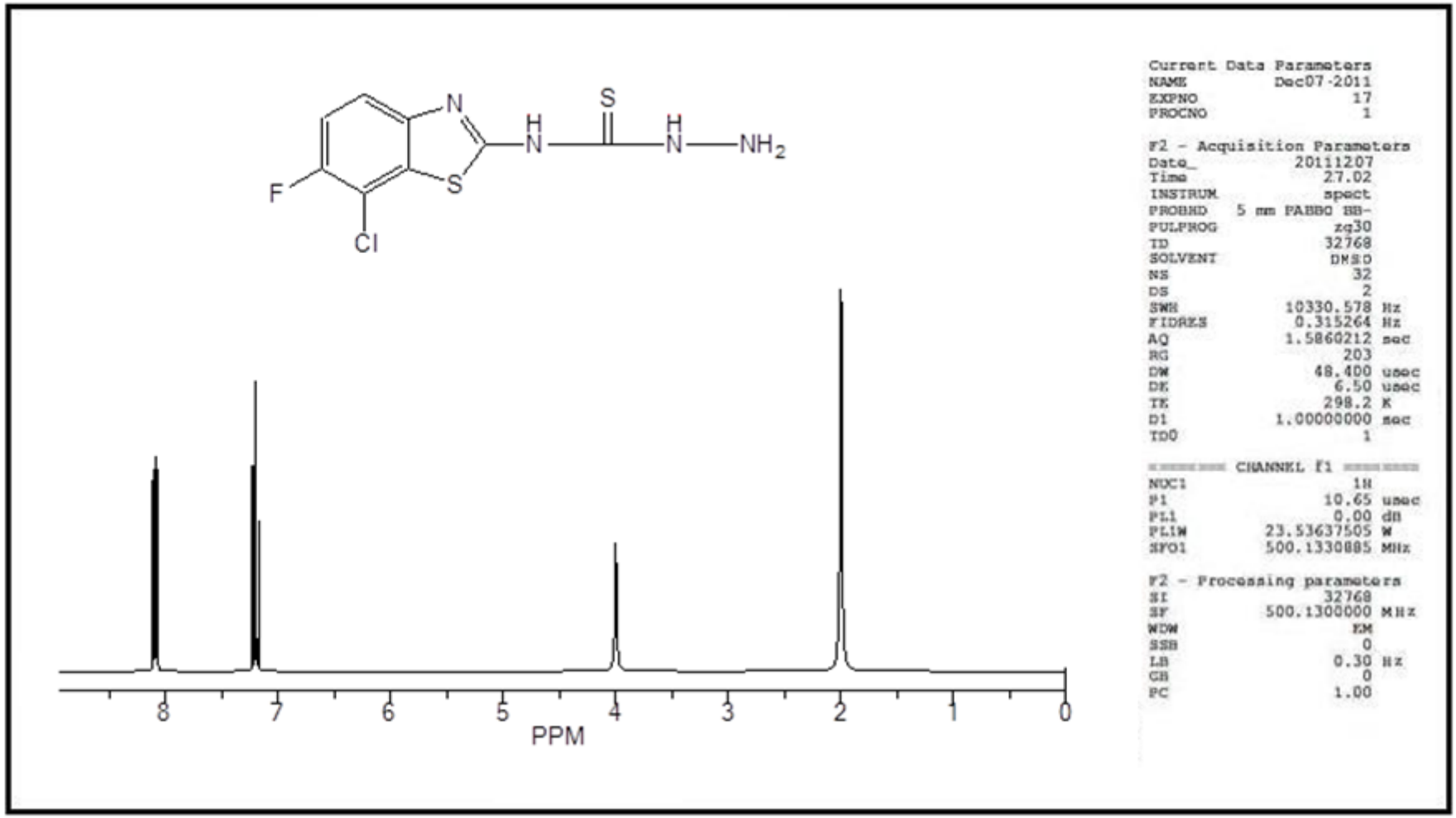


Fig-61: ^{1}H NMR Spectra of the compound SH_2

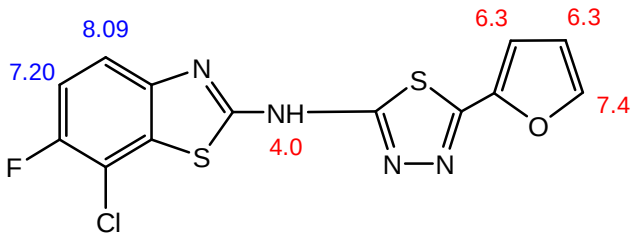
6.2.3. 7-chloro-6-fluoro-*N*-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine (SH_3)
UV: (Fig-62)

λ_{max} (MeOH) 282.0 (ε_{max} 2.6021)

IR (KBr): (Fig-63)

S. No	Wave Number (cm^{-1})	Assignment
1	3392	N – H stretching
2	3032	Aromatic C – H stretching
3	1653	C = N stretching
4	1477	Aromatic C = C ring stretching
5	1337	Aromatic C – N secondary vibrations
6	1289	C – O bending
7	1029	C – F stretching
8	844	C – Cl stretching
9	714	Aromatic C – H bending
10	642	C – S stretching

NMR (DMSO – d_6) (Fig-64)

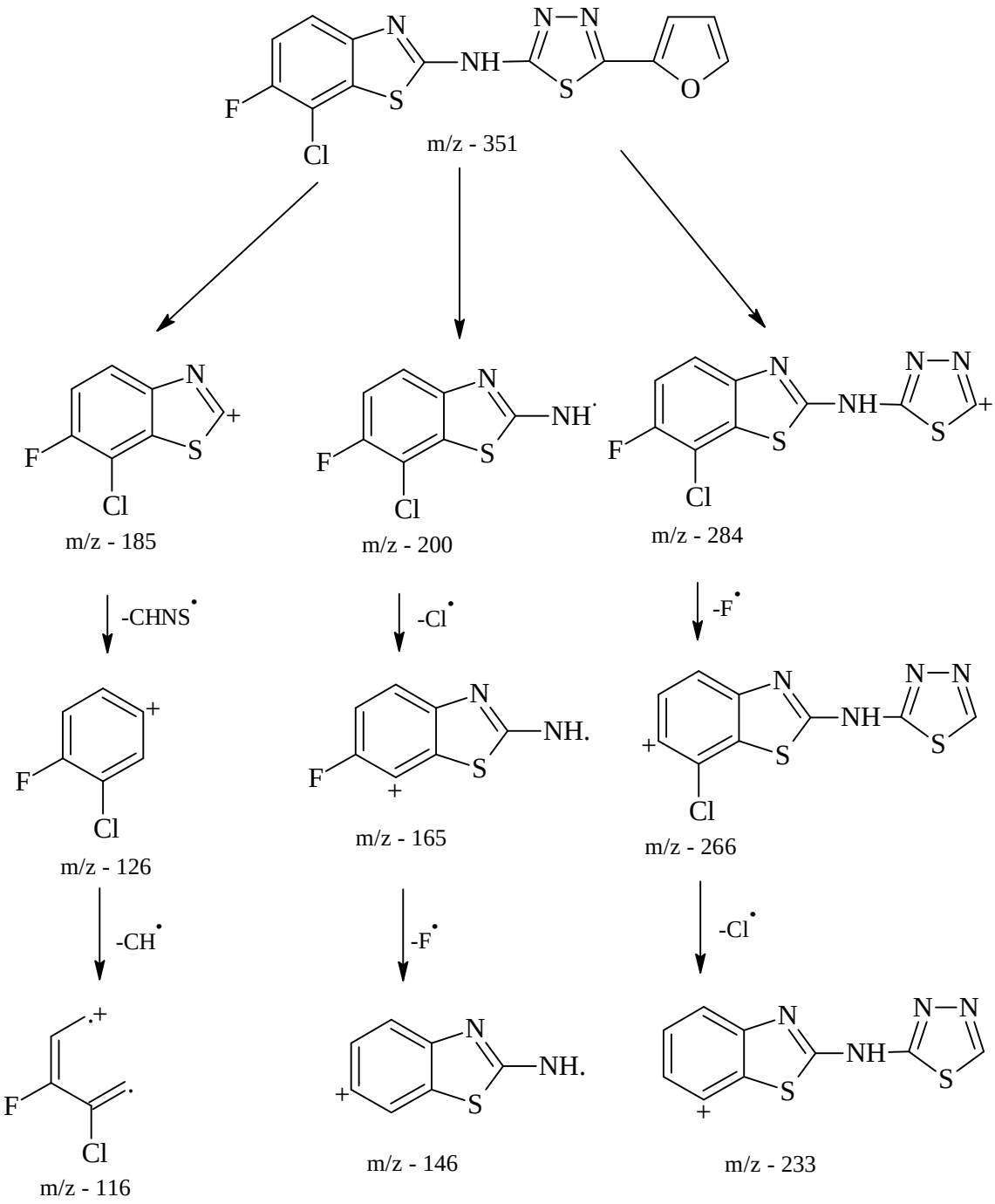


(2 aromatic protons, 3 protons of furan, 1 proton on nitrogen)

S. No	δ	Assignment
1	8.09	1H, d, Ar – H of benzothiazole
2	7.20	1H, d, Ar – H of benzothiazole
3	7.4	1H, d, CH of furan
4	6.3	2H, d, CH of furan
5	4.0	1H, s, NH

MASS: (Fig-65)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



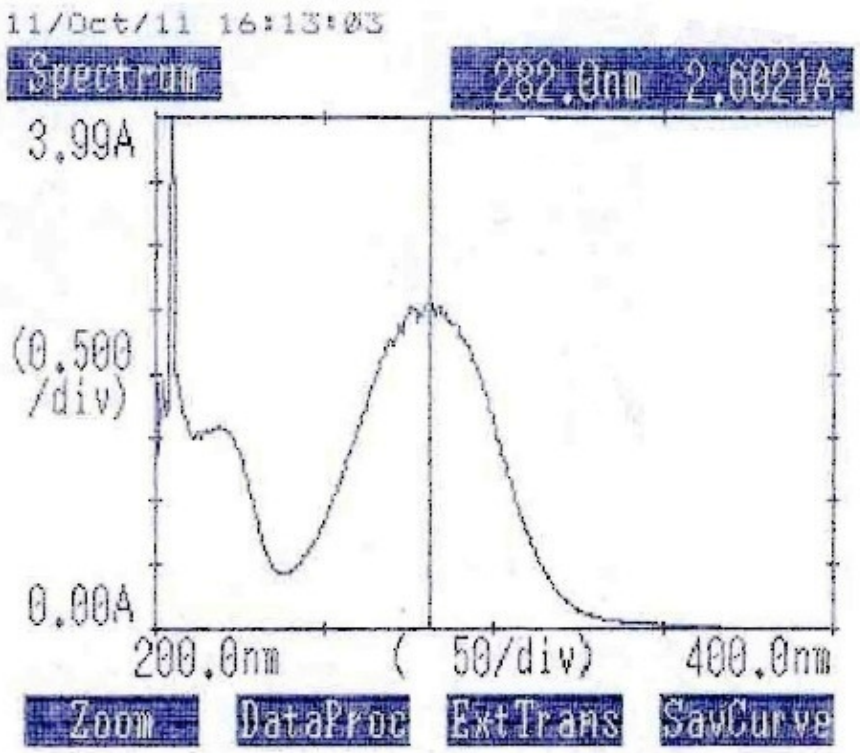


Fig.62: UV Spectrum of compound SH_3

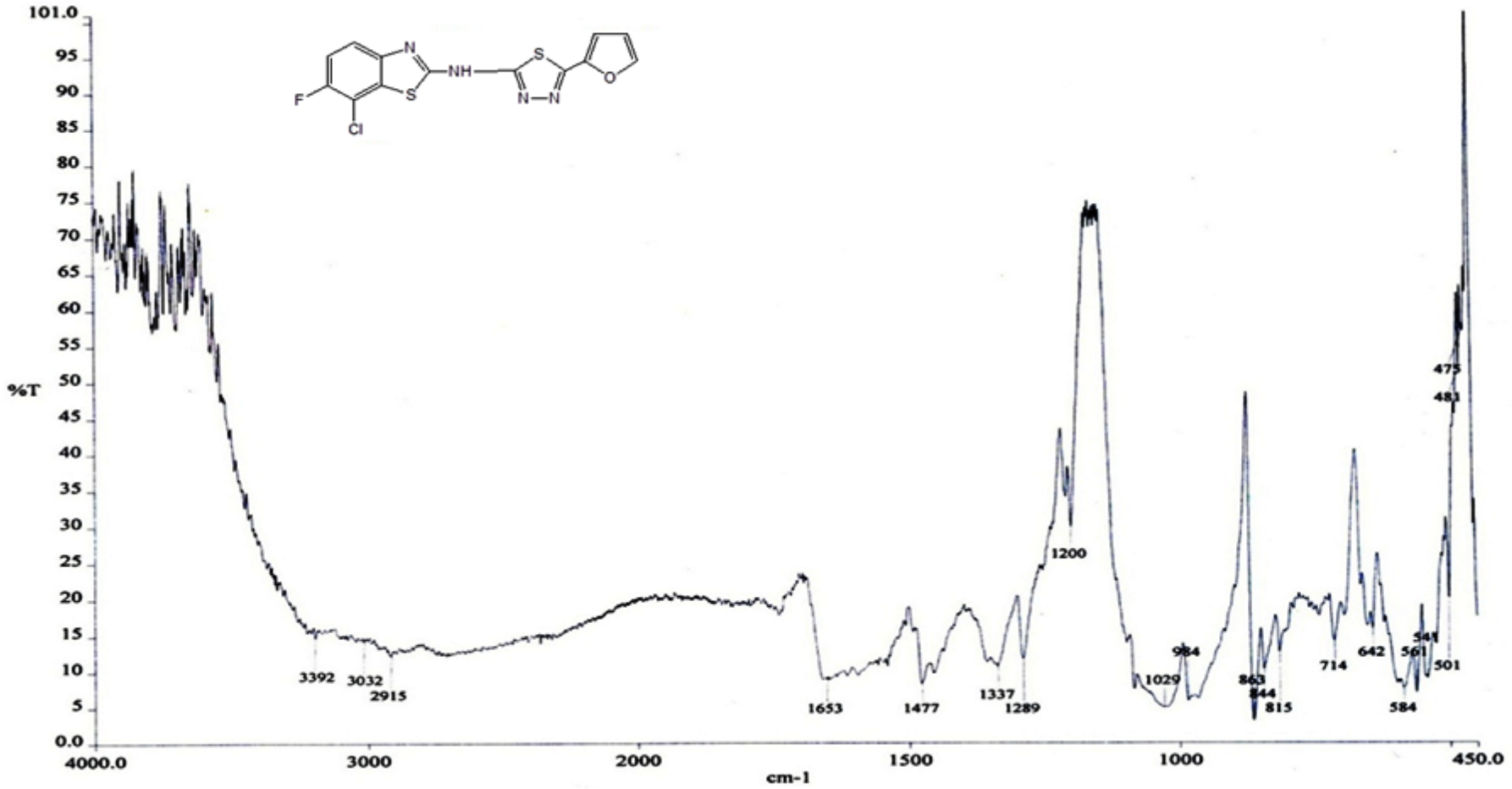


Fig.63: IR Spectrum of compound SH_3

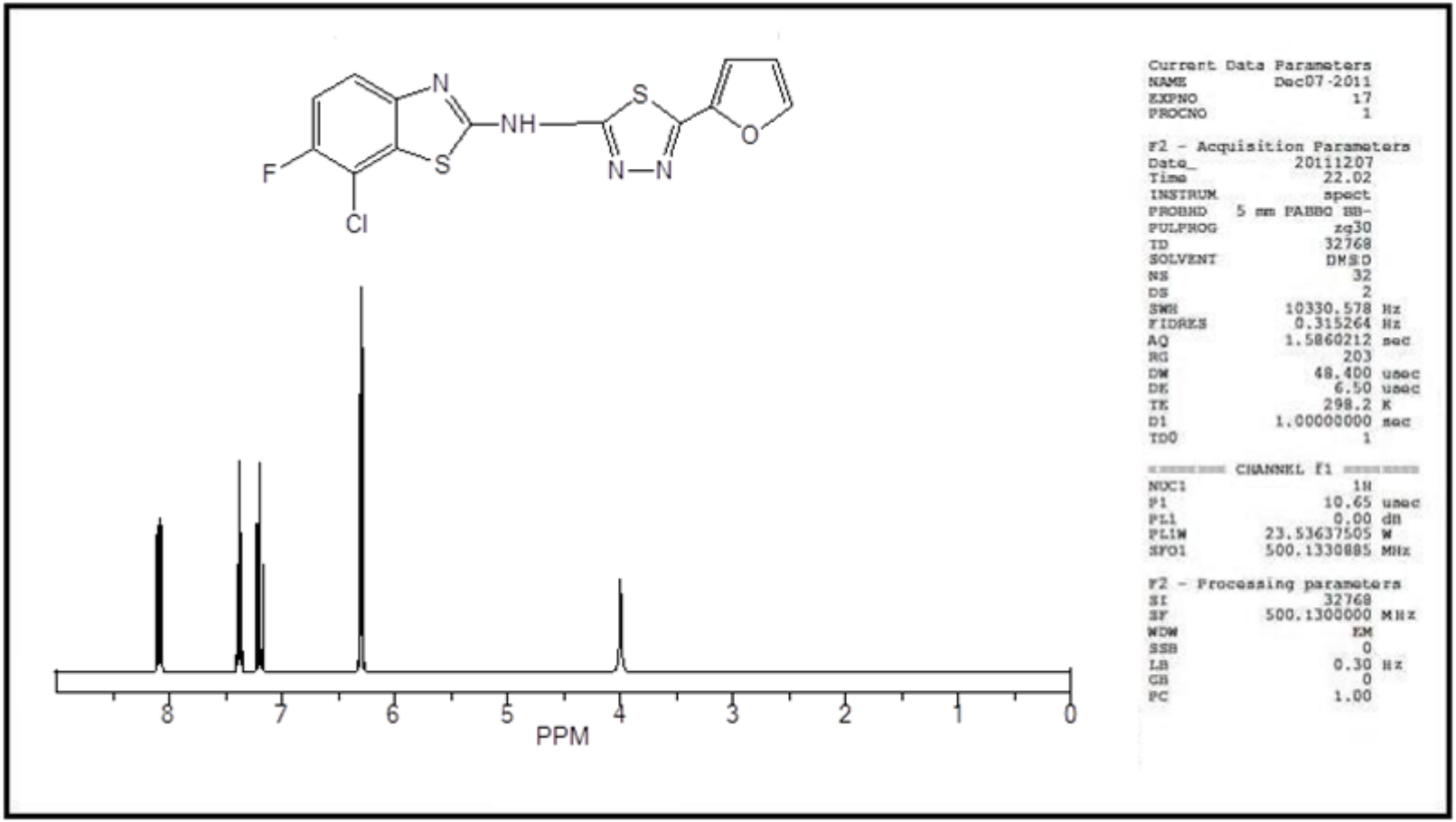


Fig-64: ^{1}H NMR Spectra of the compound SH_3

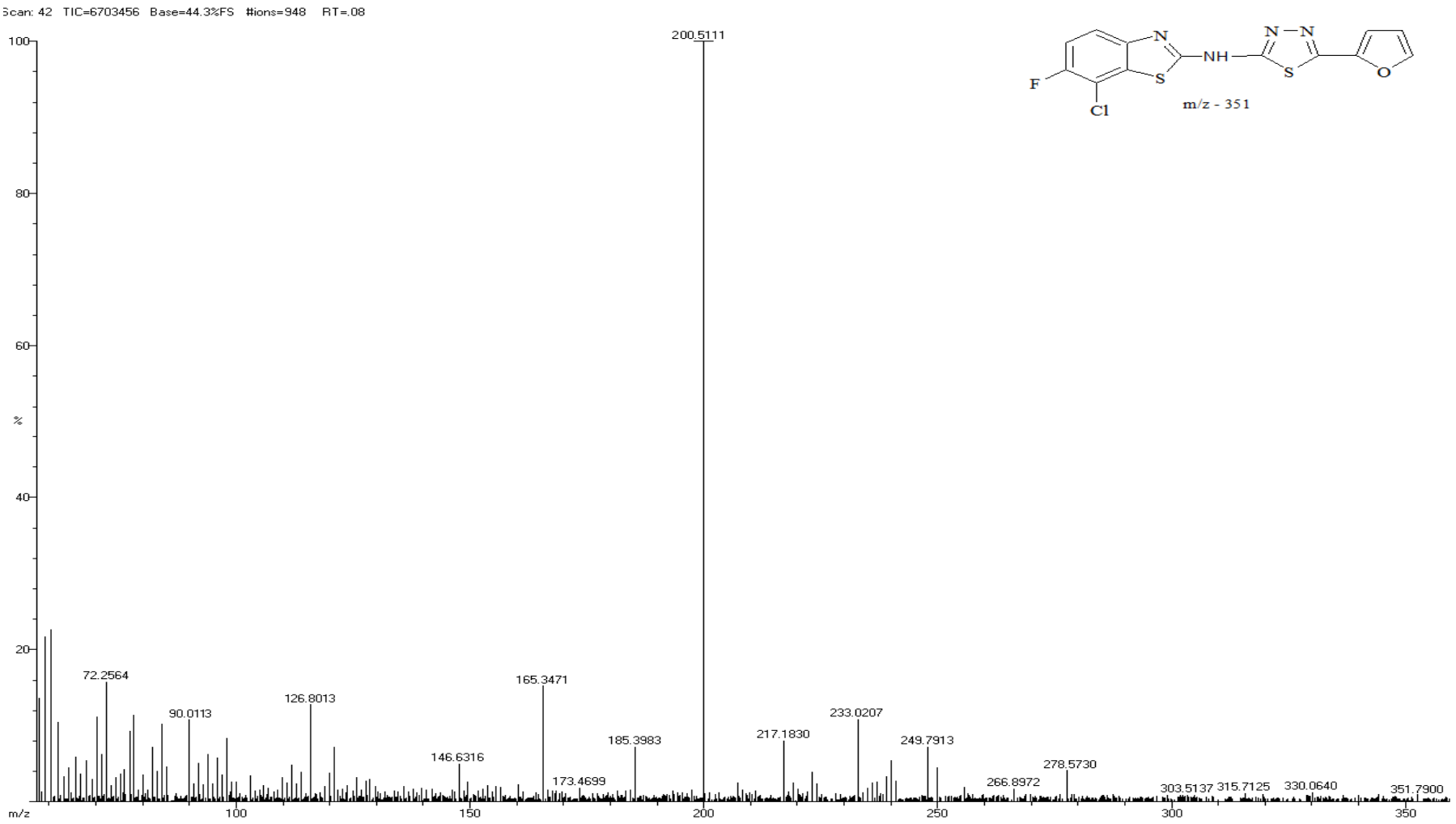


Fig-65: Mass Spectrum of the compound SH_3

6.2.4. 7-chloro-6-fluoro-*N*-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine (SH_4)

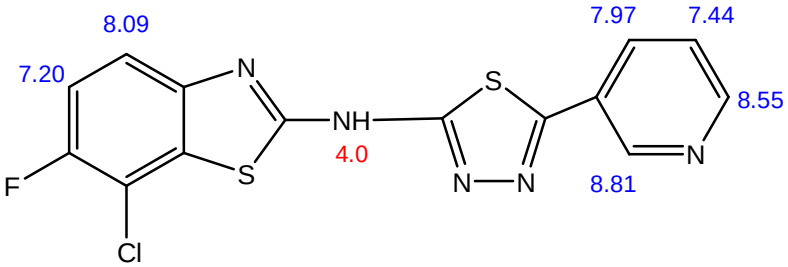
UV: (Fig-66)

λ_{max} (MeOH) 284.5 (ε_{max} 0.8198)

IR (KBr): (Fig-67)

S. No	Wave Number (cm^{-1})	Assignment
1	3292	N – H stretching
2	3090	Aromatic C – H stretching
3	3022	Aromatic Pyridine C – H stretching
4	1632	C = N stretching
5	1543	C = C, C = N ring stretching in pyridine
6	1454	Aromatic C = C ring stretching
7	1341	Aromatic C – N secondary vibrations
8	1032	C – F stretching
9	844	C – Cl stretching
10	716	Aromatic C –H bending
11	645	C – S stretching

NMR (DMSO – d_6): (Fig-68)

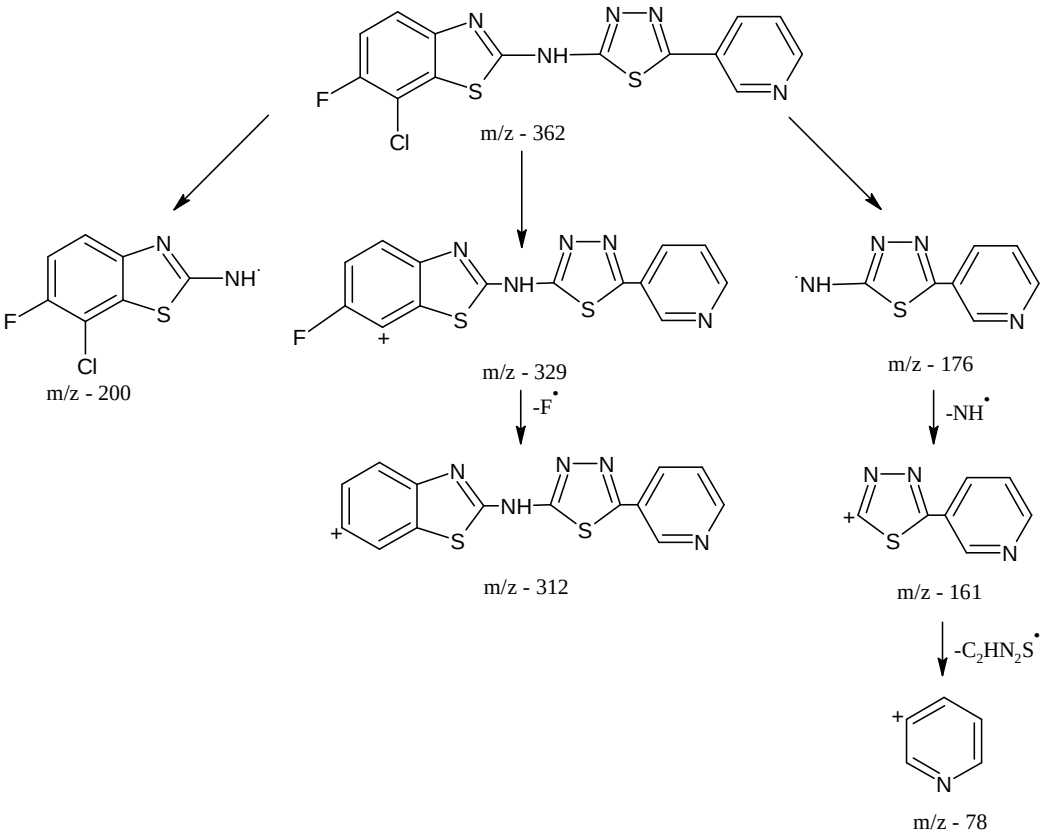


(6 aromatic protons, 1 proton on nitrogen)

S. No	δ	Assignment
1	8.85	1H, d, CH of pyridine
2	8.81	1H, s, CH of pyridine
3	8.09	1H, d, Ar – H of benzothiazole
4	7.97	1H, d, CH of pyridine
5	7.44	1H, t, CH of pyridine
6	7.20	1H, d, Ar – H of benzothiazole
7	4.0	1H, s, NH

MASS: (Fig-69)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



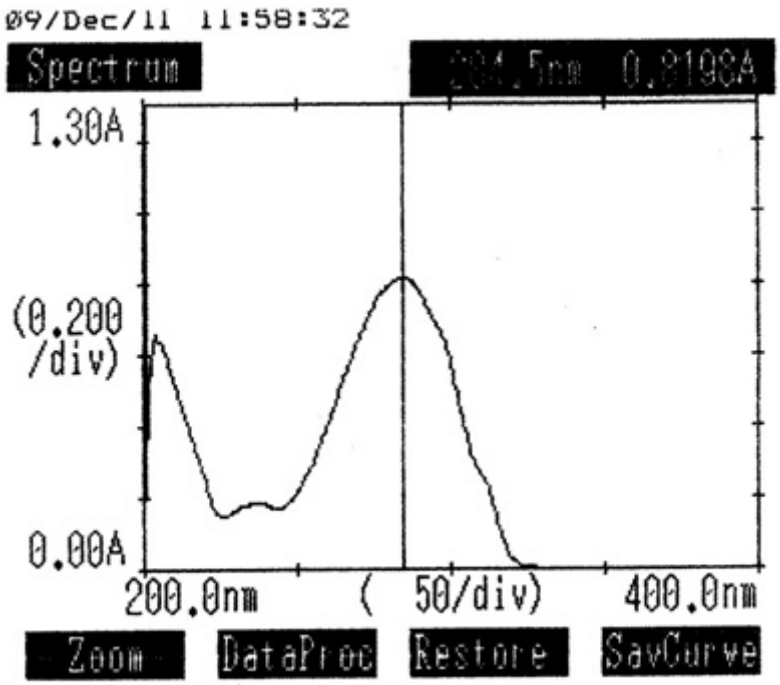


Fig.66:UV Spectrum of Compound SH_4

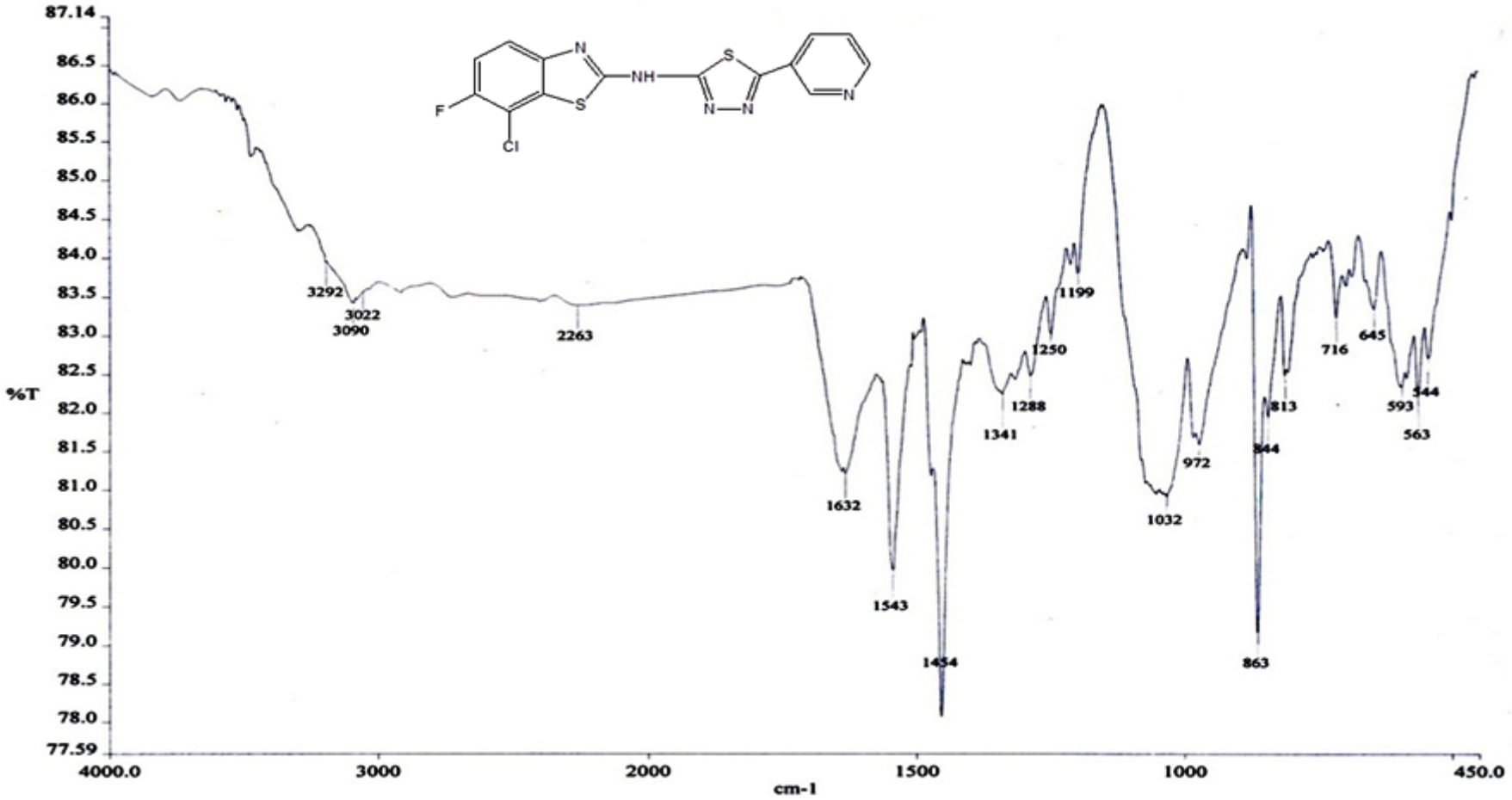


Fig.67: IR Spectrum of compound SH_4

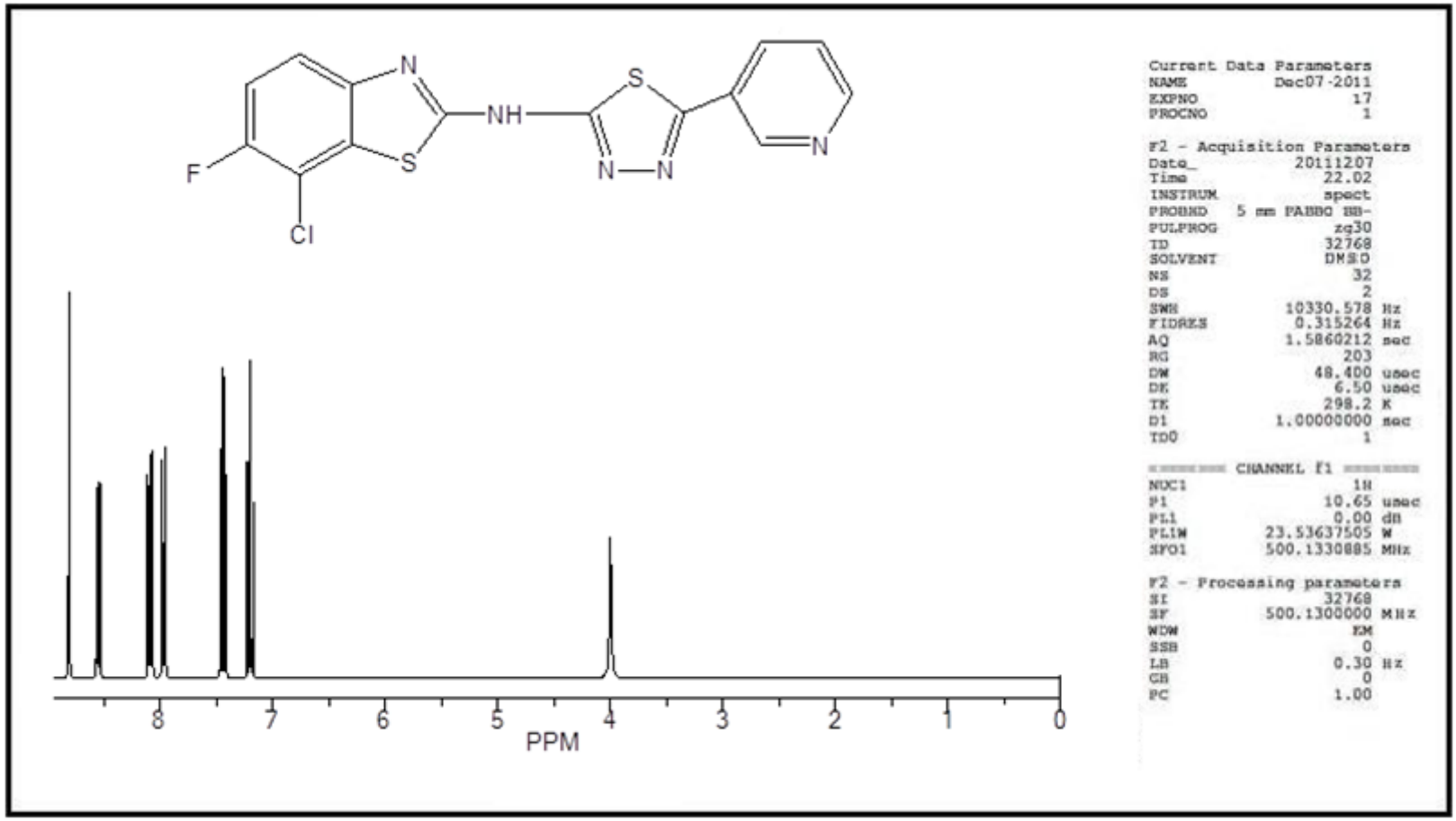


Fig-68: ^{1}H NMR Spectra of the compound SH_4

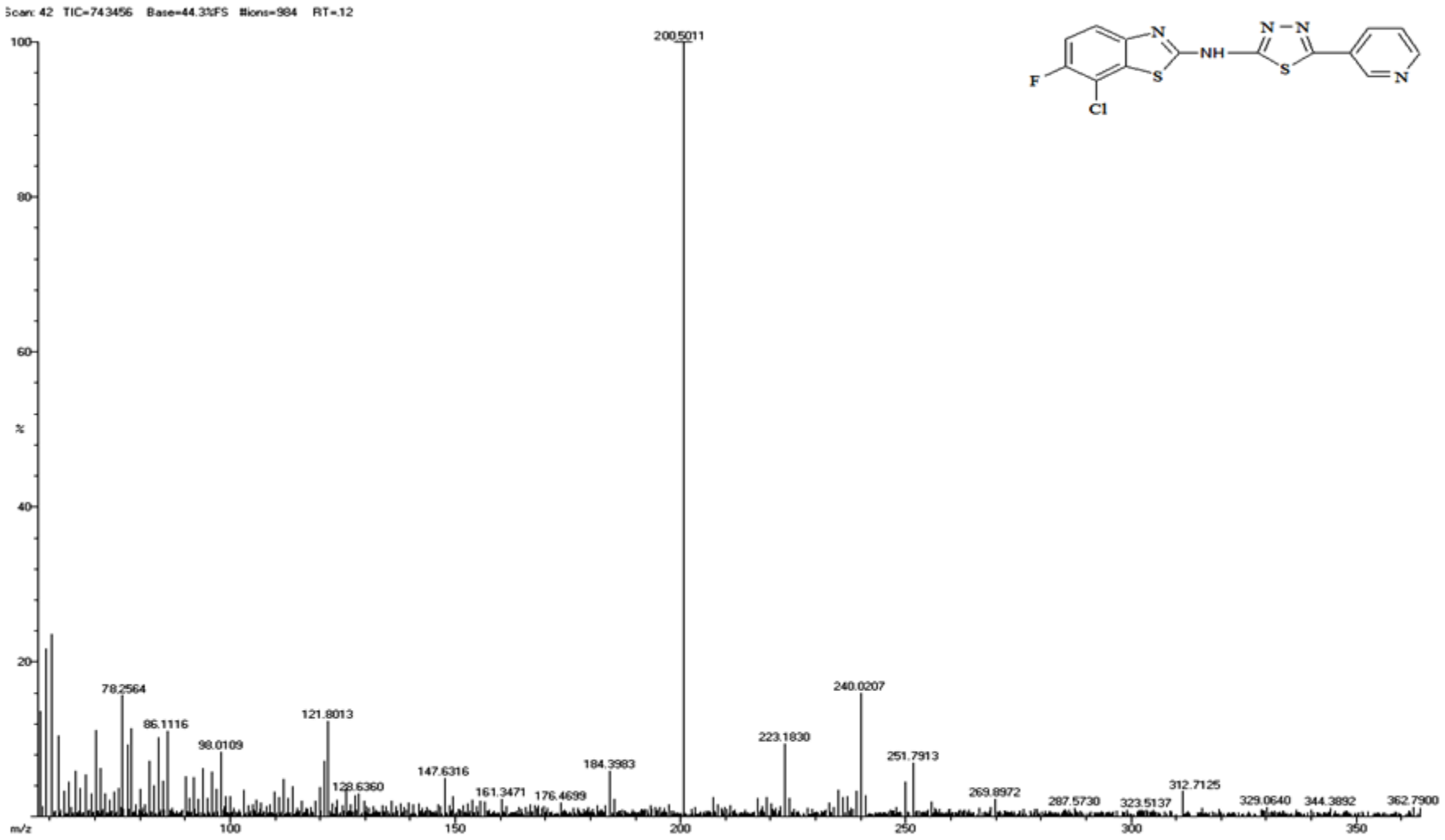


Fig-69: Mass Spectrum of the compound SH_4

6.2.5. *N*-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-7-chloro-6-fluoro-1,3-benzothiazol-2-amine (SH_5)

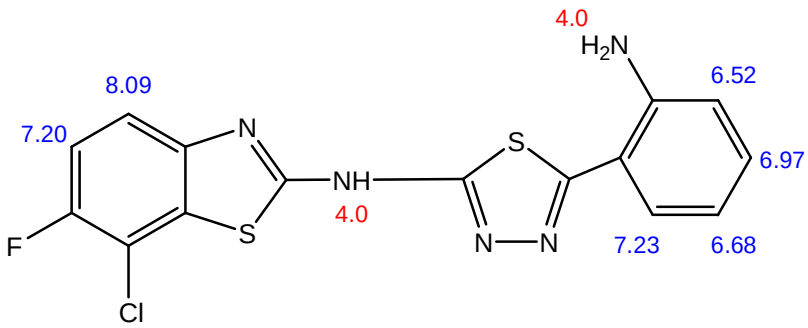
UV: (Fig-70)

λ_{max} (MeOH) 280.5 **(**ε_{max} 0.4451)

IR (KBr): (Fig-71)

S. No	Wave Number (cm^{-1})	Assignment
1	3388	N –H stretching
2	3078	Aromatic C – H stretching
3	1634	C = N stretching
4	1546	N – H bending
5	1458	Aromatic C = C ring stretching
6	1116	C – F stretching
7	809	C – Cl stretching
8	685	C – S stretching

NMR (DMSO – d_6): (Fig-72)

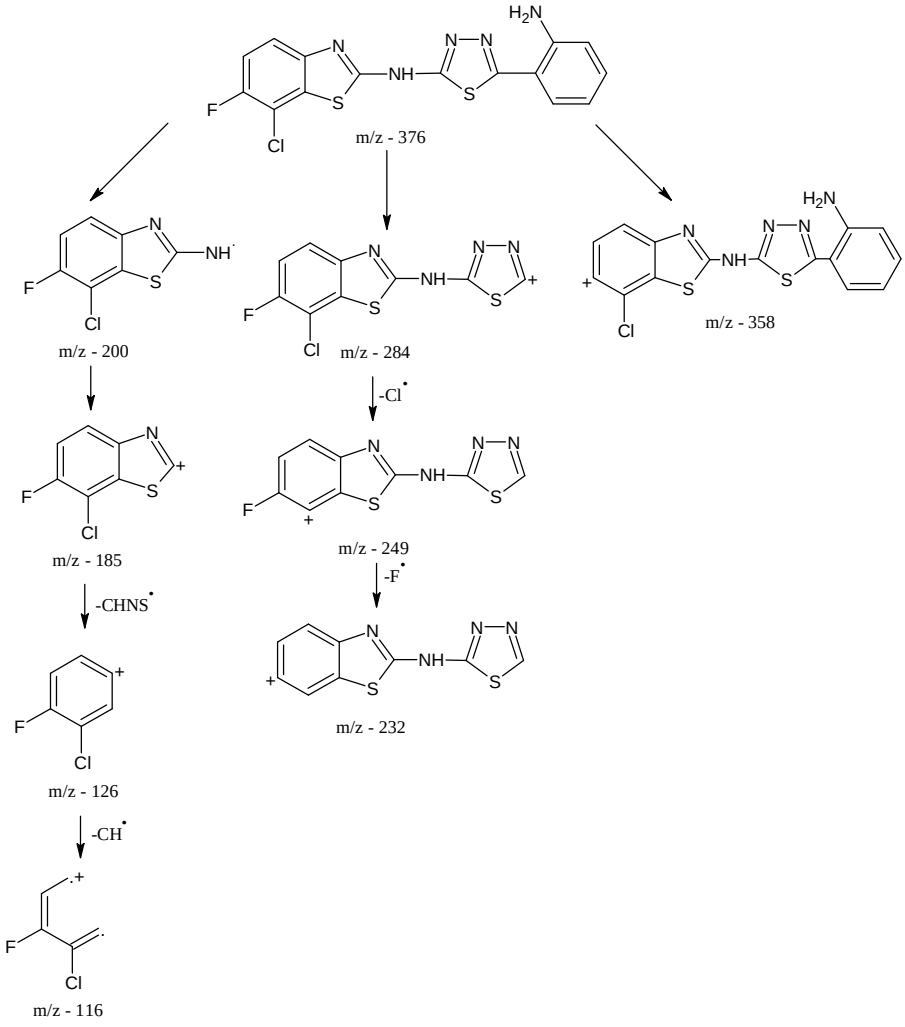


(6 aromatic protons, 3 protons on nitrogen)

S. No	δ	Assignment
1	8.09	1H, d, Ar – H of benzothiazole
2	7.23	1H, d, CH of benzene
3	6.97	1H, t, CH of benzene
4	6.68	1H, t, CH of benzene
5	6.52	1H, d, Ar – H of benzene
6	7.20	1H, d, Ar – H of benzothiazole
7	4.0	3H, s, NH

MASS: (Fig-73)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



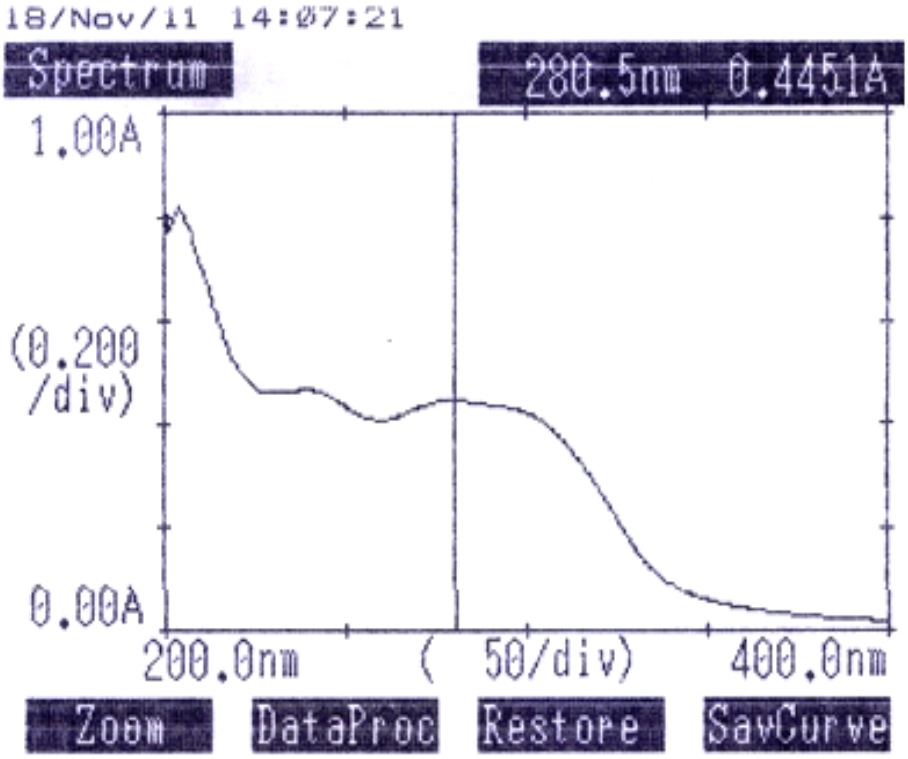


Fig.70:UV Spectrum of Compound SH_5

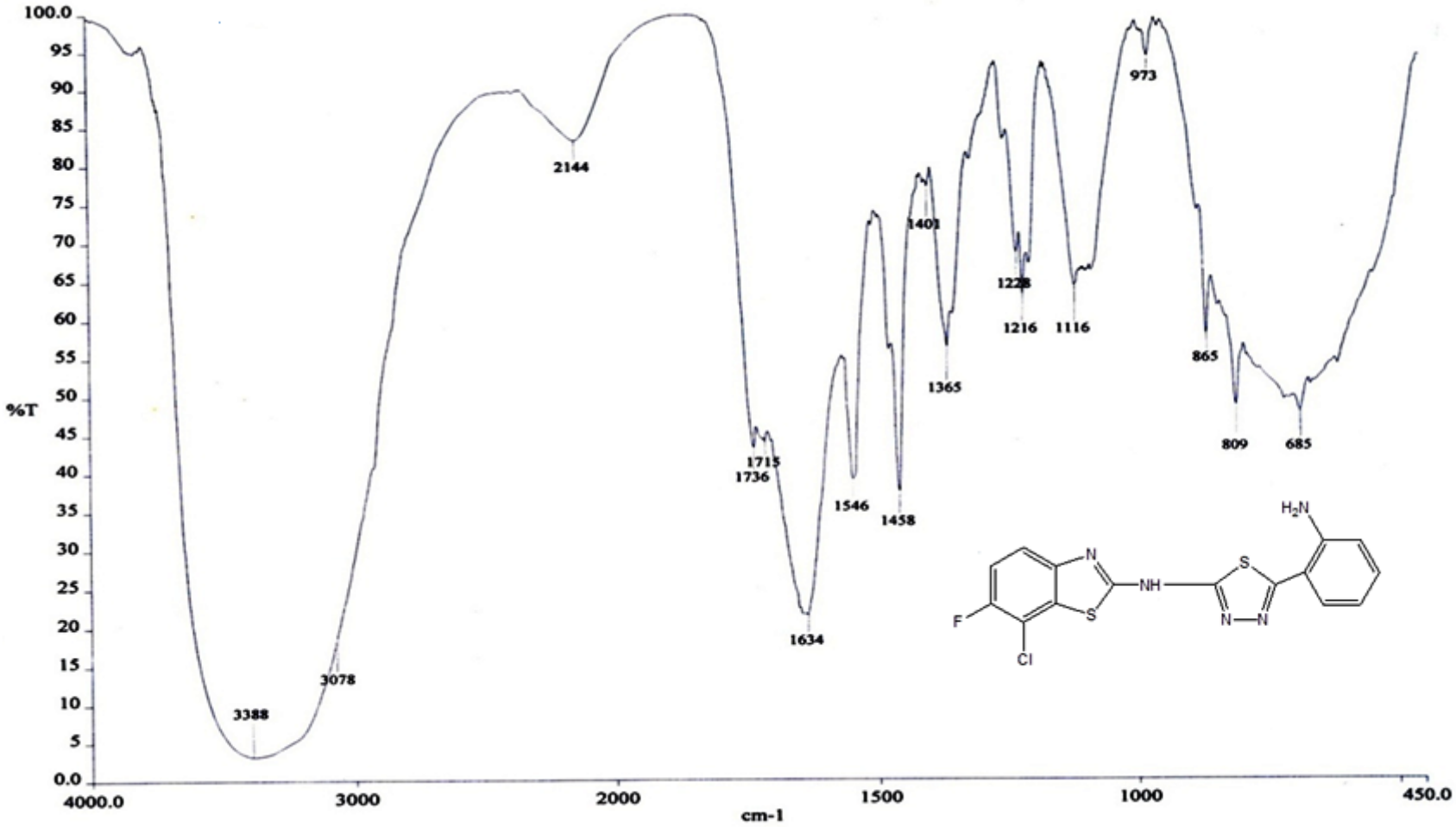


Fig.71: IR Spectrum of compound SH_5

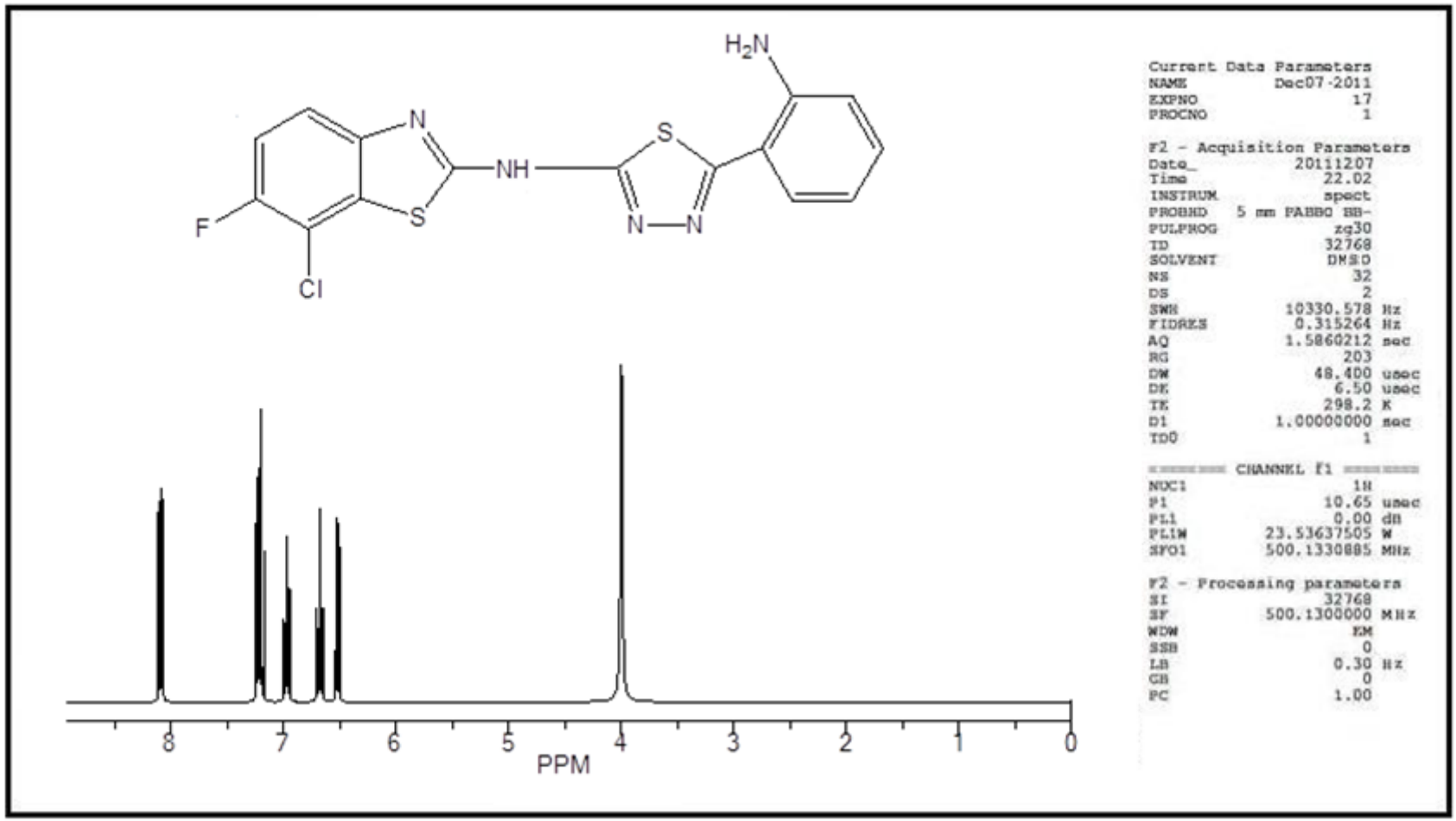


Fig-72: ^{1}H NMR Spectra of the compound SH_5

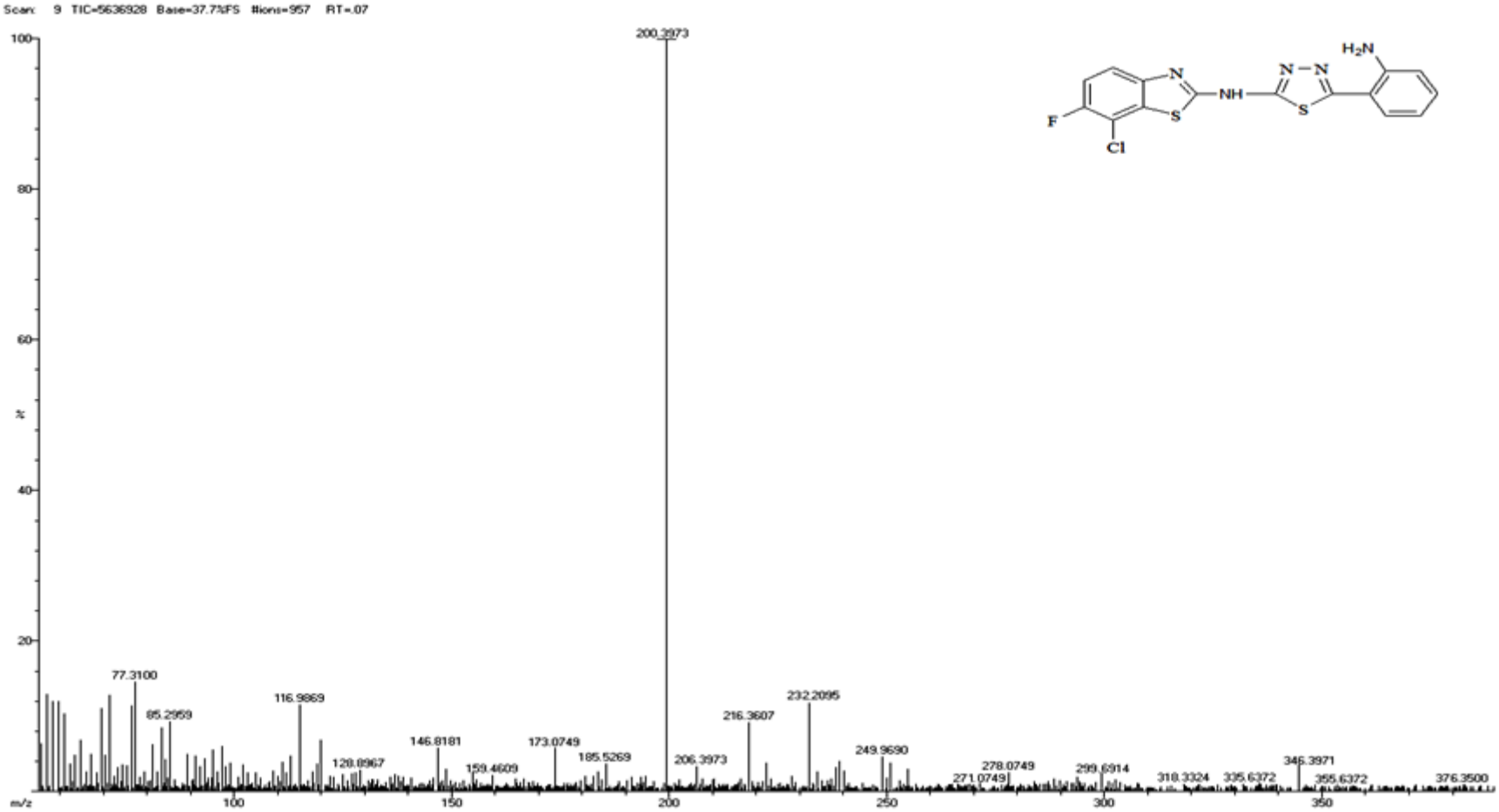


Fig-73: Mass Spectrum of the compound SH_5

6.2.6. 6-fluoro-N^2-[5-(furan-2-yl)-1, 3, 4-thiadiazol-2-yl]-N^7-(2-nitrophenyl)-1,3-benzothiazole-2, 7-diamine (SH_6)

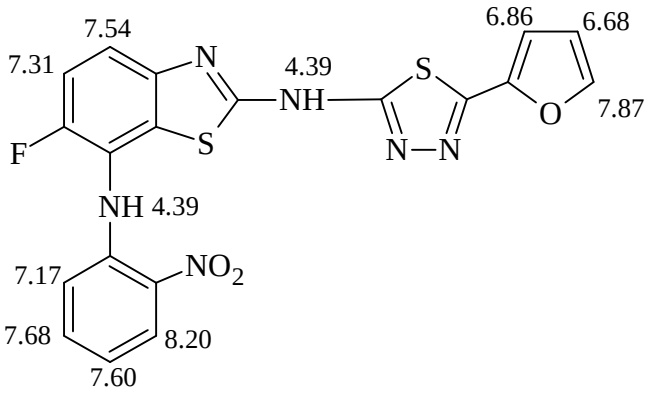
UV: (Fig-74)

λ_{max} (MeOH) 293.5 **(**ε_{max} 0.2321)

IR (KBr): (Fig-75)

S. No	Wave Number (cm^{-1})	Assignment
1	3477.49	N – H asymmetrical stretching
2	2925.18	Aromatic C – H stretching
3	1731.91	C – H out of plane bending
4	1569.79	C = N stretching
5	1507.47	NO_2 asymmetrical stretching
6	1345.48	C – N stretching
7	1169.53	N – N stretching
8	1101.20	C – O stretching
9	1016.47	2 – substituted furans
10	779.06	C – S stretching
11	742.44	C – H out of plane bending

NMR (DMSO – d_6): (Fig-76-78)

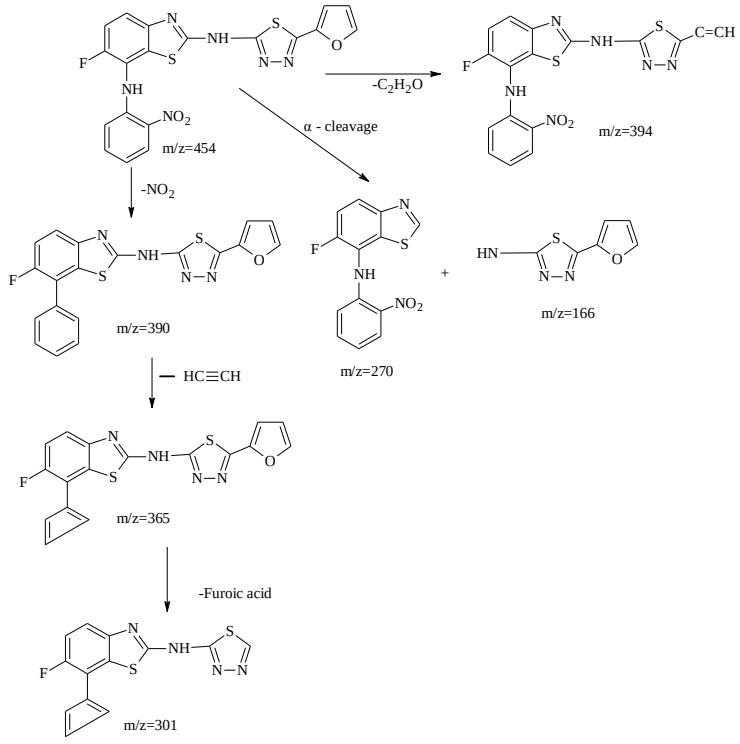


(6 aromatic protons, 3 proton of furan, 2 protons on nitrogen)

S. No	δ	Assignment
1	8.20	1H, d, Ar – H
2	7.87	1H, d, CH of furan
3	7.68	1H, t, Ar – H
4	7.60	1H, t, Ar – H
5	7.54	1H, d, Ar – H of benzothiazole
6	7.31	1H, d, Ar – H of benzothiazole
7	7.17	1H, d, Ar – H
8	6.86	1H, d, CH of furan
9	6.68	1H, t, CH of furan
10	4.39	2H, s, NH

MASS: (Fig-79)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



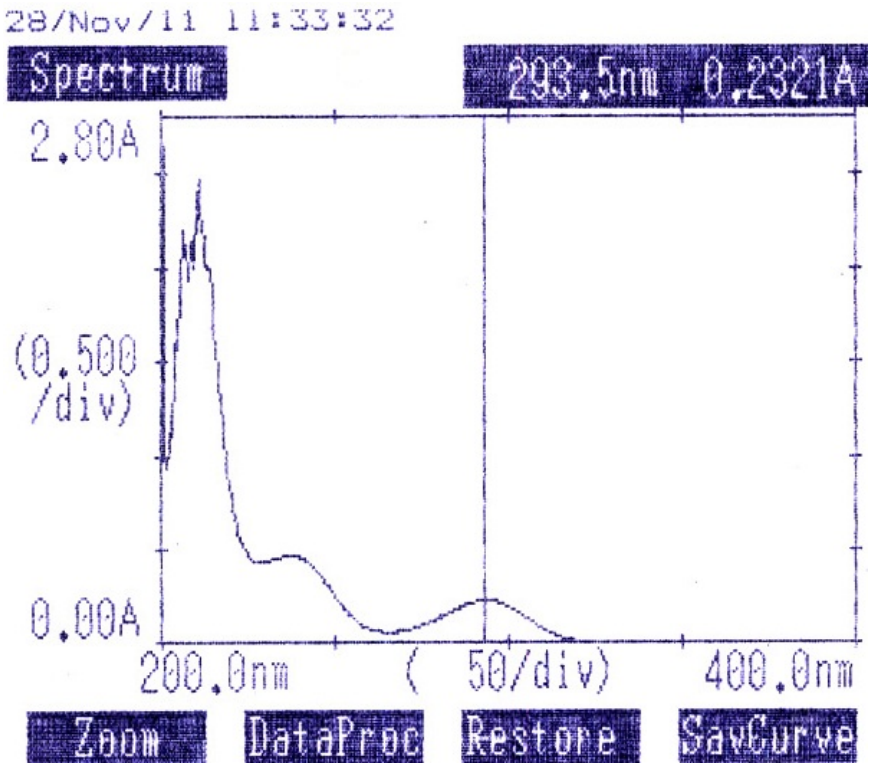


Fig.74:UV Spectrum of Compound SH_6

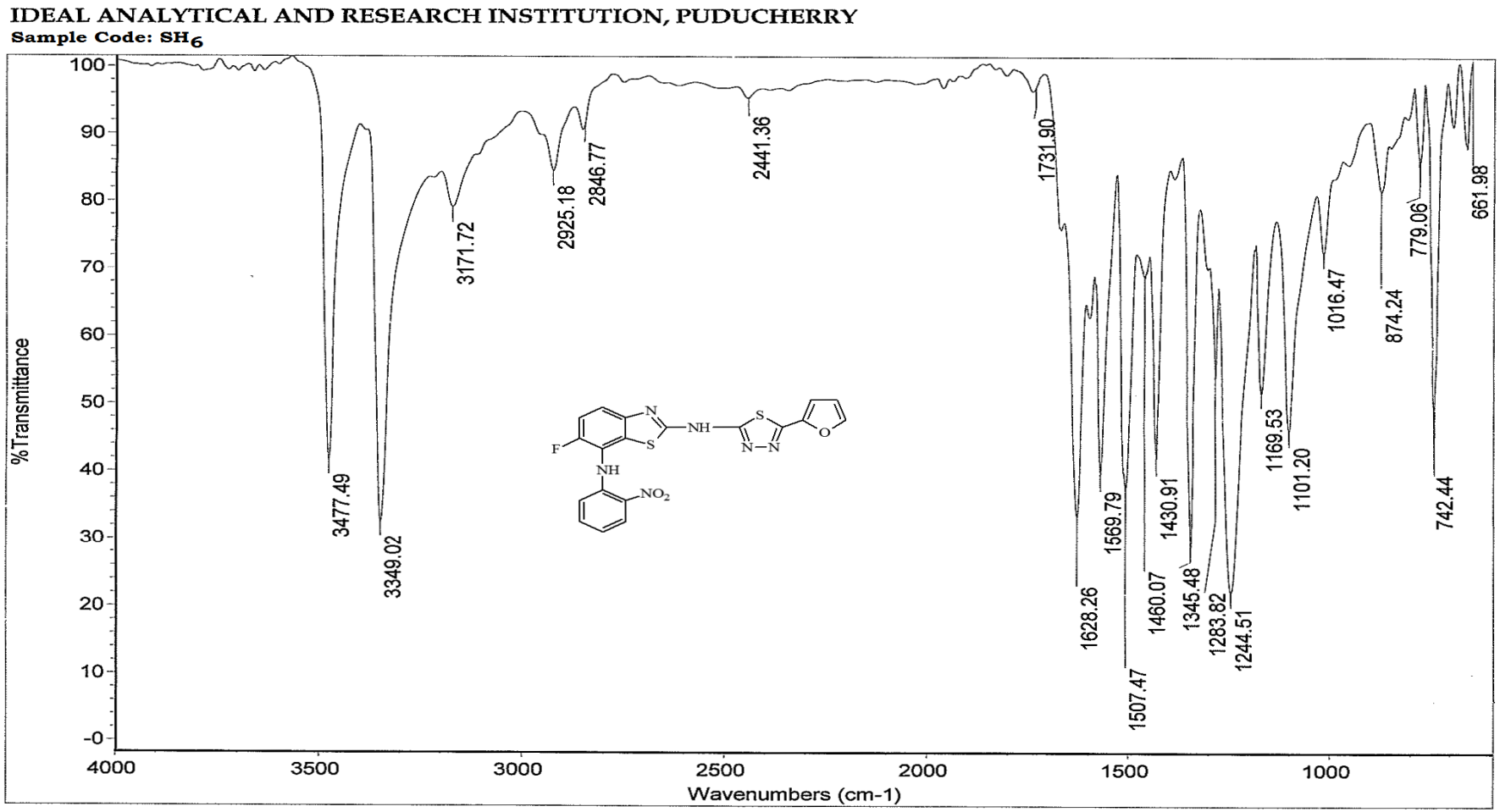


Fig.75: IR Spectrum of compound SH_6

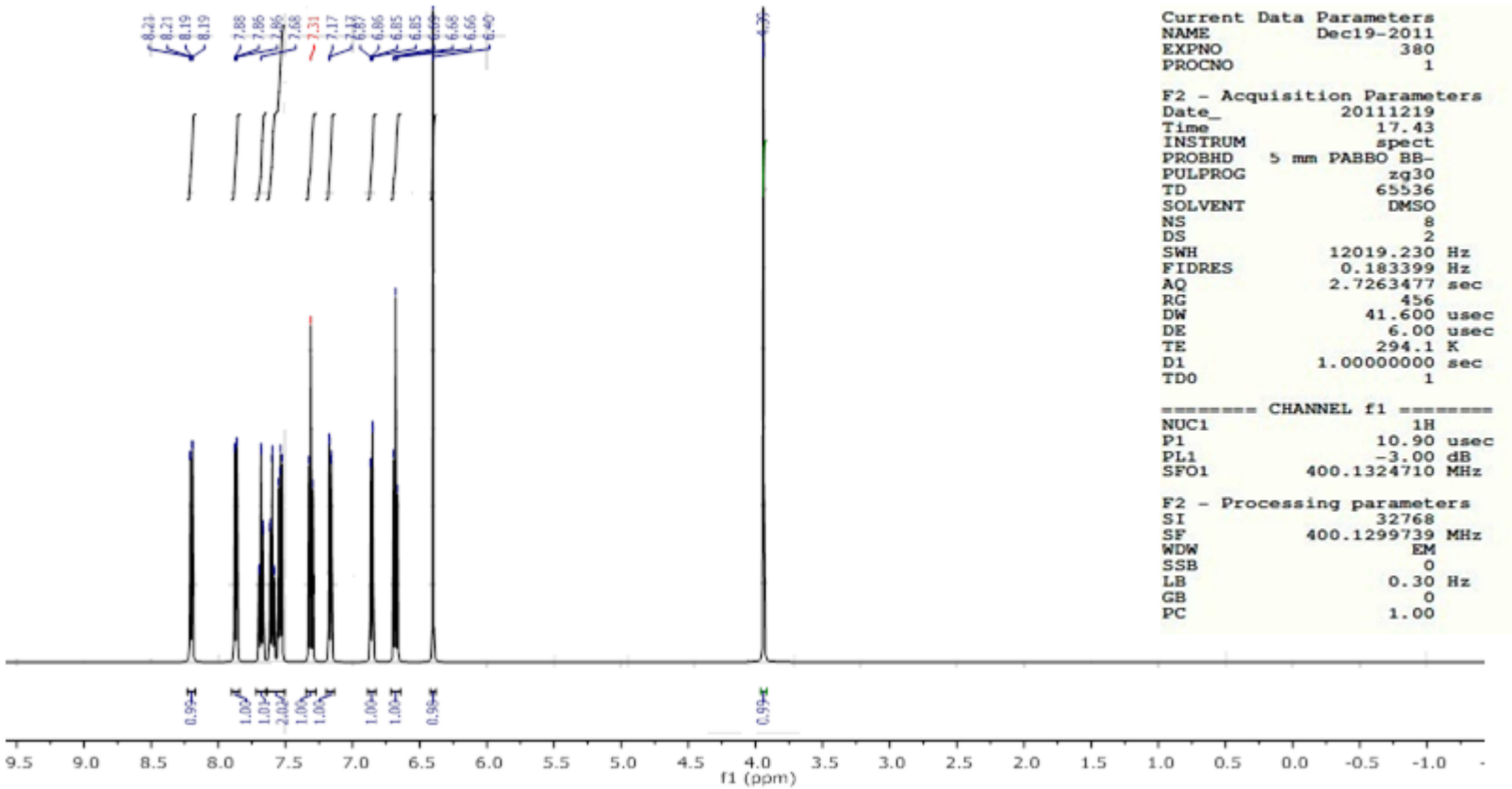


Fig-76: ^{1}H NMR Spectra of the compound SH_6

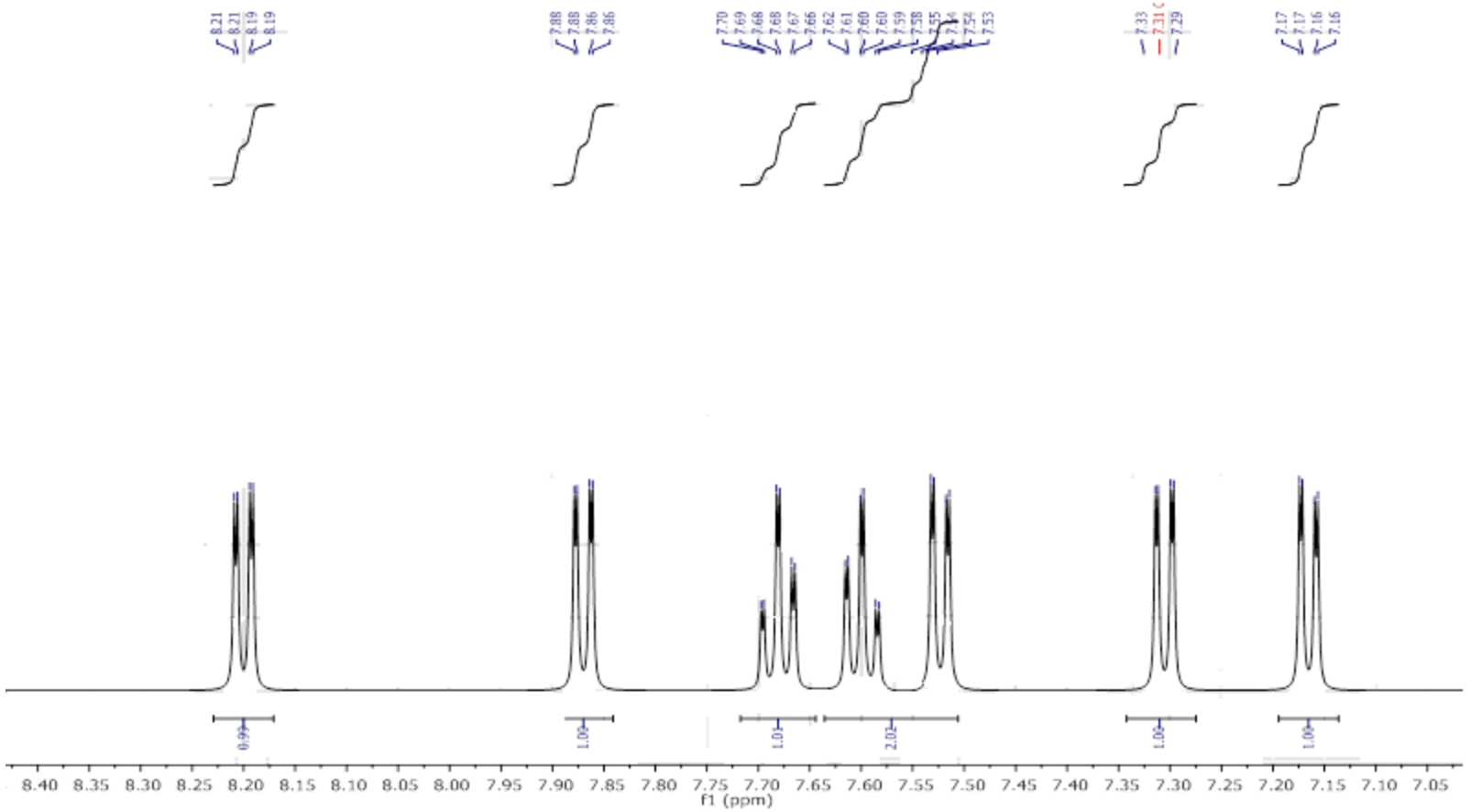


Fig-77: ^{1}H NMR Spectra of the compound SH_6 (Zoom View 1)

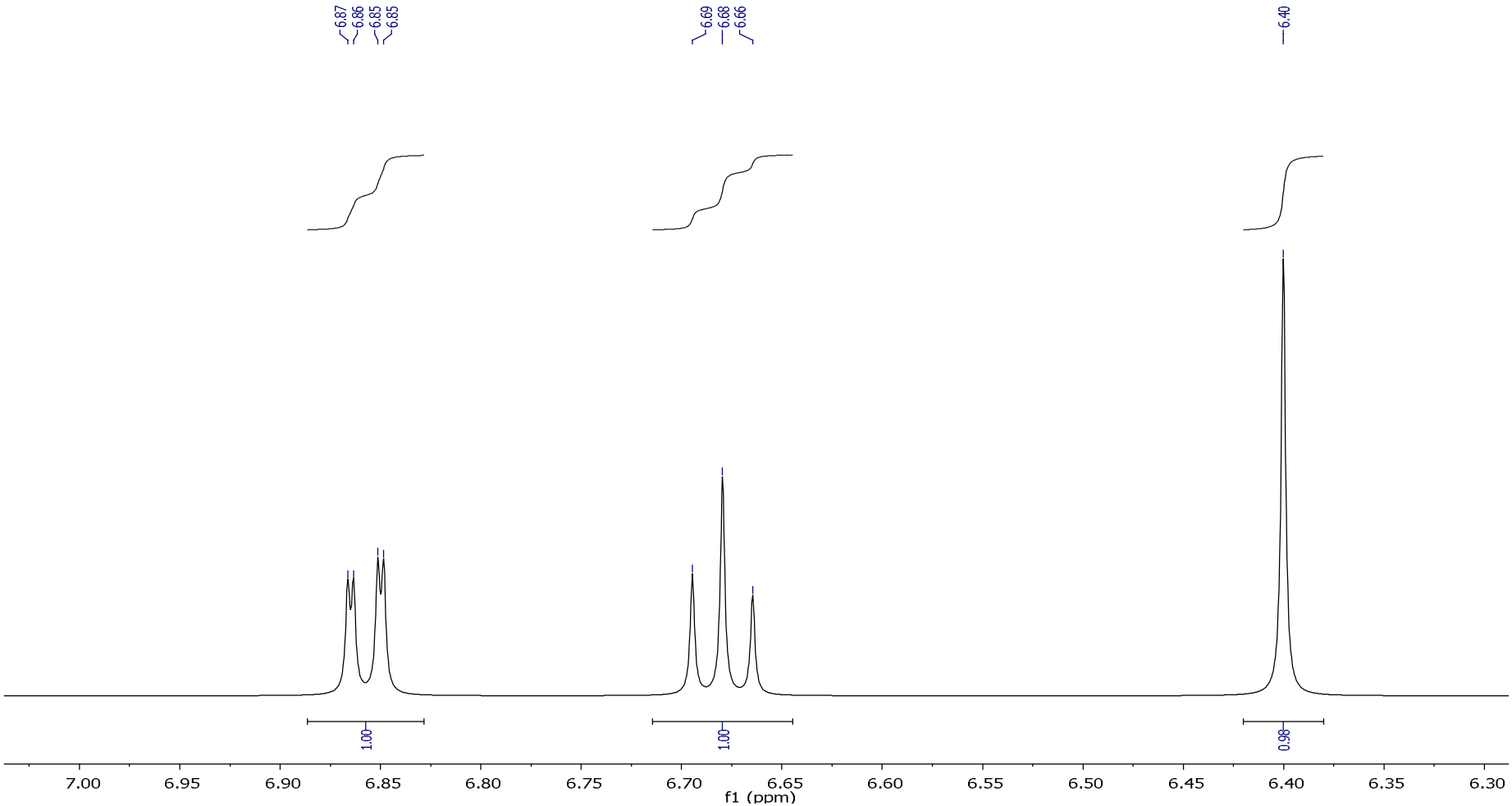


Fig-78: ^{1}H NMR Spectra of the compound SH_6 (Zoom View 2)

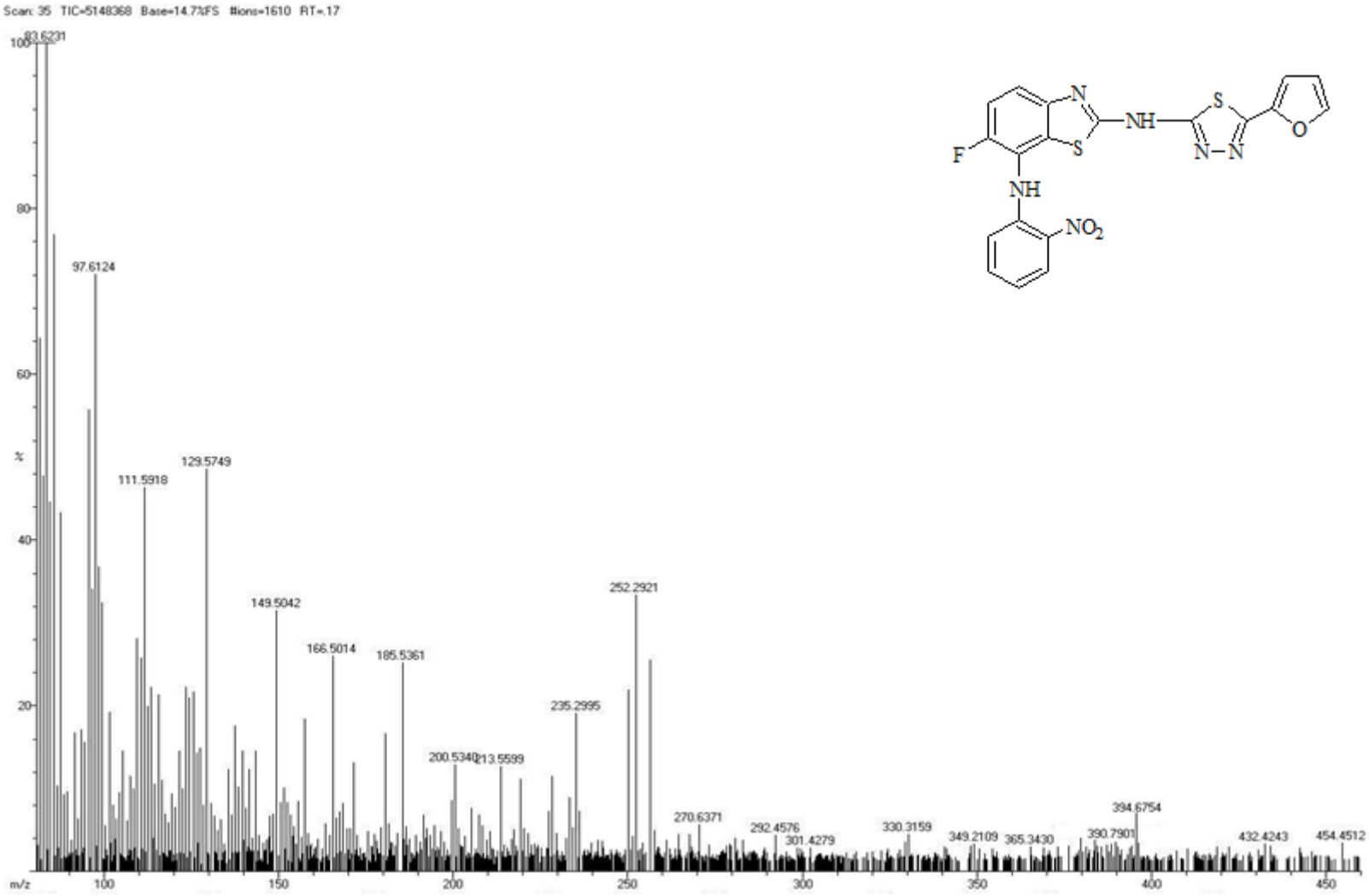


Fig.79: Mass Spectrum of the compound SH_6

6.2.7. N^7-(2,4-dinitrophenyl)-6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine(SH_7)

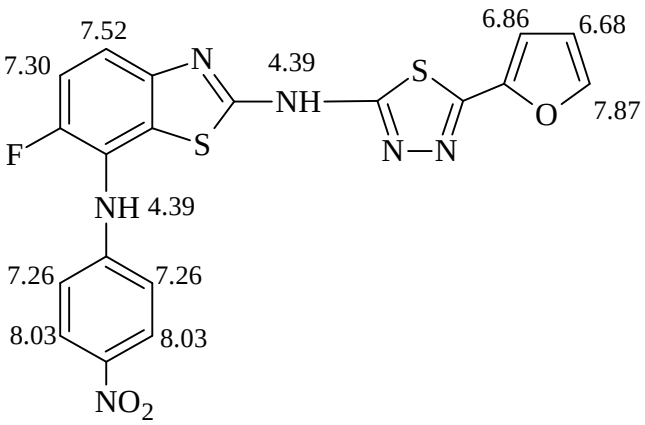
UV: (Fig-80)

λ_{max} (MeOH) 287.5 (ε $_{max}$ 0.4285)

IR (KBr): (Fig-81)

S. No	Wave Number (cm^{-1})	Assignment
1	3361.71	N – H asymmetrical stretching
2	2923.85	Aromatic C – H stretching
3	1923.56	Aromatic C – H out of plane bending
4	1505.47	C = N stretching
5	1301.98	C – H stretching
6	1281.62	C – N stretching
7	1013.55	2 – substituted furans
8	753.74	C – S stretching

NMR (DMSO – d_6): (Fig-82-84)

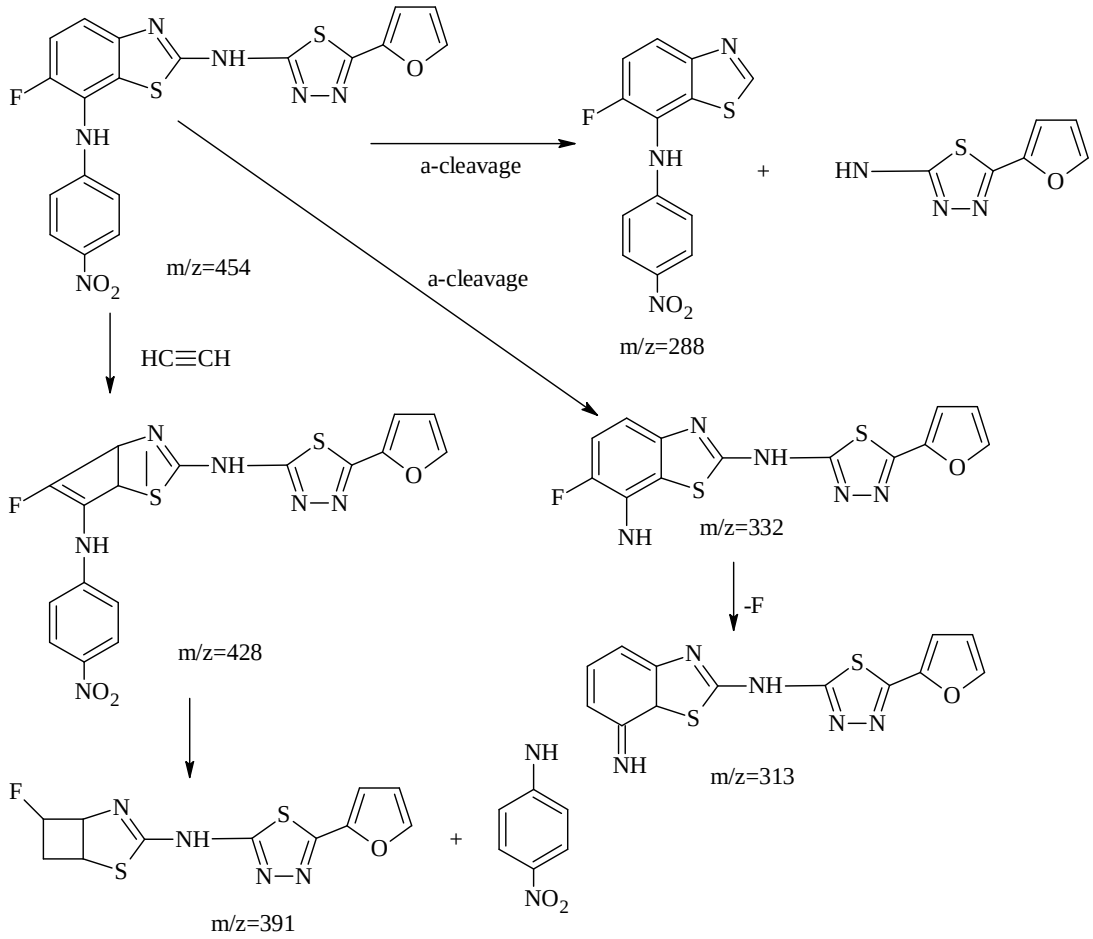


(8 aromatic protons, 3 proton of furan)

S. No	δ	Assignment
1	8.03	2H, d, Ar – H – of aniline group
2	7.87	1H, d, CH of furan
3	7.52	1H, d, Ar – H of benzothiazole
4	7.26-7.30	3H, m, Ar – H
5	6.86	1H, d, CH of furan
6	6.68	1H, t, CH of furan
7	4.39	2H, s, NH

MASS: (Fig-85)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



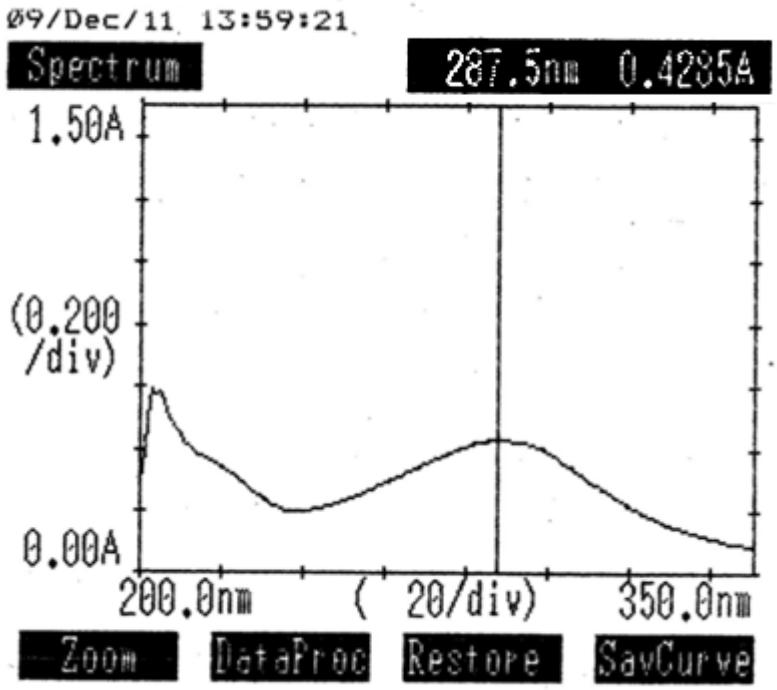


Fig.80:UV Spectrum of Compound SH_7

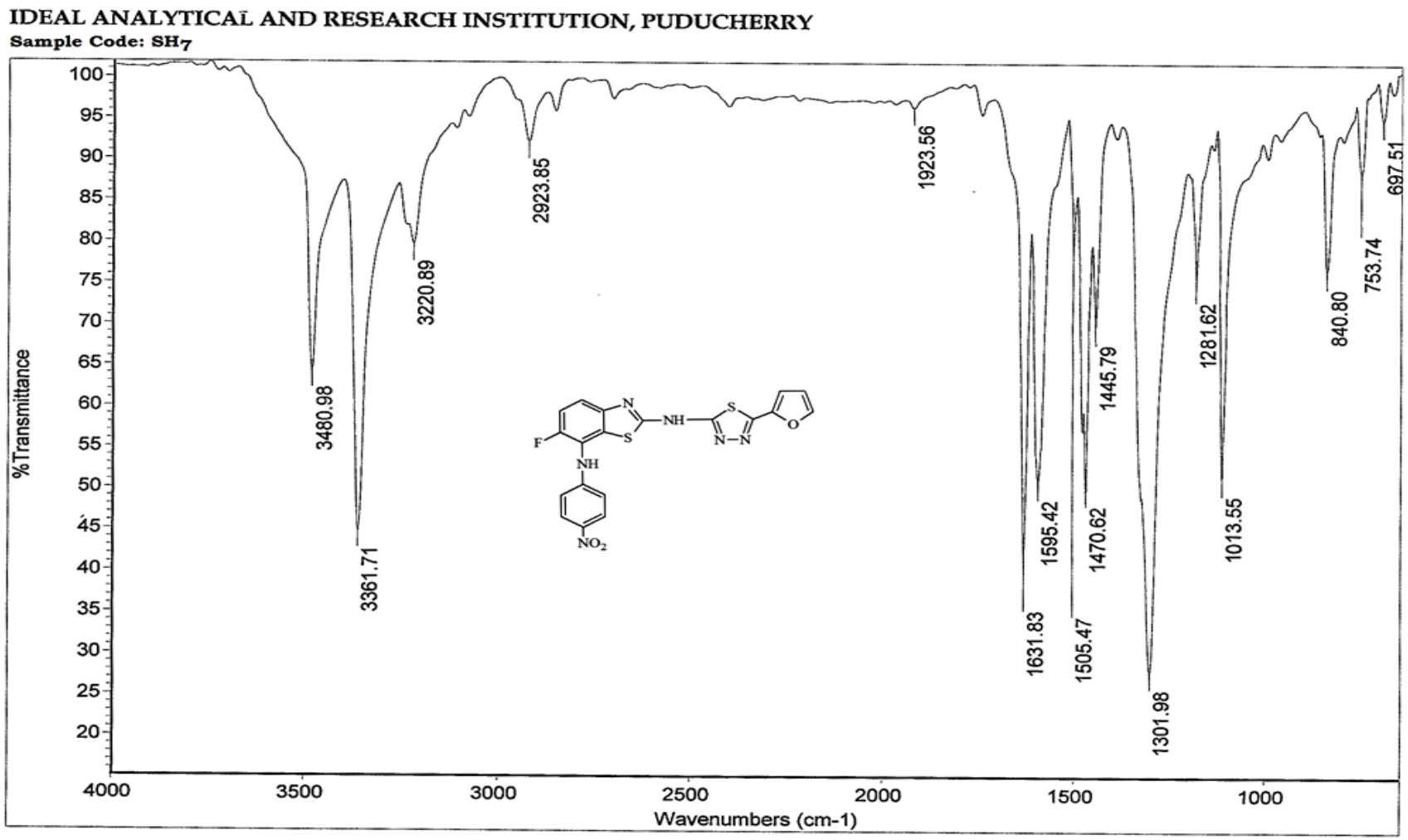


Fig.81: IR Spectrum of compound SH_7

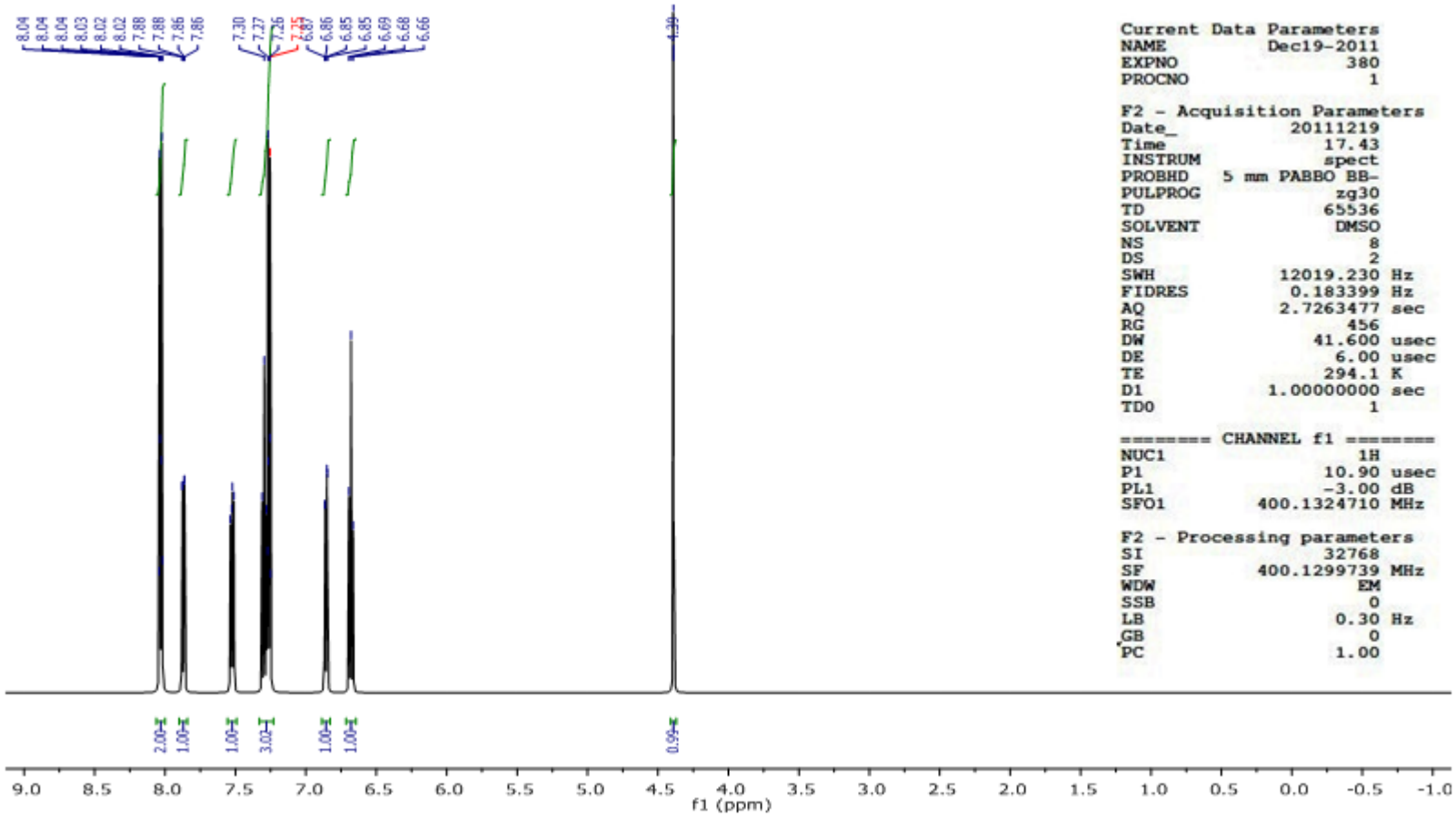


Fig-82: ^{1}H NMR Spectra of the compound SH_7

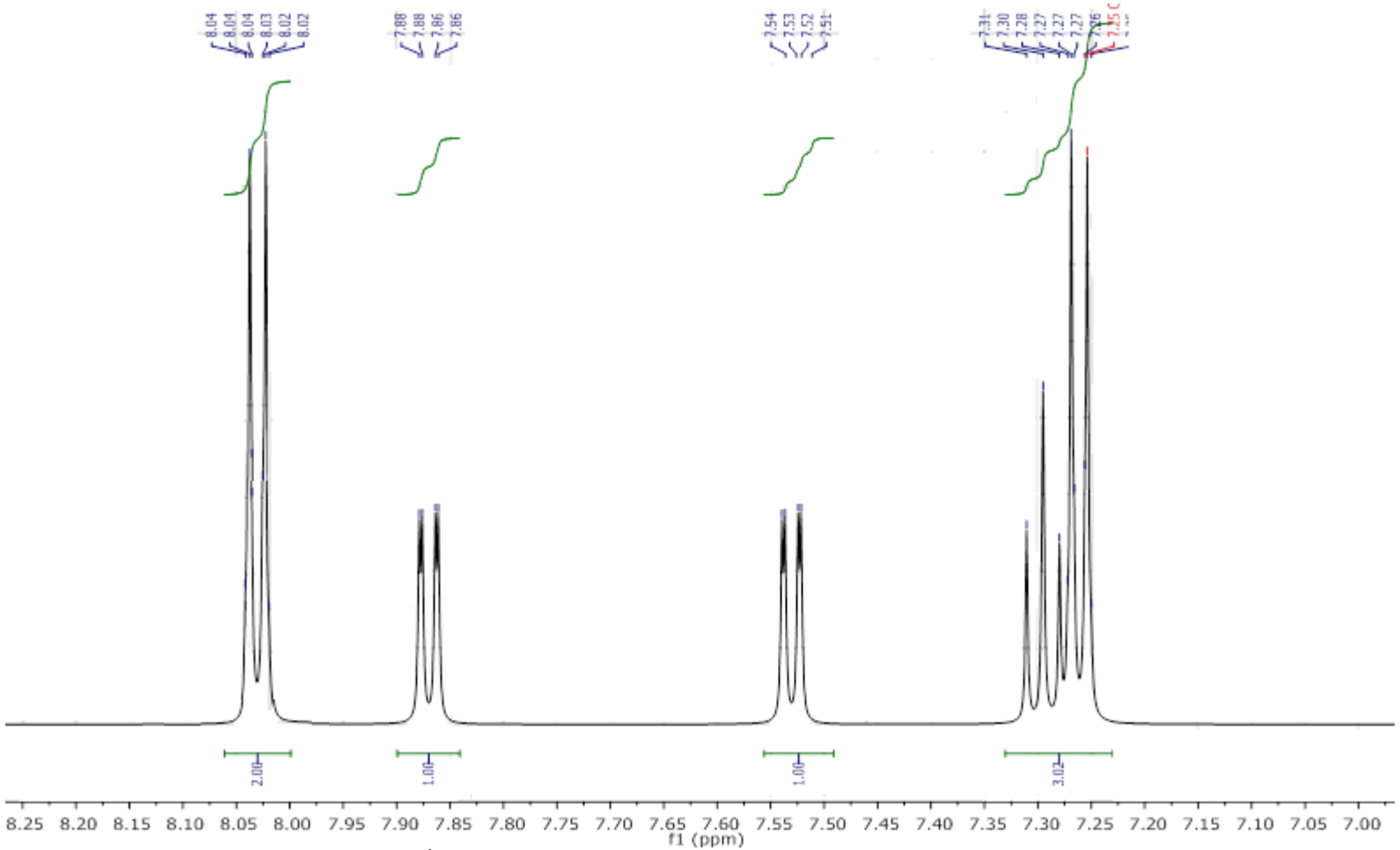


Fig-83: ^{1}H NMR Spectra of the compound SH_7 (Zoom View 1)

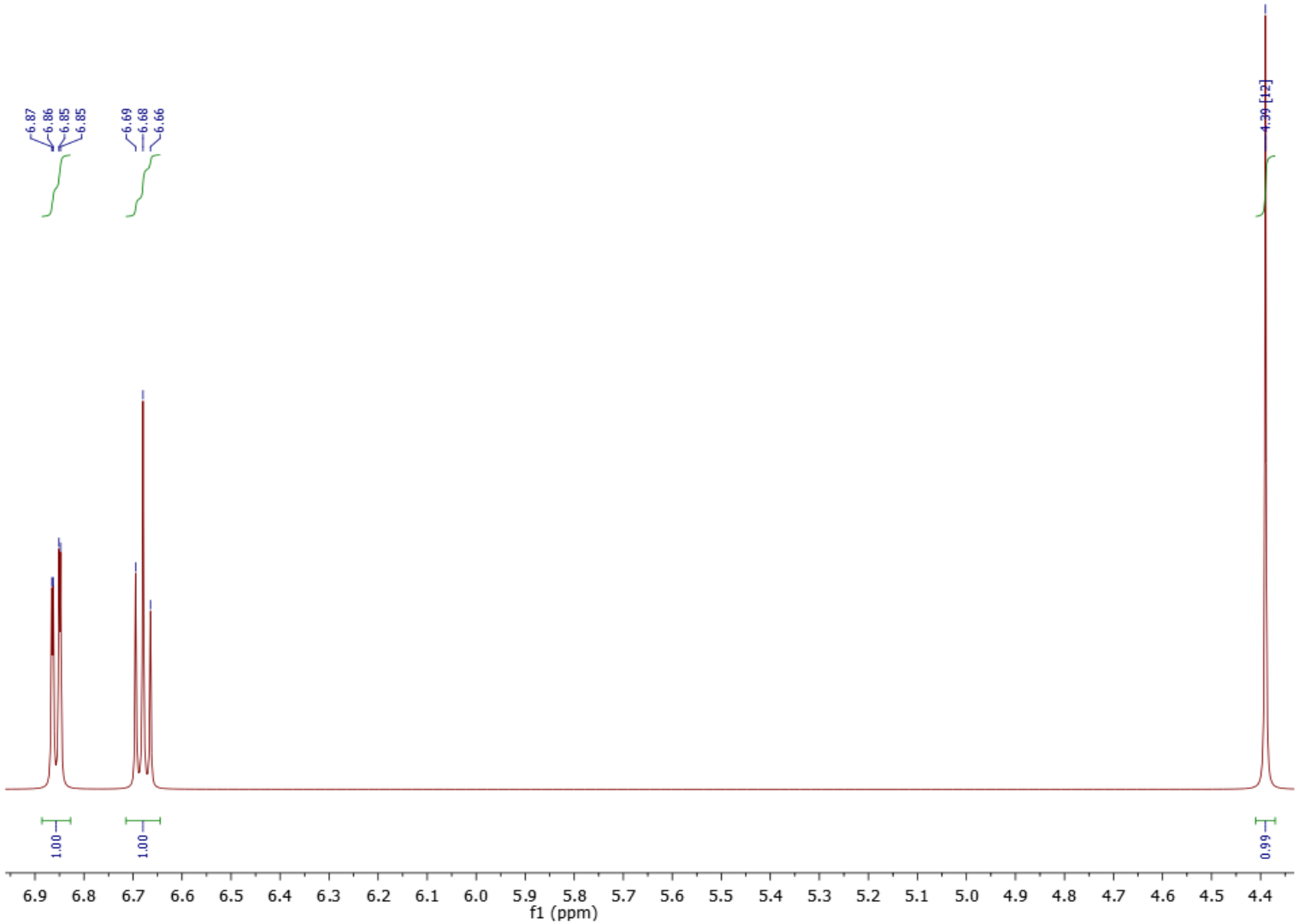


Fig-84: ^{1}H NMR Spectra of the compound SH_7 (Zoom View 2)

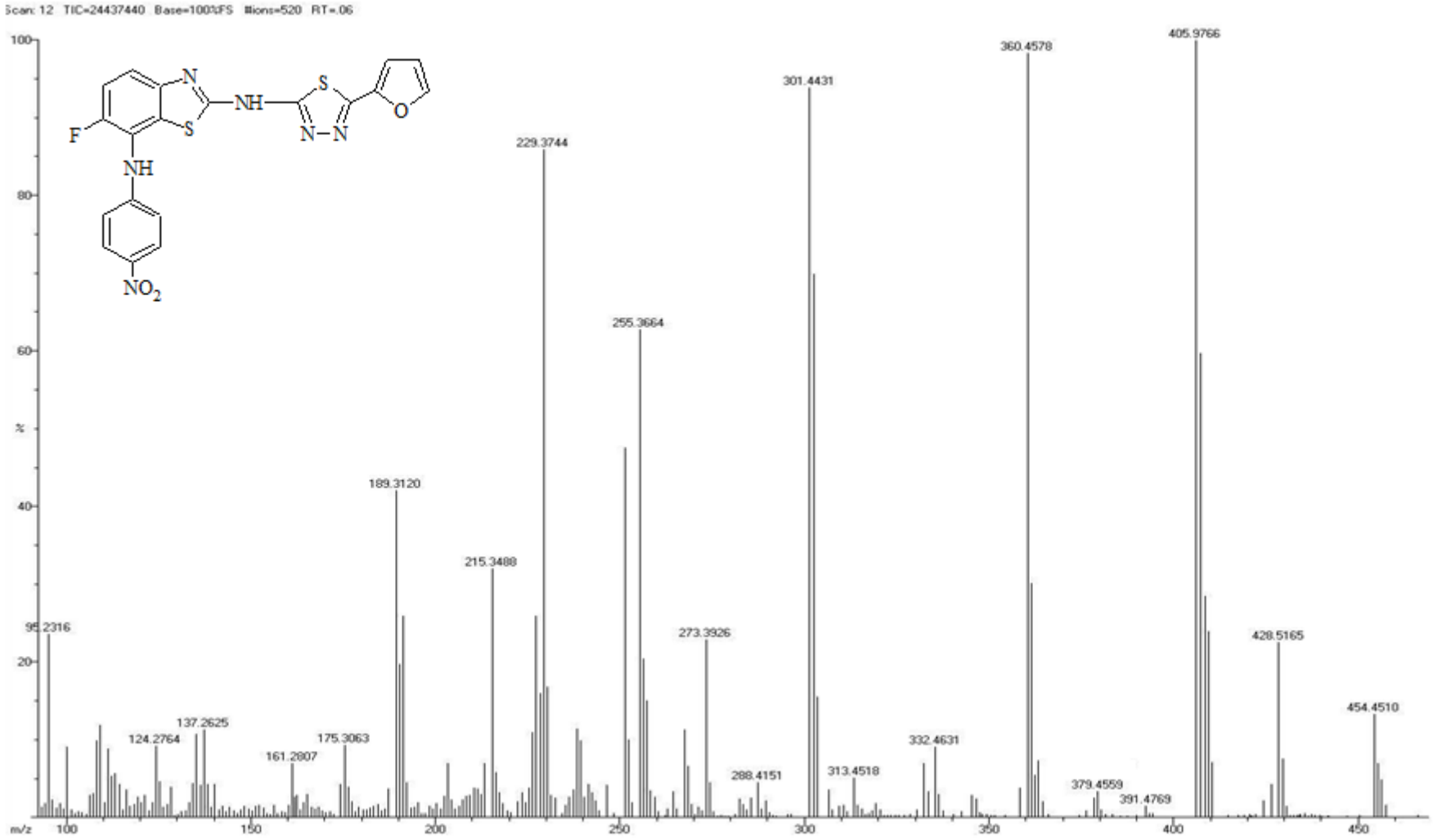


Fig.85: Mass Spectrum of the compound SH_7

6.2.8. 6-fluoro-N^7-(2-nitrophenyl)-N^2-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine(SH_8)

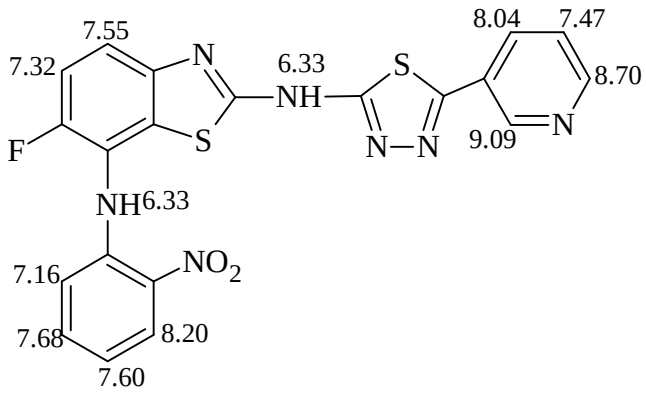
UV: (Fig-86)

λ_{max} (MeOH) 289.5 **(**ε_{max} 1.0659)

IR (KBr): (Fig-87)

S. No	Wave Number (cm^{-1})	Assignment
1	3348.87	N – H asymmetrical stretching
2	2851.37	Aromatic C – H stretching
3	1628.84	Aromatic C – H out of plane bending
4	1507.01	C = N stretching
5	1301.98	C – H stretching
6	1283.77	C – N stretching
7	1101.29	C – F stretching
8	977.00	N – N stretching
9	881.74	C – H out of plane bending
10	742.33	C – S stretching

NMR(DMSO – d_6): (Fig-88-90)

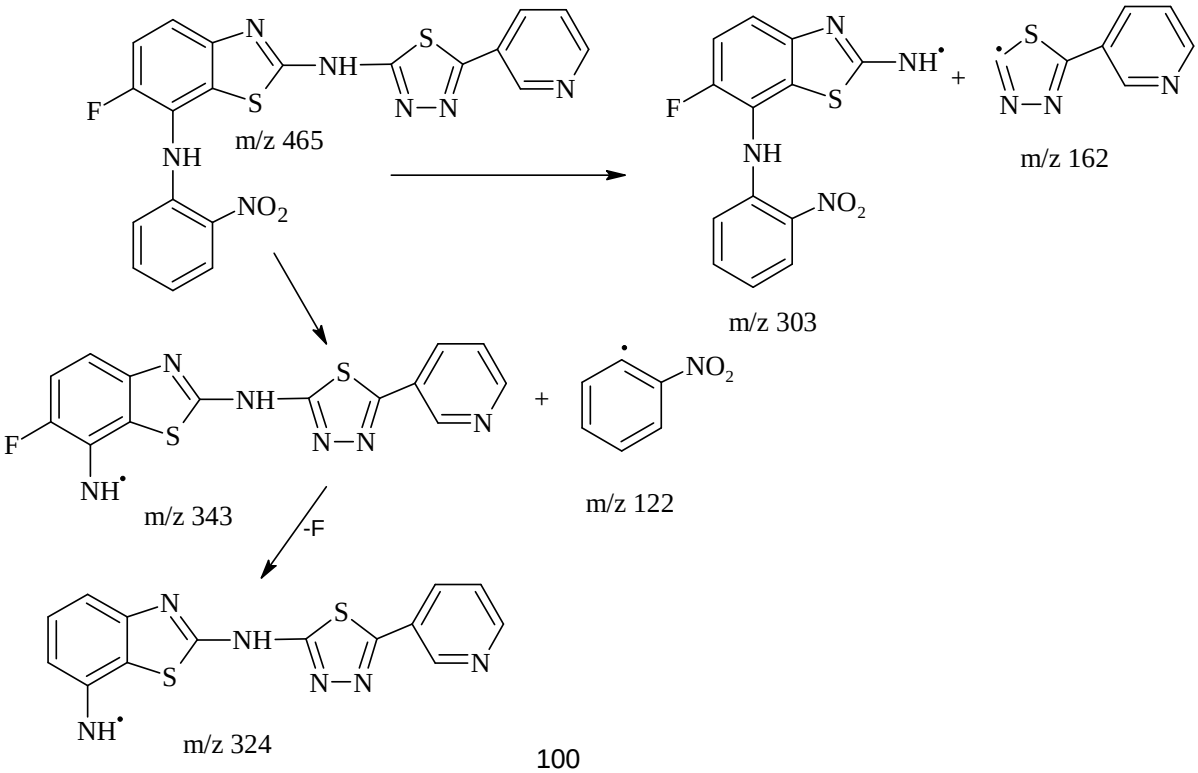


(10 aromatic protons, 2 protons on Nitrogen)

S. No	δ	Assignment
1	9.09	1H, s, CH of pyridine
2	8.70	1H, d, CH of pyridine
3	8.20	1H, d, Ar – H
4	8.04	1H, d, CH of pyridine
5	7.68	1H, t, Ar – H of aniline group
6	7.60	1H, t, Ar – H of aniline group
7	7.55	1H, d, Ar – H of benzothiazole
8	7.47	1H, t, CH of pyridine
9	7.32	1H, d, Ar – H of benzothiazole
10	7.16	1H, d, Ar – H
11	6.33	2H, s, NH

MASS: (Fig-91)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



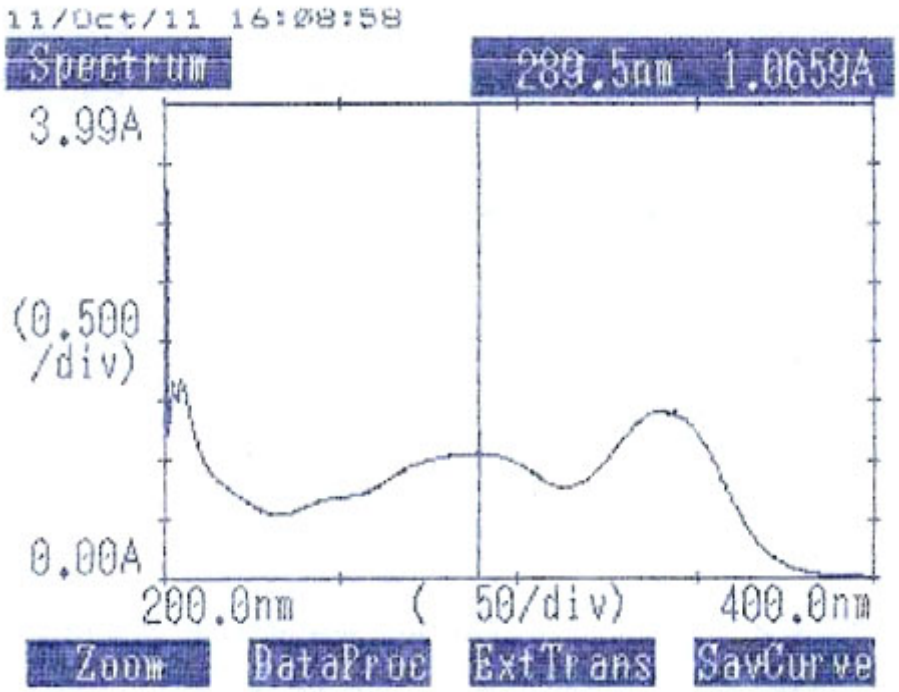


Fig.86:UV Spectrum of Compound SH_8

IDEAL ANALYTICAL AND RESEARCH INSTITUTION, PUDUCHERRY

Compound Code: SH8

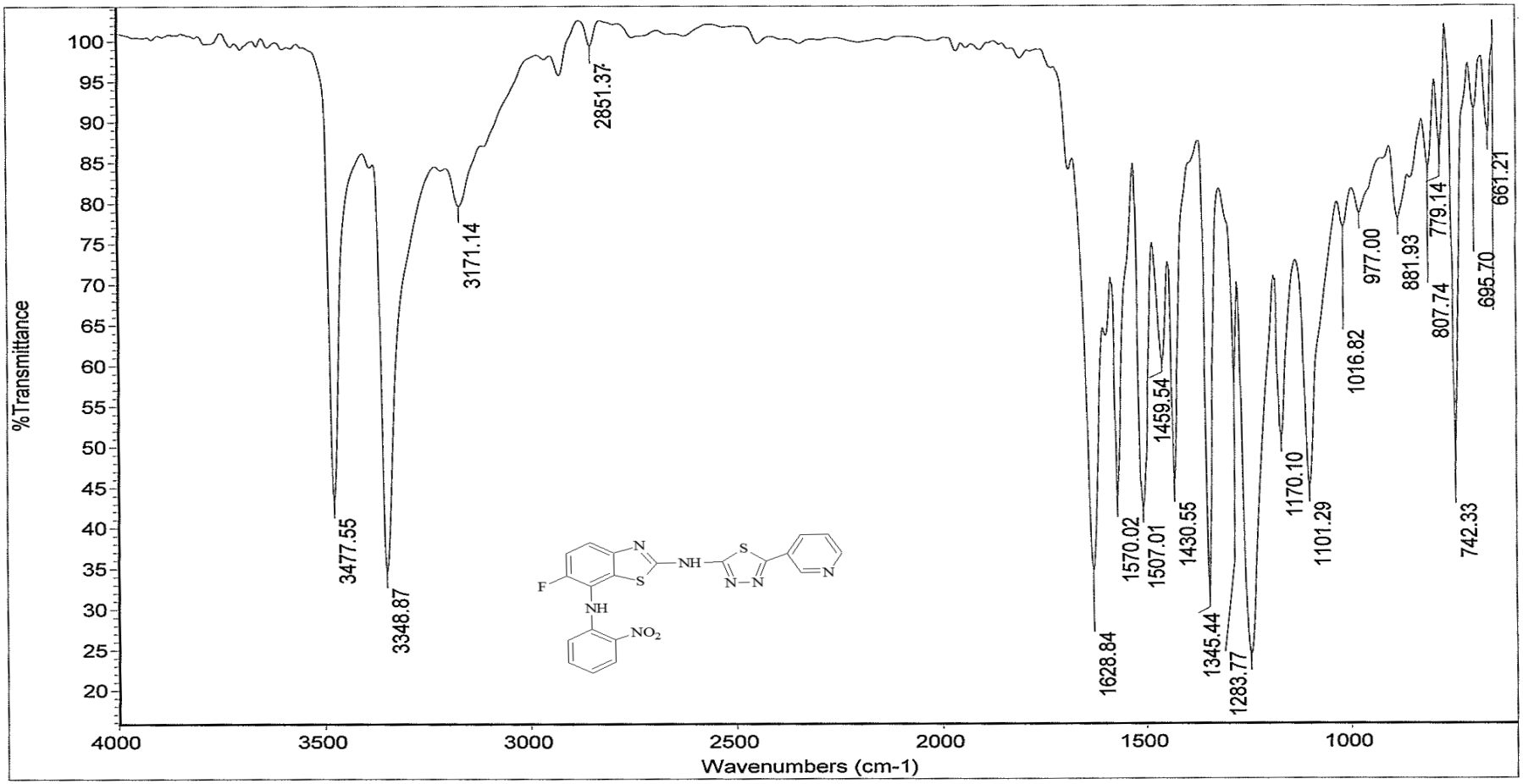


Fig.87: IR Spectrum of compound SH_8

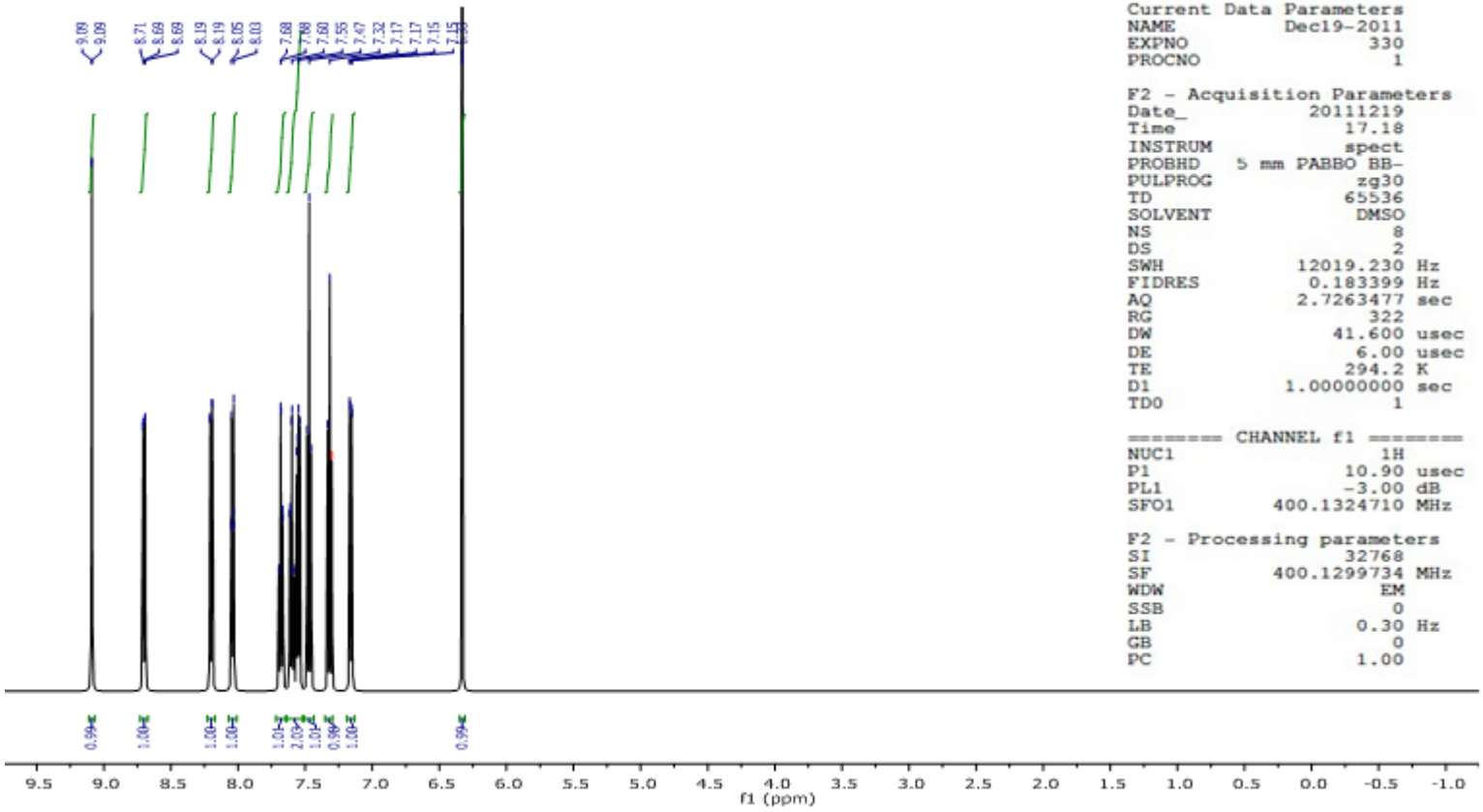


Fig-88: ^{1}H NMR Spectra of the compound SH_8

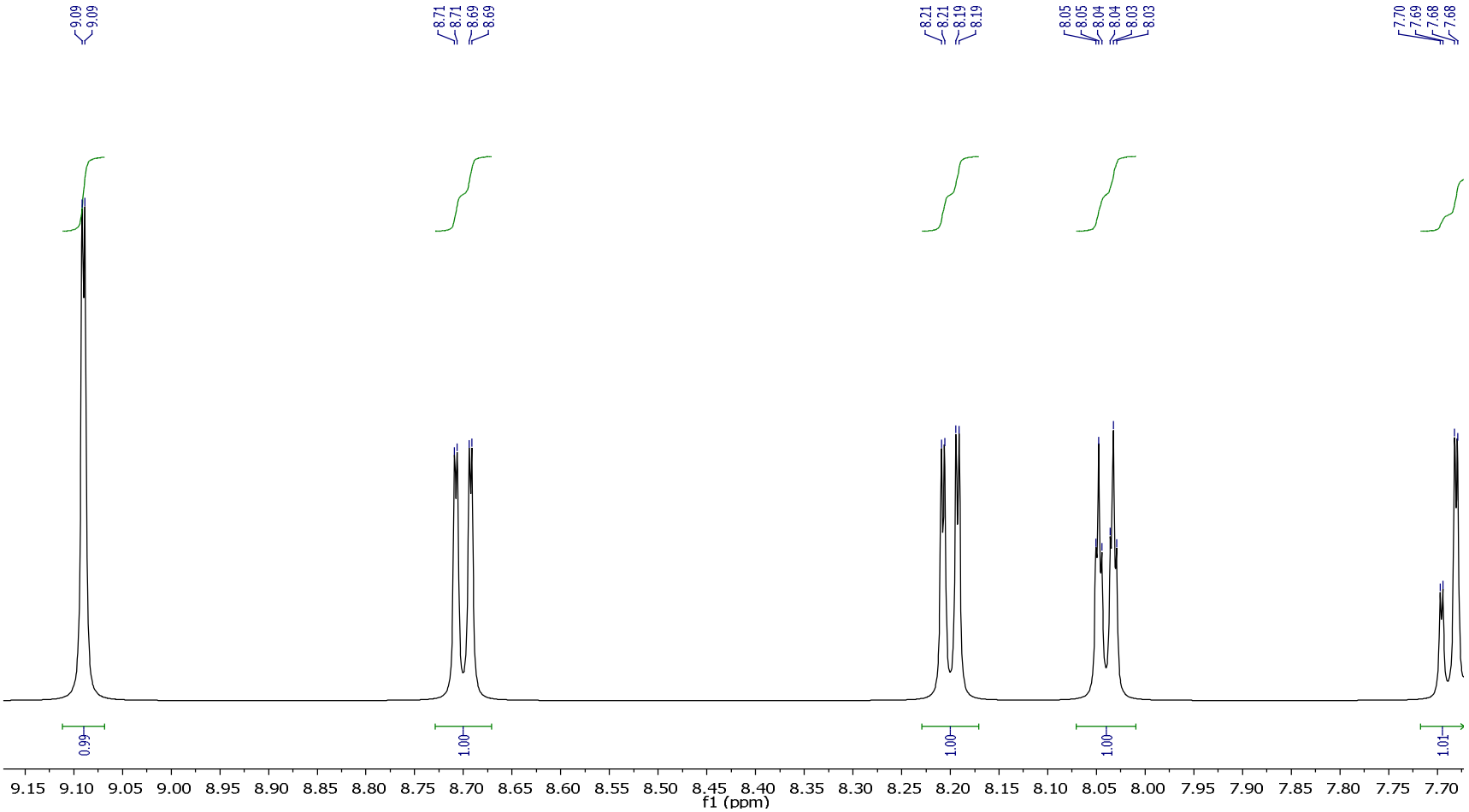


Fig-89: ^{1}H NMR Spectra of the compound SH_8 (Zoom View 1)

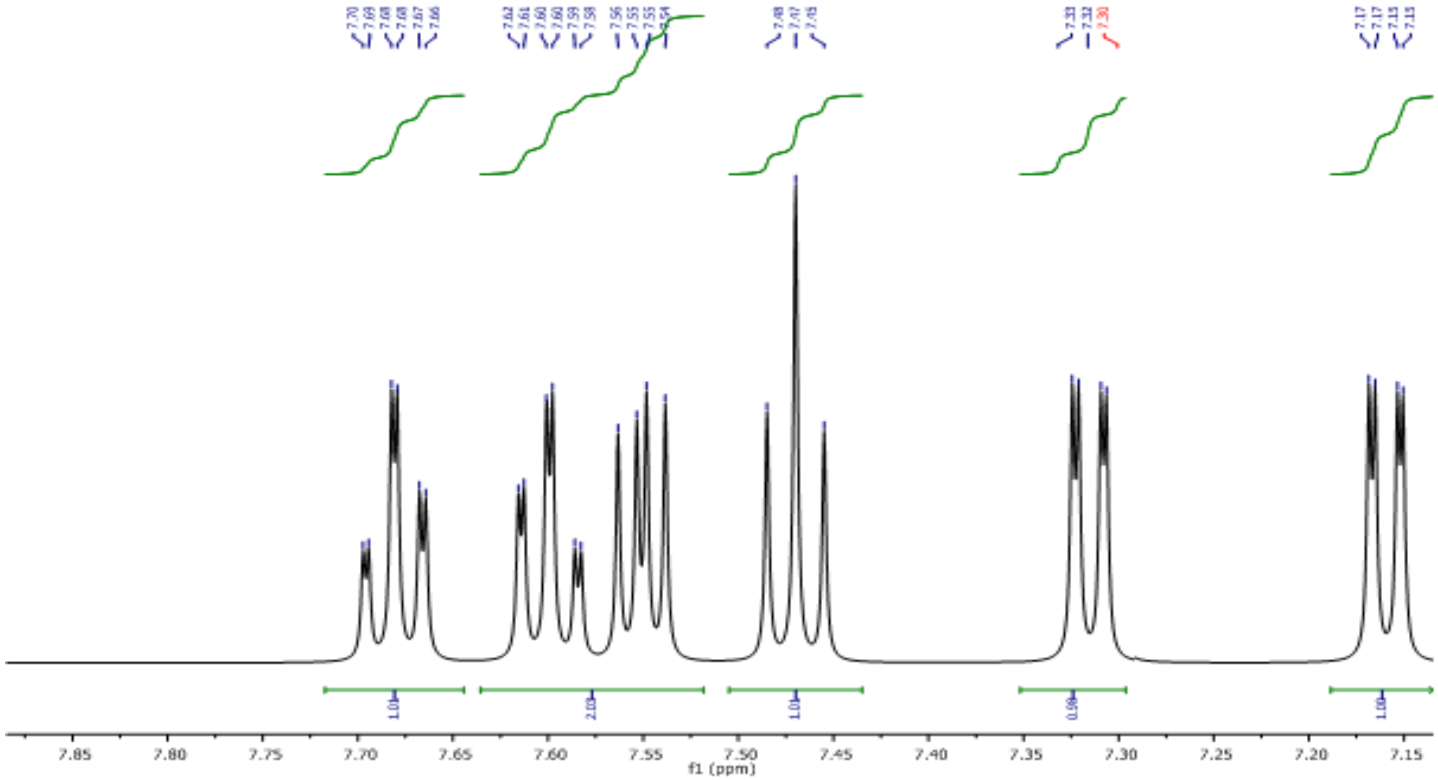


Fig-90: ^{1}H NMR Spectra of the compound SH_8 (Zoom View 2)

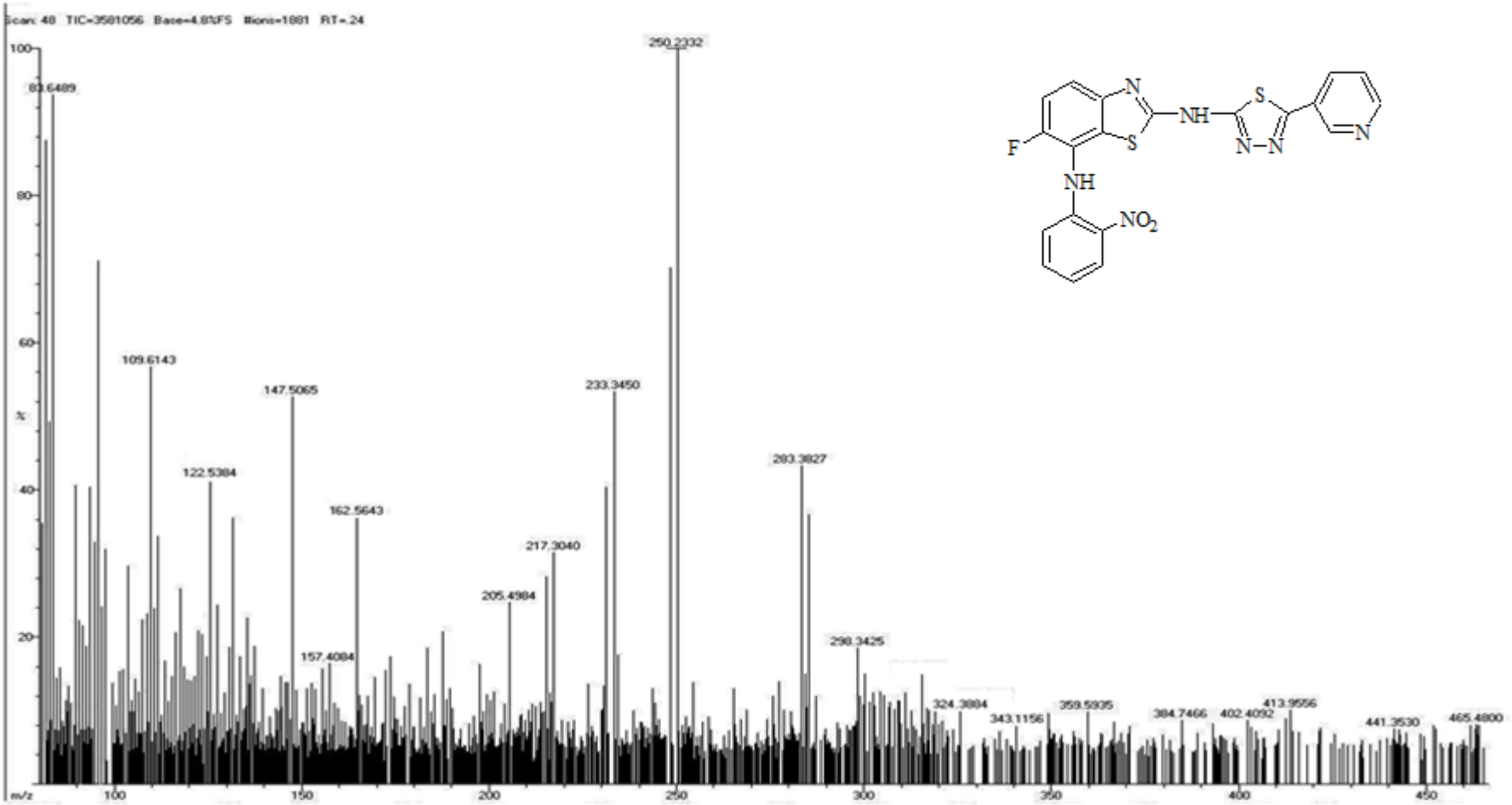


Fig.91: Mass Spectrum of the compound SH_8

6.2.9. 6-fluoro-N^7-(4-nitrophenyl)-N^2-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine(SH_9)

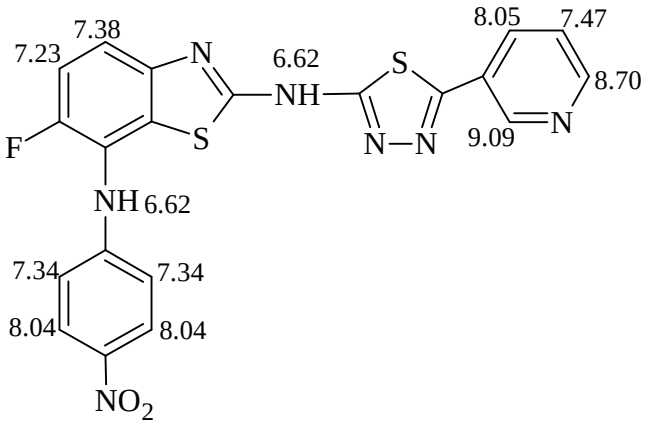
UV: (Fig-92)

λ_{max} (MeOH) 306.5 (ε_{max} 0.4948)

IR (KBr): (Fig-93)

S. No	Wave Number (cm^{-1})	Assignment
1	3345.55	N – H asymmetrical stretching
2	2857.14	Aromatic C – H stretching
3	1622.15	Aromatic C – H out of plane bending
4	1515.16	C = N stretching
5	1330.14	C – H stretching
6	1273.17	C – N stretching
7	1108.27	C – F stretching
8	970.03	N – N stretching
9	881.93	C – H out of plane bending
10	742.33	C – S stretching

NMR (DMSO – d_6): (Fig.94-96)

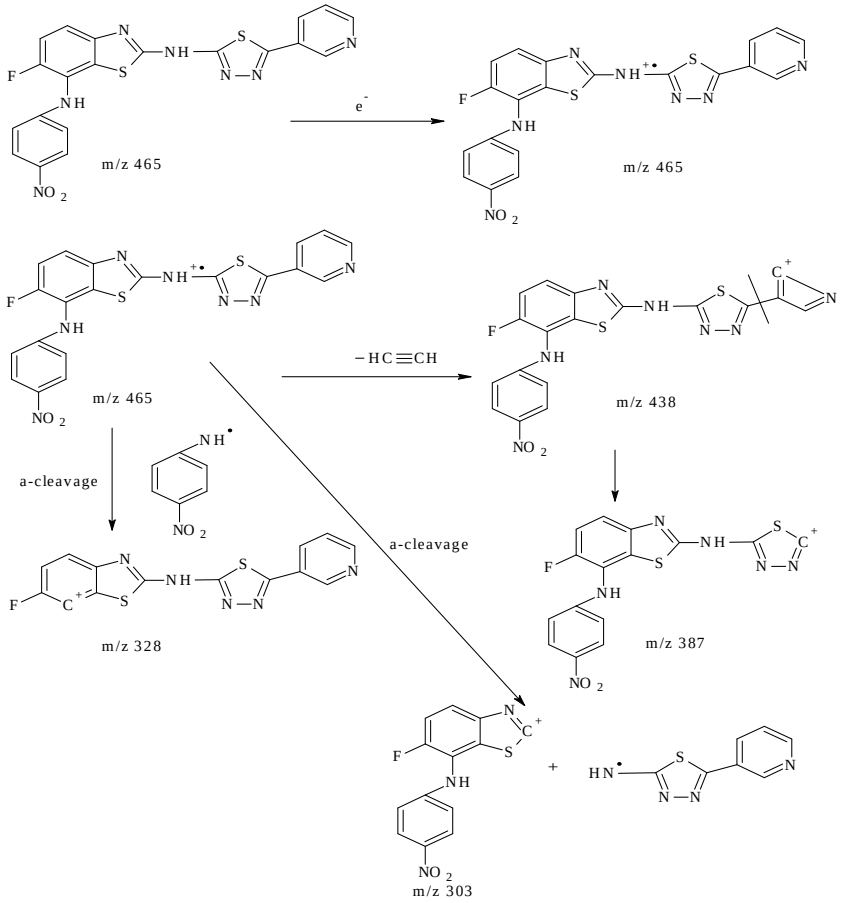


(10 aromatic protons, 2 protons on Nitrogen)

S. No	δ	Assignment
1	9.09	1H, s, Ar – CH
2	8.70	1H, d, Ar – CH
3	8.05-8.04	3H, m, Ar – H
4	7.47	1H, t, Ar – CH
5	7.38	1H, d, Ar – H of benzothiazole
6	7.34	2H, d, Ar – H
7	7.23	1H, d, Ar – CH of benzothiazole
8	6.62	1H, s, NH

MASS: (Fig-97)

The structure of the compound was further confirmed by mass spectroscopy and the molecular ion peak was found to be 463.31



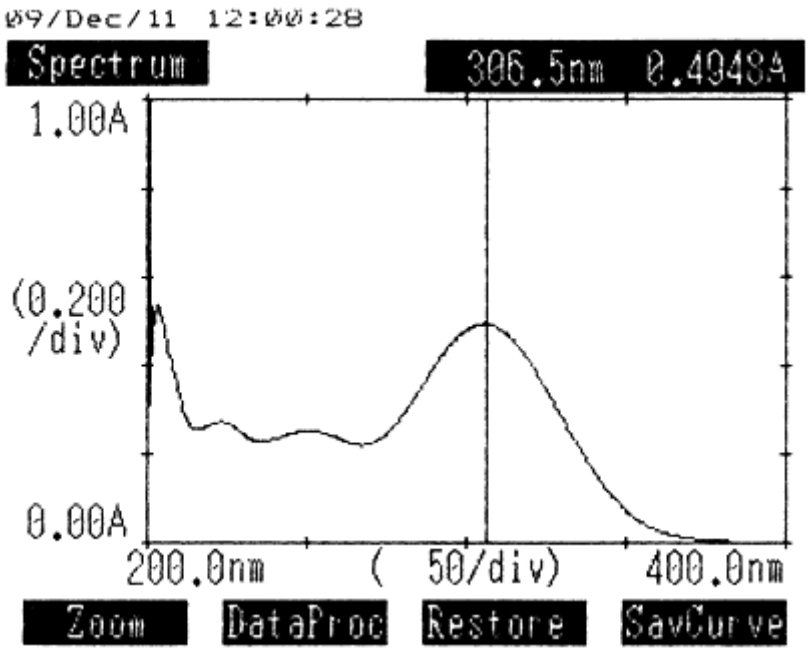


Fig.92:UV Spectrum of Compound SH_9

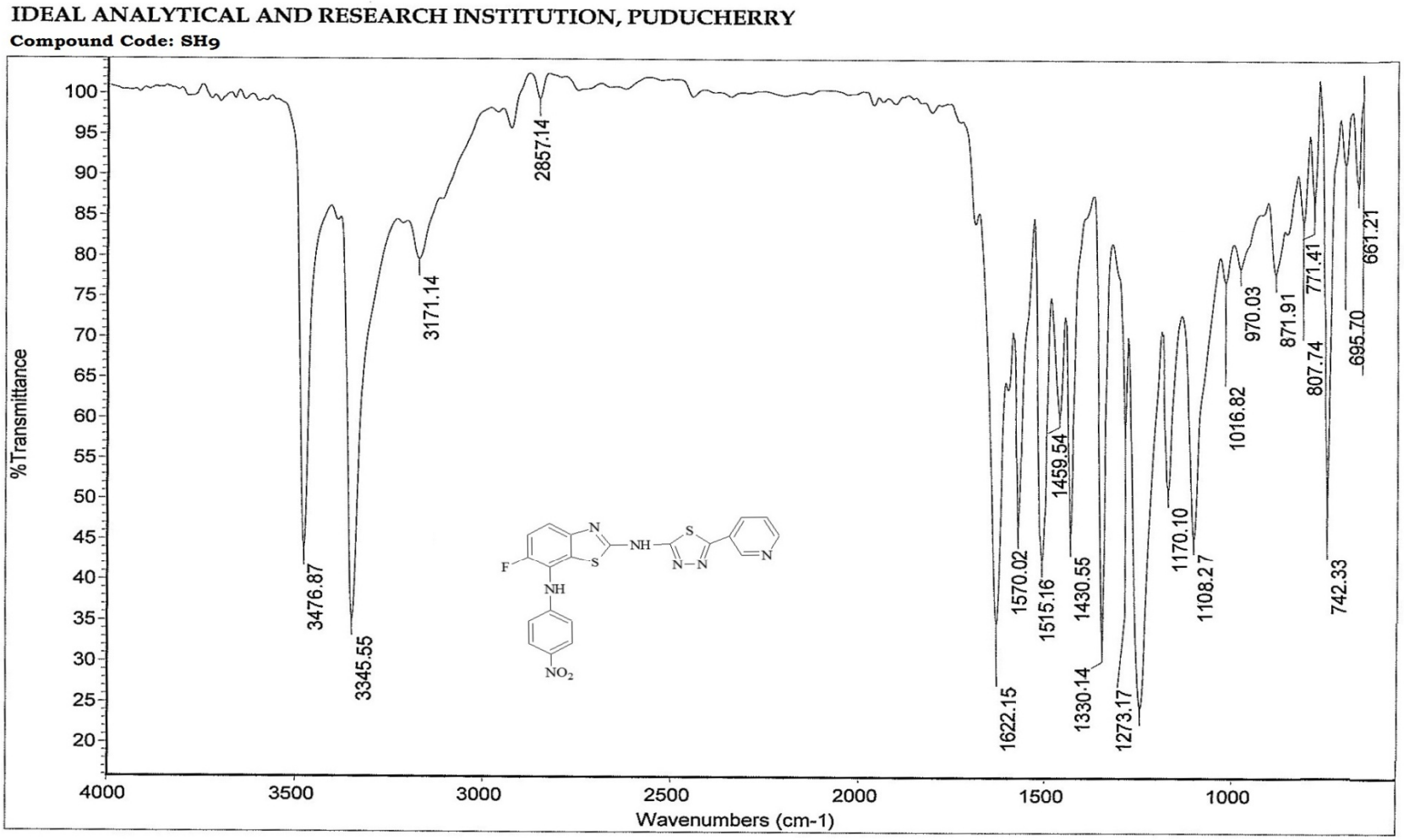


Fig.93: IR Spectrum of compound SH_9

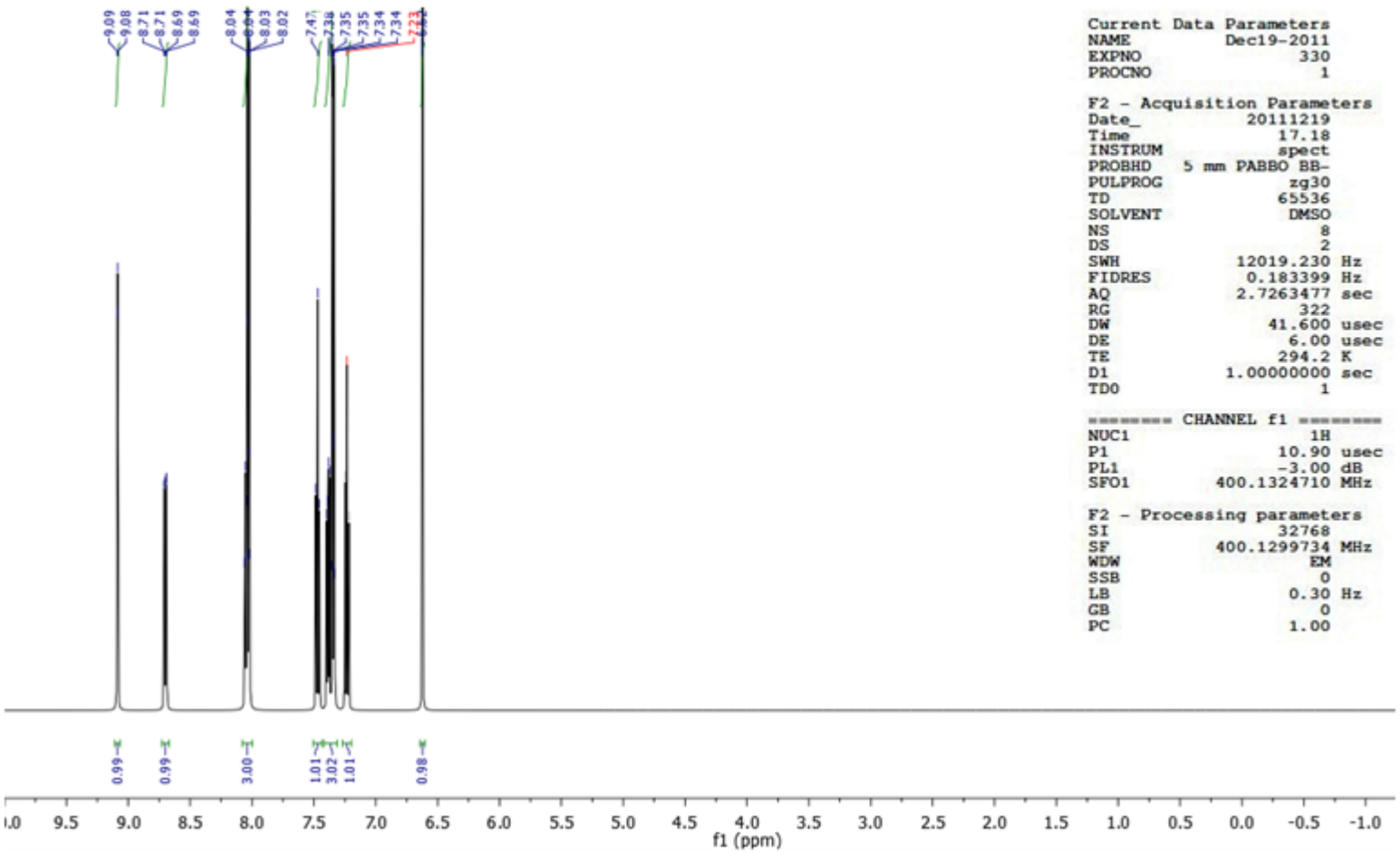


Fig-94: ^{1}H NMR Spectra of the compound SH_9

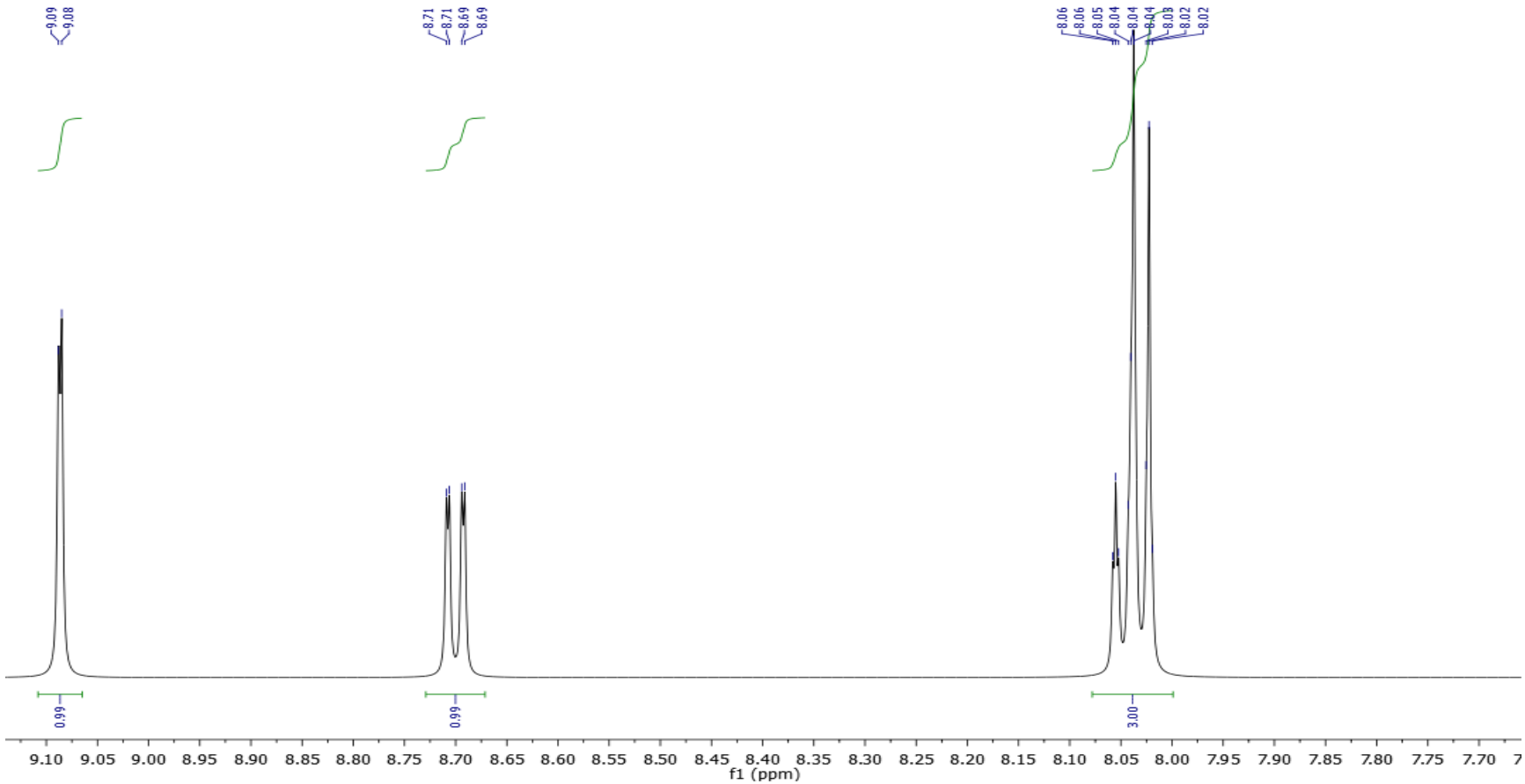


Fig-95: ^{1}H NMR Spectra of the compound SH_9(Zoom View 1)

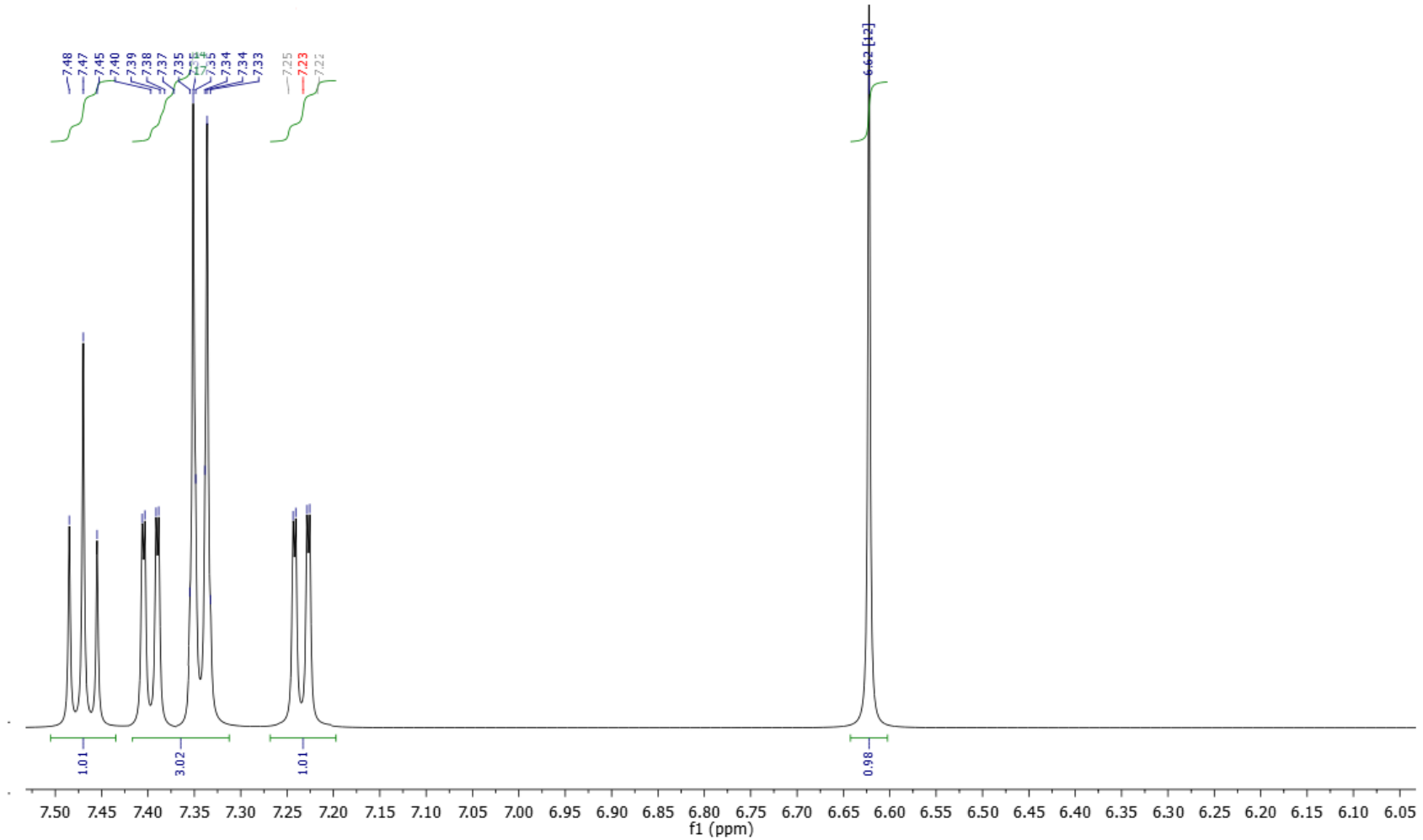


Fig-96: ^{1}H-NMR Spectra of the compound SH_9 (Zoom View 2)

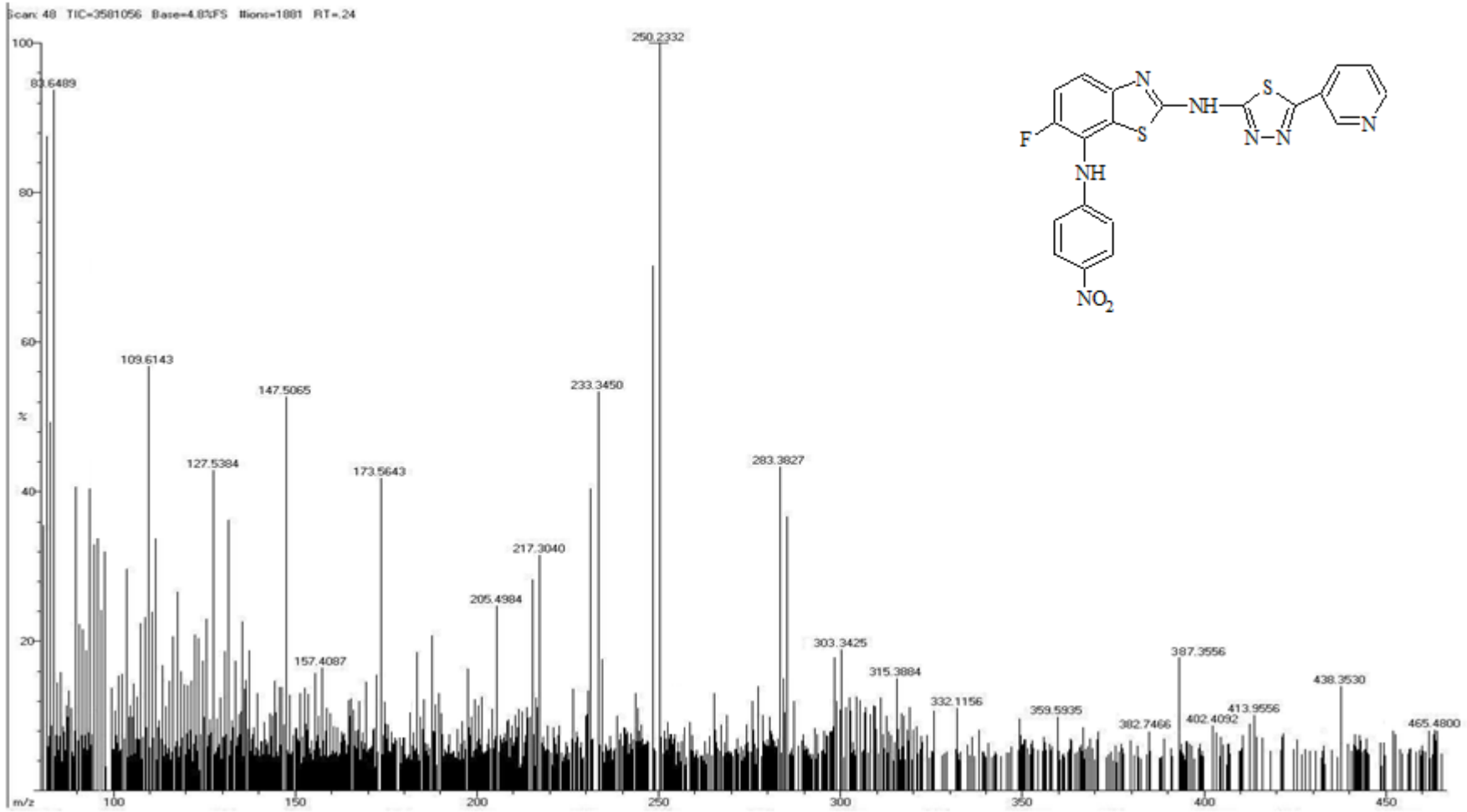


Fig.97: Mass Spectrum of the compound SH_9

6.2.10. N^2-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-N^7-(4-nitrophenyl)-1,3-benzothiazole-2,7-diamine (SH_{10})

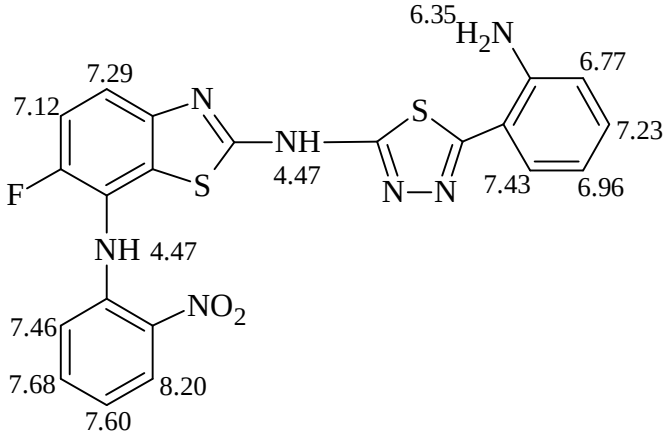
UV: (Fig.98)

λ_{max} (MeOH) 288.0 (ε_{max} 0.4660)

IR (KBr): (Fig.99)

S. No	Wave Number (cm^{-1})	Assignment
1	3351.36	N – H asymmetrical stretching
2	2925.41	Aromatic C – H stretching
3	1627.88	Aromatic C – H out of plane bending
4	1506.57	C = N stretching
5	1346.02	C – N stretching
6	1101.57	C – F stretching
7	1016.33	N – N stretching
8	694.78	C – H out of plane bending
9	743.99	C – S stretching

NMR (DMSO – d_6): (Fig-100-102)

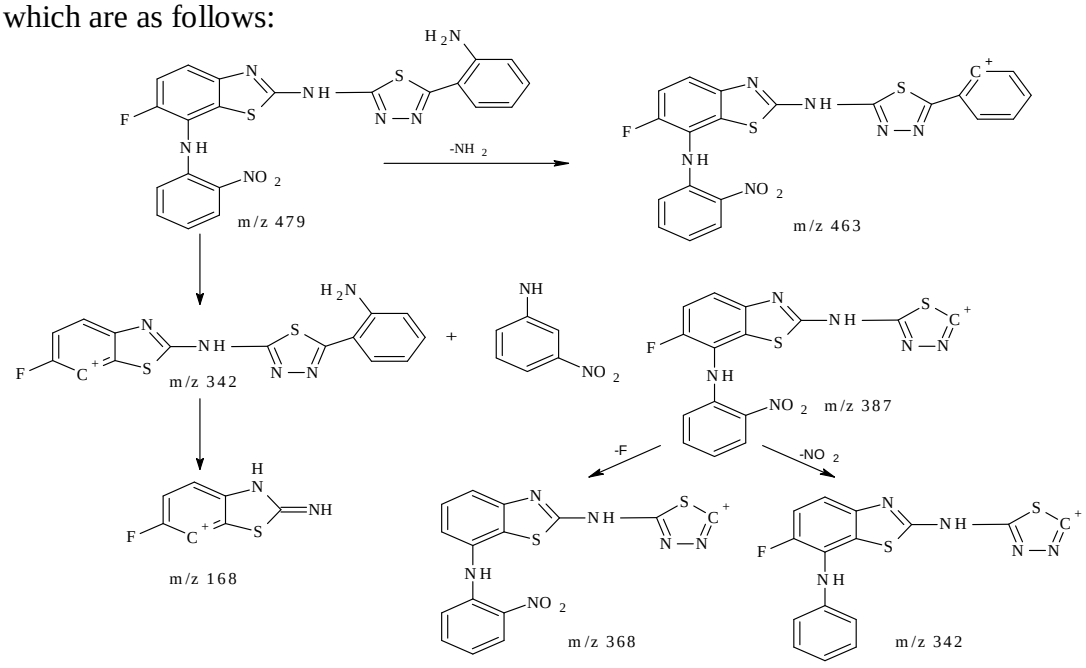


(10 aromatic protons, 4 protons on Nitrogen)

S. No	δ	Assignment
1	8.20	1H, d, of aniline group
2	7.68	1H, t, Ar – H of aniline group
3	7.60	1H, t, Ar – H of aniline group
4	7.46	1H, d, Ar – H of aniline group
5	7.43	1H, d, Ar – H
6	7.29	1H, d, Ar – H of benzothiazole
7	7.23	1H, t, Ar – H
8	7.12	1H, d, Ar – H of Benzothiazole
9	6.96	1H, t, Ar – H
10	6.77	1H, d, Ar – H
11	6.35	2H, s, NH_2
12	4.47	2H, s, NH

MASS: (Fig-103)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



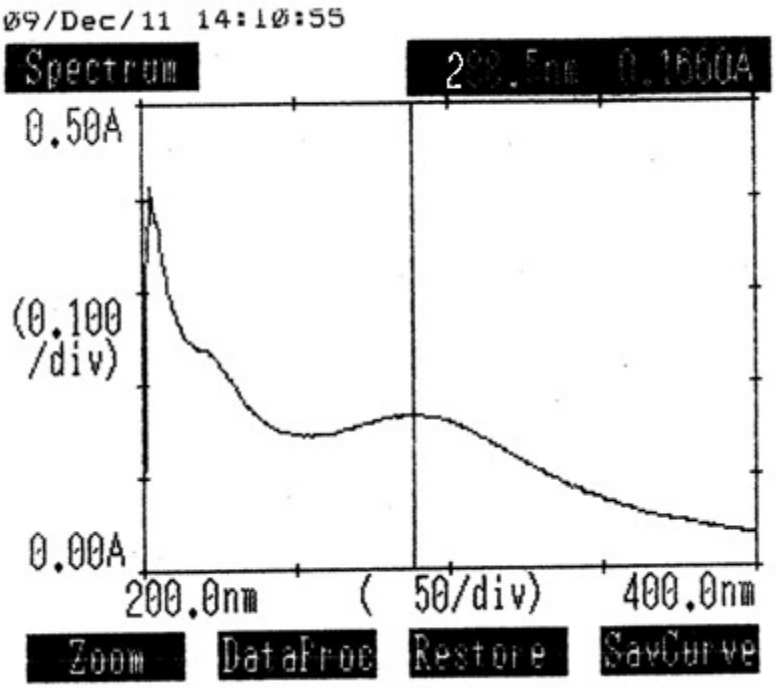


Fig.98:UV Spectrum of Compound SH_{10}

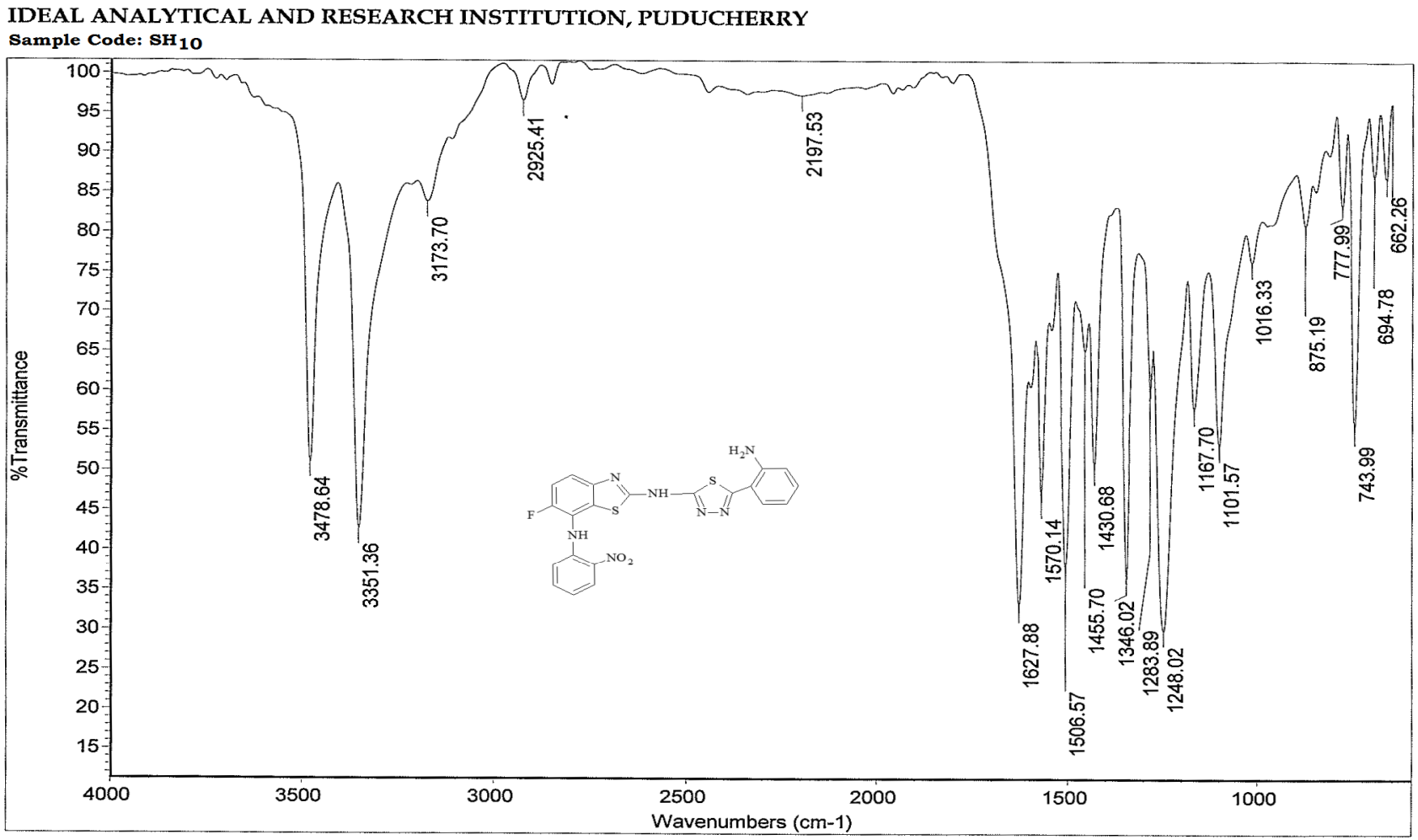


Fig.99: IR Spectrum of compound SH_{10}

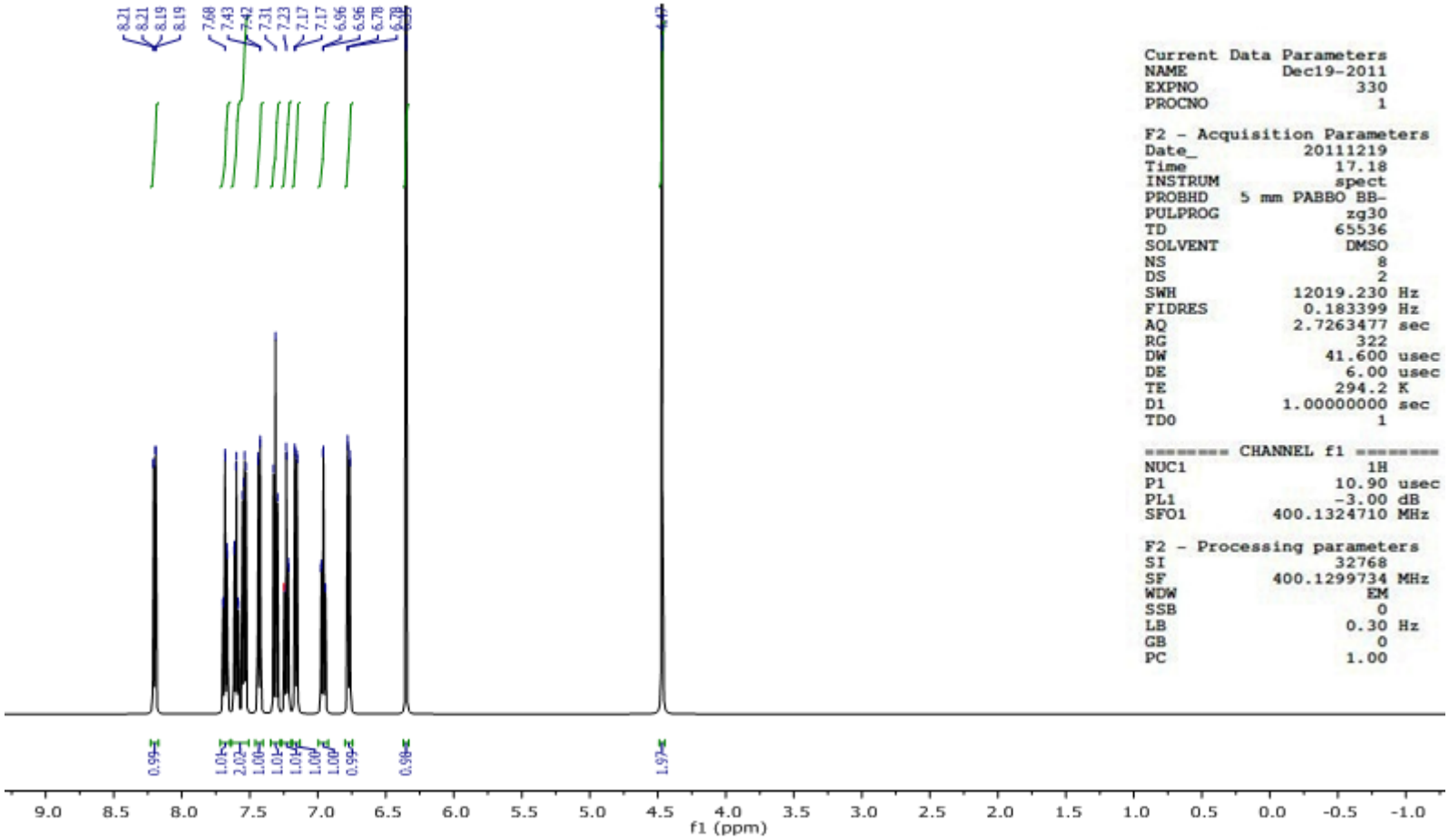


Fig-100: ^{1}H NMR Spectra of the compound SH_{10}

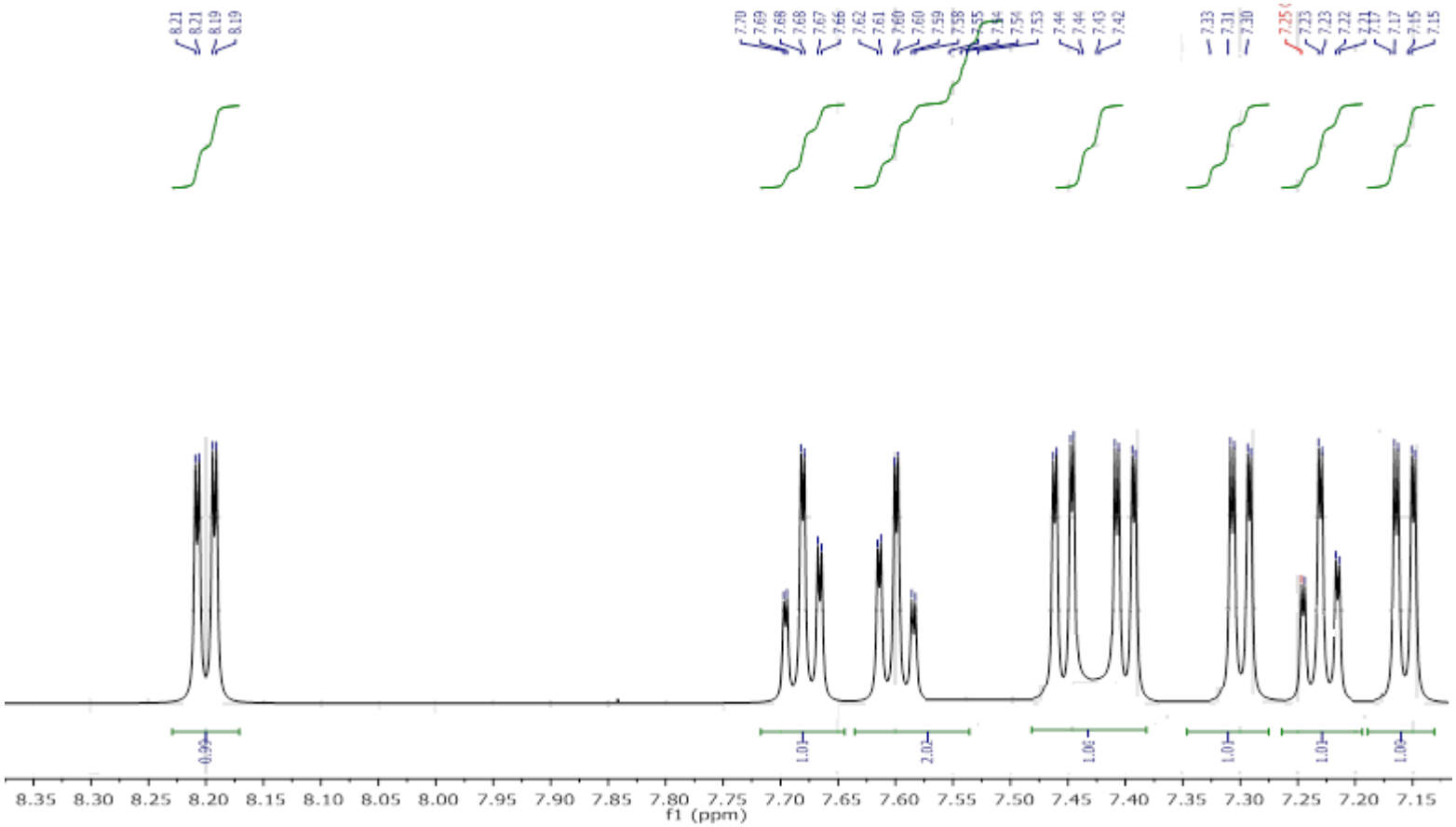


Fig-101: ^{1}H NMR Spectra of the compound SH_{10} (Zoom View 1)

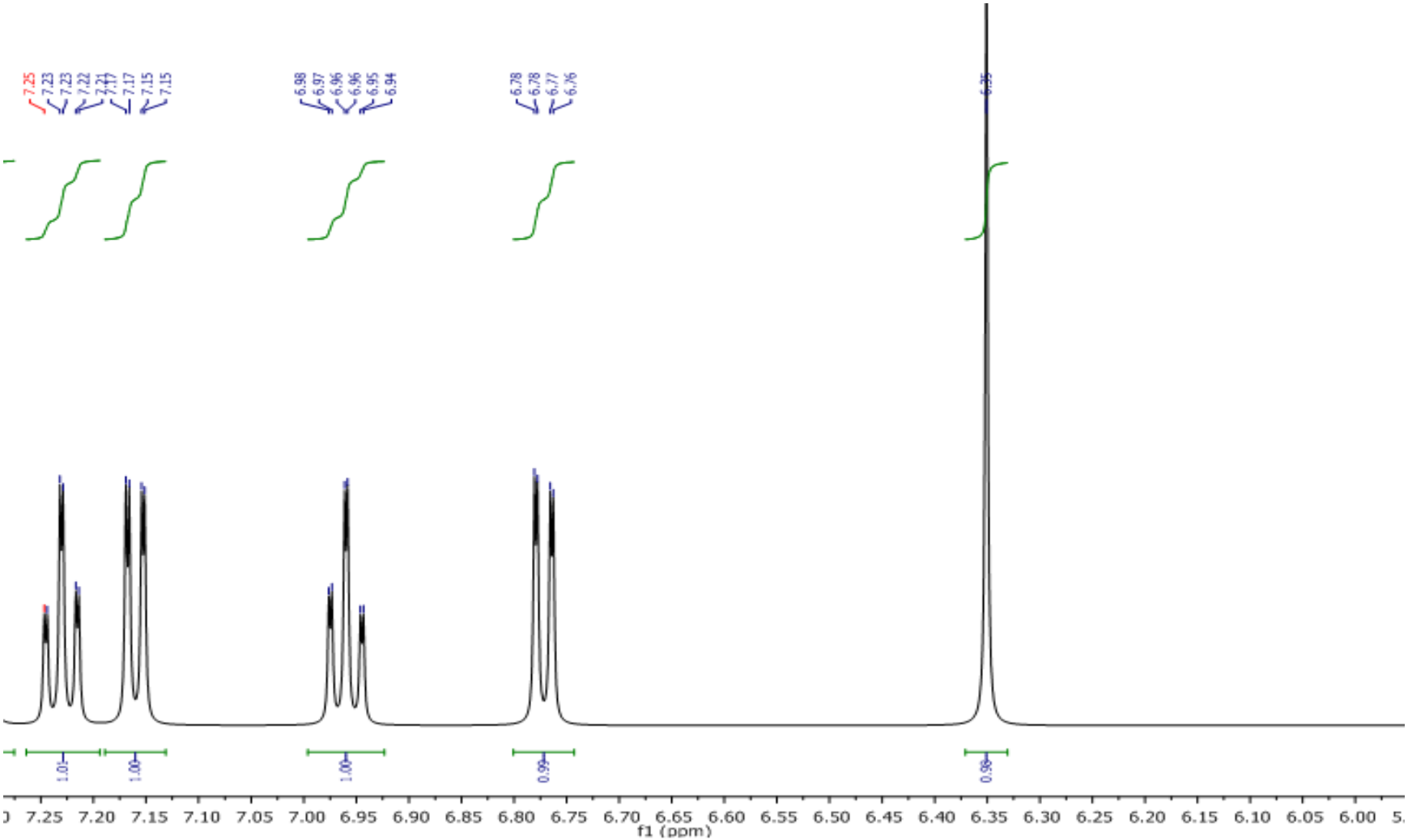


Fig-102: ^{1}H NMR Spectra of the compound SH_{10} (Zoom View 2)

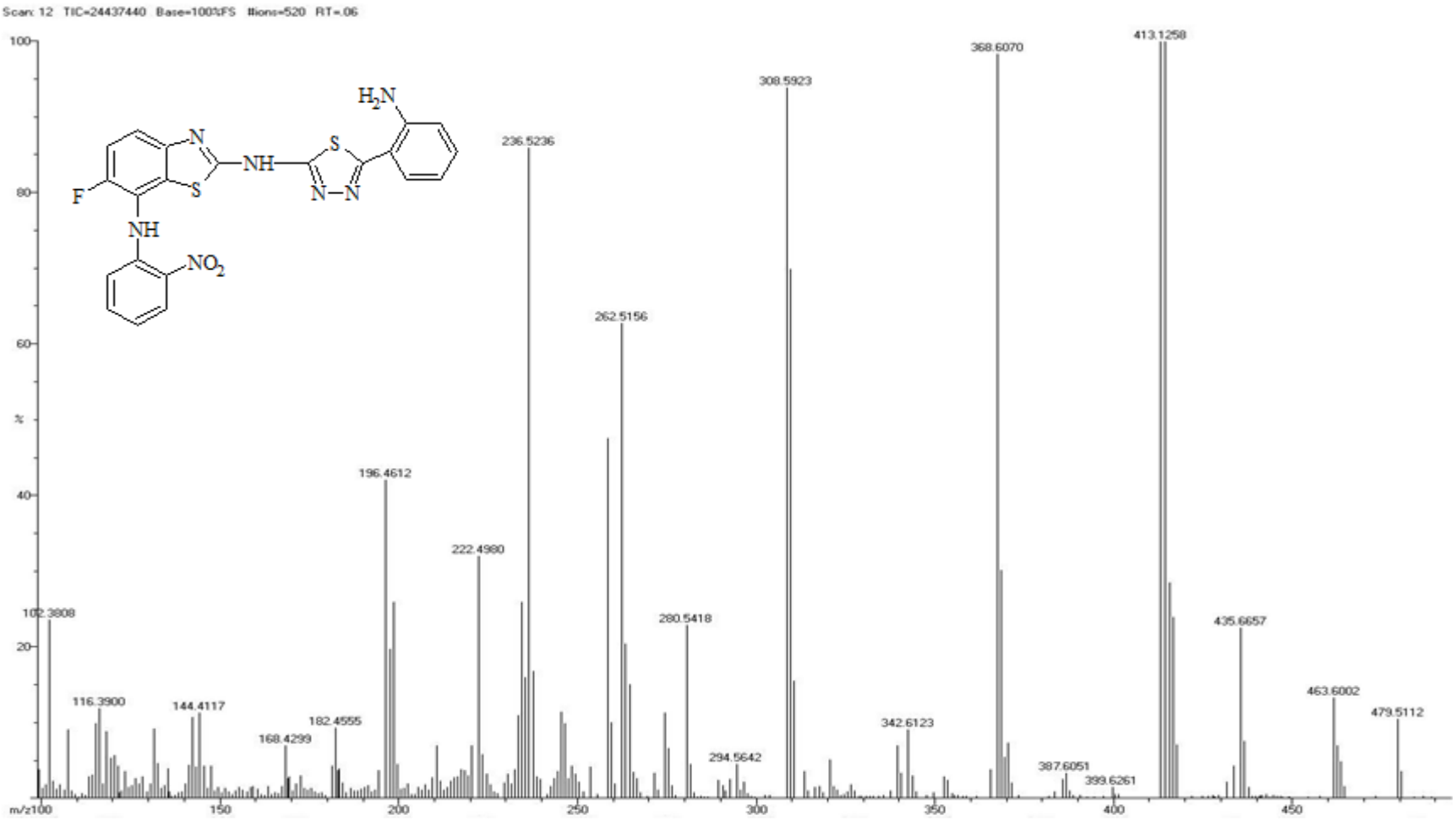


Fig.103: Mass Spectrum of the compound SH_{10}

6.2.11. N^2-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-N^7-(2-nitrophenyl)-1,3-benzothiazole-2,7-diamine (SH_{11})

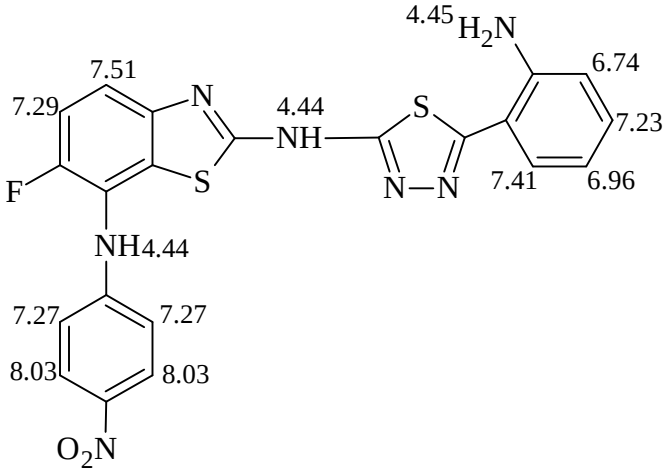
UV: (Fig-104)

λ_{max} (MeOH) 290.0 (ε_{max} 0.9673)

IR (KBr): (Fig-105)

S. No	Wave Number (cm^{-1})	Assignment
1	3482.56	N – H asymmetrical stretching
2	2919.88	Aromatic C – H stretching
3	1629.00	Aromatic C – H out of plane bending
4	1504.86	C = N stretching
5	1285.90	C – N stretching
6	1109.11	C – F stretching
7	1015.26	N – N stretching
8	842.98	C – H out of plane bending
9	751.72	C – S stretching

NMR(DMSO – d_6): (Fig-106-108)

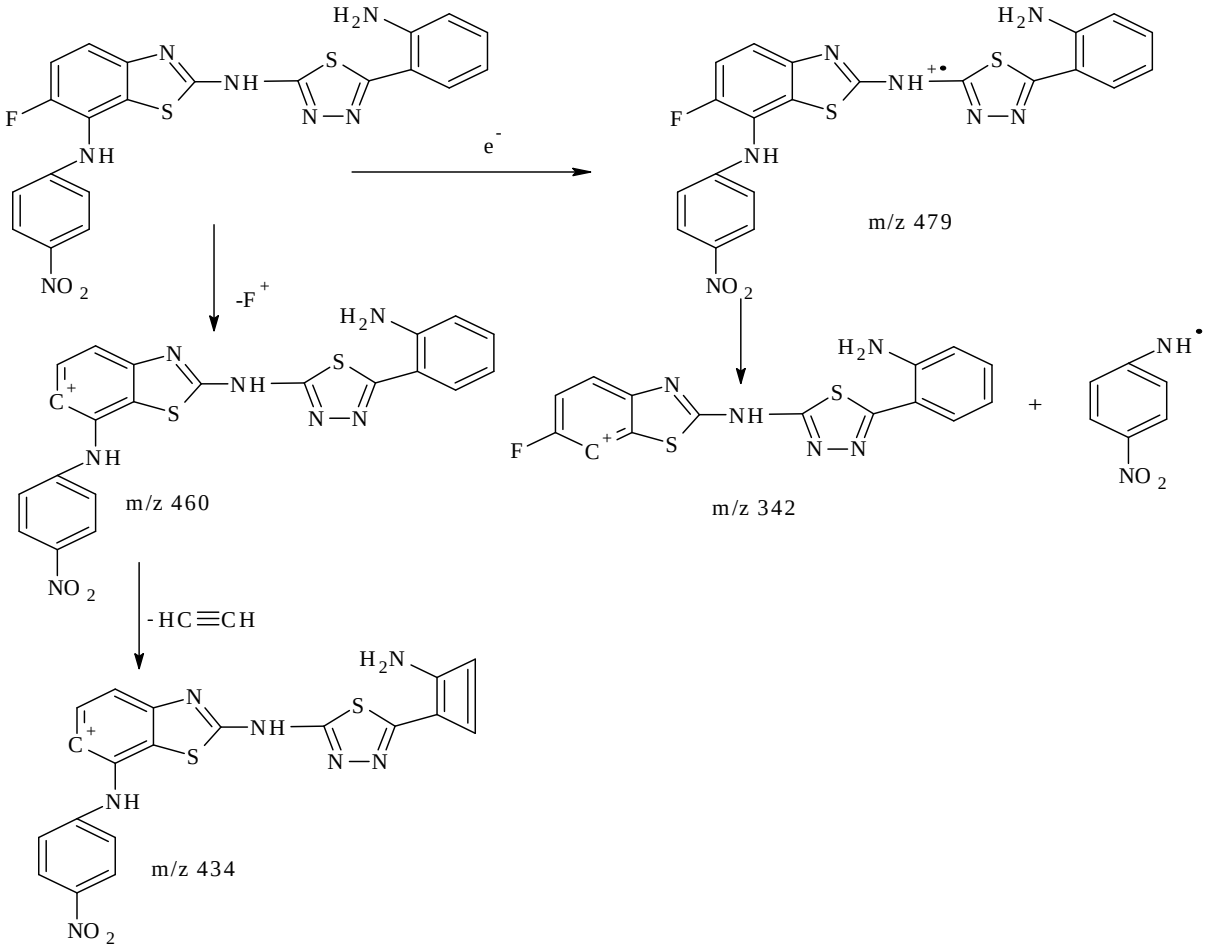


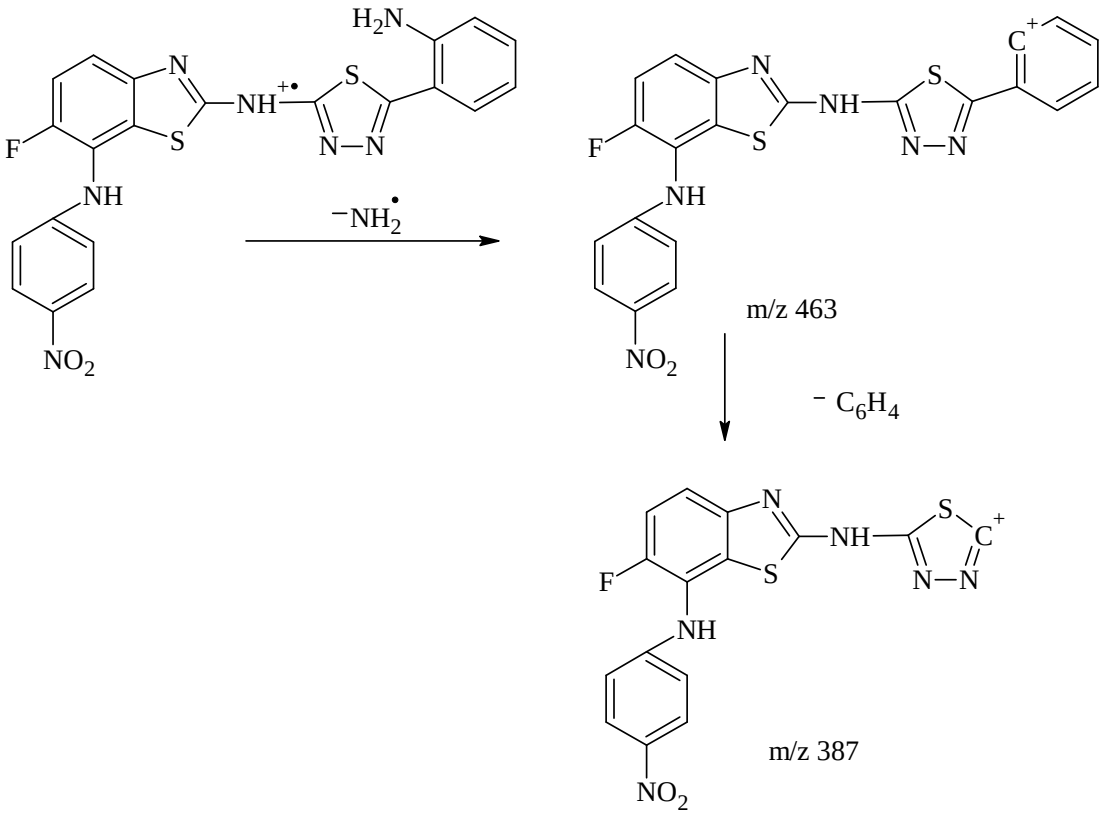
(10 aromatic protons, 4 protons on Nitrogen)

S. No	δ	Assignment
1	8.03	2H, d, Ar – H of aniline group
2	7.51	1H, d, Ar – H of benzothiazole
3	7.41	1H, d, Ar – H
4	7.23-7.29	4H, m, Ar – H
7	6.96	1H, t, Ar – H
8	6.74	1H, d, Ar – H
9	4.45	2H, s, NH_2
10	4.44	2H, s, NH

M**ASS: (Fig-109)**

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:





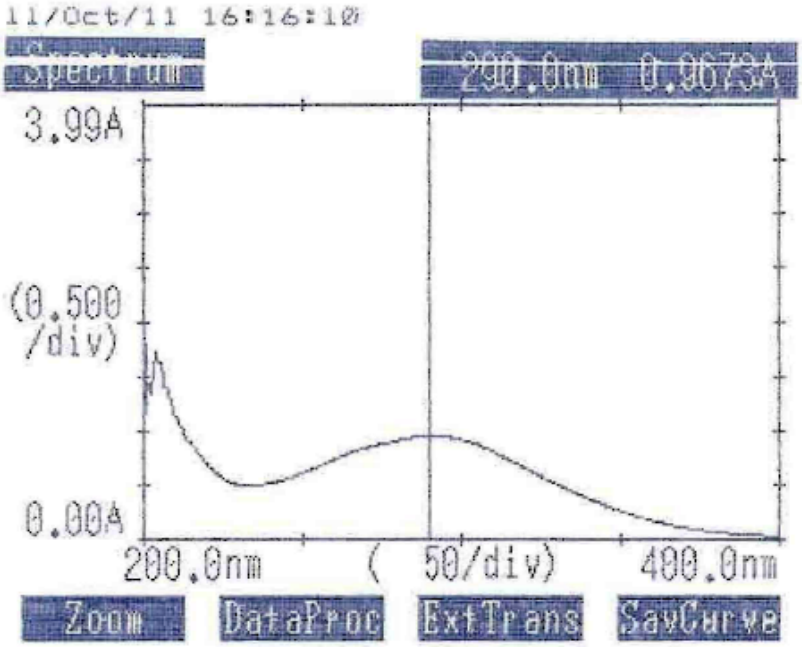


Fig.104:UV Spectrum of Compound SH_{11}

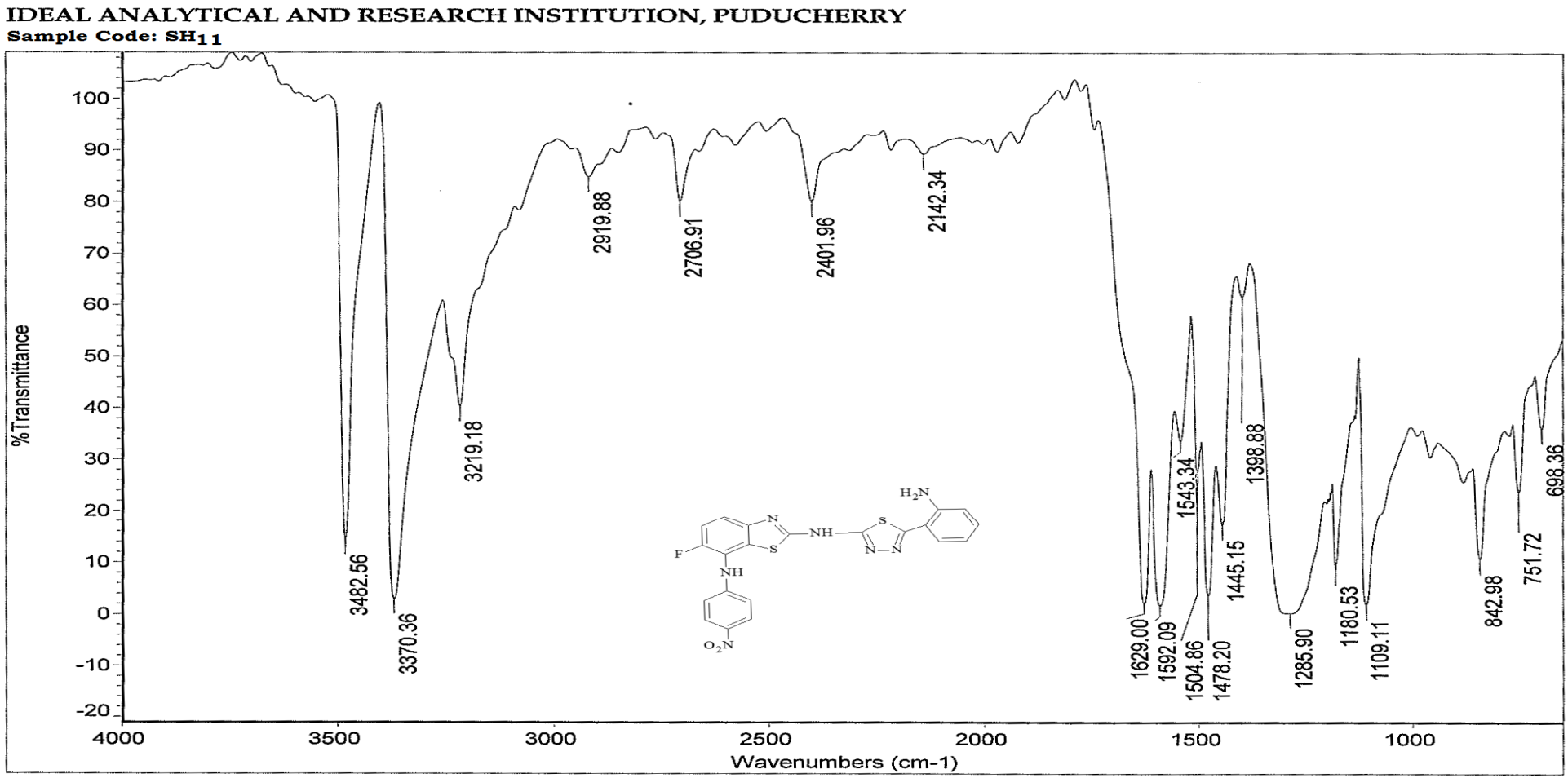


Fig.105: IR Spectrum of compound SH_{11}

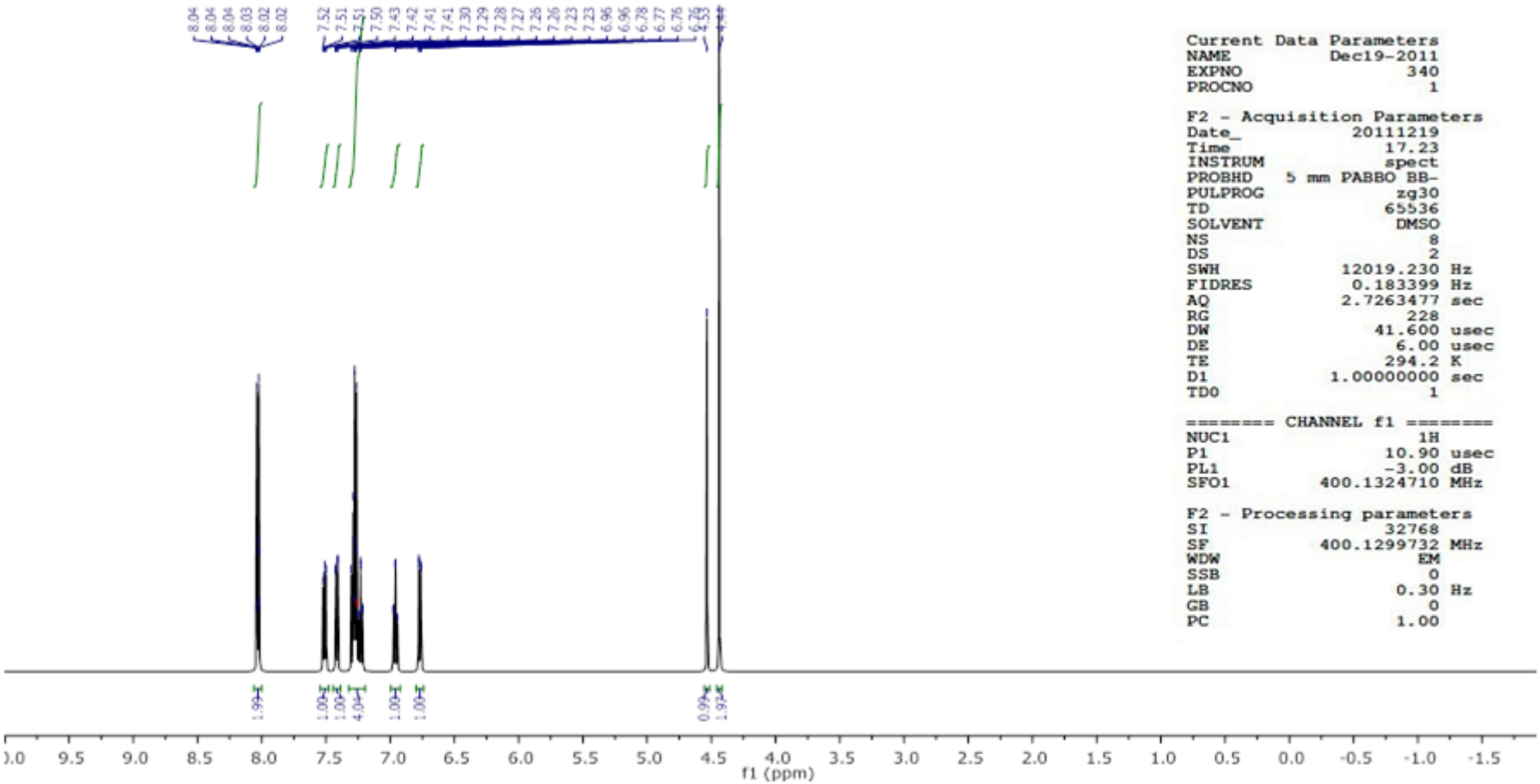


Fig-106: ^{1}H NMR Spectra of the compound SH_{11}

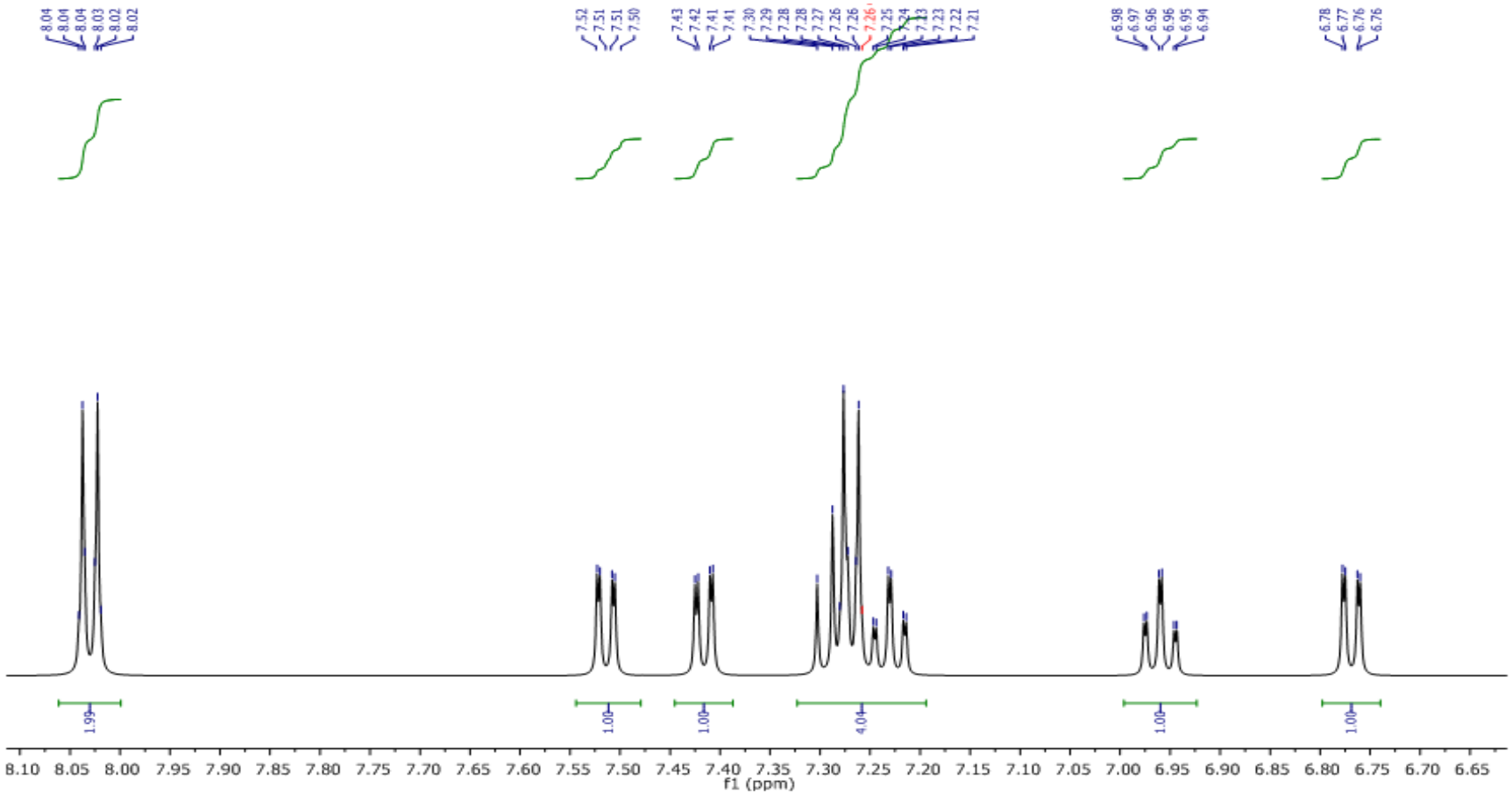


Fig-107: ^{1}H NMR Spectra of the compound SH_{11} (Zoom View 1)

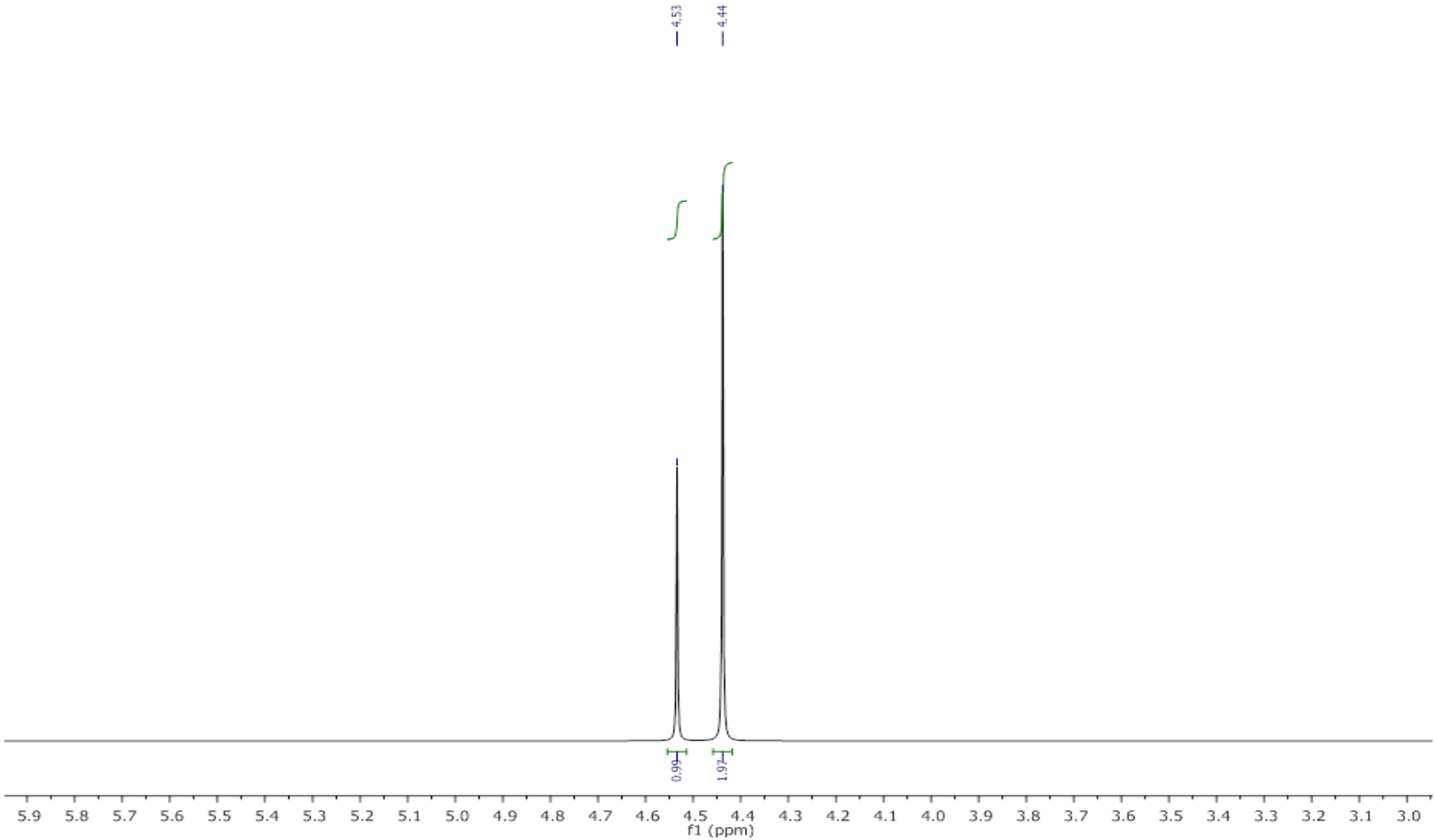


Fig-108: ^{1}H NMR Spectra of the compound SH_{11} (Zoom View 2)

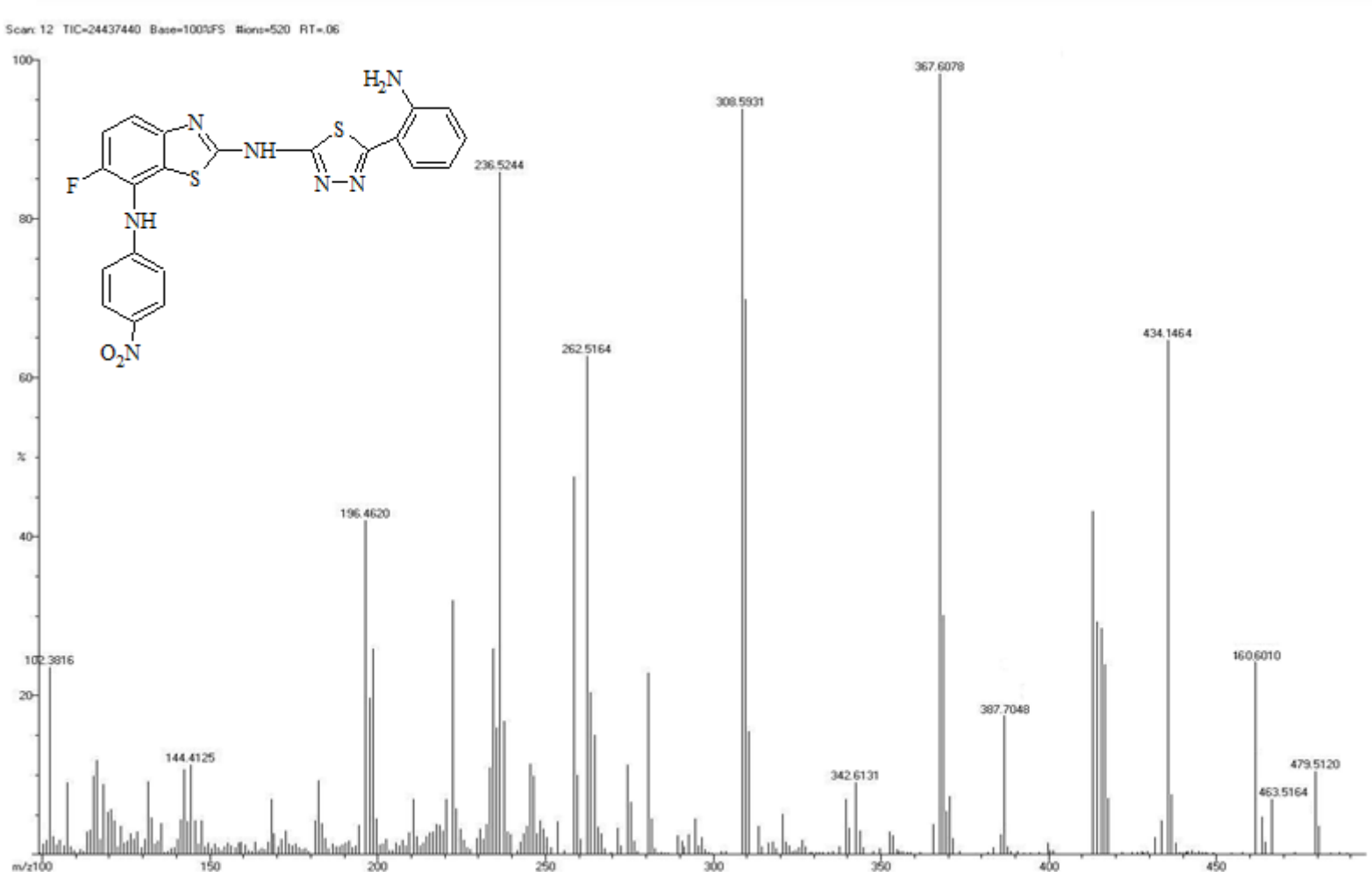


Fig.109: Mass Spectrum of the compound SH_{11}

6.3. Physical and Analytical Data of the Synthesized Compounds

Code	Name	Nature	Solubility	Molecular Weight (gm)	Molecular Formula	Melting Point (°C)	Percentage Yield (%)	R_f Value
SH_1	7-chloro-6-fluoro-1,3-benzothiazol-2-amine	Light yellow colour powder	Freely soluble: Methanol, ethyl acetate, ethanol and acetone Sparingly soluble: water, benzene and chloroform	202.63	$C_7H_4ClFN_2S$	212	47.5	0.57
SH_2	N-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)hydrazinecarbothioamide	Light brownish white colour	Freely soluble: DMF Methanol, ethanol, ethyl acetate, benzene, chloroform and acetone Sparingly soluble: water	276.74	$C_8H_6ClFN_4S_2$	241	51.63	0.69
SH_3	7-chloro-6-fluoro-*N*-[5-(2-furyl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine	Brown colour solid	Freely soluble: DMF Methanol, ethyl acetate, ethanol and acetone Sparingly soluble: chloroform and water	352.79	$C_{13}H_6ClFN_4OS_2$	224	36.75	0.62

SH_4	7-chloro-6-fluoro-*N*-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine	Pale pink colour solid	Freely soluble: DMF Methanol, ethyl acetate, benzene, chloroform and acetone Soluble: ethanol Sparingly soluble: hexane and water	362.21	$C_{14}H_7ClFN_5S_2$	192	42.19	0.57
SH_5	7-chloro-6-fluoro-*N*-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazol-2-amine	Dirty white colour powder	Freely Soluble: DMF Methanol and acetone Sparingly soluble: ethanol, ethyl acetate, hexane and water Very slightly soluble: benzene and chloroform	377.84	$C_{15}H_9ClFN_5S_2$	227	39.42	0.53
SH_6	6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-N^7-(2-nitrophenyl)-1,3-benzothiazole-2,7-diamine	Yellow colour powder	Soluble: Methanol DMF and DMSO Sparingly soluble: acetone, ethyl acetate, chloroform and ethanol	454.45	$C_{19}H_{11}FN_6O_3S_2$	210	54.79	0.81

SH_7	N^7-(2,4-dinitrophenyl)-6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine	Orange colour powder	Freely soluble: Methanol, DMF and DMSO Sparingly soluble: ethyl acetate, acetone and ethanol Slightly soluble: benzene and chloroform	454.45	$C_{19}H_{11}FN_6O_3S_2$	212	43.53	0.69
SH_8	6-fluoro-N^7-(2-nitrophenyl)-N^2-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-benzothiazole-2,7-diamine	Green colour powder	Freely soluble: Methanol, DMF and DMSO Sparingly soluble: ethanol and chloroform Slightly soluble: ethyl acetate and acetone	465.48	$C_{20}H_{12}FN_7O_2S_2$	216	54.59	0.84
SH_9	6-fluoro-N^2-[5-(furan-2-yl)-1,3,4-thiadiazol-2-yl]N^7-(2-nitrophenyl)-1,3-benzothiazole-2,7-diamine	Dark green colour powder	Soluble: Methanol, DMF and DMSO Sparingly soluble: ethanol, acetone and chloroform	465.48	$C_{20}H_{12}FN_7O_2S_2$	217	47.09	0.81

SH_{10}	6-fluoro-7 N^2-[5-(2-aminophenyl)-1, 3, 4-thiadiazol-2-yl] - N^7-(2-nitrophenyl)-1, 3-benzothiazole-2, 7-diamine	Yellow colour powder	Freely soluble: DMF, Methanol and $DMSO_4$ Slightly soluble: ethanol ethyl acetate and water.	479.51	$C_{21}H_{14}FN_7O_2S_2$	220	49.54	0.58
SH_{11}	6-fluoro-N^2-[5-(2-aminophenyl)-1,3,4-thiadiazol-2-yl] -N^7-(4-nitrophenyl)-1,3-benzothiazole-2,7-diamine	Orange colour powder	Freely soluble: DMF, methanol and $DMSO_4$ Slightly soluble: chloroform, benzene Very slightly soluble: ethanol, acetone, water	479.51	$C_{21}H_{14}FN_7O_2S_2$	217	44.04	0.78

6.4.a. Screening of Antibacterial Activity

The synthesized compounds were evaluated for *in vitro* antibacterial activity against gram negative bacteria *Proteus vulgaris* NCTC 4635 and gram positive bacteria *Micrococcus leutus* NL98. These are the agents commonly causes urinary tract infection, nosocomial infection, biliary tract infection. The gram negative organism *Proteus vulgaris* causes urinary tract infections and wound infections. The gram positive organism *Micrococcus leutus* causes endocarditis, septic arthritis, meningitis and caveating pneumonia. As per the data obtained, it was confirmed that all the tested compounds possessed antibacterial activity against both gram positive and gram negative organisms. The SH_6, SH_7, SH_{11} showed greater activity against *Micrococcus leutus* than *Proteus vulgaris*. Whereas SH_8, SH_9, SH_{10} showed greater activity against *Proteus vulgaris* than *Micrococcus leutus* However the antibacterial activity of all the synthesized compounds against the tested organism was found to be less than that of ciprofloxacin, a standard antibacterial drug at tested dose level. The results were tabulated in Table-1.

6.4. b. Screening of Antifungal Activity

The synthesized compounds were evaluated *in vitro* antifungal activity against fungal organism *Aspergillus flavus* ATCC 46646. The organism causes serious lungs infection and bronchitis. As per the data obtained, it was confirmed that all the tested compounds possessed antifungal activity. However, SH_{11} exhibited potent anti-fungal activity against fungal organism among the tested compounds. However the anti-bacterial activity of the SH_{11} against the tested organism was found to be less than that of antifungal standard drug ketoconazole at tested dose level. The results were tabulated in Table-1.

6.4. c. Acute Oral Toxicity

The acute toxicity of the synthesized compounds SH_8 & SH_{11} was determined by using albino swiss mice (20-25 gm). The animals were fasted for 24 h prior to the experiment and acute class toxic (OECD 423) method of CPCSEA was adapted for acute toxicity studies. The animals were treated up to the dose level of 300 mg and since no morbidity was observed up to that level, 1/6th of the lethal dose (50 mg/ kg *b.w*) was taken as effective dose ED_{50} (Therapeutic dose).

6.4. d. Anti-Inflammatory Activity

The synthesized derivatives, SH_8 & SH_{11} were selected for the screening of anti-inflammatory activity using carrageenan induced paw edema method. The test compounds exhibited significant activity in acute inflammatory models in rats after 1 h, 2 h and 3 h. The results were tabulated in Table-2.

- The SH_8 showed significant activity at 1 h, 2 h and 3 h and the mean paw oedema volume at these hours were 0.59±0.058, 0.69±0.057 and 0.80±0.066 respectively. The compound was found to have statistically significant activity at 1^{st} h, 2^{nd} h but it was highly significant in 3^{rd} h.
- The SH_{11} showed significant activity at 1 h, 2 h and 3 h and the mean paw edema volume at these hours were 0.53±0.066, 0.61±0.066 and 0.72±0.066 respectively. The compound was found to have statistically significant activity at 1^{st} h, 2^{nd} h but it was highly significant in 3^{rd} h.
- The standard drug diclofenac sodium was significant at 1 h, 2 h and 3 h and the activity at these hours were 0.46±0.066, 0.53±0.088 and 0.53±0.088 respectively. The compound showed significant activity at 1h and 2 h but it was highly significant at 3 h.

The compounds SH_8 & SH_{11} exhibited maximum inhibition of 45.51 % and 51.20 % respectively where as the standard diclofenac sodium showed reduction in oedema volume by 62.14 %.

Table 1: *In vitro* Antimicrobial Activity of Synthesized Compounds by Disc Diffusion Method

Microorganisms	Diameter of Zone of Inhibition in mm													
	SH_6		SH_7		SH_8		SH_9		SH_{10}		SH_{11}		Ketaconazole (µg/disc)	Ciprofloxacin (µg/disc)
	25 (µg)	100 (µg)	25 (µg)	100 (µg)	25 (µg)	100 (µg)	25 (µg)	100 (µg)	25 (µg)	100 (µg)	25 (µg)	100 (µg)	100	100
Micrococcus luteus	17	28	17	27	14	18	14	17	11	14	15	17.6	-----	32
Proteus vulgaris	14	17	12	16	22	26	17.6	19	12	14.6	12	16.1	-----	29
Aspergillus flavus	11	16	14	18.6	17	21.6	21	23	21	25	23	27	30	-----

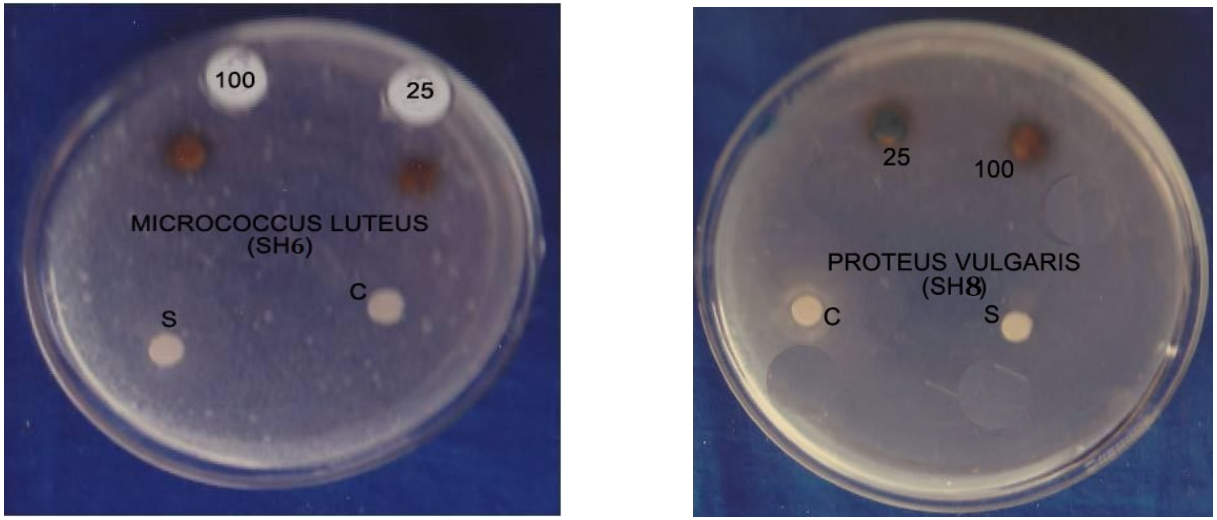


Fig-82: Antibacterial Activity of Compound SH_6 and SH_8 against Tested Organisms

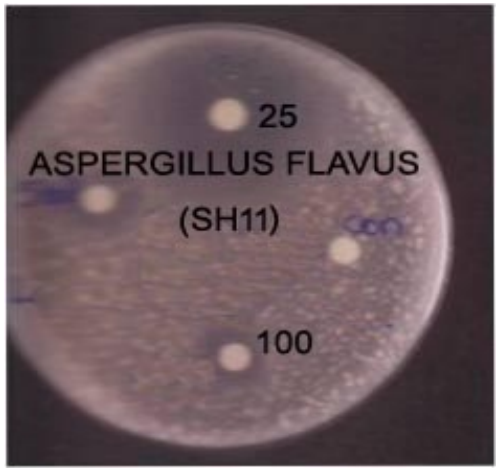


Fig-82: Antifungal Activity of Compound SH_{11} against Tested Organism

Table 2: Anti-Inflammatory Activity of Synthesized Compounds by Carrageenan Induced Paw Oedema Method

S. No.	Compound Code	Dose (mg/kg)	Paw Edema Volume (in ml) at			
			0 hour	1 hour	2 hour	3 hour
			mean±SEM	mean±SEM	mean±SEM	mean±SEM
1	Control	-	0.33±0.066	0.9±0.088	1.1±0.088	1.47±0.057
2	Diclofenac Sodium	20	0.23±0.033	0.46±0.066** (48.88)	0.53±0.088** (51.51)	0.53±0.088*** (62.14)
3	SH_8	50	0.53±0.057	0.59±0.058* (34.44)	0.69±0.057* (37.27)	0.80±0.065*** (45.51)
4	SH_{11}	50	0.46±0.066	0.53±0.066* (41.11)	0.61±0.066** (44.54)	0.72±0.066*** (51.20)

n = 3 in each group.*p< 0.05, **p < 0.01 and ***p<0.001 when compared to control (One-way ANOVA followed by Bonferroni test)

Figures in the parenthesis indicate % inhibition of paw oedema

SUMMARY & CONCLUSION

7. SUMMARY AND CONCLUSION

In recent year attention has increasingly been given to the synthesis compounds with combination of two heterocyclic nucleuses. In order to expand this we synthesized compounds which contains benzothiazole and 1,3,4 thiadiazole nucleuses. Individually benzothiazole and 1,3,4 thiadiazole nucleuses possess numerous biological activities as documented in literature. Hence this versatile biological significance inspired us to synthesize the compounds which contain combination of these two moieties in hope of getting molecules with biodynamic potentials.

Fluorine has become an essential tool in drug discovery, including fluorine atom in potential medicines can have a variety of dramatic effects on the molecular properties perhaps making them more selective, increasing efficacy. Hence the 3-chloro, 4-fluoro aniline was selected as starting molecule to synthesize fluorobenzothiazole incorporated with 1,3,4 – thiadiazole.

3-chloro 4-fluoro-aniline reacted with potassium thiocynate in the presence of glacial acetic acid and bromine at 0° C to give the corresponding 2-amino-6-fluoro-7-chloro-(1,3)- benzothiazole, which further reacts with hydrazine hydrade, ammonia, carbon disulphide, sodium chloroacetate in the presence of ethanol to form 2-thiosemicarbazide substituted 6-fluoro-7-chloro-(1,3)-benzothiazole. The third step involves acyclation of 2-thiosemicarbazide followed by dehydrative cyclization using phosphorous oxy chloride to form 1,3,4 thiadiazole moiety, in this step different aromatic acids were also substituted. Then the 1,3,4 thiadiazole substituted compound was treated with ortho and para nitro anilines, in the presence of DMF to obtain different fluorobenzothiazole incorporated 1,3,4 thiadiazole compounds. The purity of

the synthesized compounds was checked by thin layer chromatography (R_f) and determining melting point.

The structure of the synthesized compounds were established by spectral (IR, ^{1}H NMR and Mass) analysis data. In SH_1, the NH band at 3228 cm^{-1} and NH proton signal δ 4.0 of 2-amino benzothiazole in IR and ^{1}H NMR spectrum respectively confirmed the formation of benzothiazole nucleus. In SH_2, three protons singlet at δ 2.0 and one proton singlet at δ 4.0 confirmed the formation of thiosemicarbazide group.

In the compounds SH_3, SH_4, SH_5 C = N band (1653 – 1632 cm^{-1}) and C – S band (645 - 642 cm^{-1}) of IR spectrum conforms the formation of 1,3,4 thiadiazole nucleus. In SH_3, two doublets at δ 6.3 and one doublet proton at δ 7.4 indicates the formation of furan ring. In SH_4, three doublet protons at δ 8.85, δ 7.97, δ7.44 and one singlet proton at δ 8.81 conforms the formation of an aromatic nucleus. In the case of SH_5, two triplets at δ 6.97, 6.68 and two doublet at δ 7.23, 6.52 indicates the formation of an aromatic nucleus.

The presence of nitro group in SH_6-SH_{11} was ascertained from strong bands at (1584 -1510 cm^{-1}) and (1365-1335 cm^{-1}) corresponding to asymmetric and symmetric O=N=O stretching respectively. The C – Cl stretching band which appeared at SH_1-SH_5 at (809 – 683cm^{-1}) was disappeared in SH_6-SH_{11}. Instead, C – N stretching band appeared at (1346.02-1255.90cm^{-1}) in SH_6-SH_{11} indicated the attachment of ortho nitro aniline (or) and para nitro aniline group.

In compounds SH_6, SH_8, SH_{10}, two doublet for 2 protons (δ 8.20, δ 7.17-δ7.46) and two triplet protons at (δ 7.68, δ 7.60) confirmed the presence of nitro group at ortho position in the aromatic ring. In compounds SH_7, SH_9. SH_{11} two

doublet protons at (δ 7.26- δ 7.34, δ 8.03 - δ 8.04) confirmed the presence of nitro group at para position in aromatic ring.

In the Mass spectrum, synthesized compounds produced (M^+) Molecular ion peaks at 351.12, 362.21, 376.16, 454.45, 454.45, 463.48, 463.48, 479.51 and 479.51 values for SH_3, SH_4, SH_5,SH_6, SH_7, SH_8, SH_9, SH_{10},and SH_{11} respectively, corresponds to their molecular formulas. The predicted chemical structure of titled compounds was further supported by the fragmentation peaks.

All the compounds showed very good antibacterial and antifungal activity even at less concentration. From the data, it is evident that the compound SH_6 and SH_8 was the most potent candidate against *Micrococcus leutus and Proteus vulgaris* in the anti-bacterial studies and compound SH_{11} was the much potent candidate against *Aspergillus flavus* in the antifungal studies. Since a fewer species have been used in this study, it was warranted to screen these compounds with varied species and resistant strains.

The anti-inflammatory activity confirmed that the test compound SH_{11} showed superior activity in the inhibition of oedema than SH_8. However, both the test compounds were found to less activity than the standard drug.

These results suggest that the tested compounds of fluorobenzothiazole incorporated with 1,3,4 thiadiazole have excellent scope for further development as commercial antimicrobial and anti-inflammatory agents. Further experiments were needed to elucidate their exact mechanism of activity.

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8. BIBLIOGRAPHY

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ANNEXURE

CERTIFICATE

This is to certify that the project title Acute oral Toxicity studies in mice for Synthesized Fluoro Benzothiazole incorporated with 1,3,4- Thiadiazole derivative has been approved by the IAEC.

S. SHOBA
Name of Chairman/member Secretary IAEC:

Dr. P. Balakrishna murthy
Name of CPCSEA nominee:

Signature with date

S. Shoba 22/7/2011
Chairman/Member Secretary of IAEC:

22.7.11
CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

ATTESTED

Dr.T.Vetrichelvan, M.Pharm.
Professor and Principal
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CERTIFICATE

This is certify that the project title..Evaluation of Anti-inflammatory activity of Fluoro Benzothiazole incorporated with Thiadiazole derivatives. has been approved by the IAEC.

S.SHOBA

Name of Chairman/member Secretary IAEC:

Dr P. Balakrishna murthy

Name of CPCSEA nominee:

Signature with date

S. Shoba 22/7/2011

Chairman/Member Secretary of IAEC:

22/7/11

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

ATTESTED

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