STUDIES ON DIVERSED HETROCYCLIC CHEMICAL ENTITIES & THEIR APPLICATION

A THESIS

SUBMITTED TO THE

SAURASHTRA UNIVERSITY

FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

Dhairya P. Bhavsar

SUPERVISOR

Prof. Anamik Shah

DEPARTMENT OF CHEMISTRY

(DST-FUNDED, UGC-SAP SPONSORED),

SAURASHTRA UNIVERSITY

(Re-Accredited Grade **B by NAAC, CGPA 2.93),**

RAJKOT - 360 005

INDIA

April - 2011

Dedicated To My Family

Statement under *O. Ph. D. 7* of Saurashtra University

The work included in the thesis is done by me under the supervision of Prof. Anamik K. Shah and the contribution made thereof is my own work.

Date:

Place: Dhairya P. Bhavsar

CERTIFICATE

This is to certify that the present work submitted for the Ph.D. degree of Saurashtra University by Mr. Dhairya P. Bhavsar has been the result of work carried out under my supervision and is a good contribution in the field of organic chemistry.

Date:

Place: Prof. Anamik K. Shah

ACKNOWLEDGEMENTS

I bow before Almighty God for making me capable of doing all that I propose, the work leading to my PhD Thesis submission is one of them.

In fact, words fails me to express my sincere feelings of gratitude to my research guide **Prof. Anamik Shah,** his keen interest, patience and constant encouragement during course of my work on this thesis. Of course, it is my life time achievement to work with him.

Foremost I would like to bow my head with utter respect to my **Father, Mother,** my jiju Gaurav Patel, my lovely sister Riddhi and my Family members, who blessed me with their good wishes, relieving all types of stress and remaining always with me and continuing to boost my spirit.

I would like to express my feelings of gratitude to **Prof. P.H. Parsania,** Head, Department of Chemistry, for providing adequate infrastructure facilities in carrying out research work.

I am also thankful to **Dr. Yogesh Naliapara, Dr. V. H. Shah, Dr. H. S. Joshi, Dr. Shipra Baluja, Dr. Manish Shah, Dr. Bhoya and Dr. R. C. Khunt** for their constant support.

Words are inadequate to thanks my dearest friends Paresh, Bhavin, Vaibhav, Harshad, Ashish (Master), Hitesh, Hardev, Rakshit, Manisha, Mrunal, Vishwa, Nilay, Punit, Sachin, Bharat, Mahesh, Dipak, Ketki, Pratik, Dilip, Sabera, Madhve and Krunal who were always with me and helping me in all situation.

My special thanks and lots of love to my colleagues I would like to thank Shrey, Abhay, Shailesh, Jignesh, Renish and Ashish (don) for constant help and support.

I would also like to express my deep sense of gratitude to Ranjan Madam for her mother like treatment and Aditya Shah for their tremendous support during the course of the research.

I am also thankful to my colleagues Govind, Bhavesh, Ram, Kaushik, Piyush, Naimish, Amit, Piyush, Anil, Vipul, Suresh, Jignesh, Lina, Pooja, Minaxi, Dipti, Sandip, Ritesh, Ashish, Bhatt and Kataria.

I would like to thank teaching and non-teaching staff members of department for their constant help.

I am thankful to **"National Facility For drug discovery through new chemical entities development and instrumentation support to small manufacturing pharma enterprises"** for providing necessary instrumental facilities.

I am also grateful to Sophisticated Analytical Instrumentation Facility (SAIF), RSIC, Punjab University, Chandigarh for ^{1}H NMR, Dept. of Chemistry, Saurashtra University, Rajkot for IR, Mass and Elemental analysis.

Lastly I would like to thank each and every one of them who helped me directly or indirectly during this wonderful and lots of experience gaining journey.

I once again bow my head before almighty to facilitate me at every stage of my dream to accomplish this task.

Dhairya P. Bhavsar

CONTENTS

Chapter-3

A facile synthesis of some novel substituted 1-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)amine

Chapter-4

Synthesis and characterization of *N*'-(2-(substituted benzamido)thiazol-4-yl)-2-propylpentanehydrazide

Chapter-5

Biological evaluation of synthesized chemical entities

GENERAL REMARKS

1. Melting points were recorded by open capillary method and are uncorrected.
2. Infrared spectra were recorded on Shimadzu FT IR-8400 (Diffuse reflectance attachment) using KBr. Spectra were calibrated against the polystyrene absorption at 1610 cm^{-1}.
3. ^{1}H & ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer. Sample Solution ware prepared in DMSO-d_6 and $CDCl_3$ solvents and tetramethylsilane (TMS) as a internal standard.
4. Mass spectra were recorded on Shimadzu GC MS-QP 2010 spectrometer operating at 70 eV using direct injection probe technique.
5. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel-G F_{254} aluminium plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light.
6. The chemicals used for the synthesis of intermediates and end products were purchased from Spectrochem, Sisco Research Laboratories (SRL), Thomas Baker, Sd fine chemicals, Loba chemie and SU-Lab.
7. All the reactions were carried out in Samsung MW83Y microwave oven which was locally modified for carrying out chemical reactions
8. Evaporation of all the solvents was carried out under reduced pressure on Heidolph LABOROTA-400-efficient.
9. % Yield reported are isolated yields of material judged homogeneous by TLC and before recrystallization.
10. The structures and names of all compounds are given in the experimental section and physical data table were generated using ChemBio Draw Ultra 12.0.
11. Elemental analysis was carried out on Vario EL Carlo Erba 1108.

ABBREVIATIONS

AcOH	Acetic acid
PAF	Anti platelet activating factor
AIDS	Acquired immuno deficiency syndrome
$AlCl_3$	Aluminum chloride
Ar	Aromatic
CuoAC	Couper acetate
EDDA	Ethylenediammonium diacetate
TFA	Trifluoroaceitic acid
TEA	Triethyl amine
EtoAc	Ethyl acetate
[bmim]BF_4	1-Butyl-3-methylimidazolium tetrafluoroborate
BP	Boiling point
[bmim]OH	1-Butyl-3-methylimidazolium hydroxide
BuLi	Butyllithium
$BiCl_3$	Bismuth chloride
$CDCl_3$	Deuterated chloroform
CNS	Central nervous system
DNA	Deoxyribonucleic acid
MAPK	Mitogen-activated protein kinase
ATP	Adenosine triphosphate
PBMC	Peripheral blood mononuclear cells
Conc.	Concentrated
TNFR	Tumor necrosis factor R
mRNA	Messenger ribonucleic acid
DIBAL	Diisobutylaluminium hydride
$(Boc)_2O$	Di-tert-butyl dicarbonate
nm	Nano meter
µm	Micro meter
PPO	Polyphenol oxidase
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide

$KMnO_4$	Potassium permanganate
$NaHSO_4$	Sodium hydrogen sulphate
FT-IR	Fourier transform infrared
GABA	Gama-amino butyric acid
GC -MS	Gas chromatography mass spectra
IC_{50}	Inhibitory concentration
IR	Infra red
K_2CO_3	Potassium carbonate
KBr	Potassium bromide
m	Meta
MF	Molecular formula
MHz	Mega hurtz
MP	Melting point
MS	Mass spectra
MW	Microwave
MW	Molecular weight
MWI	Microwave irradiation
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
NCEs	New chemical entities
NEt_3	Tri ethylamine
NH_2NH_2	Hydrazine hydrate
NMR	Nuclear magnetic resonance
o	Ortho
p	Para
$Pd(OAc)_4$	Palladium tetraacetate
$POCl_3$	Phosphorous oxychloride
PCl_5	Phosphorus pentachloride
QSAR	Quantitative structural activity relationship
R&D	Research and development
R.T.	Room temperature
R_f	Retention factor
RNA	Ribonucleic Acid

THF	Tetrahydrofuran
TLC	Thin layer chromatography
VPA	Valproic acid
CS_2	Carbon disulphide
$FeCl_3$	Iron(III) chloride

Chapter-1

SYNTHESIS AND CHARACTERIZATION OF 4-AMINO COUMARIN DERIVATIVES USING MICROWAVE IRRADIATION

1.1 INTRODUCTION

Coumarin are the best known aromatic lactones.[1]The isolation of coumarin was first reported by Vogel[2] in Munich in1820.He associated the pleasant odor of the tonka bean from Guiana with that of clover, *Melilotous officinalis*, which gives rise to the characteristic aroma of new -mown hay. Vogel then concluded that the long colorless crystals which he discovered on slicing open Tonka beans and which crystallized as glistening needles from aqueous alcohol were identical with similar crystals he obtained, albeit in much lower yield, by extracting fresh clover blossoms.[3] The name coumarin originated[4] from a Caribbean word '*coumarou*' for the tonka tree, which was known botanically at one time as *Coumarouna odorata aubl*. Coumarin is now well, accepted trivial name. The IUPAC nomenclature of the coumarin ring system is 2*H*-1-benzopyran-2-one (I) (Figure-1).

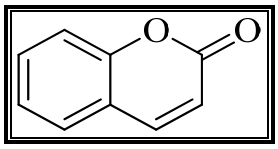


Figure-1

The coumarin ring system has an easy acceptability in the biological system compared to its isomeric chromones and flavones nucleus[5] and is widely distributed in nature.[6,9]An excellent account of these naturally occurring coumarin is presented by Murray and Brown[10]

Coumarin comprises a group of natural compounds found in a variety of plant sources. The very long association of plant coumarin with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems. The coumarins that were studied have diverse biological properties and various effects on the different cellular systems. A lot of biological parameters should be evaluated to increase our understanding of mechanisms by which these coumarin act. Coumarin has important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection. The coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral,

and anticarcinogenic activities. The hydroxycoumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The coumarin displays a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which can influence their biological activity. Vast majority of coumarin, completely innocuous, may be beneficial in a variety of human disorders, in spite of some ongoing controversy. There has been, in recent years, a major rekindling of interest in pharmacognosy. Coumarin turns out to be present in many natural therapeutically utilized products. They hold a place apart in view of their cytotoxic activity. It was suggested that alterations in the chemical structure of coumarin could change their cytotoxic properties.[11]

Coumarin and its derivatives have been prominently accepted as natural pharmaceuticals[12] worldwide, has revealed new biological activities with interesting therapeutic applications, besides their traditional employment as anticoagulants(anti-vitamin K activity),[13]antibiotics (novobiocin and analogues)[14] and anti AIDS.[15] Apart from this, they also possess anti-cancerous,[11] antibacterial,[16] neurotropic, [17]immunosuppressive,[18] anti inflammatory,[19] antiulcerous,[20] anti PAF (anti platelet activating factor)[21] and antimutagenic[22] effects.

1.2 PHARMACOLOGY

Numerous biological activities have been associated with simple coumarin and its analogues. Among them, antimicrobial, antiviral, anticancer, enzyme inhibition, anti-inflammatory, antioxidant, anticoagulant and effect on central nervous system are most prominent. Coumarin nucleus possesses diversified biological activities that can be briefly summarized as under:

1 Antimicrobial and Molluscicidal [23-45]
2 Antiviral [46-50]
3 Anticancer [51-61]
4 As Enzyme Inhibition [62-67]
5 Antioxidant [68-71]
6 Anti-inflammatory [72-76]
7 Anticoagulant and Cardiovascular [77-80]
8 Effect on Central Nervous System [81-82]

4-Hydroxycoumarin is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocyclic compounds. The synthetic versatility of 4-hydroxy coumarin has led to the extensive use of this compound in organic synthesis. 4-hydroxy coumarin shows diversified chemical reactivity.

Amide linkage containing analogues: A collection of commercial analogues based on α-aminoamide structure[83]: (-NH-CO-CH_2-N<)

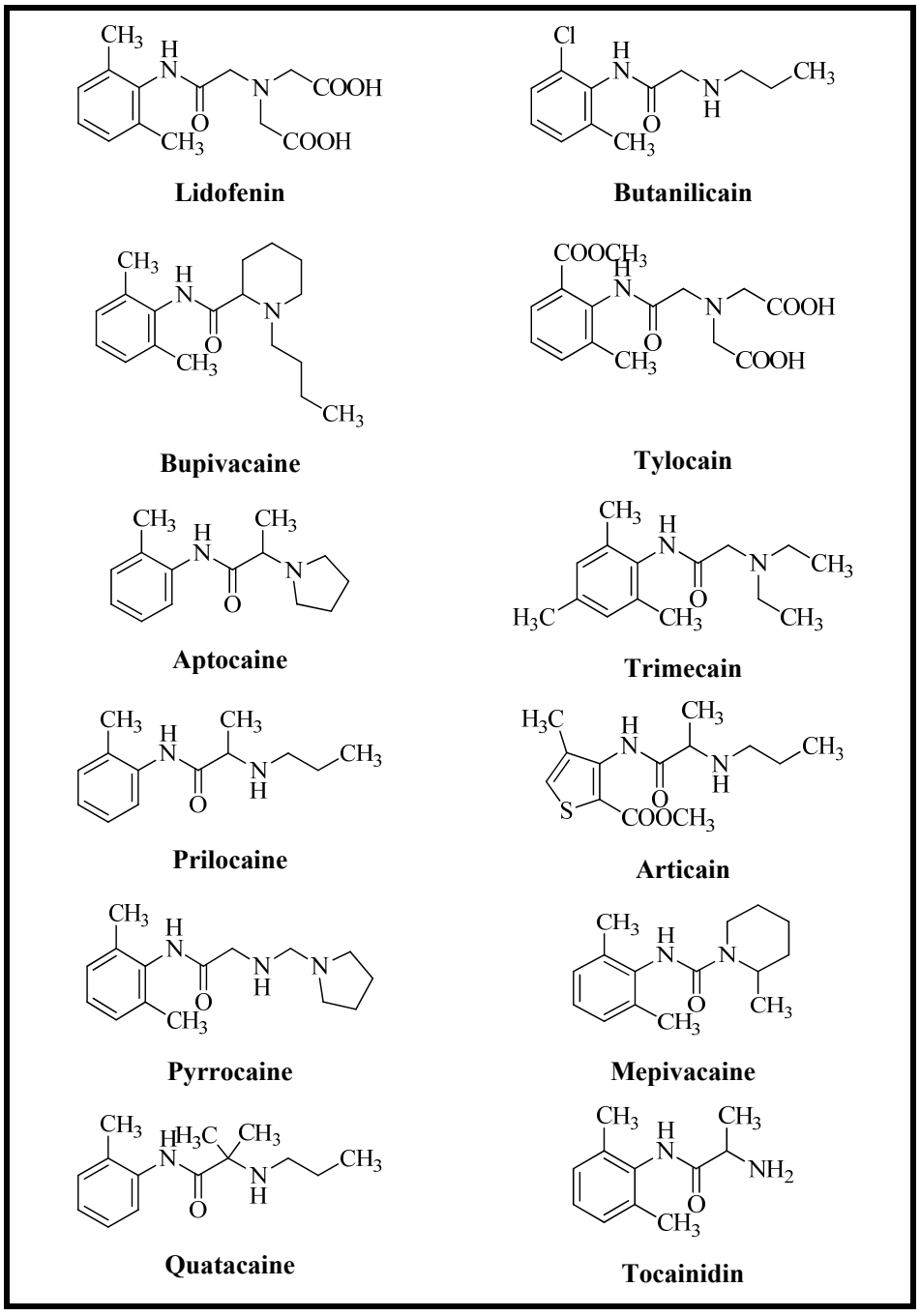


Figure-2

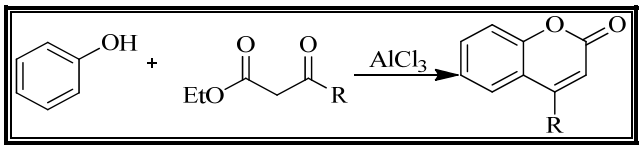
1.3 SYNTHETIC ASPECT

Coumarin and its derivatives were synthesized by many researchers using different methods.

Perkin[84] synthesized coumarin and then several methods are reported for the synthesis of 4-hydroxy coumarins and their 4-hydroxy substituted derivatives namely:

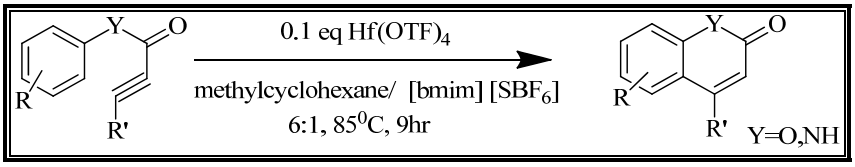
1 Anschutz method [85]
2 Pauli Lockemann synthesis [86]
3 Sonn's synthesis [87]
4 Mentzer's synthesis [88]
5 Robertson synthesis [89]
6 Ziegler and Junek method [90]
7 Garden's method [91]
8 Shah, Bose and Shah's method [92]
9 Kaneyuki method [93]
10 Resplandy's method [94]
11 Jain, Rohatagi and Sheshadri's method [95]
12 Shah, Bhatt and Thakor's method [96]

Shah *et al*[92-96] have prepared 4-hydroxy coumarin derivatives in good yield by condensation of different phenols with malonic acid in the presence of zinc chloride and phosphorous oxychloride. The method is useful as single step preparation of 4-hydroxy coumarin derivatives substituted in benzenoid part.

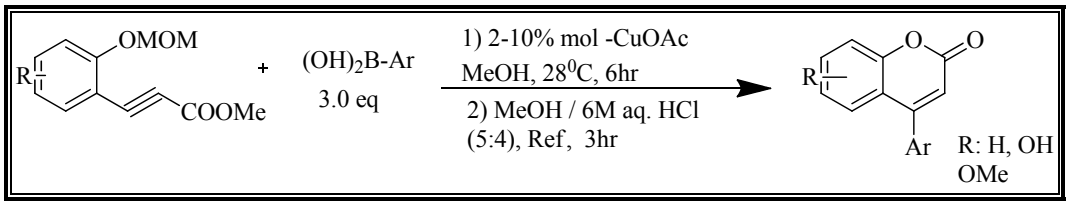


Pechmann Coumarin Synthesis

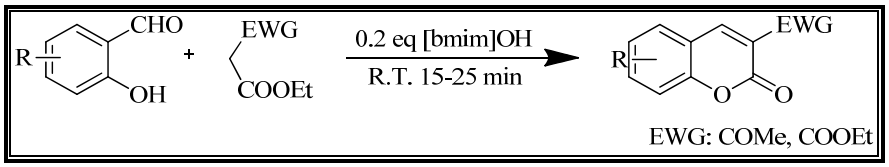
Recently many researchers[97-128] have reported synthetic strategies for 4-hydroxy coumarin.



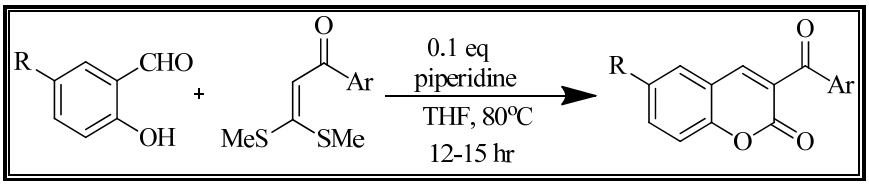
The employment of hydrophobic ionic liquids dramatically enhanced the activity of metal triflates in Friedel-Crafts alkenylations of aromatic compounds with various alkyl-and aryl-substitutedalkynes.[129]



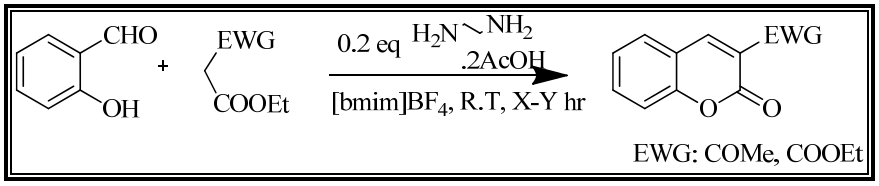
Arylpropionic acid methyl esters having a MOM-protected hydroxy group at the *ortho* position underwent hydroarylation with various arylboronic acids in MeOH at ambient temperature in the presence of a catalytic amount of CuOAc, resulting in the formation of 4-arylcoumarins in high yields after the acidic workup.[130]



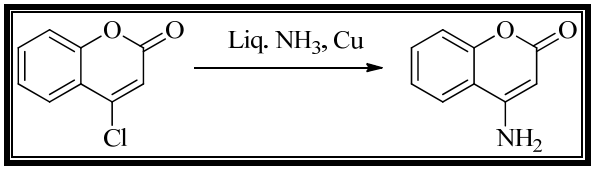
The basic ionic liquid 1-butyl-3-methylimidazolium hydroxide, [bmim]OH, efficiently catalyzes the Knoevenagel condensation of various aliphatic and aromatic aldehydes and ketones with active methylenes at room temperature without requirement of any organic solvent.[131]



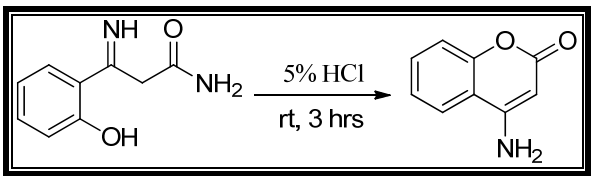
A facile, convenient, efficient and high yielding synthesis of a combinatorial library of 3-aroylcoumarins has been developed by the condensation of easily available aroylketene dithioacetals and 2-hydroxybenzaldehydes in the presence of catalytic amount of piperidine in THF reflux.[132]



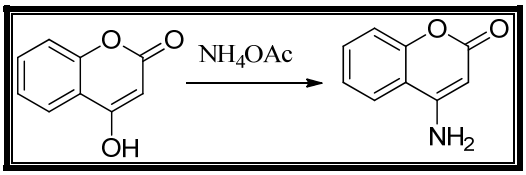
The ionic liquid 1-butyl-3-methylimidazonium tetrafluoroborate [bmim]BF_4 was used for ethylenediammonium diacetate (EDDA) catalyzed Knoevenagel condensation between aldehydes or ketones with active methylene compounds. Catalyst and solvent were recyclable.[133]



Zagorevskii, V. A. and Dudykina, N. V. was prepared 4-aminocoumarin from 4-chlorocooumarin using liq. Ammonia and copper.[134]



3-(2-Hydroxyphenyl)-3-iminopropanamide treated with 5% HCl at room temperature for 3 hours to yield 4-aminocoumarin.[135]

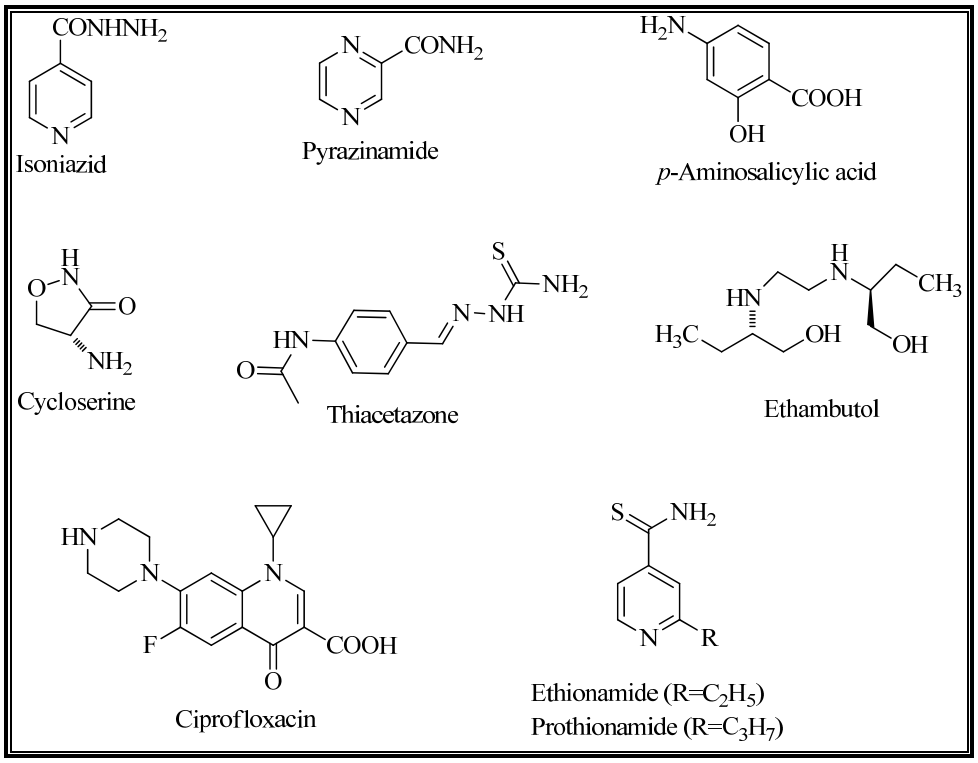


Ivanov, I. et al reports the amination of 4-hydroxycoumarin using ammonium acetate as a catalyst.[136,137]

1.4 AIM OF CURRENT WORK

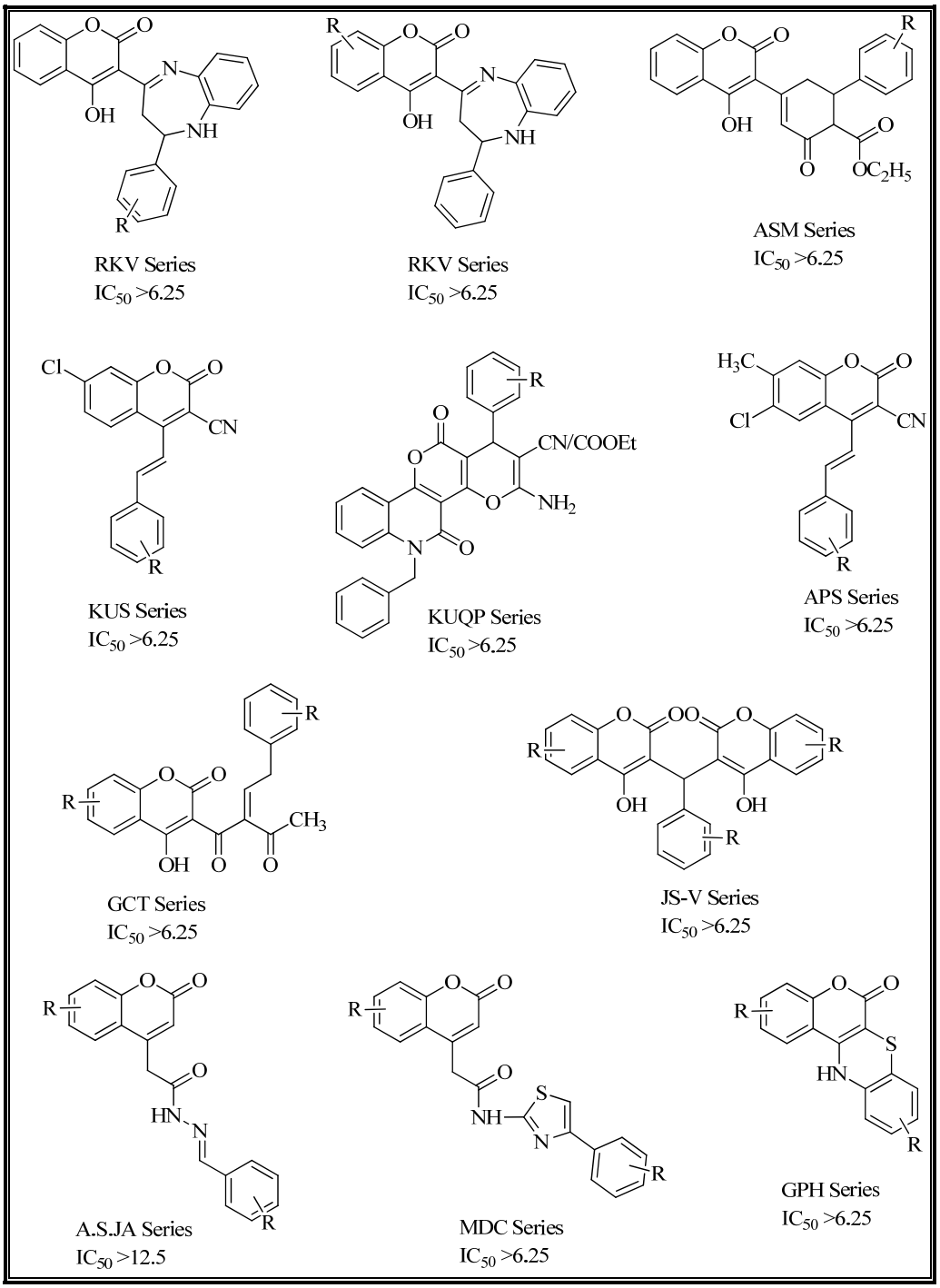
The literature survey revealed that some extensive work has been done on coumarin compounds. Also, due to the usefulness of traditional medicines like Auraptene, Ferulenol and Fraxetin, the coumarin moiety has been selected for the research criteria.

Anti-Tubercular drug in market



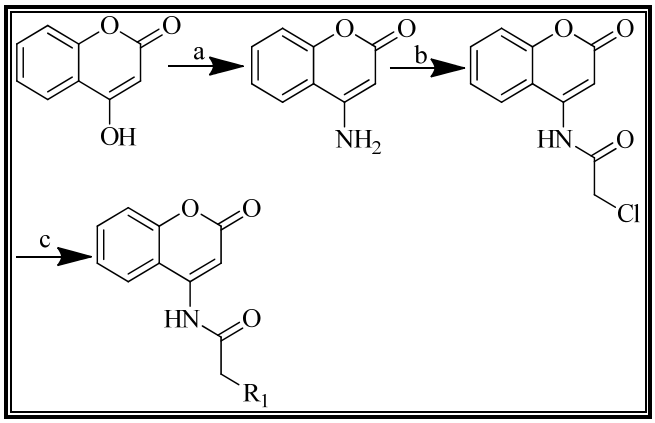
Browsing through the literature of organomedicinal chemistry the most useful moiety found was substituted 4-amino coumarin derivatives. Because of the less toxicological properties and good to moderate activities, several compounds have been synthesized by our team in the laboratory which is given as under. The work encompassed in this chapter is an extension of the aforesaid research activity.

Anti-Tubercular compounds synthesized from our laboratory



Though the chemistry of the synthesized compounds is unknown, the compounds are reported here in the first time. Biological importance of such vital compounds is described in chapter-5.

1.5 REACTION SCHEME

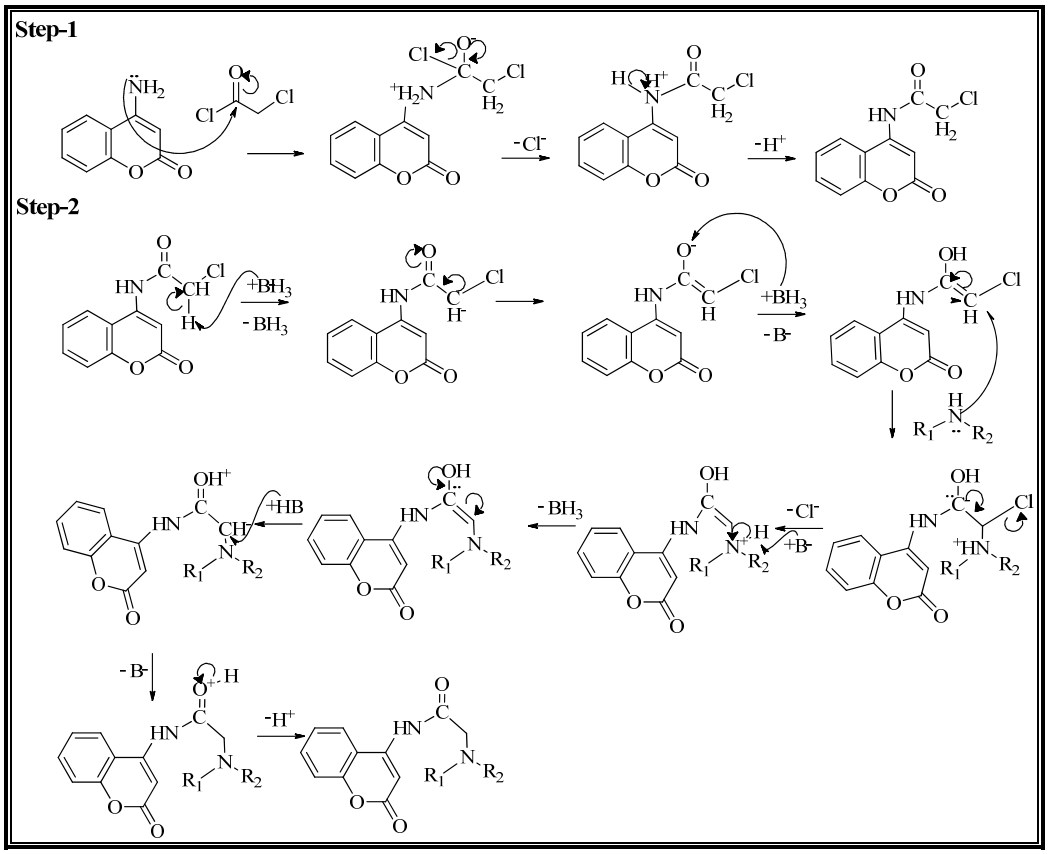


a) Ammonium acetate, MW, 300W, 3.5min

b) DMF,TEA, $ClCH_2COCl$, 0-5 °C

c) DMF, K_2CO_3, R_1= Substituted amine

1.6 PLAUSIBLE REACTION MECHANISM



1.7 EXPERIMENTAL

❖ **Preparation of 4-Amino coumarin**

A mixture of 4-hydroxy coumarin (0.1 mole) and ammonium acetate (0.3 mole) was heated on microwave at 300 W, 100 °C for 3.5min. The reaction mixture was poured in ice, forming solid yellow product. This product was washed with sat. $NaHCO_3$ to remove unreacted 4-hydroxy coumarin. The purity of the compound was checked by TLC. (EtoAC: Hexane :: 3:7). Yield : 80%

❖ **Preparation of 2-Chloro-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide**

4-Amino coumarin (0.1 mole), Tri ethyl amine (0.2 mole) and DMF were taken in a flask and stirred it. Resulting mixture was cooled at 0-5 °C then added drop wise chloracetylchloride (0.2 mole). The reaction mixture was stirred for over night. The reaction was poured into the ice and extracted with ethyl acetate. The organic layer was separated out, dried with sodium sulphate and evaporated under vaccum to give yellow oily compound. The compound was purified by column chromatography by silica gel 230-400 mash. TLC. (EtoAC: Hexane :: 3:7). Yield : 65%

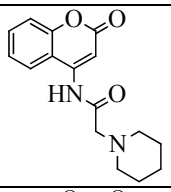
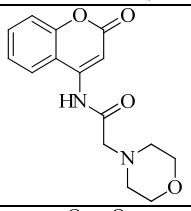
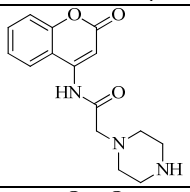
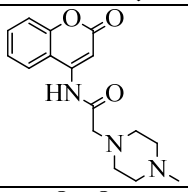
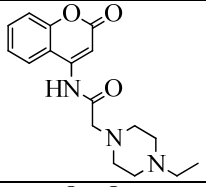
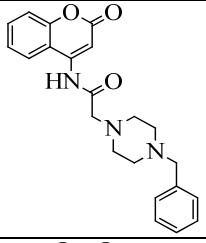
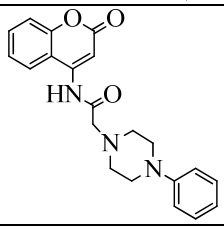
Note: The entire reaction was carried out under nitrogen atmosphere.

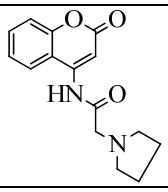
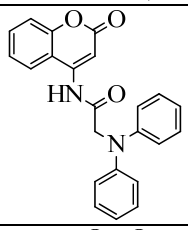
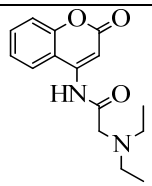
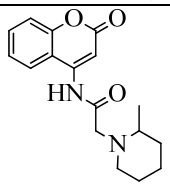
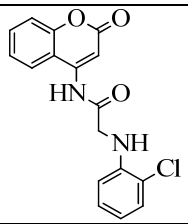
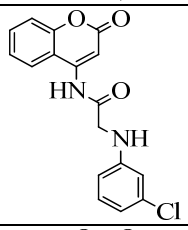
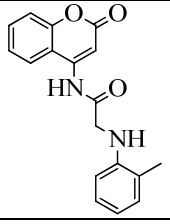
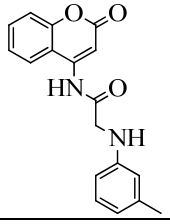
❖ **General method for preparation of *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine -1-yl)acetamide derivatives**

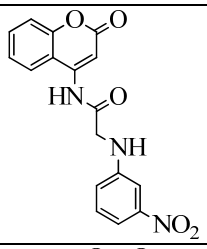
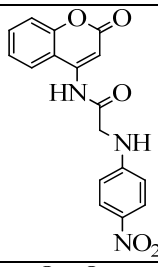
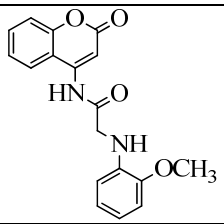
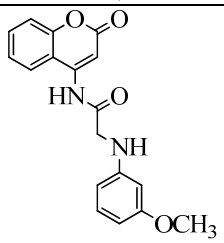
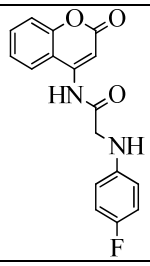
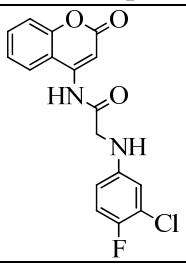
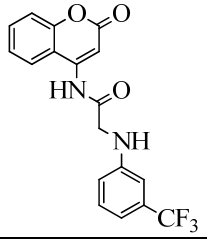
2-Chloro-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (0.1 mole), anhydrous potassium carbonate (0.2 mole) and DMF were taken in flask and stirred continuously with mechanical stirring then added dropwise substituted amine (0.1 mole) at room temperature, reaction mixture was stirred for 12 h. The reaction was poured into the ice to give solid title compound.The compound was recrystlized by ethanol. TLC. (EtoAC: Hexane :: 4:6). Yield : 45-85%

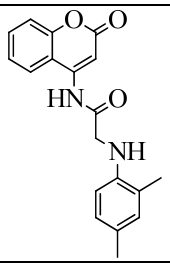
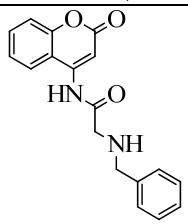
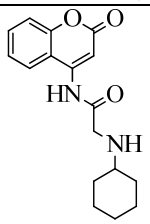
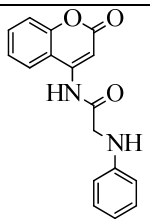
1.8 PHYSICAL DATA

TABLE: 1 PHYSICAL DATA OF *N*-(2-OXO-2*H*-CHROMEN-4-YL)-2-(SUBSTITUTED AMINE -1-YL)ACETAMIDE DERIVATIVES

Sr. No	Code	Structure	M.F.	M. P. (°C)	R_f value	% Yield
1	DPB-1		$C_{16}H_{18}N_2O_3$	210-212	0.44	62
2	DPB-2		$C_{15}H_{16}N_2O_4$	180-182	0.51	75
3	DPB-3		$C_{15}H_{17}N_3O_3$	170-172	0.42	55
4	DPB-4		$C_{16}H_{19}N_3O_3$	150-152	0.46	77
5	DPB-5		$C_{17}H_{21}N_3O_3$	220-222	0.41	81
6	DPB-6		$C_{22}H_{23}N_3O_3$	240-242	0.44	85
7	DPB-7		$C_{21}H_{21}N_3O_3$	178-180	0.52	86

8	DPB-8		$C_{15}H_{16}N_2O_3$	168-170	0.42	65
9	DPB-9		$C_{23}H_{18}N_2O_3$	154-156	0.45	74
10	DPB-10		$C_{15}H_{18}N_2O_3$	176-178	0.48	56
11	DPB-11		$C_{17}H_{20}N_2O_3$	188-190	0.51	62
12	DPB-12		$C_{17}H_{13}N_2O_3Cl$	192-194	0.40	58
13	DPB-13		$C_{17}H_{13}N_2O_3Cl$	160-162	0.44	52
14	DPB-14		$C_{18}H_{16}N_2O_3$	188-190	0.40	61
15	DPB-15		$C_{18}H_{16}N_2O_3$	179-181	0.50	63

16	DPB-16		$C_{17}H_{13}N_3O_5$	220-222	0.44	45
17	DPB-17		$C_{17}H_{13}N_3O_5$	244-246	0.48	48
18	DPB-18		$C_{18}H_{16}N_2O_4$	210-212	0.41	69
19	DPB-19		$C_{18}H_{16}N_2O_4$	220-222	0.48	73
20	DPB-20		$C_{17}H_{13}N_2O_3F$	240-242	0.44	66
21	DPB-21		$C_{17}H_{12}N_2O_3FCl$	176-178	0.46	59
22	DPB-22		$C_{18}H_{13}N_2O_3F_3$	164-166	0.44	54

23	DPB-23		$C_{19}H_{18}N_2O_3$	174-176	0.42	68
24	DPB-24		$C_{18}H_{16}N_2O_3$	184-186	0.48	42
25	DPB-25		$C_{17}H_{20}N_2O_3$	220-222	0.42	47
26	DPB-26		$C_{17}H_{14}N_2O_3$	236-238	0.51	62

R_f value was calculated using solvent system, Ethyl acetate: Hexane (4: 6)

1.9 SPECTRAL STUDY

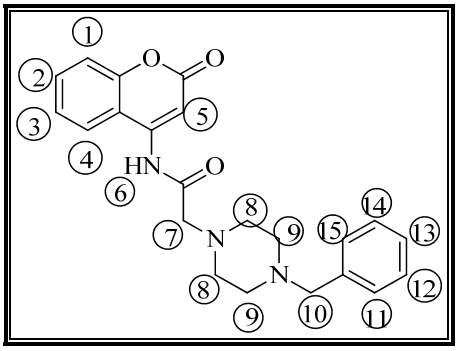
❖ IR spectra

Infra Red spectra were taken on **Shimadzu FT-IR-8400** spectrometer using KBr Pellet method. The characteristic carbonyl group in coumarin moiety is observed at 1750-1720 cm^{-1}, while carbonyl value of –CONH peaks are observed in the range 1690-1630 cm^{-1}. Amine (> NH) observed a broad peak between 3200-3000 cm^{-1}.Methylene gp ($-CH_2$) observed at 3000-2850 cm^{-1} and methyl ($-CH_3$) observed at 1350 cm^{-1}. DPB-05 and DPB-20 of IR spectra are given on page no: - 32.

❖ ^{1}H NMR spectra

^{1}H NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d_6 & $CDCl_3$ as a solvent. In the NMR spectra of *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine-1-yl)acetamide various proton values of methylene ($-CH_2$), amine (>NH), methyl ($-CH_3$) and aromatic protons (Ar-H) etc. were observed as under.

The values for methylene ($-CH_2$) proton is observed between 2.50-3.55 δ ppm. In some cases, the value of methylene proton differs 4.20-4.43 δ ppm. The -NH protons of substituted aniline was observed at 3.95-4.20 δ ppm. Aromatic protons shows the multiplet between 6.01-8.54 δ ppm. The signal due to NH proton of amide group (>CONH) was observed at 10.1-10.5 δ ppm value. DPB-02, DPB-05 and DPB-06 of ^{1}H NMR spectra are given on page no: - 27 to 31.



1. The aromatic ring, in coumarin ring proton no. 1, 2, 3 and 4 are on same atmosphere so these four protons gave a multiplet at 7.42 δ ppm-7.67 δ ppm it shows in spectra.

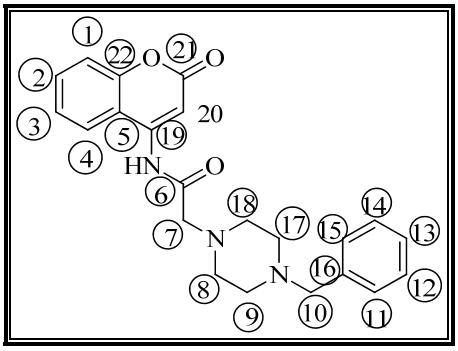
2. The deshielded proton no. 5 of coumarin ring gave a singlet and it shows on down field at 7.56 δ ppm due to the effect of carbonyl group.
3. The aromatic ring of the phenyl ring attached with methylene, in phenyl ring proton no. 11, 12, 15 and 16 are on same atmosphere so these five protons gave a multiplet at 7.26 δ ppm-7.41 δ ppm it shows in spectra.
4. The proton no. 6 of the -NH (-CONH) group gave a characteristic broad singlet at 10.4 δ ppm.
5. The proton no. 7 of the methylene group gave a characteristic singlet at 3.29 δ ppm.
6. The proton no. 8 of four protons gave a multiplet at 2.74 δ ppm due to the effect of nitrogen atom.
7. The proton no. 9 of four protons gave a multiplet at 2.90 δ ppm due to the effect of nitrogen atom.
8. The proton no. 10 of the methylene group gave a characteristic singlet at 3.60 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound DPB-06 was confirmed.

❖ **^{13}C NMR spectra**

^{13}C NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using DMSO-d_6 & $CDCl_3$ as a solvent. In the ^{13}C NMR spectra of *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine-1-yl)acetamide various carbon values of methylene (-CH_2), keto (>C=O), methyl (-CH_3) and aromatic carbon (Ar-H) etc. were observed as under.

The values for methylene (-CH_2) carbon is observed between 35-65 δ ppm. The >C=O carban observed at 160-180 δ ppm. Aromatic carbon shows between 110-140 δ ppm. DPB-02, DPB-05 and DPB-06 of ^{13}C NMR spectra are given on page no: - 27 to 31.



1. The carbon no. 8, 9, 17 and 18 of piperazine ring, appear at 39.30 δ ppm-40.56 δ ppm it shows in spectra.
2. The carbon no. 7 of methylene group, appear at 53.36 δ ppm due to the effect of nitrogen atom.
3. The carbon no. 10 of methylene group, appear at 62.66 δ ppm due to the effect of nitrogen atom.
4. The carbon no. 20 of coumarin ring, appear at 99.36 δ ppm due to the effect of carbonyl group.
5. The carbon no. 5 of coumarin ring, appear at 114.05 δ ppm
6. The carbon no. 1,2,3,4,11,12,13,14,15,16 and 22 of aromatic, appear at 114.05 δ ppm-144.74 δ ppm.
7. The carbon no. 21 of coumarin ring, appear at 153.71 δ ppm due to the effect of carbonyl group.
8. The carbon no. 6 of coumarin ring, appear at 161.92 δ ppm due to the effect of carbonyl group.
9. The carbon no. 19 of coumarin ring, appear at 169.89 δ ppm due to the effect of nitrogen atom.

❖ **Mass spectra**

The mass spectrum of compounds were recorded by **Shimadzu GC-MS-QP-2010** spectrometer. The mass spectrum of compounds was obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectral study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized. DPB- 19, DPB-20 and DPB-22 of Mass spectra are given on page no.-26 and 27.

- **Elemental analysis**

Elemental analysis of the synthesized compounds was carried out on **Vario EL-III Carlo Erba 1108** model at Saurashtra University, Rajkot which showed that calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The elemental analysis data are given for individual compounds.

1.10 SPECTRAL CHARACTERIZATION

N-(2-oxo-2H-chromen-4-yl)-2-(piperidin-1-yl)acetamide (DPB-1)

IR (KBr) cm^{-1}: 3355 (-NH), 1285 (C-N, str), 1714 (>C=O), 1680 (-CONH), 2869 ($>CH_2$,str), 1450 ($>CH_2$,ban), 3010 (-CH, str), 3052 (Ar, C-H, str), 1523 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 286. **Elemental analysis, Calculated:** C, 67.12; H, 6.34; N, 9.78 Found: C, 67.04; H, 6.30; N, 9.72.

2-Morpholino-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-2)

IR (KBr) cm^{-1}: 3368 (-NH), 1280 (C-N, str), 1710 (>C=O), 1650 (-CONH), 2850 ($>CH_2$,str), 1390 ($>CH_2$,ban), 3075 (-CH, str), 3050 (Ar, C-H, str), 1510 (Ar, C=C, str), 1120 (C-O-C). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 2.59 (m, 4H, $-CH_2$), 3.32 (s, 2H, $-CH_2$), 3.84 (m, 4H, $-CH_2$), 7.37-7.67 (m, 5H, Ar-H), 10.23 (s, broad, 1H,-NH). **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 53.6, 62.4, 67.0, 99.5, 114.1, 117.9, 120.4, 124.4, 132.5, 144.5, 153.7, 161.7, 169.6 **Mass: [m/e (%)], M. Wt.:** 288. **Elemental analysis, Calculated:** C, 62.49; H, 5.59; N, 9.72. Found: C, 62.35; H, 5.63; N, 9.65.

N-(2-oxo-2H-chromen-4-yl)-2-(piperazin-1-yl)acetamide (DPB-3)

IR (KBr) cm^{-1}: 3496 (-NH), 1275 (C-N, str), 1712 (>C=O), 1652 (-CONH), 2920 ($>CH_2$,str), 1362 ($>CH_2$,ban), 3058 (-CH, str), 3060 (Ar, C-H, str), 1550 (Ar, C=C, str), 3185 (2^{nd} amine) **Mass: [m/e (%)], M. Wt.:** 287. **Elemental analysis, Calculated:** C, 62.71; H, 5.96; N, 14.63. Found: C, 62.79; H, 5.99; N, 14.55.

2-(4-Methylpiperazin-1-yl)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-4)

IR (KBr) cm^{-1}: 3392 (-NH), 1260 (C-N, str), 1722 (>C=O), 1685 (-CONH), 2936 ($>CH_2$,str), 1374 ($>CH_2$,ban), 3023 (-CH, str), 3086 (Ar, C-H, str), 1423 (Ar, C=C, str), 1368 ($-CH_3$, str) **Mass: [m/e (%)], M. Wt.:** 301. **Elemental analysis, Calculated:** C, 63.77; H, 6.36; N, 13.94. Found: C, 63.82; H, 6.25; N, 13.87.

2-(4-Ethylpiperazin-1-yl)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-5)

IR (KBr) cm^{-1}: 3471 (-NH), 1192 (C-N, str), 1730 (>C=O), 1691 (-CONH), 2881 ($>CH_2$,str), 1377 ($>CH_2$,ban), 3027 (-CH, str), 3063 (Ar, C-H, str), 1564 (Ar, C=C, str), 1368 ($-CH_3$, str.). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 1.18 (t, 3H, $-CH_3$), 2.51 (q, 2H, $-CH_2$), 2.61 (m, 4H, $-CH_2$), 2.76(m, 4H, $-CH_2$), 3.28 (s, 2H, $-CH_2$), 7.28-7.62(m, 5H, Ar-H), 10.30 (s, broad, 1H,-NH) **^{13}C NMR 400 MHz: (DMSO-d_6, δ**

ppm): 12.0, 52.2, 53.3, 53.5, 62.0, 99.8, 114.1, 118.2, 119.7, 124.2, 132.3, 144.3, 153.6, 161.9, 169.5 **Mass: [m/e (%)], M. Wt.:** 315. **Elemental analysis, Calculated:** C, 64.74; H, 6.71; N, 13.32. Found: C, 64.70; H, 6.63; N, 13.22.

2-(4-Benzylpiperazin-1-yl)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-6)

IR (KBr) cm^{-1}: 3462 (-NH), 1320 (C-N, str), 1730 (>C=O), 1670 (-CONH), 2858 ($>CH_2$,str), 1389 ($>CH_2$,ban), 3042 (-CH, str), 3023 (Ar, C-H, str), 1544 (Ar, C=C, str). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 2.59 (m, 4H, -CH_2), 2.60 (m, 4H, -CH_2), 3.29 (s, 2H, CH_2), 3.9 (s, 2H, -CH_2) 7.26-7.67 (m, 10H, Ar-H), 10.40 (s, broad, 1H,-NH) **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 39.3, 39.9, 40.1, 40.4, 53.6, 61.9, 62.6, 99.3, 114.0, 117.9, 120.1, 124.4, 127.2, 128.2, 129.1, 132.4, 137.4, 144.7, 153.7, 161.9, 169.8 **Mass: [m/e (%)], M. Wt.:** 377. **Elemental analysis, Calculated:** C, 70.01; H, 6.14; N, 11.13. Found: C, 69.96; H, 6.08; N, 11.02.

N-(2-oxo-2H-chromen-4-yl)-2-(4-phenylpiperazin-1-yl)acetamide (DPB-7)

IR (KBr) cm^{-1}: 3420 (-NH), 1310 (C-N, str), 1700 (>C=O), 1655 (-CONH), 2848 ($>CH_2$,str), 1332 ($>CH_2$,ban), 3065 (-CH, str), 3050 (Ar, C-H, str), 1532 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 363. **Elemental analysis, Calculated:** C, 69.41; H, 5.82; N, 11.56. Found: C, 69.37; H, 5.78; N, 11.46.

N-(2-oxo-2H-chromen-4-yl)-2-(pyrrolidin-1-yl)acetamide (DPB-8)

IR (KBr) cm^{-1}: 3482 (-NH), 1269 (C-N, str), 1728 (>C=O), 1675 (-CONH), 2853 ($>CH_2$,str), 1372 ($>CH_2$,ban), 3042 (-CH, str), 3100 (Ar, C-H, str), 1498 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 272. **Elemental analysis, Calculated:** C, 66.16; H, 5.92; N, 10.29. Found: C, 66.25; H, 5.87; N, 10.20.

2-(Diphenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-9)

IR (KBr) cm^{-1}: 3462 (-NH), 1325 (C-N, str), 1736 (>C=O), 1685 (-CONH), 2858 ($>CH_2$,str), 1393 ($>CH_2$,ban), 3039 (-CH, str), 3028 (Ar, C-H, str), 1530 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 370. **Elemental analysis, Calculated:** C, 74.58; H, 4.90; N, 7.56. Found: C, 74.40; H, 4.98; N, 7.43.

2-(Diethylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-10)

IR (KBr) cm^{-1}: 3325 (-NH), 1258 (C-N, str), 1732 (>C=O), 1679 (-CONH), 2848 ($>CH_2$,str), 1370 ($>CH_2$,ban), 3052 (-CH, str), 3063 (Ar, C-H, str), 1562 (Ar, C=C,

str), 1368 (-CH3, str.). **Mass: [m/e (%)], M. Wt.:** 274. **Elemental analysis, Calculated:** C, 65.68; H, 6.61; N, 10.21. Found: C, 65.60; H, 6.51; N, 10.11.

2-(2-Methylpiperidin-1-yl)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-11)

IR (KBr) cm^{-1}: 3426 (-NH), 1325 (C-N, str), 1721 (>C=O), 1686 (-CONH), 2956 (>CH_2 ,str), 11421 (>CH_2 ,ban), 33065 (-CH, str), 3075 (Ar, C-H, str), 1523 (Ar, C=C, str), 1360 (-CH3, str.). **Mass: [m/e (%)], M. Wt.:** 300. **Elemental analysis, Calculated:** C, 67.98; H, 6.71; N, 9.33 Found: C, 67.88; H, 6.84; N, 9.09.

2-(2-Chlorophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-12)

IR (KBr) cm^{-1}: 3325 (-NH), 1258 (C-N, str), 1732 (>C=O), 1679 (-CONH), 2848 (>CH_2 ,str), 1370 (>CH_2 ,ban), 3052 (-CH, str), 3063 (Ar, C-H, str), 1562 (Ar, C=C, str), 710 (*ortho* sub.). **Mass: [m/e (%)], M. Wt.:** 328. **Elemental analysis, Calculated:** C, 62.11; H, 3.99; N, 8.52 Found: C, 62.16; H, 3.87; N, 8.45.

2-(3-Chlorophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-13)

IR (KBr) cm^{-1}: 3336 (-NH), 1265 (C-N, str), 1722 (>C=O), 1640 (-CONH), 2835 (>CH_2 ,str), 1362 (>**Mass: [m/e (%)], M. Wt.:** 328. **Elemental analysis, Calculated:** C, 62.11; H, 3.99; N, 8.52 Found: C, 61.18; H, 4.11; N, 8.60.

2-(o-Tolylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-14)

IR (KBr) cm^{-1}: 3325 (-NH), 1258 (C-N, str), 1732 (>C=O), 1679 (-CONH), 2848 (>CH_2 ,str), 1370 (>CH_2 ,ban), 3052 (-CH, str), 3063 (Ar, C-H, str), 1562 (Ar, C=C, str), 1368 (-CH3, str.), 715 (*ortho* sub.). **Mass: [m/e (%)], M. Wt.:** 308. **Elemental analysis, Calculated:** C, 70.12; H, 5.23; N, 9.09 Found: C, 70.66; H, 5.30; N, 9.15.

2-(m-Tolylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-15)

IR (KBr) cm^{-1}: 3442 (-NH), 1260 (C-N, str), 1728 (>C=O), 1688 (-CONH), 2898 (>CH_2 ,str), 1389 (>CH_2 ,ban), 3027 (-CH, str), 3096 (Ar, C-H, str), 1523 (Ar, C=C, str), 1368 (-CH_3, str.), 765 (meta sub.). **Mass: [m/e (%)], M. Wt.:** 308. **Elemental analysis, Calculated:** C, 70.12; H, 5.23; N, 9.09 Found: C, 69.95; H, 5.63; N, 9.15.

2-(3-Nitrophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-16)

IR (KBr) cm^{-1}: 3355 (-NH), 1285 (C-N, str), 1714 (>C=O), 1680 (-CONH), 2869 (>CH_2 ,str), 1450 (>CH_2 ,ban), 3010 (-CH, str), 3052 (Ar, C-H, str), 1523 (Ar, C=C,

str), 1525 (Nitro gp), 765 (meta sub.). **Mass: [m/e (%)], M. Wt.:** 339. **Elemental analysis, Calculated:** C, 60.18; H, 3.86; N, 12.38 Found: C, 59.58; H, 3.46; N, 12.09.

2-(4-Nitrophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-17)

IR (KBr) cm^{-1}: 3345 (-NH), 1221 (C-N, str), 1721 (>C=O), 1665 (-CONH), 2885 ($>CH_2$,str), 1469 ($>CH_2$,ban), 3075 (-CH, str), 3065 (Ar, C-H, str), 1536 (Ar, C=C, str), 1564 (Nitro gp), 863 (pera- sub.). **Mass: [m/e (%)], M. Wt.:** 339. **Elemental analysis, Calculated:** C, 60.18; H, 3.86; N, 12.38 Found: C, 60.25; H, 3.93; N, 12.45.

2-(2-Methoxyphenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-18)

IR (KBr) cm^{-1}: 3330 (-NH), 1261 (C-N, str), 1732 (>C=O), 1655 (-CONH), 2896 ($>CH_2$,str), 1362 ($>CH_2$,ban), 3054 (-CH, str), 3063 (Ar, C-H, str), 1562 (Ar, C=C, str), 1361 (-CH3, str.), 723 (*ortho* sub.). **Mass: [m/e (%)], M. Wt.:** 324. **Elemental analysis, Calculated:** C, 66.66; H, 4.97; N, 8.64 Found: C, 66.50; H, 4.85; N, 8.72.

2-(3-Methoxyphenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-19)

IR (KBr) cm^{-1}: 3337 (-NH), 1232 (C-N, str), 1732 (>C=O), 1652 (-CONH), 2920 ($>CH_2$,str), 1372 ($>CH_2$,ban), 3023 (-CH, str), 3055 (Ar, C-H, str), 1485 (Ar, C=C, str), 1358 (-CH3, str.), 772 (meta sub.). **Mass: [m/e (%)], M. Wt.:** 324. **Elemental analysis, Calculated:** C, 66.66; H, 4.97; N, 8.64 Found: C, 66.60; H, 4.86; N, 8.55.

2-(4-Fluorophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-20)

IR (KBr) cm^{-1}: 3319 (-NH), 1238 (C-N, str), 1720 (>C=O), 1689 (-CONH), 2847 ($>CH_2$,str), 1483 ($>CH_2$,ban), 3075 (-CH, str), 3066 (Ar, C-H, str), 1533 (Ar, C=C, str), 875 (pera- sub.). **Mass: [m/e (%)], M. Wt.:** 312. **Elemental analysis, Calculated:** C, 65.38; H, 4.20; N, 8.97 Found: C, 65.24; H, 3.26; N, 8.75.

2-(3-Chloro-4-fluorophenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide(DPB-21)

IR (KBr) cm^{-1}: 3469 (-NH), 1268 (C-N, str), 1732 (>C=O), 1678 (-CONH), 2898 ($>CH_2$,str), 1378 ($>CH_2$,ban), 3035 (-CH, str), 3082 (Ar, C-H, str), 1524 (Ar, C=C, str), 825 (di sub.). **Mass: [m/e (%)], M. Wt.:** 346. **Elemental analysis, Calculated:** C, 58.89; H, 3.49; N, 8.08 Found: C, 59.96; H, 3.53; N, 8.00.

2-(3-(Trifluoromethyl)phenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB22)

IR (KBr) cm^{-1}: 3442 (-NH), 1260 (C-N, str), 1728 (>C=O), 1688 (-CONH), 2898 ($>CH_2$,str), 1389 ($>CH_2$,ban), 3027 (-CH, str), 3096 (Ar, C-H, str), 1523 (Ar, C=C,

str), 765 (meta sub.). **Mass: [m/e (%)], M. Wt.:** 362. **Elemental analysis, Calculated:** C, 59.67; H, 3.62; N, 7.73 Found: C, 59.50; H, 4.69; N, 7.70.

2-(2,4-Dimethylphenylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-23)

IR (KBr) cm^{-1}: 3452 (-NH), 1268 (C-N, str), 1725 (>C=O), 1689 (-CONH), 2879 ($>CH_2$,str), 1393 ($>CH_2$,ban), 3035 (-CH, str), 3096 (Ar, C-H, str), 1532 (Ar, C=C, str), 1368 (-CH3, str.), 825 (di sub.). **Mass: [m/e (%)], M. Wt.:** 322. **Elemental analysis, Calculated:** C, 70.79; H, 5.63; N, 8.69 Found: C, 71.85; H, 5.45; N, 7.72

2-(Benzylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-24)

IR (KBr) cm^{-1}: IR (KBr) cm^{-1}: 3445 (-NH), 1275 (C-N, str), 1725 (>C=O), 1678 (-CONH), 2883 ($>CH_2$,str), 1356 ($>CH_2$,ban), 3058 (-CH, str), 3085 (Ar, C-H, str), 1532 (Ar, C=C, str), 1368 (-CH3, str.). **Mass: [m/e (%)], M. Wt.:** 308. **Elemental analysis, Calculated:** C, 70.12; H, 5.23; N, 9.09 Found: C, 70.23; H, 5.18; N, 9.12

2-(Cyclohexylamino)-N-(2-oxo-2H-chromen-4-yl)acetamide (DPB-25)

IR (KBr) cm^{-1}: IR (KBr) cm^{-1}: 3355 (-NH), 1285 (C-N, str), 1714 (>C=O), 1680 (-CONH), 2869 ($>CH_2$,str), 1450 ($>CH_2$,ban), 3010 (-CH, str), 3052 (Ar, C-H, str), 1523 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 300. **Elemental analysis, Calculated:** C, 67.98; H, 6.71; N, 9.33 Found: C, 67.90; H, 6.65; N, 9.38

N-(2-oxo-2H-chromen-4-yl)-2-(phenylamino)acetamide (DPB-26)

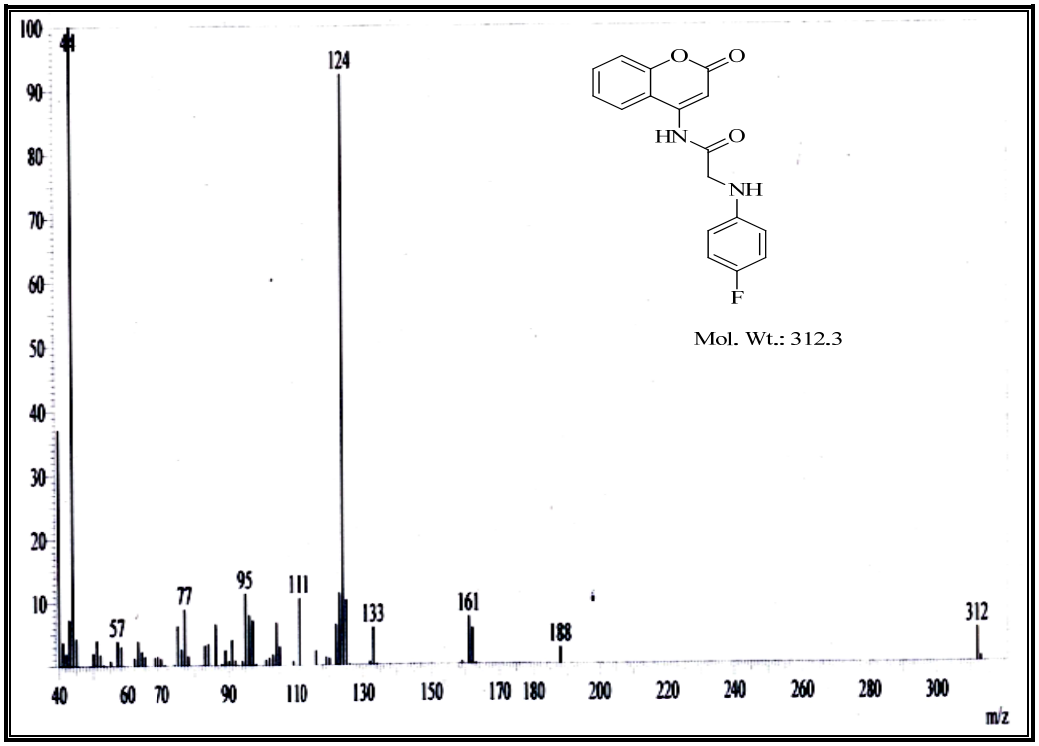
IR (KBr) cm^{-1}: IR (KBr) cm^{-1}: 3359 (-NH), 1276 (C-N, str), 1710 (>C=O), 1686 (-CONH), 2864 ($>CH_2$,str), 1455 ($>CH_2$,ban), 3011 (-CH, str), 3045 (Ar, C-H, str), 1529 (Ar, C=C, str). **Mass: [m/e (%)], M. Wt.:** 294. **Elemental analysis, Calculated:** C, 69.38; H, 4.79; N, 9.52 Found: C, 69.46; H, 4.71; N, 9.48

1.11 CONCLUSION

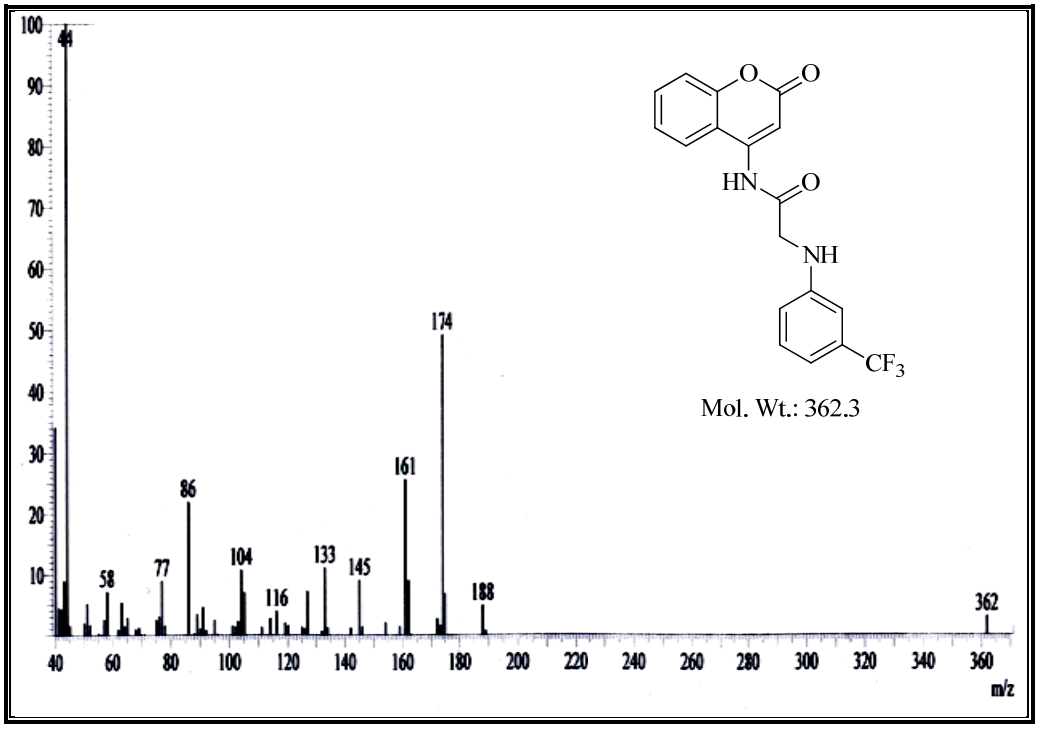
Total 26 derivatives of *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine -1-yl) acetamide were synthesized in this chapter. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C, Mass spectroscopy and Elemental analysis. In addition the newly synthesized compounds were screened for anti-tubercular activity. All the synthesized compounds found less active against tuberculosis strains *in vitro*.

1.12 REPRESENTATIVE SPECTRA

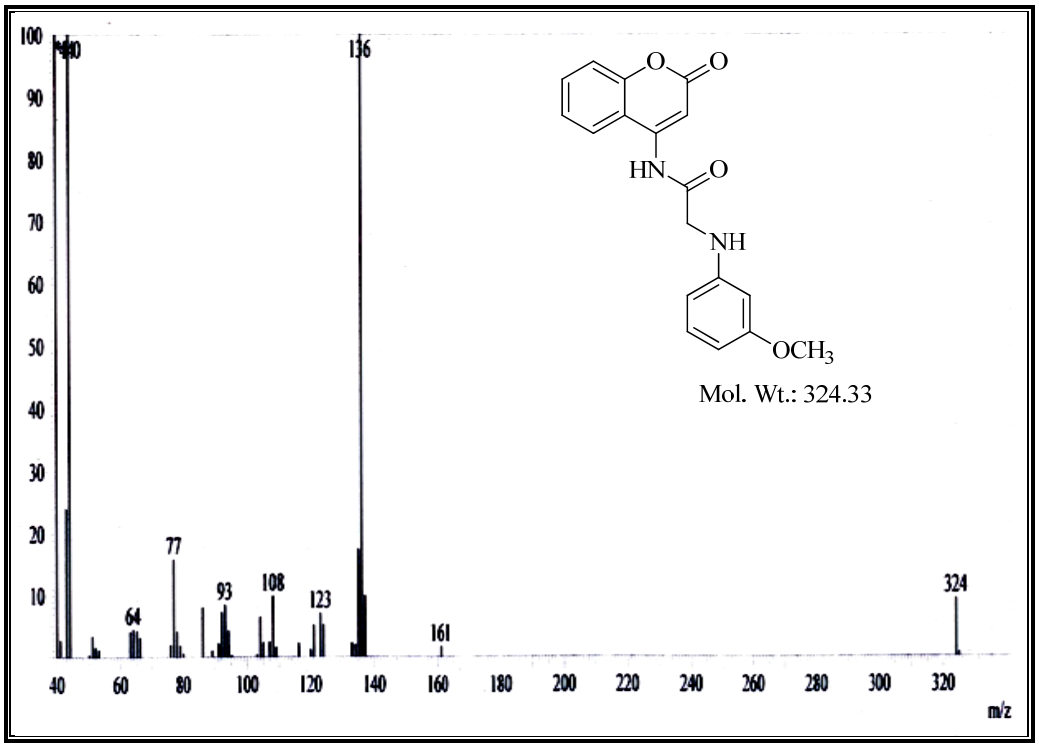
Mass spectrum of 2-(4-Fluorophenylamino)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-20)



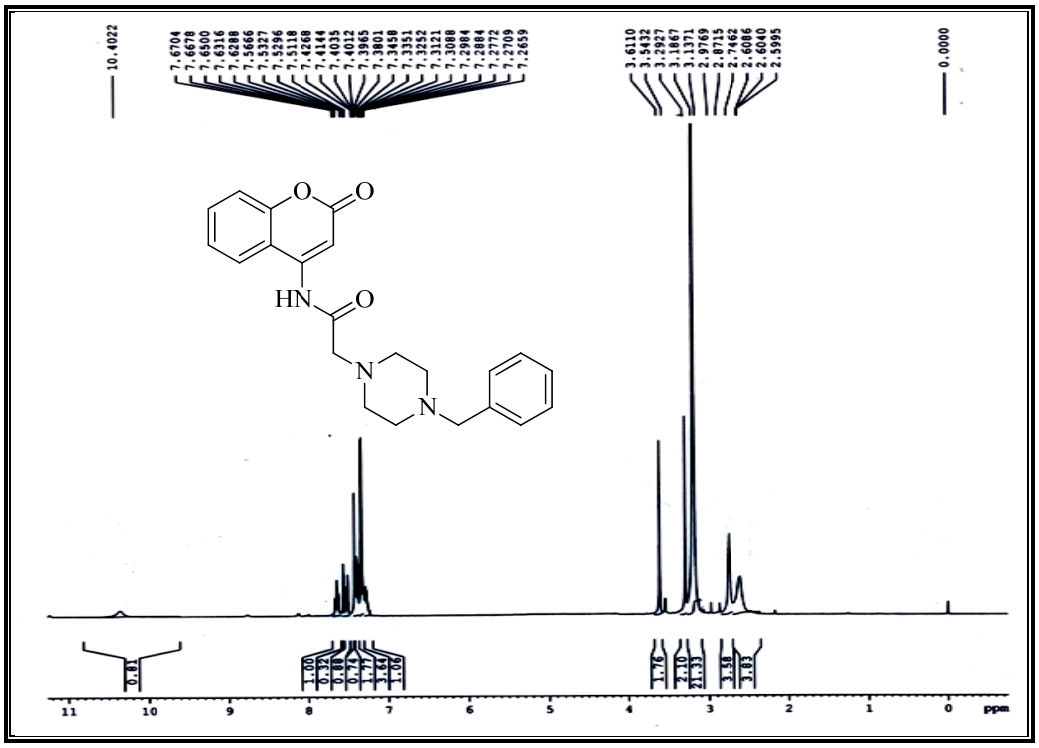
Mass spectrum of 2-(3-(Trifluoromethyl)phenylamino)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-22)

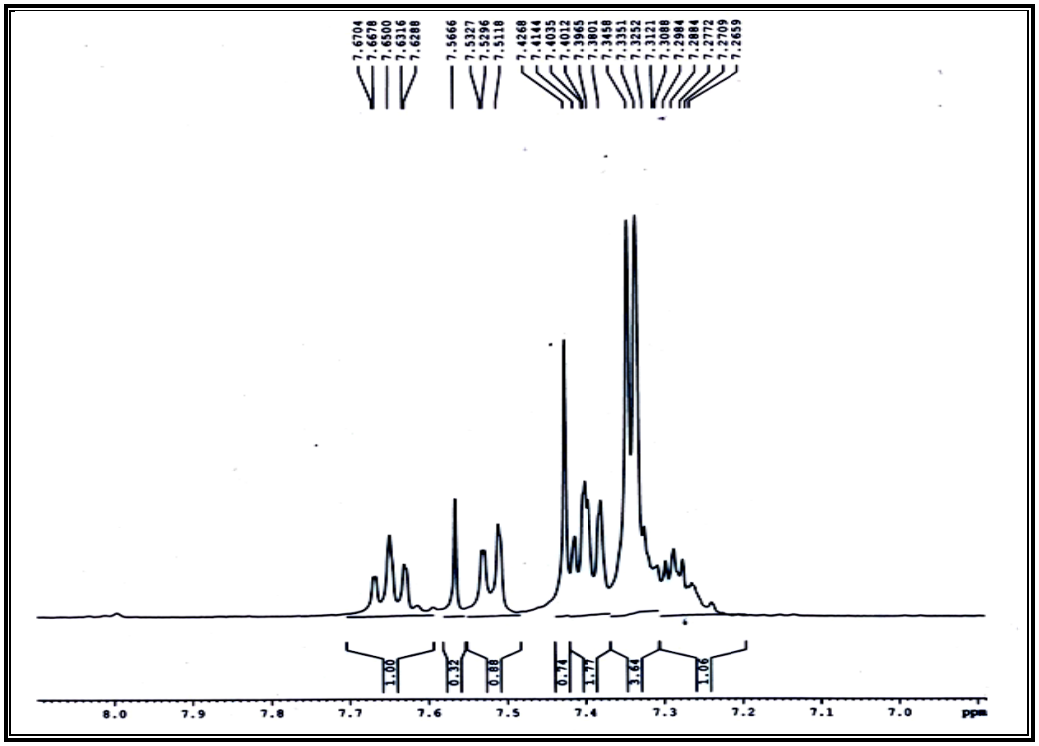


Mass spectrum of 2-(3-Methoxyphenylamino)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-19)

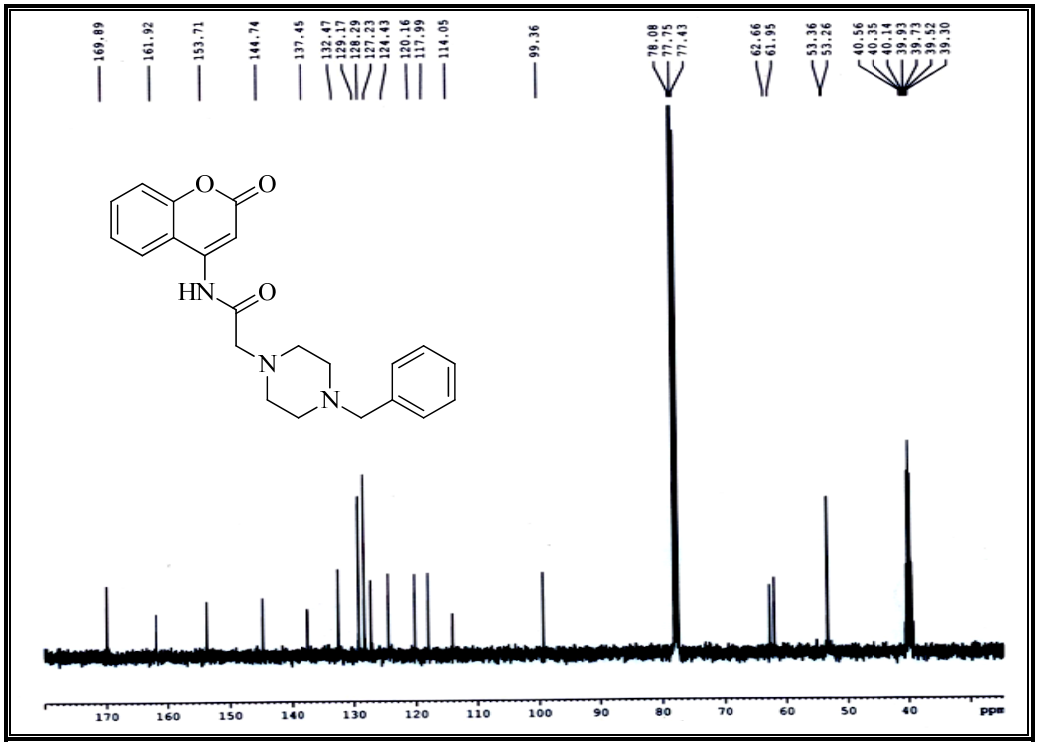


^{1}H NMR spectrum of 2-(4-Benzylpiperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-6)

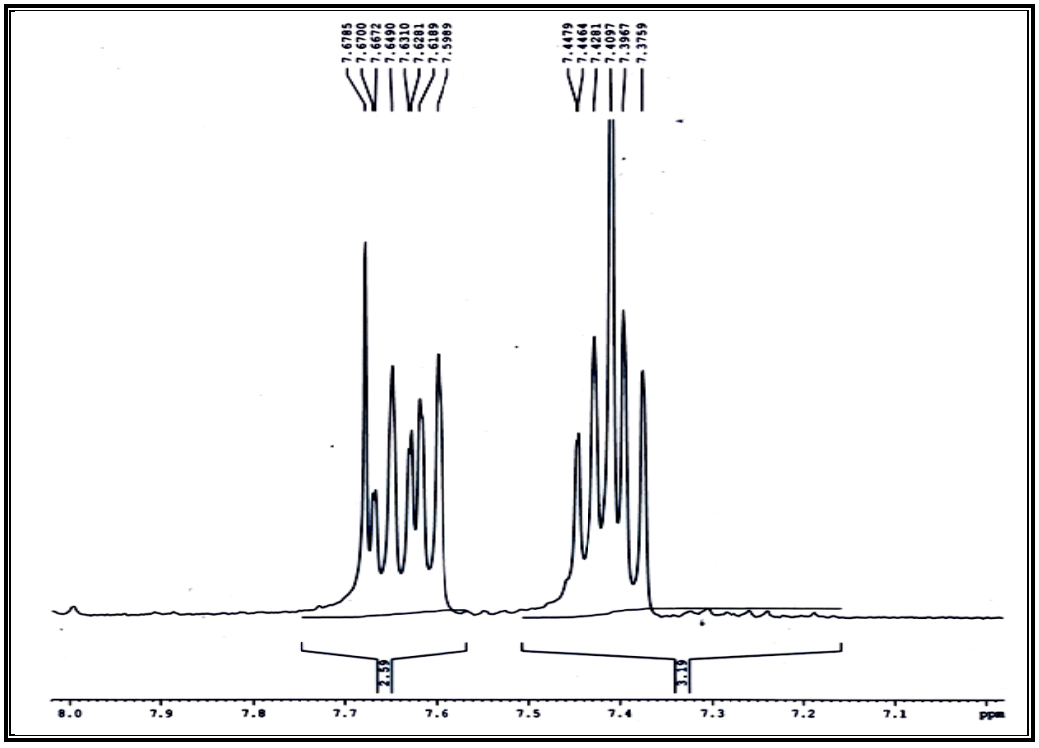




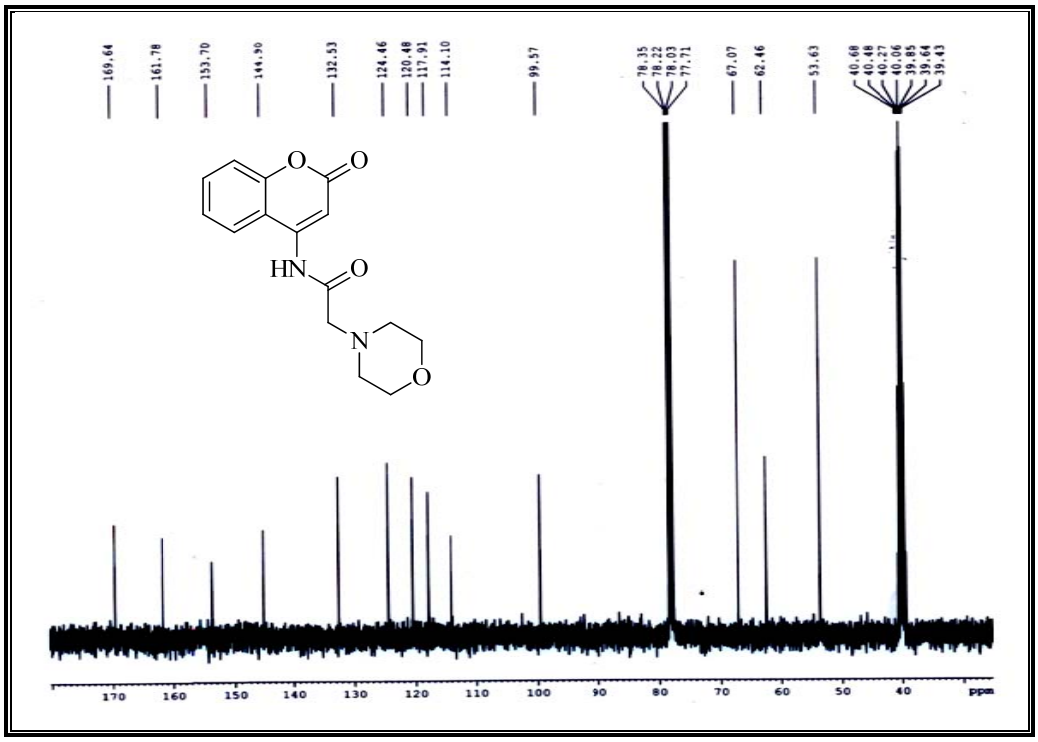
^{13}C NMR spectrum of 2-(4-Benzylpiperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-6)



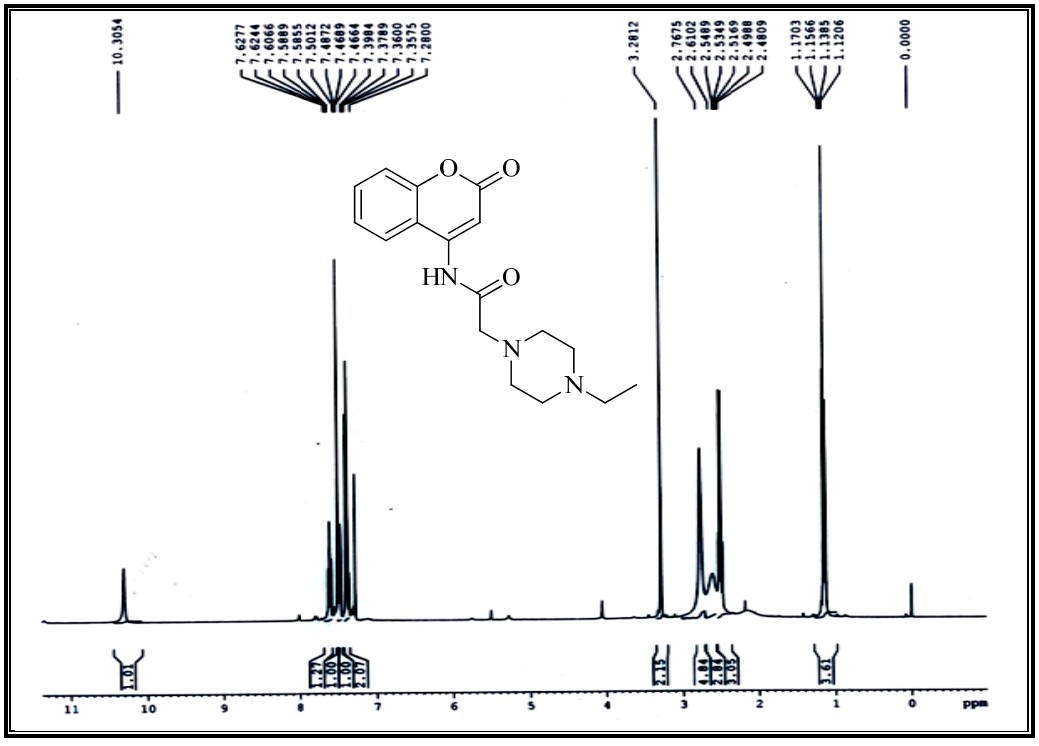
^{1}H NMR spectrum of Morpholino-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide(DPB-2)

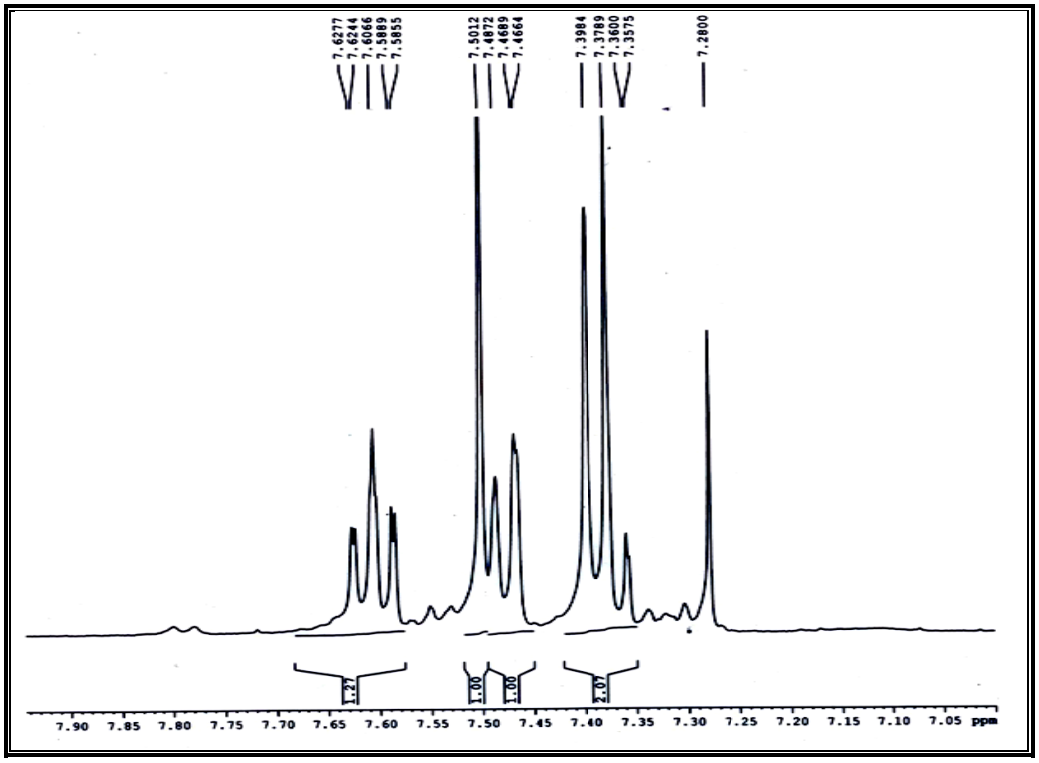


^{13}C NMR spectrum of Morpholino-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-2)

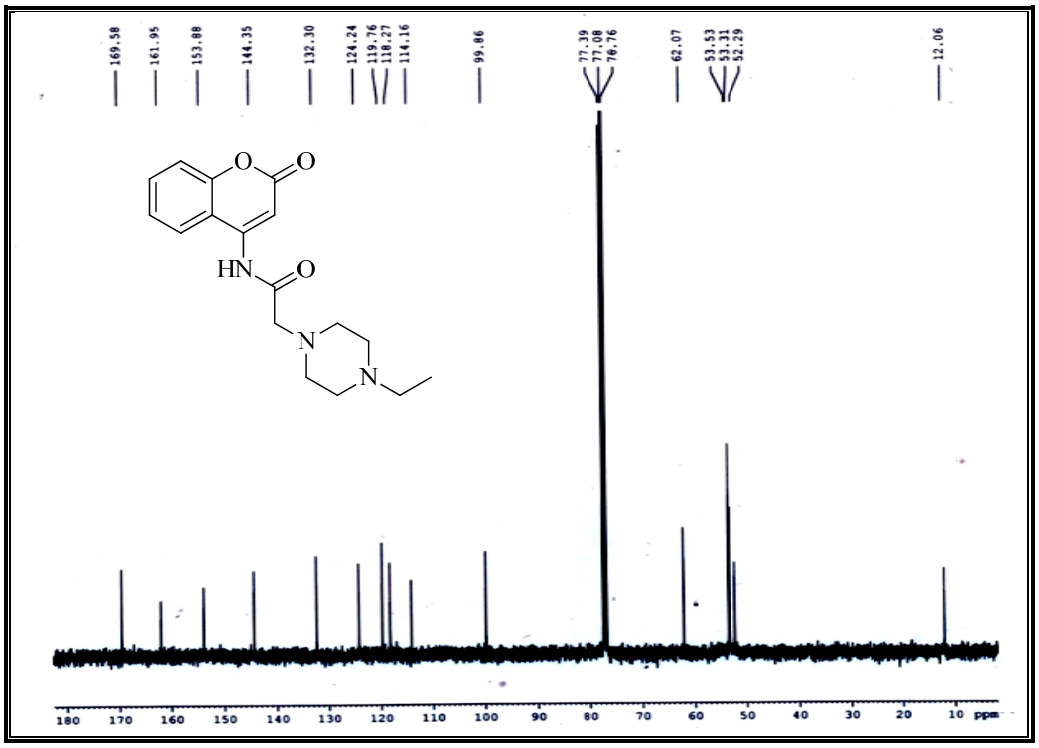


^{1}H NMR spectrum of 2-(4-Ethylpiperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-5)

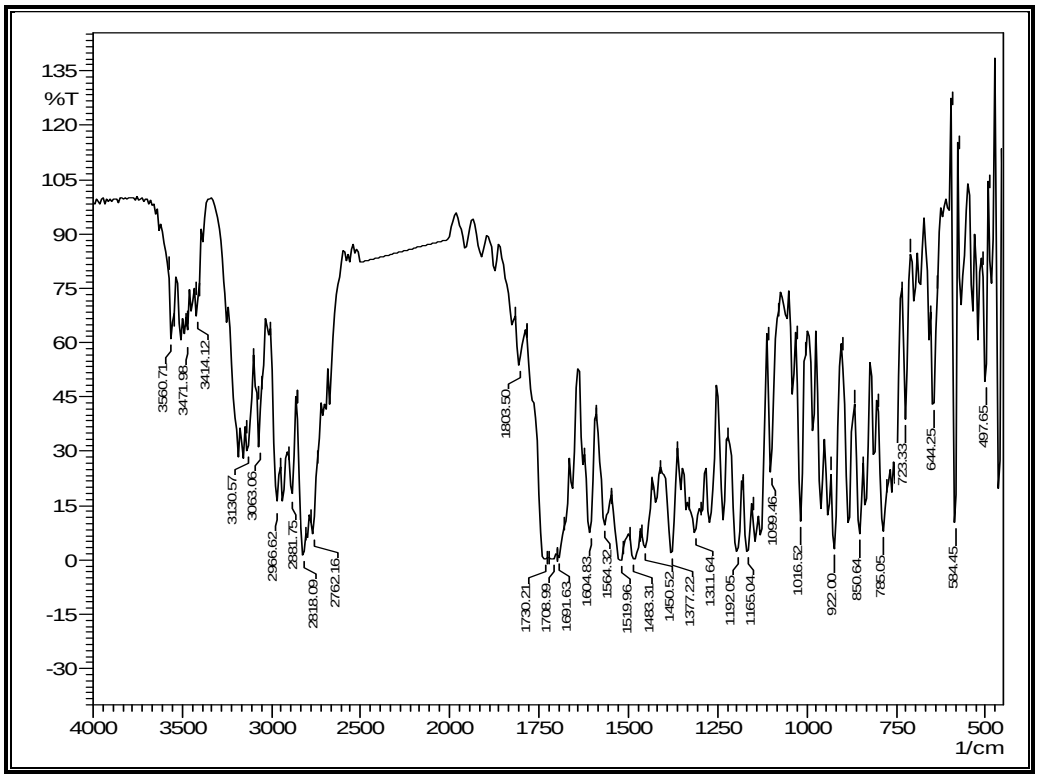




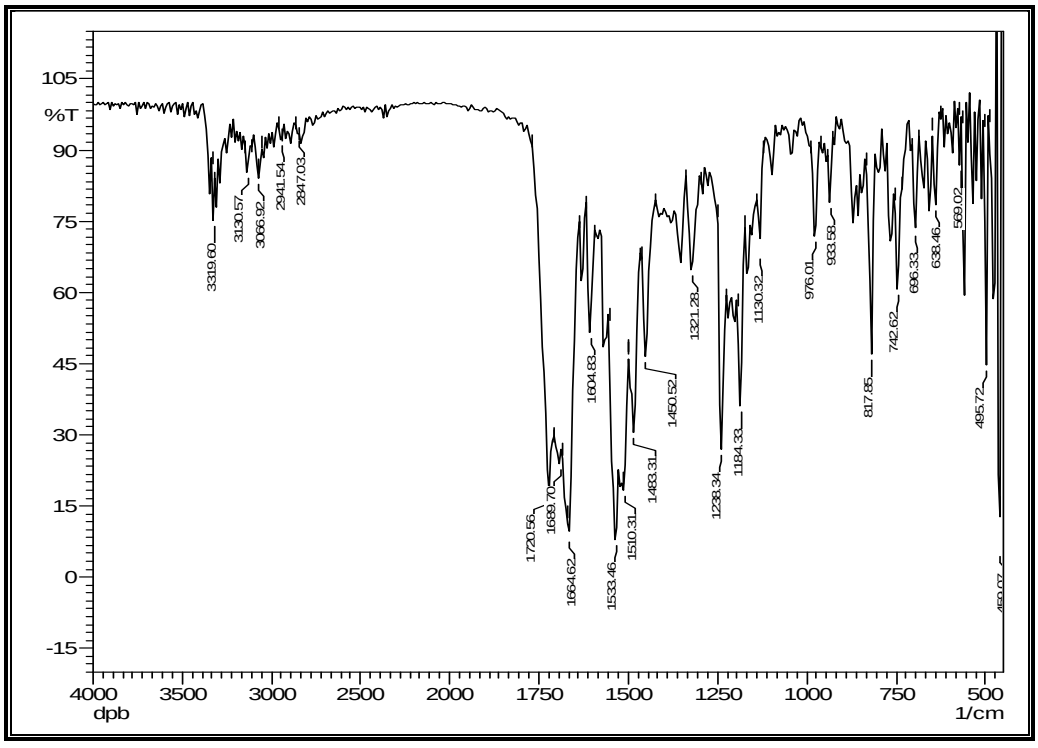
^{13}C NMR spectrum of 2-(4-Ethylpiperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-5)



IR spectrum of 2-(4-Ethylpiperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-5)



IR spectrum of 2-(4-Fluorophenylamino)-*N*-(2-oxo-2*H*-chromen-4-yl)acetamide (DPB-20)



1.13 REFERENCES

1. S. Sen, V. Srivastava, *J. Ind. Chem. Soc*, **1989**, *66*, 166.
2. A Vogel Gilbert's *Ann. Phys*., **1820**, *64*, 161.
3. J. Abernethy, *J.Chem.Edu*., **1969**, *46*, 561.
4. A. Guillemette Justus Leibigs Ann. Chem.,**1835**, *14*, 324.
5. V. Gupta , B. Sharma, R. Arora, *J.Sci.Ind.Res*. **1961**, *20B*, 300.
6. F. Dean, *Progr.Chem.Org.Nat.Prod*., **1952**, *9*, 225.
7. K.Bose, *J.Indian.Chem.Soc*., **1958**, *35*, 367.
8. T. Gexssman, E. Hineiner, *Bot.Rev*.**1952**, *18*, 77.
9. R.Robinson, "The structural relation of natural products", *Oxford* **1955**
10. D. Murray, A. Stewart "The Natural Coumarins", *John Wiley & Sons* **1982**
11. I.Kostava, *Curr. Med. Chem.- Anti Cancer Agents*, **2005**, *5*, 29
12. (a).B. Nielsen, Coumarin patterns in the Umbelliferae, in: V.H. Heywood(Ed.), *The biology and chemistry of the Umbelliferae*, Acedemic Press, London, **1971,** 325 (b). A.Estevez-Braun, A. Gonzalez, *Nat. Prod. Reps*.,**1997**, *14*, 465.
13. (a) M. Stahmann, T. Wolff, K. Link, *J. Am.Chem.Soc*., **1943**, *65*, 2285. (b) E. Renk, W. Stoll, *Prog. Drug. Res*., **1968**, *14*, 226 (c) W.Levin, In The Pharmacology Basis of Therapeutics; 4th ed., L.Goodman, A.Gilman, Eds. New York: Macmillan **1975**, 1445 (d) R. O'Reilly, *Pharmacology* **1972**, *8*, 181. (e)T. Kralt, V. Classen, Drug Design, 3, *Acedemic Press New York* **1972** (f). F. Kazmier, Mayo *Clinic. Proc*., **1974**, *49*, 918 (g) W.Levine, The Pharmacological Basis of Therapeutics, McMillan, New York **1975** (h). R. O'Reilly, *Ann.Rev.Med.*, **1976**, *27*, 245 (i). R. Silverman, *J. Am.Chem.Soc.*, **1981**, *103*, 3910 (j). I. Manolov, N. Danchev, *Arch Pharm*., **2003**, *83*, 336. (k). T.Sionae, *J.Pharm.Sci*., **1964**, *53*, 231 (l). B.Bose, P.Saxena, Entomol. Res., **1984**, *8*, 109 (m). A.Craciun, M. Groenen0van Dooren, H. Thijssen, C. Vermeer, *Biochim. Biophys. Acta*., **1998**, 75, 1380.() (n). S. Moran, Crop. Protect., **2001**, *20*, 529() (o). J.Berthelon, US Patent Appl., **1986**, 4585786 (p). A. Dubock, Plant Protect. Bull., **1980**, *22*, 223.
14. J. Hinmann, H. Hoeksema, E. Caron, W. Jackson, *J. Am.Chem.Soc*.,**1956**, 78, 1072.

15. (a) A. Bourinbaiar, X. Tan, R. Nagomy, Acta Virol., **1993**, *37*, 241 (b) P.Tummino, D. Ferguson, D. Hupe, *Biochem. Biophys. Res. Commun*., **1994**, *201*, 290 (c) Z. Ivezic, M. Trkovnik, PCT Int. Appl. **2003** WO 2003/029237 (d) H. Zhao, N. Neamati, H. Hong, A. Mazmuder, S. Wang, S. Sunder, G. Milne, Y. Pommier, T. Burke, *J.Med.Chem*., **1997**, *40*, 242 (e) A. Mazmuder, S. Wang, N. Neamati, S. Sunder, J.Chen, G. Milne, W. Rice, Y. Pommier, T. Burke, *J.Med.Chem*., **1996**, *39*, 2472 (f) S. Kirkiacharian, T. Thuy, S. Sicsic, R. Bikhchinian, R. Kurkjian, Farmaco, **2002**, *57*, 703 (g) J. Shippeck, H. Kar, L. Gosink, J. Wheatley, E. Gjerstad, S. Loftus, A. Zubiria, J. Janc., *Bioorg. Med. Chem. Lett*.,**2000**, *10*, 2639.

16. (a) P. Laurin, M. Klich, C. Dupis-Hamelin, P. Mauvais, P. Lassaigne, A. Bonnefoy, B. Musicki, *Bioorg. Med. Chem. Lett*., **1999**, *9*, 2079() (b). Y. Inoue, H. Kondo, M. Taguchi, Y. Jinbo, G. Tsukamoto, *J.Med.Chem*.,**1994**, *37*, 586.

17. V. Savelev, N. Pryanishinikova, O. Artamonova, I. Fenida, V. Zagorevskii, *Khimiko Farmatsevticheskii Zhurnal*, **1975,** 9(6), 10.

18. (a).Y.Kimura, H. Okuda, S. Arichi, K. Baba, M. Kozawa, *Biochim. Biophys. Acta*., **1985**, *224*, 834. (b) J. Hoffmanova, A. Kozubik, L. Dusek, J. Pachernik, *Eur. J. Pharmacol*., **1998**, *350*, 273 (c). U. Matern, P. Lu er, D. Kreusch, In Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives, 1st Ed., U.Sandkawa, *Ed., Elsvier*: *Great Britain*, **1999,** 1, 623.

19. (a) N. Thornberry, K. Chapman, D. Nicholson, *Methods Enzymology*, **2000,** *100*, 322. (b) T. Rano, T. Timkey, E. Peterson, J. Rotonda, D. Nicholson, J. Becker, K. Chapman, N. Thornberry, *Chem.Bio.*, **1997**, *4*, 149 (c) A. Ruwet, C. Draguet, M.Renson, *Bull.Soc.Chem.Belg*., **1970,** *79*, 639 (d) B. Chakravarty, Y. Rao, S. Gombir, K.God, *Planta Med.*, **1981**, *43*, 64. (e) R. Romen, *Res. Commun Pathol Pharmacol*., **1975**, *11*, 552. (f) I. Singh, A. Kumar, S. Gurtu, J. Sinha, K. Shanker, Arch Pharma.(Weinheim), **1984**, *317*, 984 (g). A. Kumar, M. Verma, A. Saxena, K. Shanker, *Ind.J.Chem*., **1987**, *26B*, 378.

20. V. Trapov, E. Perfanov, L. Smirnov, *Khim.-Farm. Zh*.,**1996**, *30*, 20.

21. G. Raskob, P. Comp, G. Pineo, R. Hull, In Anticoagulants: Physiologic, Pathologic band Pharmacologic, D. Green, Ed.6, Eds.; CRC Press, Boca Raton, **1994**, 231.

22. S. Pillai, S. Menon, L. Mitscher, C. Pillai, D. Shankel, *J. Nat. Prod.*,**1999**, 62, 1358.

23 W. Gao, W. Hou, M. Chang, Y. Cui, Y. Li, X. Wang, L. Tang and S. Sun; *Faming Zhuanli Shenqing Gongkai Shuomingshu, CN 101220016*, **2008**.

24. L. Schio, F. Chatreaux and M. Klich; *Tet. Lett.*, **2000**, *41*, 1543**.**

25. M. Garazd, L. Garazd, V. Shillin and P. Khliya; *Chem. Nat. Compounds*, **2000**, *36*, 485.

26. S. Schiedel, A. Briehn and P. Bauerle; *Angrew. Chem. Int. Ed.*, **2001**, *40*, 4677.

27. B. Mahantesha, K. Shivashankar, K. Manohar, R. Vijaykumar, P. Harishchandra, M. Sumit, M. Ashwini., *Eur. J. Med. Chem.*, **2010**, *45(3)*, 1151-1157.

28. I. Ivanova, V. Eremin, I. Shvets; *Tetrahedron*, **1996**, *52*, 9581.

29. F. Nahla, W. El-Tayeb, *Eur. J. Med. Chem.*, **2010**, *45(1)*, 317-325.

30. S. Govori, V. Rapic, O. Leci and I. Tabakovic; *J. Heterocyclic Chem.*, **1996**, *33*, 351.

31. I. Aziz; *J. Heteroatom Chem.*, **1996**, *7*, 137.

32. C. Majumdar, S. Saha, N. De and K. Ghosh; *J. Chem. Soc.*, **1993**, 715.

33. N. Nicolaides, C. Fylaktakidou, E. Litinas, D. Hadlipavlou-Litina, *J. Heterocyclic Chem.*, **1996**, *33*, 967.

34. A. Emmanuel-Giota, C. Fylaktakidou, D. Hadlipavlou-Litina, E. Litinas, N. Nicolaides, *J. Heterocyclic Chem.*, **2001**, 38, 717.

35. B. Oduszek and M. Uher ; *Synth. Commun.*, **2000**, *30*, 1749.

36. N. Nishizono, K. Oda, K. Ohno, M. Minami and M. Machida; *Heterocycles*, **2001**, *55*, 1897.

37. K. Ito, Y. Higuchi, C. Tame and J. Hariya, *Heterocycles*, **1993**, *35*, 937.

38. V. Hagen, S. Frings, S. Wiesner and B. Kaupp, *J. Chem. Bio. Chem.*, **2003**, *4*, 434.

39. L. Rao, K. Mukerjee, *Ind. J. Chem.*, **1994**, *55*, 14777.

40. M. Rahman, I. Gray, *Phytochemistry*, **2002**, *59*, 73.

41. A. Schinkovitz, S. Gibbons, M. Stavri, J. Cocksedge, F. Bucar, *Plant Med.*, **2003**, *69*, 369.

42. R. Chowdhury, M. Hasan and A. Rashid; *Fitoterapia*, **2003**, *74*, 155.

43. M. Kawase, T. Tanaka, Y. Sohara, S. Tan, H. Sakagami; *In vivo*, **2003**, *17*, 509.
44. A. Zaha and A. Hazem; *New Microbio*., **2002**, *25*, 213.
45. C. Gleye, G. Lewin, A. Laurens, C. Jullian and C. Loiseau; *J. Nat. Prod.*, **2003**, *66*, 323.
46. E. De Clercq ; *Med. Res. Rev.*, **2000**, *20* 323.
47. T. Makhija and M. Kulkarni; *J. Comput. Aid. Mol. Des*., **2001**, *15*, 961.
48. S. Bourinbaiar, X. Tan, R. Nagorny; *Acta Virol*., **1993**, *37*, 241.
49. H. Zhao, N. Neamati, Y. Pommier, R. Burke, Jr.; *Heterocycles*, **1997**, *45*, 2277.
50. J. Vlientick, T. De Bruyne, S. Apers and A. Pieters; *Plant Med*., **1998**, *64*, 97.
51. S. Koneni, K. Abdhesh, K. Manoj, S. Jayanta, S. Sudhir, *Bioorg. & Med. Che. Let.*, **2010**, *20(24)*, 7205-7211.
52. S. Jagadish, R. Rajapandi, T. Maity, *Asian Journal of Chemistry*, **2010**, *22(5)*, 4099-4103.
53. J. Finn, B. Creaven and A. Egan; *Melanoma Res.*, **2001**, *11*, 461.
54. S. Kawaii, Y. Tomono, K. Ogawa, M. Sugiura, M. Yano, Y. Yoshizawa, C. Ito and H. Furukawa; *Anticancer Res*., **2001**, *21*, 1905.
55. S. Kawaii, Y. Tomono, M. Ogawa, Y. Yoshizawa; *Anticancer Res*., **2001**, *21*, 917.
56. J. Wang, J. Hsieh, L. Lin, H. Tseng; *Cancer Lett.*, **2002**, *183*, 163.
57. J. Finn, E. Kenealy, S. Creaven, A. Egan; *Cancer Lett.*, **2002**, *183*, 61.
58. J. Finn, S. Creaven, A. Egan; *Eur. J. Pharmacol.*, **2003**, *481*, 159.
59. R. Edenharder, X. Tang; *Food Chem. Toxicol.*, **1997**, *35*, 357.
60. S. Ahmed, K. James, P. Owen, K. Patel; *Bioorg. & Med. Chem. Lett.*, **2002**, *12*, 1343.
61. T. Ho, A. Purohit, N. Vicker, P. Newman, J. Robinson, P. Leese, D. Ganeshapillai, L. Woo, L. Potter, J. Reed; *Biochem. Biophys. Res. Commun*., **2003**, *305*, 909.
62. C. Bruhimann, F. Ooms, A. Carrupt, B. Testa, M. Catto, F. Leonetti, C. Altomare, A. Carotti; *J. Med. Chem.*, **2001**, *44*, 3195.
63. S. Jo, L. Gyibg, K. Bae, K. Lee, H. Jun; *Plant Med.*, **2002**, *68*, 84.
64. H. Wang, B. Ternai, G. Polya; *Phytochemistry*, **1997**, *44*, 787.

65. S. Sardari, S. Nishibe, K. Horita, T. Nikaido, M. Daneshtalab; *Pharmazie*, **1999**, *54*, 554.

66. B. Yang, B. Zhao, K. Zhang and P. Mack; *Biochem. Biophys. Res. Commun.*, **1999**, *260*, 682.

67. X. Wang, B. Ng; *Plant Med.*, **2001**, *67*, 669.

68. L. Costantino, G. Rastelli, A. Albasini; *Pharmazie*, **1996**, *51*, 994.

69. T. Kaneko, N. Baba, M. Matsuo; *Cytotechnology*, **2001**, *35*, 43

70. B. Fernandez-Puntero, I. Barroso, I. Idlesias and J. Benedi, *Bio. Pharm. Bull.*, **2001**, *24*, 777.

71. G. Lazarova, I. Kostova, H. Neychev; *Fitoterapia*, **1993**, *64*, 134.

72. V. Maddi, S. Raghu, A. Rao; *J. Pharm. Sci.*, **1992**, *81*, 964.

73. N. Nicolaides, C. Fylaktakidou, E. Litinas, D. Hadlipavlou-Litina; *Eur. J. Med. Chem.*, **1998**, *33*, 715.

74. G. Delgado, S. Olivares, M. I. Chavez, T. Ramirez-Apan, E. Linares, R. Bye; *J. Nat. Prod.*, **2001**, *64*, 861.

75. M. Ghate, D. Manoher, V. Kulkarni, R. Shosbha, S. Kattimani; *Eur. J. Med. Chem.*, **2003**, *38*, 297.

76. D. Hadlipavlou-Litina; *J. Arzneim-Forsch./Drug Res.*, **2000**, *50*, 631.

77. M. Ferrer, J. Leiton, L. Zaton; *J. Protein Chem.*, **1998**, *17*, 115.

78. G. Roma, M. Di Braccio, A. Carrieri, G. Grossi, G. Leoncini, G. Signorello, A. Carotti; *Bioorg. & Med. Chem.*, **2003**, *11*, 123.

79. F. Chiou, L. Huang, F. Chen, C. Chen; *Planta Med*., **2001**, *67*, 282.

80. R. Pignatello, A. Puleo, S. Giustolisi, S. Cuzzoccrea, L. Dugo, P. Caputi, G. Puglisi; *Drug Dev. Res.*, **2002**, *57*, 115.

81. L. Santana, E. Uriarte, Y. Fall, M. Teijeira, C. Teran, E. Garcia-Martinez, R. Tolf; *Eur. J. Med. Chem.*, **2002**, *37*, 503.

82. M. Gonzalez-Gomez, L. Santana, E. Uriarte, J. Brea, M. Villlazon, I. Loza, M. De Luca, E. Rivas, Y. Montegero, A. Fontela; *Bioorg. & Med. Chem. Lett.*, **2003**, *13*, 175.

83. A. Domling, I. Ugi, *Angew. Chem,* Int. Ed., **2000,** *39*, 3168-3210

84. (a) W. H. Perkin; *J. Chem. Soc.*, **1868**, *21*, 53.; (b) W. H. Perkin; *Justus Liebigs Ann. Chem.*, **1868**, *147*, 229.

85. R. Anshutz; *Ber.*, **1903**, *36*, 465.

86. H. Pauly, K. Lokemann; *Ber.*, **1915**, *48*, 48.

87. A. Sonn; *Ber.*, **1917**, *50*, 1292.
88. C. Mentzer, G. Urbain; *Bull. Soc. Chem.*, **1944**, *11*, 171.
89. A. Robertson, J. Boyd; *J. Chem. Soc.*, **1948**, 174.
90. E. Ziegler, H. Junek; *Monatshefte fuer Chemie*, **1955**, *86*, 29.
91. J. F. Garden, N. F. Hayes, R. H. Thomso; *J. Chem. Soc.*, **1956**, 3315.
92. V. R. Shah, J. L. Bose, R. C. Shah; *J. Org. Chem.*, **1960**, *25*, 677.
93. H. Kaneyuki; *Bull. Chem. Soc. Japan*, **1962**, *35*, 579.
94. A. Resplandy; *Compat Rend.*, **1965**, *260*, 6479.
95. C. Jain, K. Rohtagi, T. R. Sheshadri; *Tet. Lett.*, **1966**, 2701.
96. A. Shah, N. Bhatt, V. M. Thakor; *Curr. Sci.*, **1984**, *53(24)*, 1289.
97. K. Sen, P. Bagchi; *J. Org. Chem.*, **1959**, *24*, 316.
98. J. Bose, R. Shah, V. Shah; *Chemistry & Industry*, **1960**, 623.
99. Y. Shaikh, K.Trivedi; *Ind. J. Chem.*, **1974**, *12(12)*, 1262.
100. W. Barz, R. Schlepphorst, J. Laimer; *Phytochemistry*, **1976**, *15(1)*, 87.
101. V. Szabo, J. Borda; *Acta Chim. Acade. Scientia. Hung.*, **1977**, *95(2-3)*, 333.
102. V. Szabo, J. Borda, E. Theisz; *Magy. Kemi. Folyoir.*, **1978**, *84(3)*, 134.
103. Z. Jerzmanowska, W. Basinski, L. Zielinska; *Pol. J. Chem.*, **1980**, *54(2)*, 383.
104. A. Ogawa, K. Kondo, S. Murai and N. Sonoda; *J. Chem. Soc., Chem. Commun.*, **1982**, *21*, 1283.
105. W. Basinski, Z. Jerzmanowska; *Pol. J. Chem.*, **1983**, *57(4-5-6)*, 471.
106. A. Ogawa, N. Kambe, S. Murai, N. Sonoda; *Tetrahedron*, **1985**, *41(21)*, 4813.
107. N. Shobanaa, P. Shanmugam; *Ind. J. Chem.*, **1986**, *25B(6)*, 658.
108. J. Chatterjea, K. Singh, I. Jha, Y. Prasad, S. Shaw; *Ind. J. Chem.*, **1986**, *25B(8)*, 796.
109. T. Mizuno, I. Nishiguchi, T. Hirashima, A. Ogawa, N. Kambe, N. Sonoda; *Synthesis*, **1988**, *3*, 257.
110. N. Shobana, M. Amirthavalli, V. Deepa, P. Shanmugam; *Ind. J. Chem.*, **1988**, *27B(10)*, 965.
111. E. Parfenov, V. L. Savel'ev, L. D. Smirnov; *Khim. Geterotsikli. Soedin.*, **1989**, *3*, 423.
112. G. Pandey, C. Muralikrishna, U. Bhalerao; *Tetrahedron*, **1989**, *45(21)*, 6867.
113. M. Badran, A. El-Ansari, S. El-Meligie; *Rev. Roum. de Chim.*, **1990**, *35(6)*, 777.
114. S. Nayak, S. Kadam, A. Banerji; *Synlett*, **1993**, *8*, 581.

115. T. Kakimoto, T. Hirai; *Jpn. Kokai Tokkyo Koho, JP 05255299*, **1993**.
116. T. Kakimoto, T. Hirai; *Jpn. Kokai Tokkyo Koho, JP 05262756*, **1993**.
117. D. Ye, Y. Zhou, Q. Su; *Faming Zhuanli Shenqing Gongkai Shuomingshu, CN 1101045*, **1995**.
118. A. Kalinin, A. Da Silva, C. Lopes, R. Lopes, V. Snieckus; *Tet. Lett.*, **1998**, *39(28)*, 4995.
119. V. Sosnovskikh, V. Kutsenko, I. S. Ovsyannikov; *Russ. Chem. Bull.*, **2000**, *49(3)*, 478.
120. J. Jung, Y. Jung, O. Park; *Synth. Commun.*, **2001**, *31(8)*, 1195.
121. X. Long; *Jiangxi Shifan Daxue Xuebao, Ziran Kexueban*, **2001**, *25(3)*, 237.
122. M. Buzariashvili, M. Tsitsagi, I. Mikadze, M. Dzhaparidze, A. Dolidze; *Sakartvelos Mecnierebata Akademiis Macne, Kimiis Seria*, **2003**, *29(3-4)*, 242.
123. Y. Ling, X. Yang, M. Yang, W. Chen; *Huaxue Tongbao*, **2004**, *67(5)*, 355.
124. Y. Takahashi, K. Kato, K. Kubota; *Jpn. Kokai Tokkyo Koho, JP 2005097140*, **2005**.
125. N. Ganguly, S. Dutta, M. Datta; *Tet. Lett.*, **2006**, *47(32)*, 5807.
126. M. Gebauer; *Bioorg. & Med. Chem.*, **2007**, *15(6)*, 2414.
127. S. Park, J. Lee, K. Lee, *Bull. Kore. Chem. Soc.*, **2007**, *28(7)*, 1203.
128. W. Gao, W. Hou, M. Chang, Y. Cui, Y. Li, X. Wang, L. Tang, S. Sun; *Faming Zhuanli Shenqing Gongkai Shuomingshu, CN 101220016*, **2008**.
129. C. Song, D. Jung, S. Choung, E. Roh, S. Lee, *Angew. Chem.*, **2004**, *116*, 6309-6311.
130. Y. Yamamoto, N. Kirai, *Org. Lett.*, **2008**, *10*, 5513-5516.
131. B. Ranu, R. Jana, *Eur. J. Org. Chem.*, **2006**, 3767-3770.
132. H. Rao, S. Sivakumar, *J. Org. Chem.*, **2006**, *71*, 8715-8723.
133. C. Su, Z. Chen, Q. Zhen, *Synthesis*, **2003**, 555-559.
134. V. A. Zagorevskii, N. V. Dudykina, *Zhurnal Obshchei Khimii*, **1962**, *32*, 2384.
135. H. Uno, M. Kurokawa, H. Nishimura, *Chem. Pharm. Bull.*, **1976,** *24*, 644.
136. I. C. Ivanov, S. K. Karagiosov, I. Manolov, *Arch. der Pharm.*, **1991**, 324, 61.
137. A. P. Chavan, *Journal of Chemical Research*, **2006**, *3*, 179.

Chapter-2

MULTISTEP SYNTHESIS OF SOME NOVEL IMIDAZO[1,2-a]PYRIDIN AND THIAZOLE DERIVATIVES

2.1 INTRODUCTION

Bridge nitrogen containing fused heterocycles represents important building blocks in both natural and synthetic bioactive compounds which have been shown to possess diverse therapeutic activities.[1] Hence they are interesting target for research as therapeutically important heterocyclic entities. Aza-indolizine are of two types, imidazo[1,2-*a*]pyridine and imidazo[1,5-a]pyridine (Figure-1).

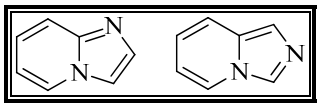


Figure-1

The aza-indolizine containing a phenyl ring fused to a imidazole ring is indicated in the structure, hence it is also known as imidazo[1,2-*a*]pyridine.[2] Several procedure for their synthesis have been extensively studied. Such studies have been stimulated by various promising applications, especially in the case of bridgehead nitrogen containing fused heterocyclic entities.

The constitution of imidazo[1,2-*a*]pyridine was reviewed by W. L. Mosby[3] in 1961. imidazo[1,2-*a*]pyridine derivatives not only known for their pharmacological applications, they are also used in disperse dyes.[4]

2.2 PHARMACOLOGY

Imidazo[1,2-*a*]pyridines are potential bioactive agents due to their wide spectrum of therapeutic importance. A large number of substituted imidazo[1,2-*a*]pyridine derivatives are prepared and tested for varieties of biological activities such as,

1. Anti-inflammatory, analgesic, antipyretic[5,6]
2. Antiviral[7,8]
3. Antianxiety[9]
4. Antiulcer[10,11]
5. Antifungal agents[12]
6. Anthelmintic[13]
7. Antibacterials[14,15]
8. Hypnotic[16]
9. Antiherpetie[17,18]
10. Gastric antisecretory[19,20]
11. Hypnoselective and anxioselective[21]
12. β-Amyloid formation inhibitors[22]
13. Benzodiazepine receptor agonists[23]
14. Nonsedative anxiolytic[24]
15. Active nonpeptide bradykinin B2 receptor antagonists[24]
16. Cardio tonic agents[26]
17. Anticytomegalo-zoster and antivaricellazoster virus[27-29]
18. Long-acting local anesthetic[30]
19. Calcium channel blockers[31]

Alexander C. Humphries and *et al*[32] have synthesized 8-fluoro imidazo[1,2-*a*]pyridine derivatives (Figure-2) and evaluated as a bioisosteric replacement for imidazo[1,2-*a*]pyridine in an allosteric modulator ligand of the GABAA receptor. Kristjan S. Gudmundsson and co-workers[33] reported the synthesis and antiviral activity of newer erythrofuranosyl imidazo[1,2-*a*]pyridine C-nucleosides. I. Aramori *et al*.[34] have been synthesized imidazo[1,2-*a*]pyridine derivatives which are highly potent and selective non-peptide bradykinin receptor antagonist (Figure-2).

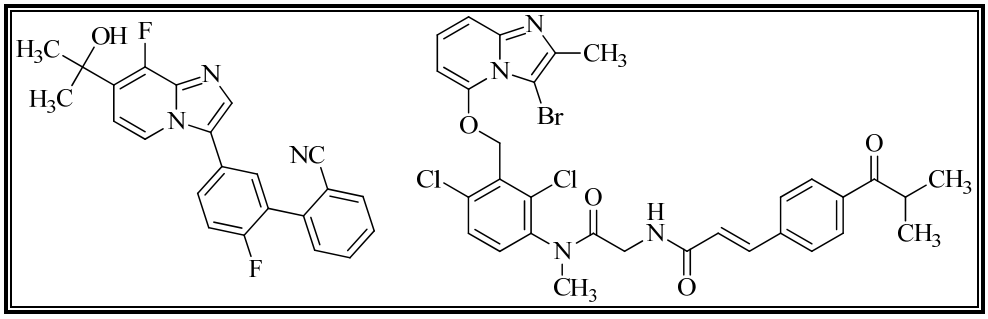


Figure-2

Several imidazo[1,2-*a*]pyridine nucleus already in market which include alpidem[35] [a ligand of both the central benzodiazepine receptors and the peripheral type (Mitochondrial) benzodiazepine receptor] has sedative and anxiolytic properties and zolpidem[35] [a selective ligand for the central benzodiazepine receptor] is a hypnotic drug (Figure-3). Both alpidem and zolpidem have higher affinity for benzodiazepine than for benzodiazepine-2 receptors[36] and their interaction with various receptors has been repoted.[37]

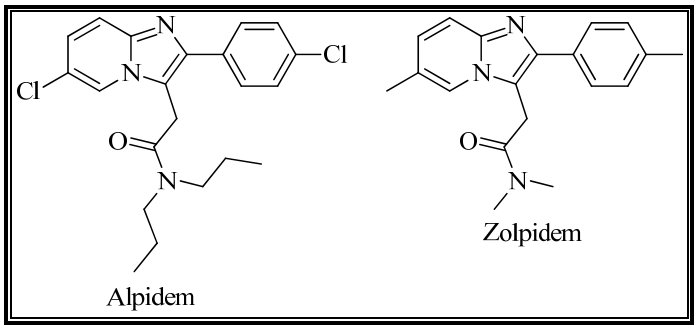


Figure-3

James J. Kaminski *et al*[38] have investigated imidazo[1,2-*a*]pyridine derivative 3-(cyanomethyl)-2-methyl-8-(phenylmethoxy)imidazo[1,2-*a*]pyridine (Figure-4) for an antiulcer activity. On the basis of the reported metabolism of zolimidine, they reported that the 3-cyanomethyl and 8-phenylmethoxy group have been established as metabolic sites.

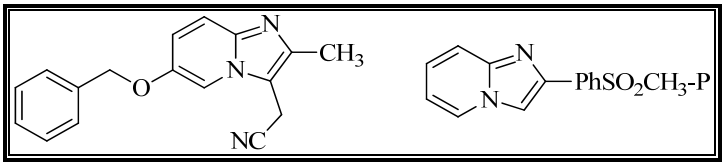


Figure-4

Brian A. Johns *et al.*[39] and Chaouni-Bendallah A. *et al.*[40] synthesized a novel imidazo[1,2-*a*]pyridines (Figure-5) with potent activity against herpes simplex viruses. Sophic Ceard *et al*[41] have synthesized some newer imidazo[1,2-*a*]pyridine derivatives (Figure-5) as bioactive agent. Imidazo[1,2-*a*]pyridine units appear as important building blocks in both natural and synthetic bioactive compounds[42-44] and recognition on DNA binding and to yield different pharmacokinetic profile.

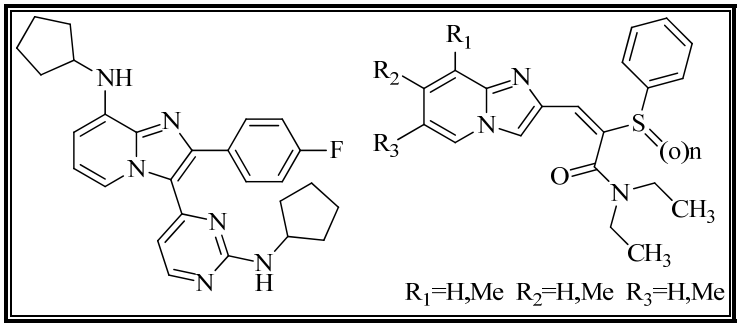


Figure-5

Mohamed A. Ismail *et al*[45] have synthesized some newer diamine imidazo[1,2-*a*]pyridine (Figure-6), 5,6,7,8-tetrahydo imidazo[1,2-*a*]pyridines and their corresponding *N*-hydroxy and *N*-methoxy analogues and evaluated against Trypanosoma B. Rhodesiense (T. B. Rhodesiense) and Plasmodium Falciparum (P. Falciparum). Aromatic diamidines exhibit broad spectrum antimicrobial activity including effectiveness against the protozoan disease caused by Trypanosoma SP and Plasmodium SP.[46]

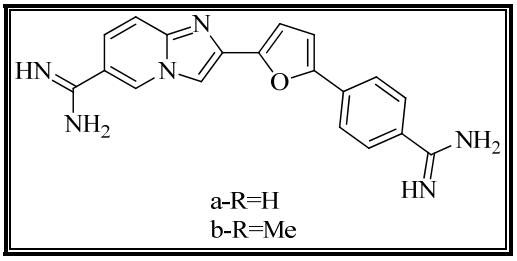


Figure-6

2.2.1 p38 MAP (Mitogen-activated protein) kinase

Laufer *et. al.*[47] reported a series of polysubstituted pyridin-4-yl imidazole inhibitors of p38 MAP kinase which was prepared as small molecular anticytokine agents and drug candidates for the treatment of chronic inflammatory diseases. The contribution of substituents at the pyridine and imidazole moiety to selective inhibition of p38

without concomitant cytochrome P450 interaction was evaluated. Placement of a 1-phenylethyl (p38: IC_{50} 0.38 μM) or acetyl substituent at the exocyclic nitrogen of several 2-aminopyridine imidazoles led to the identification of potent p38 inhibitors which exceeded the starting lead ML 3375 (p38: IC_{50} 0.63 μM) in potency (Figure-7).

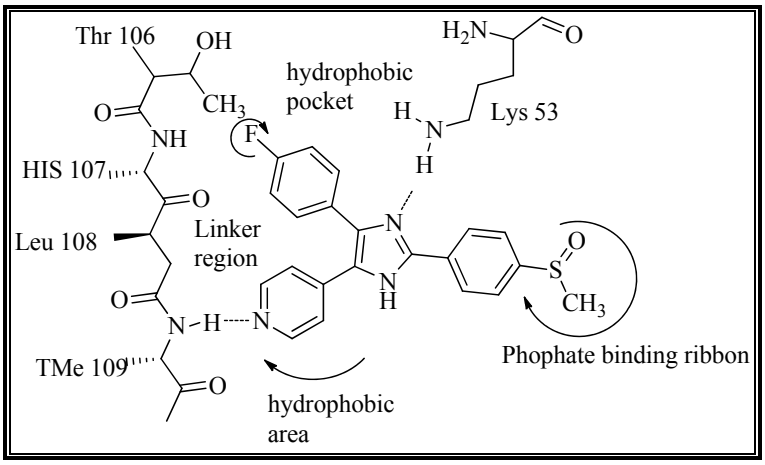


Figure-7: Schematic drawing of important interactions between the prototypicalpyridine-4-yl imidazole inhibitor of SB 203580 and the ATP binding site of p38

A preliminary modeling study related the enhanced bioactivity of 1-phenylethyl substituent to a novel interaction between its 1-phenylethylamino side chain and a hydrophobic pocket close to the linker region of p38. The most active p38 inhibitors in this series maintained their efficacy in functional PBMC (peripheral blood mononuclear cells) and whole blood assays (Figure-8).

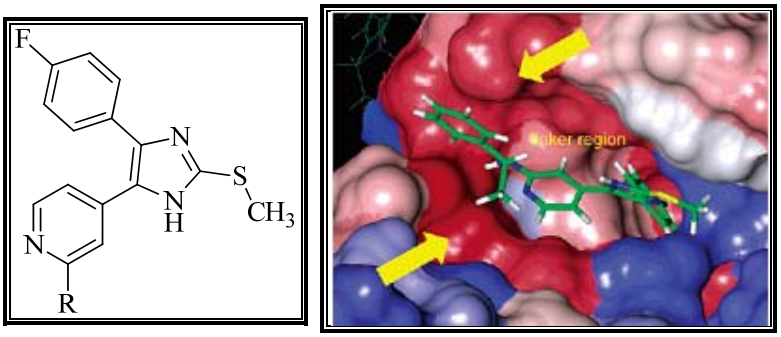


Figure-8: Banzylsulfulfanylimdazoles and modeling of 7g into the ATP cleft of p38 MAP Kinase. The arrows denote the hydrophobic area in close proximity to the linker region which stretches both above and below the pyridine ring.

Laufer *et al*[48] then prepared novel 1,2,4,5-tetrasubstituted imidazole derivatives with high anti-inflammatory activity. Systematic optimization of the imidazole *N*-1

substituent resulted in a compound that potently inhibited the mitogen activated protein kinase p38 (p38 IC_{50}) 0.218 μM) as well as the release of the proinflammatory cytokines interleukin-1α (L-1α) and tumor necrosis factor R (TNFR) from human whole blood after stimulation with LPS. Furthermore, this compound exhibited reduced cytochrome P450 interaction in comparison with SB203580. This result is particularly important, since cytochrome P450 interaction is observed for some p38 inhibitors and in turn can potentially cause drug-drug interaction or lead to other hepatic changes such as P450 enzyme induction (Figure-9).

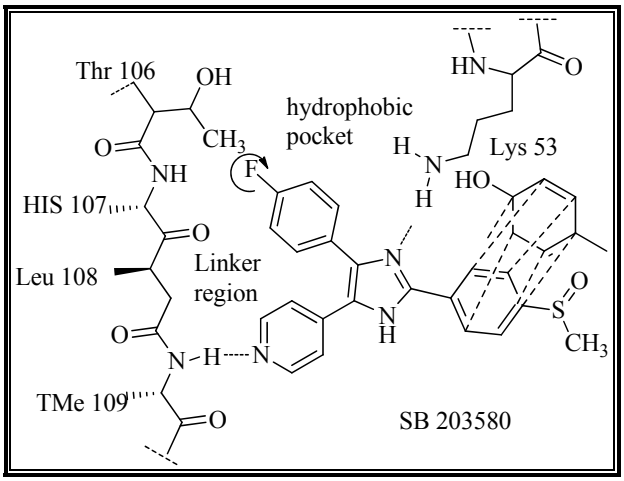


Figure-9: Representation of the active site interactions between SB 203580 and p38-MAPK

A new therapeutic drug target for the treatment of inflammatory disorders is the mitogen-activated protein kinase (MAPK) p38.[49-52] P38 is a serine/threonine kinase that is part of the stress-activated signal transduction cascade that transducers extracellular signals to intracellular response, *e.g.* cytokine production.[53,54] Activated p38 phosphorylates other kinases or transcription factors, leading to mRNA stabilization or expression of certain target genes.[55-57]

Pyridinylimidazoles (*i.e.* SB203580) are potent and selective inhibitors of p38-MAPK7[58,59] by competing with ATP for binding to the ATP pocket.[60-62] This small hydrophobic pocket near the ATP-binding site is responsible for the selectivity of SB203580 for p38 compared to most other kinases.[63,64] The pyridin-4-yl moiety is essential for the inhibitory potency and generates a pivotal hydrogen bond with the amino backbone of Met109 through its pyridinium nitrogen[65] (Figure-10).

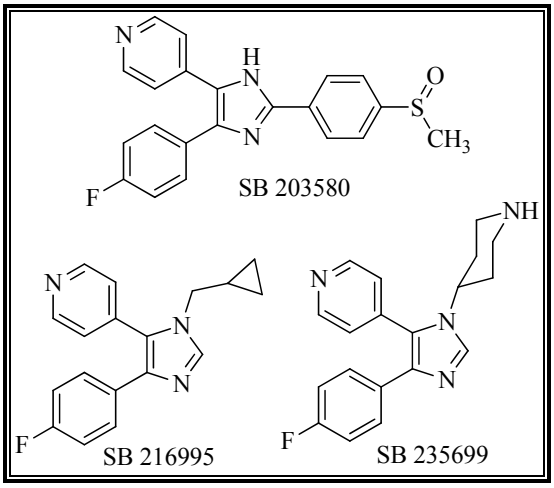
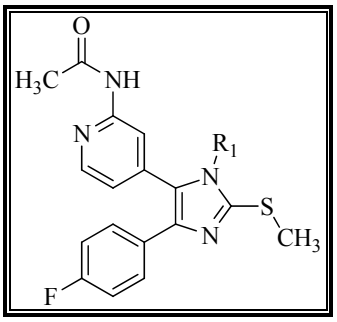
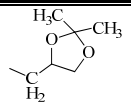
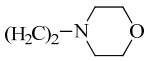
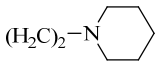
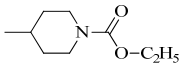


Figure-10: Pyidinylimidazole inhibitors of p-38-MAP kinase.

Table-1: Inhibition of p-38-MAP kinase by acetylaminopyridines.



Comp.	R^1	$IC_{50} \pm (\mu M)$ P38α
1	$-(CH_2)-OH$	0.398±0.037 (n=3)
2	$-(CH_2)_2-O-CH_3$	0.218±0.010 (n=3)
3	$-(CH_2)_3-OH$	0.813±0.040 (n=3)
4	$-(CH_2)_3-O-CH_3$	0.205±0.027 (n=3)
5	$-(CH_2)_2O-(CH_2)_2-OH$	8.479±0.384 (n=2)
6	$-(CH_2)-CH-(CH_3)-OH$	8.692±0.188 (n=2)
7	$-(CH_2)_2-NH-COCH$	7.29 (n=1)
8	$-(CH_2)_2-N(CH_3)_2$	2.409±0.145 (n=3)
9	$-(CH_2)_2OCH_2CH=CH_2$	1.85±0.753 (n=3)
10	$-(CH_2)_2OCH_2C\equiv CH$	0.666±0.148 (n=3)
11	$-(CH_2)_2-S-CH_3$	0.431±0.1 (n=3)
12	$-(CH_2)-CH(OCH_3)_2$	4.099±1.690 (n=2)

13		3.732±2.116 (n=2)
14		2.029±0.686 (n=2)
16		1.343±0.576 (n=2)
17		> 10
SB 203580		0.462±0.025 (n=6)

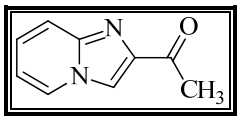
[α] Results are given as mean of two independent experiments except stated otherwise.

2.3 SYNTHETIC ASPECT

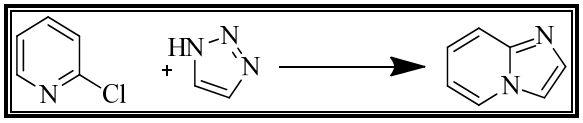
Classical methods have been reported in the literature for the synthesis of imidazo[1,2-*a*]pyridines. The procedure for synthesizing imidazo[1,2-*a*]pyridines have been described as under.

The synthesis of imidazo[1,2-*a*]pyridine from 2-aminopyridine with α-bromoacetophenone was reported by Tschitschibabine.[66]

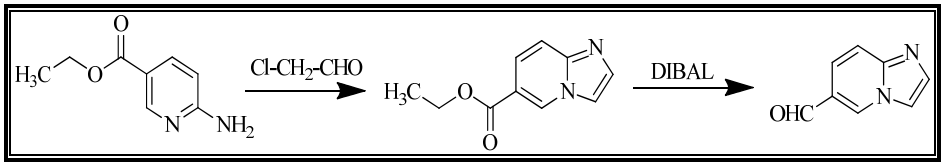
2-Acetylimidazo[1,2-*a*]pyridine[67] can be constructed by the cyclocodensation of 2-aminopyridine with bromo butanedione.



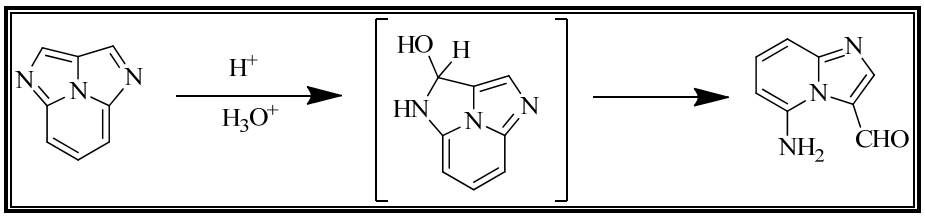
Reaction of 2-chloropyridine with 1,2,3-triazoles and subsequent elimination of nitrogens give the imidazo[1,2-*a*]pyridine.[68]



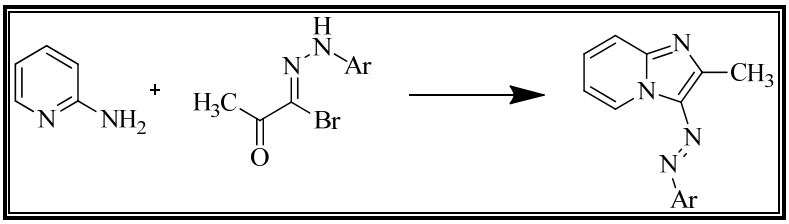
Condensation of ethyl-6-aminonicotinate with chloroacetaldehyde according to Hand's procedure gave imidazo[1,2-*a*]pyridine-6-carbaldehyde.[69]



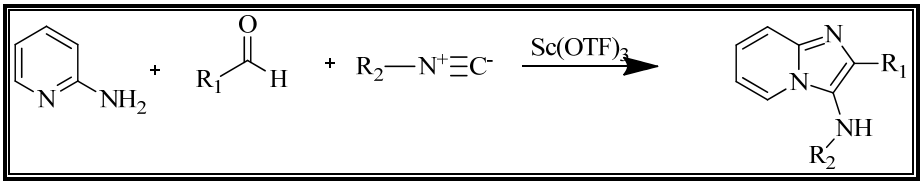
Paudler *et al*.[70] have synthesized 5-amino-3-formylimidazo[1,2-*a*]pyridine from acid catalyzed hydrolysis of 1,4-diazacycl[3,2,2]azine.



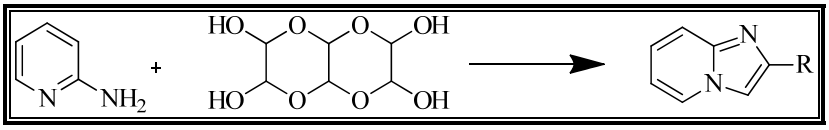
Imidazo[1,2-*a*]pyridine[71] nucleus can be also synthesized by the reaction of α-ketohydrazidoyl halide with heterocyclic amines.



Tsai *et al.*[72] have been prepared 3-amino imidazo[1,2-*a*]pyridine derivatives by a three component condensation reaction between 2-aminopyridine, aldehyde and isonitrile in the presence of scandiumtriflate as a catalyst.

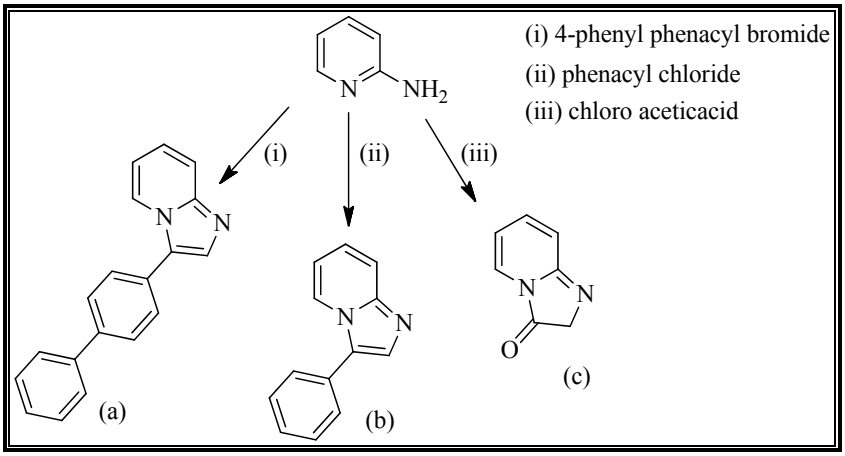


Groziak *et al.*[73] have synthesized substituted imidazo[1,2-*a*]pyridine derivatives by the condensation of 2-aminopyridine with glyoxal trimer dehydrate in aqueous $NaHSO_3$.

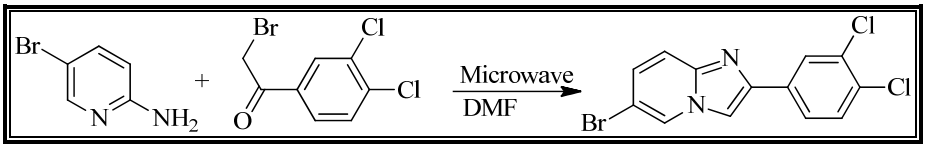


Synthesis of $Cu(OTf)_2$-catalyzed imidazo[1,2-*a*]pyridines from α-diazoketones and 2-aminopyridines by J. S. Yadav.[74]

Jumat Salimon et al.[75] synthesized imidazo[1,2-*a*]pyridine-3(2*H*)-one & 3-substituted-4-yl imidazo[1,2-*a*] pyridine from 2-aminopyridine.



Shankarappa A Biradar[76] have synthesized 6-bromo-2-(3,4-dichlorophenyl) imidazo[1,2-*a*]pyridine using microwave irradiation from 5-bromo-2-aminopyridine and 2-bromo-1-(3,4-dichlorophenyl)ethanone.

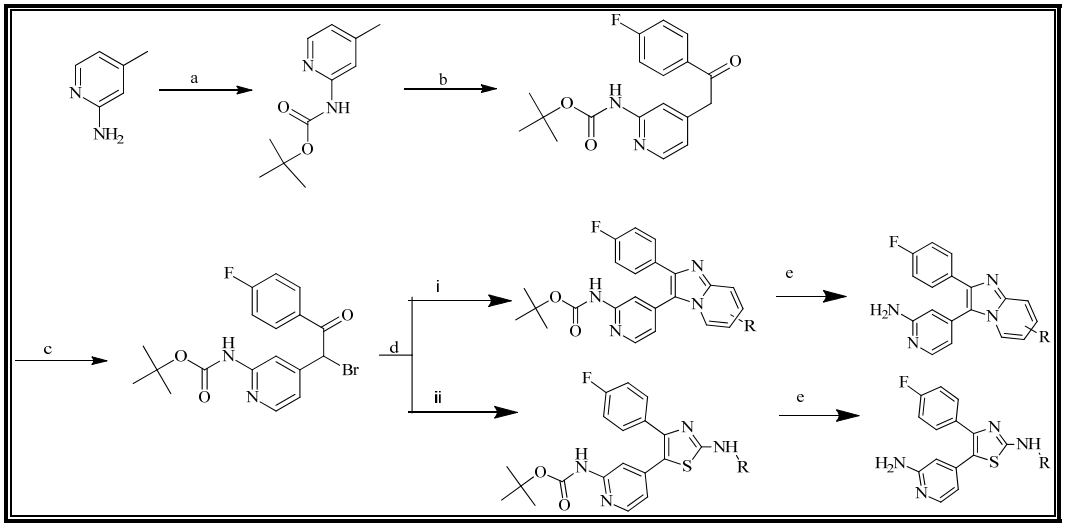


2.4 AIM OF CURRENT WORK

The literature survey revealed that some extensive work has been done on imidazo [1,2-*a*]pyridine compounds. A series of substituted imidazo[1,2-*a*]pyridine inhibitors of p38 MAP (Mitrogen-activated protein) kinase was prepared as small molecular library for the treatment of inflammatory diseases. From the literature of medicinal chemistry the most useful moiety found was substituted imidazo[1,2-*a*]pyridine derivatives, because of the less toxicological properties and good to moderate activities.

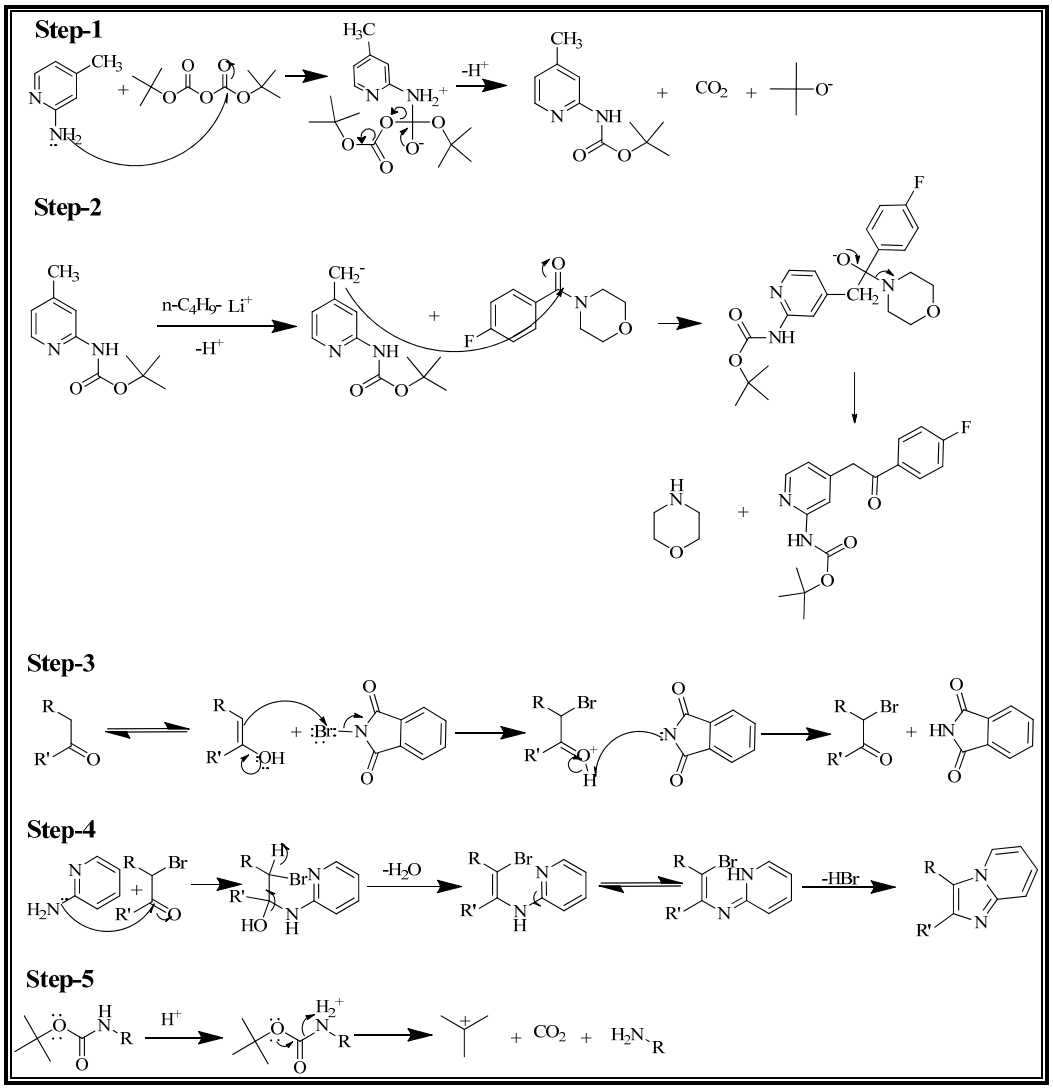
Based on these observations, a new series of 15 compounds have been synthesized by taking 4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl)pyridin-2-ylcarbamate coupled with various 2-aminopyridines and thiourea, to give 4-(2-(4-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-3-yl) pyridin-2-amine derivatives. The synthesized compounds were screened for anti-inflammatory activity and results are awaited.

2.5 REACTION SCHEME



a) $(Boc)_2O$, t-BuOH, 28 °C, 24 h
b) *N*-butyllithium, dry THF, (4-fluorophenyl)(morpholino)methanone, -76 °C
c) *N*-bromo succinamide, 27 °C, 1.5 h.
d) (i) 2-Amino pyridine derivatives
(ii) Thiourea derivatives, DMF, 75 °C
e) Acidic silica, MW

2.6 PLAUSIBLE REACTION MECHANISM



2.7 EXPERIMENTAL

❖ **Preparation of Tert-butyl 4-methylpyridin-2-ylcarbamate**

A mixture of 4-methylpyridin-2-amine (0.1 mole) was dissolved in t-butyl alcohol in to 100ml round bottom flask. Boc anhydride (0.15 mole) was added to the above flask. The reaction mixture was stirred at room temperature for 24 h. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (9:1) as a mobile phase. After the reaction was completed, the reaction was pored in to the ice and extracted with ethyl acetate. The organic layer was separated out and dried with sodium sulphate. The organic layer was evaporated under vaccum to give yellow oily compound. The compound was purified by column chromatography by silica gel 230-400 mash. Yield : 73%

❖ **Preparation of 1-(4-Fluorophenyl)-2-(2-(*N*-Boc amino)pyridin-4-yl) ethanone**

A mixture of tert-butyl 4-methylpyridin-2-ylcarbamate (0.1 mole) was dissolved in dry THF in a 250ml round bottom flask. *N*-butyl lithium (0.3 mole) was drop wise added to the above flask at -78 °C. Resulting reaction mixture was stirred at room temperature for 35 min. Now the (4-fluorophenyl)(morpholino)methanone (0.1 mole) was added drop wise into the reaction mixture at -78 °C. The reaction mixture was stirred for 2 h at room temperature. After the reaction was completed, the reaction was poured in to the ice and extracted with ethyl acetate. The organic layer was separated out and dried with sodium sulphate. The organic layer was evaporated under vaccum to give yellow oily compound. The compound was purified by column chromatography by silica gel 230-400 mash. TLC (EtoAC: Hexane, 2:8). Yield : 61%.

Note: The entire reaction was carried out under nitrogen atmosphere.

❖ **Preparation of Tert-butyl 4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl) pyridin-2-ylcarbamate**

1-(4-Fluorophenyl)-2-(2-(*N*-Boc amino)pyridin-4-yl)ethanone (0.1 mole) was dissolved in dioxane in a 100ml round bottom falsk. *N*-bromo succinamide (0.1 mole) was added to the above flask. The reaction mixture was stirred at room temperature for 1.5 h. The progress and the completion of the reaction were checked by TLC using

hexane: ethyl acetate (8:2) as a mobile phase. After the reaction to be completed, the reaction was poured in to the ice to give yellow solid compound. Yield : 85%.

- **Preparation of Tert-butyl 4-(2-(4-fluorophenyl)*H*-imidazo[1,2-a]pyridin-3-yl)pyridin-2-ylcarbamate derivatives**

Tert-butyl 4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl)pyridin-2-ylcarbamate (0.1 mole) was dissolved in DMF in a 100ml round bottom falsk. Substituted 2-amino pyridine (0.1 mole) / substituted thiourea (0.1 mole) was also added in to flask. The resulting mixture was heated at 75 °C for 1 h to give solid compound. The reaction mixture was cooled at room temperature, filtered and washed with ether. TLC (EtoAC: Hexane, 4:6), Yield : 82%.

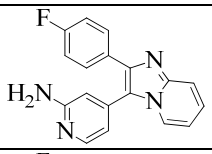
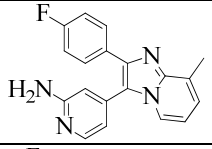
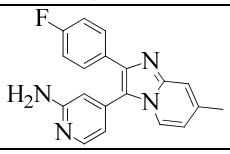
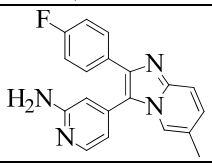
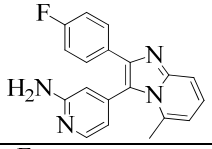
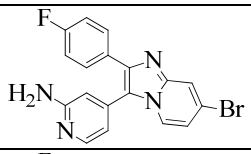
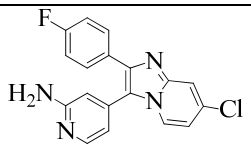
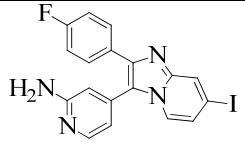
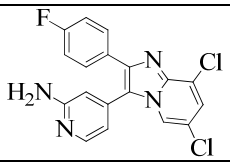
- **Preparation of 4-(2-(4-Fluorophenyl)*H*-imidazo[1,2-a]pyridin-3-yl) pyridin-2-amine derivatives**

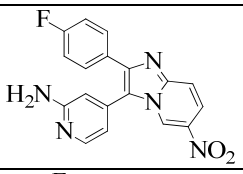
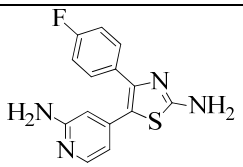
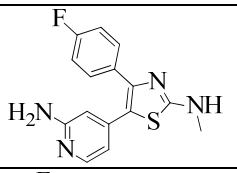
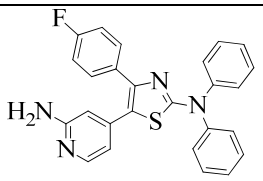
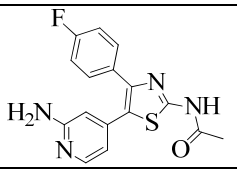
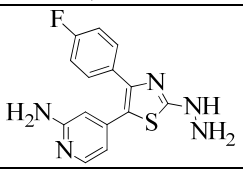
The typical procedure, *N*-BOC- 4-(3-(3-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-ylcarbamate derivatives (0.1 mole) were treated with acidic silica (0.2mole) under microwave irradiation at 250 W, 80°C in methanol. All the BOC-amine was deprotected in 2.5-5.0 min. The reaction was readily monitored by TLC. After completion of reaction, the silica was removed by simple filtration and the solvent was evaporated to dryness to give desired product. The pure 4-(3-(3-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-amine derivatives were isolated between 80-90% yield in one step. No additional purification steps were required. The final compounds were furthermore confirmed by ^{1}H NMR spectra, here *N*-Boc protected amide proton was observed at 8.0-9.0 δ ppm where *N*-Boc deprotected free amine proton was demonstrated at 5.5-6.5 δ ppm.

.

2.8 PHYSICAL DATA

TABLE: 2 PHYSICAL DATA OF 4-(3-(3-FLUOROPHENYL)*H*-IMIDAZO[1,2-*a*]PYRIDIN-2-YL)PYRIDIN-2-AMINE AND THIOUREA DERIVATIVES

Sr. No	Substituted	M.F.	M. P (°C)	R_f value	% Yield
DPB-27		$C_{18}H_{13}FN_4$	184-186	0.36	87
DPB-28		$C_{19}H_{15}FN_4$	156-158	0.39	85
DPB-29		$C_{19}H_{15}FN_4$	196-198	0.51	85
DPB-30		$C_{19}H_{15}FN_4$	210-212	0.42	87
DPB-31		$C_{19}H_{15}FN_4$	246-248	0.48	76
DPB-32		$C_{18}H_{12}BrFN_4$	188-190	0.37	88
DPB-33		$C_{18}H_{12}ClFN_4$	176-178	0.46	88
DPB-34		$C_{18}H_{12}BrFN_4$	212-214	0.33	85
DPB-35		$C_{18}H_{11}Cl_2FN_4$	276-278	0.53	87

DPB-36		$C_{18}H_{12}FN_5O_2$	222-224	0.38	72
DPB-37		$C_{14}H_{11}FN_4S$	189-191	0.41	87
DPB-38		$C_{15}H_{13}FN_4S$	256-258	0.50	85
DPB-39		$C_{26}H_{19}FN_4S$	156-158	0.48	84
DPB-40		$C_{16}H_{13}FN_4OS$	179-181	0.36	87
DPB-41		$C_{14}H_{12}FN_5S$	245-247	0.44	74

R_f value was calculated using solvent system, EtoAC**:** Hexane, 5**:**5.

2.9 SPECTRAL STUDY

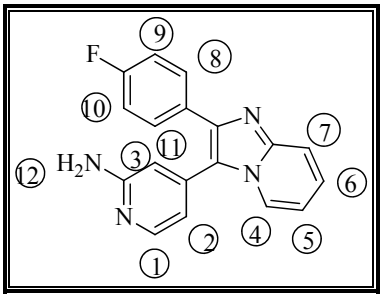
❖ IR spectra

Infra Red spectra were taken on **Shimadzu FT-IR-8400** spectrometer using KBr Pellet method. The characteristic amine in imidazol[1,2-*a*] pyridine moiety is observed at 3500-3300 cm^{-1}, while aromatic group are observed in the range 3100-3000 cm^{-1}. C-X (X=Cl,Br,I,F) observed between 1000-500 cm^{-1}. methyl ($-CH_3$) observed at 1350 cm^{-1}.The characteristic C=N in imidazol[1,2-*a*] pyridine oberseved at 1612-1590 cm^{-1}. DPB- 28 and DPB-40 of IR spectra are given on page no: - 73.

❖ ^{1}H NMR spectra

^{1}H NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d_6 & $CDCl_3$ as a solvent. In the NMR spectra of 4-(3-(3-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-amine derivatives proton values of amine ($-NH_2$), methyl ($-CH_3$) and aromatic protons (Ar-H) etc. were observed as under.

The values for $-NH_2$ protons were observed at 4.0-5.0 δ ppm. Aromatic protons shows the multiplet between δ 6.05-8.50 δ ppm. The signal due to $-CH_3$ proton was observed at 1.5-2.5 δ ppm value. Intermediate, DPB-27, DPB-28, DPB-33 and DPB-37 of ^{1}H NMR spectra are given on page no: - 65 to 72.



1. The aromatic pyridine ring proton no. 1 gave a doublet at 8.19 δ ppm. It shows in spectra due to the coupling with proton no.2 and *J* values is 8.0Hz from the *J* value, suggesting it is *ortho* coupled.
2. The aromatic ring proton no. 4 gave a doublet at 8.17 δ ppm it shows in spectrum due to the effect of nitrogen atom
3. The aromatic ring proton no. 3 gave a characteristic singlet at 7.28 δ ppm.

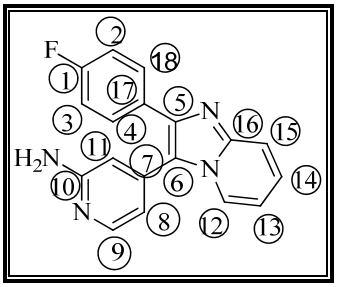
4. The aromatic ring of the phenyl ring proton no. 7, 8 and 11 are on same atmosphere so these three protons gave a multiplet at 7.63 δ ppm-7.97 δ ppm it shows in spectra.
5. The aromatic ring of the phenyl ring proton no. 9 and 10 are on same atmosphere so these three protons gave a multiplet at 7.02 δ ppm-7.06 δ ppm it shows in spectra.
6. The aromatic pyridine ring proton no. 2 gave a doublet at 6.63 δ ppm it shows in spectra due to the coupling with proton no.1 and *J* values is 8.0Hz from the *J* value, suggesting it is *ortho* coupled.
7. The aromatic ring of the phenyl ring proton no. 5 and 6 are on same atmosphere so these two protons gave a multiplet at 6.78 δ ppm-6.89 δ ppm it shows in spectra.
8. The proton no. 12 of the $-NH_2$ group gave a characteristic broad singlet at 6.12 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound DPB-27 was confirmed.

❖ **^{13}C NMR spectra**

^{13}C NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using DMSO-d_6 & $CDCl_3$ as a solvent. In the ^{13}C NMR spectra of substituted 4-(3-(3-Fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-amine various carbon values of methylene ($-CH_2$), keto (>C=O), methyl ($-CH_3$) and aromatic carbon (Ar-H) etc. were observed as under.

The values for methylene ($-CH_2$) carbon is observed between 40-60 δ ppm. The >C=O carbon observed at 160-185 δ ppm. Aromatic carban shows between 110-145 δ ppm. Intermediate, DPB-27, DPB-28, DPB-33 and DPB-37 of ^{13}C NMR spectra are given on page no: - 65 to 72.



1. The carbon no. 2, 8, 11, 13, 15 and 18 of aromatic, appear at 109.67 δ ppm-117.09 δ ppm.
2. The carbon no. 3, 4, 5, 7, 12, 14 and 17 of aromatic, appear at 123.19 δ ppm-129.76 δ ppm.
3. The carbon no. 6 of imidazole ring, appear at 142.50 δ ppm due to the effect of nitrogen atom.
4. The carbon no. 9 and 16 of imidazole or pyridine ring, appear at 144.88 δ ppm due to the effect of nitrogen atom.
5. The carbon no. 10 of pyridine ring, appear at 142.50 δ ppm due to the effect of amino group.
6. The carbon no. 1 of aromatic ring, appear at 158.30 δ ppm due to the effect of fluorine group.

❖ **Mass spectra**

The mass spectrum of compounds were recorded by **Shimadzu GC-MS-QP-2010** spectrometer (EI method). The mass spectrum of compounds was obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectral study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized. DPB- 27, DPB-29 and DPB-40 of Mass spectra are given on page no.-64 and 65.

❖ **Elemental analysis**

Elemental analysis of the compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model at Saurashtra University, Rajkot and the results are in agreement with the structures assigned.

2.10 SPECTRAL CHARACTERIZATION

4-(3-(3-Fluorophenyl)H-imidazo[1,2-a]pyridin-2-yl)pyridin-2-amine (DPB-27)

IR (KBr) cm^{-1}: 3355 (-NH, str), 1285 (C-N, str), 1580 (-NH,ban) 1028 (C-F), 3052 (Ar, C-H, str), 1523 (Ar, C=C, str). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 6.12 (s, 2H, $-NH_2$), 6.63-7.67 (m, 9H, Ar-H), 8.05 (d,1H, Ar-H), 8.19 (d,1H, Ar-H). **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 39.3, 39.9, 40.1, 40.4, 109.4, 112.6, 113.0, 114.7, 115.6, 117.0, 123.1, 123.3, 129.4, 129.6, 129.7, 142.5, 144.0, 145.0, 158.3 **Mass: [m/z (%)], M. Wt.:** 304. **Elemental analysis, Calculated:** C, 71.04; H, 4.31; N, 18.41 Found: C, 71.12; H, 4.29; N, 18.39.

4-(2-(3-Fluorophenyl)-8-methylH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-28)

IR (KBr) cm^{-1}: 3331 (-NH. str), 1290 (C-N, str), 1052 (C-F), 3021 (Ar, C-H, str), 1510 (Ar, C=C, str), 2845 ($-CH_3$, str), 1348 ($-CH_3$, ban**). ^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 1.72 (s, 3H, $-CH_3$), 4.61 (s, 2H, $-NH_2$), 6.7-7.6 (m, 8H, Ar-H), 8.01 (d, 1H, Ar-H, *J*=6.4Hz), 8.27 (d, 1H, Ar-H, J=3.2Hz**). Mass: [m/z (%)], M. Wt.:** 318. **Elemental analysis, Calculated:** C, 71.68; H, 4.75; N, 17.60 Found: C, 70.75; H, 4.69; N, 17.55.

4-(2-(3-Fluorophenyl)-7-methylH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-29)

IR (KBr) cm^{-1}: 3330 (-NH. str), 1296 (C-N, str), 1082 (C-F), 3070 (Ar, C-H, str), 1552 (Ar, C=C, str), 2860 ($-CH_3$, str), 1378 ($-CH_3$, ban). **Mass: [m/z (%)], M. Wt.:** 318. **Elemental analysis, Calculated:** C, 71.68; H, 4.75; N, 17.60 Found: C, 70.75; H, 4.70; N, 17.45.

4-(2-(3-Fluorophenyl)-6-methylH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-30)

IR (KBr) cm^{-1}: 3312 (-NH. str), 1254 (C-N, str), 1042 (C-F), 3052 (Ar, C-H, str), 1523 (Ar, C=C, str), 2872 ($-CH_3$, str), 1344 ($-CH_3$, ban). 1.65 (s, 3H, $-CH_3$), 4.53 (s, 2H, $-NH_2$), 6.65 (s, 1H, Ar-H) 6.79-7.62 (m, 9H, Ar-H). **Mass: [m/z (%)], M. Wt.:** 318. **Elemental analysis, Calculated:** C, 71.68; H, 4.75; N, 17.60 Found: C, 71.50; H, 4.66; N, 17.52.

4-(2-(3-Fluorophenyl)-5-methylH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-31)

IR (KBr) cm^{-1}: 3386 (-NH. str), 1296 (C-N, str), 1128 (C-F), 3042 (Ar, C-H, str), 1563 (Ar, C=C, str), 2828 (-CH_3, str), 1372 (-CH_3, ban). **Mass: [m/z (%)], M. Wt.:** 318. **Elemental analysis, Calculated:** C, 71.68; H, 4.75; N, 17.60 Found: C, 71.65; H, 4.66; N, 17.52.

4-(7-Bromo-2-(3-fluorophenyl)H-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-32)

IR (KBr) cm^{-1}: 3392 (-NH. str), 1268 (C-N, str), 1025 (C-F), 3056 (Ar, C-H, str), 1572 (Ar, C=C, str), 534 (C-Br**). Mass: [m/z (%)], M. Wt.:** 382. **Elemental analysis, Calculated:** C, 56.42; H, 3.16; N, 14.62 Found: C, 56.50; H, 3.25; N, 14.64.

4-(7-Chloro-2-(3-fluorophenyl)H-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-33)

IR (KBr) cm^{-1}: 3362 (-NH. str), 1242 (C-N, str), 1058 (C-F), 3072 (Ar, C-H, str), 1549 (Ar, C=C, str), 725 (C-Cl). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 4.51 (s, 2H, -NH_2), 6.58 (s, 1H, Ar-H), 6.63-7.71 (m, 9H, Ar-H), **Mass: [m/z (%)], M. Wt.:** 338. **Elemental analysis, Calculated:** C, 63.82; H, 3.57; N, 16.54 Found: C, 63.55; H, 3.51; N, 16.48.

4-(2-(3-Fluorophenyl)-7-iodoH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-34)

IR (KBr) cm^{-1}: 3382 (-NH. str), 1241 (C-N, str), 1032 (C-F), 3079 (Ar, **C**-H, str), 1585 (Ar, C=C, str), 492 (C-I). **Mass: [m/z (%)], M. Wt.:** 430. **Elemental analysis, Calculated:** C, 50.25; H, 2.81;N, 13.02 Found: C, 50.05; H, 2.62;N, 13.35.

4-(6,8-Dichloro-2-(3-fluorophenyl)H-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-35)

IR (KBr) cm^{-1}: 3346 (-NH. str), 1275 (C-N, str), 1015 (C-F), 3053 (Ar, C-H, str), 1575 (Ar, C=C, str), 742 (C-Cl). **Mass: [m/z (%)], M. Wt.:** 372. **Elemental analysis, Calculated:** C, 57.93; H, 2.97;N, 15.01 Found: C, 58.90; H, 2.87; N, 15.11.

4-(2-(3-Fluorophenyl)-6-nitroH-imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine (DPB-36)

IR (KBr) cm^{-1}: 3382 (-NH. str), 1262 (C-N, str), 1046 (C-F), 3041 (Ar, C-H, str), 1568 (Ar, C=C, str), 1545 (-NO_2). **Mass: [m/z (%)], M. Wt.:** 349. **Elemental analysis, Calculated:** C, 61.89; H, 3.46; N, 20.05 Found: C, 61.81; H, 3.52; N, 20.11.

4-(2-Amino-4-(3-fluorophenyl)thiazol-5-yl)pyridin-2-amine (DPB-37)

IR (KBr) cm^{-1}: 3356 (-NH. str), 1243 (C-N, str), 1043 (C-F), 3092 (Ar, C-H, str), 1575 (Ar, C=C, str). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 5.9 (s, 2H, -NH_2), 7.8 (s, 2H, -NH_2), 6.18 (d, 1H, Ar-H, *J*=4.8Hz), 6.2 (s, 1H, Ar-H), 7.1 (t, 2H, Ar-H), 7.4 (t, 2H, Ar-H), 7.3 (d, 1H, Ar-H, *J*=5.2Hz). **Mass: [m/z (%)], M. Wt.:** 286. **Elemental analysis, Calculated:** C, 58.73; H, 3.87; N, 19.57; S, 11.20 Found: C, 58.63; H, 3.81; N, 19.60; S, 11.10.

4-(4-(3-Fluorophenyl)-2-(methylamino)thiazol-5-yl)pyridin-2-amine (DPB-38)

IR (KBr) cm^{-1}: 3330 (-NH. str), 1295 (C-N, str), 1082 (C-F), 3070 (Ar, C-H, str), 1552 (Ar, C=C, str), 2868 (-CH_3, str), 1356 (-CH_3, ban). **Mass: [m/z (%)], M. Wt.:** 300. **Elemental analysis, Calculated:** C, 59.98; H, 4.36; N, 18.65; S, 10.68 Found: C, 59.88; H, 4.26; N, 18.73; S, 10.75.

4-(2-(Diphenylamino)-4-(3-fluorophenyl)thiazol-5-yl)pyridin-2-amine (DPB-39)

IR (KBr) cm^{-1}: 3346 (-NH. str), 1272 (C-N, str), 1016 (C-F), 3076 (Ar, C-H, str), 1582 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 438. **Elemental analysis, Calculated:** C, 71.21; H, 4.37; N, 12.78; S, 7.31Found: C, 71.18; H, 4.29; N, 12.89; S, 7.45.

N-(5-(2-aminopyridin-4-yl)-4-(3-fluorophenyl)thiazol-2-yl)acetamide (DPB-40)

IR (KBr) cm^{-1}: 3302 (-NH), 1228 (C-N, str), 1687 (-CONH), 2866 (-CH_3, str), 1361 (-CH_3, ban). **Mass: [m/z (%)], M. Wt.:** 328. **Elemental analysis, Calculated:** C, 58.52; H, 3.99; N, 17.06; O, 4.87; S, 9.77 Found: C, 58.48; H, 4.21; N, 17.12; O, 4.90; S, 9.86.

4-(2-Hydrazine-4-(3-fluorophenyl)thiazol-5-yl)pyridin-2-amine (DPB-41)

IR (KBr) cm^{-1}: 3375 (-NH. str), 1265 (C-N, str), 1056 (C-F), 3059 (Ar, C-H, str), 1547 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 301. **Elemental analysis, Calculated:** C, 55.80; H, 4.01; N, 23.24; S, 10.64 Found: C, 55.75; H, 4.11; N, 23.32; S, 10.54.

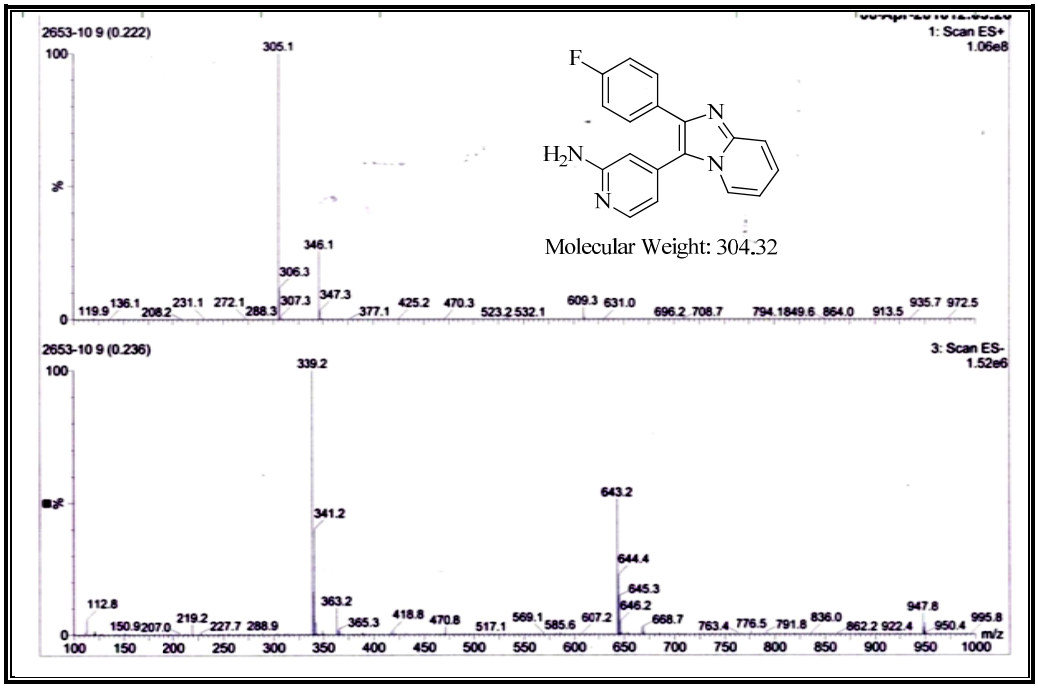
2.11 CONCLUSION

Total 15 derivatives of *N*-4-(3-(3-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-amine have been synthesized using 2-amino-4-methyl-pyridine. All the compounds were characterized by IR, ^{1}H NMR, ^{13}C, Mass spectral data and elemental analysis. The main significance of the present process is the easy work up method, excellent yield and high chemical purity of the desired compounds for biological interest.

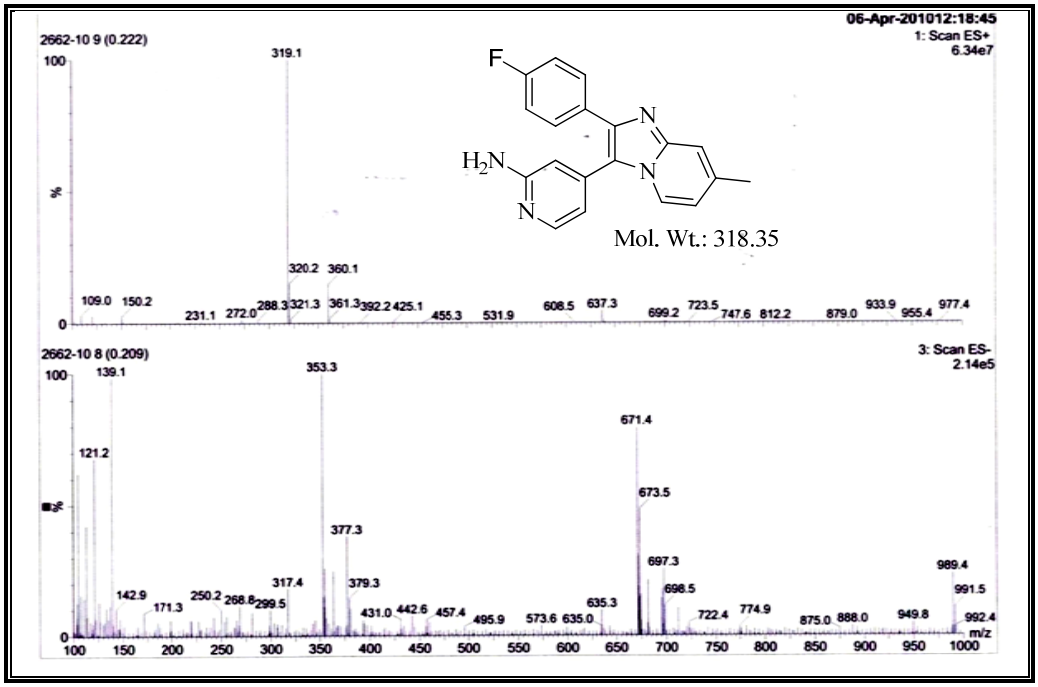
\

2.12 REPRESENTATIVE SPECTRUM

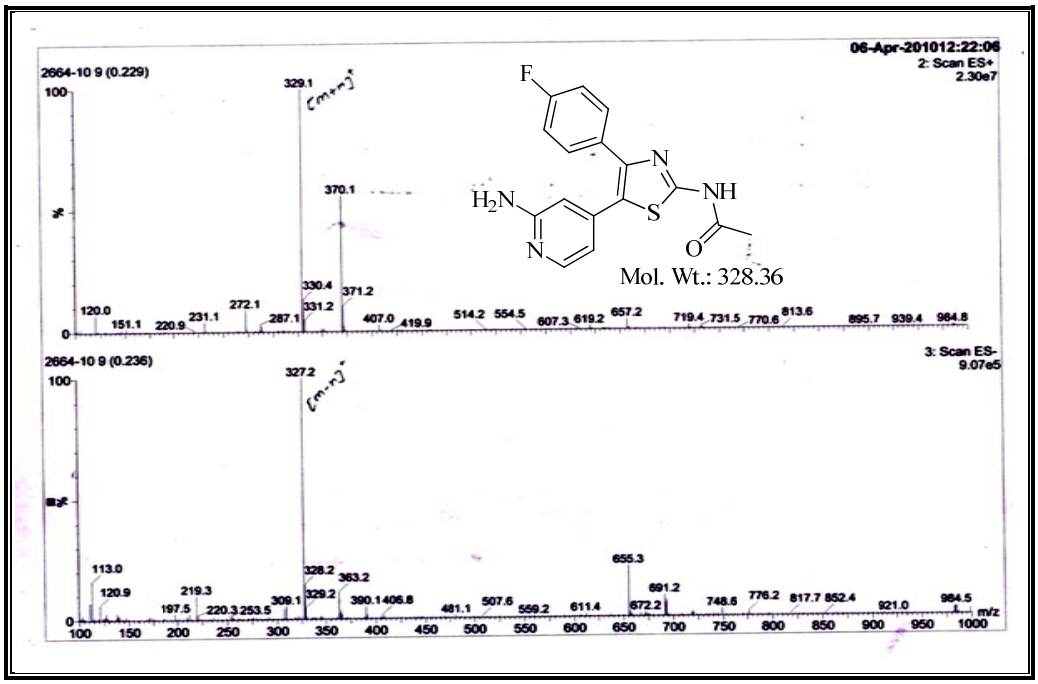
Mass spectrum of 4-(3-(3-Fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl)pyridin-2-amine (DPB-27)



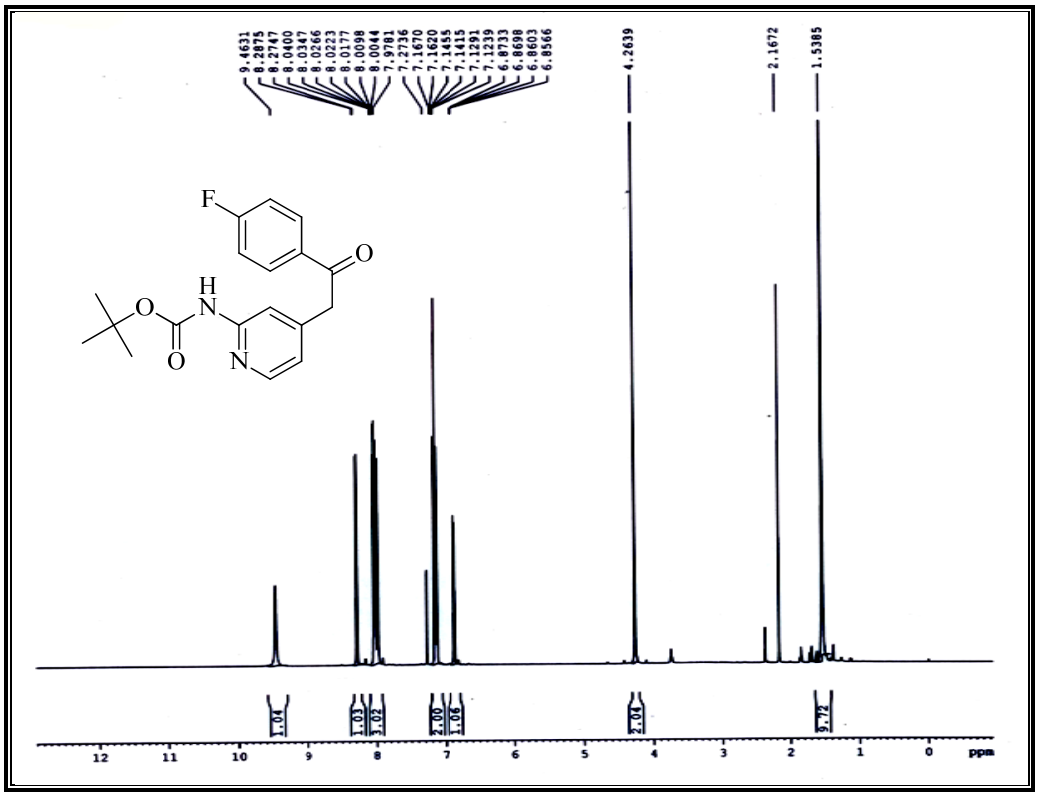
Mass spectrum of 4-(2-(3-Fluorophenyl)-7-methyl*H*-imidazo[1,2-*a*]pyridin-3-yl) pyridin-2-amine (DPB-29)

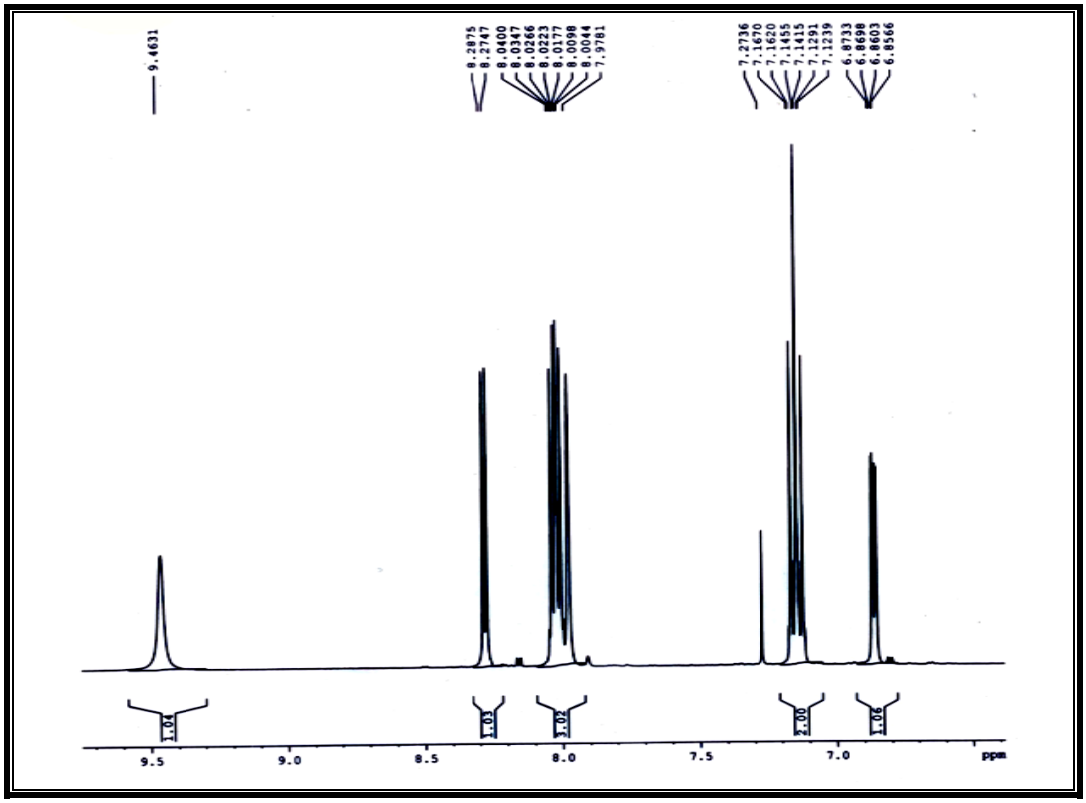


Mass spectrum of *N*-(5-(2-aminopyridin-4-yl)-4-(3-fluorophenyl)thiazol-2-yl) acetamide (DPB-40)

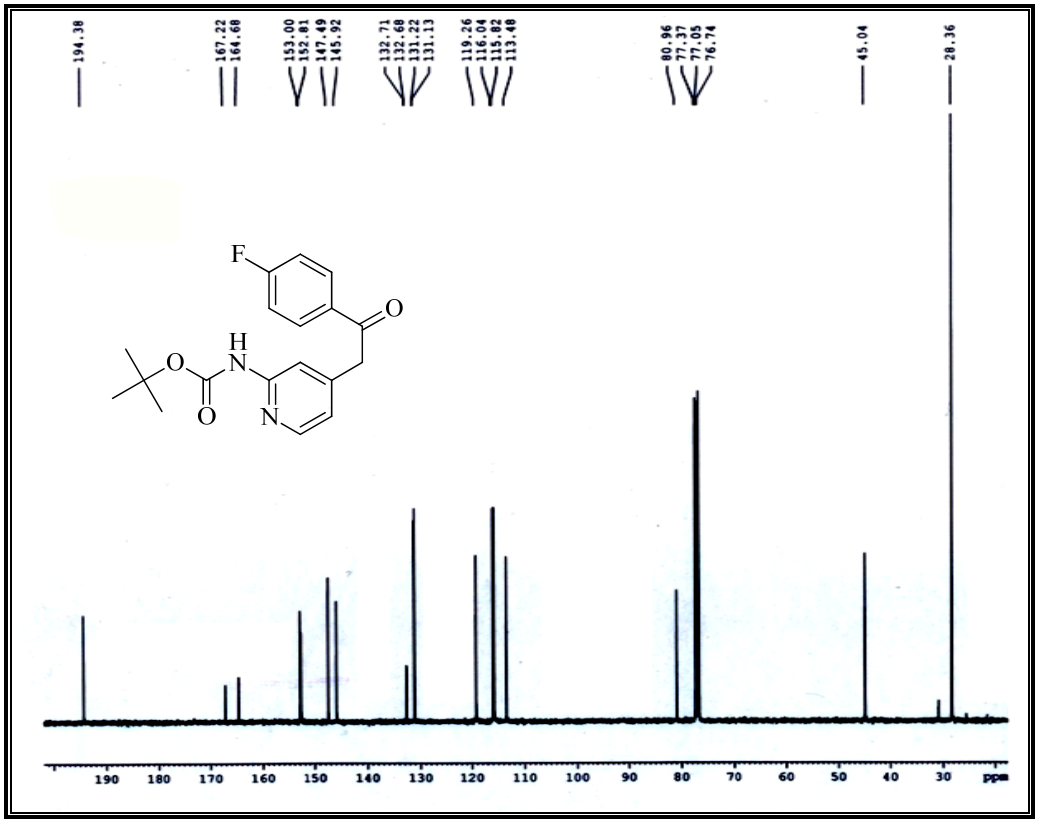


^{1}H NMR spectrum of 1-(4-Fluorophenyl)-2-(2-(*N*-Boc amino)pyridin-4-yl) ethanone (Intermediate)

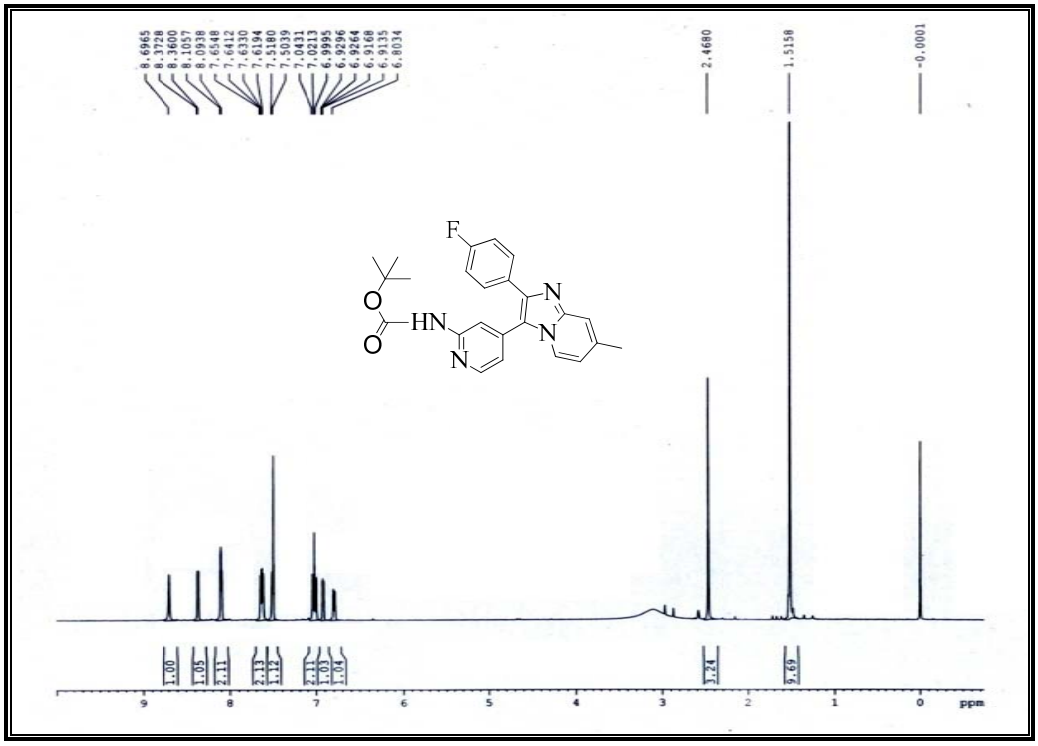


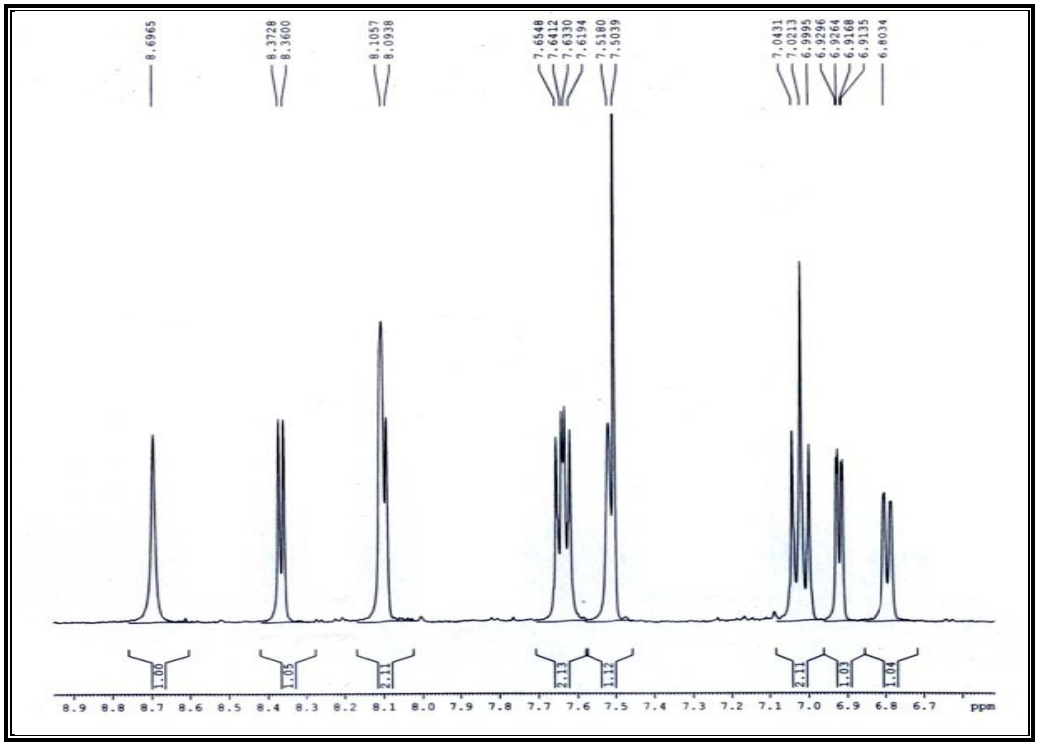


^{13}C NMR spectrum of 1-(4-Fluorophenyl)-2-(2-(*N*-Boc amino)pyridin-4-yl) ethanone

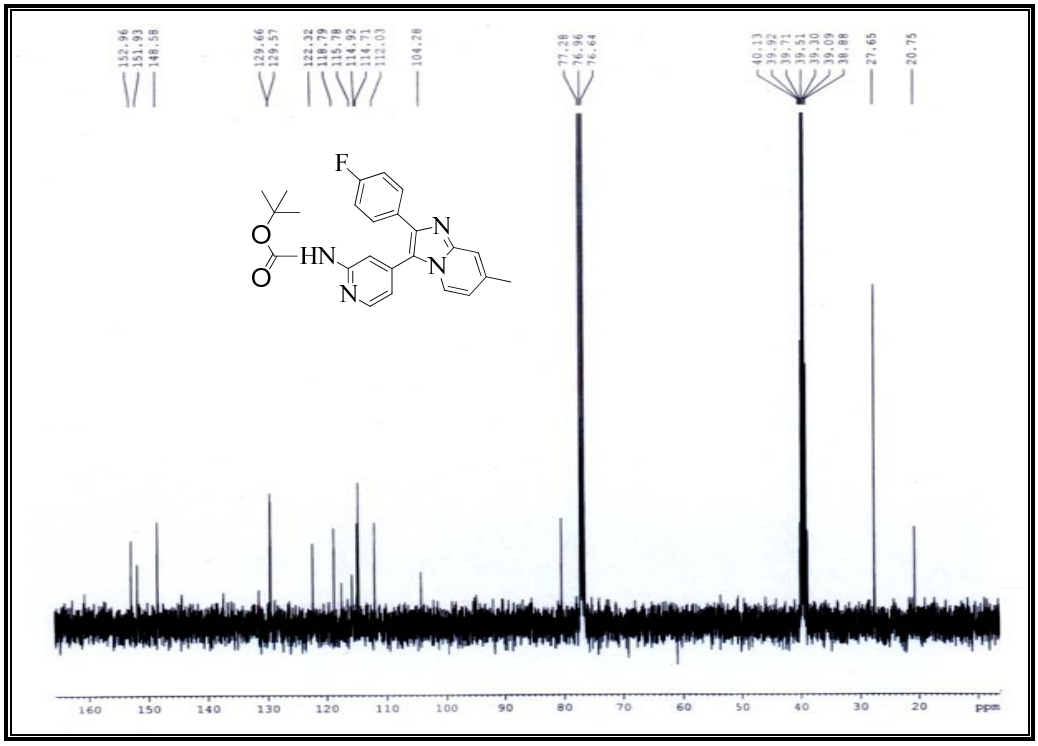


^{1}H NMR spectrum of Tert-butyl (4-(2-(4-fluorophenyl)-7-methylimidazo[1,2-*a*] pyridin-3-yl)pyridin-2-yl)carbamate (Intermediate)

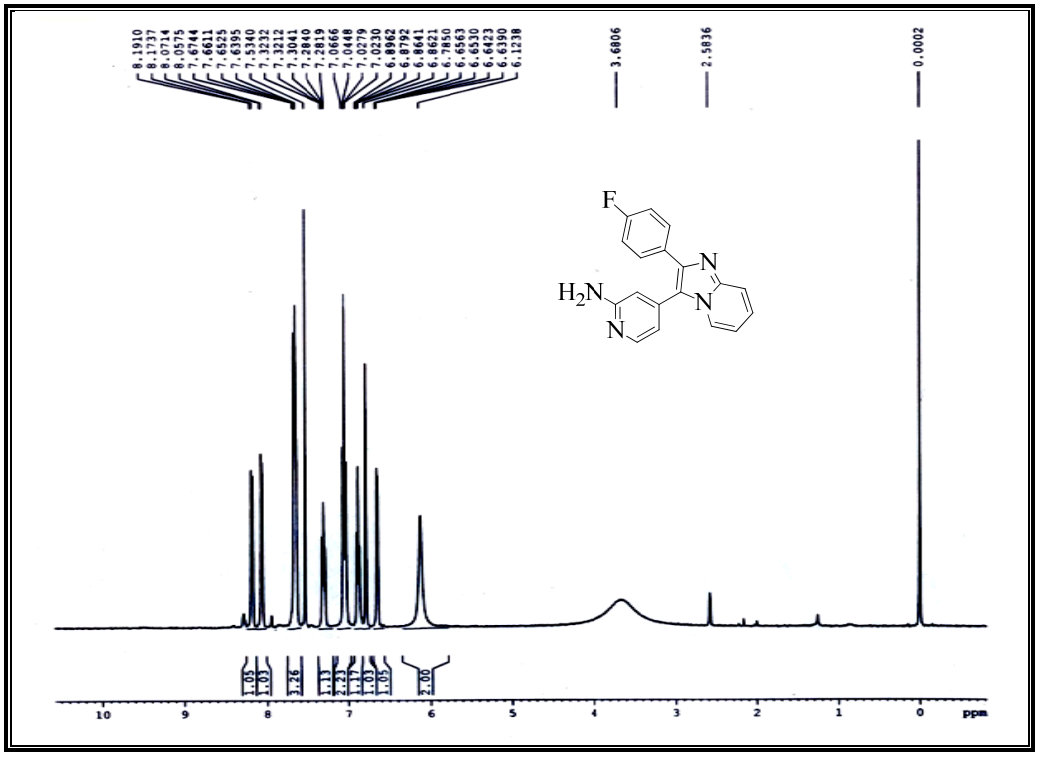


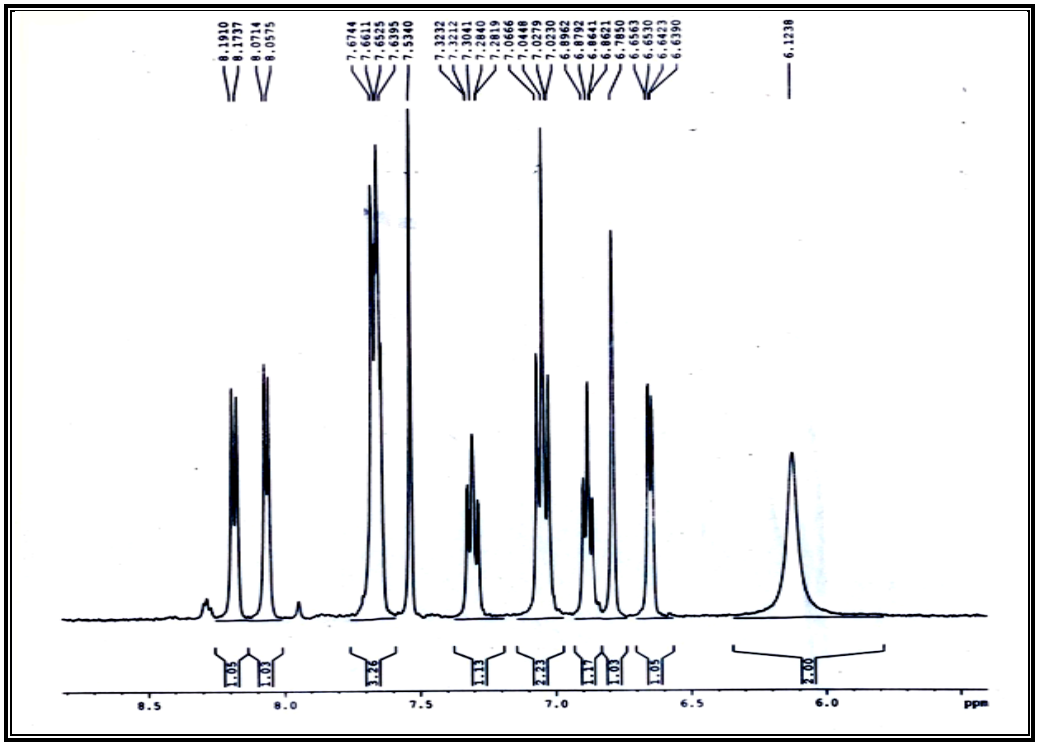


^{13}C NMR spectrum of Tert-butyl (4-(2-(4-fluorophenyl)-7-methylimidazo[1,2-*a*] pyridin-3-yl)pyridin-2-yl)carbamate (Intermediate)

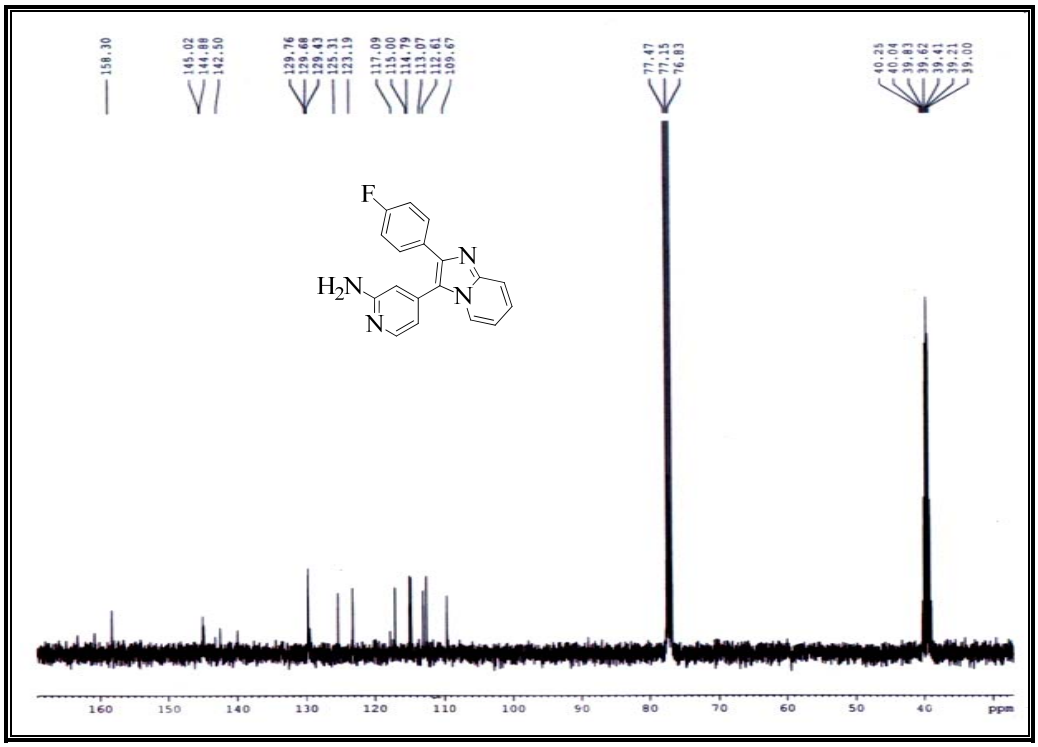


^{1}H NMR spectrum of 4-(3-(3-Fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl) pyridin-2-amine (DPB-27)

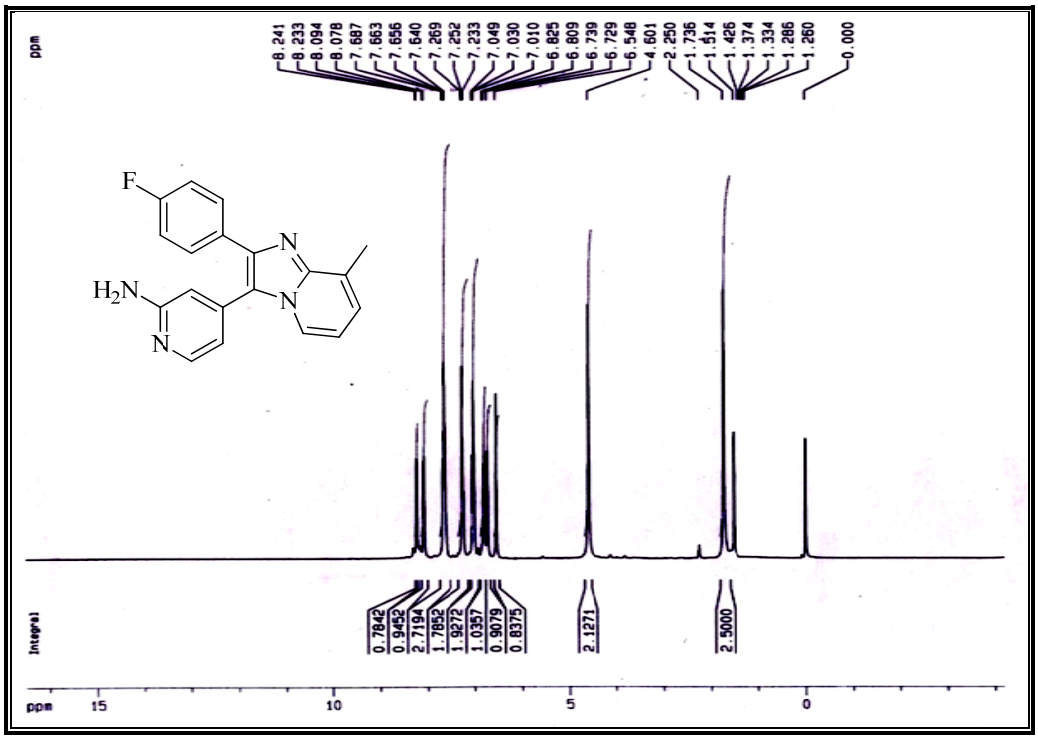


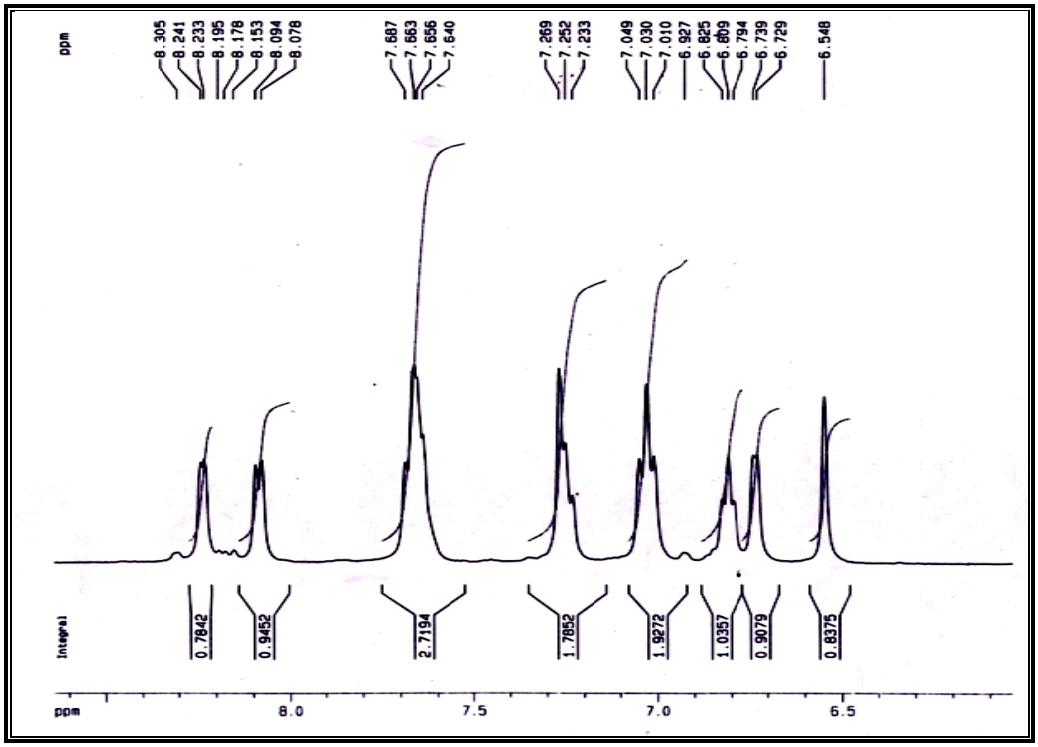


^{13}C NMR spectrum of 4-(3-(3-Fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-2-yl) pyridin-2-amine (DPB-27)

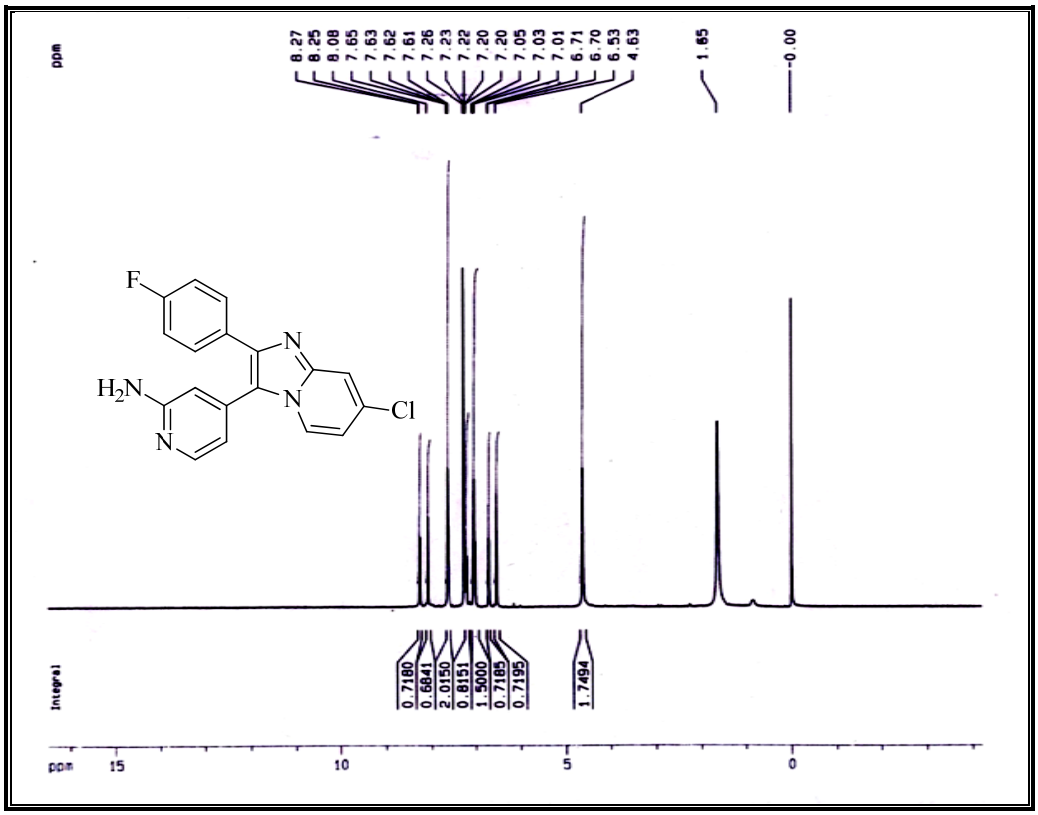


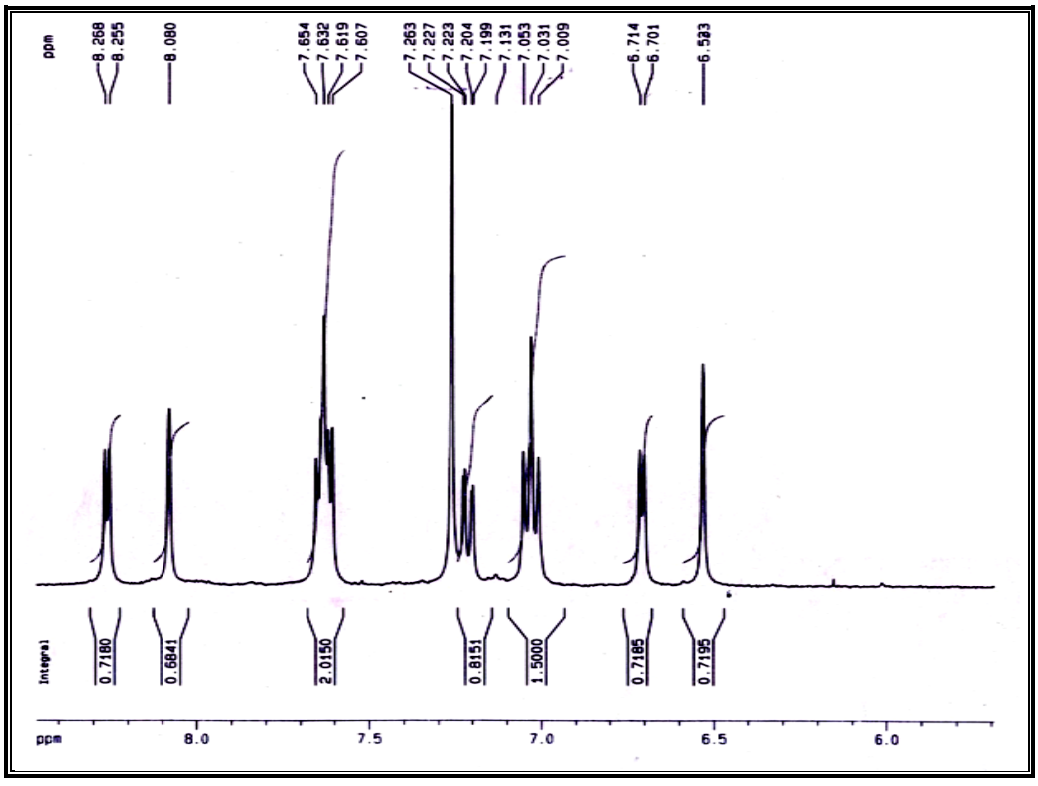
^{1}H NMR spectrum of 4-(2-(4-Fluorophenyl)-8-methyl*H*-imidazo[1,2-*a*]pyridin-3-yl) pyridin-2-amine (DPB-28)



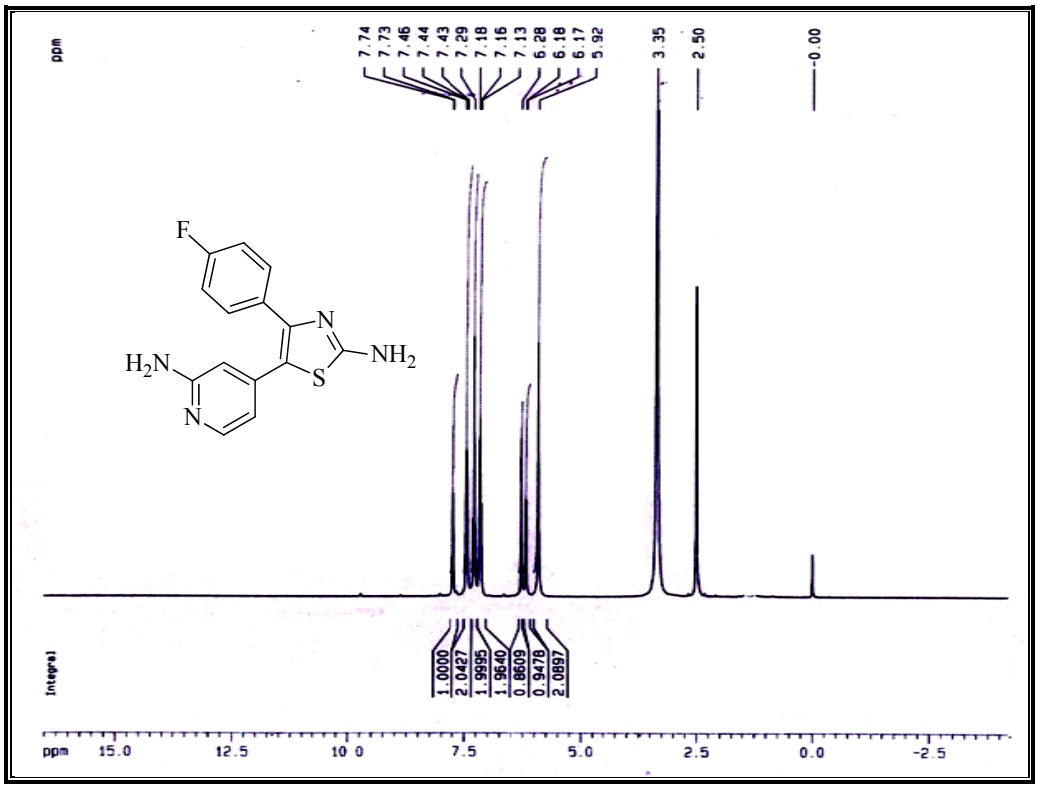


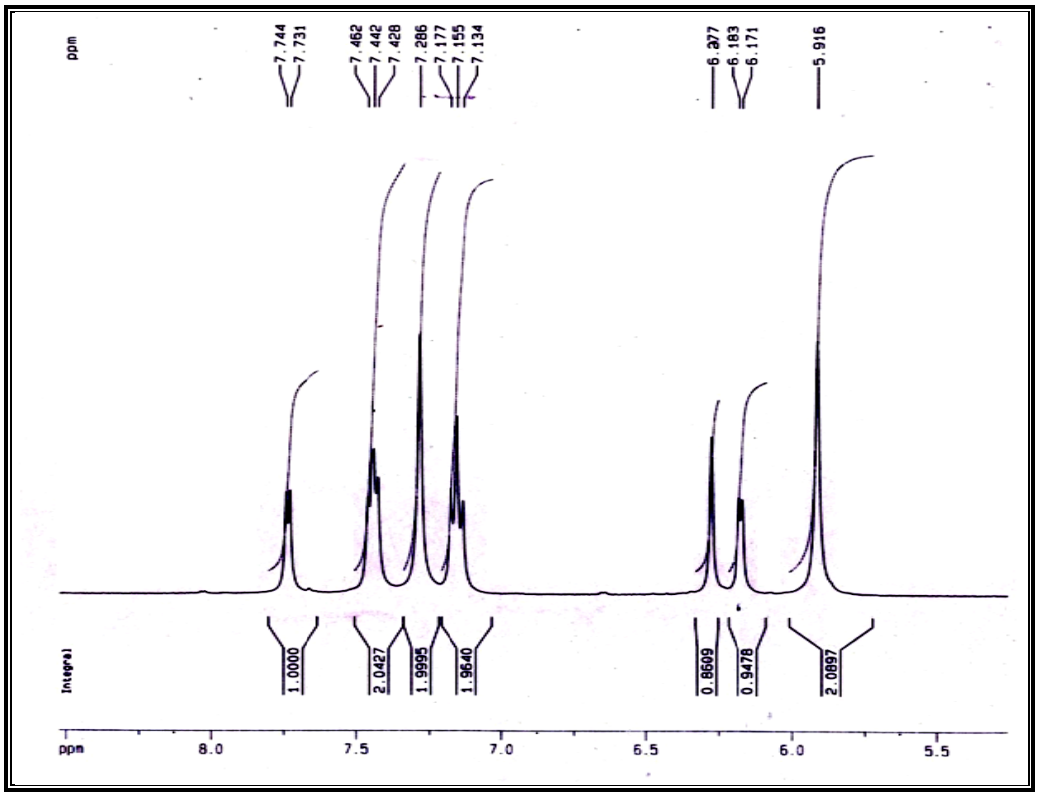
^{1}H NMR spectrum of 4-(7-Chloro-2-(4-fluorophenyl)*H*-imidazo[1,2-*a*]pyridin-3-yl)pyridin-2-amine (DPB-33)



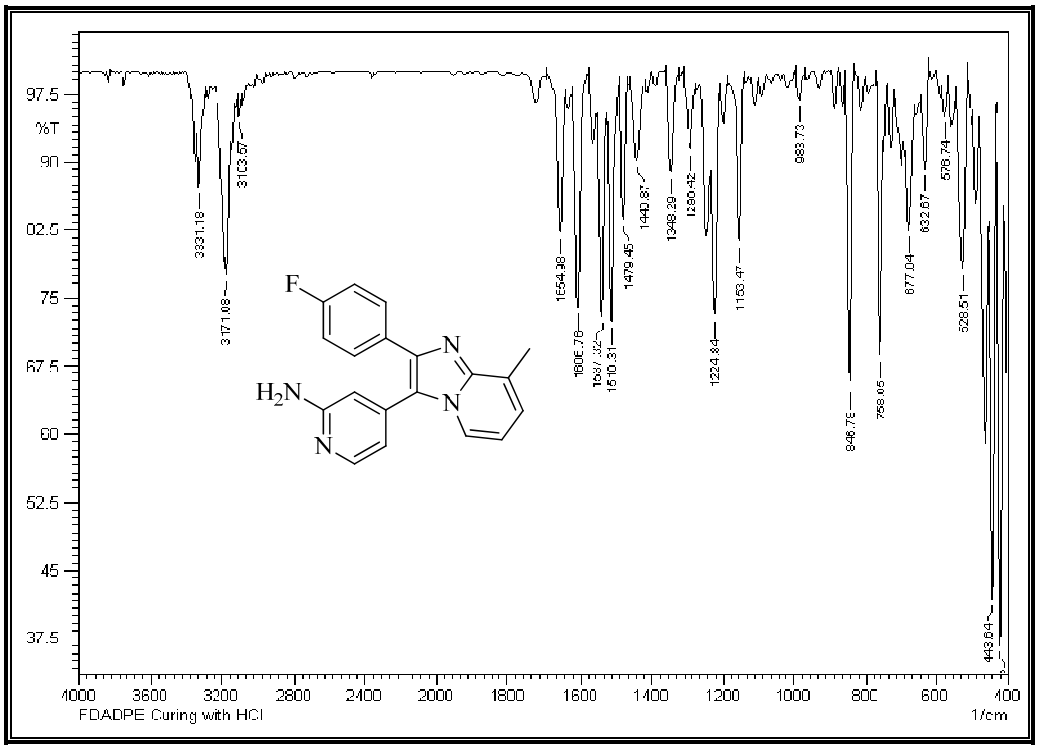


^{1}H NMR spectrum of 4-(2-Amino-4-(4-fluorophenyl)thiazol-5-yl)pyridin-2-amine (DPB-37)

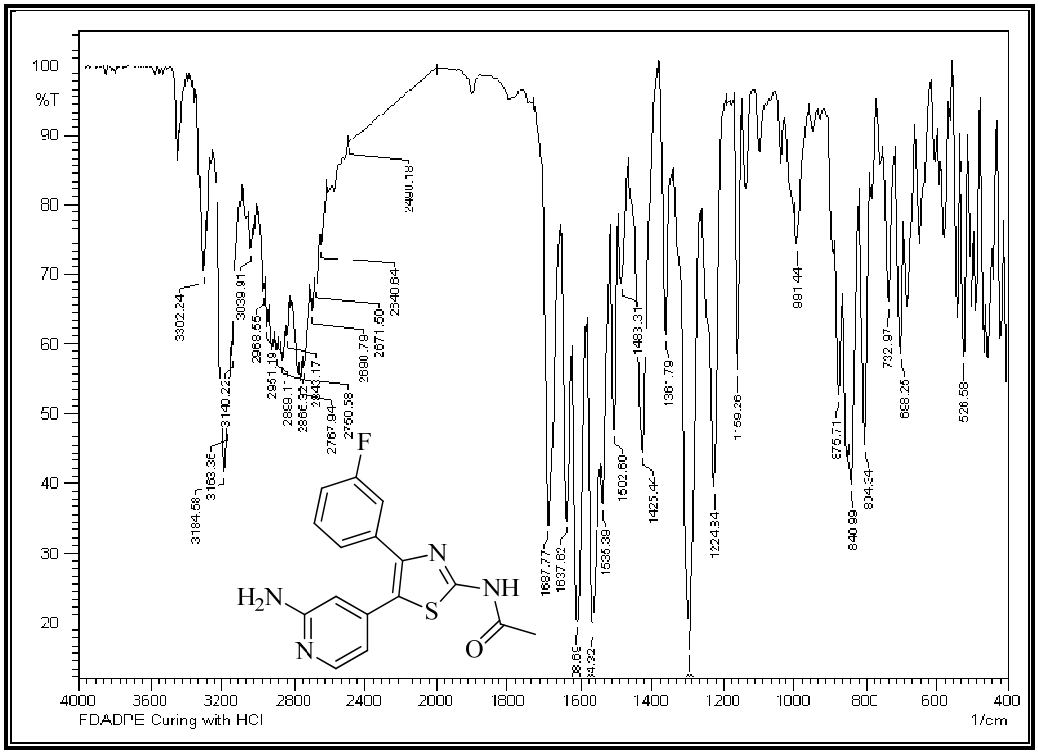




IR spectrum of 4-(2-(4-Fluorophenyl)-8-methyl*H*-imidazo[1,2-*a*]pyridin-3-yl) pyridin-2-amine (DPB-28)



IR spectrum of *N*-(5-(2-aminopyridin-4-yl)-4-(3-fluorophenyl)thiazol-2-yl) acetamide (DPB-40)



2.13 REFERENCES

1. J. Joule, K. Mills; *Heterocyclic Chemistry* 4th Ed.492.
2. J. Silvestre, P. Leeson, J. Castaner; *Drugs Fut*., **1998,** *23*, 598-601.
3. W. Mosby, *Chem. Heterocycl. Compd.*, **1961**, *15(1)*, 460.
4. F. Karci, A. Demircali; *Dyes and Pigments*, **2006**, *71(2)*, 97-102.
5. E. Abignente, *Actual. Chim. Ther.*, **1991**, *18*, 193; *Chem. Abstr.,* **1991**, *115*, 256028n.
6. E. Abignente, F. Arena, E. Luraschi, C. Saturnino, F. Rossi; *Rend. Atti. Accad. Sci. Med. Chir.*, **139**, 313 (1985); *Chem. Abstr.,* **1986,** *105*, 126822z.
7. C. Jean-Michel, J. Paeshuyse, G. Vincent, C. Damien, M. Aurelie, L. Claire, G. Alain, M. Emmanuel, *European Journal of Medicinal Chemistry*, **2010**, *45(5)*, 2044-2047.
8. M. Lhassani, O. Chavignon, A. Gueiffier; *Eur. J. Med. Chem.*, **1999,** *34*, 271-274.
9. P. George, G. Rossey, B. Zivkovic; *Eds*; *Raven Press Ltd.*; New York, **1993**, 49-59.
10. Y. Katsura, S. Nishino; *Chem. Pharma. Bull*., **1992,** *40*, 371.
11. E. Starrett, A. Montzka, L. Cavanagh; *J. Med. Chem.*, **1989,** *32*, 2204 .
12. S. Sunil, G. Kalpesh, *Pharma Chemica,* **2010**, *2(4)*, 378-388.
13. H. Fischer, A. Lusi; *J. Med. Chem.*, **1972,** *15*, 982.
14. H. Taleb, R. Al-Qawasmeh, *European journal of medicinal chemistry,* **2010**, *45(12)*, 5848-55.
15. C. Teulade, G. Grassy, P. Girard, P. Chapat; *Eur. J. Med. Chem.*, **1978,** *13*, 271.
16. P. Kaplan, P. George; *Eur. Patent* **1982**, 0050563; *Chem. Abstr.,* **2002,** *97*, 149531a.
17. A. Chaouni, C. Galtier, H. Allouchi, C. Teulade *et al*.; *Chem. Pharma. Bull.*, **2001,** *49*, 1631.
18. S. Mavel, L. Renou, C. Galtier, R. Snoeck, G. Andrei; *Arzneimittel-Forschung*, **2001,** *51*, 304.
19. J. Kaminnski, A. Bristol, T. Mcphail; *J. Med. Chem.*, **1985,** *28*, 876-892.
20. J. Kaminnski, M. Doweyko; *J. Med. Chem.*, **1997,** *40*, 427-436.
21. G. Bartholini; *L. Monogr. Ser*., **1993**, *8*, 1; *Chem. Abstr.,* **1996,** *124*, 164079n.

22. K. Fuchs, M. Romig, K. Mendla, H. Briem, K. Fechteler; *WO*, **2002,** 14 313,; *Chem. Abstr.,* **2002,** *136*, 183824r.

23. R. Tully, R. Grder, J. Gillespie , R. Westwood; *J. Med. Chem.*, **1991,** *34*, 2060.

24. Z. Langer, S. Arbilla, J. Benavides, B. Scatton; *Adv. Biochem Psychopharmacol*, **1990,** *46*, 61.

25. Y. Abe, H. Kayakiri, S. Satoh, T. Inoue, H. Tanaka; *J. Med. Chem.*, **1998,** *41*, 564.

26. D. Dvey, W. Erhardt, W. Lumma, E. Cantor; *J. Med. Chem.*, **1987,** *30(8)*, 1337-1342.

27. A. Elhakmaoui, A. Gueiffier, C. Milhavet, E. Declercq; *Bioorg. Med. Chem. Lett*., **1994,** *4*, 1937.

28. B. Townsend, C. Drach; *WO*, **27**, 205 (1997); *Chem. Abstr.,* **1997,** *127*, 190983j.

29. S. Mavel, L. Renou, C. Galtier, R. Snoeck, A. Gueiffier; *Arzneim-Forsch*., **2001,** *51*, 304; *Chem. Abstr.,* **2001,** *135*, 131730s.

30. B. Dubinsky, A. Shriver, E. Rosenthale; *Drug. Dev. Res*., **1990,** *21*, 277.

31. J. Sanfilippo, M. Urbanski, B. Moore; *J. Med. Chem.* , **1998,** *31*, 2221.

32. A. Humphries, E. Ganeia, M. Gilligan; *Bioorganic and Medicinal Chemistry Letters,* **2006,** *16(6)*, 1518-1522.

33. K. Gudmundsson, J. Williams, L. Townsend; *J. Med. Chem.*, **2003,** *46*, 1449-1455.

34. I. Aramori, J. Zenkoh, N. Morikawa, Y. Notsu; *Mol. Pharmacol*., **1997,** *51*, 171-176.

35. W. Martindale; *The Extra Pharmacopocia 29th Ed., J. E. F. Reynolds, Ed. The Pharmaceutical Press, London*, **1989,** *712*, 1630.

36. A. Wafford, J. Whitting, A. Kemp; *Mol. Pharmocol.*, **1993,** *43*, 240; *Chem. Abstr.,* **1996,** *118*, 77084.

37. C. Faure-Hally, D. Graham, S. Arbilla, Z. Langer; *Eur. J. Pharmocol. ol. Pharmacol. Sect.*, **1993,** *246*, 283.

38. J. Kaminski, G. Perkins, D. Frantz, J. Long; *J. Med. Chem.*, **1987,** *30*, 2047-2051.

39. K. Gudmundsson, B. Johns; *Org. Lett.*, **2003,** *5(8)*, 1369-1372.

40. C. Bendallah, C. Galtier, C. Teulade *et al*.; *Chem. Pharm. Bull.*, **2001,** *49*, 1631.

41. S. Ceard, M. Vivier, D. Roche, M. Madesclaire; *Sulfur Latters.*, **2002,** *25(1)*, 15-20.

42. C. Hamdouchi, J. Blass, M. Prade, L. Vance; *J. Med. Chem.*, **1999,** *42*, 50-59.

43. S. Hamama, H. Zoorob; *Tetrahedron,* **2002,** *58*, 6143-6162.

44. J. Sundberg, S. Biswas, K. Murthi, D. Rowe; *J. Med. Chem.*, **1998,** *41*, 4317-4328.

45. M. Ismail, R. Burn, D. Boykin; *J. Med. Chem.*, **2004,** *47*, 3658-3664.

46. R. Tidwell, W. Boykin, D. Wilson; *Eds.; Wiley-VCH: New York*, **2003,** *2*, 414-460.

47. A. Laufer, K. Wagner, A. Kotschenreuther, W. Albrecht, *J. Med. Chem.* **2003**, *46*, 3230-3244.

48. A. Laufer, W. Zimmermann, J. Ruff, *J. Med. Chem.* **2004**, *47*, 6311-6325.

49. C. Lee, S. Kumar, E. Griswold, C. Underwood, J. Votta, L. Adams, *Immunopharmacology,* **2000**, *47*, 185-201.

50. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heyes, J. R. *Nature (London)* **1994**, *372*, 739-746.

51. P. Young, P. McDonnell, D. Dunnington, A. Hand, J. Laydon, J. Lee, *Agents Actions,* **1993**, *39*, 67-9.

52. G. Pearson, F. Robinson, B. Gibson, E. Xu, M. Karandikar, K. Berman, H. Cobb, *Endocr. Rev.* **2001**, *22*, 153-183.

53. R. Beyaert, A. Cuenda, W. Vanden, S. Plaisance, C. Lee, G. Haegeman, P. Cohen, W. Fiers, *EMBO J.* **1996**, *15*, 1914-1923.

54. J. Raingeaud, S. Gupta, S. Rogers, M. Dickens, J. Han, J. Ulevitch, R. Davis, *J. Biol. Chem.* **1995**, *270*, 7420-7426.

55. C. Lee, S. Kassis, S. Kumar, A. Badger, L. Adams, *Pharmacol. Ther.* **1999**, *82*, 389-397.

56. R. Henry, E. Cavender, S. Wadsworth, *Drugs Future,* **1999**, *24*, 1345-1354.

57. G. Salituro, A. Germann, P. Wilson, W. Bemis, T. Fox, M. Su, *Curr. Med. Chem.* **1999**, *6*, 807-823.

58. C. Boehm, L. Adams, New inhibitors of p38 kinase. *Expert Opin. Ther. Patents* **2000**, *10*, 25-37.

59. M. Mclay, F. Halley, E. Souness, J. McKenna, V. Benning, M. Birrell, B. Burton, M. Belvisi, A. Collis, A. Constan, M. Foster, D. Hele, Z. Jayyosi, M. Kelley, *Bioorg. Med. Chem.* **2001**, *9*, 537-554.

60. L. Tong, S. Pav, M. White, S. Rogers, M. Crane, L. Cywin, L. Brown, A. Pargellis, *Nat. Struct. Biol.* **1997**, *4*, 311-316.

61. Z. Wang, J. Canagarajah, C. Boehm, S. Kassisa, H. Cobb, R. Young, S. Abdel-Meguid, L. Adams, E. Goldsmith, *Structure,* **1998**, *6*, 1117-1128.

62. K. Wilson, G. McCaffrey, K. Hsiao, S. Pazhinisamy, V. Galullo, G. Bemis, M. Fitzgibbon, P. Caron, M. Murcko, S. Su, *Chem. Biol.* **1997**, *4*, 423-431.

63. J. Gum, M. McLaughlin, S. Kumar, Z. Wang, J. Bower, C. Lee, L. Adams, P. Livi, J. Goldsmith, R. Young, *J. Biol. Chem.* **1998**, *273*, 15605-15610.

64. J. Lisnock, A. Tebben, B. Frantz, A. O'Neill, G. Croft, J. O'Keefe, B. Li, C. Hacker, S. Laszlo, A. Smith, B. Libby, N. Liverton, J. Hermes, P. LoGrasso, *Biochemistry,* **1998**, *37*, 16573-16581.

65. Z. Wang, J. Canagarajah, C. Boehm, S. Kassisa, H. Cobb, R. Young, S. Abdel-Meguid, L. Adams, J. Goldsmith, *Structure (London)* **1998**, *6*, 1117-1128.

66. E. Tschitschibabine; *Ber.*, **1925,** *58*, 1704.

67. A. Montgomery, A. Secrist; *Comprehensive Heterocyclic Chemistry*; R. Katrisky, W. Rees, T. Potts, *Eds*; *Pergamon*; Oxford, **1984,** *5*, 607.

68. A. Hubert, H. Reimlinger; *Chem. Ber.*, **1970,** *103*, 3811.

69. S. Hand, W. Paudler, *J. Org. Chem.*, **1978,** *43*, 2900.

70. W. Paudler, R.Vandahm, Y. Park; *J. Heterocyclic Chem*., **1972,** *9*, 81.

71. A. Abdelhamid, H. Hassaneen, A. Shawli; *J. Heterocyclic Chem*., **1983,** *20*, 639.

72. C. Blackburn, B. Guan, S. Tsai; *Tetrahedron Letters*, **1998**, *39(22)*, 3655-3638.

73. M. Groziak, S. Wilson, G. Clauson; *J. Am. Chem. Soc*., **1986,** *108*, 8002.

74. J. Yadav, B. Subba Reddy, Y. Gopal Rao, M. Srinivas, A. Narsaiah, *Tet. Lett.,* **2007**, *48***,** 7717-7720.

75. J. Salimon, N. Salih, H. Hussien, E. Yousif, *Eur. J. Sci. Research,* **2009**, *31(2)***,** 256-264.

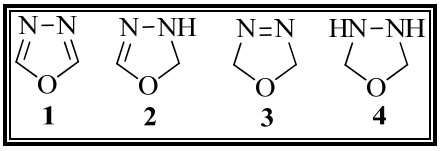
76. S. A Biradar, V. K. Bhovi, Y. D. Bodke, R. Bhavanishankar, *molbank,* **2009**, 587.

Chapter-3

A FACILE SYNTHESIS OF SOME SUBSTITUTED 1-((5-(BENZOFURAN-2-YL)-1,3,4-OXADIAZOL-2-YL)METHYL)AMINE

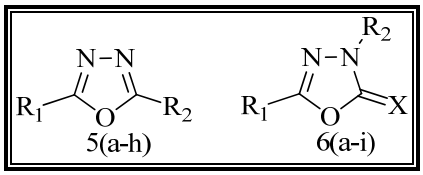
3.1 INTRODUCTION

Oxadiazoles belong to an important group of heterocyclic compounds having –N=C-O- linkage. 1,3,4-oxadiazole(**1**) is a thermally stable aromatic heterocycle and exist in two partially reduced forms; 2,3-dihydro-1,3,4-oxadiazole(1,3,4-oxadiazoline)(**2**) and 2,5-dihydro-1,3,4-oxadiazole(1,3,4-oxadiazoline)(**3**) depending on the position of the double bond. The completely reduced form of the 1,3,4-oxadiazole is known as 2,3,4,5-tetrahydro-1,3,4-oxadiazole (1,3,4-oxadiazolidine)(**4**)[1]



1,3,4-Oxadiazole is a heterocyclic molecule with oxygen atom at 1 and two nitrogen atoms at 3 and 4 position. They have been known for about 80 years, it is only in the last decade that investigations in this field have been intensified. This is because of large number of applications of 1,3,4-oxadiazoles in the most diverse areas viz. drug synthesis, dye stuff industry, heat resistant materials, heat resistant polymers and scintillators. Reviews of the relevant literature prior to 1965 are available.

Bactericidal and/or fungicidal activity was reported for oxadiazole (5a), aminooxadiazole (5b)[2] and oxadiazolinethiones (6a).[3] The tin derivatives (6b) is an effective fungicide and antimicrobial activity is shown by thiones (6c).[4] Antiinflammatory, sedative and analgesic properties were reported for aryloxadiazoles (5c).[5] Amino-oxadiazoles (5d) show analgesic activity and amino-oxadiazoles (5e) exhibit both anti-inflammatory and antiproteolytic properties[6]. Anticonvulsant and nervous system depressant activity was reported for amino-oxadiazoles (5f), where R is quinazolin-3-yl group.[7] Aminooxadiazole (5g) show local anaesthetic activity.[8] The oxadiazolinone (6d) is an orally active antiallergic agent, for example in the treatment of asthma and allergy disease and is claimed to be more potent than sodium cromoglycate.[9] Examples of the many oxadiazolones for the many herbicidal activity (week killers) are (6e,6f) and "oxadiazon"(6g), which is the subject of many regular reports in the literature. Insecticidal activity is shown by oxadiazolones (6h, 6i the later is an aphicide), and oxadiazole (5h)



	R^1		R^2		R^1	R^2	X
5a	Ar		$CH_2CONHCONHR$	6a	heteroarylOCH_2	H	S
5b	AR		OCH_2 NHCOR	6b	1-methylcyclopropyl	$Sn(Ph)_3$	O
5c	trimethoxy		3,4-dimethoxyphenyl	6c	5-Cl-2-phenylindol-3-ylNH	H	S
5d	2-pyridyl	or	NR_2HCl	6d	3-Cl-benzo[b]thiophen-2-yl	H	O
5e	4-biphenylylmethyl		NHAr	6e	4-cyclohexylphenoxy	H	O
5f	Ar		$NHCH_2CONHR$	6f	2,4-diCl-phenoxymethyl	Bn	O
5g	Ar		$NHCO(CH_2)nNRR'HCl(n=2or3)$	6g	t-Bu	2,4-diCl--5-isopropoxyphenyl	O
				6h	OCH_3	o-methoxyphenyl	O
				6i	CH_3NH	2,3-diH-2,2,4-triMebenzofuran-7-yl	O

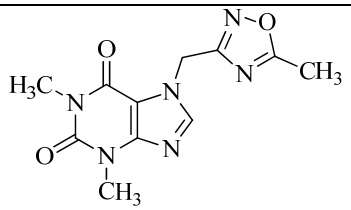
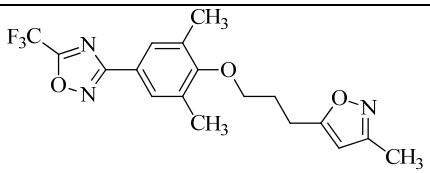
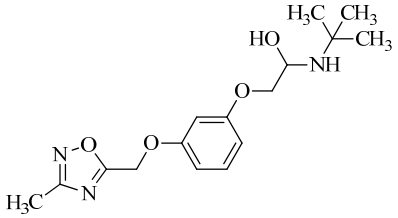
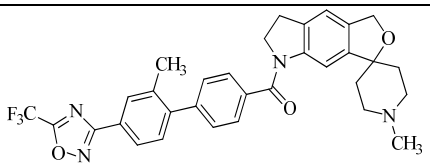
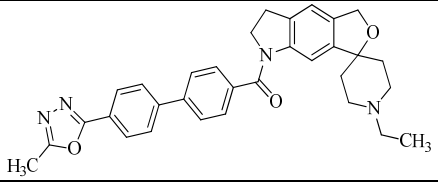
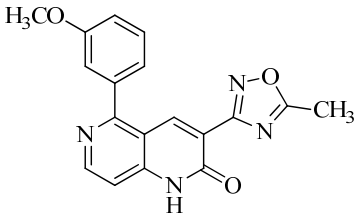
3.2 PHARMACOLOGY

1,3,4-Oxadiazole derivatives have been tested for various pharmacological activities, which have been summarized as under.

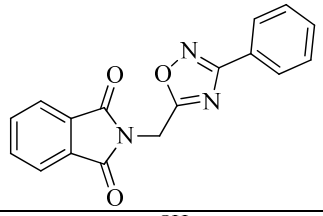
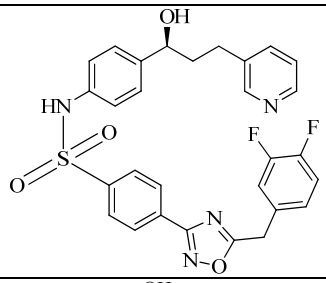
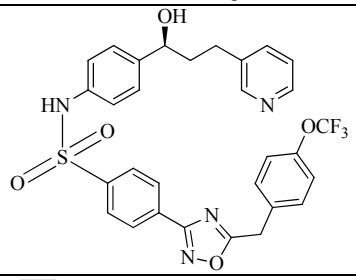
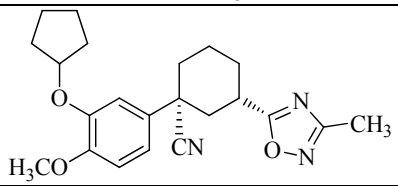
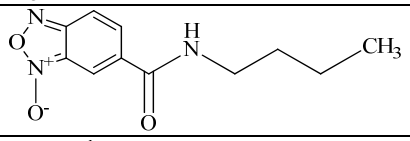
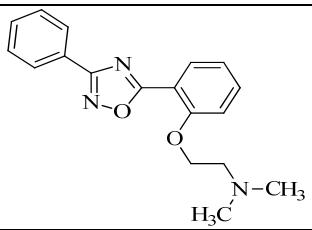
1. Antibacterial[10]
2. Antiinflammatory[11]
3. Analgesic[12]
4. Antiviral and anticancer[13]
5. Antihypertensive[14]
6. Anticonvulsant[15]
7. Antiproliferative[16]
8. Antifungal[17]
9. Cardiovascular[18]
10. Herbicidal[19]
11. Hypoglycemic[20]
12. Hypnotic and Sedative[21]
13. MAO inhibitor[22]
14. Insecticidal[23]

1,3,4-Oxadiazole is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 1,3,4-oxadiazole has led to the extensive use of this compound in organic synthesis.

Some oxadiazole drugs & derivatives under Preclinical/Phase clinical trials.

Sr. No	Chemical structure	Activity	Phase	Originator
1		Antitussive, Bronchodilator	Phase-I	Sanofi-Synthlabo
2		Antirhinoviral, Antiviral	Phase-III	Viro pharma
3		Antihypertensive, Antianginal, Antiglaucoma agent, Beta-adrenoceptor antagonist	Phase-II	Center for Chemistry of Drugs
4		Antidepressants, Anxiolytic, 5-HT1D Antagonist	Biological testing	Smithkline Beecham
5		Antidepressants, Anxiolytic, 5-HT1D Inverse agonist	Preclinical	Smithkline Beecham
6		Cognition en-hancing drug, GABA(A) recep-tor modulator, GABA(A) B2 site inverse agonist	Preclinical	Dainoppon pharma

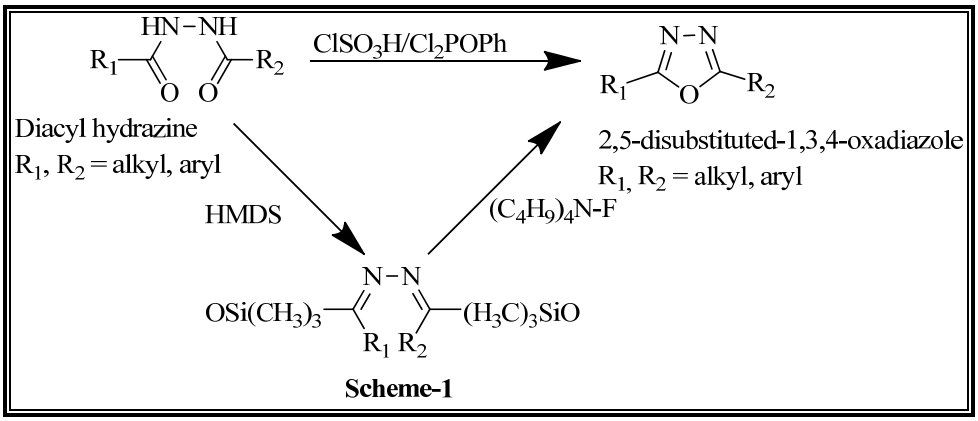
Some oxadiazole drugs & derivatives under Preclinical/Phase clinical trials.

Sr. No	Chemical structure	Activity	Phase	Originator
1		Analgesic	Preclinical	Universidade federal per-nambuco
2		Antiobesity drug, Antidiabetic drug, Beta3 adrenoce tor agonist	Preclinical	Merck
3		Antiobesity drug, Antidiabetic drug, Beta3 adrenoceptor agonist	Preclinical	Merck
4		Bronchodilator, Phosphodiesterase Inhibitor	Preclinical	Smithkline Beecham
5		Antitrypanosomal	Preclinical	Universidad de larepubli-ca
6		Antiepileptic drug,Neuronal In-jury Inhibitor, AMPA antagon-ist,Sodium channel blocker	Preclinical	Boehringer Ingelaeim

3.3 SYNTHETIC ASPECT

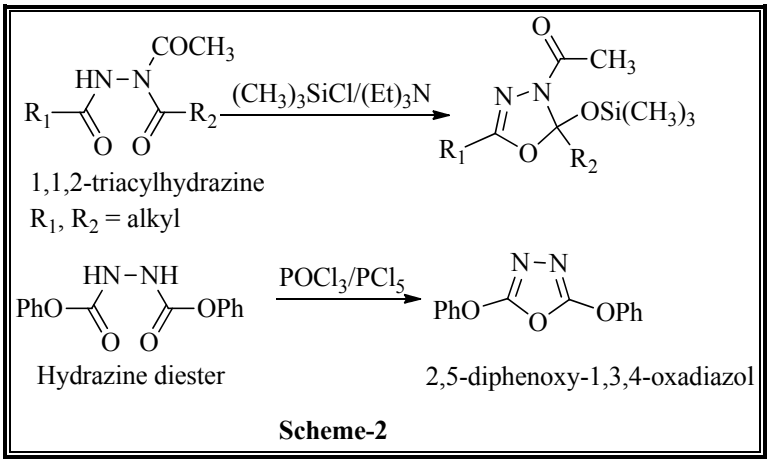
There were several routes for the synthesis of 1,3,4-oxadiazoles reported in the literature among which the most important aspects of synthesis were discussed as under.

2,5-Disubstituted 1,3,4-oxadiazole can be accomplished by cyclodehydration of 1,2-diacylhydrazine either by using chlorosulphonic acid[24] or phenyl dichorophosphite in di-methylformamide. A nonaqueous, nonacedic, route involves treatment of hydrazine with hexamethyl disilazane (HMDS) and tetrabutylammoniumfluoride, the last step presumably being fluoride catalyzed cyclization of intermediate bis silyl ether[25-26] (Scheme-1)



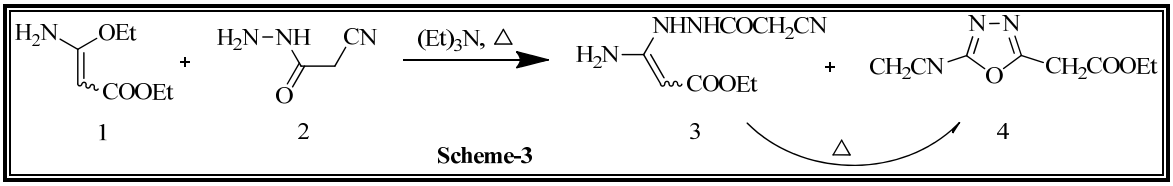
Scheme-1

In a related reaction, 1,1,2-triacetylhydrazine with trimethylsilylchloride/triethylamine gave oxadiazolinyl silylether.[27] Cyclodehydration ($PCl_5/POCl_3$) of hydrazinyl diester gave the diphenyloxyoxadiazole.[28] (Scheme-2)



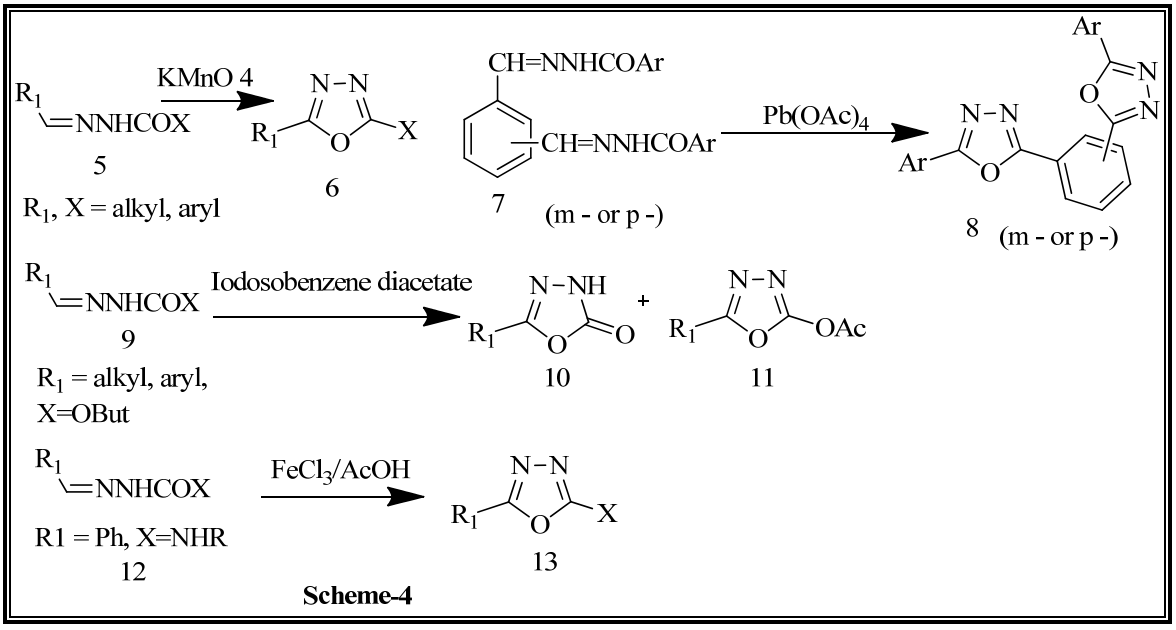
Scheme-2

The malonate derivative (1) reacted with acylhydrazine (2) to give a mixture of diacylhydrazine monoamine (3) and oxadiazole (4). The later was also formed from (3) by heating.[29] (Scheme-3)



Scheme-3

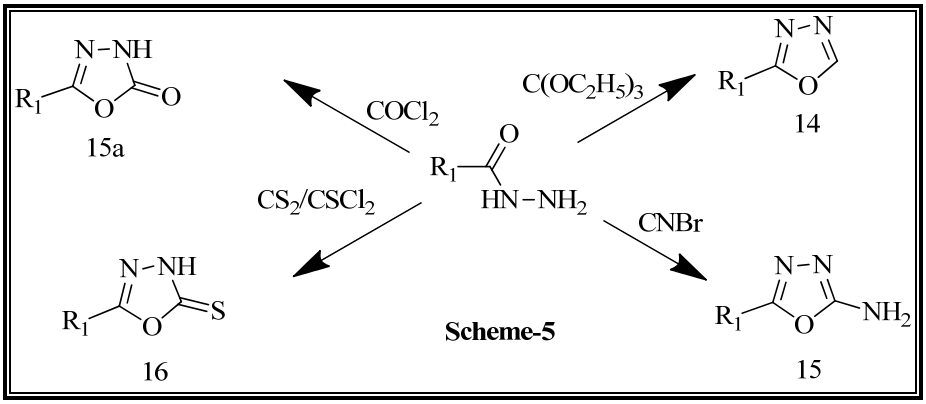
Oxidation of acylhydrazones derived (5) from aldehydes has been developed into a useful route to disubstituted oxadiazoles (6). The use of potassium permanganate with acetone as solvent was claimed to give better yields than the use of other oxidizing agents (e.g.halogens).[30] An improved synthesis of bis-oxadiazolylbenzenes (8) involved oxidation of bishydrazones (7) with lead tetraacetate.[31] Acylhydrazones (9) were oxidized by iodosobenzene diacetate to oxadiazolinones (10), with acetates (11) also being formed in some cases. A similar oxidation of ethyl esters (9, X=OEt) gave oxadiazolyl ethers (11, X=OEt).[32] Oxidative cyclization($FeCl_3$/AcOH) of semicarbazone (12) yielded amino-oxadiazoles (13).[33] (Scheme-4)



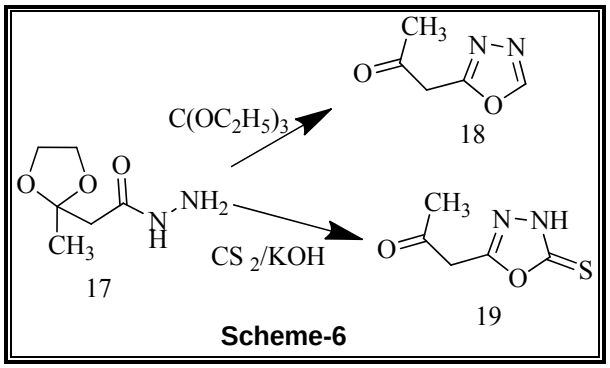
Scheme-4

Important routes to monosubstituted oxadiazoles (14), aminooxadiazoles (15), oxadiazolinones (15a) and oxadiazolinethiones (16) involve reaction of hydrazides

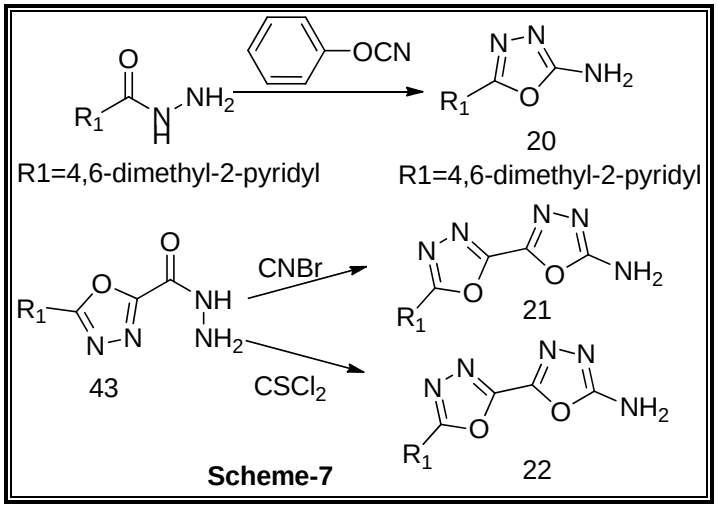
($R_1CONHNH_2$) with triethyl orthoformate, cyanogen bromide, phosgene, or carbon disulphide (or $CSCl_2$) respectively. (Scheme-5)



Reaction of hydrazide (17) with triethylorthoformate, or with CS_2/KOH, allowed the synthesis of oxadiazolyl methyl ketones (18) and (19), respectively, after hydrolysis of the acetal group.[34] (Scheme-6)

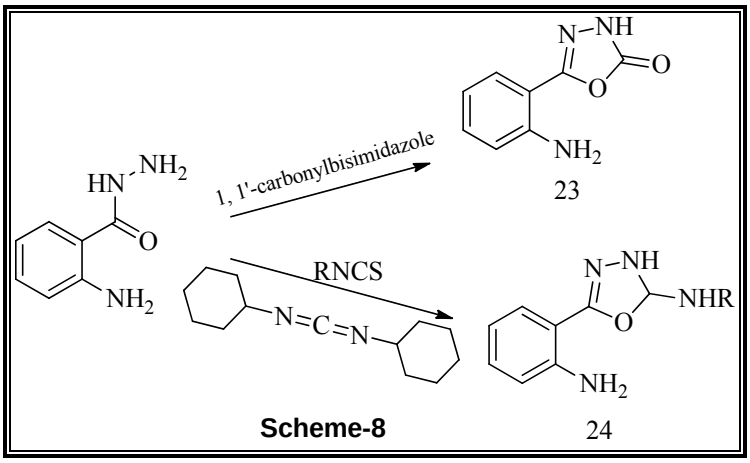


An alternative to cyanogenbromide is phenyl cyanate (PhOCN), which reacted with hydrazines ($R_1CONHNH_2$) to give aminooxadiazoles (R_1= 4,6- dimethyl-2-pyrimidyl).[35] From oxadiazol-2-carbohydrazides (20) bioxadiazolyls (21) and (22) were prepared using cyanogen bromide[36] or thiophosgene[37] respectively. (Scheme-7)



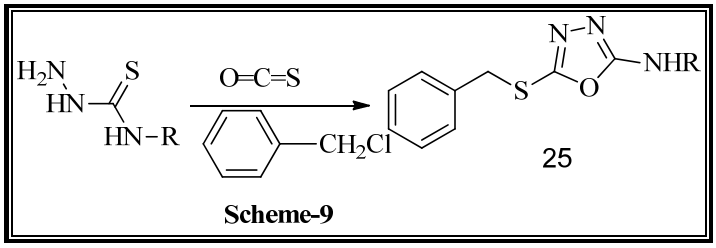
Scheme-7

It has been shown that *o*-aminobenzoylhydrazine reacted with (i) 1,1'-carbonyl- bis-imidazole(a variation of the use of phosgene) to give oxadiazolinone(23)[38] and (ii) 1,3 dicyclohexylcarbodiimide and an isothiocyanate RNCS to give aminooxadiazole (24).[39] (Scheme-8)



Scheme-8

A variation of the oxidative cyclization of acyl-thiosemicarbazides to aminooxadiazoles.[40]A variation of the reaction of acylhydrazines and carbon disulfide forming oxadiazolinethiones, is the reaction of thiosemicarbazide ($RNHCSNHNH_2$) with carbon oxysulfide and benzyl chloride, which yields amino-oxadiazolyl thioethers(25).[41] (Scheme-9)

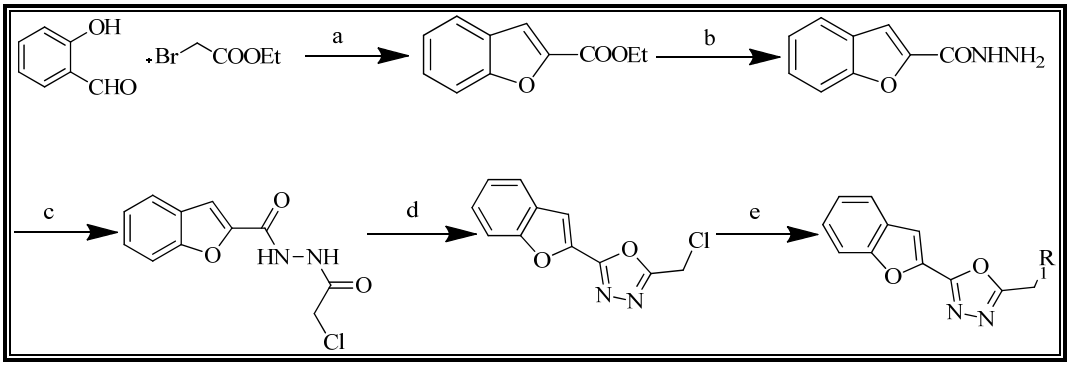


Scheme-9

3.4 AIM OF CURRENT WORK

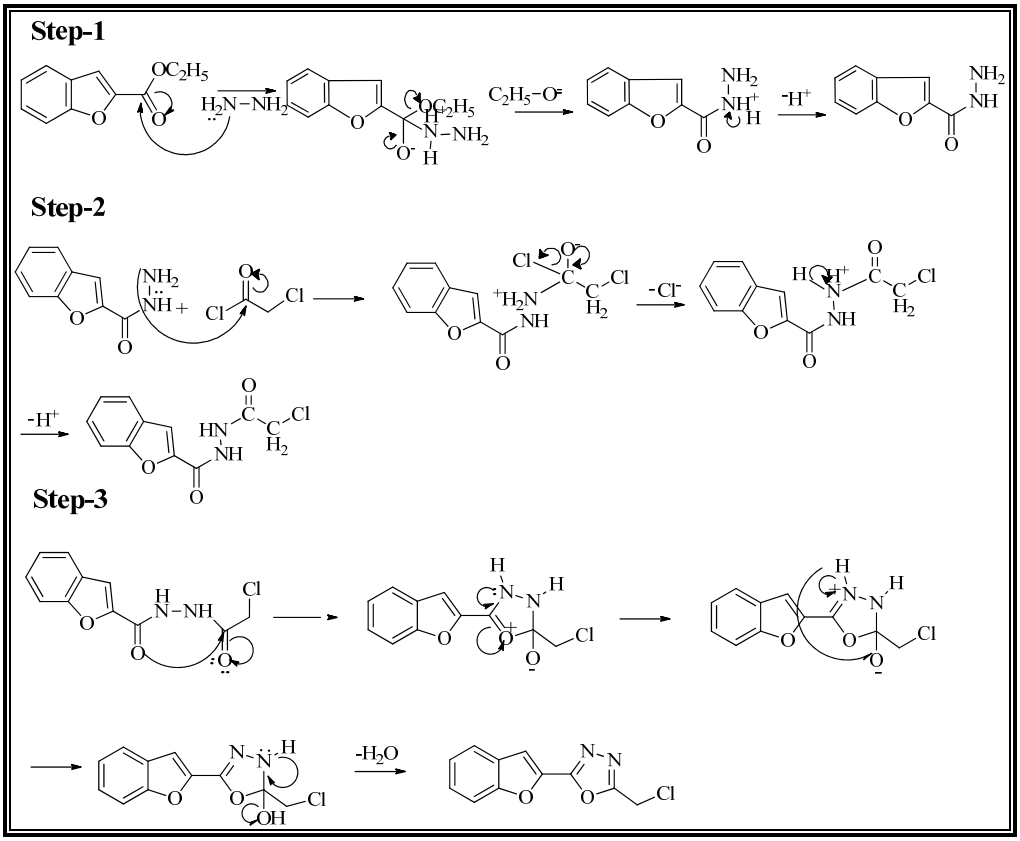
A large number of heterocyclic oxadiazole and related compounds have been reported for their anti-inflammatory activity. Some new work is also reported so far on the anti-inflammatory activity. These results promoted us to synthesize the substituted 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)amine. A series of 26, benzofuran bearing oxadiazole derivatives have been synthesized by treating different amines with benzofuran containing 1,3,4-oxadiazole in DMF.

3.5 REACTION SCHEME



a) DMF,K_2CO_3, 110 °C
b) NH_2-NH_2 , 0 °C
c) DMF,TEA, $ClCH_2COCl$, 0 °C
d) $POCl_3$,Reflux
e) DMF, K_2CO_3, R_1= Substituted amine

3.6 REACTION MECHANISM



3.7 EXPERIMENTAL

❖ Preparation of Ethyl benzofuran-2-carboxylate

Salisaldehyde (0.01 mole) was charged into 250 ml round bottom flask. 30 ml of DMF was added into the flask. Then 0.01 mole of ethylbromo acetate and K_2CO_3 (0.03 mole) was added. The reaction mixture was refluxed for 1.5 h at 110 °C on oil bath. The progress and the completion of the reaction were checked by TLC using hexane: ethyl acetate (9:1) as a mobile phase. After the reaction was completed, reaction mixture was poured into ice. Then product was extracted using ethyl acetate (50 ml × 3), the combined organic layer was washed using brine solution (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 77 %, B.P.- 276 °C.

❖ Preparation of Benzofuran-2-carbohydrazides

Ethyl benzofuran-2-carboxylate (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added drop wise at 0-5 °C in above flask. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction was completed, the mixture was stirred at room temperature to give benzofuran-2-carbohydrazide as a white colored shining product. M.P.-190-194 °C.

❖ Preparation of *N*'-(2-chloroacetyl)benzofuran-2-carbohydrazide

Benzofuran-2-carbohydrazides (0.01 mole), tri ethyl amine (0.02 mole) and DMF were taken in a flask and stirred continuously with mechanical stirring. Resulting mix was cooled at 0 °C then added drop wise chloracetyl chloride (0.02 mole). The reaction mix was stirred over night. The reaction was poured in to the ice and extracted with ethyl acetate. The organic layer was separated out and dried with sodium sulphate. The organic layer was evaporated under reduced pressure to give yellow oily compound. The compound was purified by column chromatography by silica gel 230-400 mash. TLC. (EtoAC: Hexane : 4:6). Yield : 65%.

Note: The entire reaction was carried out under nitrogen atmosphere.

❖ **Preparation of 2-(Benzofuran-2-yl)-5-(chloromethyl)-1,3,4-oxadiazole**

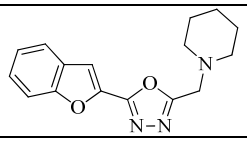
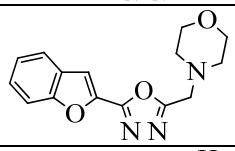
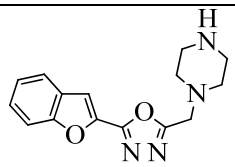
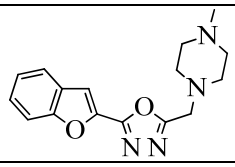
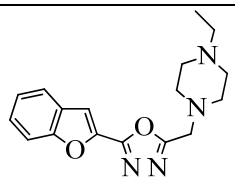
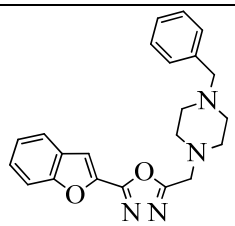
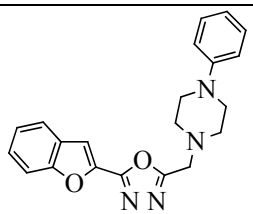
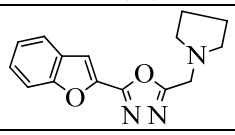
N'-(2-chloroacetyl) benzofuran-2-carbohydrazide (0.01mole), phosphorous oxychloride 10 ml was charged into 250 ml round bottom flask and reflux for 10 h at 100 °C. The progress and the completion of the reaction were checked by TLC using hexane: ethyl acetate (4: 6) as a mobile phase. After completion of the reaction, reaction was poured in to ice to give solid 2-(Benzofuran-2-yl)-5-(chloromethyl)-1,3,4-oxadiazole. Yield : 72%.

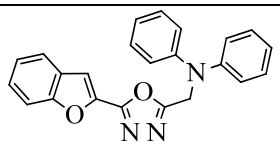
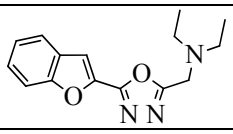
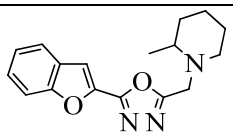
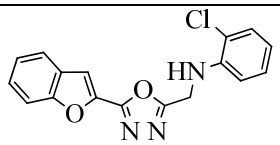
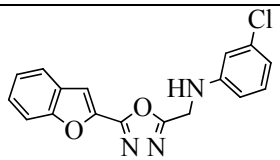
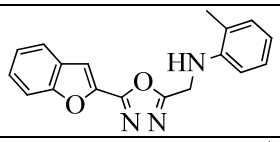
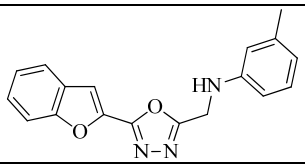
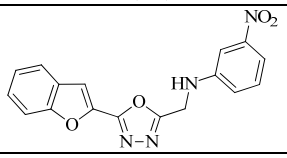
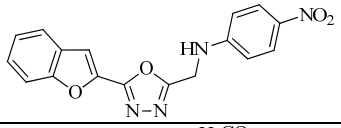
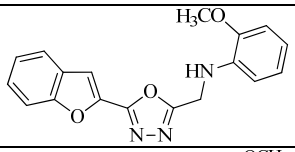
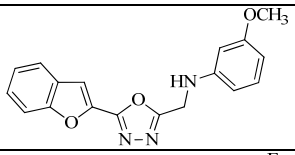
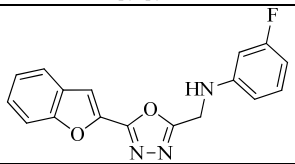
❖ **General method for preparation of substituted 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)amine**

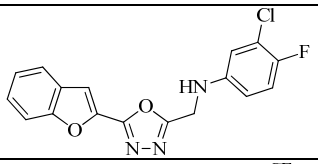
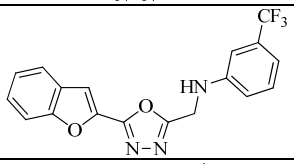
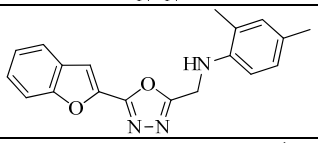
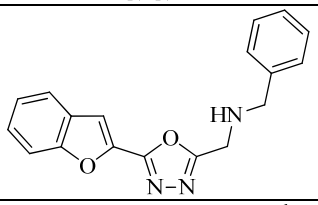
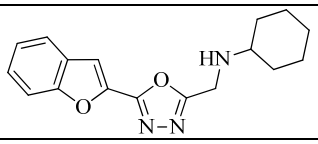
2-(Benzofuran-2-yl)-5-(chloromethyl)-1,3,4-oxadiazole (0.1 mole), anhydrous potassium carbonate (0.2 mole) and DMF were taken in flask and sitrred continuously with mechanical stirring. The substituted amine (0.1 mole) was added drop wise at room temperature, reaction mix was stirred for 12 h. The reaction was poured in to the ice to give solid title compound.The compound was recrystlized by ethanol. TLC. (EtoAC: Hexane : 3:7). Yield : 55-77%.

3.8 PHYSICAL DATA

TABLE: 1 PHYSICAL DATA OF SUBSTITUTED 1-((5-(BENZOFURAN-2-YL)-1,3,4-OXADIAZOL-2-YL)METHYL)AMINE

Sr. No	Structure	M.F	M.P. (°C)	R_f value	% Yield
DPB-42		$C_{16}H_{17}N_3O_2$	180-182	0.46	67
DPB-43		$C_{15}H_{15}N_3O_3$	224-226	0.52	63
DPB-44		$C_{15}H_{16}N_4O_2$	192-194	0.44	48
DPB-45		$C_{16}H_{18}N_4O_2$	244-246	0.48	58
DPB-46		$C_{17}H_{20}N_4O_2$	198-200	0.40	53
DPB-47		$C_{22}H_{22}N_4O_2$	202-204	0.54	68
DPB-48		$C_{21}H_{20}N_4O_2$	188-190	0.50	47
DPB-49		$C_{15}H_{15}N_3O_2$	224-226	0.52	58

DPB-50		$C_{23}H_{17}N_3O_2$	212-214	0.42	71
DPB-51		$C_{15}H_{17}N_3O_2$	200-202	0.44	55
DPB-52		$C_{17}H_{19}N_3O_2$	180-182	0.46	57
DPB-53		$C_{17}H_{12}ClN_3O_2$	188-190	0.51	48
DPB-54		$C_{17}H_{12}ClN_3O_2$	208-210	0.51	59
DPB-55		$C_{18}H_{15}N_3O_2$	222-224	0.49	57
DPB-56		$C_{18}H_{15}N_3O_2$	238-240	0.45	46
DPB-57		$C_{17}H_{12}N_4O_4$	192-194	0.48	49
DPB-58		$C_{17}H_{12}N_4O_4$	189-191	0.42	61
DPB-59		$C_{18}H_{15}N_3O_3$	198-200	0.49	53
DPB-60		$C_{18}H_{15}N_3O_3$	220-222	0.50	46
DPB-61		$C_{17}H_{12}FN_3O_2$	238-240	0.48	56

DPB-62		$C_{17}H_{11}ClFN_3O_2$	202-204	0.52	59
DPB-63		$C_{18}H_{12}F_3N_3O_2$	188-190	0.51	65
DPB-64		$C_{19}H_{17}N_3O_2$	224-226	0.47	74
DPB-65		$C_{18}H_{15}N_3O_2$	230-232	0.46	65
DPB-66		$C_{17}H_{19}N_3O_2$	190-192	0.48	61

R_f value was calculated using solvent system, EtoAC**:** Hexane : 3**:**7.

3.9 SPECTRAL STUDY

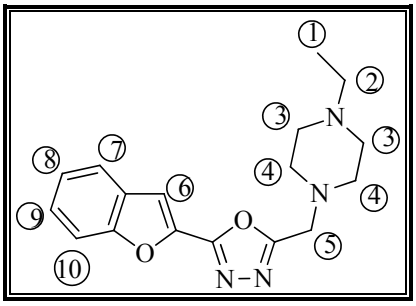
❖ IR spectra

Infra Red spectra were taken on **Shimadzu FT-IR-8400** spectrometer using KBr pellet method. The characteristic aromatic group in 1,3,4-oxadiazol moiety is observed at 3010-3090 cm^{-1}. Secondary amine (> NH) observed a broad peak between 3200-3000 cm^{-1}. Methylene group (>CH_2) observed at 3000-2850 cm^{-1}. Methyl (-CH_3) observed at 1350 cm^{-1}. DPB-42 and DPB-61 of IR spectra are given on page no: - 110.

❖ ^{1}H NMR spectra

^{1}H NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d_6 & $CDCl_3$ as a solvent. In the NMR spectra of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)amine derivatives various proton values of methylene (-CH_2), amine (-NH), methyl (-CH_3) and aromatic protons (Ar-H) etc. were observed as under.

The values for methylene (-CH_2) proton is observed between 2.50-3.55 δ ppm. In some cases, the value of methylene proton differs to 4.20 and 4.43 δ ppm. The -NH protons of substituted aniline observed at 3.95-4.20 δ ppm. Aromatic protons shows the multiplet between 6.12-8.39 δ ppm. DPB-46, DPB-47, DPB-48 and Intermediate of ^{1}H NMR spectra are given on page no: - 104 to 109.



1. The proton no.1 of methyl group gave a characteristic triplet at 0.98 δ ppm.

2. The proton no.2 of methylene group gave a characteristic quartet at 2.33 δ ppm.

3. The proton no. 3 and 4 of piperazine eight protons which is attached with nitrogen atom gave a multiplet at 2.44 δ ppm- 2.59 δ ppm. it became a deshielded due to the nitrogen atom.

4. The proton no.5 of methylene group gave a characteristic singlet at 3.88 δ ppm

5. The proton no. 6 of benzofuran ring gave a characteristic singlet at 7.26 δ ppm.

6. The aromatic ring of proton no. 10 gave a multiplet at 7.30 δ ppm.

7. The aromatic ring of proton no. 8 and 9 gave a multiplet at 7.53 δ ppm -7.62 δ ppm.

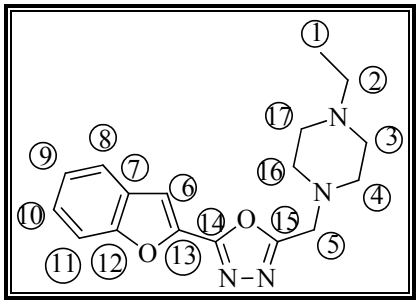
8. The aromatic ring of proton no. 7 gave a multiplet at 7.68 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound DPB-46 was confirmed.

- **^{13}C NMR spectra**

^{13}C NMR spectra were recorded on a **Bruker AC 400 MHz NMR** spectrometer using DMSO-d_6 & $CDCl_3$ as a solvent. In the ^{13}C NMR spectra of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl) amine derivatives various carbon values of methylene (-CH_2), keto (>C=O), methyl (-CH_3) and aromatic carbon (Ar-H) etc. were observed as under.

The values for methylene (-CH_2) carbon is observed between 35-65 δ ppm. The >C=O carbon observed at 160-180 δ ppm. Aromatic carbon shows between 110-140 δ ppm. DPB-46, DPB-47, DPB-48 and Intermediate of ^{13}C NMR spectra are given on page no: -104 to 109.



1. The carbon no. 1 of methyl group, appear at 11.54 δ ppm.
2. The carbon no. 2 and 5 of methylene group, appear at 40.24 δ ppm.
3. The carbon no. 3, 4, 16 and 17 methyl groups, appear at 51.12 δ ppm- 52.06 δ ppm.
4. The carbon no. 12 of benzofuran ring, appear at 155.02 δ ppm.
5. The carbon no. 13 of benzofuran ring, appear at 140.01 δ ppm.
6. The carbon no. 14 of oxadiazole ring, appear at 163.1 δ ppm.
7. The carbon no. 15 of oxadiazole ring, appear at 157.7 δ ppm.
8. The carbon no. 7, 8, 9, 10 and 11 of benzofuran ring, appear at 109.9 δ ppm- 126.8 δ ppm.

❖ **Mass spectra**

The mass spectrum of compounds were recorded by **Shimadzu GC-MS-QP-2010** spectrometer. The mass spectrum of compounds was obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectral study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized. DPB- 46, DPB-59 and DPB-61 of Mass spectra are given on page no.- 103 and 104.

❖ **Elemental analysis**

Elemental analysis of the synthesized compounds was carried out on **Vario EL-III Carlo Erba 1108** model at Saurashtra University, Rajkot which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The elemental analysis data are given for individual compounds.

3.10 SPECTRAL CHARACTERIZATION

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)piperidine (DPB-42)

IR (KBr) cm^{-1}: 1639 (>C=N, str), 1087 (C-O-C), 2852 (>CH_2 ,str), 1438 (>CH_2 ,ban), 3010 (=C-H, str), 3037 (Ar, C-H, str), 1564 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 283. **Elemental analysis, Calculated:** C, 67.83; H, 6.05; N, 14.83 Found: C, 67.79; H, 6.10; N, 14.79.

4-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)morpholine (DPB-43)

IR (KBr) cm^{-1}: 1625 (>C=N, str), 1087 (C-O-C), 2872 (>CH_2 ,str), 1455 (>CH_2 ,ban), 3015 (=C-H, str), 3042 (Ar, C-H, str), 1513 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 285. **Elemental analysis, Calculated:** C, 63.15; H, 5.30; N, 14.73 Found: C, 63.10; H, 5.28; N, 14.65.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (DPB-44)

IR (KBr) cm^{-1}: 3496 (-NH), 1616 (>C=N, str), 1077 (C-O-C), 2871 (>CH_2 ,str), 1445 (>CH_2 ,ban), 3010 (=C-H, str), 3036 (Ar, C-H, str), 1520 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 284. **Elemental analysis, Calculated:** C, 63.37; H, 5.67; N, 19.71; Found: C, 63.23; H, 5.68; N, 19.61.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-methylpiperazine (DPB-45)

IR (KBr) cm^{-1}: 1618 (>C=N, str), 1080 (C-O-C), 2862 (>CH_2 ,str), 1455 (>CH_2 ,ban), 3015 (=C-H, str), 3042 (Ar, C-H, str), 1526 (Ar, C=C, str), 1368 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 298. **Elemental analysis, Calculated:** C, 64.41; H, 6.08; N, 18.78 Found: C, 64.32; H, 6.16; N, 18.71.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-ethylpiperazine (DPB-46)

IR (KBr) cm^{-1}: 1626 (>C=N, str), 1077 (C-O-C), 2878 (>CH_2 ,str), 1463 (>CH_2 ,ban), 3026 (=C-H, str), 3047 (Ar, C-H, str), 1533 (Ar, C=C, str), 1374 (-CH_3, str.). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 3.86 (s, 2H, -CH_2), 2.31 (q, 2H, -CH_2), 1.00 (t, 3H, -CH_3), 2.46 (m, 8H, -CH_2), 7.26-7.71 (m, 5H, Ar-H). **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 11.5, 51.1, 51.5, 51.9, 52.0, 109.9, 111.4, 122.1, 123.7, 126.7, 126.8, 140.0, 155.0,

157.7, 163.1 **Mass: [m/z (%)], M. Wt.:** 312. **Elemental analysis, Calculated:** C, 65.37; H, 6.45; N, 17.94 Found: C, 65.42; H, 6.39; N, 17.88.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-benzylpiperazine (DPB-47)

IR (KBr) cm^{-1}: 1630 (>C=N, str), 1075 (C-O-C), 2885 (>CH_2 ,str), 1471 (>CH_2 ,ban), 3025 (=C-H, str), 3052 (Ar, C-H, str), 1545 (Ar, C=C, str). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 3.50 (s, 2H, -CH_2), 3.91 (s, 2H, -CH_2), 2.39-2.61 (m, 8H, -CH_2), 7.18-7.78 (m, 10H, Ar-H) **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 30.5, 51.1, 52.2, 110.0, 111.5,122.3,123.8, 126.6, 126.8, 126.9, 127.8, 128.6, 137.7, 140.0, 154.9, 157.6, 163.2**Mass: [m/z (%)], M. Wt.:** 374. **Elemental analysis, Calculated:** C, 70.57; H, 5.92; N, 14.96 Found: C, 70.49; H, 5.86; N, 14.90.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-phenylpiperazine (DPB-48)

IR (KBr) cm^{-1}: 1637 (>C=N, str), 1076 (C-O-C), 2882 (>CH_2 ,str), 1475 (>CH_2 ,ban), 3023 (=C-H, str), 3056 (Ar, C-H, str), 1547 (Ar, C=C, str). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 3.99 (s, 2H, -CH_2), 2.77 (m, 4H, -CH_2), 3.19 (m, 4H, -CH_2), 6.76-7.78 (m, 10H, Ar-H) **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 30.5, 48.3, 51.1, 52.2, 110..1, 111.5, 115.5, 119.0, 122.3, 123.8, 126.8, 128.6, 140.0, 150.7, 155.0, 157.7, 163.2 **Mass: [m/z (%)], M. Wt.:** 360. **Elemental analysis, Calculated:** C, 69.98; H, 5.59; N, 15.55 Found: C, 69.92; H, 5.65; N, 15.65.

2-(Benzofuran-2-yl)-5-((pyrrolidin-1-yl)methyl)-1,3,4-oxadiazole (DPB-49)

IR (KBr) cm^{-1}: 1640 (>C=N, str), 1078 (C-O-C), 2890 (>CH_2 ,str), 1463 (>CH_2 ,ban), 3052 (=C-H, str), 3045 (Ar, C-H, str), 1565 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 269. **Elemental analysis, Calculated:** C, 66.90; H, 5.61; N, 15.60 Found: C, 66.98; H, 5.55; N, 15.65.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-N-phenylbenzenamine (DPB-50)

IR (KBr) cm^{-1}: 1639 (>C=N, str), 1068 (C-O-C), 2896 (>CH_2 ,str), 1455 (>CH_2 ,ban), 3052 (=C-H, str), 3039 (Ar, C-H, str), 1564 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 367. **Elemental analysis, Calculated:** C, 75.19; H, 4.66; N, 11.44 **Found:** C, 75.22; H, 4.62; N, 11.48.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-N-ethylethanamine (DPB-51)

IR (KBr) cm^{-1}: 1652 (>C=N, str), 1087 (C-O-C), 2879 (>CH_2 ,str), 1462 (>CH_2 ,ban), 3041 (=C-H, str), 3062 (Ar, C-H, str), 1574 (Ar, C=C, str), 1361 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 271. **Elemental analysis, Calculated:** C, 66.40; H, 6.32; N, 15.49 **Found:** C, 66.38; H, 6.35; N, 15.57.

1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-methylpiperidine (DPB-52)

IR (KBr) cm^{-1}: 1651 (>C=N, str), 1089 (C-O-C), 2883 (>CH_2 ,str), 1450 (>CH_2 ,ban), 3049 (=C-H, str), 3063 (Ar, C-H, str), 1579 (Ar, C=C, str), 1370 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 297. **Elemental analysis, Calculated:** C, 68.67; H, 6.44; N, 14.13**Found:** C, 68.60; H, 6.38; N, 14.11.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-chlorobenzenamine (DPB-53)

IR (KBr) cm^{-1}: 1655 (>C=N, str), 1098 (C-O-C), 2879 (>CH_2 ,str), 1462 (>CH_2 ,ban), 3074 (=C-H, str), 3068 (Ar, C-H, str), 1545 (Ar, C=C, str), 3352 (N-H, str), 1626 (N-H, ban). **Mass: [m/z (%)], M. Wt.:** 325. **Elemental analysis, Calculated:** C, 62.68; H, 3.71; N, 12.90 **Found:** C, 62.65; H, 3.65; N, 12.89.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-chlorobenzenamine (DPB-54)

IR (KBr) cm^{-1}: 1656 (>C=N, str), 1087 (C-O-C), 2888 (>CH_2 ,str), 1452 (>CH_2 ,ban), 3044 (=C-H, str), 3068 (Ar, C-H, str), 1585 (Ar, C=C, str), 3351 (N-H, str), 1627 (N-H, ban).) **Mass: [m/z (%)], M. Wt.:** 325. **Elemental analysis, Calculated:** C, 62.68; H, 3.71; N, 12.90 **Found:** C, 62.65; H, 3.68; N, 12.75.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-methylbenzenamine (DPB-55)

IR (KBr) cm^{-1}: 1656 (>C=N, str), 1087 (C-O-C), 2888 (>CH_2 ,str), 1452 (>CH_2 ,ban), 3044 (=C-H, str), 3068 (Ar, C-H, str), 1585 (Ar, C=C, str), 3351 (N-H, str), 1627 (N-H, ban), 1372 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 305. **Elemental analysis, Calculated:** C, 70.81; H, 4.95; N, 13.76 **Found:** C, 70.96; H, 4.82; N, 13.71.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-methylbenzenamine (DPB-56)

IR (KBr) cm^{-1}: 1646 (>C=N, str), 1095 (C-O-C), 2886 (>CH_2 ,str), 1445 (>CH_2 ,ban), 3045 (=C-H, str), 3072 (Ar, C-H, str), 1581 (Ar, C=C, str), 3358 (N-H, str), 1630 (N-H,

ban), 1352 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 305. **Elemental analysis, Calculated:** C, 70.81; H, 4.95; N, 13.76 **Found:** C, 70.95; H, 4.83; N, 13.75.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-nitrobenzenamine (DPB-57)

IR (KBr) cm^{-1}: 1653 (>C=N, str), 1115 (C-O-C), 2872 (>CH_2 ,str), 1450 (>CH_2 ,ban), 3052 (=C-H, str), 3081 (Ar, C-H, str), 1582 (Ar, C=C, str), 3351 (N-H, str), 1632 (N-H, ban), 1515 (-NO_2, str). **Mass: [m/z (%)], M. Wt.:** 336. **Elemental analysis, Calculated:** C, 60.71; H, 3.60; N, 16.66 **Found:** C, 60.79; H, 3.45; N, 16.62.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-nitrobenzenamine (DPB-58)

IR (KBr) cm^{-1}: 1651 (>C=N, str), 1086 (C-O-C), 2870 (>CH_2 ,str), 1455 (>CH_2 ,ban), 3059 (=C-H, str), 3089 (Ar, C-H, str), 1579 (Ar, C=C, str), 3356 (N-H, str), 1636 (N-H, ban), 1525 (-NO_2, str). **Mass: [m/z (%)], M. Wt.:** 336. **Elemental analysis, Calculated:** C, 60.71; H, 3.60; N, 16.66 **Found:** C, 60.79; H, 3.45; N, 16.62.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-methoxybenzenamine (DPB-59)

IR (KBr) cm^{-1}: 1655 (>C=N, str), 1089 (C-O-C), 2873 (>CH_2 ,str), 1454 (>CH_2 ,ban), 3062 (=C-H, str), 3089 (Ar, C-H, str), 1589 (Ar, C=C, str), 3358 (N-H, str), 1636 (N-H, ban), 1358 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 321. **Elemental analysis, Calculated:** C, 67.28; H, 4.71; N, 13.08 **Found:** C, 67.32; H, 4.75; N, 13.10.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-methoxybenzenamine (DPB-60)

IR (KBr) cm^{-1}: 1649 (>C=N, str), 1093 (C-O-C), 2883 (>CH_2 ,str), 1452 (>CH_2 ,ban), 3063 (=C-H, str), 3087 (Ar, C-H, str), 1579 (Ar, C=C, str), 3362 (N-H, str), 1630 (N-H, ban), 1358 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 321. **Elemental analysis, Calculated:** C, 67.28; H, 4.71; N, 13.08 **Found:** C, 67.32; H, 4.75; N, 13.11.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-fluorobenzenamine (DPB-61)

IR (KBr) cm^{-1}: 1636 (>C=N, str), 1087 (C-O-C), 2910 (>CH_2 ,str), 1440 (>CH_2 ,ban), 3069 (=C-H, str), 3032 (Ar, C-H, str), 1564 (Ar, C=C, str), 3335 (N-H, str), 1631 (N-H, ban),1015 (>C-F, str). **Mass: [m/z (%)], M. Wt.:** 309. **Elemental analysis, Calculated:** C, 66.02; H, 3.91;N, 13.59 **Found:** C, 66.10; H, 3.98;N, 13.63.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-chloro-4-fluorobenzenamine (DPB-62)

IR (KBr) cm^{-1}: 1637 (>C=N, str), 1099 (C-O-C), 2879 (>CH_2 ,str), 1462 (>CH_2 ,ban), 3069 (=C-H, str), 3092 (Ar, C-H, str), 1597 (Ar, C=C, str), 3383 (N-H, str), 1630 (N-H, ban),1026 (>C-F, str), 652 (>C-Cl, str). **Mass: [m/z (%)], M. Wt.:** 343. **Elemental analysis, Calculated:** C, 59.40; H, 3.23; N, 12.22 **Found:** C, 59.45; H, 3.15; N, 12.29.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-(trifluoromethyl)benzenamine (DPB-63)

IR (KBr) cm^{-1}: 1639 (>C=N, str), 1119 (C-O-C), 2880 (>CH_2 ,str), 1462 (>CH_2 ,ban), 3076 (=C-H, str), 3099 (Ar, C-H, str), 1589 (Ar, C=C, str), 3389 (N-H, str), 1638 (N-H, ban),1029 (>C-F, str). **Mass: [m/z (%)], M. Wt.:** 359. **Elemental analysis, Calculated:** C, 60.17; H, 3.37; N, 11.69 **Found:** C, 60.17; H, 3.37; N, 11.69.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2,4-dimethylbenzenamine (DPB-64)

IR (KBr) cm^{-1}: 1642 (>C=N, str), 1109 (C-O-C), 2881 (>CH_2 ,str), 1461 (>CH_2 ,ban), 3078 (=C-H, str), 3089 (Ar, C-H, str), 1599 (Ar, C=C, str), 3345 (N-H, str), 1640 (N-H, ban), 1359 (-CH_3, str.). **Mass: [m/z (%)], M. Wt.:** 319. **Elemental analysis, Calculated:** C, 71.46; H, 5.37; N, 13.16 **Found:** C, 71.40; H, 5.35; N, 13.14.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)(phenyl)methanamine (DPB-65)

IR (KBr) cm^{-1}: 1649 (>C=N, str), 1109 (C-O-C), 2871 (>CH_2 ,str), 1451 (>CH_2 ,ban), 3086 (=C-H, str), 3095 (Ar, C-H, str), 1587 (Ar, C=C, str), 3346 (N-H, str), 1648 (N-H, ban). **Mass: [m/z (%)], M. Wt.:** 305. **Elemental analysis, Calculated:** C, 70.81; H, 4.95; N, 13.76 **Found:** C, 70.78; H, 4.91; N, 13.78.

N-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)cyclohexanamine (DPB-66)

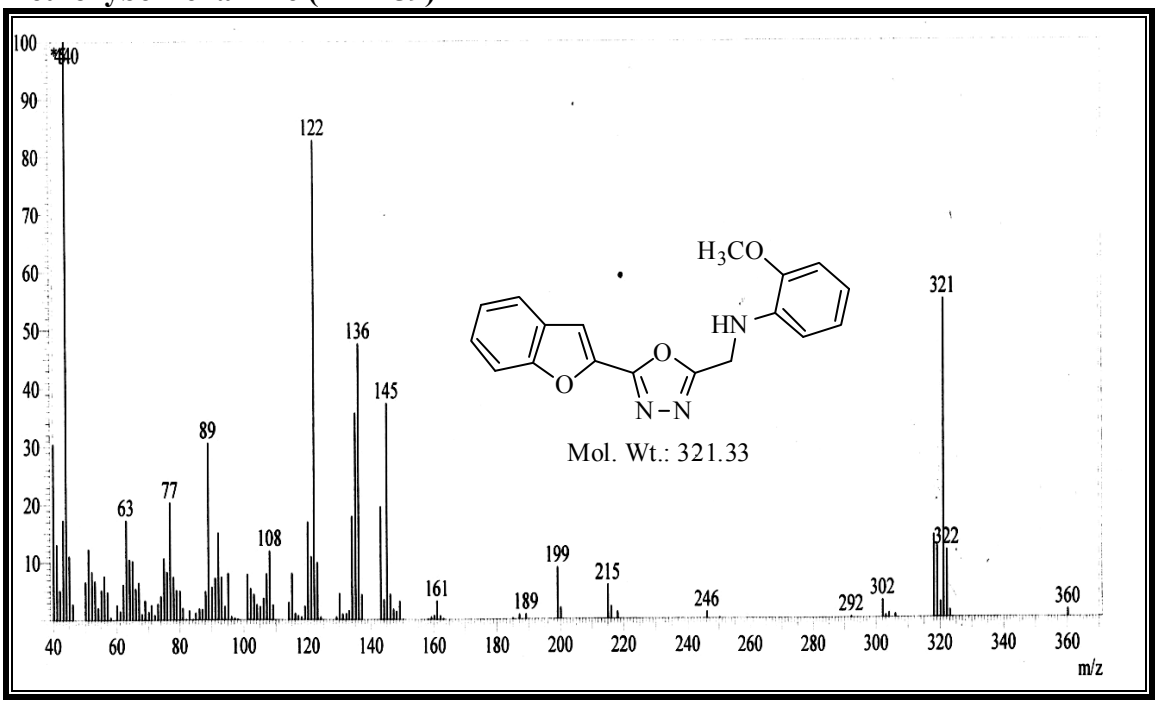
IR (KBr) cm^{-1}: 1645 (>C=N, str), 1108 (C-O-C), 2873 (>CH_2 ,str), 1456 (>CH_2 ,ban), 3075 (=C-H, str), 3010 (Ar, C-H, str), 1589 (Ar, C=C, str), 3353 (N-H, str), 1650 (N-H, ban). **Mass: [m/z (%)], M. Wt.:** 297. **Elemental analysis, Calculated:** C, 68.67; H, 6.44; N, 14.13 **Found:** C, 68.78; H, 6.36; N, 14.10.

3.11 CONCLUSION

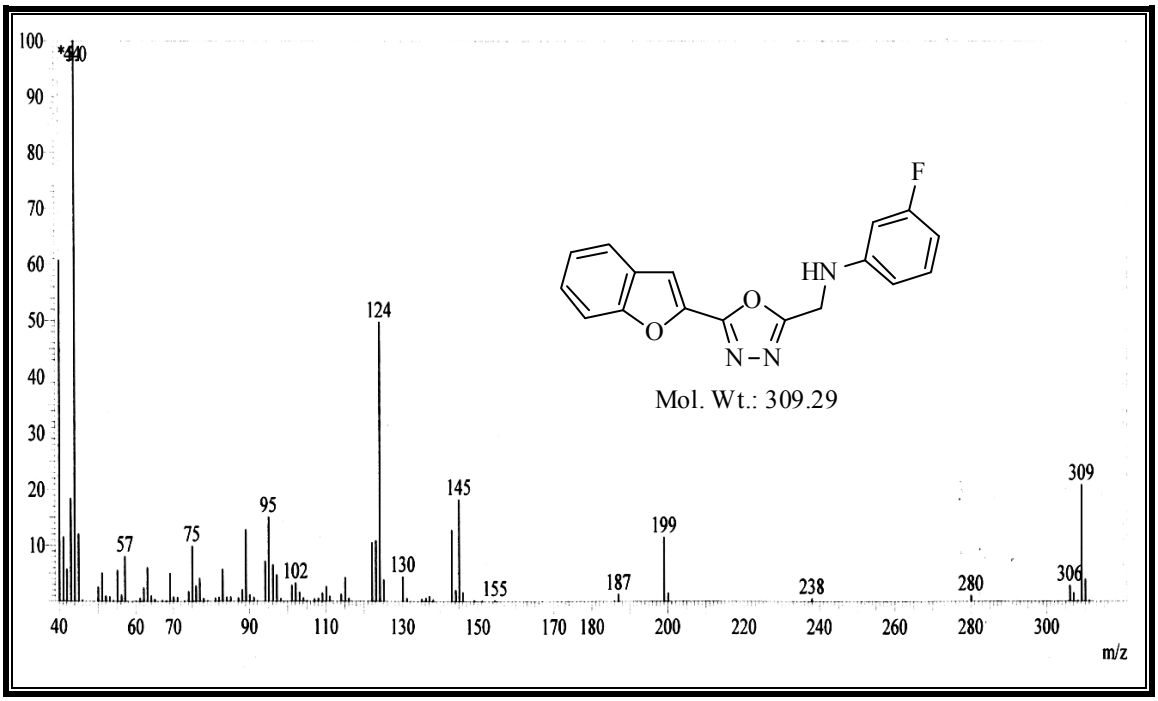
Total 25 derivatives of substituted 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl) amine were synthesized. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and elemental analysis. The synthesized compounds were screened for anti-inflammatory and anti-cancer activity and results are awaited.

3.12 REPRESENTATIVE SPECTRUM

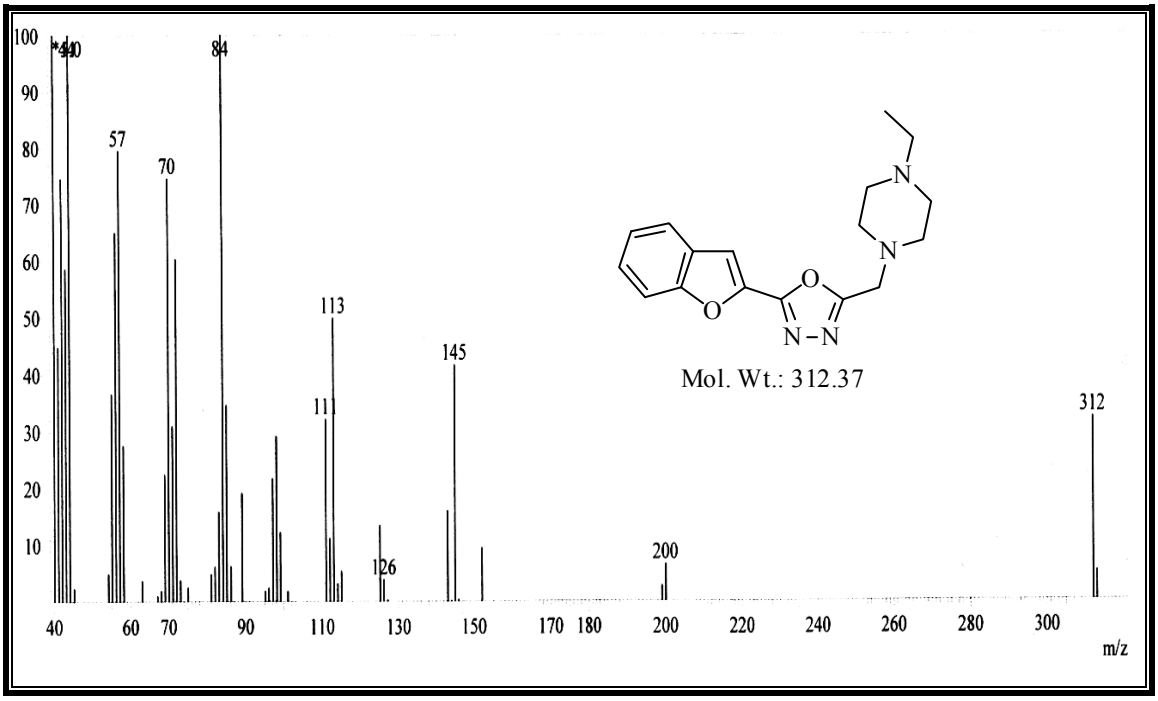
Mass spectrum of *N*-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-methoxybenzenamine (DPB-59)



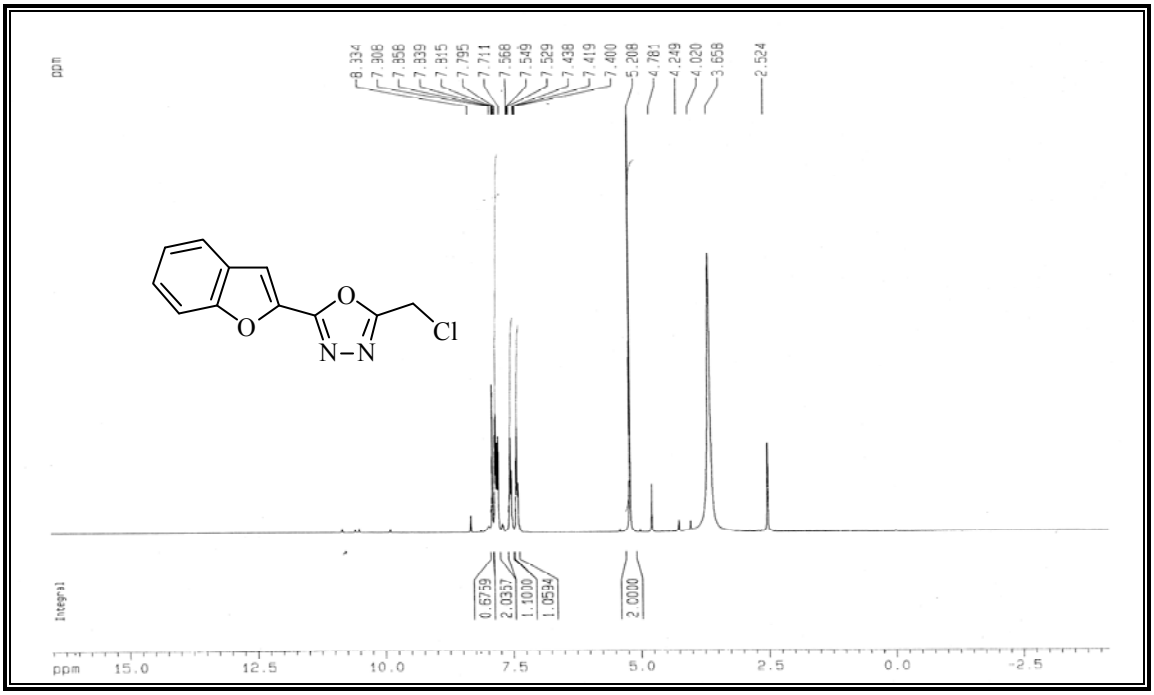
Mass spectrum of *N*-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-fluorobenzenamine (DPB-61)

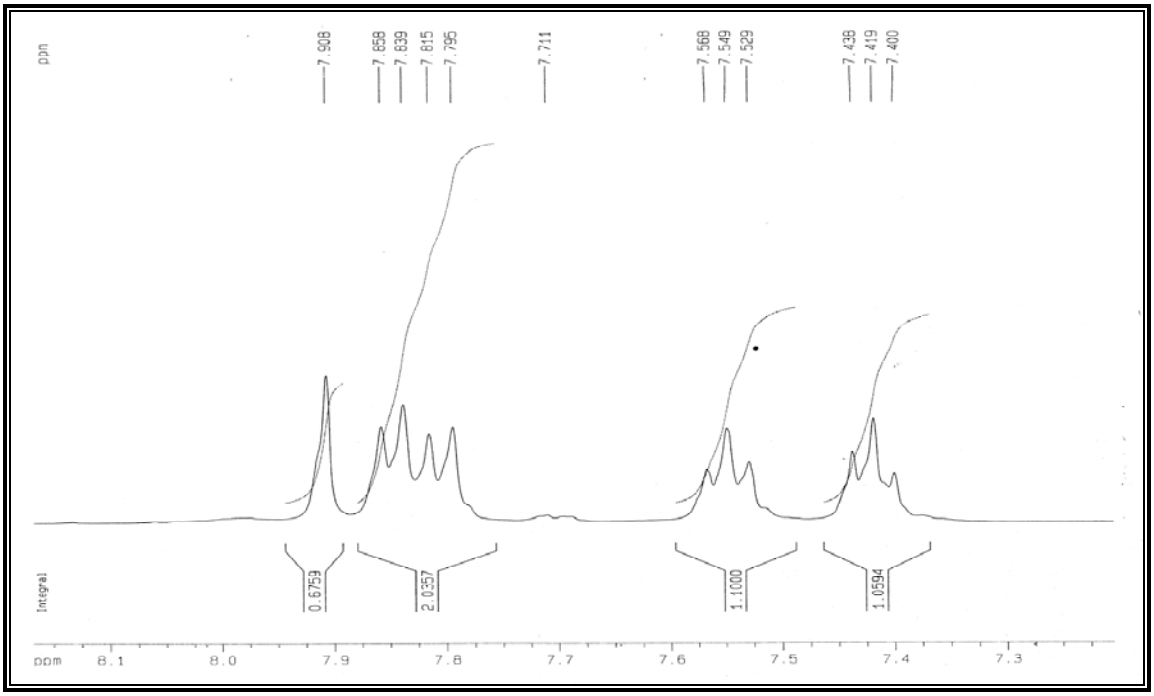


Mass spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-ethylpiperazine (DPB-46)

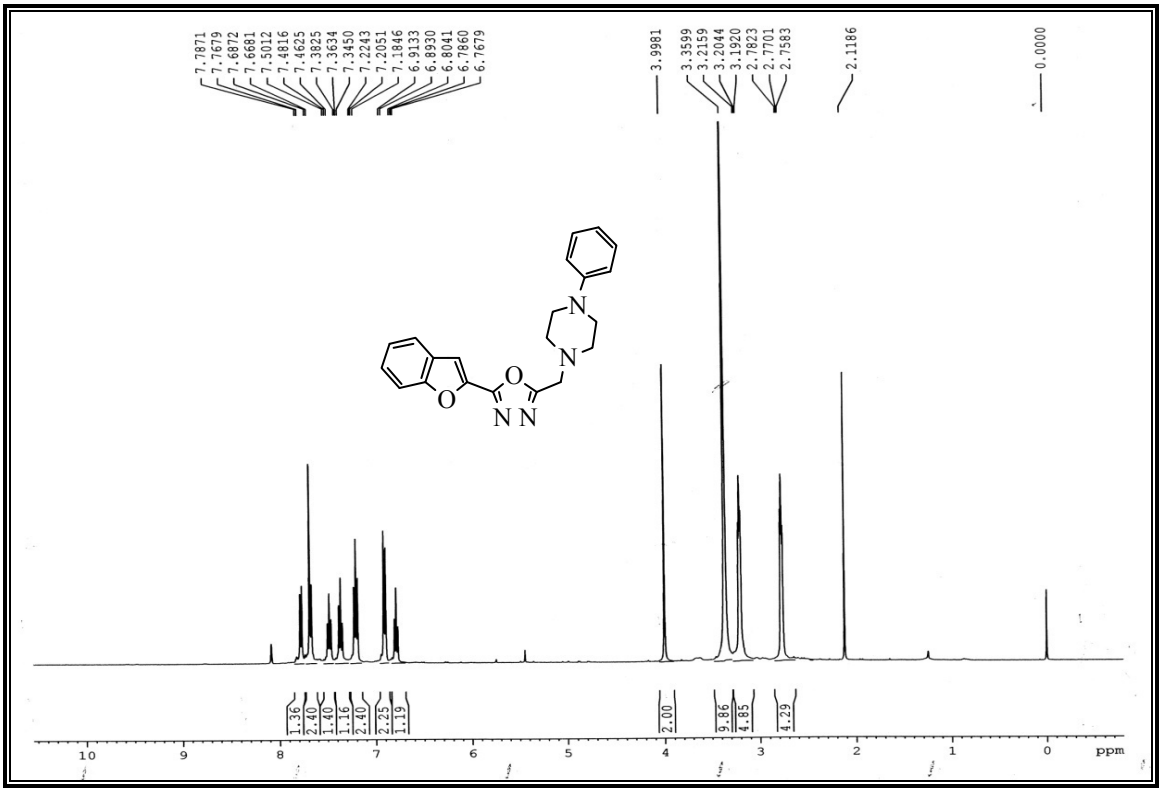


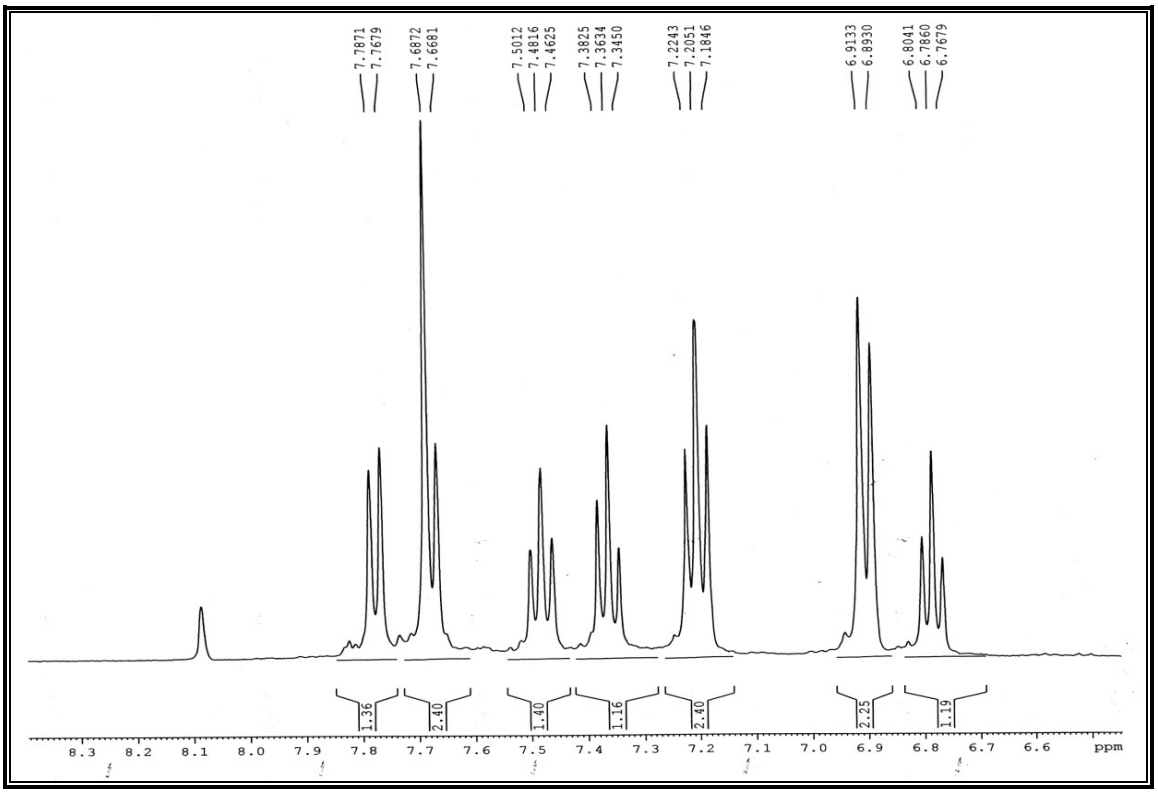
^{1}H NMR spectrum of 2-(Benzofuran-2-yl)-5-(chloromethyl)-1,3,4-oxadiazole (Intermediate)



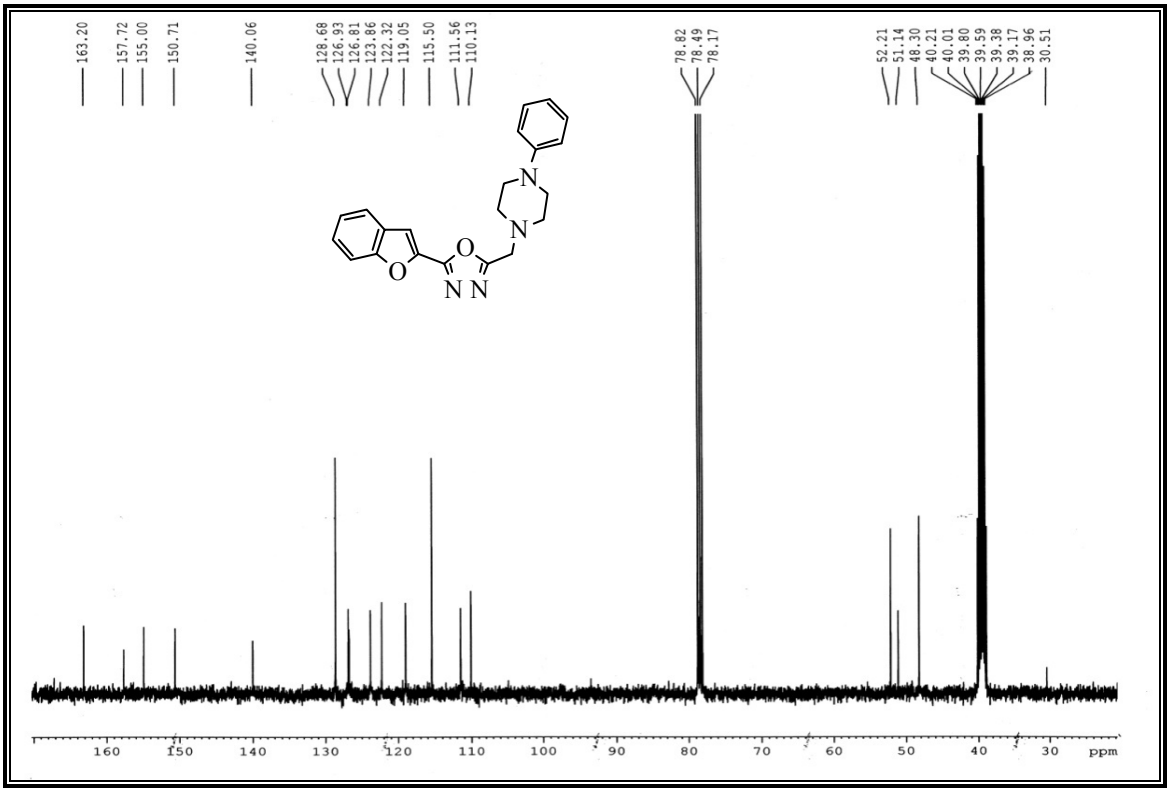


^{1}H NMR spectrum of 2-(Benzofuran-2-yl)-5-((4-phenylpiperazin-1-yl)methyl)-1,3,4-oxadiazole (DPB-

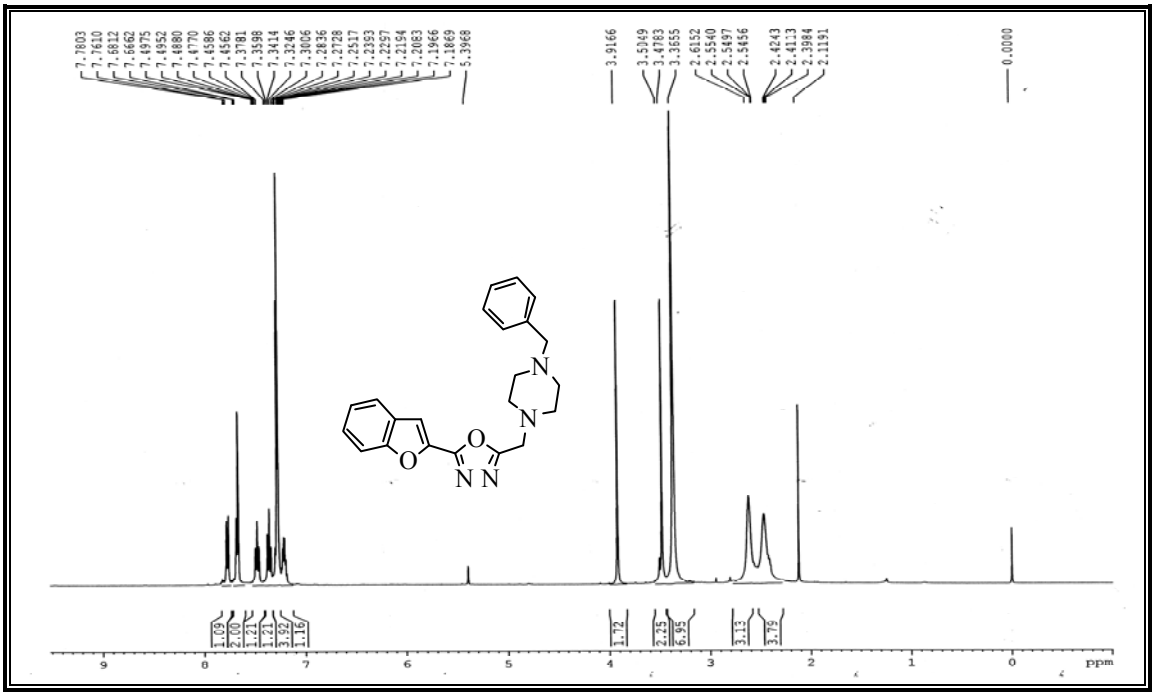


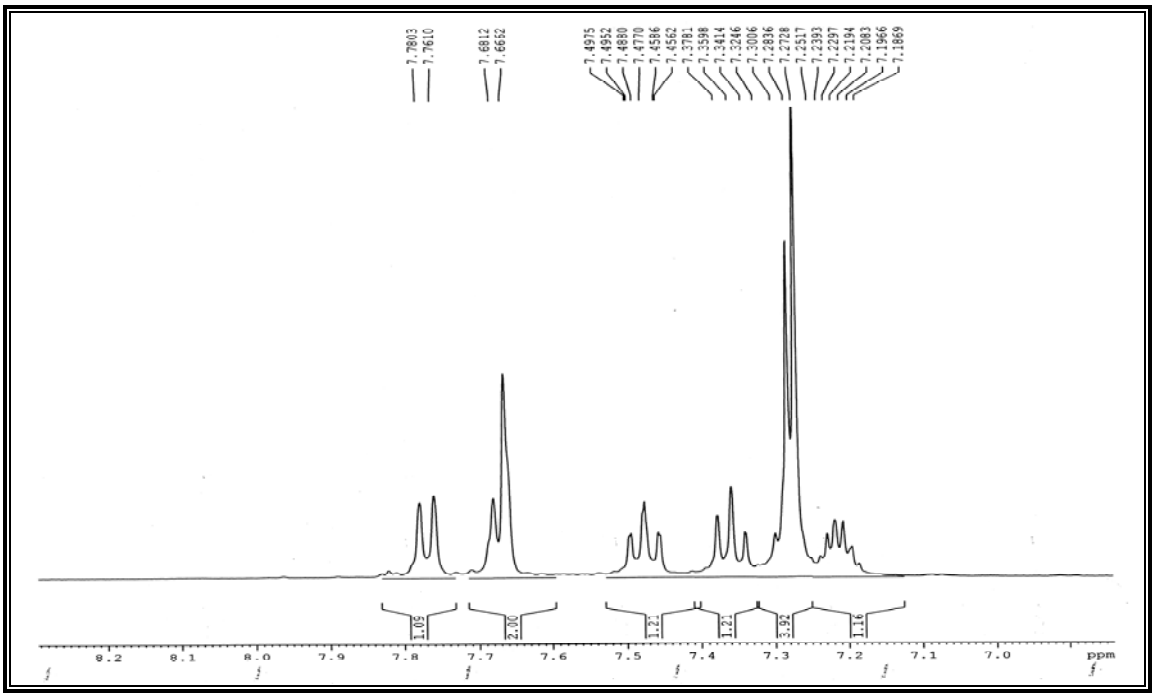


^{13}C NMR spectrum of 2-(Benzofuran-2-yl)-5-((4-phenylpiperazin-1-yl)methyl)-1,3,4-oxadiazole

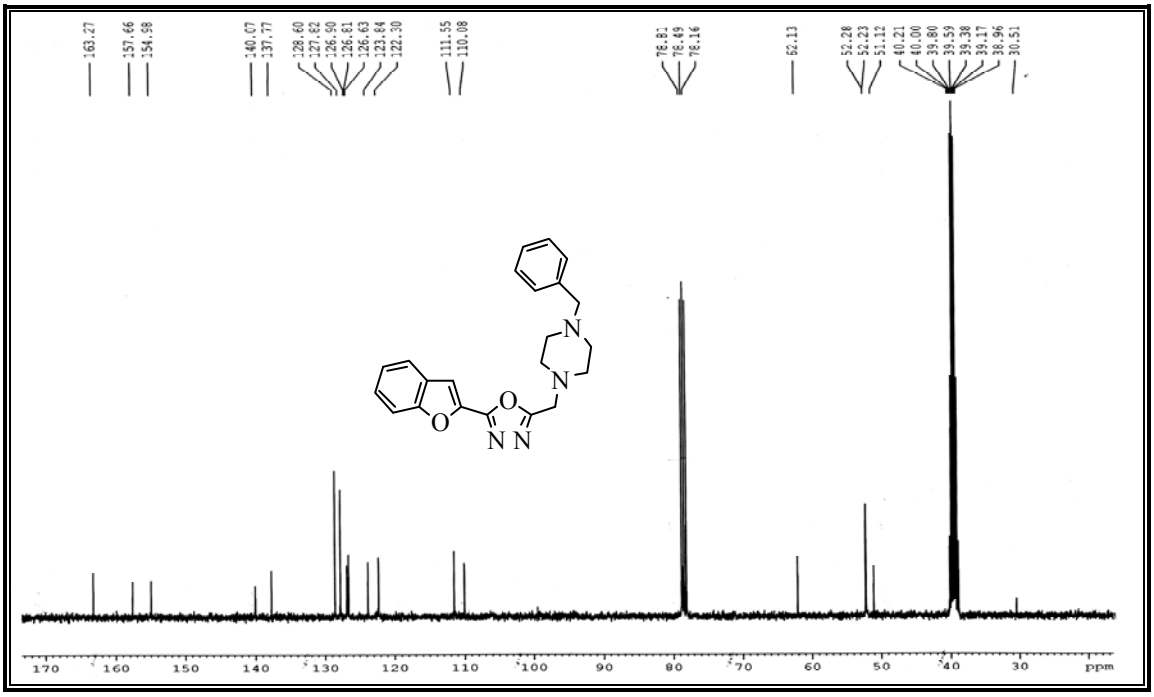


^{1}H NMR spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-benzylpiperazine (DPB-47)

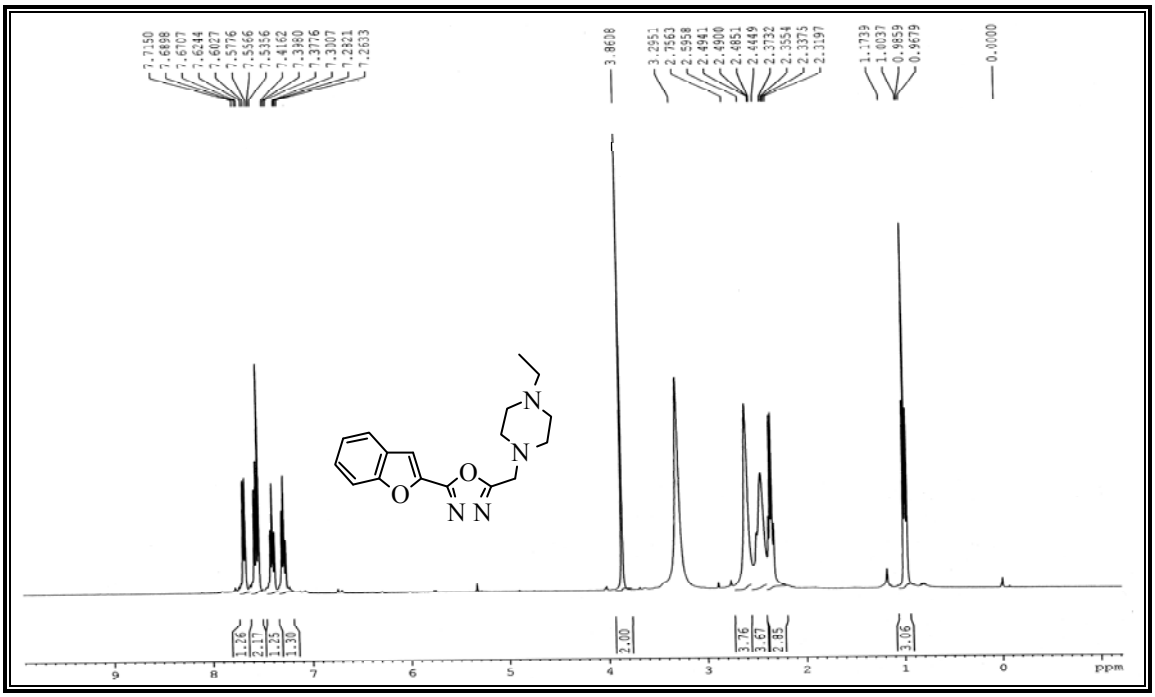


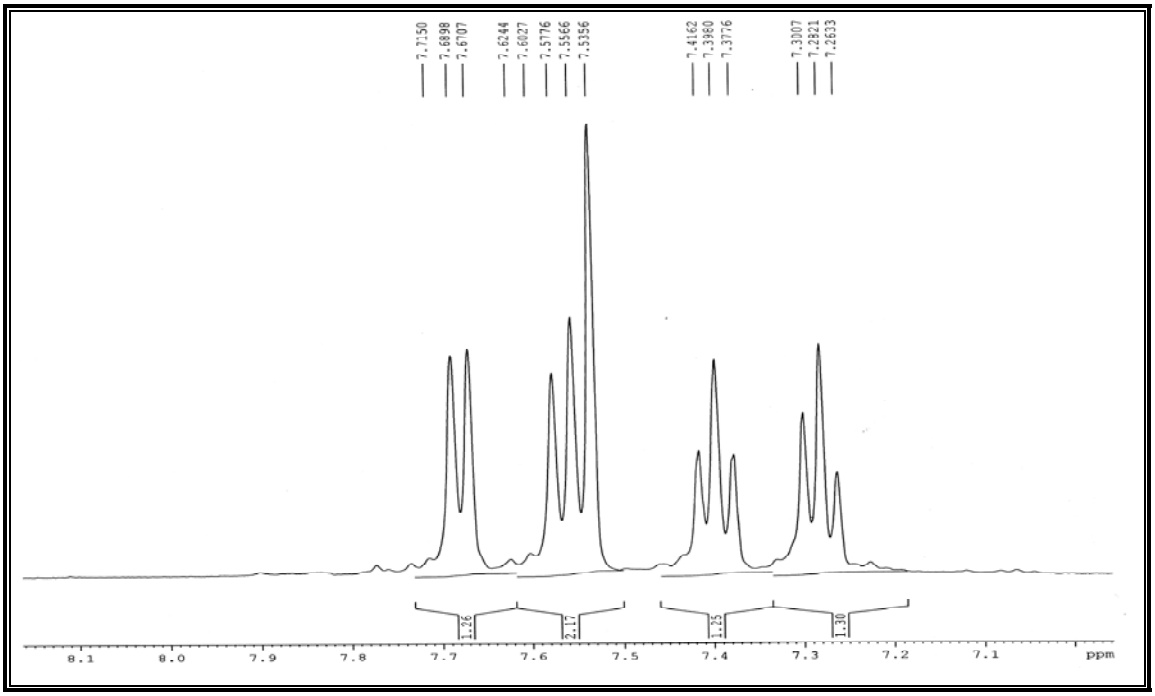


^{13}C NMR spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-benzylpiperazine (DPB-47)

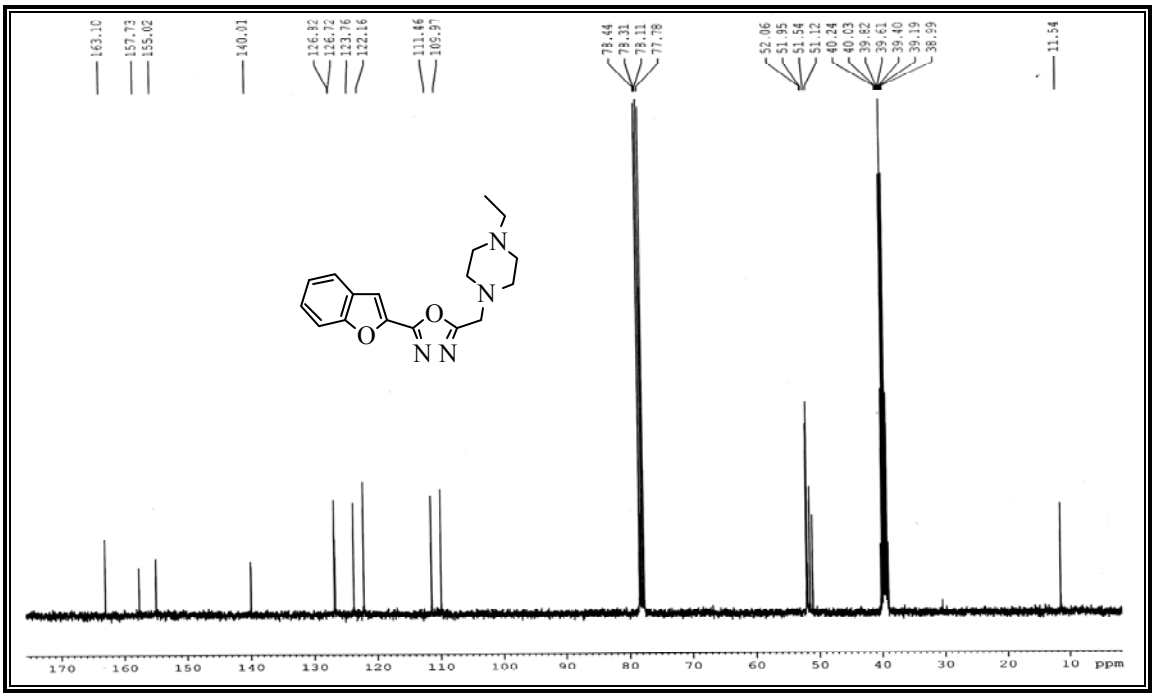


^{1}H NMR spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-ethylpiperazine (DPB-46)

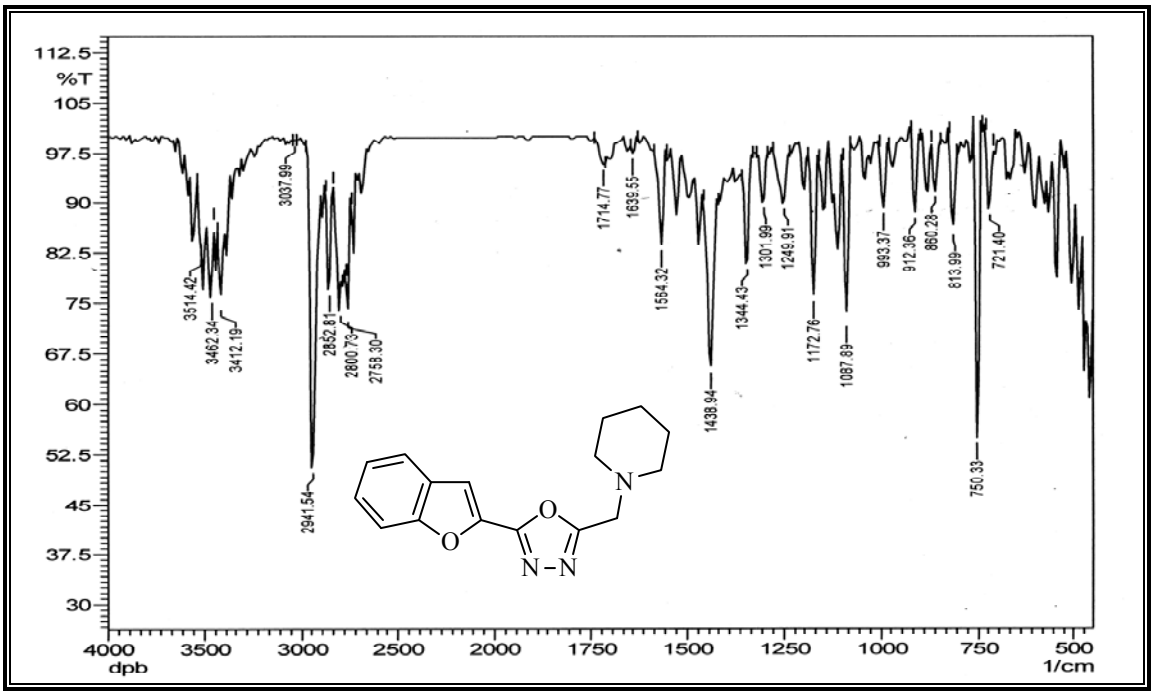




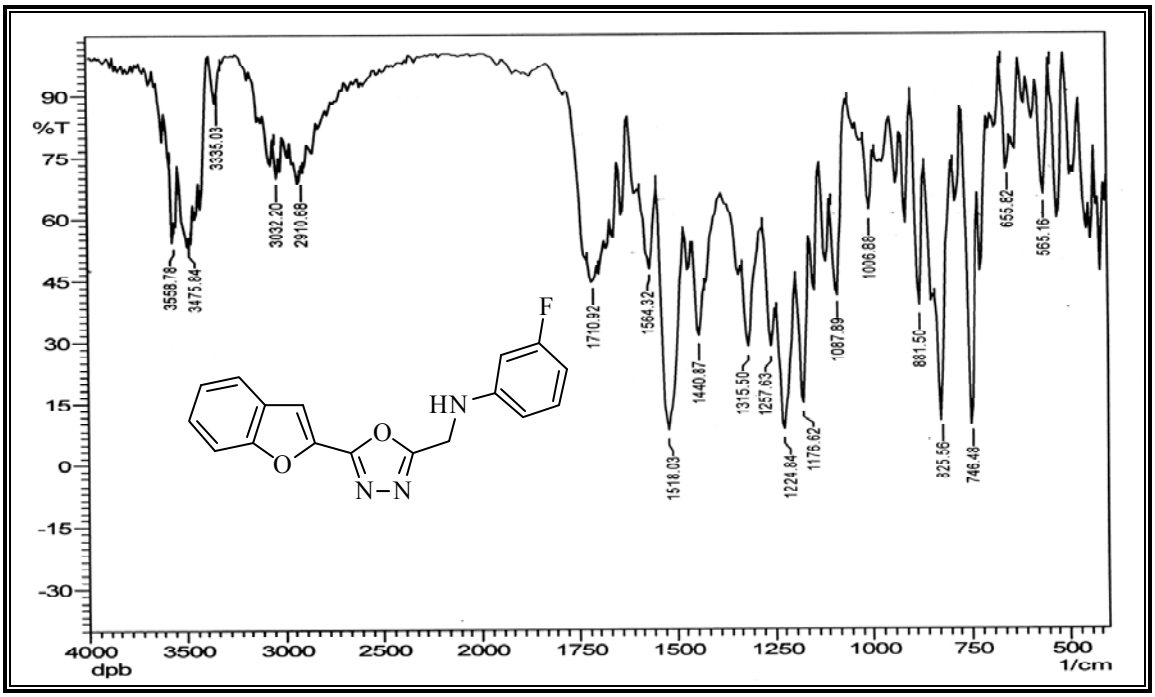
^{13}C NMR spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-4-ethylpiperazine (DPB-46)



IR spectrum of 1-((5-(Benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)piperidine (DPB-42)



IR spectrum of *N*-((5-(benzofuran-2-yl)-1,3,4-oxadiazol-2-yl)methyl)-3-fluorobenzenamine (DPB-61)



3.13 REFERENCE

1. J. Hill, *Comp.Heterocycl.Chem.*, **1984,** *1st edition*, 6427.
2. K. Roda, R. Vansdadia, H. Parekh, *J.Ind.Chem.Soc.* **1988**, 65, 807.
3. V. Adhikari, V. Badiger, *Ind.J.Chem.Sect-B,* **1988**, *27*, 542.
4. K. Manjunatha, B. Poojary, P. Lobo, J. Fernandes, N. Kumari, *European Journal of Medicinal Chemistry,* **2010**, *45(11)*, 5225-5233.
5 M. Azam,S. Afzal, A. Thomas, *Indian Journal of Heterocyclic Chemistry* **2010**, *20(1)*, 77-80.
6. K. Raman, S. Parmar, S. Salzman, *J.Pharm.Sci.*, **1989,** *78*, 999.
7. N. Ergenc, S. Buyuktimkin, G. Capan, G. baktir, S. Rollas, *Pharmazie*, **1991,** *46*, 290.
8 V. Saxena, A. Singh, R. Agarwal, S. Mehra, *J.Ind.Chem.Soc.*, **1983,** *60*, 575.
9. J. Musser *et al*., *J.Med.Chem.*, **1984,** 27, 121.
10. S. Chao, X. Li, S. Wang, *Huaxue Yanjiu Yu Yingyong*, **2010**, *22(8)*, 1066-1071.
11. S. Gilani, S. Khan, N. Siddiqui, *Bioorg. Med. Chem. Lett.*, **2010**, *20(16)*, 4762-4765.
12. S. Bhandari, J. Parikh, K. Bothara, T. Chitre, D. Lokwani, T. Devale, N. Modhave, V. Pawar, S. Panda, *Journal of enzyme inhibition and medicinal chemistry*, **2010**, *25(4)*, 520-530.
13. Gattige Vidya, *PCT Int. Appl*., WO 2009090548, **2009**, 82.
14. G. Bankar, G. Nampurath, P. Nayak, S. Bhattacharya, *Chemico-Biological Interactions,* **2010**, *183(2)*, 327-331.
15. M. Bhat, M. Al-Omar, N. Siddiqui, *Pharma Chemica*, **2010**, *2(2)*, 1-10.
16. Q. Zheng, X. Zhang, Y. Xu, K. Cheng, Q. Jiao, H. Zhu, *Bioorg. Med. Chem.,* **2010**, *18(22)*, 7836-7841.
17. L. Srikanth, U. Naik, R. Jadhav, N. Raghunandan, J. Rao, K. Manohar, *Pharma Chemica,* **2010**, *2(4)*, 231-243.
18. Z. M. Zuhair, J. Ghada, A. Elham, N. Lina, *Jord J. Chem,* **2008**, *3(3)*, 233-43.
19. R. Bankar , K. Nandakumar, G. Nayak, A. Thakur, C. Rao, N. Kutty, *Chemico-Biological Interactions,* **2009**, *181(3)*, 377-382.

20. Wang Bao-Lei, Li Zheng-Ming, Li Yong-Hong, Wang Su-Hua, *Gaodeng Xuexiao Huaxue Xuebao*, **2008**, *29(1)*, 90-94.
21. I. Fumio, K. Jun, K. Hiromi, K. Eiji, S. Morihisa, K. Tomohiro, I. Hiroki, M. Katsuhito, *PCT Int. Appl*. **2008**, 531.
22. K. Sushil, V. Gupta, V. Kashaw, P. Mishra, J. Stables, N. Jain, *Med. Chem. Research.*, **2009**, *38(2)*, 157-159.
23. U. Ghani, N. Ullah, *Bioorg. Med. Chem.*, **2010**, *18(11)*, 4042-4048.
24. C. Chiriac, *Rev.Chim.*, (Bucharest),**34**,1131(1983), *Chem.Abstr.*, **1984,** *100,*174735
25. C. Chiriac, *Rev.Chim.*,(Bucharest),**27**,935(1982), *Chem.Abstr.* **1983**, *98,*107216.
26. B. Rigo, P. Cauliez, D. Fasseur, D. Couturier, *Synth.Commun.*, **1986,** *16,*1665.
27. A. Kalinin, B. Khasapov, E. Aposav, I. Kalikhman, S. Ioffe, *Izv.Akad Nauk SSSR Ser.Khim.* **1984**, 694, *Chem.Abstr.* **1984**, *101,* 91045.
28. A. Theocharis, N. Alexandrou,*J.Heterocycl.Chem.*, **1990,** *27*,1685.
29. M. Elnagdi, N. Ibrahim, F. Abdelrazek, A. Erian, *Liebigs Ann.Chem.*, **1988**, 909.
30. P. Reddy, *Ind.J.Chem.Sect-B,* **1987,** *26,* 890.
31. S. Rekkas, N. Rodias, N. Alexandrou,*Synthesis*, **1986**, 411.
32. H. Baumgarten, D. Hwang, T. Rao, *J.Heterocycl.Chem.*, **1986,** *23*, 945.
33. S. Hiremath, N. Goudar, M. Purohit, *Ind.J.Chem.Sect-B,* **1982,** *21*,321.
34. B. Kubel, *Monatsh Chem.*, **1982,** *113*, 793.
35. A. Hetzheim, G. Mueller, P. Vainilavicius, D. Girdziunaite,*Pharmazie*, **1985,** *40*, 17.
36. J. Dost, M. Heschel, J. Stein, *J.Prakt.Chem.*, **1985,** *327,*109.
37. E. Beriger, W. Eckhardt, *Eur.Pat.*364396, **1990**, *Chem.Abstr.*, **1990,** *113*, 152432.
38. E. Tihanyi, M. Gal, P. Dvortsak, *Heterocycles,* **1983,** *20*, 571.
39. N. Peet, S. Sunder, *J.Heterocycl.Chem.*, **1984,** *21*, 1807.
40. J. Hill,*Comp.Heterocycl.Chem.,*1stEdn., **1984,** *6,* 427.
41. M. Chande, A. Karnik, I. Inamdar, S. Damle, *Ind.J.Chem.*Sect B., **1991,** *30*,430.

Chapter-4

SYNTHESIS AND CHARACTERIZATION OF N'-(2-(SUBSTITUTED BENZAMIDO)THIAZOL-4-YL)-2-PROPYLPENTANEHYDRAZIDE

4.1 INTRODUCTION

Valproic acid is simple branched chain carboxylic acid used in epilepsy. Valproic acid increases GABA (γ-amino butyric acid) synthesis and release and potentiates by this mechanism GABA ergic transmission in specific brain regions. Valproic acid also reduces the release of excitatory amino acid β-hydroxy butyric acid and attenuates neuronal excitation mediated by activation of *N*-methyl-D-aspartames glutamate receptors. Valproic acid is a broad-spectrum antiepileptic drug effective against all seizure types.

Valproic acid (chemical name 2-propylvaleric acid) was first synthesized in 1882 by Burton as an analogue of valeric acid, found naturally in valerian. A clear liquid fatty acid at room temperature, for many decades its only use was in laboratories as a "metabolically inert" solvent for organic compounds. In 1962, the French researcher Pierre Eymard serendipitously discovered the anticonvulsant properties of valproic acid while using it as a vehicle for a number of other compounds that were being screened for anti-seizure activity. He found that it prevented pentylenetetrazol-induced convulsions in rodents. Since then it has also been used for migraine and bipolar disorder.

4.2 VALPROATE: PAST, PRESENT, AND FUTURE

Preclinical studies have been carried out during the past four decades to investigate the different mechanisms of action of valproate (VPA). The mechanisms of VPA which seem to be of clinical importance include increased GABA ergic activity, reduction in excitatory neurotransmission, and modification of monoamines. These mechanisms are discussed in relation to the various clinical uses of the drug. VPA is widely used as an antiepileptic drug with a broad spectrum of activity. In patients, VPA possesses efficacy in the treatment of various epileptic seizures such as absence, myoclonic, and generalized tonic-clonic seizures. It is also effective in the treatment of partial seizures with or without secondary generalization and acutely in status epileptics. The pharmacokinetic aspects of VPA and the frequent drug interactions between VPA and other drugs are discussed. The available methods for the determination of VPA in body fluids are briefly evaluated. At present, investigations

and clinical trials are carried out and evaluated to explore the new indications for VPA in other conditions such as in psychiatric disorders, migraine and neuropathic pain. Furthermore, the toxicity of VPA, both regarding commonly occurring side effects and potential idiosyncratic reactions are described. Derivatives of VPA with improved efficacy and tolerability are in development.

4.3 CHEMICAL STRUCTURE OF VALPROIC ACID AND ITS DERIVATIVES

Molecular formula of valproic acid is $C_8H_{16}O_2$ and molecular weight is 144.2. The derivatives of valproic acid are Sodium valproate molecular formula is $C_8H_{15}NaO_2$ and molecular weight is 166.2, Semi sodium valproate molecular formula is $C_{16}H_{31}NaO_4$ and molecular weight is 310.4, Valproate pivoxil molecular formula is $C_{14}H_{26}O_4$ and molecular weight is 258.4, Valpromide molecular formula is $C_8H_{17}NO$ and molecular weight is 143.2.

Chemical names of Valproic acid:

2-Propylpentanoic acid, 2-Propylvaleric acid, Di-n-dipropylacetic acid

Chemical names of Sodium valproate:

Sodium 2-propylvalerate, Sodium 2-propylpentanoate

Chemical names of Semi sodium valproate:

2-Propylvaleric acid-sodium 2-propylvalerate, Sodium hydrogen bis(2-propylvalerate)

Chemical names of Valproate pivoxil:

Hydroxymethyl 2-propylvalerate pivalate

Chemical names of Valpromide:

Dipropylacetamide, 2-Propylvaleramide

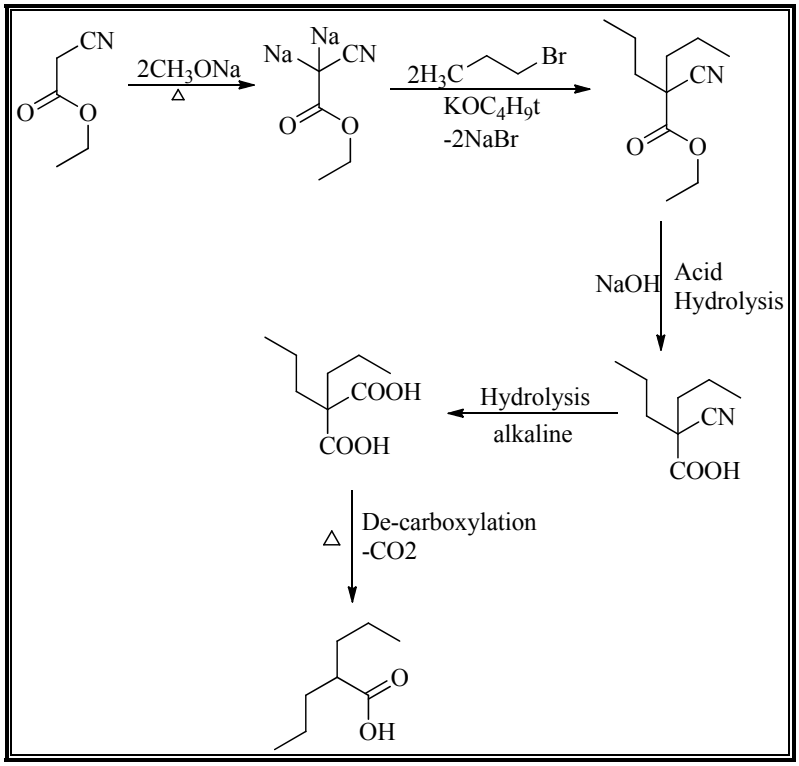
4.4 PHYSICAL PROPERTIES OF VALPROIC ACID

Valproic acid is colorless to pale yellow viscous liquid. It is slightly soluble in water (1.2 mg/mL); fully soluble in acetone, chloroform, ether and methyl alcohol. Valproic acid is stored in airtight containers and is sensitive to light. Valproic acid capsules should be stored at 15 to 30 °C and freezing should be avoided.

4.5 USES OF VALPROIC ACID

Valproic acid is used solely or in combination with other anticonvulsants in the treatment of simple (petit mal) and complex absence seizures. Valproate may be effective against myoclonic and atonics seizures in young children.

4.6 SYNTHESIS OF VALPROIC ACID



4.7 MECHANISM ACTION OF VALPROIC ACID

Valproic acid (VPA) is indicated for the treatment of epilepsy and bipolar disorder and in the prevention of migraine headaches. VPA has also become more widely prescribed due to several off-label indications such as in the treatment of neuropathic pain and cancer.[1,2] Despite VPA being well tolerated and having a low incidence of serious side effects, one concern with VPA therapy is weight gain. A prospective study identified that 37% of female patients with epilepsy developed obesity, as defined as a body mass index (BMI) greater than 25, after 1 yr of treatment with VPA.[3] Numerous retrospective and cross-sectional analyses also report that treatment with VPA is associated with a significant increase in weight ranging from 5 to 49 kg.[4,5,6,7] Studies examining VPA-induced weight gain have been conducted predominantly in adult women because VPA can induce a number of reproductive endocrine abnormalities that include hyperandrogenism, menstrual disturbances, weight gain, and/or polycystic ovaries.[8,9,10] Fifty-two percent of males treated with VPA, however, also have BMI scores within the obesity category,[11] and youth and adolescents treated with VPA are reported to have BMI scores over expected age norms.[12,13,14] Similarly, a prospective double-blind comparison of the incidence and magnitude of weight gain in patients receiving VPA. Lamotrigine monotherapy demonstrated that weight gain was greater for those patients treated with VPA and was significant within 10 wk of treatment onset.[15,16] Weight gain associated with VPA treatment is of great concern due to its physical and psychological consequences.[17] Notably, obesity leads to increase risk for numerous other diseases, such as diabetes mellitus, coronary heart disease,[18] and increased noncompliance with pharmacotherapy in psychiatric patients.[19]

The mechanism underlying VPA-induced weight gain has not been elucidated. Age, gender, medical condition, dose and serum concentrations of VPA and family history of body weight problems are not significantly correlated with the gain in weight associated with VPA treatment.[20,21] In attempts to generate animal models of VPA-induced weight gain, VPA has been shown to induce a significant increase in body weight in female rhesus monkeys;[22] however, we and others have demonstrated that VPA does not cause weight gain in rodents.[23,24,25] The etiology of VPA-induced weight gain is most likely multifactorial because weight is the output of energy homeostasis controlled by many organs that produce and secrete a variety of appetite-

regulating peptides and cytokines that act within the hypothalamus.[26] VPA treatment in humans increases the serum level of two hormones, leptin and insulin, which are produced by the adipose tissue and pancreatic β-cells, respectively. After VPA treatment for 1 yr, 37% of female patients with epilepsy who developed obesity had a 1.8-fold increase in fasting serum insulin and 3.4-fold increase in serum leptin levels. Similarly, in women receiving VPA for treatment of bipolar disorder, insulin and leptin levels were significantly elevated when compared with women receiving lithium.[27] High levels of serum leptin are commonly associated with obesity and could represent a state of leptin resistance.[26,28] The increase in serum leptin associated with weight gain after VPA treatment may be a consequence of the increase in adipose tissue; however, it is also possible that VPA may have a direct effect on leptin secretion from adiposities or may alter leptin signaling and decrease negative feedback. VPA has been shown to have direct effects on hormone secretion from other endocrine cells. For example, an *ex vivo* study using human pancreatic islet cells has shown that VPA can directly increase insulin release.[29] Moreover, VPA can also potentiate androgen production from ovarian theca cells.[30] We previously demonstrated that VPA inhibited mouse 3T3-L1 and human preadipocyte differentiation.[31] Treatment with VPA during adiposeness reduced the protein levels for several key adipocyte-specific transcription factors, including CCAAT/enhancer binding protein (C/EBP)-α, peroxisome proliferators-activated receptor (PPAR)-γ, and steroid regulatory element binding protein (SREBP) 1a.[32] The present work demonstrates that treatment with VPA in mature adiposities significantly reduces leptin mRNA levels and secretion of the leptin protein in a dose and time-dependent manner. These findings were paradoxical because treatment of patients with VPA is associated with increased serum leptin levels. The reduction in leptin secretion from adiposities was not accompanied by alterations in glucose uptake or altered intracellular free fatty acid levels, which are known regulators of leptin secretion. In addition, C/EBP α, PPAR γ, or SREBP1a protein levels did not change with VPA treatment, suggesting the levels of these transcription factors are not responsible for the effect of VPA on leptin expression. Evidence from experiments using actinomycin D (ActD) or cyclohexamide (CHX) show that VPA does not promote degradation of leptin mRNA; however, VPA can alter leptin transcription through an unknown mechanism independent of new protein synthesis. These results show that VPA can

have direct effects on adiposities that may contribute to altered energy balance in patients treated with VPA.

4.8 ANTI-CANCER ACTIVITY OF VALPROIC ACID

The short chain fatty acid valproic acid (VPA, 2-propylpetanoic acid) is approved for the treatment of epilepsia, bipolar disorders and migraine and clinically used for schizophrenia. In 1999, the first clinical anti-cancer trial using VPA was initiated. Currently, VPA is examined in numerous clinical trials for different leukemia's and solid tumor entities. In addition to clinical assessment, the experimental examination of VPA as anti-cancer drug is ongoing and many questions remain unanswered. Although other mechanisms may also contribute to VPA-induced anti-cancer effects, inhibition of histone deacetylases appears to play a central role.

4.9 THE SECOND GENERATION TO VALPROIC ACID (VPA)

Valproic acid, one of the established AEDs (Anti epilepsy drugs), is in animal models the least potent of the major AEDs. However, due to its wide spectrum of antiepileptic activity, VPA is the most prescribed AED.[33,34] Valproic acid is also an effective (and FDA-approved) drug in migraine prophylaxis and in the treatment of bipolar disorder.

Valproic acid is a simple molecule (isooctanoic acid)[35] and, thus, a useful, cheap, and readily available starting material for synthesizing an array of derivatives that can become CNS-active follow-up compounds to VPA. We believe that novel chemical modifications and further development of specific VPA analogues and derivatives will show promising potential in the areas of epilepsy, pain, bipolar disorder, and other related neurological diseases. As VPA is the least potent among the established AEDs, it is possible to develop VPA analogues that will be significantly more potent than the parent compound and will also be nonteratogenic.

There are numerous reports defining the strict structural requirements for the teratogenicity of VPA and its structurally related compounds. [36] Structure-activity relationship studies conducted in mice strains prone to VPA-associated teratogenicity indicate that to be teratogenic, and to cause neural tube defects in mice embryos, VPA analogues and derivatives should contain a tertiary carbon bound to a carboxylic

group, a hydrogen atom, and two alkyl chains. A VPA derivative lacking any one of these structural requirements has the potential to become a nonteratogenic entity.[37] For example, the corresponding CNS-active amide of VPA valpromide (VPD) that has a carboxamide moiety instead of a carboxylic group is not teratogenic. Similarly, the active metabolite of VPA is 2-ene-VPA, which does not have an α-hydrogen to the carboxylic moiety, is also nonteratogenic.[38] Valpromide and 2-ene-VPA are potent anticonvulsant compounds that may represent a novel type of second-generation VPA drug. However, as their fraction metabolized to VPA in humans is greater than 90% (for VPD) and approximately 20% (for 2-ene-VPA), their lack of teratogenicity does not offer a clinical advantage over VPA.

Unlike teratogenicity, the current thinking on VPA induced hepatotoxicity (microvesicular steatosis) is that it is not caused by the parent compound but primarily by VPA metabolite(s) with a terminal double-bond: 4-ene-VPA and 2,4-diene-VPA. These metabolites are further biotransformed to chemically reactive intermediates that bind to cellular macromolecules and enzymes involved in the metabolism of fatty acids. The first step in this cascade is the formation of an acyl-coenzyme A (CoA) thioester leading to depletion of CoA in the liver and, consequently, to hepatotoxicity.[39,42] Designing substituted aliphatic and alicyclic VPA analogues and α and β substituted VPA derivatives (amides) to block the formation of these two metabolites should prevent, or at least minimize, the VPA-induced hepatotoxicity.[43,45]

Structure–activity relationship studies mapped the structural elements of the VPA molecules responsible for the anticonvulsant activity.[46] Subsequent studies showed that constitutional isomers of VPA, such as valnoctic acid (VCA), propylisopropyl acetic acid (PIA), or diisopropylacetic acid (DIA), were less active as anticonvulsants than VPA. However, their respective corresponding amides, propylisopropylacetamide (PID) and diisopropylacetamide (DID), are more potent than VPA. Unlike valproyl esters, VPA amide derivatives act as drugs on their own and not as prodrugs to their corresponding acids.[47] Recent SAR data indicate that a pharmacokinetic-based design is an attractive and feasible approach for the development of nonteratogenic and nonhepatotoxic CNS-active second generation to VPA drugs.

4.10 AIM OF CURRENT WORK

Over and above, the known antiepileptic properties of valproic acids and its salt, renewed interest of these molecules in anticancer[1] and antiviral[2] therapy has led wide interest in newer derivatives of these molecules.

Currently, the leading compounds that are second generation to VPA can be divided into three groups (Figs. 1, 2, and 3) **1)** Alkyl analogues of VPA and their amide derivatives, including chiral and achiral constitutional isomers of VPD (Fig. 1) **2)** Amide derivatives of TMCA, a cyclopropyl analogue of VPA (FIG. 2) **3)** Conjugation products between VPA and neuroinhibitory amino acids: GABA, glycine, taurine, and their corresponding amides (Fig. 3). Some valproic acid drugs & derivatives under preclinical/phase clinical trials.[48,49]

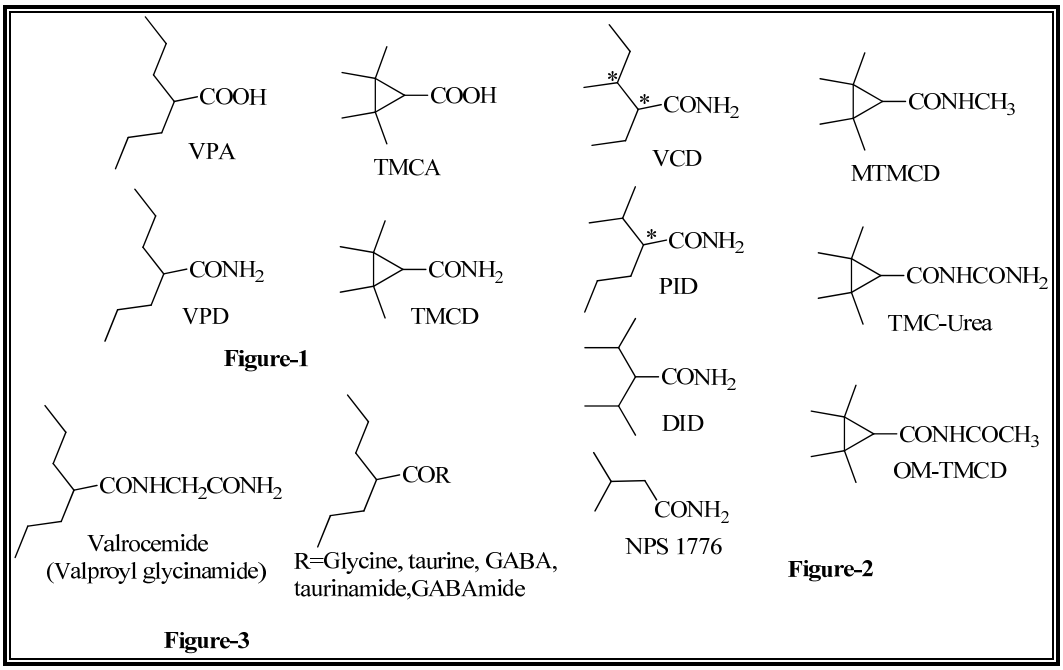
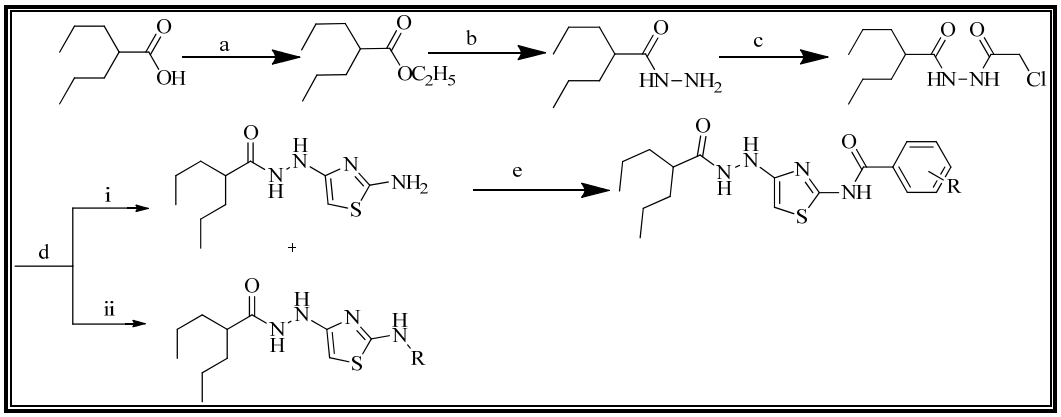


FIG. 1. Valproic acid (VPA), its cyclopropyl analogue 2,2,3,3 tetramethylcyclopropanecarboxylic acid (TMCA) and their corresponding amides valpromide (VPD) and 2,2,3,3–tetramethylcyclopropanecarboxamide (TMCD). **FIG. 2.** Chemical structures of CNS-active amides of valproic acid (VPA) analogues with the potential to become second-generation VPA drugs. Valnoctamide (VCD), propylisopropylacetamide (PID), di-isopropylacetamide (DID), *N*-methyl-2,2,3,3-tetramethylcylclopropanecarboxamide (MTMCD), 2,2,3,3-tetramethylcyclopropylcarbonyl urea (TMC-urea), *N*-methoxy–2,2,3,3-tetramethylcylclopropanecarbonylurea (OM-TMCD), and isovaleramide (NPS 1776). * Indicates the chiral center. **FIG. 3.** Valrocemide and conjugation products between valproic acid and neuroinhibitory amino acids and their corresponding amides.

[1] Current Pharmaceutical Design, **2007**, *13(33)*, 3378-3393.
[2] Clin Pharmacokinet, **1996** , *30(5)*, 385-401.

In the current chapter, the valproic 'Core' structure was used as a starting material to synthesize valproic acid containing thiazole derivatives. The reaction of valproate with hydrazine hydrates and followed by chloroacetyal chloride and thiourea afforde the desired substituted thiazole derivatives.

4.11 REACTION SCHEME



a) CH_3OH, gla.CH_3COOH, 60-70 °C

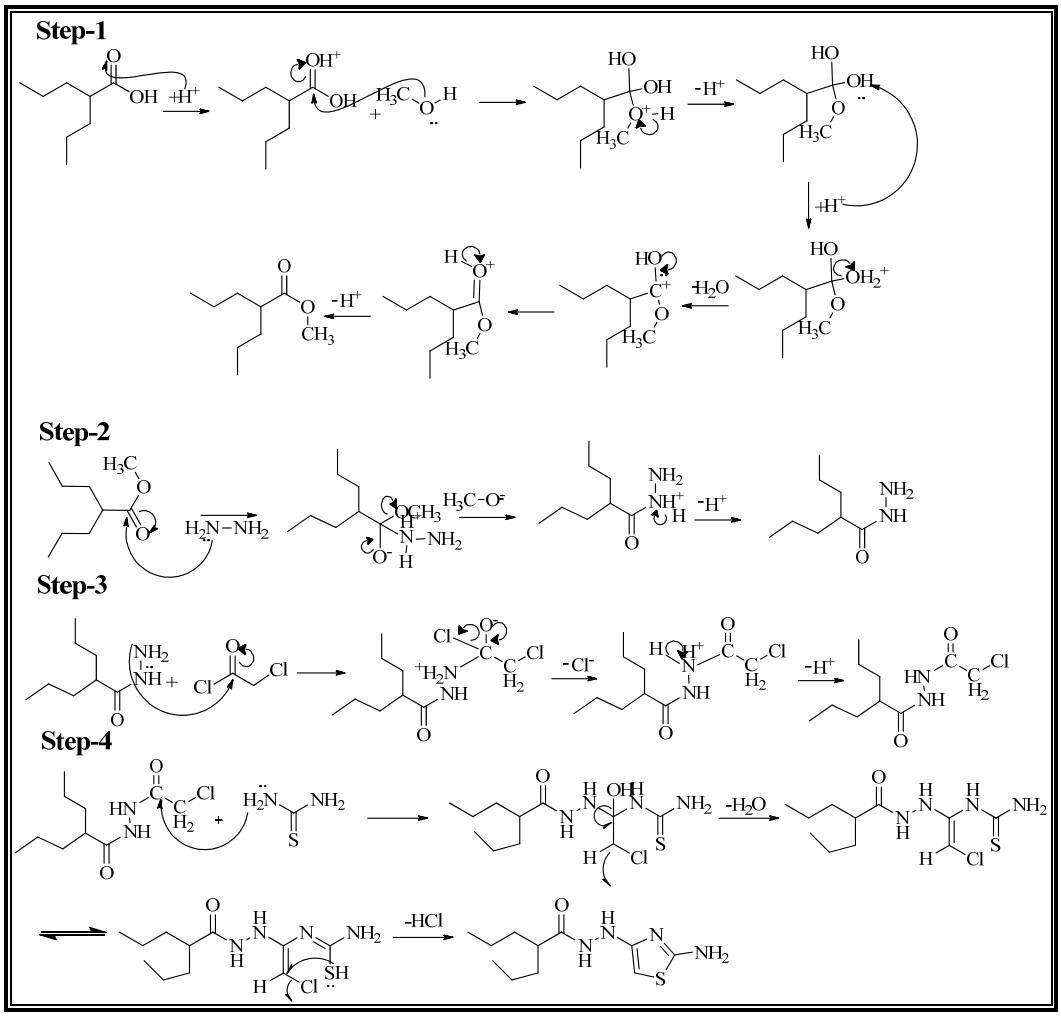
b) NH_2-NH_2, 110 °C

c) DMF,TEA, $ClCH_2COCl$, 0-5 °C

d) (i) Thiourea, CH_3OH, 65 °C
(ii) Thiourea derivatives, CH_3OH, 65 °C

e) DMF,TEA, Substituted acid chloride, 0-5 °C

4.12 REACTION MECHANISM



4.13 EXPERIMENTAL

❖ Preparation of Methyl 2-propyl pentanoate

2-Propyl pentanoic acid (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 h on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction was completed, excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml × 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml × 2) followed by water (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 90 %, B. P. - 170-172 °C.[1]

[1] I. Dostovalova; Organic Magnetic Resonance **1983**, *21(1)*, 111-19.

❖ Preparation of 2-Propyl pentanohydrazide

Methyl 2-propylpentanoate (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 h. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white colored shining fluffy product. Yield - 60 %, M. P. - 124-126 °C. [2]

[2] Benoit-Guyod, L. Jean; Chemica Therapeutica **1968**, *3(5)*, 336-42.

❖ Preparation of *N*'-(2-chloroacetyl)-2-propyl pentanehydrazide

2-Propylpentanohydrazide (0.01 mole) was charged in 10 ml of tetrahydrofuran into 250 ml round bottom flask. Then add triethylamine (0.015 mole) and chloroacetyl chloride (0.01 mole) at 0-5 °C. The reaction mixture was stirred at room temperature overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (4: 6) as a mobile phase.

After completion of the reaction, the reaction mixture was poured into crushed ice. *N*'-(2-chloroacetyl)-2-propylpentanehydrazide A brown colored solid product.

Note: The entire reaction was carried out under nitrogen atmosphere.

- **General procedure of *N*'-(substituted 2-aminothiazol-4-yl)-2-propylpentanehydrazide**

N'-(2-chloroacetyl)-2-propyl pentanehydrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of methanol was added to dissolve it. Then add 0.015 mole of substituted thiourea. Resulting reaction mixture was reflux at 2-3 h. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (3:7) as a mobile phase. After the reaction was complete the mixture was poured into crushed ice to give white solid compound.

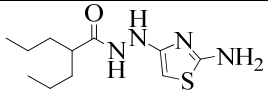
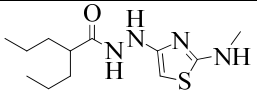
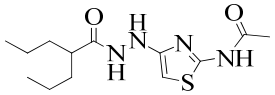
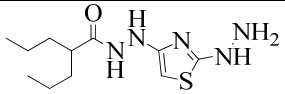
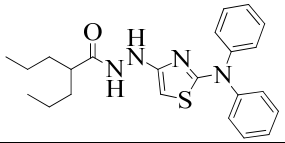
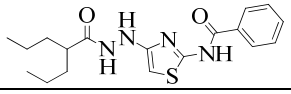
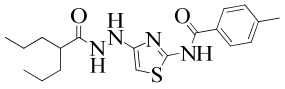
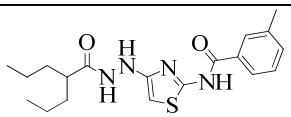
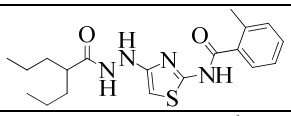
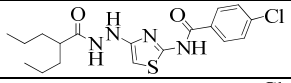
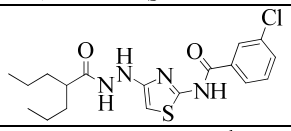
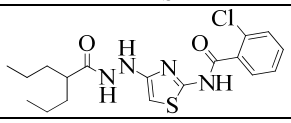
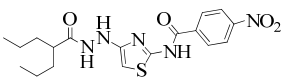
- **General procedure of *N*'-(2-(substituted benzamido)thiazol-4-yl)-2-propyl-pentanehydrazide**

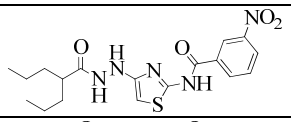
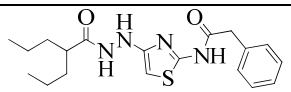
N'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of tetrahydrofuran was added to dissolve it. Add 0.015 mole of triethylamine as a catalyst then added 0.01 mole of substituted acid chlorides at 0-5 $^{\circ}$C. The reaction mixture was stirred at RT overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (3: 7) as a mobile phase. After the reaction was complete mixture poured into crushed ice. *N*'-(2-(substituted benzamido)thiazol-4-yl)-2-propylpentanehydrazide a brown colored solid product.

Note: The entire reaction was carried out under nitrogen atmosphere.

4.14 PHYSICAL DATA

TABLE: 1 PHYSICAL DATA OF *N*'-(2-(SUBSTITUTED BENZAMIDO) THIAZOL-4-YL)-2-PROPYLPENTANEHYDRAZIDE DERIVATIVES

Sr. No	Substituted	M.F.	M. P (^{o}C)	R_f value	% Yield
DPB-67		$C_{11}H_{20}N_4OS$	150-152	0.32	81
DPB-68		$C_{12}H_{22}N_4OS$	163-165	0.38	75
DPB-69		$C_{13}H_{22}N_4O_2S$	142-146	0.36	72
DPB-70		$C_{11}H_{21}N_5OS$	182-184	0.28	62
DPB-71		$C_{23}H_{28}N_4OS$	225-227	0.42	69
DPB-72		$C_{18}H_{24}N_4O_2S$	189-191	0.38	72
DPB-73		$C_{19}H_{26}N_4O_2S$	195-197	0.35	67
DPB-74		$C_{19}H_{26}N_4O_2S$	193-195	0.36	69
DPB-75		$C_{19}H_{26}N_4O_2S$	197-199	0.35	73
DPB-76		$C_{18}H_{23}ClN_4O_2S$	183-185	0.41	79
DPB-77		$C_{18}H_{23}ClN_4O_2S$	180-182	0.39	75
DPB-78		$C_{18}H_{23}ClN_4O_2S$	186-188	0.42	76
DPB-79		$C_{18}H_{23}N_5O_4S$	175-177	0.39	66

DPB-80		$C_{18}H_{23}N_5O_4S$	179-181	0.41	63
DPB-81		$C_{19}H_{26}N_4O_2S$	196-198	0.35	70

R_f value was calculated using solvent system, Toluene**:** Ethyl Acetate (3**:** 7).

4.15 SPECTRAL STUDY

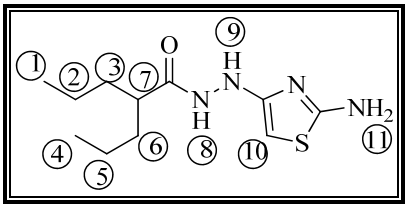
❖ IR spectra

Infra Red spectra were taken on **SHIMADZU FTIR-435** spectrometer using KBr pellet method. The characteristic carbonyl group of –CONH in valporic acid moiety was observed at 1690-1630 cm^{-1}. Amine (>NH) observed a broad peak between 3200-3000 cm^{-1}. Methylene gp (>CH_2) observed at 3000-2850 cm^{-1}. Methyl (-CH_3) observed at 1350 cm^{-1}. DPB- 68 and DPB-69 of IR spectra are given on page no: - 138 and 139.

❖ ^{1}H NMR spectra

^{1}H NMR spectra were recorded on a **Bruker AC 400 MHz FT-NMR** spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d_6 & $CDCl_3$ as a solvent. In the NMR spectra of *N*'-(2-(substituted benzamido) thiazol-4-yl)-2-propylpentanehydrazide various proton values of methylene (-CH_2), amine (-NH) and methyl (-CH_3) etc. were observed as under.

The values for methyl (-CH_3) proton is observed between 0.8-1.3 δ ppm.The values for methylene (-CH_2) proton is observed between 1.3-2.2 δ ppm. The -NH protons of amide group (>CONH) at 7.0-11.0 δ ppm. The signal due to NH proton of amide group was observed at 10.0-10.5 δ ppm value. DPB-67 and DPB-69 of ^{1}H NMR spectra are given on page no: - 135 to 138.



1. The proton no.11 of amine group gave a characteristic broad singlet at 5.38 δ ppm.

2. The proton no.10 of thiazole ring gave a characteristic singlet at 3.88 δ ppm.

3. Proton no. 1and 4 of propyl chain of two methyl group of six proton gave a multiplet at 0.85 δ ppm - 0.89 δ ppm.

4. Proton no. 2, 3, 5 and 6 of di-propyl chain gave a multiplet at 1.25 δ ppm - 1.38 δ ppm. It showed expanded spectra.

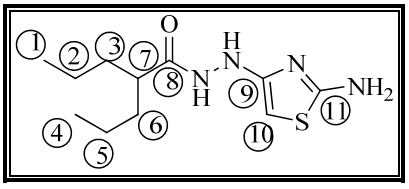
5. Proton no. 7 of di-propyl chain gave a multiplet at 2.27 δ ppm-2.32 δ ppm.

6. Two most deshielded proton no.8 and 9 of secondary amine in hydrazide linkage of two (-NH) group gave two separable singlet in the down field at 7.91 δ ppm and 10.02 δ ppm respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound DPB-67 was confirmed.

❖ **^{13}C NMR spectra**

^{13}C NMR spectra were recorded on a **Bruker AC 400 MHz FT-NMR** spectrometer using DMSO-d_6 & $CDCl_3$ as a solvent. In the ^{13}C NMR spectra of *N*'-(2-(substituted benzamido)thiazol-4-yl)-2-propylpentanehydrazide various carbon values of methylene (-CH_2), keto (>C=O), methyl (-CH_3) and aromatic carban (Ar-H) etc. were observed as under. The values for methylene (-CH_2) carban is observed between δ 35-65 ppm. The >C=O carban observed at 160-180 δ ppm. Aromatic carbon shows between 110-140 δ ppm. DPB-67 and DPB-69 of ^{13}C NMR spectra are given on page no: - 135 to 138.



1. The carbon no. 1 and 4 methyl group, appear at 13.7 δ ppm it shows in spectra.
2. The carbon no. 2 and 5 methylene group, appear at 20.12 δ ppm.
3. The carbon no. 3 and 6 methylene group, appear at 34.8 δ ppm.
4. The carbon no. 7 appears at 43.55 δ ppm it shows in spectra.
5. The carbon no. 8 appears at 176.6 δ ppm due to the effect of carbonyl group.
6. The carbon no. 9 and 11 thiazole ring appear at 173.1 δ ppm due to the effect of nitrogen atom.
7. The carbon no. 10 thiazole ring appears at 99.49 δ ppm.

❖ **Mass spectra**

The mass spectrum of compounds were recorded by **Shimadzu GC-MS-QP-2010** spectrometer (EI method). The mass spectrum of compounds was obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectral study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized. DPB- 67, DPB-68 and DPB-69 of Mass spectra are given on page no.- 134 and 135.

❖ **Elemental analysis**

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** model at Saurashtra University, Rajkot which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The elemental analysis data are given for individual compounds.

4.16 SPECTRAL CHARACTERIZATION

N'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)

IR (KBr) cm^{-1}: 3446 (N-H str), 2956 (-CH_3 str.), 2931 (-CH_2 str.), 2874 (-CH_3 str.), 1640 (-CONH), 1608 (N-H bending), 1452 (-CH_3 ben), 1367 (-CH_2 ben). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 0.89 (m, 6H, -CH_3), 1.32 (m, 6H, -CH_2), 1.57 (m, 2H, -CH_2), 2.29 (m, 1H, -CH), 3.88 (s, 1H, -CH), 5.38 (s, broad, 2H,-NH_2), 7.91 (s, broad, 1H,-NH), 10.02 (s, broad, 1H,-NH). **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 13.78, 20.12, 34.85, 43.88, 99.49, 173.1, 176.6 **Mass: [m/z (%)], M. Wt.:** 256. **Elemental analysis, Calculated:** C, 51.53; H, 7.86; N, 21.85 Found: C, 51.59; H, 7.79; N, 21.70

N'-(2-(methylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-68)

IR (KBr) cm^{-1}: 3408 (N-H str), 2924 (-CH_3 str.), 287 (-CH_2 str.), 2818 (-CH_3 str.), 1653 (-CONH), 1602 (N-H bending), 1481 (-CH_3 ben), 1369 (-CH_2 ben). **Mass: [m/z (%)], M. Wt.:** 270. **Elemental analysis, Calculated:** C, 53.30; H, 8.20; N, 20.72 Found: C, 53.32; H, 8.28; N, 20.65

N'-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)

IR (KBr) cm^{-1}: 3489 (N-H str), 2963 (-CH_3 str.), 2870 (-CH_2 str.), 2775 (-CH_3 str.), 1664 (-CONH), 1631 (N-H bending), 1447 (-CH_3 ben), 1371 (-CH_2 ben). **^{1}H NMR 400 MHz: (DMSO-d_6, δ ppm):** 0.89 (m, 6H, -CH_3), 1.33 (m, 6H, -CH_2), 1.61 (m, 2H, -CH_2), 2.32 (m, 1H, -CH), 3.20 (s, 3H, -CH_3), 3.86 (s, 1H, -CH), 7.78 (s, broad, 1H,-NH), 9.93 (s, broad, 1H,-NH), 11.66 (s, broad, 1H,-NH). **^{13}C NMR 400 MHz: (DMSO-d_6, δ ppm):** 13.70, 20.15, 34.56, 43.76, 173.2 **Mass: [m/z (%)], M. Wt.:** 298. **Elemental analysis, Calculated:** C, 52.32; H, 7.43; N, 18.78 Found: C, 52.22; H, 7.49; N, 18.85

N'-(2-hydrazinylthiazol-4-yl)-2-propylpentanehydrazide (DPB-70)

IR (KBr) cm^{-1}: 3455 (N-H str), 2947 (-CH_3 str.), 2952 (-CH_2 str.), 2849 (-CH_3 str.), 1641 (-CONH), 1625 (N-H bending), 1452 (-CH_3 ben), 1375 (-CH_2 ben). **Mass: [m/z (%)], M. Wt.:** 271. **Elemental analysis, Calculated:** C, 48.68; H, 7.80; N, 25.81 Found: C, 48.65; H, 7.77; N, 25.89

N'-(2-(diphenylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-71)

IR (KBr) cm^{-1}: 3445 (N-H str), 2951 (-CH_3 str.), 2945 (-CH_2 str.), 2852 (-CH_3 str.), 1658 (-CONH), 1630 (N-H bending), 1458 (-CH_3 ben), 1371 (-CH_2 ben), 3011 (=C-

H, str), 3045 (Ar, C-H, str), 1529 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 408. **Elemental analysis, Calculated:** C, 67.61; H, 6.91; N, 13.71 Found: C, 67.66; H, 6.85; N, 13.68

N'-(2-(benzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-72)

IR (KBr) cm^{-1}: 3440 (N-H str), 2959 (-CH_3 str.), 2947 (-CH_2 str.), 2859 (-CH_3 str.), 1648 (-CONH), 1636 (N-H bending), 1457 (-CH_3 ben), 1372 (-CH_2 ben), 3018 (=C-H, str), 3055 (Ar, C-H, str), 1535 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 360. **Elemental analysis, Calculated:** C, 59.97; H, 6.71; N, 15.54 Found: C, 59.95; H, 6.69; N, 15.50

N'-(2-(4-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-73)

IR (KBr) cm^{-1}: 3441 (N-H str), 2962 (-CH_3 str.), 2941 (-CH_2 str.), 2862 (-CH_3 str.), 1642 (-CONH), 1639 (N-H bending), 1462 (-CH_3 ben), 1370 (-CH_2 ben), 3022 (=C-H, str), 3053 (Ar, C-H, str), 1545 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 374. **Elemental analysis, Calculated:** C, 60.94; H, 7.00; N, 14.96 Found: C, 60.97; H, 7.03; N, 14.98

N'-(2-(3-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-74)

IR (KBr) cm^{-1}: 3439 (N-H str), 2955 (-CH_3 str.), 2953 (-CH_2 str.), 2849 (-CH_3 str.), 1649 (-CONH), 1645 (N-H bending), 1478 (-CH_3 ben), 1375 (-CH_2 ben), 3010 (=C-H, str), 3049 (Ar, C-H, str), 1549 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 374. **Elemental analysis, Calculated:** C, 60.94; H, 7.00; N, 14.96 Found: C, 60.98; H, 7.04; N, 14.92

N'-(2-(2-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-75)

IR (KBr) cm^{-1}: 3446 (N-H str), 2956 (-CH_3 str.), 2951 (-CH_2 str.), 2855 (-CH_3 str.), 1647 (-CONH), 1641 (N-H bending), 1470 (-CH_3 ben), 1369 (-CH_2 ben), 3015 (=C-H, str), 3050 (Ar, C-H, str), 1552 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 374. **Elemental analysis, Calculated:** C, 60.94; H, 7.00; N, 14.96 Found: C, 60.95; H, 7.05; N, 14.98

N'-(2-(4-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-76)

IR (KBr) cm^{-1}: 3442 (N-H str), 2958 (-CH_3 str.), 2956 (-CH_2 str.), 2849 (-CH_3 str.), 1648 (-CONH), 1647 (N-H bending), 1465 (-CH_3 ben), 1371 (-CH_2 ben), 3016 (=C-H, str), 3051 (Ar, C-H, str), 1549 (Ar, C=C, str), 742 (C-Cl). **Mass: [m/z (%)], M.**

Wt.: 394. **Elemental analysis, Calculated:** C, 54.74; H, 5.87; N, 14.19 Found: C, 54.76; H, 5.89; N, 14.22

N'-(2-(3-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-77)

IR (KBr) cm^{-1}: 3441 (N-H str), 2959 (-CH_3 str.), 2957 (-CH_2 str.), 2852 (-CH_3 str.), 1656 (-CONH), 1650 (N-H bending), 1462 (-CH_3 ben), 1378 (-CH_2 ben), 3010 (=C-H, str), 3056 (Ar, C-H, str), 1552 (Ar, C=C, str), 748 (C-Cl). **Mass: [m/z (%)], M. Wt.:** 394. **Elemental analysis, Calculated:** C, 54.74; H, 5.87; N, 14.19 Found: C, 54.70; H, 5.83; N, 14.20

N'-(2-(2-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-78)

IR (KBr) cm^{-1}: 3448 (N-H str), 2958 (-CH_3 str.), 2953 (-CH_2 str.), 2848 (-CH_3 str.), 1648 (-CONH), 1644 (N-H bending), 1460 (-CH_3 ben), 1376 (-CH_2 ben), 3021 (=C-H, str), 3041 (Ar, C-H, str), 1549 (Ar, C=C, str), 747 (C-Cl). **Mass: [m/z (%)], M. Wt.:** 394. **Elemental analysis, Calculated:** C, 54.74; H, 5.87; N, 14.19 Found: C, 54.69; H, 5.84; N, 14.23

N'-(2-(4-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-79)

IR (KBr) cm^{-1}: 3452 (N-H str), 2955 (-CH_3 str.), 2954 (-CH_2 str.), 2847 (-CH_3 str.), 1648 (-CONH), 1640 (N-H bending), 1463 (-CH_3 ben), 1374 (-CH_2 ben), 3025 (=C-H, str), 3047 (Ar, C-H, str), 1556 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 405. **Elemental analysis, Calculated:** C, 53.32; H, 5.72; N, 17.27 Found: C, 53.35; H, 5.70; N, 17.30

N'-(2-(3-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-80)

IR (KBr) cm^{-1}: 3442 (N-H str), 2958 (-CH_3 str.), 2956 (-CH_2 str.), 2849 (-CH_3 str.), 1652 (-CONH), 1647 (N-H bending), 1461 (-CH_3 ben), 1370 (-CH_2 ben), 3016 (=C-H, str), 3059 (Ar, C-H, str), 1549 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 405. **Elemental analysis, Calculated:** C, 53.32; H, 5.72; N, 17.27 Found: C, 53.30; H, 5.71; N, 17.29

N'-(2-(2-phenylacetamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-81)

IR (KBr) cm^{-1}: 3452 (N-H str), 2955 (-CH_3 str.), 2951 (-CH_2 str.), 2856 (-CH_3 str.), 1640 (-CONH), 1644 (N-H bending), 1461 (-CH_3 ben), 1375 (-CH_2 ben), 3022 (=C-H, str), 3045 (Ar, C-H, str), 1549 (Ar, C=C, str). **Mass: [m/z (%)], M. Wt.:** 374.

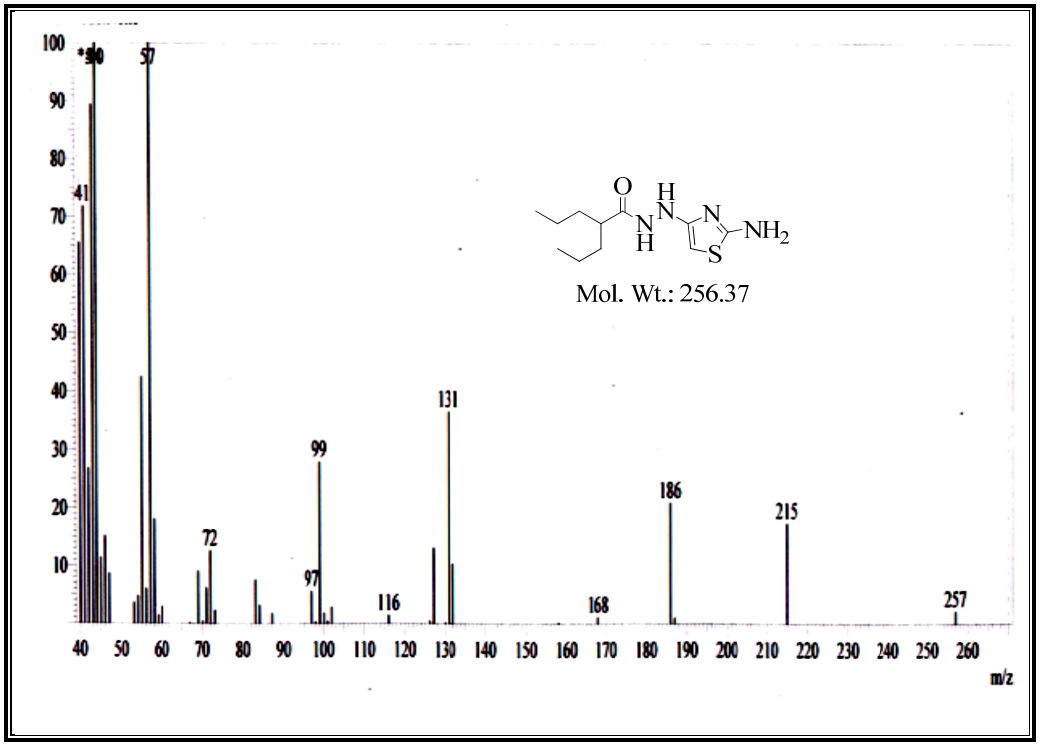
Elemental analysis, Calculated: C, 60.94; H, 7.00; N, 14.96 Found: C, 60.95; H, 7.03; N, 14.98

4.17 CONCLUSION

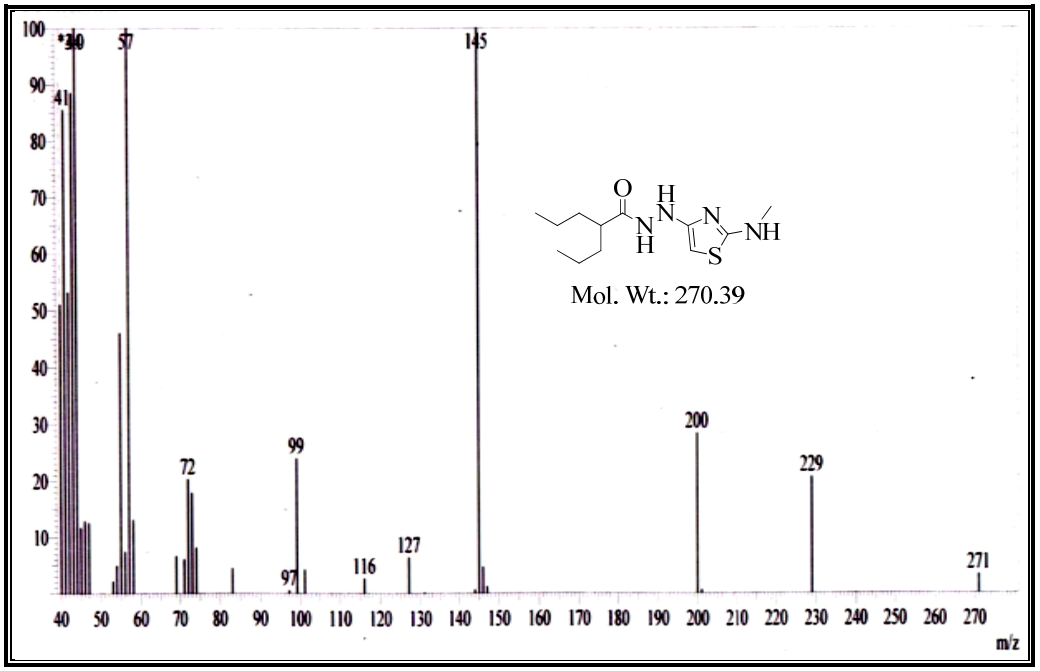
In conclusion, several novel valproate containing thiazole derivatives for biological activity were synthesized. The reaction of valproate with hydrazine hydrate afforded the 2-propyl pentanohydrazide, which on reaction with chloroacetyl chloride yielded the *N*'-(2-chloroacetyl)-2-propyl pentanehydrazide. The desired thiazole derivatives had been synthesized by the reaction of thiourea with *N*'-(2-chloroacetyl)-2-propyl pentanehydrazide. All the newly synthesized compounds were well characterized by spectroscopy techniques.

4.18 REPRESENTATIVE SPECTRUM

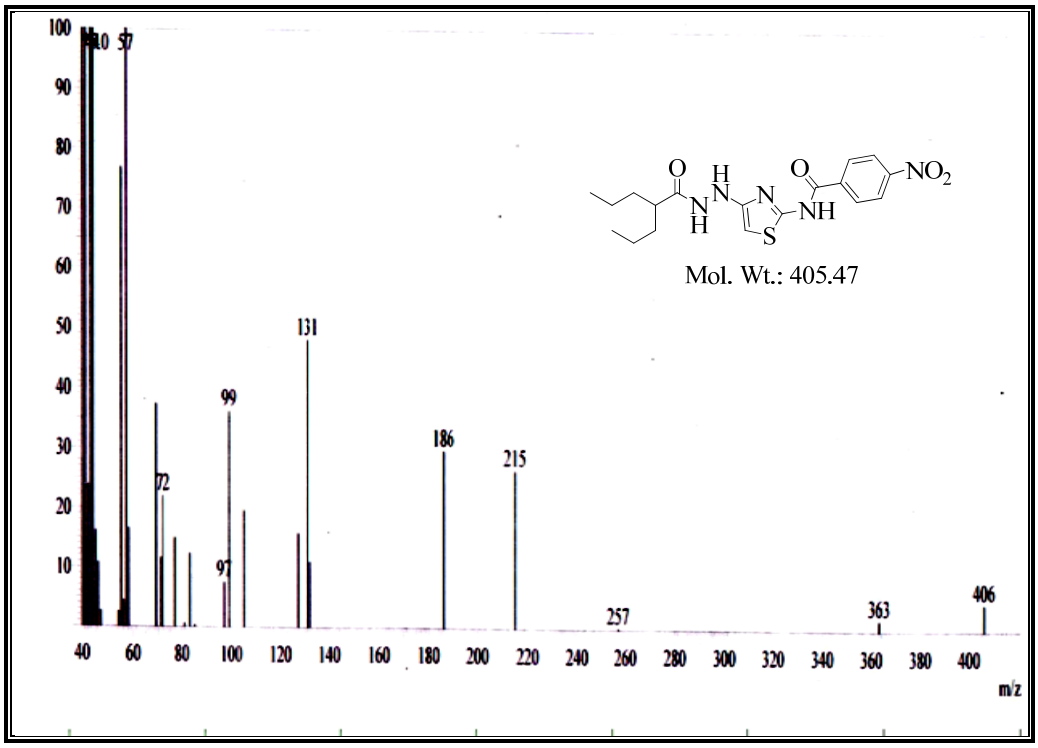
Mass spectrum of *N*'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)



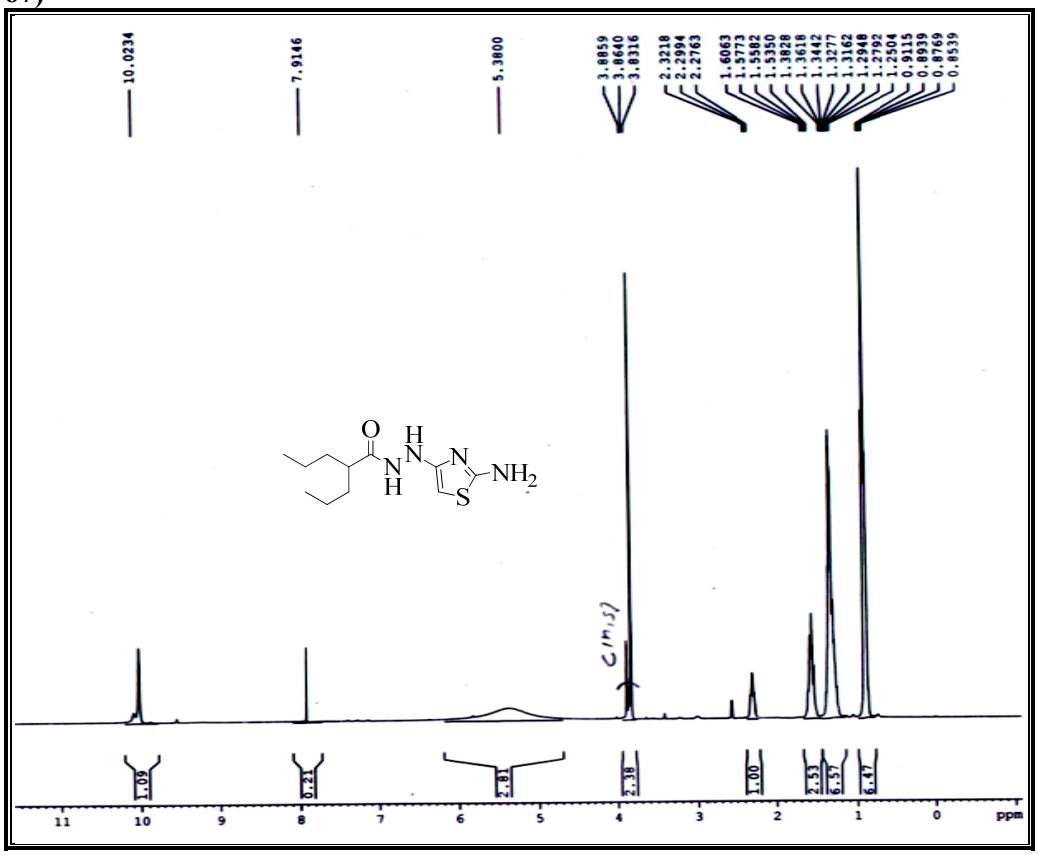
Mass spectrum of *N*'-(2-(methylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-68)

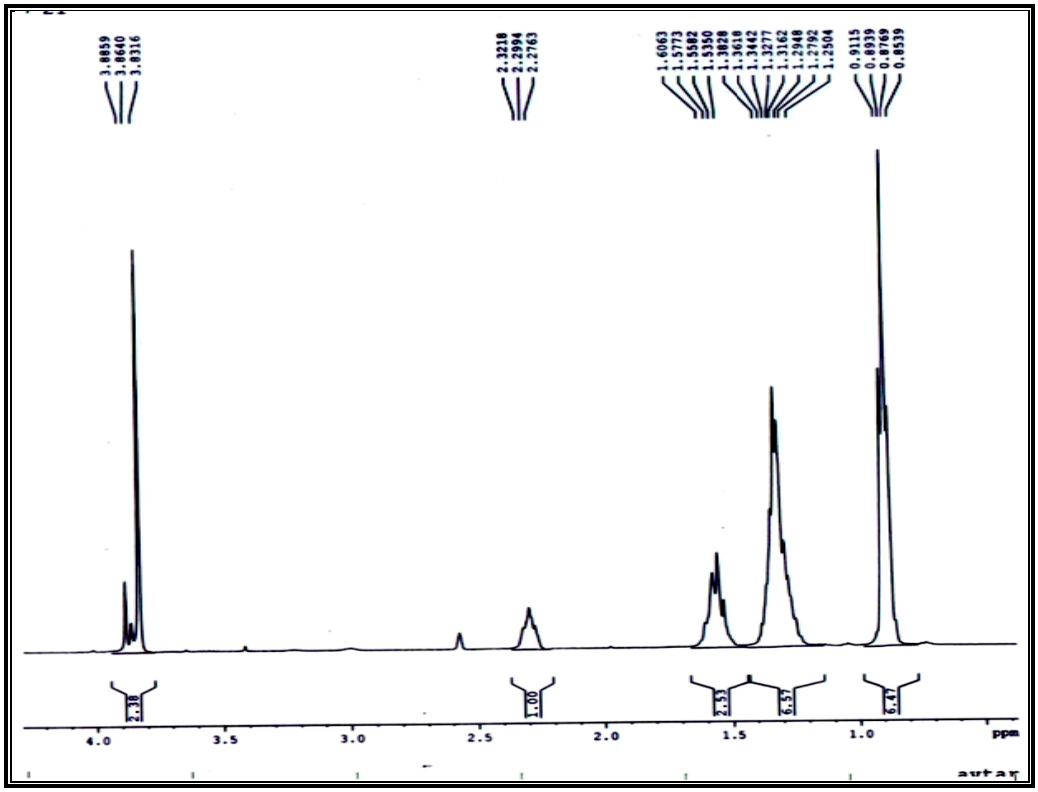


Mass spectrum of *N*'-(2-(4-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-79)

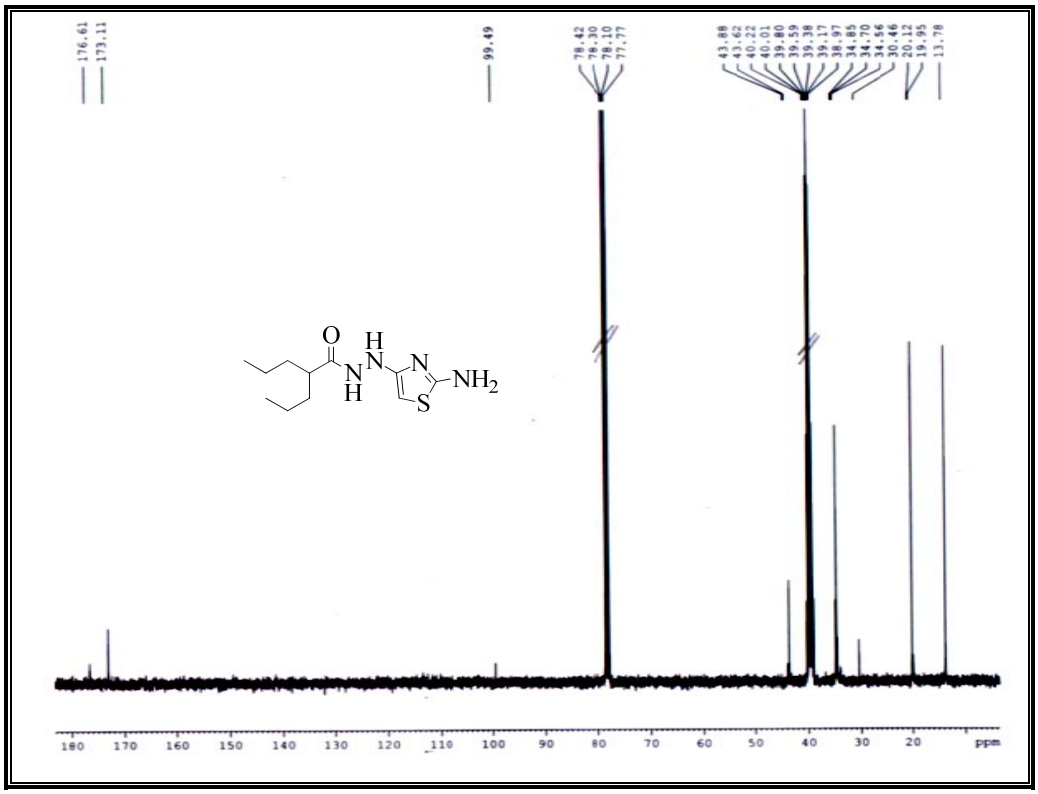


^{1}H NMR spectrum of *N*'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)

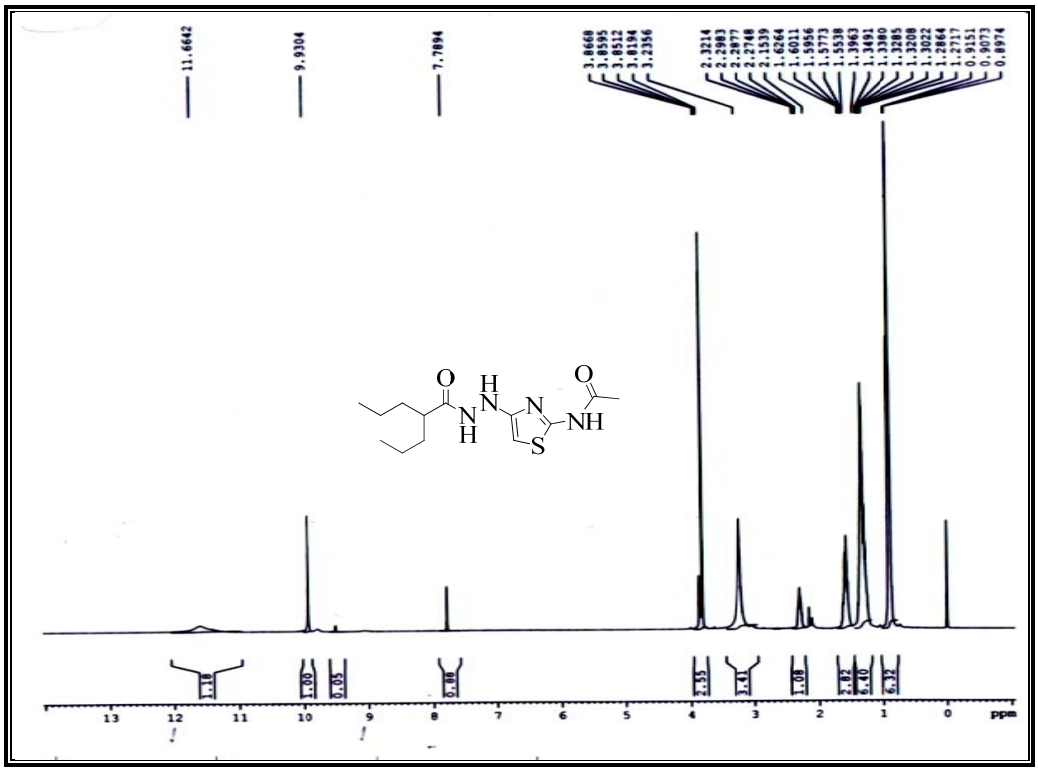


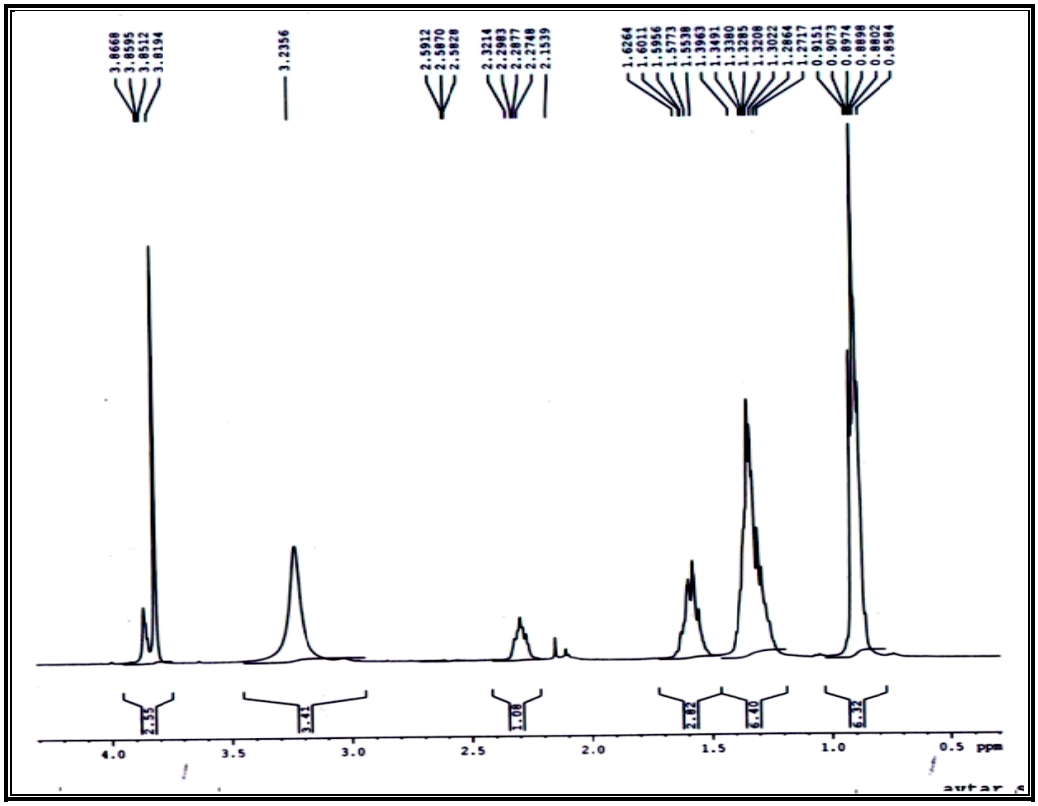


^{13}C NMR spectrum of *N*'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)

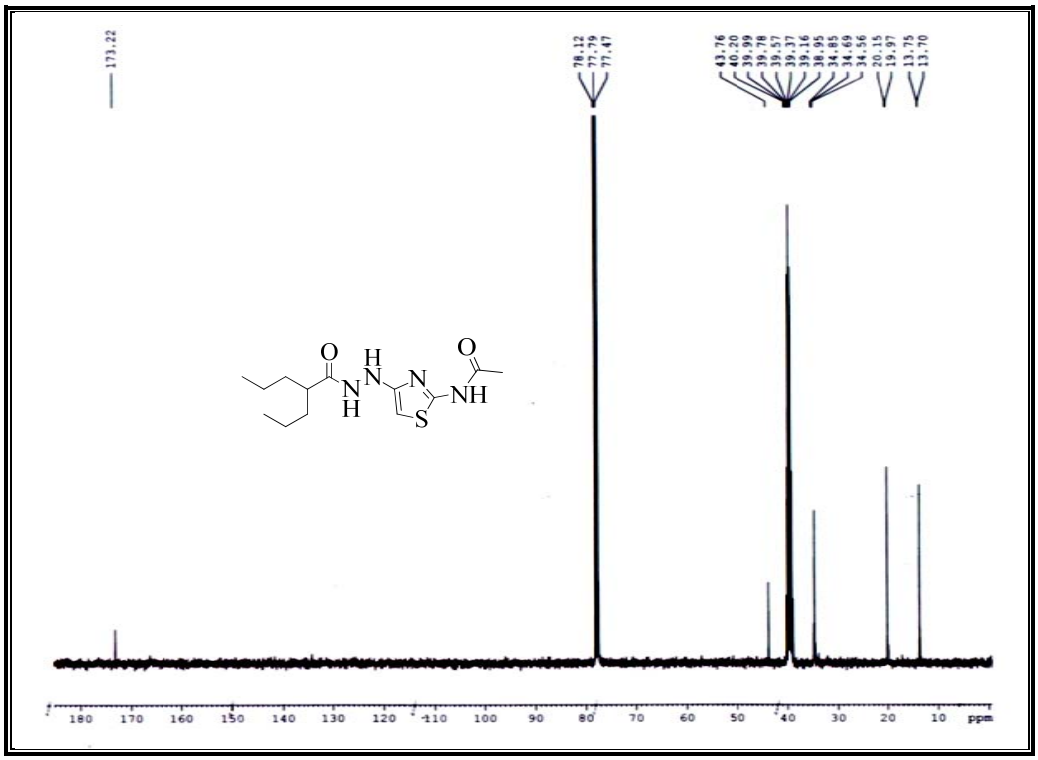


^{1}H NMR spectrum of *N*'-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)

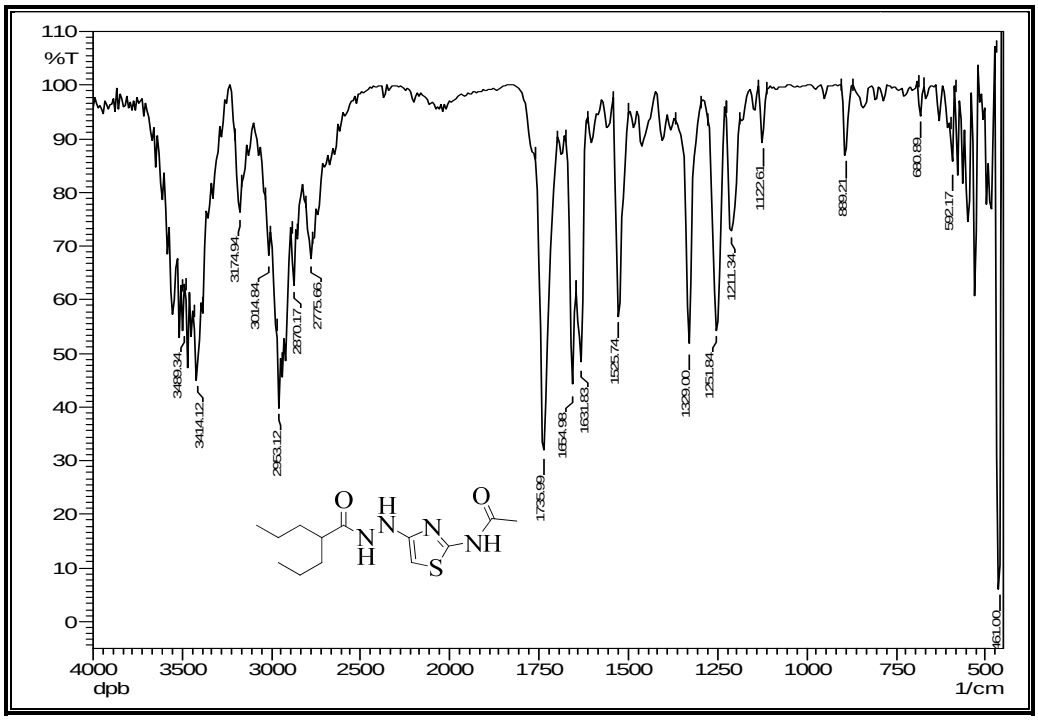




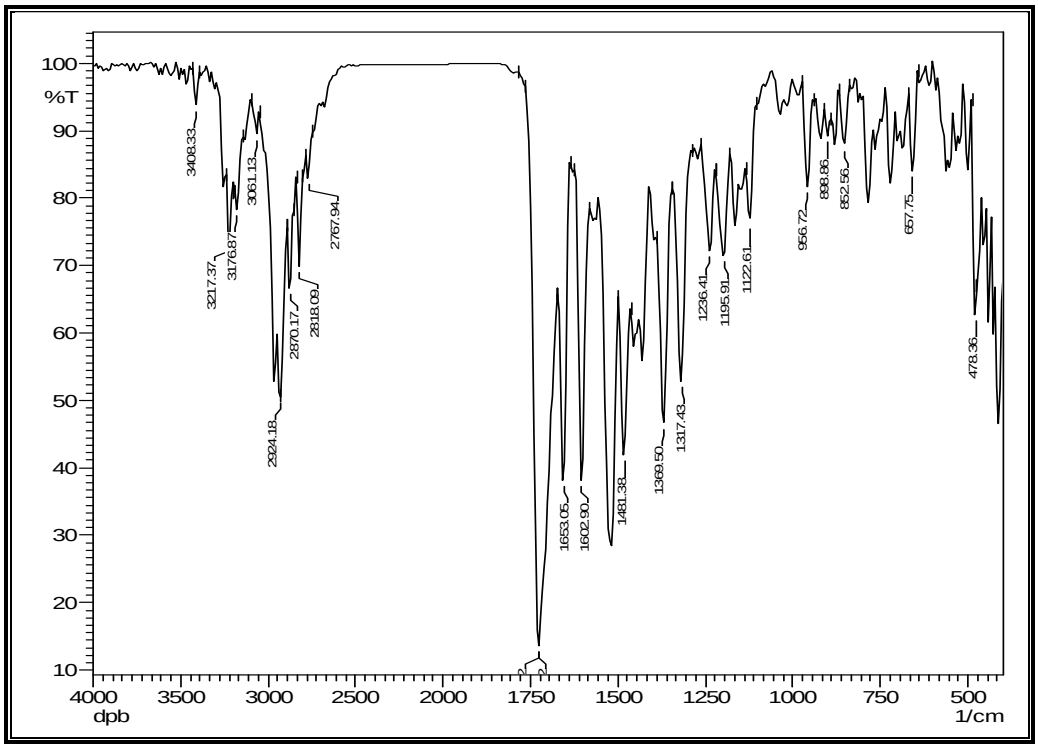
^{13}C NMR spectrum of *N*'-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)



IR spectrum of *N*'-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)



IR spectrum of *N*'-(2-(methylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-68)



4.19 REFERENCE

1. Johannessen, C.U., Johannessen, SI; *CNS Drug Rev* **2003**, *9*, 199–216.
2. Blaheta, R.A., Cinatl, Jr.; *Med Res Rev* **2002,** *22*, 492–511.
3. Verrotti, A., Basciani, F., Morresi, S., de Martino, M., Morgese, G., Chiarelli, F.; *Neurology* **1999,** *53*, 230–232.
4. Jallon, P., Picard, F.; A comparative review. Drug Saf **2001**; 24, 969–978.
5. Zimmermann, U., Kraus, T., Himmerich, H., Schuld, A.; *J Psychiatr Res* **2003,** *37*, 193–220.
6. Biton, V.; *CNS Drugs* **2003,** *17*, 781–791.
7. Nemeroff, C.B.; *J Clin Psychiatry* **2003,** *64*, 532–539.
8. Joffe, H., Hall, J.E., Cohen, L.S., Taylor, A.E., Baldessarini, R.J.; *Harv Rev Psychiatry* **2003,** *11*, 99–108.
9. Herzog, A.G., Schachter, S.C.; final thoughts. *Epilepsia* **2001,** *42*, 311–315.
10. Bauer, J., Isojarvi, J.I., Herzog, A.G., Reuber, M., Polson, D., Tauboll, E., Genton, P., van der Ven, H., Roesing, B., Luef, G. J., Galimberti, C. A., van Parys, J., Flugel, D., Bergmann, A., Elger, C. E.; *J Neurol Neurosurg Psychiatry* **2002,** *73*, 121–125.
11. Pylvanen, V., Knip, M., Pakarinen, A., Kotila, M., Turkka, J., Isojarvi, J. I.; *Epilepsia* **2002,** *43*, 514–517.
12. Novak, G. P., Maytal, J., Alshansky, A., Eviatar, L., Sy-Kho, R., Siddique, Q.; *J Child Neurol* **1999,** *14*, 490–495.
13. Demir, E., Aysun, S.; *Pediatr Neurol* **2000,** *22*, 361–364.
14. Wirrell, E. C.; *Pediatr Neurol* **2003,** *28*, 126–129.
15. Biton, V., Mirza, W., Montouris, G., Vuong, A., Hammer, A. E., Barrett, P. S.; *Neurology* **2001,** *56*, 172–177.
16. Biton, V., Levisohn, P., Hoyler, S., Vuong, A., Hammer, A. E.; *J Child Neurol* **2003,** *18*, 133–139.
17. Kawachi, I.; *J Clin Psychiatry* **1999,** *60*, 5–9.
18. Kopelman, P. G.; *Nature* **2000,** *404*, 635–643.
19. Berken, G. H, Weinstein, D. O., Stern, W. C.; *J Affect Disord* **1984,** *7*, 133–138.
20. Dinesen, H., Gram, L., Andersen, T.; *Acta Neurol Scand* **1984,** *70*, 65–69.

21. Corman, C. L., Leung, N. M., Guberman, A. H.; *Can J Neurol Sci* **1997,** *24*, 240–244.
22. Ferin, M., Morrell, M., Xiao, E., Kochan, L., Qian, F., Wright, T., Sauer, M.; *J Clin Endocrinol Metab* **2003,** *88*, 2908–2915.
23. Chapman, J. B., Cutler, M. G.; *Psychopharmacology (Berl)* **1984,** *83*, 390–396.
24. Wolden-Hanson, T., Gidal, B. E., Atkinson, R. L.; *Pharmacotherapy* **1998,** *18*, 1075–1081.
25. Lagace, D. C., Nachtigal, M. W.; *Prog Neuropsychopharmacol Biol Psychiatry* **2003,** *27*, 587–594.
26. Spiegelman, B. M.; *Cell* **2001,** *104*, 531–543.
27. McIntyre, R. S., Mancini, D. A., McCann, S., Srinivasan, J., Kennedy, S. H.; *Bipolar Disord* **2003,** *5*, 28–35.
28. Sahu, A., Minireview.; *Endocrinology* **2004,** *145*, 2613–2620.
29. Luef, G. J., Lechleitner, M., Bauer, G., Trinka, E., Hengster, P.; *Epilepsy Res* **2003,** *55*, 53–58.
30. Nelson-DeGrave, V. L., Wickenheisser, J. K., Cockrell, J. E., Wood, J. R., Legro, R. S.;*Endocrinology* **2004,** *145*, 799–808.
31. Lagace, D. C., Nachtigal, M. W.; *J Biol Chem* **2004,** *279*, 18851–18860.
32. Rosen, E. D., Spiegelman.; *Annu Rev Cell Dev Biol* **2000**, *16*,145–171.
33. Perucca, E.,*CNS Drugs;* **2002**, *16*, 695-714.
34. Loscher, W., *Switzerland: Birkhauser Verlag*; **1999**.
35. Bialer, M., *Expert Opin Investig Drugs*; **2006**, *15,* 637-647.
36. Nau, H., Hauck, R. S, Ehlers, K. *Pharmacol Toxicol;* **1991,** *69*, 310-321.
37. Nau, H., Hendrickx AG. *ISI Atlas Sci Pharmacol;* **1987,** *1*, 52-56.
38. Nau H., Loscher, W. *Fundam Appl Toxicol*; **1986**, *6,*669-676.
39. Tang, W., Palaty, J., Abbott, F. S., *J Pharmacol Exp Ther*; **1997,** *282*, 1163-1172.
40. Tang, W., Borel, AG., Fujimiya, T., Abbott, S. F., *Chem Res Toxicol*; **1995,** *8,* 671-682.
41. Neuman, M. G., Shear, N. H., Jacobson-Brown P. M, *Clin Biochem;* **2001,** *34,* 211-218.
42. Grillo, M. P., Chiellini, G., Tonelli, M., Benet, L. Z., *Drug Metab Dispos*; **2001**, *29,* 1210 -1215.

43. Isoherranen, N., Yagen, B., Blotnik, S., *Br J Pharmacol;* **2003,** *138,* 602-613.

44. Sobol, E., Bialer, M., Yagen, B. *J Med Chem;* **2004,** *47,* 4316-4326.

45. Winkler, I., Sobol, E., Yagen, B., Steinman, A., Devor, M., Bialer, M. *Neuropharmacology*; **2005,** *49,*1110 -1120.

46. Morre, M., Keane, P. E., Vernieres, J. C., Simiand, J., Roncucci, R., *Epilepsia;* **1984,** *25*, S5-S9.

47. Badir, K., Haj-Yehia, A., Vree, T. B., Kleijn, E., Bialer, M. *Pharm Res*; **1991,** *8*, 750-753.

48. Isoherranen, N., Yagen, B., Bialer, M. Curr Opin Neurol; **2003,** *16,* 203-211.

49. Bialer, M,. *Expert Opin Investig Drugs*; **2006,** *15,* 637-647.

Chapter-5

BIOLOGICAL EVALUATION OF SYNTHESIZED CHEMICAL ENTITIES

5.1 INTRODUCTION

The present chapter deals with the preliminary biological screening results of DPB 1 to DPB 26 compounds synthesized during the course of research work, which are heterocycles like *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine -1-yl)acetamide derivatives. In the current chapter, biological aspects of anti tubercular screening were described along with the activity protocols and activity data obtained by preliminary screening *In vitro*. Finally on the screening results a brief discussion is also narrated.

The anti tubercular screening of all the synthesized compounds of DPB 1 to 26 series was carried out at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Alabama, USA.

5.2 PROCEDURE FOR THE RESAZURIN MIC ASSAY

The resazurin MIC assay, developed by Collins and Franzblau (1997), is a colorimetric assay used to test compounds for antimycobacterial activity. A color change from blue to pink is observed when growth occurs. Compounds are initially tested at a single point concentration of 10 μg/ml against *Mycobacteruim tuberculosis* H37Rv (H37Rv), obtained from Colorado State University, Fort Collins, CO. If compounds are active at the 10 μg/ml level, they are further tested in an MIC assay at 8 concentrations in a dose range between 10 to 0.078 μg/ml.

- **Preparation of Test Compounds**

The test compounds were placed in a -20 °C freezer. The day of the experiment, one vial from each compound is reconstituted using the supplier's recommended solvent to achieve a stock concentration of 3.2 mg/ml.

- **Inoculum Preparation**

H37Rv was grown in Middlebrook 7H9 broth medium (7H9 medium) supplemented with 0.2% (v/v) glycerol, 10% (v/v) ADC (albumin, dextrose, catalase), and 0.05% (v/v) Tween 80. The bacteria were inoculated in 50 ml of 7H9 medium in 1 liter roller bottles

that were placed on a roller bottle apparatus in an ambient 37 °C incubator. When the cells reach an OD600 of 0.150 (equivalent to ~1.5 x 107 CFU/ml), they were diluted 200-fold in 7H9 medium.

- **Single Point Concentration Procedure**

The procedure is the same as that used for the MIC procedure described below, but only the first 2 fold dilution was made that reduces the stock solution to 1.6 mg/ml. An additional 1:10 dilution was made in water (see Step 3 below) which reduces the stock solution further to 0.16 mg/ml. Addition of 6.25 µl of the 1:10 dilution to the wells in a final volume of 100 µl gave rise to a concentration equivalent to 10 µg/ml (see Step 2 below).

- **MIC Procedure**

1. 20 µl of the 3.2 mg/ml test compound was added to a 96-well microtiter plate.
2. 2-fold dilutions were made by the addition of 20 µl of diluent.

Expected final Test Compound dose level (µg/ml) = 3.2 mg/ml

↓ Dilute 1:2

10 — 1st dilution of 8 = 1.6 mg/ml

↓ Dilute 1:2

5 — 2nd dilution of 8 = 0.8 mg/ml

↓ Dilute 1:2

2.5 — 3rd dilution of 8 = 0.4 mg/ml

↓ Dilute 1:2

1.25 — 4th dilution of 8 = 0.2 mg/ml

↓ Dilute 1:2

0.625 — 5th dilution of 8 = 0.1 mg/ml

↓ Dilute 1:2

0.312 — 6th dilution of 8 = 0.05 mg/ml

↓ Dilute 1:2

0.156 — 7th dilution of 8 = 0.025 mg/ml

↓ Dilute 1:2

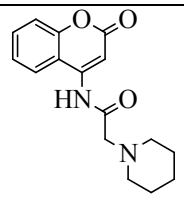
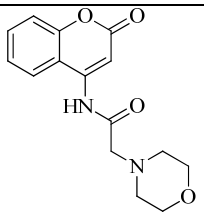
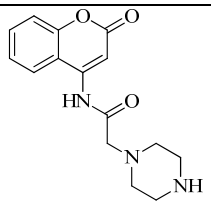
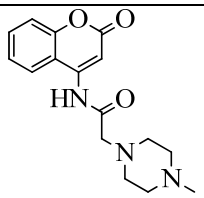
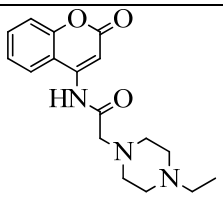
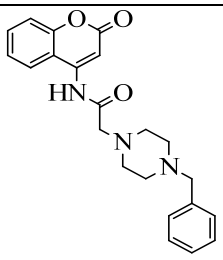
0.078 — 8th dilution of 8 = 0.0125 mg/ml

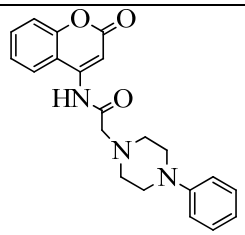
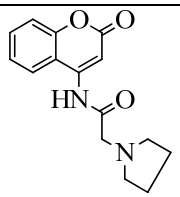
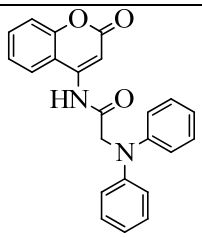
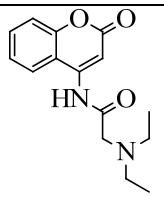
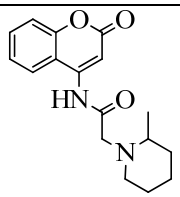
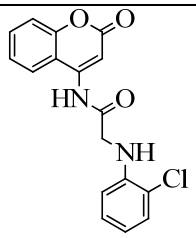
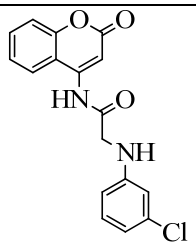
3. Each dilution was further diluted 1:10 in sterile water (10 µl of dilution to 90 µl of sterile water). **Note**: The additional 10-fold dilution in water was required when DMSO was used as solvent to minimize toxicity to the bacteria. For uniformity in

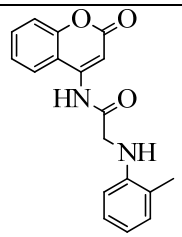
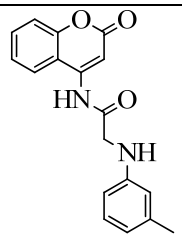
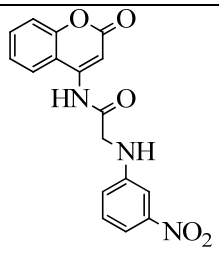
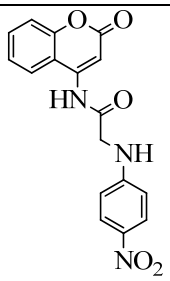
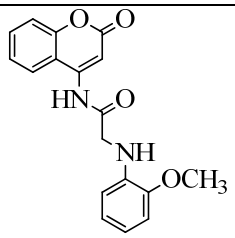
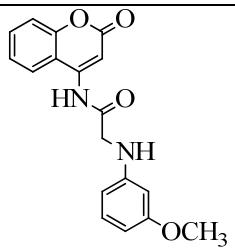
the assay procedure, this dilution step was used even if water or other solvents are used.

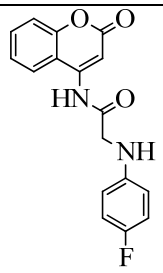
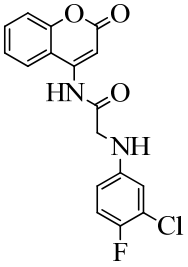
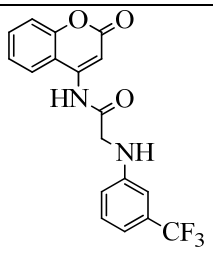
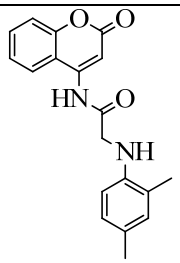
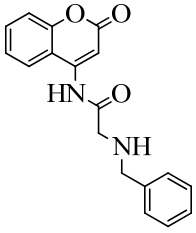
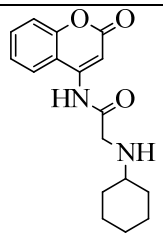
4. 6.25 µl of each dilution is transferred to duplicate 96-well test plates.
5. 93.75 µl of the cell suspension (~ 104 bacteria) in 7H9 medium was added to the test plates.
6. Positive, negative, sterility and resazurin controls ware tested.
   - **a.** Positive controls include: Rifampicin and Isoniazid
   - **b.** Negative controls include:
     i. Cell culture with solvent and water
     ii. Cell culture only
   - **c.** Sterility controls include:
     i. Media only
     ii. Media with solvent and water
   - **d.** Resazurin control includes one plate containing the diluted compounds with resazurin only. No bacterial suspension is added. This control plate is needed to verify whether the compound reacts with resazurin that could possibly elicit fluorescence.
7. The 96 well test plates were incubated in an ambient 37 ºC incubator for 6 days.
8. After the 6 day incubation, 5 µl of a 0.05% sterile resazurin solution was added to each well of the 96-well plate. The plates were placed in an ambient 37 ºC incubator for 2 days.
9. After the 2 day incubation, a visual evaluation and fluorimetric read-out was performed. The results were expressed as µg/ml (visual evaluation) and as IC_{50} and IC_{90} (fluoremetric readout)

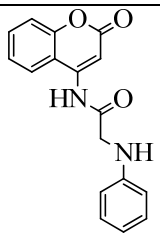
TABLE-1 – IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-1

Compound ID	Structure	Solvent	Results (µg/ml)
DPB-1		DMSO	>10
DPB-2		DMSO	>10
DPB-3		DMSO	>10
DPB-4		DMSO	>10
DPB-5		DMSO	>10
DPB-6		DMSO	>10

DPB-7		DMSO	>10
DPB-8		DMSO	>10
DPB-9		DMSO	>10
DPB-10		DMSO	>10
DPB-11		DMSO	>10
DPB-12		DMSO	>10
DPB-13		DMSO	>10

DPB-14		DMSO	>10
DPB-15		DMSO	>10
DPB-16		DMSO	>10
DPB-17		DMSO	>10
DPB-19		DMSO	>10
DPB-18		DMSO	>10

DPB-20		DMSO	>10
DPB-21		DMSO	>10
DPB-22		DMSO	>10
DPB-23		DMSO	>10
DPB-24		DMSO	>10
DPB-25		DMSO	>10

DPB-26		DMSO	>10
Rifampin		**DMSO**	**0.0125**
Isoniazid		**DMSO**	**0.063**

Reference:

Collins, L. A. and S. G. Franzblau. Microplate Alamar Blue Assay versus BACTEC 460 System for High-Throughput Screening of Compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium.* Antimicrobial Agents and Chemotherapy, 41:1004-1009 (1997).

Summary

The work represented in the thesis entitled ***"STUDIES ON DIVERSED HETEROCYCLIC CHEMICAL ENTITIES & THEIR APPLICATION"*** is divided into five chapters which can be summarized as under.

Chapter-1 relates with the introduction of oxygen heterocycle – coumarin. It deals with synthesis of 26 new molecules derived from 4-amino coumarin to arrive at variously functionalized *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine -1-yl)acetamide derivatives. The chapter covers reaction mechanism, IR, ^{1}H NMR, ^{13}C NMR, Mass spectral and other physical data to support the structure elucidation.

Chapter-2 deals with the imidazo[1,2-*a*]pyridine derivatives. In this chapter we have developed a novel, rapid and efficient methodology for the synthesis of highly functionalized imidazoles and thiazoles. This process involves protection and deprotection of amine functionality by BOC. The deprotection of amine group has been carried out using SiO_2 under microwave irradiation with short reaction time and afforded products with high chemical purity. The main advantage of this process is silica gel, which is inexpensive and non-toxic material and reusable after reaction. The process delivers very high yields of the products for biological interest. The chapter covers reaction mechanism, IR, ^{1}H NMR, ^{13}C NMR, Mass spectral and other physical data to support the structure elucidation.

Chapter-3 covers an introduction of oxadiazole. It deals with synthesis of 25 new molecules derived from 1,3,4-oxadiazole coupled with various primary and secondary amines. The process delivers very high yields of the products for biological interest. The chapter covers reaction mechanism, IR, ^{1}H NMR, ^{13}C NMR, Mass spectral and other physical data to support the structure elucidation.

Chapter-4 is an effort to modify known antiepileptic drug valproic acid to convert into various analogs, in order to explore other biological activities. Total 15 compounds are synthesized. The new molecules derived from condensation of (2-chloroacetyl)-2-propylpentanehydrazide with thiourea afforded 5 novel derivatives. Further, (2-aminothiazol-4-yl)-2-propylpentanehydrazide react with substituted acid chlorides

afforded 10 novel derivatives. The synthesized compounds were well characterized by IR, ^{1}H and ^{13}C NMR and Mass spectrometry.

Chapter-5, we have demonstrates the biological activity of newly synthesized *N*-(2-oxo-2*H*-chromen-4-yl)-2-(substituted amine-1-yl)acetamide. The biological study of the synthesized compounds revels that the tested compounds were moderate to good against *Mycobacterium tuberculosis* H37Rv. The biological activities of remaining compounds are under study.

Papers/ Presentation

PAPER

1. **Synthesis of some novel benzofuran-2-yl(4,5-dihyro-3,5-substituted diphenylpyrazol-1-yl) methanones and studies on the antiproliferative effects and reversal of multidrug resistance of human MDR1-gene transfected mouse lymphoma cells in vitro.**

   Shrey Parekh, **Dhairya Bhavsar**, Mahesh Savant, Shailesh Thakrar, Abhay Bavishi, Manisha Parmar, Hardev Vala, Ashish Radadiya, Nilay Pandya, Juliana Serly, Joseph Molnár and Anamik Shah*, *European Journal of Medicinal Chemistry*, 46(**2011**) 1942-1948.

2. **Synthesis and biological evaluation of 4-Styrylcoumarin derivatives as inhibitors of TNF-α and IL-6 with anti-tubercular activity" has been received by Bioorganic & Medicinal Chemistry Letters.**

   Kuldip Upadhyay, Abhay Bavishi, Shailesh Thakrar, Ashish Radadiya, Hardevsinh Vala, Shrey Parekh, **Dhairya Bhavsar**, Mahesh Savant, Manisha Parmar, Priti Adlakha and Anamik Shah*, *Bioorganic and Medicinal Chemistry Letters*, Doi:10.1016/j.bmcl.2011.02.016.

3. **Synthesis and In Vitro anti-HIV Activity of N-1,3-benzo[d]thiazol-2-yl-2-(2-oxo-2H-chromen-4-yl) Acetamide Derivatives using MTT method.**

   Dhairya Bhavsar, Jalpa Trivedi, Shrey Parekh, Mahesh Savant, Shailesh Thakrar, Abhay Bavishi, Ashish Radadiya, Hardev Vala, Manisha Parmar, Roberta Loddo, Anamik Shah*, *Bioorganic & Medicinal Chemistry Letters*. (**Accepted**)

4. **An efficient synthesis of highly functionalized imidazoles and thiazoles using microwave irradiation**

   Dhairya Bhavsar, Shrey Parekh, Jignesh Lunagariya, Abhay Bavishi, Shailesh Thakrar, Manisha Parmar, Hardevsinh Vala, Mahesh Savant, Anamik Shah*, *Synthetic communication*, **(Under Review)**

5. **An efficient and rapid synthesis of highly functionalized novel symmetric 1,4-dihydropyridines using glacial acetic acid as a solvent.**

   Shailesh Thakrar, Abhay Bavishi, **Dhairya Bhavsar**, Shrey Parekh, Hardev Vala, Ashish Radadiya, Manisha Parmar, Mahesh Savant, Nilay Pandya and Anamik Shah*, *Synthetic communication*, **(Accepted)**

6. **Diversity oriented design of various hydrazides and their in vitro evaluation against Mycobacterium tuberculosis H37Rv strains**.

   Atul Manvar, Abhay Bavishi, Shailesh Thakrar, **Dhairya Bhavsar**, Hardev Vala, Anamik Shah*, *Bioorganic & Medicinal Chemistry Letters* (**Under Review**)

7. **Synthesis and Biological evaluation of 1-[2,4-dimethyl-5-(5-aryl-1,3,4-oxadiazol-2-yl)-1H-pyrrol-3-yl] ethanones as potent Anti-tubercular and Antibacterial agents.**

   Kuldip Upadhyay, Abhay Bavishi, Ashish Radadiya, Shailesh Thakrar, Hardevsinh Vala, Shrey Parekh, **Dhairya Bhavsar**, Mahesh Savant, Manisha Parmar, Kena Raval, Chetna Rajyaguru, Anamik Shah*, *Bioorganic & Medicinal Chemistry Letters* (**Under Review**)

8. **In vitro anticancer evaluation against different cell lines of molecularly diverse *N*-substituted oxyindoles.**

   Abhay Bavishi, Atul Manvar, Ashish Radadiya, Shailesh Thakrar, Hardevsinh Vala, Shrey Parekh, Manisha Parmar, **Dhairya Bhavsar**, Mahesh Savant, Priti Adlakha, Manu Jaggi and Anamik Shah*, *European Journal of Medicinal Chemistry* (**Under Review**)

9. **An Efficient Microwave assisted one pot synthesis of novel 2-amino 3-cyano pyridine derivatives using Two Reusable Solid Acids as Catalysts.**

   Shailesh Thakrar, Abhay Bavishi, **Dhairya Bhavsar**, Shrey Parekh, Hardevsinh Vala, Ashish Radadiya, Mahesh Savant, Manisha Parmar, Nilay Pandya and Anamik Shah*, *Tetrahedron Letters* (**Under Review**)

10. **Microwave assisted rapid Synthesis of novel 1,5-benzodiazepines derivatives as potent antimicrobial agent.**

    Shailesh Thakrar, Abhay Bavishi, Shrey Parekh, **Dhairya Bhavsar**, Hardevsinh Vala, Ashish Radadiya, Manisha Parmar, Nilay Pandya and Anamik Shah*, *Journal of heterocyclic chemistry* (**Under Review**)

PRESENTATION

- ***A Simple and efficient cleavage of N-Boc using silica under microwave irritation***

  Dhairya Bhavsar, Shrey Parekh, Sailesh Thakrar and Anamik Shah*

Poster Presented at 15th ISCB International conference ***"Bridging gaps in discovery and development: Chemical & biological science for affordable health, wellness & sustainbility"*** on Saurashtra University, Rajkot on 4-7th Feb. 2011.

- ***Synthesis and anti-HIV Activity of N-1, 3-benzo[d]thiazol-2-yl-2-(2-oxo-2H-chromen-4-yl) acetamides***

  Dhairya Bhavsar, Jalpa Trivedi and Anamik Shah*

Poster Presented at 14th ISCB International conference ***"International conference on chemical biology for discovery: perspectives and Challenges"*** on CDRI, Lucknow on 15-18th Jan., 2010.

Conferences/ Workshops

Confrance/ Workshops attended

1. ISCB Conference ***"Bridging gaps in discovery and development: Chemical & biological science for affordable health, wellness & sustainbility"*** at Saurahtra University, Rajkot on 4-7th Feb., 2011.

2. ISCB Conference ***"International conference on chemical biology for discovery: perspectives and Challenges"*** at CDRI, Lucknow on 15-18th Jan., 2010.

3. ***"International Seminar on Recent Developments in Structure and Ligandbased Drug Design"*** jointly organized by Schrodinger LLC, USA; National Facility for Drug Discovery through New Chemicals Entities Development & Instrumentation support to Small Manufacturing Pharma Enterprises and DSTFIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot on 23rd, December, 2009.

4. ***"National seminar on Alternative Synthetic Strategies for Drugs & Drug Intermediates"*** at Institute of Pharmacy, Nirma University, Ahmedabad on 13th November, 2009.

5. ***"Two Days National Workshop on Patents & Intellectual Property Rights Related Updates"*** Sponsored by TIFAC & GUJCOST and Organized by DST-FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot on 19-20th September, 2009.

6. DST-FIST, UGC (SAP) supported and GUJCOST Sponsored ***"National Conference on Selected Topics in Spectroscopy and Stereochemistry"*** organized by the Department of Chemistry, Saurashtra University, Rajkot on 18-20th March, 2009.

7. National seminar on "***Recent Advances in Chemical Sciences & an Approach to Green Chemistry***", Rajkot on October, 2006.

8. National workshop on "***E-resources in Chemical Synthsis and Natural Products***", Rajkot on March, 2006