Studies on Some Heterocycles of Medicinal Interest

A Thesis Submitted in the Fulfillment of the Requirements of the Award of the Degree

Doctor of Philosophy

From

Saurashtra University

By

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Under the Guidance of
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India

August 2010

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

The work included in the thesis is done by me under the supervision of Dr. V. H. Shah and the contribution made thereof is my own work.

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CERTIFICATE

This is to certify that the present work submitted for the Ph. D. degree of Saurashtra University, Rajkot, Gujarat (India) by Mr. Chintan P. Vakhariya has been the result of work carried out under my supervision and is a significant contribution in the field of synthetic organic chemistry.

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Dedicated To
My Beloved Mummy-Papa
And
My Beloved Brother

Acknowledgement

It is a moment of gratification and pride to look back with a sense of contentment at the long traveled path, to be able to recapture some of the fine moments, to be think of the infinite number of people, some who were with me from the beginning, some who joined me at different stages during this journey, whose kindness, love and blessings has brought me to this day. I wish to thank each of them from the bottom of my heart.

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Vakhariya for their blessings. It was a dream of my family which has now come true.

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

Chintan P. Vakhariya

/08/2010

Rajkot

Chemistry [...] is an art, it's music, it's a style of thinking

Orbitals are for mathematicians

Chemistry is for people who like to cook!

- Alexander Shulgin (1925)

Table of Contents

Section B:

General remarks

1. ^{1}H NMR spectra were recorded on Bruker avance II 400 MHz NMR spectrometer using TMS as an internal reference.

2. Mass spectra were recorded on GC-MS QP-2010 spectrometer.

3. IR spectra were recorded on Schimadzu FT-IR-8400 spectrometer.

4. Elemental analysis was carried out on Vario EL III Carlo Erba 1108.

5. Thin layer chromatography was performed on Silica Gel (Merck 60 F_{254}).

6. The chemicals used for the synthesis of compounds were purchased from Spectrochem, Merck, Thomas-baker and SD fine chemical.

7. Melting Points were taken in open capillary and are uncorrected.

8. All the structures are drawn according to ACS Document 1996 style.

List of Abbreviations

NCEs	New Chemical Entities
R & D	Research & Development
HTS	High Throughput Screening
AIDS	Acquired Immune Deficiency Syndrome
DHFR	Dihydrofolate Reductase
UTIs	Urinary Tract Infections
IDU	Idoxuridine
AZT	Azidothymidine
ARC	AIDS - related complex
Hsv	Herpes simplex virus
HIV	Human Immunodeficiency Virus
5-HT	5-hydroxytryptamine
CNS	Central Nervous System
NSAID	Non-Steroidal Anti-Inflammatory Drug
COX	Cyclooxygenase
GnRH	Gonadotropin-Releasing Hormone Antagonist
PDE4 inhibitors	Phosphodiesterase inhibitor
FT-IR	Fourier Transform- Infrared spectroscopy
^{1}H-NMR	^{1}H- Nuclear Magnetic Resonance spectroscopy
Gl.	Glacial
TLC	Thin Layer Chromatography
R_f	Retardation factor
EtOH	Ethanol
Conc.	Concentrated
h.	Hours
GC-MS	Gas Chromatograph- Mass Spectrometry
DMSO	Dimethyl sulfoxide
mL	Milliliter
MeOH	Methanol
mp	Melting Point
Ms	Mass

Anal. Calcd.	Analytical Calculated
IR	Infrared
TMS	Trimethylsilane
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection
NCCLS	National Committee for Clinical Laboratory Standards
mg	Miligram
KDR	Kinase insert Domain Receptor
CDK-2	Cyclin-Dependent Kinase -2
PPA	Polyphosphoric Acid
DMF	Dimethylformamide
MAOS	Microwave-Assisted Organic Synthesis
MW	Microwave
Min.	Minute
W	Watt
Pd	Palladium
SiO_2	Selenium Dioxide
$InCl_3$	Indium Trichloride
PTP	Pyrazolotriazolopyrimidine
B. P.	Base Peak
M. I. P.	Molecular Ion Peak

Synopsis

The work to be presented in thesis entitled “Studies on Some Heterocycles of Medicinal Interest” is classified into following Chapters.

Chapter 1	General Introduction
Chapter 2	Biological and medicinal significance of Pyrimidines and related heterocycles
Chapter 3	Studies and biological evaluation of dihydropyrimidines
Chapter 4	Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines

Chapter 1 General Introduction

Nowadays, the entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of new chemical entities (NCEs).

Chapter 1 gives a brief introduction for the pressing need of New Chemical Entities (NCEs) for pharmaceutical industry. It also describes importance of bicyclic and tricyclic aromatic heterocycles in drug discovery. Concept of “privileged structures” is also explained in brief. Chapter 1 also describes aims and objectives of the proposed research work.

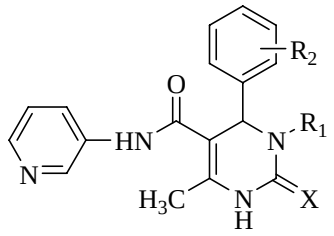
Chapter 2 Biological and medicinal significance of pyrimidines and related heterocycles

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS and many other diseases like sarcoma, malaria, tuberculosis (TB), gonorrhea etc.

Chapter 2 outlines the biological and medicinal significance of one of the most important heterocycles, the pyrimidine. An attempt has been made to cover most of the medicinally important compounds containing pyrimidine and its derivatives.

Chapter 3 Studies and biological evaluation of dihydropyrimidines

Pyrimidine represents one of the most active classes of compounds possessing a wide spectrum of biological activities *viz*. antiviral, anti-HIV, anticancer, diuretic, antitubercular, antihypertensive etc. In view of getting better therapeutic agents containing pyrimidines nucleus, it was thought worthwhile to synthesize some new pyrimidine derivatives.



CPV 101 TO 130

When R_1= H, X= O/S, R_2= 4-OCH_3, 4-CH_3, 4-Cl etc.
When R_1= CH_3, X= O, R_2= 4-OCH_3, 4-CH_3, 4-Cl etc.

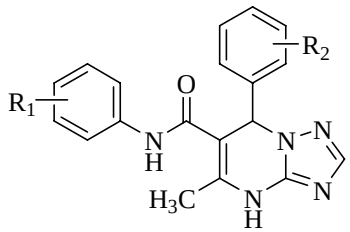
Due to various biological applications and with a view to further assess the pharmacological profile of this class of compounds, three novel series of dihydropyrimidines (CPV-101 to CPV-130) are synthesized in Chapter 3. The synthesis of dihydropyrimidines (CPV-101 to CPV-130) was achieved by the Biginelli reaction of acetoacetamide, urea derivatives and various aromatic aldehydes in the presence of catalytic amount of con.HCl. The products were characterized by various analytical techniques like FT-IR spectroscopy, Mass spectrometry, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds are subjected to various biological activities *viz*. antimicrobial, antimycobacterial, anticancer and antiviral.

Chapter 4 Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines

The biological importance of 1,2,4-triazolo[1,5-*a*]pyrimidines is well documented. Over the decades, various derivatives of these heterocycles have shown utility against a large range of biological targets. They have demonstrated antimalarial and antibronchospasmic activity and shown activity as xanthine oxidase inhibitors, coronary vasodilators, leishmanicides, antibiotics, immunosuppressant, antitumor agents, antihypertensive agents, fungicides, phosphodiesterase inhibitors and adenosine A_2a antagonists.

Section A:

In Section A of Chapter 4, synthesis of four new series of 1,2,4-triazolo [1,5-*a*] pyrimidines (CPV-201 to CPV-240) containing four acetoacetamide fragments has been undertaken.



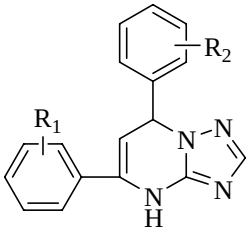
CPV 201 TO 240

Where R_1= pyridin-2-yl, pyridin-3-yl, 4-Cl, 3-Cl 4-F
R_2= 4-OCH_3, 4-CH_3, 4-F, 4-Cl etc..

Section B:

In Section B, another four new series of 1,2,4-triazolo [1,5-*a*] pyrimidines (CPV-241 to CPV-280) are synthesized by one pot condensation of different aldehydes, acetophenones and 1-H,1,2,4-triazol-3-amine. The structures of all the newly synthesized compounds are confirmed by various analytical techniques like FT-IR spectroscopy, Mass spectrometry, ^{1}H NMR spectroscopy and elemental analyses. The

newly synthesized compounds are subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.



CPV 241 TO 280

Where R_1= 4-Br, 4-Cl, 4-OCH_3, 4-NO_2
R_2= 4-CH_3, 4-F, 4-Cl, 3-Cl etc..

Chapter 1
General Introduction

1.1 Heterocycles in drug discovery

Nowadays, the entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of new chemical entities (NCEs).

The cause of this innovation deficit is definitively not the biology. Decoding of the human genome[a] has led to a wealth of drug targets. With more than 30,000 human genes[b], the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each of these genes is linked to the function of between five and ten proteins, the conclusion is that there might be 5,000–10,000 targets for new drugs [1]. Despite the successful introduction of protein therapeutics and the promise of gene therapy, major pharmaceutical companies are still focused on the discovery and development of low-molecular weight compounds. Hence, the challenge is to select the most drugable targets and to find the corresponding drug-like molecules, substances that not only interact with the target, but also have specific pharmacokinetic and toxicological properties, that allow them to be developed as a drug.

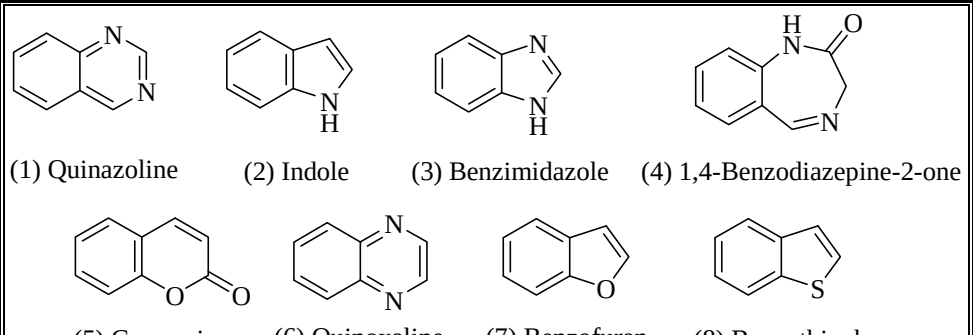
Medicinal chemistry as a scientific discipline has introduced several new techniques over the last few years in order to speed up the drug discovery process, such as combinatorial chemistry, microwave-assisted organic synthesis (MAOS) and

[a] The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in number of base pairs.
[b] According to the official Guidelines for Human Gene Nomenclature, a gene is defined as "a DNA segment that contributes to phenotype/function. In the absence of demonstrated function a gene may be characterized by sequence, transcription or homology."

high-throughput purification [2]. Despite this steady increase in R & D, the number of NCEs reaching the market has actually decreased dramatically.

It seems clear that selecting appropriate molecules to synthesize is one of the most troublesome questions. It has been estimated that the number of possible molecules with a molecular weight of less than 500 Dalton is 10^{200}, of which only 10^{60} may possess drug-like properties. The proportion of these drug-like molecules synthesized to date has been estimated as one part in 10^{57}, or roughly the ratio of the mass of one proton to the mass of the sun! The issue is therefore the selection of new molecules from this vast universe, which have the potential to be biologically active [3].

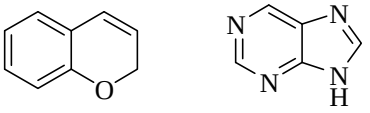
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening campaign of commercially available compound libraries against the target of interest. It became clear in recent years that combinatorial libraries are not diverse enough. As the main interest of the laboratory of medicinal chemistry lays in the synthesis and biological evaluation of aromatic heterocycles, we performed a literature survey of commercially available combinatorial libraries. This search revealed that the number of available heterocycles is mainly limited to well-known nitrogen containing compounds, such as quinazolines (1), indoles (2) and benzimidazole (3).



Examples of privileged structures

These structural classes are considered to be privileged structures. The concept of “privileged structures” was first proposed by Evans B. E. *et al.* to describe selected

structural types that bind to multiple, unrelated classes of protein receptors and enzymes as high affinity ligands [4]. These privileged structures are typically rigid, polycyclic heteroatomic systems capable of orienting the various substituents in a well-defined three-dimensional space. Well-known examples of privileged substructures include benzodiazepines (4), coumarins (5), quinoxalines (6), benzofurans (7) and benzothiophenes (8) [5]. In order to improve the hit rate in High Throughput Screening (HTS) campaigns, privileged structures provide an ideal source of lead compounds. A single library based upon privileged substructures can lead to active compounds in variety of biological assays. Several research groups have utilized these structures in such a manner. For example, Nicolau *et al.* constructed a library based on the benzopyran (9) privileged scaffold [6], whereas Schultz *et al.* made use of the purine (10) scaffold [7].



(9) Benzopyran (10) Purine

Thr benzopyran and purine privileged scaffold

1.2 Nomenclature of the fused ring system

As the following chapters deal with the synthesis of bicyclic and tricyclic fused ring systems, its nomenclature is herewith shortly reviewed. The nomenclature follows the following rules:

(1) The individual components are named without any application of fused ring system.

(2) The parent component is represented in the fusion name by citing it last in the name. The parent component is the one with highest priority according to the following criteria:

(a) A heterocyclic component containing the heteroatom occurring earliest in the order: N, F, Cl, Br, I, O, S, Se, Te, P, As, Sb, Bi, Si, Ge, Sn, Pb, B, Hg.

(b) A component containing the larger ring

(c) A component containing the greater number of heteroatoms.

(d) A component containing the greater variety of heteroatoms.

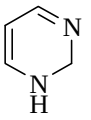
(3) The attached component is then added as a prefix to the parent component. In the name of the prefix, the terminal 'e' is changed to 'o'.

(4) The bonds of the parent component are indicated by a, b, c…starting with the bond normally occupying the 1,2 positions. The atoms of the attached component are numbered as usual, following the order of numbers in the original heterocycle.

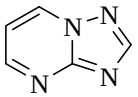
(5) The numbering of the final condensed heterocycle is carried out independently, starting at an atom adjacent to a bridged-head atom, whereby heteroatoms receive the smallest possible number.

1.3 Objectives

Our interest in the synthesis and biological evaluation of heterocycles like dihydropyrimidines (11) and 1,2,4-triazolo[1,5-*a*]pyrimidines (12) in commercial compound libraries, prompted us to elaborate this type of chemistry and to synthesize three different heterocyclic scaffolds.



(11) dihydropyrimidine



(12) 1,2,4-triazolo[1,5-*a*]pyrimidine

1.4 References and notes

[1] (a) Drews, J. *Science* **2000**, *287*, 1960; (b) Wess, G.; Urmann M.; Sickenberger, B. *Angew. Chem. Int. Ed*. **2001**, *40*, 3341.

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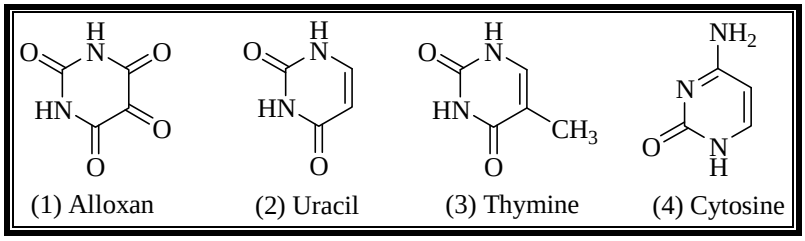
[7] Ding, S.; Gray, N. S.; Ding, Q.; Schultz, P. G. *Tetrahedron Lett*. **2001**, *42*, 8751.

Chapter 2
Biological and medicinal significance of pyrimidines and related heterocycles

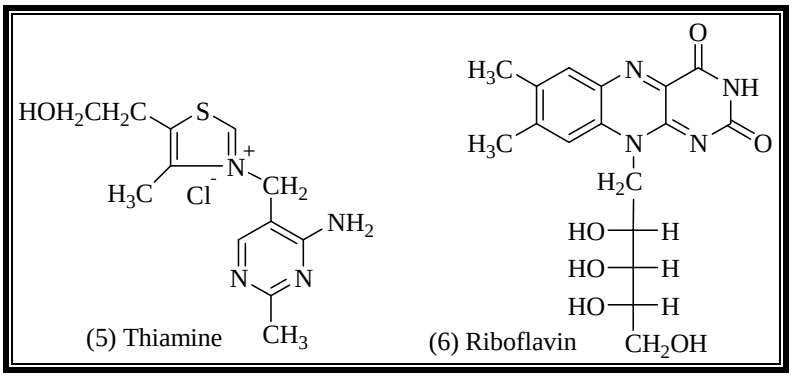
2.1 Biological significance

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of Acquired immunodeficiency syndrome (AIDS).

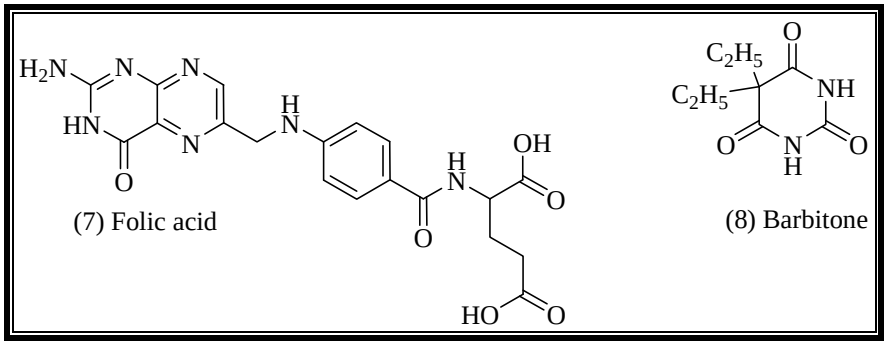
Alloxan (1) is known for its diabetogenic action[a] in a number of animals [1]. Uracil (2), thymine (3) and cytosine (4) are the three important constituents of nucleic acids.



The pyrimidine ring is found in vitamins like thiamine (5), riboflavin (6) and folic acid (7) [2]. Barbitone (8), the first barbiturate hypnotic, sedative and anticonvulsant are pyrimidine derivatives [1].



[a] The substances or compounds which cause the disease diabetes are called diabetogenic and the mechanism action of such substances is called ‘diabetogenic action’.

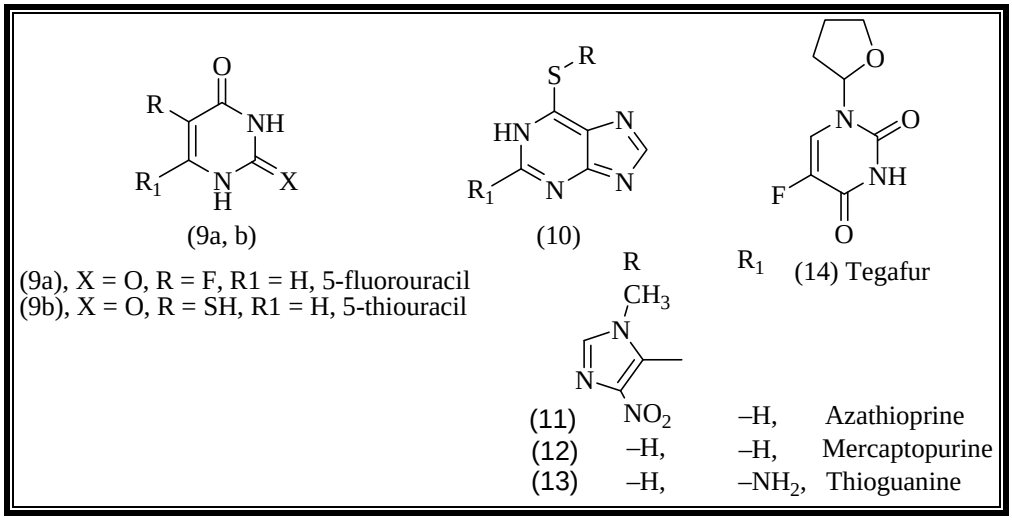


2.2 Medicinal significance

During the last two decades, several pyrimidine derivatives have been developed as chemotherapeutic agents and have found wide clinical applications.

2.2.1 Antineoplastics[b] or anticancer agents

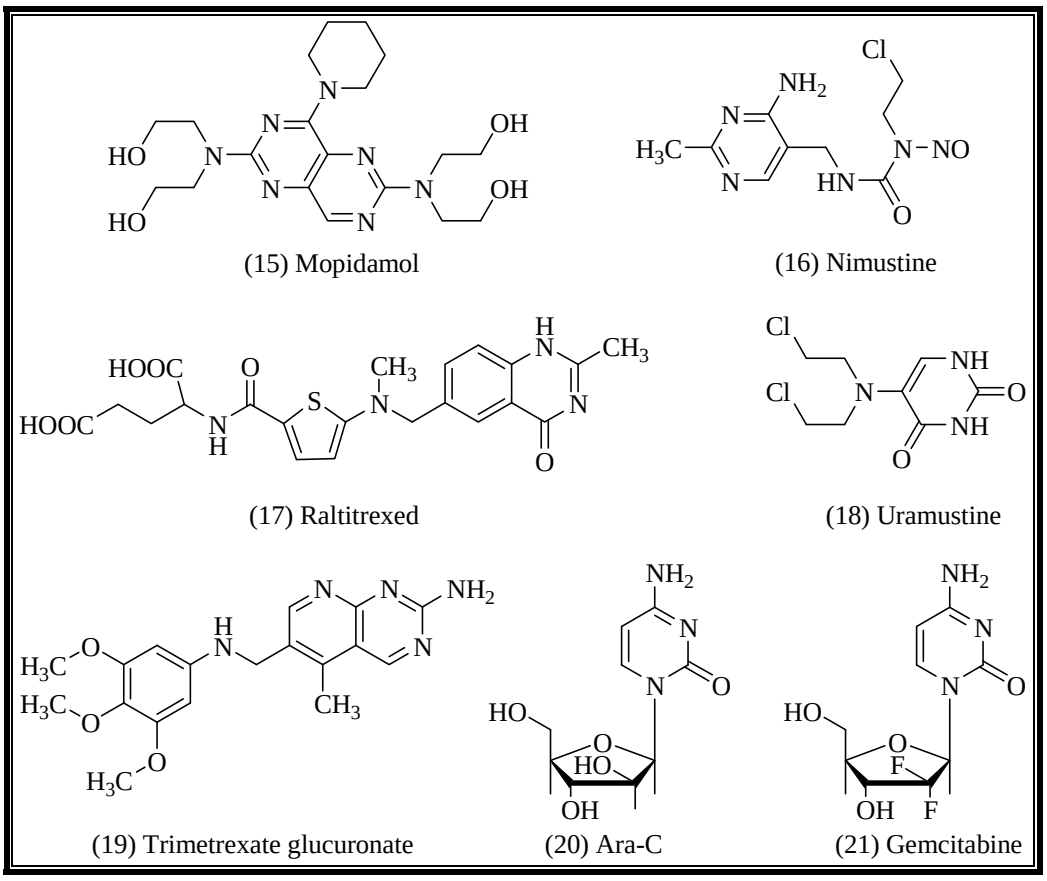
There are a large number of pyrimidine-based antimetabolites. Usually, they are structurally related to the endogenous substrates[c] that they antagonize[d]. The structural modification may be on the pyrimidine ring or on the pendant sugar groups. One of the early metabolites prepared was 5-fluorouracil (5-FU, 9a) [3, 4], a pyrimidine derivative. 5-Thiouracil (9b) also exhibits some useful antineoplastic activities [5].



[b] Drugs that inhibit and combat the development of neoplasms (an abnormal mass of tissue due to the abnormal proliferation of cells).
[c] Endogenous substrates are those that originate from within an organism, tissue, or cell.
[d] To oppose or to compete the metabolites.

The antineoplastic compounds [6] possessing the guanine nucleus (10) like azathioprine (11) [7], mercaptopurine (12) [8], thioguanine (13) [9], tegafur (14) [10], etc. were discovered after formulation of the antimetabolite theory by Woods *et al.* in 1940. These drugs prevent the utilization of normal cellular metabolites [6].

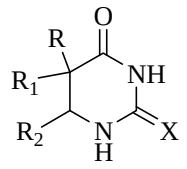
There are many more in recent times, like mopidamol (15) [11], nimustine (16) [12], raltitrexed (17) [13], uramustine (18) [14] and trimetrixate (19) [15]. 1-*β*-D-Arabinosylcytosine (Ara-C, 20) [16] is also an example of a pyrimidine antimetabolite in which the sugar is arabinose having a beta configuration. It is mainly used as an anticancer agent and also exhibits significant therapeutic effects in patients with herpes virus infections and herpes encephalitis.



Gemcitabine (21), a pyrimidine antimetabolite, shows excellent antitumour activity against murine solid tumours [17].

2.2.2 Drugs for hyperthyroidism[e]

2-Thiouracil (9c) and its alkyl analogue, thiobarbital (9e) are effective drugs against hyperthyroidism. Propylthiouracil (9d) is used as a drug for hyperthyroidism with minimum side effects [18].



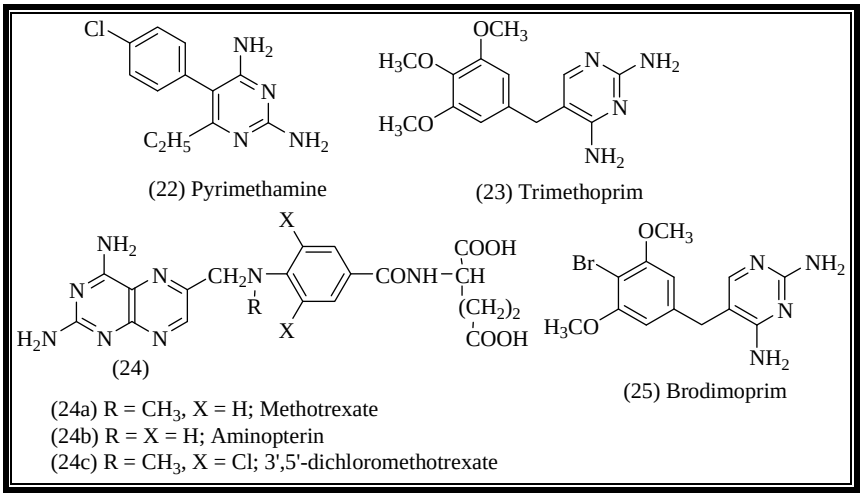
(9c–e)

(9c), R = R_1 = R_2 = H, X = S; 2-thiouracil
(9d), R = R_1 = H, R_2 = C_3H_7, X = S; propylthiouracil
(9e), R = R_1 = C_2H_5, R_2 = O, X = S; thiobarbital

2.2.3 Antifolates[f], antibacterials[g] and antiprotozoals[h]

In 1948, Hitchings G. *et al.* made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid [19]. Since then, a large number of 2,4-diaminopyrimidines have been synthesized as antifolates. It was eventually proved that these pyrimidines are inhibitors of the enzyme dihydrofolate reductase (DHFR) [20, 21]. Notable amongst the 2,4-diaminopyrimidine drugs are pyrimethamine (22), a selective inhibitor of the DHFR of malarial plasmodia; trimethoprim (23), an antibacterial drug which selectively inhibits bacterial DHFR and most importantly, the very potent but non selective DHFR inhibitors, methotrexate (24a) and aminopterin (24b), both used in cancer chemotherapy [22]. 3',5'-dichloromethotrexate (24c), which is less toxic and more readily metabolized than methotrexate, has recently been introduced for anticancer therapy [23]. Brodimoprim (25) is also found to be an effective antibacterial compound [24].

[e] The term used for overactive tissue within the thyroid gland causing an overproduction of thyroid hormones.
[f] Antifolates are drugs which impair the function of folic acids.
[g] The drugs having the capability of either to kill or to stop the growth of bacteria.
[h] An antiprotozoal agent is a class of pharmaceuticals used in treatment of protozoan infection.

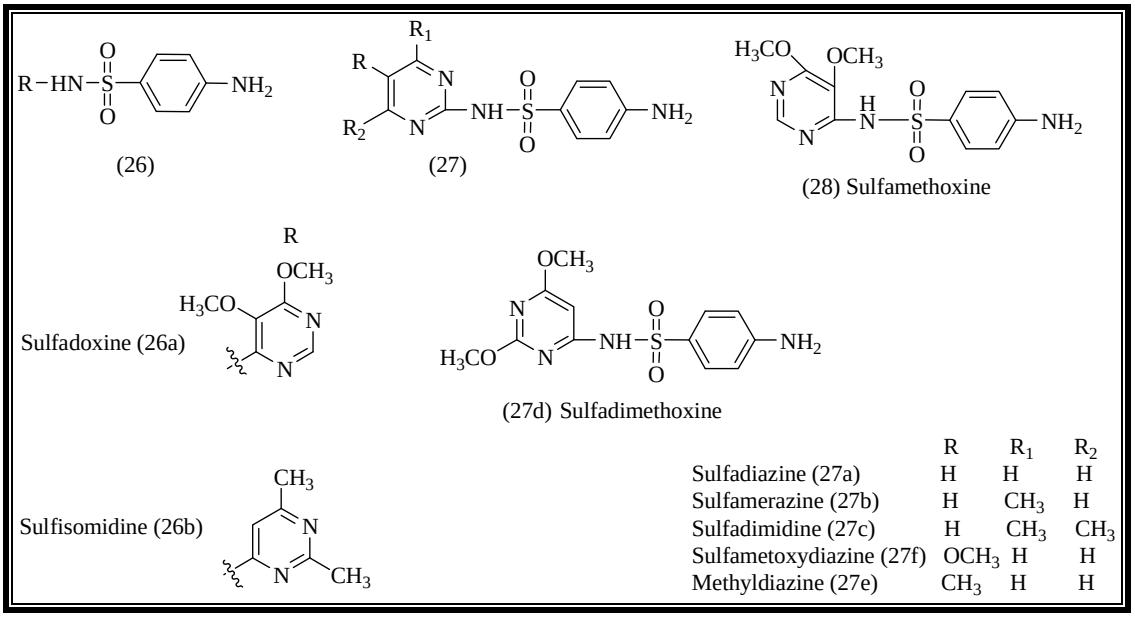


2.2.4 Sulfa drugs[i]

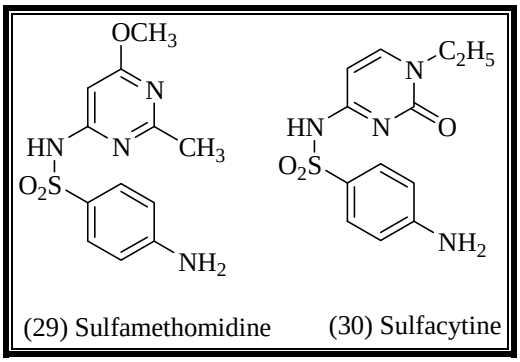
Pyrimidine derivatives of sulfa drugs, namely sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute Urinary Tract Infection (UTIs)[j], cerebrospinal meningitis and for patients allergic to penicillins [25]. Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS [26]. Sulfadoxine (26a) [27], a short and intermediate acting sulfonamide with a half-life of 7–9 days is used for malarial prophylaxis. Sulfisomidine (26b) with a half-life of 7 h is used as a combination sulfa therapy in veterinary medicine [28]. Sulfadiazine (27a), sulfamerzine (27b) and sulfadimidine (27c) possess good water solubility and therefore carry minimum risk of kidney damage, which makes them safe even for patients with impaired renal functions.

In 1959, sulfadimethoxine (27d) [29] was introduced with a half-life of approximately 40 h. The related 4-sulfonamidopyrimidine, sulfamethoxine (28) [29] having two methoxy groups at 5 and 6 positions, has by far the longest half-life of about 150 h. Methyldiazine (27e) [29] has a half-life of 65 h. Also, sulfamethoxy diazine (27f) [29] possesses good half-life.

[i] The synthetic antimicrobial agents that contain the sulfonamide group are called sulpha drugs.
[j] A Urinary Tract Infection (UTI) is a bacterial infection that affects any part of the urinary tract.



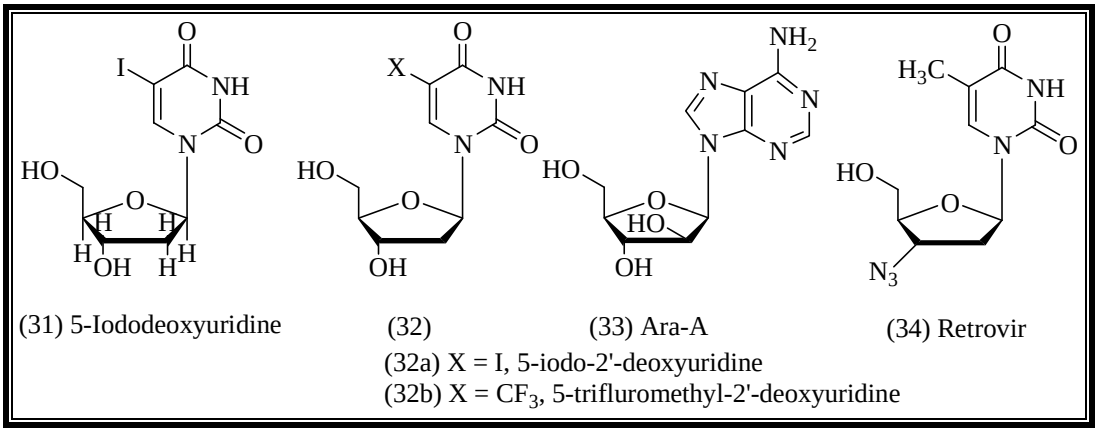
A new broad-spectrum sulfonamide, sulfamethomidine (29) [29] is relatively nontoxic and patients do not need extra fluid intake or alkalization. Sulfacytine (30) has been reported to be 3-10 times more potent than sulfaisoxazole and sulfisodimidine [29].



2.2.5 Antivirals[k] and anti-AIDS[l]

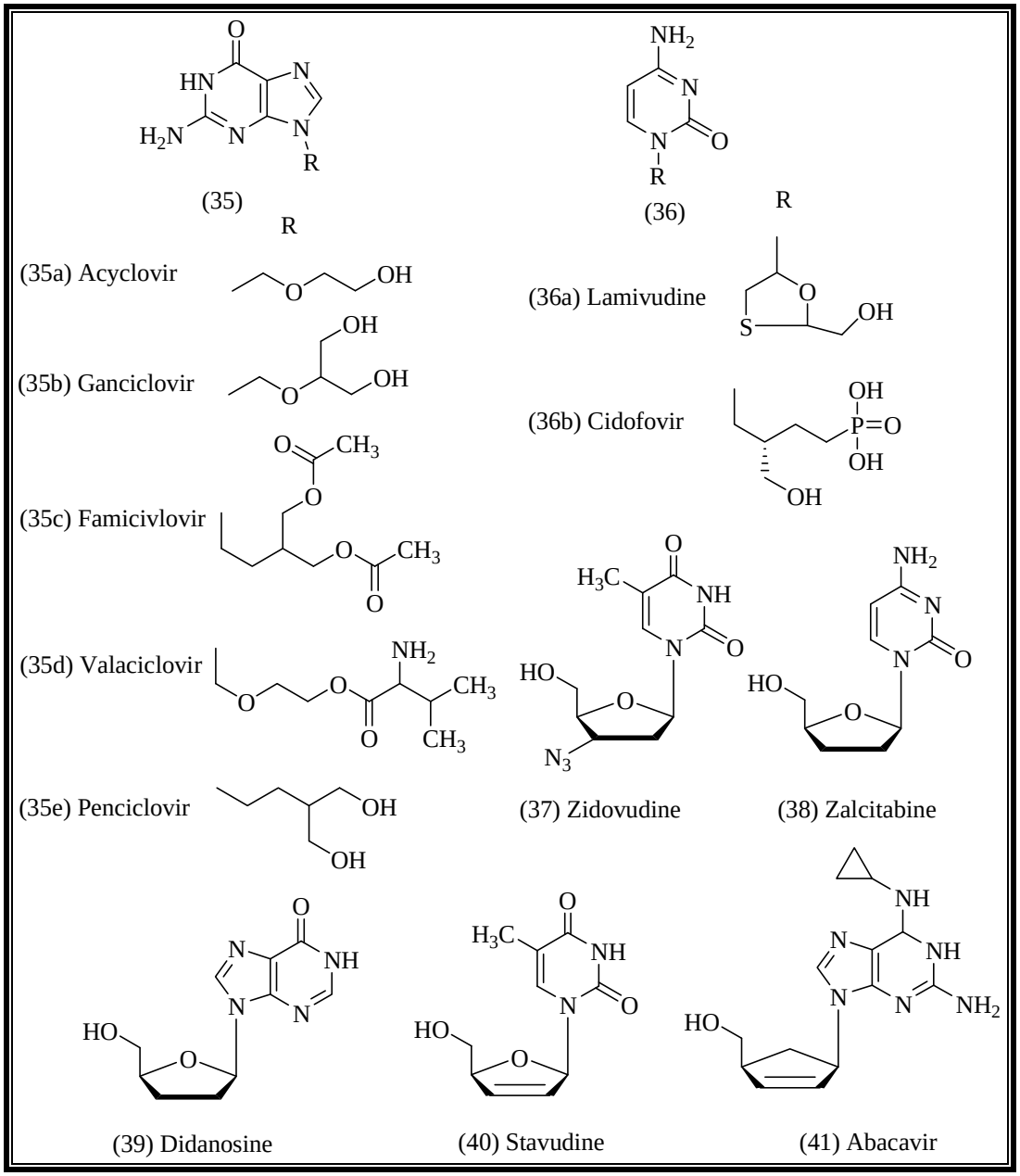
Recently, pyrimidine derivatives have generated widespread interest due to their antiviral properties. 5-Iododeoxyuridine (IDU) (31) [30] is an antiviral agent of high selectivity.

[k] Antiviral drugs are a class of medication used specifically for treating viral infections.
[l] The drugs which are used to treat the disease AIDS (acquired immunodeficiency syndrome).



5-iodo-2’-deoxyuridine (IDU) (32a) has been extensively utilized for viral infections. 5-Trifluromethyl-2’-deoxyuridine (F3 TDR, 32b) has been found useful against infections resistant to IDU therapy [30]. Ara-A, 9-*β*-D-arabinofuranosyl adenine (33), a relatively new antiviral drug, is effective against herpes infections of eye, brain and skin. It is especially effective against IDU-resistant herpes virus [30].

Some purine nucleosides are equally noteworthy. Retrovir Azidothymidine (AZT-16, 34) is a potent inhibitor of the *in vivo* replication and cytopathic effects of human immunodeficiency virus (HIV) and has been recently approved for use against AIDS and severe AIDS related complex (ARC) [31]. At present, Acyclovir (35a) is the only remedy for genital herpes. The oral formulation of Acyclovir is effective against both first and second degree recurrence genital herpes with minimal side effects [32]. Ganciclovir (35b) [33] has shown good *in vivo* activity against Hepatitis C virus ($HCV_{1\&2}$).



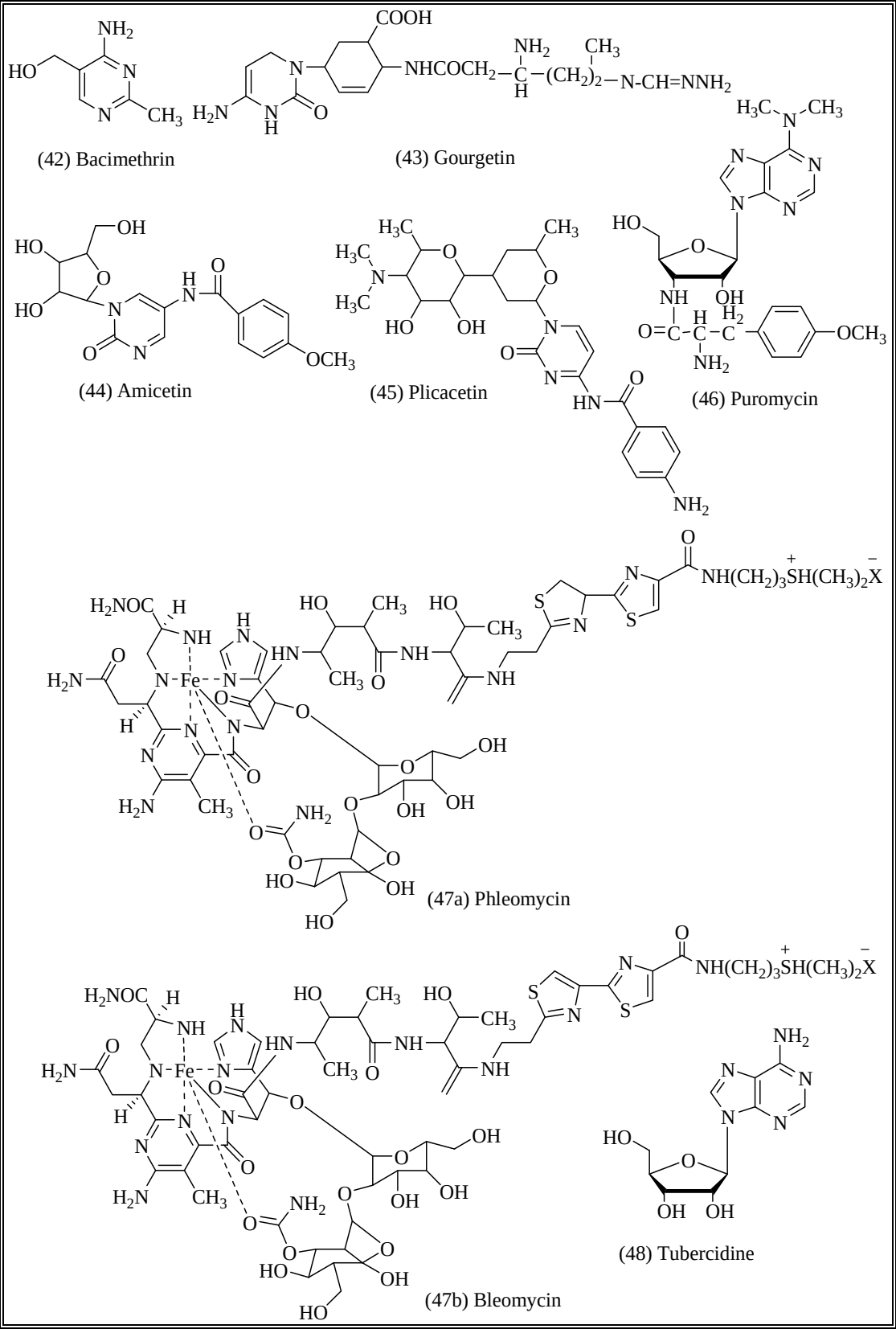
Several members of a series of acyclic nucleosides, which contain a fused pyrimidine ring (mainly purine), are found to be effective antivirals. Famiciclovir (35c) and valaciclovir (35d) are drugs used for several deoxyribonucleic acid (DNA) viruses, including Varicella-zoster virus and Epstein-Barr virus [34]. Penciclovir (35e) [35] is useful for topical treatment of recurrent herpes, *Libialis*. Cidofovir (36b) [35], an antimetabolite for deoxycytosine triphosphate is used for the treatment of cytomegalovirus (CMV) in AIDS patients. Lamivudine (36a) [35] is an effective anti-AIDS drug when used in combination with zidovudine (37) [35]. Zidovudine [36] is

an analogue of thymidine in which the azido group is substituted at the 3-position of the dideoxyribose moiety. It is active against Ribonucleic acid (RNA) tumour viruses (retroviruses) that are the causative agents of AIDS and T-cell leukaemia. It is used in AIDS and AIDS-related complex (ARC) to control opportunistic infections by raising absolute $CD4^+$ lymphocyte counts. Also, zalcitabine (38) [36] is another useful alternative drug to zidovudine. It is given in combination with zidovudine, when cluster of differentiation 4 ($CD4^+$ cell) count falls below 300 cells/mm^3. Didanosine (39) [37] is a purine dideoxynucleoside, which is an analogue of inosine. Didanosine inhibits HIV Drug Resistance Database (HIV RT) and exerts a virustatic effect on the retroviruses. Combined with zidovudine, antiretroviral activity of didanosine is increased. Stavudine (40) [37] is a pyrimidine nucleoside analogue that has significant activity against HIV-1 after intracellular conversion of the drug to a D4T-triphosphate. It is more effective than zidovudine or didenosine for treatment in patients for delaying the progression of HIV infection. It is recommended for patients with advanced HIV infection. Abacavir sulfate (41) [37] was approved in 1998 as a NRTI (Nucleoside Reverse Transcriptase Inhibitor) to be used in combination with other drugs for the treatment of HIV and AIDS. The major use of abacavir appears to be in combination with other Nucleoside reverse transcriptase inhibitor (NRTIs).

2.2.6 Antibiotics[m]

There are few examples of pyrimidine antibiotics. The simplest of all is bacimethrin (5-hydroxymethyl-2-methoxypyrimidin-4-amine) (42), which is active against several staphylococcal infections [38]. Gourgetin (43), a cytosine derivative is active against mycobacteria as well as several Gram-positive and Gram-negative bacteria [39]. There are more derivatives of cytosine, namely amicetin (44) and plicacetin (45), which exhibit activity against acid fast and Gram-positive bacteria as well as some other organisms [38]. Puromycin (46) has a wide spectrum of antitrypanosomal activity. Aminoglycoside antibiotics phleomycin (47a), bleomycin (47b) and related families are wide-spectrum antibiotics containing the pyrimidine ring.

[m] An antibiotic is a substance or compound that kills bacteria or inhibits their growth.



(42) Bacimethrin

(43) Gourgetin

(44) Amicetin

(45) Plicacetin

(46) Puromycin

(47a) Phleomycin

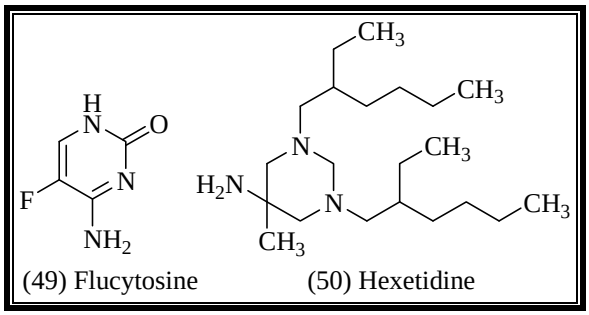
(47b) Bleomycin

(48) Tubercidine

Another antibiotic tubercidine (48) is reported to exhibit antitumour properties [39]. In addition, they have antineoplastic activity. Bleomycin is already in clinical use against certain tumours like Hodgkin's lymphoma and disseminated testicular cancer [40].

2.2.7 Antifungals[n]

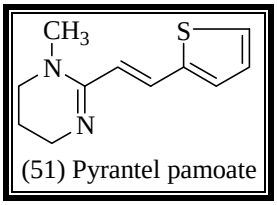
Pyrimidines also exhibit antifungal properties. Flucytosine (49) [41] is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and Cryptococcus [42]. Hexetidine (50) [43] is mainly used for the treatment of aphthous ulceration.



(49) Flucytosine (50) Hexetidine

2.2.8 Anthelmintics[o]

These drugs have the ability of ridding the body of parasitic worms. Pyrantel pamoate (51) is a depolarizing neuromuscular blocking agent that causes spastic paralysis in helminthes and is employed in the treatment of infestations caused by pinworms and roundworms [44].

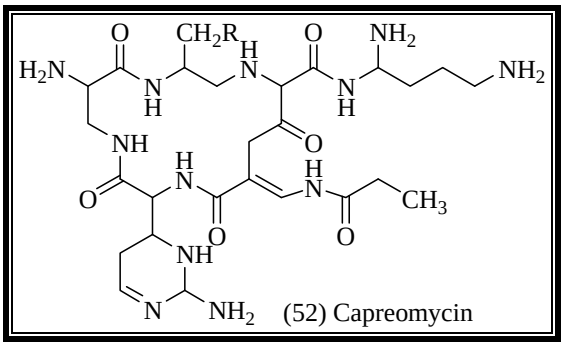


(51) Pyrantel pamoate

[n] An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis and others.

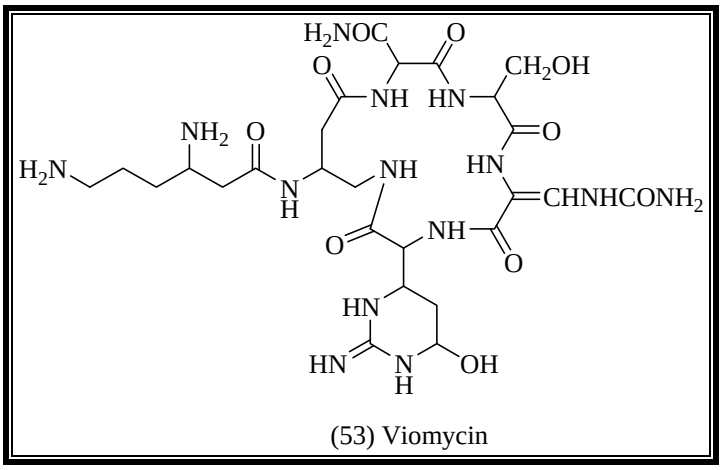
[o] Anthelmintics or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing).

2.2.9 Antitubercular drugs[p]



(52) Capreomycin

Capreomycin (52) produced by *Streptomyces capreolus* is a second-line bacteriostatic antituberculin drug containing pyrimidine [45, 46].



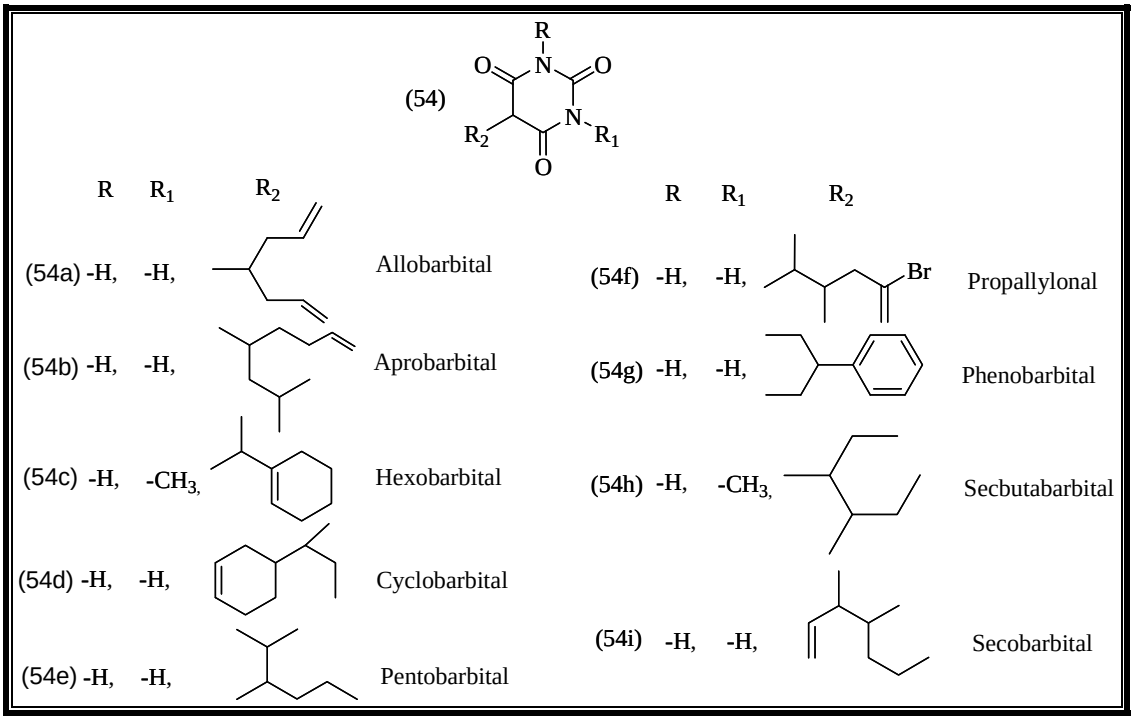
(53) Viomycin

Viomycin (53) is more tuberculostatic than *p*-aminosalicyclic acid. It is effective in the treatment of experimental tuberculosis.

[p] Antitubercular drugs are the antibiotics used in prevention and treatment of tuberculosis caused by the bacteria mycobacterium tuberculosis.

2.2.10 CNS active agents

2.2.10.1 Sedative[q]/hypnotic/antiepileptic agents

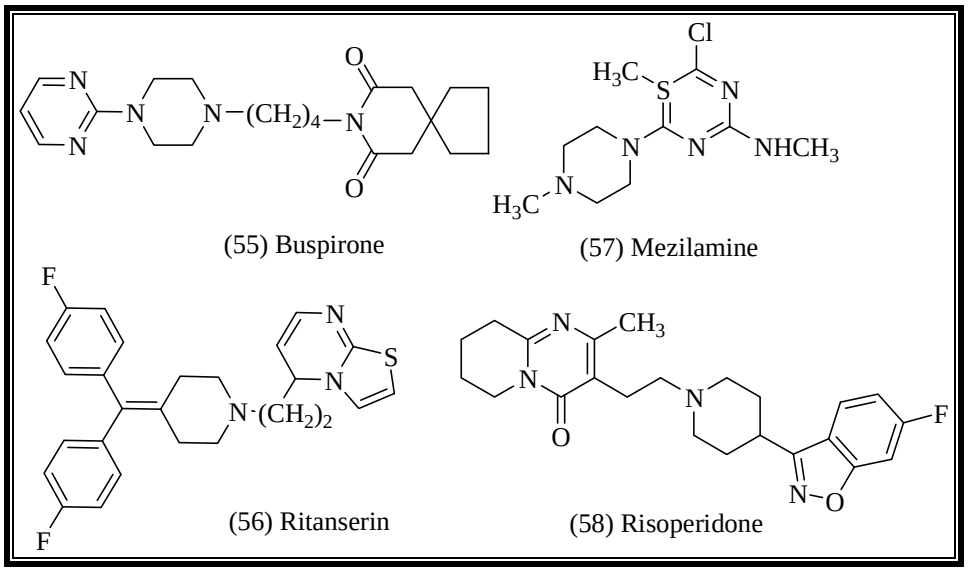


Agents of the anxiolytic, sedative and hypnotic group include a wide variety of barbiturates (54a–i) used as sedative and hypnotics and are classified as drugs having short, intermediate and long duration of action [47, 48]. Allobarbital (54a), aprobarbital (54b), pentobarbital (54e), phenobarbital (54g) and secobarbital (54i) are frequently used clinically as hypnotic barbiturates [49]. Hexobarbital (54c), cyclobarbital (54d) and propallylonal (54f) are some of the current drugs in the market used as sedative hypnotics [50]. Barbiturates as sedative hypnotics have a long and fascinating history. In fact Eli Lilly [51] patented secbutabarbital (54h) in 1932, while barbitone (8), the first of the barbiturates [1] was introduced in 1903.

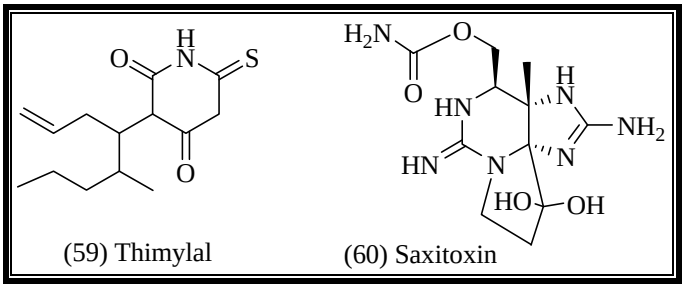
[q] A sedative is a substance that induces sedation by reducing irritability or excitement.

2.2.10.2 Anxiolytic[r] agents

Few of the pyrimidine derivatives are also used as anxiolytics. Most important of these is buspirone (55), indicated in the management of anxiety disorders accompanied with or without depression. It lacks sedative, anticonvulsant and muscle-relaxant effects and most importantly abuse potential [52]. Buspirone lacks affinity to benzodiazepine receptors, but binds avidly to one subclass of serotonin receptors, the 5-HT_{1A} subtype [53, 54]. Ritanserin (56), a $5HT_2$ antagonist with anxiolytic activity is a pyrimidine derivative [55]. A simple pyrimidine derivative, mezilamine (57) is classified as an antipsychotic agent [56]. Risoperidone (58) is an antipsychotic drug, which is a structural hybrid of butyrophenone and can be used as anxiolytic, antidepressant and antiparkinsonian drug [57].



2.2.10.3 Pyrimidine anaesthetics[s]



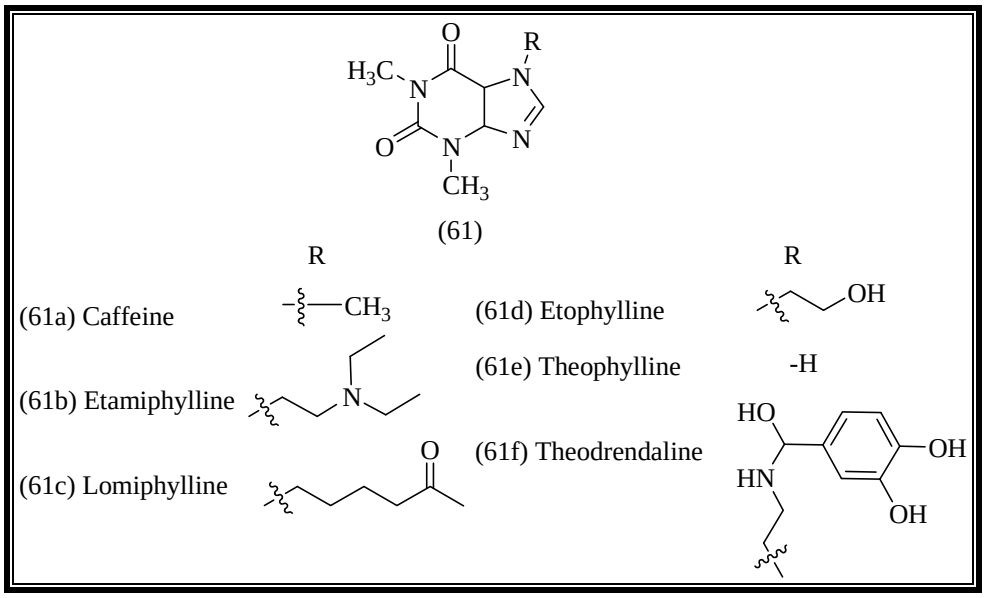
[r] An anxiolytic (also antipanic or antianxiety agent) is a drug used for the treatment of symptoms of anxiety.
[s] An anesthetic (or anaesthetic) is a drug that causes anesthesia—reversible loss of sensation.

Thimylal (59) is a short acting general anaesthetic drug, which is also a pyrimidine analogue [58, 59].

Saxitoxin (60) [58] is a naturally occurring pyrimidine containing anaesthetic agent, but is too toxic to be of clinical use. Saxitoxin is isolated from some marine dinoflagellates.

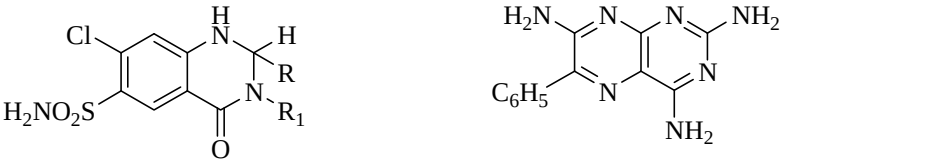
2.2.10.4 Diuretics[t] and uricosurics[u]

Several xanthine derivatives (61) containing fused pyrimidine ring systems like caffeine (61a) [60], etamiphylline (61b) [61], lomiphylline (61c) [62], etophylline (61d) [63], theophylline (61e) [60] and theodrendaline (61f) [64] are known to promote a weak diuresis by stimulation of cardiac function and by a direct action on the nephron, acting as adenosine receptor antagonists [60].



There are a few examples of diuretics which contain a pyrimidine ring. Noteworthy are quinethazine (62a), metolazone (62b) [65] and triamterene (63) [66].

[t] A diuretic is any drug that elevates the rate of urination and thus provides a means of forced diuresis.
[u] Uricosuric medications (drugs) are substances that increase the excretion of uric acid in the urine, thus reducing the concentration of uric acid in blood plasma.



(62) (63) Triamterene

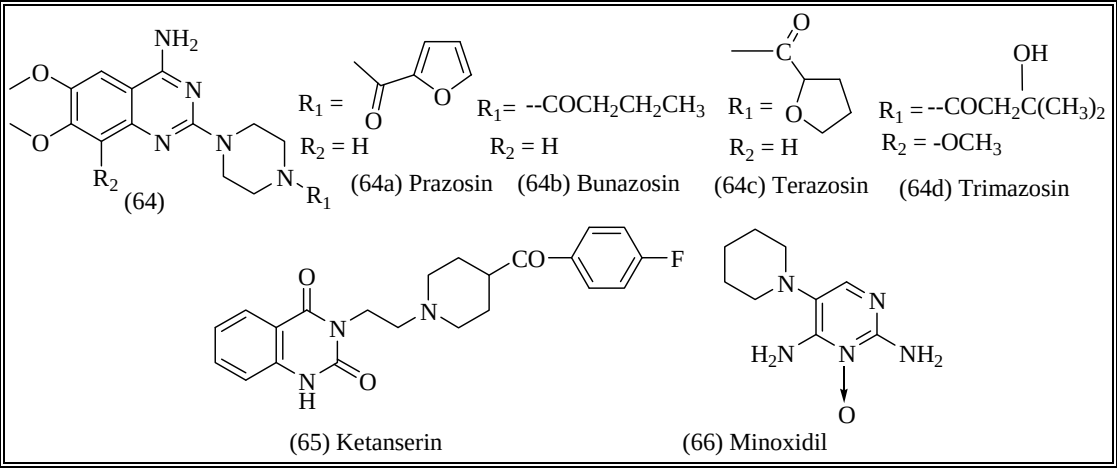
(62a) R = C_2H_5, R_1 = H; Quinethazine (62b) R = CH_3, R_1 = 2-$CH_3C_6H_4$; Metolazone

2.2.11 Cardiac agents

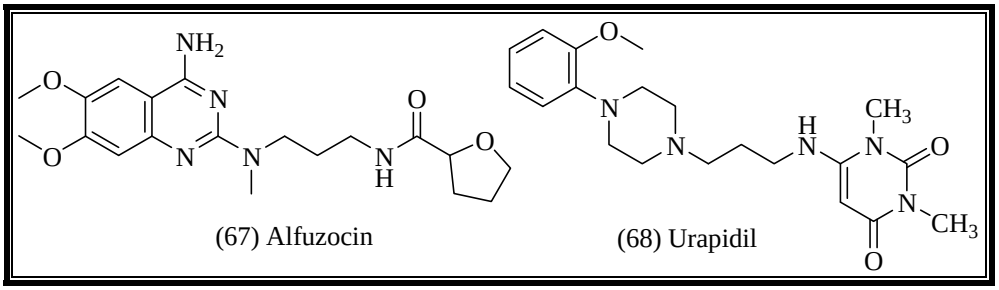
2.2.11.1 Antihypertensives[v]

Several pyrimidine ring-containing drugs have exhibited antihypertensive activity. Prazosin (64a), a quinozoline derivative, is a selective α_1-adrenergic antagonist [67, 68]. Its related analogues bunazosin (64b) [69], terazosin (64c) [70] and trimazosin (64d) [71] are potent antihypertensive agents.

Another quinazoline derivative, ketanserin (65) [72] having a similar effect is an antagonist of both a_1-adrenergic and serotonin-S_2 receptors. Its mechanism of action however is still controversial. A triaminopyrimidine derivative, minoxidil (66), whose mechanism of action and therapeutic action are similar to Prazosin, has been introduced in therapy for its side effects, in the treatment of alopecia, male baldness [73]. Besides these, some more pyrimidine derivatives given below were found to be antihypertensives [74, 75].



[v] The antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure).

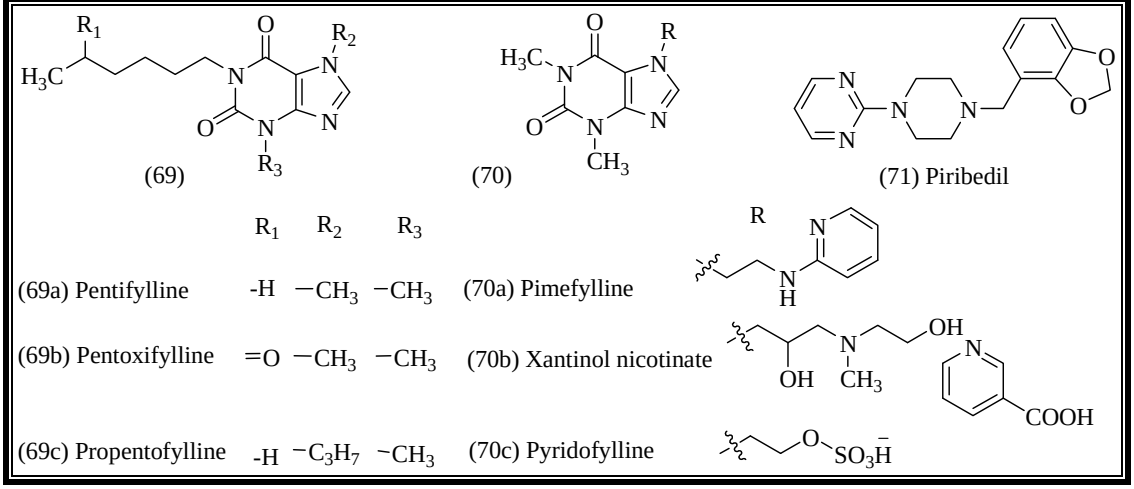


(67) Alfuzocin (68) Urapidil

Alfuzocin (67) [74], a prazosin analogue and an α_1-adrenoceptor antagonist as well as urapidil (68) [75] are used especially in urinary obstruction caused by benign prostate hyperplasia.

2.2.11.2 Vasodilators[w]

A series of xanthine derivatives are used as peripheral and cerebral vasodilators. Especially, pentifylline (69a) and pentoxifylline (69b) are used in cardiovascular disorders [76]. Other derivatives like xantinol nicotinate (70b) [77], a vasodilator with general properties like nicotinic acid used in cerebral and peripheral vascular disorders and pimefylline (70a) and pyridofylline (70c) [78] are noteworthy. A new dopamine receptor stimulant, pirebidil (71) [79] is reported to have produced significant improvement in Activity of Daily Living (ADL) in patients suffering from Parkinson's syndrome.



(69) (70) (71) Piribedil

	R_1	R_2	R_3
(69a) Pentifylline	-H	$-CH_3$	$-CH_3$
(69b) Pentoxifylline	=O	$-CH_3$	$-CH_3$
(69c) Propentofylline	-H	$-C_3H_7$	$-CH_3$

(70a) Pimefylline

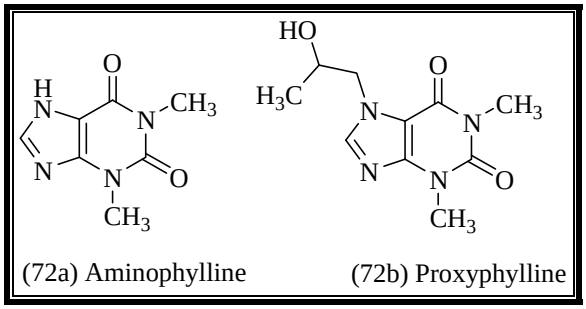
(70b) Xantinol nicotinate

(70c) Pyridofylline

[w] The term vasodilation refers to the widening of blood vessels, resulting from relaxation of smooth muscle cells within the vessel walls, and the drugs to which are used in phenomena are called vasodilators.

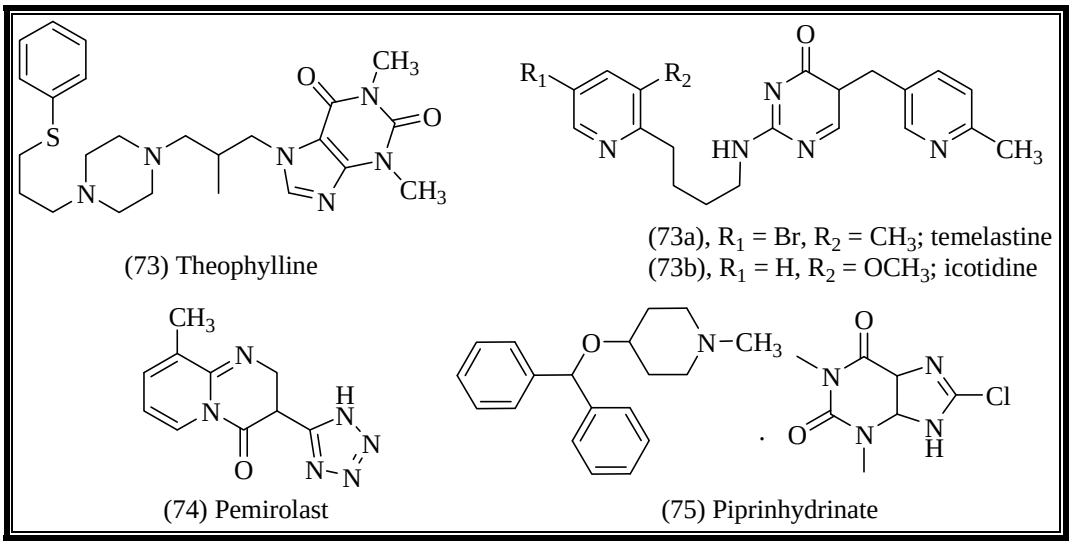
2.2.11.3 Cardiotonics[x]/bronchodialators

Several xanthine derivatives *viz.*, theophylline (61e), aminophylline (72a) [80] and proxyphylline (72b) [80] exhibit good bronchodilator activity.



2.2.12 Antihistaminic[y] pyrimidines

Theophylline (73) is ten times more potent than either astemizole or terfenadine in its affinity for H_1-histamine binding site and appears to be devoid of Central nervous system (CNS) activity [81]. Another pyrimidine containing antihistaminic drug, temelastine (73a) is comparable to mepyramine [82]. Radiolabelled studies have indicated that it does not penetrate the CNS appreciably. Icotidine (73b), a structural analogue of temelastine lacks CNS activity and is a dual antagonist of both H_1 and H_2 receptors [83].

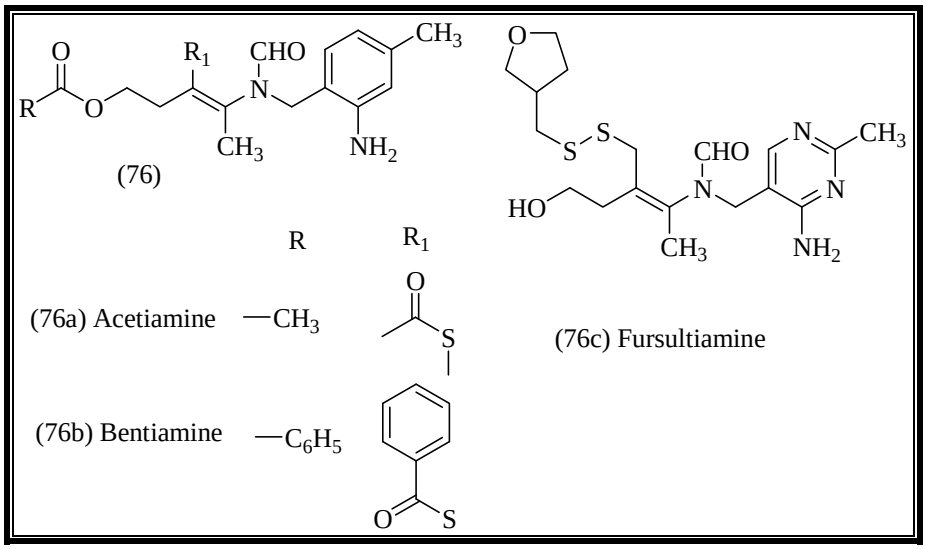


[x] Agents that have a strengthening effect on the heart or that can increase cardiac output.
[y] A histamine antagonist is an agent that inhibits action of histamine or the drug which is used to treat the allergy is called antihistaminic agent.

Pemirolast (74) [84], a new oral nonbronchodilator antihistaminic agent is also a pyrimidine derivative. It has demonstrated sufficient antihistaminic activity to warrant its use in severe asthma. Another compound, piprinhydrinate (75) [85] is also a pyrimidine derivative.

2.2.13 Analgesics[z] and NSAID[aa] drugs

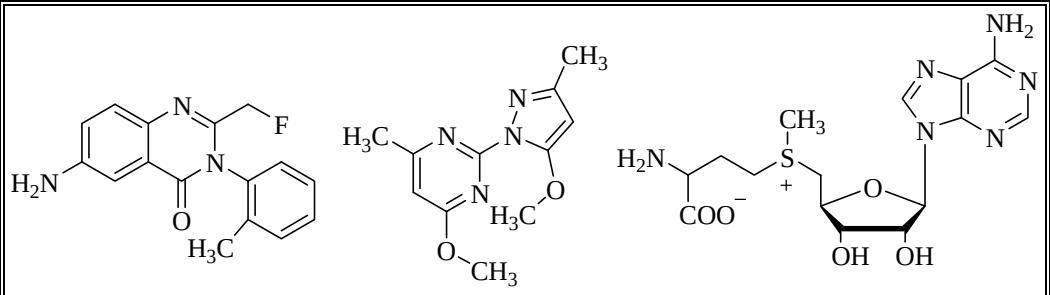
Acetiamine (76a) [86], bentiamine (76b) [86] and fursultiamine (76c) [87] are new lipid-soluble forms of thiamine (vitamin B_1) having therapeutic use in beriberi, polyneuritis, encephalopathy, pain, malnutrition and alcoholism and especially in the treatment of long-standing insulin-dependent diabetes mellitus. Fursultamine has been reported to inhibit the arachadonic acid cascade-line activation and reverse the increase in Coronary Blood Flow (CBF).



Afloqualone (77) [88] has been evaluated as a successful anti-inflammatory agent with lower back pain patients. Epirizole (78) [89], another NSAID, is suggested to be a COX-2 inhibitor. Ademetionine (79) [90] is primarily used in conjunction to glucosamine and chondroitin therapy. Octotiamine (80) [91], a vitamin B_1 derivative also exhibits anti-inflammatory activity. Proquazone (81) [92], a condensed

[z] An analgesic (also known as a painkiller) is any member of the group of drugs used to relieve pain.
[aa] Nonsteroidal anti-inflammatory drugs, usually abbreviated to NSAIDs or NAIDs, are drugs with analgesic and antipyretic effects and which have, in higher doses, anti-inflammatory effects (reducing inflammation).

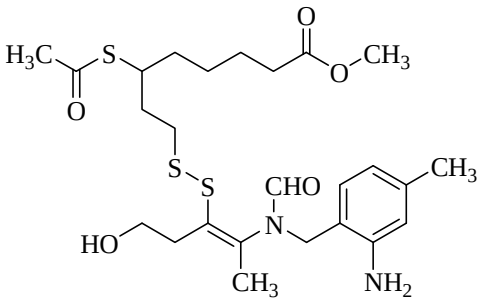
pyrimidin-2-one derivative has been reported to exhibit good Nonsteroidal anti-inflammatory drugs (NSAID) potential.



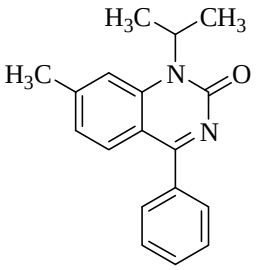
(77) Afloqualone

(78) Epirizole

(79) Ademetionine



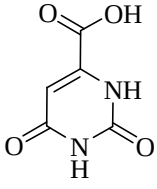
(80) Octotiamine



(81) Proquazone

2.2.14 Metabolic electrolytes

Orotic acid (82) [93], a simple pyrimidine derivative and its mineral forms are used in metabolic therapy, especially for cardiovascular patients to prevent heart failure in cardiomyopathy. Oroate is needed as a key intermediate in biosynthesis of pyrimidine nucleotides, which are building blocks for DNA and RNA required for the final protein synthesis.



(82) Orotic acid

2.3 Conclusion

Pyrimidines occupy a distinct and unique place in our life. This heterocyclic moiety has great biological and medicinal significance. A large array of pyrimidine drugs possesses a variety of medicinal properties. These properties include anticancer, antibacterial, antiprotozoal, antimicrobial, antiviral, antihypertensive, antihistaminic, anti-inflammatory, analgesic and CNS-active to metabolic adjuvant.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of bi/tricyclic aromatic heterocycles related to pyrimidines, two different heterocyclic scaffolds related to pyrimidines 1,2,3,4 tetrahydropyrimidines and 1,2,4-triazolo[1,5-*a*]pyrimidines have been synthesized in the framework of this doctoral thesis.

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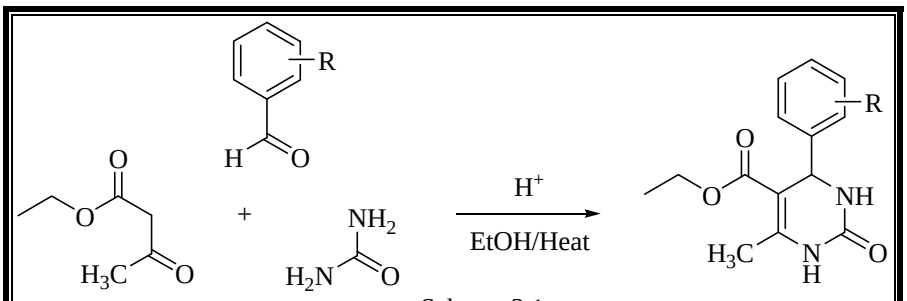
Chapter 3

Synthesis and biological evaluation of dihydropyrimidines

3.1 Introduction

Biginelli P. reported the synthesis of functionalized 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) via three-component condensation reaction of an aromatic aldehydes, urea and ethyl acetoacetate (Scheme 3.1). In the past decade, this multicomponent reaction has experienced a remarkable revival, mainly due to the interesting pharmacological properties associated with this dihydropyrimidine scaffold.



Scheme 3.1

The classical three-component Biginelli condensation is usually carried out in alcoholic solution containing a few drops of concentrated hydrochloric or sulfuric acid as catalyst, although other systems such as tetrahydrofuran/hydrochloric acid (THF/HCl), dioxane/hydrochloric acid or acetic acid/hydrochloric acid has also been employed. Multicomponent reactions (MCRs) occupy an outstanding position in organic and medicinal chemistry for their high degree of atom economy, applications in combinatorial chemistry and diversity-oriented synthesis [1].

The venerable Biginelli reaction, one pot cyclocondensation of aldehyde, 1,3-ketoester and urea or thiourea, is inarguably one of the most useful MCRs [2]. Polyfunctionalized dihydropyrimidines (DHPMs) represent a heterocyclic system of

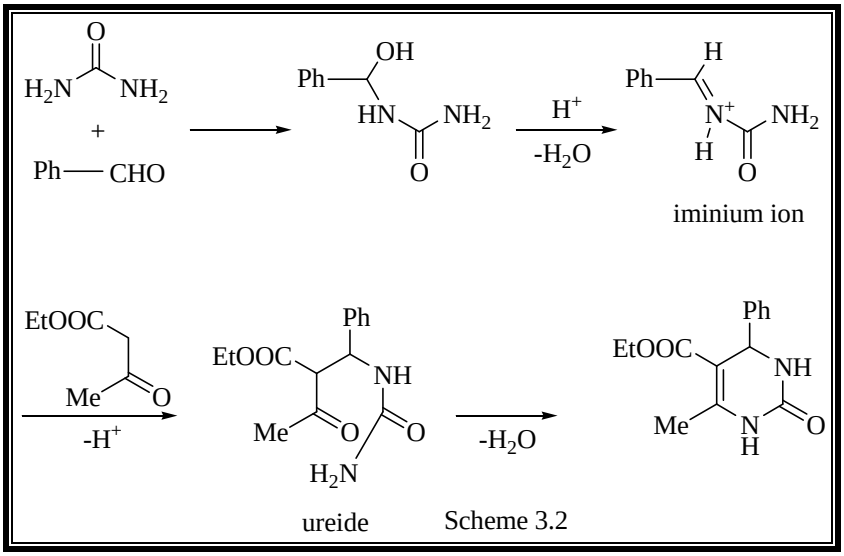
remarkable pharmacological activity.

4-Aryl-1,4-dihydropyridines of the nifedipine type are the most studied class of organic calcium channel modulators and, since their introduction into clinical medicine in 1975 have become almost indispensable for the treatment of cardiovascular diseases such as hypertension, cardiac arrhythmias or angina. In recent years research interest has also focused on aza-analogs such as dihydropyrimidines which shows similar pharmacological profile to this type of classical dihydropyridines calcium channel modulators [3].

3.1.1 Mechanistic Studies

The mechanism of the Biginelli reaction has been the subject of some debate over the past decades. Early work by Folkers K. *et al.* suggested that bisureide i.e., the primary bimolecular condensation product of benzaldehydes and urea is the first intermediate in this reaction [4]. In 1973 Sweet F. *et al.* proposed that a carbenium ion, produced by an acid-catalyzed aldol reaction of benzaldehyde with ethyl acetoacetate, is the key intermediate and is formed in the first and limiting step of the Biginelli reaction [5].

Kappe O. *et al.* reinvestigated the mechanism in 1997 using ^{1}H and ^{13}C NMR spectroscopy and have established that the first step in this reaction involves the acid-catalyzed formation of an *N*-acyliminium ion intermediate from the aldehydes and urea component (Scheme 3.2). Interception of the iminium ion by ethyl acetoacetate, possibly through its enol tautomer, produces an open-chain ureide which subsequently cyclize to dihydropyrimidine. Although the highly reactive *N*-acyliminium ion species could not be isolated or directly observed, further evidence for the proposed mechanism was obtained by isolation of intermediates, employing sterically bulky [6] or electron-deficient acetoacetates [7] respectively. The relative stereochemistry in hexahydropyrimidine was established by an X-ray analysis.



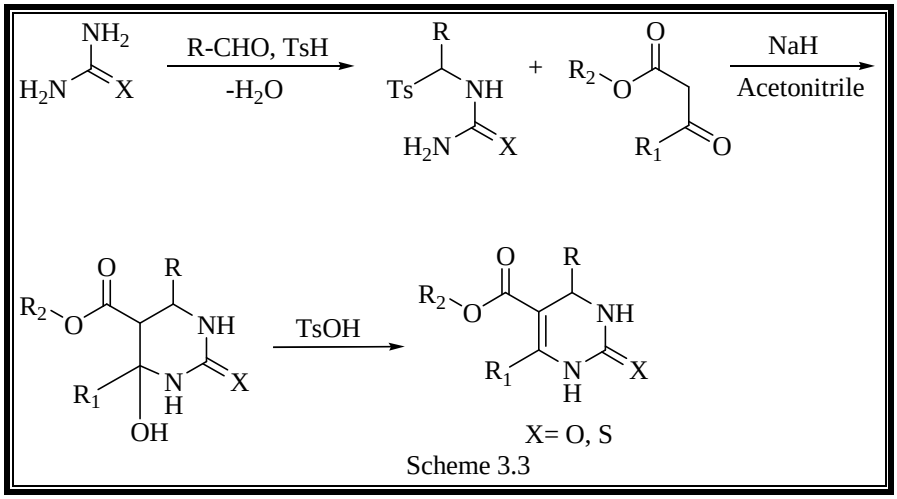
Scheme 3.2

3.1.2 Atwal alternative synthetic route

Apart from the traditional Biginelli condensation, there are only few other synthetic methods available that lead to DHPMs. Since most of these protocols lack the experimental and conceptual simplicity of the Biginelli one-pot, one-step procedure, none of these have any significance today or can compete with the original Biginelli MCR approach.

One noticeable exception is the so-called “Atwal modification” of the Biginelli reaction [8]. Here, arylidene is first condensed with a suitable protected urea or thiourea derivative under almost neutral conditions. Deprotection of the resulting 1,4-dihydropyrimidine with hydrochloric acid (HCl) or trifluoro acetic acid (TFA)/ethane thiol (EtSH) leads to the desired DHPMs. Although this method requires prior synthesis of enones (arylidenes), its reliability and broad applicability make it an attractive alternative to the traditional one step Biginelli condensation.

Another novel approach to DHPMs has been described by Shutalev *et al.* is outlined below (Scheme 3.3) [9]. This synthesis is based on the condensation of readily available R-tosyl-substituted thiourea with the (in situ prepared) enolates of acetoacetates or 1,3-dicarbonyl compounds. The resulting hexahydropyrimidines need not to be isolated and can be converted directly into DHPMs. This method works particularly well for aliphatic aldehydes and thiourea and produces high overall yields of the desired target compounds.



Scheme 3.3

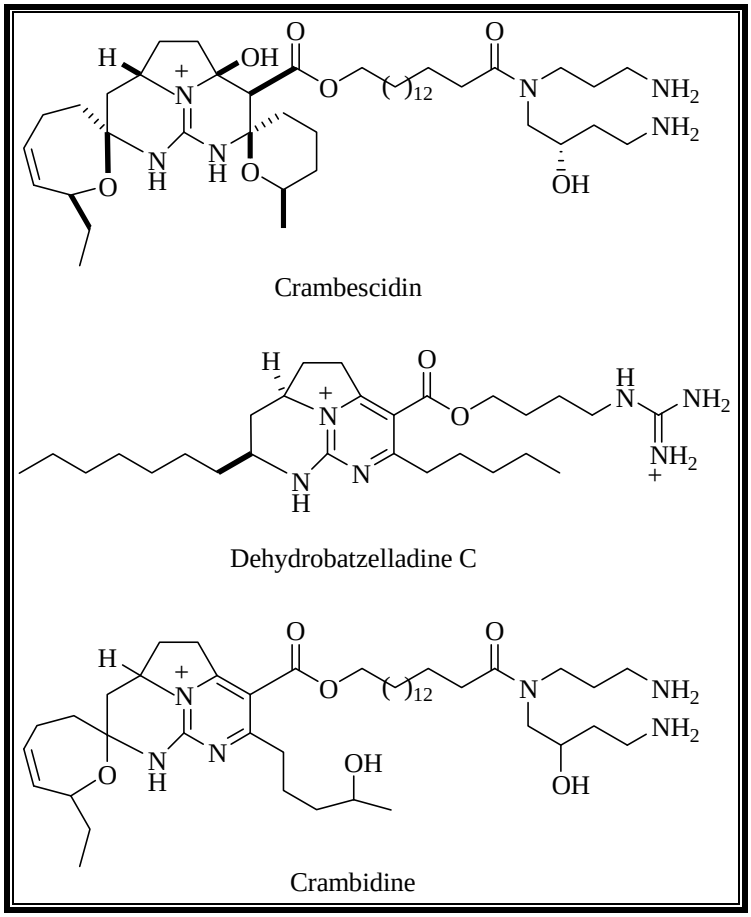
3.1.3 Pharmacological Profile

The interest in synthesis of dihydropyrimidines - Biginelli compounds stems from their close structural relationship [10] to clinically important 1,4-dihydropyridine calcium channel modulators of the type nifedipine etc. and also because of interesting biological properties of several marine alkaloids [11-13] based upon dihydropyrimidine *viz.* crambine, batzelladine and ptilomycelin A. Derivatization of the dihydropyrimidines especially [14] at C4 has led to the recognition of several lead compounds that show a very similar pharmacological profile [15-17] to 1,4-dihydropyridine based drugs.

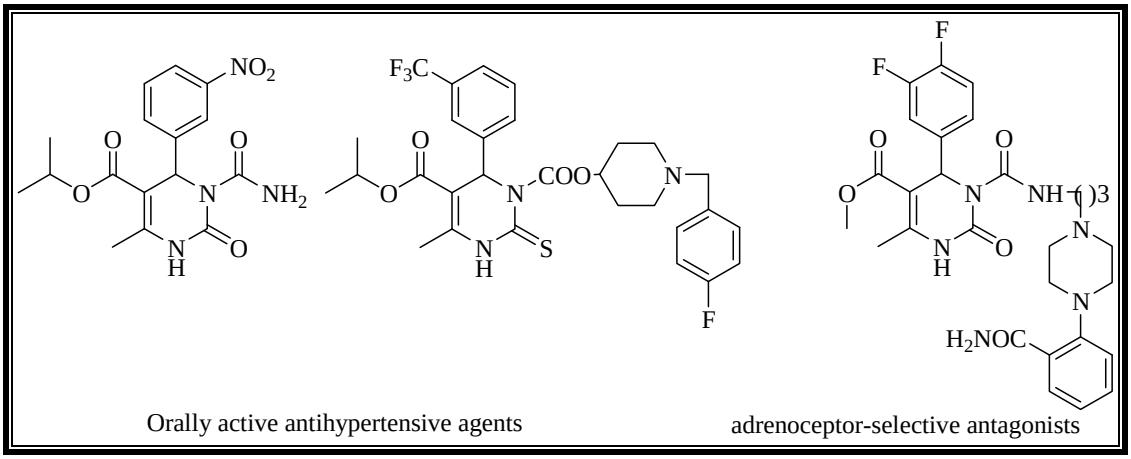
C. crambe is a bright red marine sponge, that is the most wide spread in the northwestern meditettanean [18]. Extract of C. crambe have been known to be ichthyotoxic and shown various pharmacological activities. A variety of structurally intricate guanidine alkaloids are present in marine sources [19]. Diverse biological activities are associated with many of these alkaloids, likely reflecting the multiple ways that a guanidinium cation can participate in noncovalent interactions.

Among the most notable marine alkaloids of these are the crambescidin [20] and batzelladine [21] alkaloids, which have been isolated primarily from sponges belonging to the orders Poecilosclerida and Axinellida. Diverse biological activities have been reported for these secondary metabolites, including cytotoxicity towards several cancer cell lines, antifungal and antiviral activities and inhibition of HIV-1 fusion. The novel structures of these marine alkaloids have inspired the development

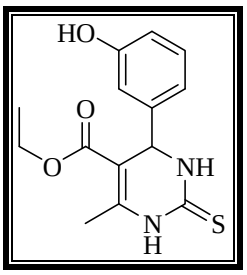
of many strategies or assembling polycyclic guanidines that contain the octahydro-5,6,6a-triazaacenaphthalene and hexahydro-5,6,6a-triazaacenaphthalene moieties common to the crambescidin and batzelladine alkaloids [22, 23].



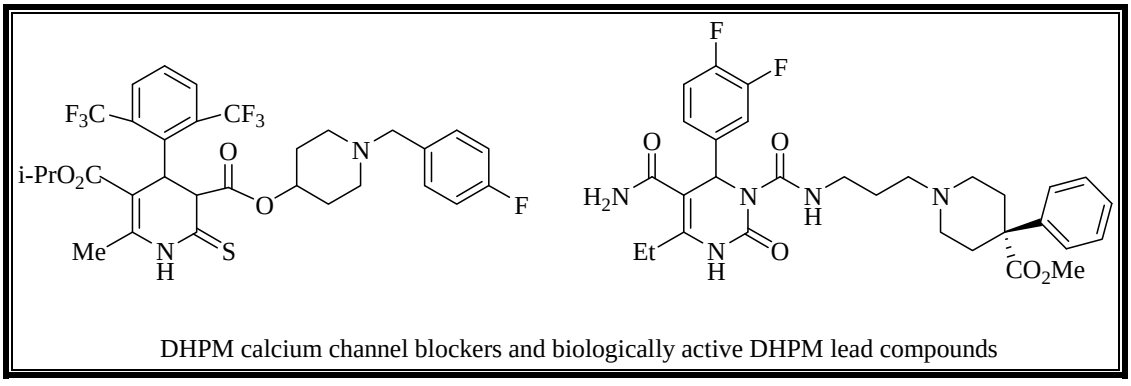
More recently, appropriately functionalized DHPMs have emerged as, e.g., orally active antihypertensive agents [24-26] or α_{1a} adrenoceptor-selective antagonists [27].



Simple DHPM monastrol as a novel cell-permeable molecule that blocks normal bipolar spindle assembly in mammalian cells and therefore causes cell cycle arrest [28].



Kappe O. *et al.* reported *N*-substituted 3,4-dihydropyrimidinones entities shows very good activity as a calcium channel blockers [29].

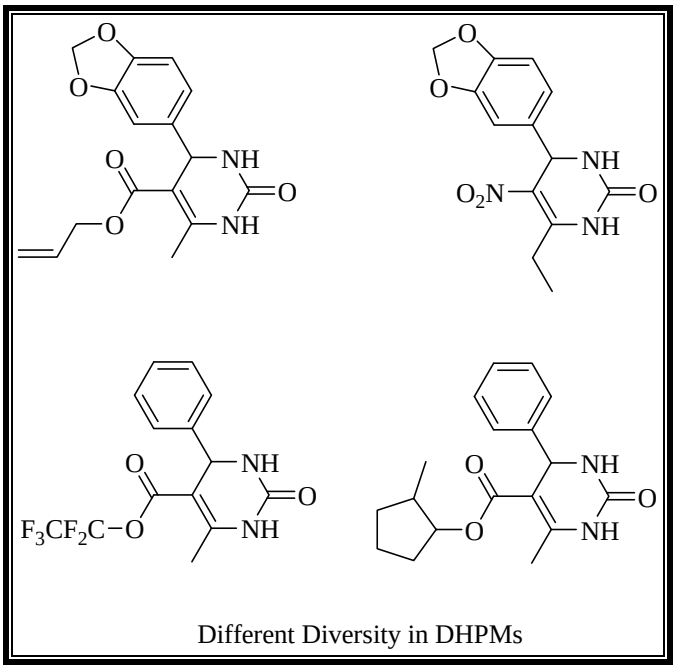


Demarest K. *et al.* reported that calcitonin, a 32 amino acid polypeptide hormone secreted by the thyroid and thymus glands, plays an important role in

inhibiting bone resorption through the mediation of osteoclasts. By inhibiting bone resorption and promoting renal calcium excretion, calcitonin has therapeutic applications in a variety of clinical disorders, including hypercalcemia associated with Paget's disease [30] and osteoporosis [31, 32].

A multiplex mimetic cell based assay was designed for high-throughput screening. In an effort to differentiate activity amongst similar G-protein coupled receptors, 6 cell lines-calcitonin receptor-2 (CTR-2; clone #33), Glucogen-like peptide 1 (GLP1-7; clone #7), Gastric inhibitory polypeptide (GIP-1; clone #1), parathyroid hormone receptor 1 and calcitonin gene related peptide-1 (CGRP1-7; clone 7) were cloned onto the human embryonic kidney (HEK 293) cell line and plated together in one assay well.

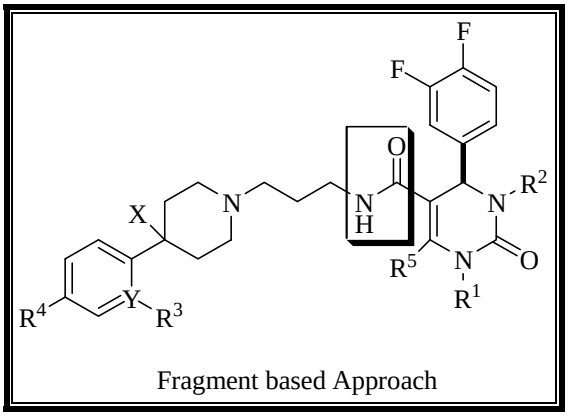
The following compounds are examples of an active series of 1,4-dihydropyrimidines that stimulated cyclic adenosine monophosphate (cAMP) accumulation in HEK 293 cells expressing the CTR-2 ligand [33].



Different Diversity in DHPMs

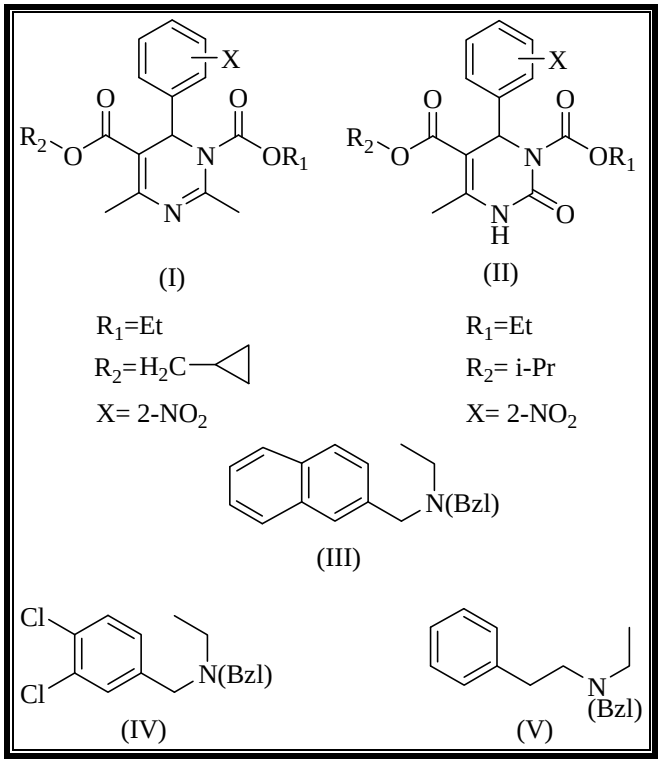
Research interest in multifunctionalized DHPMs of privileged heterocyclic core associated with several pharmacological properties. Small molecules targeting the mitotic machinery [34]. Notably, 4-aryldihydropyrimidinone heterocycles attached to an aminopropyl-4-piperidine moiety via a C5 amide linkage have proven to be excellent templates for selective α_{1a} receptor subtype antagonists to warrant

further consideration for the treatment of Benign prostatic hyperplasia (BPH) [35]. In the synthesis of these DHPM-5-carboxamides, amide bond formation between the requisite amines and the corresponding DHPM acids was performed using standard solution phase amide coupling chemistry involving carbodiimide coupling reagents [35, 36].

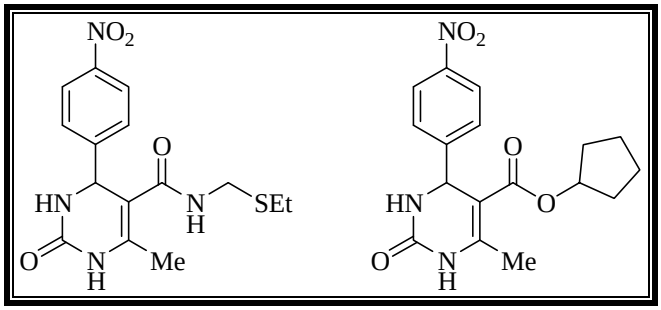


Fragment based Approach

Some dihydropyrimidines (V), (III) and (IV) were weaker in blocking atrioventricular conduction in anesthetized open-chest dogs and less toxic than the dihydropyridines [37].



Christopher B. *et al.* synthesized 3,4-dihydropyrimidinone analogues as a fatty acid transporter (FATP4). Among these some of the compounds hit by high through put screening and optimized FATP4 inhibitors. Blocking the absorption of fats (triglycerides) by administration of an anti-absorptive agent is of interest for the treatment of obesity [38].

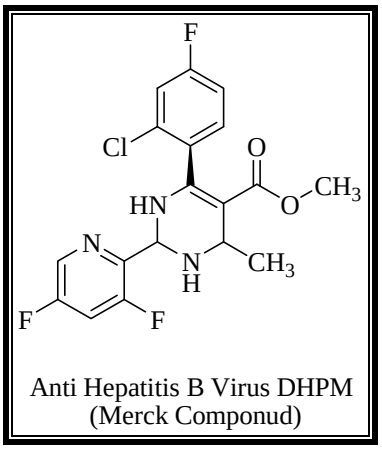


Ingested dietary triglycerides are hydrolyzed by gastric and pancreatic lipases and the resulting fatty acids are taken up by enterocytes lining the small intestine where they are re-esterified to triglycerides and then transported into the blood. The lipase inhibitor orlistat (pills used to lose weight: trade name-Xenical^{TM}), blocks fat absorption by inhibiting the hydrolysis of dietary fat to fatty acids [39] with administration leading to a concomitant decrease in body weight and improvement of blood lipid profiles. A family of proteins, termed fatty acid transport proteins (FATPs), that mediate the uptake of fatty acids into cells has been described [40, 41]. Earlier studies [42-45] provided evidence that fatty acid transport protein 4 (FATP4) mediates the transport of fatty acids from the gut into enterocytes both *in vitro* and *in vivo*.

We therefore reasoned that inhibitors of FATP4 might be expected to have benefits similar to orlistat. Since FATP4 inhibition would result in the accumulation of free fatty acids rather than triglycerides, we would also expect a different, possibly improved, side-effect profile compared to orlistat. The FATP family of proteins is most closely related in sequence to the ATP-utilizing acyl-CoA synthetase enzymes [46-49].

Moreover, Merck and Co. developed a compound which is very active as non-nucleoside inhibitors of human hepatitis B virus for reduction of HBV DNA in human hepatoma HepG 2.2.15 cells with low cytotoxicity in uninfected cells. This compound

inhibited both viral DNA and viral cores in HepG 2.2.15 cells and HBV transfected cell lines, whereas it did not affect the activity of endopolymerase and had no effect on other DNA and RNA viruses. *In vivo*, in a transgenic mouse model, oral doses of 3-100 mg/kg b.i.d. (twice a day) or t.i.d. (three times a day) for 28 days dose [50].



Anti Hepatitis B Virus DHPM
(Merck Componud)

3.1.4 Improved reaction conditions

Previous reported protocols normally required prolonged reaction times and high temperature with moderate yields, so there has been considerable interest to explore mild, rapid and higher yielding protocol. The toxicity and volatile nature of many organic solvents, particularly chlorinated hydrocarbons that are widely used in huge amounts for organic reactions have posed a serious threat to the environment. Thus, so many improved protocols have been designed for preparing these types of entities has been developed to improve and modify this reaction by several catalysts. Catalytic reaction has received tremendous attention in recent times in the area of green synthesis. However, it has been observed that the solvent and lewis acids employed are not always ecofriendly and because of this severe environmental pollution often results during the process of waste disposal. This prompted us to initiate a systematic investigation to look into the feasibility of a reaction under modified experimental conditions towards development of real green methodology for useful molecules.

Different catalysts have been employed for these types of reaction are:

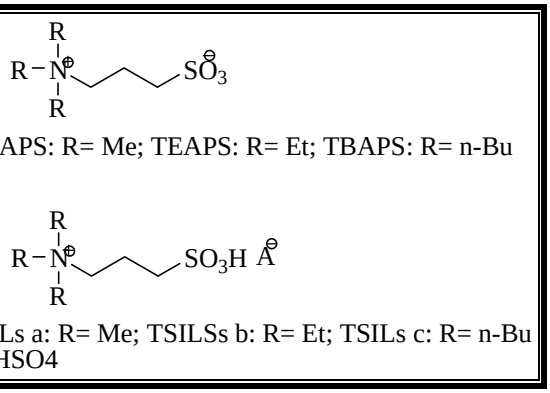
Ferric chloride ($FeCl_3$)/tetraethyl orthosilicate [51], triflates [52, 53], metal bromide [54, 55], polyoxometalate [56], strontium (II) nitrate [57], cerium (III) chloride [58], lithium trifluoromethanesulfonate or lithium triflate (LiOTf) [59], lanthanide triflates-$Ln(OTf)_3$ [60], heteropolyacids [61-65], ion exchange resins, polymer based solid

acid [66, 67], L-proline [68, 69], chiral phosphoric acid [70], trimethylsilyl chloride (TMSCl) [71], zirconium tetrachloride $ZrCl_4$ [72], dowex [73], Boron trifluoride-etharate (BF_3-etharate) [74], BF_3-etharate/cuprous chloride (CuCl) [74], vanadium trichloride (VCl_3) [75], lithium perchlorate ($LiClO_4$) [76], stannous chloride ($SnCl_2.2H_2O$) [122a], $AlCl_3$/KI [122b], $CoCl_2/MnCl_2$ [122c], $AlCl_3/AlBr_3$ [122d], P_2O_5 [123], Bismuth oxide perchlorate ($BiOClO_4.xH_2O$) [124], $CaCl_2$ [125a], 1,3-Dibromo-5,4-dimethylhydantoin [125b], Zinc tetrafluoroborate [125c].

Numerous modifications on Lewis acid adsorbed on mineral inorganic solid supports, silica, different clays are also reported and discloses a simple modification of the Biginelli DHPM synthesis. Excellent yields enhanced reaction rates, compatibility with various functional groups, environmentally friendly procedure, timesaving process, low cost and easy availability of the catalyst are some of the salient features of this reaction. This procedure will offer an easy access to substituted dihydropyrimidin-2(1*H*)-ones and thiones with different substitution patterns in high to excellent yields.

3.1.5 Ionic Liquids

Zuliang L. *et al.* used cheap and reusable task-specific ionic liquids that bear an alkanesulfonic acid group in an acyclic trialkylammonium cation were found to be effective catalysts for synthesizing 3,4-dihydropyrimidine-2-(1*H*)-ones via the one-pot three-component Biginelli reaction. The satisfactory results were obtained with good yields, short reaction time and simplicity in the experimental procedure. The catalysts could be recycled and reused six times without noticeably decrease in the catalytic activity [77].



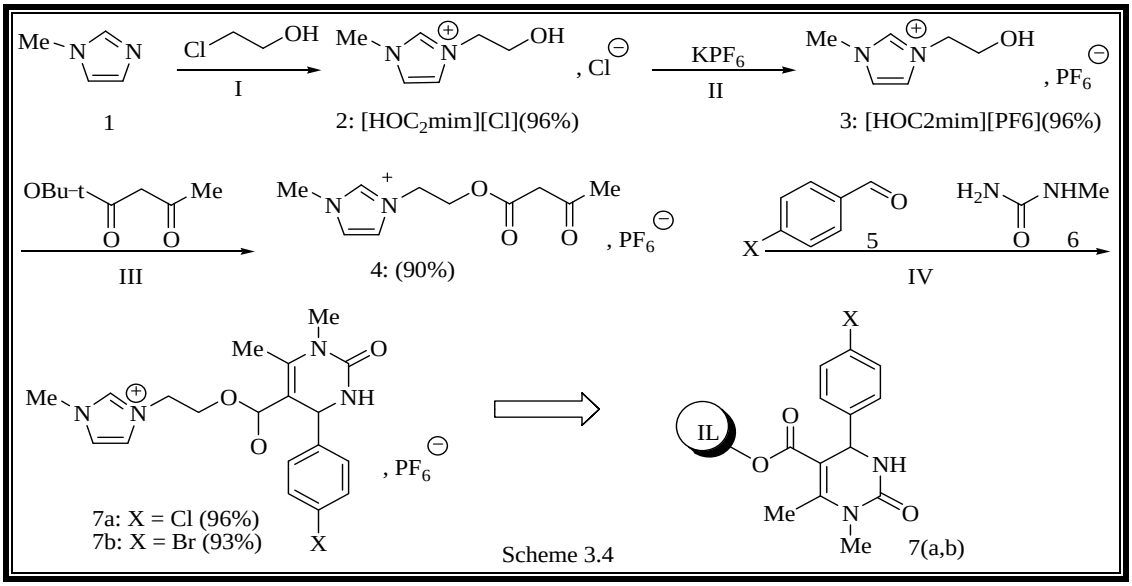
TMAPS: R= Me; TEAPS: R= Et; TBAPS: R= n-Bu

TSILs a: R= Me; TSILSs b: R= Et; TSILs c: R= n-Bu
A: HSO4

Bazureau J. P. *et al.* reported new N-3 functionalized 3,4-dihydropyrimidine-2(1*H*)-ones with 1,2,4-oxadiazole group as amide isostere were synthesized in six steps by ionic liquid-phase organic synthesis (IoLIPOS) methodology from Ionic Liquid Phase (ILP) bound acetoacetate. The 3,4-dihydropyrimidine-2(1*H*)-one (3,4-DHPM) core was prepared in the first step by one-pot three-component Biginelli condensation followed by N-alkylation with chloroacetonitrile. Then the nitrile group appended on the 3,4-dihydropyrimidine heterocycle was quantitatively transformed into amidoxime. Addition of aliphatic carboxylic anhydride or aromatic carboxylic acid to the amidoxime produced the expected 1,2,4-oxadiazole via the O-acylamidoxime intermediate grafted on the ILP bound 3,4-dihydropyrimidines using two convergent methods. After cleavage by transesterification under mild conditions, the target compounds were obtained in good overall yields. The structures and the purities of the reaction intermediates in each step were verified easily by routine spectroscopic analysis [78].

3.1.6 Preparation of ionic liquid phases bound 3,4-dihydro pyrimidine -2(1*H*)-ones

Scheme 3.4. Reagents and reaction conditions: (i) chloroethanol (1 equiv), mw, 180 ^{0}C, 60 W, 10 min; (ii) potassium hexafluorophosphate (KPF_6) (1 equiv), cyano methane (MeCN), 25 ^{0}C, 18 h; (iii) *tert*-butyl acetoacetate (2.6 equiv), μω, 170 ^{0}C, 150 W, 10 min; (iv) 100 ^{0}C, hydrochloric acid (HCl) cat., 60 min



Scheme 3.4

Zlotin S. G. *et al.* reported the synthesis of dihydropyrimidinones by condensation of aromatic (heteroaromatic) aldehydes with 1,3-dicarbonyl compounds under the 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]) ionic liquid-piperidinium acetate catalytic system (0.2 equiv. of each component) in the absence of a solvent affords, depending on the structures of the reagents, 2-arylidene derivatives of methyl acetoacetate and acetylacetone, diethyl 2,4-bis(trifluoroacetyl)-3-phenylpentanedioate or dimethyl 2-aryl-4-hydroxy-6-oxocyclohexane-1,3-dicarboxylates. The reactions of the resulting 2-arylidene derivatives with O-methylisourea in the [Bmim][BF_4] ionic liquid produced methyl 2-methoxy-4-methyl-6-aryldihydropyrimidine-5-carboxylates and 1-(2-methoxy-4-methyl-6-phenyl dihydropyrimidin-5-yl)ethanone (mixtures of 3,6- and 1,6-dihydro isomers), which were transformed into the corresponding 3,4-dihydropyrimidin-2(1*H*)-one derivatives [79].

Jingxing D. *et al.* used novel ionic liquid, 3-carboxymethyl-1-methylimidazolium bisulfate ($CmimHSO_4$) used as a recyclable catalyst for the Biginelli reaction under solvent-free conditions [80]. Bazureau J. P. *et al.* reported ionic liquid phase bound acetoacetate for the synthesis of 3,4-dihydropyrimidine-2(1*H*)-ones [81]. These compounds can also be synthesized in high yields in the presence of catalytic amounts of room temperature ionic liquids such as 1-*n*-butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$) or 1-*n*-butyl-3-methylimidazolium hexafluorophosphate ($BmimPF_6$) [82]. It has been also reported that not only trialkylammonium halides [83] but also very inexpensive and easily available ammonium chloride [84]. Gholap *et al.* reported the synthesis of DHPMs by using *N*-Butylimidazolium tetrafluoroborate ([Nbim]BF_4) [85]. Jain *et al.* used [bmim]BF_4 immobilized Cu (II) as a catalyst in synthesis of DHPMs [86]. Hua-Zheng Y. *et al.* reported non-toxic room temperature ionic liquid l-n-butyl-3-methylimidazolium saccharinate (BmimSac) [87].

In recent years, task-specific room-temperature ionic liquids (TSILs) have emerged as a powerful alternative to conventional molecular organic solvents or catalysts due to their particular properties, such as undetectable vapor pressure, wide liquid range, as well as the ease of recovery and reuse The TSILs have also been used as catalysts for Biginelli reaction [88-97].

However, TSILs with imidazole as the cation are relatively expensive, which hinders their industrial applications. Furthermore, typical ionic liquids consist of

halogen containing anions such as $[PF_6]^-$, $[BF_4]^-$, $[CF_3SO_3]^-$ and $[(CF_3SO_2)_2N]^-$, which in some regard limit their "greenness" [98-100]. Therefore, it is necessary to synthesize less expensive and halogen-free TSILs.

Shaabani A. *et al.* [101] used room-temperature ionic liquid 1,1,3,3-tetramethylguanidinium trifluoroacetate as catalyst.

3.1.7 Building blocks and diversity

Out of the three building blocks in the Biginelli reaction it is the aldehyde component, which can be varied to the largest extent. In general, the reaction works best with aromatic aldehydes. These can be substituted in the o-, m- or p- position with either electron-withdrawing or electron-donating groups. Good yields are usually obtained with m- or p- substituted aromatic aldehydes carrying electron-withdrawing substituents. For o-substituted benzaldehydes having bulky substituents, yields can be significantly lower.

Heterocyclic aldehydes derived from furan, thiophene and pyridine rings also generally furnish acceptable yields of DHPM products [102]. Aliphatic aldehydes typically provide only moderate yields in the Biginelli reaction unless special reaction conditions are employed, i.e. Lewis acid catalysts/solvent free methods or using the aldehydes in protected form [103]. The C4 unsubstituted DHPM can be prepared in a similar manner employing suitable formaldehyde synthons [103]. Of particular interest are reactions where the aldehyde component is derived from a carbohydrate. In such transformations, DHPMs having a sugar-like moiety in position 4 (C-nucleoside analogs) are obtained [104]. In a few cases, bisaldehydes have been used as synthons in Biginelli reactions [105].

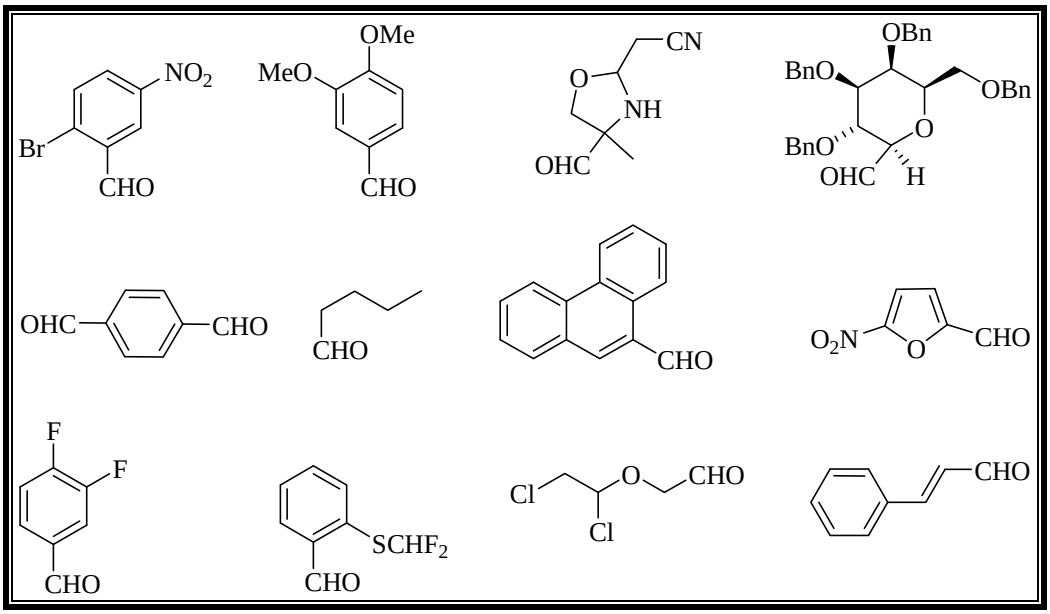
Traditionally, simple alkyl acetoacetates are employed as methine-acidic carbonyl building blocks, but other types of 3-oxoalkanoic esters or thioesters can also be used successfully. With methyl 4-chloroacetoacetate, for example, the corresponding 6-chloromethyl-substituted DHPMs which can serve as valuable templates for further synthetic transformations are obtained [106]. Benzoylacetic esters react analogously, but yields are usually significantly lower and the overall condensation process is more sluggish [102]. Primary, secondary and tertiary acetoacetamides can be used in place of esters to produce pyrimidine-5-carboxamide [102]. In addition, β-diketones serve as viable substrates in Biginelli reactions.

Condensations can also be achieved employing cyclic β-diketones such as cyclohexane-1,3-dione [107] and other cyclic β-dicarbonyl compounds [108].

If a C6-unsubstituted DHPM derivative needs to be synthesized, the corresponding 3-oxopropanoic ester derivative in which the aldehyde functional group is masked as an acetal can be employed [109]. Apart from ester-derived methine-acidic carbonyl compounds, nitroacetone also serves as a good building block, leading to 5-nitro-substituted DHPM derivatives in generally high yields [110]. The urea is the component in the Biginelli reaction that faces the most restrictions in terms of allowed structural diversity [111]. Therefore, most of the published examples involve urea itself as building block. However, simple monosubstituted alkyl ureas generally react equally well, in a regiospecific manner, to provide good yields of N1-substituted DHPMs. Thiourea and substituted thioureas follow the same general rules as ureas, although longer reaction times are required to achieve good conversions.

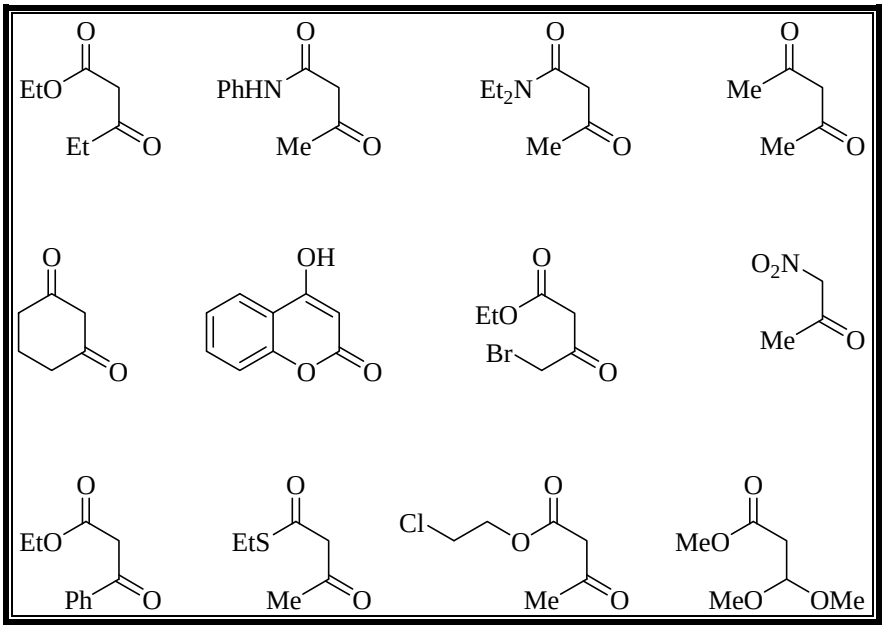
Yields are typically lower when compared to the corresponding urea derivatives. In some instances, it is also possible to react protected urea or thiourea (isourea) or guandidine under weak basic conditions with the aldehyde and methine-acidic carbonyl component (or with a precondensed Knoevenagel type enone) to yield the corresponding protected DHPMs [112, 113].

3.1.7.1 Aldehyde and protected aldehyde building blocks used in the Biginelli reaction



This latter method, using precondensed enones of type 5 has been frequently referred to as the "Atwal modification" of the Biginelli reaction [102, 103, 113]. Given the diversity in building block selection that is tolerated in the Biginelli reaction it is evident that a large number of DHPM derivatives of the general structure can be synthesized by combination of a relatively small number of (commercially available or proprietary) individual building blocks.

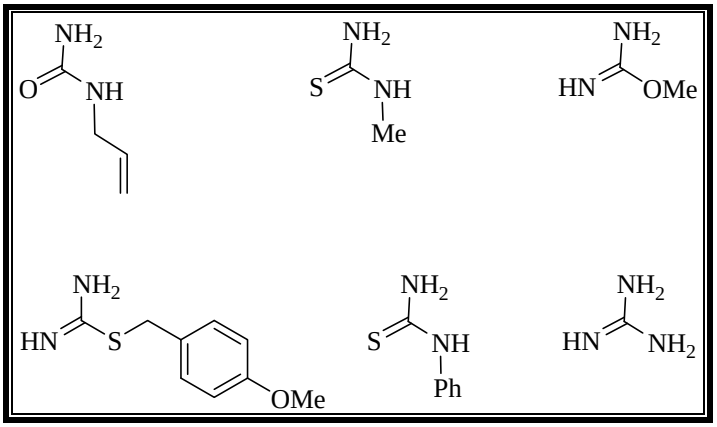
3.1.7.2 Methine-acidic carbonyl building blocks used in the Biginelli reaction



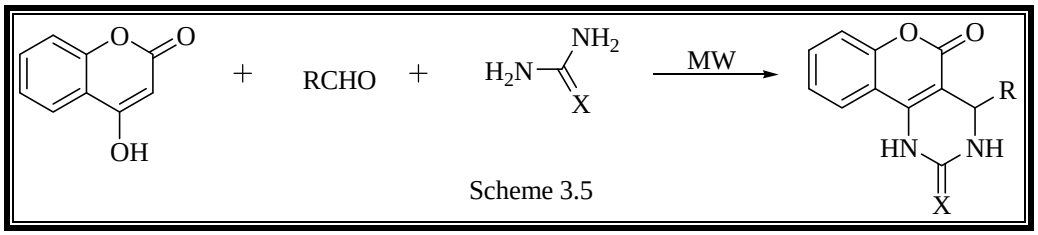
Employing different aldehydes (point of diversity at C4 position), different methine-acidic carbonyl derivatives (points of diversity at 5 and 6 position) and thiourea analogs (points of diversity at 2 position) in a Biginelli or Atwal type condensation would lead to a library of 1,000 DHPM compounds, with a total of five diversity points around the dihydropyrimidine core [114]. It is therefore not surprising that a literature search for the general DHPM structure in the Chemical Abstracts Registry database led to well over 10,000 hits [114].

It is interesting to note however, that only a small fraction of these compounds has been published in the chemical literature (<1,000) [114]. On the other hand, more than half the 10,000 structures of type are commercially available, typically from companies specializing in chemical library generation.

3.1.7.3 Urea type building blocks used in the Biginelli reaction



Kidwai M. *et al.* have been proposed an ecologically benign method for the synthesis of benzopyranopyrimidines by reactions of 4-hydroxycoumarin (instead of 1,3 diketones or β-ketoesters) with aldehydes and urea and thiourea in the absence of solvent under microwave irradiation (Scheme 3.5) [115].



Scheme 3.5

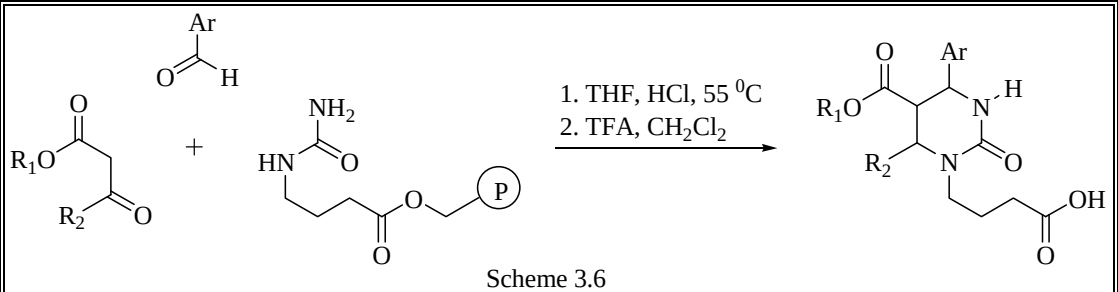
3.1.8 Solid phase synthesis

The generation of combinatorial libraries of heterocyclic compounds by solid phase synthesis is of great interest for accelerating lead discovery and lead optimization in pharmaceutical research. Multicomponent reactions (MCRs) leading to heterocycles are particularly useful for the creation of diverse chemical libraries, since the combination of n≥3 small molecular weight building blocks in a single operation leads to high combinatorial efficacy. Therefore, solid phase modifications of MCRs are rapidly becoming the cornerstone of combinatorial synthesis of small-molecule libraries. One such MCR that has attracted considerable attention in recent years is the Biginelli reaction, which involves the one pot cyclocondensation of a β-ketoester with an aryl aldehyde and a urea derivative. The resulting 4-aryl-3,4-dihydropyrimidin-

2(1*H*)-ones. Kappe O. *et al.* reported the 4-aryl-3,4-dihydropyrimidines using Resin-bound isothiourea building blocks and multidirectional resin cleavages. Solid phase organic synthesis remains one of the cornerstones of combinatorial chemistry, since this technique allows the chemist to take full advantage of the powerful principles (i.e. split and mix synthesis) offered by combinatorial technologies.

For a multicomponent reaction such as the Biginelli condensation, various solid-phase strategies can be envisaged and in fact a number of different approaches have been disclosed in recent years, utilizing different resin-bound building blocks and linker combinations. Given the regioselectivity encountered in using *N*-substituted urea building blocks in the Biginelli condensation, a solid phase modification where the urea component is linked to the solid support via the amide nitrogen is an obvious choice.

The first actual solid-phase modification of the Biginelli condensation was reported by Wipf P. *et al.* in 1995 [116]. In this sequence, *γ*-aminobutyric acid-derived urea was attached to Wang resin using standard procedures. The resulting polymer-bound urea was condensed with excess β-ketoesters and aromatic aldehydes in tetrahydrofuran (THF) at 55 ^{0}C in the presence of a catalytic amount of hydrochloric acid (HCl) to afford the corresponding immobilized DHPMs (Scheme 3.6).

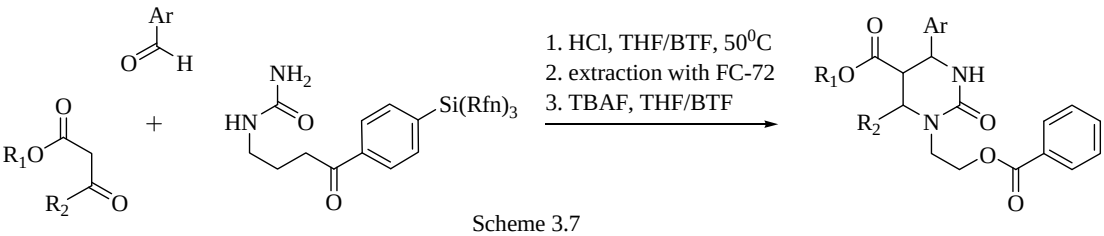


Scheme 3.6

In an interesting variation of this protocol, the Biginelli reaction was also adapted to fluorous-phase conditions by the Wipf P. *et al.* [116, 117]. In fluorous synthesis, an organic molecule is rendered soluble in fluorocarbon solvents by attachment of a suitable fluorocarbon group (“fluorous tag”). Fluorocarbon solvents are usually immiscible with organic solutions and fluorous molecules partition out of an organic phase and into a fluorous phase by standard liquid-liquid extraction.

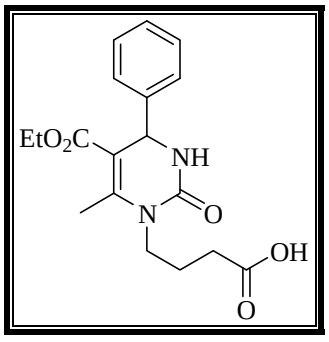
At the desired stage of the synthesis, the fluorous label is cleaved and the product is rendered “organic” again [118]. In the fluorous Biginelli reaction, the fluorous urea derivative was prepared by attachment of a suitable fluorous tag to hydroxyethylurea. The fluorous urea was then condensed with 10 equivalents each of the corresponding acetoacetates and aldehydes in tetrahydrofuran (THF)-benzotrifluoride (BTF) containing hydrochloric acid (HCl). After extraction of the fluorous DHPMs with fluorous solvent (perfluorohexanes, fluorocarbon: FC-72), desilylation with tetrabutylammonium fluoride (TBAF) followed by extractive purification provided the “organic” Biginelli products DHPMs in good overall yields.

Considering the simple experimental techniques used in this fluorous chemistry, automation should be feasible, thus allowing the preparation of DHPM libraries (Scheme 3.7) [118].



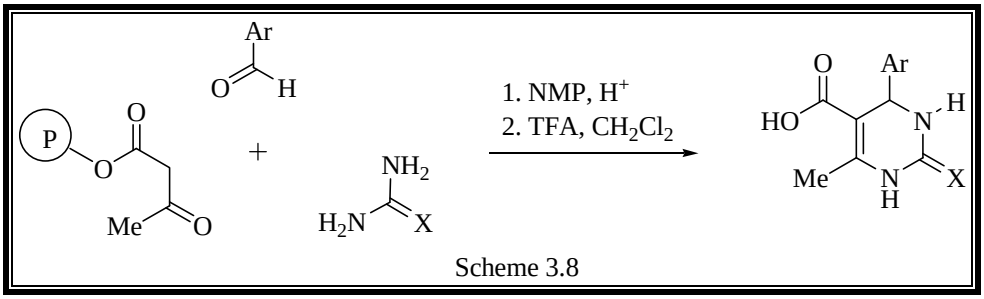
Scheme 3.7

Kappe O. *et al.* reported the procedure in which urea component is linked to the solid (or fluorous) support via the amide nitrogen, which invariably leads to the formation of N1-functionalized, so far pharmacologically active, DHPMs.



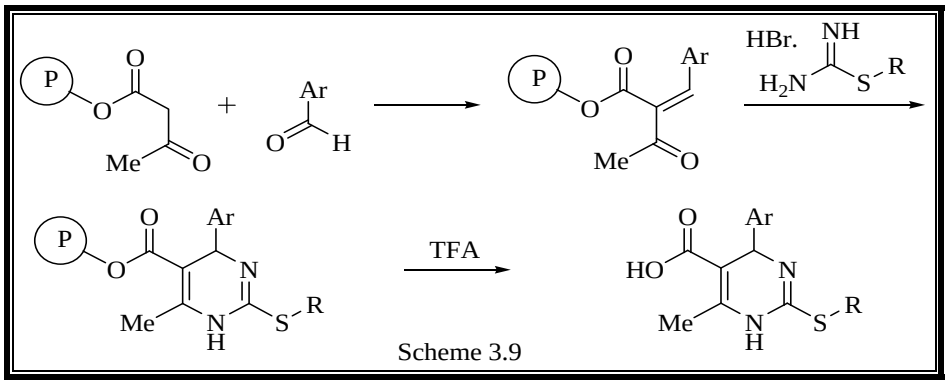
Kappe O. *et al.* has developed an alternative protocol, where the acetoacetate building block is linked to the solid support. Thus, Biginelli condensation of Wang-bound acetoacetate with excess aldehydes and urea/thiourea in NMP (*N*-methyl

pyrollidine/HCl provided the desired DHPMs on solid support. Subsequent cleavage with 50 % trifluoro acetic acid (TFA) furnished the free carboxylic acids in high over all yield (Scheme 3.8)



Scheme 3.8

In addition to solid-phase adaptions of the traditional three-component Biginelli condensation, solid-phase variations of the "Atwal modification" of the Biginelli reaction (see above) have also been reported. Kappe C. O. *et al.* have disclosed the synthesis of a 648-membered combinatorial library of 1,4-dihydropyrimidines. Towards this end, polymer-bound acetoacetate was subjected to Knoevenagel condensation with aromatic aldehydes, followed by condensation with isothioureas. The resulting polymer-bound 1,4-dihydropyrimidines were cleaved from the resin with 50 % trifluoro acetic acid (TFA) to produce carboxylic acid (Scheme 3.9) [119].



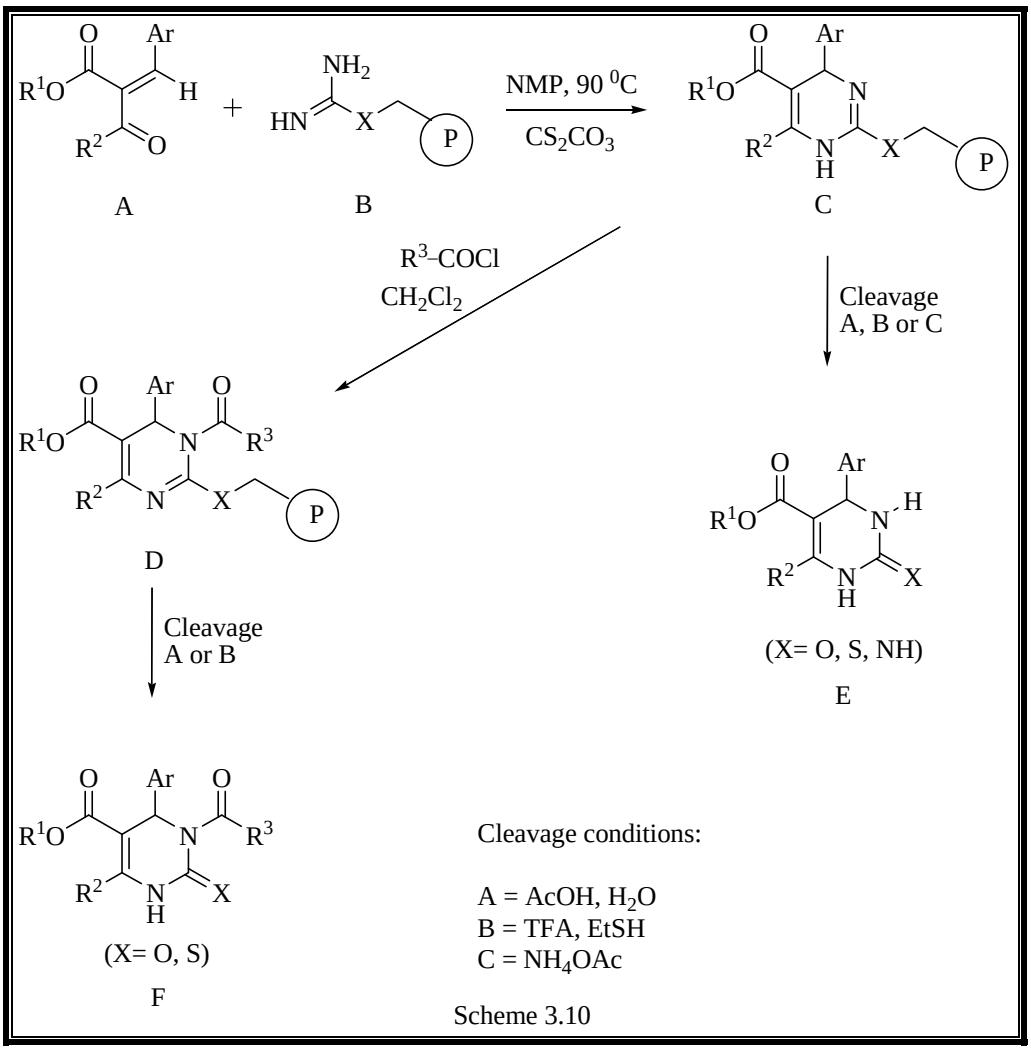
Scheme 3.9

In an effort to increase the molecular diversity in solid phase syntheses of DHPM scaffolds, a novel and versatile solid-phase approach was adopted where an isourea building block is attached to the solid support [120].

In the key step, polymer-bound (Wang) isothiourea (**B)** is condensed with enones in *N*-methylpyrrolidone (NMP) in the presence of base. Thepolymer-bound

dihydropyrimidine can then be directly cleaved from the resin (**C→E**) by employing different cleavage strategies. Therefore, three types of DHPMs **E** (X = O, S and NH) can be obtained by applying the appropriate cleaving conditions (**A), (B)** or **(C).** On the other hand, an additional element of diversity can be introduced onto the pyrimidine nucleus by regioselective N_3-acylation of the polymer-bound intermediate (**C)** with suitable electrophiles (e.g., acyl chlorides, R^3COCl). By applying different cleaving strategies to (**D)**, the corresponding N_3-functionalized DHPMs (**F)** were obtained in moderate to high overall yields. This solid-phase approach is therefore particularly attractive for the preparation of pharmacologically active N_3-acylated analogues such as DHPMs and should be useful for the generation of targeted libraries of this heterocyclic scaffold.

3.1.8.1 Diversity in solid phase DHPM synthesis

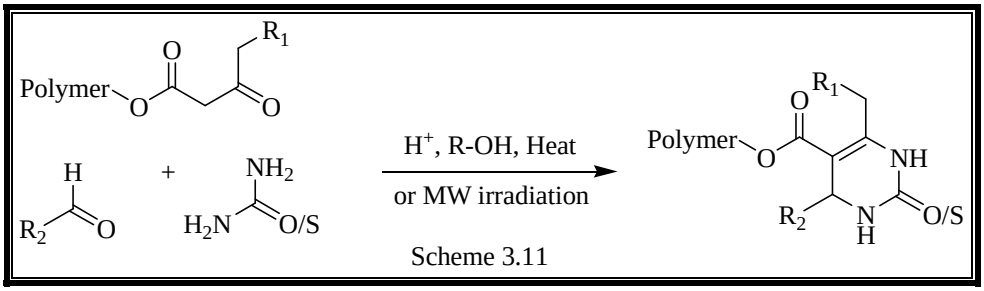


A B C D E F

Scheme 3.10

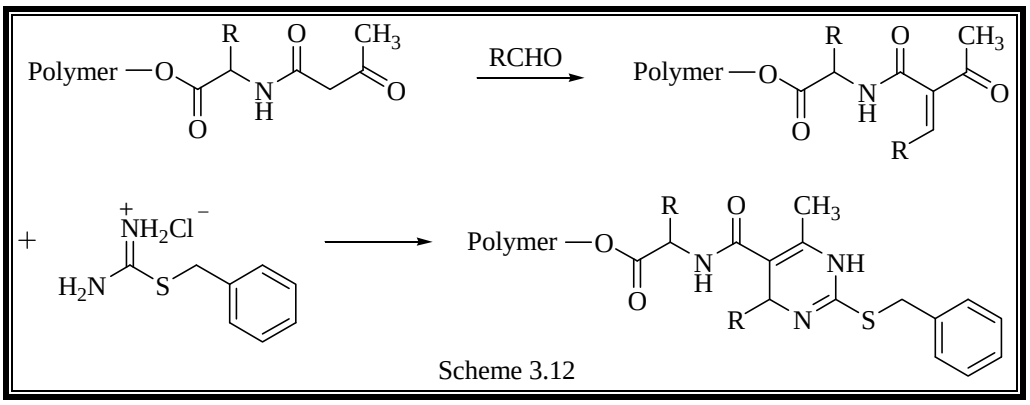
Schober A. developed synthetic protocol based on immobilized β-ketoamides to increase the diversity of DHPM derivatives by varying the substituents in position 4 in a simple manner. Depending on the building blocks for the three-component reaction, the immobilization strategies were chosen. At least three different strategies for the preparation of DHPM derivatives on solid support were described in recent literature. The first one makes use of immobilized urea or thiourea moieties. The second uses an immobilized β-ketoester and the third one uses an S-linked isothiouronium salt, Biginelli protocols depending on immobilized aldehydes were not found (Scheme 3.10) [121].

3.1.8.2 DHPM synthesis with immobilized ketoesters



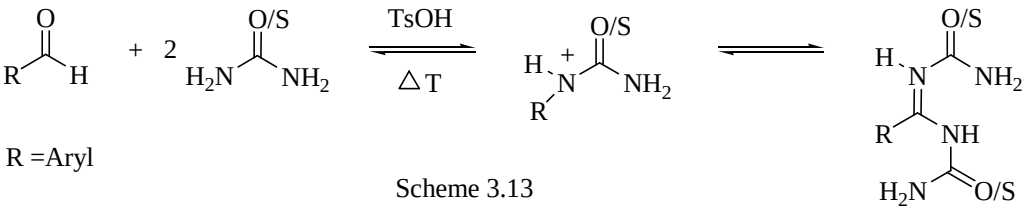
Scheme 3.11

3.1.8.3 DHPM synthesis with immobilized β-ketoamides using Atwal's route



Scheme 3.12

3.1.8.4 *N*-acyliminium ion, the essential reaction intermediate



R =Aryl

Scheme 3.13

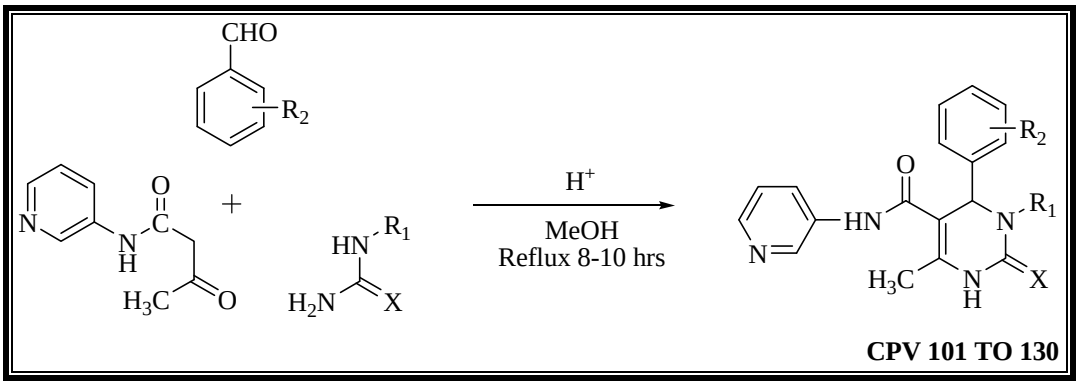
Thus by employing any of the solid-phase synthesis methods described above, libraries of DHPMs can be generated in a relatively straightforward fashion. Biginelli products are therefore contained in many commercially available small molecule libraries or compound collections and have undoubtedly been subjected to many high-throughput screening (HTS) processes. However, all of these products would still be racemic and therefore screening will not address possible enantioselective effects on molecular activity.

3.2 Current work

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. The 1,2,3,4-tetrahydropyrimidine ring system is of special biological interest because it has numerous pharmacological and medicinal applications *viz*, antitumour, antiviral, antimalarial, antitubarcular etc.

Keeping in mind various biomedical applications and with a view to further assess, the pharmacological profile of these class of compounds, three novel series of 1,2,3,4-tetrahydropyrimidine (CPV-101 to CPV-130) are synthesized. The synthesis of these thirty compounds was achieved by the Biginelli reaction of acetoacetanilide, urea derivatives and corresponding aldehydes. The reaction is catalysed by concentrated hydrochloric acid (HCl). The products were characterized by various analytical techniques like FT-IR spectroscopy, mass spectrometry, ^{1}H NMR spectroscopy and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial.

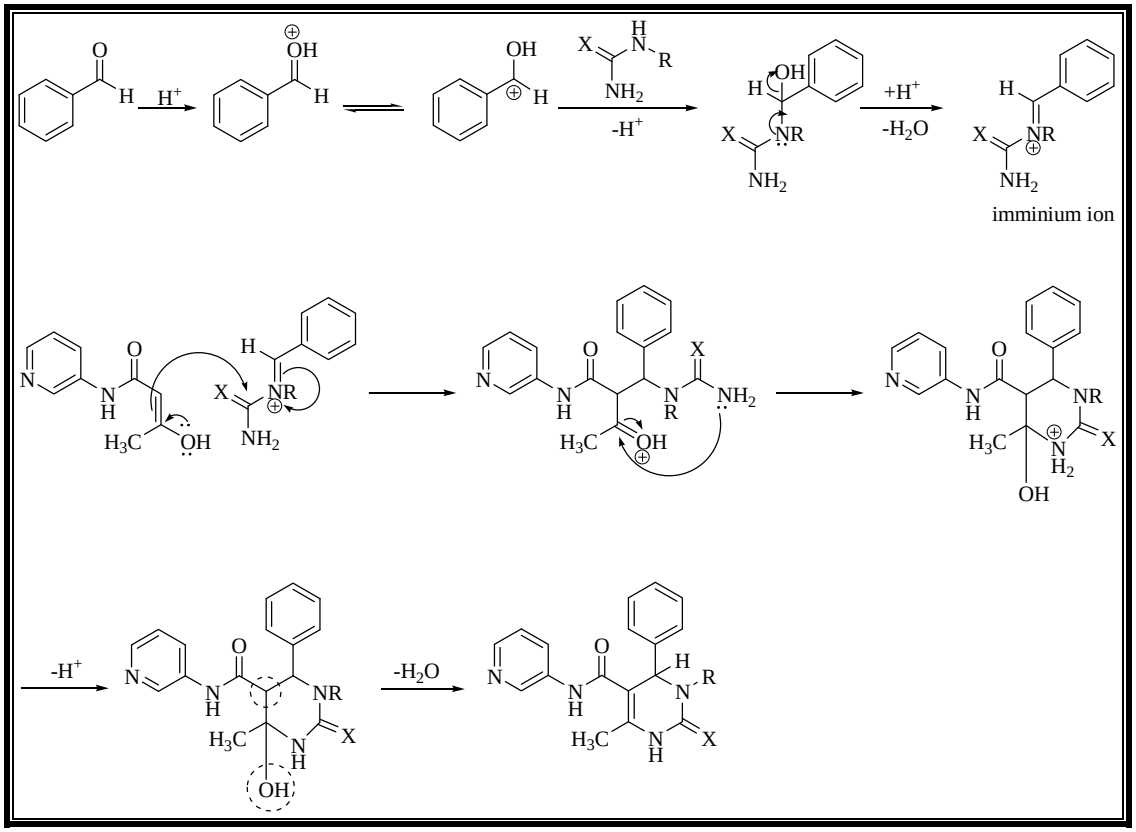
3.3 Reaction scheme



Code	R_1	X	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
CPV-101	H	O	H	$C_{17}H_{16}N_4O_2$	308	160-162	66	0.42	0.66
CPV-102	H	O	4-CH_3	$C_{18}H_{18}N_4O_2$	322	191-193	64	0.50	0.69
CPV-103	H	O	4-OCH_3	$C_{18}H_{18}N_4O_3$	338	221-223	63	0.49	0.73
CPV-104	H	O	4-Cl	$C_{17}H_{15}ClN_4O_2$	342	199-201	76	0.46	0.68
CPV-105	H	O	4-F	$C_{17}H_{15}FN_4O_2$	326	198-200	80	0.54	0.75
CPV-106	H	O	4-NO_2	$C_{17}H_{15}N_5O_4$	353	183-185	69	0.50	0.70
CPV-107	H	O	3-NO_2	$C_{17}H_{15}N_5O_4$	353	192-194	65	0.53	0.72
CPV-108	H	O	2-NO_2	$C_{17}H_{15}N_5O_4$	353	226-228	79	0.50	0.65
CPV-109	H	O	3-Cl	$C_{17}H_{15}ClN_4O_2$	342	153-155	58	0.55	0.67
CPV-110	H	O	2-Cl	$C_{17}H_{15}ClN_4O_2$	342	223-225	62	0.48	0.77
CPV-111	H	S	H	$C_{17}H_{16}N_4OS$	324	167-169	64	0.50	0.61
CPV-112	H	S	4-CH_3	$C_{18}H_{18}N_4OS$	338	231-233	74	0.58	0.67
CPV-113	H	S	4-OCH_3	$C_{18}H_{18}N_4O_2S$	354	181-183	70	0.41	0.74
CPV-114	H	S	4-Cl	$C_{17}H_{15}ClN_4OS$	358	216-218	72	0.56	0.66
CPV-115	H	S	4-F	$C_{17}H_{15}FN_4OS$	342	209-211	77	0.53	0.60
CPV-116	H	S	4-NO_2	$C_{17}H_{15}N_5O_3S$	369	236-238	65	0.50	0.58
CPV-117	H	S	3-NO_2	$C_{17}H_{15}N_5O_3S$	369	229-231	63	0.54	0.61
CPV-118	H	S	2-NO_2	$C_{17}H_{15}N_5O_3S$	369	234-236	68	0.57	0.64
CPV-119	H	S	3-Cl	$C_{17}H_{15}ClN_4OS$	358	188-190	62	0.48	0.57
CPV-120	H	S	2-Cl	$C_{17}H_{15}ClN_4OS$	358	238-240	59	0.58	0.70
CPV-121	CH_3	O	H	$C_{18}H_{18}N_4O_2$	322	244-246	78	0.49	0.55
CPV-122	CH_3	O	4-CH_3	$C_{19}H_{20}N_4O_2$	336	212-214	69	0.55	0.66
CPV-123	CH_3	O	4-OCH_3	$C_{19}H_{20}N_4O_3$	352	235-237	70	0.54	0.65
CPV-124	CH_3	O	4-Cl	$C_{18}H_{17}ClN_4O_2$	356	271-273	66	0.52	0.60
CPV-125	CH_3	O	4-F	$C_{18}H_{17}FN_4O_2$	340	261-263	66	0.48	0.53
CPV-126	CH_3	O	4-NO_2	$C_{18}H_{17}N_5O_4$	367	265-267	71	0.54	0.67
CPV-127	CH_3	O	3-NO_2	$C_{18}H_{17}N_5O_4$	367	223-225	59	0.58	0.69
CPV-128	CH_3	O	2-NO_2	$C_{18}H_{17}N_5O_4$	367	218-220	74	0.51	0.64
CPV-129	CH_3	O	3-Cl	$C_{18}H_{17}ClN_4O_2$	356	261-263	63	0.49	0.57
CPV-130	CH_3	O	2-Cl	$C_{18}H_{17}ClN_4O_2$	356	221-223	72	0.51	0.63

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4,
TLC Solvent system R_{f2}: Chloroform: Methanol – 9:1.

3.4 Plausible Reaction Mechanism



3.5 Experimental

3.5.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

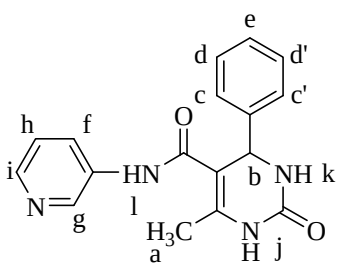
3.5.2 Synthesis of N-(pyridin-3-yl)-3-oxo-butanamide

Synthesis of *N-(pyridin-3-yl)-3-oxo-butanamide* was achieved using previously published methods [45].

3.5.3 General procedure for the synthesis of 1,2,3,4-tetrahydro-6-methyl-2-oxo-4-aryl-N-(pyridin-3-yl)pyrimidine-5-carboxamides (CPV 101-110)

A mixture of N-(pyridin-3-yl)-3-oxo-butanamide (0.01 mol), appropriate aromatic aldehyde (0.01 mol), urea (0.015 mol) and catalytical amount of concentrated hydrochloric acid in ethanol (30 ml) was heated under reflux condition for 8 to10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The product obtained was isolated and recrystallized from ethanol.

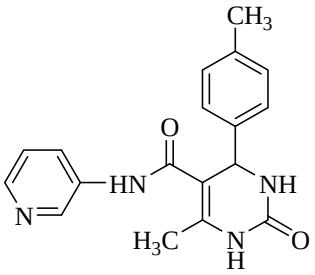
3.5.3.1 1,2,3,4-tetrahydro-6-methyl-2-oxo-4-phenyl-N-(pyridin-3-yl)pyrimidine-5-carboxamide (CPV-101)



Yield: 66%; mp 160-162 ºC; IR (cm^{-1}): 3331 (N-H stretching of primary amide), 3294 (N-H stretching of pyrimidine ring), 3059 (C-H symmetrical stretching of CH_3 group), 3024 (C-H stretching of aromatic ring), 2931 (C-H asymmetrical stretching of CH_3 group), 1699 (C=O stretching of

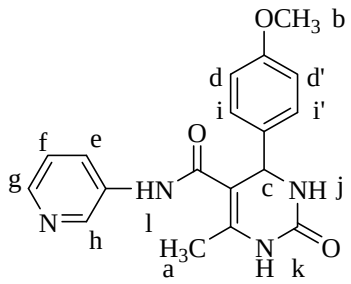
amide), 1631 and 1525 (C=C stretching of aromatic ring), 1593 (N-H deformation of pyrimidine ring), 1460 (C-H asymmetrical deformation of CH_3 group), 1342 (C-H symmetrical deformation of CH_3 group), 1323 (C-N-C stretching of pyrimidine ring), 1282 (C-N stretching of pyrimidine ring), 1234 (C-H in plane deformation of aromatic ring), 759 and 713 (C-H out of plane deformation of mono substituted benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.25 (s, 3H, H_a), 5.43 (s, 1H, H_b), 7.21-7.36 (m, 6H, $H_{cc'-f}$), 7.67 (s, 1H, H_g), 7.95-7.97 (d, 1H, H_h, J = 8.0 Hz), 8.20-8.21 (d, 1H, H_i, J = 4.0 Hz), 8.69 (s, 1H, H_k), 9.76 (s, 1H, H_l): *m/z* 308; Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17; O, 10.38. Found: C, 66.15; H, 5.20; N, 18.11; O, 10.30%.

3.5.3.2 1,2,3,4-tetrahydro-6-methyl-2-oxo-N-(pyridin-3-yl)-4-p-tolylpyrimidine-5-carboxamide (CPV-102)



Yield: 64%; mp 191-193 ºC; MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{18}N_4O_2$: C, 67.07; H, 5.63; N, 17.38; O, 9.93. Found: C, 67.02; H, 5.59; N, 17.31, O, 9.90%.

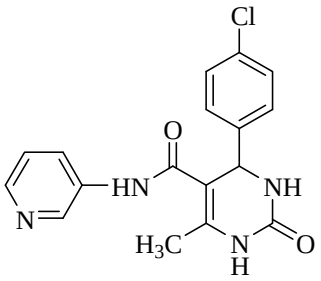
3.5.3.3 1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-6-methyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-103)



Yield: 63%; mp 221-223 ºC; IR (cm^{-1}): 3498 (N-H stretching of primary amide), 3230 (N-H stretching of pyrimidine ring), 3115 (C-H symmetrical stretching of CH_3 group), 2937 (C-H asymmetrical stretching of CH_3 group), 1712 (C=O stretching of amide), 1641 (N-H deformation of pyrimidine ring), 1525 and 1483 (C=C stretching of aromatic ring), 1435 (C-H asymmetrical deformation of CH_3 group), 1408 (C-N-C stretching of pyrimidine ring), 1340 (C-H symmetrical deformation of CH_3 group), 1276 (C-N stretching of pyrimidine ring), 1240 (C-O-C asymmetrical stretching of ether linkage), 1174 (C-H in plane deformation of aromatic ring), 1062 (C-O-C symmetrical stretching of ether linkage), 866 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.11 (s, 3H, H_a), 3.73 (s, 3H, H_b), 5.44 (s, 1H, H_c), 6.82-6.84 (d,

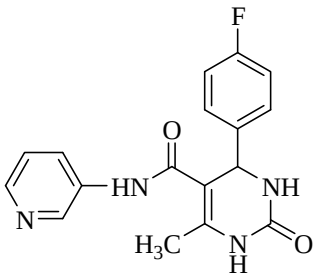
2H, $H_{dd'}$, *J* = 8.0 Hz), 7.18-7.25 (m, 3H, H_{e-g}), 7.49 (s, 1H, H_h), 7.99-8.00 (d, 2H, $H_{ii'}$, *J* = 4.0 Hz), 8.17-8.18 (d, 1H, H_j, *J* = 4.0 Hz), 8.70 (s, 2H, H_{kj}), 9.60 (s, 1H, H_l); MS: *m/z* 338; Anal. Calcd. for $C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56; O, 14.19. Found: C, 63.81; H, 5.30; N, 16.50; O, 14.11%.

3.5.3.4 4-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-104)



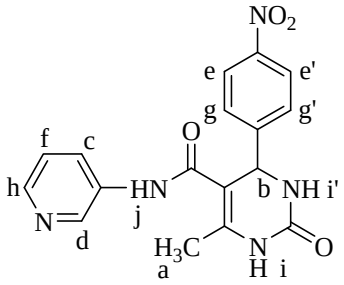
Yield: 76%; mp 199-201 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{17}H_{15}ClN_4O_2$: C, 59.57; H, 4.41; N, 16.34; O, 9.34. Found: C, 59.51; H, 4.35; N, 16.27; O, 9.25%.

3.5.3.5 4-(4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-105)



Yield: 80%; mp 198-200 ºC; MS: *m/z* 326; Anal. Calcd. for $C_{17}H_{15}FN_4O_2$: C, 62.57; H, 4.63; N, 17.17; O, 9.81. Found: C, 62.50; H, 4.57; N, 17.10; O, 9.75%.

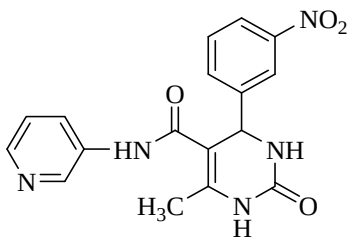
3.5.3.6 1,2,3,4-tetrahydro-6-methyl-4-(4-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-106)



Yield: 69%; mp 183-185 ºC; IR (cm^{-1}): 3298 (N-H stretching of primary amide), 3234 (N-H stretching of pyrimidine ring), 3026 (C-H symmetrical stretching of CH_3 group), 2829 (C-H asymmetrical stretching of CH_3 group), 1689 (C=O stretching of amide), 1600 and 1471 (C=C stretching of aromatic ring), 1583 (C-NO_2 symmetrical stretching), 1521 (N-H deformation of pyrimidine ring), 1423 (C-N stretching of pyrimidine ring), 1390 (C-H asymmetrical deformation of CH_3 group), 1348 (C-N-C stretching of pyrimidine ring), 1309 (C-H symmetrical deformation of CH_3 group), 1244 (C-H in plane deformation of aromatic ring), 798 (C-H out of plane deformation of 1,4-

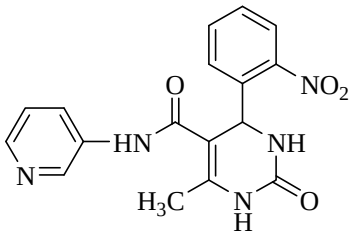
disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.19 (s, 3H, H_a), 5.63 (s, 1H, H_b), 7.18-7.22 (m, 1H, H_c), 7.49 (s, 1H, H_d), 7.59-7.61 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 8.01-8.03 (d, 1H, H_f, J = 8.0 Hz), 8.14-8.16 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 8.23-8.24 (d, 1H, H_h, J = 4.0 Hz), 8.71-8.73 (d, 2H, $H_{ii'}$, J = 8.0 Hz), 9.60 (s, 1H, H_j); MS: *m/z* 353; Anal. Calcd. for $C_{17}H_{15}N_5O_4$: C, 57.79; H, 4.28; N, 19.82; O, 18.11. Found: C, 57.69; H, 4.20; N, 19.76; O, 18.04%.

3.5.3.7 1,2,3,4-tetrahydro-6-methyl-4-(3-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-107)



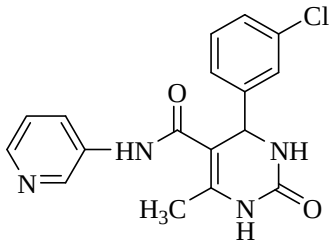
Yield: 65%; mp 192-194 ºC; MS: *m/z* 353; Anal. Calcd. for $C_{17}H_{15}N_5O_4$: C, 57.79; H, 4.28; N, 19.82; O, 18.11. Found: C, 57.71; H, 4.22; N, 19.78; O, 18.04%.

3.5.3.8 1,2,3,4-tetrahydro-6-methyl-4-(2-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-108)



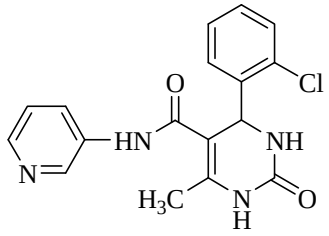
Yield: 79%; mp 226-228 ºC; MS: *m/z* 353; Anal. Calcd. for $C_{17}H_{15}N_5O_4$: C, 57.79; H, 4.28; N, 19.82; O, 18.11. Found: C, 57.70; H, 4.23; N, 19.75; O, 18.00%.

3.5.3.9 4-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-109)



Yield: 58%; mp 153-155 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{17}H_{15}ClN_4O_2$: C, 59.57; H, 4.41; N, 16.34; O, 9.34. Found: C, 59.50; H, 4.36; N, 16.25; O, 9.20%.

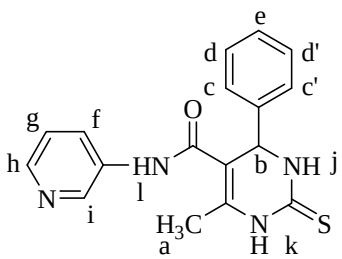
3.5.3.10 4-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-N-(pyridin-3-yl)pyrimidine-5-carboxamide (CPV-110) Yield: 62%; mp 223-225 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{17}H_{15}ClN_4O_2$: C, 59.57; H, 4.41; N, 16.34; O, 9.34. Found: C, 59.51; H, 4.30; N, 16.25; O, 9.21%.

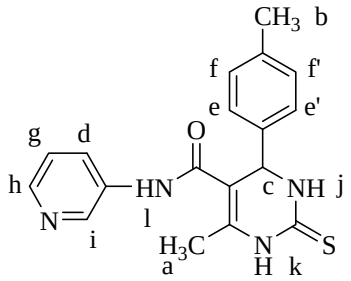


3.5.4 General procedure for the synthesis of 1,2,3,4-tetrahydro-6-methyl-4-aryl-N-(pyridin-3-yl)-2-thioxopyrimidine-5-carboxamides (CPV 111-120)

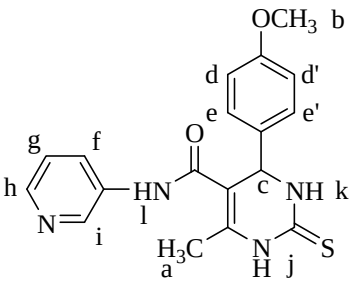
A mixture of N-(pyridin-3-yl)-3-oxo-butanamide (0.01 mol), appropriate aromatic aldehyde (0.01 mol), thiourea (0.015 mol) and catalytical amount of concentrated hydrochloric acid in ethanol (30 ml) was heated under reflux condition for 8 to10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The product obtained was isolated and recrystallized from ethanol.

3.5.4.1 1,2,3,4-tetrahydro-6-methyl-4-phenyl-N-(pyridin-3-yl)-2-thioxopyrimidine-5-carboxamide (CPV-111) Yield: 64%; mp 167-169 ºC; IR (cm^{-1}): 3290 (N-H stretching of primary amide), 3192 (N-H stretching of pyrimidine ring), 3099 (C-H symmetrical stretching of CH_3 group), 2874 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1589 (N-H deformation of pyrimidine ring), 1523 and 1471 (C=C stretching of aromatic ring), 1433 (C-H asymmetrical deformation of CH_3 group), 1338 (C-N-C stretching of pyrimidine ring), 1290 (C-H symmetrical deformation of CH_3 group), 1242 (C-N stretching of pyrimidine ring), 1201 (C=S stretching), 1031 (C-H in plane deformation of aromatic ring), 758 and 721 (C-H out of plane deformation of mono substituted benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.50 (s, 3H, H_a), 5.43 (s, 1H, H_b), 7.24-7.38 (m, 6H, $H_{cc'-f}$), 7.96-7.98 (d, 1H, H_g, J = 8.0 Hz), 8.22-8.24 (d, 1H, H_h, J = 8.0 Hz), 8.70 (s, 1H, H_i,), 9.53 (s, 1H, H_j), 9.94 (s, 1H, H_k), 10.08 (s, 1H, H_l); MS: *m/z* 324; Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; O, 4.93; S, 9.88. Found: C, 62.85; H, 4.91; N, 17.20; O, 4.83; S, 9.80%.



3.5.4.2 1,2,3,4-tetrahydro-6-methyl-N-(pyridin-3-yl)-2-thioxo-4-p-tolylpyrimidine-5-carboxamide (CPV-112) 

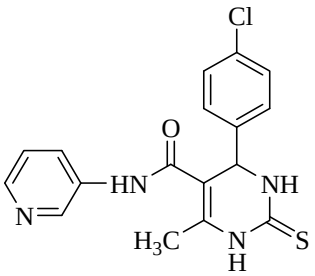
Yield: 74%; mp 231-233 ºC; IR (cm^{-1}): 3271 (N-H stretching of secondary amide), 3036 (C-H symmetrical stretching of CH_3 group), 2924 (C-H asymmetrical stretching of CH_3 group), 1708 (C=O stretching of amide), 1629 (N-H deformation of pyrimidine ring), 1591 and 1512 (C=C stretching of aromatic ring), 1408 (C-H asymmetrical deformation of CH_3 group), 1338 (C-H symmetrical deformation of CH_3 group), 1263 (C-N-C stretching of pyrimidine ring), 1236 (C-N stretching of pyrimidine ring), 1149 (C=S stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.11 (s, 3H, H_a), 2.53-2.55 (s, 3H, H_b), 5.46-5.47 (s, 1H, H_c), 7.19-7.23 (m, 1H, H_d), 7.31 (s, 4H, $H_{e\text{-}f'}$), 7.62 (s, 1H, H_g), 7.97-8.00 (m, 1H, H_h), 8.18-8.20 (m, 1H, H_i), 8.70-8.71 (d, 1H, H_j, J = 4.0 Hz), 8.80 (s, 1H, H_k), 9.68 (s, 1H, H_l); MS: *m/z* 338; Anal. Calcd. for $C_{18}H_{18}N_4OS$: C, 63.88; H, 5.36; N, 16.56; O, 4.73; S, 9.47. Found: C, 63.80; H, 5.28; N, 16.50; O, 4.68; S, 9.40%.

3.5.4.3 1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-6-methyl-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-113) 

Yield: 70%; mp 181-183 ºC; IR (cm^{-1}): 3363 (N-H stretching of primary amide), 3319 (N-H stretching of pyrimidine ring), 3099 (C-H symmetrical stretching of CH_3 group), 2966 (C-H asymmetrical stretching of CH_3 group), 1672 (C=O stretching of amide), 1566 (N-H deformation of pyrimidine ring), 1516 and 1481 (C=C stretching of aromatic ring), 1415 (C-H asymmetrical deformation of CH_3 group), 1388 (C-H symmetrical deformation of CH_3 group), 1340 (C-N-C stretching of pyrimidine ring), 1280 (C-N stretching of pyrimidine ring), 1197 (C-O-C asymmetrical stretching of ether linkage), 1187 (C=S stretching), 1033 (C-O-C symmetrical stretching of ether linkage), 954 (C-H in plane deformation of aromatic ring), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.15 (s, 3H, H_a), 3.74 (s, 1H, H_b), 5.45 (s, 1H, H_c), 6.83-6.85 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.19-7.25 (m, 3H, $H_{e\text{-}f}$), 7.99-8.01 (d, 1H, H_g, J = 8.0 Hz), 8.20-8.22 (d, 1H, H_h, J = 8.0 Hz), 8.70-8.71 (d, 1H, H_i, J = 4.0 Hz), 9.35 (s, 1H, H_j), 9.74 (s, 1H, H_k), 9.88 (s, 1H, H_l); MS: *m/z* 354; Anal. Calcd.

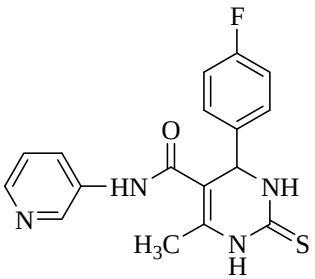
for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81; O, 9.03; S, 9.05. Found: C, 60.00; H, 5.05; N, 15.73; O, 8.95; S, 9.00%.

3.5.4.4 4-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-114)



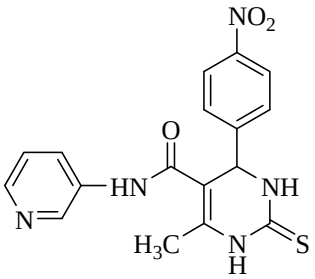
Yield: 72%; mp 216-218 ºC; MS: *m/z* 358; Anal. Calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61; O, 4.46; S, 8.94. Found: C, 56.79; H, 4.15; N, 15.55; O, 4.40; S, 8.88%.

3.5.4.5 4-(4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-115)



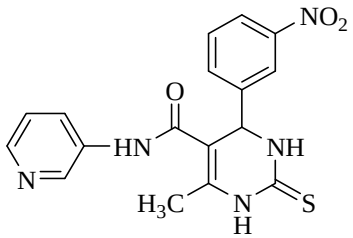
Yield: 77%; mp 209-211 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{17}H_{15}FN_4OS$: C, 59.63; H, 4.42; N, 16.36; O, 4.67; S, 9.37. Found: C, 59.57; H, 4.36; N, 16.28; O, 4.62; S, 9.30%.

3.5.4.6 1,2,3,4-tetrahydro-6-methyl-4-(4-nitrophenyl)-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-116)

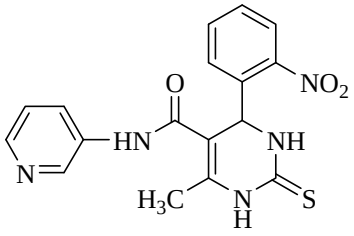


Yield: 65%; mp 236-238 ºC; MS: *m/z* 369; Anal. Calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96; O, 12.99; S, 8.68. Found: C, 55.20; H, 4.00; N, 18.90; O, 12.90; S, 8.60%.

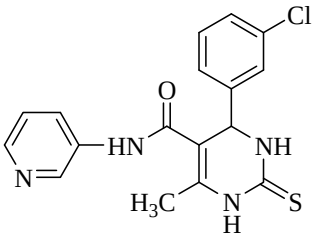
3.5.4.7 1,2,3,4-tetrahydro-6-methyl-4-(3-nitrophenyl)-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-117)



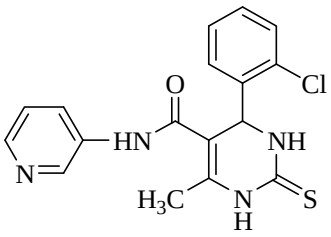
Yield: 63%; mp 229-231 ºC; MS: *m/z* 369; Anal. Calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96; O, 12.99; S, 8.68. Found: C, 55.19; H, 3.98; N, 18.88; O, 12.91; S, 8.59%.

3.5.4.8 1,2,3,4-tetrahydro-6-methyl-4-(2-nitrophenyl)-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-118) 

Yield: 68%; mp 234-236 ºC; MS: *m/z* 369; Anal. Calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96; O, 12.99; S, 8.68. Found: C, 55.17; H, 3.96; N, 18.89; O, 12.91; S, 8.58%.

3.5.4.9 4-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-119) 

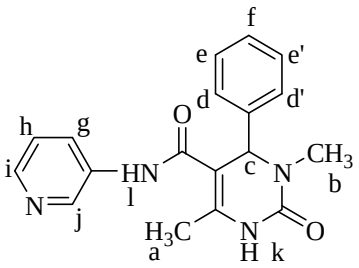
Yield: 62%; mp 188-190 ºC; MS: *m/z* 358; Anal. Calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61; O, 4.46; S, 8.94. Found: C, 56.81; H, 4.16; N, 15.54; O, 4.41; S, 8.88%.

3.5.4.10 4-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-N-(pyridin-3-yl)-2-thioxo pyrimidine-5-carboxamide (CPV-120) 

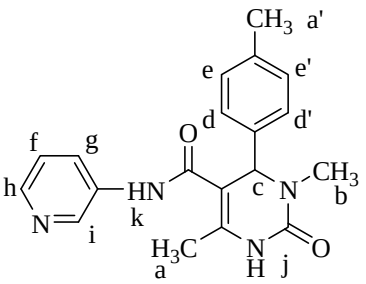
Yield: 59%; mp 238-240 ºC; MS: *m/z* 359; Anal. Calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61; O, 4.46; S, 8.94. Found: C, 56.82; H, 4.14; N, 15.54; O, 4.39; S, 8.85%.

3.5.5 General procedure for the synthesis of 1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-6-aryl-N-(pyridin-3-yl)pyrimidine-5-carboxamide (CPV 121-130)

A mixture of N-(pyridin-3-yl)-3-oxo-butanamide (0.01 mol), appropriate aromatic aldehyde (0.01 mol), N-methyl urea (0.015 mol) and catalytical amount of concentrated hydrochloric acid in ethanol (30 ml) was heated under reflux condition for 8 to10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The product obtained was isolated and recrystallized from ethanol.

3.5.5.1 1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-6-phenyl-N-(pyridin-3-yl)pyrimidine-5-carboxamide (CPV-121) 

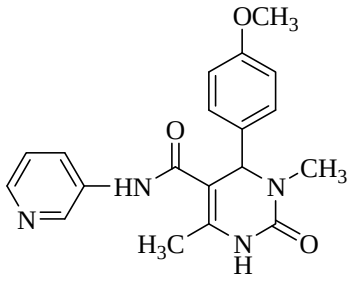
Yield: 78%; mp 244-246 ºC; IR (cm^{-1}): 3527 (N-H stretching of primary amide), 3427 (N-H stretching of pyrimidine ring), 3009 (C-H symmetrical stretching of CH_3 group), 2924 (C-H asymmetrical stretching of CH_3 group), 1693 (C=O stretching of amide), 1629 (N-H deformation of pyrimidine ring), 1471 and 1456 (C=C stretching of aromatic ring), 1421 (C-H asymmetrical deformation of CH_3 group), 1356 (C-N-C stretching of pyrimidine ring), 1334 (C-H symmetrical deformation of CH_3 group), 1263 (C-N stretching of pyrimidine ring), 1220 (C-H in plane deformation of aromatic ring), 746 and 705 (C-H out of plane deformation of mono substituted benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 3.16 (s, 3H, H_a), 3.32 (s, 3H, H_b), 5.41 (s, 1H, H_c), 7.20-7.30 (m, 6H, $H_{d\text{-}g}$), 7.67-7.68 (d, 1H, H_h, J = 4.0 Hz), 8.02-8.05 (m, 1H, H_i), 8.21-8.22 (m, 1H, H_j), 8.73-8.74 (d, 1H, H_k, J = 4.0 Hz), 9.91 (s, 1H, H_l); MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{18}N_4O_2$: C, 67.07; H, 5.63; N, 17.38; O, 9.93. Found: C, C, 67.00; H, 5.59; N, 17.30; O, 9.88%.

3.5.5.2 1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-(pyridin-3-yl)-6-p-tolylpyrimidine-5-carboxamide (CPV-122) 

Yield: 69%; mp 212-214 ºC; IR (cm^{-1}): 3435 (N-H stretching of primary amide), 3275 (N-H stretching of pyrimidine ring), 3045 (C-H symmetrical stretching of CH_3 group), 2937 (C-H asymmetrical stretching of CH_3 group), 1693 (C=O stretching of amide), 1629 (N-H deformation of pyrimidine ring), 1585 and 1471 (C=C stretching of aromatic ring), 1417 (C-H asymmetrical deformation of CH_3 group), 1354 (C-H symmetrical deformation of CH_3 group), 1332 (C-N-C stretching of pyrimidine ring), 1261 (C-N stretching of pyrimidine ring), 1186 (C-H in plane deformation of aromatic ring), 898 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.17-2.24 (d, 6H, $H_{aa'}$), 3.08 (s, 3H, H_b), 5.27 (s, 1H, H_c), 7.12 (m, 4H, $H_{dd'\text{-}ee'}$), 7.28-7.32 (m, 1H, H_f), 7.76-7.77 (d, 1H, H_g, J = 4.0 Hz), 7.98-8.00 (d, 1H, H_h, J = 8.0 Hz), 8.22-8.24 (d, 1H, H_i, J = 8.0 Hz), 8.72 (s, 1H, H_j), 10.01 (s, 1H, H_k); MS: *m/z* 336; Anal. Calcd. for

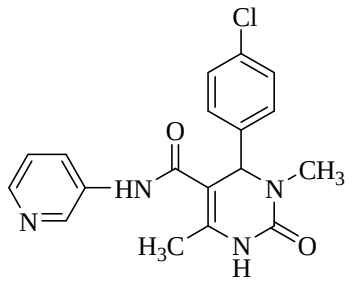
$C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66; O, 9.51. Found: C, 67.78; H, 5.90; N, 16.60; O, 9.44%.

3.5.5.3 1,2,3,6-tetraydro-6-(4-methoxyphenyl)-1,4-dimethyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-123)



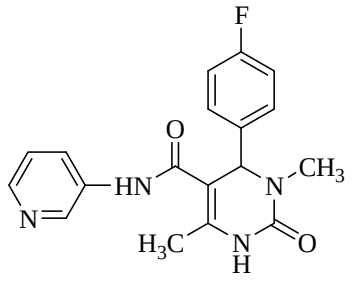
Yield: 70%; mp 235-237 ºC; MS: *m/z* 352; Anal. Calcd. for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90; O, 13.62. Found: C, 64.70; H, 5.65; N, 15.80; O, 13.55%.

3.5.5.4 6-(4-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-124)



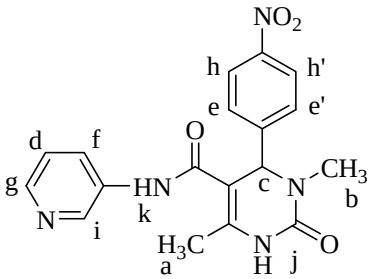
Yield: 66%; mp 271-273 ºC; MS: *m/z* 356; Anal. Calcd. for $C_{18}H_{17}ClN_4O_2$: C, 60.59; H, 4.80; N, 15.70; O, 8.97. Found: C, 60.50; H, 4.73; N, 15.63; O, 8.91%.

3.5.5.5 6-(4-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-125)



Yield: 66%; mp 261-263 ºC; MS: *m/z* 340; Anal. Calcd. For $C_{18}H_{17}FN_4O_2$: C, 63.52; H, 5.03; N, 16.46; O, 9.40. Found: C, 63.47; H, 4.95; N, 16.40; O, 9.33%.

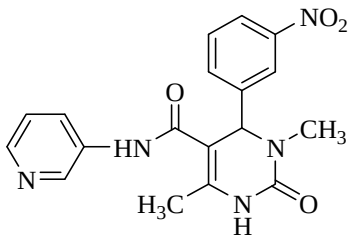
3.5.5.6 1,2,3,6-tetrahydro-1,4-dimethyl-6-(4-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-126)



Yield: 71%; mp 265-267 ºC; IR (cm^{-1}): 3477 (N-H stretching of primary amide), 3217 (N-H stretching of pyrimidine ring), 3103 (C-H symmetrical stretching of CH_3 group), 2937 (C-H asymmetrical stretching of CH_3

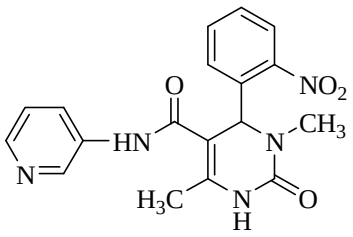
group), 1697 (C=O stretching of amide), 1626 (N-H deformation of pyrimidine ring), 1519 (C=C stretching of aromatic ring), 1481 (C-H asymmetrical deformation of CH_3 group), 1419 (C-NO_2 symmetrical stretching), 1386 (C-H symmetrical deformation of CH_3 group), 1348 (C-N-C stretching of pyrimidine ring), 1265 (C-N stretching of pyrimidine ring), 1220 (C-H in plane deformation of aromatic ring), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.26 (s, 3H, H_a), 3.17 (s, 3H, H_b), 5.50 (s, 1H, H_c), 7.21-7.24 (m, 1H, H_d), 7.55-7.57 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.90-7.92 (d, 1H, H_f, J = 8.0 Hz), 8.03-8.05 (d, 1H, H_g, J = 8.0 Hz), 8.14-8.16 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 8.23-8.24 (d, 1H, H_i, J = 4.0 Hz), 8.74 (s, 1H, H_j), 9.98 (s, 1H, H_k); MS: *m/z* 367; Anal. Calcd. For $C_{18}H_{17}N_5O_4$: C, 58.85; H, 4.66; N, 19.06; O, 17.42. Found: C, 58.80; H, 4.60; N, 18.98; O, 17.35%.

3.5.5.7 1,2,3,6-tetrahydro-1,4-dimethyl-6-(3-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-127)



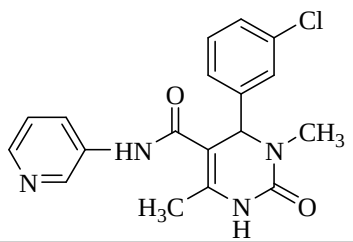
Yield: 59%; mp 223-225 ºC); MS: *m/z* 367; Anal. Calcd. For $C_{18}H_{17}N_5O_4$: C, 58.85; H, 4.66; N, 19.06; O, 17.42. Found: C, 58.78; H, 4.57; N, 18.97; O, 17.33%.

3.5.5.8 1,2,3,6-tetrahydro-1,4-dimethyl-6-(2-nitrophenyl)-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-128)



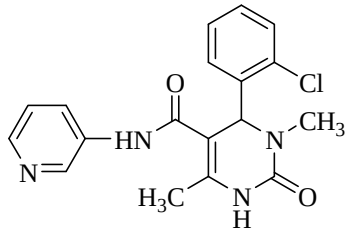
Yield: 74%; mp 218-220 ºC; MS: *m/z* 367; Anal. Calcd. For $C_{18}H_{17}N_5O_4$: C, 58.85; H, 4.66; N, 19.06; O, 17.42. Found: C, 58.75; H, 4.59; N, 18.97; O, 17.34%.

3.5.5.9 6-(3-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-129)



Yield: 63%; mp 261-263 ºC; MS: *m/z* 356; Anal. Calcd. for $C_{18}H_{17}ClN_4O_2$: C, 60.59; H, 4.80; N, 15.70; O, 8.97. Found: C, 60.51; H, 4.74; N, 15.63; O, 8.90%.

3.5.5.10 6-(2-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-(pyridin-3-yl) pyrimidine-5-carboxamide (CPV-130) Yield: 72%; mp 221-223 ºC; MS: *m/z* 356; Anal. Calcd. for $C_{18}H_{17}ClN_4O_2$: C, 60.59; H, 4.80; N, 15.70; O, 8.97. Found: C, 60.49; H, 4.75; N, 15.65; O, 8.91%.

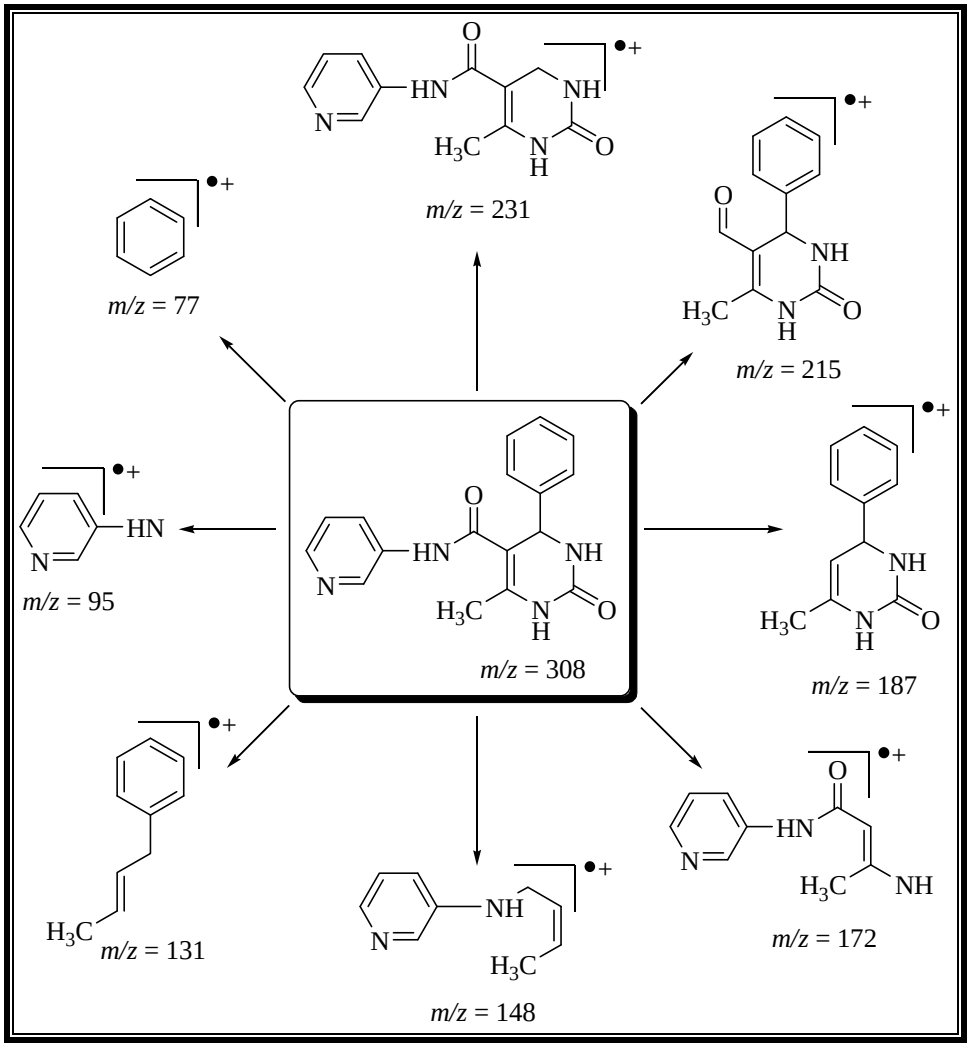


3.6 Spectral discussion

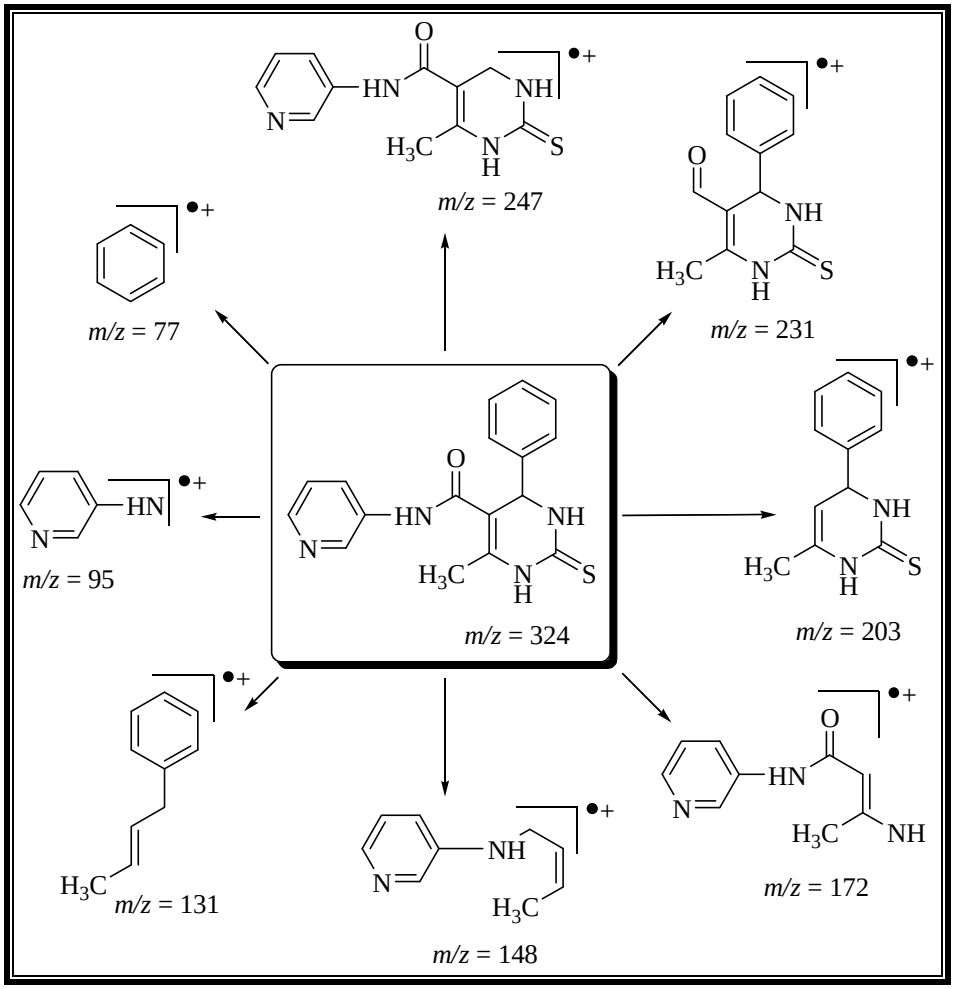
3.6.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

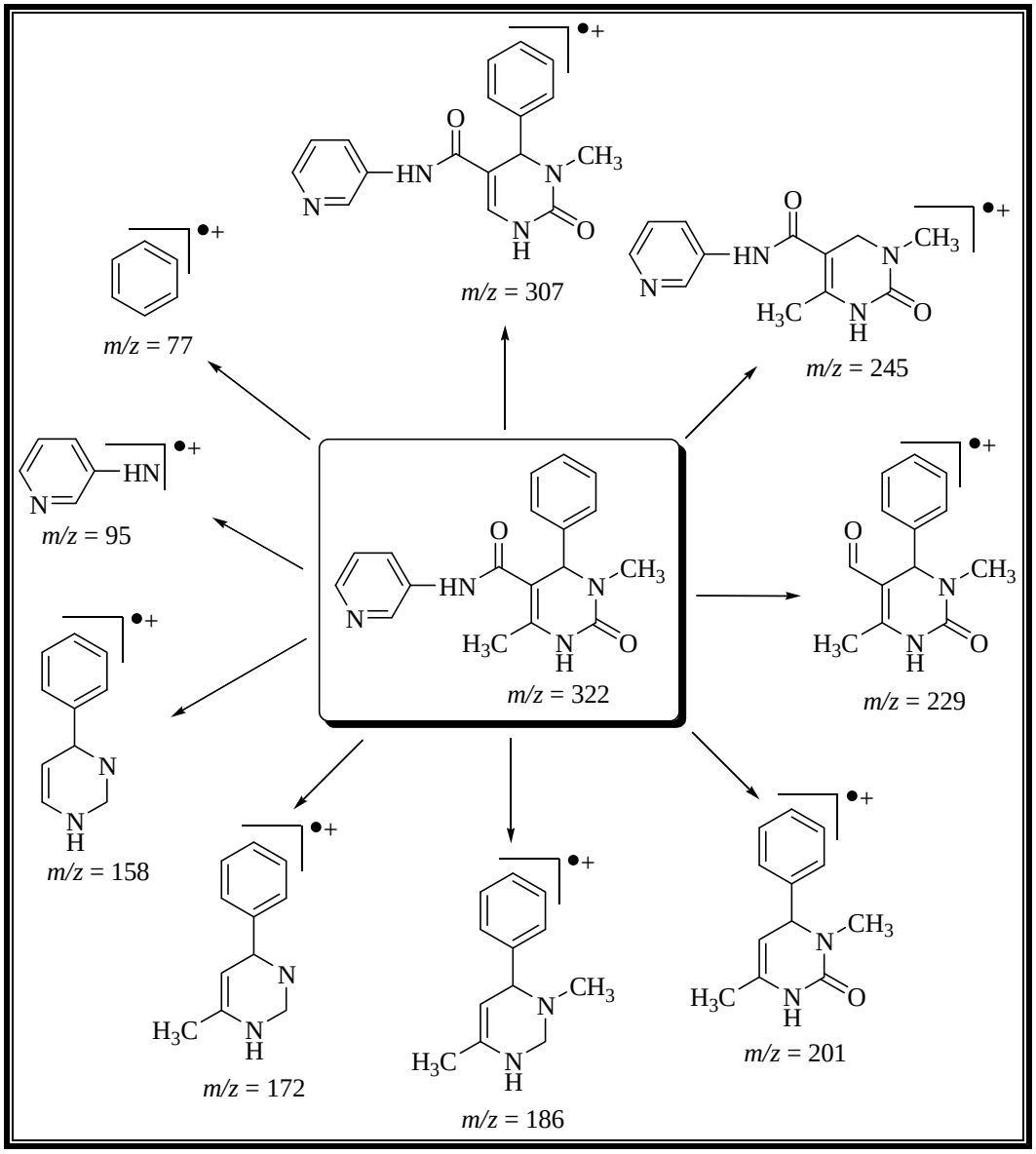
3.6.1.1 Mass fragmentation pattern for CPV-101



3.6.1.2 Mass fragmentation pattern for CPV-111



3.6.1.3 Mass fragmentation pattern for CPV-121



3.6.2 IR spectral study

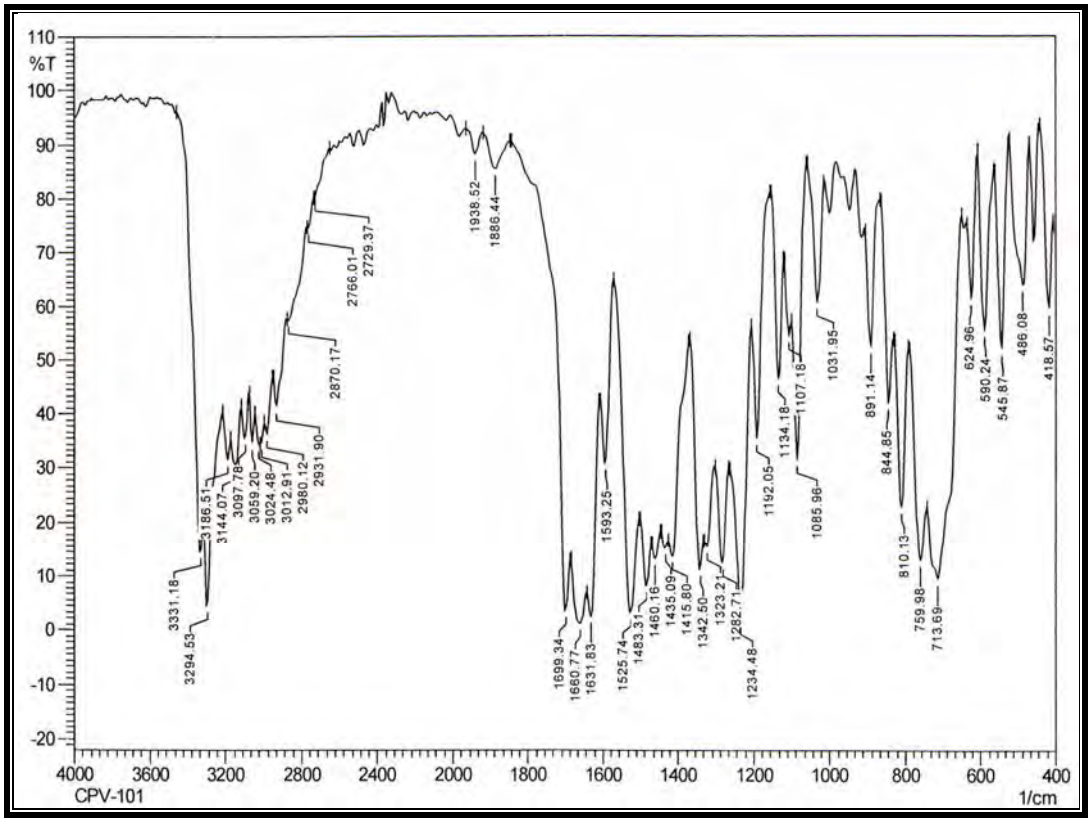
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For compounds CPV-101 to 130, confirmatory band for amidic linkage of acetoacetanilide fragment was found in the range of 3215-3530 cm^{-1} and pyrimidine nucleus (C-N-C stretching, C-N stretching) were found in the range of 1320-1410 cm^{-1} and 1260-1425 cm^{-1} respectively. Another characteristic carbonyl stretching band of pyrimidine was observed at 1662-1712 cm^{-1} suggesting formation of desired products CPV-101 to 130.

3.6.3 ^{1}H NMR spectral study

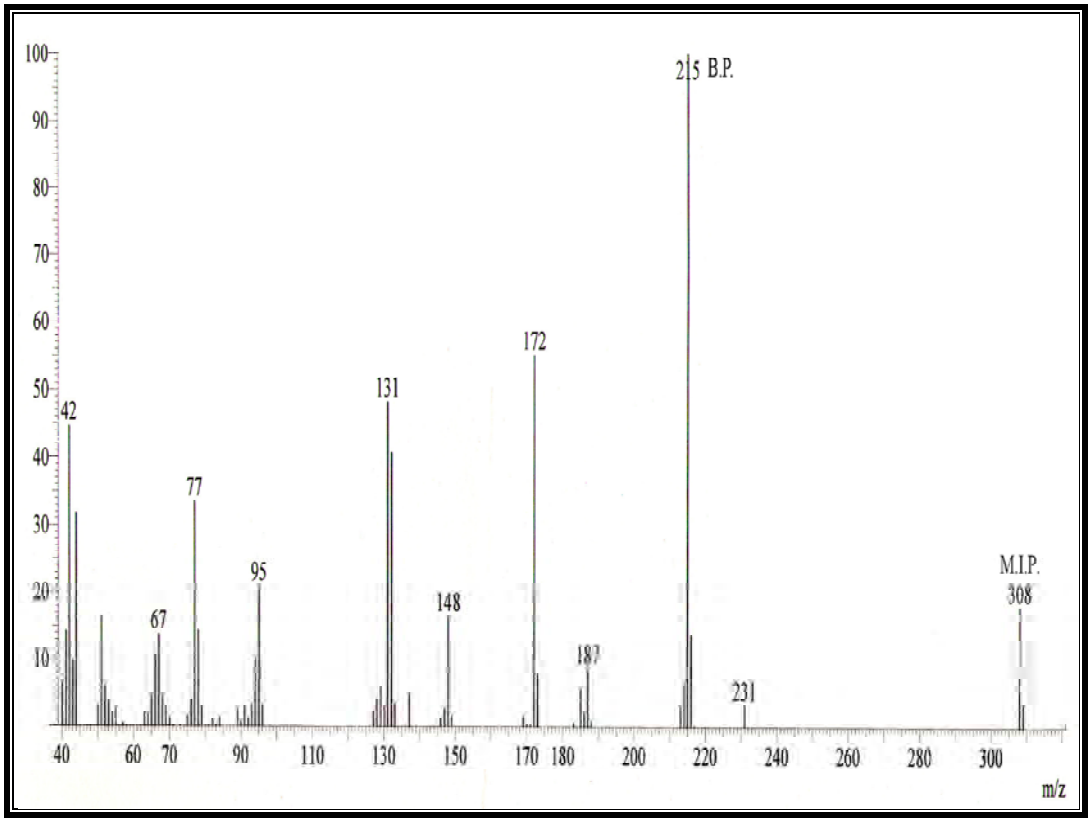
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

For CPV-101 to 130, characteristic singlets were observed for methyl group of acetoacetanilide fragment at 2.11-3.16 δ ppm. Another characteristic methine proton peak was observed at 5.27-5.63 δ ppm which further confirmed the cyclisation. The aromatic ring protons were observed at 6.82-8.70 δ ppm and J value were found to be in accordance with substitution pattern.

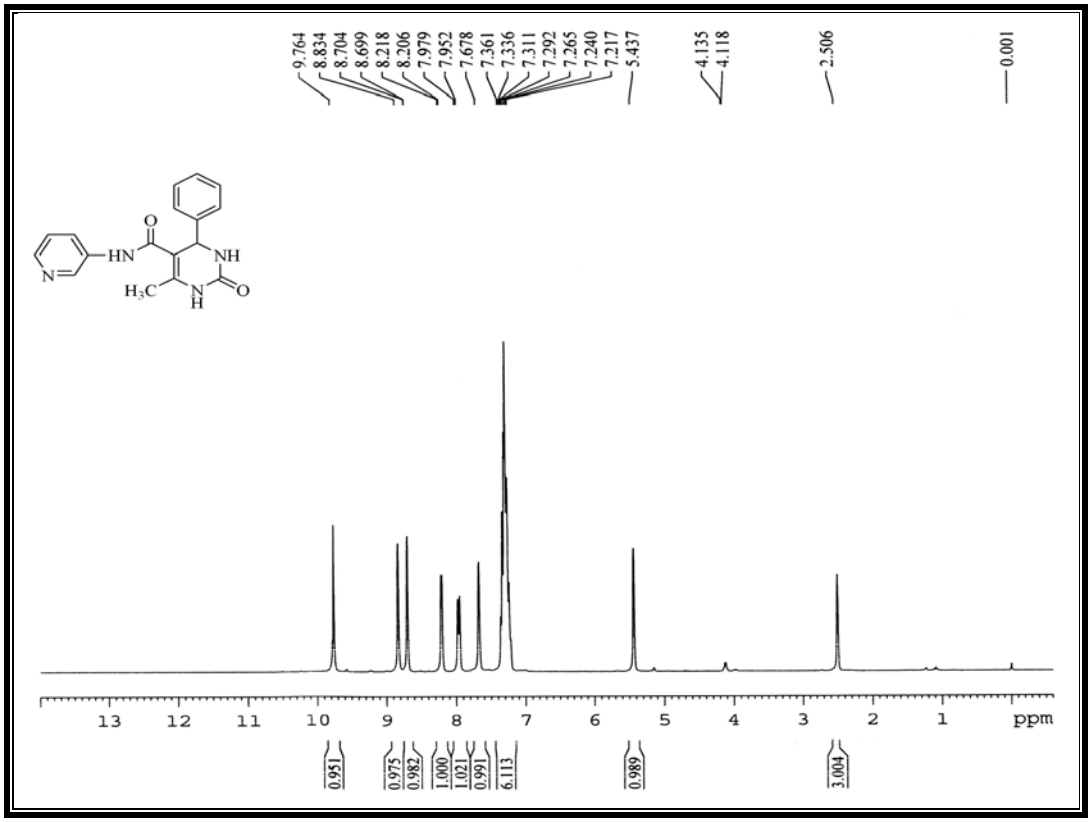
IR spectrum of CPV-101



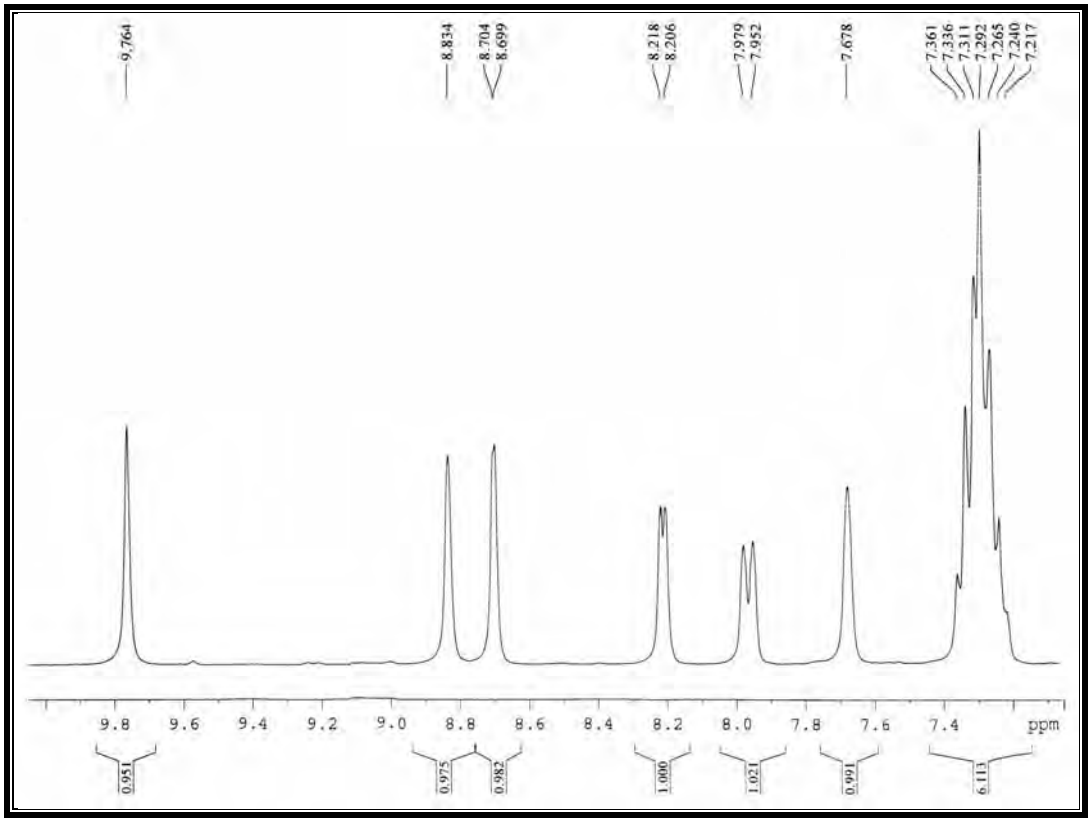
Mass spectrum of CPV-101



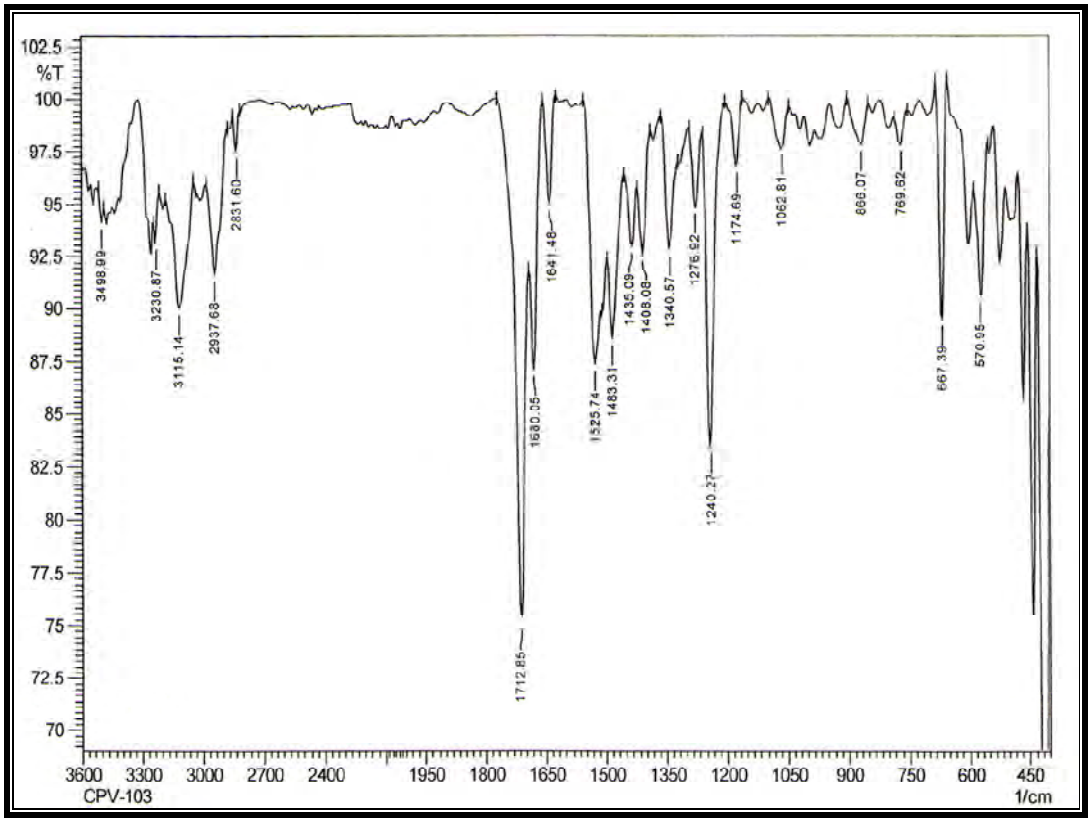
^{1}H NMR spectrum of CPV-101



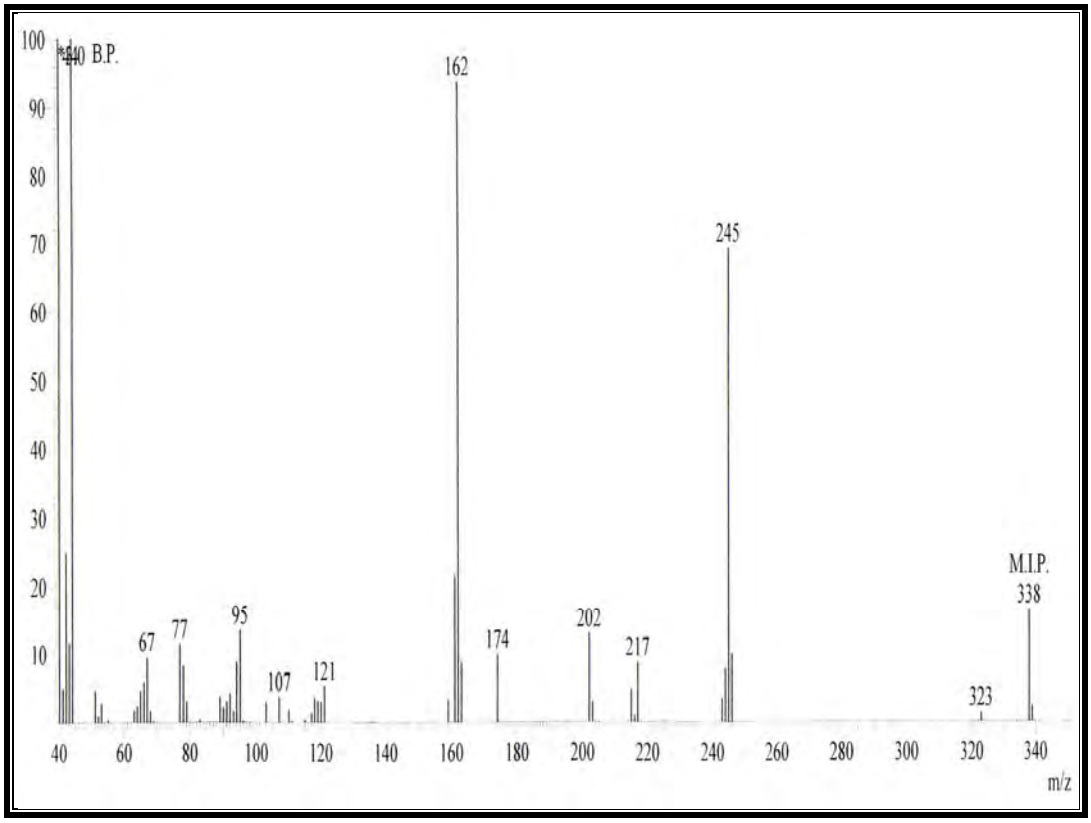
Expanded ^{1}H NMR spectrum of CPV-101



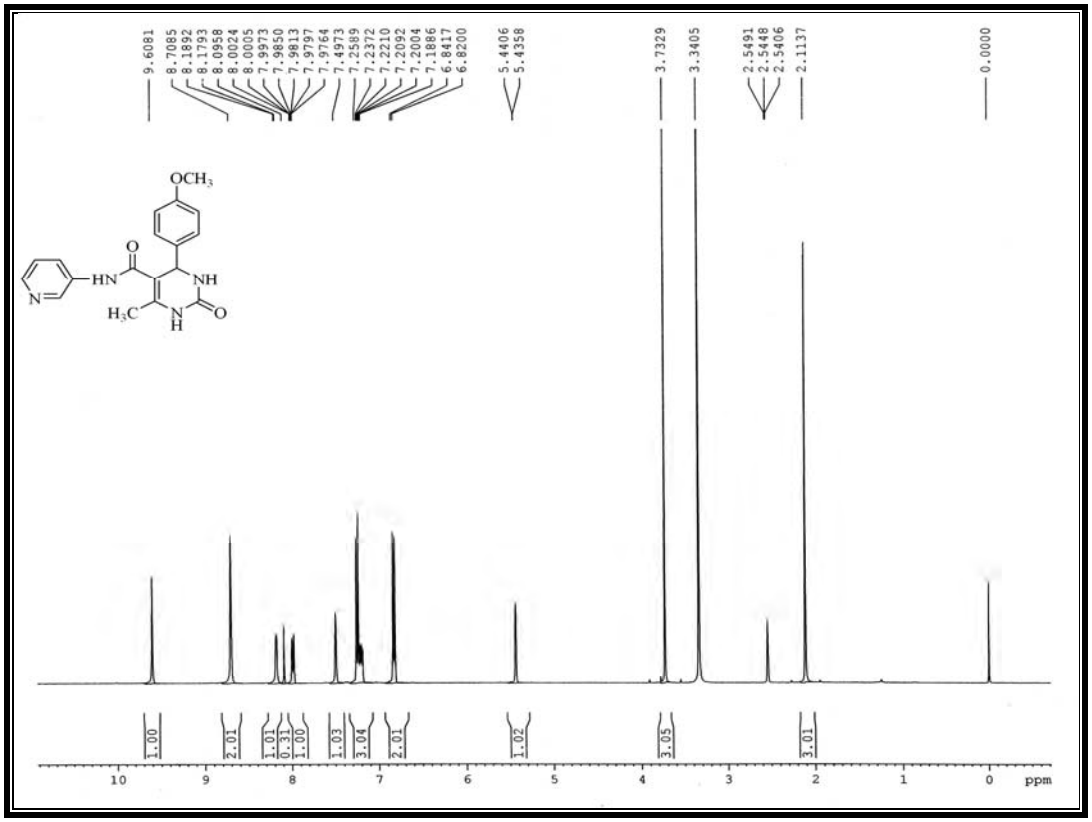
IR spectrum of CPV-103



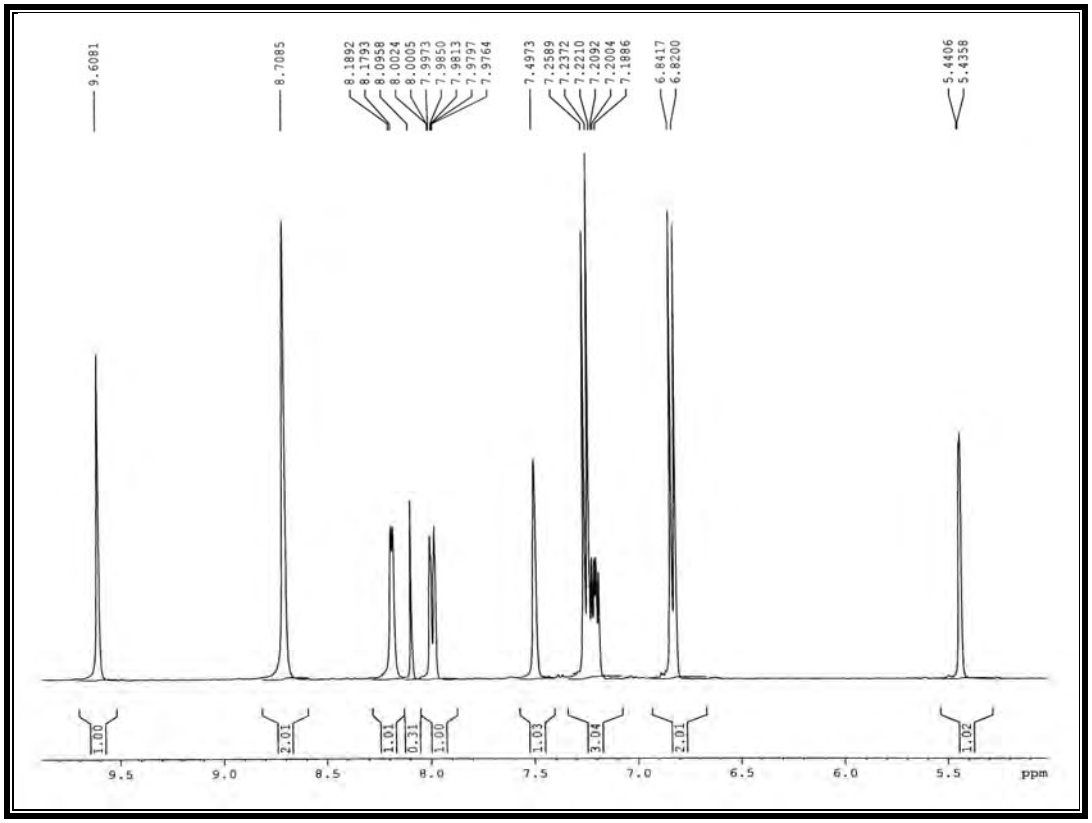
Mass spectrum of CPV-103



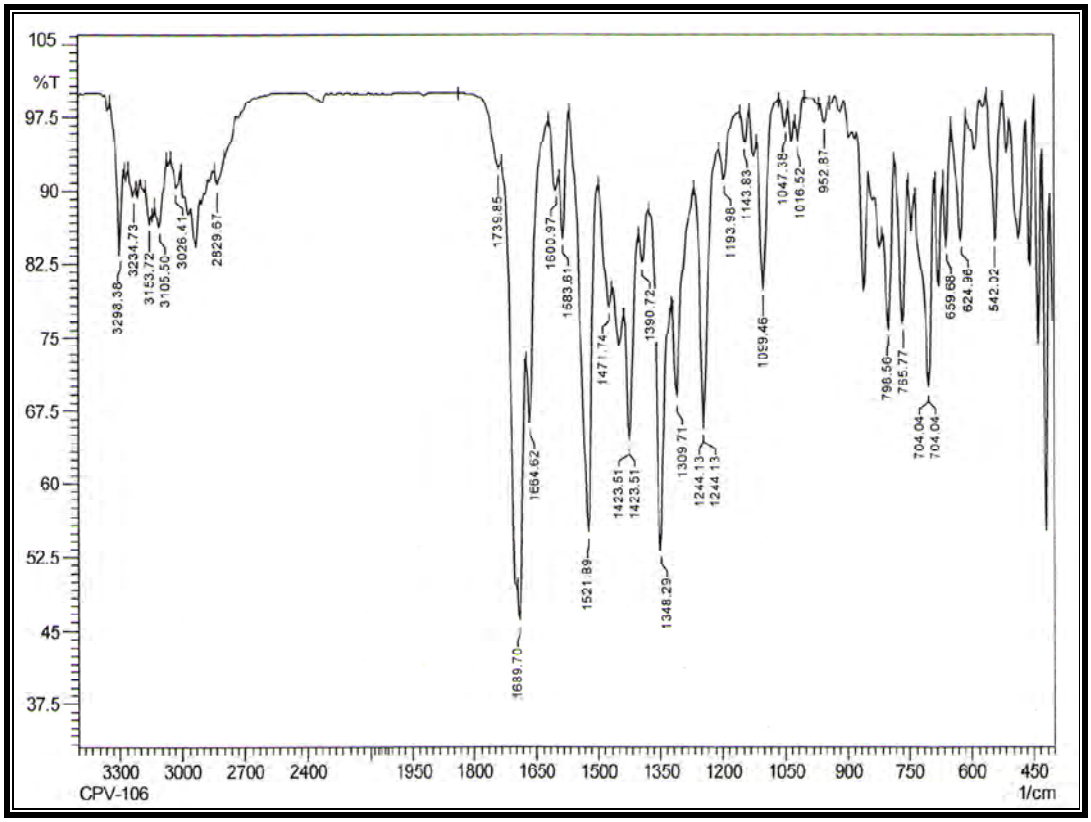
^{1}H NMR spectrum of CPV-103



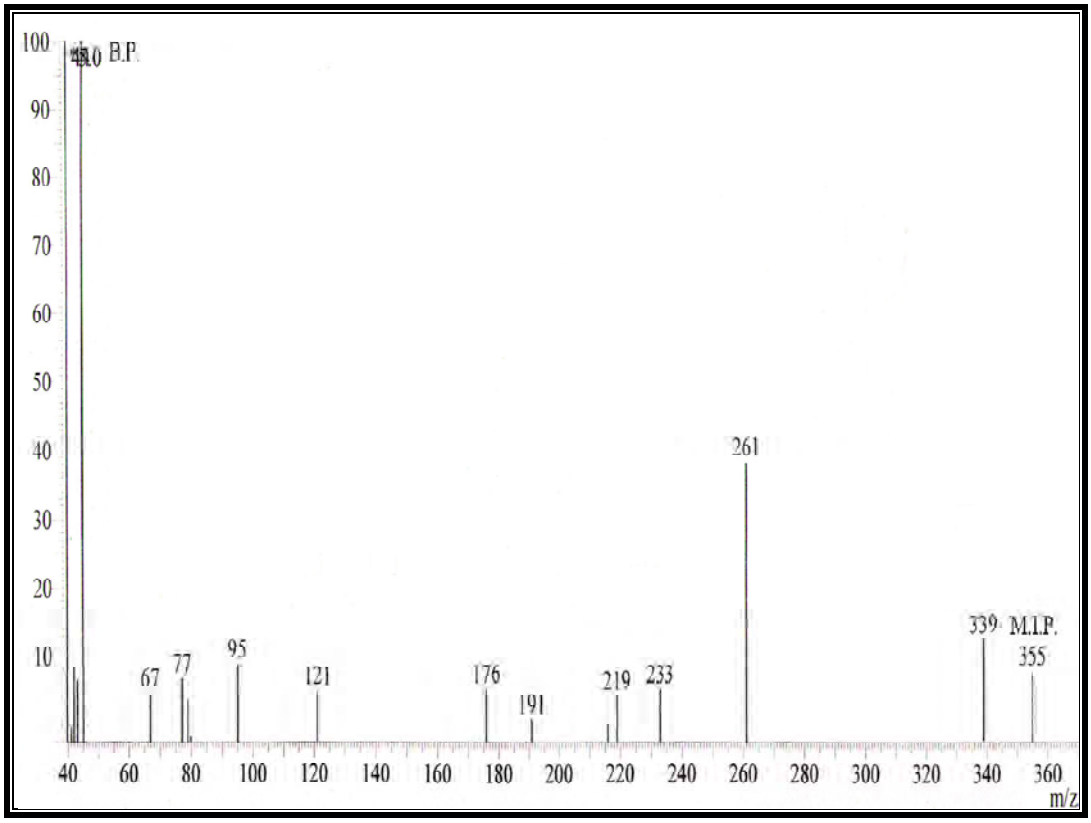
Expanded ^{1}H NMR spectrum of CPV-103



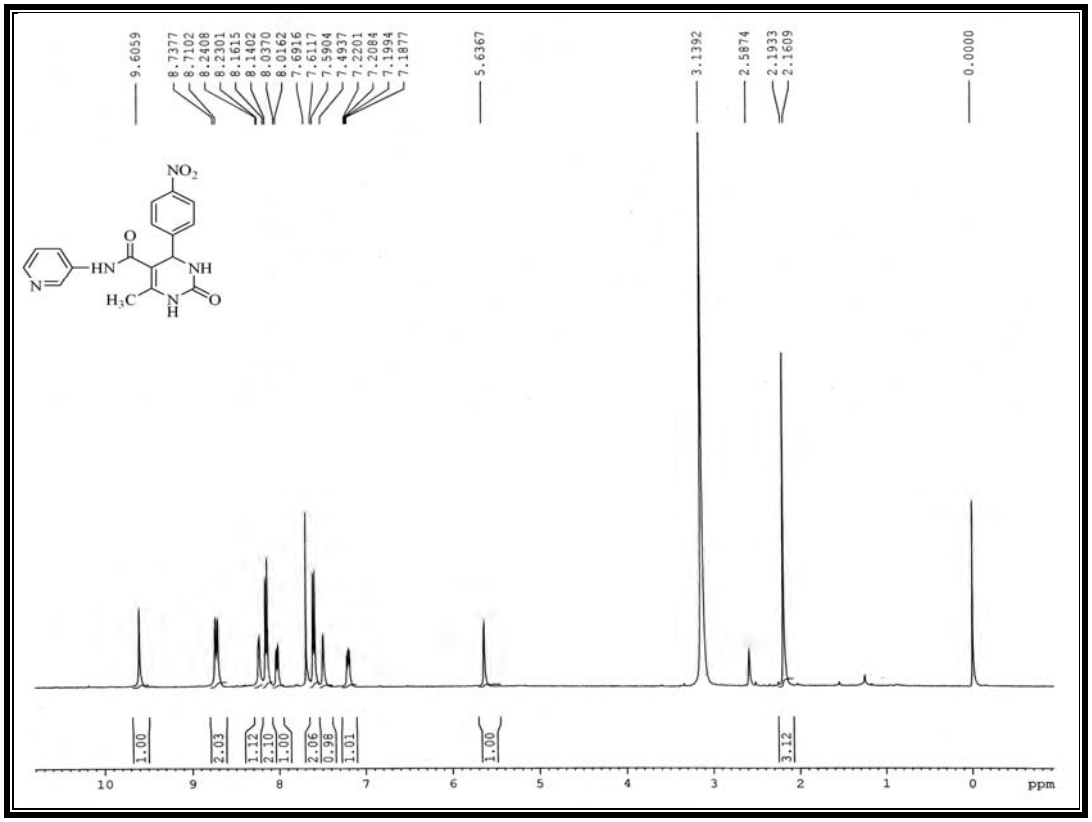
IR spectrum of CPV-106



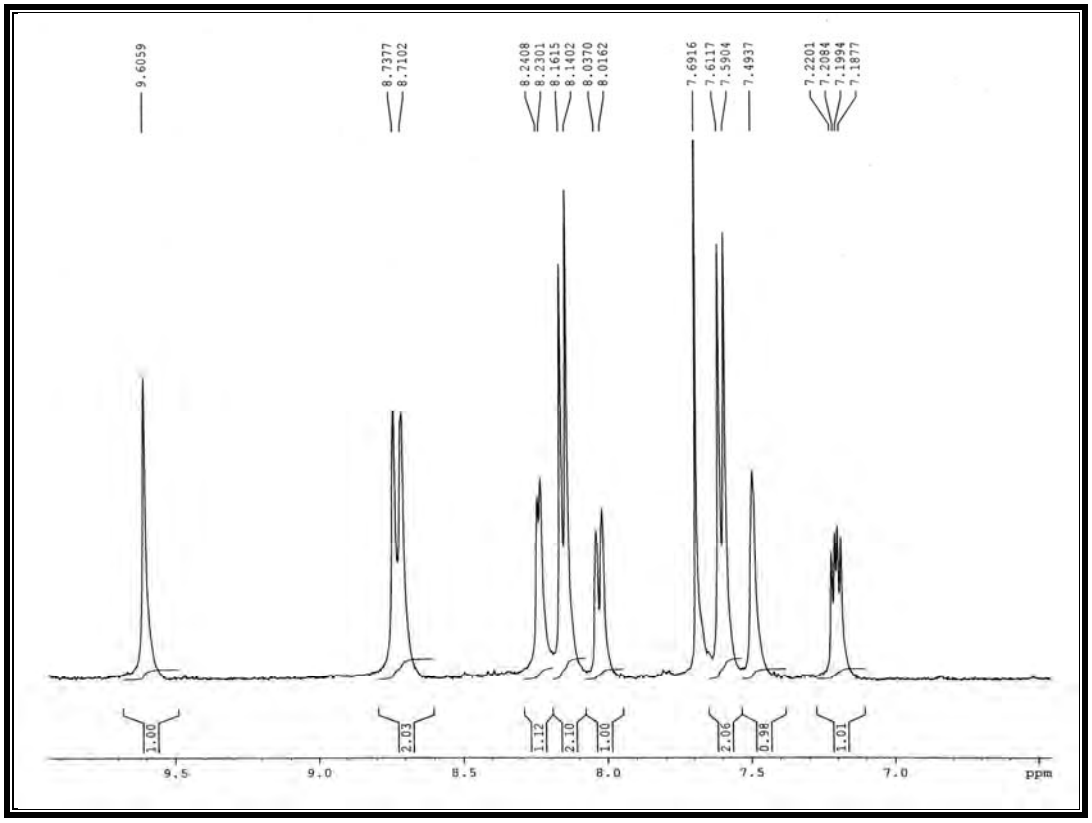
Mass spectrum of CPV-106



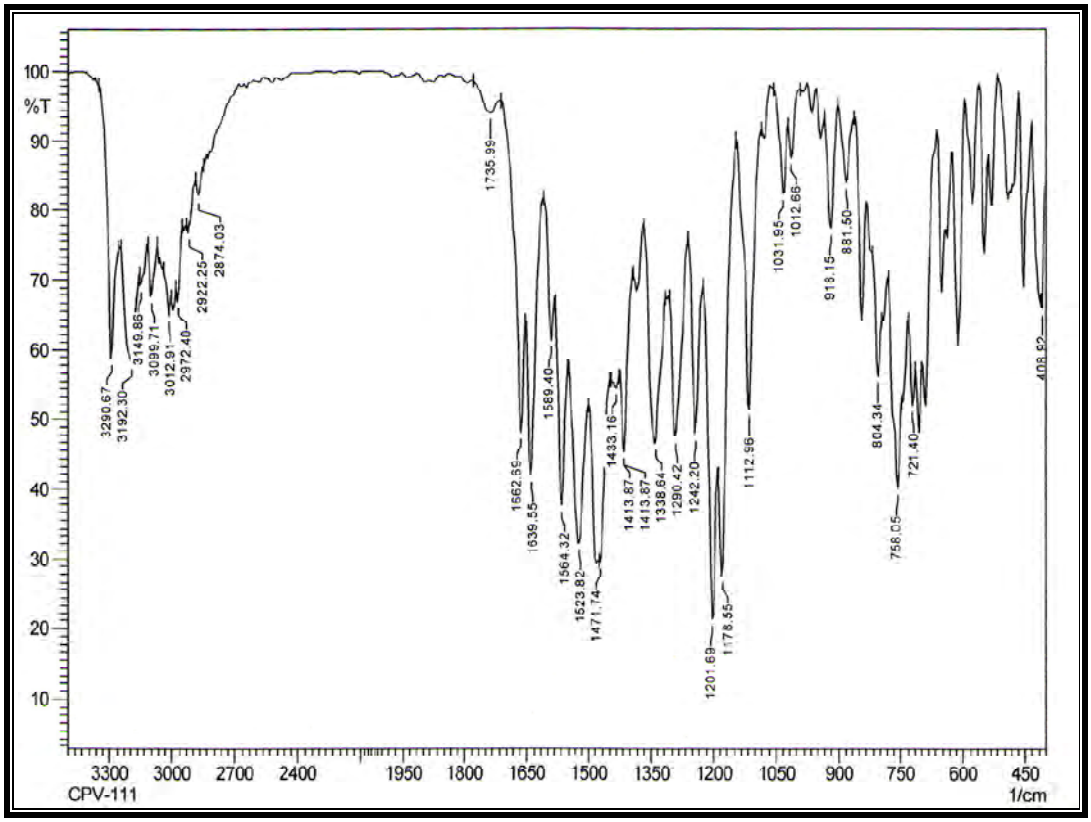
^{1}H NMR spectrum of CPV-106



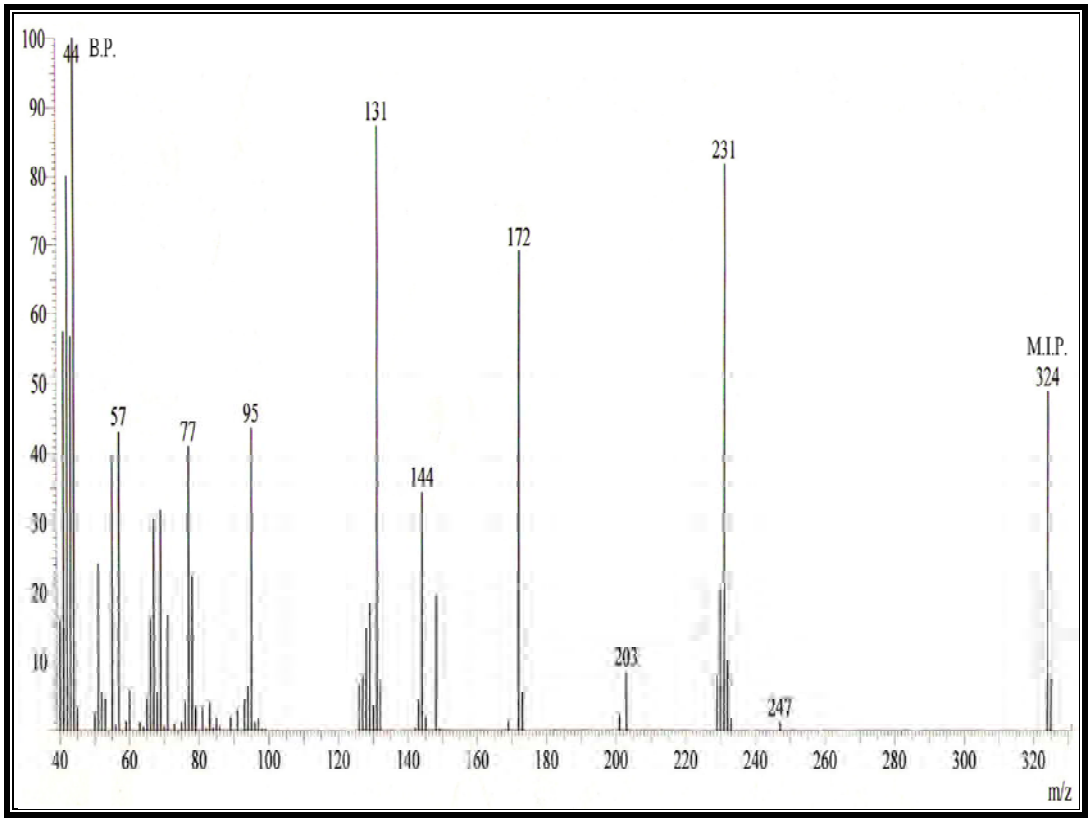
Expanded ^{1}H NMR spectrum of CPV-106



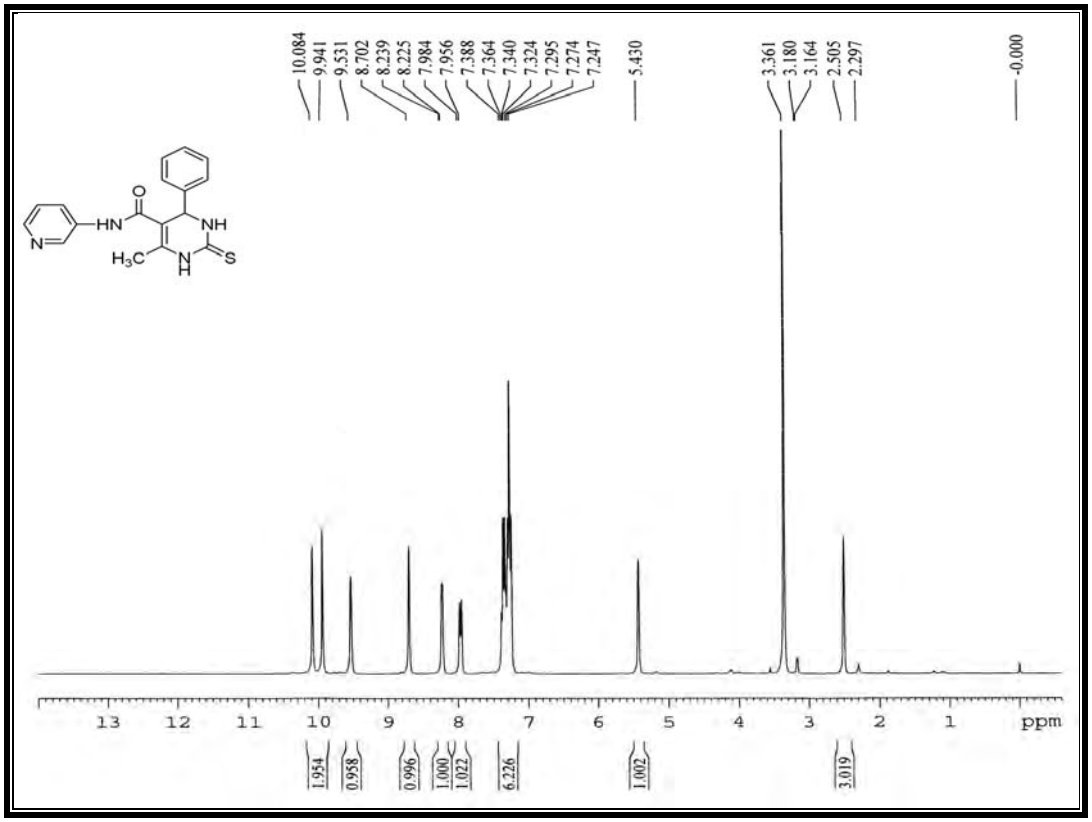
IR spectrum of CPV-111



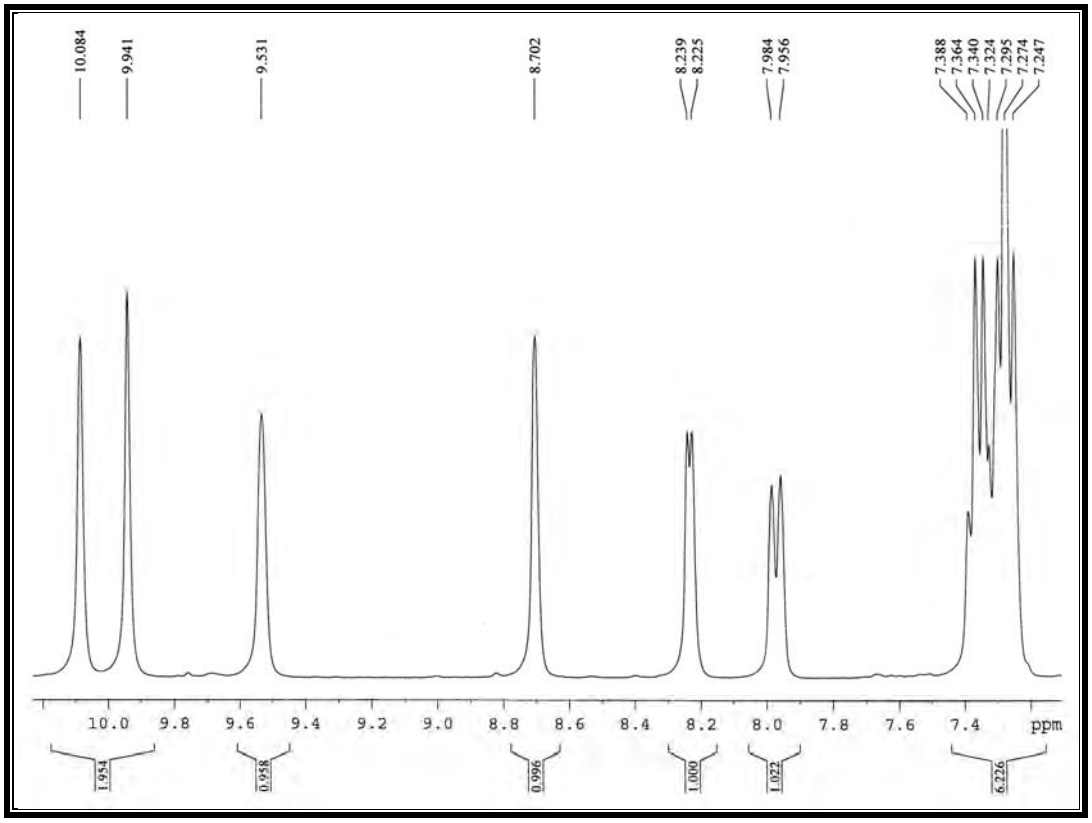
Mass spectrum of CPV-111



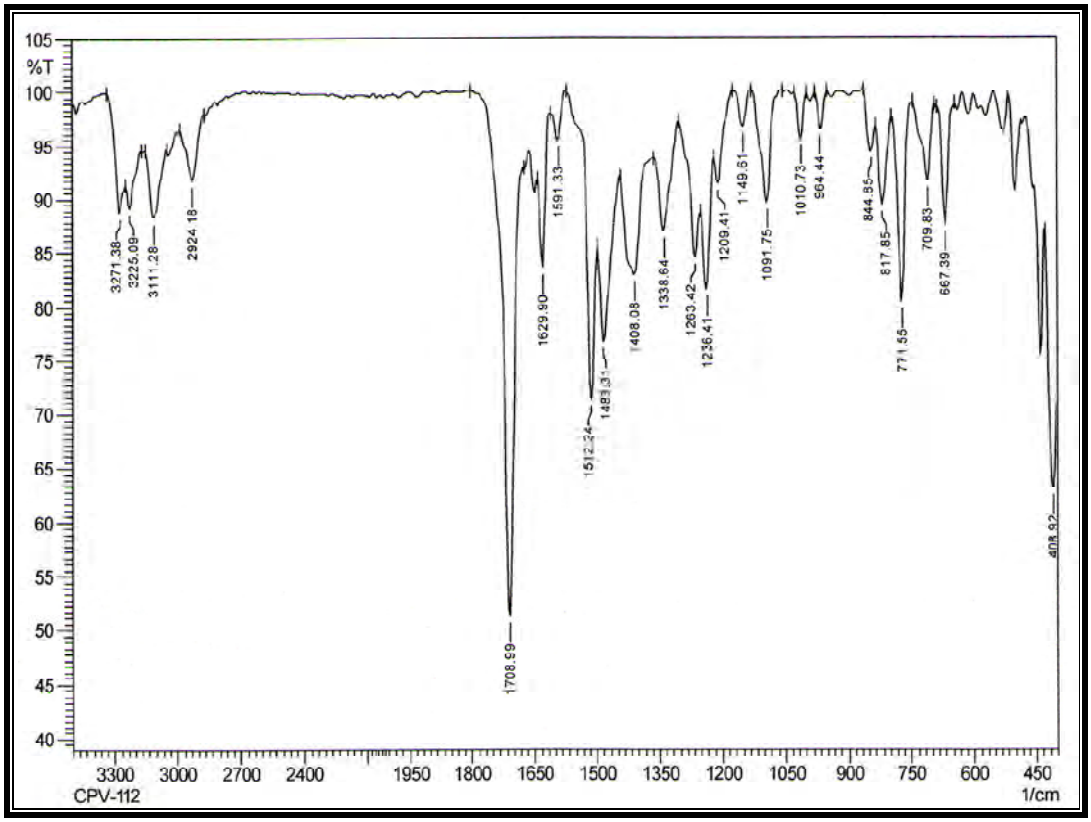
^{1}H NMR spectrum of CPV-111



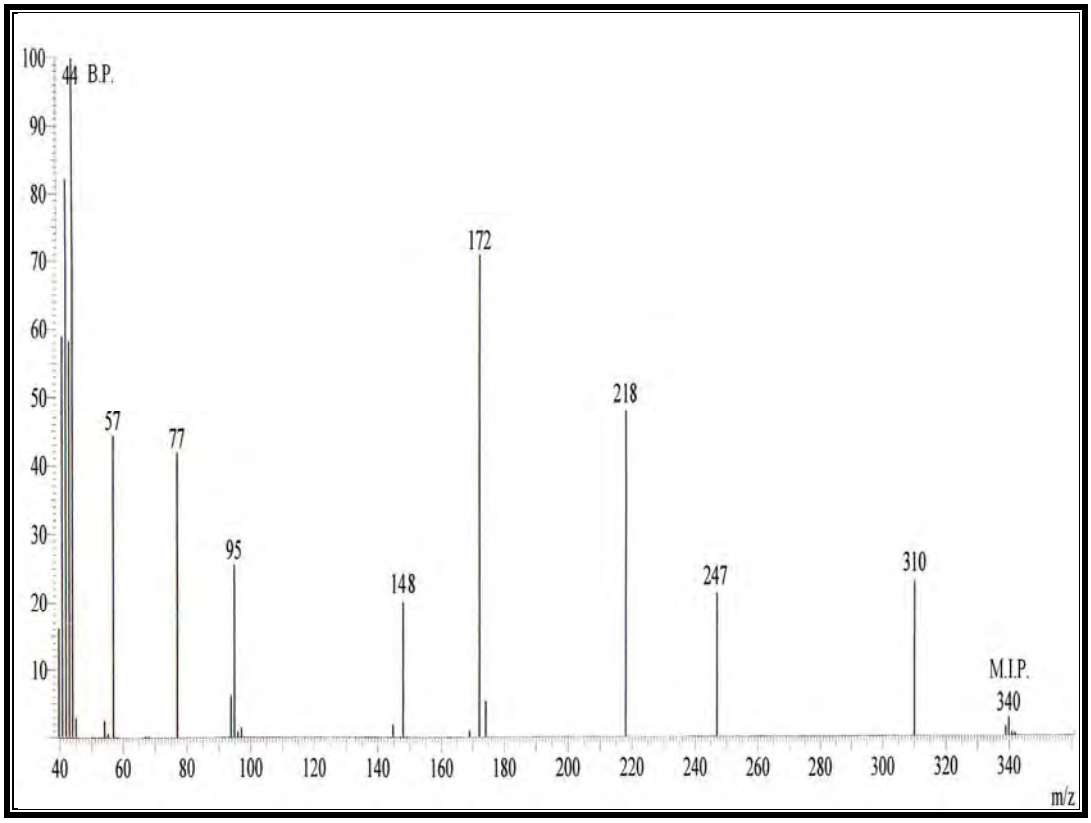
Expanded ^{1}H NMR spectrum of CPV-111



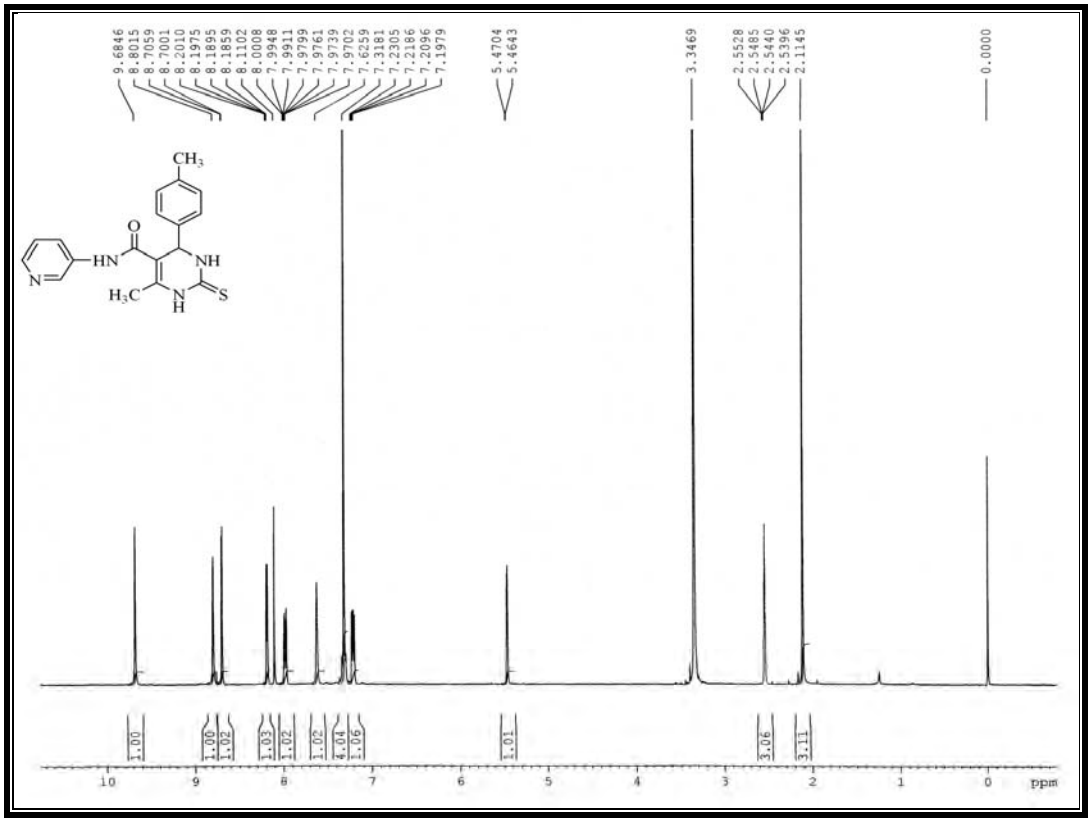
IR spectrum of CPV-112



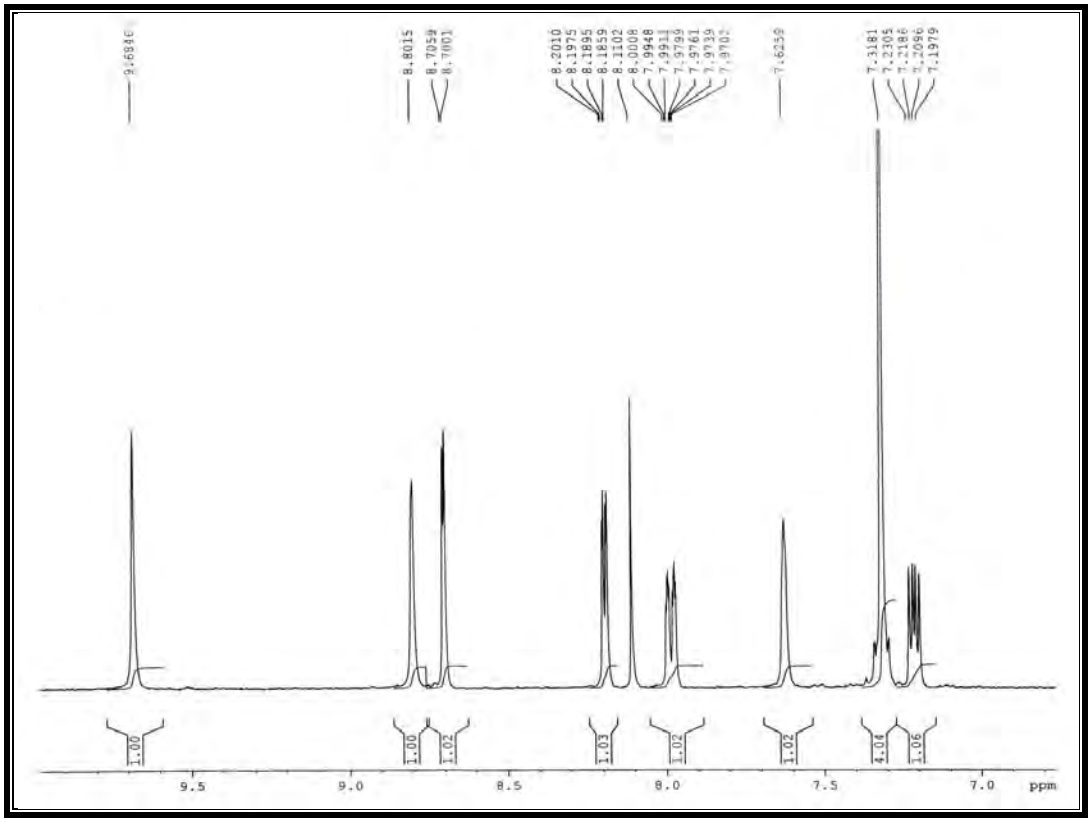
Mass spectrum of CPV-112



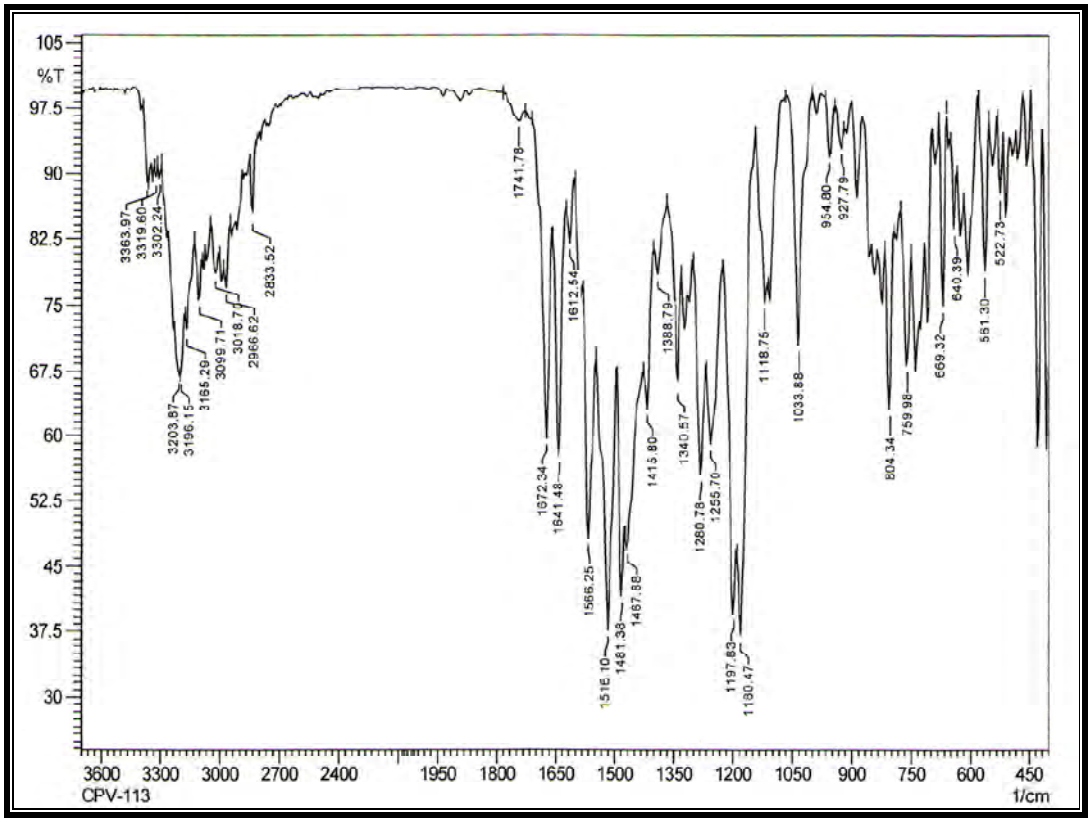
^{1}H NMR spectrum of CPV-112



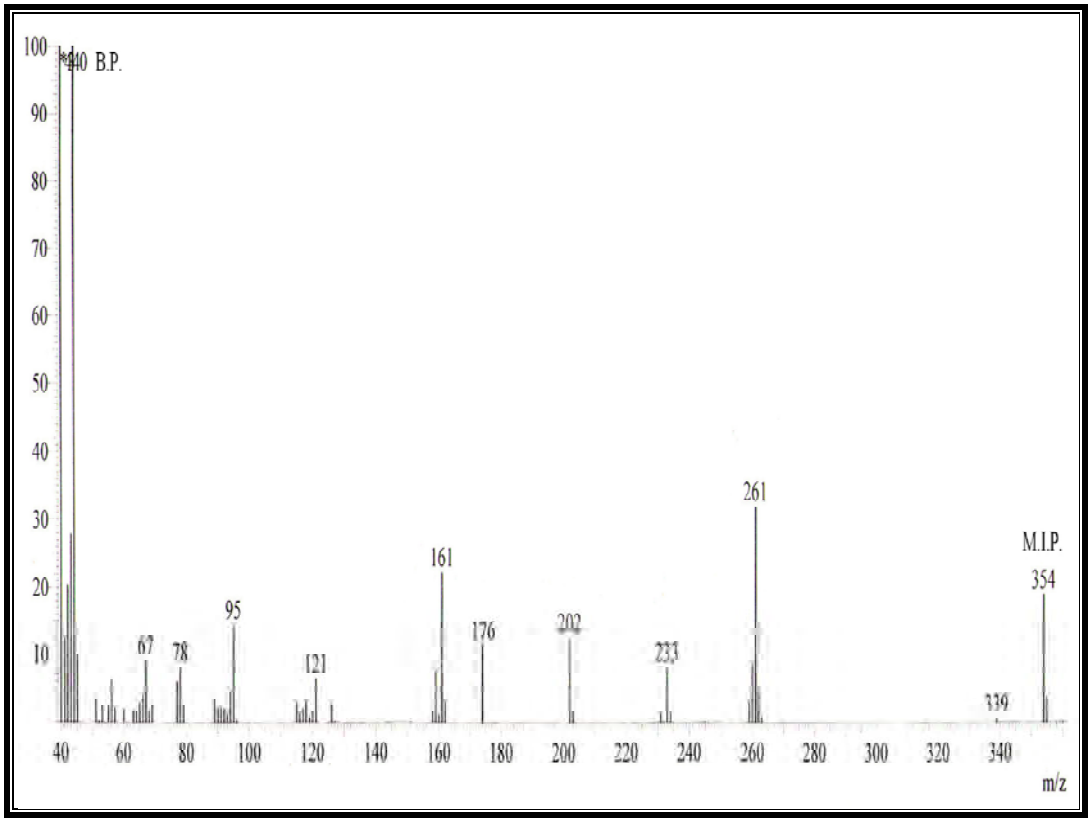
Expanded ^{1}H NMR spectrum of CPV-112



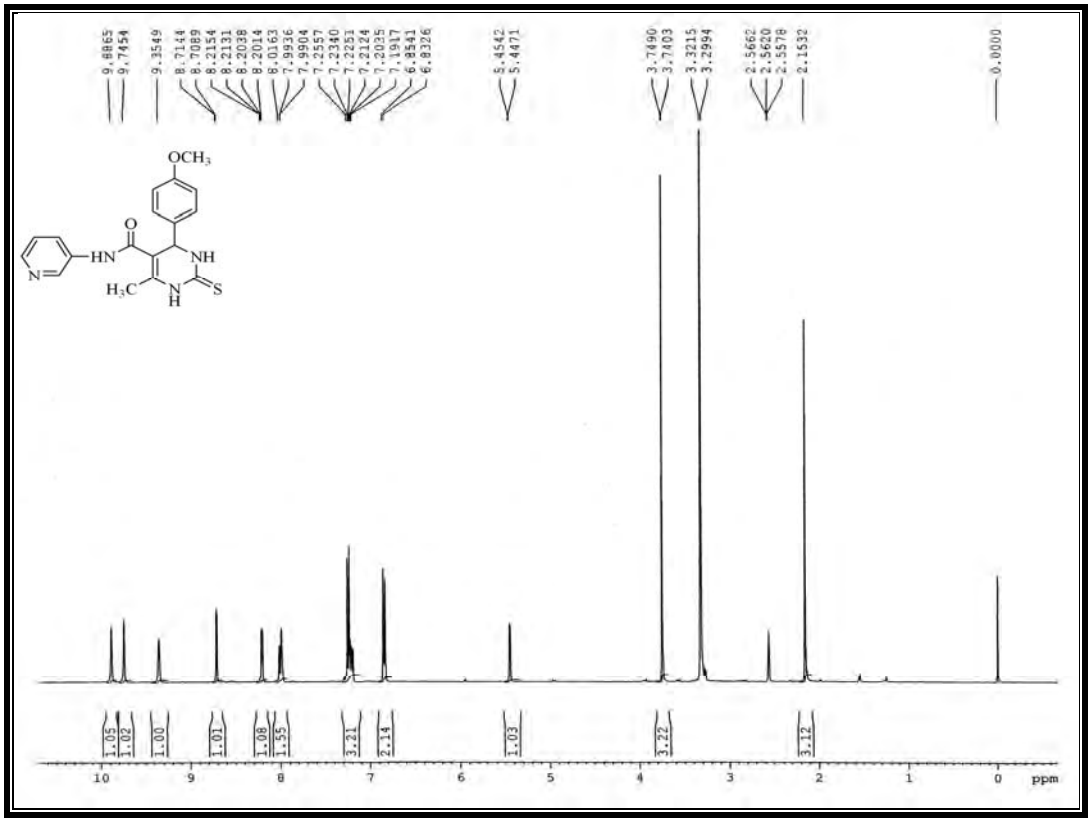
IR spectrum of CPV-113



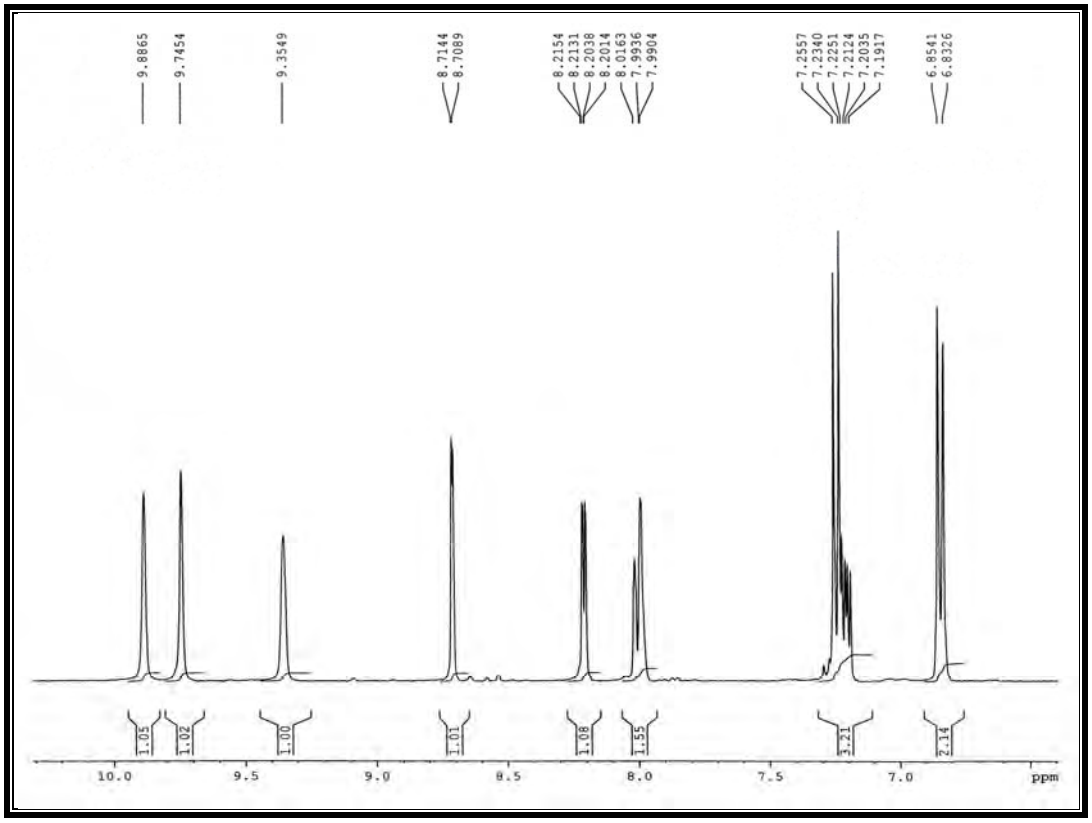
Mass spectrum of CPV-113



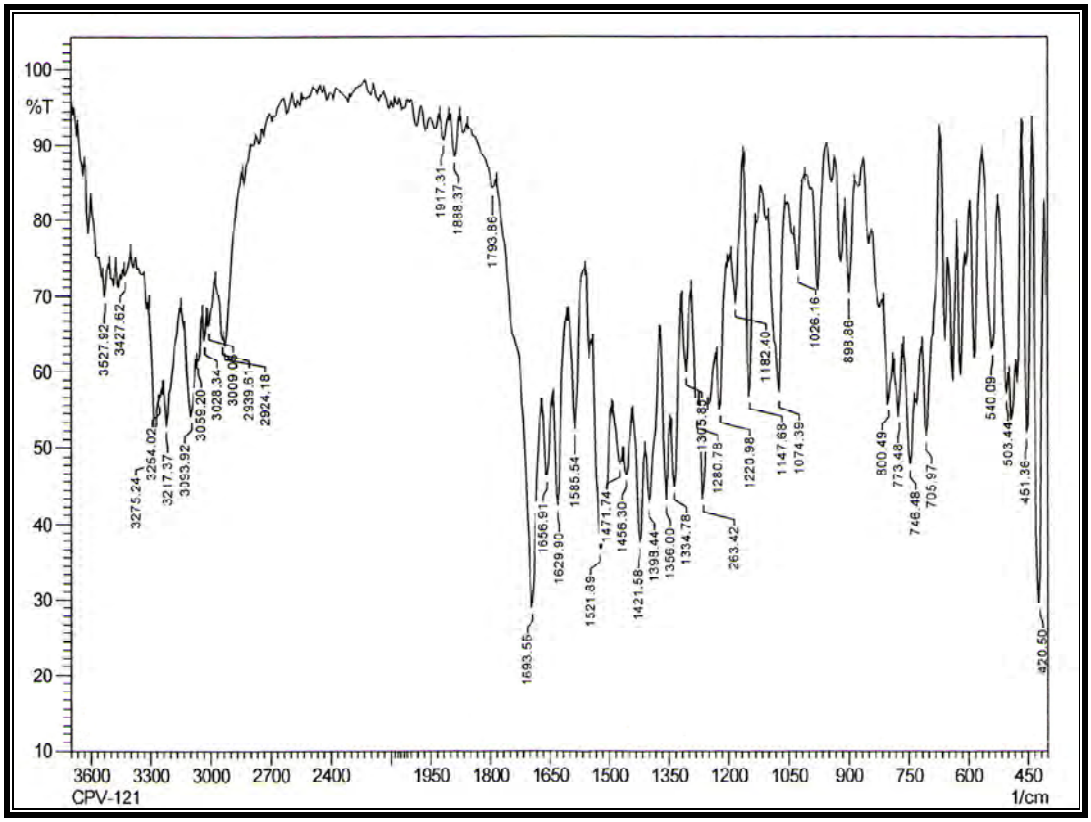
^{1}H NMR spectrum of CPV-113



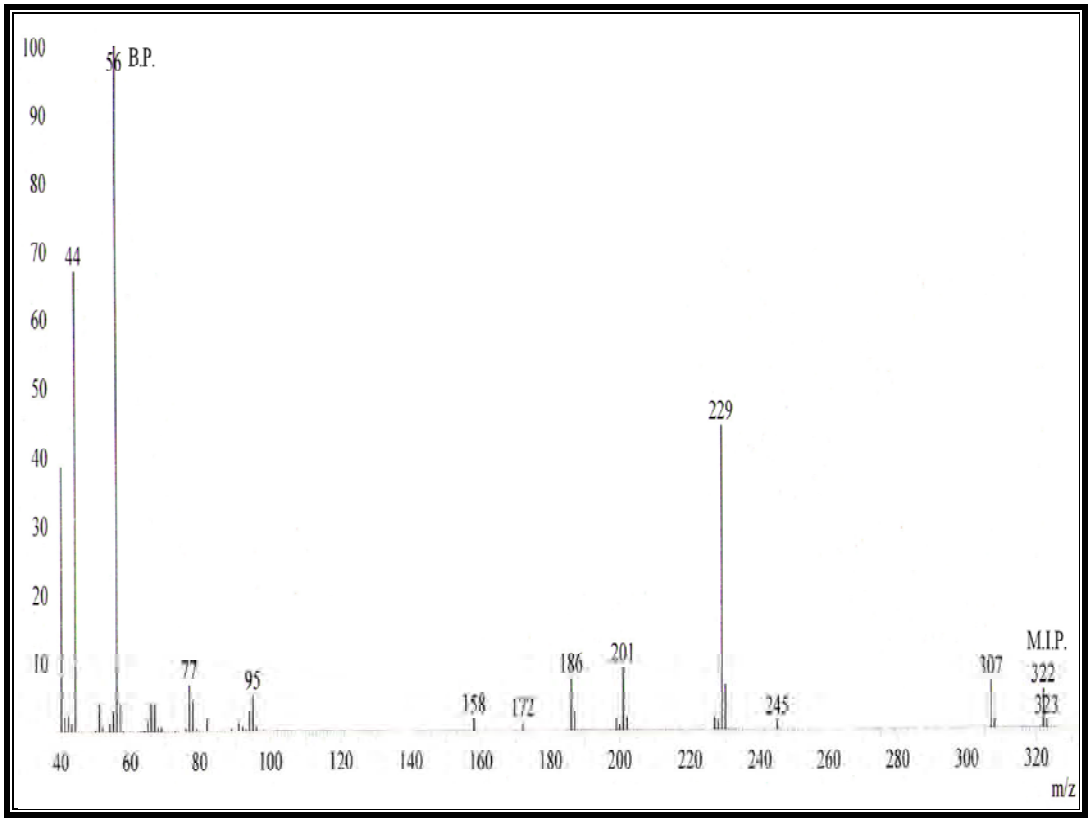
Expanded ^{1}H NMR spectrum of CPV-113



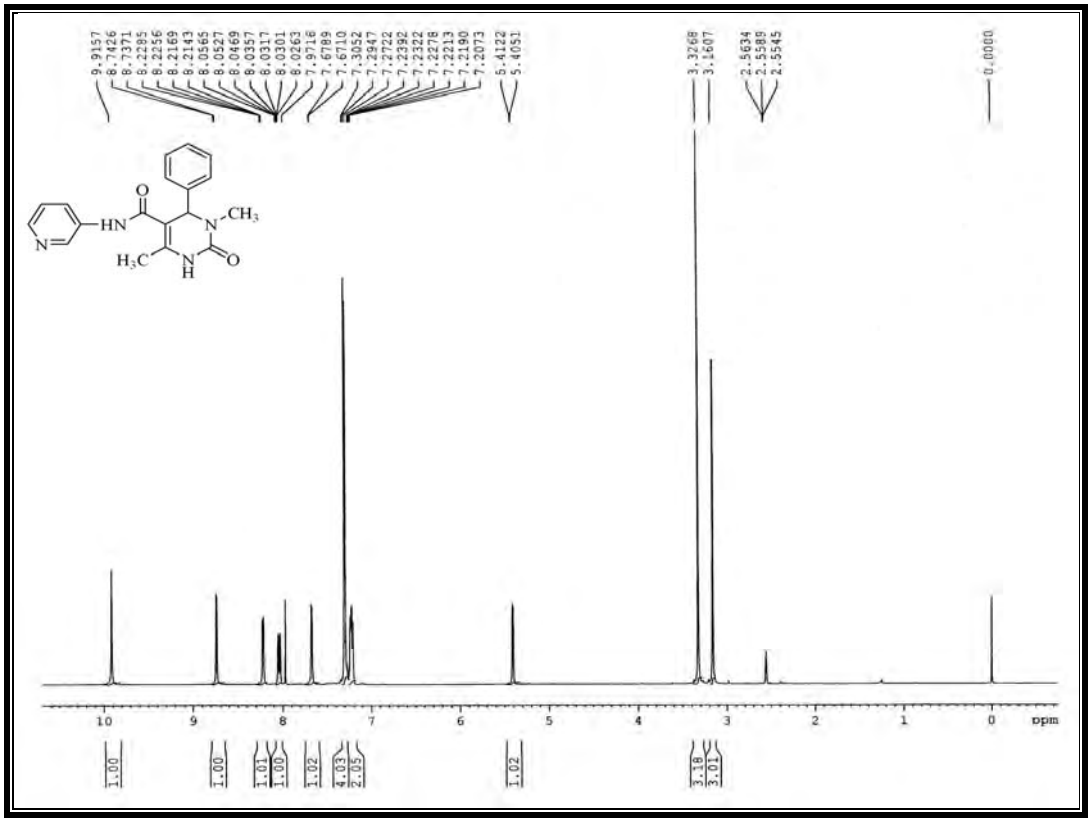
IR spectrum of CPV-121



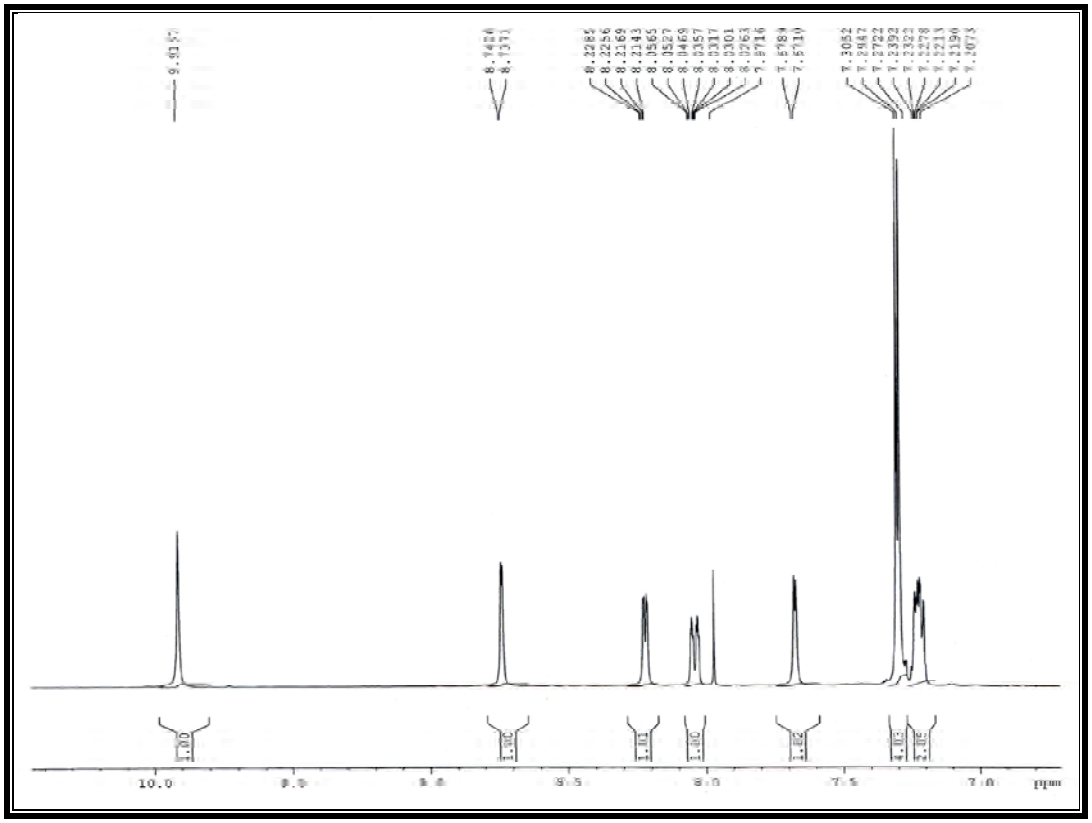
Mass spectrum of CPV-121



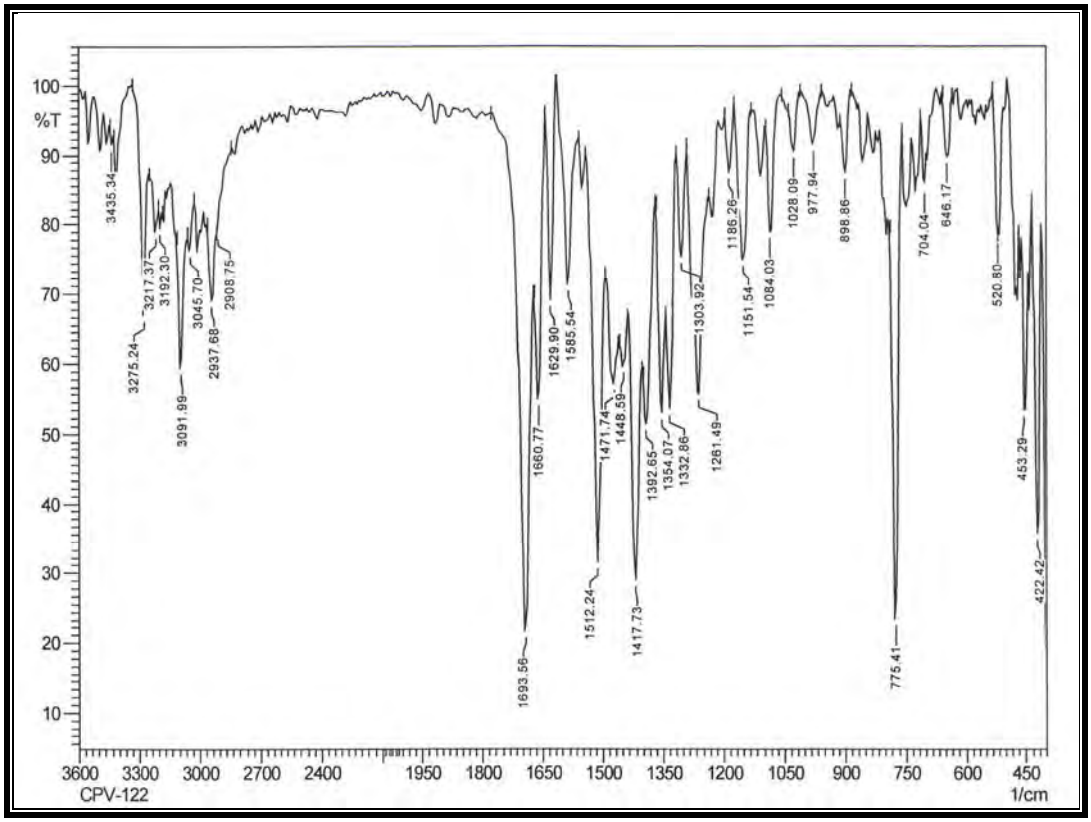
^{1}H NMR spectrum of CPV-121



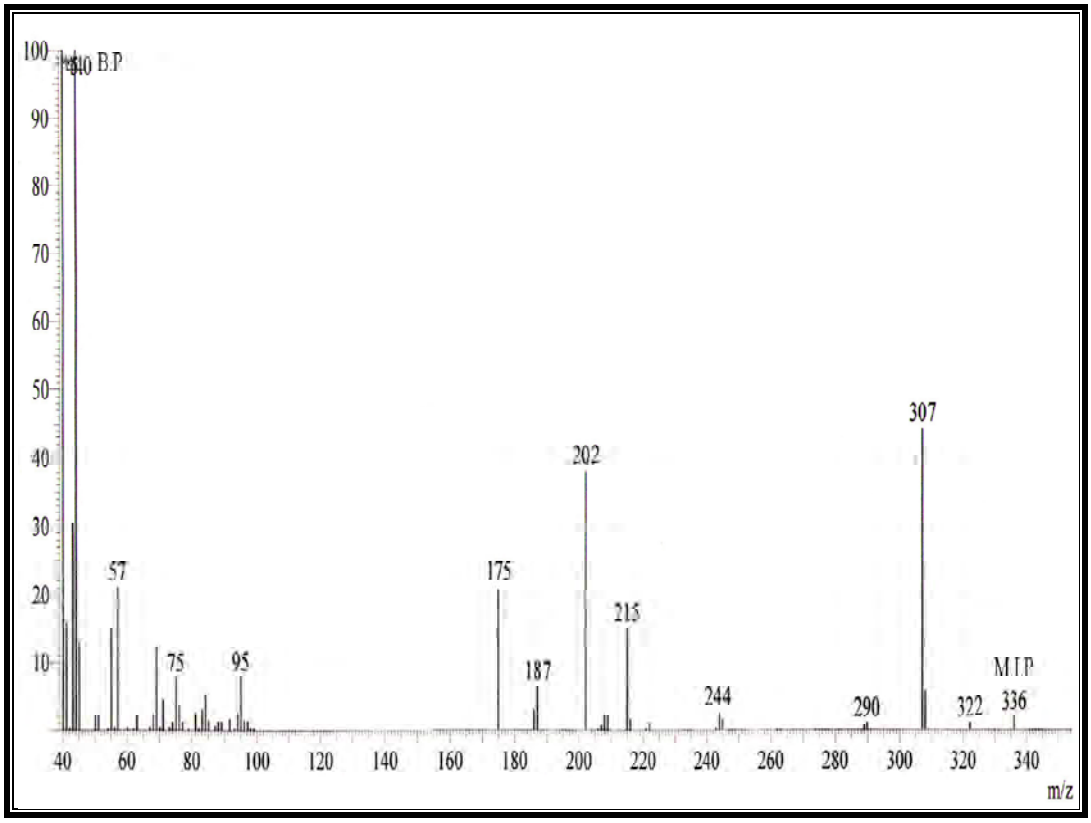
Expanded ^{1}H NMR spectrum of CPV-121



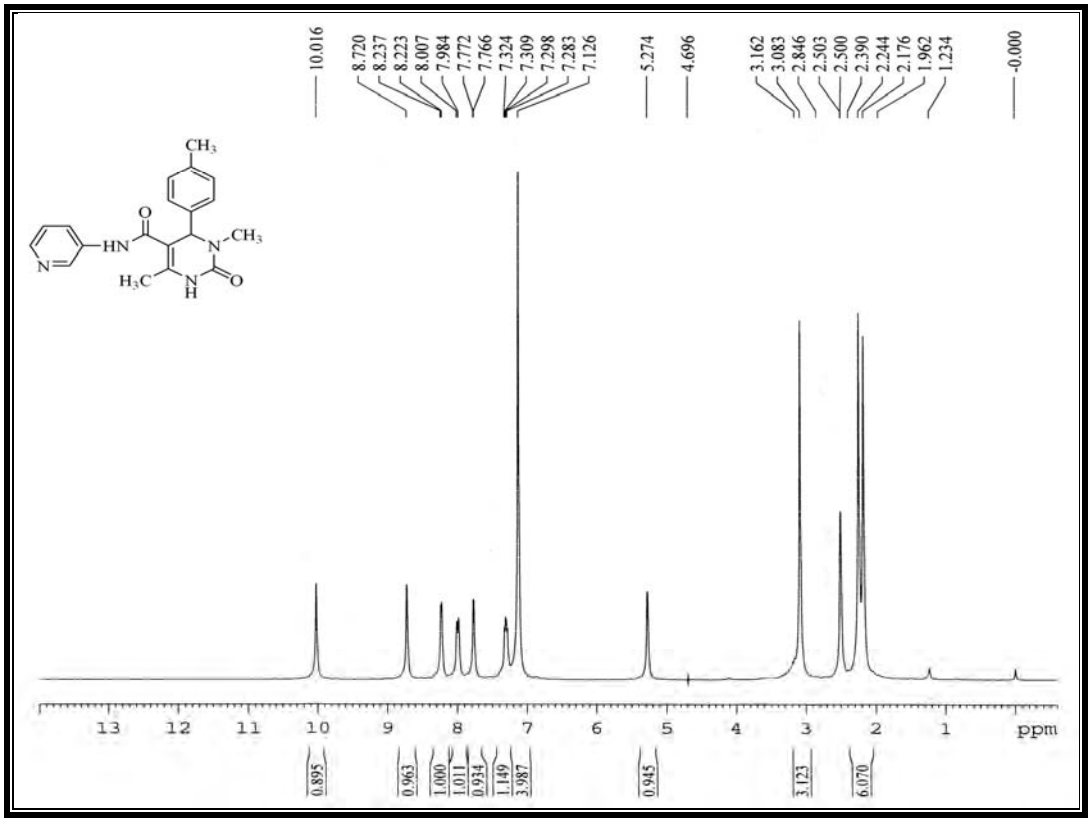
IR spectrum of CPV-122



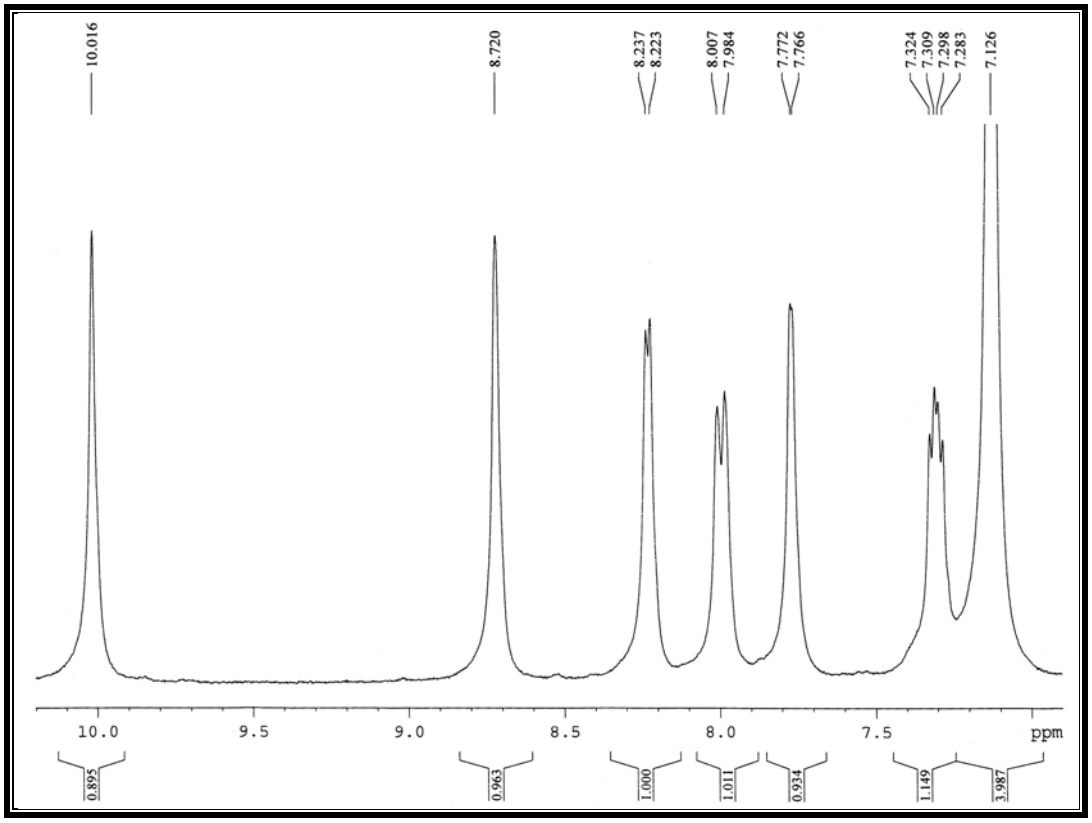
Mass spectrum of CPV-122



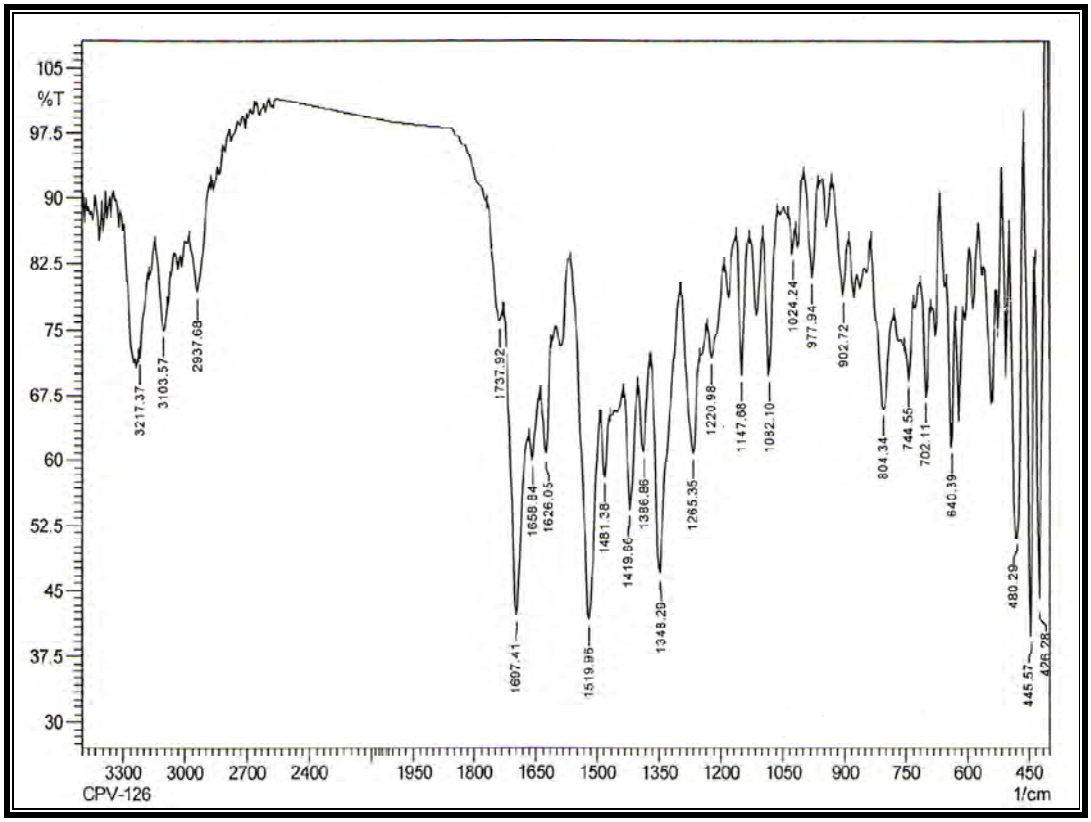
^{1}H NMR spectrum of CPV-122



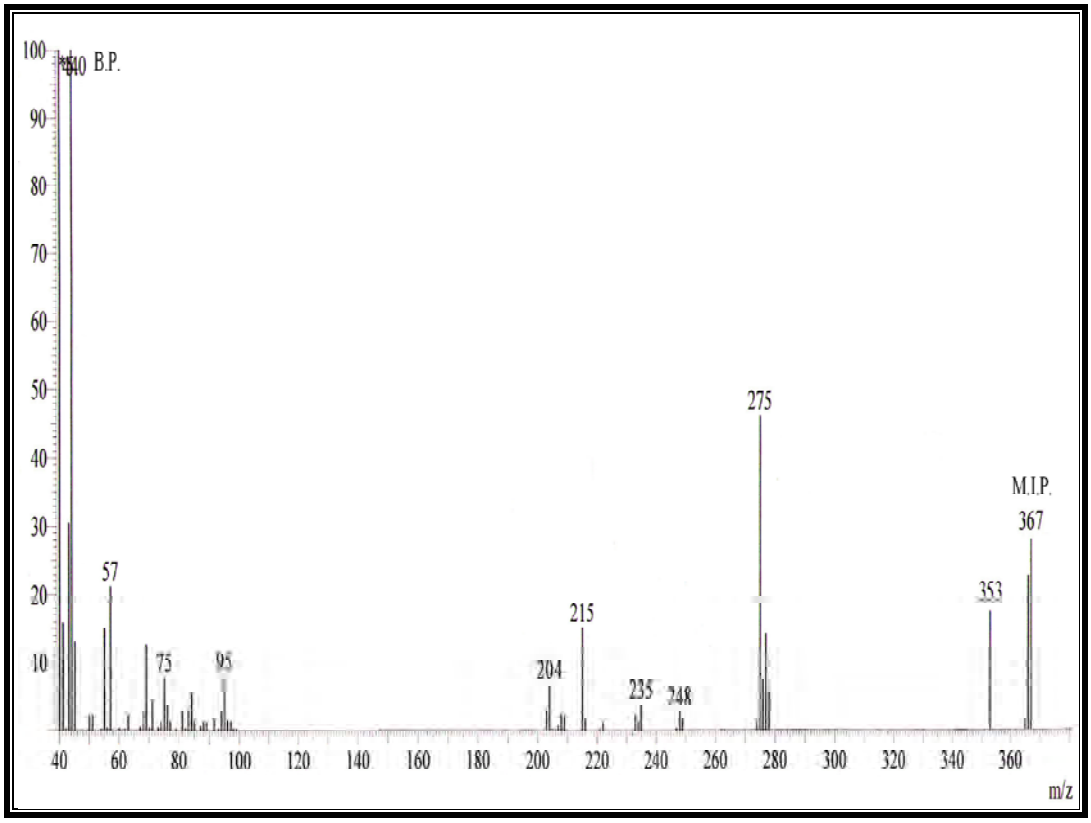
Expanded ^{1}H NMR spectrum of CPV-122



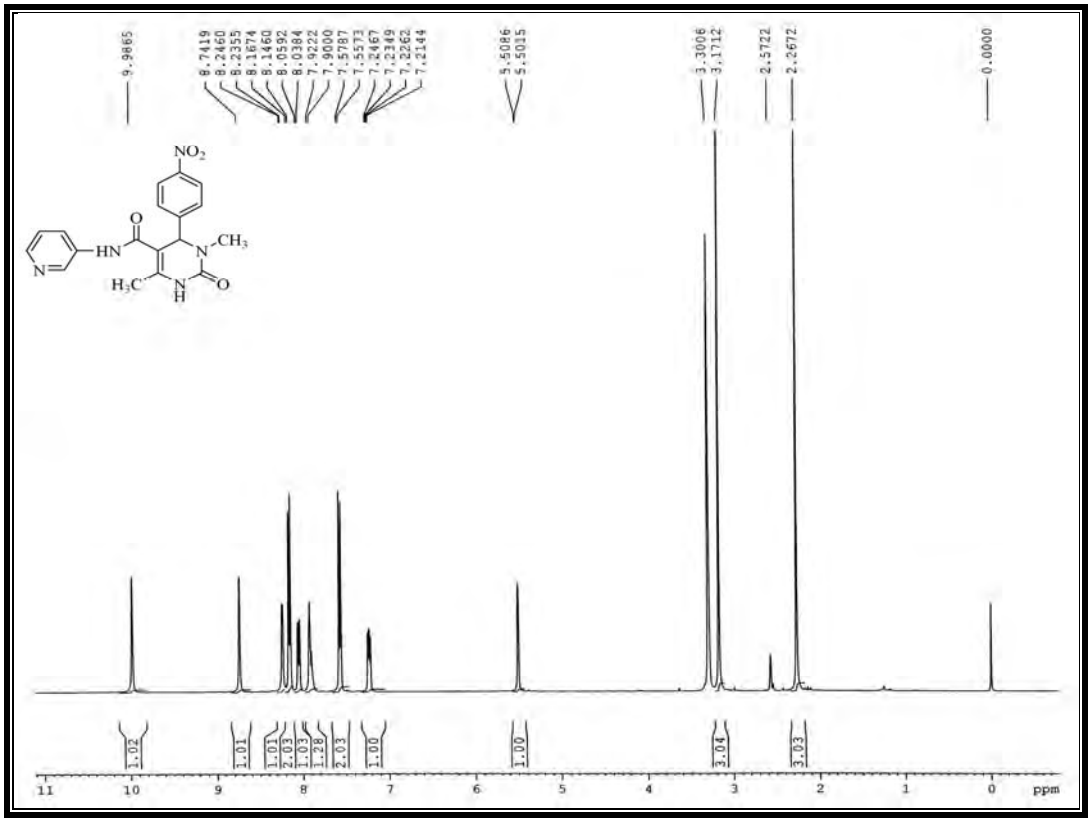
IR spectrum of CPV-126



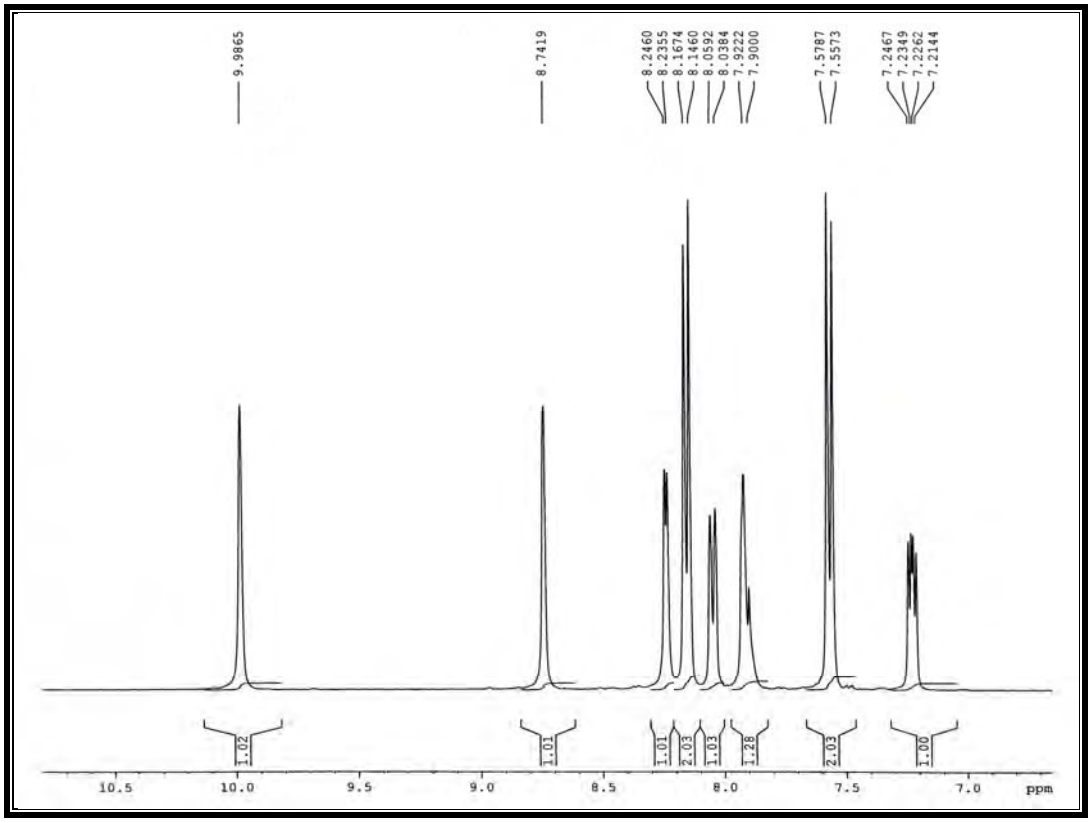
Mass spectrum of CPV-126



^{1}H NMR spectrum of CPV-126



Expanded ^{1}H NMR spectrum of CPV-126



3.7 Biological evaluation

3.7.1 Antimicrobial evaluation

All of the synthesized compounds (CPV-101 to 130) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [126-128] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards [126].

Minimal Inhibition Concentration [MIC]:-

The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at $37\ ^{0}C$ overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Methods used for primary and secondary screening: -

Each synthesized drug was diluted obtaining 2000 μg mL^{-1} concentration, as a stock solution. Inoculum size for test strain was adjusted to 10^8 cfu (colony forming unit) per milliliter by comparing the turbidity.

Primary screen: - In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: - The drugs found active in primary screening were similarly diluted to obtain 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1} and 6.250 μg mL^{-1} concentrations.

Reading Result: - The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table-1:- *In vitro* Antimicrobial Screening Results for CPV-101 to 130

Code	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
CPV-101	150	200	250	250	1000	500	500
CPV-102	100	250	200	200	500	>1000	>1000
CPV-103	150	150	62.5	100	>1000	>1000	>1000
CPV-104	250	250	100	100	1000	500	1000
CPV-105	200	200	100	100	>1000	>1000	>1000
CPV-106	500	500	62.5	200	>1000	1000	1000
CPV-107	250	500	250	250	500	1000	1000
CPV-108	500	500	200	200	1000	500	1000
CPV-109	100	62.5	250	250	>1000	500	1000
CPV-110	100	500	62.5	250	500	>1000	1000
CPV-111	200	500	250	250	>1000	>1000	>1000
CPV-112	200	250	200	100	500	1000	1000
CPV-113	250	500	250	500	>1000	1000	1000
CPV-114	250	250	100	250	500	1000	500
CPV-115	200	200	100	100	500	>1000	1000
CPV-116	250	500	62.5	200	>1000	500	>1000
CPV-117	200	100	250	62.5	500	500	>1000
CPV-118	500	500	200	250	500	1000	500
CPV-119	100	62.5	100	250	500	1000	>1000
CPV-120	200	250	100	250	>1000	>1000	>1000
CPV-121	500	500	500	200	>1000	>1000	>1000
CPV-122	500	500	250	250	>1000	>1000	>1000
CPV-123	200	250	250	150	500	>1000	500
CPV-124	250	250	100	100	>1000	>1000	>1000
CPV-125	200	200	500	500	>1000	>1000	>1000
CPV-126	500	500	250	500	>1000	>1000	>1000
CPV-127	200	200	100	150	1000	1000	250
CPV-128	250	500	62.5	250	500	1000	>1000
CPV-129	500	500	100	250	1000	1000	250
CPV-130	200	200	100	250	1000	1000	>1000
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Iprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

3.7.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds CPV-101 to CPV-130 is currently under investigation and results are awaited.

3.8 References and notes

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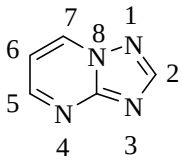
Chapter 4

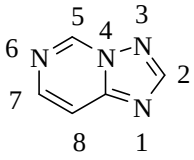
Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines

4.1 Introduction

The condensation of a ring of 1,2,4-triazole and another one of pyrimidine gives rise to the formation of bicyclic heterocycles known as 1,2,4-triazolopyrimidines. Four different possibilities exist for the relative orientation of both rings, so four different isomeric families of compounds are defined: 1,2,4-triazolo[1,5-*a*]pyrimidine (1), 1,2,4-triazolo[1,5-*c*]pyrimidine (2), 1,2,4-triazolo[4,3-*a*]pyrimidine (3) and 1,2,4-triazolo[4,3-*c*]pyrimidine (4).



(1) 1,2,4-triazolo[1,5-*a*]pyrimidine



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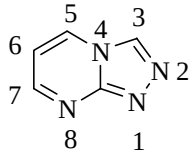

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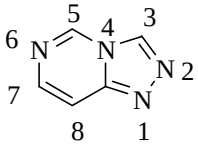
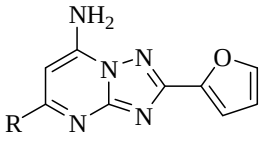


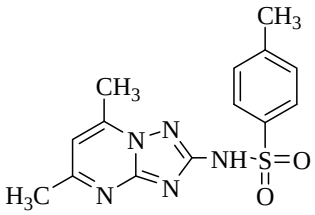
(4) 1,2,4-triazolo[4,3-*c*]pyrimidine

Among these isomeric families of compounds, 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives are thermodynamically more stable and, thus, the most studied ones [1], a few of them being commercially available. Revisions surveying the synthesis, reactivity, spectroscopic characterization and crystallographic studies of 1,2,4-triazolo[1,5-*c*]pyrimidines [2], 1,2,4-triazolo[4,3-*a*]pyrimidines [3] and 1,2,4-triazolo [4,3-*c*]pyrimidines [4] have also been published.

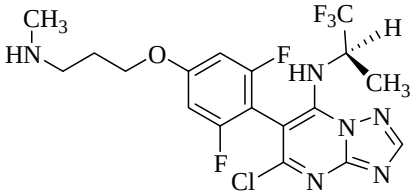
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological activities, such as antitumor potency [5, 6], inhibition of KDR kinase [7], antifungal effect [8] and macrophage activation [9]. They have proved to be promising anticancer agents with dual mechanisms of tubulin polymerization promotion [5, 6] as well as cyclin dependent kinases 2 inhibition [10]. Some examples of published derivatives of 1,2,4-triazolo[1,5-*a*]pyrimidine with their biological activities are as following.



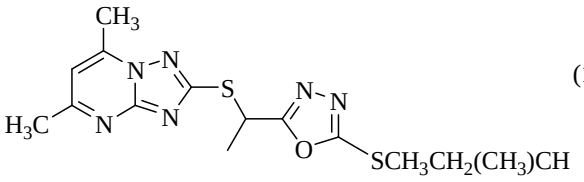
(5) Activity: A_{2A} adenosine receptor antagonists [11]



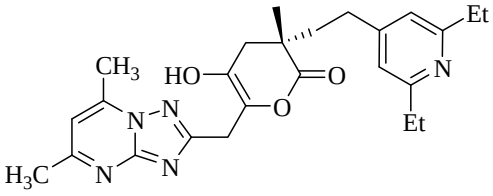
(6) Activity: latent leishmanicidal activity [12]



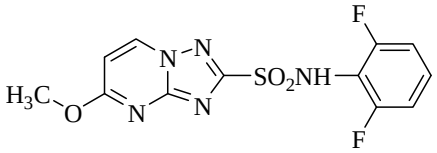
(7) Activity: Anticancer acticity [13]



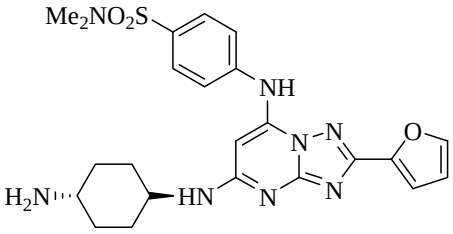
(8) Activity: antifungal activity [14]



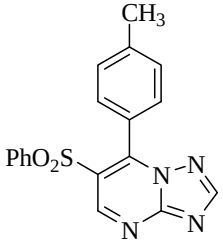
(9) Activity: Hepatitis C virus polymerase inhibitor [15]



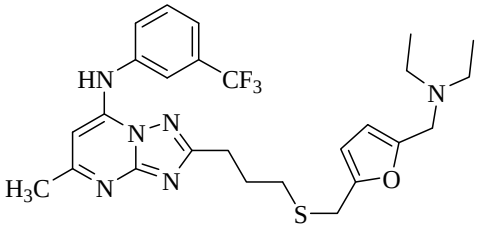
(10) Activity: Acetohydroxyacid synthase inhibitor [16]



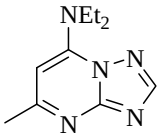
(11) Activity: CDK2 inhibitors [17]



(12) Activity: Anti-inflammatory [18]



(13) Activity: Antiproliferative activity [19]



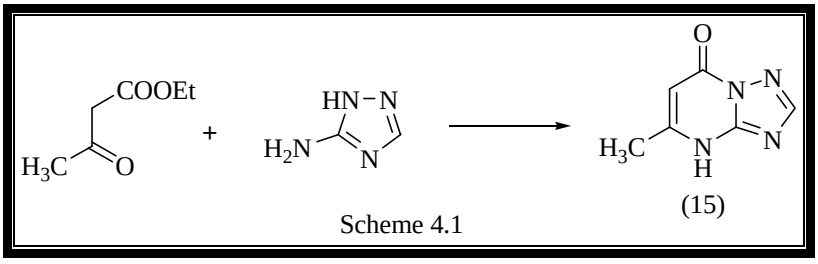
(14) Trapidil: Antiischemic and cardiatonic agent [20]

4.2 Reported synthetic strategies

4.2.1 Amino-1,2,4-triazole and 1,3-bifunctional synthons

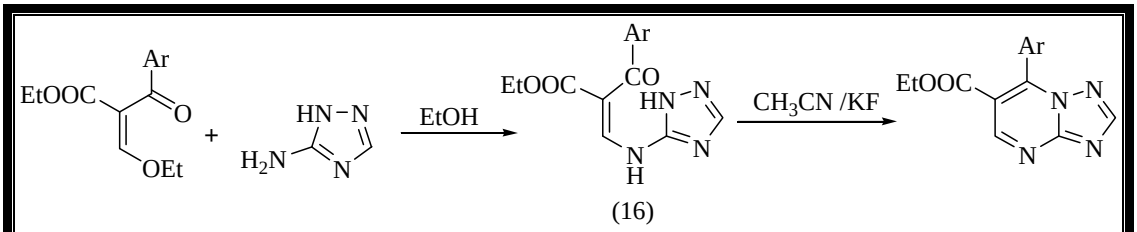
4.2.1.1 Principle and Conditions

By far the most triazolo[1,5-*a*]pyrimidine synthesis are condensations of dinucleophilic 5-amino-1,2,4-triazoles with 1,3-bifunctional synthons as shown in the formation of triazolo[1,5-*a*]pyrimidine (15) (Scheme 4.1) [21-24]. New synthetic conditions recently described involve melting under microwave irradiation, a reaction that is environmental friendly and gives higher yields than conventional heating in solvent [25]. Furthermore, certain lithium 1,3-diketonates have proven to be better synthons than the corresponding diketones [26].



Scheme 4.1

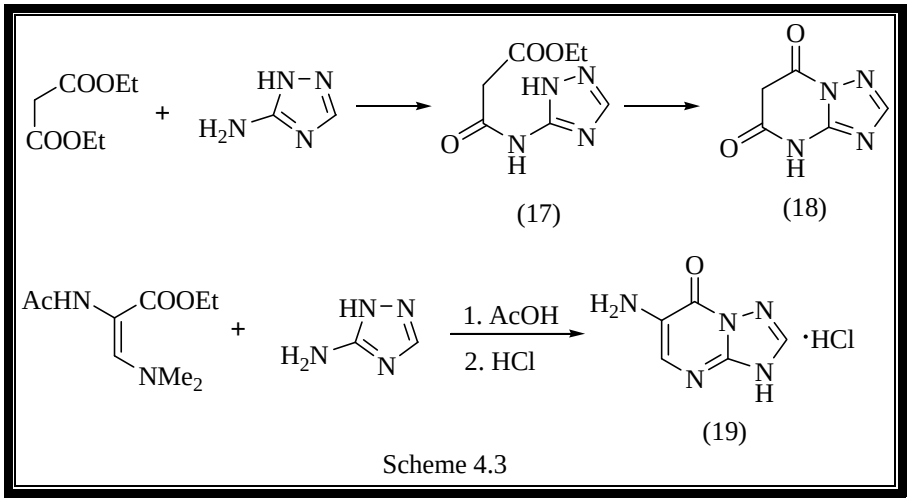
Previous mechanistic conclusions have been confirmed by isolating stable intermediate 5-amino-1,2,4-triazole derivatives such as enamine (16) (Scheme 4.2) on reacting 5-amino-1,2,4-triazoles with 3-ketovinyl ethers [27], 3-ketoenamines [28], 3-ketoaldehydes [29], enamine-2-carboxylic esters [30] or ethoxymethylene malonates [31].



Scheme 4.2

That means, the overall reaction starts with the interaction of the amino-1,2,4-triazole amino group and the enolic (or analogous) functionality of the three-carbon synthon. In the two-step examples, just mentioned, the first step proceeds under milder conditions (sometimes just in ethanol at room temperature), but the final

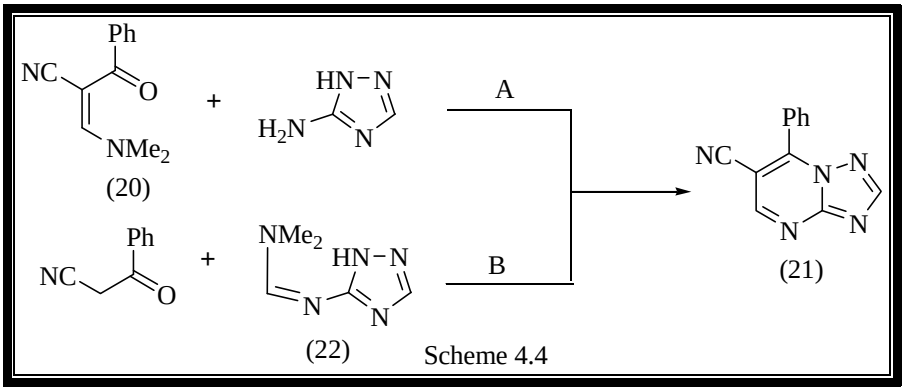
cyclization (or the one-step reaction, if the intermediate is not trapped) requires stronger means (e.g., polyphosphoric acid or boiling acetic acid). Under extreme conditions, triazolylamide (17) was subject to flash vacuum pyrolysis between 300 and 450 ºC to give about 50% triazolo[1,5-a]pyrimidine (18) (Scheme 4.3) [32]. Libraries of fused 3-aminopyrimidin-4-ones (19) and other compounds were just recently prepared by the solid-phase and by the solution-phase parallel synthesis [33]. The latter method turned out to be advantageous with respect to yield and purity.



Scheme 4.3

4.2.1.2 Use of Modified 5-Amino-1,2,4-triazoles

Scheme 4.4 shows two parallel paths of pyrimidine ring annulation: the conventional method, route A and a route B using a reactive amino-1,2,4-triazole derivative [34]. Amidine (22), formed from 5-amino-1,2,4-triazole and DMF dimethylacetal, can be regarded as the result of incorporating one carbon of the three-carbon synthon (20) into the 5-amino-1,2,4-triazole molecule; condensation with a reactive two-carbon component leads to target triazolo[1,5-*a*]pyrimidine (21).



Scheme 4.4

Path B also serves in confirming the structure of product (21). Similar syntheses of 7-aryl and 7-heterocyclyl triazolo[1,5-*a*]pyrimidines have been described [35-37], for example, that of an antipyrine derivative [38].

4.2.1.3 The diversity of 1,3-bifunctional synthons

Examples of triazolo[1,5-*a*]pyrimidine synthesis published in the relevant period are listed in Table 1, arranged according to the bifunctional synthons used and to the substituents entering the positions 5 and 7. Triazolo[1,5-*a*]pyrimidines are included in reviews dealing with heterocyclic synthesis by the use of enamines [39], enamine-2-carboxylic esters [40] and ketene mercaptals [41].

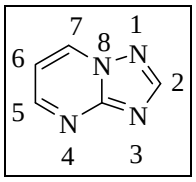


Table 1. Syntheses of triazolo[1,5-a]pyrimidines from 1,3-bifunctional synthons and 5-amino-1,2,4-triazoles

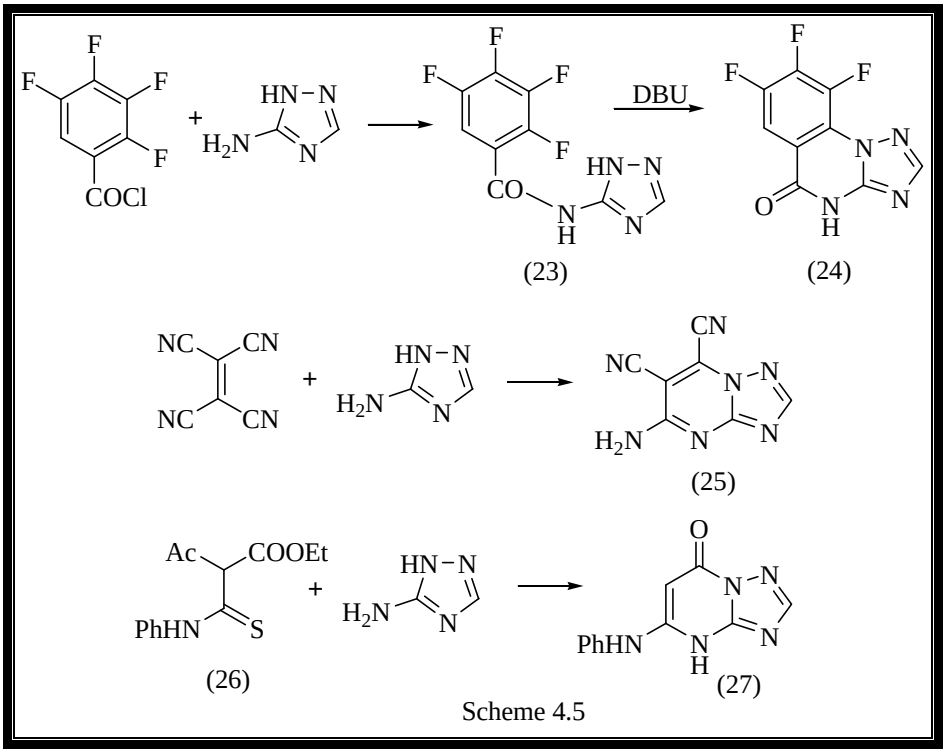
Bifunctional Synthons	R-5[b]	R-7[b]	Bifunctional Synthons	R-5[b]	R-7[b]
1,3-Dialdehyde [42]	H	H	Enamine-2-carboxylate [59]	H	OH
2-Formylacetal [43]	H	H	Acetylenedicarboxylate [60]	CO_2Me	OH
1,3-Diacetal [44]	H	H	3-Ketocarboxylate [61]	R	OH
2-Formylvinyl ether [45]	H	H	3-Alkoxyacrylate [62]	OH	R
2-Formylvinylchloride [46]	H	R	Alkoxyalkylene malonate [63]	R	OH
3-Iminiovinylchloride [47]	H	R	2-Chloroacrylate [64]	OH	R
2-Formylenamine [48]	H	R	Malonic ester [65]	OH	OH
3-Iminioenamine [49]	H	R	Malonyl chloride [66]	OH	OH
3-Ketoaldehyde [50]	R	H	2-Acylketene mercaptal [67]	SR	R’
3-Ketoacetal [51]	R	H	2-Cyanoketene mercaptal [68]	SR	NH_2
3-Ketovinyl ether [52]	H	R	Alkoxyalkylene cyanoacetate [69]	R	NH_2
3-Ketovinyl sulfone [53][c]	R	H	Alkoxyalkylene malonitrile [70]	R	NH_2
3-Ketoenamine [54]	H	R	2-Formylnitrile [71]	H	NH_2
1,3-Diketone [55]	R	R’	2-Cyanoenamine [72]	H	NH_2
3-Ketoalkyne [56]	R[d]	H	Malonitrile [73]	NH_2	NH_2
2-Formylcarboxylate [57]	R	OH	2-Thiocarbamylcarboxylate [74]	NHR	OH
2-Alkoxycarbonylacetal [58]	OH	H			

[a]or tautomeric form.
[b]Substituents on C-5 and C-7, respectively; R and R’ mean (possibly substituted) alkyl, aryl, heterocyclyl and H; OH means hydroxy or tautomeric oxo form.
[c]And regioisomeric 7-R compound.
[d]Deoxyaltrose derivative relating C-glycosides [75].

In recent years, 3-ketoenamines have growing interest as building blocks for 7-aryl triazolo[1,5-*a*]pyrimidines (Scheme 4.4, Path A) [76, 77]. They also serve to synthesize 7-heterocyclyl triazolo[1,5-*a*]pyrimidines [78, 79]. In addition to usual *N*,

N-dimethyl compounds also analogues having a free amino group can be used as in the synthesis of 7-trifluoromethyl derivatives [80]. Enaminones can be formed in situ, for instance, from dimedone and DMF dimethylacetal [81].

In the course of the cyclization of the stable tetrafluorobenzoyl derivative (23) (Scheme 4.5) fluorine at the *o*-position is involved in the reaction and is replaced to give trifluorobenzo triazolo[1,5-*a*]pyrimidine (24) [82]. Acetonyl is introduced as substituent into the 7-position by the use of triketone heptan-2,4,6-trione [83].

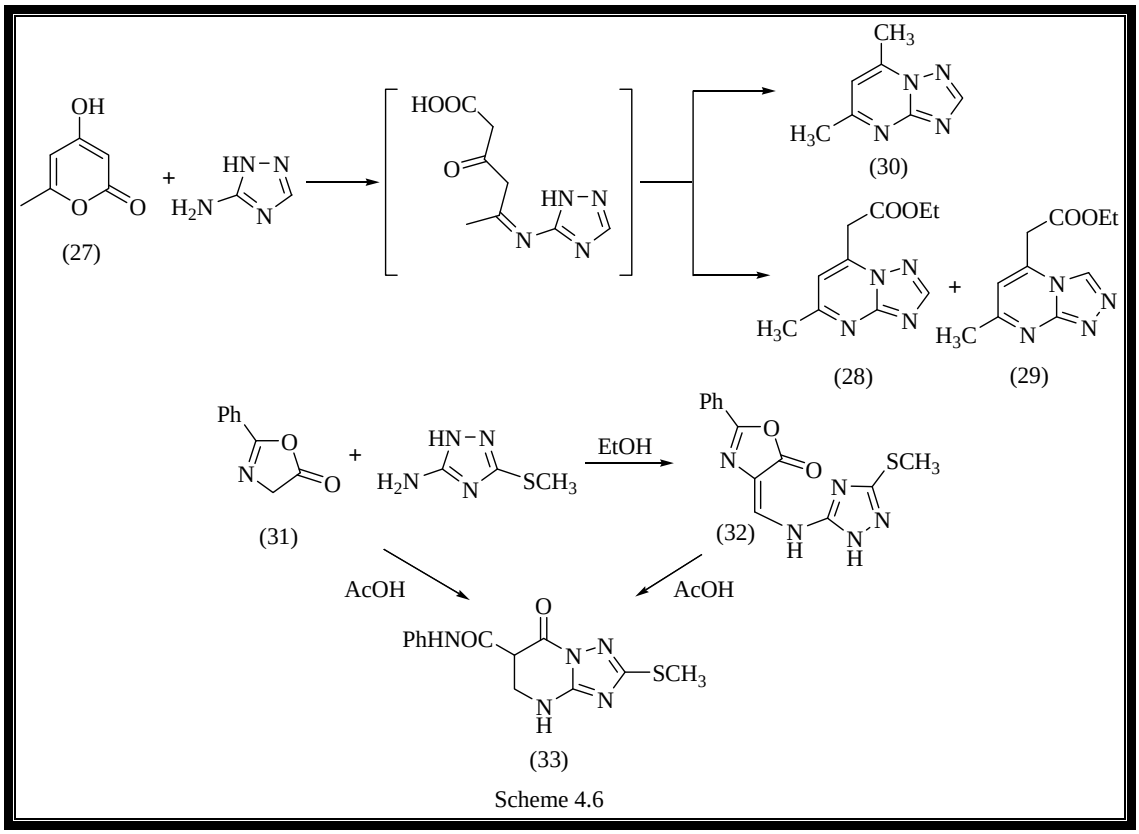


Scheme 4.5

The electron acceptor tetracyanoethylene on interaction with amino-1,2,4-triazole first forms a charge transfer complex that after loss of hydrocyanic acid is transformed into dicyano triazolo[1,5-*a*]pyrimidine (25) [84]. Fusion of 1,4-naphthoquinone or indenone onto triazolo[1,5-*a*]pyrimidine can in a similar way be performed by the use of 2,3-dicyano-1,4-naphthoquinone or dicyanomethylene indane-1,3-dione, respectively. Another indeno triazolo[1,5-*a*]pyrimidine is accessible from triketone 2-acetylindane-1,3-dione [85]. On the other hand, acetoacetic ester (26) with 5-amino-1,2,4-triazole suffers ester group cleavage to form anilino triazolo[1,5-*a*]pyrimidine (27) [86].

4.2.2 Other pyrimidine ring synthesis

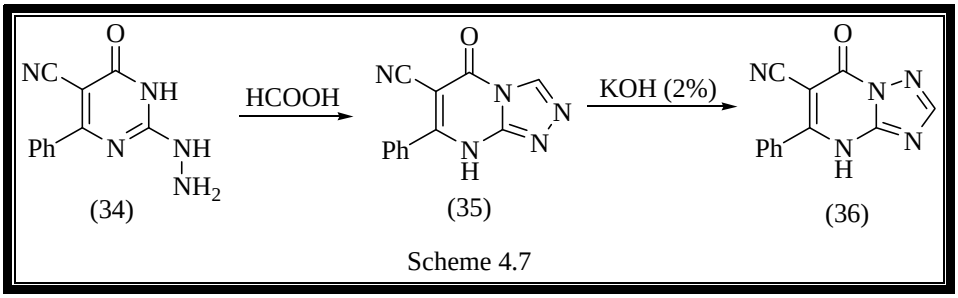
The annulation of pyrimidine onto the triazole ring can be accomplished by the use of heterocyclic precursors that can be regarded as masked 1,3-bifunctional reagents. This way, triacetic acid lactone (27) (Scheme 4.6) reacts as a masked 1,3-diketone and transforms 5-amino-1,2,4-triazole to triazolo[1,5-*a*]pyrimidine (28) together with ring isomer (29) and decarboxylation product (30) [87]. Oxazolones play a similar part [88-90]. Thus, enol ether (31) behaves as a masked 3-ethoxyacrylate and yields, through intermediate (32), benzamido triazolo1,5-*a*]pyrimidine (33) that, under harsher conditions, directly forms from compound [30].



Scheme 4.6

4.2.3 2-Hydrazinopyrimidines and one-carbon synthons

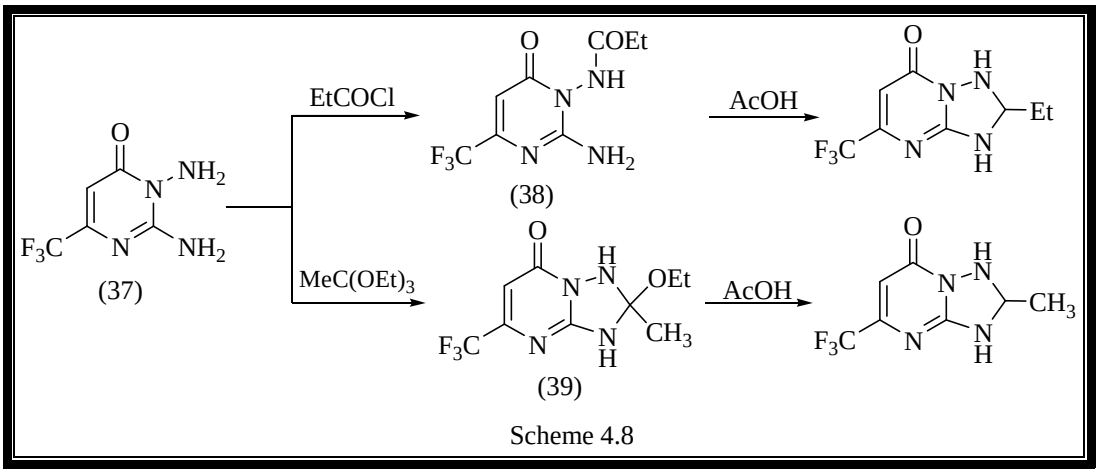
A second common triazolo[1,5-*a*]pyrimidine synthesis consists in the condensation of a C_1-synthon with a 2-hydrazinopyrimidine derivative (e.g., 34, Scheme 4.7). A triazolo[4,3-a]-pyrimidine (35) initially forms that often can be isolated [91]. Harsher conditions allow it to isomerize to the target triazolo[1,5-*a*]pyrimidine (36) by Dimroth rearrangement.



Scheme 4.7

4.2.4 Other triazole ring synthesis

Most cyclization of 2,3-diaminopyrimidones (37) [92] or corresponding quinazolones proceed with the participation of carboxylic acids or their derivatives (esters, anhydrides, chlorides, or orthoesters) as shown in Scheme 4.8. Noncyclized or saturated intermediates (38, 39) can frequently be found during synthesis of triazolo[1,5-*a*]pyrimidines.



Scheme 4.8

4.3 Current work

The biological importance of 1,2,4-triazolo[1,5-*a*]pyrimidines is well documented. Over the years, various substituted derivatives of these heterocycles have shown utility against a range of biological targets. For example, they have demonstrated activity against malaria and bronchospasm and shown activity as coronary vasodilators, antihypertensive agents, leishmanicides, antibiotics, adenosine A_{2a} antagonists, immunosuppressants, antitumor agents, fungicides, xanthine oxidase inhibitors and phosphodiesterase inhibitors.

SECTION: - A

In section A, synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidines is based on the Biginelli like cyclocondensation of aromatic aldehydes and acetoacetic acid derivatives with aminoazoles containing a guanidine fragment. There are literary data about the synthesis of triazolopyrimidines by treatment of 5-amino-1,2,4-triazole or 5-aminotetrazole with aldehydes and ethyl acetoacetate or cyclic β-diketones [93]. The cyclocondensations were realized by heating of the starting materials in ethanol with catalytic amounts of hydrochloric acid under reflux conditions [93a-c] or using DMF as solvent [93d-e]. The use of acetoacetamides in these or similar reactions has not been described.

Recognizing these facts, we have synthesised four new series of 1,2,4-triazolo[1,5-*a*]pyrimidines (CPV-201 to CPV-240) containing an acetoacetamide fragment.

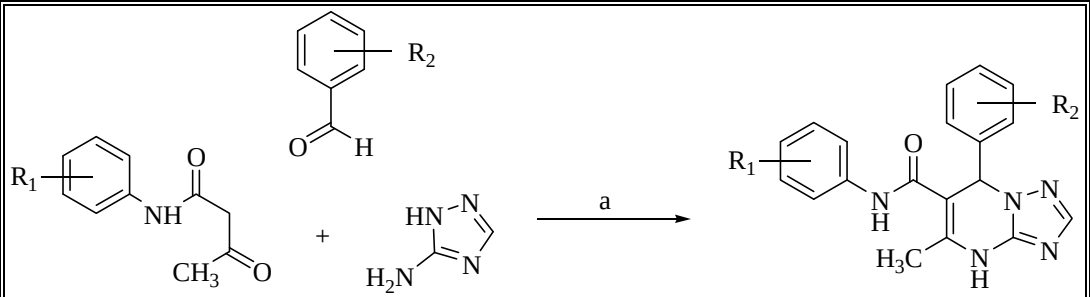
SECTION: - B

In this section B, we have synthesised another four new series of 5-substituted 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines (CPV-241 to CPV-280). The reaction is one pot cyclocondensation of aromatic aldehyde, corresponding acetophenone and 5-amino-1,2,4-triazole using glacial acetic acid as a solvent.

The structures of all the newly synthesized compounds (CPV-201 to CPV-280) were elucidated by various analytical techniques like FT-IR spectroscopy, mass spectrometry, ^{1}H NMR spectroscopy and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.

SECTION: - A

4.4 Reaction Scheme

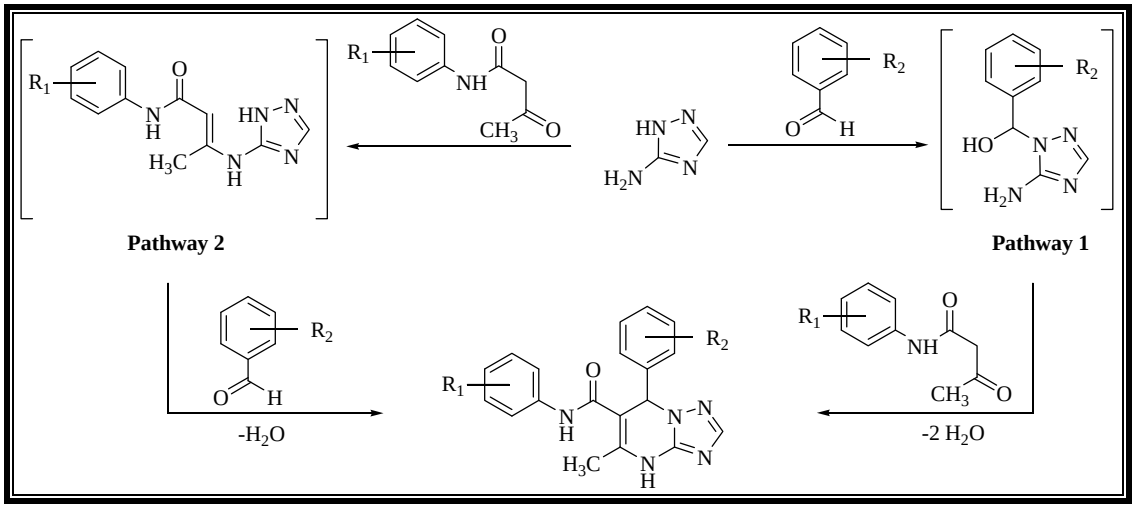


Reagents and conditions: (a) DMF, Reflux, 12-15 Minutes **CPV 201 TO 240**

Code	R_1	R_2	M.F.	M.W.	M.P. °C	Yield %	R_{f1}	R_{f2}
CPV-201	pyridin-2-yl	H	$C_{18}H_{16}N_6O$	332	189-191	76	0.54	0.72
CPV-202	pyridin-2-yl	4-CH_3	$C_{19}H_{18}N_6O$	346	179-181	74	0.53	0.69
CPV-203	pyridin-2-yl	4-OCH3	$C_{19}H_{18}N_6O_2$	362	222-224	85	0.50	0.64
CPV-204	pyridin-2-yl	4-Cl	$C_{18}H_{15}ClN_6O$	366	215-217	78	0.53	0.68
CPV-205	pyridin-2-yl	4-F	$C_{18}H_{15}FN_6O$	350	261-263	66	0.50	0.66
CPV-206	pyridin-2-yl	4-NO_2	$C_{18}H_{15}N_7O_3$	377	226-228	75	0.42	0.74
CPV-207	pyridin-2-yl	3-NO_2	$C_{18}H_{15}N_7O_3$	377	231-233	68	0.51	0.79
CPV-208	pyridin-2-yl	2-NO_2	$C_{18}H_{15}N_7O_3$	377	225-227	73	0.50	0.63
CPV-209	pyridin-2-yl	3-Cl	$C_{18}H_{15}ClN_6O$	366	212-214	81	0.43	0.62
CPV-210	pyridin-2-yl	2-Cl	$C_{18}H_{15}ClN_6O$	366	199-201	71	0.59	0.75
CPV-211	pyridin-3-yl	H	$C_{18}H_{16}N_6O$	332	256-258	75	0.52	0.68
CPV-212	pyridin-3-yl	4-CH_3	$C_{19}H_{18}N_6O$	346	202-204	80	0.56	0.65
CPV-213	pyridin-3-yl	4-OCH3	$C_{19}H_{18}N_6O_2$	362	257-259	77	0.49	0.66
CPV-214	pyridin-3-yl	4-Cl	$C_{18}H_{15}ClN_6O$	366	213-215	74	0.61	0.73
CPV-215	pyridin-3-yl	4-F	$C_{18}H_{15}FN_6O$	350	227-229	70	0.53	0.66
CPV-216	pyridin-3-yl	4-NO_2	$C_{18}H_{15}N_7O_3$	377	242-244	67	0.55	0.61
CPV-217	pyridin-3-yl	3- NO_2	$C_{18}H_{15}N_7O_3$	377	203-205	77	0.53	0.75
CPV-218	pyridin-3-yl	2-NO_2	$C_{18}H_{15}N_7O_3$	377	199-201	72	0.64	0.78
CPV-219	pyridin-3-yl	3-Cl	$C_{18}H_{15}ClN_6O$	366	207-209	68	0.45	0.63
CPV-220	pyridin-3-yl	2-Cl	$C_{18}H_{15}ClN_6O$	366	217-219	77	0.60	0.74
CPV-221	4-Cl	H	$C_{19}H_{16}ClN_5O$	365	211-213	65	0.42	0.56
CPV-222	4-Cl	4-CH_3	$C_{20}H_{18}ClN_5O$	379	233-235	70	0.52	0.62
CPV-223	4-Cl	4-OCH3	$C_{20}H_{18}ClN_5O_2$	395	241-243	83	0.50	0.69
CPV-224	4-Cl	4-Cl	$C_{19}H_{15}Cl_2N_5O$	400	235-237	78	0.57	0.75
CPV-225	4-Cl	4-F	$C_{19}H_{15}ClFN_5O$	383	248-250	70	0.50	0.58
CPV-226	4-Cl	4-NO_2	$C_{19}H_{15}ClN_6O_3$	410	236-238	65	0.43	0.54
CPV-227	4-Cl	3-NO_2	$C_{19}H_{15}ClN_6O_3$	410	239-241	64	0.51	0.66
CPV-228	4-Cl	2-NO_2	$C_{19}H_{15}ClN_6O_3$	410	218-220	72	0.52	0.65
CPV-229	4-Cl	3-Cl	$C_{19}H_{15}Cl_2N_5O$	400	255-257	67	0.56	0.60
CPV-230	4-Cl	2-Cl	$C_{19}H_{15}Cl_2N_5O$	400	220-222	63	0.45	0.63
CPV-231	3-Cl,4-F	H	$C_{19}H_{15}ClFN_5O$	383	222-224	75	0.55	0.59
CPV-232	3-Cl,4-F	4-CH_3	$C_{20}H_{17}ClFN_5O$	397	229-231	76	0.44	0.64
CPV-233	3-Cl,4-F	4-OCH3	$C_{20}H_{17}ClFN_5O_2$	413	291-293	70	0.47	0.60
CPV-234	3-Cl,4-F	4-Cl	$C_{19}H_{14}Cl_2FN_5O$	418	255-257	82	0.49	0.67
CPV-235	3-Cl,4-F	4-F	$C_{19}H_{14}ClF_2N_5O$	401	276-278	81	0.57	0.69
CPV-236	3-Cl,4-F	4-NO_2	$C_{19}H_{14}ClFN_6O_3$	428	215-217	76	0.52	0.65
CPV-237	3-Cl,4-F	3-NO_2	$C_{19}H_{14}ClFN_6O_3$	428	256-258	72	0.48	0.56
CPV-238	3-Cl,4-F	2-NO_2	$C_{19}H_{14}ClFN_6O_3$	428	275-277	78	0.46	0.59
CPV-239	3-Cl,4-F	3-Cl	$C_{19}H_{14}Cl_2FN_5O$	418	242-244	68	0.50	0.70
CPV-240	3-Cl,4-F	2-Cl	$C_{19}H_{14}Cl_2FN_5O$	418	256-258	72	0.59	0.64

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4; TLC Solvent system R_{f2}: Chloroform: Methanol - 9:1.

4.5 Plausible Reaction Mechanism



4.6 Experimental

4.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreement with the structures assigned.

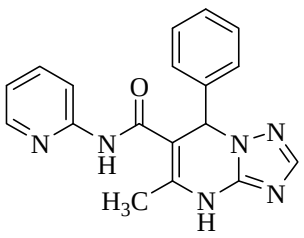
4.6.2 Synthesis of N-(aryl)-3-oxobutanamides

Synthesis of *N*-(aryl)-3-oxobutanamides was achieved using previously published methods [97].

4.6.3 General procedure for the synthesis of 4,7-dihydro-5-methyl-7-aryl-N-(pyridin-2-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (CPV 201-210)

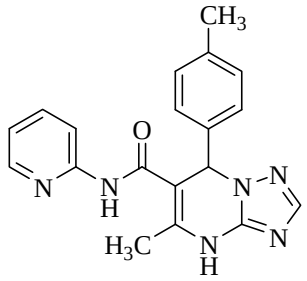
A mixture of the aminoazole (0.01 mol), 3-oxo-*N*-(pyridin-2-yl)butanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of dimethyl formamide (DMF) for 12 to 15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 201-210, which were crystallized from ethanol and subsequently dried in air.

4.6.3.1 4,7-dihydro-5-methyl-7-phenyl-N-(pyridin-2-yl)-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (CPV-201)



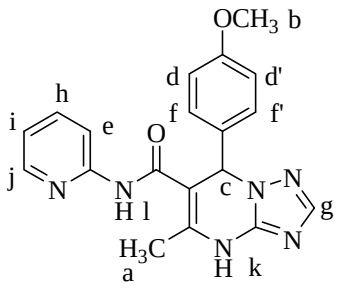
Yield: 76%; mp 189-191 ºC; MS: *m/z* 332; Anal. Calcd. for $C_{18}H_{16}N_6O$: C, 65.05; H, 4.85; N, 25.29; O, 4.81. Found: C, 64.99; H, 4.79; N, 25.22; O, 4.78%.

4.6.3.2 4,7-dihydro-5-methyl-N-(pyridin-2-yl)-7-p-tolyl-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (CPV-202)

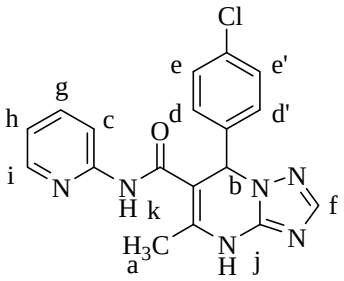


Yield: 74%; mp 179-181 ºC; MS: *m/z* 346; Anal. Calcd. for $C_{19}H_{18}N_6O$: C, 65.88; H, 5.24; N, 24.26; O, 4.62. Found: C, 65.80; H, 5.19; N, 24.17; O, 4.56%.

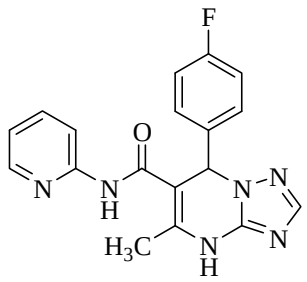
4.6.3.3 4,7-dihydro-7-(4-methoxyphenyl)-5-methyl-N-(pyridin-2-yl)[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-203)



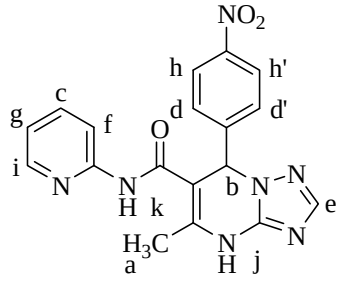
Yield: 85%; mp 222-224 ºC; IR (cm^{-1}): 3379 (N-H stretching of secondary amine), 3059 (C-H stretching of aromatic ring), 3012 (C-H symmetrical stretching of CH_3 group), 2895 (C-H asymmetrical stretching of CH_3 group), 1676 (C=O stretching of amide), 1645 (C=N stretching of triazole ring), 1552 (N-H deformation of pyrimidine ring), 1514 and 1458 (C=C stretching of aromatic ring), 1429 (C-H asymmetrical deformation of CH_3 group), 1329 (C-H symmetrical deformation of CH_3 group), 1301 (C-N stretching), 1145 (C-O-C asymmetrical stretching of ether linkage), 1064 (C-O-C symmetrical stretching of ether linkage), 1031 (C-H in plane deformation of aromatic ring), 840 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, H_a), 3.72 (s, 3H, H_b), 6.55 (s, 1H, H_c), 6.79-6.81 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 6.96-6.99 (m, 1H, H_e), 7.28-7.30 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.49 (s, 1H, H_g), 7.58-7.63 (m, 1H, H_h), 7.96-7.98 (d, 1H, H_i, J = 8.0 Hz), 8.22-8.24 (m, 1H, H_j), 9.78 (s, 1H, H_k), 10.11 (s, 1H, H_l); MS: *m/z* 362; Anal. Calcd. for $C_{19}H_{18}N_6O_2$: C, 62.97; H, 5.01; N, 23.19; O, 8.83. Found: C, 62.90; H, 4.95; N, 23.10; O, 8.76%.

4.6.3.4 7-(4-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-2-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-204) 

Yield: 78%; mp 215-217 ºC; IR (cm^{-1}): 3456 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 3012 (C-H symmetrical stretching of CH_3 group), 2922 (C-H asymmetrical stretching of CH_3 group), 1670 (C=O stretching of amide), 1591 (C=N stretching of triazole ring), 1573 (N-H deformation of pyrimidine ring), 1516 and 1492 (C=C stretching of aromatic ring), 1431 (C-H asymmetrical deformation of CH_3 group), 1334 (C-H symmetrical deformation of CH_3 group), 1300 (C-N stretching), 1087 (C-H in plane deformation of aromatic ring), 831 (C-H out of plane deformation of 1,4-disubstitution), 771 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, H_a), 6.61 (s, 1H, H_b), 6.97-7.00 (m, 1H, H_c), 7.24-7.26 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.31-7.33 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.52 (s, 1H, H_f), 7.59-7.64 (m, 1H, H_g), 7.96-7.98 (d, 1H, H_h, J = 8.0 Hz), 8.24-8.25 (d, 1H, H_i, J = 4.0 Hz), 9.85 (s, 1H, H_j), 10.20 (s, 1H, H_k); MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.87; H, 4.05; N, 22.85; O, 4.30%.

4.6.3.5 7-(4-fluorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-2-yl)[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-205) 

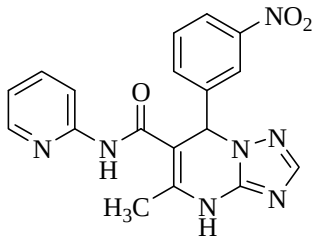
Yield: 66%; mp 261-263 ºC; MS: *m/z* 350; Anal. Calcd. for $C_{18}H_{15}FN_6O$: C, 61.71; H, 4.32; N, 23.99; O, 4.57. Found: C, 61.63; H, 4.26; N, 23.89; O, 4.53%.

4.6.3.6 4,7-dihydro-5-methyl-7-(4-nitrophenyl)-N-(pyridin-2-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-206) 

Yield: 75%; mp 226-228 ºC; IR (cm^{-1}): 3273 (N-H stretching of secondary amine), 3095 (C-H stretching of aromatic ring), 3020 (C-H symmetrical stretching of CH_3 group), 2916 (C-H asymmetrical stretching of CH_3 group), 1670 (C=O stretching of amide),

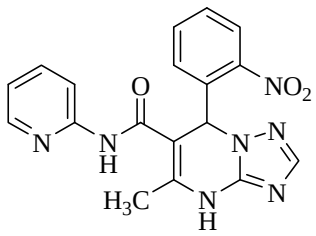
1622 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1521 (C-NO_2 stretching), 1473 (C=C stretching of aromatic ring), 1431 (C-H asymmetrical deformation of CH_3 group), 1346 (C-H symmetrical deformation of CH_3 group), 1315 (C-N stretching), 1240 (C-H in plane deformation of aromatic ring), 823 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, H_a), 6.75 (s, 1H, H_b), 6.97-7.00 (m, 1H, H_c), 7.53-7.63 (m, 4H, H_{d-f}), 7.90-7.97 (m, 1H, H_g), 8.12-8.14 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 8.24-8.25 (d, 1H, H_i, J = 4.0 Hz), 10.10 (s, 1H, H_j), 10.33 (s, 1H, H_k); MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.20; H, 3.94; N, 25.93; O, 12.66%.

4.6.3.7 4,7-dihydro-5-methyl-7-(3-nitrophenyl)-N-(pyridin-2-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-207)



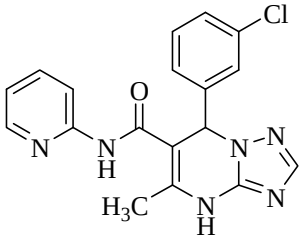
Yield: 68%; mp 231-233 ºC; MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.22; H, 3.91; N, 25.91; O, 12.62%.

4.6.3.8 4,7-dihydro-5-methyl-7-(2-nitrophenyl)-N-(pyridin-2-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-208)



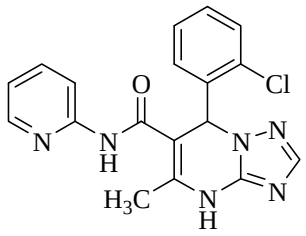
Yield: 73%; mp 225-227 ºC; MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.26; H, 3.96; N, 25.92; O, 12.67%.

4.6.3.9 7-(3-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-2-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-209)



Yield: 81%; mp 212-214 ºC; MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.89; H, 4.05; N, 22.83; O, 4.32%.

4.6.3.10 7-(2-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-2-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-210)



Yield: 71%; mp 199-201 ºC; MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.85; H, 4.07; N, 22.85; O, 4.30%.

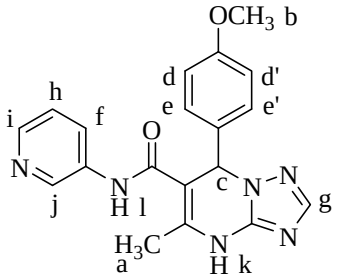
4.6.4 General procedure for the synthesis of 4,7-dihydro-5-methyl-7-aryl-N-(pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (CPV 211-220)

A mixture of the 5-amino-1,2,4-triazole (0.01 mol), 3-oxo-N-(pyridin-3-yl)butanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of dimethyl formamide (DMF) for 12 to 15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 211-220, which were crystallized from ethanol and subsequently dried in air.

4.6.4.1 4,7-dihydro-5-methyl-7-phenyl-N-(pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-211) Yield: 75%; mp 256-258 ºC; MS: *m/z* 332; Anal. Calcd. for $C_{18}H_{16}N_6O$: C, 65.05; H, 4.85; N, 25.29; O, 4.81. Found: C, 64.99; H, 4.80; N, 25.20; O, 4.75%.

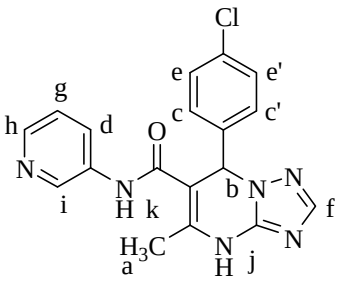
4.6.4.2 4,7-dihydro-5-methyl-N-(pyridin-3-yl)-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-212) Yield: 80%; mp 202-204 ºC; MS: *m/z* 346; Anal. Calcd. for $C_{19}H_{18}N_6O$: C, 65.88; H, 5.24; N, 24.26; O, 4.62. Found: C, 65.92; H, 5.18; N, 24.20; O, 4.55%.

4.6.4.3 4,7-dihydro-7-(4-methoxyphenyl)-5-methyl-N-(pyridin-3-yl)[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-213)



Yield: 77%; mp 257-259 ºC; IR (cm^{-1}): 3159 (N-H stretching of secondary amine), 3099 (C-H stretching of aromatic ring), 3041 (C-H symmetrical stretching of CH_3 group), 2897 (C-H asymmetrical stretching of CH_3 group), 1664 (C=O stretching of amide), 1593 (C=N stretching of triazole ring), 1533 (N-H deformation of pyrimidine ring), 1512 and 1481 (C=C stretching of aromatic ring), 1417 (C-H asymmetrical deformation of CH_3 group), 1329 (C-H symmetrical deformation of CH_3 group), 1284 (C-N stretching), 1149 (C-O-C asymmetrical stretching of ether linkage), 1093 (C-O-C symmetrical stretching of ether linkage), 1035 (C-H in plane deformation of aromatic ring), 833 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 3H, H_a), 3.71 (s, 3H, H_b), 6.56 (s, 1H, H_c), 6.80-6.82 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.19-7.22 (m, 3H, $H_{e\text{-}f}$), 7.52 (s, 1H, H_g), 7.95-7.97 (d, 1H, H_h, J = 8.0 Hz), 8.19-8.20 (d, 1H, H_i, J = 4.0 Hz), 8.68 (s, 1H, H_j), 9.80 (s, 1H, H_k), 10.17 (s, 1H, H_l); MS: *m/z* 362; Anal. Calcd. for $C_{19}H_{18}N_6O_2$: C, 62.97; H, 5.01; N, 23.19; O, 8.83. Found: C, 62.90; H, 4.95; N, 23.10; O, 8.79%.

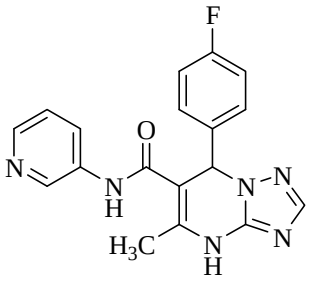
4.6.4.4 7-(4-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-214)



Yield: 74%; mp 213-215 ºC; IR (cm^{-1}): 3103 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 3005 (C-H symmetrical stretching of CH_3 group), 2974 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1595 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1535 and 1489 (C=C stretching of aromatic ring), 1419 (C-H asymmetrical deformation of CH_3 group), 1390 (C-H symmetrical deformation of CH_3 group), 1329 (C-N stretching), 1089 (C-H in plane deformation of aromatic ring), 837 (C-H out of plane deformation of 1,4-disubstitution), 771 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H, H_a), 6.60 (s, 1H, H_b), 7.21-7.27 (m, 3H, $H_{c\text{-}d}$), 7.31-7.33 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.58 (s, 1H, H_f), 7.93-7.95 (d, 1H, H_g, J = 8.0 Hz), 8.18-8.21 (m,

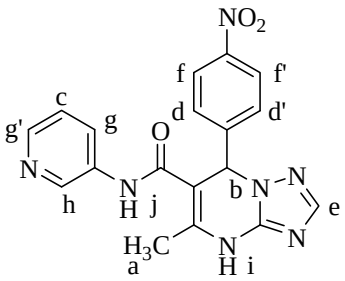
1H, H_h), 8.66-8.67 (d, 1H, H_i, J = 4.0 Hz), 9.88 (s, 1H, H_j), 10.32 (s, 1H, H_k); MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.89; H, 4.08; N, 22.88; O, 4.30%.

4.6.4.5 7-(4-fluorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-215)



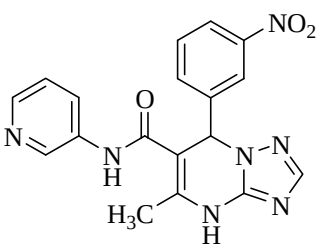
Yield: 70%; mp 227-229 ºC; MS: *m/z* 350; Anal. Calcd. for $C_{18}H_{15}FN_6O$: C, 61.71; H, 4.32; N, 23.99; O, 4.57. Found: C, 61.66; H, 4.28; N, 23.90; O, 4.51%.

4.6.4.6 4,7-dihydro-5-methyl-7-(4-nitrophenyl)-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-216)



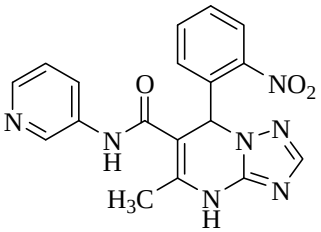
Yield: 67%; mp 242-244 ºC; IR (cm^{-1}): 3205 (N-H stretching of secondary amine), 3101 (C-H stretching of aromatic ring), 3028 (C-H symmetrical stretching of CH_3 group), 2902 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1587 (C=N stretching of triazole ring), 1533 (N-H deformation of pyrimidine ring), 1518 and 1479 (C=C stretching of aromatic ring), 1423 (C-H asymmetrical deformation of CH_3 group), 1384 (C-H symmetrical deformation of CH_3 group), 1350 (C-NO_2 stretching), 1292 (C-N stretching), 1105 (C-H in plane deformation of aromatic ring), 831 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29 (s, 3H, H_a), 6.77 (s, 1H, H_b), 7.18-7.22 (m, 1H, H_c), 7.50-7.52 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.56 (s, 1H, H_e), 7.95-7.97 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 8.14-8.16 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 8.21-8.24 (m, 1H, H_h), 8.67 (s, 1H, H_i), 9.85 (s, 1H, H_j); MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.20; H, 3.96; N, 25.92; O, 12.66%.

4.6.4.7 4,7-dihydro-5-methyl-7-(3-nitrophenyl)-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-217)



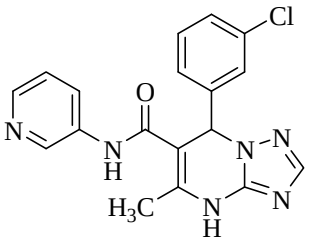
Yield: 77%; mp 203-205 ºC; MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.18; H, 3.97; N, 25.94; O, 12.65%.

4.6.4.8 4,7-dihydro-5-methyl-7-(2-nitrophenyl)-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-218)



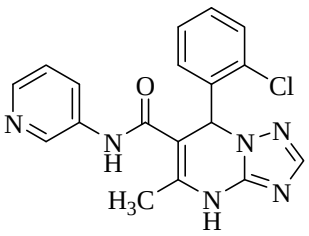
Yield: 72%; mp 199-201 ºC; MS: *m/z* 377; Anal. Calcd. for $C_{18}H_{15}N_7O_3$: C, 57.29; H, 4.01; N, 25.98; O, 12.72. Found: C, 57.22; H, 3.94; N, 25.92; O, 12.68%.

4.6.4.9 7-(3-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-219)



Yield: 68%; mp 207-209 ºC; MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.88; H, 4.02; N, 22.88; O, 4.29%.

4.6.4.10 7-(2-chlorophenyl)-4,7-dihydro-5-methyl-N-(pyridin-3-yl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-220)

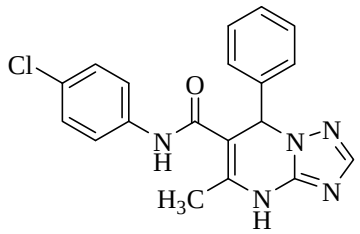


Yield: 77%; mp 217-219 ºC; MS: *m/z* 366; Anal. Calcd. for $C_{18}H_{15}ClN_6O$: C, 58.94; H, 4.12; N, 22.91; O, 4.36. Found: C, 58.87; H, 4.05; N, 22.85; O, 4.30%.

4.6.5 General procedure for the synthesis of N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-aryl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV 221-230)

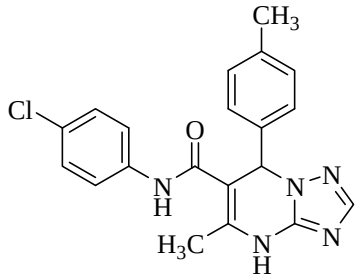
A mixture of the 5-amino-1,2,4-triazole (0.01 mol), N-(4-chlorophenyl)-3-oxobutanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of dimethyl formamide (DMF) for 12 to 15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 221-230, which were crystallized from ethanol and subsequently dried in air.

4.6.5.1 N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-221)



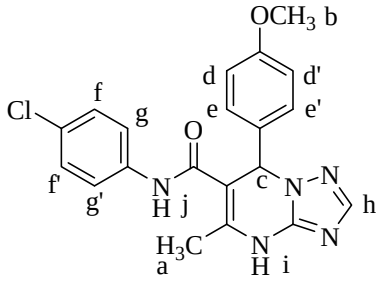
Yield: 65%; mp 211-213 ºC; MS: *m/z* 365; Anal. Calcd. for $C_{19}H_{16}ClN_5O$: C, 62.38; H, 4.41; N, 19.14; O, 4.37; Found: C, 62.30; H, 4.34; N, 19.10; O, 4.30%.

4.6.5.2 N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-222)



Yield: 70%; mp 233-235 ºC; MS: *m/z* 379; Anal. Calcd. for $C_{20}H_{18}ClN_5O$: C, 63.24; H, 4.78; N, 18.44; O, 4.21; Found: C, 63.19; H, 4.69; N, 18.39; O, 4.16%.

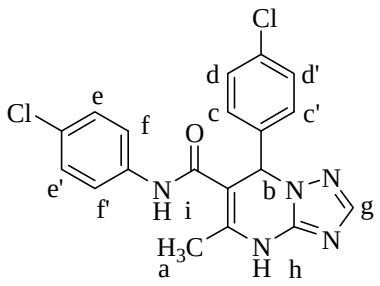
4.6.5.3 N-(4-chlorophenyl)-4,7-dihydro-7-(4-methoxyphenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-223)



Yield: 83%; mp 241-243 ºC; IR (cm^{-1}): 3261 (N-H stretching of secondary amine), 3097 (C-H stretching of aromatic ring), 3028 (C-H symmetrical stretching of CH_3 group), 2966 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of

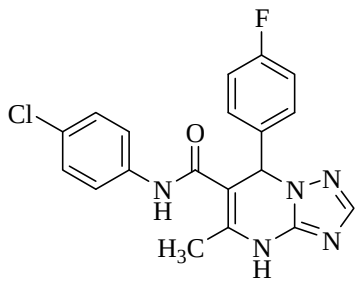
amide), 1595 (C=N stretching of triazole ring), 1556 (N-H deformation of pyrimidine ring), 1512 and 1460 (C=C stretching of aromatic ring), 1394 (C-H asymmetrical deformation of CH_3 group), 1352 (C-H symmetrical deformation of CH_3 group), 1327 (C-N stretching), 1153 (C-H in plane deformation of aromatic ring), 1091 (C-O-C asymmetrical stretching of ether linkage), 1031 (C-O-C symmetrical stretching of ether linkage), 831 (C-H out of plane deformation of 1,4-disubstitution), 786 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.17 (s, 3H, H_a), 3.68 (s, 3H, H_b), 6.49 (s, 1H, H_c), 6.83-6.85 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.13-7.15 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.29-7.31 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.54-7.56 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 7.60-7.62 (s, 1H, H_h), 9.83 (s, 1H, H_i), 10.22 (s, 1H, H_j): MS: *m/z* 395; Anal. Calcd. for $C_{20}H_{18}ClN_5O_2$: C, 60.68; H, 4.58; N, 17.69; O, 8.08. Found: C, 60.62; H, 4.50; N, 17.61; O, 8.00%.

4.6.5.4 N,7-bis(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-224)



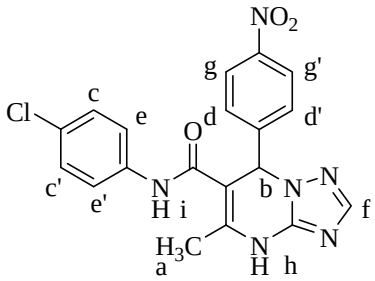
Yield: 78%; mp 235-237 ºC; IR (cm^{-1}): 3265 (N-H stretching of secondary amine), 3101 (C-H stretching of aromatic ring), 3026 (C-H symmetrical stretching of CH_3 group), 2895 (C-H asymmetrical stretching of CH_3 group), 1664 (C=O stretching of amide), 1591 (C=N stretching of triazole ring), 1554 (N-H deformation of pyrimidine ring), 1514 and 1492 (C=C stretching of aromatic ring), 1396 (C-H asymmetrical deformation of CH_3 group), 1325 (C-H symmetrical deformation of CH_3 group), 1247 (C-N stretching), 1089 (C-H in plane deformation of aromatic ring), 825 (C-H out of plane deformation of 1,4-disubstitution), 781 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.17 (s, 3H, H_a), 6.55 (s, 1H, H_b), 7.22-7.24 (d, 2H, $H_{cc'}$, J = 8.0 Hz), 7.30-7.32 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.37-7.39 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.53-7.55 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.67 (s, 1H, H_g), 9.89 (s, 1H, H_h), 10.36 (s, 1H, H_i); MS: *m/z* 400; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5O$: C, 57.01; H, 3.78; N, 17.50; O, 4.00. Found: C, 56.94; H, 3.72; N, 17.45; O, 3.96%.

4.6.5.5 N-(4-chlorophenyl)-7-(4-fluorophenyl)-4,7-dihydro-5-methyl-[1,2,4] triazolo[1,5-a]pyrimidine-6carboxamide (CPV-225)



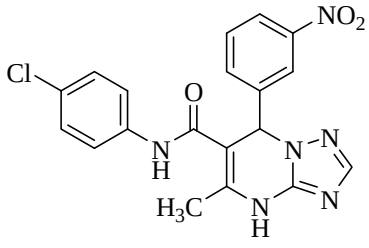
Yield: 70%; mp 248-250 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25; O, 4.17. Found: C, 59.37; H, 3.91; N, 18.20; O, 4.11%.

4.6.5.6 N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-(4-nitrophenyl)-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-226)



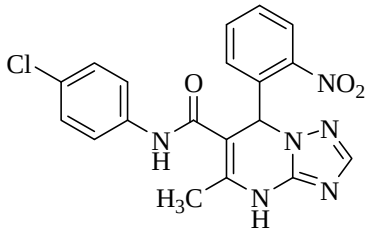
Yield: 65%; mp 236-238 ºC; IR (cm^{-1}): 3257 (N-H stretching of secondary amine), 3095 (C-H stretching of aromatic ring), 2982 (C-H symmetrical stretching of CH_3 group), 2872 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1622 (N-H deformation of pyrimidine ring), 1595 (C=N stretching of triazole ring), 1521 and 1494 (C=C stretching of aromatic ring), 1396 (C-H asymmetrical deformation of CH_3 group), 1348 (C-H symmetrical deformation of CH_3 group), 1325 (C-NO_2 stretching), 1244 (C-N stretching), 1190 (C-H in plane deformation of aromatic ring), 819 (C-H out of plane deformation of 1,4-disubstitution), 740 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.18 (s, 3H, H_a), 6.68 (s, 1H, H_b), 7.29-7.31 (d, 2H, $H_{cc'}$, J = 8.0 Hz), 7.46-7.48 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.52-7.54 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.70 (s, 1H, H_f), 8.17-8.19 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 9.92 (s, 1H, H_h), 10.47 (s, 1H, H_i); MS: *m/z* 410; Anal. Calcd. for $C_{19}H_{15}ClN_6O_3$: C, 55.55; H, 3.68; N, 20.46; O, 11.68. Found: C, 55.49; H, 3.62; N, 20.40; O, 11.61%.

4.6.5.7 N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-(3-nitrophenyl)-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-227)



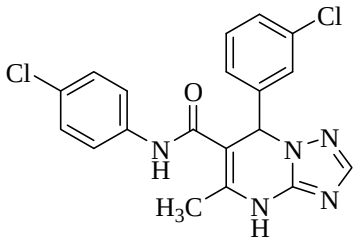
Yield: 64%; mp 239-241 ºC; MS: *m/z* 410; Anal. Calcd. for $C_{19}H_{15}ClN_6O_3$: C, 55.55; H, 3.68; Cl, 8.63; N, 20.46; O, 11.68. Found: C, 55.47; H, 3.60; N, 20.37; O, 11.61%.

4.6.5.8 N-(4-chlorophenyl)-4,7-dihydro-5-methyl-7-(2-nitrophenyl)-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-228)



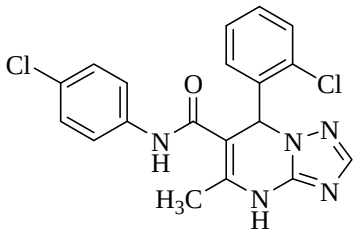
Yield: 72%; mp 218-220 ºC; MS: *m/z* 410; Anal. Calcd. for $C_{19}H_{15}ClN_6O_3$: C, 55.55; H, 3.68; N, 20.46; O, 11.68. Found: C, 55.45; H, 3.60; N, 20.39; O, 11.63%.

4.6.5.9 7-(3-chlorophenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-229)



Yield: 67%; mp 255-257 ºC; MS: *m/z* 400; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5O$: C, 57.01; H, 3.78; N, 17.50; O, 4.00. Found: C, 56.93; H, 3.73; N, 17.44; O, 3.92%.

4.6.5.10 7-(2-chlorophenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-230)



Yield: 63%; mp 220-222 ºC; MS: *m/z* 400; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5O$: C, 57.01; H, 3.78; N, 17.50; O, 4.00. Found: C, 56.95; H, 3.70; N, 17.44; O, 3.90%.

4.6.6 General procedure for the synthesis of N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-aryyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (CPV 231-240)

A mixture of the 5-amino-1,2,4-triazole (0.01 mol), *N*-(2-flourophenyl)-3-oxobuta namide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of dimethyl formamide (DMF) for 12 to 15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 231-240, which were crystallized from ethanol and subsequently dried in air.

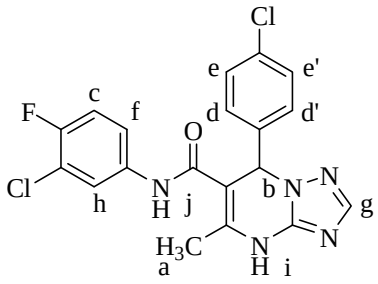
4.6.6.1 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-phenyl-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-231) Yield: 65%; mp 222-224 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25; O, 4.17. Found: C, 59.40; H, 3.89; N, 18.20; O, 4.10%.

4.6.6.2 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-p-tolyl-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (CPV-232) Yield: 76%; mp 229-231 ºC; MS: *m/z* 397; Anal. Calcd. for $C_{20}H_{17}ClFN_5O$: C, 60.38; H, 4.31; N, 17.60; O, 4.02. Found: C, 60.30; H, 4.24; N, 17.50; O, 3.92%.

4.6.6.3 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-7-(4-methoxyphenyl)-5-methyl [1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-233) Yield: 70%; mp 291-293 ºC; IR (cm^{-1}): 3281 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 3009 (C-H symmetrical stretching of CH_3 group), 2972 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1591 (C=N stretching of triazole ring), 1558 (N-H deformation of pyrimidine ring), 1541 and 1518 (C=C stretching of aromatic ring), 1438 (C-H asymmetrical deformation of CH_3 group), 1388 (C-H symmetrical deformation of CH_3 group), 1251 (C-N stretching), 1213 (C-O-C asymmetrical stretching of ether linkage), 1149 (C-H in plane deformation of aromatic ring), 1058 (C-O-C symmetrical stretching of ether linkage), 1030 (C-F stretching), 829 (C-H out of plane deformation of 1,2,4-trisubstitution), 731 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 3H, H_a), 3.72 (s, 3H, H_b), 6.55 (s, 1H, H_c), 6.79-6.81 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.05-7.09 (t, 1H, H_e), 7.19-7.21 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.39-7.43 (m, 1H, H_g), 7.51 (s, 1H, H_h), 7.79-7.82 (m, 1H, H_i), 9.70 (s, 1H, H_j), 10.10 (s, 1H, H_k); MS:

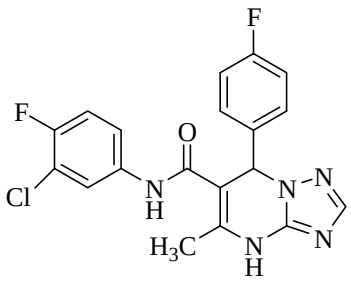
m/z 413; Anal. Calcd. for $C_{20}H_{17}ClFN_5O_2$: C, 58.05; H, 4.14; N, 16.92; O, 7.73. Found: C, 58.00; H, 4.09; N, 16.85; O, 7.67%.

4.6.6.4 N-(3-chloro-4-fluorophenyl)-7-(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-234)

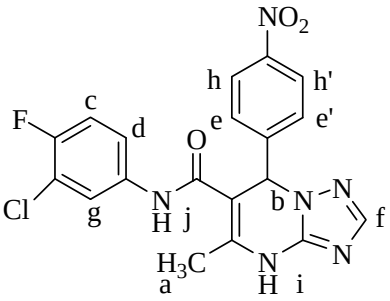


Yield: 82%; mp 255-257 ºC; IR (cm^{-1}): 3267 (N-H stretching of secondary amine), 3097 (C-H stretching of aromatic ring), 3026 (C-H symmetrical stretching of CH_3 group), 2943 (C-H asymmetrical stretching of CH_3 group), 1658 (C=O stretching of amide), 1593 (C=N stretching of triazole ring), 1633 (N-H deformation of pyrimidine ring), 1554 and 1502 (C=C stretching of aromatic ring), 1388 (C-H asymmetrical deformation of CH_3 group), 1313 (C-H symmetrical deformation of CH_3 group), 1261 (C-N stretching), 1234 (C-H in plane deformation of aromatic ring), 1089 (C-F stretching), 871 (C-H out of plane deformation of 1,2,4-trisubstitution), 686 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H, H_a), 6.59 (s, 1H, H_b), 7.09-7.14 (t, 1H, H_c), 7.23-7.31 (dd, 4H, $H_{dd'-ee'}$, J = 8.0 Hz), 7.40-7.44 (m, 1H, H_f), 7.54 (s, 1H, H_g), 7.80-7.82 (dd, 1H, H_h, J = 8.0 Hz), 9.80 (s, 1H, H_i), 10.26 (s, 1H, H_j); MS: *m/z* 418; Anal. Calcd. for $C_{19}H_{14}Cl_2FN_5O$: C, 54.56; H, 3.37; N, 16.74; O, 3.83. Found: C, 54.50; H, 3.31; N, 16.68; O, 3.77%.

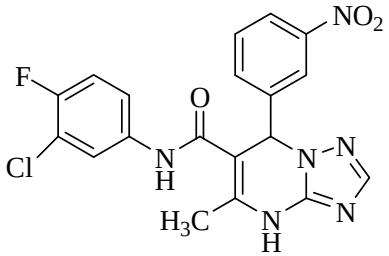
4.6.6.5 N-(3-chloro-4-fluorophenyl)-7-(4-fluorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-235)



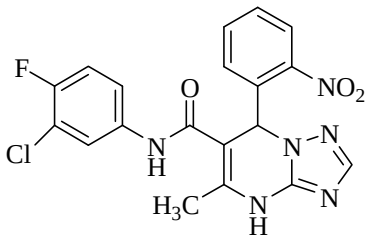
Yield: 81%; mp 276-278 ºC; MS: *m/z* 401; Anal. Calcd. for $C_{19}H_{14}ClF_2N_5O$: C, 56.80; H, 3.51; N, 17.43; O, 3.98. Found: C, 56.70; H, 3.44; N, 17.35; O, 3.92%.

4.6.6.6 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-236) 

Yield: 76%; mp 215-217 ºC; IR (cm^{-1}): 3234 (N-H stretching of secondary amine), 3099 (C-H stretching of aromatic ring), 3026 (C-H symmetrical stretching of CH_3 group), 2941 (C-H asymmetrical stretching of CH_3 group), 1664 (C=O stretching of amide), 1597 (C=N stretching of triazole ring), 1525 (N-H deformation of pyrimidine ring), 1504 and 1448 (C=C stretching of aromatic ring), 1427 (C-H asymmetrical deformation of CH_3 group), 1388 (C-H symmetrical deformation of CH_3 group), 1348 (C-N stretching), 1323 (C-NO_2 stretching), 1244 (C-H in plane deformation of aromatic ring), 1153 (C-F stretching), 866 (C-H out of plane deformation of 1,2,4-trisubstitution), 783 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 3H, H_a), 6.71 (s, 1H, H_b), 7.12-7.17 (t, 1H, H_c), 7.40-7.43 (m, 1H, H_d), 7.48-7.50 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.59 (s, 1H, H_f), 7.79-7.81 (dd, 1H, H_g, J = 8.0 Hz), 8.15-8.17 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 9.88 (s, 1H, H_i), 10.42 (s, 1H, H_j); MS: *m/z* 428; Anal. Calcd. for $C_{19}H_{14}Cl\,FN_6O_3$: C, 53.22; H, 3.29; N, 19.60; O, 11.19. Found: C, 53.16; H, 3.22; N, 19.56; O, 11.15%.

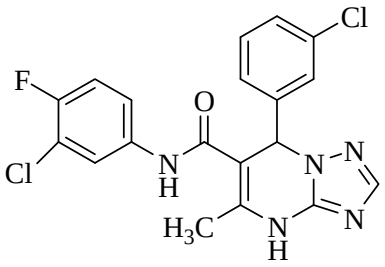
4.6.6.7 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-237) 

Yield: 72%; mp 256-258 ºC; MS: *m/z* 428; Anal. Calcd. for $C_{19}H_{14}Cl\,FN_6O_3$: C, 53.22; H, 3.29; N, 19.60; O, 11.19. Found: C, 53.14; H, 3.24; N, 19.56; O, 11.10%.

4.6.6.8 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-methyl-7-(2-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-238) 

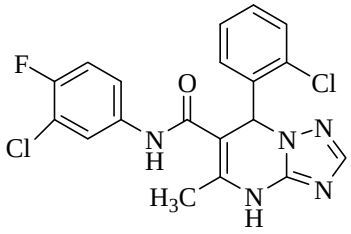
Yield: 78%; mp 275-277 ºC; MS: *m/z* 428; Anal. Calcd. for $C_{19}H_{14}Cl\,FN_6O_3$: C, 53.22; H, 3.29; N, 19.60; O, 11.19. Found: C, 53.15; H, 3.24; N, 19.56; O, 11.08%.

4.6.6.9 N-(3-chloro-4-fluorophenyl)-7-(3-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-239)



Yield: 68%; mp 242-244 ºC; MS: *m/z* 418; Anal. Calcd. for $C_{19}H_{14}Cl_2FN_5O$: C, 54.56; H, 3.37; N, 16.74; O, 3.83. Found: C, 54.51; H, 3.30; N, 16.69; O, 3.77%.

4.6.6.10 N-(3-chloro-4-fluorophenyl)-7-(2-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (CPV-240)



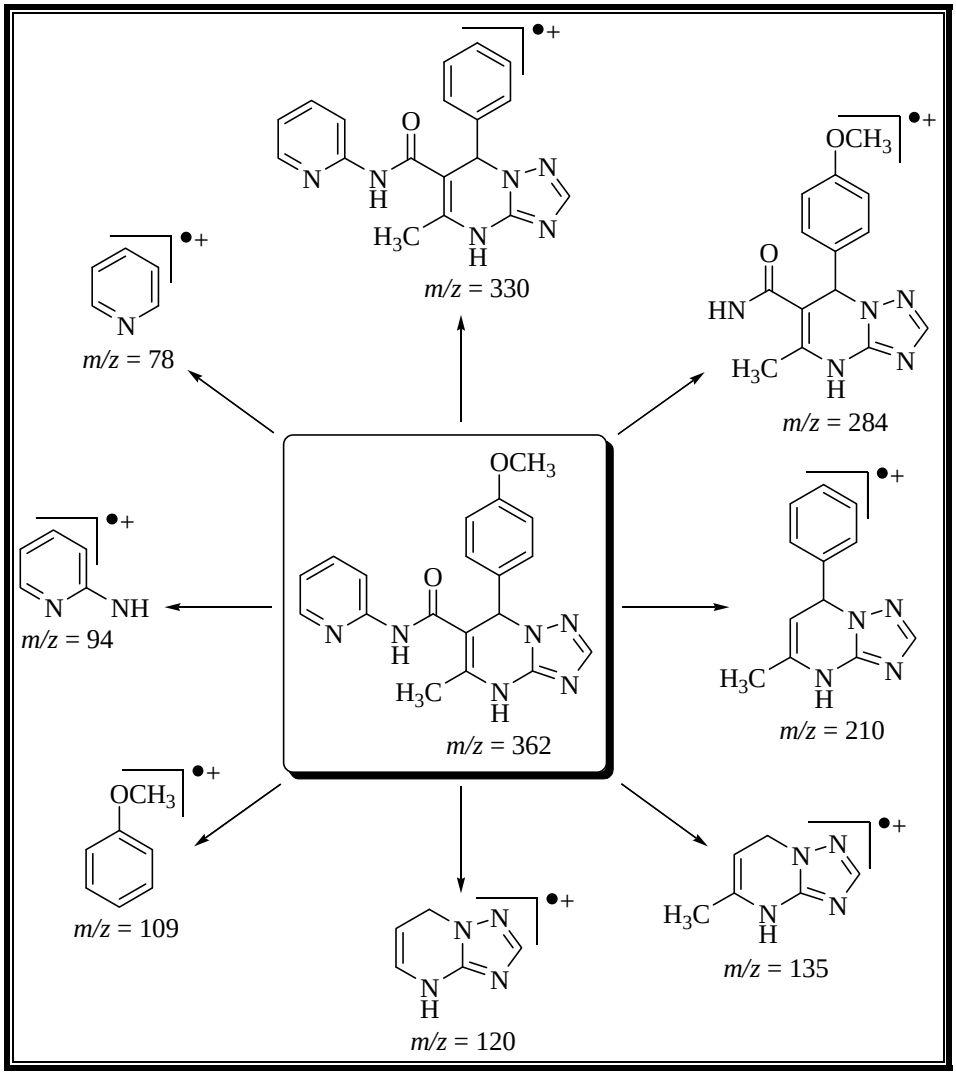
Yield: 72%; mp 256-258 ºC; MS: *m/z* 418; Anal. Calcd. for $C_{19}H_{14}Cl_2FN_5O$: C, 54.56; H, 3.37; N, 16.74; O, 3.83. Found: C, 54.50; H, 3.32; N, 16.67; O, 3.75%.

4.7 Spectral discussion

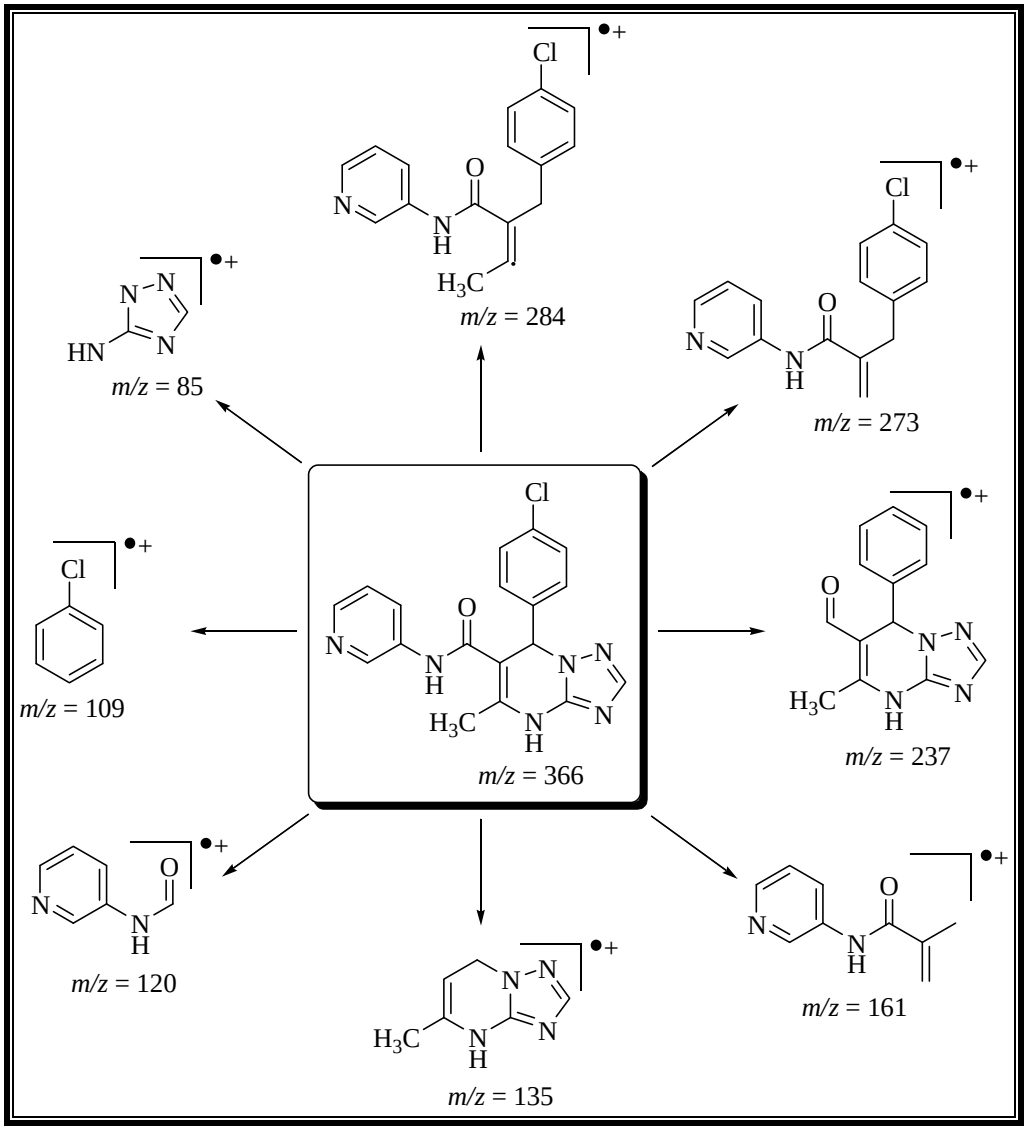
4.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

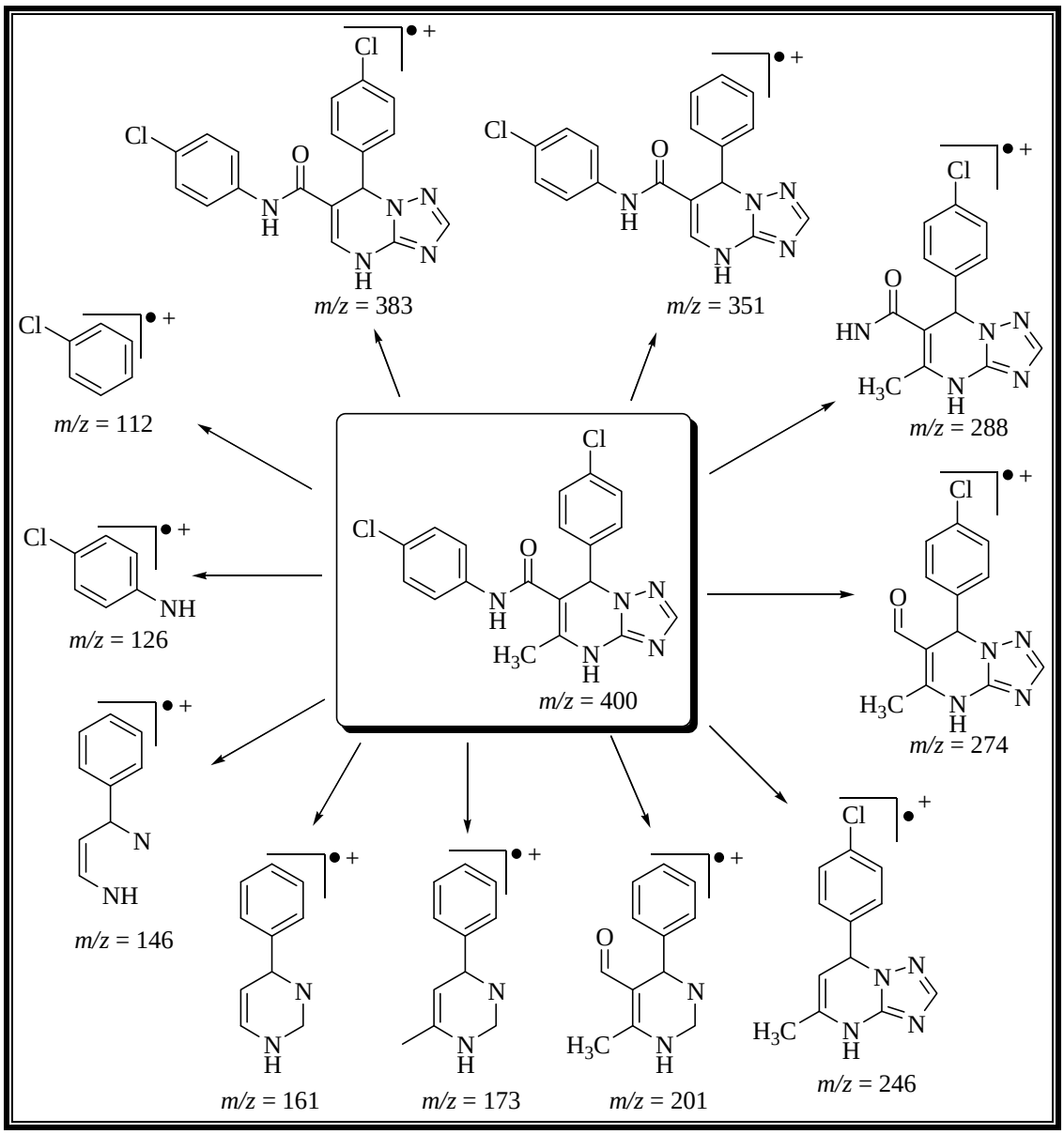
4.7.1.1 Mass fragmentation pattern for CPV-203



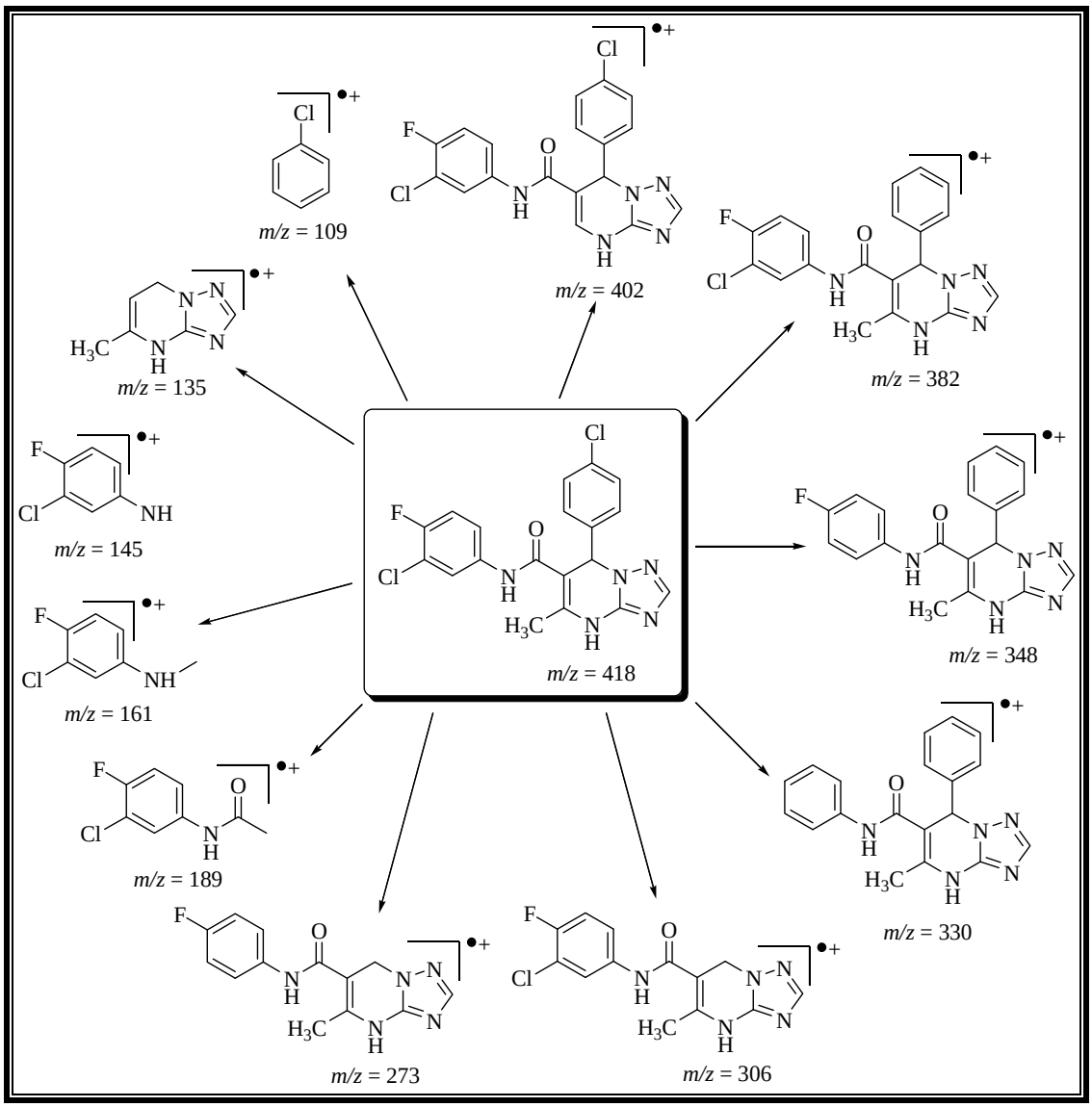
4.7.1.2 Mass fragmentation pattern for CPV-214



4.7.1.3 Mass fragmentation pattern for CPV-224



4.7.1.4 Mass fragmentation pattern for CPV-234



4.7.2 IR spectral study

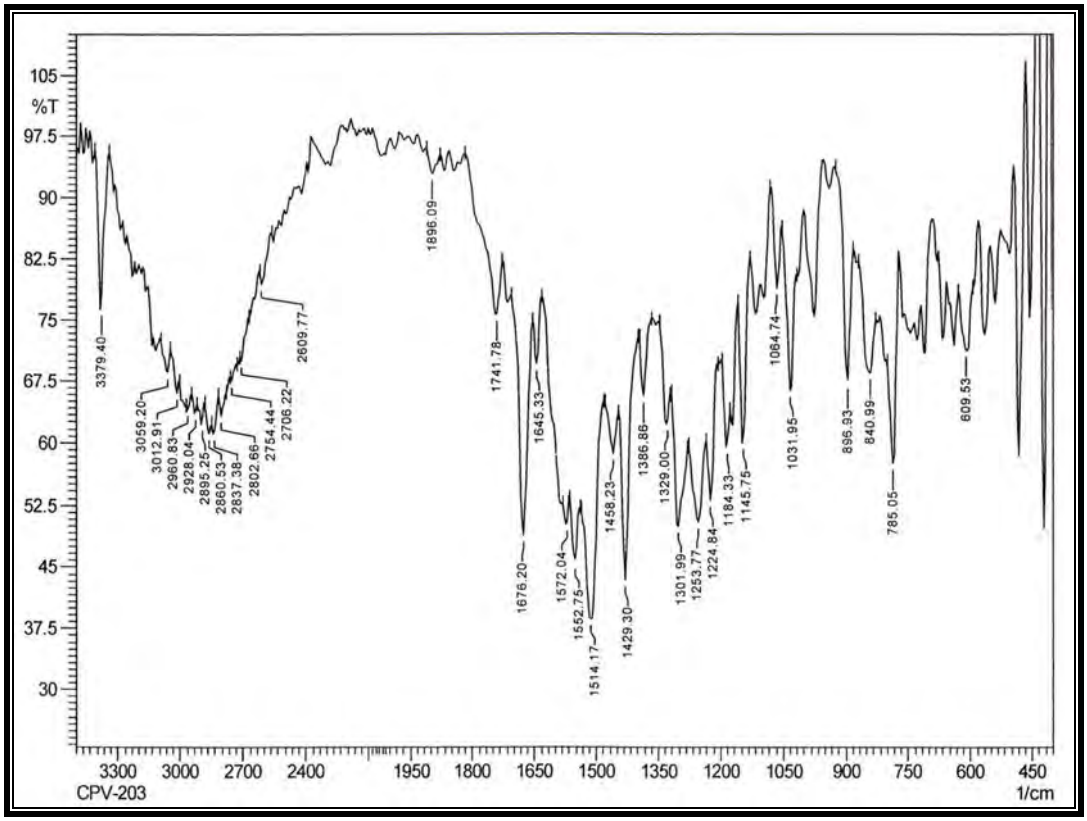
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For triazolopyrimidines CPV-201 to 240, confirmatory bands for secondary amine and amidic carbonyl groups were observed at 3103-3456 cm^{-1} and 1658-1676 cm^{-1} respectively. Another characteristic C=N stretching band of triazole ring was observed at 1587-1645 cm^{-1}, which suggested formation of desired products CPV-201 to 240.

4.7.3 ^{1}H NMR spectral study

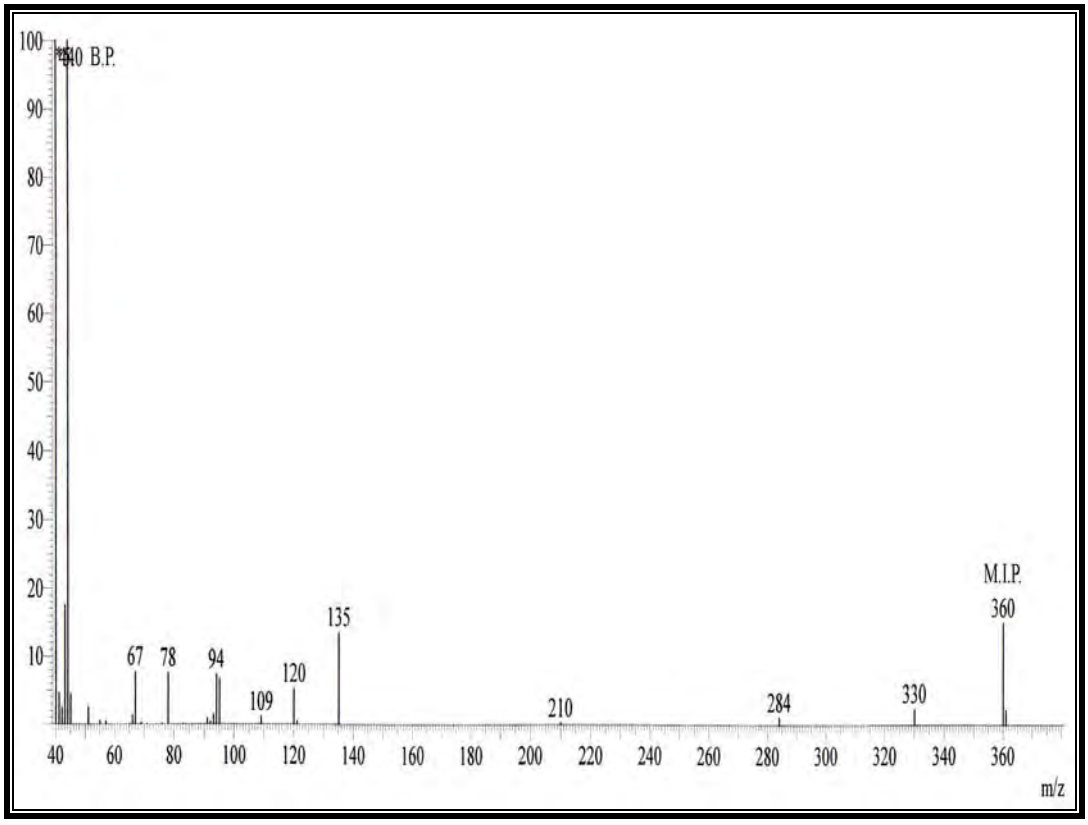
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of triazolopyrimidines CPV-201 to 240 on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 6.49-6.77 δ ppm, a singlet for the methine proton of triazole ring at 7.49-7.70 δ ppm and singlets for amino and amide group protons at 8.67-10.10 and 9.85-10.47 δ ppm, respectively. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern.

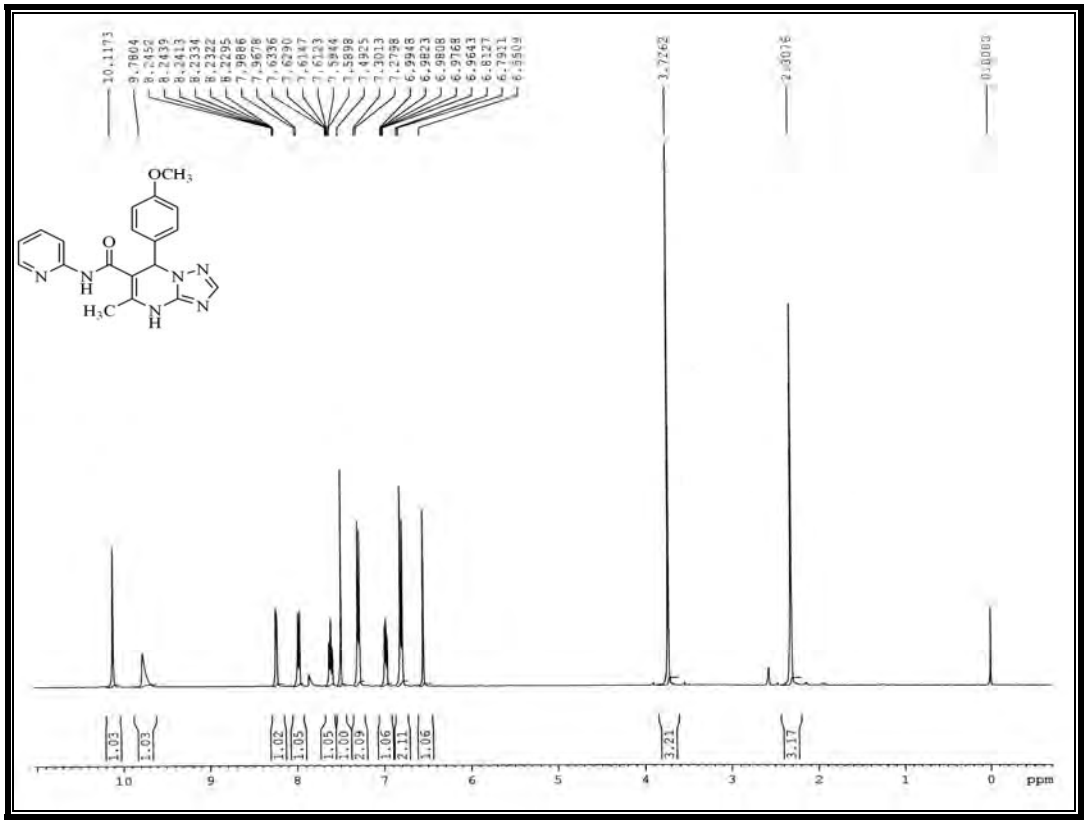
IR spectrum of CPV-203



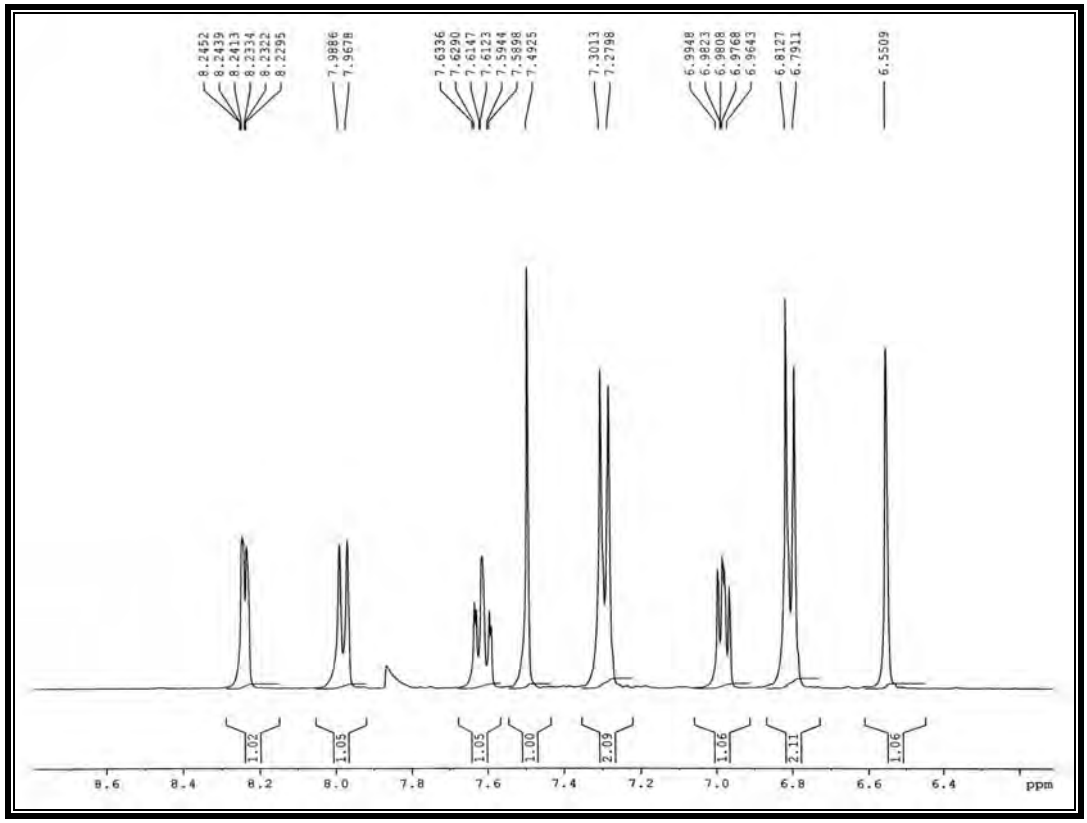
Mass spectrum of CPV-203



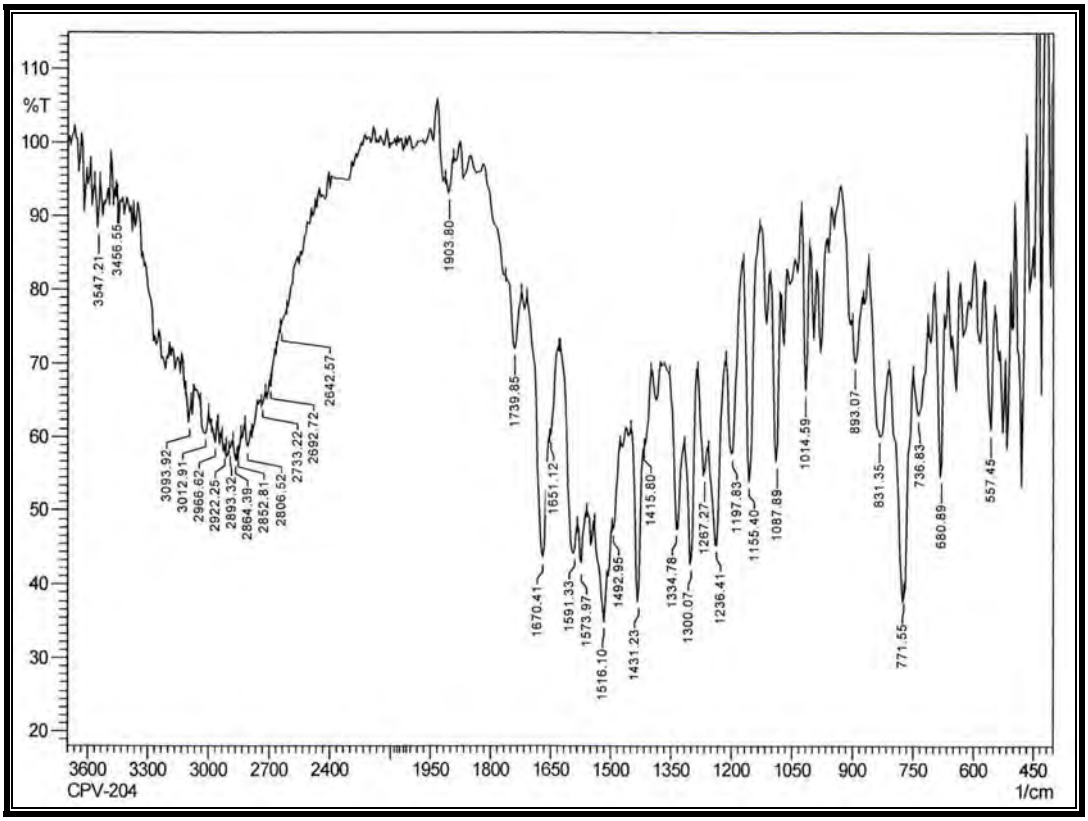
^{1}H NMR spectrum of CPV-203



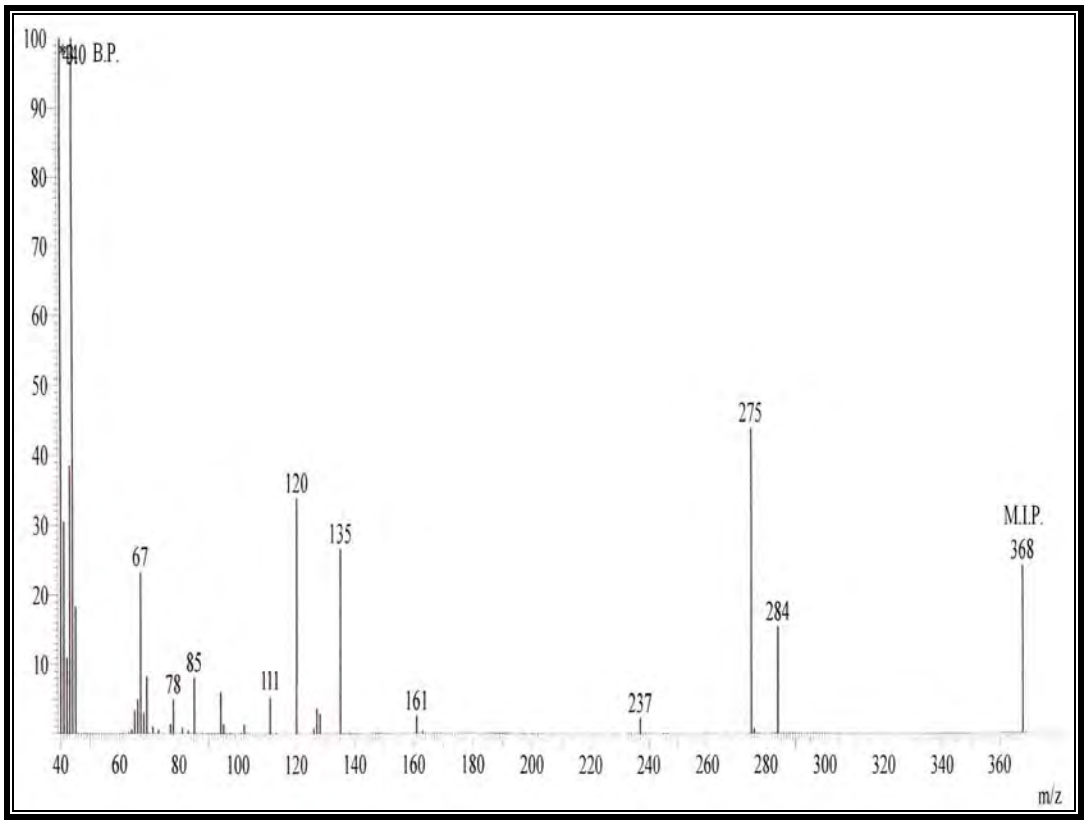
Expanded ^{1}H NMR spectrum of CPV-203



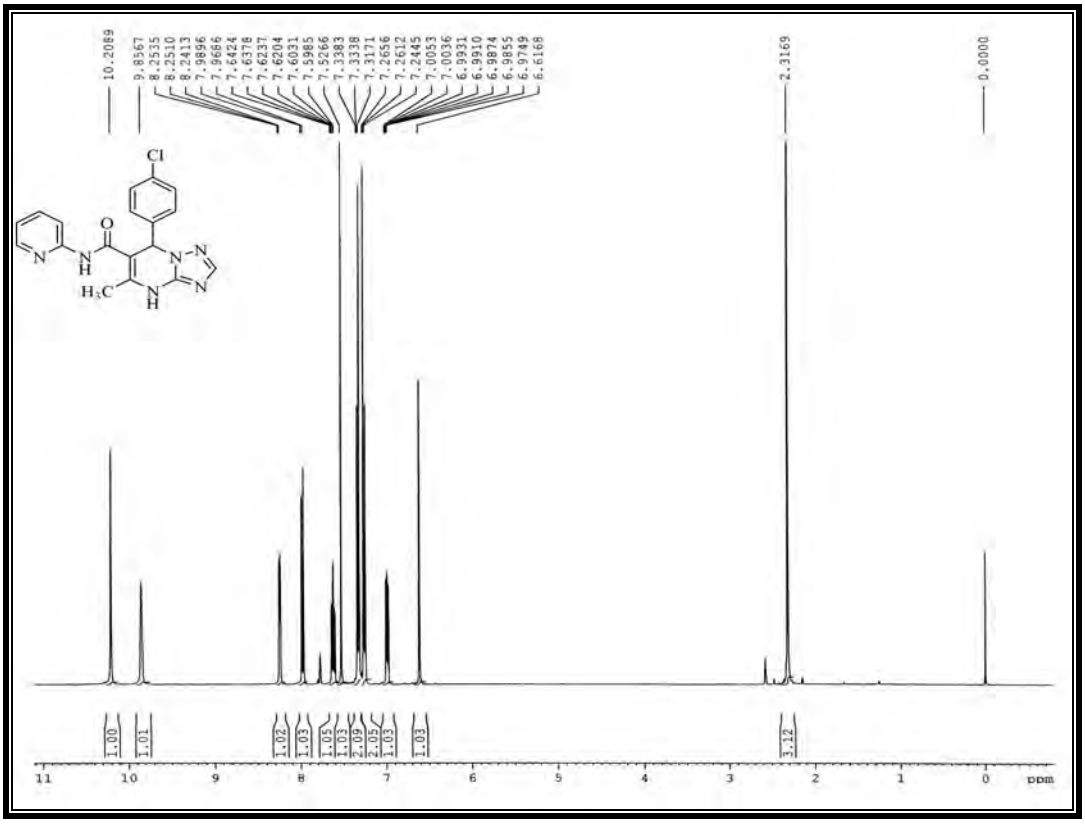
IR spectrum of CPV-204



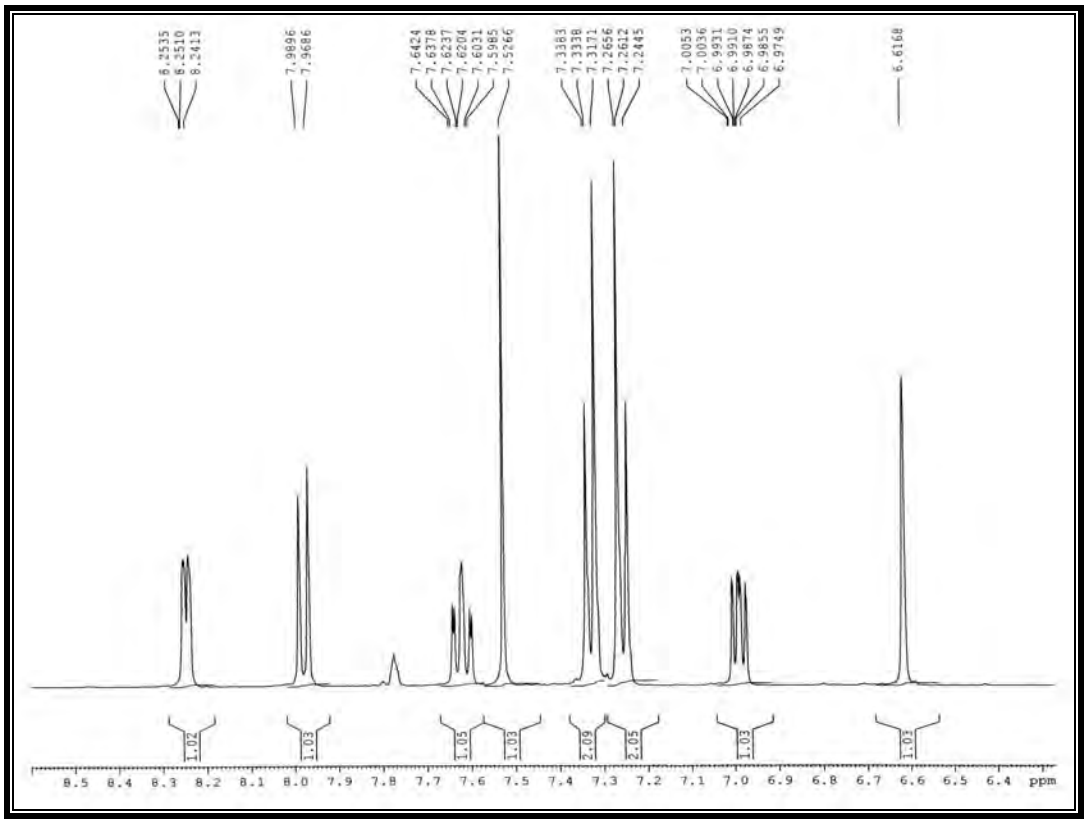
Mass spectrum of CPV-204



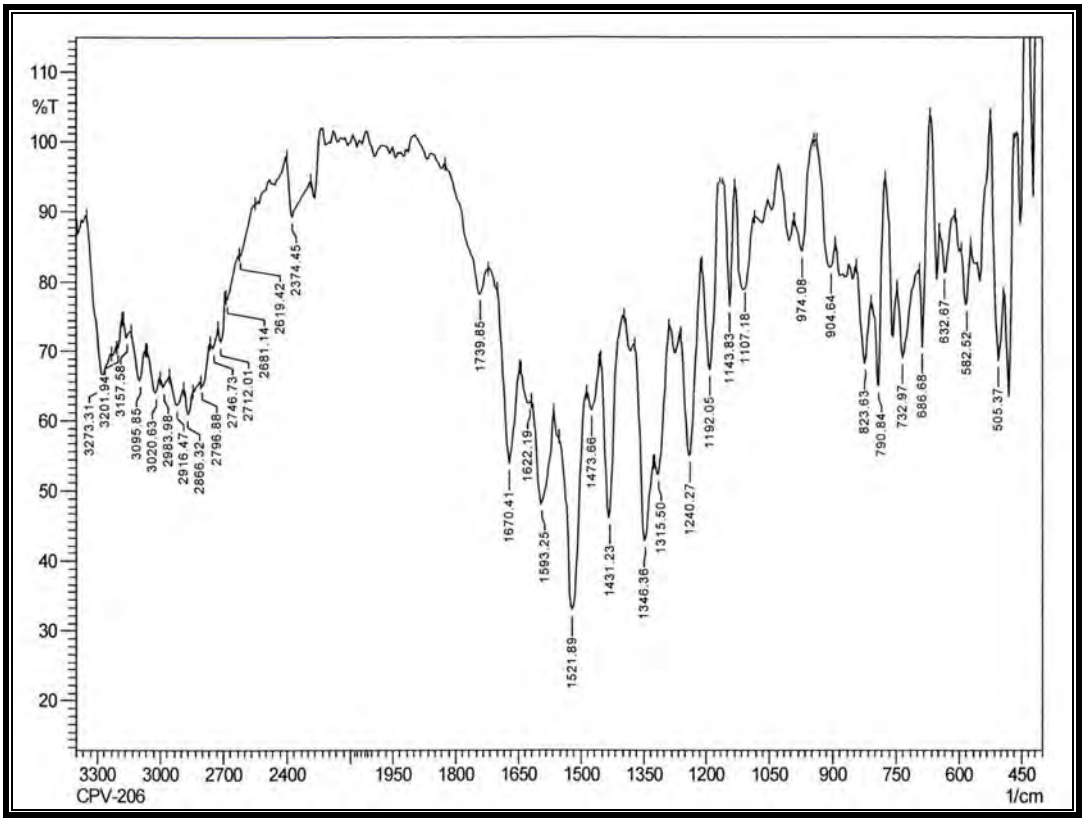
^{1}H NMR spectrum of CPV-204



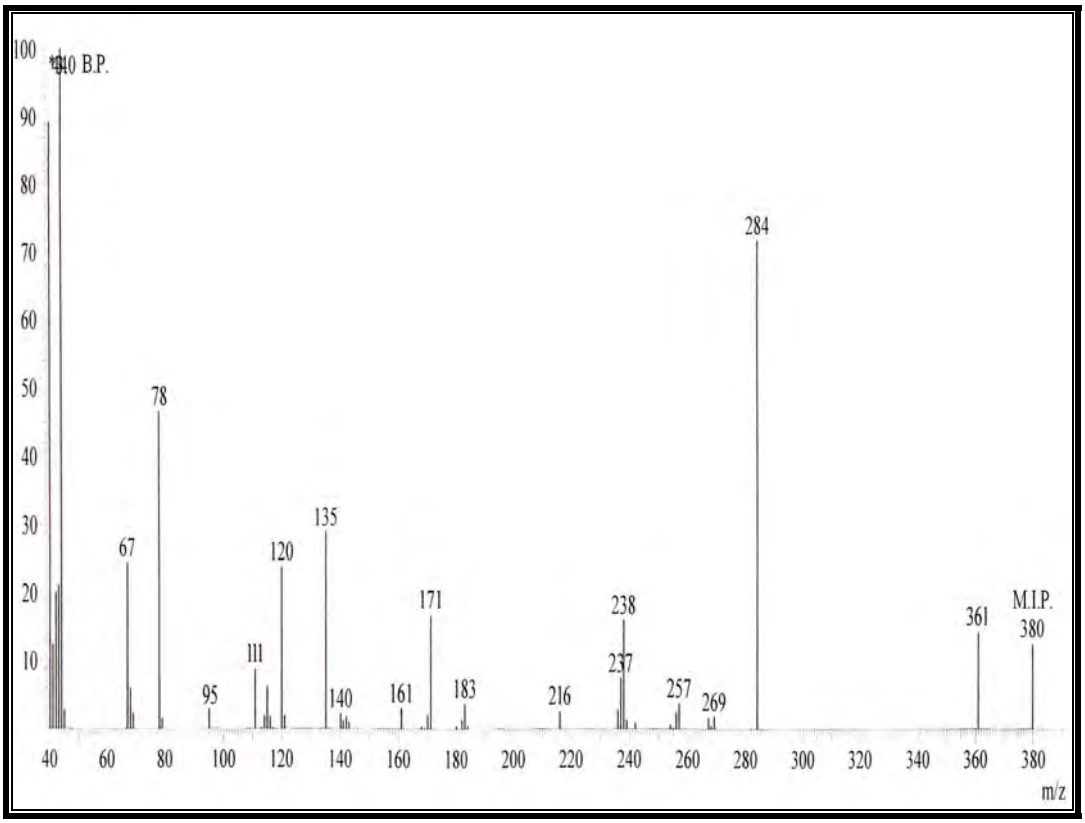
Expanded ^{1}H NMR spectrum of CPV-204



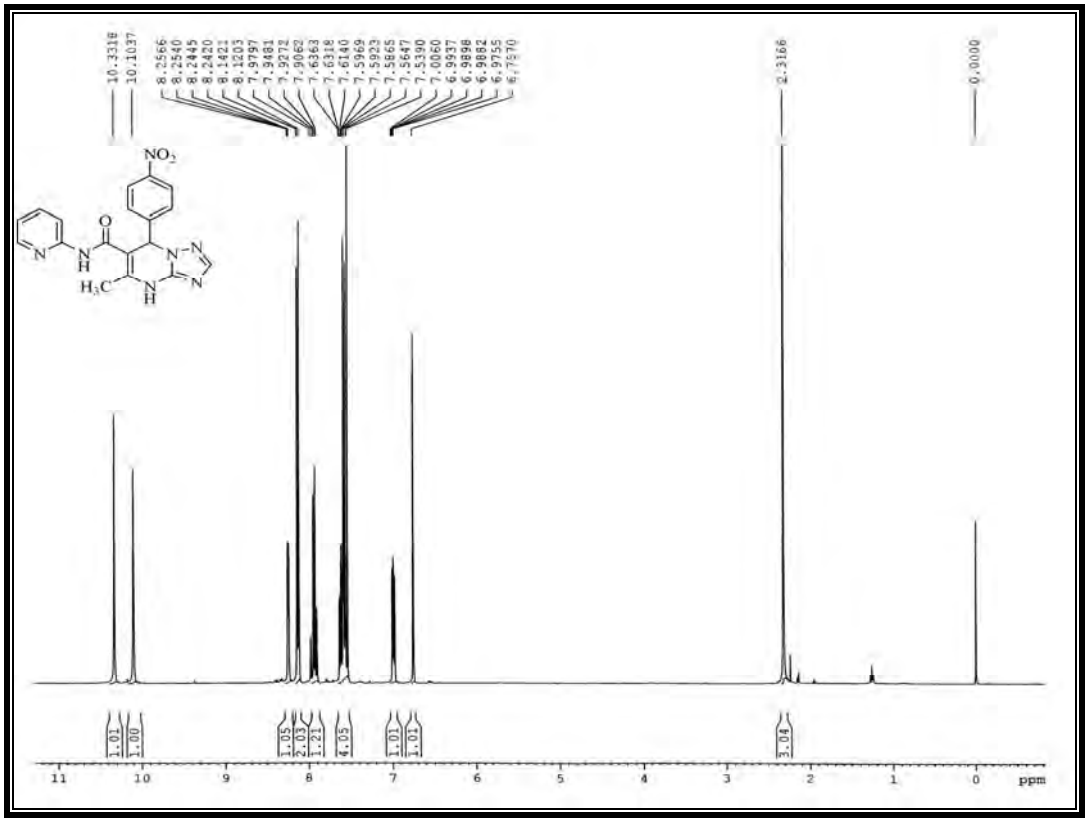
IR spectrum of CPV-206



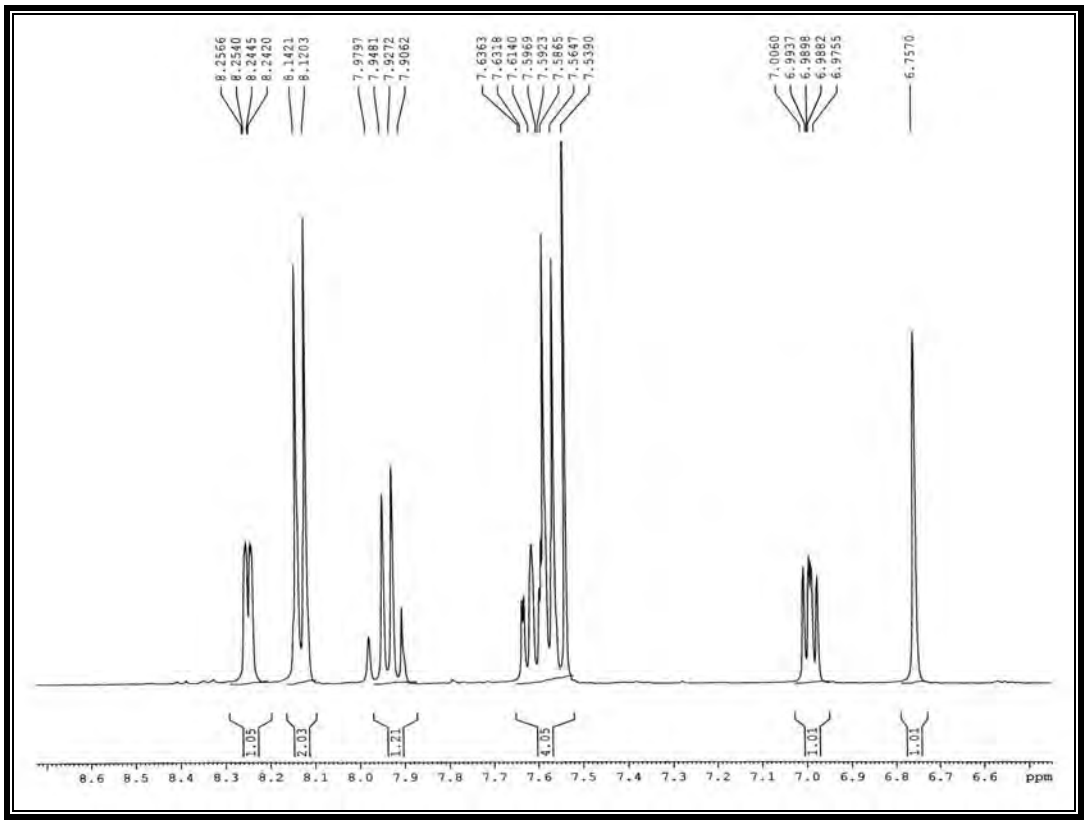
Mass spectrum of CPV-206



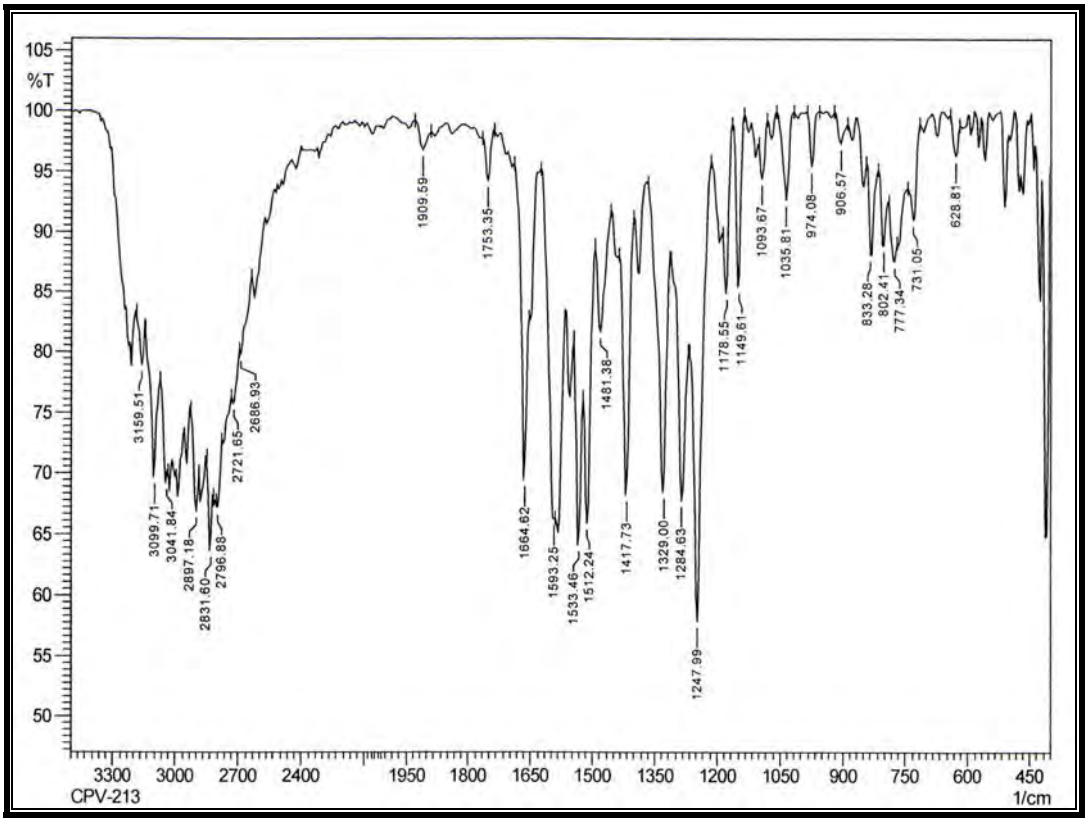
^{1}H NMR spectrum of CPV-206



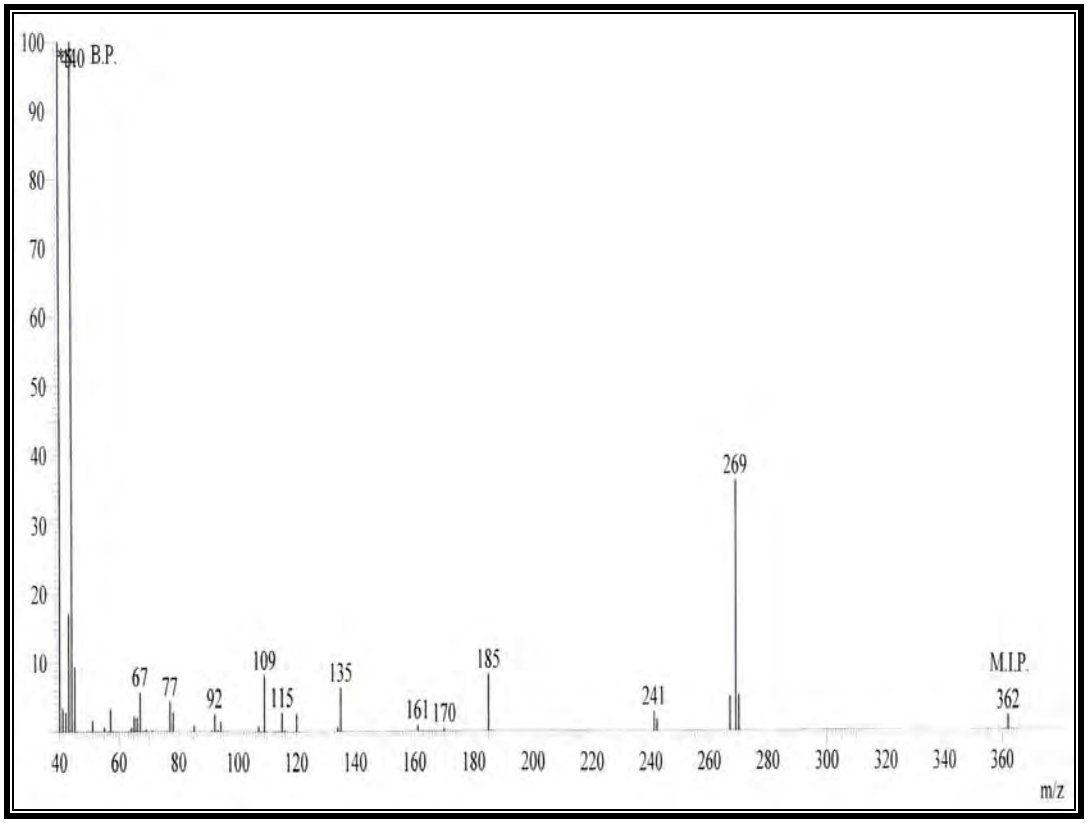
Expanded ^{1}H NMR spectrum of CPV-206



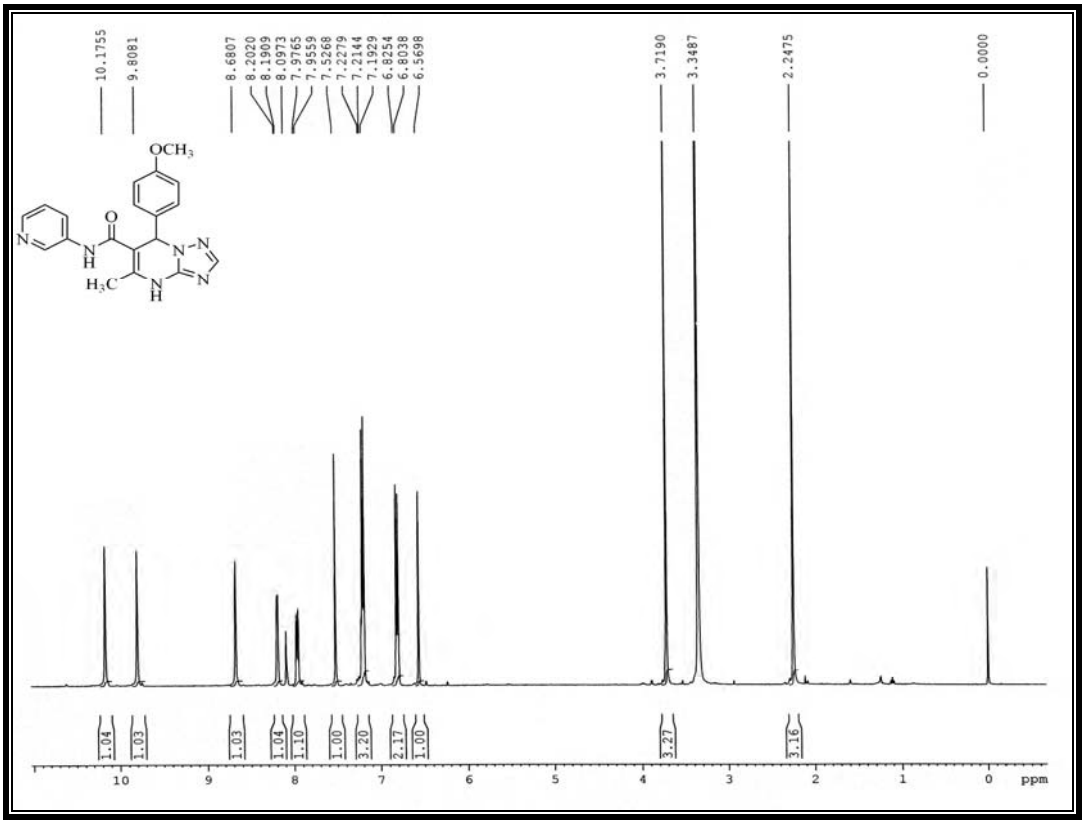
IR spectrum of CPV-213



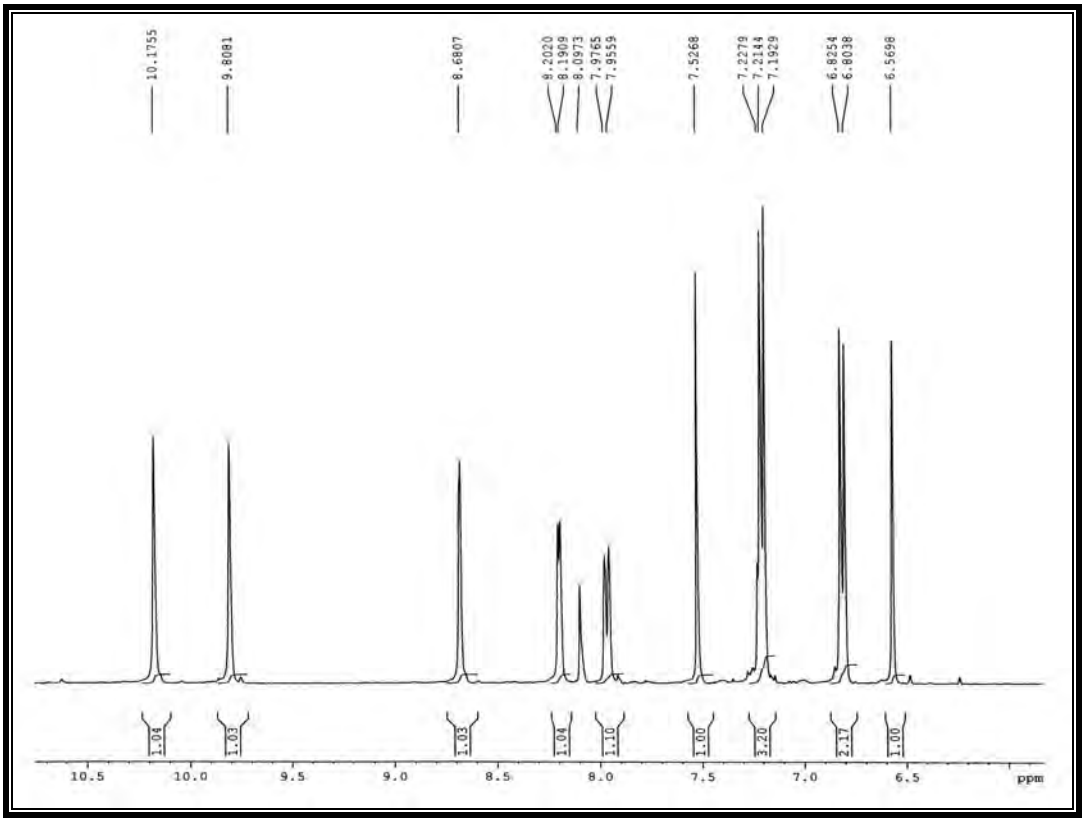
Mass spectrum of CPV-213



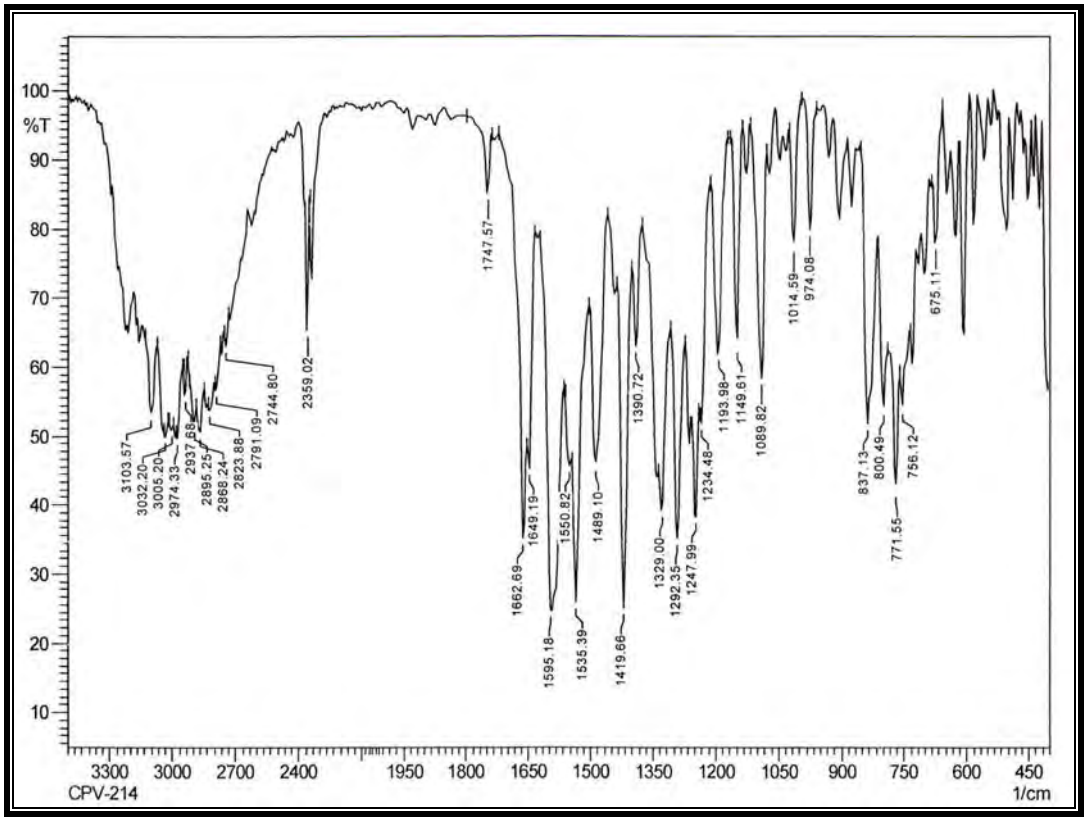
^{1}H NMR spectrum of CPV-213



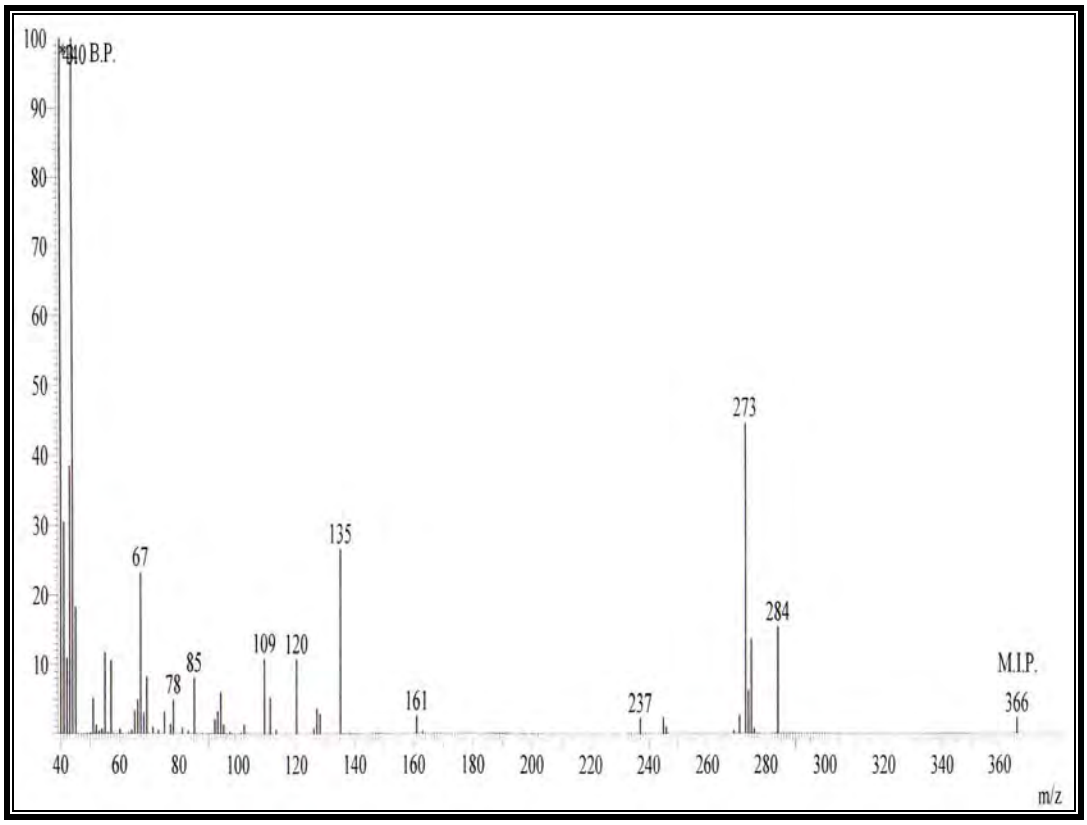
Expanded ^{1}H NMR spectrum of CPV-213



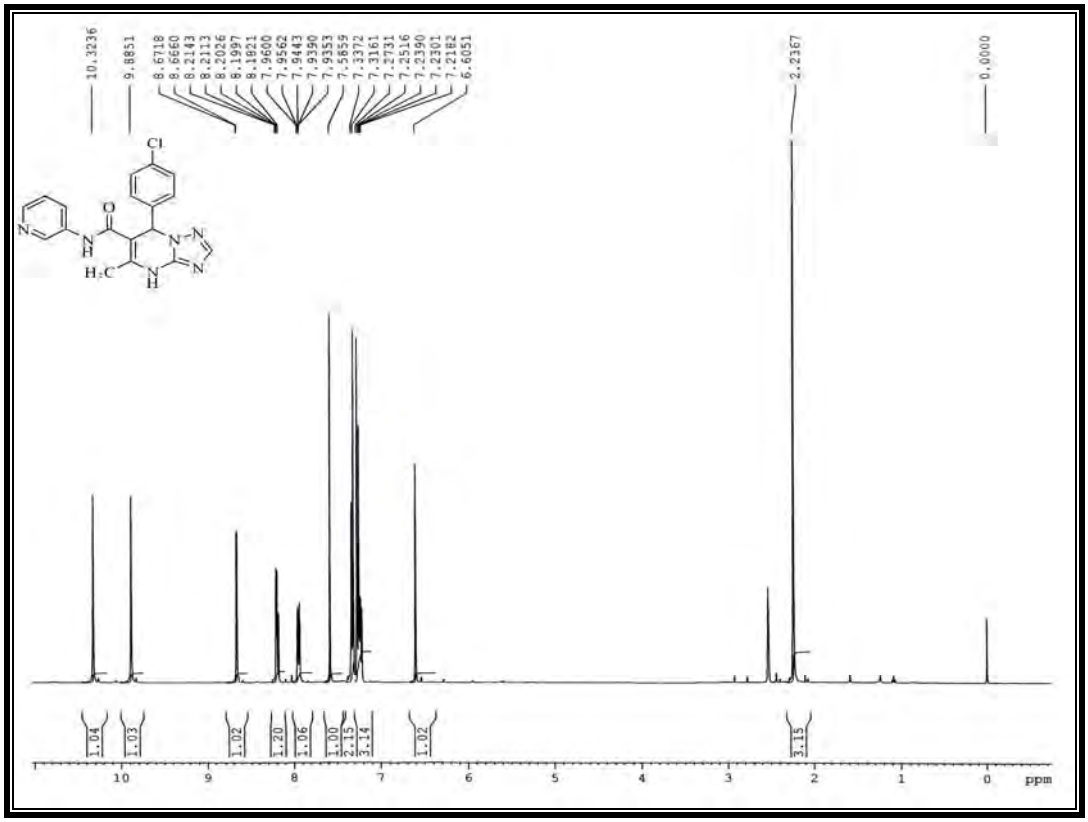
IR spectrum of CPV-214



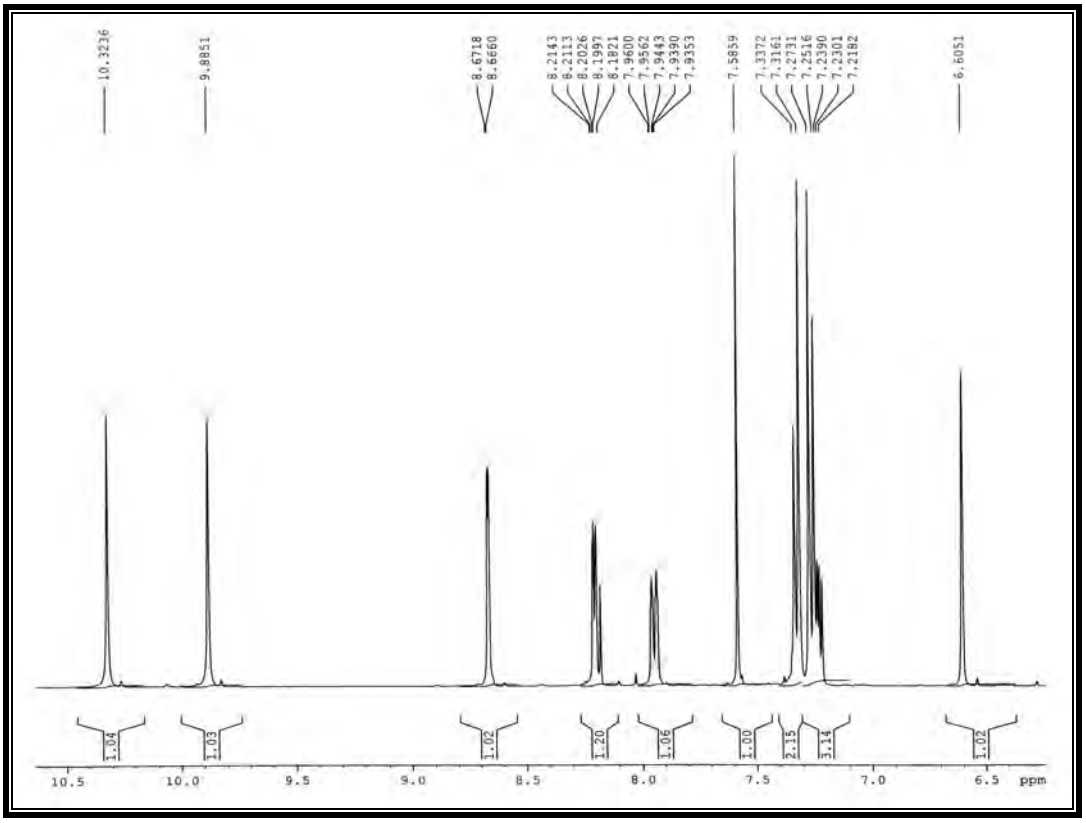
Mass spectrum of CPV-214



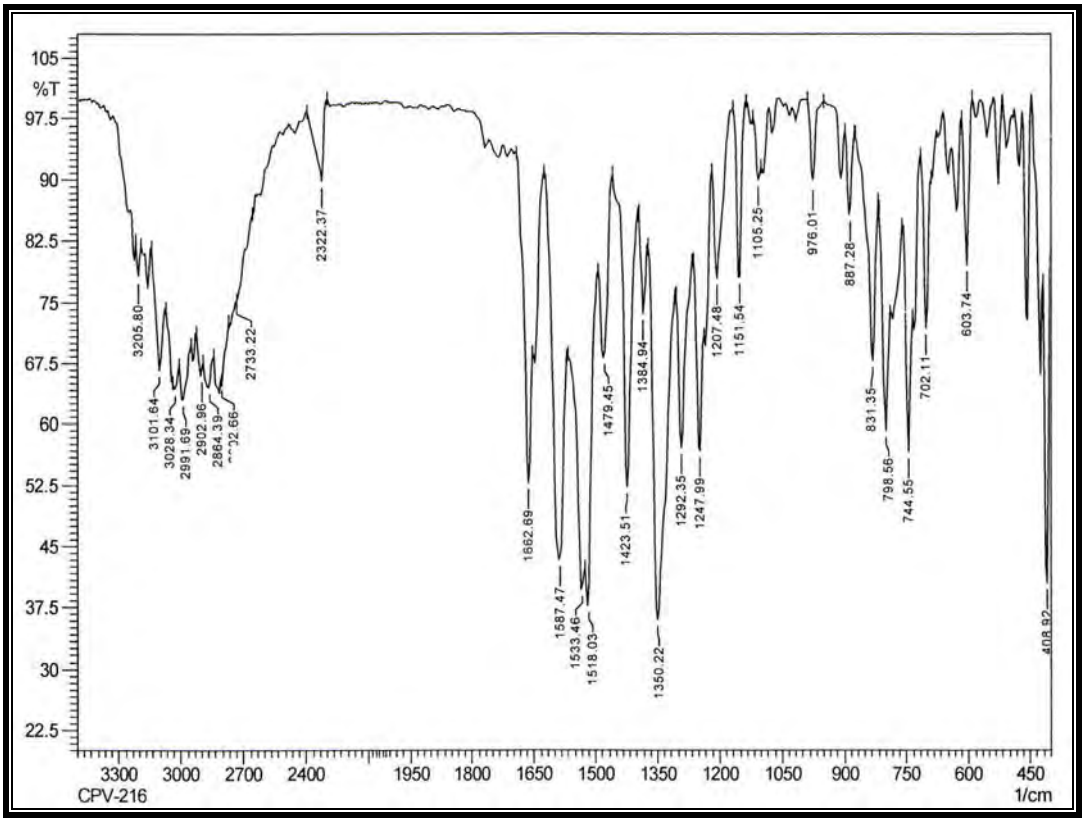
^{1}H NMR spectrum of CPV-214



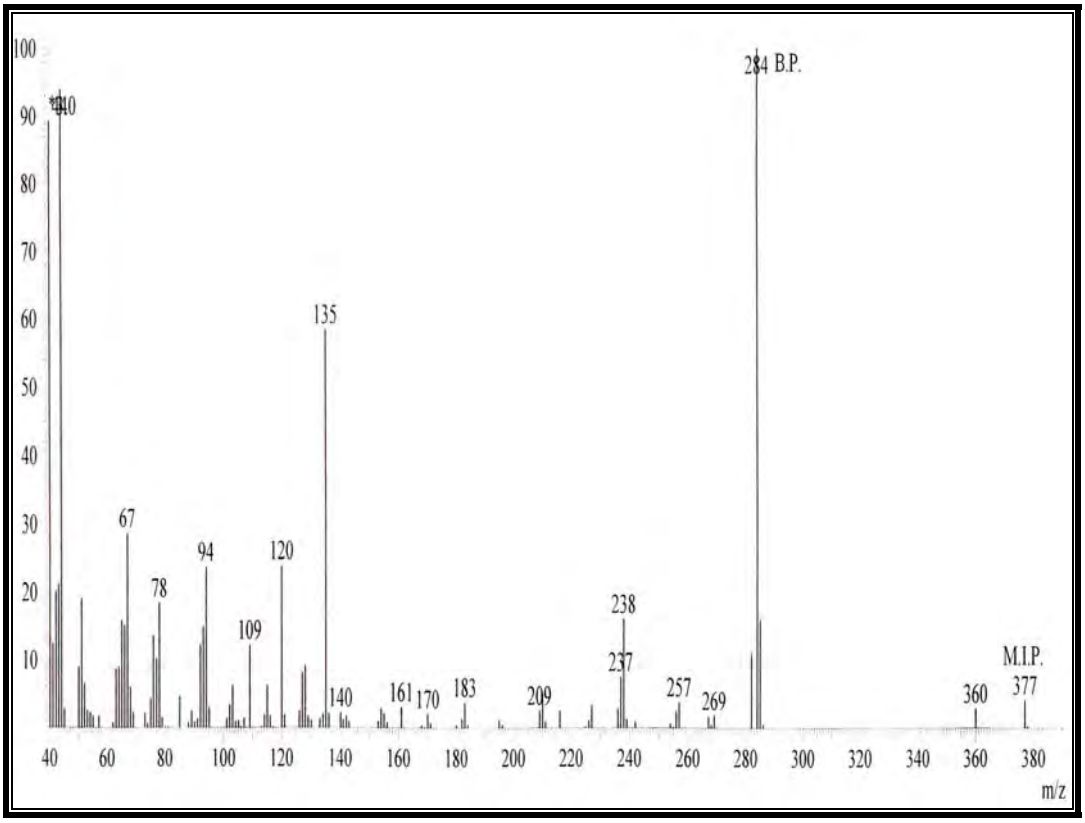
Expanded ^{1}H NMR spectrum of CPV-214



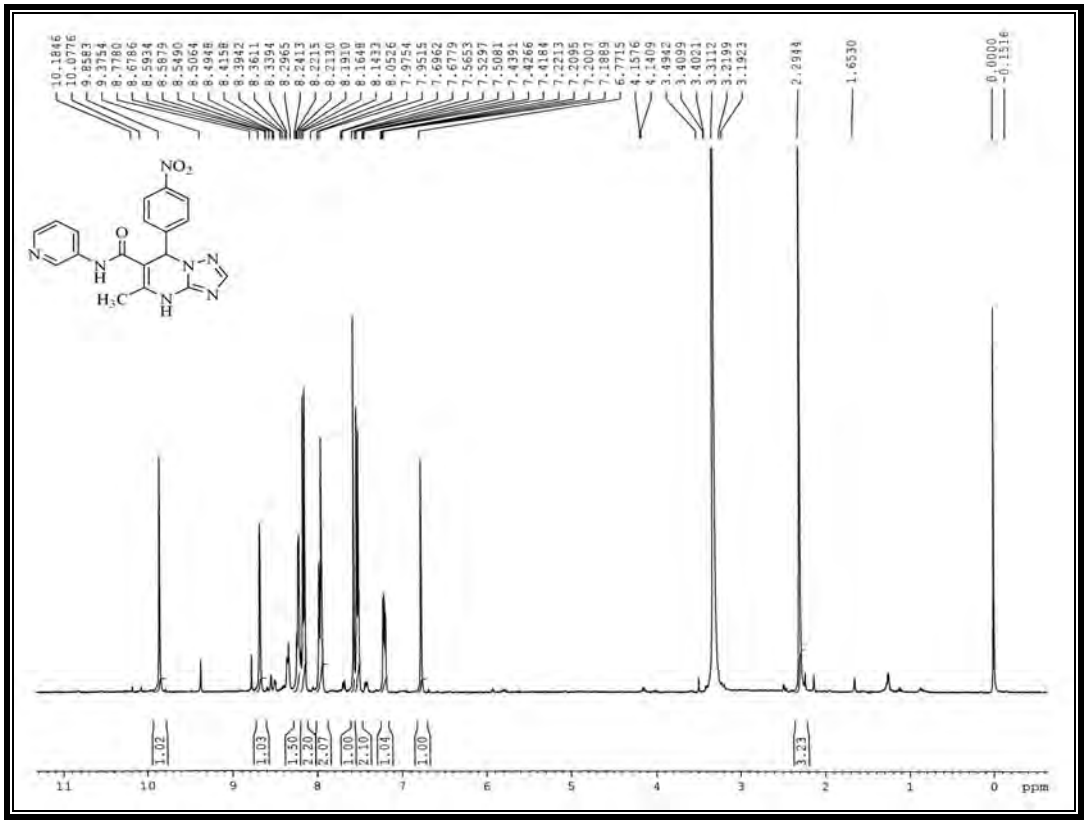
IR spectrum of CPV-216



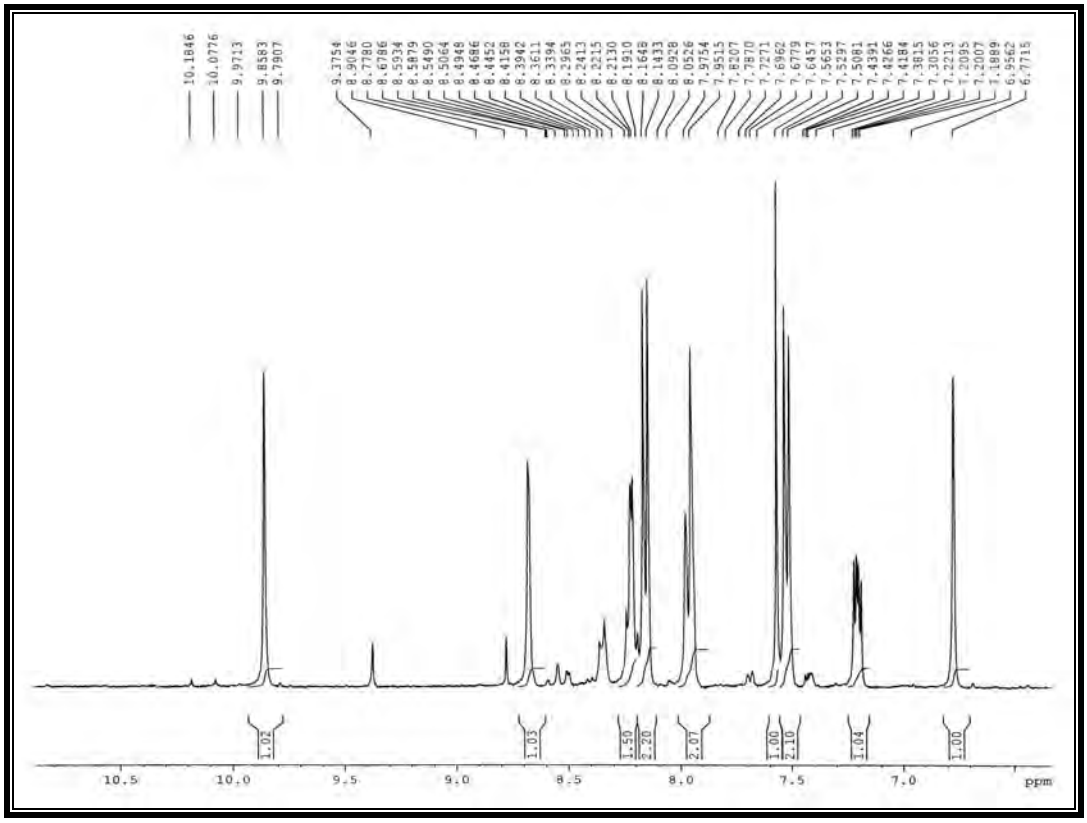
Mass spectrum of CPV-216



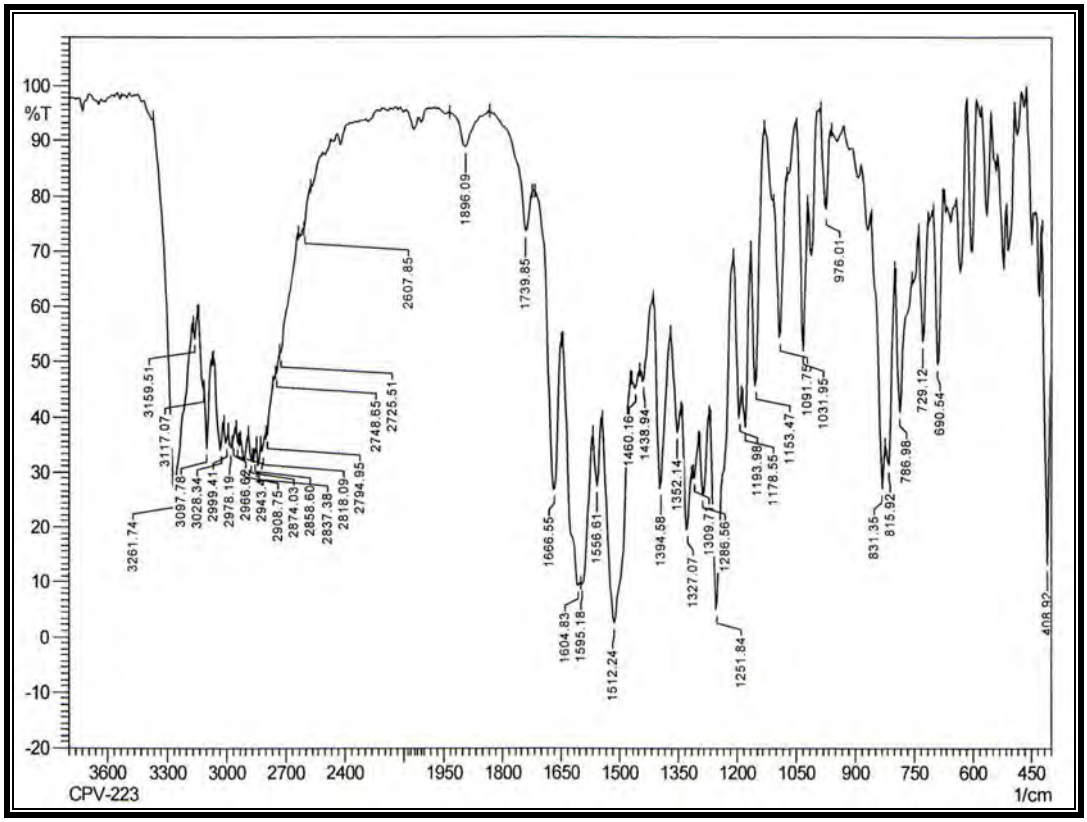
^{1}H NMR spectrum of CPV-216



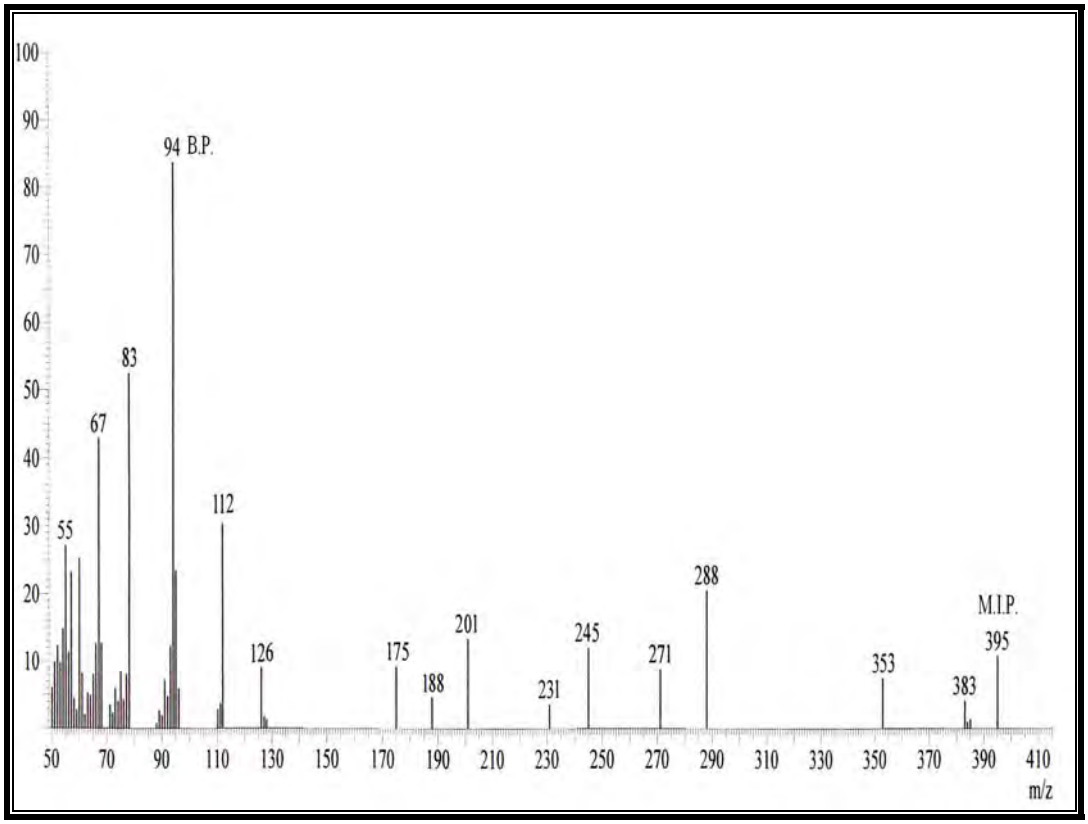
Expanded ^{1}H NMR spectrum of CPV-216



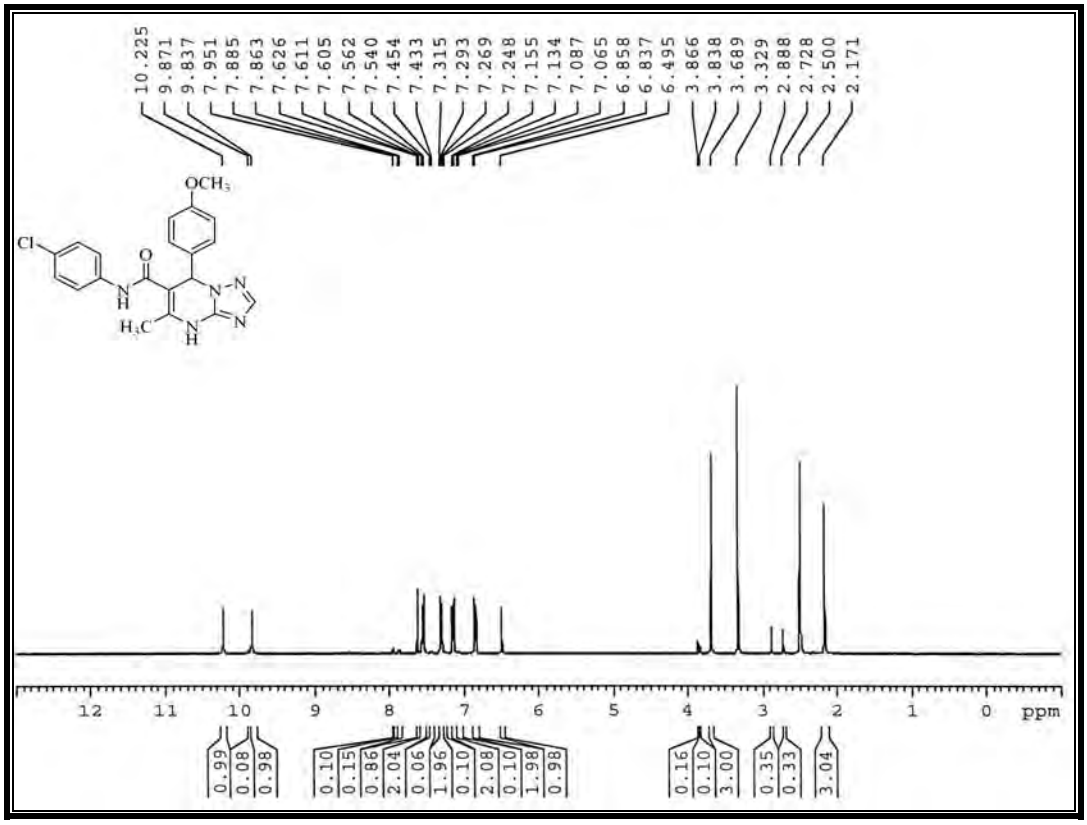
IR spectrum of CPV-223



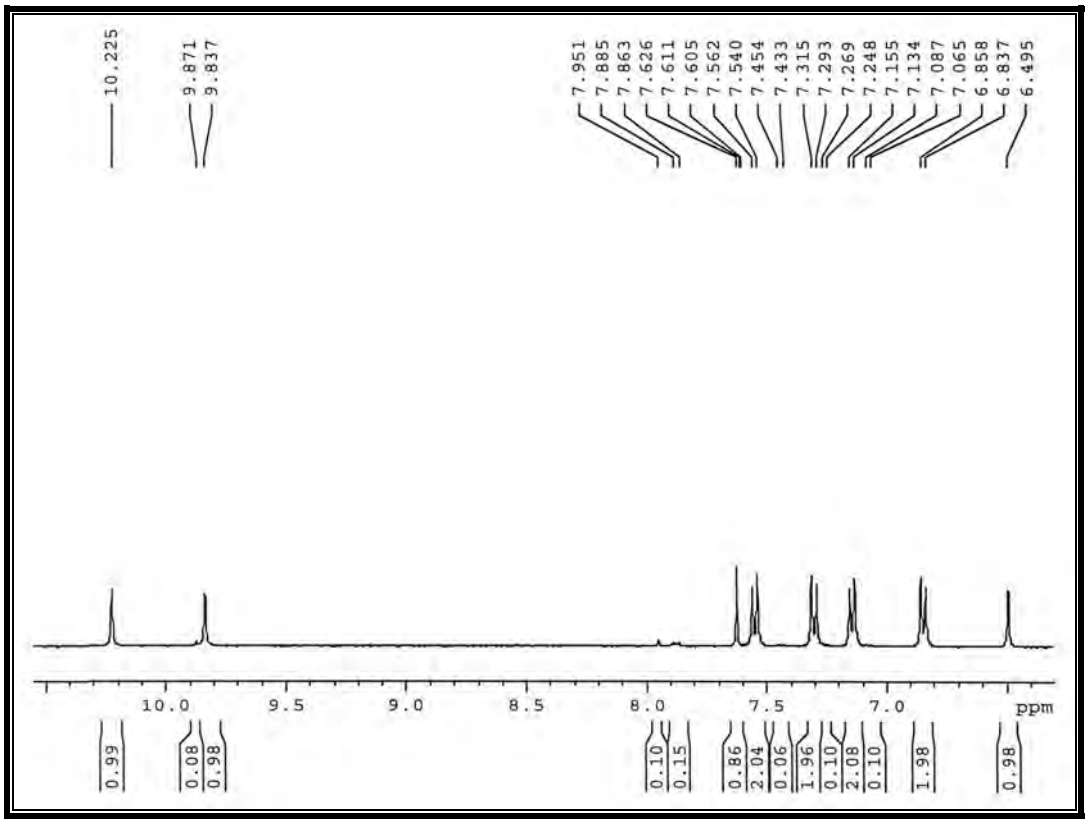
Mass spectrum of CPV-223



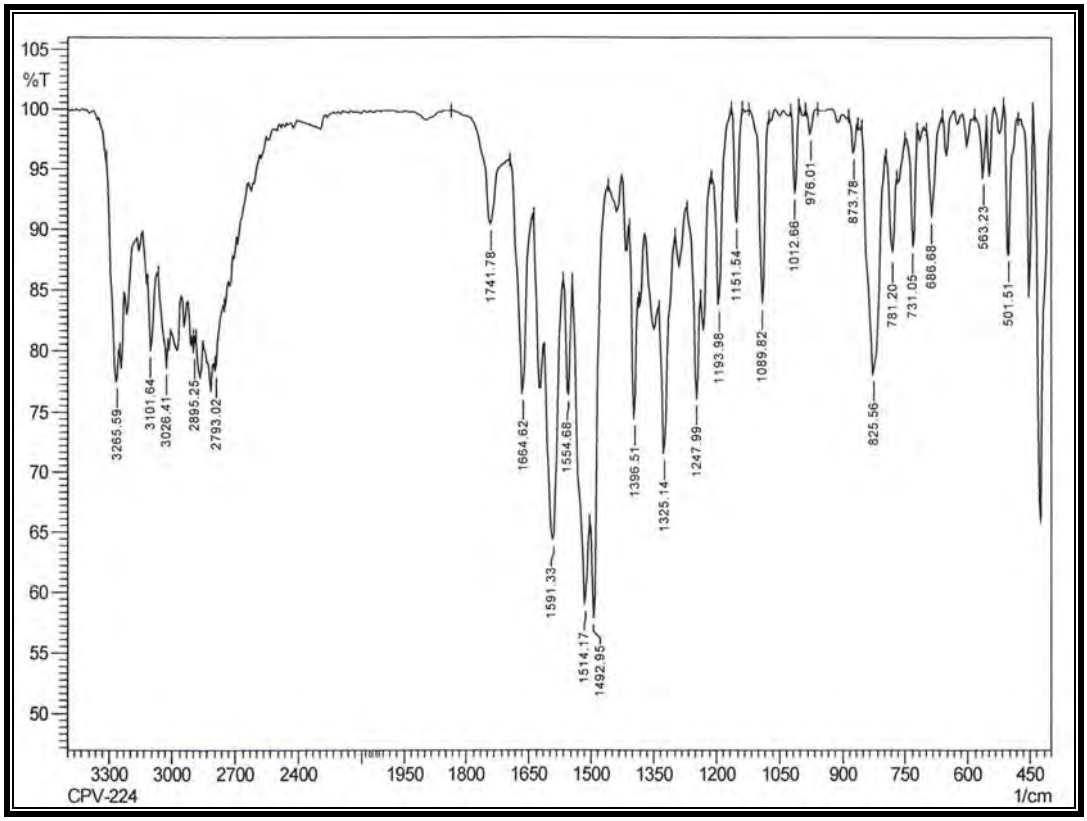
^{1}H NMR spectrum of CPV-223



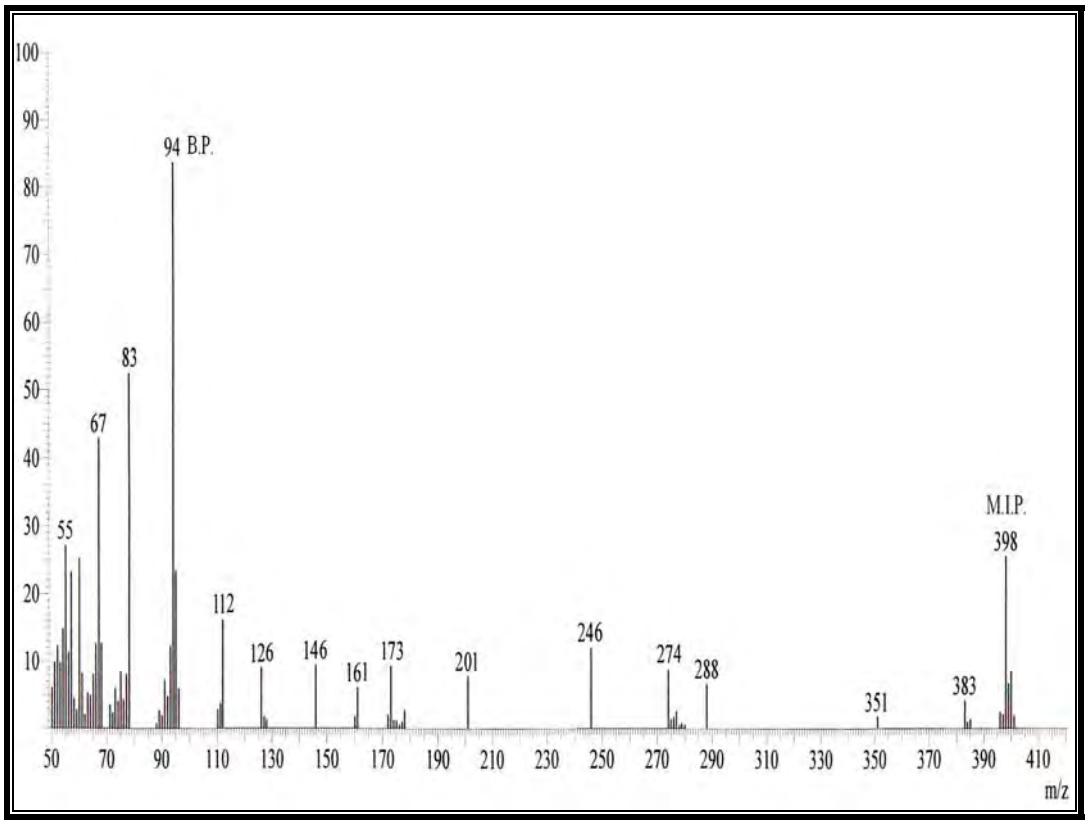
Expanded ^{1}H NMR spectrum of CPV-223



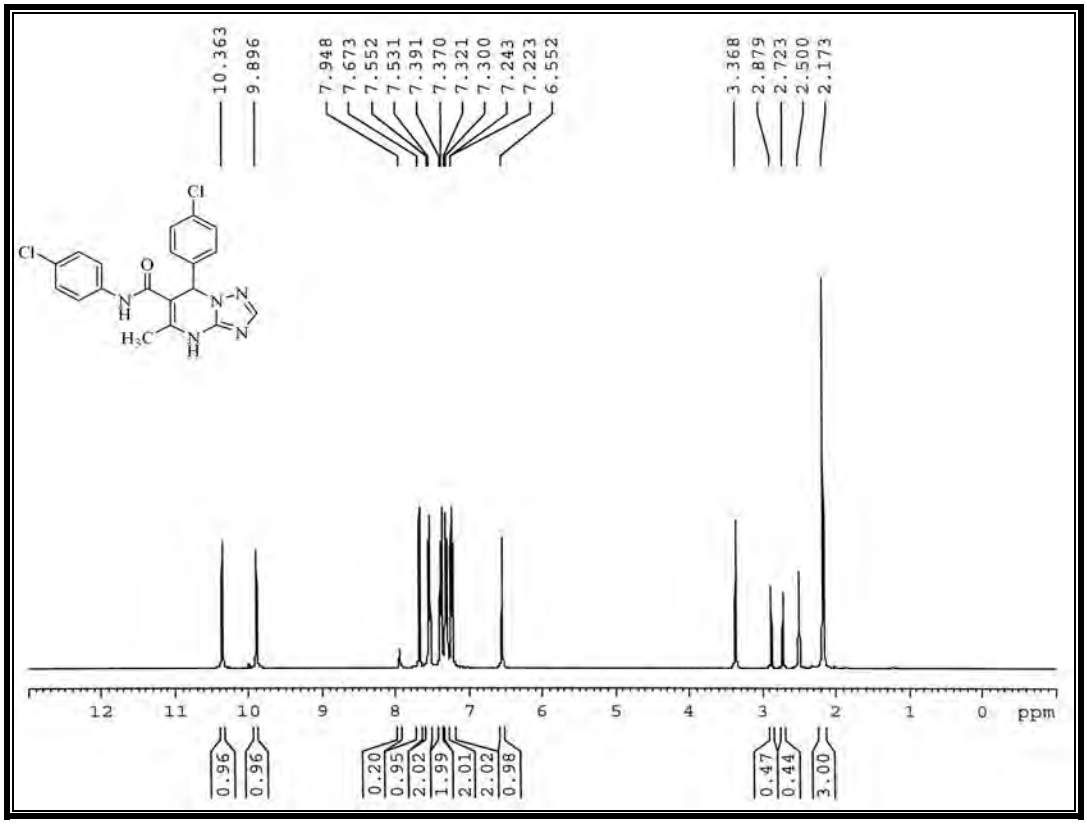
IR spectrum of CPV-224



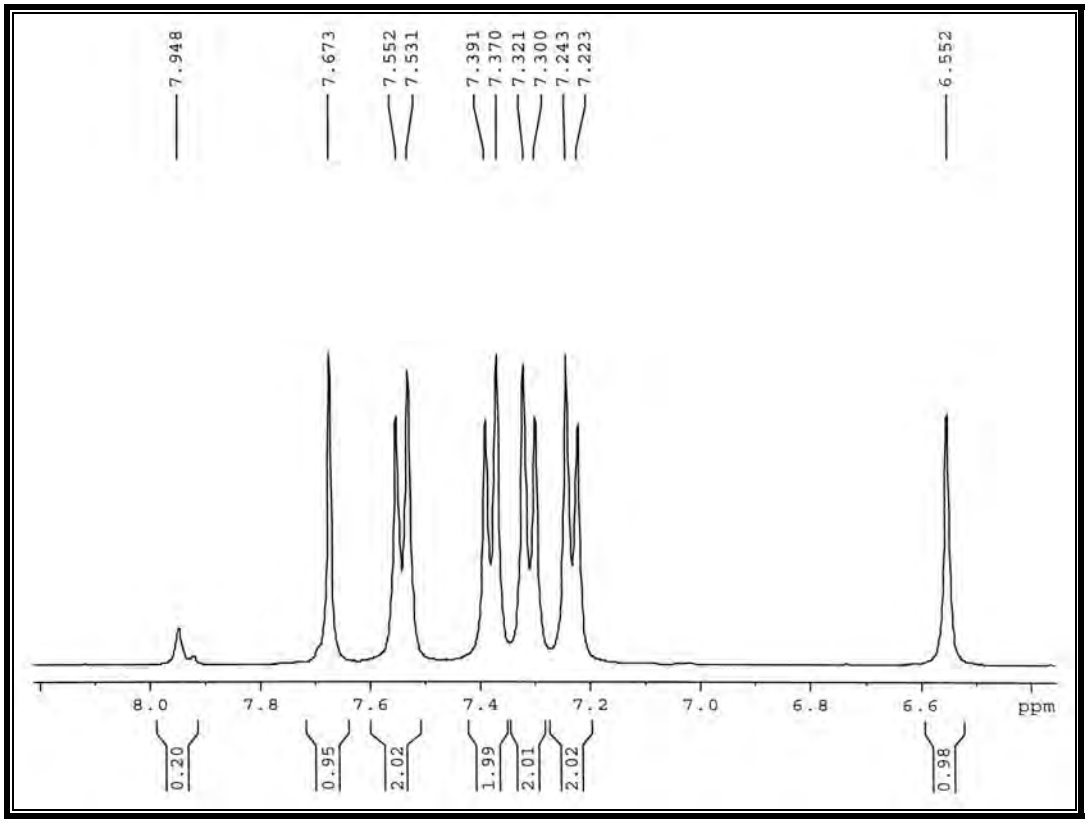
Mass spectrum of CPV-224



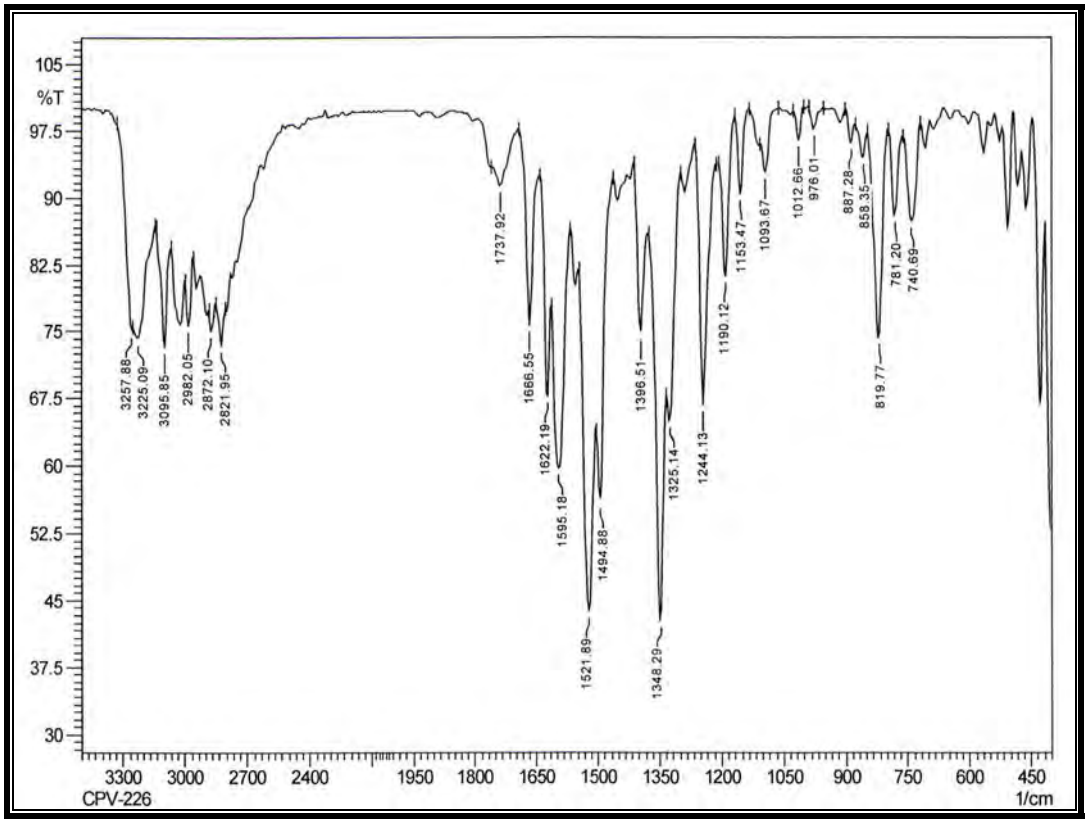
^{1}H NMR spectrum of CPV-224



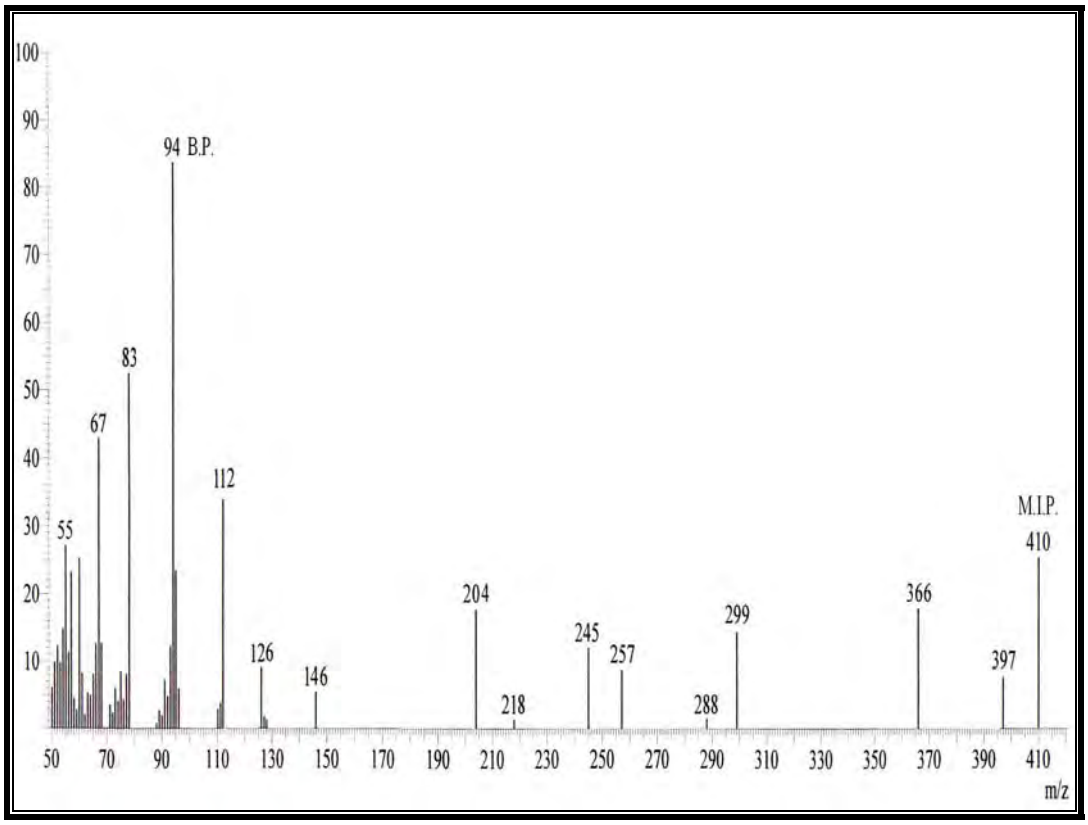
Expanded ^{1}H NMR spectrum of CPV-224



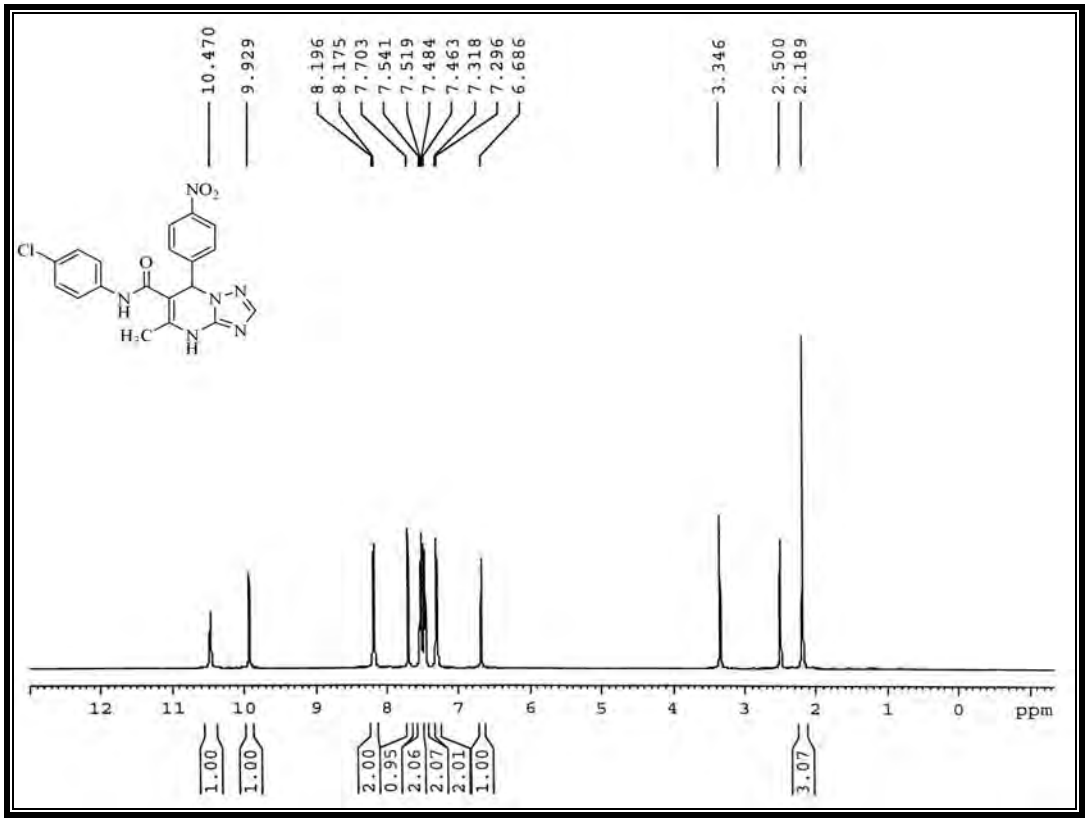
IR spectrum of CPV-226



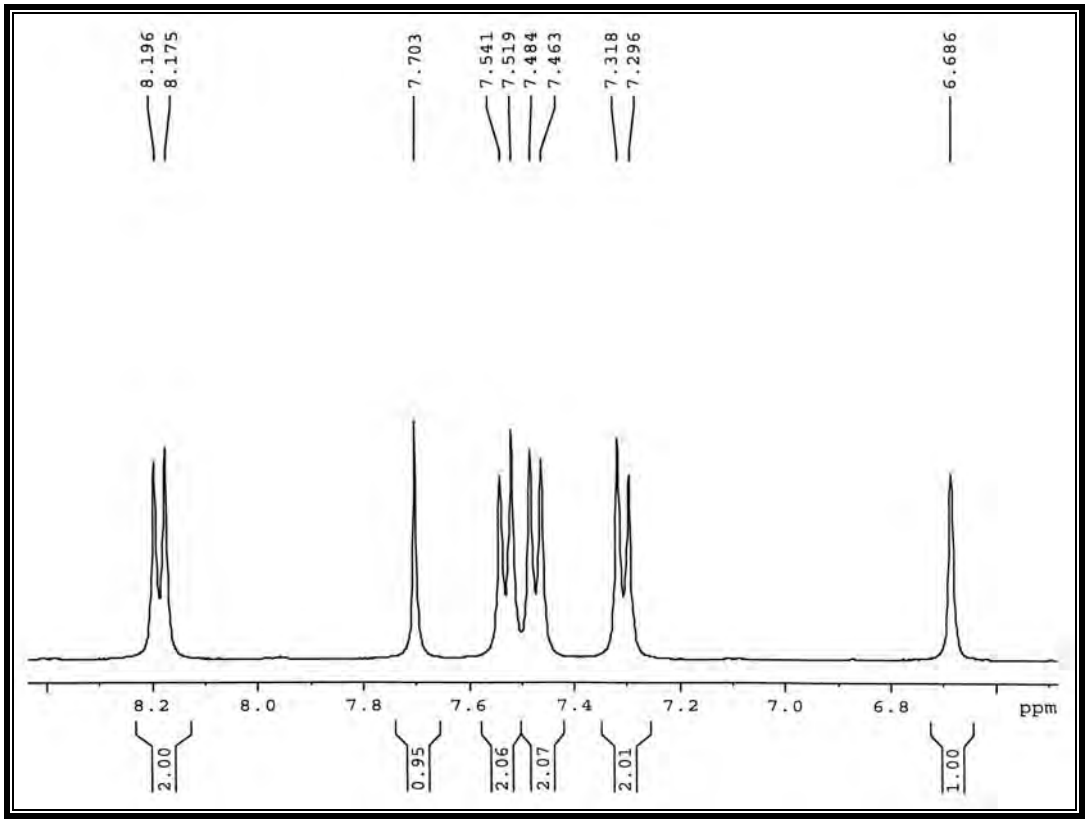
Mass spectrum of CPV-226



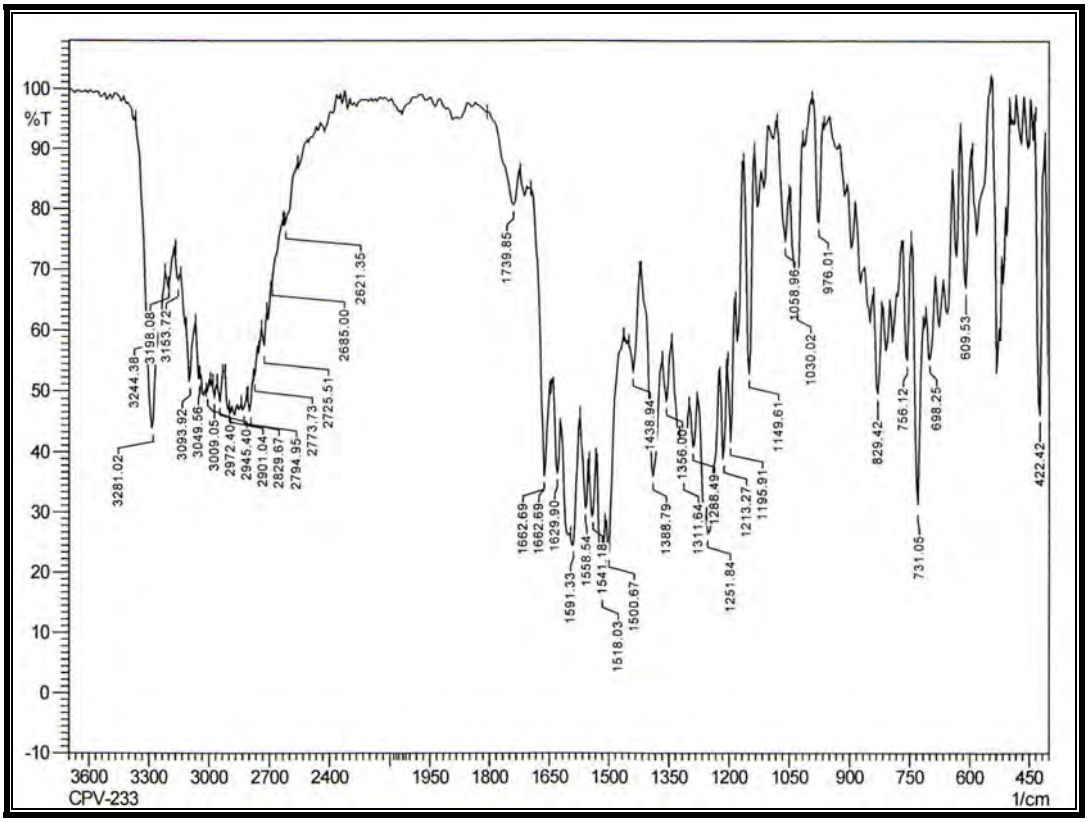
^{1}H NMR spectrum of CPV-226



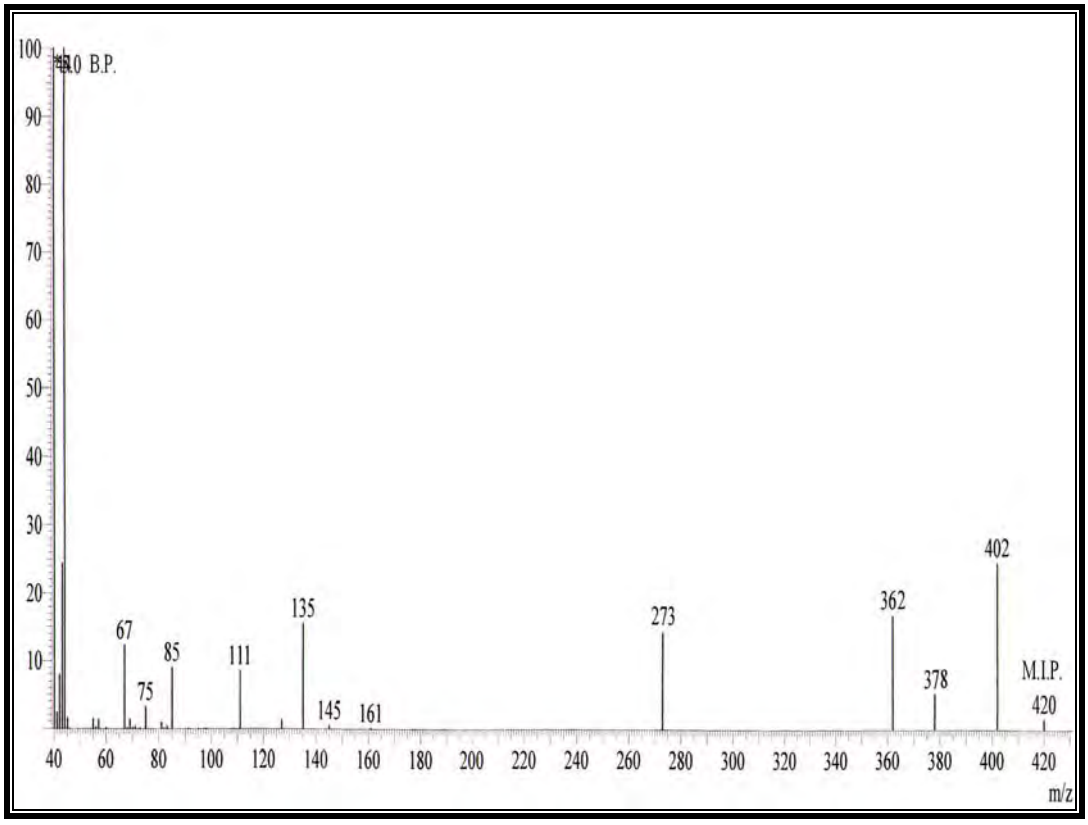
Expanded ^{1}H NMR spectrum of CPV-226



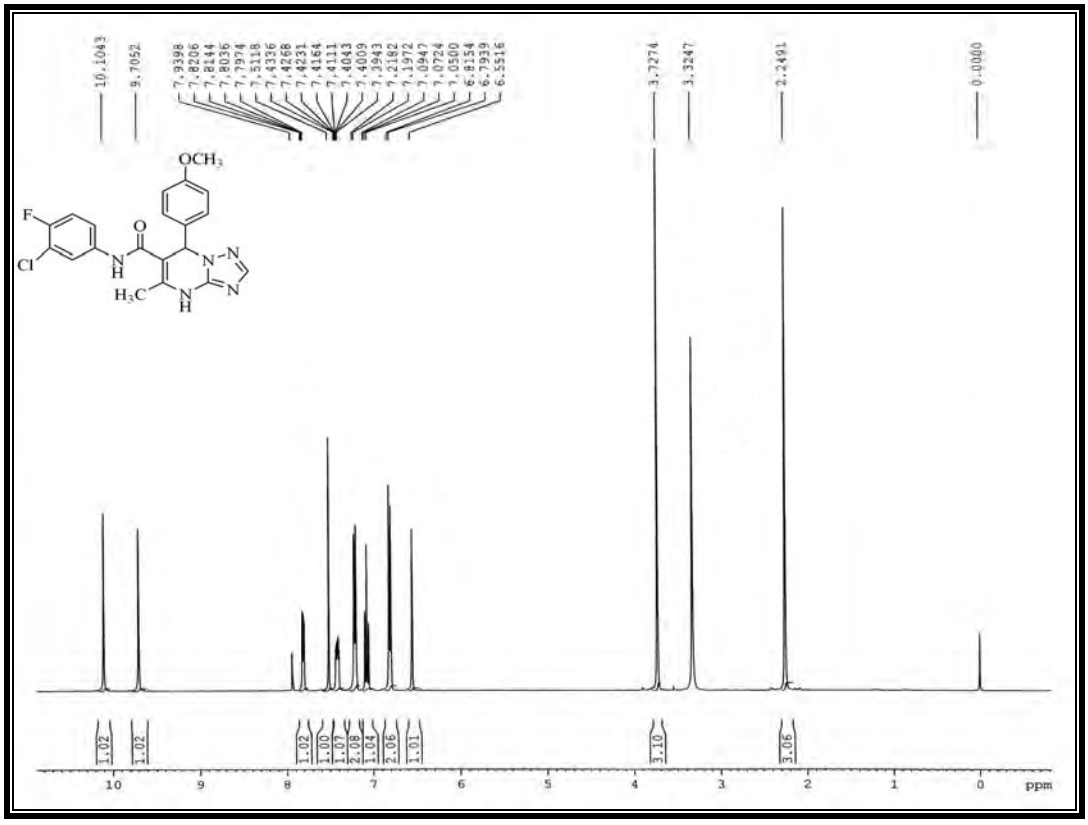
IR spectrum of CPV-233



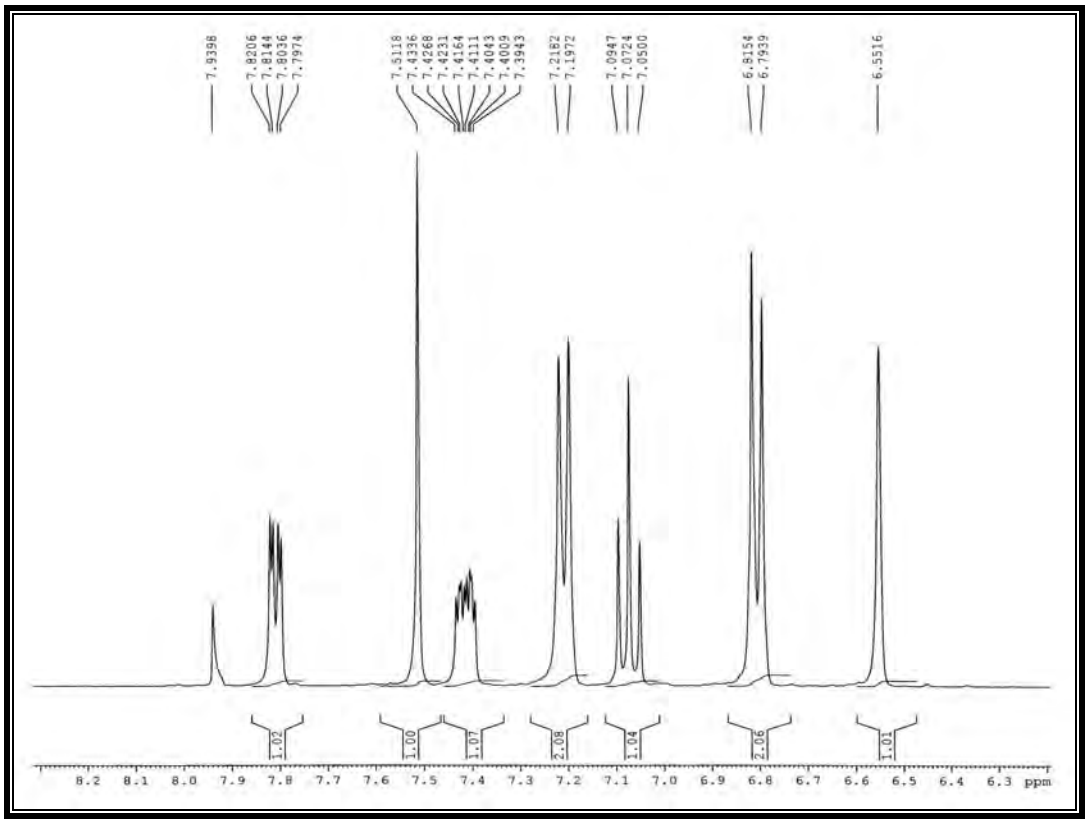
Mass spectrum of CPV-233



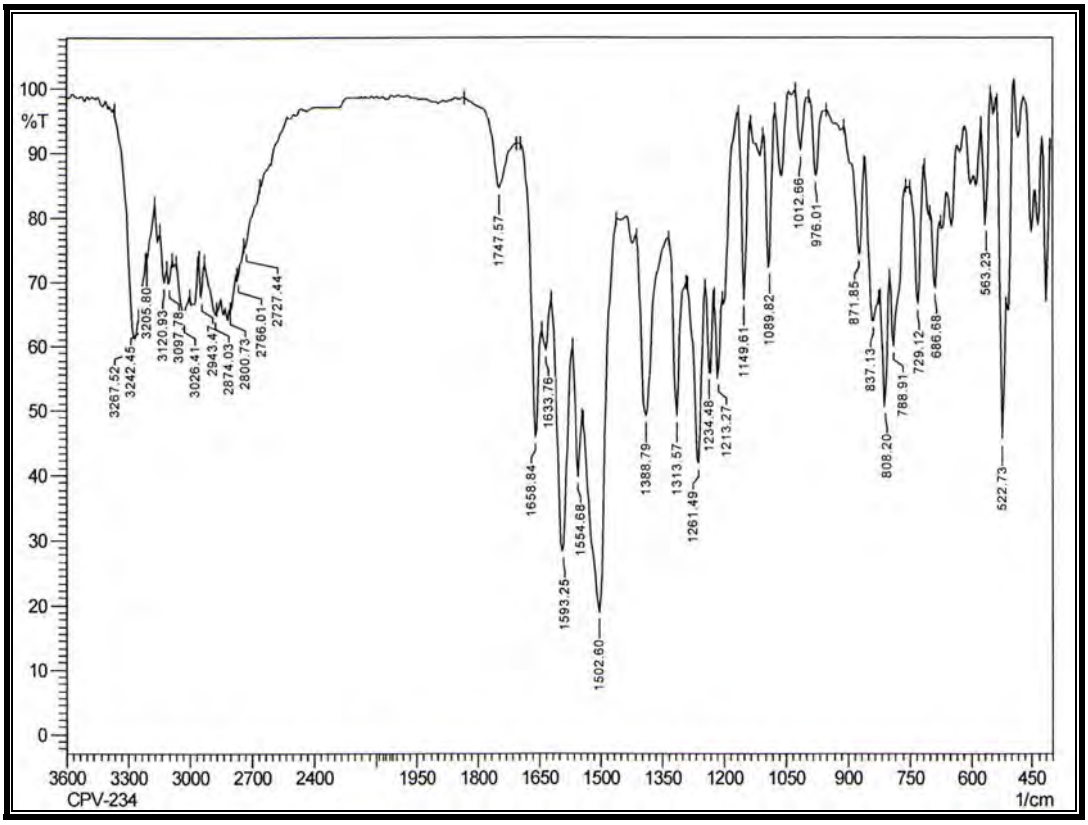
^{1}H NMR spectrum of CPV-233



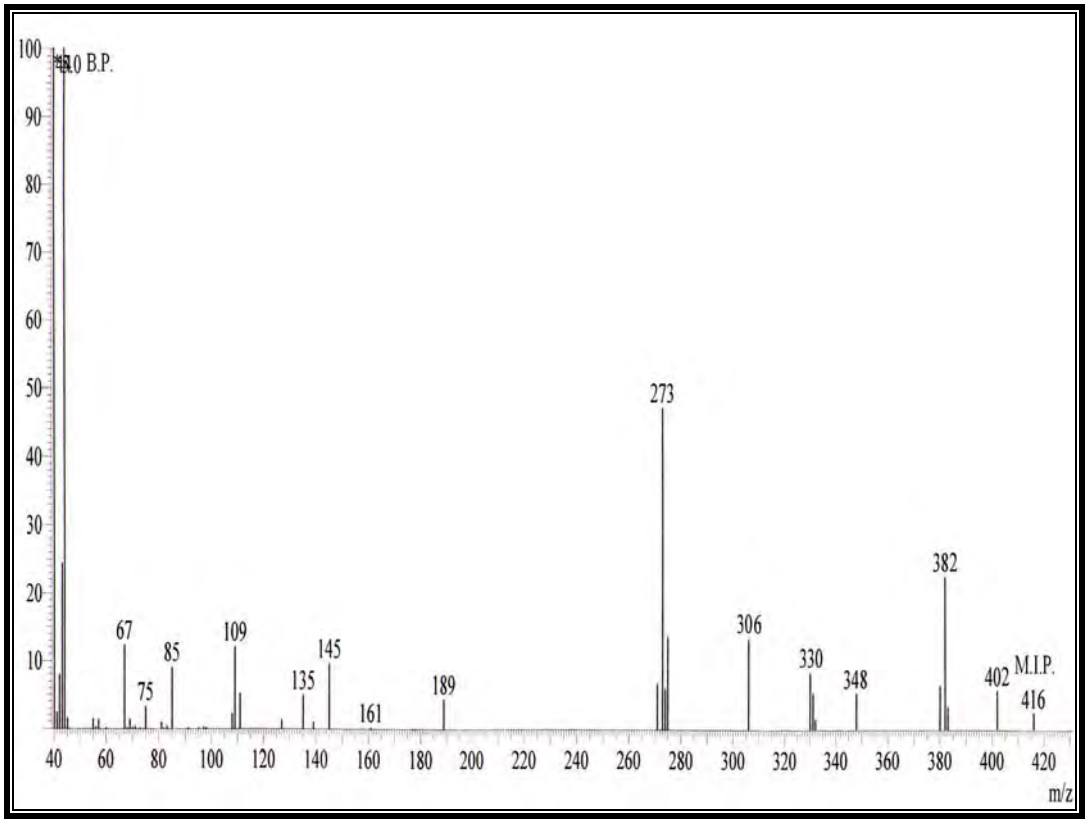
Expanded ^{1}H NMR spectrum of CPV-233



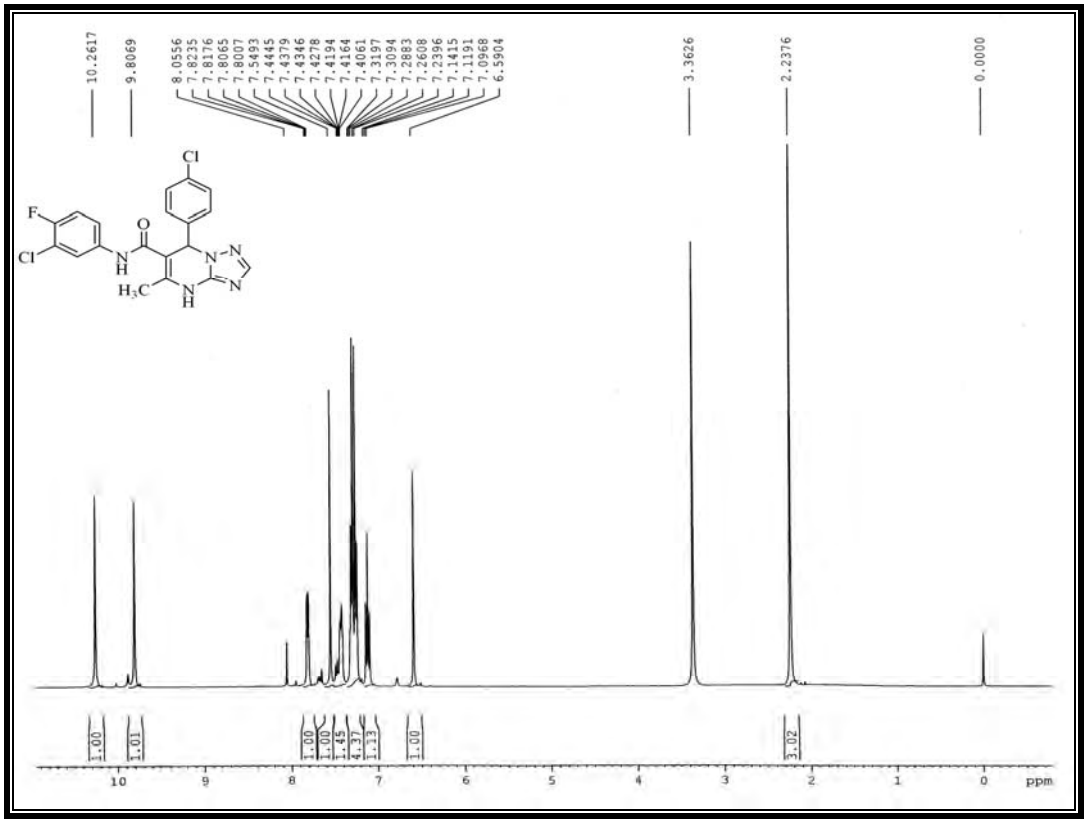
IR spectrum of CPV-234



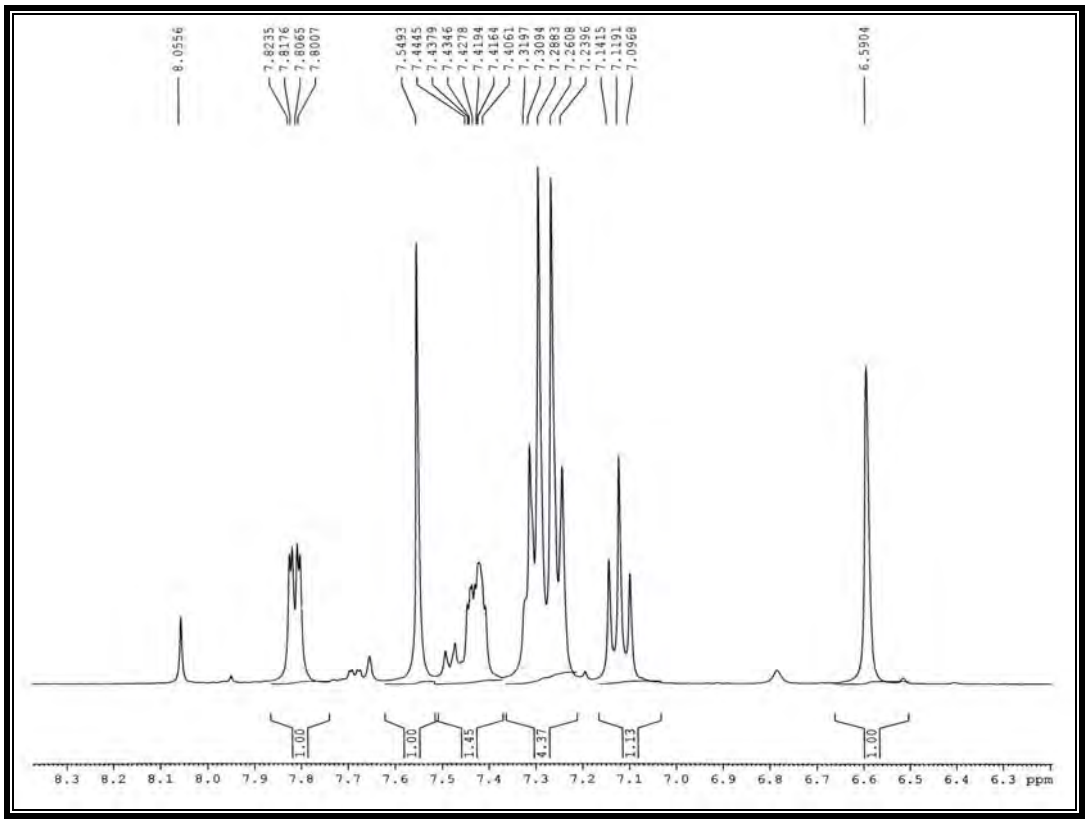
Mass spectrum of CPV-234



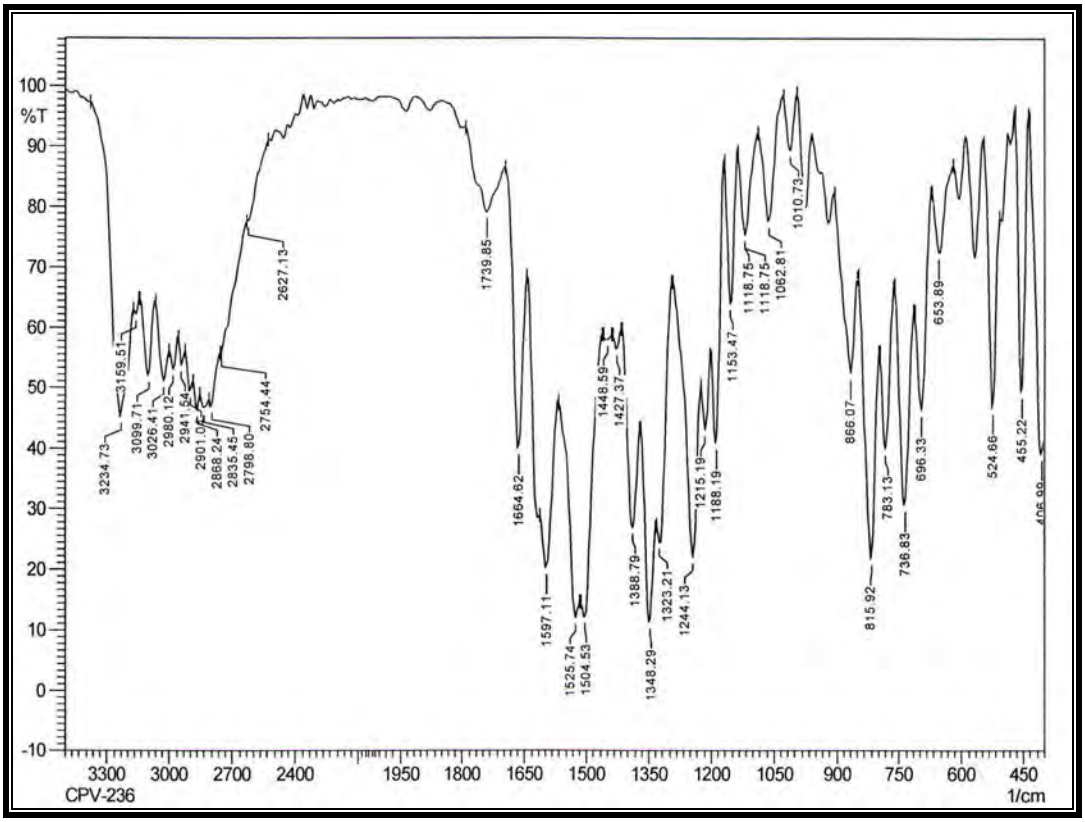
^{1}H NMR spectrum of CPV-234



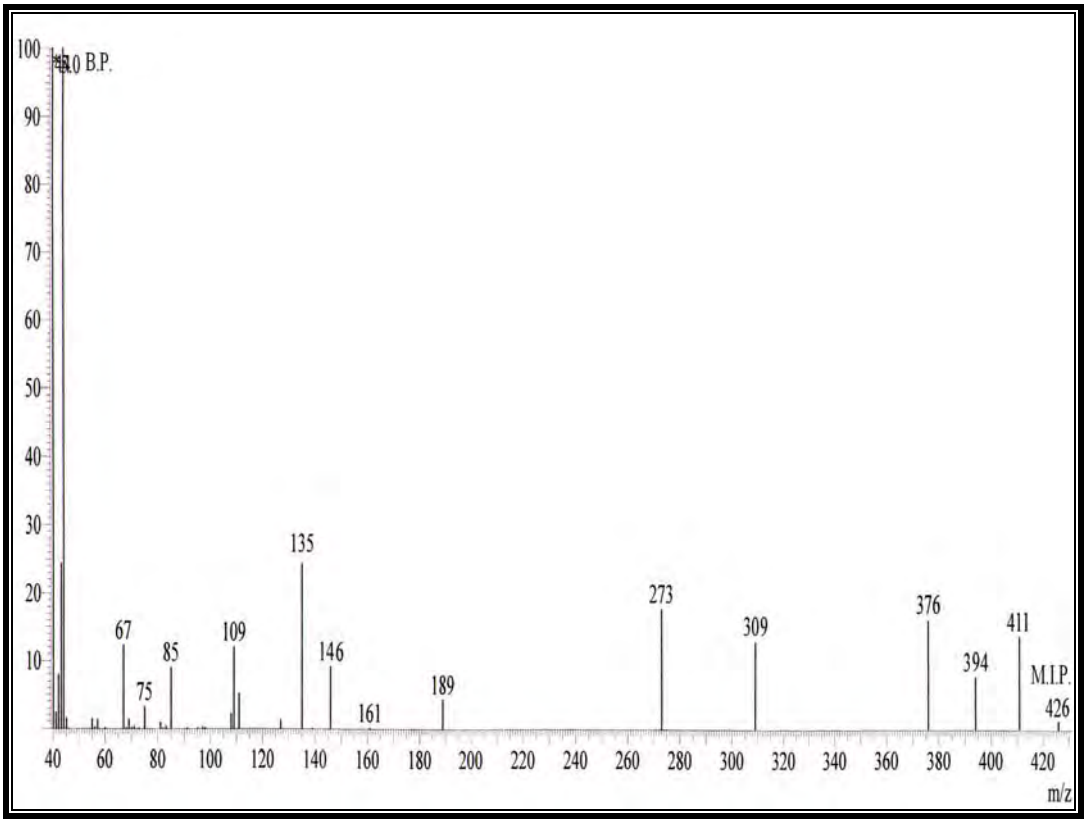
Expanded ^{1}H NMR spectrum of CPV-234



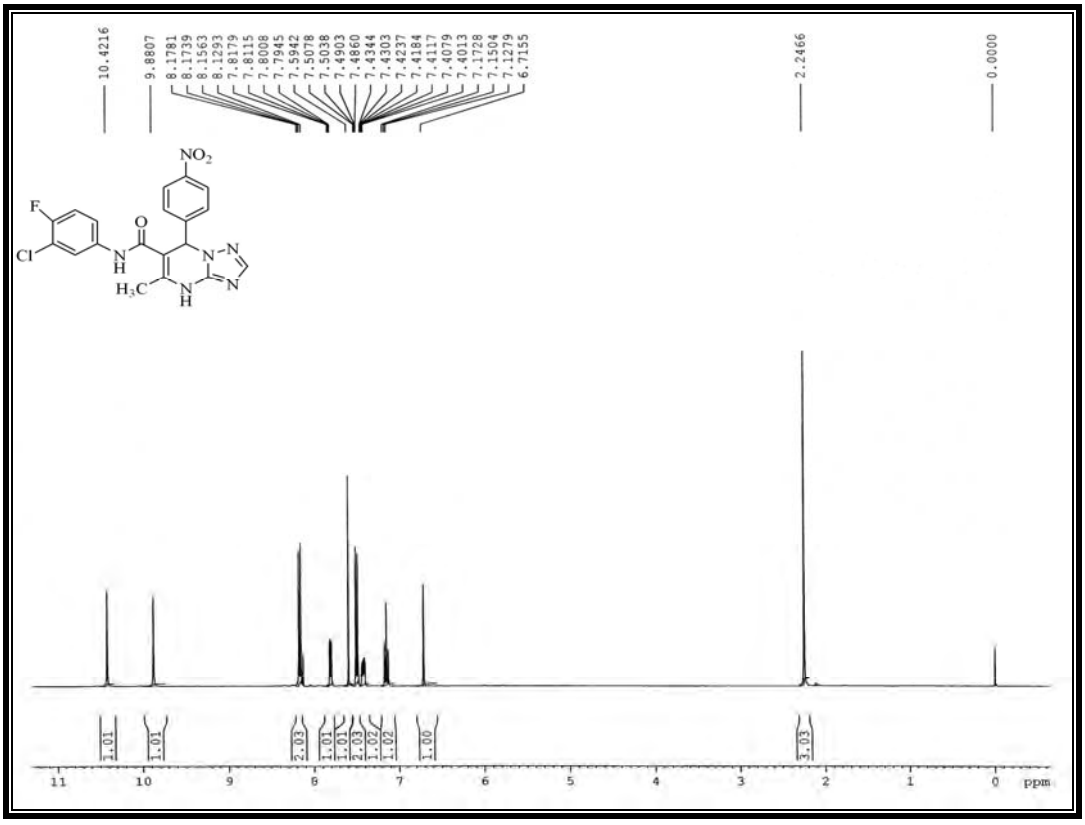
IR spectrum of CPV-236



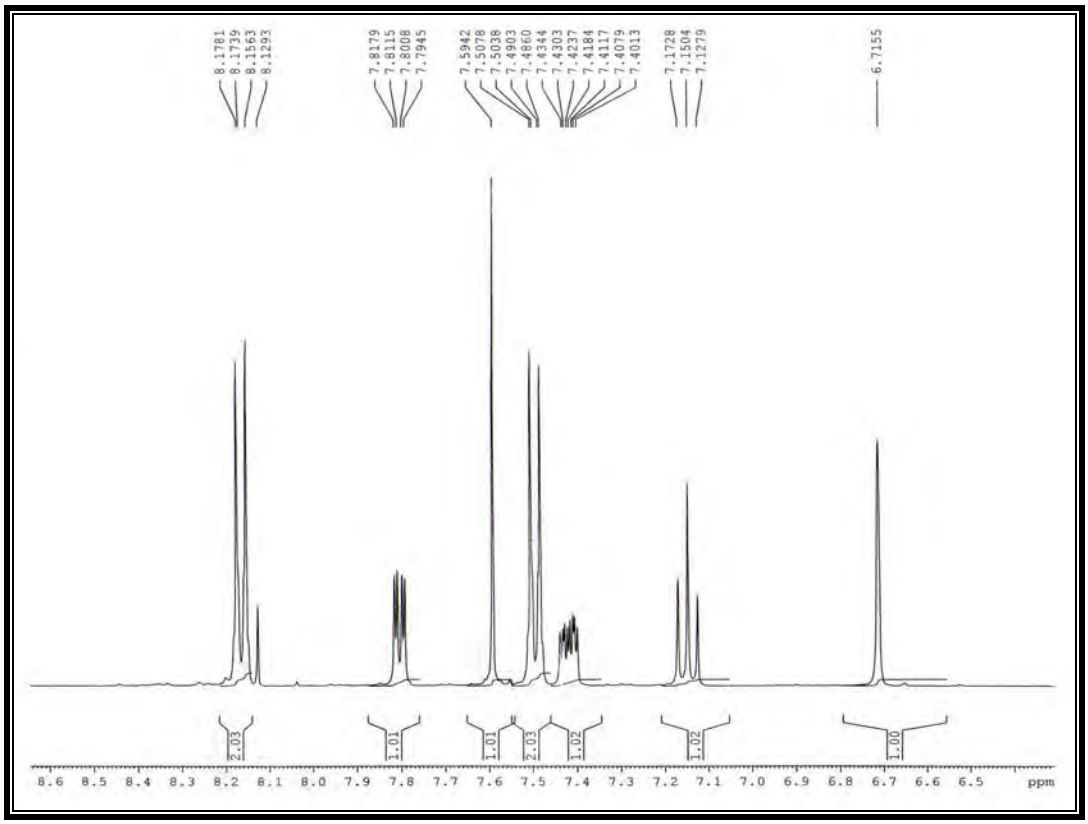
Mass spectrum of CPV-236



^{1}H NMR spectrum of CPV-236



Expanded ^{1}H NMR spectrum of CPV-236



4.8 Biological evaluation

4.8.1 Antimicrobial evaluation

All of the synthesized compounds (CPV-201 to 240) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [98-100] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards [98].

Minimal Inhibition Concentration [MIC]:-

The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Methods used for primary and secondary screening: -

Each synthesized drug was diluted obtaining 2000 μg mL^{-1} concentration, as a stock solution. Inoculum size for test strain was adjusted to 10^8 cfu (colony forming unit) per milliliter by comparing the turbidity.

Primary screen: - In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: - The drugs found active in primary screening were similarly diluted to obtain 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentrations.

Reading Result: - The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

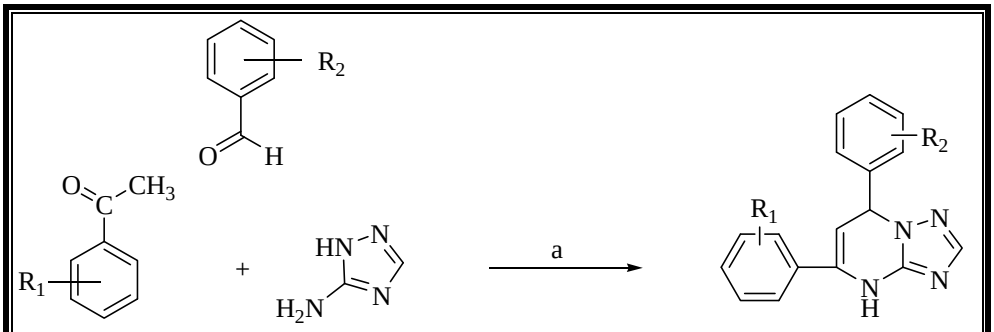
Table-1:- *In vitro* Antimicrobial Screening Results for CPV-201 to 240

Code	Minimal inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
CPV-201	200	200	150	100	500	1000	1000
CPV-202	200	200	100	150	1000	500	500
CPV-203	500	500	100	250	1000	500	1000
CPV-204	1000	500	500	500	250	1000	1000
CPV-205	100	62.5	62.5	50	500	500	1000
CPV-206	500	500	500	250	500	250	250
CPV-207	500	250	100	250	1000	1000	1000
CPV-208	1000	500	62.5	500	1000	500	500
CPV-209	1000	500	500	500	250	1000	1000
CPV-210	500	62.5	150	250	500	500	500
CPV-211	1000	250	250	500	250	1000	250
CPV-212	500	200	250	555	250	1000	1000
CPV-213	200	100	125	62.5	250	1000	1000
CPV-214	125	100	500	500	500	>1000	1000
CPV-215	500	500	500	250	500	500	1000
CPV-216	1000	500	500	500	500	>1000	>1000
CPV-217	1000	500	250	500	1000	500	>1000
CPV-218	500	500	62.5	50	1000	500	500
CPV-219	125	500	500	500	250	1000	1000
CPV-220	1000	500	500	250	500	500	500
CPV-221	62.5	200	250	500	500	1000	250
CPV-222	250	250	500	500	1000	>1000	>1000
CPV-223	500	500	500	250	1000	>1000	>1000
CPV-224	200	200	500	500	500	500	1000
CPV-225	250	500	250	250	500	1000	>1000
CPV-226	250	250	500	500	500	1000	1000
CPV-227	1000	100	500	250	1000	500	1000
CPV-228	500	62.5	62.5	500	250	500	500
CPV-229	62.5	100	125	500	500	1000	>1000
CPV-230	1000	250	100	250	1000	>1000	>1000
CPV-231	1000	500	250	555	250	1000	250
CPV-232	250	250	250	250	200	1000	1000
CPV-233	200	200	100	100	500	1000	1000
CPV-234	62.5	200	500	100	1000	1000	1000
CPV-235	200	250	200	500	1000	1000	1000
CPV-236	500	200	250	500	1000	1000	1000
CPV-237	100	62.5	150	62.5	250	500	250
CPV-238	1000	500	250	500	250	500	>1000
CPV-239	62.5	200	125	500	1000	>1000	>1000
CPV-240	1000	250	500	250	1000	500	>1000
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Iprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

4.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds CPV-201 to CPV-240 is currently under investigation and results are awaited.

4.9 Reaction Scheme

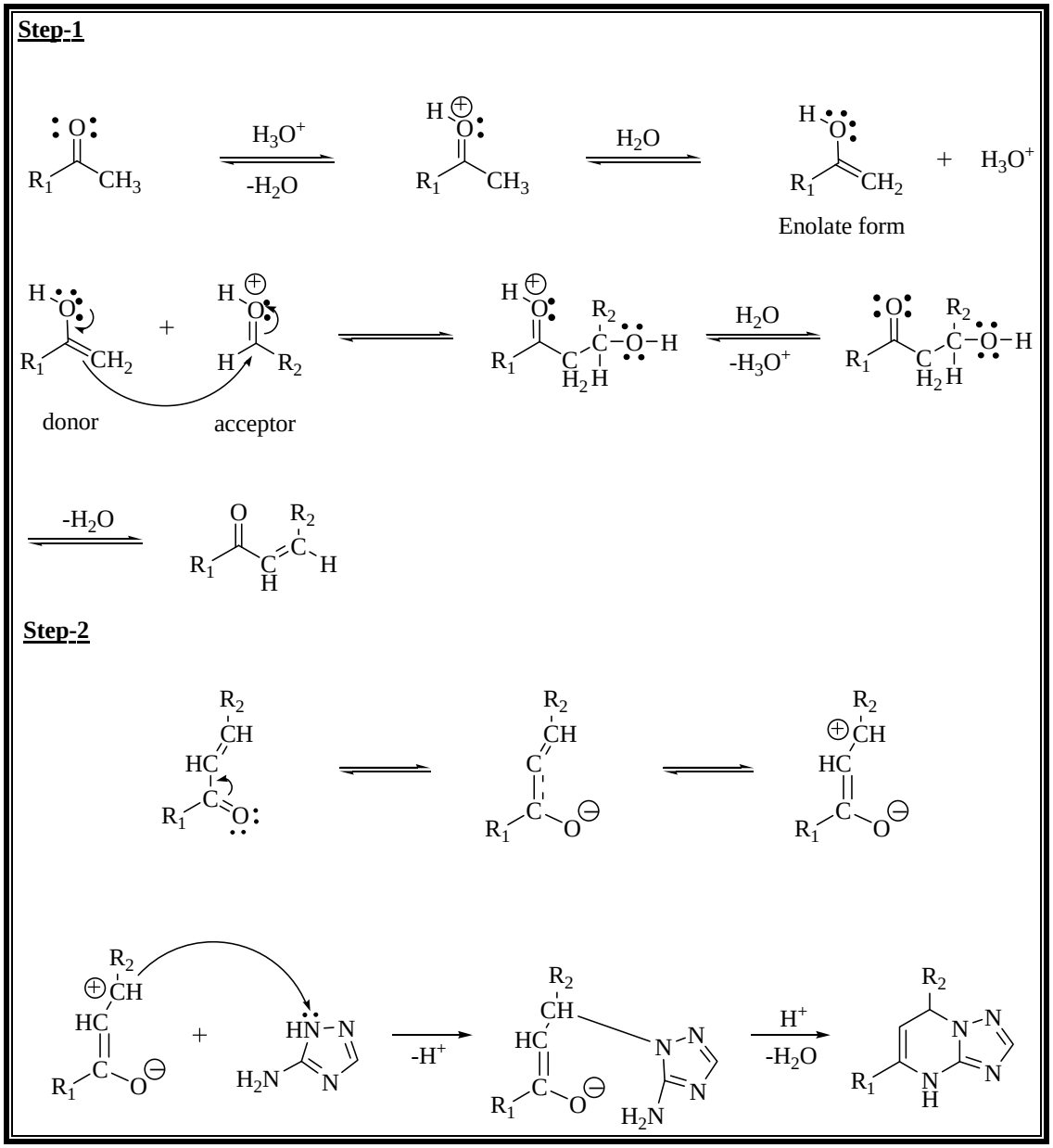


Reagents and conditions: (a) glacial HAc, Reflux, 20-24 hours **CPV 241 TO 280**

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
CPV-241	4-Br	H	$C_{17}H_{13}BrN_4$	353	185-187	70	0.56	0.71
CPV-242	4-Br	4-CH_3	$C_{18}H_{15}BrN_4$	367	179-181	75	0.51	0.69
CPV-243	4-Br	4-OCH3	$C_{18}H_{15}BrN_4O$	383	190-192	71	0.48	0.64
CPV-244	4-Br	4-Cl	$C_{17}H_{12}BrClN_4$	387	202-204	78	0.50	0.68
CPV-245	4-Br	4-F	$C_{17}H_{12}BrFN_4$	371	185-187	68	0.53	0.70
CPV-246	4-Br	4-NO_2	$C_{17}H_{12}BrN_5O_2$	398	221-223	72	0.44	0.74
CPV-247	4-Br	3-NO_2	$C_{17}H_{12}BrN_5O_2$	398	205-207	67	0.51	0.70
CPV-248	4-Br	2-NO_2	$C_{17}H_{12}BrN_5O_2$	398	193-195	81	0.50	0.63
CPV-249	4-Br	3-Cl	$C_{17}H_{12}BrClN_4$	387	207-209	76	0.41	0.62
CPV-250	4-Br	2-Cl	$C_{17}H_{12}BrClN_4$	387	185-187	69	0.49	0.74
CPV-251	4-Cl	H	$C_{17}H_{13}ClN_4$	308	221-223	66	0.52	0.69
CPV-252	4-Cl	4-CH_3	$C_{18}H_{15}ClN_4$	322	240-242	80	0.56	0.68
CPV-253	4-Cl	4-OCH3	$C_{18}H_{15}ClN_4O$	338	209-211	64	0.50	0.66
CPV-254	4-Cl	4-Cl	$C_{17}H_{12}Cl_2N_4$	343	267-269	75	0.61	0.77
CPV-255	4-Cl	4-F	$C_{17}H_{12}ClFN_4$	326	217-219	71	0.52	0.69
CPV-256	4-Cl	4-NO_2	$C_{17}H_{12}ClN_5O_2$	353	191-193	80	0.54	0.61
CPV-257	4-Cl	3- NO_2	$C_{17}H_{12}ClN_5O_2$	353	208-210	72	0.53	0.71
CPV-258	4-Cl	2-NO_2	$C_{17}H_{12}ClN_5O_2$	353	148-150	68	0.64	0.78
CPV-259	4-Cl	3-Cl	$C_{17}H_{12}Cl_2N_4$	343	164-166	77	0.48	0.62
CPV-260	4-Cl	2-Cl	$C_{17}H_{12}Cl_2N_4$	343	210-212	80	0.61	0.72
CPV-261	4-OCH_3	H	$C_{18}H_{16}N_4O$	304	205-207	76	0.45	0.56
CPV-262	4-OCH_3	4-CH_3	$C_{19}H_{18}N_4O$	318	196-198	63	0.55	0.62
CPV-263	4-OCH_3	4-OCH3	$C_{19}H_{18}N_4O_2$	334	225-227	82	0.52	0.70
CPV-264	4-OCH_3	4-Cl	$C_{18}H_{15}ClN_4O$	338	222-224	64	0.58	0.73
CPV-265	4-OCH_3	4-F	$C_{18}H_{15}FN_4O$	322	145-147	75	0.50	0.58
CPV-266	4-OCH_3	4-NO_2	$C_{18}H_{15}N_5O_3$	349	249-251	83	0.43	0.55
CPV-267	4-OCH_3	3-NO_2	$C_{18}H_{15}N_5O_3$	349	229-231	79	0.53	0.69
CPV-268	4-OCH_3	2-NO_2	$C_{18}H_{15}N_5O_3$	349	121-123	72	0.52	0.67
CPV-269	4-OCH_3	3-Cl	$C_{18}H_{15}ClN_4O$	338	212-214	74	0.53	0.61
CPV-270	4-OCH_3	2-Cl	$C_{18}H_{15}ClN_4O$	338	171-173	63	0.48	0.68
CPV-271	4-NO_2	H	$C_{17}H_{13}N_5O_2$	319	203-205	80	0.51	0.59
CPV-272	4-NO_2	4-CH_3	$C_{18}H_{15}N_5O_2$	333	227-229	77	0.45	0.63
CPV-273	4-NO_2	4-OCH3	$C_{18}H_{15}N_5O_3$	349	202-204	62	0.47	0.60
CPV-274	4-NO_2	4-Cl	$C_{17}H_{12}ClN_5O_2$	353	224-226	68	0.49	0.67
CPV-275	4-NO_2	4-F	$C_{17}H_{12}FN_5O_2$	337	264-266	60	0.57	0.64
CPV-276	4-NO_2	4-NO_2	$C_{17}H_{12}N_6O_4$	364	199-201	72	0.52	0.65
CPV-277	4-NO_2	3-NO_2	$C_{17}H_{12}N_6O_4$	364	212-214	75	0.48	0.55
CPV-278	4-NO_2	2-NO_2	$C_{17}H_{12}N_6O_4$	364	207-209	77	0.43	0.59
CPV-279	4-NO_2	3-Cl	$C_{17}H_{12}ClN_5O_2$	353	166-168	62	0.50	0.68
CPV-280	4-NO_2	2-Cl	$C_{17}H_{12}ClN_5O_2$	353	206-208	61	0.54	0.64

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4; TLC Solvent system R_{f2}: Chloroform: Methanol --9:1

4.10 Plausible Reaction Mechanism



4.11 Experimental

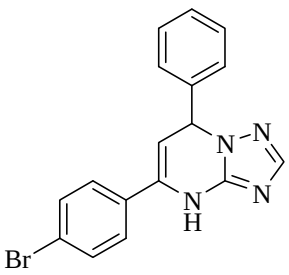
4.11.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreement with the structures assigned.

4.11.2 General procedure for the synthesis of 5-(4-bromophenyl)-4,7-dihydro-7-aryl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV 241-250)

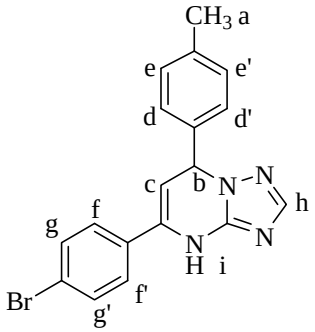
A mixture of the aminoazole (0.01 mol), 4-Bromo acetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 8 to 10 mL of glacial acetic acid for 20 to 24 hours. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 241-250, which were crystallized from ethanol and subsequently dried in air.

4.11.2.1 5-(4-bromophenyl)-4,7-dihydro-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-241)



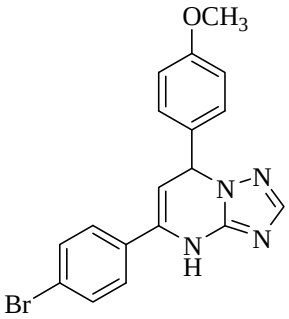
Yield: 70%; mp 185-187 ºC; MS: *m/z* 353; Anal. Calcd. For $C_{17}H_{13}BrN_4$: C, 57.81; H, 3.71; N, 15.86. Found: C, 57.72; H, 3.65; N, 15.80%.

4.11.2.2 5-(4-bromophenyl)-4,7-dihydro-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine

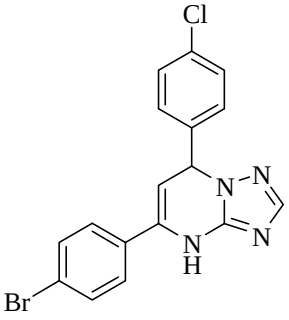


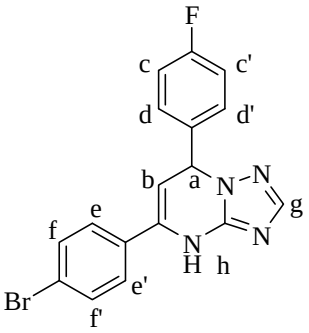
(CPV-242) Yield: 75%; mp 179-181 ºC; IR (cm^{-1}): 3090 (N-H stretching of secondary amine), 3028 (C-H stretching of aromatic ring), 2987 (C-H symmetrical stretching of CH_3 group), 2916 (C-H asymmetrical stretching of CH_3 group), 1658 (N-H deformation of pyrimidine ring), 1597 (C=N stretching of triazole ring), 1550 and 1512 (C=C stretching of aromatic ring), 1411 (C-H asymmetrical deformation of CH_3 group), 1330 (C-N stretching), 1226 (C-H symmetrical deformation of CH_3 group), 1203 (C-H in plane deformation of aromatic ring), 873 (C-H out of plane deformation of 1,4-disubstitution), 588 (C-Br stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, H_a), 5.11 (s, 1H, H_b), 6.11-6.12 (d, 1H, H_c, J = 4.0 Hz), 7.13-7.19 (dd, 4H, $H_{dd'\text{-}ee'}$, J = 8.0 Hz), 7.52-7.54 (m, 5H, $H_{f\text{-}h}$), 9.96 (s, 1H, H_i); MS: *m/z* 367; Anal. Calcd. For $C_{18}H_{15}BrN_4$: C, 58.87; H, 4.12; N, 15.26. Found: C, 58.80; H, 4.05; N, 15.20%.

4.11.2.3 5-(4-bromophenyl)-4,7-dihydro-7-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-243) Yield: 71%; mp 190-192 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{18}H_{15}BrN_4O$: C, 56.41; H, 3.95; N, 14.62; O, 4.17. Found: C, 56.35; H, 3.90; N, 14.55; O, 4.11%.

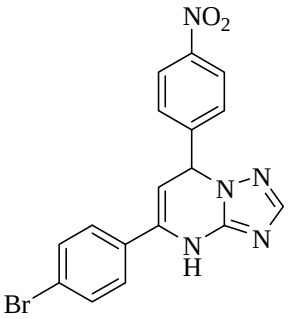


4.11.2.4 5-(4-bromophenyl)-7-(4-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-244) Yield: 78%; mp 202-204 ºC; MS: *m/z* 387; Anal. Calcd. for $C_{17}H_{12}BrClN_4$: C, 52.67; H, 3.12; N, 14.45. Found: C, 52.59; H, 3.04; N, 14.40%.

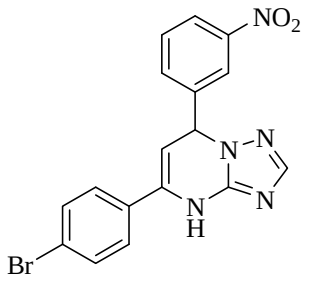


4.11.2.5 5-(4-bromophenyl)-7-(4-fluorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-245) 

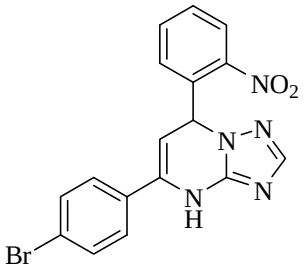
Yield: 68%; mp 185-187 ºC; IR (cm^{-1}): 3093 (N-H stretching of secondary amine), 3036 (C-H stretching of aromatic ring), 1656 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1550 and 1508 (C=C stretching of aromatic ring), 1410 (C-F stretching), 1330 (C-N stretching), 1226 (C-H in plane deformation of aromatic ring), 842 (C-H out of plane deformation of 1,4-disubstitution), 717 (C-Br stretching); ^{1}H NMR (DMSO-d_6) δ ppm: ^{1}H NMR (DMSO-d_6) δ ppm: 5.10-5.11 (d, 1H, H_a, J = 4.0 Hz), 6.18-6.19 (d, 1H, H_b, J = 4.0 Hz), 7.06-7.10 (m, 2H, $H_{cc'}$), 7.32-7.35 (m, 2H, $H_{dd'}$), 7.53-7.54 (m, 5H, H_{e-g}), 10.03 (s, 1H, H_h); MS: *m/z* 371; Anal. Calcd. for $C_{17}H_{12}BrFN_4$: C, 55.00; H, 3.26; N, 15.09. Found: C, 53.99; H, 3.20; N, 15.05%.

4.11.2.6 5-(4-bromophenyl)-4,7-dihydro-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-246) 

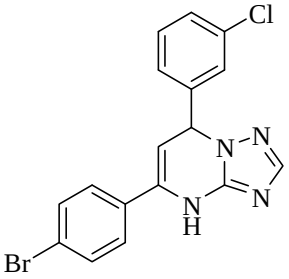
Yield: 72%; mp 221-223 ºC; MS: *m/z* 398; Anal. Calcd. for $C_{17}H_{12}BrN_5O_2$: C, 51.27; H, 3.04; N, 17.59; O, 8.04. Found: C, 51.20; H, 2.96; N, 17.50; O, 8.00%.

4.11.2.7 5-(4-bromophenyl)-4,7-dihydro-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-247) 

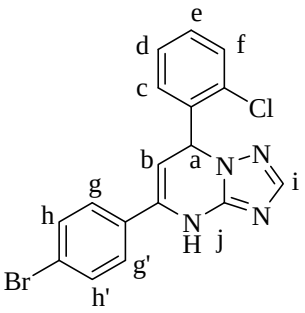
Yield: 67%; mp 205-207 ºC; MS: *m/z* 398; Anal. Calcd. for $C_{17}H_{12}BrN_5O_2$: C, 51.27; H, 3.04; N, 17.59; O, 8.04. Found: C, 51.15; H, 2.94; N, 17.52; O, 8.00%.

4.11.2.8 5-(4-bromophenyl)-4,7-dihydro-7-(2-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-248) 

Yield: 81%; mp 193-195 ºC; MS: *m/z* 398; Anal. Calcd. for $C_{17}H_{12}BrN_5O_2$: C, 51.27; H, 3.04; N, 17.59; O, 8.04. Found: C, 51.21; H, 2.91; N, 17.53; O, 7.99%.

4.11.2.9 5-(4-bromophenyl)-7-(3-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-249) 

Yield: 76%; mp 207-209 ºC; MS: *m/z* 388; Anal. Calcd. for $C_{17}H_{12}BrClN_4$: C, 52.67; H, 3.12; N, 14.45. Found: C, 52.58; H, 3.06; N, 14.39%.

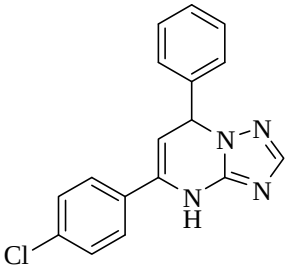
4.11.2.10 5-(4-bromophenyl)-7-(2-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-250) 

Yield: 69%; mp 185-187 ºC; IR (cm^{-1}): IR (cm^{-1}): 3254 (N-H stretching of secondary amine), 3101 (C-H stretching of aromatic ring), 1683 (N-H deformation of pyrimidine ring), 1597 (C=N stretching of triazole ring), 1473 and 1442 (C=C stretching of aromatic ring), 1269 (C-N stretching), 1199 (C-H in plane deformation of aromatic ring), 835 (C-H out of plane deformation of 1,4-disubstitution), 808 (C-Cl stretching), 758 (C-Br stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 5.08-5.09 (d, 1H, H_a, J = 4.0 Hz), 6.60-6.61 (d, 1H, H_b, J = 4.0 Hz), 7.00-7.02 (m, 1H, H_c), 7.25-7.27 (m, 2H, H_{de}), 7.38-7.41 (m, 1H, H_f), 7.48-7.53 (m, 4H, $H_{g\text{-}h'}$), 7.59 (s, 1H, H_i), 10.00 (s, 1H, H_j): MS: *m/z* 388; Anal. Calcd. for $C_{17}H_{12}BrClN_4$: C, 52.67; H, 3.12; N, 14.45. Found: C, 52.61; H, 3.06; N, 14.38%.

4.11.3 General procedure for the synthesis of 5-(4-chlorophenyl)-4,7-dihydro-7-aryl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV 251-260)

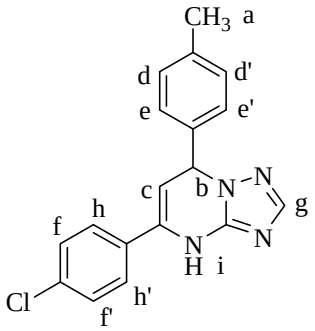
A mixture of the aminoazole (0.01 mol), 4-Chloro acetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 8 to 10 mL of glacial acetic acid for 20 to 24 hours. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 251-260, which were crystallized from ethanol and subsequently dried in air.

4.11.3.1 5-(4-chlorophenyl)-4,7-dihydro-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-251)



Yield: 66%; mp 221-223 ºC; MS: *m/z* 309; Anal. Calcd. for $C_{17}H_{13}ClN_4$: C, 66.13; H, 4.24; N, 18.15. Found: C, 66.05; H, 4.20; N, 18.09%.

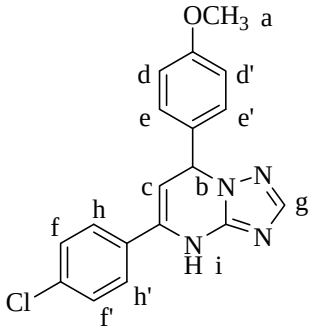
4.11.3.2 5-(4-chlorophenyl)-4,7-dihydro-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-252)



Yield: 80%; mp 240-242 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3109 (C-H stretching of aromatic ring), 3030 (C-H asymmetrical stretching of CH_3 group), 2831 (C-H symmetrical stretching of CH_3 group), 1683 (C=N stretching of triazole ring), 1622 (N-H deformation of pyrimidine ring), 1554 and 1498 (C=C stretching of aromatic ring), 1425 (C-H asymmetrical deformation of CH_3 group), 1375 (C-H symmetrical deformation of CH_3 group), 1294 (C-N stretching), 1203 (C-H in plane deformation of aromatic ring), 802 (C-H out of plane deformation of 1,4-disubstitution), 721 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, H_a), 5.11-5.12 (d, 1H, H_b, J = 4.0 Hz), 6.12-6.13 (d, 1H, H_c, J = 4.0 Hz), 7.13-7.19 (2×d, 2×2H, $H_{d\text{-}e'}$, J = 8.0 Hz), 7.39-7.41 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.53 (s, 1H, H_g), 7.59-7.61 (d, 2H, $H_{hh'}$, J = 8.0

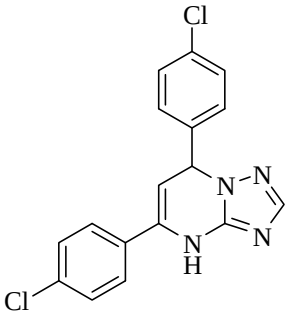
Hz), 9.97 (s, 1H, H_i): MS: *m/z* 323; Anal. Calcd. for $C_{18}H_{15}ClN_4$: C, 66.98; H, 4.68; N, 17.36. Found: C, 66.89; H, 4.62; N, 17.30%.

4.11.3.3 5-(4-chlorophenyl)-4,7-dihydro-7-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-253)

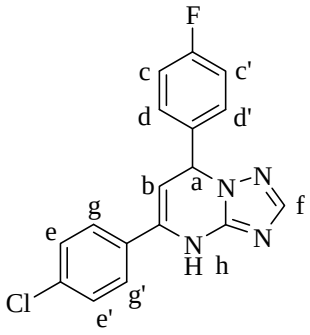


Yield: 64%; mp 209-211 ºC; IR (cm^{-1}): 3252 (N-H stretching of secondary amine), 3099 (C-H stretching of aromatic ring), 1683 (N-H deformation of pyrimidine ring), 1604 (C=N stretching of triazole ring), 1552 and 1510 (C=C stretching of aromatic ring), 1425 (C-H asymmetrical deformation of CH_3 group), 1298 (C-N stretching), 1246 (C-H in plane deformation of aromatic ring), 1199 (C-O-C asymmetrical stretching of ether linkage), 1060 (C-O-C asymmetrical stretching of ether linkage), 802 (C-H out of plane deformation of 1,4-disubstitution), 721 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 3.76 (s, 3H, H_a), 5.08-5.09 (d, 1H, H_b, J = 4.0 Hz), 6.10-6.11 (d, 1H, H_c, J = 4.0 Hz), 6.86-6.88 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.23-7.25 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.38-7.40 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.51 (s, 1H, H_g), 7.57-7.61 (m, 2H, $H_{hh'}$), 9.93 (s, 1H, H_i): MS: *m/z* 339; Anal. Calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54; O, 4.72. Found: C, 63.75; H, 4.42; N, 16.50; O, 4.63%.

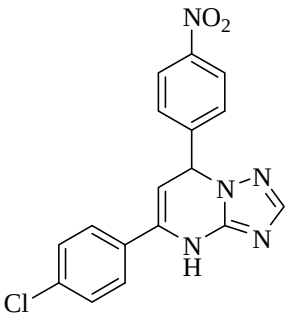
4.11.3.4 5,7-bis(4-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-254)



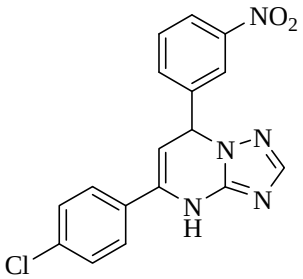
Yield: 71%; mp 217-219 ºC; MS: *m/z* 343; Anal. Calcd. for $C_{17}H_{12}Cl_2N_4$: C, 59.49; H, 3.52; N, 16.32. Found: C, 59.40; H, 3.43; N, 16.27%.

4.11.3.5 5-(4-chlorophenyl)-7-(4-fluorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-255) 

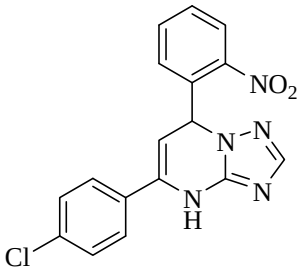
Yield: 75%; mp 267-269 ºC; IR (cm^{-1}): 3553 (N-H stretching of secondary amine), 3097 (C-H stretching of aromatic ring), 1656 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1550 and 1506 (C=C stretching of aromatic ring), 1330 (C-N stretching), 1222 (C-H in plane deformation of aromatic ring), 1134 (C-F stretching), 842 (C-H out of plane deformation of 1,4-disubstitution), 795 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 5.08-5.09 (d, 1H, H_a, J = 4.0 Hz), 6.18-6.19 (d, 1H, H_b, J = 4.0 Hz), 7.05-7.10 (m, 2H, $H_{cc'}$), 7.31-7.36 (m, 2H, $H_{dd'}$), 7.38-7.40 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.52 (s, 1H, H_f), 7.58-7.59 (d, 2H, $H_{gg'}$, J = 4.0 Hz), 10.07 (s, 1H, H_h): MS: *m/z* 327; Anal. Calcd. for $C_{17}H_{12}ClFN_4$: C, 62.49; H, 3.70; N, 17.15. Found: C, 62.40; H, 3.65; N, 17.10%.

4.11.3.6 5-(4-chlorophenyl)-4,7-dihydro-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-256) 

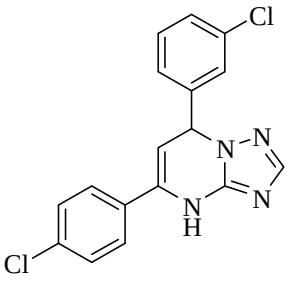
Yield: 80%; mp 191-193 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.65; H, 3.36; N, 19.76; O, 8.93%.

4.11.3.7 5-(4-chlorophenyl)-4,7-dihydro-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-257) 

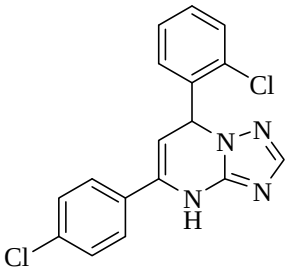
Yield: 72%; mp 208-210 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.67; H, 3.38; N, 19.74; O, 8.95%.

4.11.3.8 5-(4-chlorophenyl)-4,7-dihydro-7-(2-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-258) 

Yield: 68%; mp 148-150 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.64; H, 3.35; N, 19.73; O, 8.97%.

4.11.3.9 7-(3-chlorophenyl)-5-(4-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-259) 

Yield: 77%; mp 164-166 ºC; MS: *m/z* 343; Anal. Calcd. for $C_{17}H_{12}Cl_2N_4$: C, 59.49; H, 3.52; N, 16.32. Found: C, 59.42; H, 3.45; N, 16.22%.

4.11.3.10 7-(2-chlorophenyl)-5-(4-chlorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-260) 

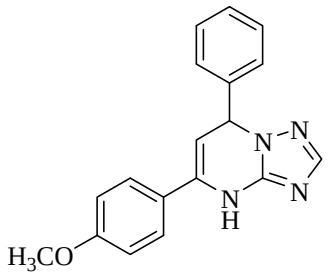
Yield: 80%; mp 210-212 ºC; MS: *m/z* 343; Anal. Calcd. for $C_{17}H_{12}Cl_2N_4$: C, 59.49; H, 3.52; N, 16.32. Found: C, 59.40; H, 3.46; N, 16.24%.

4.11.4 General procedure for the synthesis of 4,7-dihydro-5-(4-methoxyphenyl)-7-aryl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV 261-270)

A mixture of the aminoazole (0.01 mol), 4-Methoxy acetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 8 to 10 mL of glacial acetic acid for 20 to 24 hours. After cooling, methanol (~10 mL) was added. The reaction

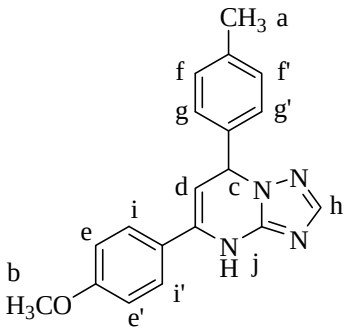
mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 261-270, which were crystallized from ethanol and subsequently dried in air.

4.11.4.1 4,7-dihydro-5-(4-methoxyphenyl)-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine



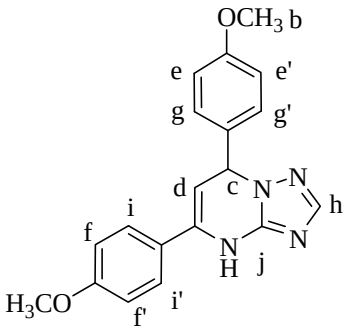
(CPV-261) Yield: 76%; mp 205-207 ºC; MS: *m/z* 304; Anal. Calcd. for $C_{18}H_{16}N_4O$: C, 71.04; H, 5.30; N, 18.41; O, 5.26. Found: C, 70.98; H, 5.24; N, 18.36; O, 5.20%.

4.11.4.2 4,7-dihydro-5-(4-methoxyphenyl)-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine



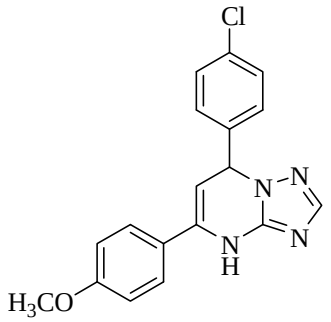
(CPV-262) Yield: 63%; mp 196-198 ºC; IR (cm^{-1}): 3265 (N-H stretching of secondary amine), 3101 (C-H stretching of aromatic ring), 3026 (C-H symmetrical stretching of CH_3 group), 2895 (C-H asymmetrical stretching of CH_3 group), 1591 (C=N stretching of triazole ring), 1554 (N-H deformation of pyrimidine ring), 1514 and 1492 (C=C stretching of aromatic ring), 1396 (C-H asymmetrical deformation of CH_3 group), 1325 (C-H symmetrical deformation of CH_3 group), 1247 (C-N stretching), 1230 (C-O-C asymmetrical stretching of ether linkage), 1089 (C-H in plane deformation of aromatic ring), 1031 (C-O-C symmetrical stretching of ether linkage), 825 (C-H out of plane deformation of 1,4-disubstitution), 781 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, H_a), 3.80 (s, 3H, H_b), 4.97-4.98 (d, 1H, H_c, J = 4.0 Hz), 6.08-6.09 (d, 1H, H_d, J = 4.0 Hz), 6.91-6.93 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.12-7.19 (2×d, 2×2H, $H_{ff'-gg'}$, J = 8.0 Hz), 7.50-7.54 (m, 3H, $H_{h-i'}$), 9.87 (s, 1H, H_j): MS: *m/z* 318; Anal. Calcd. for $C_{19}H_{18}N_4O$: C, 71.68; H, 5.70; N, 17.60; O, 5.03. Found: C, 71.62; H, 5.65; N, 17.52; O, 4.96%.

4.11.4.3 4,7-dihydro-5,7-bis(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine

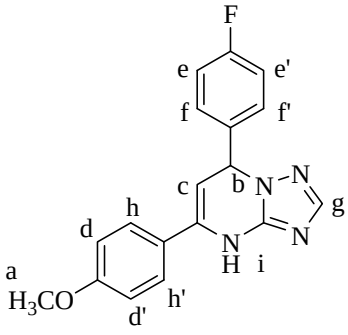


(CPV-263) Yield: 82%; mp 225-227 ºC; IR (cm^{-1}): 3198 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 1658 (N-H deformation of pyrimidine ring), 1595 (C=N stretching of triazole ring), 1548 and 1512 (C=C stretching of aromatic ring), 1336 (C-N stretching), 1298 (C-H in plane deformation of aromatic ring), 1246 (C-O-C asymmetrical stretching of ether linkage), 1035 (C-O-C symmetrical stretching of ether linkage), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.75 (s, 3H, H_a), 3.79 (s, 3H, H_b), 4.96-4.97 (d, 1H, H_c, J = 4.0 Hz), 6.07-6.08 (d, 1H, H_d, J = 4.0 Hz), 6.85-6.87 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 6.91-6.93 (d, 2H, $H_{ff'}$, J = 8.0 Hz), 7.23-7.25 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 7.48 (s, 1H, H_h), 7.53-7.55 (d, 2H, $H_{ii'}$, J = 8.0 Hz), 9.99 (s, 1H, H_j); MS: *m/z* 334; Anal. Calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76; O, 9.57. Found: C, 68.20; H, 5.36; N, 16.71; O, 9.53%.

4.11.4.4 7-(4-chlorophenyl)-4,7-dihydro-5-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-264) Yield: 75%; mp 145-147ºC; MS: *m/z* 339; Anal. Calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54; O, 4.72. Found: C, 63.71; H, 4.42; N, 16.50; O, 4.66%.

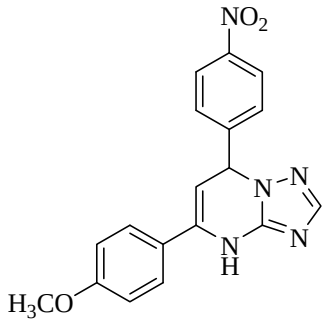


4.11.4.5 7-(4-fluorophenyl)-4,7-dihydro-5-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-265) Yield: 64%; mp 222-224 ºC; IR (cm^{-1}): 3477 (N-H stretching of secondary amine), 3091 (C-H stretching of aromatic ring), 1656 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1552 and 1512 (C=C stretching of aromatic ring), 1338 (C-N stretching),



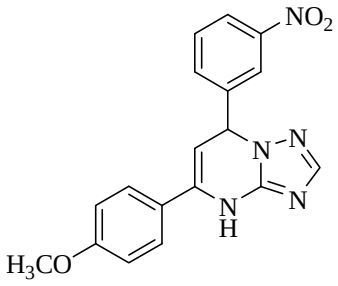
1298 (C-F stretching), 1224 (C-O-C asymmetrical stretching of ether linkage), 1136 (C-O-C stretching), 1072 (C-H in plane deformation of aromatic ring), 1031 (C-O-C symmetrical stretching of ether linkage), 842 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.82 (s, 3H, H_a), 4.95-4.96 (d, 1H, H_b, J = 4.0 Hz), 6.14-6.15 (d, 1H, H_c, J = 4.0 Hz), 6.91-6.93 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.03-7.07 (m, 2H, $H_{ee'}$), 7.33-7.36 (m, 2H, $H_{ff'}$), 7.52-7.55 (m, 3H, $H_{g-h'}$), 9.73 (s, 1H, H_i); MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{15}FN_4O$: C, 67.07; H, 4.69; N, 17.38; O, 4.96. Found: C, 67.00; H, 4.62; N, 17.30 O, 4.90%.

4.11.4.6 4,7-dihydro-5-(4-methoxyphenyl)-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-266)



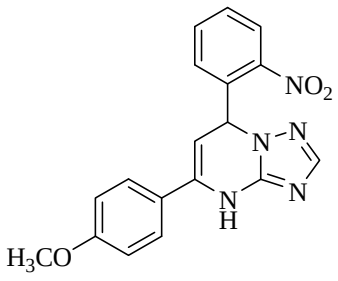
Yield: 83%; mp 249-251 ºC; MS: *m/z* 349; Anal. Calcd. for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74. Found: C, 61.81; H, 4.24; N, 19.96; O, 13.69%.

4.11.4.7 4,7-dihydro-5-(4-methoxyphenyl)-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-267)



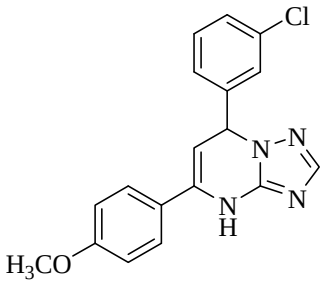
Yield: 79%; mp 229-231 ºC; MS: *m/z* 349; Anal. Calcd. for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74. Found: C, 61.80; H, 4.22; N, 19.98; O, 13.68%.

4.11.4.8 4,7-dihydro-5-(4-methoxyphenyl)-7-(2-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-268)

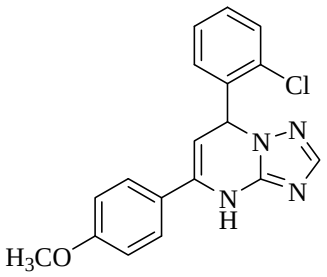


Yield: 72%; mp 121-123 ºC; MS: *m/z* 349; Anal. Calcd. for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74. Found: C, 61.81; H, 4.25; N, 19.95; O, 13.65%.

4.11.4.9 7-(3-chlorophenyl)-4,7-dihydro-5-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-269) Yield: 74%; mp 212-214 ºC; MS: *m/z* 339; Anal. Calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54; O, 4.72. Found: C, 63.75; H, 4.40; N, 16.50; O, 4.64%.

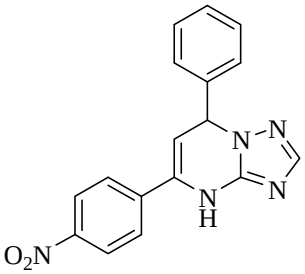


4.11.4.10 7-(2-chlorophenyl)-4,7-dihydro-5-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-270) Yield: 63%; mp 171-173 ºC; MS: *m/z* 339; Anal. Calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54; O, 4.72. Found: C, 63.77; H, 4.38; N, 16.49; O, 4.66%.

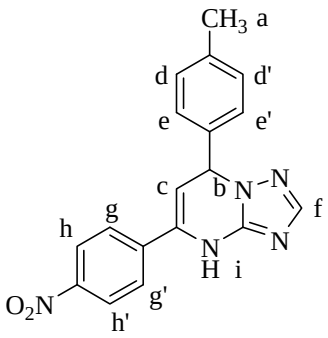


4.11.5 General procedure for the synthesis of 4,7-dihydro-5-(4-nitrophenyl)-7-aryl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV 271-280)

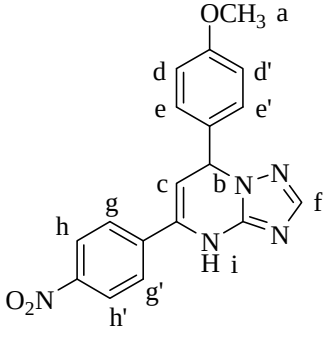
A mixture of the aminoazole (0.01 mol), 4-Nitro acetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 8 to 10 mL of glacial acetic acid for 20 to 24 hours. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products CPV 271-280, which were crystallized from ethanol and subsequently dried in air.

4.11.5.1 4,7-dihydro-5-(4-nitrophenyl)-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-271) 

Yield: 80%; mp 203-205 ºC; MS: *m/z* 319; Anal. Calcd. for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.93; O, 10.02. Found: C, 63.90; H, 4.04; N, 21.87; O, 9.92%.

4.11.5.2 4,7-dihydro-5-(4-nitrophenyl)-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-272) 

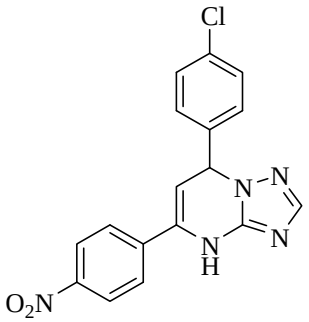
Yield: 77%; mp 227-229 ºC; IR (cm^{-1}): 3553 (N-H stretching of secondary amine), 3255 (C-H symmetrical stretching of CH_3 group), 3095 (C-H stretching of aromatic ring), 2939 (C-H asymmetrical stretching of CH_3 group), 1653 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1554 and 1475 (C=C stretching of aromatic ring), 1518 and 1377 (C-NO_2 stretching), 1344 (C-N stretching), 1315 (C-H asymmetrical deformation of CH_3 group), 1271 (C-H symmetrical deformation of CH_3 group), 1197 (C-H in plane deformation of aromatic ring), 810 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.14 (s, 3H, H_a), 5.29-5.30 (d, 1H, H_b, J = 4.0 Hz), 6.16-6.17 (d, 1H, H_c, J = 4.0 Hz), 7.15-7.21 (2×d, 2×2H, $H_{dd'\text{-}ee'}$, J = 8.0 Hz), 7.54 (s, 1H, H_f), 7.84-7.86 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 8.23-8.25 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 10.17 (s, 1H, H_i); MS: *m/z* 333; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01; O, 9.60. Found: C, 64.80; H, 4.49; N, 20.94; O, 9.52%.

4.11.5.3 4,7-dihydro-7-(4-methoxyphenyl)-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-273) 

Yield: 62%; mp 202-204 ºC; IR (cm^{-1}): 3201 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 1595 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1512 and 1464 (C=C stretching of aromatic ring), 1346 (C-N stretching), 1307 (C-NO_2

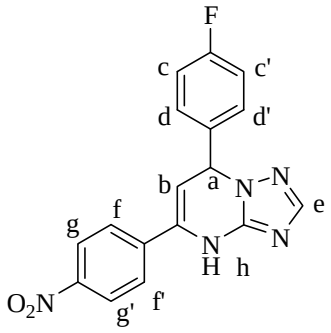
stretching), 1247 (C-H in plane deformation of aromatic ring), 1197 (C-O-C asymmetrical stretching of ether linkage), 1031 (C-O-C symmetrical stretching of ether linkage), 808 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.77 (s, 3H, H_a), 5.29-5.30 (d, 1H, H_b, J = 4.0 Hz), 6.15-6.16 (d, 1H, H_c, J = 4.0 Hz), 6.87-6.89 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.25-7.27 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.53 (s, 1H, H_f), 7.85-7.87 (d, 2H, $H_{gg'}$, J = 8.0 Hz), 8.23-8.25 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 10.16 (s, 1H, H_i); MS: *m/z* 349; Anal. Calcd. for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74. Found: C, 61.82; H, 4.25; N, 19.97; O, 13.70%.

4.11.5.4 7-(4-chlorophenyl)-4,7-dihydro-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]



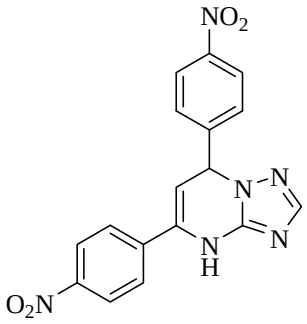
pyrimidine (CPV-274) Yield: 60%; mp 264-266 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.63; H, 3.35; N, 19.74; O, 9.00%.

4.11.5.5 7-(4-fluorophenyl)-4,7-dihydro-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]

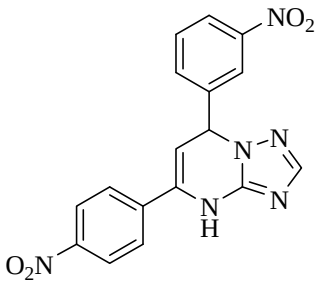


pyrimidine (CPV-275) Yield: 68%; mp 224-226 ºC; IR (cm^{-1}): 3099 (N-H stretching of secondary amine), 3010 (C-H stretching of aromatic ring), 1595 (C=N stretching of triazole ring), 1552 (N-H deformation of pyrimidine ring), 1510 and 1479 (C=C stretching of aromatic ring), 1346 (C-NO_2 stretching), 1269 (C-N stretching), 1219 (C-H in plane deformation of aromatic ring), 1134 (C-F stretching), 804 (C-H out of plane deformation of 1,4-disubstitution),; ^{1}H NMR (DMSO-d_6) δ ppm: 5.31-5.32 (d, 1H, H_a, J = 4.0 Hz), 6.24-6.25 (d, 1H, H_b, J = 4.0 Hz), 7.07-7.11 (m, 2H, $H_{cc'}$), 7.33-7.37 (m, 2H, $H_{dd'}$), 7.56 (s, 1H, H_e), 7.84-7.89 (m, 2H, $H_{ff'}$), 8.23-8.25 (m, 2H, $H_{gg'}$), 10.22 (s, 1H, H_h); MS: *m/z* 337; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$: C, 60.53; H, 3.59; N, 20.76; O, 9.49. Found: C, 60.48; H, 3.52; N, 20.70; O, 9.45%.

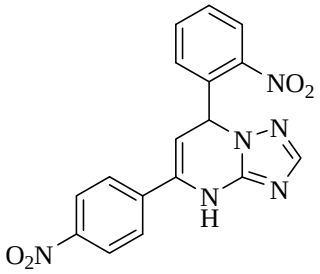
4.11.5.6 4,7-dihydro-5,7-bis(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-276) Yield: 72%; mp 199-201 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{17}H_{12}N_6O_4$: C, 56.05; H, 3.32; N, 23.07; O, 17.57. Found: C, 55.99; H, 3.27; N, 23.00; O, 17.50%.



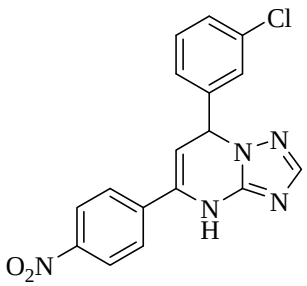
4.11.5.7 4,7-dihydro-7-(3-nitrophenyl)-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (CPV-277) Yield: 75%; mp 212-214 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{17}H_{12}N_6O_4$: C, 56.05; H, 3.32; N, 23.07; O, 17.57. Found: C, 55.98; H, 3.25; N, 23.02; O, 17.51%.

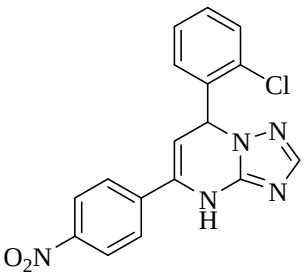


4.11.5.8 4,7-dihydro-7-(2-nitrophenyl)-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (CPV-278) Yield: 77%; mp 207-209 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{17}H_{12}N_6O_4$: C, 56.05; H, 3.32; N, 23.07; O, 17.57. Found: C, 55.95; H, 3.26; N, 23.00; O, 17.49%.



4.11.5.9 7-(3-chlorophenyl)-4,7-dihydro-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (CPV-279) Yield: 62%; mp 166-168 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.65; H, 3.37; N, 19.70; O, 8.98%.



4.11.5.10 7-(2-chlorophenyl)-4,7-dihydro-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (CPV-280) 

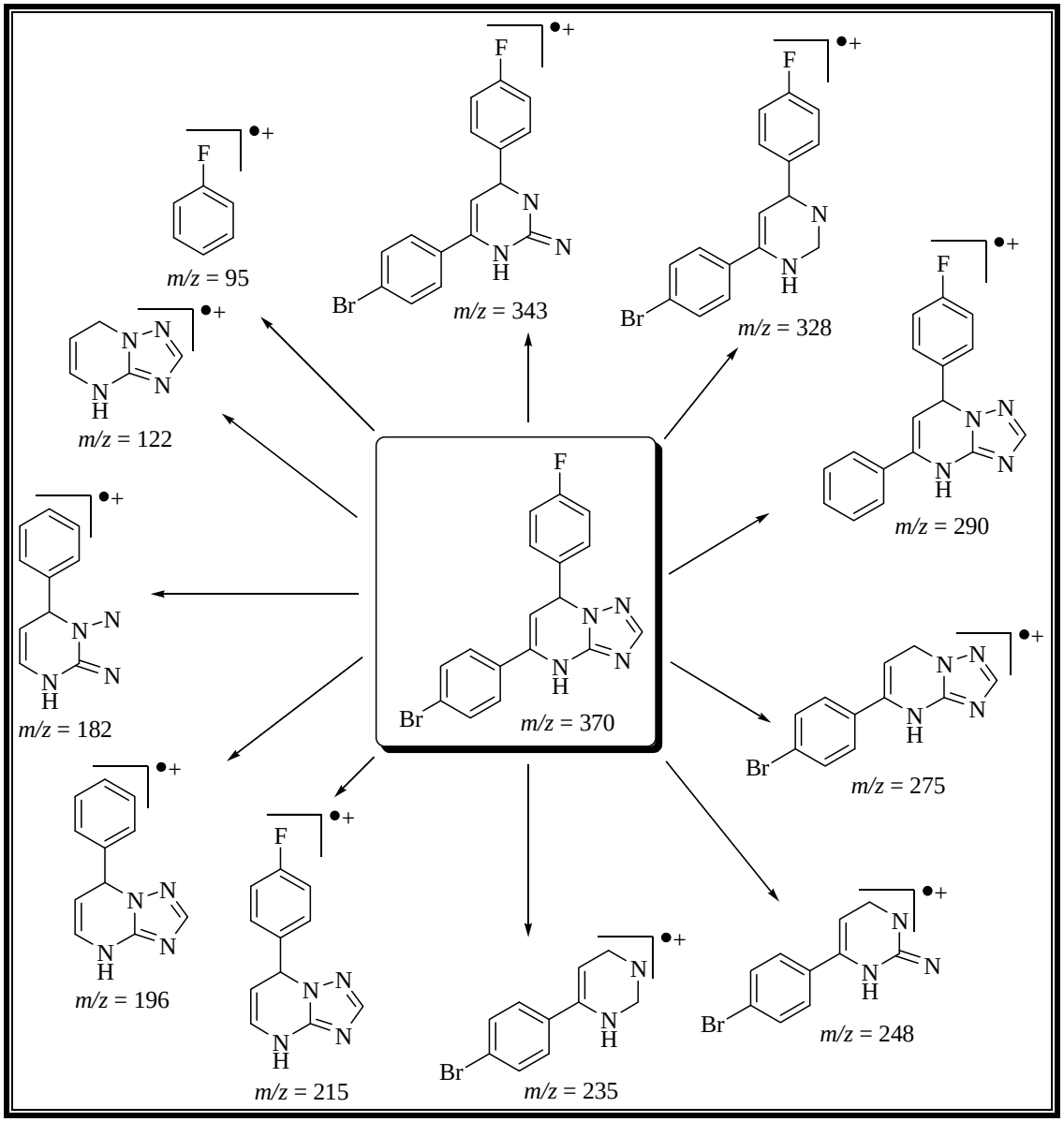
Yield: 61%; mp 206-208 ºC; MS: *m/z* 354; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; N, 19.80; O, 9.05. Found: C, 57.63; H, 3.35; N, 19.75; O, 8.97%.

4.12 Spectral discussion

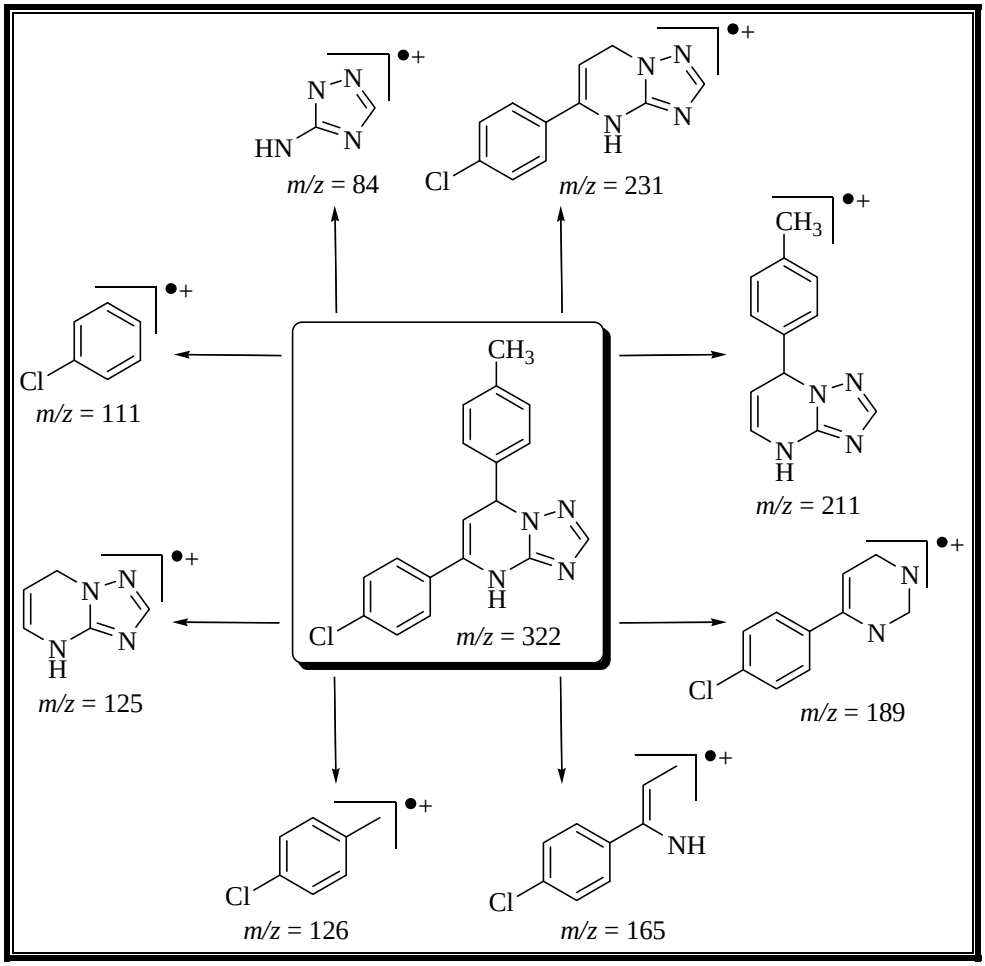
4.12.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

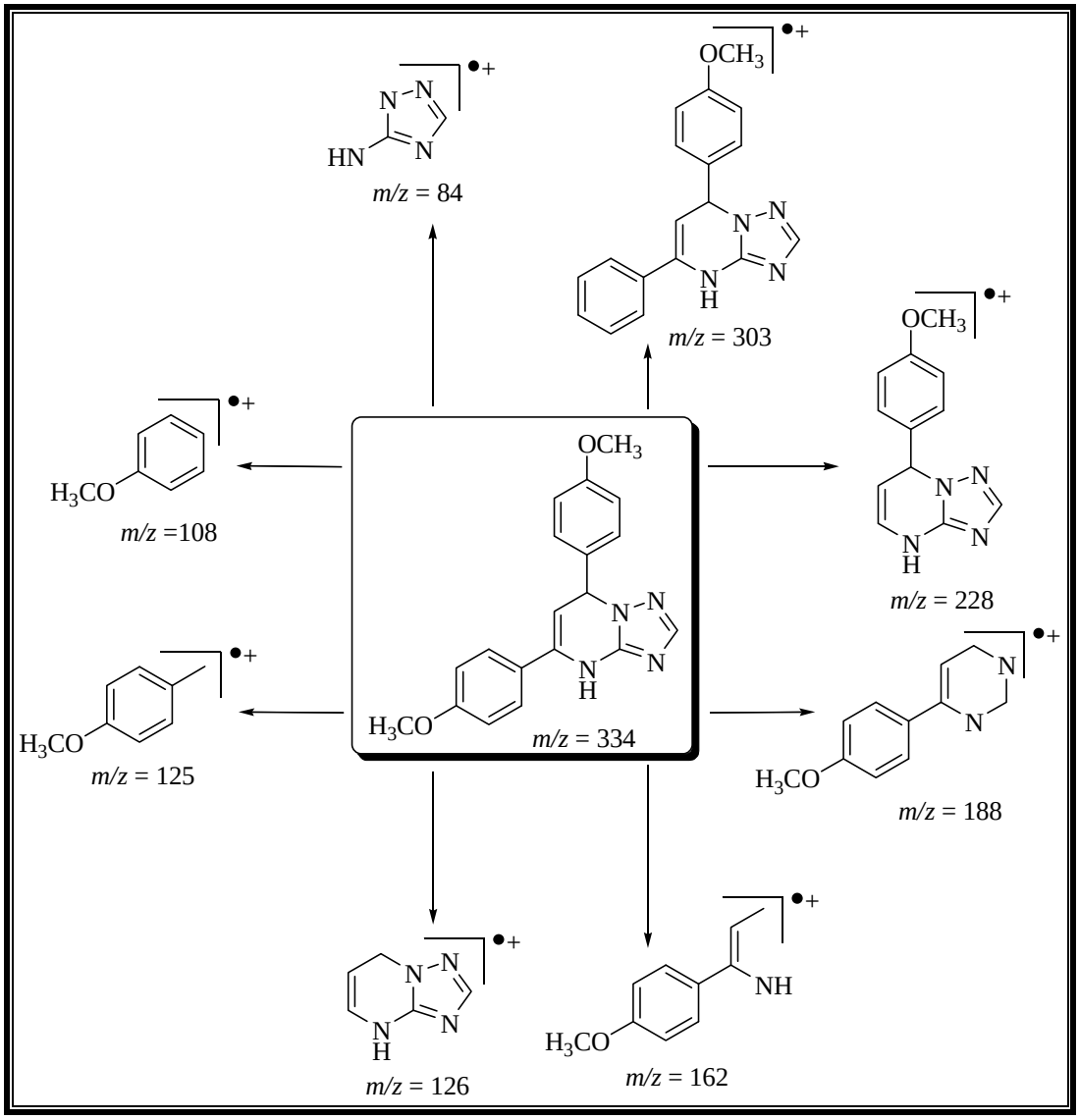
4.12.1.1 Mass fragmentation pattern for CPV-245



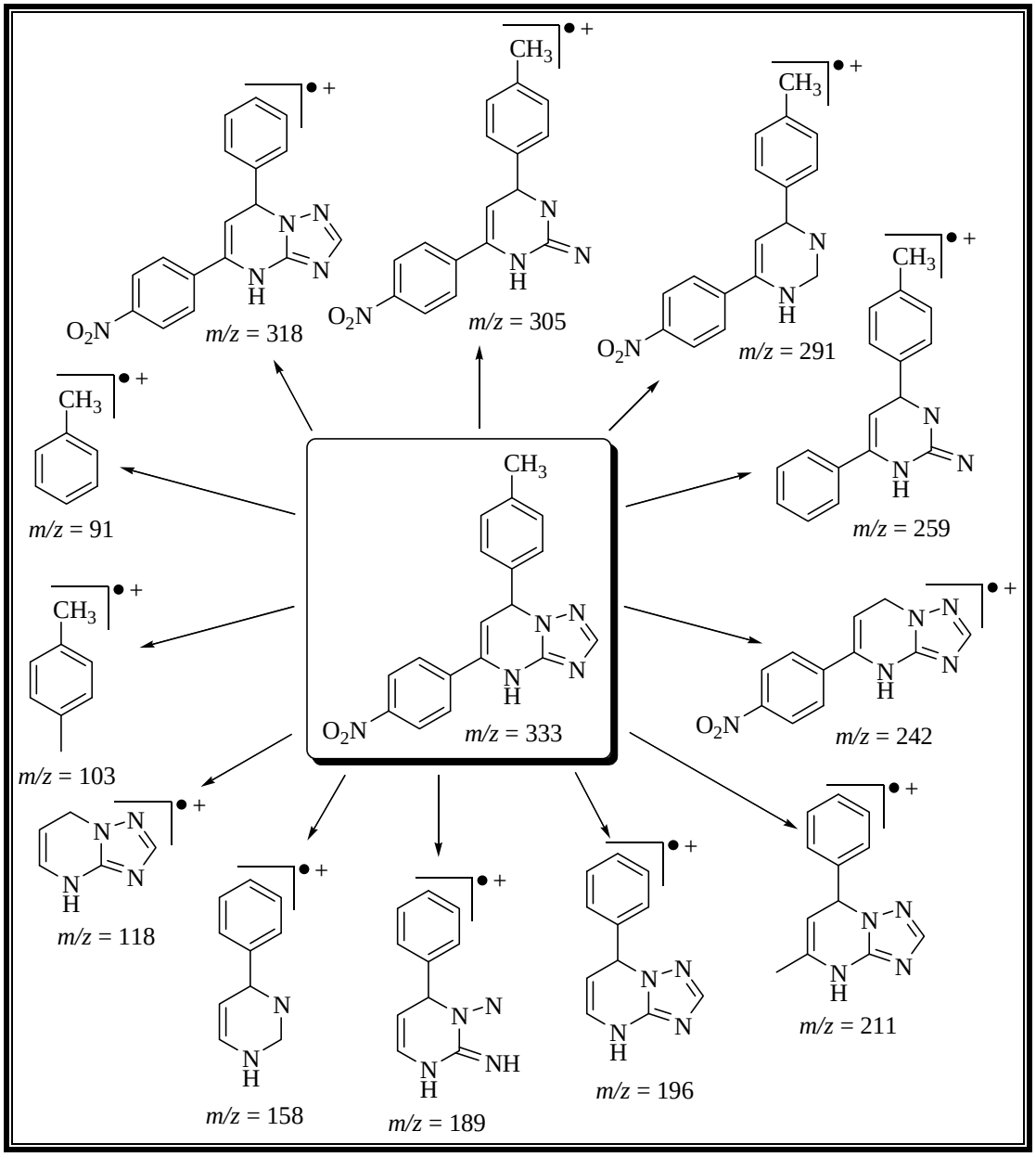
4.12.1.2 Mass fragmentation pattern for CPV-252



4.12.1.3 Mass fragmentation pattern for CPV-263



4.12.1.4 Mass fragmentation pattern for CPV-272



4.12.4.2 IR spectral study

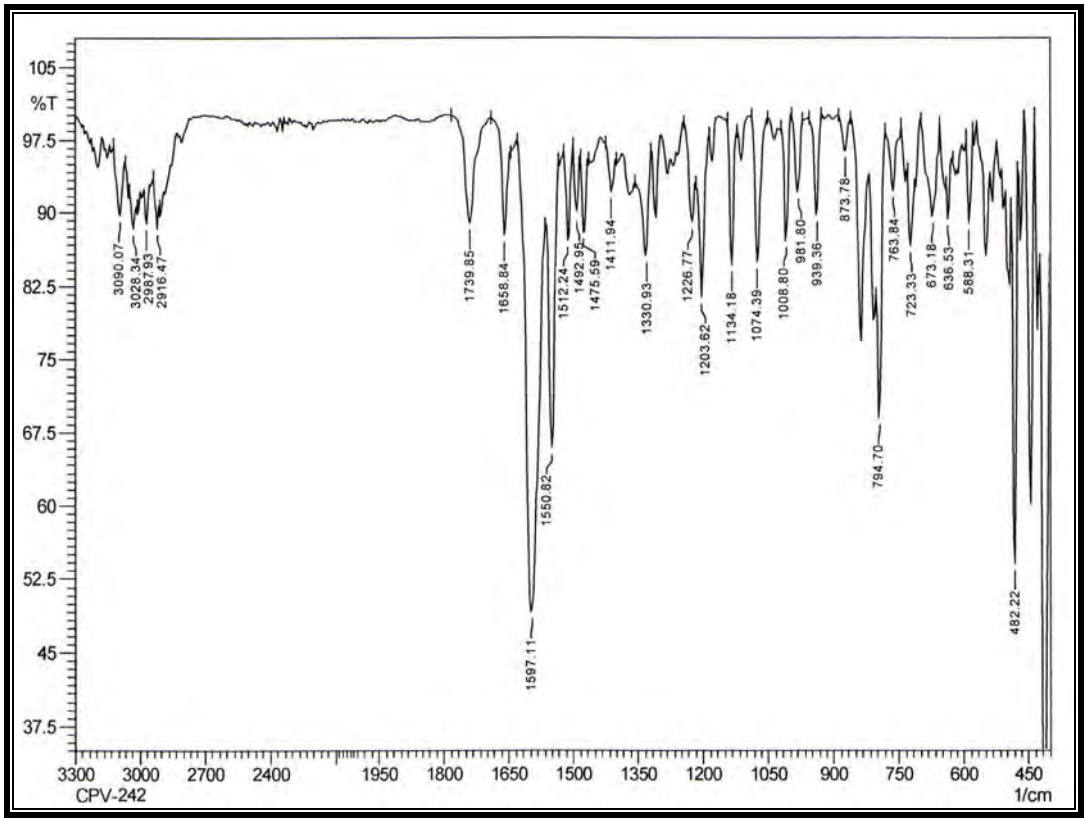
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For triazolopyrimidines CPV-241 to 280, confirmatory bands for secondary amine and C=N stretching band of triazole ring were observed at 3090-3553 cm^{-1} and 1591-1683 cm^{-1} respectively. Another characteristic band for N-H deformation and C-N stretching were observed at 1550-1683 cm-^{1} and 1247-1346 cm^{-1} respectively, which suggested the formation of pyrimidine ring.

4.12.4.3 ^{1}H NMR spectral study

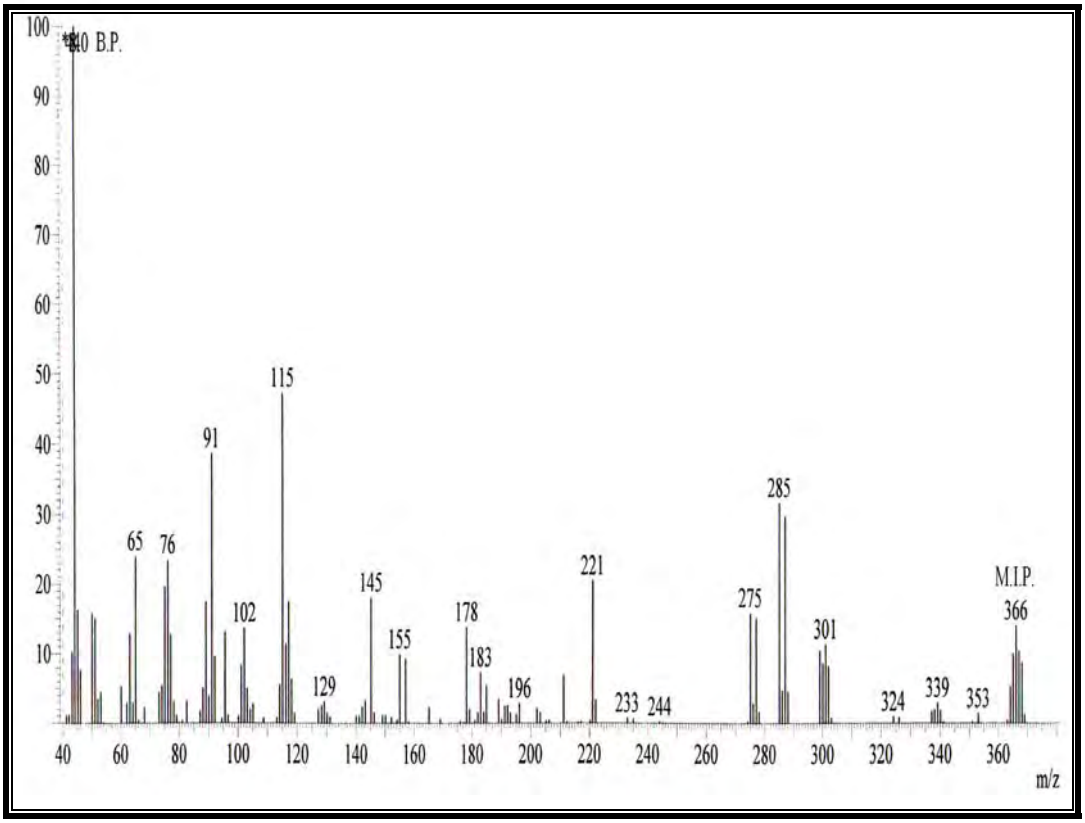
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of triazolopyrimidines CPV-241 to 280 on the basis of following signals: two characteristic peaks for the methine proton of pyrimidine ring and for the methine proton of triazole ring were observed at 4.95-5.32 δ ppm and 7.48-7.59 δ ppm respectively. And another singlet for amino group proton was observed at 9.73-10.22 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern.

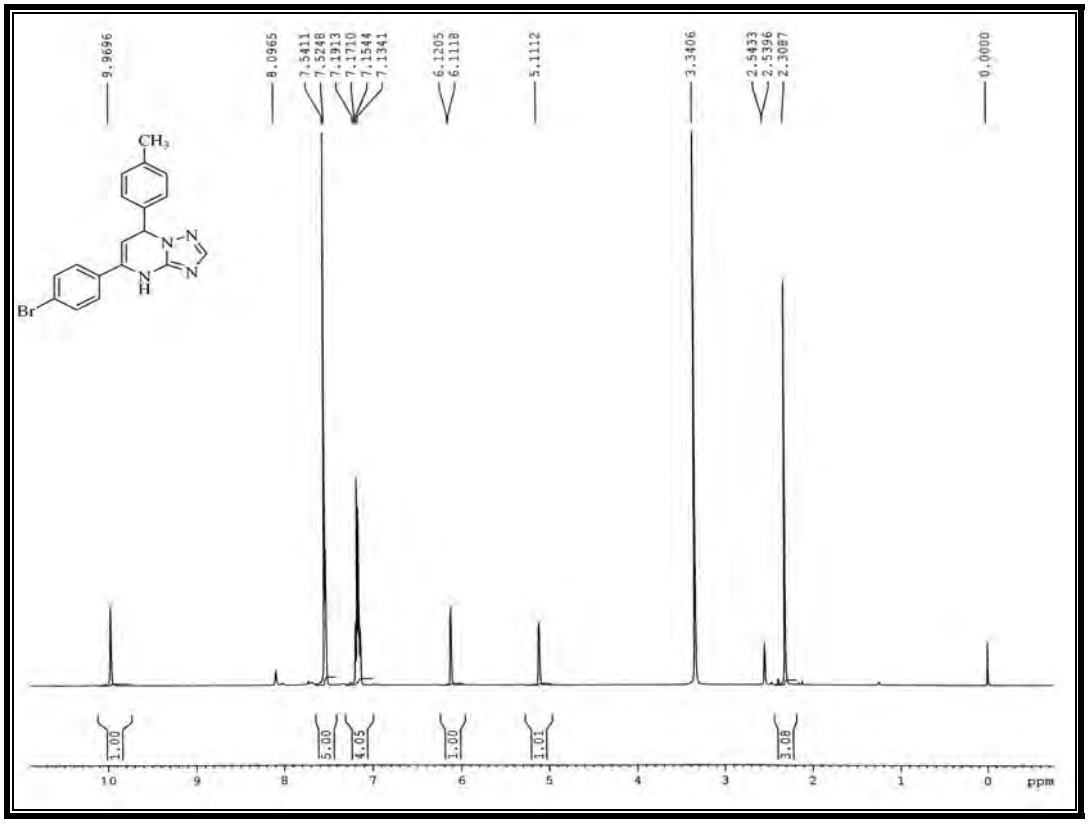
IR spectrum of CPV-242



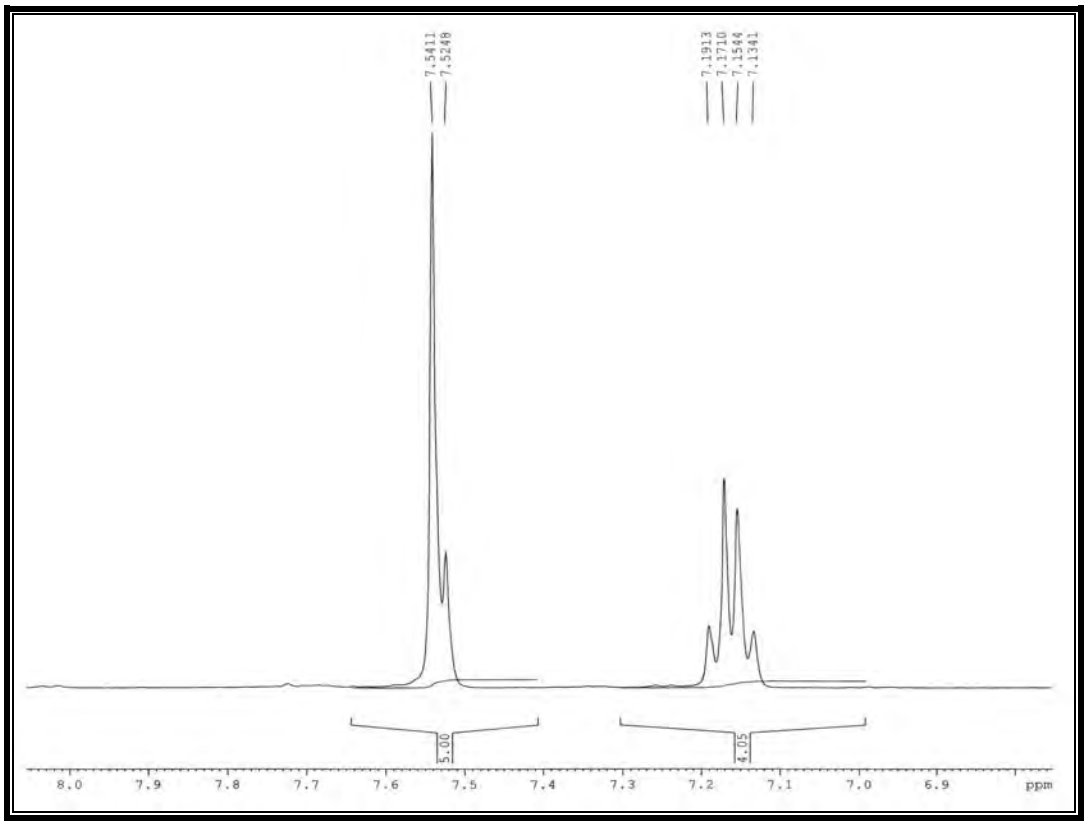
Mass spectrum of CPV-242



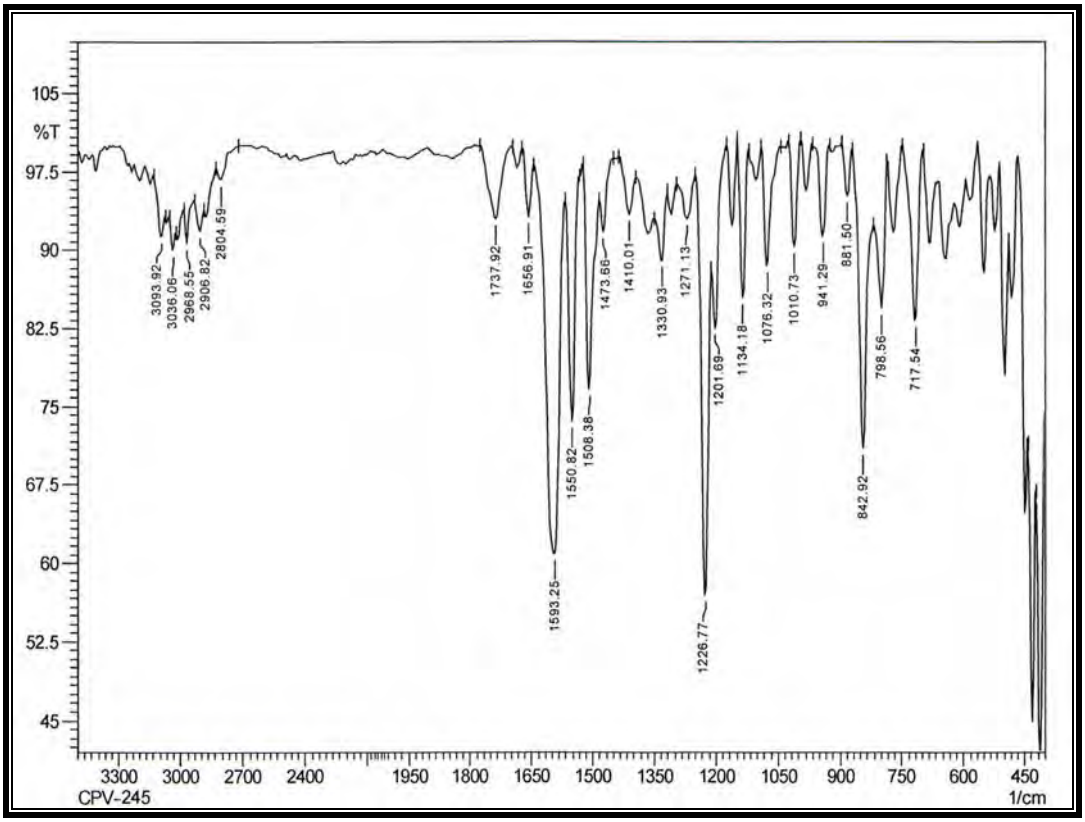
^{1}H NMR spectrum of CPV-242



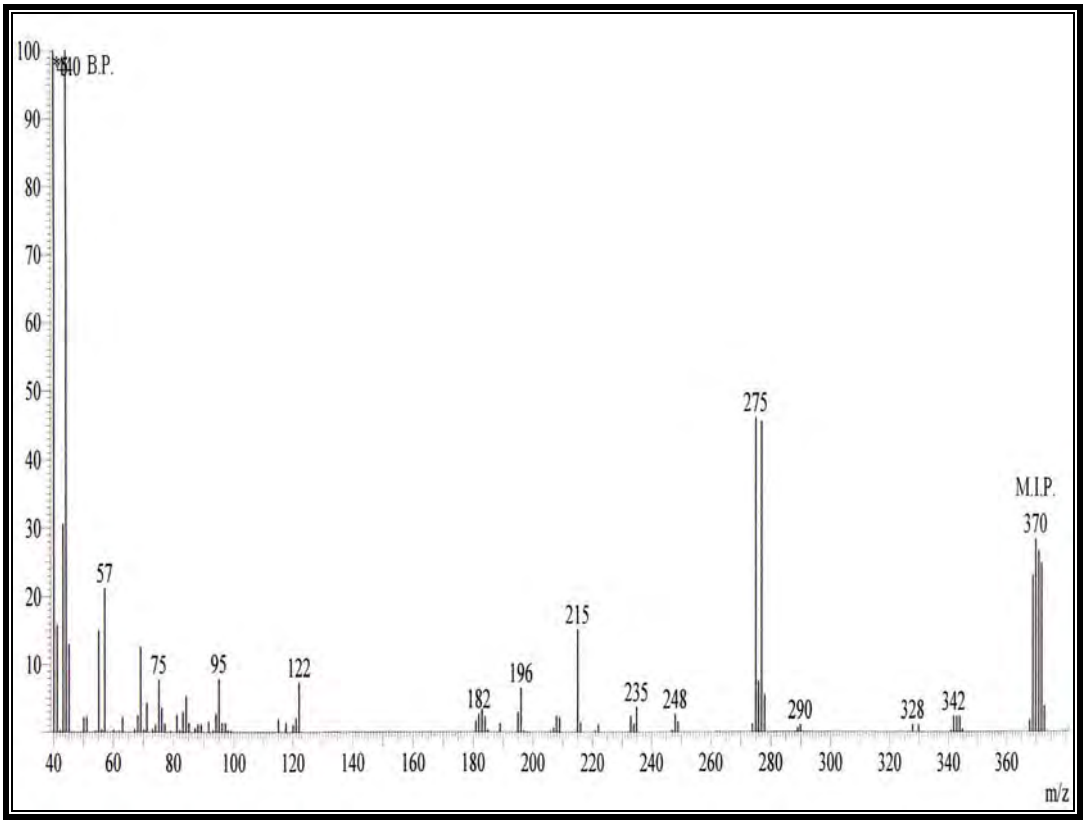
Expanded ^{1}H NMR spectrum of CPV-242



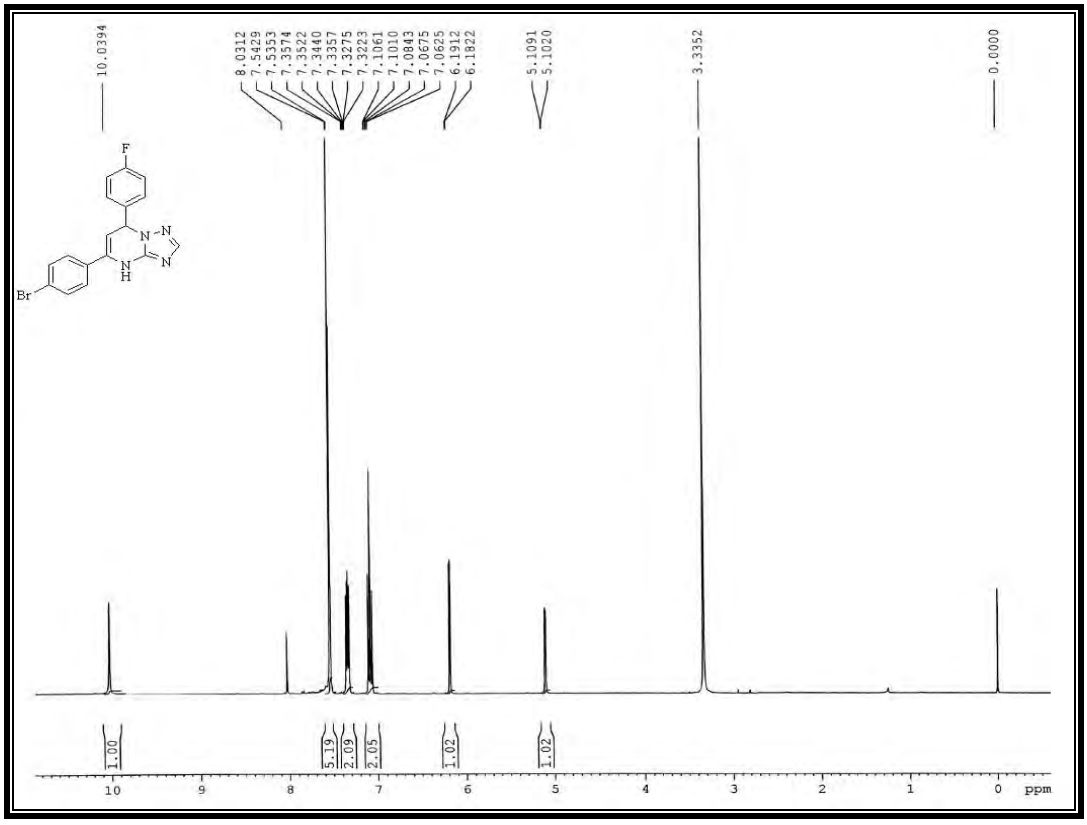
IR spectrum of CPV-245



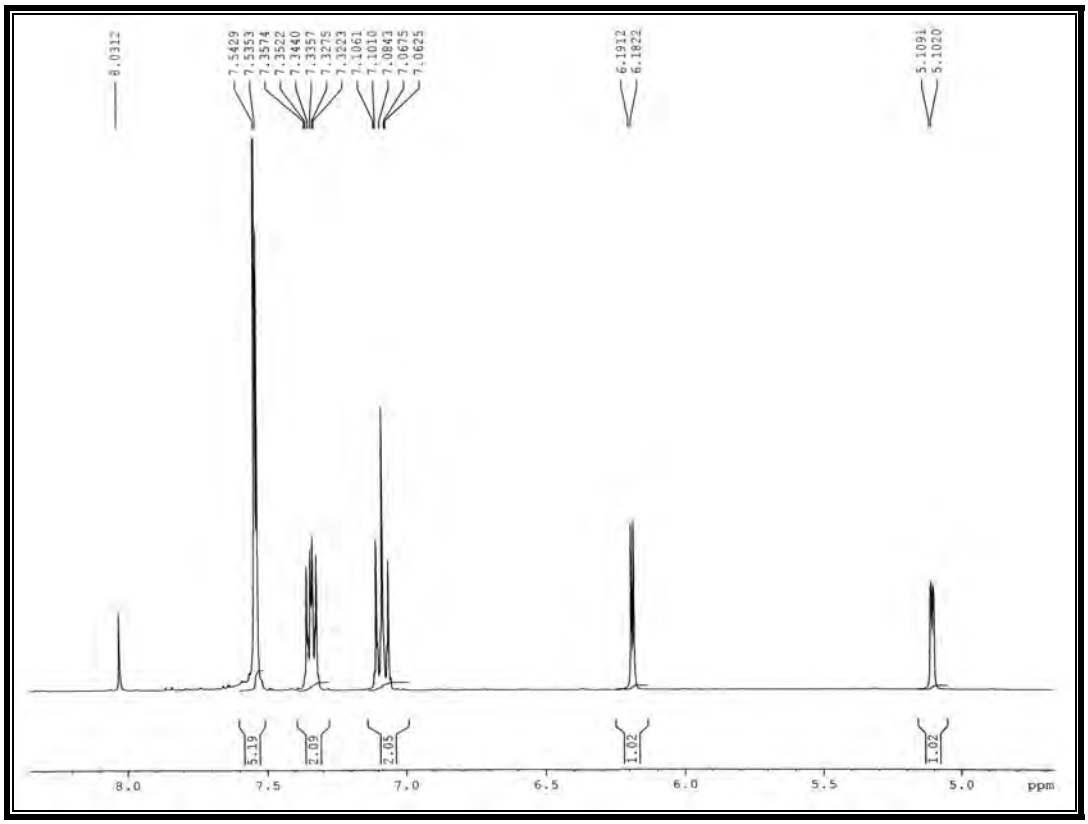
Mass spectrum of CPV-245



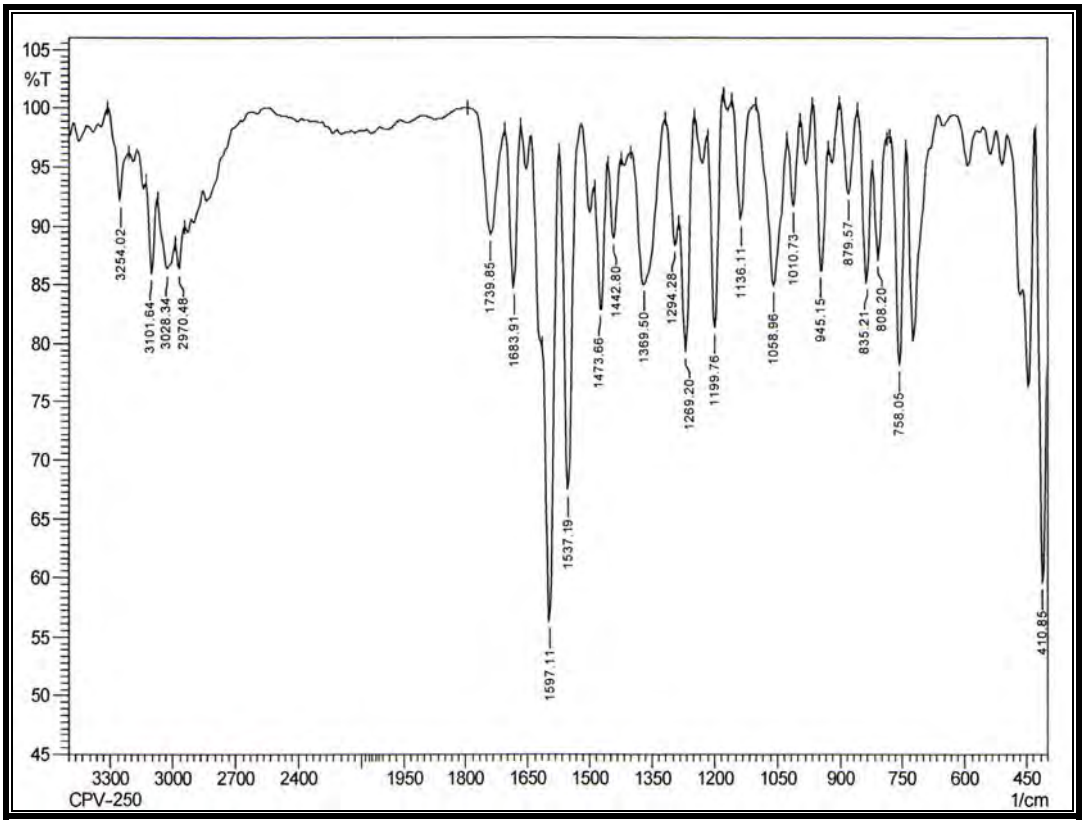
^{1}H NMR spectrum of CPV-245



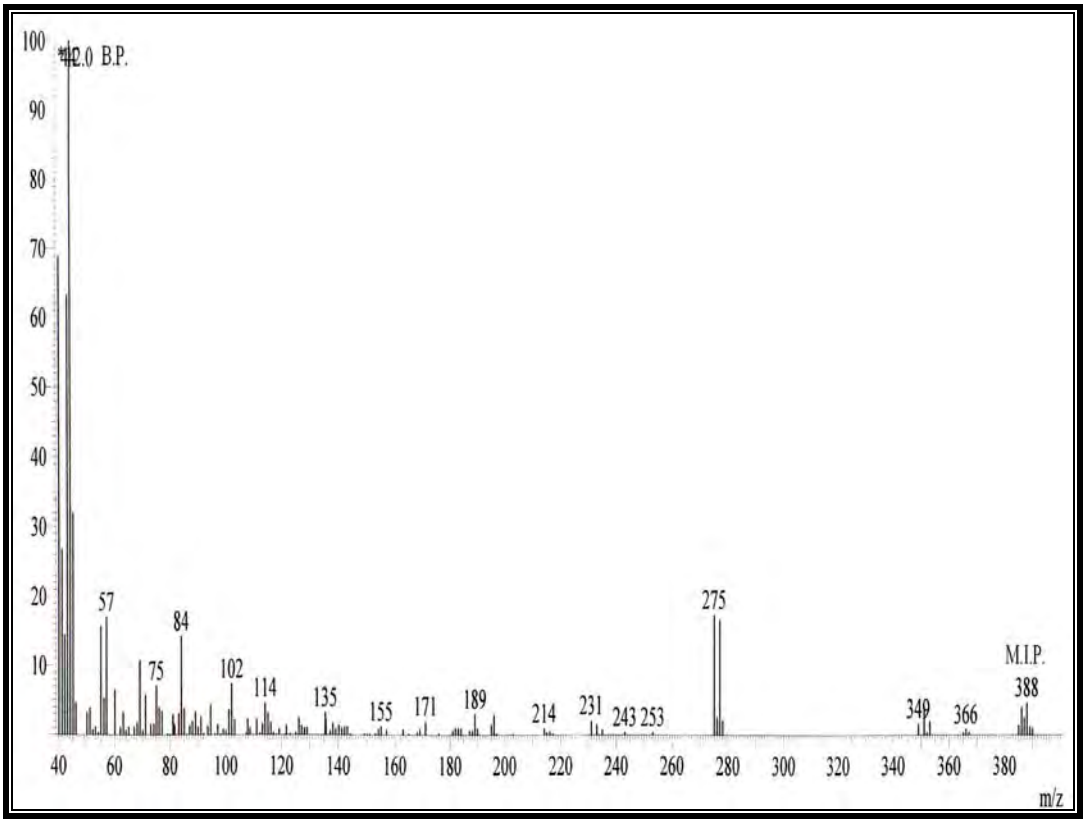
Expanded ^{1}H NMR spectrum of CPV-245



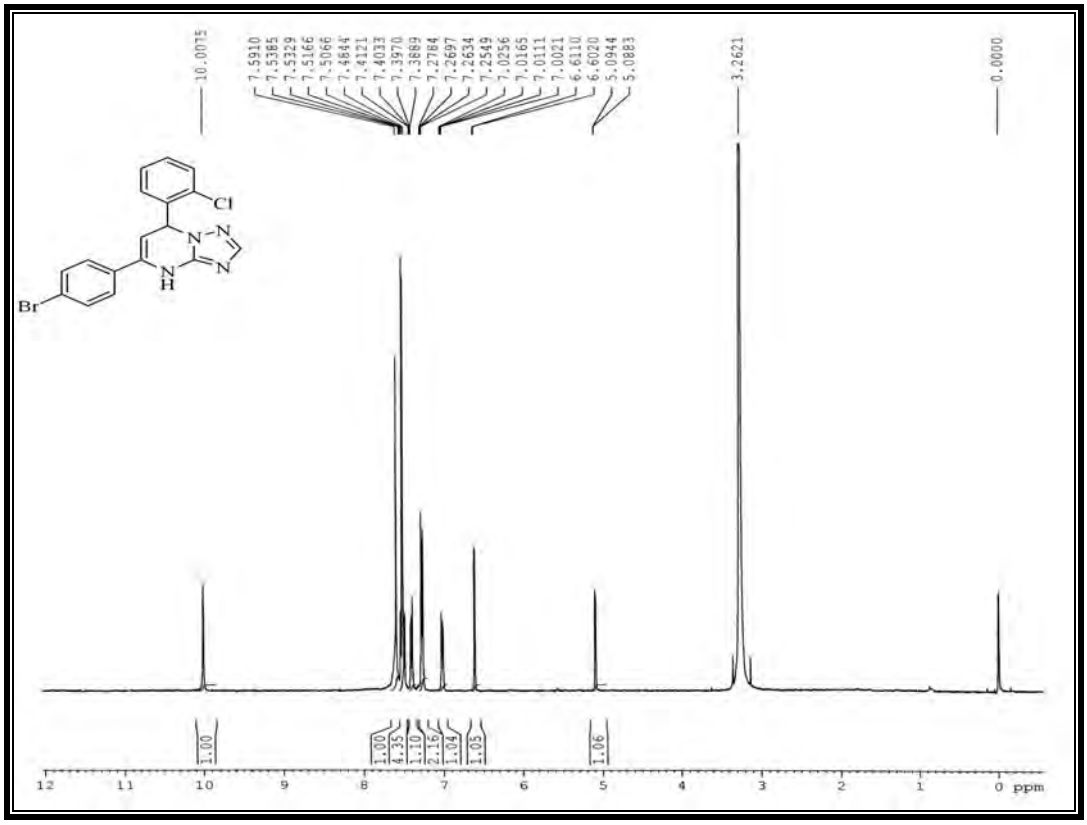
IR spectrum of CPV-250



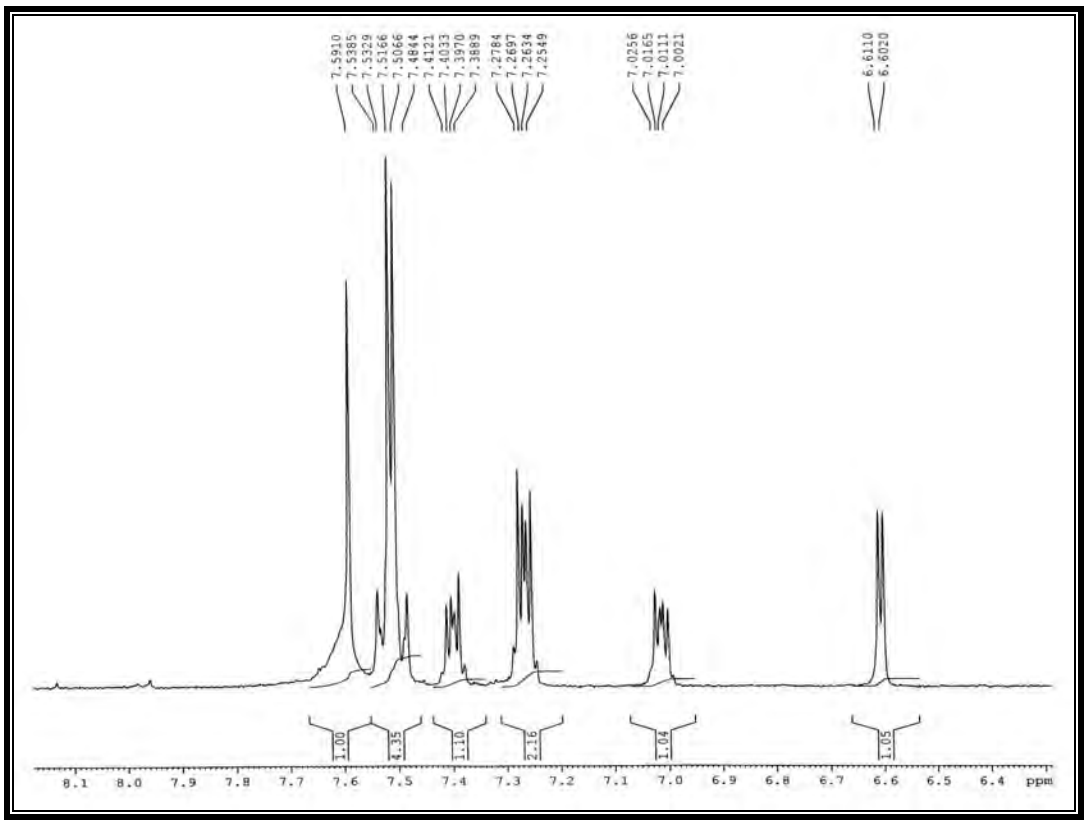
Mass spectrum of CPV-250



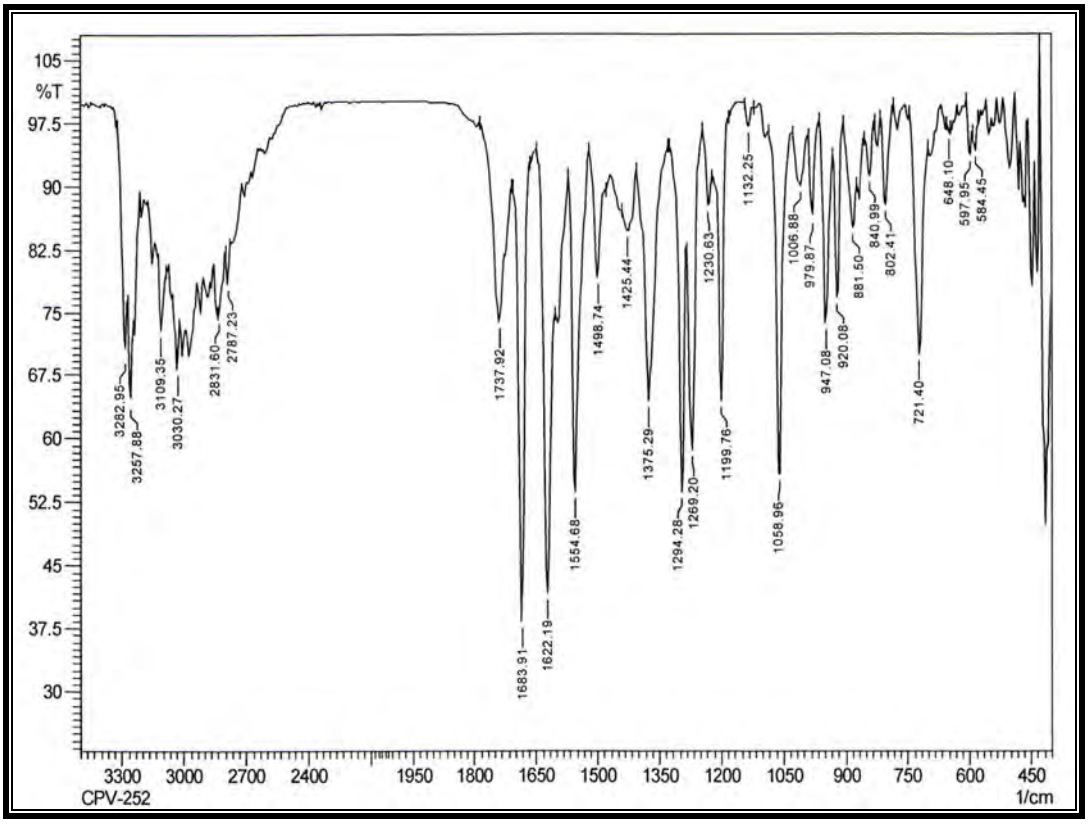
^{1}H NMR spectrum of CPV-250



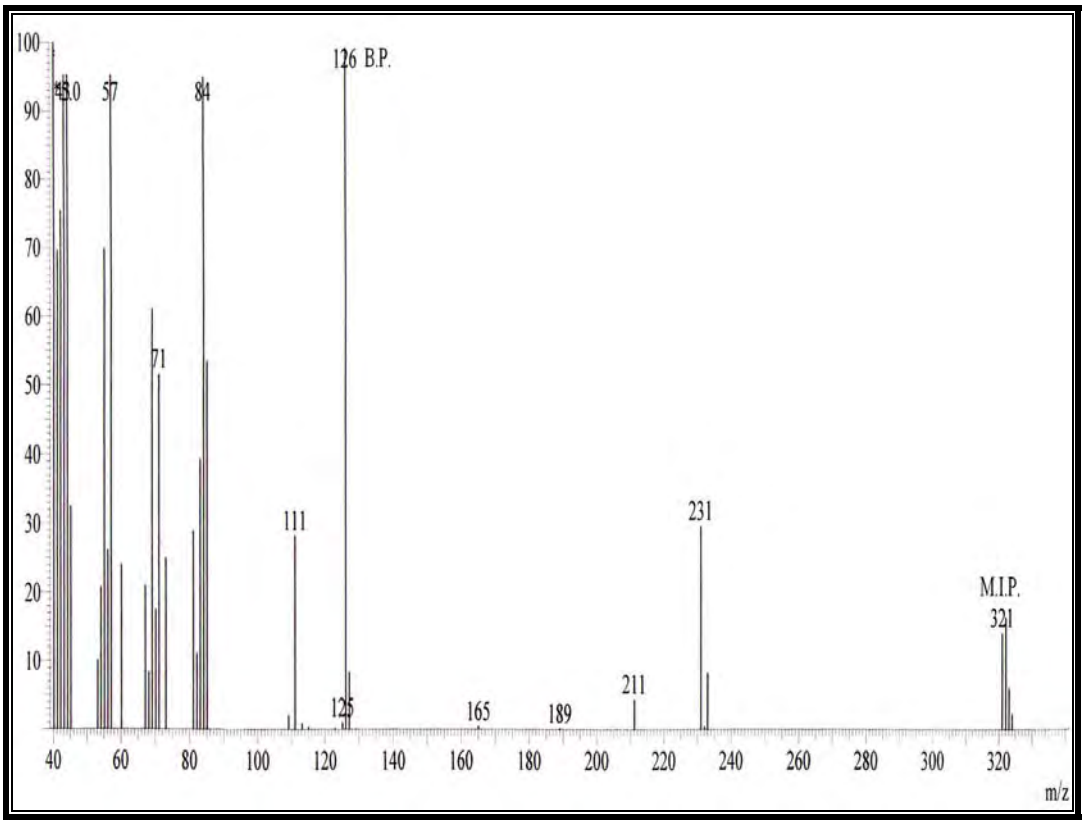
Expanded ^{1}H NMR spectrum of CPV-250



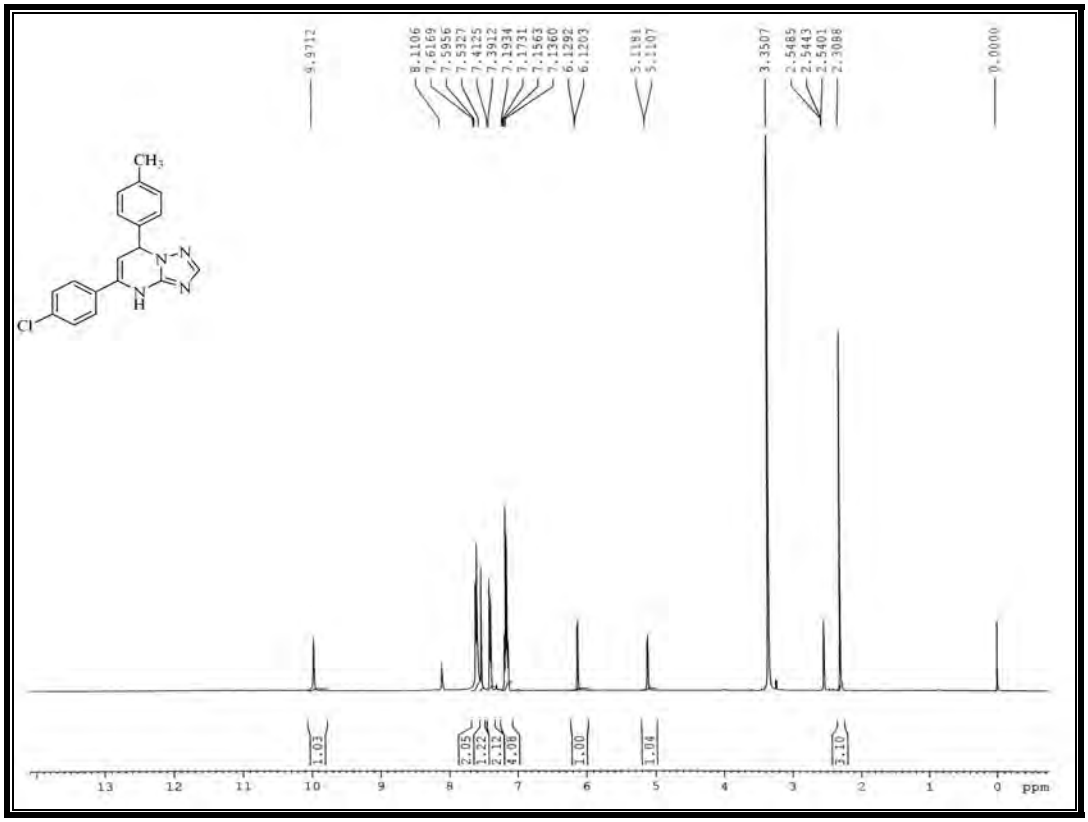
IR spectrum of CPV-252



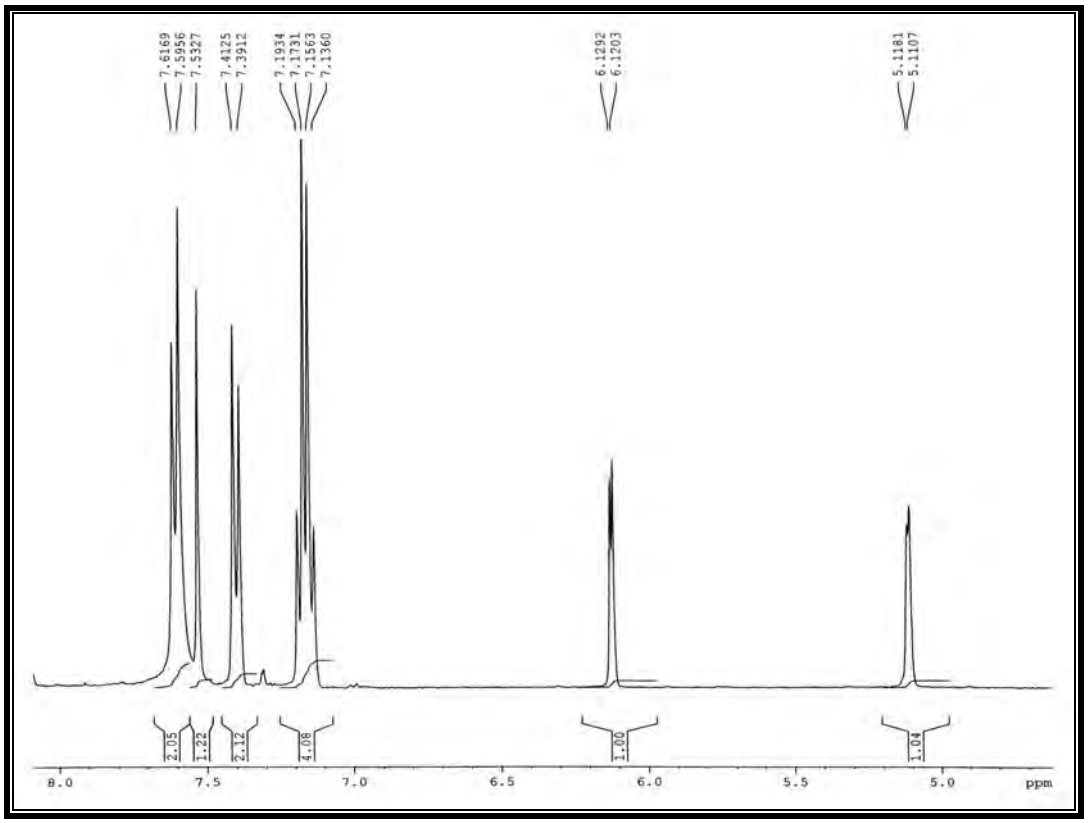
Mass spectrum of CPV-252



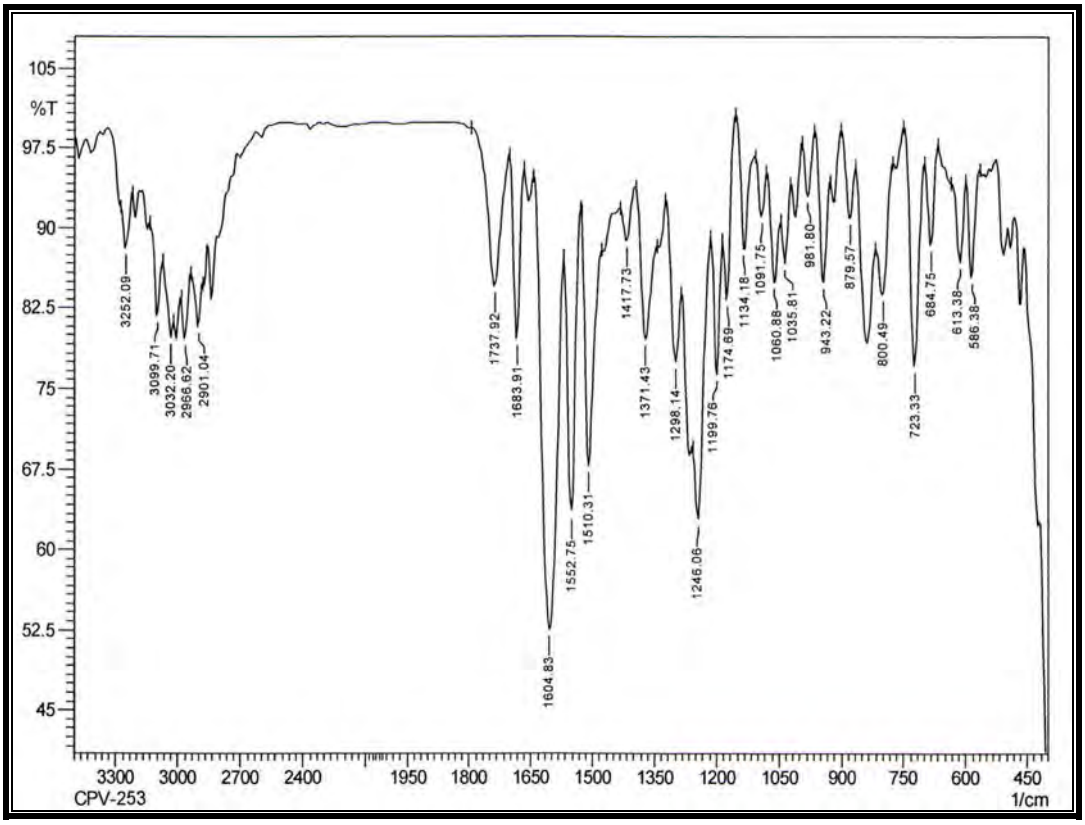
^{1}H NMR spectrum of CPV-252



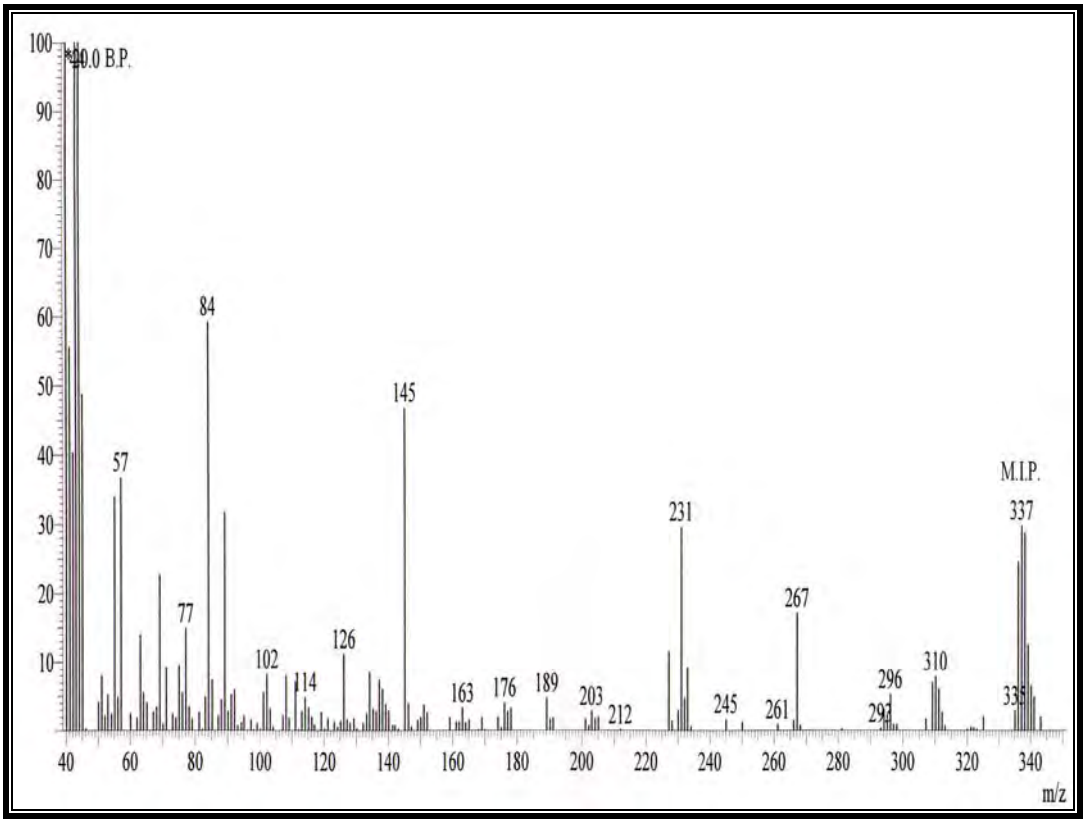
Expanded ^{1}H NMR spectrum of CPV-252



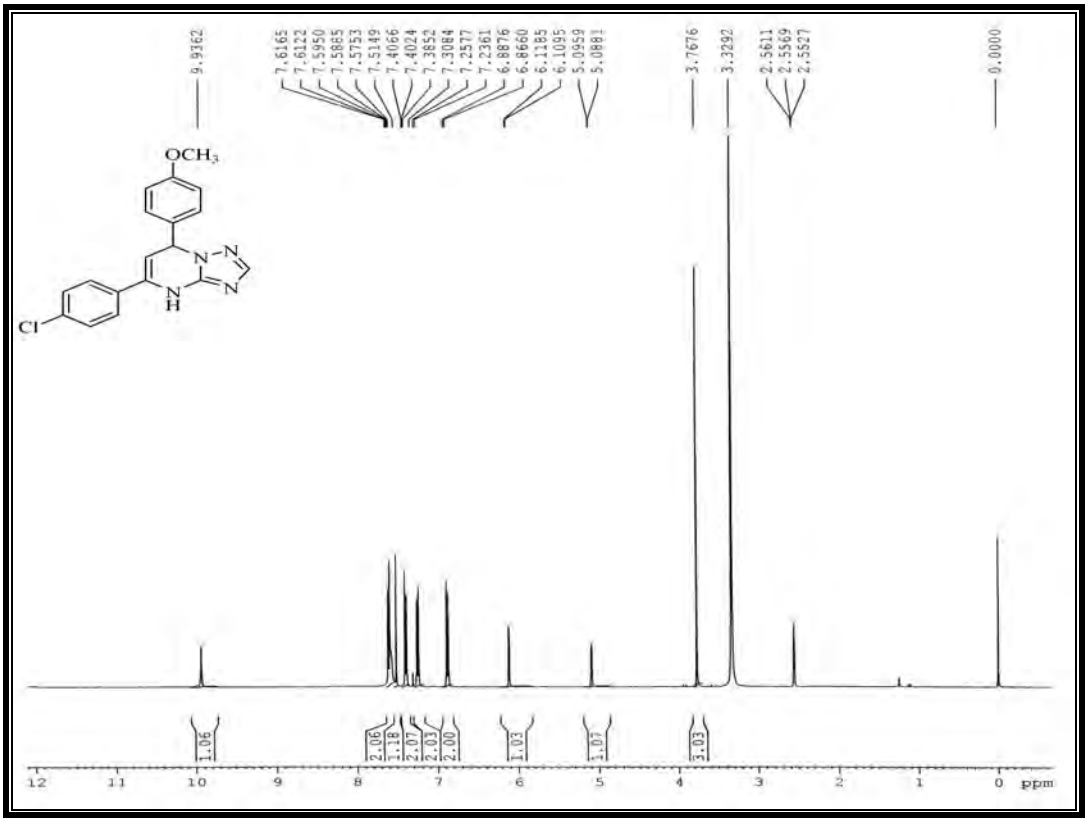
IR spectrum of CPV-253



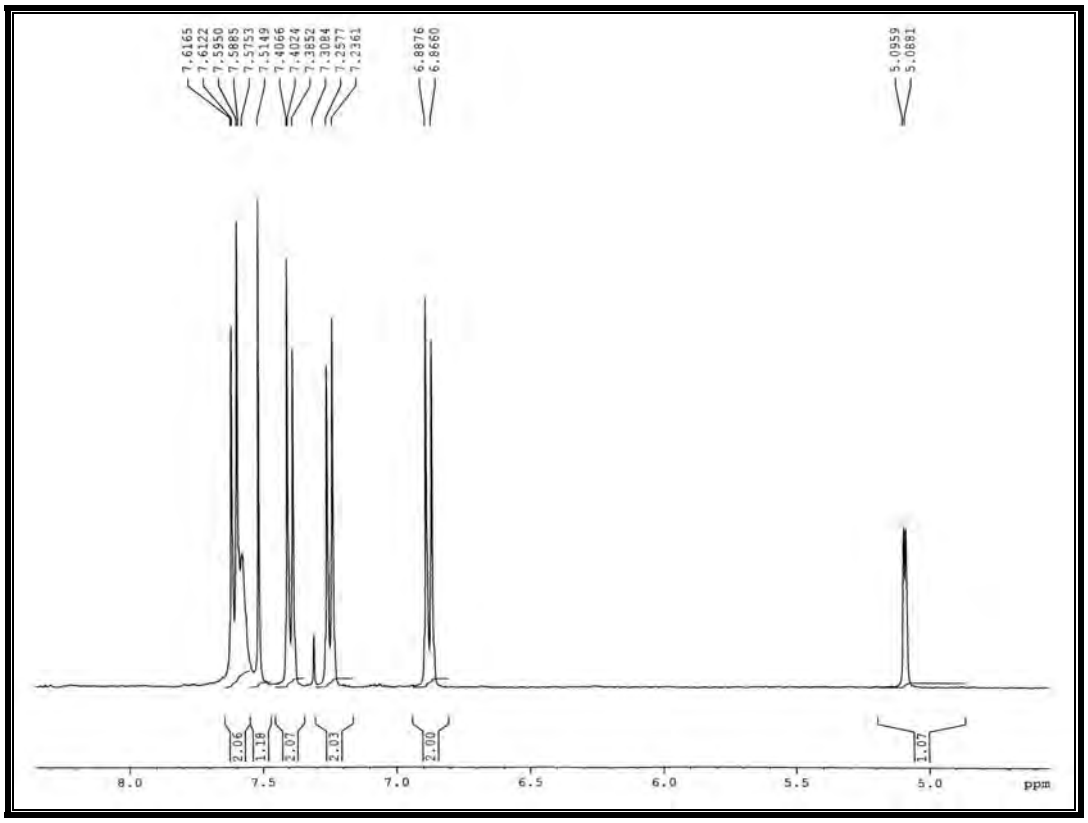
Mass spectrum of CPV-253



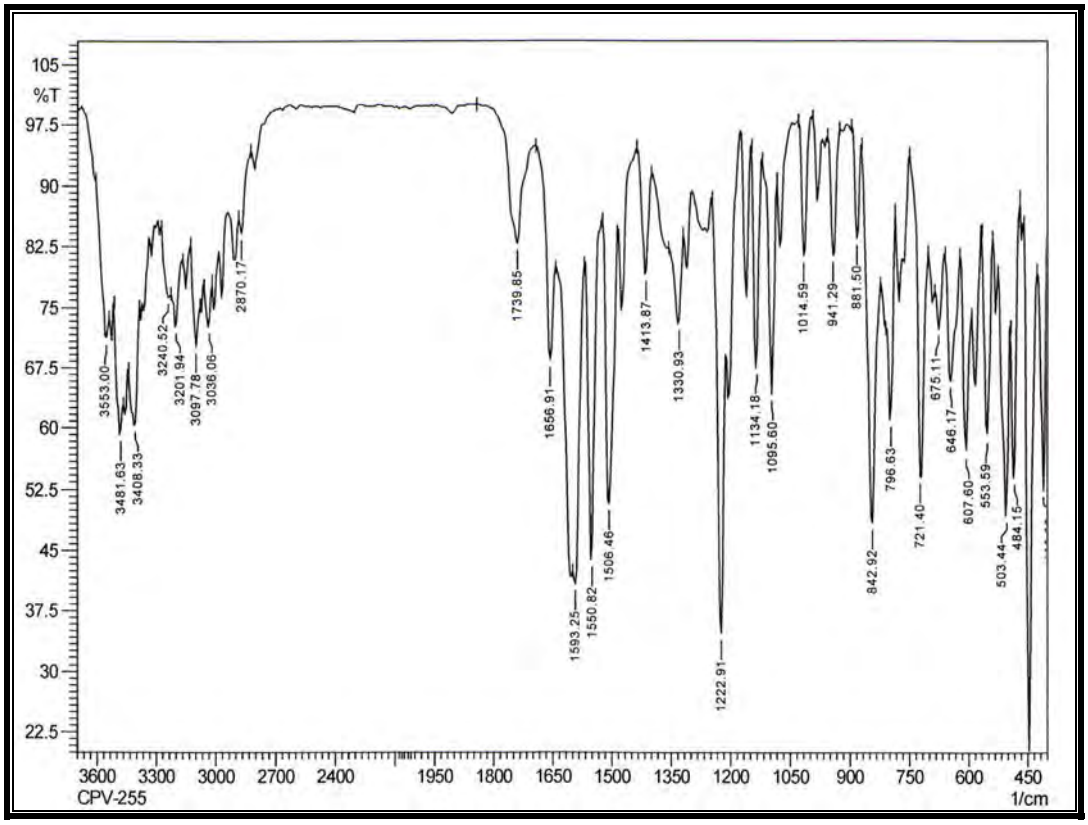
^{1}H NMR spectrum of CPV-253



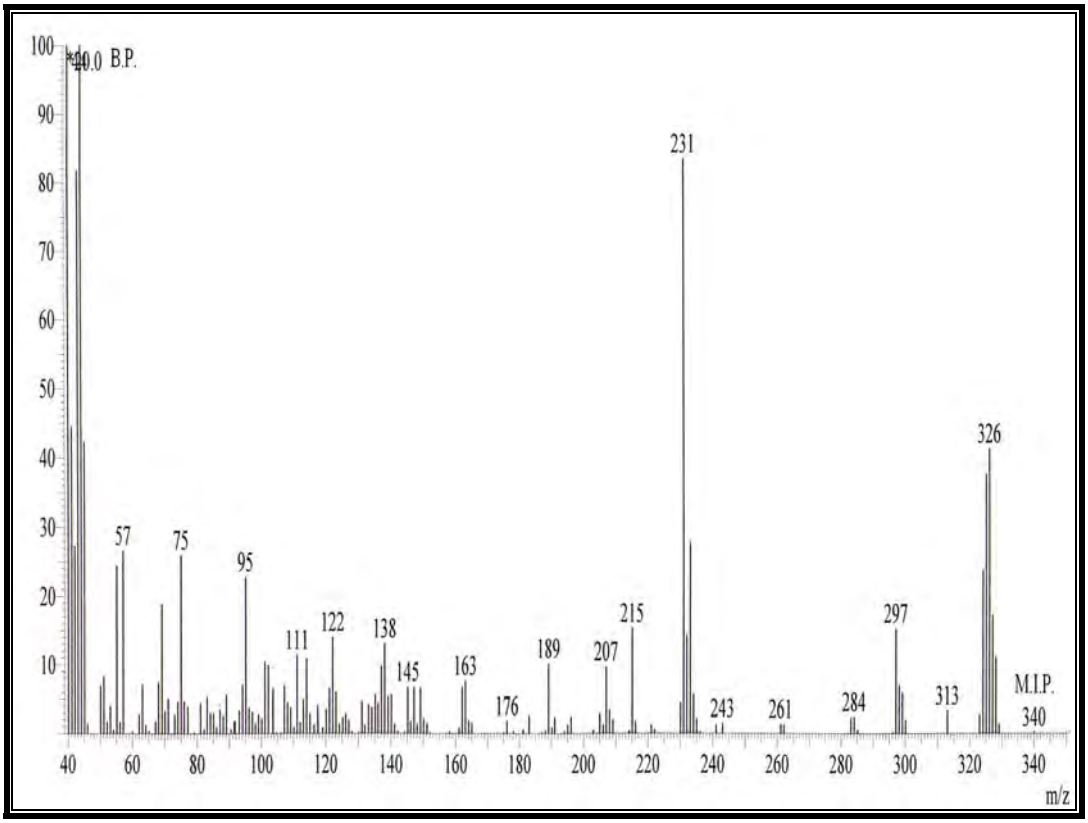
Expanded ^{1}H NMR spectrum of CPV-253



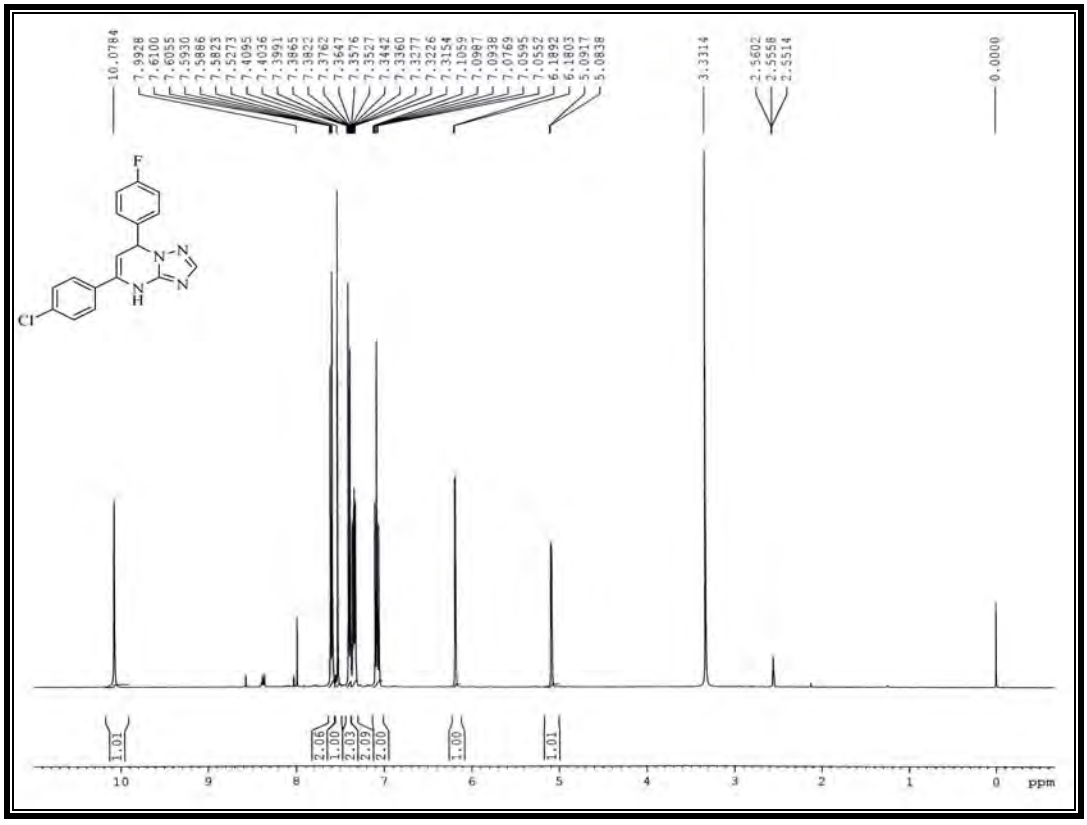
IR spectrum of CPV-255



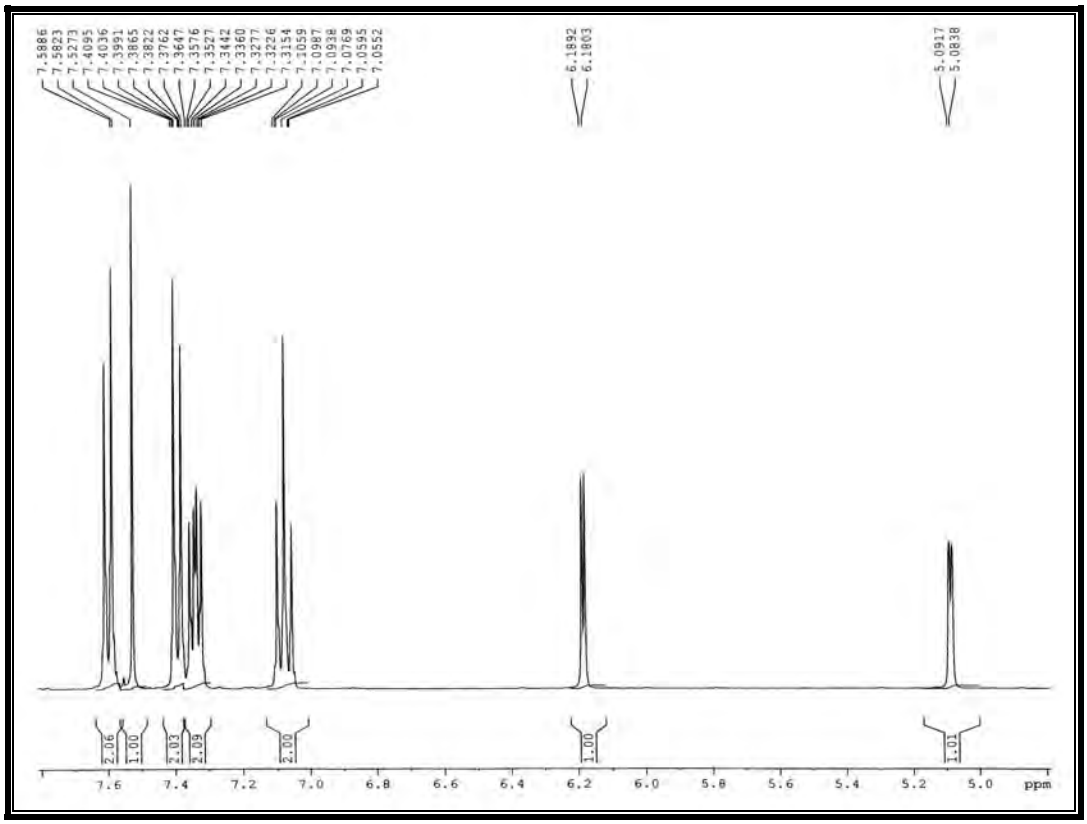
Mass spectrum of CPV-255



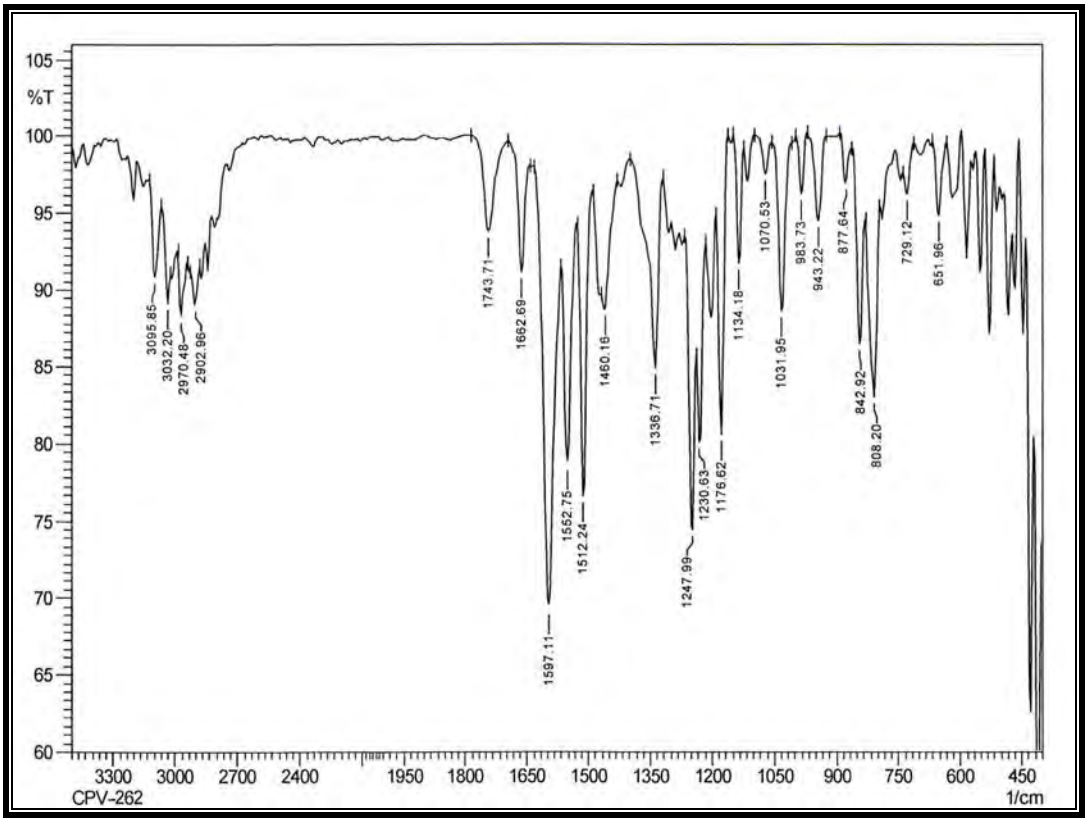
^{1}H NMR spectrum of CPV-255



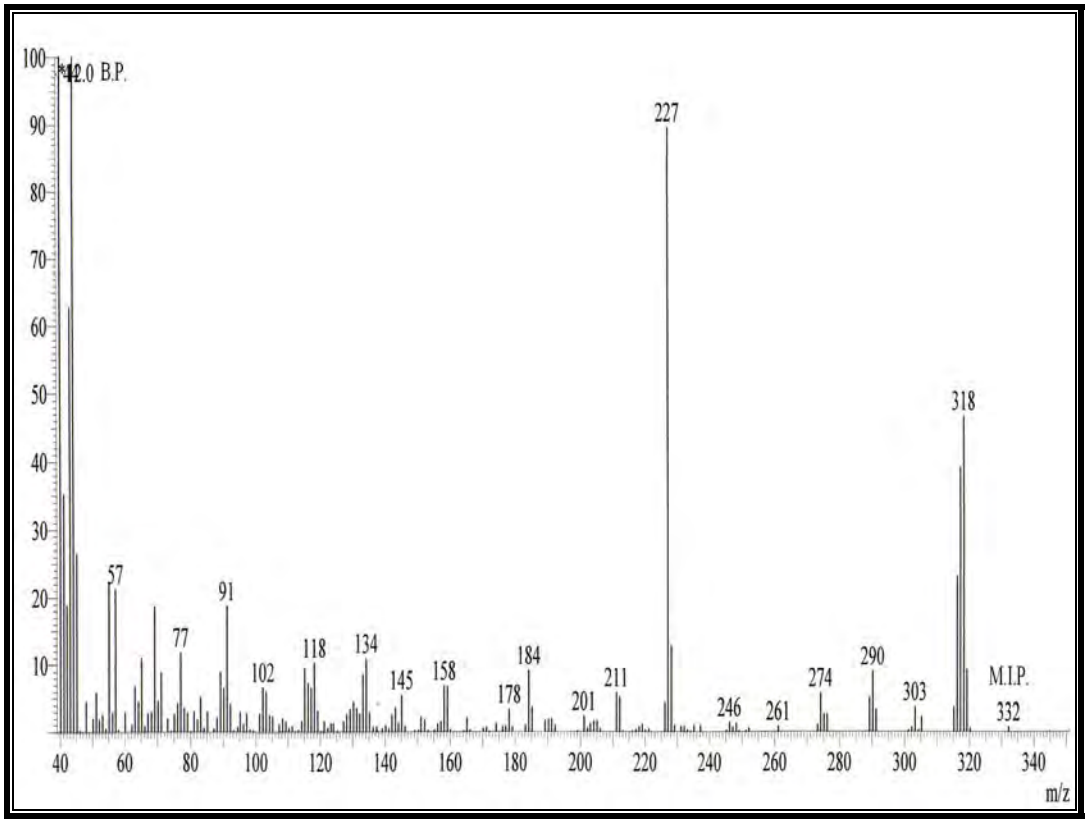
Expanded ^{1}H NMR spectrum of CPV-255



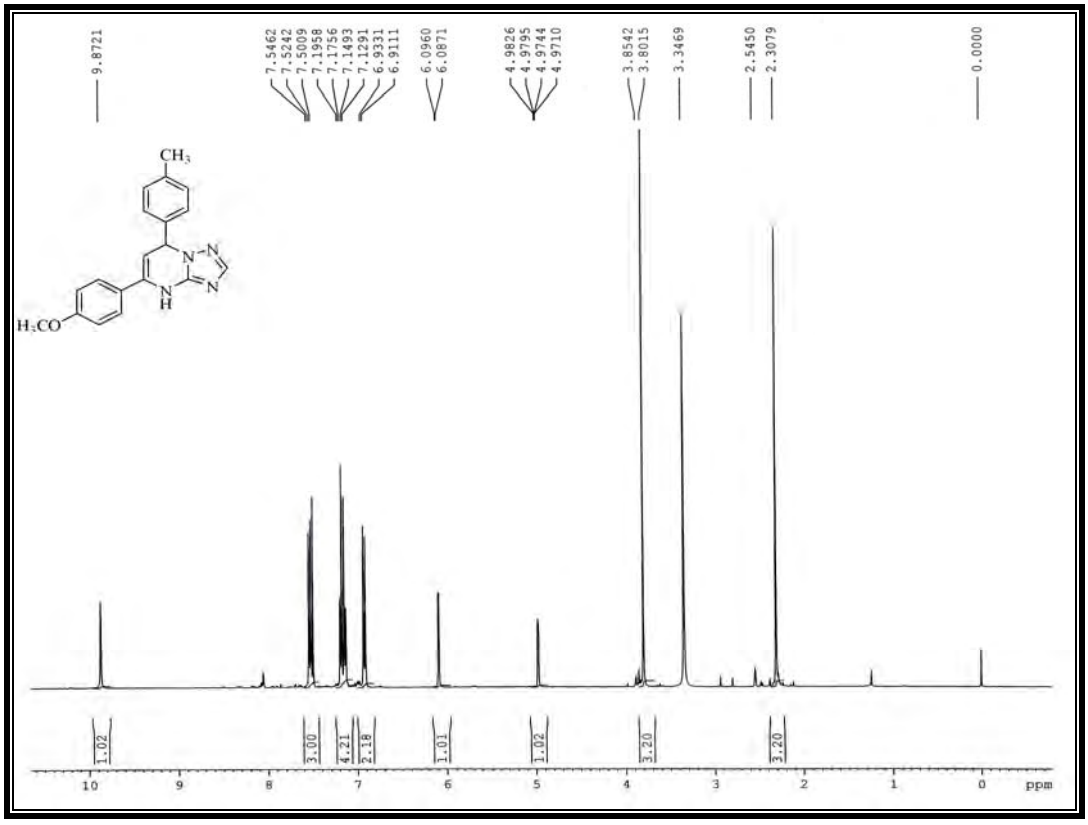
IR spectrum of CPV-262



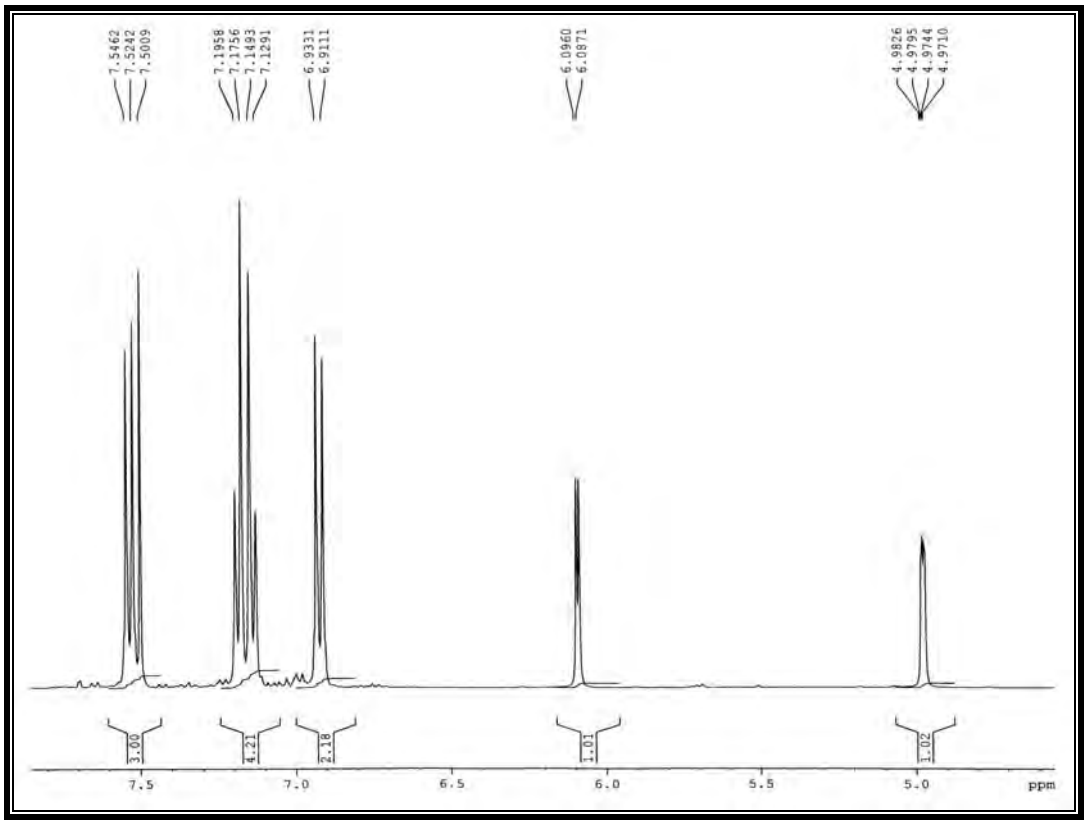
Mass spectrum of CPV-262



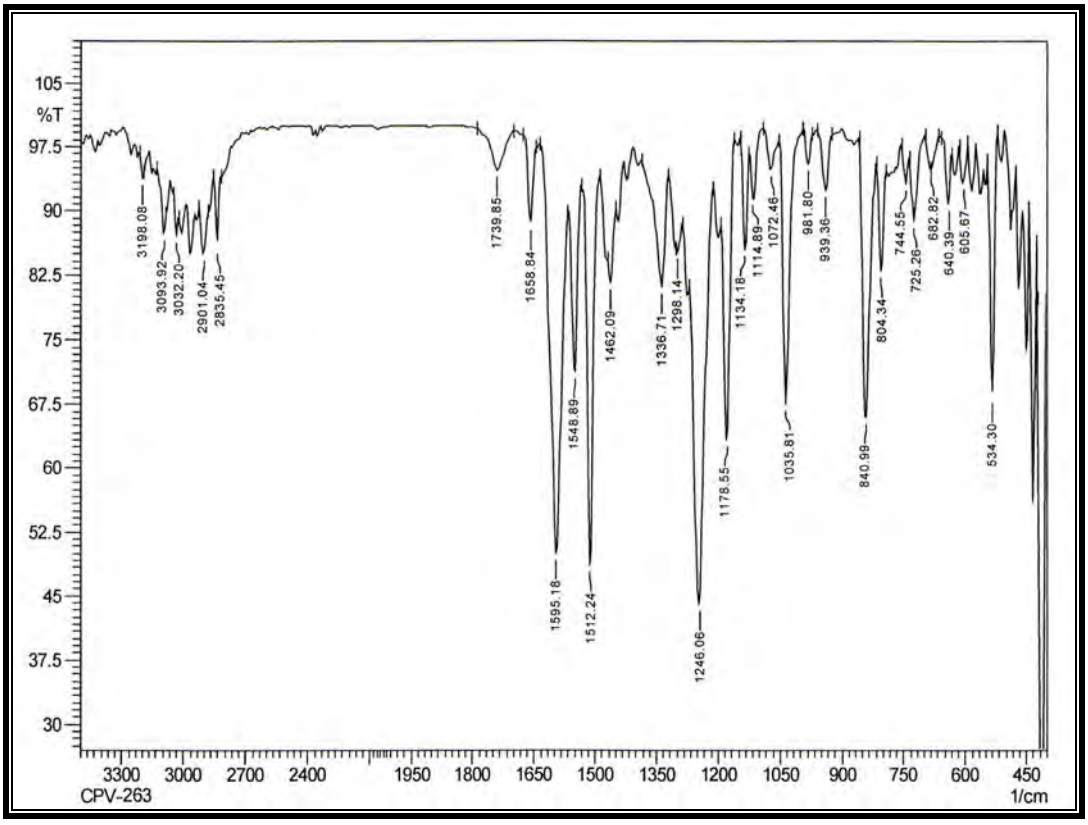
^{1}H NMR spectrum of CPV-262



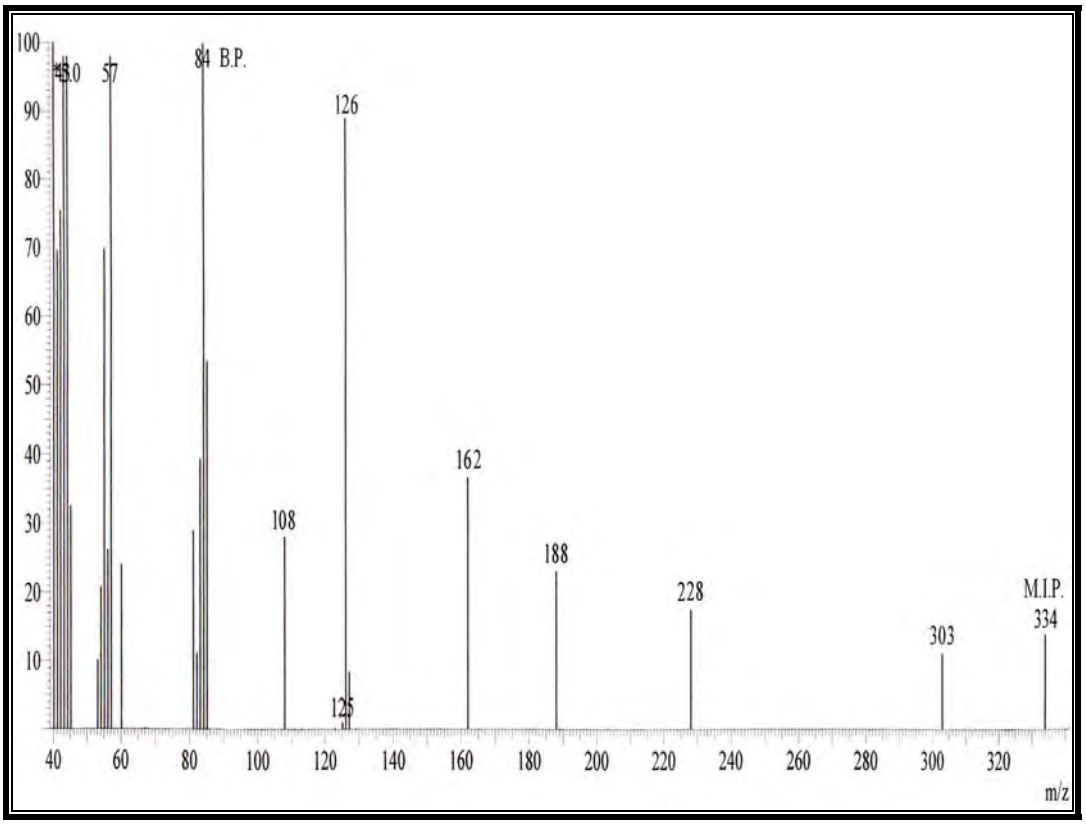
Expanded ^{1}H NMR spectrum of CPV-262



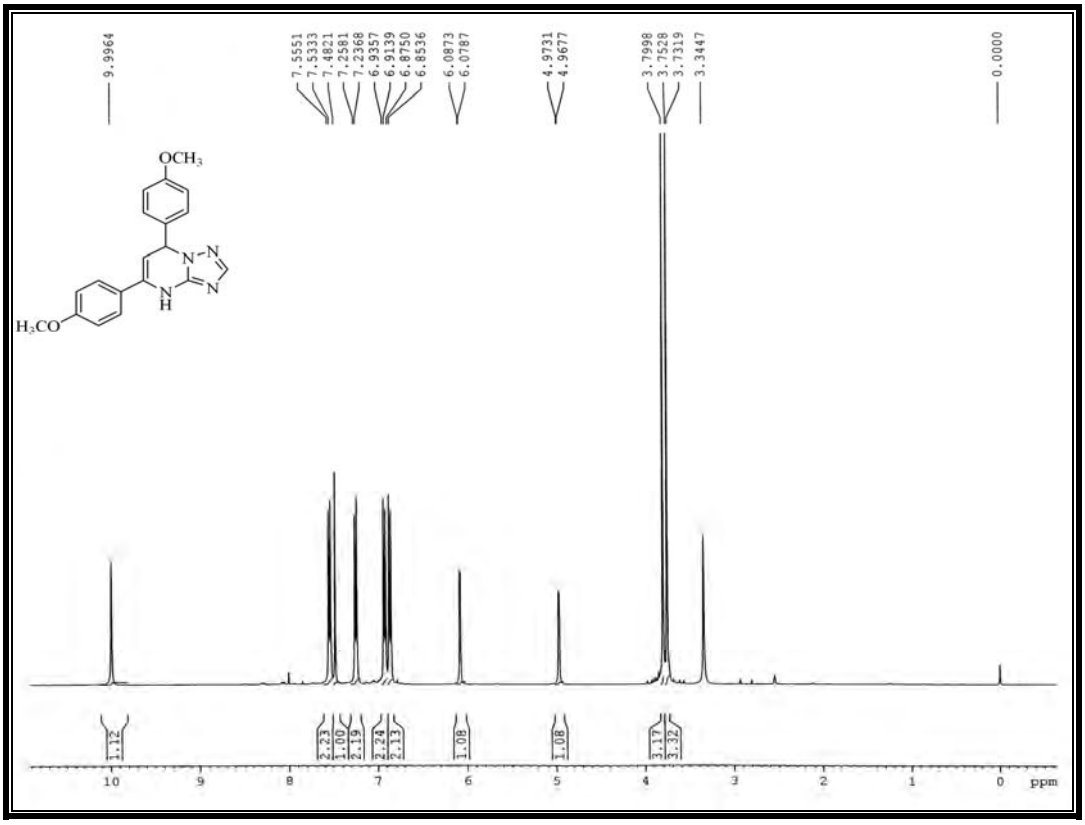
IR spectrum of CPV-263



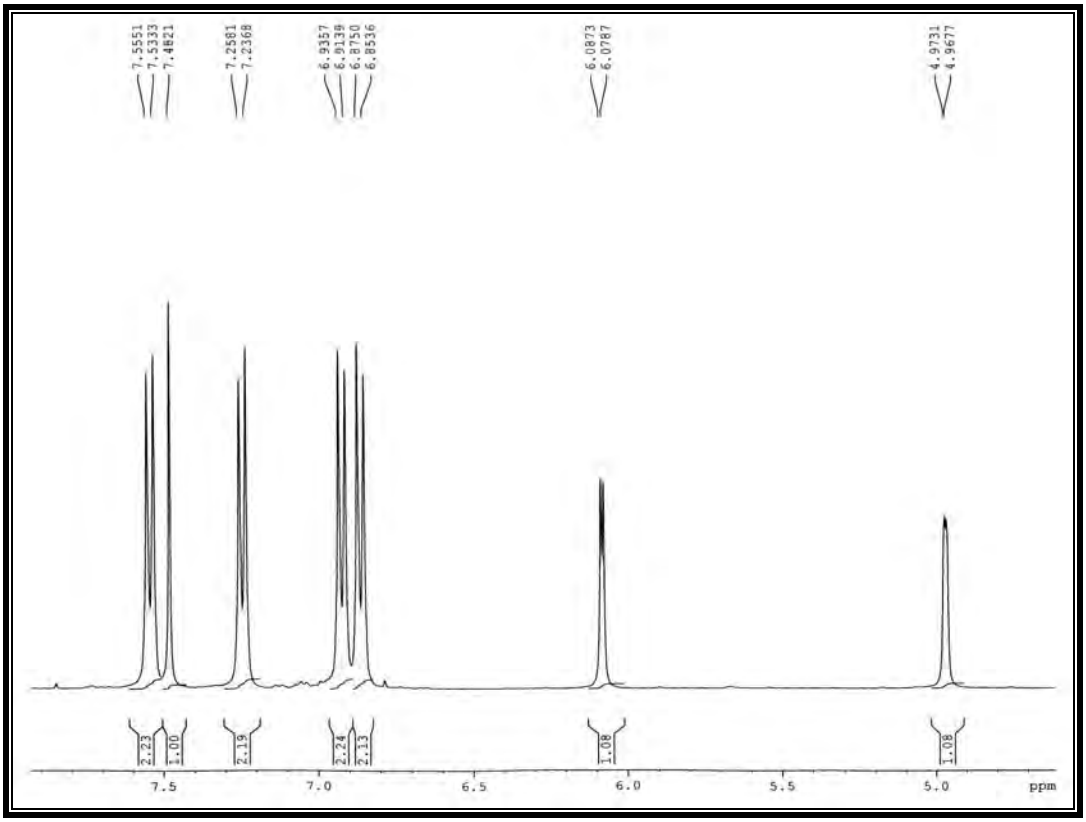
Mass spectrum of CPV-263



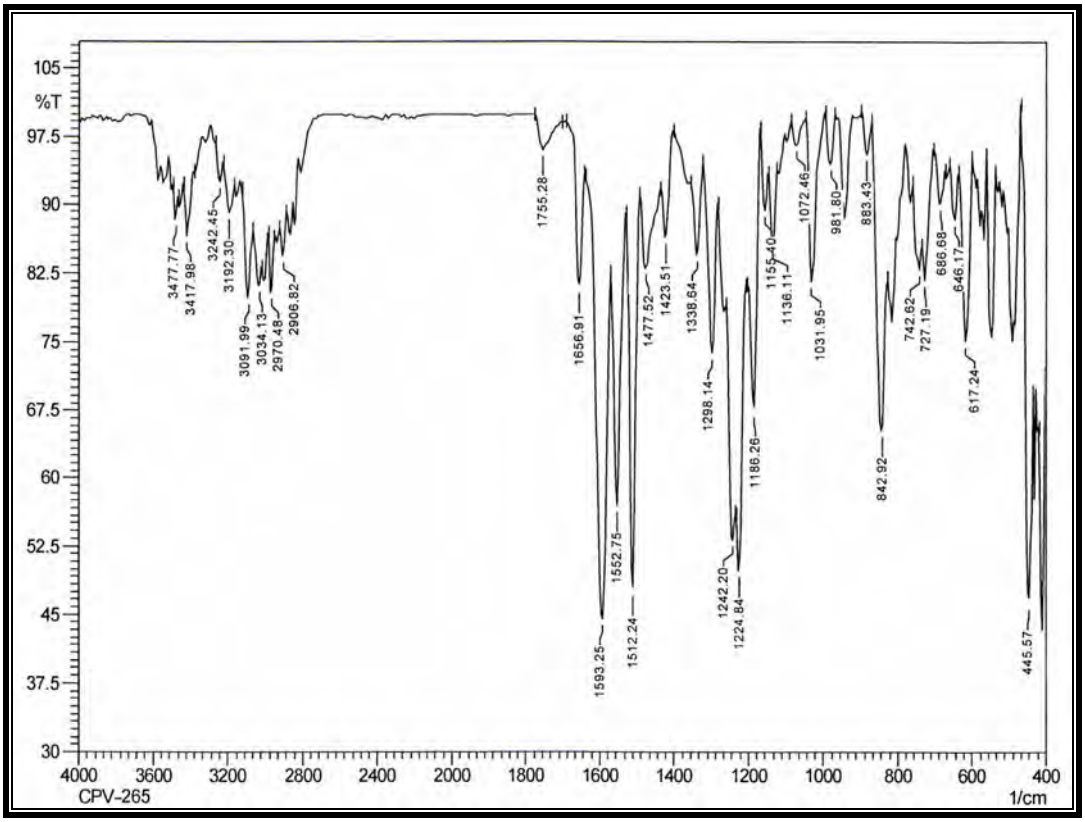
^{1}H NMR spectrum of CPV-263



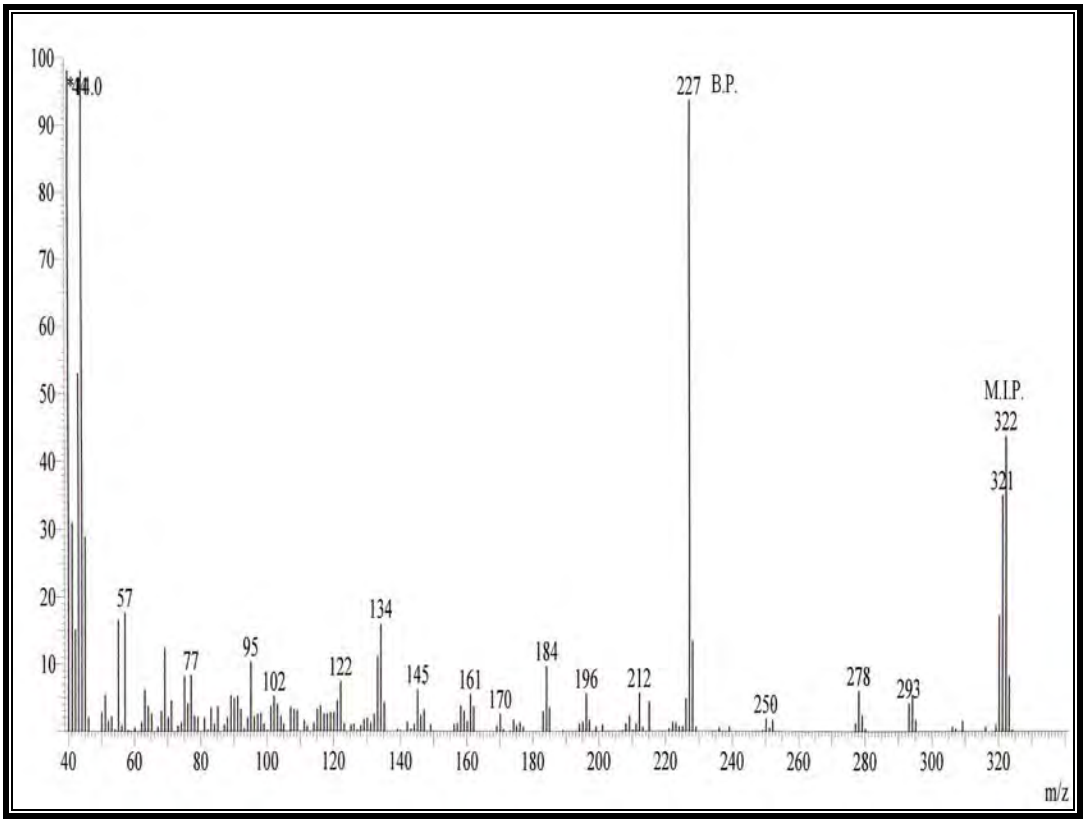
Expanded ^{1}H NMR spectrum of CPV-263



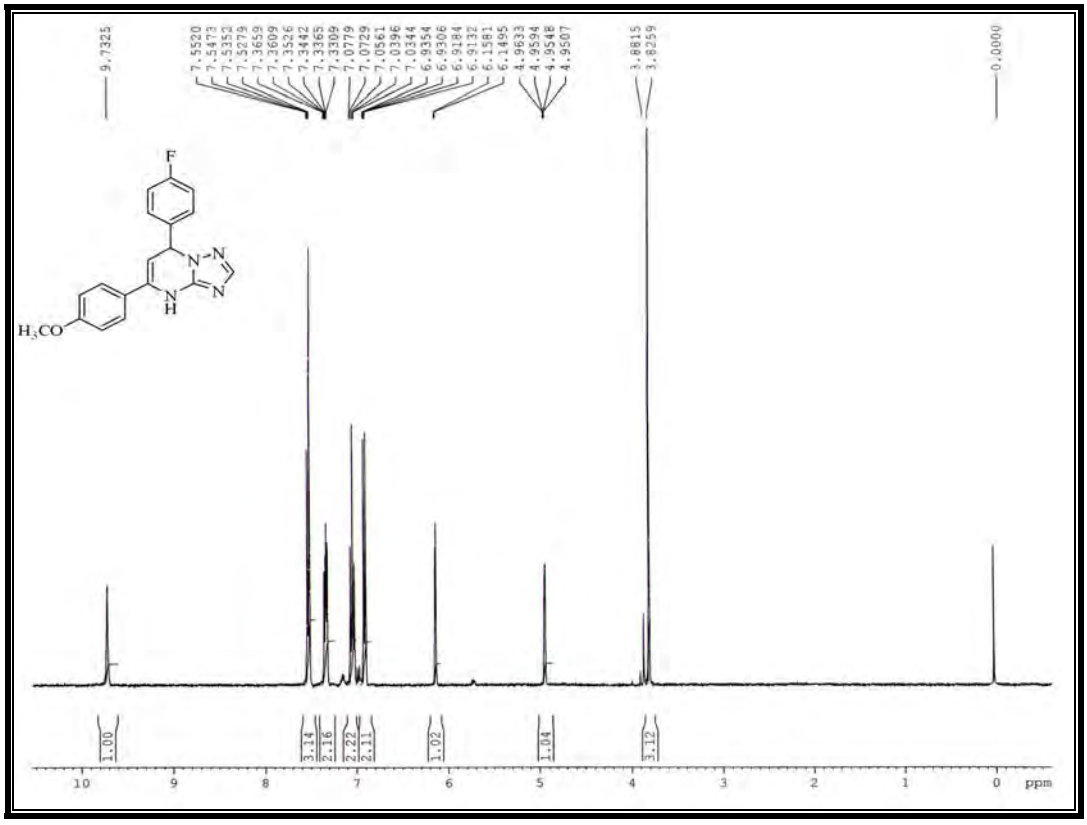
IR spectrum of CPV-265



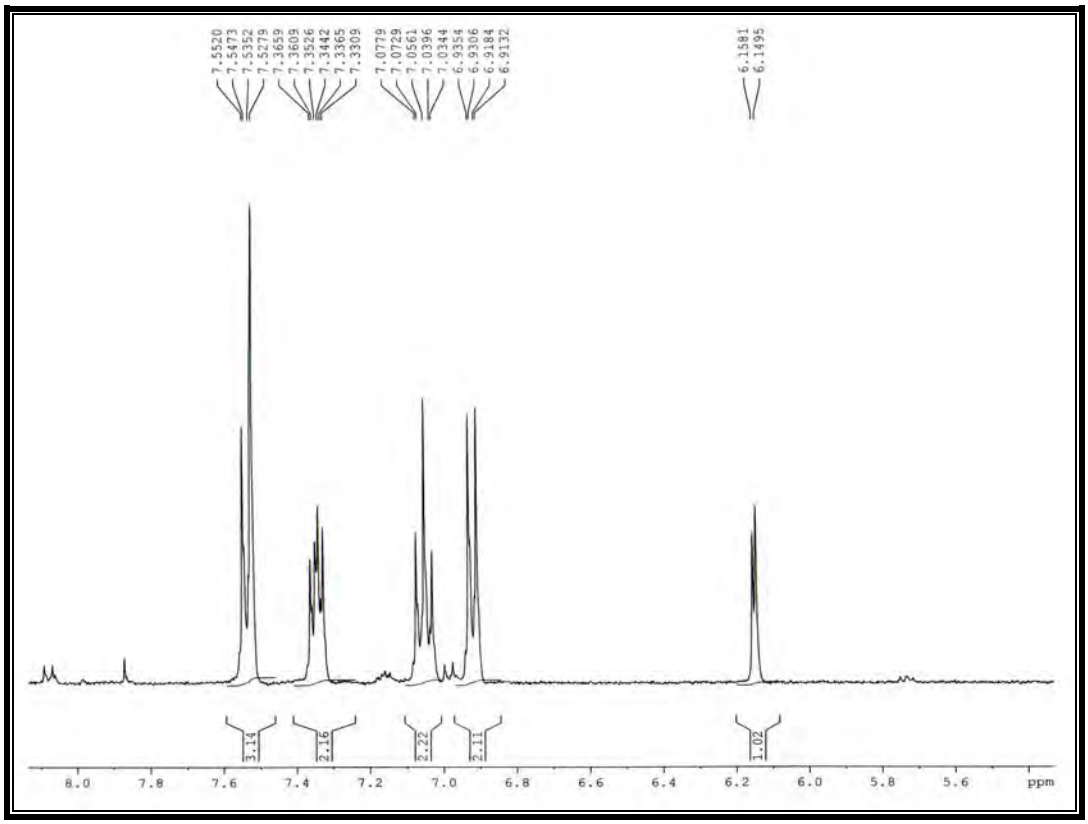
Mass spectrum of CPV-265



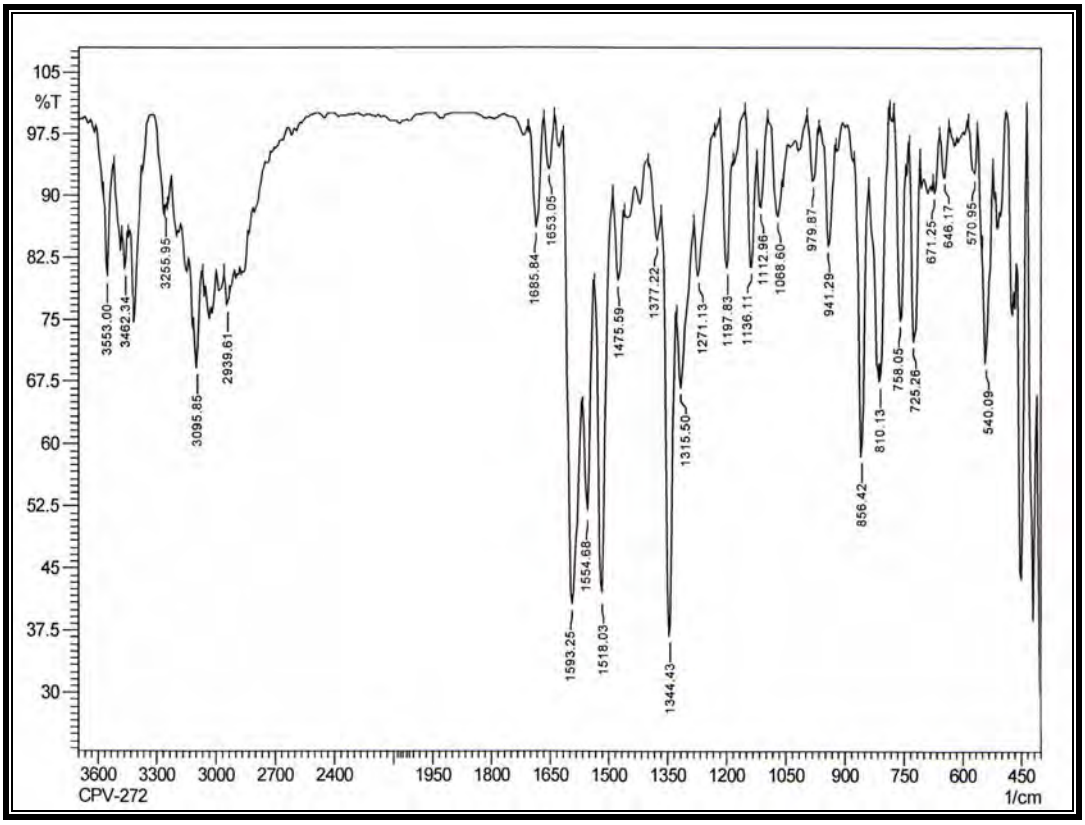
^{1}H NMR spectrum of CPV-265



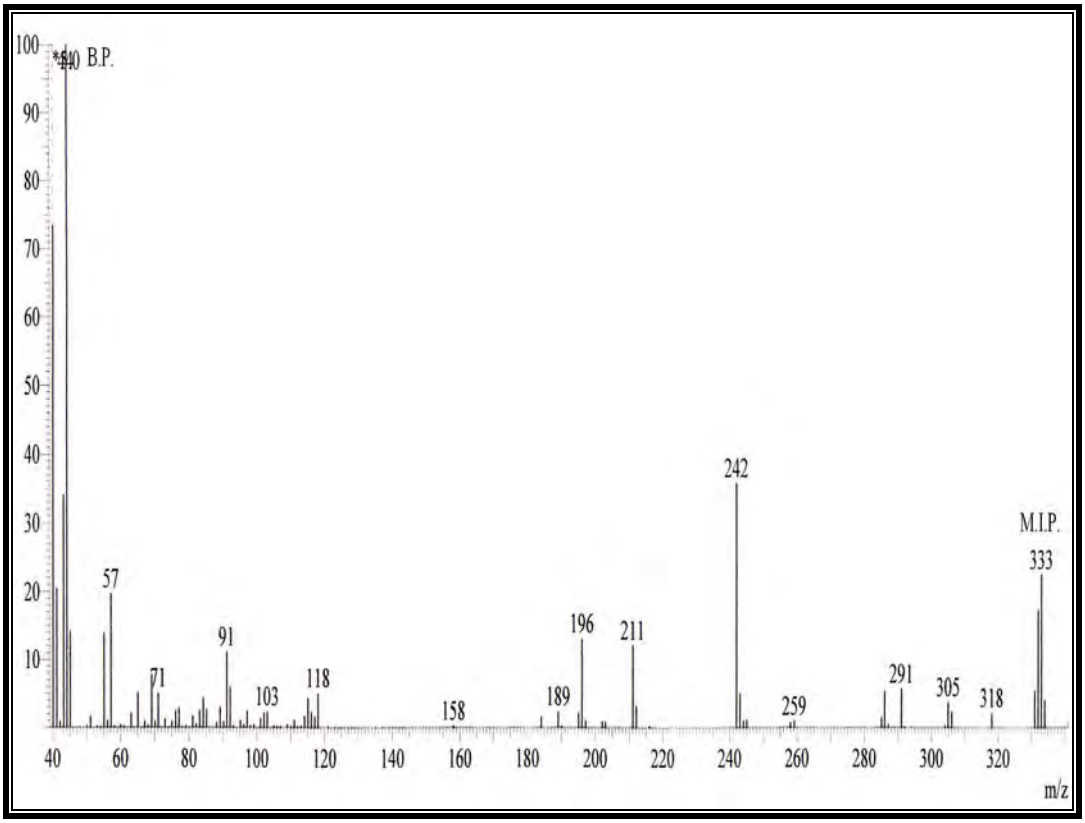
Expanded ^{1}H NMR spectrum of CPV-265



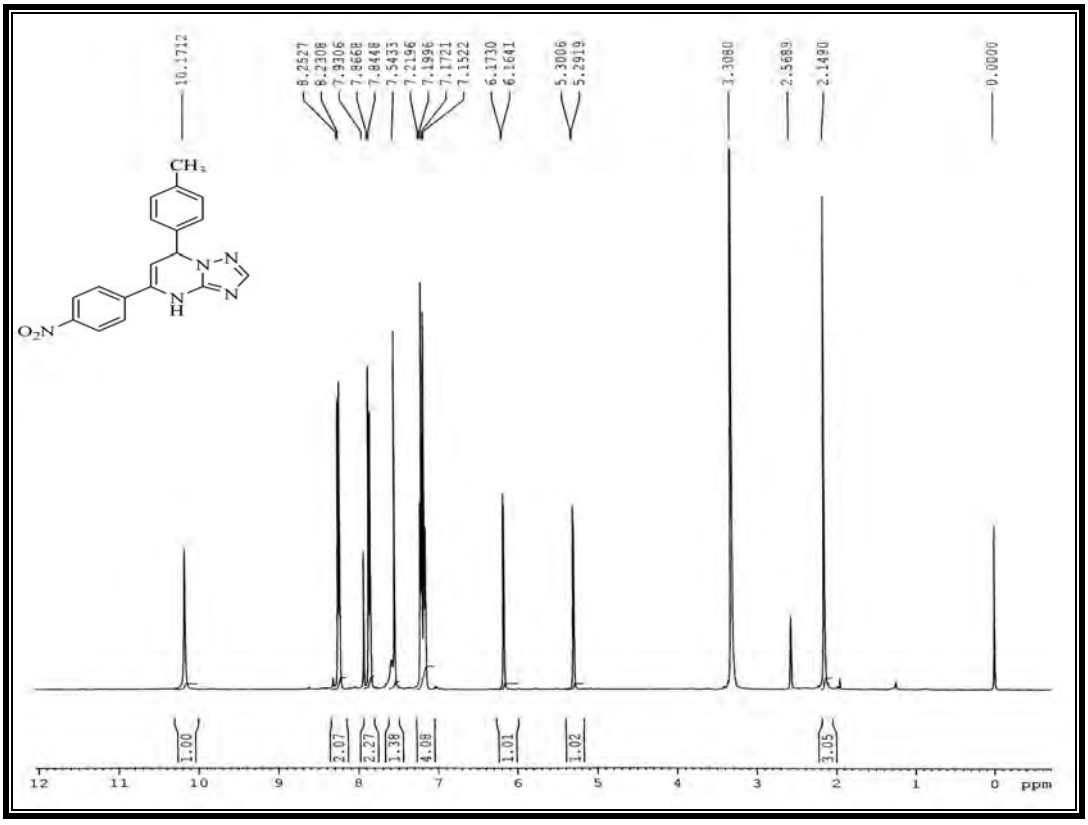
IR spectrum of CPV-272



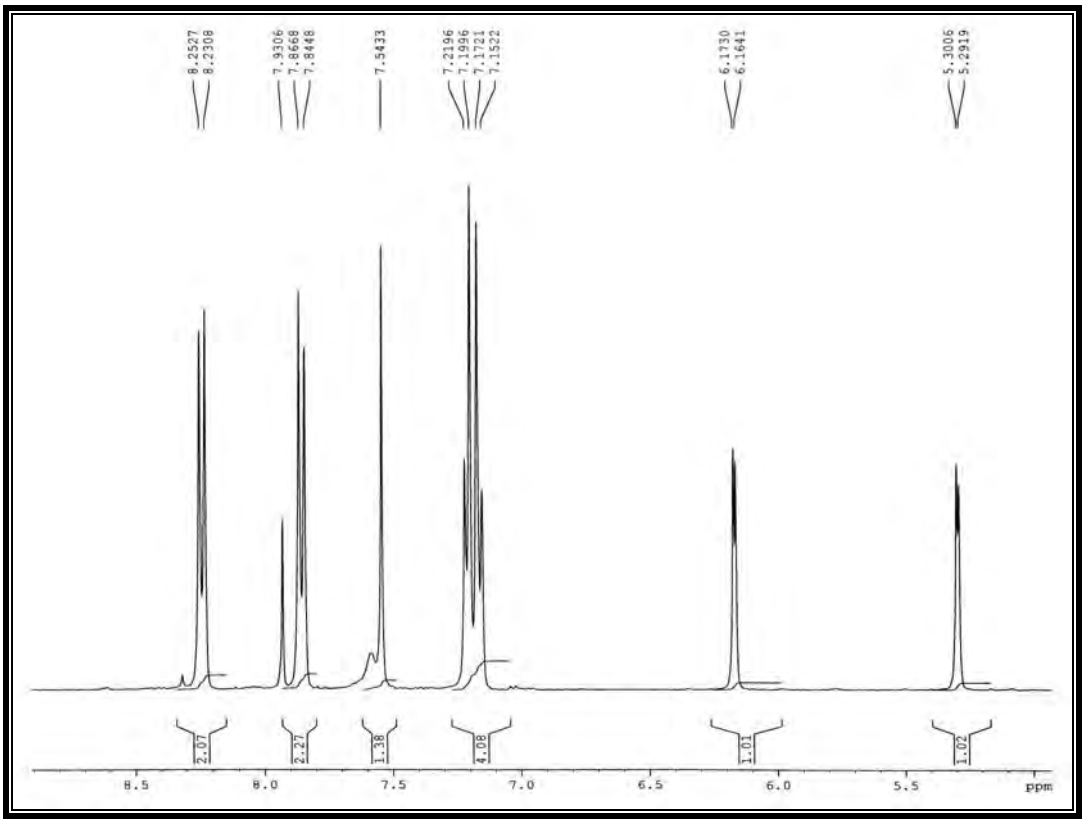
Mass spectrum of CPV-272



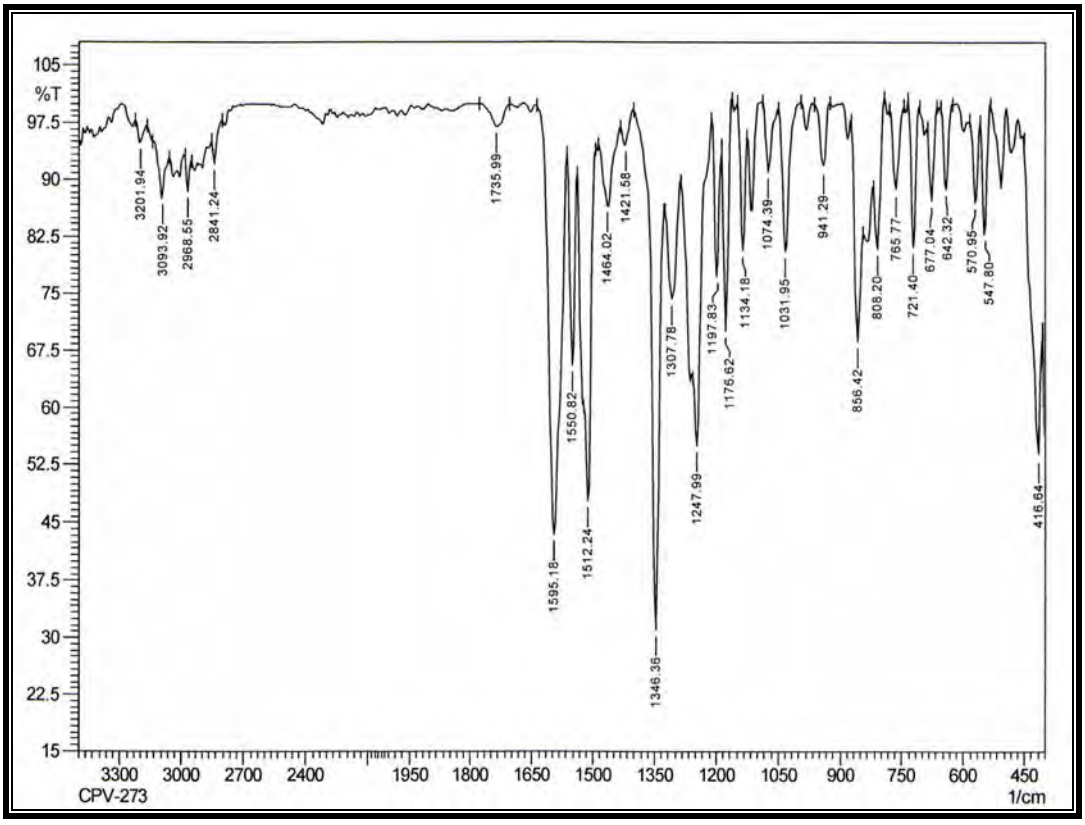
^{1}H NMR spectrum of CPV-272



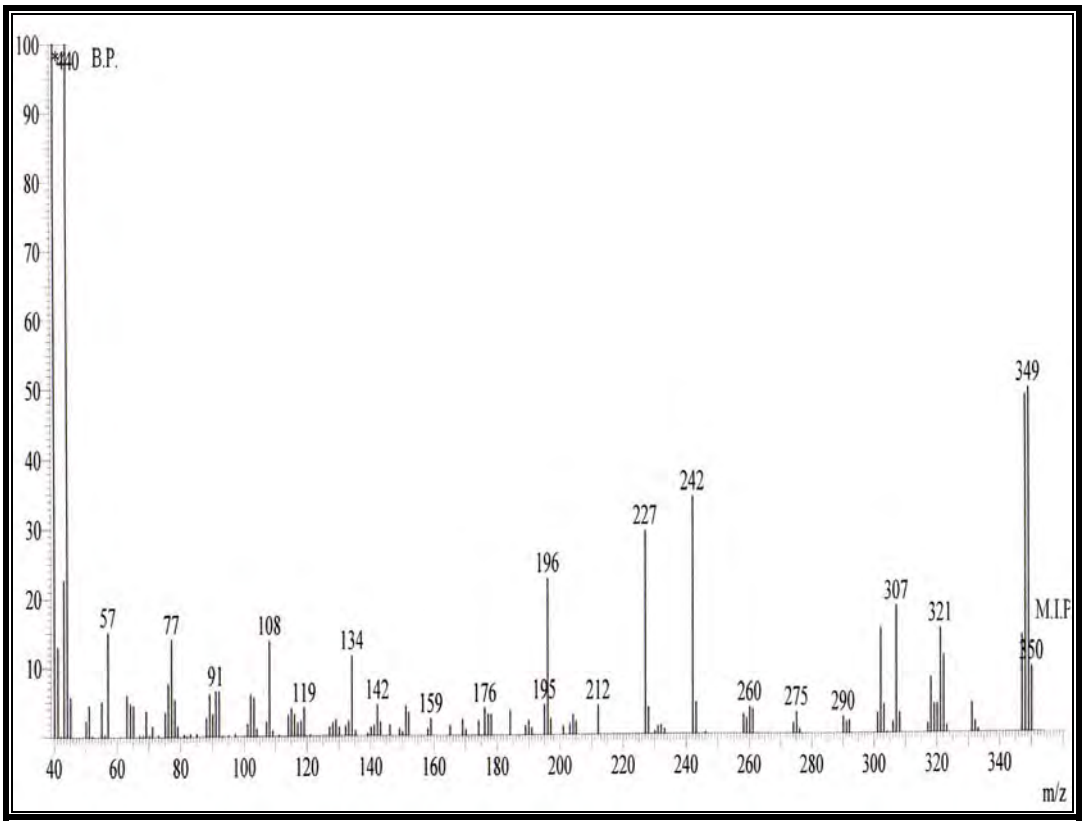
Expanded ^{1}H NMR spectrum of CPV-272



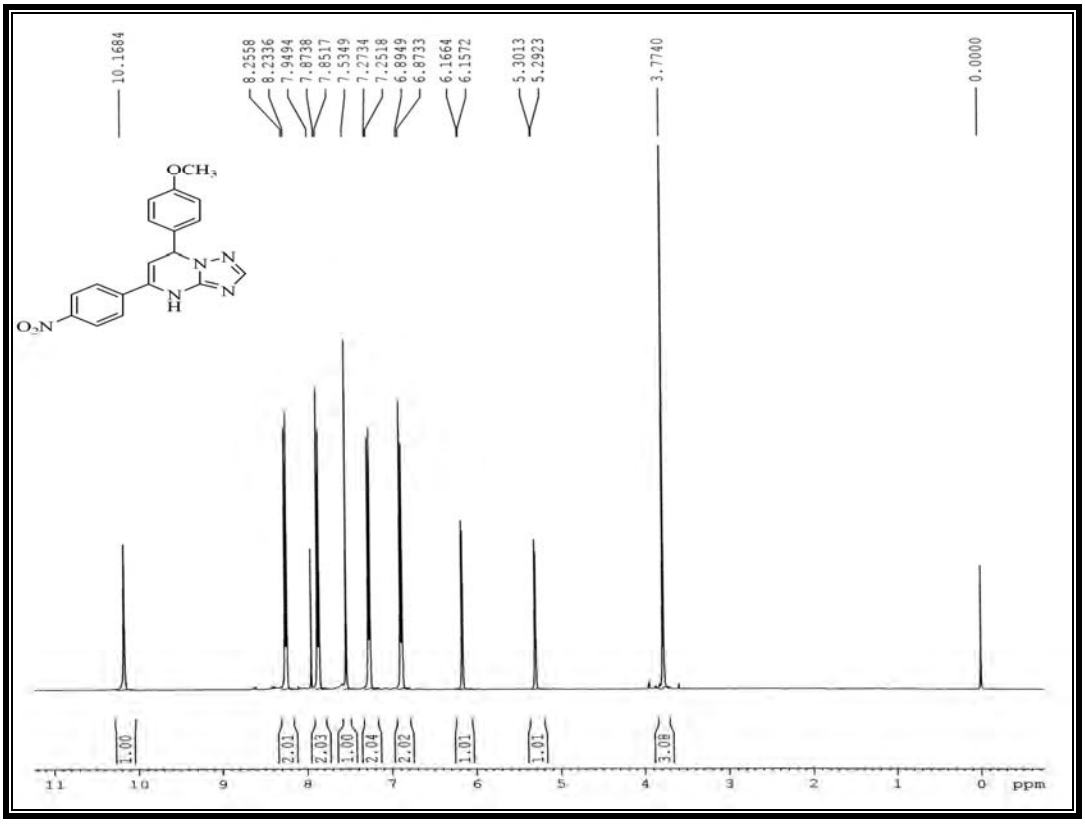
IR spectrum of CPV-273



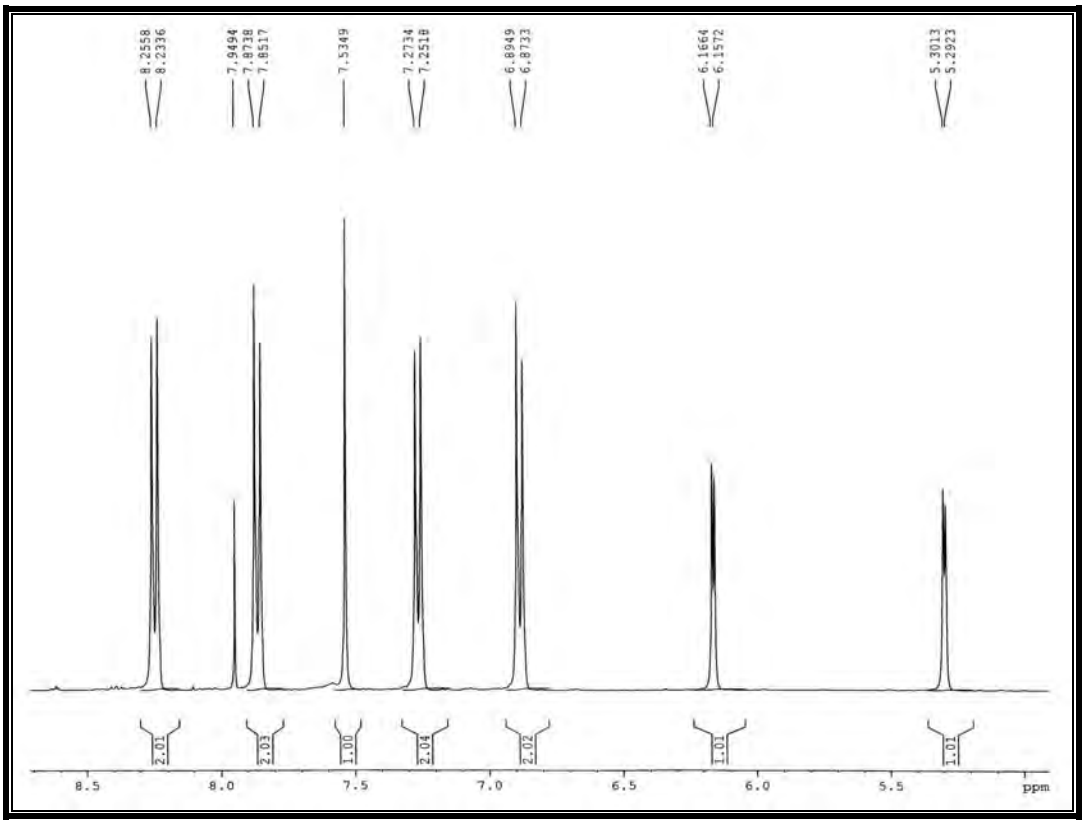
Mass spectrum of CPV-273



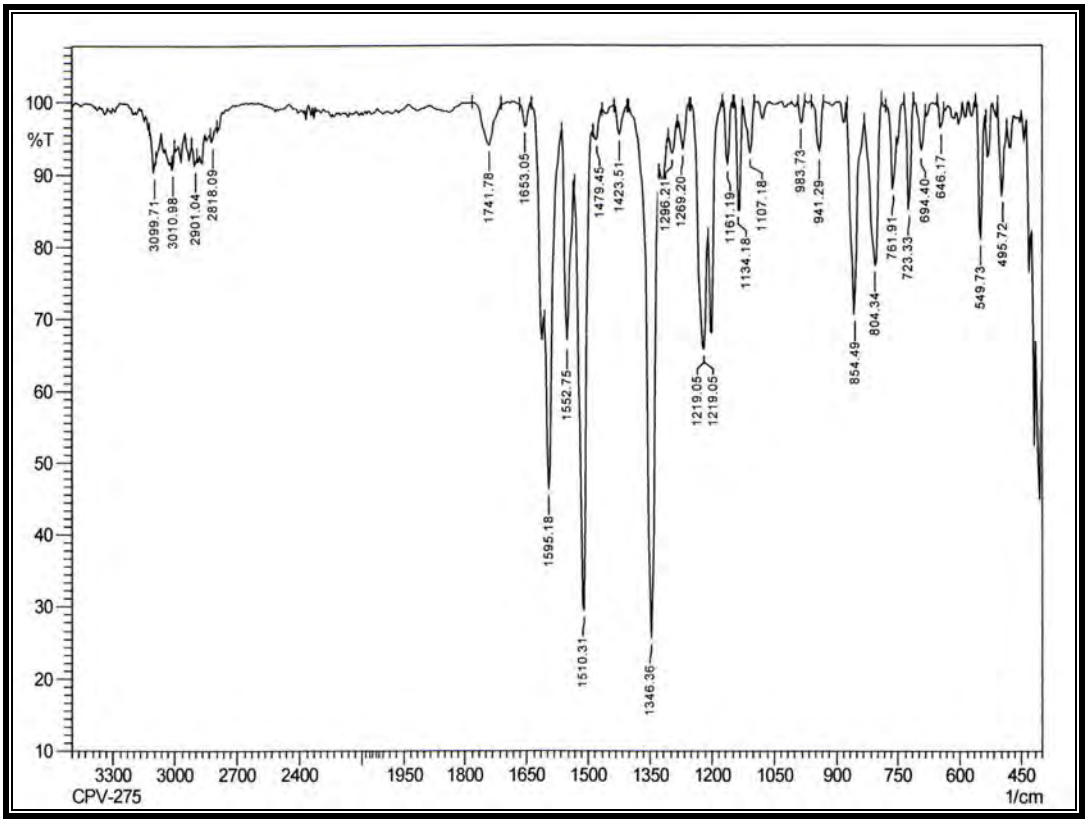
^{1}H NMR spectrum of CPV-273



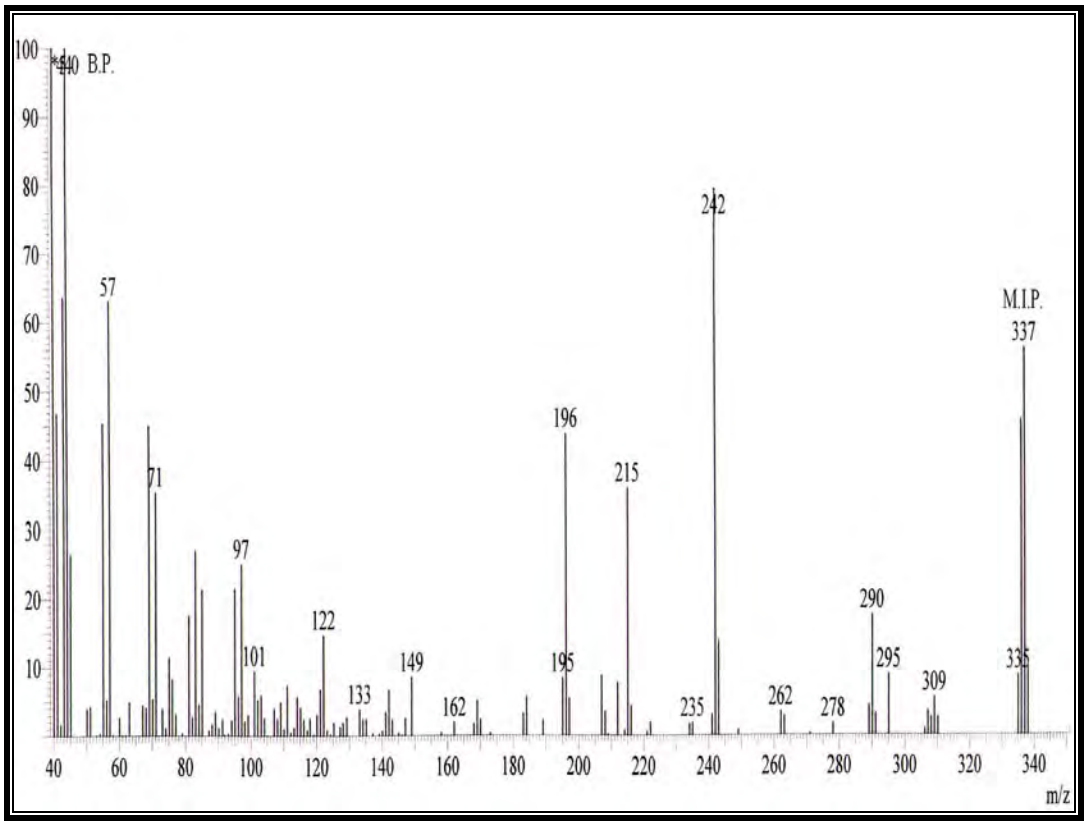
Expanded ^{1}H NMR spectrum of CPV-273



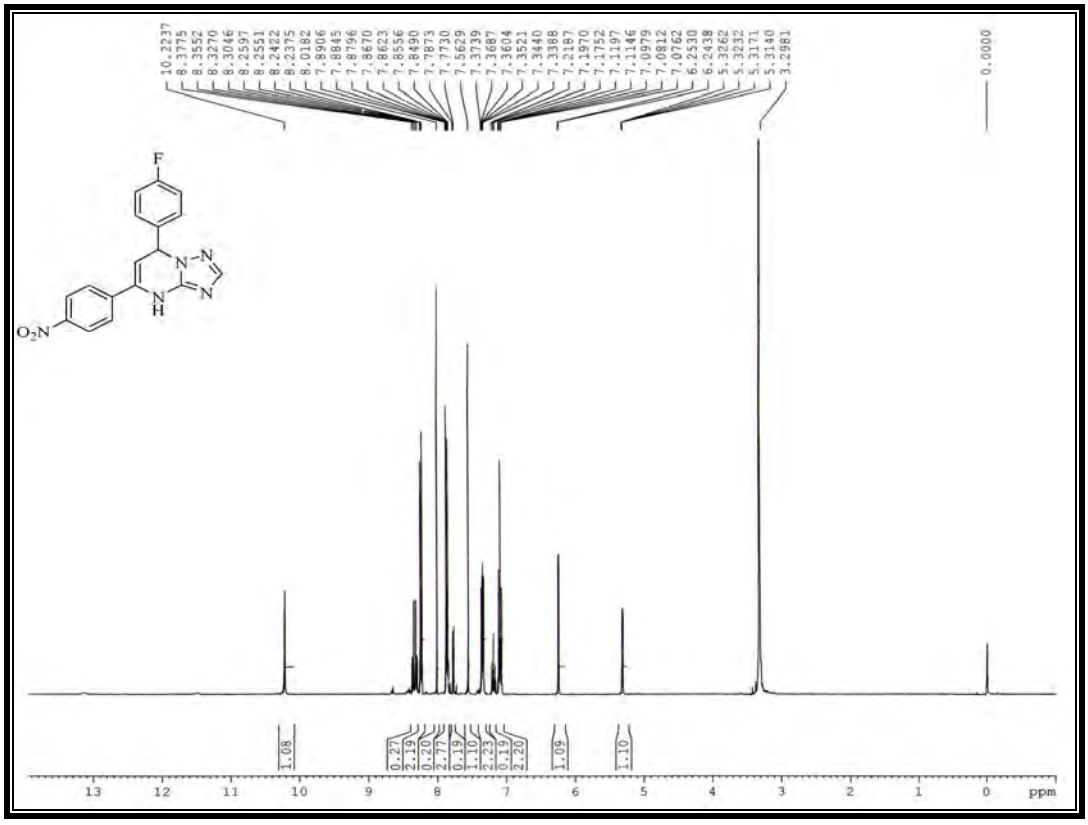
IR spectrum of CPV-275



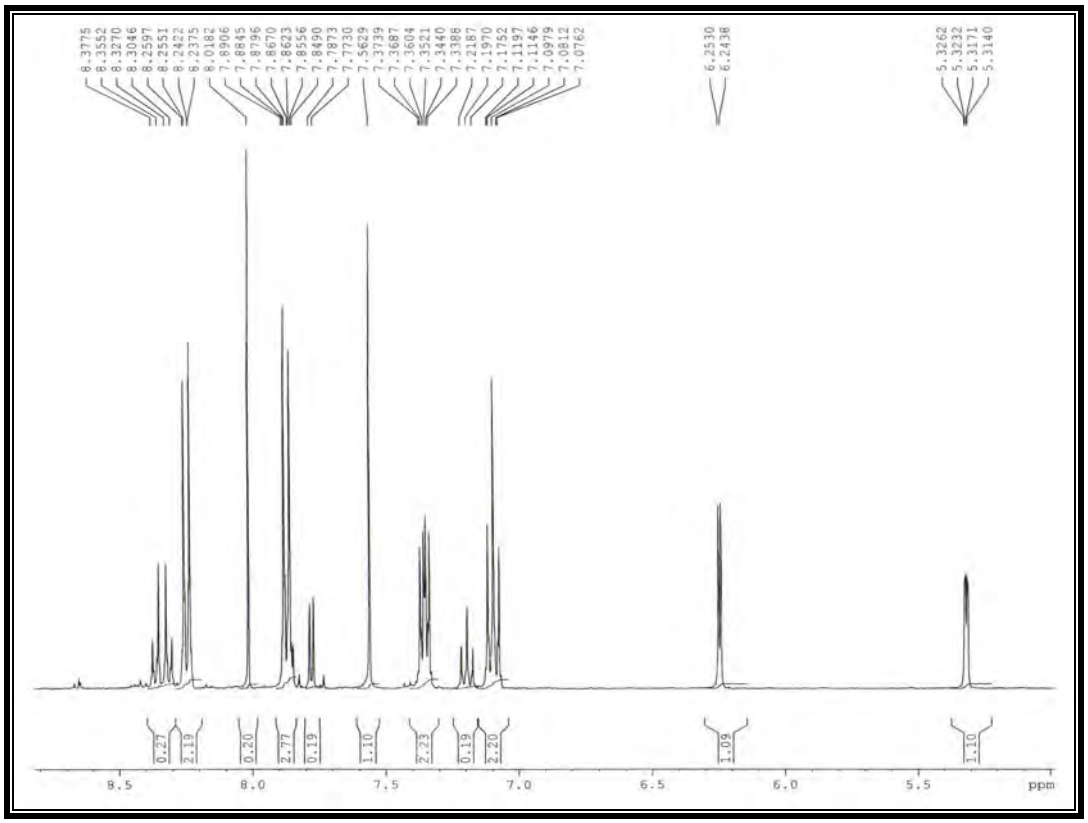
Mass spectrum of CPV-275



^{1}H NMR spectrum of CPV-275



Expanded ^{1}H NMR spectrum of CPV-275



4.13 Biological evaluation

4.13.1 Antimicrobial evaluation

All of the synthesized compounds (CPV-241 to 280) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [98-100] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards [98].

Minimal Inhibition Concentration [MIC]:-

The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Methods used for primary and secondary screening: -

Each synthesized drug was diluted obtaining 2000 μg mL^{-1} concentration, as a stock solution. Inoculum size for test strain was adjusted to 10^8 cfu (colony forming unit) per milliliter by comparing the turbidity.

Primary screen: - In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: - The drugs found active in primary screening were similarly diluted to obtain 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentrations.

Reading Result: - The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table-1:- *In vitro* Antimicrobial Screening Results for CPV-241 to 280

Code	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
CPV-241	200	200	150	100	500	1000	1000
CPV-242	500	200	500	500	500	>1000	>1000
CPV-243	250	250	250	500	1000	>1000	>1000
CPV-244	500	500	500	500	1000	>1000	>1000
CPV-245	500	500	1000	1000	1000	>1000	>1000
CPV-246	200	250	500	250	500	250	250
CPV-247	500	250	150	250	1000	1000	1000
CPV-248	1000	62.5	62.5	500	1000	500	500
CPV-249	1000	500	200	500	250	500	1000
CPV-250	500	250	250	500	500	500	>1000
CPV-251	1000	62.5	100	125	250	1000	250
CPV-252	250	500	250	250	250	200	200
CPV-253	200	250	100	250	500	500	>1000
CPV-254	100	500	250	500	500	>1000	1000
CPV-255	200	250	250	500	>1000	>1000	>1000
CPV-256	100	62.5	62.5	125	500	>1000	>1000
CPV-257	500	500	250	500	1000	500	>1000
CPV-258	500	500	62.5	500	1000	500	500
CPV-259	250	500	500	500	250	>1000	>1000
CPV-260	200	500	1000	1000	500	1000	1000
CPV-261	250	62.5	100	500	500	1000	200
CPV-262	200	250	250	250	500	500	1000
CPV-263	200	500	500	1000	250	500	500
CPV-264	500	100	62.5	100	500	500	>1000
CPV-265	500	500	500	500	200	500	200
CPV-266	250	250	500	500	1000	1000	1000
CPV-267	500	100	500	250	1000	>1000	1000
CPV-268	500	62.5	62.5	125	250	1000	500
CPV-269	100	250	150	500	500	1000	>1000
CPV-270	500	250	1000	1000	1000	>1000	>1000
CPV-271	500	62.5	62.5	100	250	1000	>1000
CPV-272	500	500	250	200	>1000	>1000	>1000
CPV-273	200	500	250	200	500	>1000	>1000
CPV-274	250	250	500	500	500	250	500
CPV-275	500	500	250	500	>100	>1000	>1000
CPV-276	500	500	250	500	250	>1000	>1000
CPV-277	250	200	150	100	250	500	250
CPV-278	500	500	250	1000	250	500	>1000
CPV-279	100	62.5	62.5	500	1000	>1000	>1000
CPV-280	500	250	500	250	>1000	>1000	>1000
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Iprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

4.13.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds CPV-241 to CPV-280 is currently under investigation and results are awaited.

4.14 References and notes

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Summary

The work presented in the current Ph.D.thesis entitled “Studies on Some Heterocycles of Medicinal Interest” can be summarized as below.

Chapter 1 briefly introduces importance of bicyclic and tricyclic aromatic heterocycles in drug discovery as well as concept of “privileged structures”. Chapter 1 further describes aims and objectives of the proposed research work.

Chapter 2 outlines the biological significance and medical significance of pyrimidines. Also, an attempt has been made to include most of the physiologically as well as medicinally important compounds containing pyrimidine and its derivatives to further elaborate the importance of these class of compounds**.**

In Chapter 3, synthesis of thirty novel dihydropyrimidines is reported, which occupy a special position among fused pyrimidines due to a very wide spectrum of their biological activities. The synthesis of these derivatives was carried out by Biginelli cyclocondensation of acetoacetamide of 3-aminopyridine, urea derivative and substituted aromatic aldehydes, using concentrated hydrochloric acid as a catalyst.

Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing from the standpoint of biological activity, due to their diverse pharmacological activities. Chapter 4 includes the brief review of the reported synthetic strategies for the synthesis of these classes of compound.

In Section A of chapter 4, forty 1,2,4-triazolo[1,5-*a*]pyrimidines were synthesized by Biginelli like cyclocondensation of aromatic aldehydes and acetoacetanilide derivatives with aminoazole. The synthesis was accomplished by refluxing in small amount of DMF within a very short period of time of just 12-15 minutes.

In Section B, another four new series of 5-substituted 4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidines were synthesized. The reaction is one pot cyclocondensation of aromatic aldehyde, corresponding acetophenone and 5-amino-1,2,4-triazole using glacial acetic acid as a solvent.

All the synthesized compounds were characterized by various analytical techniques like IR spectroscopy, Mass spectromentry, ^{1}H NMR spectroscopy and elemental analyses.

Thus, 110 compounds are synthesized and characterized in entire thesis work. The synthesized compounds are screened for antimicrobial activity, results of which are incorporated in the thesis. Looking at the antimicrobial activity results (i.e. antibacterial and antifungal), remarkable number of compounds have demonstrated excellent antimicrobial activity as compared to the standard drugs. All the newly synthesized compounds are also under antimycobacterial, anticancer and antiviral evaluation and their results are awaited.

Publication

1. Synthesis and anti-tubercular evaluation of some novel pyrazolo[3,4-*d*]pyrimidine derivatives by Amit Trivedi, Bipin Dholariya, Chintan Vakhariya, Dipti Dodiya, Haresh Ram, Vipul kataria, Arif Siddiqui and Viresh Shah*. Communicated to Medicinal Chemistry Research, Springer link.

Conferences/Seminars participated

1. National Workshop on Management and Use of Chemistry Databases and Patent Literature jointly organized by Department of Chemistry & Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar at Rajkot, India (February 27-29, 2008).
2. "National Workshop on Updates in Process & Medicinal Chemistry" jointly Organized by Department of Chemistry, Saurashtra University, Rajkot and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises and Think Pharma USA (March 3-4, 2009).
3. "National Conference on Spectroscopy & Stereochemistry" Organized by Department of Chemistry, Saurashtra University, Rajkot Sponsored by UGC, New Delhi and Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar (March 18-20, 2009).
4. "Two Days National Workshop on Patents & IPR Related Updates" Organized by Technology Information, Forecasting Assessment Council (TIFAC)-New Delhi, Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises at Department of Chemistry, Saurashtra University, Rajkot (September 19-20, 2009).
5. "International Seminar on Recent Developments in Structure and Ligand Based Drug Design" jointly organized by Schrodinger LLC, USA & Department of Chemistry and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises at Department of Chemistry, Saurashtra University, Rajkot (December 23, 2009).