Studies on Some Compounds 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

Amit R. Trivedi

Under the Guidance of
Prof. V. H. Shah

Department of Chemistry
(DST-FIST Funded & UGC-SAP Sponsored)
Saurashtra University
Rajkot, Gujarat
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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.

Date:

Place: Rajkot (Amit R. Trivedi)

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Professor

No.: Date:

Certificate

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

Date:
Place: Rajkot

Dr. Viresh H. Shah
Professor,
Department of Chemistry,
Saurashtra University,
Rajkot-360005
Gujarat (India).

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

Chapter 5 Microwave assisted synthesis and biological evaluation of pyrazolo [3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines

List of Abbreviations

NCEs	New Chemical Entities
R & D	Research & Development
Da	Dalton
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

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. Microwave assisted reaction were carried out in QPro-M microwave synthesizer.

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

Synopsis

The work to be presented in thesis entitled "Studies on Some Compounds 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	Synthesis and biological evaluation of thieno[2,3-*d*]pyrimidines
Chapter 4	Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines
Chapter 5	Microwave assisted synthesis and biological evaluation of pyrazolo [3,4-*d*][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.

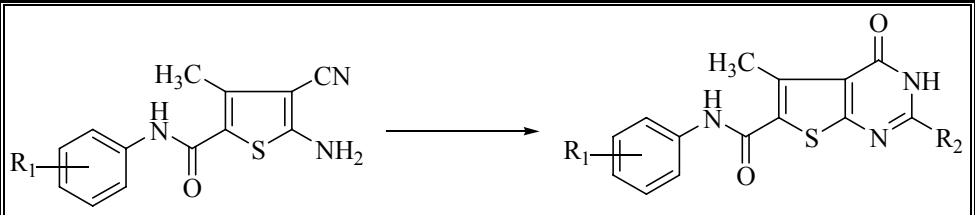
Chapter 2 Biological and medicinal significance of pyrimidines and related heterocycles

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.

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

Chapter 3 Synthesis and biological evaluation of thieno[2,3-*d*]pyrimidines

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. Due to formal isoelectronic relationship with purines, the thieno[2,3-*d*]pyrimidine ring system is of special biological interest. It has numerous pharmacological and medicinal applications *viz*, antitumour, immunodilator, tuberculosis, antiallergic and radioprotective.



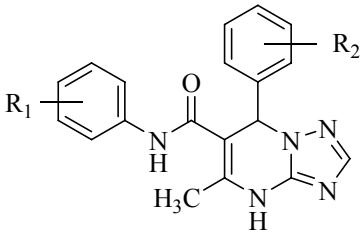
Where R_1= H, 2-OCH_3, 4-OCH_3 etc and R_2 = H, CH_3, CF_3

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 thieno[2,3-*d*]pyrimidines (ART-111 to ART-130) are synthesized in chapter 3. The synthesis of thieno[2,3-*d*]pyrimidines (ART-111 to ART-130) was achieved by acid catalysed cyclocondensation of polysubstituted 2-aminothiophenes (ART-101 to ART-110) with formic acid, gl. acetic acid and trifluoroacetic acid, respectively. Polysubstituted 2-aminothiophenes were prepared via the Gewald reaction. The products were characterized by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. 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 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.

In chapter 4, synthesis of four new series of of 1,2,4-triazolo[1,5-*a*]pyrimidines (ART-201 to ART-240) containing an acetoacetamide fragment has been undertaken. The structures of all the newly synthesized compounds are elucidated by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds are subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.



ART 201 TO 240

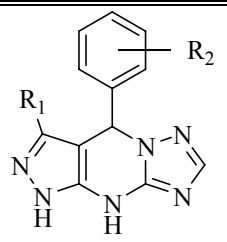
Where R_1= 4-OCH_3, 4-F, 4-CF_3, 2-F and R_2 = 4-OCH_3, 4-CH_3, 4-F, 4-Cl etc..

Chapter 5 Microwave assisted synthesis and biological evaluation of pyrazolo [3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines

Flat heterocyclic structures are fundamental moieties in anticancer compounds acting as DNA intercalating agents. In this context, pyrazolotriazolopyrimidines have been the subject of intense research due to the interesting pharmacological activities found for several of their derivatives. Among the various pyrazolotriazolopyrimidine ring systems, pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines have never been explored,

and there are no reports on the synthesis and chemical properties of these class of compounds. With the aim to extend the synthetic pathways to new planar heterocyclic ring systems we focused our studies on the pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine core structure which was found to be absent in the literature survey (Scifinder, CA, STN search).

Chapter 5 describes synthesis of pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimi dine (ART-301 to ART-330), a new ring system, which was achieved by using a one-pot, microwave-assisted, catalyst-free biginelli like condensation. All the newly synthesized compounds are characterized by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds are subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.



ART 301 TO 330

Where R_1= CH_3, NH_2 and R_2 = 4-OCH_3, 4-CH_3, 4-F, 4-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).

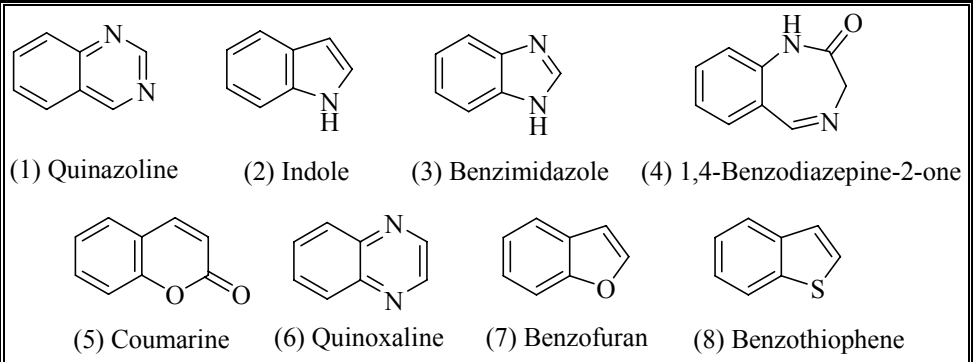
The cause of this innovation deficit is definitively not the biology. Decoding of the human genome has led to a wealth of drug targets. With more than 30,000 human genes, the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each 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 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 Da 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].

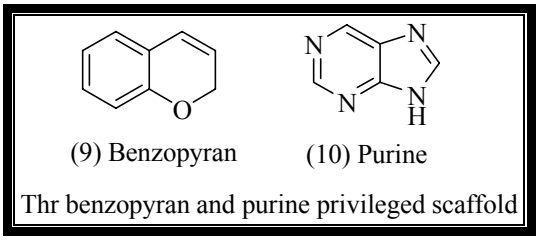
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening 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 *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 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 and co-workers constructed a library based on the benzopyran (9) privileged scaffold [6], whereas Schultz and co-workers 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.

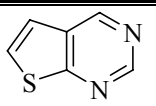
(3) The attached component is then added as a prefix to the parent component. In the name of the prefix, the terminal 'e' is changed to 'o'.

(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.

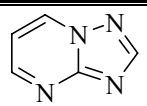
(5) The numbering of the final condensed heterocycle is carried out independently, starting at an atom adjacent to a bridged-head atom, whereby heteroatoms receive the smallest possible number.

1.3 Objectives

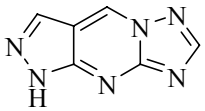
Our interest in the synthesis and biological evaluation of heterocyclic bi and tricycles and the fact that some of these compounds are not frequently used (thieno[2,3-*d*] pyrimidines (11), 1,2,4-triazolo[1,5-*a*]pyrimidines (12) and pyrazolo[3,4-*d*][1,2,4] triazolo[1,5-*a*]pyrimidines (13)) in commercial compound libraries, prompted us to elaborate this type of chemistry and to synthesize three different heterocyclic scaffolds.



(11) Thieno[2,3-*d*]pyrimidine



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



(13) Pyrazolo[3,4-*d*][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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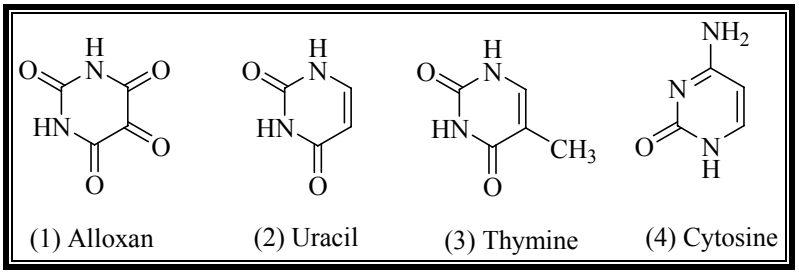
[7] Ding, S.; Gray, N. S.; Ding, Q.; Schultz, P. G. *Tetrahedron Lett*. **2001**, *42*, 8751.

Chapter 2
Biological and medicinal significance of pyrimidines and related heterocycles

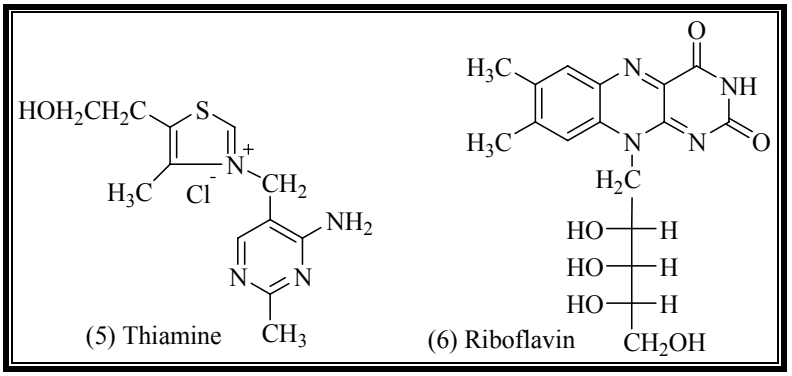
2.1 Biological significance

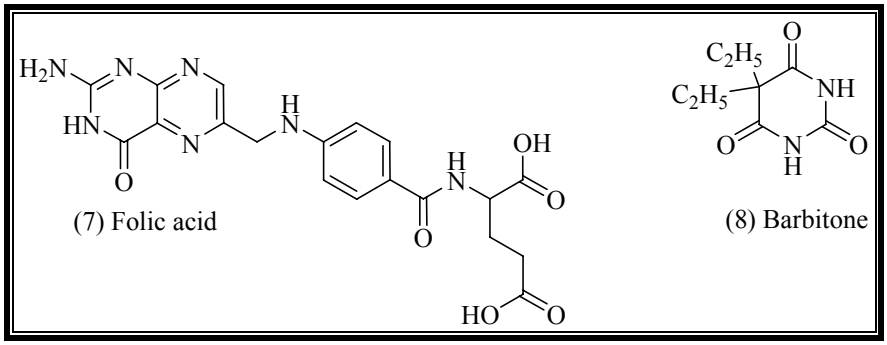
Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS.

Alloxan (1) is known for its diabetogenic action 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].





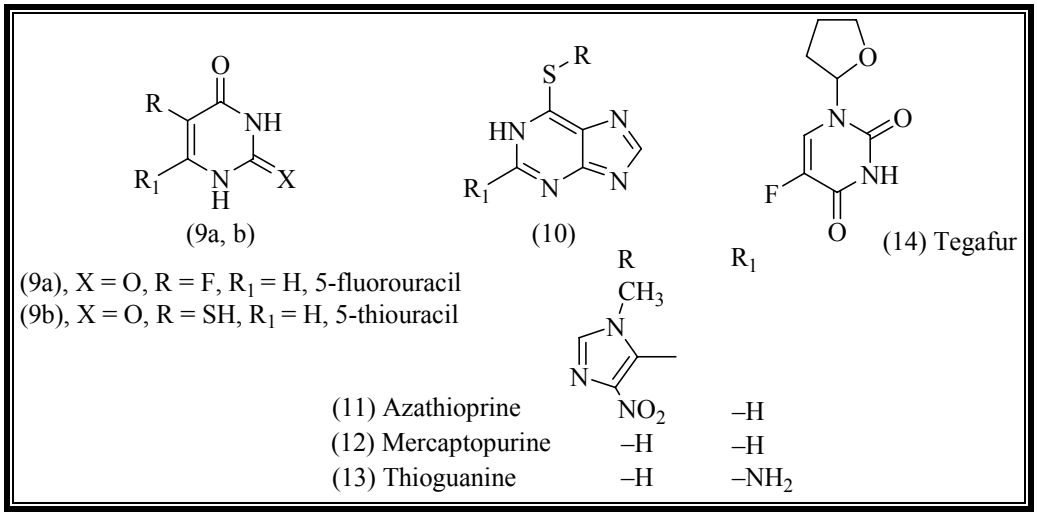
2.2 Medicinal significance

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

2.2.1 Antineoplastics and anticancer agents

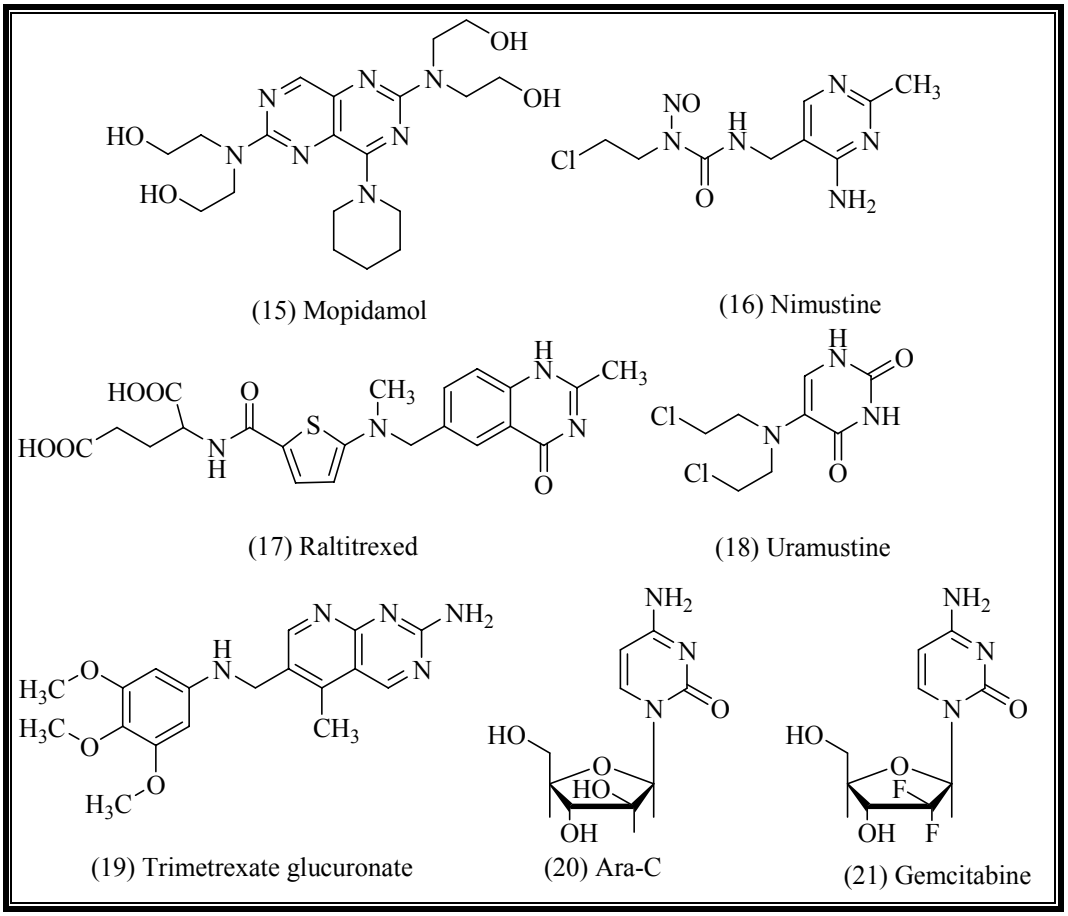
There are a large number of pyrimidine-based antimetabolites. Usually, they are structurally related to the endogenous substrates that they antagonize. The structural modification may be on the pyrimidine ring or on the pendant sugar groups. One of the early metabolites prepared was 5-fluorouracil (5-FU,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

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 and Fildes in 1940. These drugs prevent the utilization of normal cellular metabolites [6].



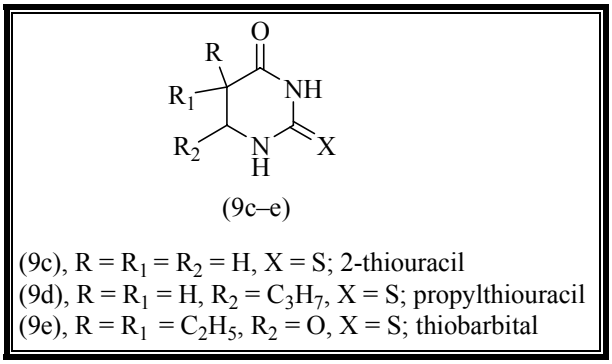
There are many more in recent times, like mopidamol (15) [11], nimustine (16) [12], raltitrexed (17) [13], uramustine (18) [14] and trimetrixate (19) [15]. 1-*β*-D-Arabinosylcytosine (Ara-C, 20) [16] is also an example of a pyrimidine antimetabolite in which the sugar is arabinose having a beta configuration. 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

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].

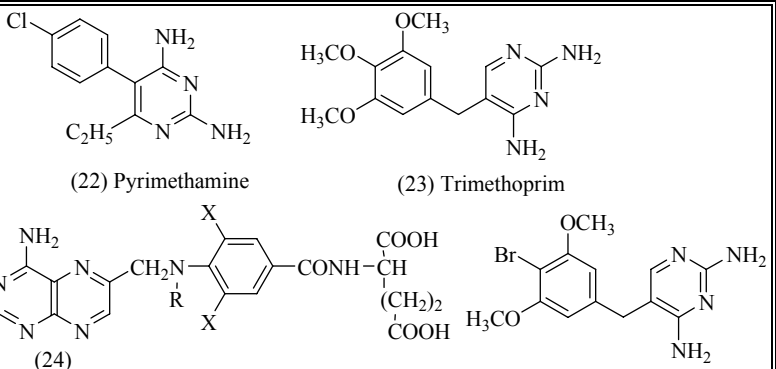


(9c–e)

(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, antibacterials and antiprotozoals

In 1948, Hitchings 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].



(22) Pyrimethamine

(23) Trimethoprim

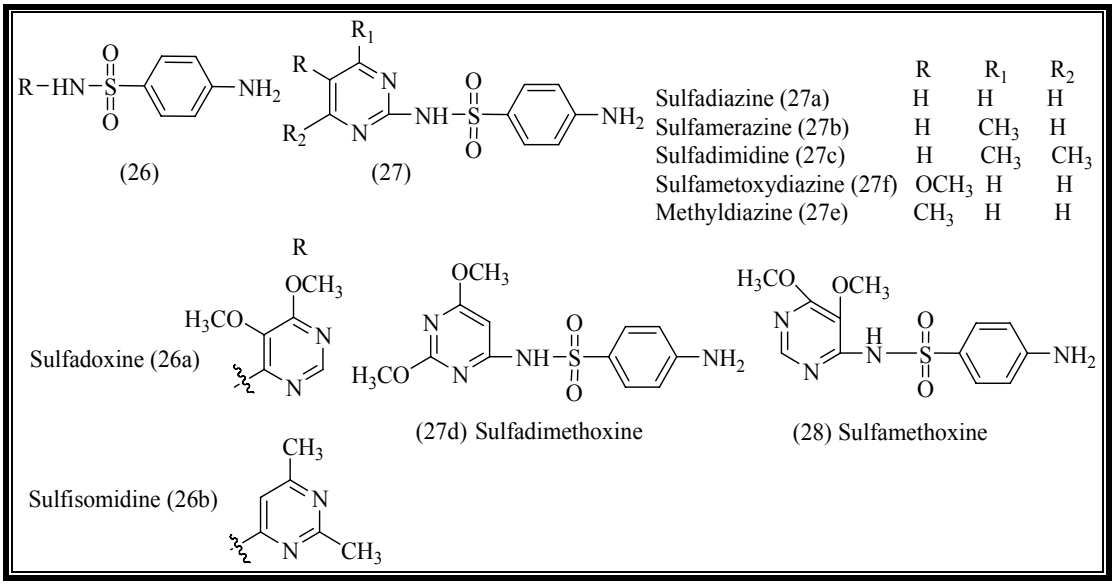
(24)

(25) Brodimoprim

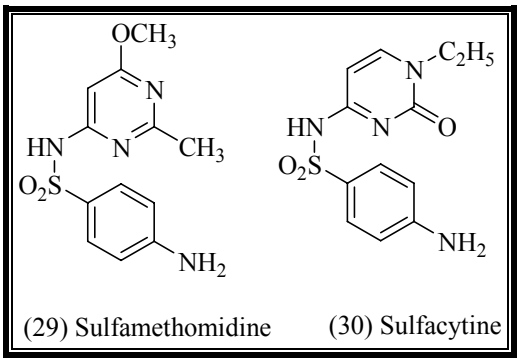
(24a) R = CH_3, X = H; Methotrexate
(24b) R = X = H; Aminopterin
(24c) R = CH_3, X = Cl; 3',5'-dichloromethotrexate

2.2.4 Sulfa drugs

Pyrimidine derivatives of sulfa drugs, namely sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute UTIs, 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.



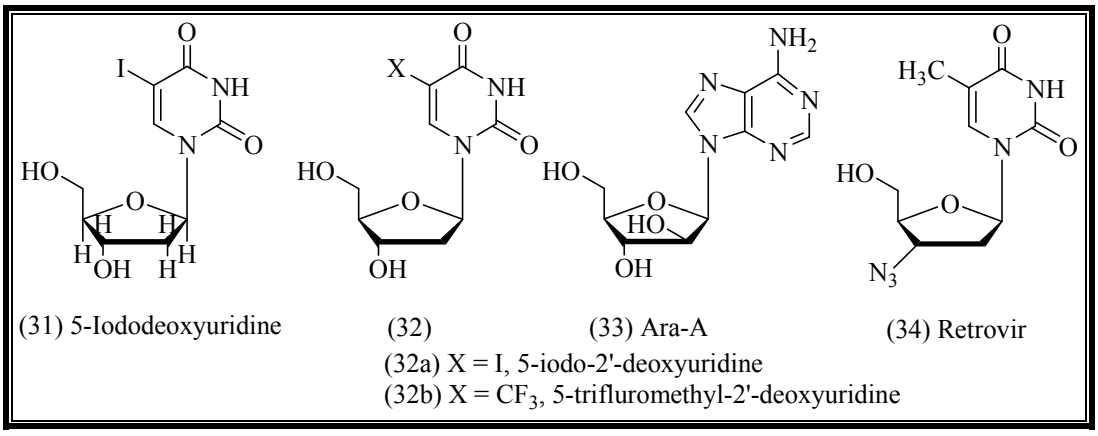
In 1959, sulfadimethoxine (27d) [29] was introduced with a half-life of approximately 40 h. The related 4-sulfonamidopyrimidine, sulfamethoxine (28) [29] having two methoxy groups at 5 and 6 positions, has by far the longest half-life of about 150 h. Methyldiazine (27e) [29] has a half-life of 65 h.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



(29) Sulfamethomidine (30) Sulfacytine

2.2.5 Antivirals and anti-AIDS

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



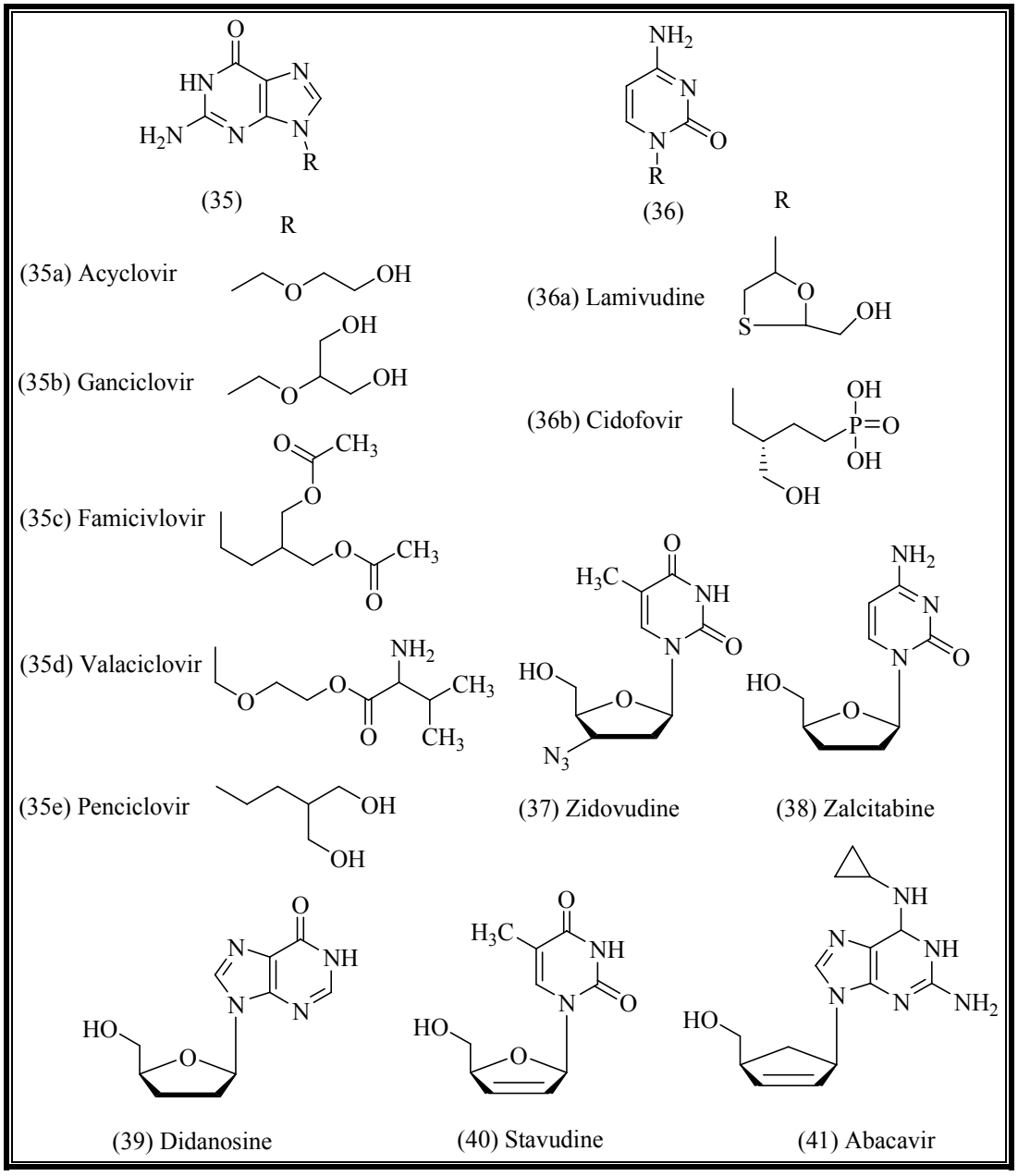
(31) 5-Iododeoxyuridine (32) (33) Ara-A (34) Retrovir

(32a) X = I, 5-iodo-2'-deoxyuridine
(32b) X = CF_3, 5-trifluromethyl-2'-deoxyuridine

IDU (5-iodo-2'-deoxyuridine) (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 (AZT-16, 34) is a potent inhibitor of the *in vivo* replication and cytopathic effects of HIV and has been recently approved for use against AIDS and severe 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 $HCV_{1\&2}$.

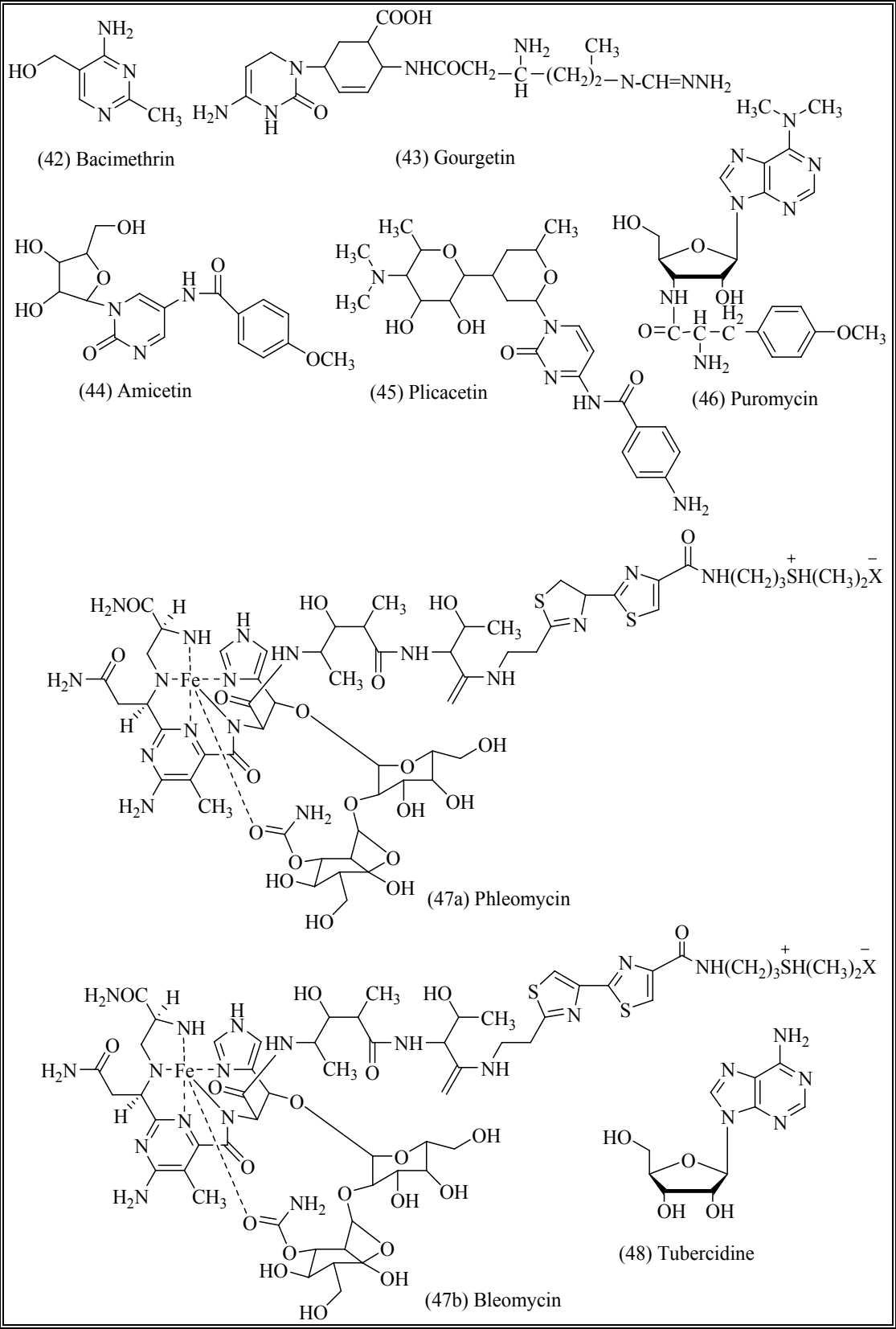


Several members of a series of acyclic nucleosides, which contain a fused pyrimidine ring (mainly purine), are found to be effective antivirals. Famiciclovir (35c) and valaciclovir (35d) are drugs used for several DNA viruses, including HSV types 1 and 2, Varicella-zoster virus and Epstein-Barr virus [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 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 $CD4^+$ cell count falls below 300 cells/mm^3. Didanosine (39) [37] is a purine dideoxynucleoside, which is an analogue of inosine. Didanosine inhibits HIV 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 NRTIs.

2.2.6 Antibiotics

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. 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].



(42) Bacimethrin

(43) Gourgetin

(44) Amicetin

(45) Plicacetin

(46) Puromycin

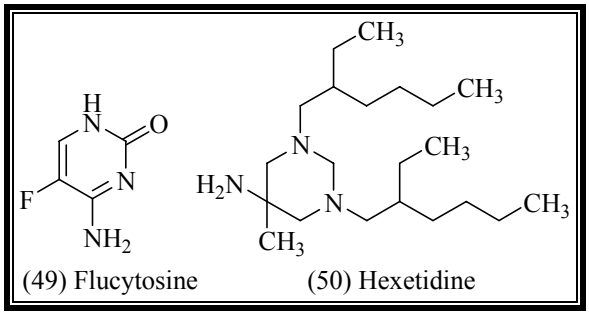
(47a) Phleomycin

(47b) Bleomycin

(48) Tubercidine

2.2.7 Antifungals

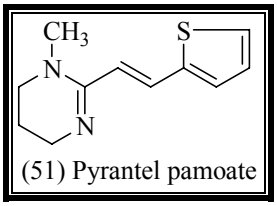
Pyrimidines also exhibit antifungal properties. Flucytosine (49) [41] is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and Cryptococcus [42]. Hexetidine (50) [43] is mainly used for the treatment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



(49) Flucytosine (50) Hexetidine

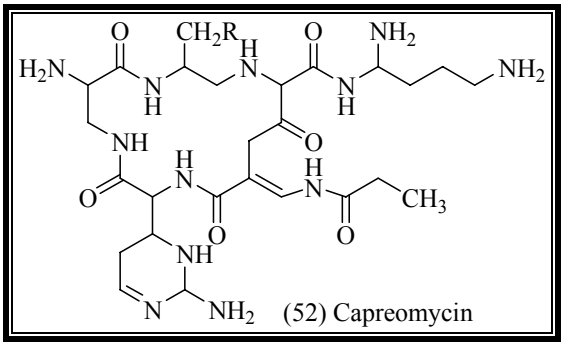
2.2.8 Anthelmentics

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].



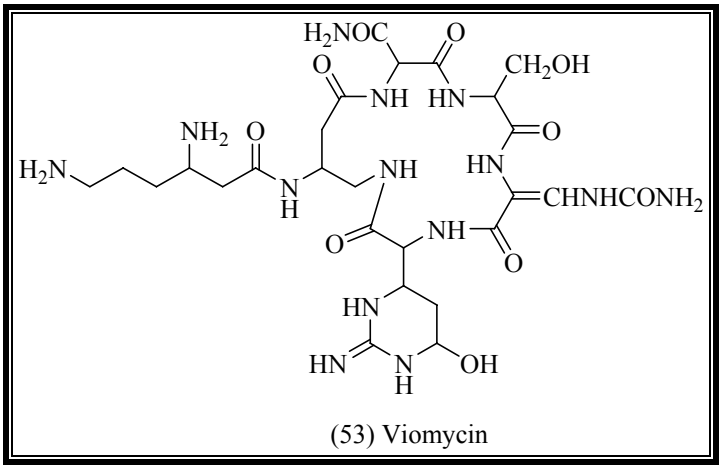
(51) Pyrantel pamoate

2.2.9 Antitubercular drugs



(52) Capreomycin

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

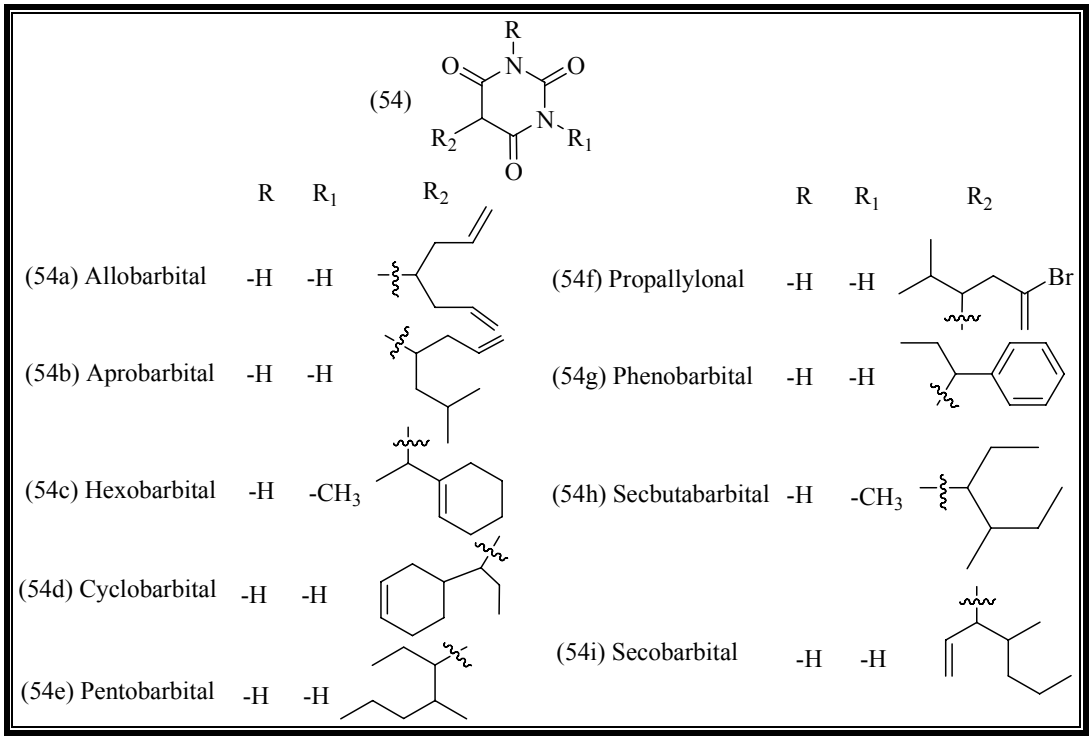


(53) Viomycin

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

2.2.10 CNS active agents

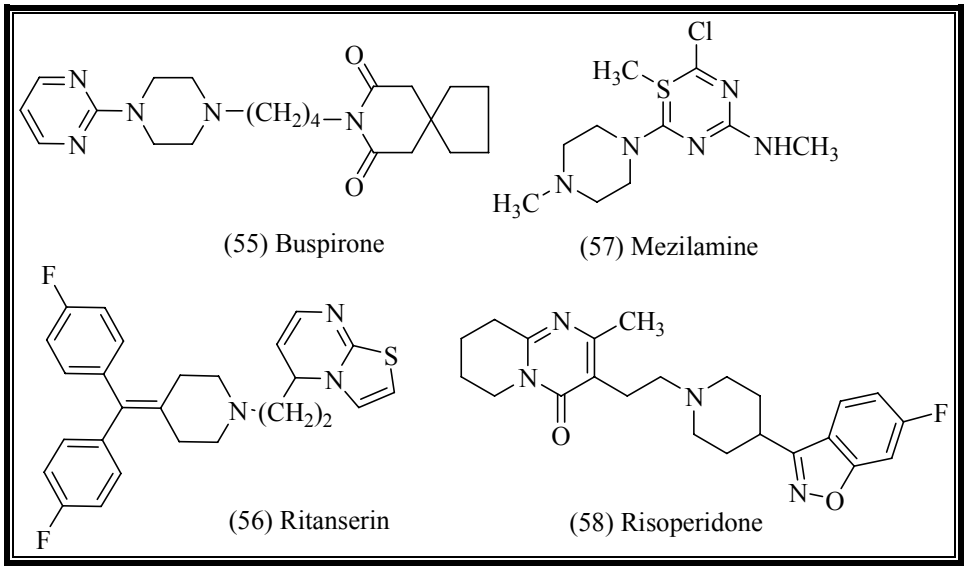
2.2.10.1 Sedative/hypnotic/antiepileptic agents



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.

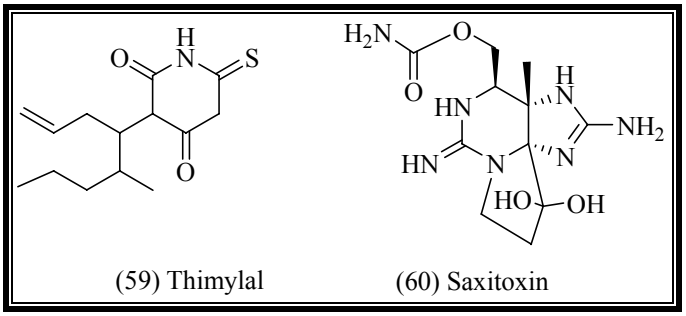
2.2.10.2 Anxiolytic agents

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

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

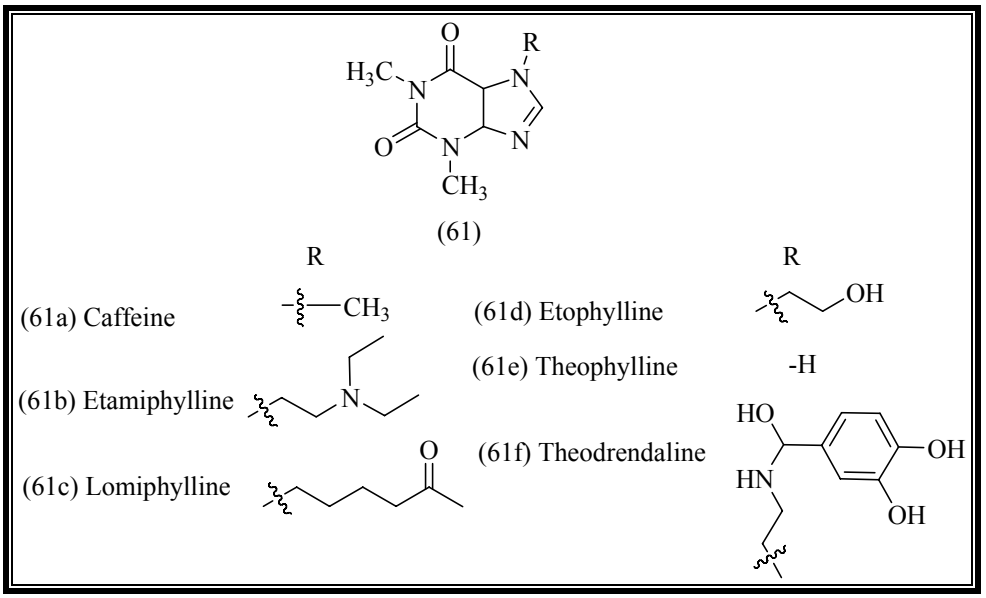


(59) Thimylal (60) Saxitoxin

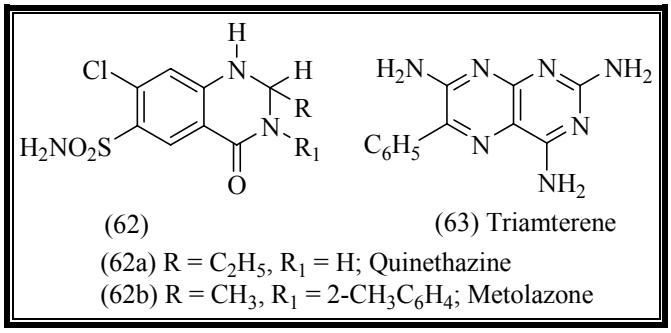
Saxitoxin (60) [58] is a naturally occurring pyrimidine containing anaesthetic agent, but is too toxic to be of clinical use. Saxitoxin is isolated from some marine dinoflagellates.

2.2.10.4 Diuretics and uricosurics

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].



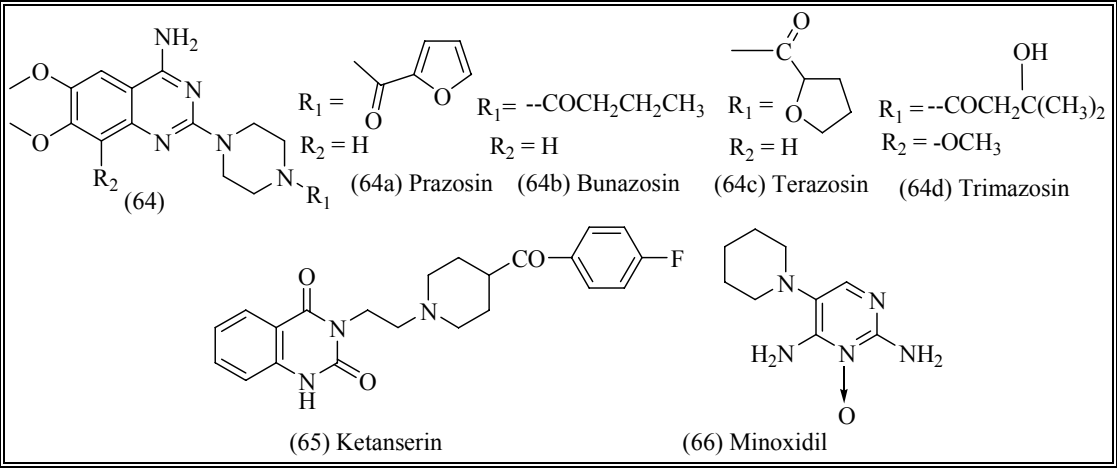
(62) (63) Triamterene

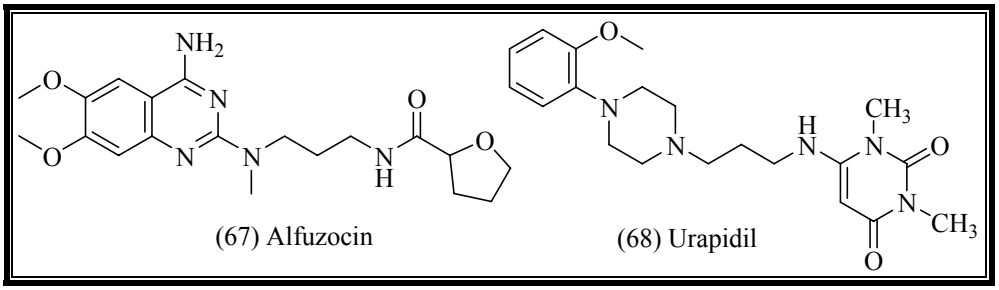
(62a) R = C_2H_5, R_1 = H; Quinethazine
(62b) R = CH_3, R_1 = 2-$CH_3C_6H_4$; Metolazone

2.2.11 Cardiac agents

2.2.11.1 Antihypertensives

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].

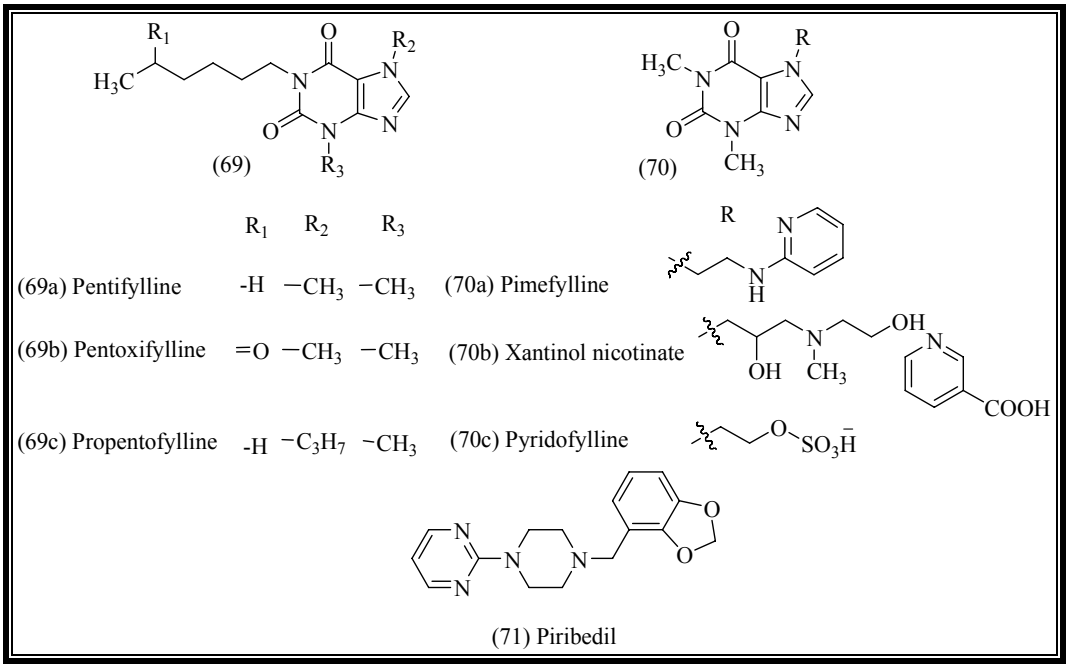




Alfuzocin (67) [74], a prazosin analogue and an α_1-adrenoceptor antagonist as well as urapidil (68) [75] are used especially in urinary obstruction caused by benign prostate hyperplasia.

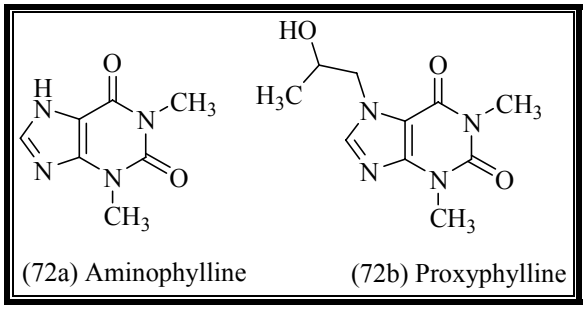
2.2.11.2 Vasodilators

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 ADL (Activity of Daily Living) in patients suffering from Parkinson's syndrome.



2.2.11.3 Cardiotonics/bronchodialators

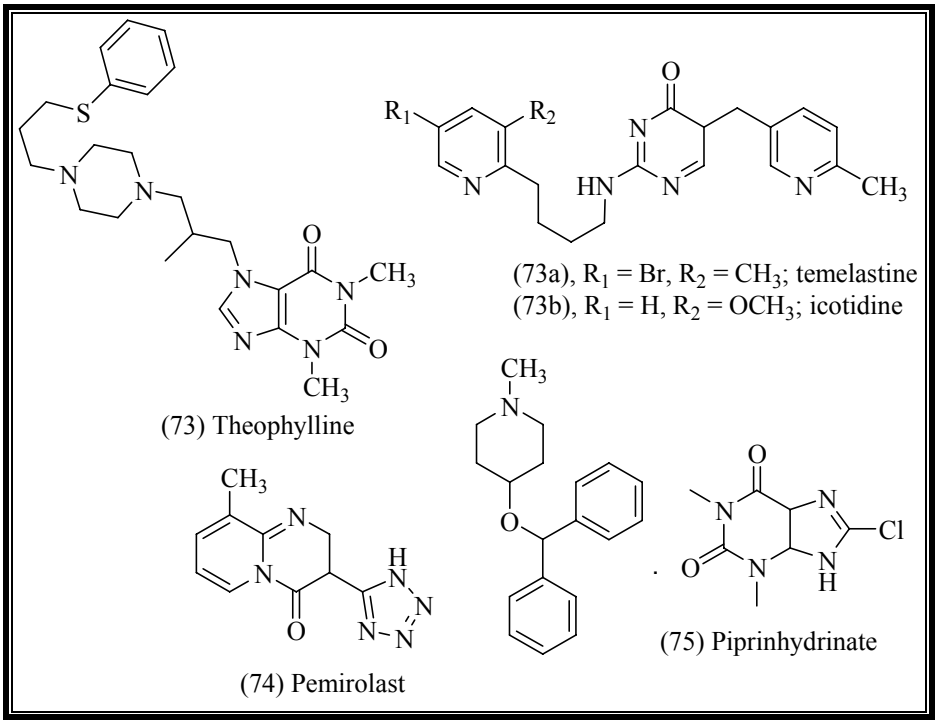
Several xanthine derivatives *viz.*, theophylline (61e), aminophylline (72a) [80] and proxyphylline (72b) [80] exhibit good bronchodilator activity.



(72a) Aminophylline (72b) Proxyphylline

2.2.12 Antihistaminic 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 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].



(73a), R_1 = Br, R_2 = CH_3; temelastine
(73b), R_1 = H, R_2 = OCH_3; icotidine

(73) Theophylline

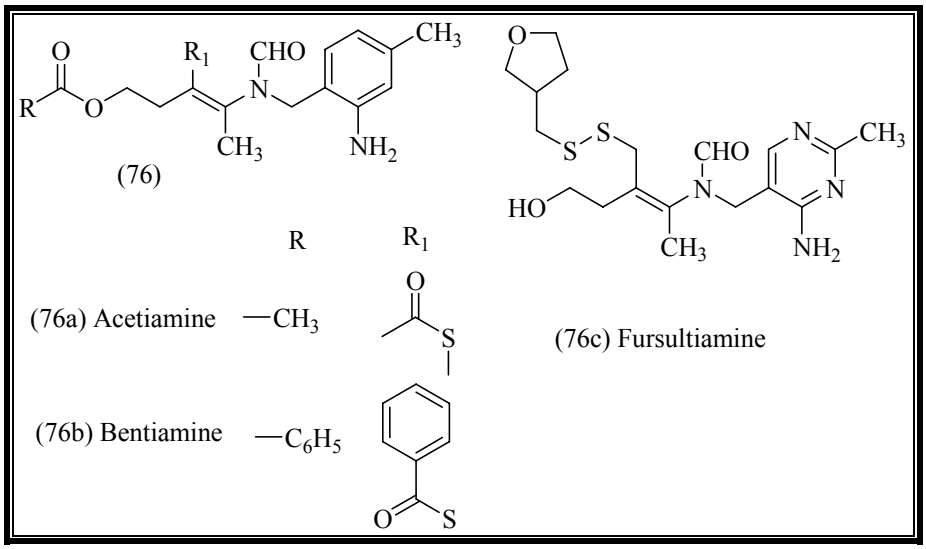
(74) Pemirolast

(75) Piprinhydrinate

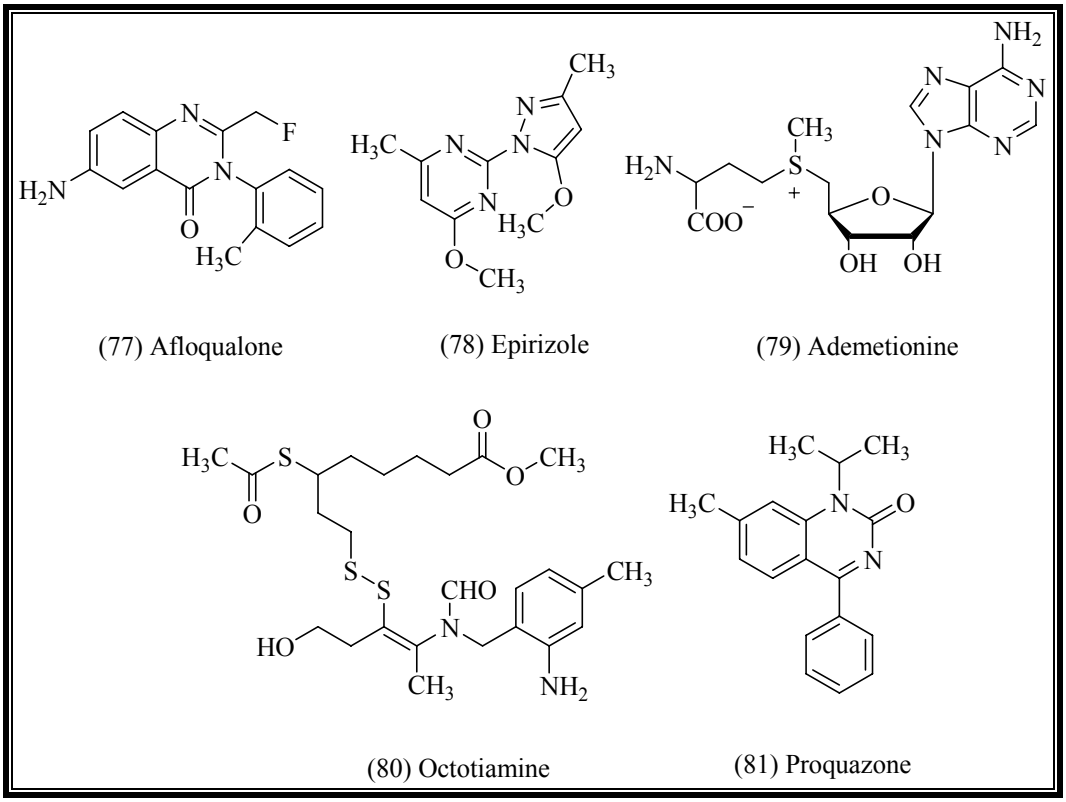
Pemirolast (74) [84], a new oral nonbronchodilator antihistaminic agent is also a pyrimidine derivative. It has demonstrated sufficient antihistaminic activity to warrant its use in severe asthma. Another compound, piprinhydrinate (75) [85] is also a pyrimidine derivative.

2.2.13 Analgesics and NSAID 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 CBF (Coronary Blood Flow).

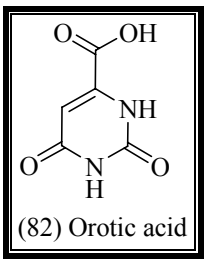


Afloqualone (77) [88] has been evaluated as a successful anti-inflammatory agent with lower back pain patients. Epirizole (78) [89], another NSAID, is suggested to be a COX-2 inhibitor. Ademetionine (79) [90] is primarily used in conjunction to glucosamine and chondroitin therapy. Octotiamine (80) [91],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



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, 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, three different heterocyclic scaffolds related to pyrimidines (thieno[2,3-*d*]pyrimidines, 1,2,4-triazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*d*][1,2,4]triazolo [1,5-a]pyrimidines) have been synthesized in the framework of this doctoral thesis.

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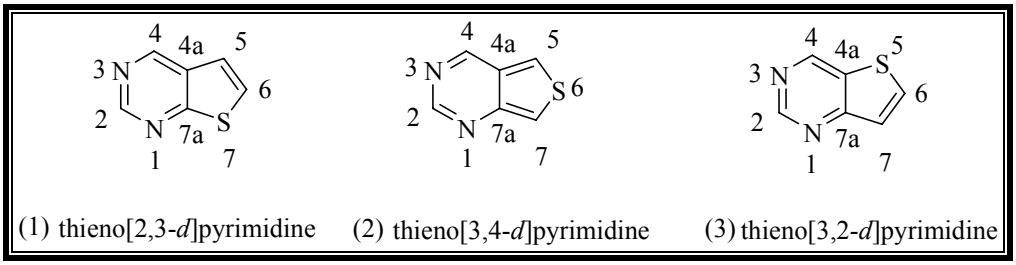
Chapter 3
Synthesis and biological evaluation of thieno[2,3-*d*]pyrimidines

3.1 Introduction

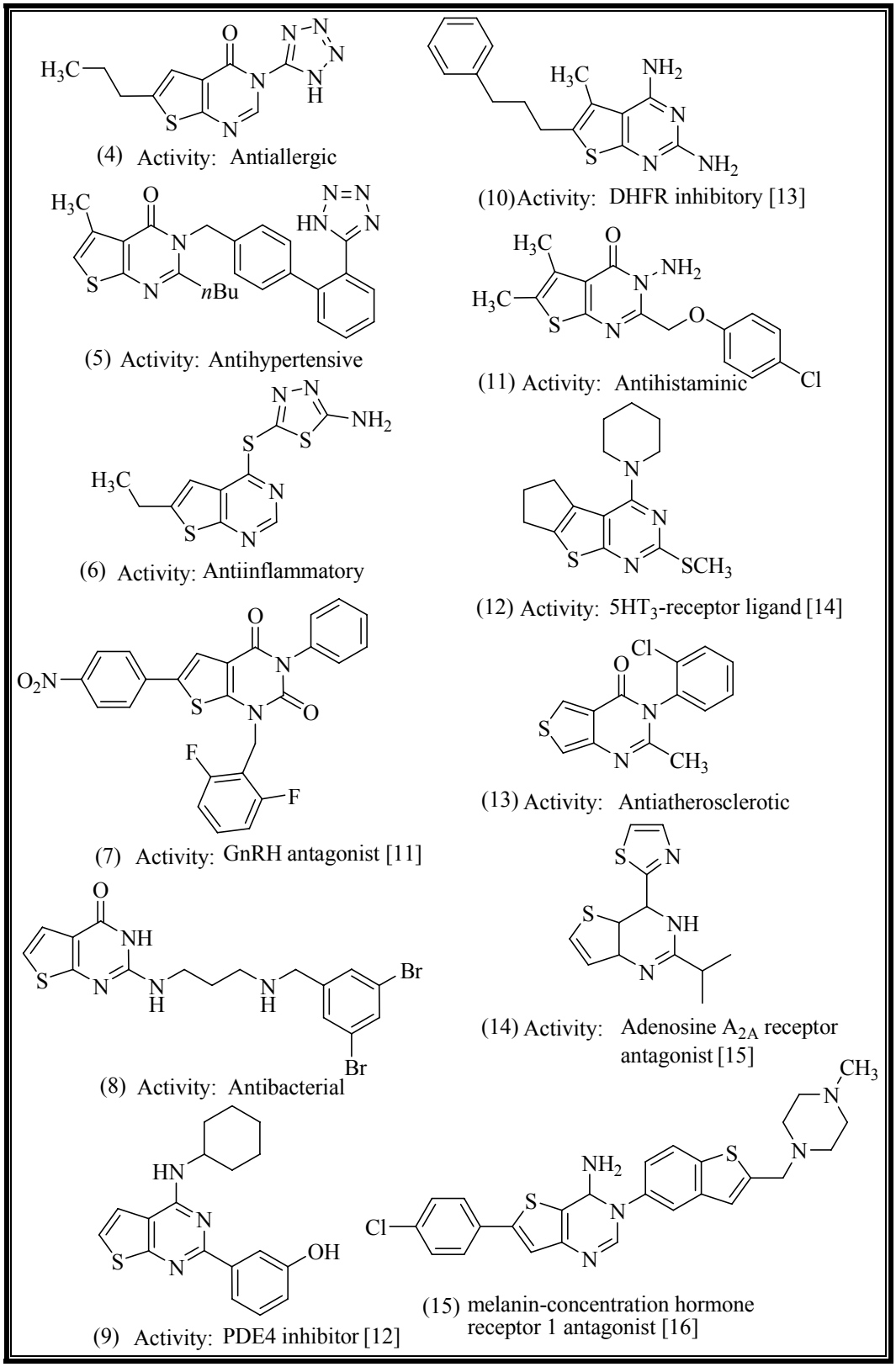
Fused pyrimidines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities. Thienopyrimidines occupy a special position among these compounds. Along with some other pyrimidine systems containing an annelated five membered hetero aromatic ring, thienopyrimidines are structural analogues of biogenic purines and can be considered as potential nucleic acid antimetabolites.

There are three isomeric thienopyrimidines corresponding to the three possible types of annulation of thiophene to the pyrimidine ring: thieno[2,3-*d*]pyrimidine (1), thieno[3,4-*d*]pyrimidine (2), and thieno[3,2-*d*]pyrimidine (3).



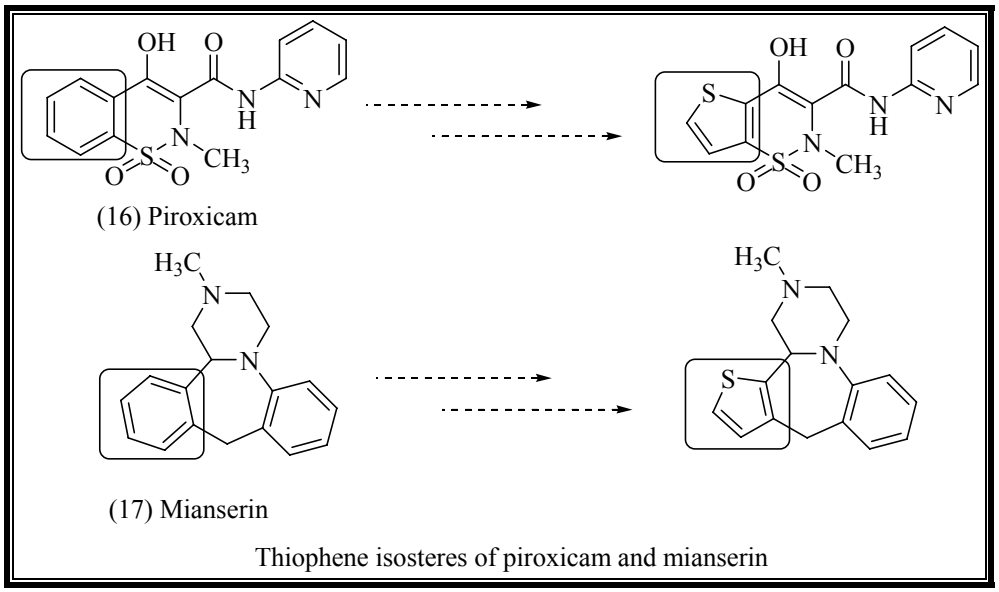
(1) thieno[2,3-*d*]pyrimidine (2) thieno[3,4-*d*]pyrimidine (3) thieno[3,2-*d*]pyrimidine

From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Antiallergic [1], antiatherosclerotic [2], antibacterial [3], antidepressive [4], antidiabetic [5], antihypertensive [6], antihistaminic [7], analgesic and anti-inflammatory [8], antiviral [9] and spasmolytic [10] activities have been reported for certain thienopyrimidine derivatives. Some examples of published derivatives of thienopyrimidine with their biological activities are as following.



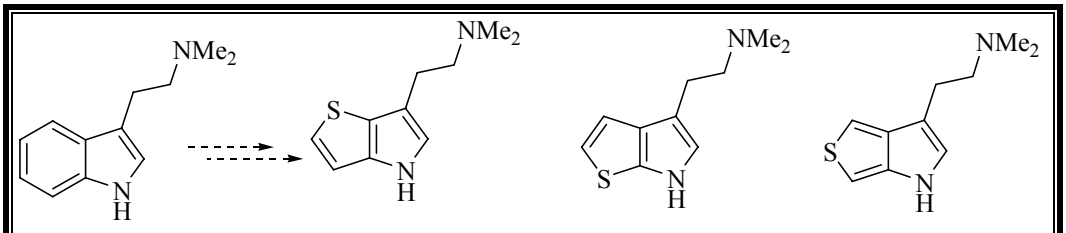
The similarity between the physicochemical properties of benzene and thiophene is striking. For example, the boiling point of benzene is 81.1 °C and the one

of thiophene is 84.4 °C (at 760 mm Hg) and therefore, thiophene and benzene is a well known example of bioisosterism. The change of a benzene moiety into a thiophene often results in superior pharmacodynamic, pharmacokinetic, or toxicological properties. For example, the thiophene analogue of piroxicam (a non-steroidal anti-inflammatory agent used in arthritis patients) has the same biological activity, with the same mechanism of action as piroxicam, and even displayed a longer plasma half-life than piroxicam [17]. Thiophene isosteres of mianserin (a tetracyclic antidepressive agent) also act as serotonin receptor (5-HT) antagonists (16 and 17) [18].



Thiophene isosteres of piroxicam and mianserin

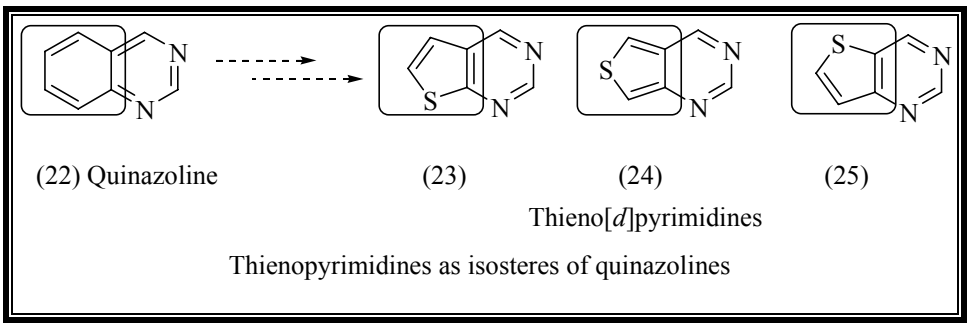
The exchange of a phenyl ring by a thiophene ring in bicyclic derivatives can generate three regioisomers. For example, Blair and co-workers described the replacement of the phenyl ring of *N*,*N*-dimethyltryptamine (18) by a thiophene moiety,



Thiophene isosteres of *N*,*N*-dimethyltryptamine

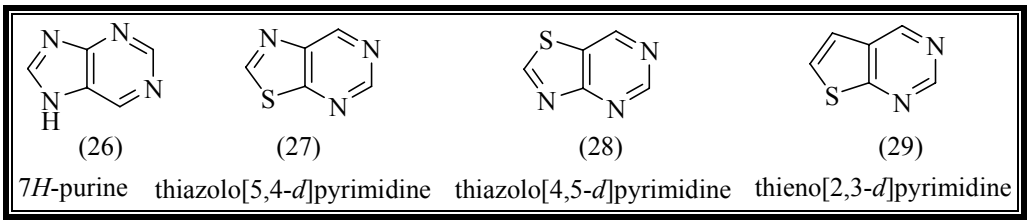
giving rise to three isomers: thieno[3,2-*b*]pyrrole (19), thieno[2,3-*b*]pyrrole (20) and thieno[3,4-*b*]pyrrole (21) [19]. Biological evaluation demonstrated that both thieno[3,2-*b*]pyrrole and thieno[2,3-*b*]pyrrole showed similar activity as the parent indole analogue, and whereas thieno[3,4-*b*]pyrrole lost activity.

As a logical consequence of thiophene – phenyl isosterism, thienopyrimidines (23, 24 and 25) can be considered as bioisosteres of quinazolines (22), which are extensively described in scientific and patent literature as displaying a plethora of biological activities. The synthesis of thienopyrimidine derivatives as potential surrogates for the quinazoline core structure has therefore become a routine strategy in modern drug design and development.



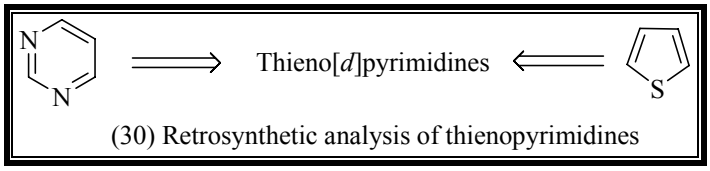
Thienopyrimidines as isosteres of quinazolines

Thienopyrimidines (29) can also be considered as structural analogues of five-membered heterocycles such as purines (26) and thiazolopyrimidines (27) and (28).



3.2 Reported synthetic strategies

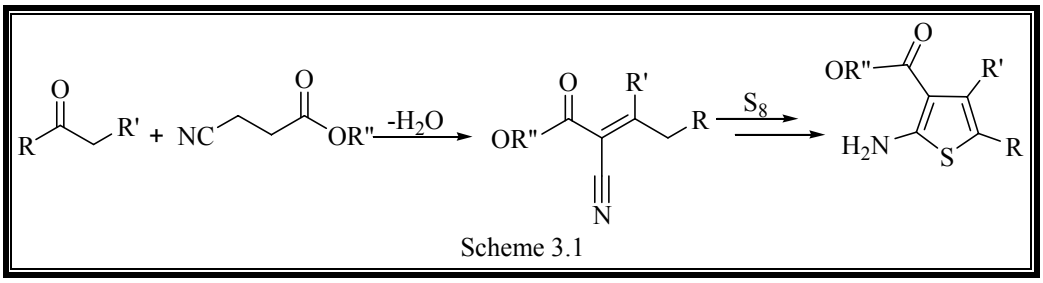
Synthetic approaches towards thienopyrimidines can be divided into two main groups according to the type of starting material. Either, the synthesis starts from a pyrimidine derivative and a thiophene ring is then constructed, or a thiophene analogue is used as starting material, followed by the formation of a pyrimidine ring (30).



(30) Retrosynthetic analysis of thienopyrimidines

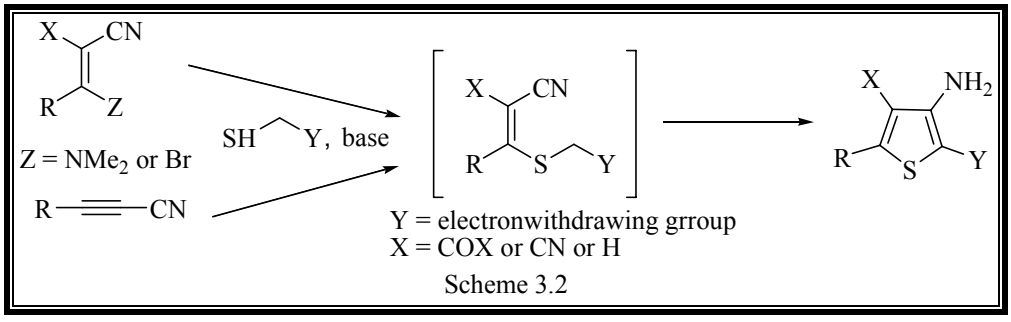
3.2.1 Synthesis of thienopyrimidines from a thiophene moiety

For the synthesis of thienopyrimidines from a thiophene moiety, an appropriately substituted aminothiophene serves as the starting material. 2-aminothiophene derivatives, which are the starting materials for thieno[2,3-*d*]pyrimidines, can be easily synthesized by Gewald reaction [20]. The first step of this multicomponent reaction is the Knoevenagel-Cope condensation of carbonyl compound (ketone or aldehyde) with an activated nitrile (*α*-cyanoester), yielding an *α*,*β*–unsaturated nitrile. This intermediate is then thiolated at the methylene group by elemental sulfur, followed by an intramolecular cyclization yielding a polysubstituted-2-aminothiophene (Scheme 3.1).



Scheme 3.1

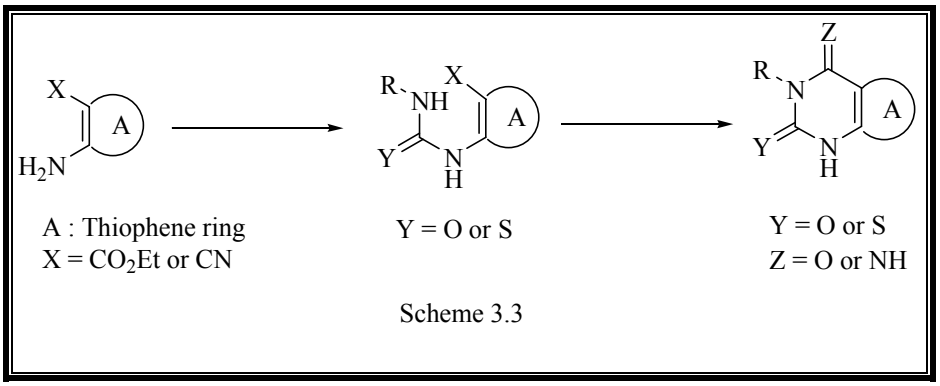
The Thorpe-Ziegler reaction is one of the most efficient methods for the synthesis of 5-membered heterocycles containing an amino group at C-3 position [21]. 3-aminothiophene derivatives are useful building blocks for the construction of thieno[3,2-*d*]pyrimidines and thieno[3,4-*d*]pyrimidines.



Scheme 3.2

The alkylthio olefin intermediates, generated by the reaction of *vic*-cyanodimethylaminoethylenes or *vic*-bromocyanoethylenes or cyanoacetylenes [22] with mercapto compounds of general formula $HSCH_2Y$ (Y is an electron withdrawing group) in the presence of base, undergo a Thorpe-Ziegler cyclization yielding polysubstituted 3-aminothiophenes.

The construction of a pyrimidine ring system from a 2-amino- or 3-amino-thiophene derivative follows the same reaction sequence. One of the most popular approaches to construct the pyrimidine ring is *via* the synthesis of thienylureas or thienylthioureas. In a first step, the amino group of the thiophene moiety is converted into a urea by treatment with an isocyanate [23], potassium cyanate hydrochloride [24], or chlorosulfonyl isocyanate [25] and into a thiourea by reaction with an isothiocyanate [26], or thiophosgene and an amine [27]. The resulting thienylureas and thienylthioureas readily undergo an intramolecular cyclization upon treatment with bases or acids to afford thienopyrimidines (Scheme 3.3).



Scheme 3.3

The synthesis of thienopyrimidin-4(3*H*)-ones is well studied and can be categorized into 4 groups according to the functional groups on the thiophene moiety and the structures of the intermediates (Scheme 3.4).

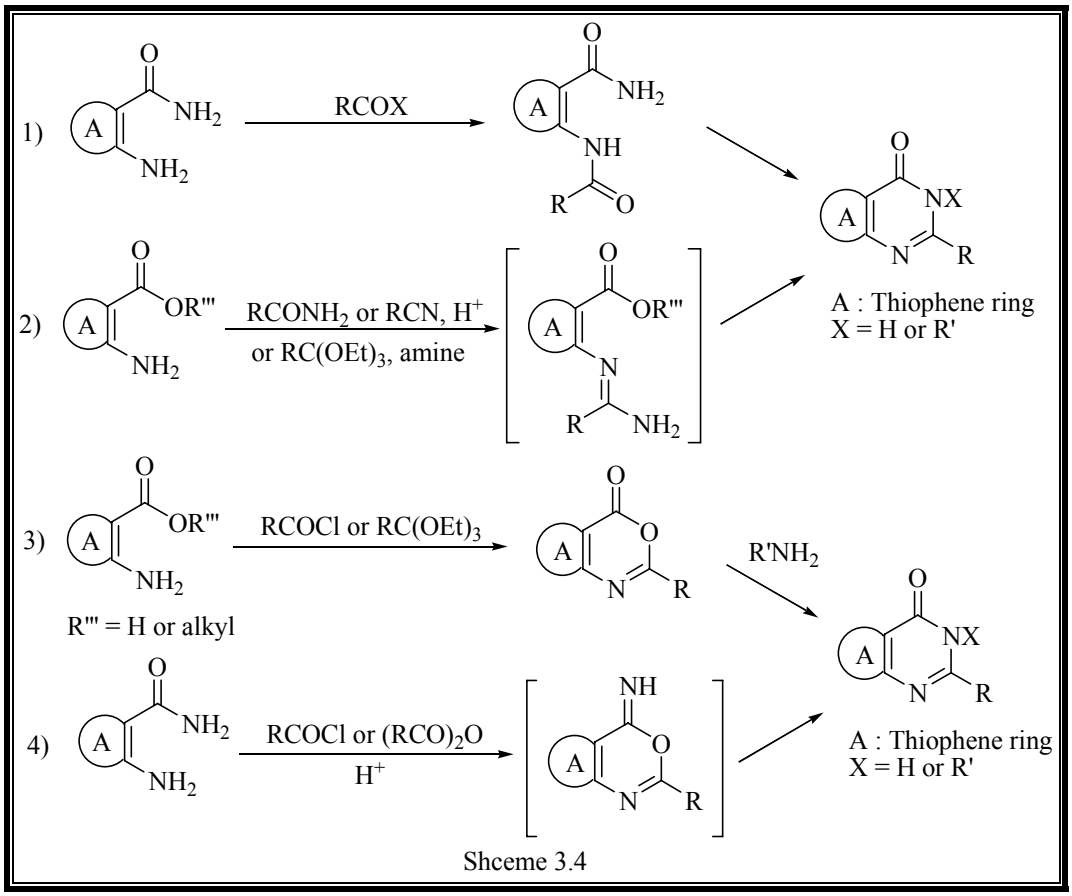
(1) Thienopyrimidinones can be prepared *via* cyclization of diamides intermediates, which are generated from *vic*-aminocarbamoylthiophenes by reaction with acylating agents such as orthoesters [28], acid anhydrides and acid chlorides [29], formic acid [30] and diethyl oxalate [31].

(2) Alternatively, the synthesis of thienopyrimidinones can be achieved from *vic*-aminoalkoxycarbonylthiophenes. Amidine intermediates, formed by the

reaction of thiophenes with amides [32], nitrites under acidic conditions [33], orthoesters and amines [34], undergo an intramolecular cyclization to afford thienopyrimidinones.

(3) A third procedure is based on the recyclization of thieno-oxazinones, which are generated by reaction of *vic*-aminocarboxylic acids or esters with acid chlorides or orthoesters [35]. The recyclization proceeds through the diamide intermediate which is generated upon treatment with amines [36].

(4) *Vic*-aminocyanothiophenes also serve as valuable starting materials for the synthesis of thienopyrimidinones. Initially, the thieno-oxazinimine interme diates are generated by the acylation of the amino group and then recyclization in the presence of an acid occurs to afford thienopyrimidinones [37].



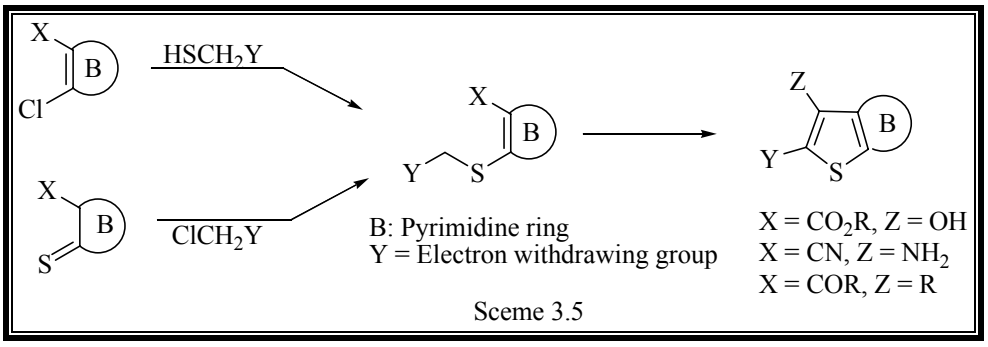
Shceme 3.4

3.2.2 Synthesis of thienopyrimidines from a pyrimidine moiety

Due to the poor availability of appropriately substituted pyrimidines, the synthesis of thienopyrimidines from pyrimidines is much less described in literature. In general,

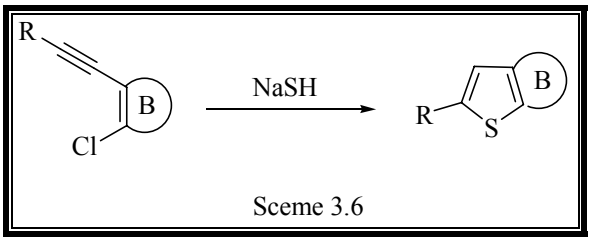
thieno[2,3-*d*]pyrimidines and thieno[3,2-*d*]pyrimidines can be obtained by the intramolecular cyclization of pyrimidine (Scheme 3.5). This pyrimidine derivative can be obtained by the substitution of the mercaptoacetic acid residue for the chlorine [38].

Alkylation of pyrimidinethiones with a chloroacetic acid derivative [39] is also a possibility. Depending on the substituents of the pyrimidine ring (X) and of mercapto side chain (Y), thienopyrimidines with a different substitution pattern on the thiophene moiety can be synthesized. When X = CO_2R, 5-hydroxythieno pyrimidines (which exist predominantly as the oxo form) are formed. In case of X = CN, the Thorpe-Ziegler reaction, affords amino substituted pyrimidines. When X and Y are a ketone or an aldehyde, the Claisen-Schmidt condensation affords R substituted thienopyrimidines.



Sceme 3.5

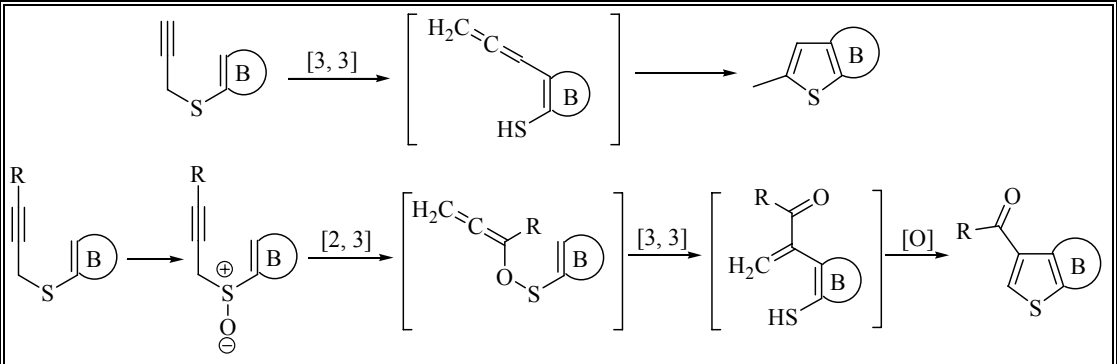
Alternatively, thienopyrimidines can be prepared by the reaction of *vic*-chloroalkynylpyrimidines with sodium sulphide [40] (Scheme 3.6). The alkynylpyrimidines are obtained *via* a Sonogashira reaction.



Sceme 3.6

Sigmatropic rearrangement is a useful method to construct 5-membered nitrogen and sulphur containing heterocycles [41]. The thio-Claisen rearrangement of propargylic sulfides affords thienopyrimidines *via* an allene intermediate followed by tautomerization, which then ring closes yielding the thiophene analogue [42] (Scheme

3.7). Another approach of sigmatropic rearrangement involves the oxidation of sulfide to sulfoxide. The sulfoxide undergoes an initial [2,3] sigmatropic rearrangement to generate the allene intermediate which then undergoes a Claisen-like [3,3] sigmatropic rearrangement through the S-O bond, followed by tautomerization to produce intermediate. An intramolecular Michael type addition followed by aromatization gives access to the desired thienopyrimidines.



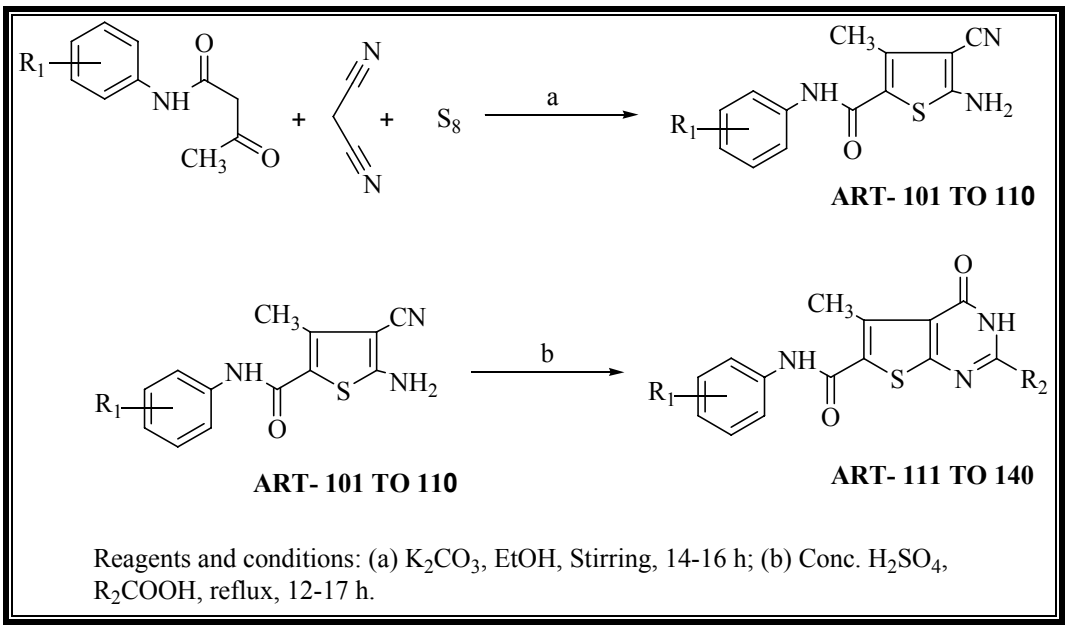
Scheme 3.7

3.3 Current work

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. Due to formal isoelectronic relationship with purines, the thieno[2,3-*d*]pyrimidine ring system is of special biological interest. It has numerous pharmacological and medicinal applications *viz*, antitumour, immunodilator, tuberculostic, antiallergic and radioprotective.

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 thieno[2,3-*d*]pyrimidines (ART-111 to ART-130) are synthesized. The synthesis of thieno[2,3-*d*]pyrimidines (ART-111 to ART-130) was achieved by acid catalysed cyclocondensation of polysubstituted 2-aminothiophenes (ART-101 to ART-110) with formic acid, gl. acetic acid and trifluoroacetic acid, respectively. Polysubstituted 2-aminothiophenes were prepared via the Gewald reaction [43]. The products were characterized by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.

3.4 Reaction scheme



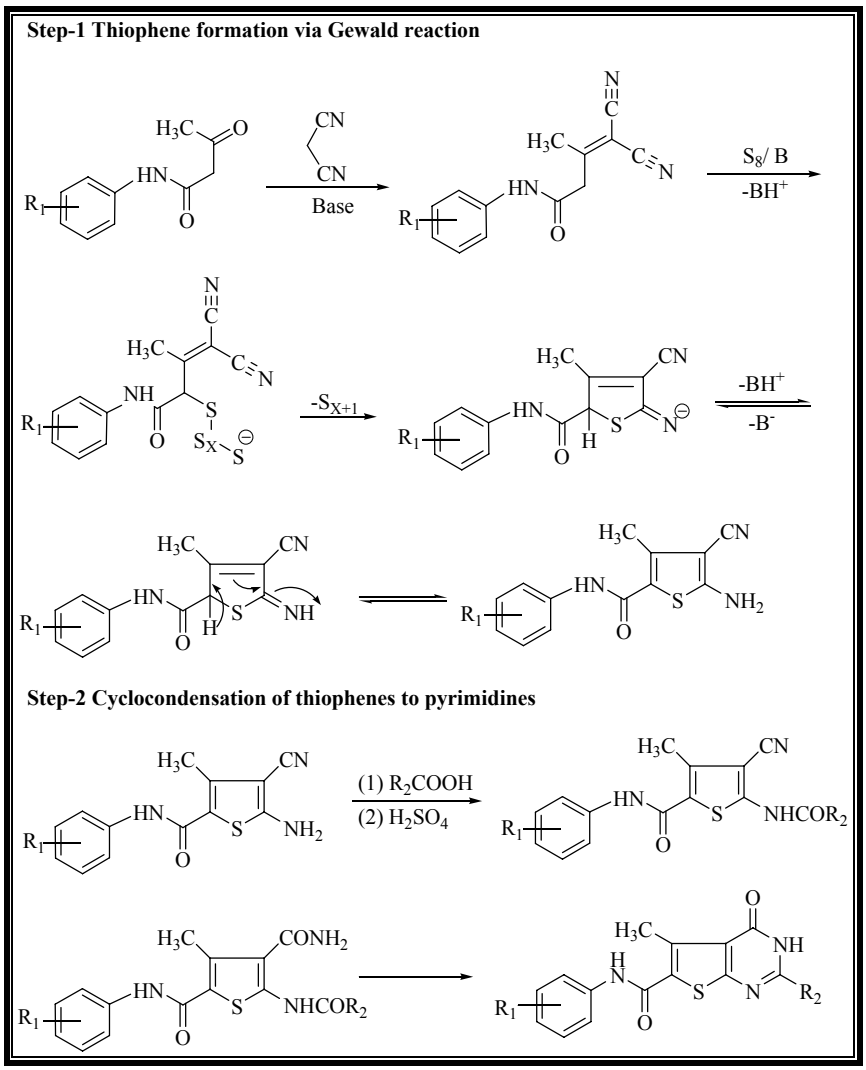
Reagents and conditions: (a) K_2CO_3, EtOH, Stirring, 14-16 h; (b) Conc. H_2SO_4, R_2COOH, reflux, 12-17 h.

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
ART-101	H	-	$C_{13}H_{11}N_3OS$	257	156-158	64	0.46	0.67
ART-102	4-OCH_3	-	$C_{14}H_{13}N_3O_2S$	287	203-205	69	0.48	0.70
ART-103	2-OCH_3	-	$C_{14}H_{13}N_3O_2S$	287	221-223	63	0.52	0.73
ART-104	4-NO_2	-	$C_{13}H_{10}N_4O_3S$	302	179-181	72	0.54	0.75
ART-105	2,5-CH_3	-	$C_{15}H_{15}N_3OS$	285	182-184	79	0.46	0.68
ART-106	4-Cl	-	$C_{13}H_{10}ClN_3OS$	291	199-201	73	0.51	0.71
ART-107	3-Cl	-	$C_{13}H_{10}ClN_3OS$	291	181-183	65	0.56	0.75
ART-108	4-F	-	$C_{13}H_{10}FN_3OS$	275	254-256	51	0.50	0.65
ART-109	2-F	-	$C_{13}H_{10}FN_3OS$	275	153-155	58	0.58	0.68
ART-110	3-CF_3	-	$C_{14}H_{10}F_3N_3OS$	325	223-225	62	0.48	0.77
ART-111	H	H	$C_{14}H_{11}N_3O_2S$	285	128-130	68	0.53	0.61
ART-112	4-OCH_3	H	$C_{15}H_{13}N_3O_3S$	315	241-243	74	0.58	0.67
ART-113	2-OCH_3	H	$C_{15}H_{13}N_3O_3S$	315	178-180	70	0.49	0.58
ART-114	4-NO_2	H	$C_{14}H_{10}N_4O_4S$	330	239-241	80	0.53	0.60
ART-115	2,5-CH_3	H	$C_{16}H_{15}N_3O_2S$	313	256-258	69	0.59	0.70
ART-116	4-Cl	H	$C_{14}H_{10}ClN_3O_2S$	319	263-265	56	0.51	0.59
ART-117	3-Cl	H	$C_{14}H_{10}ClN_3O_2S$	319	229-231	63	0.54	0.61
ART-118	4-F	H	$C_{14}H_{10}FN_3O_2S$	303	234-236	68	0.56	0.63
ART-119	2-F	H	$C_{14}H_{10}FN_3O_2S$	303	173-175	52	0.48	0.57
ART-120	3-CF_3	H	$C_{15}H_{10}F_3N_3O_2S$	353	238-240	50	0.58	0.68
ART-121	H	CH_3	$C_{15}H_{13}N_3O_2S$	299	208-210	68	0.49	0.57
ART-122	4-OCH_3	CH_3	$C_{16}H_{15}N_3O_3S$	329	243-245	78	0.51	0.63
ART-123	2-OCH_3	CH_3	$C_{16}H_{15}N_3O_3S$	329	253-255	70	0.54	0.65
ART-124	4-NO_2	CH_3	$C_{15}H_{12}N_4O_4S$	344	237-239	78	0.48	0.56
ART-125	2,5-CH_3	CH_3	$C_{17}H_{17}N_3O_2S$	327	261-263	66	0.56	0.60
ART-126	4-Cl	CH_3	$C_{15}H_{12}ClN_3O_2S$	333	265-267	71	0.52	0.65
ART-127	3-Cl	CH_3	$C_{15}H_{12}ClN_3O_2S$	333	223-225	59	0.54	0.67
ART-128	4-F	CH_3	$C_{15}H_{12}FN_3O_2S$	317	270-272	74	0.51	0.64
ART-129	2-F	CH_3	$C_{15}H_{12}FN_3O_2S$	317	261-263	59	0.49	0.57
ART-130	3-CF_3	CH_3	$C_{16}H_{12}F_3N_3O_2S$	367	256-258	60	0.53	0.61
ART-131	H	CF_3	$C_{15}H_{10}F_3N_3O_2S$	353	192-194	59	0.55	0.63
ART-132	4-OCH_3	CF_3	$C_{16}H_{12}F_3N_3O_3S$	383	198-200	68	0.53	0.65
ART-133	2-OCH_3	CF_3	$C_{16}H_{12}F_3N_3O_3S$	383	188-190	65	0.49	0.69

ART-134	4-NO_2	CF_3	$C_{15}H_9F_3N_4O_4S$	398	118-120	74	0.56	0.66
ART-135	2,5-CH_3	CF_3	$C_{17}H_{14}F_3N_3O_2S$	381	194-196	76	0.58	0.62
ART-136	4-Cl	CF_3	$C_{15}H_9ClF_3N_3O_2S$	387	108-110	68	0.54	0.60
ART-137	3-Cl	CF_3	$C_{15}H_9ClF_3N_3O_2S$	387	261-263	72	0.50	0.68
ART-138	4-F	CF_3	$C_{15}H_9F_4N_3O_2S$	371	242-244	61	0.52	0.67
ART-139	2-F	CF_3	$C_{15}H_9F_4N_3O_2S$	371	250-252	63	0.56	0.65
ART-140	3-CF_3	CF_3	$C_{16}H_9F_6N_3O_2S$	421	131-133	59	0.58	0.64

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

3.5 Mechanism



It is likely that the first step of reaction is Knoevenagel-cope condensation of a carbonyl compound with activated nitrile yielding an *α,β*-unsaturated nitrile. This intermediate is then thiolated at the methylene group by electrophilic elemental sulphur, following by ring closure to afford thiophene derivatives (step-1).

Polysubstituted 2-aminothiophenes obtained from first step are cyclised to thienopyrimidines *via* formation of two intermediates as per mechanism suggested by S. Hesse *et al*. [44] (step-2).

3.6 Experimental

3.6.1 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 agreements with the structures assigned.

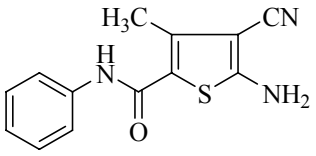
3.6.2 Synthesis of N-(aryl)-3-oxobutanamides

Synthesis of *N*-(aryl)-3-oxobutanamides was achieved using previously published methods [45].

3.6.3 General procedure for the synthesis of 5-amino-4-cyano-N-(aryl)-3-methylthiophene-2-carboxamides (ART 101-110)

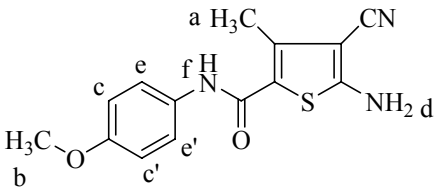
0.01 mol of an appropriate *N*-(aryl)-3-oxobutanamide was dissolved in 10 mL of EtOH and 0.01 mol each of malononitrile and powdered sulphur were added to the same solution. 1 gm of K_2CO_3 was added to the resulting solution as inorganic basic support. The heterogeneous mixture was stirred at room temperature for 14-16 h. After the completion of the reaction as monitored by TLC, the reaction mixture was filtered off to separate K_2CO_3 as residue and the filtrate was poured onto 50 mL of ice-cold water. The product got precipitated out, which was filtered and recrystallized from methanol.

3.6.3.1 5-amino-4-cyano-N-(phenyl)-3-methylthiophene-2-carboxamide (ART-101)



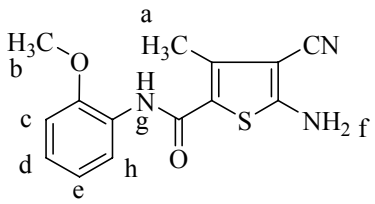
Yield: 64%; mp 156-158 ºC; MS: *m/z* 257; Anal. Calcd. for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.55; H, 4.20; N, 16.21

3.6.3.2 5-amino-4-cyano-N-(4-methoxyphenyl)-3-methylthiophene-2-carboxamide



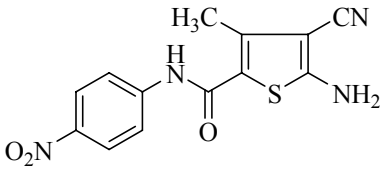
(ART-102) Yield: 69%; mp 203-205 ºC; IR (cm^{-1}): 3397 and 3335 (N-H stretching of primary amine), 3221 (N-H stretching of amide), 3032 (C-H stretching of aromatic ring), 2985 (C-H asymmetrical stretching of CH_3 group), 2837 (C-H symmetrical stretching of CH_3 group), 2210 (C≡N stretching of nitrile group), 1701 (C=O stretching of amide), 1616 (N-H deformation of NH_2 group), 1548, 1494 and 1440 (C=C stretching of aromatic ring), 1408 (C-H asymmetrical deformation of CH_3 group), 1336 (C-H symmetrical deformation of CH_3 group), 1238 (C-O-C stretching), 700 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.42 (s, 3H, H_a), 3.76 (s, 3H, H_b), 6.80-6.83 (d, 2H, $H_{cc'}$, J = 8.96 Hz), 7.28 (s, 2H, H_d) 7.48-7.51 (d, 2H, $H_{ee'}$, J = 8.92 Hz), 9.10 (s, 1H, H_f); MS: *m/z* 287; Anal. Calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.49; H, 4.30; N, 14.48%.

3.6.3.3 5-amino-4-cyano-N-(2-methoxyphenyl)-3-methylthiophene-2-carboxamide



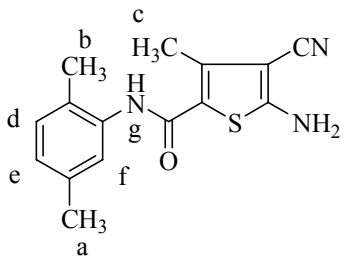
(ART-103) Yield: 63%; mp 221-223 ºC; IR (cm^{-1}): 3427 and 3323 (N-H stretching of primary amine), 3213 (N-H stretching of amide), 3020 (C-H stretching of aromatic ring), 2964 (C-H asymmetrical stretching of CH_3 group), 2839 (C-H symmetrical stretching of CH_3 group), 2206 (C≡N stretching of nitrile group), 1701 (C=O stretching of amide), 1620 (N-H deformation of NH_2 group), 1539, 1500 and 1494 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of CH_3 group), 1344 (C-H symmetrical deformation of CH_3 group), 1251 (C-O-C stretching), 740 (C-S-C stretching): ^{1}H NMR (DMSO-d_6) δ ppm: 2.49 (s, 3H, H_a), 3.91 (s, 3H, H_b), 6.89-6.97 (m, 2H, H_{cd}), 7.02-7.06 (t, 1H, H_e), 7.48 (s, 2H, H_f), 8.18 (s, 1H, H_g), 8.20 (d, 1H, H_h); MS: *m/z* 287; Anal. Calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.49; H, 4.30; N, 14.48%.

3.6.3.4 5-amino-4-cyano-N-(4-nitrophenyl)-3-methylthiophene-2-carboxamide



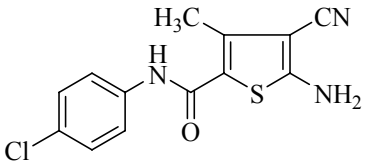
(ART-104) Yield: 72%; mp 179-181 ºC; MS: *m/z* 302; Anal. Calcd. for $C_{13}H_{10}N_4O_3S$: C, 51.65; H, 3.33; N, 18.53. Found: C, 51.55; H, 3.39; N, 18.25%.

3.6.3.5 5-amino-4-cyano-N-(2,5-methylphenyl)-3-methylthiophene-2-carboxamide



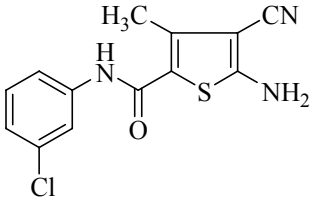
(ART-105) Yield: 79%; mp 182-184 ºC; IR (cm^{-1}): 3331 and 3300 (N-H stretching of primary amine), 3184 (N-H stretching of amide), 3025 (C-H stretching of aromatic ring), 2978 (C-H asymmetrical stretching of CH_3 group), 2914 (C-H symmetrical stretching of CH_3 group), 2208 (C≡N stretching of nitrile group), 1701 (C=O stretching of amide), 1627 (N-H deformation of NH_2 group), 1527, 1491 and 1446 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of CH_3 group), 1319 (C-H symmetrical deformation of CH_3 group), 717 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.18 (s, 3H, H_a), 2.25 (s, 3H, H_b), 2.47 (s, 3H, H_c), 6.89-6.91 (d, 1H, H_d), 7.06-7.08 (d, 1H, H_e), 7.34 (s, 1H, H_f), 8.32 (s, 1H, H_g); MS: *m/z* 285; Anal. Calcd. for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73. Found: C, 63.03; H, 5.19; N, 14.62%.

3.6.3.6 5-amino-4-cyano-N-(4-chlorophenyl)-3-methylthiophene-2-carboxamide



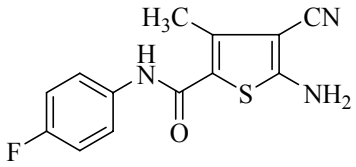
(ART-106) Yield: 73%; mp 199-201 ºC; MS: *m/z* 291; Anal. Calcd. for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40. Found: C, 53.46; H, 3.29; N, 14.30%.

3.6.3.7 5-amino-4-cyano-N-(3-chlororphenyl)-3-methylthiophene-2-carboxamide



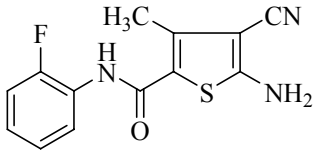
(ART-107) Yield: 65%; mp 181-183 ºC; MS: *m/z* 291; Anal. Calcd. for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40. Found: C, 53.44; H, 3.30; N, 14.32%.

3.6.3.8 5-amino-4-cyano-N-(4-fluorophenyl)-3-methylthiophene-2-carboxamide



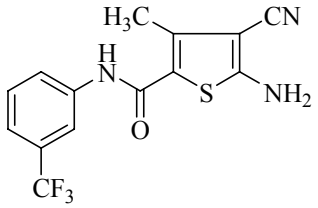
(ART-108) Yield: 51%; mp 254-256 ºC; MS: *m/z* 275; Anal. Calcd. for $C_{13}H_{10}FN_3OS$: C, 56.72; H, 3.66; N, 15.26. Found: C, 56.62; H, 3.59; N, 15.21%.

3.6.3.9 5-amino-4-cyano-N-(2-fluorophenyl)-3-methylthiophene-2-carboxamide



(ART-109) Yield: 58%; mp 153-155 ºC; MS: *m/z* 275; Anal. Calcd. for $C_{13}H_{10}FN_3OS$: C, 56.72; H, 3.66; N, 15.26. Found: C, 56.65; H, 3.60; N, 15.16%.

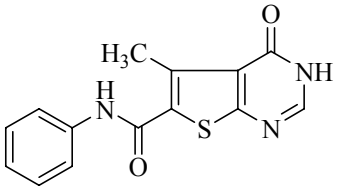
3.6.3.10 5-amino-4-cyano-N-(3-trifluorophenyl)-3-methylthiophene-2-carboxamide

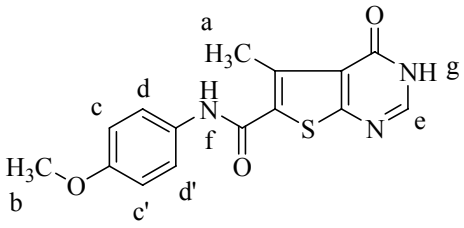


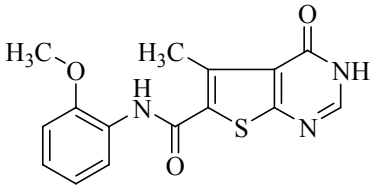
(ART-110) Yield: 62%; mp 223-225 ºC; MS: *m/z* 325; Anal. Calcd. for $C_{14}H_{10}F_3N_3OS$: C, 51.69; H, 3.10; N, 12.92. Found: C, 51.52; H, 3.03; N, 12.82%.

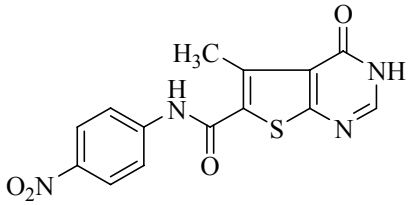
3.6.4 General procedure for the synthesis of 3,4-dihydro-N-(aryl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamides (ART 111-120)

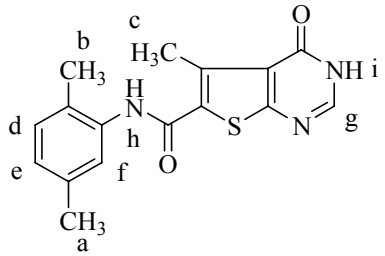
A mixture of an appropriate 5-amino-4-cyano-*N*-(aryl)-3-methylthiophe ne-2-carboxamide (0.01 mol) and formic acid (20 mL) was stirred under reflux for 12- 14 h. in the presence of catalytic amount of concentrated H_2SO_4 (under TLC analysis). The reaction mixture was allowed to cool to room temperature and was poured onto crushed ice. The solid thus formed was collected by filtration, washed with ethanol (20 mL), dried and crystallized from dimethylformamide to afford the desired products ART 111-120.

3.6.4.1 3,4-dihydro-N-(phenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-111)  Yield: 68%; mp 128-130 ºC; MS: *m/z* 285; Anal. Calcd. for $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73. Found: C, 58.56; H, 3.79; N, 14.66%.

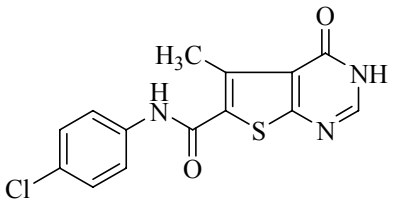
3.6.4.2 3,4-dihydro-N-(4-methoxyphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-112)  Yield: 74%; mp 241-243 ºC; IR (cm^{-1}): 3294 (N-H stretching of secondary amine), 3070 (C-H stretching of aromatic ring), 2962 (C-H asymmetrical stretching of CH_3 group), 2897 (C-H asymmetrical stretching of OCH_3 group), 2829 (C-H symmetrical stretching of CH_3 group), 1697 (C=O stretching of amide), 1660 (C=O stretching of pyrimidine ring), 1624 (C=N stretching of pyrimidine ring), 1600 and 1537 (C=C stretching of aromatic ring), 1435 (C-H asymmetrical deformation of OCH_3 group), 1415 (C-H asymmetrical deformation of CH_3 group), 1296 (C-H symmetrical deformation of CH_3 group), 1114 (C-O-C stretching), 696 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.78 (s, 3H, H_a), 3.78 (s, 3H, H_b), 6.85-6.87 (d, 2H, $H_{cc'}$, J = 9.04 Hz), 7.59-7.61 (d, 2H, $H_{dd'}$, J = 8.96 Hz), 8.02 (s, 1H, H_e), 9.87 (s, 1H, H_f), 12.45 (s, 1H, H_g); MS: *m/z* 315; Anal. Calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 57.03; H, 4.09; N, 13.29%.

3.6.4.3 3,4-dihydro-N-(2-methoxyphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-113)  Yield: 70%; mp 178-180 ºC; MS: *m/z* 315; Anal. Calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 57.06; H, 4.10; N, 13.26%.

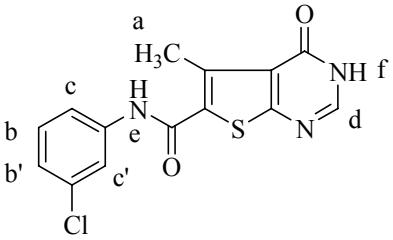
3.6.4.4 3,4-dihydro-N-(4-nitrophenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-114)  Yield: 80%; mp 239-241 ºC; MS: *m/z* 330; Anal. Calcd. for $C_{14}H_{10}N_4O_4S$: C, 50.91; H, 3.05; N, 16.96. Found: C, 50.82; H, 2.99; N, 16.88%.

3.6.4.5 3,4-dihydro-N-(2,5-methylphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-115) 

Yield: 69%; mp 256 258 ºC; IR (cm^{-1}): 3275 and 3244 (N-H stretching of secondary amine), 3022 (C-H stretching of aromatic ring), 2960 (C-H asymmetrical stretching of CH_3 group), 2860 (C-H asymmetrical stretching of CH_3 group), 1691 (C=O stretching of amide), 1670 (C=O stretching of pyrimidine ring), 1626 (C=N stretching of pyrimidine ring), 1577, 1539 and 1485 (C=C stretching of aromatic ring), 1421 (C-H asymmetrical deformation of -CH_3 group), 1313 (C-H symmetrical deformation of CH_3 group), 694 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H, H_a), 2.30 (s, 3H, H_b), 2.82 (s, 3H, H_c), 6.95-6.97 (d, 1H, H_d), 7.10-7.12 (d, 1H, H_e), 7.23 (s, 1H, H_f), 8.05 (s, 1H, H_g), 9.48 (s, 1H, H_h), 12.52 (s, 1H, H_i); MS: *m/z* 313; Anal. Calcd. for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.25; H, 4.76; N, 13.31%.

3.6.4.6 3,4-dihydro-N-(4-chlorophenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-116) 

Yield: 56%; mp 263-265 ºC; IR (cm^{-1}): MS: *m/z* 319; Anal. Calcd. for $C_{14}H_{10}ClN_3O_2S$: C, 52.59; H, 3.15; N, 13.14. Found: C, 52.50; H, 3.05; N, 13.07%.

3.6.4.7 3,4-dihydro-N-(3-chlorophenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-117) 

Yield: 63%; mp 229-231 ºC; 3259 (N-H stretching of secondary amine), 3066 (C-H stretching of aromatic ring), 2937 (C-H asymmetrical stretching of CH_3 group), 2879 (C-H symmetrical stretching of CH_3 group), 1680 (C=O stretching of amide), 1658 (C=O stretching of pyrimidine ring), 1591 (C=N stretching of pyrimidine ring), 1560, 1550 and 1502 (C=C stretching of aromatic ring), 1406 (C-H asymmetrical deformation of -CH_3 group), 1313 (C-H symmetrical deformation of CH_3 group), 692 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.78 (s, 3H, H_a). 7.01-7.06 (t, 2H,

$H_{bb'}$), 7.69-7.72 (m, 2H, $H_{cc'}$), 8.00 (s, 1H, H_d), 10.00 (s, 1H, H_e), 12.43 (s, 1H, H_f); MS: *m/z* 319; Anal. Calcd. for $C_{14}H_{10}ClN_3O_2S$: C, 52.59; H, 3.15; N, 13.14. Found: C, 52.49; H, 3.07; N, 13.03%

3.6.4.8 3,4-dihydro-N-(4-fluorophenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-118) Yield: 68%; mp 234-236 ºC; MS: *m/z* 303; Anal. Calcd. for $C_{14}H_{10}FN_3O_2S$: C, 55.44; H, 3.32; N, 13.85. Found: C, 55.36; H, 3.24; N, 13.78%.

3.6.4.9 3,4-dihydro-N-(2-fluorophenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-119) Yield: 52%; mp 173-175 ºC; MS: *m/z* 303; Anal. Calcd. for $C_{14}H_{10}FN_3O_2S$: C, 55.44; H, 3.32; N, 13.85. Found: C, 55.32; H, 3.26; N, 13.71%.

3.6.4.10 3,4-dihydro-N-(3-trifluoromethylphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-120) Yield: 50%; mp 238-240 ºC; MS: *m/z* 353; Anal. Calcd. For $C_{15}H_{10}F_3N_3O_2S$: C, 50.99; H, 2.85; N, 11.89. Found: C, 50.90; H, 2.78; N, 11.79%.

3.6.5 General procedure for the synthesis of 3,4-dihydro-N-(aryl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamides (ART 121-130)

A mixture of an appropriate 5-amino-4-cyano-*N*-(aryl)-3-methylthiophe ne-2-carboxamide (0.01 mol) and glacial acetic acid (20 mL) was stirred under reflux for 14-16 h. in the presence of catalytic amount of concentrated H_2SO_4 (under TLC analysis). The reaction mixture was allowed to cool to room temperature and was poured onto crushed ice. The solid thus formed was collected by filtration, washed with ethanol (20 mL), dried and crystallized from dimethylformamide to afford the desired products ART 121-130.

3.6.5.1 3,4-dihydro-N-(phenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-121) Yield: 68%; mp 208-210 ºC; MS: *m/z* 299; Anal. Calcd. for $C_{15}H_{13}N_3O_2S$: C, 60.18; H, 4.38; N, 14.04. Found: C, 60.10; H, 4.29; N, 13.99%.

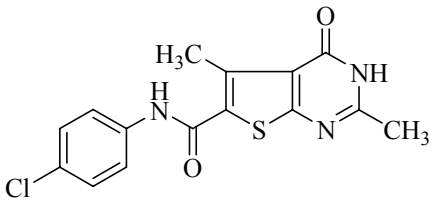
3.6.5.2 3,4-dihydro-N-(4-methoxyphenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-122) Yield: 78%; mp 243-245 ºC; MS: *m/z* 329; Anal. Calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.27; H, 4.51; N, 12.70%.

3.6.5.3 3,4-dihydro-N-(2-methoxyphenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-123) Yield: 70%; mp 253-255 ºC; MS: *m/z* 329; Anal. Calcd. For $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.28; H, 4.49; N, 12.68%.

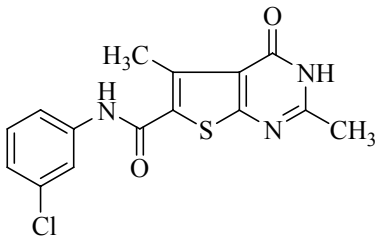
3.6.5.4 3,4-dihydro-N-(4-nitrophenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-124) Yield: 78%; mp 237-239 ºC; MS: *m/z* 344; Anal. Calcd. For $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 52.23; H, 3.45; N, 16.19%.

3.6.5.5 3,4-dihydro-N-(2,5-methylphenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-125) Yield: 66%; mp 261-263 ºC; MS: *m/z* 327; Anal. Calcd. For $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83. Found: C, 62.26; H, 5.12; N, 12.75%.

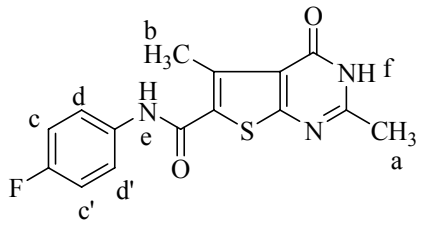
3.6.5.6 3,4-dihydro-N-(4-chlorophenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-126) Yield: 71%; mp 265-267 ºC; MS: *m/z* 333; Anal. Calcd. For $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59. Found: C, 53.88; H, 3.56; N, 12.51%.



3.6.5.7 3,4-dihydro-N-(3-chlorophenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-127) Yield: 59%; mp 223-225 ºC; MS: *m/z* 333; Anal. Calcd. For $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59. Found: C, 53.89; H, 3.58; N, 12.49%.



3.6.5.8 3,4-dihydro-N-(4-fluorophenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-128) Yield: 74%; mp 270-272 ºC; IR (cm^{-1}): 3298 (N-H stretching of secondary amine), 3066 (C-H stretching of aromatic ring), 2960 (C-H asymmetrical stretching of CH_3 group), 2833 (C-H symmetrical stretching of CH_3 group), 1685 (C=O stretching of amide), 1643 (C=O stretching of pyrimidine ring), 1608 (C=N stretching of pyrimidine ring), 1548, 1518 and 1502 (C=C stretching of aromatic ring), 1404 (C-H asymmetrical deformation of -CH_3 group), 1311 (C-H symmetrical deformation of CH_3 group), 700 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, H_a), 2.76 (s, 3H, H_b), 7.02-7.08 (t, 2H, $H_{cc'}$), 7.68-7.72 (q, 2H, $H_{dd'}$), 10.02 (s, 1H, H_e), 12.40 (s, 1H, H_f) ; MS: *m/z* 317; Anal. Calcd. for $C_{15}H_{12}FN_3O_2S$: C, 56.77; H, 3.81; N, 13.24. Found: C, 56.69; H, 3.73; N, 13.18%.



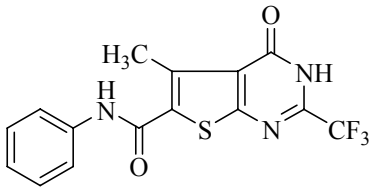
3.6.5.9 3,4-dihydro-N-(2-fluorophenyl)-2,5-dimethyl-4-oxothieno[2,3-d]pyrimidine -6-carboxamide (ART-129) Yield: 59%; mp 261-263 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3070 (C-H stretching of aromatic ring), 2885 (C-H asymmetrical stretching of CH_3 group), 2729 (C-H symmetrical stretching of CH_3 group), 1697 (C=O stretching of amide), 1639 (C=O stretching of pyrimidine ring), 1597 (C=N stretching of pyrimidine ring), 1539, 1487 and 1454 (C=C stretching of aromatic ring), 1420 (C-H asymmetrical deformation of -CH_3 group), 1332 (C-H symmetrical deformation of CH_3 group), 657 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.44 (s, 3H, H_a), 2.87 (s, 3H, H_b), 7.15-7.18 (m, 3H, $H_{c\text{-}e}$), 7.90-7.92 (m, 1H, H_f), 9.15 (s, 1H, H_g), 12.41 (s, 1H, H_h) ; MS: *m/z* 317; Anal. Calcd. for $C_{15}H_{12}FN_3O_2S$: C, 56.77; H, 3.81; N, 13.24. Found: C, 56.68; H, 3.71; N, 13.16%.

3.6.5.10 3,4-dihydro-N-(3-trifluoromethylphenyl)-2,5-dimethyl-4-oxothieno[2,3-d] pyrimidine-6-carboxamide (ART-130) Yield: 60%; mp 256-258 ºC; IR (cm^{-1}): 3408 and 3308 (N-H stretching of secondary amine), 3066 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of CH_3 group), 2891 (C-H symmetrical stretching of CH_3 group), 1718 (C=O stretching of amide), 1641 (C=O stretching of pyrimidine ring), 1593 (C=N stretching of pyrimidine ring), 1537 and 1518 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of -CH_3 group), 1320 (C-H symmetrical deformation of CH_3 group), 686 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.43 (s, 3H, H_a), 2.80 (s, 3H, H_b), 7.35-7.37 (d, 1H, H_c), 7.47-7.51 (t, 1H, H_d), 7.93-7.95 (d, 1H, H_e), 8.14 (s, 1H, H_f), 10.24 (s, 1H, H_g), 12.41 (s, 1H, H_h); MS: *m/z* 367; Anal. Calcd. for $C_{16}H_{12}F_3N_3O_2S$: C, 52.31; H, 3.29; N, 11.44. Found: C, 52.22; H, 3.20; N, 11.36%.

3.6.6 General procedure for the synthesis of 2-(trifluoromethyl)-3,4-dihydro-N-(aryl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamides (ART 131-140)

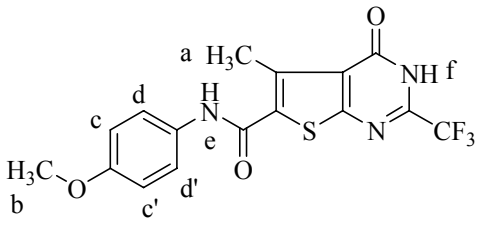
A mixture of an appropriate 5-amino-4-cyano-*N*-(aryl)-3-methylthiophe-2-carbo xamide (0.01 mol) and trifluoroacetic acid (20 mL) was stirred under reflux for 15-17 h. in the presence of catalytic amount of Concentrated H_2SO_4 (under TLC analysis). The reaction mixture was allowed to cool to room temperature and was poured onto water (100 mL). The solid thus formed was collected by filtration, washed with ethanol (20 mL), dried and crystallized from dimethylformamide to afford the desired products ART 131-140.

3.6.6.1 2-(trifluoromethyl)-3,4-dihydro-N-(phenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-131)



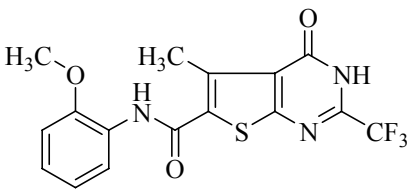
Yield: 59%; mp 192-194 ºC; MS: *m/z* 353; Anal. Calcd. For $C_{15}H_{10}F_3N_3O_2S$: C, 50.99; H, 2.85; N, 11.89. Found: C, 50.90; H, 2.76; N, 11.81%.

3.6.6.2 2-(trifluoromethyl)-3,4-dihydro-N-(4-methoxyphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide (ART-132)



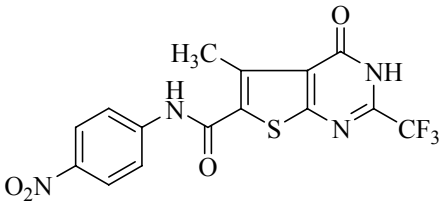
Yield: 68%; mp 198-200 ºC; IR (cm^{-1}): 3292 (N-H stretching of secondary amine), 3103 (C-H stretching of aromatic ring), 2999 (C-H asymmetrical stretching of CH_3 group), 2869 (C-H asymmetrical stretching of OCH_3 group), 2837 (C-H symmetrical stretching of CH_3 group), 1720 (C=O stretching of amide), 1647 (C=O stretching of pyrimidine ring), 1629 (C=N stretching of pyrimidine ring), 1580 and 1532 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of OCH_3 group), 1404 (C-H asymmetrical deformation of CH_3 group), 1290 (C-H symmetrical deformation of CH_3 group), 1166 (C-O-C stretching), 705 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.68 (s, 3H, H_a), 3.77 (s, 3H, H_b), 6.84-6.86 (d, 2H, $H_{cc'}$, J = 9.00 Hz), 7.57-7.59 (d, 2H, $H_{dd'}$, J = 8.96 Hz), 9.84 (s, 1H, H_e), 13.61 (s, 1H, H_f) ; MS: *m/z* 383; Anal. Calcd. for $C_{16}H_{12}F_3N_3O_3S$: C, 50.13; H, 3.16; N, 10.96. Found: C, 50.06; H, 3.09; N, 10.90%.

3.6.6.3 2-(trifluoromethyl)-3,4-dihydro-N-(2-methoxyphenyl)-5-methyl-4-oxothieno [2,3-d]pyrimidine-6-carboxamide (ART-133)



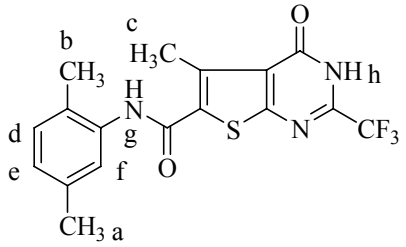
Yield: 65%; mp 188-190 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{16}H_{12}F_3N_3O_3S$: C, 50.13; H, 3.16; N, 10.96. Found: C, 50.05; H, 3.05; N, 10.92%.

3.6.6.4 2-(trifluoromethyl)-3,4-dihydro-N-(4-nitrophenyl)-5-methyl-4-oxothieno[2,3 -d]pyrimidine-6-carboxamide (ART-134)



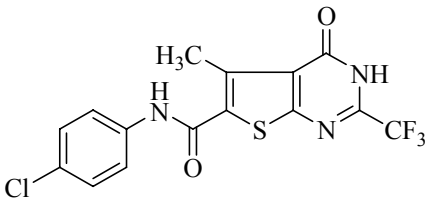
Yield: 74%; mp 118-120 ºC; MS: *m/z* 398; Anal. Calcd. for $C_{15}H_9F_3N_4O_4S$: C, 45.23; H, 2.28; N, 14.07. Found: C, 45.18; H, 2.19; N, 13.99%.

3.6.6.5 2-(trifluoromethyl)-3,4-dihydro-N-(2,5-methylphenyl)-5-methyl-4-oxothieno [2,3-d]pyrimidine-6-carboxamide (ART-135)



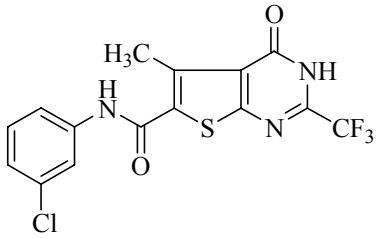
Yield: 76%; mp 194-196 ºC; IR (cm^{-1}): 3300 and 3223 (N-H stretching of secondary amine), 3020 (C-H stretching of aromatic ring), 2874 (C-H asymmetrical stretching of CH_3 group), 2820 (C-H asymmetrical stretching of CH_3 group), 1707 (C=O stretching of amide), 1647 (C=O stretching of pyrimidine ring), 1626 (C=N stretching of pyrimidine ring), 1556, 1529 and 1504 (C=C stretching of aromatic ring), 1400 (C-H asymmetrical deformation of -CH_3 group), 1301 (C-H symmetrical deformation of CH_3 group), 659 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.21 (s, 3H, H_a), 2.29 (s, 3H, H_b), 2.70 (s, 3H, H_c), 6.94-7.24 (m, 3H, $H_{d\text{-}f}$), 9.42 (s, 1H, H_g), 13.42 (s, 1H, H_h); MS: *m/z* 381; Anal. Calcd. for $C_{17}H_{14}F_3N_3O_2S$: C, 53.54; H, 3.70; N, 11.02. Found: C, 53.47; H, 3.69; N, 10.94%.

3.6.6.6 2-(trifluoromethyl)-3,4-dihydro-N-(4-chlorophenyl)-5-methyl-4-oxothieno[2, 3-d]pyrimidine-6-carboxamide (ART-136)



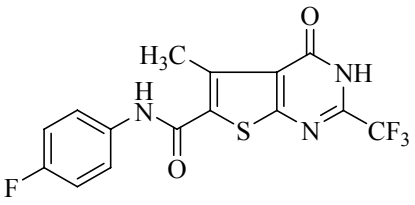
Yield: 68%; mp 108-110 ºC; MS: *m/z* 387; Anal. Calcd. for $C_{15}H_9ClF_3N_3O_2S$: C, 46.46; H, 2.34; N, 10.84. Found: C, 46.39; H, 2.27; N, 10.75%.

3.6.6.7 2-(trifluoromethyl)-3,4-dihydro-N-(3-chlorophenyl)-5-methyl-4-oxothieno[2, 3-d]pyrimidine-6-carboxamide (ART-137)



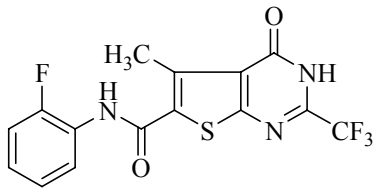
Yield: 72%; mp 261-263 ºC; MS: *m/z* 387; Anal Calcd. for $C_{15}H_9ClF_3N_3O_2S$: C, 46.46; H, 2.34; N, 10.84. Found: C, 46.70; H, 2.26; N, 10.77%.

3.6.6.8 2-(trifluoromethyl)-3,4-dihydro-N-(4-fluorophenyl)-5-methyl-4-oxothieno[2, 3-d]pyrimidine-6-carboxamide (ART-138)



Yield: 61%; mp 242-244 ºC; MS: *m/z* 371; Anal. Calcd. for $C_{15}H_9F_4N_3O_2S$: C, 53.97; H, 3.62; N, 12.59. Found: C, 53.89; H, 3.58; N, 12.49%.

3.6.6.9 2-(trifluoromethyl)-3,4-dihydro-N-(2-fluorophenyl)-5-methyl-4-oxothieno[2, 3-d]pyrimidine-6-carboxamide (ART-139)



Yield: 63%; mp 250-252 ºC; MS: *m/z* 371; Anal. Calcd. for $C_{15}H_9F_4N_3O_2S$: C, 53.97; H, 3.6; N, 2.59. Found: C, 53.90; H, 3.56; N, 12.50%.

3.6.6.10 2-(trifluoromethyl)-3,4-dihydro-N-(3-trifluoromethylphenyl)-5-methyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamid (ART-140) Yield: 59%; mp 131-133 ºC; IR (cm^{-1}): 3387 and 3300 (N-H stretching of secondary amine), 3026 (C-H stretching of aromatic ring), 2991 (C-H asymmetrical stretching of CH_3

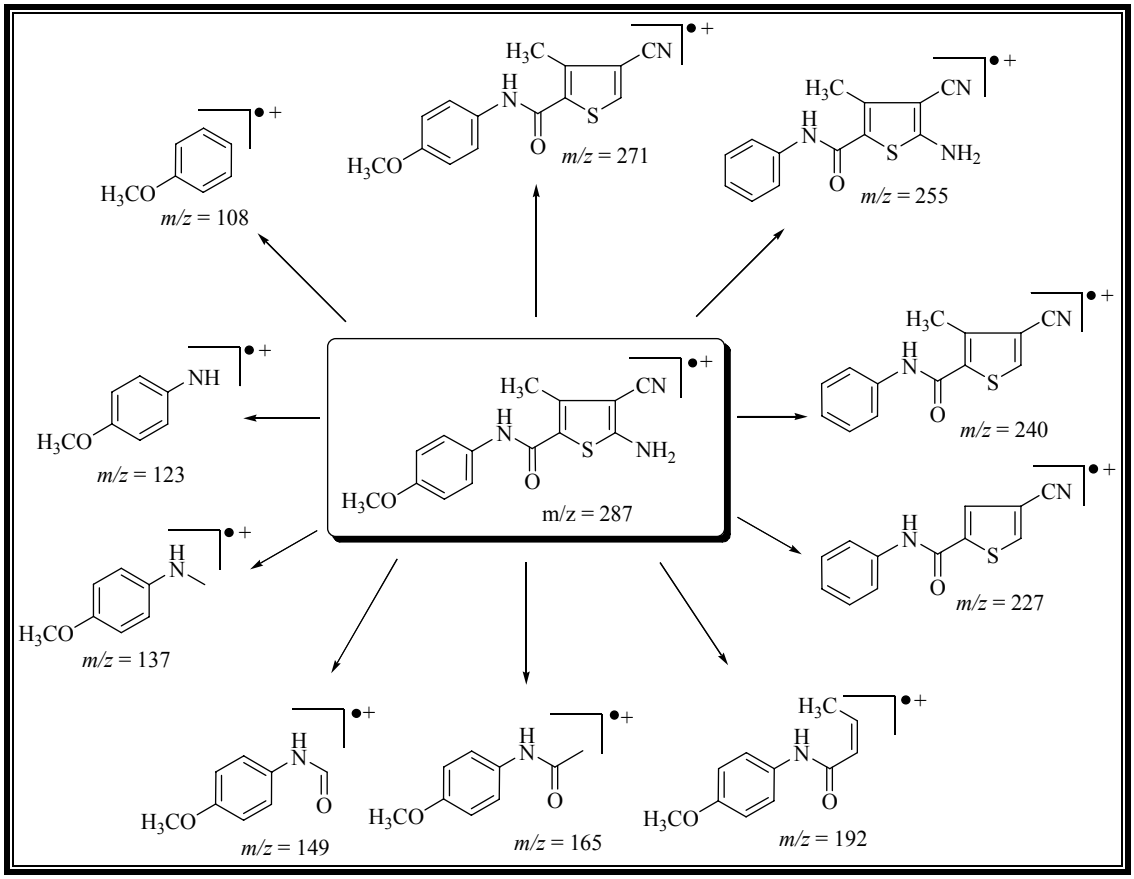
group), 2897 (C-H symmetrical stretching of CH_3 group), 1720 (C=O stretching of amide), 1647 (C=O stretching of pyrimidine ring), 1564 (C=N stretching of pyrimidine ring), 1492 and 1450 (C=C stretching of aromatic ring), 1400 (C-H asymmetrical deformation of CH_3 group), 1334 (C-H symmetrical deformation of CH_3 group), 696 (C-S-C stretching). ^{1}H NMR (DMSO-d_6) δ ppm: 2.73 (s, 3H, H_a), 7.34-7.36 (d, 1H, H_b), 7.44-7.48 (t, 1H, H_c), 7.93-7.95 (d, 1H, H_d), 8.09 (s, 1H, H_e), 10.10 (s, 1H, H_f), 13.60 (s, 1H, H_g); MS: *m/z* 421; Anal. Calcd. for $C_{16}H_9F_6N_3O_2S$: C, 45.61; H, 2.15; N, 9.97. Found: C, 45.55; H, 2.06; N, 9.89%.

3.7 Spectral discussion

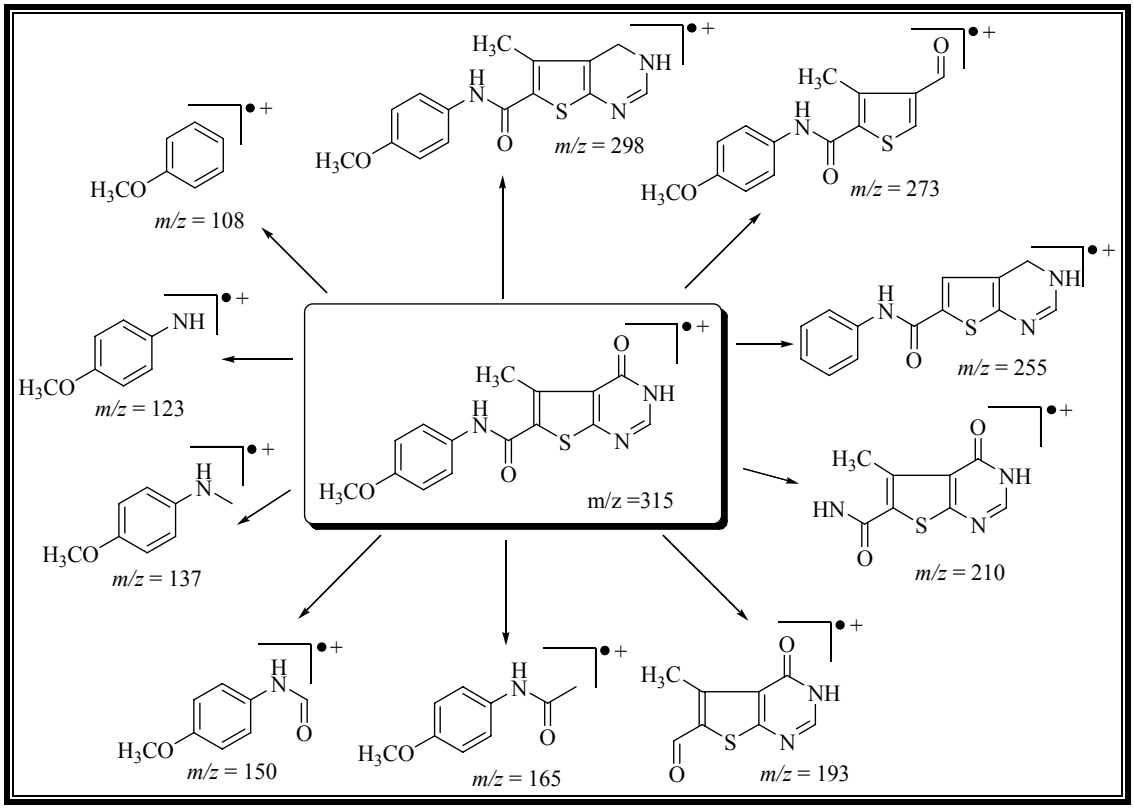
3.7.1 Mass spectral study

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.

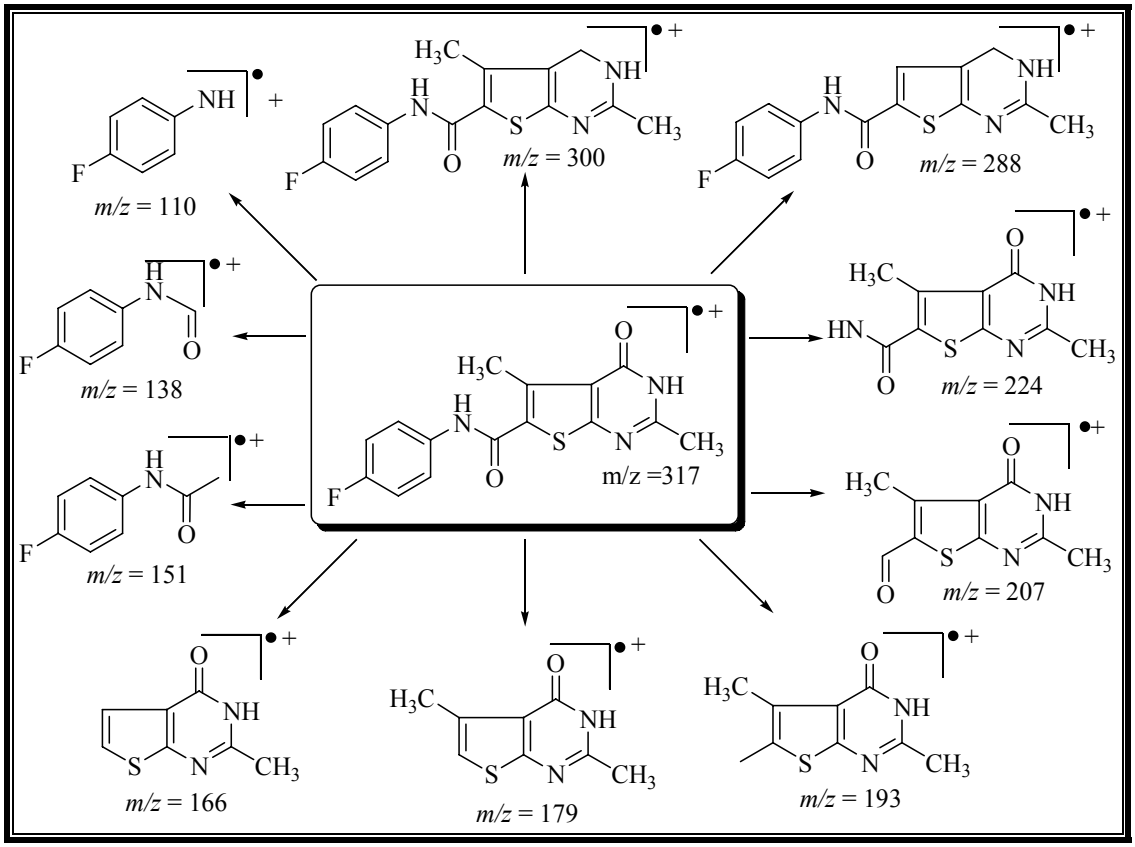
3.7.1.1 Mass fragmentation pattern for ART-102



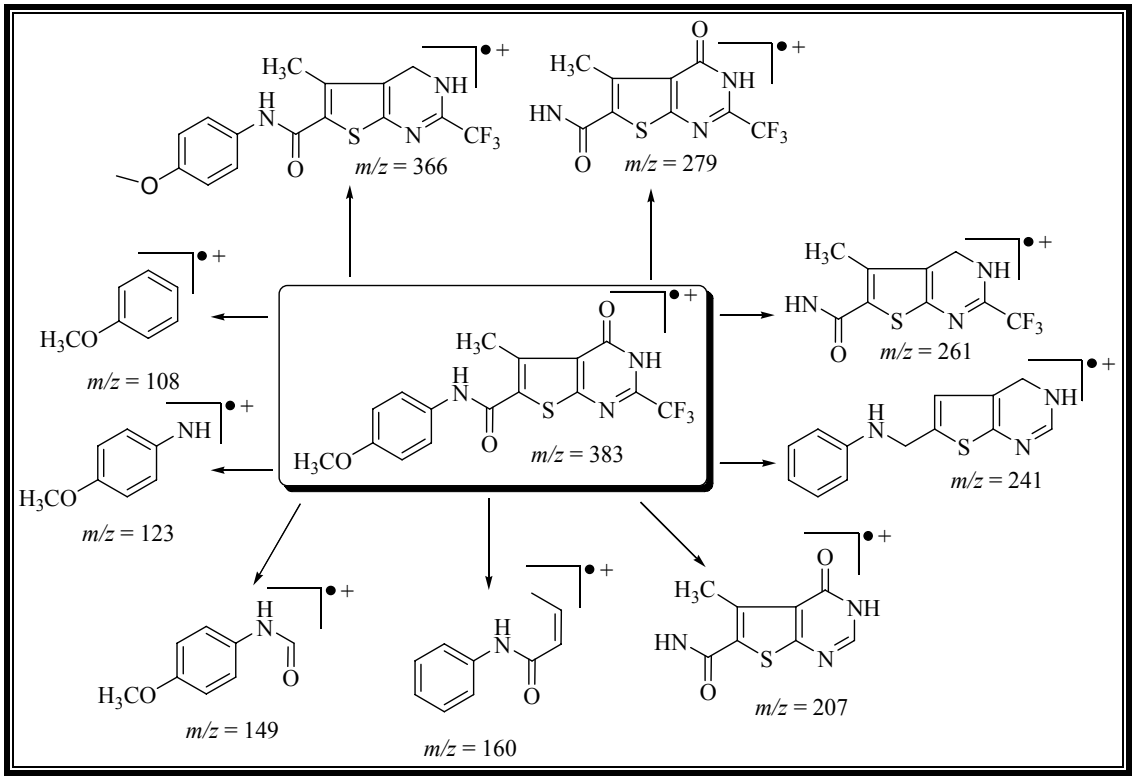
3.7.1.2 Mass fragmentation pattern for ART-112



3.7.1.3 Mass fragmentation pattern for ART-128



3.7.1.4 Mass fragmentation pattern for ART-132



3.7.2 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 thiophenes ART-101 to 110, a characteristic band of nitrile group was observed in the range of 2206-2210 cm^{-1}. Confirmatory bands for primary amine, amide and amidic carbonyl groups were observed at 3300-3427 cm^{-1}, 3184-3281 cm^{-1} and 1701 cm^{-1} respectively. For pyrimidines ART-111 to 140, the characteristic nitrile absorption band at 2206-2210 cm^{-1} was found to be absent confirming the cyclisation from corresponding thiophenes. Another characteristic carbonyl stretching band of pyrimidine was observed at 1658-1670 cm^{-1} suggesting formation of desired products ART-111 to 140.

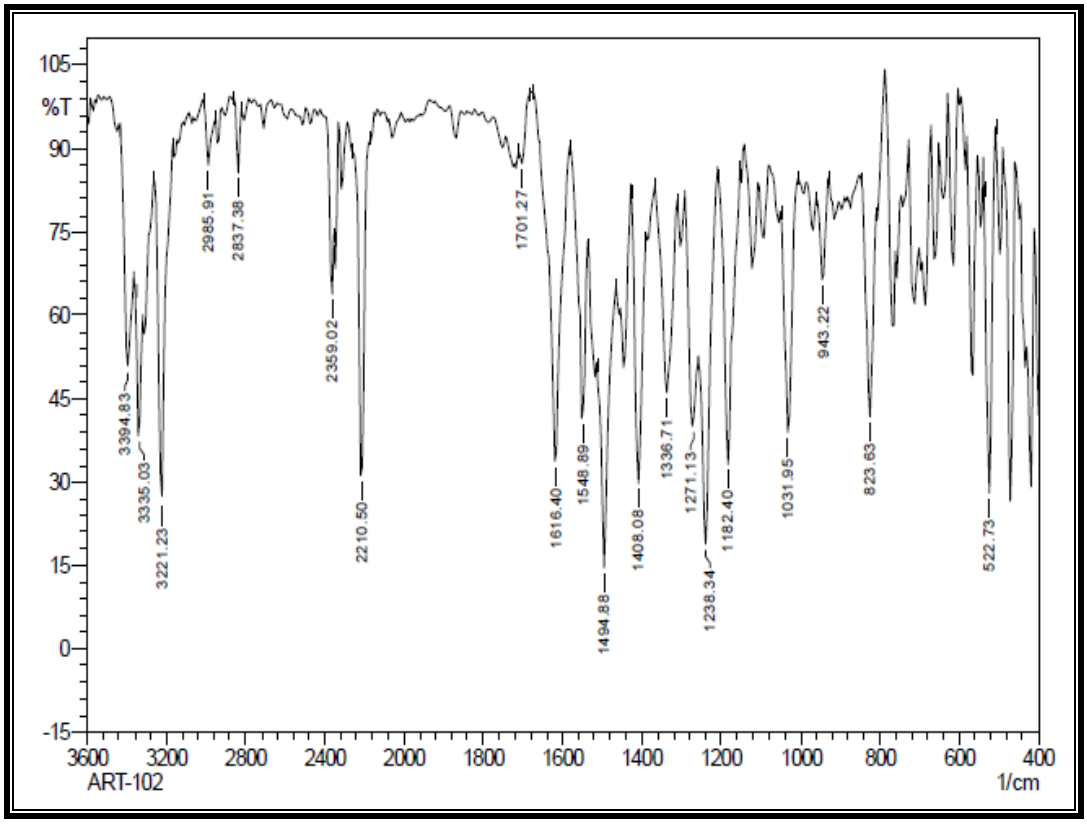
3.7.3 ^{1}H NMR spectral study

^{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.

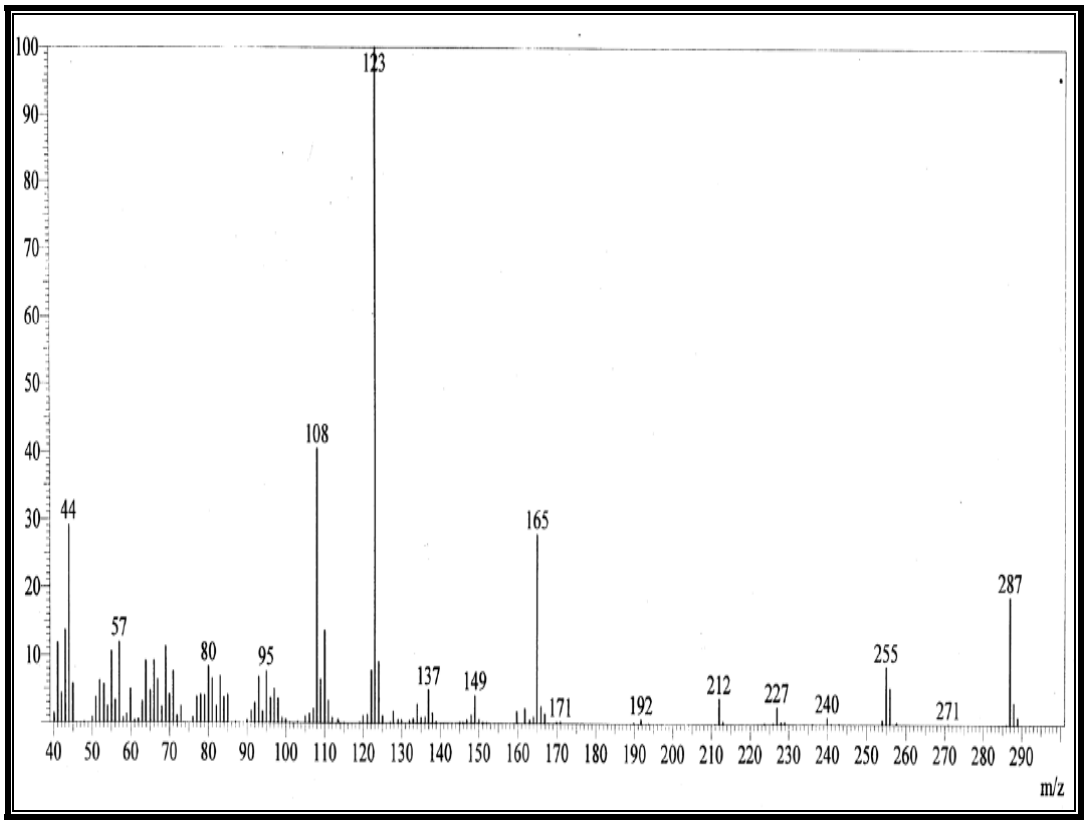
For thiophenes ART-101 to 110, characteristic singlets were observed for methyl and amide group at 2.18-2.49 δ ppm and 8.18-9.10 δ ppm respectively. The aromatic ring protons were observed at 6.80-8.32 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. The primary amine proton peak was observed as a broad singlet around 7.28-7.48 δ ppm.

While, for pyrimidines ART-111 to 140, characteristic singlets were observed for methyl, amide and secondary amine protons at 2.68-3.32, 9.15-10.24 and 12.40-12.61 δ ppm. The appearance of secondary amine proton peak confirmed the conversion of thiophenes to pyrimidines. In addition to that, characteristic methine proton peak was observed at 8.00-8.05 δ ppm for pyrimidines ART-111 to 120, which further confirmed the cyclisation. While, the appearance of a characteristic methyl singlet at 2.41-2.43 δ ppm suggested the formation of pyrimidines ART-121 to 130. The aromatic ring protons were observed at 6.84-8.14 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring.

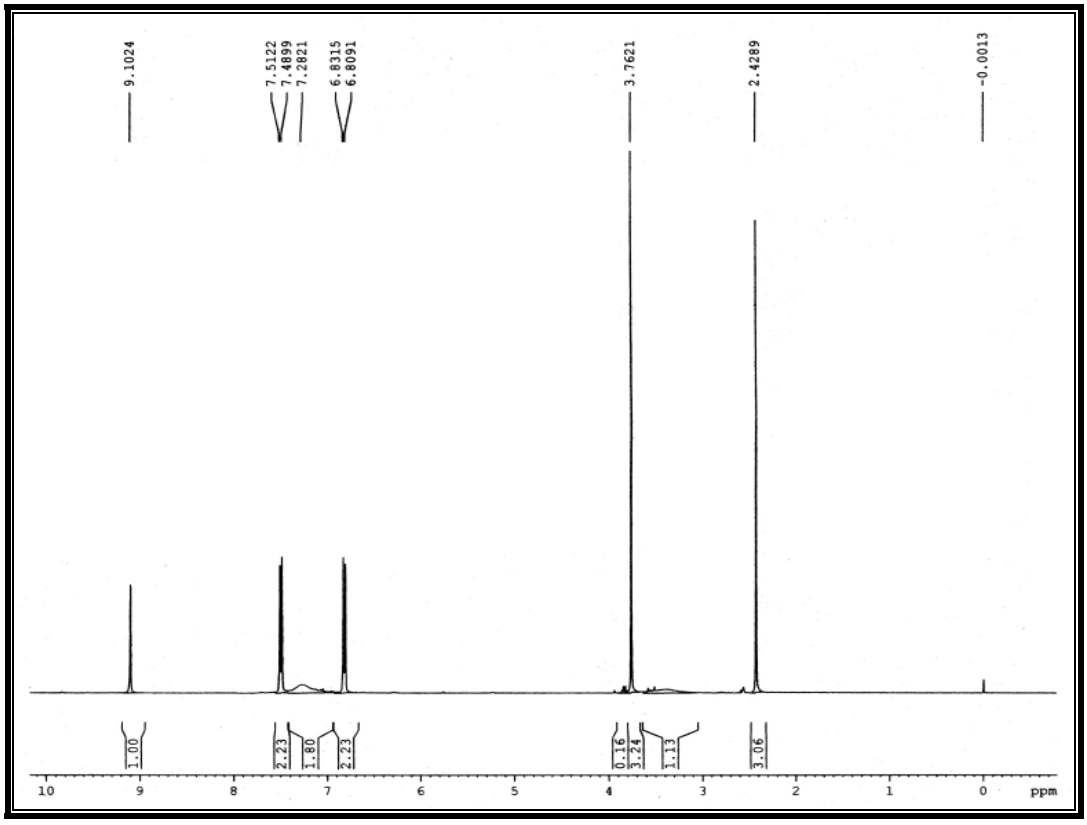
IR spectrum of ART-102



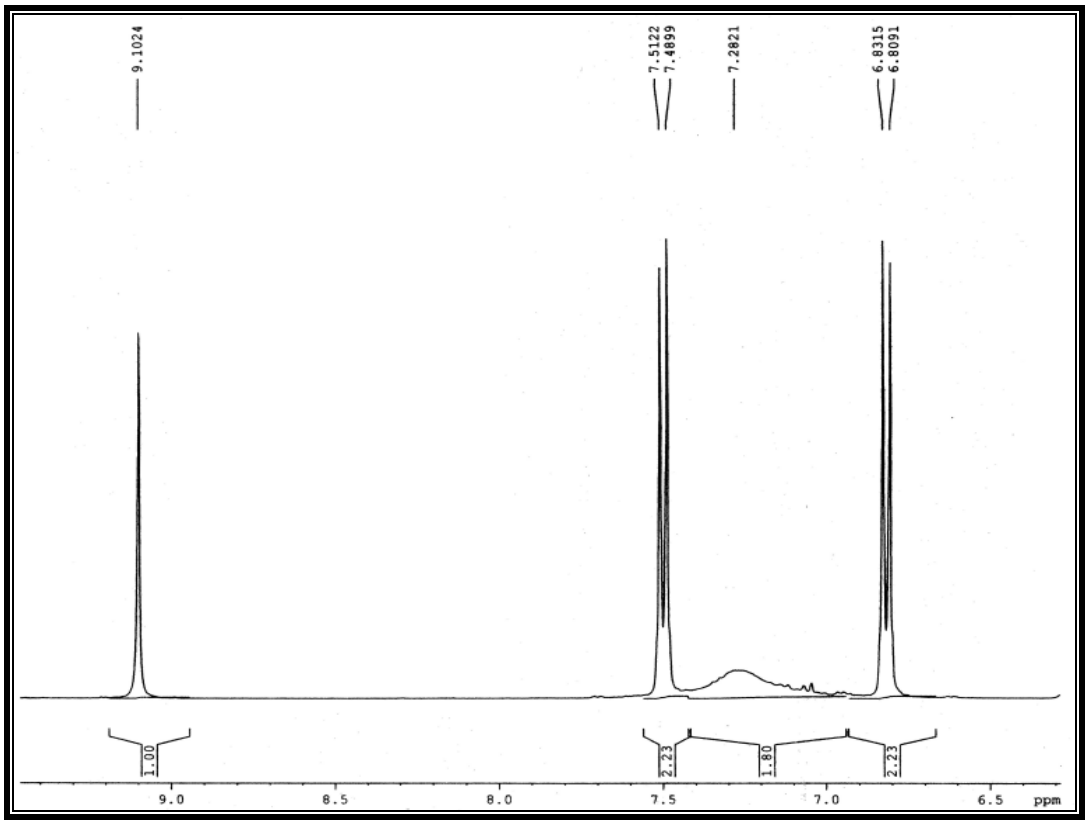
Mass spectrum of ART-102



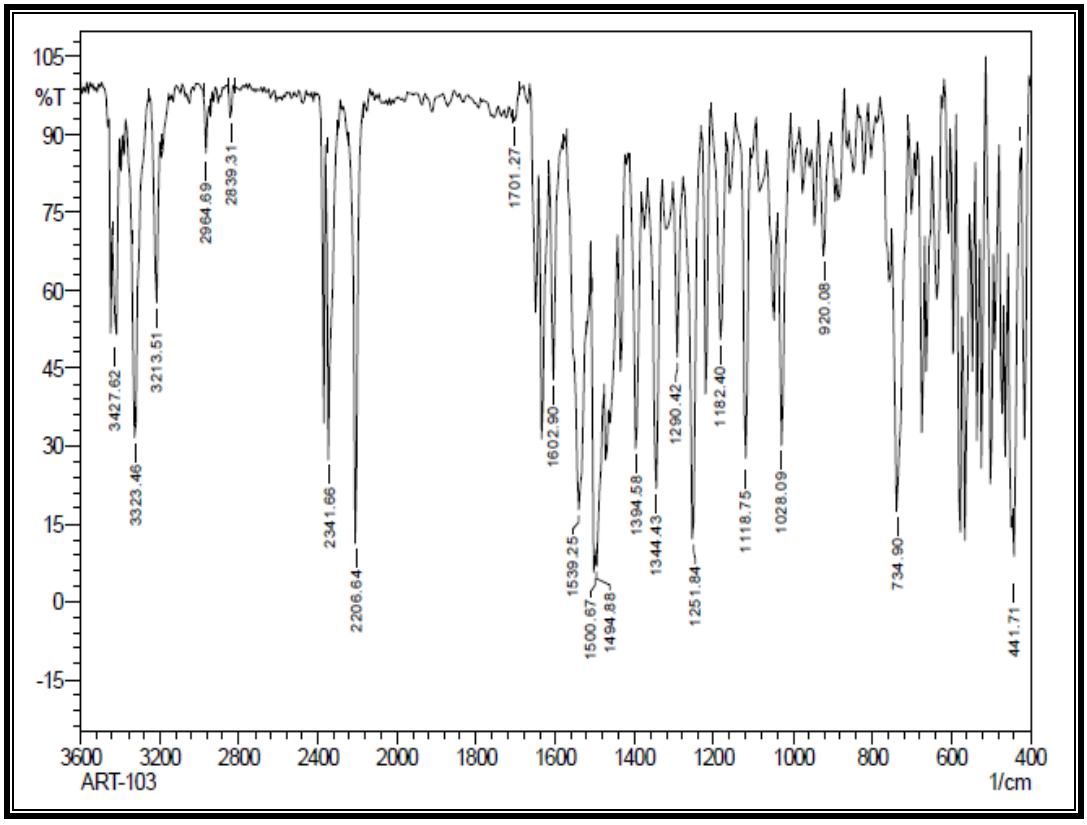
^{1}H NMR spectrum of ART-102



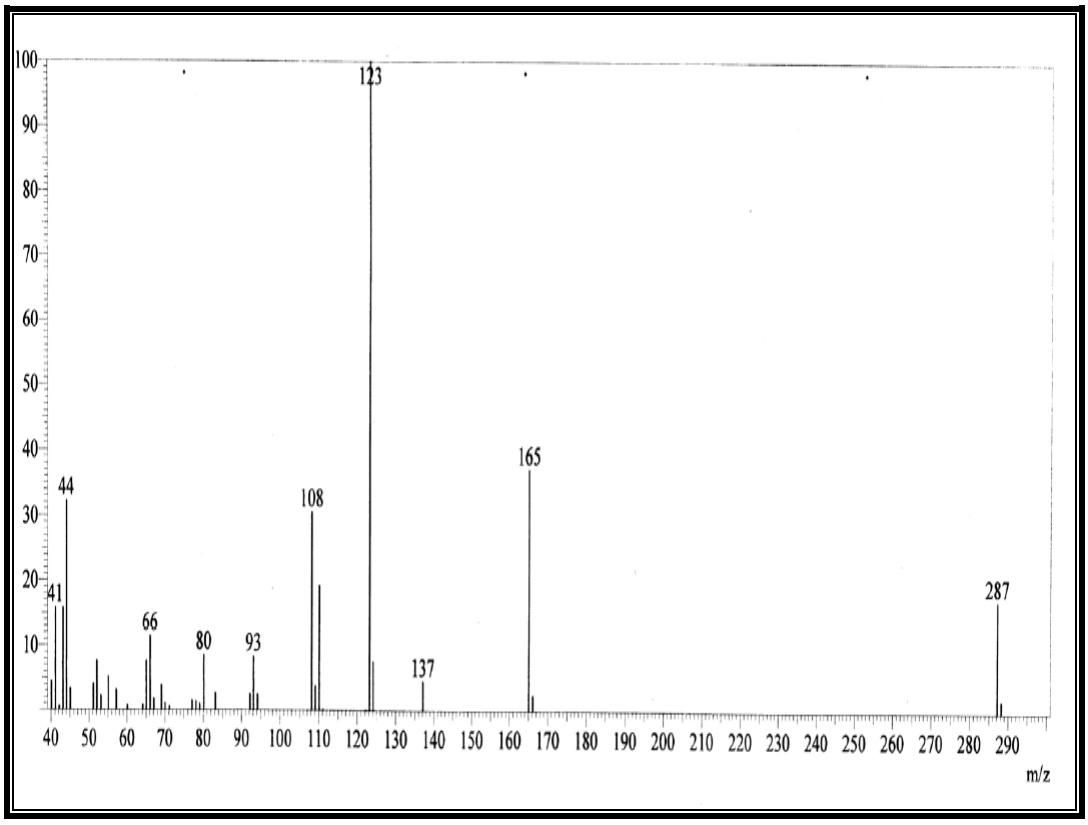
Expanded ^{1}H NMR spectrum of ART-102



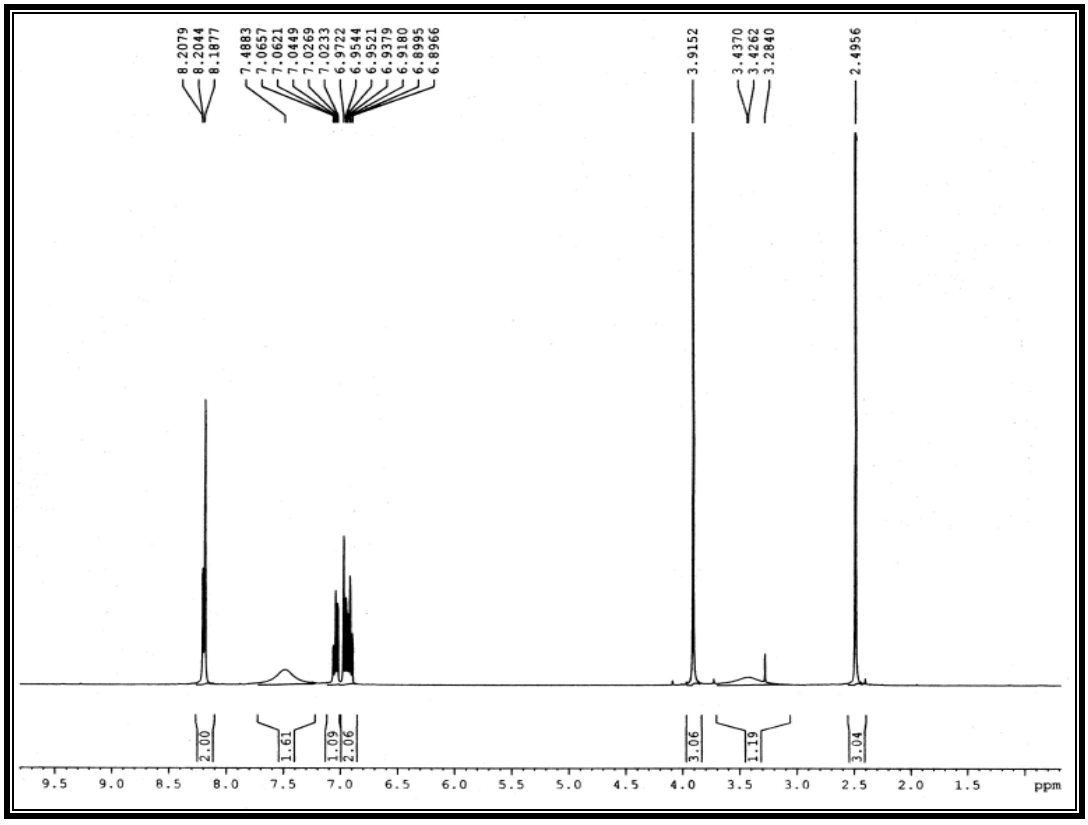
IR spectrum of ART-103



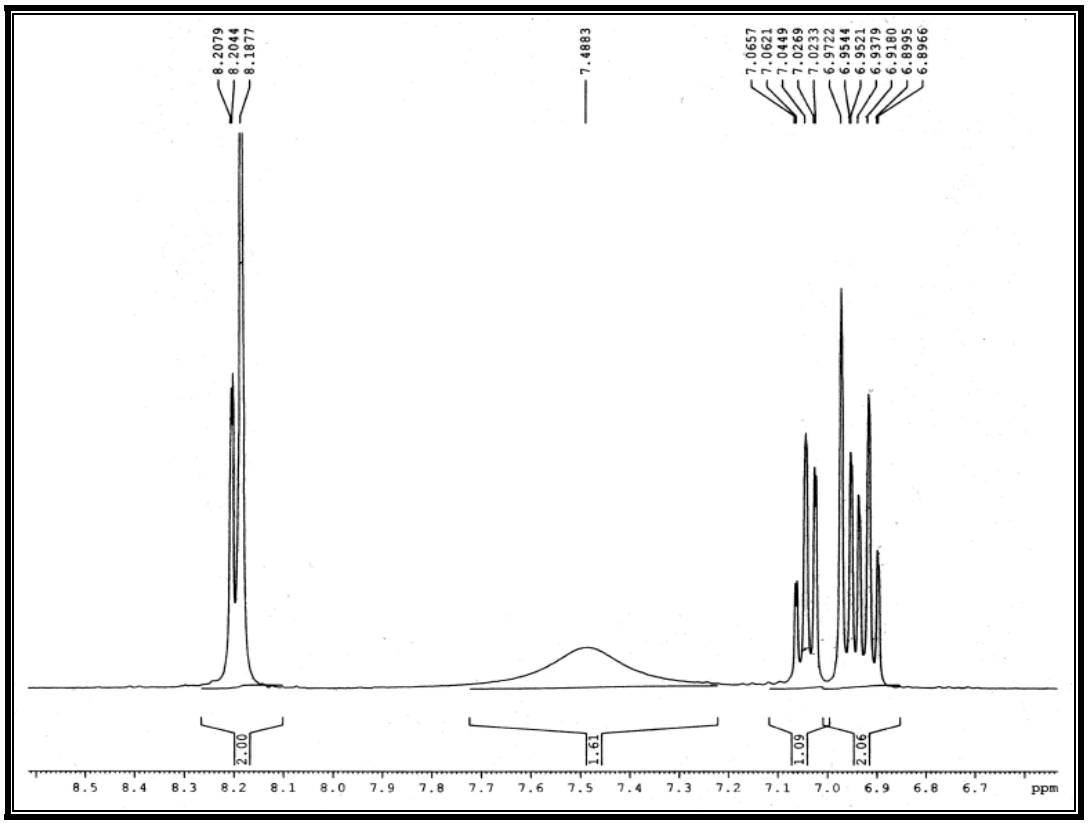
Mass spectrum of ART-103



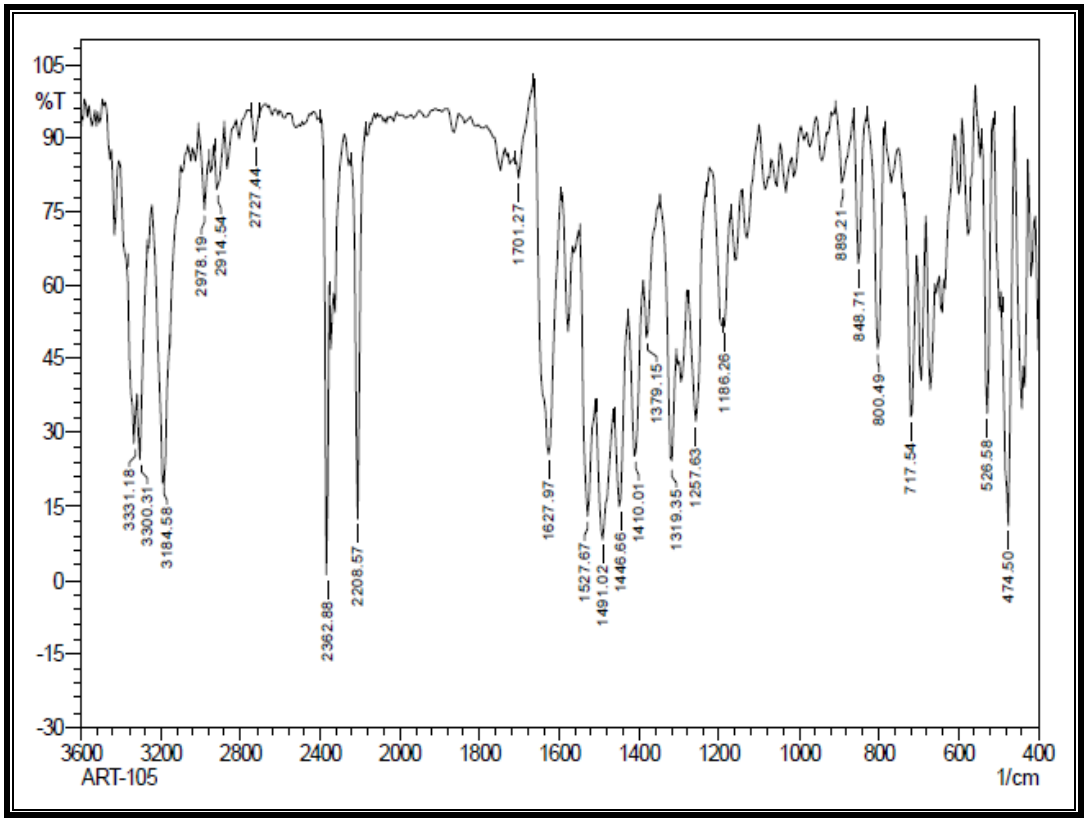
^{1}H NMR spectrum of ART-103



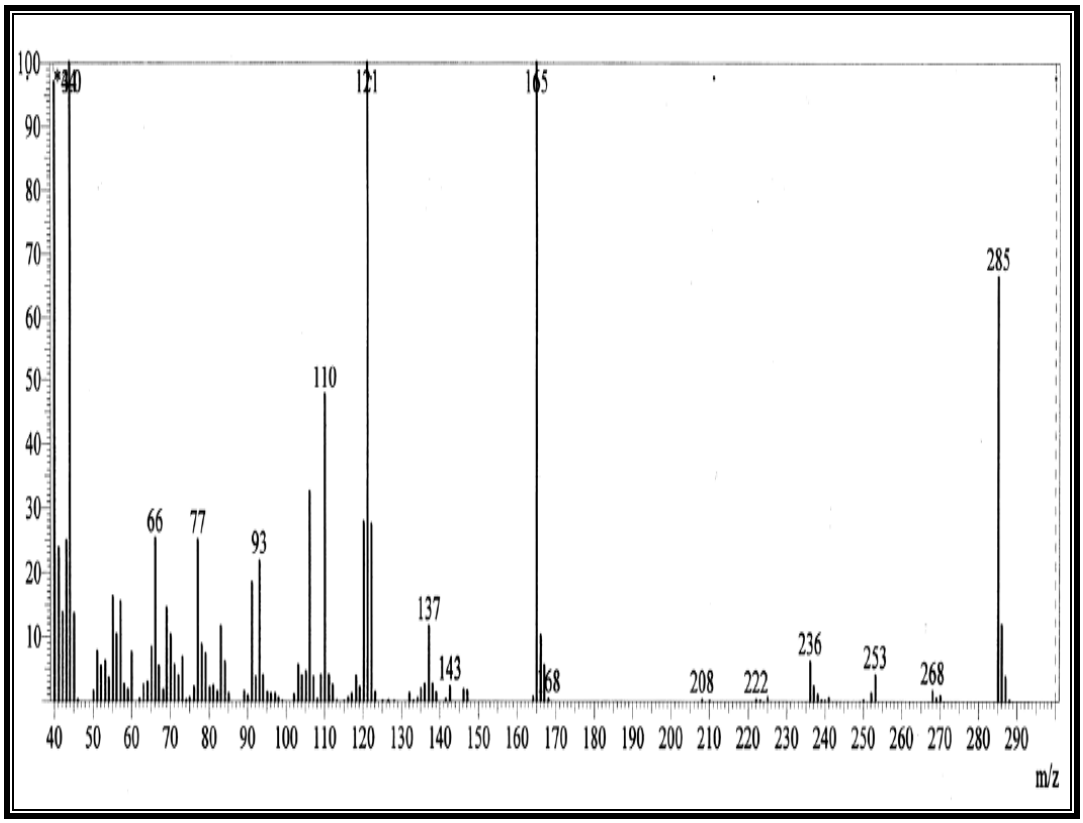
Expanded ^{1}H NMR spectrum of ART-103



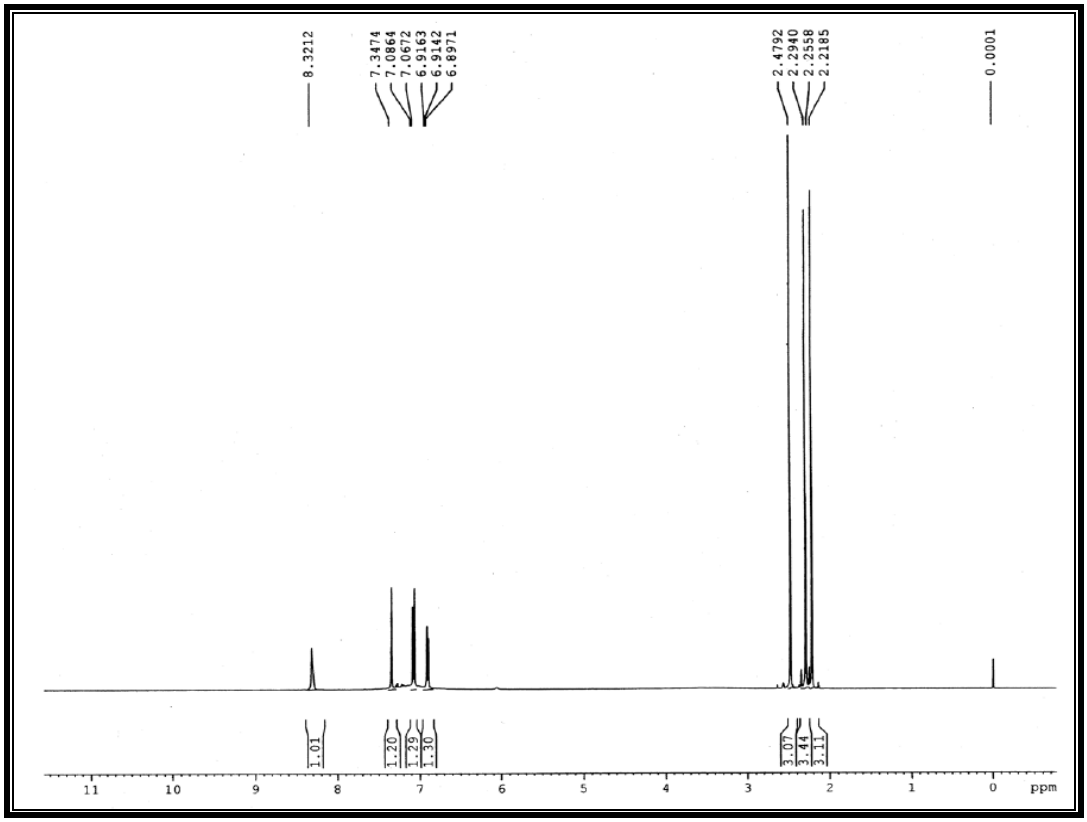
IR spectrum of ART-105



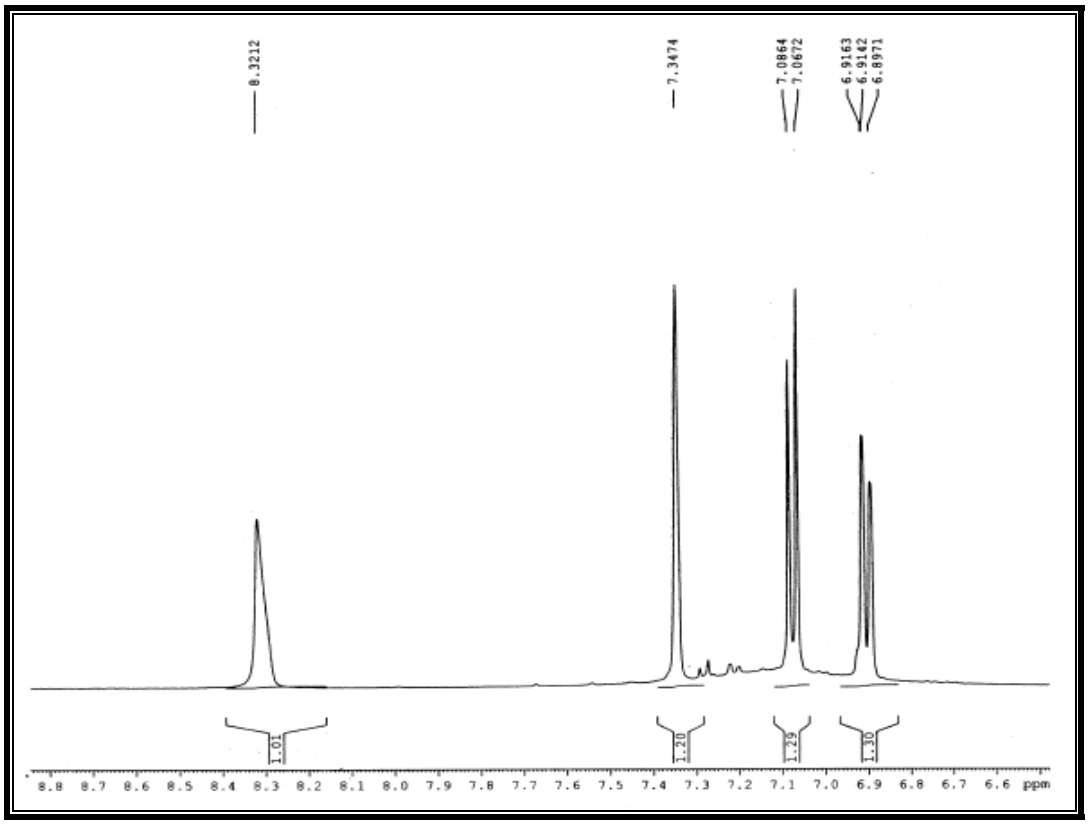
Mass spectrum of ART-105



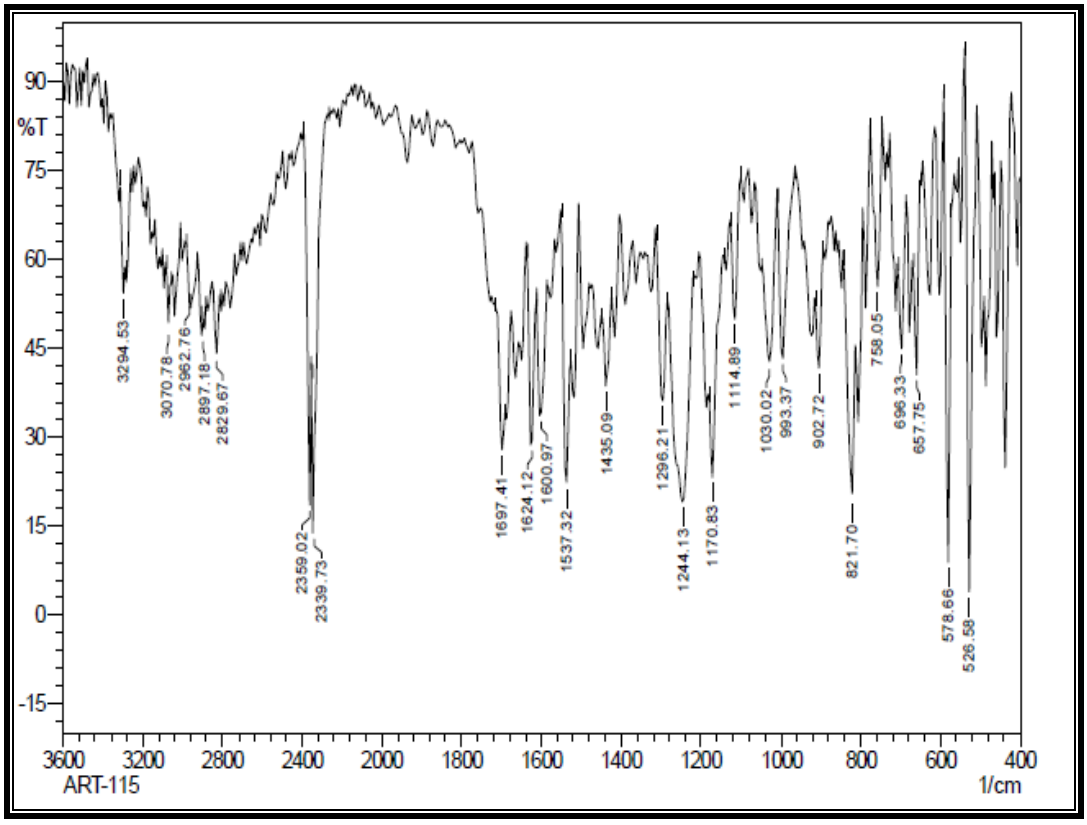
^{1}H NMR spectrum of ART-105



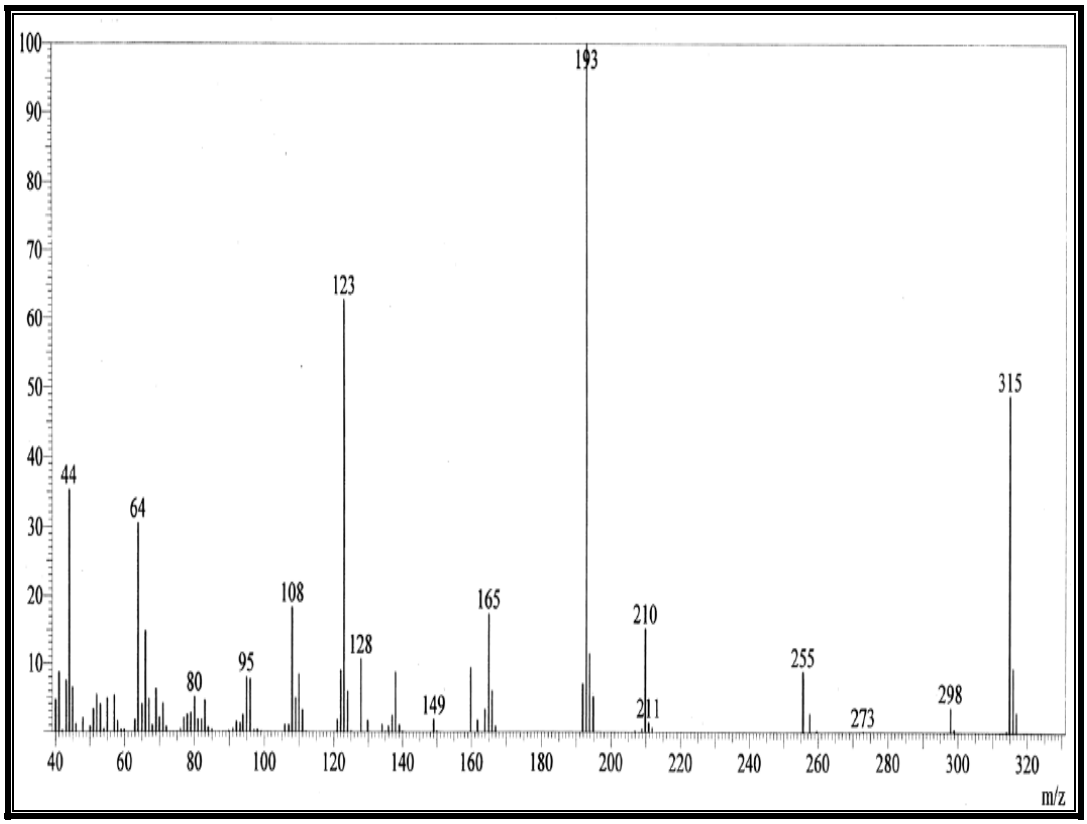
Expanded ^{1}H NMR spectrum of ART-105



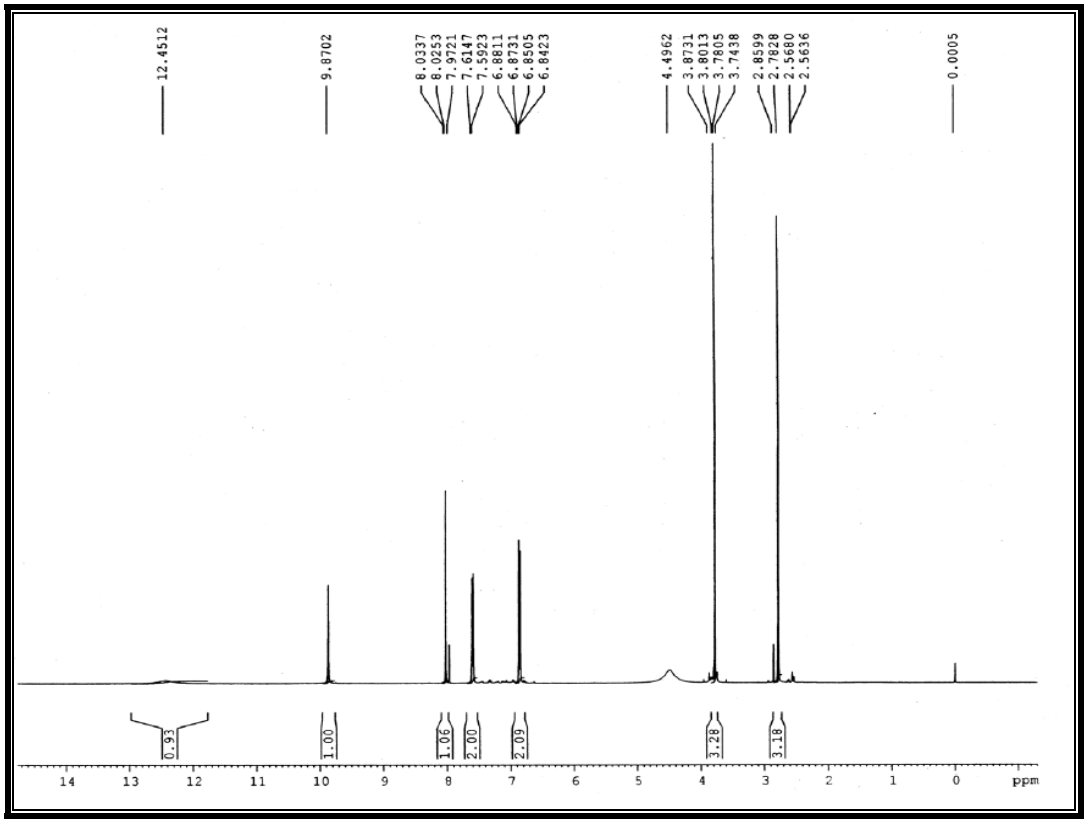
IR spectrum of ART-112



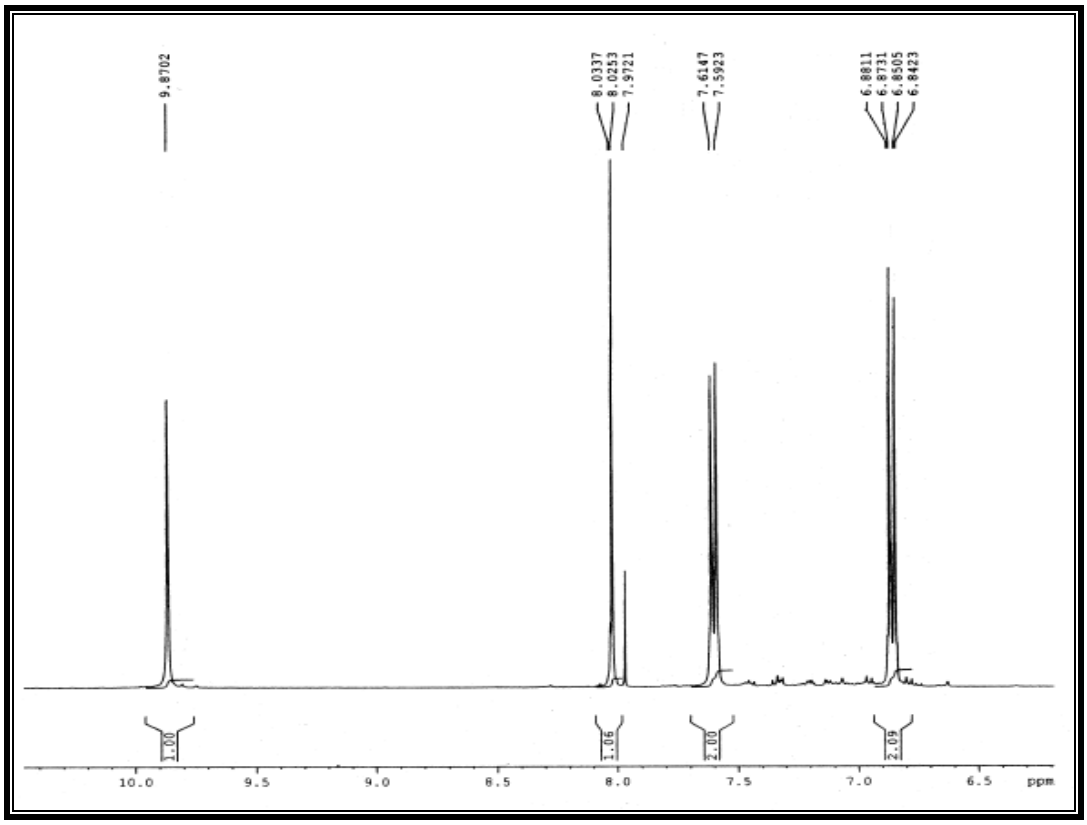
Mass spectrum of ART-112



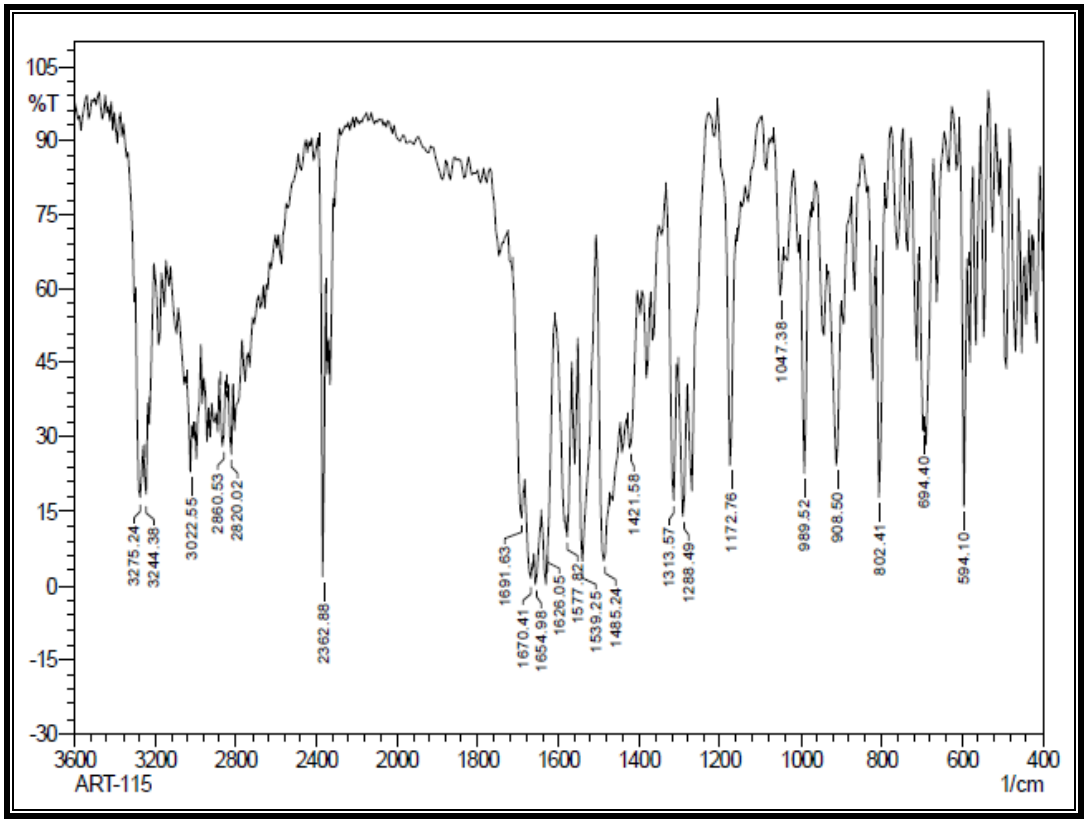
^{1}H NMR spectrum of ART-112



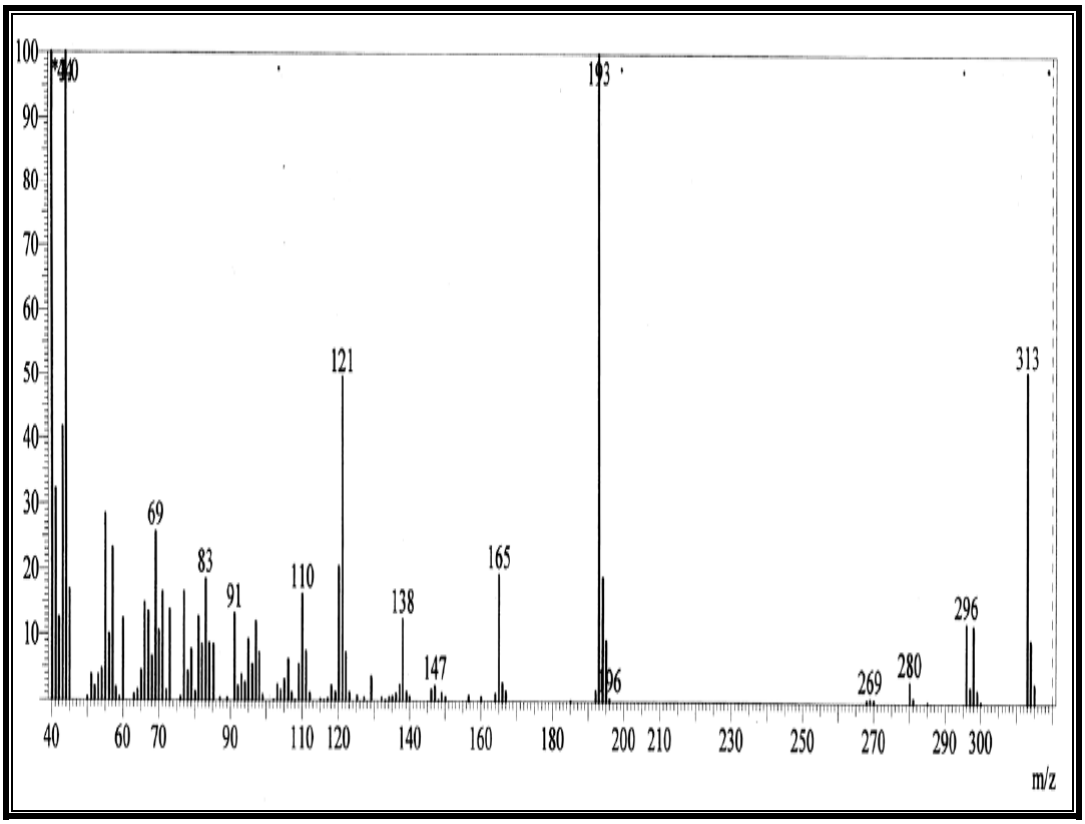
Expanded ^{1}H NMR spectrum of ART-112



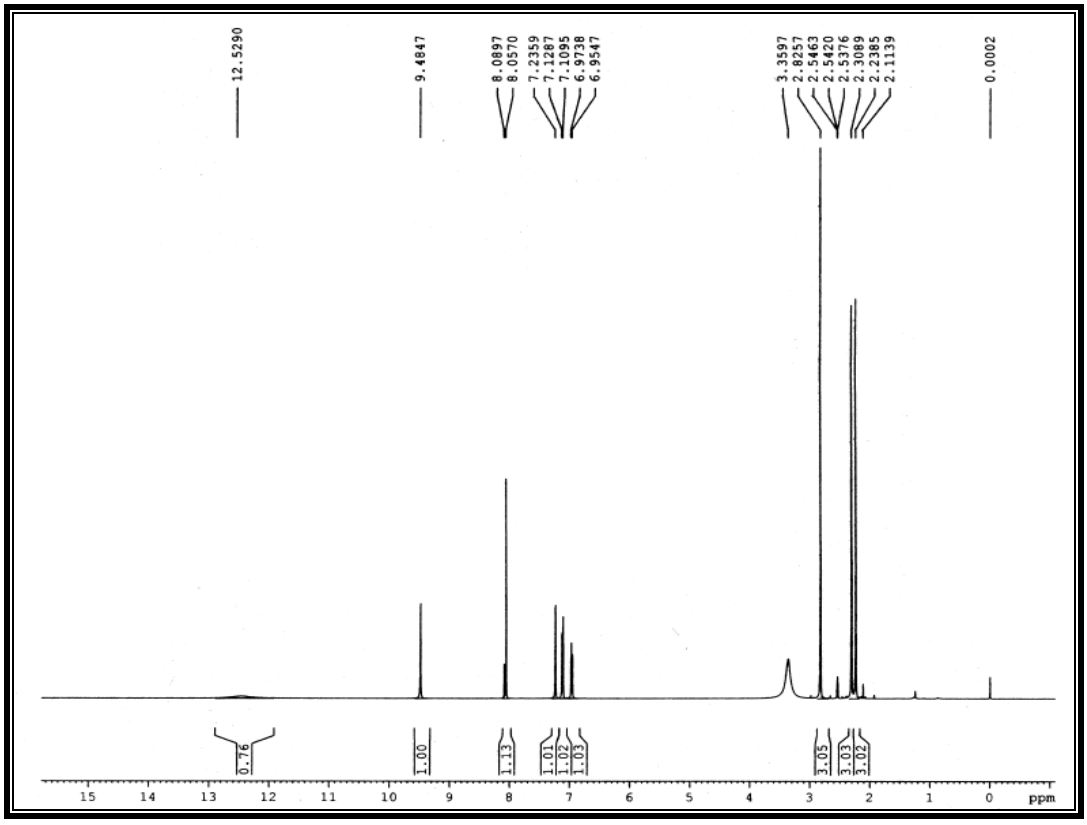
IR spectrum of ART-115



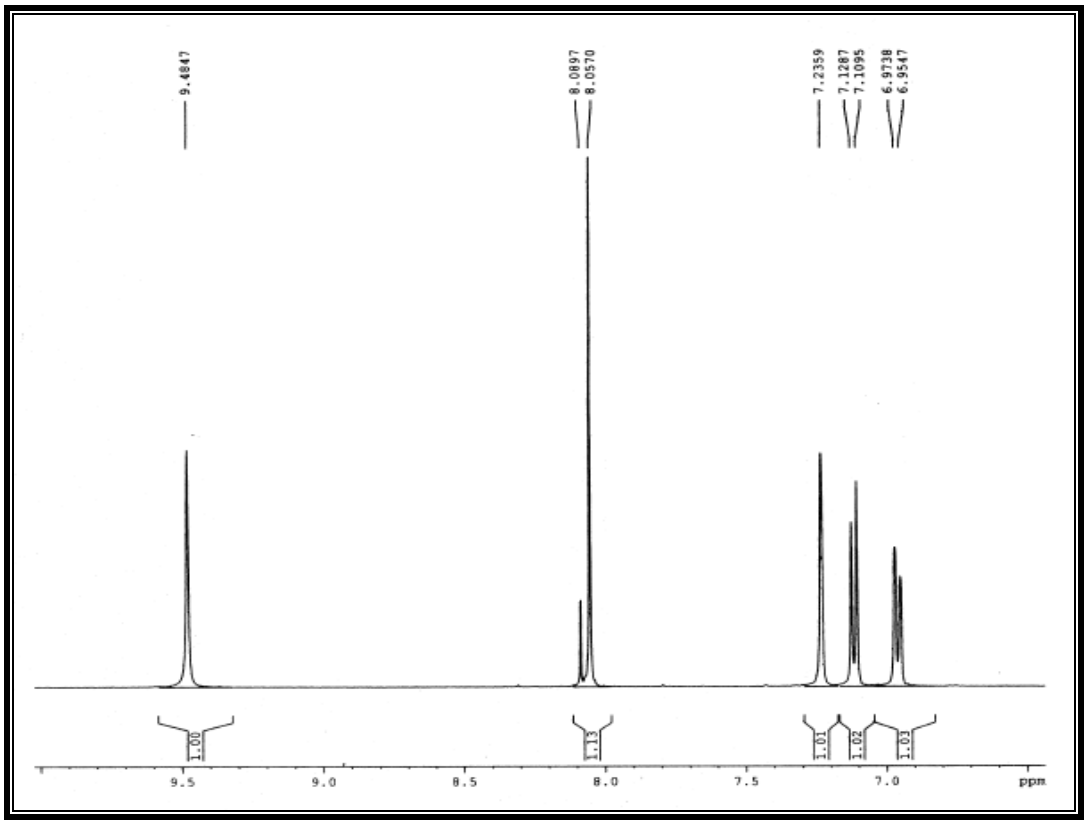
Mass spectrum of ART-115



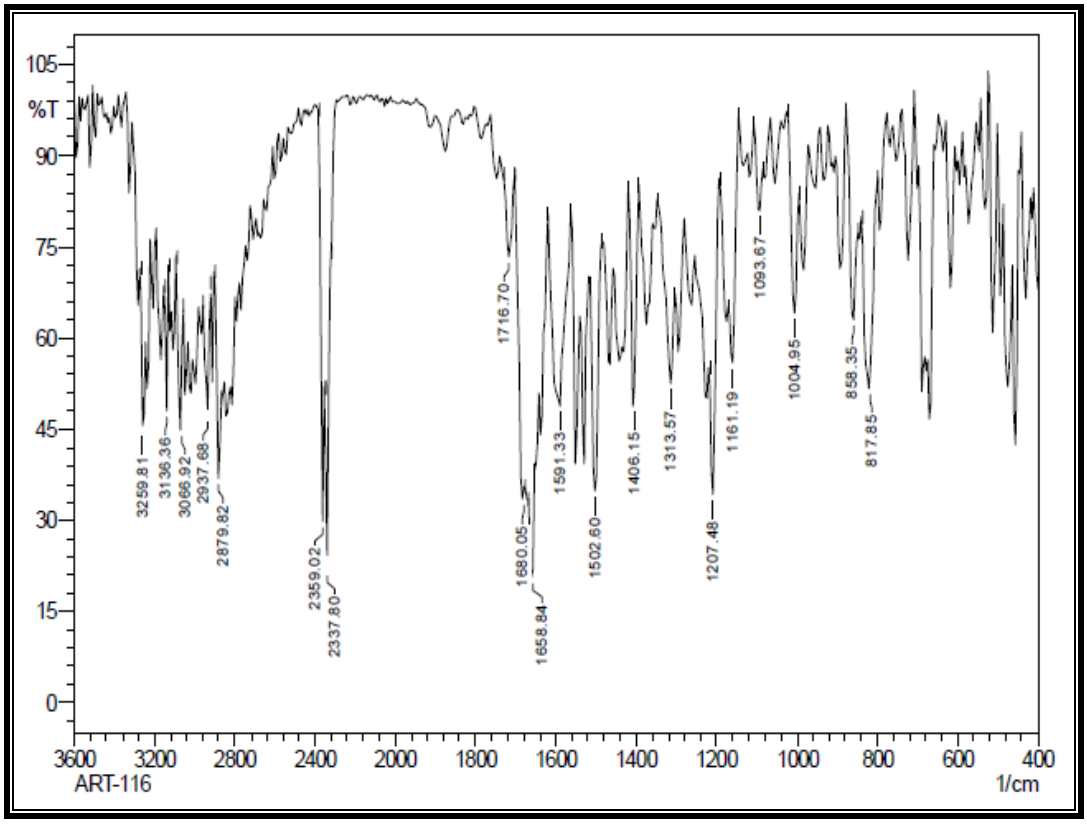
^{1}H NMR spectrum of ART-115



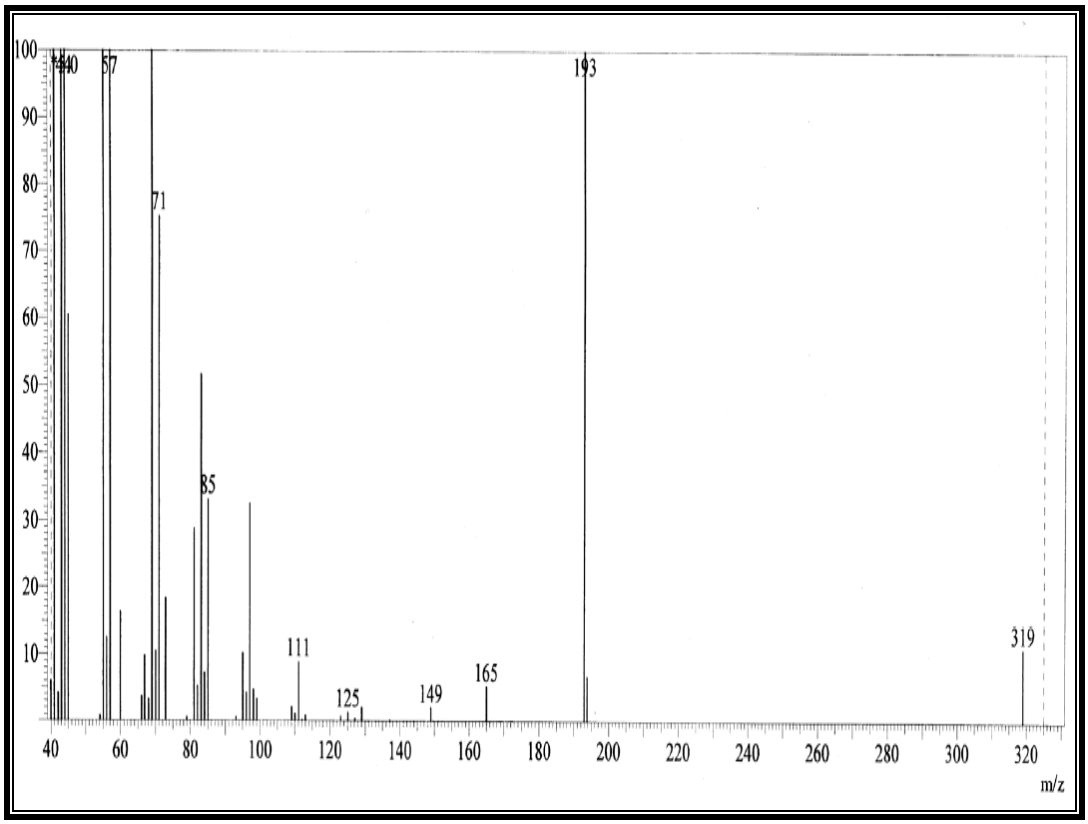
Expanded ^{1}H NMR spectrum of ART-115



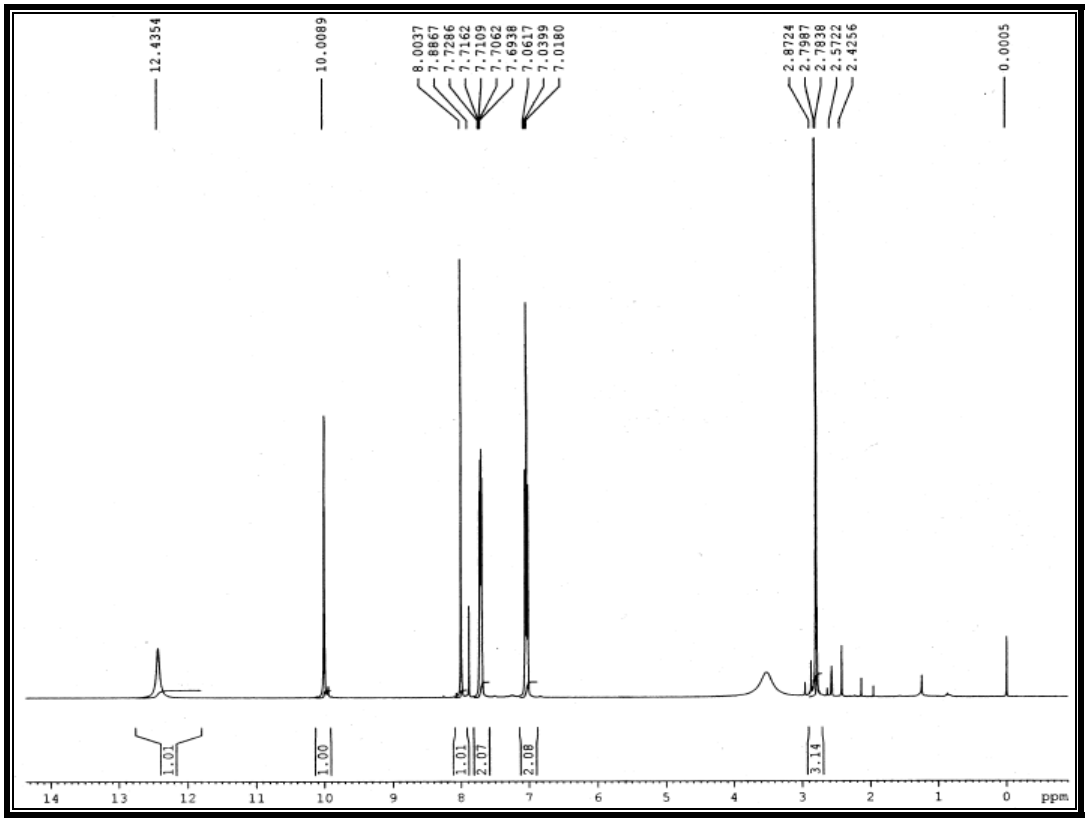
IR spectrum of ART-117



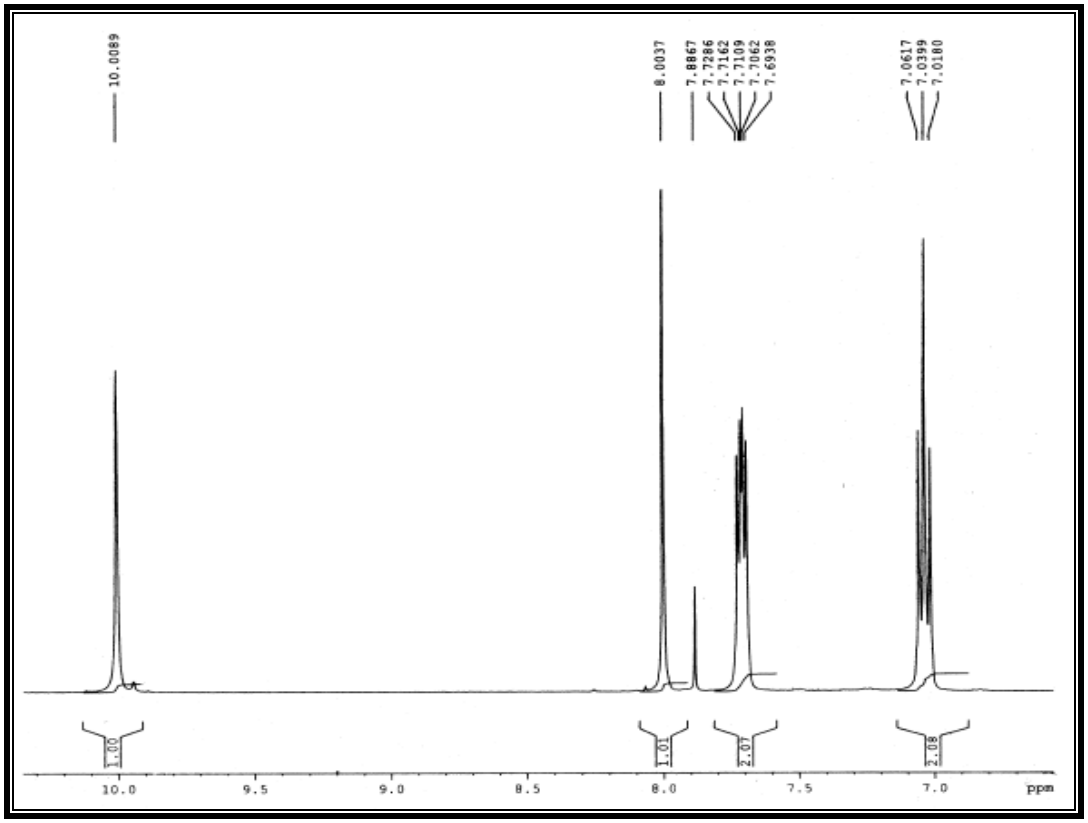
Mass spectrum of ART-117



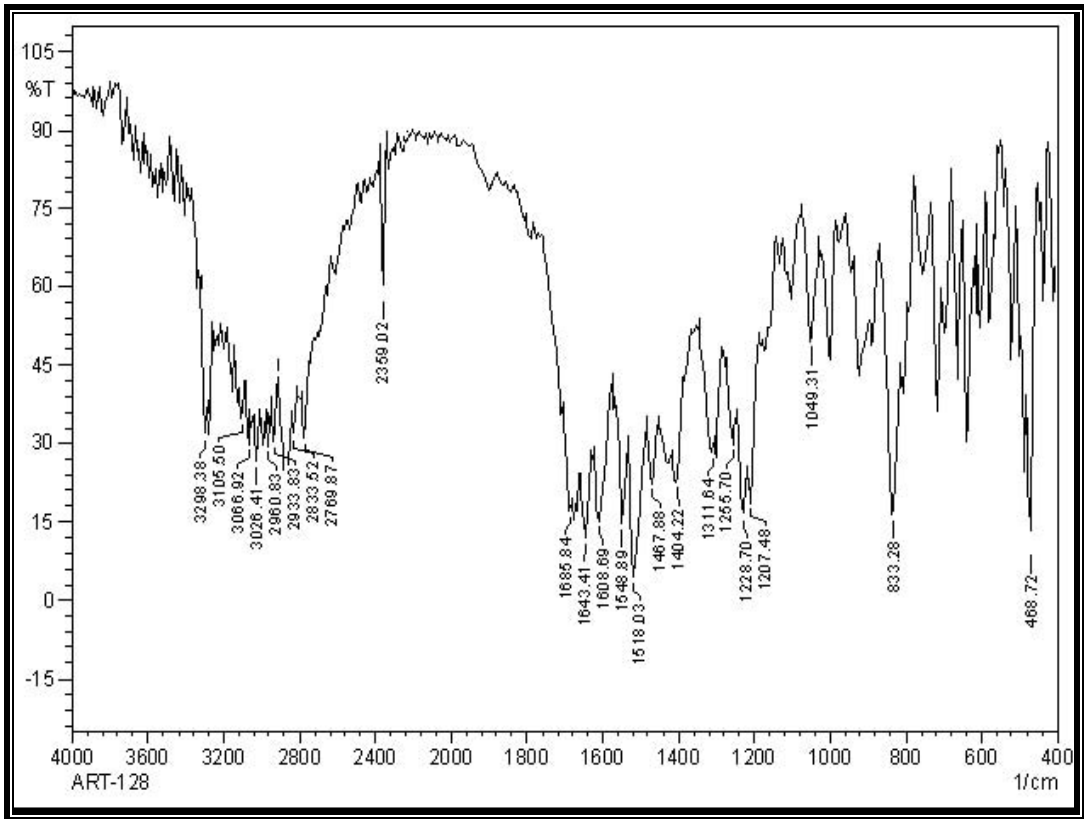
^{1}H NMR spectrum of ART-117



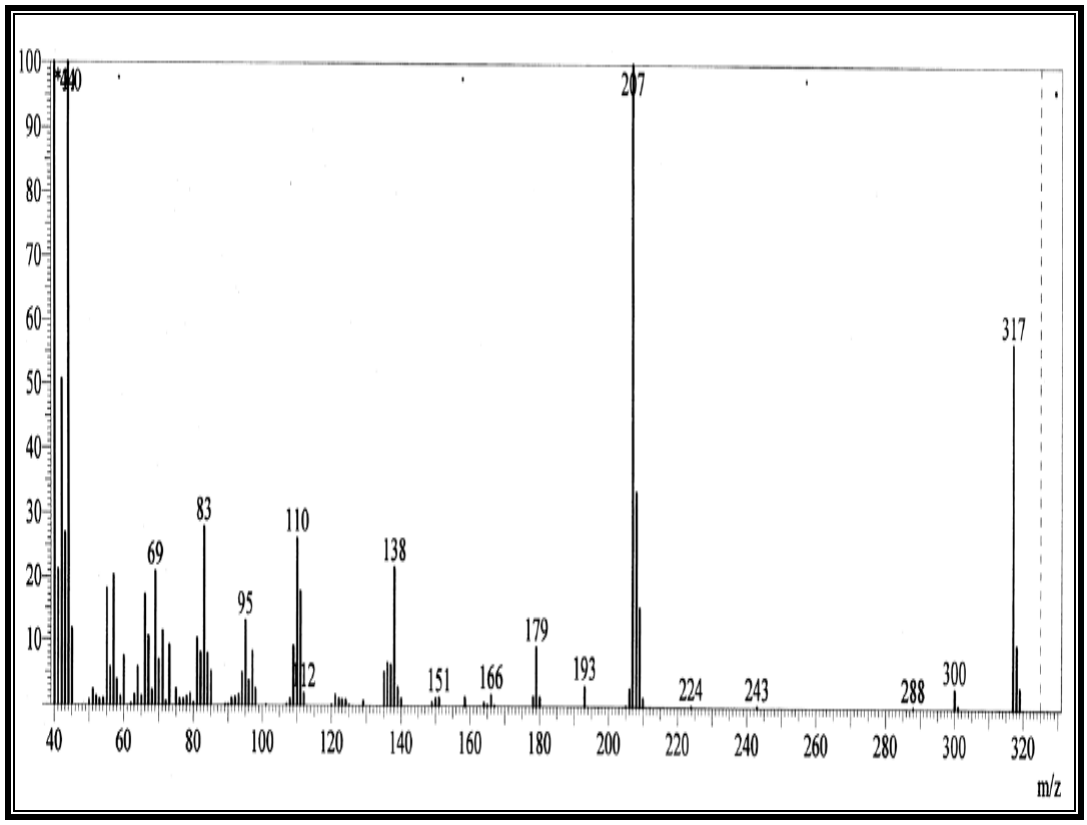
Expanded ^{1}H NMR spectrum of ART-117



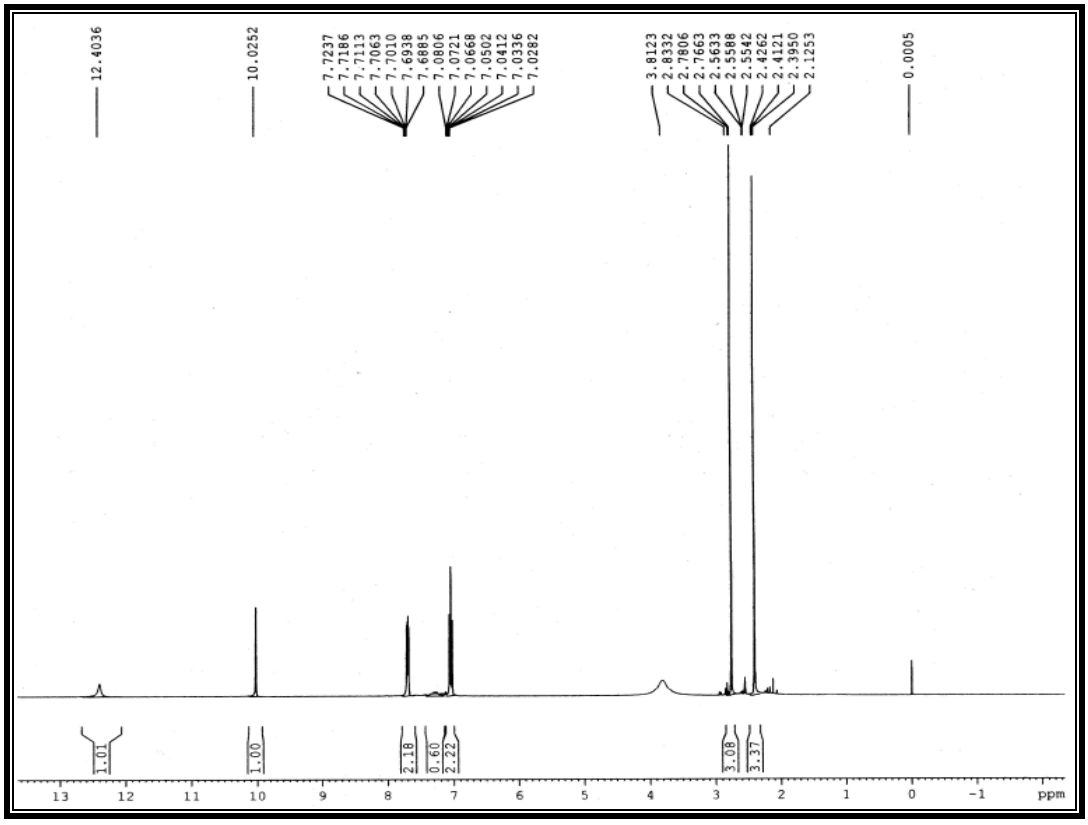
IR spectrum of ART-128



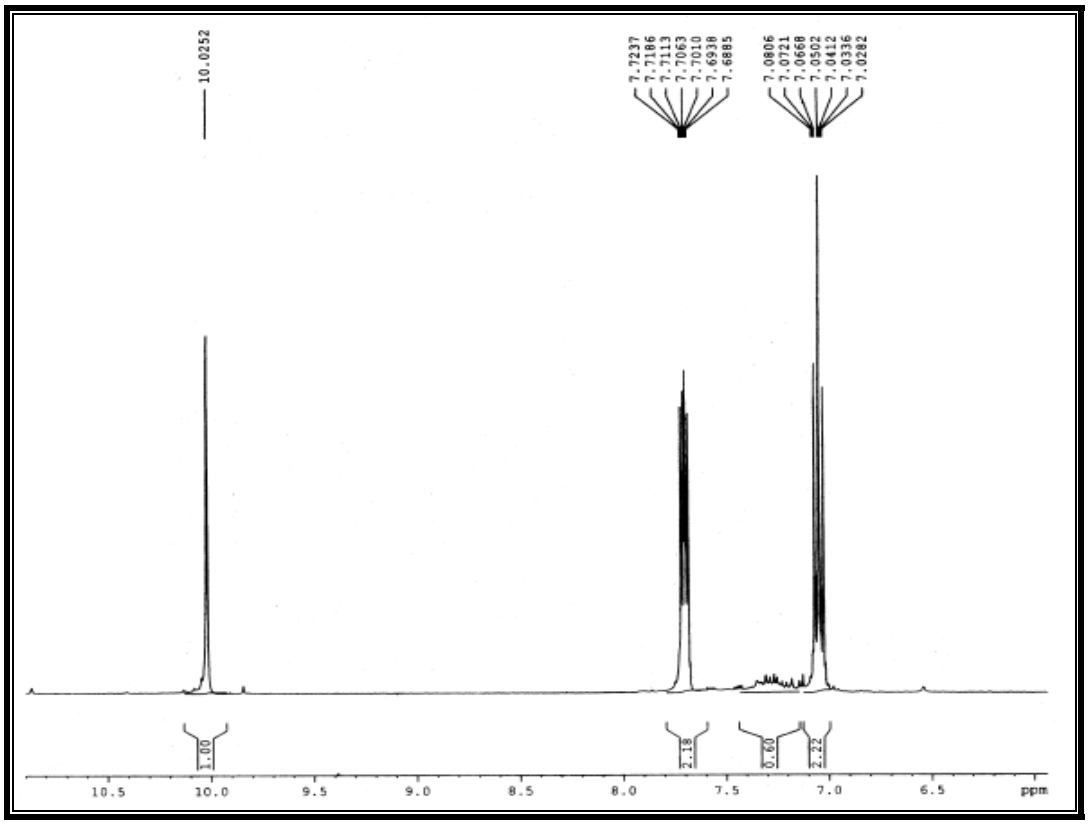
Mass spectrum of ART-128



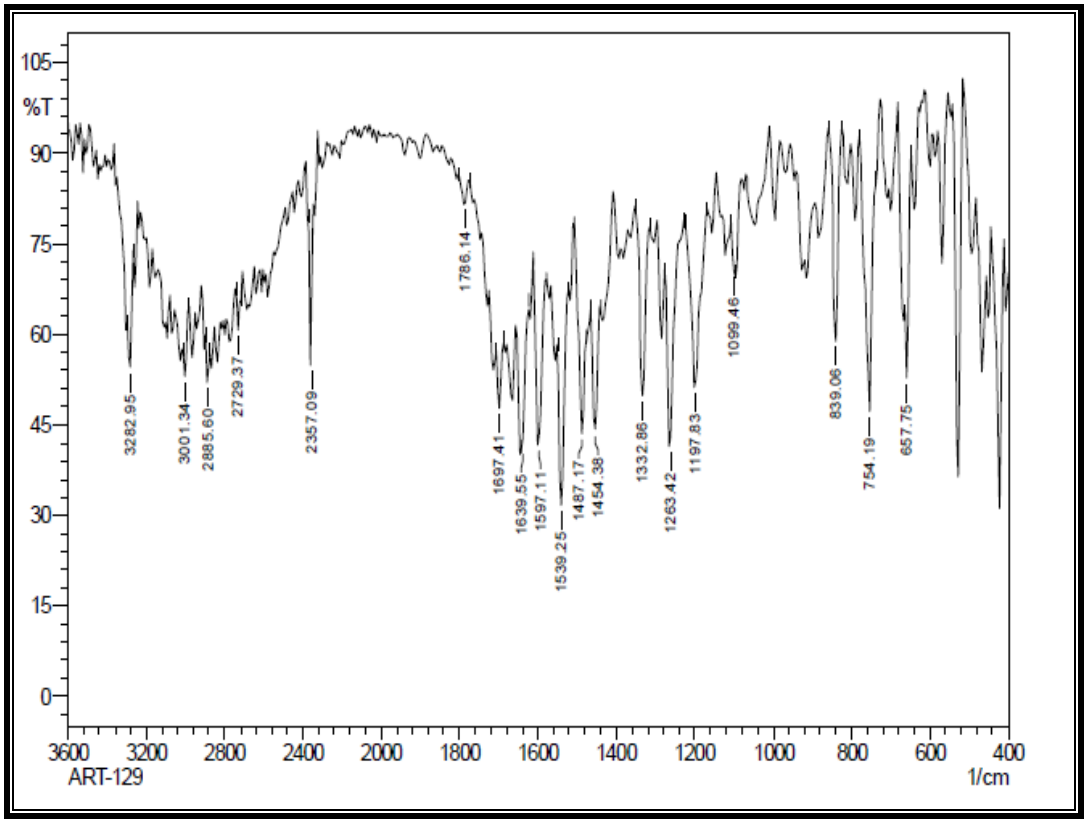
^{1}H NMR spectrum of ART-128



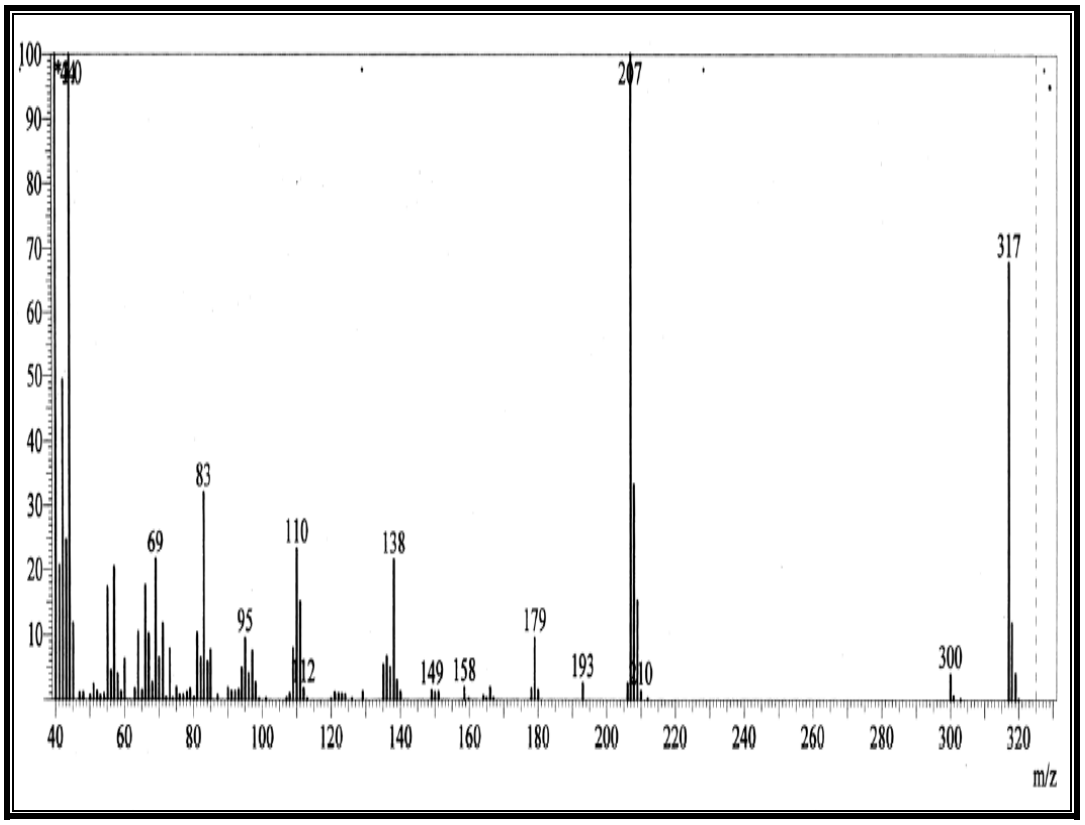
Expanded ^{1}H NMR spectrum of ART-128



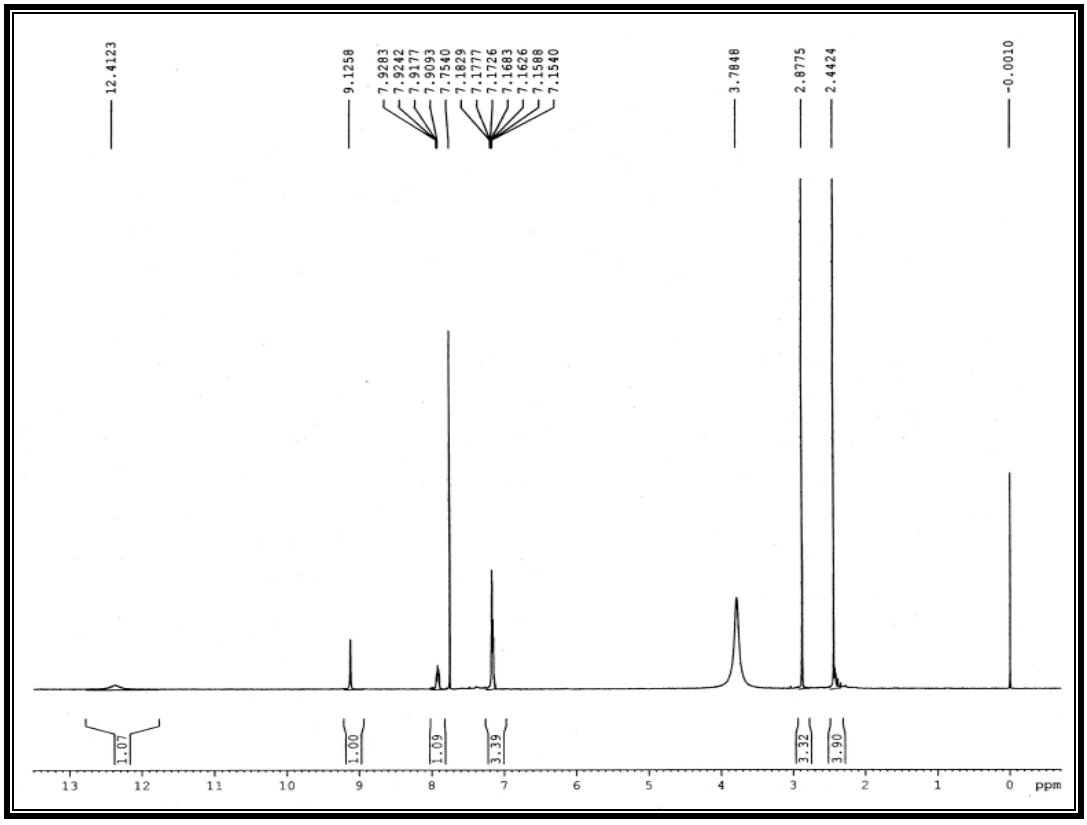
IR spectrum of ART-129



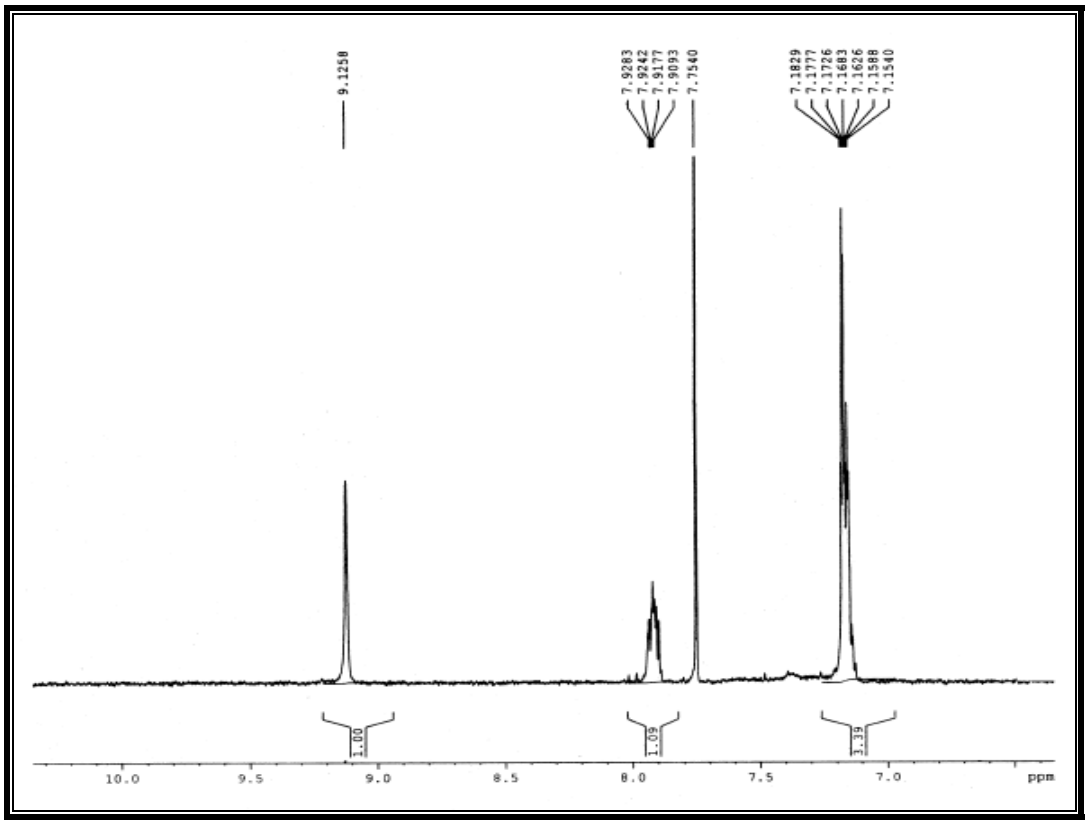
Mass spectrum of ART-129



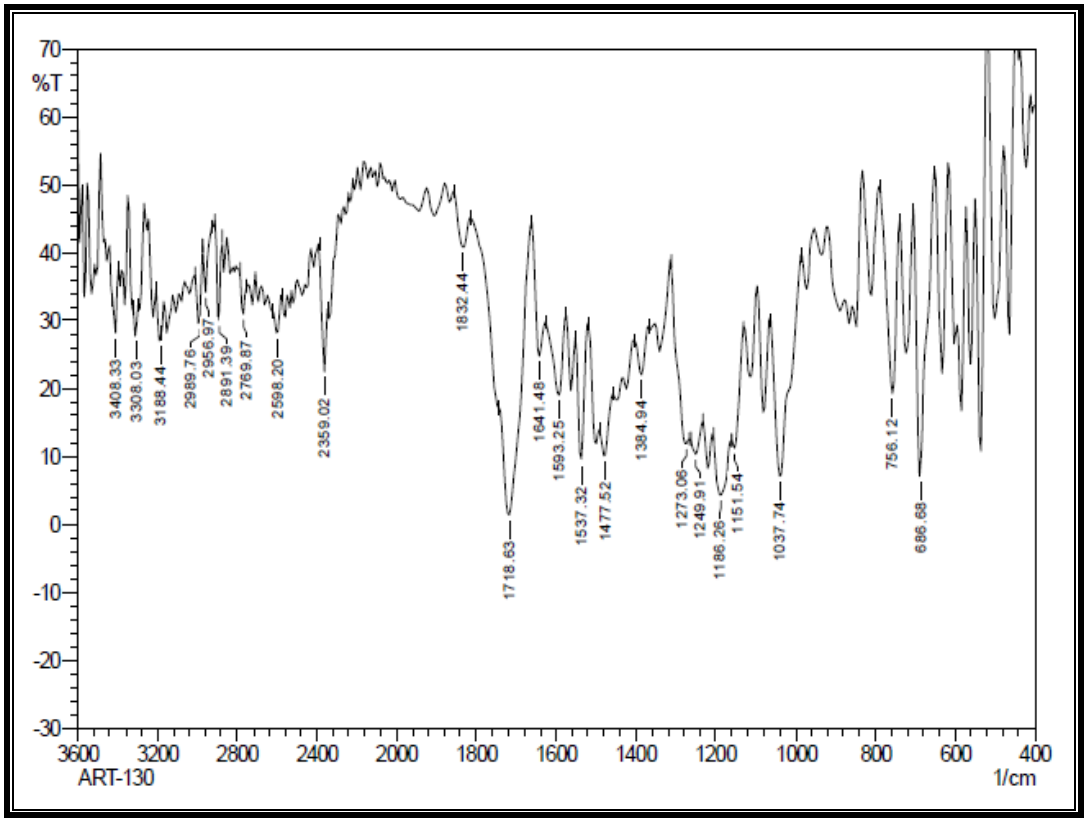
^{1}H NMR spectrum of ART-129



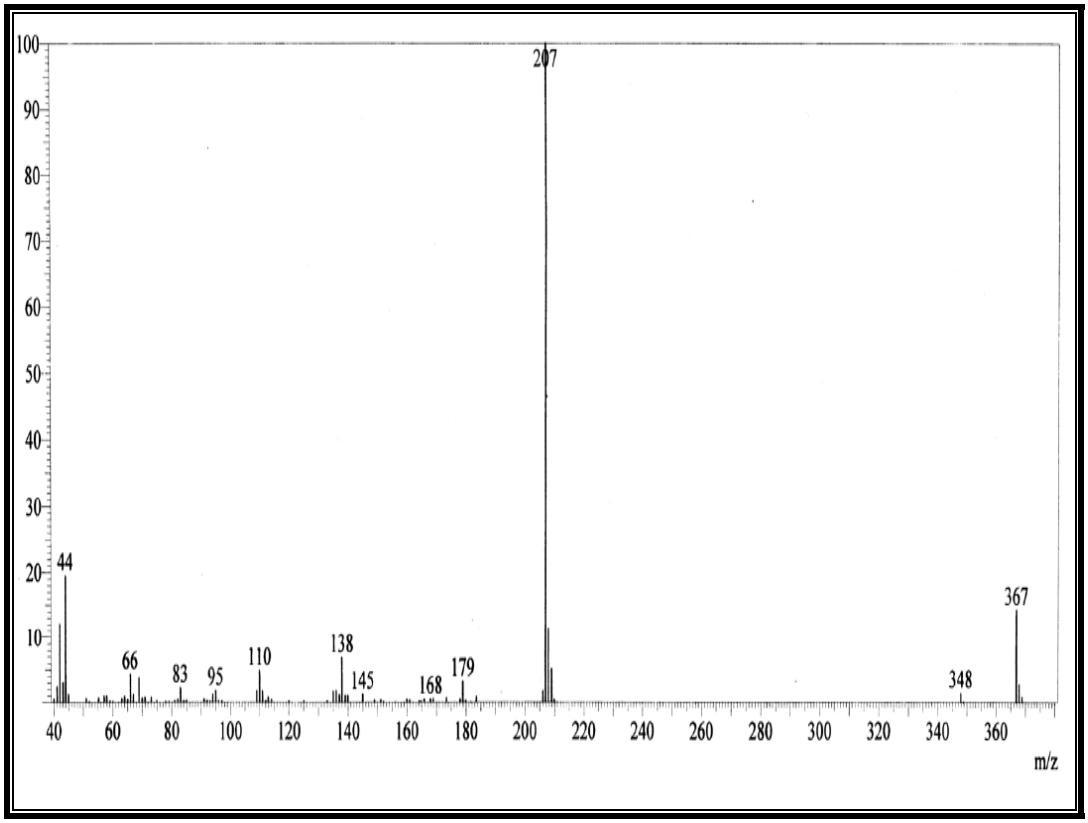
Expanded ^{1}H NMR spectrum of ART-129



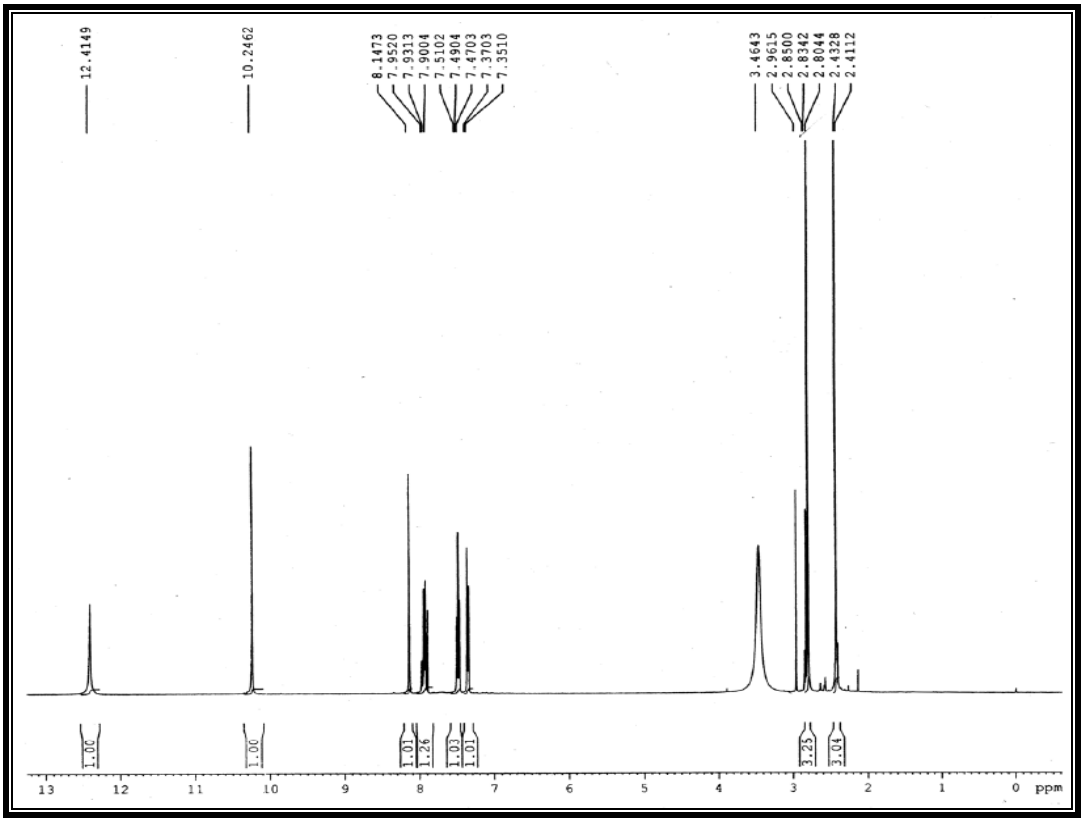
IR spectrum of ART-130



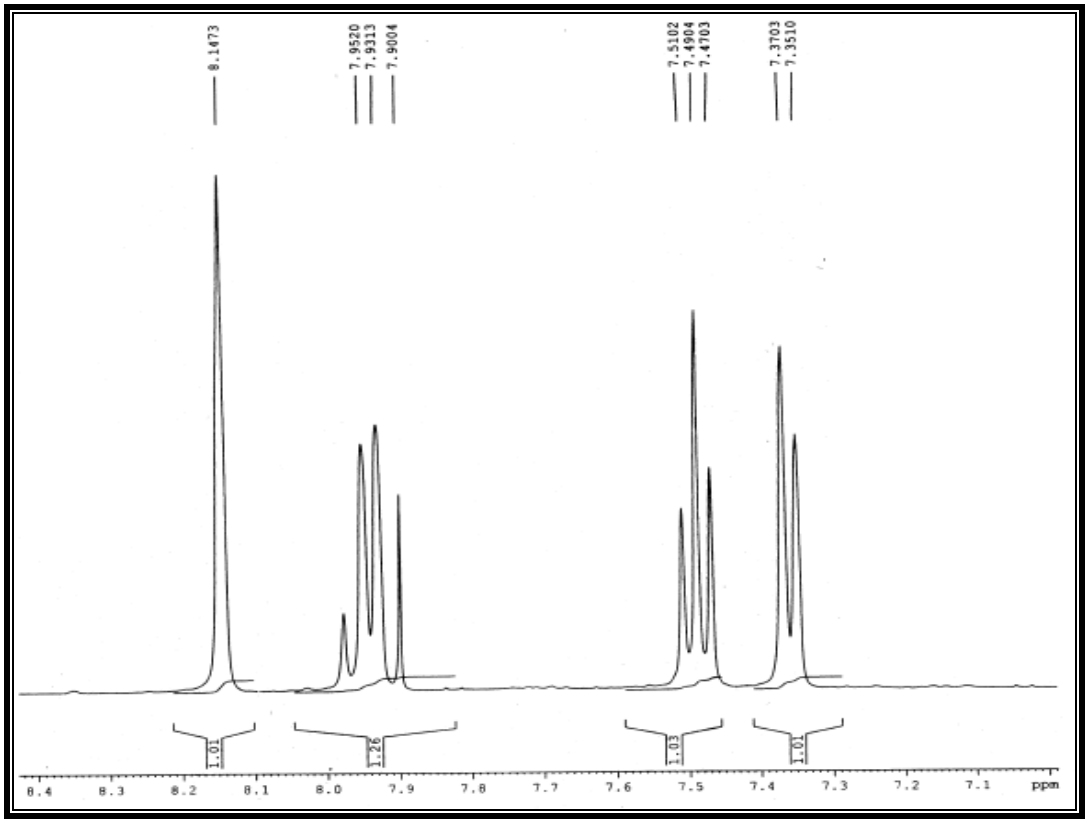
Mass spectrum of ART-130



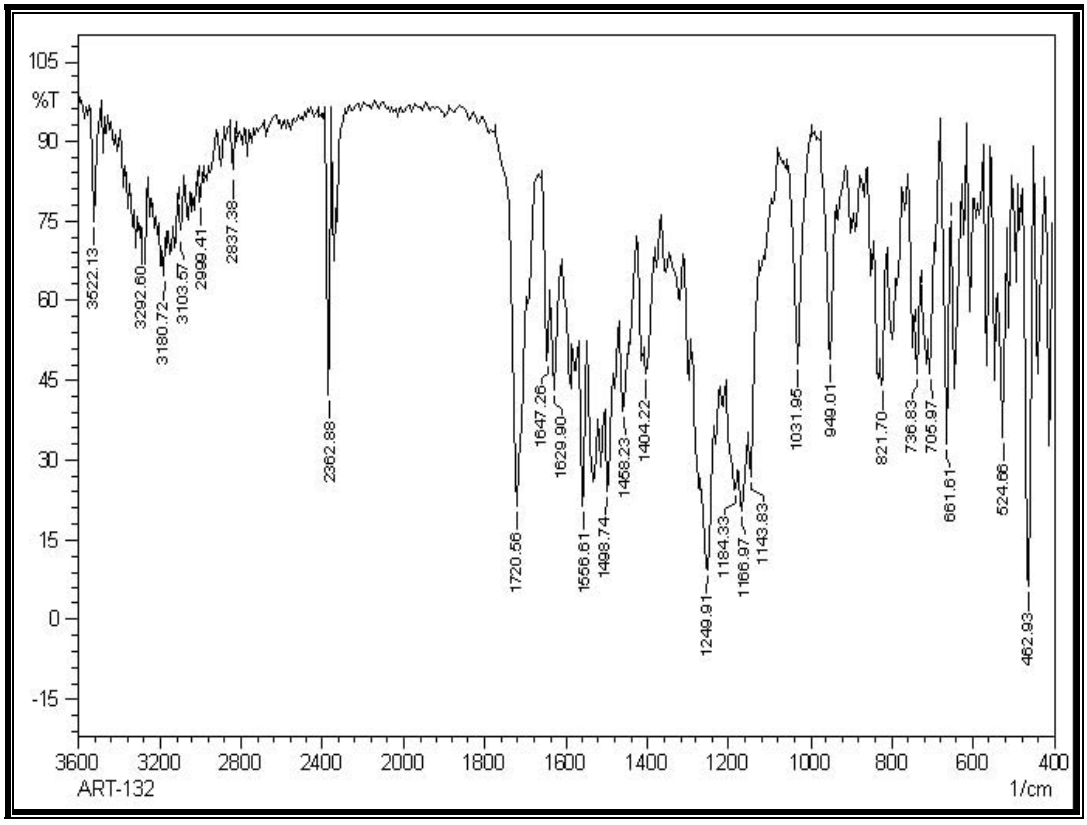
^{1}H NMR spectrum of ART-130



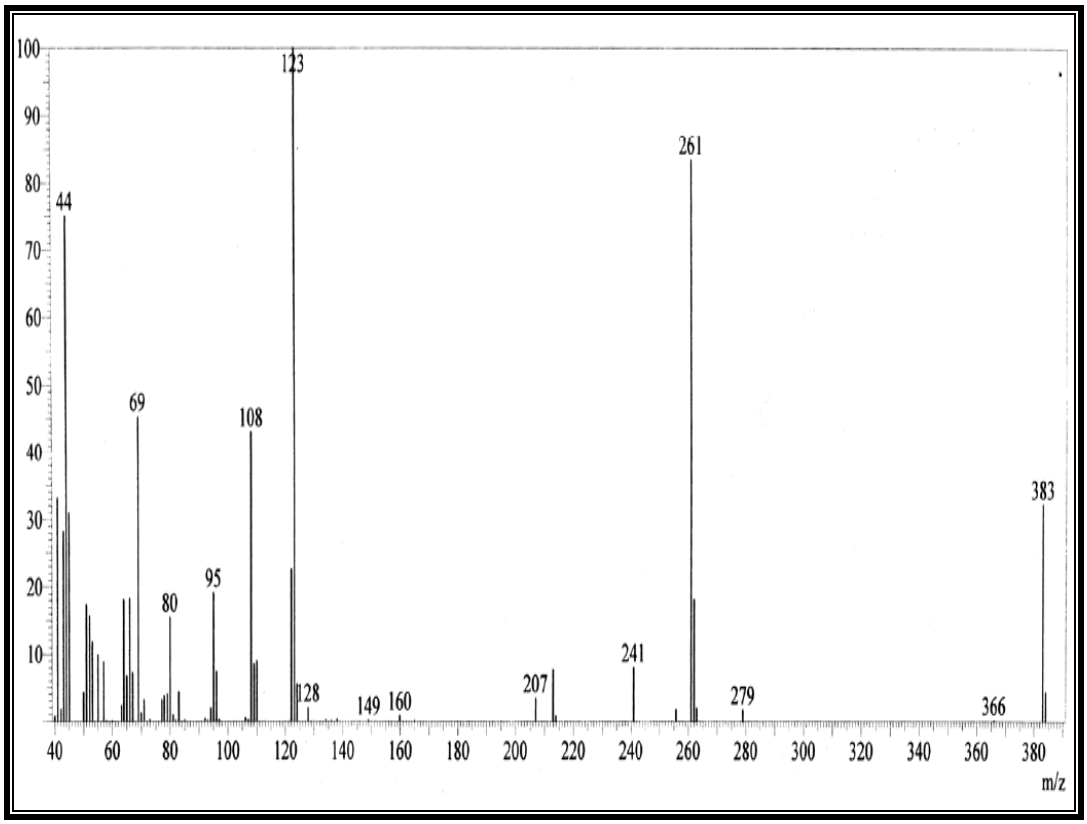
Expanded ^{1}H NMR spectrum of ART-130



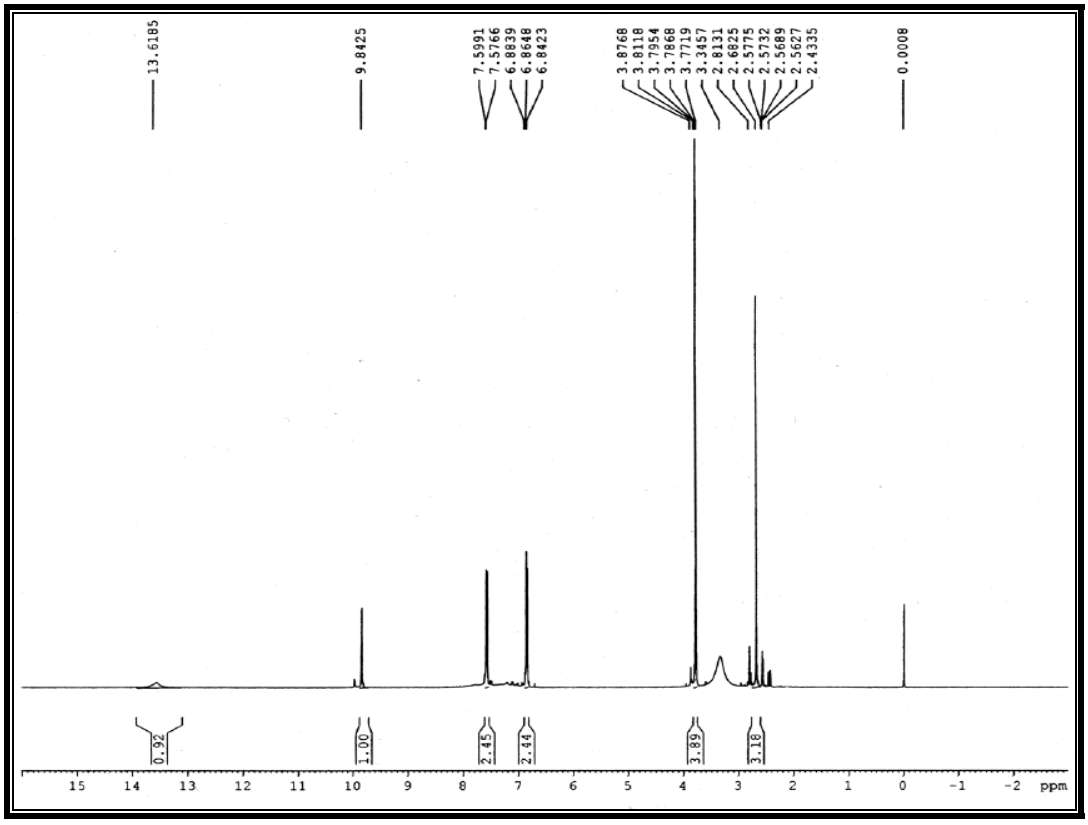
IR spectrum of ART-132



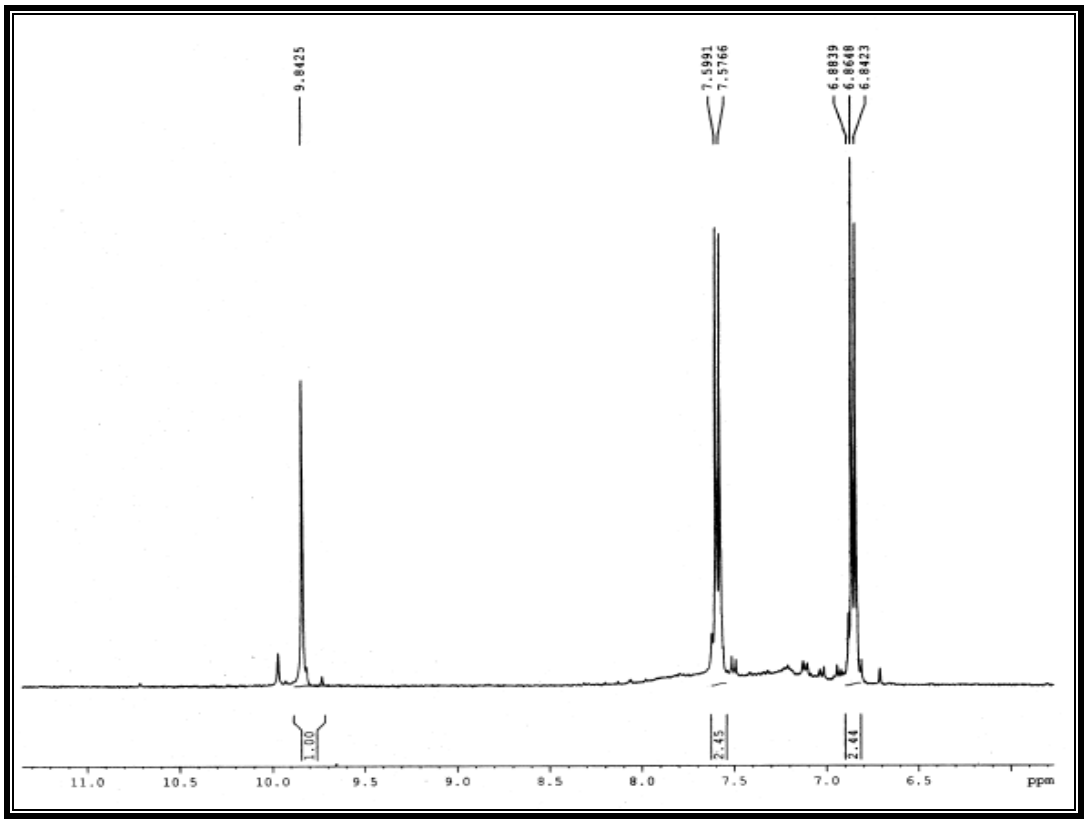
Mass spectrum of ART-132



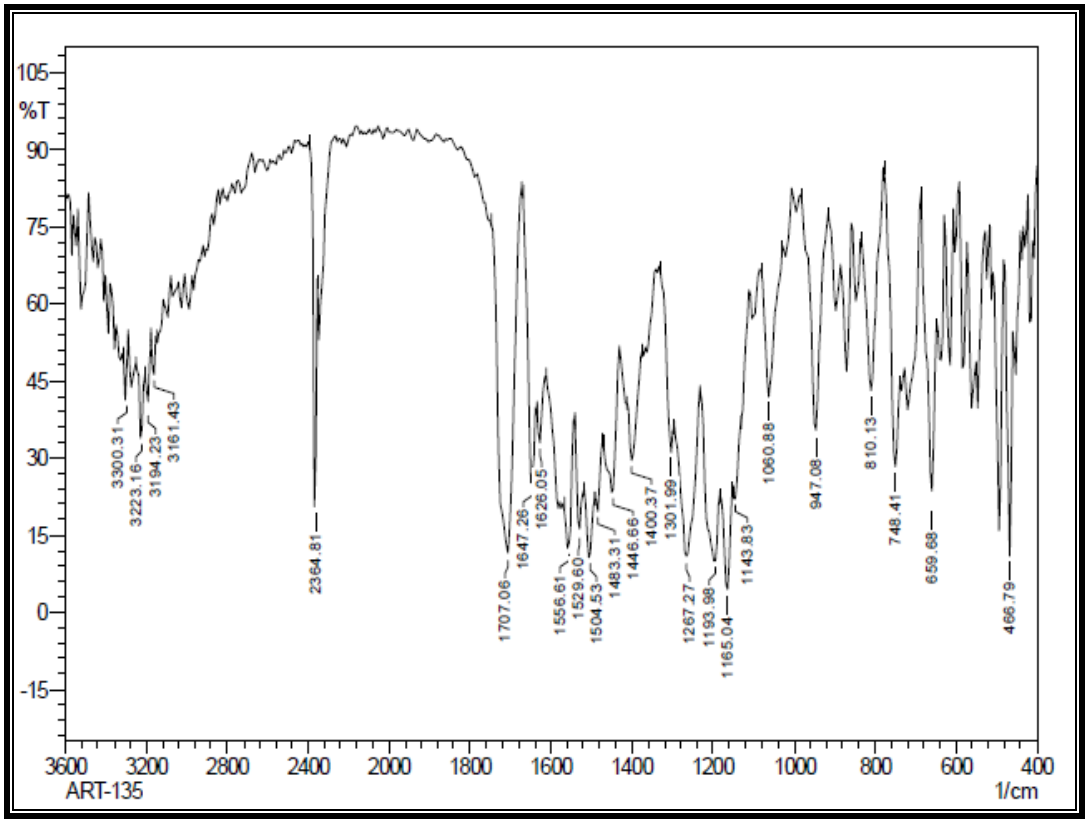
^{1}H NMR spectrum of ART-132



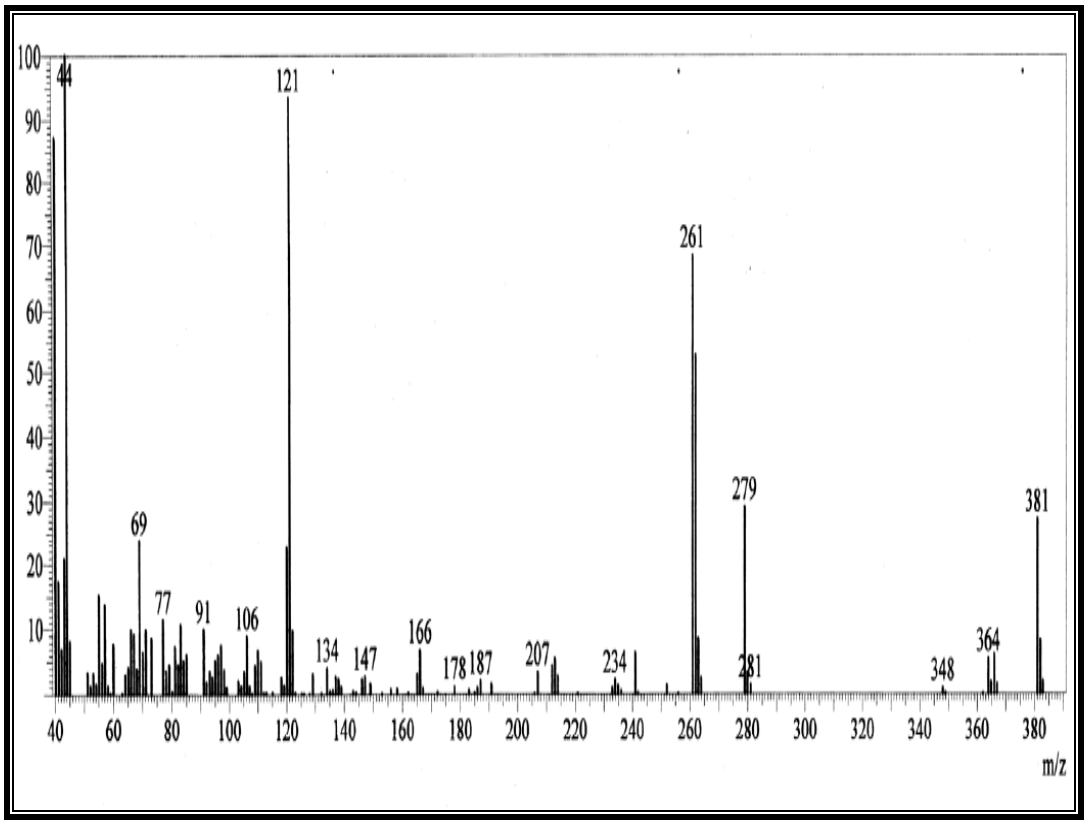
Expanded ^{1}H NMR spectrum of ART-132



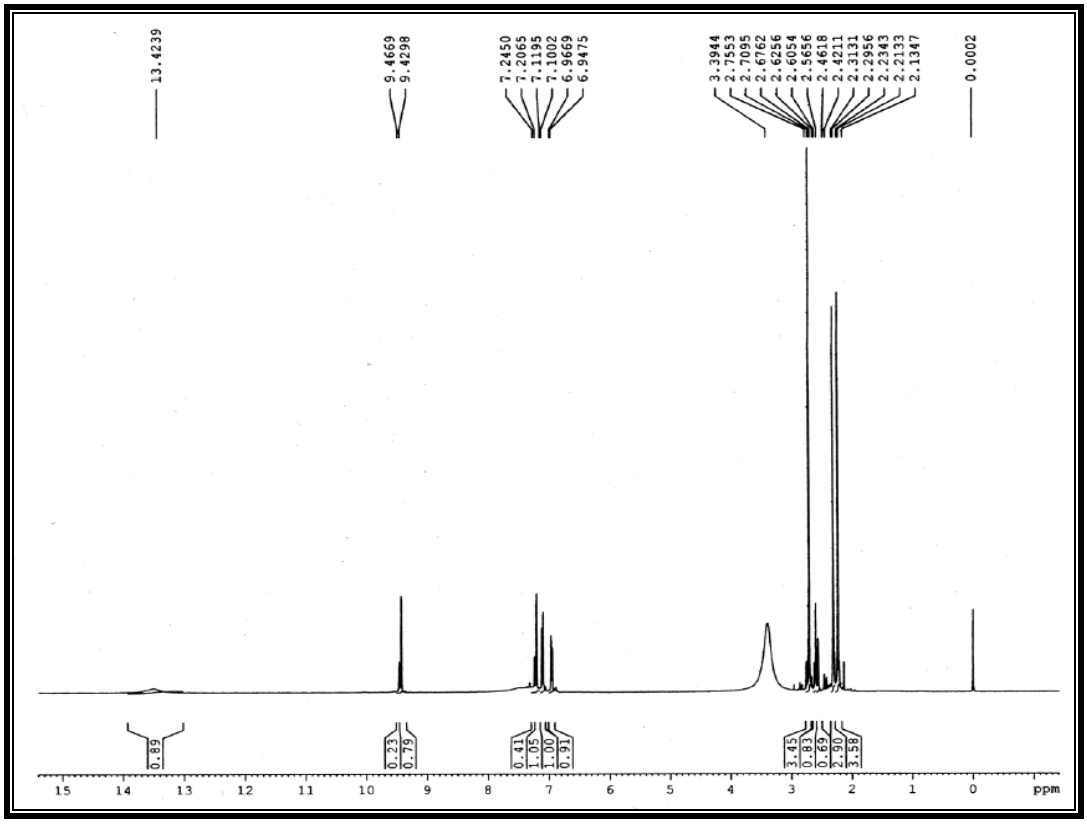
IR spectrum of ART-135



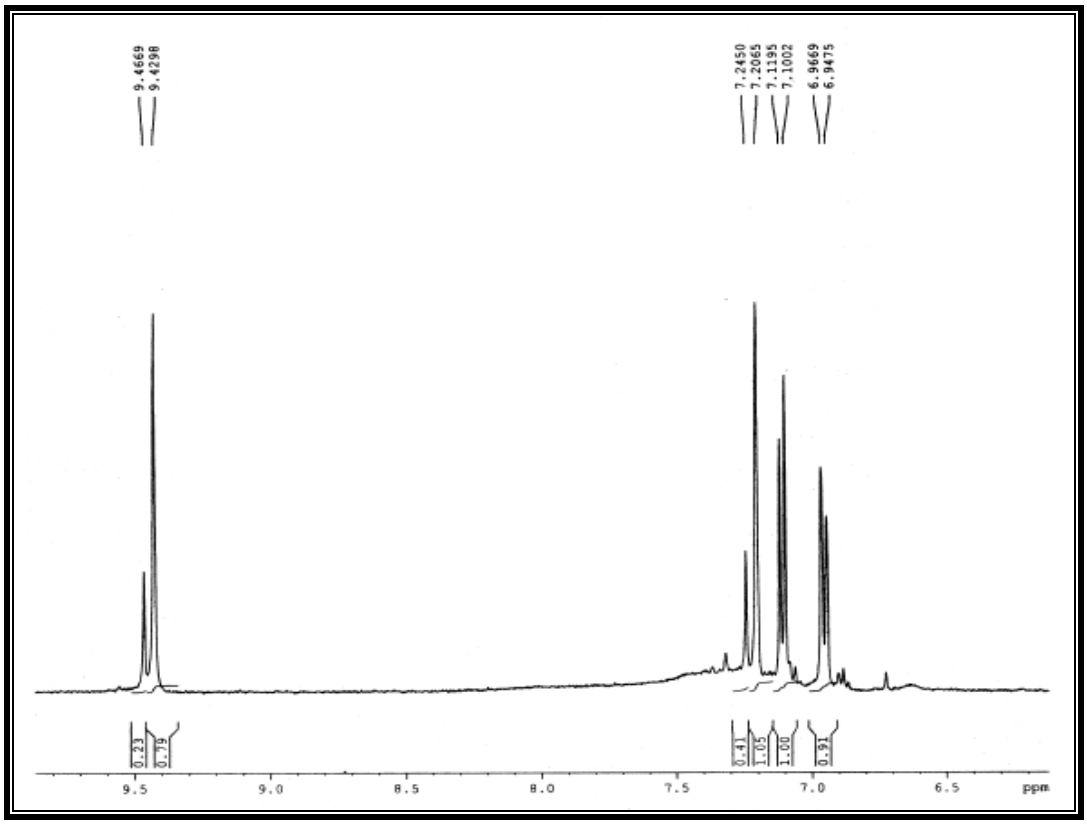
Mass spectrum of ART-135



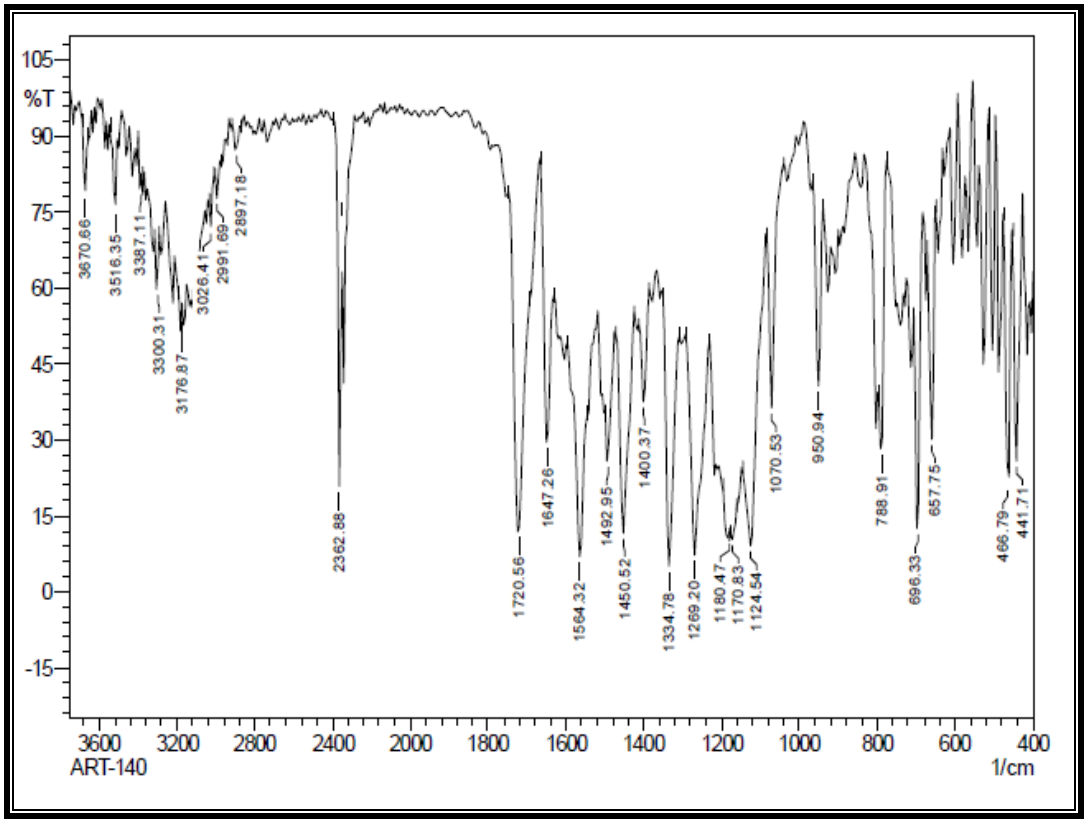
^{1}H NMR spectrum of ART-135



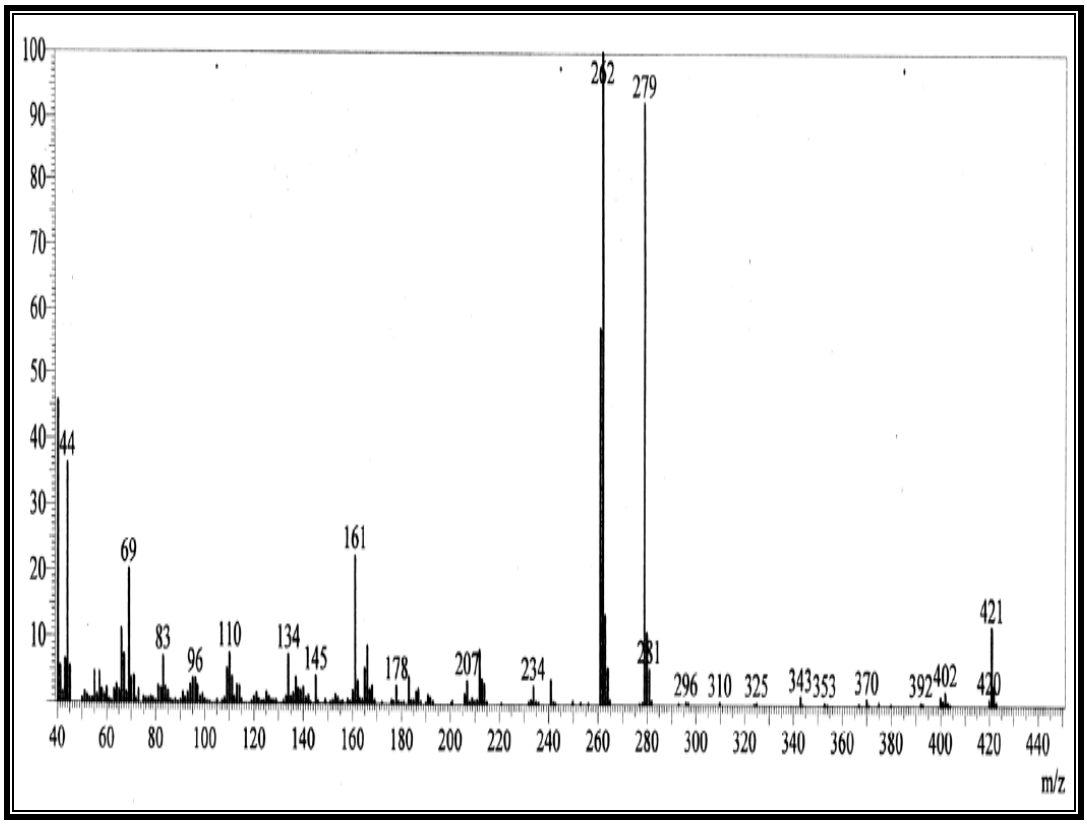
Expanded ^{1}H NMR spectrum of ART-135



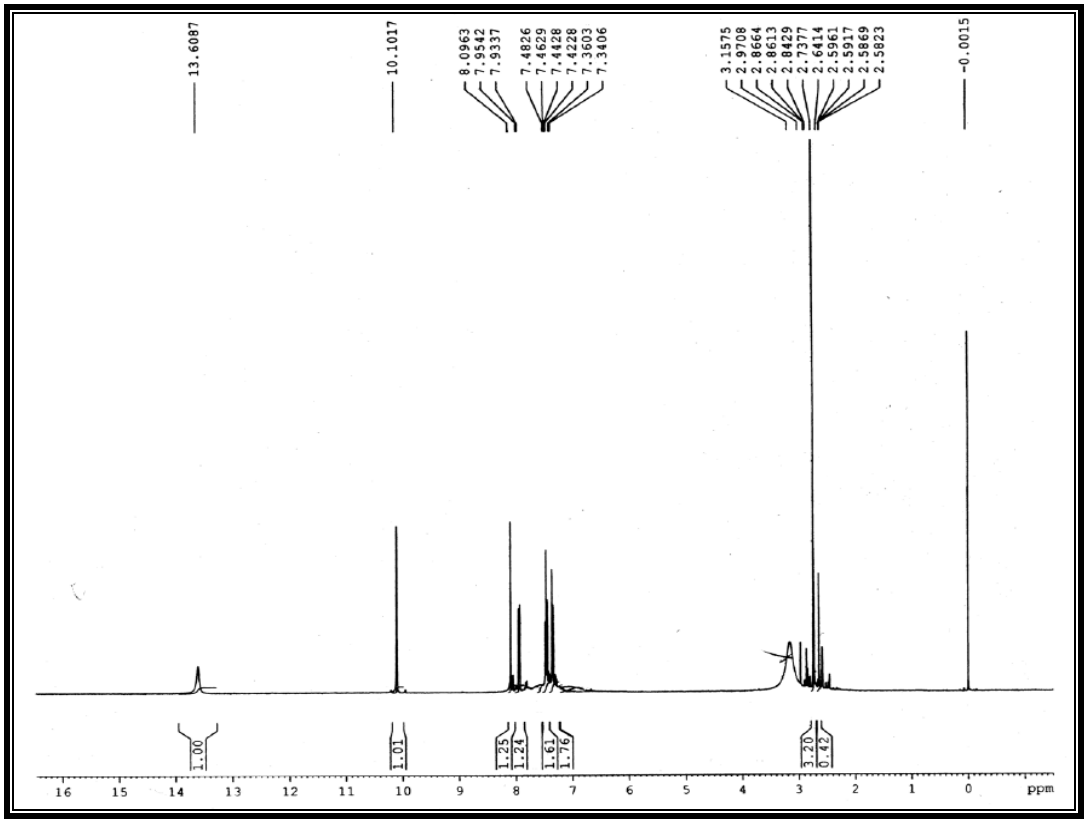
IR spectrum of ART-140



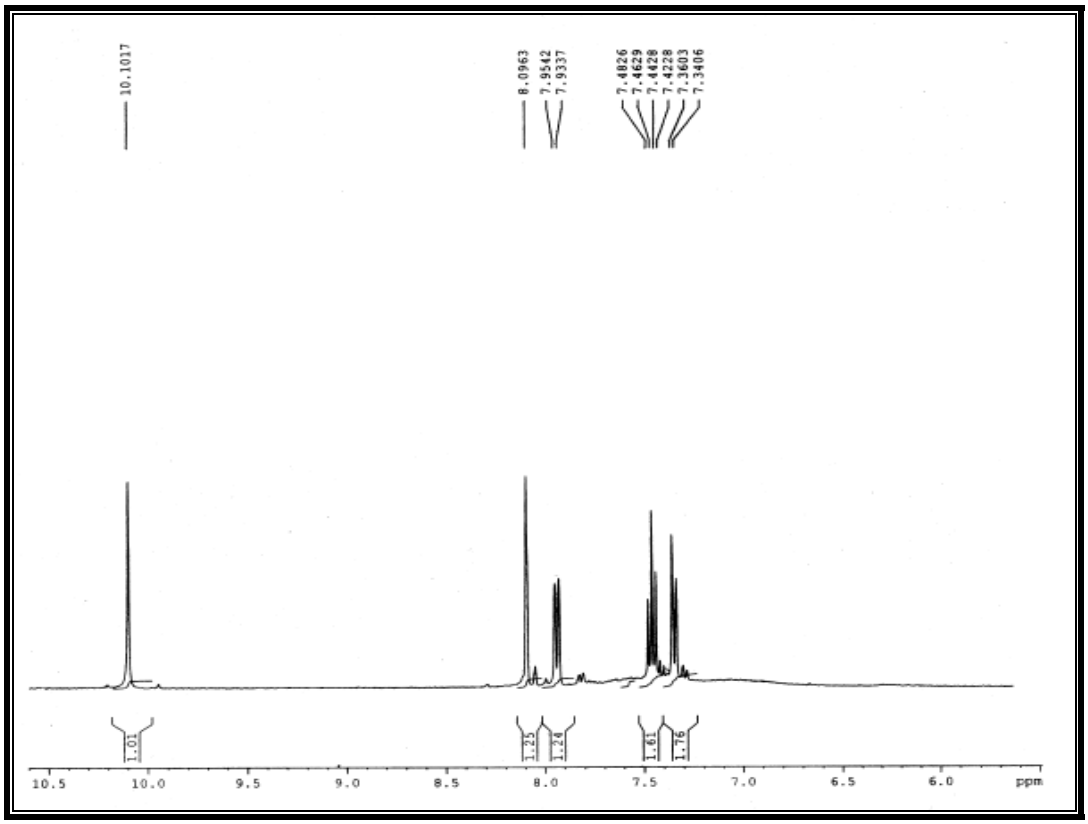
Mass spectrum of ART-140



^{1}H NMR spectrum of ART-140



Expanded ^{1}H NMR spectrum of ART-140



3.8 Biological evaluation

3.8.1 Antimicrobial evaluation

All of the synthesized compounds (ART-101 to 140) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [46-48] 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 ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, 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 microdilution broth method according to NCCLS standards [46]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 μg mL^{-1}. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds ART-101 to 140

Code	Minimum 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.***
ART-101	500	500	500	500	500	500	>1000
ART-102	1000	1000	1000	1000	1000	500	500
ART-103	>1000	500	250	500	1000	250	250
ART-104	100	62.5	125	100	500	500	500
ART-105	1000	1000	1000	1000	1000	>1000	500
ART-106	25	100	200	100	1000	500	500
ART-107	125	200	500	250	500	500	250
ART-108	100	125	100	100	1000	500	500
ART-109	50	100	25	100	>1000	500	1000
ART-110	25	50	100	50	500	500	1000
ART-111	1000	500	100	1000	>1000	500	>1000
ART-112	500	>1000	500	100	500	250	1000
ART-113	500	500	500	1000	>1000	500	125
ART-114	125	100	50	250	250	1000	500
ART-115	500	>1000	500	500	500	500	1000
ART-116	50	125	100	200	>1000	500	>1000
ART-117	125	100	250	125	500	500	>1000
ART-118	125	250	125	50	500	1000	500
ART-119	100	125	100	250	500	1000	>1000
ART-120	50	125	250	100	100	1000	500
ART-121	>1000	1000	500	500	500	1000	500
ART-122	500	500	500	500	500	500	500
ART-123	1000	250	500	1000	500	>1000	500
ART-124	250	125	100	125	1000	500	>1000
ART-125	>1000	500	>1000	>1000	500	500	500
ART-126	125	62.5	125	50	500	1000	>1000
ART-127	250	200	100	125	100	1000	250
ART-128	25	250	125	100	500	1000	>1000
ART-129	250	125	250	125	500	1000	250
ART-130	50	62.5	125	62.5	1000	1000	>1000
ART-131	500	500	500	500	1000	500	>1000
ART-132	>1000	500	500	250	500	500	1000
ART-133	500	500	125	1000	500	100	1000
ART-134	125	125	100	125	250	>1000	500
ART-135	500	1000	1000	>1000	500	100	500
ART-136	100	125	225	50	100	250	1000
ART-137	250	50	125	250	100	1000	1000
ART-138	62.5	250	125	500	500	1000	>1000
ART-139	250	125	125	100	100	1000	100
ART-140	12.5	125	62.5	25	250	500	500
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

3.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds ART-101 to ART-140 is currently under investigation and results are awaited.

3.9 References and notes

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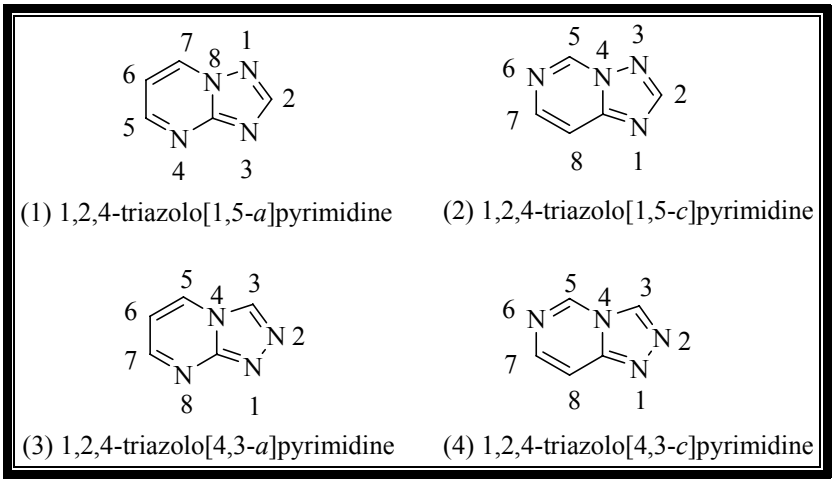
Chapter 4
Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines

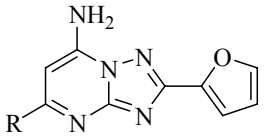
4.1 Introduction

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).

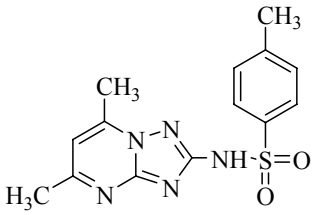


Among these isomeric families of compounds, 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives are thermodynamically more stable and, thus, the most studied ones [1], a few of them being commercially available. Revisions surveying the synthesis, reactivity, spectroscopic characterization and crystallographic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

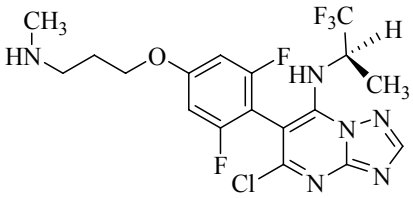
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological 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.



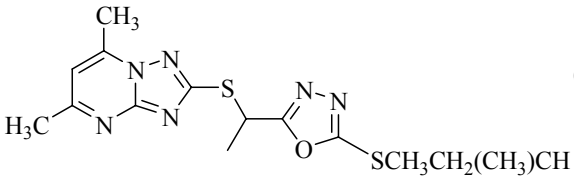
(5) Acticity: A_{2A} adenosine receptor antagonists [11]



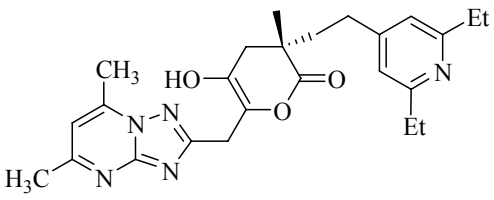
(6) Acticity: latent leishmanicidal activity [12]



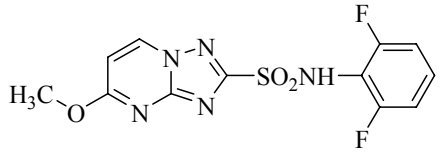
(7) Acticity: Anticancer acticity [13]



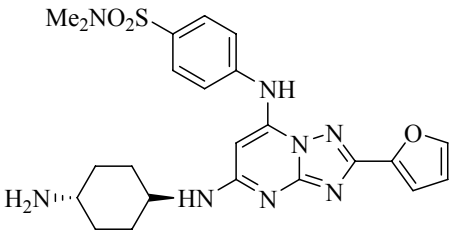
(8) Acticity: antifungal activity [14]



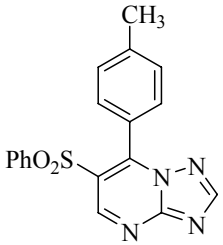
(9) Acticity: Hepatitis C virus polymerase inhibitor [15]



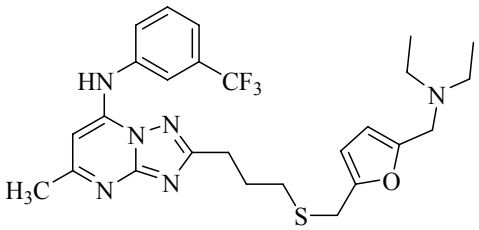
(10) Acticity: Acetohydroxyacid synthase inhibitor [16]



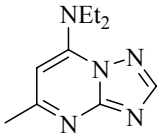
(11) Acticity: CDK2 inhibitors [17]



(12) Acticity: Anti-inflammatory [18]



(13) Acticity: Antiproliferative activity [19]



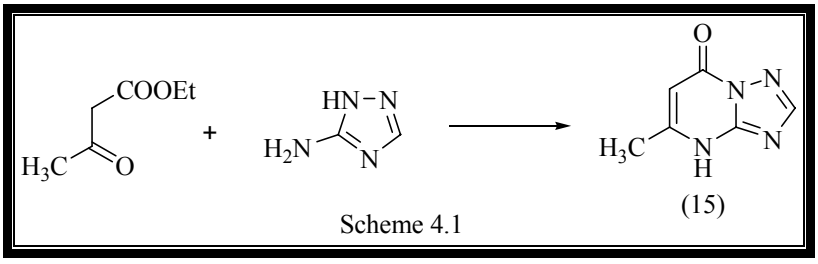
(14) Trapidil: Antiischemic and cardiatonic agent [20]

4.2 Reported synthetic strategies

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

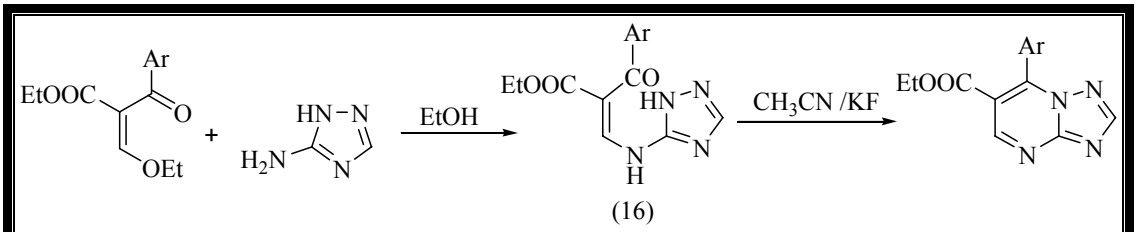
4.2.1.1 Principle and Conditions

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

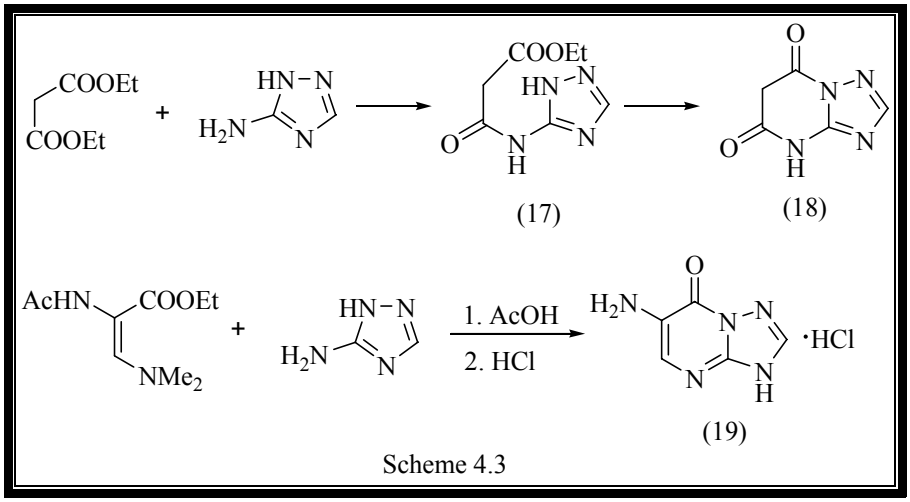
Previous mechanistic conclusions have been confirmed by isolating stable intermediate 5-amino-1,2,4-triazole derivatives such as enamine (16) (Scheme 4.2) on reacting 5-amino-1,2,4-triazoles with 3-ketovinyl ethers [27], 3-ketoenamines [28], 3-ketoaldehydes [29], enamine-2-carboxylic esters [30] or ethoxymethylene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

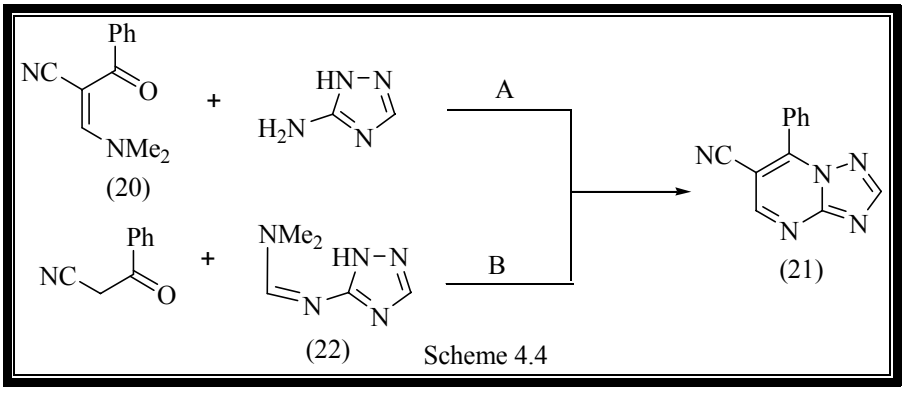
cyclization (or the one-step reaction, if the intermediate is not trapped) requires stronger means (e.g., PPA or boiling acetic acid). Under extreme conditions, triazolylamide (17) was subject to flash vacuum pyrolysis between 300 and 450 ºC to give about 50% triazolo[1,5-a]pyrimidine (18) (Scheme 4.3) [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].

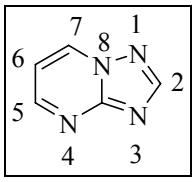


Table 1. Syntheses of triazolo[1,5-a]pyrimidines from 1,3-bifunctional synthons and 5-amino-1,2,4-triazoles

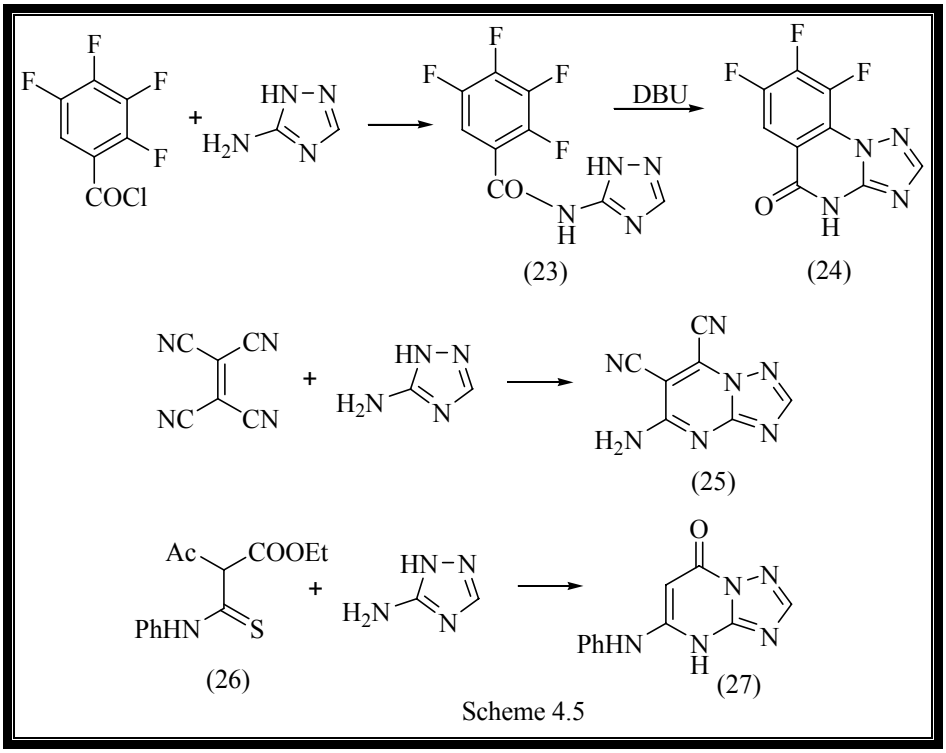
Bifunctional Synthons	R-5[b]	R-7[b]	Bifunctional Synthons	R-5[b]	R-7[b]
1,3-Dialdehyde [42]	H	H	Enamine-2-carboxylate [59]	H	OH
2-Formylacetal [43]	H	H	Acetylenedicarboxylate [60]	CO_2Me	OH
1,3-Diacetal [44]	H	H	3-Ketocarboxylate [61]	R	OH
2-Formylvinyl ether [45]	H	H	3-Alkoxyacrylate [62]	OH	R
2-Formylvinylchloride [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].

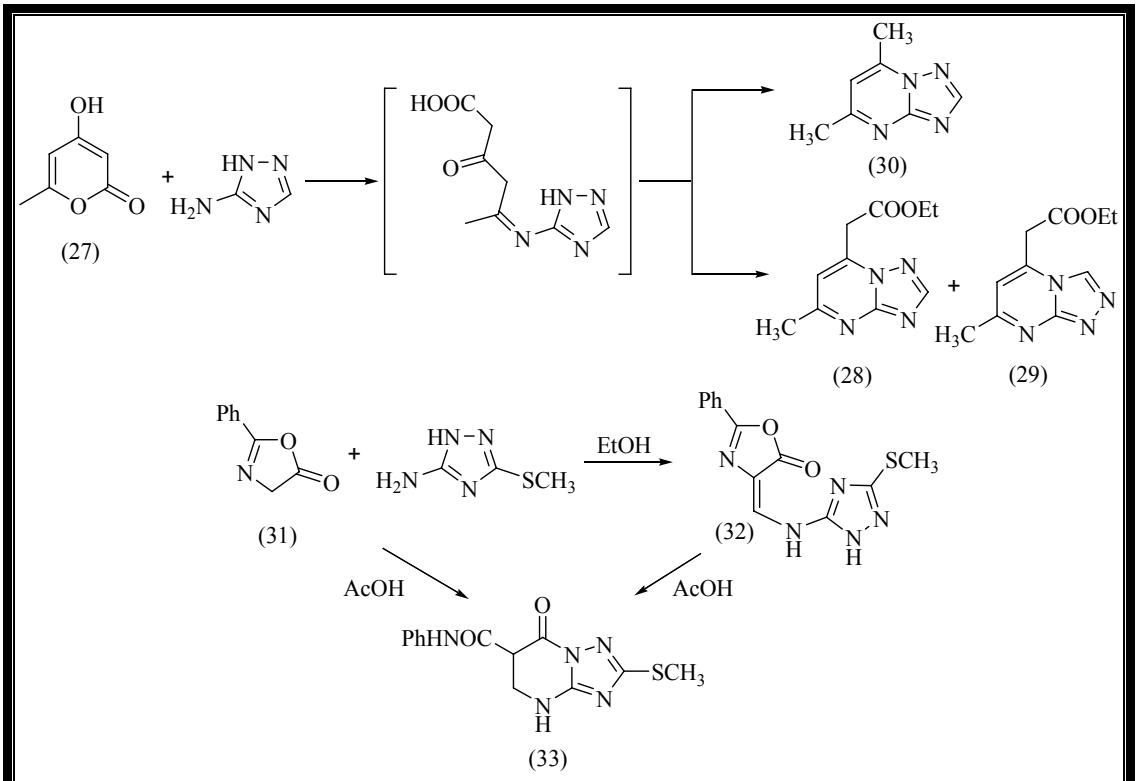


Scheme 4.5

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

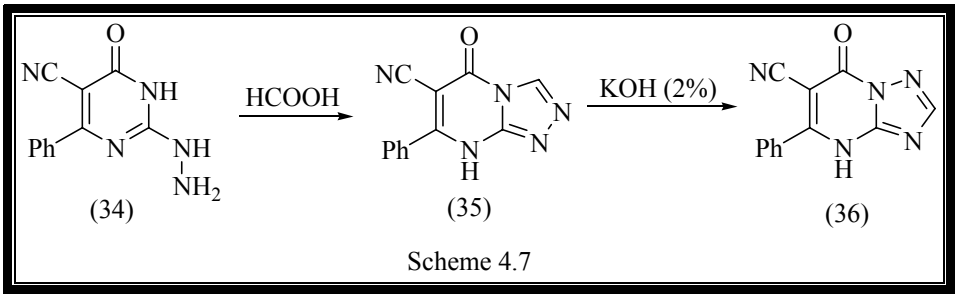
The annulation of pyrimidine onto the triazole ring can be accomplished by the use of heterocyclic precursors that can be regarded as masked 1,3-bifunctional reagents. This way, triacetic acid lactone (27) (Scheme 4.6) reacts as a masked 1,3-diketone and transforms 5-amino-1,2,4-triazole to triazolo[1,5-*a*]pyrimidine (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 TP (33) that, under harsher conditions, directly forms from compound [30].



Scheme 4.6

4.2.3 2-Hydrazinopyrimidines and one-carbon synthons

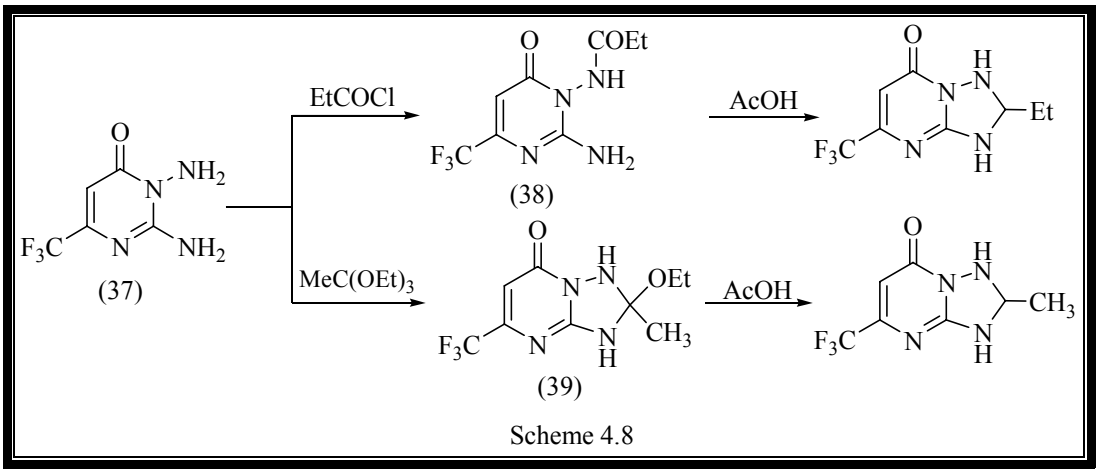
A second common triazolo[1,5-*a*]pyrimidine synthesis consists in the condensation of a C_1-synthon with a 2-hydrazinopyrimidine derivative (e.g., 34, Scheme 4.7). A triazolo[4,3-a]-pyrimidine (35) initially forms that often can be isolated [91]. Harsher conditions allow it to isomerize to the target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

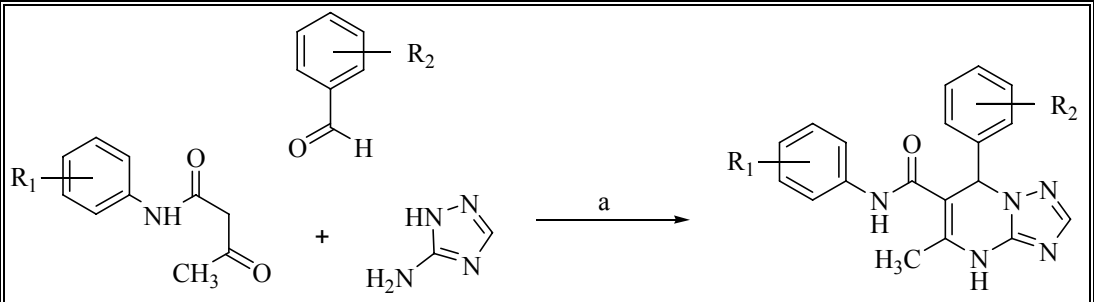
4.3 Current work

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.

One of the synthetic pathways to 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 (ART-201 to ART-240) containing an acetoacetamide fragment. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.

4.4 Reaction Scheme



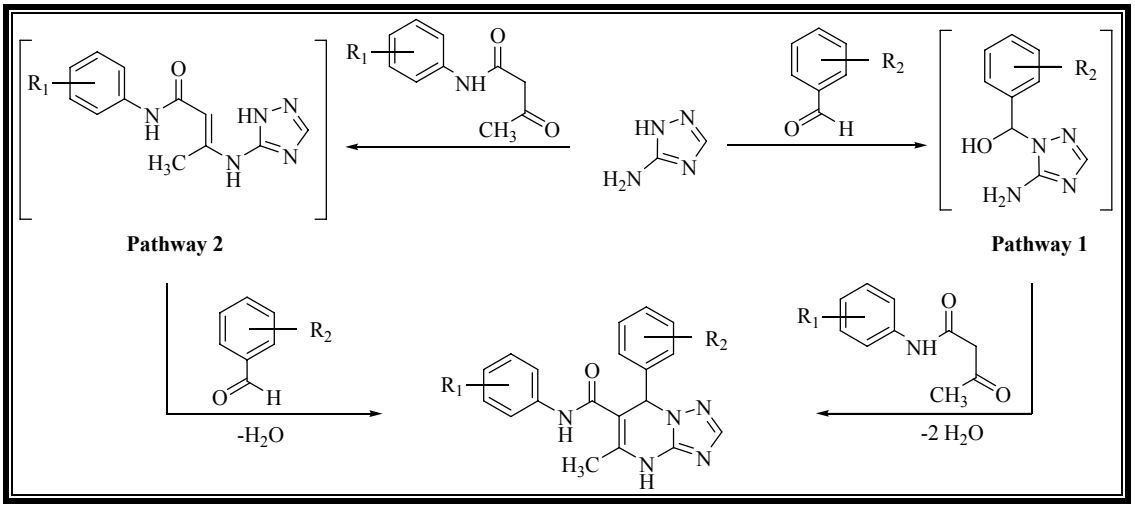
ART-201 T0 240

Reagents and conditions: (a) DMF, Reflux, 12-15 Minutes

Code	R_1	R_2	M.F.	M.W.	M.P. °C	Yield %	R_{f1}	R_{f2}
ART-201	4-OCH_3	4-OCH_3	$C_{21}H_{21}N_5O_3$	391	219-221	78	0.56	0.71
ART-202	4-OCH_3	4-CH_3	$C_{21}H_{21}N_5O_2$	375	179-181	72	0.51	0.69
ART-203	4-OCH_3	4-F	$C_{20}H_{18}FN_5O_2$	379	257-259	82	0.48	0.64
ART-204	4-OCH_3	4-Cl	$C_{20}H_{18}ClN_5O_2$	395	179-181	69	0.50	0.68
ART-205	4-OCH_3	4-NO_2	$C_{20}H_{18}N_6O_4$	406	261-263	80	0.53	0.70
ART-206	4-OCH_3	3-NO_2	$C_{20}H_{18}N_6O_4$	406	183-185	75	0.44	0.74
ART-207	4-OCH_3	3-Cl	$C_{20}H_{18}ClN_5O_2$	395	244-246	69	0.51	0.70
ART-208	4-OCH_3	3,4,5-OCH_3	$C_{23}H_{25}N_5O_5$	451	274-276	76	0.50	0.63
ART-209	4-OCH_3	3,4-OCH_3	$C_{22}H_{23}N_5O_4$	421	169-171	82	0.41	0.62
ART-210	4-OCH_3	2-Cl	$C_{20}H_{18}ClN_5O_2$	395	183-185	70	0.49	0.74
ART-211	4-F	4-OCH_3	$C_{20}H_{18}FN_5O_2$	379	256-258	73	0.52	0.69
ART-212	4-F	4-CH_3	$C_{20}H_{18}FN_5O$	363	227-229	85	0.56	0.68
ART-213	4-F	4-F	$C_{19}H_{15}F_2N_5O$	367	257-259	78	0.50	0.66
ART-214	4-F	4-Cl	$C_{19}H_{15}ClFN_5O$	383	247-249	72	0.52	0.69
ART-215	4-F	4-NO_2	$C_{19}H_{15}FN_6O_3$	394	273-275	76	0.61	0.77
ART-216	4-F	3-NO_2	$C_{19}H_{15}FN_6O_3$	394	242-244	66	0.54	0.61
ART-217	4-F	3-Cl	$C_{19}H_{15}ClFN_5O$	383	233-235	78	0.53	0.71
ART-218	4-F	2-NO_2	$C_{19}H_{15}FN_6O_3$	394	196-198	71	0.64	0.78
ART-219	4-F	2-Cl	$C_{19}H_{15}ClFN_5O$	383	207-209	65	0.48	0.62
ART-220	4-F	3,4-OCH_3	$C_{21}H_{20}FN_5O_3$	409	217-219	79	0.61	0.72
ART-221	3-CF_3	4-OCH_3	$C_{21}H_{18}F_3N_5O_2$	429	208-210	68	0.45	0.56
ART-222	3-CF_3	4-CH_3	$C_{21}H_{18}F_3N_5O$	413	233-235	74	0.55	0.62
ART-223	3-CF_3	4-F	$C_{20}H_{15}F_4N_5O$	417	215-217	82	0.52	0.70
ART-224	3-CF_3	4-Cl	$C_{20}H_{15}ClF_3N_5O$	433	248-250	71	0.50	0.58
ART-225	3-CF_3	4-NO_2	$C_{20}H_{15}F_3N_6O_3$	444	253-255	79	0.58	0.73
ART-226	3-CF_3	3-NO_2	$C_{20}H_{15}F_3N_6O_3$	444	236-238	68	0.43	0.55
ART-227	3-CF_3	3-Cl	$C_{20}H_{15}ClF_3N_5O$	433	239-241	66	0.53	0.69
ART-228	3-CF_3	2-NO_2	$C_{20}H_{15}F_3N_6O_3$	444	238-240	72	0.52	0.67
ART-229	3-CF_3	2-Cl	$C_{20}H_{15}ClF_3N_5O$	433	258-260	67	0.53	0.61
ART-230	3-CF_3	3,4-OCH_3	$C_{22}H_{20}F_3N_5O_3$	459	220-222	68	0.48	0.68
ART-231	2-F	4-OCH_3	$C_{20}H_{18}FN_5O_2$	379	228-230	75	0.51	0.59
ART-232	2-F	4-CH_3	$C_{20}H_{18}FN_5O$	363	229-231	76	0.45	0.63
ART-233	2-F	4-F	$C_{19}H_{15}F_2N_5O$	367	281-283	71	0.47	0.60
ART-234	2-F	4-Cl	$C_{19}H_{15}ClFN_5O$	383	276-278	81	0.57	0.64
ART-235	2-F	4-NO_2	$C_{19}H_{15}FN_6O_3$	394	277-279	82	0.49	0.67
ART-236	2-F	3-NO_2	$C_{19}H_{15}FN_6O_3$	394	259-261	74	0.52	0.65
ART-237	2-F	3-Cl	$C_{19}H_{15}ClFN_5O$	383	256-258	72	0.48	0.55
ART-238	2-F	2-NO_2	$C_{19}H_{15}FN_6O_3$	394	275-277	78	0.43	0.59
ART-239	2-F	2-Cl	$C_{19}H_{15}ClFN_5O$	383	221-223	68	0.50	0.68
ART-240	2-F	3,4-OCH_3	$C_{21}H_{20}FN_5O_3$	409	256-258	72	0.54	0.64

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

4.5 Reaction Mechanism



The reaction mechanism of this three-component condensation is probably similar to the described [94] mechanism for the "classical" Biginelli reaction (Pathway 1). The first step is a nucleophilic addition of N_2 of the aminoazole to a carbonyl carbon of aldehyde, followed by subsequent cyclization with acetoacetamide to form the dihydropyrimidine ring. An alternate sequence is also possible and cannot be excluded [95] (Pathway 2), which is the initial formation of an enamine by reaction of aminoazole with the acetoacetamide followed by cyclocondensation. The third alternative involving the formation of 2-benzylidene-*N*-aryl-3-oxobutanamide derivatives as intermediates requires the presence of a strong base [96] and is most likely not possible for the case described herein.

4.6 Experimental

4.6.1 Materials and Methods

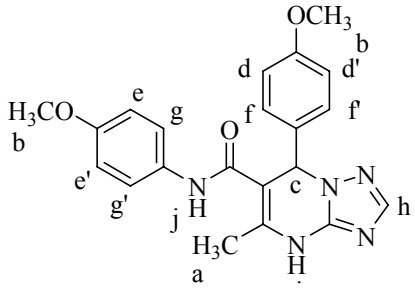
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra 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 agreements with the structures assigned.

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

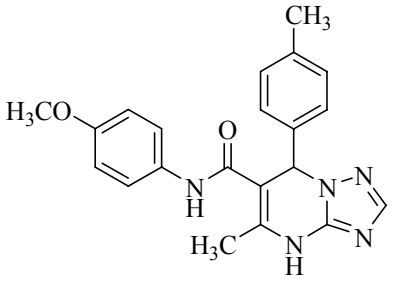
Synthesis of *N*-(aryl)-3-oxobutanamides was achieved using previously published methods [97].

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

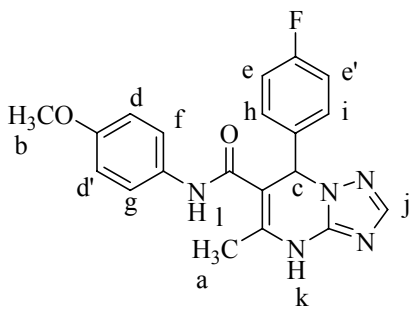
A mixture of the aminoazole (0.01 mol), *N*-(4-methoxyphenyl)-3-oxobutanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of DMF for 12-15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products ART 201-210, which were crystallized from ethanol and subsequently dried in air.

4.6.3.1 7-(4-methoxyphenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-201) 

Yield: 78%; mp 219-221 ºC; IR (cm^{-1}): 3259 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2920 (C-H asymmetrical stretching of CH_3 group), 2875 (C-H asymmetrical stretching of CH_3 group), 1668 (C=O stretching of amide), 1606 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1514 and 1480 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH_3 group), 1410 (C-H symmetrical deformation of CH_3 group), 1330 (C-N stretching), 1247 (C-O-C stretching), 1028 (C-H in plane deformation of aromatic ring), 821 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, H_a), 3.74 (s, 2×3H, H_b), 6.52 (s, 1H, H_c), 6.75-6.77 (d, 2H, $H_{dd'}$, J = 9.14 Hz), 6.80-6.82 (d, 2H, $H_{ee'}$, J = 8.68 Hz), 7.24-7.27 (d, 2H, $H_{ff'}$, J = 8.64 Hz), 7.36-7.38 (d, 2H, $H_{gg'}$, J = 8.96 Hz), 7.56 (s, 1H, H_h), 9.15 (s, 1H, H_i), 10.02 (s, 1H, H_j); MS: *m/z* 391; Anal. Calcd. for $C_{21}H_{21}N_5O_3$: C, 64.44; H, 5.41; N, 17.89. Found: C, 64.38; H, 5.29; N, 17.75%.

4.6.3.2 7-(4-methylphenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-202) 

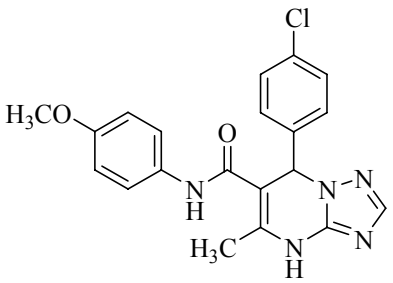
Yield: 72%; mp 179-181 ºC; MS: *m/z* 375; Anal. Calcd. for $C_{21}H_{21}N_5O_2$: C, 67.18; H, 5.64; N, 18.65. Found: C, 67.10; H, 5.54; N, 18.49%.

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

Yield: 82%; mp 257-259 ºC; IR (cm^{-1}): 3269 (N-H stretching of secondary amine), 3024 (C-H stretching of aromatic ring), 2922 (C-H asymmetrical stretching of CH_3 group), 2868 (C-H asymmetrical stretching of CH_3 group),

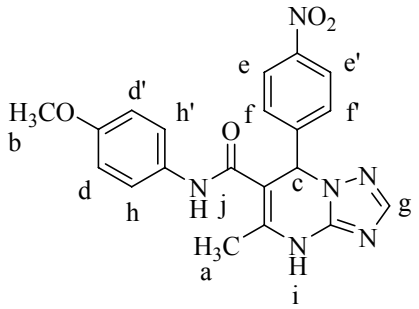
1666 (C=O stretching of amide), 1618 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1510, 1479 and 1442 (C=C stretching of aromatic ring), 1413 (C-H asymmetrical deformation of CH_3 group), 1329 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1247 (C-O-C stretching), 1033 (C-H in plane deformation of aromatic ring), 825 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.28 (s, 3H, H_a), 3.74 (s, 3H, H_b), 6.59 (s, 1H, H_c), 6.76-6.78 (d, 2H, $H_{dd'}$, J = 8.84 Hz), 6.96-7.00 (t, 2H, $H_{ee'}$), 7.32-7.38 (m, 4H, H_{f-i}), 7.57 (s, 1H, H_j), 9.26 (s, 1H, H_k), 10.06 (s, 1H, H_l); MS: *m/z* 379; Anal. Calcd. for $C_{20}H_{18}FN_5O_2$: C, 63.32; H, 4.78; N, 18.46. Found: C, 63.32; H, 4.78; N, 18.46%.

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



Yield: 69%; mp 179-181 ºC; MS: *m/z* 395; Anal. Calcd. for $C_{20}H_{18}ClN_5O_2$: C, 60.68; H, 4.58; N, 17.69. Found: C, 60.61; H, 4.50; N, 17.54%.

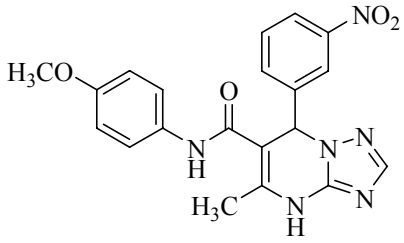
4.6.3.5 7-(4-nitrophenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-205)



Yield: 80%; mp 261-263 ºC; IR (cm^{-1}): 3217 (N-H stretching of secondary amine), 3045 (C-H stretching of aromatic ring), 2964 (C-H asymmetrical stretching of CH_3 group), 2872 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1595 (C=N stretching of triazole ring), 1516 (N-H deformation of pyrimidine ring), 1440, 1400 (C=C stretching of aromatic ring), 1411 (C-H asymmetrical deformation of CH_3 group), 1344 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1247 (C-O-C stretching), 1033 (C-H in plane deformation of aromatic ring), 819 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, H_a), 3.74 (s, 3H, H_b), 6.72 (s, 1H, H_c), 6.76-6.78 (d, 2H, $H_{dd'}$, J = 8.96 Hz Hz), 7.36-7.39 (d, 2H, $H_{ee'}$, J = 8.96 Hz), 7.50-7.52 (d,

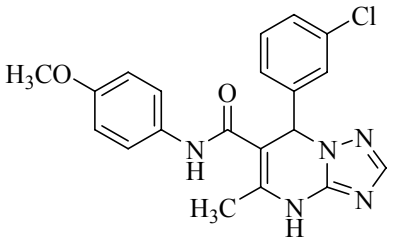
2H, $H_{ff'}$, J = 8.68 Hz), 7.57 (s, 1H, H_g), 8.12-8.14 (d, 2H, $H_{hh'}$, J = 8.68 Hz), 9.36 (s, 1H, H_i), 10.14 (s, 1H, H_j); MS: *m/z* 406; Anal. Calcd. for $C_{20}H_{18}N_6O_4$: C, 59.11; H, 4.46; N, 20.68. Found: C, 59.05; H, 4.38; N, 20.51%.

4.6.3.6 7-(3-nitrophenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-206)



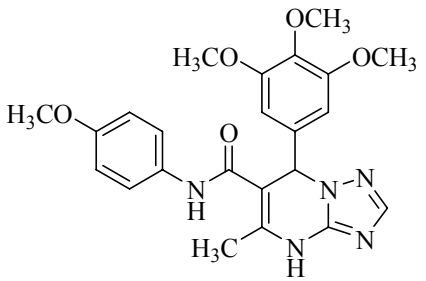
Yield: 75%; mp 183-185 ºC; MS: *m/z* 406; Anal. Calcd. for $C_{20}H_{18}N_6O_4$: C, 59.11; H, 4.46; N, 20.68. Found: C, 59.08; H, 4.39; N, 20.61%.

4.6.3.7 7-(3-chlorophenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (ART-207)



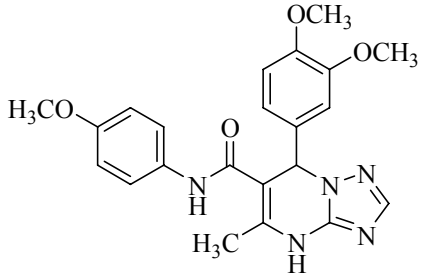
Yield: 69%; mp 244-246 ºC; MS: *m/z* 395; Anal. Calcd. for $C_{20}H_{18}ClN_5O_2$: C, 60.68; H, 4.58; N, 17.69. Found: C, 60.56; H, 4.45; N, 17.58%.

4.6.3.8 7-(3,4,5-methoxyphenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (ART-208)



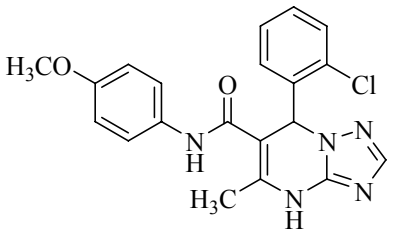
Yield: 76%; mp 274-276 ºC; MS: *m/z* 451; Anal. Calcd. for $C_{23}H_{25}N_5O_5$: C, 61.19; H, 5.58; N, 15.51. Found: C, 61.09; H, 5.48; N, 15.44%.

4.6.3.9 7-(3,4-methoxyphenyl)-4,7-dihydro-N-(4-methoxyphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (ART-209)



Yield: 82%; mp 169-171 ºC; MS: *m/z* 421; Anal. Calcd. for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.61; H, 5.39; N, 16.57%.

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

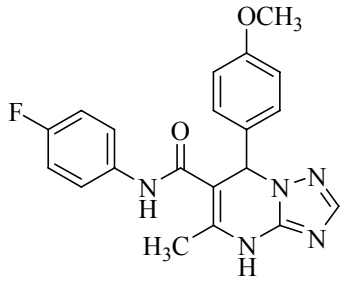


Yield: 70%; mp 183-185 ºC; MS: *m/z* 395; Anal. Calcd. for $C_{20}H_{18}ClN_5O_2$: C, 60.68; H, 4.58; N, 17.69. Found: C, 60.59; H, 4.47; N, 17.60%.

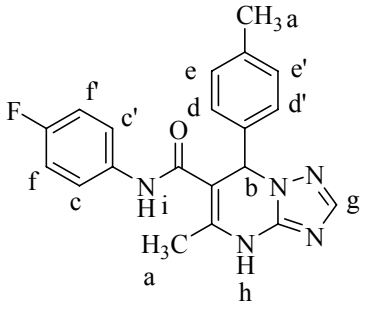
4.6.4 General procedure for the synthesis of 7-(aryl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (ART 211-220)

A mixture of the 5-amino-1,2,4-triazole (0.01 mol), *N*-(4-flourophenyl)-3-oxobutana mide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of DMF for 12-15 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 ART 211-220, which were crystallized from ethanol and subsequently dried in air.

4.6.4.1 7-(4-methoxyphenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-211) Yield: 73%; mp 256-258 ºC; MS: *m/z* 379; Anal. Calcd. for $C_{20}H_{18}FN_5O_2$: C, 63.32; H, 4.78; N, 18.46. Found: C, 63.25; H, 4.68; N, 18.39%.



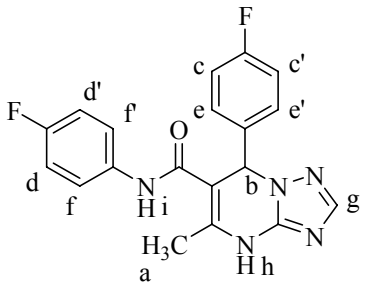
4.6.4.2 7-(4-methylphenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-212)



Yield: 85%; mp 227-229 ºC; IR (cm^{-1}): 3267 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2980 (C-H asymmetrical stretching of CH_3 group), 2862 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1602 (C=N stretching of triazole ring), 1516 (N-H deformation of pyrimidine ring), 1510 and

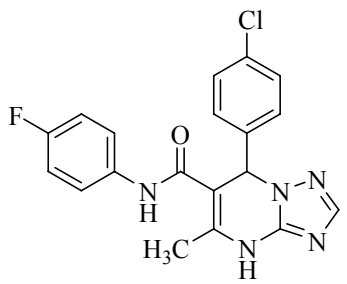
1450 (C=C stretching of aromatic ring), 1404 (C-H asymmetrical deformation of CH_3 group), 1329 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1014 (C-H in plane deformation of aromatic ring), 831 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.25-2.27 (2×s, 2×3H, H_a), 6.58 (s, 1H, H_b), 6.90-6.96 (t, 2H, $H_{cc'}$), 7.07-7.09 (d, 2H, $H_{dd'}$, J = 7.96), 7.16-7.18 (d, 2H, $H_{ee'}$, J = 8.08), 7.47-7.51 (m, 3H, $H_{ff'-g}$), 9.52 (s, 1H, H_h), 10.00 (s, 1H, H_i); MS: *m/z* 363; Anal. Calcd. for $C_{20}H_{18}FN_5O$: C, 66.10; H, 4.99; N, 19.27. Found: C, 65.99; H, 4.92; N, 19.21%.

4.6.4.3 7-(4-flourophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-213)



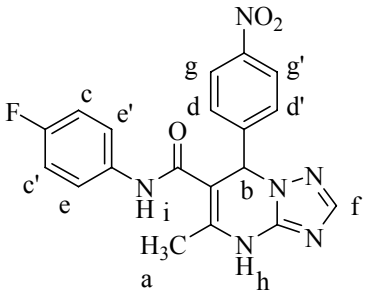
Yield: 78%; mp 257-259 ºC; IR (cm^{-1}): 3279 (N-H stretching of secondary amine), 3047 (C-H stretching of aromatic ring), 2989 (C-H asymmetrical stretching of CH_3 group), 2864 (C-H asymmetrical stretching of CH_3 group), 1658 (C=O stretching of amide), 1602 (C=N stretching of triazole ring), 1558 (N-H deformation of pyrimidine ring), 1514, 1494 and 1446 (C=C stretching of aromatic ring), 1420 (C-H asymmetrical deformation of CH_3 group), 1323 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1060 (C-H in plane deformation of aromatic ring), 827 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, H_a), 6.61 (s, 1H, H_b), 6.91-7.00 (m, 4H, $H_{cc'-dd'}$), 7.30-7.33 (m, 2H, $H_{ee'}$), 7.46-7.49 (m, 2H, $H_{ff'}$), 7.71-7.72 (s, 1H, H_g), 9.49 (s, 1H, H_h), 10.05 (s, 1H, H_i); MS: *m/z* 367; Anal. Calcd. for $C_{19}H_{15}F_2N_5O$: C, 62.12; H, 4.12; N, 19.06. Found: C, 62.03; H, 4.06; N, 19.00%.

4.6.4.4 7-(4-chlorophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-214)



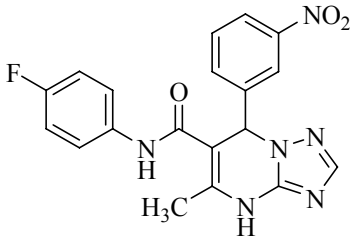
Yield: 72%; mp 247-249 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.36; H, 3.83; N, 18.17%.

4.6.4.5 7-(4-nitrophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-215)



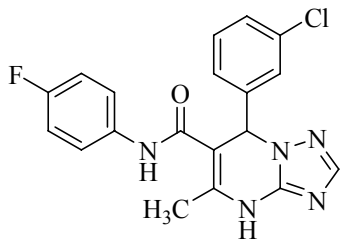
Yield: 76%; mp 273-275 ºC; IR (cm^{-1}): 3230 (N-H stretching of secondary amine), 3034 (C-H stretching of aromatic ring), 2910 (C-H asymmetrical stretching of CH_3 group), 2856 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1602 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1529, 1510 and 1450 (C=C stretching of aromatic ring), 1406 (C-H asymmetrical deformation of CH_3 group), 1344 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1014 (C-H in plane deformation of aromatic ring), 823 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.25 (s, 3H, H_a), 6.72 (s, 1H, H_b), 6.93-6.98 (t, 2H, $H_{cc'}$), 7.48-7.51 (m, 4H, $H_{dd'-ee'}$), 7.56 (s, 1H, H_f), 8.14-8.16 (d, 2H, $H_{gg'}$, J = 8.40), 9.74 (s, 1H, H_h), 10.32 (s, 1H, H_i): MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.79; H, 3.75; N, 21.19%.

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



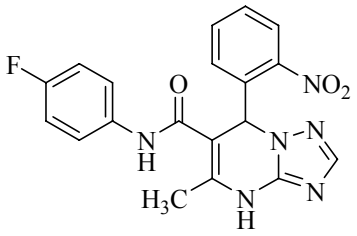
Yield: 66%; mp 242-244 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.77; H, 3.78; N, 21.21%.

4.6.4.7 7-(3-chlorophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-217)



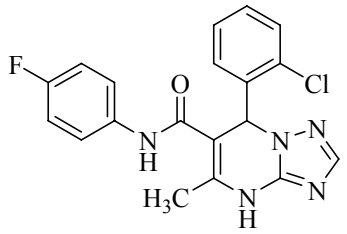
Yield: 78%; mp 233-235 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.38; H, 3.85; N, 18.15%.

4.6.4.8 7-(2-nitrophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-218)



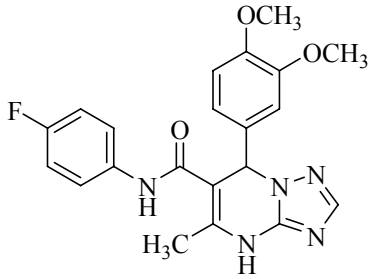
Yield: 71%; mp 196-198 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.74; H, 3.75; N, 21.23%.

4.6.4.9 7-(2-chlorophenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-219)



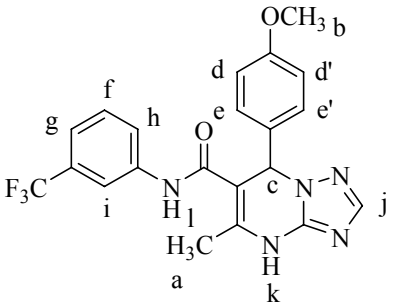
Yield: 65%; mp 207-209 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.36; H, 3.82; N, 18.17%.

4.6.4.10 7-(3,4-methoxyphenyl)-4,7-dihydro-N-(4-flourophenyl)-5-methyl[1,2,4] triazlo[1,5-a]pyrimidine-6-carboxamide (ART-220) Yield: 79%; mp 217-219 ºC; MS: *m/z* 409; Anal. Calcd. for $C_{21}H_{20}FN_5O_3$: C, 61.61; H, 4.92; N, 17.11. Found: C, 61.55; H, 4.82; N, 17.03%.

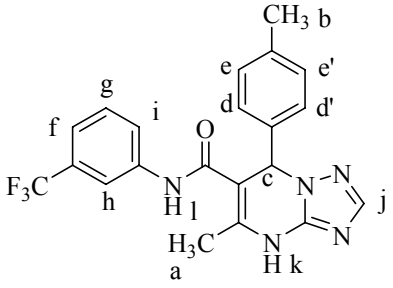


4.6.5 General procedure for the synthesis of 7-(aryl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (ART 221-230)

A mixture of the 5-amino-1,2,4-triazole (0.01 mol), *N*-(3-triflouromethylphenyl)-3-oxobutanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of DMF for 12-15 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 ART 221-230, which were crystallized from ethanol and subsequently dried in air.

4.6.5.1 7-(4-methoxyphenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-221) 

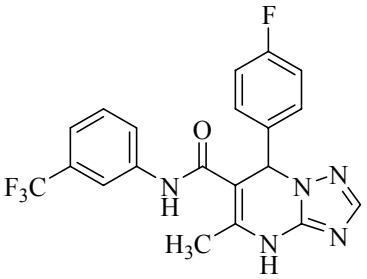
Yield: 68%; mp 208-210 ºC; IR (cm^{-1}): 3246 (N-H stretching of secondary amine), 3045 (C-H stretching of aromatic ring), 2926 (C-H asymmetrical stretching of CH_3 group), 2837 (C-H asymmetrical stretching of CH_3 group), 1668 (C=O stretching of amide), 1602 (C=N stretching of triazole ring), 1552 (N-H deformation of pyrimidine ring), 1516 and 1489 (C=C stretching of aromatic ring), 1442 (C-H asymmetrical deformation of CH_3 group), 1332 (C-H symmetrical deformation of CH_3 group), 1282 (C-N stretching), 1251 (C-O-C stretching), 1031 (C-H in plane deformation of aromatic ring), 831 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29 (s, 3H, H_a), 3.73 (s, 3H, H_b), 6.59 (s, 1H, H_c), 6.78-7.74 (m, 8H, $H_{dd'-i}$), 7.93 (s, 1H, H_j), 9.71 (s, 1H, H_k), 10.07 (s, 1H, H_l); MS: *m/z* 429; Anal. Calcd. for $C_{21}H_{18}F_3N_5O_2$: C, 58.74; H, 4.23; N, 16.31. Found: C, 58.68; H, 4.15; N, 16.26%.

4.6.5.2 7-(4-methylphenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-222) 

Yield: 74%; mp 233-235 ºC; IR (cm^{-1}): 3246 (N-H stretching of secondary amine), 3041 (C-H stretching of aromatic ring), 2924 (C-H asymmetrical stretching of CH_3 group), 2833 (C-H asymmetrical stretching of CH_3 group), 1664 (C=O stretching of amide), 1626 (C=N stretching of triazole ring), 1600 (N-H deformation of pyrimidine ring), 1525 and 1489 (C=C stretching of aromatic ring), 1444 (C-H asymmetrical deformation of CH_3 group), 1330 (C-H symmetrical deformation of CH_3 group), 1276 (C-N stretching), 1070 (C-H in plane deformation of aromatic ring), 792 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, H_a), 2.31 (s, 3H, H_b), 6.60 (s, 1H, H_c), 7.08-7.10 (d, 2H, $H_{dd'}$, J = 7.92 Hz), 7.19-7.21 (d, 2H, $H_{ee'}$, J = 8.08 Hz), 7.24-7.26 (d, 2H, H_f), 7.35-7.38 (t, 2H, H_g), 7.54 (s, 1H, H_h), 7.72-7.74 (d, 1H, H_i), 7.93 (s, 1H, H_j),

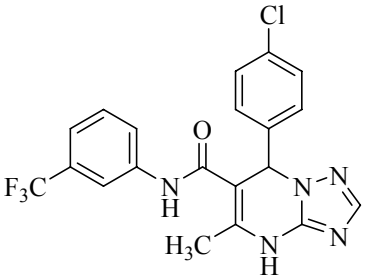
9.68 (s, 1H, H_k), 10.09 (s, 1H, H_l); MS: *m/z* 413; Anal. Calcd. for $C_{21}H_{18}F_3N_5O$: C, 61.01; H, s4.39; N, 16.94; Found: C, 61.01; H, 4.39; N, 16.94%.

4.6.5.3 7-(4-flourophenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide



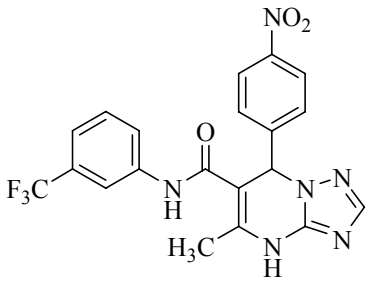
(ART-223) Yield: 82%; mp 215-217 ºC; MS: *m/z* 417; Anal. Calcd. for $C_{20}H_{15}F_4N_5O$: C, 57.56; H, 3.62; N, 16.78. Found: C, 57.48; H, 3.56; N, 16.71%.

4.6.5.4 7-(4-chlorophenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6carboxamide



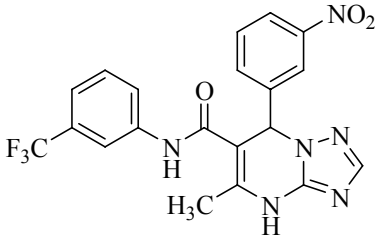
(ART-224) Yield: 71%; mp 248-250 ºC; MS: *m/z* 433; Anal. Calcd. for $C_{20}H_{15}ClF_3N_5O$: C, 55.37; H, 3.49; N, 16.14. Found: C, 55.37; H, 3.49; N, 16.14%.

4.6.5.5 7-(4-nitrophenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide



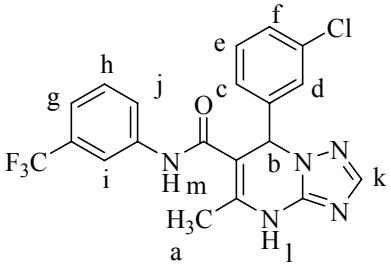
(ART-225) Yield: 79%; mp 253-255 ºC; MS: *m/z* 444; Anal. Calcd. for $C_{20}H_{15}F_3N_6O_3$: C, 54.06; H, 3.40; N, 18.91. Found: C, 53.96; H, 3.30; N, 18.85%.

4.6.5.6 7-(3-nitrophenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide



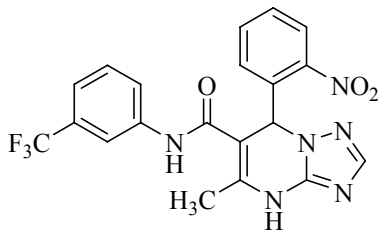
(ART-226) Yield: 68%; mp 236-238 ºC; MS: *m/z* 444; Anal. Calcd. for $C_{20}H_{15}F_3N_6O_3$: C, 54.06; H, 3.40; N, 18.91. Found: C, 53.99; H, 3.32; N, 18.81%.

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



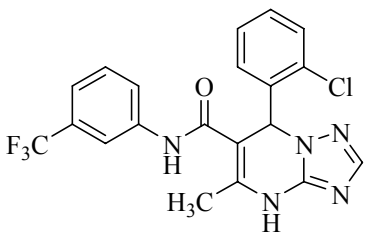
(ART-227) Yield: 66%; mp 239-241 ºC; IR (cm^{-1}): 3248 (N-H stretching of secondary amine), 3051 (C-H stretching of aromatic ring), 2972 (C-H asymmetrical stretching of CH_3 group), 2835 (C-H asymmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1626 (C=N stretching of triazole ring), 1599 (N-H deformation of pyrimidine ring), 1552, 1527 and 1514 (C=C stretching of aromatic ring), 1442 (C-H asymmetrical deformation of CH_3 group), 1330 (C-H symmetrical deformation of CH_3 group), 1274 (C-N stretching), 1070 (C-H in plane deformation of aromatic ring), 777 (C-H out of plane bending of 1,3-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.28 (s, 3H, H_a), 6.62 (s, 1H, H_b), 7.22-7.30 (m, 5H, H_{c-g}), 7.38-7.42 (t, 1H, H_h), 7.56 (s, 1H, H_i), 7.33-7.50 (d, 1H, H_j), 7.94 (s, 1H, H_k), 9.90 (s, 1H, H_l), 10.25 (s, 1H, H_m); MS: *m/z* 433; Anal. Calcd. for $C_{20}H_{15}ClF_3N_5O$: C, 55.37; H, 3.49; N, 16.14. Found: C, 55.31; H, 3.42; N, 16.06%.

4.6.5.8 7-(2-nitrophenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide



(ART-228) Yield: 72%; mp 238-240 ºC; MS: *m/z* 444; Anal. Calcd. for $C_{20}H_{15}F_3N_6O_3$: C, 54.06; H, 3.40; N, 18.91. Found: C, 54.00; H, 3.30; N, 18.79%.

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



(ART-229) Yield: 67%; mp 258-260 ºC; MS: *m/z* 433; Anal. Calcd. for $C_{20}H_{15}ClF_3N_5O$: C, 55.37; H, 3.49; N, 16.14; Found. C, 55.26; H, 3.40; N, 16.06%.

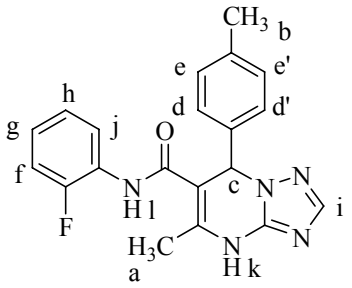
4.6.5.10 7-(3,4-methoxyphenyl)-4,7-dihydro-N-(3-triflouromethylphenyl)-5-methyl [1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-230) Yield: 68%; mp 220-222 ºC; MS: *m/z* 459; Anal. Calcd. for $C_{22}H_{20}F_3N_5O_3$: C, 57.51; H, 4.39; N, 15.24. Found: C, 57.45; H, 4.32; N, 15.16%.

4.6.6 General procedure for the synthesis of 7-(aryl)-4,7-dihydro-N-(2-flourophenyl) -5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamides (ART 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 DMF for 12-15 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 ART 231-240, which were crystallized from ethanol and subsequently dried in air.

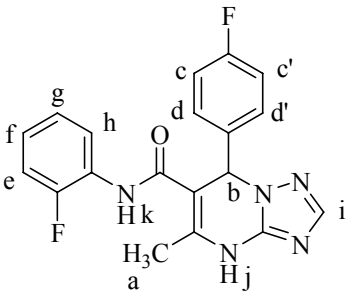
4.6.6.1 7-(4-methoxyphenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-231) Yield: 75%; mp 228-230 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3014 (C-H stretching of aromatic ring), 2966 (C-H asymmetrical stretching of CH_3 group), 2837 (C-H asymmetrical stretching of CH_3 group), 1658 (C=O stretching of amide), 1599 (C=N stretching of triazole ring), 1562 (N-H deformation of pyrimidine ring), 1516 and 1446 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of CH_3 group), 1336 (C-H symmetrical deformation of CH_3 group), 1284 (C-N stretching), 1257 (C-O-C stretching), 1028 (C-H in plane deformation of aromatic ring), 839 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.33 (s, 3H, H_a), 3.75 (s, 3H, H_b), 6.50 (s, 1H, H_c), 6.82-7.69 (m, 8H, $H_{dd'-i}$), 7.80 (s, 1H, H_j), 8.86 (s, 1H, H_k), 10.13 (s, 1H, H_l); MS: *m/z* 379; Anal. Calcd. for $C_{20}H_{18}FN_5O_2$: C, 63.32; H, 4.78; N, 18.46. Found: C, 63.29; H, 4.61; N, 18.35%.

4.6.6.2 7-(4-methylphenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-232)



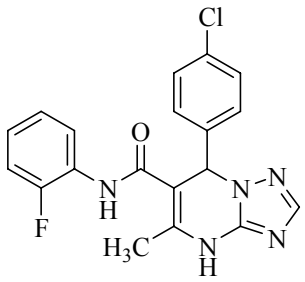
Yield: 76%; mp 229-231 ºC; IR (cm^{-1}): 3267 (N-H stretching of secondary amine), 3014 (C-H stretching of aromatic ring), 2968 (C-H asymmetrical stretching of CH_3 group), 2870 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1599 (C=N stretching of triazole ring), 1558 (N-H deformation of pyrimidine ring), 1529, 1506 and 1479 (C=C stretching of aromatic ring), 1450 (C-H asymmetrical deformation of CH_3 group), 1325 (C-H symmetrical deformation of CH_3 group), 1284 (C-N stretching), 1028 (C-H in plane deformation of aromatic ring), 839 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29 (s, 3H, H_a), 2.30 (s, 3H, H_b), 6.52 (s, 1H, H_c), 7.03-7.63 (m, 9H, $H_{dd'-j}$), 9.06 (s, 1H, H_k), 10.12 (s, 1H, H_l); MS: *m/z* 363; Anal. Calcd. for $C_{20}H_{18}FN_5O$: C, 66.10; H, 4.99; N, 19.27. Found: C, 66.01; H, 4.88; N, 19.15%.

4.6.6.3 7-(4-flourophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-233)



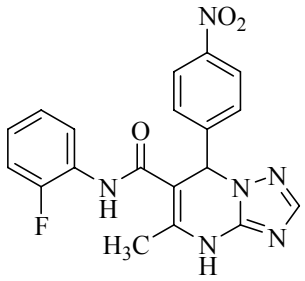
Yield: 71%; mp 281-283 ºC; IR (cm^{-1}): 3214 (N-H stretching of secondary amine), 3045 (C-H stretching of aromatic ring), 2968 (C-H asymmetrical stretching of CH_3 group), 2831 (C-H asymmetrical stretching of CH_3 group), 1662 (C=O stretching of amide), 1600 (C=N stretching of triazole ring), 1556 (N-H deformation of pyrimidine ring), 1525 and 1506 (C=C stretching of aromatic ring), 1448 (C-H asymmetrical deformation of CH_3 group), 1325 (C-H symmetrical deformation of CH_3 group), 1280 (C-N stretching), 1012 (C-H in plane deformation of aromatic ring), 823 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, H_a), 6.58 (s, 1H, H_b), 6.99-7.58 (m, 9H, $H_{cc'-i}$), 9.13 (s, 1H, H_j), 10.18 (s, 1H, H_k); MS: *m/z* 367; Anal. Calcd. for $C_{19}H_{15}F_2N_5O$: C, 62.12; H, 4.12; N, 19.06. Found: C, 62.01; H, 4.02; N, 19.03%.

4.6.6.4 7-(4-chlorophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-234)



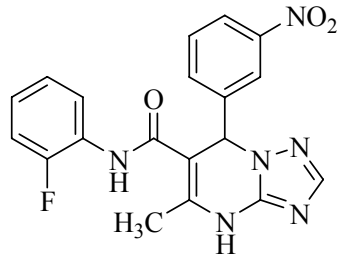
Yield: 81%; mp 276-278 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.38; H, 3.84; N, 18.14%.

4.6.6.5 7-(4-nitrophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-235)



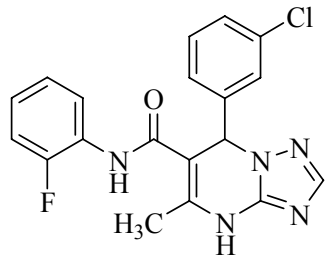
Yield: 82%; mp 277-279 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.78; H, 3.73; N, 21.17%.

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



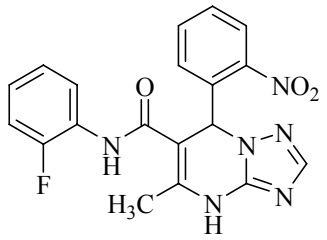
Yield: 74%; mp 259-261 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.74; H, 3.80; N, 21.25%.

4.6.6.7 7-(3-chlorophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-237)



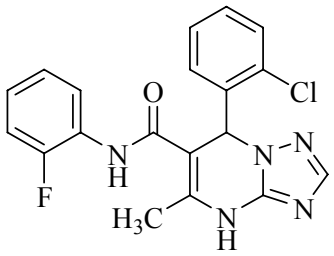
Yield: 72%; mp 256-258 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.40; H, 3.81; N, 18.12%.

4.6.6.8 7-(2-nitrophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-238)



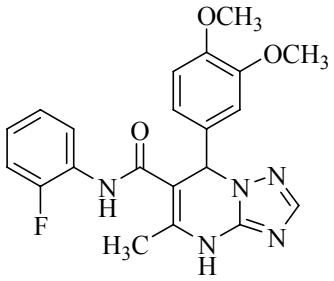
Yield: 78%; mp 275-277 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{19}H_{15}FN_6O_3$: C, 57.87; H, 3.83; N, 21.31. Found: C, 57.75; H, 3.76; N, 21.21%.

4.6.6.9 7-(2-chlorophenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (ART-239)



Yield: 68%; mp 221-223 ºC; MS: *m/z* 383; Anal. Calcd. for $C_{19}H_{15}ClFN_5O$: C, 59.46; H, 3.94; N, 18.25. Found: C, 59.39; H, 3.85; N, 18.12%.

4.6.6.10 7-(3,4-methoxyphenyl)-4,7-dihydro-N-(2-flourophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (ART-240)



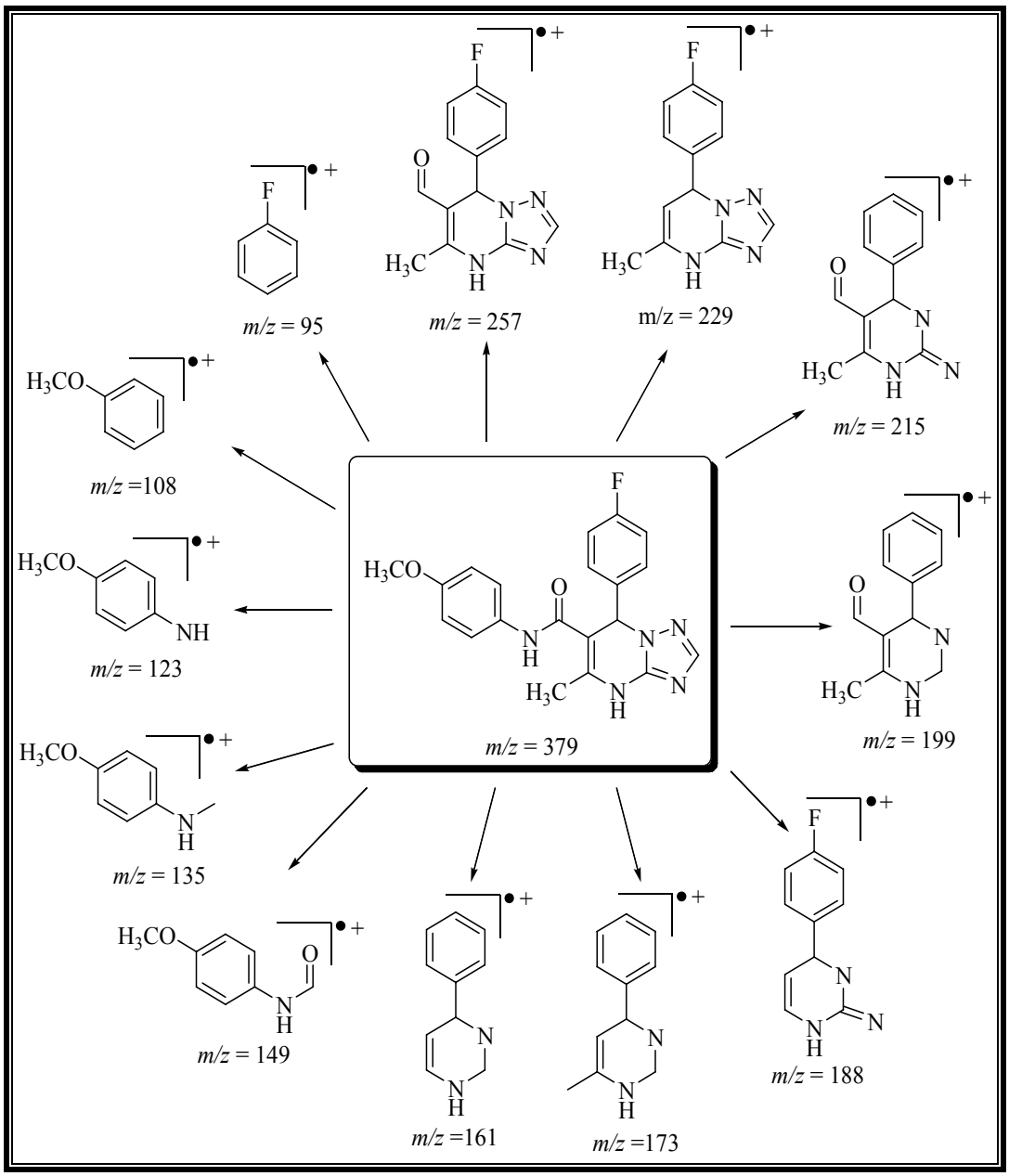
Yield: 72%; mp 256-258 ºC; MS: *m/z* 409; Anal. Calcd. for $C_{21}H_{20}FN_5O_3$: C, 61.61; H, 4.92; N, 17.11. Found: C, 61.57; H, 4.79; N, 17.01%.

4.7 Spectral discussion

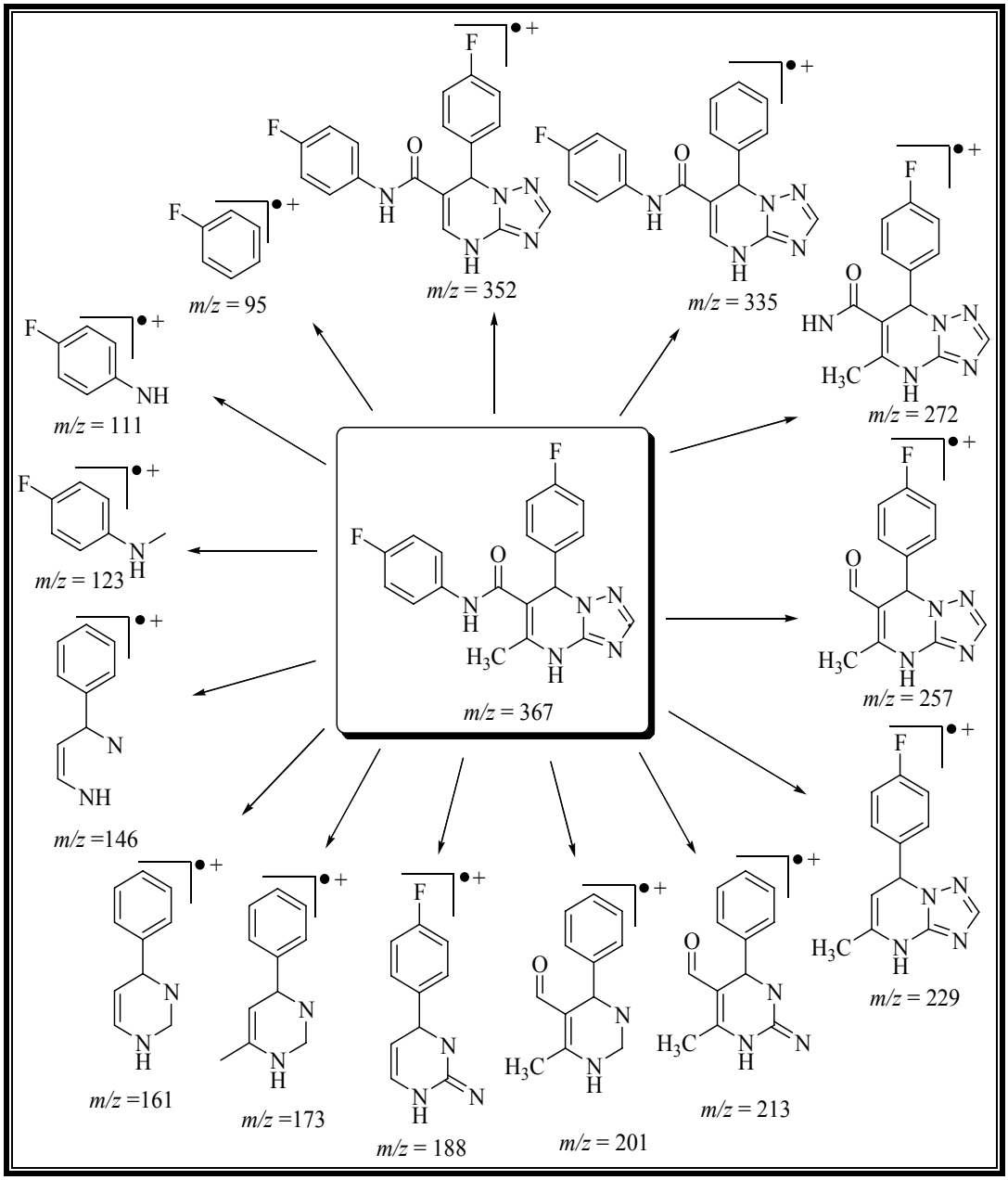
4.7.1 Mass spectral study

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.

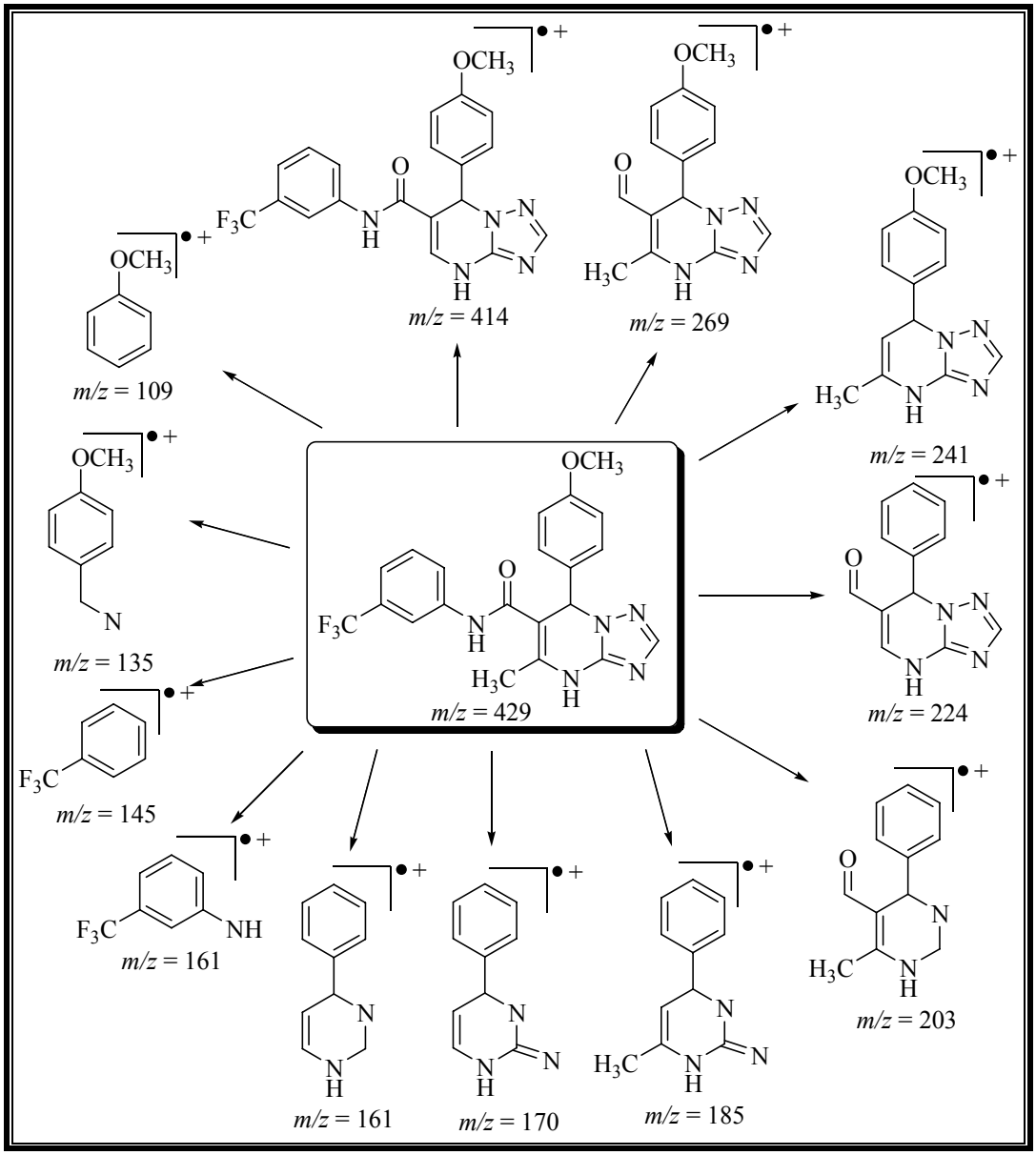
4.7.1.1 Mass fragmentation pattern for ART-203



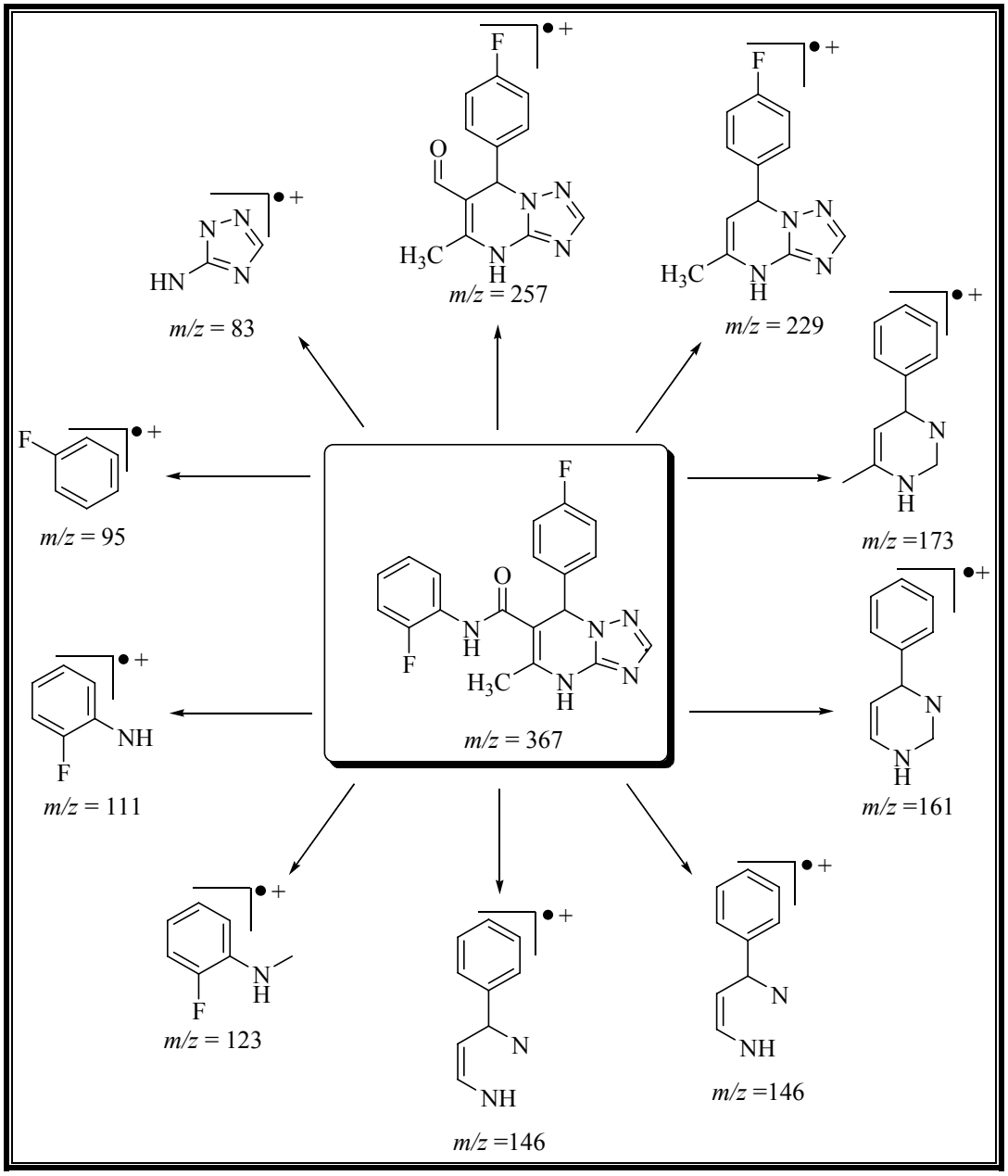
4.7.1.2 Mass fragmentation pattern for ART-213



4.7.1.3 Mass fragmentation pattern for ART-221



4.7.1.4 Mass fragmentation pattern for ART-233



4.7.2 IR spectral study

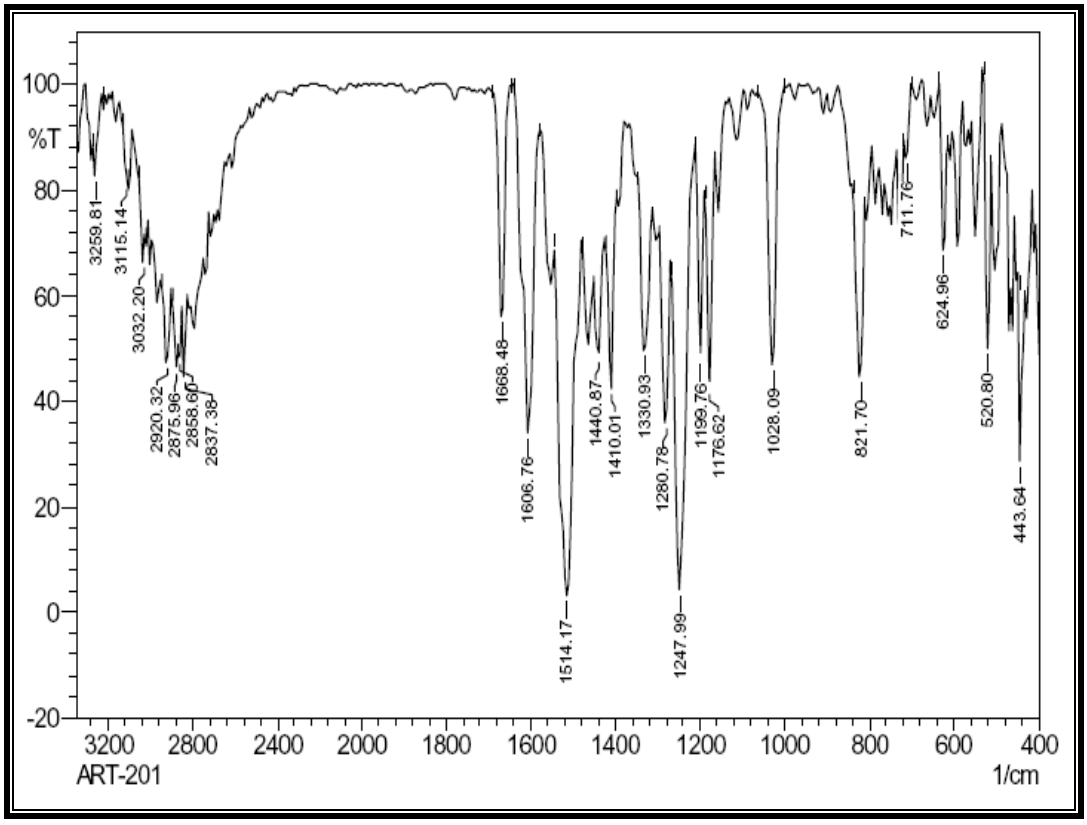
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For triazolopyrimidines ART-201 to 240, confirmatory bands for secondary amine and amidic carbonyl groups were observed at 3214-3282 cm^{-1} and 1658-1668 cm^{-1} respectively. Another characteristic C=N stretching band of triazole ring was observed at 1595-1626 cm^{-1}, which suggested formation of desired products ART-201 to 240.

4.7.3 ^{1}H NMR spectral study

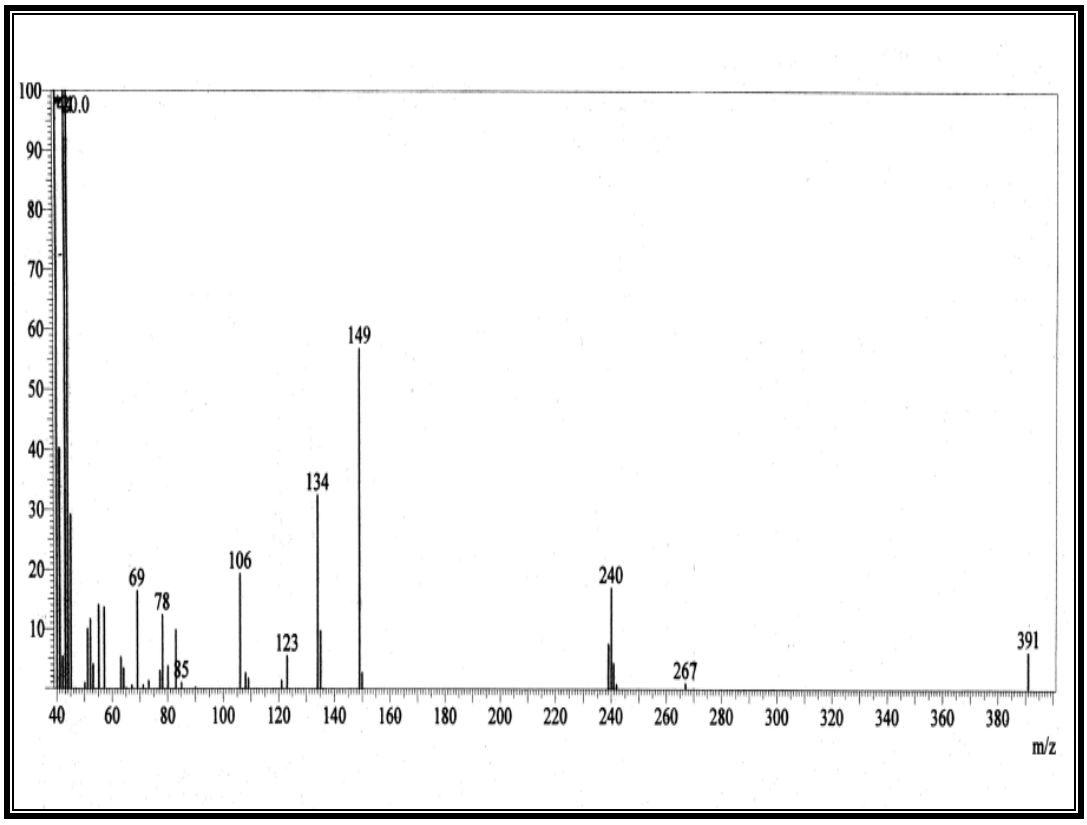
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of triazolopyrimidines ART-201 to 240 on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 6.50-6.72 δ ppm, a singlet for the methine proton of triazole ring at 7.50-7.94 δ ppm and singlets for amino and amide group protons at 8.86-9.90 and 10.02-10.32 δ ppm, respectively. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.

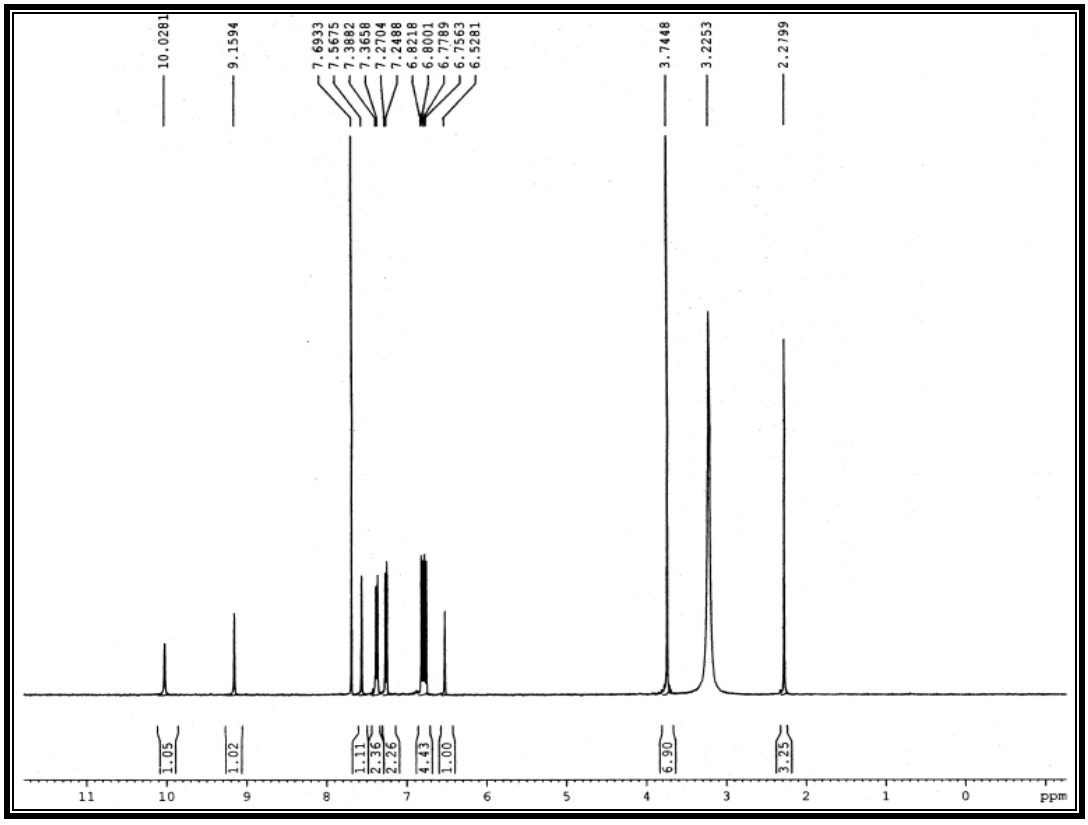
IR spectrum of ART-201



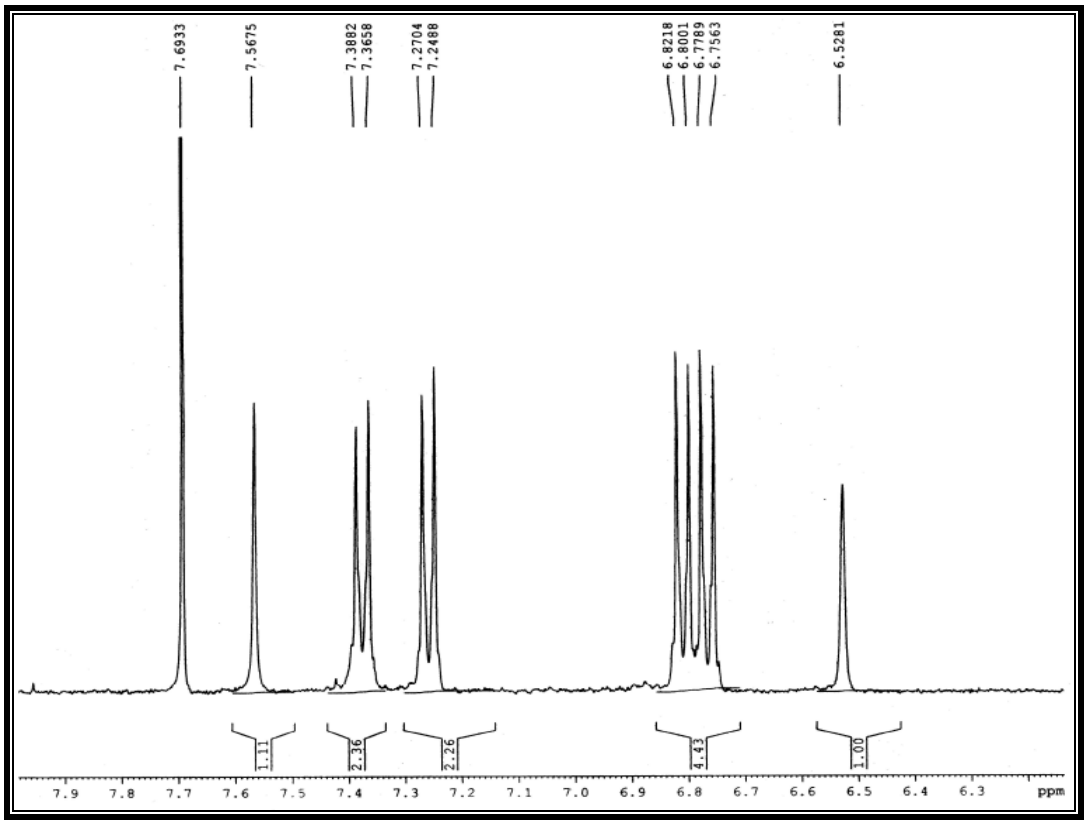
Mass spectrum of ART-201



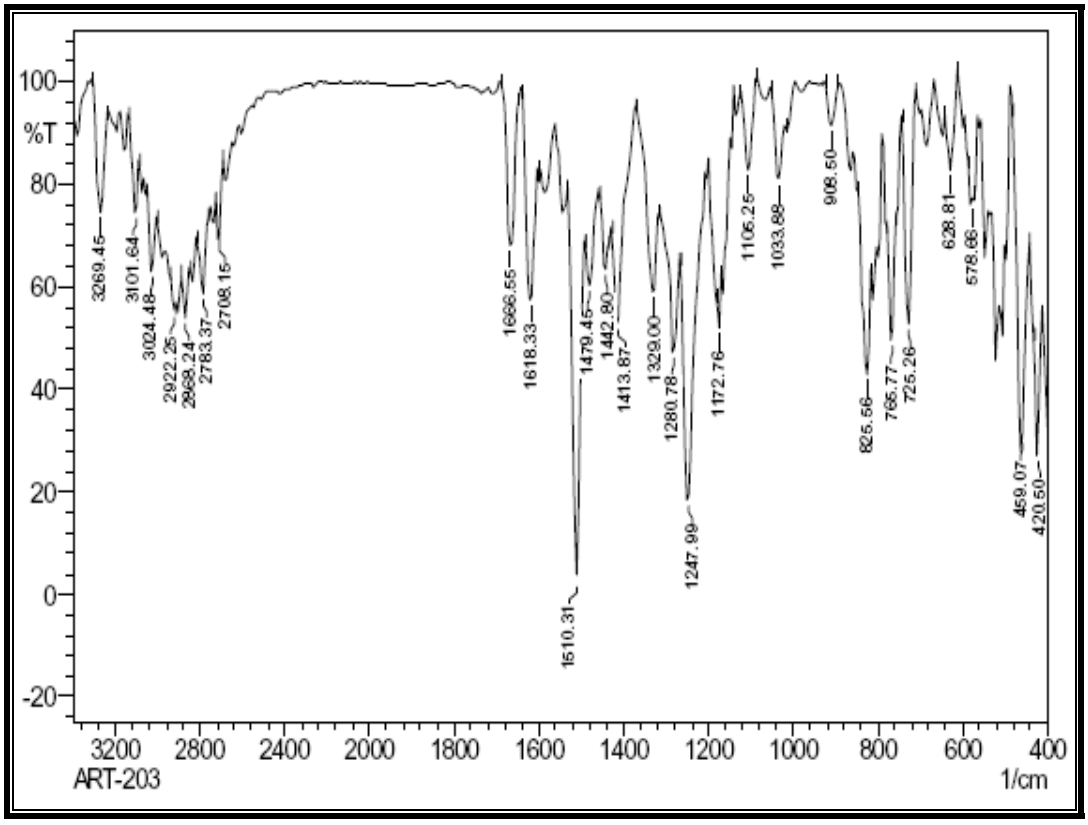
^{1}H NMR spectrum of ART-201



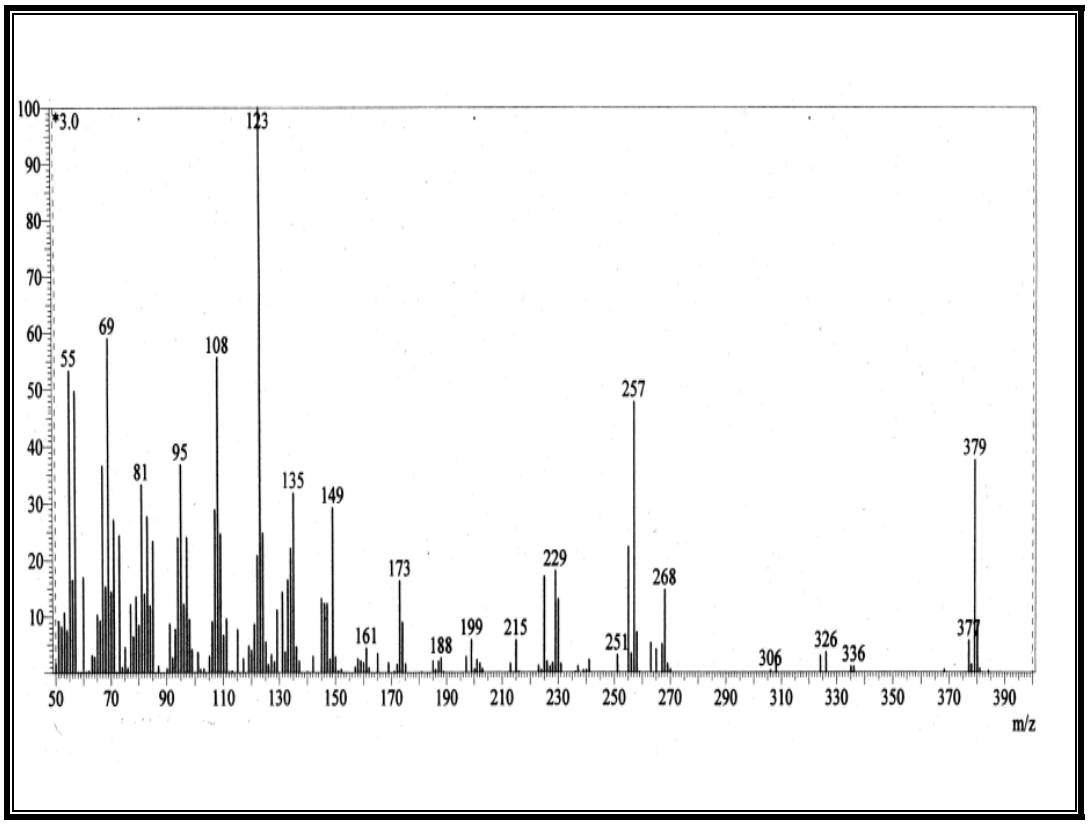
Expanded ^{1}H NMR spectrum of ART-201



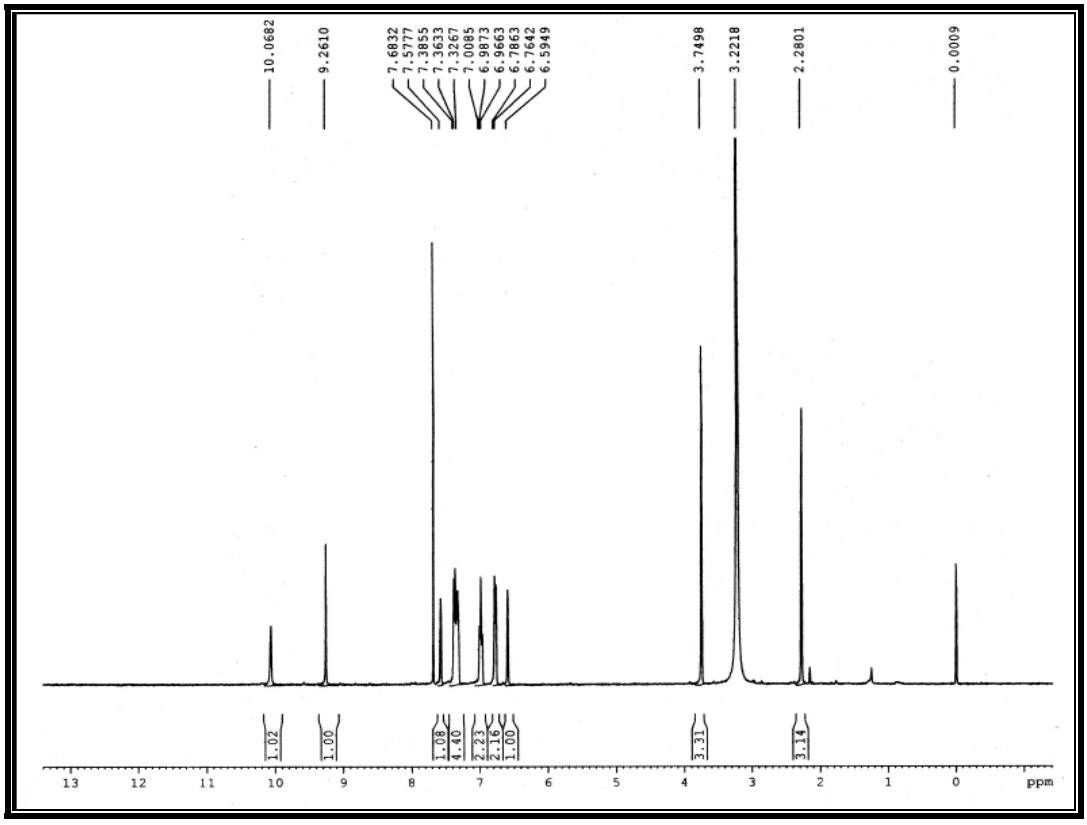
IR spectrum of ART-203



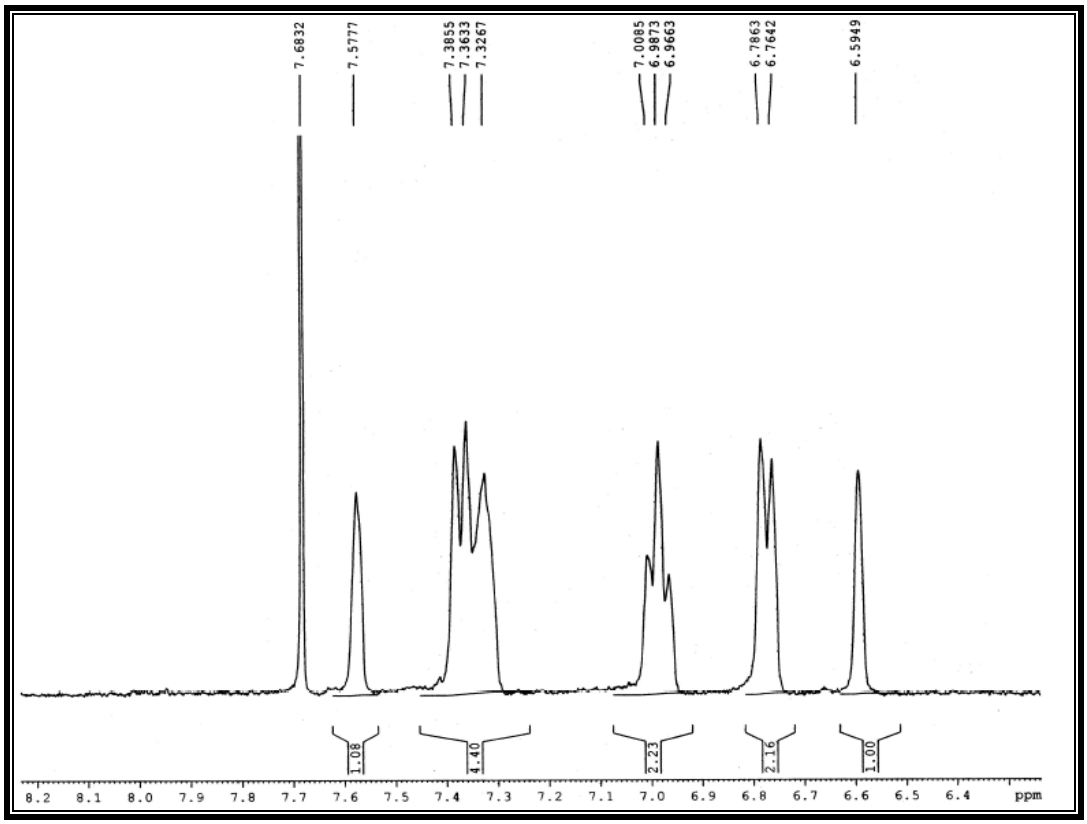
Mass spectrum of ART-203



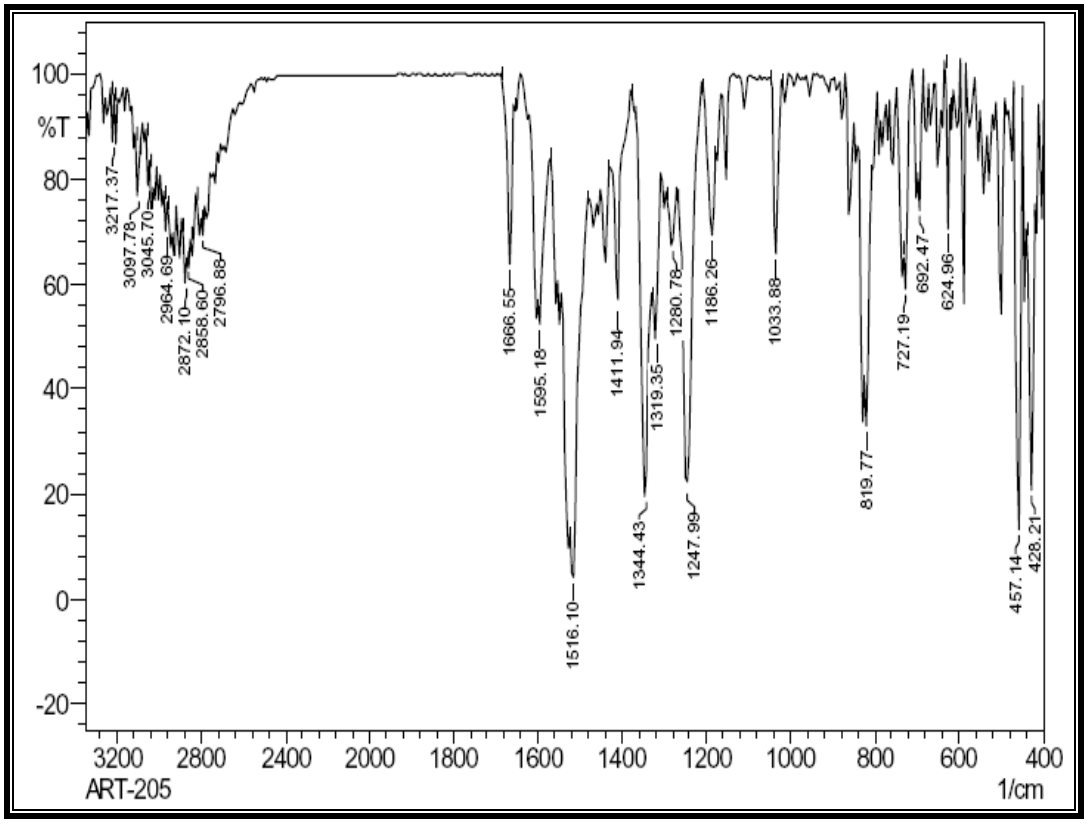
^{1}H NMR spectrum of ART-203



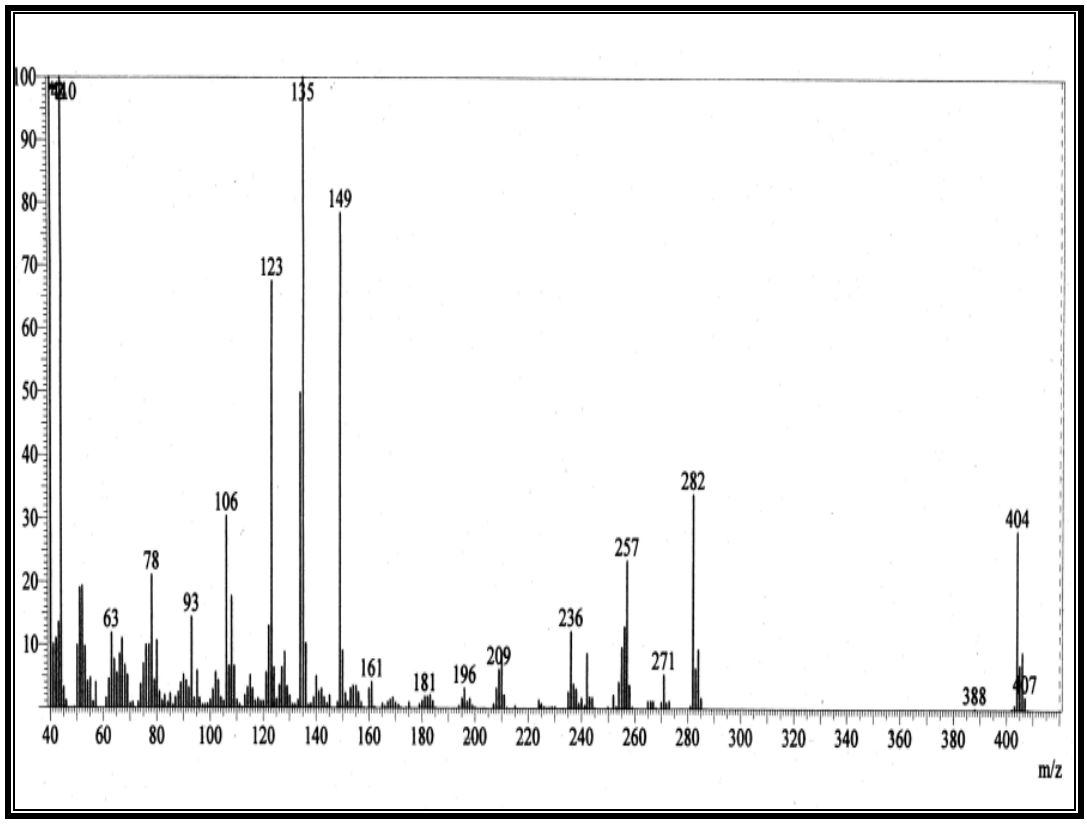
Expanded ^{1}H NMR spectrum of ART-203



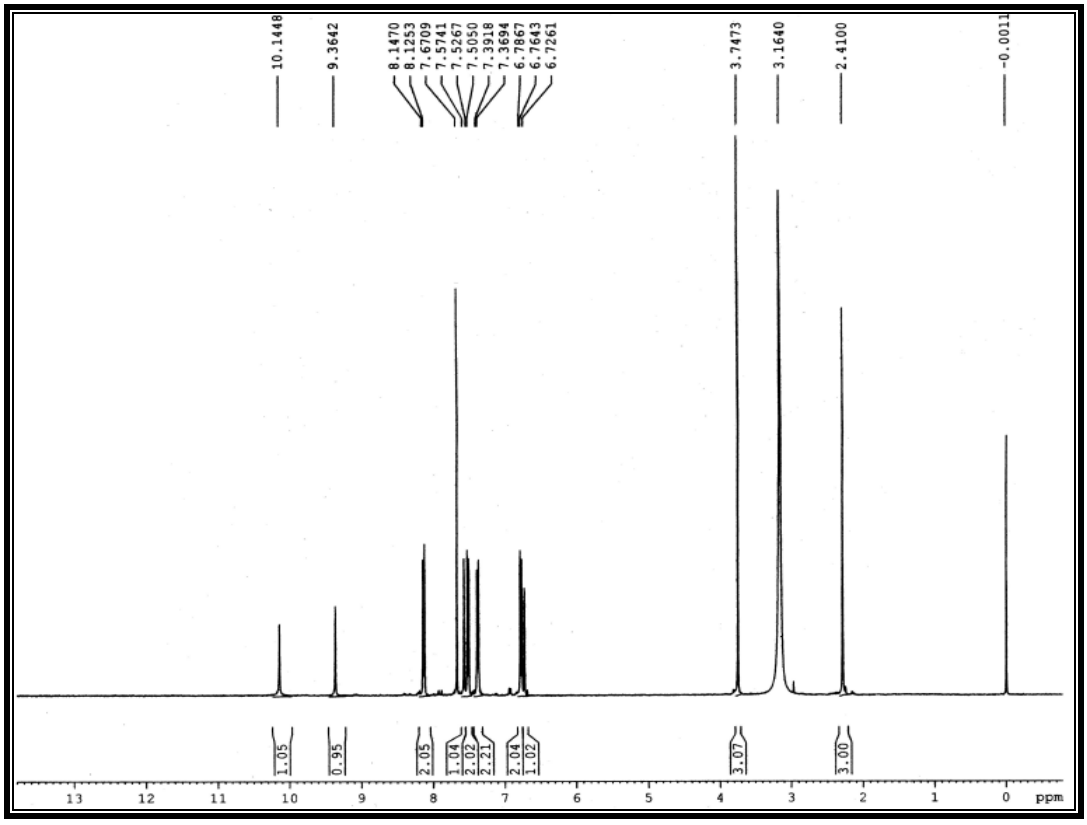
IR spectrum of ART-205



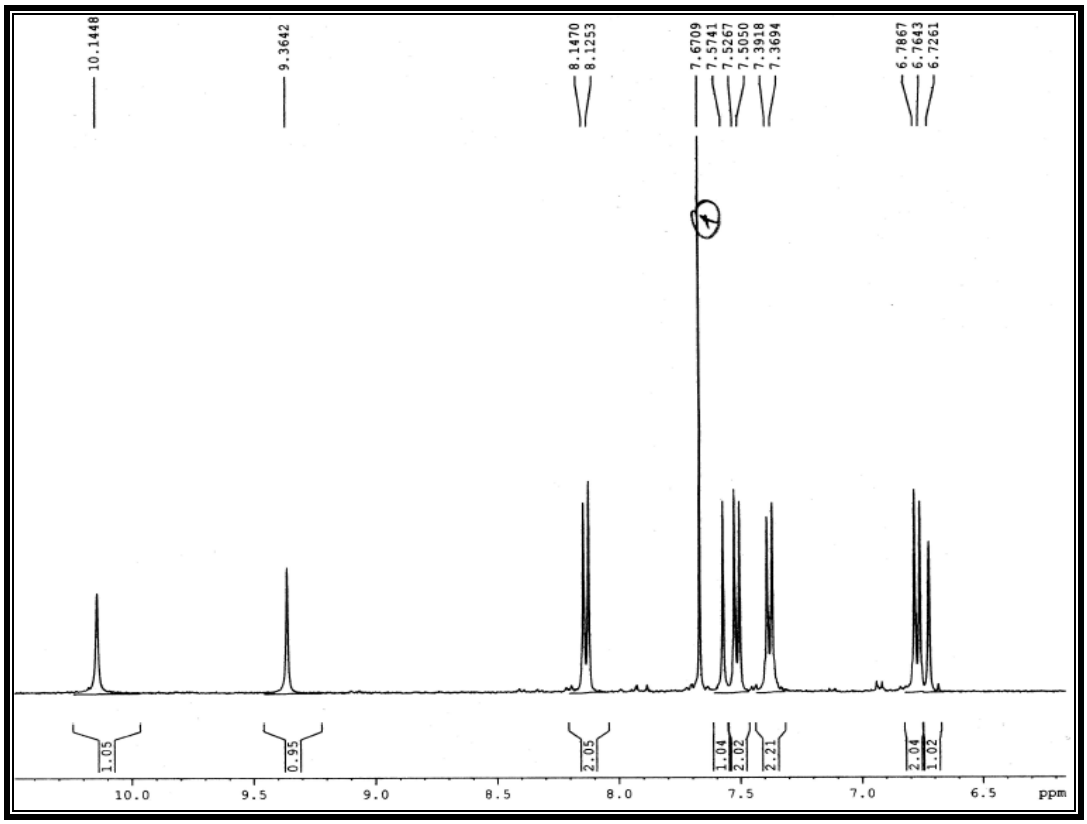
Mass spectrum of ART-205



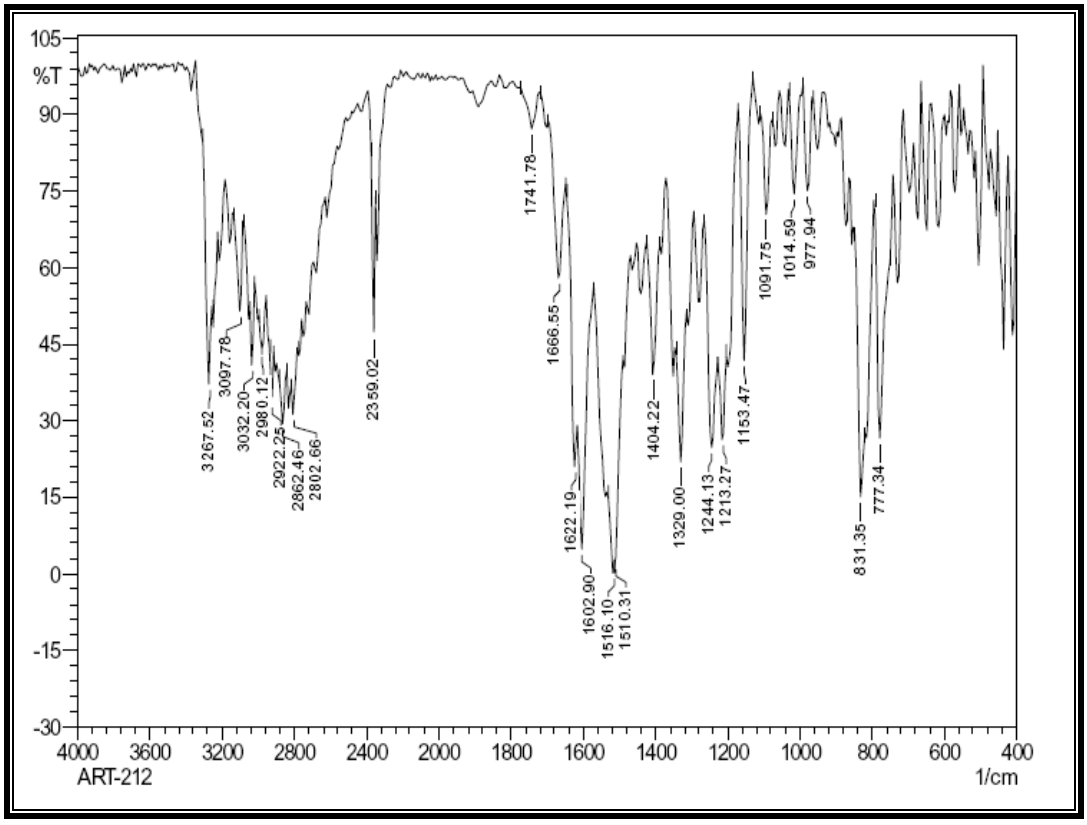
^{1}H NMR spectrum of ART-205



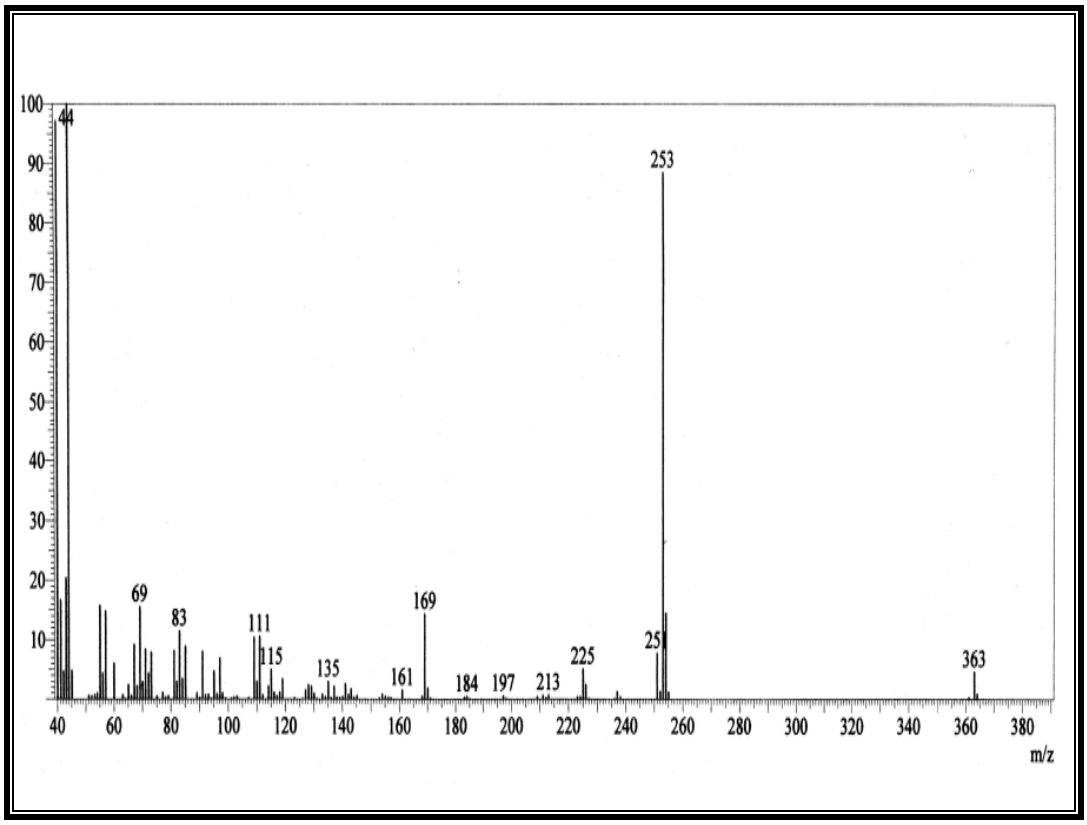
Expanded ^{1}H NMR spectrum of ART-205



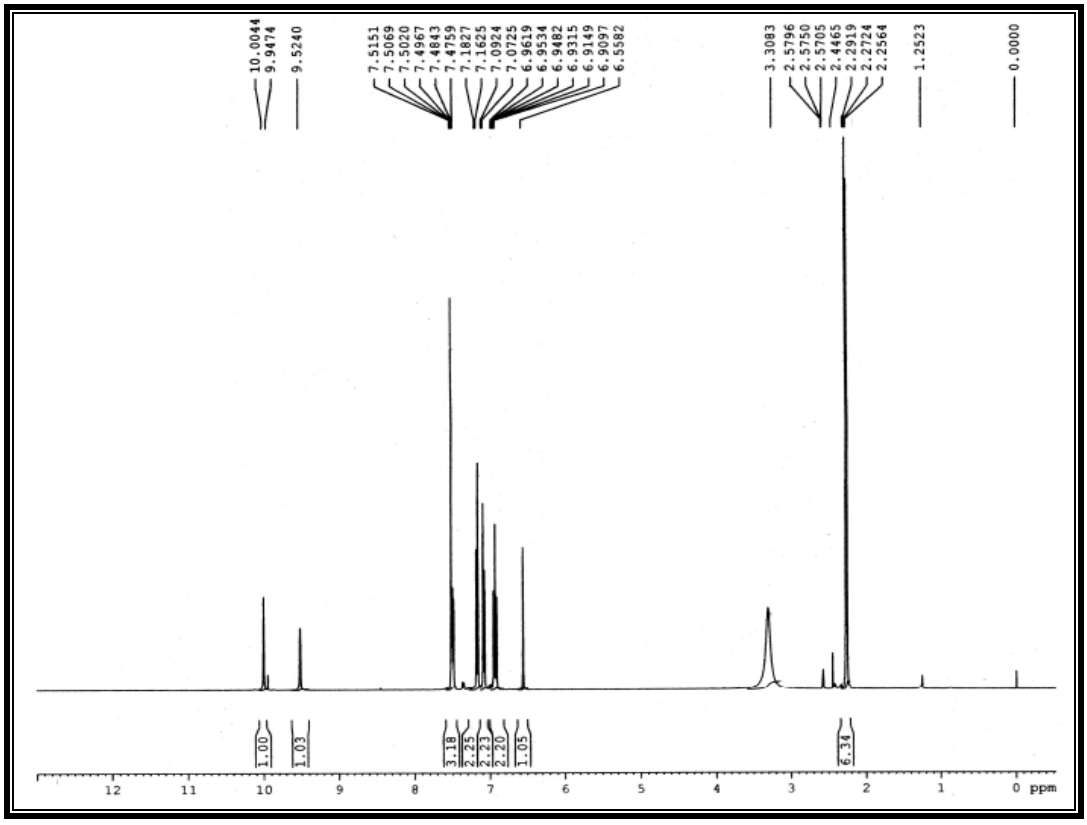
IR spectrum of ART-212



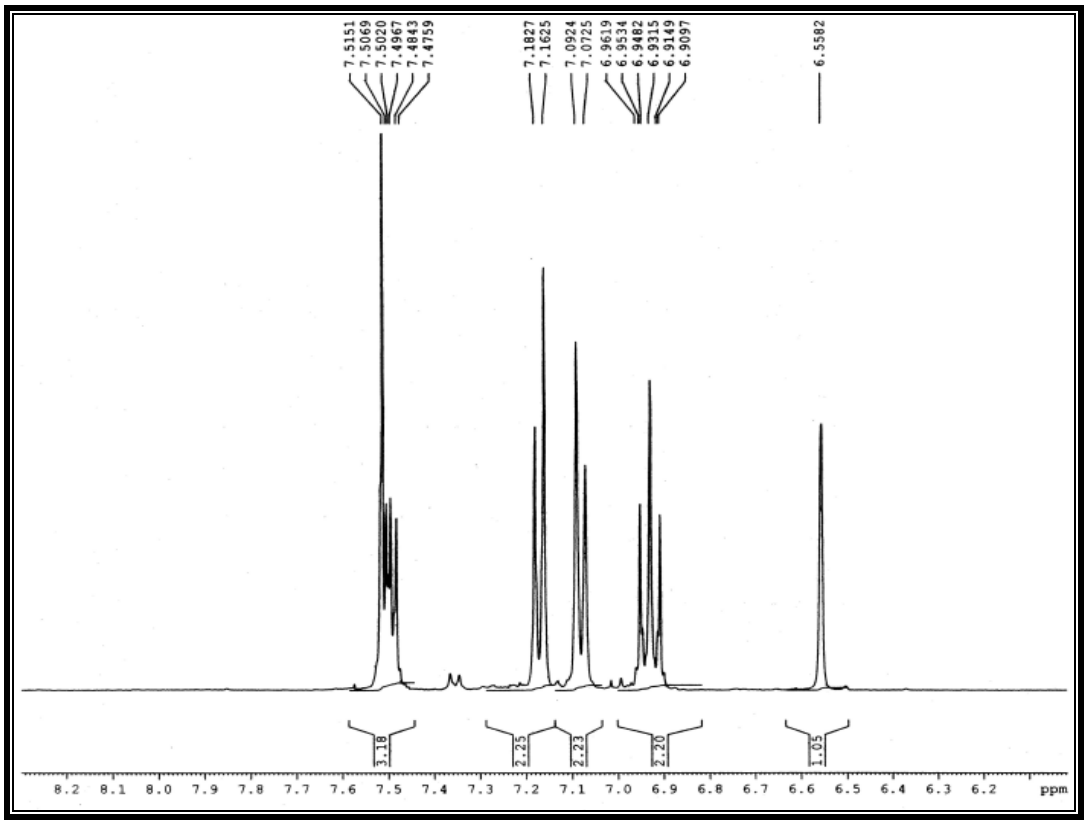
Mass spectrum of ART-212



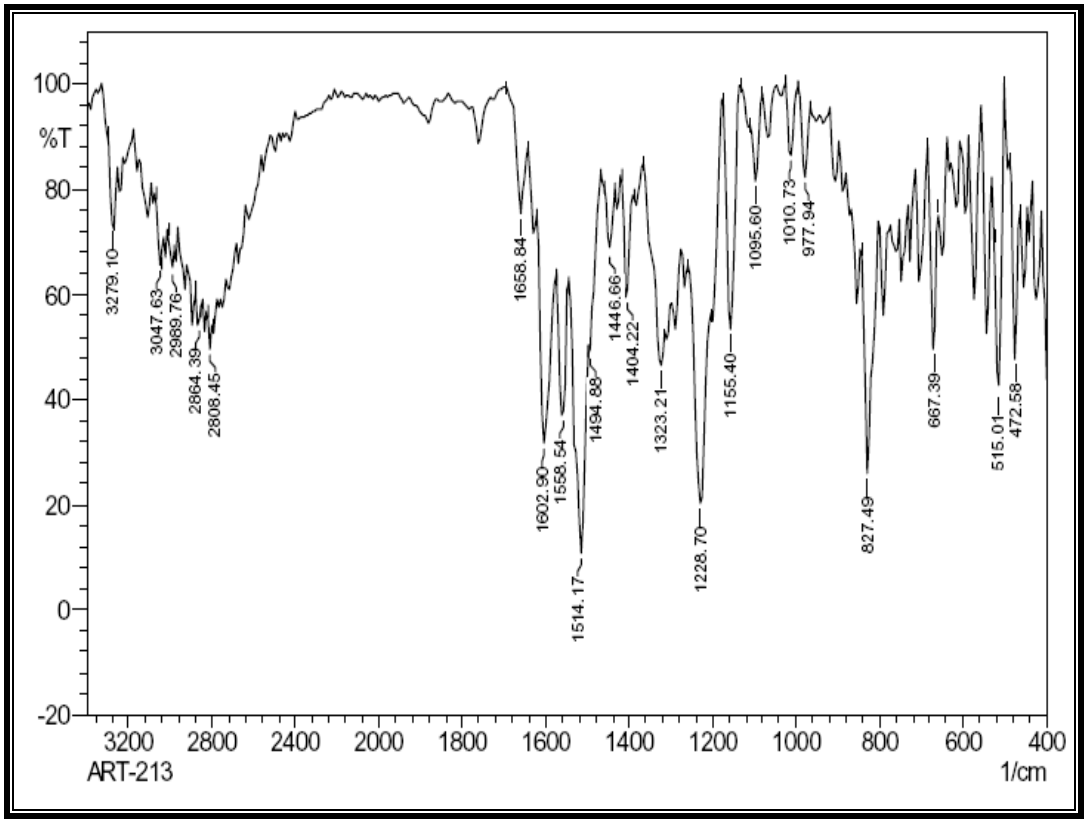
^{1}H NMR spectrum of ART-212



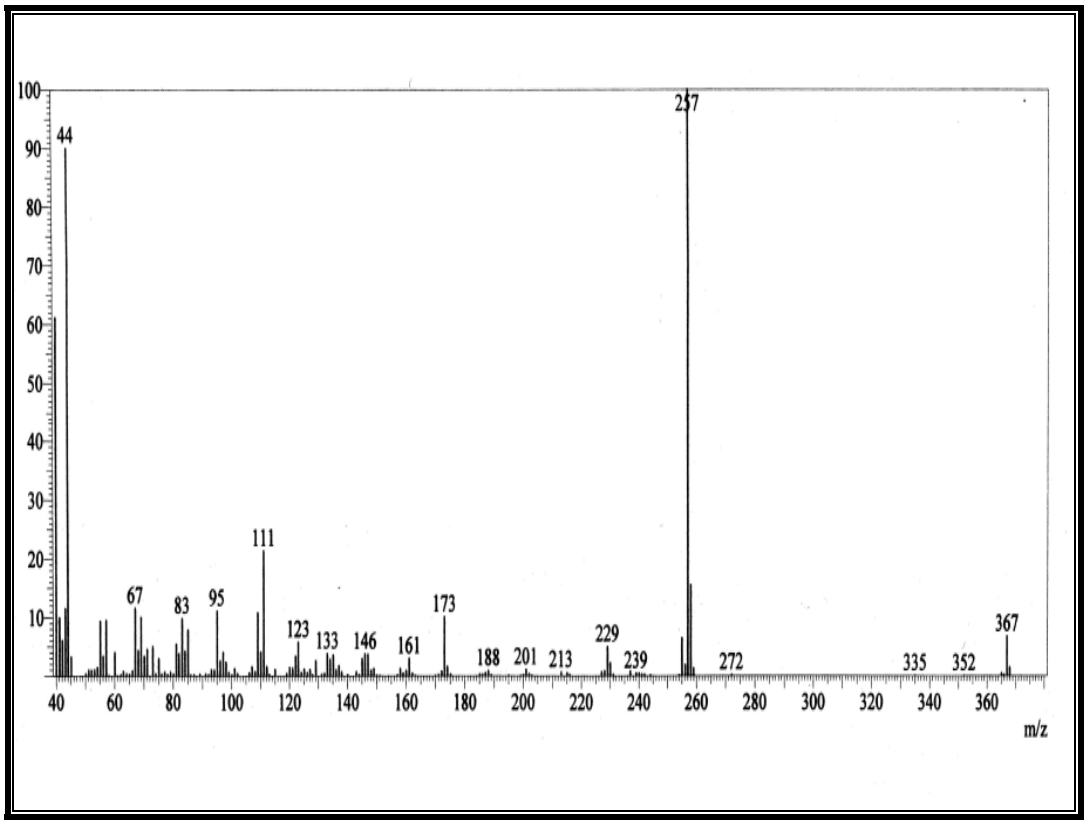
Expanded ^{1}H NMR spectrum of ART-212



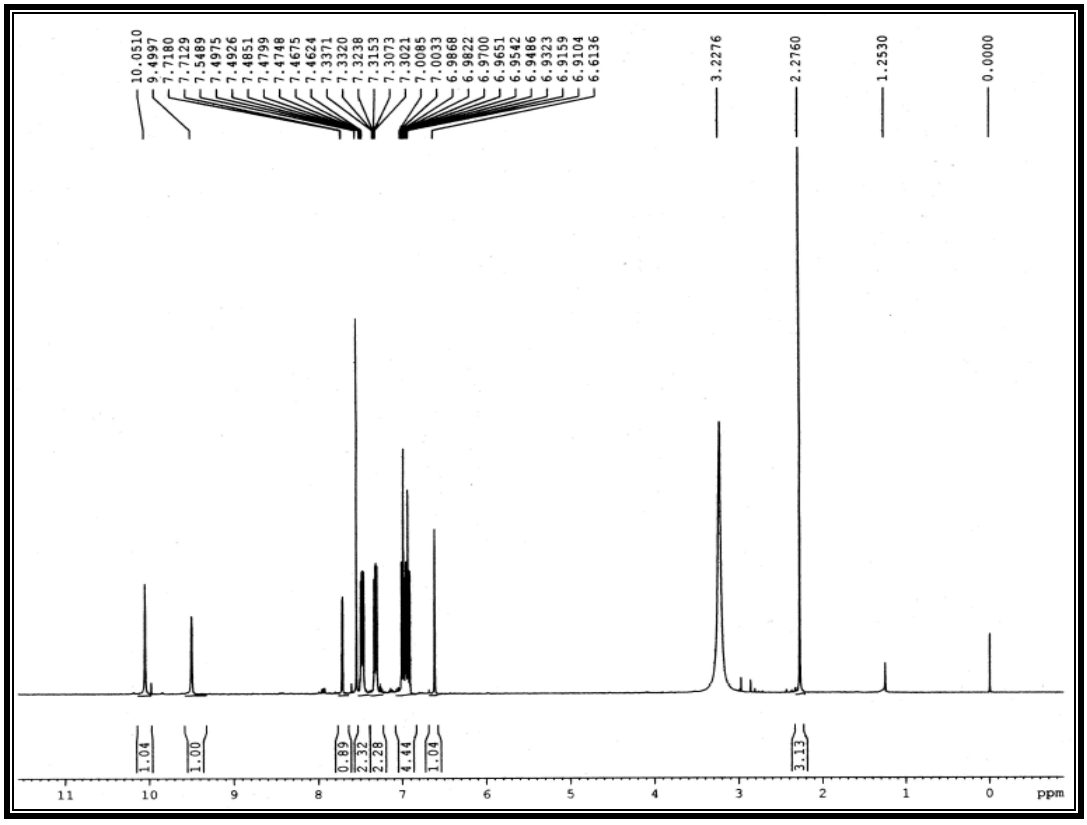
IR spectrum of ART-213



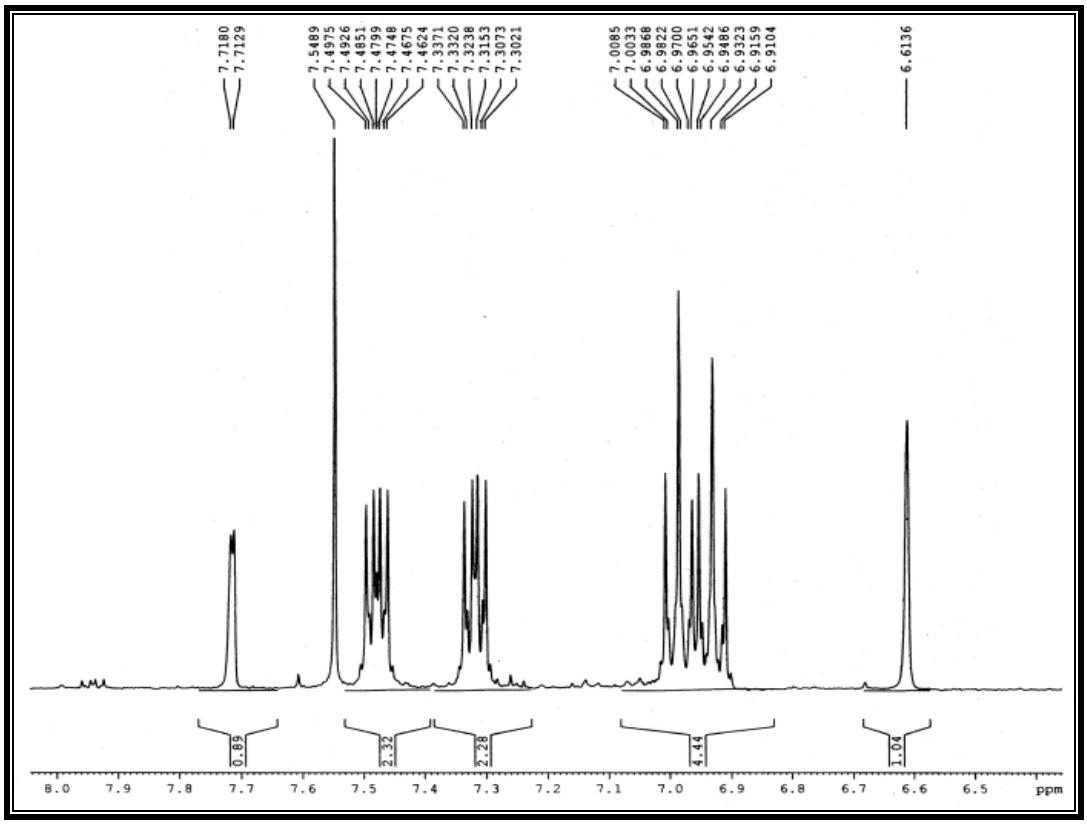
Mass spectrum of ART-213



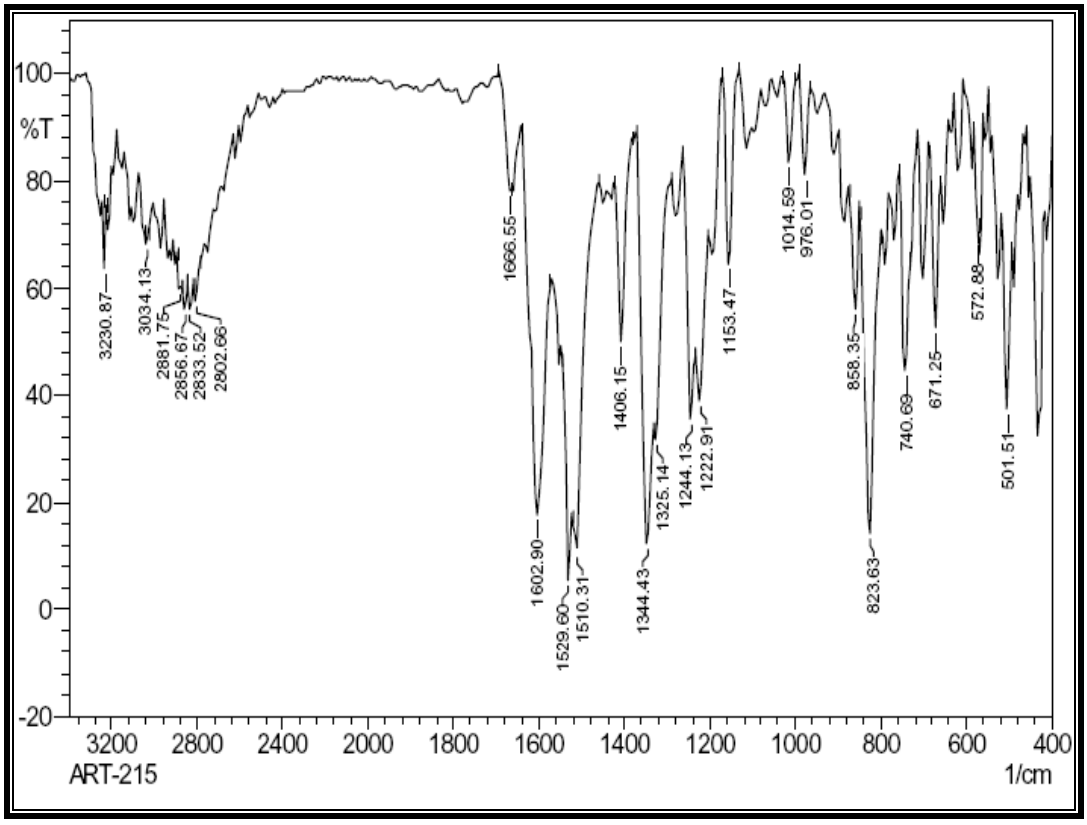
^{1}H NMR spectrum of ART-213



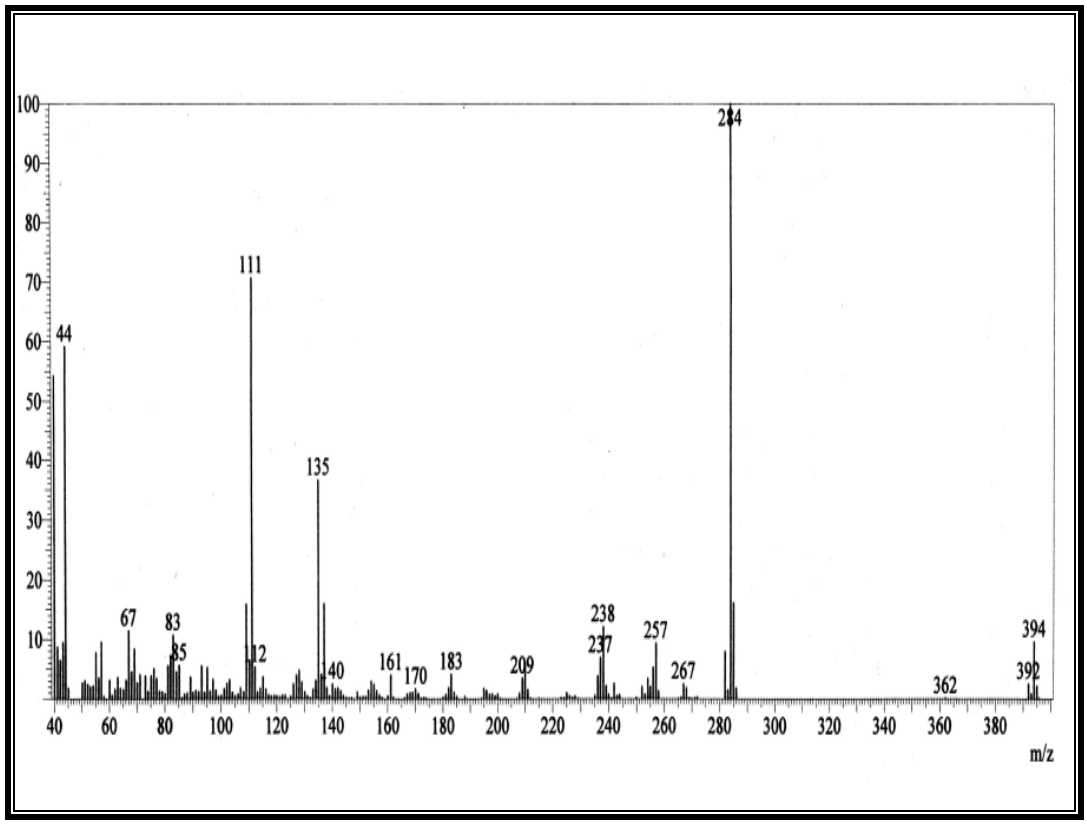
Expanded ^{1}H NMR spectrum of ART-213



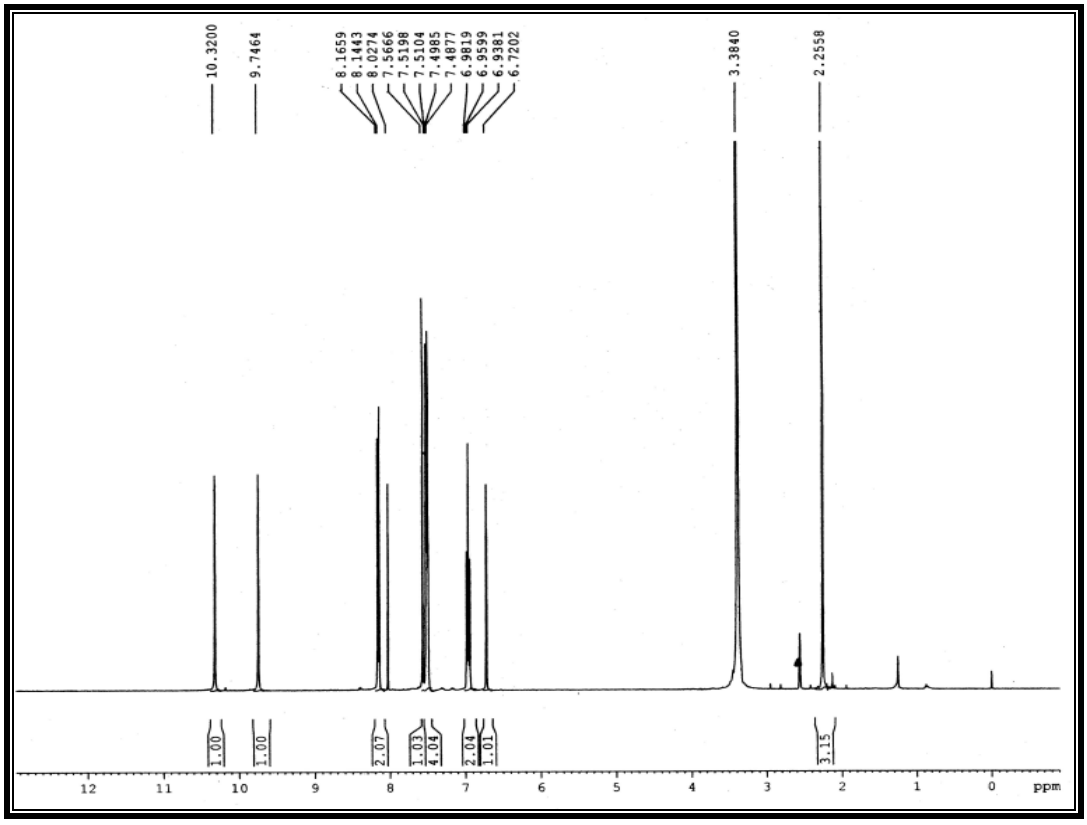
IR spectrum of ART-215



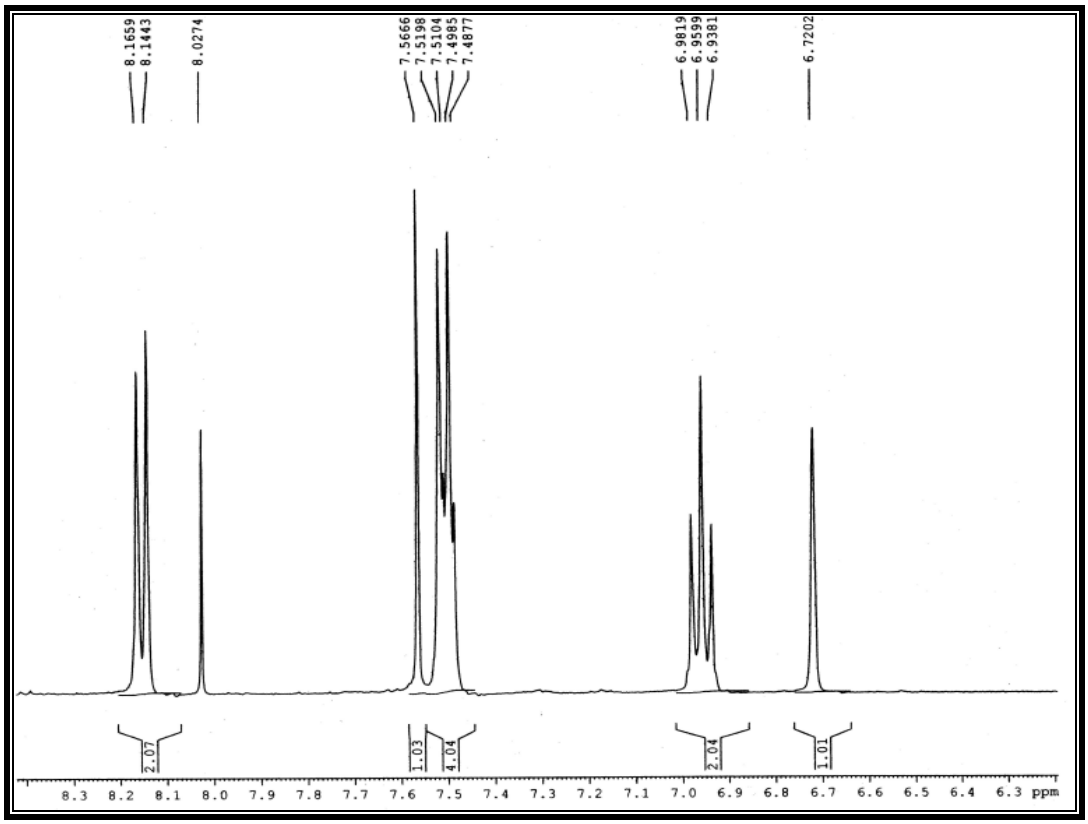
Mass spectrum of ART-215



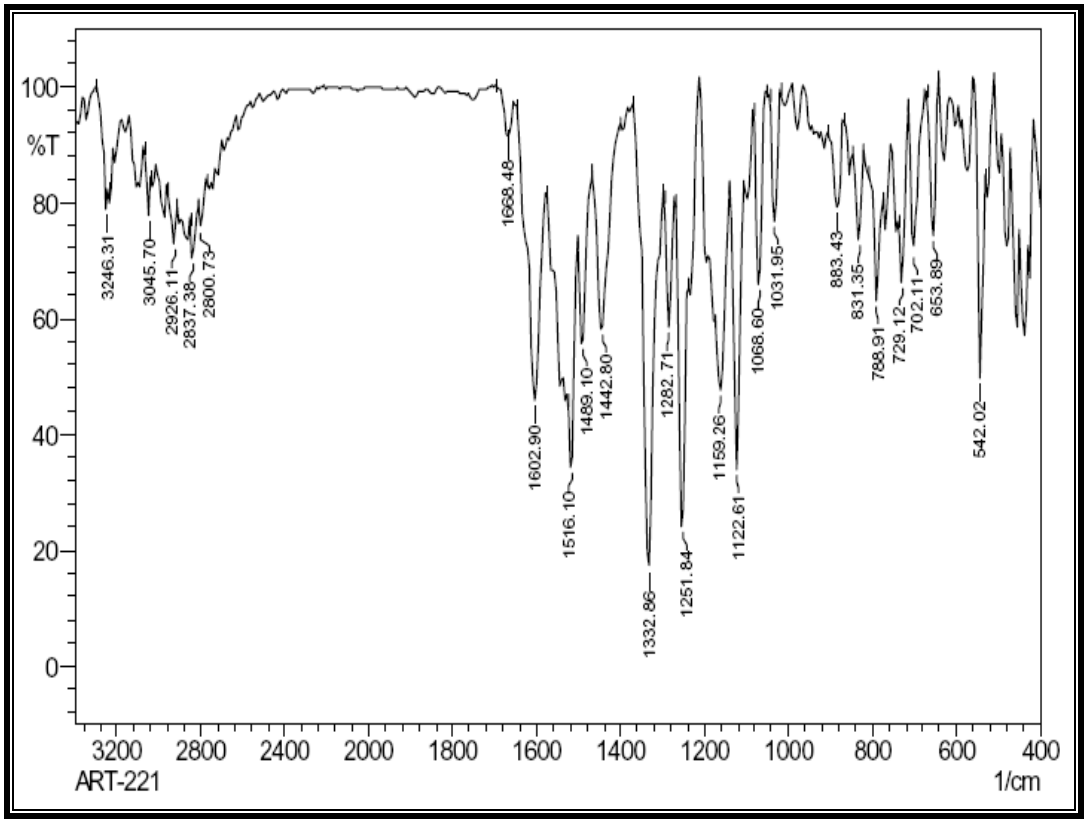
^{1}H NMR spectrum of ART-215



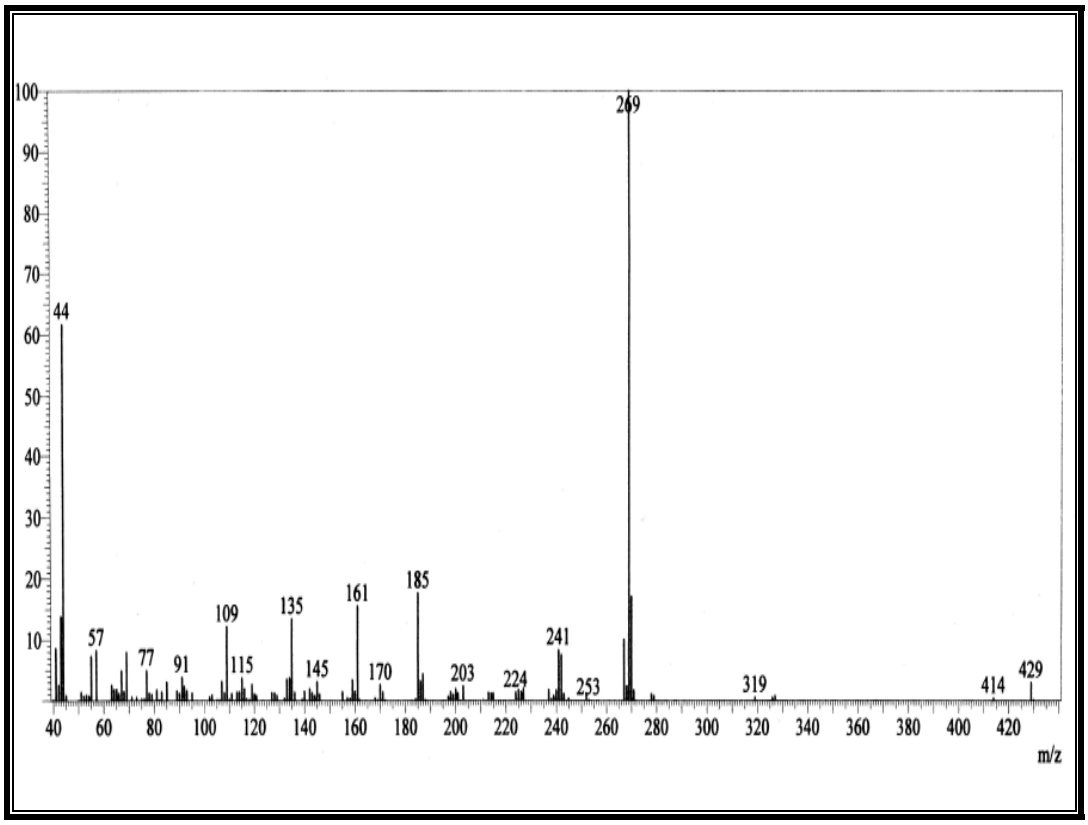
Expanded ^{1}H NMR spectrum of ART-215



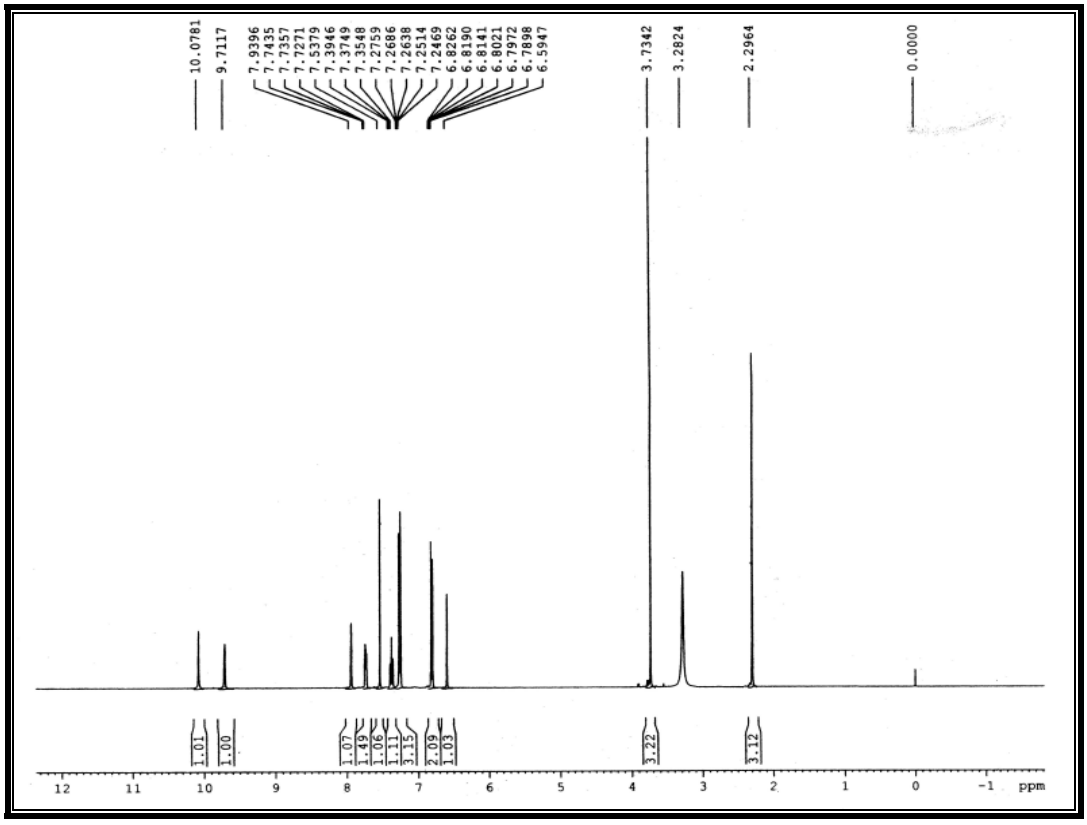
IR spectrum of ART-221



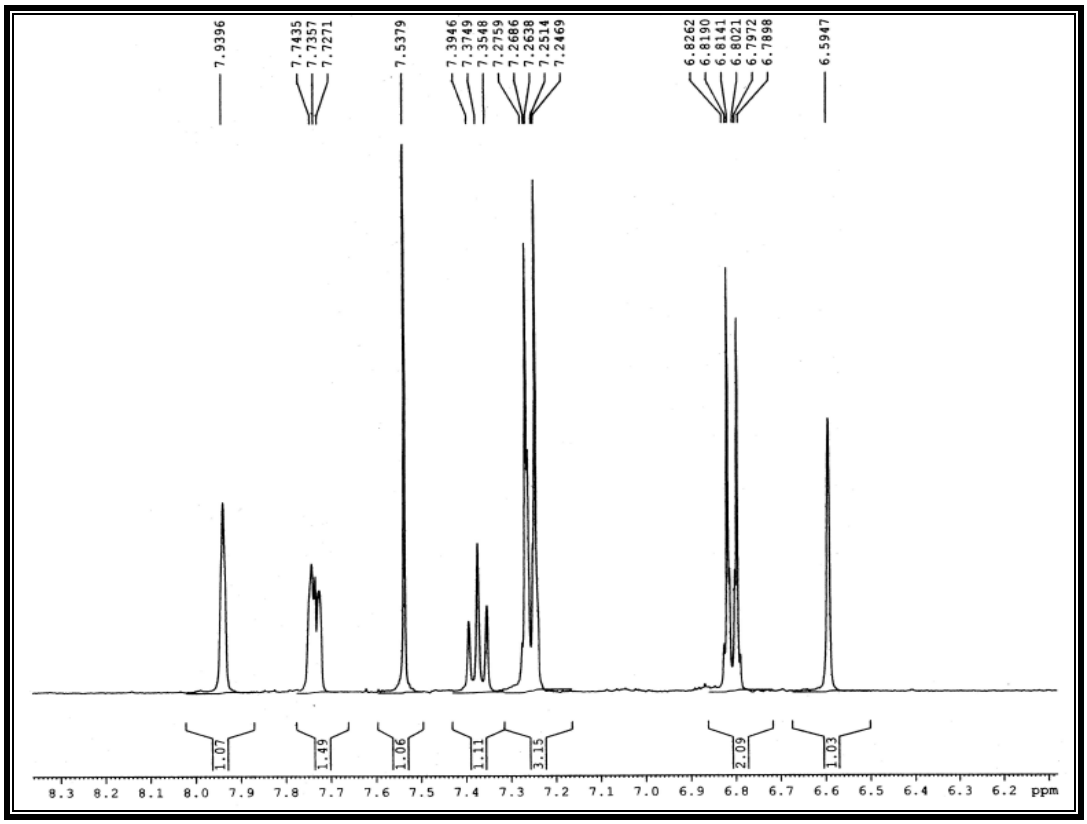
Mass spectrum of ART-221



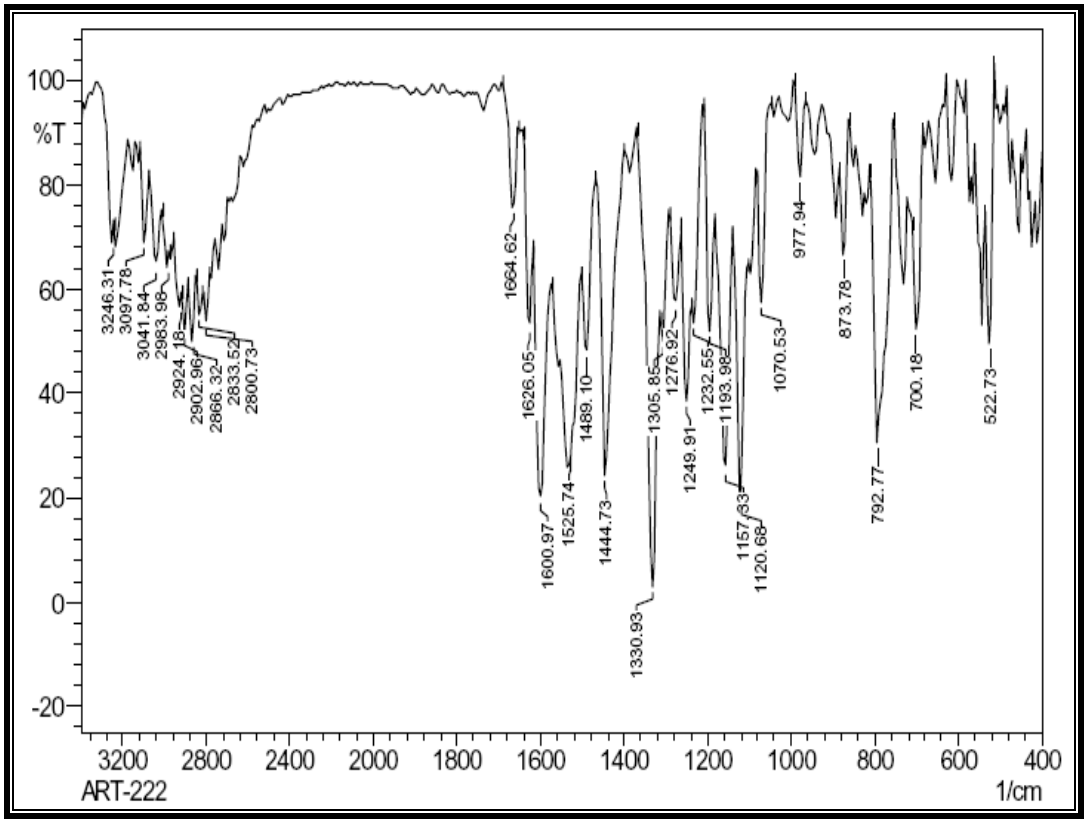
^{1}H NMR spectrum of ART-221



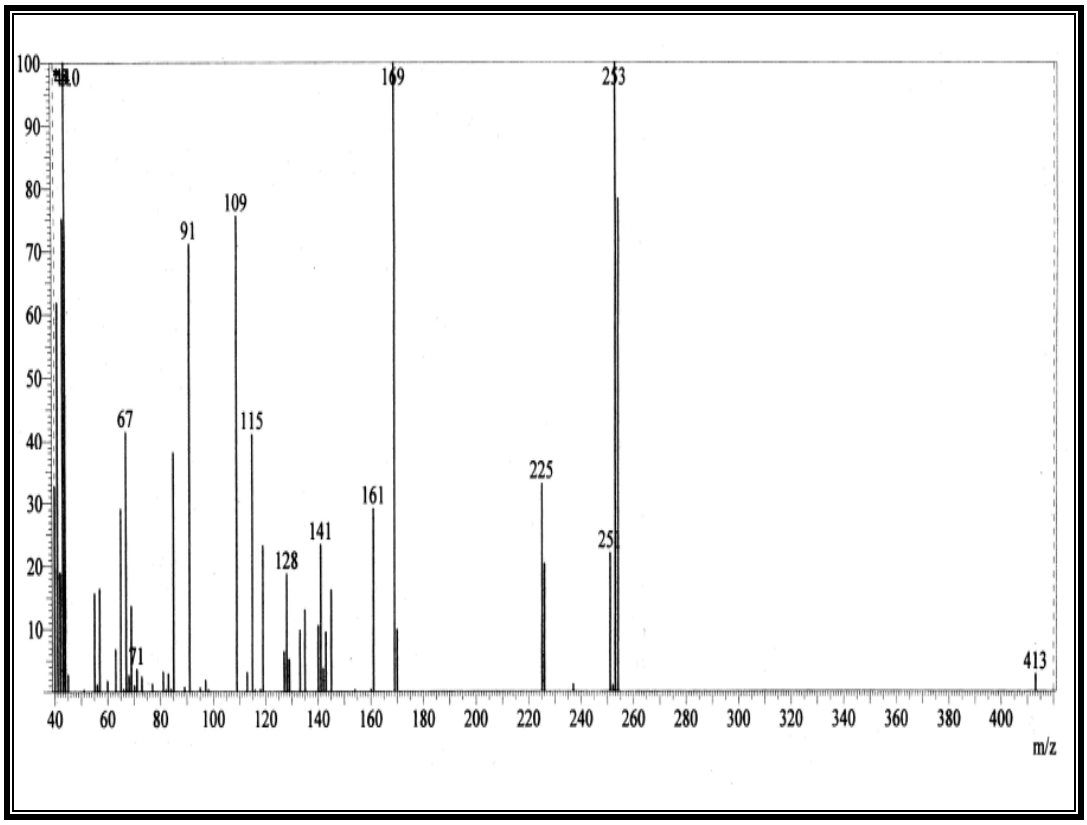
Expanded ^{1}H NMR spectrum of ART-221



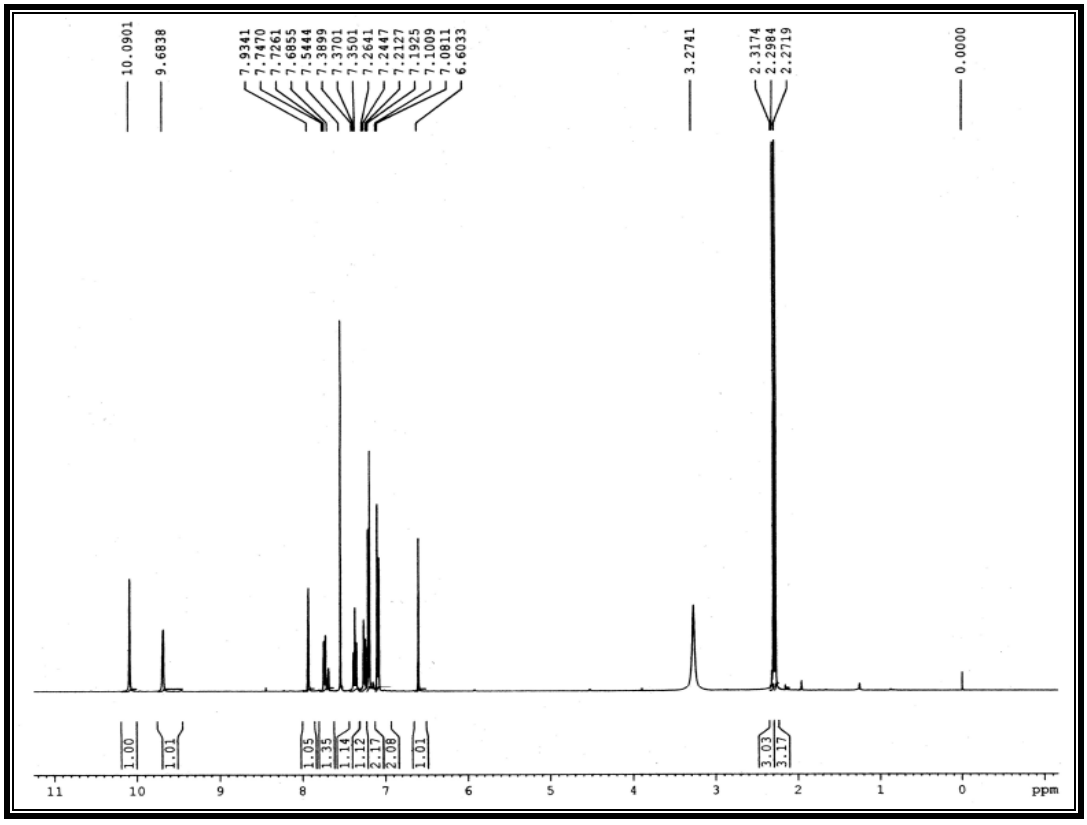
IR spectrum of ART-222



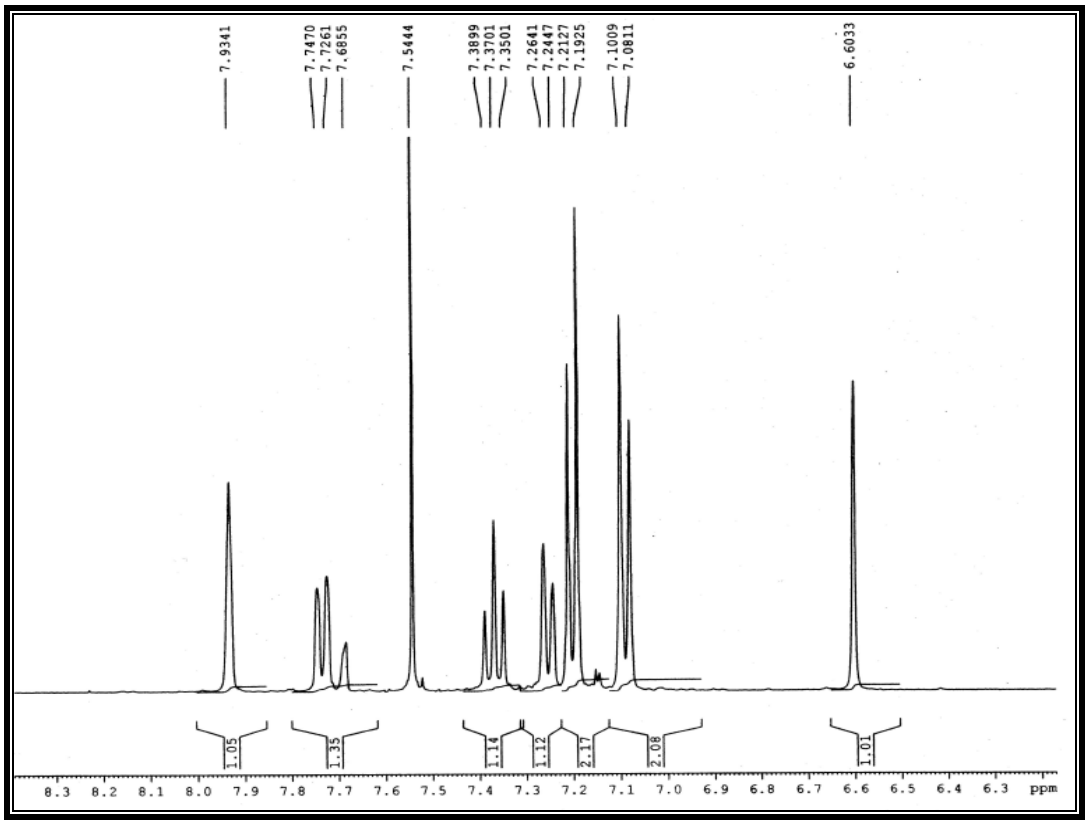
Mass spectrum of ART-222



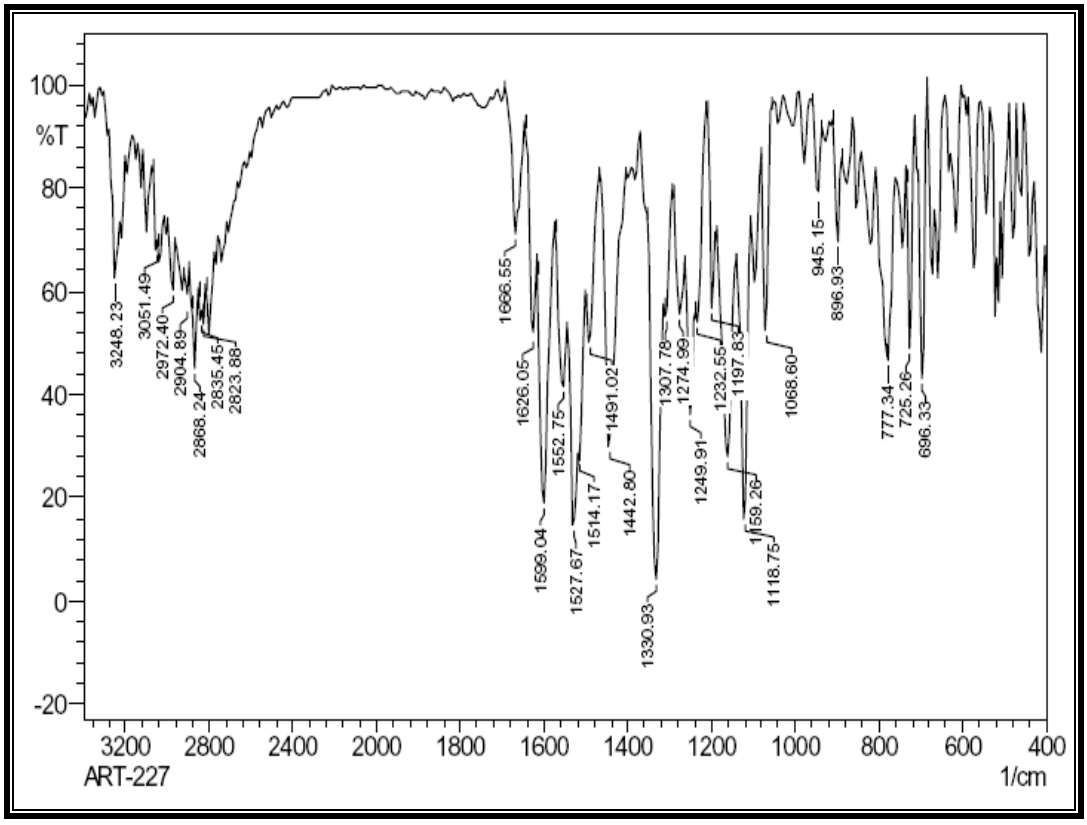
^{1}H NMR spectrum of ART-222



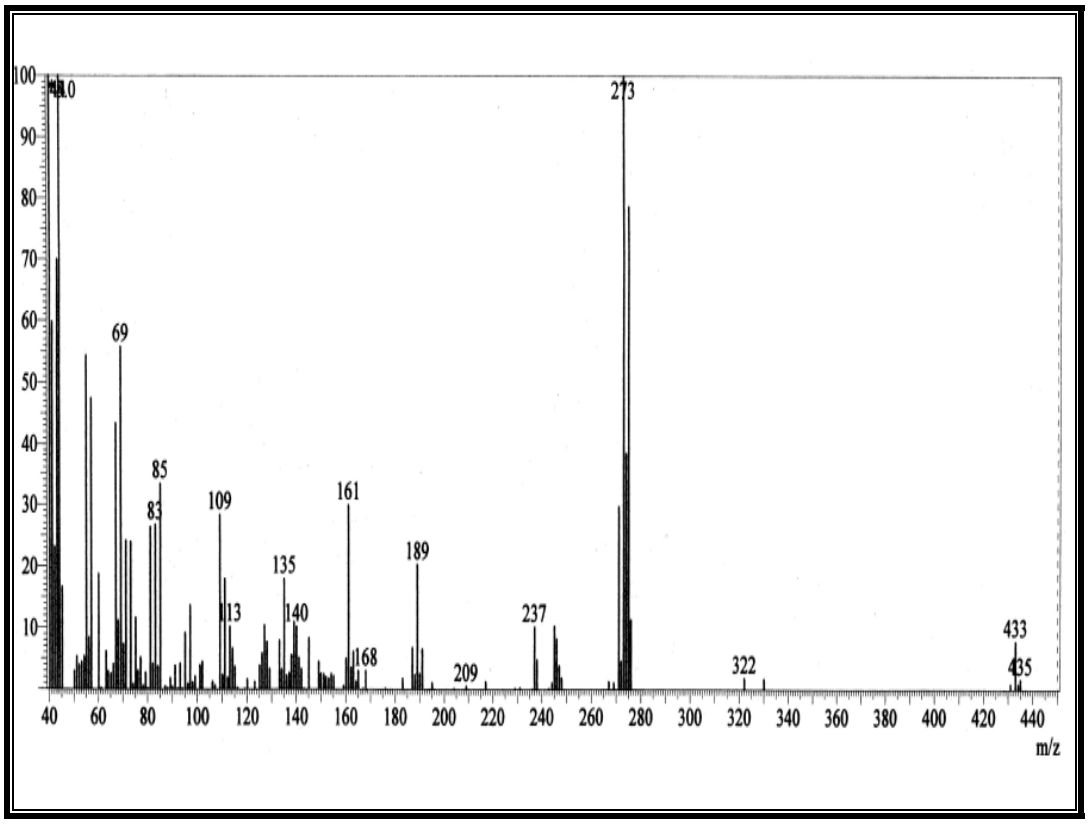
Expanded ^{1}H NMR spectrum of ART-222



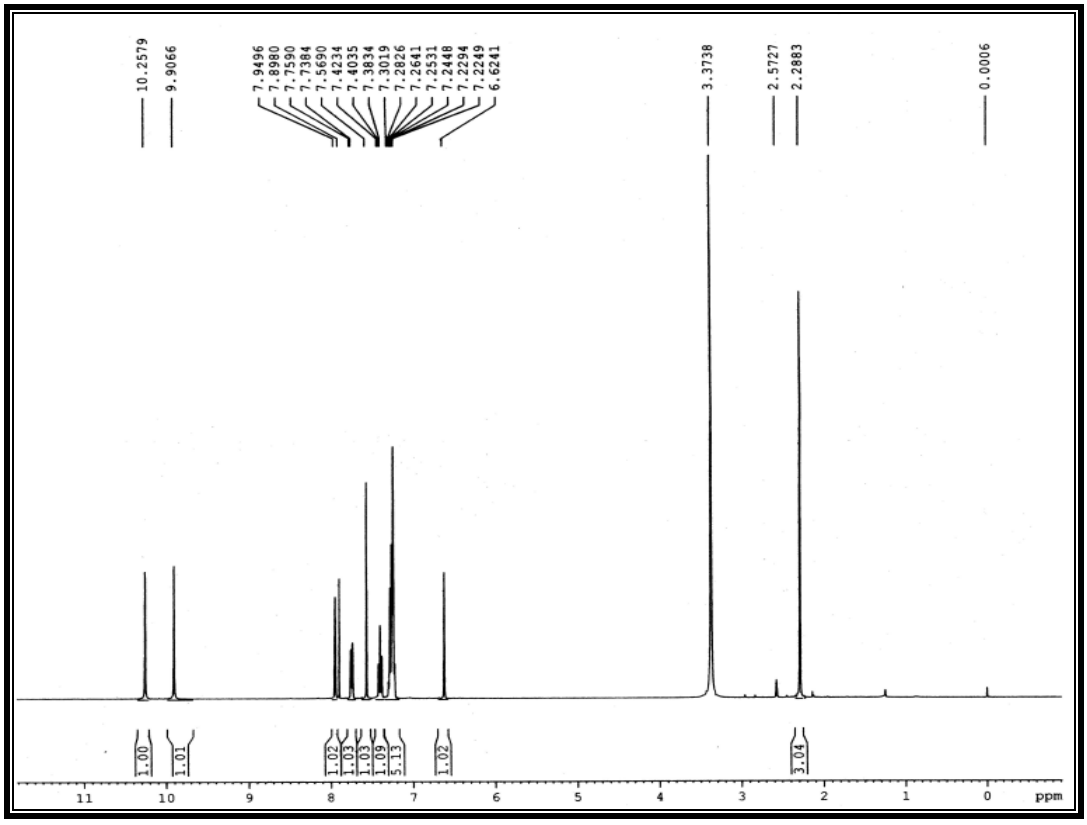
IR spectrum of ART-227



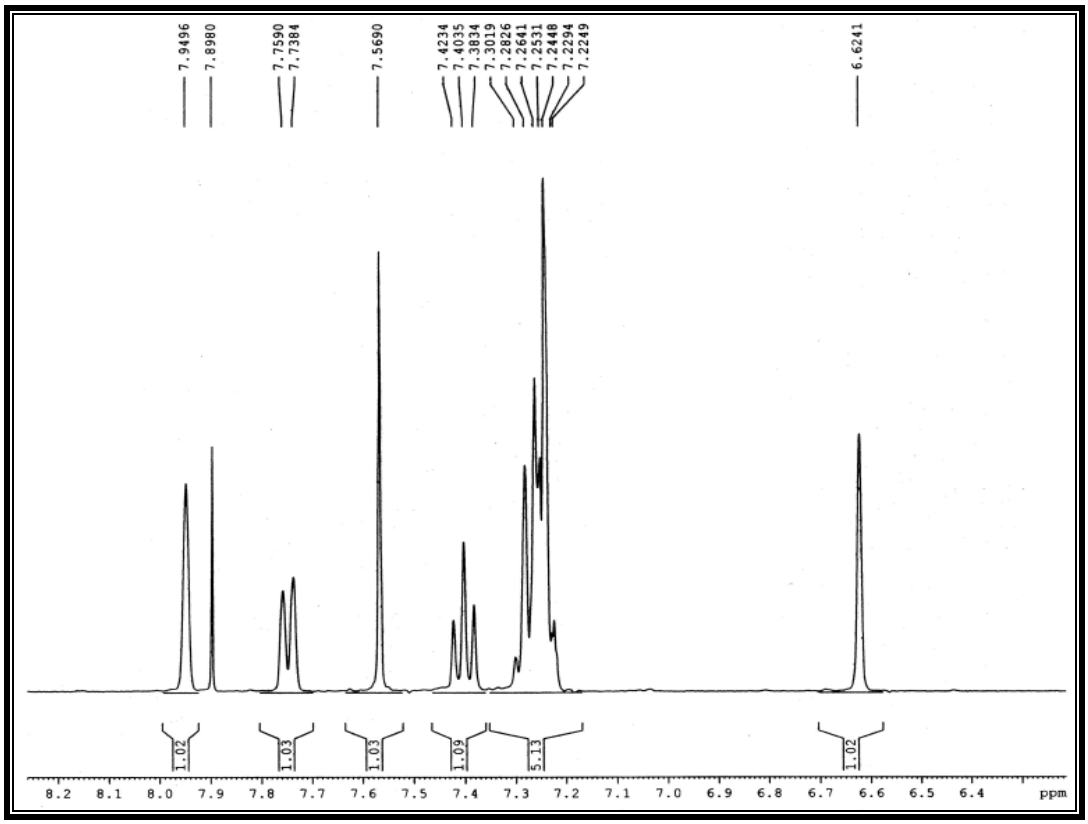
Mass spectrum of ART-227



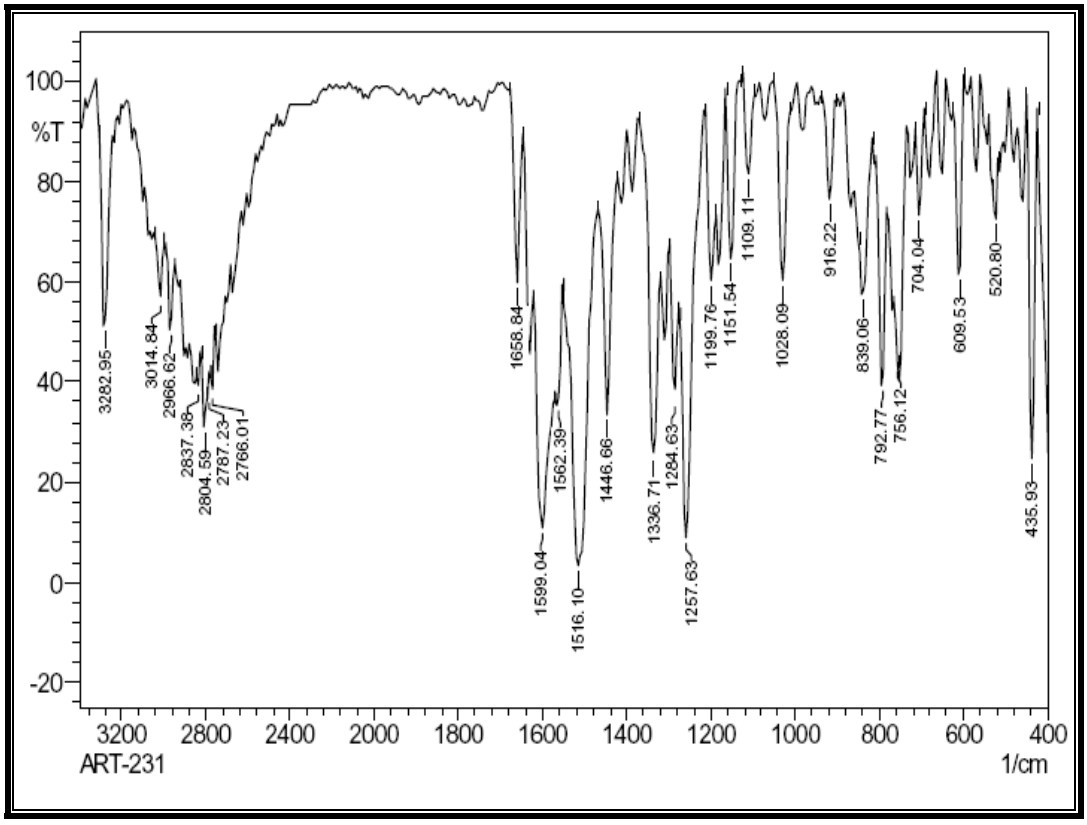
^{1}H NMR spectrum of ART-227



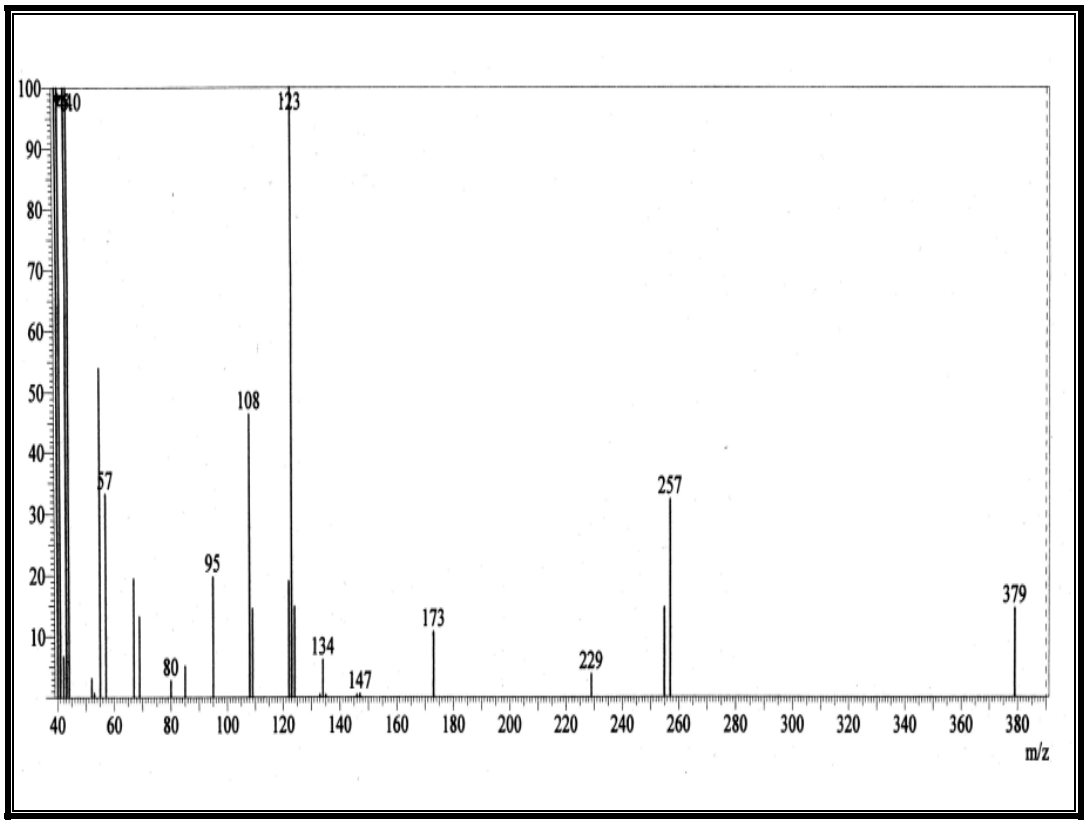
Expanded ^{1}H NMR spectrum of ART-227



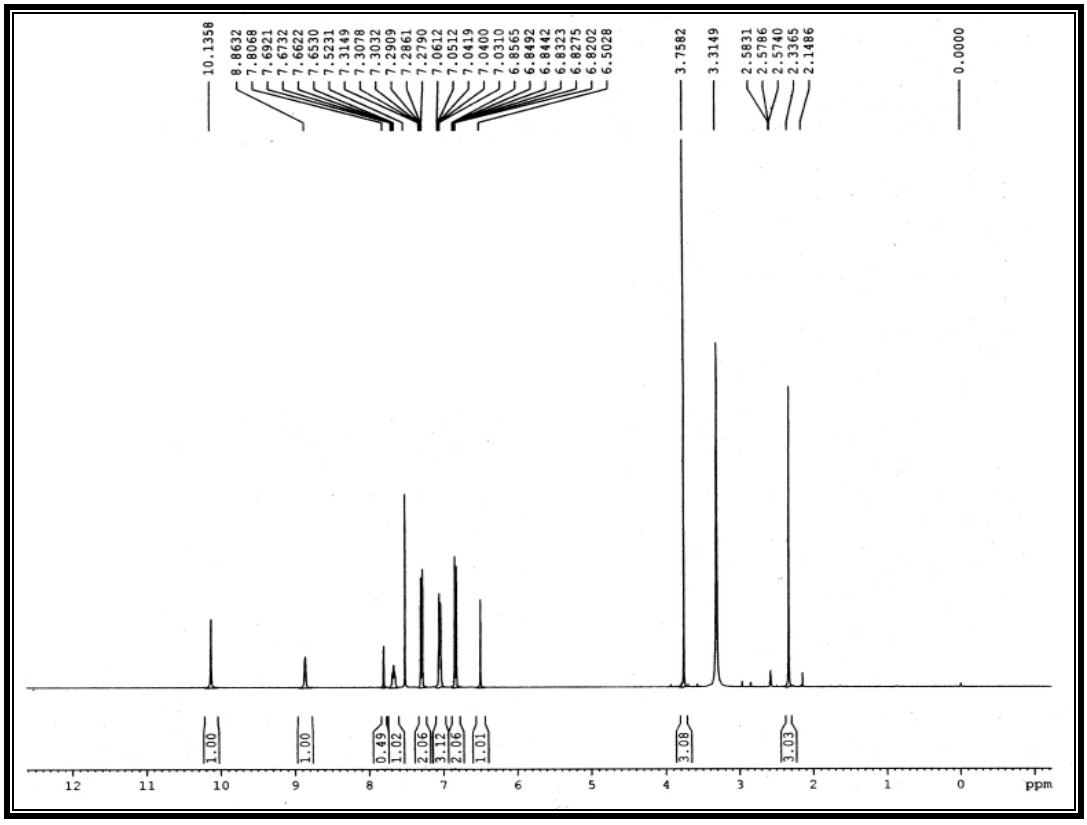
IR spectrum of ART-231



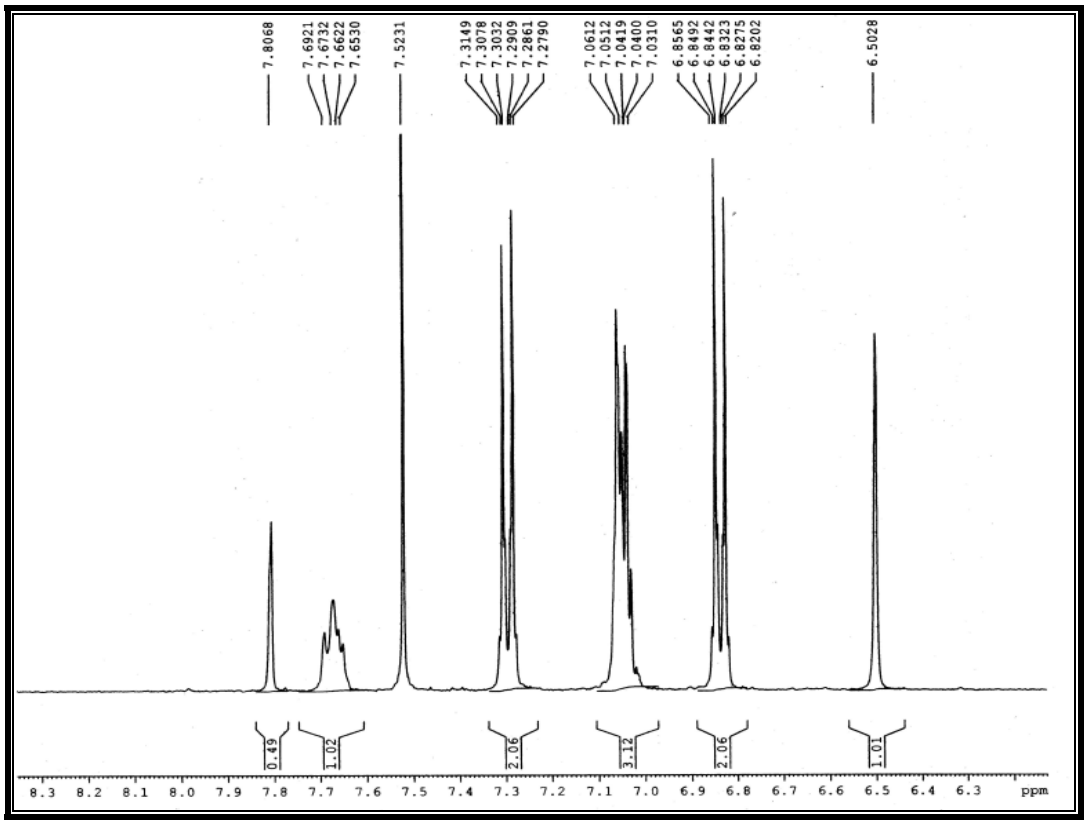
Mass spectrum of ART-231



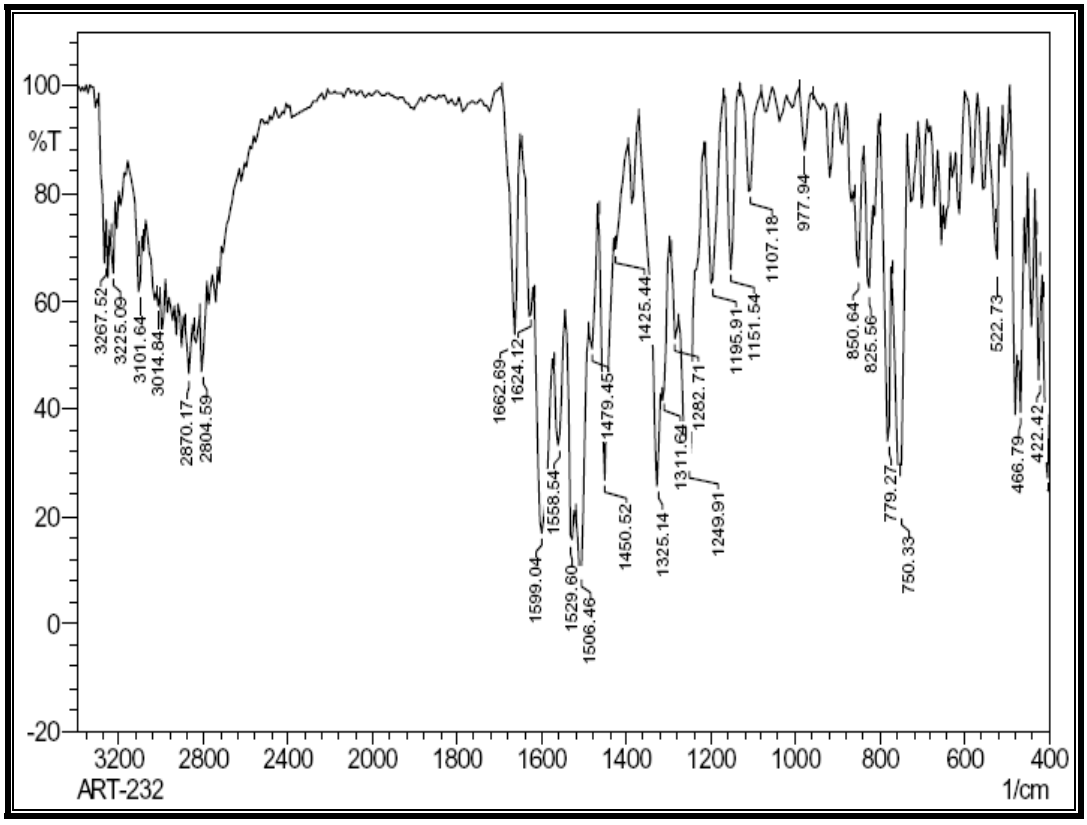
^{1}H NMR spectrum of ART-231



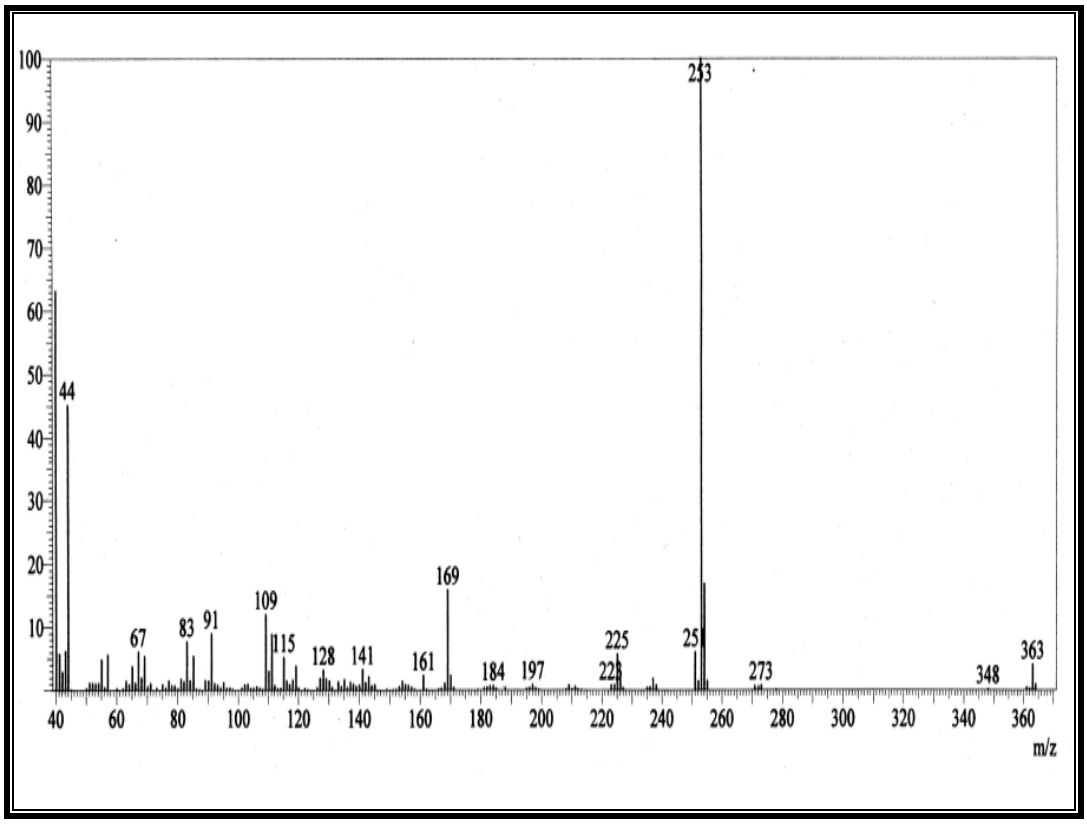
Expanded ^{1}H NMR spectrum of ART-231



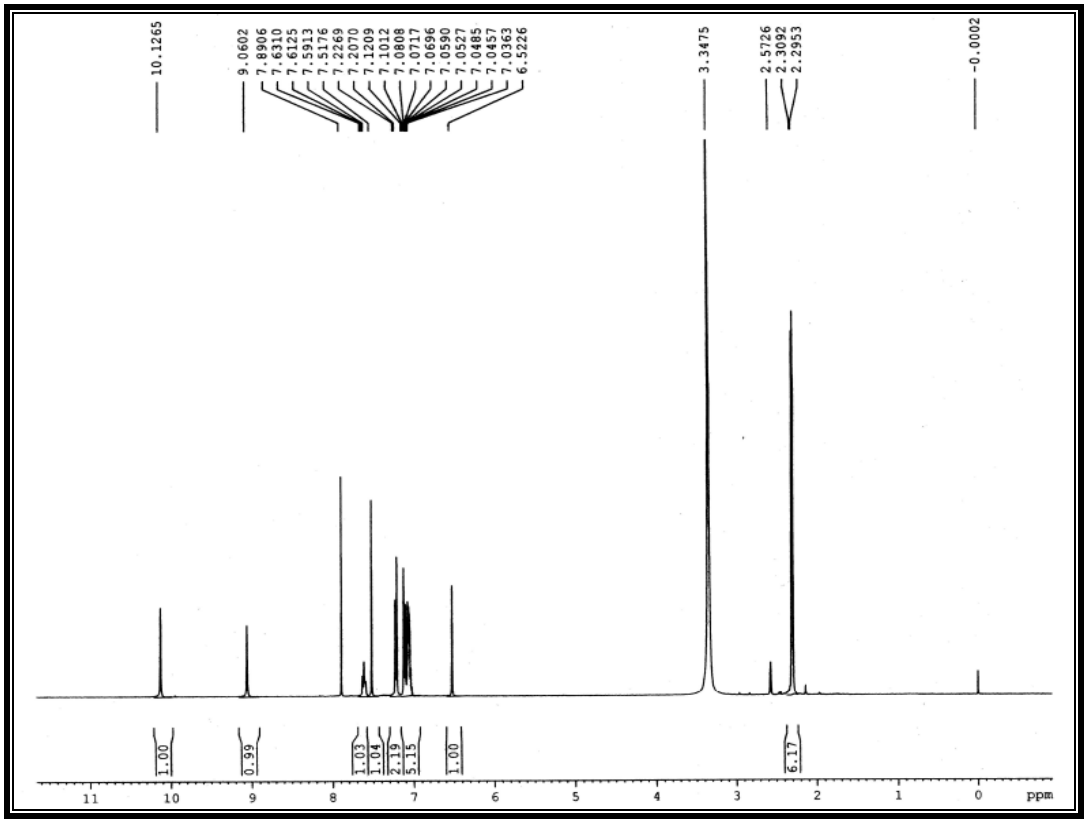
IR spectrum of ART-232



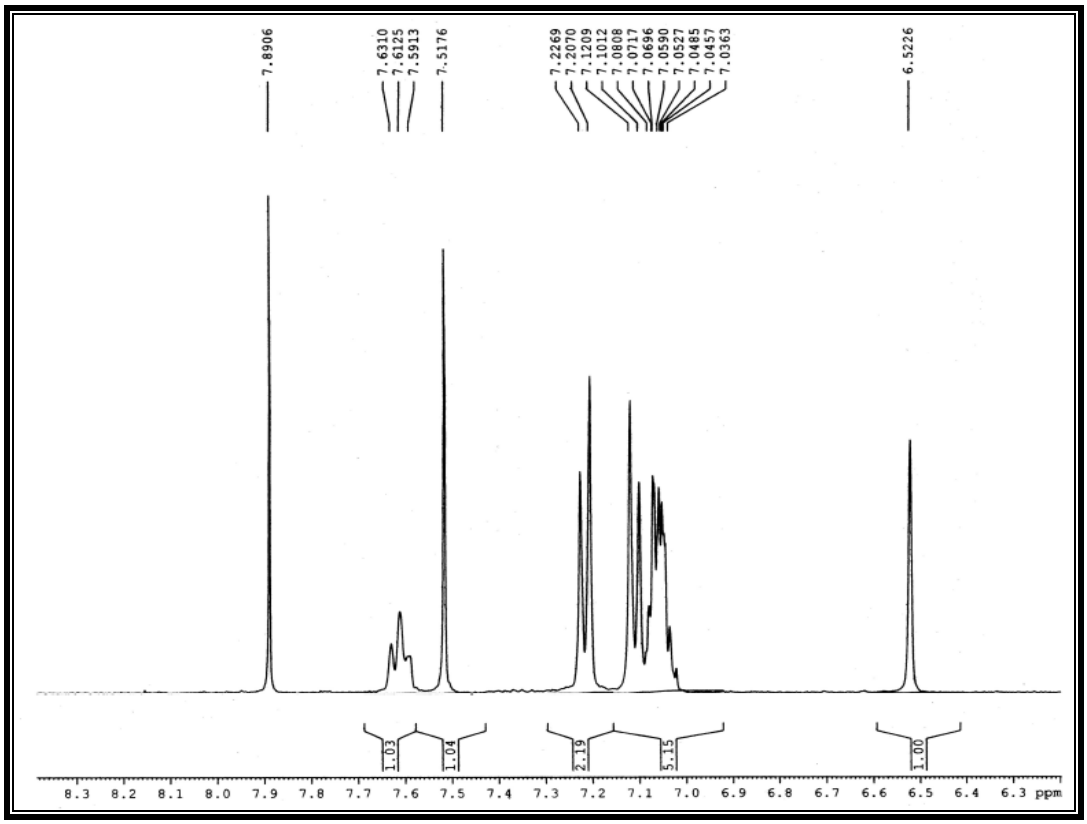
Mass spectrum of ART-232



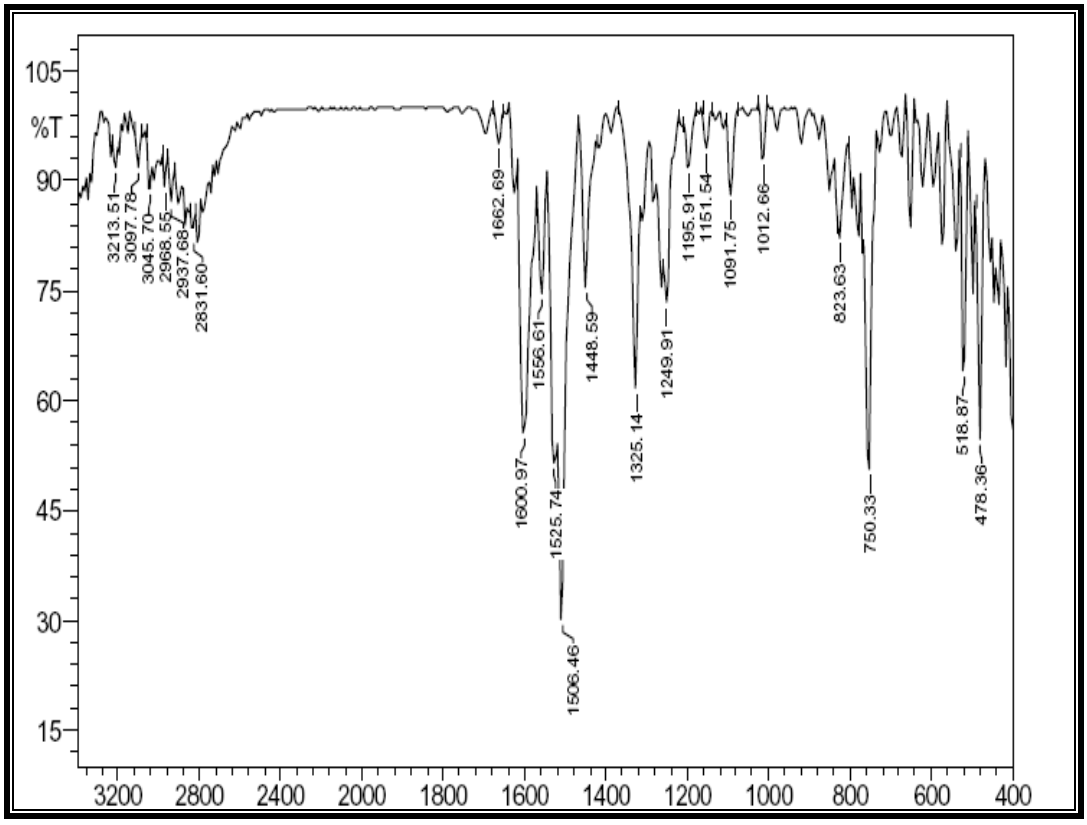
^{1}H NMR spectrum of ART-232



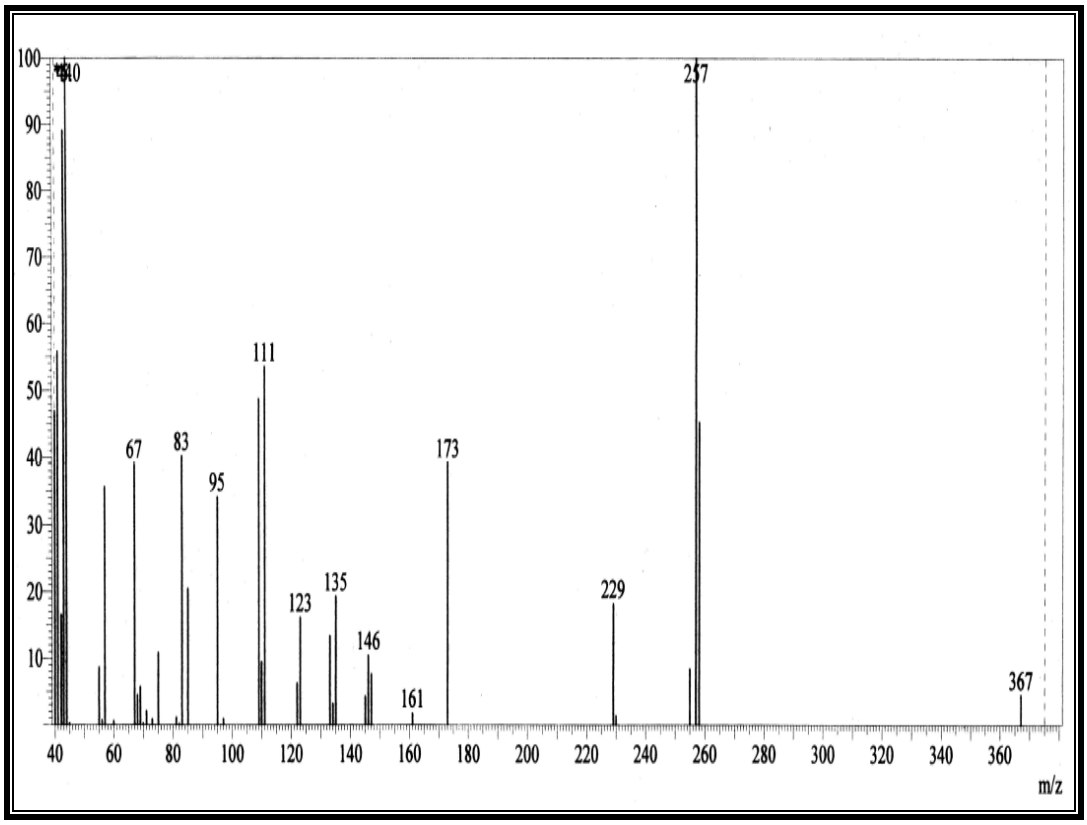
Expanded ^{1}H NMR spectrum of ART-232



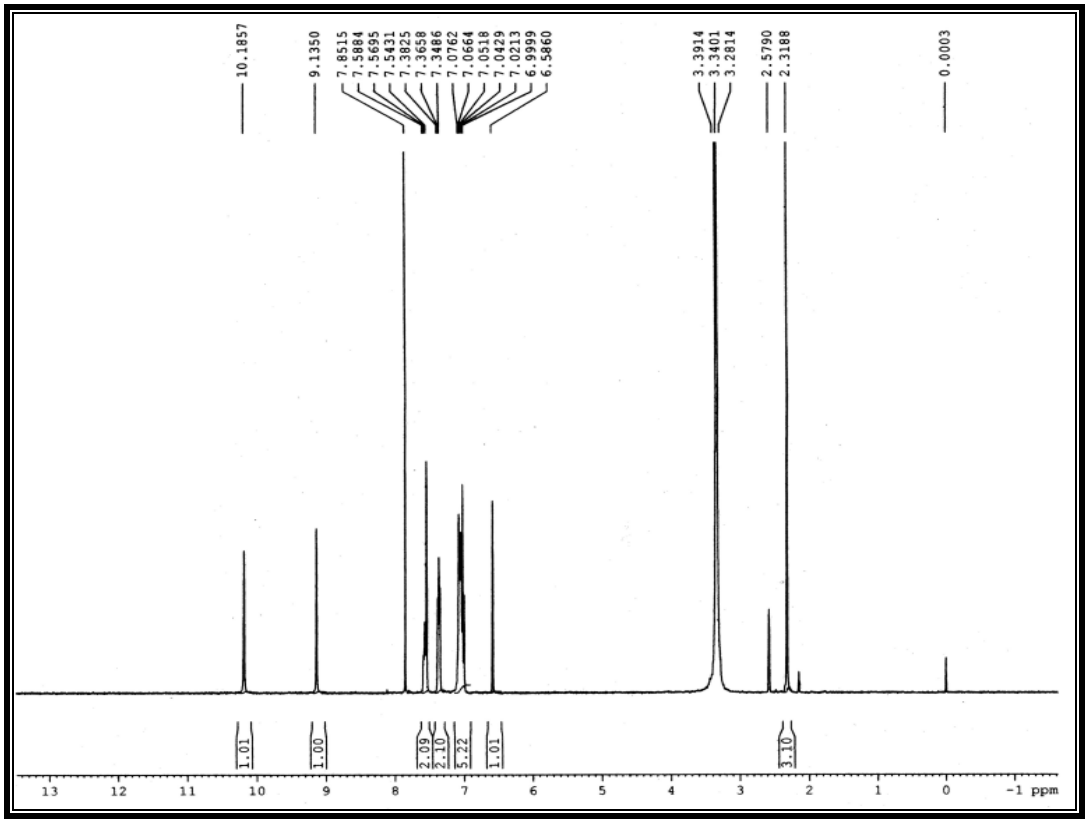
IR spectrum of ART-233



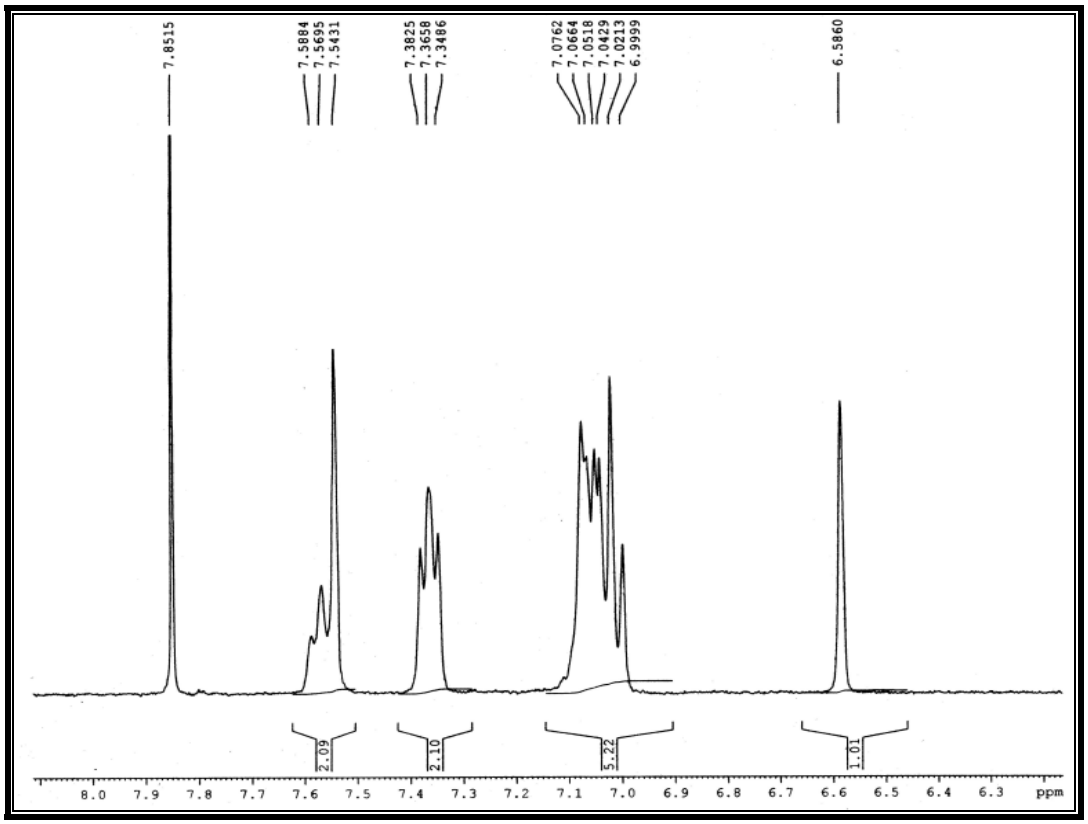
Mass spectrum of ART-233



^{1}H NMR spectrum of ART-233



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



4.8 Biological evaluation

4.8.1 Antimicrobial evaluation

All of the synthesized compounds (ART-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 ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, 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 microdilution broth method according to NCCLS standards [98]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 μg mL^{-1}. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds ART-201 to 240

Code	Minimum 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.*
ART-201	500	1000	500	100	1000	500	500
ART-202	1000	500	1000	1000	500	500	1000
ART-203	500	500	250	500	>1000	1000	500
ART-204	250	62.5	125	250	1000	500	250
ART-205	125	100	1000	500	100	1000	500
ART-206	500	1000	250	1000	500	500	>1000
ART-207	1000	250	500	500	500	100	250
ART-208	100	125	100	62.5	>1000	1000	1000
ART-209	100	>1000	500	1000	>1000	500	1000
ART-210	25	500	250	100	500	1000	>1000
ART-211	1000	100	100	500	250	100	250
ART-212	125	100	100	500	500	250	1000
ART-213	500	500	1000	>1000	1000	500	125
ART-214	125	100	50	250	500	1000	500
ART-215	100	1000	250	1000	1000	500	1000
ART-216	50	500	250	250	>1000	1000	>1000
ART-217	500	1000	500	1000	500	500	100
ART-218	125	25	100	100	500	>1000	500
ART-219	125	500	500	250	100	1000	250
ART-220	>1000	250	>1000	500	1000	1000	500
ART-221	1000	500	1000	500	>1000	1000	1000
ART-222	500	>1000	500	1000	500	500	1000
ART-223	500	1000	1000	>1000	100	>1000	>1000
ART-224	250	125	250	500	500	500	1000
ART-225	1000	500	>1000	1000	250	500	500
ART-226	50	25	125	500	1000	1000	500
ART-227	250	250	100	500	100	500	500
ART-228	100	500	125	1000	500	1000	1000
ART-229	250	1000	250	500	500	250	250
ART-230	50	125	250	62.5	1000	1000	500
ART-231	500	500	500	>1000	100	500	250
ART-232	1000	500	1000	1000	500	500	100
ART-233	500	1000	1000	500	500	100	500
ART-234	62.5	100	250	125	1000	>1000	1000
ART-235	1000	500	500	1000	500	100	500
ART-236	100	125	500	500	250	500	100
ART-237	250	1000	1000	250	500	1000	1000
ART-238	100	250	100	500	500	1000	1000
ART-239	250	125	250	250	100	1000	250
ART-240	62.5	100	100	250	250	500	250
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

4.8.2 Antimycobacterial, anticancer and antiviral evaluation

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

4.9 References and notes

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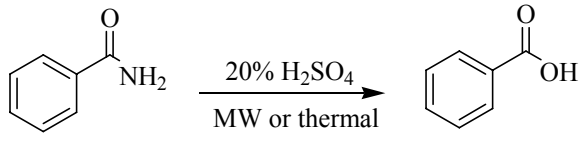
Chapter 5
Microwave assisted synthesis and biological evaluation of pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines

5.1 Microwave-Assisted Organic Synthesis (MAOS) – A Brief History

While fire is now rarely used in synthetic chemistry, it was not until Robert Bunsen invented the burner in 1855 that the energy from this heat source could be applied to a reaction vessel in a focused manner. The Bunsen burner was later superseded by the isomantle, the oil bath or the hot plate as a means of applying heat to a chemical reaction. In the past few years, heating and driving chemical reactions by microwave energy has been an increasingly popular theme in the scientific community [1, 2].

Microwave energy, originally applied for heating foodstuffs by Percy Spencer in the 1940s, has found a variety of technical applications in the chemical and related industries since the 1950s, in particular in the food-processing, drying and polymer industries. Other applications range from analytical chemistry (microwave digestion, ashing and extraction) [3] to biochemistry (protein hydrolysis, sterilization) [3], pathology (histoprocessing, tissue fixation) [4] and medical treatments (diathermy) [5]. Somewhat surprisingly, microwave heating has only been implemented in organic synthesis since the mid-1980s. The first reports on the use of microwave heating to accelerate organic chemical transformations (MAOS) were published by the groups of Richard Gedye (Scheme 5.1) [6] and Raymond J. Giguere/George Majetich [7] in 1986.



Thermal: 1h, 90% yield (reflux)
MW: 10 min, 99% yield (sealed vessel)

Scheme 5.1 Hydrolysis of benzamide: The first published example (1986) of microwave-assisted organic synthesis.

In those early days, experiments were typically carried out in sealed teflon or glass vessels in a domestic household microwave oven without any temperature or pressure measurements. The results were often violent explosions due to the rapid uncontrolled heating of organic solvents under closed-vessel conditions. In the 1990s, several groups started to experiment with solvent-free microwave chemistry (so-called dry-media reactions), which eliminated the danger of explosions [8]. Here, the reagents were pre-adsorbed onto either an essentially microwave-transparent (i.e., silica, alumina or clay) or strongly absorbing (i.e., graphite) inorganic support, that additionally may have been doped with a catalyst or reagent. Particularly in the early days of MAOS, the solvent-free approach was very popular since it allowed the safe use of domestic microwave ovens and standard open-vessel technology. While a large number of interesting transformations using "dry-media" reactions have been published in the literature [8], technical difficulties relating to non-uniform heating, mixing and the precise determination of the reaction temperature remained unresolved, in particular when scale-up issues needed to be addressed.

Alternatively, microwave-assisted synthesis has been carried out using standard organic solvents under open-vessel conditions. If solvents are heated by microwave irradiation at atmospheric pressure in an open vessel, the boiling point of the solvent typically limits the reaction temperature that can be achieved. In order to nonetheless achieve high reaction rates, high-boiling microwave-absorbing solvents have been frequently used in open-vessel microwave synthesis [9]. However, the use of these solvents presented serious challenges in relation to product isolation and recycling of the solvent. Because of the recent availability of modern microwave reactors with on-line monitoring of both temperature and pressure, MAOS in dedicated sealed vessels using standard solvents-a technique pioneered by Christopher R. Strauss in the mid-1990s [10]-has been celebrating a comeback in recent years. This is clearly evident surveying the recently published (since 2001) literature in the area of controlled microwave-assisted organic synthesis (MAOS). It appears that the combination of rapid heating by microwaves with sealed-vessel (autoclave) technology will most likely be the method of choice for performing MAOS on a laboratory scale in the future. Importantly, recent innovations in microwave reactor technology now allow controlled parallel and automated sequential

processing under sealed-vessel conditions, and the use of continuous- or stop-flow reactors for scale-up purposes.

Since the early days of microwave synthesis, the observed rate accelerations and sometimes altered product distributions compared to oil-bath experiments have led to speculation on the existence of so-called “specific” or “non-thermal” microwave effects [11]. Historically, such effects were claimed when the outcome of a synthesis performed under microwave conditions was different from that of the conventionally heated counterpart at the same apparent temperature. Reviewing the present literature [12], it appears that today most scientists agree that in the majority of cases the reason for the observed rate enhancements is a purely thermal/kinetic effect, i.e., a consequence of the high reaction temperatures that can rapidly be attained when irradiating polar materials in a microwave field, although effects that are caused by the unique nature of the microwave dielectric heating mechanism (“specific microwave effects”) clearly also need to be considered. While for the medicinal chemist in industry this discussion may seem largely irrelevant, the debate on “microwave effects” is undoubtedly going to continue for many years in the academic world. Regardless of the nature of the observed rate enhancements, microwave synthesis has now truly matured and has moved from a laboratory curiosity in the late 1980s to an established technique in organic synthesis, heavily used in both academia and industry.

The initially slow uptake of the technology in the late 1980s and 1990s has been attributed to its lack of controllability and reproducibility, coupled with a general lack of understanding of the basics of microwave dielectric heating. The risks associated with the flammability of organic solvents in a microwave field and the lack of available dedicated microwave reactors allowing for adequate temperature and pressure control were major concerns. Important instrument innovations now allow for careful control of time, temperature and pressure profiles, paving the way for reproducible protocol development, scale-up and transfer from laboratory to laboratory and from scientist to scientist. Today, microwave chemistry is as reliable as the vast arsenal of synthetic methods that preceded it. Since 2001, therefore, the number of publications related to MAOS has increased dramatically, to such a level that it might be assumed that, in a few years, most chemists will probably use microwave energy to heat chemical reactions on a laboratory scale [1, 2]. Not only is

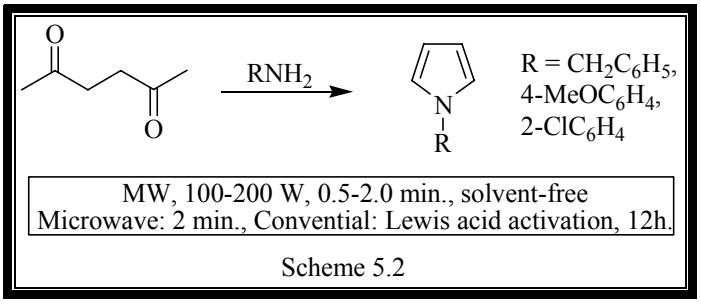
direct microwave heating able to reduce chemical reaction times significantly, but it is also known to reduce side reactions, increase yields and improve reproducibility. Therefore, many academic and industrial research groups are already using MAOS as a technology for rapid reaction optimization, for the efficient synthesis of new chemical entities or for discovering and probing new chemical reactivity.

5.2 Applications of microwaves in heterocyclic ring formation

5.2.1 Five-membered heterocyclic rings

5.2.1.1 Pyrroles

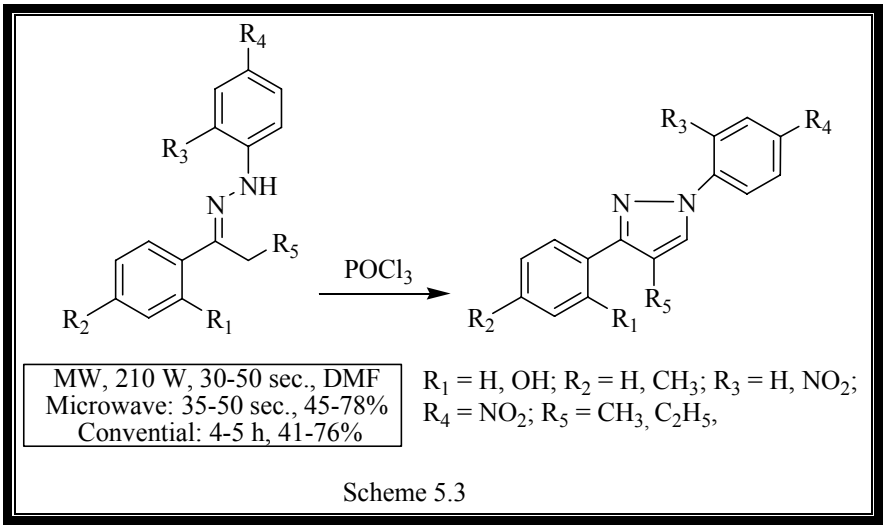
The classical Paal-Knorr cyclization of 1,4-diketones to give pyrroles is dramatically speeded- up under microwave irradiation and high yields are obtained as shown in Scheme 5.2 [13].



Scheme 5.2

5.2.1.2 Pyrazoles

Another recent application of microwaves in cyclization is the preparation of pyrazoles from hydrazones using the Vilsmeier cyclization method by treatment with

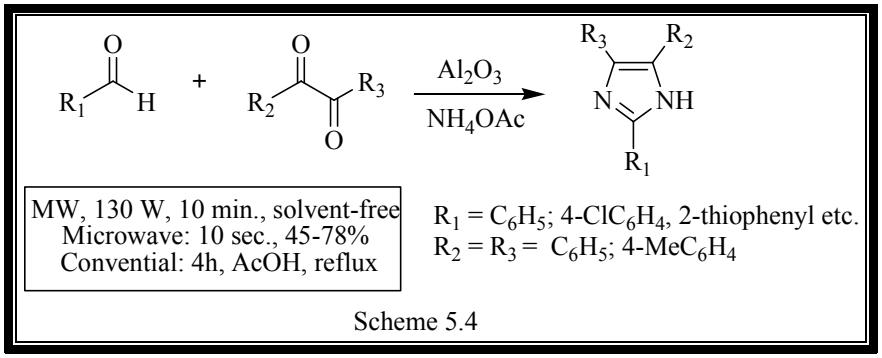


Scheme 5.3

$POCl_3$ and DMF [14]. As shown in Scheme 5.2, once again the reaction is speeded-up by factors of several 100-fold.

5.2.1.3 Imidazoles

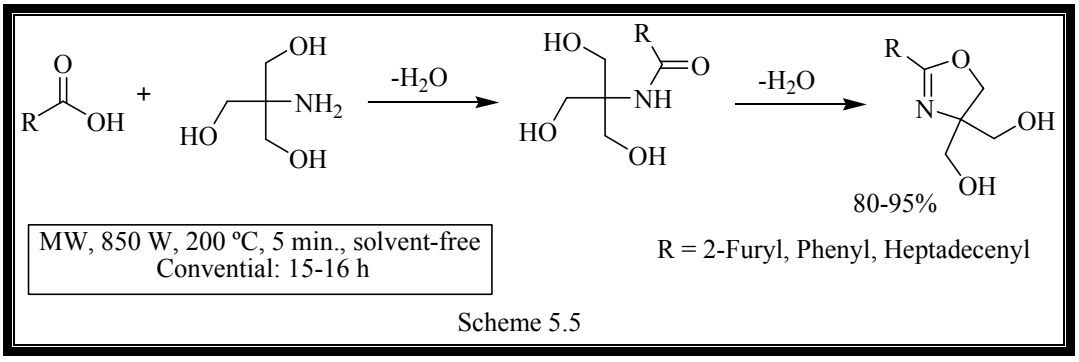
An important classical preparation of imidazoles is from an α-diketone, an aldehyde and ammonia. Here again, excellent yields can be obtained in reaction times of a few minutes as shown in Scheme 5.4 [15].



Scheme 5.4

5.2.1.4 Oxazolines

The example of Scheme 5.5, the preparation of oxazolines shows that partially saturated five-membered rings can also be prepared advantageously using microwaves [16].

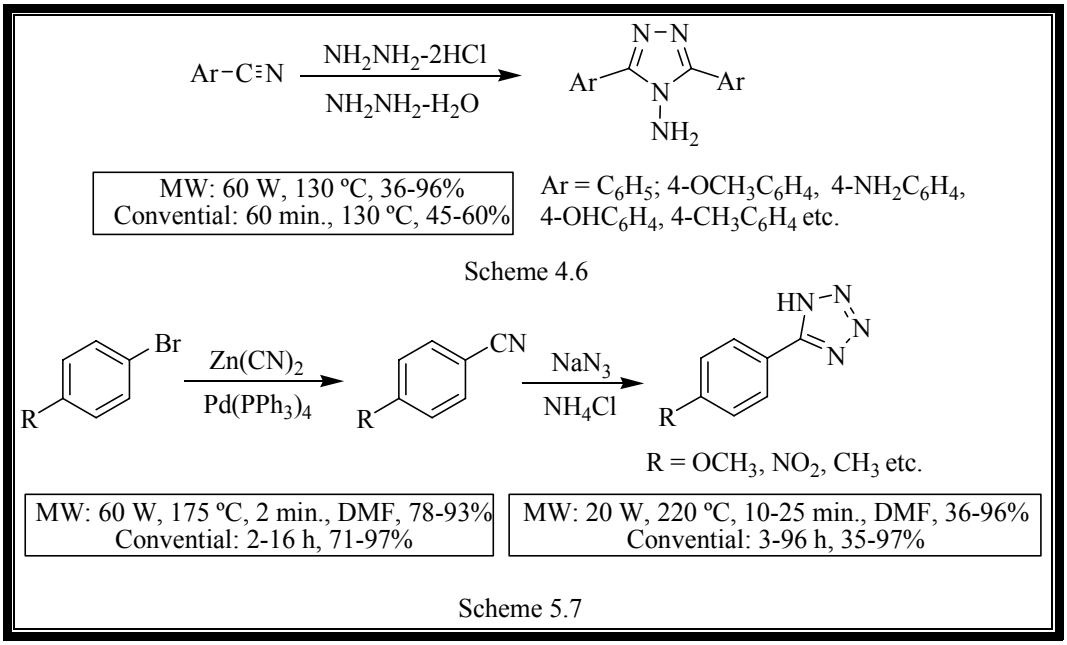


Scheme 5.5

5.2.1.5 Triazoles and Tetrazoles

Schemes 5.6 and 5.7 continue the overview of five-membered rings with illustrations of the advantageous preparation of 1,2,4–triazoles (Scheme 4.6) [17] and tetrazoles (Scheme 5.7) [18] using microwaves. Notice that in Scheme 5.6 the starting aryl

cyanides are also made by a Pd-catalyzed but microwave-enhanced replacement of aryl bromides using zinc cyanide.

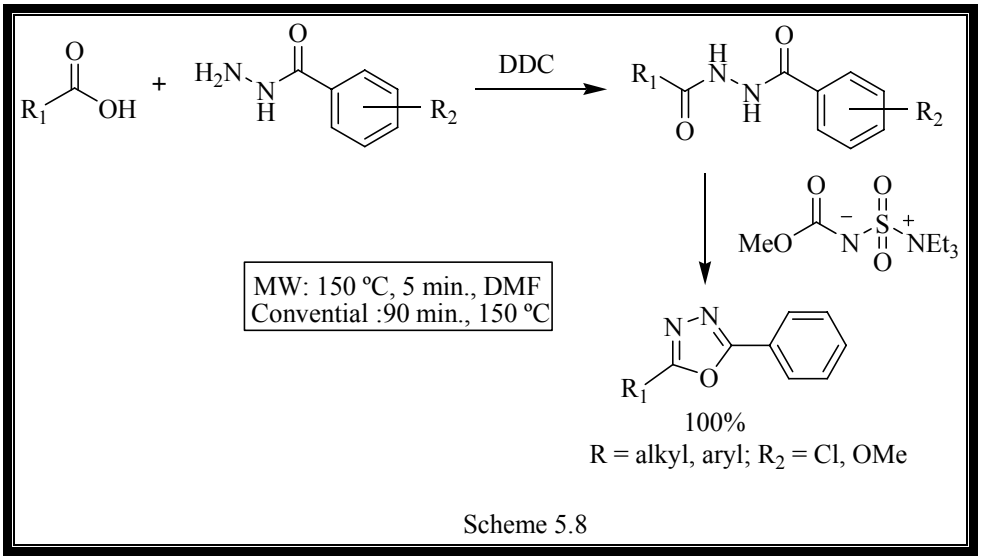


Scheme 4.6

Scheme 5.7

5.2.1.6 Oxadiazoles

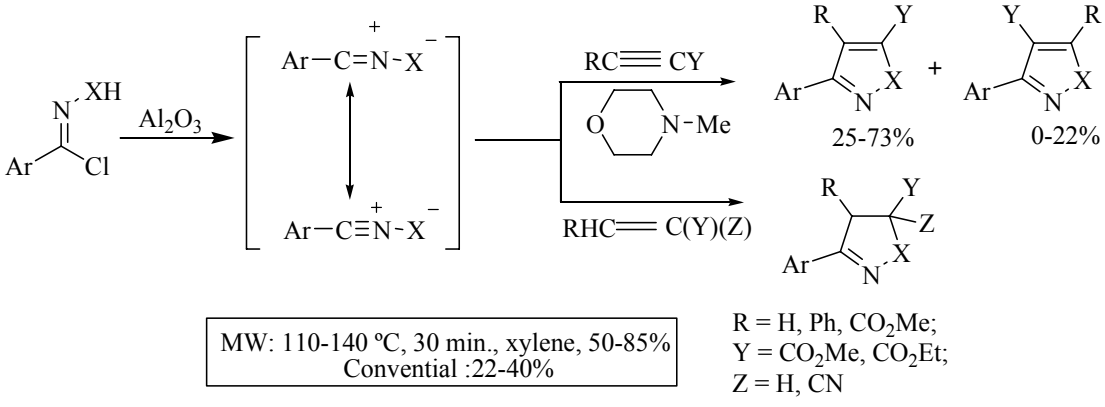
The dehydration of unsymmetrical diacylhydrazines (themselves prepared by a conventional Mitsunobu reaction) using Burgess's reagent is shown in Scheme 5.8 to give 1,3,4-oxadiazoles rapidly under microwave irradiation [19].



Scheme 5.8

5.2.1.7 Isoxazolines and pyrazolines

The acceleration of 1,3-dipolar cycloaddition reactions to give isoxazolines and pyrazolines by the addition of activated olefins to nitrile oxides or nitrile imides, respectively, is illustrated in Scheme 5.9; the resulting compounds are obtained in far high yield than under conventional conditions [20].

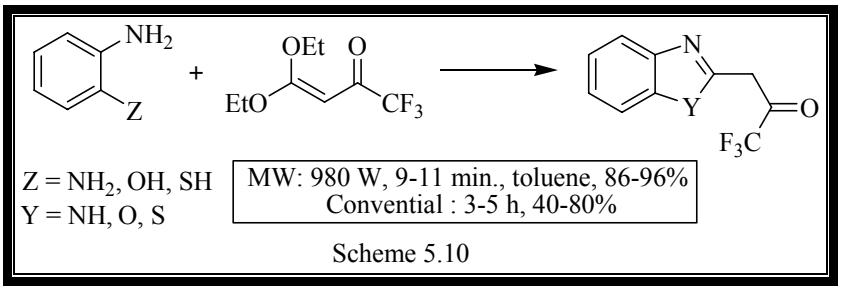


Scheme 5.9

5.2.2 Benzo-derivatives of five-membered rings

5.2.2.1 Benz-imidazoles, -oxazoles, and –thiazoles

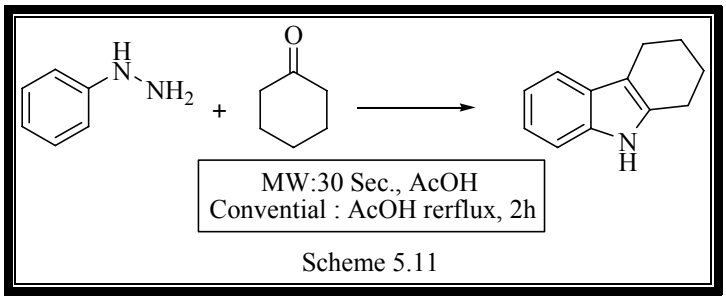
Ring closure reactions of appropriate *o*-substituted anilines to give benzimidazoles, benzoxazoles, and benzthiazoles takes place much faster and in significantly high yield under microwave conditions than conventionally [21] as shown in Scheme 5.10.



Scheme 5.10

5.2.2.2 Indoles

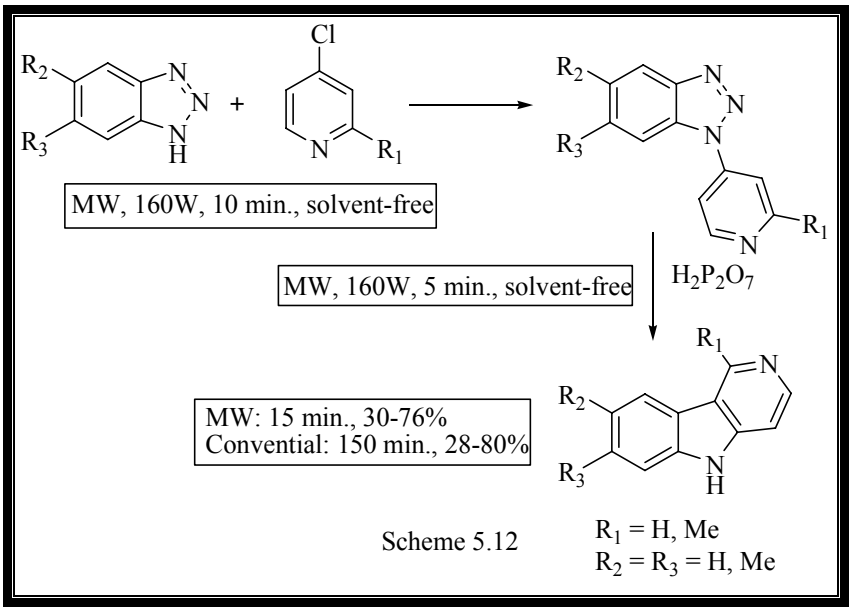
The classical Fischer-indole synthesis from an aryl hydrazine and a ketone is speeded-up by several 100-fold as documented in Scheme 5.11 [22].



Scheme 5.11

5.2.2.3 γ-Carbolines

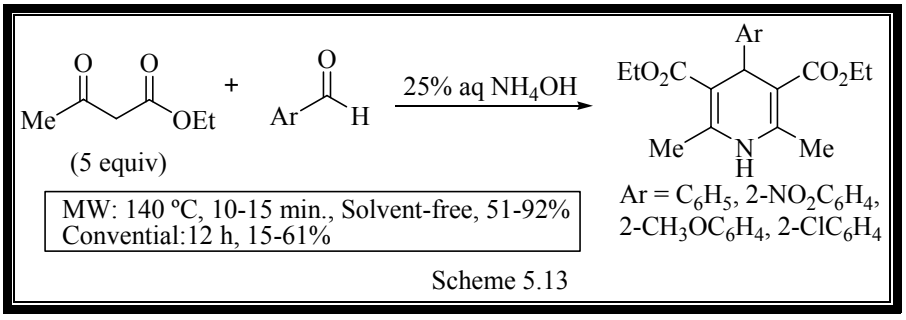
The Graebe-Ullmann synthesis which converts 1-arylbenzotriazoles into carbazoles or their heterocyclic analogs is also accelerated under microwave conditions as shown in Scheme 5.12 where the 1-(4-pyridyl)benzotriazole is converted into a γ-carboline [23].



Scheme 5.12

5.2.3 Six-membered rings

5.2.3.1 Dihydropyridines

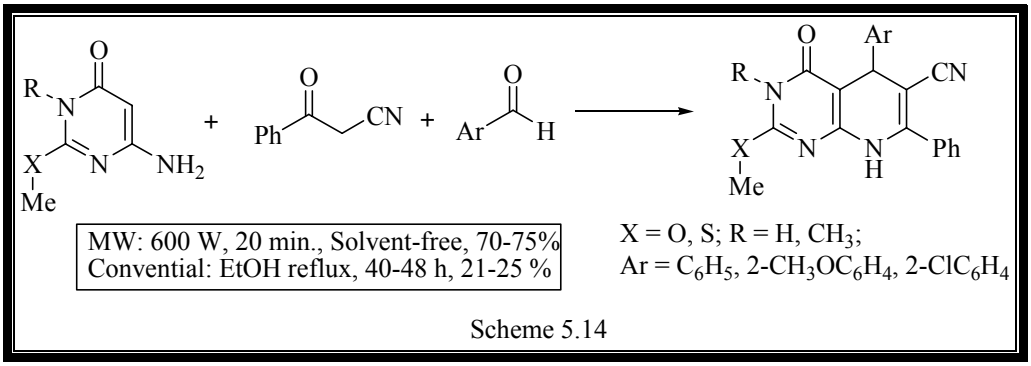


Scheme 5.13

The Hantzsch dihydropyridine synthesis remains one of the most important routes to pyridine ring systems. Under conventional conditions long periods of heating are required and yields are poor to moderate. Microwaves dramatically reduce the heating times and also significantly increase the yields as shown in Scheme 5.13 [24].

5.2.3.2 Dihydropyridopyrimidinones

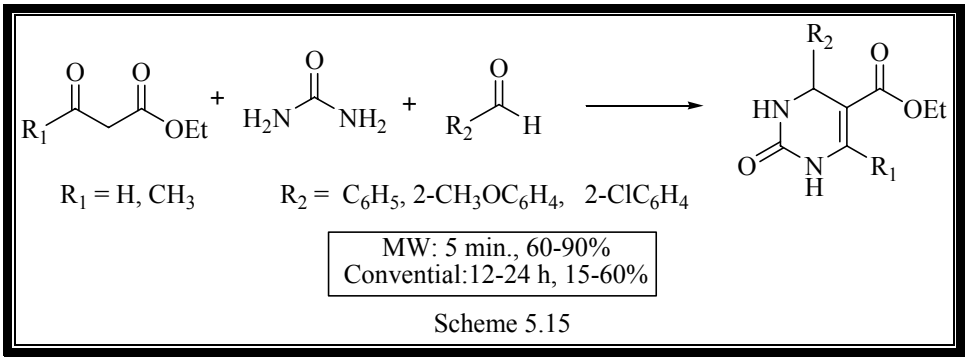
Dihydropyridopyrimidinones have been produced by ring annulations of aminopyrimidinones. Once again the reaction time is dramatically reduced and yields are much better with the solvent-free microwave conditions (Scheme 5.14) [25].



Scheme 5.14

5.2.3.3 Dihydropyrimidines

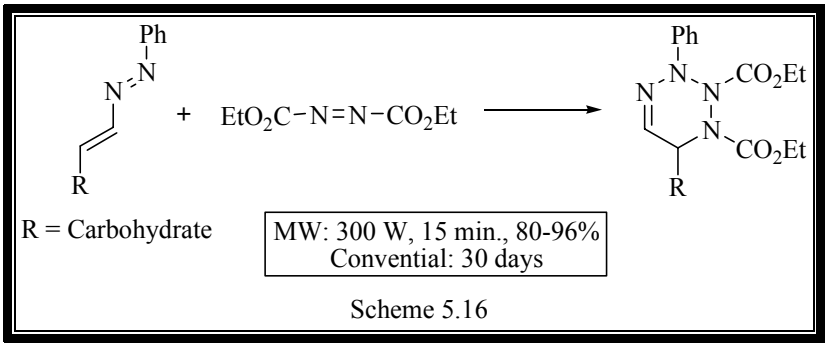
The Biginelli reaction is important for the preparation of dihydropyrimidine derivatives and excellent results are found for reactions carried out with microwave enhancement (Scheme 5.15) [19].



Scheme 5.15

5.2.3.4 Tetrazines

The Diels-Alder reaction between aza-olefins and aza-dicarboxylic ester to give tetrazines is speeded-up by a factor of 1000 by microwave enhancement as shown in Scheme 5.16 [26].

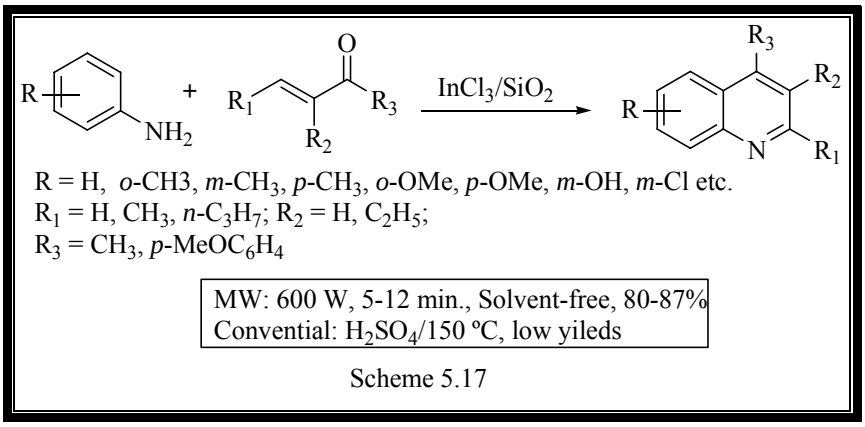


Scheme 5.16

5.2.4 Polycyclic six-membered rings

5.2.4.1 Quinolines

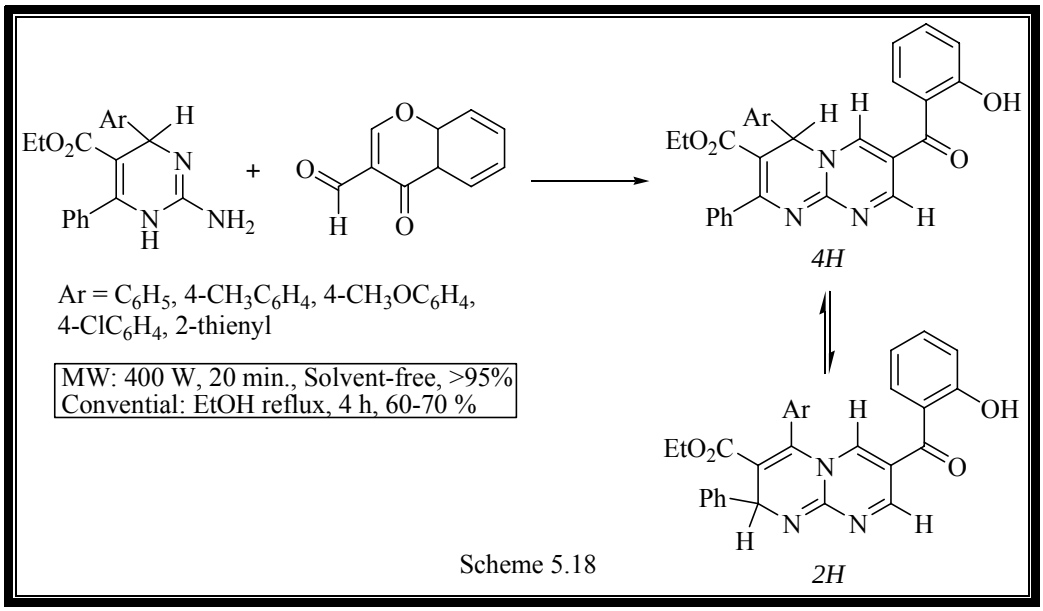
The Skraup synthesis has a bad reputation as it involves very messy conditions and gives only low yields of quinolines when carried out conventionally. Recently, it has been reported that microwave enhancement reduces the reaction time to a few minutes and allows high yields to be isolated (Scheme 5.17) [27].



Scheme 5.17

5.2.4.2 Pyrimido[1,2-*a*]pyrimidines

Pyrimido[1,2-*a*]pyrimidines are prepared from dihydroaminopyrimidines and chromone-3-aldehydes as is shown in Scheme 5.18 [28]. Although the conventional reaction must proceed in refluxing ethanol, reactions are much faster and better yields have been obtained with microwaves.

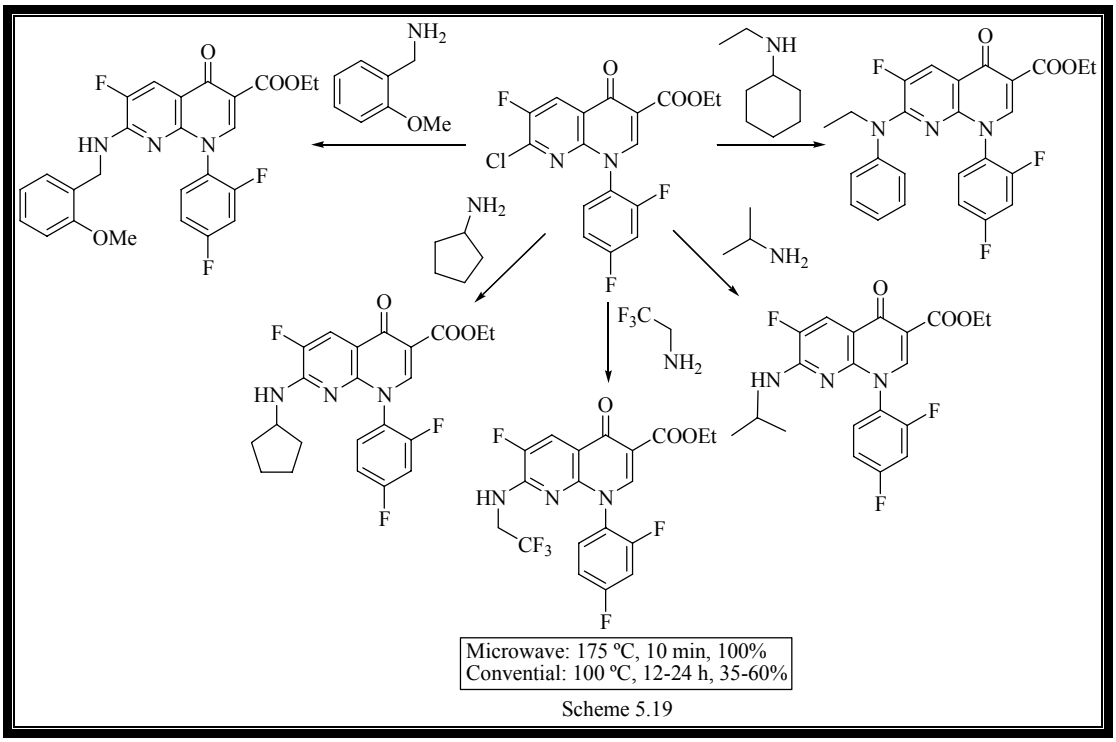


Scheme 5.18

5.2.5 Nucleophilic Substitutions

5.2.5.1 Heterocyclic *C*-alkylations

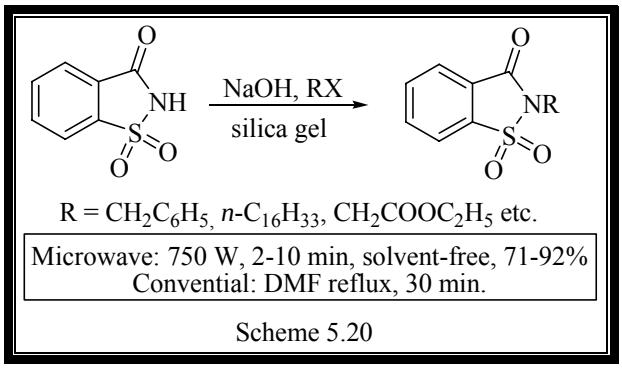
Nucleophilic substitution reactions can be speeded-up very considerably as is illustrated in Scheme 5.19 for a chloro-naphthyridine derivative [29].



Scheme 5.19

5.2.5.2 Heterocyclic *N*-alkylations

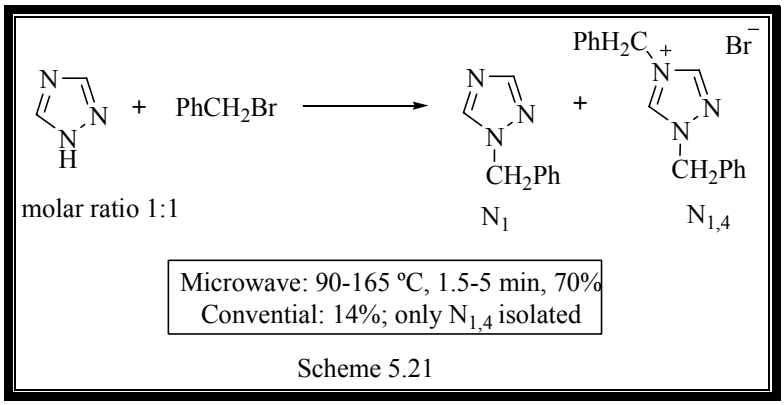
Another class of nucleophilic substitution is involved in heterocyclic *N*-alkylation which we have illustrated in Scheme 5.20. This shows that nucleophilic substitution on the nitrogen atom of saccharin is significantly speeded-up by microwave irradiation [19].



Scheme 5.20

5.2.5.3 Selective-alkylation

In Scheme 5.21, the results presented indicate that selectivity is achieved in the *N* alkylation of 1,2,4-triazole under microwave conditions where only the N_1-alkyl derivative was formed in contradistinction to the conventional conditions which give a considerable amount of the di-1,4-substituted compound [30].

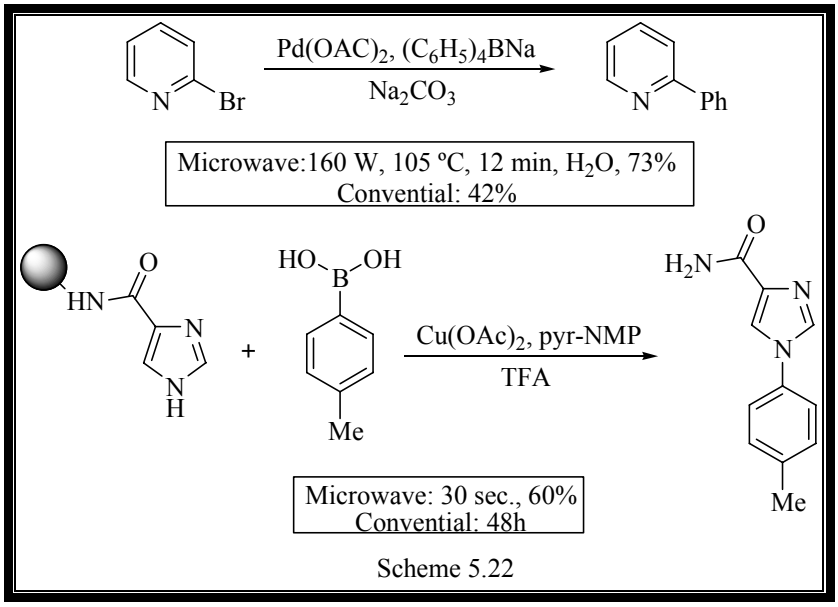


Scheme 5.21

5.2.5.4 Transition metal cross-coupling

An important type of nucleophilic substitution reactions which are recently much exploited are comprised of transition metal cross-coupling. A Suzuki coupling is shown at the top of Scheme 5.22 to give significantly better yield in the presence of

microwave irradiation [31]. At the bottom of Scheme 4.22 another Suzuki coupling is speeded-up by a factor of 100 [32].

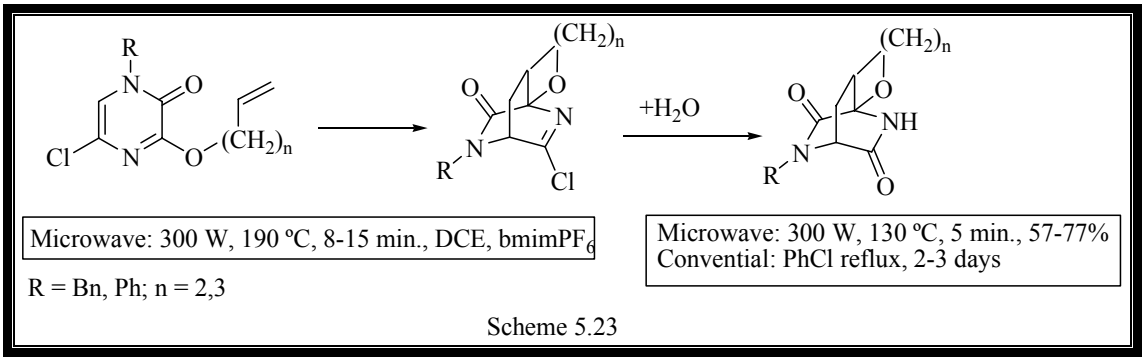


Scheme 5.22

5.2.6 Hetero-Diels−Alder reactions

5.2.6.1 Intramolecular reactions

We have already seen one example of a hetero-Diels−Alder reaction involving acyclic components. Hetero-Diels−Alder reactions involving cyclic components which lead to polycyclic ring systems are of great importance. An intramolecular example shown in Scheme 5.23 indicates that the reaction was accelerated by a factor of around 1000 by microwave irradiation [33].

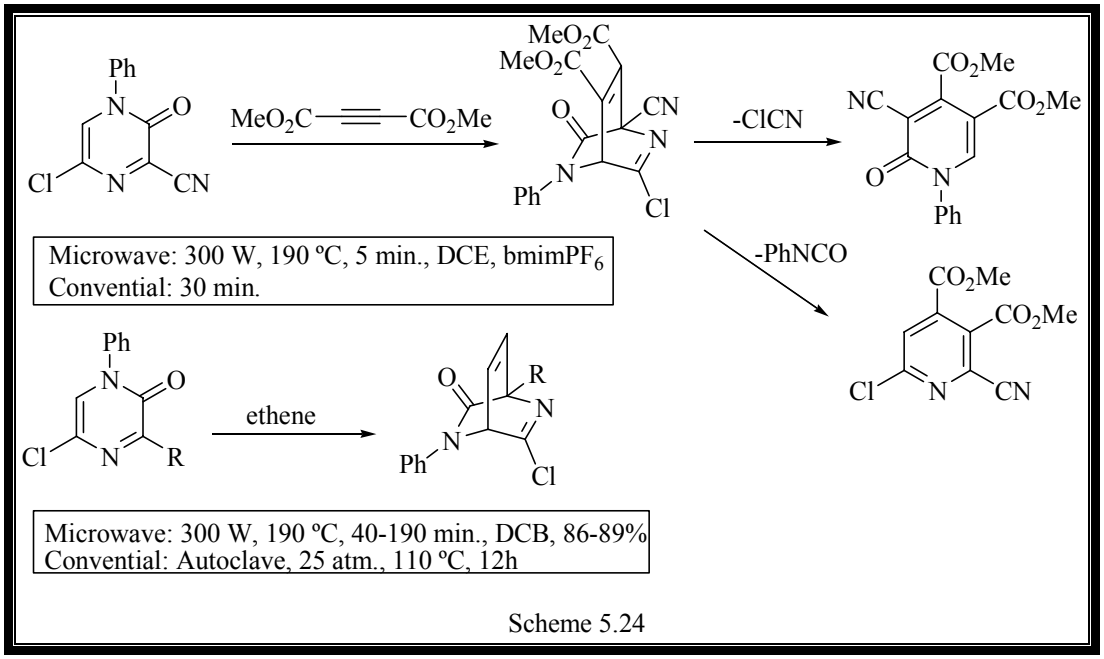


Scheme 5.23

5.2.6.2 Intermolecular reactions

Scheme 5.24 shows two impressive examples of rate enhancement for intermolecular hetero-Diels−Alder reactions [33]. In the first example on the top of Scheme 4.24 the

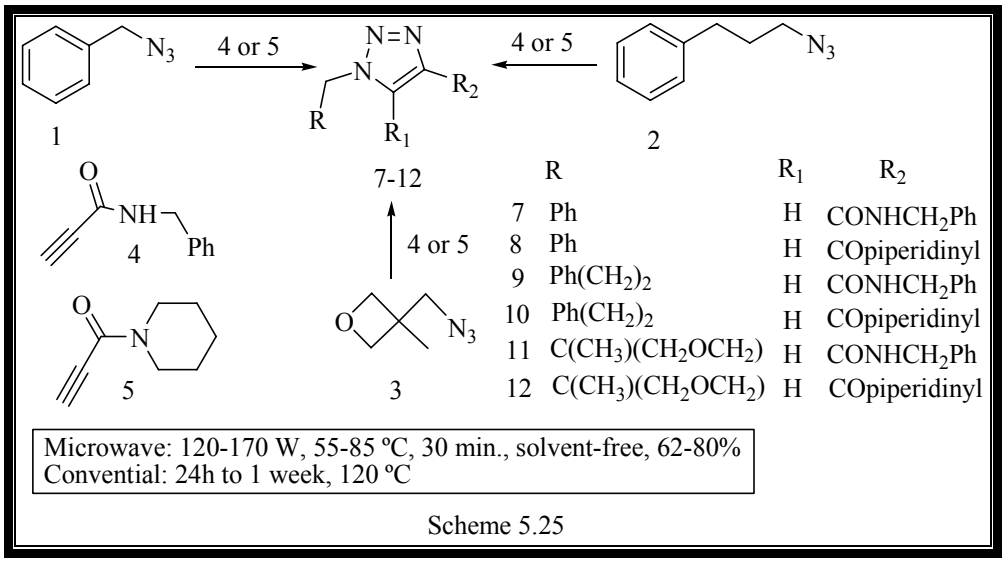
initial reaction is followed by elimination thus involving the conversion of a pyrazine derivative into a pyridine. Perhaps more impressive is the lower example in Scheme 5.24 where an autoclave is required under conventional conditions but which can be dispensed with when microwave acceleration is utilized.



Scheme 5.24

5.2.7 1,3-Dipolar cycloaddition reactions

5.2.7.1 Synthesis of *C*-carbamoyl-1,2,3-triazoles

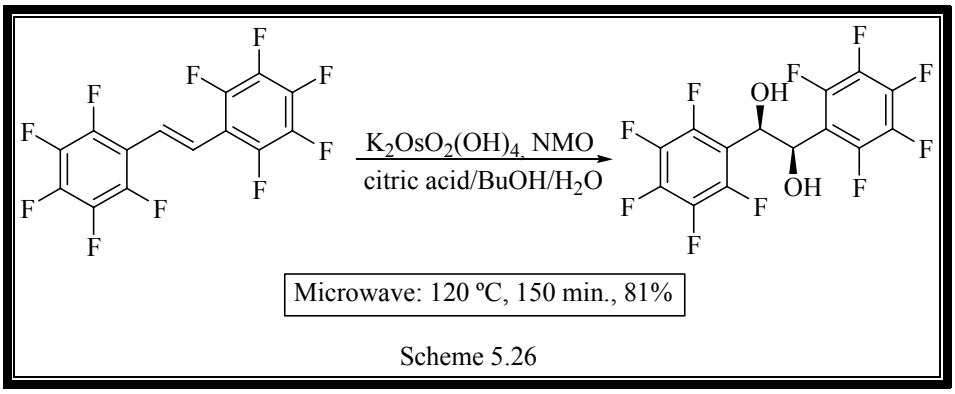


Scheme 5.25

We now turn to some of our own recent work which has involved microwave induced 1,3-dipolar cycloaddition of organic azides to acetylenic amides. As shown in Scheme 4.25 we were able to achieve these reactions under microwave conditions in a reasonable time at temperatures of around 70±15 ºC [34]. Under conventional conditions the times were roughly 100 times as long and the temperature had to be taken up to 120 ºC [35].

5.2.8 Oxidation

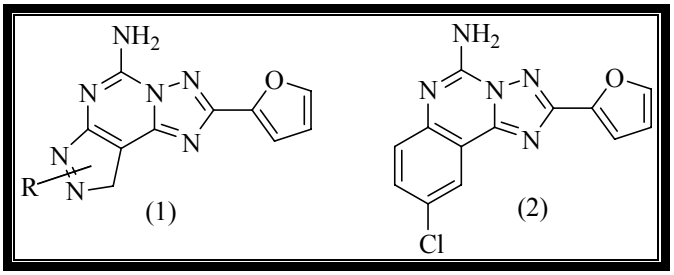
The osmium-catalyzed dihydroxylation reaction, the addition of osmium tetroxide to olefins to produce a vicinal diol, is one of the most selective and reliable organic transformations. Recent work by Sharpless, Fokin, and coworkers [36] has uncovered that electron-deficient olefins can be converted into the corresponding diols much more efficiently when the reaction medium is kept acidic (Scheme 5.26).



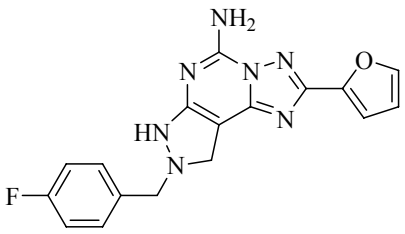
Scheme 5.26

5.3 Biological significance of pyarazlo-triazolo-pyrimidines

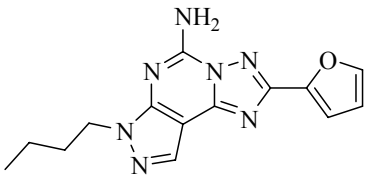
In recent years pyrazolotriazolopyrimidines have been the subject of intense research due to the interesting pharmacological activities found for several of their derivatives [37-39].



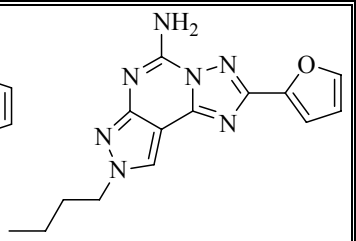
Pyrazolo-triazolo-pyrimidine nucleus (1) represents an attractive key intermediate for obtaining adenosine receptor antagonists due to its strong structural correlation with the nonselective adenosine receptor antagonist CGS15943 (2) [40]. The great advantage of this nucleus with respect to the reference compound (2) is related to a large number of substitutions that could be done on this nucleus, such as in positions N_7, N_8, N_5, C_9, or C_2 opening kaleidoscopic possibilities for different substituted heterocycles as adenosine receptor antagonists. Structures and binding affinities of various pyrazolo-triazolo-pyrimidines as adenosine receptors are as following.



(4) 8FB-PTP
K_i rA_1 = 3.3 nM
K_i rA_{2A} = 1.2 nM

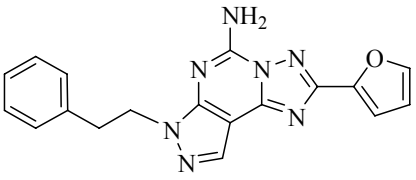


(5)
K_i rA_1 = 236 nM
K_i rA_{2A} = 8.9 nM

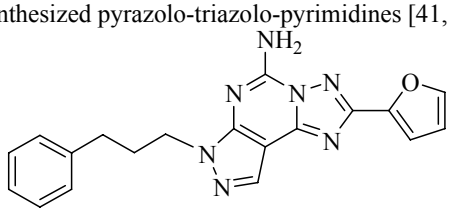


(6)
K_i rA_1 = 30.4 nM
K_i rA_{2A} = 2.4 nM

Structures and binding affinities of the first synthesized pyrazolo-triazolo-pyrimidines [41, 42]

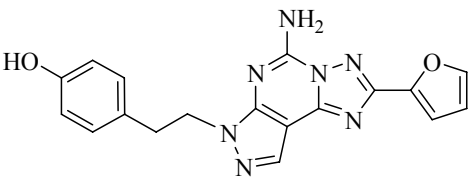


(7) SCH 58261
K_i rA_1 = 121 nM
K_i rA_{2A} = 2.3 nM
K_i hA_{2B} = >10000 nM
K_i hA_3 = >10000 nM

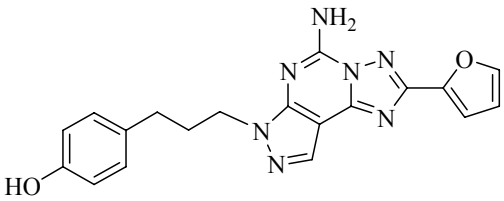


(8) SCH 63390
K_i rA_1 = 504 nM
K_i rA_{2A} = 2.4 nM
K_i hA_3 = >10000 nM

First generation of pyrazolo-triazolo-pyrimidines as potent and selective A_{2A} adenosine receptor antagonists [42-44]

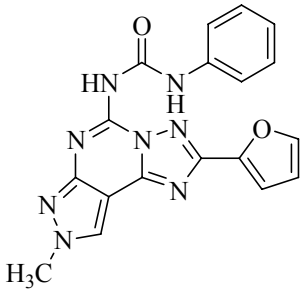


(8)
K_i rA_1 = 444 nM
K_i rA_{2A} = 1.7 nM
K_i hA_3 = >10000 nM



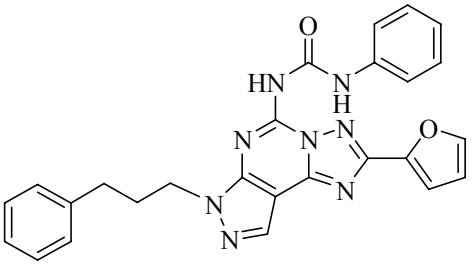
(9)
K_i rA_1 = 741 nM
K_i rA_{2A} = 0.94 nM
K_i hA_3 = >10000 nM

Hydrophilic pyrazolotriazolopyrimidines as A_{2A} adenosine receptor antagonists [43]



(10)

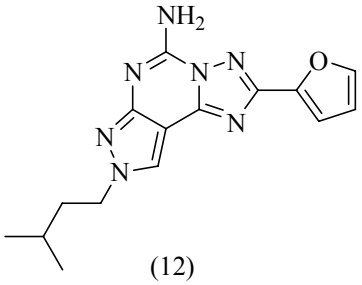
K_i hA_1 = 594 nM
K_i hA_{2A} = 381 nM
K_i hA_{2B} = 222 nM
K_i hA_3 = 0.16 nM



(11)

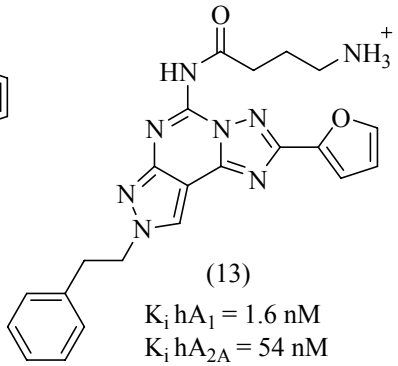
K_i hA_1 = >10000 nM
K_i hA_{2A} = 594 nM
K_i hA_{2B} = >10000 nM
K_i hA_3 = 0.16 nM

Structures and binding affinities of the most interesting compounds modified at the phenyl ring [44]



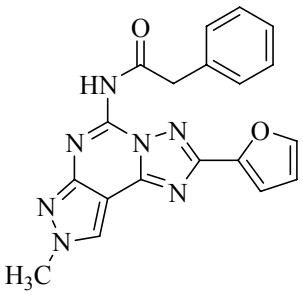
(12)

K_i hA_1 = 2 nM
K_i hA_{2A} = 0.8 nM
K_i hA_{2B} = 9 nM
K_i hA_3 = 700 nM



(13)

K_i hA_1 = 1.6 nM
K_i hA_{2A} = 54 nM
K_i hA_{2B} = 27 nM
K_i hA_3 = 65 nM



(14)

K_i hA_1 = 702 nM
K_i hA_{2A} = 423 nM
K_i hA_{2B} = 165 nM
K_i hA_3 = 0.81 nM

Structures and binding affinities of pyrazolo-triazolopyrimidines as A_{2B} adenosine receptor antagonists [45]

5.4 Current work

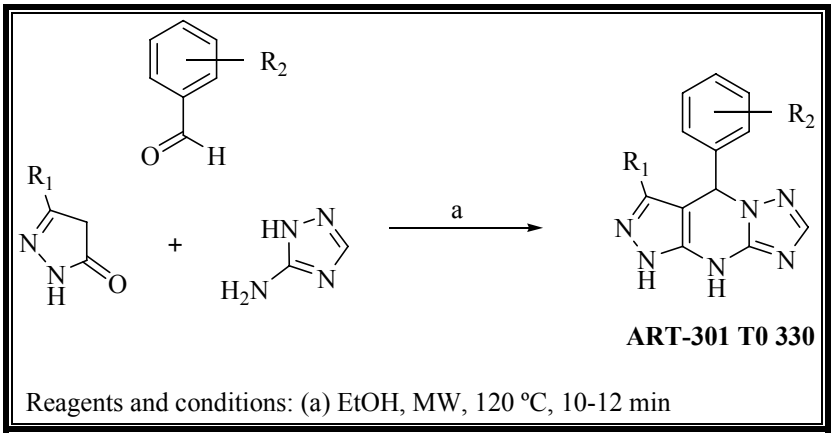
Flat heterocyclic structures are fundamental moieties in anticancer compounds acting as DNA intercalating agents [46]. In fact, they play a determinant role in the π–π stacking interactions with the DNA base pairs. Consequently, the modulation of their electronic and steric features by the presence of opportune substituents or heteroatoms, could improve this capability.

In this context, pyrazolotriazolopyrimidines have been the subject of intense research due to the interesting pharmacological activities found for several of their derivatives. Among the various pyrazolotriazolopyrimidine ring systems, pyrazolo [3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines have never been explored, and there are no reports on the synthesis and chemical properties of these class of compounds.

With the aim to extend the synthetic pathways to new planar heterocyclic ring systems, we focused our studies on the pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine core structure which was found to be absent in the literature survey (Scifinder, CA, STN search).

Synthesis of pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine (ART-301 to ART-330), a new ring system, was achieved by using a one-pot, microwave-assisted, catalyst-free biginelli like condensation. All the newly synthesized characterized by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.

5.5 Reaction Scheme



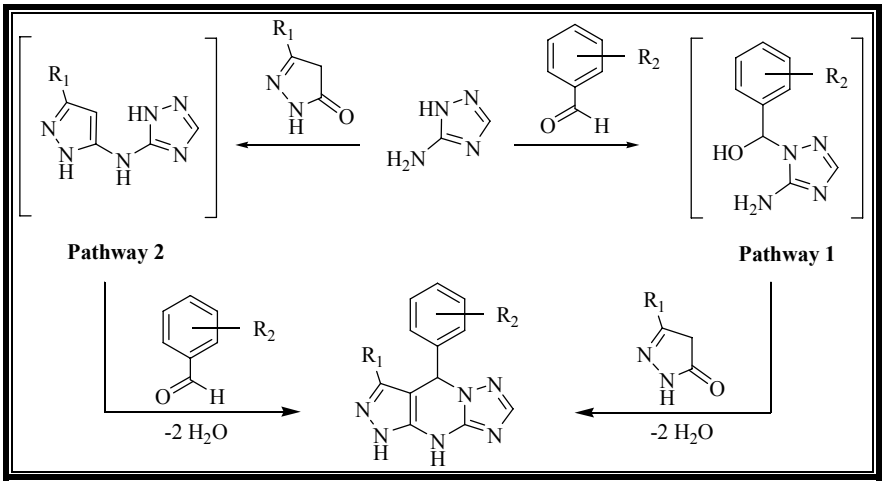
ART-301 T0 330

Reagents and conditions: (a) EtOH, MW, 120 ºC, 10-12 min

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
ART-301	CH_3	4-OCH_3	$C_{14}H_{14}N_6O$	282	204-206	69	0.52	0.68
ART-302	CH_3	4-F	$C_{13}H_{11}FN_6$	270	158-160	68	0.49	0.59
ART-303	CH_3	4- NO_2	$C_{13}H_{11}N_7O_2$	297	185-187	75	0.55	0.66
ART-304	CH_3	4-Cl	$C_{13}H_{11}ClN_6$	286	221-223	74	0.41	0.69
ART-305	CH_3	4- CH_3	$C_{14}H_{14}N_6$	266	205-207	56	0.43	0.59
ART-306	CH_3	4-OH	$C_{13}H_{12}N_6O$	268	216-218	72	0.42	0.69
ART-307	CH_3	3-NO_2	$C_{13}H_{11}N_7O_2$	297	226-228	58	0.49	0.66
ART-308	CH_3	3-Cl	$C_{13}H_{11}ClN_6$	286	188-190	68	0.54	0.62
ART-309	CH_3	3-OH	$C_{13}H_{12}N_6O$	268	198-200	75	0.43	0.68
ART-310	CH_3	3-Br	$C_{13}H_{11}BrN_6$	331	212-214	72	0.50	0.70
ART-311	CH_3	3,4-OCH_3	$C_{15}H_{16}N_6O_2$	312	148-150	68	0.42	0.59
ART-312	CH_3	2-NO_2	$C_{13}H_{11}N_7O_2$	297	235-237	72	0.51	0.66
ART-313	CH_3	2-Cl	$C_{13}H_{11}ClN_6$	286	208-210	69	0.55	0.65
ART-314	CH_3	2-OH	$C_{13}H_{12}N_6O$	268	201-203	70	0.39	0.66
ART-315	CH_3	H	$C_{13}H_{12}N_6$	252	193-195	59	0.44	0.64
ART-316	NH_2	4-OCH_3	$C_{13}H_{13}N_7O$	283	238-240	66	0.40	0.62
ART-317	NH_2	4-F	$C_{12}H_{10}FN_7$	271	213-215	71	0.41	0.63
ART-318	NH_2	4- NO_2	$C_{12}H_{10}N_8O_2$	298	218-220	63	0.38	0.70
ART-319	NH_2	4-Cl	$C_{12}H_{10}ClN_7$	287	221-223	79	0.39	0.68
ART-320	NH_2	4- CH_3	$C_{13}H_{13}N_7$	267	179-181	78	0.42	0.70
ART-321	NH_2	4-OH	$C_{12}H_{11}N_7O$	269	167-169	82	0.45	0.66
ART-322	NH_2	3-NO_2	$C_{12}H_{10}N_8O_2$	298	148-150	78	0.51	0.66
ART-323	NH_2	3-Cl	$C_{12}H_{10}ClN_7$	287	207-209	75	0.48	0.65
ART-324	NH_2	3-OH	$C_{12}H_{11}N_7O$	269	208-210	71	0.37	0.64
ART-325	NH_2	3-Br	$C_{12}H_{10}BrN_7$	332	229-231	61	0.46	0.71
ART-326	NH_2	3,4-OCH_3	$C_{14}H_{15}N_7O_2$	313	177-179	72	0.44	0.67
ART-327	NH_2	2-NO_2	$C_{12}H_{10}N_8O_2$	298	183-185	68	0.41	0.65
ART-328	NH_2	2-Cl	$C_{12}H_{10}ClN_7$	287	213-215	68	0.40	0.71
ART-329	NH_2	2-OH	$C_{12}H_{11}N_7O$	269	238-240	75	0.39	0.68
ART-330	NH_2	H	$C_{12}H_{11}N_7$	253	186-188	70	0.36	0.63

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

5.6 Reaction Mechanism



The reaction mechanism of this three-component condensation is probably similar to the described [47] mechanism for the "classical" Biginelli reaction (Pathway 1). The first step is a nucleophilic addition of $N_{(2)}$ of the aminoazole to a carbonyl carbon of aldehyde, followed by subsequent cyclization with 3-sub-1*H*-pyrazol-5(4*H*)-one to form the dihydropyrimidine ring. An alternate sequence is also possible and cannot be excluded [48] (Pathway 2), which is the initial formation of an enamine by reaction of aminoazole with the 3-sub-1*H*-pyrazol-5(4*H*)-one followed by cyclocondensation. The third alternative involving the formation of 3-sub-4-benzylidene-1*H*-pyrazol-5(4*H*)-one derivatives as intermediates requires the presence of a strong base [49] and is most likely not possible for the cases described herein.

5.7 Experimental

5.7.1 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. Microwave assisted reaction were carried out in QPro-M microwave synthesizer. 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.

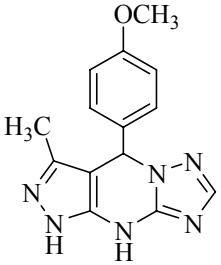
5.7.2 Synthesis of 3-sub-1H-pyrazol-5(4H)-ones

Syntheses of 3-substituted-1*H*-pyrazol-5(4*H*)-ones were achieved using previously published methods [50].

5.7.3 General procedure for the synthesis of 4-(aryl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a]pyrimidines (ART 301-315)

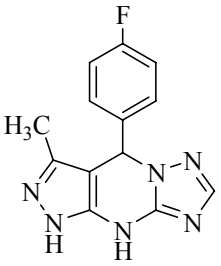
A mixture of the aminoazole (0.01 mol), 3-methyl-1*H*-pyrazol-5(4*H*)-one (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in ethanol (5 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. The reaction mixture was allowed to stand overnight at room temperature and was then filtered to give the solid triazolopyrazolopyrimidine products ART 301-315, which were washed with ethanol and dried in air. Triazolopyrazolopyrimidine were obtained in high purity and did not require further purification by recrystallization.

5.7.3.1 4-(4-methoxyphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



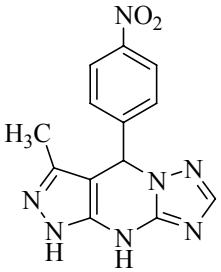
[1,5-a]pyrimidine (ART-301) Yield: 69%; mp 204-206 ºC; MS: *m/z* 282; Anal. Calcd. for $C_{14}H_{14}N_6O$: C, 59.56; H, 5.00; N, 29.77. Found: C, 59.45; H, 4.92; N, 29.70%.

5.7.3.2 4-(4-fluorophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



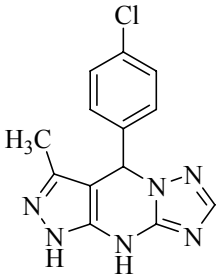
[1,5-a]pyrimidine (ART-302) Yield: 68%; mp 158-160 ºC; MS: *m/z* 270; Anal. Calcd. for $C_{13}H_{11}FN_6$: C, 57.77; H, 4.10; N, 31.10. Found: C, 57.66; H, 4.00; N, 31.01%.

5.7.3.3 4-(4-nitrophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo

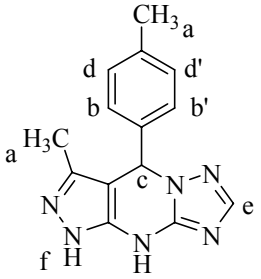


[1,5-a]pyrimidine (ART-303) Yield: 75%; mp 185-187 ºC; MS: *m/z* 297; Anal. Calcd. for $C_{13}H_{11}N_7O_2$: C, 52.52; H, 3.73; N, 32.98. Found: C, 52.40; H, 3.66; N, 32.89%.

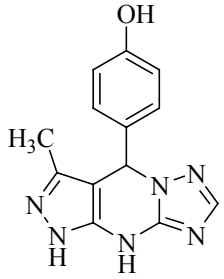
5.7.3.4 4-(4-chlorophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



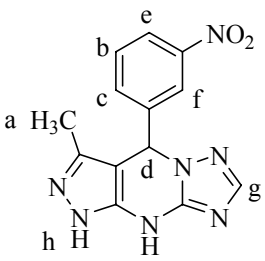
[1,5-a]pyrimidine (ART-304) Yield: 74%; mp 221-223 ºC; MS: *m/z* 286; Anal. Calcd. for $C_{13}H_{11}ClN_6$: C, 54.46; H, 3.87; N, 29.31. Found: C, 54.32; H, 3.76; N, 29.22%.

5.7.3.5 4-(4-methylphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-305) 

Yield: 56%; mp 205-207 ºC; IR (cm^{-1}): 3356 (N-H stretching of secondary amine), 3095 (C-H stretching of aromatic ring), 2974 (C-H asymmetrical stretching of CH_3 group), 2889 (C-H asymmetrical stretching of CH_3 group), 1693 (C=N stretching), 1606 (N-H deformation of pyrimidine ring), 1506, 1471 and 1400 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH_3 group), 1352 (C-H symmetrical deformation of CH_3 group), 1352 (C-N stretching), 812 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.37 (s, 2×3H, H_a), 7.18-7.20 (d, 2H, $H_{bb'}$, J = 8.00 Hz), 7.39 (s, 1H, H_c), 7.51-7.53 (d, 2H, $H_{dd'}$, J = 7.96 Hz), 7.93 (s, 1H, H_e), 11.47 (s, aH, H_f); MS: *m/z* 266; Anal. Calcd. for $C_{14}H_{14}N_6$: C, 63.14; H, 5.30; N, 31.56. Found: C, 63.01; H, 5.22; N, 31.48%.

5.7.3.6 4-(4-hydroxyphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-306) 

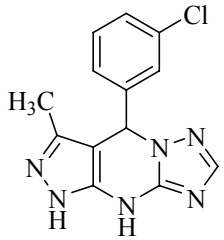
Yield: 72%; mp 216-218 ºC; MS: *m/z* 268; Anal. Calcd. for $C_{13}H_{12}N_6O$: C, 58.20; H, 4.51; N, 31.33. Found: C, 58.10; H, 4.39; N, 31.25%.

5.7.3.7 4-(3-nitrophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-307) 

Yield: 58%; mp 226-228 ºC; IR (cm^{-1}): 3261 (N-H stretching of secondary amine), 3080 (C-H stretching of aromatic ring), 2999 (C-H asymmetrical stretching of CH_3 group), 2862 (C-H asymmetrical stretching of CH_3 group), 1699 (C=N stretching), 1614 (N-H deformation of pyrimidine ring), 1566, 1525 and 1494 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH_3 group), 1388 (C-H symmetrical deformation of CH_3 group), 1350 (C-N stretching), 705 (C-H out of plane bending of 1,3-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 2.57 (s, 3H, H_a), 7.63-7.68 (t, 1H, H_b), 8.04-8.06 (d, 1H, H_c), 8.10

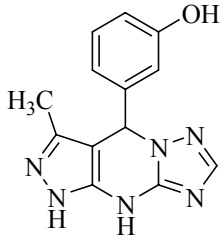
(s, 1H, H_d), 8.20-8.22 (d, 2H, H_e), 8.30 (s, 1H, H_f,), 8.45-8.46 (s, 1H, H_g), 11.95-12.04 (s, 1H, H_h); MS: *m/z* 297; Anal. Calcd. for $C_{13}H_{11}N_7O_2$: C, 52.52; H, 3.73; N, 32.98. Found: C, 52.45; H, 3.64; N, 32.90%.

5.7.3.8 4-(3-chlorophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



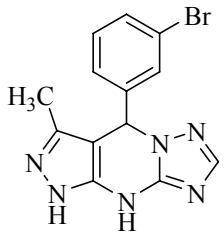
[1,5-a]pyrimidine (ART-308) Yield: 68%; mp 188-190 ºC; MS: *m/z* 286; Anal. Calcd. for $C_{13}H_{11}ClN_6$: C, 54.46; H, 3.87; N, 29.31. Found: C, 54.34; H, 3.77; N, 29.20%.

5.7.3.9 4-(3-hydroxyphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



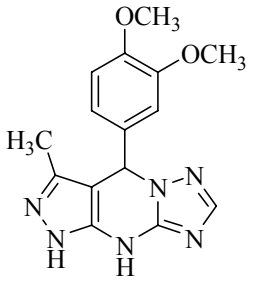
[1,5-a]pyrimidine (ART-309) Yield: 75%; mp 198-200 ºC; MS: *m/z* 268; Anal. Calcd. for $C_{13}H_{12}N_6O$: C, 58.20; H, 4.51; N, 31.33. Found: C, 58.12; H, 4.41; N, 31.22%.

5.7.3.10 4-(3-bromophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo



[1,5-a]pyrimidine (ART-310) Yield: 72%; mp 212-214 ºC; MS: *m/z* 331; Anal. Calcd. for $C_{13}H_{11}BrN_6$: C, 47.15; H, 3.35; N, 25.38. Found: C, 47.02; H, 3.21; N, 25.57%.

5.7.3.11 4-(3,4-methoxyphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triaz



olo[1,5-a]pyrimidine (ART-311) Yield: 68%; mp 148-150 ºC; MS: *m/z* 312; Anal. Calcd. for $C_{15}H_{16}N_6O_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.59; H, 5.02; N, 26.82%.

5.7.3.12 4-(2-nitrophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-312) Yield: 72%; mp 235-237 ºC; MS: *m/z* 297; Anal. Calcd. for $C_{13}H_{11}N_7O_2$: C, 52.52; H, 3.73; N, 32.98. Found: C, 52.41; H, 3.66; N, 32.89%.

5.7.3.13 4-(2-chlorophenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-313) Yield: 69%; mp 208-210 ºC; MS: *m/z* 286; Anal. Calcd. for $C_{13}H_{11}ClN_6$: C, 54.46; H, 3.87; N, 29.31. Found: C, 54.38; H, 3.74; N, 29.24%.

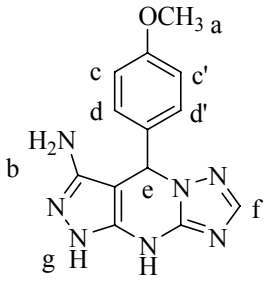
5.7.3.14 4-(2-hydroxyphenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-314) Yield: 70%; mp 201-203 ºC; MS: *m/z* 268; Anal. Calcd. for $C_{13}H_{12}N_6O$: C, 58.20; H, 4.51; N, 31.33. Found: C, 58.10; H, 4.43; N, 31.20%.

5.7.3.15 4-(phenyl)-3-methyl-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a] pyrimidine (ART-315) Yield: 59%; mp 193-195 ºC; IR (cm^{-1}): 3213 (N-H stretching of secondary amine), 3080 (C-H stretching of aromatic ring), 2949 (C-H asymmetrical stretching of CH_3 group), 2882 (C-H asymmetrical stretching of CH_3 group), 1689 (C=N stretching), 1602 (N-H deformation of pyrimidine ring), 1560, 1520 and 1491 (C=C stretching of aromatic ring), 1448 (C-H asymmetrical deformation of CH_3 group), 1384 (C-H symmetrical deformation of CH_3 group), 1340 (C-N stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.53 (s, 3H, H_a), 7.37-7.73 (m, 6H, $H_{bb'-f}$), 11.73 (s, 1H, H_g); MS: *m/z* 252; Anal. Calcd. for $C_{13}H_{12}N_6$: C, 61.89; H, 4.79; N, 33.31. Found: C, 61.80; H, 4.68; N, 33.21%.

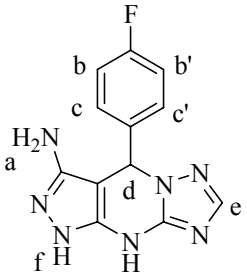
5.7.4 General procedure for the synthesis of 4-(aryl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a]pyrimidines (ART 316-330)

A mixture of the aminoazole (0.01 mol), 3-amino-1*H*-pyrazol-5(4*H*)-one (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in ethanol (5 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. The reaction mixture was allowed to stand overnight at room temperature and was then filtered to give the solid triazolopyrazolopyrimidine products ART 316-330, which were washed with ethanol and dried in air. Triazolopyrazolopyrimidine were obtained in high purity and did not require further purification by recrystallization.

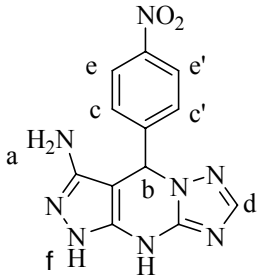
5.7.4.1 4-(4-methoxyphenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-316)



Yield: 66%; mp 238-240 ºC; IR (cm^{-1}): 3412 (N-H stretching of primary amine), 3215 (N-H stretching of secondary amine), 3090 (C-H stretching of aromatic ring), 2964 (C-H asymmetrical stretching of CH_3 group), 2835 (C-H asymmetrical stretching of CH_3 group), 1691 (C=N stretching), 1668 (N-H deformation of primary amine), 1608 (N-H deformation of pyrimidine ring), 1568, 1516, 1465 (C=C stretching of aromatic ring), 1425 (C-H asymmetrical deformation of CH_3 group), 1384 (C-H symmetrical deformation of CH_3 group), 1336 (C-N stretching), 837 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 3.84 (s, 3H, H_a), 3.94 (s, 2H, H_b), 6.90-6.92 (d, 2H, $H_{cc'}$, J = 8.8 Hz), 7.57-7.59 (d, 2H, $H_{dd'}$, J = 8.8 Hz), 7.71 (s, 1H, H_e), 7.93 (s, 1H, H_f), 11.62 (s, 1H, H_g); MS: *m/z* 283; Anal. Calcd. for $C_{13}H_{13}N_7O$: C, 55.12; H, 4.63; N, 34.61. Found: C, 55.02; H, 4.56; N, 34.52%.

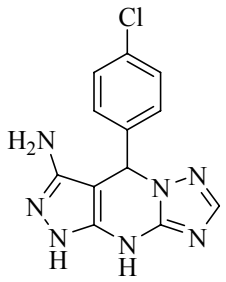
5.7.4.2 4-(4-fluorophenyl)-3 amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-317) 

Yield: 71%; mp 213-215 ºC; IR (cm^{-1}): 3466 (N-H stretching of primary amine), 3290 (N-H stretching of secondary amine), 3090 (C-H stretching of aromatic ring), 1691 (C=N stretching), 1674 (N-H deformation of primary amine), 1602 (N-H deformation of pyrimidine ring), 1564, 1546, 1510 (C=C stretching of aromatic ring), 1430 (C-H asymmetrical deformation of CH_3 group), 1373 (C-H symmetrical deformation of CH_3 group), 1340 (C-N stretching), 837 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 3.94 (s, 3H, H_a), 7.35-7.38 (d, 2H, $H_{bb'}$), 7.58-7.62 (d, 2H, $H_{cc'}$), 7.65 (s, 1H, H_d), 7.96 (s, 1H, H_e), 11.79 (s, 1H, H_f); MS: *m/z* 271; Anal. Calcd. for $C_{12}H_{10}FN_7$: C, 53.13; H, 3.72; N, 36.15. Found: C, 53.01; H, 3.61; N, 36.03%.

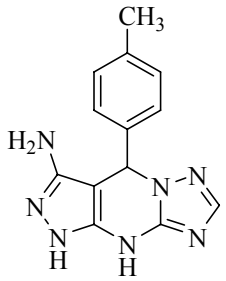
5.7.4.3 4-(4-nitrophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-318) 

Yield: 63%; mp 218-220 ºC; IR (cm^{-1}): 3412 (N-H stretching of primary amine), 3254 (N-H stretching of secondary amine), 3086 (C-H stretching of aromatic ring), 1695 (C=N stretching), 1589 (N-H deformation of pyrimidine ring), 1518 and 1483 (C=C stretching of aromatic ring), 1450 (C-H asymmetrical deformation of CH_3 group), 1377 (C-H symmetrical deformation of CH_3 group), 1346 (C-N stretching), 852 (C-H out of plane bending of 1,4-disubstituion); ^{1}H NMR (DMSO-d_6) δ ppm: 3.96 (s, 2H, H_a), 7.55 (s, 1H, H_b), 7.82-7.85 (d, 2H, $H_{cc'}$), 8.08 (s, 1H, H_d), 8.23-8.25 (d, 2H, $H_{ee'}$, J = 7.68 Hz), 12.07 (d, 2H, H_f); MS: *m/z* 298; Anal. Calcd. for $C_{12}H_{10}N_8O_2$: C, 48.32; H, 3.38; N, 37.57. Found: C, 48.21; H, 3.29; N, 37.48%.

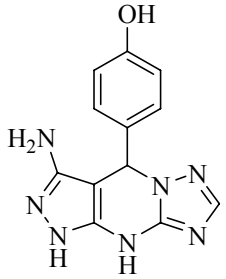
5.7.4.4 4-(4-chlorophenyl)-3- amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-319) Yield: 79%; mp 221-223 ºC; MS: *m/z* 287; Anal. Calcd. for $C_{12}H_{10}ClN_7$: C, 50.10; H, 3.50; N, 34.08. Found: C, 50.00; H, 3.39; N, 34.00%.



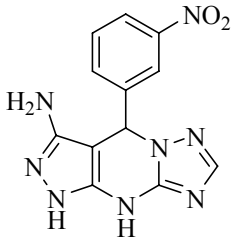
5.7.4.5 4-(4-methylphenyl)-3- amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-320) Yield: 78%; mp 179-181 ºC; MS: *m/z* 267; Anal. Calcd. for $C_{13}H_{13}N_7$: C, 58.42; H, 4.90; N, 36.68. Found: C, 58.31; H, 4.88; N, 36.59%.



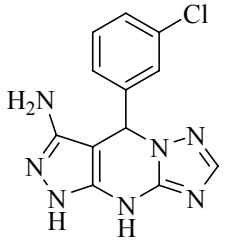
5.7.4.6 4-(4-hydroxyphenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-321) Yield: 82%; mp 167-169 ºC; MS: *m/z* 269; Anal. Calcd. for $C_{12}H_{11}N_7O$: C, 53.53; H, 4.12; N, 36.41. Found: C, 53.48; H, 4.01; N, 36.29%.



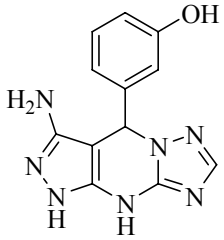
5.7.4.7 4-(3-nitrophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-322) Yield: 78%; mp 148-150 ºC; MS: *m/z* 298; Anal. Calcd. for $C_{12}H_{10}N_8O_2$: C, 48.32; H, 3.38; N, 37.57. Found: C, 48.23; H, 3.27; N, 37.49%.



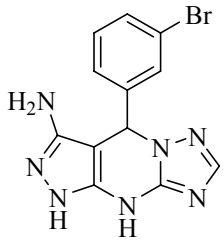
5.7.4.8 4-(3-chlorophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-323) Yield: 75%; mp 207-209 ºC; MS: *m/z* 287; Anal. Calcd. for $C_{12}H_{10}ClN_7$: C, 50.10; H, 3.50; N, 34.08. Found: C, 50.01; H, 3.39; N, 33.99%.



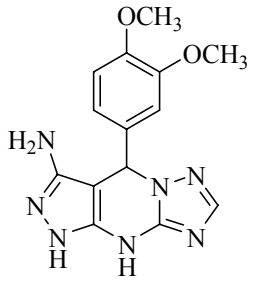
5.7.4.9 4-(3-hydroxyphenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-324) Yield: 71%; mp 208-210 ºC; MS: *m/z* 269; Anal. Calcd. for $C_{12}H_{11}N_7O$: C, 53.53; H, 4.12; N, 36.41. Found: C, 53.44; H, 4.03; N, 36.30%.



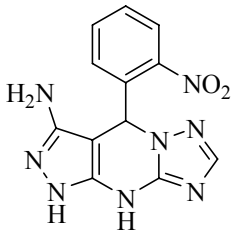
5.7.4.10 4-(3-bromophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-325) Yield: 61%; mp 229-231 ºC; MS: *m/z* 332; Anal. Calcd. for $C_{12}H_{10}BrN_7$: C, 43.39; H, 3.03; N, 29.52. Found: C, 43.27; H, 2.95; N, 29.40%.



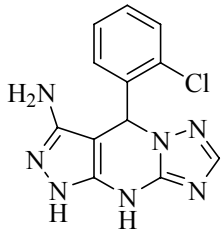
5.7.4.11 4-(3,4-methoxyphenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a]pyrimidine (ART-326) Yield: 72%; mp 177-179 ºC; MS: *m/z* 313; Anal. Calcd. for $C_{14}H_{15}N_7O_2$: C, 53.67; H, 4.83; N, 31.29. Found: C, 53.56; H, 4.77; N, 31.21%.



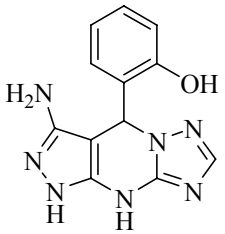
5.7.4.12 4-(2-nitrophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-327) Yield: 68%; mp 183-185 ºC; MS: *m/z* 298; Anal. Calcd. for $C_{12}H_{10}N_8O_2$: C, 48.32; H, 3.38; N, 37.57. Found: C, 48.22; H, 3.27; N, 37.49%.



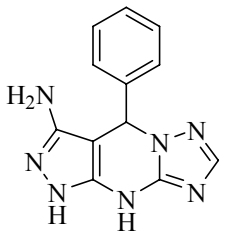
5.7.4.13 4-(2-chlorophenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-328) Yield: 68%; mp 213-215 ºC; MS: *m/z* 287; Anal. Calcd. for $C_{12}H_{10}ClN_7$: C, 50.10; H, 3.50; N, 34.08. Found: C, 50.01; H, 3.39; N, 33.99%.



5.7.4.14 4-(2-hydroxyphenyl)-3-amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a]pyrimidine (ART-329) Yield: 75%; mp 238-240 ºC; MS: *m/z* 269; Anal. Calcd. for $C_{12}H_{11}N_7O$: C, 53.53; H, 4.12; N, 36.41. Found: C, 53.42; H, 4.01; N, 36.29%.



5.7.4.15 4-(phenyl)-3- amino-4,9-dihydro-1H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a] pyrimidine (ART-330) Yield: 70%; mp 186-188 ºC; MS: *m/z* 253; Anal. Calcd. for $C_{12}H_{11}N_7$: C, 56.91; H, 4.38; N, 38.71. Found: C, 56.79; H, 4.26; N, 38.60%.

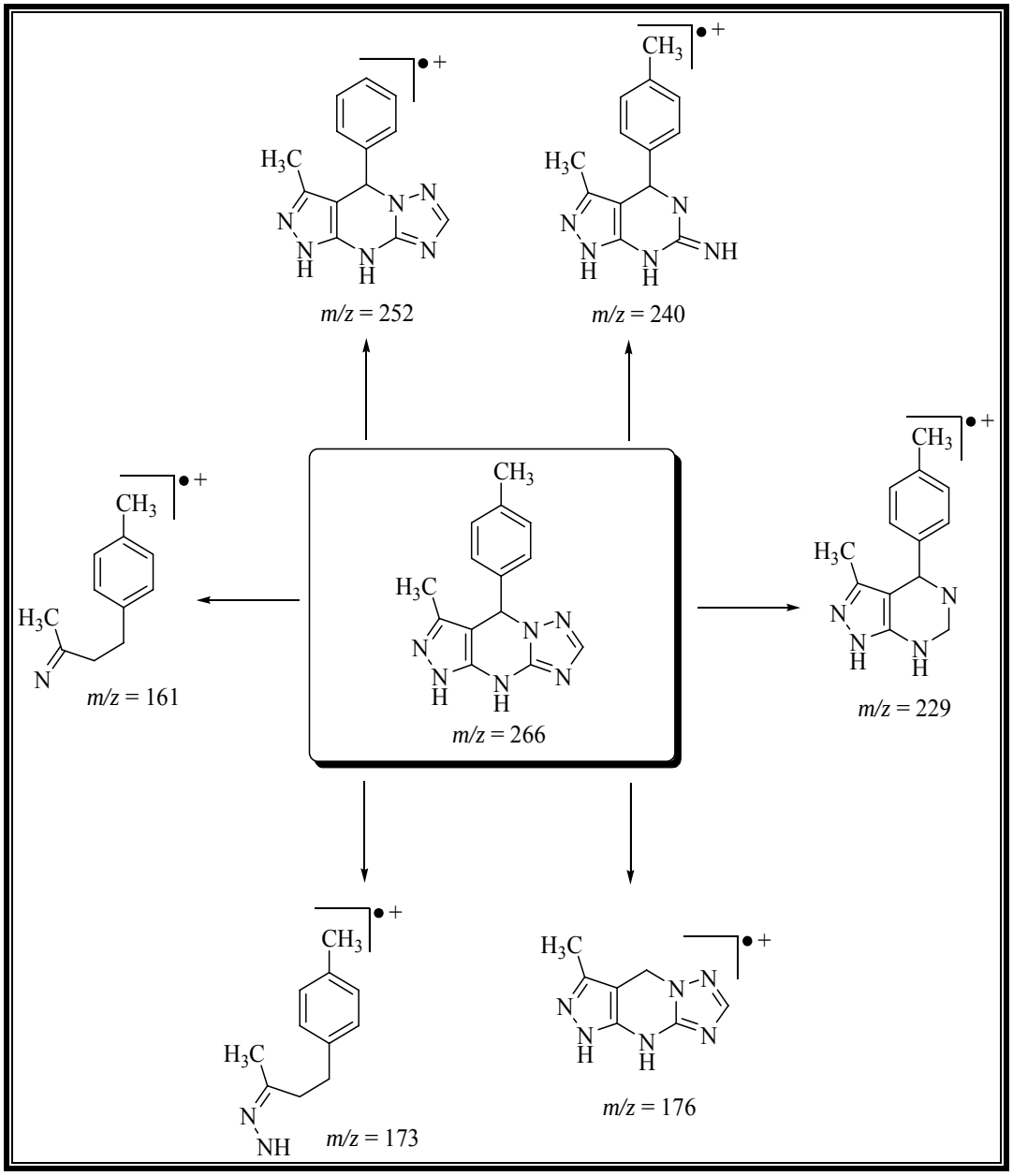


5.8 Spectral discussion

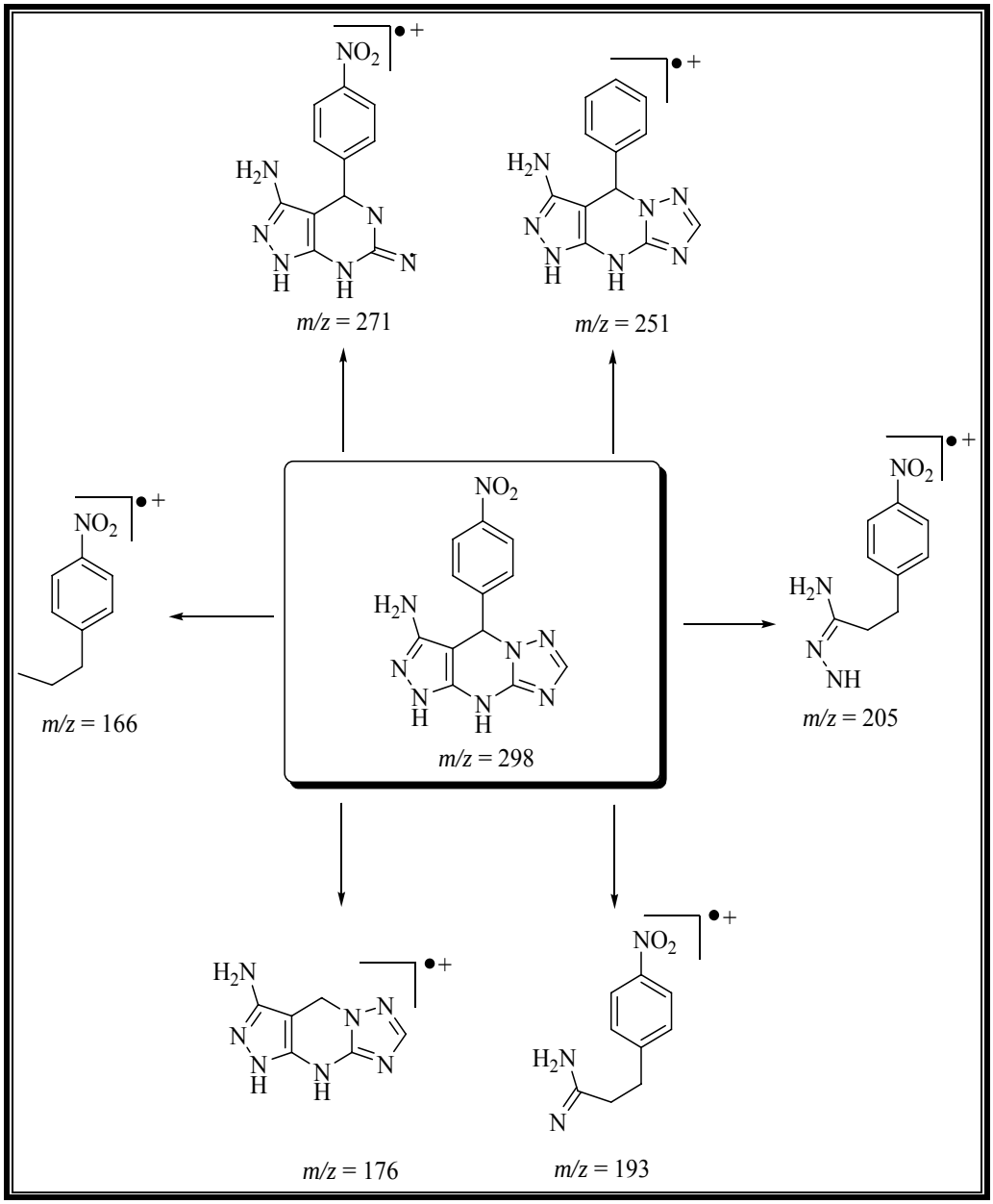
5.8.1 Mass spectral study

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.

5.8.1.1 Mass fragmentation pattern for ART-305



5.8.1.2 Mass fragmentation pattern for ART-318



5.8.2 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 pyrazolo-triazolo-pyrimidines ART-301 to 315, confirmatory bands for secondary amine and C=N stretching band was observed at 3213-3356 cm^{-1} and 1689-1693 cm^{-1} respectively, which suggested formation of desired products ART-301 to 315.

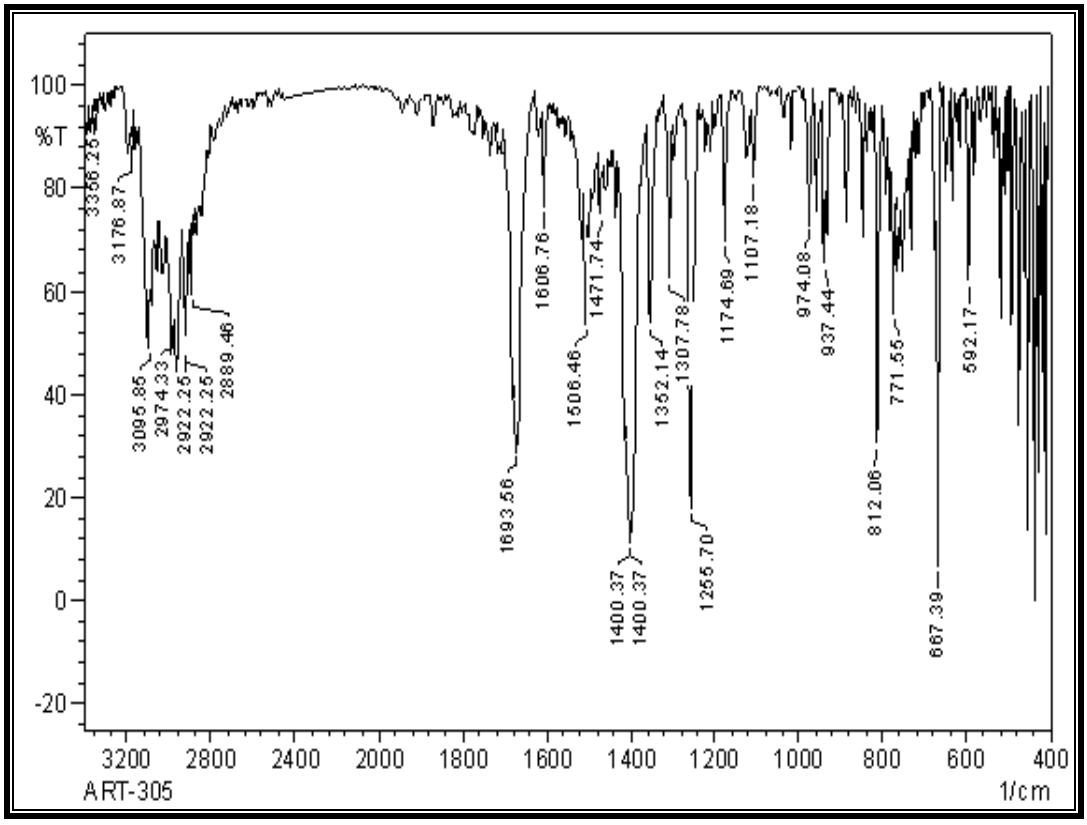
While, for pyrazolo-triazolo-pyrimidines ART-316 to 330 confirmatory bands for secondary amine and C=N stretching band was observed at 3215-3290 cm^{-1} and 1691-1695 cm^{-1} respectively, along with a characteristic stretching band for primary amine at 3412-366 cm^{-1} suggested formation of desired products ART-316 to 330.

5.8.3 ^{1}H NMR spectral study

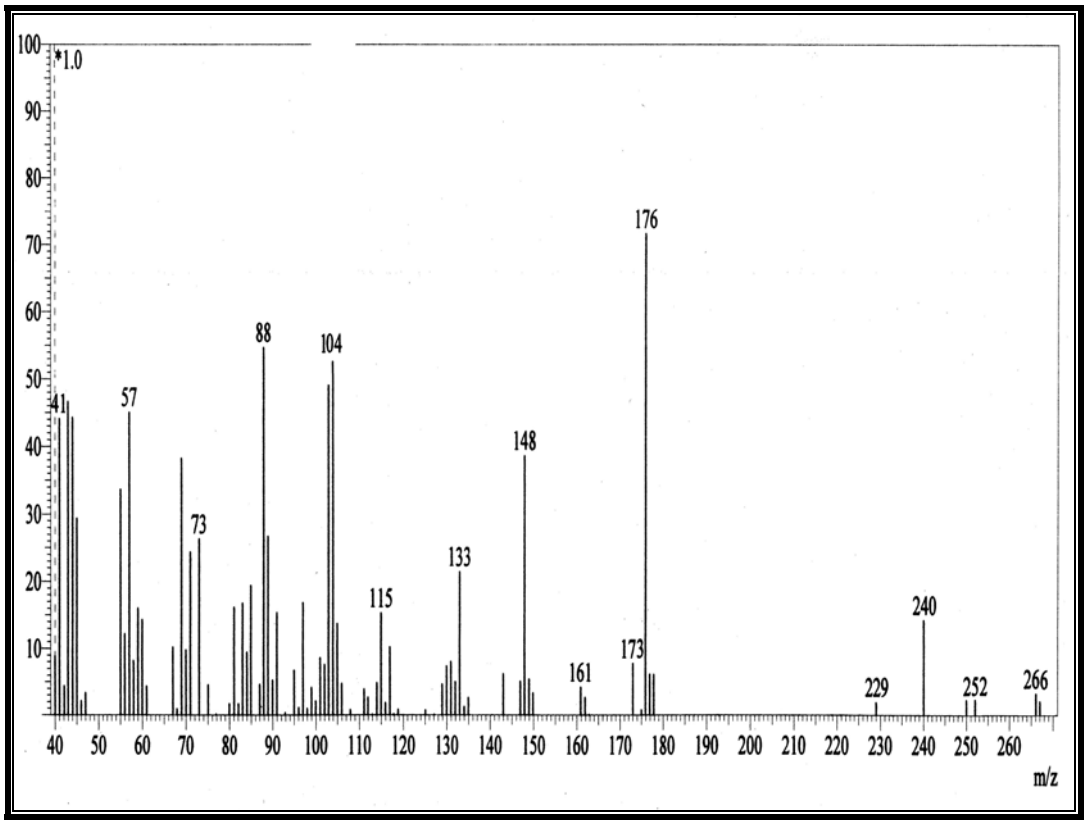
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of pyrazolo-triazolo-pyrimidines ART-301 to 330 on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 7.39-8.10 δ ppm and a singlet for the methine proton of triazole ring at 7.93-8.30 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.

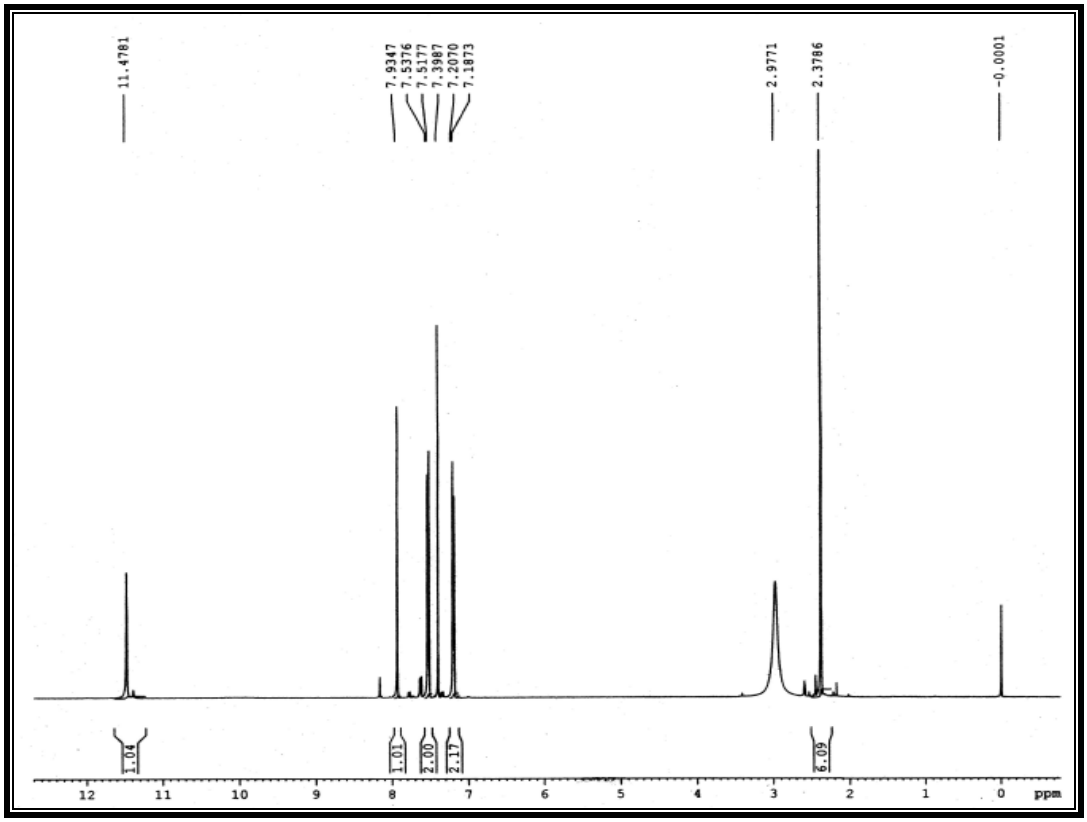
IR spectrum of ART-305



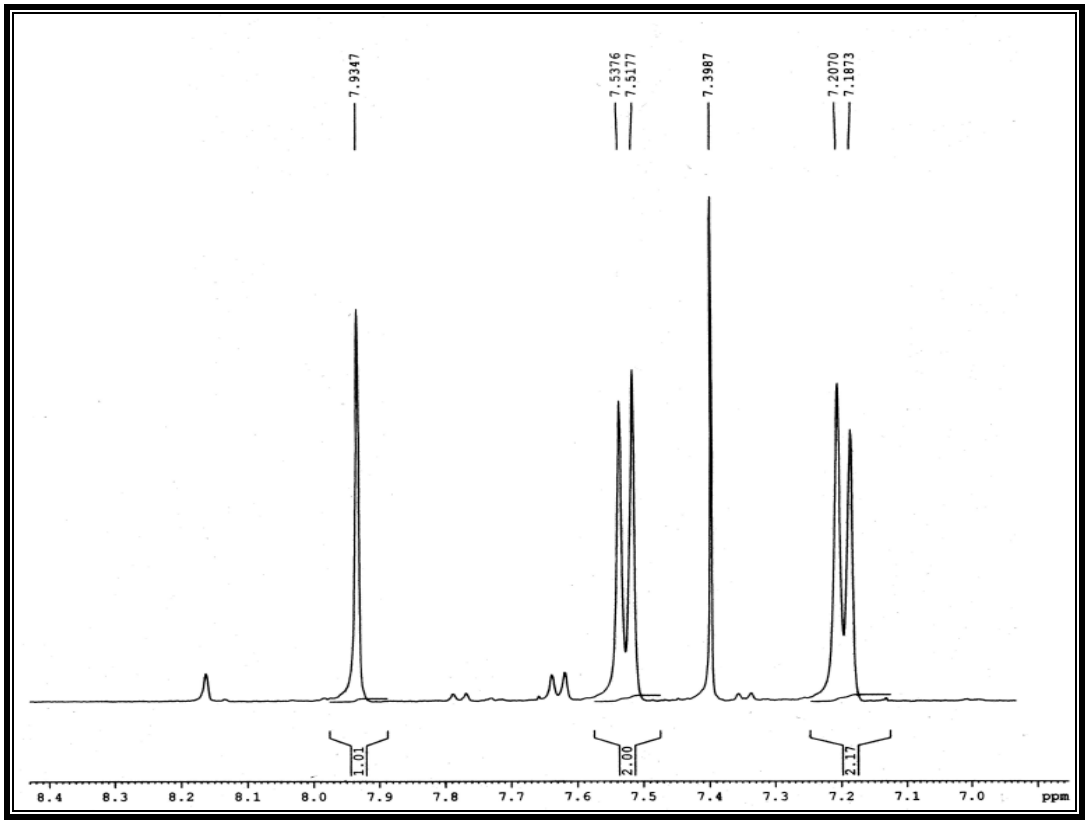
Mass spectrum of ART-305



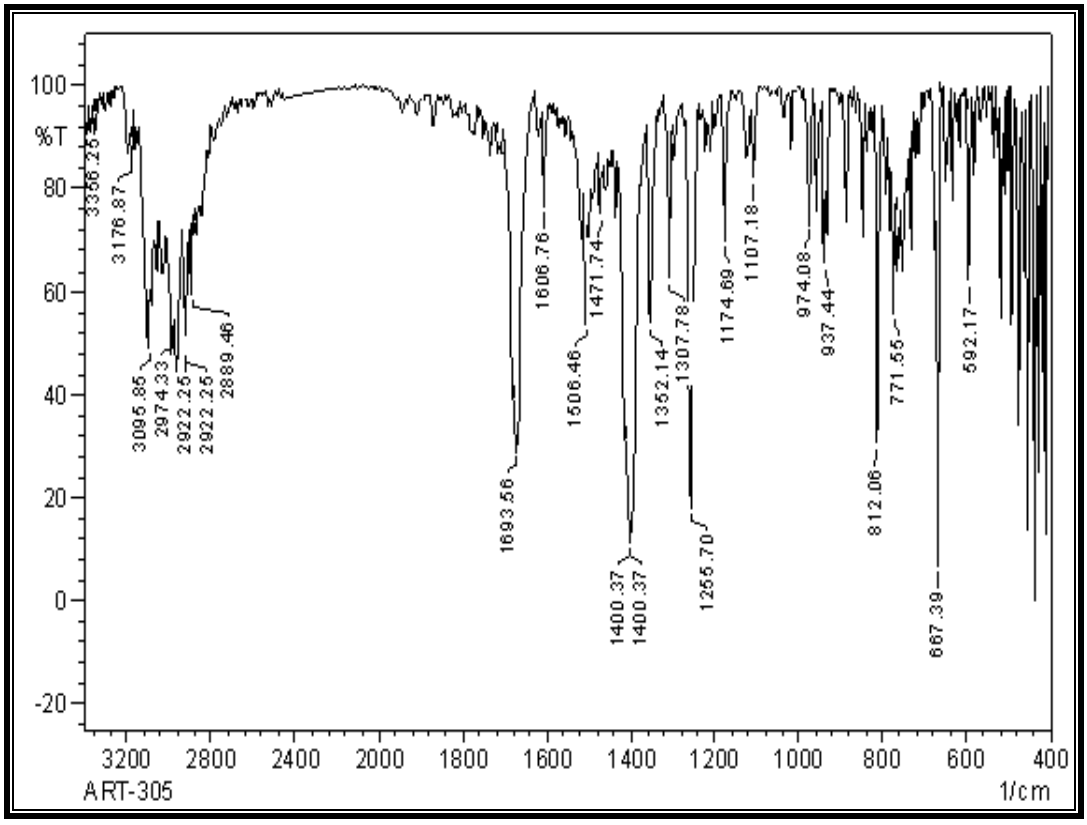
^{1}H NMR spectrum of ART-305



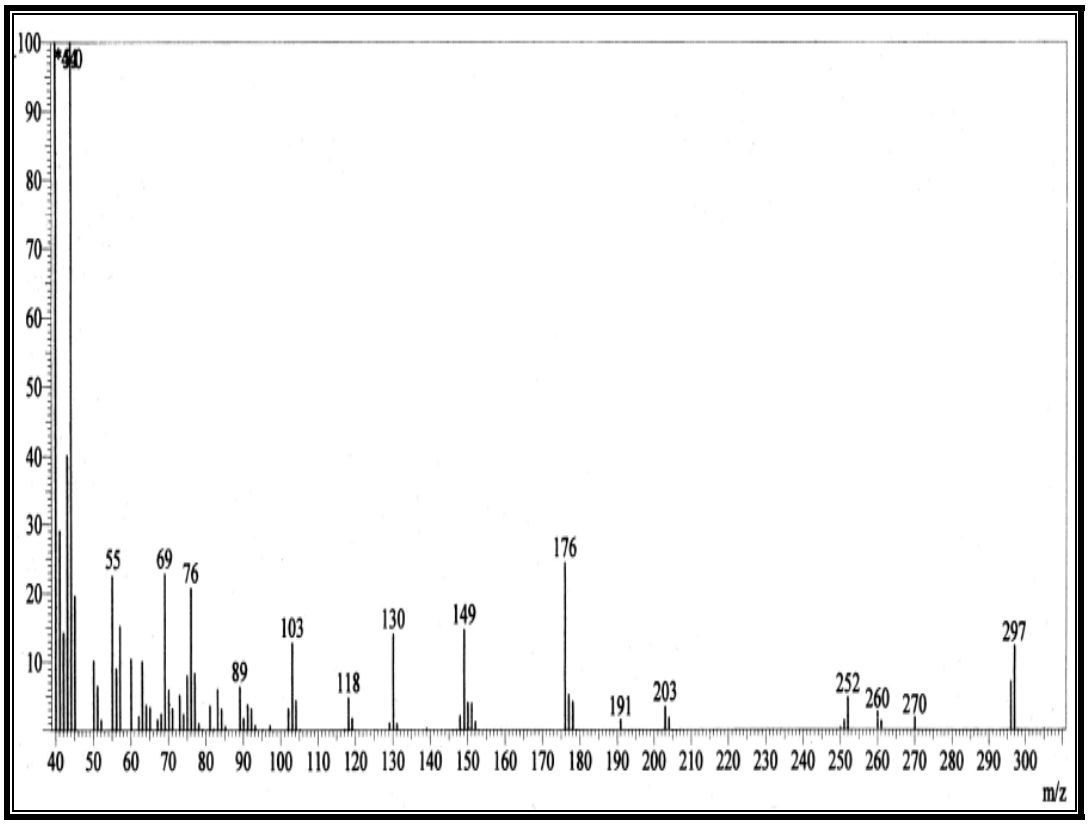
Expanded ^{1}H NMR spectrum of ART-305



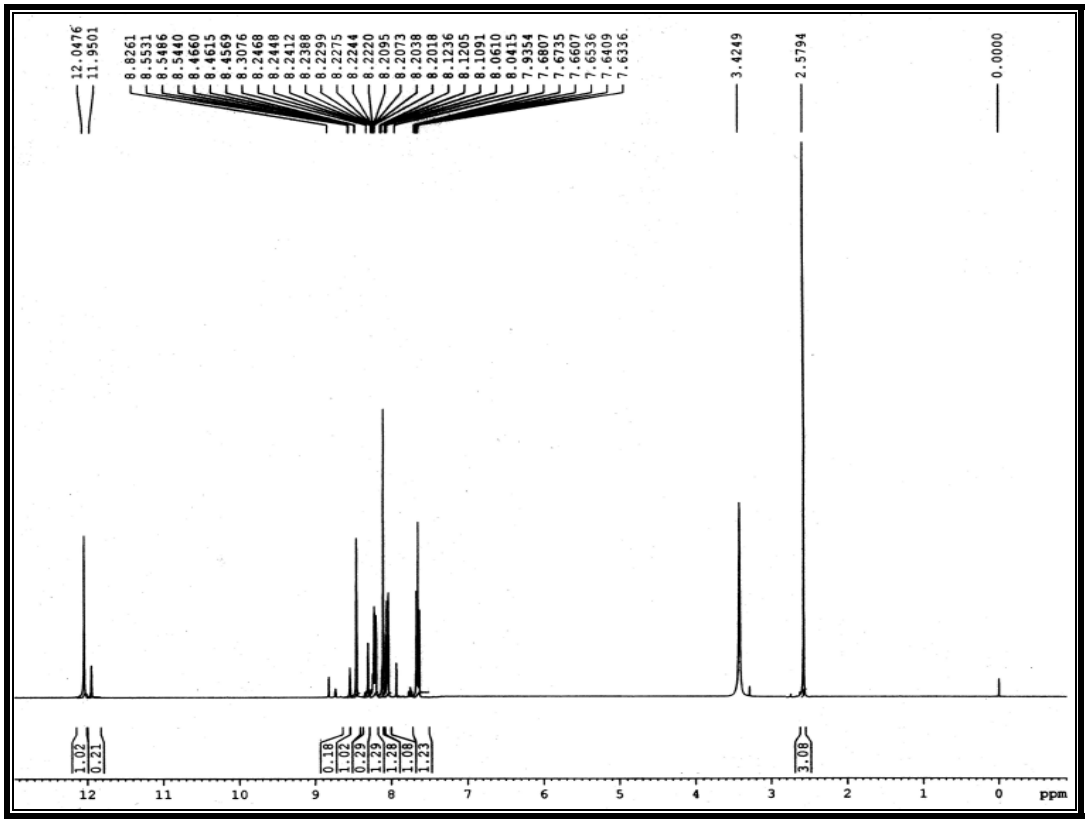
IR spectrum of ART-307



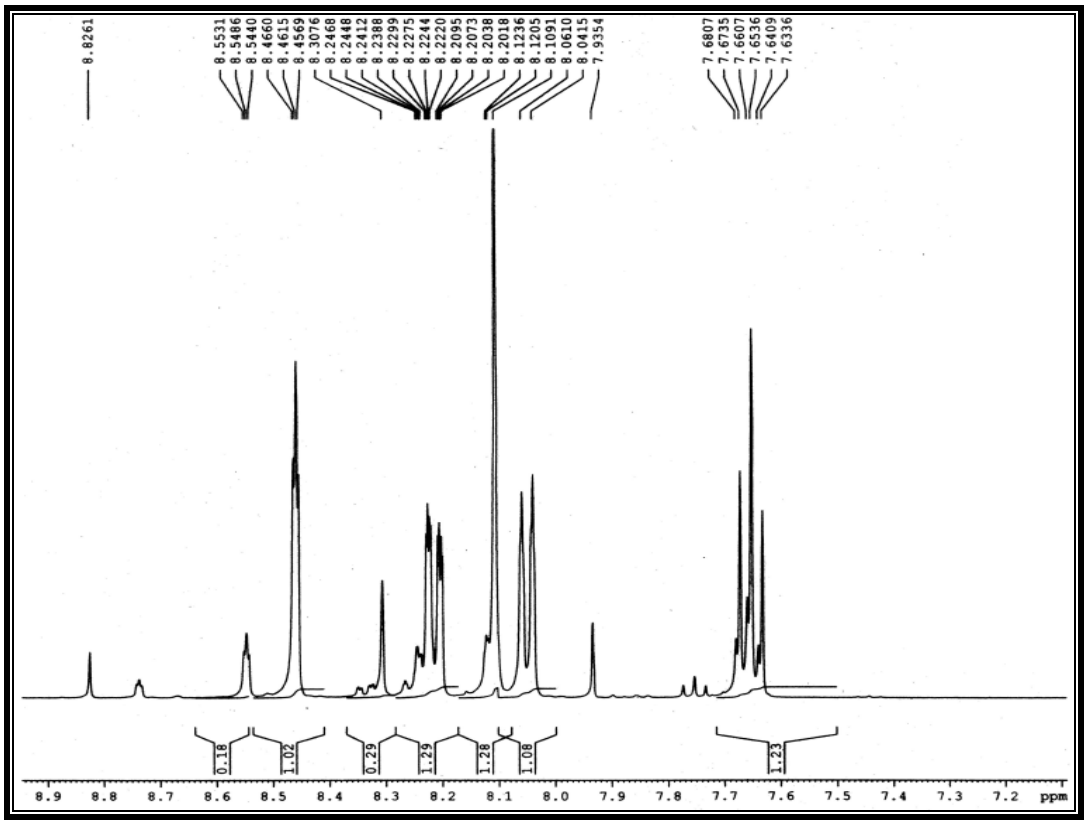
Mass spectrum of ART-307



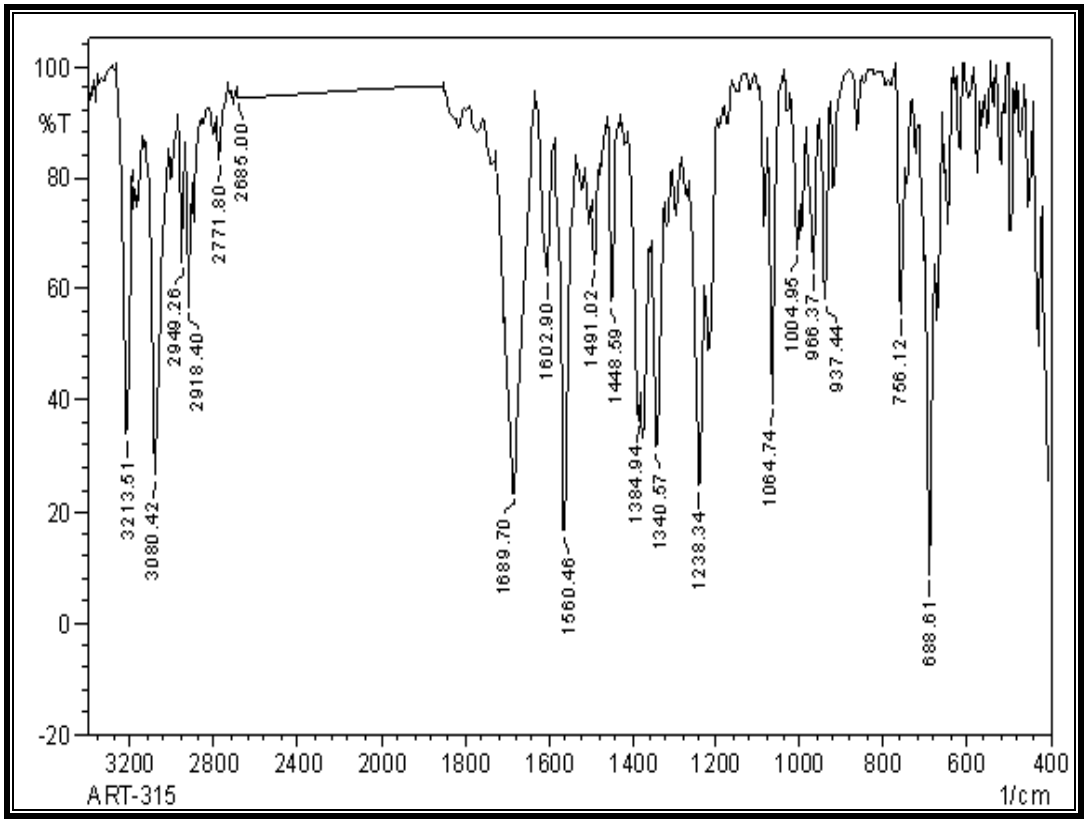
^{1}H NMR spectrum of ART-307



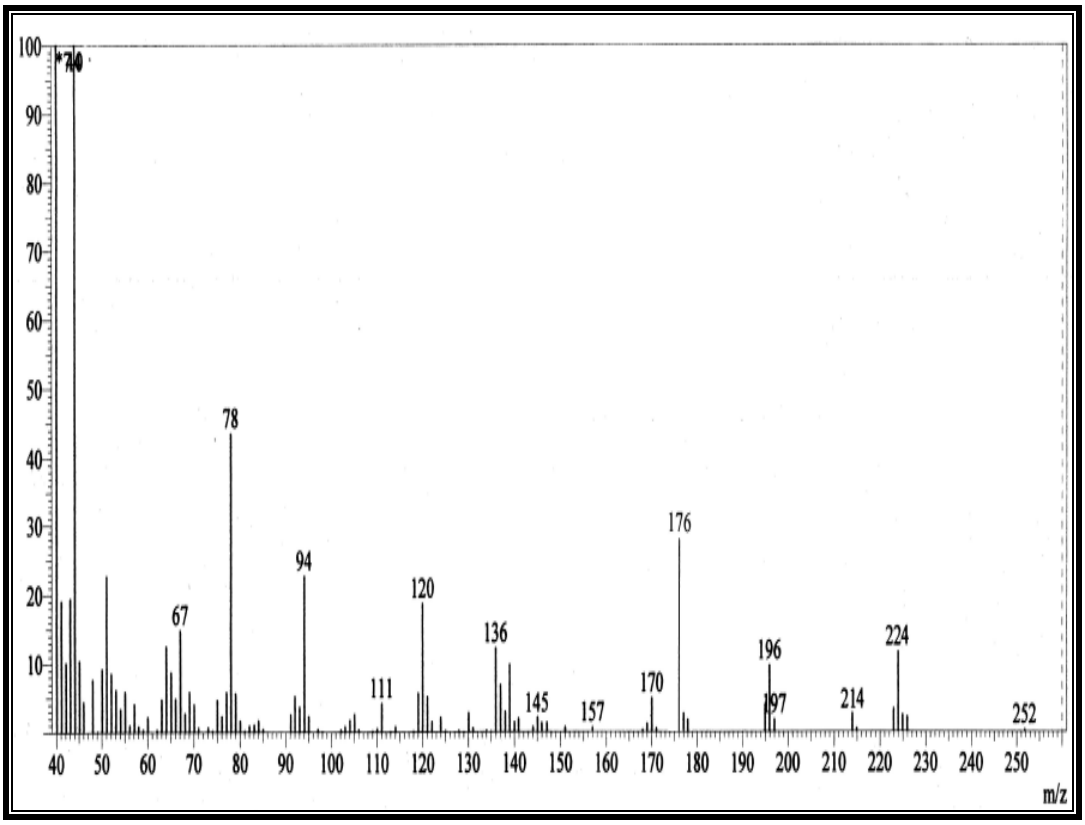
Expanded ^{1}H NMR spectrum of ART-307



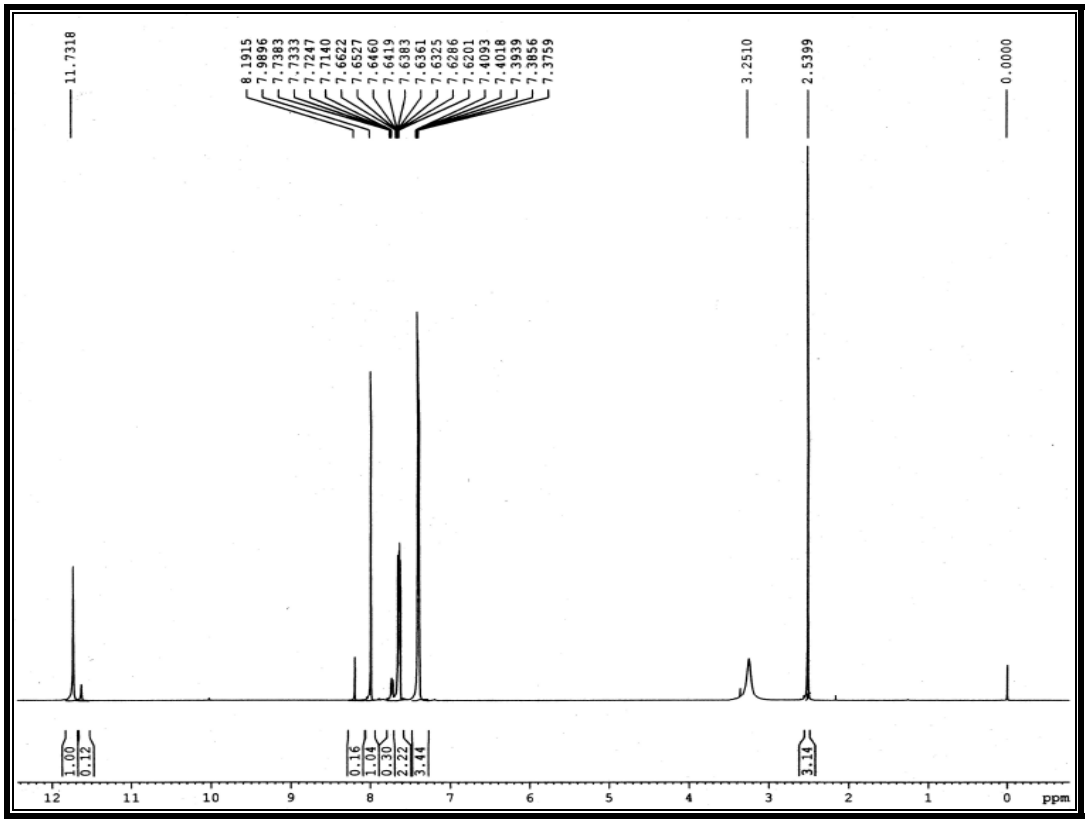
IR spectrum of ART-315



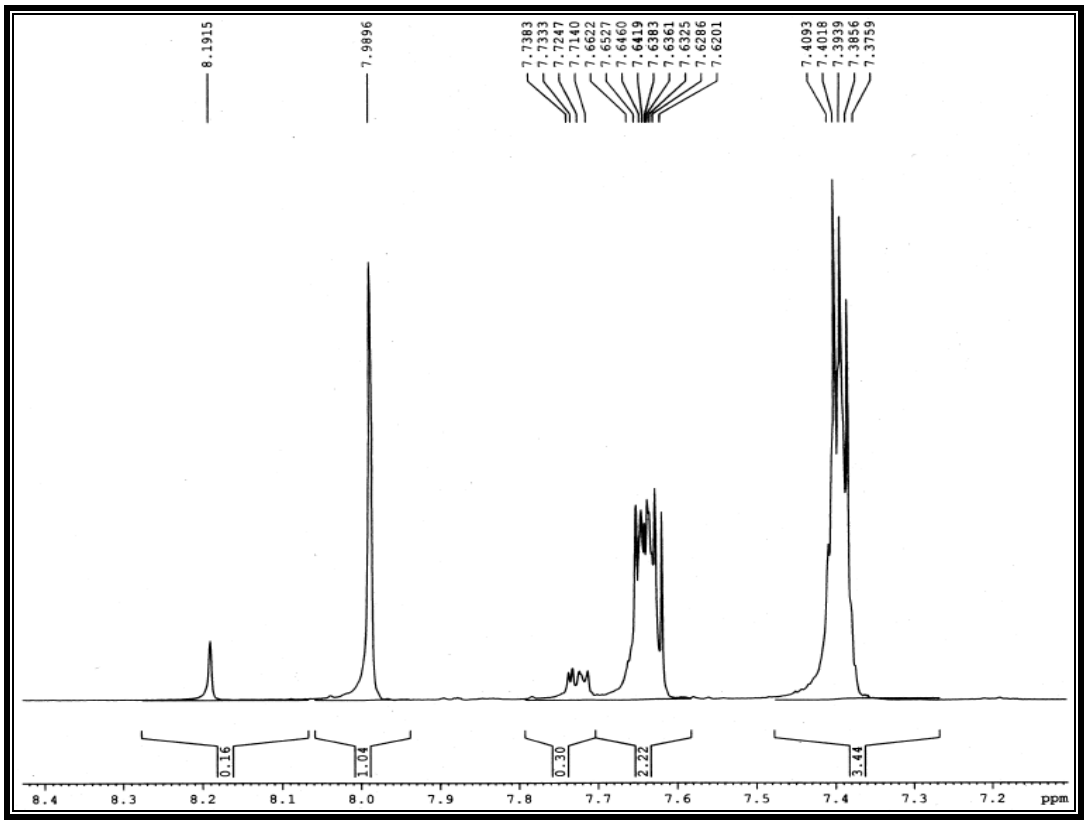
Mass spectrum of ART-315



^{1}H NMR spectrum of ART-315



Expanded ^{1}H NMR spectrum of ART-315



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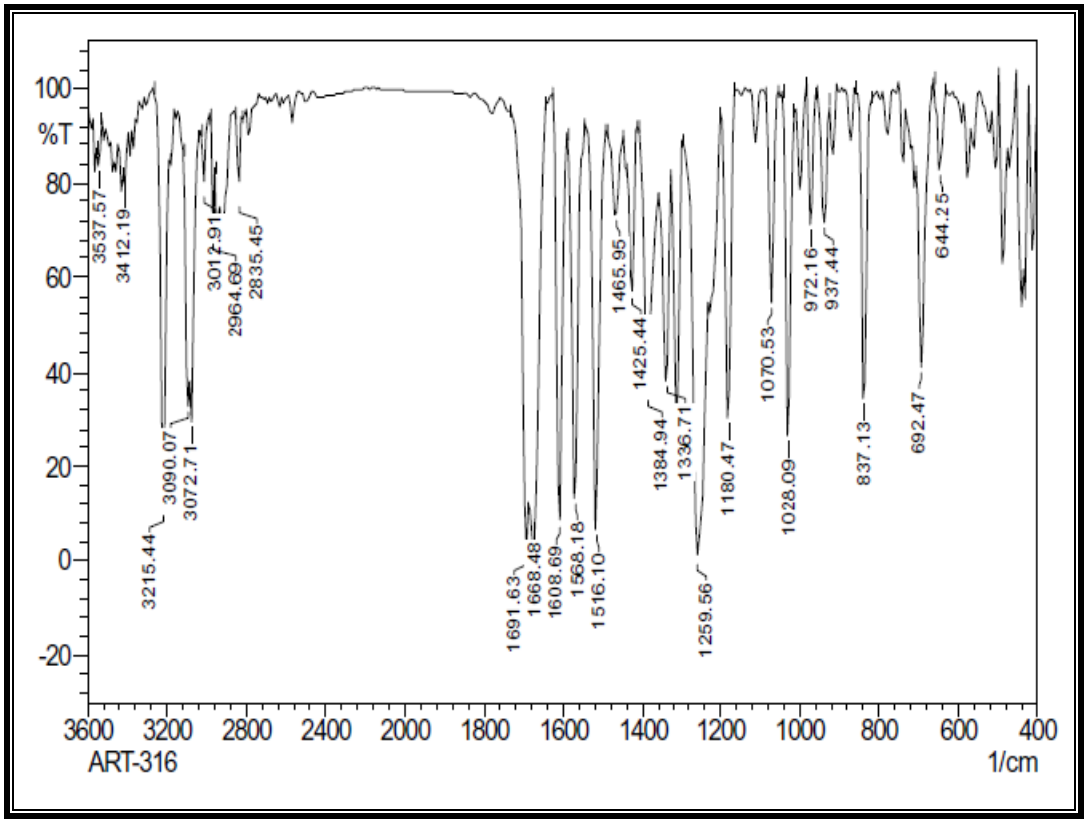

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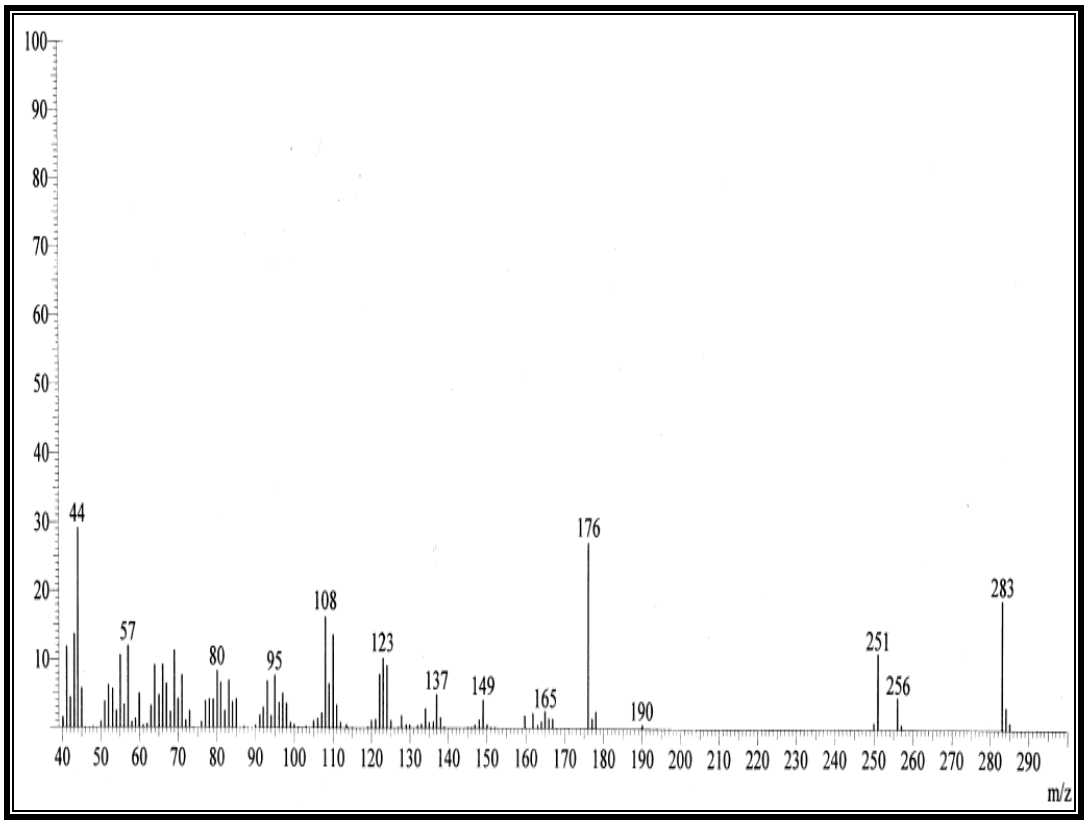

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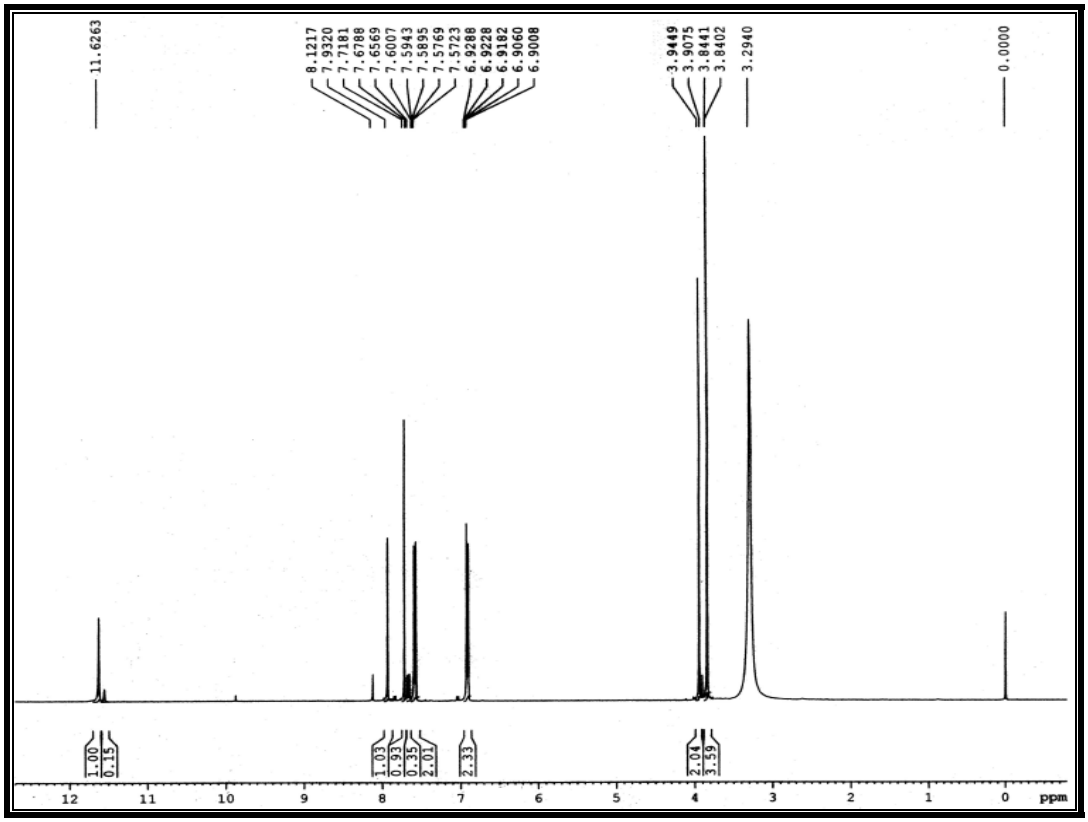
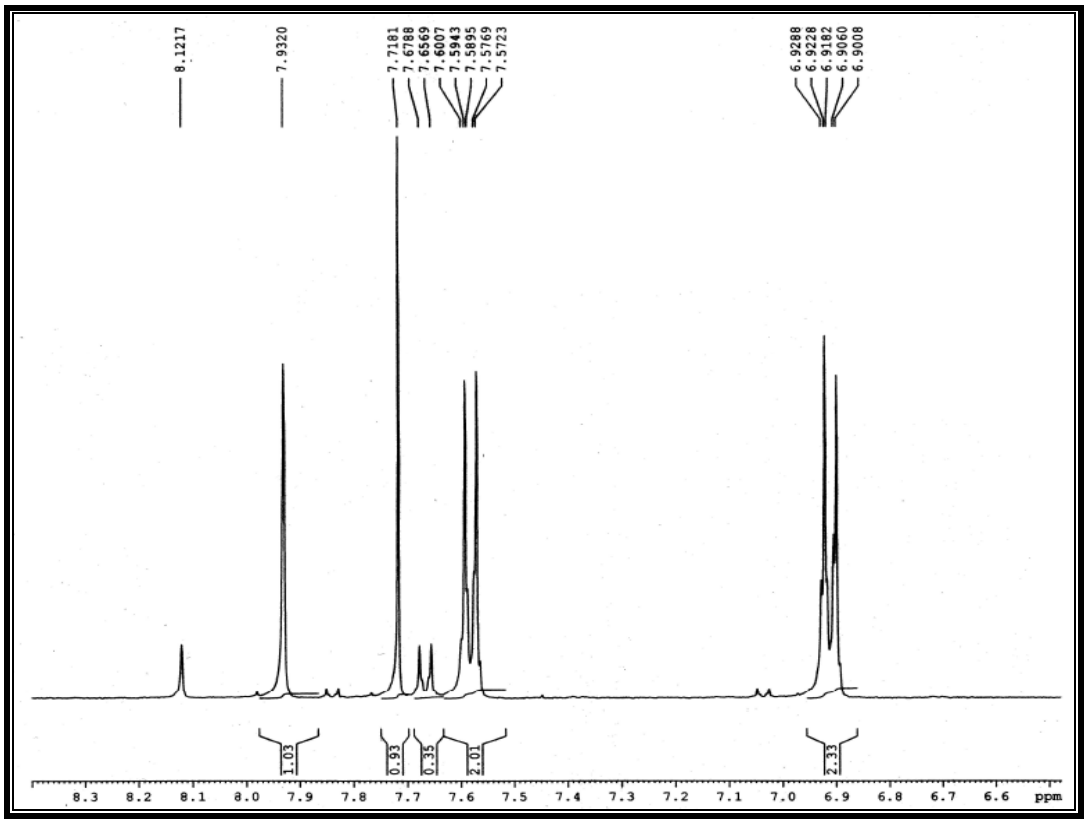


Expanded ^{1}H NMR spectrum of ART-316



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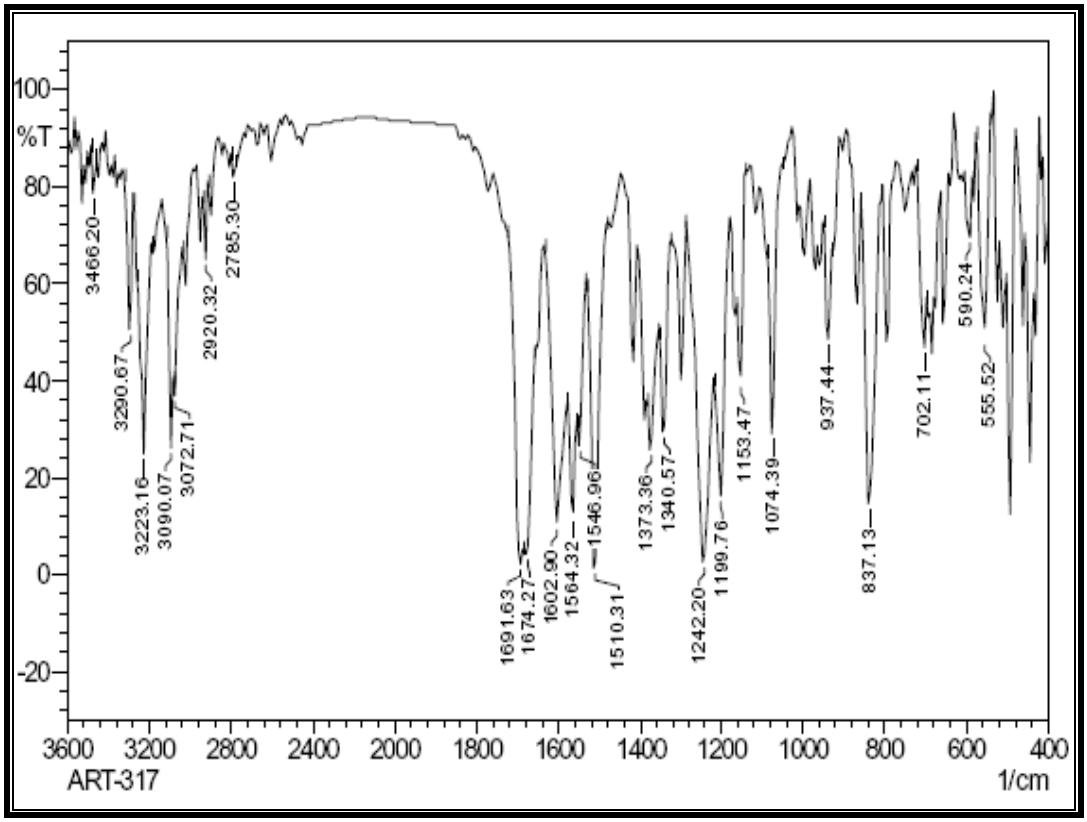
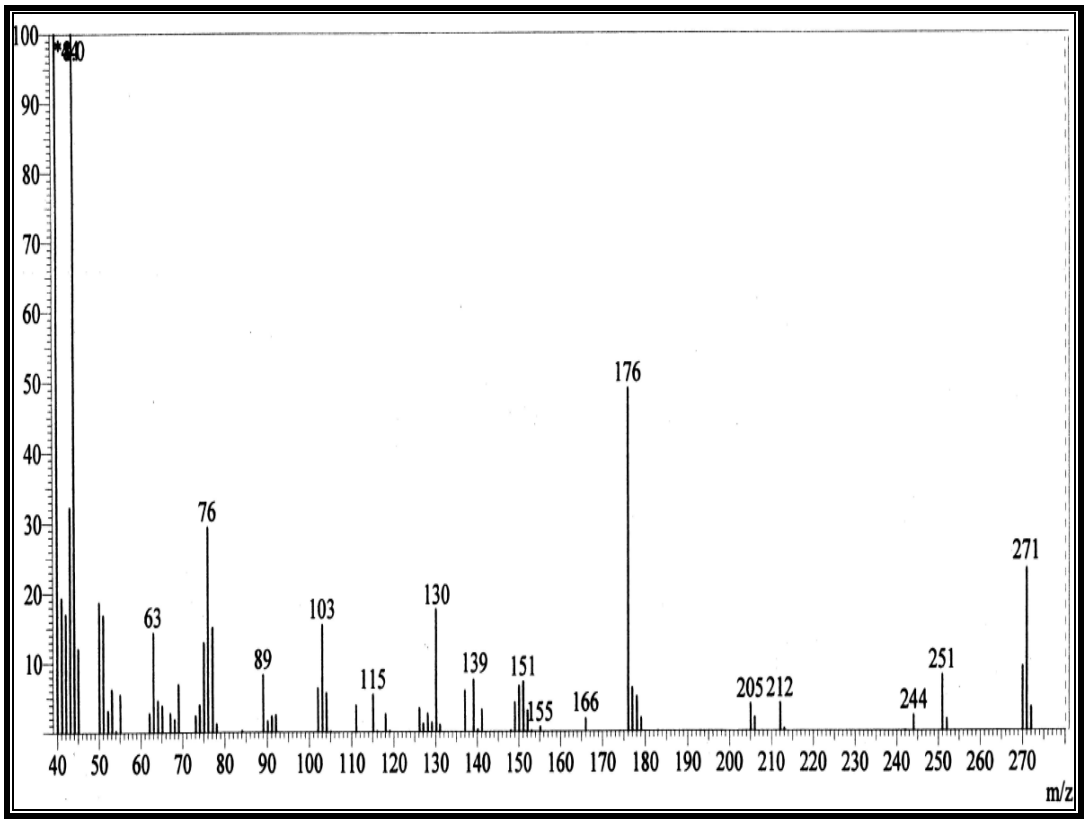
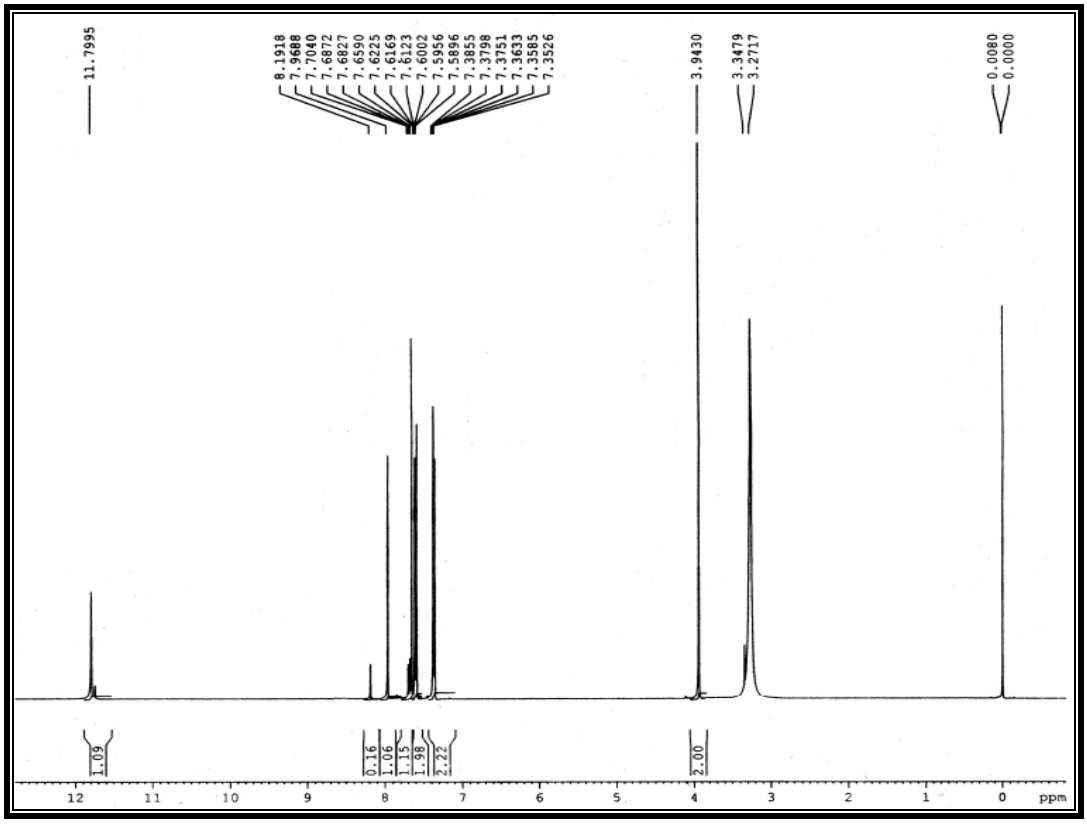


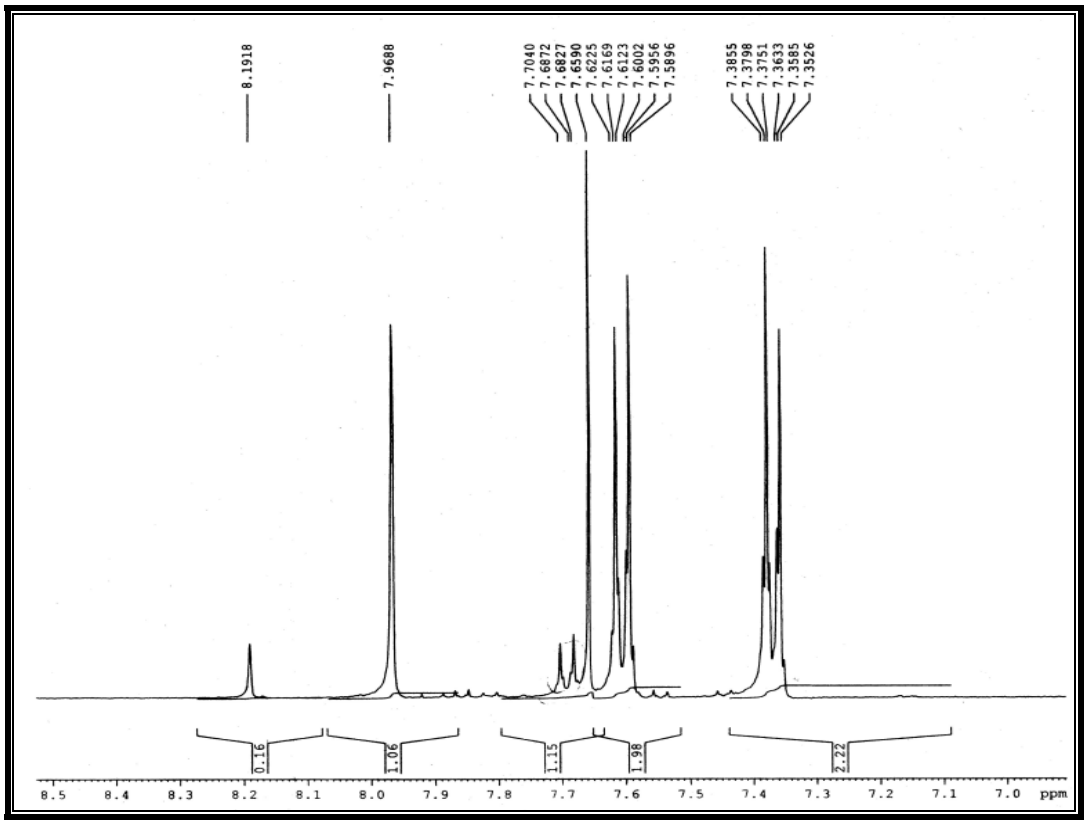
Mass spectrum of ART-317



^{1}H NMR spectrum of ART-317



Expanded ^{1}H NMR spectrum of ART-317



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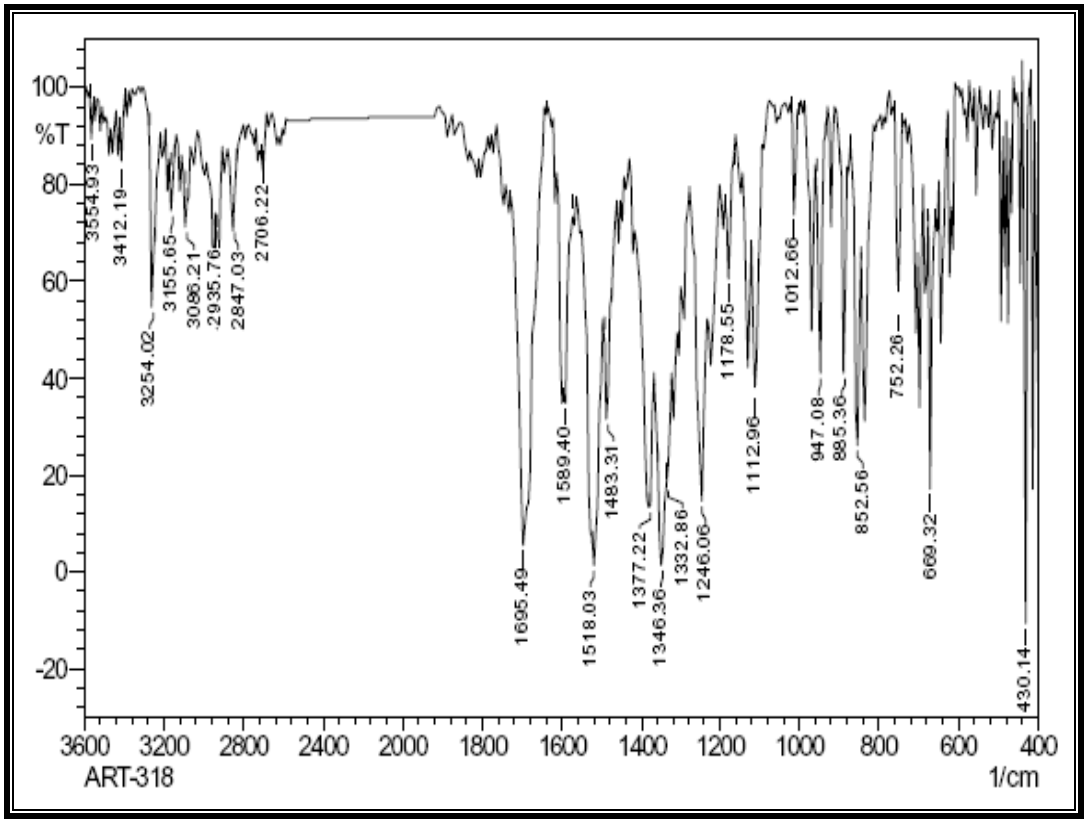
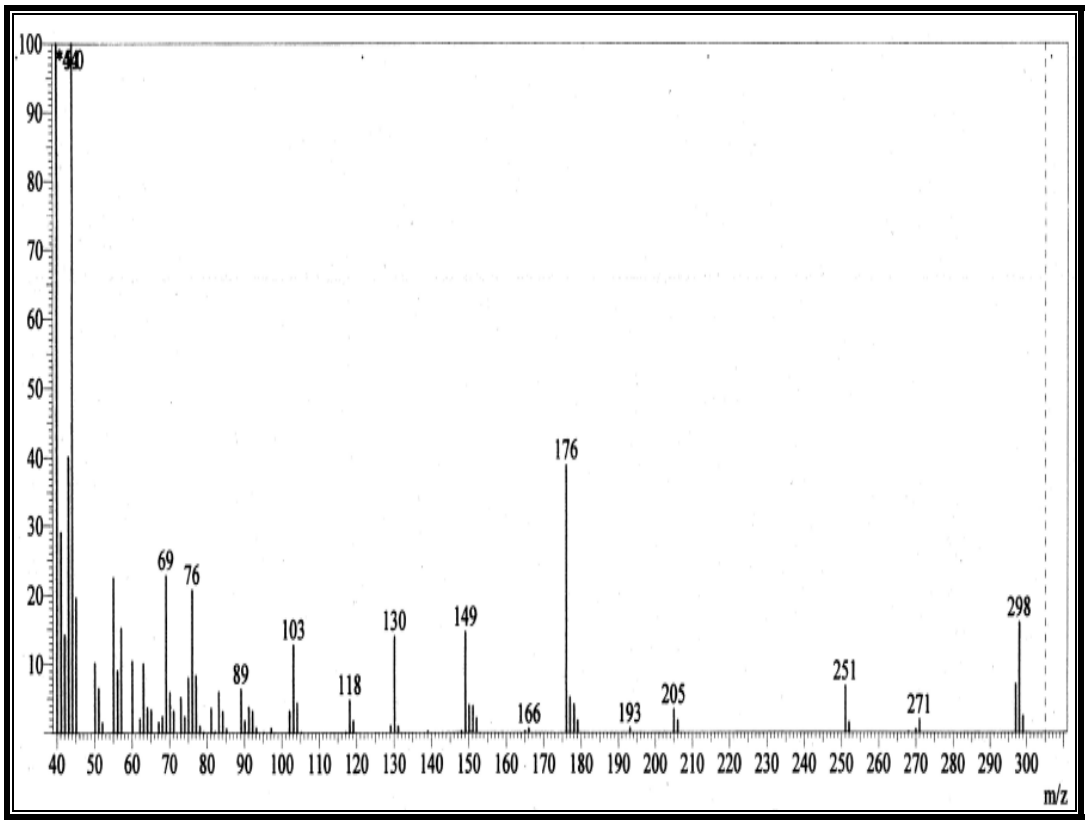
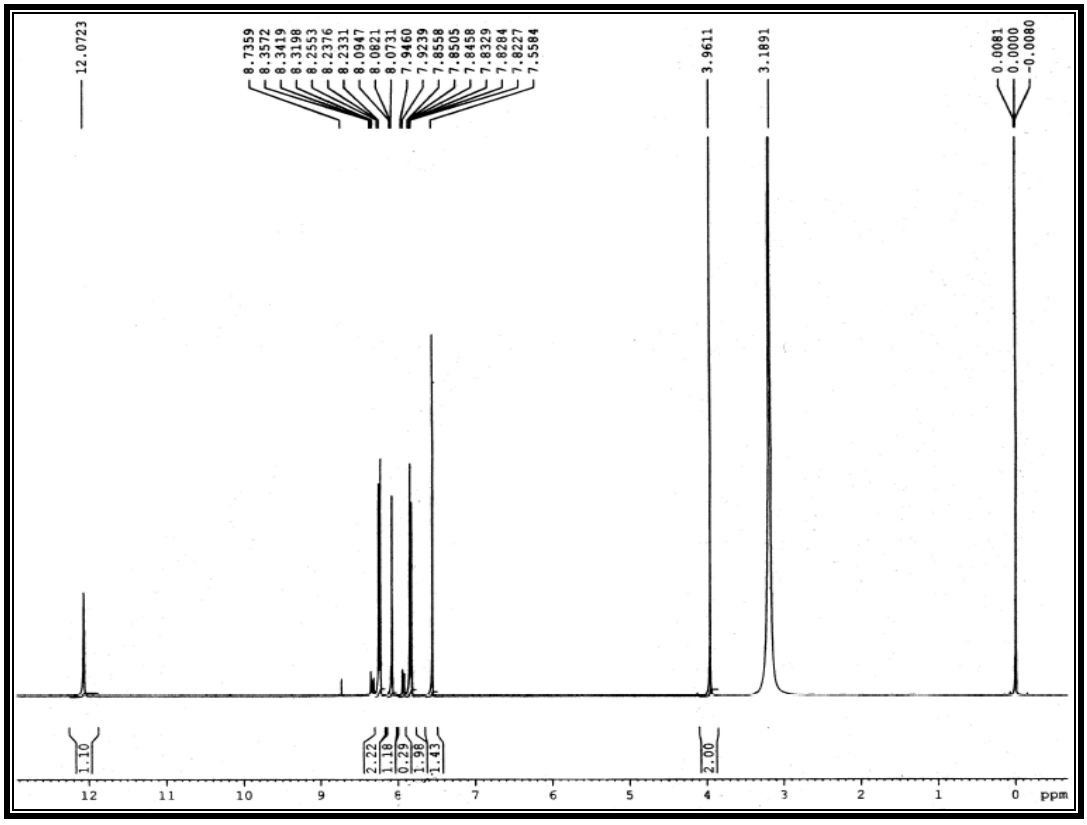


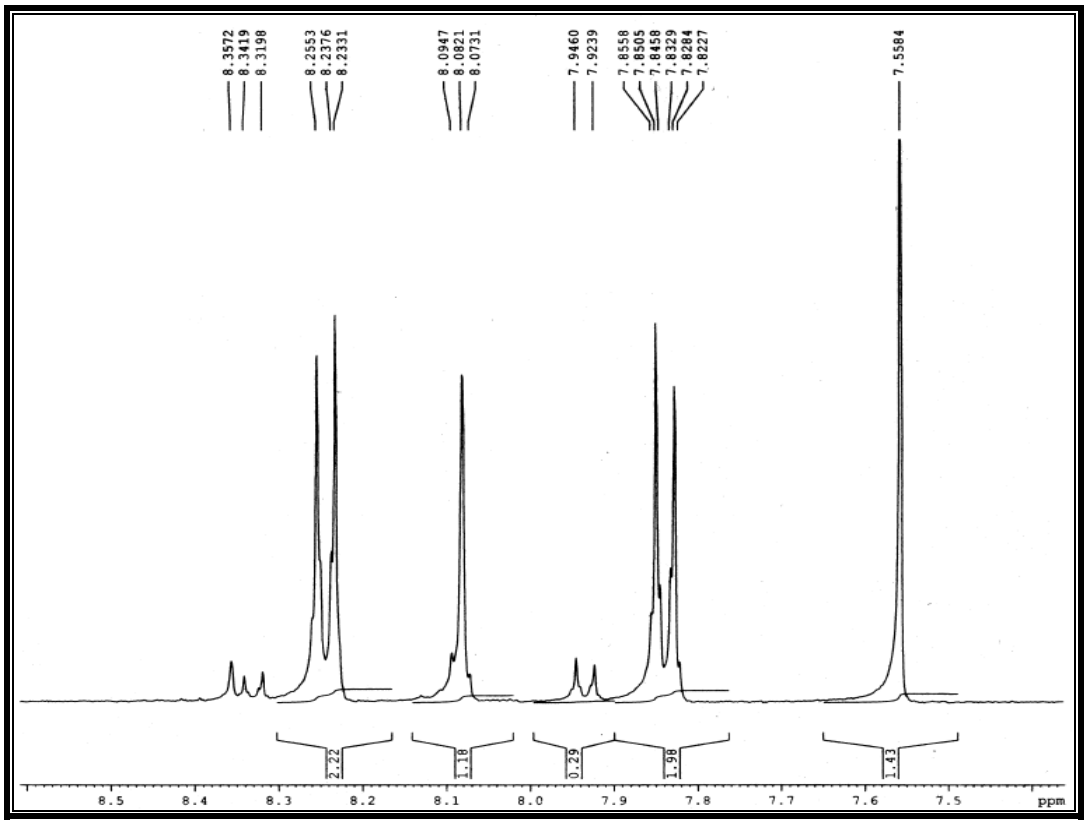
Mass spectrum of ART-318



^{1}H NMR spectrum of ART-318



Expanded ^{1}H NMR spectrum of ART-318



5.9 Biological evaluation

5.9.1 Antimicrobial evaluation

All of the synthesized compounds (ART-301 to 330) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [51-53] 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 ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, 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 microdilution broth method according to NCCLS standards [51]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 μg mL^{-1}. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds ART-301 to 330

Code	Minimum 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.***
ART-301	1000	500	500	1000	250	1000	500
ART-302	250	1000	100	500	500	250	>1000
ART-303	50	500	250	500	>1000	100	500
ART-304	125	100	500	125	250	500	500
ART-305	500	500	1000	500	1000	1000	250
ART-306	500	500	>1000	1000	500	1000	>1000
ART-307	62.5	250	500	125	1000	100	500
ART-308	100	500	1000	1000	500	500	100
ART-309	1000	1000	250	>1000	500	250	1000
ART-310	250	250	500	500	1000	100	1000
ART-311	250	1000	1000	250	>1000	1000	500
ART-312	125	500	>1000	1000	500	250	500
ART-313	100	500	500	500	125	250	250
ART-314	500	1000	250	1000	500	1000	500
ART-315	250	100	500	>1000	1000	500	1000
ART-316	1000	500	250	500	500	500	1000
ART-317	100	100	1000	250	1000	100	1000
ART-318	250	250	1000	1000	250	>1000	500
ART-319	500	25	500	125	100	100	250
ART-320	>1000	500	1000	1000	500	1000	500
ART-321	1000	500	250	250	>1000	>1000	1000
ART-322	250	500	500	1000	250	500	250
ART-323	1000	1000	500	>1000	1000	1000	500
ART-324	>1000	1000	250	500	500	500	500
ART-325	1000	500	1000	500	125	250	1000
ART-326	500	250	500	100	500	100	250
ART-327	250	100	1000	500	250	1000	500
ART-328	1000	500	500	500	500	500	>1000
ART-329	250	1000	62.5	250	500	250	500
ART-330	500	250	250	500	>1000	500	500
Ampicillin	250	100	1000	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

5.9.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds ART-301 to ART-330 is currently under investigation and results are awaited.

5.10 References and notes

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Summary

The work presented in the Thesis entitled "Studies on Some Compounds 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 thieno[2,3-*d*]pyrimidines 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 affected from novel 2-aminothiophenes containing an acetoacetamide fragment by acid catalysed cyclocondensation with formic acid, gl. acetic acid and trifluoroacetic acid, respectively.

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. 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 min.

Chapter 5 describes applications of microwaves in heterocyclic ring formation. Pyrazolo-triazolo-pyrimidines have been the subject of intense research due to the interesting pharmacological activities found for several of their derivatives. Chapter 5 includes synthesis of thirty novel pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines with the aim to extend the synthetic pathways to new planar heterocyclic ring systems, chemistry of which have never been explored, and there are no reports on the synthesis and chemical properties of these class of compounds. The synthesis was achieved by using a one pot, microwave-assisted, catalyst-free biginelli like

condensation of aminoazole, 3-substituted-pyrazol-5(4*H*)-ones, and an appropriate aromatic aldehyde in ethanol. Thus, a new green chemistry approach was developed leading to the improvement in the reaction time, yield and simplicity of work up procedure.

All the synthesized compounds were characterized by IR, Mass, ^{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.

Publications

1. Synthesis and antimycobacterial evaluation of various 6-substituted pyrazolo[3,4-*d*]pyrimidine derivatives, by Amit R. Trivedi, Shailesh J. Vaghasiya, Bipin H. Dholariya, Dipti Dodiya and Viresh H. Shah. *Journal of Enzyme Inhibition and Medicianl Chemistry*, Accepted, In Press.
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Conferences/Seminars participated

1. National seminar on "Recent Advances in Chemical Sciences & An Approach to Green Chemistry" jointly organized by Department of Chemistry & Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar at Rajkot, India (October 11-13, 2006).
2. 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).
3. "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).
4. "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).
5. "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).
6. "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).

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