# *Studies on Some Organic Compounds of Therapeutic Interest*

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

# *Doctor of Philosophy*

**From**

**Saurashtra University**

**By**

**Haresh K. Ram**

**Under the Guidance of**
**Prof. V. H. Shah**
**(*M.Sc. Ph.D. FIC*)**

**Department of Chemistry**
**Saurashtra University**
**Rajkot, Gujarat**
**India**

**August 2010**

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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 (Haresh K. Ram)

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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. Haresh K. Ram 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-360 005
Gujarat (India).

*Dedicated*
*to my*
*beloved parents*

# ***Acknowledgement***

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

*First and foremost I bow my head with absolute respect and pleasantly convey my heartily thankfulness to my research guide and thesis supervisor, most respectable* ***Prof. Viresh Shah****, who has helped me at each and every stage of my research work with patience and enthusiasm. I am much indebted to him for his inspiring guidance, affection, generosity and everlasting supportive nature throughout the tenure of my research work. I can never forget what Viresh sir has done for me.*

*I would like to bow my head with utter respect and convey my pleasant regards to the most adorable personalities in the world, my mummy-papa for giving me permission and chance to undertake this project and also for their blessings, constant support, courage and enthusiasm, they have shown throughout my work without which the thesis would not have been appeared in the present form. I am equally thankful to my younger brother* ***Mehulbhai and jagdishbhai*** *for their moral support and courage in each moment.*

*It was a dream of my family which has now come true. I bow my head humbly before* ***jay shree Lakhanaimataji*** *for making me much capable that I could adopt and finish this huge task.*

*I would like to convey my pleasant heartily thankfulness to my dearest friend* ***Dodiya bhavesh, Joshi kaushik, Ram vijay, Kher gopal, pada ranjit, Nandaniya ramesh, Chavda rajendra Vala hardev, bhatt mehul, Bavishi abhay, Tada rakesh, Odish vipul, and Shailesh,*** *for his time being help and moral support. I will never forget their all kind concern, help, best wishes and that they have done for me. I am really very much thankful to God for giving me such nice friend.*

*Words are inadequate to thank my most beloved friends and colleagues* ***Trivedi amit, Dodiya dipti and Vakhariya chintu*** *who were always with me during Ph. D., helping me in all situations. Their constant support, care and moral boost always kept me encouraged in all*

*the difficult situations. I am really very much thankful to God for giving me such nice friends. I would like to express my deep sense of gratitude to my dearest friend* ***hashi****.*

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*I am also grateful to Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CDRI), Punjab University, Chandigarh and CDRI, Lucknow for* $^{1}$*H NMR analysis, for Elemental, Mass and IR analysis- Department of Chemistry, Saurashtra University, Rajkot. My sincere thanks go to Microcare Laboratory, Surat for antimicrobial evaluation of the synthesized compounds.*

*I would also like to thank High Authority Commands,* ***University Grants Commission (UGC)****, New Delhi and* ***Saurashtra University****, Rajkot for providing* ***state of the art laboratory facility*** *and other infrastructure facilities.*

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

***Haresh K. Ram***

***/08/2010***

***Rajkot***

# ***Table of Contents***

# ***General remarks***

1. $^{1}$H NMR spectra were recorded on Bruker avance II 400 MHz NMR spectrometer using TMS as an internal reference.
2. Mass spectra were recorded on GC-MS QP-2010 spectrometer.
3. IR spectra were recorded on Schimadzu FT-IR-8400 spectrometer.
4. Elemental analysis was carried out on Vario EL III Carlo Erba 1108.
5. Thin layer chromatography was performed on Silica Gel (Merck 60 $F_{254}$).
6. The chemicals used for the synthesis of compounds were purchased from Spectrochem, Merck, Thomas-baker and SD fine chemical.
7. Melting Points were taken in open capillary and are uncorrected.
8. All the structures are drawn according to ACS Document 1996 style.

# ***Synopsis***

The work to be presented in thesis entitled "Studies on Some organic Compounds of Therapeutic 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 1,2,3,4 tetra hydro pyrimidines |
| Chapter 4 | Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines |
| Chapter 5 | Synthesis and biological evaluation of 1,4-dihydropyrimido[1,2-*a*] benzimidazole |

## 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 entity (NCE) 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 objective of the proposed research work.

## Chapter 2 Biological and medicinal significance of pyrimidines and related heterocycles

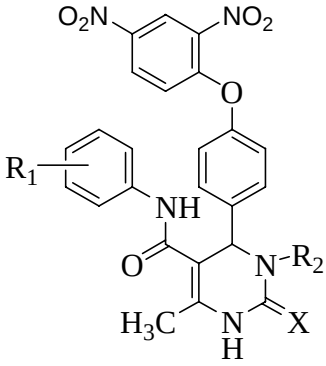
Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS.

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**.**

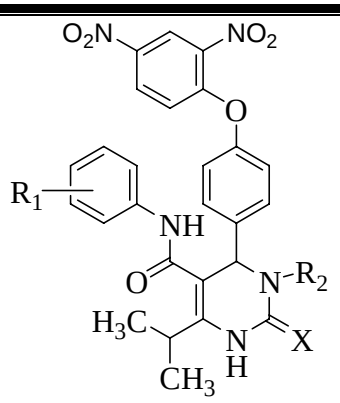
## Chapter 3 Synthesis and biological evaluation of 1,2,3,4 tetra hydro pyrimidines

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities against unrelated DNA and RNA viruses including antitubercular, antibacterial, immunodilator, antiallergic etc. The 1,2,3,4-tetrahydro pyrimidine ring system is of special biological interest because it has numerous pharmacological and medicinal applications.




**RHK 101 TO 130**

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



**RHK 131 to 160**

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

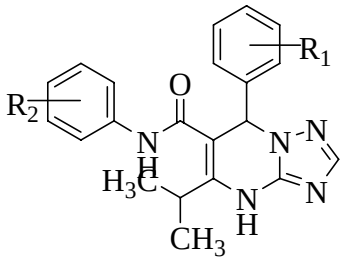
Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of these class of compounds, six novel series of 1,2,3,4-tetrahydro pyrimidines **(RHK- 101 to 160)** are synthesized in chapter 3. The synthesis of 1,2,3,4-tetrahydro pyrimidines **(RHK- 101 to 160)** was achieved by acid catalysed undergoing the Biginelli reaction of acetoacetamide, urea derivatives and the corresponding aldehydes. 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 $A_2$a 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 **(RHK- 161 to 190)**. 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.



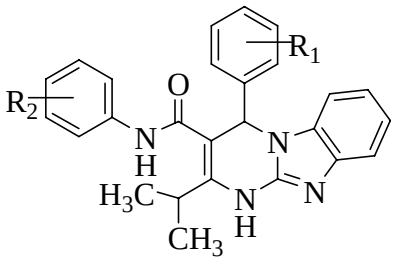
**RHK 161 to 190**

Where $R_1$= **4-$OCH_3$, 4-Cl, 4-$CH_3$** etc..
Where $R_2$= **4-Cl,4-F,3-Cl 4-F**.

## Chapter 5 Synthesis and biological evaluation of 1,4-dihydropyrimido[1,2-*a*] benzimidazole

Regioselectivity in the cyclizations of α-amino azoles with carbonyl 1,3-biselectrophiles is the subject of a series of studies. However, work in this area has not lost current interest because of the many reagents used in similar reactions. Moreover, flat heterocyclic structures are fundamental moieties in anticancer compounds acting as DNA intercalating agents. In this context, 1,4-dihydropyrimido[1,2-*a*]benzimidazole have been little explored, and there are very few 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 1,4-dihydropyrimido[1,2-*a*]benzimidazole.

Chapter 5 describes synthesis of 1,4-dihydropyrimido[1,2-*a*]benzimidazole **(RHK-190 to 220)**, which was achieved by using a one-pot 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 are subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral




**RHK = 191 to 120**

Where $R_1$= **4-$OCH_3$, 4-Cl, 4-$CH_3$** etc..
When $R_2$= **4-Cl, 4-F, 3-Cl 4-F.**

# CHAPTER 1

# General Introduction

## 1.1 Heterocycles in drug discovery

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

The cause of this innovation deficit is definitively not the biology. Decoding of the human genome[a] has led to a wealth of drug targets. With more than 20,000 human genes[b], the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each of these genes is linked to the function of between five and ten proteins, the conclusion is that there might be 5,000-10,000 targets for new drugs [1]. Despite the successful introduction of protein therapeutics and the promise of gene therapy, major pharmaceutical companies are still focused on the discovery and development of low-molecular weight compounds. Hence, the challenge is to select the most drugable targets and to find the corresponding drug-like molecules, substances that not only interact with the target, but also have specific pharmacokinetic and toxicological properties, that allow them to be developed as a drug.

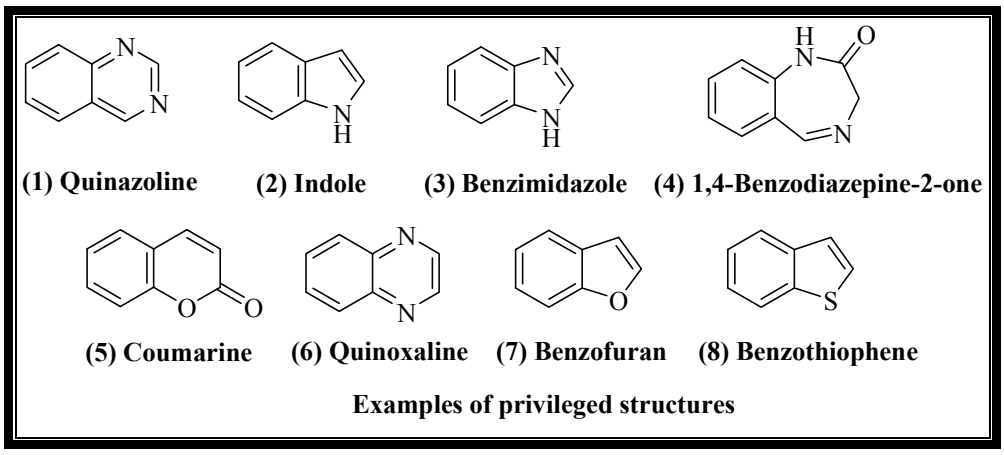
Medicinal chemistry as a scientific discipline has introduced several new techniques over the last few years in order to speed up the drug discovery process, such as combinatorial chemistry, microwave-assisted organic synthesis, and high-throughput purification [2]. Despite this steady increase in R & D, the number of new chemical entities (NCEs) reaching the market has actually decreased dramatically.

[a] The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in number of basepairs.

[b] According to the official Guidelines for Human Gene Nomenclature, a gene is defined as "a DNA segment that contributes to phenotype/function. In the absence of demonstrated function a gene may be characterized by sequence, transcription or homology."

It seems clear that selecting the 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 atomic mass unit (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 that have the potential to be biologically active [3].

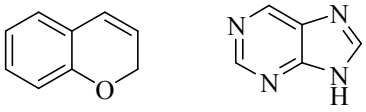
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening 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 bicyclic aromatic heterocycles, we performed an in-house survey of commercially available combinatorial libraries. This search revealed that the number of available bicyclic heterocycles is mainly limited to well-known nitrogen containing compounds, such as quinazolines (1), indoles (2) and benzimidazoles (3).




**Examples of privileged structures**

These structural classes are considered to be privileged structures. The concept of "privileged structures" is first proposed by Evans B. E. et al. to describe select 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 K. C. et al. constructed a library based on the benzopyran (9) privileged scaffold [6], whereas Schultz P. G. et al. made use of the purine (10) scaffold [7].




**(9) Benzopyran** **(10) Purine**

**The benzopyran and purine privileged scaffold**

## 1.2 Nomenclature of the fused ring system

As the following chapters deal with the synthesis of bicyclic 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.

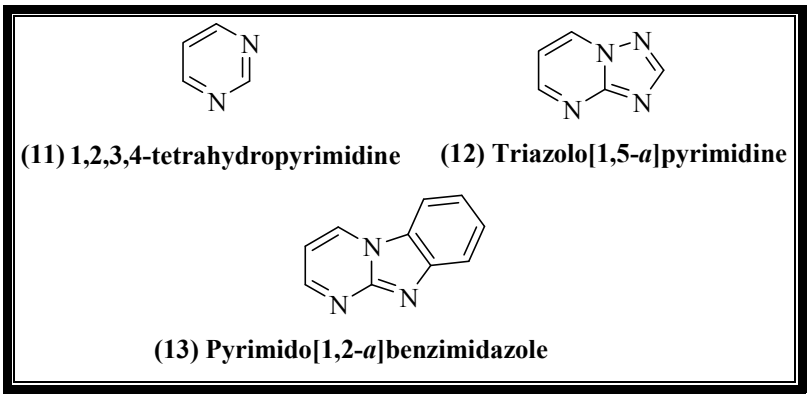
(3) The attached component is then added as a prefix to the parent component. In the name of the prefix, the terminal 'e' is changed to 'o'.

(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/tricycles and the fact that some of these compounds are not frequently used 1,2,3,4-tetrahydropyrimidines (11), triazolo[1,5-*a*]pyrimidines (12) and pyrimido[1,2-*a*]benzimidazoles (13) in commercial compound libraries, prompted us to elaborate this type of chemistry and to synthesize three different heterocyclic scaffolds.




**(11) 1,2,3,4-tetrahydropyrimidine** **(12) Triazolo[1,5-*a*]pyrimidine**

**(13) Pyrimido[1,2-*a*]benzimidazole**

Combinatorial and parallel chemistry [c] are powerful tools for medicinal chemists in drug discovery programs. It has encouraged chemists to work out

[c] Parallel chemistry is used in medicinal chemistry to synthesis a number of analogues of compounds at the same time. This is typically done by performing the same type of reaction and applying the same reaction conditions to a number of reactors (flasks/vials/tubes etc.) but varying the substrates or reagents.

synthetic strategies and approaches that can be used for the construction of libraries. Combinatorial chemistry can be done on a solid support or in solution. Although solid-phase chemistry definitively has some advantages (mainly related to purification), we opted for the construction of libraries using the solution-phase approach.

## 1.4 References and notes

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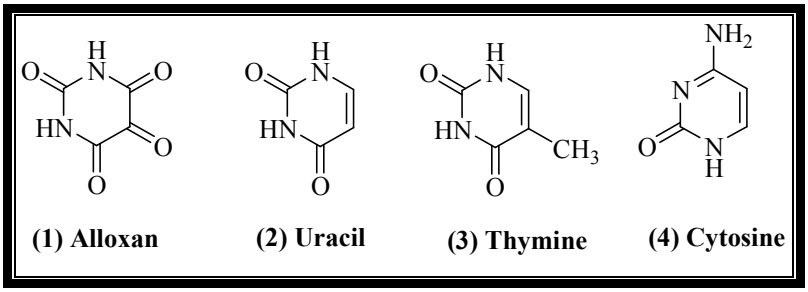
# CHAPTER 2
# Biological and medicinal significance of pyrimidines and related heterocycles

## 2.1 Biological significance

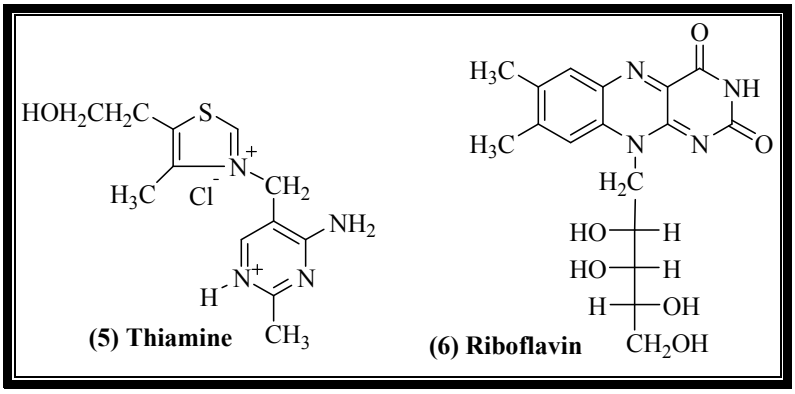
Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of Acquired Immunodeficiency Syndrome (AIDS).

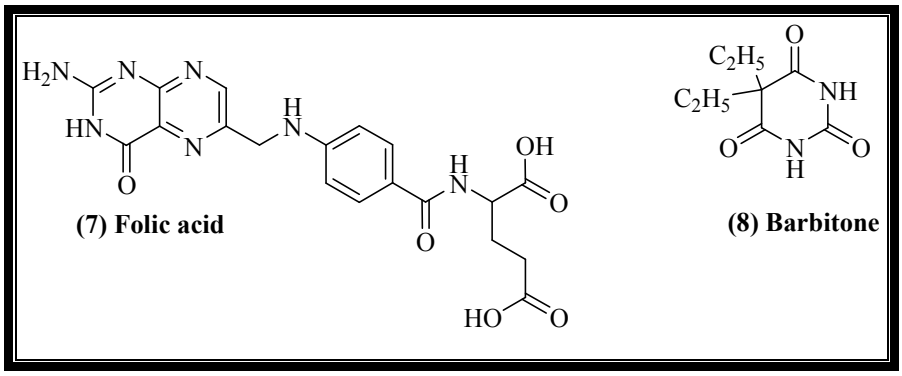
Alloxan (1) is known for its diabetogenic[a] action in a number of animals [1]. Uracil (2), thymine (3) and cytosine (4) are the three important constituents of nucleic acids.




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 is a pyrimidine derivative [1].

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




## 2.2 Medicinal significance

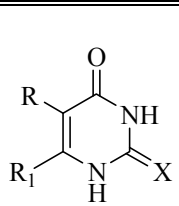
During the last two decades, several pyrimidine derivatives have been developed as chemotherapeutic agents and have found wide clinical applications.

### 2.2.1 Antineoplastics[b] and anticancer agents

There are a large number of pyrimidine-based antimetabolites. They are usually structurally related to the endogenous[c] substrates that they antagonize[d]. The structural modification may be on the pyrimidine ring or on the pendant sugar groups. One of the early metabolites prepared was 5-fluorouracil (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

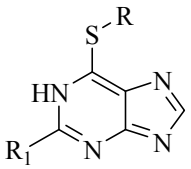
[b] Drugs that inhibit and combat the development of neoplasms (an abnormal mass of tissue due to the abnormal proliferation of cells).
[c] Endogenous substrates are those that originate from within an organism, tissue, or cell.
[d] To oppose or to compete the metabolites.

discovered after formulation of the antimetabolite theory by Woods and Fildes in 1940. These drugs prevent the utilization of normal cellular metabolites [6].

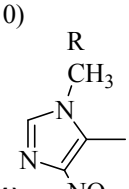


(9a, b)

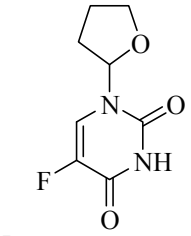
**(9a) X = O, R = F, $R_1$ = H, 5-fluorouracil**
**(9b) X = O, R = SH, $R_1$ = H, 5-thiouracil**



(10)



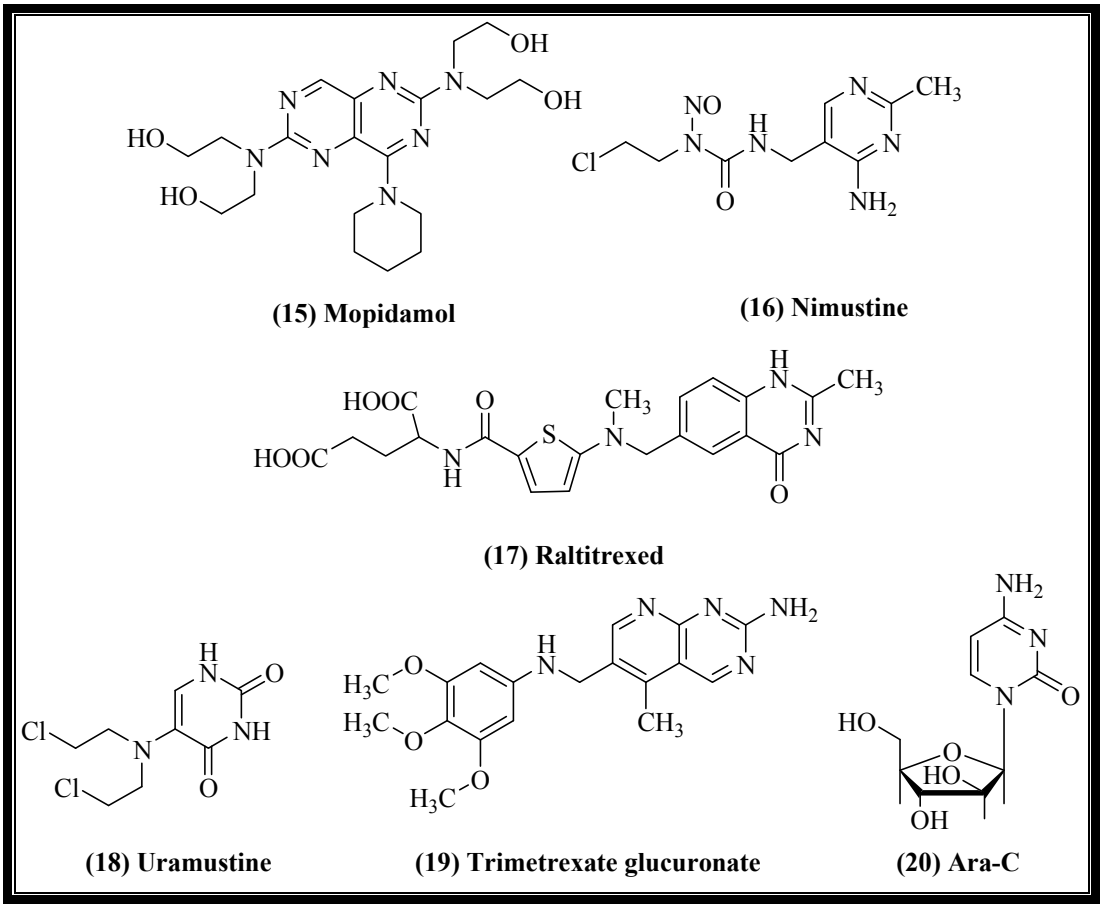
| | | | |
|---|---|---|---|
| **(11)** | **–H,** | **–H,** | **Azathioprine** |
| **(12)** | **–H,** | **–H,** | **Mercaptopurine** |
| **(13)** | **–H,** | **–$NH_2$,** | **Thioguanine** |



**(14) Tegafur**

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].



**(15) Mopidamol**

**(16) Nimustine**

**(17) Raltitrexed**

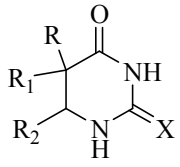
**(18) Uramustine**

**(19) Trimetrexate glucuronate**

**(20) Ara-C**

## 1.2.2 Drugs for hyperthyroidism[e]

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



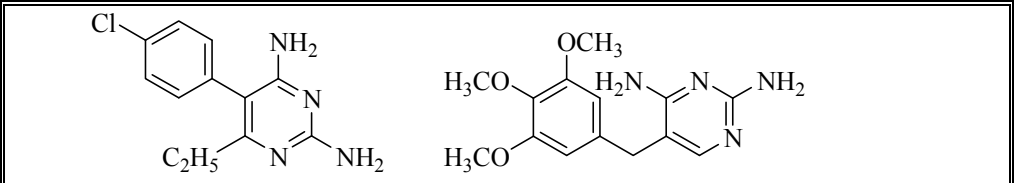
(9c–e)

**(9c)** R = $R_1$ = $R_2$ = H, X = S; **2-thiouracil**
**(9d)** R = $R_1$ = H, $R_2$ = $C_3H_7$, X = S; **Propylthiouracil**
**(9e)** R = $R_1$ = $C_2H_5$, $R_2$ = O, X = S; **Thiobarbital**

[e] The term used for overactive tissue within the thyroid gland causing an overproduction of thyroid hormones

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

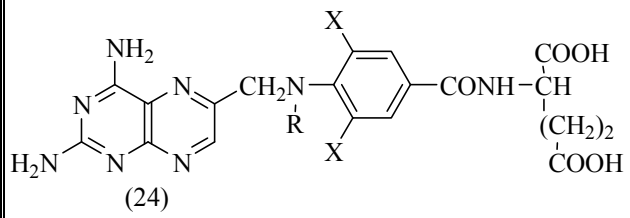
In 1948, Hitchings made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid [19]. Since then, a large number of 2,4-diaminopyrimidines have been synthesized as antifolates. It was eventually proved that these pyrimidines 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 Dihydrofolate reductase (DHFR) of malarial plasmodia; trimethoprim (23), an antibacterial drug which selectively inhibits bacterial Dihydrofolate reductase (DHFR) and most importantly, the very potent but non selective Dihydrofolate reductase (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**

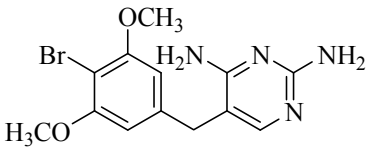


**(23) Trimethoprim**



(24)

**(24a) R = CH3, X = H; Methotrexate**
**(24b) R = X = H; Aminopterin**
**(24c) R = CH3, X = Cl; 3',5'-dichloromethotrexate**

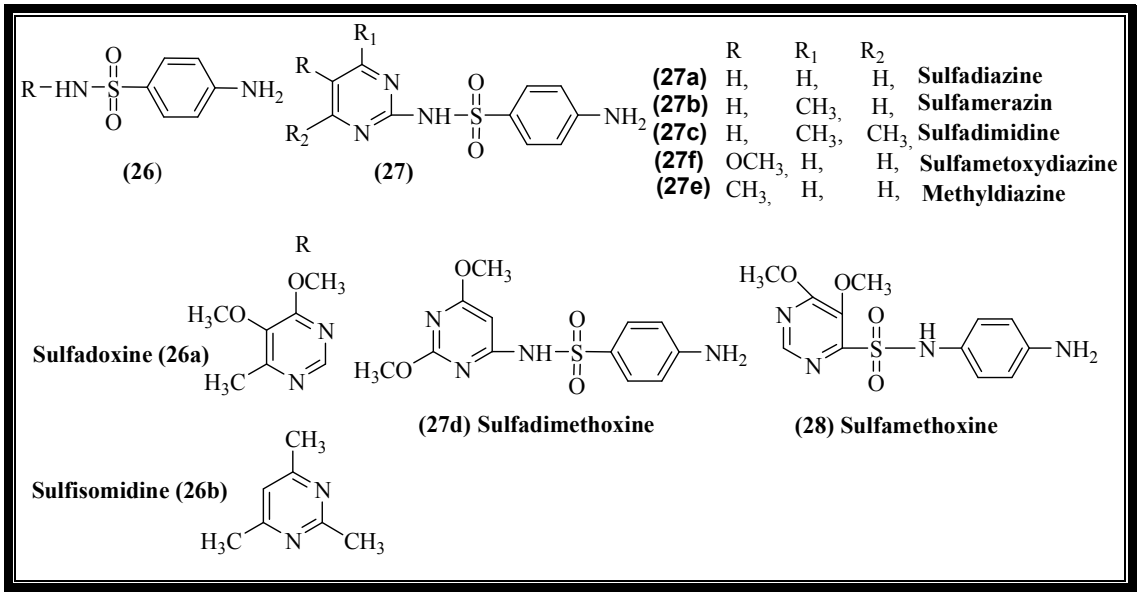


**(25) Brodimoprim**

[f] Antifolates are drugs which impair the function of folic acids.
[g] The drugs having the capability of either to kill or to stop the growth of bacteria.
[h] An antiprotozoal agent is a class of pharmaceuticals used in treatment of protozoan infection.

## 2.2.4 Sulfa drugs[i]

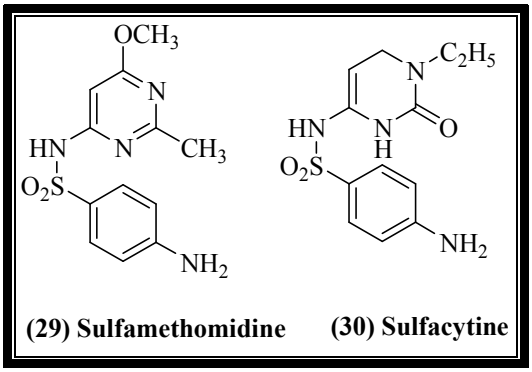
Pyrimidine derivatives of sulfa drugs, Viz; sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute Urinary Tract Infection (UTIs[j]) infections, cerebrospinal meningitis and for patients allergic to pencillins [25]. Sulfonamide-trimethoprim combinations are used extensively for opportunistic infections in patients with acquired immunodeficiency syndrome (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 in 5 and 6 positions, has by far the longest half-life of about 150 h. Methyldiazine (27e) has a half-life of 65 h. Also, sulfamethoxydiazine (27f) [29] possesses good half-life. A new broad-spectrum sulfonamide,

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

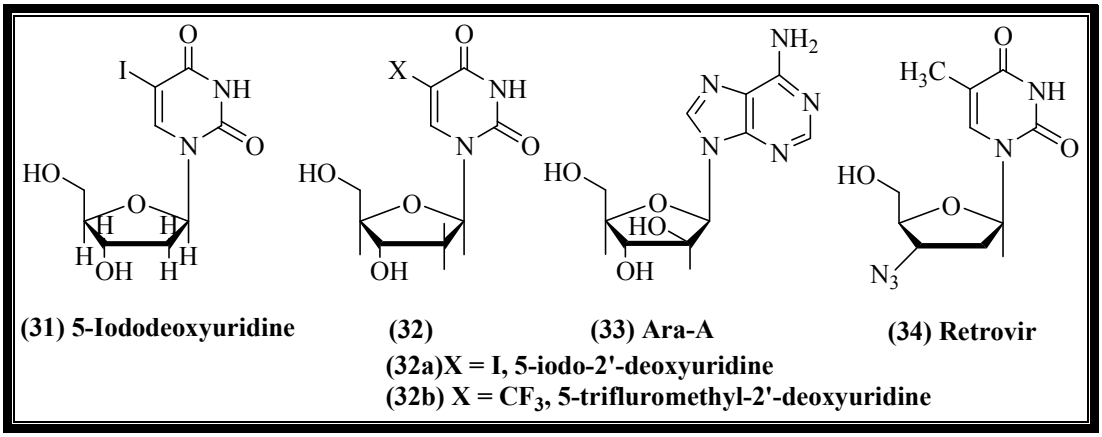
sulfamethomidine (29) [29] is relatively nontoxic and patients do not need extra fluid intake or alkalization. Sulfacytine (30) [29] has been reported to be 3–10 times more potent than sulfaisoxazole and sulfisodimidine [29].



**(29) Sulfamethomidine** **(30) Sulfacytine**

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

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



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

**(32a)X = I, 5-iodo-2'-deoxyuridine**

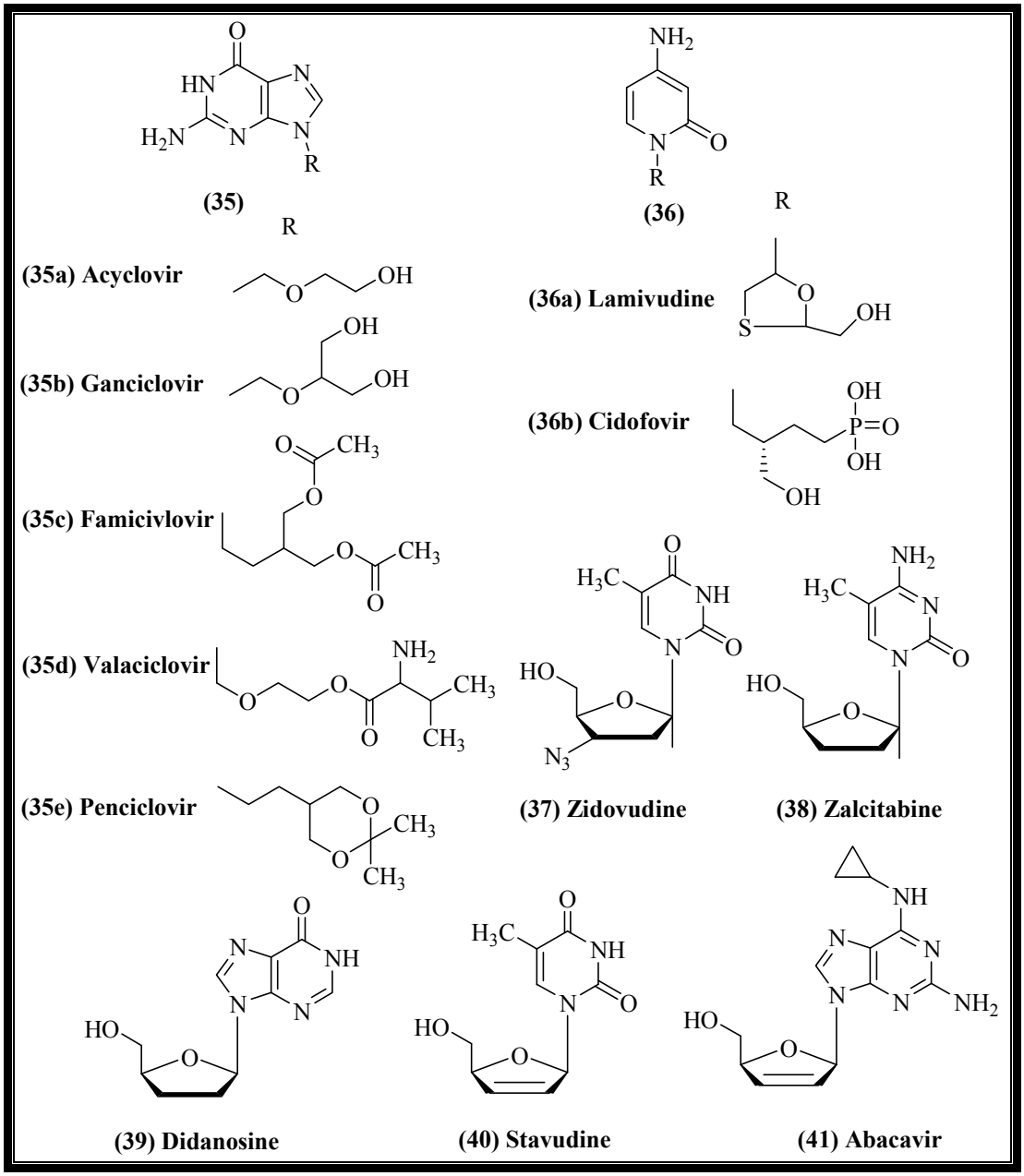
**(32b) X = $CF_3$, 5-trifluromethyl-2'-deoxyuridine**

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

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

herpes infections of eye, brain and skin. It is especially effective against 5-iodo-2'-deoxyuridine (IDU)-resistant herpes virus [30].

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




Several members of a series of acyclic nucleosides, which contain a fused pyrimidine ring (mainly purine), are found to be effective antivirals. Famiciclovir (35c) and valacyclovir (35d) are drugs used for several deoxyribonucleic acid (DNA) viruses, including Varicella-zoster virus and Epstein-Barr virus [34]. Penciclovir (35e) [35] is useful for topical treatment of recurrent herpes, Libialis. Cidofovir (36b) [35], an antimetabolite for deoxycytosine triphosphate is used for treatment of Cytomegalovirus (CMV) in AIDS patients. Lamivudine (36a) [35] is an effective anti-AIDS drug when used in combination with zidovudine (37) [35]. Zidovudine [36] is an analogue of thymidine in which the azido group is substituted at the 3-position of

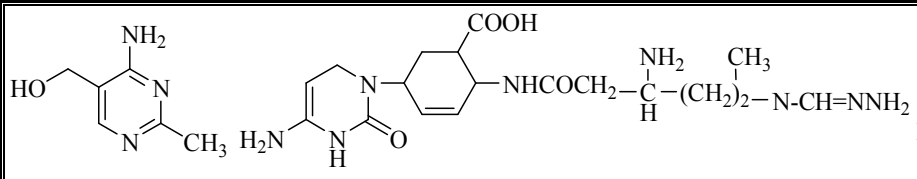
the dideoxyribose moiety. It is active against Ribonucleic acid (RNA) tumour viruses (retroviruses) that are the causative agents of Acquired immunodeficiency syndrome (AIDS) and T-cell leukaemia. It is used in Acquired immunodeficiency syndrome (AIDS) and Acquired immunodeficiency syndrome (AIDS)-related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

### 2.2.6 Antibiotics[m]

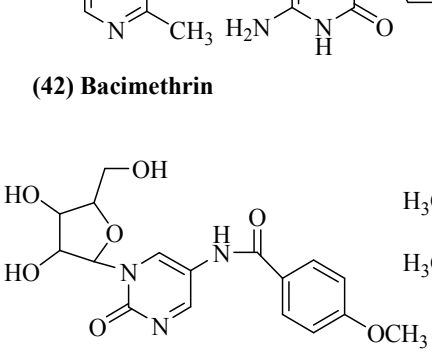
There are few examples of pyrimidine antibiotics. The simplest of all is bacimethrin (5-hydroxymethyl-2-methoxypyrimidin-4-amine) (42), which is active against several staphylococcal infections [38]. Gourgetin (43), a cytosine derivative is active against mycobacteria as well as several Gram-positive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

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

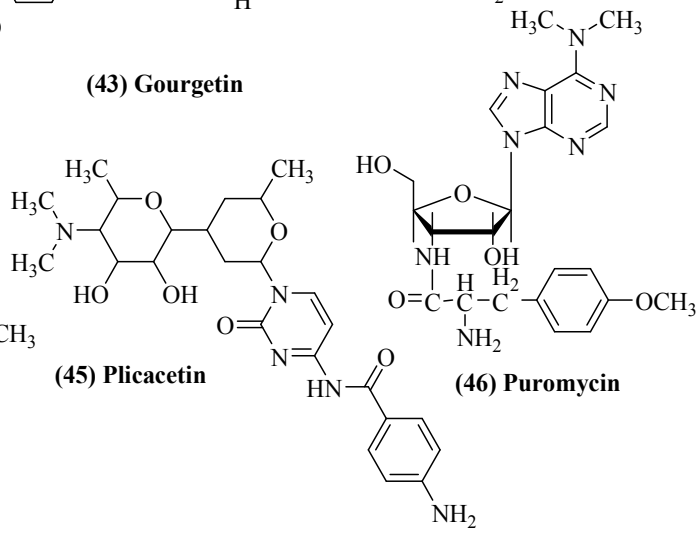
(47b) and related families are wide-spectrum antibiotics containing the pyrimidine ring. Another antibiotic tubercidine (48) is reported to exhibit antitumour properties [39]. In addition, they have antineoplastic activity. Bleomycin is already in clinical use against certain tumours like Hodgkin's lymphoma and disseminated testicular cancer [40].



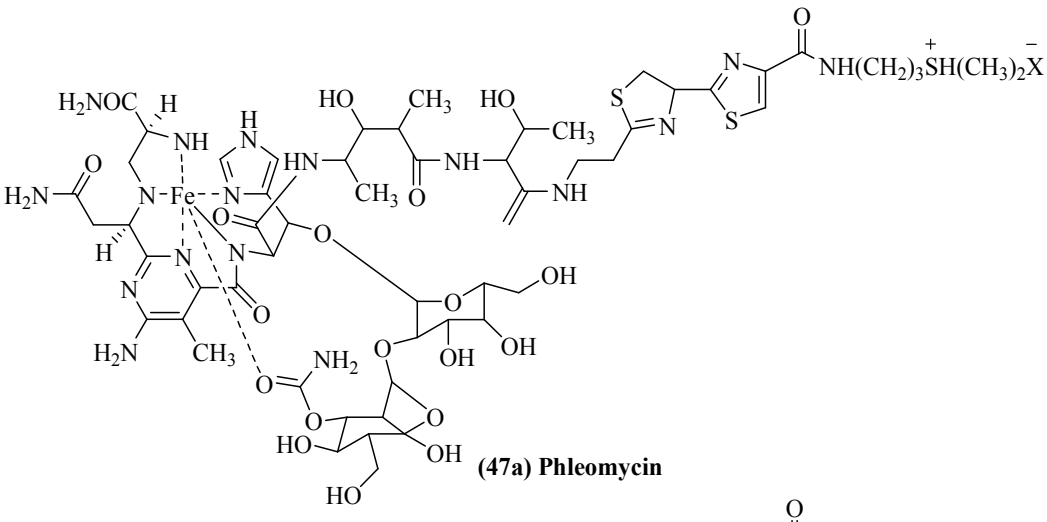
**(42) Bacimethrin** **(43) Gourgetin**



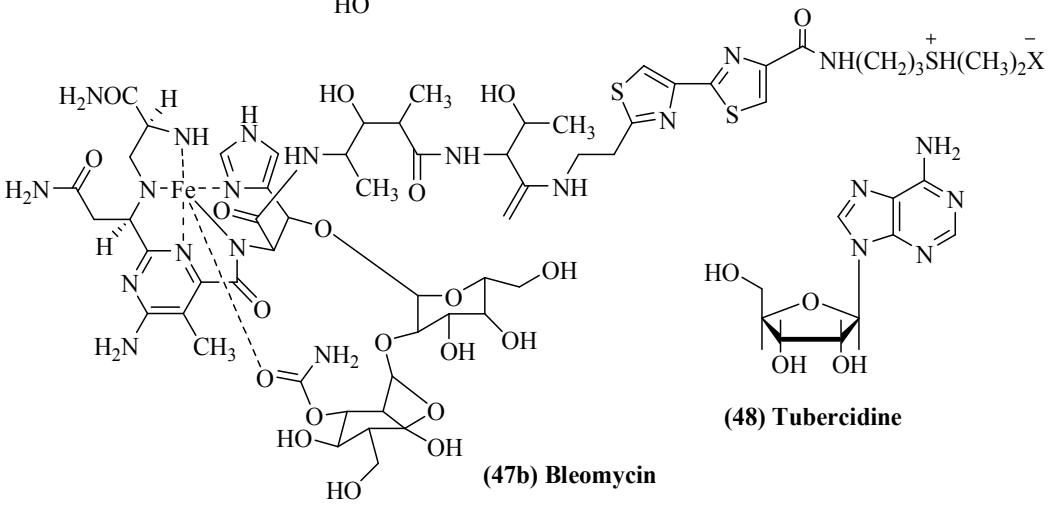
**(44) Amicetin**



**(45) Plicacetin** **(46) Puromycin**



**(47a) Phleomycin**

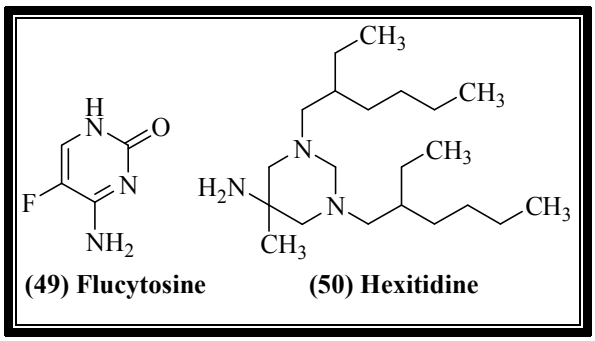


**(48) Tubercidine**

**(47b) Bleomycin**

### 2.2.7 Antifungals[n]

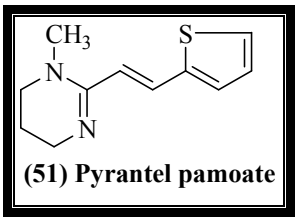
Pyrimidines also exhibit antifungal properties. Flucytosine (49) [41] is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and Cryptococcus [42]. Hexitidine [43] (50) is mainly used for the treatment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




**(49) Flucytosine** **(50) Hexitidine**

### 2.2.8 Anthelmentics[o]

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

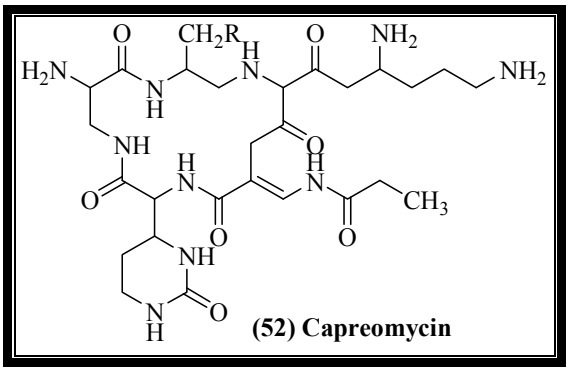



**(51) Pyrantel pamoate**

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

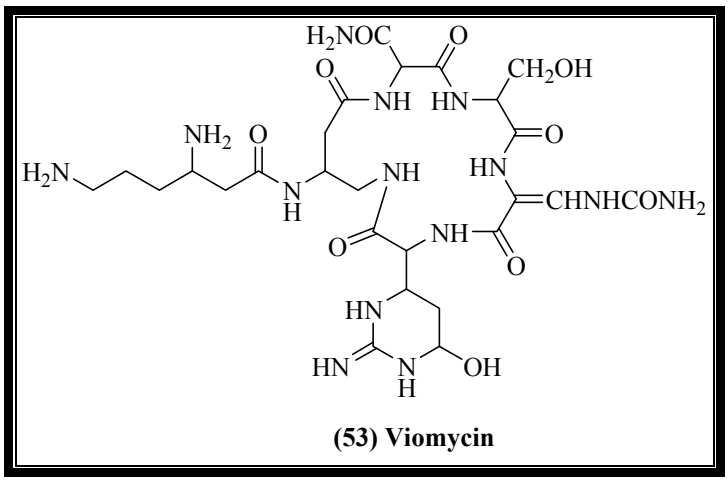
[o] Anthelmintics or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing).

## 2.2.9 Antitubercular drugs[p]



**(52) Capreomycin**

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



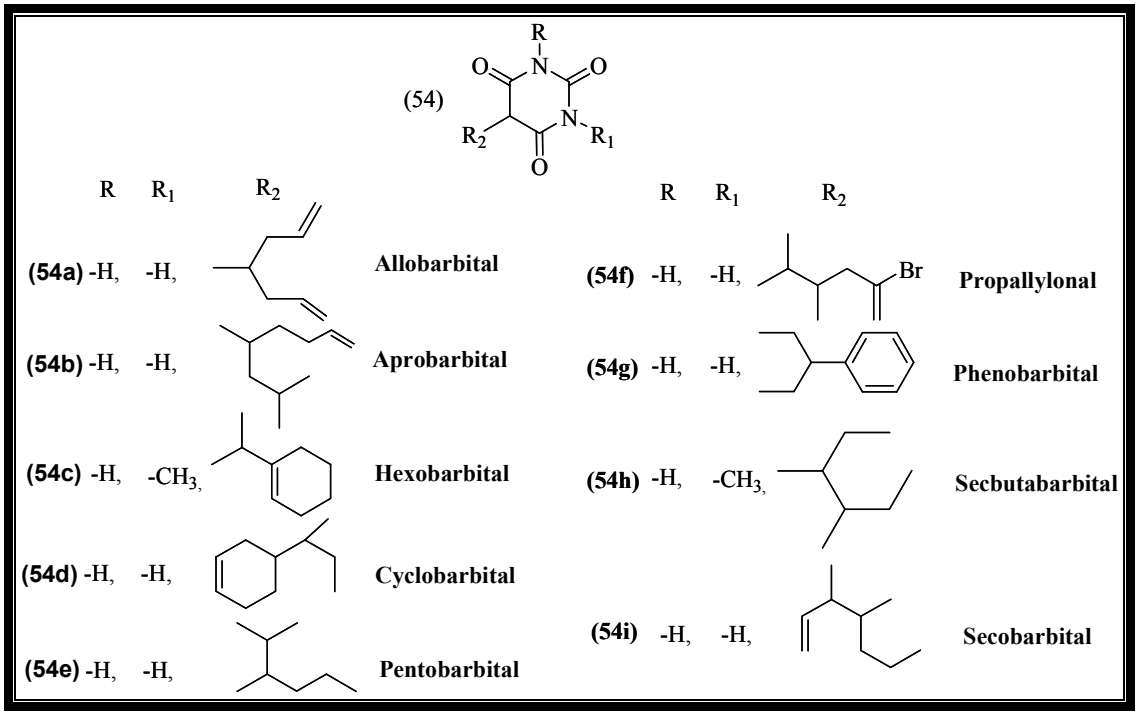
**(53) Viomycin**

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

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

## 2.2.10 CNS active agents

### 2.2.10.1 Sedative[q]/Hypnotic/Antiepileptic agents

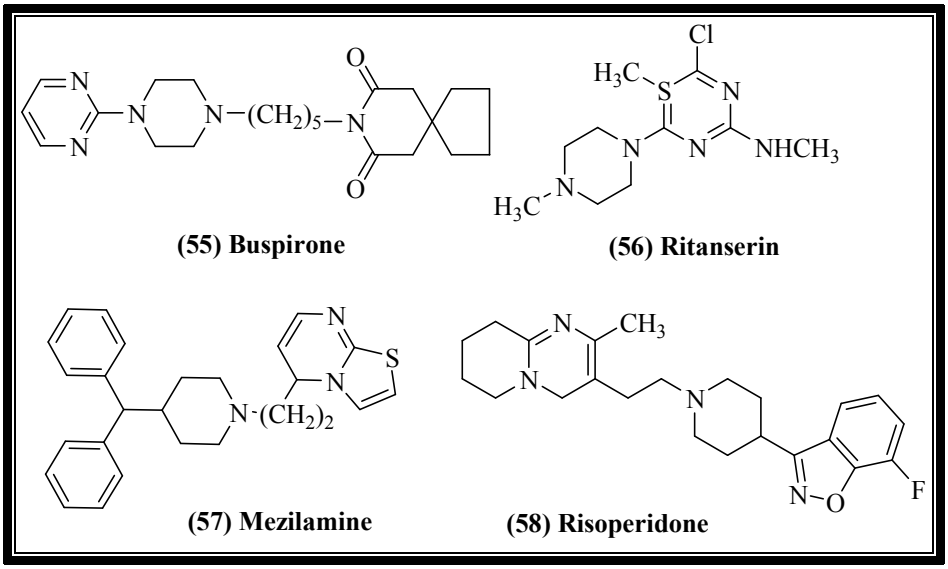



Agents of the anxiolytic, sedative and hypnotic group include a wide variety of barbiturates (54a–i) used as sedative and hypnotics and are classified as drugs having short, intermediate and long duration of action [47, 48]. Allobarbital (54a), aprobarbital (54b), pentobarbital (54e), phenobarbital (54g)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

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

### 2.2.10.2 Anxiolytic[r] agents

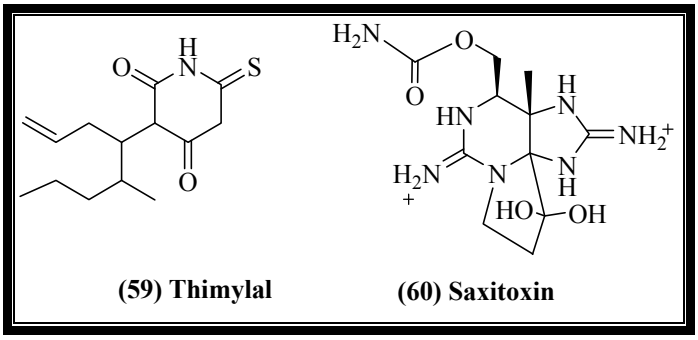
Few of the pyrimidine derivatives are also used as anxiolytics. Most important of these is buspirone (55), indicated in the management of anxiety disorders accompanied with or without depression. It lacks sedative, anticonvulsant and muscle-relaxant effects and most importantly abuse potential [52].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




### 2.2.10.3 Pyrimidine anaesthetics[s]

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

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

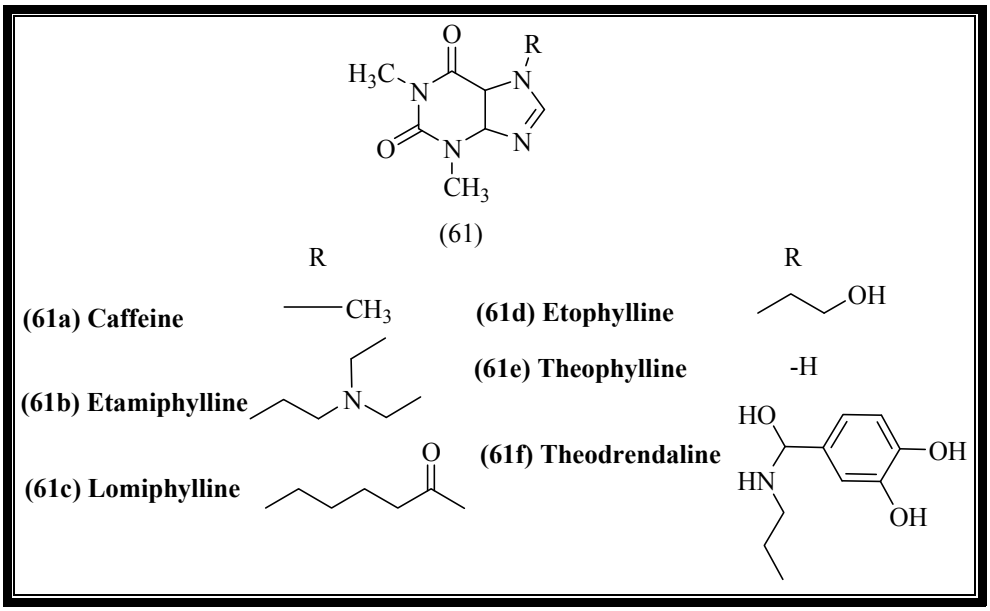


**(59) Thimylal** **(60) Saxitoxin**

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[t] and uricosurics[u]

Several xanthine derivatives (61) containing fused pyrimidine ring systems like caffeine (61a) [60], etamiphylline (61b) [61], lomiphylline (61c) [62], etophylline [63] (61d), theophylline (56e) [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].



(61)

**(61a) Caffeine** R = $CH_3$

**(61b) Etamiphylline**

**(61c) Lomiphylline**

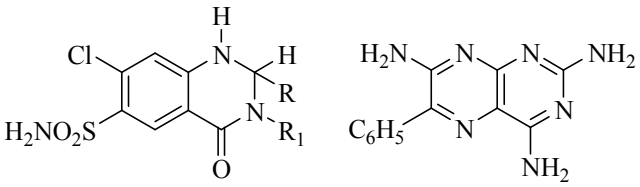
**(61d) Etophylline** R = OH

**(61e) Theophylline** R = -H

**(61f) Theodrendaline**

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

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**

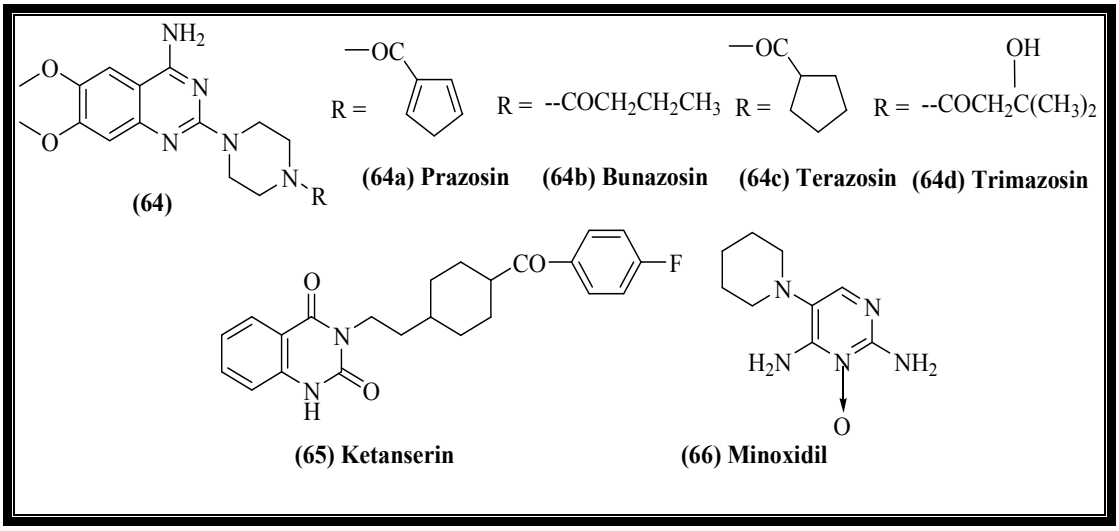
**(62a) R = $C_2H_5$, $R_1$ = H; Quinethazine**
**(62b) R = $CH_3$, $R_1$ = 2-$CH_3C_6H_4$; Metolazone**

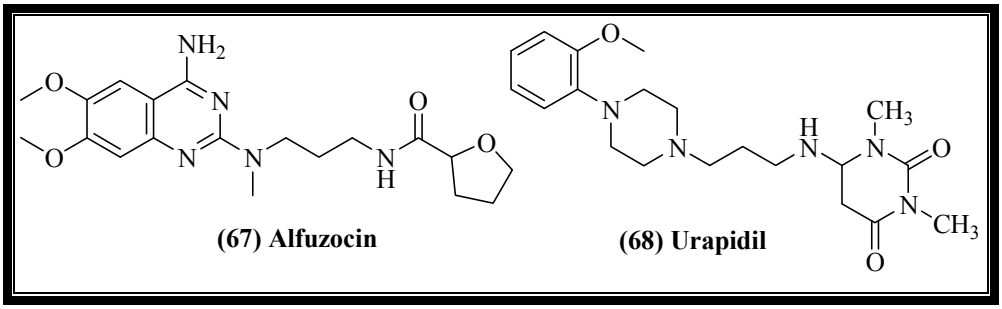
## 2.2.11 Cardiac agents

### 2.2.11.1 Antihypertensives[v]

Several pyrimidine ring-containing drugs have exhibited antihypertensive activity. Prazosin (64a), a quinozoline derivative, is a selective $\alpha_1$-adrenergic antagonist [67, 68]. Its related analogues bunazosin (64b) [69], terazosin (64c) [70] and trimazosin (64d) [71] are potent antihypertensive agents. Another quinazoline derivative, ketanserin (65) [72] having a similar effect is an antagonist of both $a_1$-adrenergic and serotonin-$S_2$ receptors. Its mechanism of action however is still controversial. A triaminopyrimidine derivative, minoxidil (66), whose mechanism of action and therapeutic action are similar to Prazosin, has been introduced in therapy for its side effects, in the treatment of alopecia, male baldness [73]. Besides these, some more pyrimidine derivatives given below were found to be antihypertensives [74, 75].

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



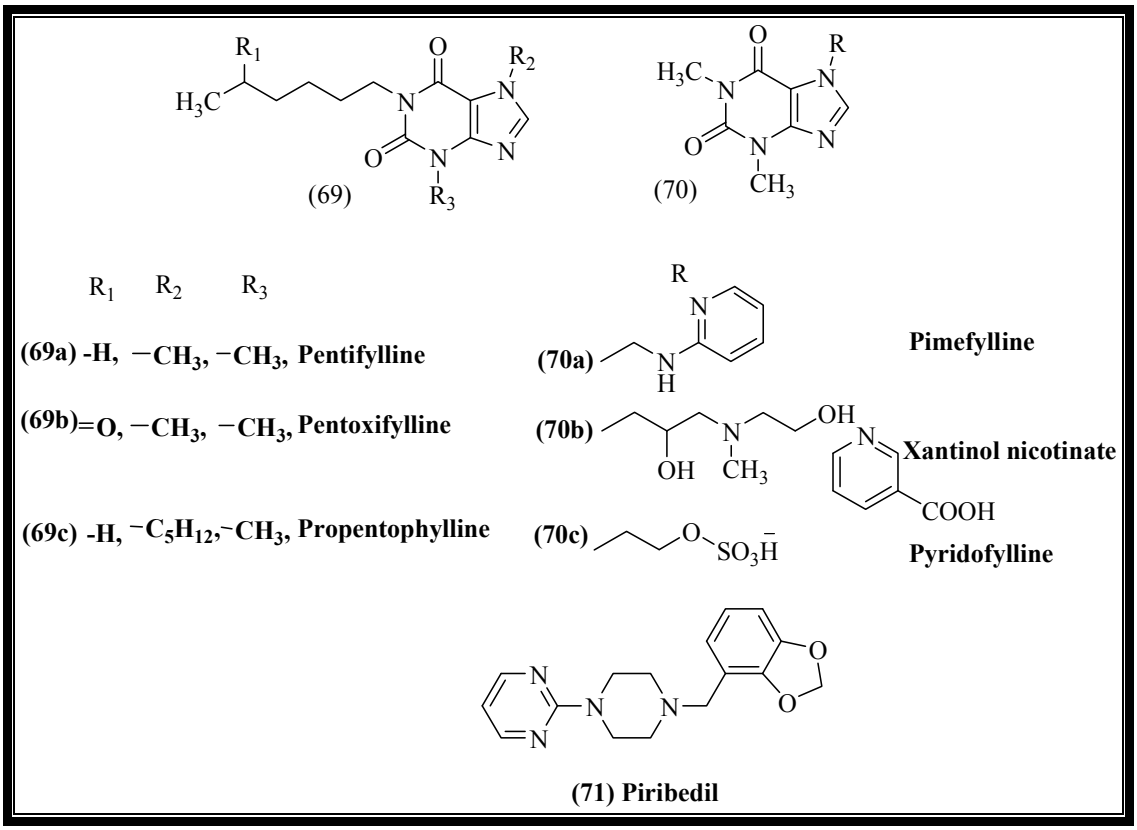

Alfuzocin (67) [74], a prazocin analogue and an $\alpha_1$-adrenergic receptor antagonist as well as urapidil (68) [75] are used especially in urinary obstruction caused by benign prostate hyperplasia.

### 2.2.11.2 Vasodilators[w]

A series of xanthine derivatives are used as peripheral and cerebral vasodilators. Especially, pentifylline (69a) and pentoxyphilline (69b) are used in cardiovascular disorders [76]. Other derivatives like xantinol nicotinate [77] (70b), a vasodilator with general properties like nicotinic acid used in cerebral and peripheral vascular disorders and pimephylline (70a) and pyridophylline [78] (70c) are noteworthy. A new dopamine receptor stimulant, pirebidil (71) [79] is reported to

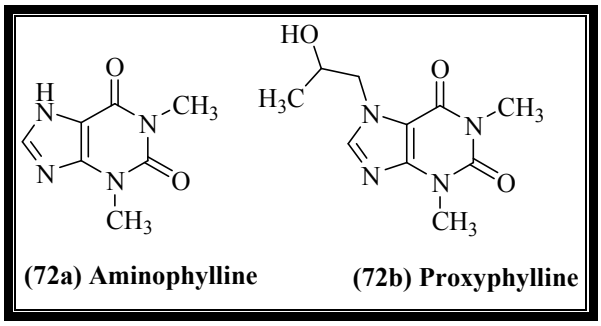
[w] The term vasodilation refers to the widening of blood vessels, resulting from relaxation of smooth muscle cells within the vessel walls, and the drugs to which are used in phenomena are called vasodilators.

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




### 2.2.11.3 Cardiotonics[x]/Bronchodialators

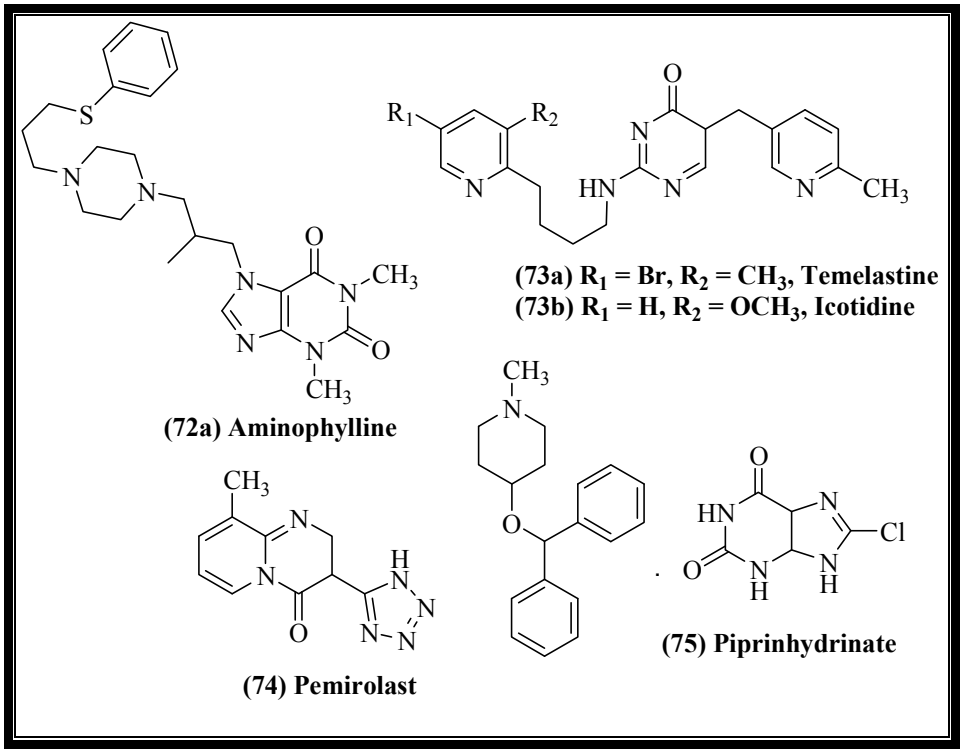
Several xanthine derivatives theophylline (61e), aminophylline (72a) [80] and proxyphylline (72b) [80] exhibit good bronchodilator activity.




[x] Agents that have a strengthening effect on the heart or that can increase cardiac output.

## 2.2.12 Antihistaminic[y] pyrimidines

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

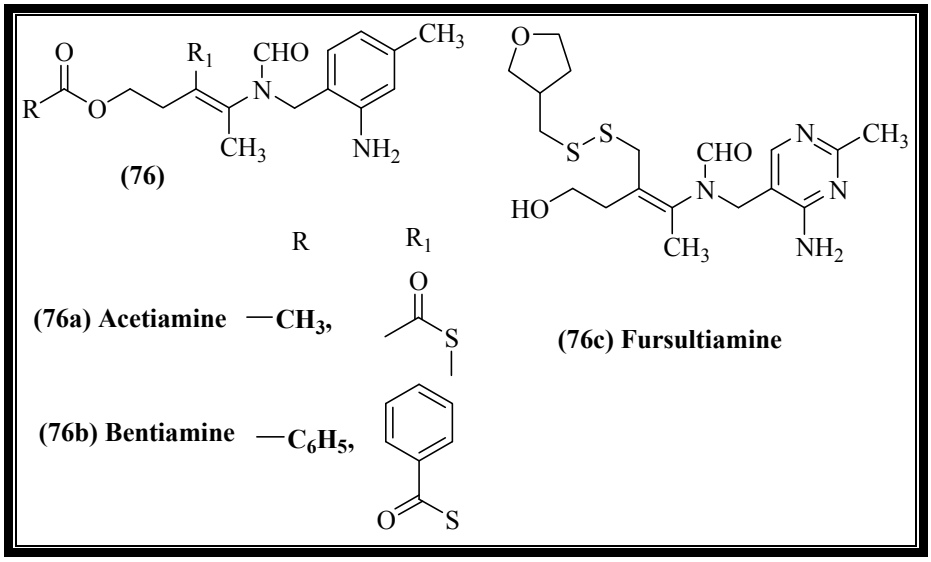



Pemirolast (74) [84], a new oral nonbronchodilator antihistaminic agent is also a pyrimidine derivative. It has demonstrated sufficient antihistaminic activity to warrant its use in severe asthma. Another compound, piprinhydrinate (75) [85] is also a pyrimidine derivative.

[y] A histamine antagonist is an agent that inhibits action of histamine or the drug which is used to treat the allergy is called antihistaminic agent.

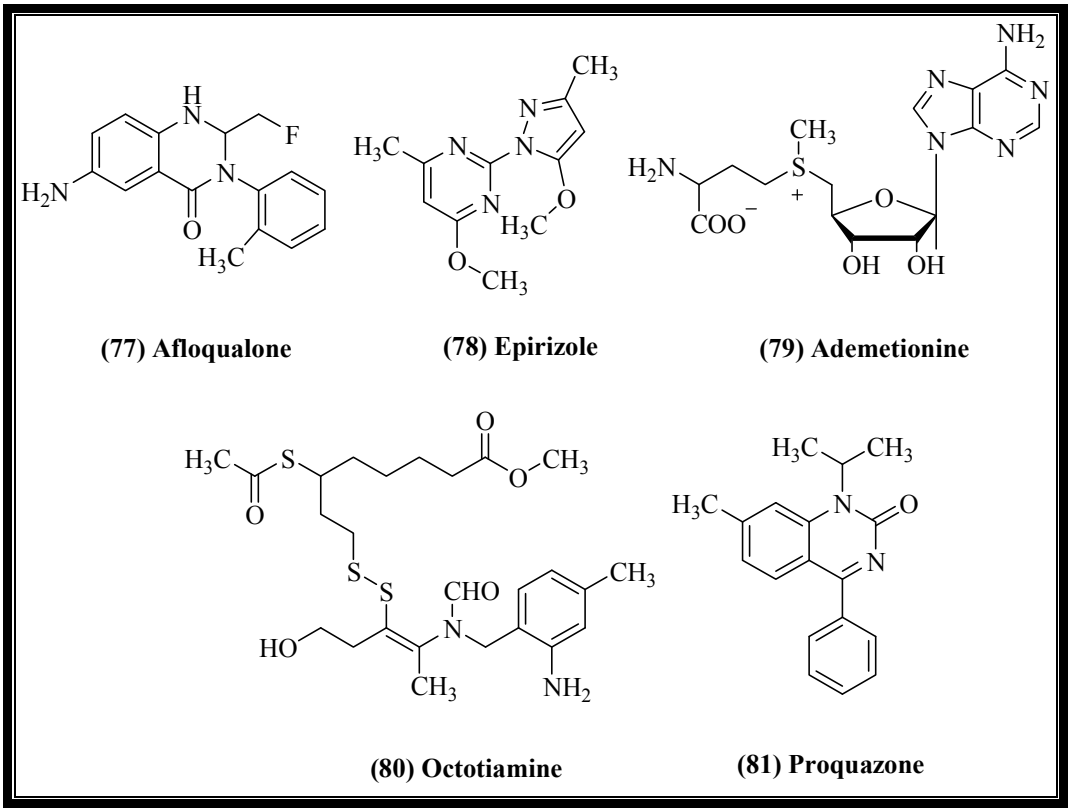
## 2.2.13 Analgesics[z] and NSAID drugs[aa]

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




Afloqualone (77) [88] has been evaluated as a successful anti-inflammatory agent with lower back pain patients. Epirazole (78) [89], another Nonsteroidal anti-inflammatory drugs (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 Nonsteroidal anti-inflammatory drugs (NSAID) potential.

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

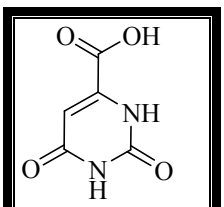


**(77) Afloqualone** **(78) Epirizole** **(79) Ademetionine**

**(80) Octotiamine** **(81) Proquazone**

## 2.2.14 Metabolic electrolytes

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



**(82) Orotic acid**

## 2.3 Conclusion

Pyrimidines occupy a distinct and unique place in our life. This heterocyclic moiety has great biological and medicinal significance. A large array of pyrimidine drugs possesses a variety of medicinal properties. These properties include anticancer, antibacterial, antiprotozoal, antimicrobial, antiviral, antihypertensive, antihistaminic, anti-inflammatory, analgesic, and Central nervous system (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 (1,2,3,4-tetrahydropyrimidines, 1,2,4-triazolo[1,5-*a*]pyrimidines and pyrimido[1,2-*a*]benzimidazole) have been synthesized in the work of this doctoral thesis.

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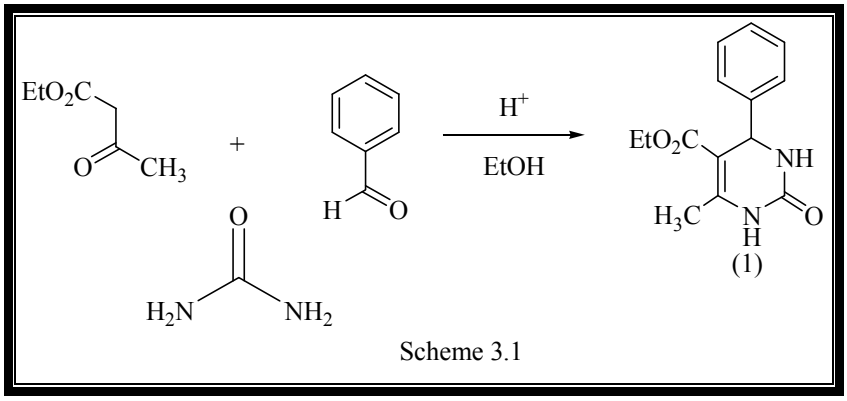
# CHAPTER 3

# Synthesis and biological evaluation of 1,2,3,4-tetrahydro pyrimidines

## 3.1 Introduction

In 1893, Italian chemist Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehydes and urea [1]. The reaction was carried out simply by heating a mixture of the three components dissolved in ethanol with a catalytic amount of hydrochloric acid (HCl) at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydro pyrimidin-2(1*H*)-one.

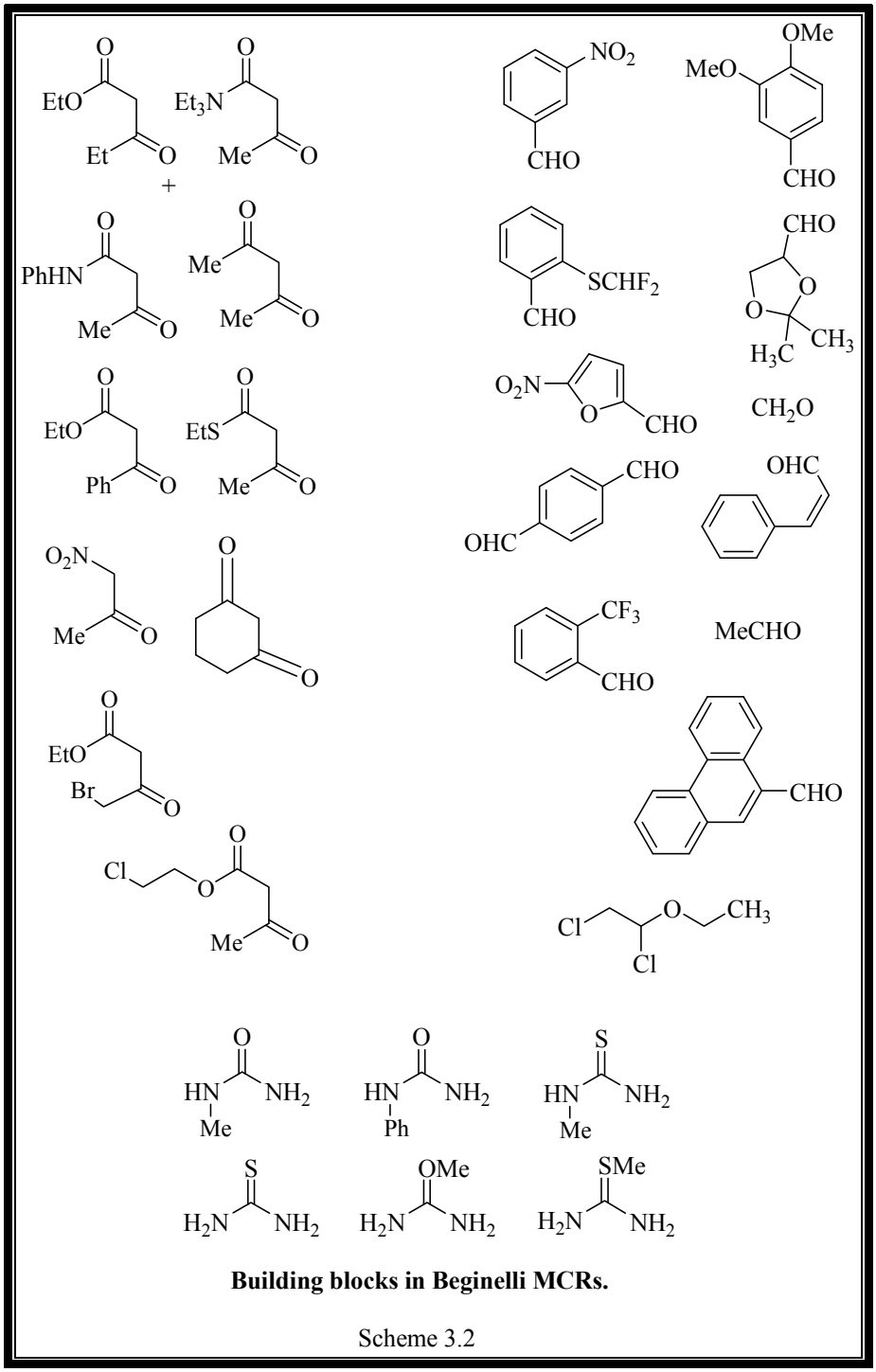



Scheme 3.1

**The Biginelli dihydropyrimidine synthesis.**

A part from a series of publications by the late Folkers K. et al. [2] in the mid 1930s, the "Biginelli reaction" or "Biginelli condensation" as it was henceforth called was largely ignored in the early part of the 20th century. The synthetic potential of this new heterocycle synthesis therefore remained unexplored for quite some time. In the 1970s and 1980s, interest slowly increased, and the scope of the original cyclocondensation reaction shown in (Scheme 3.1) was gradually extended by

variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines (1) [3].

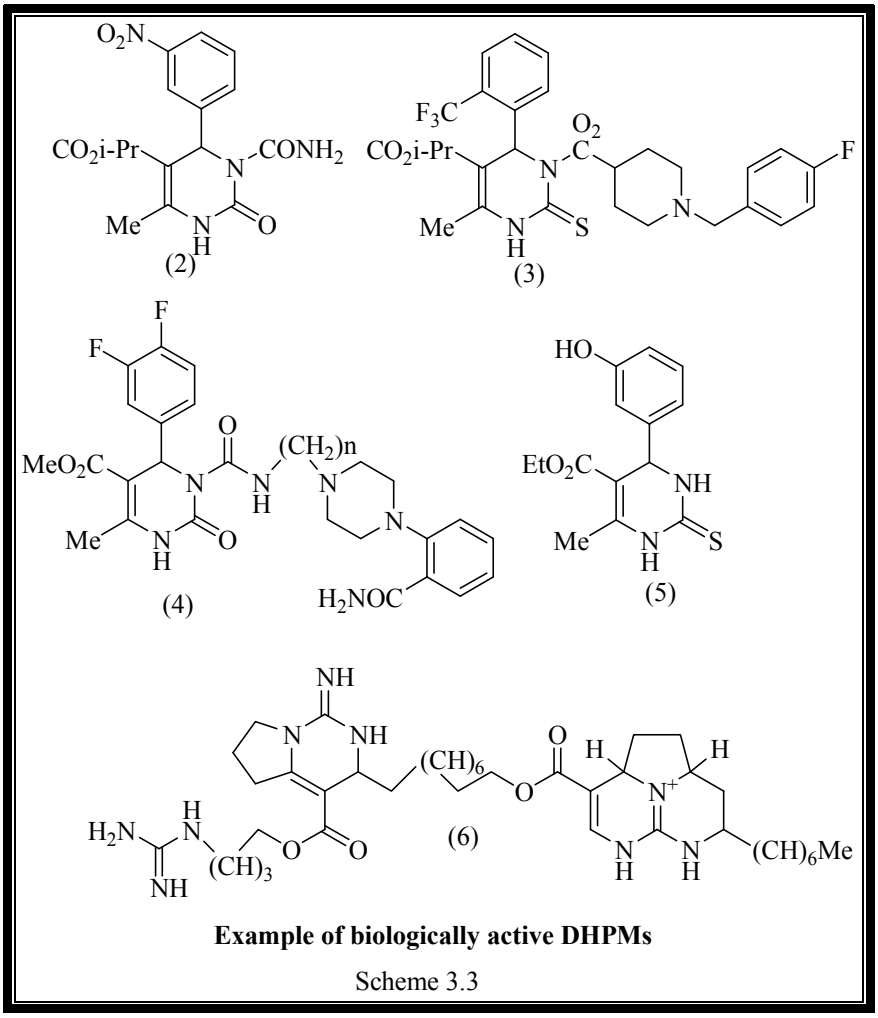



**Building blocks in Beginelli MCRs.**

Scheme 3.2

## 3.2 Biological importance

The tremendously growing number of publications and patents on the dihydropyrimidine is mainly due to the fact that the multifunctionalized dihydropyrimidine scaffold (DHPMs, "Biginelli compounds") represents a heterocylic system of remarkable pharmacological efficiency. In the past decades, a broad range of biological effects, including antiviral, antitumor, antibacterial, and antiinflammatory activities, has been ascribed to these partly reduced pyrimidine derivatives. More recently, appropriately functionalized DHPMs have emerged as, e.g., orally active antihypertensive agents (2) [4-6] or adrenoceptor-selective antagonists (3) [7,8]. A very recent highlight in this context has been the identification of the structurally rather simple dihydropyrimidine (DHPM) monastrol as a novel cell-permeable molecule that blocks normal bipolar spindle assembly in mammalian cells and therefore causes cell cycle arrest (4) [9]. Monastrol specifically inhibits the mitotic kinesin motor protein and can be considered as a new lead for the development of anticancer drugs (5) [10]. Furthermore, apart from synthetic DHPM derivatives, several marine natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate have recently been isolated [11]. Most notable among these are the batzelladine alkaloids A and B, which inhibit the binding of Human immunodeficiency virus (HIV) envelope glycoprotein (protein gp-120) to human cluster of differentiation 4 ($CD^4$) cells and, therefore, are potential new leads for Acquired immunodeficiency syndrome (AIDS) therapy (6) [12].

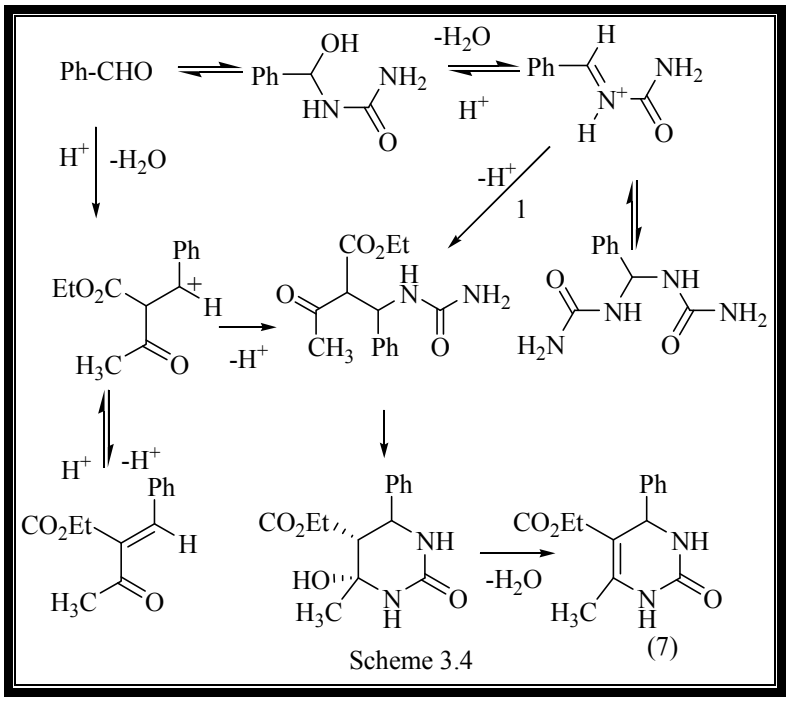



**Example of biologically active DHPMs**

Scheme 3.3

## 3.3 Mechanism of Biginelli reaction

The first mechanistic studies of the Biginelli reaction were conducted by Folkers and Johnson forty years after Biginelli's initial report [13]. A second mechanistic proposal was suggested by Sweet F. et al. forty years after Folkers K. et al. [14]. Kappe C. O. et al. further explored the mechanism of the Biginelli reaction using Nuclear magnetic resonance (NMR) spectroscopy and trapping experiments [15]. Kappe C. O. et al. proposal is currently the accepted mechanism for the Biginelli reaction.




Scheme 3.4

## 3.4 Reaction advancements

### 3.4.1 Improved Reaction Conditions

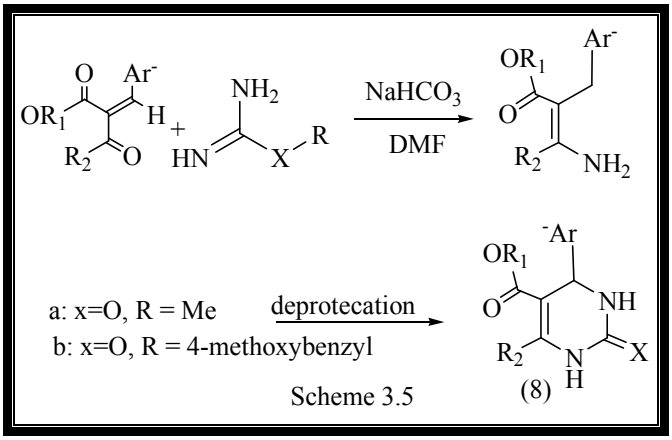
A number of improved variants in traditional Biginelli reaction employing new reagents, catalyst, methodologies and technique have emerged. Numerous synthetic method for the preparation of these compounds have been reported using indium(III) chloride ($InCl_3$) [16], lanthanide triflate [17], diethoxytrifluoroborane ($BF_3$•$OEt_2$) [18], polyphosphate ester (PPE) [19], KSF clay [20], lanthanum(III) chloride ($LaCl_3$) [21], sulphuric acid ($H_2SO_4$) [22], ceric ammonium nitrate($CeNO_2$) [23], manganese(III)acetate ($Mn(OAc_3)$ [24], ion-exchange resin [25], indium(III)bromide ($InBr_3$) [26], ferric chloride ($FeCl_3$) [27], cadmium(II)chloride ($CdCl_2$) [28], 1-n-butyl-3- methyl imidazoliumtetrafluoroborate [29], ytterbium triflates [30], silicond ioxide ($SiO_2$)/sodium bisulphate ($NaHSO_4$) [31], bismuth chloride ($BiCl_3$) [32], Lithium per chlorate ($LiClO_4$) [33], zirconium chloride ($ZrCl_4$) [34], copper bistrifluoromethane-sulfonicacid ($Cu(OTf)_2$) [35], bismuththri-strifluoromethane-sulfonicacid ($Bi(OTf)_3$) [36], lithum bromide (LiBr) [37], ammonium chloride ($NH_4Cl$) [38], stenus chloride ($SnCl_2.2H_2O$) [39], aluminium chloride ($AlCl_3$)/potassium iodide (KI) [40], cobalt chloride ($CoCl_2$)/manganese

chloride ($MnCl_2$) [41], aluminium chloride $AlCl_3$/ aluminium bromide $AlBr_3$ [42], Phosphorus pentoxide ($P_2O_5$) [43], BiO $ClO_4.H_2O$ [44], calcium chloride ($CaCl_2$) [45], 1,3-Dibromo-5,4-dimethylhydantoin [46], zinc tetrafluoro borate [47].

## 3.5 Alternative synthetic strategies

One noticeable alternate is the so-called "Atwal modification" of the Biginelli reaction. [48-50]. Here, an enone of type is first condensed with a suitable protected urea or thiourea derivative under almost neutral conditions. Deprotection of the resulting 1,4-dihydropyrimidine with hydrochloric acid (HCl) or trifluoroacetic acid (TFA) leads to the desired DHPMs.

Although this method requires prior synthesis of enones, its reliability and broad applicability make it an attractive alternative to the traditional one step Biginelli condensation.

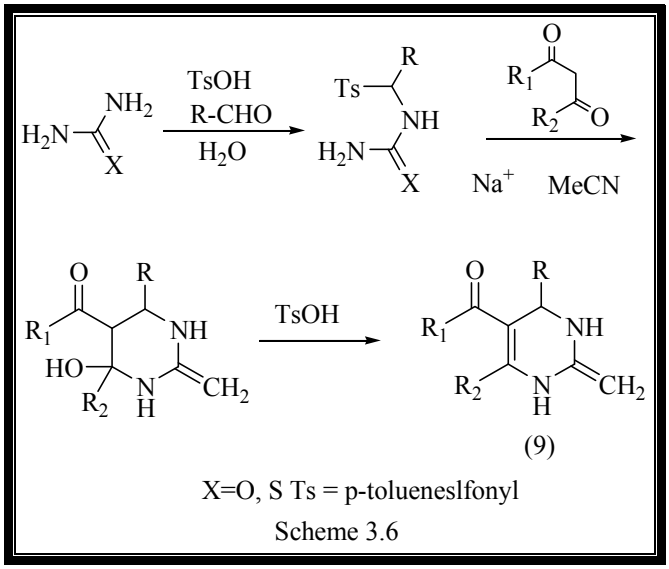



Scheme 3.5

(8)

One other novel approach to DHPMs has been described by Shutalev A. D. et al. [51]. This synthesis is based on the condensation of readily available *R*-tosyl-substituted thioureas with the (*in situ* prepared) enolates of acetoacetates or 1,3-dicarbonyl compounds. The resulting hexahydropyrimidines need not to be isolated and can be converted directly into DHPMs.

This method works particularly well for aliphatic aldehydes and thioureas and produces high overall yields of the desired target compounds.

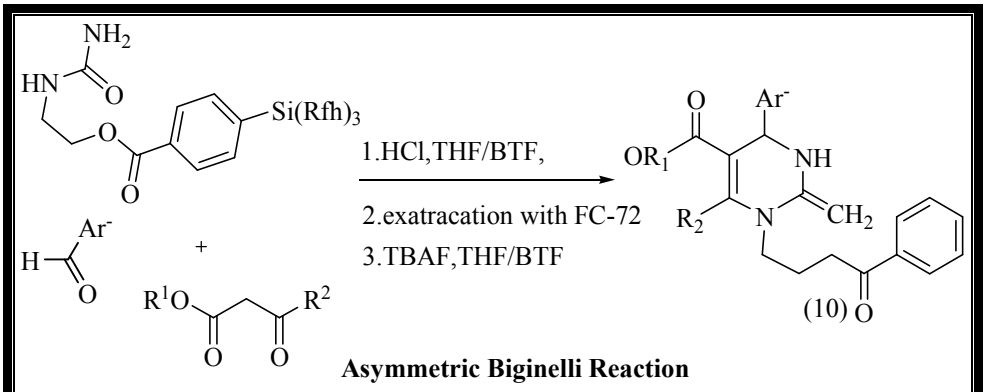



X=O, S Ts = p-toluenesulfonyl

Scheme 3.6

The interesting and diverse biological activity of dihydropyrimidines has been explored through the generation of libraries of compounds via microwave, solid-phase, and fluorous-phase technologies. Significant rate and yield enhancements were also reported for Biginelli reactions carried out under microwave irradiation [52-54]. Kappe C. O. et al. have recently demonstrated that, by using neat polyphosphate ester (PPE) as reaction mediator coupled with microwave irradiation, excellent yields of variously substituted DHPMs can be obtained.

The first actual solid-phase modification of the Biginelli condensation was reported by Wipf P. et al. in 1995 [55]. In this sequence, amino butyric acid-derived urea was attached to wang resin (the wang resin is the most commonly used resin for peptides with C-terminal carboxylic acids if a C-terminal amide is desired, the rink) using standard procedures. The resulting polymer-bound urea was condensed with excess ketoesters and aromatic aldehydes in tetra hydro furan (THF) at 55°C in the presence of a catalytic amount of hydrochloric acid (HCl) to afford the corresponding immobilized DHPMs. Subsequent cleavage of product from the resin by 50% trifluoroacetic acid (TFA) provided DHPMs in high yields and excellent purity.




**Asymmetric Biginelli Reaction**

Scheme 3.7

Several methods have been developed for the asymmetric synthesis of enantioenriched dihydropyrimidines. The first of these methods to give synthetically useful enantiomeric ratios was reported by Zhu C. et al. in 2005, over one-hundred years after discovery of the Biginelli reaction [56]. Zhu C. et al. found that the use of chiral ytterbium catalyst allowed for dihydropyrimidines to be synthesized in high yield and enantioselectivity. The ytterbium catalyst is recoverable and can be recycled several times without diminishing the product. A second protocol for the synthesis of enantioenriched dihydropyrimidines was introduced by Gong L. et al. [57].

The frontier of the Biginelli reaction will continue to be developed as new methods are reported and as the biological importance of this class of compounds is explored in greater detail.

## 3.6 Current Work

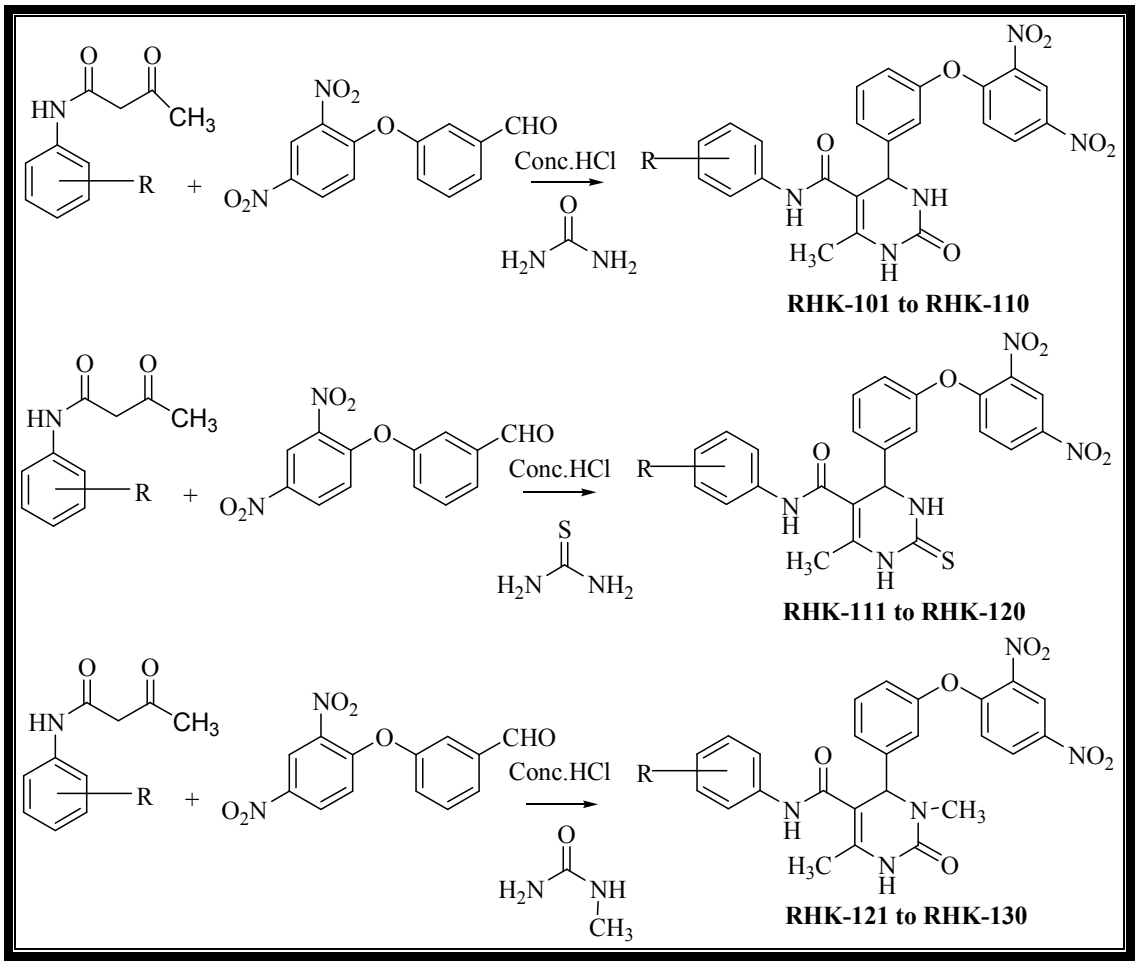
The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. The 1,2,3,4-tetrahydropyrimidines ring system is of special biological interest because it has numerous pharmacological and medicinal applications *viz*:antimicrobial, immunodilator, tuberculosis, antiallergic and radioprotective.

In **section-I** 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 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(substituted phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide **(RHK- 101 to 130)** are synthesized. The synthesis of **(RHK- 101 to 130)** was achieved by acid catalysed cyclocondensation of N-(substituted)-3-oxobutanamide, substituted urea and 4-(2,4-dinitrophenoxy)-benzaldehydes. The products were characterized by FT-IR, mass spectra, $^{1}$H NMR and elemental analyses. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial.

In **section-II** 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 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-substitutedphenyl)-1,2,3,4-tetrahydro -6-isopropyl-2-oxopyrimidine-5-carboxamide **(RHK- 131 to 160)** are synthesized. The synthesis of **(RHK- 131 to 160)** was achieved by acid catalysed cyclocondensation of N-(substituted phenyl)-4-methyl-3-oxopentanamide, substituted urea and 4-(2,4-dinitrophenoxy)benzaldehydes. The products were characterized by FT-IR, mass spectra, $^{1}$H NMR and elemental analyses. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial.

# 3.7 Section: - I

## 3.7.1 Reaction scheme



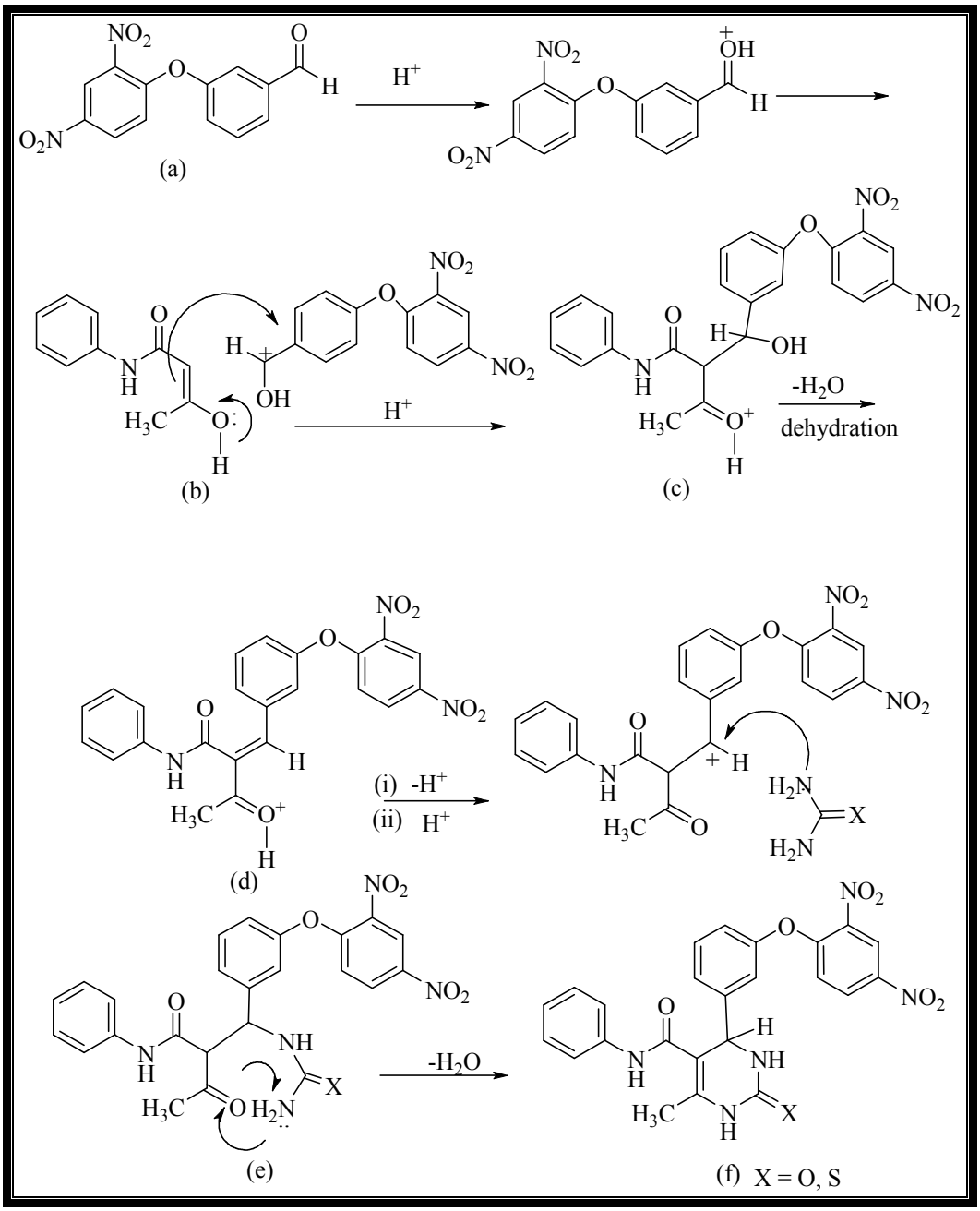
| Code | $R_1$ | M.F. | M.W. | M.P.$^0$C | Yield % | $R_{f1}$ | $R_{f2}$ |
|---|---|---|---|---|---|---|---|
| RHK-101 | 2-$OCH_3$ | $C_{25}H_{21}N_5O_8$ | 519 | 207 | 68 | 0.48 | 0.65 |
| RHK-102 | 3-Cl | $C_{24}H_{18}ClN_5O_7$ | 523 | 217 | 63 | 0.53 | 0.61 |
| RHK-103 | 2-F | $C_{24}H_{18}FN_5O_7$ | 507 | 202 | 59 | 0.49 | 0.58 |
| RHK-104 | 3-Cl 4-F | $C_{24}H_{17}ClFN_5O_7$ | 541 | 212 | 70 | 0.50 | 0.66 |
| RHK-105 | 4-$OCH_3$ | $C_{25}H_{21}N_5O_8$ | 519 | 205 | 75 | 0.55 | 0.70 |
| RHK-106 | 4-Cl | $C_{24}H_{18}ClN_5O_7$ | 523 | 224 | 71 | 0.41 | 0.68 |
| RHK-107 | 4-$CH_3$ | $C_{25}H_{21}N_5O_7$ | 503 | 201 | 69 | 0.59 | 0.68 |
| RHK-108 | 4-F | $C_{24}H_{18}FN_5O_7$ | 507 | 195 | 55 | 0.55 | 0.71 |
| RHK-109 | 2-Cl | $C_{24}H_{18}ClN_5O_7$ | 523 | 185 | 70 | 0.40 | 0.75 |
| RHK-110 | H | $C_{24}H_{19}N_5O_7$ | 489 | 235 | 75 | 0.55 | 0.70 |
| RHK-111 | 2-$OCH_3$ | $C_{25}H_{21}N_5O_7S$ | 535 | 245 | 61 | 0.41 | 0.69 |
| RHK-112 | 3-Cl | $C_{24}H_{18}ClN_5O_6S$ | 539 | 233 | 58 | 0.58 | 0.68 |
| RHK-113 | 2-F | $C_{24}H_{18}FN_5O_6S$ | 523 | 211 | 68 | 0.58 | 0.65 |
| RHK-114 | 3-Cl 4-F | $C_{24}H_{17}ClFN_5O_6S$ | 557 | 200 | 79 | 0.49 | 0.72 |
| RHK-115 | 4-$OCH_3$ | $C_{25}H_{21}N_5O_7S$ | 535 | 213 | 67 | 0.57 | 0.63 |
| RHK-116 | 4-Cl | $C_{24}H_{18}ClN_5O_6S$ | 539 | 218 | 73 | 0.44 | 0.64 |
| RHK-119 | 2-Cl | $C_{24}H_{18}ClN_5O_6S$ | 539 | 202 | 80 | 0.43 | 0.63 |
| RHK-120 | H | $C_{24}H_{19}N_5O_6S$ | 505 | 255 | 73 | 0.50 | 0.70 |
| RHK-121 | 2-$OCH_3$ | $C_{26}H_{23}N_5O_8$ | 533 | 233 | 80 | 0.42 | 0.62 |
| RHK-122 | 3-Cl | $C_{25}H_{20}ClN_5O_7$ | 537 | 229 | 72 | 0.56 | 0.66 |
| RHK-123 | 2-F | $C_{25}H_{20}FN_5O_7$ | 521 | 224 | 76 | 0.48 | 0.58 |
| RHK-124 | 3-Cl 4-F | $C_{25}H_{19}ClFN_5O_7$ | 555 | 219 | 68 | 0.52 | 0.72 |
| RHK-125 | 4-$OCH_3$ | $C_{26}H_{23}N_5O_8$ | 533 | 227 | 82 | 0.59 | 0.69 |
| RHK-126 | 4-Cl | $C_{25}H_{20}ClN_5O_7$ | 537 | 222 | 59 | 0.54 | 0.64 |
| RHK-127 | 4-$CH_3$ | $C_{26}H_{23}N_5O_7$ | 517 | 226 | 64 | 0.52 | 0.72 |
| RHK-128 | 4-F | $C_{25}H_{20}FN_5O_7$ | 521 | 197 | 62 | 0.44 | 0.64 |
| RHK-129 | 2-Cl | $C_{25}H_{20}ClN_5O_7$ | 537 | 243 | 74 | 0.40 | 0.70 |
| RHK-130 | H | $C_{25}H_{21}N_5O_7$ | 503 | 250 | 60 | 0.48 | 0.68 |

TLC Solvent system $R_{f1}$:- Hexane : Ethyl acetate – 6:4,

$R_{f2}$:- Chloroform : methanol – 9:1.

### 3.7.2 Plausible Reaction Mechanism

The reaction mechanism of pyrimidine formation can be depicted as under:



## 3.7.3 Experimental

### 3.7.3.1 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 potassium bromide (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.

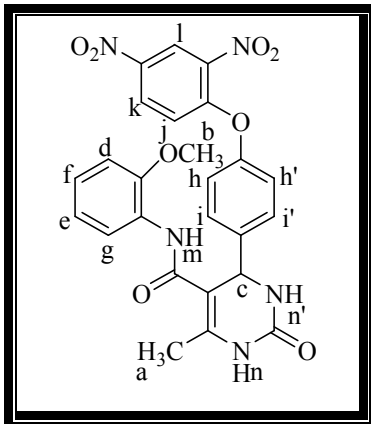
### *3.7.3.2 Synthesis of N-(substituted phenyl)-3-oxobutanamides*

Syntheses of *N*-(substituted phenyl)-3-oxobutanamides were achieved using previously published methods [58].

### *3.7.3.3 General procedure for the synthesis of 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(substituted phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-101 to110)*

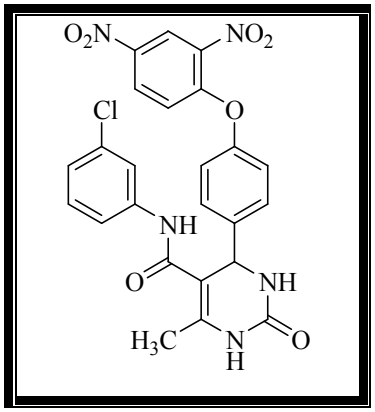
A mixture of *N*-(substituted phenyl)-3-oxobutanamides (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), urea (0.015 M) and catalytic amount of conc. hydrochloric acid (HCl) in ethanol (30 ml) was heated under reflux condition for 8 to 10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

### *3.7.3.3.1 4-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-N-(2-methoxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-101)*



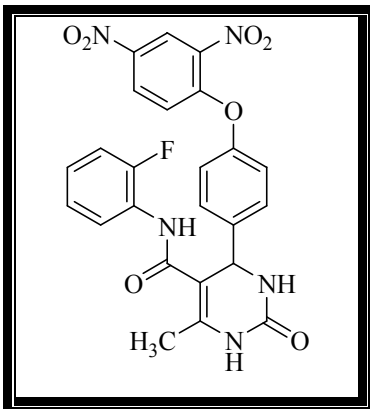

Yield: 68%; mp 207ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_8$: C, 57.80; H, 4.07; N, 13.48; O, 24.64 Found: C, 57.65; H, 4.00; N, 13.21; O, 24.29%; IR ($cm^{-1}$): 3410 (N-H stretching of amide), 3070 (C-H stretching of aromatic ring), 2935 (C-H asymmetrical stretching of $CH_3$ group), 2900 (C-H symmetrical stretching of $CH_3$ group), 1693 (C=O stretching of amide), 1693 (C=O stretching of cyclic) 1600 (N-H deformation of pyrimidine ring), 1525 (C=C stretching of aromatic ring), 1525 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1469 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of $CH_3$ group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1344 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1278 (C-N stretching), 1247 (C-O-C asymmetrical stretching $OCH_3$), 1074 (C-H in plane deformation of aromatic ring), 1030 (C-O-C symmetrical stretching $OCH_3$) 824 (para-substituted); MS: *m/z* 519; $^1$H NMR (DMSO-$d_6$) δ ppm: 2.19 (s, 3H, $H_a$), 3.72 (s, 3H, $H_b$), 5.35 (s, 1H, $H_c$), 6.84-6.86 (d, 1H, $H_d$, $J$ = 7.60 Hz), 6.87-7.14 (m, 3H, $H_{e\text{-}g}$), 7.29-7.31 (d, 2H, $H_{hh'}$, $J$ = 8.40 Hz), 7.44 (d, 2H, $H_{ii'}$), 7.68 (s, 1H, $H_j$), 7.75-7.78 (d, 1H, $H_k$, $J$ = 12.00), 8.37 (s, 1H, $H_l$).

### *3.7.3.3.2 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-102)*



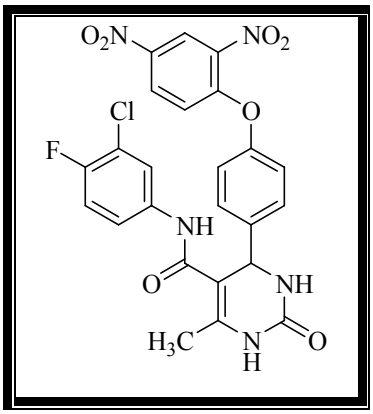

Yield: 63%; mp 217ºC; Anal. Calcd. for $C_{24}H_{18}ClN_5O_7$: C, 55.02; H, 3.46; Cl, 6.77; N, 13.37; O, 21.38; Found: C, 54.96; H, 3.25; Cl, 6.63; N, 13.13; O, 21.22%; MS: *m/z* 523.

***3.7.3.3.3 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-103)***



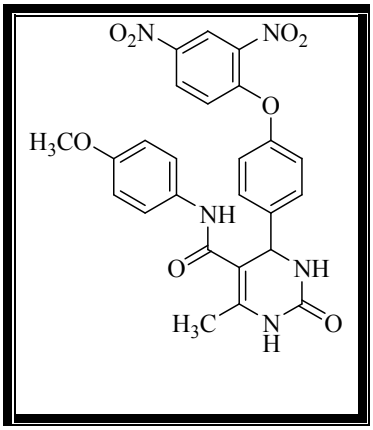
Yield: 59%; mp 202ºC; Anal. Calcd. for $C_{24}H_{18}FN_5O_7$: C, 56.81; H, 3.58; F, 3.74; N, 13.80; O, 22.07; Found: C, 56.74; H, 3.45; F, 3.67; N, 13.72; O, 22.00%; MS: *m/z* 507.

***3.7.3.3.4 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chloro-4-fluorophenyl)-1,2,3,4-tetr-ahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-104)***



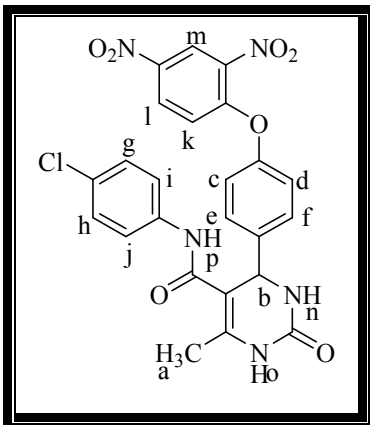
Yield: 70%; mp 212ºC; Anal. Calcd. for $C_{24}H_{17}ClFN_5O_7$: C, 53.20; H, 3.16; Cl, 6.54; F, 3.51; N, 12.92; O, 20.67; Found: C, 53.11; H, 3.08; Cl, 6.42; F, 3.43; N, 12.78; O, 20.56%; MS: *m/z* 441.

***3.7.3.3.5 4-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-N-(4-methoxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-105)***



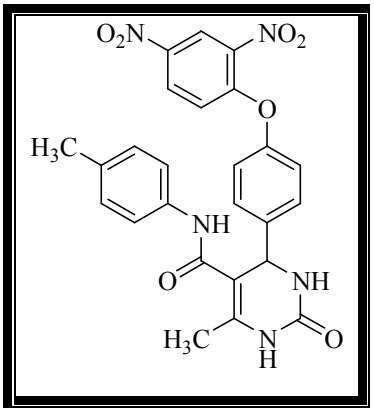
Yield: 75%; mp 205ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_8$: C, 57.80; H, 4.07; N, 13.48; O, 24.64; Found: C, 57.71; H, 4.01; N, 13.32; O, 24.52%; MS: *m/z* 519.

### *3.7.3.3.6 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-106)*



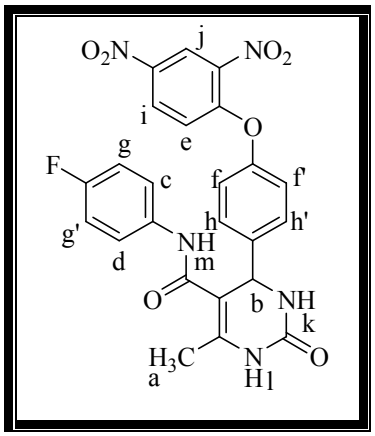

Yield: 71%; mp 224ºC; Anal. Calcd. for $C_{24}H_{18}ClN_5O_7$: C, 55.02; H, 3.46; Cl, 6.77; N, 13.37; O, 21.38; Found: C, 55.00; H, 3.35; Cl, 6.61; N, 13.23; O, 21.22%; IR ($cm^{-1}$): 3311 (N-H stretching of amide), 3101 (C-H stretching of aromatic ring), 2945 (C-H asymmetrical stretching of $CH_3$ group), 2945 (C-H symmetrical stretching of $CH_3$ group), 1703 (C=O stretching of amide), 1681 (C=O stretching of cyclic) 1589 (N-H deformation of pyrimidine ring), 1531 (C=C stretching of aromatic ring), 1531 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1438 (C-H asymmetrical deformation of $CH_3$ group), 1398 (C-H symmetrical deformation of $CH_3$ group), 1342 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1342 (C-N-C stretching vibration of pyrimidine ring), 1240 (C-N stretching), 1089 (C-H in plane deformation of aromatic ring), 824 (para-substituted), 761 (C-H in out plane deformation of aromatic ring), 709 (C-Cl stretching) ; MS: *m/z* 523; $^1$H NMR (DMSO-*d6*) δ ppm: 2.02 (s, 3H, $H_a$), 5.43 (s, 1H, $H_b$), 6.96-6.99 (d, 1H, $H_c$, J = 9.20Hz), 7.10 (s, 1H, $H_d$), 7.14-7.17 (m, 1H, $H_e$), 7.26-7.28 (d, 3H, $H_{fh}$ J = 8.80Hz), 7.51-7.54 (m, 3H, $H_{i-k}$, J = 12.40Hz), 7.67 (s, 1H, $H_l$), 8.20-8.23 (m, 1H, $H_m$, J = 12.00Hz), 8.82 (s, 1H, $H_n$), 8.86 (s, 1H, $H_o$), 9.70 (s, 1H, $H_p$).

### *3.7.3.3.7 4-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-N-p-tolylpyrimidine-5-carboxamide (RHK-107)*



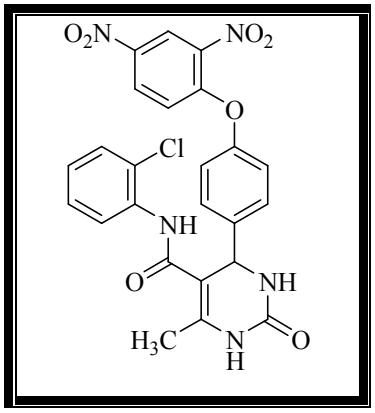

Yield: 69%; mp 201ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_7$: C, 59.64; H, 4.20; N, 13.91; O, 22.25; Found: C, 59.54; H, 4.11; N, 13.75; O, 22.14%; MS: *m/z* 503.

### *3.7.3.3.8 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-108)*



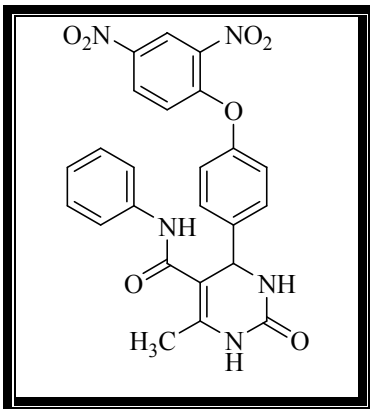

Yield: 55%; mp 195ºC; Anal. Calcd. for $C_{24}H_{18}FN_5O_7$: C, 56.81; H, 3.58; F, 3.74; N, 13.80; O, 22.07; Found: C, 56.77; H, 3.44; F, 3.65; N, 13.71; O, 22.00%; IR ($cm^{-1}$): 3410 (N-H stretching of amide), 3070 (C-H stretching of aromatic ring), 2935 (C-H asymmetrical stretching of $CH_3$ group), 2830 (C-H symmetrical stretching of $CH_3$ group), 1710 (C=O stretching of amide), 1670 (C=O stretching of cyclic) 1608 (N-H deformation of pyrimidine ring), 1521 (C=C stretching of aromatic ring), 1521 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1456 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of $CH_3$ group), 1313 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1313 (C-N-C stretching vibration of pyrimidine ring), 1244 (C-N stretching), 1089 (C-H in plane deformation of aromatic ring), 1012 (C-F stretching), 810 (para-substituted), 744 (C-H in out plane deformation of aromatic ring); MS: *m/z* 503; $^1$H NMR (DMSO-*d6*) δ ppm: 2.06 (s, 3H, $H_a$), 5.42-5.43 (s, 1H, $H_b$), 7.07-7.11 (m, 3H, $H_{c\text{-}e}$), 7.24-7.26 (d, 2H, $H_{ff'}$, J = 8.80Hz), 7.40-7.42 (d, 2H, $H_{gg'}$, J = 8.40Hz), 7.54-7.57 (m, 3H, $H_{hh'}$), 7.68 (s, 1H, $H_i$), 8.43-8.46 (m, 1H, $H_j$, J = 12.00Hz), 8.81 (m, 1H, $H_k$), 8.88-8.89 (d, 1H, $H_l$, J = 2.80Hz), 9.65 (s, 1H, $H_m$).

### *3.7.3.3.9 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-109)*




Yield: 70%; mp 185ºC; Anal. Calcd. for $C_{24}H_{18}ClN_5O_7$ C, 55.02; H, 3.46; Cl, 6.77; N, 13.37; O, 21.38; Found: C, 54.89; H, 3.35; Cl, 6.54; N, 13.26; O, 21.22%; MS: *m/z* 523.

***3.7.3.3.10 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide (RHK-110)***

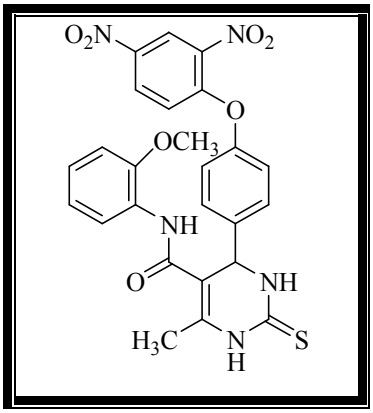


Yield: 75%; mp 235ºC; Anal. Calcd. for $C_{24}H_{19}N_5O_7$: C, 58.90; H, 3.91; N, 14.31; O, 22.88; Found: C, 58.79; H, 3.81; N, 14.21; O, 22.74%; MS: *m/z* 489.

***3.7.3.4 General procedure for the synthesis of 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(substituted phenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-111 to120)***

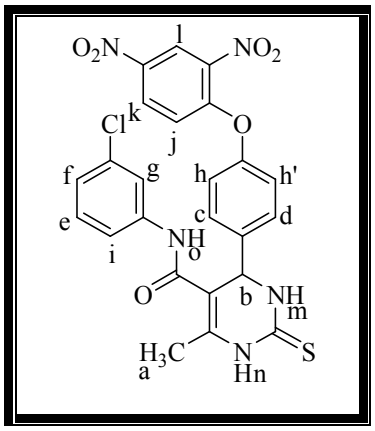
A mixture of *N*-(substituted phenyl)-3-oxobutanamides (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), thiourea (0.015 M) and catalytic amount of conc. acid in ethanol (30 ml) was heated under reflux condition for 11 to 12 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

***3.7.3.4.1 4-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-N-(2-methoxyphenyl)-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-111)***



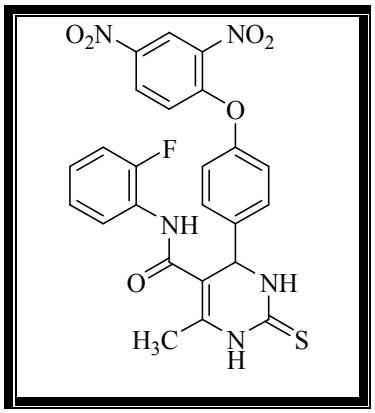
Yield: 61%; mp 245ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_8$: C, 56.07; H, 3.95; N, 13.08; O, 20.91; S, 5.99; Found: C, 56.00; H, 3.85; N, 13.01; O, 20.81; S, 5.86%; MS: *m/z* 535.

***3.7.3.4.2*** ***4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-112)***



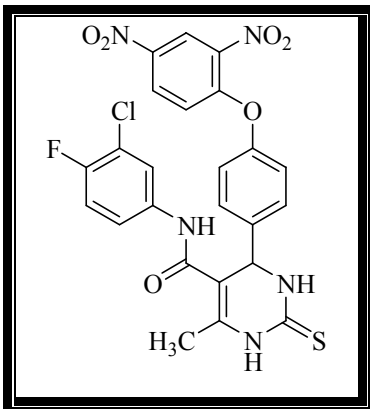
Yield: 58%; mp 233ºC; Anal. Calcd. for $C_{24}H_{18}ClN_5O_6S$: C, 53.28; H, 3.26; Cl, 6.46; N, 12.88; O, 17.71; S, 5.82; Found: C, 54.96; H, 3.25; Cl, 6.63; N, 13.13; O, 21.22%; IR ($cm^{-1}$): 3363 (N-H stretching of amide), 3109 (C-H stretching of aromatic ring), 2955 (C-H asymmetrical stretching of $CH_3$ group), 2871 (C-H symmetrical stretching of $CH_3$ group), 1703 (C=O stretching of amide), 1662 (N-H deformation of pyrimidine ring), 1597 (C=C stretching of aromatic ring), 1523 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1423 (C-H asymmetrical deformation of $CH_3$ group), 1342 (C-H symmetrical deformation of $CH_3$ group), 1342 (C-N-C stretching vibration of pyrimidine ring), 1269 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1246 (C-N stretching), 1163 (C=S stretching of cyclic), 1070 (C-H in plane deformation of aromatic ring), 833 (para-substituted), 777 (C-H in out plane deformation of aromatic ring), 677 (C-Cl stretching) ; MS: *m/z* 523; $^1$H NMR (DMSO-*d6*) δ ppm: 2.10 (s, 3H, $H_a$), 5.43-5.44 (s, 1H, $H_b$), 7.07-7.10 (m, 1H, $H_c$, J = 9.20Hz), 7.12-7.14 (d, 1H, $H_d$), 7.27-7.32 (m, 3H, $H_{e-g}$), 7.38-7.40 (d, 2H, $H_{hh'}$ J = 8.40Hz), 7.45-7.47 (d, 1H, $H_i$, J = 9.20Hz), 7.75-7.76 (s, 1H, $H_j$), 8.43-8.46 (m, 1H, $H_k$, J = 12.00Hz), 8.88-8.89 (s, 1H, $H_l$), 9.57 (s, 1H, $H_m$) 9.93 (s, 1H, $H_n$), 10.13 (s,1H,$H_o$).

***3.7.3.4.3 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-113)***



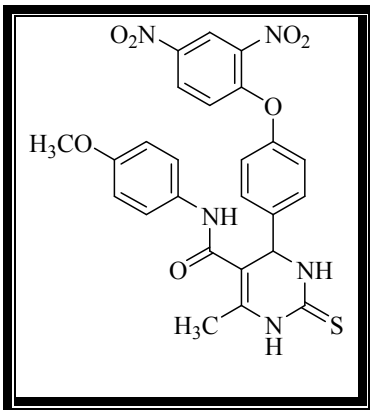
Yield: 68%; mp 211ºC; Anal. Calcd. for $C_{24}H_{18}FN_5O_6S$ : C, 55.00; H, 3.34; F, 3.55; N, 13.25; O, 18.23; S, 6.04; Found: C, 56.74; H, 3.45; F, 3.67; N, 13.72; O, 22.00%; MS: *m/z* 507.

***3.7.3.4.4 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chloro-4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-114)***



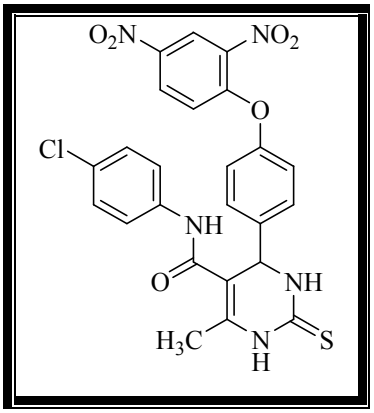
Yield: 79%; mp 200ºC; Anal. Calcd. for $C_{24}H_{17}ClFN_5O_6S$: C, 51.66; H, 3.07; Cl, 6.35; F, 3.41; N, 12.55; O, 17.21; S, 5.75; Found: C, 51.56; H, 3.00; Cl, 6.24; F, 3.32; N, 12.42; O, 17.11; S, 5.66%; MS: *m/z* 557.

***3.7.3.4.5* 4*-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-N-(4-methoxyphenyl)-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-115)***



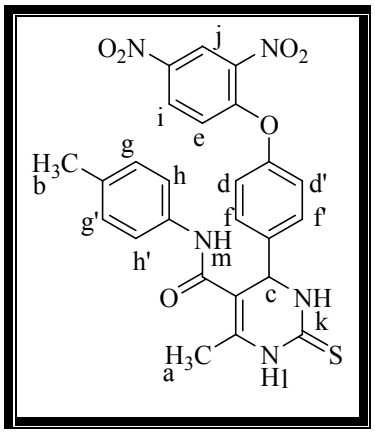
Yield: 67%; mp 213ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_7S$: C, 56.07; H, 3.95; N, 13.08; O, 20.91; S, 5.99; Found: C, 56.00; H, 3.84; N, 13.01; O, 20.82; S, 5.87 %; MS: *m/z* 535.

***3.7.3.4.6 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-116)***



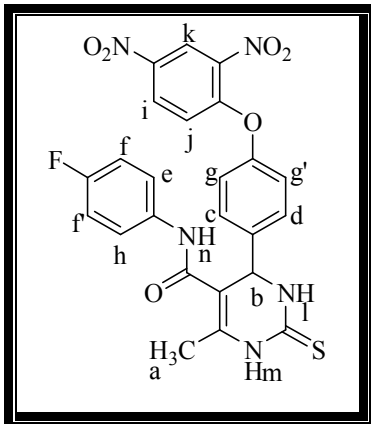
Yield: 74%; mp 218ºC; Anal. Calcd. for $C_{24}H_{18}ClN_5O_6S$: C, 53.39; H, 3.36; Cl, 6.57; N, 12.97; O, 17.78; S, 5.94; Found: C, 53.27; H, 3.28; Cl, 6.49; N, 12.88; O, 17.65; S, 5.86%; MS: *m/z* 539.

### *3.7.3.4.7 4-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxo-N-p-tolylpyrimidine-5-carboxamide (RHK-117)*



Yield: 71%; mp 203ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_6S$: C, 57.80; H, 4.07; N, 13.48; O, 18.48; S, 6.17; Found: C, 57.71; H, 4.00; N, 13.33; O, 18.34; S, 6.11%; IR ($cm^{-1}$): 3377 (N-H stretching of amide), 3109 (C-H stretching of aromatic ring), 2949 (C-H asymmetrical stretching of $CH_3$ group), 2844 (C-H symmetrical stretching of $CH_3$ group), 1680 (C=O stretching of amide), 1600 (N-H deformation of pyrimidine ring), 1589 and 1521 (C=C stretching of aromatic ring), 1521 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1467 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of $CH_3$ group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1344 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1267 (C-N stretching), 1192 (C=S stretching of cyclic), 1111 (C-H in plane deformation of aromatic ring), 817 (para-substituted), 740 (C-H in out plane deformation of aromatic ring); MS: *m/z* 519; $^1$H NMR (DMSO-*d6*) δ ppm: 2.08 (s, 3H, $H_a$), 2.22 (s, 3H, $H_b$), 5.42 (s, 1H, $H_{c,}$), 7.05-7.08 (d, 2H, $H_{dd'}$, J = 8.40Hz), 7.10-7.13 (d, 1H, He, J = 9.2Hz), 7.26-7.29 (d, 2H, $H_{ff'}$ J = 8.80Hz), 7.39-7.44 (m, 4H, $H_{g\text{-}g',h\text{-}h'}$), 8.41-8.45 (m, 1H, $H_i$), 8.88-8.89 (s, 1H, $H_j$), 9.49 (s, 1H, $H_k$), 9.68 (s, 1H, $H_l$), 10.05 (s, 1H, $H_m$).

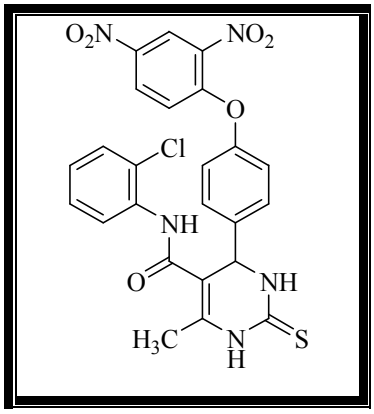
### *3.7.3.4.8 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-118)*



Yield: 65%; mp 213ºC; Anal. Calcd. for $C_{24}H_{18}FN_5O_7$: C, 56.81; H, 3.58; F, 3.74; N, 13.80; O, 22.07; Found: C, 56.77; H, 3.44; F, 3.65; N, 13.71; O, 22.00%; IR ($cm^{-1}$): 3246 (N-H stretching of amide), 3115 (C-H stretching of aromatic ring), 2902 (C-H asymmetrical stretching of $CH_3$ group), 2842 (C-H symmetrical stretching of $CH_3$ group), 1676 (C=O stretching of amide), 1606 (N-H deformation of pyrimidine ring), 1562 (C=C stretching of aromatic ring), 1512 (C-H asymmetrical deformation of $CH_3$ group), 1512 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1469 (C-H symmetrical deformation of $CH_3$ group), 1342 (C-N-C

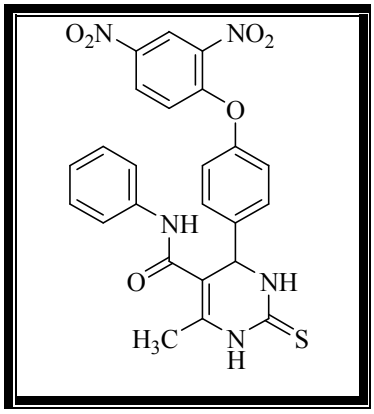
stretching vibration of pyrimidine ring), 1342 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1269 (C-N stretching), 1201 (C=S stretching of cyclic), 1064 (C-H in plane deformation of aromatic ring), 1016 (C-F stretching), 827 (para-substituted), 777 (C-H in out plane deformation of aromatic ring); MS: *m/z* 523; $^1$H NMR (DMSO-*d6*) δ ppm: 2.09 (s, 3H, $H_a$), 5.42-5.43 (s, 1H, $H_b$), 7.12-7.14 (m, 3H, $H_{c\text{-}e}$, J = 9.2), 7.27-7.29 (d, 2H, $H_{ff'}$, J = 8.40Hz), 7.39-7.41 (d, 2H, $H_{gg'}$, J = 8.4Hz), 7.54-7.58 (m, 2H, $H_{hi}$, J = 14.00Hz), 8.44-8.47 (m, 4H, $H_{g\text{-}g',h\text{-}h'}$), 8.41-8.45 (d, 1H, $H_j$), 8.88-8.89 (s, 1H, $H_k$), 9.53 (s, 1H, $H_l$), 9.82 (s, 1H, $H_m$), 10.08 (s, 1H, $H_n$).

### *3.7.3.4.9 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-119)*




Yield: 73%; mp 202ºC; Anal. Calcd. for $C_{24}H_{18}ClN5O_6S$: C, 53.39; H, 3.36; Cl, 6.57; N, 12.97; O, 17.78; S, 5.94; Found: C, 53.28; H, 3.29; Cl, 6.46; N, 12.84; O, 17.70; S, 5.88%; MS: *m/z* 539.

### *3.7.3.4.10 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxamide (RHK-120)*

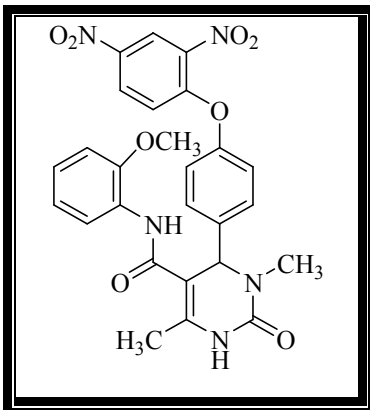



Yield: 80%; mp 255 ºC; Anal. Calcd. for $C_{24}H_{19}N_5O_6S$: C, 57.02; H, 3.79; N, 13.85; O, 18.99; S, 6.34; Found: C, 56.86; H, 3.67; N, 13.75; O, 18.88; S, 6.22%; MS: *m/z* 505.

### *3.7.3.5 General procedure for the synthesis of 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(substituted phenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-121 to130)*

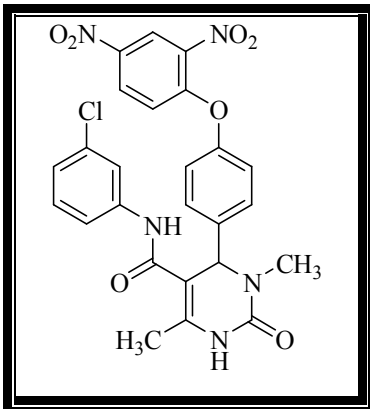
A mixture of *N*-(substituted phenyl)-3-oxobutanamides (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), N-methyl urea (0.015 M) and catalytic amount of conc. acid in ethanol (30 ml) was heated under reflux condition for 12 to 13 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

#### *3.7.3.5.1 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(2-methoxyphenyl) -1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-121)*



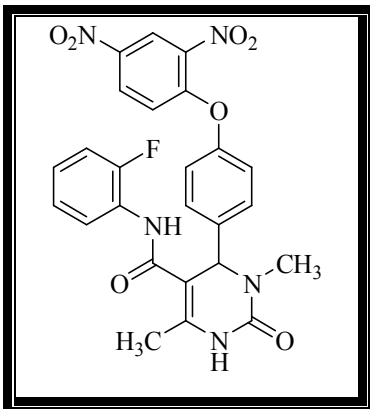
Yield: 72%; mp 233ºC; Anal. Calcd. for $C_{26}H_{23}ClN_5O_8$: C, 58.54; H, 4.35; N, 13.13; O, 23.99; Found: C, 58.46; H, 4.26; N, 13.06; O, 23.92%; MS: *m/z* 533.

#### *3.7.3.5.2 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(3-chlorophenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-122)*



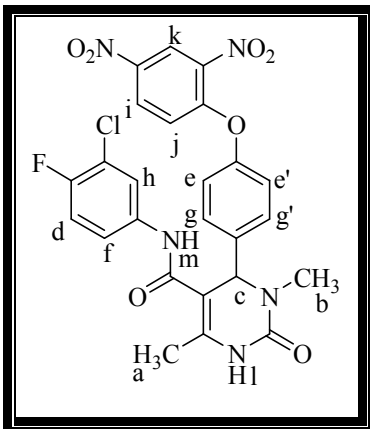
Yield: 76%; mp 229ºC; Anal. Calcd. for $C_{25}H_{20}ClN_5O_7$: C, 55.82; H, 3.75; Cl, 6.59; N, 13.02; O, 20.82; Found: , 55.76; H, 3.68; Cl, 6.50; N, 13.00; O, 20.68%; MS: *m/z* 537.

### *3.7.3.5.3 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-flurophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-123)*



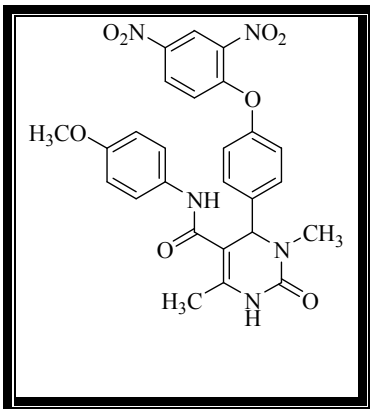
Yield: 59%; mp 202ºC; Anal. Calcd. for $C_{24}H_{18}FN_5O_7$: C, 56.81; H, 3.58; F, 3.74; N, 13.80; O, 22.07; Found: C, 56.74; H, 3.45; F, 3.67; N, 13.72; O, 22.00%; MS: *m/z* 507.

### *3.7.3.5.4 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chloro-3-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-124)*



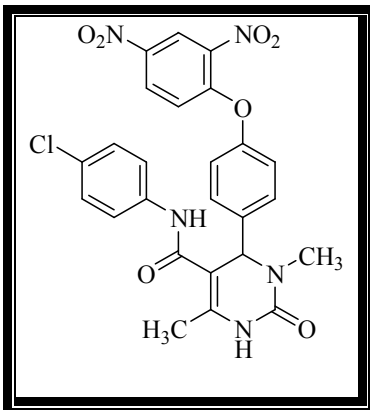
Yield: 68%; mp 224ºC; Anal. Calcd. for $C_{25}H_{19}ClFN_5O_7$: C, 54.01; H, 3.45; Cl, 6.38; F, 3.42; N, 12.60; O, 20.15; Found: C, 54.00; H, 3.33; Cl, 6.27; F, 3.35; N, 12.51; O, 20.02%; IR ($cm^{-1}$): 3315 (N-H stretching of amide), 3070 (C-H stretching of aromatic ring), 2923 (C-H asymmetrical stretching of $CH_3$ group), 2868 (C-H symmetrical stretching of $CH_3$ group), 1660 (C=O stretching of amide), 1600 (C=O stretching of cyclic) 1600 (N-H deformation of pyrimidine ring), 1529 (C=C stretching of aromatic ring), 1529 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1491 (C-H asymmetrical deformation of $CH_3$ group), 1406 (C-H symmetrical deformation of $CH_3$ group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1344 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1267 (C-N stretching), 1163 (C-F stretching), 1062 (C-H in plane deformation of aromatic ring), 835 (para-substituted), 736 (C-H in out plane deformation of aromatic ring), 653 and 526 (C-Cl stretching); MS: *m/z* 555; $^1$H NMR (DMSO-*d6*) δ ppm: 2.19 (s, 3H, $H_a$), 3.09 (s, 3H, $H_b$), 5.31-5.32 (s, 1H, $H_c$), 7.08-7.10 (d, 1H, $H_d$, J = 9.20Hz), 7.23-7.32 (d, 2H, $H_{e\text{-}e'}$, J = 8.80Hz), 7.34-7.36 (d, 1H, $H_f$,J = 9.20Hz), 7.37-7.39 (m, 1H, $H_{g\text{-}g'}$, J = 8.80Hz), 7.47-7.51(m, 1H, $H_h$), 7.88-7.90 (m, 2H, $H_{ij}$, J = 9.20Hz), 8.43-8.46 (m, 1H, $H_k$), 8.88-8.89 (d, 1H, $H_l$, J = 2.80Hz), 10.07 (s, 1H, $H_m$).

### *3.7.3.5.5 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(4-methoxyphenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-125)*



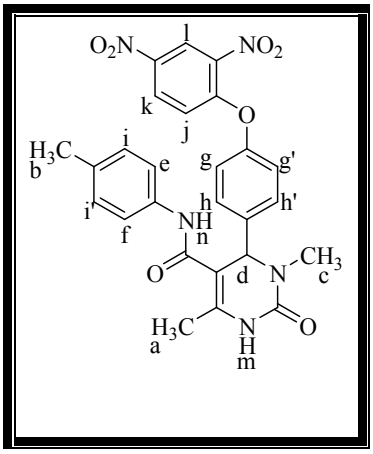
Yield: 59%; mp 227ºC; Anal. Calcd. for $C_{26}H_{23}N_5O_8$: C, 58.54; H, 4.35; N, 13.13; O, 23.99; Found: C, 58.42; H, 4.24; N, 13.00; O, 23.87%; MS: *m/z* 533.

### *3.7.3.5.6 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(4-chlorophenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-126)*



Yield: 64%; mp 222ºC; Anal. Calcd. for $C_{25}H_{20}ClN_5O_7$: C, 55.82; H, 3.75; Cl, 6.59; N, 13.02; O, 20.82; Found: C, 55.78; H, 3.66; Cl, 6.46; N, 13.00; O, 20.68%; MS: *m/z* 537.

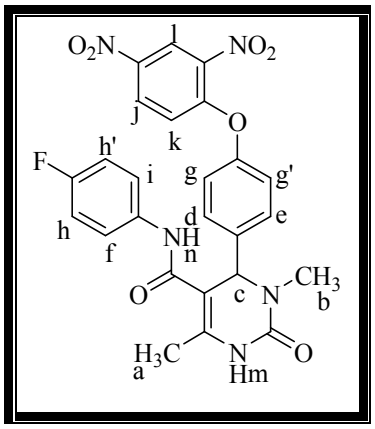
### *3.7.3.5.7 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-p-tolylpyrimidine-5-carboxamide (RHK-127)*



Yield: 62%; mp 226 ºC; Anal. Calcd. for $C_{26}H_{23}N_5O_7$: C, 60.34; H, 4.48; N, 13.53; O, 21.64; Found: C, 60.28; H, 4.44; N, 13.43; O, 21.60%; IR ($cm^{-1}$): 3425 (N-H stretching of amide), 3097 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of $CH_3$ group), 2892 (C-H symmetrical stretching of $CH_3$ group), 1695 (C=O stretching of amide), 1635 (C=O stretching of cyclic) 1597 (N-H deformation of pyrimidine ring), 1529 (C=C stretching of aromatic ring), 1529 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1489 (C-H asymmetrical deformation of $CH_3$ group), 1409 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring),

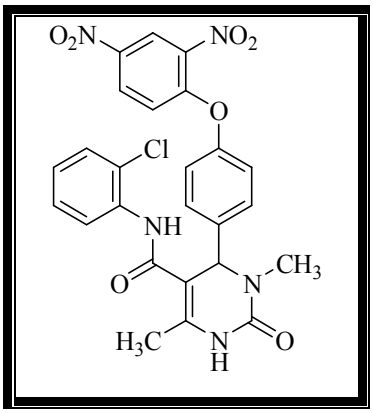
1346 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1273 (C-N stretching), 1072 (C-H in plane deformation of aromatic ring), 827 (para-substituted), 748 (C-H in out plane deformation of aromatic ring); MS: *m/z* 517; $^1$H NMR (DMSO-*d6*) δ ppm: 2.08 (s, 3H, $H_a$), 2.22 (s, 3H, $H_b$), 3.45 (s, 3H, $H_c$), 5.42-5.43 (s, 1H, $H_d$), 7.07-7.11 (m, 2H, $H_{ef}$), 7.24-7.26 (d, 2H, $H_{g\text{-}g'}$, J = 8.80Hz), 7.40-7.42 (d, 2H, $H_{h\text{-}h'}$, J = 8.40Hz), 7.54-7.57 (d, 2H, $H_{i\text{-}i'}$, J = 14.00), 7.68 (s, 1H, $H_j$) 8.43-8.46 (m, 1H, Hk, J = 12.00), 8.81 (s, 1H, $H_m$), 8.88-8.89 (d, 1H, $H_l$, J = 2.80Hz), 10.07 (s, 1H, $H_m$).

### *3.7.3.5.8 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-128)*



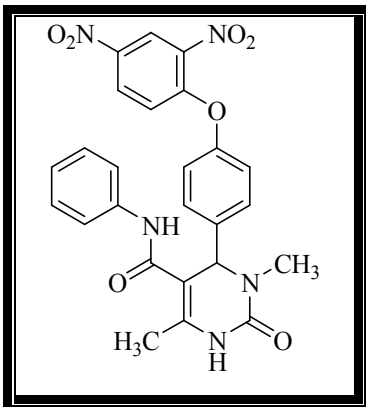

Yield: 74%; mp 197ºC; Anal. Calcd. for $C_{25}H_{20}FN_5O_7$: C, 57.58; H, 3.87; F, 3.64; N, 13.43; O, 21.48; Found: C, 57.46; H, 3.75; F, 3.55; N, 13.35; O, 21.32%; IR ($cm^{-1}$): 3284 (N-H stretching of amide), 3053 (C-H stretching of aromatic ring), 2952 (C-H asymmetrical stretching of $CH_3$ group), 2844 (C-H symmetrical stretching of $CH_3$ group), 1676 (C=O stretching of amide), 1693 (C=O stretching of cyclic) 1599 (N-H deformation of pyrimidine ring), 1539 (C=C stretching of aromatic ring), 1539 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1411 (C-H asymmetrical deformation of $CH_3$ group), 1346 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1265 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1265 (C-N stretching), 1161 (C-F stretching), 1068 (C-H in plane deformation of aromatic ring), 829 (para-substituted), 663 (C-F stretching); MS: *m/z* 521; $^1$H NMR (DMSO-*d6*) δ ppm: 2.19 (s, 3H, $H_a$), 3.09 (s, 3H, $H_b$), 5.40 (m, 1H, $H_c$), 7.08-7.14 (m, 3H, $H_{d\text{-}f}$), 7.27-7.30 (d, 2H, $H_{gg'}$ J = 11.20Hz), 7.39-7.41 (d, 2H, $H_{hh'}$ J = 8.00Hz), 7.54-7.58 (m, 1H, $H_i$ J = 4.80Hz), 8.44-8.47 (m, 1H, $H_j$, J = 12.00Hz), 8.88-8.89 (d, 1H, $H_k$, J = 2.80Hz), 9.53 (s, 1H, $H_{l,}$), 9.82 (s, 1H, $H_m$), 10.07 (s, 1H, $H_n$).

### *3.7.3.5.9 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-129)*



Yield: 60%; mp 243ºC; Anal. Calcd. for $C_{25}H_{20}ClN_5O_7$: C, 55.82; H, 3.75; Cl, 6.59; N, 13.02; O, 20.82; Found: C, 55.75; H, 3.67; Cl, 6.46; N, 13.00; O, 20.75%; MS: *m/z* 537.

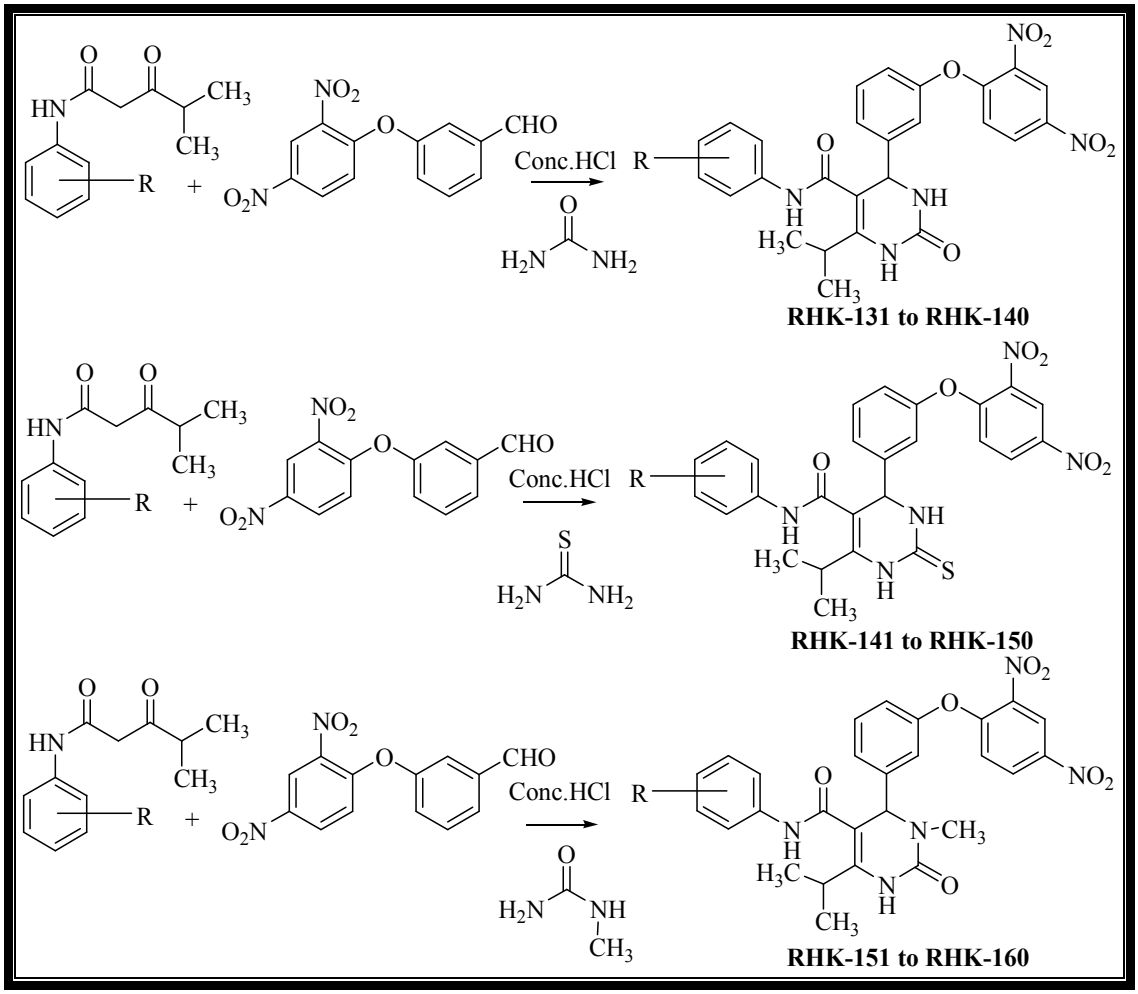
### *3.7.3.5.10 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(phenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-130)*



Yield: 68%; mp 250ºC; Anal. Calcd. for $C_{25}H_{21}N_5O_7$: C, 58.90; H, 3.91; N, 14.31; O, 22.88; Found: C, 58.79; H, 3.81; N, 14.21; O, 22.74%; MS: *m/z* 503.

## 3.8 Section: - II

### 3.8.1 Reaction scheme




| Code | $R_1$ | M.F. | M.W. | M.P.$^0$C | Yield % | $R_{f1}$ | $R_{f2}$ |
|---|---|---|---|---|---|---|---|
| RHK-131 | 2-$OCH_3$ | $C_{27}H_{25}N_5O_8$ | 547 | 223 | 69 | 0.47 | 0.66 |
| RHK-132 | 3-Cl | $C_{26}H_{22}ClN_5O_7$ | 551 | 219 | 65 | 0.52 | 0.62 |
| RHK-133 | 2-F | $C_{26}H_{22}FN_5O_7$ | 535 | 210 | 70 | 0.48 | 0.54 |
| RHK-134 | 3-Cl 4-F | $C_{26}H_{21}ClFN_5O_7$ | 569 | 201 | 75 | 0.49 | 0.65 |
| RHK-135 | 4-$OCH_3$ | $C_{27}H_{25}N_5O_8$ | 543 | 198 | 71 | 0.54 | 0.71 |
| RHK-136 | 4-Cl | $C_{26}H_{22}ClN_5O_7$ | 551 | 220 | 72 | 0.43 | 0.65 |
| RHK-137 | 4-$CH_3$ | $C_{27}H_{25}N_5O_7$ | 531 | 200 | 69 | 0.55 | 0.63 |
| RHK-138 | 4-F | $C_{26}H_{22}FN_5O_7$ | 535 | 199 | 59 | 0.54 | 0.76 |
| RHK-139 | 2-Cl | $C_{26}H_{22}ClN_5O_7$ | 551 | 195 | 72 | 0.44 | 0.73 |
| RHK-140 | H | $C_{26}H_{23}N_5O_7$ | 517 | 232 | 74 | 0.52 | 0.72 |
| RHK-141 | 2-$OCH_3$ | $C_{27}H_{25}N_5O_7S$ | 563 | 241 | 66 | 0.43 | 0.69 |
| RHK-142 | 3-Cl | $C_{26}H_{22}ClN_5O_6S$ | 567 | 232 | 59 | 0.57 | 0.67 |
| RHK-143 | 2-F | $C_{26}H_{22}FN_5O_6S$ | 551 | 201 | 63 | 0.56 | 0.63 |
| RHK-144 | 3-Cl 4-F | $C_{26}H_{21}ClFN_5O_6S$ | 585 | 222 | 75 | 0.47 | 0.71 |
| RHK-145 | 4-$OCH_3$ | $C_{27}H_{25}N_5O_7S$ | 563 | 212 | 64 | 0.58 | 0.60 |
| RHK-146 | 4-Cl | $C_{26}H_{22}ClN_5O_6S$ | 567 | 219 | 74 | 0.43 | 0.62 |

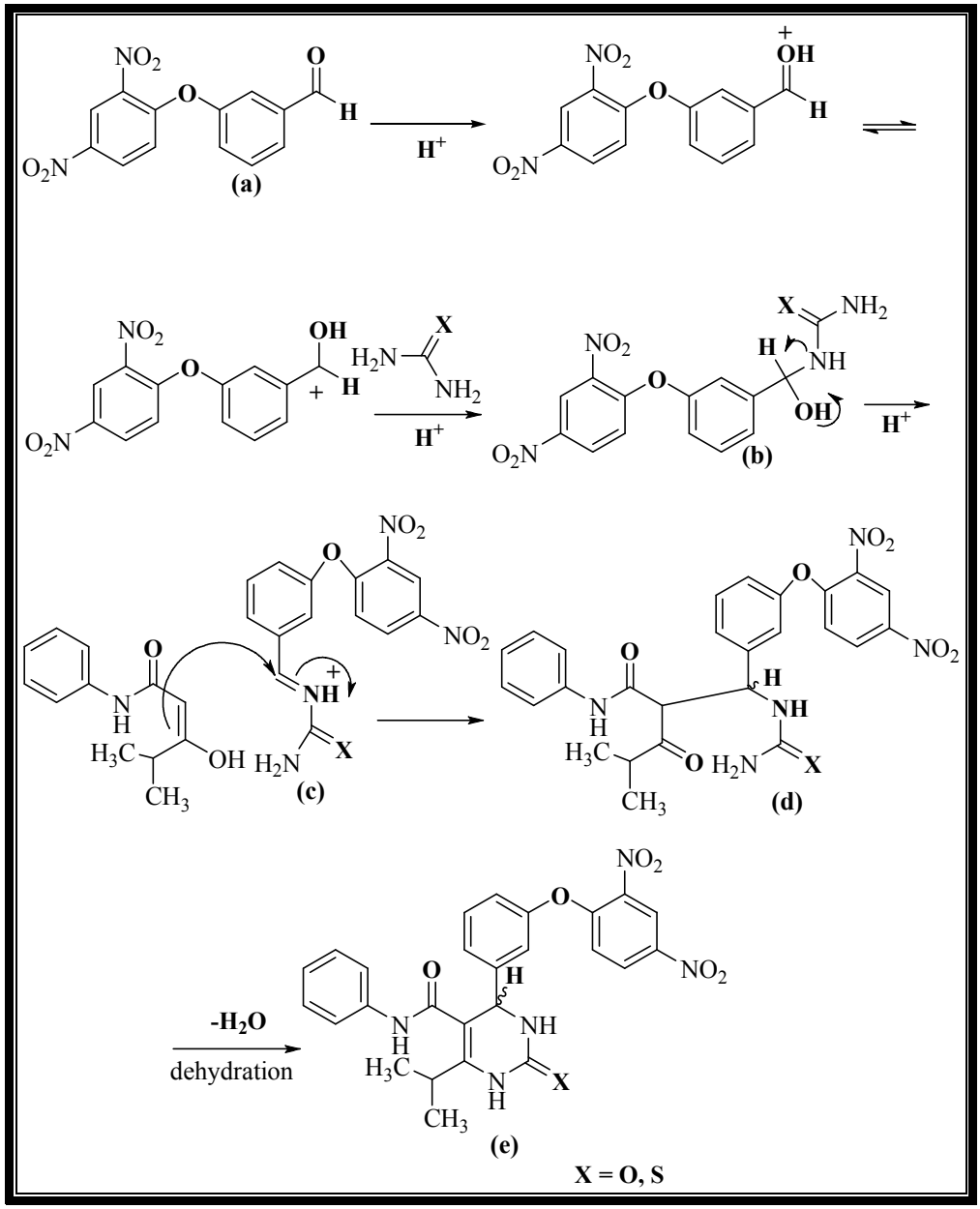
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| RHK-147 | 4-$CH_3$ | $C_{27}H_{25}N_5O_6S$ | 547 | 206 | 71 | 0.52 | 0.75 |
| RHK-148 | 4-F | $C_{26}H_{22}FN_5O_6S$ | 551 | 223 | 60 | 0.44 | 0.65 |
| RHK-149 | 2-Cl | $C_{26}H_{22}ClN_5O_6S$ | 567 | 241 | 70 | 0.43 | 0.63 |
| RHK-150 | H | $C_{26}H_{23}N_5O_6S$ | 533 | 250 | 81 | 0.50 | 0.70 |
| RHK-151 | 2-$OCH_3$ | $C_{28}H_{27}N_5O_8$ | 561 | 230 | 77 | 0.46 | 0.63 |
| RHK-152 | 3-Cl | $C_{27}H_{24}ClN_5O_7$ | 565 | 218 | 76 | 0.53 | 0.65 |
| RHK-153 | 2-F | $C_{27}H_{24}FN_5O_7$ | 549 | 216 | 68 | 0.47 | 0.55 |
| RHK-154 | 3-Cl 4-F | $C_{27}H_{23}ClFN_5O_7$ | 583 | 227 | 59 | 0.53 | 0.70 |
| RHK-155 | 4-$OCH_3$ | $C_{28}H_{27}N_5O_8$ | 561 | 229 | 64 | 0.58 | 0.65 |
| RHK-156 | 4-Cl | $C_{27}H_{24}ClN_5O_7$ | 565 | 218 | 61 | 0.52 | 0.62 |
| RHK-157 | 4-$CH_3$ | $C_{28}H_{27}N_5O_7$ | 545 | 220 | 62 | 0.53 | 0.70 |
| RHK-158 | 4-F | $C_{27}H_{24}FN_5O_7$ | 549 | 200 | 74 | 0.44 | 0.62 |
| RHK-159 | 2-Cl | $C_{27}H_{24}ClN_5O_7$ | 565 | 241 | 60 | 0.49 | 0.71 |
| RHK-160 | H | $C_{27}H_{25}N_5O_7$ | 531 | 249 | 70 | 0.42 | 0.63 |

TLC Solvent system $R_{f1}$:- Hexane : Ethyl acetate – 6:4,

$R_{f2}$:- Chloroform : methanol – 9:1.

### 3.8.2 Plausible Reaction Mechanism

The reaction mechanism of pyrimidine formation can be depicted as under:



### 3.8.3 Experimental

#### 3.8.3.1 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 potassium bromide (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 analyses of the all the synthesized compounds were carried out on elemental vario EL III carlo erba 1108 model and the results are in agreements with the structures assigned.

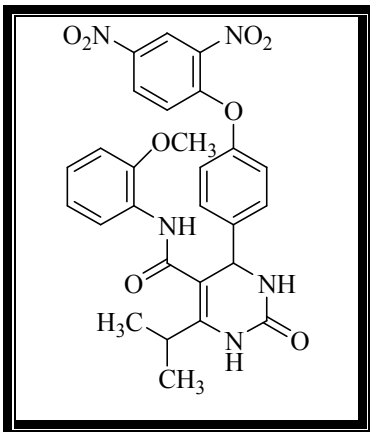
#### *3.8.3.2 Synthesis of N-(substituted phenyl)-4-methyl-3-oxopentanamide*

Synthesis of *N*-(substituted phenyl)-4-methyl-3-oxopentanamide were achieved using previously published methods [60].

#### *3.8.3.3 General procedure for the synthesis of 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-substitutedphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carbox-amide (RHK-131 to140)*

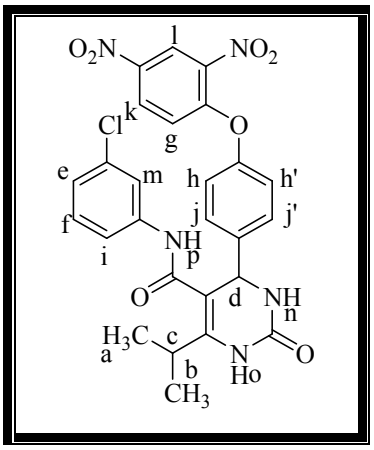
A mixture of *N*-(substituted phenyl)-4-methyl-3-oxopentanamide (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), urea (0.015 M) and catalytic amount of conc. hydrochloric acid in ethanol (30 ml) was heated under reflux condition for 8 to 10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

### *3.8.3.3.1 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-methoxyphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-131)*



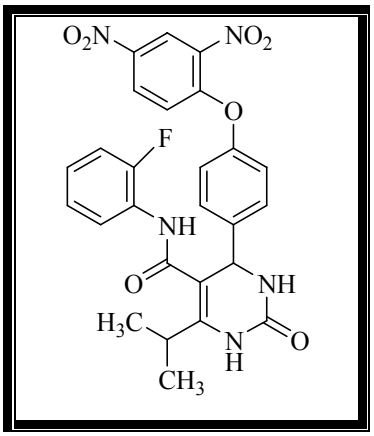
Yield: 69%; mp 223ºC; MS: *m/z* 547; Anal. Calcd. for $C_{27}H_{25}N_5O_8$: C, 59.23; H, 4.60; N, 12.79; O, 23.38; Found: C, 59.11; H, 4.51; N, 12.70; O, 23.31%; MS: *m/z* 547.

### *3.8.3.3.2 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-132)*



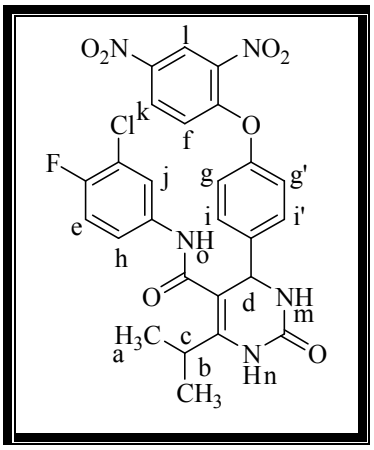
Yield: 65%; mp 219ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_7$: C, 56.58; H, 4.02; Cl, 6.42; N, 12.69; O, 20.29; Found: C, 56.45; H, 4.00; Cl, 6.36; N, 12.55; O, 20.12%; IR ($cm^{-1}$): 3425 (N-H stretching of amide), 3107 (C-H stretching of aromatic ring), 2951 (C-H asymmetrical stretching of $CH_3$ group), 2887 (C-H symmetrical stretching of $CH_3$ group), 1653 (C=O stretching of amide), 1589 (C=O stretching of cyclic) 1589 (N-H deformation of pyrimidine ring), 1529 (C=C stretching of aromatic ring), 1529 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1479 (C-H asymmetrical deformation of $CH_3$ group), 1417 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1305 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1287 (C-O-C stretching), 1278 (C-N stretching), 1068 (C-H in plane deformation of aromatic ring), 837 (para-substituted), 786 (C-H in out plane deformation of aromatic ring), 671 (C-Cl stretching); MS: *m/z* 551; $^1$H NMR (DMSO-*d6*) δ ppm: 1.48 (s, 3H, $H_a$), 1.58 (s, 1H, $H_b$), 3.87 (s, 1H, $H_c$), 4.82-4.83 (s, 1H, $H_d$), 7.13-7.15 (d, 1H, $H_e$, J = 6.80Hz), 7.15 (d, 1H, $H_f$), 7.21-7.22 (d, 2H, $H_g$, J = 4.80Hz), 7.27-7.30 (d, 2H, $H_{hh'}$, J = 8.80Hz), 7.35-7.39 (m, 1H, $H_i$), 7.44-7.47 (d, 2H, $H_{jj'}$, J = 8.80Hz), 7.48-7.51 (d, 1H, $H_k$, J = 9.20Hz), 7.83-7.84 (d, 1H, $H_l$, J = 4.00Hz), 7.91 (s, 1H, $H_m$), 8.47-8.50 (m, 1H, $H_n$, J = 12.00), 8.90 (d, 1H, $H_o$), 10.08 (s, 1H, $H_p$).

### *3.8.3.3.3 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-flurophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-133)*



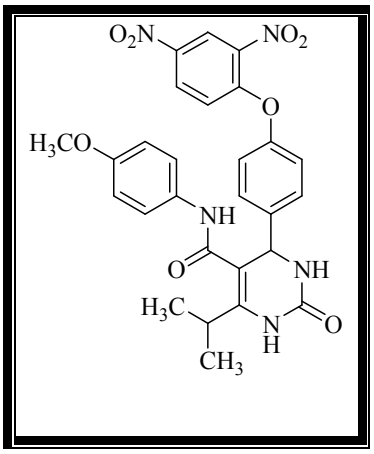
Yield: 70%; mp 210ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_7$: C, 58.32; H, 4.14; F, 3.55; N, 13.08; O, 20.92; Found: C, 58.16; H, 4.09; F, 3.35; N, 13.00; O, 20.85%; MS: *m/z* 535.

### *3.8.3.3.4 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chloro 4-flurophenyl)-1,2,3,4-tetra -hydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-134)*



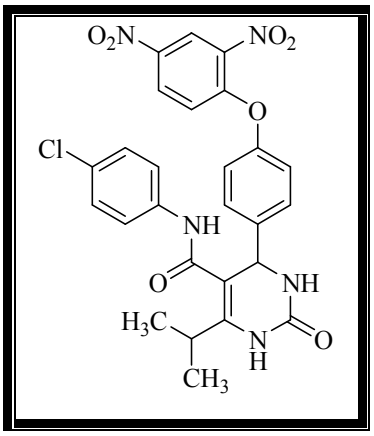
Yield: 75%; mp 207ºC; Anal. Calcd. For $C_{26}H_{21}ClFN_5O_7$: C, 54.79; H, 3.71; Cl, 6.22; F, 3.33; N, 12.29; O, 19.65; Found: C, 54.64; H, 3.62; Cl, 6.14; F, 3.24; N, 12.22; O, 19.51% IR ($cm^{-1}$): 3439(N-H stretching of amide), 3013 (C-H stretching of aromatic ring), 2985 (C-H asymmetrical stretching of $CH_3$ group), 2855 (C-H symmetrical stretching of $CH_3$ group), 1651 (C=O stretching of amide), 1597 (C=O stretching of cyclic) 1597 (N-H deformation of pyrimidine ring), 1529 (C=C stretching of aromatic ring), 1529 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1475 (C-H asymmetrical deformation of $CH_3$ group), 1406 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1274 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1274 (C-N stretching), 1134 (C-F stretching), 1064 (C-H in plane deformation of aromatic ring), 835 (para-substituted), 777 (C-H in out plane deformation of aromatic ring), 682 and 592 (C-Cl stretching); MS: *m/z* 569; $^1$H NMR (DMSO-*d6*) δ ppm: 1.47 (s, 3H, $H_a$), 1.58 (s, 1H, $H_b$), 3.85 (s, 1H, $H_c$), 4.83-4.84 (s, 1H, $H_d$), 7.09-7.11 (d, 1H, $H_e$, J = 9.60Hz), 7.20-7.21 (d, 1H, $H_f$ J = 4.80Hz), 7.27-7.29 (d, 2H, $H_{g\text{-}g'}$, J = 8.80Hz), 7.38-7.40 (d, 1H, $H_h$, J = 9.20Hz), 7.43-7.44 (d, 2H, $H_{ii'}$, J = 3.60Hz), 7.50-7.54 (m, 1H, $H_j$), 7.90 (s, 1H, $H_k$), 7.93-7.95 (m, 1H, $H_l$ J = 9.60Hz), 8.47-8.50 (m, 1H, $H_m$, J = 12.40), 8.90 (d, 1H, $H_n$), 10.09 (s, 1H, $H_o$).

***3.8.3.3.5 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-methoxyphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-135)***



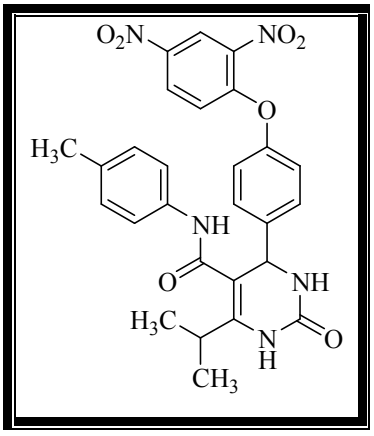
Yield: 71%; mp 198ºC; Anal. Calcd. for $C_{27}H_{25}N_5O_8$: C, 59.23; H, 4.60; N, 12.79; O, 23.38; Found: C, 59.13; H, 4.55; N, 12.69; O, 23.28%; MS: *m/z* 543.

***3.8.3.3.6 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-136)***



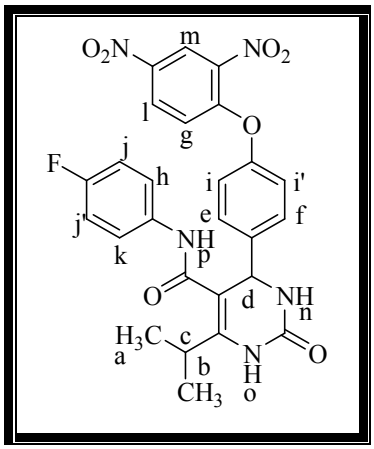
Yield: 72%; mp 220ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_7$: C, 56.58; H, 4.02; Cl, 6.42; N, 12.69; O, 20.29; Found: C, 56.50; H, 4.00; Cl, 6.34; N, 12.60; O, 20.20% MS: *m/z* 551.

***3.8.3.3.7 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-methylphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-137)***



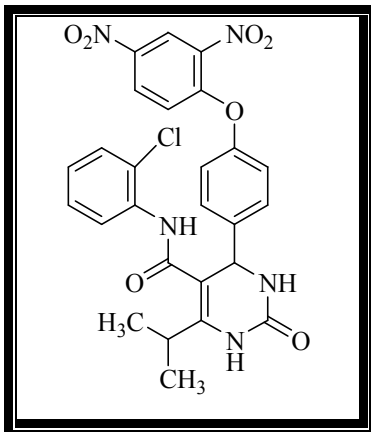
Yield: 69%; mp 200ºC; Anal. Calcd. for $C_{27}H_{25}N_5O_7$: C, 61.01; H, 4.74; N, 13.18; O, 21.07; Found: C, 59.31; H, 4.61; N, 13.08; O, 21.00%; MS: *m/z* 531.

***3.8.3.3.8 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-flurophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-138)***



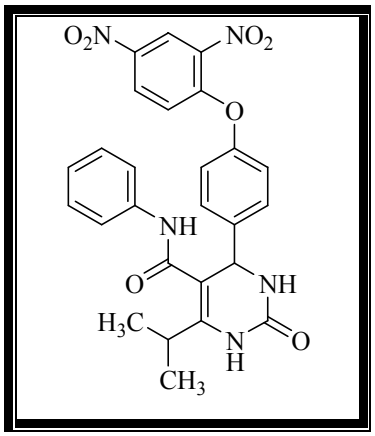
Yield: 59%; mp 199ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_7$: C, 58.32; H, 4.14; F, 3.55; N, 13.08; O, 20.92; Found: C, 58.24; H, 4.08; F, 3.45; N, 13.01; O, 20.85%; IR ($cm^{-1}$): 3313 (N-H stretching of amide), 3101 (C-H stretching of aromatic ring), 2944 (C-H asymmetrical stretching of $CH_3$ group), 2841 (C-H symmetrical stretching of $CH_3$ group), 1658 (C=O stretching of amide), 1602 (C=O stretching of cyclic) 1602 (N-H deformation of pyrimidine ring), 1535 (C=C stretching of aromatic ring), 1535 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1471 (C-H asymmetrical deformation of $CH_3$ group), 1404 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1346 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1269 (C-N stretching), 1205 (C-O-C stretching), 1151 (C-F stretching), 1051 (C-H in plane deformation of aromatic ring), 835 (para-substituted), 772 (C-H in out plane deformation of aromatic ring); MS: *m/z* 535; $^1$H NMR (DMSO-*d6*) δ ppm: 1.48 (s, 3H, $H_a$), 1.58 (s, 1H, $H_b$), 3.86 (s, 1H, $H_c$), 4.82-4.86 (s, 1H, $H_d$), 7.09-7.11 (d, 1H, $H_e$, J = 9.20Hz), 7.15-7.21 (m, 3H, $H_{fgh}$), 7.27-7.29 (d, 2H, $H_{i\text{-}i'}$, J = 8.40Hz), 7.43-7.44 (d, 2H, $H_{jj'}$, J = 8.80Hz), 7.61-7.65 (m, 2H, $H_{kl}$ J = 14.00Hz), 7.88 (s, 1H, $H_m$), 8.47-8.50 (d, 1H, $H_n$, J = 12.00), 8.90 (d, 1H, $H_o$ J = 2.8Hz), 9.93 (s, 1H, $H_p$).

***3.8.3.3.9 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-139)***



Yield: 72%; mp 195ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_7$: C, 56.58; H, 4.02; Cl, 6.42; N, 12.69; O, 20.29; Found: C, 56.46; H, 4.00; Cl, 6. 35; N, 12.56; O, 20.13%; MS: *m/z* 551.

***3.8.3.3.10 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(phenyl)-1,2,3,4-tetrahydro-6-iso-pro -pyl-2-oxopyrimidine-5-carboxamide (RHK-140)***

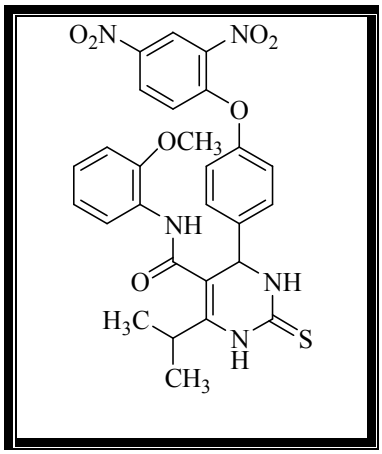


Yield: 74%; mp 232ºC; Anal. Calcd. for $C_{26}H_{23}N_5O_7$: C, 60.34; H, 4.48; N, 13.53; O, 21.64; Found: C, 60.24; H, 4.33; N, 13.42; O, 21.51%; MS: *m/z* 517.

***3.8.3.4 General procedure for the synthesis of 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-substitutedphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-thioxopyrimidine-5-carbox-amide (RHK-141 to150)***

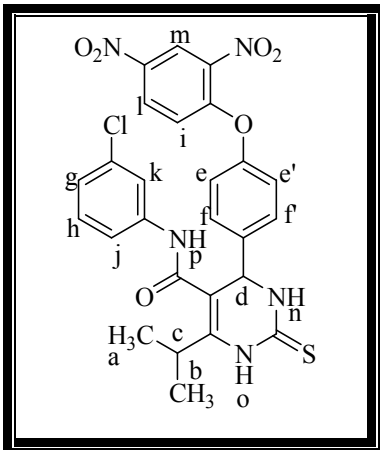
A mixture of *N*-(substituted phenyl)-4-methyl-3-oxopentanamide (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), thiourea (0.015 M) and catalytic amount of conc. acid in ethanol (30 ml) was heated under reflux condition for 11 to 12 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

***3.8.3.4.1 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-methoxyphenyl)-1,2,3,4 tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-141)***



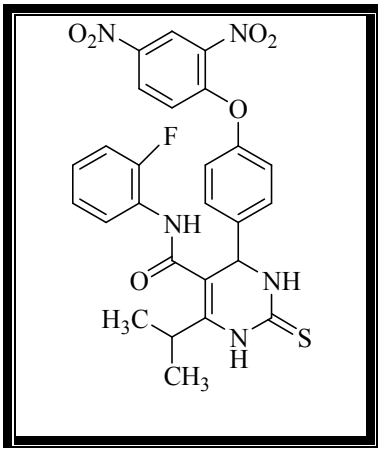
Yield: 66%; mp 241ºC; Anal. Calcd. for $C_{27}H_{25}N_5O_7S$: C, 57.54; H, 4.47; N, 12.43; O, 19.87; S, 5.69; Found: C, 57.44; H, 4.37; N, 12.33; O, 19.77; S, 5.57%; MS: *m/z* 563.

***3.8.3.4.2 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-142)***



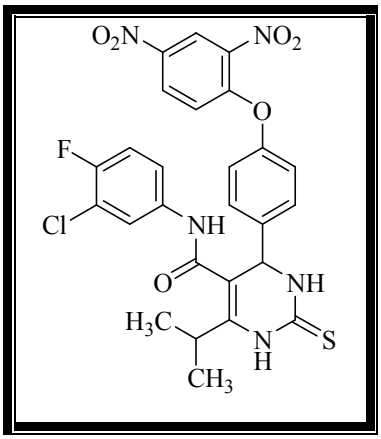

Yield: 59%; mp 232ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_6S$: C, 54.98; H, 3.90; Cl, 6.24;N, 12.33; O, 16.90; S, 5.65; Found: C, 54.87; H, 3.82; Cl, 6.12; N, 12.24; O, 16.81; S, 5.60%; IR ($cm^{-1}$): 3404 (N-H stretching of amide), 3095 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of $CH_3$ group), 2958(C-H symmetrical stretching of $CH_3$ group), 1676 (C=O stretching of amide), 1597 (N-H deformation of pyrimidine ring), 1518 (C=C stretching of aromatic ring), 1518 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1410 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of $CH_3$ group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1344 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1269 (C-N stretching), 1165 (C=S stretching of cyclic), 1064 (C-H in plane deformation of aromatic ring), 823 (para-substituted), 723 (C-H in out plane deformation of aromatic ring), 545 (C-Cl stretching); MS: *m/z* 567; $^1$H NMR (DMSO-*d6*) δ ppm: 1.49 (s, 3H, $H_a$), 1.67 (s, 1H, $H_b$), 3.92 (s, 1H, $H_c$), 4.92-4.93 (s, 1H, $H_d$), 7.16 (d, 2H, $H_{ee'}$), 7.29-7.31 (d, 2H, $H_{ff'}$, J = 8.00Hz), 7.35-7.39 (m, 3H, $H_{ghi}$), 7.48-7.50 (m, 1H, $H_j$, J = 8.40Hz), 7.81-7.82 (s, 1H, $H_k$), 8.43-8.50 (m, 1H, $H_l$, J = 12.00Hz), 8.90 (d, 1H, $H_m$, J = 2.90), 8.94-8.95 (m, 1H, $H_n$, J = 5.60Hz), 9.15 (s, 1H, $H_o$), 10.06 (s, 1H, $H_p$).

***3.8.3.4.3 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-fluorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-143)***



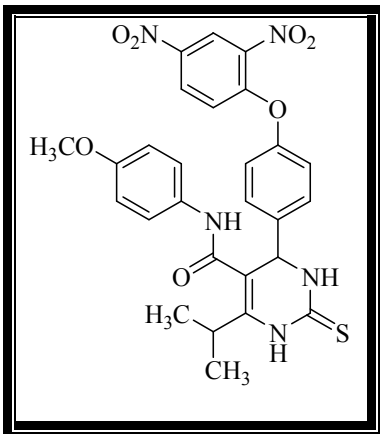

Yield: 63%; mp 201ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_6S$: C, 56.62; H, 4.02; F, 3.44; N, 12.70; O, 17.40; S, 5.81; Found: C, 56.54; H, 4.00; F, 3.34; N, 12.64; O, 17.34; S, 5.72%; MS: *m/z* 551.

***3.8.3.4.4 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(3-chloro 4-flurophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-144)***



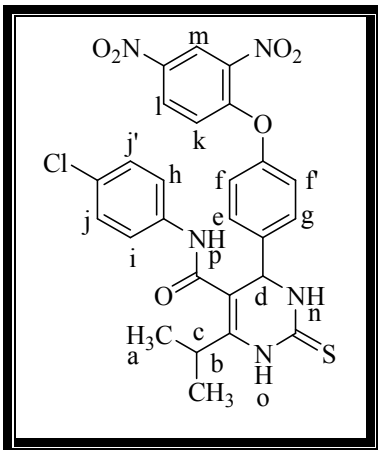
Yield: 75%; mp 222ºC; Anal. Calcd. for $C_{26}H_{21}ClFN_5O_6S$: C, 53.29; H, 3.61; Cl, 6.05; F, 3.24; N, 11.95; O, 16.38; S, 5.47; Found: C, 53.16; H, 3.49; Cl, 6.00; F, 3.12; N, 11.82; O, 16.24; S, 5.33%; MS: *m/z* 585.

***3.8.3.4.5 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-methoxyphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-145)***



Yield: 64%; mp 212 ºC; Anal. Calcd. for $C_{27}H_{25}N_5O_7S$: C, 57.54; H, 4.47; N, 12.43; O, 19.87; S, 5.69; Found: C, 57.45; H, 4.38; N, 12.33; O, 19.75; S, 5.54 %; MS: *m/z* 563.

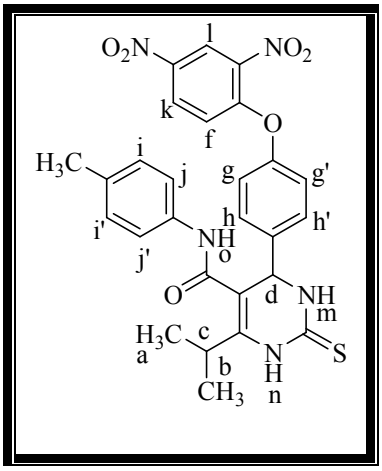
***3.8.3.4.6 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-146)***



Yield: 74%; mp 219ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_6S$: C, 54.98; H, 3.90; Cl, 6.24; N, 12.33; O, 16.90; S, 5.65; Found: C, 54.84; H, 3.80; Cl, 6.11; N, 12.21; O, 16.81; S, 5.57%; IR ($cm^{-1}$): 3412 (N-H stretching of amide), 3051 (C-H stretching of aromatic ring), 2951 (C-H asymmetrical stretching of $CH_3$ group), 2855 (C-H symmetrical stretching of $CH_3$ group), 1658 (C=O stretching of amide), 1597 (N-H deformation of pyrimidine ring), 1527 (C=C stretching of aromatic ring), 1527 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1483 (C-H asymmetrical deformation of $CH_3$ group), 1346 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C

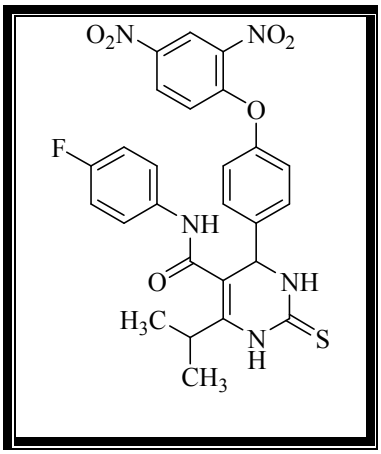
stretching vibration of pyrimidine ring), 1346 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1273 (C-N stretching), 1165 (C=S stretching of cyclic), 1060 (C-H in plane deformation of aromatic ring), 827 (para-substituted), 740 (C-H in out plane deformation of aromatic ring), 671 (C-Cl stretching); MS: *m/z* 567; $^1$H NMR (DMSO-*d6*) δ ppm: 1.49 (s, 3H, $H_a$), 1.66 (s, 3H, $H_b$) 3.91 (s, 1H, $H_c$), 4.92-4.93 (s, 1H, $H_d$), 7.08-7.11 (d, 1H, $H_e$, J = 9.20Hz), 7.28-7.30 (d, 2H, $H_{ff'}$, J = 8.80Hz), 7.36-7.41 (m, 3H, $H_{g\text{-}i'}$), 7.47-7.50 (d, 2H, $H_{jj'}$, J = 8.80Hz), 8.63-8.65 (d, 1H, $H_k$, J = 8.80Hz), 8.28-8.33 (m, 1H, $H_l$), 8.47-8.50 (m, 1H, $H_m$, J = 12.40Hz), 8.93-8.94 (m, 1H, $H_n$), 9.13 (s, 1H, $H_o$), 10.01 (s, 1H, $H_p$).

### *3.8.3.4.7 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-methylphenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamid (RHK-147)*



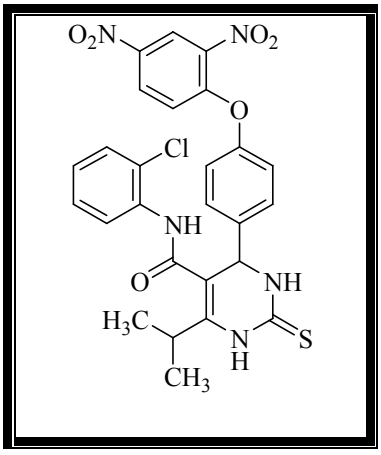

Yield: 71%; mp 206ºC; Anal. Calcd. For $C_{27}H_{25}N_5O_6S$: C, 59.22; H, 4.60; N, 12.79; O, 17.53; S, 5.86; Found: C, 59.11; H, 4.54; N, 12.67; O, 17.45; S, 5.77%; IR ($cm^{-1}$): 3421 (N-H stretching of amide), 3099 (C-H stretching of aromatic ring), 2949 (C-H asymmetrical stretching of $CH_3$ group), 2839 (C-H symmetrical stretching of $CH_3$ group), 1681 (C=O stretching of amide), 1597 (N-H deformation of pyrimidine ring), 1521 (C=C stretching of aromatic ring), 1521 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1464 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of $CH_3$ group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1344 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1269 (C-N stretching), 1063 (C-H in plane deformation of aromatic ring), 835 (para-substituted), 740 (C-H in out plane deformation of aromatic ring); MS: *m/z* 547; $^1$H NMR (DMSO-*d6*) δ ppm: 1.07 (s, 3H, $H_a$), 1.50 (s, 3H, $H_b$), 2.26 (s, 3H, $H_c$), 3.90 (s, 1H, $H_d$), 4.90-4.91 (s, 1H, $H_e$), 7.08-7.10 (d, 1H, $H_f$, J = 9.20Hz), 7.12-7.15 (d, 2H, $H_{gg'}$, J = 8.40Hz), 7.28-7.30 (d, 2H, $H_{hh'}$, J = 8.80Hz), 7.47-7.49 (d, 2H, $H_{jj'}$, J = 8.40Hz), 8.47-8.50 (d, 1H, $H_k$, J = 12.00Hz), 8.90 (d, 1H, $H_l$, J = 3.20Hz), 8.93-8.94 (d, 1H, $H_m$, J = 5.20Hz), 9.10 (s, 1H, $H_n$), 9.75 (s, 1H, $H_o$).

***3.8.3.4.8 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-flurophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-148)***



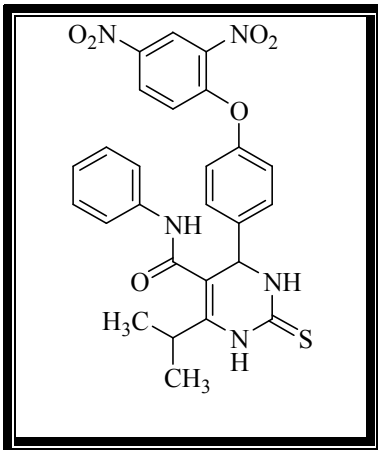
Yield: 60%; mp 223ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_6S$: C, 56.62; H, 4.02; F, 3.44; N, 12.70; O, 17.40; S, 5.81; Found: C, 56.46; H, 3.89; F, 3.31; N, 12.64; O, 17.34; S, 5.74%; MS: *m/z* 551.

***3.8.3.4.9 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-isopropyl-2-oxopyrimidine-5-carboxamide (RHK-149)***



Yield: 76%; mp 241ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_6S$: C, 54.98; H, 3.90; Cl, 6.24; N, 12.33; O, 16.90; S, 5.65; Found: C, 54.84; H, 3.82; Cl, 6.12; N, 12.21; O, 16.81; S, 5.55%; MS: *m/z* 567.

***3.8.3.4.10 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(phenyl)-1,2,3,4-tetrahydro-6-iso-pro -pyl-2-oxopyrimidine-5-carboxamide (RHK-150)***

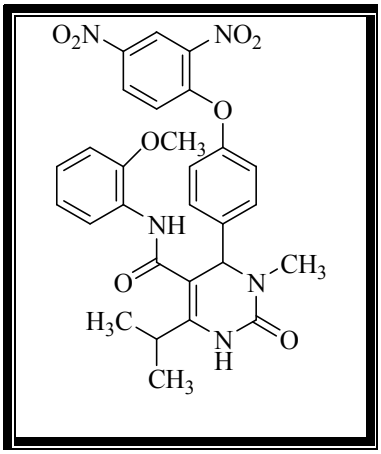


Yield: 81%; mp 250ºC; Anal. Calcd. for $C_{26}H_{23}N_5O_6S$: C, 58.53; H, 4.34; N, 13.13; O, 17.99; S, 6.01; Found: C, 58.46; H, 4.12; N, 13.00; O, 17.85; S, 5.89%; MS: *m/z* 533.

### *3.8.3.5 General procedure for the synthesis of 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(substitutedphenyl)-1,2,3,6-tetrahydro-4-isopropyl-1-methyl-2-oxopyrimidine-5-carboxamide (RHK-151 to160)*

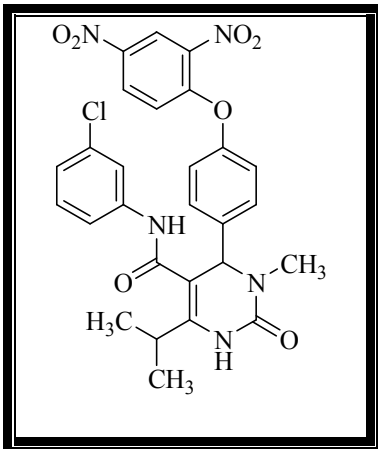
A mixture of *N*-(substitutedphenyl)-4-methyl-3-oxopentanamide (0.01 M), 4-(2,4-dinitrophenoxy)benzaldehydes (0.01 M), N-methyl urea (0.015 M) and catalytic amount of conc. acid in ethanol (30 ml) was heated under reflux condition for 12 to 13 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

#### *3.8.3.5.1 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(2-methoxyphenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-151)*



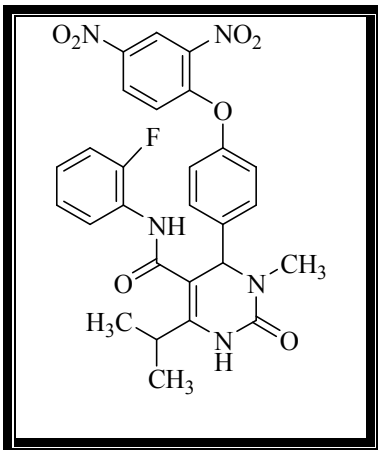

Yield: 77%; mp 230ºC; Anal. Calcd. For $C_{28}H_{27}ClN_5O_8$: C, 59.89; H, 4.85; N, 12.47; O, 22.79; Found: C, 59.78; H, 4.74; N, 12.35; O, 22.64 %; MS: *m/z* 561.

#### *3.8.3.5.2 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(3-chlorophenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-152)*



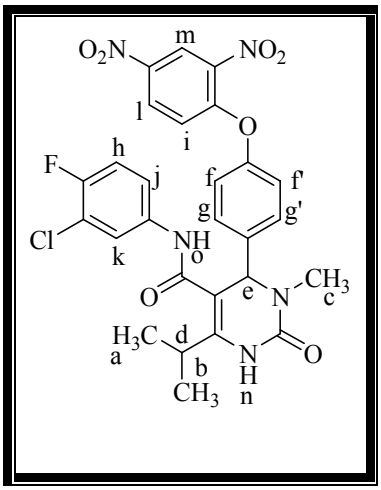

Yield: 76%; mp 218ºC; Anal. Calcd. for $C_{27}H_{24}ClN_5O_7$: C, 57.30; H, 4.27; Cl, 6.26; N, 12.37; O, 19.79; Found: , C, 57.21; H, 4.19; Cl, 6. 13; N, 12.28; O, 19.67 %; MS: *m/z* 565.

### *3.8.3.5.3 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-flurophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-153)*



Yield: 68%; mp 216ºC; Anal. Calcd. for $C_{26}H_{24}FN_5O_7$: C, 59.01; H, 4.40; F, 3.46; N, 12.74; O, 20.38; Found: C, 59.00; H, 4.31; F, 3.35; N, 12.67; O, 20.23 %; MS: *m/z* 549.

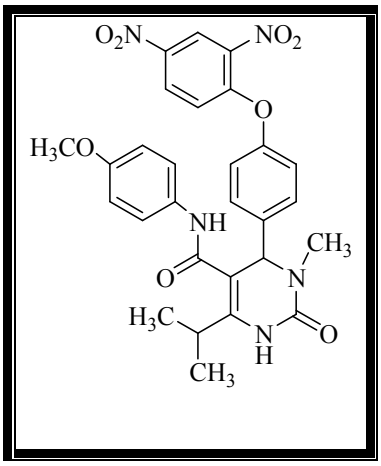
### *3.8.3.5.4 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-chloro-3-fluorophenyl)-1,2,3,6-tetrahydro1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-154)*



Yield: 59%; mp 227ºC; Anal. Calcd. for $C_{27}H_{23}ClFN_5O_7$: C, 55.53; H, 3.97; Cl, 6.07;F, 3.25; N, 11.99; O, 19.18; Found: C, 55.46; H, 3.88; Cl, 6.00;F, 3.13; N, 1186; O, 19.03 %; IR ($cm^{-1}$): 3475 (N-H stretching of amide), 3070 (C-H stretching of aromatic ring), 2959 (C-H asymmetrical stretching of $CH_3$ group), 2874 (C-H symmetrical stretching of $CH_3$ group), 1653 (C=O stretching of amide), 1600 (C=O stretching of cyclic) 1541 (N-H deformation of pyrimidine ring), 1541 (C=C stretching of aromatic ring), 1491 (C-H asymmetrical deformation of $CH_3$ group), 1491 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1396 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1346 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1276 (C-N stretching), 1087 (C-F stretching), 1087 (C-H in plane deformation of aromatic ring), 825 (para-substituted), 746 (C-H in out plane deformation of aromatic ring), 692 and 532 (C-Cl stretching); MS: *m/z* 583; $^1$H NMR (DMSO-*d6*) δ ppm: 1.26 (s, 3H, $H_a$), 1.61 (s, 3H, $H_b$), 2.95-2.96 (s, 3H, $H_c$), 3.75 (s, 1H, $H_d$), 4.97 (s, 1H, $H_e$), 7.03-7.05 (d, 2H, $H_{ff'}$, J = 9.20Hz), 7.23-7.25 (d, 2H, $H_{gg'}$, J = 8.80Hz), 7.38-7.42 (m, 3H, $H_{h-j}$), 7.52-7.53 (m, 1H, $H_k$, J = 6.40Hz), 8.91-8.92 (m,

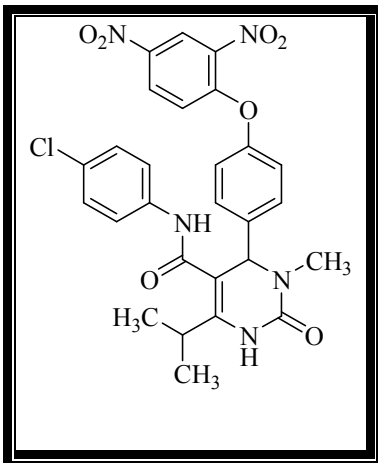
1H, $H_l$, J = 6.80Hz), 8.50-8.53 (d, 1H, $H_m$, J = 11.60Hz), 8.90 (s, 1H, $H_n$), 10.00 (s, 1H, $H_o$).

***3.8.3.5.5 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(4-methoxyphenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-155)***



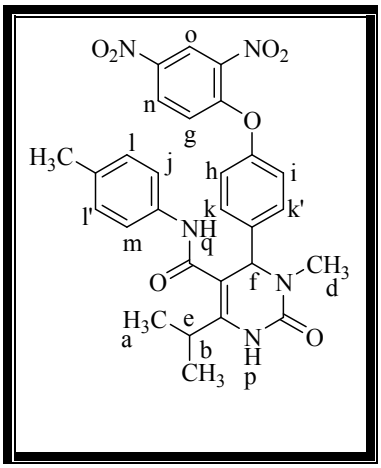
Yield: 64%; mp 229ºC; Anal. Calcd. for $C_{28}H_{27}N_5O_8$: C, 59.89; H, 4.85; N, 12.47; O, 22.79; Found: C, 59.79; H, 4.75; N, 12.34; O, 22.64 %; MS: *m/z* 561; MS: *m/z* 561

***3.8.3.5.6 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-N-(4-chlorophenyl)-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-156)***



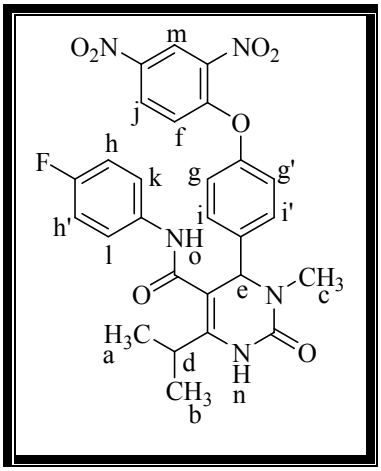
Yield: 61%; mp 218ºC; Anal. Calcd. for $C_{27}H_{24}ClN_5O_7$: C, 57.30; H, 4.27; Cl, 6.26; N, 12.37; O, 19.79; Found: C, 57.24; H, 4.15; Cl, 6.13; N, 12.23; O, 19.68 %; MS: *m/z* 565.

### *3.8.3.5.7 6-(4-(2,4-dinitrophenoxy)phenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxo-N-p-tolylpyrimidine-5-carboxamide (RHK-157)*



Yield: 62%; mp 220ºC; Anal. Calcd. for $C_{28}H_{27}N_5O_7$: C, 61.64; H, 4.99; N, 12.84; O, 20.53; Found: C, 61.52s; H, 4.87; N, 12.74; O, 20.44 %; IR ($cm^{-1}$): 3471 (N-H stretching of amide), 3057 (C-H stretching of aromatic ring), 2980 (C-H asymmetrical stretching of $CH_3$ group), 2852 (C-H symmetrical stretching of $CH_3$ group), 1716 (C=O stretching of amide), 1654 (C=O stretching of cyclic) 1600 (N-H deformation of pyrimidine ring), 1537 (C=C stretching of aromatic ring), 1537 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1496 (C-H asymmetrical deformation of $CH_3$ group), 1396 (C-H symmetrical deformation of $CH_3$ group), 1346 (C-N-C stretching vibration of pyrimidine ring), 1346 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1274 (C-N stretching), 1066 (C-H in plane deformation of aromatic ring), 829 (para-substituted), 742 (C-H in out plane deformation of aromatic ring); MS: *m/z* 545; $^1$H NMR (DMSO-*d6*) δ ppm: 1.48 (s, 3H, $H_a$), 1.58 (s, 3H, $H_b$), 2.72 (s, 3H, $H_c$) 3.09 (s, 1H, $H_d$), 3.73 (s, 1H, $H_e$), 5.27-5.28 (s, 1H, $H_f$), 6.86-6.89 (m, 1H, $H_g$), 6.99-7.01 (d, 1H, $H_h$, J = 8.00Hz), 7.06-7.08 (d, 1H, $H_i$, J = 7.20Hz), 7.10-7.14 (m, 1H, $H_j$, J = 14.80Hz), 7.27-7.29 (d, 2H, $H_{kk'}$, J = 8.40Hz), 7.46-7.48 (d, 2H, $H_{ll'}$, J = 8.80Hz), 7.67-7.69 (d, 1H, $H_m$, J = 7.60Hz), 7.82-7.83 (d, 1H, $H_n$, J = 3.20Hz), 8.44-8.47 (m, 1H, $H_o$, J = 12.00Hz), 8.71 (s, 1H, $H_p$), 8.89-8.90 (s, 1H, $H_q$).

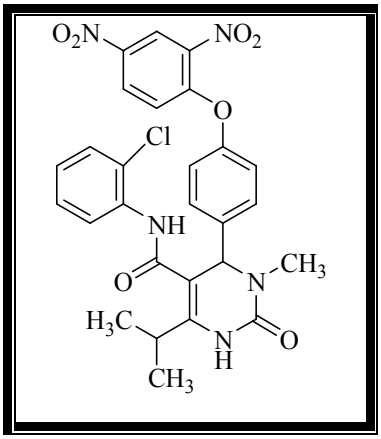
### *3.8.3.5.8 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-158)*



Yield: 74%; mp 200ºC; Anal. Calcd. for $C_{27}H_{24}FN_5O_7$: C, 59.01; H, 4.40; F, 3.46; N, 12.74; O, 20.38; Found: C, 58.79; H, 4.34; F, 3.33; N, 12.63; O, 20.24 %; IR ($cm^{-1}$): 3362 (N-H stretching of amide), 3103 (C-H stretching of aromatic ring), 2933 (C-H asymmetrical stretching of $CH_3$ group), 2838 (C-H symmetrical stretching of $CH_3$ group), 1705 (C=O stretching of amide), 1662 (C=O

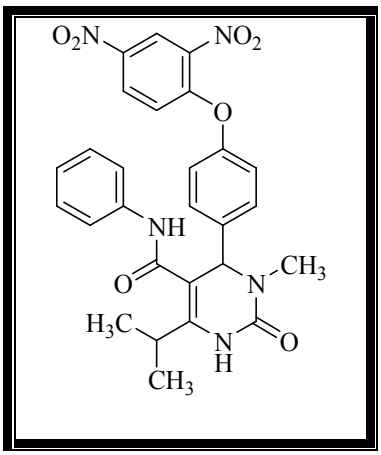
stretching of cyclic) 1593 (N-H deformation of pyrimidine ring), 1527 (C=C stretching of aromatic ring), 1527 (C-$NO_2$ asymmetrical deformation of $NO_2$ group), 1477 (C-H asymmetrical deformation of $CH_3$ group), 1429 (C-H symmetrical deformation of $CH_3$ group), 1342 (C-N-C stretching vibration of pyrimidine ring), 1342 (C-$NO_2$ symmetrical deformation of $NO_2$ group), 1244 (C-N stretching), 1099 (C-F stretching), 1070 (C-H in plane deformation of aromatic ring), 839 (para-substituted), 786 (C-H in out plane deformation of aromatic ring); MS: *m/z* 549; $^1$H NMR (DMSO-*d6*) δ ppm: 1.21 (s, 3H, $H_a$), 1.55 (s, 3H, $H_b$), 3.06 (s, 3H, $H_c$), 5.31-5.32 (s, 1H, $H_d$), 7.08-7.01 (d, 1H, $H_e$), 7.23-7.25 (d, 2H, $H_{ff'}$, J = 8.40Hz), 7.34-7.36 (d, 2H, $H_{gg'}$, J = 9.60Hz), 7.37-7.39 (d, 2H, $H_{hh'}$, J = 9.20Hz), 7.47-7.51 (m, 1H, $H_i$, J = 16.00Hz), 7.88-7.94 (m, 2H, $H_{jk}$), 8.43-8.46 (m, 1H, $H_l$, J = 12.80Hz), 8.89-8.90 (d, 1H, $H_m$, J = 2.40Hz), 10.06 (s, 1H, $H_n$).

### *3.8.3.5.9 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-159)*




Yield: 60%; mp 241ºC; Anal. Calcd. for $C_{27}H_{24}ClN_5O_7$: C, 57.30; H, 4.27; Cl, 6.26; N, 12.37; O, 19.70; Found: C, 57.20; H, 4.15; Cl, 6.13; N, 12.23; O, 19.64 %; MS: *m/z* 565

### *3.8.3.5.10 6-(4-(2,4-dinitrophenoxy)phenyl)-N-(phenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (RHK-160)*



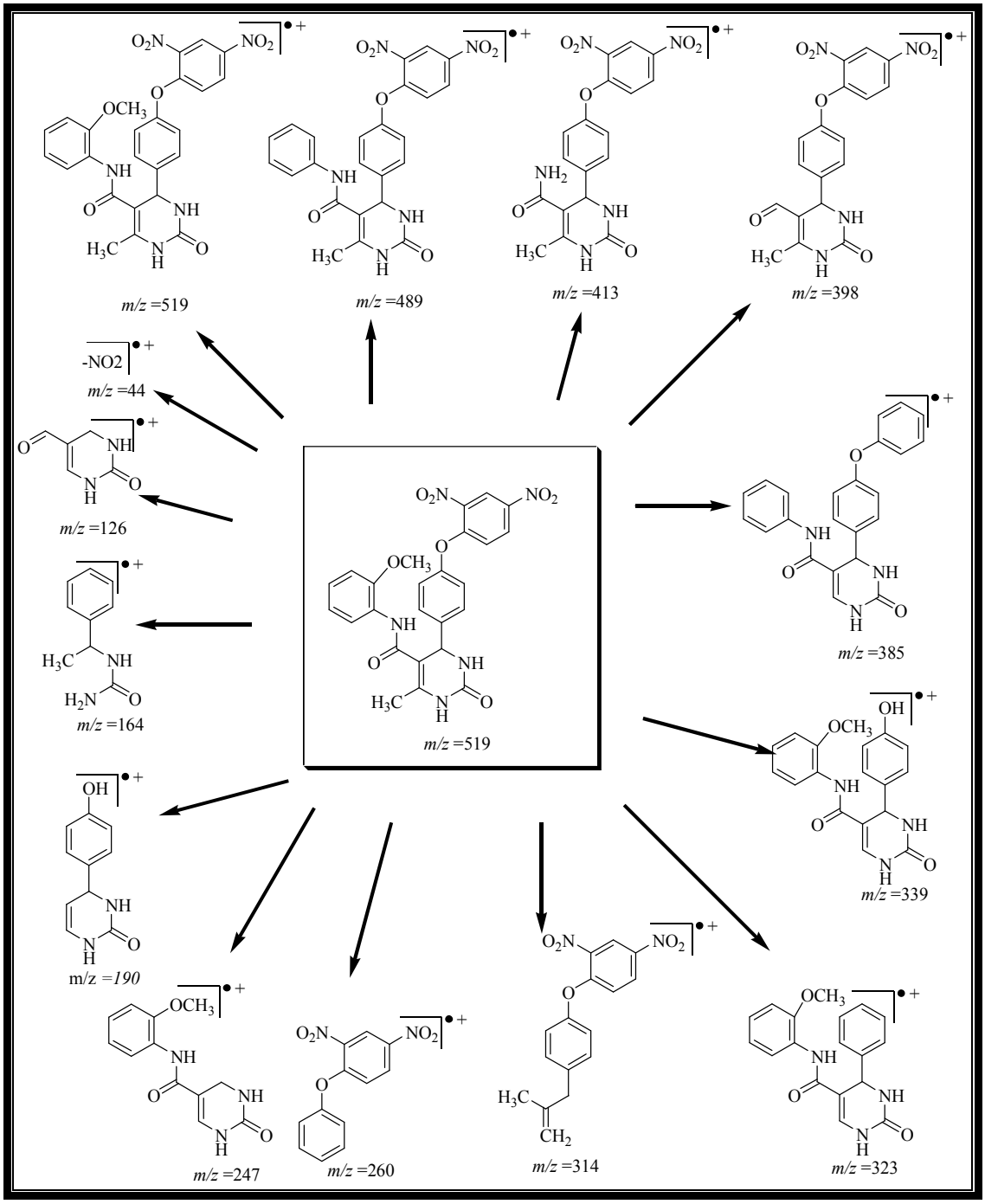

Yield: 70%; mp 249ºC; Anal. Calcd. for $C_{27}H_{25}N_5O_7$: C, 61.01; H, 4.74; N, 13.18; O, 21.07; Found: C, 59.89; H, 4.65; N, 13.02; O, 21.00 %; MS: *m/z* 531.

## 3.9 Spectral discussion

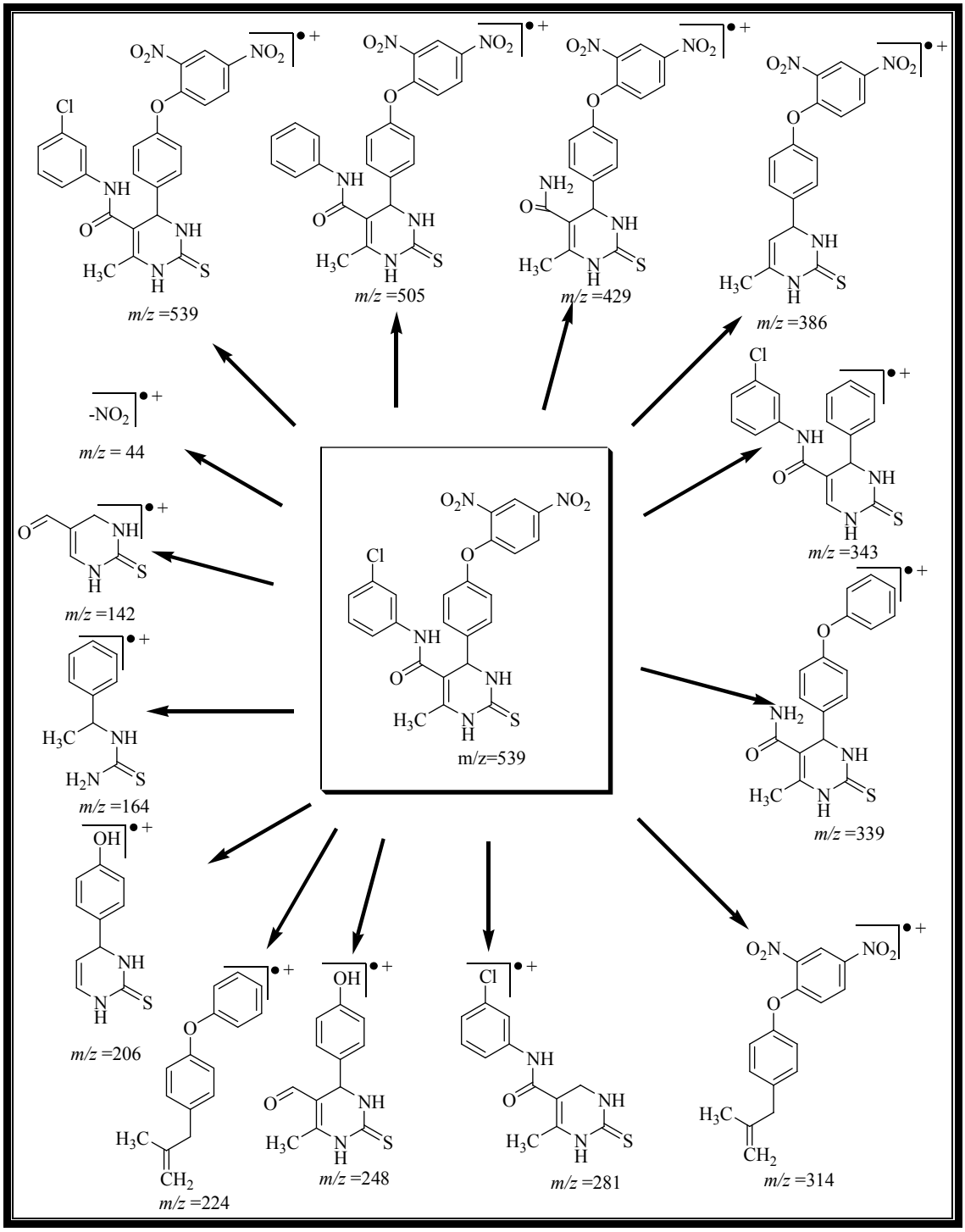
### 3.9.1 Mass spectral study

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.

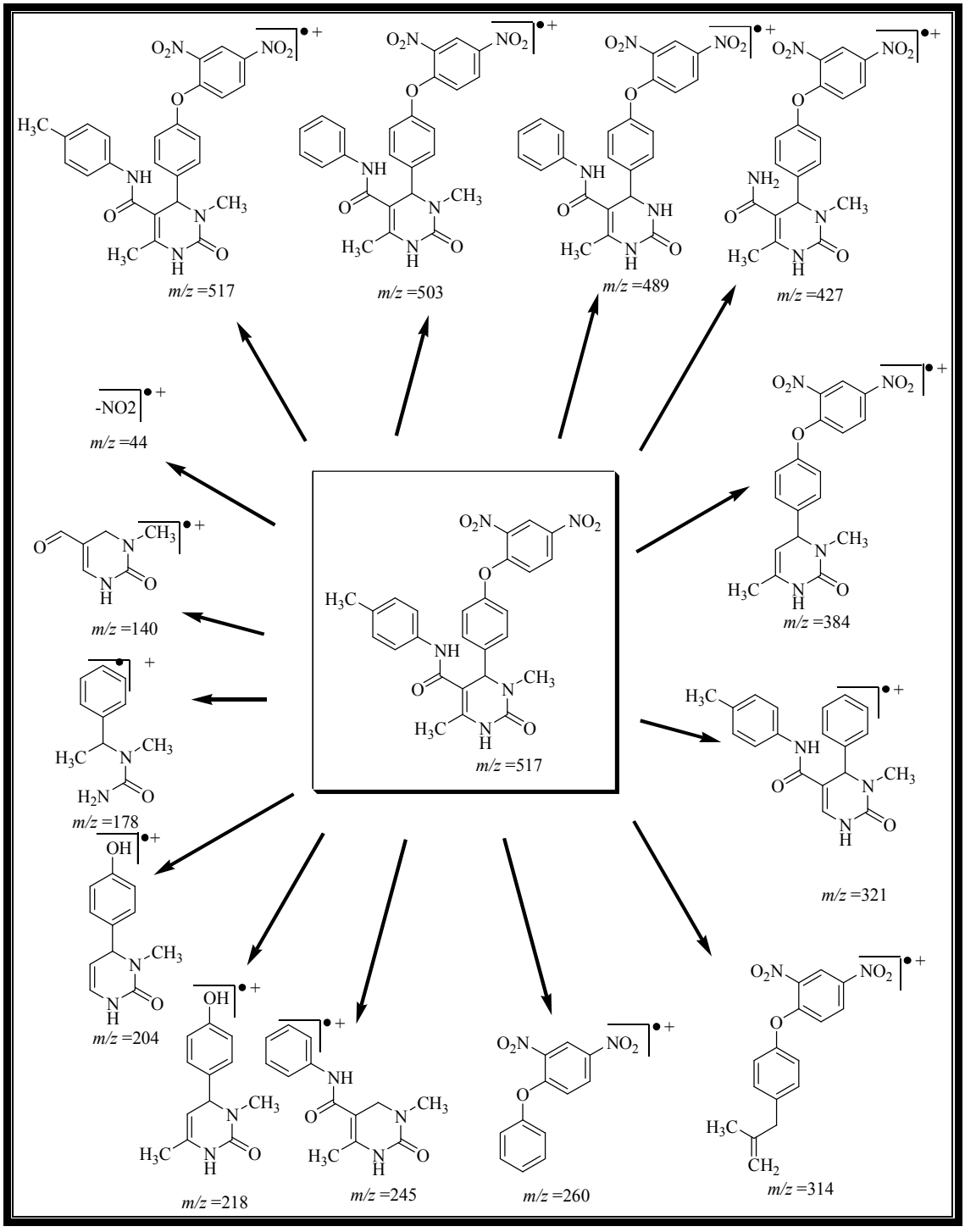
### *3.9.1.1 Mass fragmentation pattern for RHK-101*



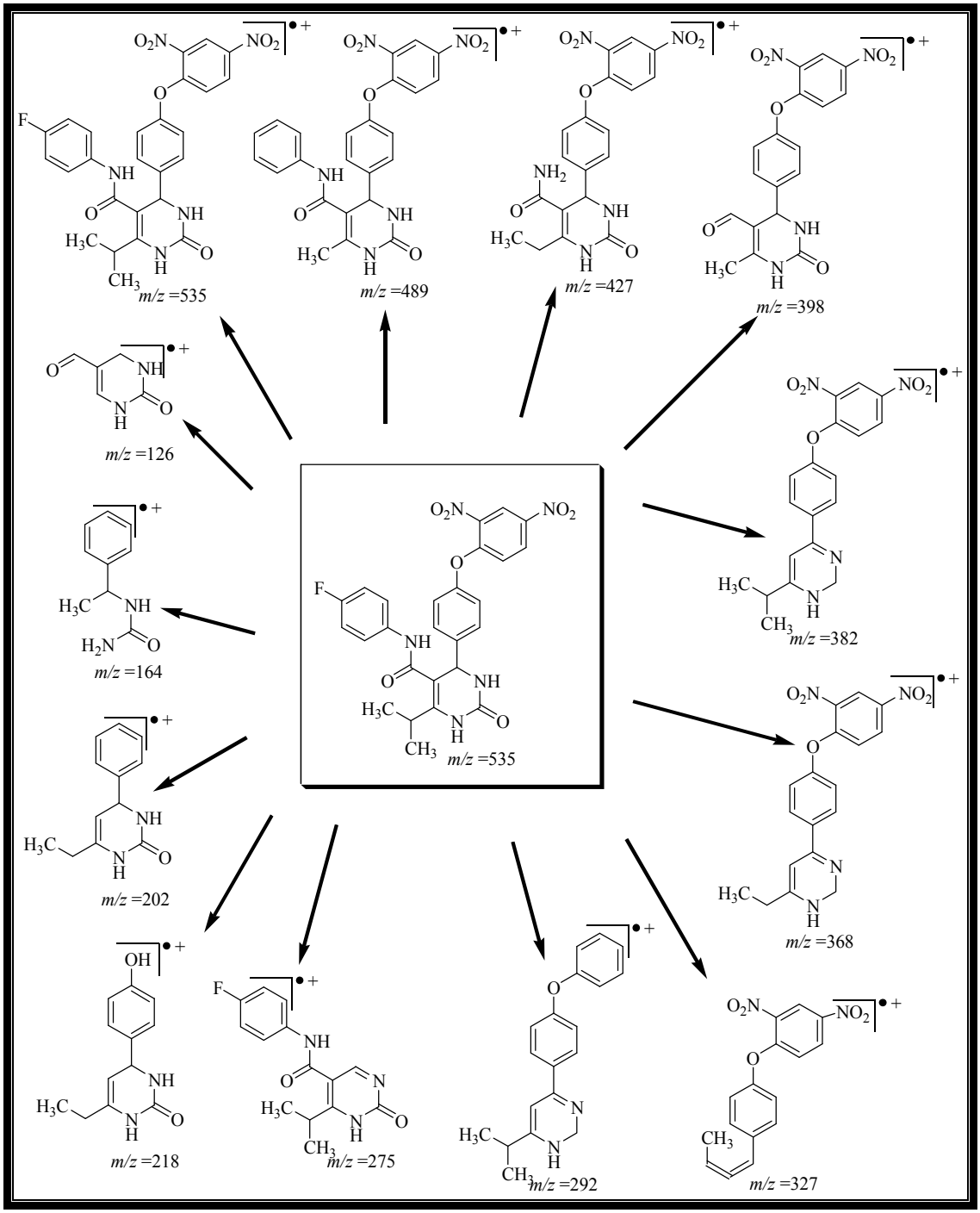
### *3.9.1.2 Mass fragmentation pattern for RHK-112*



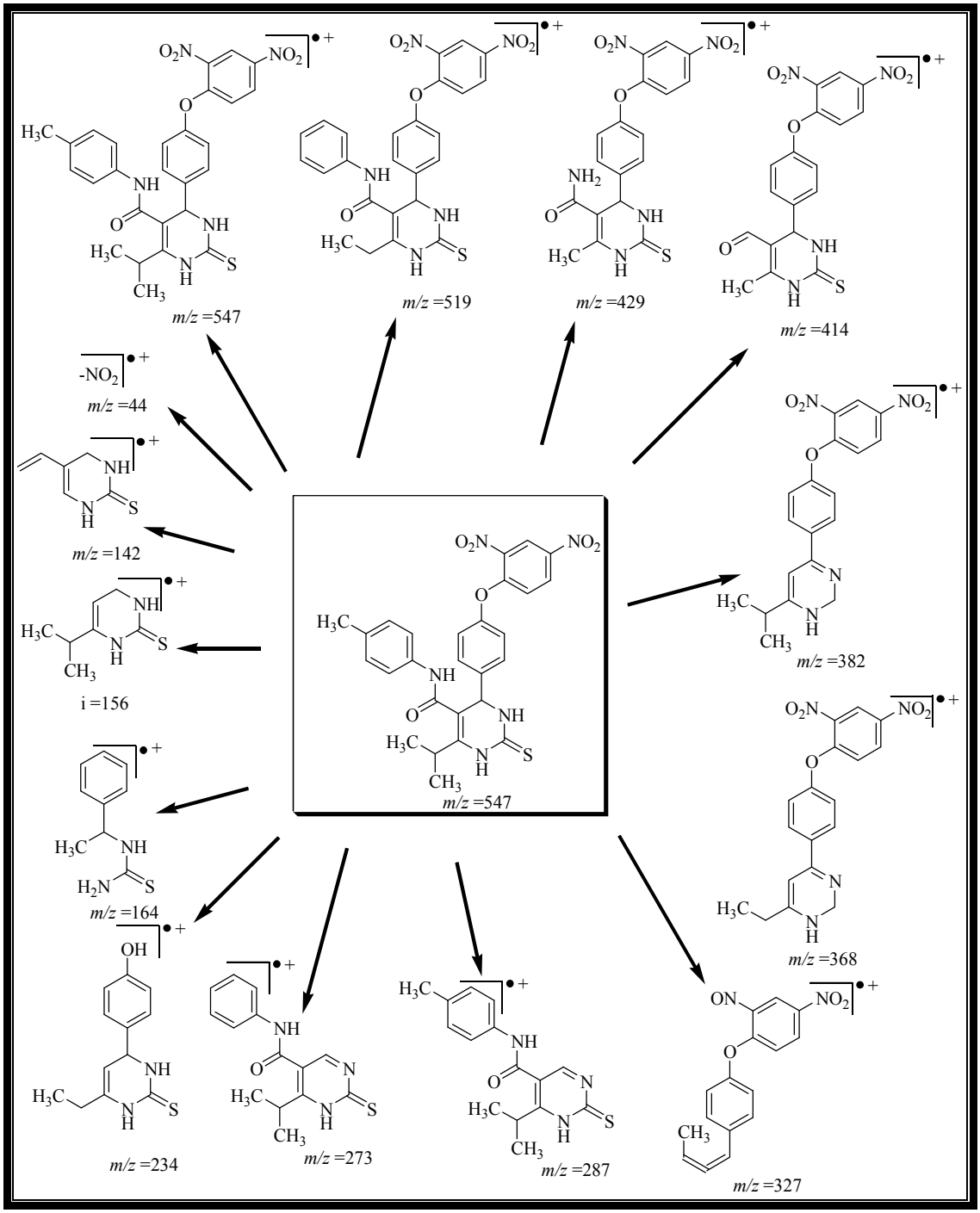
### *3.9.1.3 Mass fragmentation pattern for RHK-127*



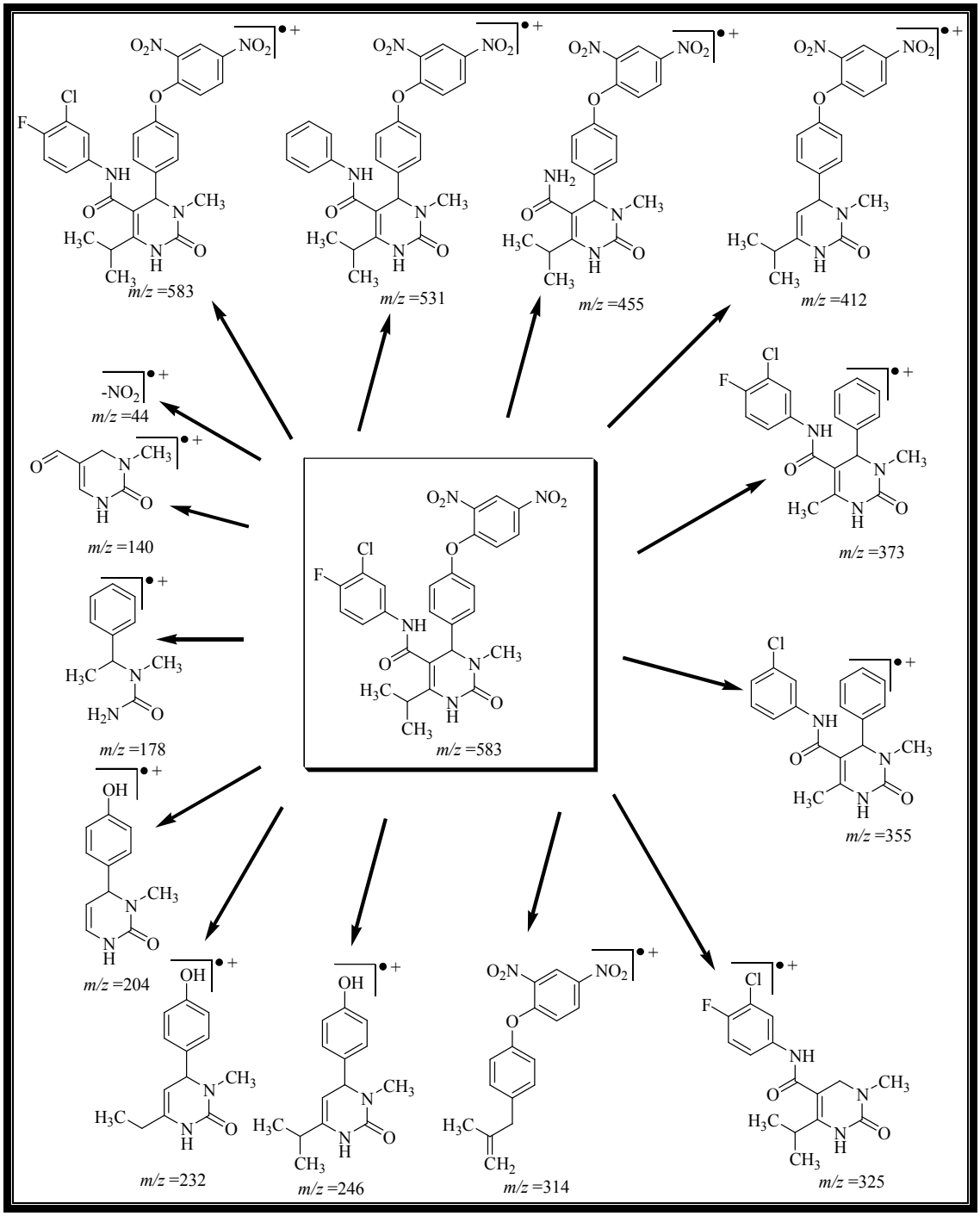
### 3.9.1.4 *Mass fragmentation pattern for RHK-138*



### *3.9.1.5 Mass fragmentation pattern for RHK-147*



### *3.9.1.6 Mass fragmentation pattern for RHK-154*



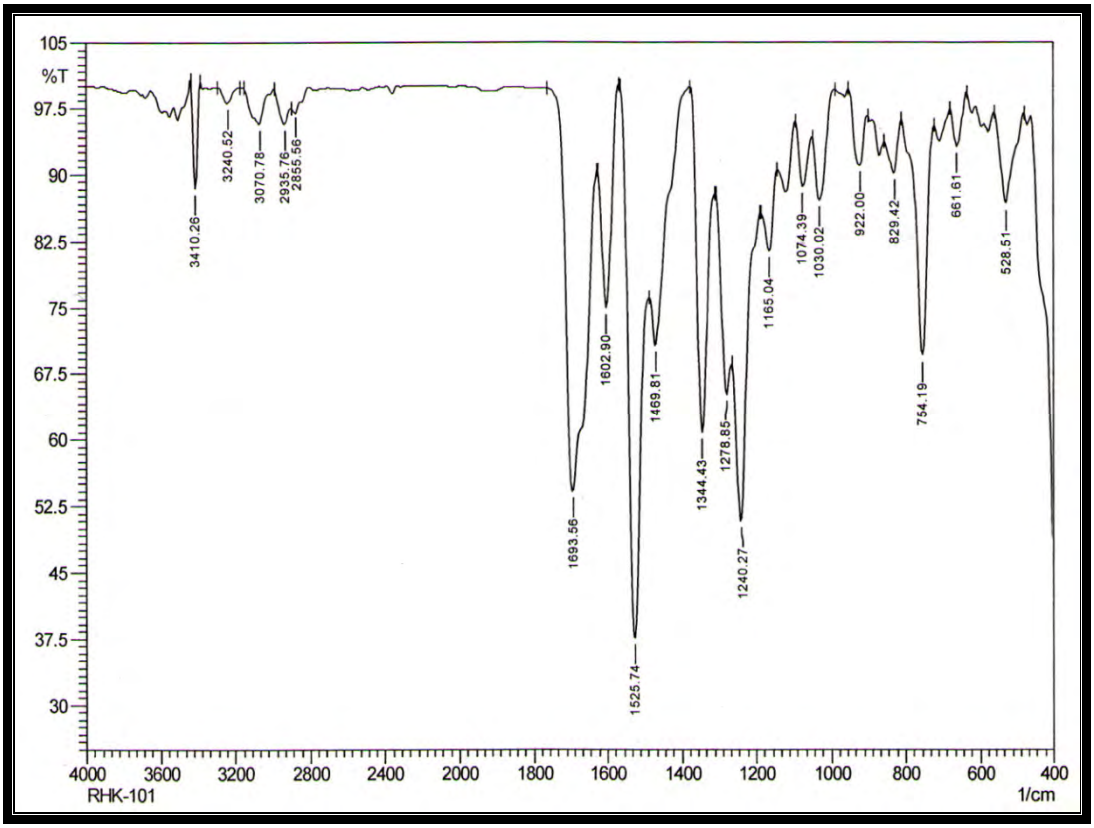
### 3.9.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using potassium bromide (KBr) pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For tetrahydropyrimidines **RHK-101 to 160**, confirmatory bands for secondary amine and amidic carbonyl groups were observed at 3450-3200 $cm^{-1}$ and 1715-1600 $cm^{-1}$ respectively. Another characteristic C-N-C (stretching vibration of pyrimidine ring) was observed at 1360-1300 $cm^{-1}$, which suggested formation of desired products **RHK- 101 to 160**.

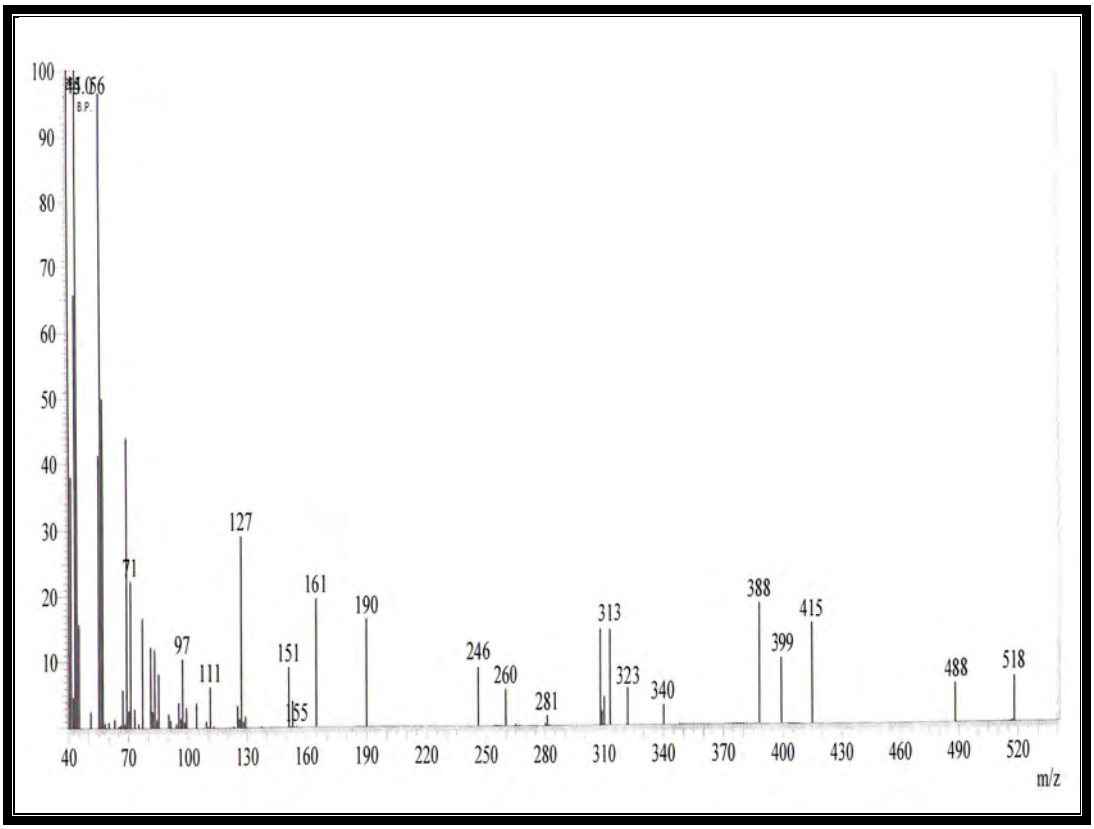
### 3.9.3 $^{1}$H NMR spectral study

$^{1}$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 tetrahydropyrimidines **RHK- 101 to 160** on the basis of following signals: a singlet for the methane proton of pyrimidine ring at 5.90-4.30 δ ppm, and singlets for N-H of primidine at 7.80-9.60 δ ppm and amide group protons at 9.10-10.32 δ ppm, respectively. The aromatic ring protons and J value were found to be in accordance with substitution pattern on phenyl ring.

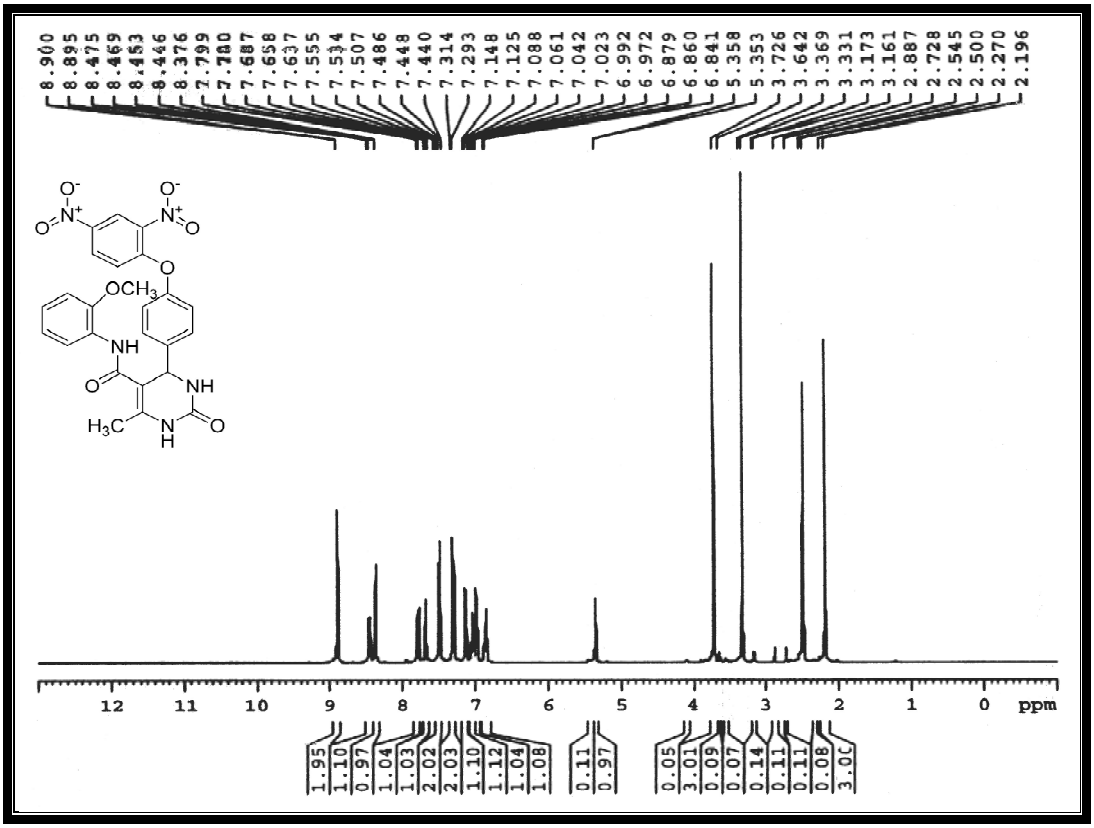
## *IR spectrum of RHK - 101*



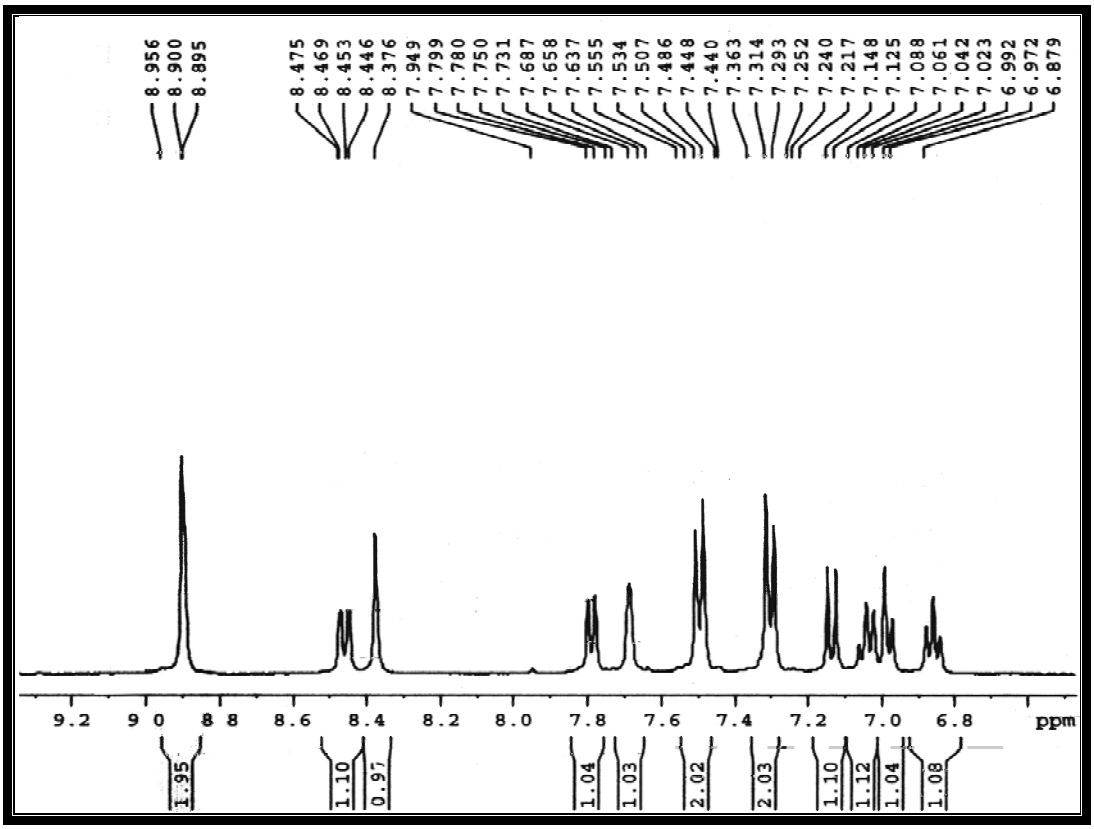

## *Mass spectrum of RHK - 101*



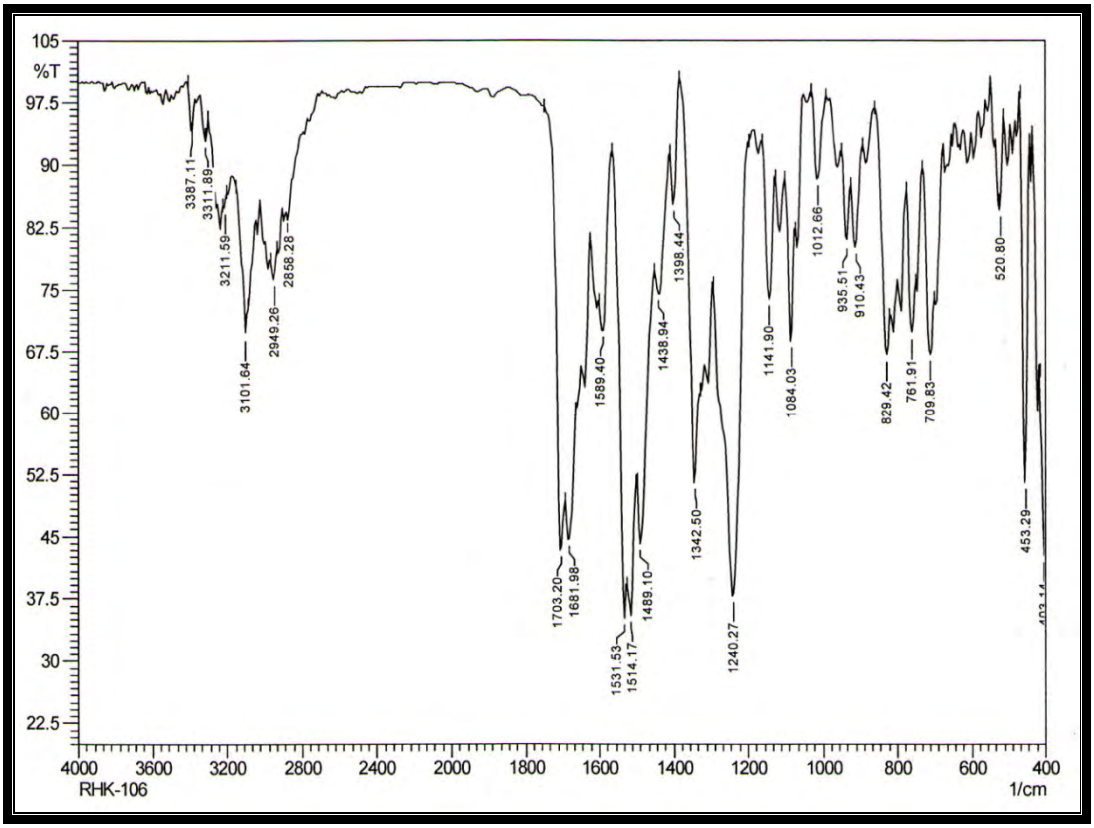
## *$^1$H NMR spectrum of RHK - 101*



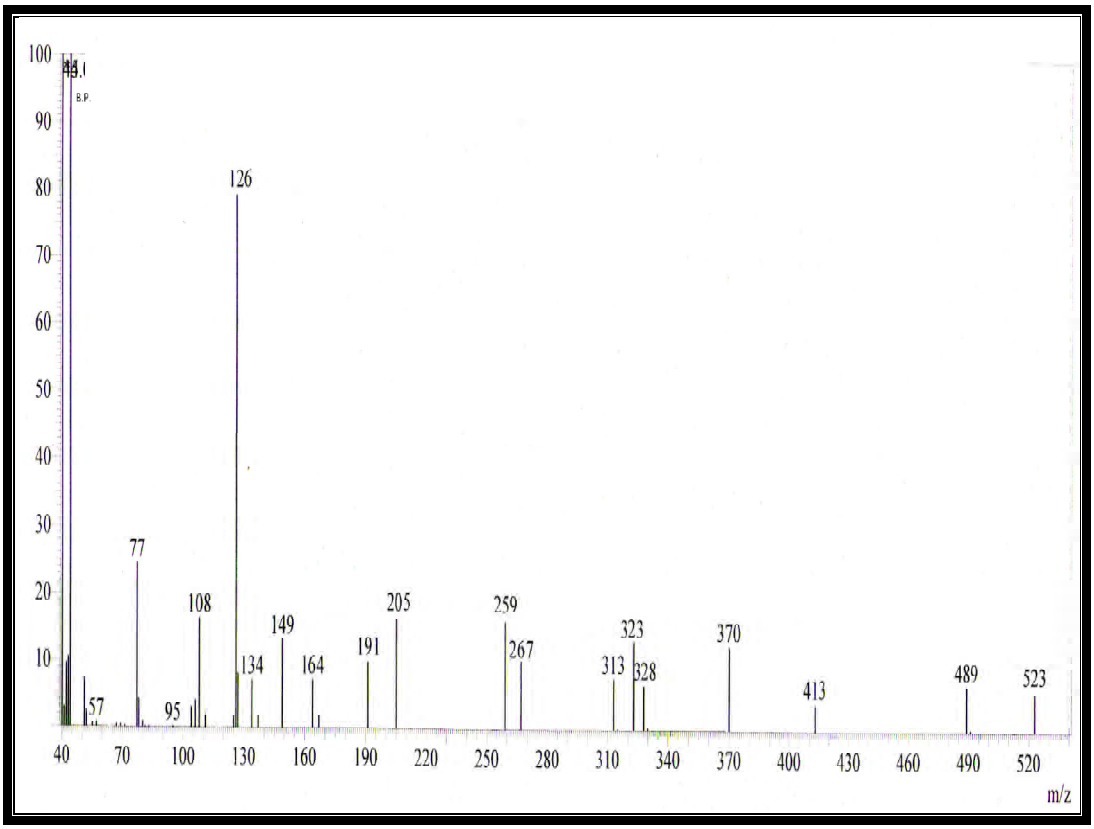

## *Expanded $^1$H NMR spectrum of RHK - 101*



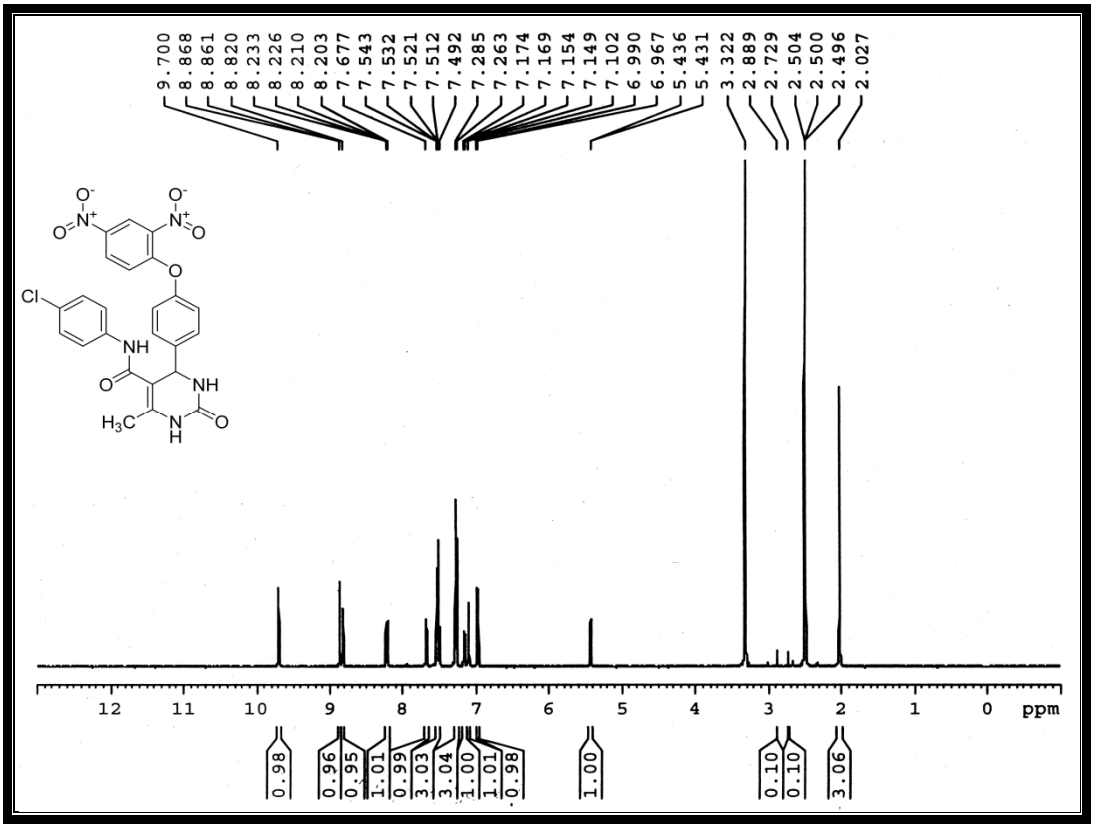
## *IR spectrum of RHK - 106*



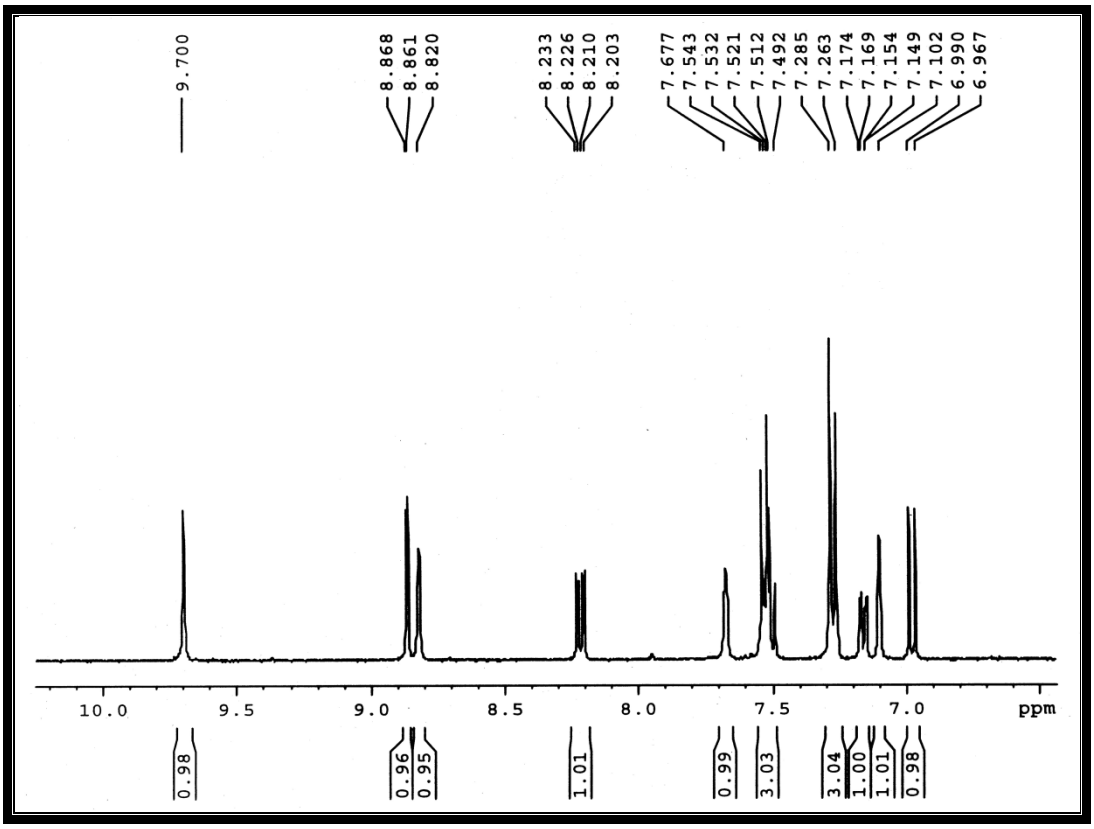

## *Mass spectrum of RHK - 106*



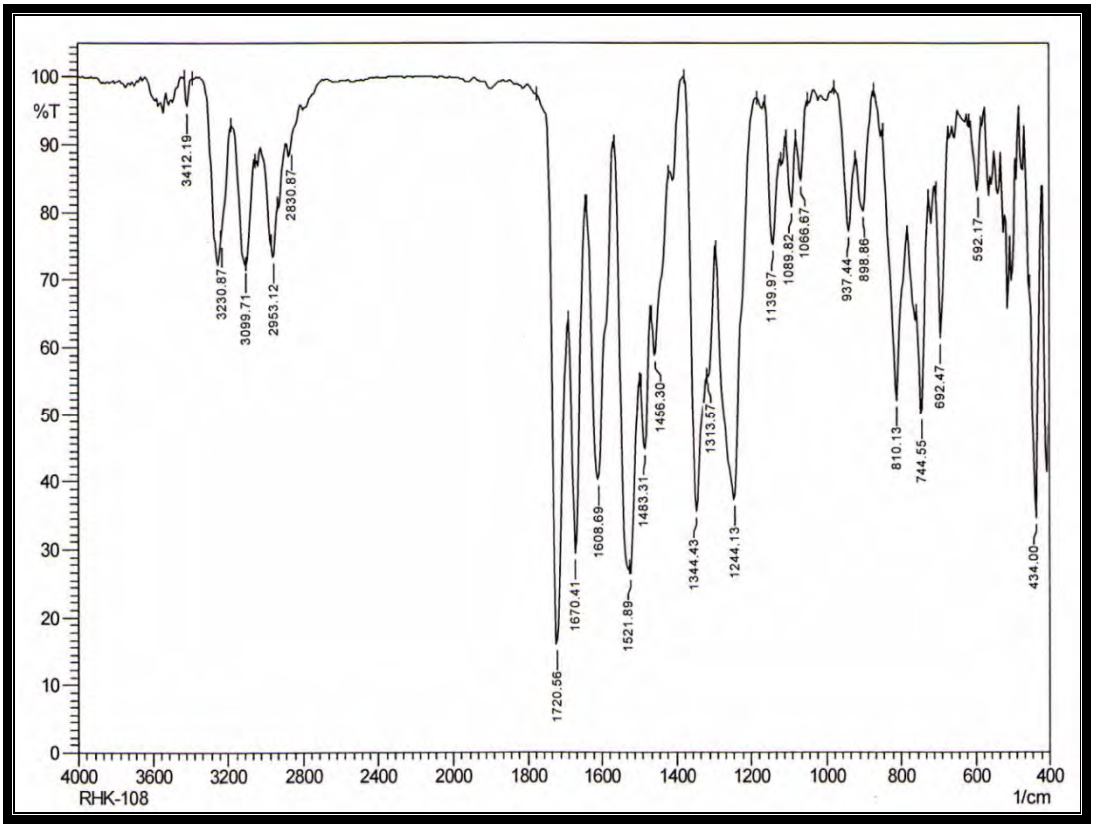
## *$^1$H NMR spectrum of RHK - 106*



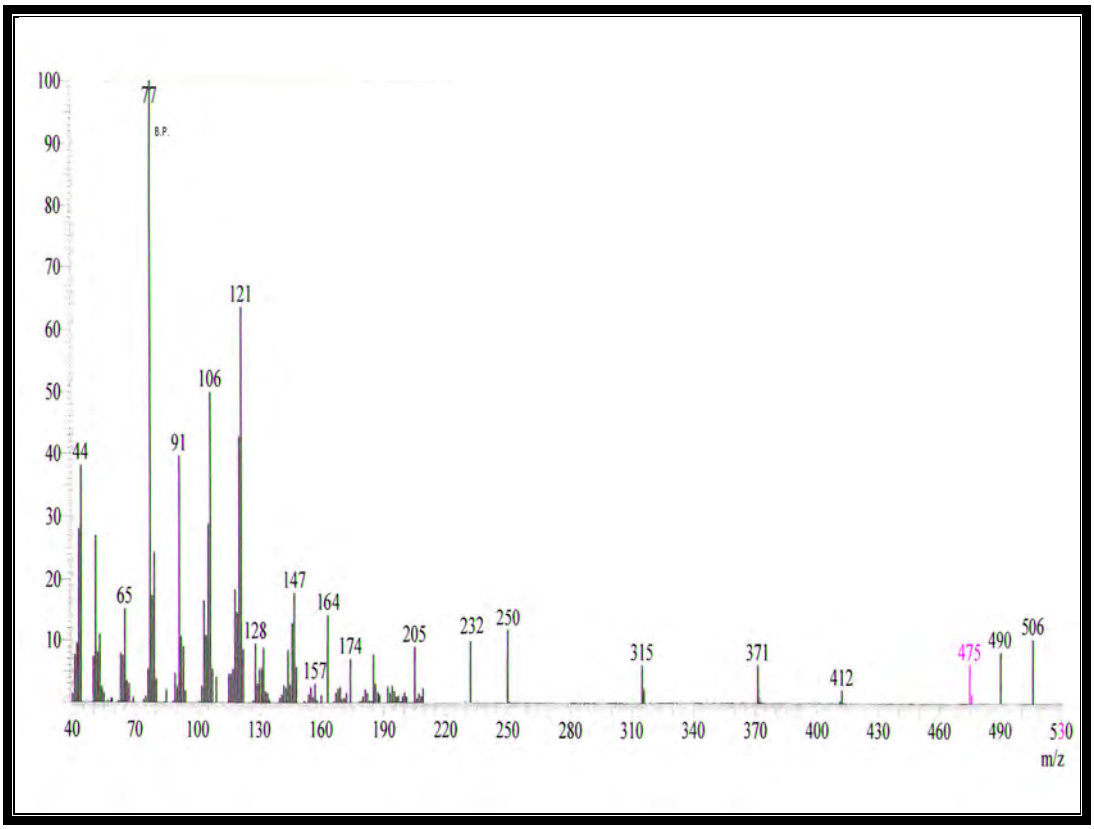

## *Expanded $^1$H NMR spectrum of RHK - 106*



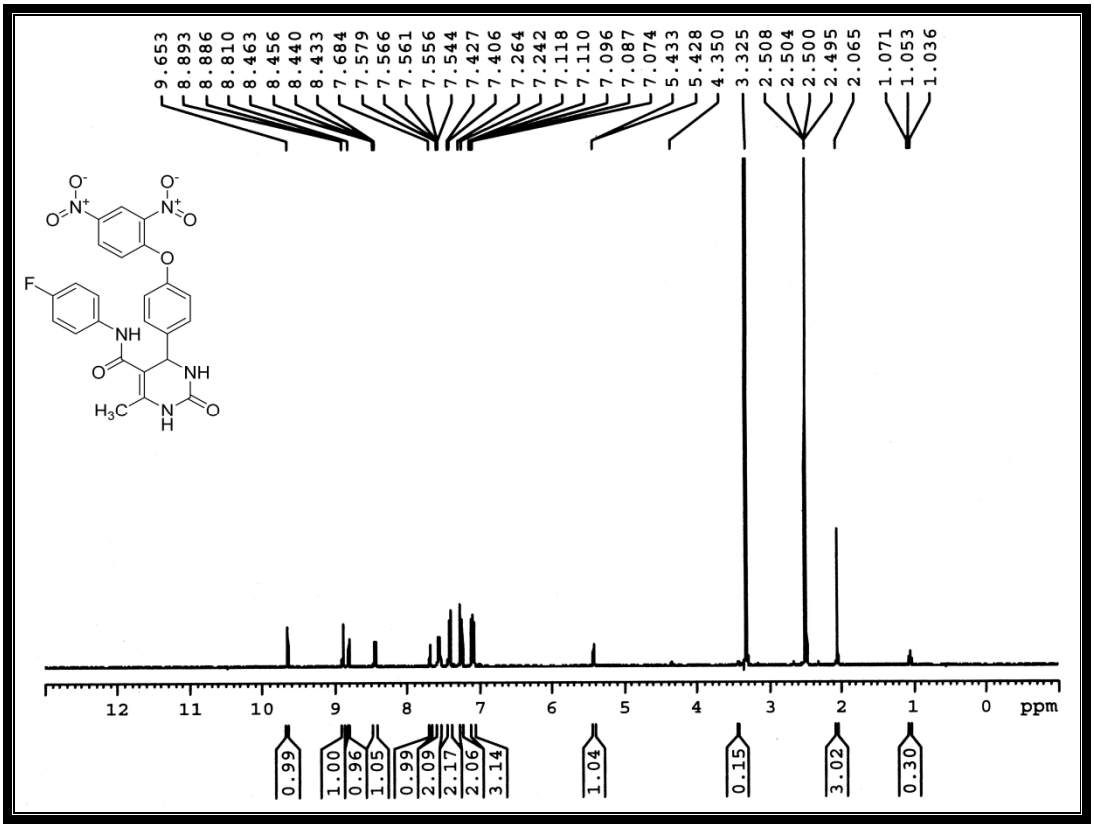
## *IR spectrum of RHK - 108*



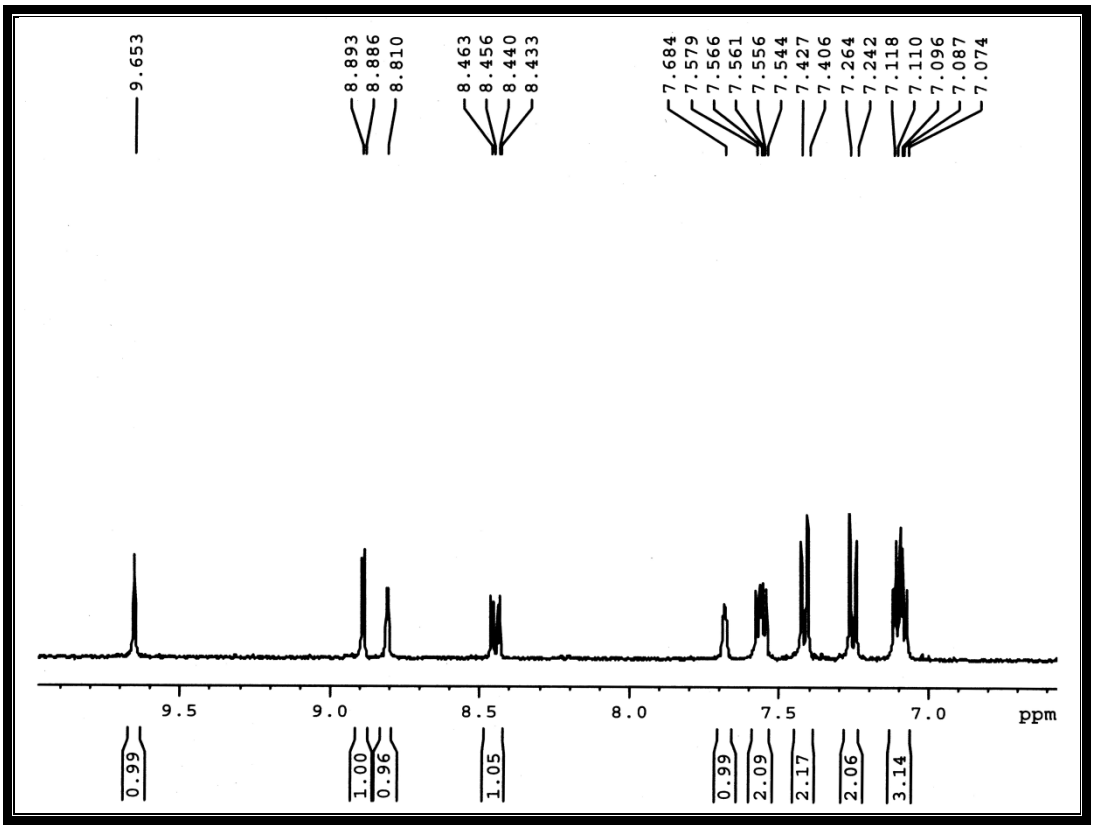

## *Mass spectrum of RHK - 108*



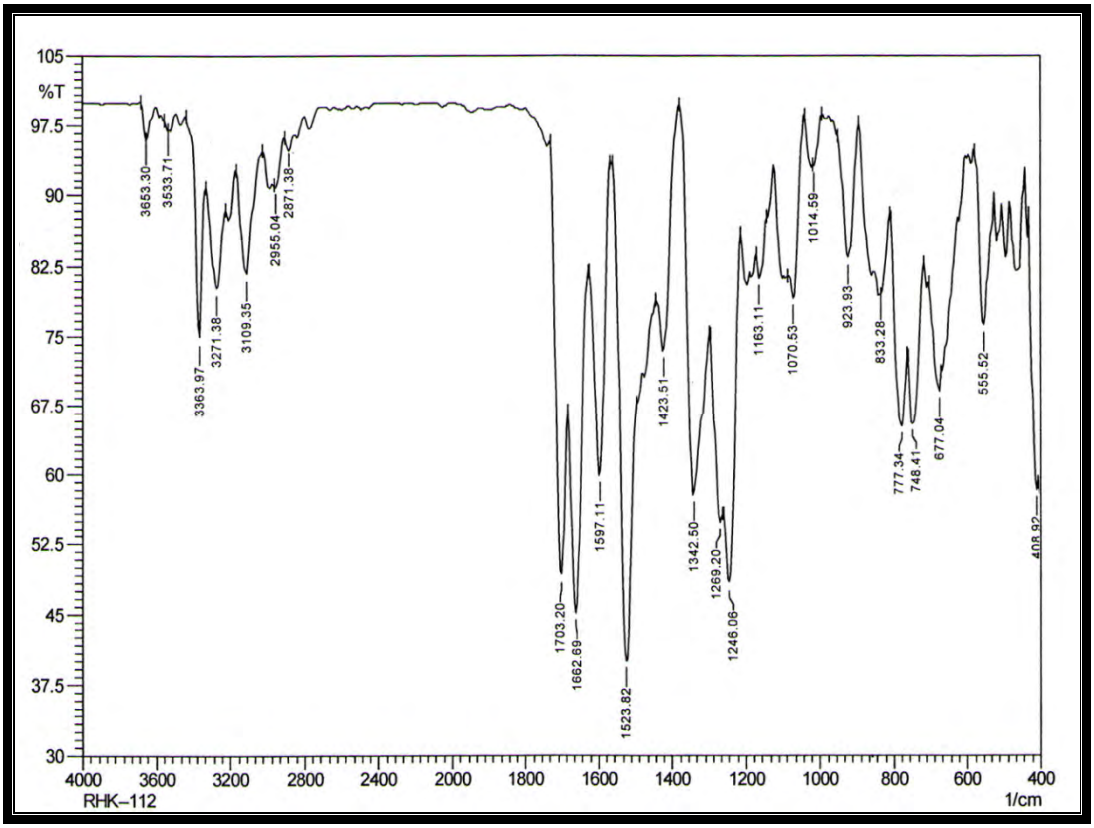
## *$^1$H NMR spectrum of RHK - 108*



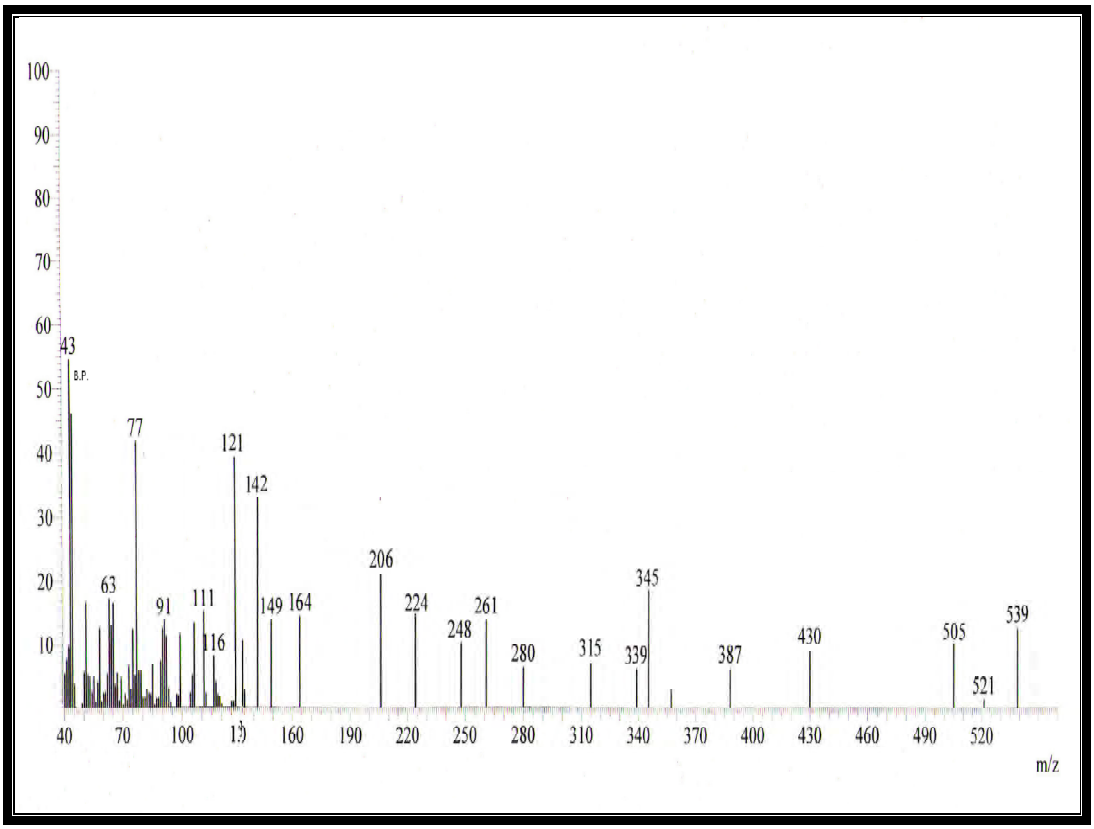

## *Expanded $^1$H NMR spectrum of RHK - 108*



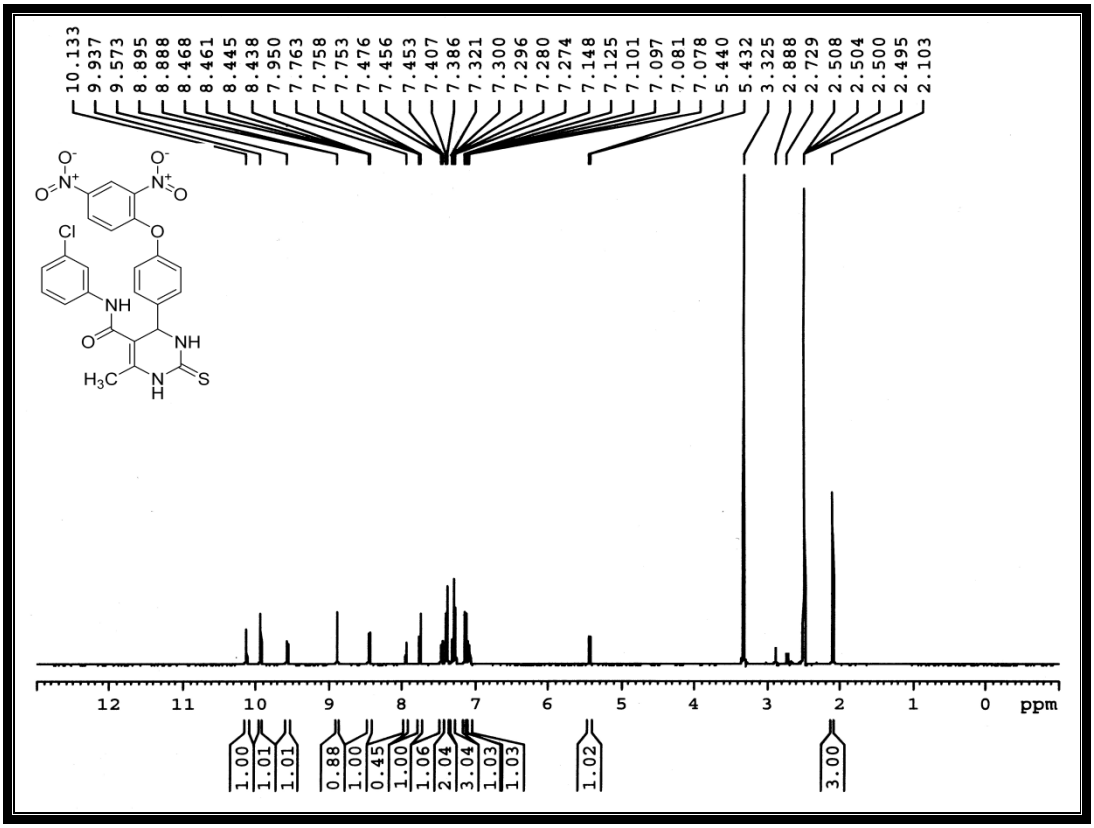
## *IR spectrum of RHK - 112*



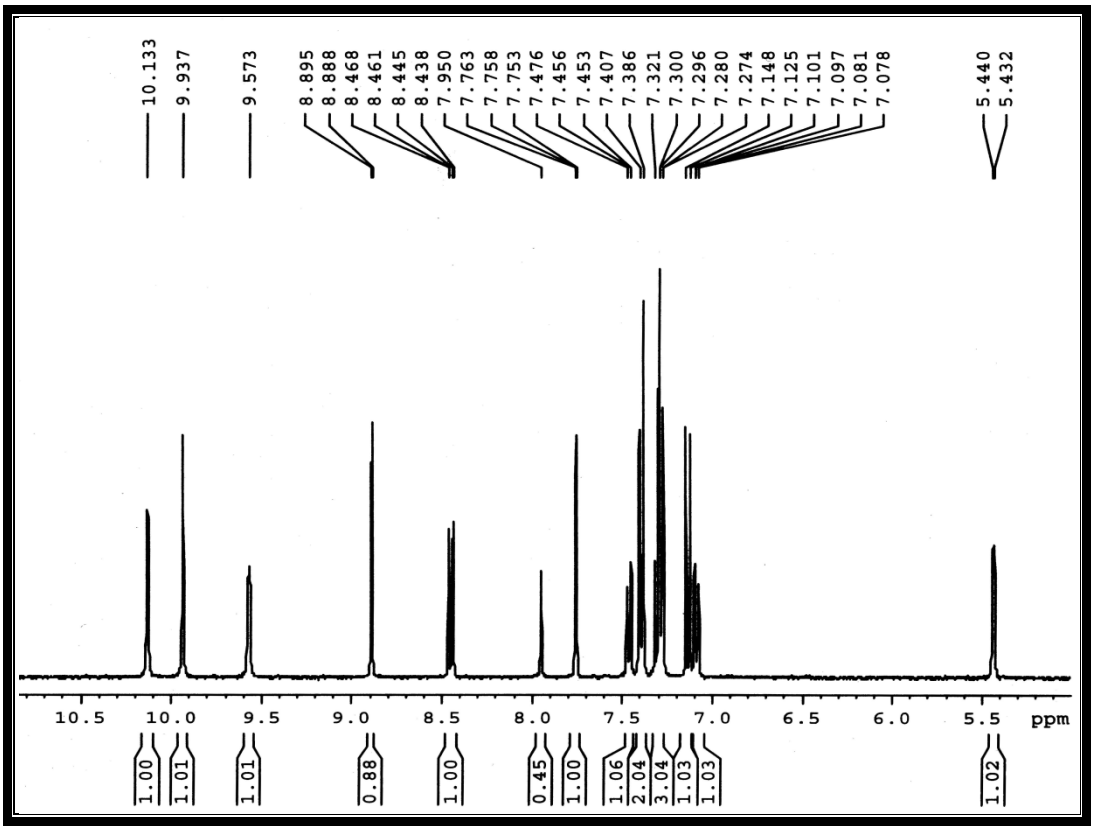

## *Mass spectrum of RHK - 112*



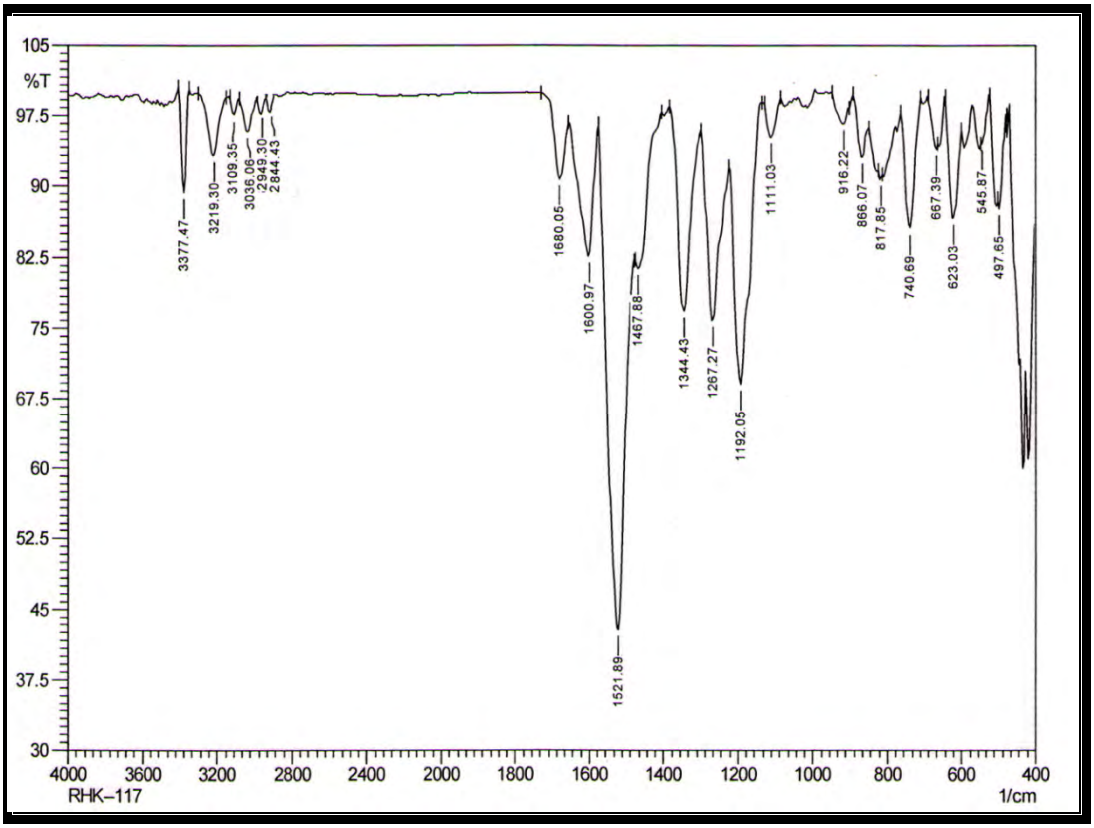
## *$^1$H NMR spectrum of RHK - 112*



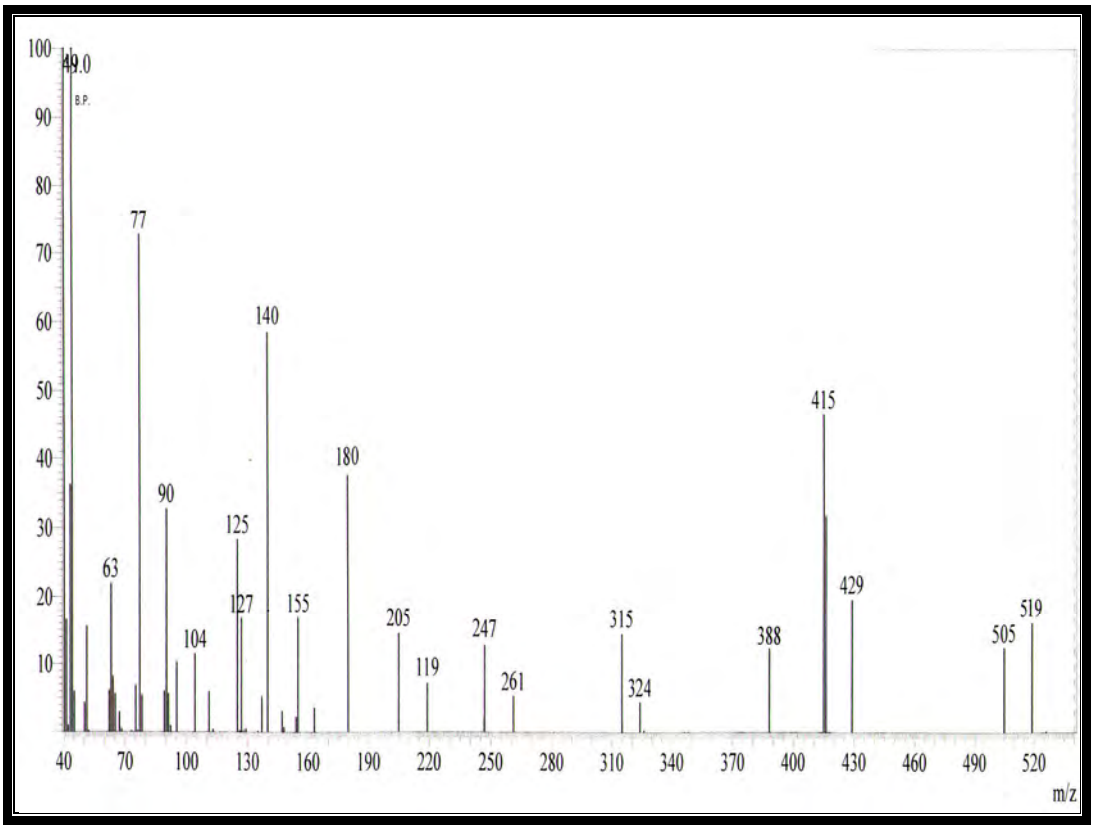

## *Expanded $^1$H NMR spectrum of RHK - 112*



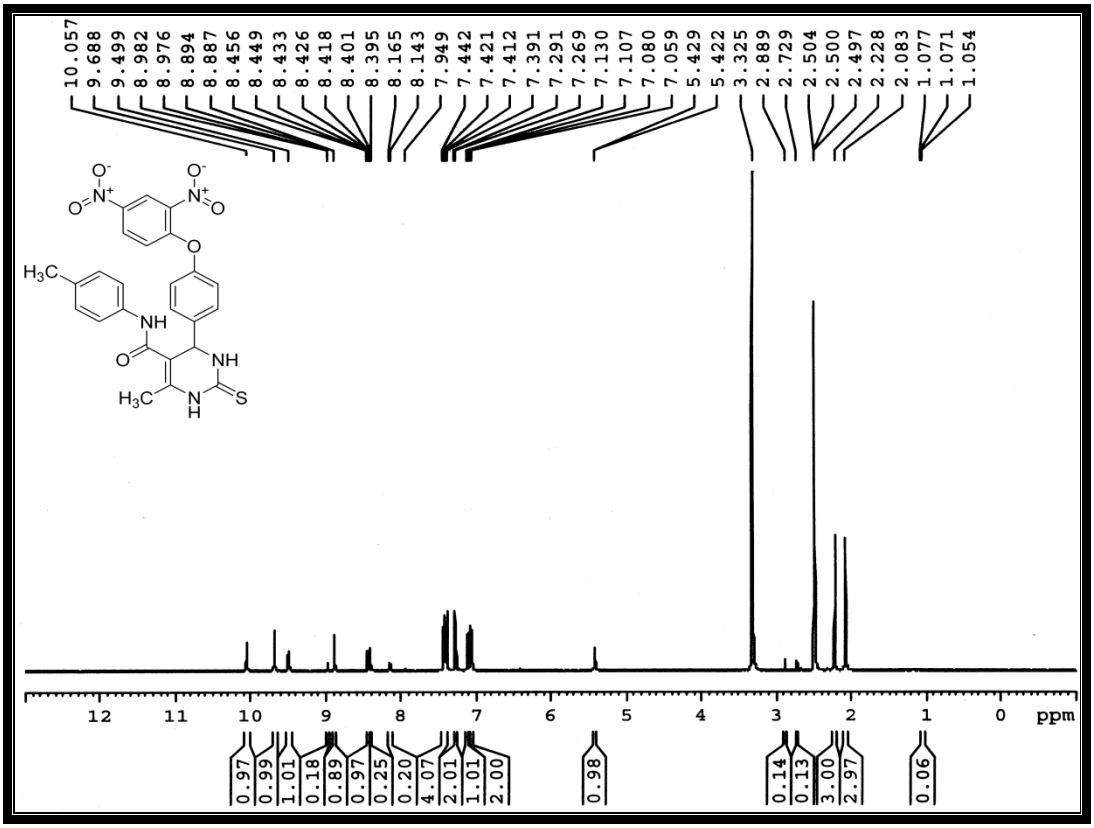
## *IR spectrum of RHK - 117*



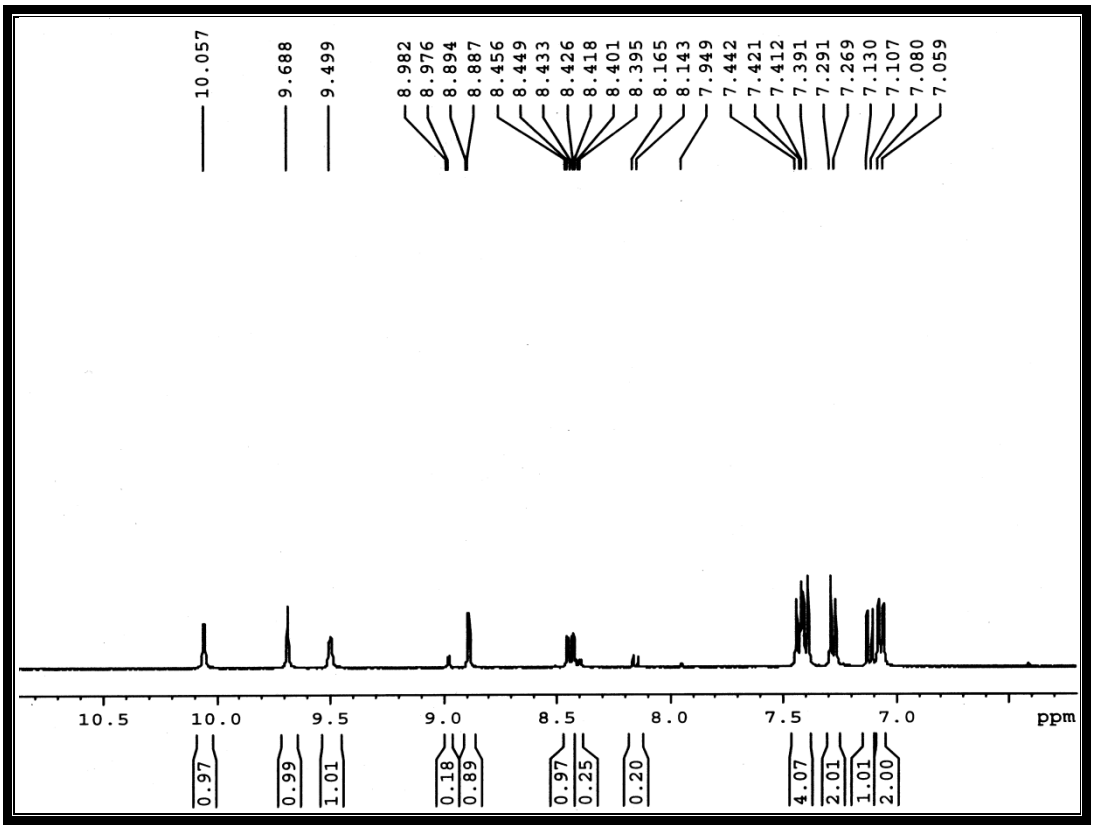

## *Mass spectrum of RHK - 117*



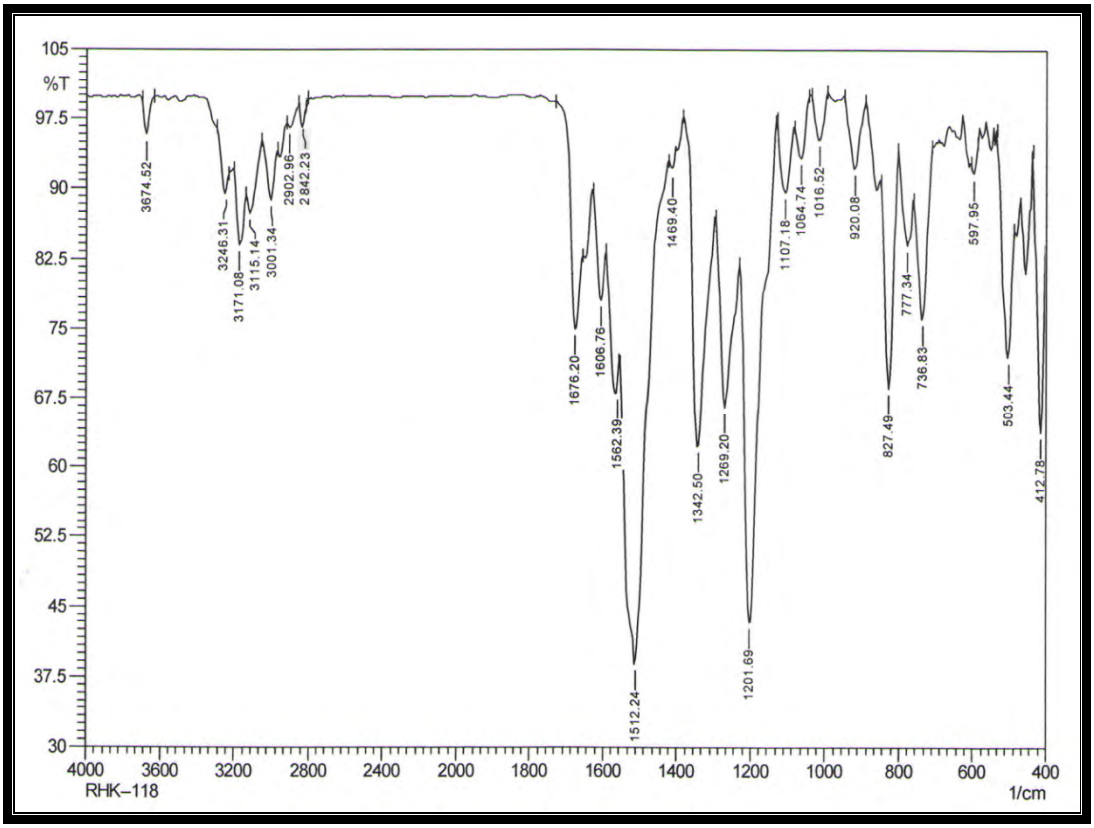
## *$^1$H NMR spectrum of RHK - 117*



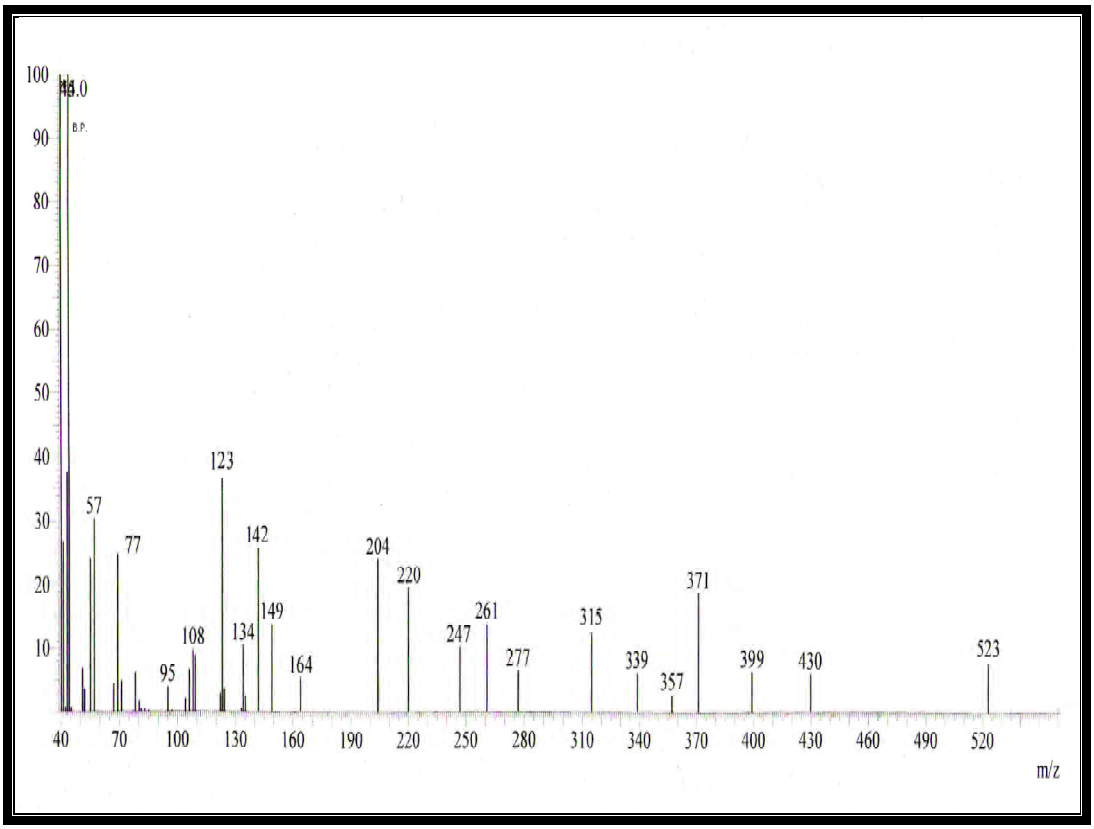

## *Expanded $^1$H NMR spectrum of RHK - 117*



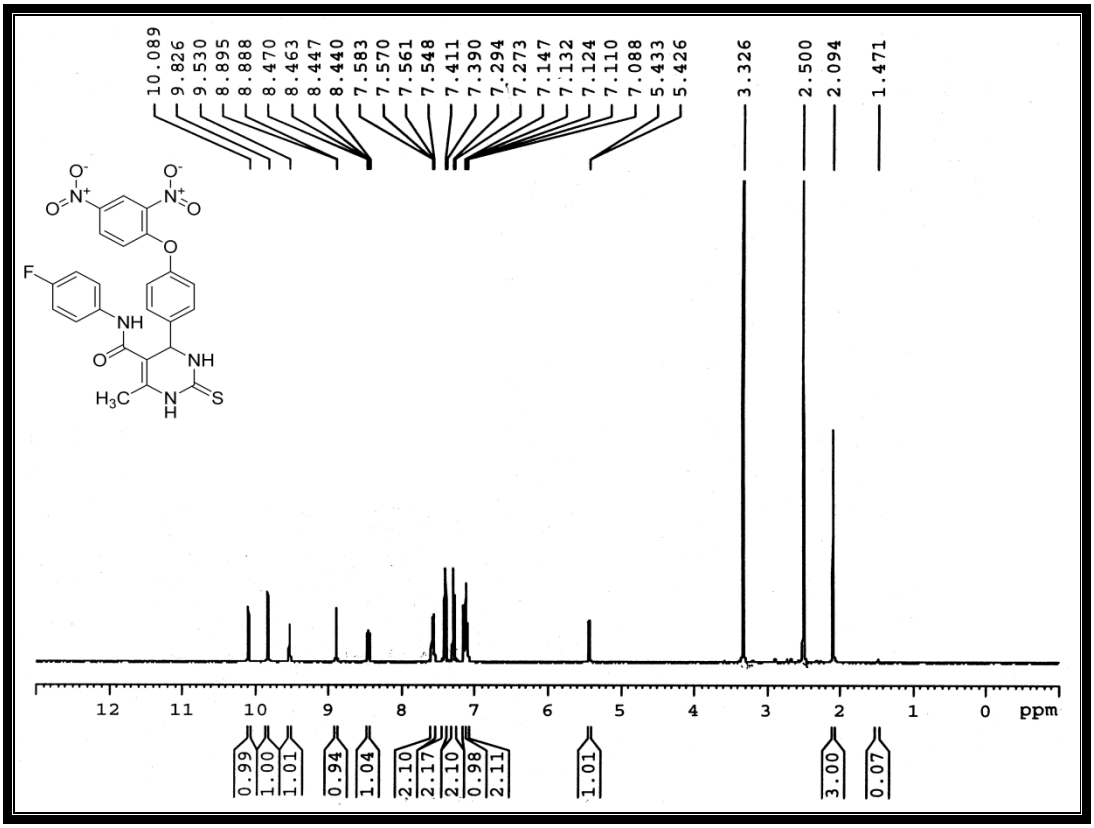
## *IR spectrum of RHK - 118*



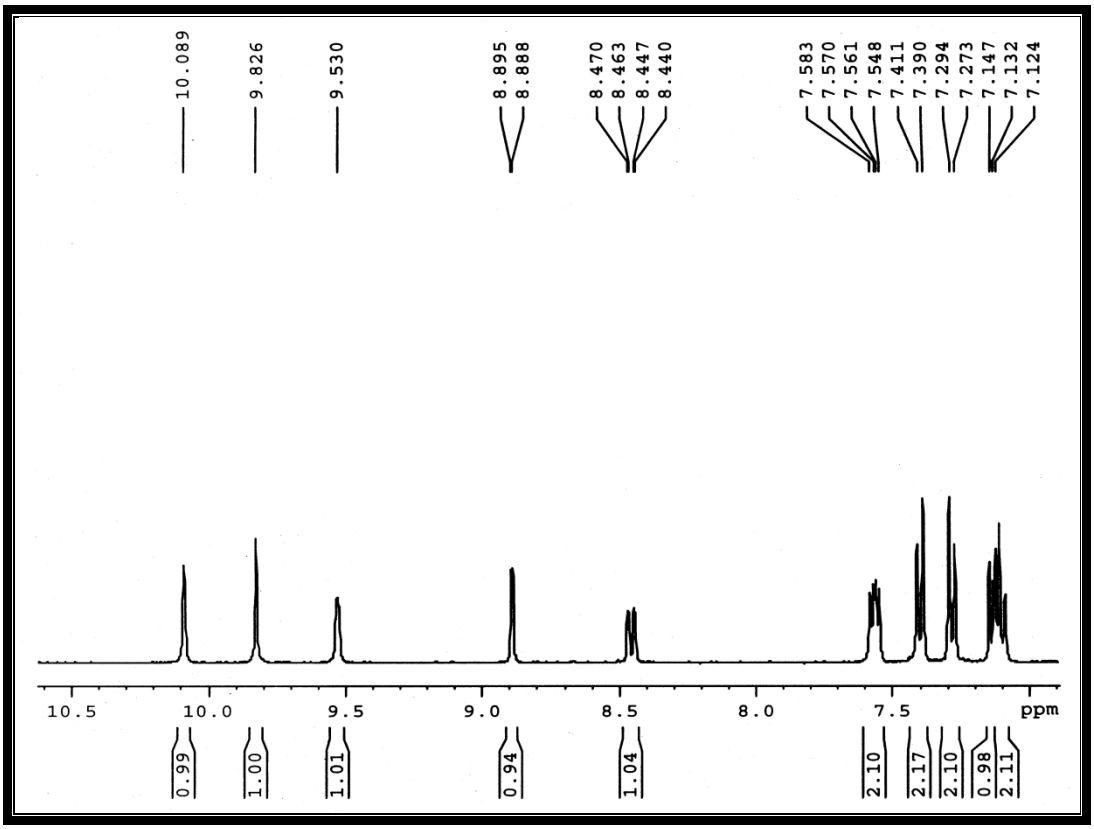

## *Mass spectrum of RHK - 118*



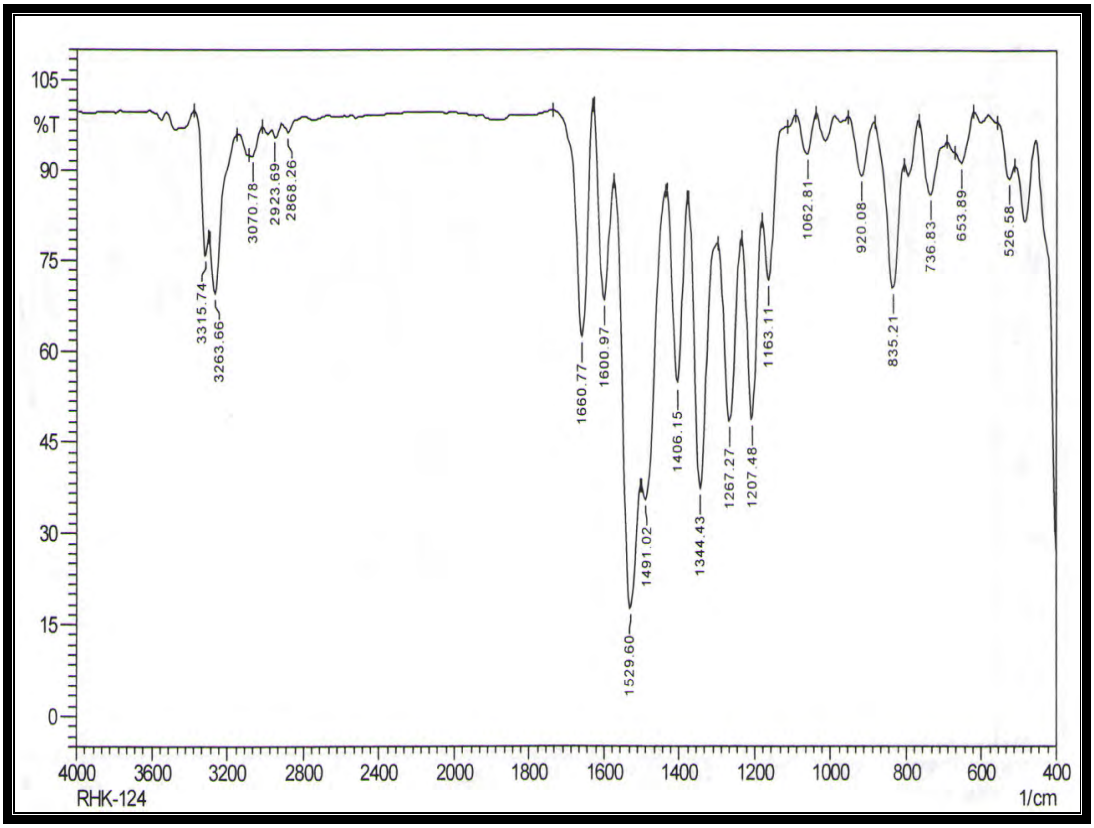
## *$^1$H NMR spectrum of RHK - 118*



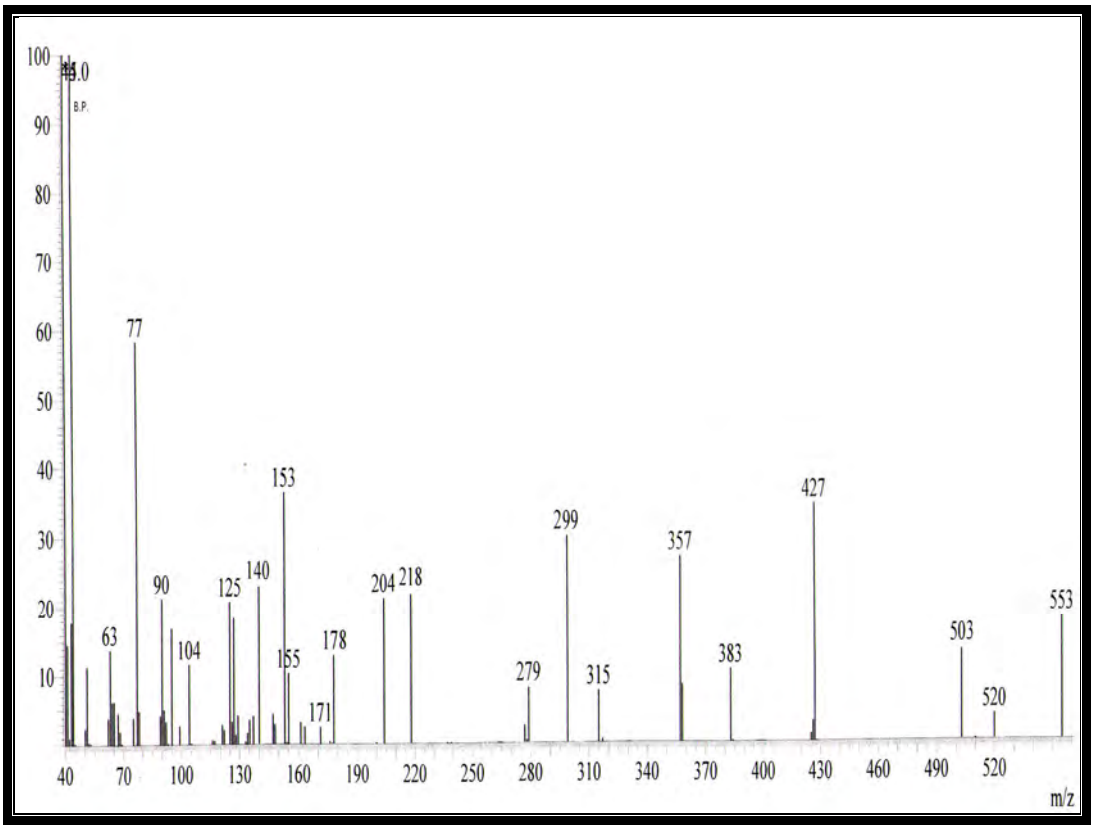

## *Expanded $^1$H NMR spectrum of RHK - 118*



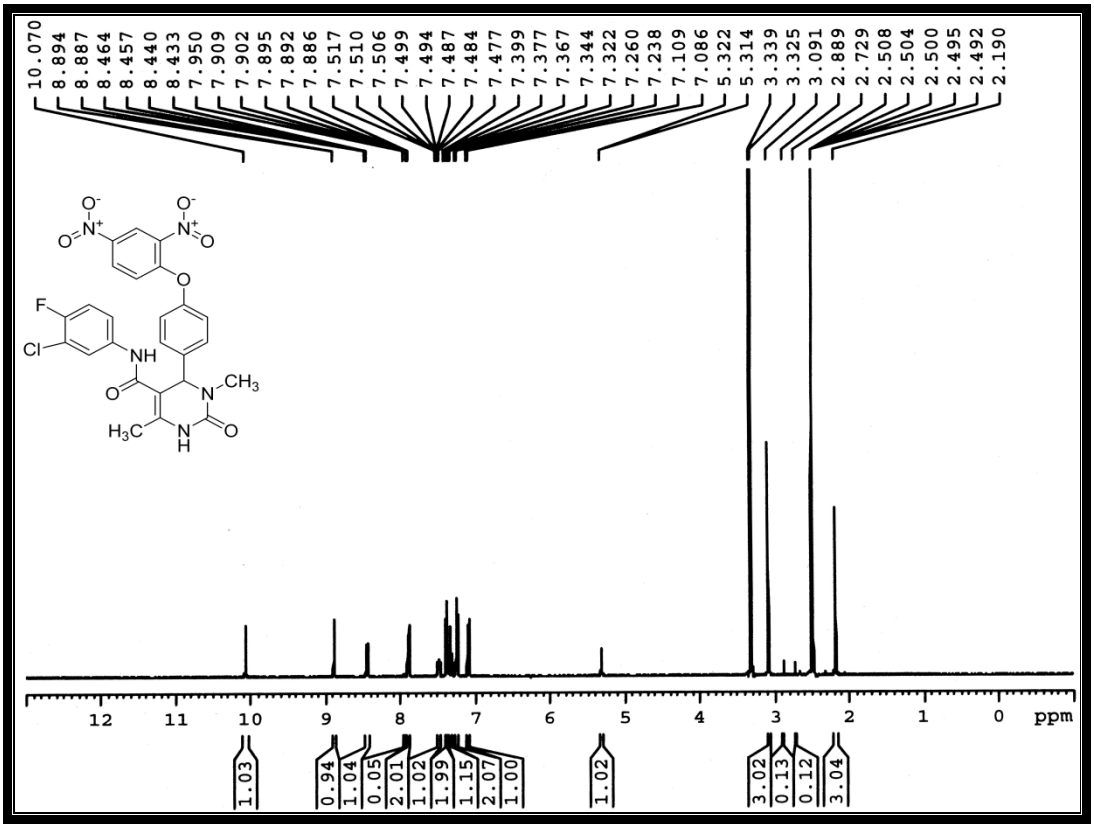
## *IR spectrum of RHK - 124*



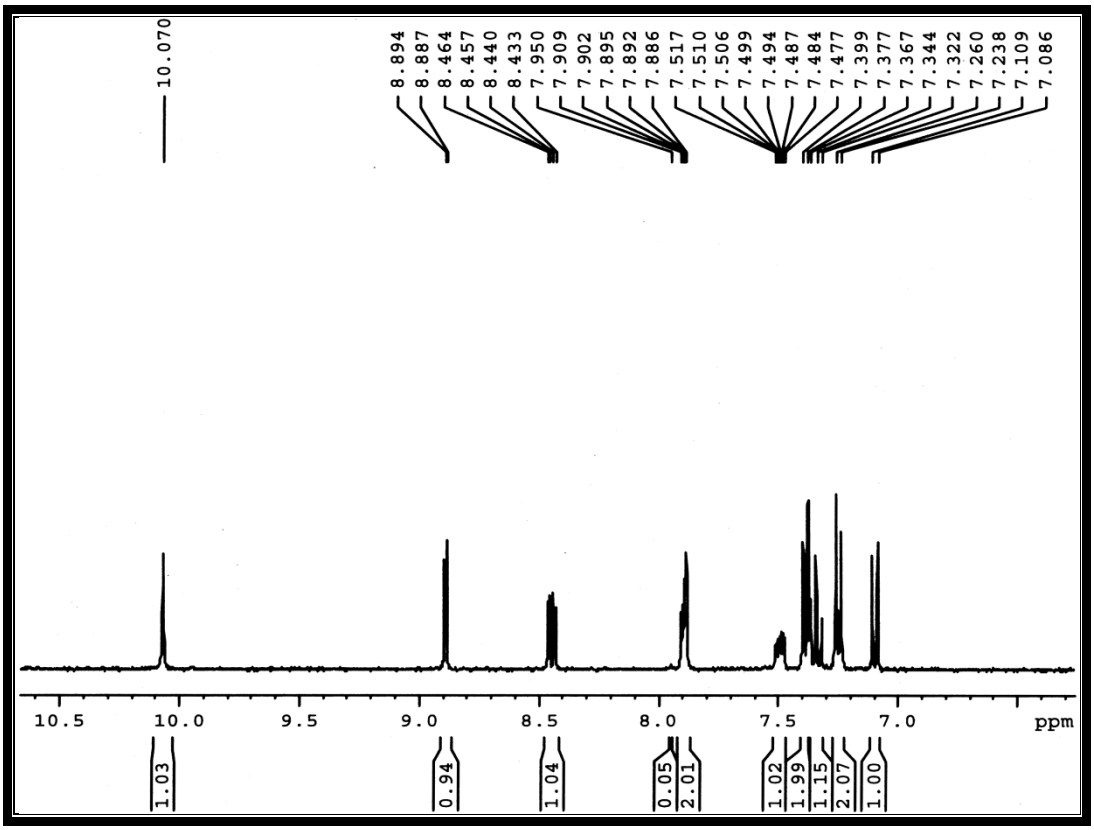

## *Mass spectrum of RHK - 124*



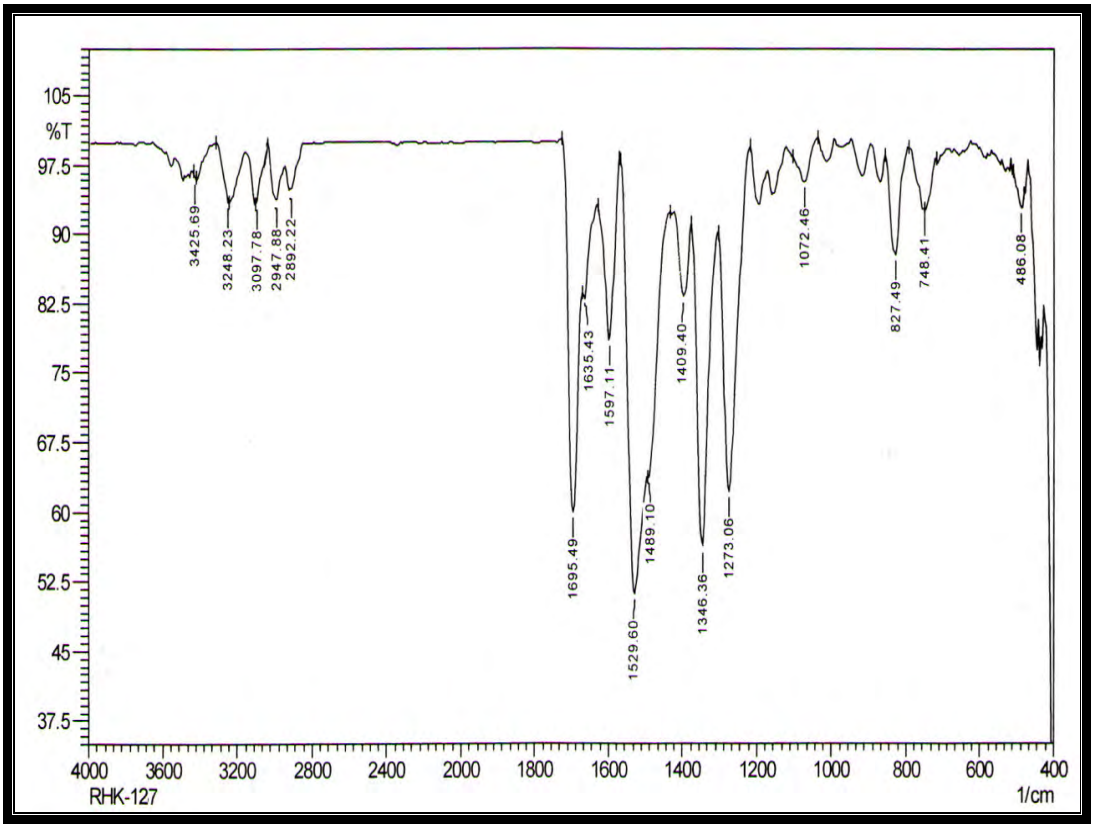
## *$^1$H NMR spectrum of RHK - 124*



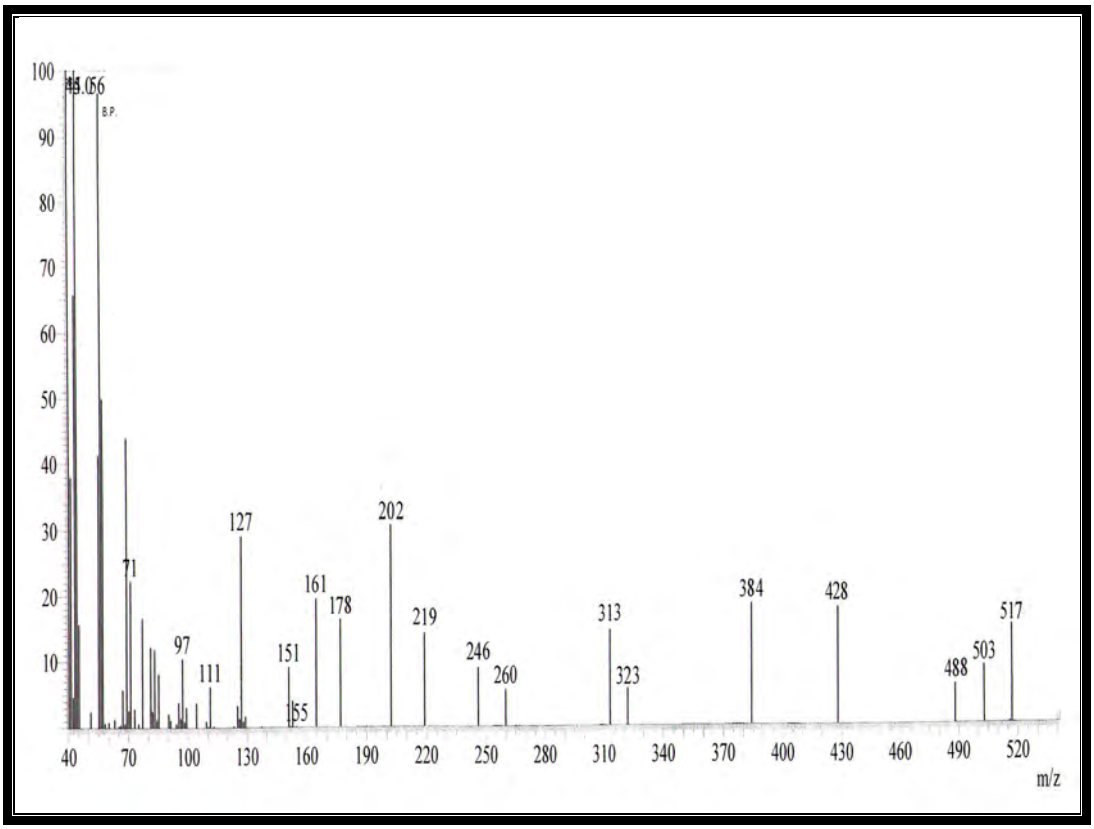

## *Expanded $^1$H NMR spectrum of RHK - 124*



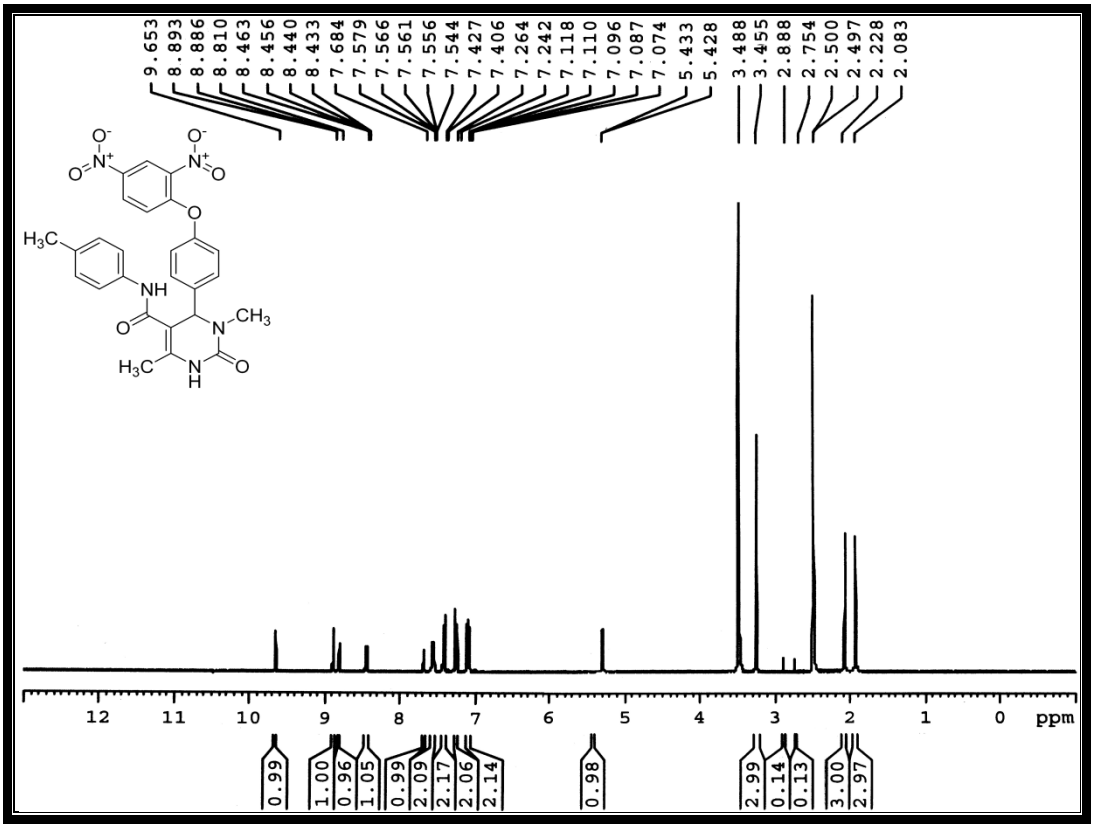
## *IR spectrum of RHK - 127*



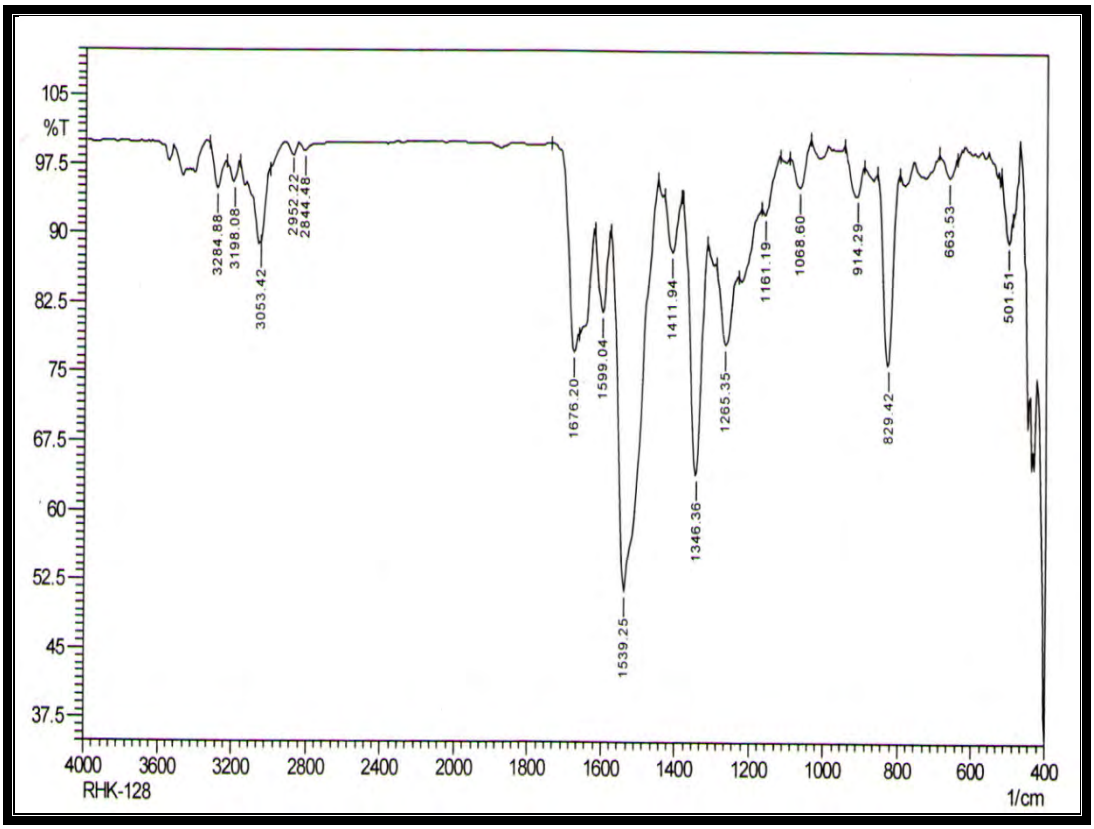

## *Mass spectrum of RHK - 127*



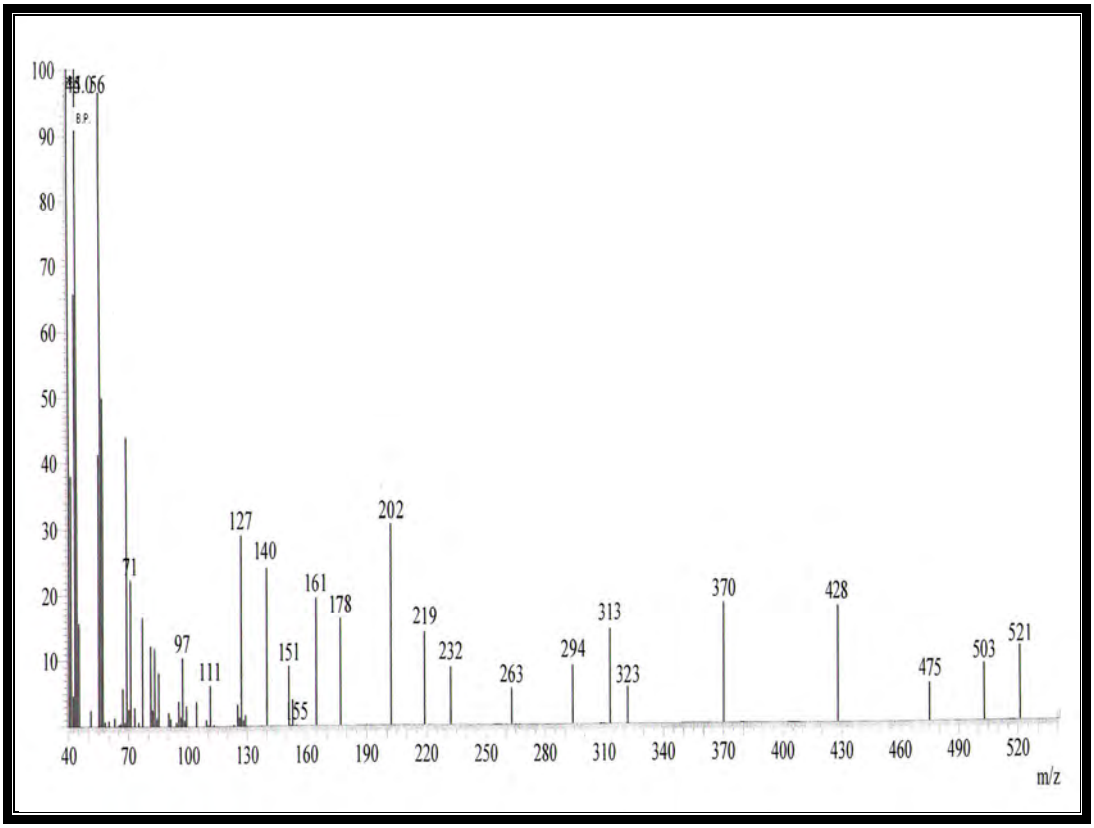
## *$^1$H NMR spectrum of RHK - 127*



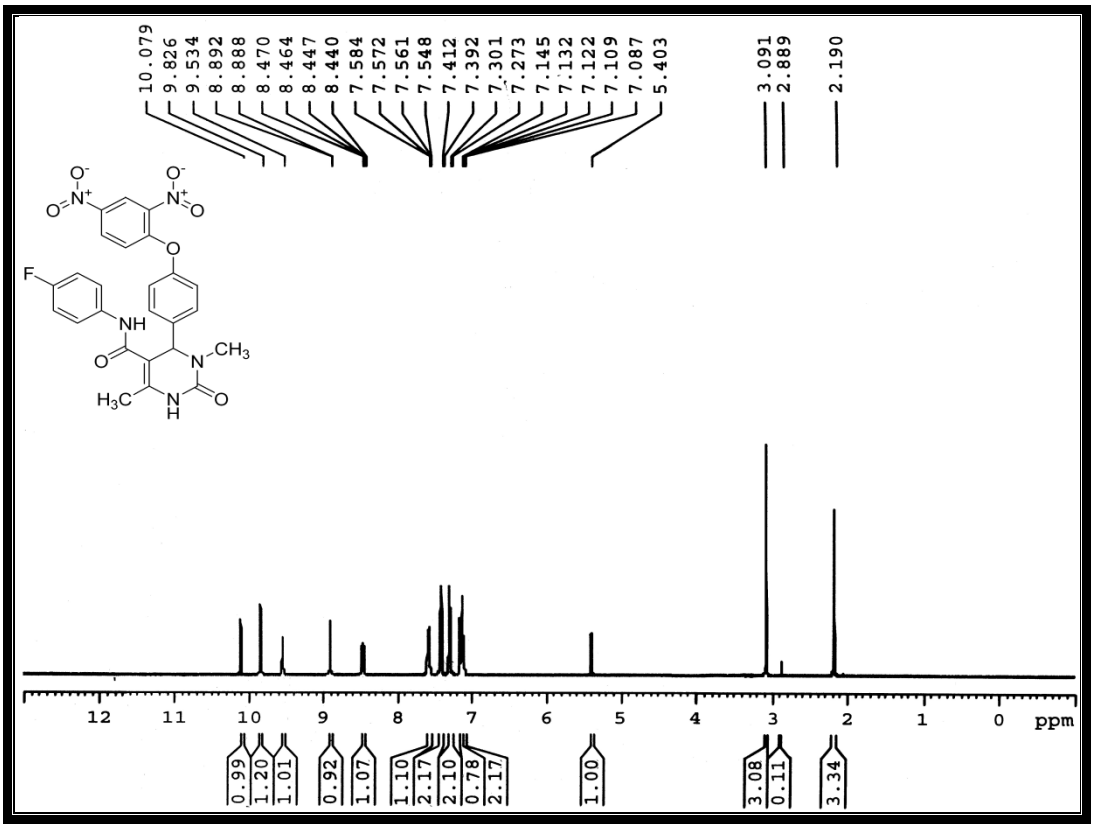

## *IR spectrum of RHK - 128*



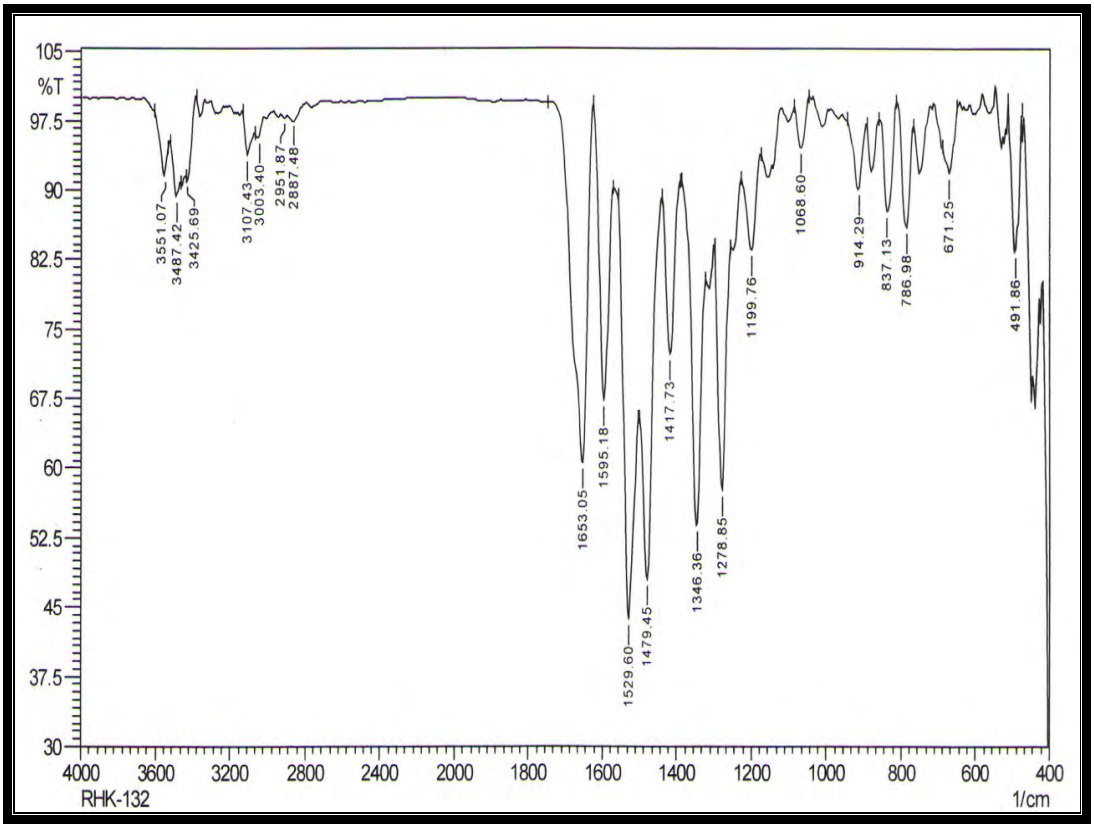
## *Mass spectrum of RHK - 128*



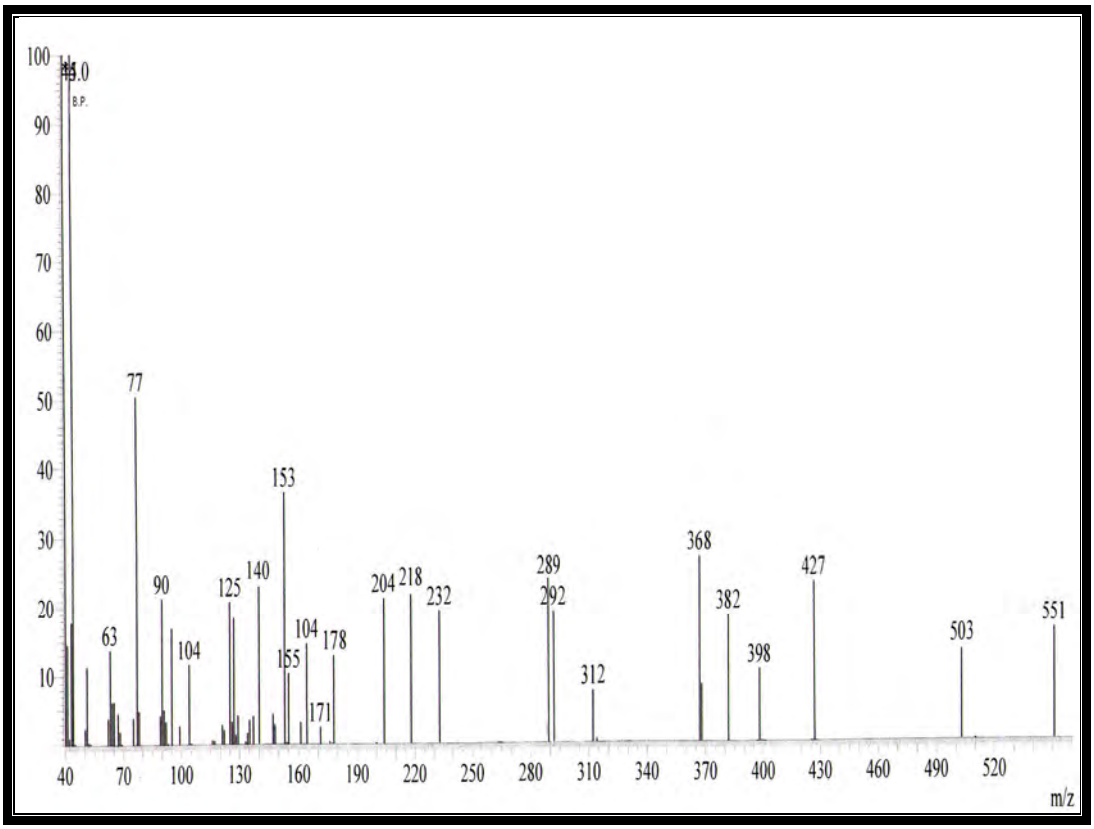

## *$^{1}$H NMR spectrum of RHK - 128*



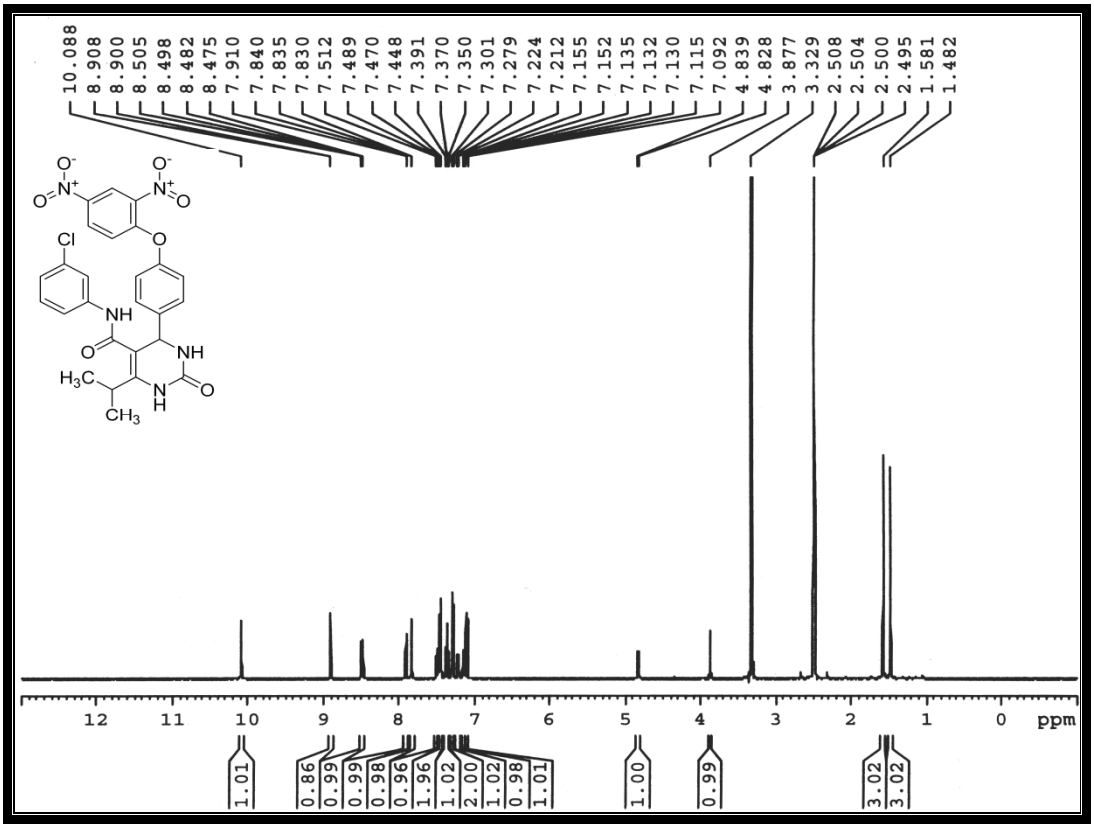
## *IR spectrum of RHK - 132*



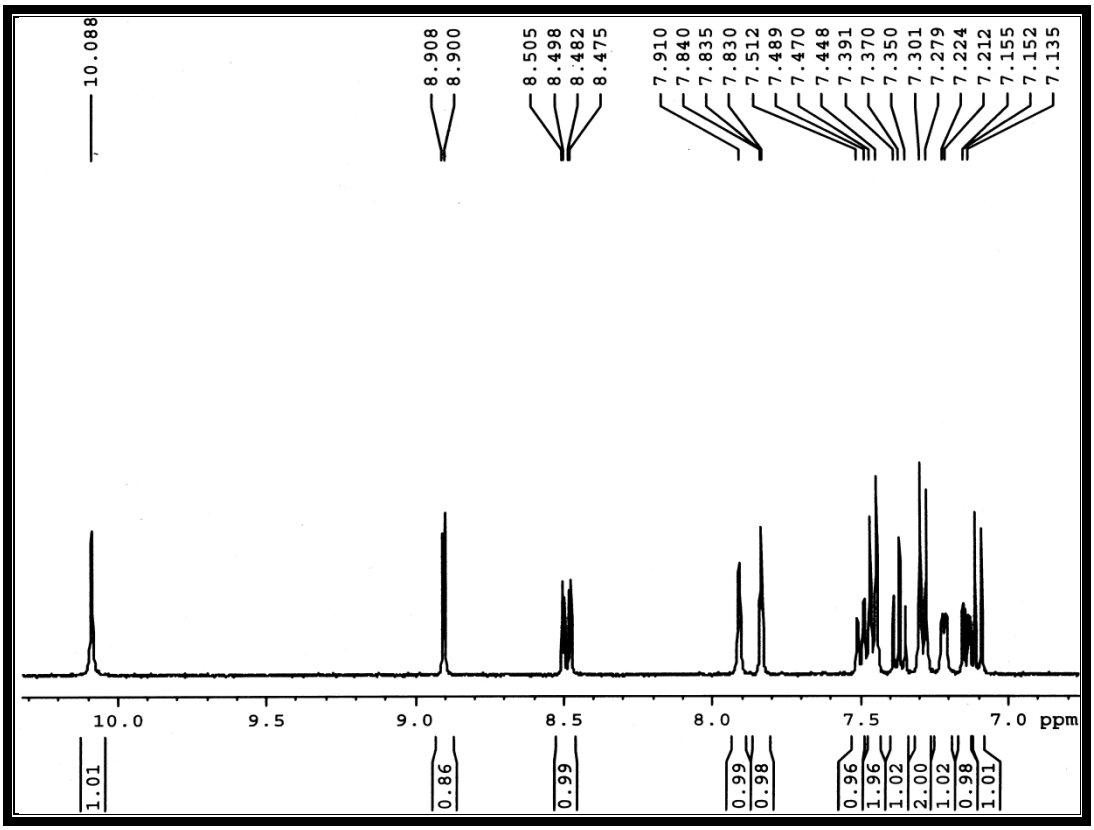

## *Mass spectrum of RHK - 132*



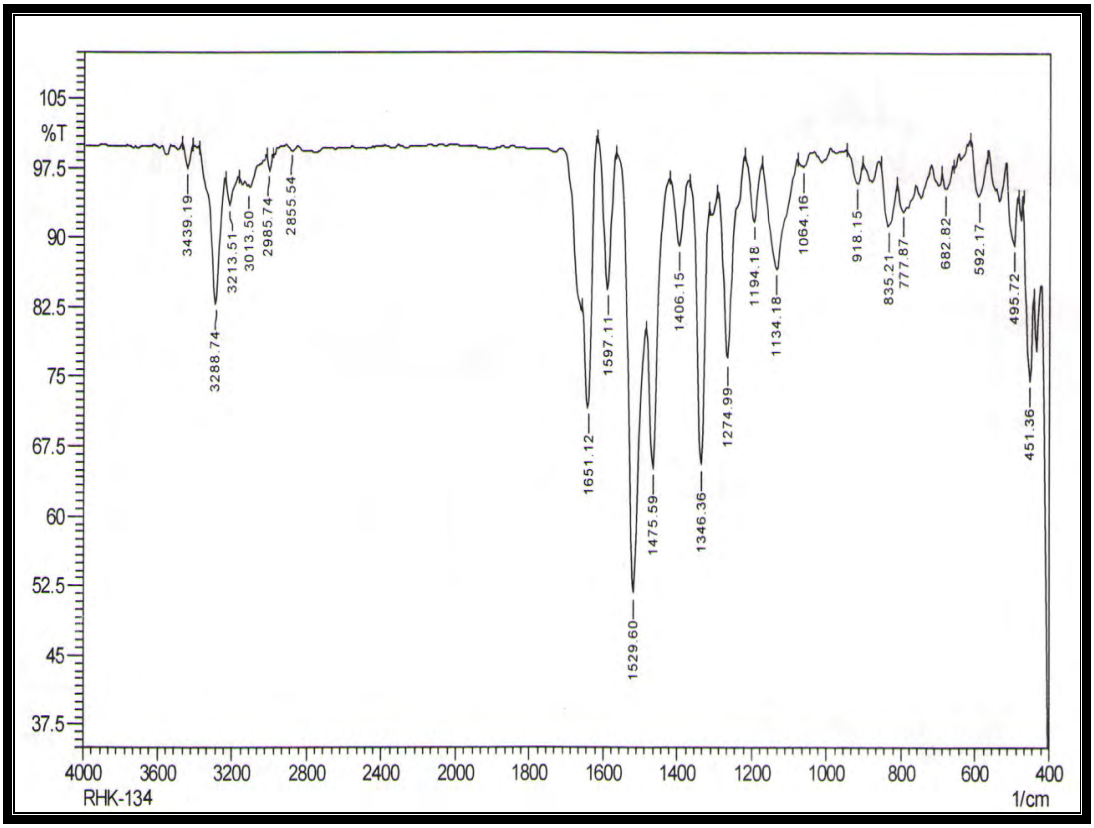
## *$^1$H NMR spectrum of RHK - 132*



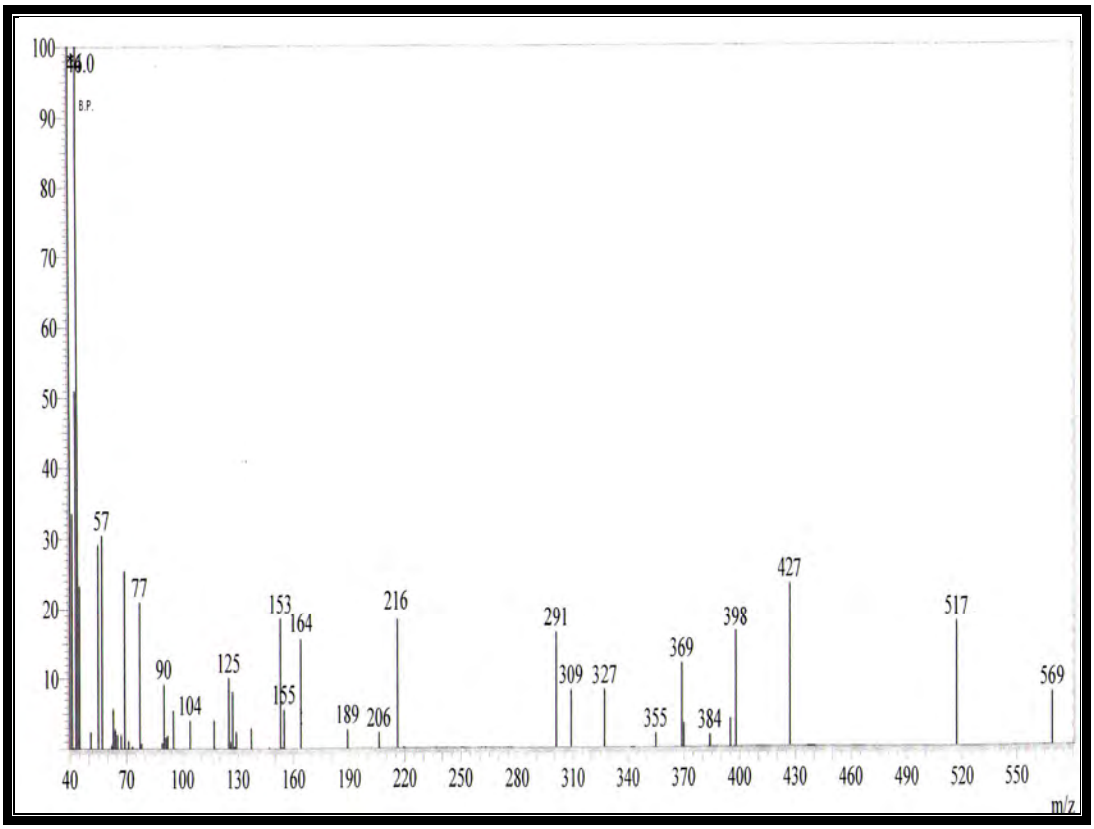

## *Expanded $^1$H NMR spectrum of RHK - 132*



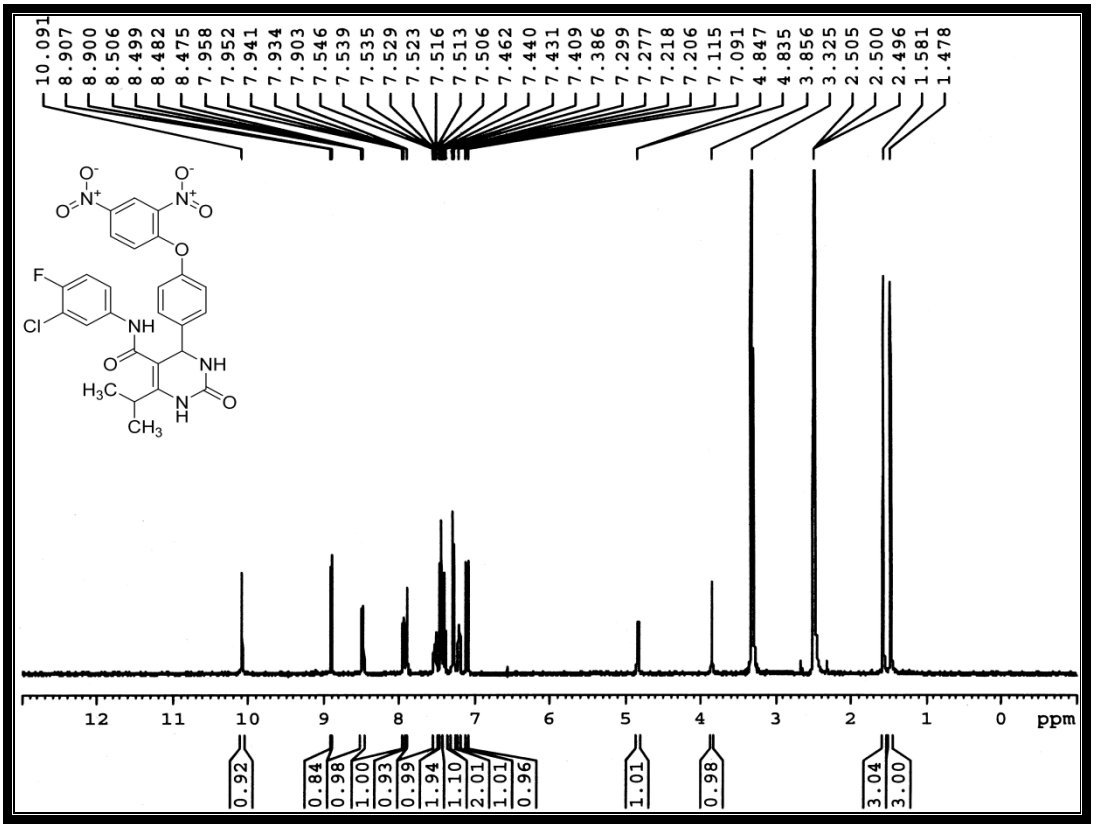
## *IR spectrum of RHK - 134*



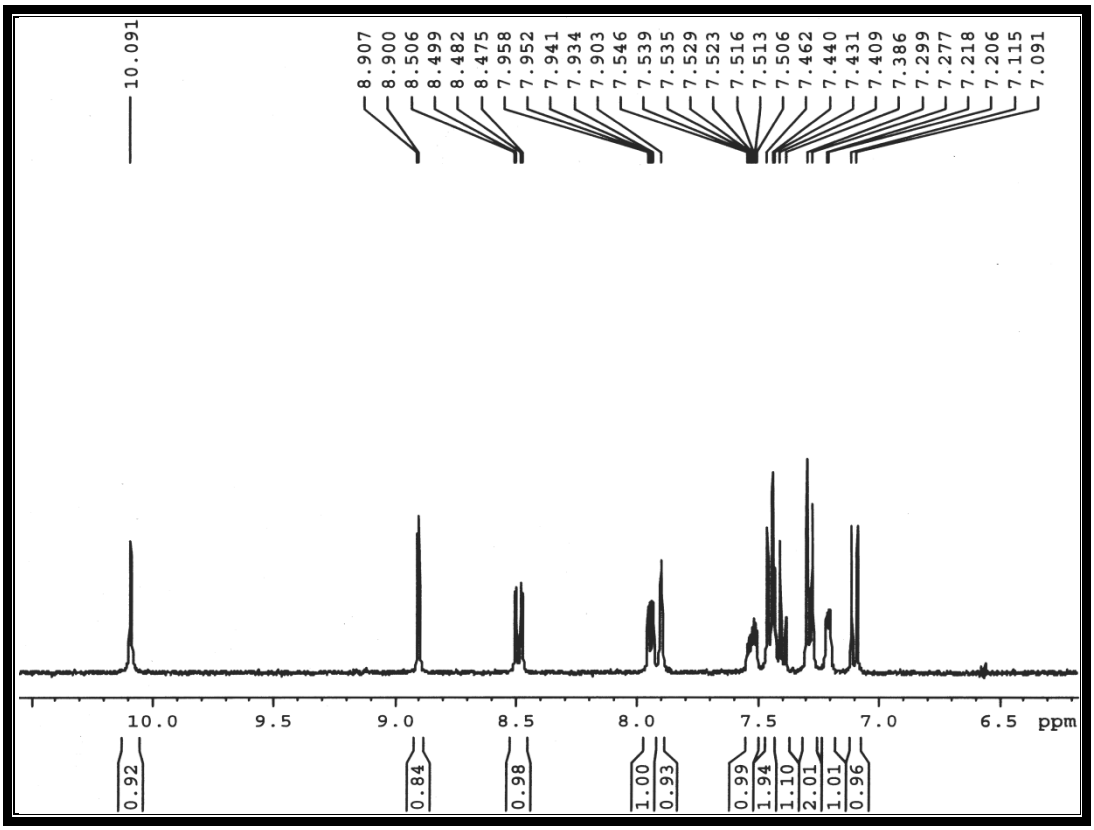

## *Mass spectrum of RHK - 134*



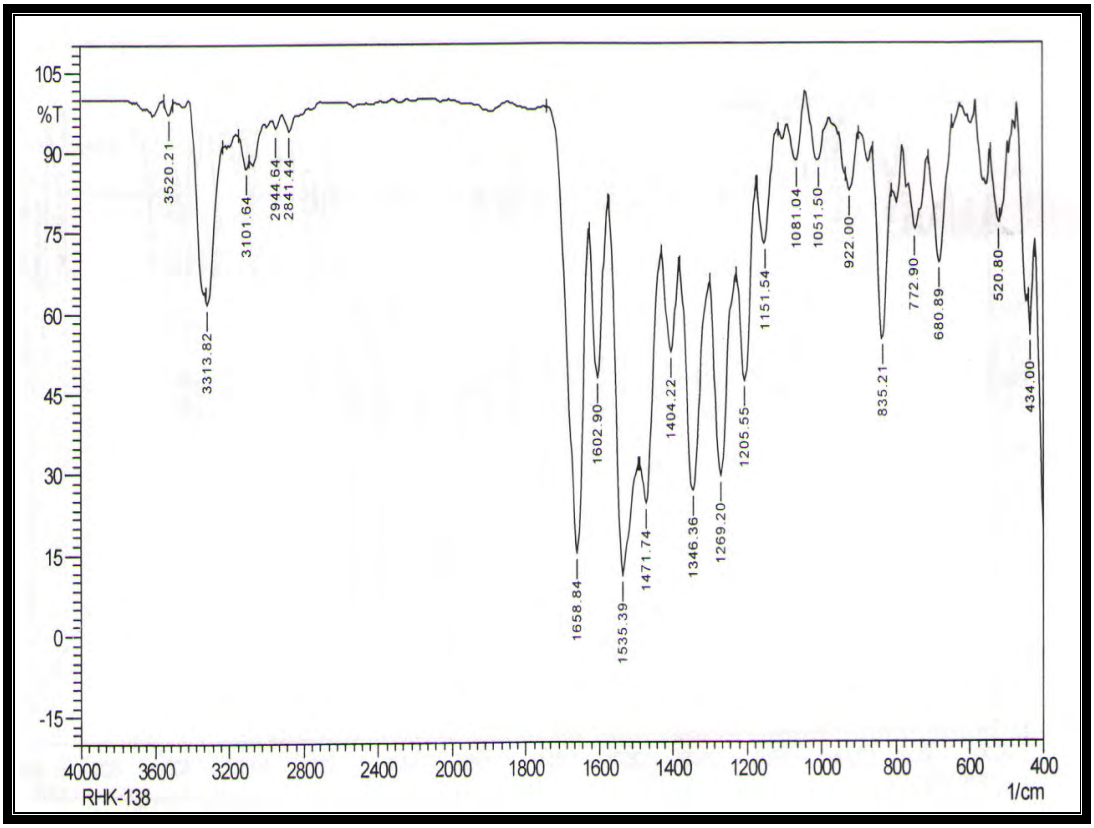
## *$^1$H NMR spectrum of RHK - 134*



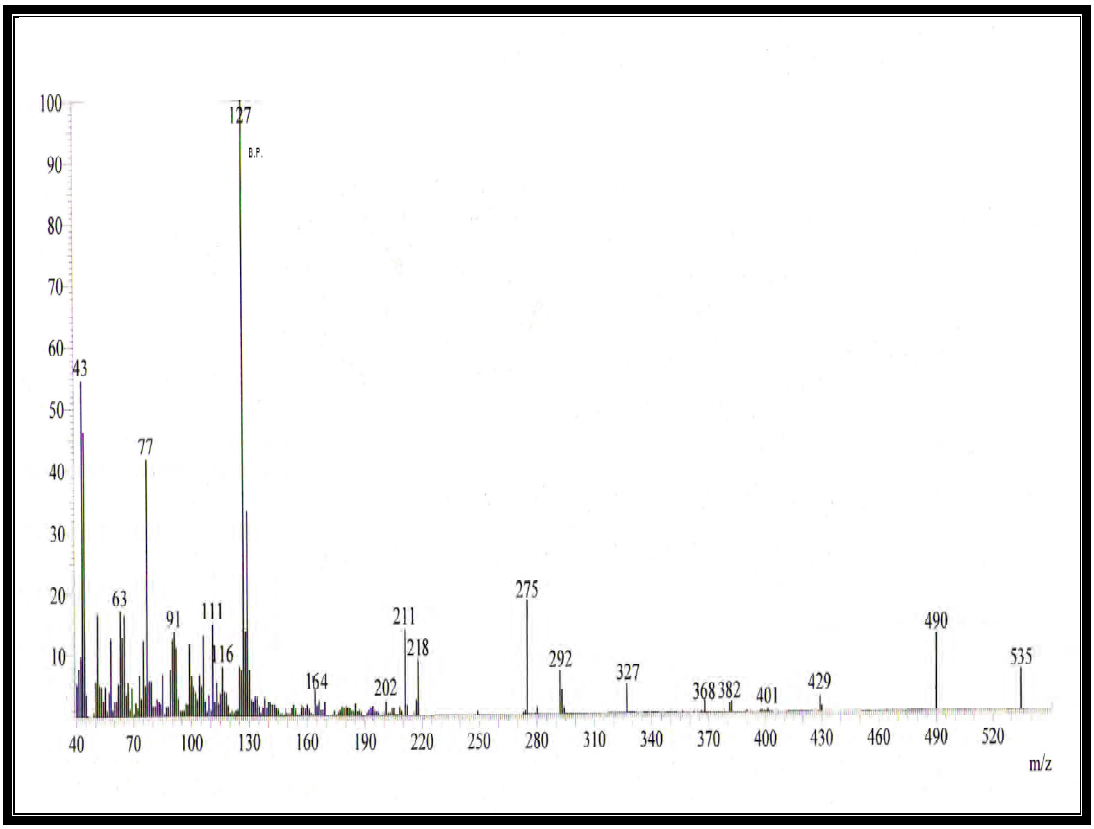

## *Expanded $^1$H NMR spectrum of RHK - 134*



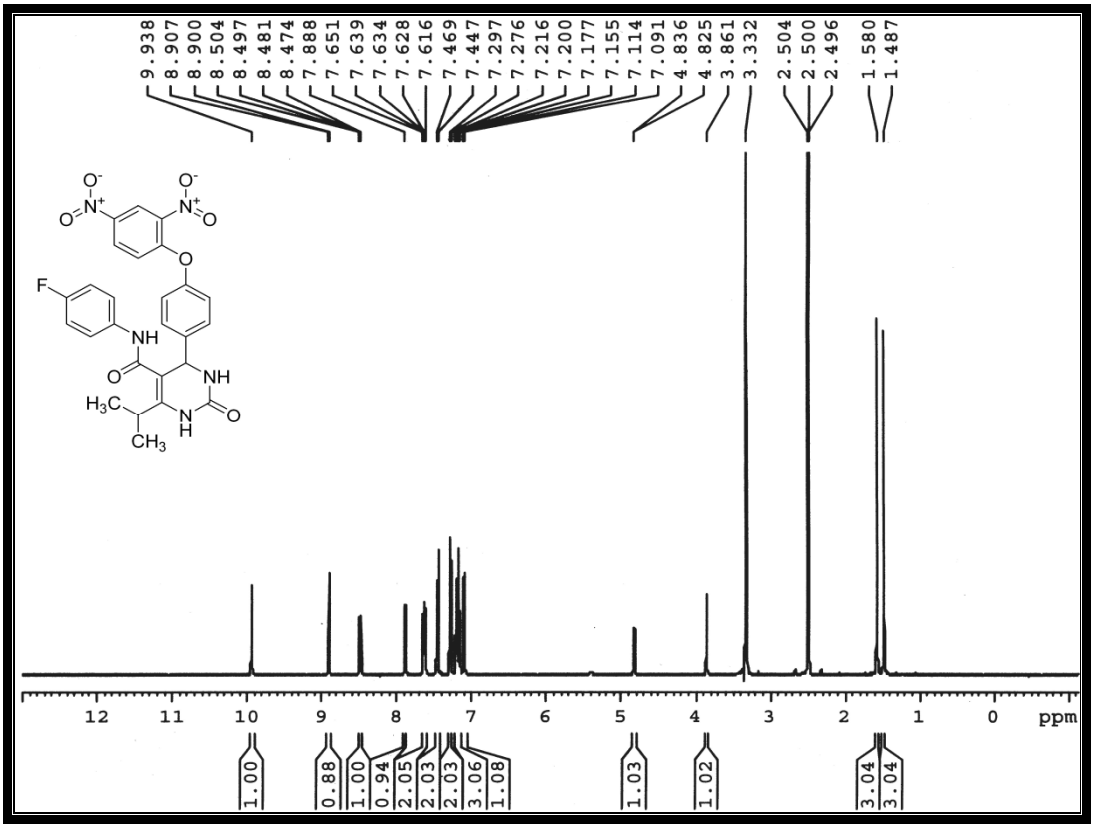
## *IR spectrum of RHK - 138*



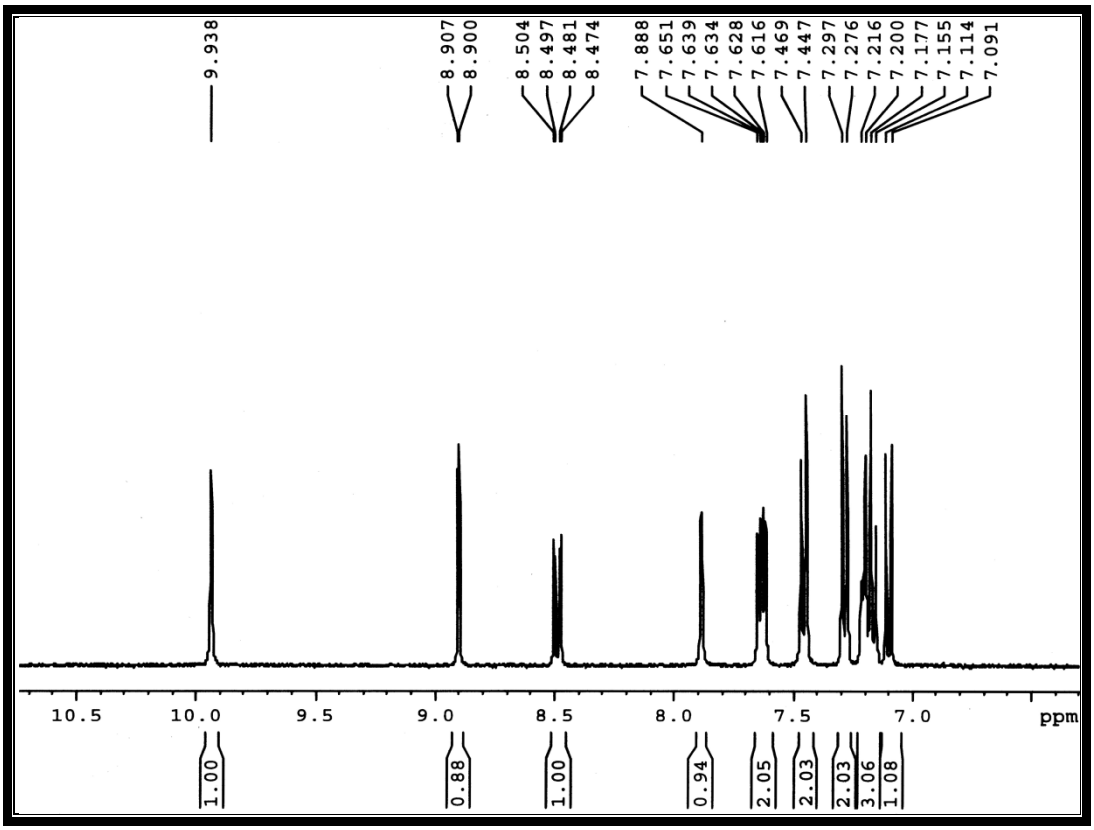

## *Mass spectrum of RHK - 138*



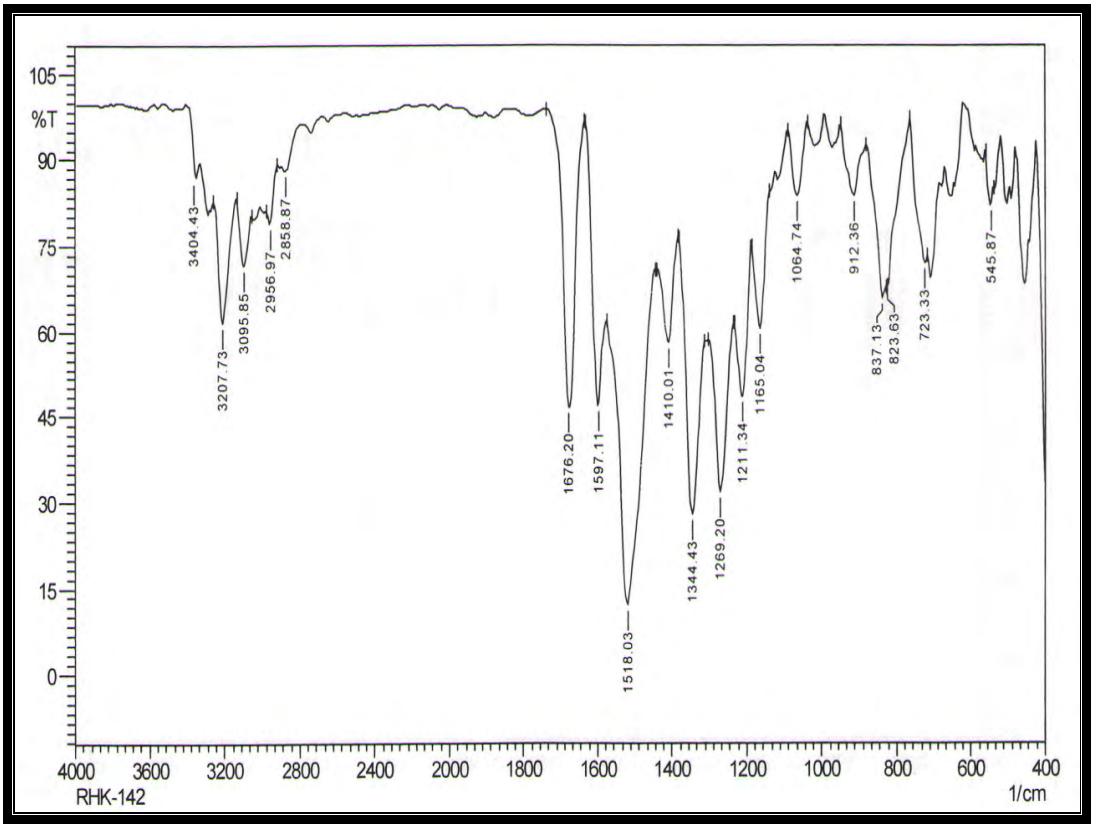
## *$^1$H NMR spectrum of RHK - 138*



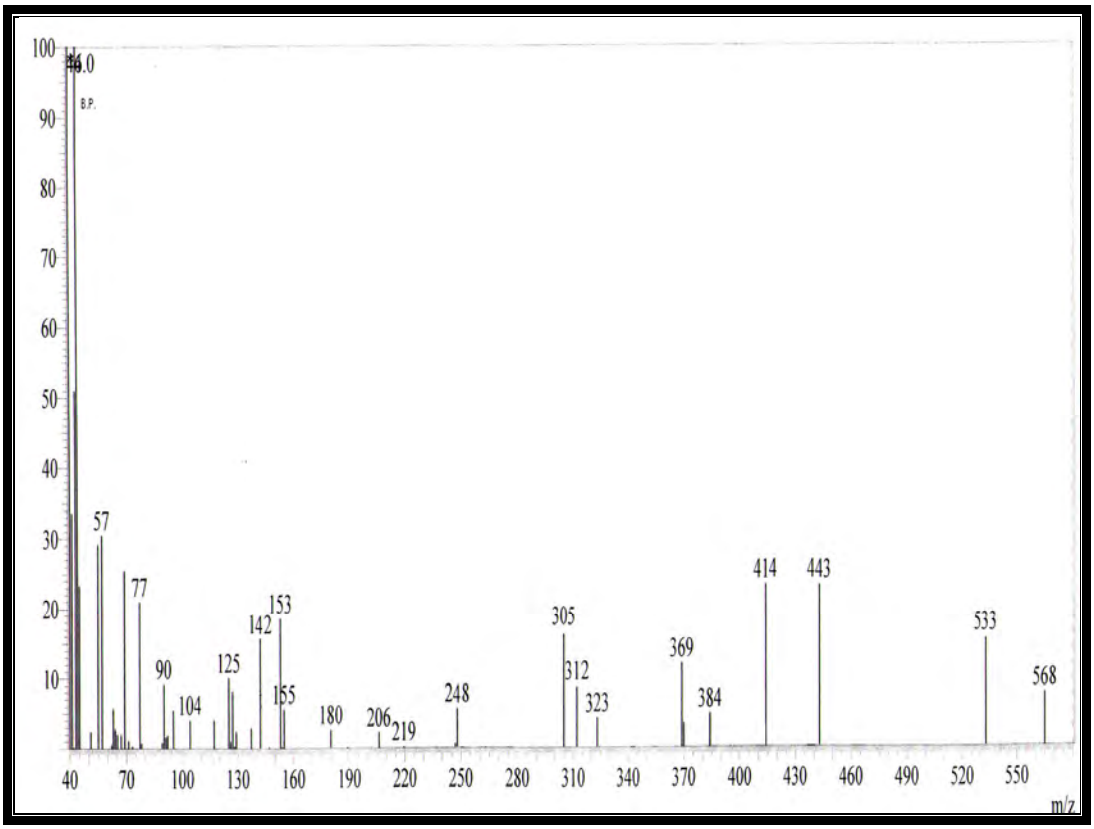

## *Expanded $^1$H NMR spectrum of RHK - 138*



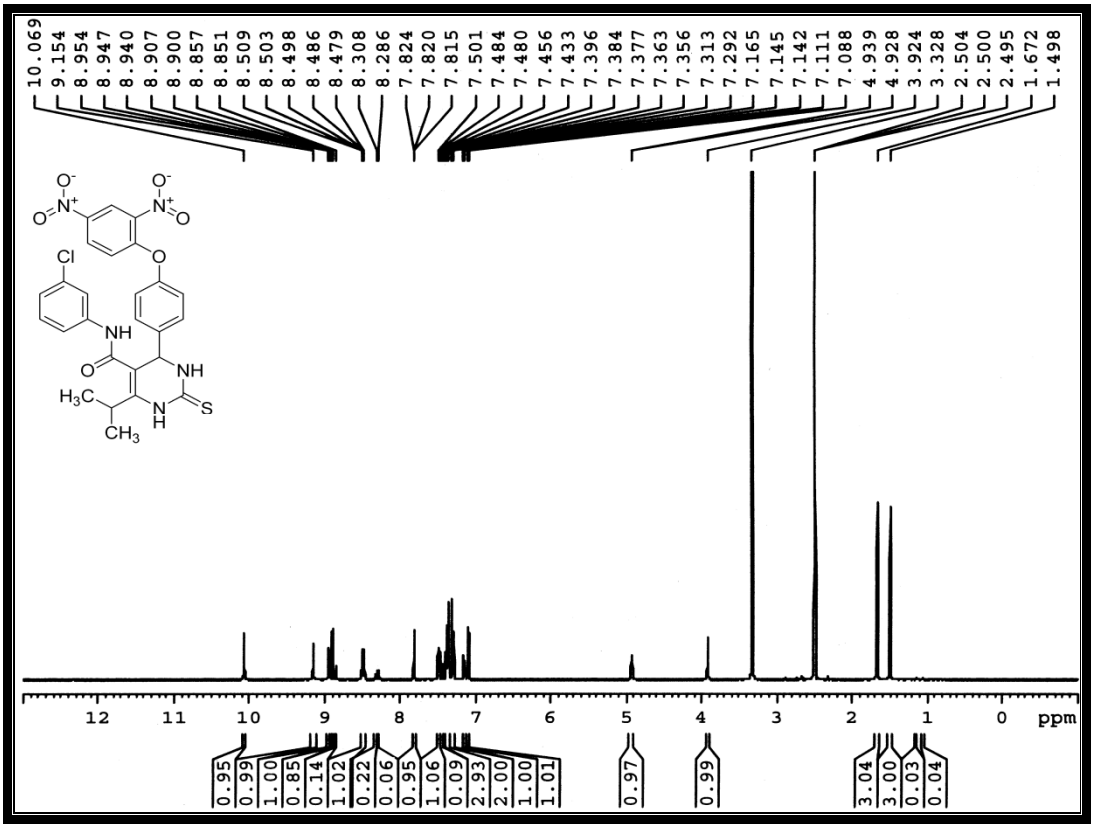
## *IR spectrum of RHK - 142*



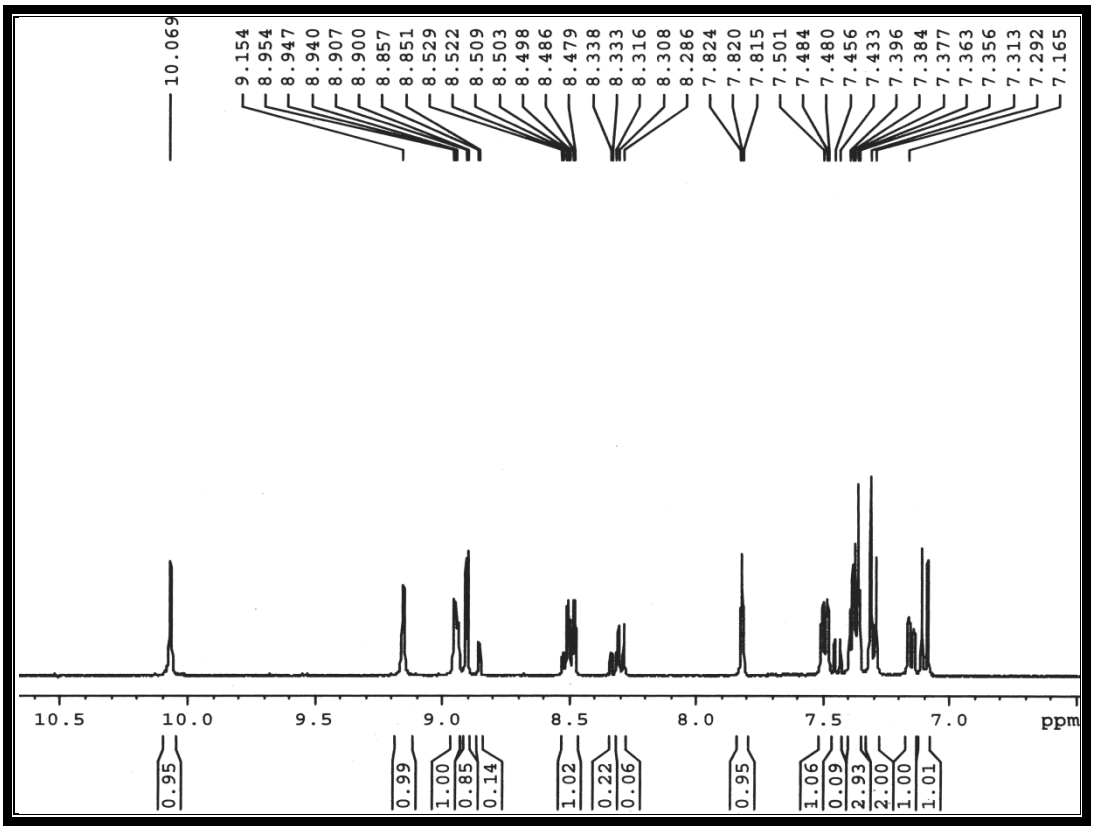

## *Mass spectrum of RHK - 142*



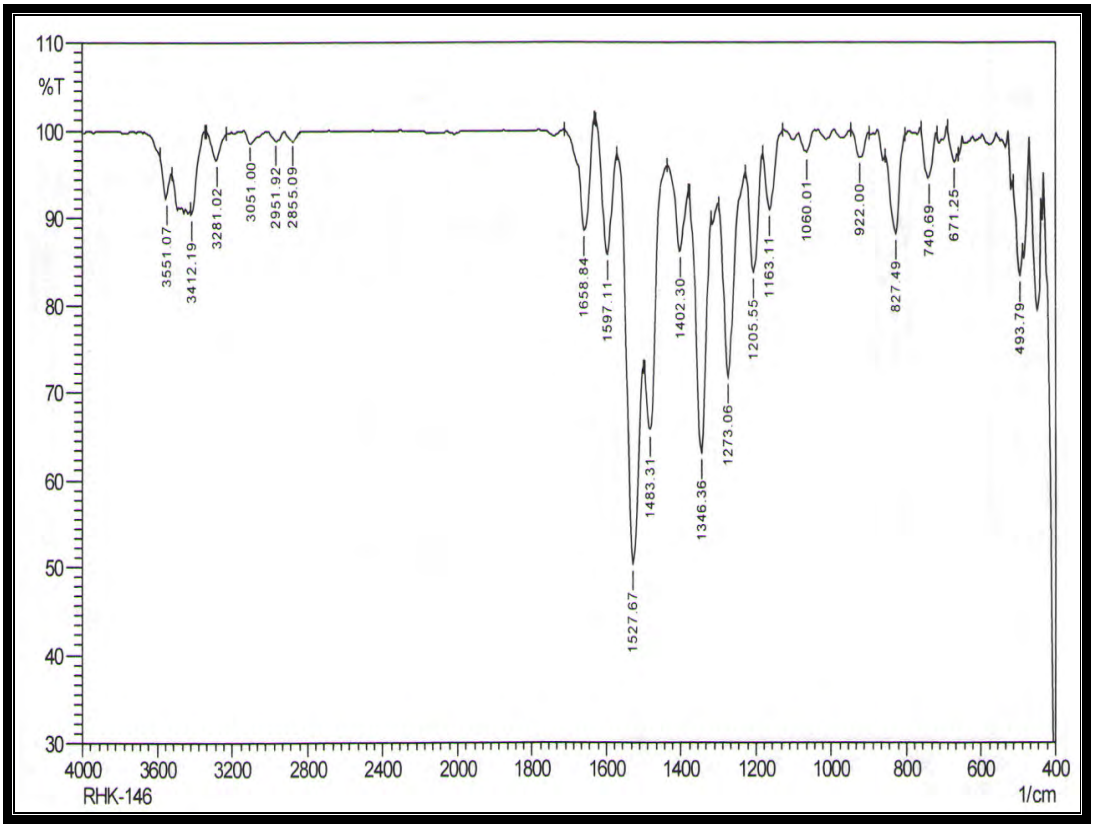
## *$^1$H NMR spectrum of RHK - 142*



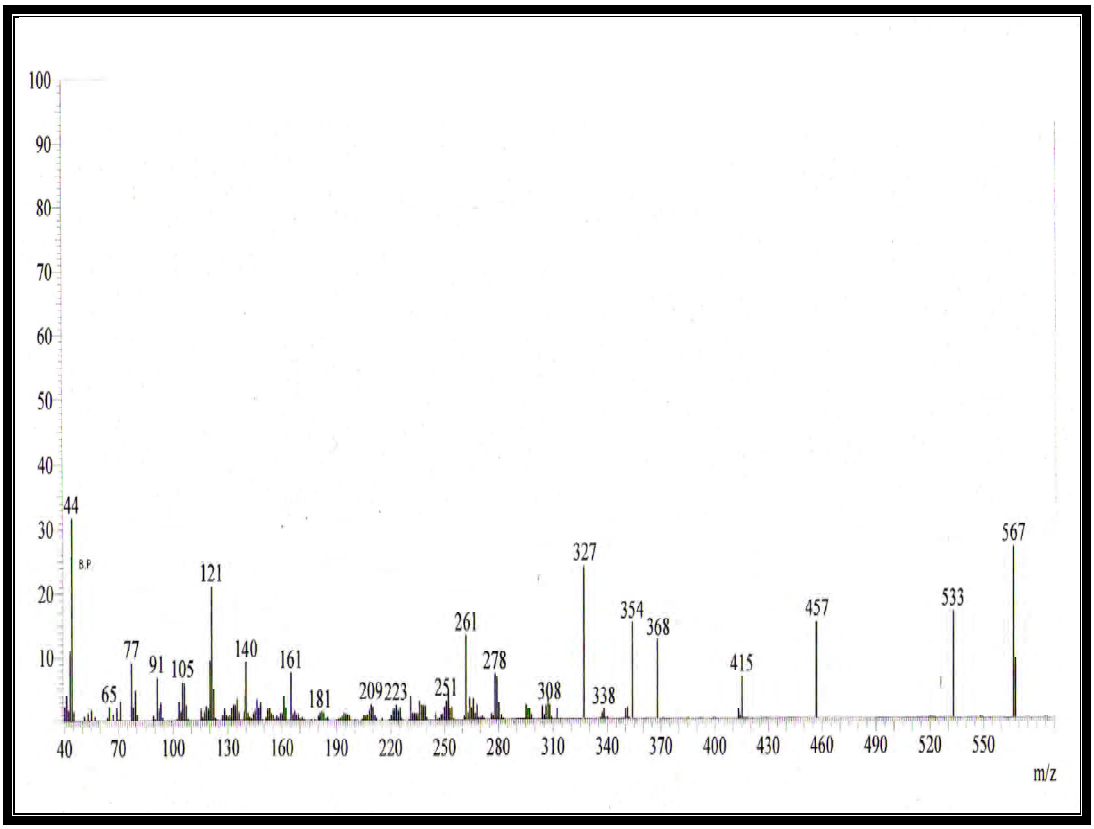

## *Expanded $^1$H NMR spectrum of RHK - 142*



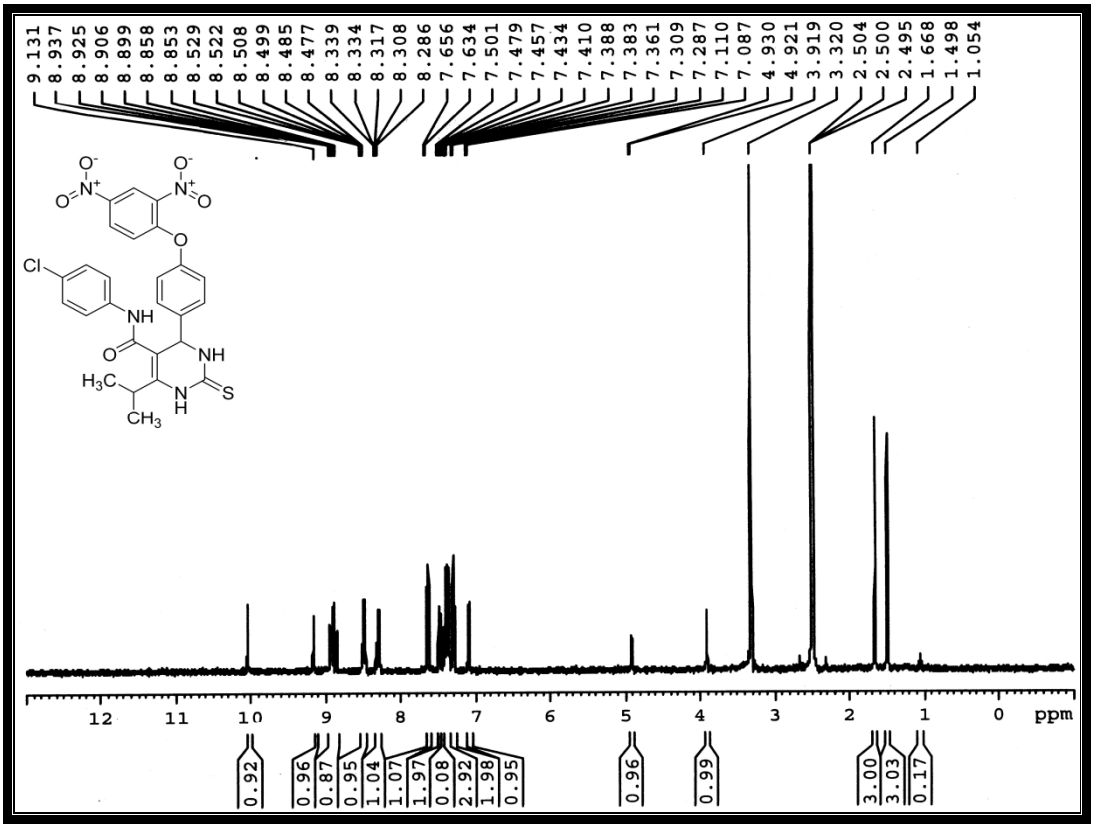
## *IR spectrum of RHK - 146*



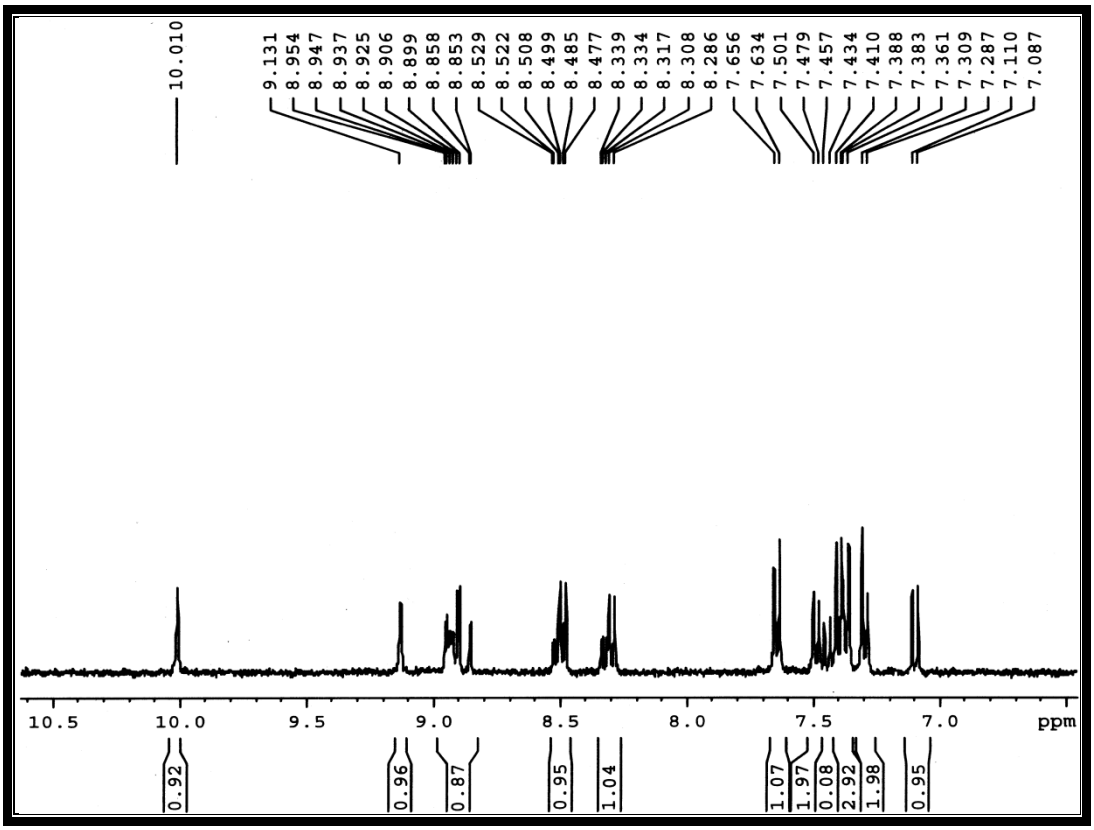

## *Mass spectrum of RHK - 146*



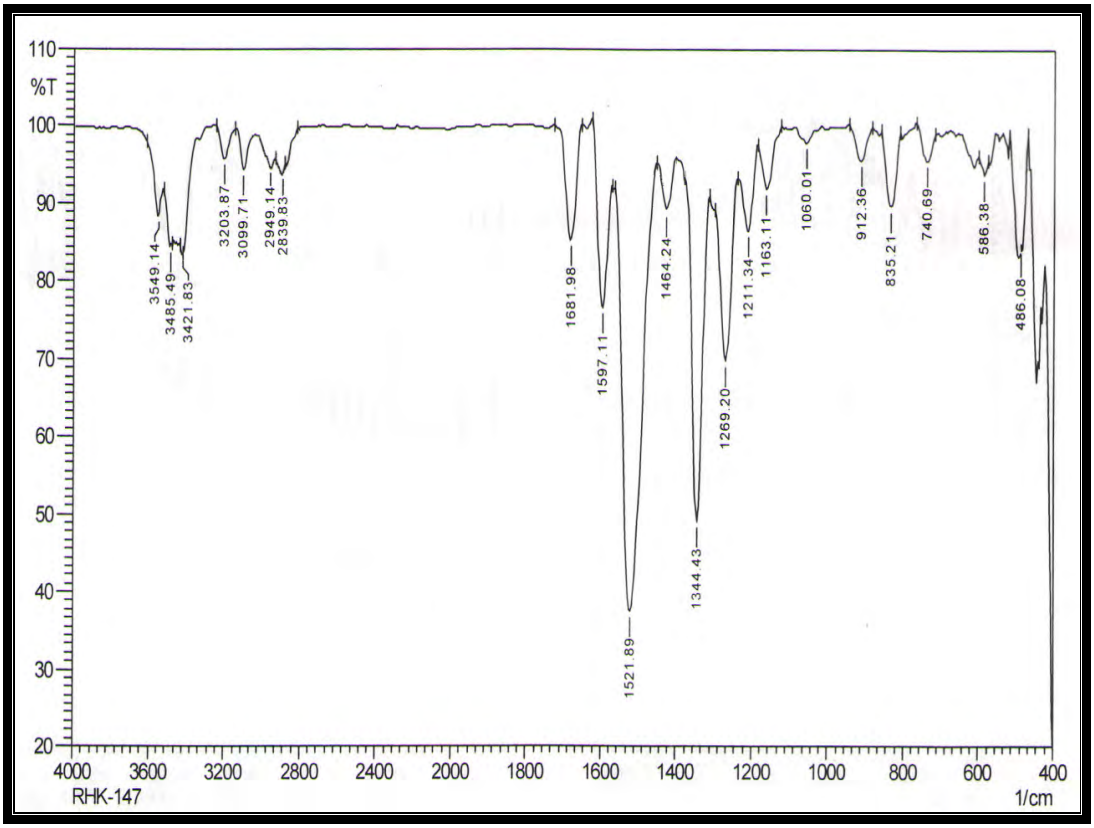
## *$^1$H NMR spectrum of RHK - 146*



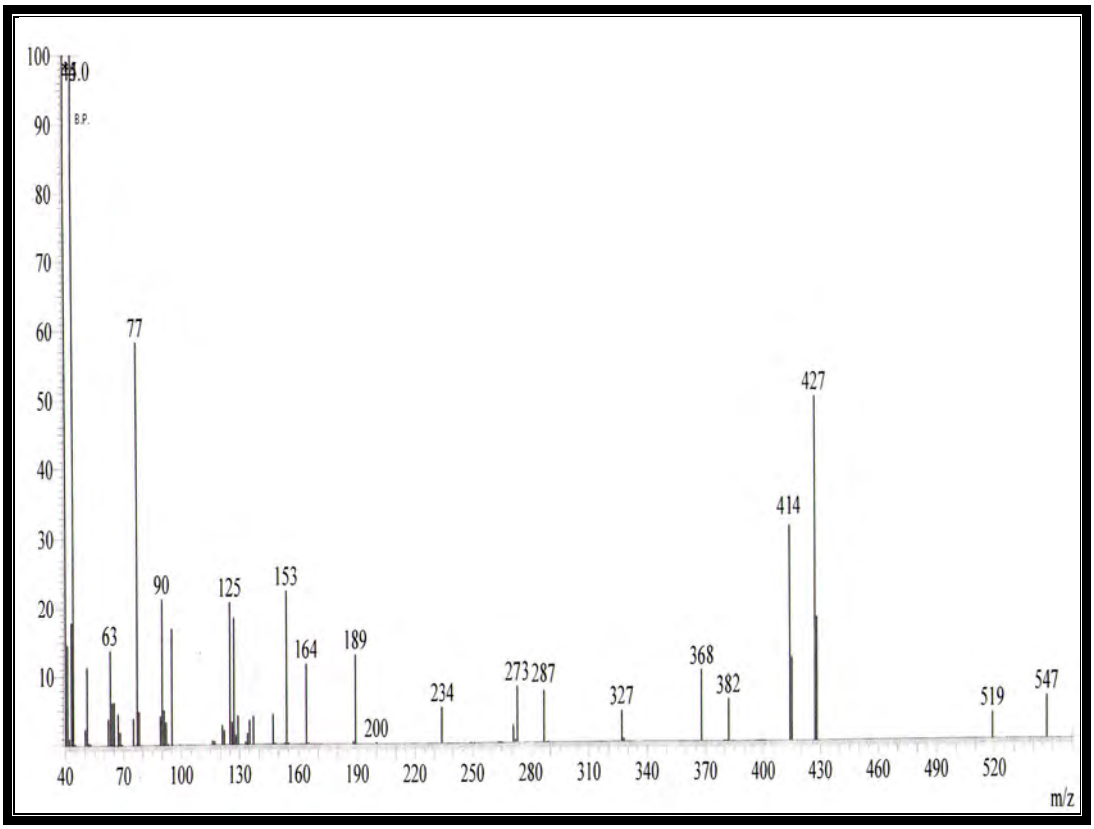

## *Expanded $^1$H NMR spectrum of RHK - 146*



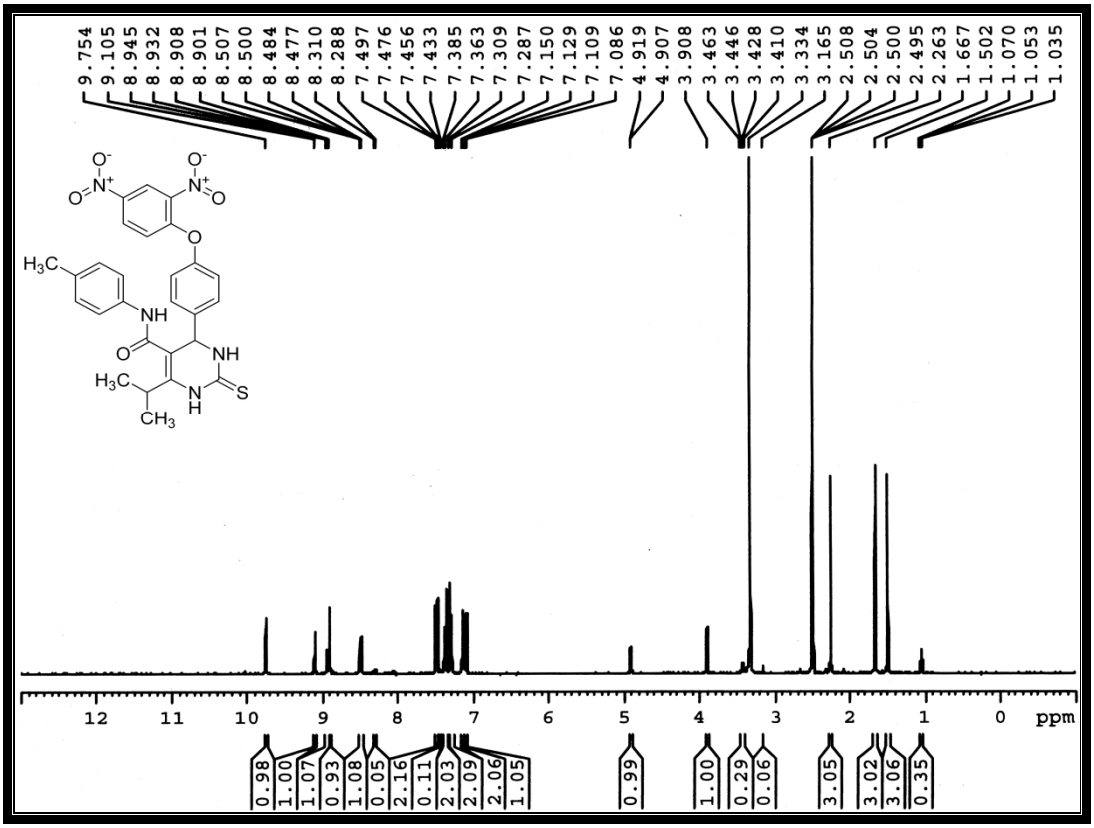
## *IR spectrum of RHK - 147*



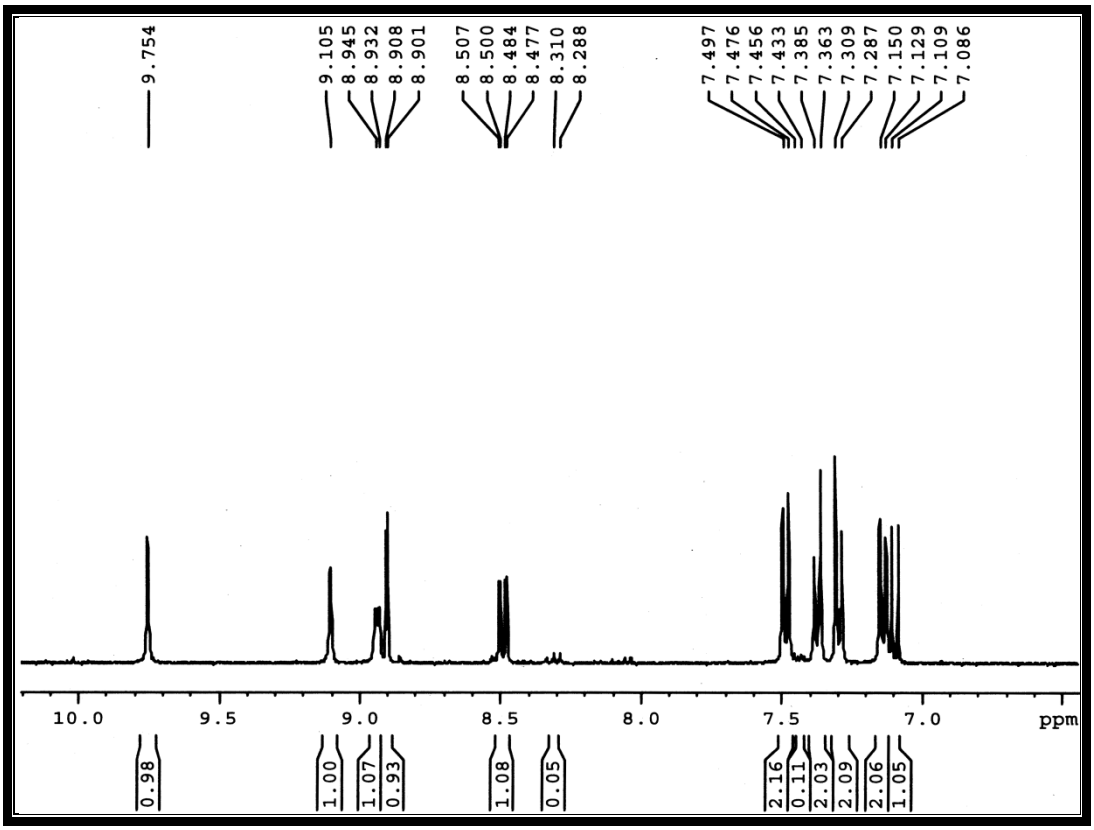

## *Mass spectrum of RHK - 147*



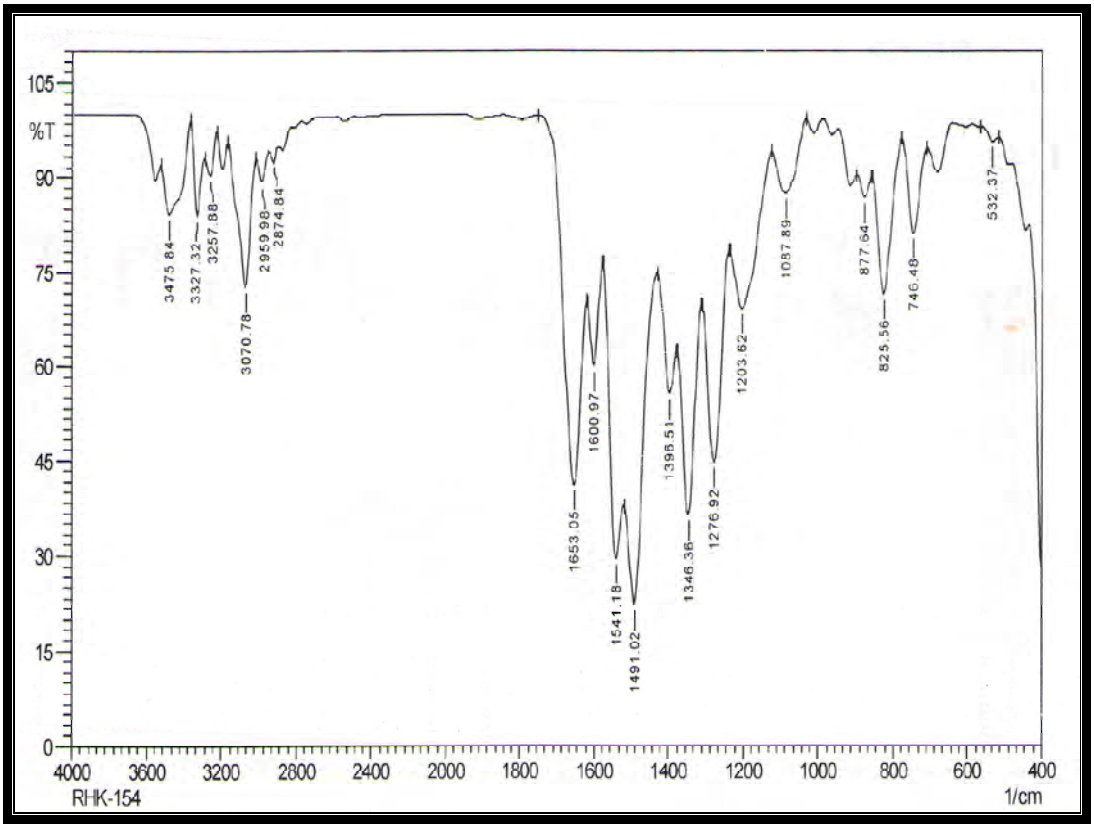
## *$^1$H NMR spectrum of RHK - 147*



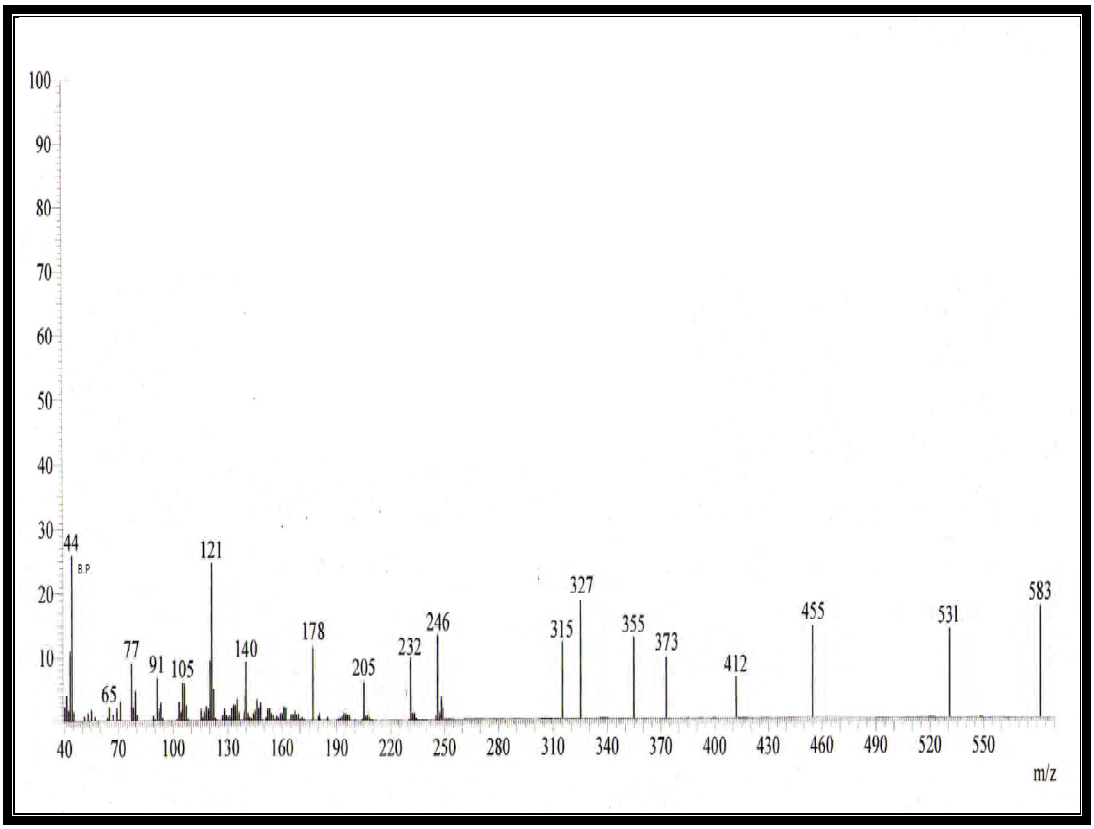

## *Expanded $^1$H NMR spectrum of RHK - 147*



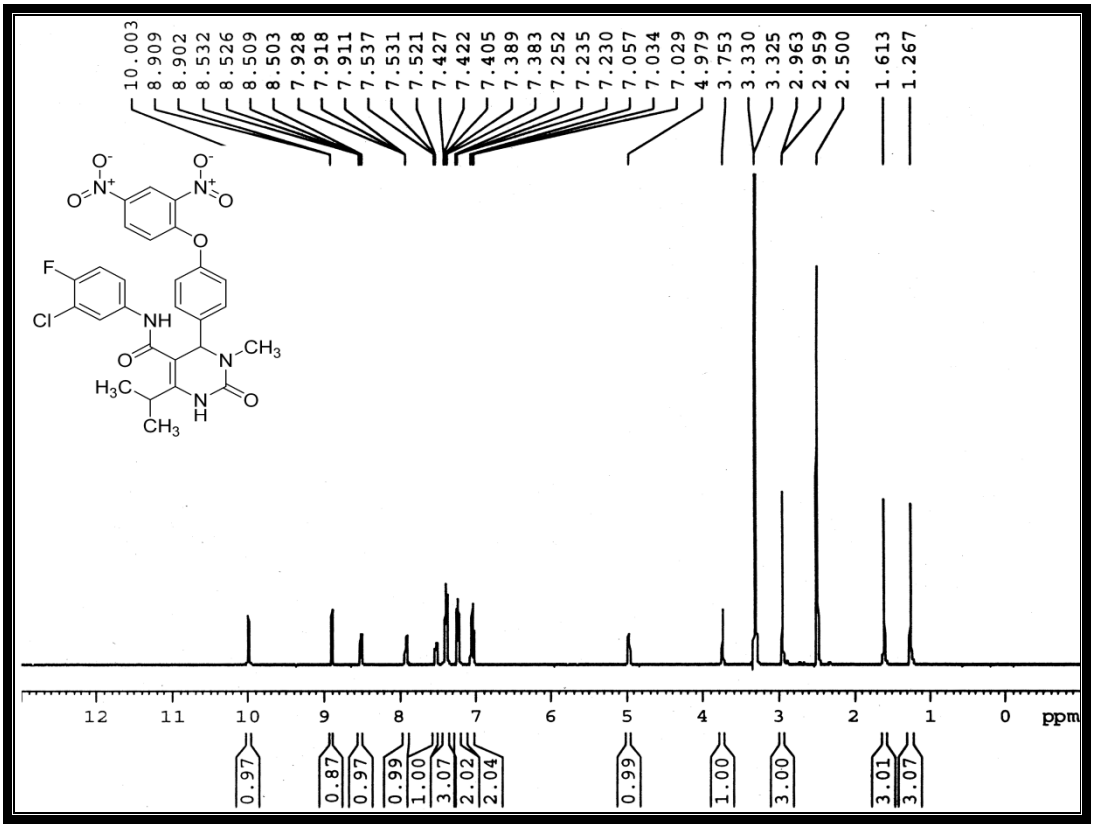
## *IR spectrum of RHK - 154*



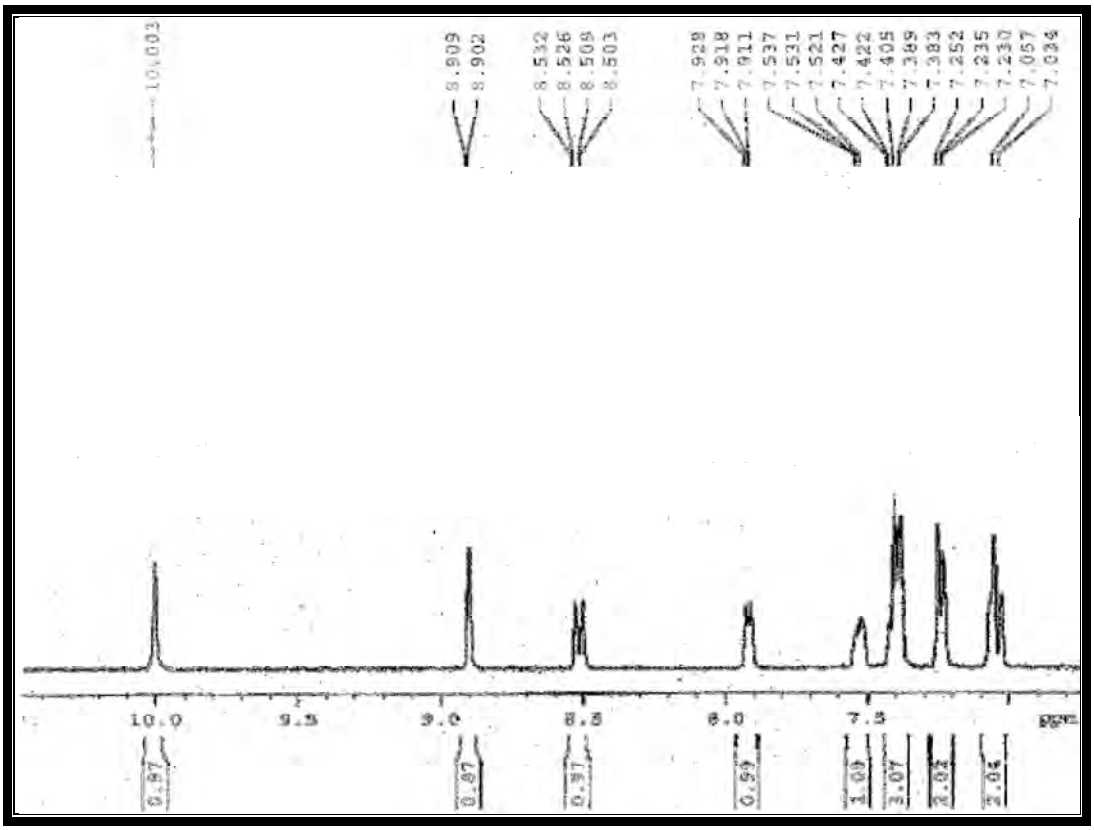

## *Mass spectrum of RHK - 154*



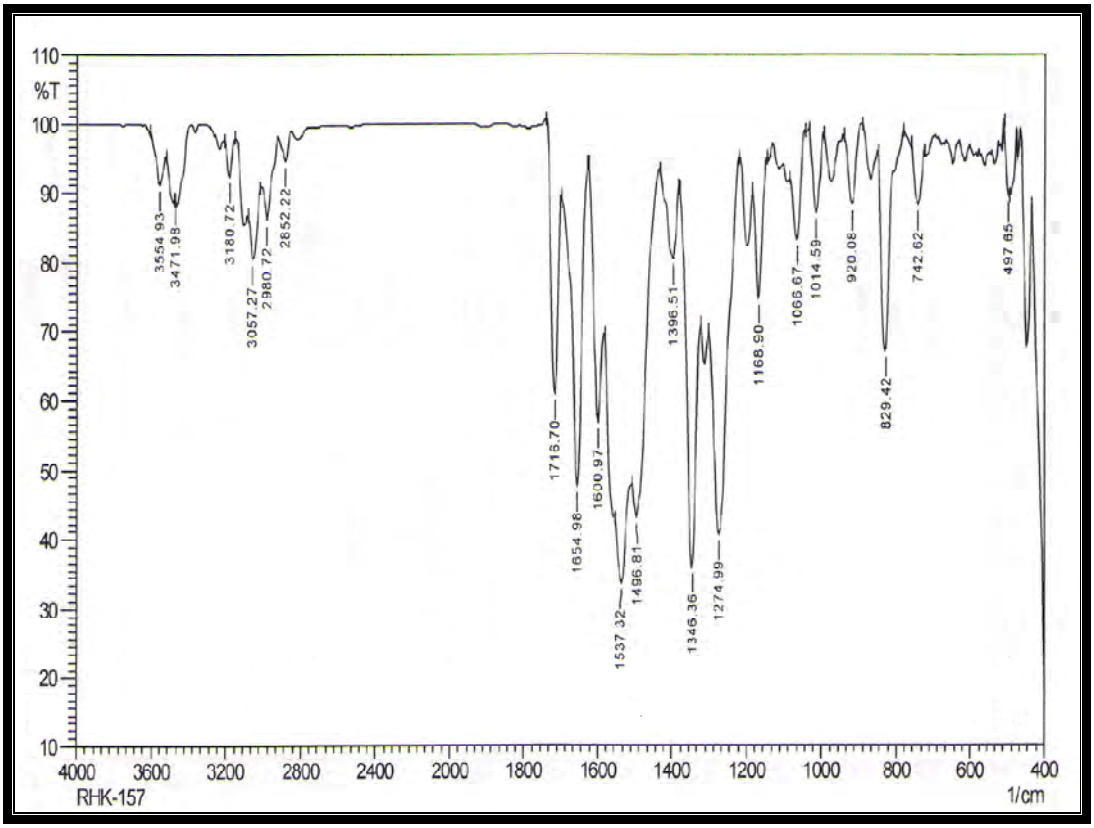
## *$^1$H NMR spectrum of RHK - 154*



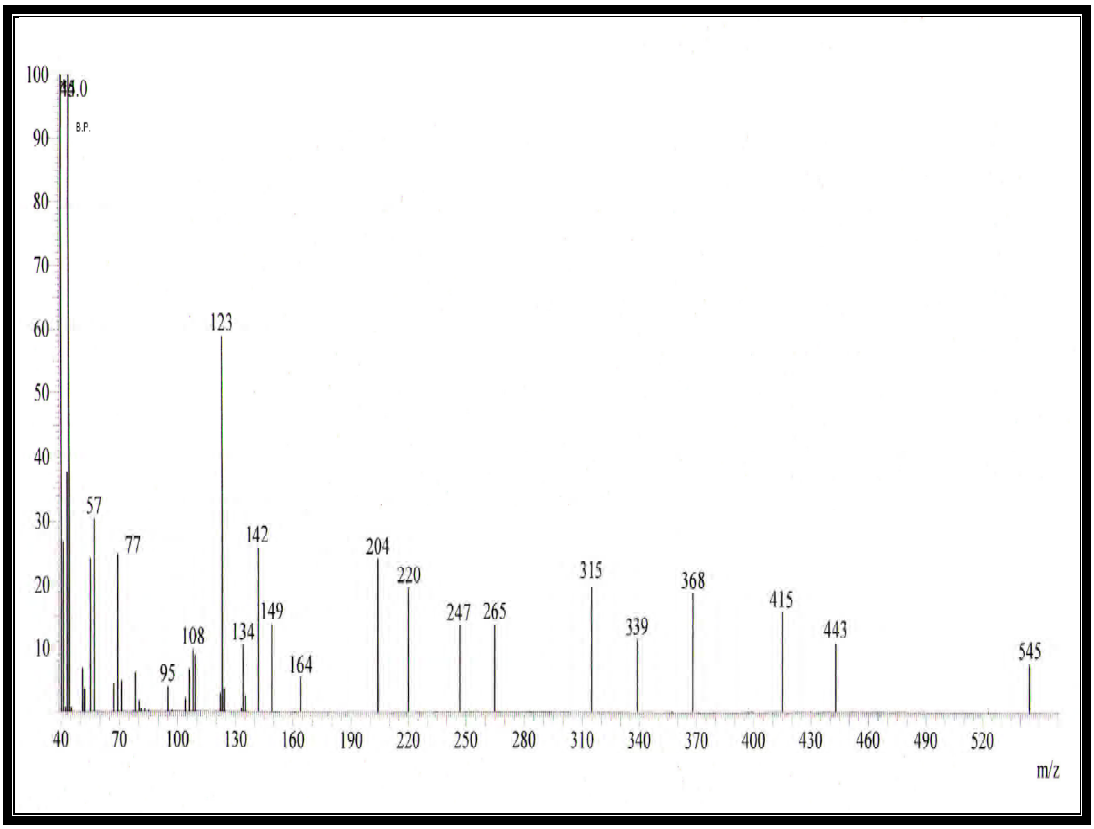

## *Expanded $^1$H NMR spectrum of RHK - 154*



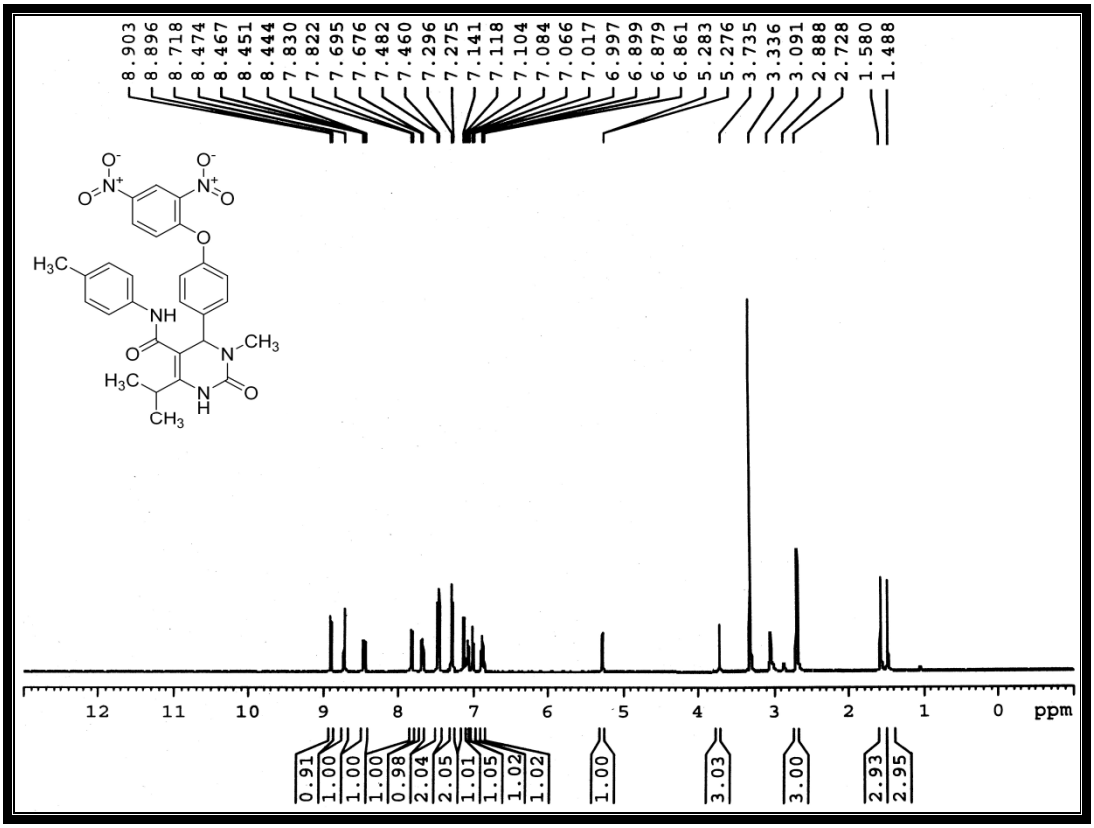
## *IR spectrum of RHK - 157*



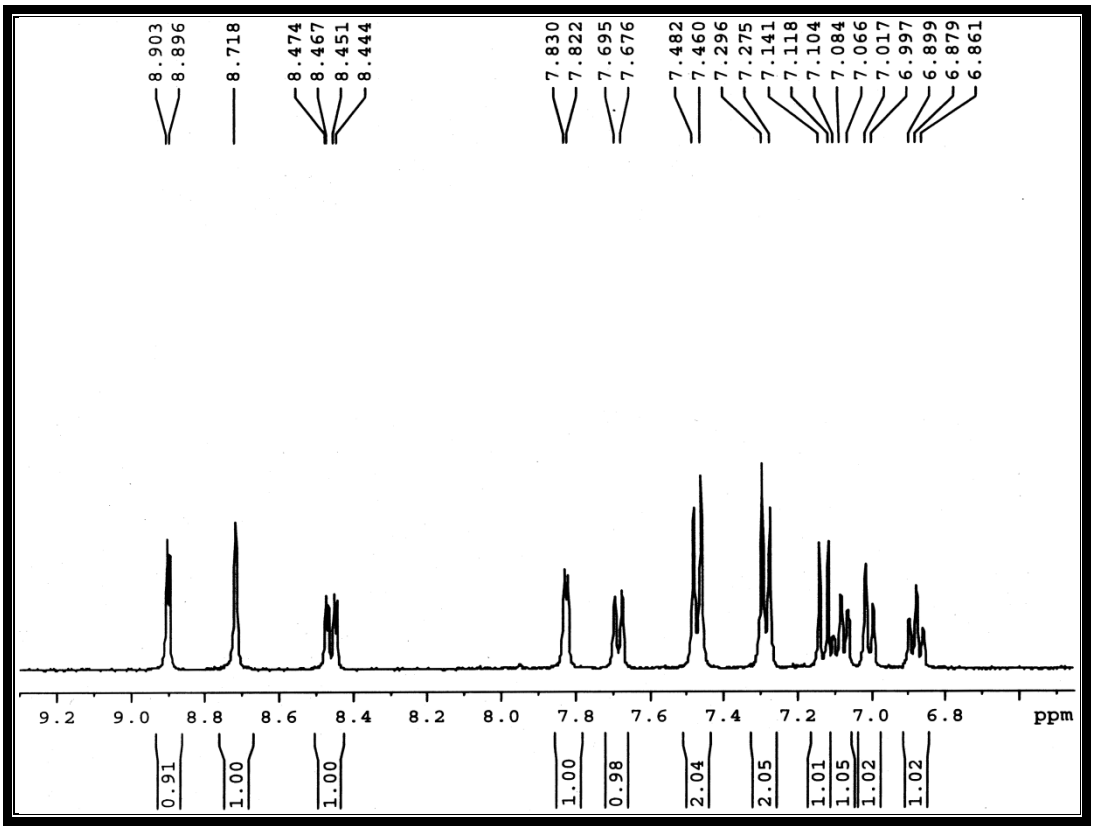

## *Mass spectrum of RHK - 157*



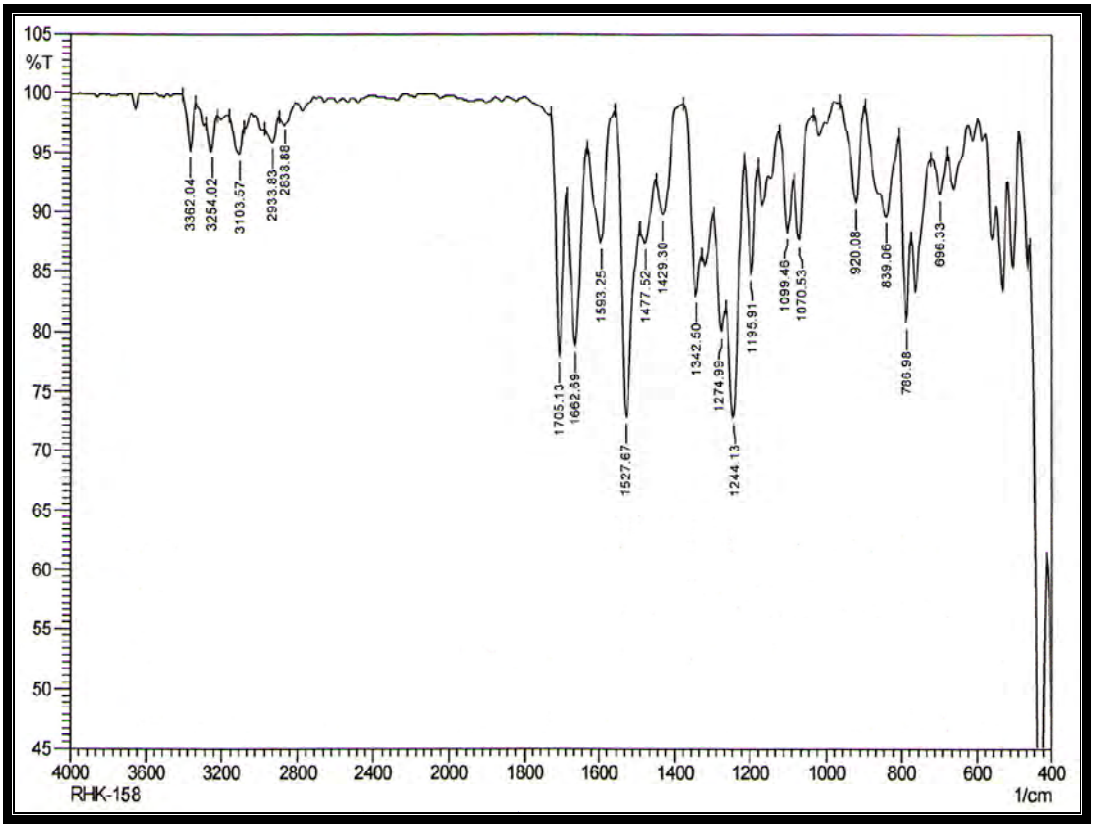
## *$^1$H NMR spectrum of RHK - 157*



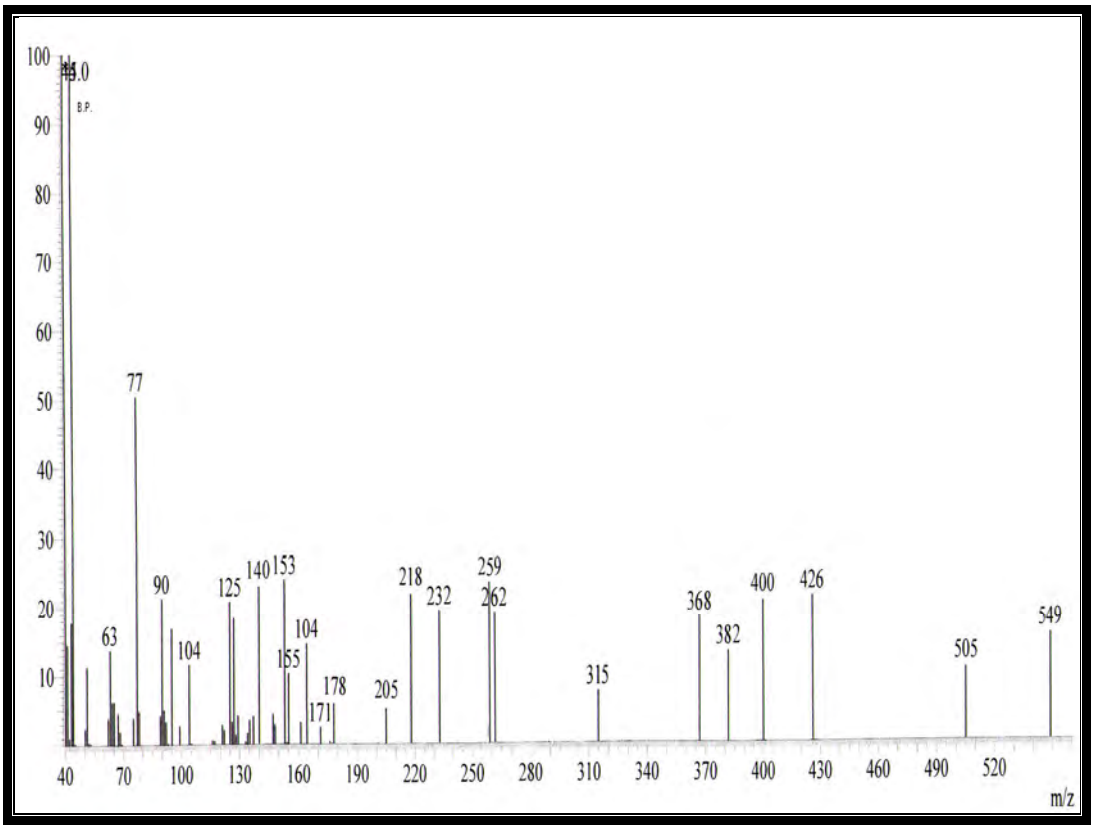

## *Expanded $^1$H NMR spectrum of RHK - 157*



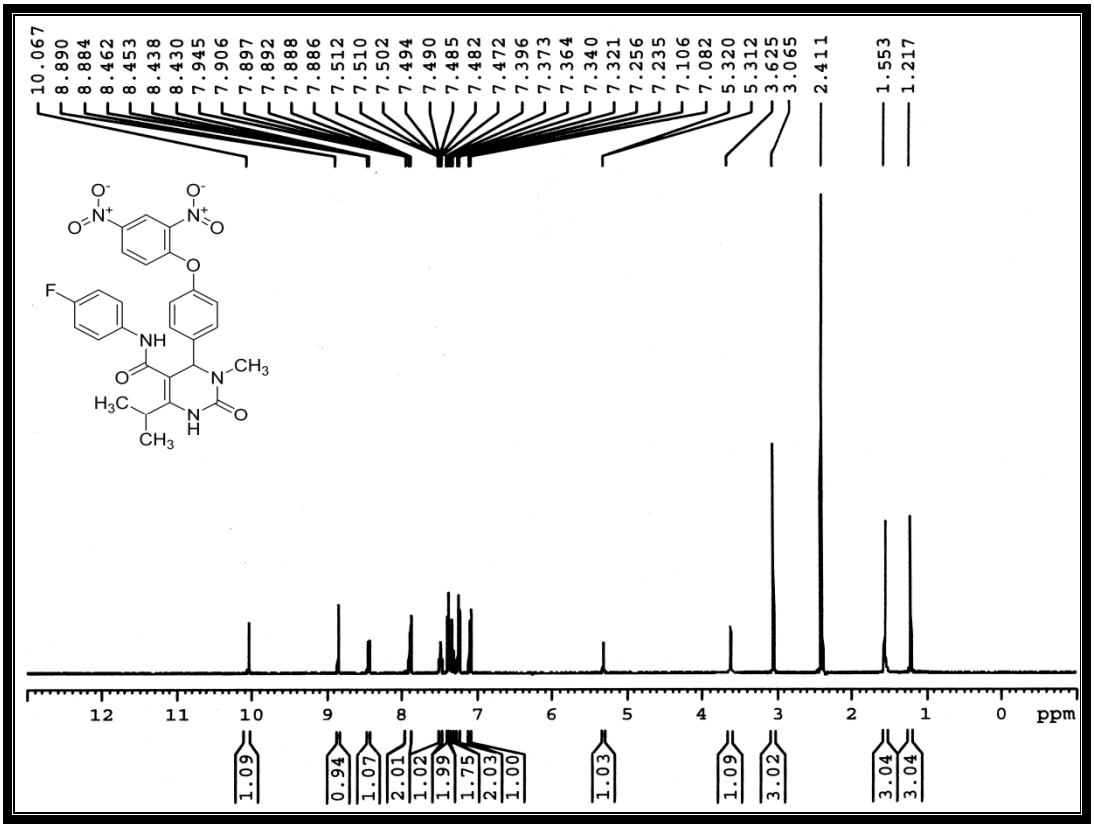
## *IR spectrum of RHK - 158*




## *Mass spectrum of RHK - 158*



*$^1$H NMR spectrum of RHK - 158*




## 3.10 Biological evaluation

### 3.10.1 Antimicrobial evaluation

All of the synthesized compounds **(RHK- 101 to 160)** were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [98-100] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound

preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards [98].

***Minimal Inhibition Concentration [MIC]:-***

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

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

***Methods used for primary and secondary screening: -***

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

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

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

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

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

**Table-1:-** ***in vitro Antimicrobial Screening Results for RHK-101 to 160***

| Code | Minimal inhibition concentration (µg $mL^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-positive | | Gram-negative | | Fungal species | | |
| | *S.a.* | *S. p.* | *E.c.* | *P.a.* | *C. a.* | *A. n.* | *A.c.* |
| RHK-101 | 500 | 500 | 500 | 500 | 250 | 1000 | 500 |
| RHK-102 | 500 | 1000 | 1000 | 1000 | >1000 | >1000 | >1000 |
| RHK-103 | 100 | 100 | 250 | 200 | 1000 | 500 | 500 |
| RHK-104 | 1000 | 500 | 1000 | 1000 | 1000 | 500 | 1000 |
| RHK-105 | 200 | 100 | 100 | 200 | 250 | 1000 | 1000 |
| RHK-106 | 1000 | 1000 | 500 | 500 | 250 | 1000 | 1000 |
| RHK-107 | 500 | 500 | 250 | 250 | 250 | 1000 | 1000 |
| RHK-108 | 100 | 100 | 200 | 250 | 1000 | 500 | 1000 |
| RHK-109 | 62.5 | 1000 | 200 | 1000 | 500 | >1000 | 1000 |
| RHK-110 | 150 | 250 | 100 | 150 | 500 | 500 | 1000 |
| RHK-111 | 1000 | 500 | 62.5 | 62.5 | >1000 | >1000 | >1000 |
| RHK-112 | 200 | 200 | 100 | 100 | >1000 | 1000 | 500 |
| RHK-113 | 500 | 1000 | 500 | 500 | 500 | >1000 | >1000 |
| RHK-114 | 150 | 250 | 100 | 150 | 500 | 500 | 500 |
| RHK-115 | 100 | 62.5 | 200 | 250 | 500 | 500 | 500 |
| RHK-116 | 200 | 200 | 200 | 250 | 500 | 500 | 500 |
| RHK-117 | 200 | 200 | 100 | 200 | 250 | 500 | 500 |
| RHK-118 | 100 | 100 | 250 | 500 | 500 | 250 | 250 |
| RHK-119 | 1000 | 500 | 1000 | 500 | 500 | 500 | 500 |
| RHK-120 | 1000 | 1000 | 1000 | 500 | 1000 | 1000 | 1000 |
| RHK-121 | 250 | 500 | 500 | 250 | 500 | 500 | 500 |
| RHK-122 | 250 | 500 | 200 | 250 | 250 | 200 | 200 |
| RHK-123 | 100 | 62.5 | 500 | 250 | 100 | 250 | 250 |
| RHK-124 | 250 | 250 | 500 | 500 | 200 | >1000 | >1000 |
| RHK-125 | 62.5 | 500 | 200 | 1000 | 500 | 500 | 500 |
| RHK-126 | 250 | 500 | 250 | 250 | 250 | >1000 | >1000 |
| RHK-127 | 200 | 200 | 100 | 100 | 250 | >1000 | >1000 |
| RHK-128 | 150 | 200 | 250 | 150 | 1000 | 500 | 500 |
| RHK-129 | 62.5 | 500 | 500 | 1000 | 1000 | 500 | 1000 |
| RHK-130 | 250 | 500 | 1000 | 1000 | 250 | 1000 | 1000 |
| RHK-131 | 1000 | 250 | 62.5 | 62.5 | 250 | 1000 | 1000 |
| RHK-132 | 200 | 200 | 62.5 | 100 | 500 | >1000 | >1000 |
| RHK-133 | 500 | 500 | 250 | 500 | 1000 | 500 | 500 |
| RHK-134 | 200 | 200 | 100 | 200 | >1000 | >1000 | >1000 |
| RHK-135 | 250 | 250 | 100 | 250 | 250 | >1000 | >1000 |
| RHK-136 | 62.5 | 100 | 1000 | 250 | 250 | >1000 | >1000 |
| RHK-137 | 250 | 250 | 500 | 500 | 500 | 500 | >1000 |
| RHK-138 | 100 | 62.5 | 250 | 250 | 100 | 250 | 250 |
| RHK-139 | 250 | 500 | 500 | 1000 | >1000 | 250 | 250 |
| RHK-140 | 200 | 500 | 62.5 | 62.5 | >1000 | 1000 | 1000 |
| RHK-141 | 150 | 500 | 100 | 1000 | 500 | 500 | 500 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| RHK-142 | 62.5 | 100 | 200 | 250 | 500 | 500 | 500 |
| RHK-143 | 500 | 100 | 250 | 250 | 250 | 1000 | 500 |
| RHK-144 | 250 | 500 | 250 | 1000 | >1000 | >1000 | >1000 |
| RHK-145 | 62.5 | 250 | 200 | 100 | 1000 | 500 | 500 |
| RHK-146 | 250 | 500 | 250 | 500 | 1000 | 1000 | 1000 |
| RHK-147 | 250 | 250 | 100 | 100 | 500 | 500 | 500 |
| RHK-148 | 200 | 250 | 62.5 | 100 | 500 | 500 | 500 |
| RHK-149 | 1000 | 500 | 500 | 1000 | 250 | 1000 | 1000 |
| RHK-150 | 250 | 500 | 100 | 100 | 500 | 250 | 250 |
| RHK-151 | 62.5 | 200 | 200 | 62.5 | 500 | 200 | 200 |
| RHK-152 | 100 | 1000 | 200 | 250 | >1000 | 500 | 250 |
| RHK-153 | 100 | 500 | 1000 | 1000 | 500 | 500 | 500 |
| RHK-154 | 150 | 200 | 100 | 150 | 250 | 250 | 250 |
| RHK-155 | 250 | 250 | 100 | 250 | 500 | 500 | 500 |
| RHK-156 | 500 | 500 | 250 | 250 | 500 | 500 | 500 |
| RHK-157 | 200 | 200 | 62.5 | 250 | 500 | 250 | 250 |
| RHK-158 | 1000 | 500 | 500 | 250 | 500 | 200 | 200 |
| RHK-159 | 62.5 | 200 | 250 | 1000 | >1000 | 500 | 250 |
| RHK-160 | 250 | 250 | 500 | 200 | 500 | 500 | 500 |
| Gentamycin | 0.25 | 0.5 | 0.05 | 1 | - | - | - |
| Ampicillin | 250 | 100 | 100 | 100 | - | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| Iprofloxacin | 50 | 50 | 25 | 25 | - | - | - |
| Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| Nystatin | - | - | - | - | 100 | 100 | 100 |
| Greseofulvin | - | - | - | - | 500 | 100 | 100 |

### 3.10.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds **RHK-101 to 160** is currently under investigation and results are awaited.

## 3.11 References and Notes

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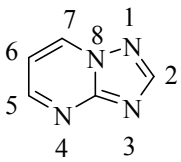
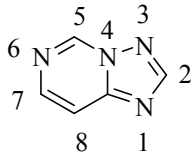
# CHAPTER 4
# Synthesis and biological evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines

## 4.1 Introduction

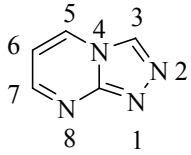
The condensation of a ring of 1,2,4-triazole and another one of pyrimidine gives rise to the formation of bicyclic heterocycles known as 1,2,4-triazolopyrimidines. Four different possibilities exist for the relative orientation of both rings, so four different isomeric families of compounds are defined: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



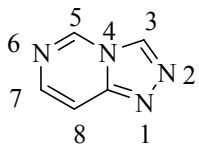
**(1) 1,2,4-triazolo[1,5-*a*]pyrimidine**



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



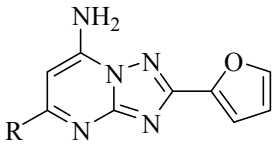
**(3) 1,2,4-triazolo[4,3-*a*]pyrimidine**



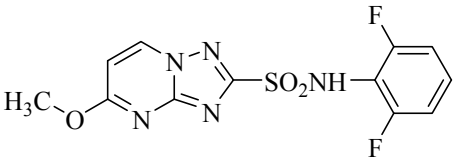
**(4) 1,2,4-triazolo[4,3-*c*]pyrimidine**

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

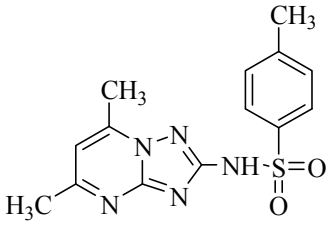
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological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



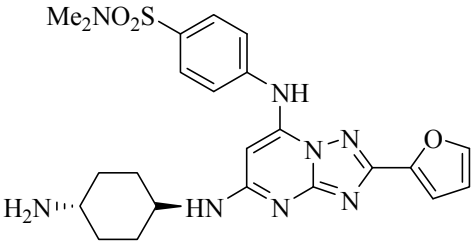
**(5) Activity: $A_{2A}$ adenosine receptor antagonists**



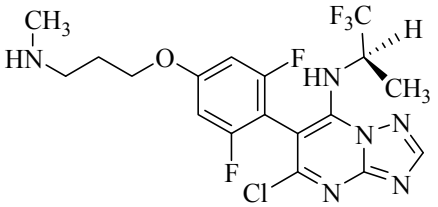
**(10) Activity: Acetohydroxyacid synthase inhibitor**



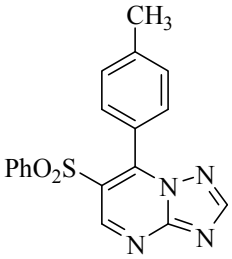
**(6) Activity: latent leishmanicidal activity**



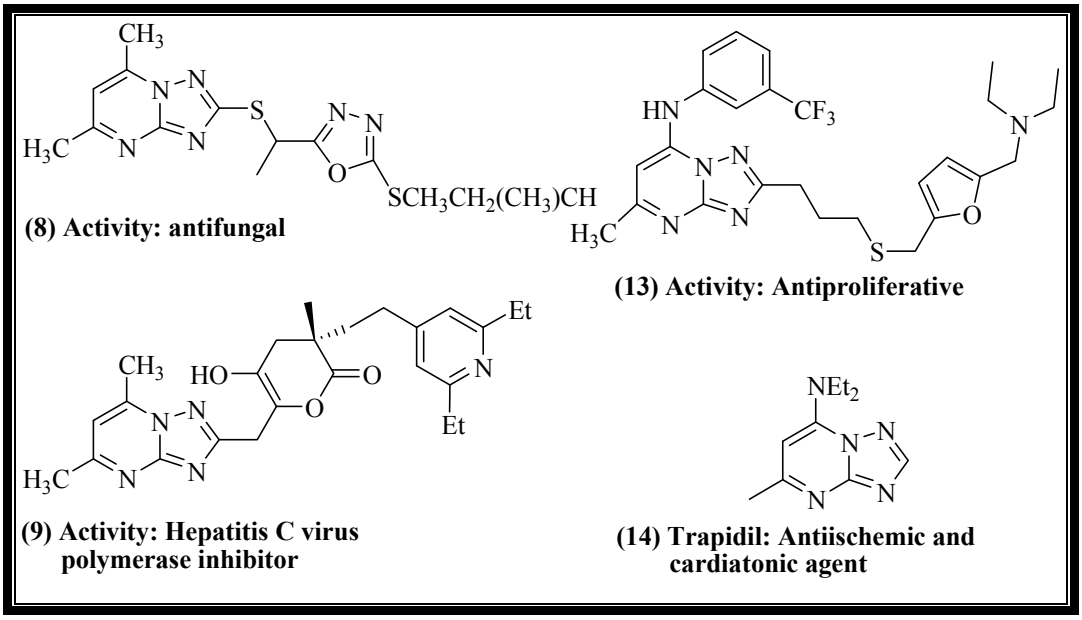
**(11) Activity: CDK-2 inhibitors**



**(7) Activity: Anticancer acticity**



**(12) Actvity: Antiinflammatory**




**(8) Activity: antifungal**

**(13) Activity: Antiproliferative**

**(9) Activity: Hepatitis C virus polymerase inhibitor**

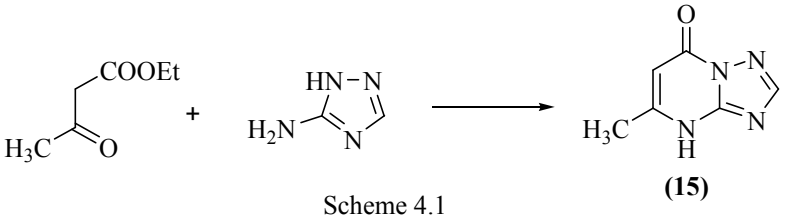
**(14) Trapidil: Antiischemic and cardiatonic agent**

## 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

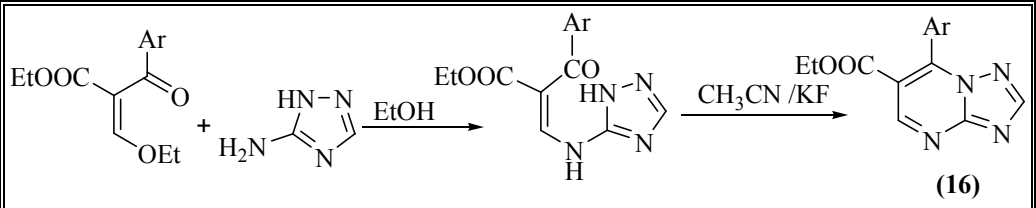
By far the most triazolo[1,5-*a*]pyrimidine synthesis are condensations of dinucleophilic 5-amino-1,2,4-triazoles with 1,3-bifunctional synthons as shown in the formation of triazolo[1,5-*a*]pyrimidine (15) (Scheme 4.1) [21-24]. New synthetic conditions recently described involve melting under microwave 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].




**(15)**

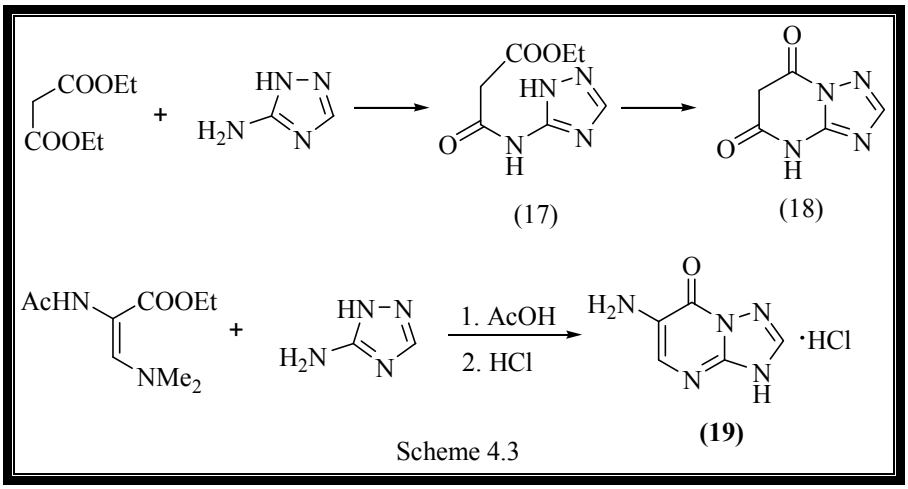
Scheme 4.1

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

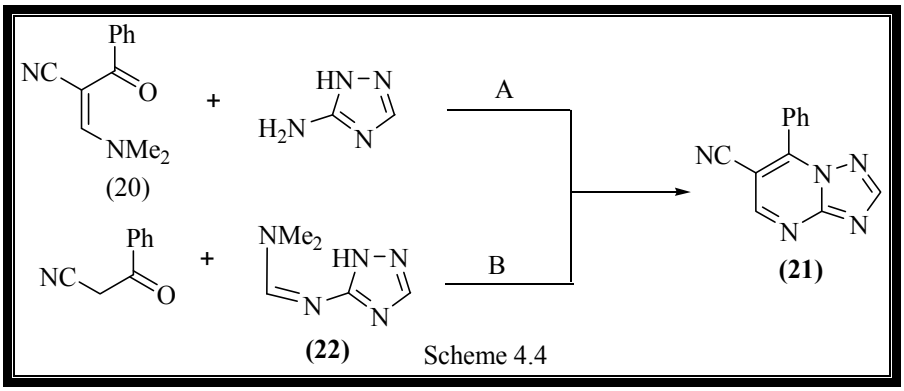
That means, the overall reaction starts with the interaction of the 5-amino-1,2,4-triazoles amino group and the enolic (or analogous) functionality of the three-carbon synthon. In the two-step examples, just mentioned, the first step proceeds under milder conditions (sometimes just in ethanol at room 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 5-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 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].

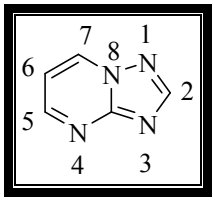


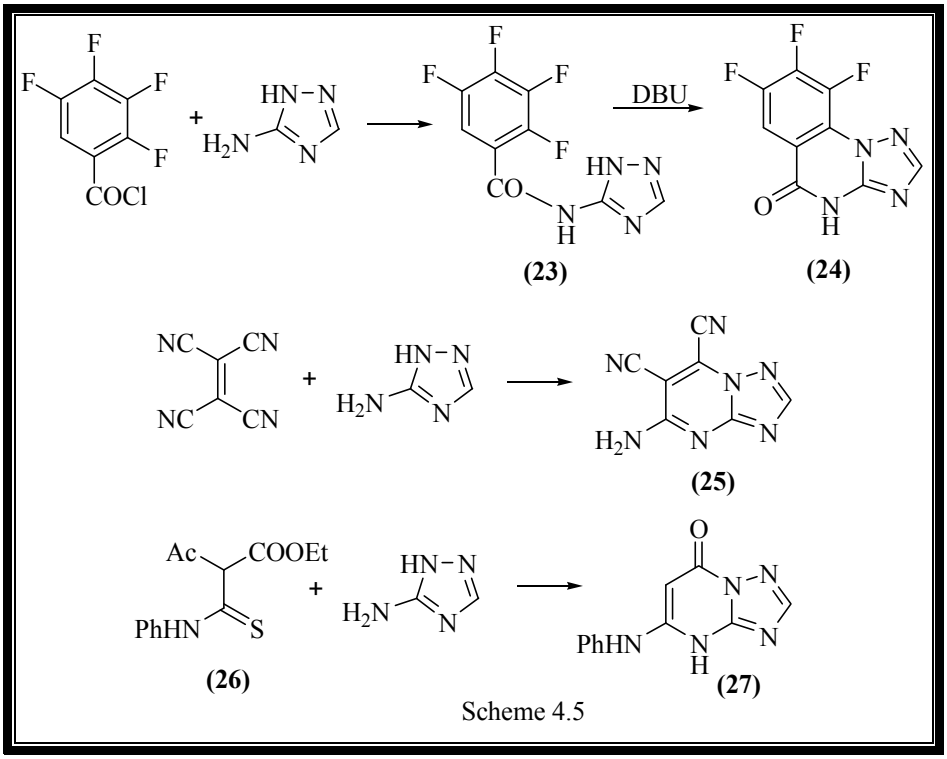
Table 1. Syntheses of triazolo[1,5-a]pyrimidines from 1,3-bifunctional synthons and 5-amino-1,2,4-triazoles

| 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].



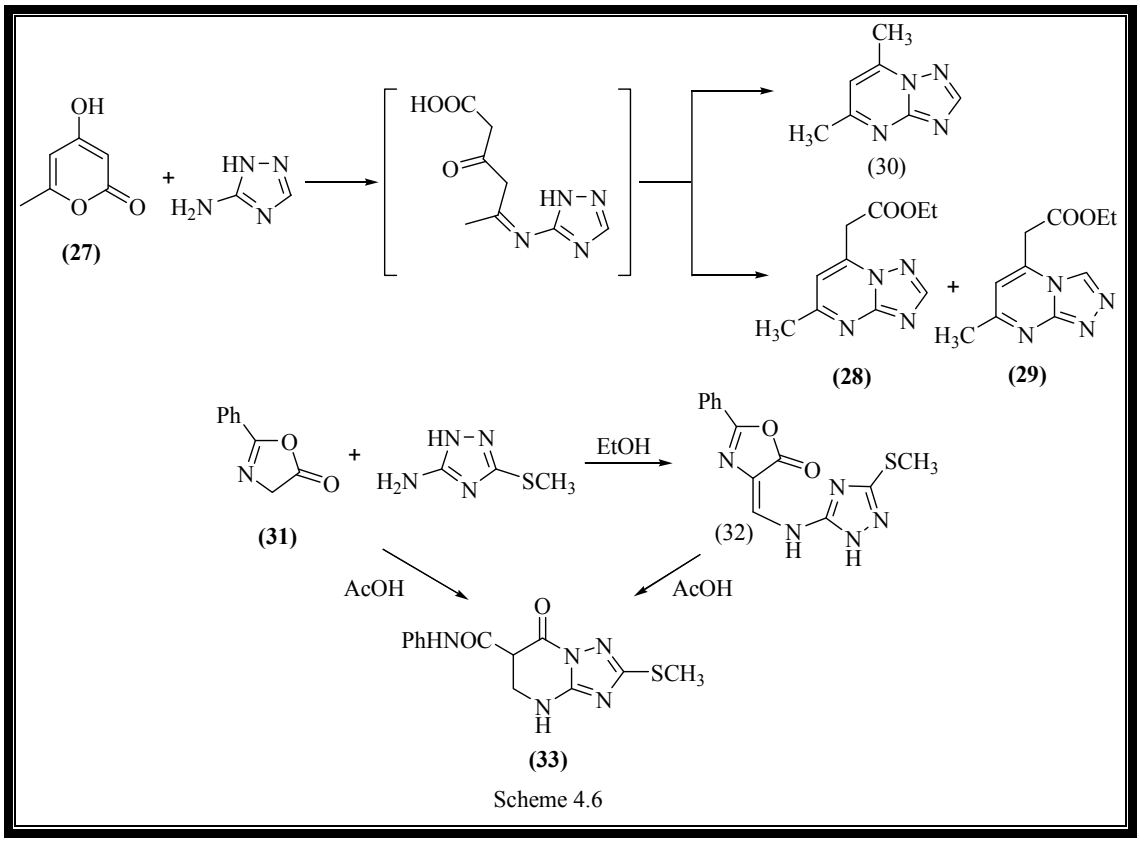

Scheme 4.5

The electron acceptor tetracyanoethylene on interaction with 5-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 [88-90]. Thus, enol ether (31) behaves as a masked 3-ethoxyacrylate

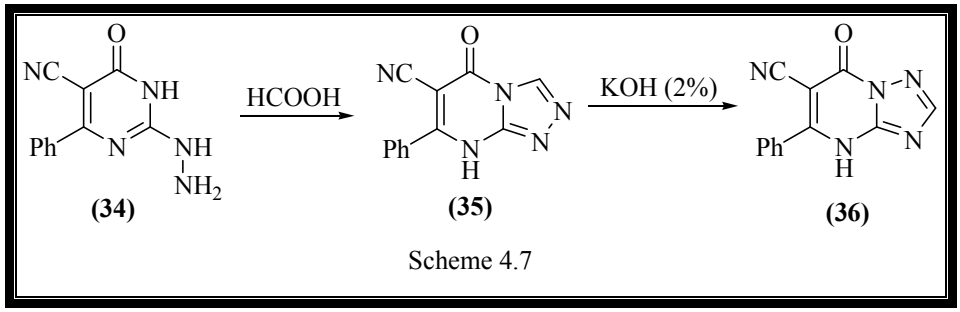
and yields, through intermediate (32), benzamido-4,5,6,7-tetrahydro-2-(methylthio)-7-oxo-N-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carbox- amide (TP) (33) that, under harsher conditions, directly forms from compound [30].




Scheme 4.6

### 4.2.3 2 Hydrazinopyrimidines and one-carbon synthons

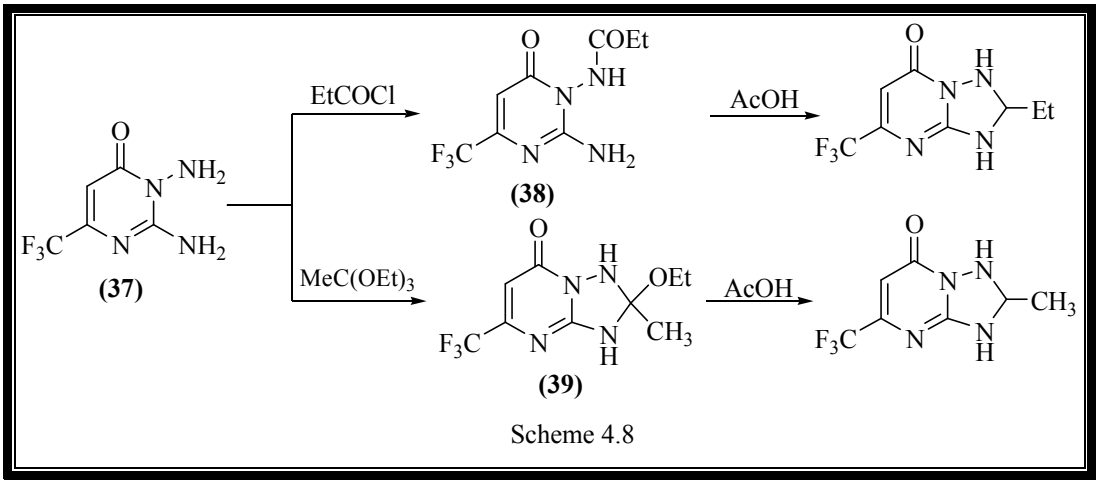
A second common triazolo[1,5-*a*]pyrimidine synthesis consists in the condensation of a $C_1$-synthon with a 2-hydrazinopyrimidine derivative (e.g., 34, Scheme 4.7). A triazolo[4,3-*a*]-pyrimidine (35) initially forms that often can be isolated [91]. Harsher conditions allow it to isomerize to the target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

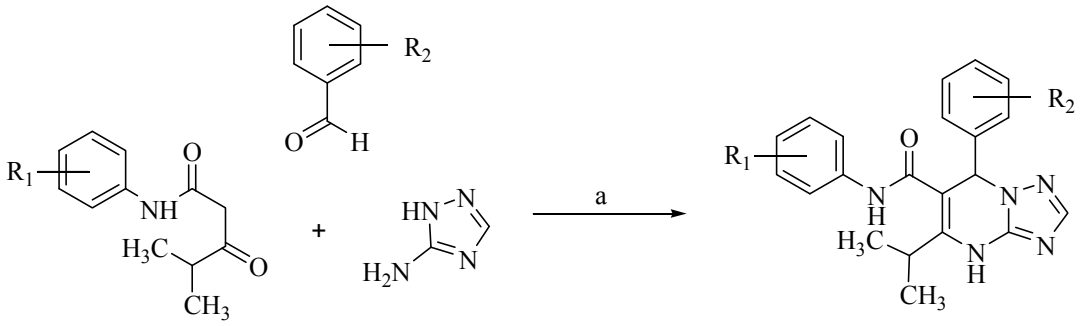
## 4.3 Current work

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 $\beta$-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 (**RHK-161 to 190**) containing an acetoacetamide fragment. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass spectra, $^1$H NMR and elemental analyses. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial.

## 4.4 Reaction Scheme



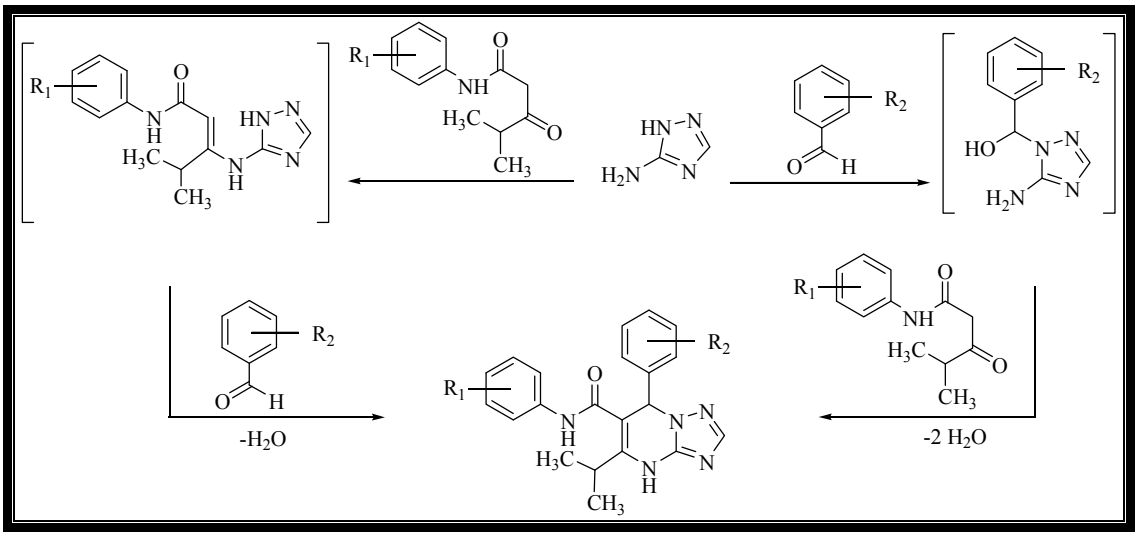

Reagents and conditions: (a) DMF, Reflux, 20 Minutes

**RHK- 161 to 190**

| Code | $R_1$ | $R_2$ | M.F. | M.W. | M.P. °C | Yield % | $R_{f1}$ | $R_{f2}$ |
|---|---|---|---|---|---|---|---|---|
| RHK-161 | 4-Cl | 4-Cl | $C_{21}H_{19}C_{l2}N_5O$ | 428 | 202 | 66 | 0.52 | 0.70 |
| RHK-162 | 4-Cl | 4-$CH_3$ | $C_{22}H_{22}ClN_5O$ | 407 | 181 | 69 | 0.49 | 0.72 |
| RHK-163 | 4-Cl | 4-OCH3 | $C_{22}H_{22}ClN_5O_2$ | 423 | 257 | 78 | 0.45 | 0.66 |
| RHK-164 | 4-Cl | 4-$NO_2$ | $C_{21}H_{19}ClN_6O_3$ | 438 | 222 | 70 | 0.48 | 0.59 |
| RHK-165 | 4-Cl | 4-F | $C_{21}H_{19}ClFN_5O$ | 411 | 261 | 81 | 0.50 | 0.69 |
| RHK-166 | 4-Cl | 3-$NO_2$ | $C_{21}H_{19}ClN_6O_3$ | 438 | 201 | 71 | 0.49 | 0.71 |
| RHK-167 | 4-Cl | 3-Cl | $C_{21}H_{19}C_{l2}N_5O$ | 428 | 244 | 59 | 0.50 | 0.69 |
| RHK-168 | 4-Cl | 2-$NO_2$ | $C_{21}H_{19}ClN_6O_3$ | 438 | 216 | 64 | 0.46 | 0.63 |
| RHK-169 | 4-Cl | 4-OH | $C_{21}H_{20}ClN_5O_2$ | 409 | 199 | 67 | 0.45 | 0.62 |
| RHK-170 | 4-Cl | 3-Br | $C_{21}H_{19}BrClN_5O$ | 472 | 183 | 70 | 0.50 | 0.74 |
| RHK-171 | 3-Cl,4-F | 4-Cl | $C_{21}H_{18}Cl_2FN_5O$ | 445 | 256 | 68 | 0.48 | 0.70 |
| RHK-172 | 3-Cl,4-F | 4-$CH_3$ | $C_{22}H_{21}ClFN_5O$ | 425 | 227 | 66 | 0.46 | 0.66 |
| RHK-173 | 3-Cl,4-F | 4-OCH3 | $C_{22}H_{21}ClFN_5O_2$ | 441 | 257 | 62 | 0.45 | 0.65 |
| RHK-174 | 3-Cl,4-F | 4-$NO_2$ | $C_{21}H_{18}ClFN_6O_3$ | 456 | 247 | 71 | 0.52 | 0.71 |
| RHK-175 | 3-Cl,4-F | 4-F | $C_{21}H_{18}ClF_2N_5O$ | 429 | 233 | 72 | 0.60 | 0.68 |
| RHK-176 | 3-Cl,4-F | 3-$NO_2$ | $C_{21}H_{18}ClFN_6O_3$ | 456 | 246- | 70 | 0.54 | 0.79 |
| RHK-177 | 3-Cl,4-F | 3-Cl | $C_{21}H_{18}Cl_2FN_5O$ | 445 | 235 | 63 | 0.53 | 0.71 |
| RHK-178 | 3-Cl,4-F | 2-$NO_2$ | $C_{21}H_{18}ClFN_6O_3$ | 456 | 198 | 66 | 0.54 | 0.78 |
| RHK-179 | 3-Cl,4-F | 4-OH | $C_{21}H_{19}ClFN_5O_2$ | 427 | 209 | 80 | 0.42 | 0.61 |
| RHK-180 | 3-Cl,4-F | 3-Br | $C_{21}H_{18}BrClFN_5O$ | 489 | 219 | 73 | 0.59 | 0.72 |
| RHK-181 | 4-F | 4-Cl | $C_{21}H_{19}ClFN_5O$ | 411 | 208 | 70 | 0.44 | 0.56 |
| RHK-182 | 4-F | 4-$CH_3$ | $C_{22}H_{22}FN_5O$ | 391 | 233 | 68 | 0.49 | 0.66 |
| RHK-183 | 4-F | 4-OCH3 | $C_{22}H_{22}FN_5O_2$ | 407 | 217 | 62 | 0.55 | 0.68 |
| RHK-184 | 4-F | 4-$NO_2$ | $C_{21}H_{19}FN_6O_3$ | 422 | 250 | 70 | 0.52 | 0.59 |
| RHK-185 | 4-F | 4-F | $C_{21}H_{19}F_2N_5O$ | 395 | 222 | 79 | 0.51 | 0.74 |
| RHK-186 | 4-F | 3-$NO_2$ | $C_{21}H_{19}FN_6O_3$ | 422 | 218 | 68 | 0.44 | 0.66 |
| RHK-187 | 4-F | 3-Cl | $C_{21}H_{19}ClFN_5O$ | 411 | 241 | 69 | 0.52 | 0.75 |
| RHK-188 | 4-F | 2-$NO_2$ | $C_{21}H_{19}FN_6O_3$ | 422 | 228 | 67 | 0.53 | 0.74 |
| RHK-189 | 4-F | 4-OH | $C_{21}H_{20}FN_5O_2$ | 393 | 258 | 70 | 0.55 | 0.71 |
| RHK-190 | 4-F | 3-Br | $C_{21}H_{19}BrFN_5O$ | 456 | 213 | 65 | 0.46 | 0.70 |

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

## 4.5 Plausible Reaction Mechanism




## 4.6 Experimental

### 4.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using potassium bromide (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.

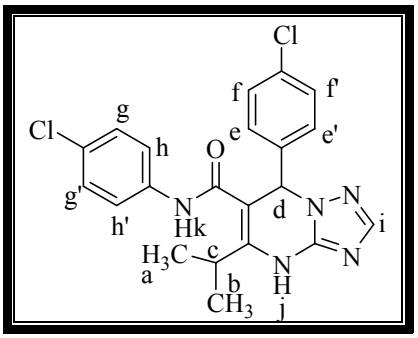
### *4.6.2 Synthesis of N-(substituted phenyl)-4-methyl-3-oxopentanamide*

Synthesis of *N*-(substituted phenyl)-4-methyl-3-oxopentanamide was achieved using previously published methods [97].

### *4.6.3 General procedure for the synthesis of N,7-bis(substitutedphenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK - 161 to 190)*

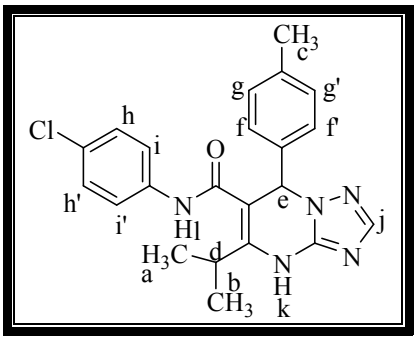
A mixture of the 3-amino-1,2,4-triazole (0.01 M), *N*-(substituedphenyl)-4-methyl-3-oxopentanamide (0.01 M) and an appropriate aromatic aldehydes (0.01 M) was refluxed in DMF (4 ml) for 20 min. After cooling, methanol (~10 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products (**RHK- 161 to 170**), which were recrystallized from ethanol.

#### *4.6.3.1 N,7-bis(4-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo-[1,5-a] pyrimidine-6-carboxamide (RHK-161)*



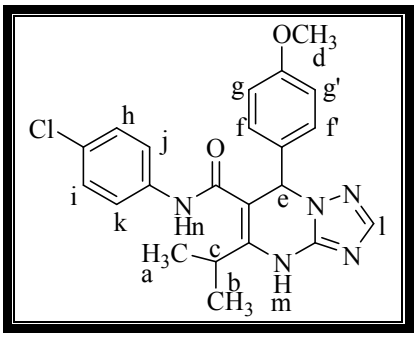

Yield: 66%; mp 202ºC; Anal. Calcd. for $C_{21}H_{19}C_{l2}N_5O$: C, 58.89; H, 4.47; Cl, 16.55; N, 16.35; O,3.74. Found: C, 58.79; H, 4.37; Cl, 16.33; N, 16.24; O,3.66%; IR ($cm^{-1}$): 3344 (N-H stretching of secondary amine), 3070 (C-H stretching of aromatic ring), 2901 (C-H asymmetrical stretching of $CH_3$ group), 2802 (C-H asymmetrical stretching of $CH_3$ group), 1687 (C=O stretching of amide), 1599 (C=N stretching of triazole ring), 1531 (N-H deformation of pyrimidine ring), 1492 (C=C stretching of aromatic ring), 1381 (C-H asymmetrical deformation of $CH_3$ group), 1309 (C-H symmetrical deformation of $CH_3$ group), 1247(C-N stretching), 1093 (C-H in plane deformation of aromatic ring), 833 (C-H out of plane bending of 1,4-disubstituion), 661 (C-Cl stretching); MS: *m/z* 428; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.15-117 (d, 3H, $H_a$, $J$ = 10.00 Hz), 1.25-1.27 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.20-3.27 (m, 1H, $H_c$), 6.50 (s, 1H, $H_d$), 7.12-7.16 (m, 2H, $H_{ee'}$ ), 7.21-7.25 (m, 2H, $H_{ff'}$, $J$ = 14.00 Hz), 7.29-7.32 (d, 2H, $H_{gg'}$, $J$ = 8.80 Hz), 7.49-7.52 (d, 2H, $H_{hh'}$, $J$ = 8.80 Hz), 7.66 (s, 1H, $H_i$), 10.02 (s, 1H, $H_j$),10.09 (s, 1H, $H_k$).

### *4.6.3.2 N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-7-p-tolyl-[1,2,4]triazolo-[1,5-a]pyrimidine-6-carboxamide (RHK-162)*




Yield: 69%; mp 181ºC; Anal. Calcd. for $C_{21}H_{19}C_{l2}N_5O$: C, 58.89; H, 4.47; Cl, 16.55; N, 16.35; O, 3.74; Found: C, 58.79; H, 4.37; Cl, 16.33; N, 16.24; O, 3.66%; IR ($cm^{-1}$): 3383 (N-H stretching of secondary amine), 3030 (C-H stretching of aromatic ring), 2970 (C-H asymmetrical stretching of $CH_3$ group), 2872 (C-H asymmetrical stretching of $CH_3$ group), 1674 (C=O stretching of amide), 1550 (C=N stretching of triazole ring), 1519 (N-H deformation of pyrimidine ring), 1492 (C=C stretching of aromatic ring), 1402 (C-H asymmetrical deformation of $CH_3$ group), 1342 (C-H symmetrical deformation of $CH_3$ group), 1244 (C-N stretching), 1138 (C-H in plane deformation of aromatic ring), 827(C-H out of plane bending of 1,4-disubstituion), 661 (C-Cl streching); MS: *m/z* 407; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.00-119 (d, 3H, $H_a$), 1.25-1.27 (d, 3H, $H_b$, $J$ = 6.80 Hz),2.22 (s, 3H, $H_c$) 3.24-3.30 (m, 1H, $H_d$), 6.47 (s, 1H, $H_e$), 7.05-7.10 (m, 4H, $H_{ff'-gg'}$ ), 7.29-7.31 (d, 2H, $H_{hh'}$, $J$ = 8.40 Hz), 7.53-7.55 (d, 2H, $H_{ii'}$, $J$ = 8.40 Hz), 7.64 (s, 1H, $H_j$), 9.96 (s, 1H, $H_k$) 10.09 (s, 1H, $H_l$).

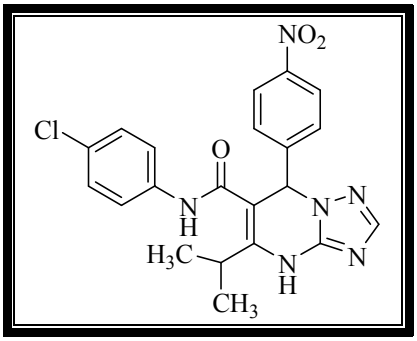
### *4.6.3.3 N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-7-(4-methoxyphenyl)-[1,2,4]-triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-163)*




Yield: 78%; mp 257ºC; Anal. Calcd. for $C_{22}H_{22}ClN_5O_2$: C, 62.34; H, 5.23; Cl, 8.36; N, 16.52; O, 7.55; Found: C, 62.22; H, 5.00; Cl, 8.20; N, 16.40; O, 7.42%; IR ($cm^{-1}$): 3292 (N-H stretching of secondary amine), 3070 (C-H stretching of aromatic ring), 2994 (C-H asymmetrical stretching of $CH_3$ group), 2868 (C-H asymmetrical stretching of $CH_3$ group), 1631 (C=O stretching of amide), 1593 (C=N stretching of triazole ring), 1558 (N-H deformation of pyrimidine ring), 1491 (C=C stretching of aromatic ring), 1398 (C-H asymmetrical deformation of $CH_3$ group), 1342 and 1303 (C-$NO_2$ stretching), 1300 (C-H symmetrical deformation of $CH_3$ group), 1244 (C-O-C asymmetrical stretching of $OCH_3$), 1236 (C-N stretching), 1206 (C-O-C asymmetrical stretching of $OCH_3$), 1033 (C-H in plane

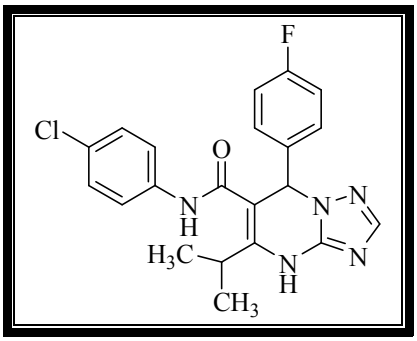
deformation of aromatic ring), 1012 (C-O-C symmetrical stretching of $OCH_3$), 825 (C-H out of plane bending of 1,4-disubstituion), 661 (C-Cl stretching); MS: *m/z* 423;$^1$H NMR (DMSO-$d_6$) δ ppm: 1.12-1.17 (d, 3H, $H_a$), 1.28-1.29 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.29-3.31 (m, 1H, $H_c$, $J$ = 7.20 Hz), 3.68 (s, 3H, $H_d$) 6.50 (s, 1H, $H_e$), 6.84-6.87 (d, 2H, $H_{ff'}$, $J$ = 8.80 Hz), 7.13-7.15 (d, 2H, $H_{gg'}$, $J$ = 8.40 Hz), 7.54-7.58 (d, 1H, $H_h$, $J$ = 8.40 Hz), 7.65 (s, 1H, $H_i$), 7.85-7.89 (m, 2H, $H_{jk}$) 8.54 (s, 1H, $H_l$) 10.03 (s, 1H, $H_m$) 10.41 (s, 1H, $H_n$).

### *4.6.3.4 N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-7-(4-nitrophenyl)-[1,2,4]- triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-164)*



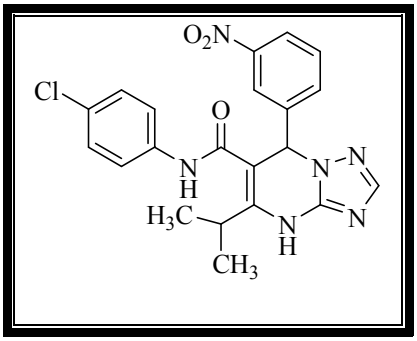
Yield: 70%; mp 222ºC; Anal. Calcd. for $C_{21}H_{19}ClN_6O_3$: C, 57.47; H, 4.36; Cl, 8.08; N, 19.15; O, 10.94; Found: C, 57.35; H, 4.25; Cl, 8.01; N, 19.00; O, 10.86%; MS: *m/z* 438.

### *4.6.3.5 N-(4-chlorophenyl)-7-(4-fluorophenyl)-4,7-dihydro-5-isopropyl- [1,2,4]- triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-165)*



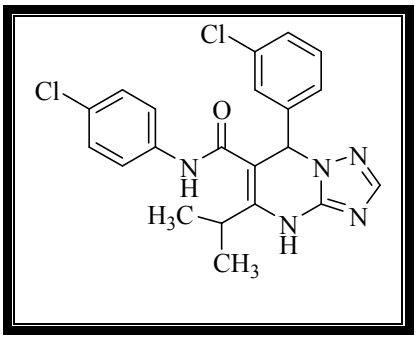
Yield: 81%; mp 261ºC; Anal. Calcd. for $C_{21}H_{19}ClFN_5O$: C, 61.24; H, 4.65; Cl, 8.61; F, 4.61; N, 17.00; O, 3.88; Found: C, 61.14; H, 4.54; Cl, 8.45; F, 4.50; N, 16.89; O, 3.75; MS: *m/z* 411.

### *4.6.3.6 N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-7-(3-nitrophenyl)-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-166)*



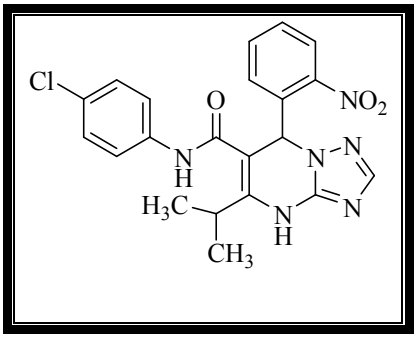
Yield: 71%; mp 201ºC; Anal. Calcd. for $C_{21}H_{19}ClN_6O_3$: C, 57.47; H, 4.36; Cl, 8.08; N, 19.15; O, 10.94; Found: C, 57.35; H, 4.25; Cl, 8.01; N, 19.00; O, 10.86%; MS: *m/z* 438.

### *4.6.3.7 7-(3-chlorophenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]- triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-167)*



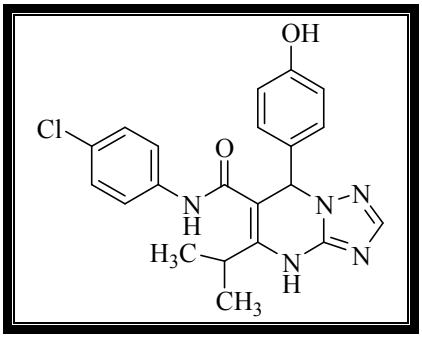
Yield: 59%; mp 244ºC; Anal. Calcd. for $C_{21}H_{19}C_{l2}N_5O$: C, 58.89; H, 4.47; Cl, 16.55; N, 16.35; O, 3.74; Found: C, 58.74; H, 4.34; Cl, 16.45; N, 16.24; O, 3.64%; MS: *m/z* 428.

### *4.6.3.8 N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-7-(2-nitrophenyl)-[1,2,4]triazo-lo[1,5-a]pyrimidine-6-carboxamide (RHK-168)*



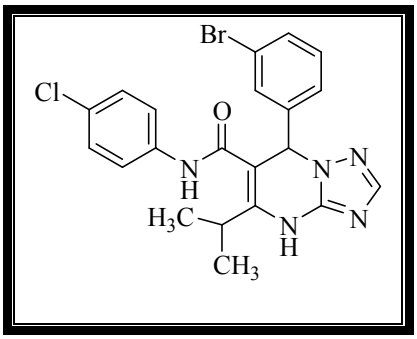
Yield: 64%; mp 216ºC; Anal. Calcd. for $C_{21}H_{19}ClN_6O_3$: C, 57.47; H, 4.36; Cl, 8.08; N, 19.15; O, 10.94; Found: C, 57.33; H, 4.23; Cl, 8.00; N, 19.05; O, 10.88%; MS: *m/z* 438.

### *4.6.3.9 N-(4-chlorophenyl)-4,7-dihydro-7-(4-hydroxyphenyl)-5-isopropyl-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-169)*



Yield: 67%; mp 199 ºC; Anal. Calcd. for $C_{21}H_{20}ClN_5O_2$ : C, 61.54; H, 4.92; Cl, 8.65; N, 17.09; O, 7.81; Found: C, 61.35; H, 4.85; Cl, 8.53; N, 17.00; O, 7.71%; MS: *m/z* 409.

***4.6.3.10 7-(3-bromophenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4] tria -zolo[1,5-a]pyrimidine-6-carboxamide (RHK-170)***

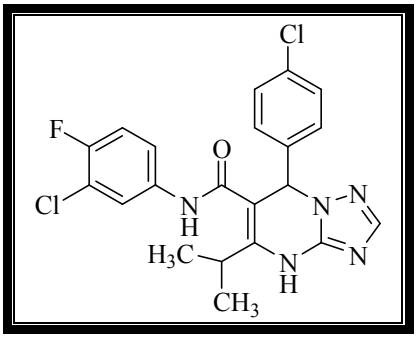



Yield: 70%; mp 183ºC; Anal. Calcd. for $C_{21}H_{19}BrClN_5O$: C, 53.35; H, 4.05; Br, 16.90; Cl, 7.50; N, 14.81; O, 3.38; Found: C, 53.13; H, 4.00; Br, 16.81; Cl, 7.41; N, 14.72; O, 3.23%; MS: *m/z* 472.

***4.6.4 General procedure for the synthesis of N-(3-chloro-4-fluorophenyl)-7-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-171to180)***

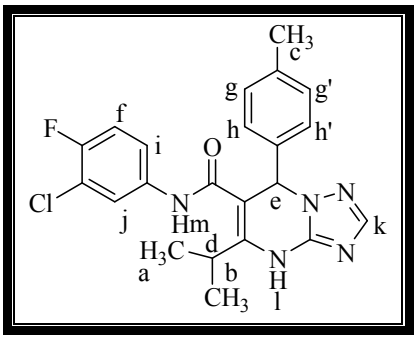
A mixture of the 3-amino-1,2,4-triazole (0.01 mol), *N*-(3-chloro-4-fluorophenyl)-4-methyl-3-oxopentanamide (0.01 mol) and an appropriate aromatic aldehydes (0.01 mol) was refluxed in 0.4 ml of DMF for 12-15 min. After cooling, methanol (~10 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products **RHK- 171 to 180**, which were crystallized from ethanol.

***4.6.4.1 N-(3-chloro-4-fluorophenyl)-7-(4-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4] tria-zolo[1,5-a]pyrimidine-6-carboxamide (RHK-171)***



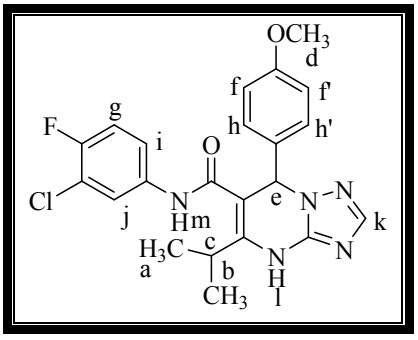

Yield: 68%; mp 256ºC; Anal. Calcd. for $C_{21}H_{18}Cl_2FN_5O$: C, 56.51; H, 4.07; Cl, 15.89; F, 4.26; N, 15.69; O, 3.58; Found: C, 56.40; H, 4.00; Cl, 15.75; F, 4.15; N, 15.56; O, 3.42%; MS: *m/z* 445.

### *4.6.4.2 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-172)*




Yield: 66%; mp 227ºC; Anal. Calcd. for $C_{22}H_{21}ClFN_5O$: C, 62.04; H, 4.97; Cl, 8.32; F, 4.46; N, 16.44; O, 3.76; Found: C, 61.89; H, 4.82; Cl, 8.20; F, 4.31; N, 16.36; O, 3.62; IR ($cm^{-1}$): 3377 (N-H stretching of secondary amine), 3022 (C-H stretching of aromatic ring), 2970 (C-H asymmetrical stretching of $CH_3$ group), 2870 (C-H asymmetrical stretching of $CH_3$ group), 1666 (C=O stretching of amide), 1577 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1529 and 1500 (C=C stretching of aromatic ring), 1408 (C-H asymmetrical deformation of $CH_3$ group), 1307 (C-H symmetrical deformation of $CH_3$ group), 1282 (C-N stretching), 1078 (C-H in plane deformation of aromatic ring), 1141 (C-F stretching) 815 (C-H out of plane bending of 1,4-disubstituion), 667 (C-Cl stretching); MS: *m/z* 425; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.15-1.17 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.27-1.29 (d, 3H, $H_b$, $J$ = 7.20 Hz), 2.20 (s, 3H, $H_c$) 3.28-3.33 (m, 1H, $H_d$),) 6.42 (s, 1H, $H_d$), 6.93-6.97 (m, 1H, $H_f$), 7.01-7.03 (d, 2H, $H_{gg'}$, $J$ = 8.00 Hz), 7.09-7.11 (d, 1H, $H_{hh'}$, $J$ = 8.00 Hz), 7.25-7.29 (m, 1H, $H_i$), 7.49 (m, 1H, $H_j$), 7.62 (s, 1H, $H_k$), 9.65 (s, 1H, $H_l$), 9.74 (s, 1H, $H_m$).

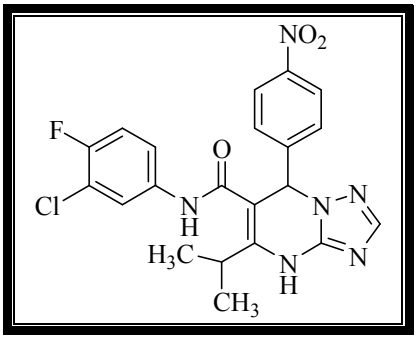
### *4.6.4.3 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-173)*




Yield: 62%; mp 257ºC; Anal. Calcd. for $C_{22}H_{21}ClFN_5O_2$: C, 59.80; H, 4.79; Cl, 8.02; F, 4.30; N, 15.85; O, 7.24; Found: C, 59.70; H, 4.66; Cl, 7.89; F, 4.21; N, 15.74; O, 7.11; IR ($cm^{-1}$): 3369 (N-H stretching of secondary amine), 3084 (C-H stretching of aromatic ring), 2970 (C-H asymmetrical stretching of $CH_3$ group), 2868 (C-H asymmetrical stretching of $CH_3$ group), 1664 (C=O stretching of amide), 1664 (C=N stretching of triazole ring), 1573 (N-H deformation of pyrimidine ring), 1550 (C=C stretching of aromatic ring), 1468 (C-H asymmetrical deformation of $CH_3$ group), 1307 (C-H symmetrical deformation of $CH_3$ group), 1280 (C-N stretching),

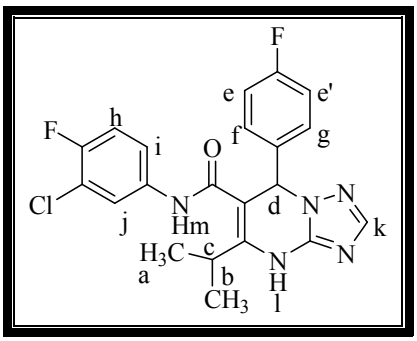
1247 (C-O-C stretching),1035 (C-F stretching), 1076 (C-H in plane deformation of aromatic ring), 779 (C-H out of plane bending of 1,4-disubstituion), 663 (C-Cl stretching); MS: *m/z* 441; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.23-1.25 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.36-1.38 (d, 3H, $H_b$, $J$ = 7.20 Hz), 3.38-3.45 (m, 1H, $H_c$), 3.73-3.75 (s, 3H, $H_d$) 6.49 (s, 1H, $H_e$), 6.81-6.84 (d, 2H, $H_{ff'}$, $J$ = 13.60 Hz), 7.00-7.04 (m, 1H, $H_g$), 7.24-7.27 (d, 2H, $H_{hh'}$, $J$ = 11.60 Hz), 7.31-7.35 (m, 1H, $H_i$), 7.58 (s, 1H, $H_j$), 7.64 (s, 1H, $H_k$), 9.66 (s, 1H, $H_l$), 9.84 (s, 1H, $H_m$).

### *4.6.4.4 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-174)*




Yield: 71%; mp 247ºC; Anal. Calcd. for $C_{21}H_{18}ClFN_6O_3$: C, 55.21; H, 3.97; Cl, 7.76; F, 4.16; N, 18.40; O, 10.51; Found: C, 55.12; H, 3.84; Cl, 7.61; F, 4.03; N, 18.34; O, 10.41; MS: *m/z* 456.

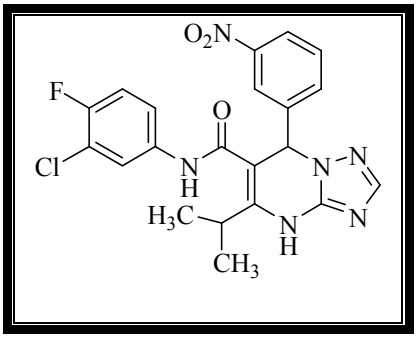
### *4.6.4.5 N-(3-***chloro***-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(4-fluorophenyl) -[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-175)*




Yield: 72%; mp 233ºC; Anal. Calcd. for $C_{21}H_{18}ClF_2N_5O$: C, 58.68; H, 4.22; Cl, 8.25; F, 8.84; N, 16.29; O, 3.72; Found: C, 58.68; H, 4.11; Cl, 8.10; F, 8.68; N, 16.12; O, 3.62; IR (cm$^{-1}$): 3421 (N-H stretching of secondary amine), 3024 (C-H stretching of aromatic ring), 2924 (C-H asymmetrical stretching of $CH_3$ group), 2875 (C-H asymmetrical stretching of $CH_3$ group), 1668 (C=O stretching of amide), 1575 (C=N stretching of triazole ring), 1552 (N-H deformation of pyrimidine ring), 1498 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of $CH_3$ group), 1305 (C-H symmetrical deformation of $CH_3$ group), 1242 (C-N stretching), 1087 (C-H in plane deformation of aromatic ring), 1012 (C-F stretching), 819 (C-H out of plane bending of 1,4-disubstituion), 686 (C-Cl stretching); MS: *m/z* 429; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.15-1.17 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.27-1.29 (d, 3H, $H_b$, $J$

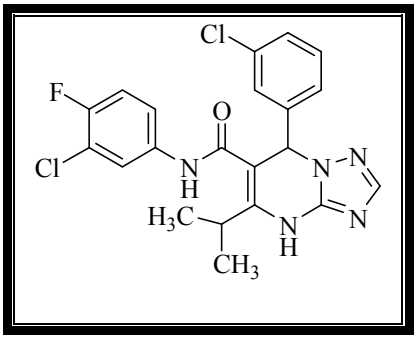
= 7.20 Hz), 3.30-3.37 (m, 1H, $H_c$), 6.41 (s, 1H, $H_d$), 6.73-6.75 (d, 2H, $H_{ee'}$, $J$ = 8.80 Hz), 6.82-6.87 (m, 2H, $H_{fg}$), 7.16-7.18 (d, 1H, $H_h$, $J$ = 8.40 Hz), 7.36-7.40 (m, 1H, $H_i$), 7.48 (s, 1H, $H_j$), 7.67 (s, 1H, $H_k$) ,9.50 (s, 1H, $H_l$), 9.71 (s, 1H, $H_m$).

### *4.6.4.6 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-176)*



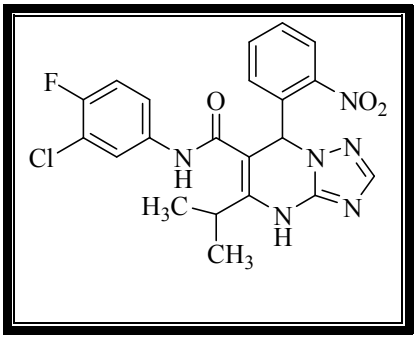
Yield: 70%; mp 247ºC; Anal. Calcd. for $C_{21}H_{18}ClFN_6O_3$: C, 55.21; H, 3.97; Cl, 7.76; F, 4.16; N, 18.40; O, 10.51; Found: C, 55.21; H, 3.97; Cl, 7.76; F, 4.16; N, 18.40; O, 10.51%; MS: *m/z* 456.

### *4.6.4.7 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(3-chlorophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-177)*



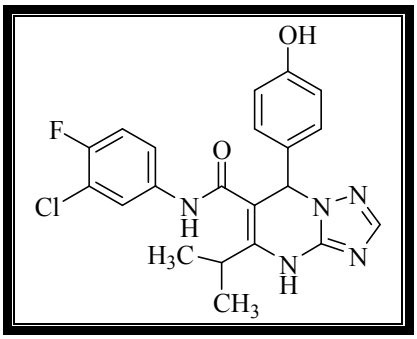
Yield: 63%; mp 235ºC; Anal. Calcd. for $C_{21}H_{18}Cl_2FN_5O$: C, 56.51; H, 4.07; Cl, 15.89; F, 4.26; N, 15.69; O, 3.58; Found: C, 56.43; H, 4.00; Cl, 15.72; F, 4.10; N, 15.54; O, 3.42%; MS: *m/z* 445.

### *4.6.4.8 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(2-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-178)*



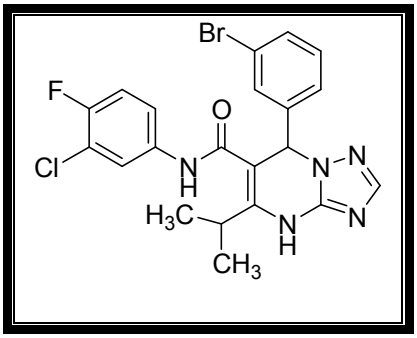
Yield: 66%; mp 198ºC; Anal. Calcd. for $C_{21}H_{18}ClFN_6O_3$: C, 55.21; H, 3.97; Cl, 7.76; F, 4.16; N, 18.40; O, 10.51;Found: C, 55.10; H, 3.88; Cl, 7.64; F, 4.05; N, 18.31; O, 10.42%; MS: *m/z* 456.

### *4.6.4.9 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-179)*




Yield: 80%; mp 209 ºC; Anal. Calcd. for $C_{21}H_{19}ClFN_5O_2$: C, 58.95; H, 4.48; Cl, 8.29; F, 4.44; N, 16.37; O, 7.48; Found: C, 58.87; H, 4.34; Cl, 8.10; F, 4.34; N, 16.23; O, 7.34%; MS: *m/z* 427.

### *4.6.4.10 N-(3-chloro-4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-(3-bromophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-180)*

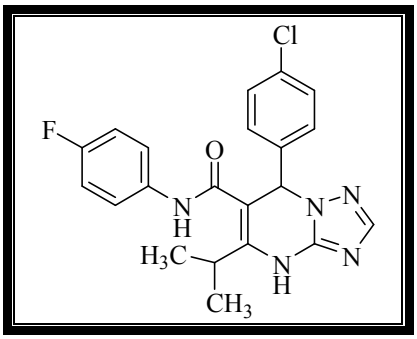



Yield: 73%; mp 219 ºC; Anal. Calcd. for $C_{21}H_{18}BrClFN_5O$: C, 51.40; H, 3.70; Br, 16.28; Cl, 7.22; F, 3.87; N, 14.27; O, 3.26; Found: C, 51.31; H, 3.61; Br, 16.15; Cl, 7.10; F, 3.76; N, 14.14; O, 3.12%; MS: *m/z* 489.

### *4.6.5 General procedure for the synthesis of N-7-(4-chlorophenyl)-N-(4-fluorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK 181 to190)*

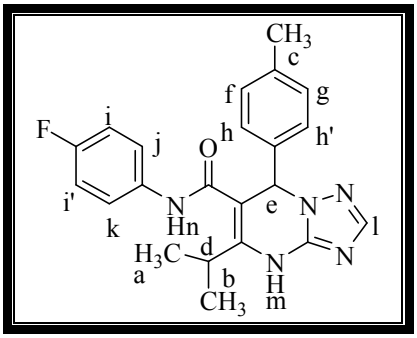
A mixture of the 5-amino-1,2,4-triazole (0.01 mol), *N*-(4-fluorophenyl)-3-oxobutanamide (0.01 mol) and an appropriate aromatic aldehydes (0.01 mol) was refluxed in 0.4 ml of DMF for 20 min. After cooling, methanol (~10 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products **RHK- 181 to 190**, which were crystallized from ethanol and subsequently dried in air.

### *4.6.5.1 7-(4-chlorophenyl)-N-(4-fluorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamid (RHK-181)*



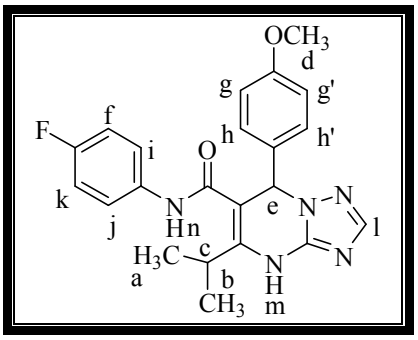
Yield: 70%; mp 208ºC; Anal. Calcd. for $C_{21}H_{19}ClFN_5O$: C, 61.24; H, 4.65; Cl, 8.61; F, 4.61; N, 17.00; O, 3.88; Found: C, 61.13; H, 4.56; Cl, 8.52; F, 4.54; N, 16.86; O, 3.76%;

### *4.6.5.2 N-(4-fluorophenyl)-4,7-dihydro-5-isopropyl-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-182)*



Yield: 68%; mp 233ºC; Anal. Calcd. for $C_{22}H_{22}FN_5O$: C, 67.50; H, 5.66; F, 4.85; N, 17.89; O, 4.09; Found: C, 67.44; H, 5.54; F, 4.72; N, 17.70; O, 4.00; IR ($cm^{-1}$): 3389 (N-H stretching of secondary amine), 3020 (C-H stretching of aromatic ring), 2928 (C-H asymmetrical stretching of $CH_3$ group), 2864 (C-H asymmetrical stretching of $CH_3$ group), 1668 (C=O stretching of amide), 1581 (C=N stretching of triazole ring),1566 (N-H deformation of pyrimidine ring), 1510 and 1475 (C=C stretching of aromatic ring), 1404 (C-H asymmetrical deformation of $CH_3$ group), 1305 (C-H symmetrical deformation of $CH_3$ group), 1220 (C-N stretching), 1139 (C-F stretching), 1072 (C-H in plane deformation of aromatic ring), 827 (C-H out of plane bending of 1,4-disubstituion); MS: *m/z* 391;$^1$H NMR (DMSO-$d_6$) δ ppm: 1.15-1.16 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.26-1.28 (d, 3H, $H_b$, $J$ = 7.20 Hz), 2.21 (s, 3H, $H_c$) 3.27-3.34 (m, 1H, $H_d$),) 6.43 (s, 1H, $H_d$), 6.85-6.90 (m, 2H, $H_{fg}$), 7.01-7.03 (d, 2H, $H_{hh'}$, $J$ = 8.00 Hz), 7.08-7.10 (d, 1H, $H_{ii'}$, $J$ = 8.00 Hz), 7.40-7.47 (m, 2H, $H_{jk}$), 7.86 (m, 1H, $H_l$), 9.69 (s, 1H, $H_m$), 9.65 (s, 1H, $H_l$), 9.72 (s, 1H, $H_n$).

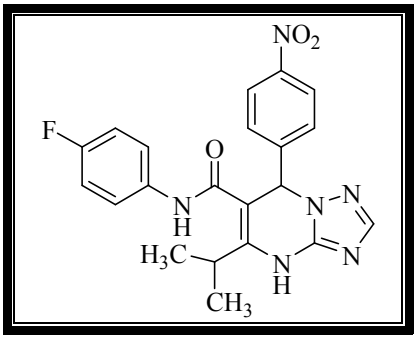
### *4.6.5.3 7-(4-chlorophenyl)-N-(4-methoxyphenyl)-4,7-dihydro-5-isopropyl-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-183)*




Yield: 62%; mp 217 ºC; Anal. Calcd. for $C_{22}H_{22}FN_5O_2$: C, 64.85; H, 5.44; F, 4.66; N, 17.19; O, 7.85; Found: C, 64.72; H, 5.33; F, 4.54; N, 17.10; O, 7.72%; IR ($cm^{-1}$): 3375 (N-H stretching of secondary amine), 3020 (C-H stretching of aromatic ring), 2929 (C-H asymmetrical stretching of $CH_3$ group), 2877 (C-H asymmetrical stretching of $CH_3$ group), 1664 (C=O stretching of amide), 1606 (C=N stretching of triazole ring), 1577 (N-H deformation of pyrimidine ring), 1533 and 1508 (C=C stretching of aromatic ring), 1475 (C-H asymmetrical deformation of $CH_3$ group), 1311 (C-H symmetrical deformation of $CH_3$ group), 1244 (C-O-C asymatrical stretching of OCH3), 1217 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 1031 (C-F stretching), 831 (C-H out of plane bending of 1,4-disubstituion); MS: *m/z* 407; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.15-1.17 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.27-1.29 (d, 3H, $H_b$, $J$ = 7.20 Hz), 3.28-3.33 (m, 1H, $H_c$), 3.76 (s, 3H, $H_d$) 6.42 (s, 1H, $H_e$), 6.93-6.97 (m, 1H, $H_f$), 7.01-7.03 (d, 2H, $H_{gg'}$, $J$ = 8.00 Hz), 7.09-7.11 (d, 2H, $H_{hh'}$, $J$ = 8.00 Hz), 7.25-7.29 (m, 2H, $H_{ij}$), 7.49 (s, 1H, $H_k$), 7.62 (s, 1H, $H_l$), 9.65 (s, 1H, $H_m$), 9.74 (s, 1H, $H_n$).

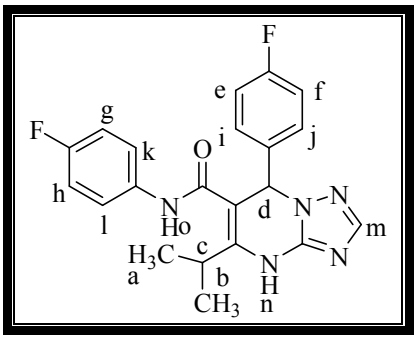
.

### *4.6.5.4 7-(4-chlorophenyl)-N-(4-nitrophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]tria-zolo[1,5-a]pyrimidine-6-carboxamide (RHK-184)*



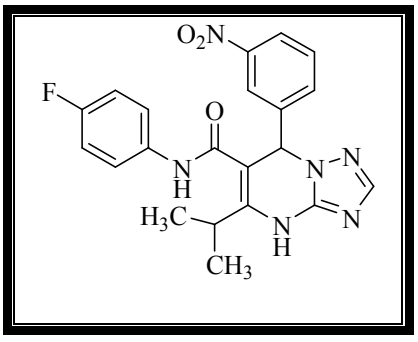

Yield: 70%; mp 250ºC; Anal. Calcd. for $C_{21}H_{19}FN_6O_3$: C, 59.71; H, 4.53; F, 4.50; N, 19.90; O, 11.36; Found: C, 59.64; H, 4.46; F, 4.43; N, 19.87; O, 11.23%; MS: *m/z* 422.

### *4.6.5.5 N,7-bis(4-fluorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-185)*



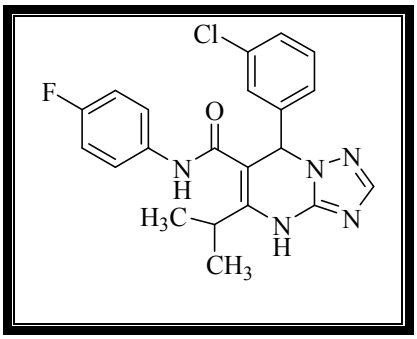
Yield: 79%; mp 222ºC; Anal. Calcd. for $C_{21}H_{19}F_2N_5O$: C, 63.79; H, 4.84; F, 9.61; N, 17.71; O, 4.05 Found: C, 63.64; H, 4.76; F, 9.51; N, 17.60; O, 4.00%; IR ($cm^{-1}$): 3281 (N-H stretching of secondary amine), 3030 (C-H stretching of aromatic ring), 2931 (C-H asymmetrical stretching of $CH_3$ group), 2875 (C-H asymmetrical stretching of $CH_3$ group), 1658 (C=O stretching of amide), 1635 (C=N stretching of triazole ring), 1587 (N-H deformation of pyrimidine ring), 1512 (C=C stretching of aromatic ring), 1408 (C-H asymmetrical deformation of $CH_3$ group), 1319 (C-H symmetrical deformation of $CH_3$ group), 1226 (C-N stretching), 1085 (C-H in plane deformation of aromatic ring), 1014 (C-F stretching), 831 (C-H out of plane bending of 1,4-disubstituion); MS: *m/z* 411; $^1$H NMR (DMSO-*d*$_6$) δ ppm: 1.15-1.17 (d, 3H, $H_a$, *J* = 7.20 Hz), 1.27-1.29 (d, 3H, $H_b$, *J* = 7.20 Hz), 3.26-3.34 (m, 1H, $H_c$), 6.48 (s, 1H, $H_d$), 6.84-6.95 (m, 2H, $H_{ef}$), 7.22-7.26 (m, 2H, $H_{gh}$), 7.37-7.40 (m, 2H, $H_{ij}$, *J* = 11.20 Hz), 7.50-7.54 (m, 2H, $H_{kl}$), 7.73 (s, 1H, $H_m$), 9.66 (s, 1H, $H_n$), 9.79 (s, 1H, $H_o$).

### *4.6.5.6 7-(4-chlorophenyl)-N-(3-nitrophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo [1,5-a]pyrimidine-6- carboxamide (RHK-186)*



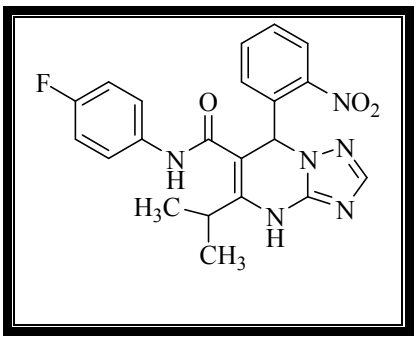
Yield: 68%; mp 218ºC; Anal. Calcd. for $C_{21}H_{19}FN_6O_3$: C, 59.71; H, 4.53; F, 4.50; N, 19.90; O, 11.36; Found: C, 59.62; H, 4.41; F, 4.40; N, 19.79; O, 11.12%; MS: *m/z* 422.

***4.6.5.7 7-(4-chlorophenyl)-N-(3-chlorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4 triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-187)***



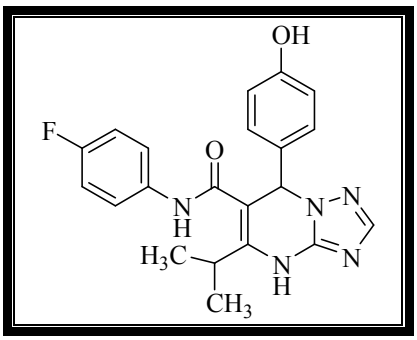
Yield: 69%; mp 241ºC; Anal. Calcd. for $C_{21}H_{19}ClFN_5O$: C, 61.24; H, 4.65; Cl, 8.61; F, 4.61; N, 17.00; O, 3.88; Found: C, 61.12; H, 4.52; Cl, 8.54; F, 4.51; N, 16.88; O, 3.74%; MS: *m/z* 411.

***4.6.5.8 7-(4-chlorophenyl)-N-(2-nitrophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-188)***



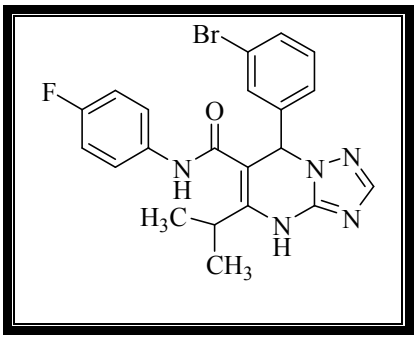
Yield: 67%; mp 228ºC; Anal. Calcd. for $C_{21}H_{19}FN_6O_3$: C, 59.71; H, 4.53; F, 4.50; N, 19.90; O, 11.36; Found: C, 59.64; H, 4.43; F, 4.42; N, 19.80; O, 11.21%; MS: *m/z* 422.

***4.6.5.9 7-(4-chlorophenyl)-N-(4-hydroxyphenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-189)***



Yield: 70%; mp 258ºC; Anal. Calcd. for $C_{21}H_{20}FN_5O_2$: C, 64.11; H, 5.12; F, 4.83; N, 17.80; O, 8.13; Found. C, 64.11; H, 5.12; F, 4.83; N, 17.80; O, 8.13%; MS: *m/z* 393.

***4.6.5.10 7-(4-chlorophenyl)-N-(3-bromophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (RHK-190)***



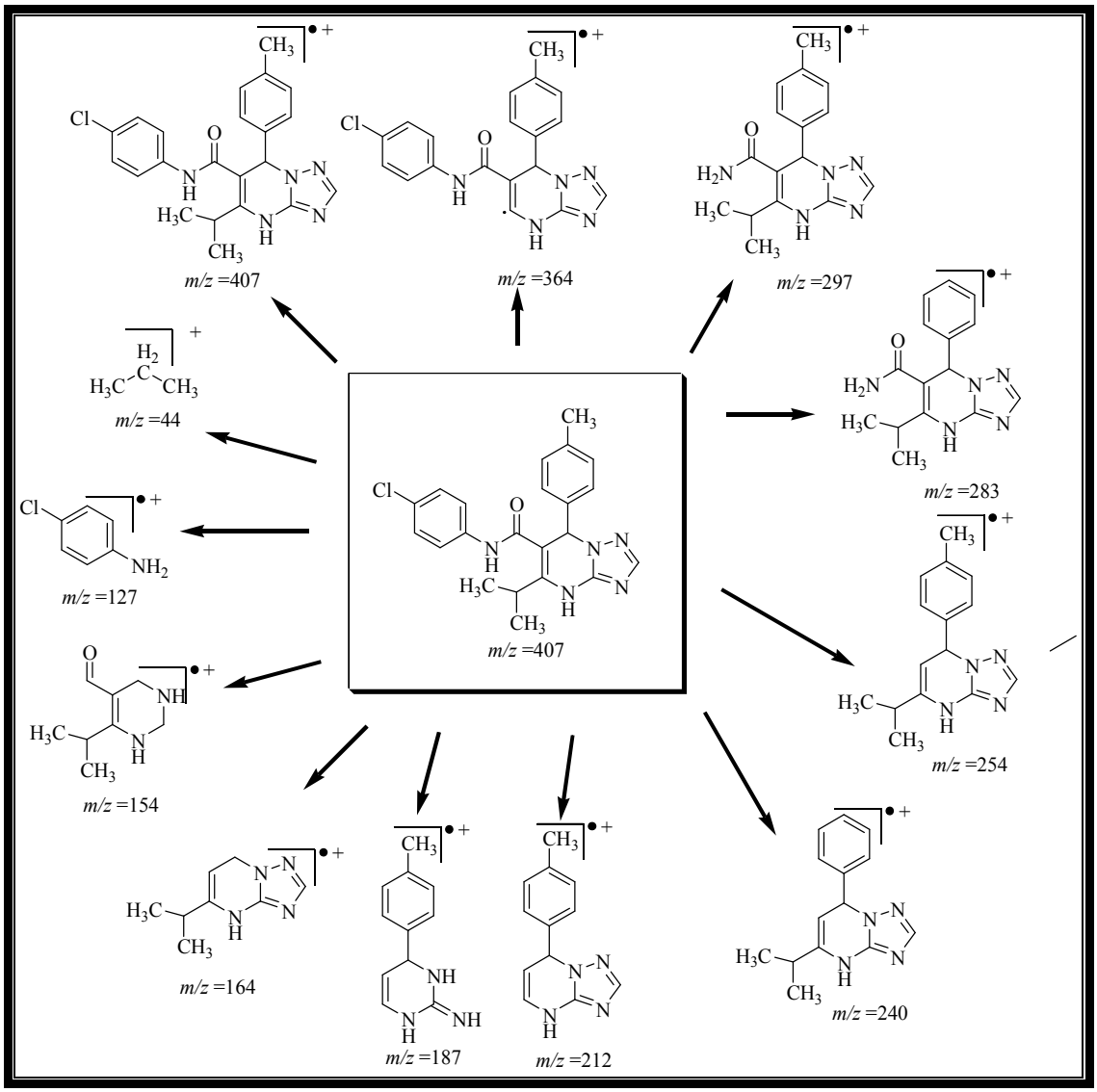

Yield: 65%; mp 213ºC; Anal. Calcd. for $C_{21}H_{19}BrFN_5O$: C, 55.27; H, 4.20; Br, 17.51; F, 4.16; N, 15.35; O, 3.51; Found: C, 55.27; H, 4.20; Br, 17.51; F, 4.16; N, 15.35; O, 3.51%; MS: *m/z* 456

## 4.7 Spectral discussion

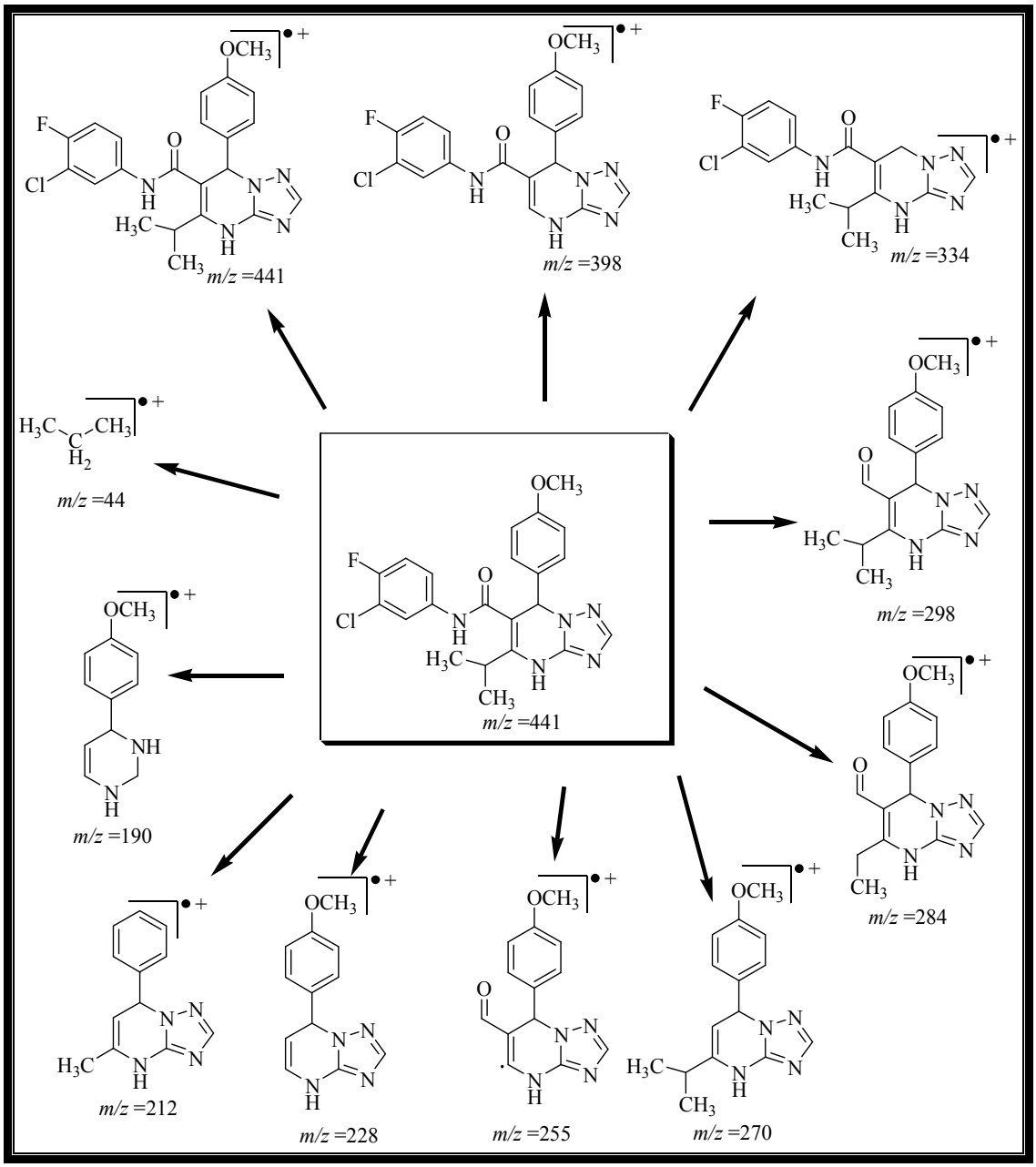
### 4.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analyses. 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.

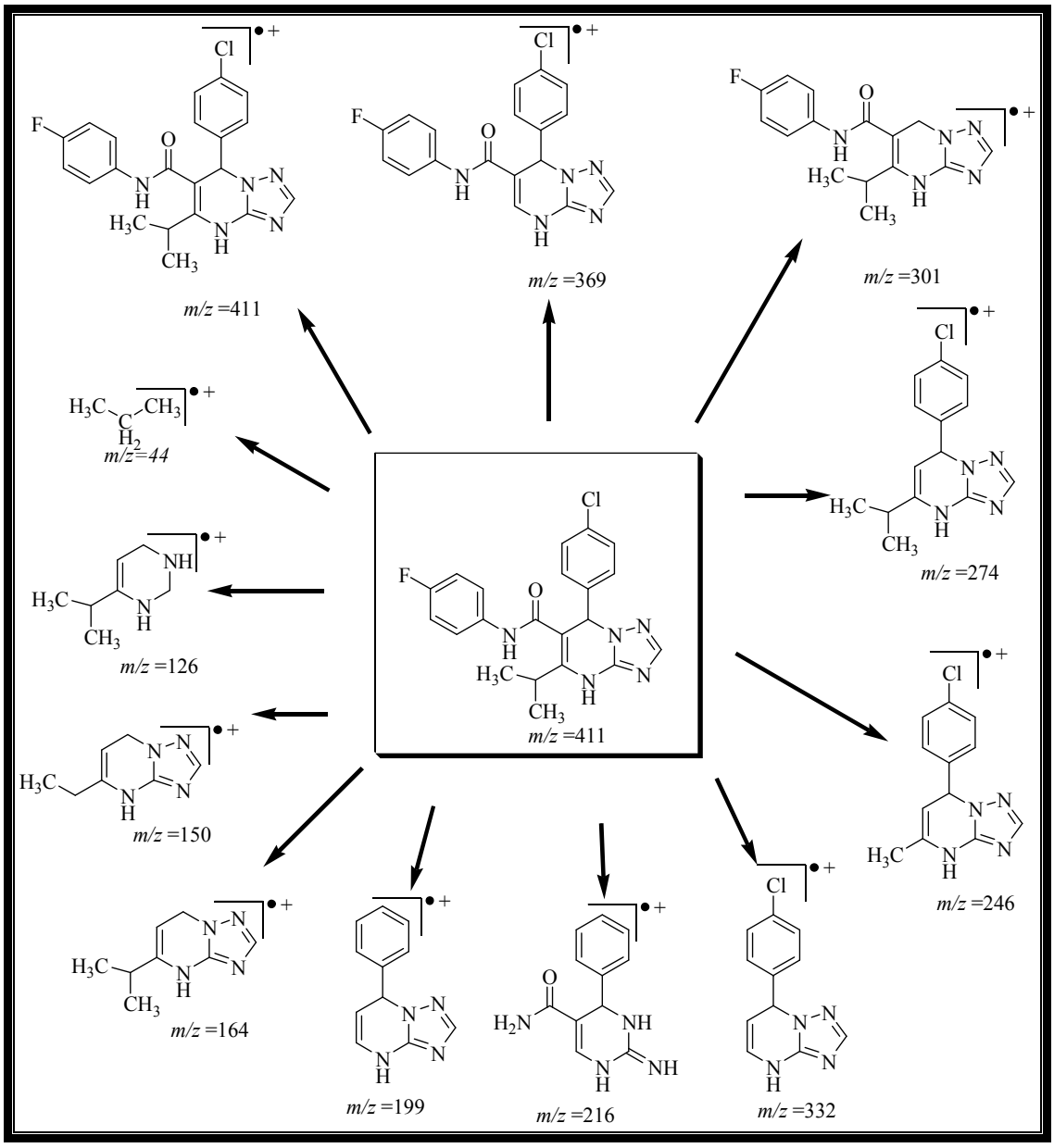
### 4.7.1.1 *Mass fragmentation pattern for RHK-162*



### 4.7.1.2 *Mass fragmentation pattern for RHK-173*



### 4.7.1.2 *Mass fragmentation pattern for RHK-181*



## 4.7.2 IR spectral study

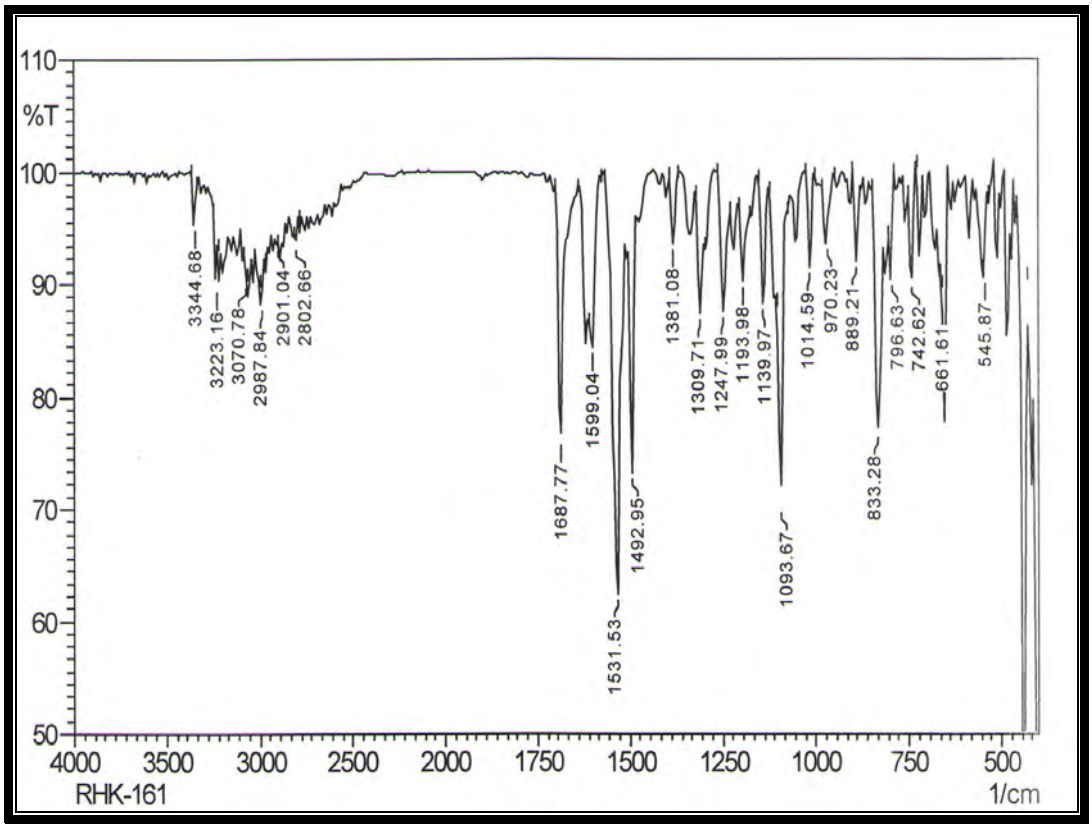
IR spectra were recorded on Shimadzu FT-IR-8400 model using potassium bromide (KBr) pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For triazolopyrimidines RHK-161to190, confirmatory bands for secondary amine and amidic carbonyl groups were observed at 3414-3282 $cm^{-1}$ and 1690-1600 $cm^{-1}$ respectively. Another characteristic C=N stretching band of triazole ring was observed at 1626-1500 $cm^{-1}$, which suggested formation of desired products **RHK- 161 to 190**.

### 4.7.3 $^1$H NMR spectral study

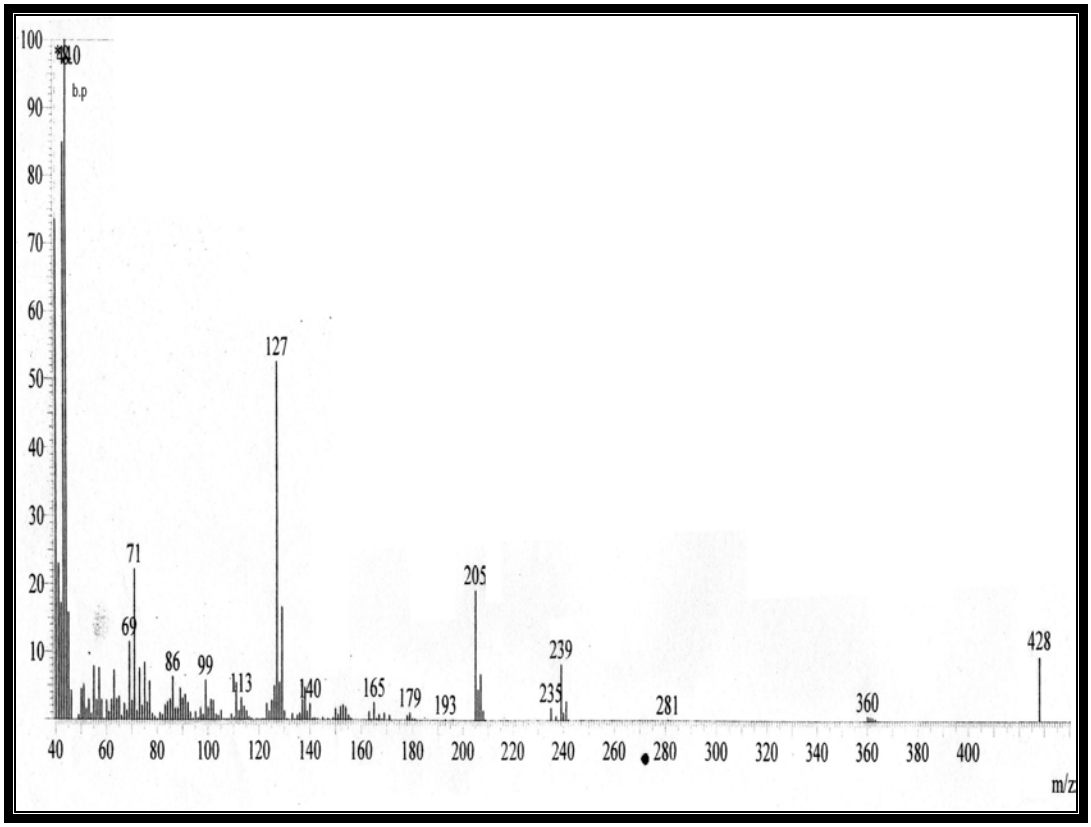
$^1$H NMR spectra were recorded in DMSO-$d_6$ solution on a bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

$^1$H NMR spectra confirmed the structures of triazolopyrimidines (**RHK- 161 to 190**) on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 6.40-6.82 δ ppm, a singlet for the methine proton of triazole ring at 7.20-8.30 δ ppm and singlets for amino and amide group protons at 7.50-9.90 and 9.45-10.50 δ ppm, respectively. The aromatic ring protons and J value were found to be in accordance with substitution pattern on phenyl ring.

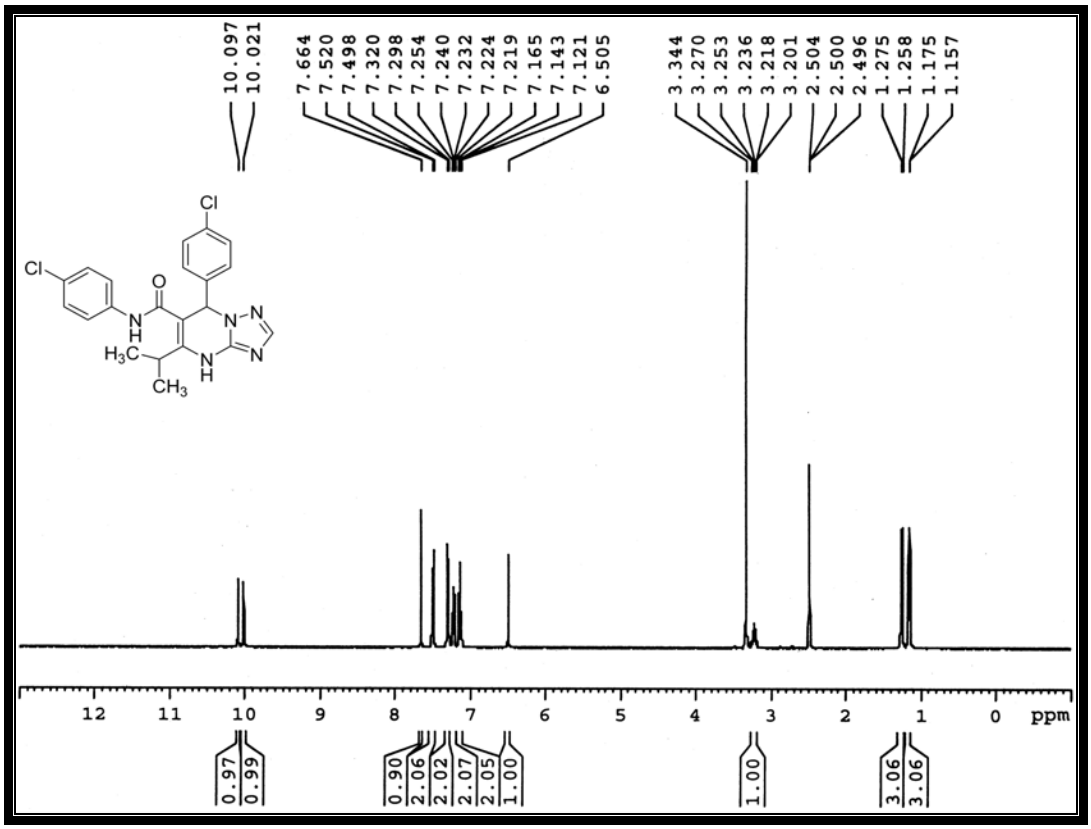
***IR spectrum of RHK - 161***



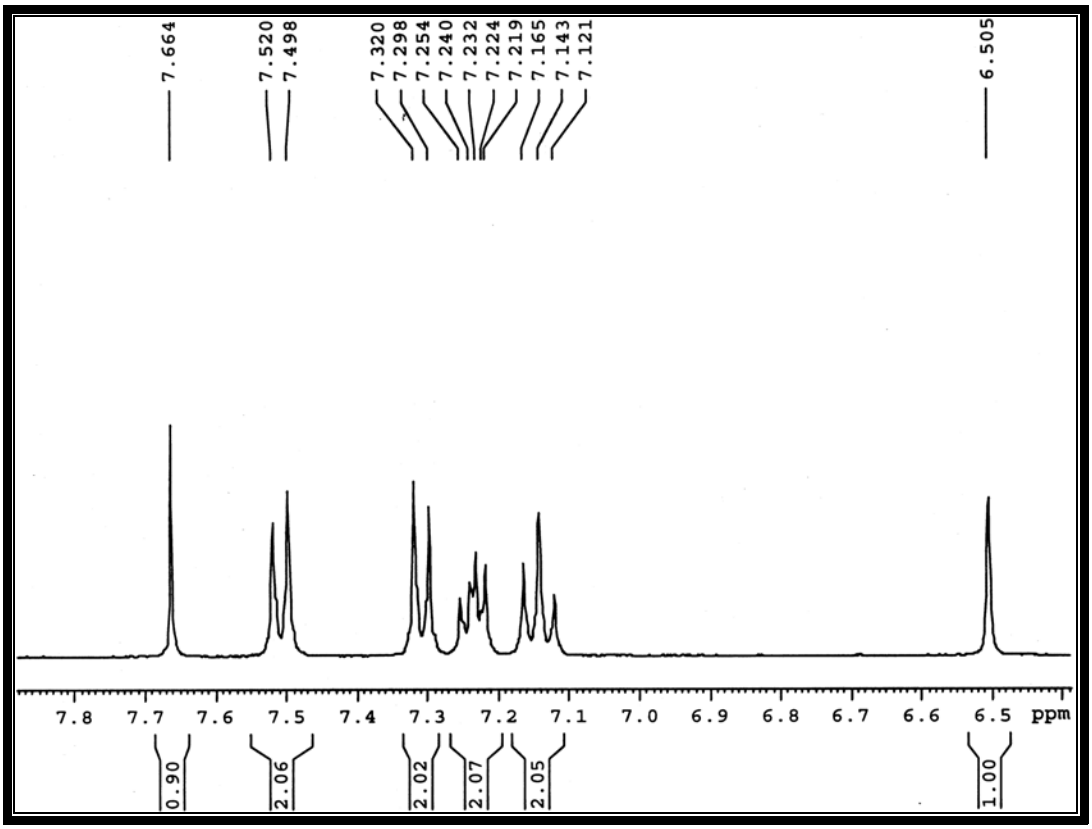

***Mass spectrum of RHK - 161***



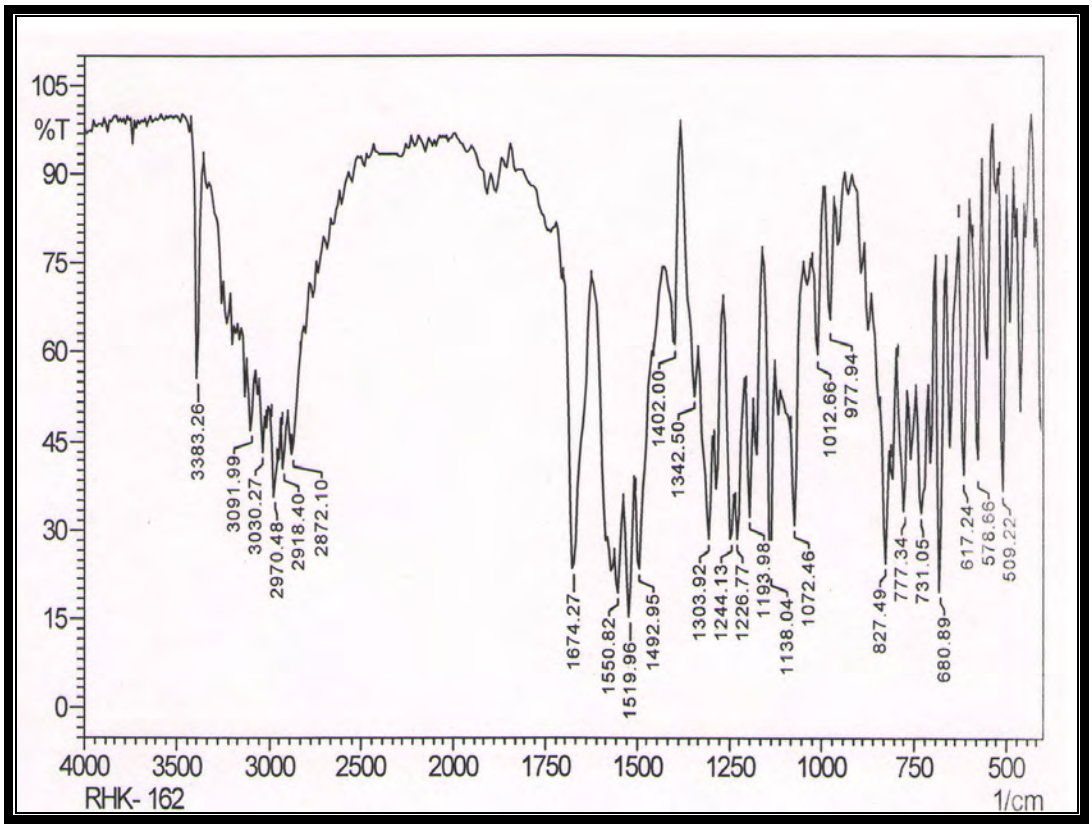
## *$^1$H NMR spectrum of RHK - 161*



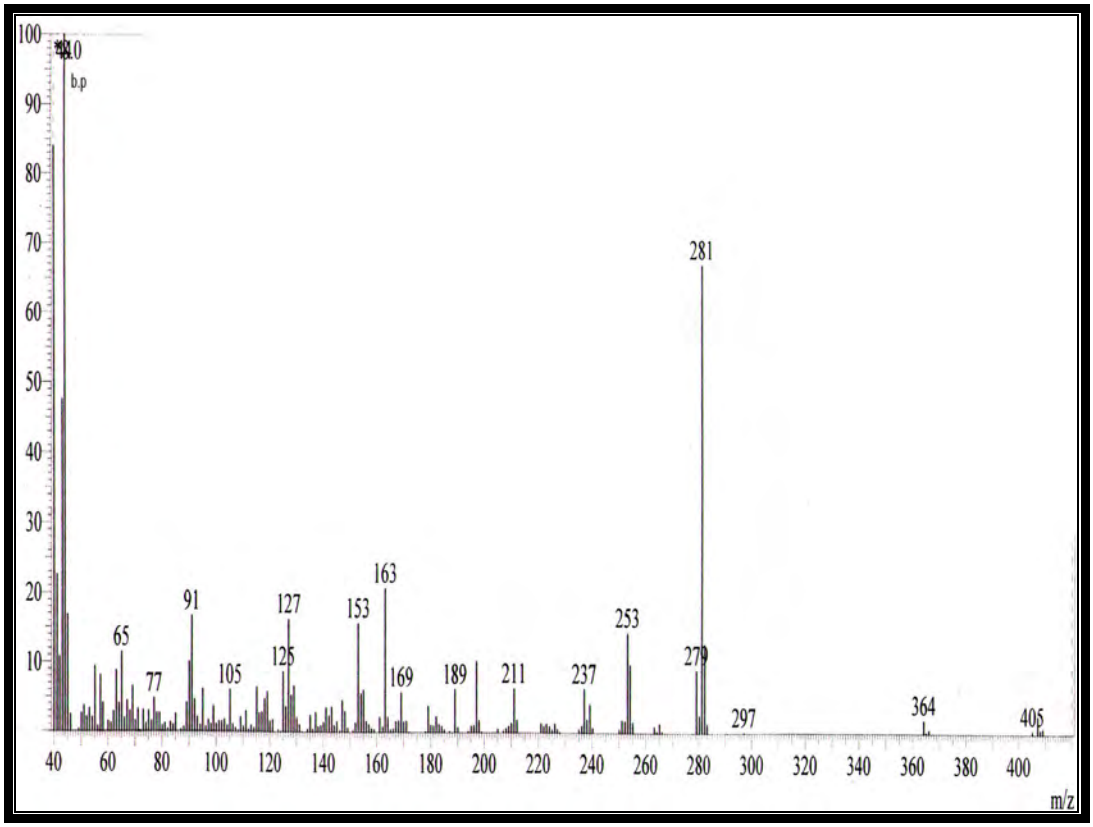

## *Expanded $^1$H NMR spectrum of RHK - 161*



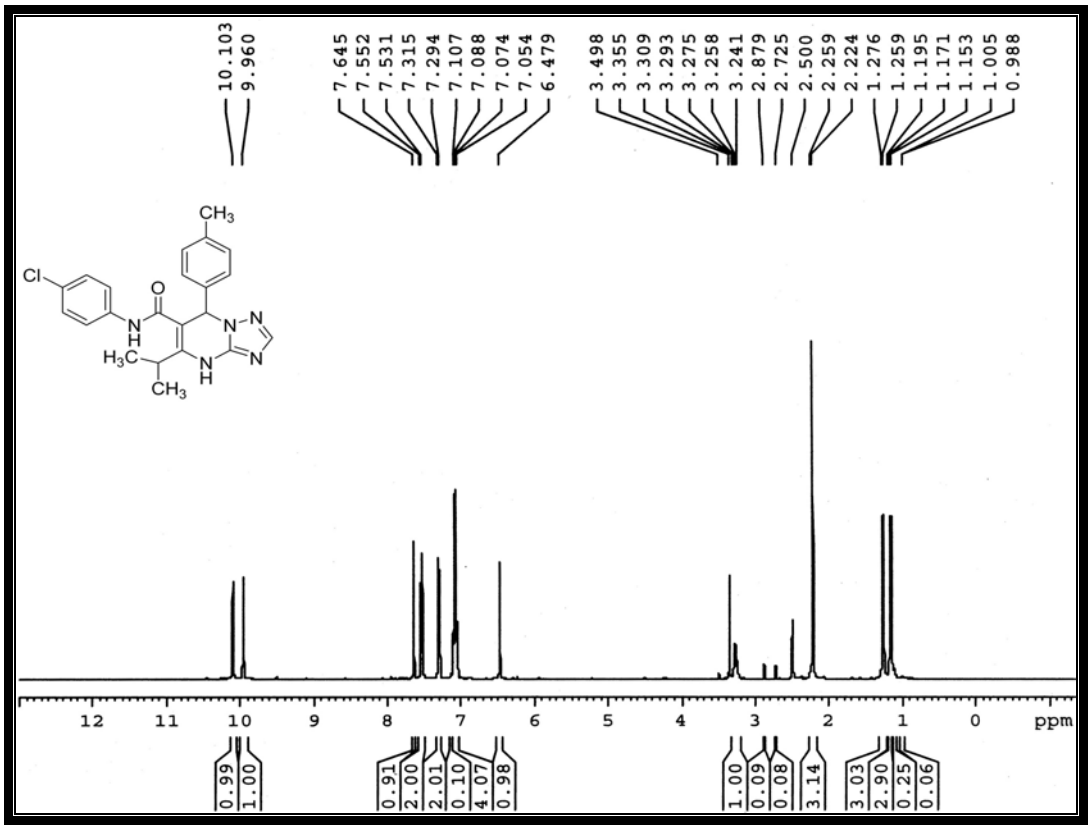
***IR spectrum of RHK - 162***



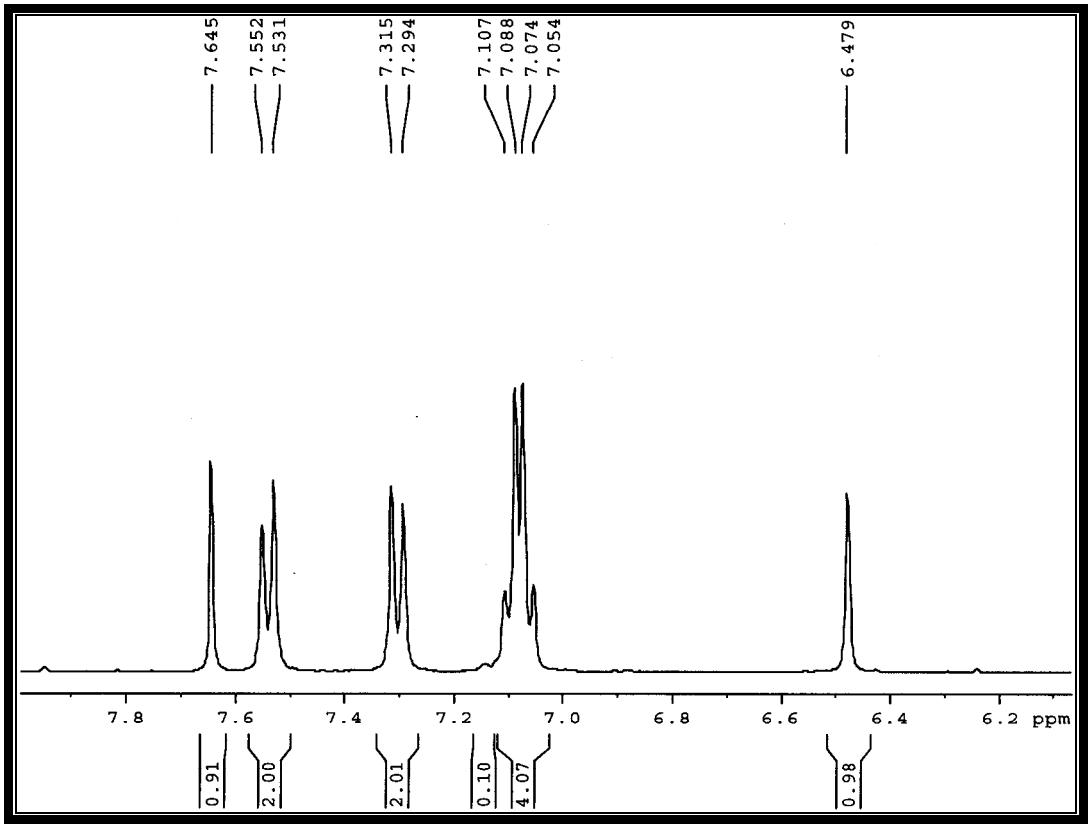

***Mass spectrum of RHK - 162***



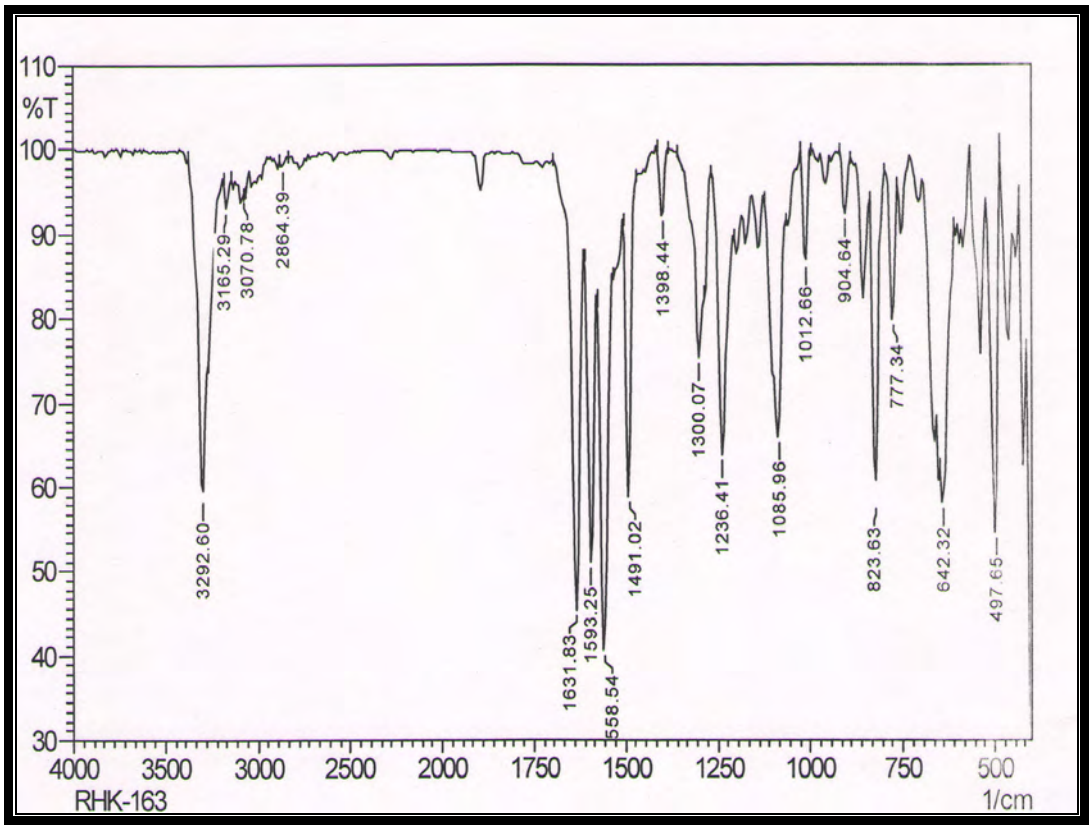
## *$^1$H NMR spectrum of RHK - 162*



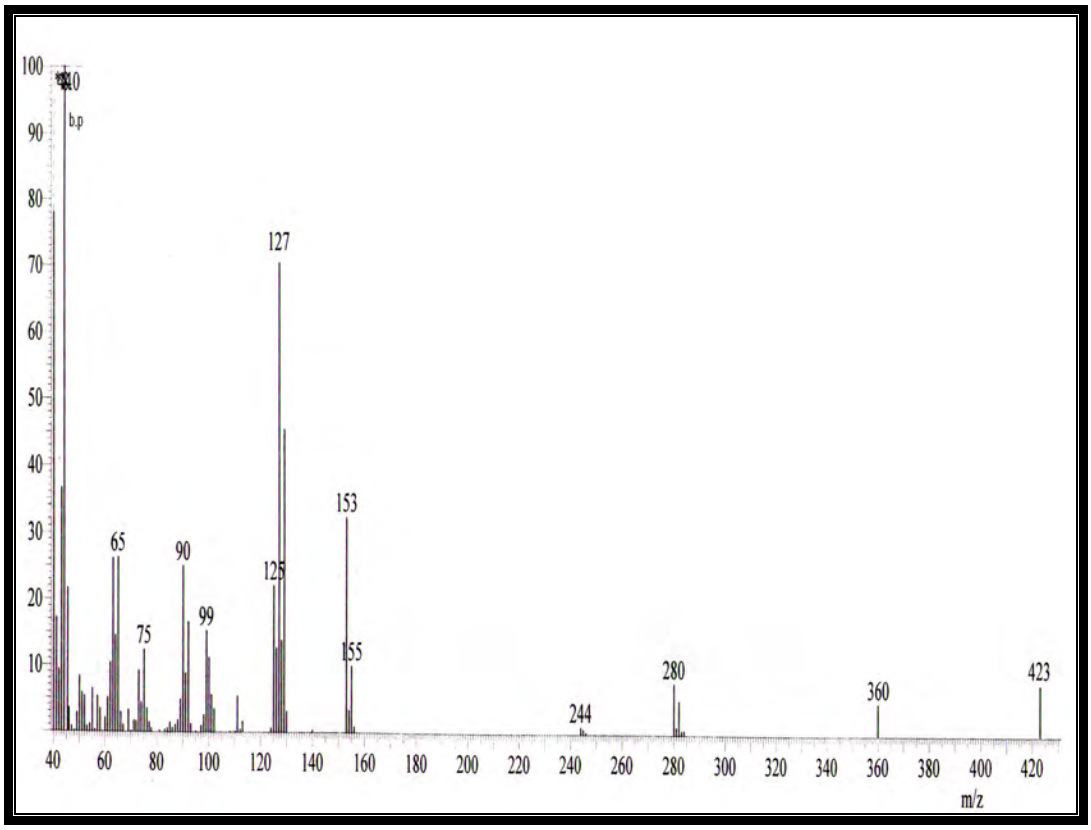

## *Expanded $^1$H NMR spectrum of RHK - 162*



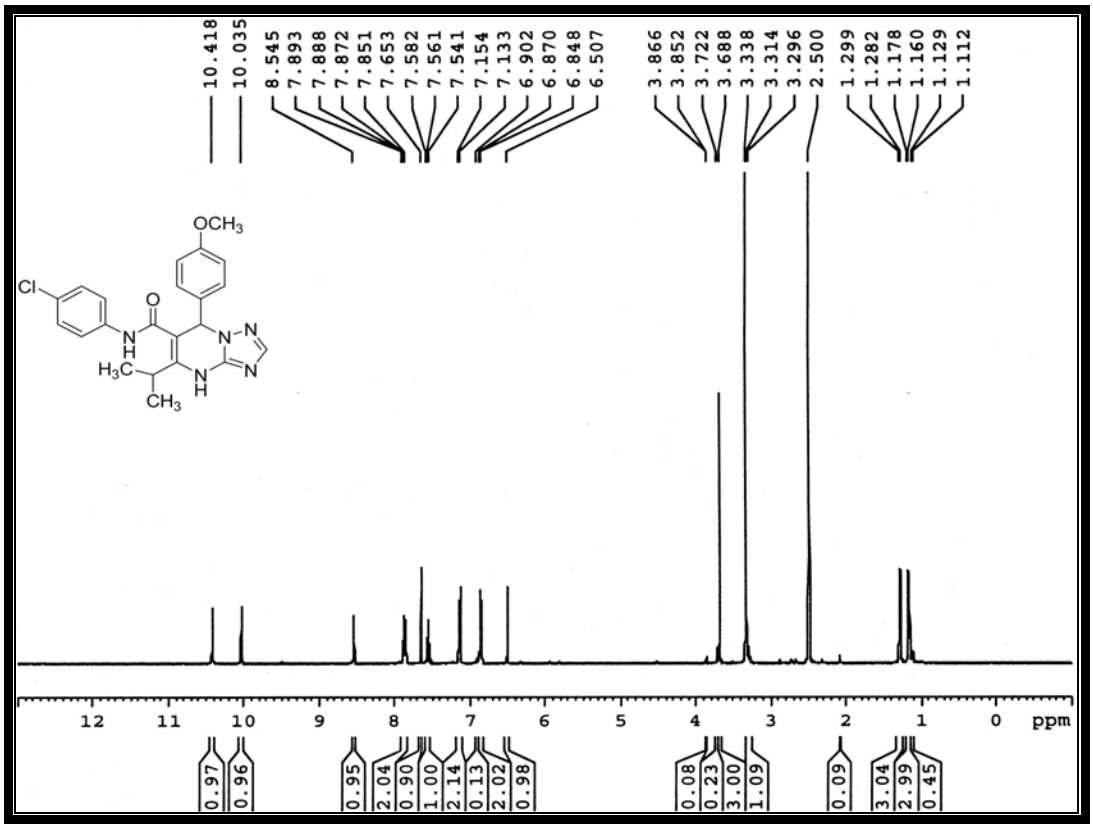
***IR spectrum of RHK - 163***



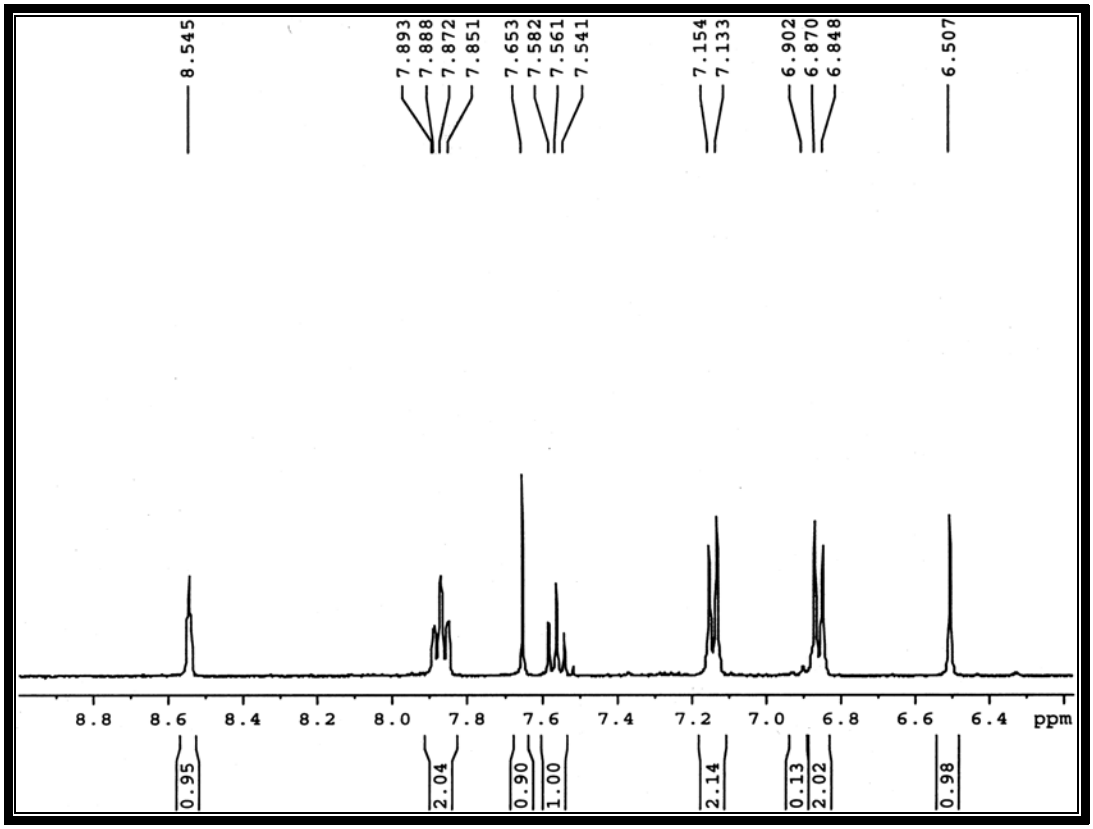

***Mass spectrum of RHK - 163***



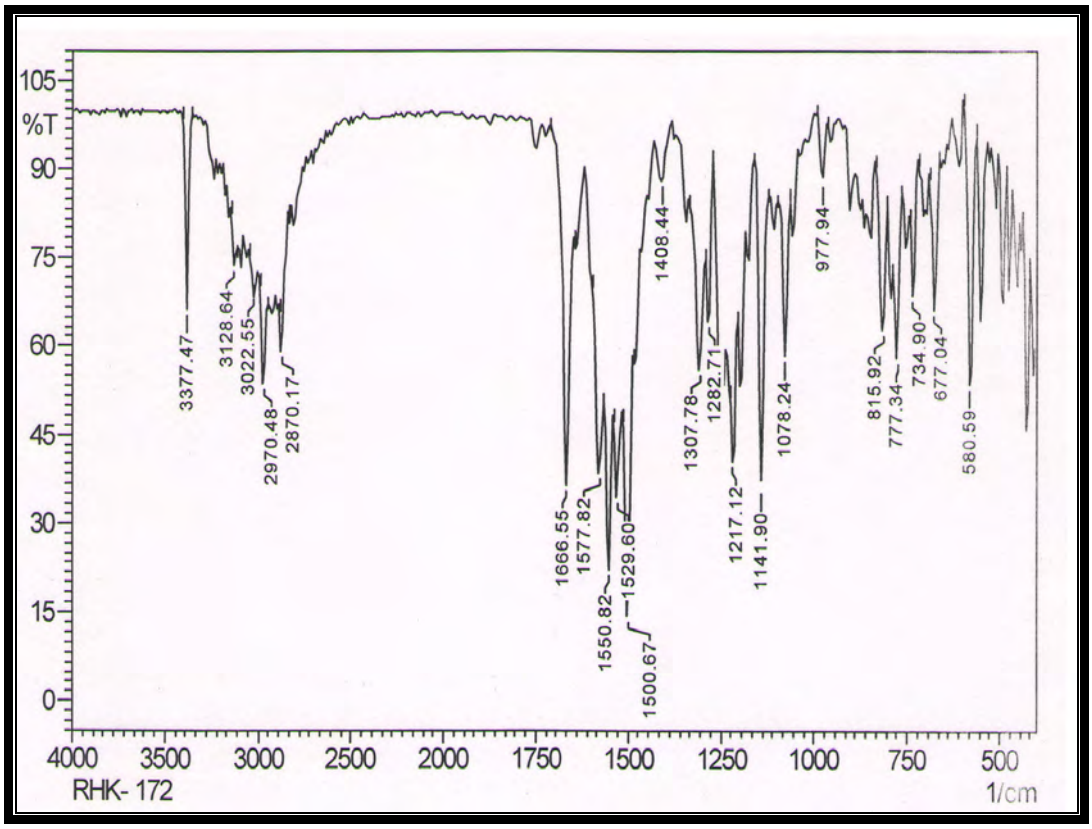
## *$^1$H NMR spectrum of RHK - 163*



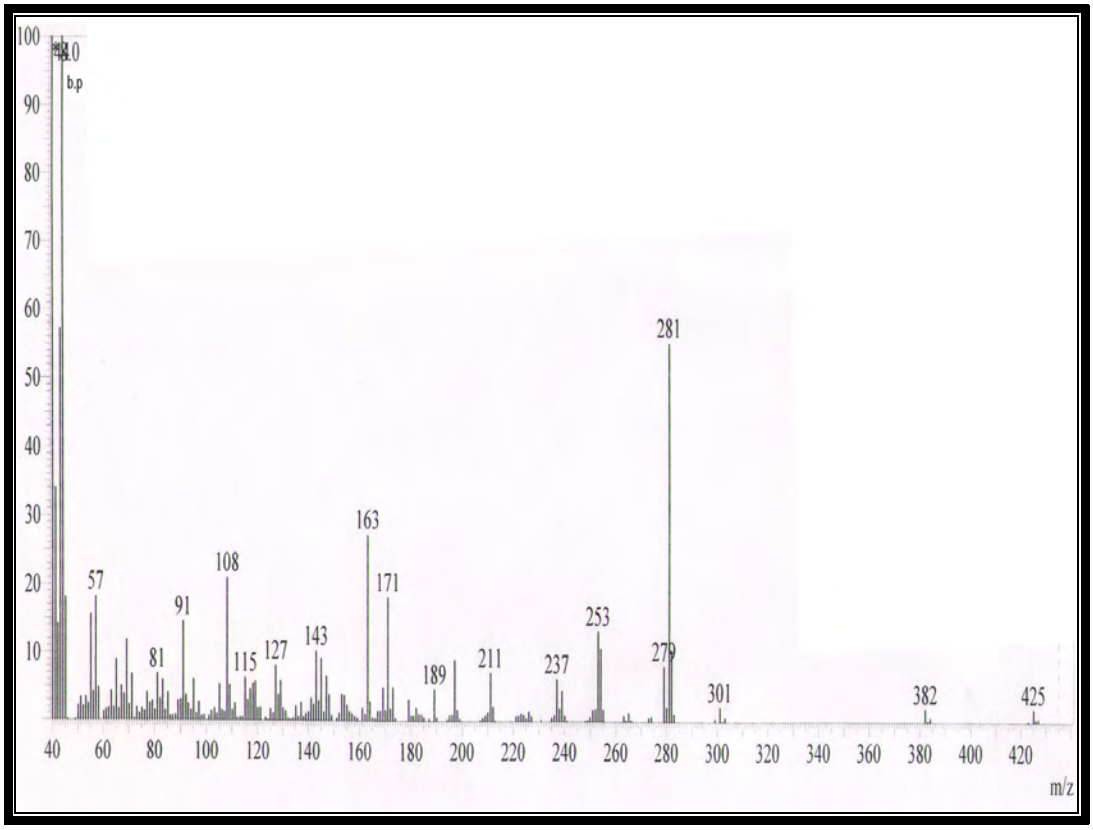

## *Expanded $^1$H NMR spectrum of RHK - 163*



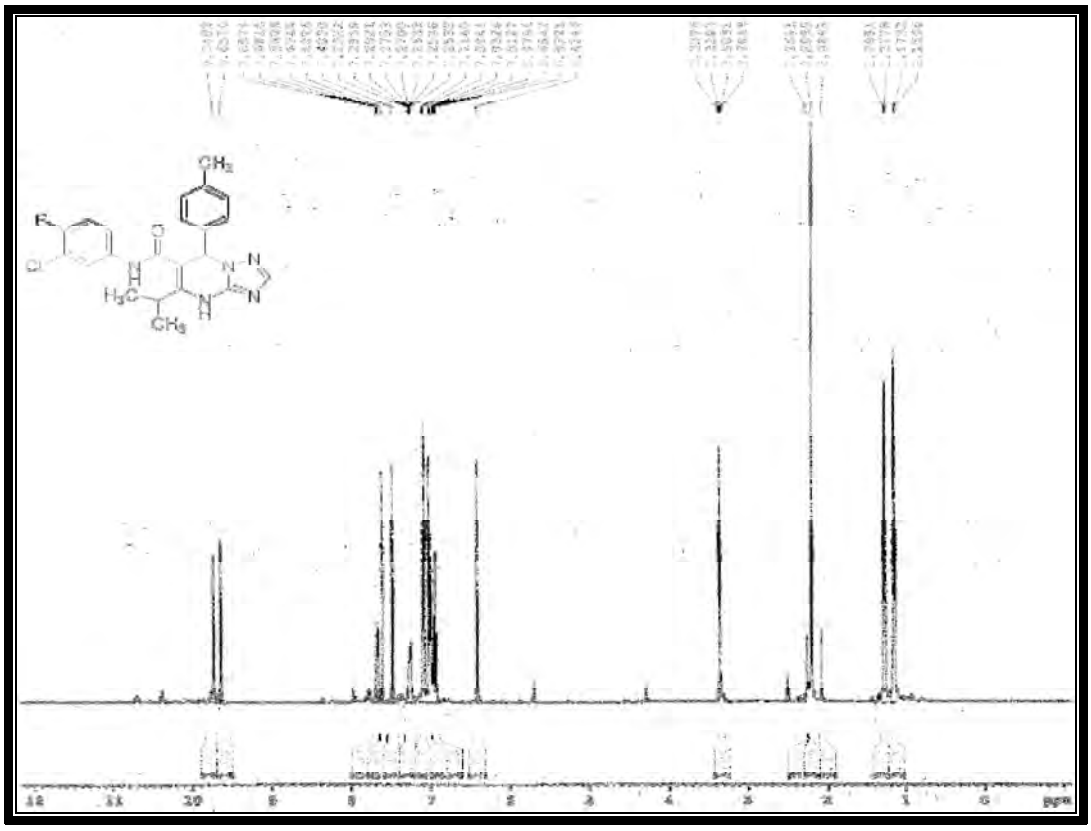
***IR spectrum of RHK - 172***



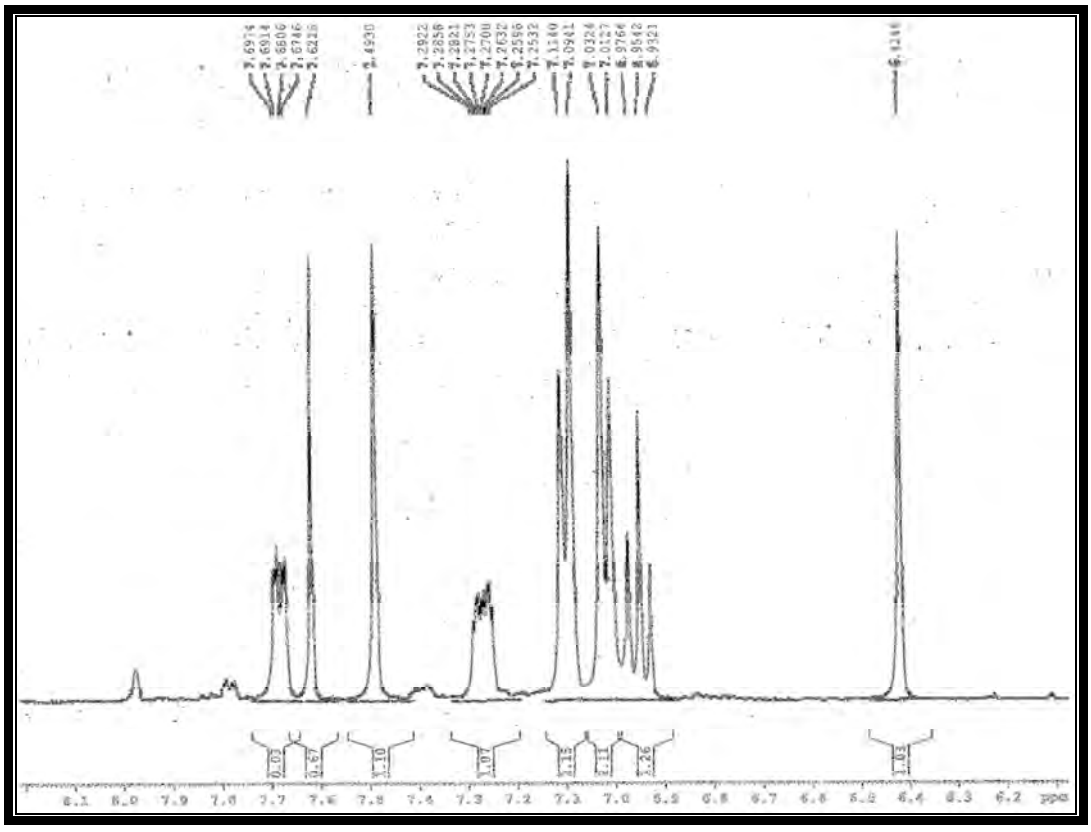

***Mass spectrum of RHK - 172***



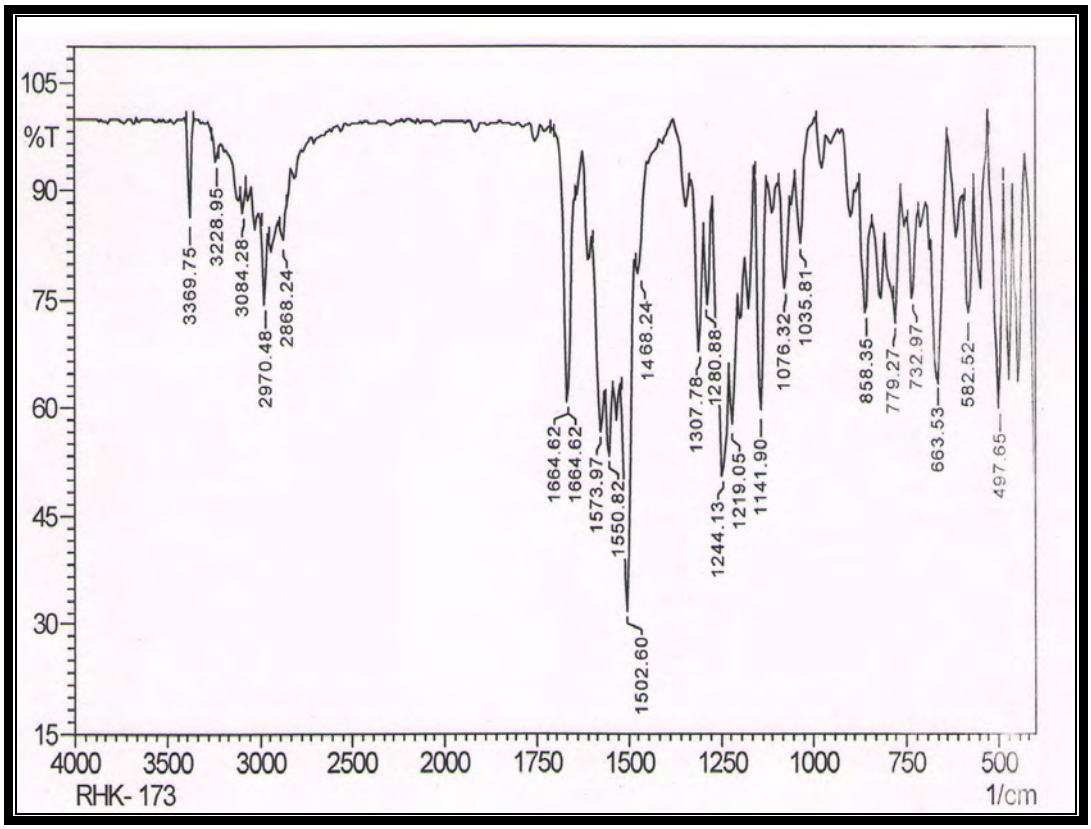
## *$^1$H NMR spectrum of RHK - 172*



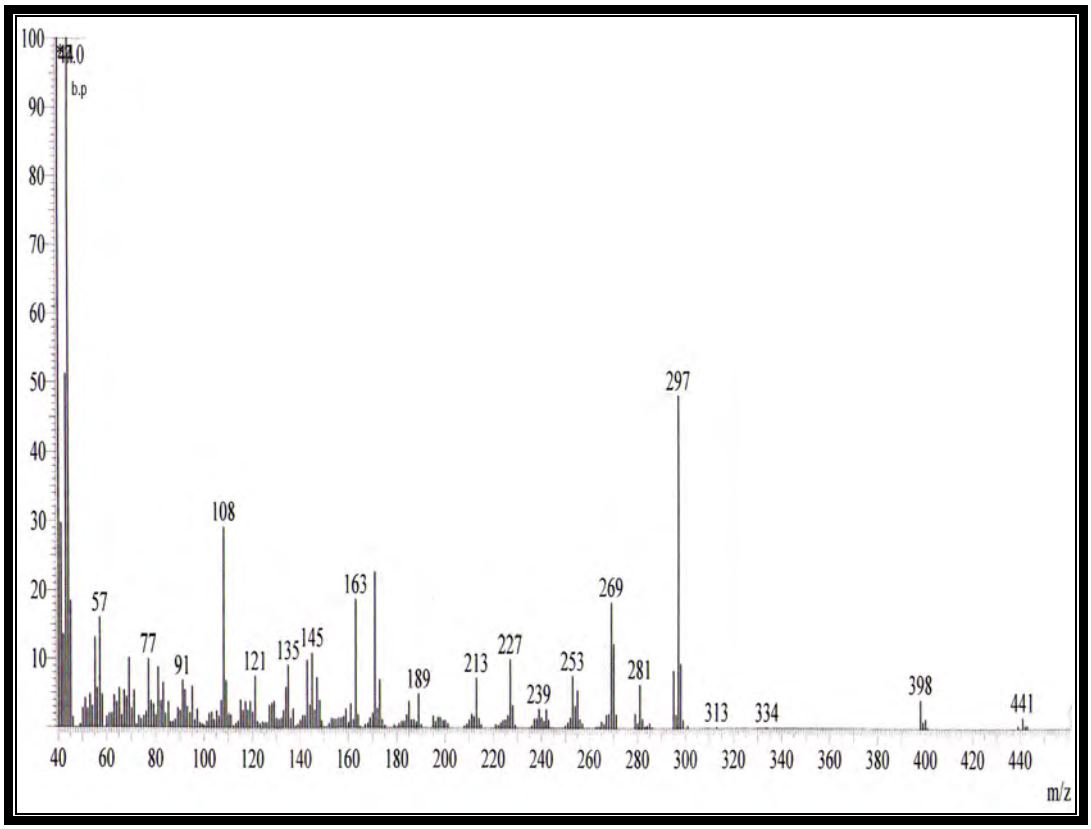
## *Expanded $^1$H NMR spectrum of RHK - 172*



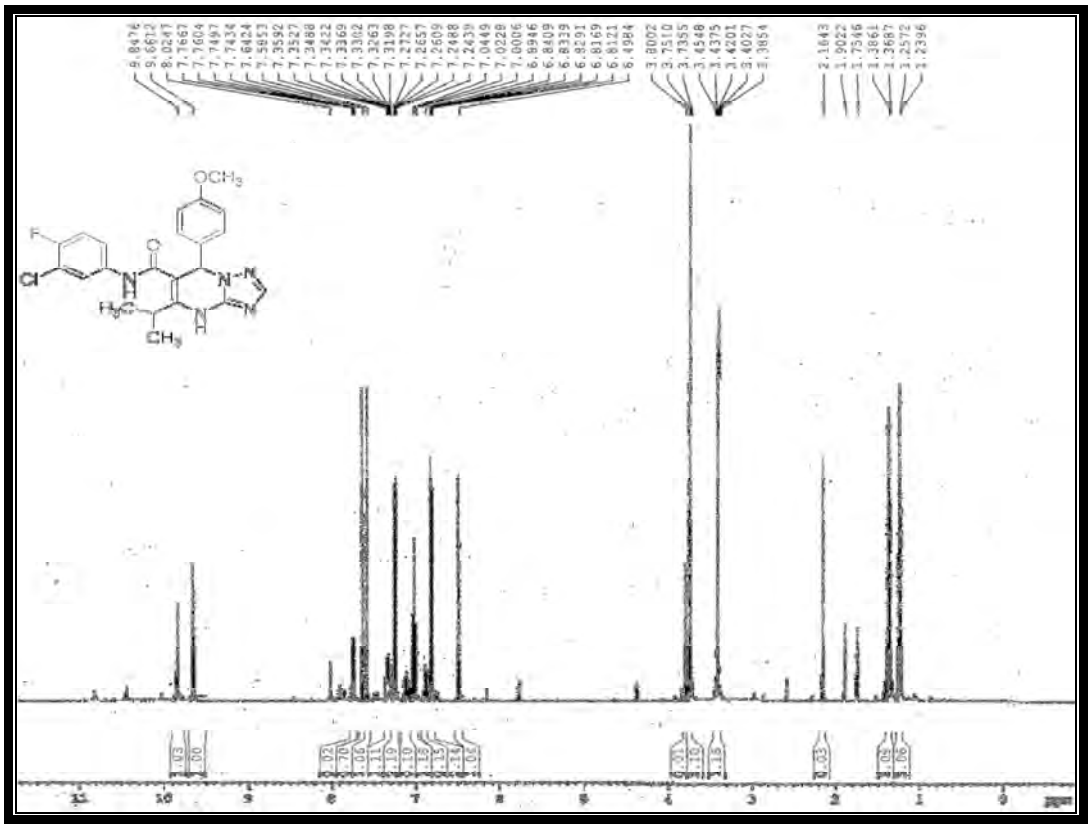
***IR spectrum of RHK - 173***



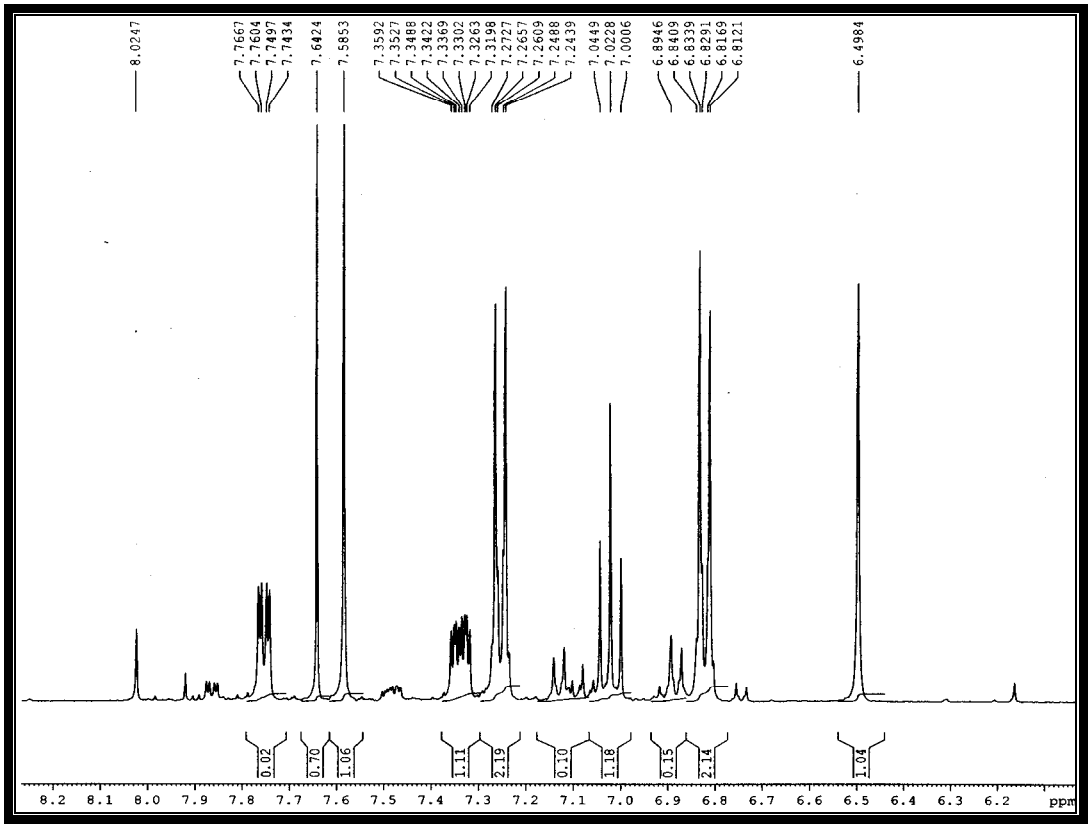

***Mass spectrum of RHK - 173***



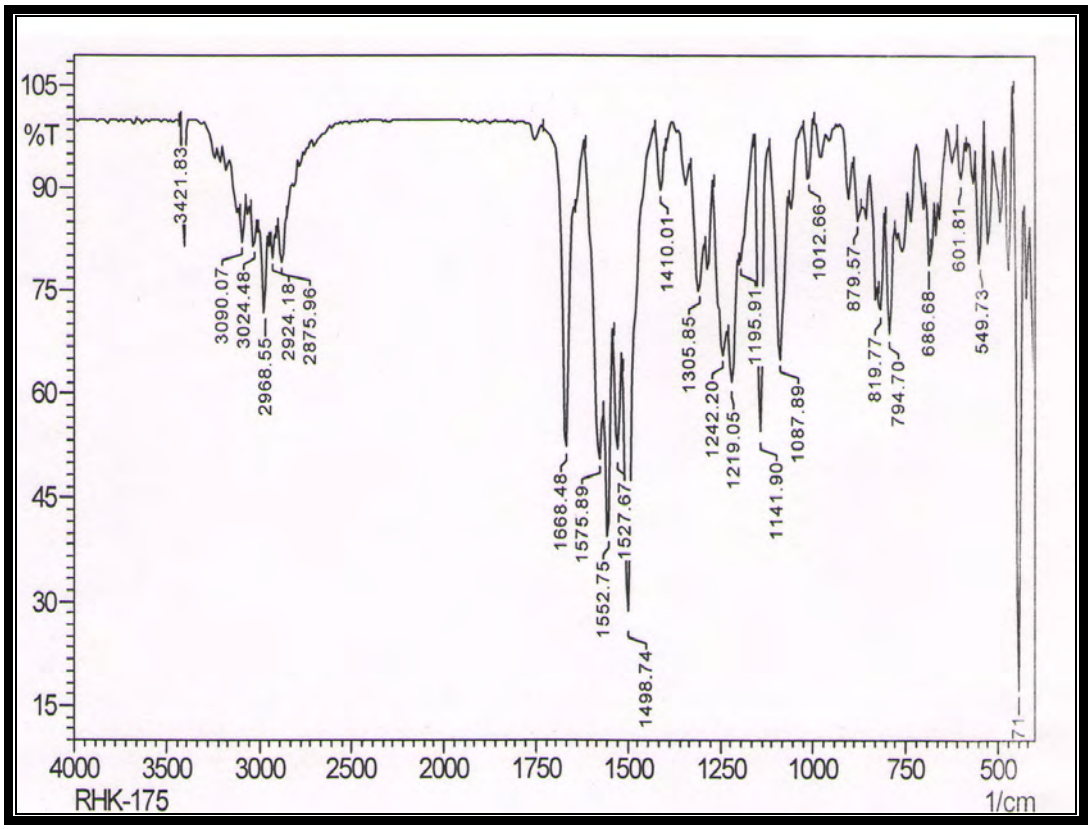
## *$^1$H NMR spectrum of RHK – 173*



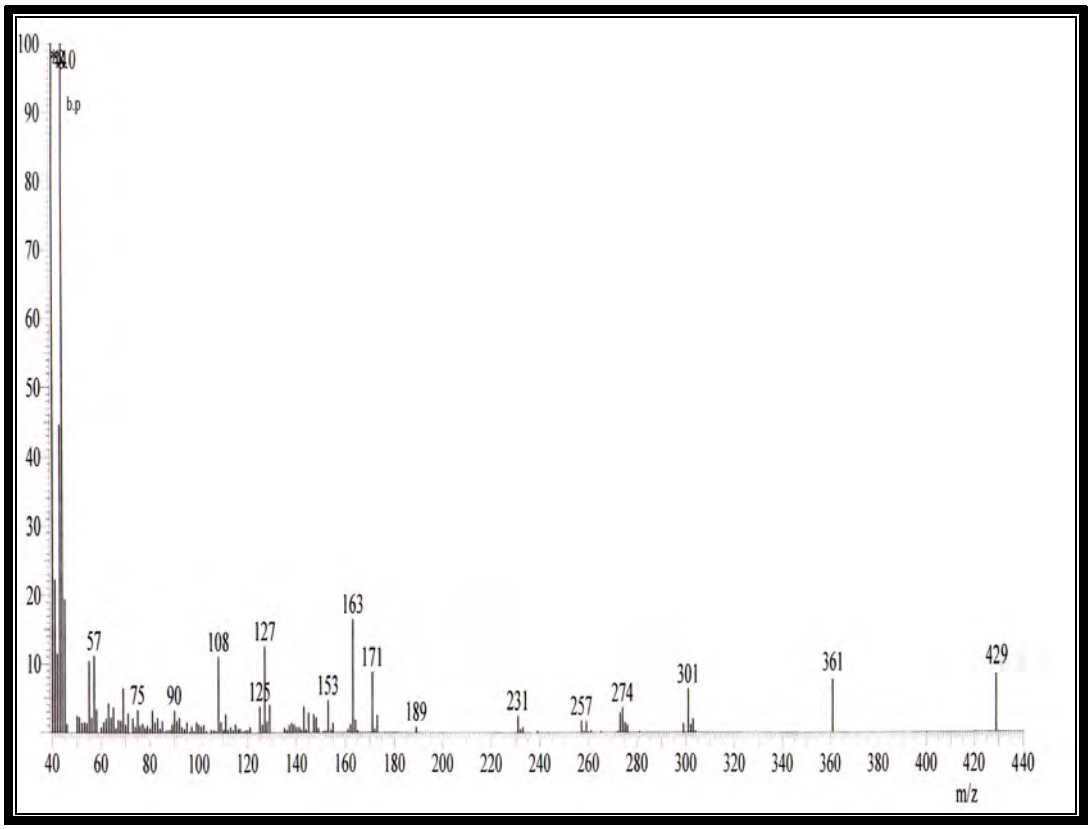
## *Expanded $^1$H NMR spectrum of RHK - 173*



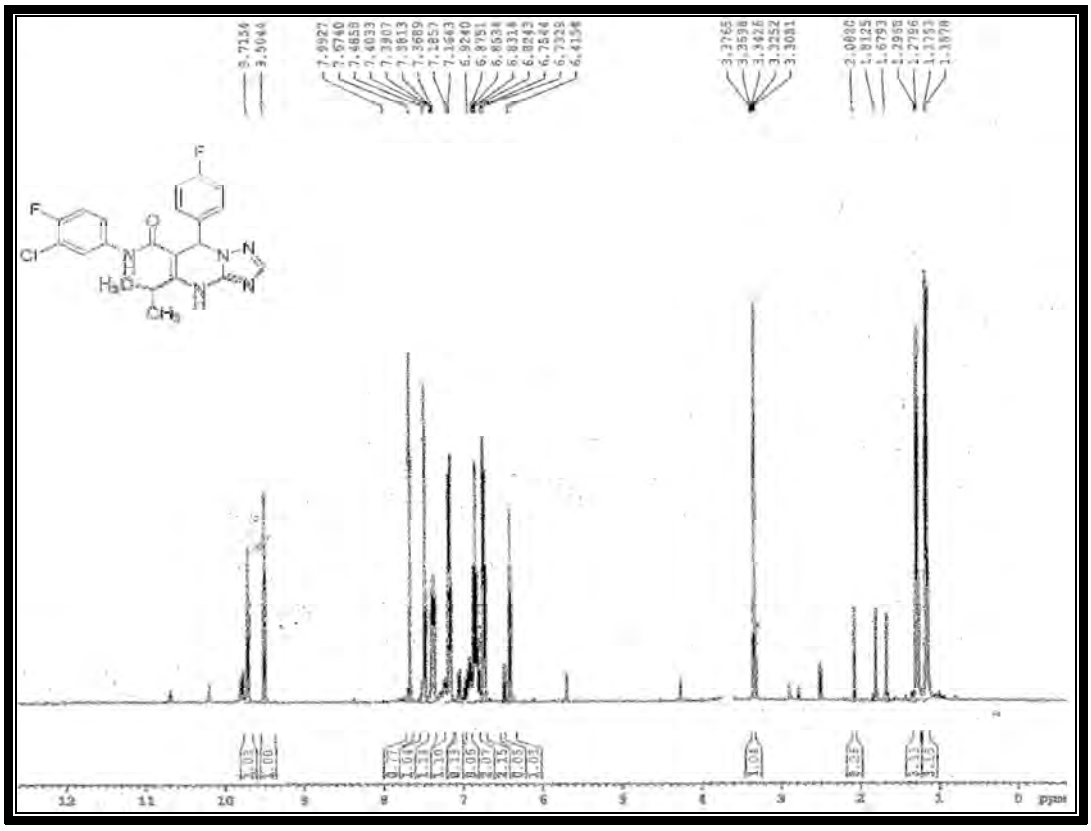
***IR spectrum of RHK - 175***



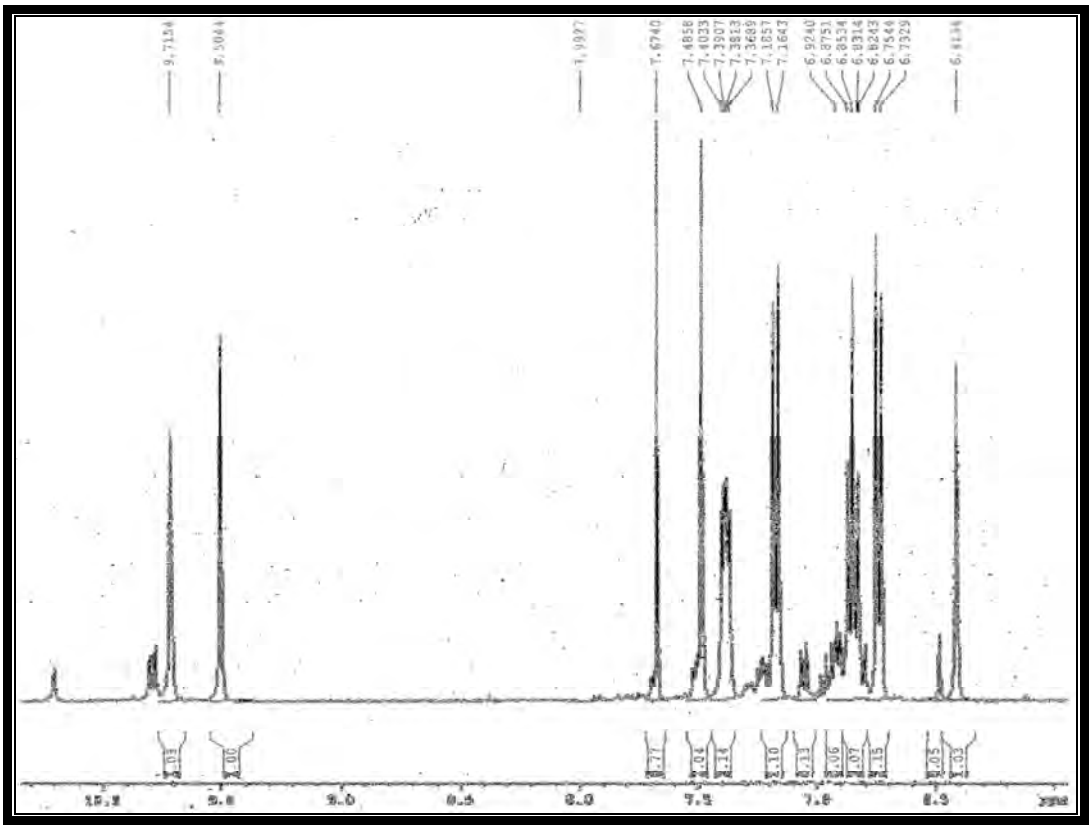

***Mass spectrum of RHK - 175***



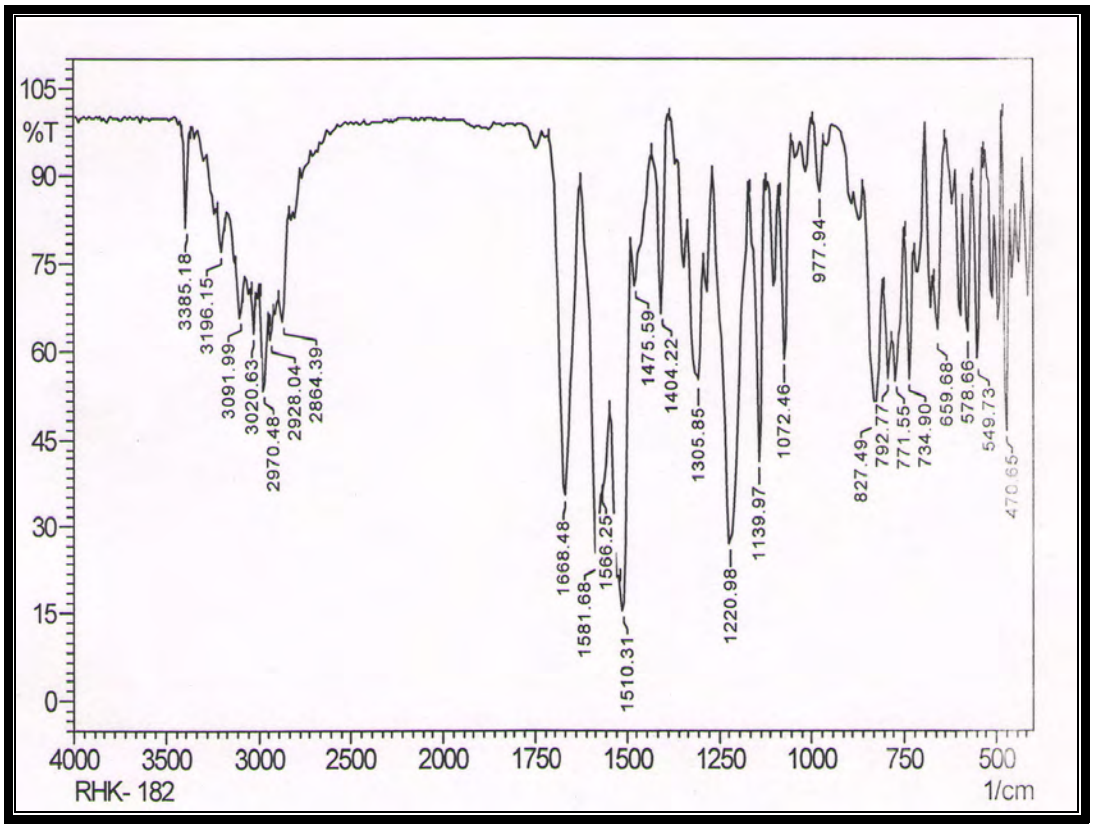
## *$^1$H NMR spectrum of RHK - 175*



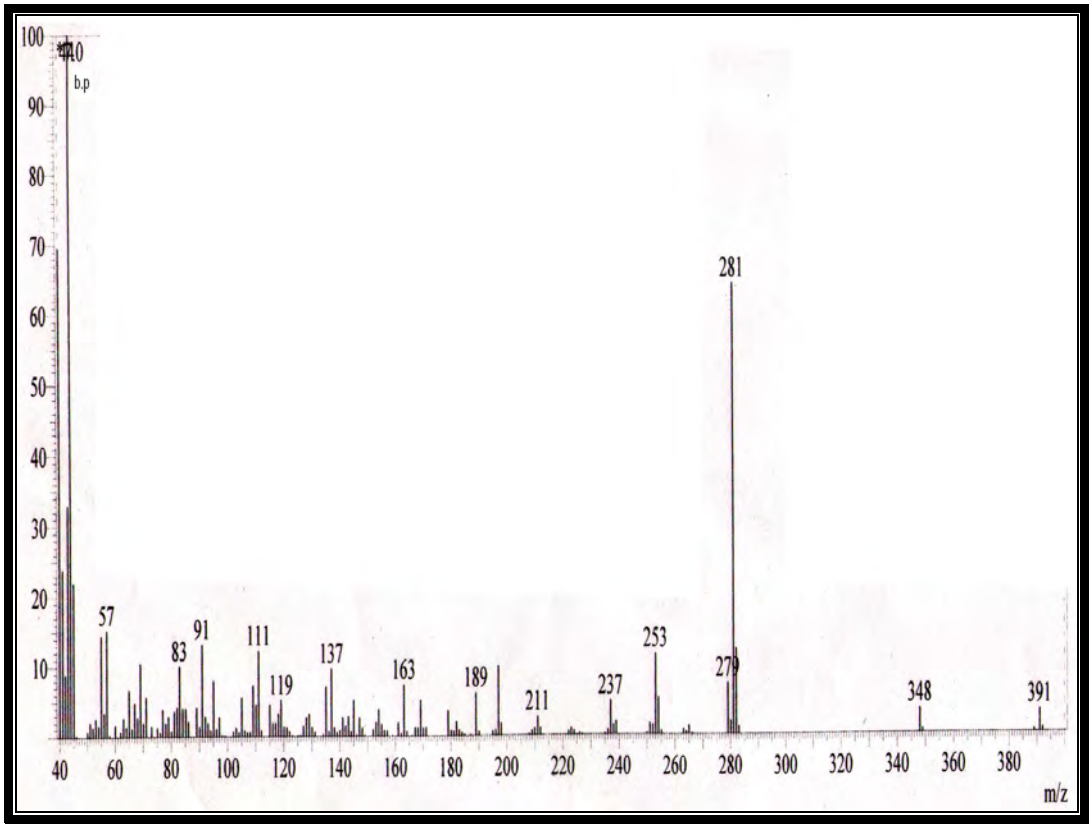
## *Expanded $^1$H NMR spectrum of RHK - 175*



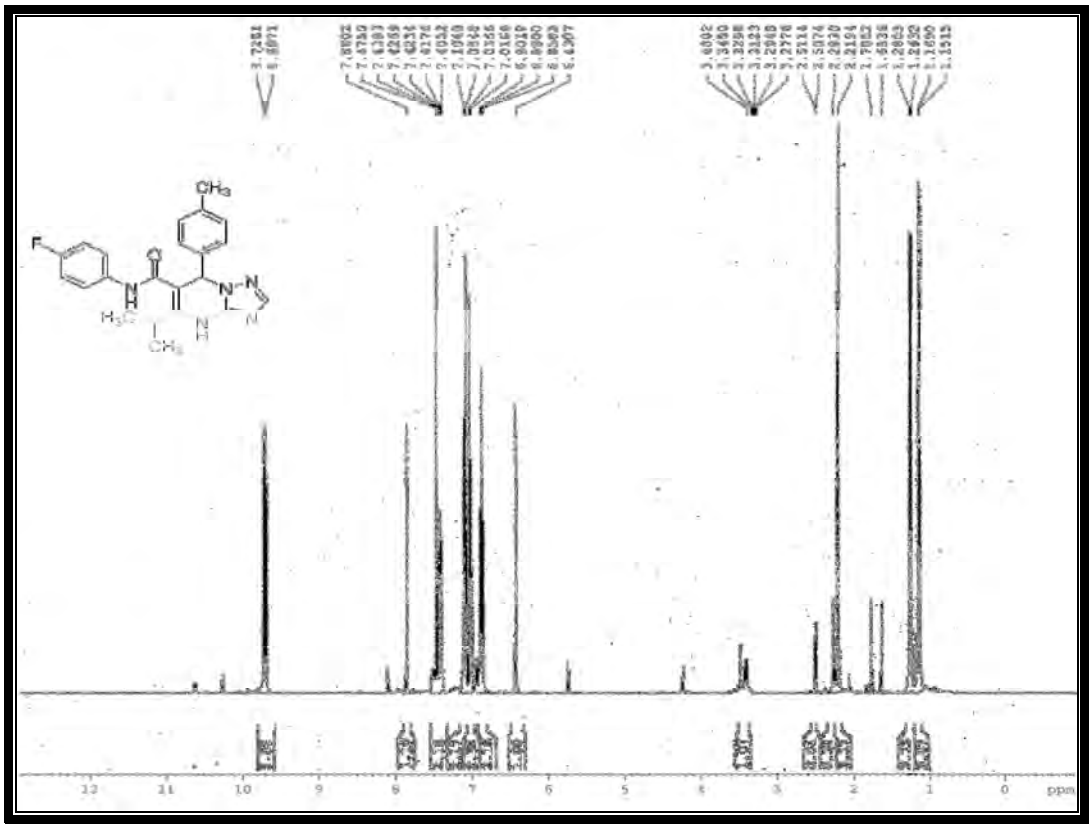
***IR spectrum of RHK - 182***



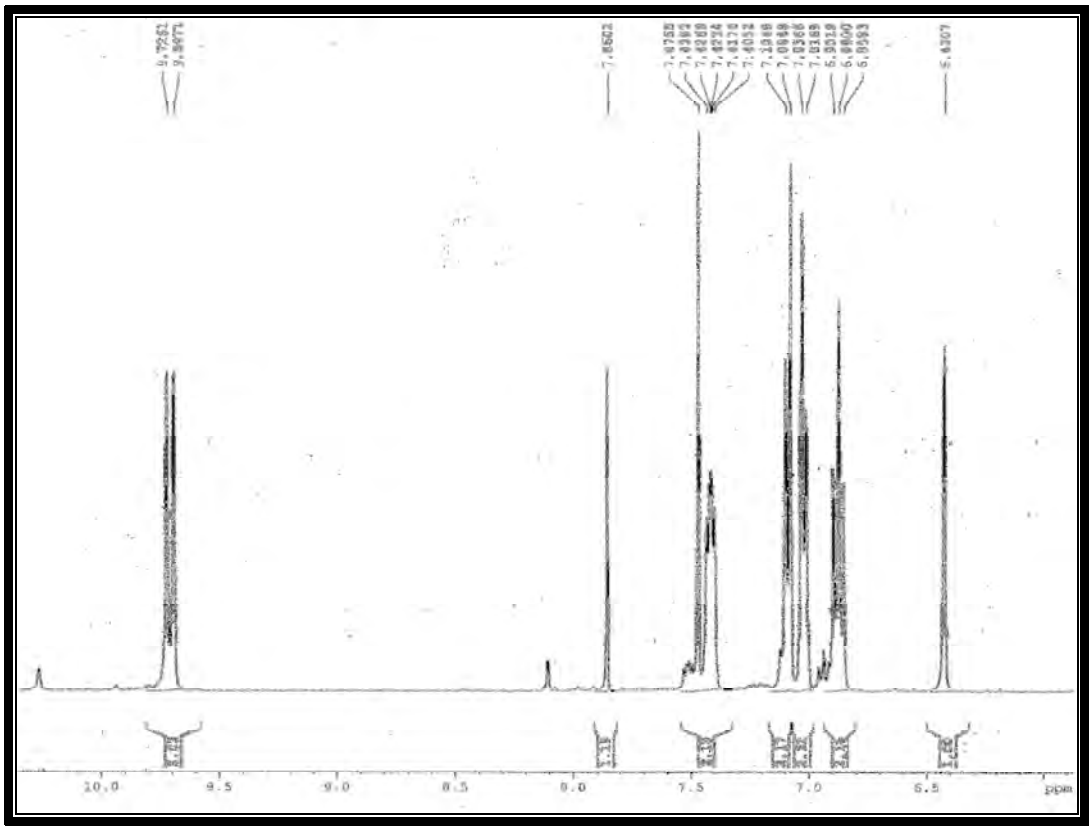

***Mass spectrum of RHK - 182***



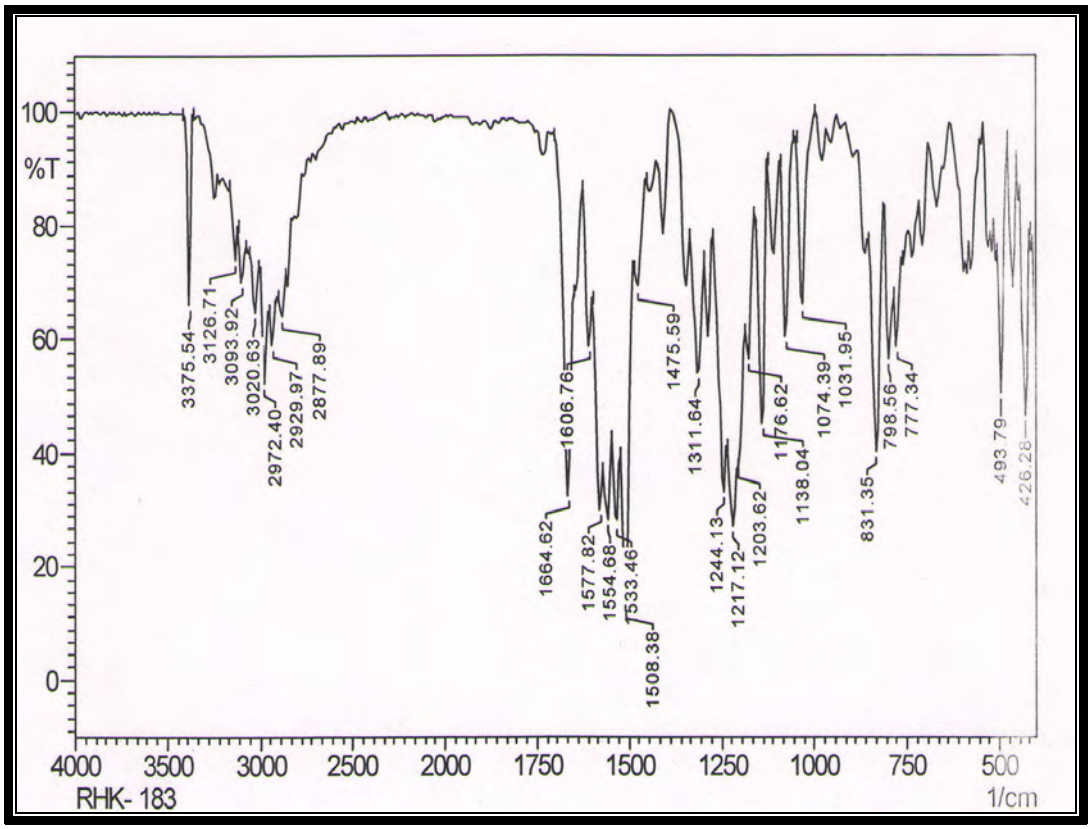
***$^1$H NMR spectrum of RHK - 182***



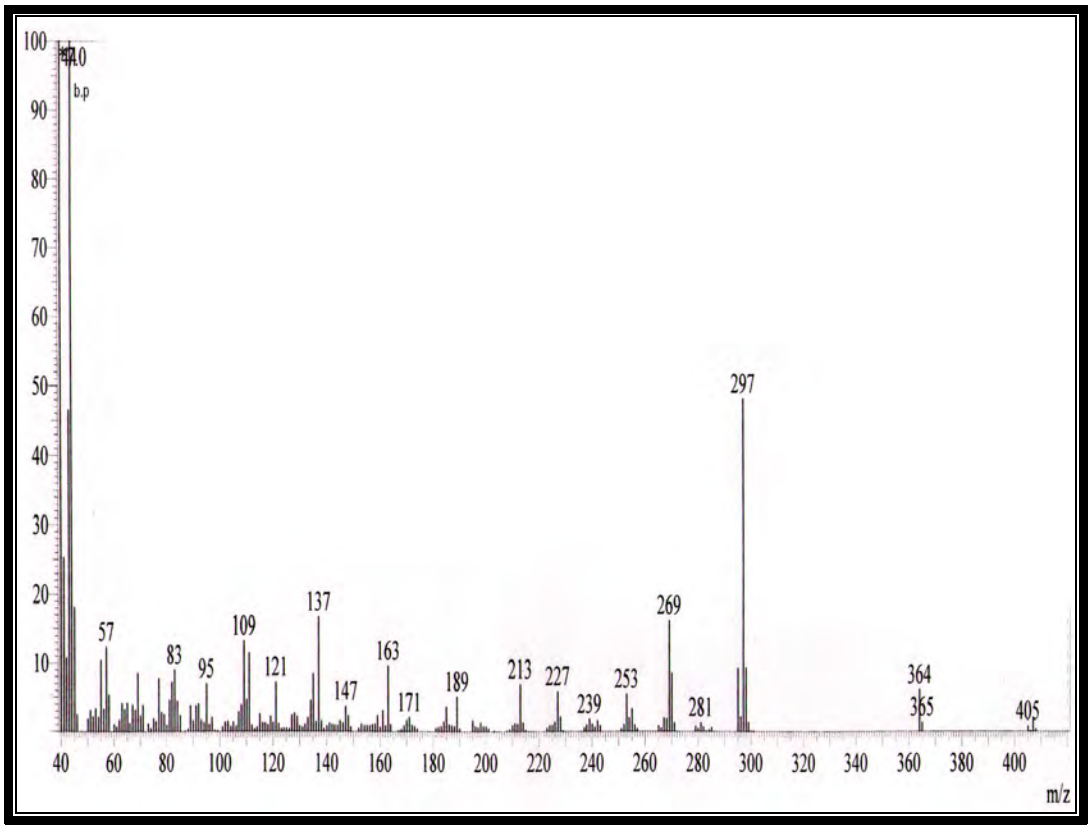
***Expanded $^1$H NMR spectrum of RHK - 182***



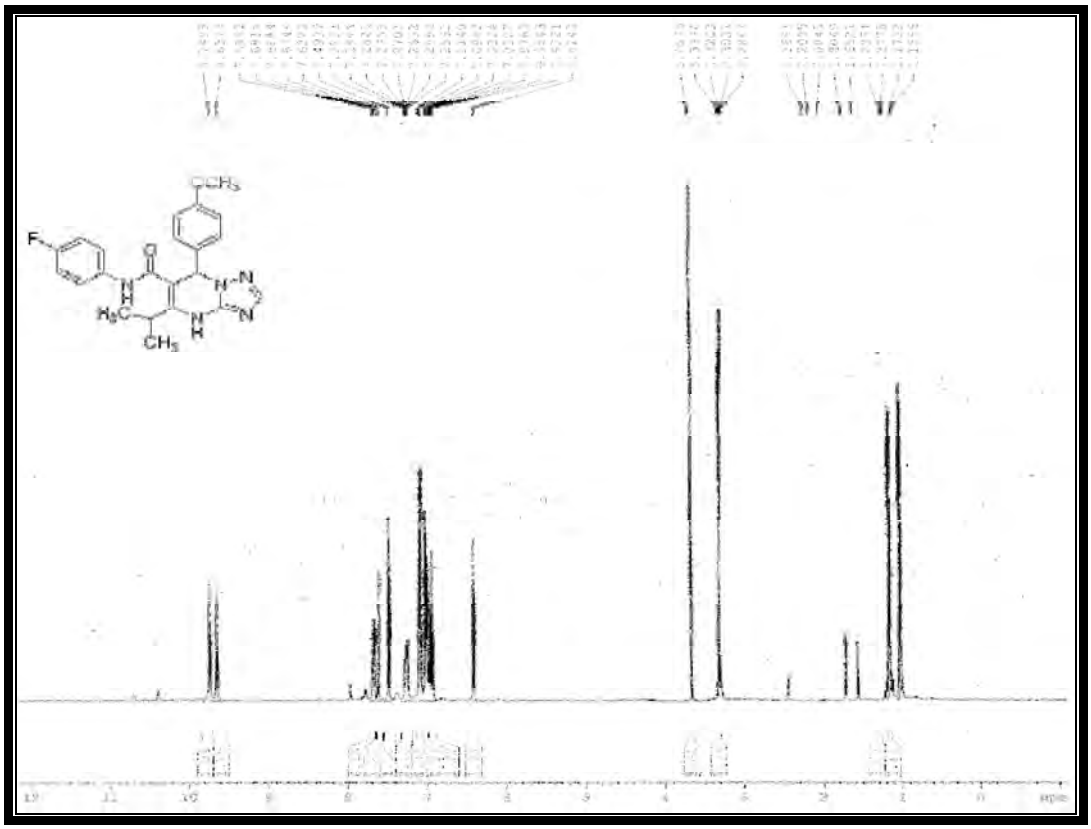
***IR spectrum of RHK - 183***



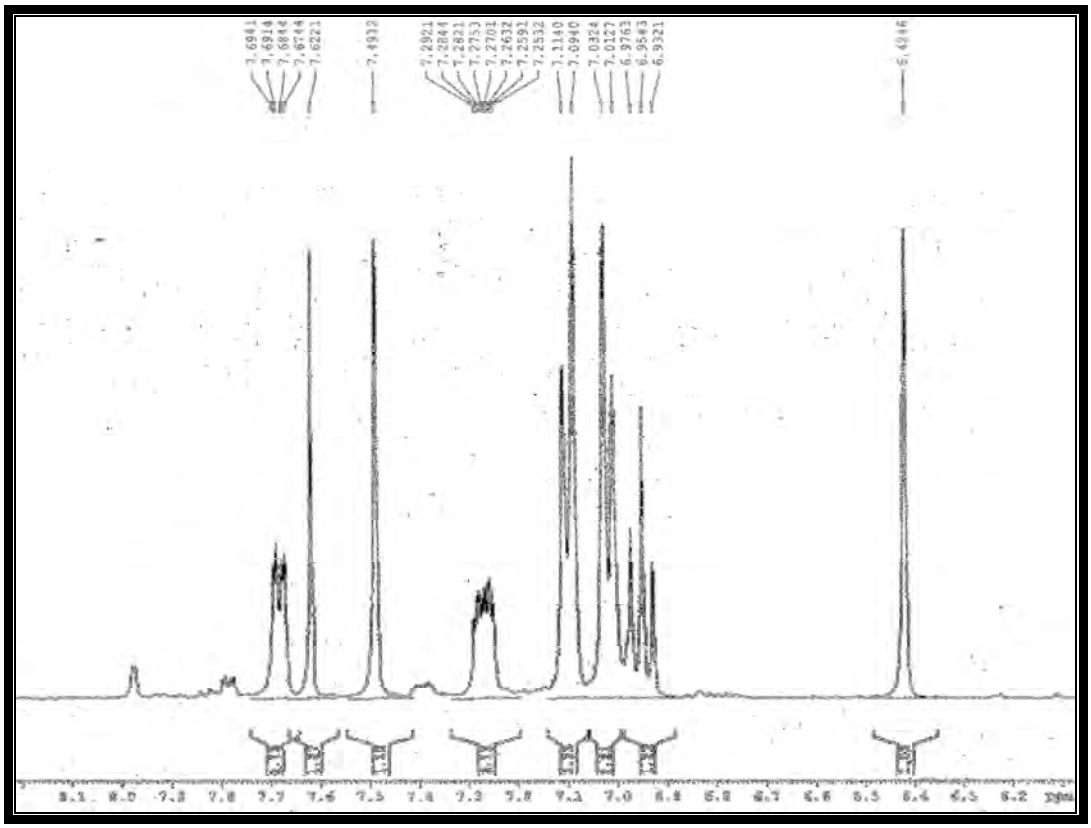

***Mass spectrum of RHK - 183***



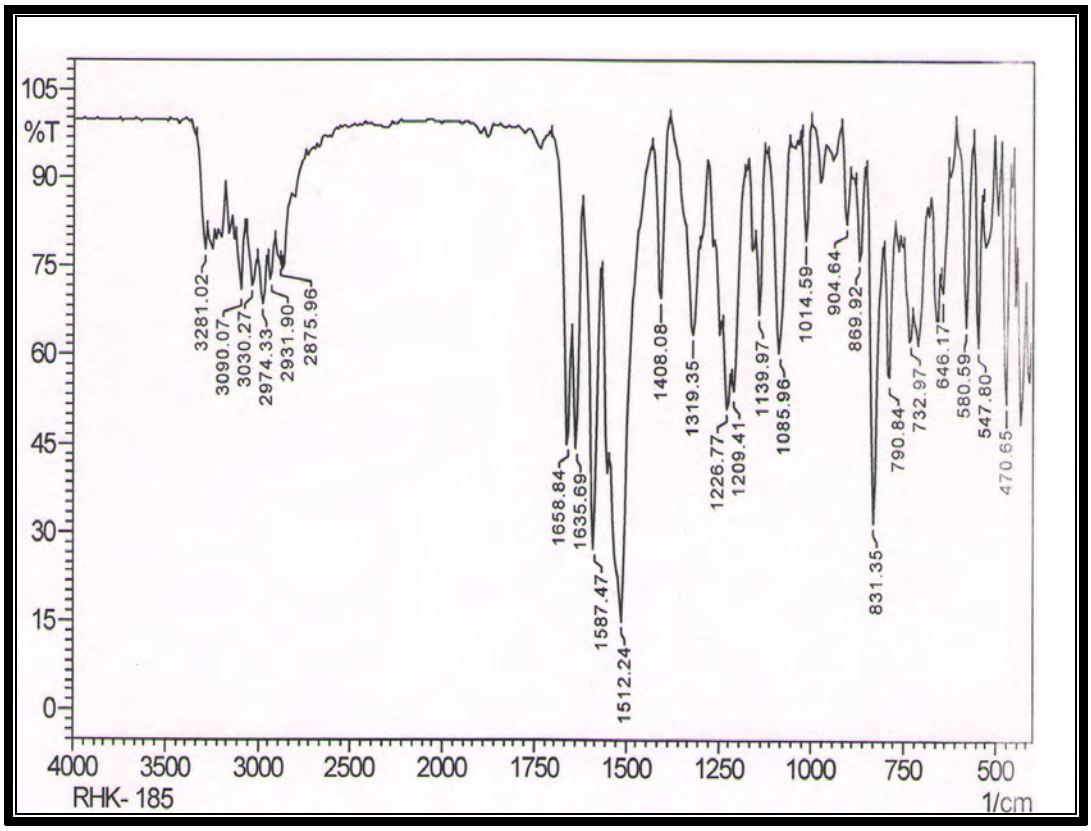
***$^1$H NMR spectrum of RHK - 183***



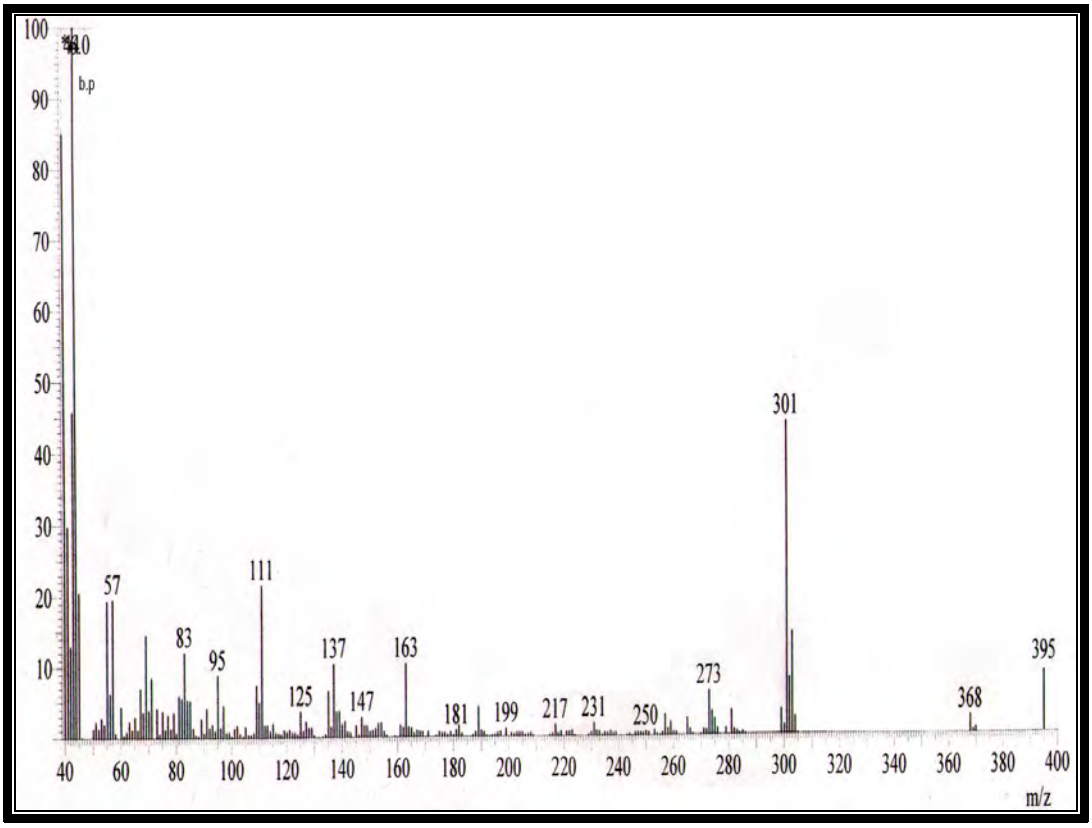
***Expanded $^1$H NMR spectrum of RHK - 183***



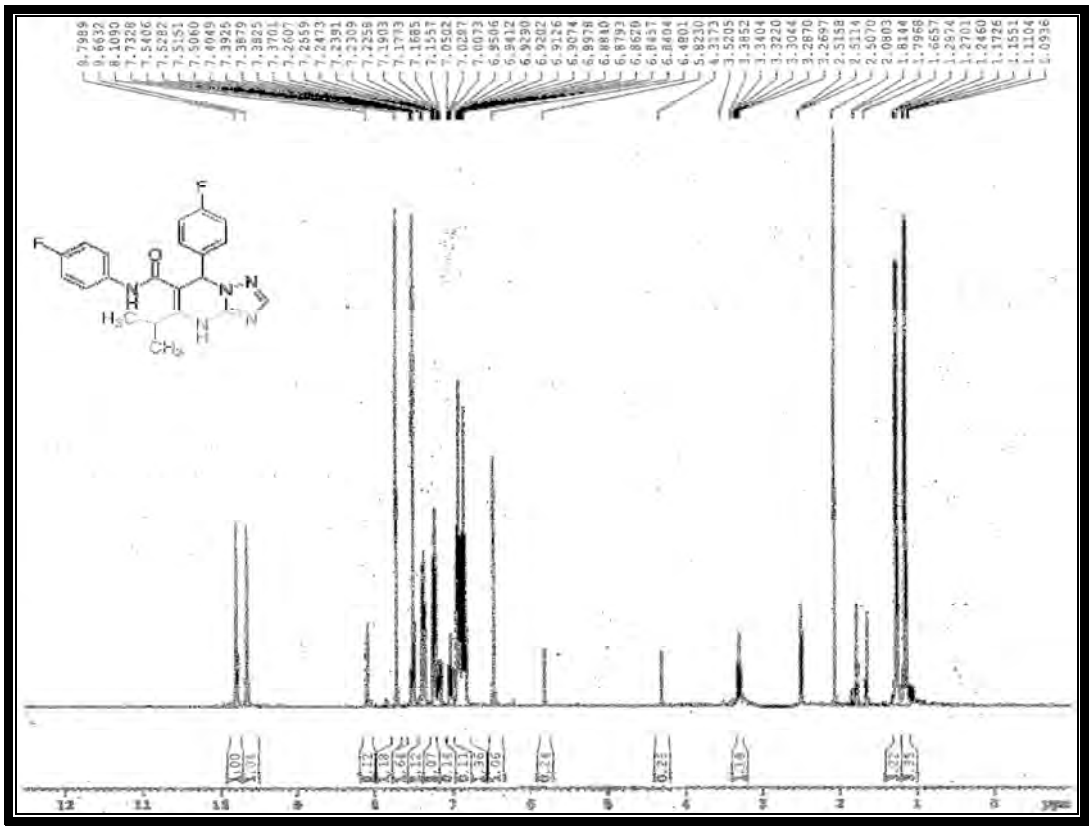
***IR spectrum of RHK - 185***



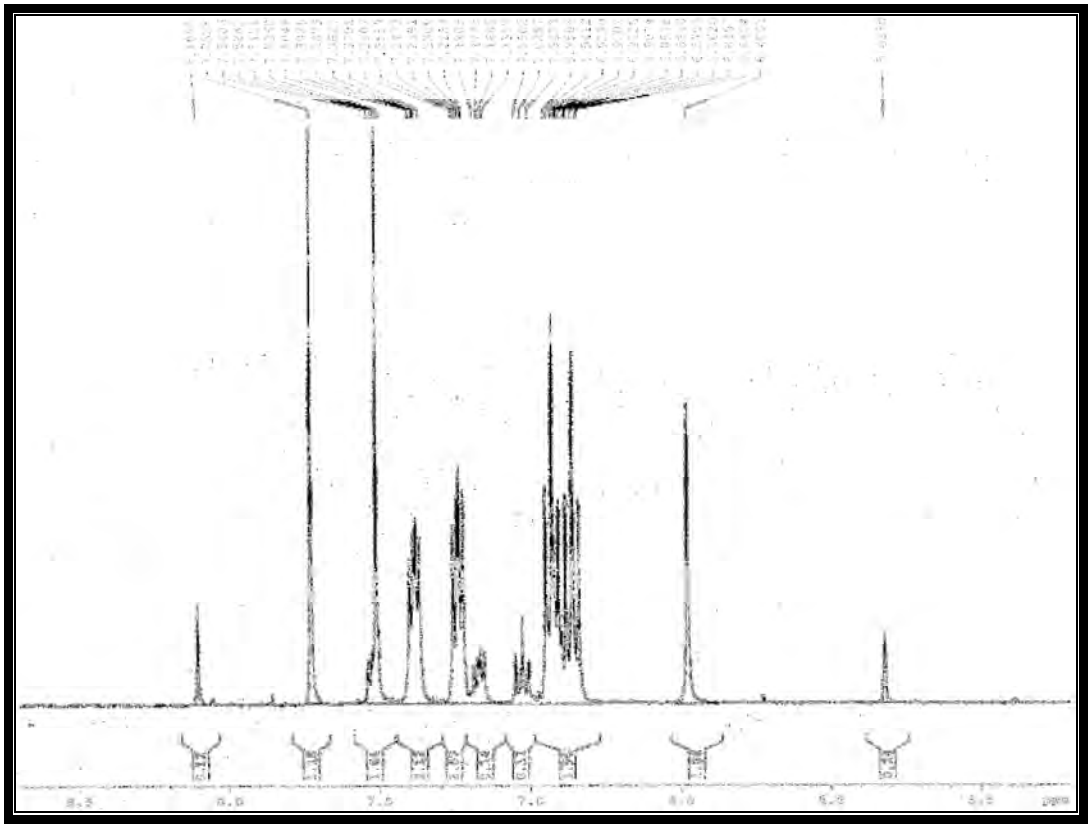

***Mass spectrum of RHK - 185***



*$^1$H NMR spectrum of RHK - 185*



*Expanded $^1$H NMR spectrum of RHK - 185*



## 4.8 Biological evaluation

### 4.8.1 Antimicrobial evaluation

All of the synthesized compounds (**RHK - 161 TO 190**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [98-100] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

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

***Minimal Inhibition Concentration [MIC]:-***

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

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

5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

***Methods used for primary and secondary screening: -***

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

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

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

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

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

**Table 1. Antibacterial and antifungal activity of synthesized compounds RHK-161 to 190**

| Code | Minimal inhibition concentration (μg $mL^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-positive | | Gram-negative | | Fungal species | | |
| | *S.a.* | *S. p.* | *E.c.* | *P.a.* | *C. a.* | *A. n.* | *A.c.* |
| RHK-161 | 1000 | 1000 | 500 | 500 | 250 | 1000 | 1000 |
| RHK-162 | 100 | 200 | 100 | 100 | 500 | 500 | 500 |
| RHK-163 | 250 | 250 | 200 | 250 | >1000 | 250 | 250 |
| RHK-164 | 1000 | 500 | 500 | 100 | 250 | >1000 | >1000 |
| RHK-165 | 250 | 250 | 250 | 250 | >1000 | 1000 | 1000 |
| RHK-166 | 150 | 200 | 200 | 100 | 1000 | 500 | 500 |
| RHK-167 | 1000 | 500 | 250 | 500 | 1000 | 500 | 1000 |
| RHK-168 | 150 | 250 | 250 | 250 | 250 | 1000 | 1000 |
| RHK-169 | 150 | 200 | 250 | 100 | 500 | 500 | 1000 |
| RHK-170 | 62.5 | 500 | 250 | 1000 | 500 | 250 | 250 |
| RHK-171 | 250 | 250 | 250 | 250 | 500 | 500 | 500 |
| RHK-172 | 200 | 200 | 50 | 100 | 250 | 1000 | 1000 |
| RHK-173 | 250 | 250 | 500 | 500 | 500 | 250 | 250 |
| RHK-174 | 250 | 500 | 100 | 200 | 500 | 200 | 200 |
| RHK-175 | 100 | 200 | 100 | 250 | 1000 | >1000 | >1000 |
| RHK-176 | 250 | 100 | 250 | 500 | 1000 | >1000 | >1000 |
| RHK-177 | 200 | 50 | 50 | 100 | 500 | 500 | 1000 |
| RHK-178 | 250 | 500 | 150 | 200 | 500 | 1000 | >1000 |
| RHK-179 | 500 | 1000 | 500 | 500 | 500 | 1000 | 1000 |
| RHK-180 | 100 | 100 | 500 | 250 | 200 | 1000 | 1000 |
| RHK-181 | 150 | 200 | 250 | 250 | >1000 | 500 | 250 |
| RHK-182 | 100 | 200 | 200 | 250 | 500 | 500 | 500 |
| RHK-183 | 200 | 250 | 250 | 250 | >1000 | >1000 | >1000 |
| RHK-184 | 250 | 250 | 500 | 500 | >1000 | >1000 | >1000 |
| RHK-185 | 1000 | 1000 | 500 | 250 | 1000 | 250 | 250 |
| RHK-186 | 250 | 500 | 150 | 500 | 1000 | 250 | 500 |
| RHK-187 | 1000 | 1000 | 1000 | 500 | 1000 | 500 | 500 |
| RHK-188 | 200 | 250 | 200 | 100 | 250 | >1000 | >1000 |
| RHK-189 | 500 | 250 | 500 | 250 | >1000 | 1000 | 1000 |
| RHK-190 | 200 | 250 | 62.5 | 100 | 1000 | 500 | 500 |
| Gentamycin | 0.25 | 0.5 | 0.05 | 1 | - | - | - |
| Ampicillin | 250 | 100 | 100 | 100 | - | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| Iprofloxacin | 50 | 50 | 25 | 25 | - | - | - |
| Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| Nystatin | - | - | - | - | 100 | 100 | 100 |
| Griseofulvin | - | - | - | - | 500 | 100 | 100 |

### 4.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (**RHK- 161 to 190**) is currently under investigation and results are awaited.

## 4.10 References and notes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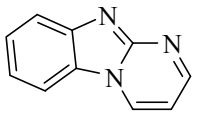
# CHAPTER 5
# Synthesis and biological evaluation of 1,4-dihydropyrimido[1,2-*a*]benz-imidazole

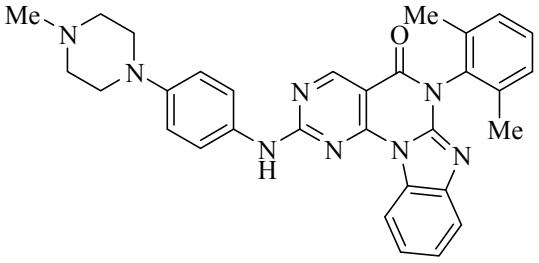
## 5.1 Introduction

Polysubstituted pyrimido[1,2-*a*]benzimidazoles possess a wide spectrum of biological activities and they are structurally related to natural purine bases.

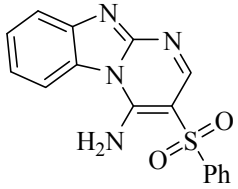


**(1) Pyrimido[1,2-*a*]benzimidazole**

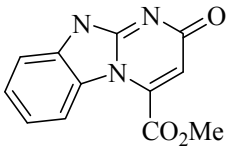
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Antimicrobial [1-4], antimalarial [5], antiproliferative [6], protein kinase inhibitor, [7], T cell activation, [8], angioprotein receptors and/or vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitory activities. [9], hypotensive, spasmolytic, and antiaggregant activities [10], anesthetic activity [11] and diuretic [12], antiInflammatory (2) [13, 14], antiamoebic [15], substance P receptor binding activity [16], antiarrhythmic [17], central nervous system-depressing [18], antidiabetics [19], virucidal [20], neurotropic [21], benzodiazepine receptor binding activity (4) [22], antiparasitic (6) [23], herbicidal [24], DNA-topoisomerase I inhibitory activity (7) [25], immunotropic activity (12) [26], antineoplastic activity (8) [27] etc. activities have been reported for certain pyrimido[1,2-*a*]benzimidazole derivatives. Some examples of published derivatives of certain pyrimido[1,2-*a*]benzimidazole with their biological activities are as following.



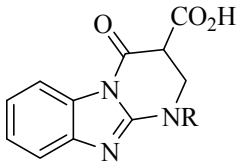
**(2) Antiinflammatory**



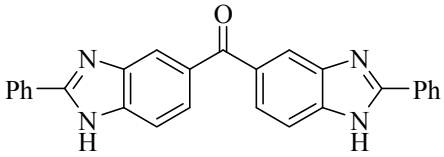
**(3) Antimicrobial**



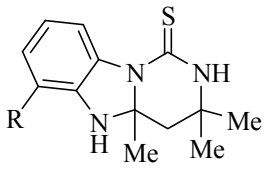
**(4) Benzodiazepine receptor binding activity**



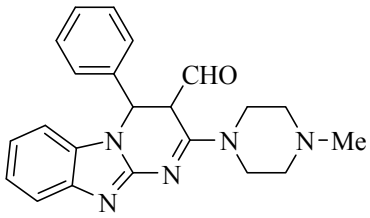
**(5) Antibacterial**



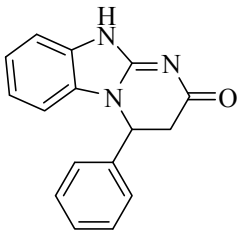
**(6) Antiparasitic**



**(7) DNA-topoisomerase I inhibitory activity**



**(8) Antineoplastic activity**



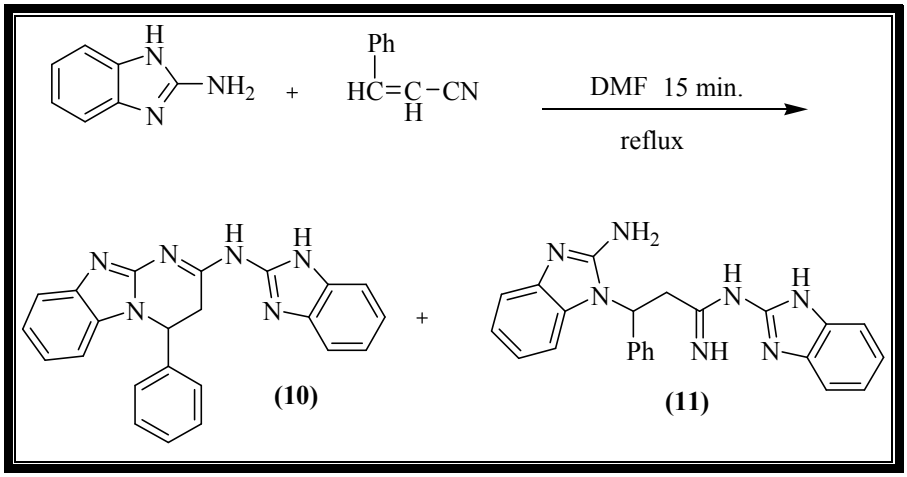
**(9) Immunotropic activity**

## 5.2 Reported synthetic strategies

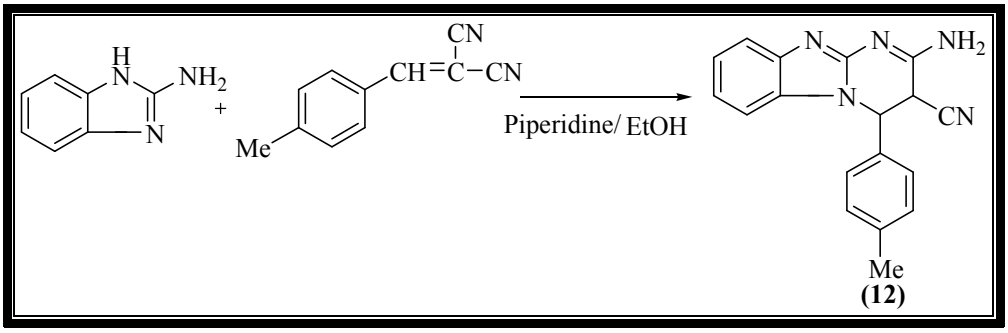
### 5.2.1 From 2-aminobenzimidazole

#### 5.2.1.1 Use of α, β- unsaturated compounds

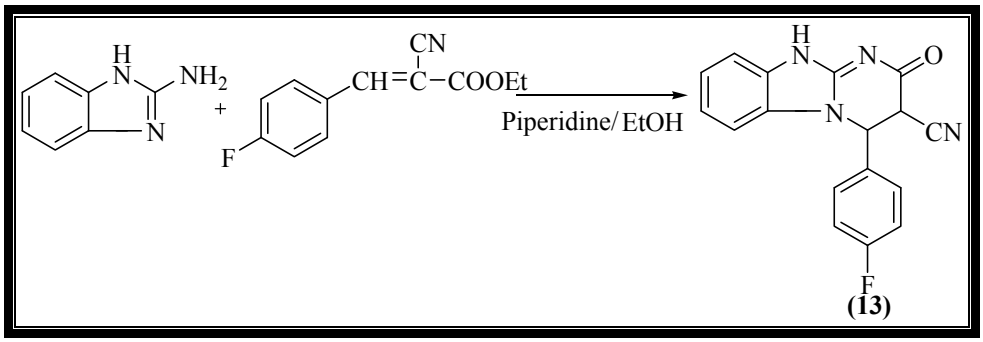
Literature survey revealed number of reports involving reaction of 2-aminobenzimidazole with α, β-unsaturated nitriles, especially arylidene malononitriles and arylidene cyanoacetates. Komykhov A. et al. have reported chemo and regioselective reaction of 2-aminobenzimidazole with arylidene malononitriles [28]. The structures of products were determined by modern nuclear magnetic resonance (NMR) techniques [including Correlation spectroscopy (COSY), Nuclear overhauser effect spectroscopy (NOESY), Heteronuclear multiple quantum coherence (HMQC) and Heteronuclear multiple bond coherence (HMBC) techniques].




Nofal Z. M. et al. have taken the synthesis pyrimido[1,2-*a*]benzimidazole derivatives by reaction of 2-aminobenzimidazole with arylidene malononitriles and arylidene cyanoacetates and tested them for antimicrobial and molluscicidal activities [29].

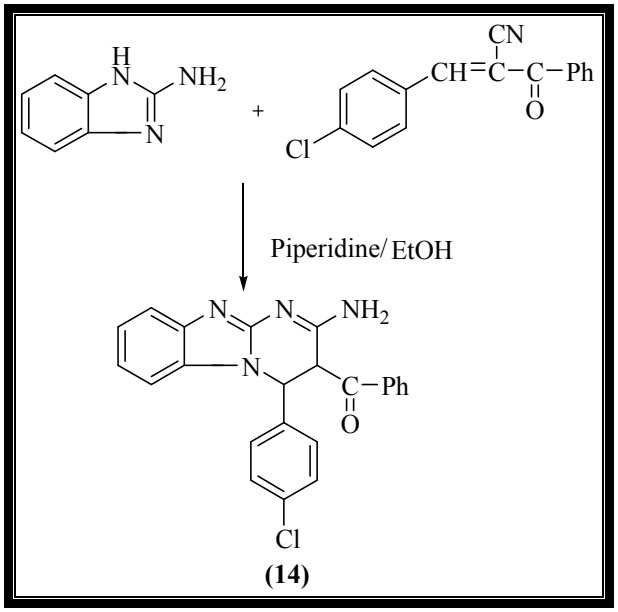



**(12)**




**(13)**

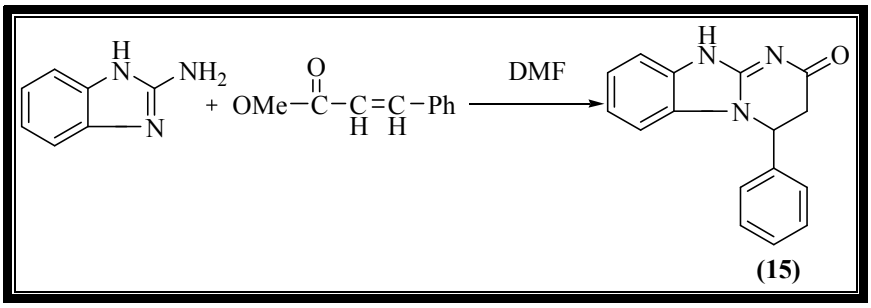
Several new pyrimidol[1,2-*a*]benzimidazole derivatives have synthesized by reaction of 2-aminobenzimidazole with benzoylacetonitrile derivatives [30].



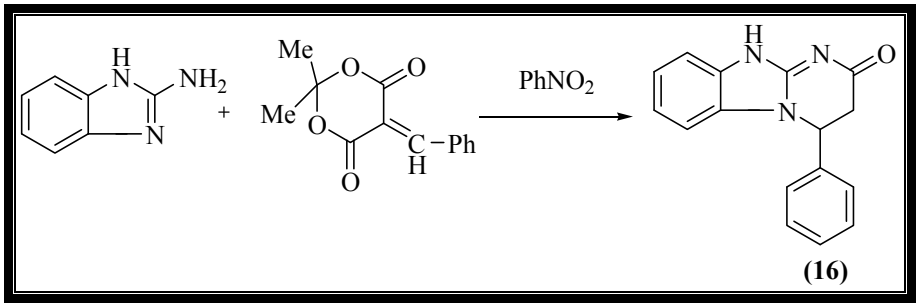

**(14)**

The reaction of 2-aminobenzimidazole with esters of substituted cinnamic acids and arylidene derivatives of 2,2-dimethyl-1,3-dioxane-4,6-dione (meldrum's acid) have been studied by Lipson V. et al. [31]. Similar reaction via one-pot two-

component thermal of cyclization of 2-aminobenzimidazole and aryl-substituted methyl cinnamates by Abdel H. et al. [27].

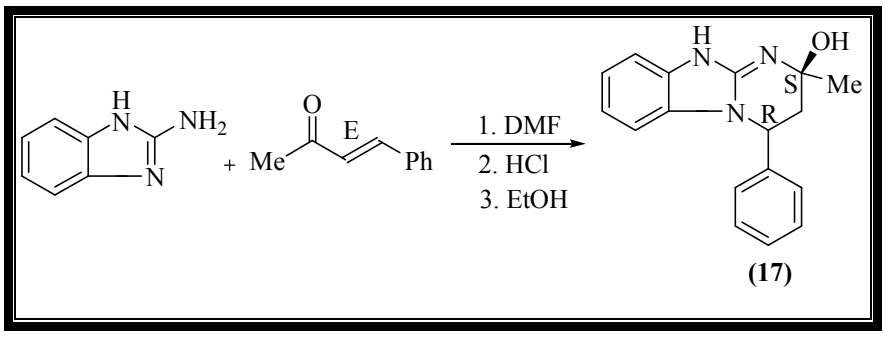



**(15)**

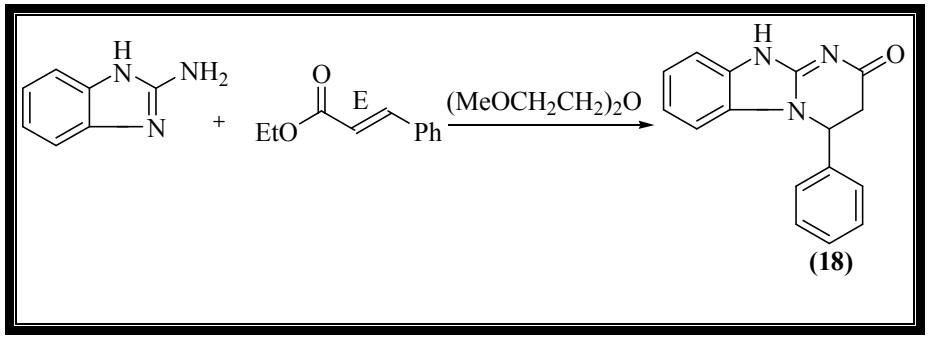



**(16)**

Synthesis and immunotropic activity of pyrimido[1,2-*a*]benzimidazole have been reported by Nawrocka W. et al. The have achieved by reaction of 2-aminobenzimidazole with different α,β-unsaturated acids and α,β-unsaturated ketones [26]. Recent literature survey also revealed number of synthesis for pyrimido [1,2-*a*]benzimidazoles other reports involving reaction of 2-aminobenzimidazoles with chalcones [32, 33] and other α,β-unsaturated ketones [34, 36].

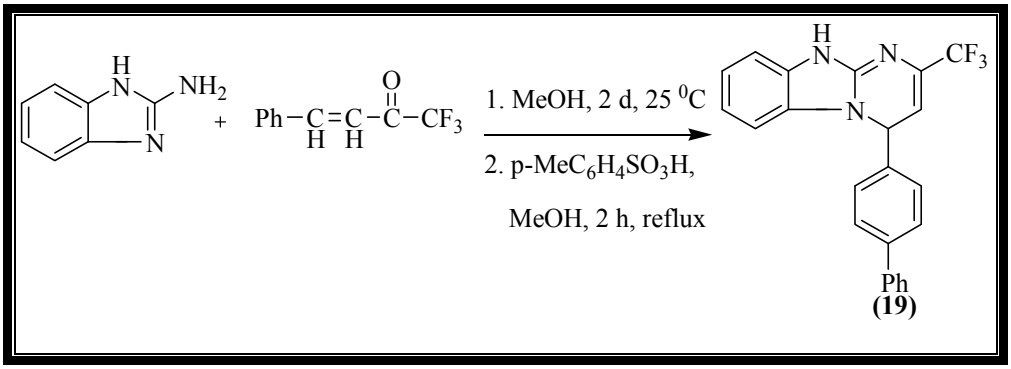



**(17)**



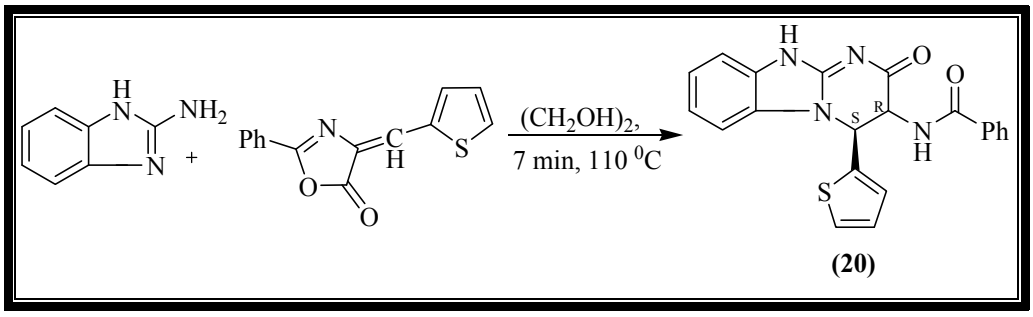

**(18)**

Similar reaction with trifluromethyl-substituted α,β-unsaturated ketone have been reported by Desenko S. M. et al. [37].




**(19)**

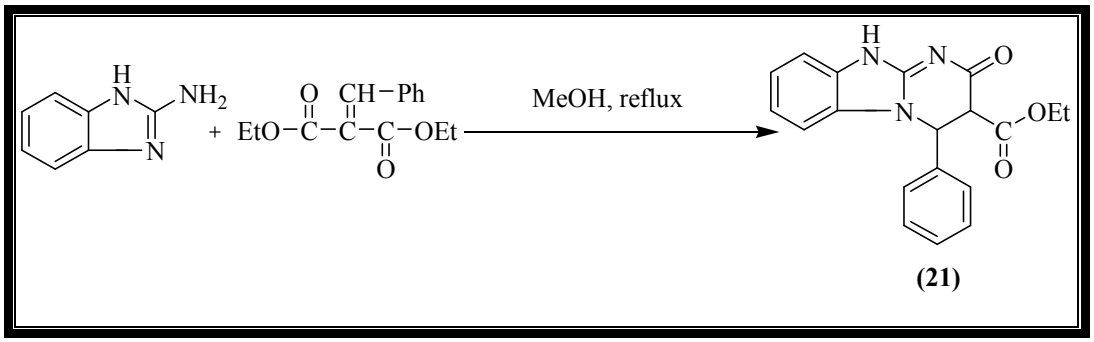
Zhuang Q. et al. have reported a microwave assisted rapid and efficient method for synthesis pyrimido[1,2-*a*]benzimidazole derivatives by the reaction of 2-aminobenzimidazole with 4-arylidene-2-phenyloxazol-5(4H)-one in glycol [38]. Similar reaction of, 2-aminobenzimidazole with 2-aryl-4-arylidene-4H-oxazol-5-ones (azlactones) was studied under classical heating by Chebanov V. A. et al. [39].




**(20)**

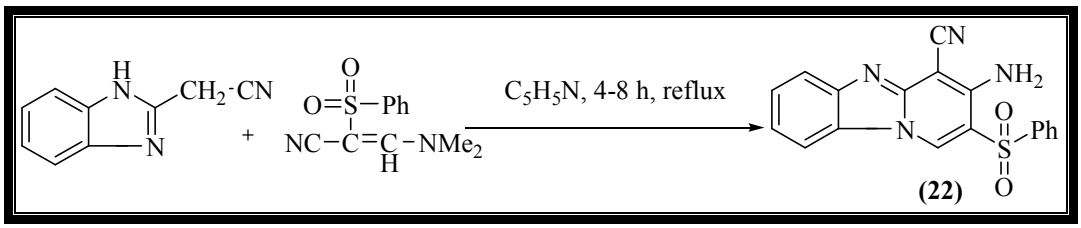
Lipson V. V. et al. have reported reaction of 2-aminobenzimidazole with diethyl benzylidenemalonate furnishing pyrimido[1,2-*a*]benzimidazole [40]. Similar

reaction of 2-aminobenzimidazole with diethyl benzylidenemalonate, diethyl ethoxymethylene malonate and ethyl 2-cyano-3-ethoxyacrylate have been reported by Kovigin Y. A. et al. [41].




**(21)**

Shaabani. M. et al. have reported reaction of 2-aminobenzimidazole with 3-(dimethylamino)-2-(phenylsulfonyl) acrylonitrile furnishing pyrimido [1,2-*a*] benzimidazole (26 ) [42].

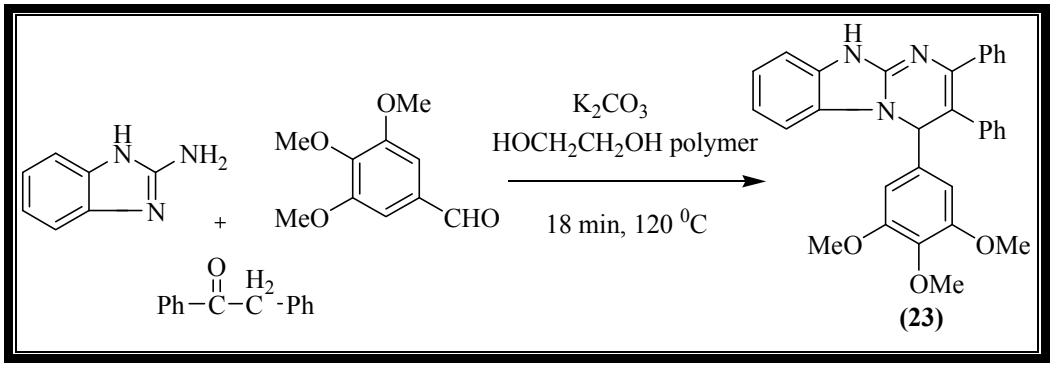



**(22)**

### 5.2.1.3 Use of 1,3-dicarbonyl compounds

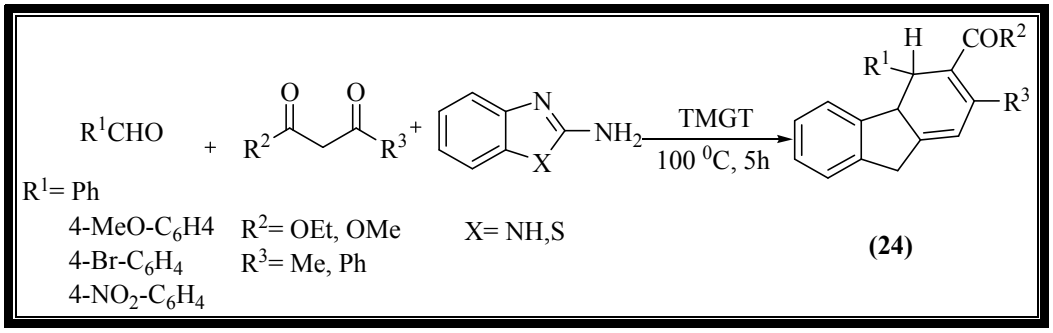
There are Several reports in literature involving reaction of 2-aminobenzimidazole with 1,3-dicarbonyl compounds among 1,3-dicarbonyl compounds, 1,3-diketones [43-49], 3-oxo esters and their derivatives [43] are generally employed.

A microwave-assisted, one-pot, multi-component reaction of aromatic aldehydes with 2-aminobenzimidazole and 1,2-diphenylethanone in polyethylene glycol in the presence of potassium carbonate ( $K_2CO_3$ ) was reported recently [50].



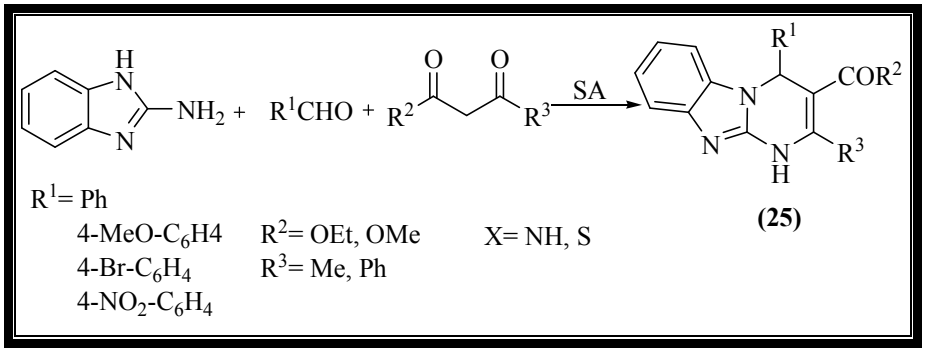

**(23)**

Shikhaliev K. S. et al. have reported the three-component condensation reaction of an aromatic aldehydes, ethylacetoacete and 2-aminobenzimidazole affording the pyrimido[1,2-*a*]benzimidazoles using ethanol as solvent [51]. Shaabani A. et al. performed the three-component reaction of 2-aminobenzimidazole with different β-ketoesters in *N*,*N*,*N*′,*N*′-tetramethylguanidinium trifluoroacetate (TMGT) as ionic liquid and obtained the pyrimido[1,2-*a*]benzimidazoles, with the limitation that the benzaldehydes must be substituted in the para-position [52].



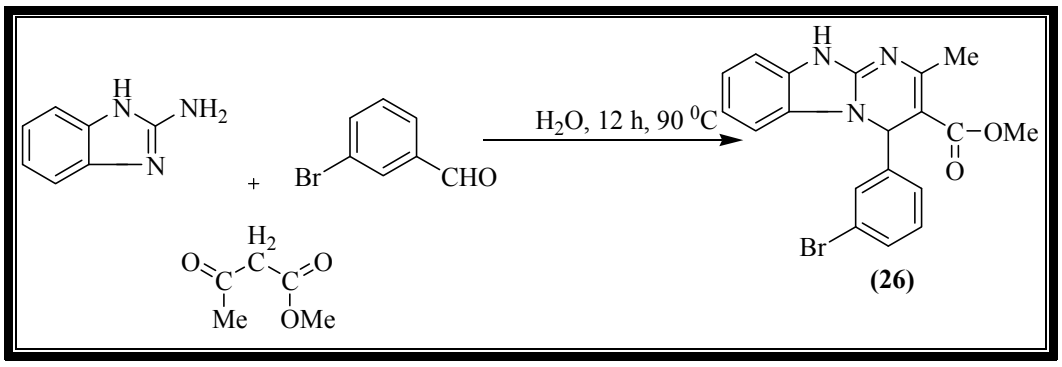

**(24)**

A simple convenient procedure for the synthesis of benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives was developed through a three-component reaction of aromatic aldehyde, different β-dicarbonyl compounds and 2-aminobenzimidazole catalyzed by sulfamic acid in a solvent-free condition [53].

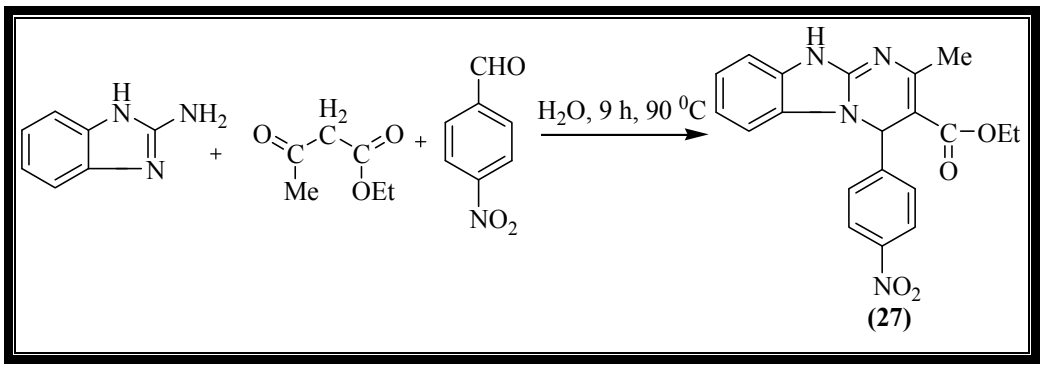



**(25)**

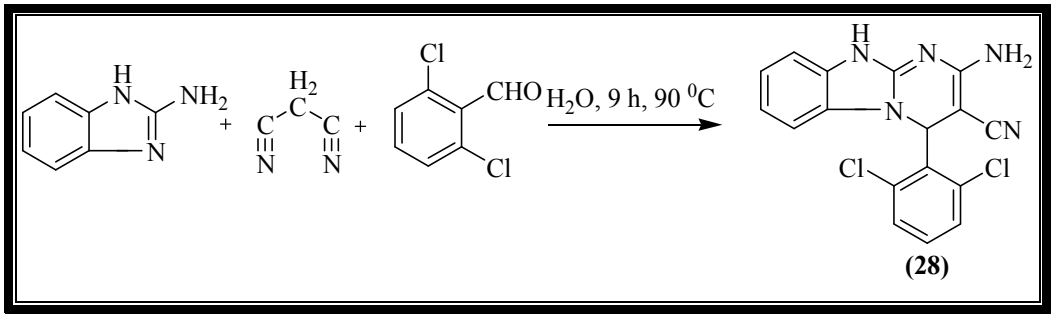
An environment-friendly three-component condensation of 3-amino-1,2,4-triazole or 2-aminobenzimidazole, an aromatic aldehydes such as β-dicarbonyl compounds and malononitrile, affording benz[4,5]imidazo[1,2-*a*]pyrimidne have been reported by Shaabani K. S. et al. [54].




**(26)**

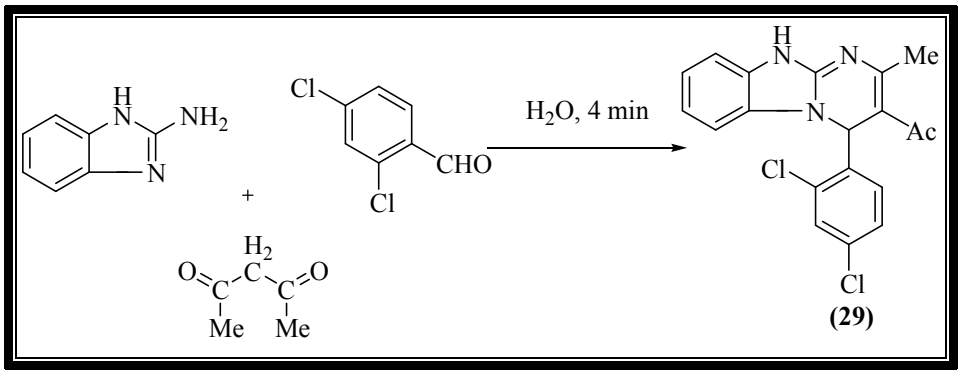



**(27)**

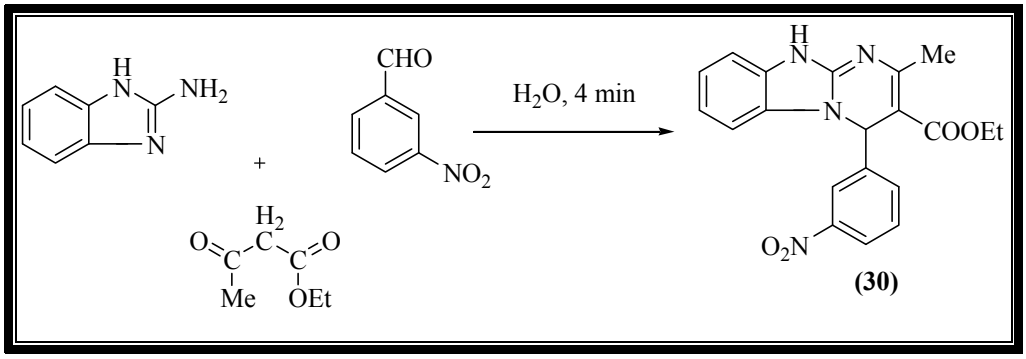



**(28)**

Tu W. et al. have reported microwave-assisted efficient synthesis of benz[4,5]imidazo[1,2-*a*]pyrimidine derivatives in water under catalyst-free conditions using 1,3-diketones and β-ketoesters [55].

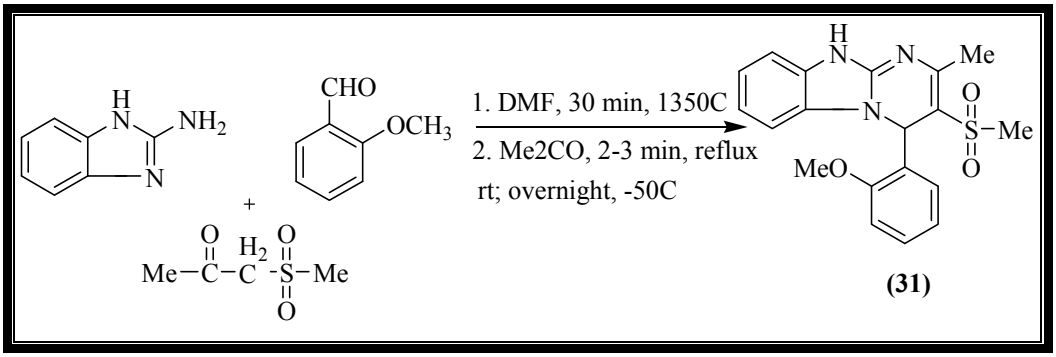



**(29)**

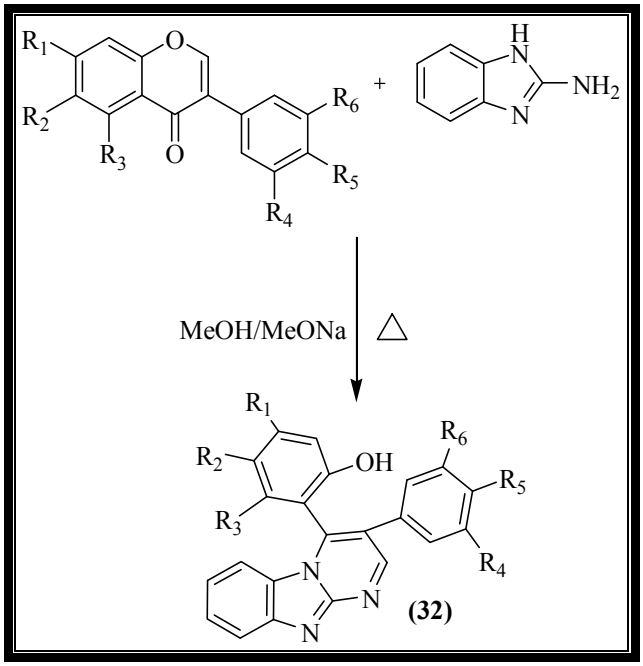



**(30)**

The multicomponent reaction of (methylsulfonyl) acetone [or α-(methylsulfonyl) acetophenone] with aromatic aldehydes and aminoazoles (or urea) under microwave irradiation to yield 5,8-dihydroimidazolo[1,2-*a*]pyrimidines [56].

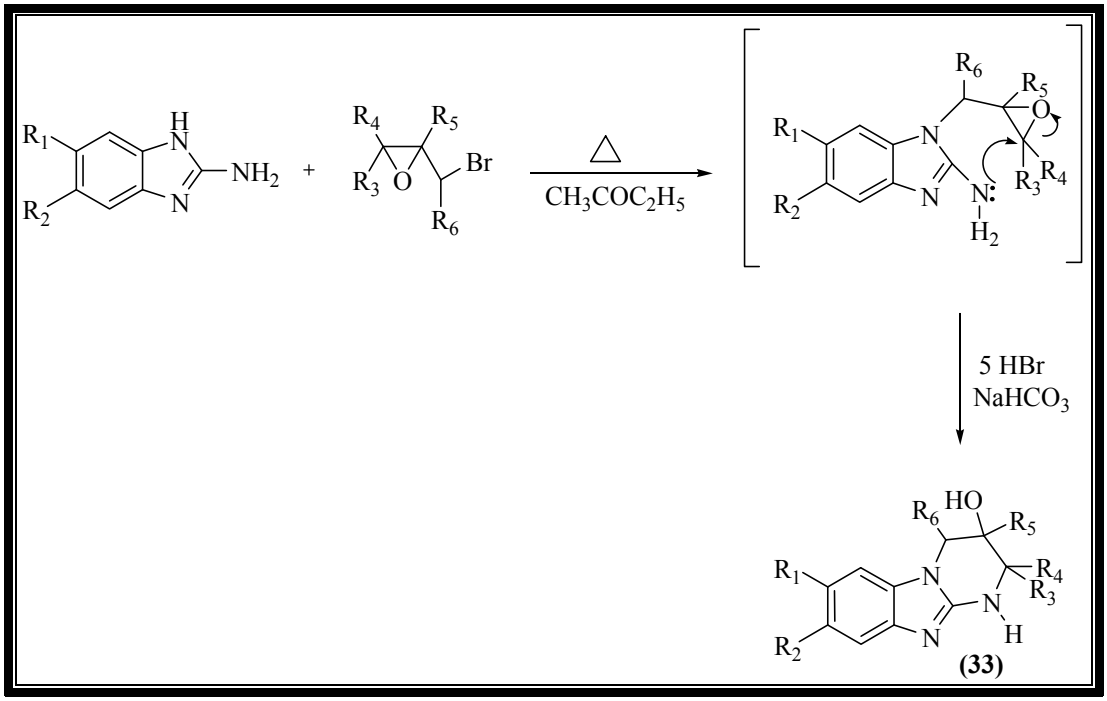



Synthesis of 2,3-Diarylpyrimido[1,2-*a*]benzimidazole by the cyclo-condensations of 2-aminobenzimidazole with isoflavone have been recently reported [57].

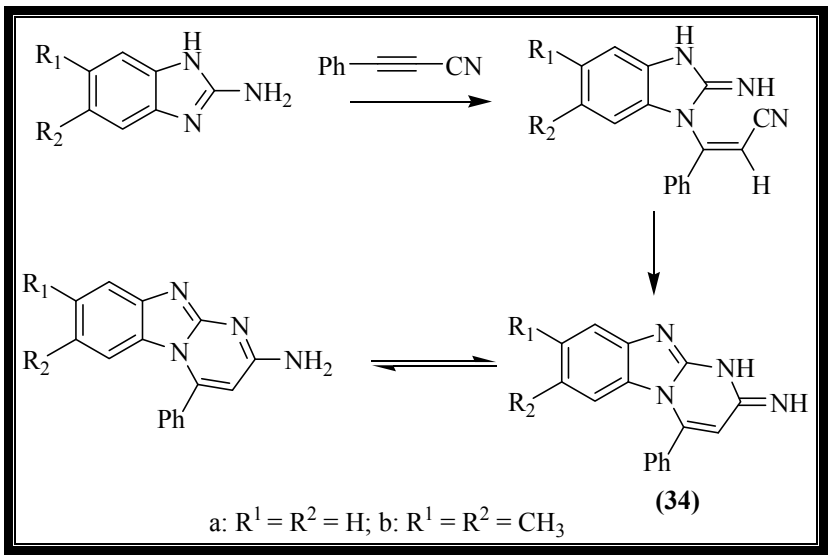



## 5.2.2 Miscellaneous

Reaction of 2-aminobenzimidazole and 2-amino-5,6-dimethylbenzimidazol with various epoxy bromides is reported to afford 3-hydroxy-1,2,3,4-tetrahydropyrimido [1,2-*a*]benzimidazoles [58].




Halene W. et al. have synthesized pyrimido[1,2-*a*]benzimidazoles from substituted acetylenic nitriles and 2-aminobenzimidazoles [59].



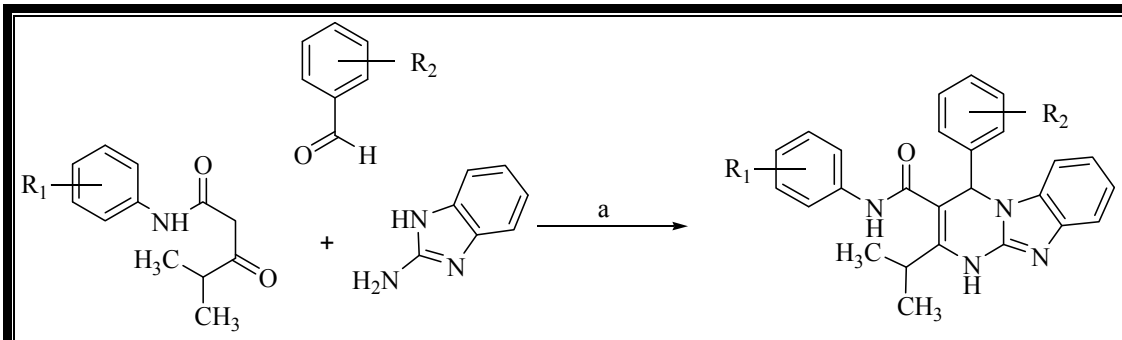
## 5.3 Current work

The biological importance of 1,4-dihydropyrimido[1,2-*a*]benzimidazoles is well documented. Over the years, various substituted derivatives of these heterocycles have shown utility against a range of biological targets.

One of the synthetic pathways to 1,4-dihydropyrimido[1,2-*a*]benzimidazoles is based on the Biginelli like cyclocondensation of aromatic aldehydes and acetoacetic acid derivatives with 2-amino benzimidazole containing a guanidine fragment. There are literary data about the synthesis of 1,4-dihydropyrimido[1,2-*a*]benzimidazoles by treatment of 2-amino benzimidazole with aldehydes and ethyl acetoacetate or cyclic $\beta$-diketones. The cyclocondensations were achieved by heating of the starting materials in ethanol with catalytic amounts of hydrochloric acid under reflux conditions or using dimethylformamide (DMF) as solvent. The use of acetoacetamides in these or similar reactions has not been described.

Recognizing these facts, we have synthesised four new series of 1,4-dihydropyrimido[1,2-*a*]benzimidazoles (**RHK-191 to 220**) containing an acetoacetamide fragment. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass spectra, $^1$H NMR and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

## 5.4 Reaction Scheme



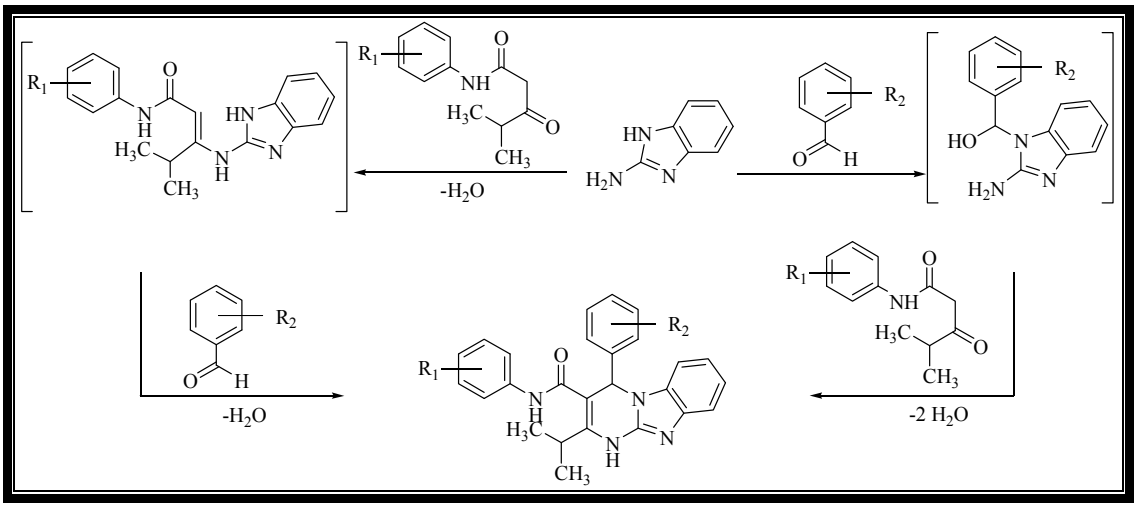

**RHK-191 to 220**

Reagents and conditions: (a) DMF, Reflux, 30 Minutes

| Code | $R_1$ | $R_2$ | M.F. | M.W. | M.P. °C | Yield % | $R_{f1}$ | $R_{f2}$ |
|---|---|---|---|---|---|---|---|---|
| RHK-191 | 4-Cl | 4-OCH3 | $C_{27}H_{25}ClN_4O_2$ | 472 | 198 | 68 | 0.49 | 0.68 |
| RHK-192 | 4-Cl | 4-$CH_3$ | $C_{27}H_{25}ClN_4O$ | 456 | 185 | 66 | 0.45 | 0.62 |
| RHK-193 | 4-Cl | 4-Cl | $C_{26}H_{22}Cl_2N_4O$ | 476 | 250 | 76 | 0.43 | 0.68 |
| RHK-194 | 4-Cl | 4-F | $C_{26}H_{22}ClFN_4O$ | 460 | 211 | 71 | 0.47 | 0.69 |
| RHK-195 | 4-Cl | 3-Br | $C_{26}H_{22}BrClN_4O$ | 520 | 240 | 80 | 0.49 | 0.68 |
| RHK-196 | 4-Cl | 3-Cl | $C_{26}H_{22}Cl_2N_4O$ | 476 | 200 | 77 | 0.59 | 0.61 |
| RHK-197 | 4-Cl | 4-$NO_2$ | $C_{26}H_{22}ClN_5O_3$ | 487 | 203 | 58 | 0.40 | 0.68 |
| RHK-198 | 4-Cl | 3-$NO_2$ | $C_{26}H_{22}ClN_5O_3$ | 487 | 201 | 60 | 0.48 | 0.66 |
| RHK-199 | 4-Cl | 2-$NO_2$ | $C_{26}H_{22}ClN_5O_3$ | 487 | 189 | 72 | 0.45 | 0.66 |
| RHK-200 | 4-Cl | 4-OH | $C_{26}H_{23}ClN_4O_2$ | 458 | 186 | 70 | 0.50 | 0.78 |
| RHK-201 | 4-F | 4-OCH3 | $C_{27}H_{25}FN_4O_2$ | 456 | 221 | 74 | 0.58 | 0.60 |
| RHK-202 | 4-F | 4-$CH_3$ | $C_{27}H_{25}FN_4O$ | 440 | 204 | 69 | 0.42 | 0.68 |
| RHK-203 | 4-F | 4-Cl | $C_{26}H_{22}ClFN_4O$ | 460 | 224 | 66 | 0.45 | 0.65 |
| RHK-204 | 4-F | 4-F | $C_{26}H_{22}F_2N_4O$ | 444 | 212 | 68 | 0.53 | 0.76 |
| RHK-205 | 4-F | 3-Br | $C_{26}H_{22}BrFN_4O$ | 504 | 211 | 64 | 0.54 | 0.68 |
| RHK-206 | 4-F | 3-Cl | $C_{26}H_{22}ClFN_4O$ | 460 | 221 | 59 | 0.51 | 0.79 |
| RHK-207 | 4-F | 4-$NO_2$ | $C_{26}H_{22}FN_5O_3$ | 471 | 201 | 64 | 0.50 | 0.71 |
| RHK-208 | 4-F | 3-$NO_2$ | $C_{26}H_{22}FN_5O_3$ | 471 | 199 | 66 | 0.46 | 0.78 |
| RHK-209 | 4-F | 2-$NO_2$ | $C_{26}H_{22}FN_5O_3$ | 471 | 200 | 80 | 0.42 | 0.66 |
| RHK-210 | 4-F | 4-OH | $C_{26}H_{23}FN_4O_2$ | 442 | 209 | 73 | 0.59 | 0.62 |
| RHK-211 | 3-Cl,4-F | 4-OCH3 | $C_{27}H_{24}ClFN_4O_2$ | 490 | 202 | 70 | 0.44 | 0.56 |
| RHK-212 | 3-Cl,4-F | 4-$CH_3$ | $C_{27}H_{24}ClFN_4O$ | 474 | 221 | 68 | 0.49 | 0.68 |
| RHK-213 | 3-Cl,4-F | 4-Cl | $C_{26}H_{21}Cl_2FN_4O$ | 494 | 212 | 61 | 0.55 | 0.68 |
| RHK-214 | 3-Cl,4-F | 4-F | $C_{26}H_{21}ClF_2N_4O$ | 478 | 251 | 72 | 0.52 | 0.56 |
| RHK-215 | 3-Cl,4-F | 3-Br | $C_{26}H_{21}BrClFN_4O$ | 538 | 218 | 64 | 0.54 | 0.76 |
| RHK-216 | 3-Cl,4-F | 3-Cl | $C_{26}H_{21}Cl_2FN_4O$ | 494 | 211 | 72 | 0.54 | 0.68 |
| RHK-217 | 3-Cl,4-F | 4-$NO_2$ | $C_{26}H_{21}ClFN_5O_3$ | 505 | 242 | 71 | 0.45 | 0.76 |
| RHK-218 | 3-Cl,4-F | 3-$NO_2$ | $C_{26}H_{21}ClFN_5O_3$ | 505 | 213 | 57 | 0.50 | 0.74 |
| RHK-219 | 3-Cl,4-F | 2-$NO_2$ | $C_{26}H_{21}ClFN_5O_3$ | 505 | 246 | 65 | 0.44 | 0.78 |
| RHK-220 | 3-Cl,4-F | 4-OH | $C_{26}H_{22}ClFN_4O_2$ | 476 | 210 | 66 | 0.56 | 0.78 |

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

## 5.5 Plausible Reaction Mechanism




## 5.6 Experimental

### 5.6.1 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 potassium bromide (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.

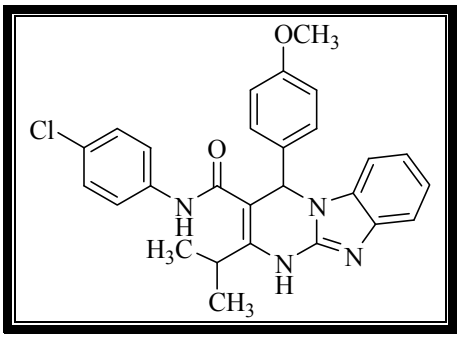
### *5.6.2 Synthesis of N-(substituted phenyl)-4-methyl-3-oxopentanamides*

Synthesis of *N*-(substituted phenyl)-4-methyl-3-oxopentanamide was achieved using previously published method [60].

### *5.6.3 General procedure for the synthesis of N-(chlorophenyl)-2-isopropyl-4-(4-sustitutedphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK- 191 to 200)*

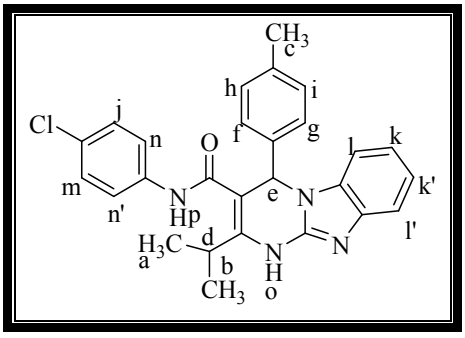
A mixture of the 2-amino benzimidazole (0.01 M), *N*-(substituted phenyl)-4-methyl-3-oxopentanamide (0.01 M) and appropriate aromatic aldehydes (0.01 M) was refluxed in DMF (4 ml) for 30 min. After cooling, methanol (~12 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid 1,4-dihydro pyrimido[1,2-*a*]benzimidazoles products (**RHK- 191 to 220**), which were recrystallized from ethanol.

#### *5.6.3.1 N-(4-chlorophenyl)-2-isopropyl-4-(4-methoxyphenyl)-1,4-dihydropyrimido-[1,2-a]benzimidazole-3-carboxamide (RHK-191)*




Yield: 68%; mp 198ºC; Anal. Calcd. for $C_{27}H_{25}ClN_4O_2$: C, 68.56; H, 5.33; Cl, 7.50; N, 11.85; O, 6.77; Found: C, 68.42; H, 5.13; Cl, 7.46; N, 11.76; O, 6.64%; MS: *m/z* 472;

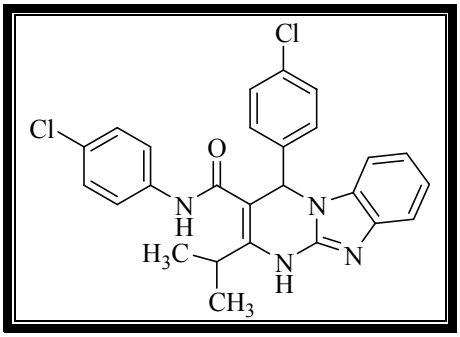
#### *5.6.3.2 N-(4-chlorophenyl)-2-isopropyl-4-(4-methlyphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-192)*




Yield: 66%; mp 185ºC; Anal. Calcd. for $C_{27}H_{25}ClN_4O$: C, 70.97; H, 5.51; Cl, 7.76; N, 12.26; O, 3.50. Found: C, 70.84; H, 5.41; Cl, 7.64; N, 12.12; O, 3.41%; IR ($cm^{-1}$): 3227 (N-H stretching of secondary amine), 3049 (C-H stretching of aromatic ring), 2975 (C-H asymmetrical stretching of $CH_3$ group), 2883 (C-H asymmetrical stretching of $CH_3$ group), 1651 (C=O stretching of amide), 1631 (N-H deformation of pyrimidine ring), 1622, 1564 and 1519 (C=C stretching of aromatic ring), 1456 (C-H asymmetrical deformation of $CH_3$ group), 1392 (C-H symmetrical deformation of $CH_3$ group), 1298 and 1247 (C-N stretching), 1078 (C-H

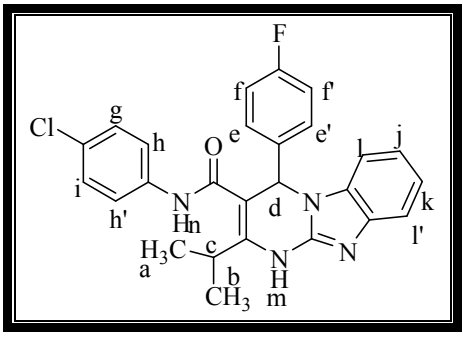
in plane deformation of aromatic ring), 825 (C-H out of plane bending of 1,4-disubstituion) 738 and 680 (C-Cl stretching); MS: *m/z* 456; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.24-1.26 (d, 3H, $H_a$, *J* = 7.20 Hz), 1.34-1.36 (d, 3H, $H_b$, *J* = 7.20 Hz), 2.24 (s, 3H, $H_c$), 3.39-3.46 (m, 1H, $H_d$), 6.52 (s, 1H, $H_e$), 6.89-6.98 (m, 2H, $H_{fg}$), 7.03-7.08 (m, 3H, $H_{hij'}$, *J* = 16.40 Hz), 7.10-7.22 (m, 4H, $H_{kk'-ll'}$), 7.39-7.41 (d, 1H, $H_m$, *J* = 7.60 Hz), 7.50-7.52 (d, 2H, $H_{nn'}$, *J* = 8.80 Hz), 9.35 (s, 1H, $H_o$), 9.56 (s, 1H, $H_p$).

### *5.6.3.3 N-(4-chlorophenyl)-2-isopropyl-4-(4-chlorophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-193)*




Yield: 76%; mp 250ºC; Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; Cl, 14.85; N, 11.74; O, 3.35; Found: C, 65.34; H, 4.60; Cl, 14.75; N, 11.64; O, 3.24%; MS: *m/z* 476.

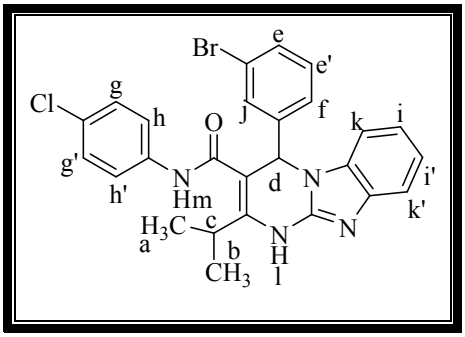
### *5.6.3.4 N-(4-chlorophenyl)-2-isopropyl-4-(4-fulorophenyl)-1,4-dihydro pyrimido - [1,2-a]benzimidazole-3-carboxamide (RHK-194)*




Yield: 71%; mp 211ºC; Anal. Calcd. for $C_{26}H_{22}ClFN_4O$: C, 67.75; H, 4.81; Cl, 7.69; F, 4.12; N, 12.16; O, 3.47; Found: C, 67.61; H, 4.71; Cl, 7.51; F, 4.04; N, 12.10; O, 3.40%; IR ($cm^{-1}$): 3238 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2974 (C-H asymmetrical stretching of $CH_3$ group), 2887 (C-H asymmetrical stretching of $CH_3$ group), 1637 (C=O stretching of amide), 1606 (N-H deformation of pyrimidine ring), 1573,1556 and 1508 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of $CH_3$ group), 1394 (C-H symmetrical deformation of $CH_3$ group), 1294 and 1246 (C-N stretching), 1084 (C-H in plane deformation of aromatic ring), 1012 (C-F stretching), 827 (C-H out of plane bending of 1,4-disubstituion), 734 (C-Cl stretching); MS: *m/z* 460; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.19-1.19 (d, 3H, $H_a$, *J* = 2.00 Hz), 1.24-1.26 (d, 3H, $H_b$, *J* = 6.40 Hz), 3.35-3.40 (m, 1H, $H_c$), 6.59 (s, 1H, $H_d$) 6.90-6.98 (m, 4H, $H_{ee'-ff'}$), 7.03-7.07 (m, 1H, $H_g$), 7.20-7.22 (d, 2H, $H_{hh'}$, *J* = 8.80 Hz),

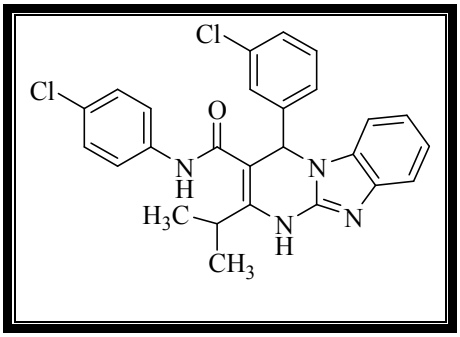
7.27-7.30 (m, 2H, $H_{ij}$, $J$ = 14.00 Hz), 7.37-7.39 (d, 1H, $H_k$, $J$ = 8.00 Hz), 7.52-7.54 (d, 2H, $H_{ll'}$, $J$ = 8.80 Hz), 9.68 (s, 1H, $H_m$), 9.84 (s, 1H, $H_n$).

### *5.6.3.5* N-*(4-chlorophenyl)-2-isopropyl-4-(3-bromophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-195)*



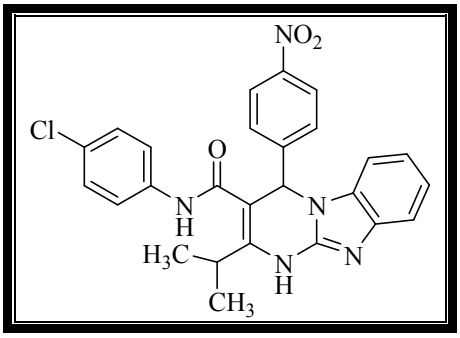

Yield: 80%; mp 240ºC; Anal. Calcd. for $C_{26}H_{22}BrClN_4O$: C, 59.84; H, 4.25; Br, 15.31; Cl, 6.79; N, 10.74; O, 3.07; Found: C, 59.75; H, 4.13; Br, 15.24; Cl, 6.62; N, 10.64; O, 3.00%; IR ($cm^{-1}$): 3294 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2958 (C-H asymmetrical stretching of $CH_3$ group), 2854 (C-H asymmetrical stretching of $CH_3$ group), 1649 (C=O stretching of amide), 1641 (N-H deformation of pyrimidine ring), 1560, 1521 and 1506 (C=C stretching of aromatic ring), 1456 (C-H asymmetrical deformation of $CH_3$ group), 1388 (C-H symmetrical deformation of $CH_3$ group), 1294 and 1236 (C-N stretching), 1078 (C-H in plane deformation of aromatic ring), 833 (C-H out of plane bending of 1,4-disubstituion), 729 (C-Cl stretching), 678 (C-Br stretching); MS: *m/z* 520; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.24-1.26 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.34-1.36 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.37-3.40 (m, 1H, $H_c$), 6.58 (s, 1H, $H_d$) 6.92-6.93 (m, 2H, $H_{ee'}$, $J$ = 4.00 Hz), 7.05-7.08 (m, 1H, $H_f$, $J$ = 12.40 Hz) 7.18-7.24 (m, 6H, $H_{gg'\text{-}hh'\text{-}ii'}$, $J$ = 8.80 Hz), 7.39-7.41 (d, 1H, $H_j$, $J$ = 8.00 Hz), 7.52-7.54 (d, 2H, $H_{kk'}$, $J$ = 8.80 Hz), 9.69 (s, 1H, $H_l$), 9.84 (s, 1H, $H_m$).

### *5.6.3.6 N-(4-chlorophenyl)-2-isopropyl-4-(4-cholorophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-196)*



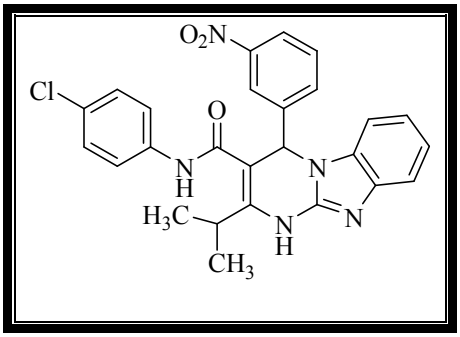

Yield: 77%; mp 200ºC; Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; Cl, 14.85; N, 11.74; O, 3.35; Found: C, 65.30; H, 4.56; Cl, 14.42; N, 11.65; O, 3.23%; MS: *m/z* 476.

### *5.6.3.7 N-(4-chlorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-197)*



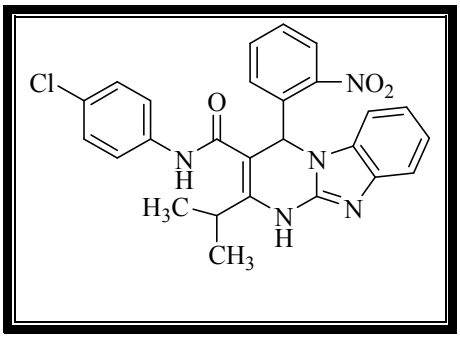
Yield: 58%; mp 203ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$: C, 64.00; H, 4.54; Cl, 7.27; N, 14.35; O, 9.84; Found: C, 63.84; H, 4.54; Cl, 7.10; N, 14.21; O, 9.72%; MS: *m/z* 487.

### *5.6.3.8 N-(4-chlorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-198)*



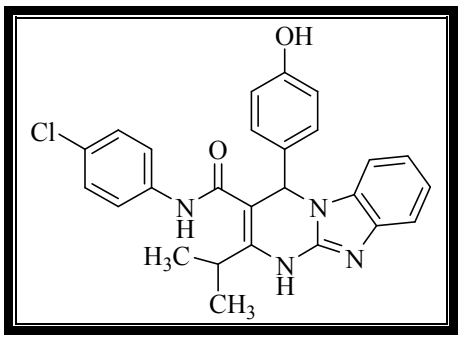
Yield: 60%; mp 201ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$: C, 64.00; H, 4.54; Cl, 7.27; N, 14.35; O, 9.84; Found: C, 63.79; H, 4.42; Cl, 7.13; N, 14.24; O, 9.74%; MS: *m/z* 487.

### *5.6.3.9 N-(4-chlorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-199)*



Yield: 72%; mp 189ºC; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$ C, 64.00; H, 4.54; Cl, 7.27; N, 14.35; O, 9.84; Found: C, 63.95; H, 4.45; Cl, 7.13; N, 14.24; O, 9.75%; MS: *m/z* 487.

***5.6.3.10*** N-***(4-chlorophenyl)-2-isopropyl-4-(4-hydrooxyphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-200)***

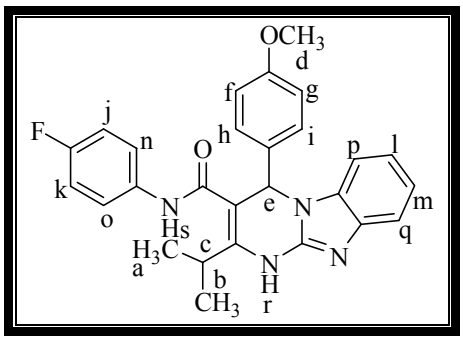



Yield: 70%; mp 186ºC; Anal. Calcd. for $C_{26}H_{23}ClN_4O_2$: C, 68.04; H, 5.05; Cl, 7.72; N, 12.21; O, 6.97; Found: C, 68.00; H, 5.00; Cl, 7.64; N, 12.08; O, 6.85%; MS: *m/z* 458.

***5.6.4 General procedure for the synthesis of N-(fluorophenyl)-2-isopropyl-4-(4-sustitutedphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK - 201 to 210)***

A mixture of the 2-amino benzimidazole (0.01mol), *N*-(4-chlorophenyl)-4-methyl-3-oxopentanamide (0.01mol) and an appropriate aromatic aldehydes (0.01mol) was refluxed in 4 ml of DMF for 30 min. After cooling, methanol (~12 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid 1,4-dihydropyrimido[1,2-*a*]benzimidazoles products **RHK- 201 to 210**, which were crystallized from ethanol.

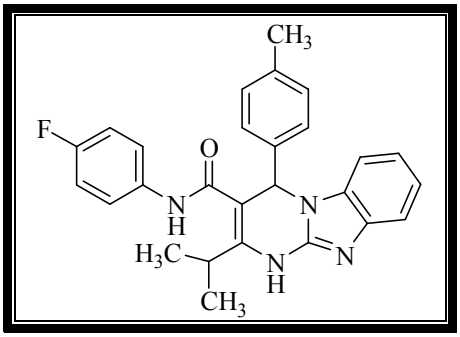
***5.6.4.1 N-(4-fluorophenyl)-2-isopropyl-4-(4-methoxyphenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-201)***




Yield: 79%; mp 221ºC; Anal. Calcd. for $C_{27}H_{25}FN_4O_2$: C, 71.04; H, 5.52; F, 4.16; N, 12.27; O, 7.01; Found: C, 71.00; H, 5.34; F, 4.05; N, 12.10; O, 6.79%; IR ($cm^{-1}$): 3157 (N-H stretching of secondary amine), 3051 (C-H stretching of aromatic ring), 2958 (C-H asymmetrical stretching of $CH_3$ group), 2899 (C-H asymmetrical stretching of $CH_3$ group), 1683 (C=O stretching of amide), 1627 (N-H deformation of pyrimidine ring), 1575 and 1510 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of $CH_3$ group), 1365 (C-H symmetrical deformation of $CH_3$ group), 1261 (C-O-C asymmetrical stretching of $OCH_3$), 1174 (C-N stretching), 1095 (C-H in plane

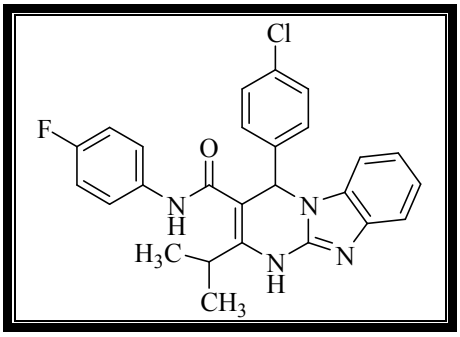
deformation of aromatic ring), 1030 (C-F stretching), 825 (C-H out of plane bending of 1,4-disubstituion); MS: *m/z* 456; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.24-1.26 (d, 3H, $H_a$, *J* = 6.80 Hz), 1.34-1.36 (d, 3H, $H_b$, *J* = 6.80 Hz), 3.38-3.43 (m, 1H, $H_c$), 3.73 (s, 1H, $H_d$), 5.96-5.97 (s, 1H, $H_e$), 6.78-6.80 (m, 3H, $H_{fgh}$, *J* = 8.40 Hz), 6.82-6.86 (m, 3H, $H_{ijk}$, *J* = 15.20 Hz), 6.95-6.99 (m, 2H, $H_{lm}$, *J* = 16.40 Hz), 7.21-7.23 (m, 3H, $H_{nop}$), 7.27-7.29 (s, 1H, $H_q$), 9.27 (s, 2H, $H_s$).

### *5.6.4.2 N-(4-fluorophenyl)-2-isopropyl-4-(4-methlyphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-202)*



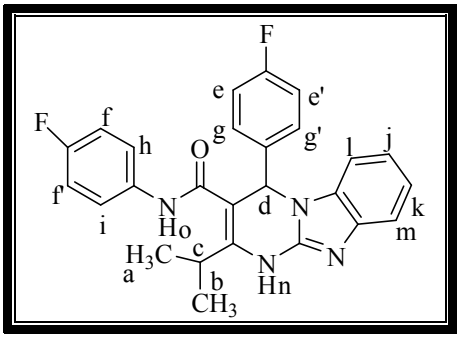
Yield: 69%; mp 204ºC; Anal. Calcd. for $C_{27}H_{25}FN_4O$: C, 73.62; H, 5.72; F, 4.31; N, 12.72; O, 3.63; Found: C, 73.50; H, 5.61; F, 4.21; N, 12.61; O, 3.54%; MS: *m/z* 440.

### *5.6.4.3 N-(4-fluorophenyl)-2-isopropyl-4-(4-chlorophenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-203)*



Yield: 66%; mp 224ºC; Anal. Calcd. for $C_{26}H_{22}ClFN_4O$: C, 67.75; H, 4.81; Cl, 7.69; F, 4.12; N, 12.16; O, 3.47; Found: C, 67.64; H, 4.76; Cl, 7.64; F, 4.04; N, 12.00; O, 3.30%; MS: *m/z* 460.

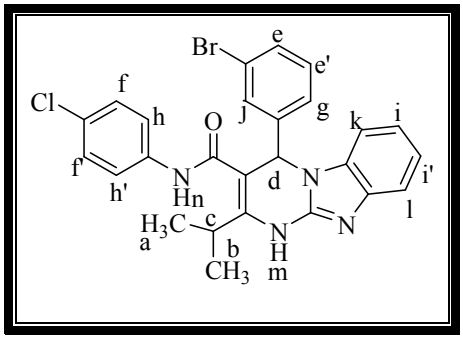
### *5.6.4.4 N-(4-fluorophenyl)-2-isopropyl-4-(4-fulorophenyl)-1,4-dihydropyrimido-[1,2-a]benzimidazole-3-carboxamide (RHK-204)*



Yield: 68%; mp 212ºC; Anal. Calcd. for $C_{26}H_{22}F_2N_4O$: C, 70.26; H, 4.99; F, 8.55; N, 12.61; O, 3.60; Found: C, 70.11; H, 4.87; F, 8.44; N, 12.52; O, 3.46%; IR (cm$^{-1}$): 3273 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2970 (C-H asymmetrical stretching

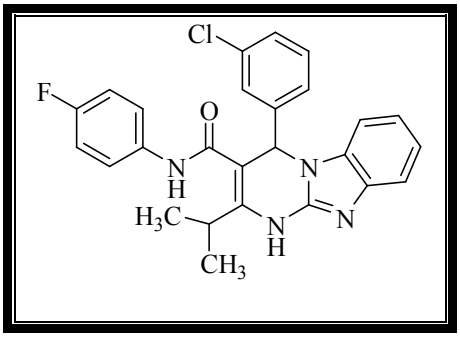
of $CH_3$ group), 2895 (C-H asymmetrical stretching of $CH_3$ group), 1637 (C=O stretching of amide), 1688 (N-H deformation of pyrimidine ring), 1508 and 1467 (C=C stretching of aromatic ring), 1410 (C-H asymmetrical deformation of $CH_3$ group), 1357 (C-H symmetrical deformation of $CH_3$ group), 1294 and 1228 (C-N stretching), 1087 (C-H in plane deformation of aromatic ring), 1012 (C-F stretching), 819 (C-H out of plane bending of 1,4-disubstituion); MS: *m/z* 444; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.24-1.26 (d, 3H, $H_a$, *J* = 6.80 Hz), 1.34-1.36 (d, 3H, $H_b$, *J* = 6.80 Hz), 3.38-3.42 (m, 1H, $H_c$, *J* = 14.00 Hz), 6.59 (s, 1H, $H_d$) 6.90-6.98 (m, 6H, $H_{ee'\text{-}ff'\text{-}gg'}$), 7.05-7.07 (m, 1H, $H_h$, *J* = 16.40 Hz), 7.28-7.31 (m, 2H, $H_{ij}$, *J* =12.00 Hz), 7.37-7.39 (d, 1H, $H_k$, *J* = 7.60 Hz), 7.48-7.52 (m, 2H, $H_{lm}$, *J* = 14.00 Hz), 9.53(s, 1H, $H_l$), 9.72 (s, 1H, $H_m$).

### *5.6.4.5* N-*(4-fluorophenyl)-2-isopropyl-4-(3-bromophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-205)*



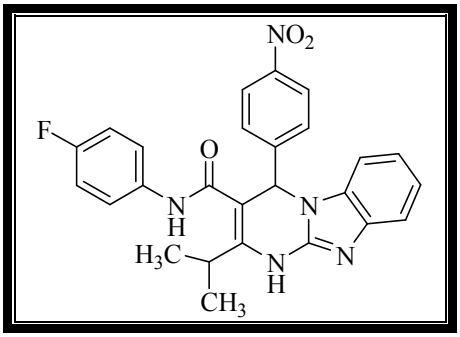

Yield: 64%; mp 211ºC; Anal. Calcd. for $C_{26}H_{22}BrFN_4O$: C, 61.79; H, 4.39; Br, 15.81; F, 3.76; N, 11.09; O, 3.17; Found: C, 61.66; H, 4.24; Br, 15.71; F, 3.70; N, 11.00; O, 3.07; IR ($cm^{-1}$): 3288 (N-H stretching of secondary amine), 3055 (C-H stretching of aromatic ring), 2978 (C-H asymmetrical stretching of $CH_3$ group), 2824 (C-H asymmetrical stretching of $CH_3$ group), 1651 (C=O stretching of amide), 1624 (N-H deformation of pyrimidine ring), 1562 and 1510 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of $CH_3$ group), 1280 (C-H symmetrical deformation of $CH_3$ group), 1228 (C-N stretching), 1076 (C-H in plane deformation of aromatic ring), 833 (C-H out of plane bending of 1,4-disubstituion), 738 (C-Cl stretching), 682 (C-Br stretching); MS: *m/z* 504; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.25-1.26 (d, 3H, $H_a$, *J* = 7.20 Hz), 1.35-1.36 (d, 3H, $H_b$, *J* = 6.80 Hz), 3.37-3.42 (m, 1H, $H_c$), 6.57 (s, 1H, $H_d$), 6.90-6.99 (m, 4H, $H_{ee'\text{-}ff'}$), 7.06-7.10 (m, 1H, $H_g$), 7.17-7.23 (m, 4H, $H_{hh'\text{-}ii'}$), 7.41-7.43 (d, 1H, $H_j$, *J* = 8.00 Hz), 7.48-7.51 (m, 2H, $H_{kl}$), 9.53 (s, 1H, $H_m$), 9.69 (s, 1H, $H_n$).

### *5.6.4.6 N-(4-fluorophenyl)-2-isopropyl-4-(4-cholorophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-206)*



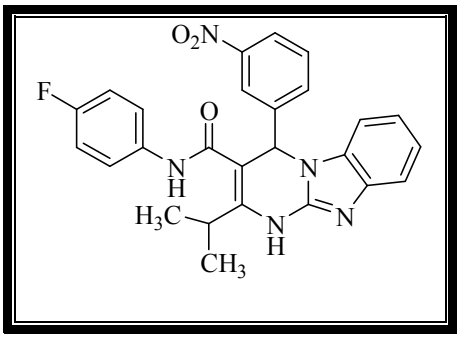

Yield: 59%; mp 221ºC; Anal. Calcd. for $C_{26}H_{22}ClFN_4O$: C, 67.75; H, 4.81; Cl, 7.69; F, 4.12; N, 12.16; O, 3.47; Found: C, 67.62; H, 4.77; Cl, 7.53; F, 4.01; N, 12.12; O, 3.21%; MS: *m/z* 460.

### *5.6.4.7 N-(4-fluorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-207)*



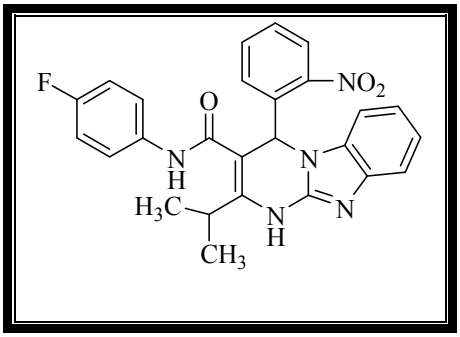

Yield: 64%; mp 201ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$: C, 66.23; H, 4.70; F, 4.03; N, 14.85; O, 10.18; Found: C, 66.00; H, 4.45; F, 4.00; N, 14.75; O, 10.00%; MS: *m/z* 471.

### *5.6.4.8 N-(4-fluorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-208)*



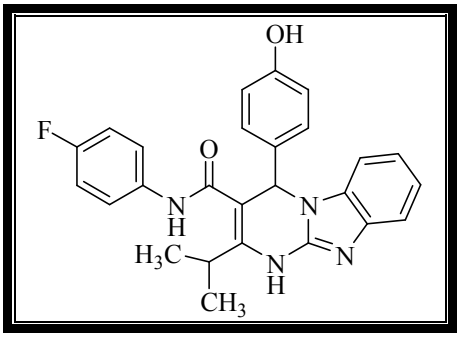

Yield: 66%; mp 199ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$: C, 66.23; H, 4.70; F, 4.03; N, 14.85; O, 10.18; Found: C, 66.15; H, 4.66; F, 3.79; N, 14.80; O, 10.00%; MS: *m/z* 471.

### *5.6.4.9 N-(4-fluorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-209)*




Yield: 80%; mp 200ºC; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$ : C, 66.23; H, 4.70; F, 4.03; N, 14.85; O, 10.18; Found: C, 66.15; H, 4.66; F, 3.89; N, 14.80; O, 10.10%; MS: *m/z* 471.

### *5.6.4.10* N-*(4-fluorophenyl)-2-isopropyl-4-(4-hydrooxyphenyl)-1,4-dihydropyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-210)*

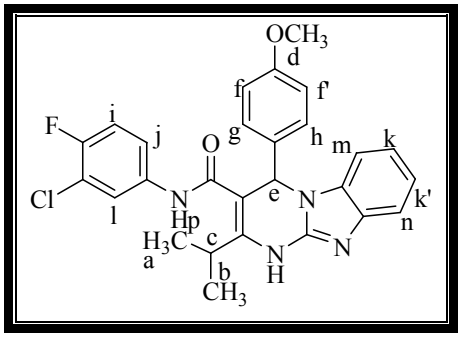



Yield: 73%; mp 209ºC; Anal. Calcd. for $C_{26}H_{23}FN_4O_2$: C, 70.57; H, 5.24; F, 4.29; N, 12.66; O, 7.23; Found: C, 70.45; H, 5.11; F, 4.13; N, 10.66; O, 7.12%; MS: *m/z* 442.

### *5.6.5 General procedure for the synthesis of N-(fluorophenyl)-2-isopropyl-4-(4-sustitutedphenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK - 211 to 220)*

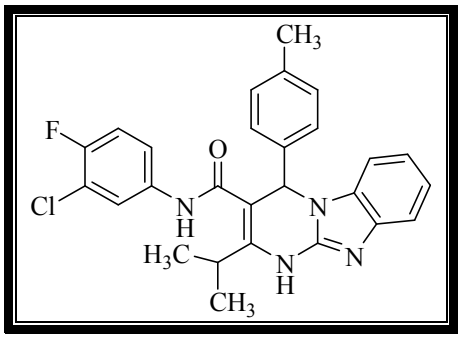
A mixture of the 2-amino benzimidazole (0.01mol), *N*-(4-chlorophenyl)-4-methyl-3-oxopentanamide (0.01mol) and an appropriate aromatic aldehydes (0.01mol) was refluxed in 4 ml of DMF for 30 min. After cooling, methanol (~12 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid 1,4-dihydropyrimido[1,2-*a*]benzimidazoles products **RHK- 211 to 220**, which were crystallized from ethanol.

### *5.6.5.1 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-methoxyphenyl)-1,4-dihydro-pyrimido [1,2-a]benzimidazole-3-carboxamide (RHK-211)*



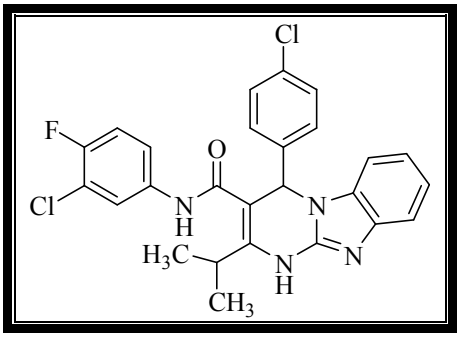
Yield: 70%; mp 202ºC; Anal. Calcd. for $C_{27}H_{24}ClFN_4O_2$: C, 66.05; H, 4.93; Cl, 7.22; F, 3.87; N, 11.41; O, 6.52; Found: C, 66.00; H, 4.88; Cl, 7.11; F, 3.76; N, 11.31; O, 6.42%; IR ($cm^{-1}$): 3308 (N-H stretching of secondary amine), 3051 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of $CH_3$ group), 2827 (C-H asymmetrical stretching of $CH_3$ group), 1670 (C=O stretching of amide), 1618 (N-H deformation of pyrimidine ring), 1583 and 1508 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of $CH_3$ group), 1386 (C-H symmetrical deformation of $CH_3$ group), 1296 (C-N stretching), 1249 (C-O-C asymmetrical stretching of $OCH_3$), 1045 (C-F stretching), 1045 (C-H in plane deformation of aromatic ring), 798 (C-H out of plane bending of 1,4-disubstituion), 736 (C-Cl stretching); MS: *m/z* 490; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.25-1.26 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.35-1.36 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.37-3.41 (m, 1H, $H_c$), 3.71 (s, 1H, $H_d$), 6.59 (s, 1H, $H_e$), 6.75-6.77 (m, 2H, $H_{ff'}$, $J$ = 8.80), 6.92-6.97 (m, 2H, $H_{gh}$), 7.103-7.08 (m, 2H, $H_{ij}$), 7.22-7.24 (d, 2H, $H_{kk'}$, $J$ = 8.80 Hz), 7.39-7.45 (m, 2H, $H_{lm}$),7.81-7.83 (m, 1H, $H_n$, $J$ = 9.60), 9.59 (s, 1H, $H_o$), 10.21 (s, 1H, $H_p$).

### *5.6.5.2 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-methlyphenyl)-1,4-dihy-dropyri mido[1,2-a]benzimidazole-3-carbo-xamide (RHK-212)*



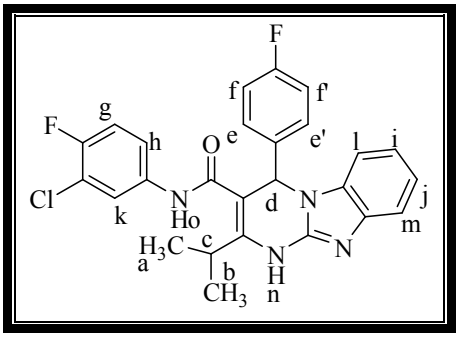
Yield: 68%; mp 221ºC; Anal. Calcd. for $C_{27}H_{24}ClFN_4O$: C, 68.28; H, 5.09; Cl, 7.46; F, 4.00; N, 11.80; O, 3.37. Found: C, 68.12; H, 5.00; Cl, 7.34; F, 3.88; N, 11.71; O, 3.30%; MS: *m/z* 474.

### *5.6.5.3 N-(4-fluoro,3-chlorophenyl) -2-isopropyl-4-(4-chlorophenyl)-1,4-dihydro-pyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-213)*



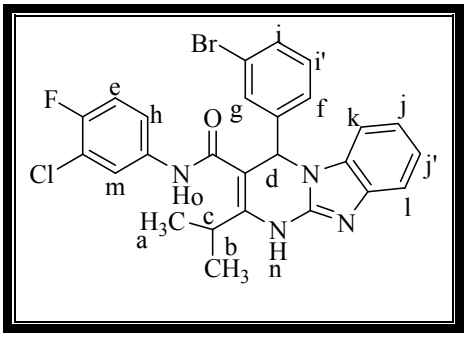
Yield: 61%; mp 212ºC; Anal. Calcd. for $C_{26}H_{21}Cl_2FN_4O$: C, 63.04; H, 4.27; Cl, 14.31; F, 3.84; N, 11.31; O, 3.23; Found: C, 62.89; H, 4.13; Cl, 14.21; F, 3.75; N, 11.21; O, 3.11%; MS: *m/z* 494.

### *5.6.5.4 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-fulorophenyl)-1,4-dihydropyr-imido[1,2-a]benzimidazole-3-carboxamide (RHK-214)*



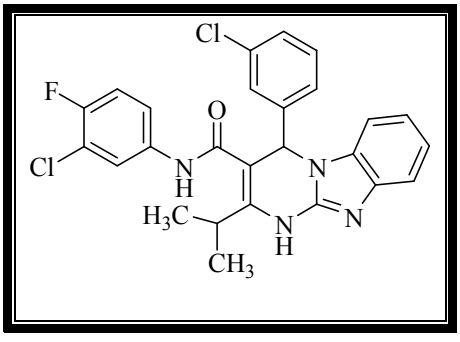
Yield: 72%; mp 251ºC; Anal. Calcd. for $C_{26}H_{21}ClF_2N_4O$: C, 57.85; H, 3.92; Br, 14.80; Cl, 6.57; F, 3.52; N, 10.38; O, 2.96 Found: C, 57.76; H, 3.84; Br, 14.70; Cl, 6.45; F, 3.42; N, 10.24; O, 2.87%; IR ($cm^{-1}$): 3274 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of $CH_3$ group), 2845 (C-H asymmetrical stretching of $CH_3$ group), 1651 (C=O stretching of amide), 1608 (N-H deformation of pyrimidine ring), 1560,1529 and 1504 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of $CH_3$ group), 1392 (C-H symmetrical deformation of $CH_3$ group), 1280 and 1253 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 1074 (C-F stretching), 812 (C-H out of plane bending of 1,4-disubstituion), 731 (C-Cl stretching); MS: *m/z* 478; $^1$H NMR (DMSO-$d_6$) δ ppm: 1.24-1.26 (d, 3H, $H_a$, $J$ = 7.20 Hz), 1.34-1.36 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.35-3.41 (m, 1H, $H_c$), 6.60 (s, 1H, $H_d$), 6.88-7.00 (m, 4H, $H_{ee'-ff'}$), 7.03-7.08 (m, 1H, $H_g$), 7.10-7.12 (m, 1H, $H_h$, $J$ = 9.20 Hz), 7.27-7.32 (d, 1H, $H_k$, $J$ = 8.00 Hz), 7.40-7.44 (m, 1H, $H_l$), 7.79-7.82 (m, 1H, $H_m$, $J$ = 9.60), 9.77 (s, 1H, $H_n$), 9.97 (s, 1H, $H_o$).

### *5.6.5.5 N-(4-fluoro,3-chlorophenyl) -2-isopropyl-4-(3-bromophenyl)-1,4-dihydropy - -rimido[1,2-a]benzimidazole-3-carboxamide (RHK-215)*



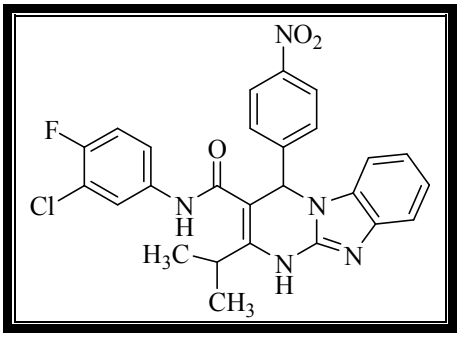

Yield: 64%; mp 218ºC; Anal. Calcd. for $C_{26}H_{21}BrClFN_4O$: C, 57.85; H, 3.92; Br, 14.80; Cl, 6.57; F, 3.52; N, 10.38; O, 2.96; Found: C, 57.76; H, 3.84; Br, 14.76; Cl, 6.46; F, 3.42; N, 10.23; O, 2.87%; IR ($cm^{-1}$): 3288 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of $CH_3$ group), 2888 (C-H asymmetrical stretching of $CH_3$ group), 1651 (C=O stretching of amide), 1624 (N-H deformation of pyrimidine ring), 1562,1529 and 1502 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of $CH_3$ group), 1394 (C-H symmetrical deformation of $CH_3$ group), 1257 and 1220 (C-N stretching), 1078 (C-H in plane deformation of aromatic ring), 1012 (C-F stretching), 817 (C-H out of plane bending of 1,4-disubstituion), 740 (C-Cl stretching), 590 (C-Br stretching); MS: *m/z* 538; $^1$H NMR (DMSO-$d_6$) δ ppm:1.25-1.26 (d, 3H, $H_a$, $J$ = 6.80 Hz), 1.34-1.36 (d, 3H, $H_b$, $J$ = 6.80 Hz), 3.34-3.43 (m, 1H, $H_c$), 6.60 (s, 1H, $H_d$), 6.93-6.95 (m, 2H, $H_{ef}$, $J$ = 9.20 Hz), 7.04-7.12 (m, 2H, $H_{gh}$) 7.19-7.27 (m,4H, $H_{ii'-jj'}$), 7.39-7.44 (m, 2H, $H_{kl}$),7.79-7.81 (m,1H,$H_m$, $J$ = 9.20), 9.87 (s, 1H, $H_n$), 9.98 (s, 1H, $H_o$).

### *5.6.5.6 N-(4-fluoro,3-chlorophenyl) -2-isopropyl-4-(4-cholorophenyl)-1,4-dihydro-pyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-216)*



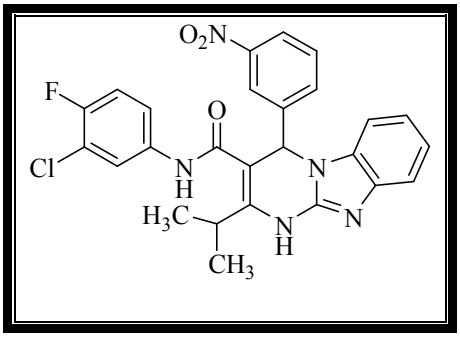

Yield: 72%; mp 211ºC; Anal. Calcd. for $C_{26}H_{21}Cl_2FN_4O$: C, 63.04; H, 4.27; Cl, 14.31; F, 3.84; N, 11.31; O, 3.23; Found: C, 63.00; H, 4.13; Cl, 14.22; F, 3.74; N, 11.24; O, 3.12%; MS: *m/z* 494.

### *5.6.5.7 N-(4-fluoro,3-chlorophenyl) -2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-217)*



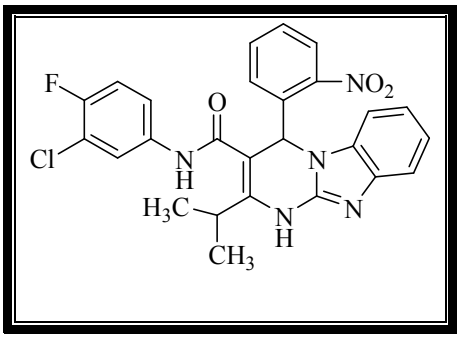
Yield: 71%; mp 242ºC; Anal. Calcd. for $C_{26}H_{21}ClFN_5O_3$: C, 61.72; H, 4.18; Cl, 7.01; F, 3.76; N, 13.84; O, 9.49; Found: C, 61.61; H, 4.11; Cl, 6.87; F, 3.62; N, 13.72; O, 9.34%; MS: *m/z* 505.

### *5.6.5.8 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-218)*



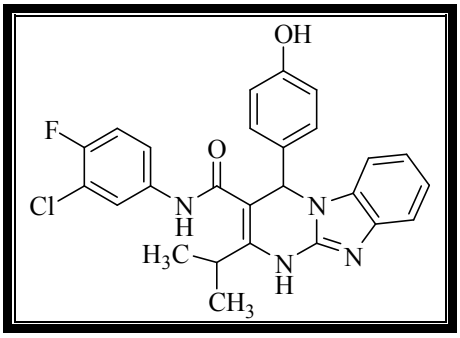
Yield: 57%; mp 213ºC; Anal. Calcd. for $C_{26}H_{21}ClFN_5O_3$: C, 61.72; H, 4.18; Cl, 7.01; F, 3.76; N, 13.84; O, 9.49; Found: C, 61.64; H, 4.02; Cl, 6.89; F, 3.64; N, 13.77; O, 9.33%; MS: *m/z* 505.

### *5.6.5.9 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-nitrophenyl)-1,4-dihydro-pyrimido[1,2-a] benzimidazole-3-carboxamide (RHK-219)*



Yield: 65%; mp 246ºC; Anal. Calcd. for $C_{26}H_{21}ClFN_5O_3$: C, 61.72; H, 4.18; Cl, 7.01; F, 3.76; N, 13.84; O, 9.49; FoundC, 61.61; H, 4.03; Cl, 7.00; F, 3.66; N, 13.75; O, 9.34%; MS: *m/z* 505.

***5.6.5.10 N-(4-fluoro,3-chlorophenyl)-2-isopropyl-4-(4-hydrooxyphenyl)-1,4-dihydro -pyrimido[1,2-a]benzimidazole-3-carboxamide (RHK-220)***



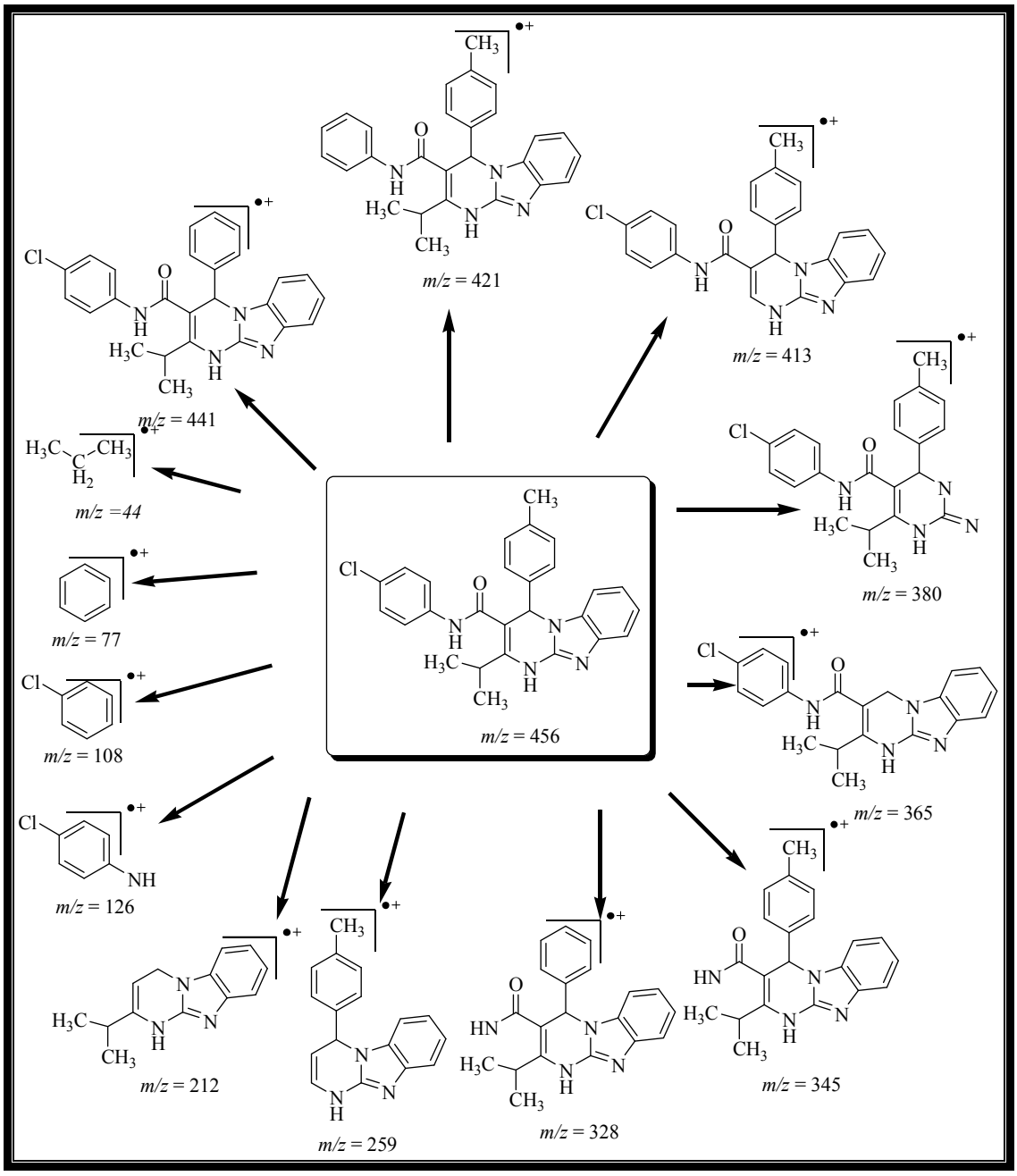

Yield: 66%; mp 210ºC; Anal. Calcd. for $C_{26}H_{22}ClFN_4O_2$: C, 65.48; H, 4.65; Cl, 7.43; F, 3.98; N, 11.75; O, 6.71. Found: C, 65.34; H, 4.54; Cl, 7.31; F, 3.87; N, 11.61; O, 6.64%; MS: *m/z* 476.

## 5.7 Spectral discussion

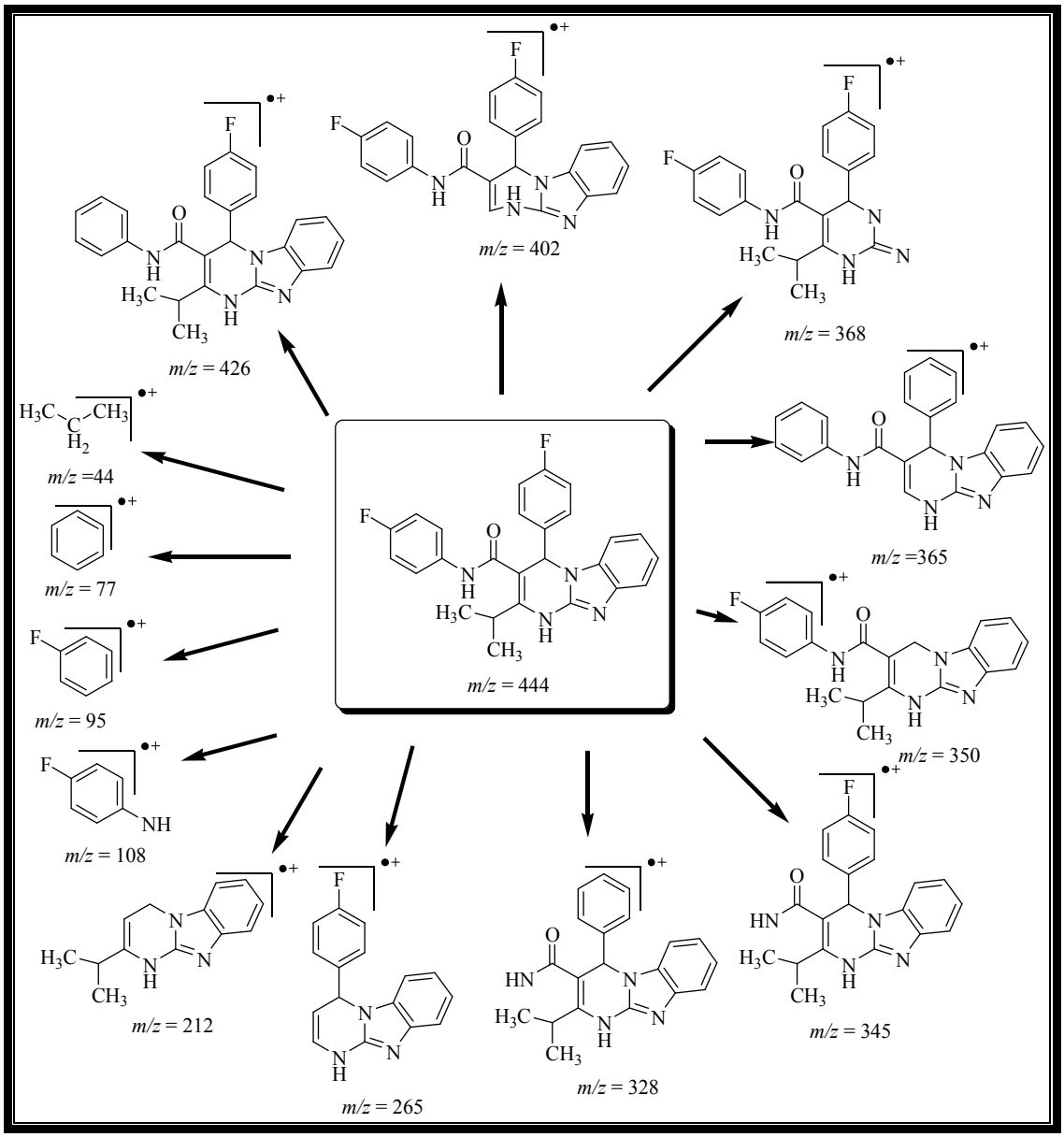
### 5.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analyses. 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.

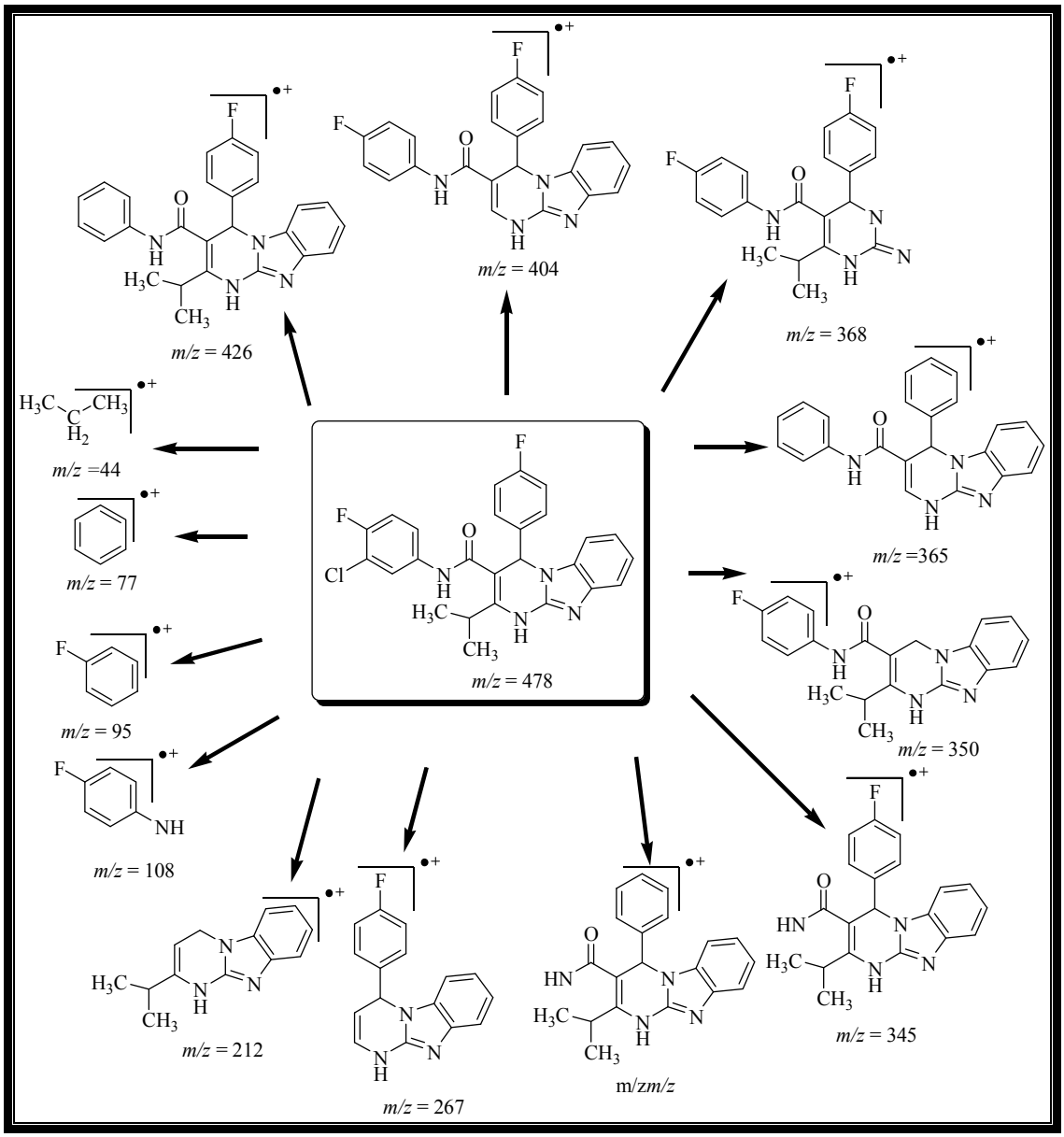
### 5.7.1.1 *Mass fragmentation pattern for RHK-192*



### 5.7.1.2 *Mass fragmentation pattern for RHK-204*



### 5.7.1.3 *Mass fragmentation pattern for RHK-214*



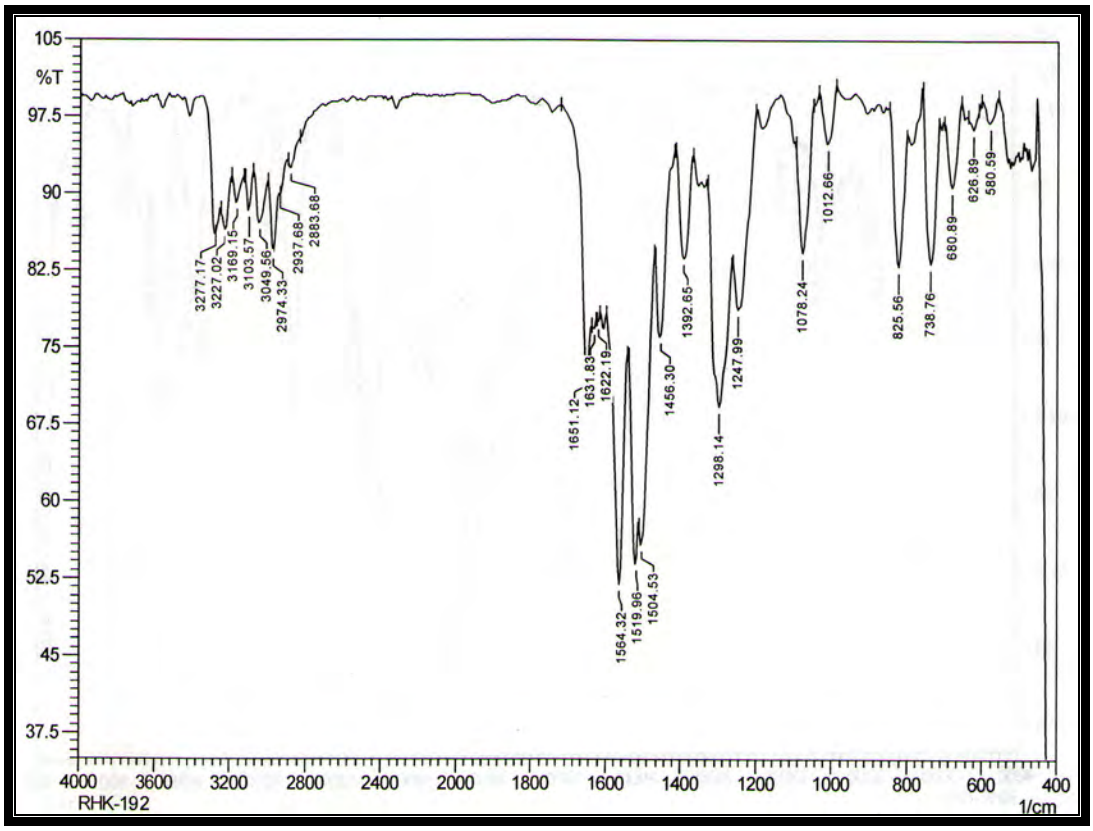
### 5.7.2 IR spectral study

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 1,4-dihydropyrimido[1,2-*a*] benzimidazoles **RHK- 191 to 220**, confirmatory bands for secondary amine and amidic carbonyl groups were observed at 3414-3282 $cm^{-1}$ and 1690-1600 $cm^{-1}$ respectively. Another characteristic C=N stretching band of triazole ring was observed at 1626-1500 $cm^{-1}$, which suggested formation of desired products **RHK-191 to 220**.

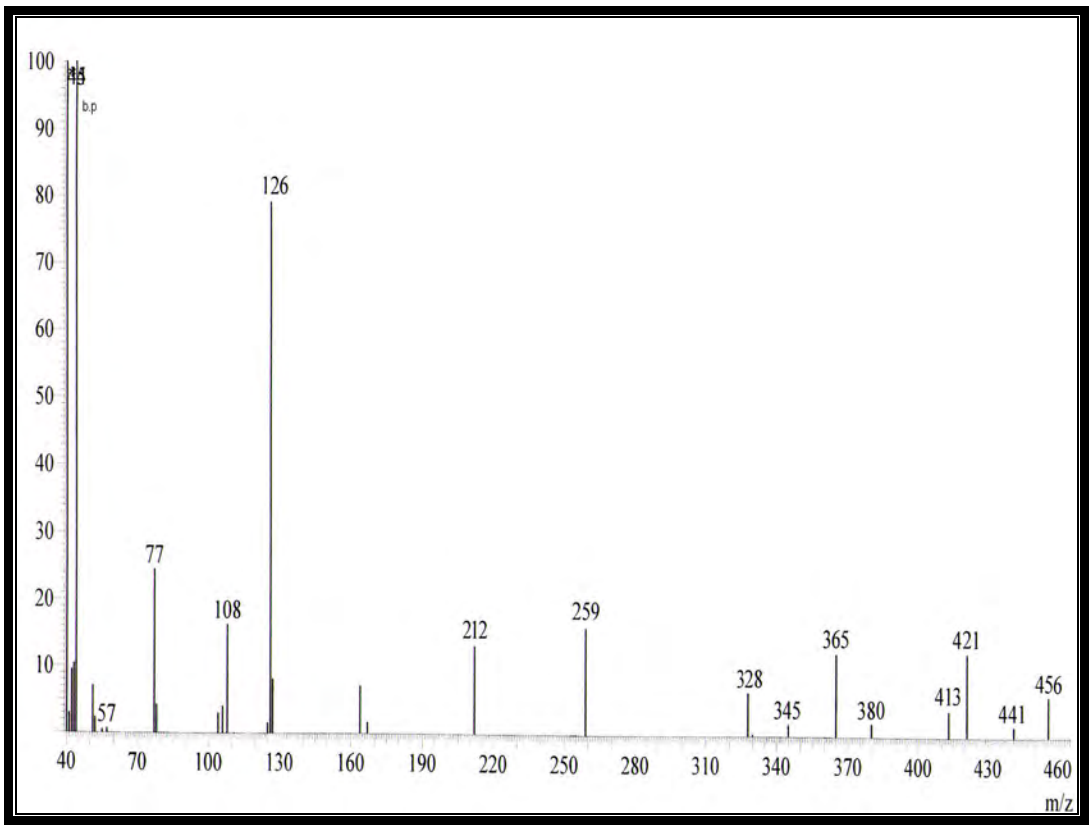
### 5.7.3 $^{1}$H NMR spectral study

$^{1}$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 1,4-dihydro-pyrimido[1,2-*a*] benzimidazoles **RHK- 191 to 220** on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 6.00-6.90 δ ppm, and singlets for amino and amide group protons at 7.50-9.90 and 9.45-10.50 δ ppm, respectively. The aromatic ring protons and J value were found to be in accordance with substitution pattern on phenyl ring.

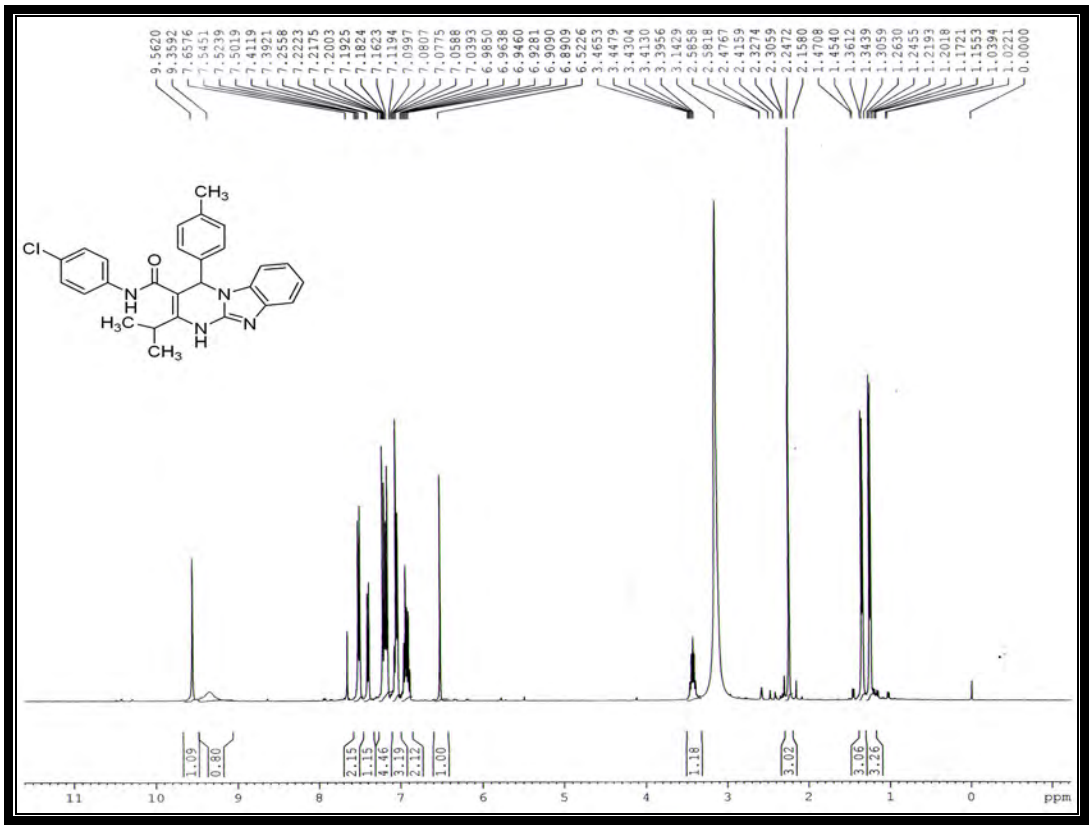
## *IR spectrum of RHK-192*




## *Mass spectrum of RHK-192*



## *$^1$H NMR spectrum of RHK-192*

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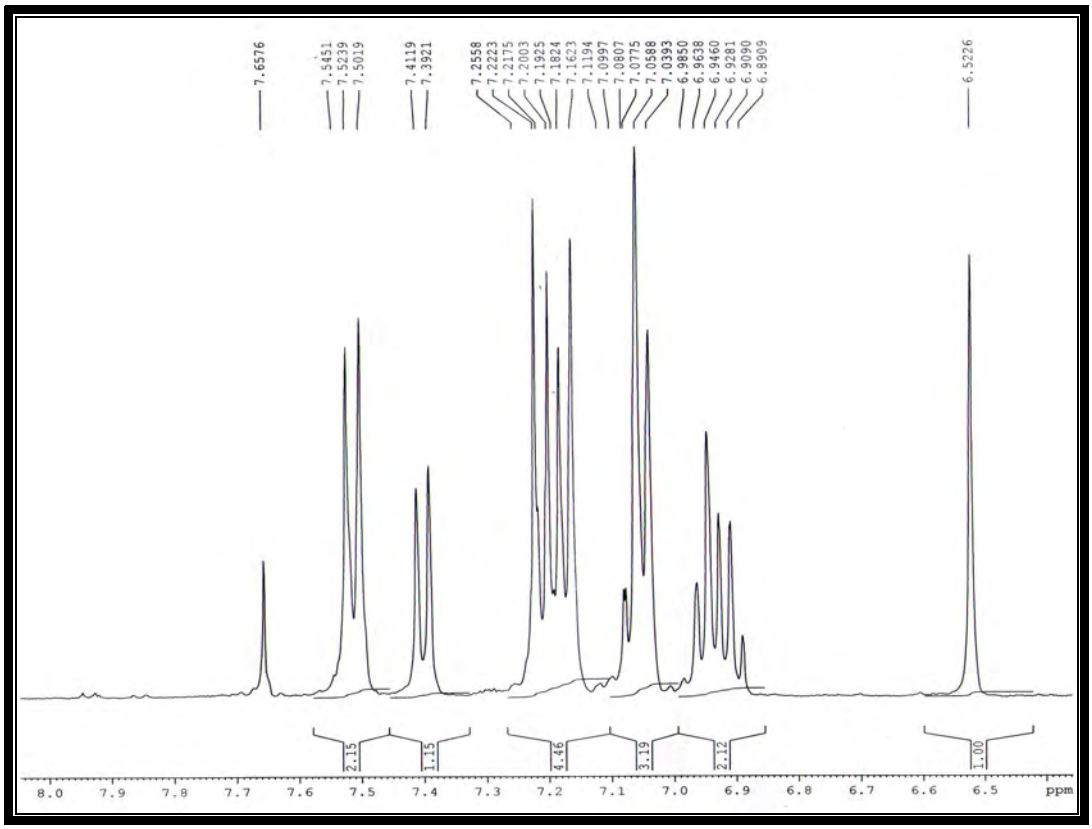

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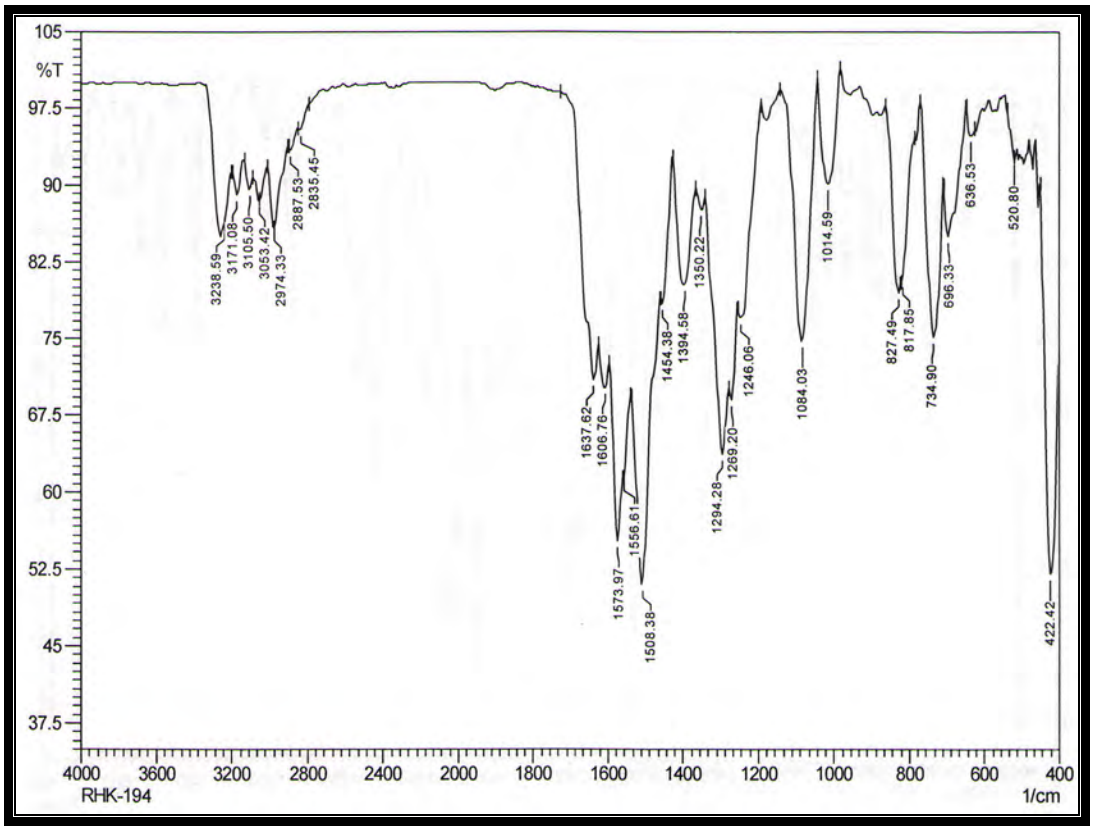


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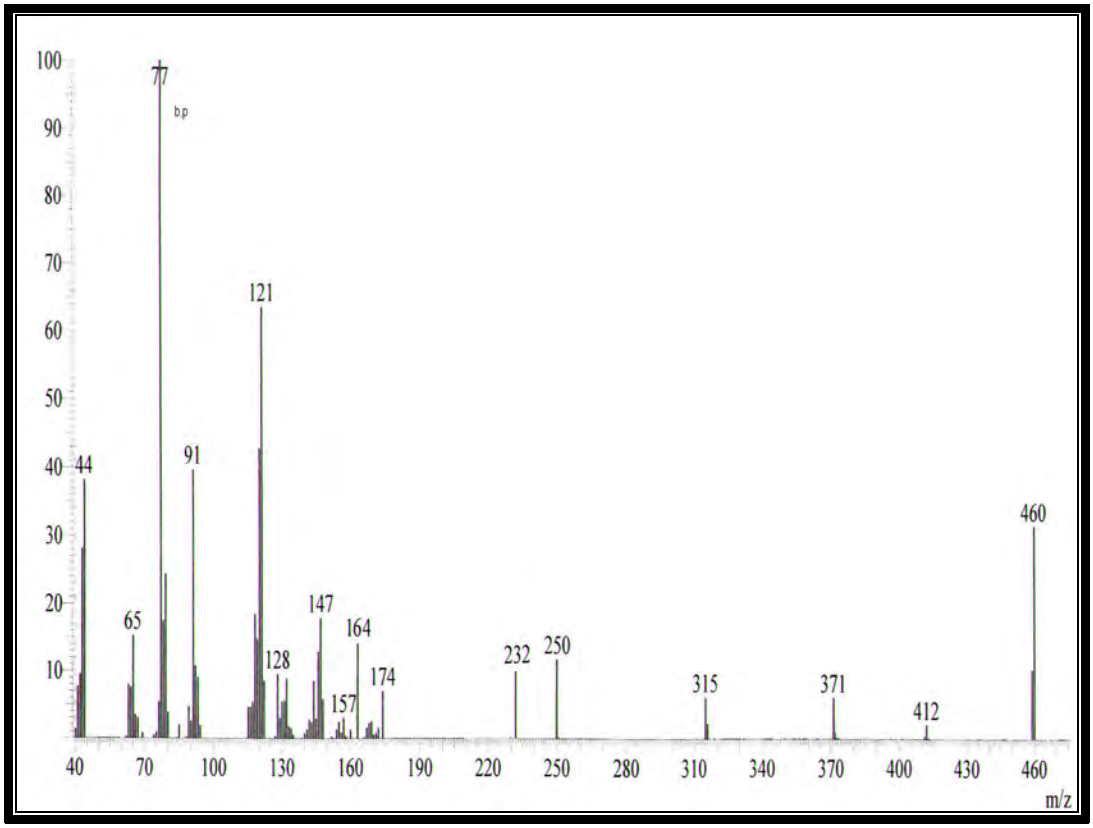

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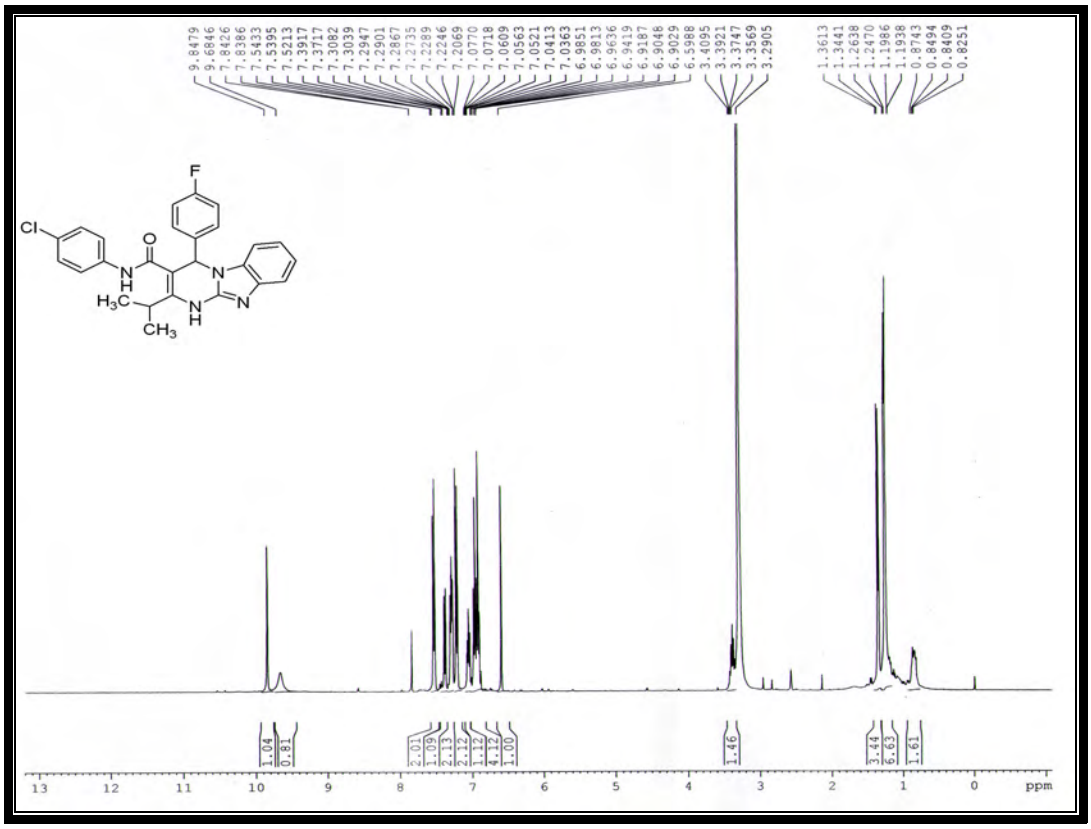


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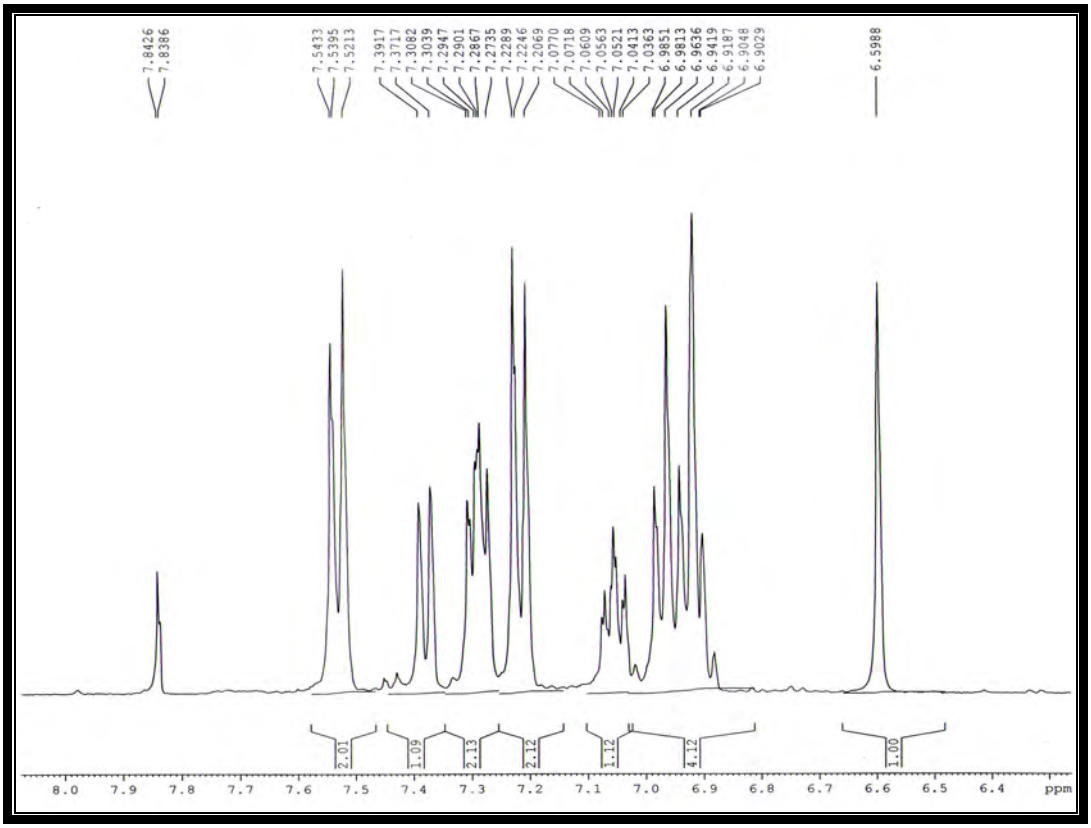

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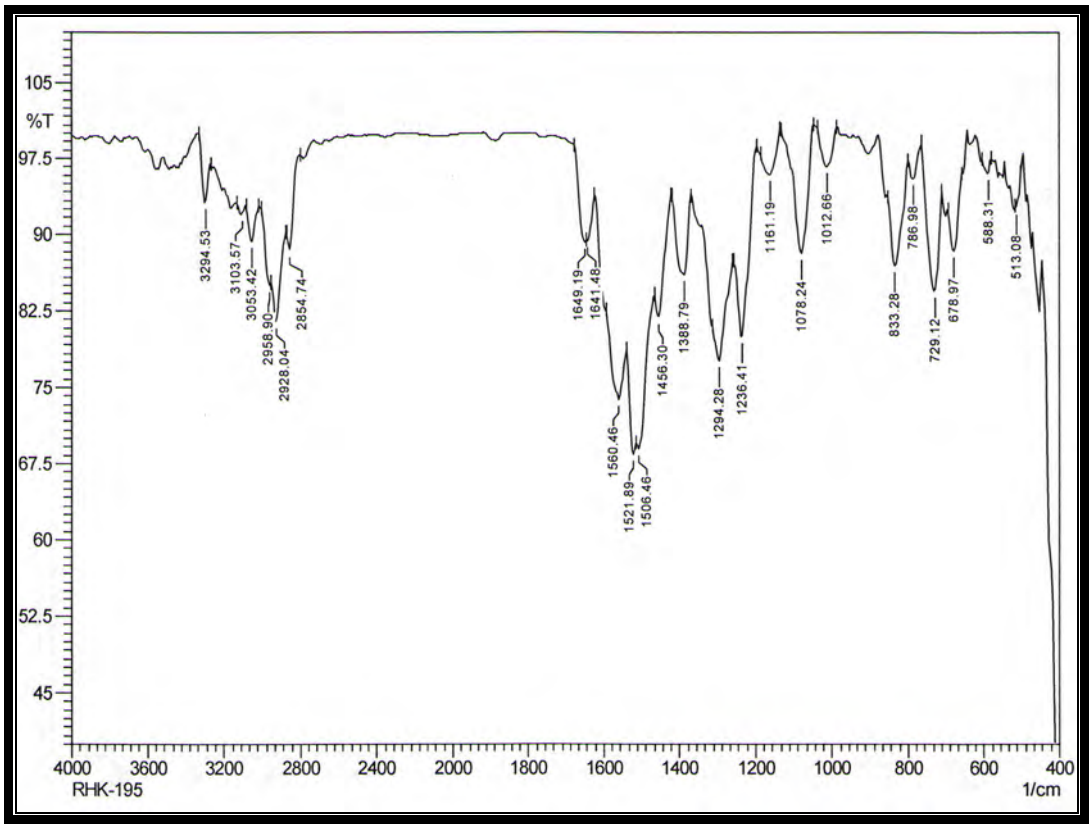
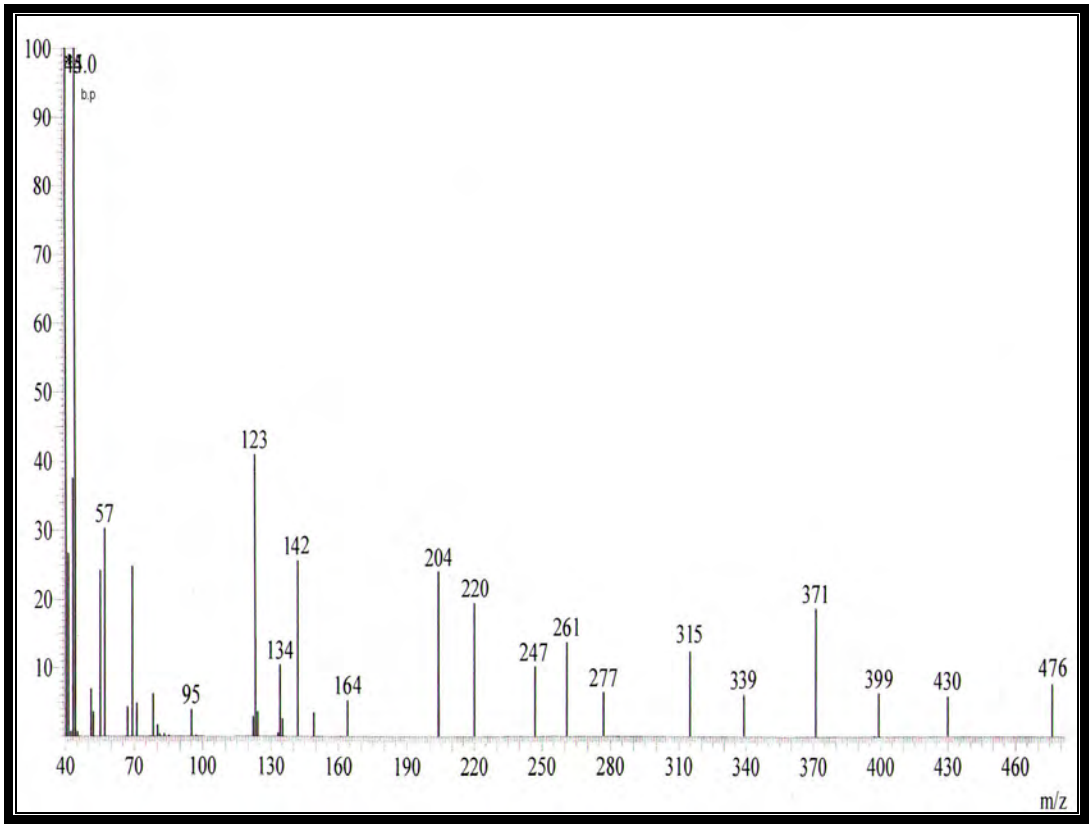



## *Mass spectrum of RHK-195*



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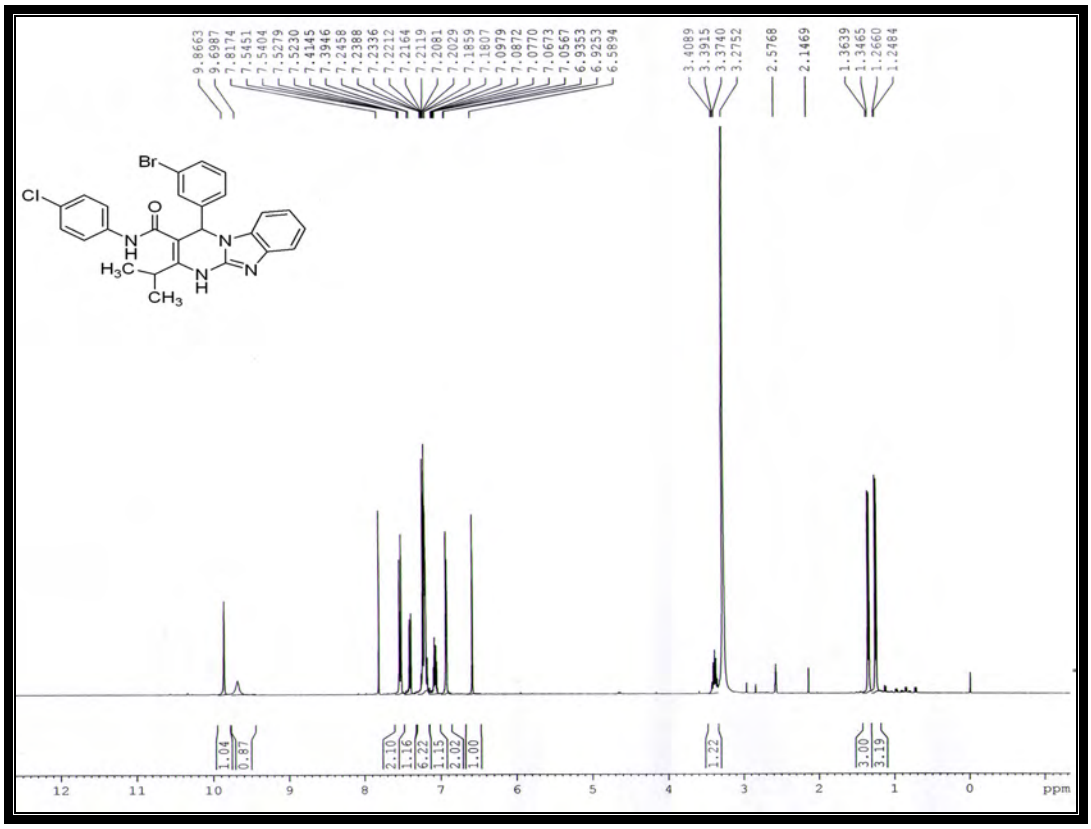


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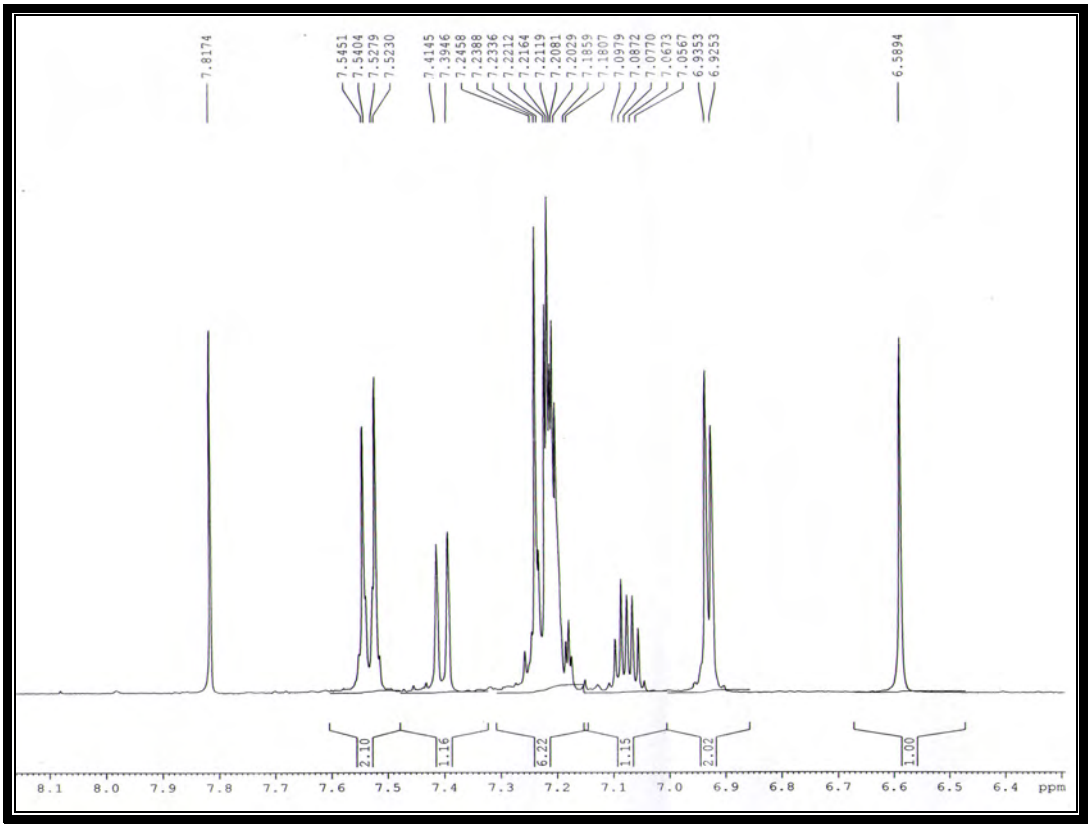

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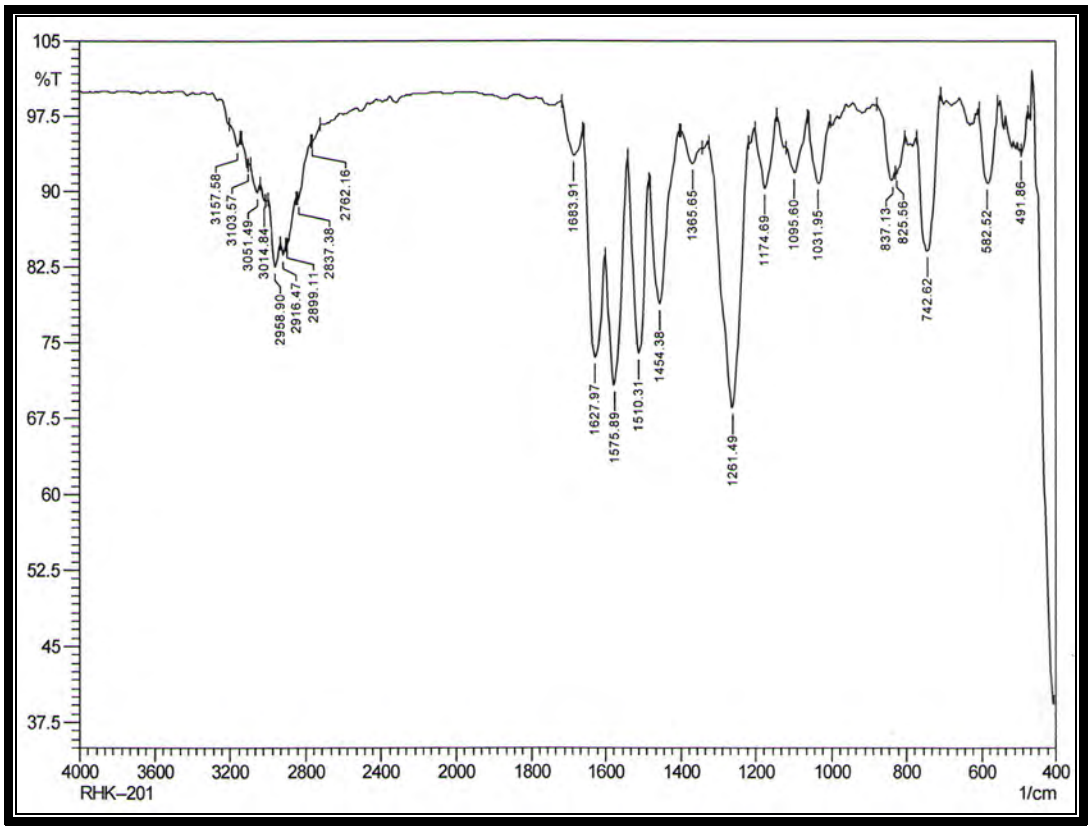
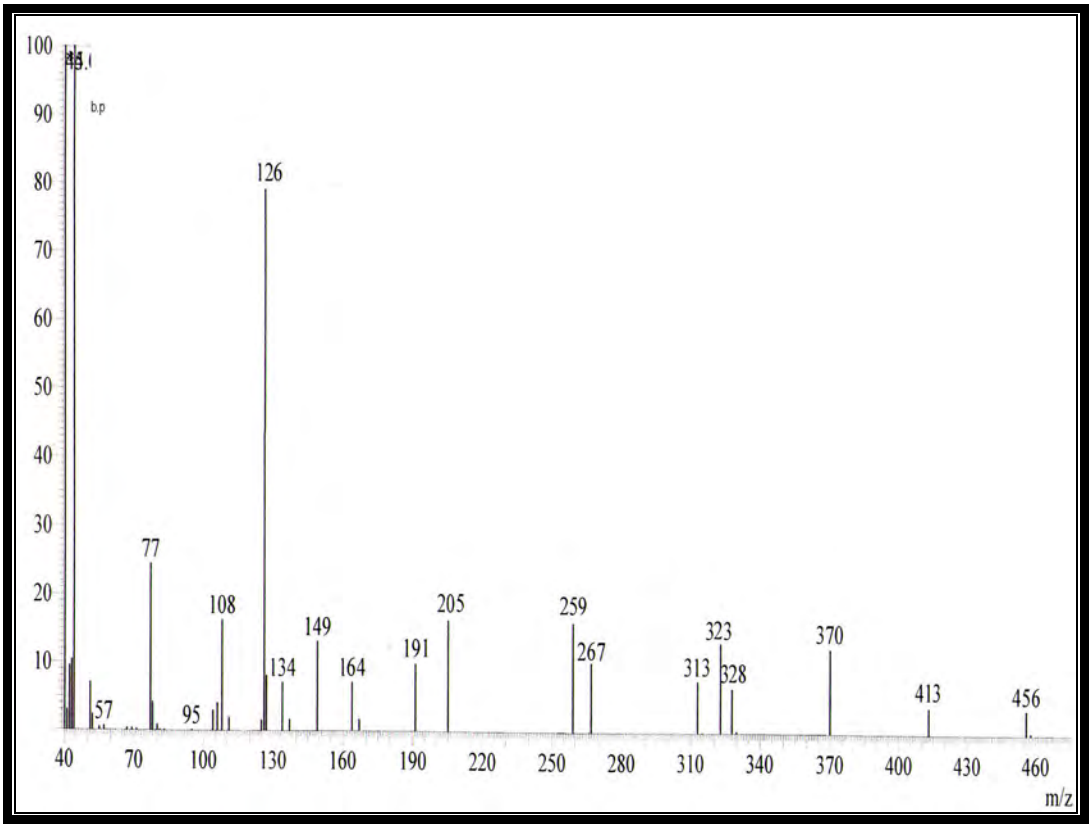
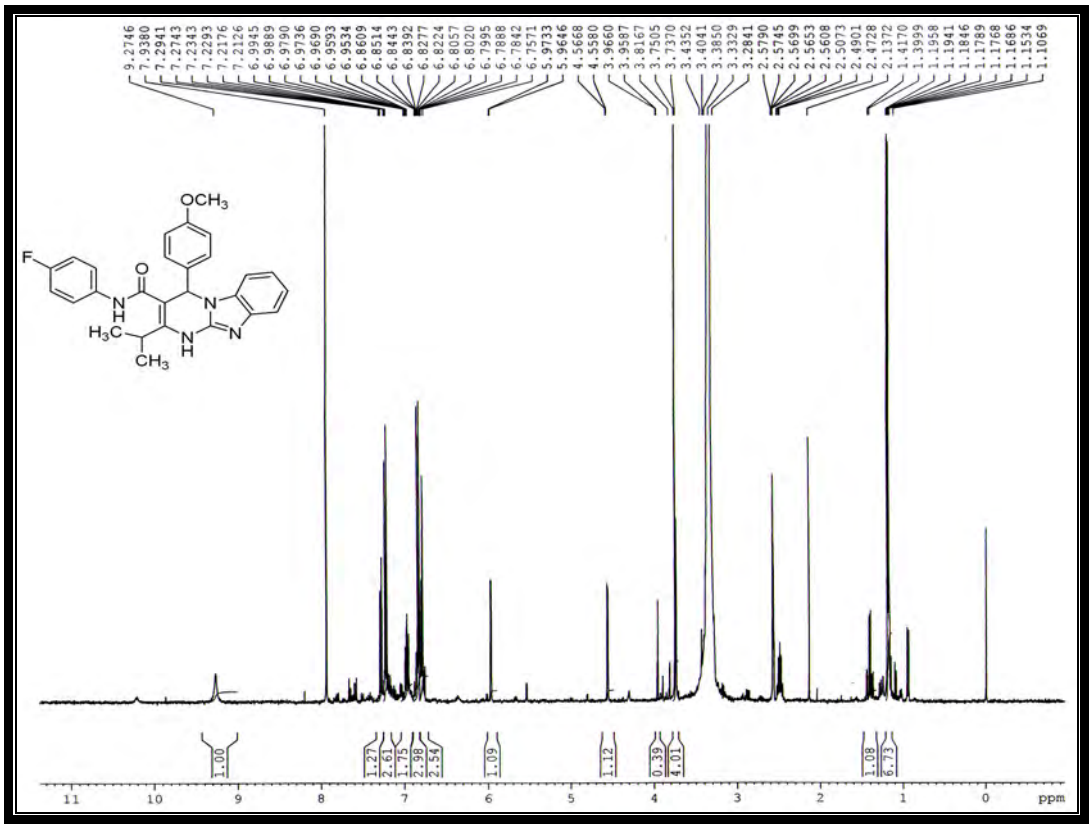


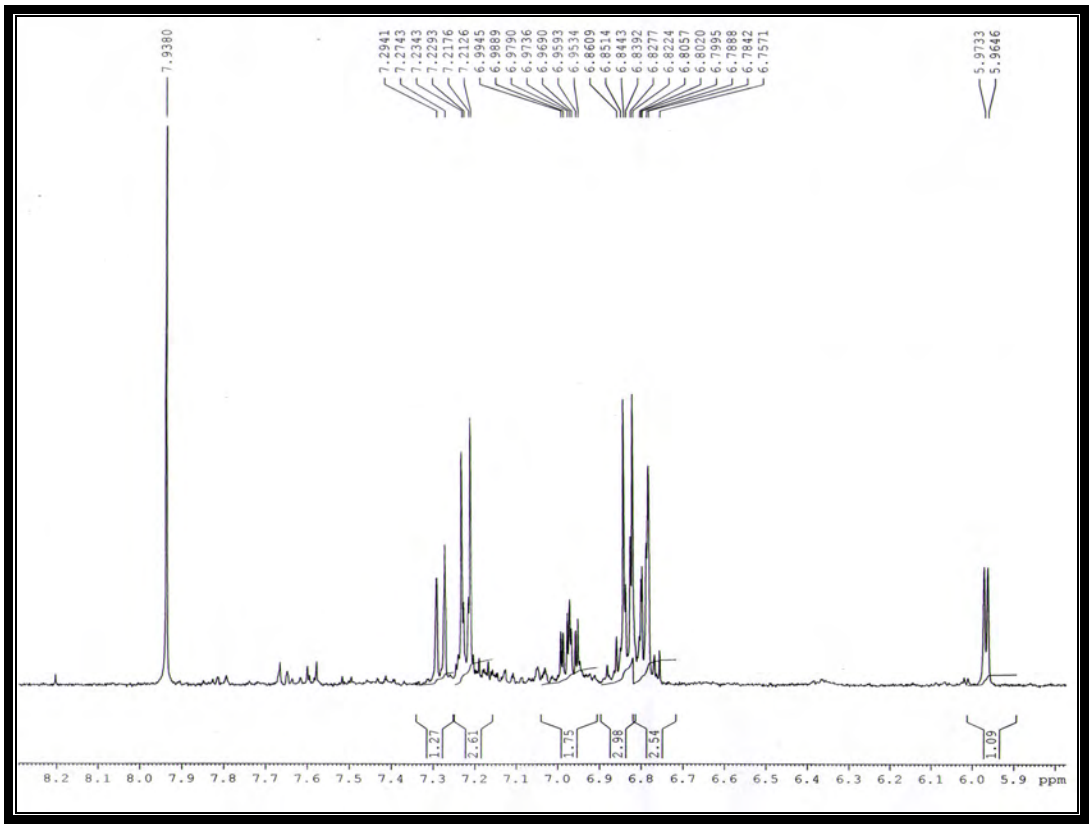

***Mass spectrum of RHK-201***



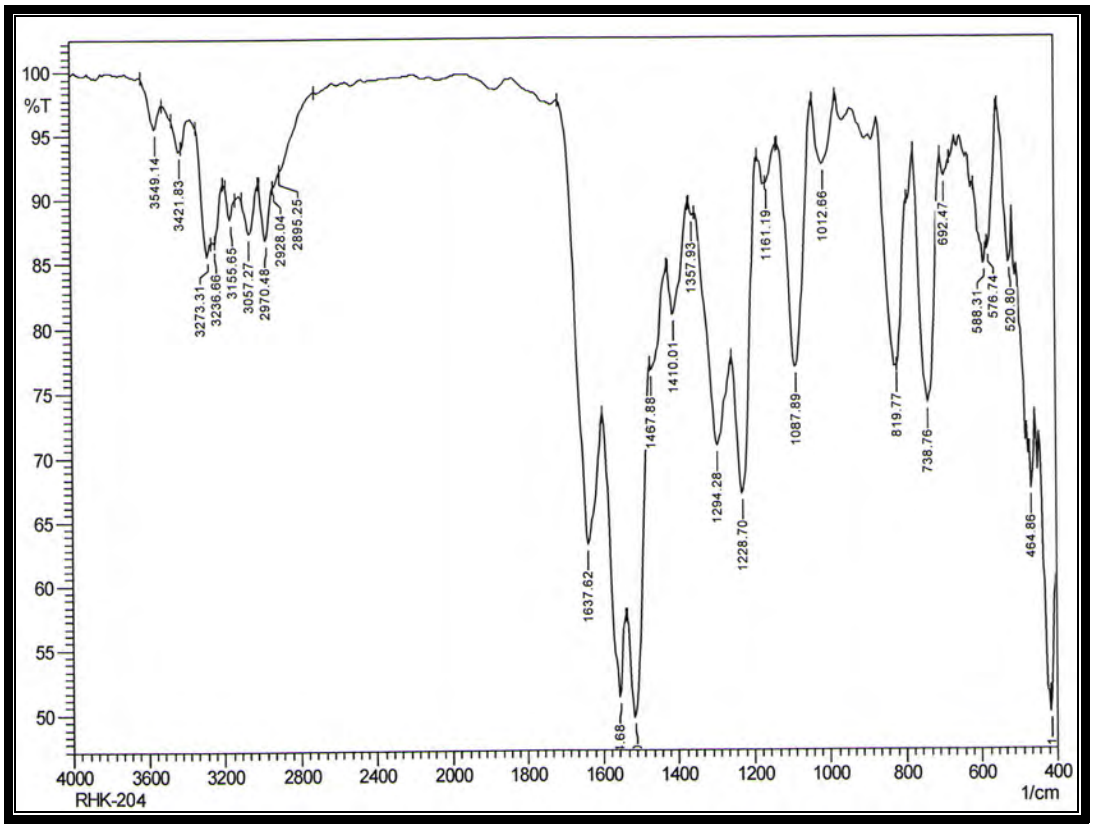
## *$^1$H NMR spectrum of RHK-201*



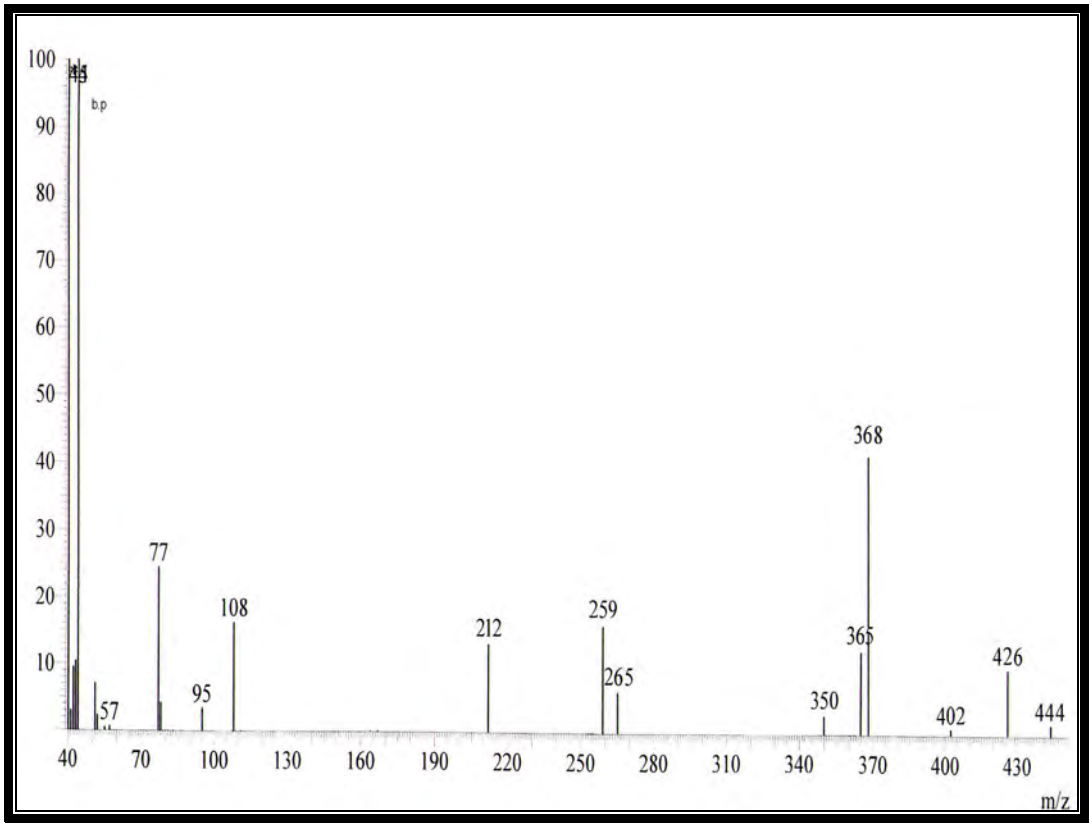

## *Expanded $^1$H NMR spectrum of RHK-201*



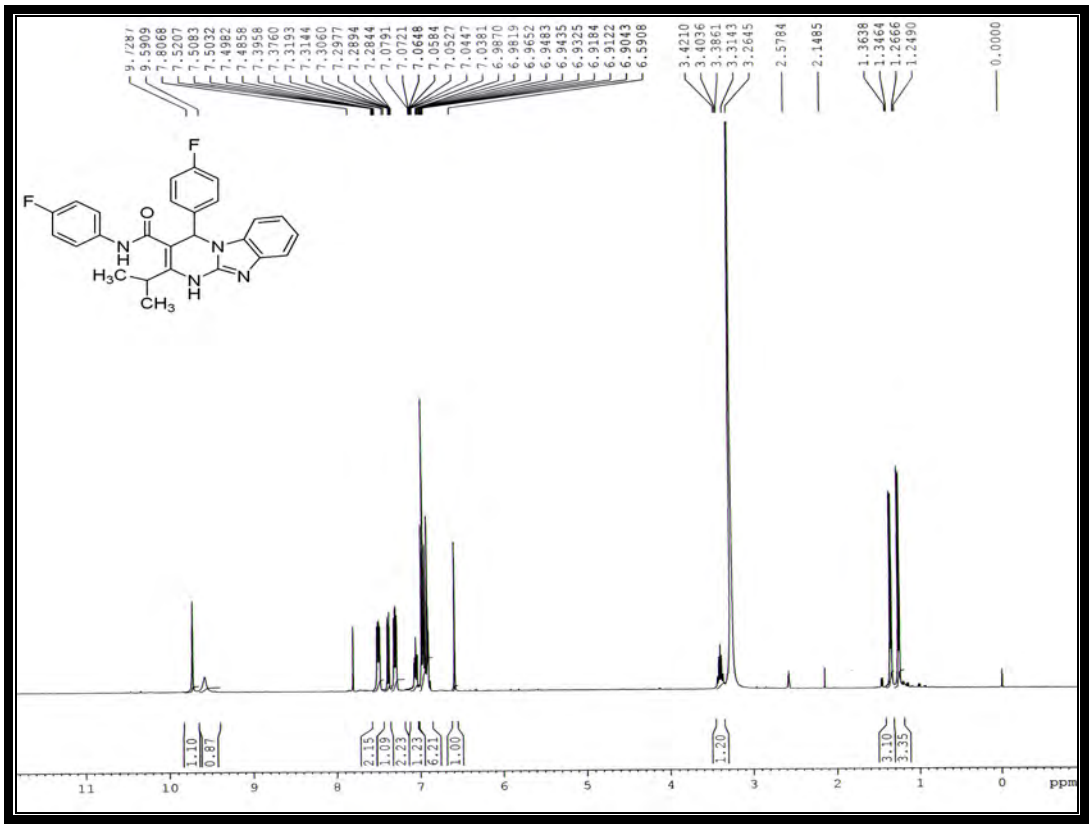
***IR spectrum of RHK-204***



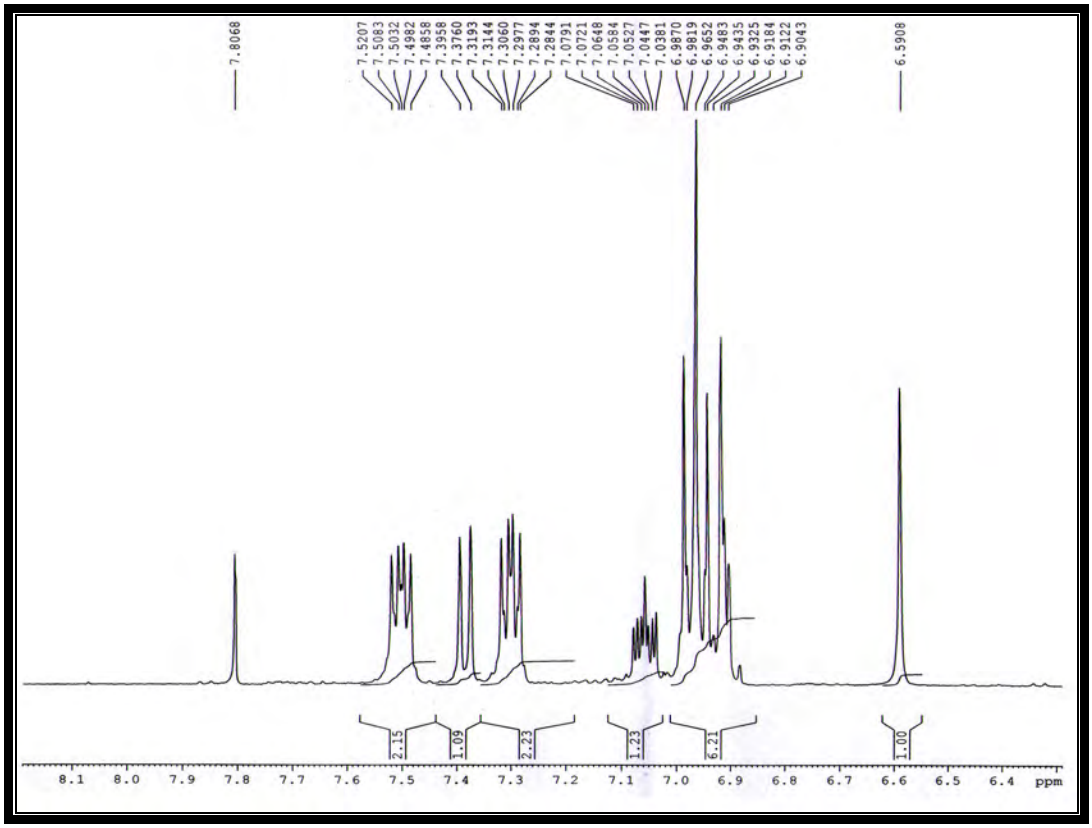

***Mass spectrum of RHK-204***



## *$^1$H NMR spectrum of RHK-204*




## *Expanded $^1$H NMR spectrum of RHK-204*



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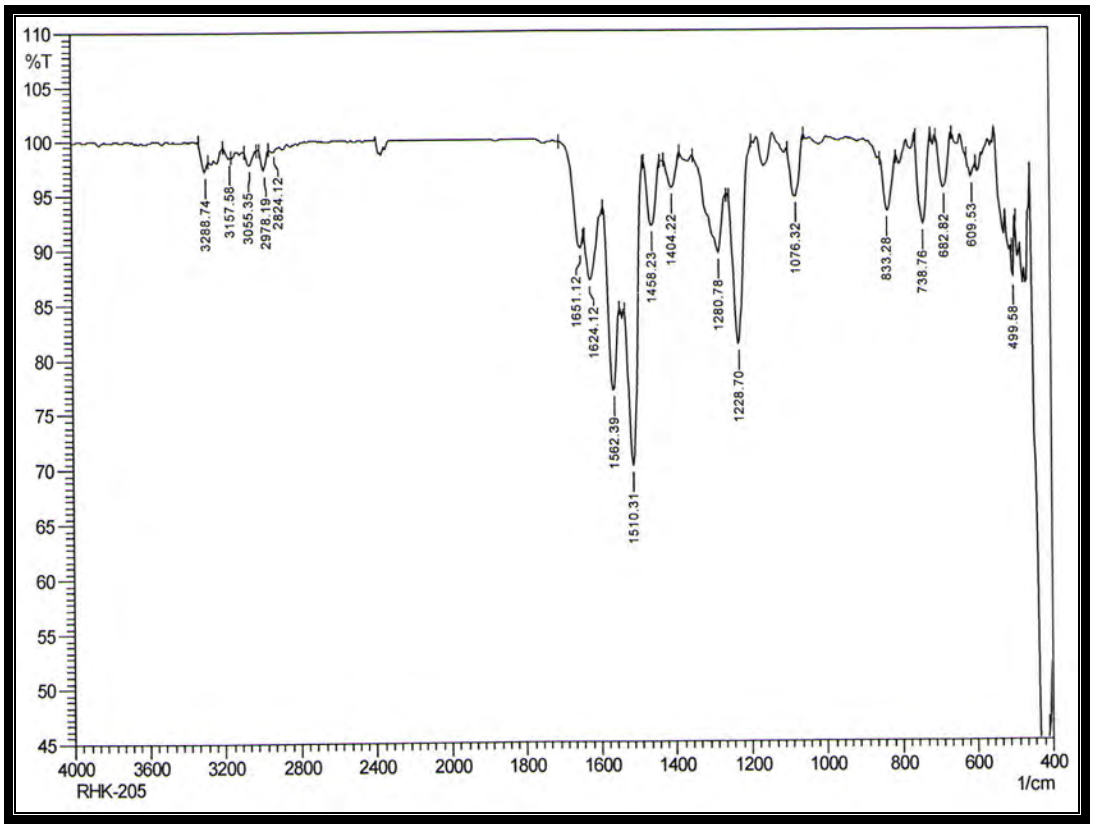
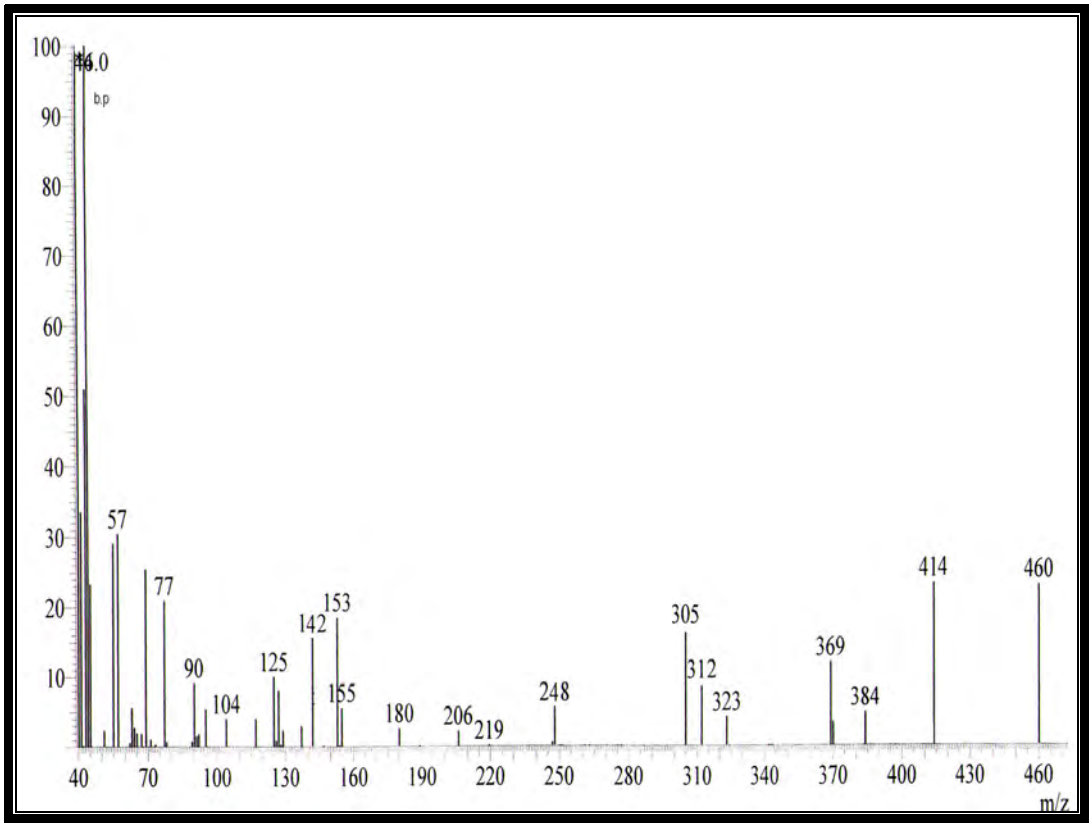
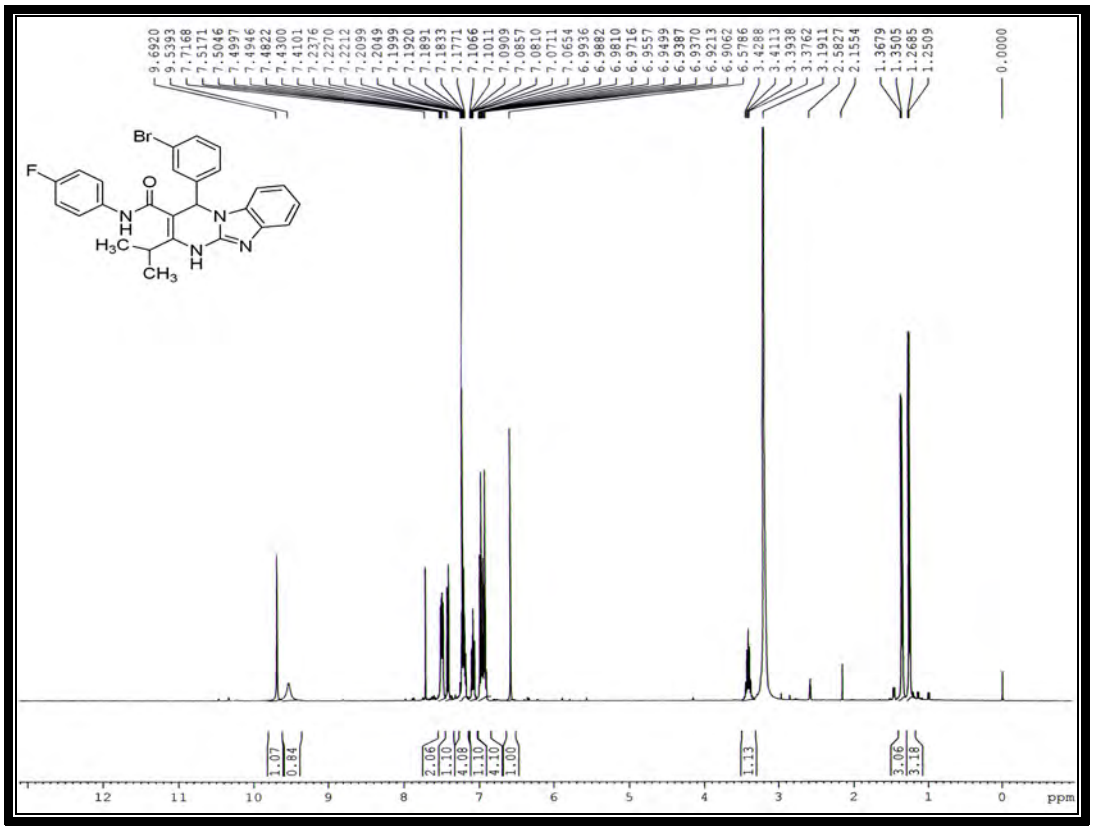


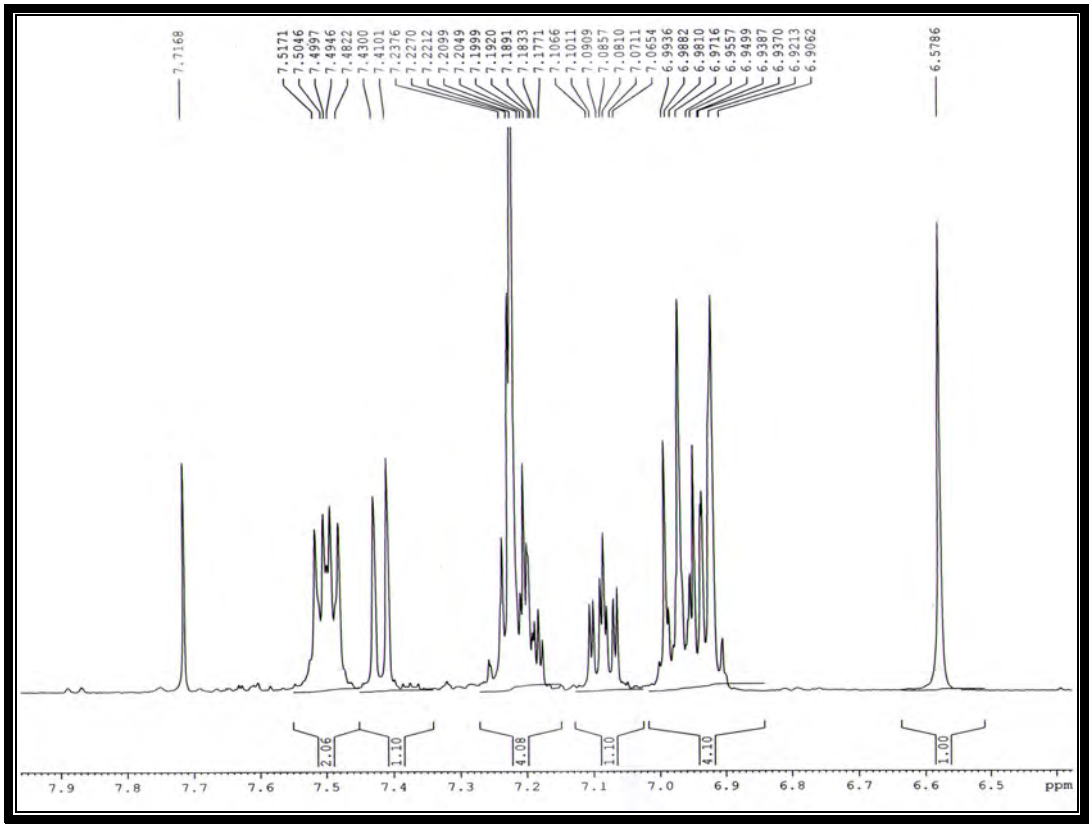

## *Mass spectrum of RHK-205*



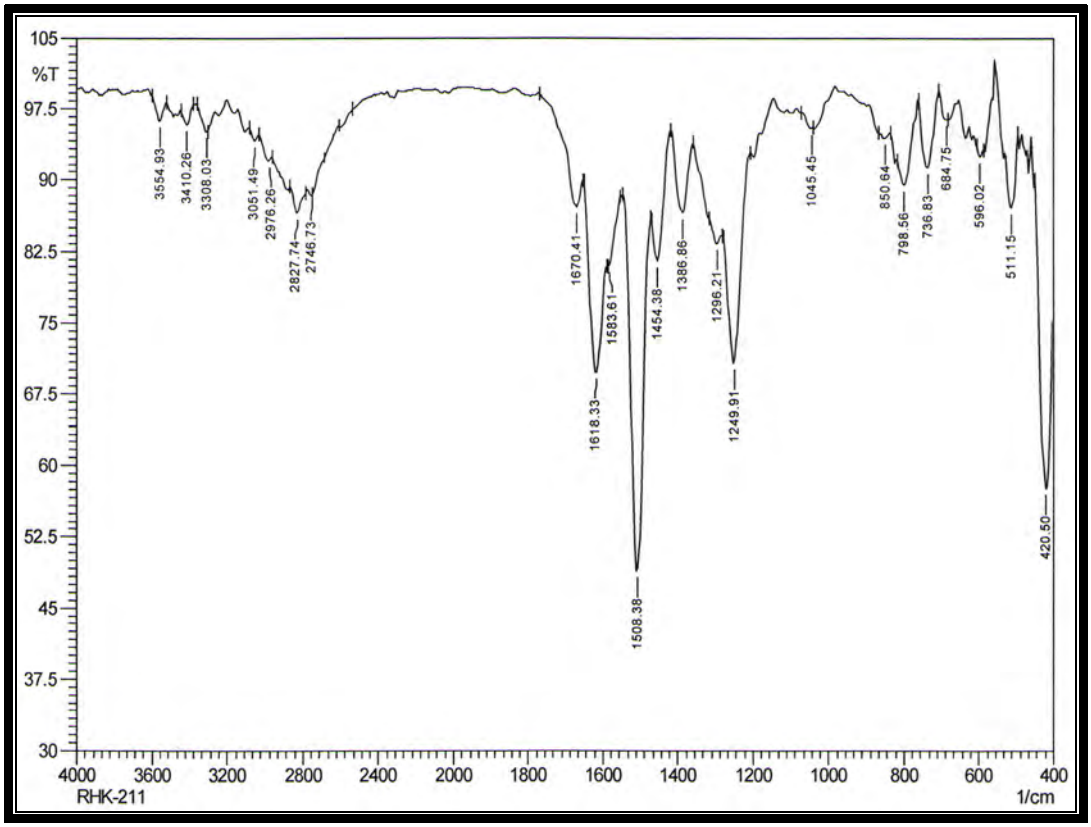
## *$^1$H NMR spectrum of RHK-205*



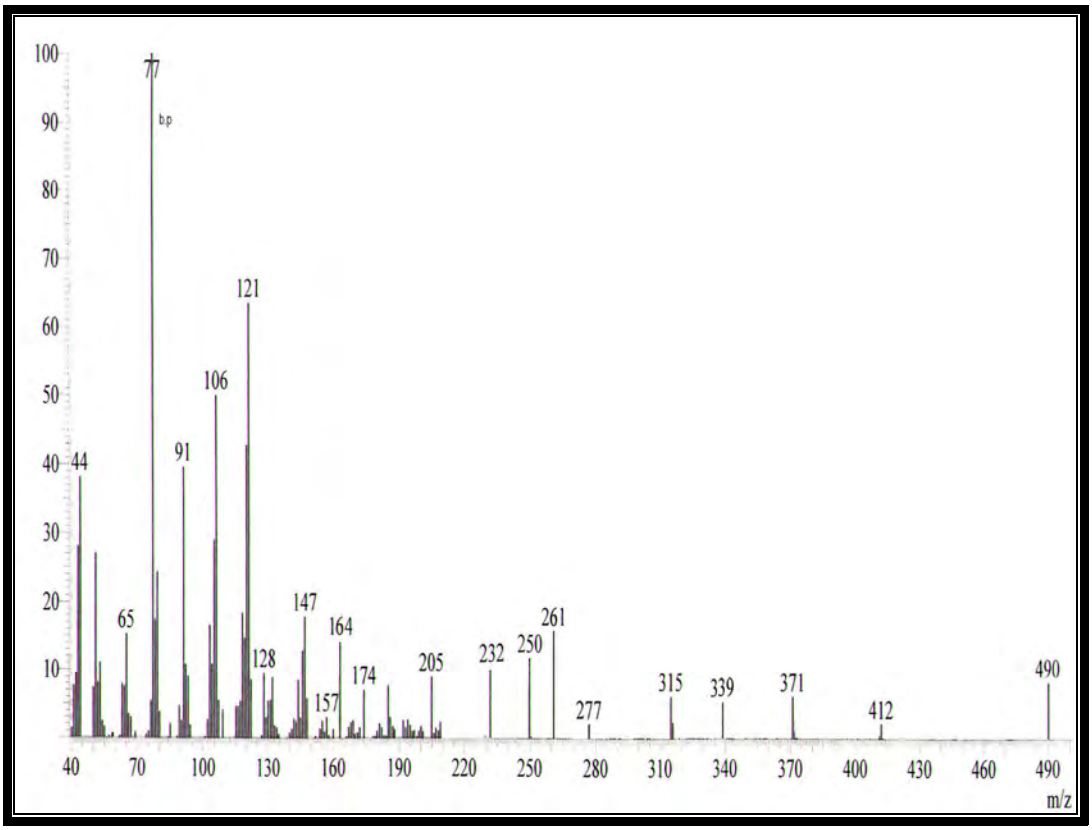

## *Expanded $^1$H NMR spectrum of RHK-205*



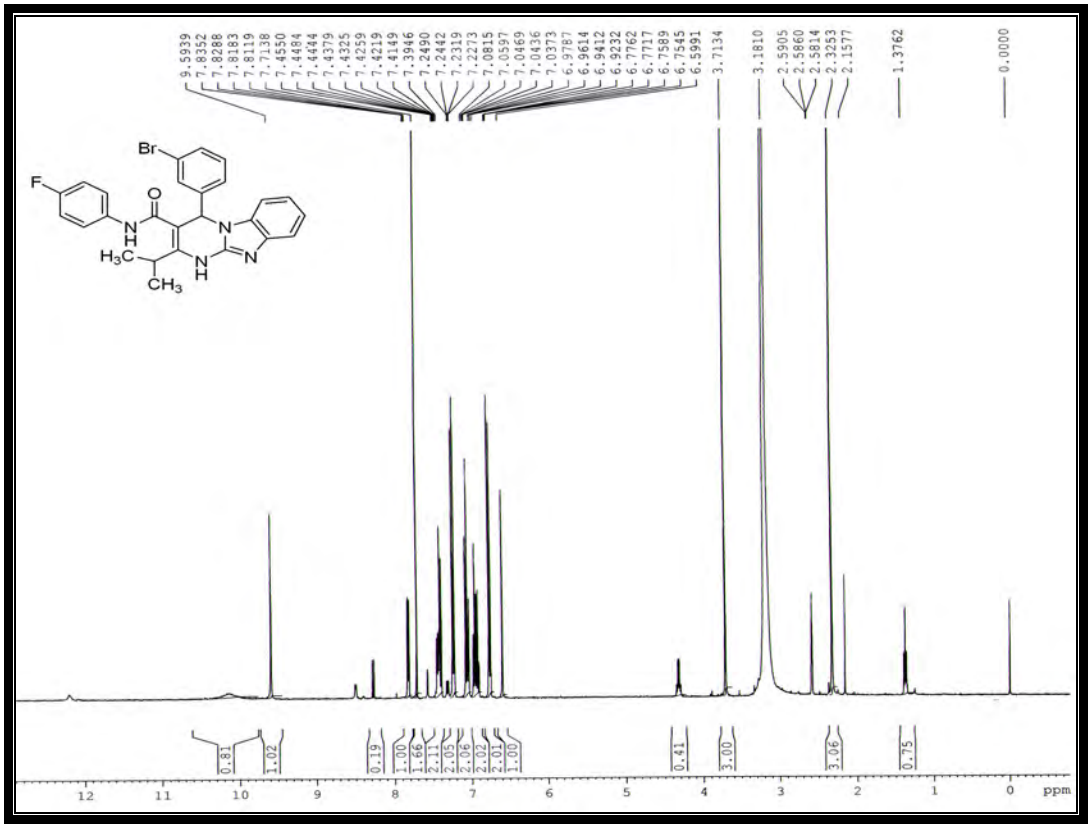
## *IR spectrum of RHK-211*



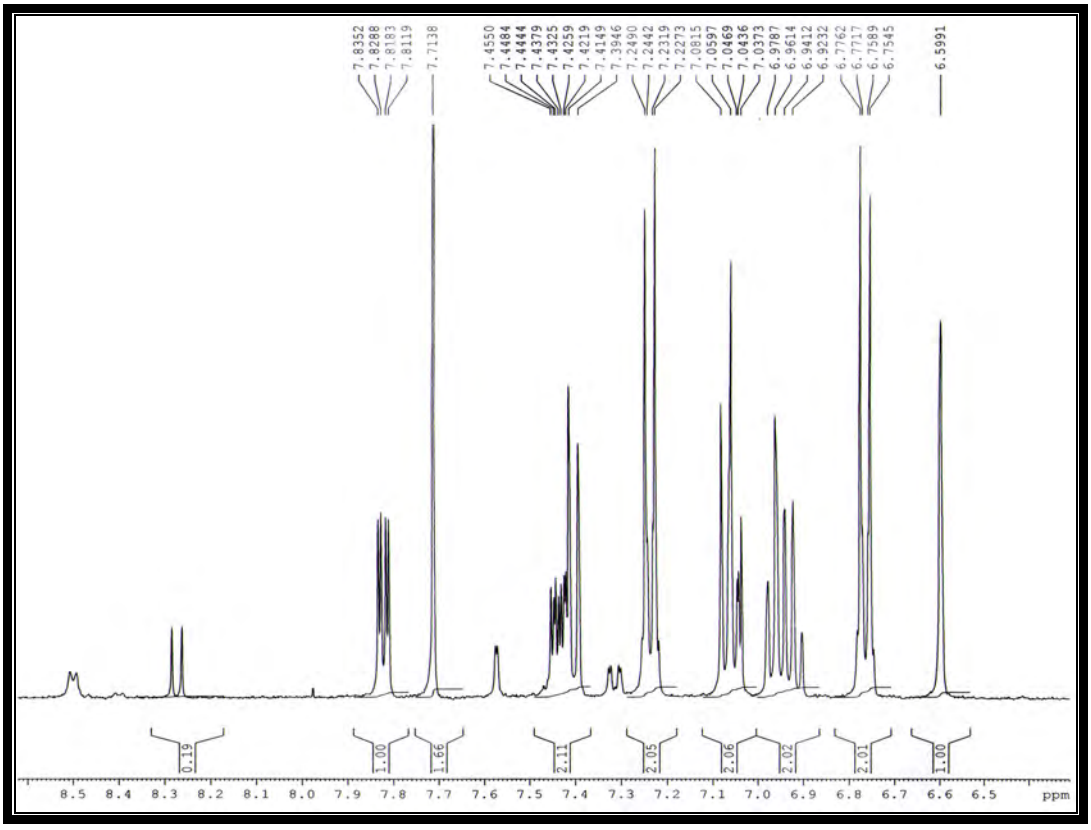

## *Mass spectrum of RHK-211*



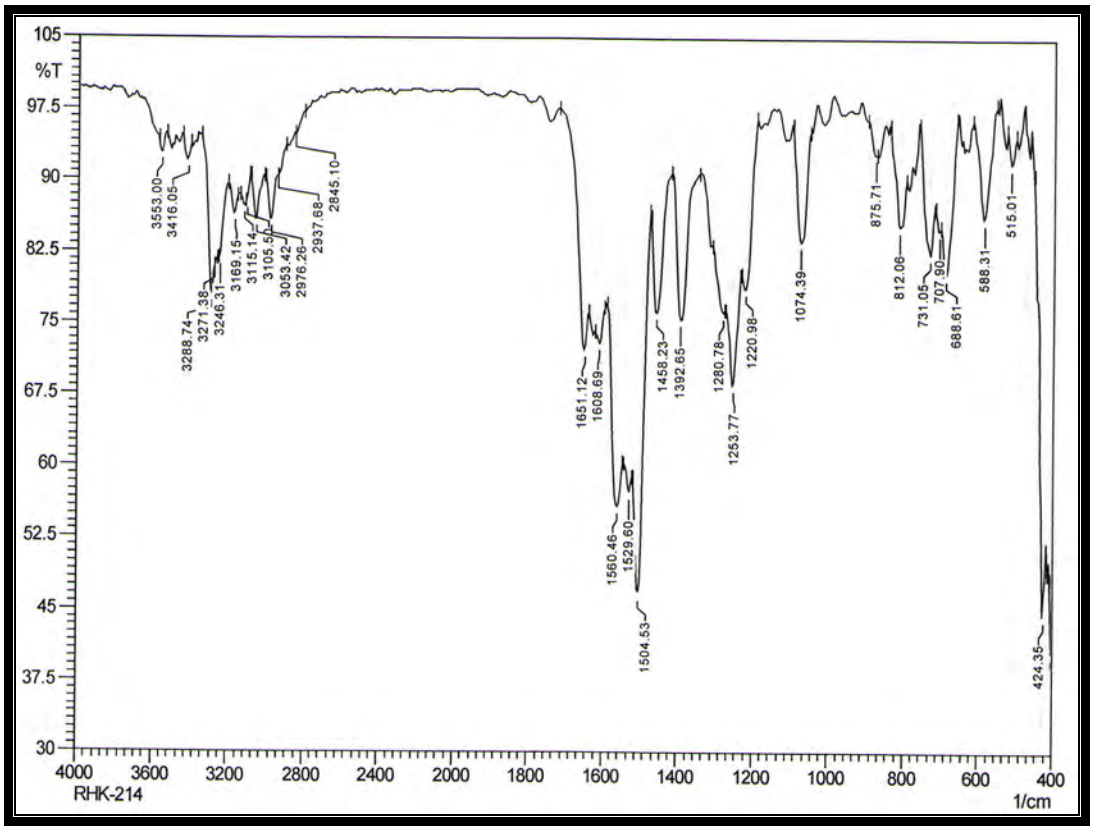
## *$^1$H NMR spectrum of RHK-211*



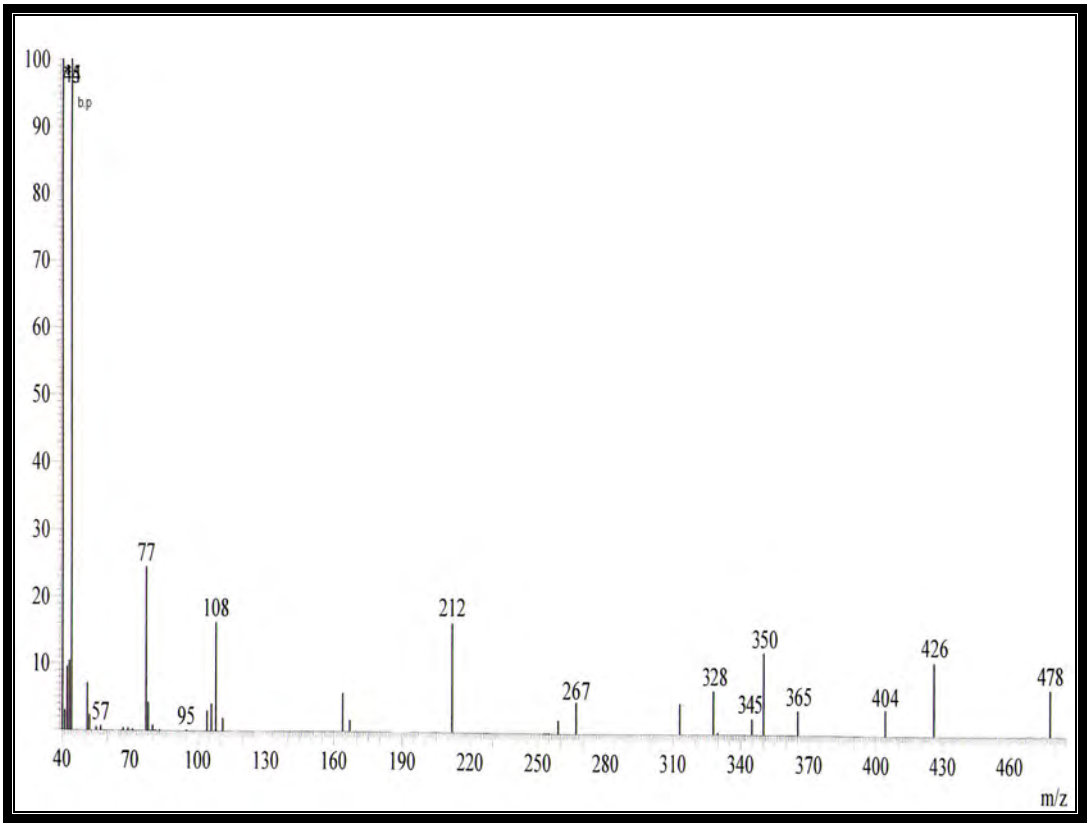

## *Expanded $^1$H NMR spectrum of RHK-211*



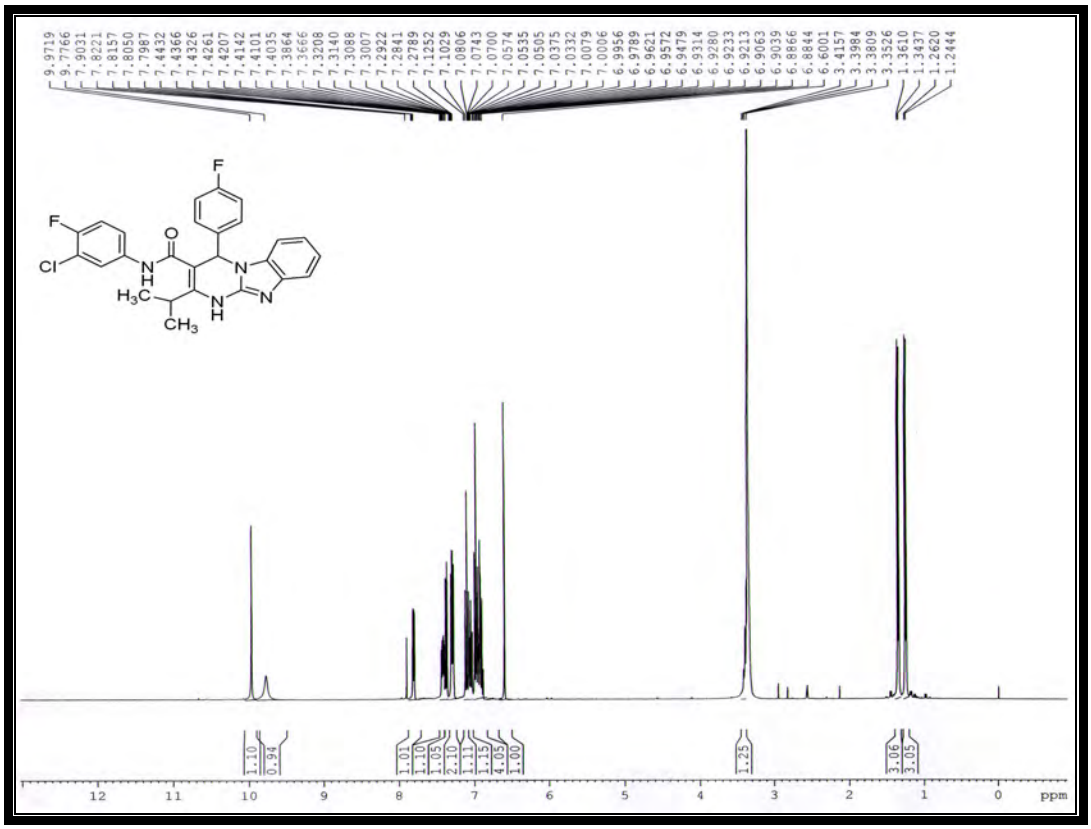
## *IR spectrum of RHK-214*



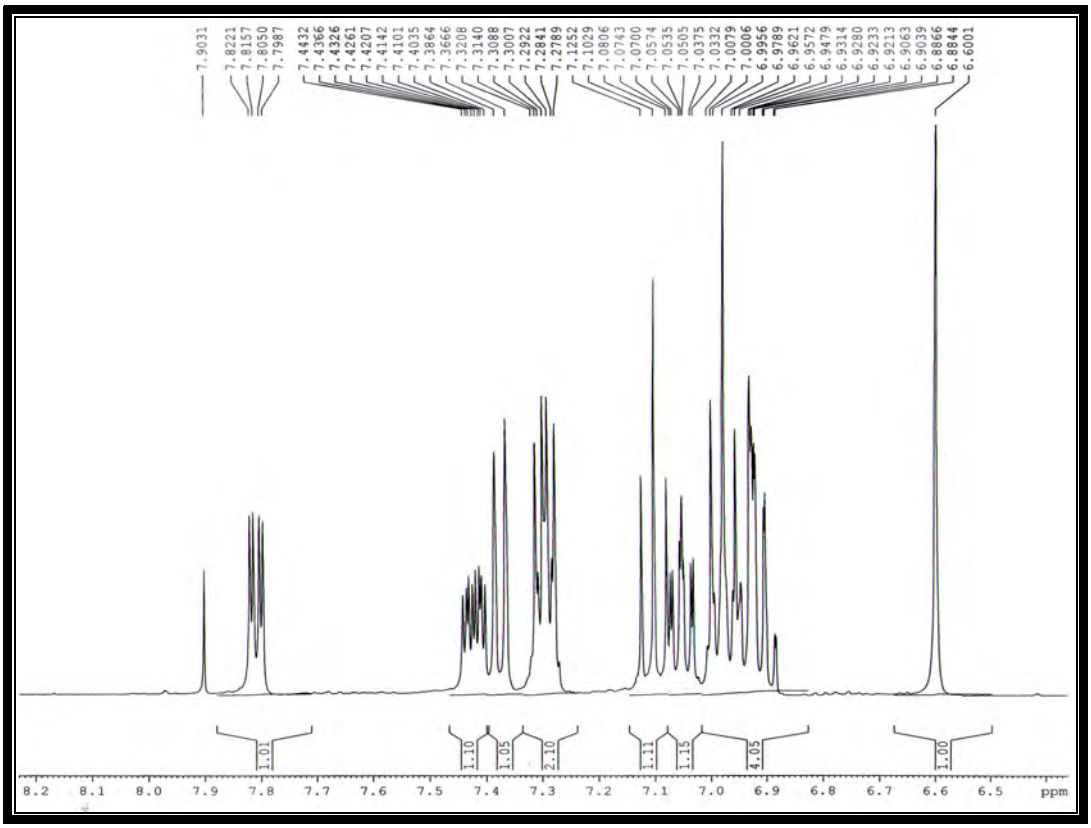

## *Mass spectrum of RHK-214*



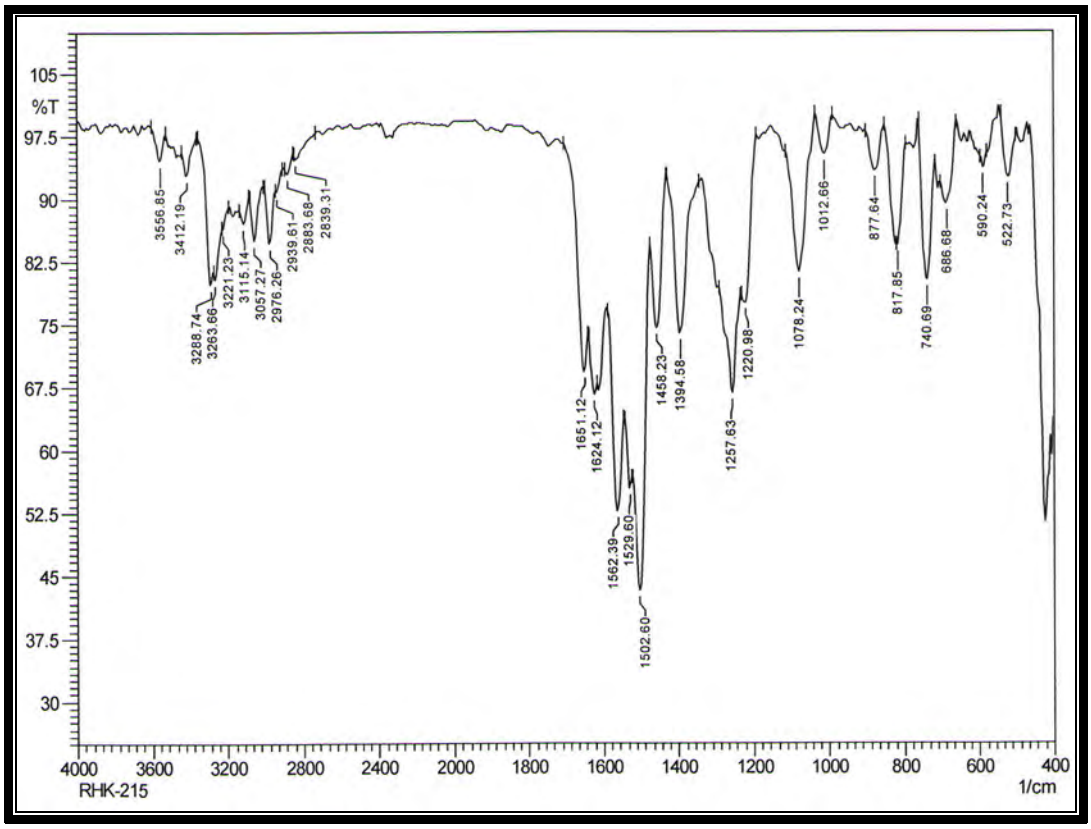
## *$^1$H NMR spectrum of RHK-214*



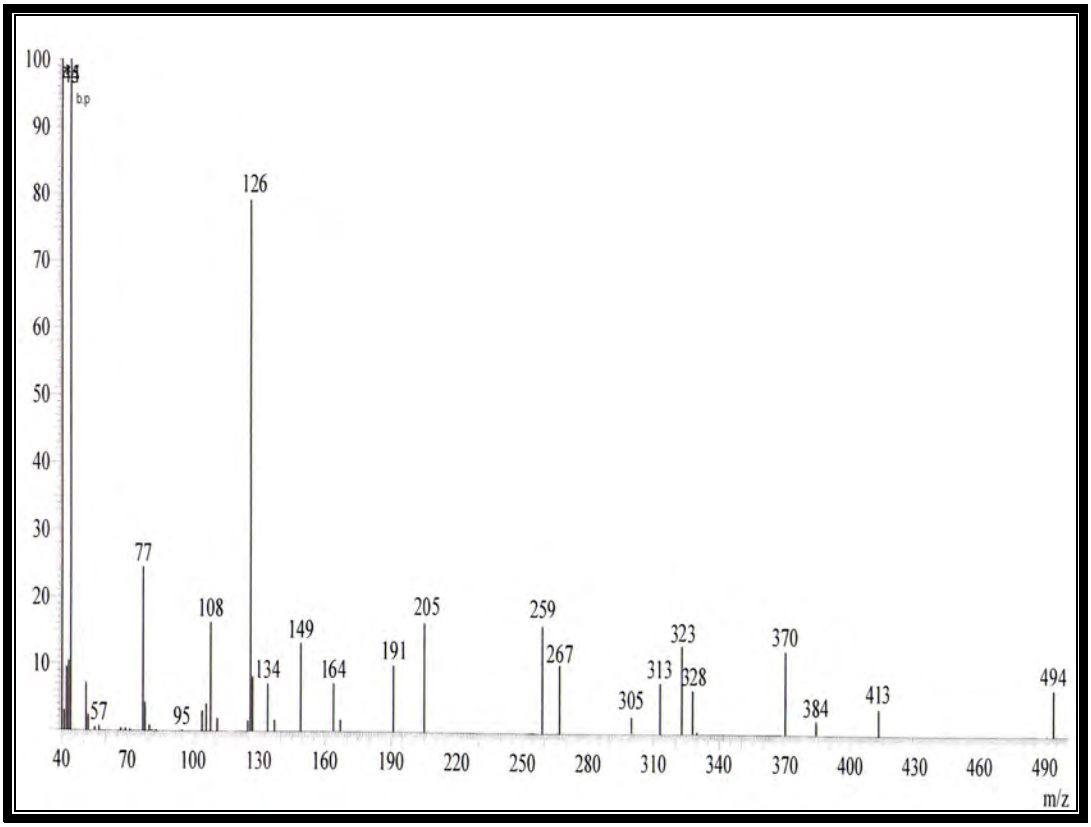

## *Expanded $^1$H NMR spectrum of RHK-214*



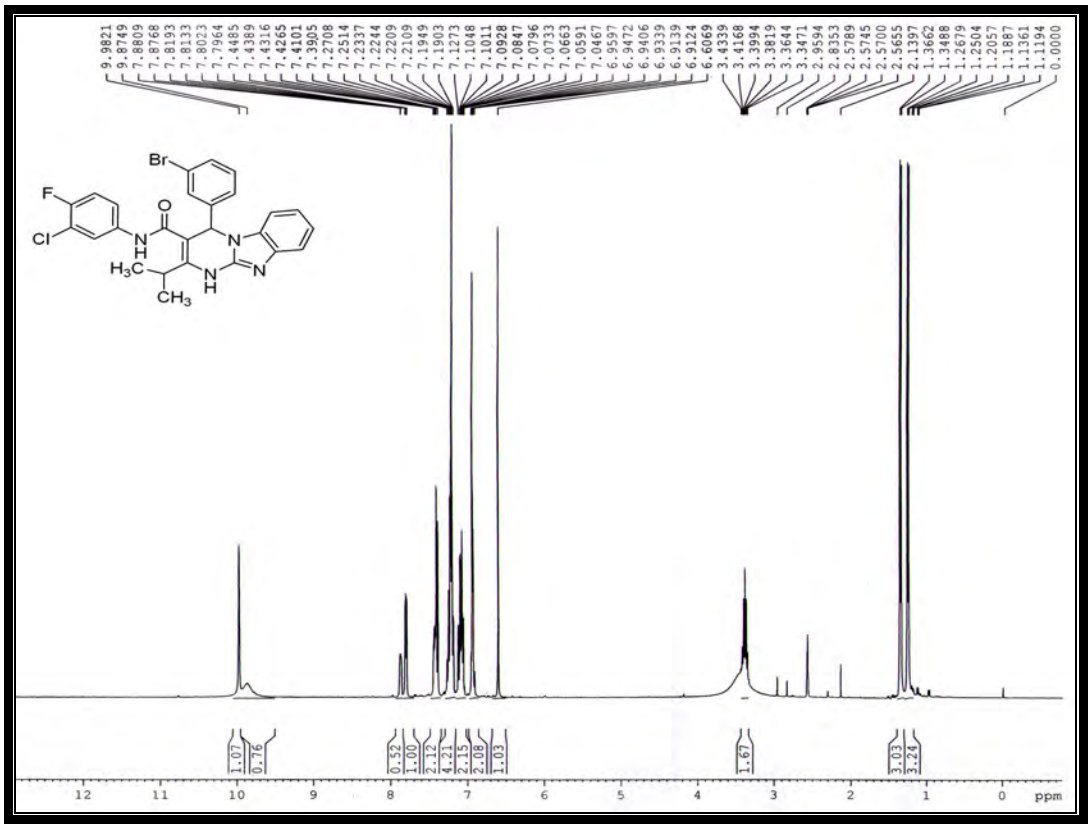
## *IR spectrum of RHK-215*



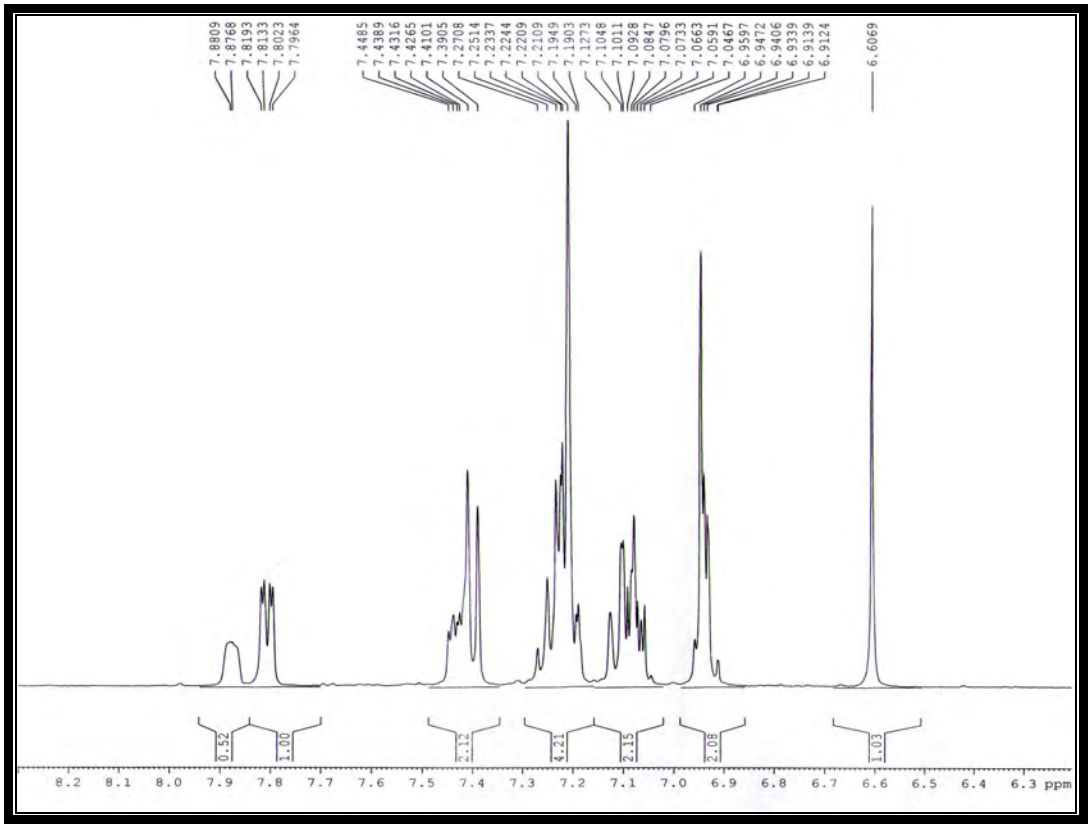

## *Mass spectrum of RHK-215*



## *$^1$H NMR spectrum of RHK-215*




## *Expanded $^1$H NMR spectrum of RHK-215*



## 5.8 Biological evaluation

### 5.8.1 Antimicrobial evaluation

All of the synthesized compounds **(RHK- 191 to 220**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [126-128] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

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

***Minimal Inhibition Concentration [MIC]:-***

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

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 $^0$C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.

4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

## *Methods used for primary and secondary screening: -*

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

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

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

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

**Table-1:-** ***in vitro*** **Antimicrobial Screening Results for RHK-191 to 220**

| Code | Minimal inhibition concentration (μg $mL^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-positive | | Gram-negative | | Fungal species | | |
| | *S.a.* | *S. p.* | *E.c.* | *P.a.* | *C. a.* | *A. n.* | *A.c.* |
| RHK-191 | 200 | 200 | 62.5 | 200 | >1000 | >1000 | >1000 |
| RHK-192 | 250 | 250 | 50 | 200 | >1000 | >1000 | >1000 |
| RHK-193 | 500 | 500 | 250 | 250 | >1000 | >1000 | >1000 |
| RHK-194 | 100 | 100 | 250 | 250 | >1000 | 500 | 500 |
| RHK-195 | 200 | 250 | 100 | 100 | >1000 | >1000 | >1000 |
| RHK-196 | 500 | 500 | 100 | 200 | 250 | 1000 | 1000 |
| RHK-197 | 1000 | 250 | 1000 | 500 | 250 | 1000 | 1000 |
| RHK-198 | 150 | 250 | 500 | 250 | 500 | >1000 | 1000 |
| RHK-199 | 100 | 200 | 100 | 250 | 500 | 500 | 1000 |
| RHK-200 | 150 | 500 | 250 | 250 | 500 | >1000 | >1000 |
| RHK-201 | 500 | 500 | 250 | 250 | 500 | 500 | 500 |
| RHK-202 | 500 | 500 | 250 | 250 | 500 | 500 | 500 |
| RHK-203 | 1000 | 1000 | 500 | 500 | 500 | 500 | 500 |
| RHK-204 | 250 | 500 | 250 | 500 | 500 | 500 | 500 |
| RHK-205 | 500 | 500 | 500 | 500 | 1000 | 250 | 250 |
| RHK-206 | 150 | 500 | 1000 | 1000 | 250 | 1000 | 1000 |
| RHK-207 | 1000 | 250 | 250 | 500 | 250 | 1000 | 1000 |
| RHK-208 | 1000 | 150 | 1000 | 500 | 250 | 1000 | 1000 |
| RHK-209 | 250 | 250 | 150 | 150 | 1000 | 500 | 1000 |
| RHK-210 | 1000 | 500 | 500 | 250 | 500 | >1000 | 1000 |
| RHK-211 | 200 | 500 | 150 | 200 | 1000 | 250 | 500 |
| RHK-212 | 250 | 500 | 100 | 200 | 1000 | 500 | 500 |
| RHK-213 | 500 | 1000 | 250 | 500 | 1000 | 1000 | >1000 |
| RHK-214 | 500 | 500 | 250 | 200 | 1000 | >1000 | >1000 |
| RHK-215 | 200 | 250 | 200 | 200 | 1000 | >1000 | >1000 |
| RHK-216 | 1000 | 250 | 500 | 150 | 1000 | 500 | 500 |
| RHK-217 | 150 | 500 | 100 | 150 | 500 | >1000 | >1000 |
| RHK-218 | 1000 | 250 | 100 | 500 | >1000 | >1000 | >1000 |
| RHK-219 | 200 | 62.5 | 62.5 | 500 | >1000 | >1000 | >1000 |
| RHK-220 | 500 | 500 | 1000 | 1000 | 500 | 500 | 1000 |
| Gentamycin | 0.25 | 0.5 | 0.05 | 1 | - | - | - |
| Ampicillin | 250 | 100 | 100 | 100 | - | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| Iprofloxacin | 50 | 50 | 25 | 25 | - | - | - |
| Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| Nystatin | - | - | - | - | 100 | 100 | 100 |
| Greseofulvin | - | - | - | - | 500 | 100 | 100 |

## 5.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (**RHK- 191 to RHK- 220**) is currently under investigation and results are awaited.

## 5.8 References and notes

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## Summary

The work presented in the Thesis entitled "Studies on Some Heterocycles of Medicinal Interest" can be summarized as below.

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

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

In Chapter 3, synthesis of six novel 1,2,3,4-tetrahydropyrimidines is reported, which occupy a special position among fused pyrimidines due to a very wide spectrum of their biological activities.

In **section-I** 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 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(substituted phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide are synthesized. The synthesis of was achieved by acid catalysed cyclocondensation of N-(substituted)-3-oxobutanamide, substituted urea and 4-(2,4-dinitrophenoxy)benzaldehydes.

In **section-II** 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 4-(4-(2,4-dinitrophenoxy)phenyl)-N-(4-substitutedphenyl)-1,2,3,4-tetrahydro -6-isopropyl-2-oxopyrimidine-5-carboxamide are synthesized. The synthesis of was achieved by acid catalysed cyclocondensation of N-(substituted phenyl)-4-methyl-3-oxopentanamide, substituted urea and 4-(2,4-dinitrophenoxy)benzaldehydes

In chapter- 4 mixture of the 3-amino-1,2,4-triazole, *N*-(substituted phenyl)-4-methyl-3-oxopentanamide and an appropriate aromatic aldehydes was refluxed in DMF (4 ml) for 20 min. After cooling, methanol was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products.

In chapter- 5 recognizing these facts, we have synthesised four new series of 1,4-dihydropyrimido[1,2-*a*]benzimidazoles containing an acetoacetamide fragment. The structures of all the newly synthesized compounds were elucidated by FT-IR,

mass spectra, $^1$H NMR and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

A mixture of the 2-amino benzimidazole *N*-(substituted phenyl)-4-methyl-3-oxopentanamide and appropriate aromatic aldehydes was refluxed in DMF (4 ml) for 30 min. After cooling, methanol was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid 1,4-dihydro pyrimido[1,2-*a*]benzimidazoles products which were recrystallized from ethanol.

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

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

All the newly synthesized compounds are also under antimycobacterial, anticancer and antiviral evaluation and their results are awaited.

## Publication

1. Synthesis, characterization and biological screening of some novel tetrahydroquinazoline derivatives, by S. J. Vaghasia, D. K. Dodiya, A. R. Trivedi, H. K. Ram and V. H. Shah. *Indian Journal of Chemistry (Section B), Accepted, In Press.*

## Conferences/Seminars participated

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