STUDIES ON THE SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SOME NOVEL PIPERAZINE AND TRIAZOLE DERIVATIVES

A thesis
submitted
In fulfillment of the requirement for the award of the Degree
of Doctor of Philosophy (Engineering)

Jadavpur University
2017

By

DEBKIRON MUKHERJEE

Department of Chemical Engineering
Jadavpur University
Kolkata 700032

Declaration

I hereby declare that the work presented in this thesis entitled" Studies on the synthesis, characterization and biological activities of some novel piperazine and triazole derivatives " has been carried out by me and neither the thesis nor any part has been submitted for any other degree whatsoever.

Date:

Place:

(Signature of the candidate)

Certificate from the supervisors

This is to certify that the thesis entitled " Studies on the synthesis, characterization and biological activities of some novel piperazine and triazole derivatives " submitted by Sri Debkiron Mukherjee, who got his name registered on 01/11/2010 for the award of Ph.D. (Engg.) degree of Jadavpur University is absolutely based upon his own work under the supervision of Prof. Alakananda Mukhopadhyay; Chemical Engg. Department, Jadavpur University and Dr. K Shridhara; ShCub Laboratories, Bangalore and that neither his thesis nor any part of the thesis has been submitted for any degree/ diploma or any other academic award anywhere before.

1._____

Signature of the Supervisor and

date with Office Seal

2._____

Signature of the Supervisor and

date with Office Seal

(if more than one supervisor)

ACHARYA & BM REDDY COLLEGE OF PHARMACY
(Affiliated to RGUHS, Recognised by Govt. of Karnataka, Approved by AICTE & Pharmacy Council of India)

ACHARYA GROUP
b.premnath reddy Initiative

Prof. Goli Divakar
M.Pharma, Ph.D., PGDCA
Principal

Acharya & B. M. Reddy College of Pharmacy
Soldevanahalli, Bangalore - 560107

Institutional Animal Ethics Committee

Date: 11/03/2014

Certificate

This is to certify that the project entitled "Evaluation of Anticonvulsant activity" submitted by **Skanda Life Sciences Pvt Ltd, Bangalore** has been approved by IAEC.

Principal Investigator: **Dr. Manjunath. PM**

Co-Investigator: **Dr. Yogisha.S**

Divaks

IAEC-Chairman

Acharya Dr. Sarvepalli Radhakrishnan Road, Soldevanahalli, Hesaraghatta Main Road, Bangalore 560 090, Karnataka India • www.acharya.ac.in/abmrcp
• Ph: +91-80-283 960 11 Extn.: 2301 • Fax: +91-80-283 935 41 • E-mail: principalabmrcp@acharya.ac.in, abmrcp@acharya.ac.in

Acknowledgement

Moments come in life when words become insufficient to express the deepest feelings of the heart. I have the opportunity to express the deep sense of gratitude to all, whose heartfelt inspiration supported me to execute my research work.

I feel exceedingly delightful to express my immeasurable regards and deepest sense of gratitude to honourable Supervisor Prof. Alakananda Mukhopadhyay, Department of Chemical Engineering, Jadavpur University, Kolkata; for providing in estimate inspiration, constructive criticism, valuable and timely suggestions throughout the course of investigation and also for extending his tireless efforts in spite of his busy schedule in finalising the thesis manuscript.

At the same time, I am grateful to Dr. K. Sridhara Bhat (Alkem Laboratories Limited), my honourable co- Supervisor; for his wholehearted support and for providing all necessary facilities of the Department during the course of my investigation.

I avail myself of this unique opportunity to express my earnest gratitude to Dr. K. S. Rao (Alkem Laboratories Limited), for his unstinted help and valuable suggestions from time to time.

My deep appreciation and gratitude are going to Dr. A. M. Sridhara, Dr. Shashiprabha, Dr. Srinivas; Alkem Laboratories Limited, for their suggestions and advice during the period of my research work.

My heartiest thanks to Srikanth Sir, Padma Madam, Sukumar, Thimmaraju, Prakash, Arun and Shivaji for their care and timely help to complete my thesis work.

I do express my sincere thanks to Alkem Laboratories Limited, Bangalore; for providing all sorts of facilities to conduct the research work.

Special thanks are extended to Prof. Asim Chatterjee and my aunt Suparna Ghosal for their help during the course of my research work..

I am highly satisfied with Bishnupriya Printers, Kalyani for computerized printing of the thesis manuscript.

Finally my gratitude to my respected parents, my beloved wife, my dear sister and parent in laws with all other members of my family for their blessings and inspirations that have helped me in achieving the goal in my life till date.

And last but not the least my whole hearted regards to late Sri Arun Roy Chowdhury . Without his advice and help this endeavour might not have been possible.

Though many are not mentioned but they are remembered and thanked forever.

Date:

Jadavpur, Kolkata **(Debkiron Mukherjee)**

Abstract

A large number of nitrogen containing heterocyclic building blocks have several applications in pharmaceutical research and drug discovery. Among nitrogen containing heterocyclic compounds, piperazines and triazoles have been extensively studied because of their varied physiological properties and extensive therapeutic applications.

The present work has two main parts. The first part is synthesis, characterization and evaluation of molecules containing either piperazine or triazole or both rings as anticonvulsant agents. This part consists of synthesis, characterization and anticonvulsant activity of 4-chloro-2-(4-substituted-piperazin-1-yl) quinazoline, 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted- piperazin-1-yl) methanones, [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted piperazin-1-yl) methanones, substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles and substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles. The second part consists of synthesis, characterization and evaluation of molecules containing piperazine ring as antibacterial agents. This part comprises of synthesis, characterization and antibacterial activity of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H- [1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid.

The synthesized compounds were characterized by using methods like thin layer chromatography, melting points, infra-red spectroscopy, ^{1}HNMR spectroscopy and mass spectroscopy techniques.

The synthesized compounds were tested for either anticonvulsant activity or antibacterial activity based on their design and structure. The biological activities were tested using standard screening methods viz. subcutaneous Pentylenetetrazole induced threshold seizure method for determining anticonvulsant activity and evaluation of antibacterial activity using Disk diffusion method. It can be inferred that 4-chloro-2-(4- substituted- piperazin-1-yl) quinazolines with aryl and glycol ether substitution act as anticonvulsant agent. In the case of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole derivatives with aryl, benzothiazole, methoxy ethanol and ethanol substituted piperazines, aryl and alkyl substitutions in the case of oxadiazoles offered protection against subcutaneous Pentylenetetrazole induced seizure. Hence showing anticonvulsant activity. N-substituted 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid derivatives with piperazine ring substituted with quinoline ring and with the halogen, cyano substituted phenyl ring has increased inhibitory activity on Gram +ve bacteria.

List of Patent And Publications

1. **List of patent:**

- Shashiprabha, Kanakamajalu S., Mukherjee Debkiron, Padmashree B., Ksundarraja R., Kuppuswamy N. Process for the preparation of Ziprasidone. U.S.Pat.No. 8,410,268B2. 2013.

2. **List of publications:**

- Mukherjee Debkiron, Mukhopadhyay A., Bhat K.S., Shridhara A.M., Rao K.S. Synthesis, Characterization and Anticonvulsant Activity of substituted 4-chloro-2-(4-piperazin-1-yl) quinazolines. *International Journal of Pharmacy & Pharmaceutical Sciences*. 6(5): 567- 571, 2014.

- Mukherjee Debkiron, Mukhopadhyay A., Bhat K.S., Shridhara A.M. Synthesis , characterization and antibacterial activity of some N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid. *International Journal of Pharmacy & Pharmaceutical Sciences*. 6(5): 616- 622, 2014.

Content

List of Tables

List of Figures

List of Images

Symbols and Abbreviations Used

%	:	Percentage
/	:	per
eg.	:	Example gratia (=for instance)
et al.	:	*et alia* (and others)
i.e.	:	*id est* (that is)
viz.	:	videlicet (= namely)
kg	:	kilogram
mg	:	Milligram
g	:	Gram
M	:	Molar
L or l	:	Liter
ml	:	milliliter
℃	:	Degree Celsius
pH	:	Negative logarithm of hydrogen ion activity
TLC	:	Thin Layer Chromatography
w/v	:	weight/ volume
R_f	:	Retardation factor
®	:	Trademark
CADD	:	Computer Aided Drug Design
QSAR	:	Quantitative Structural Activity Relationship
NMR	:	Nuclear Magnetic Resolance
hr	:	Hour
RT	:	Room Temperature
DMSO	:	Dimethylsulfoxide
Pd/ C	:	Palladium/ Carbon
DMF	:	Dimethylformamide
TEA	:	Triethylamine
IR	:	Infrared
PI	:	Protective Index
ED_{50}	:	Effective Dose, 50%
LD_{50}	:	Lethal Dose, 50%
TDM	:	Therapeutic Drug Monitoring

CHAPTER I: INTRODUCTION

Foundations of biotechnology, biochemistry and medicine are constructed with organic substances. The important role of the organic substances in life processes, in the form of enzymes, vitamins, proteins, fats and carbohydrates, make existence of life possible on earth.

Organic chemistry is a large branch of chemistry that deals with the structure, properties, and reaction ability of compounds that contain carbon. Carbon is a very special element due to its location in the periodic table. As it is situated in the middle of the second row of the periodic table, carbon is able to form stable organic compounds by sharing its electrons with other elements and formation of persistent covalent bonds.

Heterocyclic chemistry is an important sub branch of organic chemistry.

A large number of nitrogen containing heterocyclic building blocks have several applications in pharmaceutical research and drug discovery. Most of the organic compounds containing nitrogen show better biological activity than non-nitrogen compounds.

Nitrogen containing heterocyclic compounds are the key building blocks used to develop compounds of biological and medicinal interest. The synthesis of heterocyclic compounds has always drawn the attention of researchers over the years mainly because of their important biological properties.

The search for newer drugs and modification in the existing drug molecules for better therapeutic action is a continuous and endless effort. The molecular manipulation of promising leading compounds is still a major line of approach for the discovery of new drugs. Subtle modifications in the structure of the compounds result in gradual changes of physicochemical properties of drugs and thus the biological activity of the compounds.

Among nitrogen containing heterocyclic compounds, piperazines and triazoles have been extensively studied because of their varied physiological properties and extensive therapeutic applications.

This prompted us to undertake the synthesis of piperazines and triazoles and hence study the biological activity of the synthesized compounds.

1.1 SIGNIFICANCE OF THE STUDY

The title of this thesis contains the terms synthesis, characterization, biological activity, piperizine and triazole derivatives, which are used in many broad scientific fields.

Synthesis and characterization can be referred to as general terms whereas biological activity, piperizine and triazole derivatives need an explanation in context of the research work.

1.2 BIOLOGICAL ACTIVITY

In pharmacology, biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living being. Where a drug is a complex chemical mixture, this activity is exerted mainly by the substance's active ingredient or pharmacophore but can be modified by the other constituents also. Among the various properties of chemical compounds, pharmacological/ biological activity plays a crucial role since it suggests uses of the compounds in the medical applications.

The molecular structure of an organic compound determines its chemical, physical and biological properties. Therefore, an indirect approach is used which consists of two main parts: (a) representing each compound’s molecular structure with calculated structural descriptors, and (b) choosing subsets of the descriptors and building good models that predict the property or activity of interest.

The practice of medicinal chemistry is devoted to the discovery of new agents for treating disease. An important aspect of medicinal chemistry is to establish a relationship between chemical structure and pharmacological activity. There are many functional groups, also called as pharmacophores which are responsible for pharmacological activity.

In this dissertation the focus is on the **anticonvulsant** and **antimicrobial activity**.

1.3 ANTICONVULSANT ACTIVITY

Anticonvulsant activity is also known as antiepileptic activity.

Epilepsy

Epilepsy is characterised by the presence of recurrent seizures. A seizure can be defined as "an episodic disturbance of movement, feeling, or consciousness caused by sudden synchronous, inappropriate, and excessive electrical discharges in the cerebral cortex" [1].

Epileptic convulsions are expected to have negative consequences on the patient's psychological and social life such as relationships, education and employment. Uncontrolled seizures are associated with physical and psychosocial morbidity, dependent behaviour, poor quality of life and an increased risk of sudden unexpected death.

History of Epilepsy

Epilepsy is thought to be one of the oldest recorded diseases that appeared in humans as it was reported in the earliest medical documents.

The earliest record of epilepsy was found in ancient Indian medicine (4500-1500 B.C.). In Ayurvedic literature of Charaka Samhita (literature of traditional medicine in India), epilepsy was described as "Apasmara" which means "loss of consciousness".

Probably, the Greek physician Hippocrates, the Father of Medicine in 400 B.C., was the first to discuss epilepsy using scientific explanations as he connected this disease to the brain.

Epidemiology of epilepsy

This devastating neurological disease affects more than 50 million people in the world. The cost of treatment and loss of productivity to the society are also staggering. Epilepsy usually begins in childhood and is a lifelong disease. Therefore treatment of epilepsy is very important. Anticonvulsant drug treatment and rarely brain surgery are the main therapeutic options [2, 3].

Age specific incidence of epilepsy is characterised by a "U-shaped curve" in which the incidence is high in childhood and the elderly after the age of 55.

With regard to gender, there is a broad agreement worldwide that females have a lower incidence rate of epilepsy compared to males.

Among developing countries that have a higher incidence of epilepsy compared to developed ones, Latin America and several African countries show a particularly high incidence of epilepsy, possibly due to certain parasitic infections with brain involvement, prenatal brain damage or hereditary factors [4].

Treatment of epilepsy: History of Anticonvulsant drug development

Since the exact mechanisms underlying this brain pathology is not clear yet, the drug treatment of epilepsy remains mainly symptomatic [5, 6]. An ideal anticonvulsant drug should control the seizures without causing any side and toxic effects to improve the quality of life for patients. Although there are many anticonvulsant drugs in the market, none of them completely meet these expectations [7]. Uncontrolled and breakthrough seizures, dose-related severe side effects, long-term toxicity and drug-drug interactions are the main problems associated with anticonvulsant drugs therapy [8].

Seeking a treatment for epilepsy began as early as the discovery of the disease itself.

Primitive procedures, materials and herbs have been employed since ancient times. In 1857, Sir Charles Lococock advocated the use of potassium bromide for the treatment of epilepsy citing a German report and making bromide the first drug to be used against this disease [9]. But unfortunately, the side effects associated with bromide have limited its efficacy.

The modern pharmacotherapy of epilepsy was started in 1912 when the anticonvulsant properties of phenobarbital were discovered accidentally by Hauptmann (1881-1948) when studying the anxiolytic effects of various drugs used to sedate a ward of noisy psychiatric patients and those with epilepsy during the night [9]. Therefore, phenobarbital is considered as the oldest among all the antiepileptic drugs available today [10]. It was initially synthesised in 1904 by a German chemist Fischer and was known to possess sedative and hypnotic properties but it was only in 1912 that its anticonvulsant effects were discovered.

In 1908, phenytoin (sodium diphenyl hydantoinate) was synthesised and in 1938, it was applied in clinical practice following the studies of Merritt and Putnam who

showed favourable anticonvulsant efficacy of this agent against various seizure types without the sedative effect associated with phenobarbital [11].

Carbamazepine was synthesised in 1953 by Schindler at the Geigy laboratories in Switzerland [12]. Initially in 1962, it was marketed to treat trigeminal neuralgia, and then in 1963, it was applied clinically to treat epilepsy in the United Kingdom (UK).

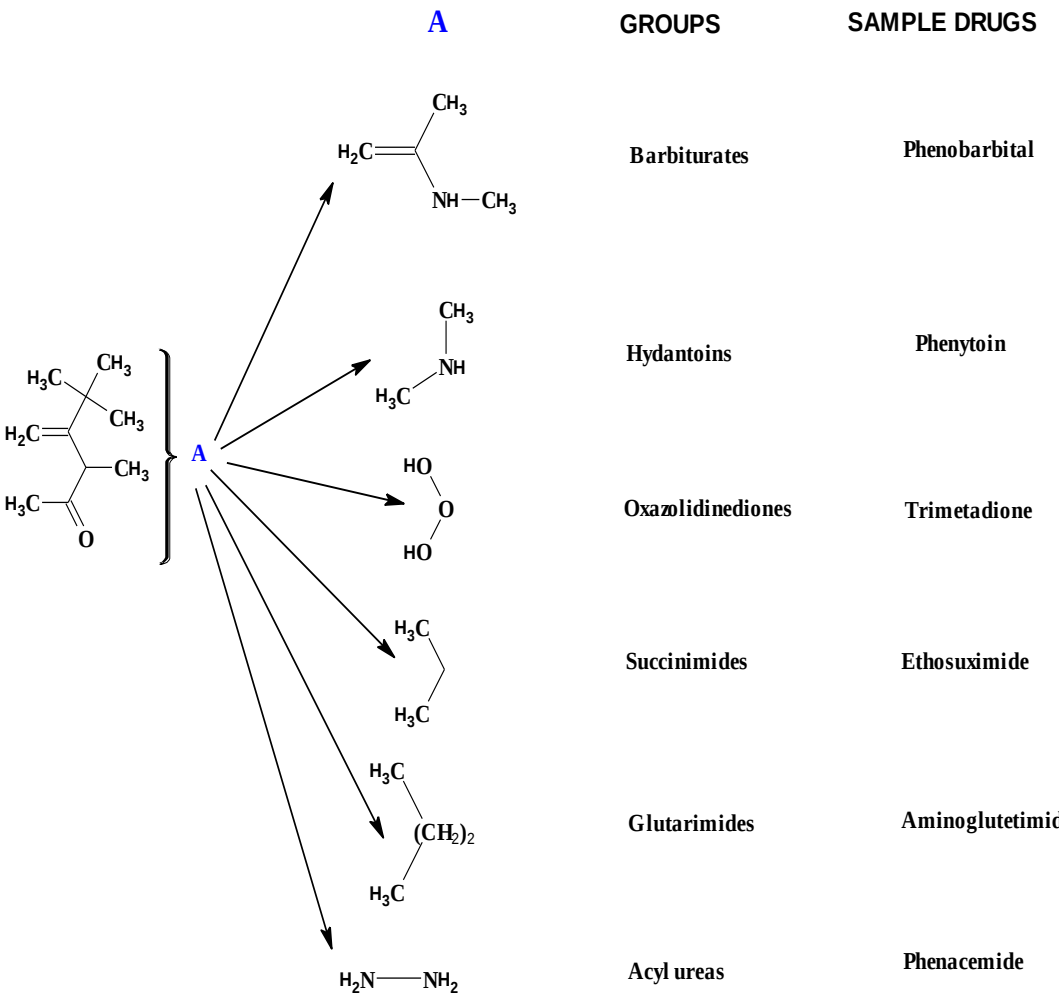
Sodium valproate was first synthesised in 1882 by Burton and for many decades was used as a solvent for organic compounds in research laboratories [13]. Its Anticonvulsant properties were discovered accidentally in 1963 by Pierre Eymard [14].

Classification

Anticonvulsants can be classified on a chronological basis e.g. date of discovery or date of approval for clinical practice. Also, as there are several mechanisms of action by which anticonvulsants exert their anticonvulsant activity, other classifications may be made based on the primary mechanism.

They can also be classified under their chemical structure.

Classification of anticonvulsant drugs according to chemical structure



Classification of anticonvulsant drugs on a chronological basis e.g. date of discovery or date of approval for clinical practice

First Generation Anticonvulsant Drugs (1960-1989)

Before 1993, the management of epilepsy was limited to six major anticonvulsant drugs. These were referred to as the older or traditional anticonvulsant drugs and consisted of Phenobarbital, Primidone, Phenytoin, Valproate, Carbamazepine and Ethosuximide. While all of the older anticonvulsant drugs were efficacious, their long-term safety was questionable. Development of newer antiepileptic medications with few serious adverse effects, minimal drug interactions and broader spectrums of activity was needed [15].

Second Generation Anticonvulsant Drugs (1990-2005)

The current second generation antiepileptic drugs include Felbamate, Gabapentin, Lamotrigine, Topiramate, Tiagabine, Zonisamide, Levetiracetam, Oxcarbazepine and Pregabalin. For this review five of the newer anticonvulsant drugs (Lamotrigine, Oxcarbazepine, Topiramate, Pregabalin, and Tiagabine) were selected based on their spectrum of use and potential place in epilepsy treatment [15].

Third Generation Anticonvulsant Drugs (2000-2010)

The third-generation anticonvulsant drugs consist of 20 novel drugs including Brivaracetam, Carabersat, Carisbamate, DP-valproic acid, Eslicarbazepine acetate, Fluorofelbamate, Fosphenytoin, Ganaxolon, Lacosamide, Losigamone, Pregabaline, Remacemide Hydrochloride, Retigabine, Rufinamide, Safinamide, Seletracetam, Soretolide, Stiripentol, Talampanel and Valrocemide. These anticonvulsant drugs have multiple diverse molecular mechanisms of action and thus may offer a novel and advantageous approach to the treatment of epilepsy, especially in patients with refractory seizures. Moreover, third-generation anticonvulsant drugs may offer better tolerability, milder adverse effects, less drug or hormonal interactions and improved pharmacokinetic characteristics compared to the first and second generation anticonvulsant drugs [15].

Animal seizure models

Two tests are commonly applied to evaluate the anticonvulsant activity of new anticonvulsant agents, namely, the Maximal Electroshock test and the subcutaneous Pentylenetetrazole test. The test is a model of seizure spread; capable of identifying drugs with activity against partial and generalised tonic clonic seizures. In contrast, the test is a model of seizure threshold that can predict agents effective against generalised absence and myoclonic seizures.

In this research work, the subcutaneous Pentylenetetrazole technique have been used to ascertain the activity of the synthesized molecules.

Generalised mechanism of action: Anticonvulsant drugs

Although the actions of each anticonvulsant drug have unique characteristics and some drugs may act by multiple mechanisms, the anticonvulsant actions of these drugs can be grouped into four broad categories:

- Modulation of voltage-dependent sodium, calcium or potassium channels.
- Increase in GABA (γ-Amino butyric acid) inhibition via actions on GABA (γ-Amino butyric acid) A receptors or on GABA (γ-Amino butyric acid) synthesis, reuptake or degradation.
- Decreased synaptic excitation via actions on ionotropic glutamate receptors.
- Modulation of neurotransmitter release via presynaptic mechanisms.

For some anticonvulsant drugs, the mechanism remains partially unknown.

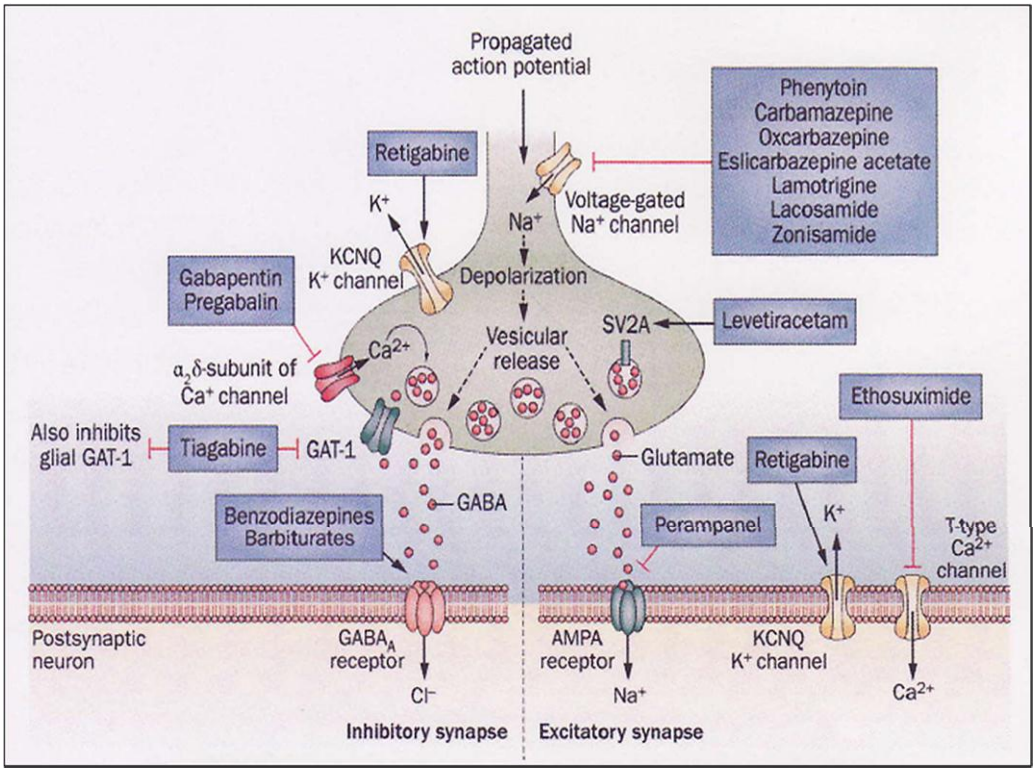


Figure 1: The mechanism of action of anticonvulsant drugs like modulation of voltage-gated ion channels (eg. Phenytoin), increase in GABAergic inhibition (eg. Benzodiazepines), decrease in glutamatergic excitation (eg. Perampanel), multiple mechanisms (eg. Valproate), modulation of neurotransmitter release via presynaptic mechanisms (eg. Gapapentin)

1.4. ANTIMICROBIAL AGENTS

Following their 20th century triumph in human medicine, antimicrobials have also been used increasingly for the treatment of bacterial disease in animals, fish and plants. In addition, they became an important element of intense animal husbandry because of their observed growth enhancing effect, when added in sub-therapeutic doses to animal feed. Antimicrobials are also used in industry, eg. to eliminate bacterial growth on the inside of oil pipelines. It is estimated that about half of the total amount of antimicrobials produced globally is used in food animals. In Europe, all classes of antimicrobials licensed for disease therapy in humans are also registered for use in animals, a situation comparable with other regions in the world where comprehensive registration data are much more difficult to obtain.

Antimicrobial drug resistance

After more than 50 years of widespread use, evolution of disease causing microbes has resulted in many antimicrobials losing their effectiveness. Microbes, such as bacteria, viruses, fungi and parasites, are living organisms that evolve over time. Their primary function is to reproduce, thrive and spread, quickly and efficiently. Therefore, microbes adapt to their environment and change in ways that ensure their survival. If something stops them from growing and spreading such as an antimicrobial, they evolve new mechanisms to resist the antimicrobials by changing their genetic structure. Changing the genetic structure ensures that the offspring of the resistant microbes are also resistant [16].

Concomitant with the upward trend in antimicrobial drug resistance a considerable downward trend in the intent and determination of pharmaceutical companies to develop new antimicrobials has become apparent.

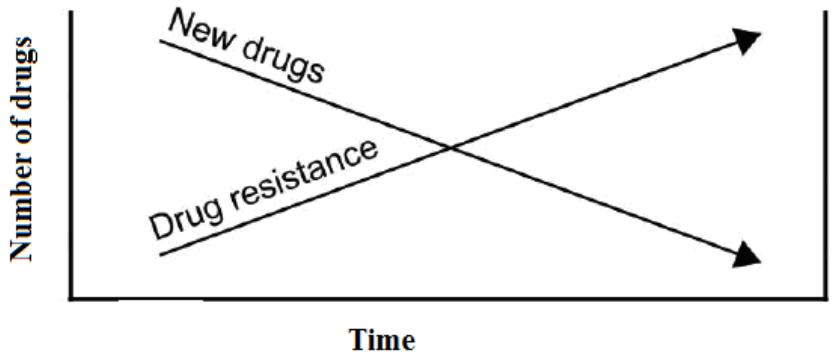


Figure 2: Graphical representation of the downward trend of new antibacterial drug discovery with respect to increasing drug resistance. It can be inferred that increase in drug resistance has resulted in the decline of new drug discovery.

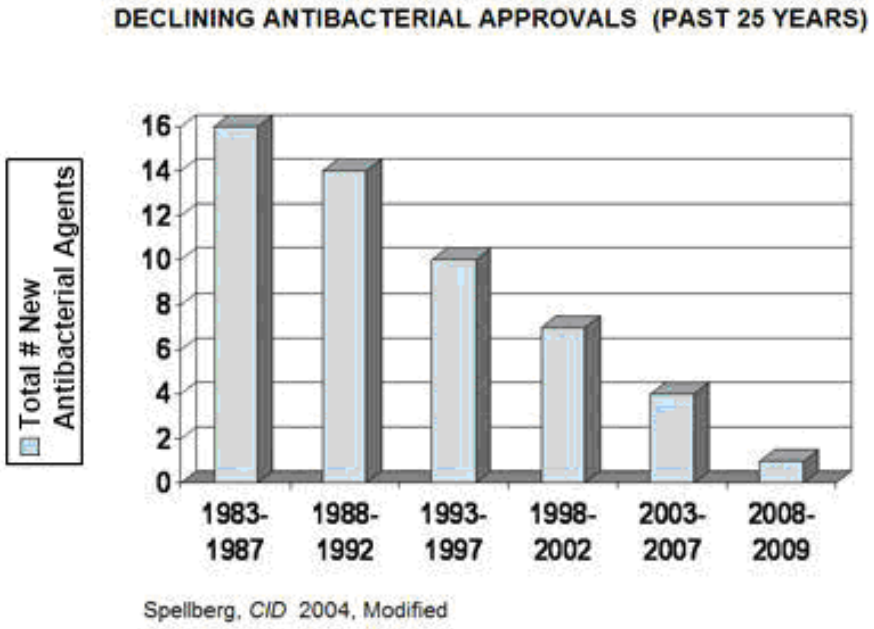


Figure 3: Graphical representation of declining antibacterial approvals. A steady decline is noticed in the field of antibacterial approvals in the last two decades.

As a consequence of this downward trend, only a limited number of new antibacterial drugs have been introduced into the market in the last three decades.

1.5 PIPERAZINES AND TRIAZOLES

Heterocycles are organic cyclic compounds [17] with an element other than carbon as a part of the ring structure.

Heteroatom can play an extremely important role in determining the properties of these compounds. Chemical difference between heterocyclic and their all carbon analogues may usually be traced one or both the following facts.

- Electro negativity difference between the heteroatom and the carbon atom results in the presence of polarized bonds in molecule.
- The heteroatom possesses nonbonding electron pair [18].

Heterocycles make up an exceedingly important class of compounds. In fact more than half of all known organic compounds are heterocycles. Many natural drugs such as Quinine, Papaverine, Emetine, Theophylline, Atropine, Procaine, Codeine, Morphine and Reserpine are heterocycles.

Almost all the compounds we know as synthetic drugs such as Diazepam, Chlorpromazine, Isoniazid, Metronidazole, Azidothymidine, Barbiturates, Antipyrine, Captopril and Methotrexate are also heterocycles. Some dyes (eg. Mauveine), luminophores, (eg. Acridine orange), pesticides (eg. Diazinon) and herbicides (eg. Paraquat) are also heterocyclic in nature [19]. All these natural and synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Furthermore, all biological processes are chemical in nature. Nitrogen containing heterocyclic structures has been associated with a wide range of biological activities. Two such important compounds are piperazine and triazoles. The varied therapeutic properties of piperazine and triazole related drugs have encouraged the medicinal chemists to synthesize a large number of new chemotherapeutic agents. In our present work we have synthesized some new chemical entities containing the triazole and piperazine nucleus.

Piperazines

Piperazine is an organic compound that consists of a six member ring containing two nitrogen atoms at opposite positions in the ring. Piperazine exists as small

alkaline deliquescent crystals with a saline taste. The piperazines are a broad class with many important pharmacological properties.

Piperazine and substituted piperazine nuclei had constituted an attractive pharmacological scaffold present in various potent marketed drugs. The incorporation of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, proper alkalinity, water solubility, the capacity for the formation of hydrogen bonds and adjustment of molecular physicochemical. This di-nitrogen moiety has been an inseparable component of plethora of drugs.

A few important marketed drug and their associated biological activities are given in Table 1. These drugs have various alterations conducted on piperazine ring.

Table 1: Name of some important drugs prepared by alterations on piperazine ring

Serial number	Name of the drug	Uses	Reference
1.	Ranolazine	Antianginals	Hale SL *et al*. [20]
2.	Trimetazidine	Antianginals	Fragasso G *et al*. [21]
3.	Buspirone	Antidepressant	Rivedi MH *et al*. [22]
4.	Nefazodone	Antidepressant	Cusack B *et al*. [23]
5.	Meclozine	Antihistaminic	Dahl E *et al*. [24]
6.	Cinnarizine	Antihistaminic	Nicholson *et al*. [25]
7.	Fluphenazine	Antipsychotic	Davis *et al*. [26]
8.	Perphenazine	Antipsychotic	Rees *et al*. [27]
9.	Antrafenine	Anti Inflammatory-Analgesic	Manoury *et al*. [28]
10.	Imatinib	Anticancer	Gambacorti *et al*. [29]
11.	Ziprasidone	Atypical antipsychotic	Hagop S *et al*. [30]
12.	Ciprofloxacin	Antibiotic	Drusano GL *et al*. [31]
13.	Levofloxacin	Antibiotic	Nelson JM *et al*. [32]
14.	Flunarizine	Anticonvulsant	Fischer W *et al*. [33]
15.	Cinnarizine	Anticonvulsant	L K Desmedt *et al*. [34]

From the above examples we can clearly understand the importance of the piperazine heterocycle in medicinal chemistry.

Triazoles

Several five membered aromatic systems having hetero atoms at different positions such as triazoles have been studied extensively owing to their interesting biological activities. The five member triazole ring exists in two tautomeric forms i.e. 1, 2, 3-triazole and 1, 2, 4-triazole. The triazole derivatives possess wide spectrum of biological activities such as antibacterial, antifungal, antitubercular, anticancer, anticonvulsant, antidepressants, analgesic, anti-inflammatory, antioxidant, antiviral, anthelmintic, antitumor and hypoglycaemic.

Given below is a brief account of few important marketed drug and their associated biological activities. These drugs have various substitutions conducted on triazole ring.

Table 2: Name of some drugs with triazole ring

Serial number	Name of the drug	Uses	Reference
1.	Rufinamide	Anticonvulsant	Hakimian S *et al*. [35]
2.	Fluconazole	Antifungal	"WHO Model List of EssentialMedicines". World Health Organization. October 2013. Retrieved 22 April 2014 (a) [36]
3.	Ribavirin	Antiviral	"WHO Model List of EssentialMedicines". World Health Organization. October 2013. Retrieved 22 April 2014 (b) [37]
4.	Dapiprazole	An alpha blocker	Doughty *et al*. [38]
5.	Nefazodone	Antidepressant	Lexi-Comp (September 2008). "Nefazodone". The Merck Manual Professional. Retrieved on November 29, 2008. [39]
6.	Etoperidone	Antidepressant	Ganellin CR *et al*. [40]

Based on the literature review on piperizine and triazole derivatives it was indicated that, they possessed significant chemical and biological properties. Encouraged by the above facts we designed a new scaffold containing piperizine and triazole pharmacophore compositions in a compact system. We synthesised various substituted piperizine and triazole derivatives and also studied the

potential additive effect of the integrated moiety within a frame work towards pharmacological activities. In view of continuous interest to develop new agents we believed that it is worth to investigate and design different scaffolds for the studies.

1.6 OBJECTIVE AND SCOPE

In the present research work synthetic transformation of analogues of two different classes of heterocycles namely piperazine and triazole was carried out to generate novel compounds. The compounds are to be characterised by using physical and chemical methods. Next to that, the possibilities of the synthesised compounds to acts as anticonvulsant or antimicrobial agents were determined by their respective biological activities.

In search of new compounds, we identified piperizine and triazole based scaffolds which may be important in discovery of new drugs. The biological significance and activity profile of piperizine and triazole pharmacophores prompted us to synthesize some new derivative heterocycles in identifying new lead agents.

The present work has two main parts. The first part is synthesis, characterization and evaluation of molecules containing either piperazine or triazole or both rings as anticonvulsant agents. The second part consists of synthesis, characterization and evaluation of molecules containing piperazine ring as antibacterial agents.

1.7 SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MOLECULES AS ANTICONVULSANT AGENTS

Strategies to develop new anticonvulsant drugs

There is a wide variety of strategies to develop new anticonvulsant drugs. Figure 4 describes a few of them, ranging from molecular modifications to gene therapy [41- 43].

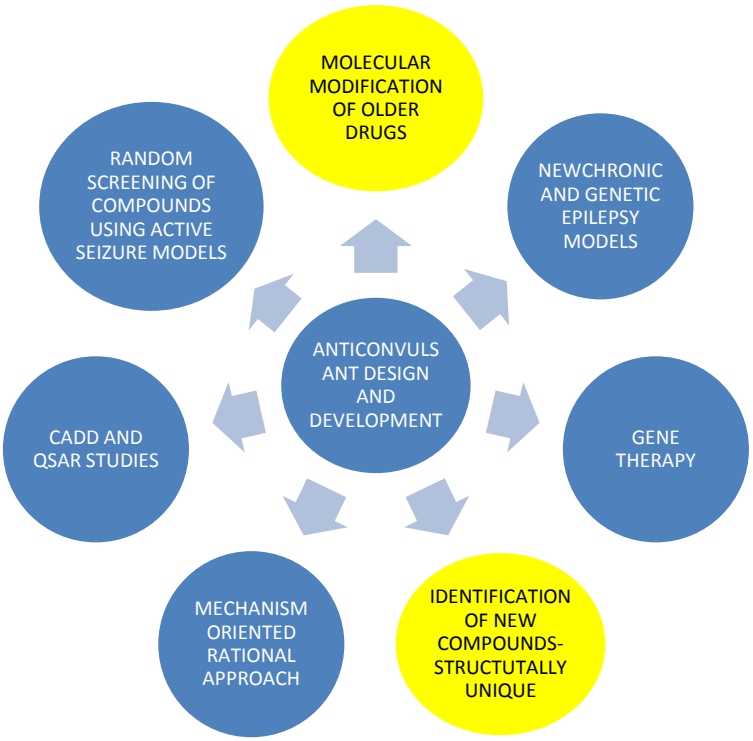


Figure 4: The design and development of anticonvulsant drugs can be broadly divided into the seven categories. Those are molecular modification of older drugs, new chronic and genetic epilepsy models, gene therapy, identification of new compounds-structurally unique, mechanism oriented rational approach, CADD & QSAR studies and random screening of compounds using active seizure models.

In the present work we have focussed on two strategies:

- Strategy I: Identification of new compounds with less structural resemblance to the existing drugs followed by random screening of the synthesized compounds using active seizure models.
- Strategy II: Molecular modification of existing drugs followed by random screening of the synthesized compounds using active seizure models.

Strategy I:

Entirely new compounds bearing little or no resemblance to the existing drugs and therapies in the treatment of convulsion is a popular pathway in drug discovery.

With the intention to develop new compounds and supported by various research works in this field a series of new molecules were synthesized containing quinazoline and piperazine rings.

Quinazoline is heterocyclic compound consisting of two fused six member aromatic rings- benzene & pyrimidine. The development of research on biological activity of quinazoline compounds started when the compound 2-methyl-1, 3-aryl-4-quinazoline derivative was synthesized. In 1968 only two derivatives were used, soporific & anticonvulsant- methaqualone and diuretic quinathazone. By 1980, about 50 kinds of derivatives of this class were included with different biological actions like soporific, sedative, tranquilizing, analgesic, anticonvulsant, antitussive, myorelexant, antirheumatic, hypotensive, antiallergic, bronchodilating, antidiabetic, cholagogue, diuretic, cystatic, antimalarial, spermicidal etc [44]. The anticonvulsant activity was attributed to its ability to bind the non-competitive site of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In a previous report [45], compounds were synthesized and tested for their anticonvulsant activity, which was comparable to that of Diazepam. As a result, these compounds were identified as potential leads for further design of more active compounds. Since the discovery of methaqualone as a sedative hypnotic [46, 47], the search for new anticonvulsant drugs with reduced toxicity and fewer side effects has been continuous. It has been reported that replacement of the methyl group by some other functionalities such as alkylthiomethyl or alkyloxymethyl groups reportedly yielded structural analogues which retained the anticonvulsant activity [48, 49].

To identify new active molecules, research work was carried on 4-chloro-2-(4-substituted-piperazin-1-yl) quinazolines.

All the compounds were screened for their anticonvulsant activity by subcutaneous Pentylenetetrazole induced seizure method.

Strategy I: New compounds with less structural resemblance to the existing drugs

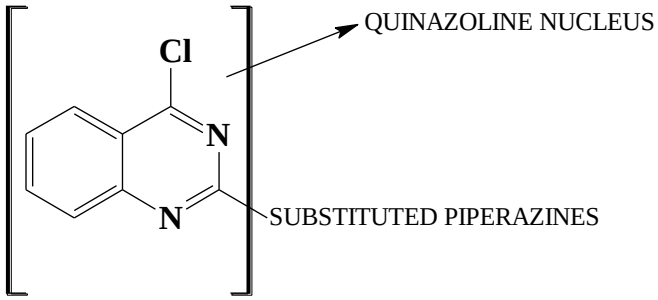


Figure 5: Generalized structure of 4-chloro-2-(4-substituted-piperazin-1-yl) quinazolines.

Strategy II:

Rufinamide was selected as the drug. Structural modifications were to be performed on the 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole moiety of Rufinamide.

Rufinamide, 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole -4 carboxamide, is a new broad spectrum anticonvulsant/ antiepileptic drug that is structurally unique [50]. It is a triazole derivative structurally unrelated to any currently marketed antiepileptic drug.

It offers various advantages:

- The ability to rapidly escalate dosing and obtain a clinical response.
- Few drug interactions.
- A good cognitive and psychiatric adverse event profile.
- Rufinamide is well absorbed after oral administration. The extent of absorption decreases slightly as the dose is increased; however the effect is negligible at most clinical doses [51].
- Rufinamide does not have significant pharmacokinetic interactions with Benzodiazepines, Carbamazepine, Lamotrigine, Phenytoin, Phenobarbital, Valproate, Topiramate, Vigabatrin, Oxcarbazepine or Primidone.
- Based on the clinical trials, Rufinamide appears to be well tolerated. A small number of Rufinamide treated patients (9% against 4% for placebo) discontinued treatment because of adverse effects [52].

The antiepileptic effect of Rufinamide has been assessed in several animal models of generalized and partial seizures. For instance, oral Rufinamide exhibited acute anticonvulsive activity in mice and rat models, suppressing maximal electroshock-induced tonic clonic seizures in both species and Pentylenetetrazol induced clonic seizures in mice [53].

In the present work 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole moiety, of the Rufinamide drug was modified by including various substituted piperazines and substituted oxadiazoles.

The following paragraphs states the rationale behind choosing piperazine and oxadiazoles as the prime substituent in the modification of the 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole moiety.

Oxadiazole is a five membered heterocyclic ring which is a versatile lead compound for designing potent bioactive agents. 1,3,4-Oxadiazoles are a class of the 5-membered heterocyclic compounds that are endowed with a wide range of biological activities [54, 55], as well as polymer and material science applications [56, 57]. They are frequently used as ester or amide substitutes in medicinal chemistry [58]. Vadrin [59] a leprostatic drug and Eudromil [60] a hypnotic drug are a few examples. They have been found to possess antiviral [61], antibacterial [62-64], antimalarial [65, 66], antifungal [67], anticonvulsant [68], anti-inflammatory and analgesic properties [69-71].

Anticonvulsant studies of piperazines derivatives, other triazoles and oxadiazoles are reviewed in the later sections.

New compounds were synthesized and characterized by incorporating modifications on the 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole nucleus.

Listed below are the four groups of compounds synthesized in line with the stated objective:

- ✓ 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted- piperazin-1-yl) methanones
- ✓ [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted piperazin-1-yl) methanones

- ✓ Substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles
- ✓ Substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles

Schematic representation of the concept in strategy II

Substituted Piperazines and Oxadiazoles in 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole nucleus:

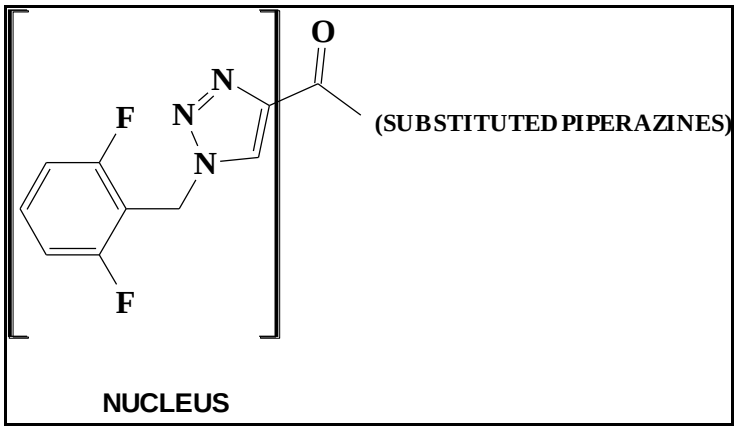


Figure 6: Generalized structure of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted- piperazin-1-yl) methanones.

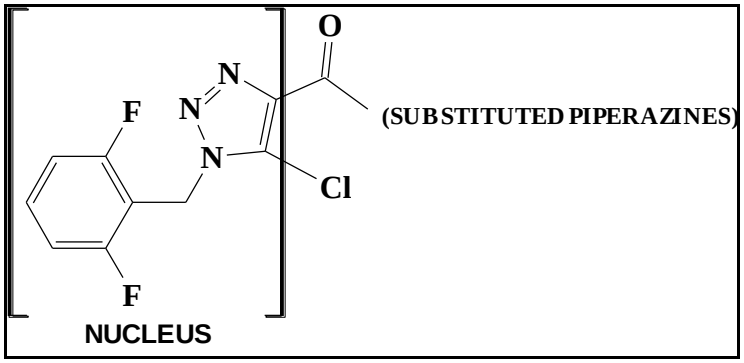


Figure 7: Generalized structure of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted- piperazin-1-yl) methanones.

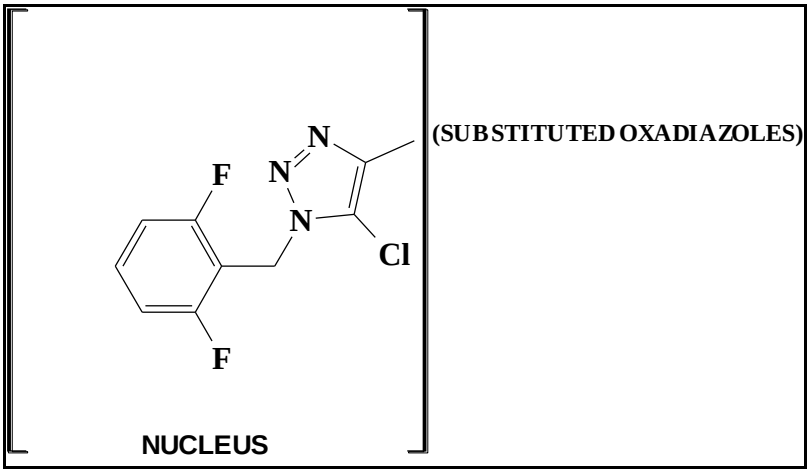


Figure 8: Generalized structure of substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles.

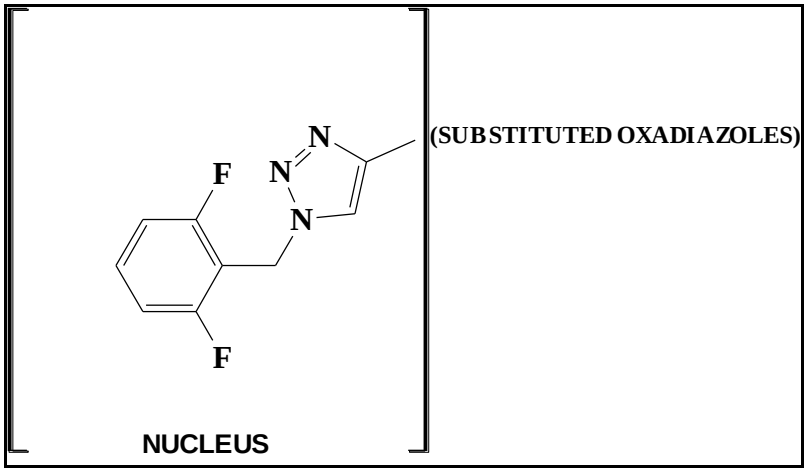


Figure 9: Generalized structure of substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles.

1.8 SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MOLECULES AS ANTIBACTERIAL AGENTS

Quinolones are unusual among antimicrobials in that they were not isolated from living organisms, rather synthesized by chemists. The first quinolone, nalidixic acid, was derived from the antimalarial drug Chloroquine [72]. Subsequent agents were derived through side chain and nuclear manipulation [73]. The development of the fluoroquinolone class may be described in generational terms, with each generation sharing similar features or antimicrobial spectra (Table 3) [72-74].

First-generation agents possess activity against aerobic Gram negative bacteria, but little activity against aerobic Gram positive bacteria or anaerobes. Second-

generation agents are the original fluoroquinolones, named for the addition of a fluorine atom at position C-6. These agents offer improved coverage against Gram negative bacteria and moderately improved Gram positive coverage. Third-generation agents achieve greater potency against Gram positive bacteria, particularly pneumococci, in combination with good activity against anaerobes. Fourth-generation fluoroquinolones have superior coverage against pneumococci and anaerobes.

Regarding antimicrobial activity, fluoroquinolones interfere with bacterial cell replication, transcription and DNA repair by disabling two bacterial enzymes crucial to these processes, DNA gyrase (formerly topoisomerase II) and topoisomerase IV. These enzymes are necessary for bacteria to manage the topological challenge of containing their genetic material. Using *Escherichia coli* as an example, a bacterial cell that is 1 to 3 mm long must accommodate a chromosome that is a double stranded DNA circle longer than 1000 mm. Chromosomal volume is reduced via tertiary folding and compaction. These processes must be reversed in order for bacterial replication to occur; DNA topoisomerases facilitate this [75].

Table 3: Evolution of the fluoroquinolone class of antimicrobials

Generation	Agent	Comment
First generation	Nalidixic acid	Generic form available
	Cinoxacin	Discontinued
Second generation	Norfloxacin	Available as Noroxin
	Ciprofloxacin	Available as Cipro and generic form
	Lomefloxacin	Discontinued
	Ofloxacin	Available as Floxin and generic form
	Levofloxacin	Available as Levaquin and generic form
Third generation	Sparfloxacin	Discontinued
	Gatifloxacin	Discontinued
	Grepafloxacin	Discontinued
Fourth generation	Trovafloxacin	Discontinued
	Moxifloxacin	Available as Avelox
	Gemifloxacin	Available as Factive
	Garenoxacin	Not approved

[Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed April 18, 2011]

The active centre of Fluoroquinolones:

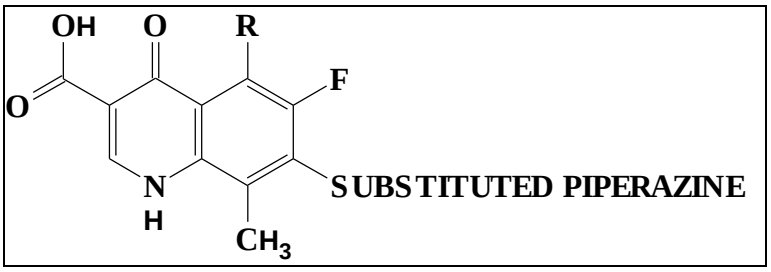


Figure 10: The biologically active centre of fluroquinolones. It is clearly evident that piperazine plays an integral role in the antebacterial activity of fluoroquinolones.

Our present work forays into the field of research which is not studied extensively i.e. substitution of the piperazine nucleus of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H- [1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid.

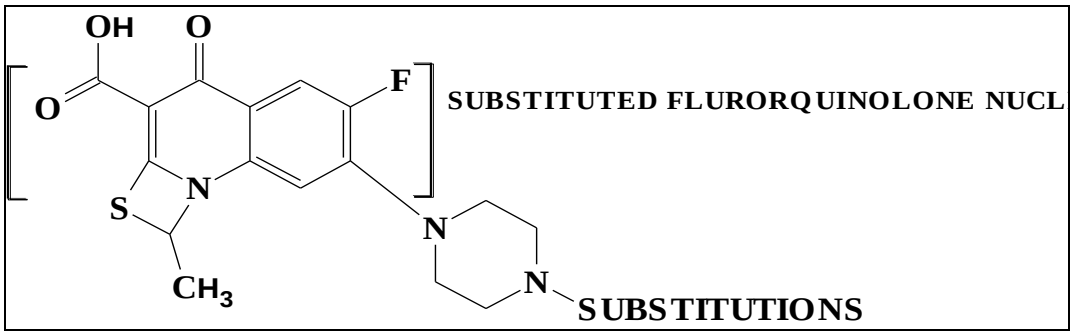


Figure 11: Generalized structure of N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H- [1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid.

From the N-substituted 4-fluoro phenyl ring at fluoroquinolone nucleus in Sarafloxacin [76] and similar 2, 4-difluoro phenyl substitutions in Trovafloxacin [77], Tosufloxacin [78] and Temafloxacin [79], it can be inferred that substituted phenyl ring pertains to enhance the antimicrobial activity of fluoroquinolones. A quinoline substitution at the fluoroquinolone nucleus in case

of Rosoxacin [80] also indicates good antimicrobial activity. Pyridines [81] and pyrazoles have been reported to exhibit substantial antimicrobial activity.

In our work we have tried to explore the effect of the phenyl, biphenyl, quinoline and pyrazole substitutions at the piperizinyl nucleus of the fluoroquinolone ring. The synthesized molecules were characterized and checked for antibacterial activity.

1.9 SUMMARY OF THE WORK PLAN

The present research work was performed based on the mentioned plan of work.

- The research work leading to this thesis initiated with the identification of the heterocycles on which the experimentations would be performed. Piperazines and triazoles were chosen based on the literature reviews and available resources.
- Following the selection of the heterocycles, the need was to decide on basis of the biological activity. Data from consulted research works, relevant importance in the field of medicinal chemistry and the availability of resources led to the selection of anticonvulsant activity and antibacterial activity as the biological activity of interest.
- Decision on the synthetic scheme and the molecules to be prepared was done thorough literature search. A synthetic work plan was designed based on the various strategies available in reviews.
- The next step was to synthesize the molecules. Synthesis was carried out in the laboratory using available chemicals following synthetic methods available in literature. Occasional modifications in the literature methods were made to facilitate the reaction to go for completion which in turn led to increase the purity of the product and yield of the reaction.
- The molecules synthesized were purified using procedures like recrystalization, column chromatography and solvent washing.
- The synthesized molecules were characterized using techniques like melting point, thin layer chromatography and spectroscopy.
- All the synthesized molecules were screened for their biological activity. The test activity varied based on the design and structure of the molecule.

- An inference was derived based on the activity of the molecules and their chemical structure.
- Based on the inference, future scope of research work is proposed.

Figure 12: Pictorial representation of the work plan. The activities are given based on the order they were performed.

1.10 OUTLINE OF RESEARCH WORK

The research work "**Studies on the synthesis, characterization and biological activities of some novel piperazine and triazole derivatives"** is divided into two major sections based on the biological activity which are anticonvulsant activity and antibacterial activity. These sections are divided into subsections based on the design and chemical structures.

Anticonvulsant Activity

1. 4-chloro-2-(4-substituted-piperazin-1-yl) quinazolines
2. 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted piperazin-1-yl) methanones
3. [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted piperazin-1-yl) methanones
4. Substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles
5. Substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles

Antibacterial activity

1. N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

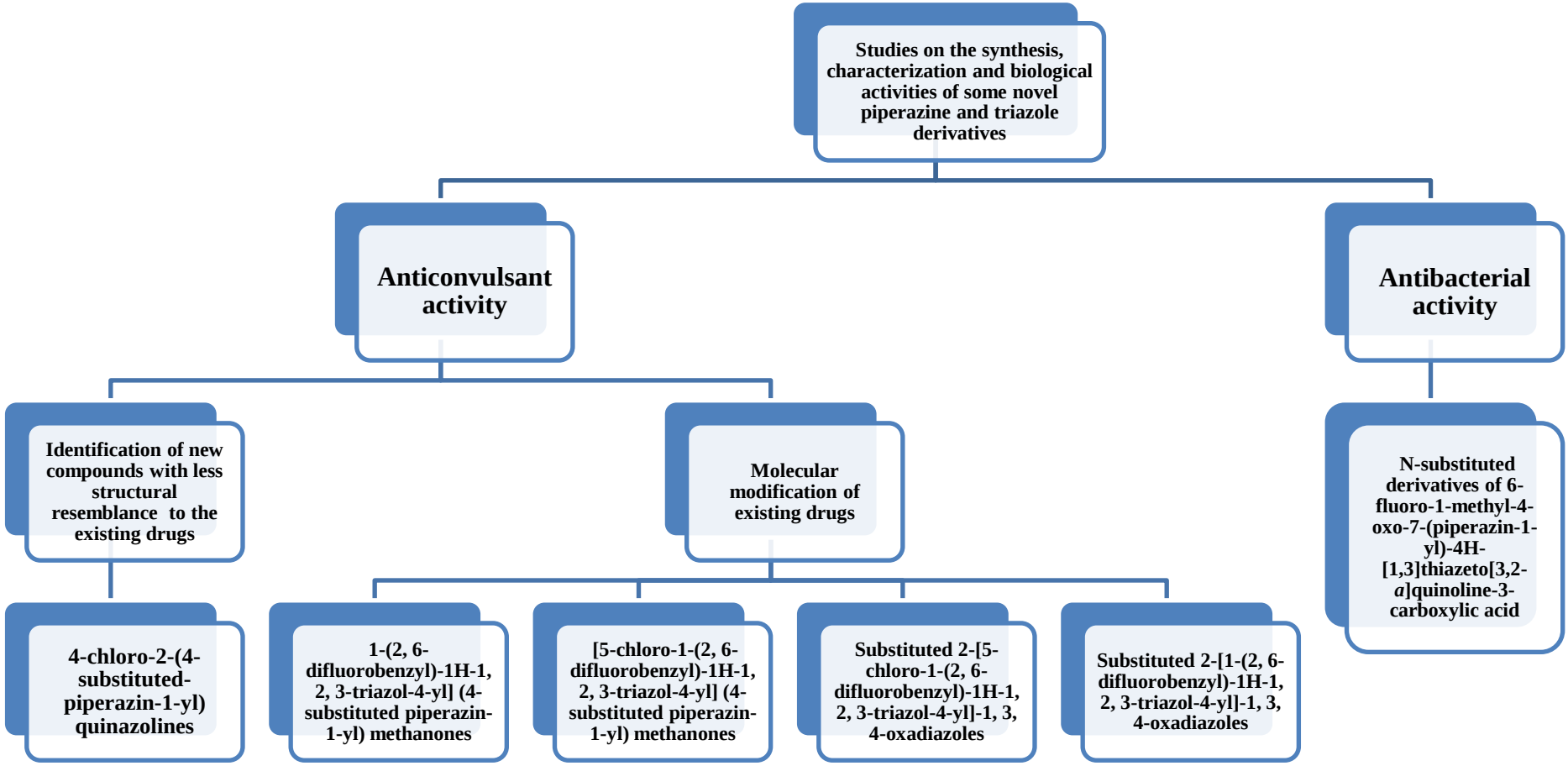


Figure 13: Flowchart representing the outline of research work.

1.11 AN OVERVIEW OF THE THESIS

1. **Chapter one: Introduction**

   The introduction lays the foundation of the research work done. The chapter describes the background on which the research work is performed along with the significance, objective, scope and outline of the research work. The introduction ends with a summary of the work plan and an overview of the contents of the thesis.

2. **Chapter two: Review of literature**

   In the review of literature, a list of articles which provided the rationale behind the research topic at that moment in time is given. A compilation of those reviews forms the core content of the research work, with examples including reviews, the research work from different journals and website citations.

3. **Chapter Three: Materials, methods and results**

   In the methodology a systematic description of the methods, synthetic and biological, applied in this study are mentioned. Typically, it encompasses the synthetic, quantitative and qualitative techniques associated with research work. The results are the outcome of the experimentation performed. This chapter also includes the characterization data.

4. **Chapter Four: Summary and conclusions**

   Summary and conclusion is the documentation of the main findings of the whole research experimentation.

5. **Chapter Five: Scope of future research work**

   This chapter provides a pathway to the research works that can be done in continuation to this study.

1.12 REFERENCES

[1] Brodie M.J., French J.A. Management of epilepsy in adolescents and adults. *Lancet*. 356: 323-329. 2000.

[2] Cavazos J.E. Seizures and Epilepsy, Overview and Classification. *Treatment & Medication*. 2010.

[3] Nadkarni S., La Joie J., Devinsky O. Current treatments of epilepsy. *Neurology*. 64: 2-11, 2005.

[4] Senanayake N., Roman G.C. Epidemiology of Epilepsy in Developing-Countries. *Bulletin of the World Health Organization*. 71: 247-258. 1993.

[5] Bromfield E.B., Cavazos J.E., Sirven J.I. (Eds.) An introduction to epilepsy: Basic mechanisms underlying seizures and epilepsy. *Am. Epilepsy Society*. 2006.

[6] Wahab, A. Difficulties in treatment and management of epilepsy and challenges in new drug development. *Pharmaceuticals*. 3: 2090-2110, 2010.

[7] Eadie M.J. Can anticonvulsant drug therapy "cure" epilepsy? *CNS Drugs*. 15: 679-690, 2001.

[8] Perucca E., French J., Bialer M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. *Lancet Neurol*. 6(9): 793-804, 2007.

[9] Pearce J.M.S. Bromide, the first effective antiepileptic agent. *Journal of Neurology Neurosurgery and Psychiatry*. 72: 412. 2002.

[10] Hauptmann A. Luminal bei epilepsie. *Munchiner Medizin Wochenschrift*. 59: 1907-1909. 1912.

[11] Merritt H.H., Putnam T.J. Landmark Article - Sodium Diphenyl Hydantoinate in the Treatment of Convulsive Disorders (Reprinted). *Jama-Journal of the American Medical Association*. 251: 1062-1067. 1984.

[12] Schmutz M. Carbamazepine. Frey H.H.J.D. (Ed.) Handbook of experimental pharmacology: Antiepileptic drugs. Springer-Verlag, Berlin, pp. 479-506. 1985.

[13] Burton B.S. 1882. On the propyl derivatives and decomposition products of ethylacetoacetate. *American Chemical Journal*. 3: 385-395. 1882.

[14] Meunier H., Carraz G., Meunier Y., Eymard P., Aimard M. Propriétés pharmacodynamiques de l'acide n-dipropylacetique. *Therapie*. 18: 435-438. 1963.

[15] Richey J. An update on antiepileptic agents: focus on second generation treatment options. *Pharma note*. 24(1): 11-14. 2008

[16] Howard S., Gold M.D., Robert C., Moellering M.D. Antimicrobial-Drug Resistance. *The new England Journal of Medicine*. 35: 1445-1453. 1996.

[17] Joule A., Mills K. Heterocyclic Chemistry.John Willey and Sons. Fifth edition. 2010.

[18] Eicher T., Hauptmann S., Speicher A. The Chemistry of heterocycles: Structure, reactions, syntheses, and applications, Second edition . 2004.

[19] Mahmud T. H. K. Publisher: Springer; First edition, Bioactive Heterocycles III Topics in Heterocyclic Chemistry. 2007.

[20] Hale S.L., Kloner R.A. Ranolazine. An inhibitor of the late sodium channel current, reduces postischemic myocardial dysfunction in the rabbit. *J Cardiovasc Pharmacol Ther*. 11(4): 249-255, 2006.

[21] Fragasso G., Palloshi A., Puccetti P., Silipigni C., Rossodivita A., Pala M., Calori G., Alfieri O., Margonato A. A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. *J Am Coll Cardiol*. 48(5): 992-998, 2006.

[22] Rivedi M.H., Fava M., Wisniewski S.R., Thase M.E., Quitkin F., Warden D., Ritz L., Nierenberg A.A., Lebowitz B.D., Biggs M.M., Luther J.F., Shores-Wilson K., Rush A.J. Medication augmentation after the failure of SSRIs for depression. *N Engl J Med*. 354(12): 1243-1252, 2006.

[23] Cusack B., Nelson A., Richelson E. Binding of Antidepressants to Human Brain Receptors: Focus on Newer Generation Compounds. *Psychopharmacology (Berl)*. 114(4): 559-565, 1994.

[24] Dahl E., Offer-Ohlsen D., Lillevold P.E., Sandvik L. Transdermal scopolamine, oral meclozine, and placebo in motion sickness. *Clinical Pharmacology And Therapeutics*. 36(1): 116-120, 1984.

[25] Nicholson A.N. *et al*. Central effects of cinnarizine: restricted use in aircrew. *Aviat Space Environ Med*. 73(6): 570-574, 2002.

[26] Davis's Drug Guide for Nurses, Eighth Edition. F.A. Davis Company, 2005.

[27] Rees L. Chlorpromazine and allied phenothiazine derivatives. *British Medical Journal*. 2(5197): 522-525, 1960.

[28] Manoury P.M., Dumas A.P., Najer H., Branceni D., Prouteau M., Lefevre-Borg F.M. Synthesis and analgesic activities of some (4-substituted phenyl-1-

piperazinyl)alkyl 2-aminobenzoates and 2-aminonicotinates. *Journal of Medicinal Chemistry*. 22(5): 554-559, 1979.

[29] Gambacorti Passerini C. Part I: Milestones in personalised medicine--imatinib. *Lancet Oncology*. 9: 600, 2008.

[30] Hagop S. Akiskal, Mauricio Tohen. Caring for the Patient. *Bipolar Psychopharmacotherapy*. John Wiley & Sons. p. 209, 2011.

[31] Drusano G.L., Standiford H.C., Plaisance K., Forrest A., Leslie J., Caldwell J., G.L. Absolute oral bioavailability of ciprofloxacin. *Antimicrob Agents Chemother*. 30(3): 444-446, 1986.

[32] Nelson J.M., Chiller T.M., Powers J.H., Angulo F.J. Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clin Infect Dis*. 44(7): 977-980, 2007.

[33] Fischer W., Kittner H., Regenthal R., De Sarro G. Anticonvulsant profile of flunarizine and relation to Na(+) channel blocking effects. *Basic Clin Pharmacol Toxicol*. 94(2): 79-88, 2004.

[34] Desmedt L.K., Niemegeers C.J., Janssen P.A. Anticonvulsive properties of cinnarizine and flunarizine in rats and mice. *Arzneimittel Forschung*. 25(9): 1408-1413, 1975.

[35] Hakimian S., Cheng Hakimian A., Anderson G.D., Miller J.W. Rufinamide: a new anti-epileptic medication. *Expert Opin Pharmacother*. 8(12): 1931-1940, 2007.

[36] WHO Model List of Essential Medicines. World Health Organization. October 2013. Retrieved 22 April 2014.(a)

[37] WHO Model List of Essential Medicines. World Health Organization. October 2013. Retrieved 22 April 2014.(b)

[38] Doughty, Michael J., Lyle, William M. A Review of the Clinical Pharmacokinetics of Pilocarpine, Moxisylyte (Thymoxamine), and Dapiprazole in the Reversal of Diagnostic Pupillary Dilation. *Optometry & Vision Science*. 69: 5, 1922.

[39] Lexi Comp. Nefazodone. *The Merck Manual Professional*. Retrieved on November 29, 2008.

[40] Ganellin C.R., Triggle D.J., Macdonald F. *Dictionary of pharmacological agents*. CRC Press. p. 859, Retrieved 27 November 2011.

[41] Jacobs M.P., Leblanc G.G., Brooks Kayal, A., Jensen F.E., Lowenstein D.H., Noebels J.L., Spencer D.D., Swann J.W. Curing epilepsy: Progress and future directions. *Epilepsy Behav*. 14(3): 438-445, 2009.

[42] Brodie M.J. Antiepileptic drug therapy the story so far. *Seizure*. 19: 650-655, 2010.

[43] Adam S.S., Cliffe E.E., Lessel B., Nicholson J.S. *J Pharmceutical Sci*. 56: 1986-1987, 1967.

[44] Kuroda T., Suzuki F., Tamura T., Ohmori K., Hosoe H. *J Med Chem.* 35: 1130, 1992.

[45] Zquez G.N.V., Mari G., Salinas M., Zetel D.F., Javier V.V., Samuel E.S., Francisco G.S., Emanuel H.N., Salvador S.F. *Biorg Med Chem*. 15: 5502, 2007.

[46] Selvam T.P., Kumar P.V. Quinazoline Marketed drugs – A Review. *Research in Pharmacy*. 1(1): 1-21, 2011.

[47] Abbas S.E. Synthesis of Some Novel 2,3-Disubstituted-3,4-dihydro-4-quinazolinones as Potential Anticonvulsant Agents. *Bull Fac Pharm Cairo Univ*. 45: 119-129, 2007.

[48] Kacker I.K., Zaheer S.H. Potential Analgesics Part I. Synthesis of Substituted 4-quinazolones. *J Ind Chem Soc.* 28: 344-346, 1951.

[49] Arroyo S. Rufinamide. *Neurotherapeutics*. 4: 155-162, 2007.

[50] Perucca E., Cloyd J., Critchley D., Fuseau E. Rufinamide: Clinical Pharmacokinetics and concentration-response relationships in patients with epilepsy. *Epilepsia*. 49: 1123-1141, 2008.

[51] Wheless J., Conry J., Krauss G., Mann A., LoPresti A., Narurkar M. Safety and tolerability of rufinamide in children with epilepsy: A pooled analysis of seven clinical trials. *J Child Neurol*. 50: 1158-1166, 2009.

[52] White H.S., Franklin M.R., Kupferberg H.J., Schmutz M., Stables J.P., Wolf H.H. The anticonvulsant profile of rufinamide (CGP33101) in rodent seizure models. *Epilepsia*. 49: 1213-1220, 2008.

[53] Gyoten M., Nagaya H., Fukuda S., Ashida Y., Kawano Y. Synthesis of Eosinophil Infiltration Inhibitors with Antihistaminic Activity. *Chem Pharm Bull Tokyo*. 51(2): 122-133, 2003.

[54] Kalluraya B., Chimbalkar R., Sivarama H.B. *Indian J Heterocycl Chem*. 5: 37, 1995.

[55] Meng H., Hung W. *J Org Chem*. 65: 3894, 2000.

[56] Bottino F.A., Pasquale G.D., Lannelli P. *Macromolecules*. 34: 33, 2001.

[57] Chen Z.K., Meng H., Lai X.H., Huang W. *Macromolecules*. 32: 4351, 1999.

[58] Perez M.A., Bermejo J.M, *J Org Chem*. 58: 2628, 1993.

[59] Lee D.W., Kwon K.Y., Jin J.I., Park Y., Kim Y.R., Hwang I.W., *Chem Mater*. 13: 565, 2001.

[60] Rajapakse H.A., Zhu H., Young M.B., Mott B.T. *Tetrahedron Lett*. 47: 4827, 2006.

[61] Wilder Smith A.E. *Arzneim Forsch*. 12: 275, 1962.

[62] Vincent M., Mailard J., Bernard M. *Bull Soc Chim Fr*. 1962.

[63] Chin T.T., ChenY., Kong K.H., Bai J., Li Y., Lim S.G., Ang T.H., Lam Y. *Antiviral Research*. 71: 7, 2006.

[64] Shah V.R., Vadodaria M., Parikh A.R. *Indian J Chem*. 36B: 101, 1997.

[65] Brown P., Rest D.J., Broom N.J.P., Casse R., Bhanol P.J., Mitchell J.J., Osborne N.F., Wilson J.M. *J Med Chem*. 40: 2563, 1997.

[66] Kadi A.A., Nasser R., Brollosy E., Omar A., Deeb A., Elsayed E.H., Ibrahim T.M., Eman A.A. *Eur J Med Chem*. 42: 235, 2007.

[67] Balin G.B., Tan W.L. *Aust J Chem*. 37: 1065, 1984.

[68] Chen B., Qin W., Shen Z., Lei Y. *Yiyago Gongye*. 16: 305, 1985.

[69] Singh H., Yadav V.L.D.S., Chaudhary J.P. *Acta Chem Hung*. 11: 118, 1985.

[70] Omar A., Mohsen M.E., Aboulwafa O.M. *J Hetercycl Chem*. 21: 1415, 1984.

[71] Andriole V.T. The quinolones: past, present, and future. *Clin Infect Dis*. 41(2): 113-119, 2005.

[72] Ball P. Adverse drug reactions: implications for the development of fluoroquinolones. *J Antimicrob Chemother*. 51(1): 21-27, 2003.

[73] Drugs@ FDA page. Available at: http://www.accessdata.fda.gov/scripts/ cder/ drugsatfda/index.cfm. Accessed April 18, 2011.

[74] Hawkey P.M. Mechanisms of quinolone action and microbial response. *J Antimicrob Chemother*. 51(1): 29-35, 2003.

[75] Drugs.com.Available at http://www.drugs.com/international/ sarafloxacin.html. Accessed on 08.04.2014.

[76] Gootz T.D., Zaniewski R., Haskell S., Schmieder B., Tankovic J., Girard D., Courvalin P., Polzer R.J. Activity of the new fluoroquinolone trovafloxacin (CP-99,219) against DNA gyrase and topoisomerase IV mutants of *Streptococcus pneumoniae* selected in vitro. *Antimicrob Agents Chemother*. 40 (12): 2691-2700, 2006.

[77] Drugs.com .Available at http://www.drugs.com/international/ tosufloxacin.html. Accessed on 08.04.2014.

[78] Rubinstein E. History of quinolones and their side effects. *Chemotherapy*. 47(3): 3-8.

[79] Drugs.com. Available at http://www.drugs.com/international /rosoxacin.html. Accessed on 08.04.2012.

[80] Deborah M.R., Youjun Y., Mary J.W., Karen B., May D. Inhibition of metallo-β-lactamases by pyridine monothiocarboxylic acid analogs. *The Journal of Antibiotics*. 63: 255-257, 2010.

[81] Browne S.G. Trial of a long-acting sulfonamide sulfaphenazole in the treatment of leprosy. *International journal of Leprosy*. 29: 502-505, 1961.

CHAPTER II: REVIEW OF LITERATURE

2.1 PIPERAZINES AND ANTICONVULSANT ACTIVITY

Piperazine is a heterocyclic compound consisting of a six member ring containing two opposing nitrogen atoms. Slight change in substitution pattern in piperazine nucleus causes distinguishable difference in their pharmacological activities.

Many chemical compounds containing the piperazine nucleus are reported to possess anticonvulsant activity.

Few examples firming the stated fact are provided below.

In 1986, Zopiclone [1] was developed and first introduced by Rhône-Poulenc S.A., now part of Sanofi-Aventis, the main worldwide manufacturer. Zopiclone is hypnotic, anti anxiety, anticonvulsant and muscle relaxing in its effects.

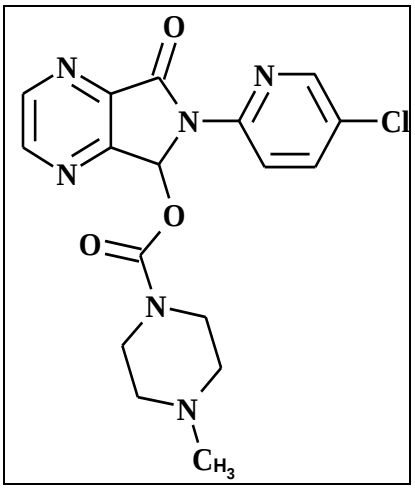


Figure 14: Zopiclone

Watkins *et al*. (1985) [2], tested two di carboxylic piperazine derivatives, 1- (p-chlorobenzoyl) piperazine-2, 3-dicarboxylic acid and 1-(p-bromobenzoyl) piperazine-2, 3-dicarboxylic acid. These compounds were expected to block excitation at glutamate receptors and were evaluated as anticonvulsants in rodent models of epilepsy by intracervical or intraperitoneal injection.

Mutlu Dilsiz Aytemir *et al*. (2004) [3], reported the synthesis and evaluation of anticonvulsant activity of 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one

derivatives. Among the compounds, 4-(3-trifluoromethylphenyl) piperazin-1-yl, showed good activity.

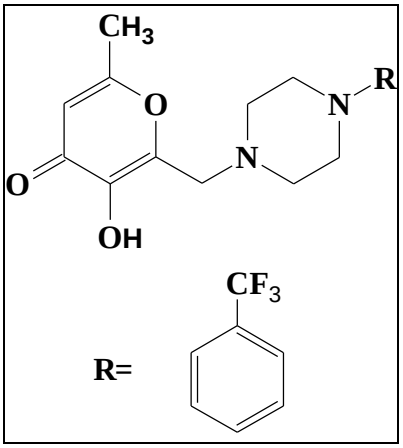


Figure 15: 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one with 4-(3-trifluoromethylphenyl) piperazin-1-yl substitution; Mutlu Dilsiz Aytemir *et al*.

J. Obniska *et al*. (2006) [4], reported the synthesis, physicochemical and pharmacological properties of new *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro [4.4] nonane and [4.5] decane-1, 3-dione derivatives. The antiepileptic effects of those compounds were examined by a maximal electroshock and a subcutaneous Pentylenetetrazole tests, and their neurotoxicity was determined using a rota-rod test. Compounds with a CF_3 group at the 3-position of the 4-arylpiperazine fragment exhibited anti-seizure properties in the maximal electro shock model. The 2-CH_3 and 2-OCH_3 analogues were inactive in both the tests used.

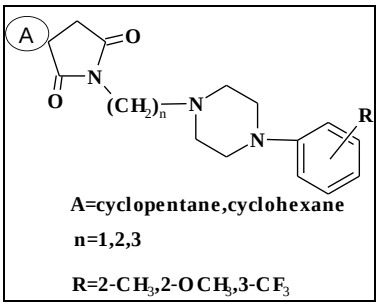


Figure 16: *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro [4.4] nonane and [4.5] decane-1, 3-dione derivatives; J. Obniska *et al*.

J. Obniska *et al*. (2009) [5], also synthesized a new series of *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl) and 3-(2-trifluoromethyl-phenyl)-pyrrolidine-2, 5-diones and tested for anticonvulsant activity using the maximal electroshock

and subcutaneous Pentylenetetrazole screens. In this series, the most active were *N*-[(4-phenylpiperazin-1-yl)-methyl]-3-(2-trifluoromethylphenyl) - pyrrolidine-2, 5- dione.

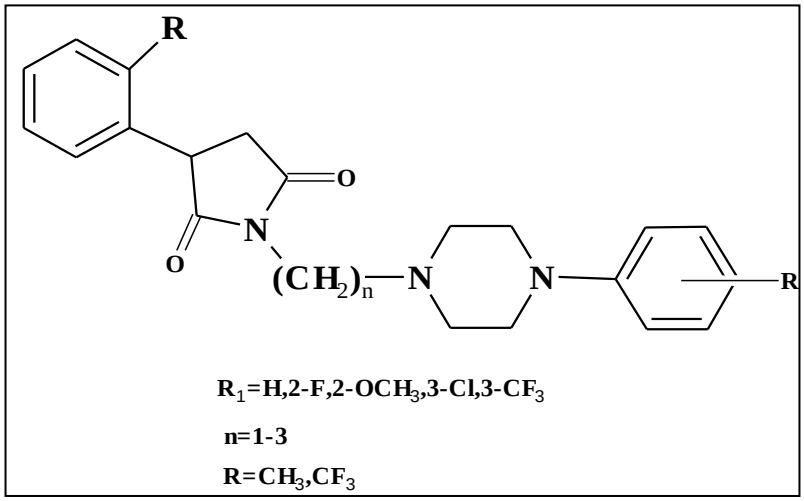


Figure 17: *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl) and 3-(2-trifluoromethyl-phenyl)-pyrrolidine-2, 5-diones as stated by J. Obniska *et al*.

Mutlu Dilsiz Aytemir *et al*. (2010) [6], reported the synthesis and anticonvulsant activity of new kojic acid derivatives. Among the compounds 3-hydroxy-6hydroxyethyl-2-[4-(2-methyl phenyl) piperazin-1-yl methyl]-4-pyran-4-one was found to be highly selective and most active.

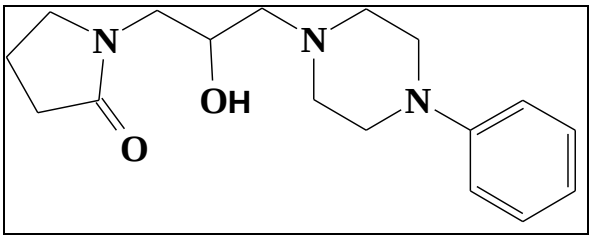


Figure 18: An example of kojic acid derivatives as mentioned in the research work done by Mutlu Dilsiz Aytemir *et al*. (2010).

Krzysztof *et al*. (2011) [7], synthesized twenty two new 1-[2-oxo-2-(4-phenylpiperazin-1-yl) ethyl] pyrrolidine-2, 5-dione and tested them for anticonvulsant activity. Anticonvulsant screening was performed using standard maximal electroshock and subcutaneous Pentylenetetrazole screens in mice.

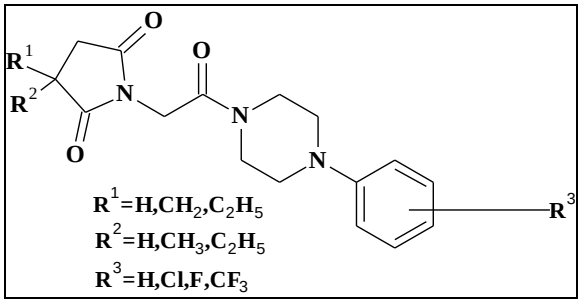


Figure 19: 1-[2-oxo-2-(4-phenylpiperazin-1-yl) ethyl] pyrrolidine-2, 5-dione derivatives as mentioned in the research work of Krzysztof *et al*. (2011).

Kikkeri *et al*. (2013) [8], synthesized a series of novel 1-[5-(4- methoxy- phenyl)-[1, 3, 4] oxadiazol-2-yl] piperazine derivatives. The synthesized compounds were screened for their anticonvulsant activity against maximal electroshock seizure model in male wistar rats and compared with the standard drug phenytoin.

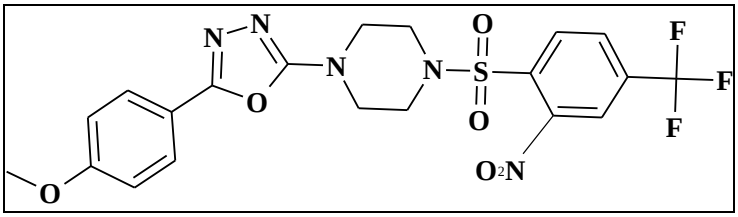


Figure 20: 1-[5-(4- methoxy- phenyl)-[1, 3, 4] oxadiazol-2-yl] piperazine derivatives; Kikkeri *et al*.

In the present study, few novel chemical entities containing the piperazine ring have been synthesized and were tested for their anticonvulsant activity using subcutaneous Pentylenetetrazole screening.

2.2 TRIAZOLES AND ANTICONVULSANT ACTIVITY

Triazoles are an important type of nitrogen containing heterocyclic compound. Triazole derivatives possess wide spectrum of biological activities such as antibacterial, antifungal, antitubercular, anticancer, anticonvulsant, antidepressants, analgesic, anti-inflammatory, antioxidant, antiviral, anthelmintic, antitumor and hypoglycaemic activities.

Due to their extensive biological activity, they find successful applications in medicine and agrochemicals.

Applications of triazoles are also seen in industries namely dye stuffs, fluorescent whiteners, photo stabilizers for polymers, optical brightening agents, corrosion inhibitors and photographic photoreceptors.

Triazoles have emerged as a new generation of anticonvulsant agents that show great promise as potentially useful anticonvulsant drugs.

The objective of the current work is to synthesize new molecules which will exhibit anticonvulsant activity. In this review presents few examples from the various works done by various researchers with relation to triazoles molecules exhibiting anticonvulsant activity are mentioned.

Perucca E *et al*. (2008) [9] explains the anticonvulsant activity of the drug Rufinamide. This molecule has been considered in Strategy II.

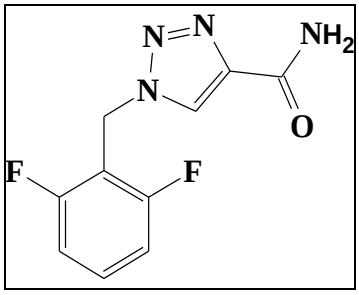


Figure 21: Rufinamide [9]

Kadaba *et al*. (1998) [10], performed pioneering studies which led to the emergence of the delta 2-1,2,3-triazolines (4,5-dihydro-1H-1,2,3-triazoles) and the closely related 1H-1,2,3-triazoles, as a unique family of anticonvulsant agents. They evaluated several groups of 1-aryl-5-pyridyl-substituted triazolines and triazoles with particular reference to structure-activity relationship. Anticonvulsant activity was determined, after intraperitoneal administration, in two standard seizure models in the mouse, the maximal electroshock and subcutaneous maximal electroshock tests. It can be inferred from the research carried out by Kadaba *et al*. that trazolines and triazoles can possess anticonvulsant activity comparable to the prototype antiepileptic drugs like Ethosuximide and Valproate.

Moreau *et al*. (1998) [11], synthesized the analogues of 3-amino-7-(2, 6-dichlorobenzyl)-6- methyl triazolo [4, 3-b] pyridazine. The compounds with amide or carboxylic acid functional groups were synthesized and tested for anticonvulsant activity. The maximum activity was reported to be generally associated with a 2, 6-dichlorobenzyl substitution pattern.

Küçükgüzel *et al.* (2004) [12], reported the synthesis of a series of novel 3-{[(substituted phenyl) methyl] thio}-4-alkyl/aryl-5-(4-aminophenyl)-4H-1, 2, 4-triazoles and several related Schiff's bases. All compounds were evaluated for their anticonvulsant activity by maximal electroshock, subcutaneous Pentylenetetrazole and neurotoxicity screens. Three compounds were subjected to oral maximal electroshock screening in rats at 30 mg/ kg and were observed to protect 50% of the animals employed in the experiment.

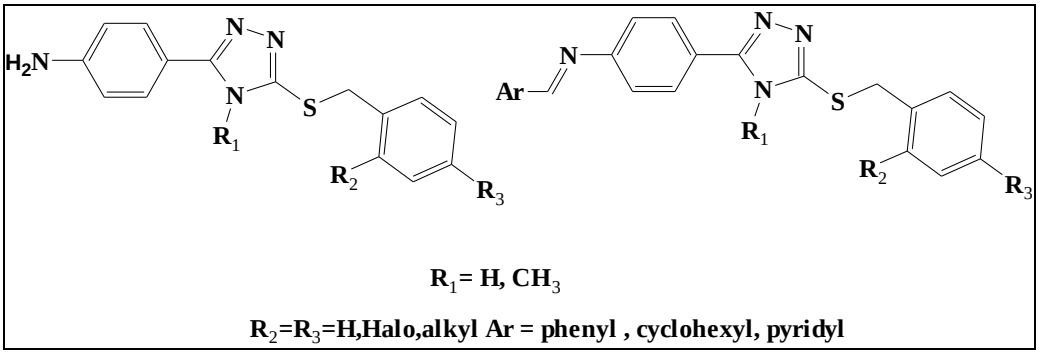


Figure 22: 3-{[(substituted phenyl) methyl] thio}-4-alkyl/aryl-5-(4-aminophenyl)-4H-1, 2, 4-triazoles and related Schiff's bases: Küçükgüzel *et al.*

Xie *et al*. (2005) [13], reported the synthesis of a series of 7-alkoxyl-4, 5-dihydro-[1, 2, 4] triazolo [4, 3- a] quinoline derivatives. The anticonvulsant activities were evaluated by the maximal electroshock test and the subcutaneous Pentylenetetrazole test and their neurotoxicity was evaluated by the rota rod neurotoxicity test. Maximal electroshock test and subcutaneous maximal electroshock tests showed that 7-(4-fluorobenzyloxy)-4, 5-dihydro-[1, 2, 4] triazolo[4,3-a] quinoline was found to be the most potent compound of the series.

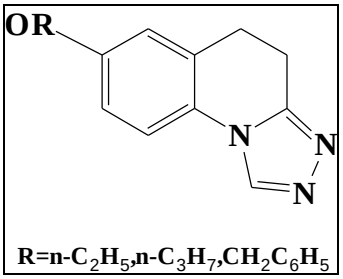


Figure 23: 7-alkoxyl-4, 5-dihydro-[1, 2, 4] triazolo [4, 3- a] quinoline derivatives: Xie *et al*.

Aiyalu *et al*. (2006) [14], reported the synthesis of several novel 1-[2-(1H-tetrazol-5-yl) ethyl]-1H-benzo[d] [1, 2, 3] triazoles. The synthesized compounds were characterized and their anticonvulsant activity was evaluated by the maximal electroshock induced convulsion method in mice. Some compounds exhibited excellent anticonvulsant activity.

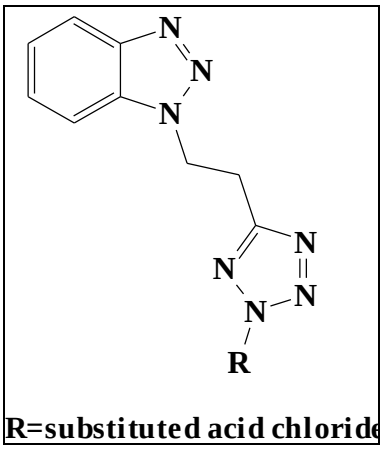


Figure 24: 1-[2-(1H-tetrazol-5-yl) ethyl]-1H-benzo[d] [1, 2, 3] triazoles: Aiyalu *et al*.

Synthesis of a series of 4-(4-alkoxylphenyl)-3-ethyl-4H-1,2,4-triazole derivatives as open-chain analogues of 7-alkoxyl-4,5-dihydro[1,2,4] triazolo[4,3-a]quinolines was reported by Chen *et al*. (2007) [15]. The anticonvulsant activities of the compounds were evaluated by the maximal electroshock test and their neurotoxicity was evaluated by the rota rod neurotoxicity test. Maximal electroshock test showed that 3-ethyl-4-(4-octyloxyphenyl)-4H-1, 2, 4-triazole was the most potent compound.

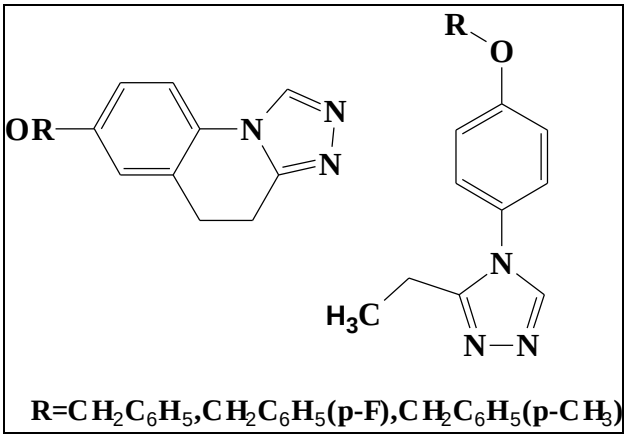


Figure 25: 4-(4-alkoxylphenyl)-3-ethyl-4H-1, 2, 4-triazole derivatives: Chen *et al*.

Narayana *et al.* (2006) [16], experimented on the synthesis of some novel 8-chloro-6-(2-fluorophenyl)-1- (aryl)- 4H -[1,2,4] triazolo[4,3-a] [1,4] benzo diazepines which were prepared by treating 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-thione with various aromatic acid hydrazides. The compounds were tested for anticonvulsant activity. Four of the tested compounds exhibited excellent anticonvulsant activity in comparison with standard drug, Diazepam.

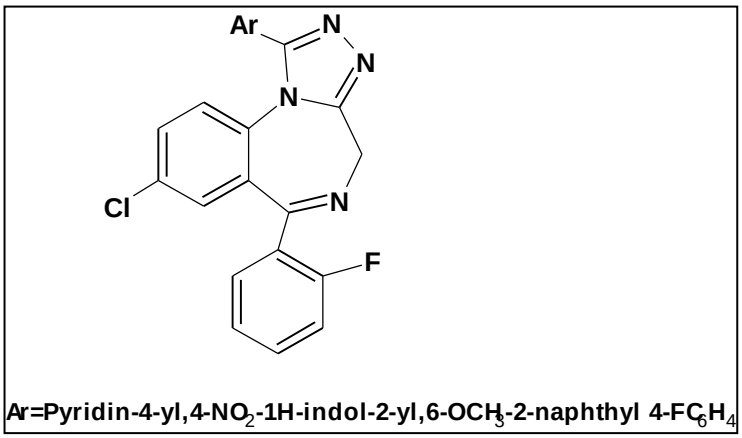


Figure 26: 8-chloro-6-(2-fluorophenyl)-1- (aryl) - 4H - [1, 2, 4] triazolo[4,3-a] [1,4] benzodiazepines: Narayana *et al.*

Shalini *et al.* (2009) [17], synthesized a new series of 4,5-diphenyl-2H-1,2,4-triazol-3(4H)-one and studied the effect of cyclization of the semicarbazone moiety of aryl semicarbazones on the anticonvulsant activity. All compounds were tested for their anticonvulsant activity in four animal models of seizures, viz. maximal electroshock seizure, subcutaneous Pentylenetetrazole, subcutaneous

strychnine, and subcutaneous Picrotoxin - induced seizure threshold tests. The compounds were also evaluated for neurotoxicity. Eight compounds exhibited anticonvulsant activity in all the four animal models of seizure.

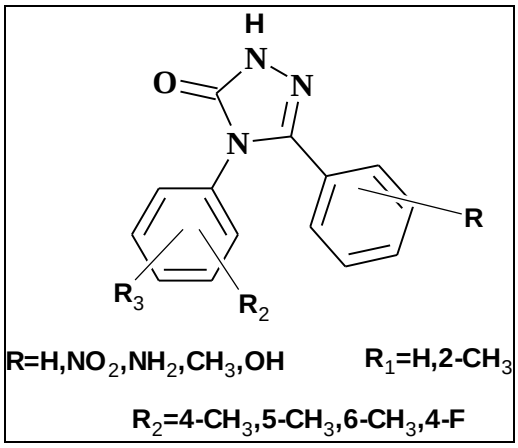


Figure 27: series of 4,5-diphenyl-2H-1,2,4-triazol-3(4H)-ones: Shalini *et al.*

In the research work carried out, some new piperazine and triazole derivatives were synthesized, characterized and tested for their anticonvulsant activity. In the present research work quinazoline and oxadiazole heterocycles have been used in conjugation with either piperazine or triazole and even in some cases with both of them to obtain novel biologically active molecules. Evidences supporting the effectiveness of that quinazoline and oxidiazole derivatives as anticonvulsant agents are provided below.

2.3 QUINAZOLINES AS ANTICONVULSANT AGENTS

In the search of new anticonvulsants agents bearing little or no resemblance in structure with the currently available drugs, new molecules were designed and synthesized with quinazoline and piperazine heterocycles. Some examples from the research works performed on quinazolines as anticonvulsant agents are provided as evidence in support of our approach.

Varsha *et al*. (2008) [18], synthesized a series of new 3-[5-substituted phenyl-1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. The synthesized compounds were evaluated them for anticonvulsant, sedative, hypnotic and central nervous system depression activities.

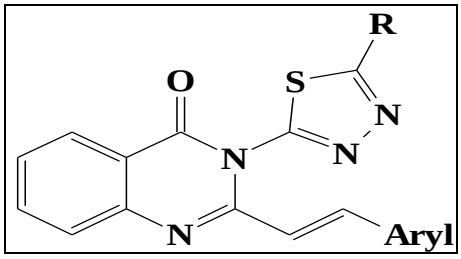


Figure 28: 3-[5-substituted phenyl-1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-one derivatives: Varsha *et al*.

Abdel Ghany *et al*. (2003) [19], synthesized a new series of 3-substituted (methyl, ethyl or phenyl) -3H-quinazolin-4-one derivatives through condensation reaction of their potassium salts with methyl, ethyl and phenyl isocyanate. The newly synthesized derivatives were evaluated for anticonvulsant activity. Reduced anticonvulsant activity was recorded in those cases. Phenobarbitone sodium was used as a reference drug.

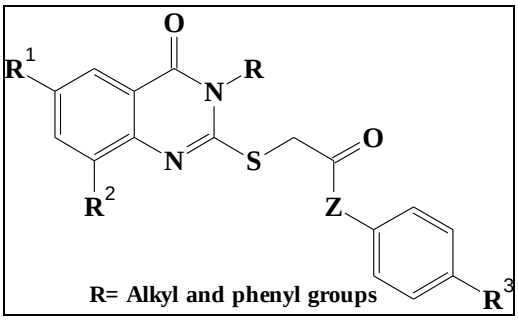


Figure 29: 3-substituted (methyl, ethyl or phenyl) -3H-quinazolin-4-one derivatives: Abdel Ghany *et al*.

A new series of 2, 3, 8-trisubstituted-4(3H)-quinazoline derivatives were synthesized by Adbel *et al*. (2012) [20]. Those were evaluated for their anticonvulsant activity against electrically and chemically (Pentylenetetrazole, Picrotoxin and Strychnine) induced seizures and compared with the standard drugs like Methaqualone and Sodium valproate.

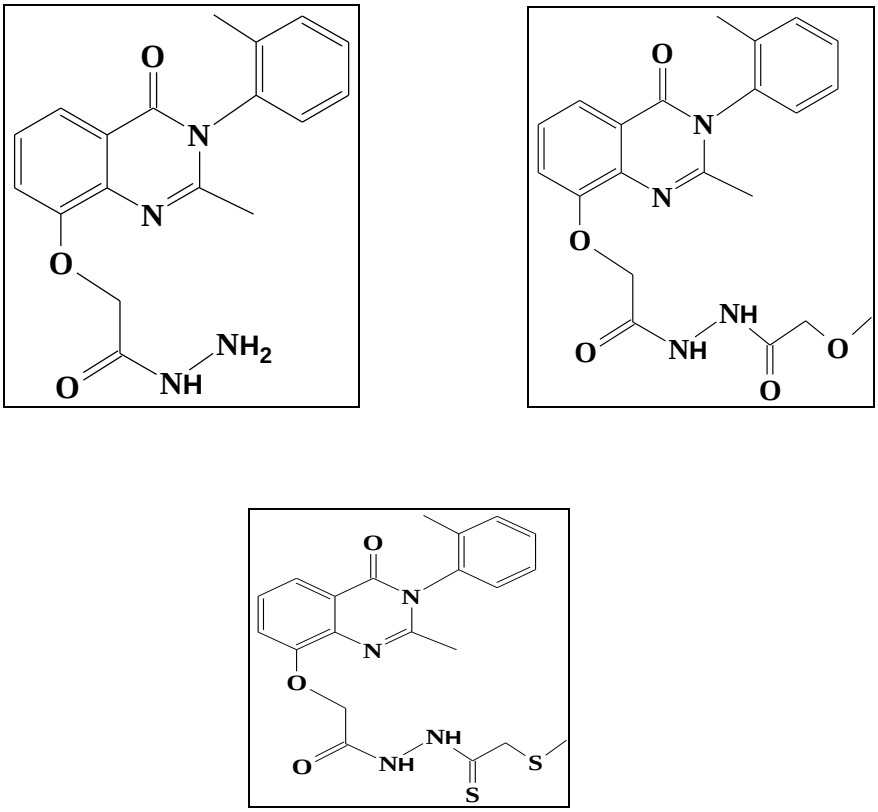


Figure 30: 2, 3, 8-trisubstituted-4(3H)-quinazoline derivatives: Adbel *et al*.

Adel S *et al*. (2012) [21], also reported the design and synthesis of a novel series of 7-substituted-4(3H)-quinazolinones and evaluated them for their antitumor and anticonvulsant activity. Some compounds showed advanced anticonvulsant activity as well as lower neurotoxicity than reference drugs.

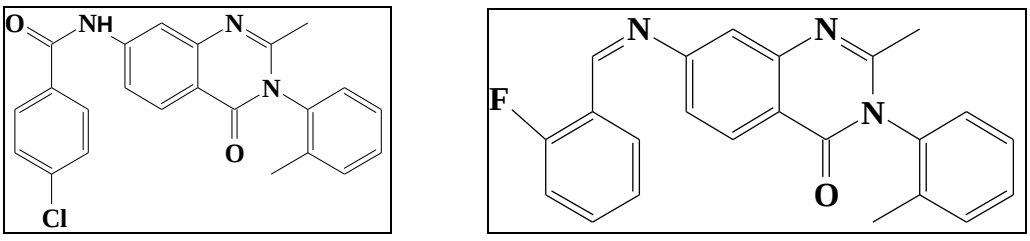


Figure 31: 7-substituted-4(3H)-quinazolinones: Adel S *et al*.

2.4 OXADIAZOLES AS ANTICONVULSANT AGENTS

Similar to the piperazine, triazole and quinazoline heterocycle oxadiazole derivatives are reported to exhibit anticonvulsant activity. Few examples of the research work performed on this subject have been discussed below.

Afsin *et al*. (2005) [22], reported the synthesis of a new series of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles and evaluated them for anticonvulsant activity. Few compounds showed considerable anticonvulsant activity both in Pentylenetetrazole and maximal electroshock seizure models.

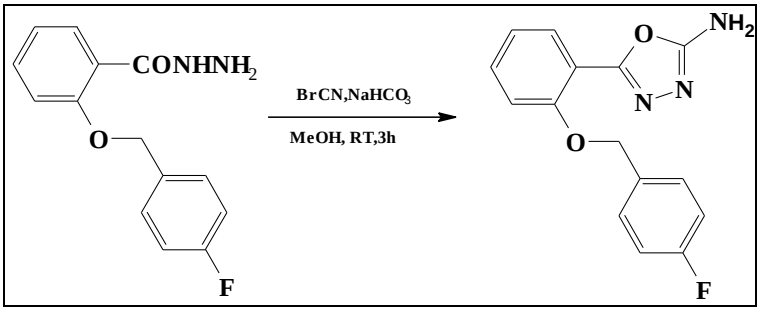


Figure 32: Synthesis of a new series of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles: Afsin *et al*.

A similar series of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1, 3, 4-oxadiazoles was synthesized and screened for their anticonvulsant activities by Ali Almasirad *et al*. (2004) [23]. Some synthesized compounds exhibited considerable anticonvulsant activity both in subcutaneous Pentylenetetrazole and maximal electroshock seizure models.

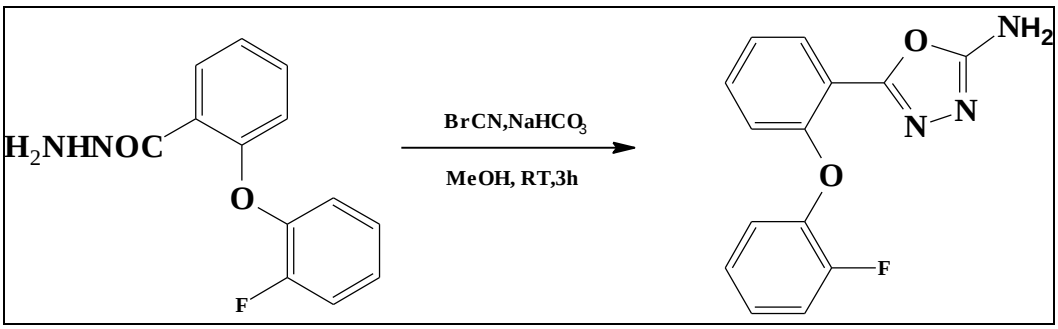


Figure 33: Synthesis of 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1, 3, 4-oxadiazoles: Ali Almasirad *et al*.

A series of 3- and 5-aryl-1, 2, 4-oxadiazole derivatives were prepared by Katrin *et al*. (2007) [24], and were tested for anticonvulsant activity in a variety of models. These 1,2,4-oxadiazoles exhibited considerable activity in both subcutaneous Pentylenetetrazole and maximal electroshock seizure models.

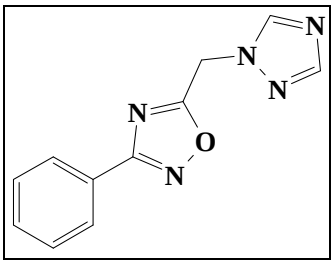


Figure 34: 3- and 5-aryl-1, 2, 4-oxadiazole derivatives: Katrin *et al*.

Kashaw *et al*. (2010) [25], Synthesized a new series of 3-[5-(4-substituted)-phenyl-1, 3, 4-oxadiazole-2-yl]-2-styrylquinazoline-4(3H)-one oxadiazoles and tested for their anticonvulsant activity.

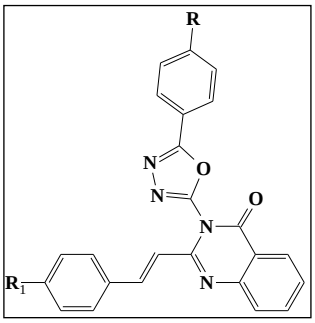


Figure 35: 3-[5-(4-substituted)-phenyl-1, 3, 4-oxadiazole-2-yl]-2-styrylquinazoline-4(3H)-one oxadiazoles: Kashaw *et al*.

Some new 2-substituted-5-(2-benzylthiophenyl)-1, 3, 4-oxadiazole derivatives were designed and synthesized as anticonvulsant agents by Zarghi *et al*. (2005) [26]. The authors found that introduction of an amino group at position 2 of the 1, 3, 4-oxadiazole ring and a fluorine substitute at the para position of the benzylthio group improves anticonvulsant activity.

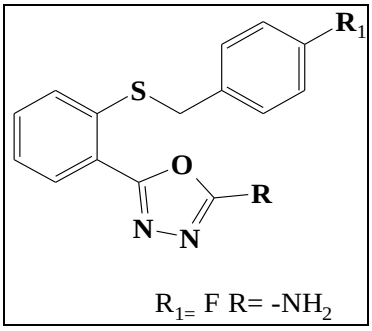


Figure 36: 2-substituted-5-(2-benzylthiophenyl)-1, 3, 4-oxadiazole derivatives: Zarghi *et al*.

The above sections containing the review articles on the anticonvulsant activities of piperazine, triazole, quinazoline and oxadizaole heterocycles support the objective of the thesis.

2.5 ANTIMICROBIAL ACTIVITIES OF SOME N-SUBSTITUTED DERIVATIVES OF 6-FLUORO-1-METHYL-4-OXO-7-(PIPERAZIN-1-YL)-4H-[1, 3] THIAZETO [3, 2-A] QUINOLINE-3-CARBOXYLIC ACID

The majority of the quinolones in clinical use belong to the subset fluoroquinolones which have a fluorine atom attached to the central ring system.

Fluoroquinolones are broad spectrum antibiotics (effective for both Gram negative and Gram positive bacteria) that play an important role in the treatment of serious bacterial infections.

Fluoroquinolones exhibit concentration dependent bactericidal activity by inhibiting the activity of DNA gyrase and topoisomerase enzymes, essential for bacterial DNA replication.

Few examples of fluoroquinolones are provided in the review. It is to be noted that the majority of the drug molecules have a piperazine ring attached to the fluoroquinolone nucleus.

Ciprofloxacin [27]

Ciprofloxacin is synthetic broad spectrum antimicrobial agent for oral administration. Ciprofloxacin hydrochloride, USP is the mono hydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It is well available in the market as different brand names.

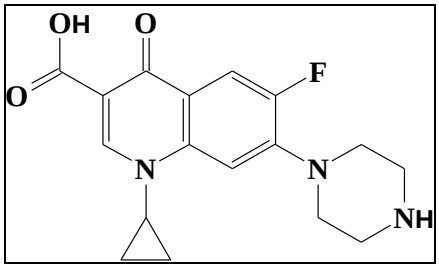


Figure 37: Ciprofloxacin

Levofloxacin [28]

Levofloxacin [trade names Levaquin (US), Tavanic (EU), and others] is a broad spectrum antibiotic of the fluoroquinolone drug class, and the levo isomer of its predecessor ofloxacin. Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration and for their availability in both oral and intravenous formulations. Levafloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia, urinary tract infections and abdominal infections.

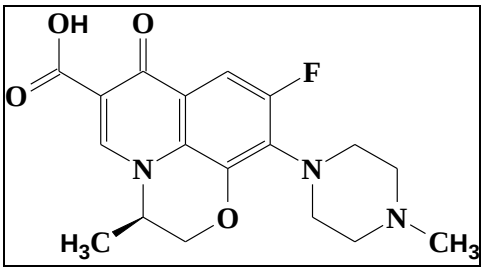


Figure 38: Levofloxacin

Trovafloxacin [29]

Gootz *et al*. reported about Trovafloxacin which is sold as Trovan by Pfizer. It is a broad spectrum antibiotic that inhibits the uncoiling of supercoiled DNA in various bacteria by blocking the activity of DNA gyrase and topoisomerase IV. It has better gram positive bacterial coverage and less gram negative coverage with respect to other fluoroquinolones.

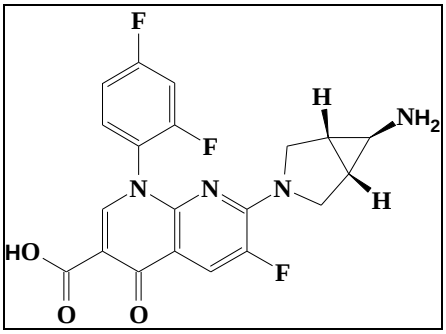


Figure 39: Trovafloxacin

Rosoxacin [30]

Rosoxacin (also known as Acrosoxacin, trade name is Eradacil) is a quinolone antibiotic indicated for the treatment of urinary tract infections and certain sexually transmitted diseases.

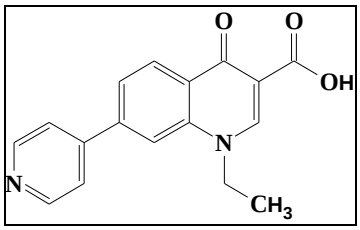


Figure 40: Rosoxacin

Temafloxacin [31]

It is a fluoroquinolone antibiotic drug (marketed by Abbott Laboratories as Omniflox).

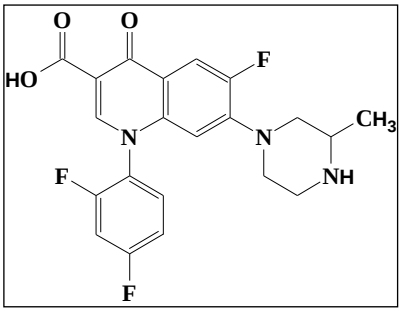


Figure 41: Temafloxacin

Tosufloxacin [32]

The drug is basically a fluoroquinolone antibiotic.

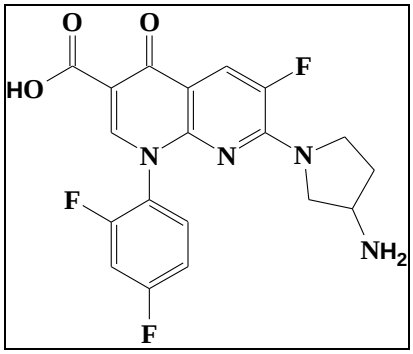


Figure 42: Tosufloxacin

Sarafloxacin [33]

Sarafloxacin hydrochloride is a fluoroquinolone antibacterial agent. It inhibits bacterial Topo II α (DNA gyrase, topoisomerase). Sarafloxacin hydrochloride is effective against *Mycobacterium tuberculosis*.

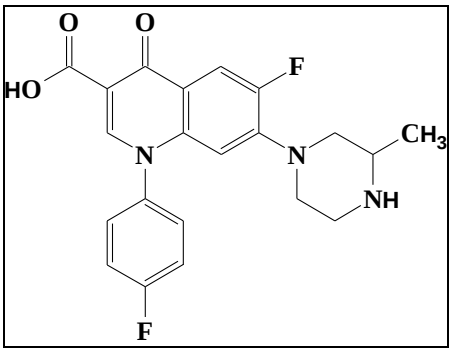
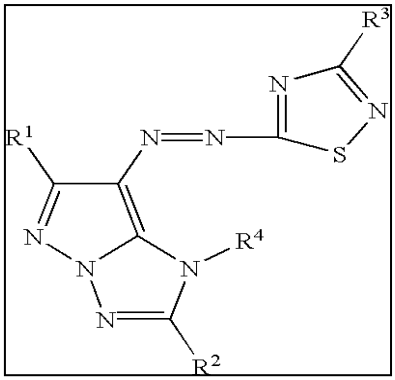


Figure 43: Sarafloxacin

2.6 TRIAZOLES IN DYE STUFF, FLUORESCENT WHITENERS, AGROCHEMICALS AND MEDICINES

Hisashi *et al*. (1999) [34] reported a yellow dye compound having 1, 2, 4-triazole as the Azo component. The dye compound was found to be excellent in transfer property and colour fastness against light, excellent in light absorption property, excellent in solubility and can be synthesized with ease. The dye compound of the invention was expressed by the general formula



Optical brighteners, optical brightening agents (OBAs), fluorescent brightening agents (FBAs) or fluorescent whitening agents (FWAs) are dyes that absorb light in the UV and violet region (usually 340-370 nm) of the EM spectrum and re-emit light in the blue region (typically 420-470 nm).

These additives are often used to enhance the appearance of colour of fabric and paper causing a "whitening" effect, making materials look less yellow by increasing the overall amount of blue light reflected. FBA production for paper, textiles and detergents is dominated by just a few di- and tetra-sulfonated triazole-stilbenes and a di-sulfonated stilbene-biphenyl.

Jing-Pei Huo *et al.* (2013) [35] reported the synthesis and characterization of Fluorescent Brightening Agents with Chiral 2(5H)-Furanone and Bis-1, 2, 3-triazole structure.

Paul A. Worthington (1987) [36] reported that using a knowledge of the mode of action of the plant growth regulator paclobutrazol, it was possible to design a series of 1, 2, 4 triazole containing tertiary alcohols which have high levels of plant fungicidal activity. From this group flutriafol and hexaconazole have been introduced into crop protection.

Lass-Flörl C (2011) [37] inferred that invasive fungal disease continues to be a problem associated with significant morbidity and high mortality in immunocompromised and, to a lesser extent, immunocompetent individuals. He also stated in his review that triazole antifungal agents have emerged as front-line drugs for the treatment and prophylaxis of many systemic mycoses. Fluconazole plays an excellent role in prophylaxis, empirical therapy, and the treatment of both superficial and invasive yeast fungal infections. Voriconazole is strongly recommended for pulmonary invasive aspergillosis. Posaconazole shows a very wide spectrum of activity and its primary clinical indications are as salvage

therapy for patients with invasive aspergillosis and prophylaxis for patients with neutropenia and haematopoietic stem-cell transplant recipients. Itraconazole also has a role in the treatment of fungal skin and nail infections as well as dematiaceous fungi and endemic mycoses. Fluconazole and voriconazole are well absorbed and exhibit high oral bioavailability, whereas the oral bioavailability of itraconazole and posaconazole is lower and more variable. Posaconazole absorption depends on administration with a high-fat meal or nutritional supplements. Itraconazole and voriconazole undergo extensive hepatic metabolism involving the cytochrome P450 system. The therapeutic window for triazoles is narrow, and inattention to their pharmacokinetic properties can lead to drug levels too low for efficacy or too high for good tolerability or safety. This makes these agents prime candidates for therapeutic drug monitoring (TDM).

2.7 REFERENCES

[1] Van, Der, Kleijn E. Effects of zopiclone and temazepam on sleep, behaviour and mood during the day. *European journal of clinical pharmacology*. 36(3): 247–251, 1989.

[2] Watkins J.C. Anticonvulsant activity of 2 novel piperazine derivatives with potent kainate antagonist Activity. *Neuroscience Letters*. Pp 325-330, 1985.

[3] Mutlu Dilsiz Aytemir, Unsal Calis, Meral Ozalp. Synthesis and evaluation of anticonvulsant and antimicrobial activities of 3-hydroxy-6-methyl-2-substituted -4H-pyran -4-one derivatives. *Arch. Pharm. Pharmaceutical Medicinal Chemistry*. 337: 281-288, 2004.

[4] Obniska J., Kołaczkowski M., Bojarski A.J., Duszyńska B. Synthesis, anticonvulsant activity and 5-HT1A, 5-HT2A receptor affinity of new N-[(4-arylpiperazin-1-yl)-alkyl] derivatives of 2-azaspiro [4.4] nonane and [4.5] decane-1,3dione. *European Journal of Medicinal Chemistry*. 41(7): 874-881, 2006.

[5] Jolanta Obniska, Krzysztof Kaminski, Dorota Skrzynska, Joanna Pichor. Synthesis and anticonvulsant activity of new N-[(4-arylpiperazin-1-yl)-alkyl] derivatives of 3-phenyl-pyrrolidine-2,5-dione. *European Journal of Medicinal Chemistry*. 44(5): 2224-2233, 2009.

[6] Mutlu Dilsiz Aytemir, Ebukekir Septioglu, Unsal Calis. Synthesis and anticonvulsant activity of new kojic acid derivatives. *Arzneimittelforschung*. 60(1): 22-29, 2010.

[7] Krzysztof Kamiński, Sabina Rzepka, Jolanta Obniska. Synthesis and anticonvulsant activity of new 1-[2-oxo-2-(4-phenylpiperazin-1-yl)ethyl]pyrrolidine-2,5 diones. *Bioorganic & Medicinal Chemistry Letters*. 21(19): 5800-5803, 2011.

[8] Kikkeri P., Harish, Kikkeri N., Mohana, Lingappa Mallesha, Basavapatna N., Prasanna kumar. Synthesis of novel 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives and evaluation of their in vivo anticonvulsant activity. *European Journal of Medicinal Chemistry*. 65: 276-283, 2013.

[9] Perucca E., Cloyd J., Critchley D., Fuseau E. Rufinamide: Clinical Pharmacokinetics and concentration-response relationships in patients with epilepsy. *Epilepsia*. 49: 1123-1141, 2008.

[10] Kadaba P.K. Triazolines. 14. 1, 2, 3-Triazolines and triazoles, a new class of anticonvulsants. Drug design and structure-activity relationships. *J Med Chem*. 31(1): 196-203, 1988.

[11] Moreau S., Coudert P., Rubat C., Vallee Goyet D., Gardette D., Gramain J.C., Couquelet J. Synthesis and Anticonvulsant Properties of Triazolo- and Imidazo pyridazinyl Carboxamides and Carboxylic Acids. *Bioorganic and Medicinal Chemistry*. 6: 983-991, 1998.

[12] Küçükgüzel I., Küçükgüzel S.G., Rollas S., Ötük-Sanıs G., Özdemir O., Bayrak I., Altug T., Stables J.P. Synthesis of some 3-(arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazole derivatives and their anticonvulsant activity. *IL Farmaco*. 59: 893-901, 2004.

[13] Xie Z.F., Chai K.Y., Piao H.R., Kwak K.C., Quan Z.S. Synthesis and anticonvulsant activity of 7-alkoxyl-4,5-dihydro-[1,2,4] triazolo[4,3-a]quinolines, *Bioorganic and Medicinal Chemistry Letters*. 15: 4803-4805, 2005.

[14] Aiyalu R., Shankarnarayan M., Kalasalingaam A. Antibacterial, Antifungal and Anticonvulsant Evaluation of Novel Newly Synthesized 1-[2- (1H-tetrazol-5-yl) ethyl]-1H-benzo[d] [1,2,3] triazoles. *Arch Pharm Res*. 29(7): 535-540, 2006.

[15] Chen J., Sun X.Y., Chai K.Y., Lee J.S., Song M.S., Quana Z.S. Synthesis and anticonvulsant evaluation of 4- (4-alkoxylphenyl)- 3- ethyl- 4H- 1,2,4- triazoles as open-chain analogues of 7-alkoxyl- 4,5- dihydro[1,2,4] triazolo[4,3-a] quinolines. *Bioorganic and Medicinal Chemistry*. 15: 6775-6781, 2007.

[16] Narayana B., Raj K.K.V., Ashalatha B.V., Kumari N.S. Synthesis of some new substituted triazolo[4,3-a][1,4]benzodiazepine derivatives as potent anticonvulsants. *European Journal of Medicinal Chemistry*. 41: 417-422, 2006.

[17] Shalini M., Yogeeswari P., Sriram D., Stables J.P. Cyclization of the semicarbazone template of aryl semicarbazones: synthesis and anticonvulsant activity of 4, 5-diphenyl-2H-1, 2, 4-triazol-3(4H)-one. *Biomedicine Pharmacotherapy*. 63: 187-193, 2009.

[18] Varsha Jatava, Pradeep Mishraa, Sushil Kashawa. CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. *European Journal of Medicinal Chemistry*. 43(9): 1945-1954, 2008.

[19] Abdel Ghany Aly El-Helby, Mohammed Hemeda Abdel Wahab. Design and synthesis of some new derivatives of 3H-quinazolin-4-one with promising anticonvulsant activity. *Acta Pharm*. 53: 127-138, 2003.

[20] Adel S. El-Azab, Kamal E.H. El Tahirb. Synthesis and anticonvulsant evaluation of some new 2,3,8-trisubstituted-4(3H)-quinazoline derivatives. *Bioorganic & Medicinal Chemistry Letters*. 22(1): 327-333, 2012.

[21] Adel S. El-Azaba, Kamal E.H. El Tahir. Design and synthesis of novel 7-aminoquinazoline derivatives: Antitumor and anticonvulsant activities. *Bioorganic & Medicinal Chemistry Letters*. 22(5): 1879-1885, 2012.

[22] Afshin Zarghi, Sayyed A. Tabatabai, Mehrdad Faizi, Avideh Ahadian, Parisa Navabi, Vahideh Zanganeh, Abbas Shafiee. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles. *Bioorganic & Medicinal Chemistry Letters*. 15(7): 1863-1865, 2005.

[23] Ali Almasirad, Sayyed A. Tabatabai, Mehrdad Faizi, Abbas Kebriaeezadeh, Nazila Mehrabi, Afshin Dalvandi, Abbas Shafiee. Synthesis and anticonvulsant activity of new 2-substituted-5- [2-(2-fluorophenoxy)

phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. *Bioorganic & Medicinal Chemistry Letters*. 14(24): 6057-6059, 2004.

[24] Hans Joachim Lankau, Klaus Unverferth, Christian Grunwald, Helge Hartenhauer, Kristina Heinecke, Katrin Bernöster, Rita Dost, Ute Egerland, Chris Rundfeldt. New GABA-modulating 1,2,4-oxadiazole derivatives and their anticonvulsant activity. *European Journal of Medicinal Chemistry*. 42(6): 873-879, 2007.

[25] Kashaw S.K., Gupta V., Kashaw V., Mishra P., Stables J.P., Jain N.K. Anticonvulsant andsedative-hypnotic activity of some novel 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2-styrylquinazoline-4(3H)-ones. *Medicinal Chemistry Research*. 19: 250-261, 2010.

[26] Zarghi A., Tabatabai S.A., Faizi M., Ahadian A., Navabi P., Zanganeh V., Shafiee A. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles. *Bioorganic & Medicinal Chemistry Letters*. 15: 1863-1865, 2005.

[27] Oliphant C.M., Green G.M. Quinolones: a comprehensive review. *Am Fam Physician*. 65 (3): 455-464, 2002.

[28] Nelson J.M., Chiller T.M., Powers J.H., Angulo F.J. Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clin Infect Dis*. 44 (7): 977-980, 2007.

[29] Gootz T.D., Zaniewski R., Haskell S., Schmieder B., Tankovic J., Girard D., Courvalin P., Polzer R.J. Activity of the new fluoroquinolone trovafloxacin against DNA gyrase and topoisomerase IV mutants of *Streptococcus pneumoniae* selected in vitro. *Antimicrob Agents Chemother*. 40(12): 2691-2697, 2006.

[30] Przybilla B., Georgii A., Bergner T., Ring J. Demonstration of quinolone phototoxicity in vitro. *Dermatologica*. 181(2): 98-103, 1990.

[31] Ozex., Rubinstein E. History of quinolones and their side effects. *Chemotherapy*. 47(3): 3-8, 2001.

[32] Ozex., Rubinstein E. History of quinolones and their side effects. *Chemotherapy*. 47(3): 3-8, 2001.

[33] Evaluation of certain veterinary drug residues in food. Fiftieth report of the joint FAO/WHO Expert Committee on Food Additives. *World Health Organ Tech Rep Ser*. 888: i-vii; 1-95, 1999.

[34] Hisashi Mikoshiba, Minami-ashigara, Takayoshi Kamio. Azo Dye Compound having 1’2’4'triazole as the Azo component. *US 6458194 B1*, Oct. 1, 2002.

[35] Jing-Pei Huo, Jian-Cheng Luo, Wei Wu, Jin-Feng Xiong, Guang-Zhen Mo, and Zhao-Yang Wang. Synthesis and Characterization of Fluorescent Brightening Agents with Chiral 2(5H)-Furanone and Bis-1, 2, 3-triazole Structure . *Ind. Eng. Chem. Res*. 52 (34): 11850–11857, 2013.

[36] Paul A. Worthington. Synthesis and Fungicidal Activity of Triazole Tertiary Alcohols. *Synthesis and Chemistry of Agrochemicals*. (32): 302-317, 1987.

[37] Lass-Flörl C. Triazole antifungal agents in invasive fungal infections: a comparative review. *Drugs*. (18) : 2405-2419, 2011.

CHAPTER III: MATERIALS, METHODS AND RESULTS- SYNTHESIS AND CHARACTERIZATION

The chapter materials, methods and results have been divided into two sub sections

- Synthesis and characterization

  In this section the route of synthesis, method of preparation, isolation and of purification has been discussed. This information is supplemented with identification and characterization data. Spectroscopic data of few representative compounds are provided for reference.
- Biological activity

  In this section the procedure followed to determine the biological activity of the synthesized molecules is discussed.

3.0 SYNTHESIS AND CHARACTERIZATION

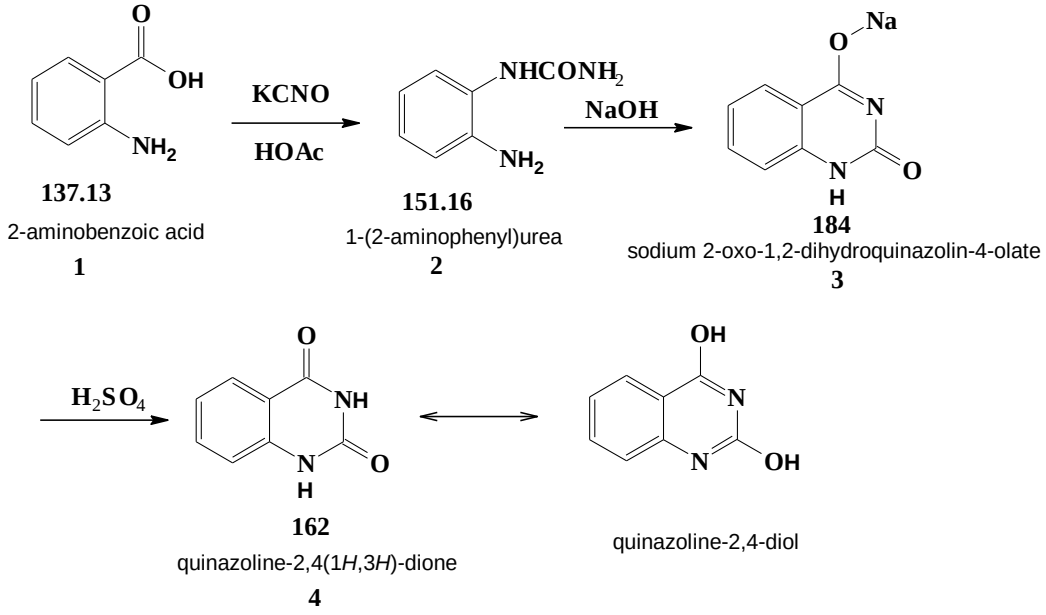
The preparation of the novel piperazine and triazole derivatives involved multiple steps. The synthetic steps have been elaborately discussed under each section along with the scheme.

3.1 Strategy I: Identification of new compounds with less structural resemblance to the existing drugs followed by random screening of the synthesized compounds using active seizure models

3.1a SYNTHESIS OF 4-CHLORO-2-(4- SUBSTITUTED -PIPERAZIN-1-YL) QUINAZOLINES

StepI: Preparation of quinazoline -2,4(1H,3H)-dione[Benzoylene urea]

Reaction



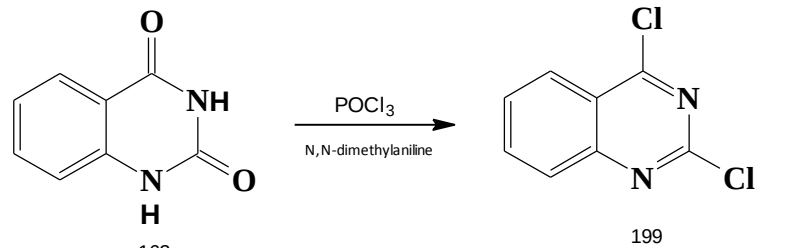
Procedure

In a 3-Litre round bottomed reactor set up , along with a condenser attachment and a heating/cooling provision, a mixture of 20 g (0.146 mole) of anthranilic acid, 700 ml of warm water (35°C) and 11 ml (11.6g, 0.19 mole) of glacial acetic acid was stirred mechanically and cooled to 25°C to 30°C. A freshly prepared solution of 15g (0.185 mole) of potassium cyanate in 50ml of water was added drop wise with stirring over a period of fifteen to twenty minutes. The resulting pasty mixture was stirred for twenty minutes and then 200g (5 moles) of flaked sodium hydroxide was added slowly in small portions. During this addition the reaction mixture was kept below 40°C, by cooling in a cold water bath. A clear solution was obtained momentarily, but in a short time a fine granular hydrated monosodium salt of benzoylene urea precipitated out. The reaction mixture was cooled overnight in an ice box. The precipitated sodium salt was collected on a büchner funnel, using a Whatman filter paper. The colourless salt was dissolved in 1 litre of hot water (90-95°C) and the solution was filtered and heated to boiling in a 3-Litre beaker. The benzoylene urea was precipitated by adding dilute sulphuric acid (1:1) with vigorous stirring until the liquor was

acidic. The product separated as a hydrate which forms small, lustrous, colourless needles. The material was collected on a büchner funnel, using a Whatman filter paper, washed with 200ml of water and dried in an oven at 100°C. Weight of the dried material was 16.5g. Melting point of the crude product was found to be 350-360°C (The result matches with the reported data).

Step II: Preparation of 2, 4-dichloroquinazoline

Reaction



162
quinazoline-2,4(1*H*,3*H*)-dione
4

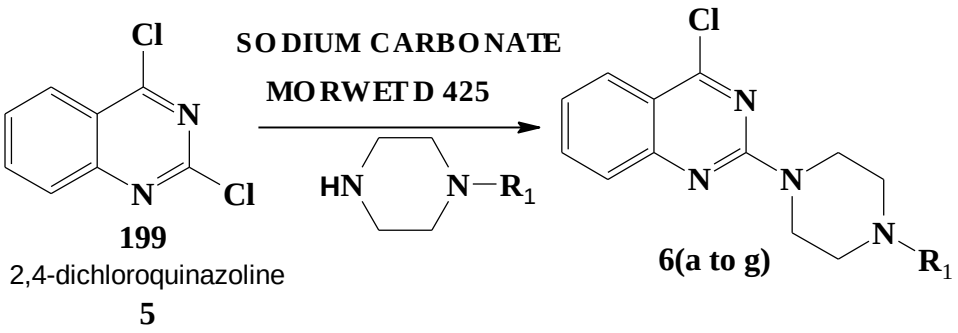
199
2,4-dichloroquinazoline
5

Procedure

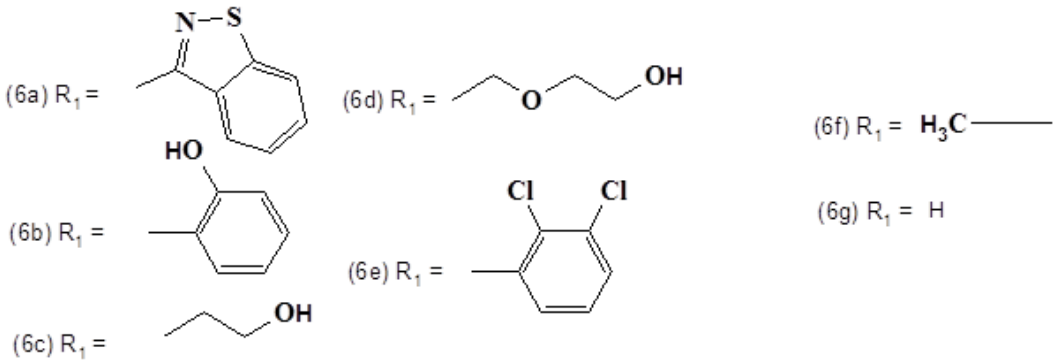
In a 500 ml round bottomed reactor 10.0g (0.061 mole) quinazoline-2, 4(1H, 3H)-dione (benzoylene urea), 14.2g (0.092 mole) of phosphorous oxychloride and 7.4g (0.061 mole) N, N-dimethylaniline were stirred mechanically and heated to 105° to 110°C. The progress of the reaction was monitored by thin layer chromatography (Mobile phase used, ethyl acetate: hexane =50:50). After the completion, the reaction mixture was cooled and poured onto ice water (0 to 5°C) under stirring. This resulted in the formation of a white precipitate. The nature of the precipitate was sticky. The resultant mass was basified with aqueous 20% w/v of potassium carbonate solution till pH 8.0. The mass was extracted with 200ml dichloromethane. The dichloromethane layer was given a water wash, dried over sodium sulphate and hence distilled on a Büchi rota evaporator to obtain 7g of 2, 4-dichloroquinazoline. Melting point of product was recorded at 118-121°C.

StepIII: Synthesis and characterization of a series of 4-chloro-2-(4-substituted-piperazin-1-yl) quinazolines [compounds 6(a- g)]

Reaction



The substitutions are,



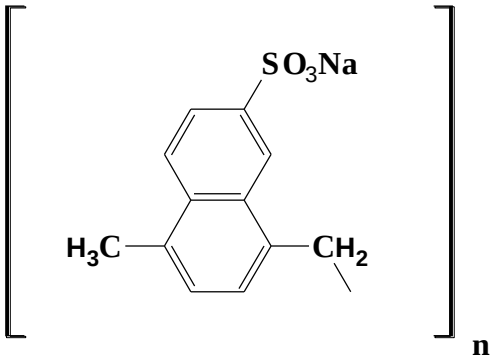
Procedure

1.5 moles of substituted piperazines, 1 mole of 2, 4-dichloroquinazoline, 3.3 moles of sodium carbonate, 5.2 times of water and 1% of dispersing agent MORWET® D-425, based on 2,4-dichloroquinazoline weight, were charged in to a round bottom glass reactor, stirred and refluxed under nitrogen for 12-16 hours. The progress of the reaction was monitored by thin layer chromatography (Mobile phase used, ethyl acetate: hexane=8:2). After the completion of the reaction, the reaction mass was cooled to 25°C to 30°C and the resulting mass was filtered. It was slurried in water, then in isopropyl alcohol and afterwards isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran to obtain pure compounds.

Note: The reaction between 2, 4-dichloroquinazoline and different substituted piperazines was initially done by using 5 volumes of DMF and 3.2 equivalents of

potassium carbonate. The reaction did not go for completion. Later the reaction was performed with 3.3 mole of sodium carbonate, 5.2 times of water based on the weight of 2, 4-dichloroquinazoline and 1% of dispersing agent, MORWET® D-425, based on the weight of 2, 4-dichloroquinazoline. This resulted in better yield and good purity.

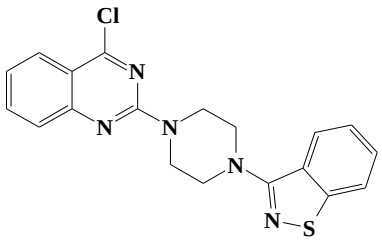
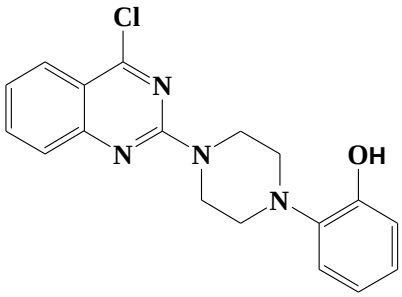
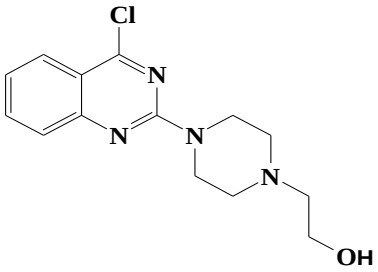
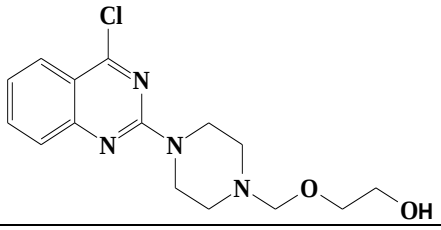
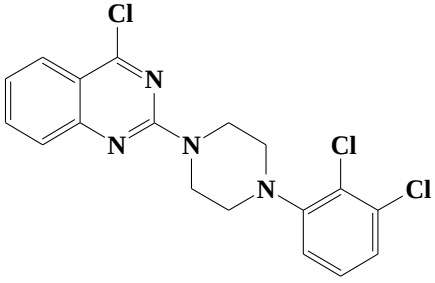
Dispersing agents like MORWET® D-425 are sodium salts of alkyl naphthylsulfonic acid/ formaldehyde condensates and sodium lignosulfonate.

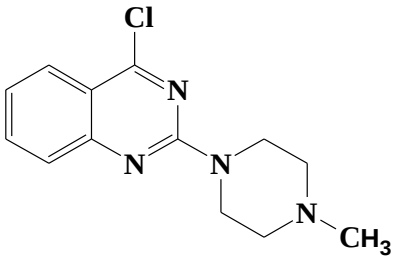
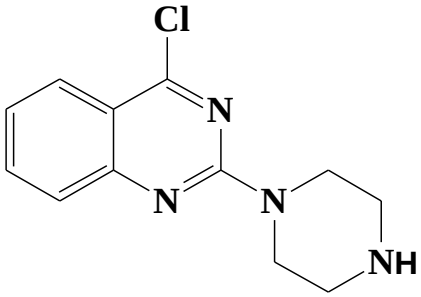


Wherein n ranges from 2 to 9

U.S.Pat.No.8, 410,268B2 disclosed a process for the preparation of Ziprasidone, which involves the same procedure [1].

Table 4: List of 4-chloro-2-(4- substituted-piperazin-1-yl) quinazolines [compounds 6(a-g)]

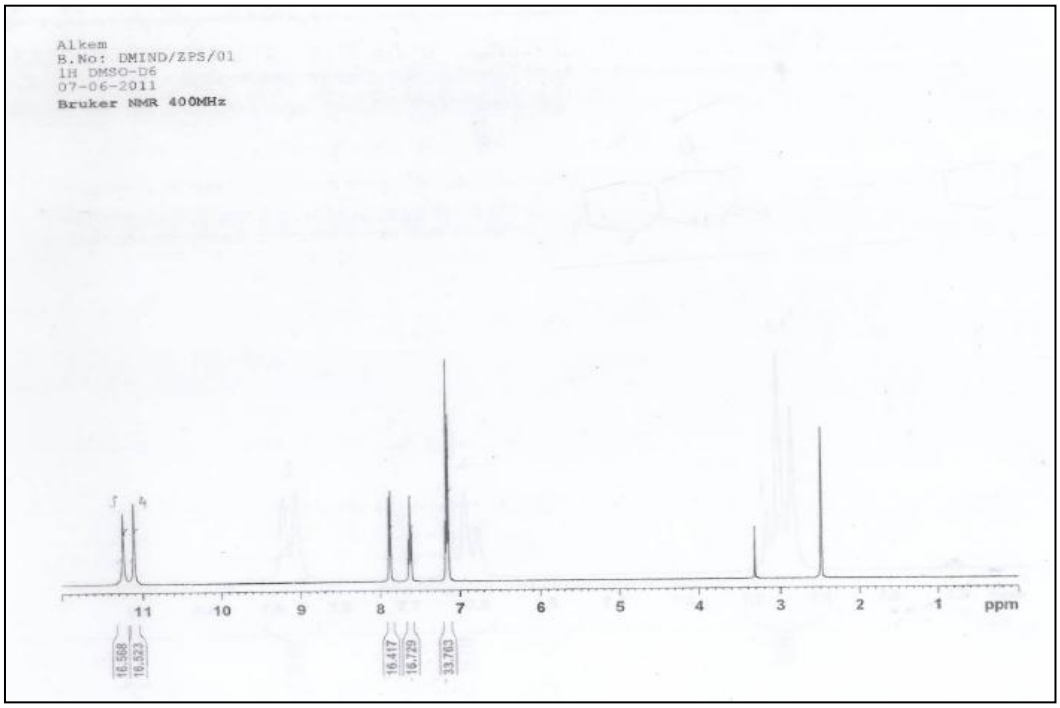
Compound code	Structure	Name	Mol Wt	MP (°C)
6a		**2-[4-(1,2-benzothiazol-3-yl)piperazin-1-yl]-4-chloroquinazoline**	**381**	**272**
6b		**2-[4-(4-chloroquinazolin-2-yl)piperazin-1-yl]phenol**	**340**	**202**
6c		**2-[4-(4-chloroquinazolin-2-yl)piperazin-1-yl]ethanol**	**292**	**198**
6d		**2-{[4-(4-chloroquinazolin-2-yl)piperazin-1-yl]methoxy}ethanol**	**322**	**201**
6e		**4-chloro-2-[4-(2,3-dichlorophenyl)piperazin-1-yl]quinazoline**	**393**	**218**

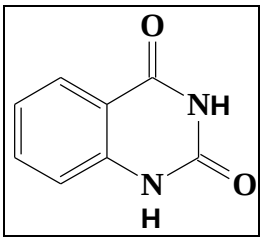
6 f		**4-chloro-2-(4-methylpiperazin-1-yl)quinazoline**	**263**	**189**
6 g		**4-chloro-2-(piperazin-1-yl)quinazoline**	**249**	**180**

3.1b CHARACTERIZATION OF 4-CHLORO-2-(4- SUBSTITUTED -PIPERAZIN-1-YL) QUINAZOLINES

Spectral data of representative compounds:

^{1}HNMR of quinazoline -2, 4(1H, 3H)-dione [benzoylene urea]:



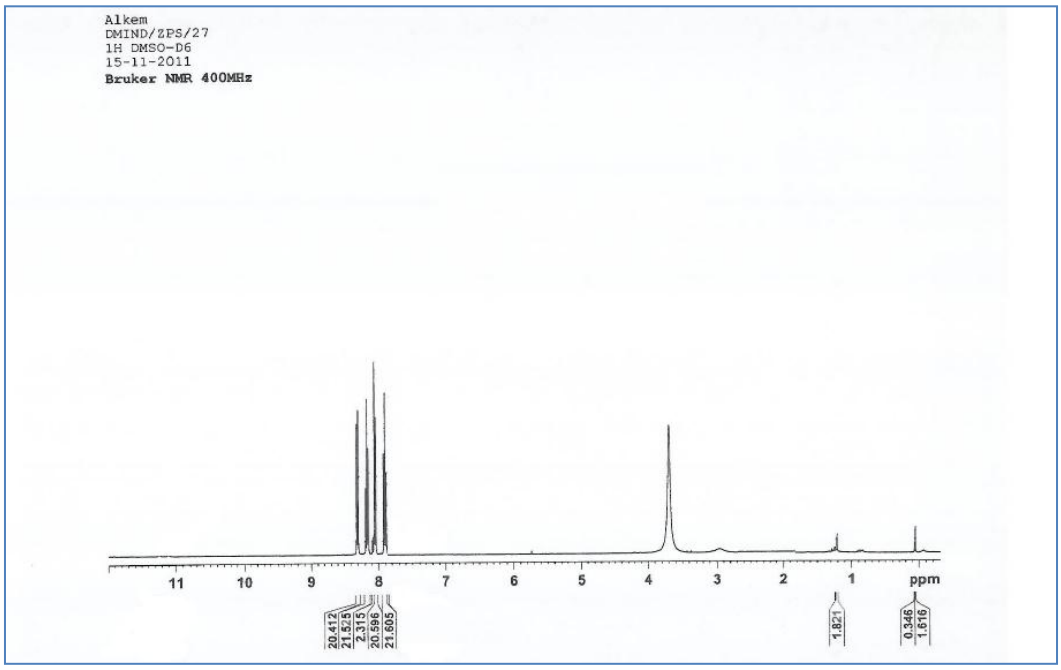


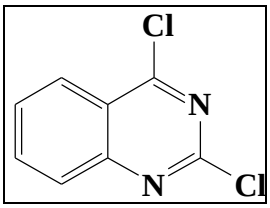
<u>The NMR depicts quinazoline-2,4-diol (Tautomer)</u>

<u>Interpretation:</u>

<u>^{1}HNMR (DMSO): 11.3 (1H,s,-OH); 11.1(1H,s,-OH); 7.9 (1H,d, -aromatic -CH); 7.6(1H,d,-aromatic CH);7.2(2H,t,-aromatic CH)</u>

[1]HNMR of 2,4-dichloroquinazoline:

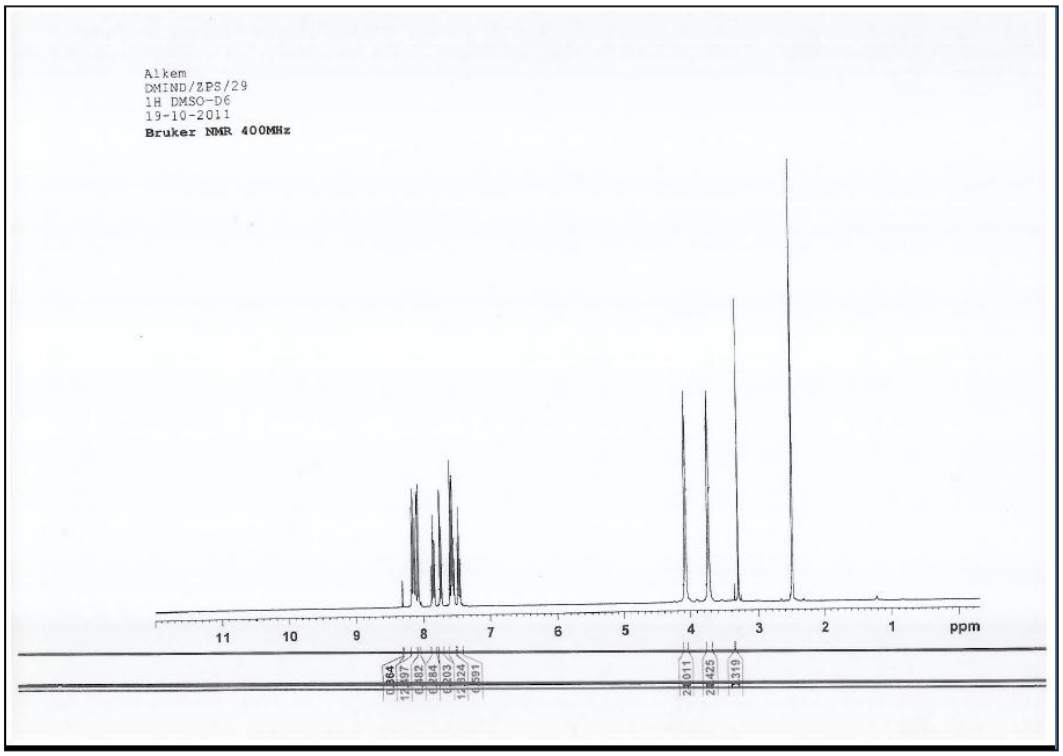


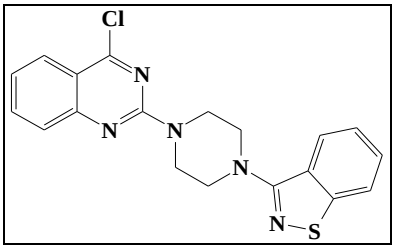


Interpretation:

[1]HNMR (DMSO D[6]): 8.3 (1H,d, -aromatic -CH); 8.2 (1H,d,-aromatic CH) ; 8.1 (1H,t,-aromatic CH); 7.9 (1H,t,-aromatic CH)

^{1}HNMR of 2-[4-(1, 2-benzoisothiazol-3-yl) piperazin-1-yl]-4-chloroquinazoline (6a):

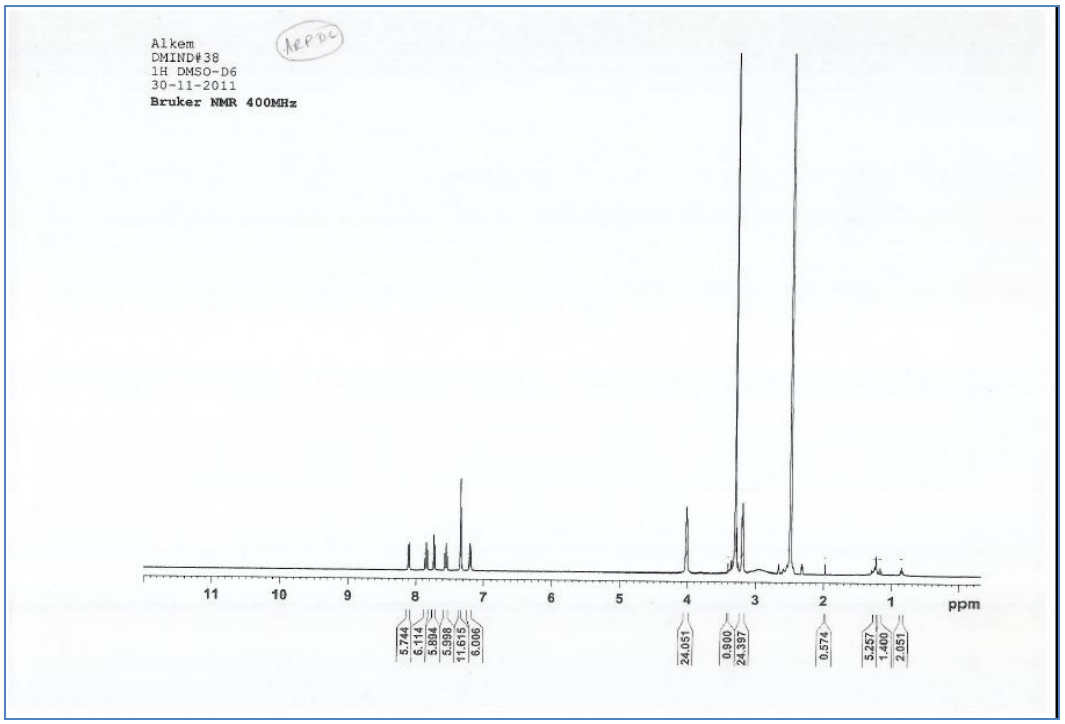


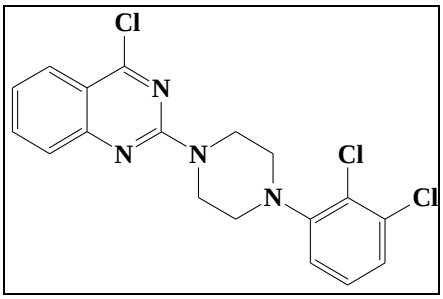


Interpretation:

^{1}HNMR(DMSO D^6): δ=8.30 (2H,m,quinazoline aromatic CH); 8.16 (1H,d,quinazoline aromatic CH); 7.86 (1H,m,quinazoline aromatic CH); 7.74 (1H,d, benzisothiazole aromatic CH); 7.55 (2H,m, benzisothiazole aromatic CH); 7.48 (1H,d, benzisothiazole aromatic CH);4.08(4H,t,piperazine-CH_2);3.70 (4H,t,piperazine CH_2)

[1]HNMR 4-chloro-2-[4-(2, 3-dichlorophenyl) piperazin-1-yl] quinazoline (6e):

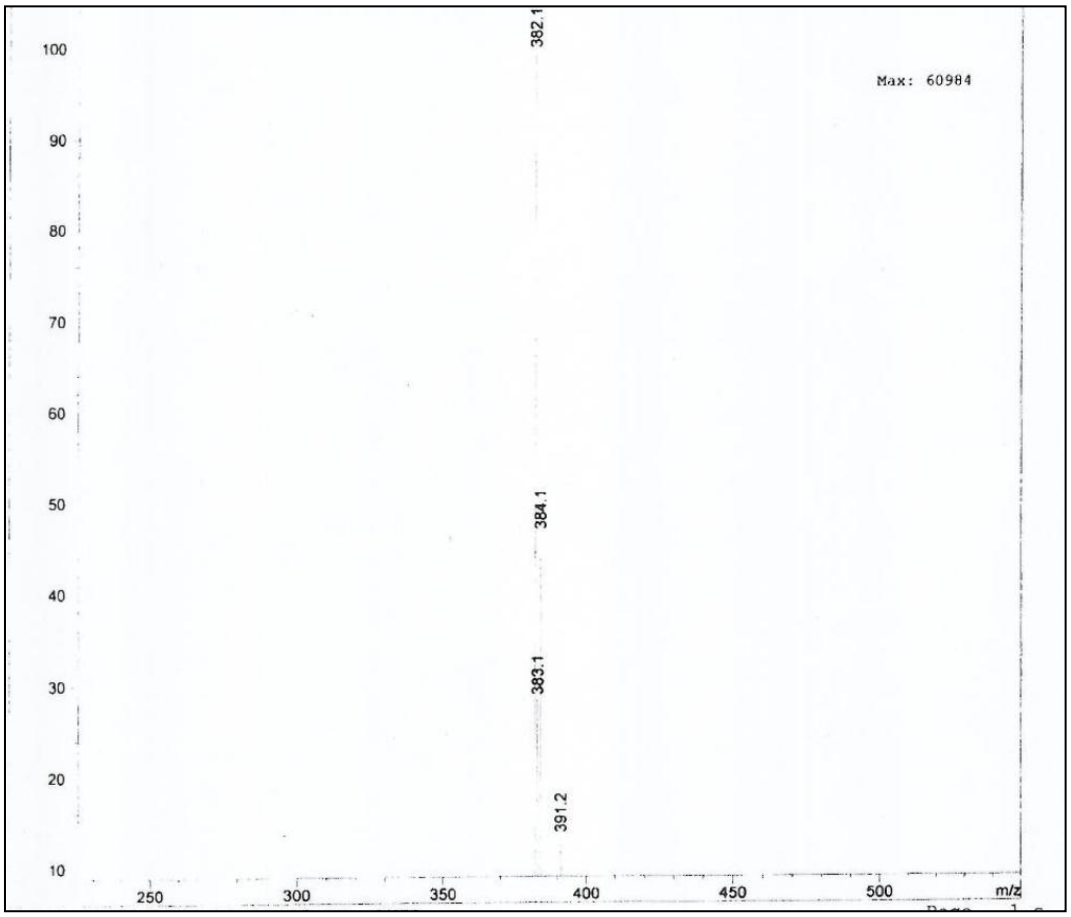




Interpretation:

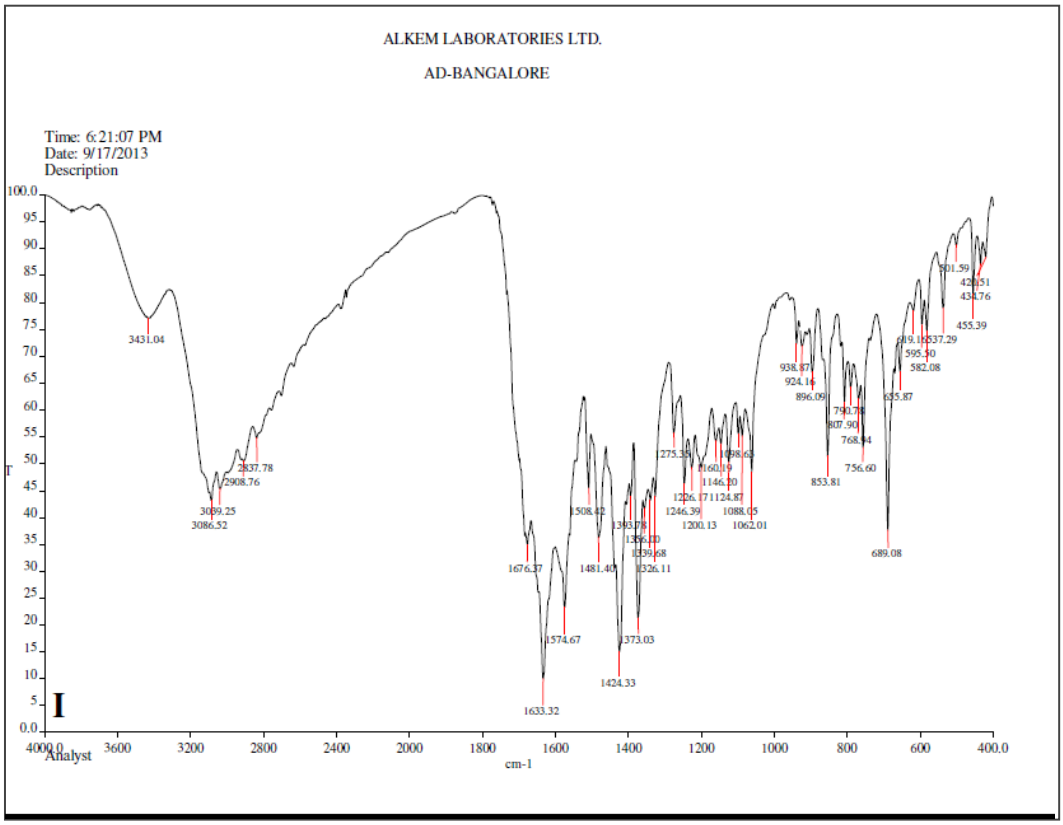
[1]HNMR(DMSO D^6): δ=8.1(1H,d,quinazoline aromatic CH); 7.85 (1H,t,quinazoline aromatic CH); 7.73 (1H,d,quinazoline aromatic CH); 7.55 (1H,t,quinazoline aromatic CH); 7.35 (2H,d,phenyl aromatic CH); 7.2 (1H,m,-aromatic -CH); 4.0 (4H,m,piperazine CH_2); 3.35 (4H,m,piperazine CH_2)

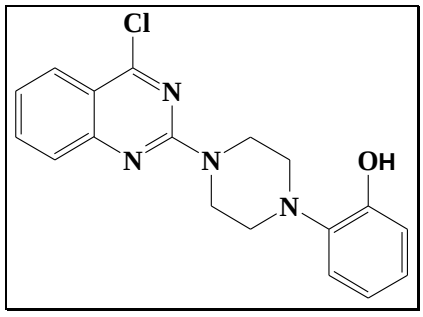
Mass Spectrum of 2-[4-(1, 2-benzoisothiazol-3-yl) piperazin-1-yl]-4-chloroquinazoline (6a):



The mass spectrum conforms to the molecular weight (381) of the synthesized compound

Infrared spectrum of 2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] phenol (6b):

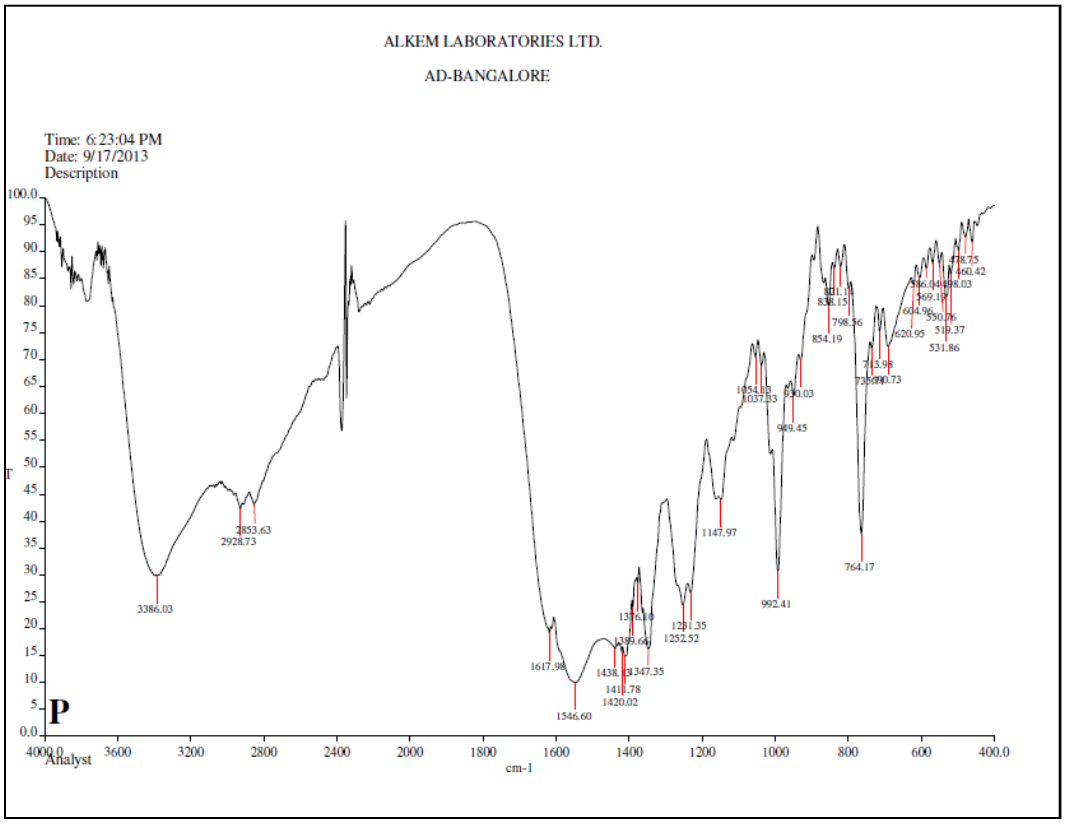


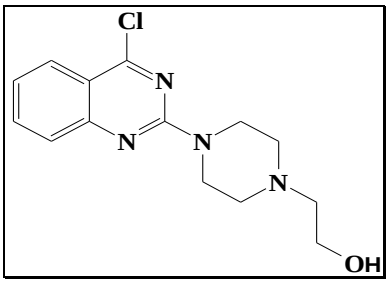


Interpretation:

IR max cm^{-1}: 3431.04 (-OH); 3039.25 (Ar-CH); 1481.40 (Ar C=C); 1676.37 (HC=N); 1226 (C-O); 756.60 (C-Cl)

Infrared spectrum of 2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] ethanol (6c):

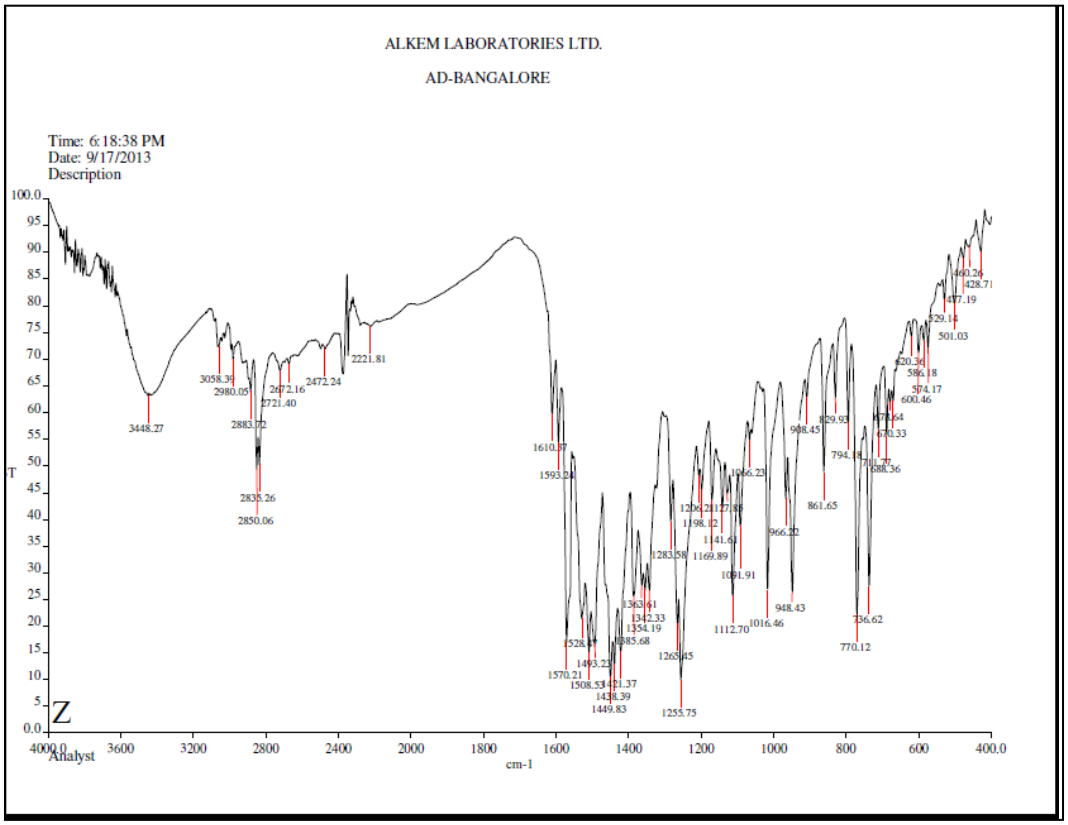


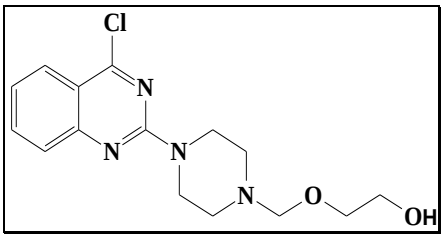


Interpretation:

IR max cm^{-1}: 3386.03 (-OH); 2928.73 (Ar-CH); 1617.98 (HC=N); 1546.60 (Ar C=C); 770.12 (C-Cl)

Infrared spectrum of 2-{[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] methoxy} ethanol (6d):





Interpretation:

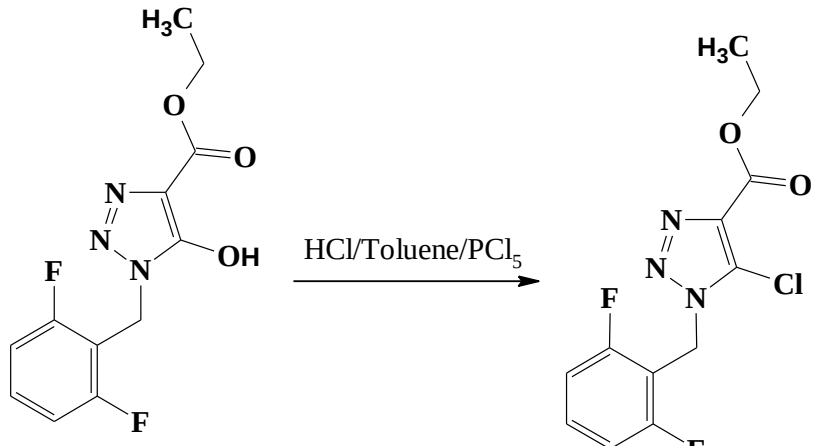
<u>IR max cm^{-1}: 3448.27 (-OH); 3058.39 (Ar-CH); 1508.53 (Ar C=C); 1066.23 (C-O); 770.12 (C-Cl)</u>

3.2 Strategy II: <u>Molecular modification of existing drugs followed by random screening of the synthesized compounds using active seizure models</u>

SYNTHESIS OF 2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOLE DERIVATIVES

<u>Step I: Preparation of ethyl-5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carboxylate</u>

<u>Reaction</u>



283.23
Ethyl -(2,6-difluorobenzyl)-5-hydroxy-1*H*-1,2,3-triazole-4-carboxylate
(1)

301.67
Ethyl 5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate
(2)

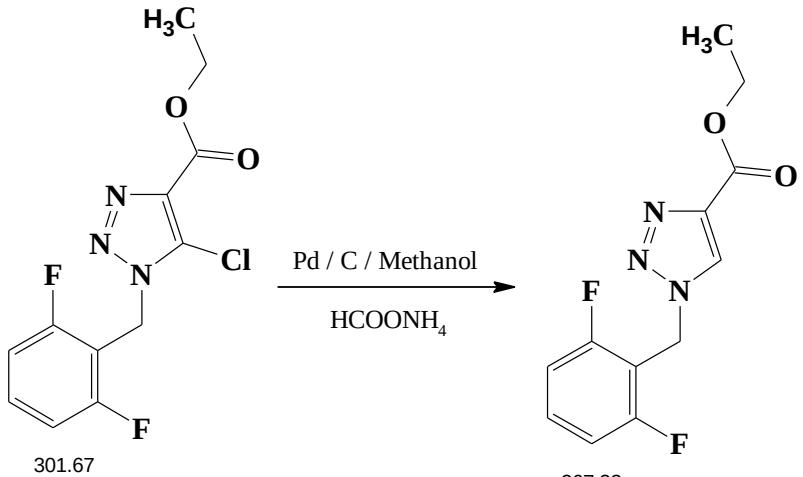
<u>Procedure</u>

170ml of toluene was added to a 250 ml glass round bottom reactor at 25°C to 30°C. 17g (0.06 mole) of ethyl-(2, 6-difluorobenzyl)-5-hydroxy-1H-1, 2, 3-triazole-4-carboxylate was added. 34ml of water and 34ml of concentrated hydrochloric acid was added to the reaction mixture. The reaction mass was heated to 65-70^{o}C and stirred for the next 2 hours. The progress of the reaction was monitored by thin layer chromatography (Mobile phase used, ethylacetate: hexane=3:7). After completion of the reaction, it was cooled to 25-30^{o}C. The reaction mass was transferred into a 500.0 ml glass separating flask and thereby the bottom aqueous layer was separated after 15 minutes. 17ml of toluene was added to the separated aqueous layer and was stirred for 15 minutes followed by

layer separation for another 15 minutes. The top organic layer was collected and mixed with earlier separated organic layer. The entire organic layer was taken for azeotropic distillation with the aid of a dean stark apparatus. The distillation was performed till no more water collected in the receiver flask of the dean stark apparatus. After completion of the distillation, the residue was cooled to 30-35^{o}C. To the residue, 17g (0.080 mole) of phosphorous pentchloride was added in 3-4 lots in course of about 20-30 minutes. After addition, the reaction mass was heated to 110-115^{o}C and was maintained for 2 hours. After completion, the reaction mass was cooled to 10-20^{o}C and later 57.5 ml of cold water (10-15^{o} C) was added. The reaction mass was stirred for 15 minutes and was transferred to 500.0 ml glass separating flask. The bottom aqueous layer was separated after 15 minutes. Again 51 ml of water was added and stirred for 15 minutes. The mass was allowed to separate into layers. The bottom aqueous layer was separated. 20 ml of 10 % (w/v) sodium carbonate solution was added gradually to the combined organic layer and was made to pH~8. Gradual addition was done to counter the effervescence. Layer separation was performed and the bottom aqueous layer was separated after 10 minutes. Then 51 ml of water was added to the organic layer and stirred for 15 minutes. The layer separation was allowed and the bottom aqueous layer was separated. The top organic layer was isolated and was taken for distillation in a rota evaporator (Büchi) till no more solvent distilled out. After distillation, the reaction mixture was cooled to 10-15^{o}C. 17ml of methanol was added and the reaction mixture was stirred at 10-15^{o}C for 10 minutes. The resultant precipitate was filtered over a thickened filter cloth kept on a büchner funnel, given a cold methanol wash (10-15^{o}C). The material was suck dried for 1 hour. The isolated material was taken for the next step.

Step II: Preparation of ethyl - (2, 6-difluorobenzyl)-1H- 1, 2, 3-triazole-4-carboxylate

Reaction



301.67

Ethyl 5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate

(2)

267.23

Ethyl -(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate

(3)

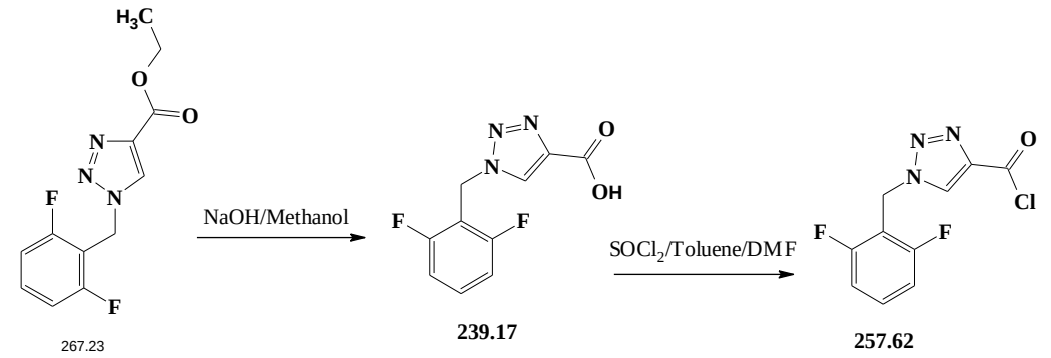
Procedure

150 ml of methanol was charged into a 500.0 ml round bottom glass reactor at 25°C to 30°C. 8.75g (0.03 mole) of ethyl-5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carboxylate was added to the reaction mixture under stirring. 5.26g (0.08 mole) of ammonium formate at 25°C to 30°C followed by a slurry of 10 % w/v of Pd/C slurry in methanol (0.43g in 5 ml). The reaction mass was heated to 45-50^{o}C and maintained for 3 hours.The reaction was monitored using thin layer chromatography at every hours interval using chloroform :methanol = 9:1 as the mobile phase . The reaction mixture was filtered after three hours. The filtration was done on a high flow-super cell bed to remove the Pd/C catalyst. Later the filtrate was distilled to 80% of the volume under vacuum (600 mm Hg) and at a temperature of 60-70^{o} C using a rota evaporator. After distillation, the residue was cooled to 10-15^{o} C and stirred for 15 minutes. The precipitated material was filtered. The material was dried in an air drier at 50-60^{o} C for 5 hours.

The dried material was taken for the next step.

Step III: preparation of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbonyl chloride

Reaction



267.23

Ethyl -(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate **(3)**

239.17

1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid **(4)**

257.62

1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbonyl chloride **(5)**

Procedure

A mixture of 5.0 g (0.02 mole) of ethyl - (2, 6-difluorobenzyl)-1*H*- 1, 2, 3-triazole-4-carboxylate and 5 volumes of methanol was taken in a 100.0 ml round bottomed glass reactor, stirred at 25°C to 30°C till dissolution. To this reaction mass 2.0 equivalent of 1.0 M sodium hydroxide solution was added gradually under stirring. After addition, the reaction mass was heated to 40°C and was maintained for two hours. The reaction was monitored by thin layer chromatography using silica gel plates and chloroform: methanol=9:1 as the mobile phase. The reaction was continued until the complete consumption of the starting ester ethyl - (2, 6-difluorobenzyl)-1*H*- 1, 2, 3-triazole-4-carboxylate was observed. The reaction mixture was then poured onto ice water and acidized to pH 3 with dilute hydrochloric acid. The white precipitate formed was filtered on a Whatman filter paper. The precipitate was washed with ice cold water till the washings showed pH 7. The isolated acid was dried under vacuum at 40°C for 7 hours.

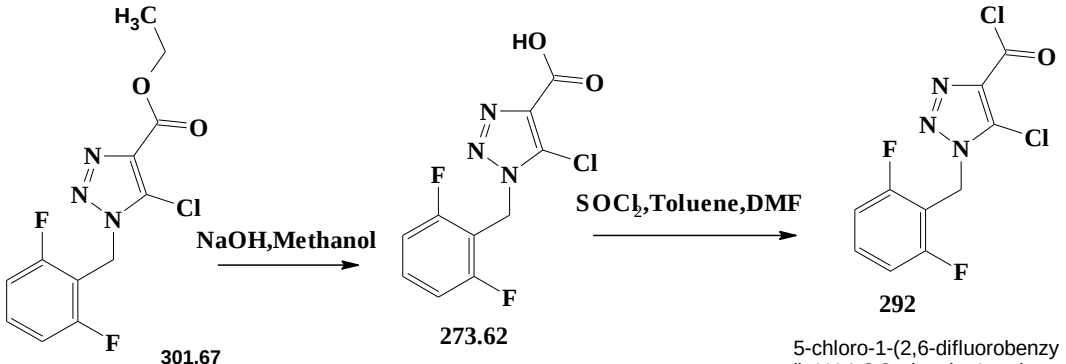
Preparation of the acid chloride, 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbonyl chloride

0.2 g (0.001) mole of the acid 1-(2, 6-diflurobenzyl)-1H-1, 2, 3-tiazole-4 carboxylic acid, 10.0 ml toluene was charged into a completely dried 100.0 ml round bottom flask fitted with a condenser whose vent is attached to a calcium chloride guard tube. The other side of the guard tube vent was in turn connected to a Teflon pipe which ended in a beaker of 10% sodium hydroxide solution. This was done to prevent the escape of excess thionyl chloride to the atmosphere. 4.75 g (0.04) mole of thionyl chloride was gradually added to the reaction mass with the help of a glass addition funnel. A catalytic amount of DMF was added to facilitate the completion. The reaction mass was then heated to 70°C. The agitation was continued for another 1 hour at the same temperature. The solvent and the excess thionyl chloride were distilled off under vacuum at 60°C. The residue was dissolved in toluene. The toluene was stripped off under vacuum. The acid chloride was again dissolved in toluene and the insoluble portions were filtered and the filtrate was evaporated to an oily mass in vacuum at not more than 60°C. Repetitive dissolution in toluene followed by concentration of the mass was done to ensure maximum removal of thionyl chloride. The formed acid chloride was dissolved in 10.0 ml DMF and hence taken for the subsequent steps to make 8 (a-g).

Note: Acid chlorides are unstable. Hence the formed acid chlorides were immediately taken for the subsequent steps.

Step IV: Preparation of 5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbonyl chloride

Reaction



Ethyl-5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate **(2)**

5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid **(6)**

5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbonyl chloride **(7)**

Procedure

A mixture of 6.0 g (0.02 mole) of ethyl -5-chloro-1- (2, 6-difluorobenzyl)-1*H*- 1, 2, 3-triazole-4-carboxylate and 5 volumes of methanol was taken in a 100.0 ml round bottomed glass reactor, stirred at 25°C to 30°C till dissolution. To this reaction mass 2.0 equivalent of 1.0 M sodium hydroxide solution was added gradually under stirring. After addition, the reaction mass was heated to 40°C and was maintained for two hours. The reaction was monitored by thin layer chromatography using silica gel plates and chloroform: methanol=9:1 as the mobile phase. The reaction was continued until the complete consumption of the starting ester, ethyl -5-chloro-1- (2, 6-difluorobenzyl)-1*H*- 1, 2, 3-triazole-4-carboxylate was observed. The reaction mixture was then poured onto ice water and acidized to pH 3 with dilute hydrochloric acid. The white precipitate formed was filtered on a thickened filter paper. The precipitate was washed with ice cold water till the washings showed pH 7. The isolated acid was dried under vacuum at 40°C for 7 hours.

Preparation of acid chloride

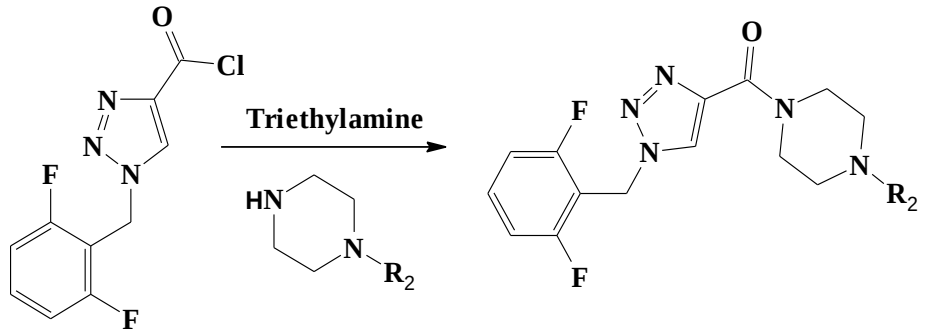
0.3 g (0.001) mole of the acid 5-chloro1-(2, 6-diflurobenzyl)-1H-1, 2, 3-tiazole-4 carboxylic acid, 10.0 ml toluene was charged into a completely dried 100.0 ml round bottom flask fitted with a condenser whose vent is attached to a calcium chloride guard tube. The other side of the guard tube vent was in turn connected to a Teflon pipe which ended in a beaker of 10% sodium hydroxide solution. This was done to prevent the escape of excess thionyl chloride to the atmosphere. 4.75 g (0.04) mole of thionyl chloride was gradually added to the reaction mass with the help of a glass addition funnel. A catalytic amount of DMF was added to facilitate the completion. The reaction mass was then heated to 70°C. The agitation was continued for another 1 hour at the same temperature. The solvent and the excess thionyl chloride were distilled off under vacuum at 60°C. The residue was dissolved in toluene. The toluene was stripped off under vacuum. The acid chloride was again dissolved in toluene and the insoluble portions were filtered and the filtrate was evaporated to an oily mass in vacuum at not more than 60°C. Repetitive dissolution in toluene followed by concentration of the mass was done to ensure maximum removal of thionyl chloride. The formed acid chloride was dissolved in 10.0 ml DMF and hence taken for the subsequent steps to make 9 (a-g).

Note: Acid chlorides are not stable for a long time hence it was prepared in lots and consumed immediately.

3.2.1a SYNTHESIS OF 1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4- SUBSTITUTED- PIPERAZIN-1-YL) METHANONES

<u>Step V:</u> Preparation of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted- piperazin-1-yl) methanones

<u>Reaction</u>

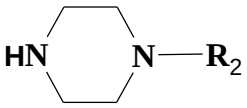


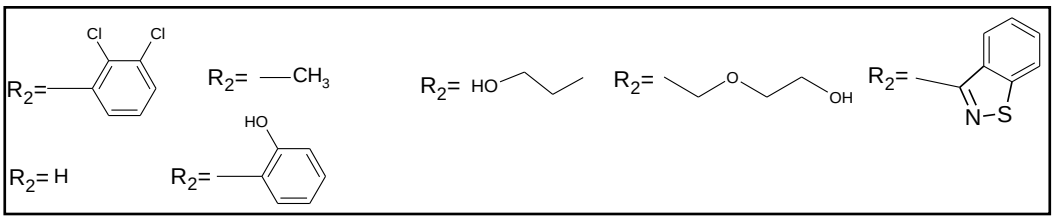
257.62

1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbonyl chloride **(5)**

[1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]methanone (piperazin-1-yl) substituted derivatives 8 (a-g)

Substituted piperazines in synthesis of series 8 (a-g)

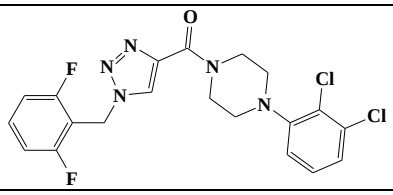
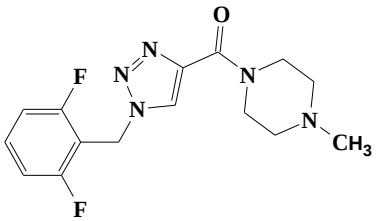
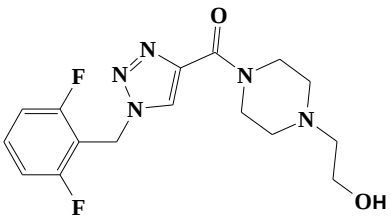


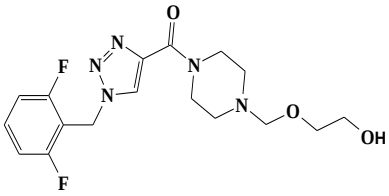
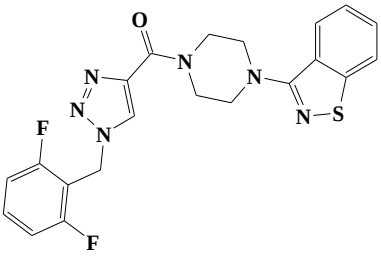
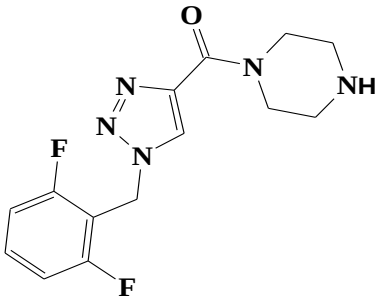
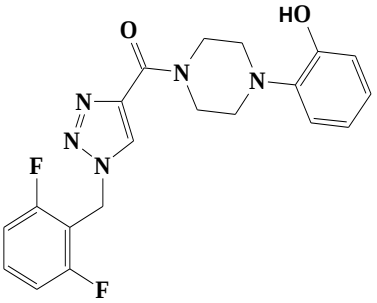


Procedure

A 10.0 ml solution of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbonyl chloride in DMF was taken in a 100.0 ml glass round bottom reactor attached to a condenser and a mechanical stirrer. 1.5 equivalents (0.0015 moles) of substituted piperazines were added. Triethylamine was added to the reaction mixture and the reaction mass was stirred for 5 hours at 80°C. A cold water circulation was run through the condenser to prevent the escape of triethylamine. The progress of the reaction was monitored by thin layer chromatography (mobile phase used was chloroform: methanol=9:1) Upon completion of the reaction, the mass was cooled to 25°C -30°C and poured to ice water (5-10°C) under stirring. The solid thrown was filtered and given cold water slurry to remove triethylamine hydrochloride. Finally the material was washed with diisopropylether and was dried under vacuum (600 mm Hg) to obtain substantially pure 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted- piperazin-1-yl) methanones.

Table 5: List of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted-piperazin-1-yl) methanones [8 (a-g)]

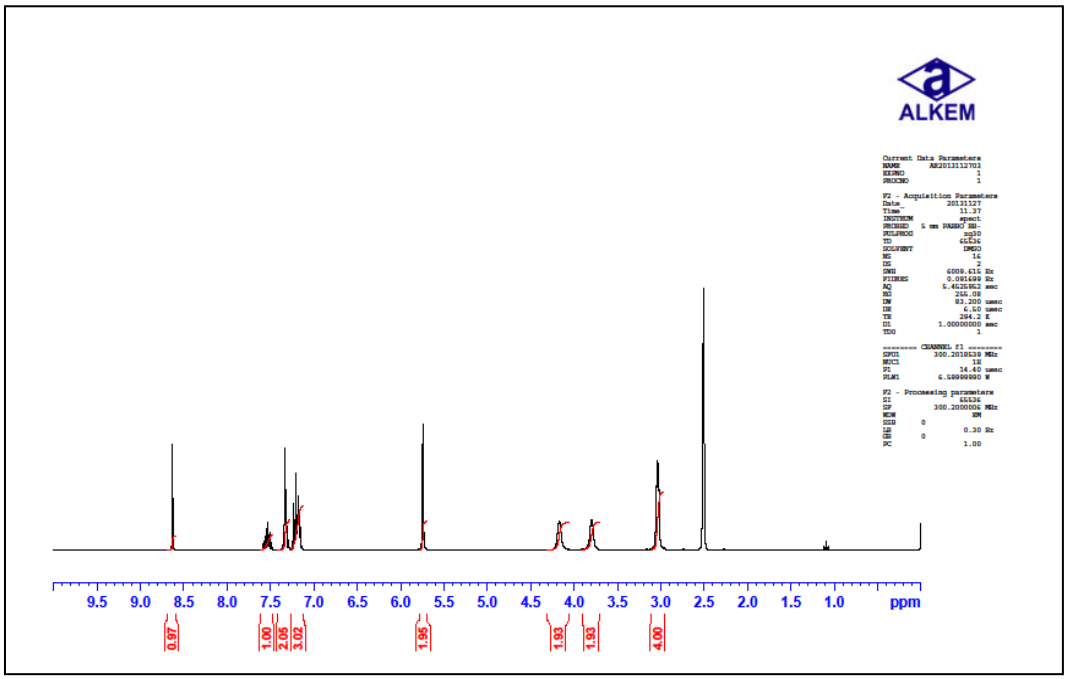
Compound code	Structure	Name	Mol. Wt	MP (°C)
8 a		**[4-(2,3-dichlorophenyl)piperazin-1-yl][1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]methanone**	**451**	**136**
8 b		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl](4-methylpiperazin-1-yl)methanone**	**321**	**159**
8 c		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl][4-(2-hydroxyethyl)piperazin-1-yl]methanone**	**351**	**158**

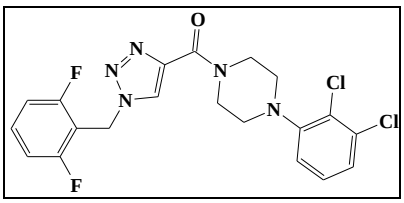
8 d		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]{4-[(2-hydroxyethoxy)methyl]piperazin-1-yl}methanone**	**381**	**164**
8 e		**[4-(1,2-benzothiazol-3-yl)piperazin-1-yl][1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]methanone**	**440**	**124**
8 f		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl](piperazin-1-yl)methanone**	**307**	**162**
8 g		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl][4-(2-hydroxyphenyl)piperazin-1-yl]methanone**	**399**	**173**

3.2.1b CHARACTERIZATION OF 1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4- SUBSTITUTED- PIPERAZIN-1-YL) METHANONES

Spectral data of representative compounds:

^{1}HNMR of [4-(2, 3-dichlorophenyl) piperazin-1-yl] [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (8 a):

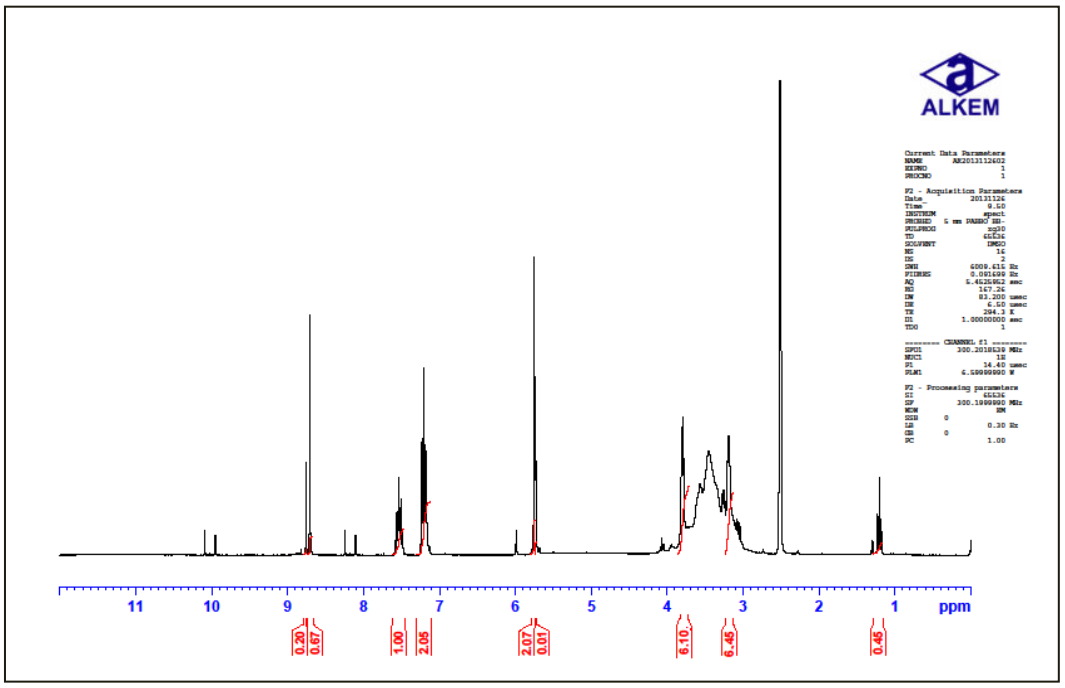


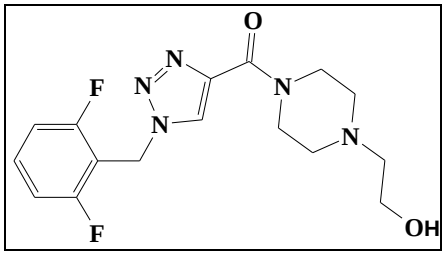


Interpretation:

^{1}HNMR (DMSO-d6): 8.7(1H, s, triazole CH); 7.6(1H, t, aromatic -CH in di fluoro phenyl ring);7.3(2H, aromatic CH of dichlorobenzene ring);7.1(3H,aromatic CH of the substituted phenyl rings);5.7(2H,s,-CH_2);4.2(2H,t,-CH_2 of piperazine); 3.8 (2H,t,-CH_2 of piperazine); 3.0 (4H,t,-CH_2 of piperazine)

[1]HNMR of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyethyl) piperazin-1-yl] methanone (8 c):

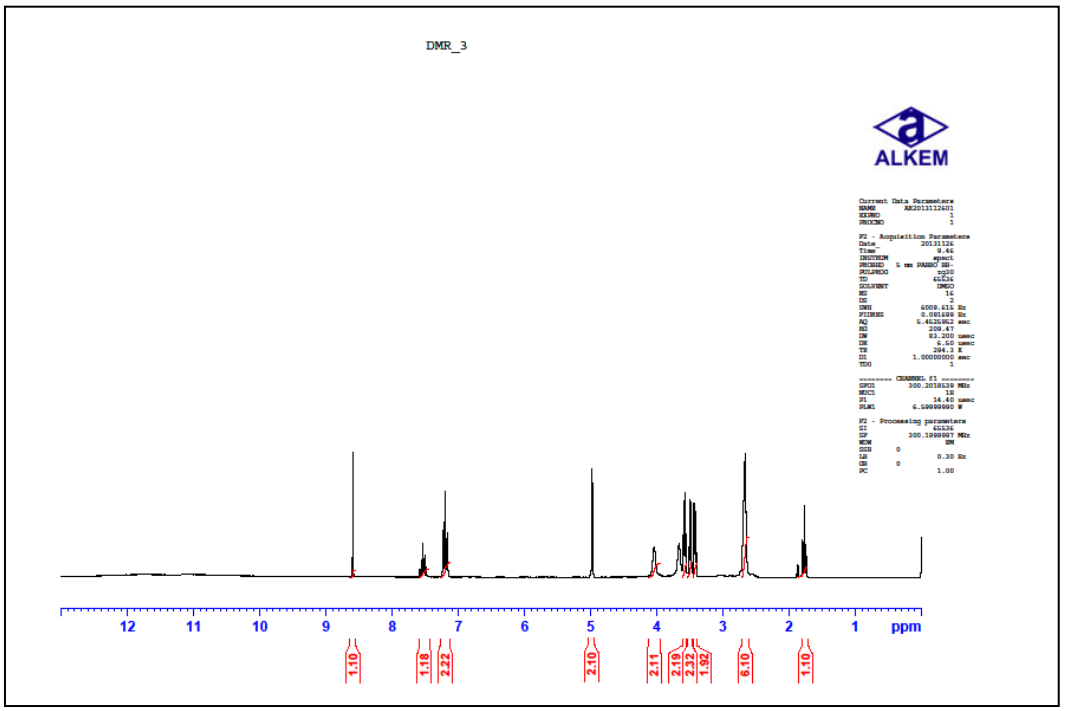


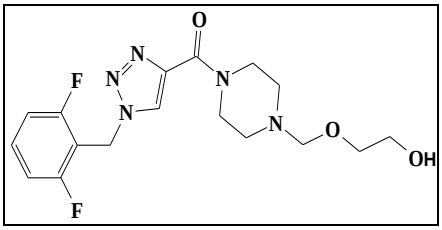


Interpretation:

[1]HNMR (DMSO-d6): 8.7 (1H, s, triazole CH); 7.5 (1H, t, aromatic -CH of di fluoro phenyl ring); 7.2 (2H, d, aromatic CH of di fluoro phenyl ring); 5.8 (2H, s,-CH_2); 3.8 (4 H,-CH_2 and 2 H of -CH_2 of piperazine) ; 3.2 (6H,-CH_2 of piperazine); 1.3 (1H, t,-OH)

[1]HNMR of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] {4-[(2-hydroxyethoxy) methyl] piperazin-1-yl} methanone (8 d):

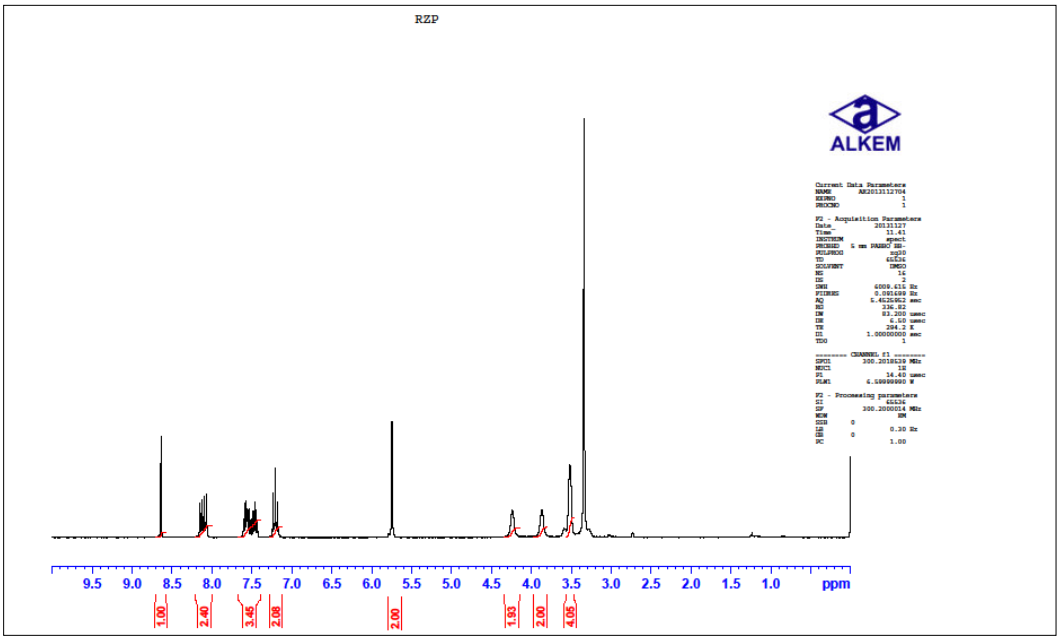


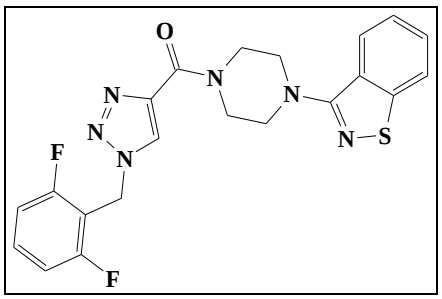


Interpretation:

[1]HNMR (DMSO-d6): 8.6 (1H, s, triazole CH); 7.5 (1H, t, aromatic -CH of di fluoro phenyl ring); 7.2 (2H, d, aromatic CH of di fluoro phenyl ring); 5.0 (2H, s,-CH_2); 4.0 (2H, t,-CH_2); 3.7 (2H, t,-CH_2); 3.5 (2H,t,-CH_2) ; 3.4 (2H of -CH_2 of piperazine) 2.8 (6H,-CH_2 of piperazine);1.9(1H,t,-OH)

[1]HNMR of [4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (8 e):

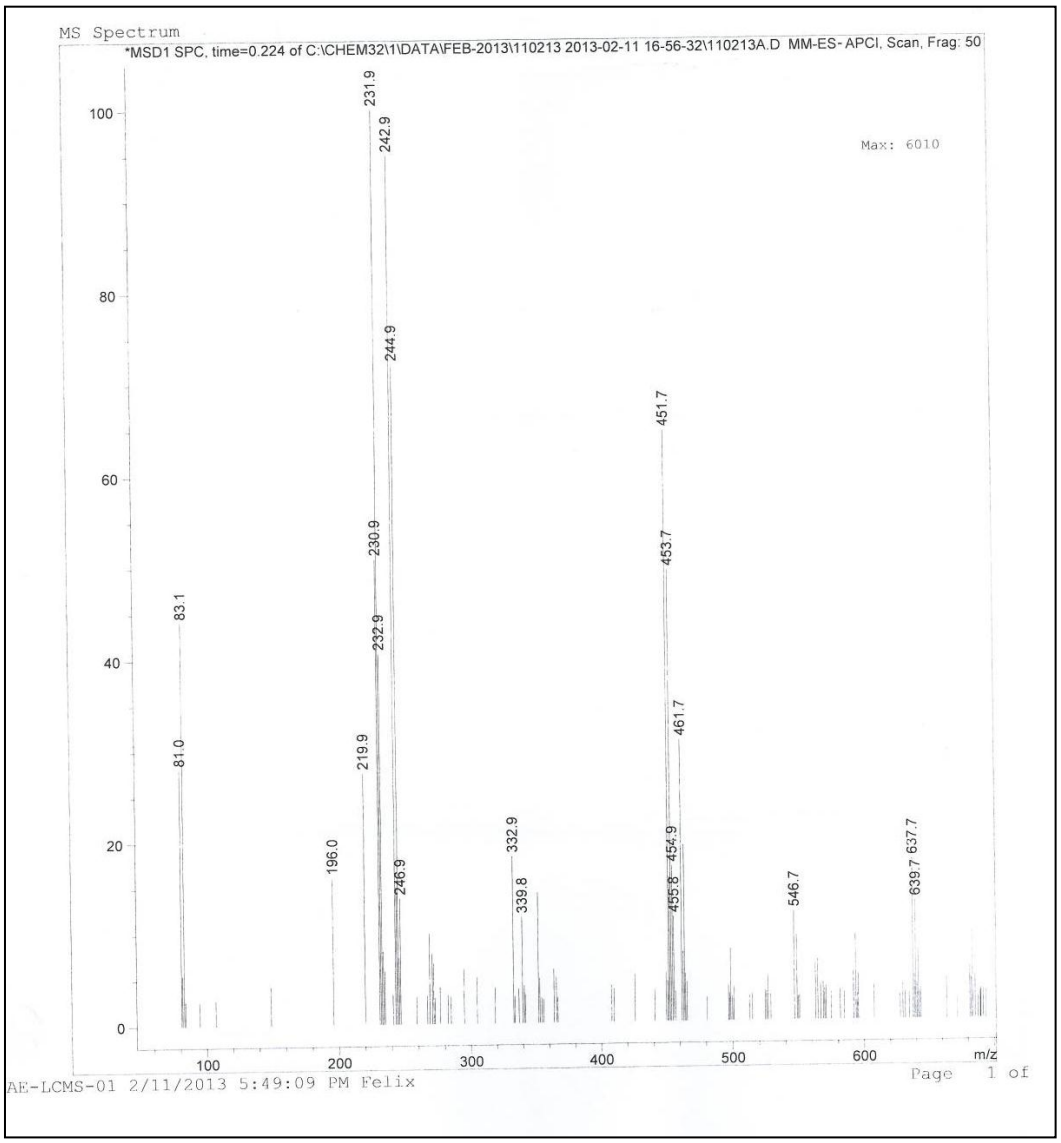




Interpretation:

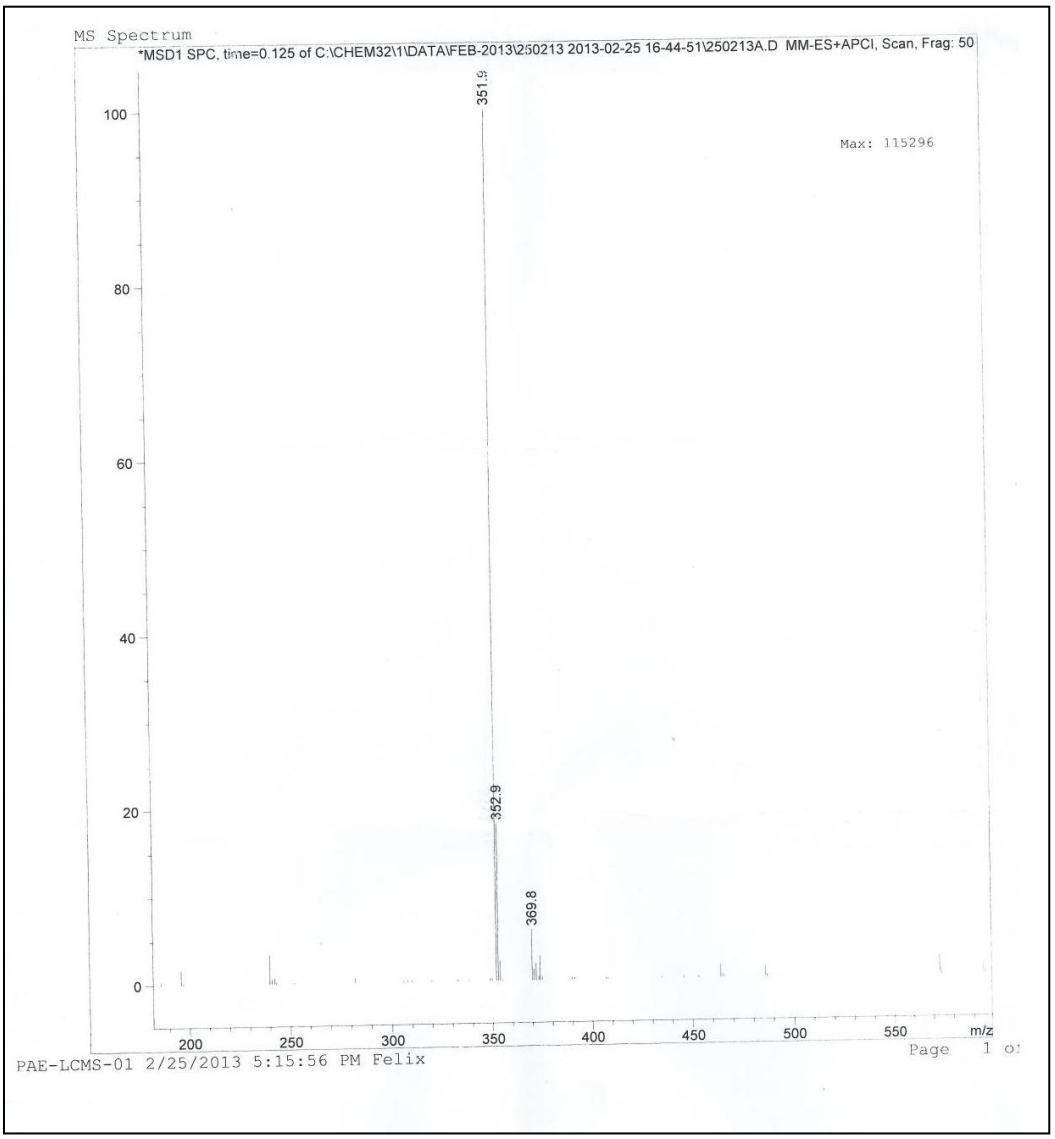
[1]HNMR (DMSO-d6): 8.7(1H, s, triazole CH); 8.1 (2H, t, aromatic -CH in benzo isothiazole ring);7.6 (3H, aromatic CH);7.2 (2H, Aromatic CH of di fluoro phenyl rings);5.8 (2H,s,-CH_2);4.3(2H,t,-CH_2 of piperazine); 3.9 (2H,t,-CH_2 of piperazine); 3.5 (4H,t,-CH_2 of piperazine)

Mass spectrum of [4-(2, 3-dichlorophenyl) piperazin-1-yl] [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (8a):



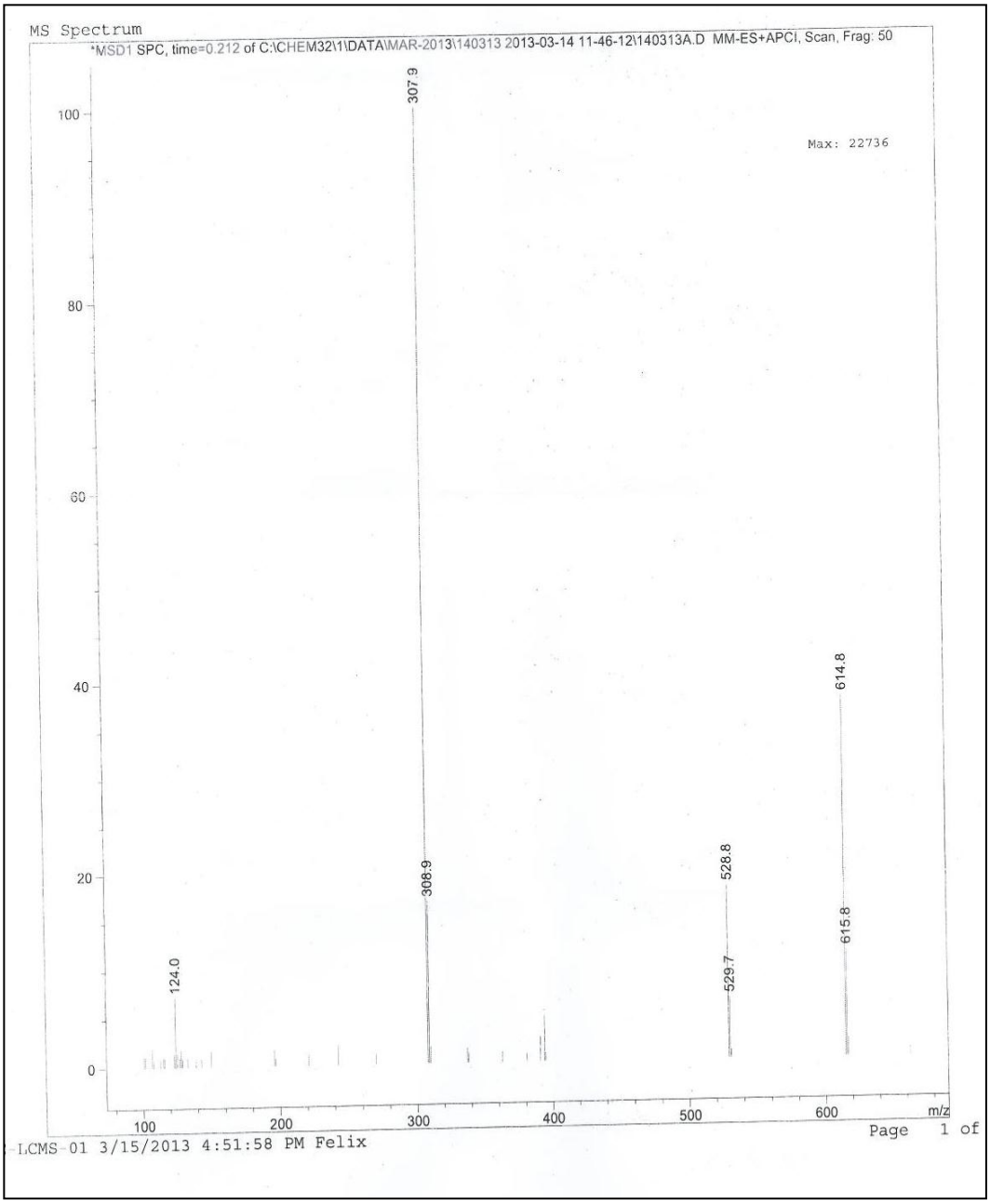
The mass spectrum conforms to the molecular weight (451) of the synthesized compound

Mass spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyethyl) piperazin-1-yl] methanone (8c):



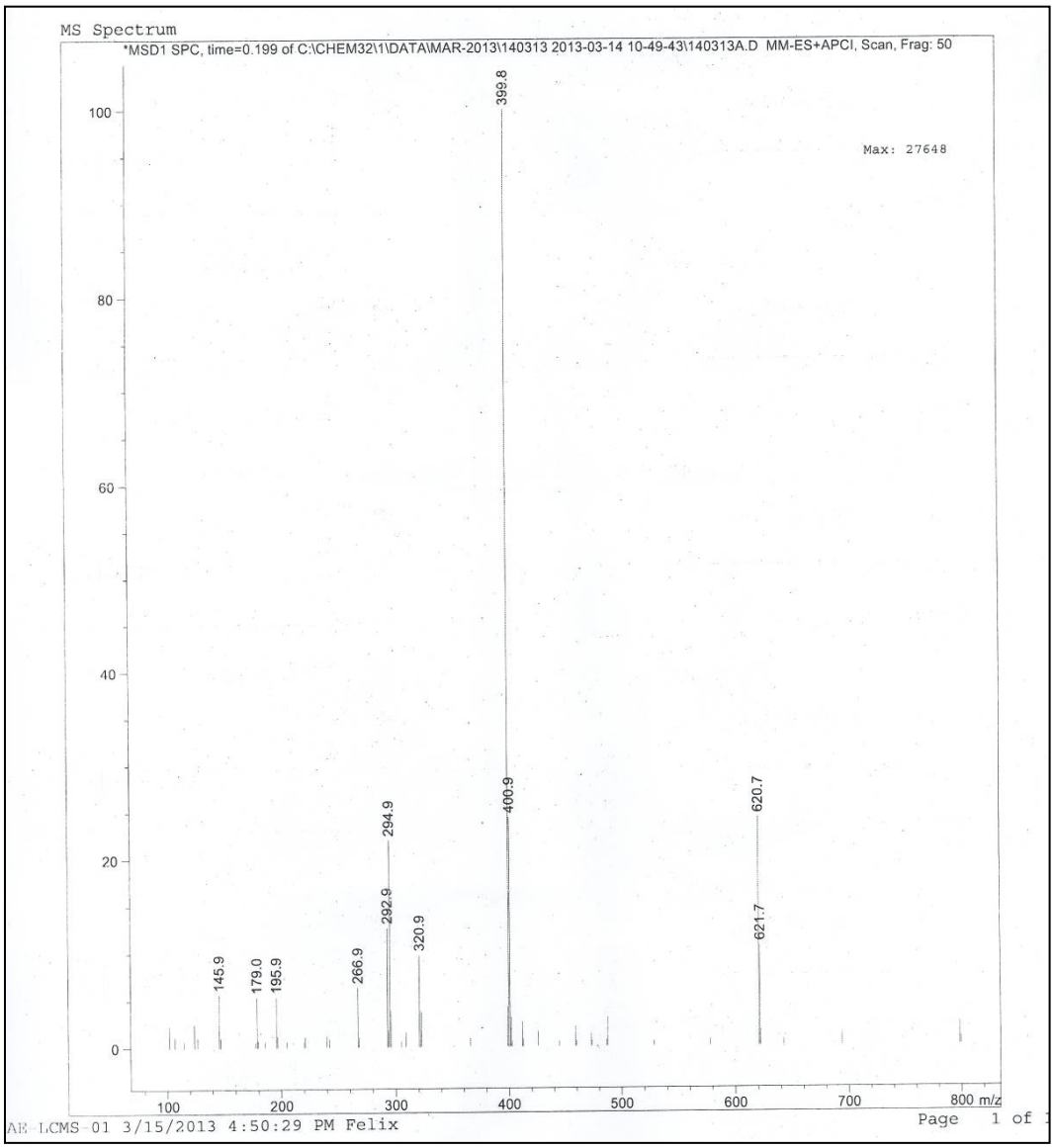
The mass spectrum conforms to the molecular weight (351) of the synthesized compound

Mass spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (piperazin-1-yl) methanone (8f):



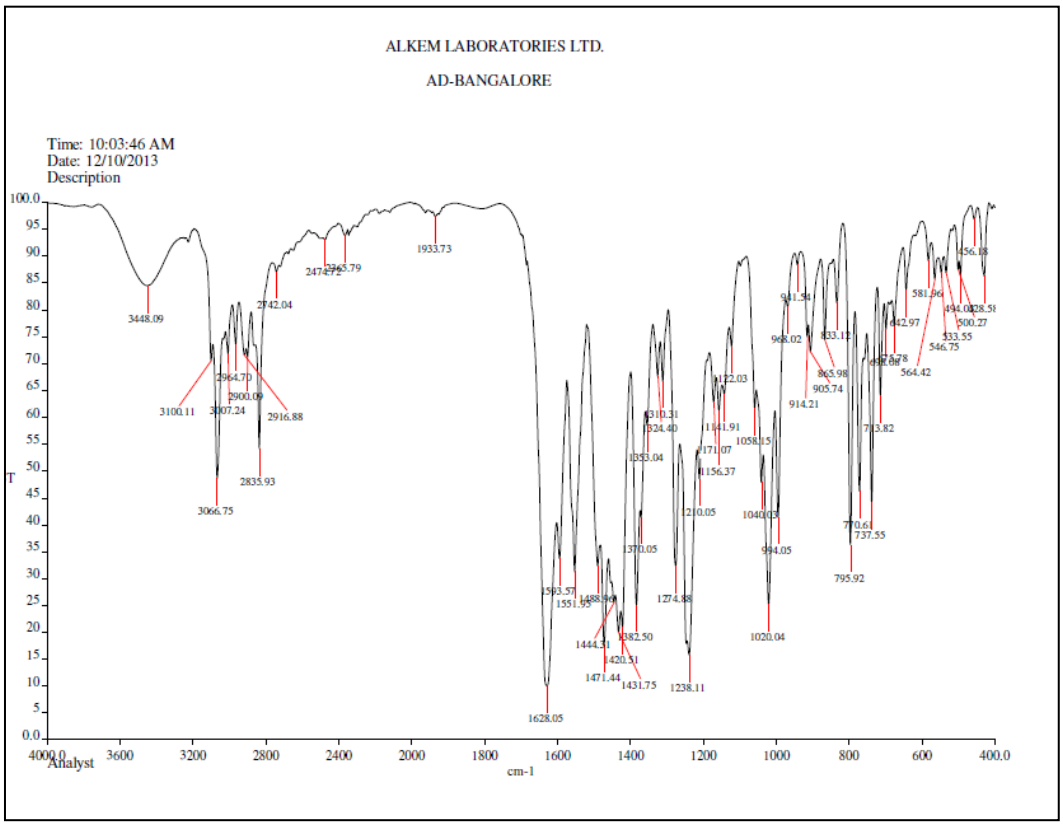
The mass spectrum conforms to the molecular weight (307) of the synthesized compound

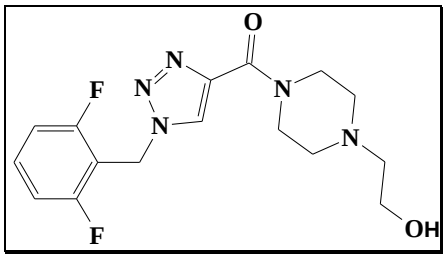
Mass spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyphenyl) piperazin-1-yl] methanone (8g):



The mass spectrum conforms to the molecular weight (399) of the synthesized compound

Infrared spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyethyl) piperazin-1-yl] methanone (8c):

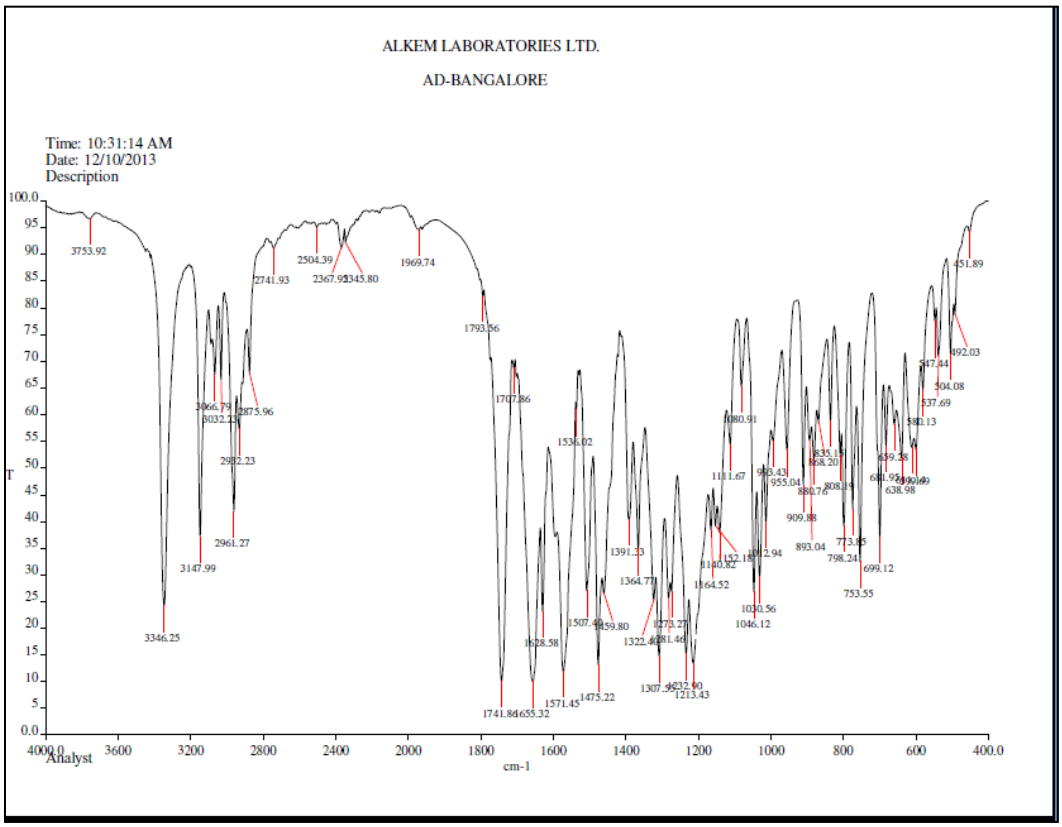


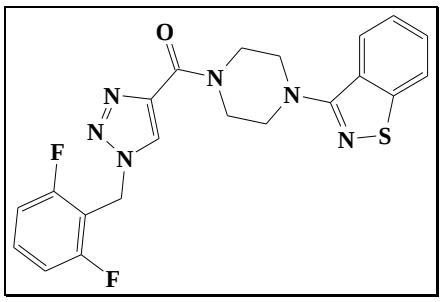


Interpretation:

IR max cm^{-1}: 3448.09 (O-H stretch H-bonded); 3066.75 (aromatic sp^2 C-H stretch); 795.92(aromatic out of plane bending); 1628.05 (-C=O stretch); 1488.90 (-C=N); 1471.44(-CH_2 stretch); 1020.04 (C-F stretch)

Infrared spectrum of [4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (8e):

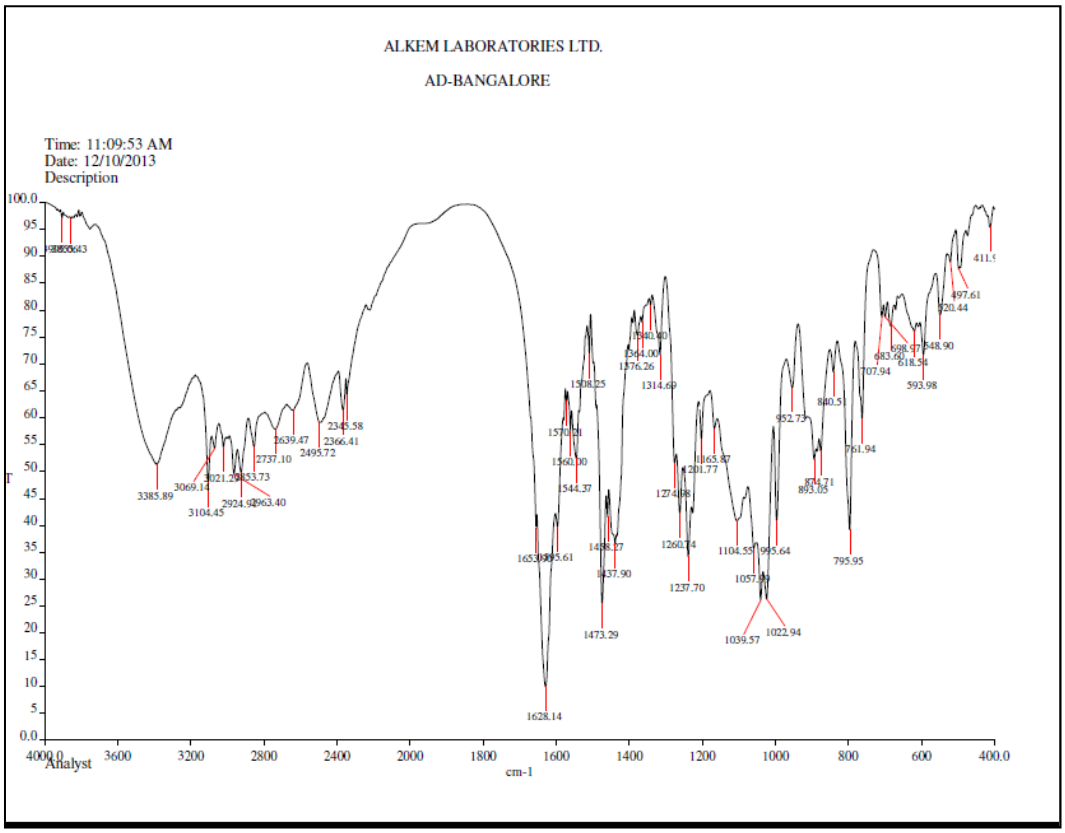


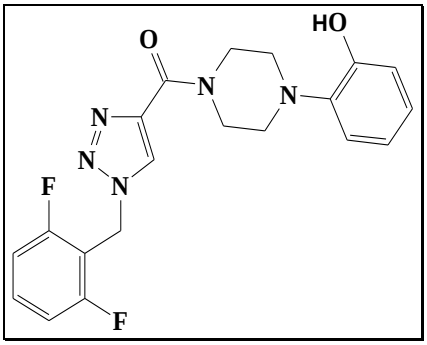


Interpretation:

IR max cm^{-1}: 3147.99 (aromatic sp^2 C-H stretch); 1655.32 (-C=O stretch); 1475.22(-CH_2 stretch); 1507.40 (-C=N); 1232.90 (C-S-C); 1046.12 (C-F stretch); 798.24(aromatic out of plane bending)

Infrared spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyphenyl) piperazin-1-yl] methanone (8g):





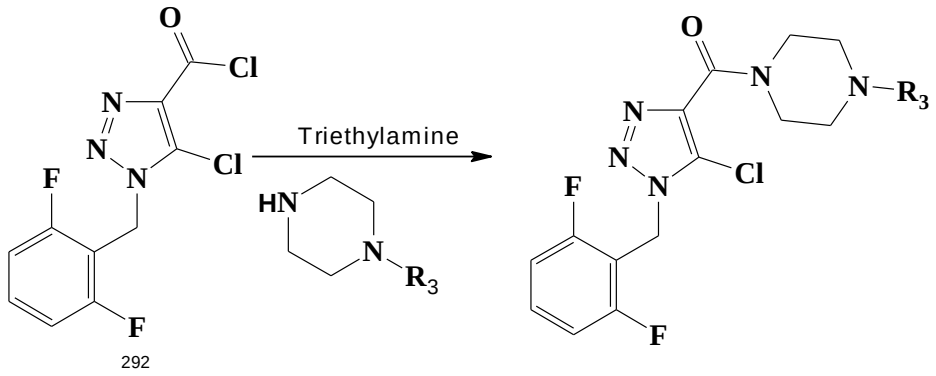
Interpretation:

IR max cm^{-1}: 3385.89 (O-H stretch H-bonded); 2924.92 (aromatic sp^2 C-H stretch); 795.95 (aromatic out of plane bending); 1628.14 (-C=O stretch); 1544.37 (-C=N); 1473.29 (-CH_2 stretch); 1237.70(-C-O stretch); 1039.57 (C-F stretch)

3.2.2a SYNTHESIS OF [5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4-SUBSTITUTED- PIPERAZIN-1-YL) METHANONES

Step VI: Preparation of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted- piperazin-1-yl) methanones

Reaction

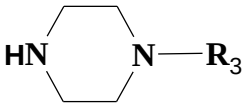


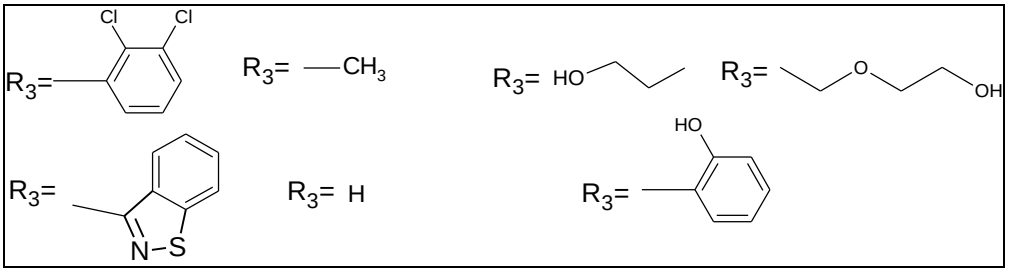
292

5-chloro-1-(2,6-difluorobenzyl)-1 *H*-1,2,3-triazole-4-carbonyl chloride**(7)**

[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2, 3-triazol-4-yl]methanone piperazin-1-yl derivatives 9 (a-g)

Substituted piperazines in synthesis of series 9 (a-g)

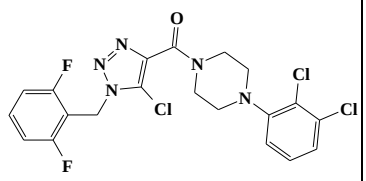
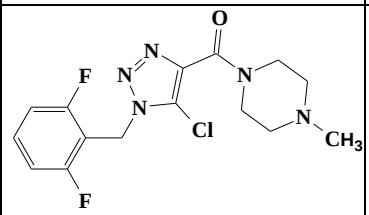
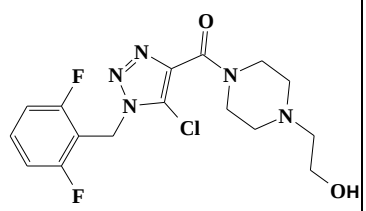


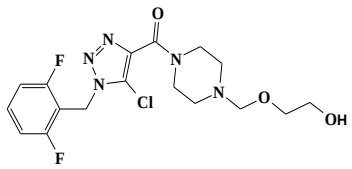
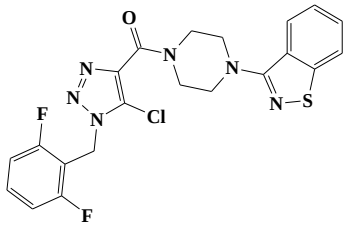
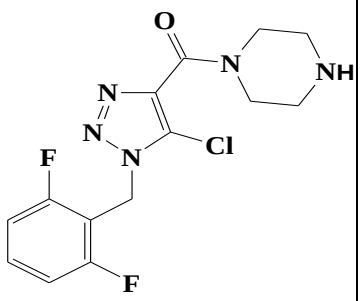
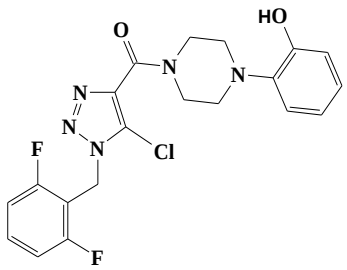


Procedure

A 10.0 ml solution of 5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbonyl chloride in DMF was taken in a 100.0 ml glass round bottom reactor attached to a condenser and a mechanical stirrer. 1.5 equivalents (0.0015 moles) of substituted piperazines were added. Triethylamine was added to the reaction mixture and the reaction mass was stirred for 5 hours at 80°C. A cold water circulation was run through the condenser to prevent the escape of triethylamine. The progress of the reaction was monitored by thin layer chromatography (mobile phase used was chloroform: methanol=9:1) Upon completion of the reaction, the mass was cooled to 25°C -30°C and poured to ice water (5-10°C) under stirring. The solid thrown was filtered and given cold water slurry to remove triethylamine hydrochloride. Finally the material was washed with diisopropylether and was dried under vacuum (600 mm Hg) to obtain substantially pure 5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4- substituted- piperazin-1-yl) methanones.

Table 6: List of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-substituted- piperazin-1-yl) methanones [9(a-g)]

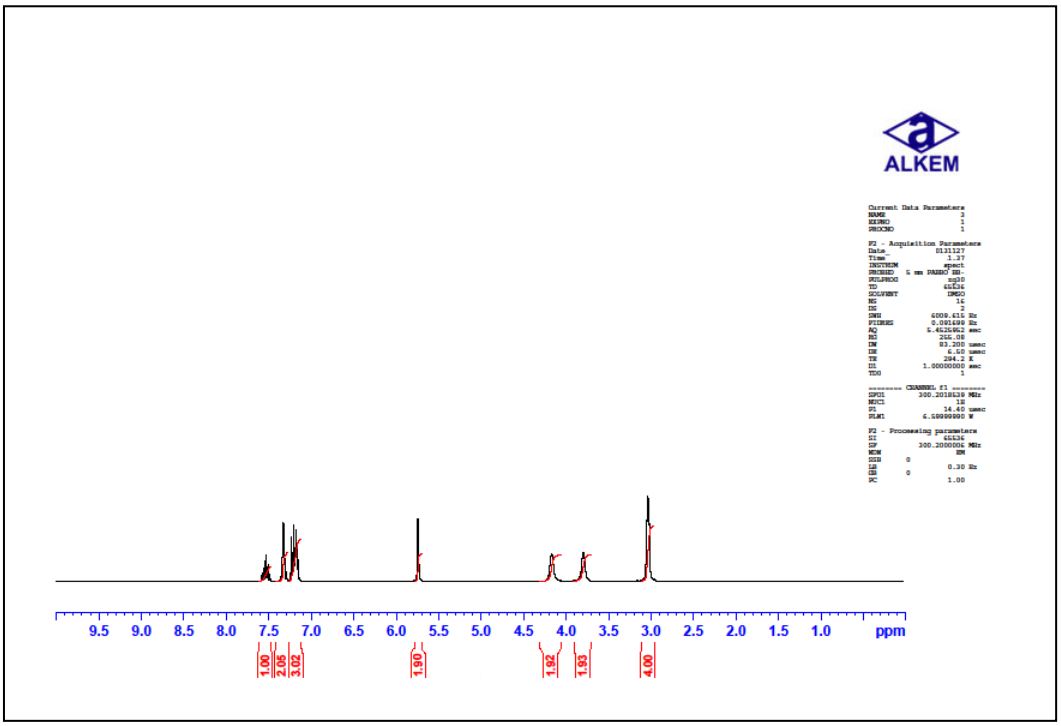
Compound code	Structure	Name	Mol wt	MP (°C)
9 a		**[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl][4-(2,3-dichlorophenyl)piperazin-1-yl]methanone**	**486**	**145**
9 b		**[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl](4-methylpiperazin-1-yl)methanone**	**355**	**208**
9 c		**[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl][4-(2-hydroxyethyl)piperazin-1-yl]methanone**	**386**	**165**

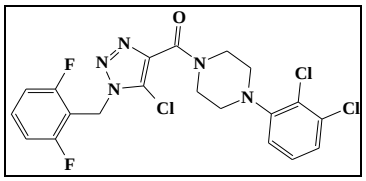
9d		**[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]{4-[(2-hydroxyethoxy)methyl]piperazin-1-yl}methanone**	**416**	**173**
9e		**[4-(1,2-benzothiazol-3-yl)piperazin-1-yl][5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]methanone**	**475**	**133**
9 f		**[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl](piperazin-1-yl)methanone**	**341**	**162**
9 g		**[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl][4-(2-hydroxyphenyl)piperazin-1-yl]methanone**	**399**	**184**

3.2.2b CHARACTERIZATION OF [5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4-SUBSTITUTED-PIPERAZIN-1-YL) METHANONES

Spectral data of representative compounds:

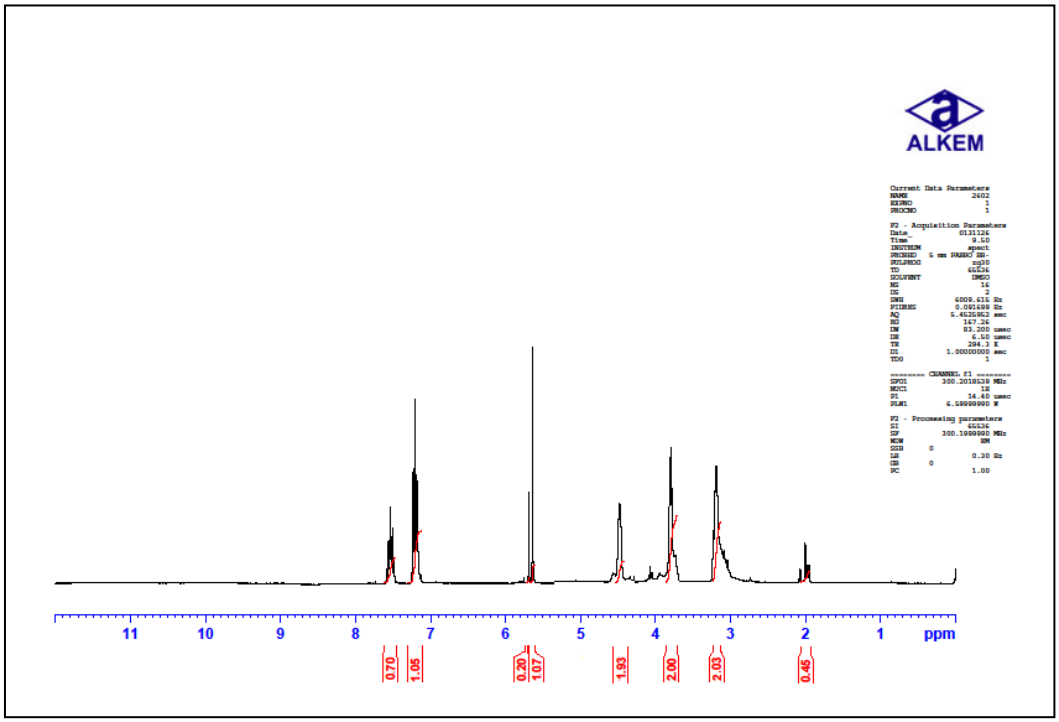
^{1}HNMR of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2, 3-dichlorophenyl) piperazin-1-yl] methanone (9a):

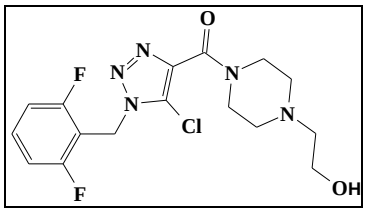




^{1}HNMR (DMSO-d6): 7.5 (1H, t, aromatic -CH in di fluoro phenyl ring);7.3(2H, aromatic CH of dichlorobenzene ring);7.2 (3H,aromatic CH of the substituted phenyl rings); 5.7 (2H,s,-CH_2); 4.3 (2H,t,-CH_2 of piperazine); 3.7 (2H,t,-CH_2 of piperazine); 3.0 (4H,t,-CH_2 of piperazine)

[1]HNMR of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyethyl) piperazin-1-yl] methanone (9c):

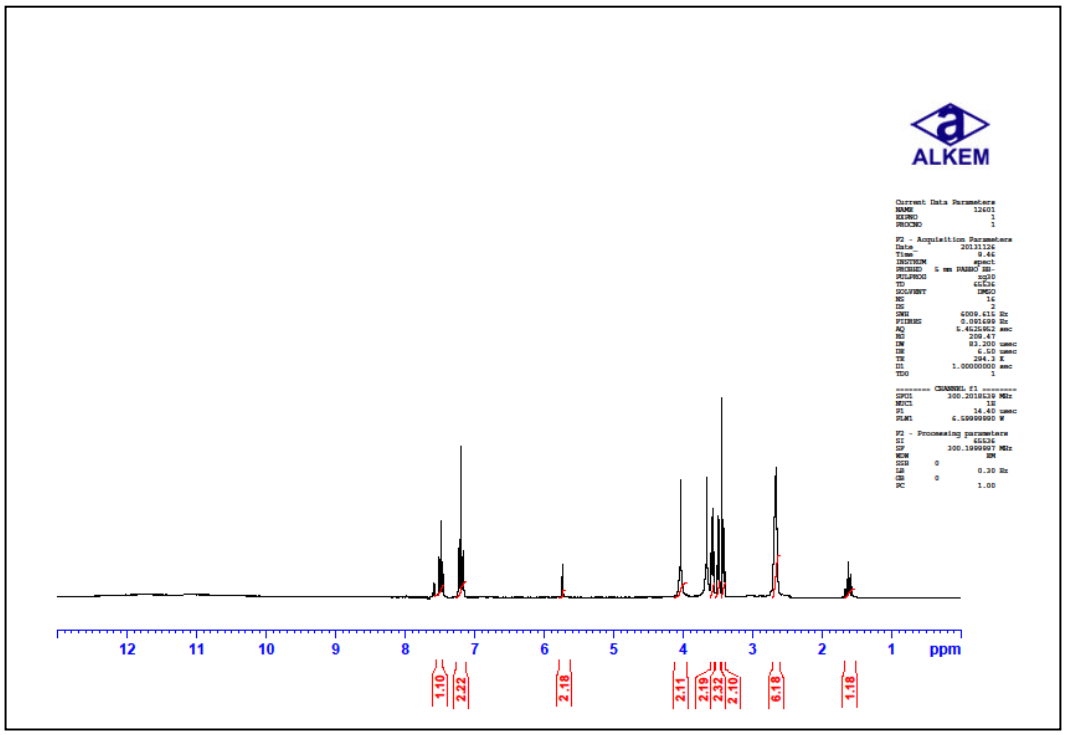


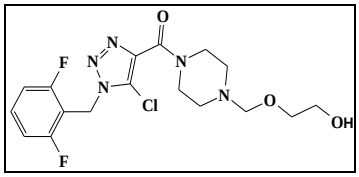


Interpretation:

[1]HNMR (DMSO-d6): 7.5 (1H, t, aromatic -CH of di fluoro phenyl ring); 7.2 (2H, d, aromatic CH of di fluoro phenyl ring); 5.8 (2H, s,-CH_2); 4.6 (4H, -CH_2); 3.8 (4 H, -CH_2 of piperazine) ; 3.2 (4H,-CH_2 of piperazine); 1.3 (1H, t,-OH)

[1]HNMR of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] {4-[(2-hydroxy ethoxy) methyl] piperazin-1-yl} methanone (9d):

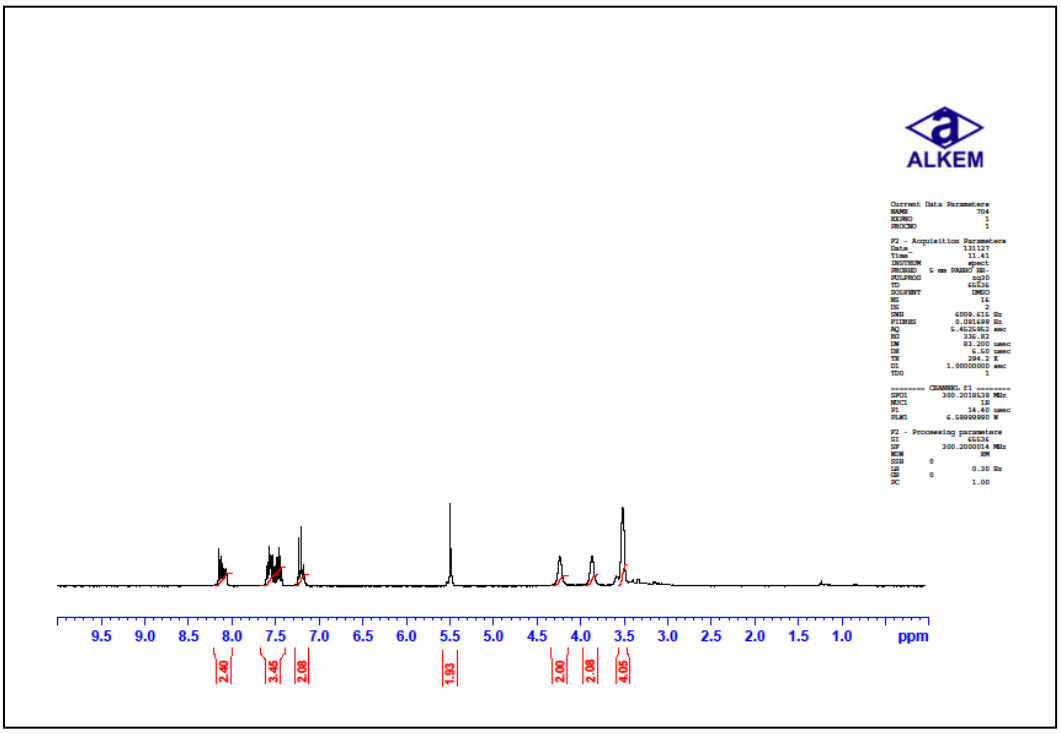


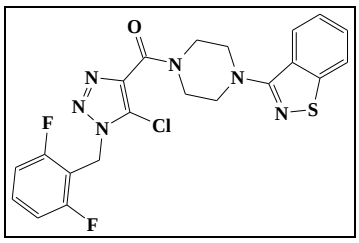


Interpretation:

[1]HNMR (DMSO-d6): 7.5 (1H, t, aromatic -CH of di fluoro phenyl ring); 7.2 (2H, d, aromatic CH of di fluoro phenyl ring); 5.7 (2H, s,-CH_2); 4.0 (2H, t,-CH_2); 3.7 (2H, t,-CH_2); 3.5 (2H,t,-CH_2) ; 3.4 (2H of -CH_2 of piperazine) ; 2.8 (6H,-CH_2 of piperazine);1.6 (1H,t,-OH)

[1]HNMR of [4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (9e):

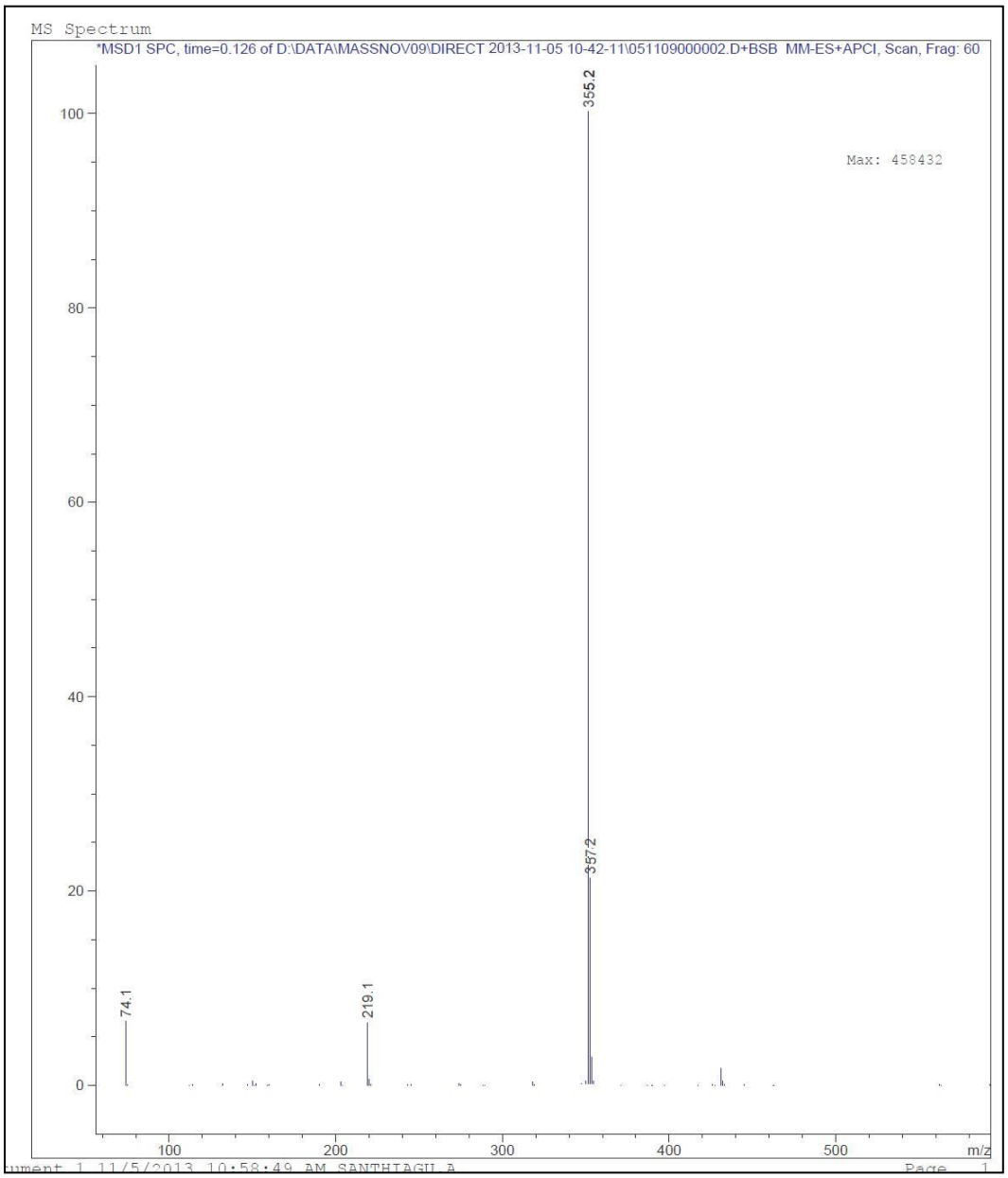




Interpretation:

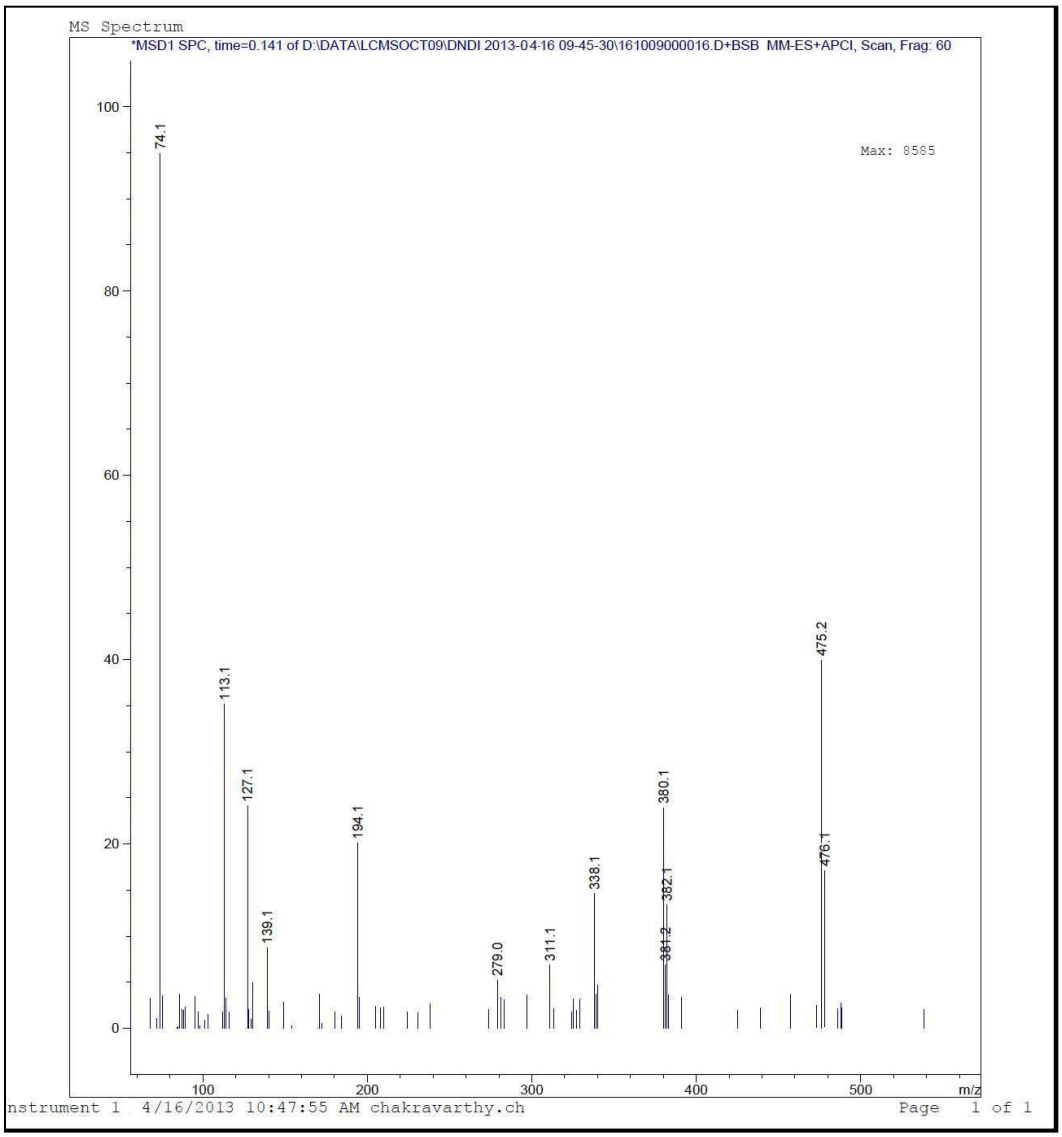
[1]HNMR (DMSO-d6): 8.1 (2H, t, aromatic -CH in benzoisothiazole ring); 7.5 (3H, aromatic CH); 7.3 (2H, aromatic CH of di fluoro phenyl rings); 5.8 (2H, s,-CH_2); 4.3(2H, t,-CH_2 of piperazine); 3.8 (2H, t,-CH_2 of piperazine); 3.5 (4H, t,-CH_2 of piperazine)

Mass spectrum of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (4-methylpiperazin-1-yl) methanone (9b):



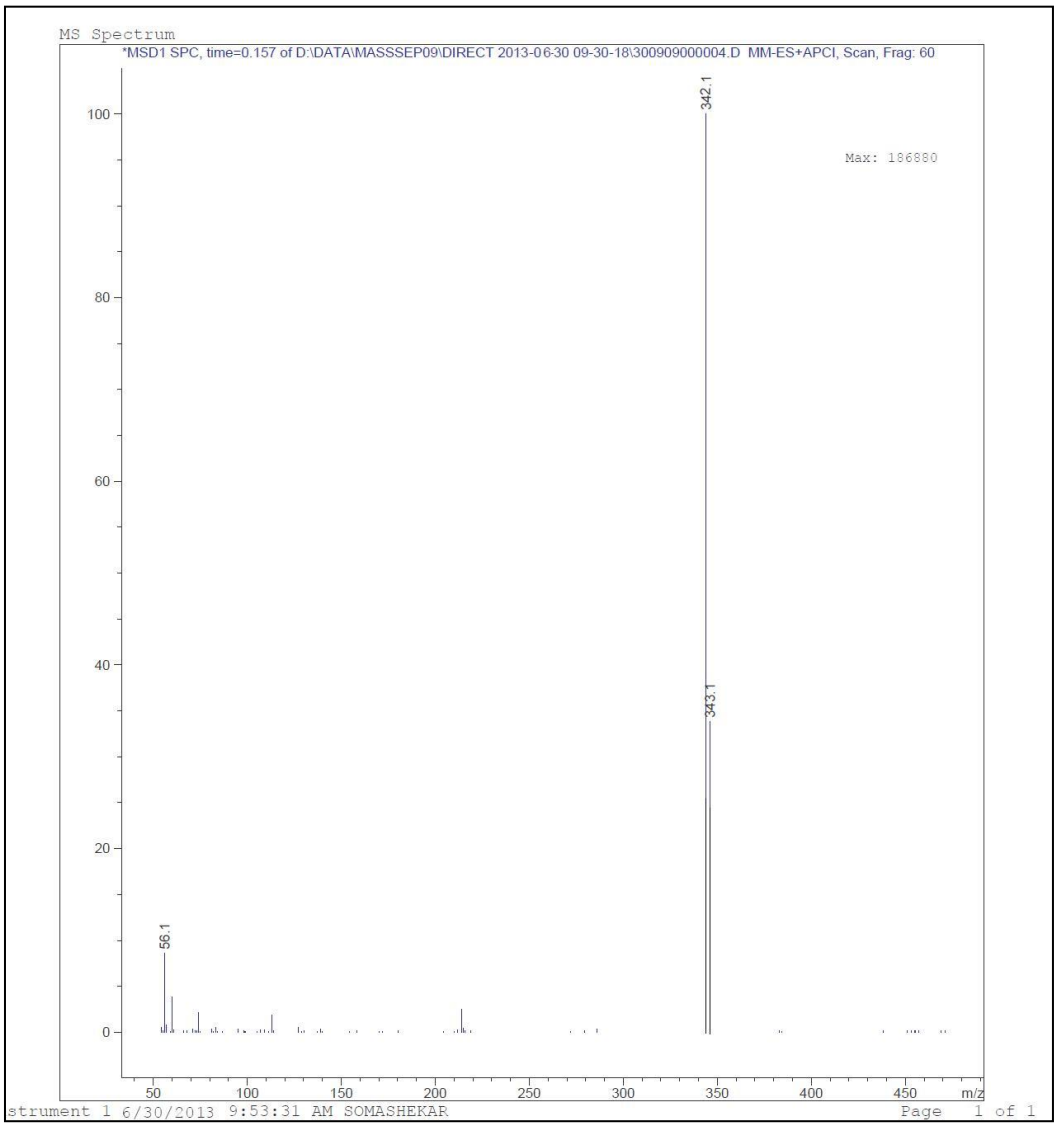
The mass spectrum conforms to the molecular weight (355) of the synthesized compound

Mass spectrum of [4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] methanone (9e):



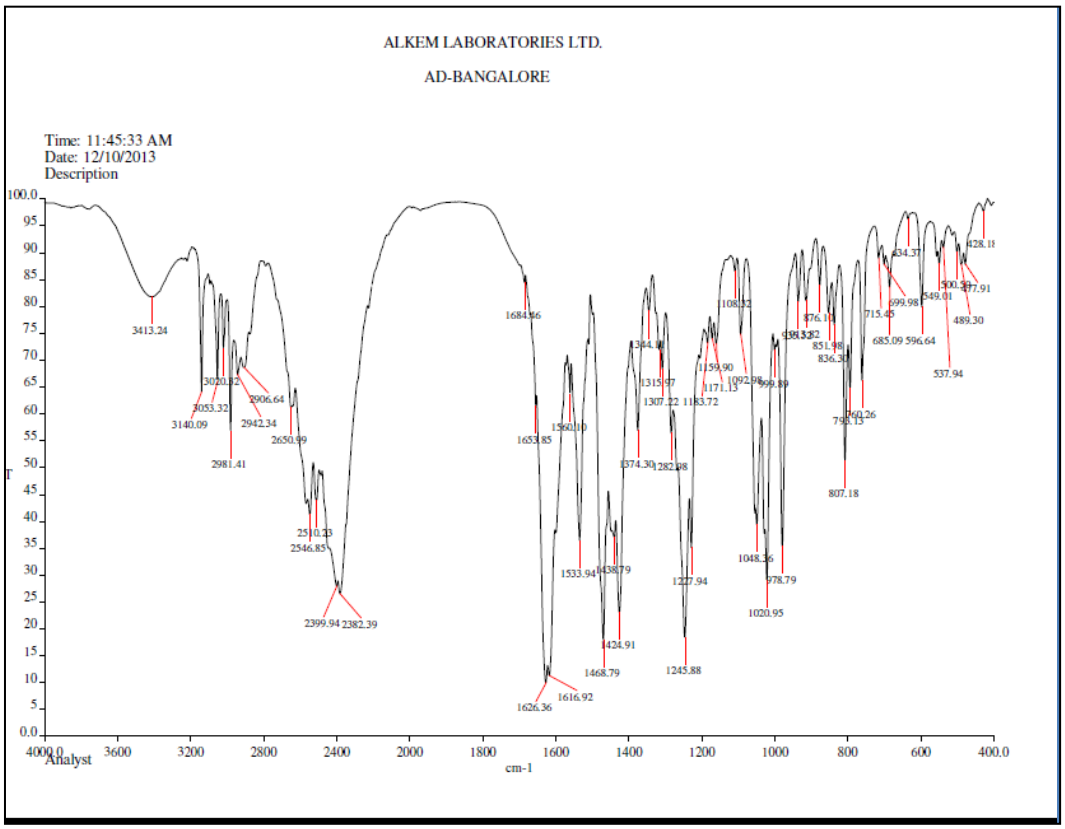
The mass spectrum conforms to the molecular weight (475) of the synthesized compound

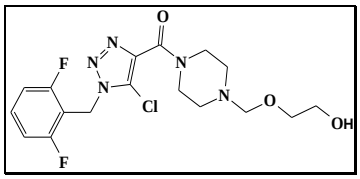
Mass spectrum of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] (piperazin-1-yl) methanone (9f):



The mass spectrum conforms to the molecular weight (341) of the synthesized compound

Infrared spectrum of [5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] {4-[(2-hydroxy ethoxy) methyl] piperazin-1-yl} methanone (9d):

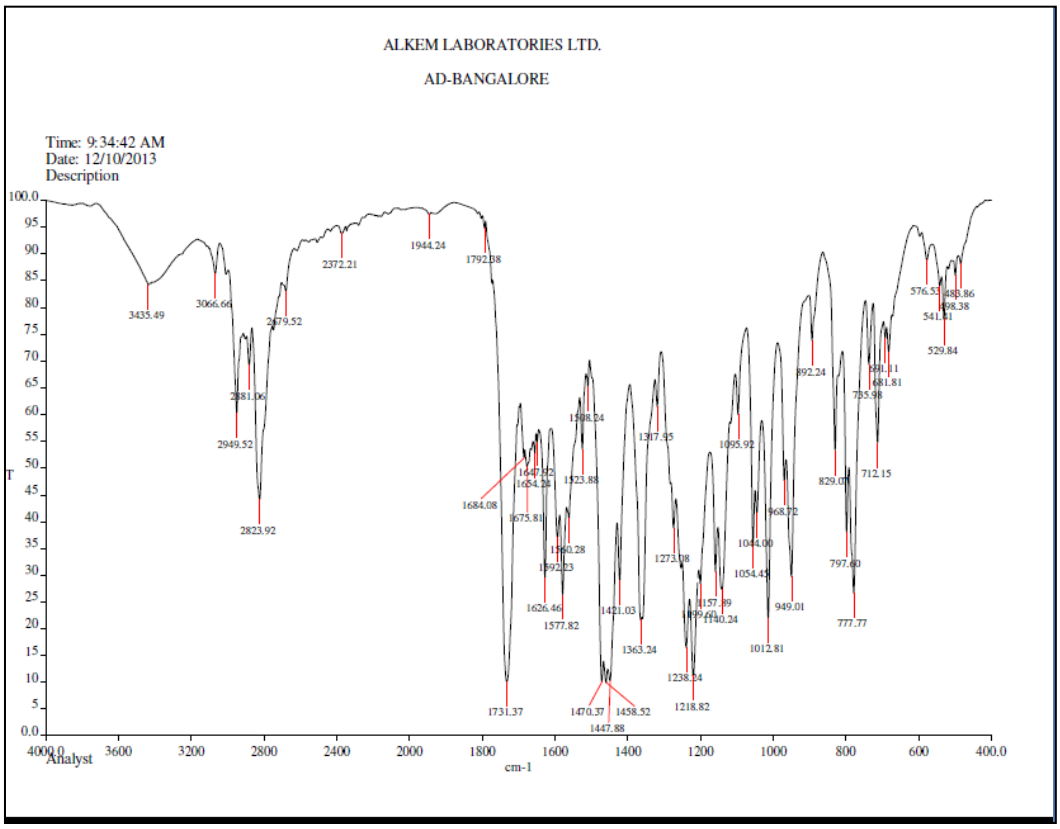


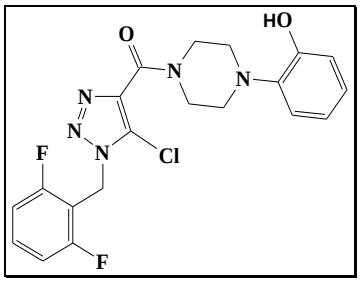


Interpretation:

IR max cm^{-1}: 3413.24 (O-H stretch H-bonded); 2981.41 (aromatic sp^2 C-H stretch); 2546.85 (sp^2 C-H stretch); 2382.39(sp^3 C-H stretch); 807.18 (aromatic out of plane bending); 1626.36 (-C=O stretch); 1533.94 (-C=N); 1468.79 (-CH_2 stretch); 1020.95 (C-F stretch)

Infrared spectrum of [1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl] [4-(2-hydroxyphenyl) piperazin-1-yl] methanone (9g):





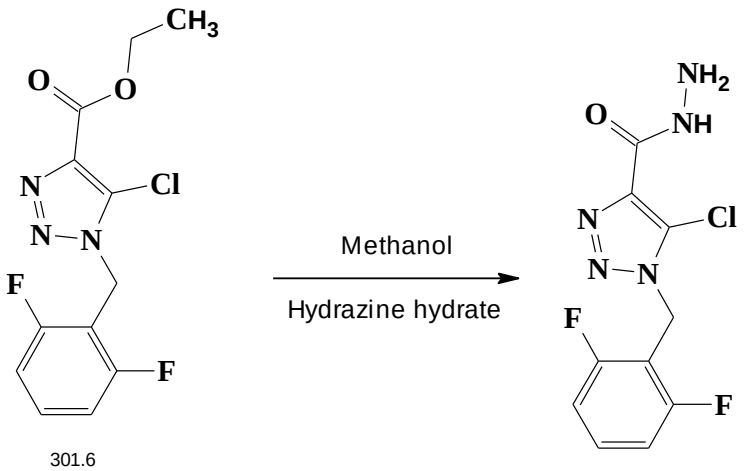
Interpretation:

IR max cm^{-1}: 3435.49 (O-H stretch H-bonded); 2823.92 (aromatic sp^2 C-H stretch); 777.77 (aromatic out of plane bending); 1626.46 (-C=O stretch); 1560.28 (-C=N); 1470.37 (-CH_2 stretch); 1238.24 (-C-O stretch); 1012.81 (C-F stretch)

3.2.3a SYNTHESIS OF SUBSTITUTED 2-[5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Step VII: Preparation of 5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbohydrazide

Reaction



301.6

Ethyl 5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate **(2)**

287.65

5-chloro-1-(2,6-difluorobenzyl)-1*H*-1, 2,3-triazole-4-carbohydrazide **(10)**

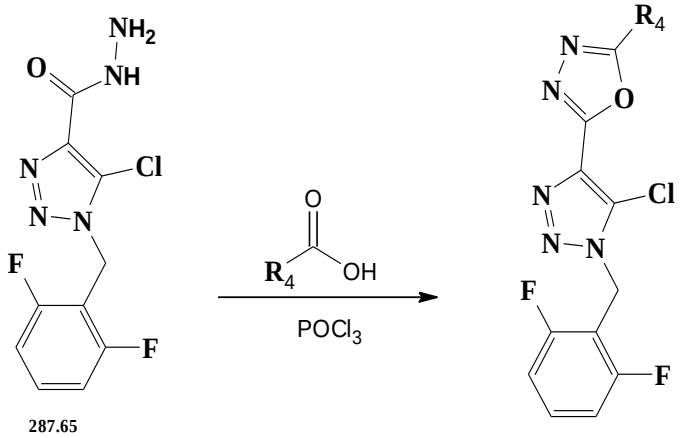
Procedure

5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carbohydrazide was obtained by refluxing 2g (0.07 mole) of ethyl 5-chloro-1-(2,6-difluorobenzyl)-1H- 1,2,3-triazole-4-carboxylate ester with 0.01 mole of hydrazine hydrate in the presence of methanol for 4 hours. The reaction mass was cooled to 10° C under mild stirring and later filtered on a Whatman filter paper to get white coloured product. The white solid obtained was dried in a hot air oven at 50°C for 4 hours.

The reaction was performed in a 100.0 ml glass round bottom reactor, fit to a condenser and a magnetic stirrer. The reaction was monitored by thin layer chromatography using chloroform: methanol=9:1 as the mobile phase.

Step VIII: Preparation of substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles [11(a-i)]

Reaction



287.65

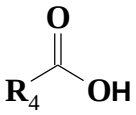
5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2, 3-triazole-4-carbohydrazide

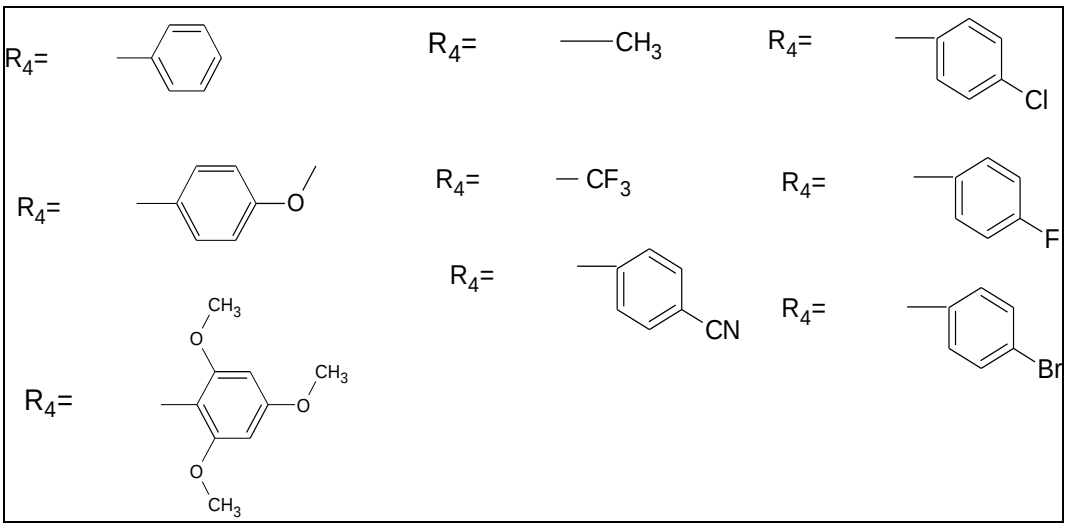
10

substituted 2-[5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2, 3-triazol-4-yl]-1,3,4-oxadiazoles

11(a-i)

Substitutions in 11 (a to i)



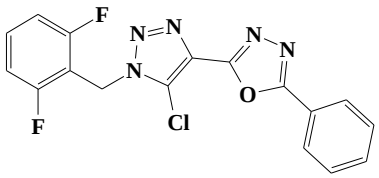
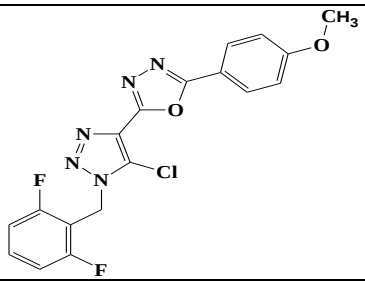
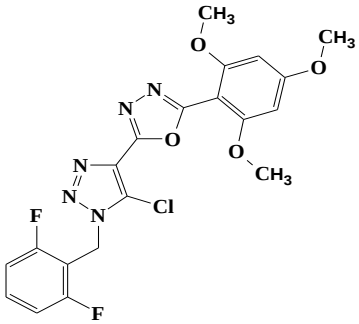
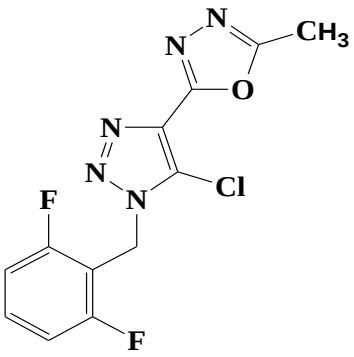
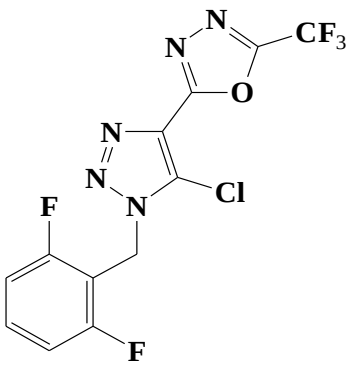


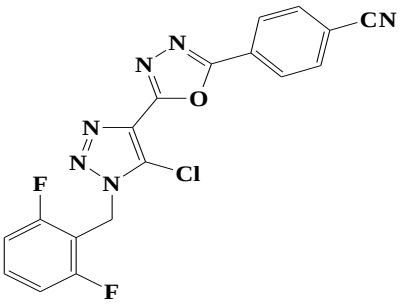
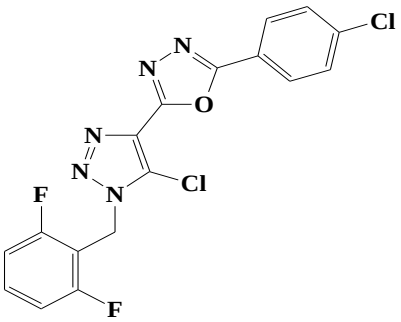
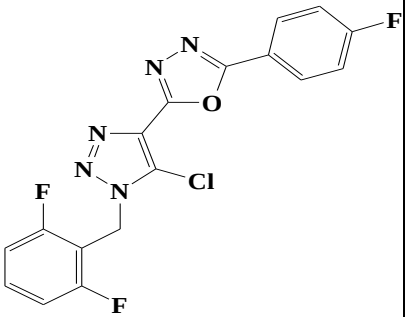
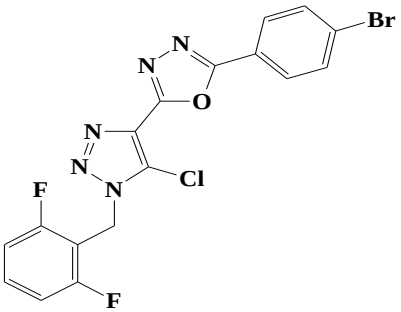
General procedure

A completely dried 100.0 ml round bottom flask fitted with a mechanical stirrer and a condenser, whose vent is attached to a calcium chloride guard tube, was taken. The guard tube vent was in turn connected to a Teflon pipe which ended in a beaker of 10% sodium hydroxide solution. This was done to prevent the escape of excess thionyl chloride to the atmosphere. Substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles were prepared by the gradual addition phosphoryl chloride (10g, 0.065 mole) through a stopper glass addition funnel to 5-chloro-1-(2,6- difluorobenzyl) -1H- 1, 2, 3- triazole -4-carbohydrazide (2g, 0.007 mole) and substituted carboxylic acids (0.01 mole). The reaction mixture was refluxed at 80°C for 2 hours. The disappearance of 5-chloro-1- (2, 6-difluorobenzyl) - 1H- 1, 2, 3- triazole-4-carbohydrazide was monitored by thin layer chromatography using hexane: ethyl acetate= 7:3 as the mobile phase. After the completion of the reaction; the excess phosphoryl chloride was distilled under vacuum and the residue was quenched with ice water. The solid separated was filtered and washed with diisopropylether to yield crude substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazole.

Purification of the crude 2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-1,3,4-oxadiazole were done by column chromatography using silica gel (230/400# mesh) and 7:3= hexane : ethyl acetate as the mobile phase. The pure fractions were collected and hence concentrated to afford the mentioned compounds 2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-1,3,4-oxadiazole [11 (a-i)].

Table 7: List of substituted 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles [11(a-i)]

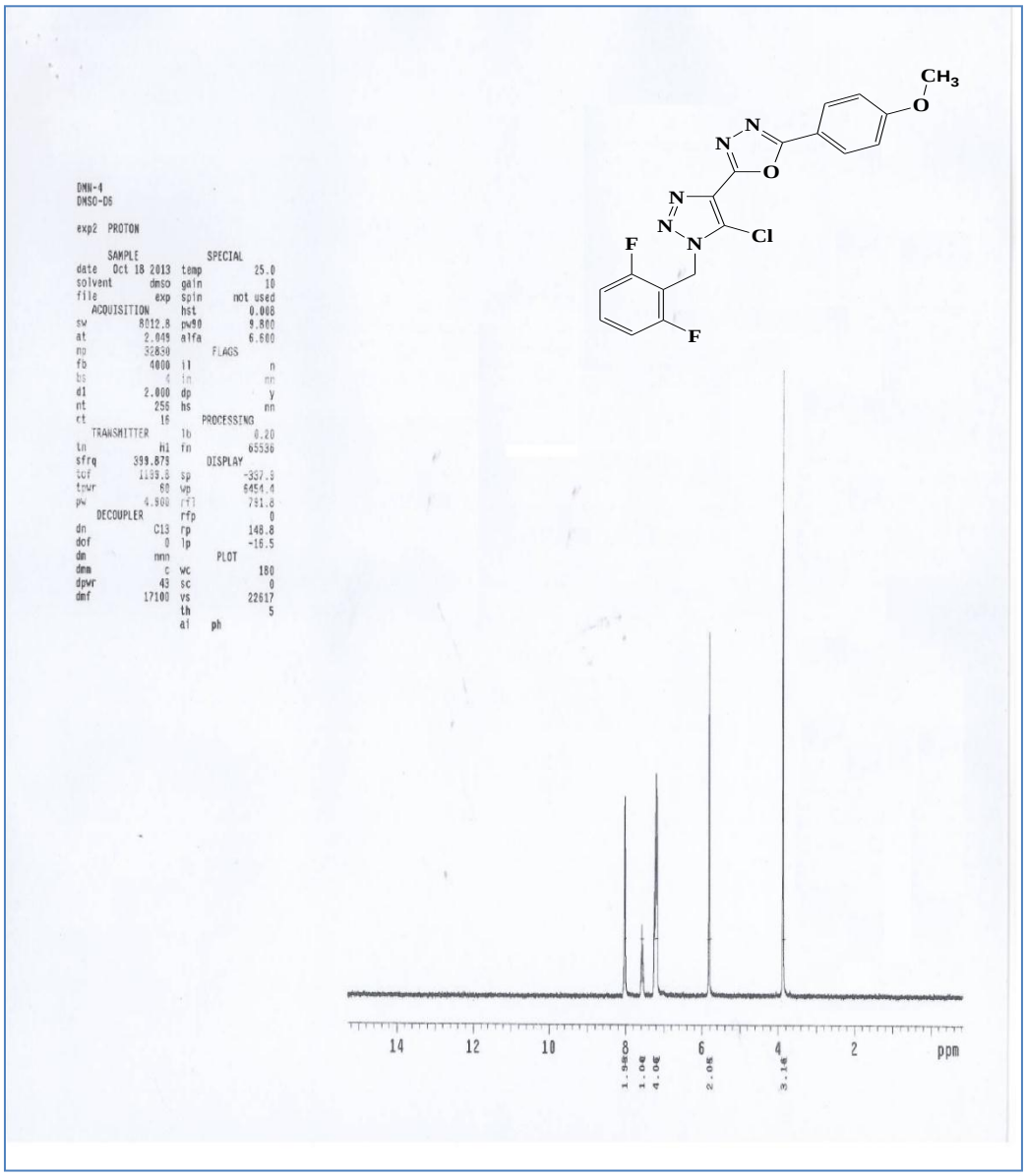
Compound code	Structure	Name	Mol Wt	MP (°C)
11 a		**2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-phenyl-1,3,4-oxadiazole**	**373**	**127**
11 b		**2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(4-methoxyphenyl)-1,3,4-oxadiazole**	**403**	**145**
11 c		**2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole**	**463**	**164**
11 d		**2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-methyl-1,3,4-oxadiazole**	**311**	**130**
11 e		**2-[5-chloro-1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(trifluoromethyl)-1,3,4-oxadiazole**	**365**	**136**

11 f		**4-{5-[5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazol-2-yl}benzonitrile**	**398**	**187**
11 g		**2-[5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-chlorophenyl)-1,3,4-oxadiazole**	**408**	**191**
11 h		**2-[5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-fluorophenyl)-1,3,4-oxadiazole**	**391**	**165**
11 i		**2-(4-bromophenyl)-5-[5-chloro-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazole**	**453**	**181**

3.2.3b CHARACTERIZATION OF SUBSTITUTED 2-[5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Spectral data of representative compounds:

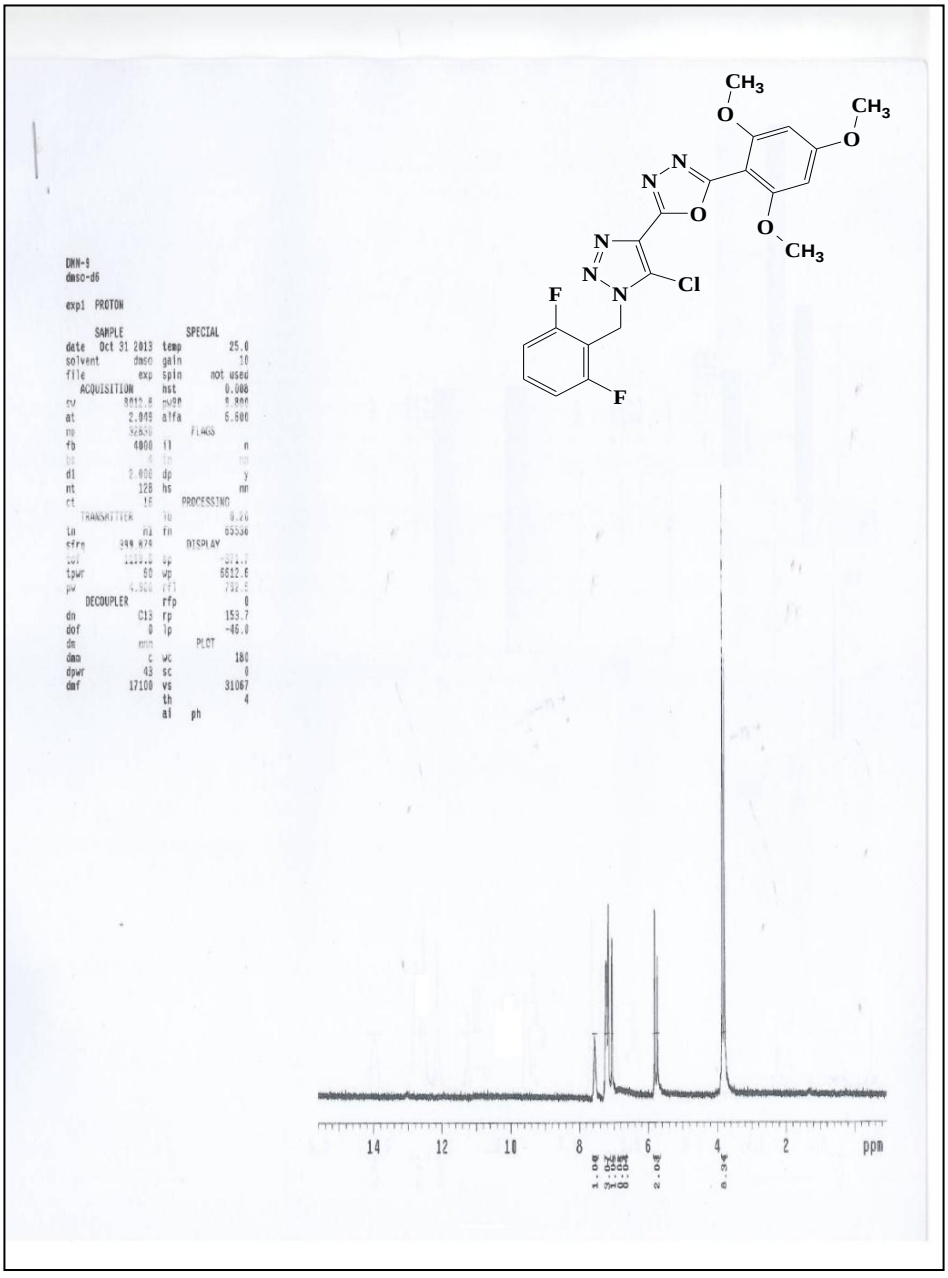
^{1}HNMR of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-(4-methoxyphenyl)-1, 3, 4-oxadiazole (11b):



Interpretation

^{1}HNMR (DMSO-d6): 8.0(2H, d, aromatic protons of the 4-methoxy phenyl ring), 7.6 (1H, t, aromatic proton of the di -fluoro phenyl ring), 7.2 (4H, m, 2 aromatic protons of the 4-methoxy phenyl ring and 2 aromatic protons of the di -fluoro phenyl ring), 5.8(2H, s, -CH_2), 3.8(3H, s, -OCH_3)

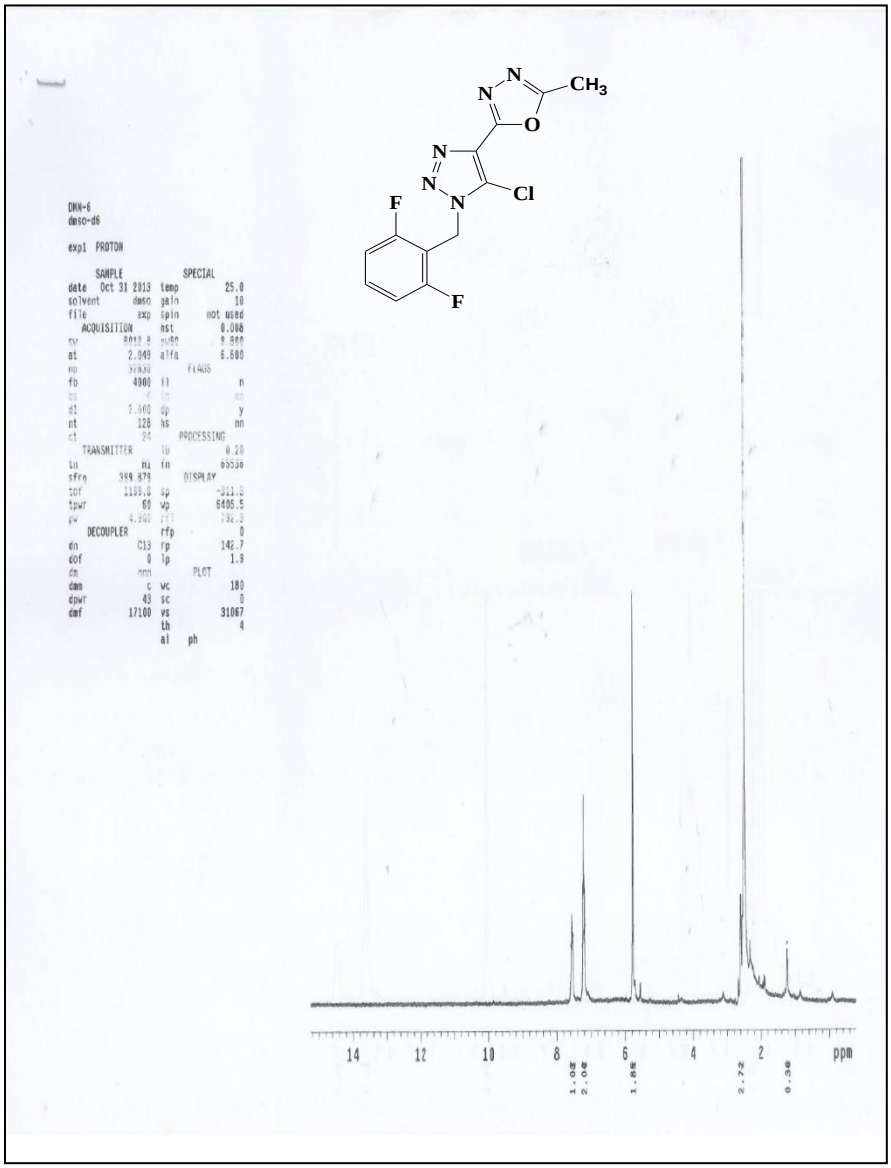
[1]HNMR of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-(2, 4, 6-trimethoxyphenyl)-1, 3, 4-oxadiazole (11c):



Interpretation

[1]HNMR (DMSO-d6): 7.6 (1H, t, aromatic proton of the di -fluoro phenyl ring), 7.2 (3H, 2 aromatic protons of the di -fluoro phenyl ring and 1 aromatic proton of tri methoxy phenyl ring); 7.0(1H, m, 1 aromatic proton of tri methoxy phenyl ring); 5.8(2H, s, -CH_2), 3.8 (9H, s, -OCH_3)

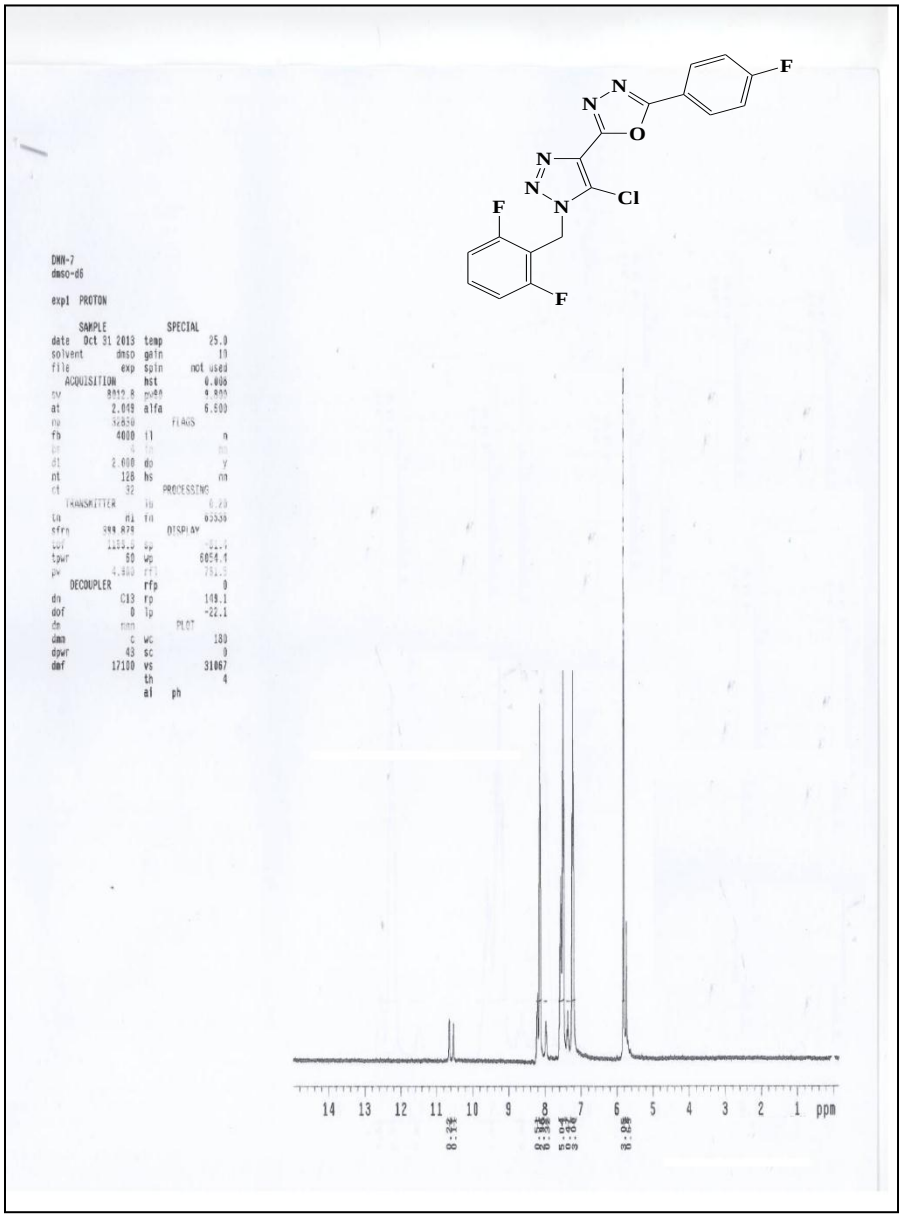
[1]HNMR of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-methyl-1, 3, 4-oxadiazole (11d):



Interpretation

[1]HNMR (DMSO-d6): 7.6 (1H, t, aromatic proton of the di -fluoro phenyl ring), 7.2 (2H, d, aromatic protons of the di -fluoro phenyl ring), 5.8(2H, s, -CH_2), 2.6 (3H, s, -CH_3)

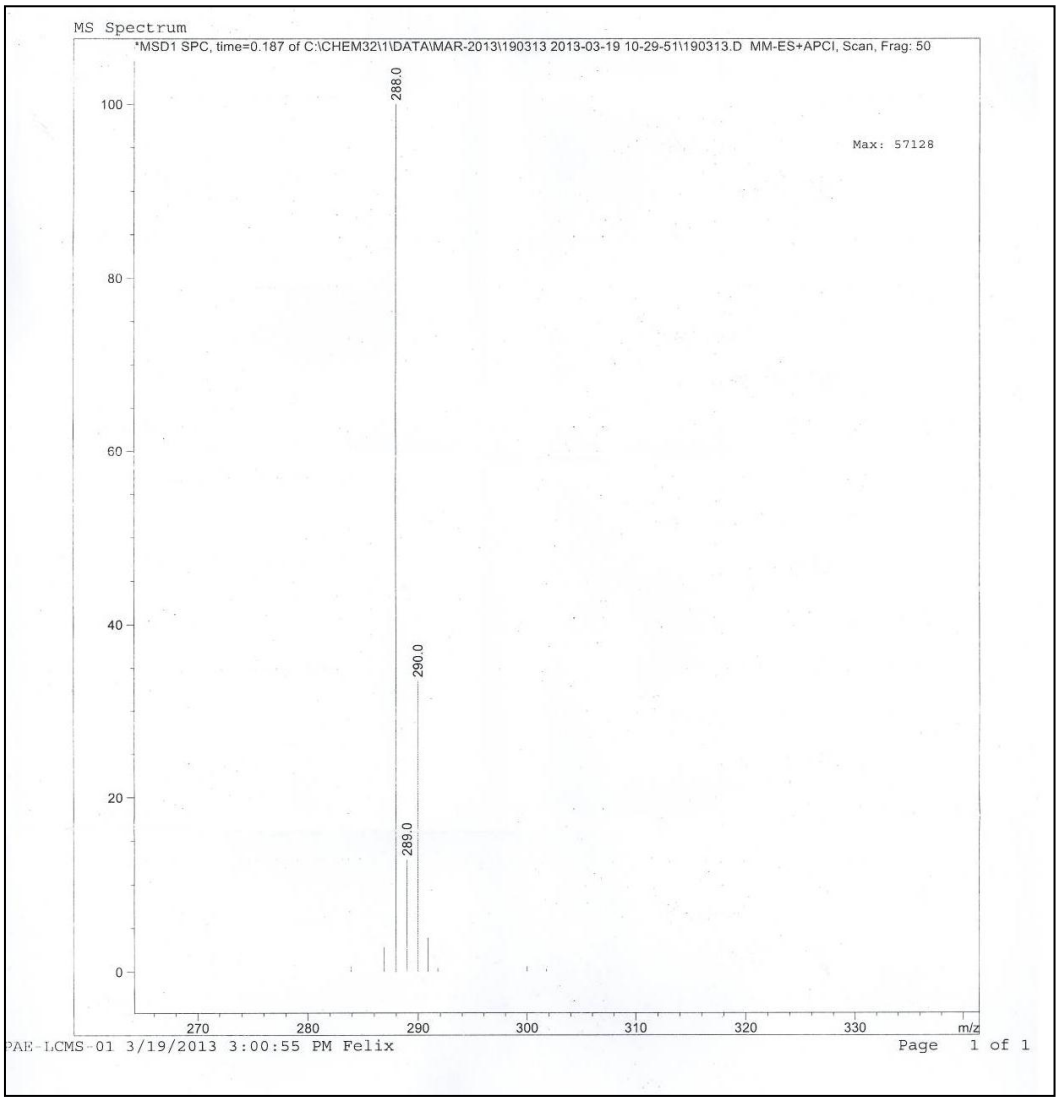
[1]HNMR of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (11h):



Interpretation

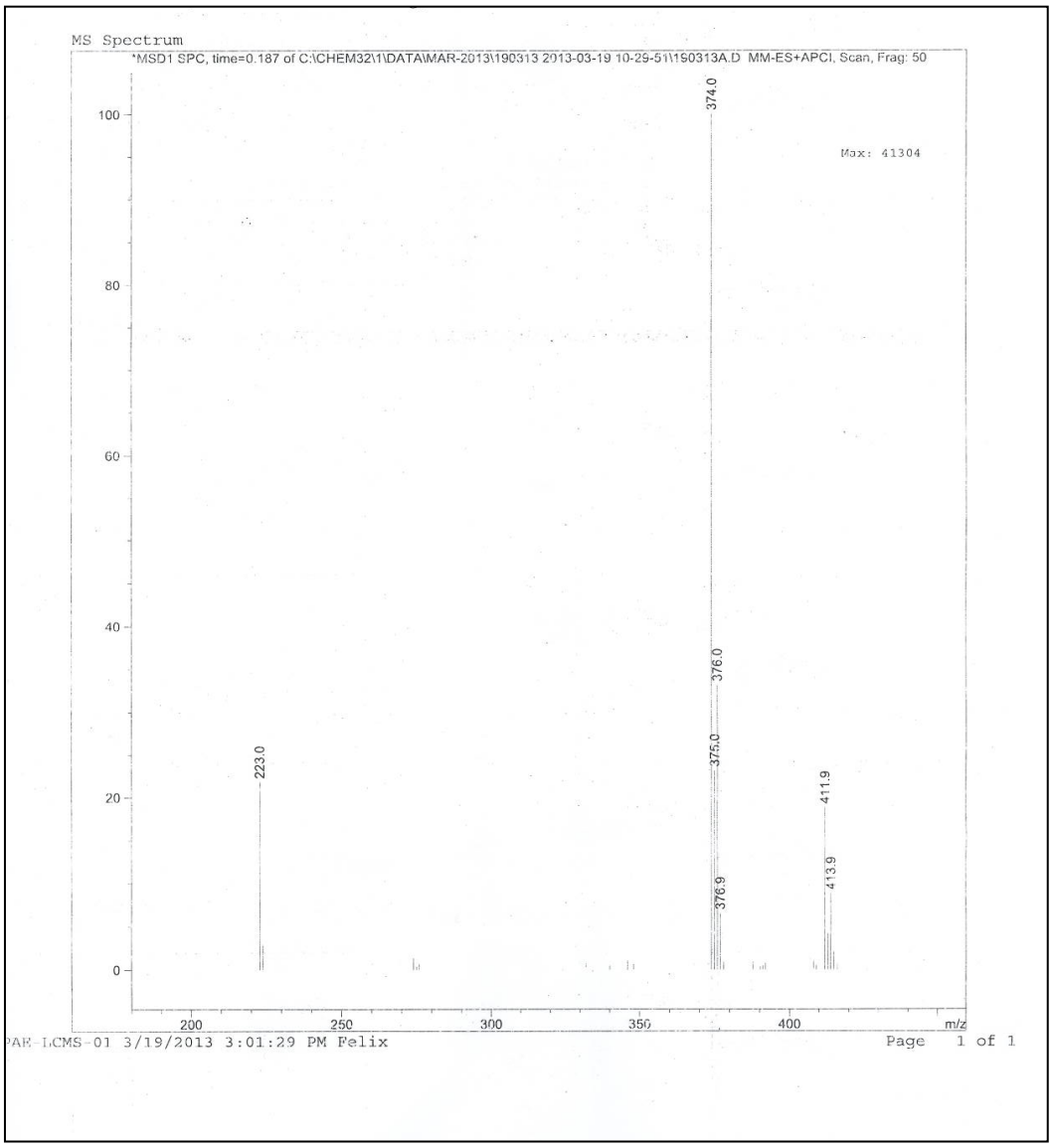
[1]HNMR (DMSO-d6): 8.2 (2H, d, aromatic protons of the fluoro phenyl ring), 7.6 (3H, m, 2 aromatic protons of the fluoro phenyl ring and 1 aromatic proton of di fluoro phenyl ring), 7.2 (2H, d, 2 aromatic protons of the di fluoro phenyl ring), 5.8(2H, s, -CH_2)

Mass spectrum of 5-chloro-1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazole-4-carbohydrazide (10):



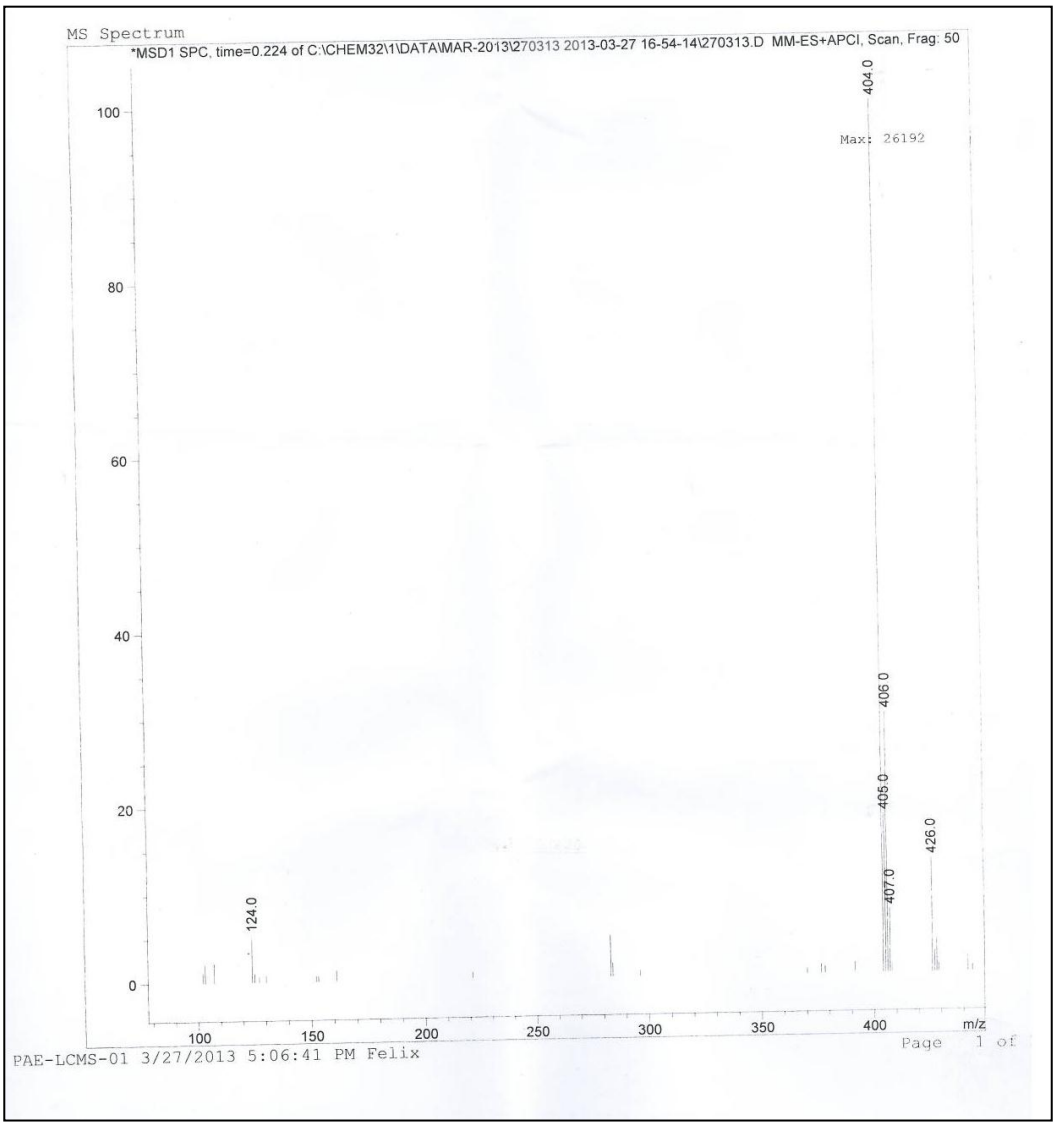
The mass spectrum conforms to the molecular weight (287) of the synthesized compound

Mass spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-phenyl-1, 3, 4-oxadiazole (11a):



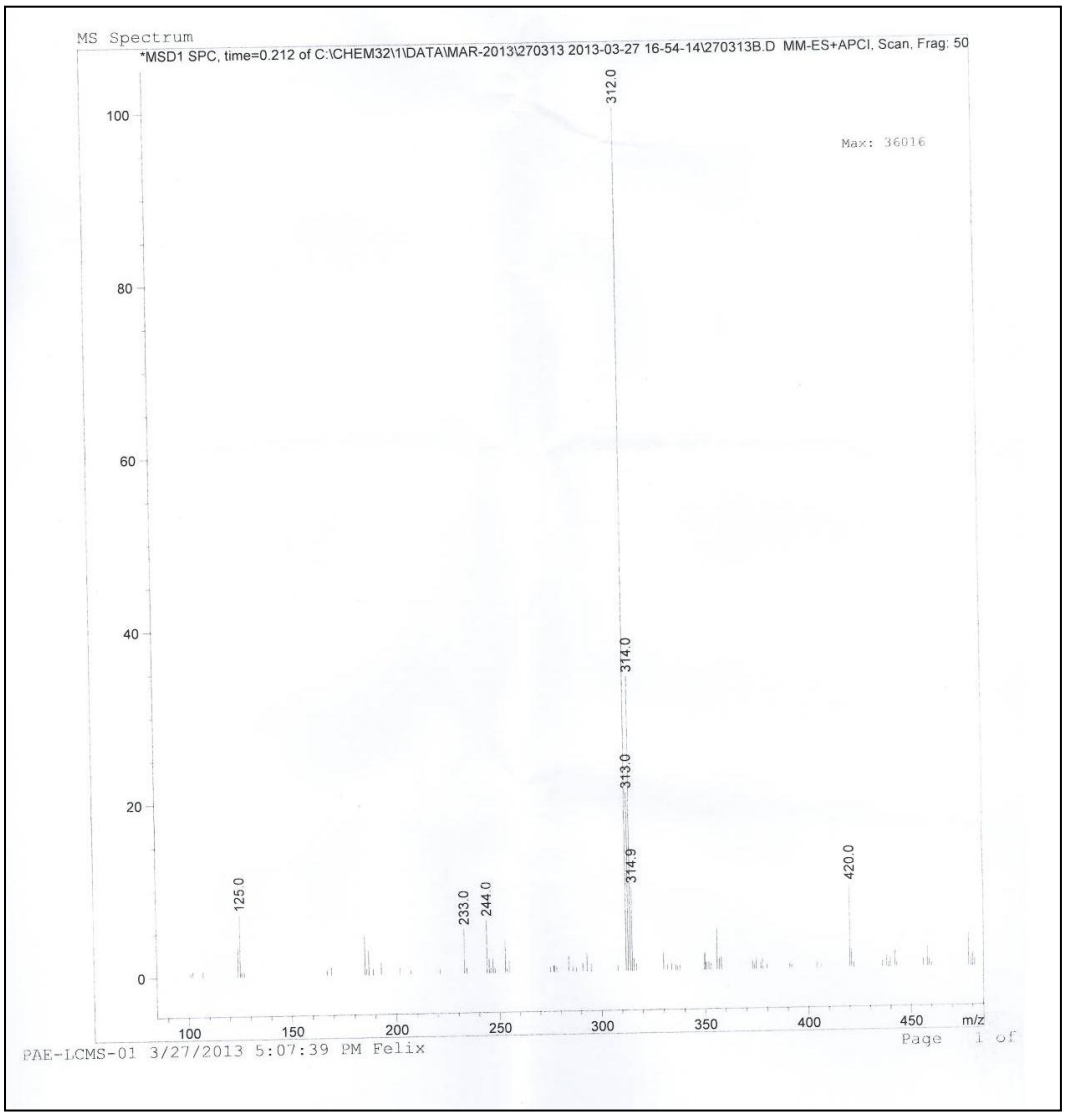
The mass spectrum conforms to the molecular weight (373) of the synthesized compound

Mass spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-(4-methoxyphenyl)-1, 3, 4-oxadiazole (11b):



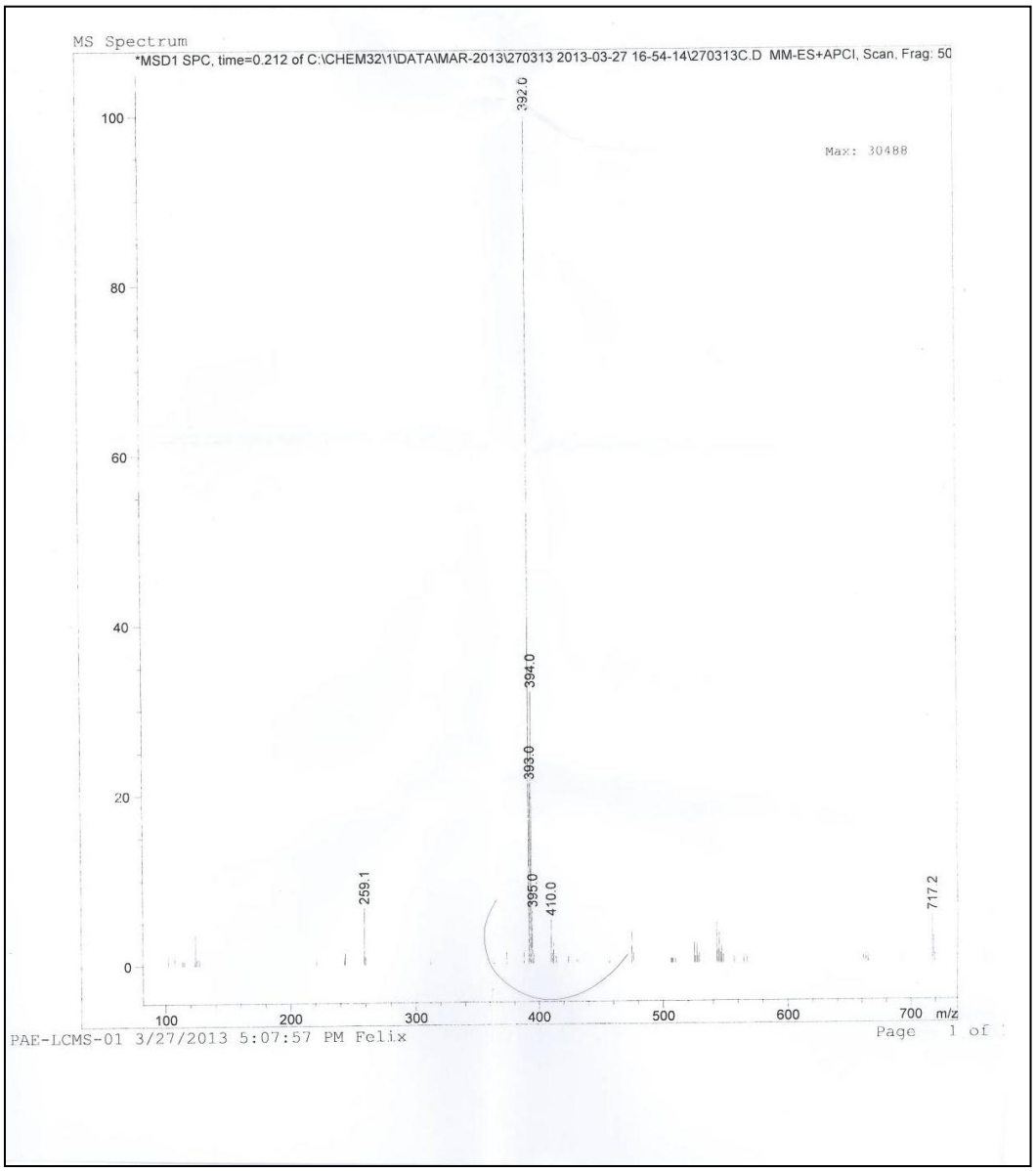
The mass spectrum conforms to the molecular weight (403) of the synthesized compound

Mass spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-methyl-1, 3, 4-oxadiazole (11d):



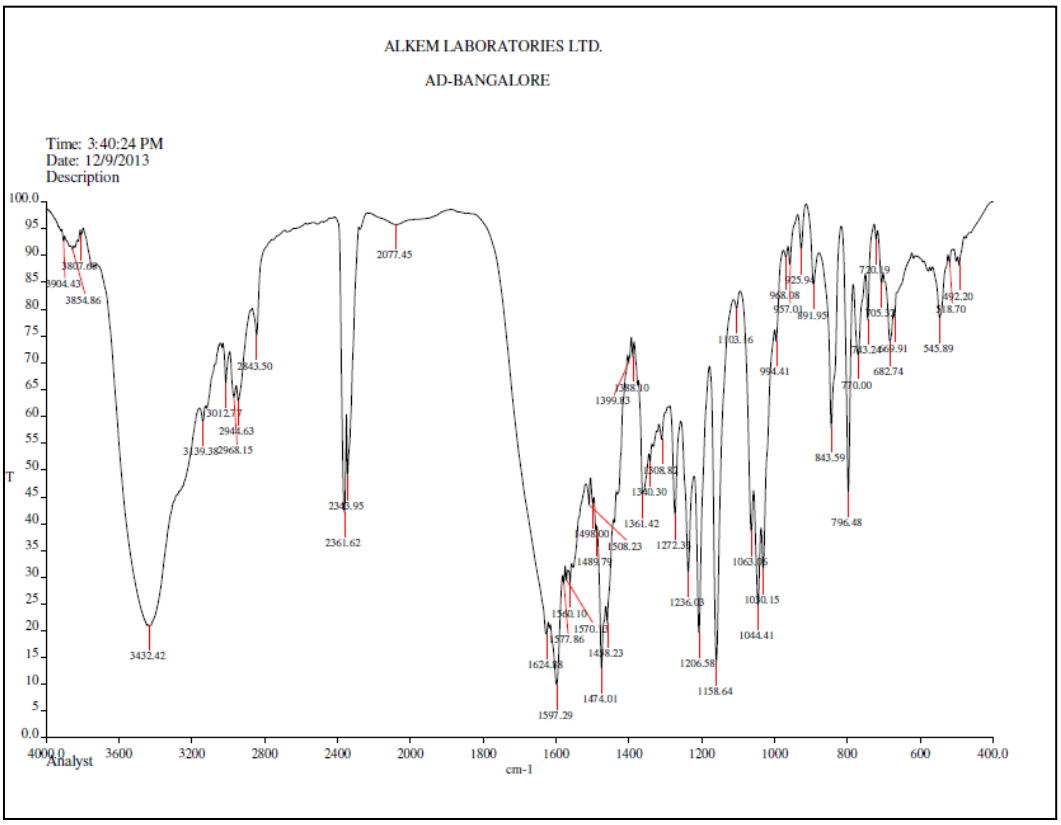
The mass spectrum conforms to the molecular weight (311) of the synthesized compound

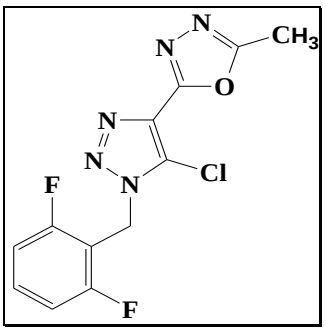
Mass spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (11h):



The mass spectrum conforms to the molecular weight (391) of the synthesized compound

Infrared spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-methyl-1, 3, 4-oxadiazole (11d):

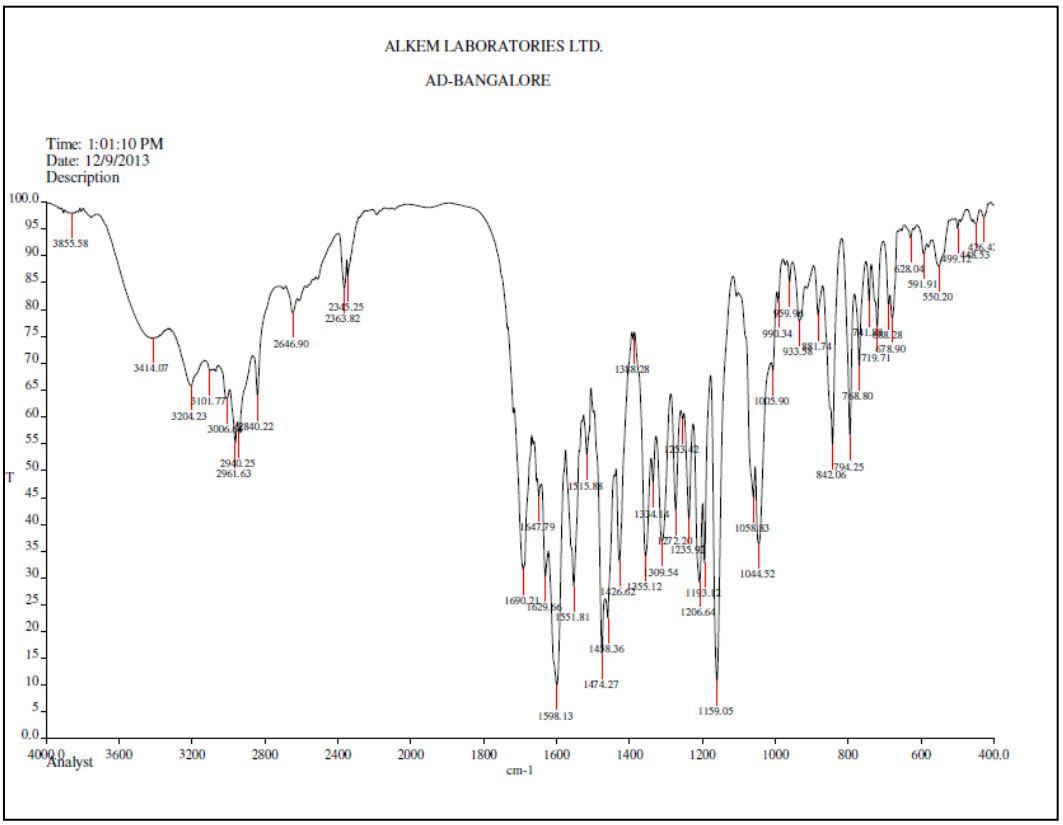


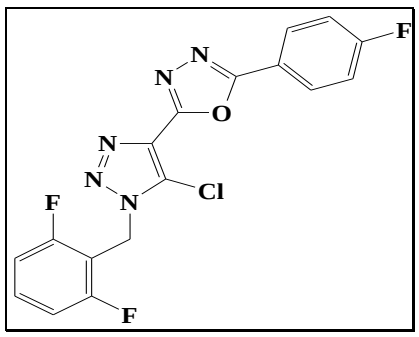


Interpretation:

IR max cm^{-1}: 3012.77 (aromatic sp^2 C-H stretch); 2968.15 (sp^3 C-H stretch); 1474.01 (-CH_2 stretch); 1560.10 (-C=N); 1158.64 (C-O stretch); 1044.41 (C-F stretch); 843.59 (aromatic out of plane bending); 796.48 (C-Cl stretch)

Infrared spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (11h):





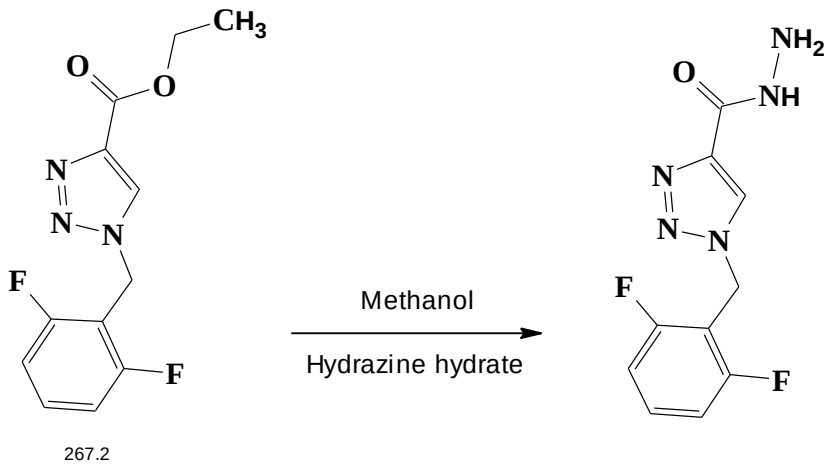
Interpretation

IR max cm^{-1}: 2961.63 (aromatic sp^2 C-H stretch); 1474.27 (-CH_2 stretch); 1551.81 (-C=N); 1159.05 (C-O stretch); 1044.52 (C-F stretch); 842.06 (aromatic out of plane bending); 794.25 (C-Cl stretch)

3.2.4a SYNTHESIS OF SUBSTITUTED 2-[1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Step IX: Preparation of 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carbohydrazide

Reaction



267.2

Ethyl-1-(2,6-difluorobenzyl)-1*H*-1,2, 3-triazole-4-carboxylate **(3)**

253.2

1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4 -carbohydrazide **(12)**

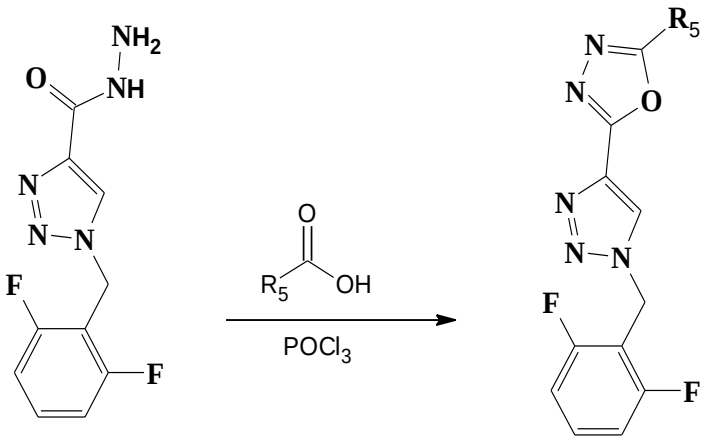
Procedure

1- (2,6- difluorobenzyl)- 1H- 1 ,2, 3- triazole-4-carbohydrazide was obtained by refluxing 2g (0.07 mole) of ethyl-1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylate with 0.01 mole of hydrazine hydrate in the presence of methanol for 4 hours. The reaction mass was cooled to 10° C under mild stirring and later filtered on a Whatman filter paper to get white coloured product. The white solid obtained was dried in a hot air oven at 50°C for 4 hours.

The reaction was performed in a 100.0 ml glass round bottom reactor, fit to a condenser and a magnetic stirrer. The reaction was monitored by thin layer chromatography using chloroform: methanol=9:1 as the mobile phase.

Step XII: Preparation substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles [13(a-i)]

Reaction

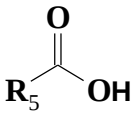


253.2

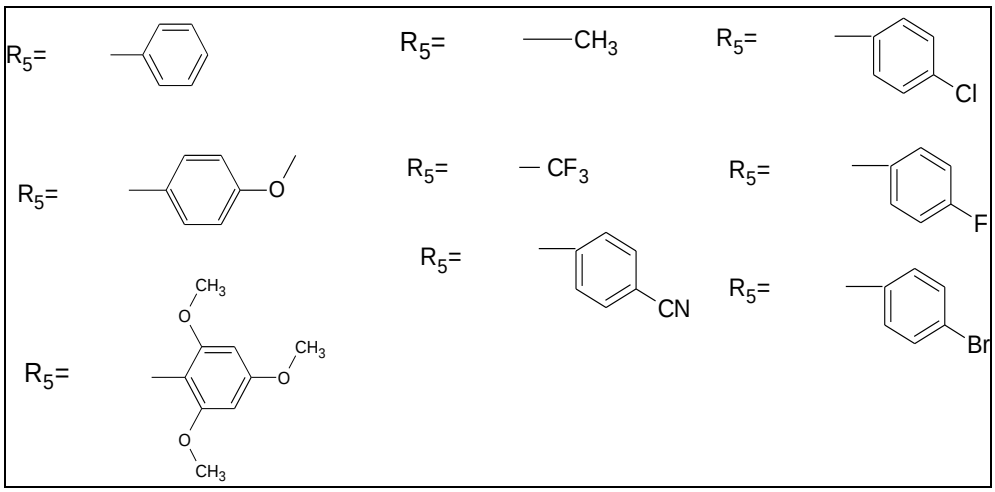
1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbohydrazide **(12)**

substituted 2-[1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazole**[13(a-i)]**

Substitutions in 13 (a to i)



Substitutions:

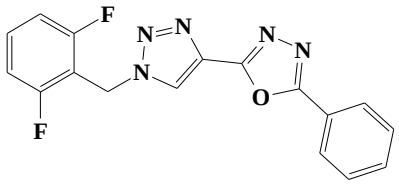
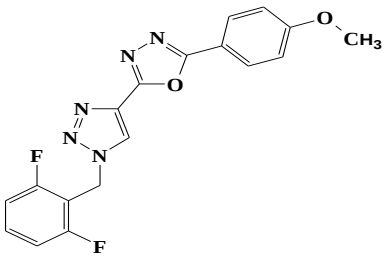
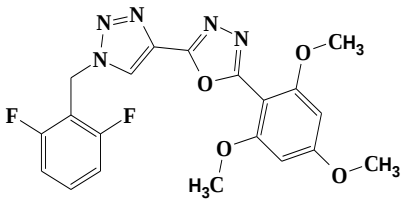
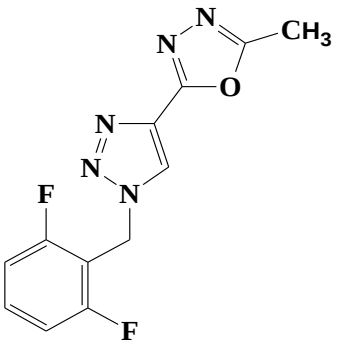
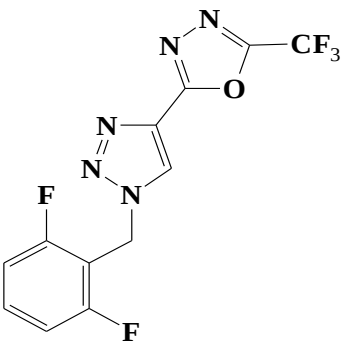


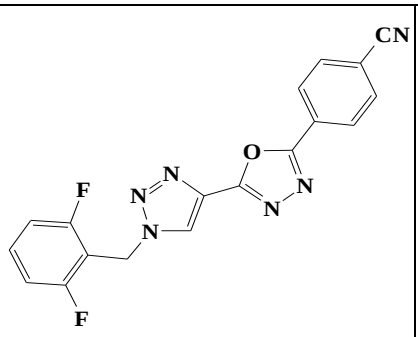
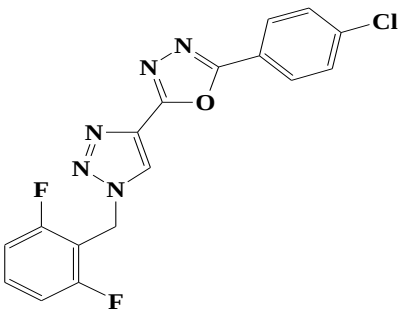
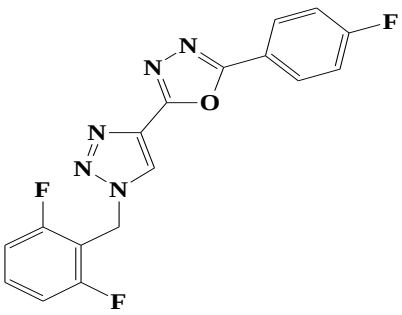
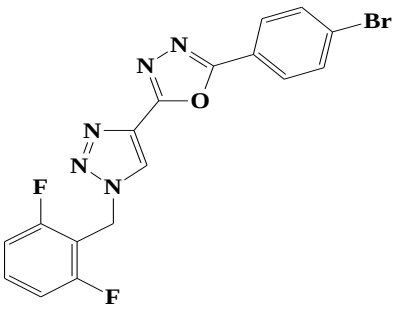
Procedure

A completely dried 100.0 ml round bottom flask fitted with a mechanical stirrer and a condenser, whose vent is attached to a calcium chloride guard tube, was taken. The guard tube vent was in turn connected to a Teflon pipe which ended in a beaker of 10% sodium hydroxide solution. This was done to prevent the escape of excess thionyl chloride to the atmosphere. Substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles were prepared by the gradual addition of phosphoryl chloride (10g, 0.065 mole) through a stopper glass addition funnel to 1-(2,6- difluorobenzyl) -1H- 1, 2, 3- triazole -4- carbohydrazide (2g, 0.007 mole) and substituted carboxylic acids (0.01 mole). The reaction mixture was refluxed at 80°C for 2 hours. The disappearance of 1- (2, 6-difluorobenzyl) - 1H- 1, 2, 3- triazole-4-carbohydrazide was monitored by thin layer chromatography using hexane: ethyl acetate= 7:3 as the mobile phase. After the completion of the reaction; the excess phosphoryl chloride was distilled under vacuum and the residue was quenched with ice water. The solid separated was filtered and washed with diisopropylether to yield crude substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles.

Purification of the crude substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles were done by column chromatography using silica gel (230/400# mesh) as stationary phase and 7:3= hexane: ethyl acetate as the mobile phase. The pure fractions were collected and hence concentrated to afford the mentioned compounds substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazoles [13 (a-i)].

Table 8: List of substituted 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazole derivatives [13(a-i)]

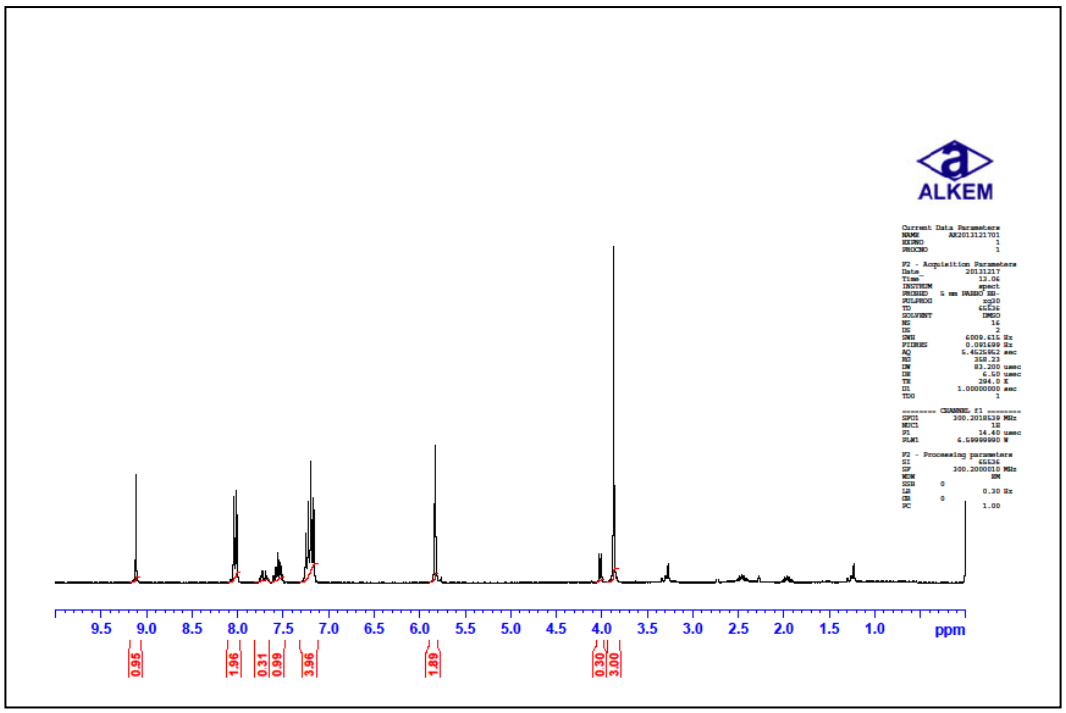
Compound code	Structure	Name	Mol Wt	MP (°C)
13 a		2-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-phenyl-1,3,4-oxadiazole	339	137
13 b		2-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(4-methoxyphenyl)-1,3,4-oxadiazole	369.32	154
13 c		2-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole	429	156
13 d		2-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-methyl-1,3,4-oxadiazole	277	120
13 e		2-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-5-(trifluoromethyl)-1,3,4-oxadiazole	331	125

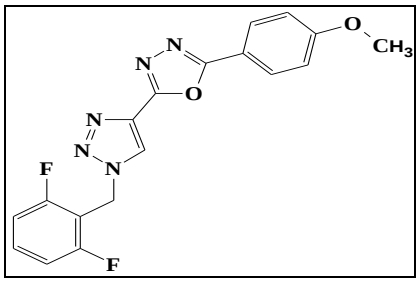
13 f		**4-{5-[1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazol-2-yl}benzonitrile**	**364**	**179**
13 g		**2-(4-chlorophenyl)-5-[1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazole**	**374**	**168**
13 h		**2-[1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-fluorophenyl)-1,3,4-oxadiazole**	**357**	**171**
13 i		**2-bromo-5-[1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl]-1,3,4-oxadiazole**	**418**	**193**

3.2.4b CHARACTERIZATION OF SUBSTITUTED 2-[1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Spectral data of representative compounds:

^{1}HNMR of 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-(4-methoxyphenyl)-1, 3, 4-oxadiazole (13b):

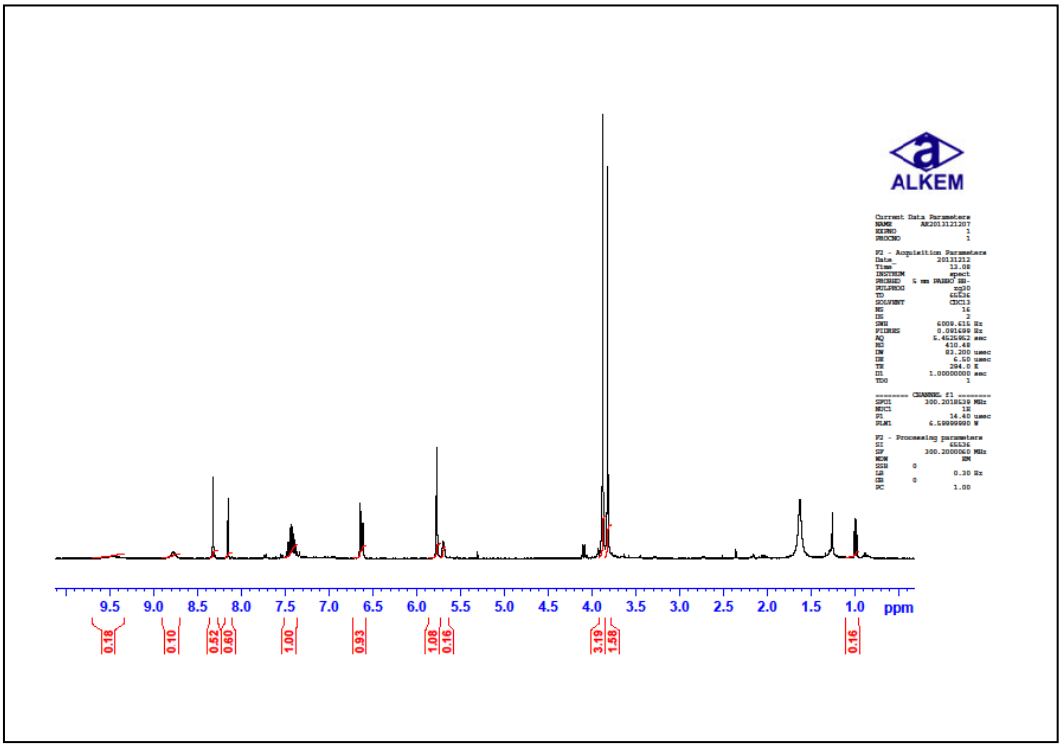


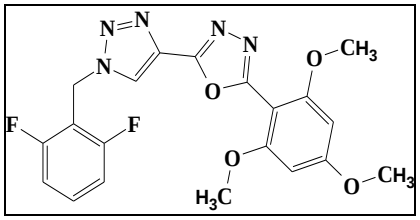


Interpretation

^{1}HNMR (DMSO-d6): 9.1(1H, s, aromatic proton of the triazole ring); 8.0 (2H, d, aromatic proton of the methoxy phenyl ring); 7.6 (1H, 1 aromatic proton of the di -fluoro phenyl ring); 7.2 (4H, 2 aromatic protons of the di -fluoro phenyl ring and 2 aromatic protons of methoxy phenyl ring); 5.8(2H, s, -CH_2), 3.9 (3H, s, -OCH_3)

[1]HNMR of 2-[1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazol-4-yl]-5-(2, 4, 6-trimethoxyphenyl)-1, 3, 4-oxadiazole (13c):

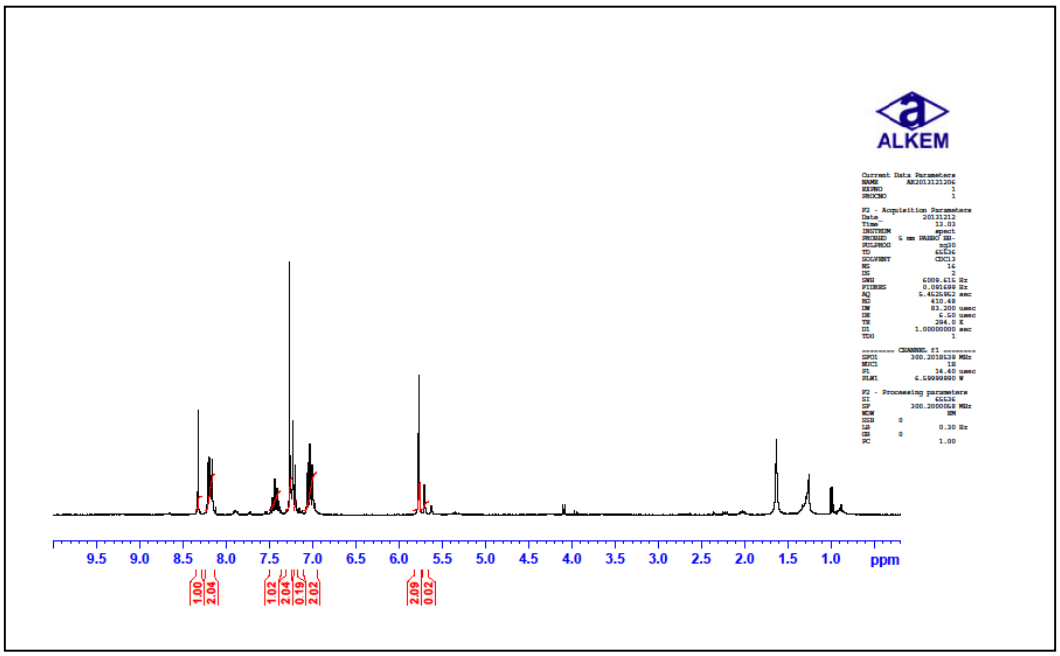


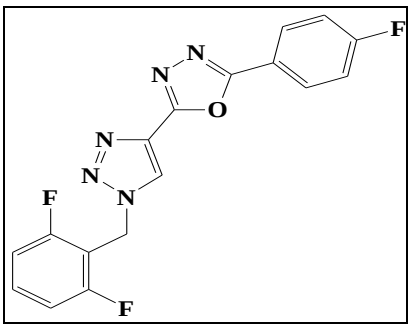


Interpretation

[1]HNMR (DMSO-d6): [1]HNMR (DMSO-d6): 8.4 (1H, s, aromatic proton of the triazole ring); 8.2 (1H, t, aromatic proton of the di -fluoro phenyl ring), 7.4 (2H,d 2 aromatic protons of the di -fluoro phenyl ring); 6.7 (2H, d , 2 aromatic protons of tri methoxy phenyl ring); 5.8(2H, s, -CH_2), 3.9 (6H, s, -OCH_3); 3.8 (3H, s, -OCH_3)

^{1}HNMR of 2-[1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (13h):

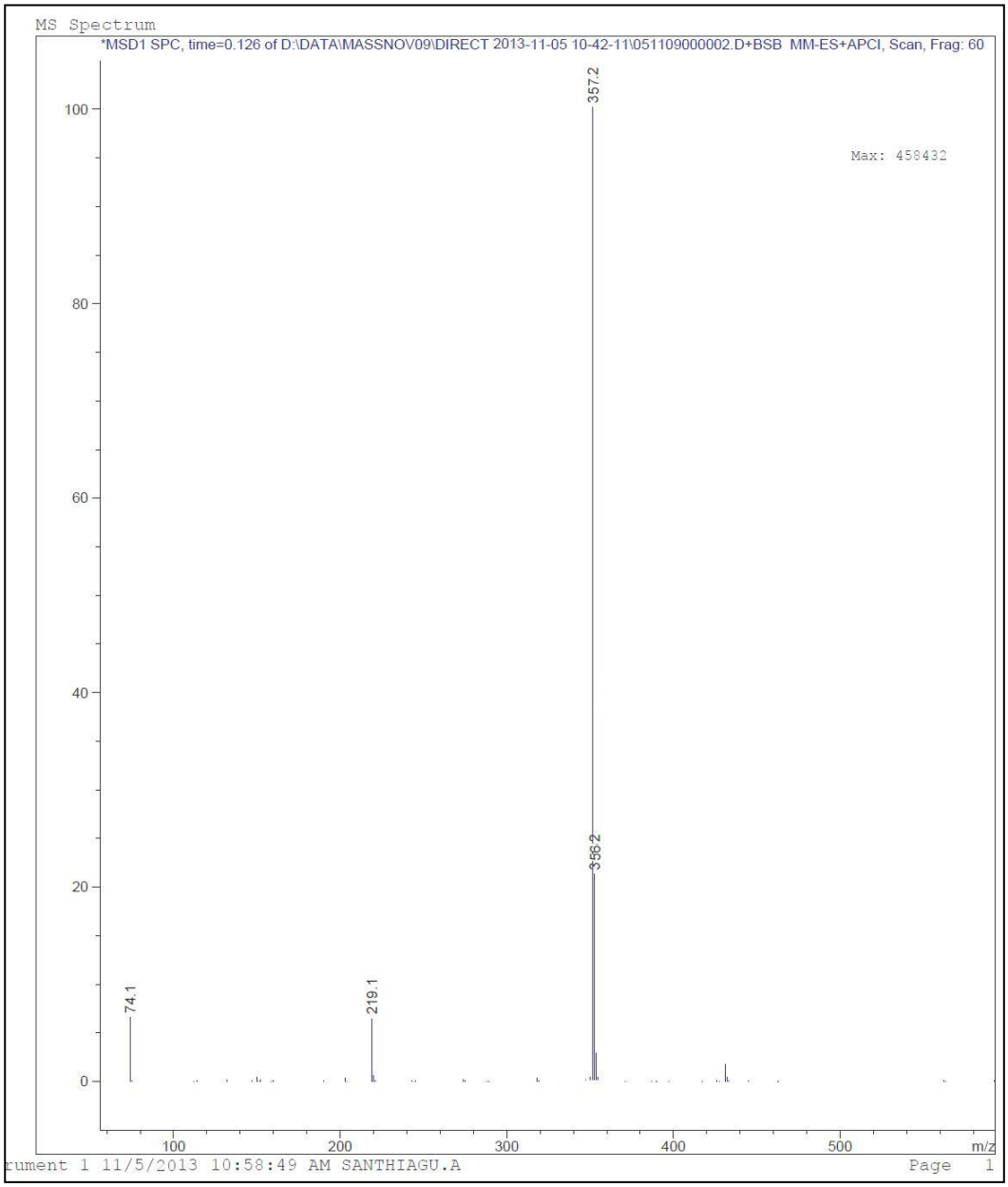




Interpretation

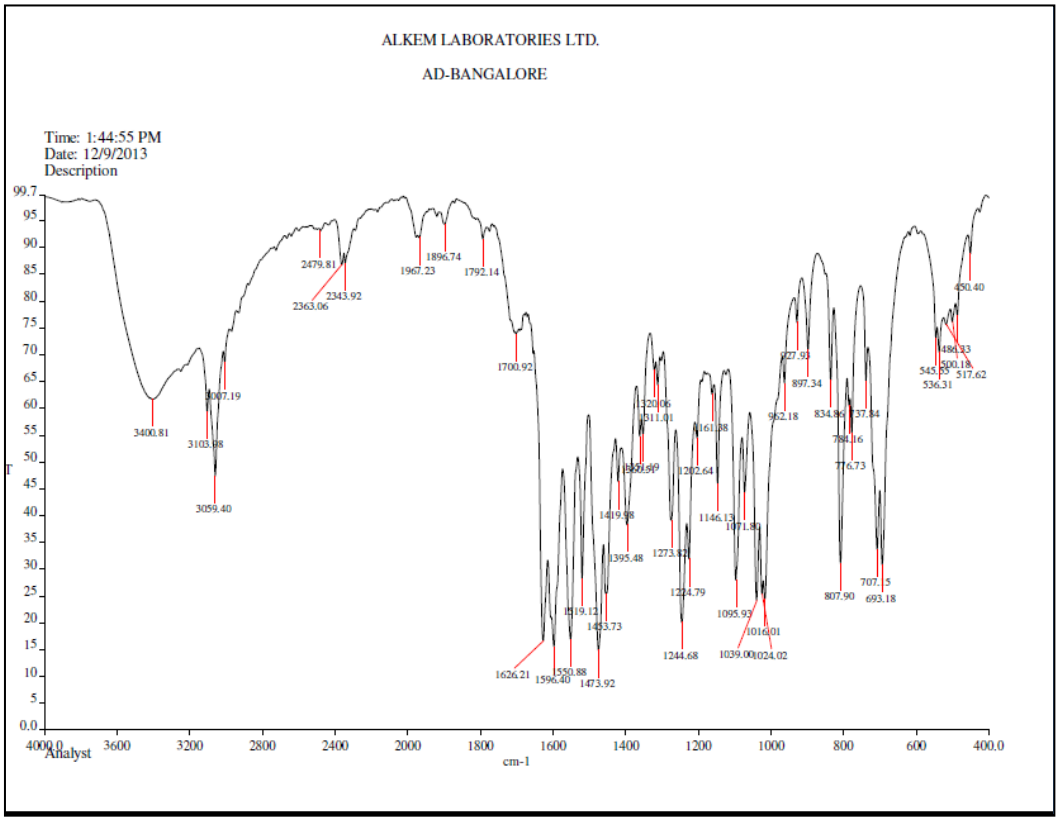
^{1}HNMR (DMSO-d6): 8.4 (1H, s, aromatic proton of the triazole ring);8.2 (2H, d, aromatic protons of the fluoro phenyl ring), 7.4 (1H, t, 1 aromatic proton of di fluoro phenyl ring); 7.3 (2H, d, 2 aromatic protons of the fluoro phenyl ring); 7.2 (2H, d, 2 aromatic protons of the di fluoro phenyl ring), 5.8(2H, s, -CH_2)

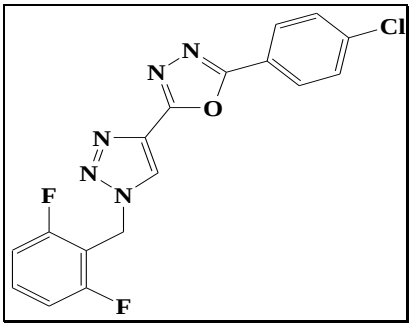
Mass spectrum of 2-[1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (13h):



The mass spectrum conforms to the molecular weight (357) of the synthesized compound

Infrared spectrum of 2-(4-chlorophenyl)-5-[1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-1, 3, 4-oxadiazole (13g):

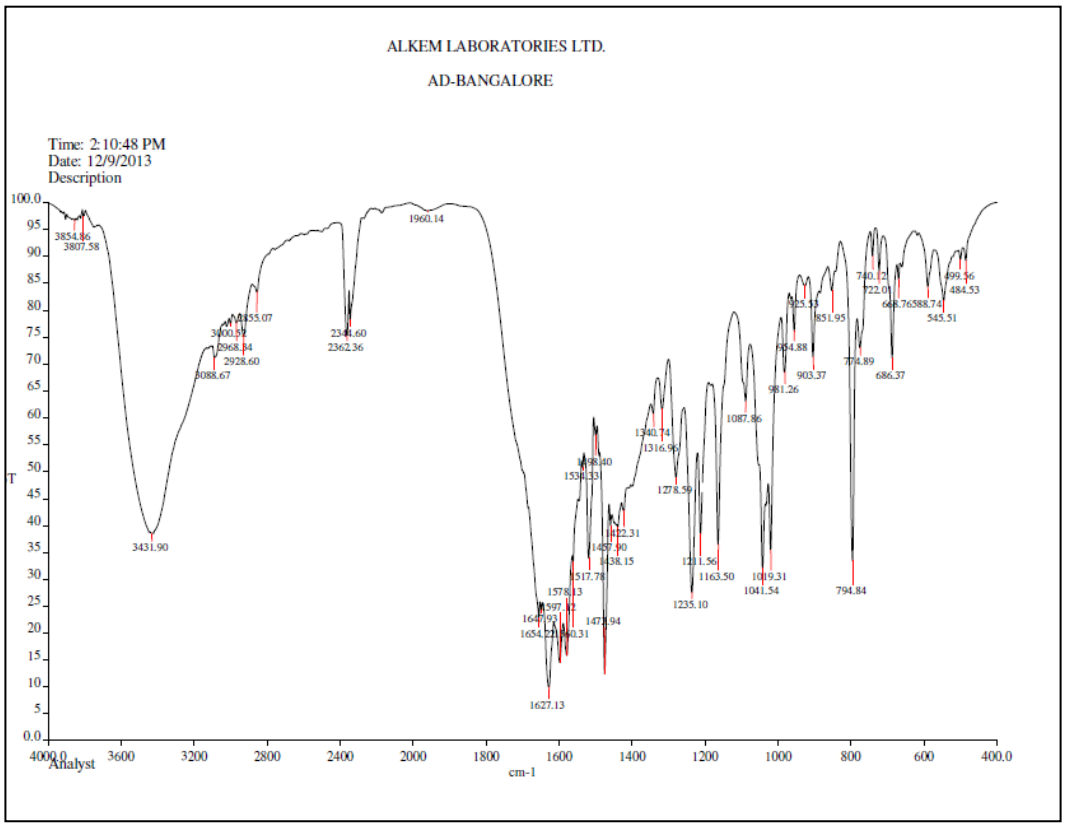


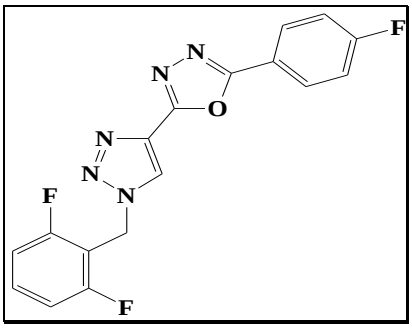


Interpretation

IR max cm^{-1}: 3059.40 (Aromatic sp^2 C-H stretch); 1473.92 (-CH_2 stretch); 1550.88 (-C=N); 1095.93 (C-F stretch); 807.90 (Aromatic out of plane bending); 707.15 (C-Cl stretch)

Infrared spectrum of 2-[5-chloro-1-(2, 6-difluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl]-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (13h):





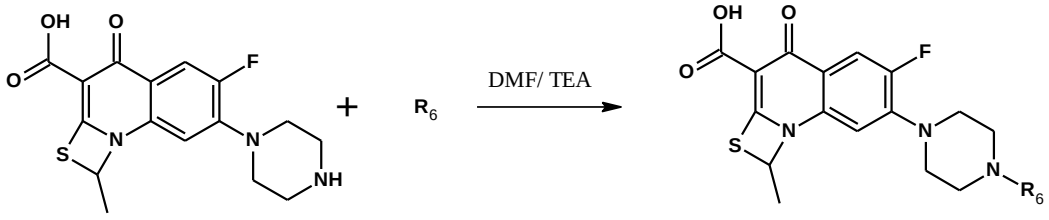
Interpretation

IR max cm^{-1}: 2928.60 (aromatic sp^2 C-H stretch); 1471.94 (-CH_2 stretch); 1517.78 (-C=N); 1163.50 (C-O stretch); 1041.54 (C-F stretch); 794.84 (aromatic out of plane bending)

3.3a SYNTHESIS OF SOME N-SUBSTITUTED DERIVATIVES OF 6-FLUORO-1-METHYL-4-OXO-7-(PIPERAZIN-1-YL)-4H-[1, 3] THIAZETO [3, 2-*A*] QUINOLINE-3-CARBOXYLIC ACID

The synthetic schemes have been divided into three parts depending on the condition of the reaction:

Part 1



349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1 ,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

(I)

II (a,d and e)

R_6: 2-(bromomethyl)-1, 3-difluorobenzene (for II a),
4'(bromomethyl)biphenyl-2-carbonitrile(for II d) and
benzyl 2-(benzyloxy)-4-(bromocarbonyl)benzoate(for II e)

General Experimental scheme for the synthesis of II (a, d and e)

Synthesis of 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-a):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid , 0.0086 mole of 2-(bromomethyl)-1,3-difluorobenzene, 0.0143 mol of triethylamine, 5 times of dimethylformamide based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl) -4H-[1,3] thiazeto[3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom reactor attached to a condenser and a mechanical stirrer. The reaction mass was stirred under nitrogen atmosphere and maintained at 85-90°C kept under for 4-6 hours. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was monitored by thin layer chromatography using ethyl acetate: hexane= 50:50 as the mobile phase. After the completion of the reaction, the mass was cooled to 25°C to 30°C. Later it was poured onto ice cold water (0-5°C) and stirred mechanically for 30 minutes. The resultant precipitate was filtered using a Whatman filter

paper. The isolated solid was stirred in 20 volumes of hot water (50-60°C), filtered and suction dried. The dried solid was stirred in 15 volumes of isopropyl alcohol. The solid was isolated by filtration, suction dried and later dried at 95-100°C. The isolated solids were slurried again in di isopropyl ether to obtain pure compounds.

Synthesis of 7-(4-((2'-cyano- [1,1'-biphenyl]-4-yl)methyl)piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H,4H-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid (II-d):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid , 0.0086 mole of 4' (bromomethyl) biphenyl-2-carbonitrile, 0.0143 mole of triethylamine, 5 times of dimethylformamide based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom reactor attached to a condenser and a mechanical stirrer. The reaction mass was stirred under nitrogen atmosphere and maintained at 85-90°C kept under for 4-6 hours. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was monitored by thin layer chromatography using ethyl acetate: hexane= 50:50 as the mobile phase. After the completion of the reaction, the mass was cooled to 25°C to 30°C. Later it was poured onto ice cold water (0-5°C) and stirred mechanically for 30 minutes. The resultant precipitate was filtered using a Whatman filter paper. The isolated solid was stirred in 20 volumes of hot water (50-60°C), filtered and suction dried. The dried solid was stirred in 15 volumes of isopropyl alcohol. The solid was isolated by filtration, suction dried and later dried at 95-100°C. The isolated solids were slurried again in di isopropyl ether to obtain pure compounds.

Synthesis of 7-(4-(1-(4-(benzyloxy)-2-((benzyloxy) carbonyl) phenyl) vinyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-e):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, 0.0086 mole of benzyl 2-(benzyloxy)-4-(bromocarbonyl) benzoate, 0.0143 mole of triethylamine, 5 times of dimethylformamide based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3] thiazeto [3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom reactor attached to a condenser and a mechanical stirrer. The reaction mass was stirred under nitrogen atmosphere and maintained at 85-90°C kept under for 4-6 hours. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was monitored by thin layer chromatography using ethyl acetate: hexane= 50:50 as the mobile phase. After the completion of the reaction, the mass was cooled to 25°C to 30°C. Later it was poured onto ice cold water (0-5°C) and stirred mechanically for 30 minutes. The resultant precipitate was filtered using a Whatman filter paper. The isolated solid was stirred in 20 volumes of hot water (50-60°C), filtered and suction dried. The dried solid was stirred in 15 volumes of isopropyl alcohol. The solid was isolated by filtration, suction dried and later dried at 95-100°C. The isolated solids were slurried again in di isopropyl ether to obtain pure compounds.

Part 2

349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1 ,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

(I)

II (b,g and h)

R_7: 5-[4'-(bromomethyl)biphenyl-2-yl]-1-trityl- l-1H-tetra(for II b) , 2-(chloromethyl)-4-(3-methoxypropoxy)-3-methylpyridine (for II g) and 1-[3-(trifluoromethyl)benzyl]-1H-pyrazole-4-carbonyl chloride(II h)

General Experimental scheme for the synthesis of II (b, g and h)

Synthesis of 6-fluro-1-methyl-4-oxo-7-(4-((2'-(1-trityl-1H-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl) methyl) piperazin-1-yl)-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-b):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid , 0.0086 mole of 5-[4'-(bromomethyl)biphenyl-2-yl]-1-trityl- l-1H-tetrazole, 0.0143 mole of triethylamine, 5% tetra butyl ammonium bromide with respect to the weight of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 5 times of dimethylformamide based on 6-fluoro-1- methyl-4- oxo-7- (piperazin-1-yl)-4H-[1,3] thiazeto[3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-6 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (eluent:: ethyl acetate: hexane=50: 50). After the completion of the reaction, the reaction mass was cooled to 25°C to 30°C and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-g):

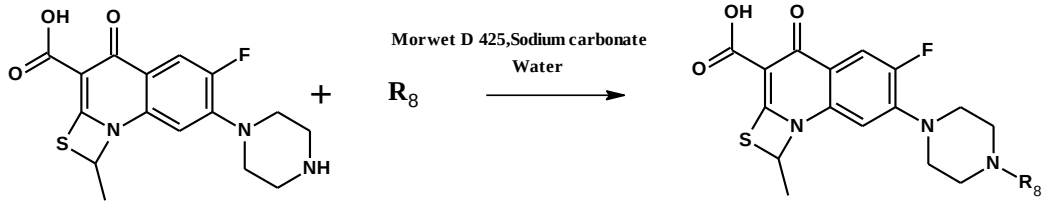
0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, 0.0086 mole of 2-(chloromethyl)-4-(3-methoxypropoxy)-3-methylpyridine, 0.0143 mole of triethylamine, 5% tetra butyl ammonium bromide with respect to the weight of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3] thiazeto[3,2-a]quinoline-3-carboxylic acid, 5 times of dimethylformamide based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-5 hours. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (eluent:: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to 25°C to 30°C and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 6-fluro-1-methyl-4-oxo-7-(4-(1-(3-(triflurомethyl)benzyl)-1H, 4H-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid(II-h):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, 0.0086 mole of 1-[3-(trifluoromethyl)benzyl]-1H-pyrazole-4-carbonyl chloride, 0.0143 mole of triethylamine, 5% tetra butyl ammonium bromide with respect to the weight of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid , 5 times of dimethylformamide based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid weight, were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-5 hours. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (eluent: ethyl acetate: hexane=50: 50). After the completion of the reaction, the

reaction mass was cooled to 25°C to 30°C and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Part 3



349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1 ,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

(I)

II (c and f)

R_8: 6-chloro-5-(2-chloroethyl)-1, 3-dihydro-2H-indol-2-one, 2-(4-chlorobutoxy)-1, 2, 3, 4-tetrahydroquinolin-7-ol,

General Experimental scheme for the synthesis of II (c and f)

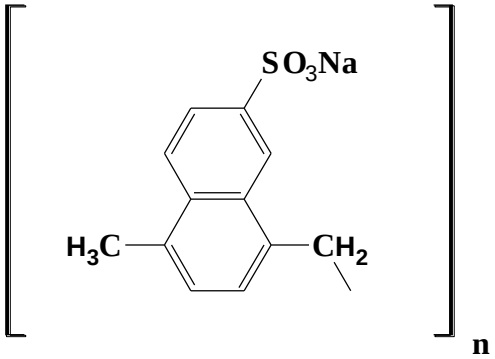
Synthesis of 7-(4-(5-(2-chloroethyl)-1, 3-dihydro-2H-indol-2-one) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-c):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, 0.0086 mole of 6-chloro-5-(2-chloroethyl)-1,3-dihydro-2H-indol-2-one, 0.024 mole of sodium carbonate, 5.2 times of water based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 7-8 hrs. The progress of the reaction was checked by thin layer chromatography (eluent:: ethyl acetate: hexane=50: 50). After the completion of the reaction, the reaction mass was cooled to 25°C to 30°C and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran to obtain pure compounds.

Synthesis of 6-fluro-7-(4-(5-(7-hydroxy-1, 2, 3, 4-tetrahydroquinolin-2-yl) pentyl) piperazin-1-yl)-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid (II-f):

0.0072 mole of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, 0.0086 mole of 2-(4-chlorobutoxy)-1,2,3,4-tetrahydroquinolin-7-ol, 0.024 mole of sodium carbonate, 5.2 times of water based on of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 7-9 hours. The progress of the reaction was checked by thin layer chromatography (eluent:: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to 25°C to 30°C and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran to obtain pure compounds.

Note: Dispersing agents like MORWET® D-425 are sodium salts of alkyl naphthylsulfonic acid/ formaldehyde condensates and sodium lignosulfonate.



Wherein n ranges from 2 to 9

U.S.Pat.No.8, 410,268B2 disclosed a process for the preparation of Ziprasidone, which involves the same procedure [1].

Melting and yield obtained:

a) 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (a)]:

M.P:228°C

b) 6-fluro-1-methyl-4-oxo-7-(4-((2'-(1-trityl-1H-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl) methyl) piperazin-1-yl)-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (b)]:

M.P:231°

c) 7-(4-(5-(2-chloroethyl)-1, 3-dihydro-2H-indol-2-one) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II(c)]

M.P:233°

d) 7-(4-((2'-cyano- [1,1'-biphenyl]-4-yl)methyl)piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 H,4H-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid [II(d)]:

M.P: 211° C

e) 7-(4-(1-(4-(benzyloxy)-2-((benzyloxy) carbonyl) phenyl) vinyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (e)]:

M.P: 220° C

f) 6-fluro-7-(4-(5-(7-hydroxy-1, 2, 3, 4-tetrahydroquinolin-2-yl) pentyl) piperazin-1-yl)-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (f)]:

M.P: 212° C

g) 6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (g)]:

M.P: 233° C

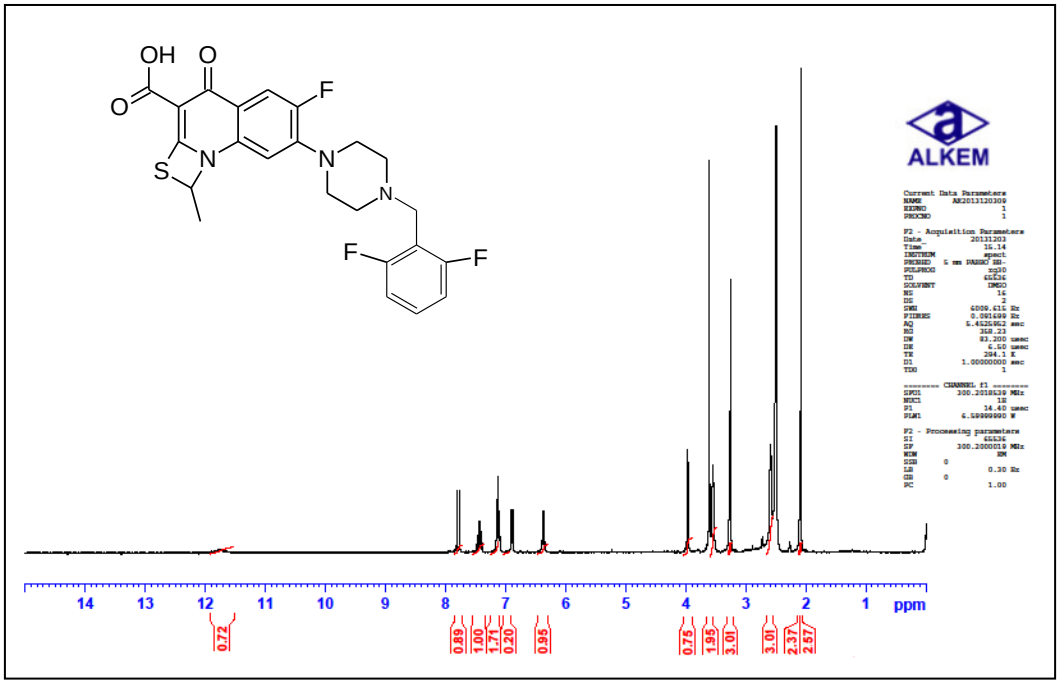
h) 6-fluro-1-methyl-4-oxo-7-(4-(1-(3-(triflurомethyl) benzyl)-1H, 4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid [II (h)]

M.P: 223° C

3.3b CHARACTERIZATION OF SOME N-SUBSTITUTED DERIVATIVES OF 6-FLUORO-1-METHYL-4-OXO-7-(PIPERAZIN-1-YL)-4H-[1, 3] THIAZETO [3, 2-*A*] QUINOLINE-3-CARBOXYLIC ACID

Spectral data of representative compounds:

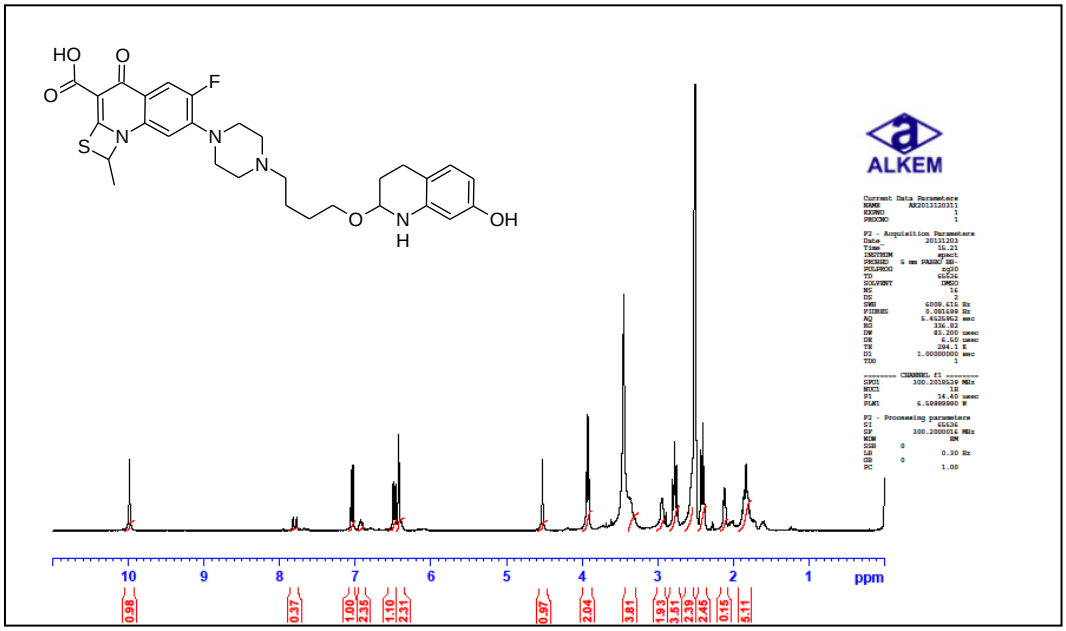
^{1}HNMR spectrum of 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (a)]:



Interpretation

^{1}HNMR (DMSO D^6): δ= 11.8(-OH,s,1H) , 7.8 (aromatic CH,S,1H),7.5 (aromatic CH,m,1H), 7.3 (aromatic CH,d,2H) 6.5 (aromatic CH,s,1h),4.0(CH,m,1H), 3.7(CH,s, 2H),3.4(-CH_2 piperazine ring,t, 4H), 2.7(-CH_2 piperazine ring,t,4H), 2.2(-CH_3,6H)

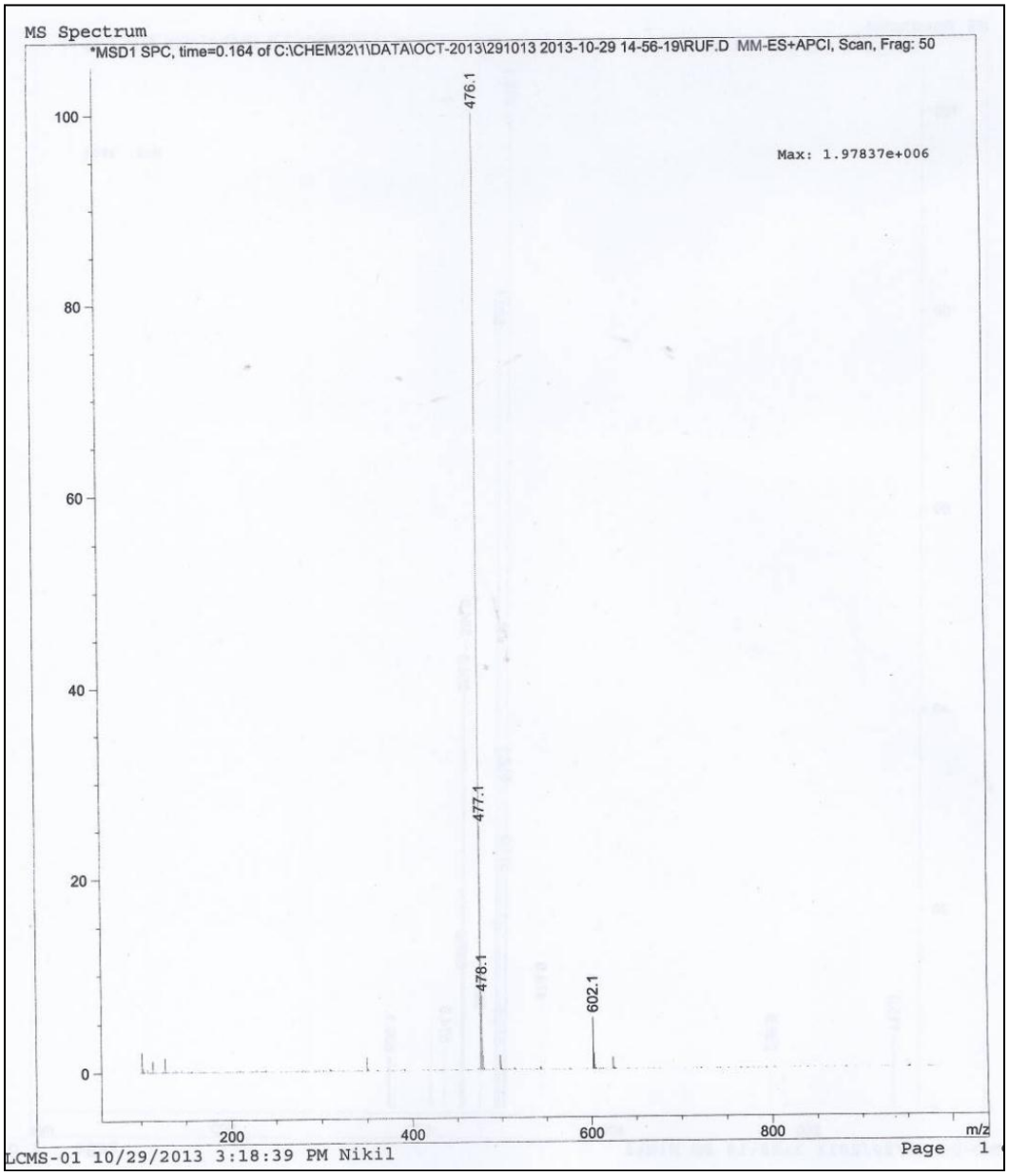
^{1}HNMR spectrum of 6-fluro-7-(4-(5-(7-hydroxy-1, 2, 3, 4-tetrahydroquinolin-2-yl) pentyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (f)]:



Interpretation

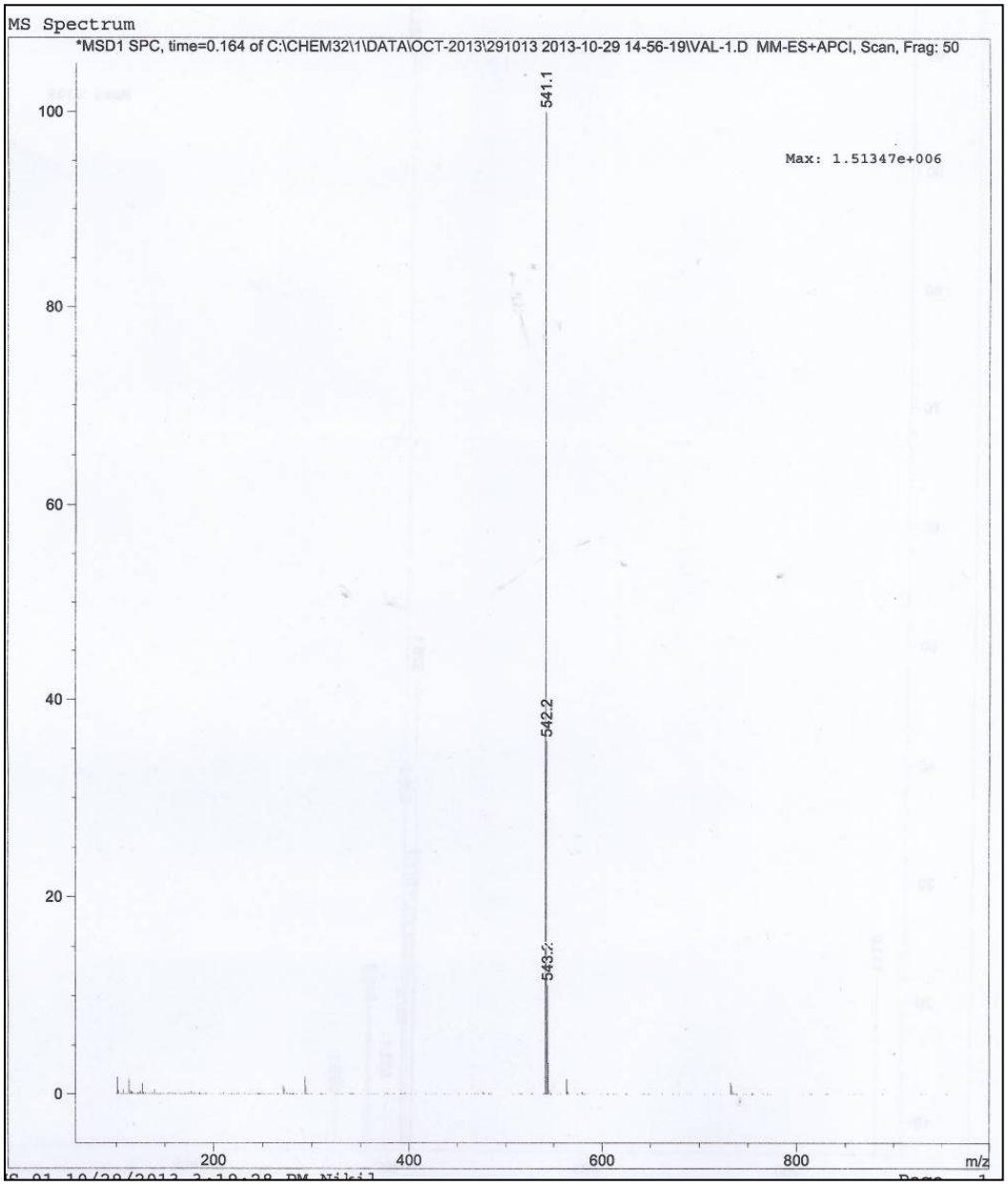
^{1}HNMR (DMSO D^6): δ= 10.0(CH$_2$-OH,s,1H),7.12(aromatic CH,S,1H),6.85 (aromatic CH,m, 2H), 6.5 (aromatic CH,t,1H) 6.4 (aromatic CH,d,2h), ,4.45 (NH,d,1H), 3.9 (-CH,m,2H), 3.5 (-CH$_2$,m,4H), 3.0(-CH$_2$,t,2H), 2.8 (-CH$_2$,t,4H), 2.4-2.5 (-CH$_2$,5H), 1.8-2.2(-CH$_2$,5H)

Mass spectrum of 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (II-a):



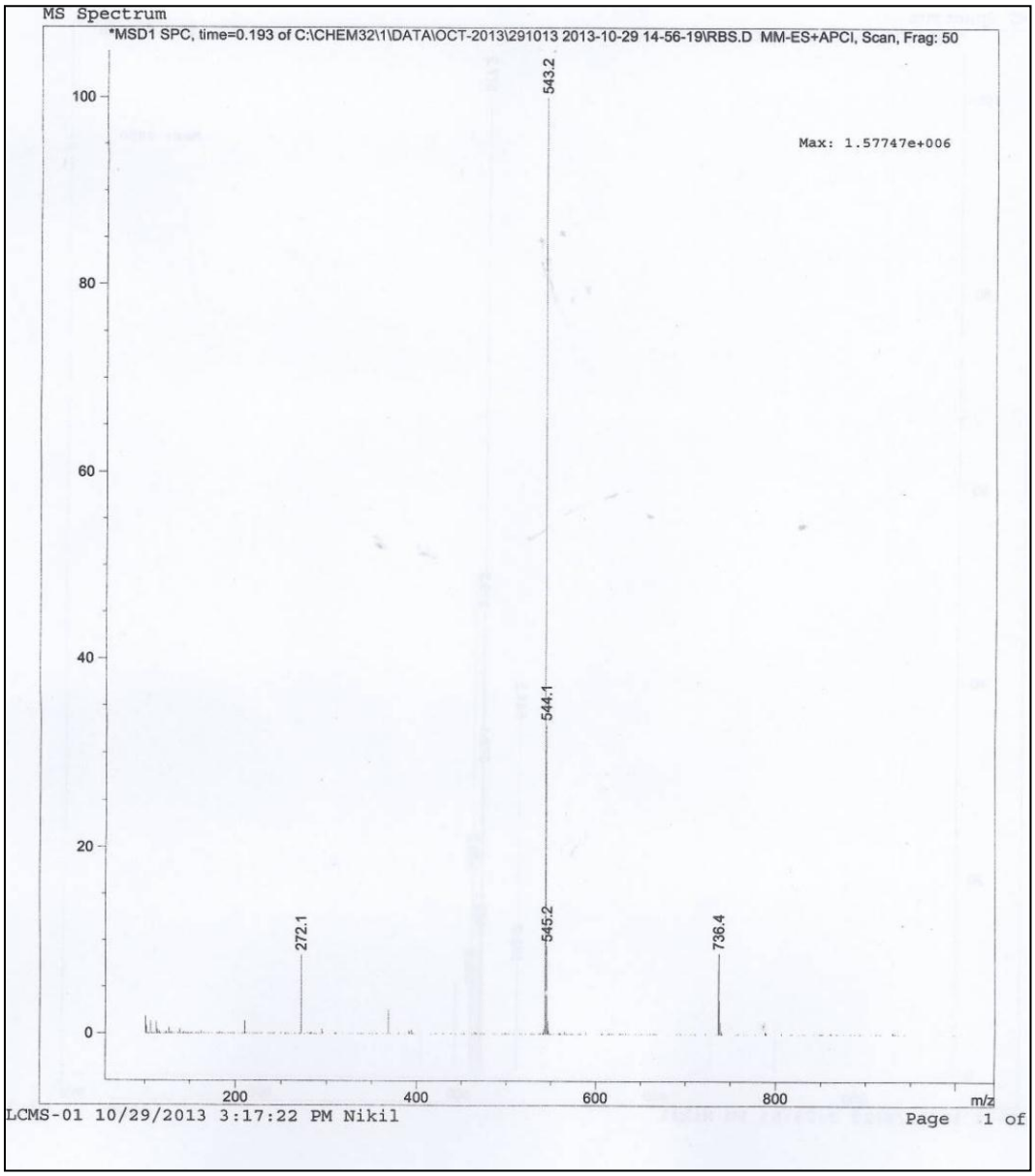
The mass spectrum corresponds to the molecular weight (475) of the compound

Mass spectrum of 7-(4-((2'-cyano- [1,1'-biphenyl]-4-yl)methyl)piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*,*4H*-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid [II(d)]:



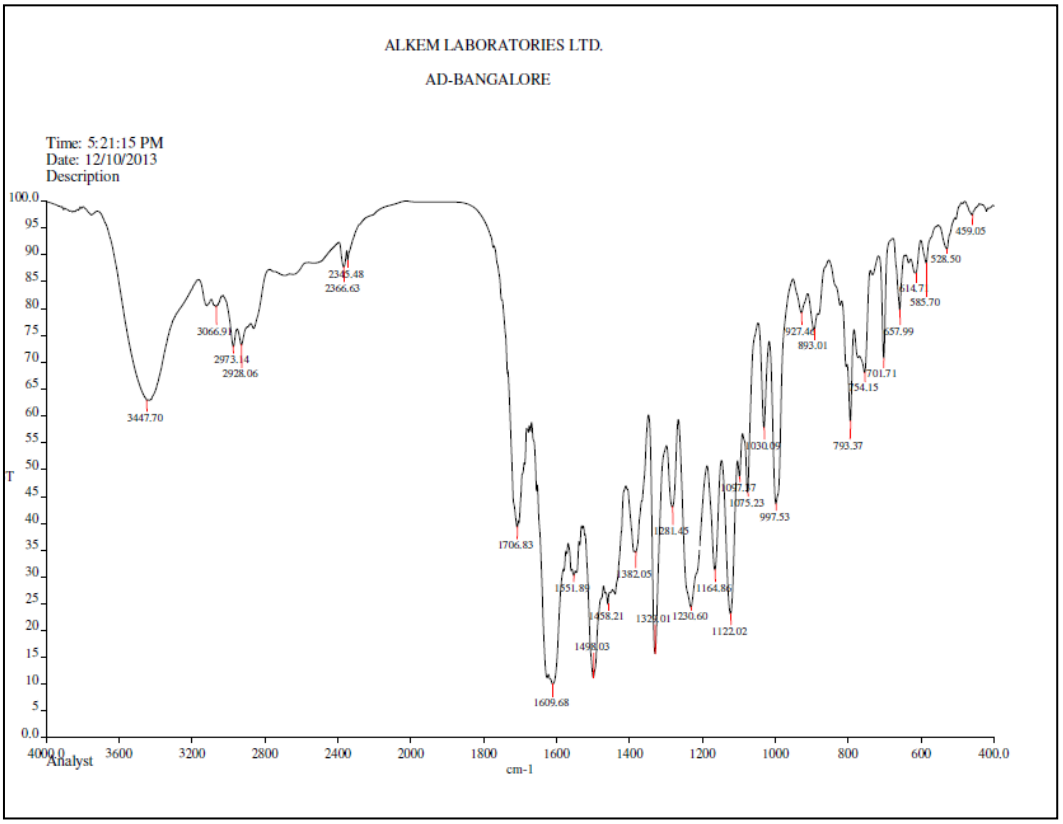
The mass spectrum corresponds to the molecular weight (540) of the compound

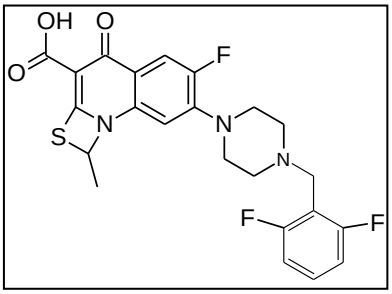
Mass spectrum of 6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (g)]:



The mass spectrum corresponds to the molecular weight (542) of the compound

Infrared spectrum of 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (a)]:

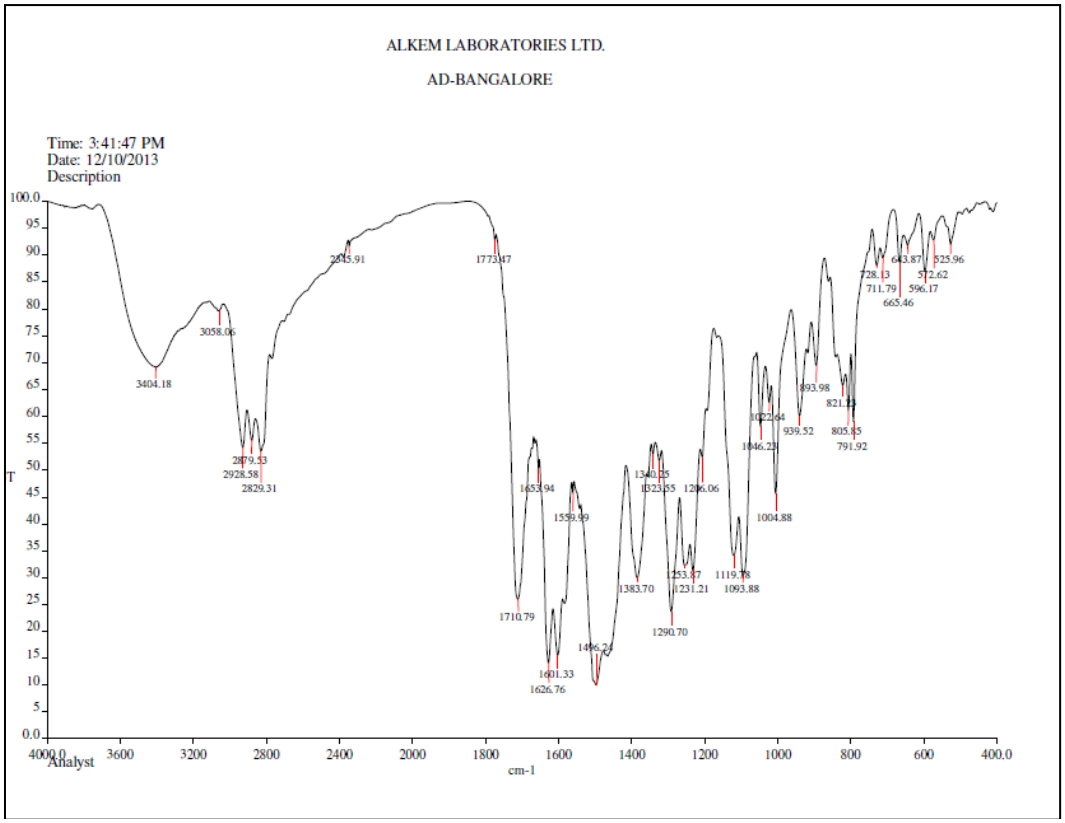


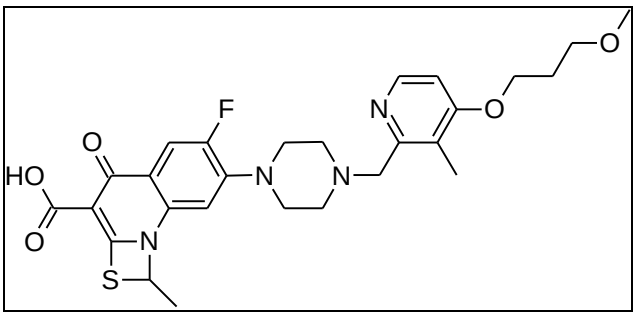


Interpretation

IR max cm^{-1}: 3448 (Ar C-H) , 3070 (O-H stretch); 1706.83 (C=O stretch); 1609.68-1498.03 (Ar C=C); 1230.60 (C-F stretch)

Infrared spectrum of 6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (g)]:

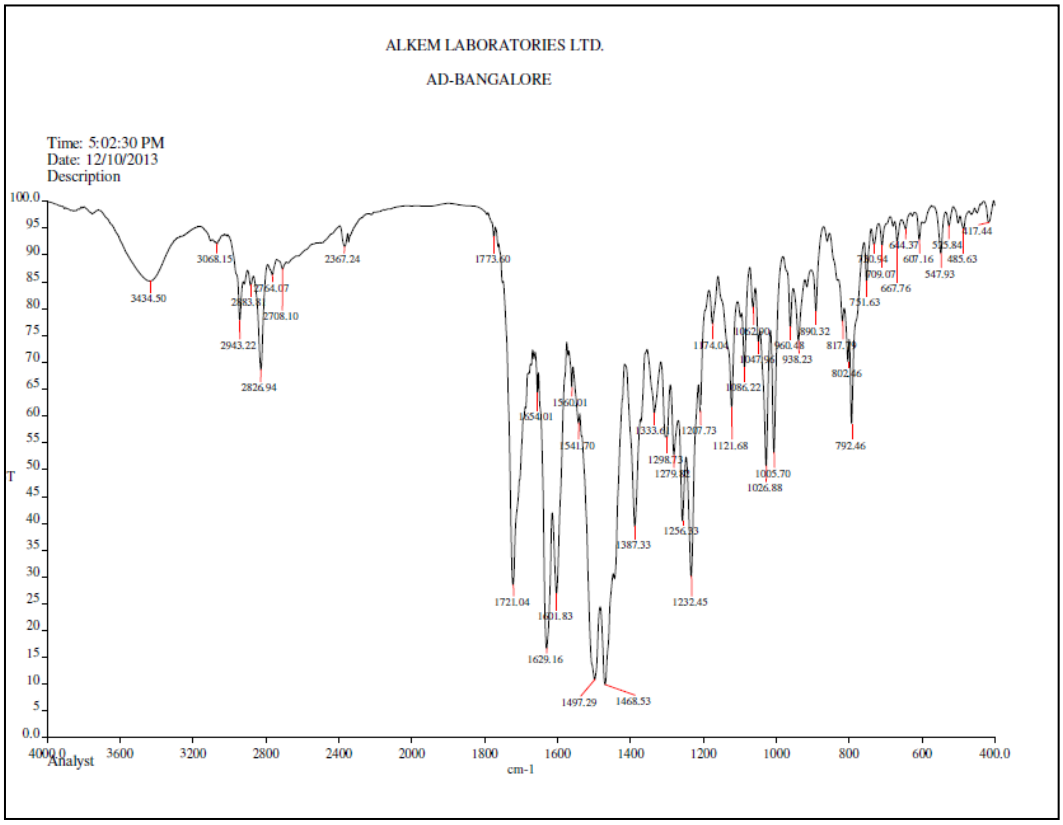


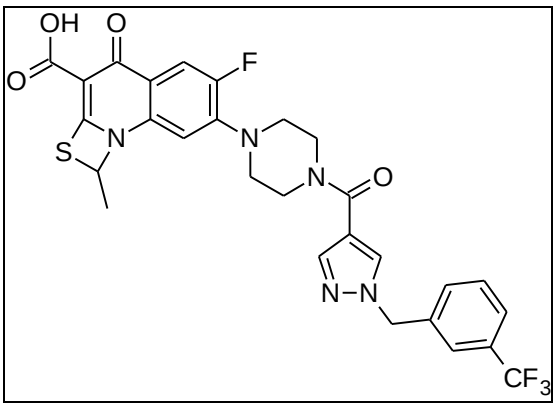


Interpretation

<u>IR max cm^{-1}: 3405 - 3059 (O-H stretch); 1710.79 (C=O stretch); 1601.33 and 1496.24 (Aromatic C=C stretch); 1231.21 (C-F stretch)</u>

Infrared spectrum of 6-fluro-1-methyl-4-oxo-7-(4-(1-(3-(trifluromethyl) benzyl)-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [II (h)] :





Interpretation

IR max cm^{-1}: 3435 - 3069 (O-H stretch); 1721.04 (C=O stretch); 1601.83 and 1468.53 (Aromatic C=C stretch); 1232.45 (C-F stretch)

3.4 REFERENCE

[1] Shashiprabha, Kanakamajalu S., Mukherjee Debkiron, Padmashree B., Ksundarraja R., Kuppuswamy N. Process for the preparation of Ziprasidone. U.S.Pat.No. 8,410,268B2. 2013.

CHAPTER IV: MATERIALS, METHODS AND RESULTS- BIOLOGICAL ACTIVITY

4.0 BIOLOGICAL ACTIVITY

4.1 Strategy I: ANTICONVULSANT ACTIVITY OF 4-CHLORO-2-(4-SUBSTITUTED- PIPERAZIN-1-YL) QUINAZOLINES

Evaluation of Anticonvulsant Activity

Experimental animals

Male albino Swiss mice, weighing 20- 25g, were used to study the effect of the synthesized compounds on subcutaneous Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that oestrous cycle could influence their activity threshold. The animals were housed in a standard cage at room temperature in a 12/ 12 hours light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000mg/ kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e*. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

2 -Pentylenetetrazole induced seizure test

- Forty five adult albino mice were randomly divided into groups of five mice each.
- Standard group: This group of mice was administered with Rufinamide. Dose: 40 mg/kg of body weight, intraperitoneal.
- Control group: This group of mice was given Pentylenetetrazole. Dose: 40 mg/ kg of body weight, subcutaneously.
- Test/ treated groups: These groups were administered with the synthesized compounds 6(a-g). Dose: 50 mg/kg of body weight intraperitoneal.

Thirty minutes later, a freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse at a dose of 50 mg/kg of body weight.

The onset of action, number of rats showing tonic convulsion as well as mortality was recorded in each group.

RESULTS

Acute toxicity study

The subcutaneous LD_{50} of the drugs was found to be 100 mg kg^{-1}.

Effects of the synthesized compounds on the convulsive activity of 40 mg/Kg of subcutaneous Pentylenetetrazole in mice

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.
The observations are tabulated in Table 9.

Table 9: Effect of the compounds (6a-6g) on Pentylenetetrazole– induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. Of animals convulsed/ No. of animals used	Animals protected (%)
6a	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
6b	1.33±0.14	0.27±0.03	No onset	No clonus	0/5	100%
6c	1.20±0.19	0.24±0.04	No onset	No clonus	0/5	100%
6d	1.41±0.13	0.28±0.02	No onset	No clonus	0/5	100%
6e	1.25±0.07	0.25±0.01	No onset	No clonus	0/5	100%
6f	1.26±0.25	0.25±0.05	82.6±49.92	No clonus	0/5	100%
6g	1.54±0.11	0.30±0.02	45.6±22.26	5.2±5.21	3/5	40%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

4.2 Strategy II:

4.2a ANTICONVULSANT ACTIVITY OF 1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4- SUBSTITUTED- PIPERAZIN-1-YL) METHANONES

Evaluation of Anticonvulsant Activity

Experimental animals

Male albino Swiss mice, weighing 20- 25 g, were used to study the effect of the synthesized compounds on subcutaneous Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that oestrous cycle could influence their activity threshold. The animals were housed in a standard cage at room temperature in a12/ 12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e*. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

2 -Pentylenetetrazole induced seizure test

- Forty five adult albino mice were randomly divided into groups of five mice each.
- Standard group: This group of mice was administered with Rufinamide. Dose: 40 mg/kg of body weight, intraperitoneal.
- Control group: This group of mice was given Pentylenetetrazole. Dose: 40 mg/kg of body weight, subcutaneous.
- Test/ treated groups: These groups were administered with the synthesized compounds 8 (a-g). Dose: 50 mg/kg of body weight intraperitoneal.

Thirty minutes later, a freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse at a dose of 50 mg/kg of body weight.

The onset of action, number of rats showing tonic convulsion as well as mortality was recorded in each group.

RESULTS

Acute toxicity study

The subcutaneously LD_{50} of the drugs was found to be 100 mg kg^{-1}.

Effects of the synthesized compounds on the convulsive activity of 40 mg/kg of subcutaneous Pentylenetetrazole in mice

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 10.

Table 10: Effect of the compounds (8a-8g) on Pentylenetetrazole– induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/ No. of animals used	Animals protected (%)
8a	1.30±0.19	0.26±0.04	No onset	No clonus	0/5	100%
8b	1.54±0.11	0.30±0.02	45.6±22.26	5.2±5.21	3/5	40%
8c	1.31±0.19	0.24±0.04	No onset	No clonus	0/5	100%
8d	1.52±0.13	0.28±0.02	No onset	No clonus	0/5	100%
8e	1.36±0.07	0.25±0.01	No onset	No clonus	0/5	100%
8f	1.26±0.25	0.25±0.05	82.6±49.92	6.3±3.21	4/5	20%
8g	1.44±0.14	0.27±0.03	No onset	No clonus	0/5	100%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

4.2b ANTICONVULSANT ACTIVITY OF [5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL] (4-SUBSTITUTED PIPERAZIN-1-YL) METHANONES

Evaluation of Anticonvulsant Activity

Experimental animals

Male albino Swiss mice, weighing 20- 25 g, were used to study the effect of the synthesized compounds on subcutaneous Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that oestrous cycle could influence their activity threshold. The animals were housed in a standard cage at room temperature in a12/ 12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e*. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

2 -Pentylenetetrazole induced seizure test

- Forty five adult albino mice were randomly divided into groups of five mice each.
- Standard group: This group of mice was administered with Rufinamide. Dose: 40 mg/kg of body weight, intraperitoneal.
- Control group: This group of mice was given Pentylenetetrazole. Dose: 40 mg/kg of body weight, subcutaneous.
- Test/ treated groups: These groups were administered with the synthesized compounds 9 (a-g). Dose: 50 mg/kg of body weight intraperitoneal.

  Thirty minutes later, a freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse at a dose of 50 mg/kg of body weight.

The onset of action, number of rats showing tonic convulsion as well as mortality was recorded in each group.

RESULTS

Acute toxicity study

The subcutaneously LD_{50} of the drugs was found to be 100 mg kg^{-1}.

Effects of the synthesized compounds on the convulsive activity of 40 mg/kg of subcutaneous Pentylenetetrazole in mice

All the control animals exhibited threshold seizures. The synthesized compounds exhibited anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 11.

Table 11: Effect of the compounds (9a-9g) on Pentylenetetrazole– induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/ No. of animals used	Animals protected (%)
9a	1.26±0.15	0.28±0.02	No onset	No clonus	0/5	100%
9b	1.50±0.07	0.24±0.04	51.6±25.16	7.1±5.21	3/5	40%
9c	1.27±0.19	0.30±0.02	No onset	No clonus	0/5	100%
9d	1.48±0.13	0.26±0.04	No onset	No clonus	0/5	100%
9e	1.32±0.07	0.27±0.03	No onset	No clonus	0/5	100%
9f	1.22±0.25	0.30±0.02	74.6±45.12	7.3±4.25	4/5	20%
9g	1.40±0.14	0.25±0.03	No onset	No clonus	0/5	100%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

4.2c ANTICONVULSANT ACTIVITY OF SUBSTITUTED 2-[5-CHLORO-1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Evaluation of Anticonvulsant Activity

Experimental animals

Male albino Swiss mice, weighing 20- 25 g, were used to study the effect of the synthesized compounds on subcutaneous Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that oestrous cycle could influence their activity threshold. The animals were housed in a standard cage at room temperature in a12/ 12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e*. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

2 -Pentylenetetrazole induced seizure test

- Fifty five adult albino mice were randomly divided into groups of five mice each.
- Standard group: This group of mice was administered with Rufinamide. Dose: 40 mg/kg of body weight, intraperitoneal.
- Control group: This group of mice was given Pentylenetetrazole. Dose: 40 mg/kg of body weight, subcutaneous.
- Test/ treated groups: These groups were administered with the synthesized compounds 11 (a-i). Dose: 50 mg/kg of body weight intraperitoneal.

  Thirty minutes later, a freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse at a dose of 50 mg/kg of body weight.

The onset of action, number of rats showing tonic convulsion as well as mortality was recorded in each group.

RESULTS

Acute toxicity study

The subcutaneously LD_{50} of the drugs was found to be 100 mg kg^{-1}.

Effects of the synthesized compounds on the convulsive activity of 40 mg/kg of subcutaneous Pentylenetetrazole in mice

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 12.

Table 12: Effect of the compounds (11a-11i) on Pentylenetetrazole– induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/No. of animals used	Animals protected (%)
11a	1.20±0.15	0.25±0.02	No onset	No clonus	0/5	100%
11b	1.44±0.07	0.21±0.04	No onset	No clonus	0/5	100%
11c	1.21±0.19	0.27±0.02	No onset	No clonus	0/5	100%
11d	1.42±0.13	0.23±0.04	No onset	No clonus	0/5	100%
11e	1.26±0.07	0.24±0.03	No onset	No clonus	0/5	100%
11f	1.16±0.25	0.27±0.02	No onset	No clonus	0/5	100%
11g	1.44±0.11	0.28±0.02	No onset	No clonus	0/5	100%
11h	1.44±0.11	0.28±0.02	No onset	No clonus	0/5	100%
11i	1.26±0.07	0.24±0.03	No onset	No clonus	0/5	100%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

4.2d ANTICONVULSANT ACTIVITY OF SUBSTITUTED 2-[1-(2, 6-DIFLUOROBENZYL)-1H-1, 2, 3-TRIAZOL-4-YL]-1, 3, 4-OXADIAZOLES

Evaluation of Anticonvulsant Activity

Experimental animals

Male albino Swiss mice, weighing 20- 25 g, were used to study the effect of the synthesized compounds on subcutaneous Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that oestrous cycle could influence their activity threshold. The animals were housed in a standard cage at room temperature in a12/ 12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non lethal dose *i.e*. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

2 -Pentylenetetrazole induced seizure test

- Fifty five adult albino mice were randomly divided into groups of five mice each.
- Standard group: This group of mice was administered with Rufinamide. Dose: 40 mg/kg of body weight, intraperitoneal.
- Control group: This group of mice was given Pentylenetetrazole. Dose: 40 mg/kg of body weight, subcutaneous.
- Test/ treated groups: These groups were administered with the synthesized compounds 13 (a-i). Dose: 50 mg/kg of body weight intraperitoneal.
  Thirty minutes later, a freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse at a dose of 50 mg/kg of body weight.

The onset of action, number of rats showing tonic convulsion as well as mortality was recorded in each group.

RESULTS

Acute toxicity study

The subcutaneously LD_{50} of the drugs was found to be 100 mg kg^{-1}.

Effects of the synthesized compounds on the convulsive activity of 40 mg/kg of subcutaneous Pentylenetetrazole in mice

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 13.

Table 13: Effect of the compounds (13a-13i) on Pentylenetetrazole– induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/ No. of animals used	Animals protected (%)
13a	1.35±0.15	0.32±0.02	No onset	No clonus	0/5	100%
13b	1.59±0.07	0.28±0.04	No onset	No clonus	0/5	100%
13c	1.36±0.19	0.34±0.02	No onset	No clonus	0/5	100%
13d	1.57±0.13	0.30±0.04	No onset	No clonus	0/5	100%
13e	1.41±0.07	0.31±0.03	No onset	No clonus	0/5	100%
13f	1.31±0.25	0.34±0.02	No onset	No clonus	0/5	100%
13g	1.59±0.11	0.35±0.02	No onset	No clonus	0/5	100%
13h	1.59±0.11	0.35±0.02	No onset	No clonus	0/5	100%
13i	1.36±0.19	0.34±0.02	No onset	No clonus	0/5	100%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

Effect of the compounds on Pentylenetetrazole induced seizures in mice- An overview

Pentylenetetrazol (PTZ) which is a CNS stimulant is used as the seizure inducer. Its epileptogenic properties have been used to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. As a non-competitive GABA antagonist, PTZ is specifically used in seizure assays as a method of assessing the excitability of the central nervous system and GABA activity. In the current study 30 minutes after administration of an I.P. dose of the synthesized compounds , the mice injected with PTZ (Pentylenetetrazol) and are observed for latencies to twitches and seizures (tonic, and clonic). Seizure profiles are assessed through 30 minutes post-PTZ administration, evaluated every 10 minutes for signs such as immobility, abnormal limb splay, ataxia, straub tail (The Straub tail reaction in mice is an S-shaped dorsiflexion of the mouse tail. It is based on a contraction of the sacro-coccygeal dorsalis muscles.), clonic/ tonic seizures (Tonic-clonic seizures are a type of generalized seizure. Tonic seizures involve sudden stiffening and contraction of the muscles. Clonic seizures involve rhythmic twitching or jerking of one or several muscles.), muscle fasciculation, loss of righting reflex, and status epilepticus (a dangerous condition in which epileptic fits follow one another without recovery of consciousness between them). Here it was noted that in most cases, similar to drugs used clinically as anticonvulsants for the treatment of epilepsy, the onset of PTZ-induced seizure activity was delayed and lesser mortality as a result of these seizures.

4.3 ANTIBACTERIAL ACTIVITY OF N-SUBSTITUTED DERIVATIVES OF 6-FLUORO-1-METHYL-4-OXO-7-(PIPERAZIN-1-YL)-4H- [1, 3]THIAZETO [3, 2-A] QUINOLINE-3-CARBOXYLIC ACID

Evaluation of antibacterial activity and determination of minimum inhibitory concentration by disk diffusion method

1. **Test organisms**: *Bacillus subtilis* and *Escherichia coli*
2. **Test compounds*:*** Eight synthesized molecules and Ciprofloxacin as positive control
3. **Inoculum:** Cell suspension prepared from cultures grown on Trypticose soya broth adjusted to 1-2 x 10^8 cells/mL
4. **Drug concentrations: drug concentration prepared: (a)** Test compounds: 8 - 1024 µg/mL in 1% DMSO **(b)** Control: 1% DMSO in Sterile water
5. **Procedure: (a) Determination of Antibacterial activity: (i)** 100 µl Inoculum of test cultures was inoculated on Muller Hinton Agar plates (90 mm). **(ii)** Test compounds (5µl, 1024 µg/mL) and ciprofloxacin (5 µl, 1 mg/mL) was impregnated on 6mm sterile Whatmann No. 1 Disks. **(iii)** Test compounds and standard disks were placed on Agar plates. **(iv)** The plates were Incubated @ 35 °C for 24-48 hrs and observe for zone of inhibition around the disk. **(v)** The compounds showing activity were further tested for determination of Minimum Inhibitory Concentration.

   (b) Determination of minimum Inhibitory Concentration: (i) 100 µl Inoculum of test cultures was inoculated on Muller Hinton Agar plates (90 mm). **(ii)** Test compounds (5 µl, different test concentrations, 8, 32, 128, 512 and 1024 µg/ mL) and ciprofloxacin (5 µl, 1 mg/ mL) was impregnated on 6mm sterile Whatmann No. 1 Disks. **(iii)** Test compounds and standard disks were placed on Agar plates. **(iv)** The plates were incubated @ 35°C for 24-48 hrs and observe for zone of inhibition around the disk. **(v)** Lowest concentration of test compound showing zone of inhibition is considered as MIC.
6. **Results: Antibacterial activity of test compounds:**

   Among the tested compounds seven compounds show antibacterial activity against *Bacillus subtilis* (Gram positive bacteria). None of the compounds

show zone of inhibition of *Escherichia coli* (Gram negative bacteria). The zone of inhibition observed for different test compounds is tabulated in Table 14.

7. **Determination of Minimum Inhibitory concentration:** The zone of inhibition and MIC determined for each test compounds in summarized in Table 14**.**

Antibacterial screening Images

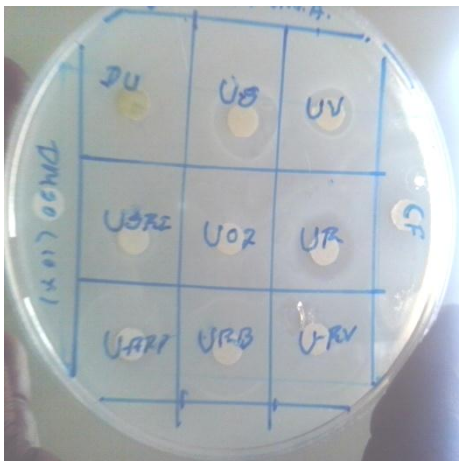


Image 1

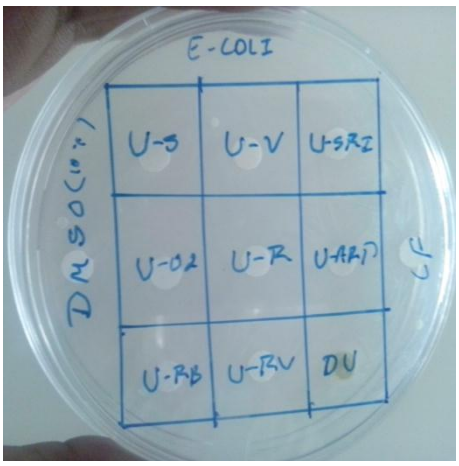


Image 2

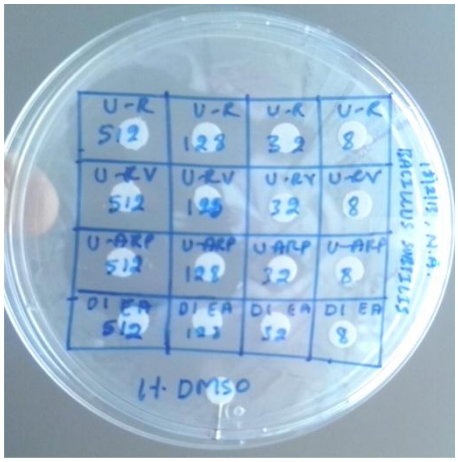


Image 3

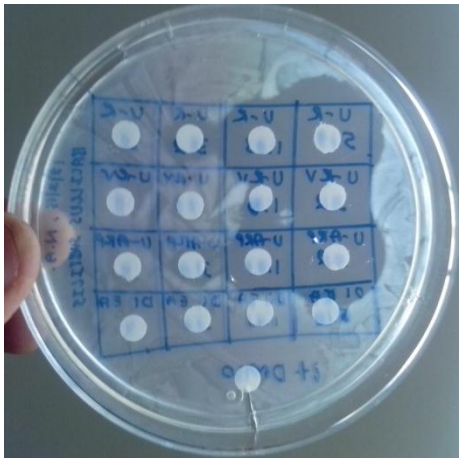


Image 4

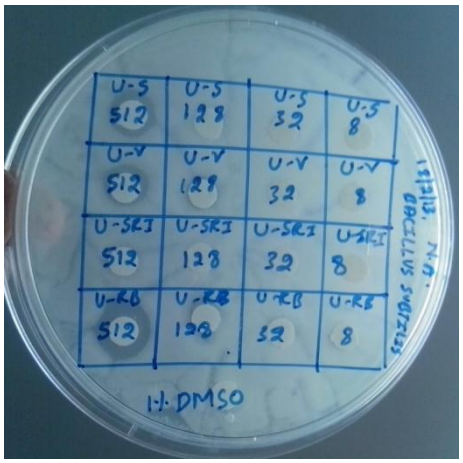


Image 5

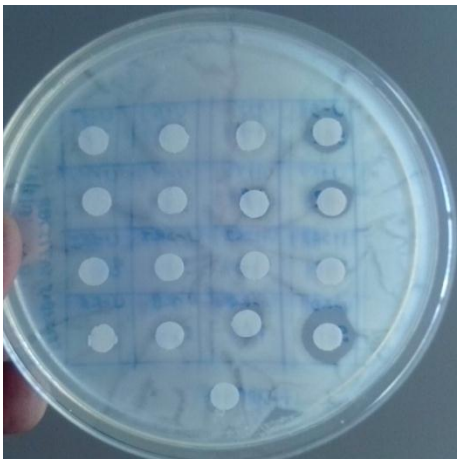


Image 6

The Images 1 and 2 represent the experiments done with *Escherichia coli* (Gram negative bacteria). These clearly demonstrate the inactivity of the compounds II (a-h) on *Escherichia coli* (Gram negative bacteria).

The Image 3 to 6 represents the experiments performed with *Bacillus subtilis* (Gram positive bacteria). The zone of inhibition developed by the action of the compounds II (a-h) can be viewed clearly.

Table 14: Zone of inhibition of test compounds and Minimum inhibitory concentration of test compounds against *Bacillus subtilis*

Sl.No	Test Compound	Concentration (µg/mL)	Zone of Inhibition (in mm) *Test organism: Bacillus subtilis*	Minimum Inhibitory Concentration (µg/mL)
1	II(a)	1024	21.00	32
		512	17.00	
		128	14.00	
		32	11.00	
		8	-	
2	II(b)	1024	19.50	128
		512	16.00	
		128	7.00	
		32	-	
		8	-	
3	II(c)	1024	10.00	512
		512	8.0	
		128	-	
		32	-	
		8	-	
4	II(d)	1024	20.00	32
		512	14.00	
		128	12.00	
		32	10.0	
		8	-	
5	II(e)	1024	13.00	512
		512	9.00	
		128	-	
		32	-	
		8	-	
6	II(f)	1024	21.00	32
		512	26.00	
		128	18.00	
		32	11.00	
		8	-	
7	II(g)	1024	10.00	128
		512	7.00	
		128	7.00	
		32	-	
		8	-	
8	II(h)	1024	19.00	32
		512	17.00	
		128	14.00	
		32	8.00	
		8	-	
9	Ciprofloxacin Positive Control	1024	14.00	-
10	DMSO Negative Control	1%	0.00	-

CHAPTER V: SUMMARY AND CONCLUSION

In the present research work, an investigation was carried out on the design, synthesis, characterisation and biological activities of some novel piperazine and triazole derivatives. The synthesized compounds were tested for either anticonvulsant activty or antibacterial activity based on their design and structure. The biological activities were tested using standard screening methods viz. subcutaneous Pentylenetetrazole induced threshold seizure method for determining anticonvulsant activity and evaluation of antibacterial activity using Disk diffusion method.

The salient findings of the experimentation are summarised below.

- **Identifying new compounds with less structural resemblance to the existing anticonvulsant drugs.**

  4-chloro-2-(4-substituted-piperazin-1-yl) quinazolines 6(a-g) [chapter reference: Synthesis and Characterization 3.1a], were synthesized and characterized. The compounds with phenyl substitutions, benzothiazole and methoxy ethanol offered protection against subcutaneous Pentylenetetrazole induced threshold seizure. So it can be inferred that 4-chloro-2-(4- substituted- piperazin-1-yl) quinazolines with aryl and glycol ether substitution can act as anticonvulsant agents. These compounds are not widely studied with respect to their anticonvulsant activities.

- **The 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole moiety, of Rufinamide "a well known anticonvulsant drug", was modified by including various substituted piperazines and substituted oxadiazoles.**

  Protection was offered by the synthesized compounds 8a, 8c, 8d, 8e, 8g [chapter reference: Synthesis and Characterization 3.2.1a], 9a, 9c, 9d, 9e and 9g [chapter reference: Synthesis and Characterization 3.2.2a] against subcutaneous Pentylenetetrazole induced threshold seizure.

  It can be inferred from the results that the compounds with piperazines substituted with aryl, benzothiazole, methoxy ethanol and ethanol moieties offered protection against subcutaneous Pentylenetetrazole induced threshold seizure.

The synthesized oxadiazoles 11(a-i) and 13(a-i) exhibited protection against subcutaneous Pentylenetetrazole induced threshold seizure. Both aryl and alkyl substitutions in the case of oxadiazoles showed similar actions.

The aforementioned work provides an idea on the structural requirements for the mentioned class of compounds to act as anticonvulsant agents.

- **Some N-substituted 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid derivatives were prepared.**

  The derivatives involving substituted phenyl and substituted quinoline ring showed substantial activity against *Bacillus subtilis* (Gram positive bacteria). Among the tested compounds seven compounds showed antibacterial activity against *Bacillus subtilis* (Gram positive bacteria).

  The main finding is that 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid derivatives with piperazine ring substituted with quinoline ring and with the halogen, cyano substituted phenyl ring has increased inhibitory activity on Gram positive bacteria.

CHAPTER VI: SCOPE OF FUTURE RESEARCH WORK

The idea introduced in this dissertation provides a natural guide to future research.

- Typically, a new domain of 4-chloro-2-(4- substituted- piperazin-1-yl) quinazolines can be initially be studied as anticonvulsant agents. Various aryl and glycol ether derivatives can be incorporated and screened for the anticonvulsant activity.
- The research on piperazine and oxadiazole substituted 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole moiety can be taken forward by synthesizing new chemical entities with various other substitutions. Main focus should be kept on the aryl substituted compounds.
- Additionally, in the context of 1-[(2, 6-difluorophenyl)-1H-1, 2, 3-triazole moiety, it is not yet completely clear how mechanisms should be evaluated. Thus, future research on this area will also involve developing a general theory for such evaluation and to conclude a mechanism of action.
- 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4H-[1, 3] thiazeto [3, 2-a] quinoline-3-carboxylic acid derivatives has exhibited substantial activity on Gram positive bacteria. As already discussed, the piperazine substitutions on the mentioned fluoroquinolone moiety is rare, future research can be carried out to study the effect of substituted piperazines on the potency and spectrum of activity of the mentioned fluoroquinolone antibiotics.

Addendum

The addendum to the thesis addresses the queries raised by the examiners during the review:

1. **In the Section 1.6 "Objective of the present study" it is suggested by the reviewer that the objective of the work be properly defined and sequence of the work should be written as per the study undertaken.**

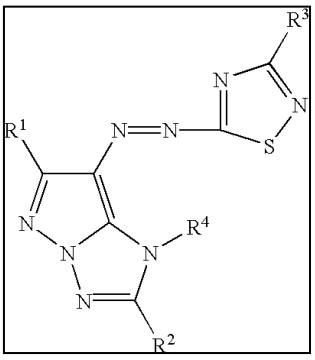
   According to the above suggestion I propose to combine the sections 1.6 and 1.7 and rename the same as "Objective and Scope". This will provide a clear definition on the work conducted and will conform to the suggestion on proper definition of objective.

   In addition to that, it may be noted that the sequence of the work is mentioned in detail in the same chapter in subsection 9 named "Summary of the work plan"

2. **In the section 2.2 "Triazoles and anticonvulsant activity" a recommendation was given by the reviewer to include references for Triazoles in medicines, agrochemicals, dye stuffs.**

   As suggested by the reviewer examples on triazoles in dye stuff, fluorescent whiteners, agrochemicals and medicines are being mentioned in the subsequent paragraphs.

   Hisashi *et al*. (1999) [1] reported a yellow dye compound having 1, 2, 4-triazole as the Azo component. The dye compound was found to be excellent in transfer property and colour fastness against light, excellent in light absorption property, excellent in solubility and can be synthesized with ease. The dye compound of the present invention is expressed by the general formula



Optical brighteners, optical brightening agents (OBAs), fluorescent brightening agents (FBAs) or fluorescent whitening agents (FWAs) are dyes that absorb light in the UV and violet region (usually 340-370 nm) of the EM spectrum and re-emit light in the blue region (typically 420-470 nm).

These additives are often used to enhance the appearance of colour of fabric and paper causing a "whitening" effect, making materials look less yellow by increasing the overall amount of blue light reflected. FBA production for paper, textiles and detergents is dominated by just a few di- and tetra-sulfonated triazole-stilbenes and a di-sulfonated stilbene-biphenyl.

Jing-Pei Huo *et al.* (2013) [2] reported the Synthesis and Characterization of Fluorescent Brightening Agents with Chiral 2(5H)-Furanone and Bis-1, 2, 3-triazole structure.

Paul A. Worthington (1987) [3] reported that using a knowledge of the mode of action of the plant growth regulator paclobutrazol, it was possible to design a series of 1,2,4 triazole containing tertiary alcohols which have high levels of plant fungicidal activity. From this group flutriafol and hexaconazole have been introduced into crop protection.

Lass-Flörl C (2011) [4] inferred that invasive fungal disease continues to be a problem associated with significant morbidity and high mortality in immunocompromised and, to a lesser extent, immunocompetent individuals. He also stated that triazole antifungal agents have emerged as front-line drugs for the treatment and prophylaxis of many systemic mycoses. Fluconazole plays an excellent role in prophylaxis, empirical therapy, and the treatment of both superficial and invasive yeast fungal infections. Voriconazole is strongly recommended for pulmonary invasive aspergillosis. Posaconazole shows a very wide spectrum of activity and its primary clinical indications are as salvage therapy for patients with invasive aspergillosis and prophylaxis for patients with neutropenia and haematopoietic stem-cell transplant recipients. Itraconazole also has a role in the treatment of fungal skin and nail infections as well as dematiaceous fungi and endemic mycoses. Fluconazole and voriconazole are well absorbed and exhibit high oral bioavailability, whereas the oral bioavailability of itraconazole and posaconazole is lower and more variable. Posaconazole absorption depends on administration with a high-fat meal or nutritional supplements. Itraconazole and voriconazole undergo extensive hepatic metabolism involving the cytochrome P450 system. The therapeutic window for triazoles is narrow, and inattention to

their pharmacokinetic properties can lead to drug levels too low for efficacy or too high for good tolerability or safety. This makes these agents prime candidates for therapeutic drug monitoring (TDM).

3. **The title of Chapter 3" Methodology and results" is suggested to be replaced by "Materials and methods".**

   The suggestion is acknowledged, hence Methodology and Results will be replaced by Materials, Methods and Results. As the section includes the outcome of the various processes performed, the term "Results" is being included in the title.

4. **In Chapter 3, in various reaction procedures the cooling range of 25 to 30 degree C is mentioned. The reviewer recommended putting the cooling temperature instead of the range**.

   The intention was to cool the reaction mass to SATP - Standard Ambient Temperature and Pressure (IUPAC) which is 25 degree C. During the optimization process it was observed that an increase of 5 deg C did not have any influence on the yield and purity of the isolated compounds. Considering the aforementioned study a temperature range 25 to 30 degree C is considered as the cooling range in multiple reaction work up procedures of the current work. Any temperature in the given range yielded consistent and expected results.

5. **The details and specification of the Filter paper (hardened filter paper) is asked for in Chapter 3: Materials, methods and results: Synthesis and characterization.**

   Whatman® quantitative filter paper, hardened low-ash, Grade 50 was used to do the filtrations during reaction work up and isolations. As per the suggestion the term hardened filter paper will be replaced with Whatman filter paper.

6. **In Chapter III Materials, methods and results: Synthesis and characterization the reviewer asked for clearer interpretation IR, mass and NMR.**

   The interpretation was done based on the spectral data and the values are interpreted below the spectrum. For better clarity, the structure of the compound has been incorporated below the spectrum. As for mass fragmentations, soft ionization was followed, with the prime intention of identifying the molecular ion peak.

7. **In Chapter III Materials, methods and results: Synthesis and characterization, the reviewer asked for more justification of the methodology.**

   Efforts were made to arrive at an efficient and systematic decision-making approach driving the optimization strategies to give the best possible results.
   Procedures stated in each section were the outcome of several trials taken to perfect the identified variables like molar ratios of reactants, solvent ratio, reaction time, isolation method and purification techniques. The trials were done with the identified variables taking one factor at a time. The yield and purity were studied and the best reaction parameters were identified. The optimized procedures are mentioned in the section "Materials, methods and results-Synthesis and characterization"

8. **In Chapter III, Materials, methods and results: Synthesis and characterization, the reviewer recommended the study of microwave assisted synthesis of the title compounds and its derivatives.**

   The suggestion is very good and useful and study would have yielded interesting results. Microwave assisted synthesis was not pursued due to the absence of laboratory/industrial grade microwave in the lab. It is to be noted that the synthesis of all the compounds were done following conventional methods as mentioned in the procedures. Microwave assisted organic synthesis is a well-known fact that microwave energy enhances organic reactions in turn reducing chemical reaction times—in the best cases, hours are even reduced to minutes. The suggestion can be taken up during future endeavours on the subject.

9. **On Chapter III, Materials, methods and results: Synthesis and characterization, reviewer asked for characterization of title compounds and its derivatives using XRD.**

   The XRD technique is mainly used to find the polymorphic nature of compounds. Single crystal XRD can be used to know the structure and isomeric forms. In the case of our research, the polymorphic study was not included and neither the title compounds nor its derivatives exhibit any kind of optical isomerism.

10. **Reproducibility of chemical synthesis**

    Validating an experiment′s reproducibility and accuracy are the foundations of scientific research. In the present work, the lead compounds were prepared more than once in order to build sufficient quantity for

preparation of derivatives. Even the processes for making derivatives were repeated for making up sample quantities required for animal and microbiological studies. Ability to replicate procedures with consistent results has been the highlight of the work.

11. **Chapter IV, Materials, methods and results: Biological activity, question is asked on whether the animals were administered a same dose with the synthesized compounds for toxicity studies.**

    The compounds were administered subcutaneously in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals.

12. **In Chapter IV, Materials, methods and results: Biological activity, explanation on the table 9 and 10 is asked for.**

    Effect of the compounds on Pentylenetetrazole induced seizures in mice- An overview

    Pentylenetetrazol (PTZ) which is a CNS stimulant is used as the seizure inducer. Its epileptogenic properties have been used to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. As a non-competitive GABA antagonist, PTZ is specifically used in seizure assays as a method of assessing the excitability of the central nervous system and GABA activity. In the current study 30 minutes after administration of an I.P. dose of the synthesized compounds , the mice injected with PTZ (Pentylenetetrazol) and are observed for latencies to twitches and seizures (tonic, and clonic). Seizure profiles are assessed through 30 minutes post-PTZ administration, evaluated every 10 minutes for signs such as immobility, abnormal limb splay, ataxia, straub tail (The Straub tail reaction in mice is an S-shaped dorsiflexion of the mouse tail. It is based on a contraction of the sacro-coccygeal dorsalis muscles.), clonic/ tonic seizures (Tonic-clonic seizures are a type of generalized seizure. Tonic seizures involve sudden stiffening and contraction of the muscles. Clonic seizures involve rhythmic twitching or jerking of one or several muscles.), muscle fasciculation, loss of righting reflex, and status epilepticus (a dangerous condition in which epileptic fits follow one another without recovery of consciousness between them). Here it was noted that in most cases, similar to drugs used clinically as anticonvulsants for the treatment of epilepsy, the onset of PTZ-induced seizure activity was delayed and lesser mortality as a result of these seizures.

    Based on the above discussion it is to be noted in the tables 9, 10, 11 and 12 that the latency of tonic convulsion, duration of clonus, number of animals convulsed were observed and reported.

13. **Effect of the compounds on Pentylenetetrazole induced seizures in mice- An overview,** will hold good for the **Tables 11, 12, 13** of Chapter 4 Materials, methods and results: Biological activity.

14. **In Chapter IV, the reviewer recommended studies with other gram negative bacteria.**

    In response to the above suggestion it can be mentioned that the study was performed with the available strains of bacteria in the contract laboratory. Further studies can be included during future research on the subject.

REFERENCES

1) Hisashi Mikoshiba, Minami-ashigara, Takayoshi Kamio. Azo Dye Compound having 1'2'4'triazole as the Azo component. *US 6458194 B1*, Oct. 1, 2002

2) Jing-Pei Huo, Jian-Cheng Luo, Wei Wu, Jin-Feng Xiong, Guang-Zhen Mo, and Zhao-Yang Wang. Synthesis and Characterization of Fluorescent Brightening Agents with Chiral 2(5H)-Furanone and Bis-1, 2, 3-triazole Structure . *Ind. Eng. Chem. Res*. 52 (34),: 11850–11857, 2013

3) Paul A. Worthington. Synthesis and Fungicidal Activity of Triazole Tertiary Alcohols. *Synthesis and Chemistry of Agrochemicals*. (32), : 302-317, 1987

4) Lass-Flörl C. Triazole antifungal agents in invasive fungal infections: a comparative review. *Drugs*. (18),: 2405-2419, 2011

Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

SYNTHESIS, CHARACTERIZATION AND ANTICONVULSANT ACTIVITY OF SUBSTITUTED 4-CHLORO-2-(4-PIPERAZIN-1-YL) QUINAZOLINES

DEBKIRON MUKHERJEE[a]*, A.MUKHOPADHYAY[b], K.SHRIDHARA BHAT[c], A.M.SHRIDHARA[d], K.S.RAO[e]

[A, b]Chemical Engineering Department, Jadavpur University, Kolkata-700032, India, [c,d,e] Alkem Laboratories Limited, 473D2, 13thCross,4thPhase, Peenya Industrial Area, Bangalore-560058.
Email: debkironmukherjee@gmail.com

Received: 07Apr 2014 Revised and Accepted: 12 May 2014

ABSTRACT

Objective: Substituted 4-chloro-2-(4-piperazin-1-yl) quinazolines: Synthesis and anticonvulsant activity.

Methods: In the present study, the 2, 4-dichloroquinazoline (5) was synthesized and the compound was reacted with different N-substituted piperazines to obtain a series of title compounds [6(A-G)]. All the new title compounds were characterized by spectral data and were screened for anticonvulsant activity.

Results: The reported compounds were synthesized using the process disclosed by us in U.S.Pat.No.8, 410,268B2. In our present work, we have achieved substantially good yields and purity.

Conclusion: Aryl substituted piperazines exhibited better protection against subcutaneous (s.c.) Pentylenetetrazol induced seizures.

Keywords: Synthesis, Quinazolines, Piperazines, Characterization, Anticonvulsant activity.

INTRODUCTION

Quinazoline heterocycle consists of two fused six membered aromatic rings benzene & pyrimidine. The research on biological activity of quinazoline compounds started when the compound 2-methyl-1,3-aryl-4-quinazoline derivative was synthesized. In 1968 only two derivatives were used, Methaqualone as soporific & anticonvulsant and Quinathazone as diuretic. By 1980, about 50 kinds of derivatives of this class with different medicinal and biological actions like soporific, sedative, tranquilizing, analgesic, anticonvulsant, antitussive, myorelexant, antirheumatic, hypotensive, antiallergic, bronchodilating, antidiabetic, cholagogue, diuretic, cystatic, antimalarial, spermicidal etc [1] were identified . The anticonvulsant activity was attributed to its ability to bind the non-competitive site of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In a previous report [2], compounds were synthesized and tested for their anticonvulsant activity, which was comparable to that of diazepam. As a result, these compounds are potential leads for further design of more active compounds. Since the discovery of methaqualone as a sedative hypnotic [3-4], the search for new anticonvulsant drugs with reduced toxicity and fewer side effects has been continuous. It has been reported that replacement of the methyl group by some other functionalities such as alkylthiomethyl or alkyloxymethyl groups reportedly yielded structural analogues which retained the anticonvulsant activity [5-6].

Piperazines are a broad class of chemical compounds with many important pharmacological properties. Piperazine and substituted piperazine nuclei had constituted an attractive pharmacological scaffold present in various potent marketed drugs. The incorporation of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, proper alkalinity, water solubility, the capacity to form hydrogen bonds and adjustment of molecular physicochemical properties. This di-nitrogen moiety has been an inseparable component of plethora of drugs. A number of substituted piperazines possess significant pharmacological action such as antihistamic [7-8], antimicrobial [9], acetylcholinesterae inhibitors [10], antimalarial [11], dopamine transporter [12-13], D2/D4 antagonist [14], MC4Receptor [15], and HIV-protease inhibitor [16-17]. It has been reported that several of piperazine derivatives showed anticonvulsant properties in several models of seizures. Some piperazine derivatives displayed protection against electroshock (MES) induced seizures, low neurotoxicity (TOX) and little protection in subcutaneous pentylenetetrazole induced seizures (ScPTZ). Some of them, i.e. 1, 4-bis[(4-chloro-3-methyl)-phenoxyethyl]-piperazine dihydrochloride prevent maximal electroshock seizures in mice with an ED50 of 115.9 mg/kg and protective index PI = 2.05 in the MES test in mice which is higher than that of valproate (PI = 1.7) [18]. The present study is a continuation to the various efforts aiming to locate novel synthetic anticonvulsant lead compound(s). Some new quinazoline analogues prepared in our study possessed remarkable anticonvulsant activity. The new series of quinazoline analogues is designed to accommodate N -substituted piperazines and benzoisothiazole rings at C-2. These structure alterations and modifications are expected to contribute to the anticonvulsant activity of the quinazoline nucleus.

MATERIALS AND METHODS

Chemicals and Instrument

Anthranilic acid, potassium cyanate, N, N-dimethyl aniline, Phosphorous oxychloride were obtained from local dealer. Piperazine, 3-(piperazinyl-1-yl) benzo[d] isothiazole, 2-(piperazin-1-yl) phenol, 2-(piperazin-1-yl) ethanol, 2-[(piperazin-1-yl) methoxy] ethanol, 1-(2, 3-dichlorophenyl) piperazine and Morwet D425 were provided by Alkem Laboratories limited. Analytical TLC was performed on Silica plates- GF254 (Merck) with visualization by UV or in iodine. Melting points were determined by MP50 (Mettler Toledo) and are uncorrected. The IR spectra (KBr, λ Max, cm^{-1}) were run on Perkin Elmer FTIR Spectrophotometer. 1H-NMR (in $CDCl_3$ / DMSO-*d*6) spectra were recorded using Bruker -400 with TMS as internal standard. MS spectra were recorded on Brucker DPX 200. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of theoretical values. All the chemicals used were of Laboratory grade.

Synthesis of Quinazoline -2,4(1H,3H)-dione[Benzoylene urea] (4)

In a 3-l. round bottom reactor, a mixture of 20 g (0.146 mole) of anthranilic acid, 700 ml of warm water (35°C) and 11 ml (11.6 g., 0.19 mole) of glacial acetic acid were stirred mechanically and allowed to cool to room temperature. A freshly prepared solution of 15 g (0.185 mole) of potassium cyanate in 50 ml of water was then added drop wise with stirring over a period of fifteen to twenty minutes. The resulting pasty mixture was stirred for

twenty minutes and then 200 g (5 moles) of flaked sodium hydroxide was added slowly in small portions. During this addition the reaction mixture was kept below 40°C by cooling in a cold-water bath. A clear solution was obtained momentarily, but in a short time a fine granular precipitate of the hydrated Benzoylene urea precipitated. The mixture was cooled overnight in an ice box. The precipitated sodium salt was collected on a Büchner funnel, using a hardened filter paper. The colourless salt was dissolved in 1 l. of hot water (90–95°C), and the solution was filtered and heated to boiling in a 3-l. beaker. The Benzoylene urea was precipitated by adding dilute sulphuric acid (1:1) with vigorous stirring until the liquor was acid to litmus. The product separates as a hydrate which forms small, lustrous, colourless needles. The material was collected on a Büchner funnel, washed with 200 ml of water, and dried in an oven at 100°C. The yield was 19.5–20.5 g. Melting point: Above 300°C

Synthesis of 2, 4-dichloroquinazoline (5)

2,4-dichloroquinazoline was obtained by refluxing 10.0g (0.061 mole) of quinazoline-2,4(1H,3H)-dione(Benzoylene urea) in 14.2g(0.092 mole) of Phosphorous oxychloride with 7.4g(0.061mole)N,N-dimethylaniline at 108°C . The progress of the reaction was monitored by TLC (Eluent: ethyl acetate: hexane=8:2). After the completion, the reaction mass was cooled to room temperature and hence poured onto ice water under stirring. An off white viscous precipitate formed. The resultant mass was basified with Aqueous 20% w/v of Potassium carbonate to ph 8.0. After reaching the mentioned pH, the reaction mass was extracted with 200.0 ml Dichloromethane. The dichloromethane layer was given a water wash, dried over sodium sulphate and hence distilled to obtain 7.0 g 2, 4-dichloroquinazoline. Melting point: 118-120°C

Synthesis of substituted 4-chloro-2-(4-piperazin-1-yl) quinazoline derivatives [6(A-G)]

0.0075 mole of substituted piperazines, 0.005 mole of 2, 4-dichloroquinazoline, 0.017 mole of Sodium carbonate, water 5.2 times based on 2,4-dichloroquinazoline weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 12-16 hr. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=8:2). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran (THF) to obtain pure compounds.

2-[4-(1, 2-benzoisothiazol-3-yl) piperazin-1-yl]-4-chloroquinazoline, 6A

M.P.272°C; Yield: 89%; MS: 381.08(100.0%), 383.08(37.1%), 382.08(23.2%), 384.08(8.4%); IR max cm^{-1}: 3074.32 (Ar-CH); 1490.56 (Ar C=C); 1668.42 (HC=N); 720.15(C-Cl);

^{1}HNMR(DMSO D^{6}): δ=8.30 (2H,m,quinazoline aromatic CH); 8.16 (1H,d,quinazoline aromatic CH); 7.86 (1H,m,quinazoline aromatic CH); 7.74 (1H,d, benzisothiazole aromatic CH); 7.55 (2H,m, benzisothiazole aromatic CH); 7.48 (1H,d, benzisothiazole aromatic CH);4.08(4H,t,piperazine-CH_2);3.70 (4H,t,piperazine CH_2); ^{13}CNMR: 50.2(4C, piperazine -CH_2); 120-131(9C, Aromatic CH); 138.2(1C, quinazoline aromatic CH); 152.9(1C, quinazoline C-N); 157.4(1C, benzisothiazole N=C-N); 161.6(1C, quinazoline C-Cl); 165.4(1C, benzisothiazole C-S); 185.0(1C, quinazoline N=C-N); Elemental analysis:C-59.76%,H-4.22%,Cl-9.28%,N-18.34%,S-8.40%

2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] phenol, 6B

M.P.202°C; Yield: 81%; MS: 340.1(100.0%), 342.1(32.5%), 343.1(6.2%); IR max cm^{-1}: 3415.12(-OH); 3014.12 (Ar-CH); 1470.56 (Ar C=C); 1678.12 (HC=N); 1215(C-O); 750.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=7.95(1H,d,quinazoline aromatic CH);7.8-7.85 (2H,m,quinazoline aromatic CH); 7.58(1H,t,quinazoline aromatic CH); 6.4-6.6 (4H,4, aromatic CH);5.2(1H,s,-OH);3.64 (4H,m,piperazine CH_2); 3.34 (4H,m,piperazine CH_2)

^{13}CNMR: 49.2(4C, piperazine -CH_2); 115-123(4C, Aromatic CH); 116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH); 142.4(1C, C-OH); 152.8(1C, quinazoline C-N); 146.8(1C, phenylring C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C, quinazoline N=C-N); Elemental analysis:C-63.44%,H-5.03%,Cl-10.40%,N-16.44%,O-4.69%

2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] ethanol, 6C

M.P198°C; Yield: 84%; MS: 292.1(100.0%), 294.1(32.5%), 293.11(16.7%), 295.11(4.9%); IR max cm^{-1}: 3315.12(-OH); 3014.12 (Ar-CH); 1470.56 (Ar C=C); 1678.12 (HC=N); 1065(C-O); 750.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.13 (1H, d, quinazoline aromatic CH); 7.85 (1H, t, quinazoline aromatic CH); 7.75(1H, d, quinazoline aromatic CH); 7.58(1H, t, quinazoline aromatic CH); 3.82(2H,m,-CH_2); 3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.44 (2H,m,-CH_2); 2.0(1H,s,-OH)

^{13}CNMR: 50.0-53.6 (8C, piperazine -CH_2); 57.0-59.4 (4C, -CH_2); 116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH); 142.4(1C, C-OH); 152.8(1C, quinazoline C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C, quinazoline N=C-N); Elemental analysis: C-57.44%,H-5.85%,Cl-12.11%,N-19.14%,O-5.46%

2-{[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] methoxy} ethanol, 6D

M.P.201°C; Yield: 81%; MS: 336.14(100.0%), 338.13(32.0%), 337.14(17.7%), 339.14(5.7%); IR max cm^{-1}: 3321.12(-OH); 3023.12 (Ar-CH); 1466.56 (Ar C=C); 1678.12 (HC=N); 1084(C-O); 761.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.15 (1H,d,quinazoline aromatic CH); 7.82 (1H,t,quinazoline aromatic CH); 7.7(1H,d,quinazoline aromatic CH); 7.53 (1H,m,quinazoline aromatic CH);3.55-3.82(6H,m,-CH_2);3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.44 (2H,m,-CH_2);2.0(1H,s,-OH)

^{13}CNMR: 50.6-55.6 (4C, piperazine -CH_2); 54.8 (1C, -CH_2); 61.4 (1C, C-OH); 68.4 (1C, C-O); 72.6 (1C, C-O)116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH);152.8(1C,quinazoline C-N);160.6(1C,quinazoline C-Cl);184.2(1C,quinazoline N=C-N);Elemental analysis:C-57.06%,H-6.28%,Cl-10.53%,N-16.63%,O-9.50%

4-chloro-2-[4-(2, 3-dichlorophenyl) piperazin-1-yl] quinazoline, 6E

M.P.218°C; Yield: 84%; MS: 392.04 (100.0%), 394.03(30.9%)

; IR max cm^{-1}: 3023.12 (Ar-CH); 1466.56 (Ar C=C); 1678.12 (HC=N); 759.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.1(1H,d,quinazoline aromatic CH); 7.85 (1H, t, quinazoline aromatic CH); 7.73 (1H, d, quinazoline aromatic CH); 7.55 (1H, t, quinazoline aromatic CH); 7.35 (2H, d, phenyl aromatic CH); 7.2 (1H, m, -aromatic -CH); 4.0 (4H, m, piperazine CH_2); 3.35 (4H, m, piperazine CH_2);

^{13}CNMR: 49.1-49.6 (4C, piperazine -CH_2);113.8,119.8,129.2(3C,Phenyl ring)116.2(1C, quinazoline aromatic CH);123.8(1C,C-Cl);134.3(1C,C-Cl); 125-139 (4C, quinazoline aromatic CH); 152.1(1C, C-N); 152.8(1C, quinazoline C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C,quinazoline N=C-N);Elemental analysis:C-54.91%,H-3.84%,Cl-27.02%,N-14.23%

4-chloro-2-(4-methylpiperazin-1-yl) quinazoline, 6F

M.P.188°C; Yield: 84%; MS: 262.10 (100.0%), 264.10(32.2%); IR max cm^{-1}: 3028.52 (Ar-CH); 1476.56 (Ar C=C); 1678.02 (HC=N); 756.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.01(1H,d,quinazoline aromatic CH);7.8-7.85 (2H,m,quinazoline aromatic CH); 7.61 (1H,m,quinazoline aromatic CH);3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.3(3H,s,-CH_3)

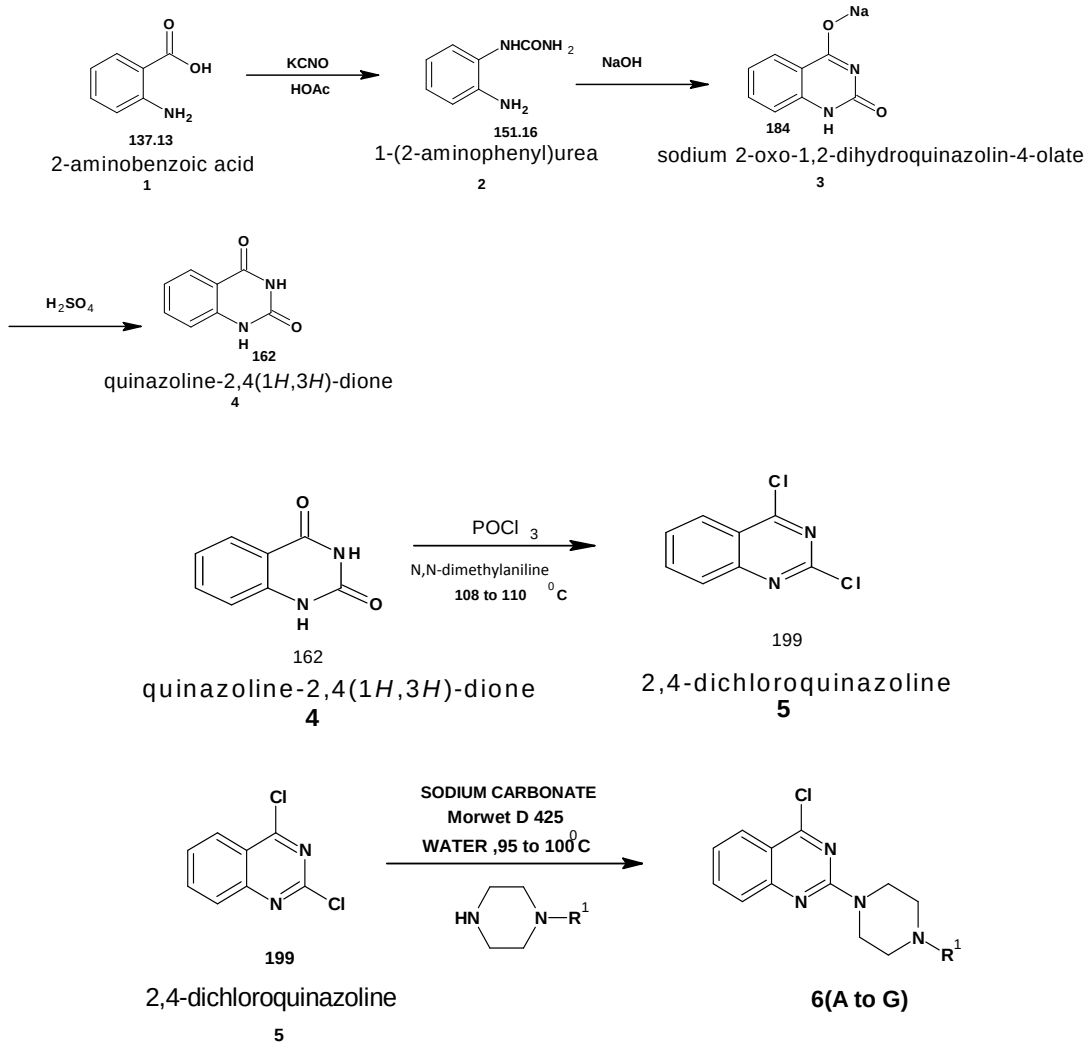
^{13}CNMR: 43.1(1C,-CH_3)49.1-49.6 (4C, piperazine -CH_2);116.2(1C, quinazoline aromatic CH);123.8(1C,C-Cl);134.3(1C,C-Cl); 125-139 (4C, quinazoline aromatic CH);152.1(1C,C-N);152.8(1C,quinazoline C-N);160.6(1C,quinazoline C-Cl);184.2(1C,quinazoline N=C-N);Elemental analysis:C-59.43%,H-5.75%,Cl-13.49%,N-21.32%,

4-chloro-2-(4-(4-chloroquinazolin-2-yl) piperazin-1-yl) quina zoline, 6G: M.P.249°C; Yield: 80%; MS: 410.08 (100.0%); ; IR max cm^{-1}: 3028.52 (Ar-CH); 1476.56 (Ar C=C); 1678.02 (HC=N); 756.15(C-Cl)

^{1}HNMR(DMSO D^6): δ=8.1(2H,d,quinazoline aromatic CH);7.8-7.9 (4H,m,quinazoline aromatic CH); 7.58 (2H,m,quinazoline aromatic CH);3.24 (8H,m,piperazine CH_2);

^{13}CNMR: 49.6 (4C, piperazine -CH_2);116.5(2C, quinazoline CH); 125-139 (8C, quinazoline aromatic CH);

152.8(2C,quinazoline C-N);161.0(2C, quinazoline C-Cl);184.1(2C,quinazoline N=C-N);Elemental analysis:C-58.41%,H-3.92%,Cl-17.24%,N-20.43%,



R^1=3-(piperazinyl-1-yl)benzo[d]isothiazole,2-(piperazin-1-yl)phenol,2-(piperazin-1-yl) nethanol, 2-[(piperazin-1-yl) methoxy] ethanol,1-(2,3-dichlorophenyl) piperazine,N-Methyl piperazine, Piperazine

Scheme 1: Synthesis of substituted 4- chloro-2-(4-piperazin-1-yl) quinazolines 6A-G

Evaluation of anticonvulsant activity

Experimental animals

Male albino Swiss mice, weighing 20 - 25 g, were used to study the effect of the synthesized compounds on subcutaneous (s.c.) Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that estrous cycle could influence their activity threshold.

The animals were housed in a standard cage at room temperature in a12 /12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously (s.c.) in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD50 was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e.* the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded [19].

2 -Pentylenetetrazole (s.c.PTZ) induced seizure test

Forty five adult albino mice were randomly divided into five mice each. Group one (Standard) received 40 mg/kg, body weight of Rufinamide intraperitonealy (i.p.), and group two (Control) was given Pentylenetetrazole subcutaneously. **(**Dose: 40 mg/kg, body weight, s .c**).** The synthesized compounds 6(A-G) were administered to group's three to nine (treated groups) intraperitonealy, 50 mg/kg of body weight. Thirty minutes later, 40 mg/kg of freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse. The onset of action, number of rats showing tonic convulsion as well as mortality where recorded in each group [20].

Table 1: Effect of the compounds (6A-6G) on Pentylenetetrazole (PTZ) – induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/ No. of animals used	Animals protected (%)
6A	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
6B	1.33±0.14	0.27±0.03	No onset	No clonus	0/5	100%
6C	1.20±0.19	0.24±0.04	No onset	No clonus	0/5	100%
6D	1.41±0.13	0.28±0.02	No onset	No clonus	0/5	100%
6E	1.25±0.07	0.25±0.01	No onset	No clonus	0/5	100%
6F	1.26±0.25	0.25±0.05	82.6±49.92	No clonus	0/5	100%
6G	1.54±0.11	0.30±0.02	45.6±22.26	5.2±5.21	3/5	40%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

RESULTS

Acute toxicity study

The subcutaneously (s.c.) LD50 of the drugs was found to be 100 mg Kg^{-1}.

Study on the effects of the synthesized compounds on the convulsive activity of 40 mg/Kg of subcutaneous Pentylene tetrazole in mice.

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 1.

DISCUSSION

The 2-chloro position of the 2, 4-dichloroquinazoline was replaced by substituted piperazines. The reaction was facilitated by the use of Morwet-D425. The major problems faced in this type of reactions were the incompletion of the reaction, the formation of sticky material and difficult stirrability of the reaction mass. These problems are evident in smaller scales but especially acute in large scale manufacturing. This results in lesser purity and lower yields. In U.S.Pat.No.8,410,268B2 [21] we have disclosed a process for the preparation of Ziprasidone, which involves the same procedure. In our present work, we have achieved substantially good yields and purity. All the compounds were screened for anticonvulsant activity. The substitutions involving aryl substituted piperazines showed better protection against subcutaneous (s.c.) Pentylenetetrazole induced seizures.

CONCLUSION

The protection offered by the synthesized compounds 6A, 6B, 6C and 6D against subcutaneous (s.c.) Pentylenetetrazole induced threshold seizure, the prevention of the onset of seizure and its protective effect against mortality in mice suggests that the synthesized compounds may be effective in the management of petit mal epilepsy since all antiepileptic drugs that are effective in the treatment of petit mal epilepsy exhibit dose dependent suppression of seizure induced by subcutaneous (s.c.) Pentylenetetrazole [22].

ACKNOWLEDGEMENT

The authors are thankful to Alkem Laboratories Limited and Skanda Life Sciences Private Limited for providing necessary facilities.

REFERENCES

1. Selvam TP and Kumar PV. Quinazoline Marketed drugs – A Review. Research in Pharmacy 2011; 1(1): 1-21.
2. Abbas SE. Synthesis of Some Novel 2,3-Disubstituted-3,4-dihydro-4-quinazolinones as Potential Anticonvulsant Agents. Bull. Fac. Pharm. Cairo Univ. 2007; 45: 119-129.
3. Kacker IK and Zaheer SH. Potential Analgesics Part I. Synthesis of Substituted 4-quinazolones. J. Ind. Chem. Soc. 1951; 28: 344-346.
4. Angelos SA and Meyers JA. The Isolation and Identification of Precursors and Reaction Products in the Clandestine Manufacture of Methaqualone and Mecloqualone. J. Forensic Sci. 1985; 30: 1022-1047.
5. Ager R, Harrison DR, Kennwell PD and Taylor JB. Synthesis and Central Nervous System Activity of Quinazolones Related to 2-Methyl- 3-(*o*- tolyl) -4 (3*H*) - quinazolone (Methaqualone). J. Med. Chem. 1977; 20: 379-386.
6. Wolfe JF, Rathman TL, Sleevi MC, Campbell JA and Grenn Wood TD. Synthesis and Anticonvulsant Activity of Some New 2-Substituted 3-aryl-4(3*H*)-quinazolinones. J. Med. Chem. 1990; 33: 161-166.
7. Gyoten M, Nagaya H, Fukuda S, Ashida Y and Kawano Y. Synthesis of Eosinophil Infiltration Inhibitors with Antihistaminic Activity. Chem. Pharm. Bull. Tokyo 2003; 51(2): 122-133.
8. Magid AG, John A, Moyer Susan TN, Michael W and Usha P. New Antihistamines: Substituted Piperazine and Piperidine Derivatives as Novel H1-Antagonists J. Med. Chem. 1995; 38: 4026- 4032.
9. Chaudhary P, Kumar R, Verma AK and Singh D. Synthesis and antimicrobial activity of *N*-alkyl and *N*-aryl piperazine derivatives. Bioorg. Med. Chem. 2006; 14: 1819-1826.
10. Hachiro S, Hiroo O, Yasuo A, Youichi I and Yoshiharu Y. Synthesis and Antimicrobial Activity of Amino Acids Conjugated Diphenyl methylpiperazine Derivatives. Japanese J. Pharmacol. 2002; 89(1): 7-20.
11. Rebecca DP and Patricia M. Combinatorial Chemistry & High Throughput Screenings, 1, 4-Bis(3-Aminopropyl) Piperazine Libraries: From the Discovery of Classical Chloroquine-Like Antimalarials to the Identification of New Targets, 2005; 8: 39-48.
12. Makoto K, Tomoko M, Koji Y, Masaki M, Nobuo K, Nobuyuki K, Kenichi K, Masato I, Yuji K, Katsuji O and Takayuki N. Novel diphenylalkyl piperazine derivatives with high affinities for the dopamine transporter. Bioorg. & Med. Chem. 2003; 11: 3953-3963.
13. Makoto K, Tomoko M, Koji Y, Masaki M, Nobuo K, Nobuyuki K, Kenichi K, Masato I, Yuji K, Katsuji O and Takayuki N. Efficient asymmetric syntheses, determination of absolute configurations and biological activities of 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine as a novel potent dopamine uptake inhibitor in the central nervous system. Bioorg. & Med. Chem. 2004; 12: 3069-3078.
14. He Zhao, Xiaoshu He, Andrew T, Diane H, Andrzej K, Robbin B, Renee P and Jan WF. Indoline and piperazine containing derivatives as a novel class of mixed D2/D4 receptor antagonists. Part 2: Asymmetric synthesis and biological evaluation. Bioorg. & Med. Chem. Lett. 2002; 12: 3111-3115.
15. Brian D, Jessica P, Teresa P, Lee C, Brian M, Robin S, Julia H, Tracy B, Mary C, John S and Val G. Aryl piperazine melanocortin MC4 receptor agonists. Bioorg. & Med. Chem. Lett. 2003; 13: 3793-3796.
16. Rossen K, Steven AW, Sager J, Reamer RA, Askin D, Volante RP and Reider PJ. Asymmetric hydrogenation of tetrahydro pyrazines: Synthesis of (S)-piperazine-2-tert-butylcarboxamide, an intermediate in the preparation of the HIV protease inhibitor indinavir. Tetrahedron Lett. 1995; 36: 6419-6422.

17. David A, Kan KE, Kai R, Robert MP, Kenneth MW, Volante RP and Paul JR. Highly diastereo selective reaction of a chiral, non-racemic amide enolate with (S)-glycidyl tosylate. Synthesis of the orally active HIV-1 protease inhibitor L-735,524, Tetrahedron Lett. 1994; 35(5): 673-676.
18. Marona H, Korona R and Szneler E. Boll. Chim. Farmac. 2004; 143: 329 .
19. Lorke DA. A New Approach to practical acute toxicity testing. Arch. Toxicol. 1983; 54: 275 -287.
20. Swinyard EA, Woodhead JH, White HS and Franklin MR. General Principle; Experimental, Selection Quantification and Evaluation of Anticonvulsants. In: Antiepileptic Drugs. Levy RH, Mattson RH, Melrum B, Penry JK, Dreifuss H. (eds). Raven press, New York, 1989; Pp. 85-102.
21. Shashiprabha, Kanakamajalu S, Debkiron M, Padmashree B, Ksundarraja R and Kuppuswamy N. Process for the preparation of Ziprasidone. U.S.Pat.No. 8,410,268B2. 2013.
22. Raza M, Farzane S, Choudhary MI, Amin S. Attaur –Rahman, S, Sompong S and De-lorenzo RJ. Phytother. Res. 2001; 15: 426-430.

Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME N-SUBSTITUTED DERIVATIVES OF 6-FLUORO-1-METHYL-4-OXO-7-(PIPERAZIN-1-YL)-4*H*-[1,3]THIAZETO[3,2-*A*]QUINOLINE-3-CARBOXYLIC ACID

DEBKIRON MUKHERJEE[a*], A.MUKHOPADHYAY[b], K.SHRIDHARA BHAT[c], A.M.SHRIDHARA[d]

[*a,b]Chemical Engineering Department, Jadavpur University, Kolkata-700032, India, [c,d] Alkem Laboratories Limited, 473D2, 13[th]Cross,4[th]Phase, Peenya Industrial Area, Bangalore-560058.
Email: debkironmukherjee@gmail.com

Received: 14 Apr 2014 Revised and Accepted: 20 May 2014

ABSTRACT

Objective: N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid: Synthesis and antibacterial activity.

Methods: In the present study N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid were prepared by using Triethylamine and DMF. This procedure was modified by adding tetra butyl ammonium bromide (TBAB) to facilitate completion in few reactions. Other reactions which did not proceed in both the aforementioned ways were facilitated by the use of Morwet-D425. All the new title compounds were characterized by their spectral data and were screened for antibacterial activity.

Results: Some of the reported compounds were synthesized using the process disclosed by us in U.S.Pat.No.8, 410,268B2. In our present work, we have achieved good yields and purity. Many compounds exhibited substantial antibacterial activity.

Conclusion: It can be inferred that the title compounds with substituted phenyl and quinoline rings exhibited comparable antibacterial activity with respect to the standard.

Keywords: Synthesis, Quinoline, Piperazine, Phenyl, Biphenyl, Pyridine, Pyrazole, Characterization, Antibacterial activity.

INTRODUCTION

Quinolones are unusual among antibacterial agents in the fact that they are not isolated from living organisms, but rather synthesized by chemists. The first quinolone, Nalidixic acid, was derived from the anti malarial drug Chloroquine[1]. Subsequent agents were derived through side chain and nuclear manipulation [2]. The development of the fluoroquinolone class may be described in generational terms, with each generation sharing similar features or antibacterial spectra. First-generation agents possess activity against aerobic gram-negative bacteria, but little activity against aerobic gram-positive bacteria or anaerobes. Second-generation agents are the original fluoroquinolones, named for the addition of a fluorine atom at position C-6 .These agents offer improved coverage against gram-negative bacteria and moderately improved gram-positive coverage. Third-generation agents achieve greater potency against gram-positive bacteria, particularly pneumococci, in combination with good activity against anaerobes. Fourth-generation fluoroquinolones have superior coverage against pneumococci and anaerobes. This article focuses on the fluoroquinolone agents.

Regarding antibacterial activity, fluoroquinolones interfere with bacterial cell replication, transcription, and DNA repair by disabling two bacterial enzymes crucial to these processes, DNA gyrase (formerly topoisomerase II) and topoisomerase IV. These enzymes are necessary for bacteria to manage the topological challenge of containing their genetic material. Using *Escherichia coli* as an example, a bacterial cell that is 1 to 3 mm long must accommodate a chromosome that is a double-stranded DNA circle longer than 1000 mm. Chromosomal volume is reduced via tertiary folding and compaction. These processes must be reversed in order for bacterial replication to occur; DNA topoisomerases facilitate this [4].

Piperazines are a broad class of chemical compounds with many important pharmacological properties. Piperazine and substituted piperazine nuclei have constituted an attractive pharmacological scaffold present in various potent marketed drugs. The incorporation of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, proper alkalinity, water solubility, the capacity for the formation of hydrogen bonds and adjustment of molecular physicochemical parameters. This di - nitrogen moiety has been an inseparable component of plethora of drugs. A number of substituted piperazines posses significant pharmacological action such as antihistamic [5-6], antibacterial [7], acetylcholinesterae inhibitors [8], antimalarial [9], dopamine transporter [10-11], D2/D4 antagonist [12], MC4Receptor [13], and HIV-protease inhibitor [14-15].

Our present work forays into the field of research which has not been studied extensively i.e. substitution of the piperazine nucleus of 6-fluoro-4-oxo-7-(piperazin-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid.

From the N-substituted 4-fluoro phenyl ring at fluoroquinolone nucleus in Sarafloxacin(16) and similar 2, 4-difluoro phenyl substitutions in Trovafloxacin(17), Tosufloxacin(18) and Temafloxacin(19),it can be inferred that substituted phenyl ring pertains to enhance the antibacterial activity of fluoroquinolones. Quinoline substitutions at the fluoroquinolone nucleus in case of Rosoxacin(20) also demonstrates good antibacterial activity. Pyridines (21) and pyrazoles (22) have been reported to exhibit prominent antibacterial activity.

We have tried to explore the effect of the substituted phenyl, biphenyl, quinoline and pyrazole substitutions at the piperizine ring of the fluoroquinolone nucleus in 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid . The synthesized molecules were characterized and checked for antibacterial activity.

MATERIALS AND METHODS

Chemicals and Instrument

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 2-(bromomethyl)-1,3-difluorobenzene, 5-[4'-(bromomethyl) biphenyl-2-yl]-1-trityl- l-1*H*-tetrazole, 6-chloro-5-(2-chloroethyl)-1,3-dihydro-2*H*-indol-2-one, 4' (bromomethyl)

biphenyl-2-carbonitrile, benzyl 2-(benzyloxy)-4-(bromocarbonyl) benzoate, 2-(4-chlorobutoxy)-1,2,3,4-tetrahydroquinolin-7-ol, 2-(chloromethyl)-4-(3-methoxypropoxy)-3-methylpyridine, 1-[3-(trifluoromethyl) benzyl]-1*H*-pyrazole-4-carbonyl chloride were synthesized and characterized in our lab. Morwet®D425 was provided by Alkem laboratories limited. Analytical TLC was performed on Silica plates- GF254 (Merck) with visualization by UV or in iodine. Melting points were determined by MP50 (Mettler Toledo). The IR spectra (KBr, λ Max, cm^{-1}) were run on Perkin Elmer FTIR Spectrophotometer. 1H-NMR (in $CDCl_3$ / DMSO-*d6*) spectra were recorded using Bruker - 400 with TMS as internal standard. MS spectra were recorded on Brucker DPX 200. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of theoretical values. All the chemicals used were of Laboratory grade.

Synthesis of 7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-a):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 2-(bromomethyl)-1,3-difluorobenzene, 0.0143 mol of Triethylamine, dimethylformamide (DMF) 5 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-6 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 6-fluro-1-methyl-4-oxo-7-(4-((2'-(1-trityl-1*H*-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl) methyl) piperazin-1-yl)-1*H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-b):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 5-[4'-(bromomethyl)biphenyl-2-yl]-1-trityl- l-1*H*-tetrazole, 0.0143 mol of Triethylamine, 5% Tetra butyl ammonium bromide with respect to the weight of **I**, dimethylformamide (DMF) 5 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-6 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 7-(4-(5-(2-chloroethyl)-1, 3-dihydro-2*H*-indol-2-one) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-c):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 6-chloro-5-(2-chloroethyl)-1,3-dihydro-2*H*-indol-2-one, 0.024 mole of Sodium carbonate, water 5.2 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 7-8 hrs. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran (THF) to obtain pure compounds.

Synthesis of 7-(4-((2'-cyano- [1,1'-biphenyl]-4-yl)methyl)piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*,4*H*-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid (I-d):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 4' (bromomethyl)biphenyl-2-carbonitrile, 0.0143 mol of triethylamine, dimethylformamide (DMF) 5 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-6 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 7-(4-(1-(4-(benzyloxy)-2-((benzyloxy) carbonyl) phenyl) vinyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-e):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of benzyl 2-(benzyloxy)-4-(bromocarbonyl) benzoate, 0.0143 mol of Triethylamine, dimethylformamide (DMF) 5 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-6 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 6-fluro-7-(4-(5-(7-hydroxy-1, 2, 3, 4-tetrahydroquinolin-2-yl) pentyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-f):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 2-(4-chlorobutoxy)-1,2,3,4-tetrahydroquinolin-7-ol, 0.024 mol of sodium carbonate, water 5 .2 times based on of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 7-9 hrs. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran(THF) to obtain pure compounds.

Synthesis of 6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I-g):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 2-(chloromethyl)-4-(3-methoxypropoxy)-3-methylpyridine, 0.0143 mol of triethylamine, 5% tetra butyl ammonium bromide with respect to the weight of **I**, dimethylformamide (DMF) 5 times based

on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-5 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

Synthesis of 6-fluro-1-methyl-4-oxo-7-(4-(1-(3-(trifluromethyl)benzyl)-1*H*, 4*H*-[1,3] thiazeto[3,2-*a*] quinoline-3-carboxylic acid(I-h):

0.0072 mol of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, 0.0086 mol of 1-[3-(trifluoromethyl)benzyl]-1*H*-pyrazole-4-carbonyl chloride, 0.0143 mol of triethylamine, 5% tetra butyl ammonium bromide with respect to the weight of **I**, dimethylformamide (DMF) 5 times based on 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid weight,were charged in to a round bottom flask and heated to 85-90°C under nitrogen, under stirring for 4-5 hrs. A chilled water circulation was maintained in the condenser to prevent the escape of triethylamine during the course of the reaction. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=50:50). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was poured onto ice cold water (0-5°C). It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were slurried again in diisopropyl ether to obtain pure compounds.

7-(4-(2, 6-diflurobenzyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [I (a)]:

M.P:228°C; Yield: 78%; MS: 475.12(100%) 476.12(26.0%); IR max cm^{-1}: 3275-2800 (O-H stretch); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 11.8(-OH,s,1H), 7.8 (Aromatic CH,S,1H),7.5 (Aromatic CH,m,1H),7.3(Aromatic CH,d,2H)6.5(Aromatic CH,s,1h),4.0(CH,m,1H),3.7(CH,s,2H),3.4(-CH_2,t,4H),2.7(-CH_2,t,4H),2.2(-CH_3,6H)

^{13}CNMR: 23.6(1C,-CH_3);38.5(1C,-CH_2);50.2(2C, piperazine -CH_2); 53.2(2C, piperazine -CH_2); 91.4(1C, Aromatic C=C);110.8-118.2 (6C,Aromatic CH); 130.5-144.6 (4C,Aromatic CH); 163.0(2C,Aromatic C-F); 166.1(1C,carboxylic acid); 175.1(1C,Aromatic S-C-N); 177.5 (1C,Aromatic C=O);Elemental analysis: C-58.10 %, H-4.24 %,F-11.99 %, N-8.84 %,O-10.09%;S-6.74%

6-fluro-1-methyl-4-oxo-7-(4-((2'-(1-trityl-1*H*-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl) methyl) piperazin-1-yl)-1*H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [I (b)]:

M.P:231°; Yield: 71%, MS: 825.29(100%), 826.29(56.5%), 827.30(14.1%); IR max $cm^{-1:}$ 3275-2800 (O-H stretch); 1630.22(C=N); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 11.0(-OH,s,1H), 7.0-7.30 (Aromatic CH, 17 H, Phenyl rings),7.3-7.6 (Aromatic CH,4H,Phenyl rings), 7.12(Aromatic CH,s,1H of fluoroquinolone ring),7.3(Aromatic CH,d,2H), 6.5(Aromatic CH,s,1h),5.93(1H,s,Aromatic CH, fluoroquinolone ring)3.84 (CH,m,1H),3.7(CH,s,2H),3.62(CH_2, s,2H) 3.45(-CH_2,t,4H),2.59 (-CH_2,t,4H),2.0 (-CH_3,6H)

^{13}CNMR: 22.9(1C,-CH_3); 50.0(2C, piperazine -CH_2); 53.2(2C, piperazine -CH_2); 60.1(1C,-CH_2); 67.2 (1C,-CH_2); 91.4(1C, Aromatic C=C, fluoroquinolone ring);100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 128.3-129.3 (23C,Aromatic C, phenyl rings); 134.5-141.9 (8C,Aromatic C, phenyl rings) 154.1 (1C,triazole ring)166.3(1C,carboxylic acid); 175.5(1C,Aromatic S-C-N); 177.5 (1C,Aromatic C=O);Elemental analysis:C-71.25%,H-4.88%,F-2.30%,N-11.87%,O-5.81%,S-3.88%

7-(4-(5-(2-chloroethyl)-1, 3-dihydro-2*H*-indol-2-one) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*, 4H-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [I(c)]

M.P:233°; Yield: 71%, MS: 542.12(100%), 544.12(33.45%), 543.12(30.6%); IR max cm^{-1}: 3275-2800 (O-H stretch); 1712.30(NH bend) 1648.25(Carbonyl of oxindole); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 11.0(-OH,s,1H), 8.0 (NH, s,1 H, Oxindole ring),7.48 (Aromatic CH,s,1H,Oxindole), 7.12(Aromatic CH,s,1H of fluoroquinolone ring),6.84(Aromatic CH,s,1H of Oxindole ring),5.93(1H,s,Aromatic CH, fluoroquinolone ring)3.84 (CH,m,1H),3.7(CH,s,2H),3.62(CH_2, s,2H) 3.41(-CH_2,t,4H,piperazine ring),2.59 (-CH_2,t,4H,piperazine ring),2.6-2.7 (-CH_2,4H),1.58(-CH_3,3H)

^{13}CNMR: 23.6 (1C,-CH_3); 25.6 (1C,-CH_2);36.6(-CH_2, 1C,oxindole ring);50.0(2C, piperazine -CH_2); 53.2(2C, piperazine -CH_2); 60.1(1C,-CH_2); 67.2 (1C,-CH); 91.4(1C, Aromatic C=C, fluoroquinolone ring);100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 122.3-132.2 (4C,Aromatic C, oxindole ring); 139.8-143.0 (2C,Aromatic C, oxindole rings),165.8(1C,carboxylic acid); 175.1(1C,Aromatic S-C-N); 176.6 and 177.5 (2C, C=O)Elemental analysis:C-57.51 %,H-4.45%,Cl-6.53%,F=3.50%,N=10.32 %, O=11.79%S-5.91%

7-(4-((2'-cyano- [1,1'-biphenyl]-4-yl)methyl)piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1 *H*,4*H*-[1,3] thiazeto[3,2-a] quinoline-3-carboxylic acid [I(d)]:

M.P: 211° C, Yield: 78%; MS: 540.16(100%), 541.17(32.80%), 542.17(6.1%); IR max cm^{-1}: 3275-2800 (O-H stretch); 2310.32(Nitrile group); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 11.0(-OH,s,1H), 7.8 (Aromatic CH, d,1 H, biphenyl ring),7.6-7.7 (Aromatic CH,2H,Biphenyl ring), 7.2 (Aromatic CH,d,2H,Biphenyl ring), 7.4 (Aromatic CH,3H,Biphenyl ring),7.12(Aromatic CH,s,1H of fluoroquinolone ring), 5.93(1H,s,Aromatic CH, fluoroquinolone ring)3.91 (CH,m,1H),3.75 (CH,s,2H),3.62(CH_2, s,2H) 3.45(-CH_2,t,4H,piperazine ring),2.61 (-CH_2,t,4H,piperazine ring),1.85 (-CH_3,3H)

^{13}CNMR: 23.6 (1C,-CH_3) ; 50.0(2C, piperazine -CH_2); 53.2(2C, piperazine -CH_2); 60.7(1C,-CH); 67.2 (1C,-CH_2); 91.4(1C, Aromatic C=C, fluoroquinolone ring);100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 104.5 (1C,-C-CN); 115.4(1C,CN),127.6-129.4 (6C, biphenyl rings),132.7-135.0(4H,biphenyl ring),166.3 (1C,carboxylic acid); 175.1(1C,Aromatic S-C-N);177.5 (2C, C=O) Elemental analysis:C-66.65 %,H-4.66%,Cl-6.53%,F=3.50%,N=10.36 %, O=8.88 %S-5.93%

7-(4-(1-(4-(benzyloxy)-2-((benzyloxy) carbonyl) phenyl) vinyl) piperazin-1-yl)-6-fluro-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [1(e)]:

M.P: 220° C, Yield: 58%; MS: 693.19 (100%), 694.20 (41.7 %), 695.20(10.3 %); IR max cm^{-1}: 3275-2800 (O-H stretch); 1648.25(Carbonyl of oxindole); 1215.12(C-O); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 11.0(-OH,s,1H), 8.04 (Aromatic CH, d,1 H, phenyl ring),7.62 (Aromatic CH,d,1H,phenyl ring), 7. 57 (Aromatic CH,s,1H, phenyl ring), 7.2-7.3 (Aromatic CH,10 H, phenyl ring),7.12(Aromatic CH,s,1H of fluoroquinolone ring), 5.93(1H,s,Aromatic CH, fluoroquinolone ring),5.3-5.4(-CH_2, 4H),3.81 (CH,m,1H),3.65(CH_2, t,4H,piperazine ring) 3.42(-CH_2,t,4H,piperazine ring),1.85 (-CH_3,3H)

^{13}CNMR: 23.6 (1C,-CH_3) ; 47.5(2C, piperazine -CH_2); 49.5(2C, piperazine -CH_2); 60.7(1C,-CH); 68.1 (1C,-CH_2); 70.2 (1C,-CH_2); 101.0(1C, Aromatic C=C, fluoroquinolone ring); 100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 111.3-119.4 (3C,phenyl ring); 127.2-130.7(11C,phenyl ring),140.5-141.2 (3C, phenyl rings),161.0(1H,-C-

O),165.8(1C,-C=O);164.3 (1C,carboxylic acid); 167.3(1C,Aromatic S-C-N);183.5 (2C, C=O) Elemental analysis:C-65.79 %,H-4.66%,F=2.74%,N=6.06 %, O=16.14 % S-4.62 %

6-fluro-7-(4-(5-(7-hydroxy-1, 2, 3, 4-tetrahydroquinolin-2-yl) pentyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [1(f)]:

M.P: 212° C, Yield: 6 8%; MS: 538.24 (100%), 539.29 (33.6 %), 540.25 (5.5 %); IR max cm^{-1}: 3275-2800 (O-H stretch); 1712.30(NH bend); 1215.12(C-O); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ= 10.0(CH_2-OH,s,1H),7.12(Aromatic CH,S,1H),6.85(Aromatic CH,m,2H),6.5 (Aromatic CH,t,1H) 6.4(Aromatic CH,d,2h),,4.45 (NH,d,1H),3.9 (-CH,m,2H),3.5 (-CH_2,m,4H),3.0(-CH_2,t,2H), 2.8 (-CH_2,t,4H), 2.4-2.5 (-CH_2,5H), 1.8-2.2(-CH_2,5H)

^{13}CNMR: 23.6 (1C,-CH_3) ;24.0(1C,-CH_2);24.7-27.8(2C,-CH_2);41.9(1C,-CH_2); 50.0(2C, piperazine -CH_2); 52.6(2C, piperazine -CH_2); 54.8(1C,-CH_2);60.7(1C,-CH); 68.1 (1C,-CH_2); 70.2 (1C,-CH_2);88.6(1C,-CH); 101.0(1C, Aromatic C=C, fluoroquinolone ring);100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 113.6-126.9 (5C,phenyl ring); 145.6(1C,phenyl ring);166.3 (1C,carboxylic acid); 167.3(1C,Aromatic S-C-N);183.5 (2C, C=O) Elemental analysis:C-64.66 %,H-6.55%,F=3.53%,N=10.40 %, O=8.91 % S-5.95 %

6-fluro-7-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl) methyl) piperazin-1-yl)-1-methyl-4-oxo-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [1(g)]:

M.P: 233° C, Yield: 61%; MS: 528.22 (100%), 529.22 (31.6 %), 530.22 (6.0 %); IR max cm^{-1}: 3275-2800 (O-H stretch); 1215.12(C-O); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ=10.2(-OH,s,1H),8.3 (Aromatic CH,d,1H),7.85 (Aromatic CH,s,1H),6.8 (Aromatic CH,d,1H)6.4 (Aromatic CH,s,1h),4.0-4.2 (CH_2,4H), 3.8 (-CH_2,t,1H),3.6 (-CH and -CH_2,m,3H), 3.4-3.5(-CH_2, 3H),3.3(-CH_2,m,2H),(-CH_2,t,4H), 2.8 (-CH_2,t,4H), 2.4-2.5 (-CH_3,3H), 2.1-2.3(-CH_3,6H)

^{13}CNMR: 10.6(1C,-CH_3);23.6 (1C,-CH_3) ;29.0(1C,-CH_2); 50.0(2C, piperazine -CH_2); 52.6(2C, piperazine -CH_2); 54.8(1C,-CH_2);59.3(1C,-CH_3); 60.7(1C,-CH); 68.1 (1C,-CH_2); 70.2 (1C,-CH_2);88.6(1C,-CH); 101.0(1C, Aromatic C=C, fluoroquinolone ring);100-118.2 (3C,Aromatic CH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 103.6-111.9 (2C,phenyl ring); 147.6(1C,phenyl ring); 160.3 (1C,phenyl ring); 165.3 (1C,phenyl ring);166.3 (1C,carboxylic acid); 167.3(1C,Aromatic S-C-N);183.5 (2C, C=O)

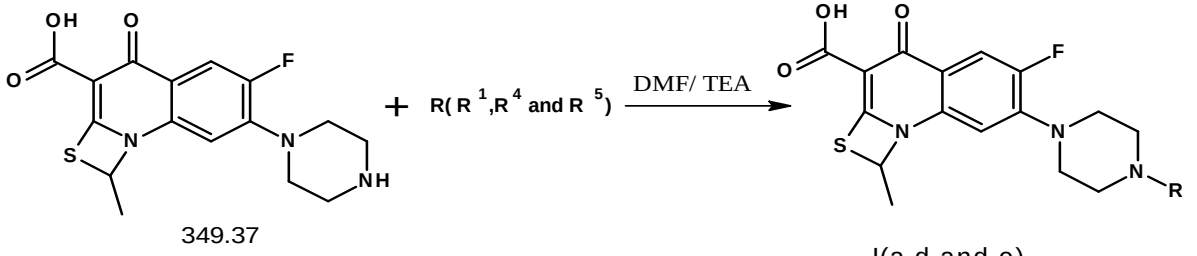
Elemental analysis:C-61.34 %,H-6.29%,F=3.59%,N=10.60 %, O= 12.11 % S-6.07 %

6-fluro-1-methyl-4-oxo-7-(4-(1-(3-(triflurомethyl) benzyl)-1*H*, 4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid [1(h)]

M.P: 223° C, Yield: 51%; MS: 587.16 (100%), 588.16 (32.9 %), 589.16 (5.4 %); IR max cm^{-1}: 3275-2800 (O-H stretch); 1215.12(C-O); 1490.56 (Ar C=C); 1220.15(C-F)

^{1}HNMR (DMSO D^{6}): δ=10.2(-OH,s,1H),7.5 (Aromatic CH of pyrazole ring,s,1H),7.4(Aromatic CH of pyrazole ring,s,1H),7.26(Aromatic CH,d,2H),7.10(Aromatic CH,m,2H),6.8(Aromatic CH,d,1H),6.4(Aromatic CH,s,1h),4.26 (CH_2,s,2H),3.73 (-CH,m,1H of thiazeto ring),3.34(-CH_2,m,4H of piperazine ring), 2.84 (-CH_2,m,4H of piperazine ring), 2.34 (-CH_3,s,3H)

^{13}CNMR: 22.8 (1C,-CH_3) ; 47.1(2C, piperazine -CH_2); 50.6(2C, piperazine -CH_2); 58.8(1C,-CH_2); 60.7(1C,-CH); 89.6(1C,-CH); 101.0(1C, Aromatic C=C, fluoroquinolone ring);100-118.2(3C,AromaticCH,fluoroquinolone ring); 140.6-144.6 (3C,Aromatic CH,fluoroquinolone ring); 103.6(1C,pyrazol); 122.2-129.2(3C,Phenyl ring);124.5(1C,CF_3);131.9-139.6(1C,pyrazol ring); 130.5-136.5 (3C,phenyl ring);166.3 (1C,carboxylic acid); 167.3(1C,Aromatic S-C-N);183.5 (1C, C=O) ; Elemental analysis:C-57.23 %,H-4.29%,F=12.93 %,N=11.92 %, O= 8.17 % S-5.46 %

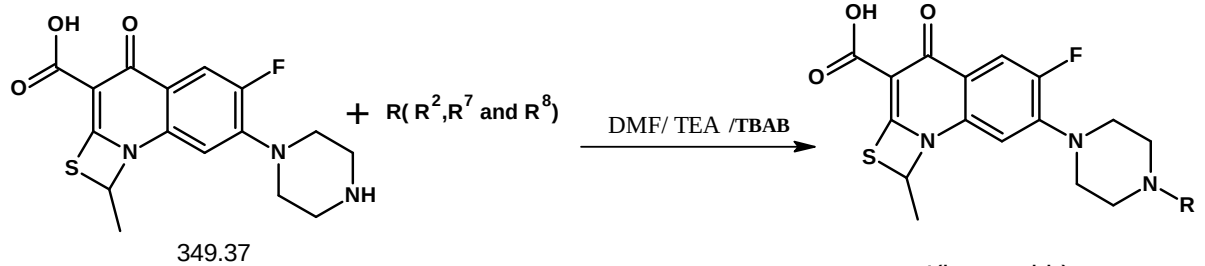


349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

I(a,d and e)

I

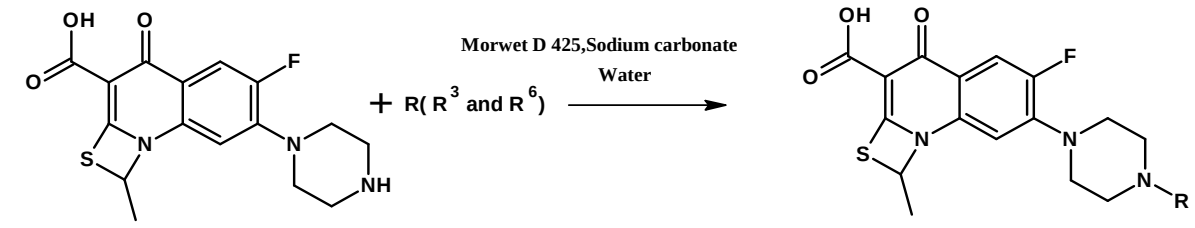


349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

I(b,g and h)

I



349.37

6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1 ,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

I

I(c and f)

R (R¹ to R⁸): 2-(bromomethyl)-1, 3-difluorobenzene, 5-[4'-(bromomethyl) biphenyl-2-yl]-1-trityl-l-1*H*-tetrazole,6-chloro-5-(2-chloroethyl)-1,3-dihydro-2*H*-indol-2-one,4' (bromomethyl) biphenyl-2- carbonitrile, benzyl 2-(benzyloxy)-4-(bromocarbonyl) benzoate, 2-(4-chlorobutoxy)-1,2,3,4-tetrahydroquinolin-7-ol, 2-(chloromethyl)-4-(3-methoxypropoxy)-3-methylpyridine, 1-[3-(trifluoromethyl) benzyl]-1*H*-pyrazole-4-carbonyl chloride

Scheme 1: Synthesis of N-substituted derivatives of 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid I (a-h)

Table 1: Zone of inhibition and Minimum inhibitory concentration of test compounds against *Bacillus subtilis*

S. No.	Test Compound	Concentration (µg/mL)	Zone of Inhibition (in mm) *Test organism: Bacillus subtilis*	Minimum Inhibitory Concentration (µg/mL)
1	I(a)	1024	21.00	32
		512	17.00	
		128	14.00	
		32	11.00	
		8	-	
2	I(b)	1024	19.50	128
		512	16.00	
		128	7.00	
		32	-	
		8	-	
3	I(c)	1024	10.00	512
		512	8.0	
		128	-	
		32	-	
		8	-	
4	I(d)	1024	20.00	32
		512	14.00	
		128	12.00	
		32	10.0	
		8	-	
5	I(e)	1024	13.00	512
		512	9.00	
		128	-	
		32	-	
		8	-	
6	I(f)	1024	21.00	32
		512	26.00	
		128	18.00	
		32	11.00	
		8	-	
7	I(g)	1024	10.00	128
		512	7.00	
		128	7.00	
		32	-	
		8	-	
8	I(h)	1024	19.00	32
		512	17.00	
		128	14.00	
		32	8.00	
		8	-	
9	Ciprofloxacin Positive Control	1024	14.00	-
10	DMSO Negative Control	1%	0.00	-

Evaluation of antibacterial activity and determination of minimum inhibitory concentration by disk diffusion method

1. Test organisms: *Bacillus subtilis and Escherichia coli*

2. Test compounds*:* Eight synthesized molecules and Ciprofloxacin as positive control

3. Inoculum: Cell suspension was prepared from cultures grown on Trypticose soya broth adjusted to 1-2 x 10^8 cells/mL

4. Drug concentrations: drug concentration prepared: (a) Test compounds: 8 - 1024 µg/mL in 1% DMSO **(b)** Control: 1% DMSO in Sterile water

5. Procedure: (a) Determination of Antibacterial activity: (i)100 µl Inoculum of test cultures was inoculated on Muller Hinton Agar plates (90 mm).**(ii)**Test compounds (5µl, 1024 µg/mL) and ciprofloxacin (5 µl, 1 mg/mL) were impregnated on 6mm sterile Whatmann No. 1 Disks.**(iii)**Test compounds and standard disks were placed on Agar plates.**(iv)** The plates were Incubated @ 35°C for 24-48 hrs and observed for zone of inhibition around the disk.**(v)** The Compounds showing activity were further tested for determination of Minimum Inhibitory Concentration.

(b)Determination of minimum inhibitory Concentration: (i) 100 µl Inoculum of test cultures was inoculated on Muller Hinton Agar plates (90 mm).**(ii)**Test compounds (5ul, Different test concentrations, 8, 32, 128, 512 and 1024 ug/mL) and ciprofloxacin (5 ul, 1 mg/mL) were impregnated on 6mm sterile Whatmann No. 1 Disks.**(iii)**Test compounds and standard disks were placed on Agar plates.**(iv)**The plates were Incubated @ 35 0C for 24-48 hrs and observe for zone of inhibition around the disk.**(v)**Lowest concentration of test compound showing zone of inhibition was considered as MIC.

Determination of Minimum Inhibitory concentration: The zone of inhibition and MIC determined for each test compound is summarized in Table 1**.**

RESULTS

Eight derivatives were synthesized and their structure was confirmed by IR, NMR, mass spectrum and elemental analysis. All the eight derivatives were subjected to antibacterial activity. Among the tested compounds seven compounds showed antibacterial activity against *Bacillus subtilis* (Gram positive bacteria). None of the compounds showed zone of inhibition against *Escherichia coli* (Gram negative Bacteria). The zone of inhibition observed for different test compounds is tabulated in Table. 1. The five derivatives [1(f), 1(d), 1(a) and 1(h)] showed comparable antibacterial activity, in test conditions, with respect to ciprofloxacin.

DISCUSSION

The N-substituted 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid derivatives were prepared by three different methods. The general procedure, which involved the use of Triethylamine and DMF, was modified by the addition of tetra butyl ammonium bromide (TBAB) to facilitate completion in few reactions. Other reactions which did not proceed in both the aforementioned ways were facilitated by the use of Morwet-D425. The problem faced in these reactions was the incompletion of the reaction or the formation of sticky material and difficult stirrability of the reaction mass which in turn results in lesser purity and lower yields. In U.S.Pat.No.8, 410,268B2 [16] we have disclosed a process for the preparation of Ziprasidone, involved the same procedure. In our present work, we have achieved substantially good yields and purity. All the compounds were screened for antibacterial activity. The derivatives involving substituted phenyl and substituted quinoline ring showed substantial activity against *Bacillus subtilis* (Gram positive bacteria).

CONCLUSION

The preparation procedures followed in this work offers reduction in the reaction time, operation simplicity, cleaner reaction and easy work-up. Observation has showed the importance of electronic environment on antibacterial activity. The quinoline substitution along with the halogen and cyano substituted phenyl ring has increased the activity of the compounds compared to those with other substituent's. This may be due to the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules and thus facilitate the crossing through the biological membrane of the microorganisms and thereby inhibit their growth .The title compounds [I(a-h)] were prepared from the starting material 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-4*H*-[1, 3] thiazeto [3, 2-*a*] quinoline-3-carboxylic acid (I) and were screened for antibacterial activity. Among all compounds it was found that derivatives with 2-(4-chlorobutoxy)-1,2,3,4-tetrahydroquinolin-7-ol, 4' (bromomethyl)biphenyl-2-carbonitrile, 2-(bromomethyl)-1,3-difluorobenzene and 1-[3-(trifluoromethyl)benzyl]-1*H*-pyrazole-4-carbonyl chloride showed comparable antibacterial activity when compared to ciprofloxacin .

ACKNOWLEDGEMENT

The authors are thankful to Alkem Laboratories Limited and Skanda Life Sciences Private Limited for providing necessary facilities.

REFERENCES

1. Andriole VT. The quinolones: past, present, and future. Clin. Infect. Dis. 2005; 41(2): 113–119.
2. Ball P. Adverse drug reactions: implications for the development of fluoroquinolones. J. Antimicrob. Chemother. 2003; 51(1): 21–27.
3. Drugs@FDA page. Available at: http:/ /www.accessdata.fda.gov /scripts/cder/ drugsatfda/index.cfm. Hawkey PM. Mechanisms of quinolone action and microbial response. J. Antimicrob. Chemother. 2003; 51(1): 29–35.
4. Gyoten M, Nagaya H, Fukuda S, Ashida Y and Kawano Y. Synthesis of Eosinophil Infiltration Inhibitors with Antihistaminic Activity. Chem. Pharm. Bull. Tokyo 2003; 51(2): 122-133.
5. Magid AG, John A, Moyer Susan TN, Michael W and Usha P. New Antihistamines: Substituted Piperazine and Piperidine Derivatives as Novel H1-Antagonists. J. Med. Chem. 1995; 38: 4026- 4032.
6. Chaudhary P, Kumar R, Verma AK and Singh D. Synthesis and antibacterial activity of *N*-alkyl and *N*-aryl piperazine derivatives. Bioorg. Med. Chem. 2006; 14: 1819-1826.
7. Hachiro S, Hiroo O, Yasuo A, Youichi I and Yoshiharu Y. Synthesis and Antibacterial Activity of Amino Acids Conjugated Diphenyl methylpiperazine Derivatives. Japanese J. Pharmacol. 2002; 89(1): 7-20.
8. Rebecca DP and Patricia M. Combinatorial Chemistry & High Throughput Screenings, 1, 4-Bis(3-Aminopropyl)Piperazine Libraries: From the Discovery of Classical Chloroquine-Like Antimalarials to the Identification of New Targets 2005; 8: 39-48.
9. Makoto K, Tomoko M, Koji Y, Masaki M, Nobuo K, Nobuyuki K, Kenichi K, Masato I, Yuji K, Katsuji O and Takayuki N. Novel diphenylalkyl piperazine derivatives with high affinities for the dopamine transporter. Bioorg. & Med. Chem. 2003; 11: 3953-3963.
10. Makoto K, Tomoko M, Koji Y, Masaki M, Nobuo K, Nobuyuki K, Kenichi K, Masato I, Yuji K, Katsuji O and Takayuki N. Efficient asymmetric syntheses, determination of absolute configurations and biological activities of 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3 (phenylamino) propyl] piperazine as a novel potent dopamine uptake inhibitor in the central nervous system. Bioorg. & Med. Chem. 2004; 12: 3069-3078.
11. He Zhao, Xiaoshu He, Andrew T, Diane H, Andrzej K, Robbin B, Renee P and Jan WF. Indoline and piperazine containing derivatives as a novel class of mixed D2/D4 receptor antagonists. Part 2: Asymmetric synthesis and biological evaluation. Bioorg. & Med. Chem. Lett. 2002; 12: 3111-3115.
12. Brian D, Jessica P, Teresa P, Lee C, Brian M, Robin S, Julia H, Tracy B, Mary C, John S and Val G. Aryl piperazine melanocortin MC4 receptor agonists. Bioorg. & Med. Chem. Lett. 2003; 13: 3793-3796.
13. Rossen K, Steven AW, Sager J, Reamer RA, Askin D, Volante RP and Reider PJ. Asymmetric hydrogenation of tetrahydro pyrazines: Synthesis of (S)- piperazine-2-tert- butyl carboxamide, an intermediate in the preparation of the HIV protease inhibitor indinavir. Tetrahedron Lett. 1995; 36: 6419-6422.

14. David A, Kan KE, Kai R, Robert MP, Kenneth MW, Volante RP and Paul JR. Highly diastereo selective reaction of a chiral, non-racemic amide enolate with (S)-glycidyl tosylate. Synthesis of the orally active HIV-1 protease inhibitor L-735,524, Tetrahedron Lett. 1994; 35(5): 673-676.
15. Drugs.com. Available at http:/ /www.drugs.com /international / sarafloxacin.html.
16. Gootz, TD, Zaniewski R, Haskell S, Schmieder B, Tankovic J, Girard D, Courvalin P and Polzer RJ. Activity of the new fluoroquinolone trovafloxacin (CP-99,219) against DNA gyrase and topoisomerase IV mutants of Streptococcus pneumoniae selected in vitro. Antimicrob. Agents Chemother. 1996; 40 (12): 2691–2700.
17. Drugs.com. Available at http:/ /www.drugs.com /international / tosufloxacin.html.
18. Rubinstein, E. History of quinolones and their side effects. Chemotherapy 2001; 47(3): 3–8.
19. Drugs.com. Available at http://www.drugs.com/ international /rosoxacin.html.
20. Deborah MR, Youjun Y, Mary J W, Karen B and May D. Inhibition of metallo-β-lactamases by pyridine monothiocarboxylic acid analogs. The Journal of A ntibiotics 2010; 63: 255-257.
21. Browne, SG. Trial of a long-acting sulfonamide sulfaphenazole in the treatment of leprosy. International journal of Leprosy 1961; 29: 502–505.
22. Shashiprabha, Kanakamajalu S, Debkiron M, Padmashree B, Ksundarraja R and Kuppuswamy N. Process for the preparation of Ziprasidone. U.S.Pat.No. 8,410,268B2. 2013.

US008410268B2

(12) **United States Patent**
Shashiprabha et al.

(10) **Patent No.: US 8,410,268 B2**
(45) **Date of Patent: Apr. 2, 2013**

(54) **PROCESS FOR THE PREPARATION OF ZIPRASIDONE**

(75) Inventors: **Shashiprabha**, Bangalore (IN); **Kanakamajalu Shridhara**, Bangalore (IN); **Debkiron Mukherjee**, Bangalore (IN); **Padmashree Badraje**, Bangalore (IN); **K Sundarraja Rao**, Bangalore (IN); **Kuppuswamy Nagarajan**, Bangalore (IN)

(73) Assignee: **Alkem Laboratories Limited**, Mumbai (IN)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 161 days.

(21) Appl. No.: **12/921,566**

(22) PCT Filed: **Mar. 9, 2009**

(86) PCT No.: **PCT/IN2009/000164**

§ 371 (c)(1),
(2), (4) Date: **Sep. 8, 2010**

(87) PCT Pub. No.: **WO2009/116085**

PCT Pub. Date: **Sep. 24, 2009**

(65) **Prior Publication Data**

US 2011/0003995 A1 Jan. 6, 2011

(30) **Foreign Application Priority Data**

Mar. 11, 2008 (IN) 499/MUM/2008

(51) **Int. Cl.**
C07D 417/12 (2006.01)

(52) **U.S. Cl.** **544/368**; 514/254.04

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,831,031	A *	5/1989	Lowe et al.	514/254.02
5,206,366	A *	4/1993	Bowles	544/368
5,312,925	A *	5/1994	Allen et al.	544/368
5,338,846	A *	8/1994	Busch et al.	544/368
6,150,366	A *	11/2000	Arenson et al.	514/254.04

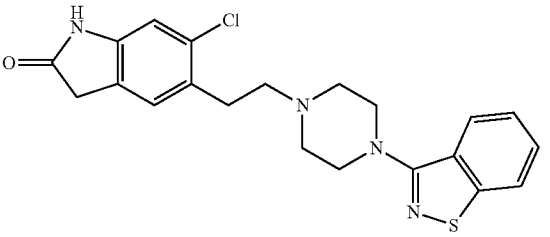
* cited by examiner

Primary Examiner — Emily Bernhardt
(74) *Attorney, Agent, or Firm* — George W. Moxon, II; Brian P. Harrod

(57) **ABSTRACT**

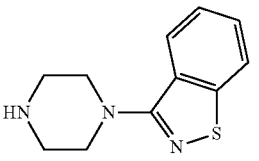
The present invention relates to a process for preparing Ziprasidone of formula I,

(I)



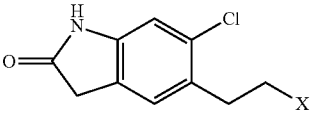
or a pharmaceutically acceptable salt or a solvate or a hydrate thereof;
comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl) piperazine of formula II or its salt:

(II)



with 5-(2-haloethyl)-6-chloro-oxindole of formula III:

(III)



wherein X is leaving groups like fluoro, chloro, bromo, iodo or sulphonyl;
in the presence of a dispersing agent and a base in a solvent to form ziprasidone of formula I; and optionally converting the ziprasidone formed into a pharmaceutically acceptable acid addition salts of ziprasidone; or a solvate or a hydrate thereof.

9 Claims, No Drawings

PROCESS FOR THE PREPARATION OF ZIPRASIDONE

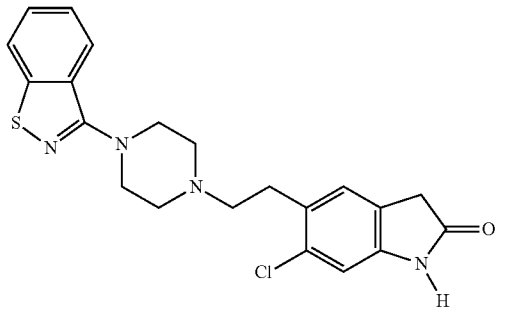
FIELD OF THE INVENTION

The present invention relates to an improved process for the preparation of ziprasidone or pharmaceutically acceptable salts thereof.

BACKGROUND OF THE INVENTION

Ziprasidone is an antipsychotic agent with the following chemical name: 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one of formula (I)

(I)



Ziprasidone is disclosed in U.S. Pat. Nos. 4,831,031 and 5,312,925 (assigned to Pfizer). Ziprasidone inhibits synaptic reuptake of serotonin and norepinephrine. No appreciable affinity was exhibited for other receptor/binding sites tested, including the cholinergic muscarinic receptor. The mechanism of action of ziprasidone, as with other drugs having efficacy in schizophrenia, is unknown. However, it has been proposed that this drug's efficacy in schizophrenia is mediated through a combination of dopamine type 2 (D 2) and serotonin type 2 (5HT 2) antagonism. Ziprasidone's antagonism of histamine H receptors may explain the somnolence observed with this drug.

U.S. Pat. No. 5,312,925 (Pfizer Inc.) describes a process for the synthesis of monohydrate of 5-(2-(4-(1,2-benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one hydrochloride and its characterization based on IR, XRD and moisture content. The '925 patent also discloses that the hemihydrate may be obtained by the process described in Example 16 of U.S. Pat. No. 4,831,031 and its characterization by IR, XRD and moisture content. It also discloses the IR, XRD and moisture content of anhydrous Ziprasidone hydrochloride. According to the invention in the '925 patent, Ziprasidone of water content of 3.97, 2.55 and 0.37% were used for the IR and XRD study of Ziprasidone hydrochloride monohydrate, hemihydrate and anhydrous. In this invention, the monohydrate of Ziprasidone hydrochloride was prepared by reacting anhydrous 5-(2-(4-(1,2-benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one with aqueous hydrochloric acid. The temperature range of the reaction was maintained between 60 to 65° C. and aqueous hydrochloride used for salt formation was around 0.7 M. Depending on the reaction temperature and other conditions, the reaction times were set around 3 to 24 hours. The final product thus obtained was dried carefully in monitored conditions to make certain that water content was from about 3.8% to about 4.5% to obtain the stable monohydrate.

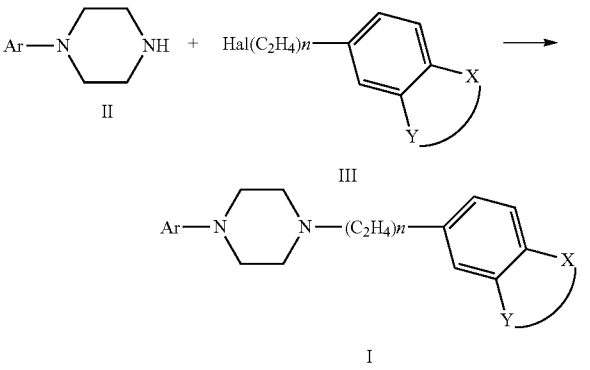
U.S. Pat. No. 6,150,366, discloses a manufacturing process of ziprasidone hydrochloride monohydrate, comprises: 1) dissolving, ziprasidone free base in a solvent comprising THF and water, in a volume ratio of about 22-35 unit volumes of THF to about 1.5-8 volumes of water; 2) heating the solution resulting from step (1); 3) adding HCl to the solution resulting from step (2); and 4) cooling the solution resulting from step (3) and crystals collected by filtration and drying.

U.S. Pat. No. 5,206,366 and U.S. Pat. No. 5,338,846 describe a process for preparing ziprasidone by reacting 1-(1,2-benzisothiazol-3-yl) piperazine with 5-(2-chloroethyl)-6-chloro-oxindole in water with a neutralizing agent such as sodium carbonate under reflux.

J. Med. Chem. 1996, 39, 143-148 discloses preparation of ziprasidone by reacting 1-(1,2-benzisothiazol-3-yl)piperazine with 5-(2-bromoethyl)-6-chloro-oxindole in isoamyl alcohol solvent in the presence of sodium carbonate.

Some salts of ziprasidone, and in particular, its hydrochloride salt is a potent commercial antipsychotic agent useful in the treatment of various disorders, including schizophrenia and anxiety diseases. Ziprasidone hydrochloride is currently marketed under the proprietary name of Geodon. Other salts of ziprasidone are also reported to be effective for the treatment of the same type of diseases.

Some of the processes described in the aforementioned patents necessitate the use of ion-exchange catalyst (i.e. sodium iodide) and/or phase transfer catalysts (for example tetra butyl ammonium bromide or tetra butyl phosphoriium bromide) in order for the coupling reaction producing ziprasidone to take place. For example, U.S. Pat. No. 4,831,031 indicates that arylpiperazinyl-ethyl (or butyl)-heterocydic compounds may be prepared by reacting piperazines of the formula II with compounds of the formula III as follows in [Scheme 1]:



Wherein Hal is fluoro, chloro, bromo or iodo; and Ar, n, X and Y are as defined therein with reference to formula I. According to the '031 patent the coupling reaction is generally conducted in a polar solvent, such as a lower alcohol, dimethylformamide or methylisobutylketone, and in the presence of a weak base and that, preferably, the reaction is carried out in the presence of a catalytic amount of sodium iodide, hydrogen chloride and neutralizing agent such as sodium carbonate.

In some instances, the ziprasidone obtained was purified by column chromatography, thus making the process impractical for large-scale preparations. Another process uses potentially explosive gases such as hydrogen in the presence of catalysts, for example zinc, palladium on carbon, followed by acid treatment to carry out a reduction and cyclization of an intermediate, in order to obtain ziprasidone.

Despite various processes disclosed in the prior art for the preparation of ziprasidone and salts thereof, still there is a need for a good process for producing ziprasidone and pharmaceutically acceptable acid addition salts of ziprasidone thereof, in high purity. One of the major problems faced in the prior art is formation of sticky material and difficult stirrability of the reaction mass. This problem is especially acute in large scale manufacturing.

The present invention provides a process for the preparation of ziprasidone in high yields and purity, suitable for large-scale manufacturing, which helps to overcome some of the deficiencies of the prior art.

OBJECT OF THE INVENTION

It is an object of the present invention to provide an improved process for the preparation of ziprasidone of formula I or a pharmaceutically acceptable salt or a solvate or a hydrate thereof.

It is further object of the present invention to provide a process for the preparation of ziprasidone in high yields and purity, suitable for large-scale manufacturing, which helps to overcome some of the deficiencies of the prior art.

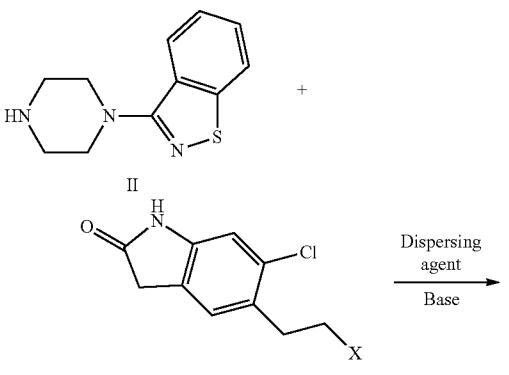
At least one of the preceding objects is met, in whole or in part, by the improved process of the invention, for preparing ziprasidone, or a pharmaceutically acceptable salt or a solvate or a hydrate thereof, by reacting 1-(1,2-benzisothiazol-3-yl) piperazine or its salt with 5-(2-haloethyl)-6-chloro-oxindole in the presence of dispersing agent and a base in a solvent.

SUMMARY OF THE INVENTION

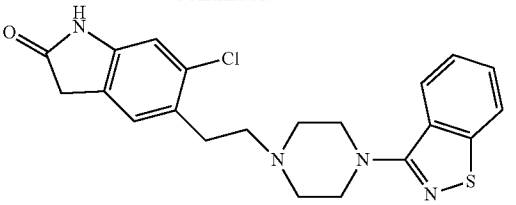
According to first aspect of the present invention is provided an improved process for the preparation of ziprasidone of formula I or a pharmaceutically acceptable salt or a solvate or a hydrate thereof.

According to another aspect of the present invention is provided an improved process for preparing ziprasidone of formula I, or a pharmaceutically acceptable salt or a solvate or a hydrate thereof, comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl) piperazine of formula II or its salts with 5-(2-haloethyl)-6-chloro-oxindole of formula III in the presence of dispersing agent and a base in a solvent [Scheme 2]. This surprisingly enabled production of pharmaceutical grade ziprasidone in an efficient manner with a yield and purity higher than the prior art processes.

Scheme 2:



-continued



Ziprasidone (I)

The ziprasidone thus obtained is optionally converted into pharmaceutically acceptable acid addition salts or a solvate or a hydrate thereof.

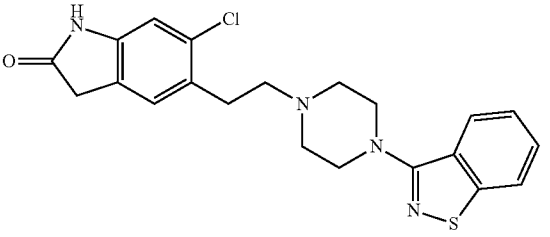
The purpose of using dispersing agent in the reaction is to overcome the problem of sticky mass formation during the reaction. This sticky mass is not easily soluble in water and causes problems during the reaction and also in cleaning of the reactors. The sticky mass formation has its negative impact on the yields and also on product quality. Additionally the use of a dispersing agent results in pharmaceutical grade ziprasidone in an efficient manner with a yield and purity higher than the prior art processes.

The process of the present invention provides ziprasidone or a pharmaceutically acceptable salt or a solvate or a hydrate in a yield and purity higher than the prior art processes. It is preferred that the ziprasidone or the pharmaceutically acceptable salt thereof obtained by the present invention has a purity of at least 98%.

The invention may be summarized as given below:

A. A process for preparing ziprasidone of formula I,

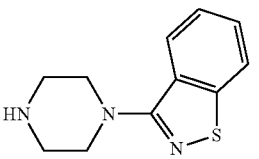
(I)



or a pharmaceutically acceptable salt or a solvate or a hydrate thereof;

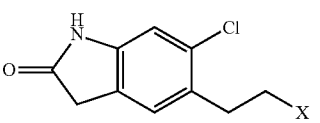
comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl) piperazine of formula II or its salt:

(II)



with 5-(2-haloethyl)-6-chloro-oxindole of formula III:

(III)



wherein X is leaving groups like fluoro, chloro, bromo, iodo or sulphonyl;

in the presence of a dispersing agent and a base in a solvent to form ziprasidone of formula I; and optionally converting the ziprasidone formed into a pharmaceutically acceptable acid addition salts of ziprasidone; or a solvate or a hydrate thereof.

B. Process as in step A above, wherein the dispersing agent is selected from modified sodium lignosulfonates such as BORRESPERSE™ Na, UFOXANE™ 3 and ULTRAZINE™ Na; Kraft sodium lignosulfonates such as REAX® 88B; naphthalene-formaldehyde condensates such as DISPERSOGEN® SI, MORWET® D-425 (naphthalene sulfonic acid formaldehyde) and GALORYL® DT 201 (naphthalene-sulfonic acid formaldehyde condensate); and the like or mixtures thereof.

C. Process as in step B above, wherein the dispersing agent is selected from naphthalene-formaldehyde condensates such as DISPERSOGEN® SI, MORWET® D-425 (naphthalene sulfonic acid formaldehyde) and GALORYL® DT 201 (naphthalenesulfonic acid formaldehyde condensate).

D. Process as in step C above, wherein the dispersing agent is MORWET® D-425 (naphthalene sulfonic acid formaldehyde).

E. Process as in step A above, wherein the base is selected from alkali metal salt, hydroxides, organic tertiary bases and the like or mixtures thereof.

F. Process as in step A above, wherein the base is selected from sodium carbonate or bicarbonate or mixtures thereof.

G. Process as in step A above, wherein the solvent is selected from the group comprising substituted, unsubstituted, cyclic, bicyclic, saturated, or unsaturated, straight or branched hydrocarbon but not limited to aliphatic or aromatic hydrocarbon, having C_6-C_{10} atoms, water, alcohols, ketones, esters, ethers and chlorinated solvents, and the like or mixtures thereof.

H. Process as in step G above, wherein the solvent is selected from the group comprising toluene; esters such as ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate and isobutyl acetate; water; alcohols such as methanol, ethanol and isopropanol; ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone and diethyl ketone; ethers such as tetrahydrofuran and dioxane; and chlorinated solvents such as methylene chloride, chloroform, carbontetrachloride and ethylene dichloride; and the like or mixtures thereof.

I. Process as in step H above, wherein the solvent is selected from the group comprising alcohols and water and the like or mixtures thereof.

J. Process as in step A above, wherein the prepared ziprasidone or pharmaceutically acceptable salt or a solvate or a hydrate thereof has a purity of at least 98%.

Further objects of the present invention together with additional features contributing thereto and advantages accruing there from will be apparent from the following description of preferred embodiments of the invention which are shown in the accompanying drawing figures wherein

Scheme 1 is a schematic representation of an embodiment for prior art process for preparing arylpiperazinyl-ethyl (or butyl)-heterocyclic compounds, or a pharmaceutically acceptable salt, by reacting piperazines of the formula II with compounds of the formula III.

Scheme 2 is a schematic representation of an embodiment for process where a process for preparing ziprasidone of formula I, or a pharmaceutically acceptable salt or a solvate or a hydrate thereof, comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl) piperazine/salt of formula II with 5-(2-haloethyl)-6-chloro-oxindole of formula III in the presence of dispersing agent and a base in a solvent.

DETAILED DESCRIPTION OF THE INVENTION

Before the present process and methods are described, it is to be understood that this invention is not limited to particular compounds, formulas or steps described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes a plurality of such compounds and reference to "the step" includes reference to one or more step and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

In accordance with the present embodiment, there is provided a process for the preparation of ziprasidone of formula I or a pharmaceutically acceptable salt or a solvate or a hydrate thereof.

In accordance with the present embodiment, there is provided a process for preparing ziprasidone of formula I, or a pharmaceutically acceptable salt or a solvate or a hydrate thereof, comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl) piperazine of formula II or its salt with 5-(2-haloethyl)-6-chloro-oxindole of formula III in the presence of dispersing agent and a base in a solvent and optionally converting the ziprasidone formed into a pharmaceutically acceptable acid addition salts of ziprasidone; or a solvate or a hydrate thereof.

Though all pharmaceutically acceptable acid addition salts of formula II can be used, those salts in which the anion does not contribute significantly to toxicity of pharmacological activity of the organic cation, may be preferred. Examples of

organic acids useful for making salt of compound of formula II are carboxylic acids such as maleic acid, acetic acid, tartaric acid, propionic acid, fumaric acid, isethionic, succinic acid, pamoic acid, cyclamic acid, pivalic acid, and the like; inorganic acids used are hydrohalide acids such as HCl, HBr, HI; sulfuric acid; phosphoric acid; and the like.

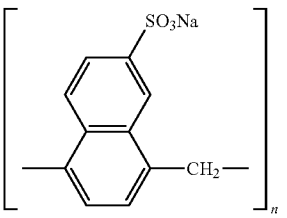
In the process of the present invention, the (1,2-benzisothiazol-3-yl) piperazine of formula II or its salt and 5-(2-haloethyl)-6-chloro-oxindole of formula III are used in the range of about 1:1 to about 10:1 of molar ratio to improve the yield of ziprasidone of formula I or a pharmaceutically acceptable salt or a solvate or a hydrate thereof.

The purpose of using dispersing agent in the reaction of the present invention is to overcome the problem of sticky mass that is formed during the reaction. This sticky mass is not easily soluble in water and causes problems in the reaction. Additionally the use of a dispersing agent results in pharmaceutical grade ziprasidone in an efficient manner with a yield and purity higher than the prior art processes. It enhances the mixing of the reaction mass and results in cleaner product.

The compounds that can be used as dispersing agent are for example, polymers of the arylsulphonate type, in particular the alkaline polynaphthalene sulphonates obtained by condensation of (alkyl) aryl sulphonate derivatives with formaldehyde, lignosulphonates (for example: sodium lignosulfonate and calcium lignosulphate), the polyphenol sulphonates, the salts of polyacrylic acids, the salts of lignosulphonic acids (for example: the sodium salt of polymerized lignosulphonic acids of the Kraft type), the salts of sulphonic phenol acids or sulphonic naphthalenes, the phosphoric esters of alcohols or of polyethoxylated phenols, the esters of fatty acids and of polyols, derivatives with a sulphates, sulphonates and phosphates function of the preceding compounds and the like or mixtures thereof.

Suitable for use as dispersants are, for example, modified sodium lignosulfonates, such as BORRESPERSE™ Na, UFOXANE™ 3A, and ULTRAZINE™ Na (manufactured by Borregard); Kraft sodium lignosulfonates, such as REAX® 88B (manufactured by Westvaco) or naphthalene-formaldehyde condensates, such as DISPERSOGEN® SI (manufactured by Clariant GmbH), MORWET® D-425 (manufactured by Witco Corporation) and GALORYL® DT 201 (manufactured by CFPI) and the like or mixtures thereof.

Preferred dispersants are sodium salts of alkylnaphthylsulfonic acid/formaldehyde condensates and sodium lignosulfonate which are commercially available, for example, under the trade names MORWET® D-425 and having the structure below:



Wherein n ranges from 2 to 9,

Therefore, based on the object of discovering that the dispersing agents particularly MORWET® D-425 was found to give a product with lesser impurities, an efficient, high-yield and being a significantly cheaper raw material, it becomes an excellent source for the production of 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one. The invention accordingly provides, use of dispersing agent such as MORWET® D-425 in the process of the invention in presence of a base such as for example sodium hydroxide or sodium carbonate.

In accordance with the present invention, there is provided a reaction that is carried out in the presence of bases like alkali metal hydroxides, carbonates, bicarbonates and organic bases like tertiary amines like triethyl amine, benzisothiazolyl piperazine, pyridine etc. Alkali metal carbonates like sodium carbonate, potassium carbonate and more specifically sodium carbonate.

In accordance with the present invention, there is provided a process for preparing ziprasidone of formula I, wherein the solvent is selected from the group comprising alcohols, ketones, water, hydrocarbons, esters, ethers and chlorinated solvents, or mixtures thereof. The solvent may be selected from the group comprising alcohols, ketones, hydrocarbons, esters, ethers and chlorinated solvents, or mixtures thereof. The solvent used in the present invention is selected from the group consisting of substituted, unsubstituted, cyclic, bicyclic, saturated, or unsaturated, straight or branched hydrocarbon but not limited to aliphatic or aromatic hydrocarbon, having C_6-C_{10} atoms, Suitable solvents are generally alcohols such as methanol, ethanol, isopropanol; ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, diethyl ketone; hydrocarbon such as toluene, ester, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate; water; ethers such as tetrahydrofuran, and dioxane; chlorinated solvents such as methylene chloride, chloroform, carbontetrachloride, ethylene dichloride and the like or mixtures thereof.

In an embodiment of the present invention, there is provided a process for preparation of ziprasidone by reacting piperazine benzisothiazole hydrochloride, 5-(2-chloroethyl)-6-chlorooxindole, sodium carbonate in which water is added 20 times based on oxindole weight and 1% of dispersing agent MORWET® D-425. All these reactants are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is then cooled to room temperature and the resulting mass is filtered. It is slurried in water and then in IPA and isolated by filtration. The solid is dried at 95-100° C.

In another embodiment of the present invention, there is provided a process for preparation of ziprasidone by reacting piperazine benzisothiazole hydrochloride, 5-(2-chloroethyl)-6-chlorooxindole, and dispersing agent MORWET® D-425 and a base. All these reactants are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is then cooled to room temperature and the resulting mass is filtered. It is slurried in IPA and then in water and isolated by filtration. The solid is dried at 95-100° C.

The ziprasidone prepared by the embodiments of the present process may be converted into a pharmaceutically acceptable acid addition salt; or a solvate or a hydrate thereof.

The process of the present invention provides ziprasidone or a pharmaceutically acceptable salt or a solvate or a hydrate in a yield and purity higher than the prior art process. It is preferred that the ziprasidone or the pharmaceutically acceptable salt thereof obtained by the present invention has a purity of at least 98%.

The following example illustrates the preparation of ziprasidone and is not to be construed as limiting the scope of the invention in any manner.

Example 1

2.2 moles of piperazine Benzisothiazole hydrochloride, 1 mol of 5-(2-chloroethyl)-6-chlorooxindole, 2.2 mol of

Sodium hydroxide, water 20 times based on oxindole weight and 1% of dispersing agent MORWET® D-425. All the reactants are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is cooled to room temperature and the resulting mass is filtered. It is slurried in IPA and then in water and isolated by filtration. The solid is dried at 95-100° C.

Yield: 90%; Purity: 98%

Example 2

1 mole of piperazine Benzisothiazole hydrochloride, 1 mole of 5-(2-chloroethyl)-6-chlorooxindole, 3.3 mole of Sodium carbonate, water 5.2 times based on oxindole weight and 1% of dispersing agent MORWET® D-425 are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is cooled to room temperature and the resulting mass is filtered. It is slurried in water and then in IPA and isolated by filtration. The solid is dried at 95-100° C.

Yield: 90%; Purity: 100.30%

Example 3

2 moles of Piperazine Benzisothiazole hydrochloride, 1 mole of 5-(2-chloroethyl)-6-chlorooxindole in water (20 times based on benzisothiazole) and in the presence of dispersing agent. All the reactants are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is cooled to room temperature and the resulting mass is filtered. It is slurried in IPA and then in water and isolated by filtration. The solid is dried at 95-100° C.

Yield: 90-92%, Purity 98% (min).

Example 4

2 moles of Piperazine Benzisothiazole, 1 mole of 5-(2-chloroethyl)-6-chlorooxindole in water (20 times based on benzisothiazole) and in the presence of dispersing agent. All the reactants are charged in to the flask and refluxed under nitrogen, under stirring for 12-16 hr. After the completion of the reaction, the reaction mass is cooled to room temperature and the resulting mass is filtered. It is slurried in water and then in IPA and isolated by filtration. The solid is dried at 95-100° C.

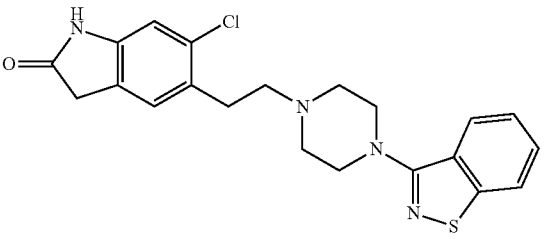
Yield: 92%, Purity 99% (min).

Although the invention has been described in terms of particular embodiments and applications, one of ordinary skill in the art, in light of this teaching, can generate additional embodiments and modifications without departing from the spirit of or exceeding the scope of the claimed invention. It should be emphasized that the above-described embodiments of the present invention, particularly any "preferred" embodiments, are merely possible examples of the invention of implementations, merely set forth for a clear understanding of the principles of the invention. Accordingly, it is to be understood that the drawings and descriptions herein are proffered by way of example to facilitate comprehension of the invention and should not be construed to limit the scope thereof.

We claim:

1. A process for preparing ziprasidone of formula I,

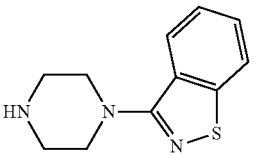
(I)



or a pharmaceutically acceptable salt or a solvate or a hydrate thereof;

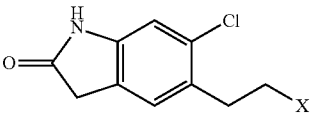
comprising the steps of reacting 1-(1,2-benzisothiazol-3-yl)piperazine of formula II or its salt:

(II)



with 5-(2-haloethyl)-6-chloro-oxindole of formula III:

(III)



wherein X is a leaving group selected from fluoro, chloro, bromo, iodo or sulphonyl leaving group; in the presence of a dispersing agent and a base in a solvent to form ziprasidone of formula I; and optionally converting the ziprasidone formed into a pharmaceutically acceptable acid addition salts of ziprasidone; or a solvate or a hydrate thereof.

2. The process according to claim **1**, wherein the dispersing agent is selected from the group consisting of modified sodium lignosulfonates kraft sodium lignosulfonates; naphthalene-formaldehyde condensates; naphthalenesulfonic acid formaldehyde condensate, or mixtures thereof.

3. The process according to claim **2**, wherein the dispersing agent is naphthalene sulfonic acid formaldehyde.

4. The process according to claim **1**, wherein the base is selected from alkali metal salt, hydroxides, organic tertiary bases or mixtures thereof.

5. The process according to claim **1**, wherein the base is selected from sodium carbonate or bicarbonate or mixtures thereof.

6. The process according to claim **1**, wherein the solvent is selected from the group consisting of substituted, unsubstituted, cyclic, bicyclic, saturated, or unsaturated, straight or branched hydrocarbon but not limited to aliphatic or aromatic hydrocarbon, having C_6-C_{10} atoms, water, alcohols, ketones, esters, ethers and chlorinated solvents, or mixtures thereof.

7. The process according to claim 6, wherein the solvent is selected from the group consisting of toluene; ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate and isobutyl acetate; water; methanol, ethanol and isopropanol; acetone, methyl ethyl ketone, methyl isobutyl ketone and diethyl ketone; tetrahydrofuran and dioxane; and methylene chloride, chloroform, carbontetrachloride and ethylene dichloride; or mixtures thereof.

8. The process according to claim 7, wherein the solvent is selected from the group consisting of alcohols and water or mixtures thereof.

9. The process according to claim 1, wherein the prepared ziprasidone or pharmaceutically acceptable salt or a solvate or a hydrate thereof has a purity of at least 98%.

* * * * *