SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS

Thesis submitted to the
University of Calicut in partial fulfillment of the
requirements for the award of the degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY
under the Faculty of Sciences

By

MUNEER C.P.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALICUT
KERALA
2013

CERTIFICATE

This is to certify that the thesis entitled "**Synthesis and Biological Activity of Some Heterocyclic Compounds** " submitted herewith is a *bonafide* record of the research work carried out by **Muneer C.P.** under my supervision and guidance in partial fulfillment of the requirements for the award of **Doctor of Philosophy in Chemistry** under the Faculty of Sciences, University of Calicut, Kerala. The contents of this thesis have not been submitted to any other Institute or University for the award of any degree or diploma.

University of Calicut,
December, 2013

Dr. P.Mohamed Shafi
(Supervising Teacher)

DECLARATION

I, **Muneer C. P**., hereby declare that this thesis is an authentic record of original work carried out by me under the guidance and supervision of **Dr. P. Mohamed Shafi**, Professor (Retired), Department of Chemistry, University of Calicut. No part of this thesis has previously formed the basis for the award of any degree or diploma as stipulated in the statutes of Calicut University.

University of Calicut,
December, 2013

Muneer C. P.

ACKNOWLEDGEMENTS

I express my heartfelt gratitude to Dr. P. Mohamed Shafi, Professor (Retired), Department of Chemistry, University of Calicut for his sincere guidance, constant encouragement and inspiring discussions for the completion of my work well in time. I can never forget the immense love and care, I enjoyed with him.

I thank Dr.V. M. Abdul Mujeeb, Head of the Department of Chemistry, and Dr. P. Ganga Devi, former Head of the Department, for extending necessary facilities to carry out my research work.

I also thank all the teaching faculties of the Department for their continuous advice and valuable suggestions. I am grateful to all the non-teaching staff of the Department for their wholehearted cooperation and help.

I would like to record my profound thanks to Dr. S. Mohankumar and Dr. MuthuTamizh, SRM University, Kattamkulathur, Mr. Abdul Raheem, NIIST, Thiruvananthapuram, Dr. Manoj, CUSAT, Cochin and Miss Resmi Thomas, IISER, Thiruvananthapuram for providing spectral data.

I am immensely thankful to Dr. Ruby John Anto, RGCD, Thiruvananthapuram, Dr. Meera Mahesh, St. Marys College, Thrissur and Mr. Sarath, Department of Botany, for helping me to carry out biological studies and Dr. Parameswaran, NIT, Calicut for realizing the computational study.

I extend my sincere thanks to the Principal and all the teaching and non-teaching staff of Govt. College, Malappuram, for their cooperation and moral support.

I owe sincere gratitude to UGC for rendering the opportunity to avail teacher fellowship (FIP) for successfully completing my research.

I am glad to acknowledge the help and encouragement provided by Dr. Shalina Begum, Mohanakrishnan, Mitty, Dr. Shinu, Pramitha, Arun, Bini, Soumya,

Jithin, Jency, Nihmath, Sreejith, Aparna, Arun, Saifunnisa, Jasna and all my fellow researchers and friends.

I am thankful to all my family members for their encouragement and support. With deep sense of respect I commemorate all my teachers including my late father, without them I would not have done this task.

I thank Bina Photostat for the neat processing of my thesis.

Last but the most, I thank the Almighty for his endless blessings.

Muneer. C. P.

CONTENTS

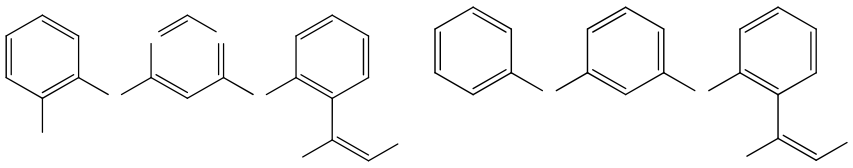
Chapter	Title	Page No.
	General Introduction	**1-5**
Chapter 1		
	Synthesis, Anticancer and Antibacterial Studies of (E)-4-(Amino (aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones	**6-40**
	1.1 Synthesis of (E)-4-(Amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones	**6-28**
	1.1.1 Introduction	6
	1.1.2 Review	8
	1.1.3 Present Work	15
	1.1.4 Result and Discussion	16
	1.1.5 Experimental	25
	1.2 Anticancer Study of (E)-4-(Amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones	**29-34**
	1.3 Antibacterial Study of (E)-4-(Amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones	**34-37**
	References	**38**
Chapter 2		
	Synthesis and Anticancer Study of (Z)-5-(Amino (aryl)methylene)-2-arylthiazol-4(5*H*)-ones	**41-80**
	2.1 Synthesis of (Z)-5-(Amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones	**41-75**
	2.1.1 Introduction	41
	2.1.2 Review	43
	2.1.3 Present Work	47
	2.1.4 Result and Discussion	49
	2.1.5 Theoretical Study	66
	2.1.6 Experimental	68
	2.2 Anticancer Study of (Z)-5- (Amino(aryl) methylene)-2-arylthiazol- 4(5*H*)-ones	**75-77**
	References	**78**
Chapter 3		**81-97**
	Synthesis and Antibacterial Study of(Z)-5-Arylidene-2-aryl-5H-thiazol-4-ones	
	3.1 Synthesis of (Z)-5-Arylidene-2-aryl-5H-thiazol-4-ones	**81-96**
	3.1.1 Introduction	81
	3.1.2 Present Work	82
	3.1.3 Result and Discussion	83
	3.1.4 Experimental	91

	3.2	**Antibacterial study of (Z)-5-Arylidene-2-aryl-5H-thiazol-4-ones**	**96**
	References		**97**
Chapter 4			**98-139**
	Synthesis and Antioxidant Property of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione		
	4.1	**Synthesis of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione**	**98-121**
		4.1.1 Introduction	98
		4.1.2 Review	100
		4.1.3 Present Work	105
		4.1.4 Result and Discussion	106
		4.1.5 Experimental	114
	4.2	**Antioxidant Study of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione**	**121-135**
	References		**136**
Chapter 5			
	Synthesis, Antibacterial and Antifungal Studies of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione Derivatives		**140-176**
	5.1	**Synthesis of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione Derivatives**	**140-170**
		5.1.1 Introduction	140
		5.1.2 Review	142
		5.1.3 Present Work	145
		5.1.4 Result and Discussion	145
		5.1.5 Theoretical Study	162
		5.1.6 Experimental	166
	5.2.1	**Antibacterial Study of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione Derivatives**	**170-172**
	5.2.2	**Antifungal Study of 3-Substituted-1H-1,2,4-triazol-5(4H)-thione Derivatives**	**172-173**
	References		**174**

GENERAL INTRODUCTION

Heterocyclic compounds have constituted one of the largest areas of research in organic chemistry, for more than a century. They have greatly contributed to the development of society from both biological and industrial point of view, to the understanding of life processes and to improve the quality of life. About half of the known organic compounds have structures with atleast one heterocyclic component. Every homocycle can give rise to many heterocycles by substitution of one or more CH links by the same or different heteroatoms. Hence, the chemistry of heterocyclic compounds is potentially of greater diversity and complexity than that of homocyclic compounds.

The widespread use of heterocyclic compounds is due to the fact that their structure can be effectively manipulated and controlled to achieve required modifications in their function. An example of the way in which heterocycles are used is provided by an account of the development of a new systemic fungicide (I)[1].



I **II**

The pyrimidine ring was incorporated into this structure because a related compound (II) proved to be too lipophilic. The water solubility and transport of the fungicide through the plant are improved by replacing a benzene ring by the more polar heterocycle.

It is amazing to know that nature has a preference for heterocyles over homocycles in various biological systems. It can be attributed to the fact that heterocycles are able to get involved in a wide range of reaction types. Depending upon P^H of the medium, they will behave either as acids or bases, forming anions or cations. Some of them interact readily with electrophilic reagents, some with nucleophiles and others with both. Similarly, some are easily oxidized but resist reduction, while others can be readily hydrogenated but are stable towards the action of oxidizing agents. Certain amphoteric heterocyclic systems simultaneously demonstrate all of the above mentioned properties. The ability of many heterocycles to produce stable complexes with metal ions has got biochemical significance. The presence of different heteroatoms makes tautomerism very common in heterocyclic series. Such versatile reactivity is linked to the electronic distributions in heterocyclic molecules. Thus heterocycles are chemically more flexible and capable to respond to many demands of biochemical systems.

A large number of heterocyclic compounds both synthetic and natural are pharmacologically active and are in medical use. Many natural drugs[2-5] such as papaverine, theobromine, quinine, emetine, theophylline, atropine, procaine, codeine,reserpine and morphine are heterocycles. Almost all the compounds identified as synthetic drugs such as diazepam, chlorpromazine, isoniazid, azidothymidine, barbiturates, antipyrine, and captopril are also heterocycles.

Synthetic heterocycles have widespread therapeutic uses such as antibacterial, antifungal, anti-HIV, antitubercular, antimalarial, analgesic, anti-inflammatory, anticonvulsant, anticancer and antidepressant agents. There are a large number of synthetic heterocycles with other important applications like fungicides, herbicides, anticorrosive agents, photostabilizers,

agrochemicals, dyestuffs, photographic developers, sensitizers, antioxidant in rubber and flavouring agents.

Nucleic acid bases pyrimidine and purine derivatives are monocyclic and bicyclic heterocycles with two and four nitrogen atoms respectively. Vitamins in the B group such as thiamine, folic acid, riboflavin, cyanocobalamine are nitrogen containing heterocycles[6] and function either as coenzymes or their precursors. Other vitamins such as ascorbic acid (vitamin C)[7] and α-tocopherol (vitamin E) are oxygen heterocycles[8]. The essential amino acids proline, histidine and tryptophan[9], photosynthesizing pigment chlorophyll, the oxygen transporting pigment haemoglobin[10], the hormones kinetin, heteroauxin, cytokinins[11], neurotransmitter serotonim, histamine are all heterocyclic compounds. They are extensively used as ligands for synthesizing useful metal chelates. They are also used as intermediates in organic synthesis as they can be carried through a number of synthetic steps and then cleaved at the required stage.

Among various heterocycles, sulphur and nitrogen containing compounds have fascinated researchers owing to their interesting biological properties. Due to the above importance of heterocycles, interest has been generated among the organic chemists throughout the world towards the synthesis of newer heterocycles especially those containing nitrogen and sulphur.

In the present investigation, it has been planned to synthesize some novel five membered heterocycles belonging to the classes imidazole, thiazole and 1,2,4-triazole and also to study the biological activities of a selected few.

Shafi and Jyothi[12] synthesized a number of 4-(amino (pyridyl)methylene)-2-pyridyl-2-imidazol-5-one, by the reaction between imidic acid ester of cyanopyridine and glycine ester in the molar ratio

2:1.Quinoline known to be the critical part of the well- known antimalarial drug quinine, similar imdazolinone is prepared using 2-cyanoquinoline and its biological properties are investigated in this work. We synthesized two more of the series starting from 3-nitrobenzonitrile and 4-nitrobenzonitrile. In addition, we improved the yield of 4-(amino(pyrazinyl)methylene)-2-pyrazinyl-2-imidazolin-5-one, which was first reported by Shafi and Shalina[13], from our lab (chapter I). The anticancer and antibacterial activities of a few compounds are also explored.

Since sulphur is expected to modify various biological properties,sulphur analogues of these imidazolones, i.e. (Z)-5-(amino(aryl)methylene)-2-arylthiazol-4(5H)-ones are synthesized by treating thioamide of pyridine-2-carbonitrile/pyrazine-2-carbonitrile, imidic ester of various nitriles and ethylbromoacetate in equimolar proportion. The computational study and anticancer screening of these compounds are also being done (chapter II).

A series of (*Z*)-5-arylidine-2-aryl-5*H*-thiazol-4-ones are prepared and subjected to antibacterial screening (chapter III).

A number of 1,2,4-triazoles are synthesized by a novel route developed in our lab. Based on this work, an article entitled "A new synthetic route to 5-substituted-2*H*-1,2,4-triazole-3(4*H*)-thiones" has been published in *Indian Journal of Heterocyclic Chemistry*, **2013**, 22, 297-300. We investigated the antioxidant properties of a few triazoles as well (chapter IV).

We also synthesized two types of derivatives of 1,2,4-triazoles such as long chain alkyl derivatives and Mannich bases and examined their antibacterial as well as antifungal properties. We conducted the computational calculation of a selected compound (chapter V).

References

1. Clough, J. M.; Godfrey, C. R. A. *Chem. Br*., **1995**, 466
2. Chi, Y.W., Balunas, M. J.; Chai, H. B.; Kinghorn, A. D.. Drug Discovery From Natural Sources. AAPS J., **2006,** 8(2), 239-253.
3. Kohen, F.E.; Carter, G. T.. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov*., **2005,** 4(3), 206-20.
4. Cordell, G.A., Quinn-Beattie, M. L.; Farnsworrth, N. R.. The potential of alkaloids in drug discovery. *Phytother Res*., **2001,** 15(3), 183-205.
5. Hughes, E.H.; Shanks, J. V. Metabolic engineering of plants for alkaloid production. *Metab. Eng*., **2002,** 4(1), 41-48.
6. National Academy of Sciences. Institute of Medicine. Food and Nutrition Board., ed**1998**. Chapter 9 – Vitamin B_{12}. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin $B_{6,}$Folate, Vitamin B_{12}, Pantothenic acid, Biotin and Choline. Washington, D.C.: National Academy Press. Pp: 346.
7. Davies, M.B., Austin, J.; Partridge, D. A. **1991**. Vitamin C: Its Chemistry and Biochemistry. The Royal Society of Chemistry, pp: 48.
8. Evans, H.M., Emerson, O. H.; Emerson, G. A.. The isolation from wheat germ oil of an alcohol, alpha tocopherol, having the properties of vitamin E. *J. Biological Chemistry*, **1936,** 113(1), 319-332.
9. Frust, P.; Stehle, P. What are the essential elements needed for the determination of amino acid requirements in humans ?.*J. Nutrition*, **2004,** 134(6 Suppl): 1558S-1565S.
10. Perutz, M.F. Structure of haemoglobin. Brookhaven symposia in Biol. **1960,** 13: 165-83.
11. Brian, P.W. Review Lecture: Hormones in Healthy and Diseased Plants Proceedings of the Royal Society of London. Series B, *Biological Sci*. **1978,** 200(1140): 231-243.
12. Jyothi, P. Ph. D. Thesis, University of Calicut, Kerala, **2009.**
13. Begum, T. S.; Jaleel, U. C. A.; Shafi, P. M. *Int J Pharm Biomed Sci* **2013**, 4(1), 40-45.

.

CHAPTER-I

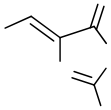
SYNTHESIS, ANTICANCER AND ANTIBACTERIAL STUDIES OF (E)-4-(AMINO(ARYL)METHYLENE)-2-ARYL-1*H*-IMIDAZOL-5(4*H*)-ONES

1.1. SYNTHESIS OF (E)-4-(AMINO(ARYL)METHYLENE)-2-ARYL-1*H*-IMIDAZOL-5(4*H*)-ONES

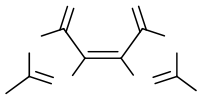
1.1.1. Introduction

Imidazoles are cyclic amidines, isomeric with pyrazoles. They are differently called glyoxaline, iminazole, and 1,3-diazole. The name imidazole is due to Hantzsch[1]. The alternative name glyoxaline derives from the first synthesis of the parent compound of the group from glyoxal and ammonia in 1858 by Debus[2]. It was not until 25 years after its discovery that the structure of the imidazole nucleus was established. The first chemical study of imidazole was carried out by Wyss[3] who substantiated the work of Debus.

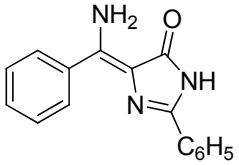
2-Imidazolin-5-ones with an exocyclic double bond in the 4th position are generally called unsaturated-2,4-disubstited-2-imidazolin-5-ones. They are also called unsaturated 2,4-disubstituted-5-ketodihydroglyoxalines.



The reaction between benzimidic acid ester and glycine ester resulted in the formation of some red coloured products[4,5]. Ekeley and Ronzio[6,7] suggested that the red colour is due to the formation of glyoxalin red.



A reinvestigation of this reaction between benzimidic acid ester and glycine ester by Shafi and Shobha[8] led to the isolation and structural elucidation of a new class of imidazolinones called 4-(amino(aryl)methylene)-2-aryl-2-imidazolin-5-one.



Similar series of aminoimidazolinones were prepared from cyanopyridines by Shafi and Jyothi[9] and from cyanopyrazine by Shalina Begum and coworkers[10].

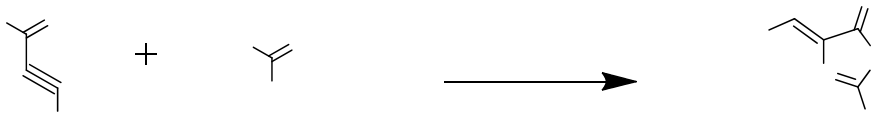
4-Arylidine-2-aryl-1*H*-imidazol-5(4*H*)-ones have been found to possess various biological properties like immunomodulatory[11], anticancer[12,13], anti-inflammatory[14], leishmanicidal[15], antibacterial and antifungal[16-18] activities besides their actions as dual specificity tyrosine-regulated kinase-1A inhibitors[19] and potential COX-2 inhibitors[20]. They are also reported to have significant antioxidant activity[21] and therefore effective for the treatment of oxidative-induced diseases. Hence the synthesis and bioassay of this type of compounds have gained much attention during the last few decades. In this perspective, synthesis and anticancer screening of (E)-4-(amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones are of great relevance.

1.1.2. Review

Since the work discussed in this chapter deals with the synthesis and anticancer studies of unsaturated 2-imidazolin-5-ones, which include aminoimidazolinones, synthetic methods available for the construction of these classes of compounds are being briefly reviewed.

Synthesis of unsaturated 2-imidazolin-5-ones

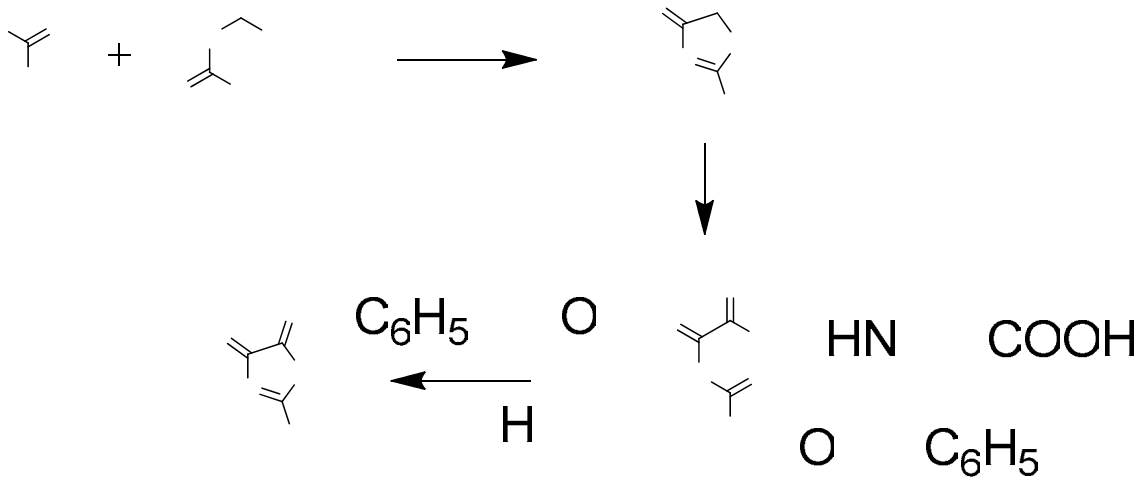
The synthesis of unsaturated 2-imidazolin-5-one was first reported in 1899 by Ruheman and Cunnington[22,23]. They synthesized 2-Phenyl-4-benzylidine-2-imidazolin-5-ones by condensing phenyl propiolic ester with benzamidine hydrochloride in presence of sodium ethoxide.



By now, four general methods are known for the synthesis of unsaturated 2-imidazolin-5-ones namely azlactone, amidine-glyoxal, amidine-haloacetic ester and imidic acid ester-glycine ester methods.

1. Azlactone method

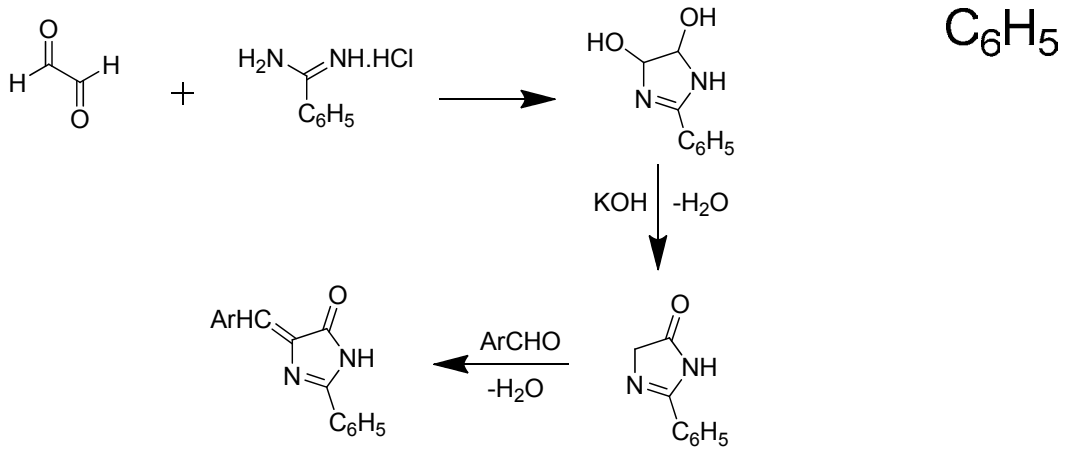
Erlenmeyer[24-32] prepared 2-phenyl-4-arylidine-2-imidazolin-5-ones from azlactone. On heating a mixture of benzaldehyde and hippuric acid in presence of fused sodium acetate and acetic anhydride, the azlactone of α-benzoylaminocinnamic acid is formed. On heating with concentrated ammonia in the presence of alcohol, this azlactone readily affords α-benzoylaminocinnamic acid amide. The amide then cyclises to give 2-phenyl-4-benzylidene-2-imidazolin-5-ones under the influence of hot dilute sodium hydroxide solution.



This method to synthesise 2,4-disubstituted-2-imidazolin-5-ones was further extended by various workers[33-44].

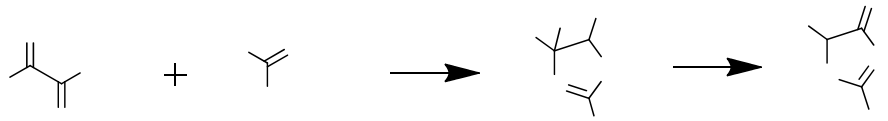
2. Amidine-Glyoxal method

In 1935 Ekeley and Ronzio[6] synthesised 2-aryl-4-arylidine-2-imidazolin-5-one by condensing aromatic aldehydes with aromatic amidine-glyoxal addition products. Thus on treating a mixture of glyoxal and benzamidine hydrochloride with KOH, a labile basic substance is formed, which on condensation with aromatic aldehyde in presence of NaOH or KOH, gave 2-phenyl-4-arylidine-2-imidazolin-5-ones.



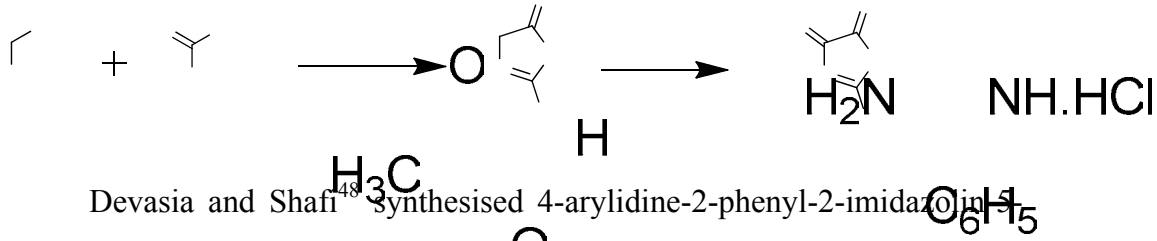
Ekeley and co-workers[6,45] prepared a number of 2-aryl-4-arylidine-2-imidazolin-5-ones by this method using different aromatic amidines like benzamidine, p-toluamidine, m-toluamidine etc.

In 1948 Cornforth[34,46] synthesised saturated 2,4-disubstituted 2-imidazolin-5-ones by the condensation of aromatic amidines and substituted glyoxals.

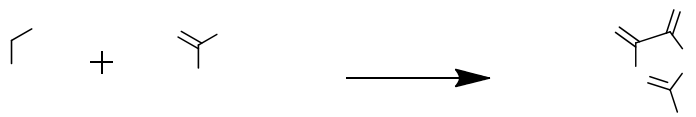


3. Amidine-haloacetic ester method

In 1976 Devasia[47] developed the amidine-chloroacetic ester method for the synthesis of unsaturated 2-imidazolin-5-one. By condensing aromatic aldehydes with a mixture of benzamidine hydrochloride and ethyl chloroacetate in presence of sodium bicarbonate in n-propanol at reflux temperature, he obtained moderately good yields of 4-arylidine-2-phenyl-2-imidazolin-5-ones.



Devasia and Shafi[48] synthesised 4-arylidine-2-phenyl-2-imidazolin-5-ones by condensing aromatic aldehydes with a mixture of chloroacetyl chloride and benzamidine in presence of sodium bicarbonate.

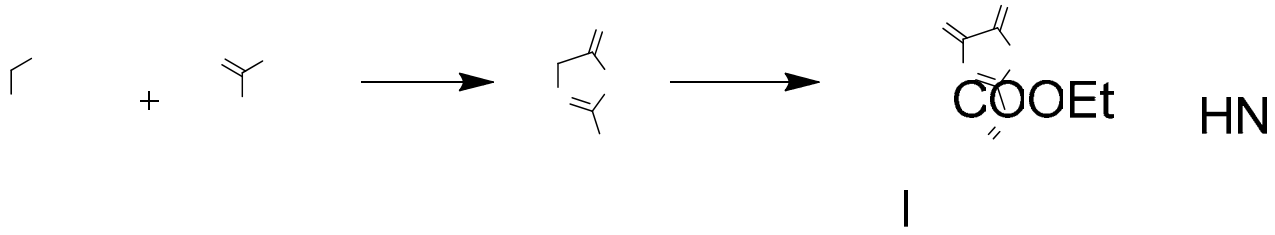


In1981 Devasia and Shafi[49] synthesised a large number of unsaturated-2,4-disubstited-2-imidazolin-5-ones by means of amidine-haloacetic ester method.

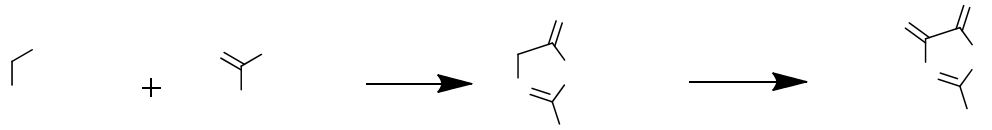
In 1985 Shafi[50] prepared 2-aryl-4-arylidine-2-imidazolin-5-ones in quantitative yield with benzamidine hydrochloride and ethyl iodoacetate in presence of $NaHCO_3$.

4. Imidic acid ester-Glycine ester method

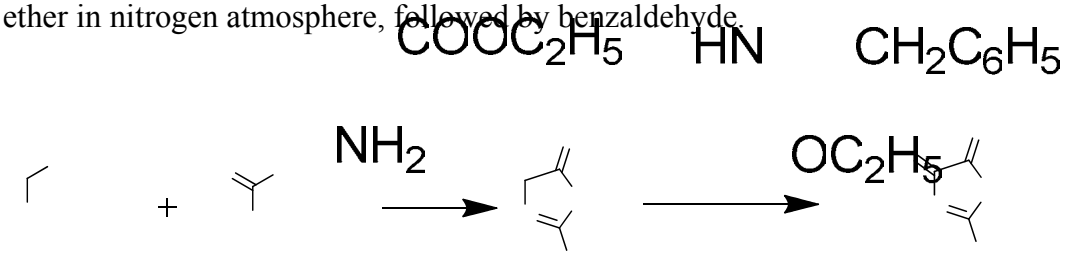
In 1907, Finger[51] synthesised 2-Methyl-2-imidazolin-5-one by condensing glycine ester with acetimidic acid ester at room temperature, which further condensed with two molecules of benzaldehyde to form 2-benzylidinemethyl-4-benzylidine-2-imidazolin-5-one.



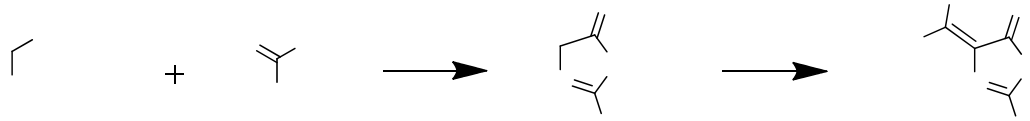
Finger and Zeh[52] synthesised 2-benzyl-2-imidazolin-5-one by treating phenylacetimidic ester with glycine ester and the imidazolinone so obtained was condensed with benzaldehyde in presence of dilute alkali to get 2-benzyl-4-benzylidine-2-imidazolin-5-one.



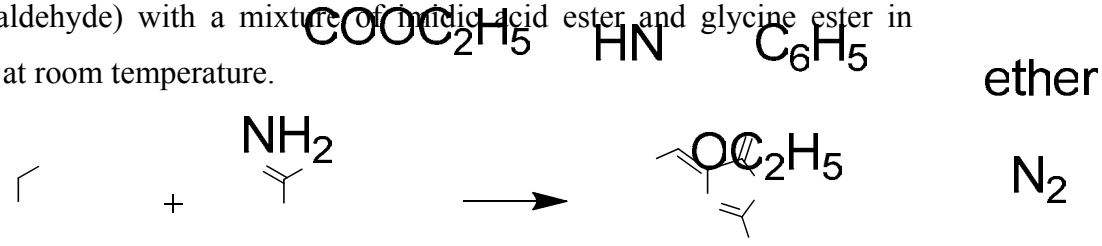
Kjaer[53] obtained 4-benzylidine-2-phenyl-2-imidazolin-5-one by condensing benzimidic acid ester with glycine ester in presence of anhydrous ether in nitrogen atmosphere, followed by benzaldehyde.



In 1953, Lehr and co-workers[54] obtained 2-substituted 4-isopropylidine-2-imidazolin-5-one instead of the expected 2-substituted 2-imidazolin-5-one, when imidic acid ester was condensed with glycine ester using acetone as solvent.



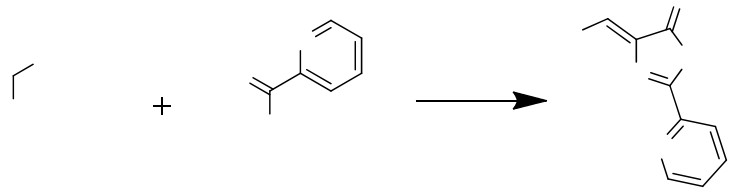
In 1962, Kidwai and Devasia[55] synthesised a number of unsaturated 2-imidazolin-5-ones by condensing aldehyde (aromatic aldehydes and isobutyraldehyde) with a mixture of imidic acid ester and glycine ester in benzene at room temperature.



In 1975, Devasia and Pillai[56] synthesised a few 2-Phenyl-4-arylidine-2-imidazolin-5-ones employing the method of Kidwai and Devasia.

In 1994, Griffiths and co-workers[57] prepared imidazolinones by cyclocondensation of glycine ester hydrohalide with imidic ester in presence of NaOH, which were chlorinated with $POCl_3$or $SOCl_2$ and subsequently treated with DMF and $POCl_3$ to form their formyl derivatives.

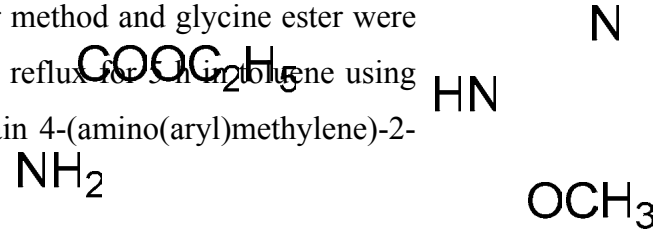
In 2009 Shafi and Jyothi[9] obtained 4-arylidine-2-pyridyl-2-imidazolin-5-ones by refluxing imidic ester of various cyanopyridines with glycine ester and aromatic aldehydes using $NaHCO_3$ as the base, in benzene. They prepared imidate of cyanopyridines using sodium methoxide (a method suggested by Fred and Grace[58]).



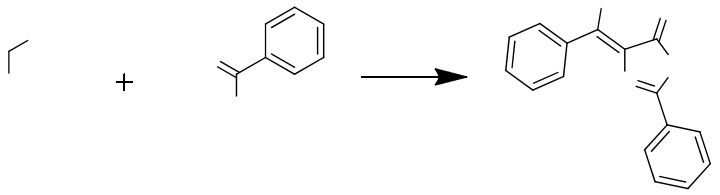
Shalina Begum and co-workers[10] prepared similar a series of compounds from pyrazine carbonitrile and hydroxybenzonitrile using their imidates , glycine ester and different aromatic aldehydes using toluene as solvent.

Synthesis of 4-(Amino(aryl)methylene)-2-aryl-2-imidazolin-5-ones

In 1998 Shafi and Shobha[8], while reinvestigating the reaction between benzimidic acid ester and glycine ester yielding red coloured products, isolated a new class of unsaturated 2,4-disubstituted-2-imidazolin-5-ones formed as a result of a tandem reaction. In their method imidic acid esters of benzonitrile and toluonitrile formed by Pinner method and glycine ester were taken in the molar ratio 2:1 and heated under reflux for 5 h in toluene using anhydrous sodium acetate as the base to obtain 4-(amino(aryl)methylene)-2-

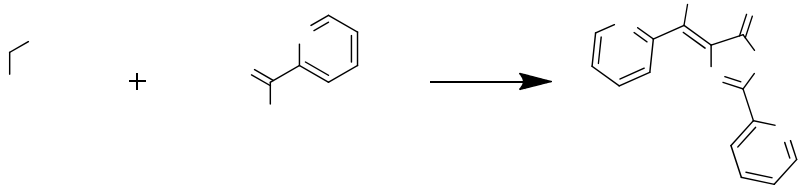


aryl-2-imidazolin-5-ones. They also prepared acetylated products of these compounds.



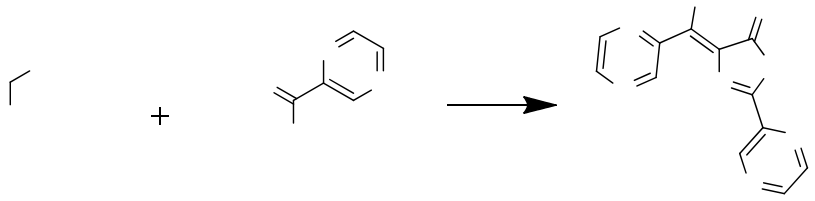
In 2001 Shafi and Basheer[59] investigated the antibacterial activity of 4-(amino(aryl)methylene)-2-aryl-2-imidazolin-5-ones and their acetylated and benzoylated derivatives.

In 2007 Shafi and Jyothi[9] prepared 4-(amino(pyridyl)methylene)-2-pyridyl-2-imidazolin-5-ones by the tandem reaction between imidic acid ester of both 2-cyanopyridine and 4-cyanopyridine and glycine ester in the molar ratio 2:1 in toluene under reflux for 2 h.

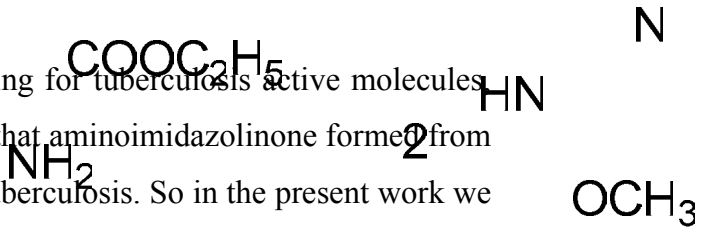


Shafi and Shalina[60] conducted the anticancer studies of 4-(amino,pyridylmethylene)-2-pyridyl-2-imidazolin-5-ones and their hydrochlorides.

In 2012, Shalina Begum and co-workers[10] synthesized 4-(amino(2-pyrazinyl)methylene)-2-(2-pyrazinyl)-2-imidazolin-5-one by refluxing imidic acid ester of pyrazine-2-carbonitrile and glycine ester in benzene for one hour, but in poor yield.



1.1.3 Present Work



During the computational screening for tuberculosis active molecules, Shalina Begum and co-workers[10] found that aminoimidazolinone formed from pyrazine carbonitrile is active towards tuberculosis. So in the present work we thought of improving the method for the synthesis of (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one to prepare it in better yield for its biological evaluation.

Secondly, quinoline known to be the critical part of the well-known antimalarial drug quinine, it was proposed to synthesise (E)-4-(amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one from quinoline-2-carbonitrile and examine its biological activity.

Further, since there are quite a few drugs which contain nitro group such as azathioprine(a potent immune-suppressant), chloramphenicol(a wide-spectrum antibiotic), clonazepam(an antianxiety agent)[61] etc. we tried to incorporate nitro group in the aminoimidazolinone system, by taking imidic acid ester of nitrobenzonitriles to reflux with glycine ester in suitable solvent, anticipating enhanced biological properties.

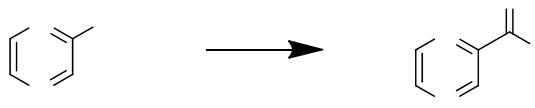
1.1.4. Results and Discussion

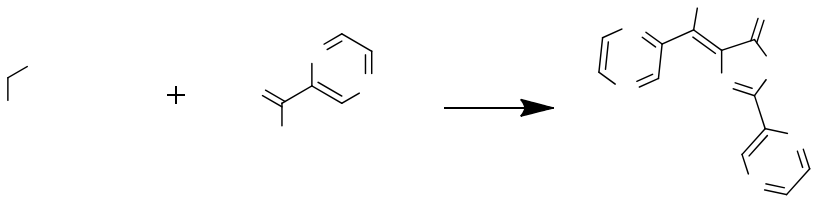
Improved Synthesis of(E)-4-(Amino(Pyrazin-2-yl)Methylene)-2-(Pyrazin-2-yl)-1*H*-Imidazol-5(4*H*)-one

Synthesis of (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one has been recently reported from our lab[10]. The method involved conversion of pyrazine-2-carbonitrile into its imidic acid ester using sodium methoxide in methanol and subsequenty refluxing the imidate formed with glycine ester hydrochloride in 2:1 molar ratio in benzene using sodium bicarbonate as the base. But the yield was very poor (37 percent).

In our attempt to improve the yield, a number of experiments were carried out by changing the proportion of metallic sodium in preparing sodium methoxide, taking different solvents and under different conditions such as nitrogen atmosphere etc. It was found that only catalytic amount of sodium is sufficient to prepare the imidate in methanol and that the best solvent for the reaction is dry THF. Thus we improved the method as follows.

Pyrazine-2-carbonitrile was converted into its imidic acid ester by dissolving in methanol containing only a catalytic amount of metallic sodium. The imidate formed was refluxed with glycine ester hydrochloride in dry THF using sodium bicarbonate as the base. The product was filtered, washed with water and then with ethanol and dried. The compound was recrystallized from distilled pyridine, to get orange red crysals which melted at 338 ^{o}C with decomposition. The reaction can be depicted as follows **(Scheme 1).**





Scheme 1

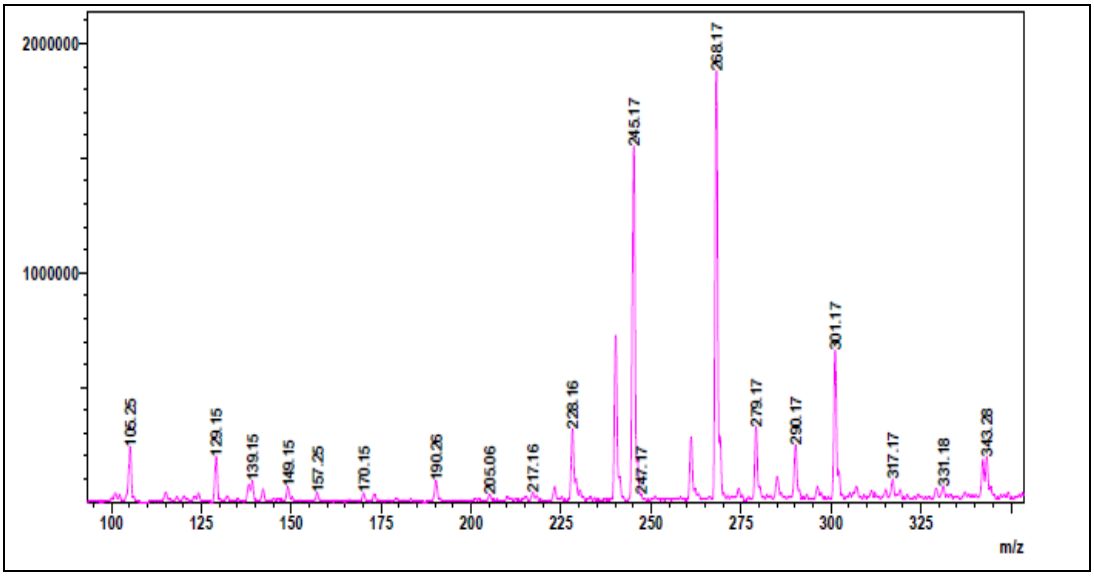
The mass spectrum **(Spectrum 1)** recorded using LCMS(ESI) method, of (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one had a peak at m/z = 268, which is the quasi molecular ion peak $[M+1]^+$, justifying the molecular mass of the compound to be 267. The odd mass supported the fact that the compound contains odd number of nitrogen atoms.

The ^{1}H NMR spectrum **(Spectrum 2,3)** showed absorption of NH proton of imidazolinone ring at δ 12.13. The six aromatic protons absorbed in the region from δ 8.6 to 10.2. The two protons of the amino group absorbed at different δ values due to different chemical environments brought about by conjugation as well as intramolecular hydrogen bonding with CO group of the imidazolinone ring. One proton absorbed at δ 9.2, which is the hydrogen bonded proton and the other at δ 8.5.

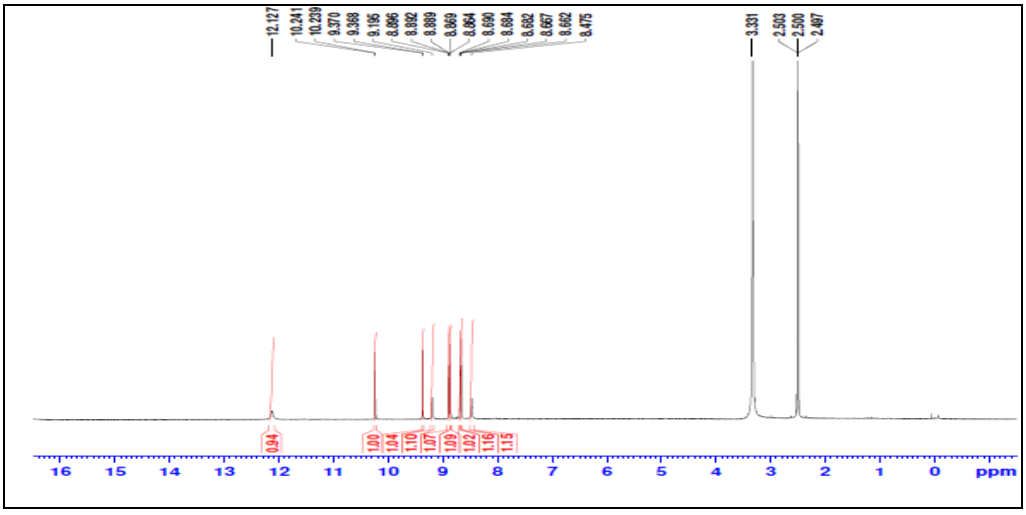
The ^{13}C NMR spectrum **(Spectrum 4)** exhibited 11 peaks in the region δ 118.1 to 170.3. The peak at δ 170.3 accounts for the carbonyl carbon of the imidazolinone ring. All other carbons are found to absorb between δ 118.1 and 149.2. It is to be noted that one carbon is not observed in the spectrum, which may be one of the quarternary carbon possessing weakest NOE effect.

The IR spectrum is also in agreement with the above observations. Three medium intensity peaks are found at 3204 cm^{-1}, 3275 cm^{-1} and 3445 cm^{-1}, which are due to symmetric and asymmetric vibrations of NH_2 and the vibration corresponding to NH of the imidazolinone ring. As expected, the carbonyl absorption is at a lower frequency of 1684 cm^{-1} due to

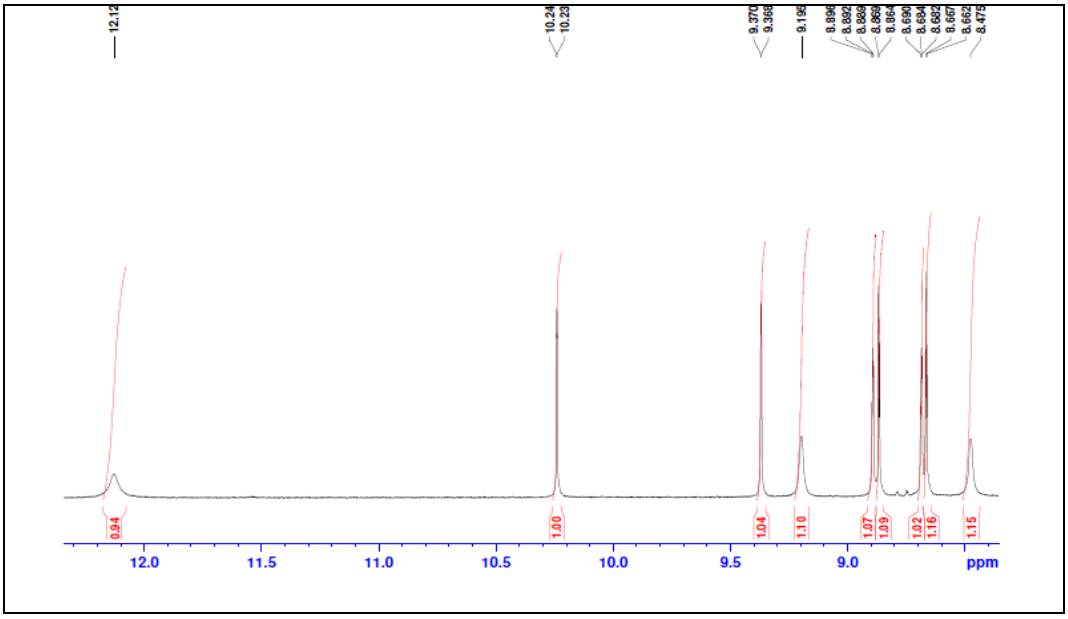
intramolecular hydrogen bonding. The UV absorption (λ_{max}) was recorded at 437nm.



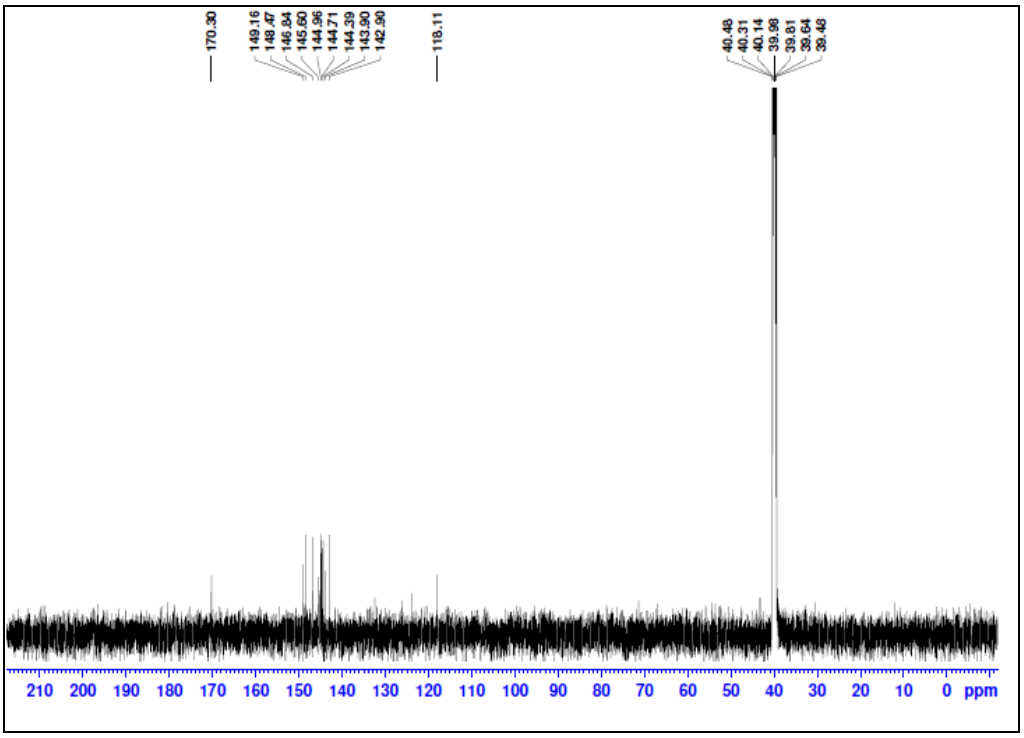
Spectrum 1: Mass Spectrum of (E)-4-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one



Spectrum 2: ^{1}H NMR Spectrum of (E)-4-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one



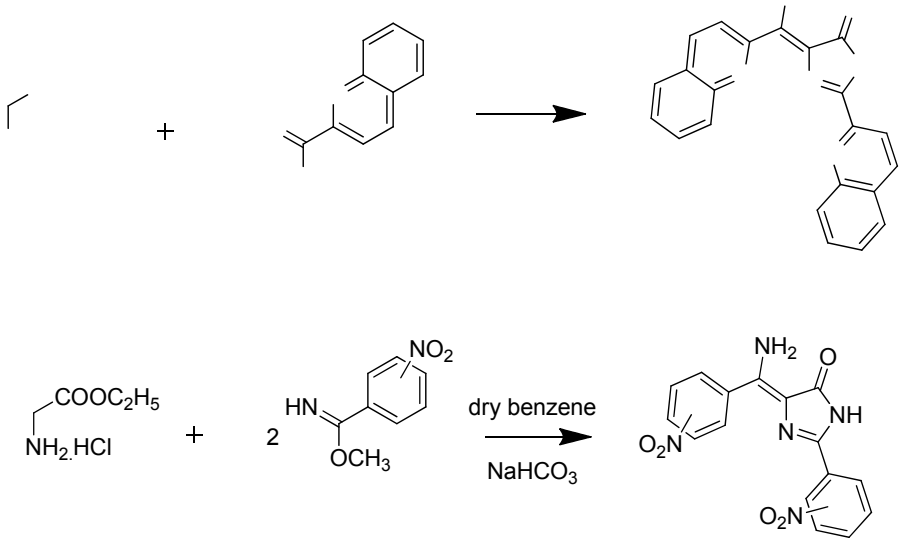
Spectrum 3: ^{1}H NMR Spectrum of (E)-4-(Amino(pyrazin-2-l)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one



Spectrum 4: ^{13}C NMR Spectrum of (E)-4-(Amino(pyrazin-2-l)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one

Synthesis of Some New (E)-4-(Amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones

Aminoimidazolinones were prepared from quinolone-2-carbonitrile, 3-nitrobenzonitrile and 4-nitrobenzonitrile by similar method. All the three nitriles were converted into corresponding imidic acid esters using sodium methoxide in methanol, which was then refluxed with glycine ester in 2:1 molar ratio in dry benzene using sodium bicarbonate as the base. The compounds were filtered, washed with water and then with alcohol and dried. The aminoimidazolinones were obtained in 60-70% yield. All the three compounds are reported for the first time **(Table I)**. The reactions are shown below **(Scheme 2)**.



(Scheme 2)

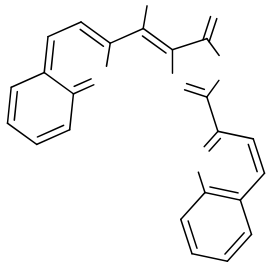
COOC2H5 N

Table 1: Characterization data of (E)-4-(Amino(aryl)methylene)-2-aryl-1*H*-imidazol-5(4*H*)-ones

Sl. No.	Name	Molecular Formula	Yield (%)	M.P. (^{0}C)	υ_{co} ($_{cm}^{-1}$)	Λ_{max} (nm)	Elemental analysis found (calcd.)		
							C	H	N
1	(E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one	$C_{22}H_{15}N_5O$	70	263	1661	433	72.29 (72.32)	4.10 (4.14)	19.22 (19.17)
2	(E)-4-(Amino(3-nitrophenyl)methylene)-2-(3-nitrophenyl)-1*H*-imidazol-5(4*H*)-one	$C_{16}H_{11}N_5O_5$	62	320	1681	400	54.35 (54.39)	3.18 (3.14)	19.80 (19.82)
3	(E)-4-(Amino(4-nitrophenyl)methylene)-2-(4-nitrophenyl)-1*H*-imidazol-5(4*H*)-one	$C_{16}H_{11}N_5O_5$	65	332	1664	460	54.41 (54.39)	3.12 (3.14)	1 (19.82)

(E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one

The mass spectrum **(Spectrum 5)** of (E)-4-(amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one showed a peak at 365.92, which is likely the quasimolecular ion peak $[M+1]^+$, indicating the molecular mass to be 365.The molecular mass is in agreement with the proposed structure. The odd mass justified the odd number of nitrogen atoms in the molecule.

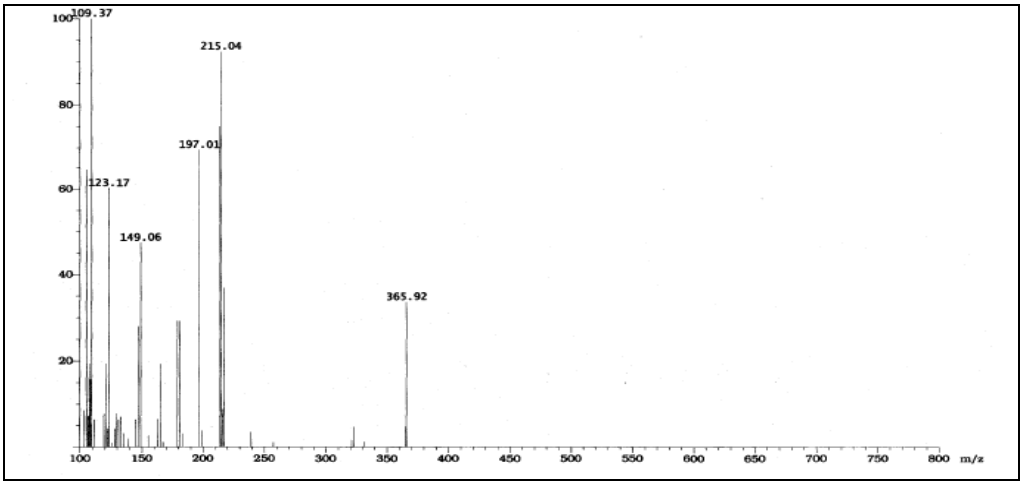


In the ^{1}H NMR spectrum, **(Spectrum 6,7)** NH proton of the imidazolinone ring absorbed at δ 12.05. The twelve aromatic protons showed absorption in the region δ 7.76 to 9.42. The two H atoms of the amino group being chemically non-equivalent absorbed at different δ values (8.5 and 9.35) and are mutually coupled. The higher value corresponds to the proton which is intramolecularly H-bonded to CO of the imidazolinone ring.

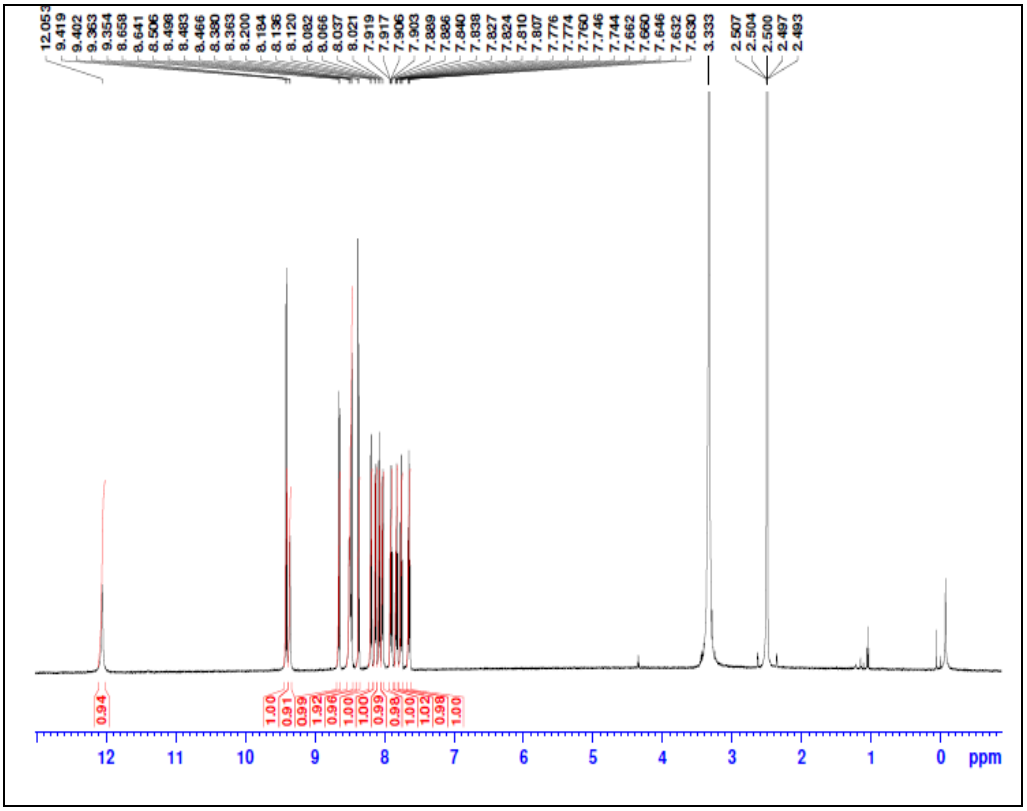
The ^{13}C NMR spectrum **(Spectrum 8)** exhibited 22 peaks in the region δ 118.3 to 170.3 as expected. The carbonyl carbon of the imidazolinone ring showed a characteristic peak at δ 170.3. All other carbons are found to absorb between δ 118.3 and 149.9.

IR spectrum consists of a broad peak in the region 3600 cm^{-1} to 3100 cm^1 with the maximum at 3418.2 cm^{-1}. The carbonyl absorption of imidazolinone ring is at 1664cm^{-1}.

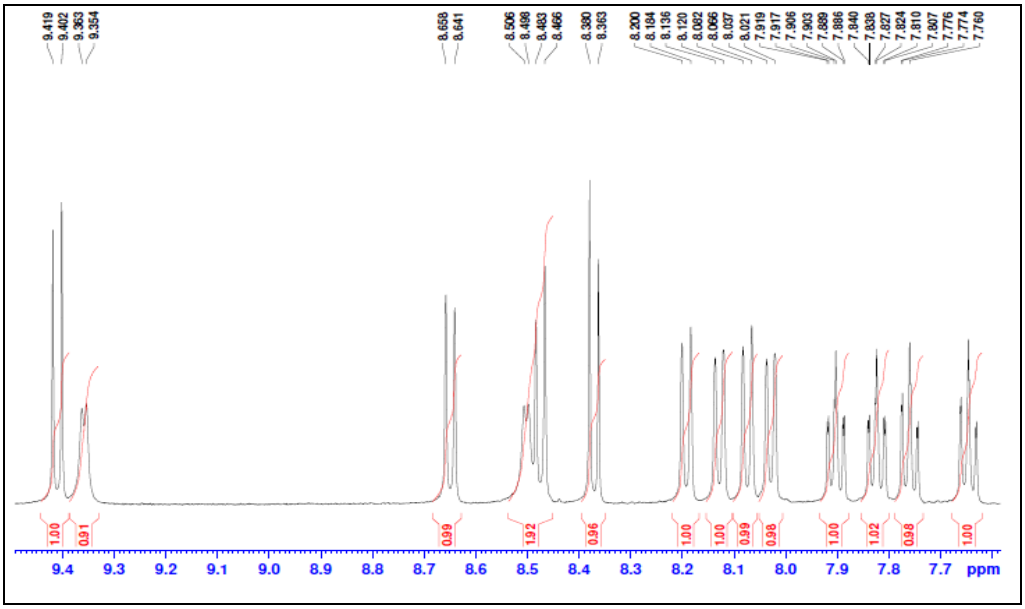
N



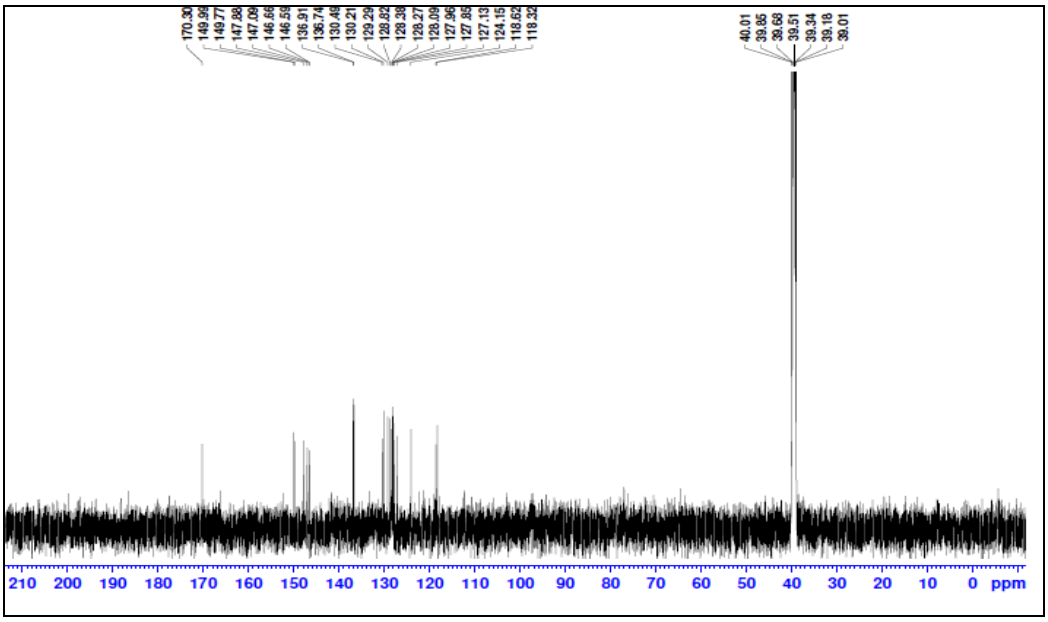
Spectrum 5: Mass spectrum of (E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one



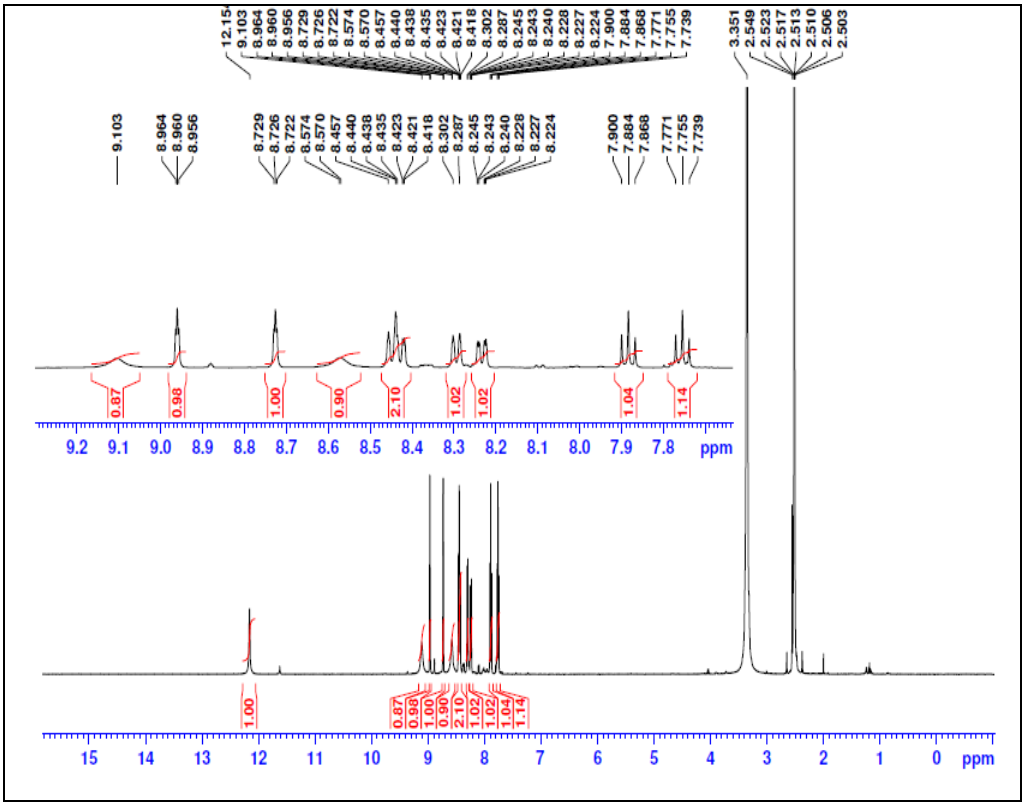
Spectrum 6: ^{1}H NMR spectrum of (E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one



Spectrum 7: ^{1}H NMR spectrum of (E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one



Spectrum 8: ^{13}C NMR spectrum of (E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one



Spectrum 9: ^{1}H NMR spectrum of (E)-4-(Amino(3-nitrophenyl) methylene)-2-(3-nitrophenyl)-1*H*-imidazol-5(4*H*)-one

1.1.5. Experimental

Melting points were recorded on a Toshniwal capillary melting point apparatus and are uncorrected. The mass spectra were recorded on LCMS-2020 Shimadzu equipment as well as JEOL JMS 600H Mass Spectrometer under FAB-MS mode. The NMR spectra were registered on Bruker Avance III 500 MHz FT-NMR instrument using TMS as internal standard. IR spectra (in KBr pellets) were recorded using Shimadzu 8101A FTIR equipment. The UV spectrometer used was JASCO V-550 UV/VIS Spectrophotometer.

Preparation of starting materials

Glycine ethyl ester hydrochloride

Glycine ethyl ester hydrochloride was prepared according to the method developed by Curtius and Geoble[62] and improved by others[63-65].

In a one litre round bottomed flask with ground joint a mixture of glycine (37.5 g, 0.5 mol) and absolute ethanol (375 mL) were placed and the flask was fitted with a rubber cork carrying an inlet tube and calcium chloride guard tube. HCl gas dried by bubbling through concentrated sulphuric acid was passed into the mixture till 50 g (1.4 mol) of the gas was absorbed. The flask was fitted with a reflux condenser carrying a calcium chloride guard tube and the mixture was refluxed for two hours. The solution was transferred into a 500 mL conical flask and allowed to cool. The solution was seeded to induce crystallization when a lot of glycine ester hydrochloride crystals were separated. The flask was tightly stoppered and placed in the refrigerator overnight to effect complete crystallization. The crystals were quickly filtered on a large Buchner funnel, washed with two 25 mL portions of ice cold absolute ethanol and dried at 80 ^{o}C for 1 hour. The colourless glycine ethyl ester hydrochloride weighed 67 g (96%) and melted at 144-145 ^{0}C.

(E)-4-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrazine-2-carbonitrile (0.9 mL, 0.01 mol) was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Glycine ester hydrochloride (0.7 g, 0.005 mol) and anhydrous sodium bicarbonate (0.9 g) were ground together and added to the imidate in the RB flask. To this mixture 10 mL of dry THF was added and refluxed for 1 hour and kept overnight. (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-

imidazol-5(4*H*)-one was filtered, washed with water, and then with ethanol and dried. The reddish brown product weighed 0.93 g (70%). The compound was recrystallized from dry pyridine which melted at 338 ^{0}C with decomposition.

Table 2: Elemenatal analysis of (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one

Molecular Formula	Analysis	C%	H%	N%
$C_{12}H_9N_7O$	Calculated	53.93	3.39	36.69
	Found	53.91	3.33	36.55

(E)-4-(Amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Quinoline-2-carbonitrile (1.54 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Glycine ester hydrochloride (0.7 g, 0.005 mol) and anhydrous sodium bicarbonate (0.9 g) were ground together and added to the imidate in the RB flask. To this mixture 10 mL of dry benzene was added and refluxed for 1 hour and kept overnight. (E)-4-(amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one was filtered, washed with water, and then with ethanol and dried. The orange red product weighed 1.27 g (70%). The compound was recrystallized from isobutanol to get dark red shining crystals, which melted at 263 ^{0}C.

(E)-4-(Amino(3-nitrophenyl)methylene)-2-(3-nitrophenyl)-1*H*-imidazol-5(4*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard

tube. 3-Nitrobenzonitrile (1.48 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Glycine ester hydrochloride (0.7 g, 0.005 mol) and anhydrous sodium bicarbonate (0.9 g) were ground together and added to the imidate in the RB flask. To this mixture 10 mL of dry benzene was added and refluxed for 1.5 hour and kept overnight. (E)-4-(amino(3-nitrophenyl)methylene)-2-(3-nitrophenyl)-1*H*-imidazol-5(4*H*)-one was filtered, washed with water, and then with ethanol and dried. The dark brown product weighed 1.09 g (62%). The compound was recrystallized from DMSO-ethyl acetate mixture to get orange red crystals, which melted at 320 ^{0}C.

(E)-4-(Amino(4-nitrophenyl)methylene)-2-(4-nitrophenyl)-1*H*-imidazol-5(4*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. 4-Nitrobenzonitrile (1.48 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Glycine ester hydrochloride (0.7 g, 0.005 mol) and anhydrous sodium bicarbonate (0.9 g) were ground together and added to the imidate in the RB flask. To this mixture 10 mL of dry benzene was added and refluxed for 1.5 hour and kept overnight. (E)-4-(amino(4-nitrophenyl)methylene)-2-(4-nitrophenyl)-1*H*-imidazol-5(4*H*)-one was filtered, washed with water, and then with ethanol and dried. The dark brown product weighed 1.15 g (65%). The compound was recrystallized from DMSO-ethyl acetate mixture to get red crystals, which melted at 332 ^{0}C.

1.2. ANTICANCER STUDY OF (E)-4-(AMINO(ARYL)METHYLENE)-2-ARYL-1*H*-IMIDAZOL-5(4*H*)-ONES

Introduction

Cancer[66] is a collective term used for a group of diseases that are characterized by the loss of control of the growth, division and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. Metastasis[67] is a process by which the primary tumor colonizes new tumor at sites distant from its point of origin. It now appears that certain malignant cells of primary tumor (metastatic cells) penetrate the extracellular barrier around the tumor and travel through tissues to either the nearest blood vessel or lymph channel. Then by secreting basement membrane degrading enzymes, these cells enter the lymphatic system or blood stream.

There are over 100 different types of cancer and each is classified by the type of cell that is initially affected. In 2007 cancer claimed the lives of about 7.6 million people in the world.

It is recently reported[68] that spreading of cancer has something to do with the adhesion properties of cancer cells. Certain molecular interactions between cells and the scaffolding that holds them in place (extracellular matrix) cause them to become unstuck at the original tumor site, they become dislodged, move on and then reattach themselves at a new site. This discovery is important because cancer mortality is mainly due to metastatic tumors and finding a way to stop cancer cells from sticking to new sites could interfere with metastatic disease, and halt the growth of secondary tumors.

Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not

experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grow out of control.

Cells can experience uncontrolled growth if there are damages or mutations to DNA, and so, damages to the genes involved in cell division. Four key types of genes are responsible for the cell division process They are oncogenes, which tell cells when to divide, tumor suppressor genes, which tell cells when not to divide, suicide genes that control apoptosis and tell the cell to kill itself if something goes wrong, and DNA-repair genes that instruct a cell to repair damaged DNA. Cancer occurs when a cell's gene mutations make the cell unable to correct DNA damage and unable to commit suicide. Similarly, cancer is a result of mutations that inhibit oncogene and tumor suppressor gene function, leading to uncontrollable cell growth.

Cancer can be the result of a genetic predisposition that is inherited from family members. It is possible to be born with certain genetic mutations or a fault in a gene that makes one statistically more likely to develop cancer later in life.

Carcinogens are a class of substances that are directly responsible for damaging DNA, promoting or aiding cancer. Tobacco, asbestos, arsenic, radiation such as gamma and X-rays, the sun and compounds in car exhaust fumes are all examples of carcinogens. When our bodies are exposed to carcinogens, free radicals are formed that try to steal electrons from other molecules in the body. These free radicals damage cell and affect their ability to function normally.

Cancer treatment depends on the type of cancer, the stage of the cancer, age, health status and additional personal characteristics. There is no single treatment for cancer, and patients often receive a combination of therapies and palliative care. Treatments include surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or gene therapy.

Chemotherapy utilizes chemicals that interfere with the cell division process- damaging proteins or DNA- so that cancer cells will commit suicide. These treatments targets any rapidly dividing cells (not necessarily just cancer cells), but normal cells usually can recover from any chemical-induced damage while cancer cells cannot. Chemotherapy is generally used to treat cancer that has spread or metastasized because the medicines travel throughout the entire body. It is a necessary treatment for some forms of leukemia and lymphoma. Chemotherapy treatment occurs in cycles so the body has time to heal between doses. However, there are still common side effects such as hair loss, nausea, fatigue and vomiting.

Chemotherapeutic drugs currently in clinical use are to kill malignant tumor cells by inhibiting some of the mechanism implied in cellular division. Accordingly the antitumor compounds developed through this approach are cytostatic or cytotoxic. However, the knowledge of tumor biology has exploded during the past decades and this may pave the way for more active, targeted anticancer drugs.

Cancer chemotherapy is infact a very difficult task. One of its main problem is the nonspecific toxicity of most anticancer drugs due to their bio distribution throughout the body, which requires the administration of a large total dose to achieve high local concentration in a tumor cell. Drug targeting aims at preferred drug accumulation in the target cells independently on the method and route of drug administration. One approach that allows to improve the selectivity of cytotoxic compounds is the use of prodrugs that are selectively activated in tumor tissues taking advantage of some unique aspects of tumor physiology, such as selective enzyme expression, hypoxia and low extra-cellular p^H.

Another problem in cancer chemotherapy is drug resistance. After the development of a resistance mechanism in response to a single drug, cells can display coss-resistance to other structurally and mechanistically unrelated

drugs,a phenomenon called multi drug resistance (MDR) in which ATP – dependant transporters have a significant role.

Present Study

The compounds (E)-4-(amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one (A) and (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one (B) were analyzed for their cytotoxicity studies against five human cancer cell lines of various origins.

Experimental

The five cancer cell lines used for the study were cervical cancer cell line HeLa, lung cancer cell line A549, melanoma cell line A375, breast cancer cell line MD-AMB-231 and brain cell line T98G. All these cell lines were obtained from NCCS, Pune and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, along with 100 units/ml penicillin, 50 µg/ml streptomycin and 1 µg/ml of amphotericin-B and were maintained at 37 ^{0}C in a humidified atmosphere of 5% CO_2 and 95% air.

For the cytotoxicity experiments, cells ($3x10^3$ / well) were seeded in o.2 ml of the medium (DMEM with 10% FBS) in 96-well plates. After overnight incubation, various concentrations of the compounds (10-100 µM) were added to the cells and after 72 h, the percentage of viable cells in the wells was determined by MTT assay[69].

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is based on the ability of mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT to form dark blue formazan crystals, which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is directly proportional to the level of the formazan product formed.

Briefly, 72 h after the drug treatment, the drug containing media was removed and fresh media containing 25 µl of MTT solution (5 mg/ml in PBS)

and 75 µl of complete medium were added to wells and incubated for 2 h. At the end of incubation, MTT lysis buffer (20% sodium dodecyl sulphate in 50% dimethyl formamide) was added to the wells (100 µl/well) and incubated for another 1 h at 37 ^{0}C. At the end of incubation, the optical densities at 570 nm were measured using a plate reader (Bio-Rad). The relative cell viability in percentage was calculated as (A_{570} of treated samples/ A_{570} of untreated samples) X 100.

Result and Dscussion

The results of the cytotoxic activity in vitro is expressed as IC_{50} – the concentration of the compound (µM) that inhibits proliferation of the cells by 50% as compared to the untreated control cells. Between the two compounds, A showed more cytotoxic activity than B, particularly against cervical cancer cell line HeLa (IC_{50} = 54.1). All other cell lines showed only weak cell growth inhibition activity against A **(Table – 3).** The antiproliferative activity of A can be attributed to the presence of heterocycles such as imidazole and quinoline. Moreover, due to planar nature and presence of many heteroatoms, quinoline and imidazole-based anticancer compounds are known to exhibit DNA intercalation[70,71], by inserting between the base pairs of the double helix and causing a significant change of DNA conformation.

Table-3: Cytotoxic activity of compound A

Concentration (µM)	**Percentage viability over untreated control**				
	HeLa	**A549**	**A375**	**MD-AMB-231**	**T98G**
25	75.9	96.4	92.4	87.0	92.3
50	53.8	96.2	86.2	86.4	86.4
100	29.5	88.0	77.2	70.0	56.3
200	24.9	49.0	38.7	63.2	42.7
IC_{50}	**54.1**	**196.0**	**163.1**	**271.7**	**114.4**

The percentage viability or the cell lines showed that the cytotoxicity of the compound B is less. There was a slight increase in the cell growth inhibition activity in MD-AMB-231, A375 and T98G, with the concentration **(Table – 4)**. However, the cell lines HeLa and A549 were found to be more resistant on increasing the concentration.

Table 4: Cytotoxic activity of compound B

Concentration (µM)	Percentage viability over untreated control				
	HeLa	A549	A375	MD-AMB-231	T98G
25	72.2	72.2	107.2	101.5	91.8
50	74.6	74.6	89.9	93.2	76.3
100	79.1	79.1	88.3	91.4	73.4
200	72.3	72.3	75.3	89.8	71.6

1.3. ANTIBACTERIAL STUDY OF (E)-4-(AMINO(ARYL) METHYLENE) -2-ARYL-1*H*-IMIDAZOL-5(4*H*)-ONES

Introduction

The search for effective remedies against pathogenic microbes is as old as mankind itself. Mankind's earliest therapeutic success against infecting organisms came in the form of natural products like honey that could be applied topically to infected lesions, or infusions that expelled worms visible to naked eye. Herbal anthelminthic known since antiquity include extract of male fern (Dryopteris filix-mas), which is an effective vermifuge for tapeworms and two compounds that expel intestinal roundworms: santonin (obtained from the seed-heads of Artemisia cina, wormseed) and oil of chenopodium (chenopodium ambrosioides; American wormseed)[72].

Observations that substances controlled the spectacular symptoms of certain diseases indeed led to the initial recognition of two other ancient

remedies : quinine, obtained from the bark of the cinchona tree, and emetine, an alkaloid obtained from ipecacuanha root; both are active against protozoa (the parasite of malaria and amoebic dysentery respectively), and both have survived into present-day use.

The first antimicrobial agent in the world was salvarsan[73], a remedy for syphilis that was synthesized by Ehrlich in 1910. In 1935, sulfonamides were developed by Domagk and others. In 1928, Fleming discovered pencillin. He found that the growth of staphylococcus aureus was inhibited in a zone surrounding a contaminated blue mold (a fungus from the penicillium genus) in culture dishes, leading to the finding that a microorganism would produce substances that could inhibit the growth of other microorganisms. The antibiotic was named pencillin and it came into clinical use in the 1940s. It led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldiers during World War II.

During the subsequent two decades, new classes of antimicrobial agents were developed one after another, leading to a golden age of antimicrobial chemotherapy. In 1944, streptomycin, an aminoglycoside antibiotic, was obtained from the soil bacterium Streptomyces griscis. Thereafter, chloramphenicol, tetracyclin, macrolide and glycopeptide (eg. vanomycin) were discovered from soil bacteria. The synthesized antimicrobial agent nalidixic acid, a quinolone antimicrobial drug was obtained in 1962.

Antibacterial chemotherapy has been quite helpful to modern humanity. The practice of administering chemical substances to treat and cure infectious diseases and disorders has been successful on a large scale. More human lives have been saved by this discipline than any other area of the pharmaceutical sciences[74].

Within the past half century, a wide variety of antibacterial substances have been discovered, designed and synthesized. During the past decades, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world[75]. There fore, new classes of antibacterial agents with novel mechanism are crucial need to combat with the multi-drug-resistant infections.

Present Study

Three compounds namely (E)-4-(amino(quinolin-2-yl)methylene)-2-(quinolin-2-yl)-1*H*-imidazol-5(4*H*)-one (A_1) (E)-4-(amino(3-nitrophenyl) ethylene)-2-(3-nitrophenyl)-1*H*-imidazol-5(4*H*)-one (A_2) and (E)-4-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)-1*H*-imidazol-5(4*H*)-one (A_3) were tested for their *in vitro* antibacterial activity against four bacterial strains. The test organisms included two Gram positive bacteria; *Staphylococcus* (MTCCNO 3103)*, Streptococcus* (MTCCNO 1938) and two Gram negative bacteria; *Escherichia coli* (MTCCNO 68) *and Pseudomonas* (MTCCNO 2642) *sp.*

Experimental

The antibacterial test was carried out by Kirby-Bauer disc diffusion method[76], with some modifications. The pure cultures purchased from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, were maintained in nutrient broth. Each culture was uniformly distributed on nutrient agar plates using sterile swabs. Sterile filter paper discs of 3 mm diameter were placed on the surface of agar plates at a distance of 2 cm using sterile forceps. 2% DMSO was used to dissolve the drug, which was found to have no adverse effect on the bacterial cultures. Drugs of different concentrations (100, 250, 500 μgmL-1) were

added on each disc with a micropipette. Disc with DMSO but without drug was used as control. Then the plates were incubated at 370C for 24-48 hrs. After incubation zone diameter was measured.

Result and Discussion

The antibacterial effects were tested against Gram positive and Gram negative bacteria by disc diffusion method. After incubation the diameter of zone of inhibition was measured. The results of disc diffusion method are shown in Table-1. The compound A_1 showed activity only against *Staphylococcus sp.* at the maximum tested dose of 500 µg/mL. Drug A_3 also showed inhibition against *E. coli and Pseudomonas sp.* (Gram negative bacteria) only at the maximum tested dose of 500 µg/mL (**Table-5**).

Table – 5: Antibacterial Activity of Compound A_1 and A_3

Compounds and conc. in (µg/ml)	Zone diameter (mm)			
	E.coli	***Pseudomonas***	***Streptococcus***	***Staphylococcus***
DMSO	NA*	NA*	NA*	NA*
A1 (500)	NA*	NA*	NA*	9
A3(500)	10	11	NA*	NA*

NA*= No activity, n = 2

References

1. Hantzsch, A. *Ann.* **1888**, 249, 2.
2. Debus, H. *Ann.* **1858**, 107, 199.
3. Wyss, G. *Ber.* **1877**, 10, 1365.
4. Finger, H. *J. Prakt. Chem.* **1907**, 76, 93.
5. Kjaer, A. *Acta Chem. Scand.* **1910**, 7, 1030.
6. Ekeley, J. B.; Ronzio, A. R. *J. Am. Chem. Soc.* **1935**, 57, 1353.
7. Ekeley, J. B.; Ronzio, A. R. *J. Am. Chem. Soc.* **1937**, 59, 1313.
8. Shafi, P. M.; Shobha, T. D.; Basheer, P. A. M. *Ind. J. Chem.* **2005**, 44B, 1298.
9. Jyothi, P. Ph. D. Thesis, University of Calicut, Kerala, **2009.**
10. Begum, T. S.; Jaleel, U. C. A.; Shafi, P. M. *Int J Pharm Biomed Sci* **2013**, 4(1), 40-45.
11. Mesaik, M. A.; Khan, K. M.; Rahat, S.; Zia-Ullah; Choudhary, M. I.; Murad, S.; Abdullah, N. R.; Ismail, Z.; Atta-ur-Rahman; Ahmad, A.; Sidhiqui, R. A. *Lett. Drug Des. Discov*. **2005**, 2, 490.
12. Solankee, A.; Kapadia, K.; Upadhyay, K.; Patel, J. *Orient J. Chem*. **2001**, 17, 315.
13. Prabhavat, M. D. *Asian J. Chem*. **2001**, 13, 226.
14. Kuchar, M.; Brunova, B.; Grimova, J.; Holubek, J.; Nemecek, O. *Cesko-Slovenska Farmacie* **1975**, 24, 287.
15. Khan, K. M.; Mughal, U. R.; Ambreen, N.; Samreen; Perveen, S.; Choudhary, M. I. *J. Enzyme Inhib. Med. Chem*. **2010**, 25, 29.
16. Khan, K. M.; Mughal, U. R.; Khan, S.; Perveen, S.; Choudhary, M. I. *Lett. Drug Des. Discov*. **2009**, 6, 69.
17. Shafi, P. M.; Basheer, P. A. M.; Jirovetz, L. *Asian J. Chem*. **2006**, 18, 3188.
18. Solankee, A.; Kapadia, K.; Patel, J.; Thakor, I. *Asian J. Chem*. **2002**, 14, 699.
19. Carreaux, F.; Bazureau, J. P.; Renault, S.; Meijer, L.; Lozach, O. **2009**, WO 2009050352 A2 20090423 CAN 150:472956.
20. Hassanein, H. H.; Khalifa, M. M.; El-Samaloty, O. N.; Abd-El-Rahim, M.; Taha, R. A.; Magda; Ismail, M. F. *Arch. Pharmacal. Res*. **2008**, 31, 562.
21. Shi, F.; Zeng, X.-N.; Wu, F.-Y.; Yan, S.; Zheng, W.-F.; Tu, S.-J. *J. Hetercycl. Chem*. **2012**, 49, 59.
22. Ruheman, S.; Cunnington, A. V. *J. Chem. Soc.* **1899**, 75, 954.
23. Hofmann, K." The Chemistry of Heterocyclic Compouds" , Vol.VI, Edited by A. Weissberger (Interscience, New York), **1953**, 93.

24. Erlenmeyer, E. *Ann.* **1893**, 275, 1.
25. Erlenmeyer, E. *Ber.* **1900**, 33, 2036.
26. Erlenmeyer, E.; Hasley, *Ann.* **1899**, 307, 138.
27. Erlenmeyer, E.; Kunlin, *Ann.* **1901**, 316, 145.
28. Erlenmeyer, E.; Matter, O. *Ann.* **1904**, 337, 271.
29. Erlenmeyer, E.; Stadlin, W. *Ann.* **1904**, 337, 283.
30. Erlenmeyer, E.; Wittenberg, F. *Ann.* **1904**, 337, 294.
31. Erlenmeyer, E.; Hasley, *Ber.* **1897**, 30, 2981.
32. Erlenmeyer, E.; Kunlin, *Ber.* **1902**, 35, 384.
33. Williams, D. L.; Ronzio, A. R. *J. Am. Chem. Soc.* **1948**, 68, 647.
34. Cornforth, J. W.; Huang, H. T. *J. Chem. Soc.* **1948**, 731.
35. Kjaer, A. *Acta Chem. Scand.* **1953**, 7, 900.
36. Badr, M.Z.; El-Sharief, H. A. H.; Tadros, M. E. *Ind. J. Chem.* **1981**, 20B, 209.
37. Mukerji; Dev Das; Nautiyal; Sudha Rani; Prasad; Chinta Ram Pol. *J. Parmacol. Pharm.* **1981**, 33(6), 633.
38. Nalepa Kavel; Metala; Leopold; Acta. Univ. Dalacki; Olomac, *Fac. Rerun. Nat.* **1981**, 69, (Chem. 20) 95.
39. Guptha; Anil, K. Sen Gupta; Anurag Ateet, *J. Ind. Chem. Soc.* **1981**, 58, 279.
40. Kidwai; Muzahir; M. Khan; Nazeem, A. *J. Nepal Chem. Soc.* **1981**, 1, 39.
41. El-Shareif, A. M. S.; Harb, A. A. *Ind. J. Chem*. **1982**, 21B (5), 499.
42. Misra; Upma; Pathk, A.K.; Tiwari, D.C.; Singh, *Amerika. Indian Drugs.* **1990**, 27, 607.
43. Mukerjee; Arya, K. Joseph; Kiran, *Ind. J. Chem.* **1993**, B32, 973-4.
44. Shafi, P. M.; Shobha, T. D. *Asian J. Chem.* **1997**, 9, 881.
45. Ekeley, J.B.; Elliot, J.L. *J. Am. Chem. Soc.* **1936**, 58, 163.
46. Waugh; Ekeley; Ronzio; *J. Am. Chem. Soc.* **1942**, 64, 2008.
47. Devasia, G.M. *Tetrahedron Lett.* **1976**, 571.
48. Devasia, G.M.; Shafi, P. M. *Ind. J. Chem.* **1979**, 17B, 526.
49. Devasia, G. M.; Shafi, P. M. *Ind. J. Chem.***1981**, 20B, 657.
50. Shafi, P. M. *Curr. Sci.* **1985**, 54, 1231.
51. Finger, H. *J. Prakt. Chem.*(2), **1907**, 76, 93.
52. Finger, H.; Zeh, W. *J. Prakt. Chem*. **1910**, 82, 502.
53. Kjaer, A. *Acta Chem. Scand.* **1953**, 7, 1030.
54. Lehr, H.; Karlan, S.; Goldberg, M. W. *J. Am. Chem. Soc.* **1953**, 75, 3640.
55. Kidwai, A. R.; Devasia, G. M. *J. Org. Chem.* **1962**, 27, 4527.
56. Devasia, G. M.; Pillai, C. R. *Tetrahedron Lett.* **1975**, 225.

57. Griffiths; Gareth; Inwinkelried, Rana; Gosteli; Jacques; (Lonza AG) Evr. Pat. Appl. EP 614892(ClCo 7D 233/68), 14 Sep **1994** CH Appl. 93/749, 12 March, **1993**, 8pp, C.A. Vol. 121, **1994**, 2307722.
58. Fred, C. Schaefer; Grace, A. Peters, *J. Org. Chem.* **1961**, Vol. 26, 412-418.
59. Basheer, P. A. M. Ph. D. Thesis, University of Calicut, Kerala, **2001**.
60. Shalina Begum, T. Ph. D. Thesis, University of Calicut, Kerala, **2012**.
61. Corey, E. J.; Barbara Czako; Laszlo Kurti, "Molecules and Medicine", Wiley-Interscience **2007**.
62. Curtus, T.; Goebel, F. *J. Prakt. Chem*. **1888**, 37, 150.
63. Kidwai, A. R.; Devasia, G. M. *J. Org. Chem*. **1962**, 27, 4527.
64. Harries, C.; Wiess, M. *Ann*. **1903**, 327, 355.
65. Chambers, R. W.; Carpenter, F. H. *J. Am. Chem. Soc*. **1955**, 77, 1522.
66. Carmen, Avendaro; J. Carlos, Menendez, "Medicinal Chemistry of Anticancer Drugs" **2011**, Vol. 14.
67. Alex, Gringauz, "Introduction to Medicinal Chemistry-How drugs act and why" **2011.**
68. Nathan, E. R.-F.; David, F. B. M.; Monte, M. W.; John, M. L.; Mary, J. X.; Gregory, H. U.; Richard, O. H.; Tyler, E. J.; Sangeetha, N. B. *Nat. Commun*. **2012**, 3, 1122.
69. Bava, S. V.; Puliappadamba, V. T.; Deepti, A. Nair; Karunagaran, D.; Anto, R. J. *J. Bio. Chem*. **2005**, 280, 6301.
70. Özkay, Y.; Işikdağ, İ.; İncesu, Z.; Akalin, G. *Eur. J. Med. Chem*. **2010**, 45, 3320-3328.
71. Wang, L.; Świtalska, M.; Mei, Z.-W; Lu, W.- J.; Takahara, Y.; Feng, X.-W.; El-Sayed, I. E.-T.; Wietrzyk, J.; Inokuchi, T. *Bioorg. Med. Chem*. **2012**, 20, 4820-4829.
72. Greenwood, D.; Finch, R.; Davey, P.; Wilcox, M. "Antimicrobial Chemotherapy" Fifth Edition, Oxford University Press **2007**.
73. *JMAJ*, March/April **2009**, Vol.52, No.2.
74. Dax, S. L. "Antibacterial Chemotherapeutic Agents" Blackie Academic and Professional, Chapman and Hall London **1997**.
75. Bayrak, H.; Demirbas, A.; Demirbas, N.; Karaoglu, S. A. *Eur. J. Med. Chem*. **2009**, 44, 4362-4366.
76. Meera C.R., Syama C., Rakhi J., Wilsy W., Anjana J.C., Ruveena T.N., Antimicrobial and anti-oxidant activities of polysaccharides isolated from an edible mushroom, *Pleurotus florida. Advanced Biotech* **2010**, 10*,* 9-11.

CHAPTER II

SYNTHESIS AND ANTICANCER STUDY OF (Z)-5-(AMINO (ARYL)METHYLENE)-2-ARYLTHIAZOL-4(5*H*)-ONES

2.1 SYNTHESIS OF (Z)-5-(AMINO(ARYL)METHYLENE)-2-ARYLTHIAZOL-4(5*H*)-ONES

2.1.1 Introduction

Thiazoles have been studied extensively and many compounds containing this eterocycle are of industrial and biological importance. Thiazole is structurally related to thiophene as well as pyridine, but in most of its reactions, it resembles the latter.

Among the dihydrothiazolines, 2-thiazolines have been immensely studied, especially those containing substituents at the 4 and 5 positions.

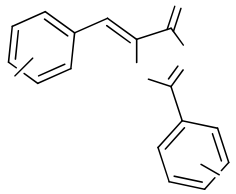
The history of true thiazole series began in 1879 with the work of Hofmann[1], who prepared derivatives of benzothiazole such as 2-chlorobenzothiazole and 2-phenylbenzothiazole. Compounds containing the simple thiazole nucleus were first reported by Hantzsch and co-workers[2] in 1887.

Important naturally occurring thiazole derivatives include Vitamin B_1 (thiamine) and the pencillins. Sulphathiazole, one of the sulphanilamide group

of drugs, is effective against certain bacterial infections, while mercaptothiazoles are used as accelerators in vulcanization of rubber[3].

Thiazolone and derivatives have attracted continuing interest over the years because of their varied biological activities such as anti-inflammatory, antimicrobial, antiproliferative, antiviral, anticonvulsant, antifungal and antibacterial[4-6]. In recent years, thiazolone derivatives with their antitumor activity have become a hot area. Havrylyuk and coworkers[7] reported that thiazolidinones containing benzothiazole moiety has anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines.

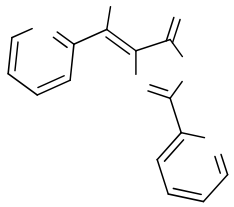
Recently[8-10], a novel class of 5-benzylidine-2-phenylthiazolinones has been synthesized. On biological evaluation, these compounds are found to be potent 5-lipoxygenase inhibitors which are supposed to be of therapeutic value for the treatment of asthma, allergic rhinitis, atherosclerosis, and certain types of cancer[11, 12].



A novel series of quinolinyl-methylene-thiazolinones[13] has been synthesized, which are identified as potent and selective cyclin-dependent kinase 1 inhibitors and therefore attractive therapeutic targets for cancer therapy. Structure activity relationship (SAR) studies of these compounds revealed that the double bond exocylic to the thiazolinone ring is required for the activity.

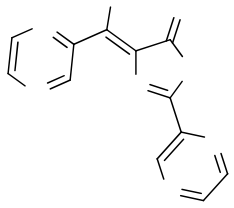
In 2007 Shafi and Jyothi[14] prepared 4-(amino(pyridinyl)methylene)-2-pyridinyl-2-imidazolin-5-ones by the tandem reaction between imidic acid

ester of both 2-cyanopyridine and 4-cyanopyridine and glycine ester in the molar ratio 2:1 in toluene under reflux for 2 hrs.



Shafi and Shalina[15] conducted the anticancer studies of 4-(amino(pyridinyl)methylene)-2-pyridinyl-2-imidazolin-5-ones and their hydrochlorides.

In 2012, Shalina Begum and co-workers[16] synthesized 4-(amino(2-pyrazinyl)methylene)-2-(2-pyrazinyl)-2-imidazolin-5-one by refluxing imidic acid ester of pyrazine-2-carbonitrile and glycine ester in toluene for two hours.



In this context, synthesis and anticancer screening of a novel class of (E)-5-(amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones formed from pyridine-2-carbonitrile and pyrazine-2-carbonitrile, would be of great interest as to how the replacement of nitrogen by sulphur affect the anticancer properties.

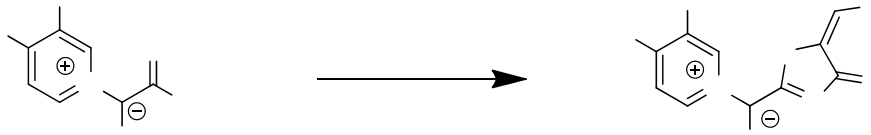
2.1.2 Review

Since this chapter and the next chapter (III) deal with the synthesis and biological evaluation of disubstituted thiazolinones, it is worthwhile to briefly

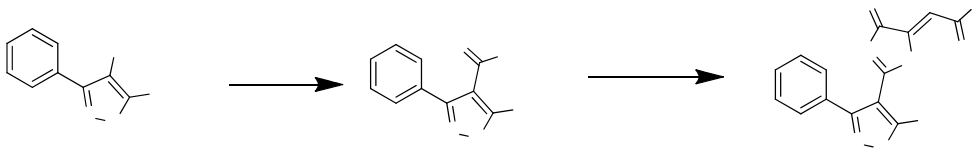
review various synthetic routes available for the construction of this class of compounds.

Usually, the synthesis of the thiazole core involves the condensation of amino thiols with either nitrile[17,18], carboxylic acid[19], or ester[20] as well as by intramolecular dehydration of β-hydroxy thioamides under Mitsunobu conditions[21] or with Burgess reagent[22]. Other methods exploit intramolecular cyclization of β-hydroxy amides with P_2S_5[23] or Lawesson's reagent[24], by the reaction of amino sugar derivatives with aryl isothiocyanates[25], or by deselenylation of thioamido selenides[26].

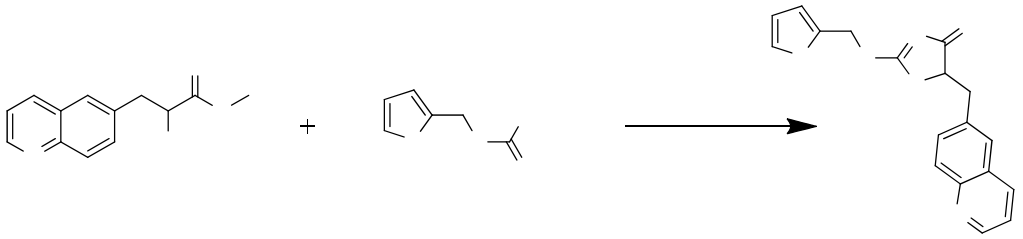
In 1993, Berseneva and co-workers[27] showed that the reaction of thiocarbamoyl pyridinium and isoquinolinium ylides with dimethyl acetylene dicarboxylate (DMAD) leads to the formation of ylides containing thiazolinone moiety.



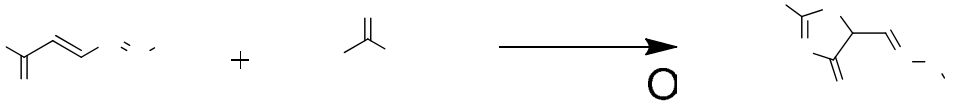
In 2001, they further obtained bis-heterocyclic compounds[28] containing thiazoline moiety by refluxing the corresponding heteroaromatic thioamides such as those of isoxazoles, imidazoles, 1,2,3-triazoles and thiadiazoles with DMAD in ethanol. The thioamides were prepared by treating respective nitriles with H_2S.



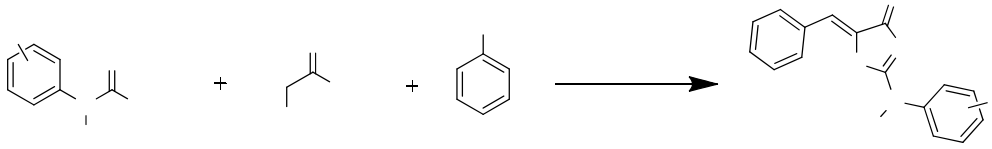
Quinolinyl-methylene-thiazolinones[29] were synthesized by refluxing beta-bromo ester of quinolone with substituted thiourea, 2-methylethanol and sodium acetate at 100 ^{o}C for 5h.



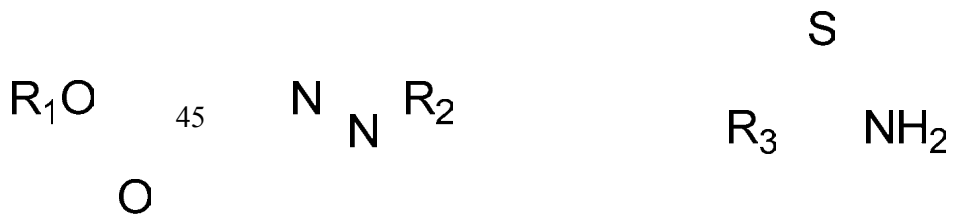
In 2008, Attanasi and co-workers[29, 30] synthesized substituted 2-thiazolin-4-ones by the reaction between 1,2-diaza-1,3-butadienes and thioamides.

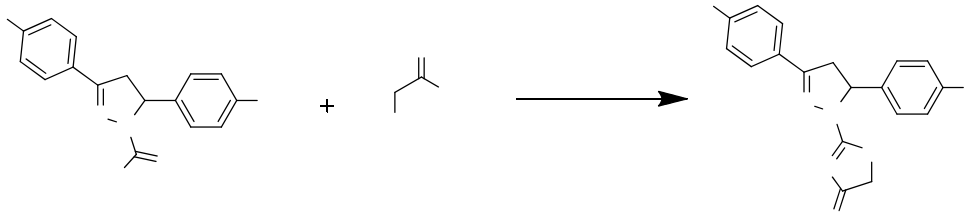


In 2010, Subtel'na and co-workers[31] reported the synthesis of 5-arylidene-2-arylaminothiazol-4(5H)-ones, by the reaction of aryl thiourea, aromatic aldehyde, chloroacetic acid and fused sodium acetate in refluxing acetic acid.

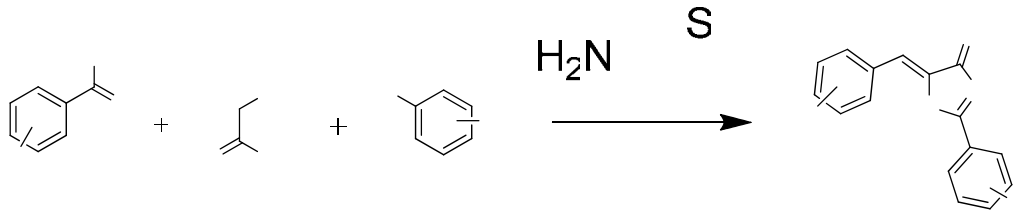


In 2012, Qiu and co-workers[32] reported the synthesis of pyrazolyl-thiazolinone derivatives obtained by the reaction between pyrazole derivative containing thiourea skeleton, bromoacetic acid, acetic anhydride and sodium acetate on stirring at 80 ^{o}C for 6-8 h.



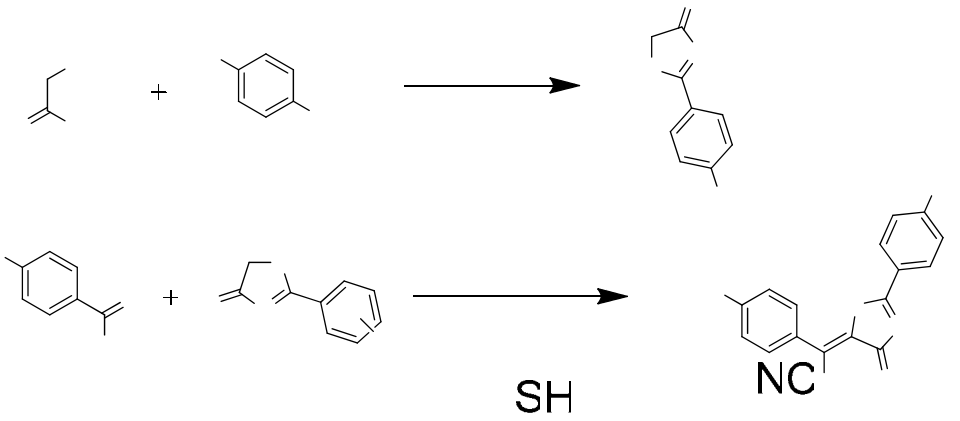


5-Benzylidine-2-phenylthiazolinones[33-35] were synthesized by refluxing overnight, aromatic nitriles, thioglycolic acid, aldehydes and triethylamine in methanol. The reaction mixture was evaporated under reduced pressure and the pure product was recrystallized from ethanol and washed with acetone.



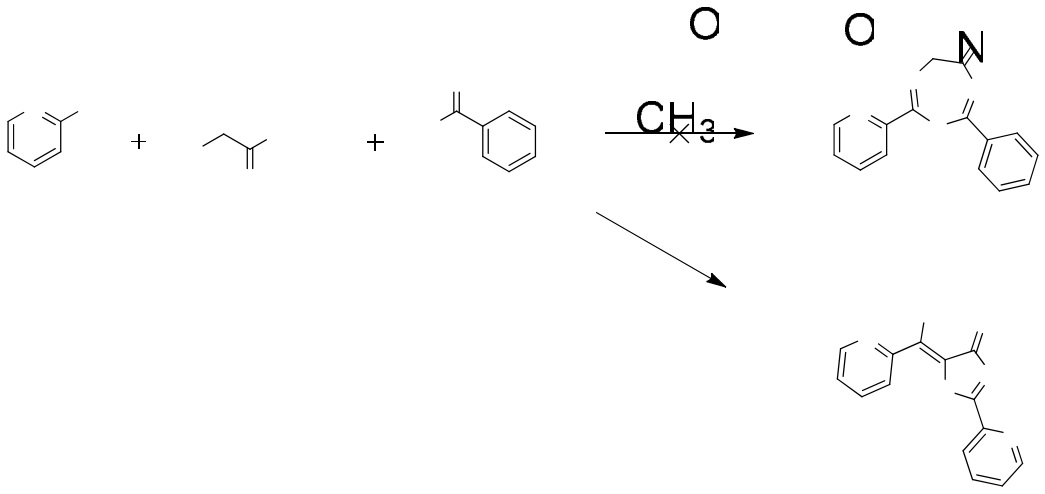
In 2012, Barzen and co-workers[36] extended the above synthesis by taking more differently substituted aromatic nitriles, aromatic aldehyde and refluxed with thioglycolic acid in presence of triethyl amine in methanol.

In the case of less reactive acetophenone derivatives, they performed the reaction in two-step synthesis route. First thioglycolic acid and the aromatic nitrile were refluxed for 1h in ethanol in presence of triethylamine to yield the thiazolinone core and then it was condensed with p-methoxyacetophenone by heating with ammonium acetate in toluene for 90 min at 130 ^{o}C under microwave irradiation.



2.1.3 Present Work

While attempting to construct a seven membered heterocyclic ring by a three-component reaction between imidic acid ester of pyridine-2-carbonitrile, thiobenzamide and ethyl chloroacetate, we could isolate and characterize a novel compound (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one **(Scheme 3).**



Scheme 3

We recognized that the unexpected compound was formed by the reaction between prydine-2-carbothioamide and ethylchloroacetate in 2:1 molar ratio, a reaction analogous to the tandem reaction between imidate of pyridine-2-carbonitrile and glycine ester as discussed in chapter I. On running

the reaction in two steps, we deduced that the pyridine-2-carbothioamide is eventually formed in situ by a kind of sulphur exchange reaction[37] between imidic acid ester of pyridine-2-carbonitrile and thiobenzamide and that thiobenzamide has just the role of donating sulphur atom to the imidate.

On further investigation with different nitrogen containing heteroaromatics, we found that the cyclisation effected only when the thioamide has an ortho nitrogen in the ring to cause strong electron withdrawing effect. Thus, in the present work, we converted pyridine-2-carbonitrile and pyrazine-2-carbonitrile to their thioamides by the reported method[38], and stirred with different heteroaromatic nitriles and ethylchloroacetate in dry benzene. It is to be mentioned that pyrimidine-2-carbothioamide, in spite of having two ortho nitrogen atoms did not undergo cyclization to form thiazolinone ring.

The method was improved in such a way that, instead of taking two moles of thioamide, one mole each of thioamide and imidate of the parent nitrile were taken for the reaction, thereby introducing a better leaving group -OMe than –SH and enhancing the yield. Besides, it enabled to incorporate different heteroaromatic residues at 5-position in terms of their imidates and also those having meta/para nitrogen, which otherwise couldn't cyclize with ethylchloroacetate, thereby synthesizing more compounds in the series. In such cases, one mole each of thioamide and imidate of the interested nitrile were taken so that two products were formed, one by the condensation between two molecules of the thioamide itself (direct product) and the other by that between one molecule each of thioamide and imidate of the nitrile (cross product). Their R_f values were so close that they required preparatory TLC method for separation.

2.1.4 Result and Discussion

Synthesis of novel (E)-5-(Amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones

In the present work, we synthesized a novel series of (E)-5-(amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones, which are reported for the first time, by stirring overnight, thioamide of pyridine-2-carbonitrile/pyrazine-2-carbonitrile, imidic acid ester of various nitriles and ethylbromoacetate in 1:1:1 molar ratio, in presence of anhydrous sodium acetate in dry benzene. Pyridine-2-carbonitrile and pyrazine-2-carbonitrile were converted to their thioamides by passing H_2S through an ice-cold solution of these nitriles in alcoholic ammonia[38] and employed for cyclization with ethylbromoacetate. Different nitriles were converted to imidates by the method suggested by Fred and Grace (chapter I). The compounds were filtered, washed with water and then with ethanol and dried. The aminothiazolinoes were obtained in 40-60% yield **(Table 6)**. In many of the cases, purification of the compound needed column chromatography with gradient elution using petroleum ether-ethyl acetate mixture.

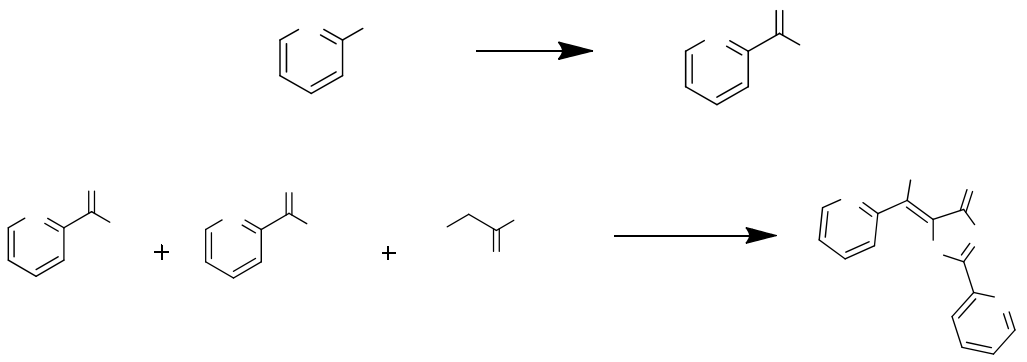
Table 6: Characterization data of (E)-5-(Amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones

Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Λ_{max} (nm)	Elemental analysis found (calcd.)		
						C	H	N
1	(E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{14}H_{10}N_4OS$	60	230	438	59.52 (59.56)	3.51 (3.57)	19.88 (19.85)
2	(E)-5-(amino(pyridin-3-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{14}H_{10}N_4OS$	42	228	426	59.60 (59.56)	3.54 (3.57)	19.81 (19.85)
3	(E)-5-(amino(pyridin-4-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{14}H_{10}N_4OS$	40	232	420	59.54 (59.56)	3.61 (3.57)	19.82 (19.85)
4	(E)-5-(amino(pyrimidin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{13}H_{10}N_5OS$	40	236	446	55.09 (55.11)	3.18 (3.20)	24.70 (24.72)
5	(E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{13}H_{10}N_5OS$	50	235	438	55.15 (55.11)	3.16 (3.20)	24.70 (24.72)
6	(E)-5-(amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one	$C_{18}H_{11}N_4OS$	55	255	448	65.02 (65.04)	3.60 (3.64)	16.82 (16.86)
7	(E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{12}H_{10}N_6OS$	48	220	460	50.75 (50.70)	2.81 (2.84)	29.58 (29.56)
8	(E)-5-(amino(pyridin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{13}H_{10}N_5OS$	50	238	458	55.08 (55.11)	3.16 (3.20)	24.68 (24.72)

Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Λ_{max} (nm)	Elemental analysis found (calcd.)		
						C	H	N
9	(E)-5-(amino(pyridin-3-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{13}H_{10}N_5OS$	40	236	429	55.15 (55.11)	3.22 (3.20)	24.70 (24.72)
10	(E)-5-(amino(pyridin-4-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{13}H_{10}N_5OS$	40	234	422	55.16 (55.11)	3.24 (3.20)	24.68 (24.72)
11	(E)-5-(amino(pyrimidin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{12}H_{10}N_6OS$	42	252	462	50.66 (50.70)	2.81 (2.84)	29.58 (29.56)
12	(E)-5-(amino(quinolin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one	$C_{17}H_{11}N_5OS$	52	260	454	61.28 (61.25)	3.30 (3.33)	21.03 (21.01)

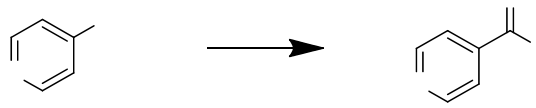
The procedure was optimized as above, after conducting a number of experiments using different reagents (ethyl chloroacetate, chloroacetyl chloride, ethyl bromoacetate and ethyl iodoacetate), solvents (absolute alcohol, isopropanol and dry benzene), reaction mode (stirring as well as refluxing) etc. When cyclization was carried out using ethyl chloroacetate, the yield was very poor. The best reagent was found to be ethyl bromoacetate. The reaction took place under reflux conditions, but there had been a lot of side reactions. The conversion of pyridine-2-carbonitrile and pyrazine-2-carbonitrile were first attempted using P_2S_5/Na_2SO_3 reagent[39] , but the conversion was not very efficient. The reactions are depicted below **(Scheme 4, 5).**

a) Direct product

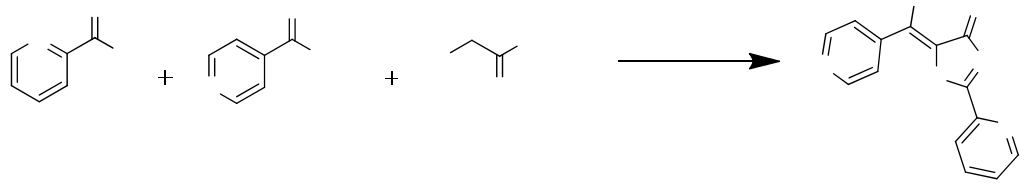


Scheme 4

b) Cross product



N CN Na



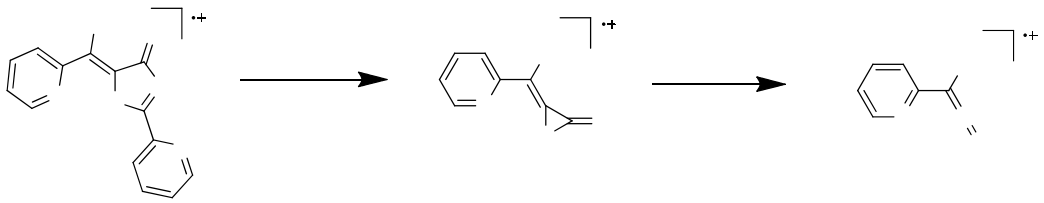
Scheme 5

All the compounds synthesized were verified by elemental analysis. The mass spectrum, ^{1}H NMR and ^{13}C NMR spectra of a few compounds are discussed here.

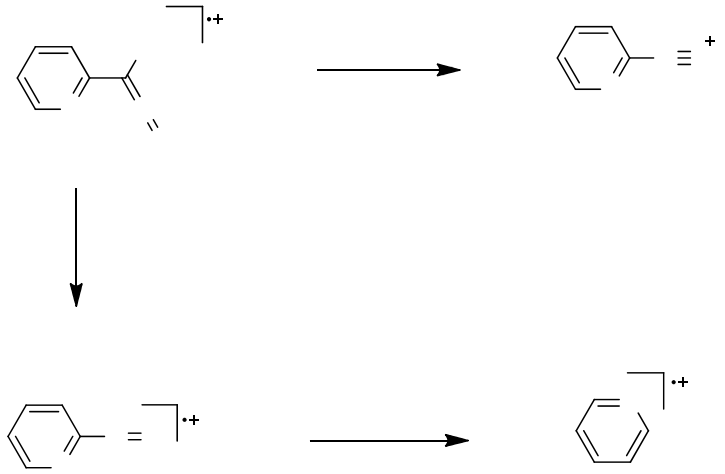
(E)-5-(Amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

The mass spectrum of the title compound isolated in this series, recorded under EIMS mode (**Spectrum 10**) gave a mass peak at m/z = 282, which is the molecular ion peak corresponding to the proposed structure. The even mass supported the fact that the compound contains even number of nitrogen atoms. Other important peaks in the mass spectrum were at m/z 178, 150,123, 105 and 79, which further supported the proposed structure. The mass spectral fragmentation of the compound can be interpreted as given below.

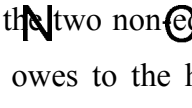
Removal of a neutral molecule of pyridine-2-carbonitrile from the molecular ion results in the peak at m/z = 178, which eliminates a neutral molecule of CO, giving the fragment at m/z = 150.



The resulting fragment can further undergo two alternate cleavages as follow, explaining the occurrence of peaks m/z 123 and 105. The former can undergo subsequent fragmentation leading to the peaks at m/z = 79.

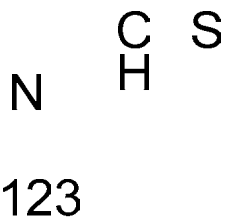


The ^{1}H NMR spectrum (**Spectrum 11, 12**) also supported this structure. The two singlets at δ 11 and 9.1 are due to the two non-equivalent protons of the amino group. The absorption at δ 11 owes to the hydrogen bonded proton. The eight aromatic protons of the two pyridinyl rings marked their absorption between δ 8.8 and 7.5.

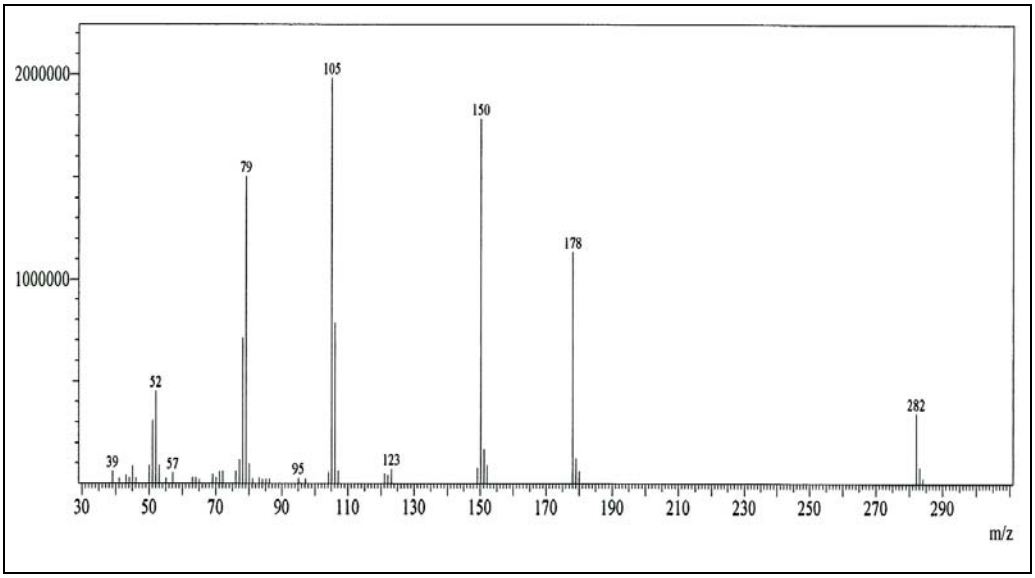


In ^{13}C NMR spectrum (**Spectrum 13**), there are fourteen peaks recorded between δ 92.5 and 182.7, which is in full agreement with the proposed structure. The peak at 182.7 can be accounted for the carbonyl carbon.

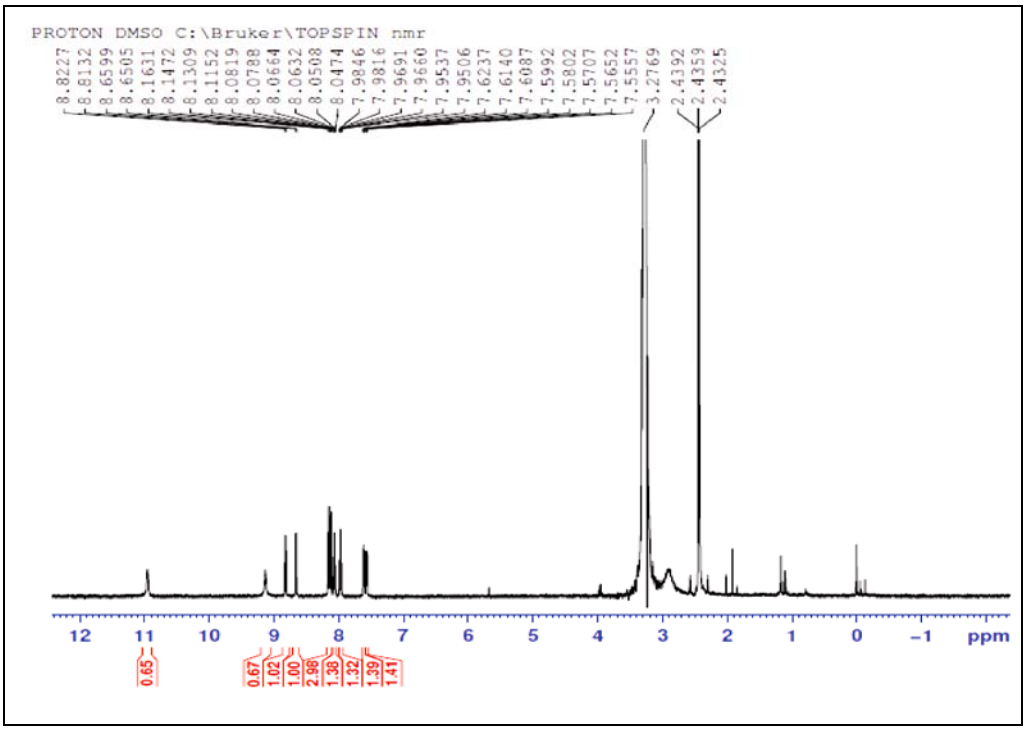
The IR spectrum was also in agreement with the above observations. A broad peak with a maximum at 3421 cm^{-1} corresponds to the symmetric and asymmetric vibrations of NH_2.



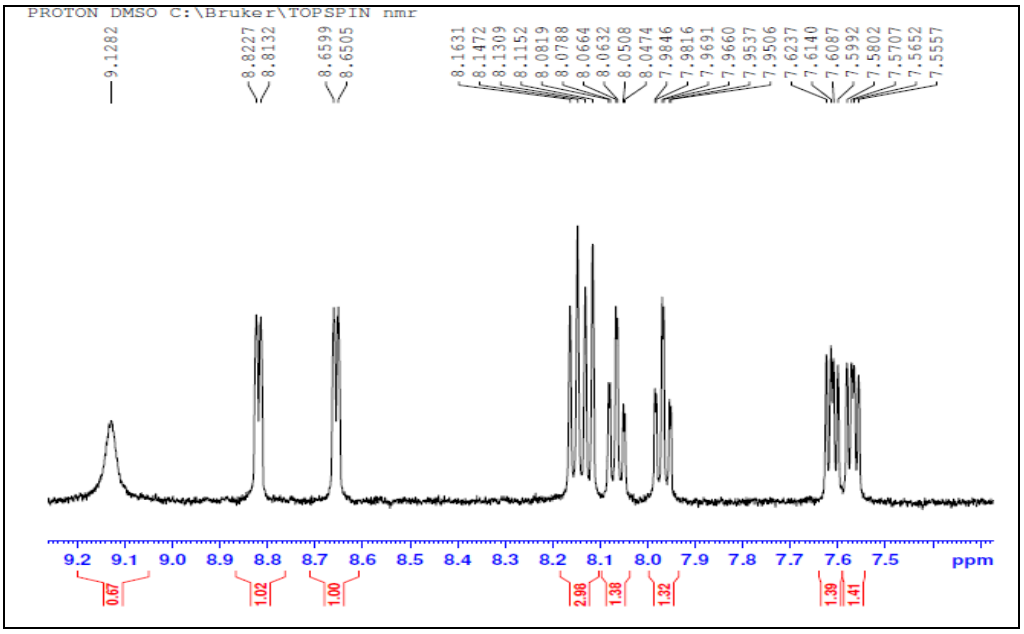
123



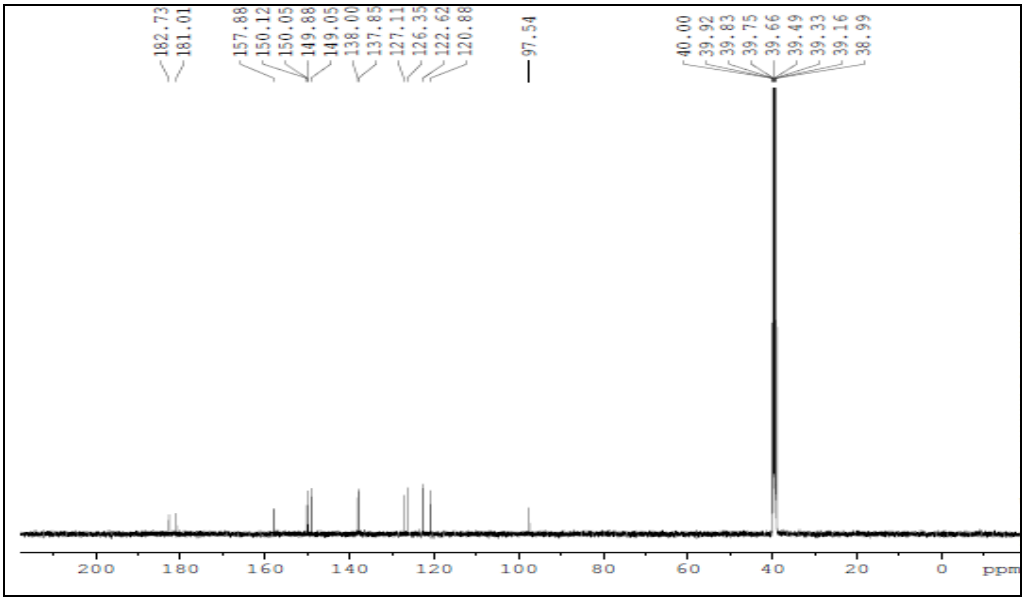
Spectrum 10: Mass spectrum of (E)-5-(Amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



Spectrum 11: ^{1}H NMR spectrum of (E)-5-(Amino(pyridin-2-yl) methylene)- 2-(pyridin-2-yl)thiazol-4(5*H*)-one



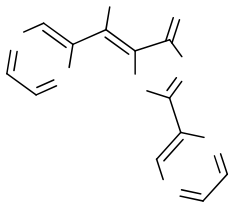
Spectrum 12: ^{1}H NMR spectrum of (E)-5-(Amino(pyridin-2-yl) methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



Spectrum 13: ^{13}C NMR spectrum of (E)-5-(Amino(pyridin-2-yl) methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

(E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

The mass spectrum of this compound (**Spectrum 14**) showed a peak at m/z = 285, which is supposed to be the quasimolecular ion peak $[M+1]^+$, indicating the molecular mass to be 284. This is in agreement with the proposed structure. The even molecular mass justified the even number of nitrogen atoms in the molecule.

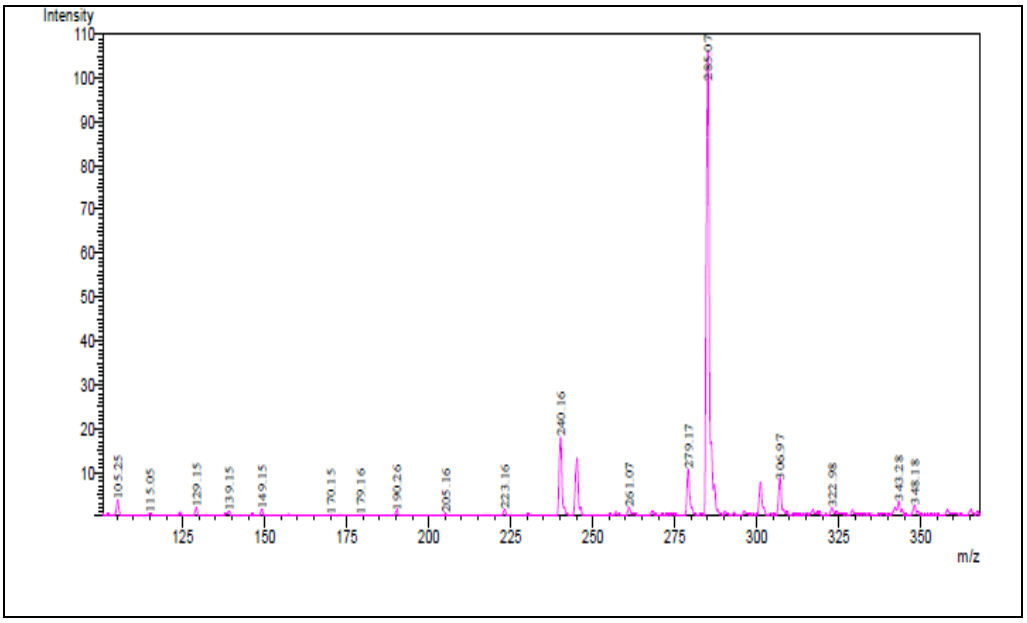


The ^1H NMR spectrum (**Spectrum 15, 16**) also supported the structure. The two protons of NH_2 group being non-equivalent due to H-bonding with CO of the thiazolinone ring absorbed at δ 11 and 9.5. Here the higher absorption is due to the H-bonded proton. The six aromatic protons of the two pyrazinyl rings showed absorption in the region δ 9.4 to 8.8.

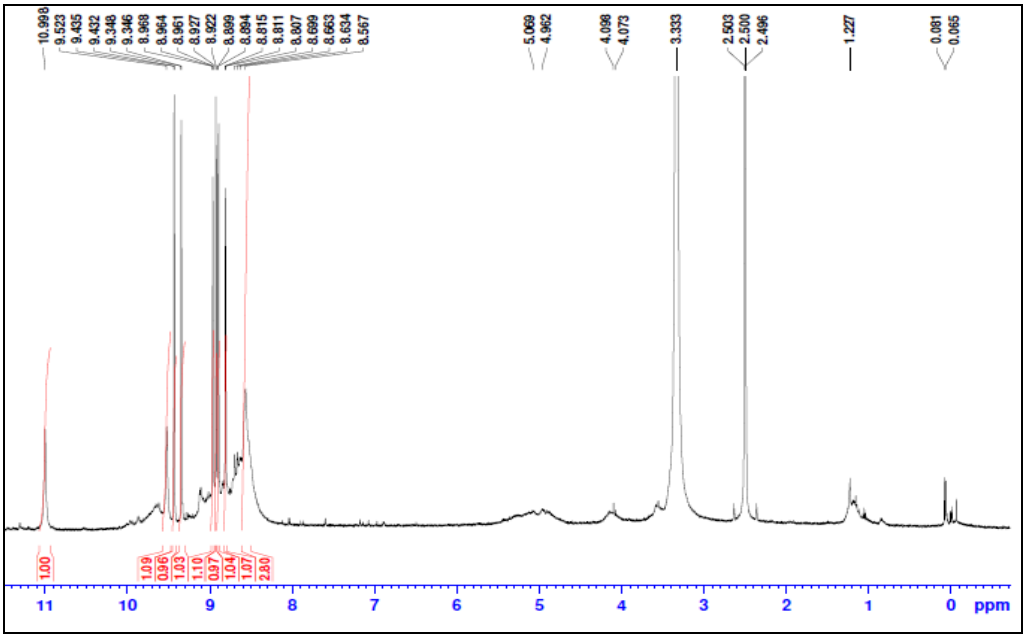
The IR spectrum is also in line with the above observations. A broad peak with a maximum at 3434 cm^{-1} corresponds to the symmetric and asymmetric vibrations of NH_2. The carbonyl absorption of the thiazolinone is seen at a lower frequency of 1688 cm^{-1}, due to conjugation as well as the hydrogen bonding with the amino group.

N

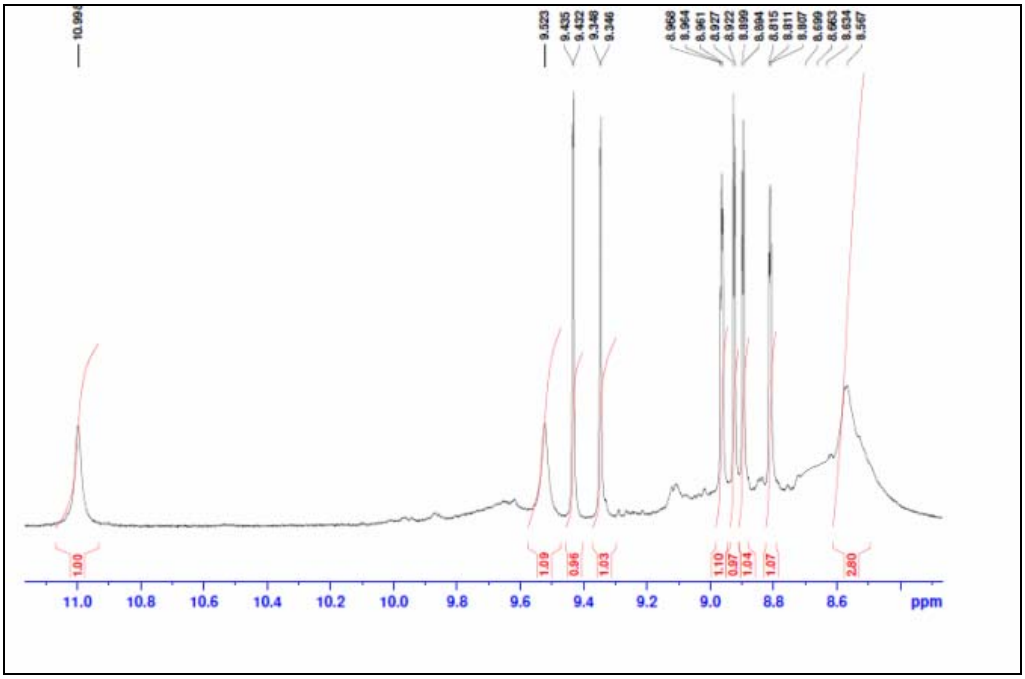
N



Spectrum 14: Mass spectrum of (E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one



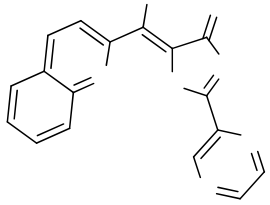
Spectrum 15: ^{1}H NMR spectrum of (E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one



Spectrum 16: ^{1}H NMR spectrum of (E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

(E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

The mass spectrum (**Spectrum 17**) exhibited a peak at m/z = 333, which is the quasimolecular ion peak $[M+1]^{+}$, suggesting that the molecular mass of the proposed structure to be true (332). The nitrogen rule is justified by the even molecular mass, as the molecule has four nitrogen atoms.

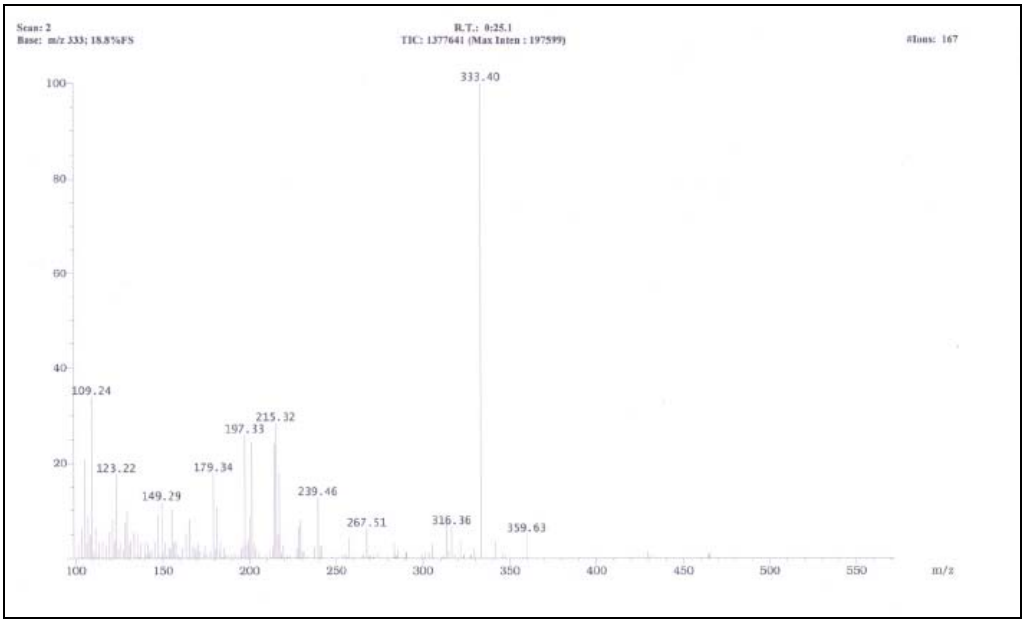


The two singlets at δ 11.1 and 9.4 in the ^{1}H NMR spectrum (**Spectrum 18, 19**) accounted for the two non-equivalent protons of the amino group. The absorption at δ 11.1 is due to the proton H-bonded to the CO of the

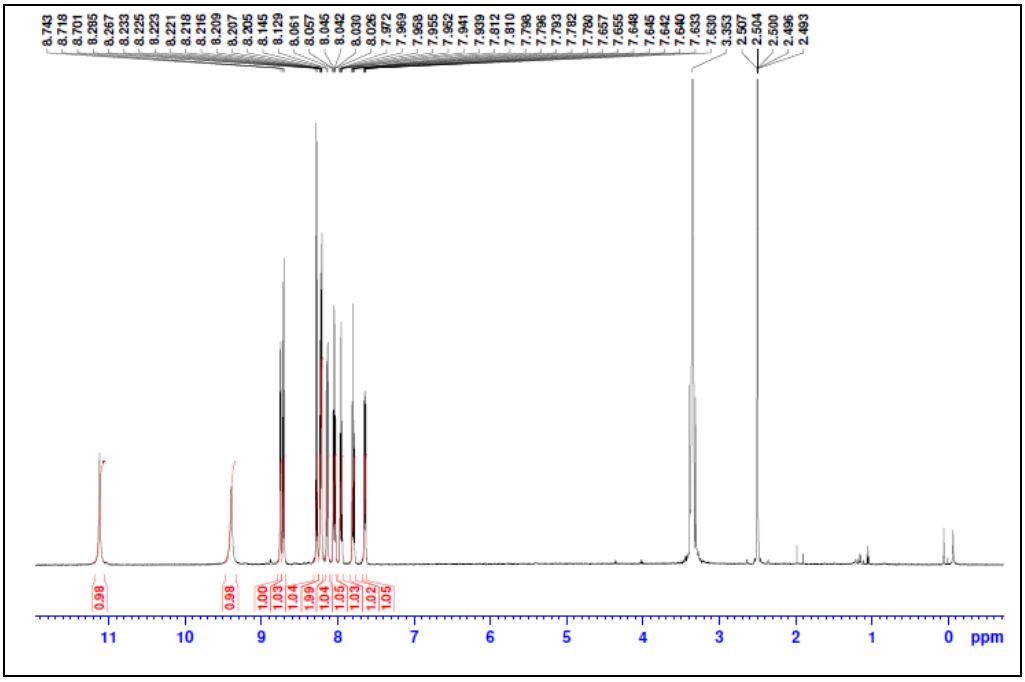
thiazolinone ring. The ten aromatic protons on the pyridine and quinoline rings are recorded in the region δ 8.75 to 7.64.

^{13}C NMR spectrum (**Spectrum 20**) showed 17 peaks in the region δ 98.16 to 182.6. The peak at δ 182.6 is due to the carbonyl absorption and all other carbons absorbed between δ 181.3 and 98.16. The absence of one peak can be accounted for the weakest NOE effect of one of the quaternary carbons.

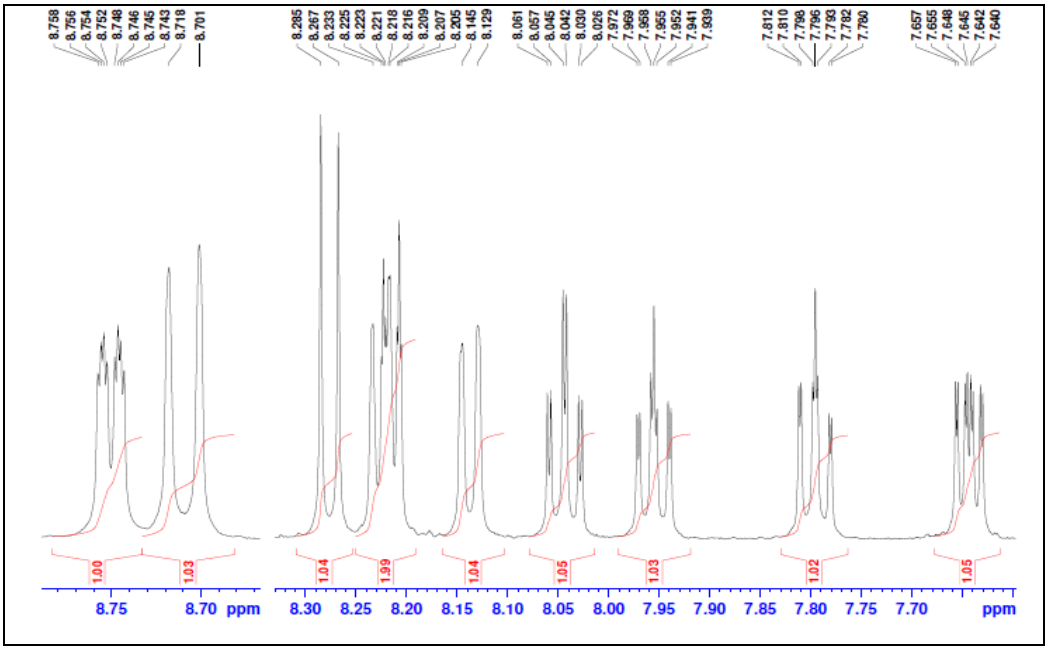
The IR spectrum also supported the above structure. The NH_2 absorption appeared as a broad peak with a maximum at 3418 cm^{-1}, as expected.



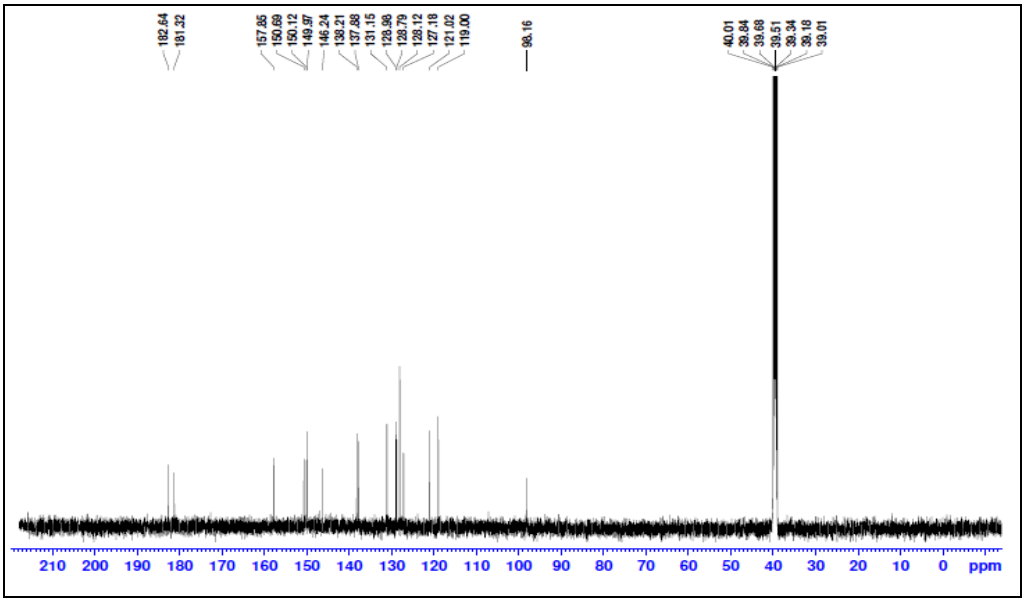
Spectrum 17: Mass spectrum of (E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



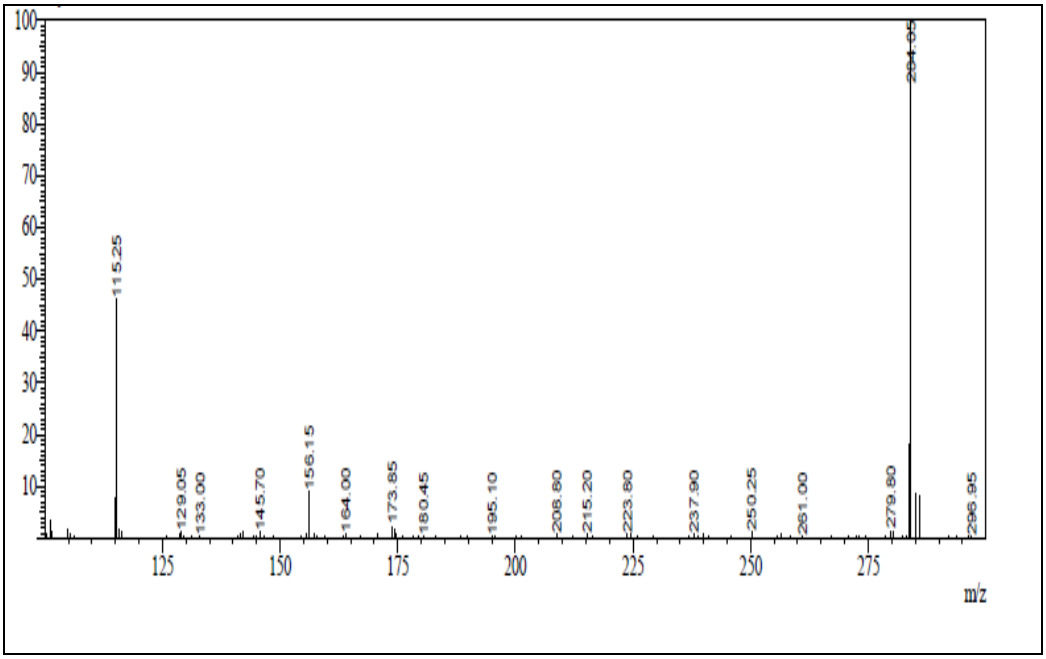
Spectrum 18: ^{1}H NMR spectrum of (E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



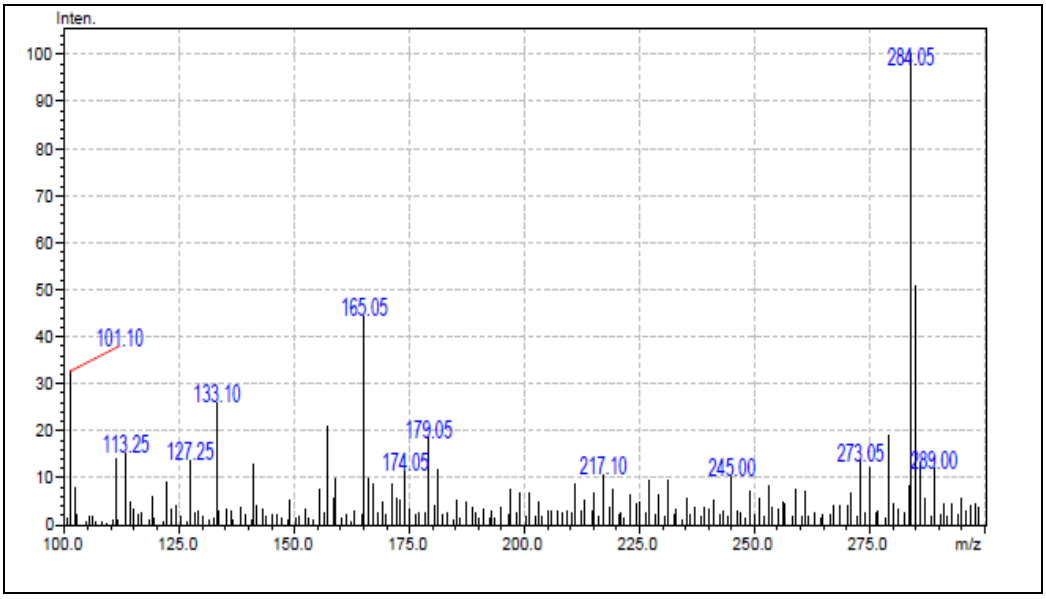
Spectrum 19: ^{1}H NMR spectrum of (E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



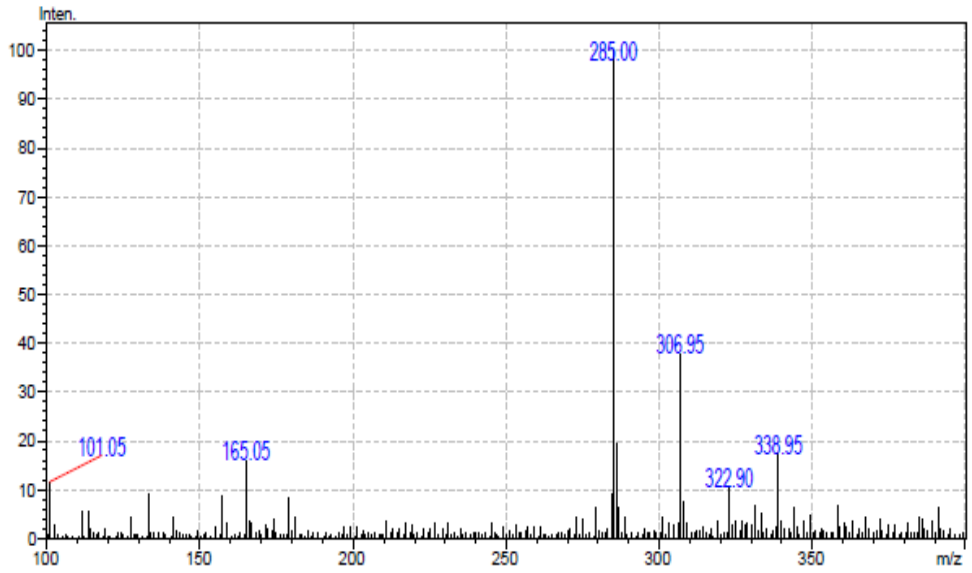
Spectrum 20: ^{13}C NMR spectrum of (E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



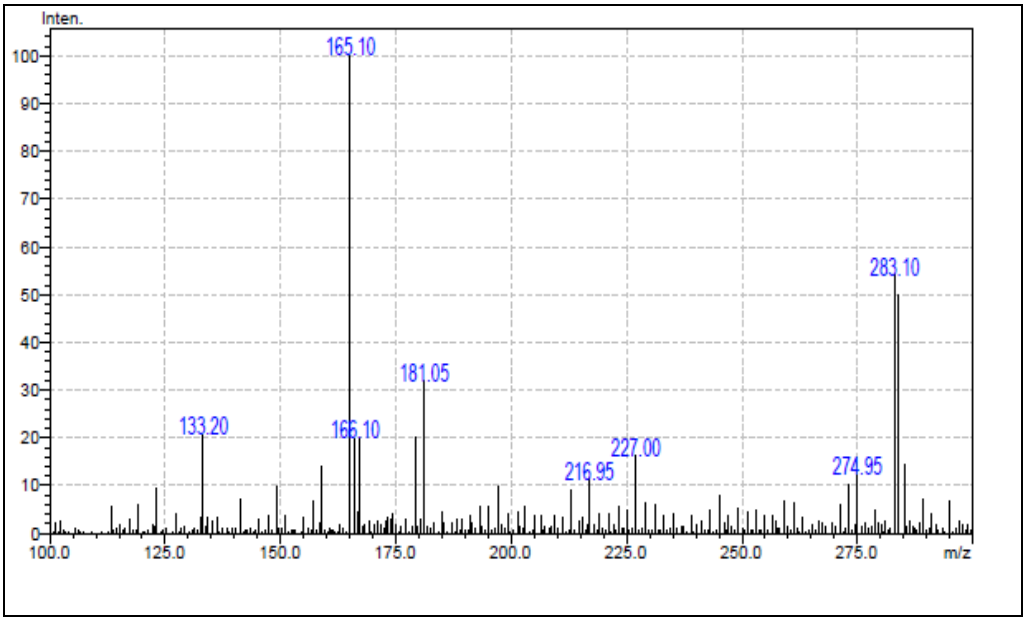
Spectrum 21: Mass spectrum of (E)-5-(Amino(pyrimidin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one



Spectrum 22: Mass spectrum of (E)-5-(Amino(pyridin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one



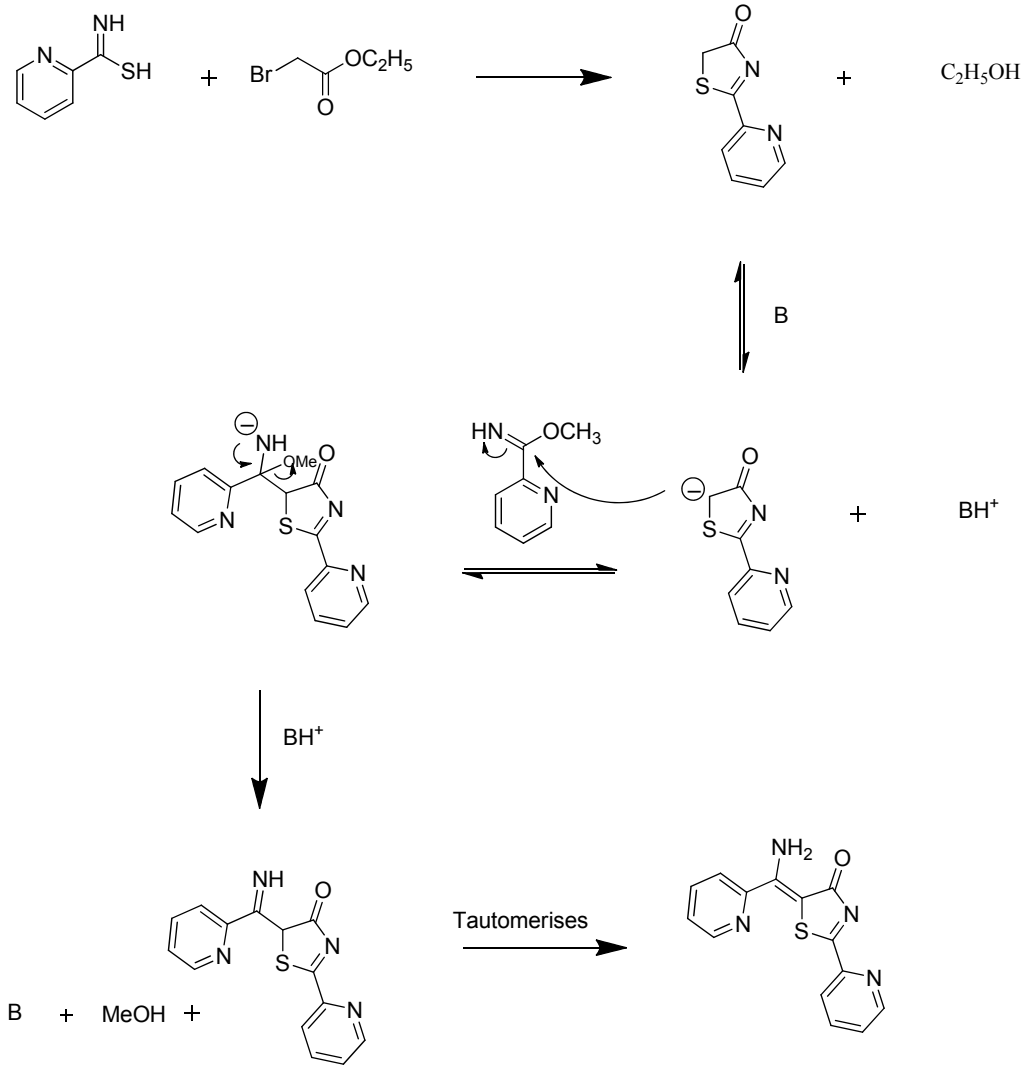
Spectrum 23: Mass spectrum of (E)-5-(Amino(pyrimidin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one



Spectrum 24: Mass spectrum of (E)-5-(Amino(pyridin-3-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

Mechanism

The following mechanism could be proposed for the reaction, which is analogous to that suggested for the formation of aminoimidazolinones[40] **(Scheme 6)**.



Scheme 6

Between the two geometrical isomers possible, the (E) isomer is thermodynamically more stable owing to the formation of intramolecular hydrogen bonding **(Figure 1)**

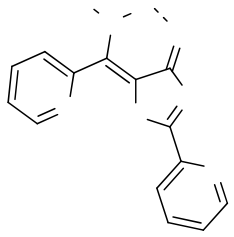


Figure 1

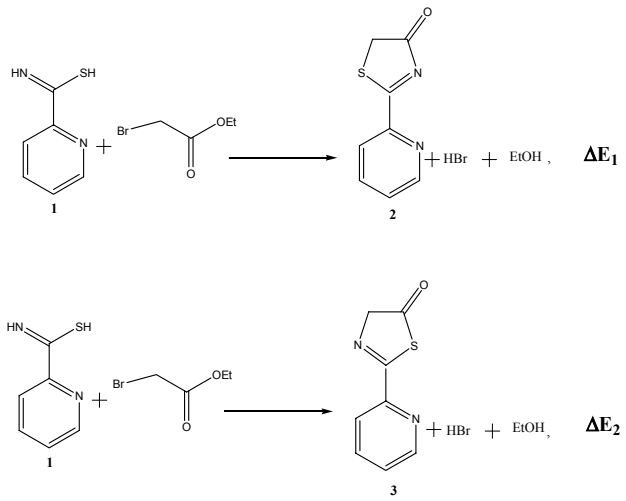
2.1.5 Theoretical Study

In order to investigate the thermodynamic and kinetic preference for the formation of the two possible isomers of the thiazolone ring (Scheme 2), computational calculations were carried out using the Gaussian 09 program package.

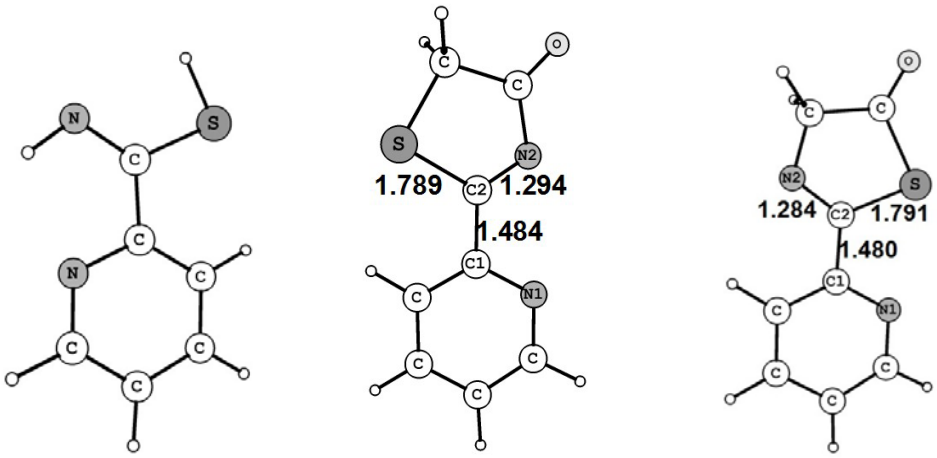
Computational Methodology

The geometries were optimized at the DFT level of theory using the exchange functional of Becke in conjunction with the correlation functional of Perdew (BP86)[41]. The basis sets used was def2-TZVPP.[42] This level of theory is denoted as BP86/TVPP. The calculations were carried out with the Gaussian 09 program package[43]. Single point calculations on the BP86/TZVPP optimized geometries have also been carried out using meta-GGA exchange correlation functional M06 with def2-TZVPP basis set[44]. Natural bond order (NBO) calculations were computed at the same level of theory[45]. The energies at M06/def2-TZVPP level were corrected by adding the zero point energies from the BP86/def2-TZVPP level of theory. This level of theory is indicated as M06/TZVPP//BP86/TZVPP.

Result and Discussion



Scheme 7: Proposed reaction pathway for the condensation reaction of 1.



1 2 3

(0 kcal/mol) (-0.005 kcal/mol)

Figure 2: Optimized geometries of 1 at the BP86/TZVPP level of theory and the relative energies between the isomers 2 and 3 at the M06/TZVPP//BP86/TZVPP (in kcal/mol) are given in the parenthesis.

Two possible condensation products (2 and 3) of 1 with ethyl -2-bromo acetate is shown in the **Scheme 7**. The optimized geometries of 1, 2 and 3 at the BP86/TZVPP level of theory are shown in the **Figure 2**. The reaction energy for the condensation reaction indicates both the products are

thermodynamically feasible with the release of 152.6 kcal/mol at the M06/TZVPP//BP86/TZVPP level of theory (Scheme 7). The mulliken charge at the CH2 group adjacent to the carbonyl oxygen of the five membered ring indicates a more positive charge for the hydrogen atoms (+0.184 and +0.182) in 2 compared to 3 with +0.171 charge. It can be concluded that there is a slight kinetic preference for electrophilic substitution of hydrogen atoms in 2.

2.1.6 Experimental

Melting poinst recorded on a Toshniwal capillary melting point apparatus are uncorrected. The mass spectrum was recorded on LCMS-2020 Shimadzu equipment as well as JEOL JMS 600H Mass Spectrometer under FAB-MS mode. The NMR experiments were conducted using Bruker Avance III 500 MHz FT-NMR instrument. IR spectra were recorded as KBr pellets using Shimadzu 8101A FTIR equipment. The UV spectrometer used was JASCO V-550 UV/VIS Spectrophotometer. TLC was performed on the glass-backed silica gel sheets (BSS 350). Column chromatography was performed using silica gel of 100-200 mesh and eluted with petroleum ether and ethyl acetate.

Preparation of starting materials

Pyridine-2-carbothioamide

0.07 mol (8.05 mL) of pyridine-2-carbonitrile was added to 100 mL of saturated alcoholic solution of ammonia and kept in an ice-bath. Dry H_2S was passed through the solution till saturation (about 45 min), so that yellow crystals of the product started appearing. The mixture was kept overnight at room temperature, for complete crystallization. The product of pyridine was filtered, washed with cold alcohol and dried. The yellow crystals of pyridine-2-carbothioamide weighed 9.3 g (96.2%) and melted at 135 ^{0}C.

Pyrazine-2-carbothioamide

0.07 mol (6.3 mL) of pyrazine-2-carbonitrile was added to 100 mL of saturated alcoholic solution of ammonia and kept in an ice-bath. Dry H_2S was passed through the solution till saturation (about 2 h), so that yellow crystals of the product started appearing. The mixture was kept overnight at room temperature, for complete crystallization. The product of was filtered, washed with cold alcohol and dried. The yellow crystals of pyrazine-2-carbothioamide weighed 9 g (92.5%) and melted at 200 ^{0}C.

Synthesis of (E)-5-(Amino(aryl)methylene)-2-arylthiazol-4(5*H*)-ones

(E)-5-(Amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-2-carbonitrile (1.15 mL, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 ml, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.69 g(60%). The compound was purified by column chromatography using petroleum ether-ethyl acetate mixture in 1:2 ratio.

(E)-5-(Amino(pyridin-3-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard

tube. Pyridine-3-carbonitrile (1.04 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-3-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.18 g(42%), which also contained some amount of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one. The separation was carried out by means of preparatory TLC, using ethyl acetate as eluent.

(E)-5-(Amino(pyridin-4-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-4-carbonitrile (1.0 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-4-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.13 g(40%), which also contained some amount of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one. The separation was carried out by means of preparatory TLC, using ethyl acetate as eluent.

(E)-5-(Amino(pyrimidin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrimidine-2-carbonitrile (1.0 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyrimidin-4-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.13 g(40%), which also contained some amount of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one. The separation was carried out by means of preparatory TLC, using ethyl acetate as eluent.

(E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-2-carbonitrile (0.9 mL, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.4 g(50%). The

compound was purified by column chromatography using petroleum ether-ethyl acetate mixture in 1:2 ratio.

(E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in10 mL RB flask, fitted with calcium chloride guard tube. Quinoline-2-carbonitrile (1.54 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The orange yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one was 1.8 g(55%). The compound was purified by column chromatography using petroleum ether-ethyl acetate mixture in 1:2 ratio.

(E)-5-(Amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrazine-2-carbonitrile (1.54 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The dark brown product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyrazin-2-

yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.4 g(48%). The compound was recrystallized from DMSO-ethanol mixture.

(E)-5-(Amino(pyridin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-2-carbonitrile (1.15 mL, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The dark brown product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.4 g(50%). The compound was recrystallized from DMSO-ethanol mixture.

(E)-5-(Amino(pyridin-3-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-3-carbonitrile (1.04 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The dark brown product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-3-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.1 g(40%), which also

contained some amount of (E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one. The separation was carried out by means of preparatory TLC, using ethyl acetate as eluent.

(E)-5-(Amino(pyridin-4-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-4-carbonitrile (1.0 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The dark brown product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-4-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.1 g(40%), which also contained some amount of (E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one. The separation was carried out by means of preparatory TLC, using ethyl acetate as eluent.

(E)-5-(Amino(pyrimidin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrimidine-2-carbonitrile (1.0 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethylbromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this

mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The dark brown product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(pyridin-4-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.2g(42%). The compound was recrystallized from DMSO-ethanol mixture.

(E)-5-(Amino(quinolin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one

A catalytic amount of sodium metal (0.1 g) was dissolved in 10 mL absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Quinoline-2-carbonitrile (1.54 g, 0.01 mol), was added to it and allowed to stand overnight at room temperature. Added 5 drops of glacial acetic acid and methanol was completely removed under vacuum. Pyrazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.2 mL, 0.01 mol) and anhydrous sodium acetate (2 g) were added to the imidate in the flask. To this mixture, 10 mL of dry benzene was added and stirred overnight at room temperature. The orange yellow product was filtered, washed with water, ethyl acetate and then with ethanol and dried. The yield of (E)-5-(amino(quinolin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one was 1.7 g(52%). The compound was purified by column chromatography using petroleum ether-ethyl acetate mixture in 1:2 ratio.

2.2 ANTICANCER STUDY OF (Z)-5-(AMINO (ARYL)METHYLENE)-2-ARYLTHIAZOL-4(5*H*)-ONES

Present Study

The compounds (E)-5-(amino(pyridin-2-yl)methylene)-2-(pyridin-2-yl)thiazol-4(5*H*)-one (C) and (E)-5-(amino(pyrazin-2-yl)methylene)-2-(pyrazin-2-yl)thiazol-4(5*H*)-one (D) were subjected for cytotoxicity studies against the same five human cancer cell lines as discussed in chapter 1.

Experimental

The MTT assay employed for the analysis remained the same as discussed in chapter 1.

Result and Discussion

Both the compounds showed only weak cytotoxic activity against all the cell lines. The percentage cell viability data showed that, of the two compounds, C is relatively more active than D. C exhibited better activity in cervical cancer cell line HeLa and brain cell line T98G **(Table 7)**. It is worthy to mention that the cytotoxic studies on imidazoline analogue of C ((E)-4-(amino(pyridine-2-yl)methylene)-2-(pyridine-2-yl)-1H-imidazol-5(4H)-one), gave better results[15], showing that presence of sulphure in place of nitrogen has reduced the cytotoxic activity.

Table 7: Cytotoxic activity of compounds C

Concentration (μM)	**Percentage viability over untreated control**				
	HeLa	A549	A375	MD-AMB-231	T98G
25	75.9	96.4	95.0	81.5	78.5
50	69.1	93.2	84.9	79.6	60.7
100	66.4	93.1	82.8	76.5	56.8
200	48.3	87.3	78.8	73.2	49.3
IC_{50}	**193.4**	**787.4**	**471.6**	**373.1**	**115.7**

The compound D did not show any significant cytotoxic activity against any of the cell lines **(Table 8)**. In fact, the percentage viability over the cell lines showed that the cell lines HeLa and A549 became more resistant towards D, on increasing the concentration.

Table 8: Cytotoxic activity of compounds C

Concentration (μM)	**Percentage viability over untreated control**				
	HeLa	A549	A375	MD-AMB-231	T98G
25	81.5	85.5	99.1	95.6	86.7
50	85.7	92.4	91.4	61.5	78.7
100	100.9	107.0	86.4	59.1	74.9
200	112.1	115.1	83.3	55.1	61.3

References

1. Hofmann, *Ber*. **1879**, 12, 1126, 2359; **1880**, 13, 8.
2. Hantzsch, A.; Weber, J. H. *Ber*. **1887**, 20, 3118.
3. Heilbron, S. I. *J. Chem. Soc*. **1949**, 2099.
4. Ottana, R.; Maccari, R.; Barreca, M. L.; Bruno, G.; Rotondo, A.; Chiricosta, G.; Di Paola, R.; Sautebin, L.; Cuzzocrea, S.; Vigorita, M. G. *Bioorg. Med. Chem*. **2005**, 13, 4243.
5. Gududuru, V.; Hurh, E.; Dalton, J. T.; Miller, D. D. *Bioorg. Med. Chem. Lett*. **2004**, 14, 5289.
6. Rydzik, E.; Zadowska, A.; Kaminska, A. *Acta Pol. Pharm*. **1984**, 41, 459.
7. Havrylyuk, D.; Mosula, L.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. *Eur. J. Med. Chem*. **2010**, 45(5012), e5021.
8. Hofmann, B.; Franke, L.; Proschak, E.; Tanrikulu, Y.; Schneider, P.; Steinhilber, D.; Schneider, G. *ChemMedChem* **2008**, 3, 1535-1538.
9. Hofmann, B.; Barzen, S.; Rodl, C, B,; Kiehl, A.; Borig, J.; Zivkovic, A.;Stark, H.; Schneider, G.; Steinhilber, D. *J. Med. Chem*. **2011**, 54, 1943.
10. Barzen, S.; Rodl, C. B.; Lill, A.; Steinhilber, D.; Stark, H.; Hofmann, B. *Bioorg. Med. Chem*. **2012**, 20, 3575-3583.
11. Werz, O.; Steinhilber, D. *Pharmacol. Ther*. **2006**, 112, 701-718.
12. Radmark, O.; Samuelsson, B. *J. Intern. Med*. **2010**, 268, 5-14.
13. Chen, S.; Chen, L.; Le, N. T.; Zhao, C.; Sidduri, A.; Lou, J. P.; Michoud, C.; Portland, L.; Jackson, N.; Liu, J.-J.; Konzelmann, F.; Chi, F.; Tovar, C.; Xiang, Q.; Chen, Y.; Wen, Y.; Vassilev, L. T. *Bioorg. Med. Chem. Lett*. **2007**, 17, 2134-2138.
14. Jyothi, P.; Ph. D. Thesis, University of Calicut, Kerala, **2009.**
15. Shalina Begum, T. Ph. D. Thesis, University of Calicut, Kerala, **2012**.
16. Begum, T. S.; Jaleel, U. C. A.; Shafi, P. M. *Int. J. Pharm. Biomed. Sci.* **2013**, 4(1), 40-45.
17. Brown, R. S.; Dowden, J.; Moreau, C.; Potter, V. L. *Tetrahedron Lett*. **2002**, 43, 6561-6562.
18. Mulqueen, G.; Pattenden, G.; Whiting, D. *Tetrahedron* **1993**, 49,5359-5364.
19. Vorbrugen, H.; Kroliewick, K. *Tetrahedron* **1993**, 49, 9353-9372.
20. Busacca, C.; Dong, Y.; Spinelli, E. *Tetrahedron Lett*. **1996**, 37,2935-2938.
21. Galeotti, N.; Montagne, C.; Pioncet, J.; Join, P. *Tetrahedron Lett*. **1992**, 33, 2807-2810.
22. Ino, A.; Murabayashi, A. *Tetrahedron* **2001,** 57, 1897-1902.

23. Aitken, R. A.; Armstrong, D. P.; Galt, R. H. B.; Mesher, S. T. E. *J. Chem. Soc., Perkin Trans. I* **1997**, 935-942.
24. Lu, S.-F.; Du, D.-M.; Zhang, S.-W.; Xu, J.*Tetrahedron Asymmetry* **2004**, 15, 3433-3441.
25. Abdel-Jalil, R. J.; Saeed, M.; Voelter, W. *Tetrahedron Lett*. **2001**, 42, 2435-2437.
26. Tiecco, M.; Testaferri, L.; Santi, C.; Tomassini, C.; Marini, F.; Bagnoli, L.; Temperini, A. *Tetrahedron Asymmetry* **2002**, 13, 429-435.
27. Berseneva, V. S.; Birucheva, N. Yu.; Bakulev, V. A. *Khim. Geterosikl. Soedin*. **1993,** 1688.
28. Berseneva, V. S.; Morzherin, Y. Y.; Dehaen, W.; Luyten, I.; Bakulev, V. A. *Tetrahedron* **2001**, 57, 2179-2184.
29. Attanasi, O. A.; De Crescentini, L.; Foresti, E.; Galarini, R.; Santeusanio, S.; Serra Zanetti, F. *Synthesis* **1995**, 1397-1400.
30. Attanasi, O. A.; De Cresentini, L.; Favi, G.; Filippone, P.; Lillini, S.; Mantellini, F.; Santeusanio, S. *Org. Lett*. **2005**, 7, 2469-2471.
31. Subtel'na, I.; Atamanyuk, D.; Szymanska, E.; Fruzinski, A.; Kiec-Kononowicz, B.; Zimenkovsky, O.; Vasylenko, O.; Gzella, A.; Lesyk, R. *Bioorg. Med. Chem*. **2010**, 18, 5090-5102.
32. Qiu, K.-M.; Wang, H.-H.; Wang, L.-M.; Luo, Y.; Yang, X.-H.; Wang, X.-M.; Zhu, H.-L. *Bioorg. Med. Chem***. 2012**, 20, 2010-2018.
33. Zayed, E. M.; Elbannany, A. A. A.; Ghozlan, S. A. S. *Pharmazie* **1985**, 40, 194-196.
34. Hofmann, B.; Franke, L.; Proschak, E.; Tanrikulu, Y.; Schneider, P.; Steinhilber, D.; Scheider, G. *ChemMedChem* **2008**, 3, 1535-1538.
35. Hofmann, B.; Barzen, S.; Rodl, C, B,; Kiehl, A.; Borig, J.; Zivkovic, A.;Stark, H.; Schneider, G.; Steinhilber, D. *J. Med. Chem*. **2011**, 54, 1943.
36. Barzen, S.; Rodl, C. B.; Lill, A.; Steinhilber, D.; Stark, H.; Hofmann, B. *Bioorg. Med. Chem*. **2012**, 20, 3575-3583.
37. Jacob Zabicky, " The Chemistry of amides", Interscience Publishers, 1970, 445-446.
38. Liboska, R.; Zyka, D.; Bobek, M. *Synthesis*, **2002**, 2, 1649-1651.
39. Goswami, S.; Maity, A. C.; Das, N. K. *J. Sulfur Chem***. 2007**, 28, 3, 233-237.
40. Shafi, P. M.; Shobha, T. D.; Basheer, P. A. M. *Ind. J. Chem.* **2005**, 44B, 1298.

41. Becke, A. D. *Phys. Rev. A,* **1988**, 38, 3098–3100; (b) Perdew, J. P. *Phys. Rev. B*, **1986**, 33, 8822–8824; (c) Perdew, J. P. *Phys. Rev. B*, **1986**, 34, 7406–7406.
42. Weigend, F.; Ahlrichs, R. *Phys. Chem. Chem. Phys.,* **2005**, 7, 3297–3303.
43. Frisch, M. J.; Trucks, G. W. ; Schlegel, H. B. ; Scuseria, G. E. ; Robb, M. A. ; Cheeseman, J. R. ; Scalmani, G. ; Barone, V.; Mennucci, B.;. Petersson, G. A; Nakatsuji, H.; Caricato, M. ; Li, X.; Hratchian, H. P. ; Izmaylov, A. F. ; Bloino, J.; Zheng, G. ; Sonnenberg, J. L. ; Hada, M.; Ehara, M. ; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M. Nakajima, T. ; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A. ; Jr. J. A., Peralta, J. E. ; Ogliaro, F.; Bearpark, M.;. Heyd, J. J; Brothers, E.; Kudin, K. N. ; Staroverov, V. N. ; Kobayashi, R.; Normand, J.; Raghavachari, K. ; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W. Martin, Morokuma, K. ; Zakrzewski, V. G. ; Voth, G. A. ; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D. ; Farkas, Ö.; Foresman, J. B. ; Ortiz, J. V. ; Cioslowski, J. and Fox, D. J. Gaussian 09 (*Revision B.01*), Gaussian, Inc., Wallingford CT, **2009**.
44. Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.,* **2008**, 120, 215–241.
45. Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.,* **1988**, 88, 899–926; (b) Glendening, E. D.; Reed, A. E.; Carpenter, J. E.; F.Weinhold, F. *NBO Version 5.9*.

CHAPTER III

SYNTHESIS AND ANTIBACTERIAL STUDY OF (Z)-5-ARYLIDENE-2-ARYL-5H-THIAZOL-4-ONES

3.1 SYNTHESIS OF (Z)-5-ARYLIDENE-2-ARYL-5H-THIAZOL-4-ONES

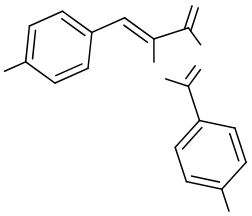
3.1.1 Introduction

Thiazoline derivatives represent a family of compounds with great industrial interest, which have found applications in food and flavor chemistry[1-4]. Thiazolines have also attracted significant biochemical interest, owing to the presence of the thiazoline moiety in the structures of several naturally occurring molecules with important pharmacological properties such as antibiotic[5], antihelmintic[6], antifungal[7] or antitumor drugs[8].

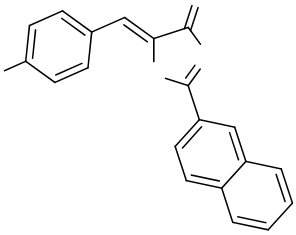
5-Lipoxygenase (5-LO), a non-heme-iron-containing enzyme, catalyzes the biosynthesis of leukotrienes, which are lipid mediators of inflammatory and allergic responses and play a key role in host defense mechanism. Leukotrienes exert their biological effects via specific G-protein-coupled receptors and play a pivotal role in inflammatory and allergic disorders and cardiovascular diseases and cancer[9,10]. Because of the key role of 5-LO in the biosynthesis of leukotrienes, 5-LO inhibitors are supposed to be of therapeutic value for the treatment of asthma, allergic rhinitis, atherosclerosis, and certain types of cancer[10,11].

Hofmann and co-workers[12] synthesized a series of 5-benzylidene-2-phenyl-5H-thiazol-4-ones, by refluxing aromatic nitrile, thioglycolic acid, aromatic aldehyde, and triethylamine in methanol and identified them to be potent direct 5-LO inhibitors. From structure-activity relationship (SAR)

studies, they recognized (Z)-2-(4-chlorophenyl)-5-(4-methoxybenzylidine)-5H-thiazol-4-one, as the most potent derivative of this class.



In 2012, they synthesized[13] an additional set of 5-benzylidene-2-phenyl-thiazolinones with wide range of molecular modifications exemplified by (Z)-5-(4-methoxybenzylidene)-2-(naphthalene-2-yl)-5H-thiazol-4-one and explored the influence of various substituents on 5-LO inhibitory activity.



H_3CO

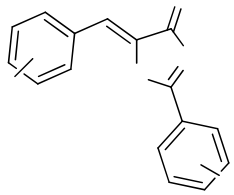
At this juncture, synthesis and biological evaluation of a series of (Z)-5-benzylidene-2-(pyridin-2-yl)-5H-thiazol-4-ones and (Z)-5-benzylidene-2-(pyrazin-2-yl)-5H-thiazol-4-ones, would be quite relevant.

3.1.2 Present Work

In the recent report[12, 13] on the synthesis and biological evaluation of 5-benzylidene-2-phenyl-thiazolinones as potent 5-lipoxygenase inhibitors, the influence of various substituents at the 2-position as well as the 5-position on the 5-LO inhibitory activity has been investigated. They found that 2-phenyl residue is crucial for the activity, though different groups were tolerated at 5-position. They also observed that the activity diminished when the 2-phenyl

H_3CO

moiety was replaced by different aliphatic heterocyles such as pyrrolidine, piperidine and azepane, linked through N.



To the best of our knowledge, this class of compounds containing aromatic heterocyclic residues such as pyridinyl and pyrazinyl moieties at the 2-position are not yet been reported. So, we became interested in the synthesis of a series of 5-benzylidene-2-phenyl-thiazolinones containing 2-pyridinyl and 2-pyrazinyl moieties at 2-position, by a more convenient method.

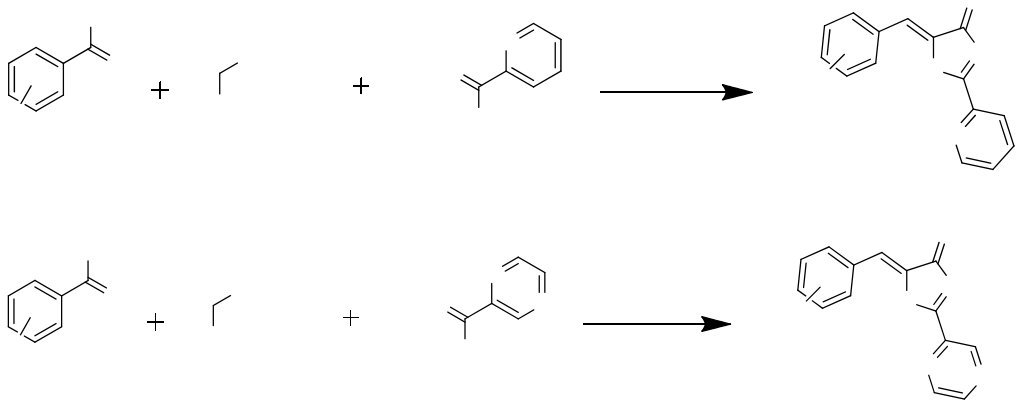
3.1.3 Result and Discussion

Synthesis of (Z)-5-Arylidene-2-aryl-5H-thiazol-4-ones

In the present work, we synthesized a new series of (Z)-5-benzylidene-2-(pyridin-2-yl)-5H-thiazol-4-ones and (Z)-5-benzylidene-2-(pyrazin-2-yl)-5H-thiazol-4-ones, by refluxing thioamide of pyridine-2-carbonitrile/ pyrazine-2-carbonitrile, aromatic aldehydes and ethylbromoacetate in 1:1.5:1.5 molar ratio, in presence of anhydrous sodium acetate in dry benzene.

All the compounds synthesized are reported for the first time. Pyridine-2-carbonitrile and pyrazine-2-carbonitrile were converted to their thioamides by passing H_2S through an ice-cold solution of these nitriles in alcoholic ammonia (chapter II) and employed for cyclization with ethyl bromoacetate. The compounds were filtered, washed with water, ethyl acetate and finally ethanol and dried. The disubstituted thiazolinones were obtained in 50-60% yield. The compounds were recrystallized from DMF-acetic acid mixture.

It is worth to note that, when the reaction was conducted by overnight stirring, aminothiazolinone (chapter II) was also formed in minor amounts along with the product. To do away this, aldehyde and ethyl bromoacetate were taken in excess and the reaction was carried out under reflux conditions. The reactions can be shown as follows **(Scheme 8)**.



Scheme 8

All the compounds gave satisfactory elemental data **(Table 9)**. Mass and NMR spectra of some representative compounds are discussed here.

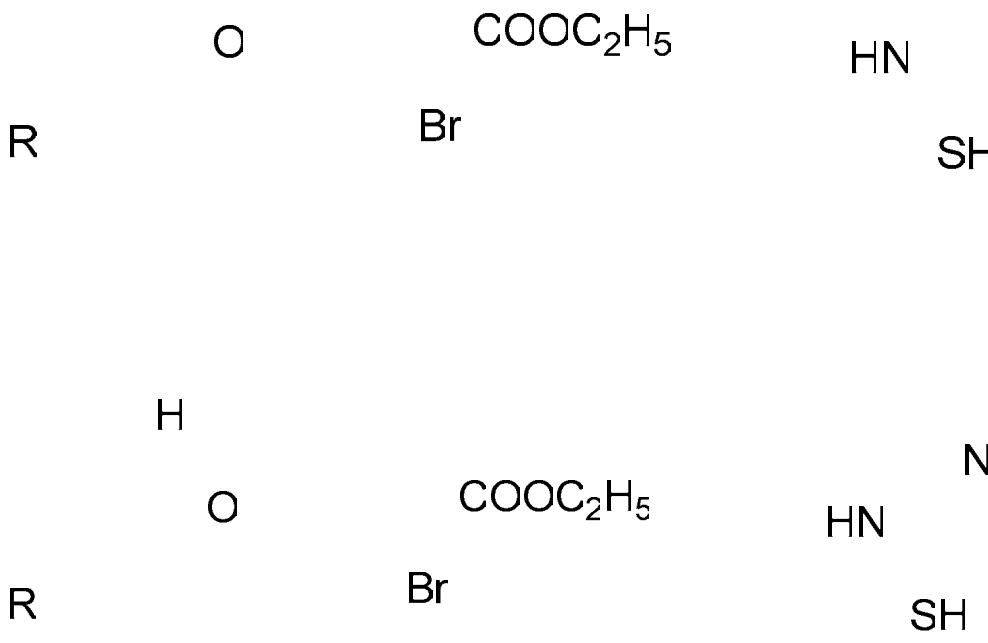
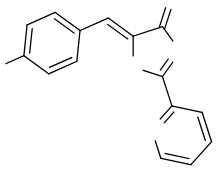


Table 9: Charecteriztion data of Synthesis of (Z)-5-arylidene-2-Aryl-5H-thiazol-4-ones

Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Λ_{max} (nm)	Elemental analysis found (calcd.)		
						C	H	N
1	(Z)-5-benzylidene-2-(pyridin-2-yl)-5H- thiazol-4-one	$C_{15}H_{10}N_2OS$	75	230	386	67.60 (67.65)	3.75 (3.78)	10.50 (10.52)
2	(Z)-5-(2-chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one	$C_{15}H_9ClN_2OS$	42	220	406	59.92 (59.90)	3.05 (3.02)	9.29 (9.31)
3	(Z)-5-(4-chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one	$C_{15}H_9N_2OS$	76	222	389	59.86 (59.90)	2.99 (3.02)	9.34 (9.31)
4	(Z)-5-(4-methylphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one	$C_{16}H_{12}N_2OS$	54	224	390	68.50 (68.55)	4.33 (4.31)	9.96 (9.99)
5	(Z)-5-(4-methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one	$C_{16}H_{12}N_2O_2S$	68	248	455	64.81 (64.85)	4.04 (4.08)	9.41 (9.45)
6	(Z)-5-(2-hydroxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one	$C_{15}H_{10}N_2O_2S$	40	212	392	63.85 (63.81)	3.59 (3.57)	9.90 (9.92)
7	(Z)-5-benzylidene-2-(pyrazin-2-yl)-5H- thiazol-4-one	$C_{15}H_{10}N_2O_2S$	52	246	400	62.95 (62.91)	3.41 (3.39)	15.68 (15.72)
8	(Z)-5-(2-chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one	$C_{14}H_8ClN_3OS$	42	262	395	55.69 (55.72)	2.65 (2.67)	13.95 (13.93)
9	(Z)-5-(4-chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one	$C_{14}H_8ClN_3OS$	80	240	397	55.74 (55.72)	2.64 (2.67)	13.95 (13.93)
10	(Z)-5-(4-methylphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one	$C_{15}H_{11}N_3OS$	40	244	396	64.02 (64.04)	3.97 (3.94)	14.90 (14.94)
11	(Z)-5-(4-methoxyphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one	$C_{15}H_{11}N_3O_2S$	45	190	395	60.61 (60.59)	3.77 (3.73)	14.11 (14.13)
12	(Z)-5-(2-hydroxyphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one	$C_{14}H_9N_3O_2S$	50	236	367	59.32 (59.35)	3.24 (3.20)	14.80 (14.83)

(Z)-5-(4-Methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

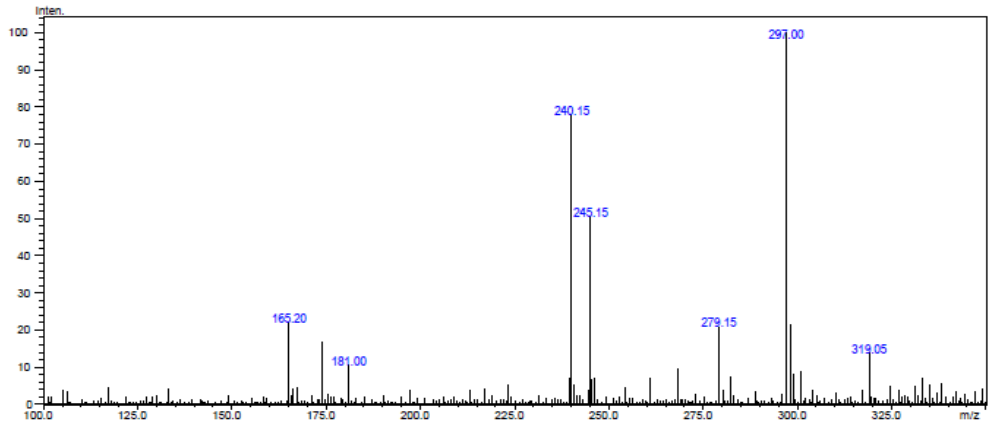
The mass spectrum **(Spectrum 25)** of this compound showed a quasimolecular ion peak $[M+1]^+$ at m/z = 297, suggesting the molecular mass to be 296, which is in support of the proposed structure. The even molecular mass justified the even number of nitrogen atoms in the molecule.



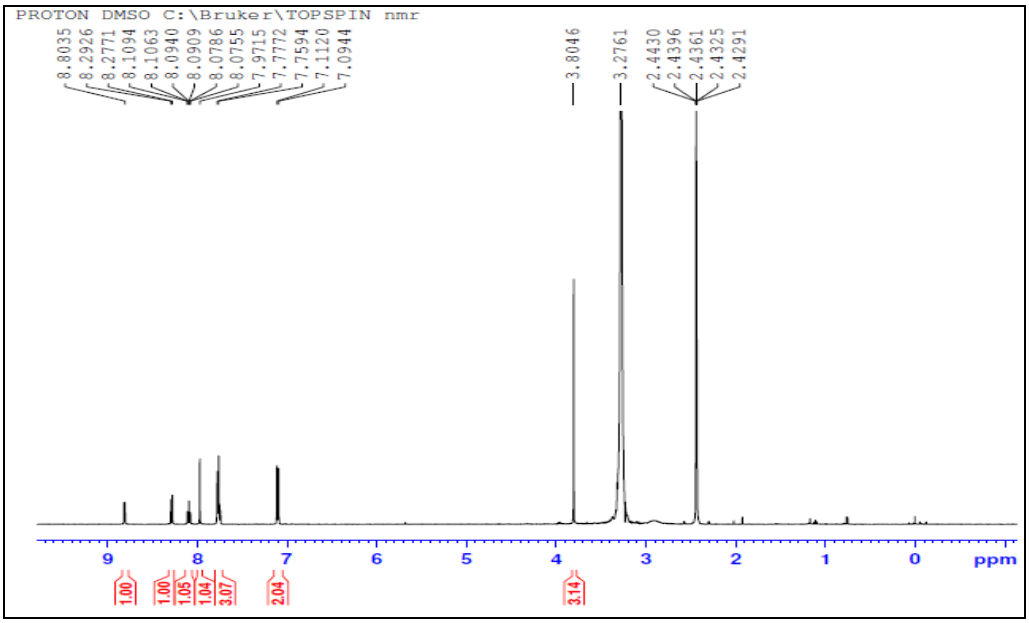
In the ^1H NMR spectrum **(Spectrum 26, 27)**, a singlet at δ 7.9 accounted for the benzylidene proton. The four pyridinyl protons showed absorption between δ 8.8 and 7.7. The four phenyl protons appeared as a pair of doublets between δ 7.7 and 7.1. It is interesting to note that one of the doublets of the phenyl ring and a triplet of the pyridinyl ring got overlapped at around δ 7.7. The tall singlet at δ 3.8 is accounted for the methoxy group.

The IR spectrum showed a lowered carbonyl absorption at 1697 cm^{-1}, which is due to the extended conjugation of CO group to the aromatic ring through the exocyclic double bond at α,β- position.

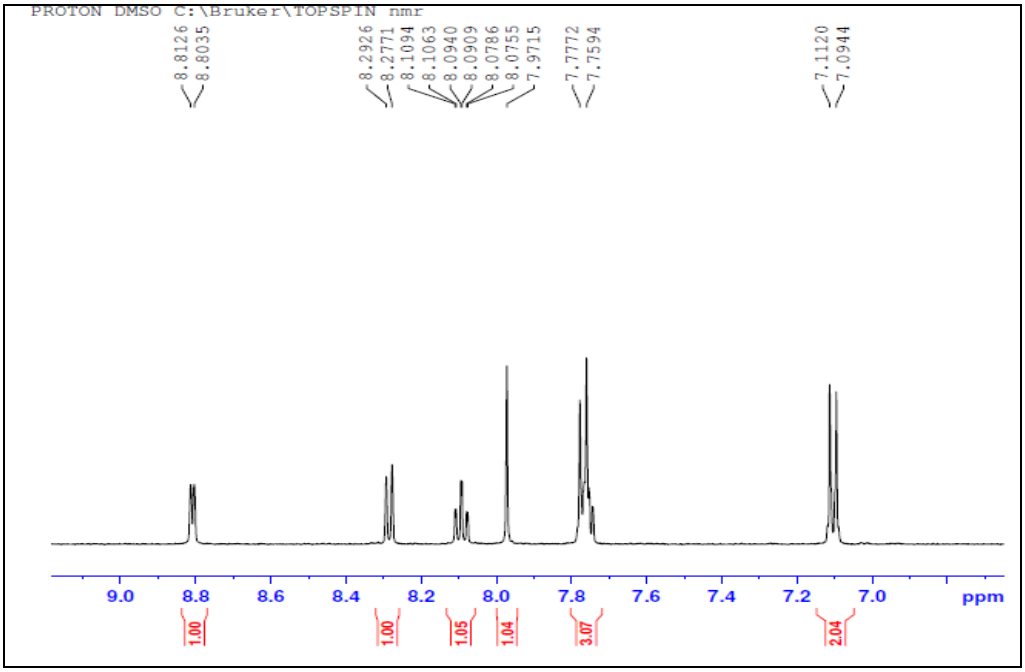
H_3CO



Spectrum 25: Mass Spectrum of (Z)-5-(4-Methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one



Spectrum 26: ^{1}H NMR Spectrum of (Z)-5-(4-Methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

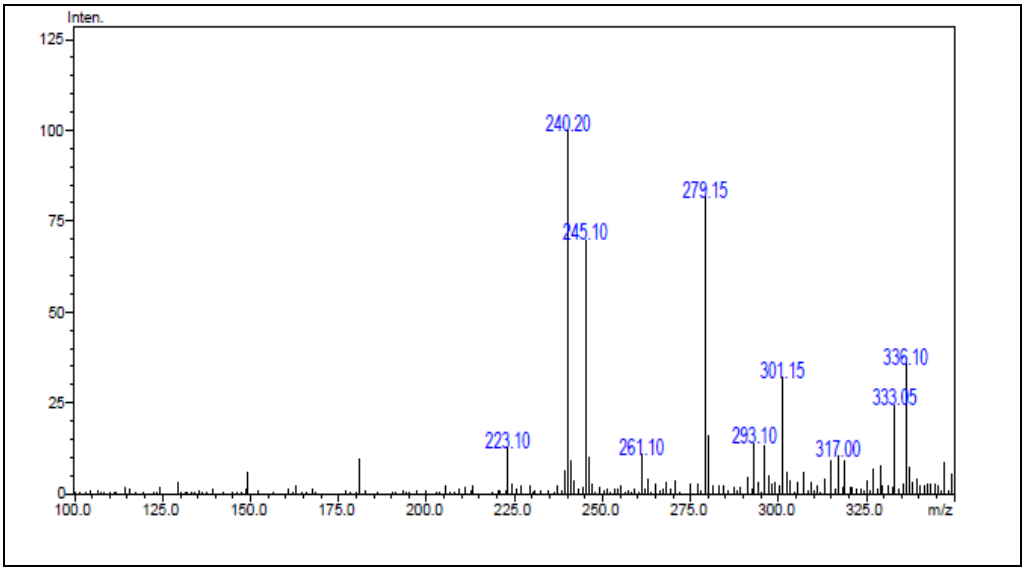


Spectrum 27: ^{1}H NMR Spectrum of (Z)-5-(4-Methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

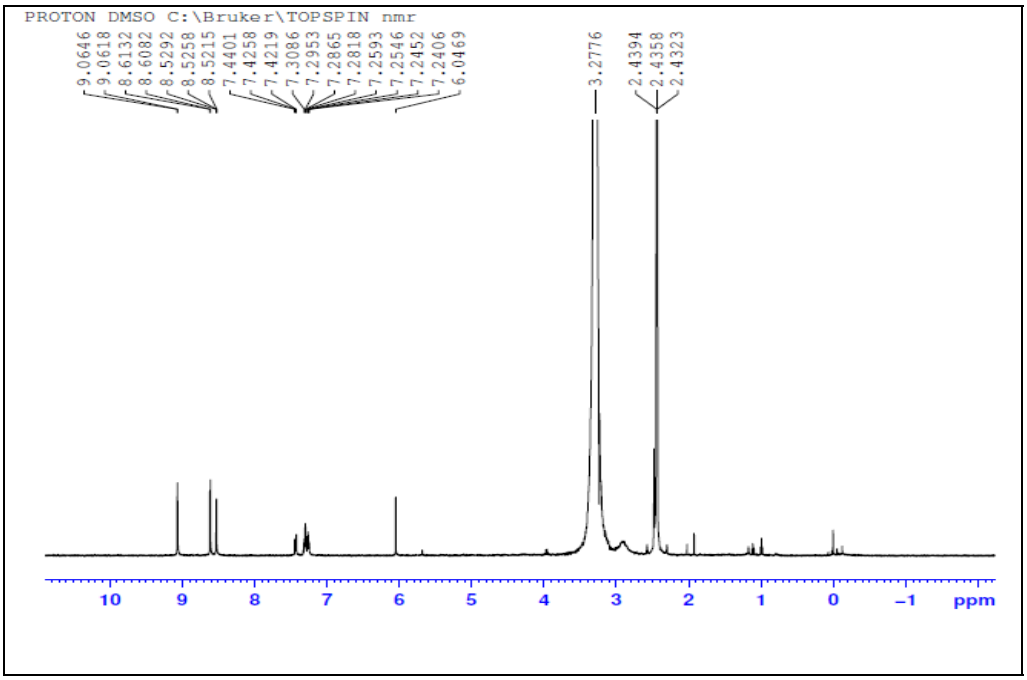
(Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

In the mass spectrum **(Spectrum 28)** the compound exhibited the molecular ion peak at m/z = 301.15, which is in accordance with the proposed structure. The odd molecular mass followed the nitrogen rule as there are three nitrogen atoms in the molecule.

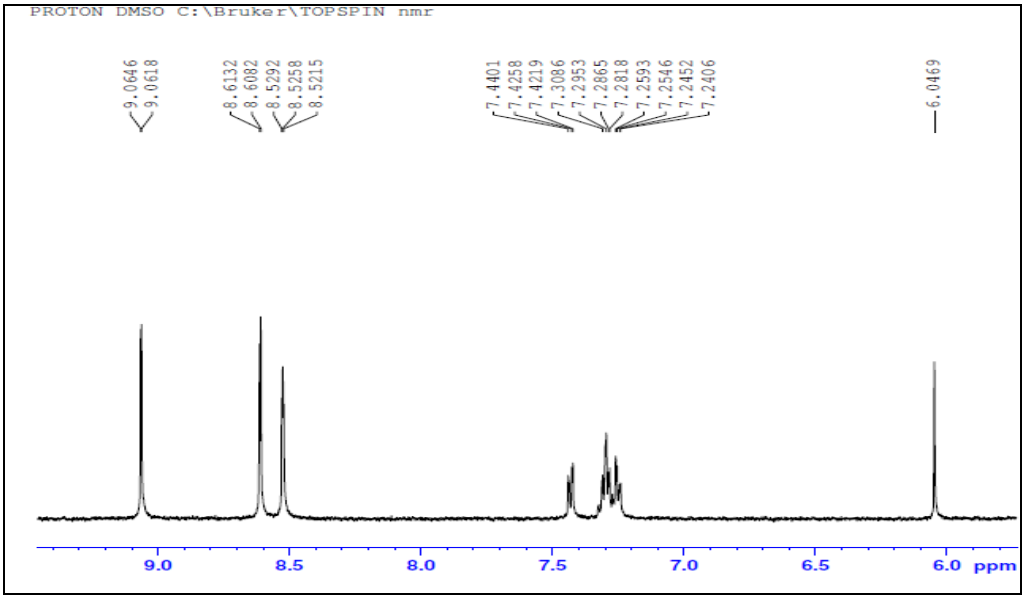
The ^{1}H NMR spectrum **(Spectrum 29, 30)** showed a singlet at δ 6, which corresponded to the benzylidene proton. The three protons of the pyrazinyl ring marked their absorption between δ 9 and 8.5. The four protons of the phenyl ring showed absorption between δ 7.4 and 7.2. ^{13}C NMR spectrum **(Spectrum 31)** also gave satisfactory spectral data.



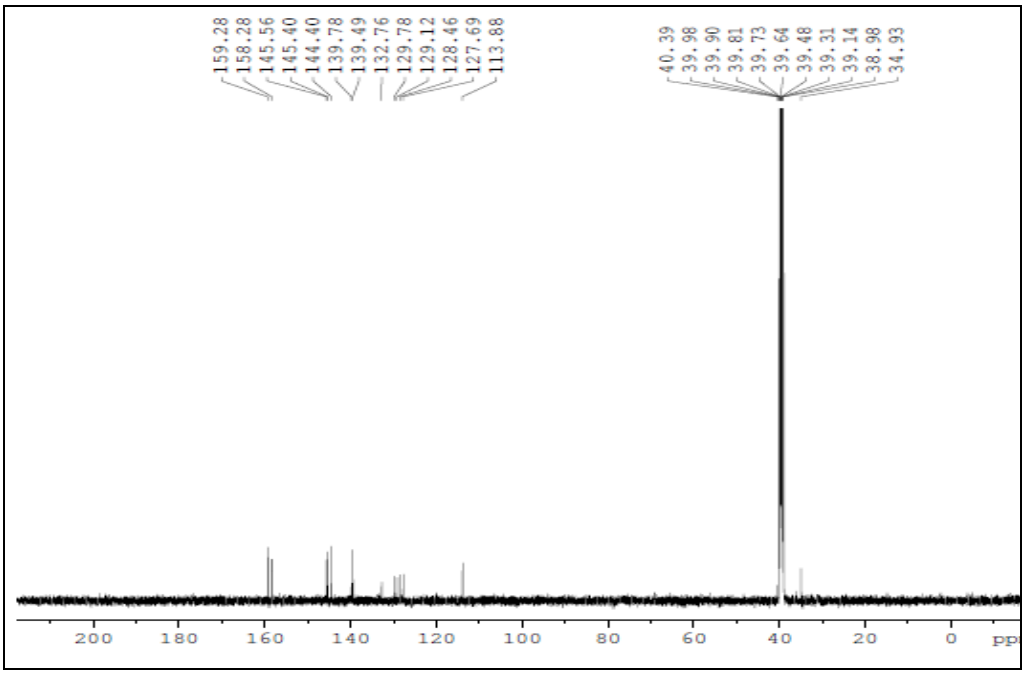
Spectrum 28: Mass Spectrum of (Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one



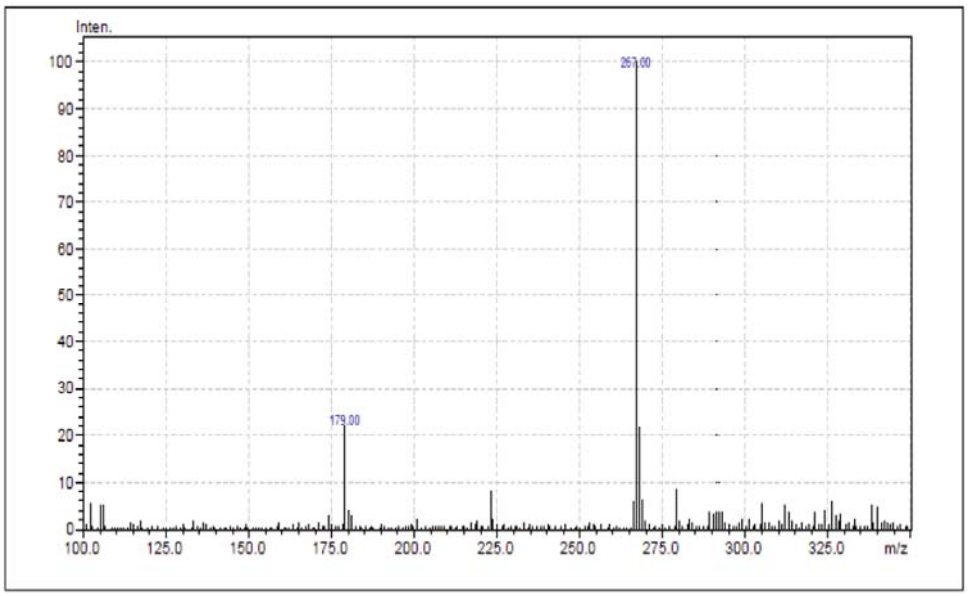
Spectrum 29 : ^{1}H NMR Spectrum of (Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one



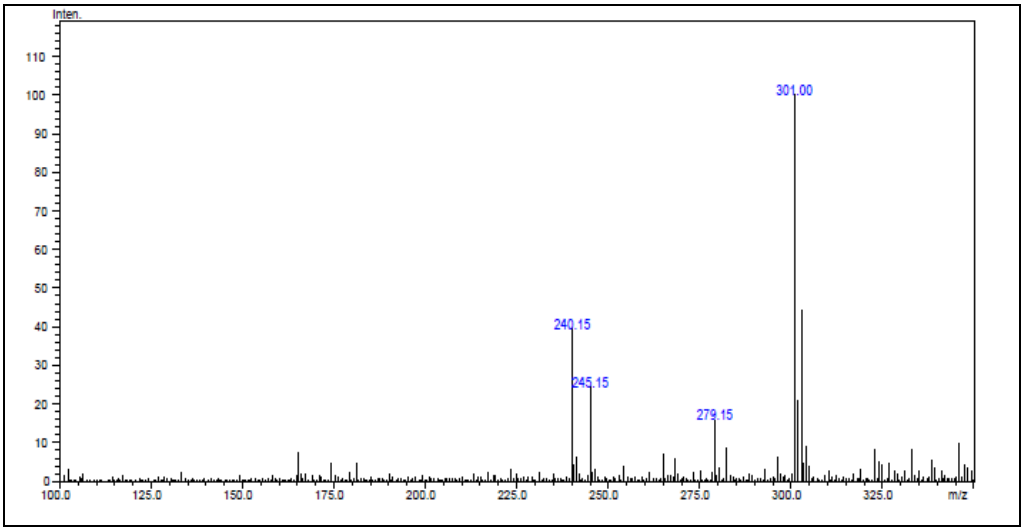
Spectrum 30: ^{1}H NMR Spectrum of (Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one



Spectrum 31: ^{13}C NMR Spectrum of (Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one



Spectrum 32: Mass Spectrum of (Z)-5-(Phenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one



Spectrum 33: Mass Spectrum of (Z)-5-(2-Chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

3.1.4 Experimental

Melting point recorded on a Toshniwal capillary melting point apparatus are uncorrected. The mass spectrum was recorded on LCMS-2020 Shimadzu machine. The NMR experiments were conducted using Bruker Avance III 500 MHz FT-NMR instrument. IR spectra were recorded as KBr pellets using Shimadzu 8101A FTIR equipment. The UV spectrometer used was JASCO V-550 UV/VIS Spectrophotometer. TLC was performed on the glass-backed silica gel sheets (BSS 350).

Preparation of starting materials

Pyridine-2-carbothioamide

0.07 mol (8.05 mL) of pyridine-2-carbonitrile was added to 100 mL of saturated alcoholic solution of ammonia and kept in an ice-bath. Dry H_2S was passed through the solution till saturation (about 45 min), so that yellow crystals of the product started appearing. The mixture was kept overnight at room temperature, for complete crystallization. The product was filtered,

washed with cold alcohol and dried. The yellow crystals of pyridine-2-carbothioamide weighed 9.3 g (96.2%) and melted at 135 ^{o}C.

Pyrazine-2-carbothioamide

0.07 mol (6.3 mL) of pyrazine-2-carbonitrile was added to 100 ml of saturated alcoholic solution of ammonia and kept in an ice-bath. Dry H_2S was passed through the solution till saturation (about 2 h), so that yellow crystals of the product started appearing. The mixture was kept overnight at room temperature, for complete crystallization. The product was filtered, washed with cold alcohol and dried. The yellow crystals of pyrazine-2-carbothioamide weighed 9 g (92.5%) and melted at 200 ^{o}C.

Synthesis of new 5-Benzylidene-2-phenyl-thiazolinones

(Z)-5-Benzylidene-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), benzaldehyde (1.5 mL, 0.015 mol), anhydrous sodium acetate (2 g) and dry benzene (10 mL) were taken in a 100 mL RB flask and refluxed for 8 h on a water bath. After cooling, the pale yellow product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-benzylidene-2-(pyridin-2-yl)-5H-thiazol-4-one was 2 g(75%). The compound was recrystallized from DMF-acetic acid mixture in 1:2 ratio.

(Z)-5-(2-Chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 2-chlorobenzaldehyde (1.8 mL, 0.015 mol), anhydrous sodium acetate (2 g) and dry benzene (10 mL) were taken in a 100 mL RB flask and refluxed for 8 h on a water bath. After cooling, the yellow product was filtered, washed with water, followed by ethyl acetate and

finally with ethanol, and dried. The yield of (Z)-5-(2-chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one was 1.3 g(42%). The compound was recrystallized from DMF-acetic acid mixture in 1:2 ratio.

(Z)-5-(4-Chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-chlorobenzaldehyde (2.1 g, 0.015 mol), anhydrous sodium acetate (2 g) and dry benzene (10 mL) were taken in a 100 mL RB flask and refluxed for 8 h on a water bath. After cooling, the pale yellow product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-chlorophenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one was 2.3 g(76%). The compound was recrystallized from DMF-acetic acid mixture in 1:2 ratio.

(Z)-5-(4-Methylphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-methylbenzaldehyde (1.8 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 8 h on a water bath. After cooling, the pale yellow product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-methylphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one was 1.5 g(54%). The compound was recrystallized from DMF-acetic acid mixture in 1:2 ratio.

(Z)-5-(4-Methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-methoxybenzaldehyde (2 mL, 0.015 mol), anhydrous sodium acetate (2 g) and dry benzene (10 mL) and refluxed for 8 h on a water bath. After cooling, the greenish yellow product was

filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one was 2 g(68%). The compound was recrystallized from isobutanol.

\(Z)-5-(2-Hydroxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one

A mixture of pyridine-2-carbothioamide (1.38 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 2-hydroxybenzaldehyde (2 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 8 h on a water bath. After cooling, the yellow product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(2-hydroxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one was 1.1 g(40%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-Benzylidene-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), benzaldehyde (1.5 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the pale brown product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-benzylidene-2-(pyazin-2-yl)-5H-thiazol-4-one was 1.4 g(52%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-(2-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 2-chlorobenzaldehyde (1.8 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the pale brown product was filtered, washed with water, followed by ethyl acetate and finally

with ethanol, and dried. The yield of (Z)-5-(2-chlorophenyl)-2-(pyazin-2-yl)-5H-thiazol-4-one was 1.3 g(42%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-(4-Chlorophenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-chlorobenzaldehyde (2.1 g, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the pale brown product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-chlorophenyl)-2-(pyazin-2-yl)-5H-thiazol-4-one was 2.4 g(80%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-(4-Methylphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-methylbenzaldehyde (1.8 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the pale brown product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-methylphenyl)-2-(pyazin-2-yl)-5H-thiazol-4-one was 1.1 g(40%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-(4-Methoxyphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 4-methoxybenzaldehyde (2 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the brown product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(4-methoxyphenyl)-2-(pyazin-2-yl)-5H-

thiazol-4-one was 1.3 g(45%). The compound was recrystallized from DMSO-ethanol mixture.

(Z)-5-(2-Hydroxyphenyl)-2-(pyrazin-2-yl)-5H-thiazol-4-one

A mixture of pyazine-2-carbothioamide (1.4 g, 0.01 mol), ethyl bromoacetate (1.8 mL, 0.015 mol), 2-hydroxybenzaldehyde (2 mL, 0.015 mol) and anhydrous sodium acetate (2 g) was dissolved in dry benzene (10 mL) and refluxed for 6 h on a water bath. After cooling, the brown product was filtered, washed with water, followed by ethyl acetate and finally with ethanol, and dried. The yield of (Z)-5-(2-hydroxyphenyl)-2-(pyazin-2-yl)-5H-thiazol-4-one was 1.4 g(50%). The compound was recrystallized from DMSO-ethanol mixture.

3.2. ANTIBACTERIAL STUDY OF (Z)-5-ARYLIDENE-2-ARYL-5H-THIAZOL-4-ONES

Present Study

Two compounds namely (Z)-5-(4-methoxyphenyl)-2-(pyridin-2-yl)-5H-thiazol-4-one (T_1) and (Z)-5-(2-chlorophenyl)-2-(pyazin-2-yl)-5H-thiazol-4-one (T_2) were tested for their *in vitro* antibacterial activity against the same bacterial strains as discussed in chapter I.

Experimental

The antibacterial test carried out remained the same Kirby-Bauer disc diffusion method, as discussed in chapter I.

Result and Discussion

Both the compounds T_1 and T_2 did not show any antibacterial activity against the tested organisms.

References

1. Mac Leod, G.; Ames, J. *J. Food Sci.* **1987**, 52, 42-46.
2. Ong, P.; Acree, T. *J. Agric. Food Chem*. **1998**, 46, 2282-2286.
3. Hartman, G.; Jin, Q. Z.; Collins, G.; Lee, K.; Ho, C. T.; Chang, S. J. *J. Agric. Food Chem*. **1983**, 31, 1030-1033.
4. Fuganti, C.; Gatti, F. G.; Serra, S. *Tetrahedron* 2007, 63, 4762-4767.
5. Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett*. **2007**, 48, 1331-1335.
6. Caujolee, R.; Baziard-Mouysset, G.; Favrot, J. D.; Payard, M.; Louiseau, P. R.; Amarouch, H.; Linas, M. D.; Seguela, J. P.; Louiseau, P. M.; Bories, C.; Gayral, P. *J. Med. Chem*. **1993**, 28, 29-35.
7. Hayashi, K.; Sato, C.; Hiki, S.; Kumagai, T.; Tamai, S.; Abe, T.; Nagao, Y. *Tetrahedron Lett*. **1999**, 40, 3761-3764.
8. Rostom, S. A. F. *Bioorg. Med. Chem.* **2006**, 14, 6475-6485.
9. Peters-Golden, M.; Henderson, W. R.; Jr. Leokotrienes. N. *Engl. J. Med.* **2007**, 357, 1841-1854.
10. Werz, O.; Steinhilber, D. *Pharmacol. Ther*. **2006**, 112, 701-718.
11. Radmark, O.; Samuelsson, B. *J. Intern. Med*. **2010**, 268, 5-14.
12. Hofmann, B.; Barzen, S.; Rodl, C, B,; Kiehl, A.; Borig, J.; Zivkovic, A.;Stark, H.; Schneider, G.; Steinhilber, D. *J. Med. Chem*. **2011**, 54, 1943.
13. Barzen, S.; Rodl, C. B.; Lill, A.; Steinhilber, D.; Stark, H.; Hofmann, B. *Bioorg. Med. Chem*. **2012**, 20, 3575-3583.

CHAPTER IV

SYNTHESIS AND ANTIOXIDANT PROPERTY OF A FEW 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONES

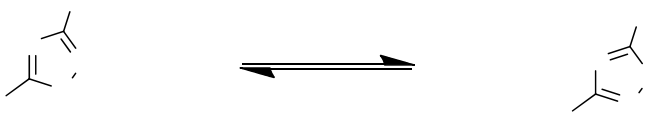
4.1. SYNTHESIS OF A FEW 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE -5 (4*H*)-THIONES

4.1.1 Introduction

Aromatic ring systems with three nitrogen and two carbon atoms are called triazoles. The two possible combinations of the five atoms account for vicinal or 1,2,3-triazole and symmetrical or 1,2,4-triazole. 1,2,4-Triazoles can be considered as cyclic hydrazidines with H atom (or substituent) on either hydrazide nitrogen (I) or on amide nitrogen (II), of which the former is more preferred.

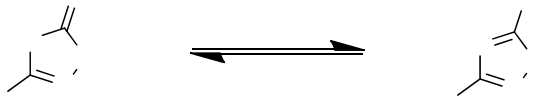
I II

N-Unsubstituted 1,2,4-triazoles exist in two tautomeric forms (if substituents R_3 and R_5 are different) with the predominance of 1H- or 2H-form depending on the conditions.

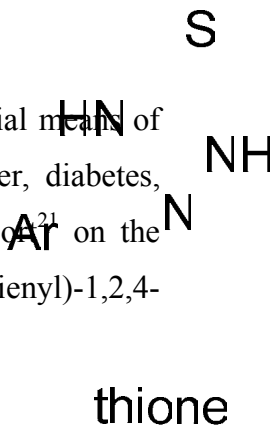


1,2,4-Triazole and its derivatives are reported to exhibit various pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anticancer and antioxidant properties[1-4]. Some present day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agent) are examples of potent molecules possessing a triazole nucleus[5-7].

Spectral studies show that 3-aryl-5-mercapto-1,2,4-triazoles exist as thione and thiol form in both solid state and solutions[8].



3-Substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, generally called triazolethiones have received considerable attention owing to their synthetic and effective biological importance. Derivatives of the mercapto- and thione-substituted 1,2,4-triazole ring systems have variety of biological properties such as antiinflammatory[9,10], antithyroid[11], antibacterial[12,13], analgesic[10,14], antitubercular[15,16], and antidepressant[16]. Triazole-3-thiol derivatives are potential blood platelet aggregation inhibitors[17] and tyrosine kinase inhibitors[18]. In addition, they are used in photosensitive materials[19], as corrosion inhibitors[20] and also in synthesis of several active heterocyclic compounds.



These days, antioxidants are gaining attention as a potential means of treating a large number of life style diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases. In a recent report[21] on the synthesis and biological evaluation of 4-substituted-5-(2-thienyl)-1,2,4-

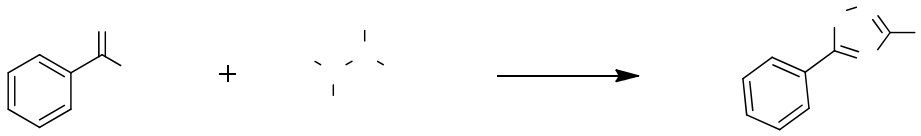
triazole-3-thiones, the antioxidant activity of 1,2,4- triazoles bearing thienyl moiety has been discussed.

In the light of the above facts, synthesis and evaluation of antioxidant activity of some 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones are of great significance.

4.1.2 Review

Since this chapter describes the synthesis of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, a brief review of various synthetic methods available for the construction of mercapto and thione substituted 1,2,4-triazoles are being discussed. Triazolethiones can be synthesized by both intramolecular and intermolecular condensation[22].

In 1949, Hoggarth[23] obtained 3-phenyl-1,2,4-1*H*-thiol by strirring benzoyl isothiocyanate with hydrazinehydrate in ethanol for 15 hours at room temperature.



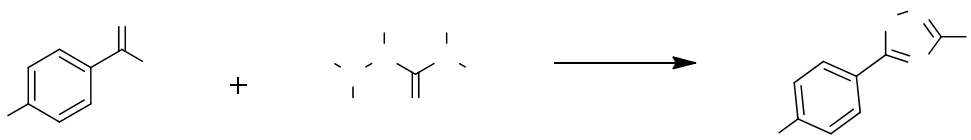
Hoggarth further improved his method by using benzoyl derivatives. Phenyl derivatives were obtained by treating phenylhydrazine in ethanol-HCl with NH_4CNS for 18 hours. Gentle refluxing of benzoyl derivatives of thiosemicarbazide in sodium ethoxide for 12 h followed by neutralization with 10% acetic acid gave 3-phenyl-1*H*,1,2,4-triazole-5-thiol. Piperidine instead of sodium ethoxide gave a similar result but pyridine gave a slower reaction.

Sugii and Yamazaki[20] reported the synthesis of 3-phenyl-1*H*,1,2,4-triazole-5-thiol, obtained by heating a solution of NaOH with 1-benzoylthiosemicarbazide for 3 h and acidifying the mixture. They also found that these compounds were useful for the detection of copper and bismuth.

In 1966, Willems and Vandenberghe[24] synthesized a series of 5-substituted-1,2,4-triazole-5-thiol by refluxing the appropriate carboxylic acid esters with thiosemicarbazide in sodium methoxide-methanol for 16 h, followed by acidification.

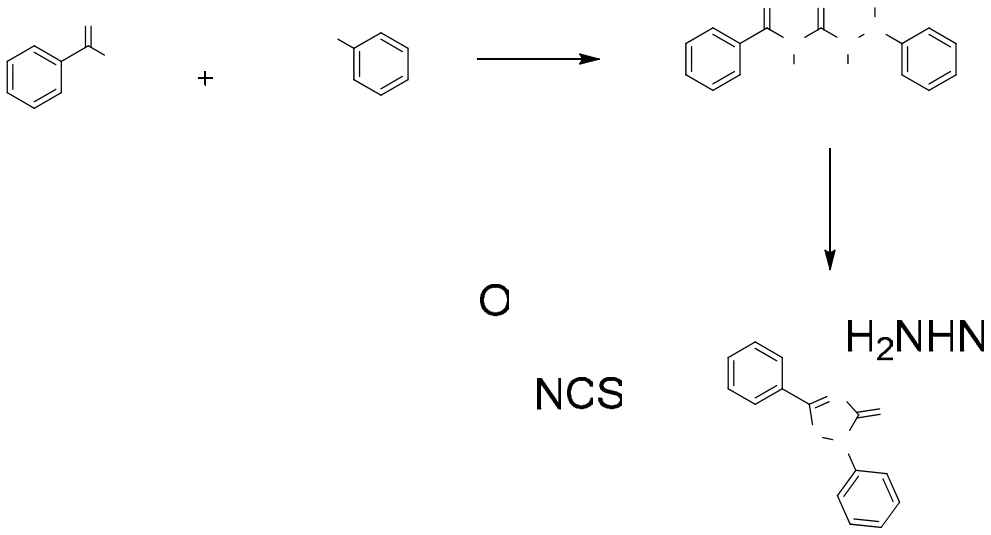
Dutta and co-workers[17] prepared 1,2,4-triazole-5-thiol by cyclization of acylthiosemicarbazide in sodium ethoxide. In some cases hydrazides were heated with thiourea. Isonicotinic acid hydrazide and thiourea were heated for 6 h at 125-30 ^{o}C and then stirred with NaOH and neutralized with HCl to give 3-(4-pyridyl)-1,2,4-triazole-5-thiol.

In 1976, Boyle and Saunders[25] obtained 1,2,4-tiazole-3-thiols by condensing substituted acid chlorides with thiosemicarbazide.



In 1980, Barnikow and Ebeling[26] treated N-substituted isothiocyanate with hydrazine to give triazolinethiones with loss of amine in 34-66% yield.

Uher and co-workers[27] synthesized substituted 1,2,4-triazoline-5-thiones by the condensation of aroyl isothiocyanate with hydrazinehydrate and arylhydrazine.

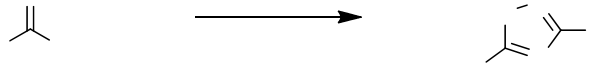


In 1981, Dubina and co-workers[28] reported the synthesis of 3-phenyl-1,2,4-triazole-5-thiol by condensing an acylisothiocyanate with hydrazinehydrate in an organic solvent. The yield was increased and the reaction time was decreased by using benzenesulphonyl benzimidoyl isothiocyanate as the acyl isothiocyanate and benzene or toluene as the solvent.

Malbec and co-workers[29] obtained 4,5-disubstituted-2,4-dihydro-1,2,4,-triazole-3-thiones by the reaction between primary amines, arylhydrazines or aroylhdrazines and thiosemicarbazones of esters. Thermolysis of these thiosemicarbazones gave triazolethiones as well as aminothiadiazoles.

Somarai and co-workers[30] reported the synthesis of triazolinethiones by the cyclization of the thiosemicarbazides with acid halides in benzene. The mixture was stirred for 80-90 ^{o}C for 90 minutes, followed by the addition of aqueous NaOH and methanol for phase separation to give triazolinethiones.

In 1985, Andrae and Schmitz[31] prepared triazolethiols by the reaction of substituted acid halides with a metal thiocyanate and hydrazine in a solvent at -70 to 200 ^{o}C. 15% Hydrazine was treated with reaction product of lead thiocyanate with benzoyl chloride in toluene to give 58% of 3-phenyl-1,2,4-1*H*-triazole-5-thiol.



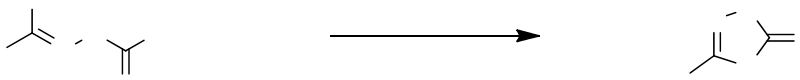
In 1992, Kalluraya and co-workers[32] synthesized 5-aryl-1,2,4-triazole-3-thiones by refluxing appropriate aroylthiosemicarbazide and KOH in ethanol, followed by acidification with acetic acid. The aroylthiosemicarbazides employed in these reactions were obtained by refluxing the corresponding aroylhydrazine with ammonium thiocyanate in presence of aqueous HCl for 3 hours.

Zhang and Lin[33] reported the synthesis of triazolinethiones by the addition reaction of 2,4-dichlorophenoxyacetic acid with aroylisothiocyanate to give aroylthiosemicarbazides, followed by cyclization in presence of KOH.

Pb(SC

H_2N-NH

Modzelewska and Szumilo[34] prepared substituted 1,2,4-triazoline-5-thione by heating derivatives of thiosemicarbazone acid amides in butanol. The acid amides were obtained by the reaction of N^3-substituted amidrazones with ammonium thiocyanate in HCl.

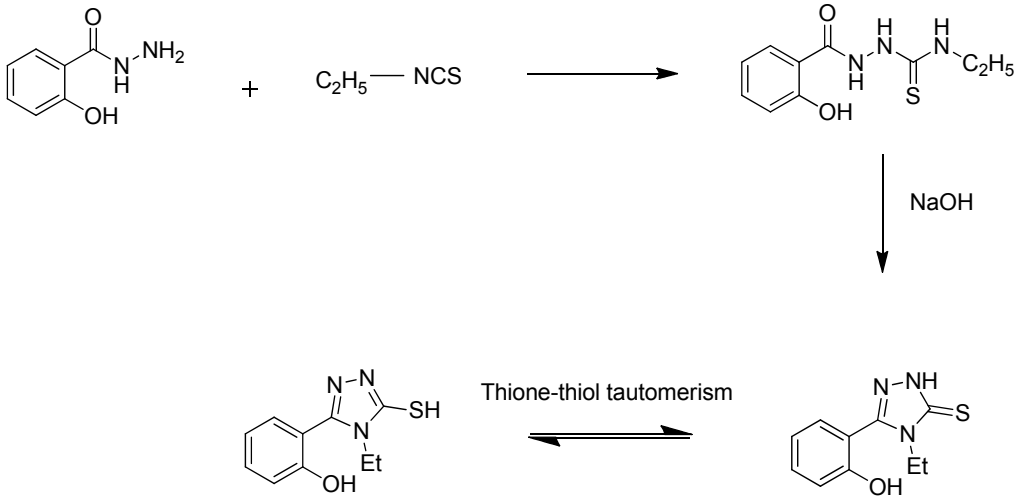


In 1997, Wang and co-workers[35]synthesized 3-aryl-1,2,4-triazoline-5-thione by microwave irradiation of aroyl derivatives of thiosemicarbazide in aqueous NaOH for several minutes. These compounds showed antibacterial activity[36].

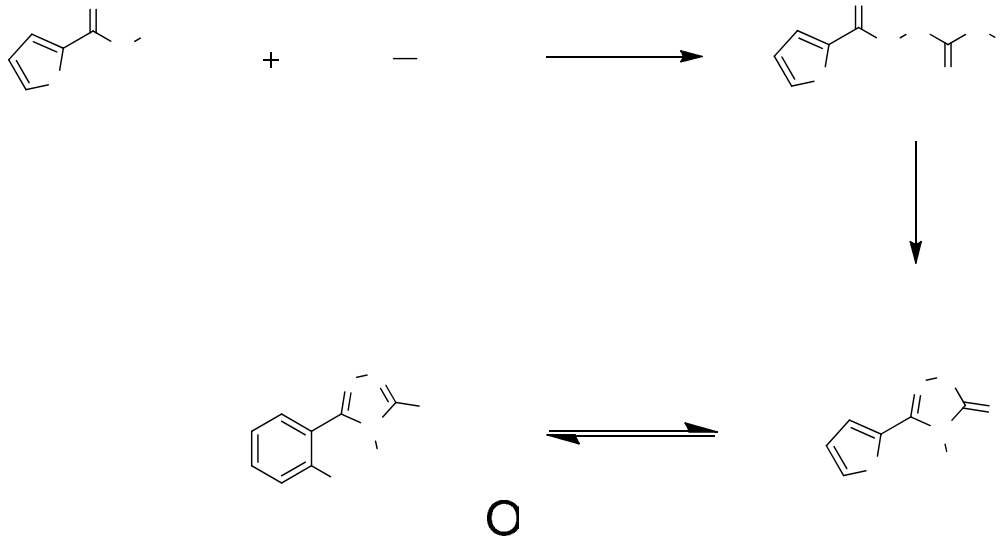
In 2002, Foks and co-workers[37] synthesized substituted 1,2,4-triazole-3-thiones starting from N-methylhydrazine of 4-chlorobenzoic acid.

In 2009, Cansiz and co-workers[38] reported the synthesis of 5-(2-hydroxyphenyl)-4-substituted-3*H*-1,2,4-triazole-3-thione by the reaction of salicylic acid hydrazide with isothiocyanate followed by cyclisation of 1,4-substituted thiosemicarbazides.

Quite recently in 2013, Koparir co-workers[39] reported the synthesis of 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione obtained by refluxing 2-hydroxybenzohydrazide with ethyl isothiocyanate in absolute ethanol for 8 h to get 1(2-hydroxybenzoyl)-4-ethyl thiosemicarbazide, which was further refluxed with NaOH solution for 4 h followed by acidification with HCl. They also investigated the antimicrobial and antioxidant activities of the synthesized compounds.



Koparir and co-workers[40] further synthesized a novel series of 4-substituted-5-(2-thienyl)-1,2,4-triazole-3-thiones, by similar procedure and examined the antimicrobial and antioxidant activities of the title compound as well as their Mannich bases.



4.1.3 Present Work

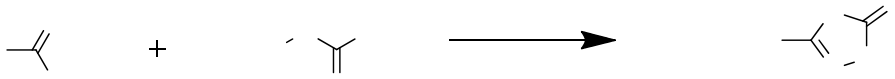
1,2,4-Triazolethiones can be prepared by various methods[22-24,33,35]. However, the known methods mostly require long reaction time and the starting materials are not easily available. In the present work, we developed a novel method for the preparation of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, from easily available compounds, the nitriles and thiosemicarbazide, which is cost effective, more handy and required short reaction time.

We also thought of investigating the antioxidant activity of a few triazoles, bearing biologically active rings such as pyridinyl, pyrazinyl, pyrimidine and quinolinyl.

4.1.4 Result and Discussion

In the present investigation, we synthesized six 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones from aromatic nitriles by two new methods (**method A and method B**) and fully characterized.

In **method A**, corresponding nitriles were first converted to their imidic acid esters by the method adopted by Pinner[41] and improved by others[42-44] or the method suggested by Fred and Grace[45] (**Chapter I**) as the case may be, and subsequently heated with equimolar amount of thiosemicarbazide in a wax bath in a long boiling tube and kept at 140-200 ^{0}C for 15 minutes. Evolution of ammonia was observed during the reaction (**Scheme 9**). After completion of the reaction, the product was filtered, washed with water and then with methanol. Six triazolethiones were synthesized by this method and fully characterized (**Table 10**).

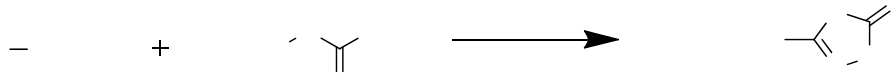


Scheme 9

Table 10: Characterization data of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones (Method A)

Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Elemental analysis found (calcd.)		
					C	H	N
1	3-(pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_6H_5N_5S$	54	258	40.18 (40.21)	2.83 (2.81)	39.06 (39.08)
2	3-(quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{11}H_8N_4S$	59	285	57.86 (57.88)	3.55 (3.53)	24.58 (24.54)
3	3-(3-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_8H_6N_4O_2S$	60	220	43.22 (43.24)	2.68 (2.72)	25.24 (25.21)
4	3-(4-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_8H_6N_4O_2S$	58	240	43.20 (43.24)	2.69 (2.72)	25.18 (25.21)
5	3-(naphthalen-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{12}H_9N_3S$	40	210	63.39 (63.41)	3.95 (3.99)	18.52 (18.49)
6	3-(2-carbamoylphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_9H_8N_4OS$	60	245	49.06 (49.08)	3.64 (3.66)	25.40 (25.44)

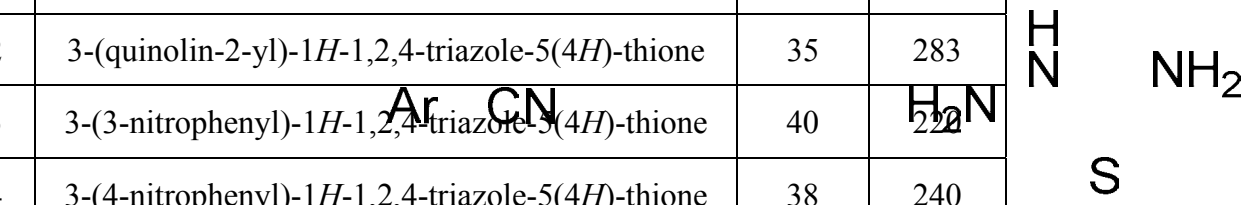
In **method B**, nitriles were directly heated with equimolar amount of thiosemicarbazide in a wax bath in a long boiling tube and kept at 140-200 ^{0}C for 15 minutes. Evolution of ammonia was observed during the reaction (**Scheme 10**). After completion of the reaction, the product was filtered, washed with water and then with methanol (**Table 11**).



Scheme 10

Table 11. Characterization data of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones (Method B)

Sl. No.	Name	Yield %	m.p(^{0}C)
1	3-(pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	30	258
2	3-(quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	35	283
3	3-(3-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione	40	222
4	3-(4-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione	38	240
5	3-(naphthalen-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	27	210
6	3-(2-cyanophenyl)-1H-1,2,4-triazole-5(4H)-thione	40	225

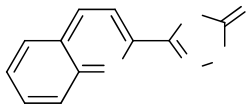


It is obvious that, the yield obtained using the method A is more than that in the case of the method B. It can be accounted for the fact that imidate form of nitrile is more reactive towards nucleophilic attack than nitrile itself. We tried to improve the yield of the method B by acid catalysis using acetic acid, but did not work out.

An interesting pattern was seen in the case of phthalonitrile. Only one of the cyano group underwent cyclization for steric reason. The other cyano group either remained as such (m/z (M+1) = 203) or got converted to carboxamide (m/z = 220), probably during the course of conversion to imidic acid ester (**Spectrum 41**).

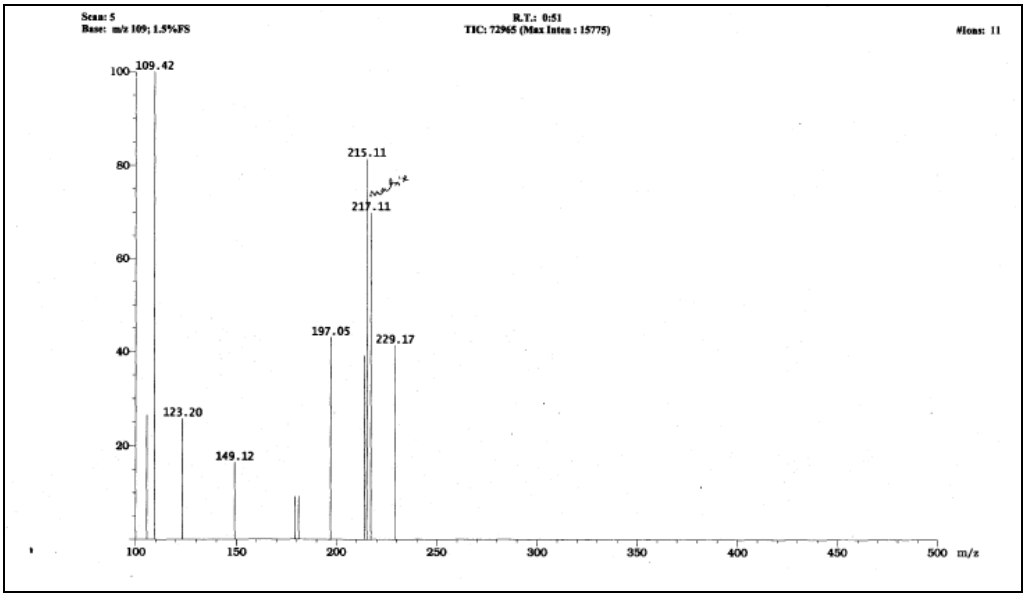
All the componds synthesized gave satisfactory analytical data. The mass, ^{1}H NMR and ^{13}C NMR spectra of a few representative compounds are being discussed here.

3-(Quinolin-2-yl)-1H-1,2,4-triazole-5(4H)-thione

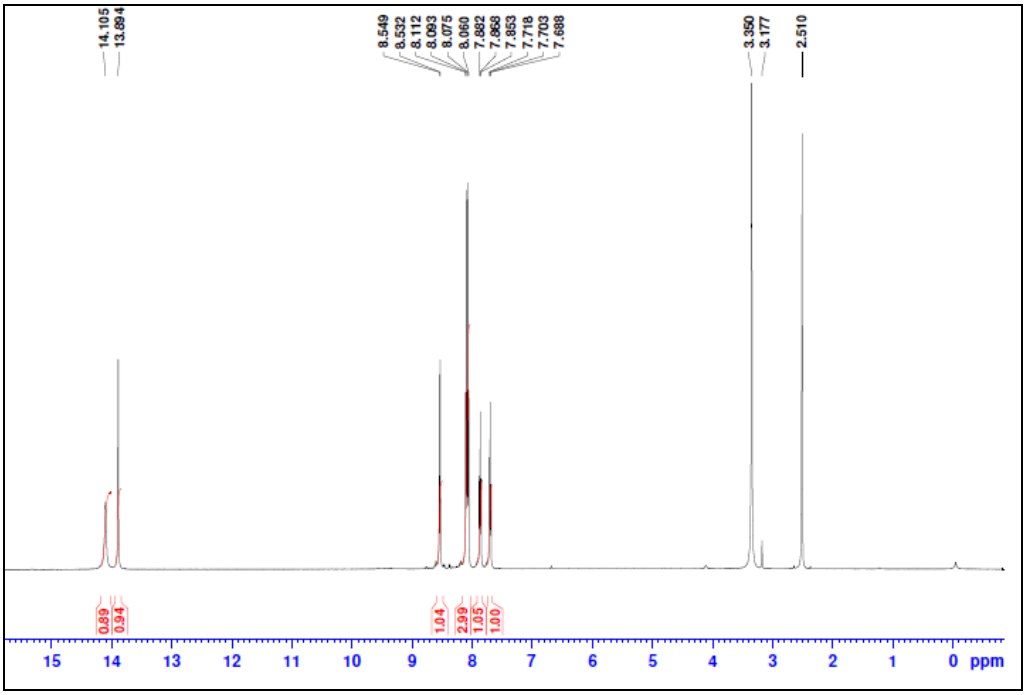


The mass spectrum (**Spectrum 34**) exhibited a peak at m/z = 180, which is the quasimolecular ion $[M+1]^{+}$ peak, suggesting that the molecular mass of the proposed structure to be true (179). The nitrogen rule is justified by the odd molecular mass, as the molecule has four nitrogen atoms. In the ^{1}H NMR spectrum (**Spectra 35, 36**), two singlets appeared at δ 13.9 and 14.1, which are due to the two protons in the triazole ring. The six aromatic protons of quinoline ring were found to absorb between δ 7.7 and 8.5.

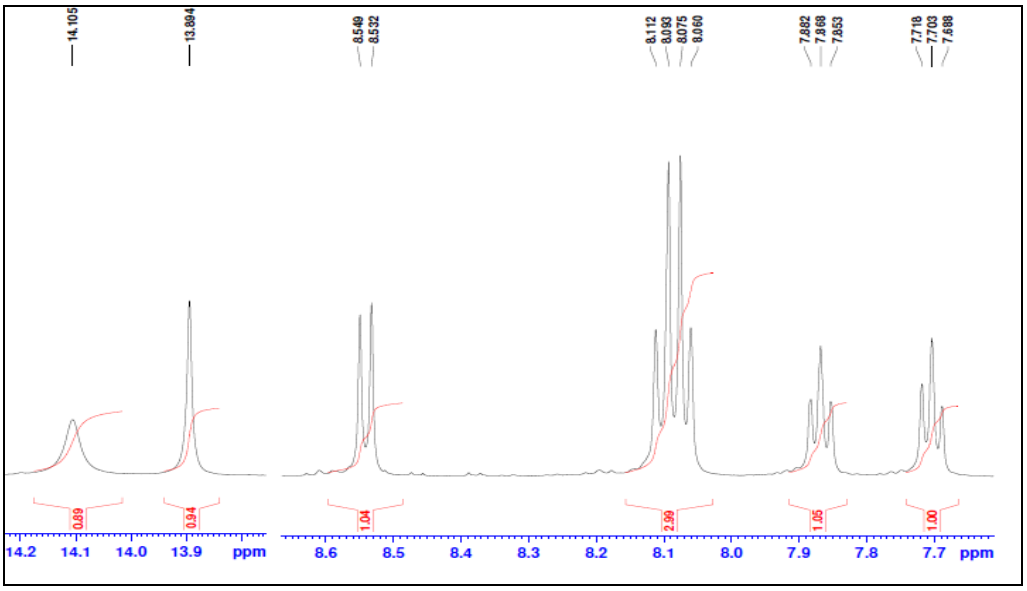
The ^{13}C NMR spectrum (**Spectrum 37**) also supported the findings. It displayed eleven peaks between δ 118 and 167.9 as expected.



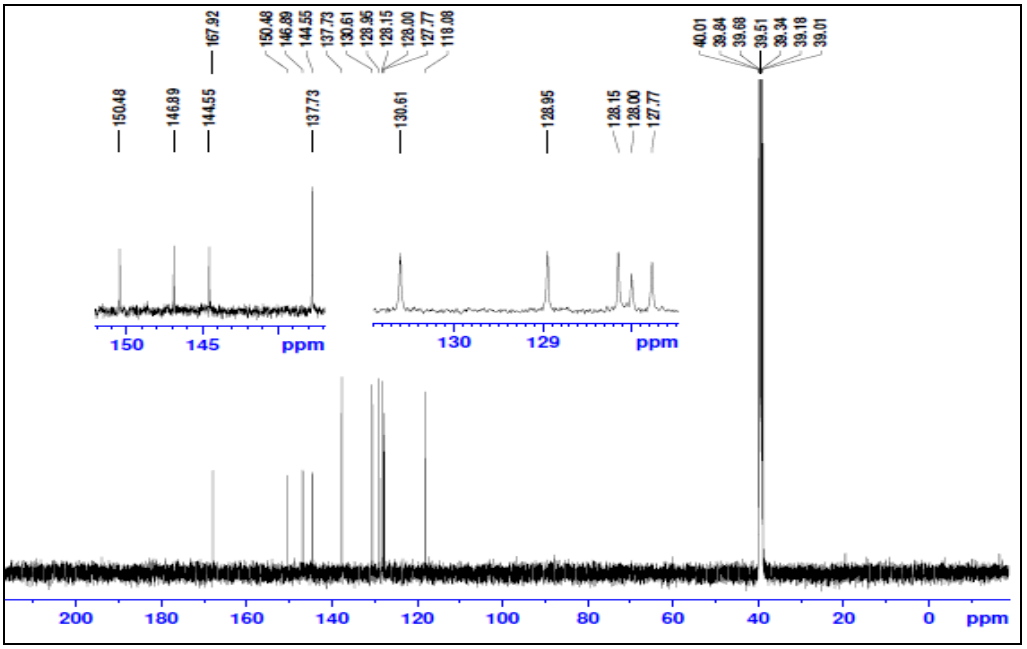
Spectrum 34: Mass spectrum of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 35: ^{1}H NMR spectrum of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

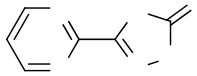


Spectrum 36: ^{1}H NMR spectrum of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 37: ^{13}C NMR spectrum of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

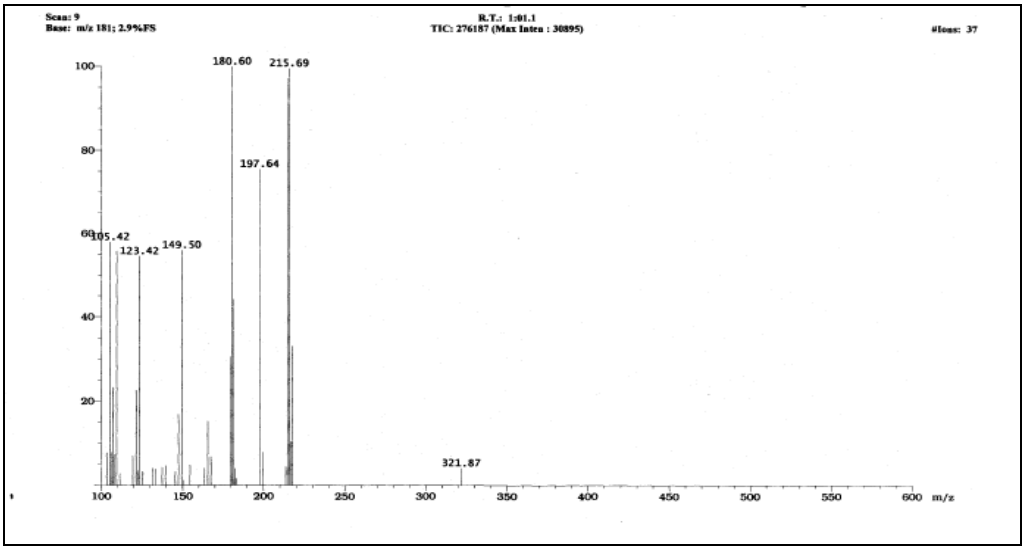
3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



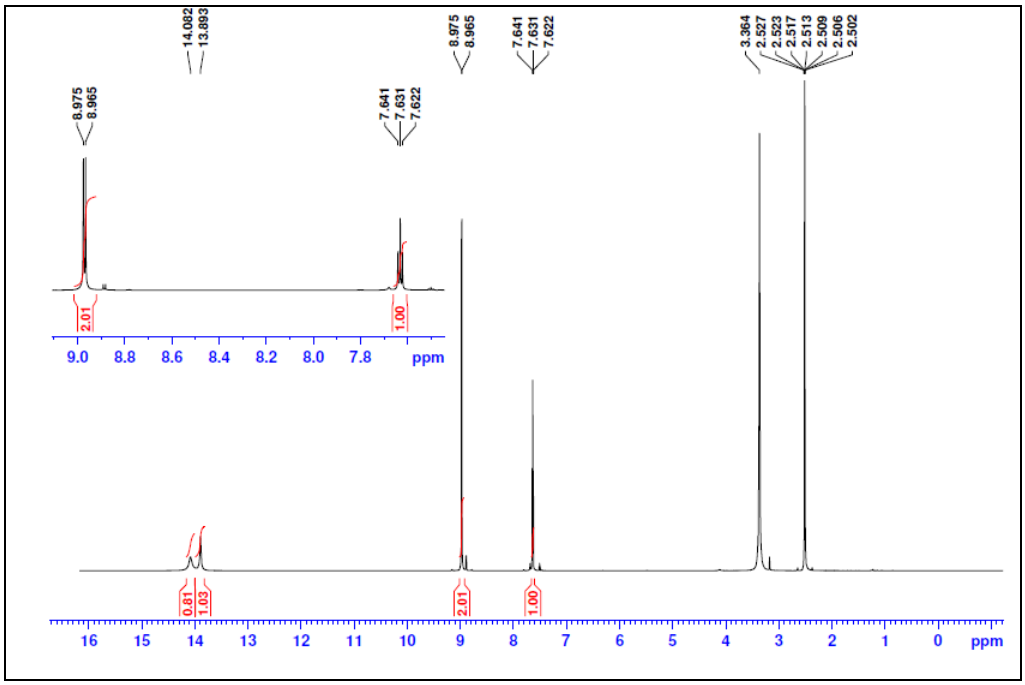
The mass spectrum of this compound (**Spectrum 38**) showed a peak at m/z = 229, which corresponds to the quasimolecular ion $[M+1]^+$ peak, suggesting that the molecular mass of the compound is 228. This is in line with the proposed structure. The even molecular mass agreed with the even number of nitrogen atoms in the molecule.

In the ^{1}H NMR spectrum (**Spectra 39**), two singlets due to the two NH protons of the triazole ring appeared at δ 13.9 and 14.1. The three aromatic protons of pyrimidine ring absorbed between δ 7.6 and 8.9.

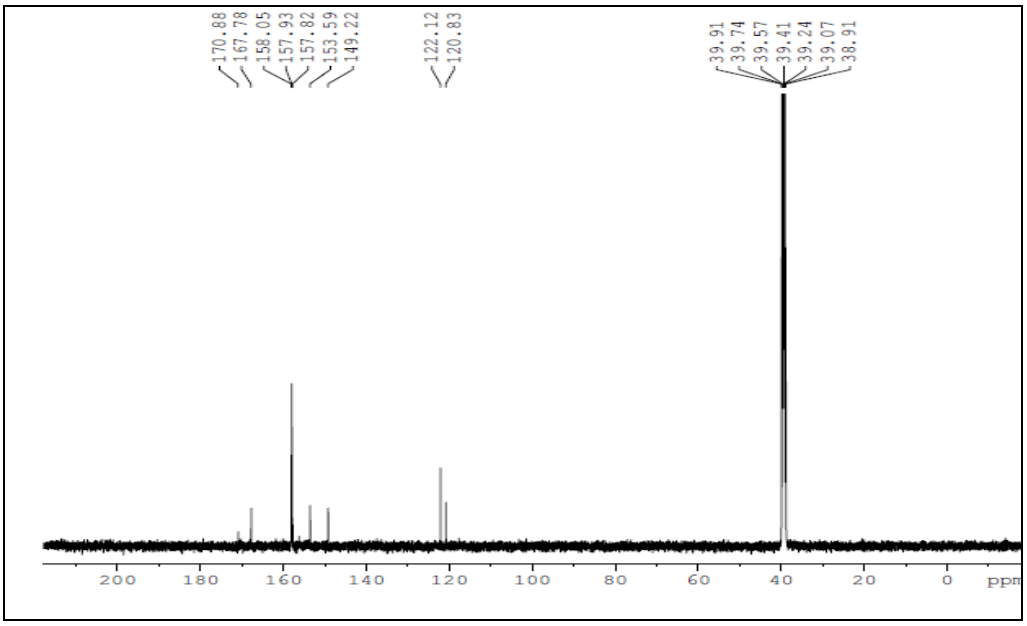
The ^{13}C NMR spectrum (**Spectrum 40**) also supported the findings. It displayed characteristic peaks between δ 120.8 and 170.9.



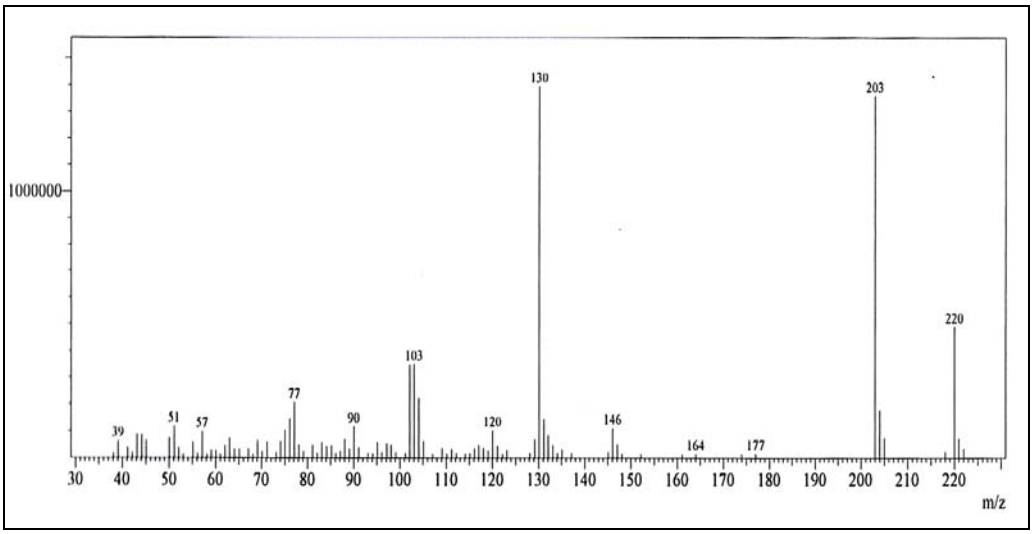
Spectrum 38: Mass spectrum of 3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 39: ^{1}H NMR spectrum of 3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 40: ^{13}C NMR spectrum of 3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 41: Mass spectrum of 3-(2-Carbamoylphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione

4.1.5 Experimental

Melting point recorded on a Toshniwal capillary melting point apparatus are uncorrected. The mass spectrum was recorded on JEOL JMS 600H Mass Spectrometer under FAB-MS mode. The NMR experiments were conducted using Bruker Avance III 500 MHz FT-NMR instrument. IR spectra were recorded as KBr pellets using Shimadzu 8101A FTIR equipment. TLC was performed on the glass-backed silica gel sheets (BSS 350).

SECTION A

SYNTHESIS OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONES FROM IMIDIC ACID ESTER

Preparation of starting materials

Imidic acid ester hydrochlorides of 1-cyanonaphthalene and phthalonitrile were prepared according to the method adopted by Pinner[41] and improved by others[42-44]. The preparation of imidic acid ester of pyrimidine-2-carbonitrile, quinolone-2-carbonitrile, 3-nitrobenzonitrile and 4-nitrobenzonitrile were carried out following the method suggested by Fred and Grace[45].

Naphthimidic acid ethyl ester hydrochloride

In a 500 ml filter flask with a cork carrying an inlet tube was placed a mixture of 1-cyanonaphthalene (3.06 g, 0.02 mol) and absolute ethanol (10 ml). A calcium chloride guard tube was attached to the side arm. HCl gas dried by bubbling through concentrated sulphuric acid was passed into the mixture till 1 g (0.03 mol) of the gas was absorbed.The flask was stoppered and kept in freezing mixture. After 3 hours the flask was placed in the refrigerator overnight. Since the imidate didn't solidify even after adding dry ether, the ethanolic solution was as such taken for the reaction.

Phthalimidic acid methyl ester hydrochloride

In a 500 ml filter flask with a cork carrying an inlet tube was placed a mixture of phthalonitrile (5.3 g, 0.02 mol) and absolute methanol (20 ml). A calcium chloride guard tube was attached to the side arm. HCl gas dried by bubbling through concentrated sulphuric acid was passed into the mixture till 1 g (0.03 mol) of the gas was absorbed.The flask was stoppered and kept in freezing mixture. After 3 hours the flask was placed in the refrigerator overnight. The solid product was carefully broken up and taken in a 500 ml conical flask. Dry ether (50 ml) was added and the flask was stoppered and allowed to stand in the refrigerator overnight. The imidic acid ester

hydrochloride was filtered, washed with two portions of 20 ml dry ether. It was dried in vacuum desiccator over KOH pellets. The phthalimidic acid methyl ester hydrochloride weighed 3.2 g (83%). Being not very stable at room temperature, it was stored in tightly stoppered bottle and kept in refrigerator.

Synthesis of 3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrimidine-2-carbonitrile (1.03 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 140 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid was filtered, washed with water and then with methanol and dried. The yield of 3-(pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.967 g (54%). The compound was recrystallized from methanol. It melted at 258 ^{0}C.

Synthesis of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Quinoline-2-carbonitrile (1.54 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To

the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 170 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The yellow solid was filtered, washed with water and then with methanol and dried. The yield of 3-(quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.35 g (59%). The compound was recrystallized from methanol. It melted at 285 ^{0}C.

Synthesis of 3-(3-Nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. 3-Nitrobenzonitrile (1.48 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 180 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid was filtered, washed with water and then with methanol and dried. The yield of 3-(3-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.32 g (60%). The compound was recrystallized from methanol. It melted at 220 ^{0}C.

Synthesis of 3-(4-Nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. 4-Nitrobenzonitrile (1.48 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was , the boiling tube was plugged with cotton and slowly heated in a wax bath upto 180 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid was filtered, washed with water and then with methanol and dried. The yield of 3-(4-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.29 g (58%). The compound was recrystallized from methanol. It melted at 240 ^{0}C.

Synthesis of 3-(Naphthalen-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

The ethanolic solution of the naphthimidic acid ester hydrochloride (0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in a long boiling tube plugged with cotton. It was then heated slowly up to 180 ^{o}C for 15 min. It was stirred with a little methanol and cooled. The white solid was filtered, washed with water, and then methanol and dried. The yield of 3-(naphthalen-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.91 g (40%). The compound was recrystallized from methanol. It melted at 210 ^{0}C.

Synthesis of 3-(2-Carbamoylphenyl)-1H-1,2,4-triazole-5(4H)-thione

Phthalimidic acid ester hydrochloride 1.92 g (0.01 mol) was weighed and ground well with thiosemicarbazide 0.9 g (0.01 mol). The mixture was

then transferred to long boiling tube, plugged with cotton and heated in a wax bath slowly up to 200 ^{o}C for 15 min. It was stirred with a little methanol and cooled. The yellow solid was filtered, washed with water and then with methanol and dried. The yield of 3-(2-carbamoylphenyl)-1H-1,2,4-triazole-5(4H)-thione was 1.32 g (60%). The compound was recrystallized from methanol. It melted at 245 ^{0}C.

SECTION B

SYNTHESIS OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONES FROM NITRILES

Synthesis of 3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

Pyrimidine-2-carbonitrile (1.03 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to 140 ^{o}C for 15 min. The reddish brown product was stirred with a little methanol and cooled. The pale yellow product was filtered, washed with water and then with methanol and dried. The yield of 3-(pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.537 g (30%). The compound was recrystallized from methanol. It melted at 258 ^{0}C.

Synthesis of 3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

Quinoline-2-carbonitrile (1.54 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to 170 ^{o}C for 15 min. The reddish brown product was stirred with a little methanol and cooled. The yellow product was filtered, washed with water and then with methanol and dried. The yield of 3-(quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.798 g (35%). The compound was recrystallized from methanol. It melted at 283 ^{0}C.

Synthesis of 3-(3-Nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione

3-Nitrobenzonitrile (1.48 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to 180 ^{o}C for 15 min. The reddish brown product was stirred with a little methanol and cooled. The pale yellow product was filtered, washed with water and then with methanol and dried. The yield of 3-(3-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.89 g (40%). The compound was recrystallized from methanol. It melted at 220 ^{0}C.

Synthesis of 3-(4-Nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione

4-Nitrobenzonitrile (1.48 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to 180 ^{o}C for 15 min. The reddish brown product was stirred with a little methanol and cooled. The pale yellow product was filtered, washed with water and then with methanol and dried. The yield of 3-(4-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.84 g (38%). The compound was recrystallized from methanol. It melted at 240 ^{0}C.

Synthesis of 3-(Naphthalen-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

1-Cyanonaphthalene (1.53 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to ^{o}C for 15 min. The pale brown product was stirred with a little methanol and cooled. The white product was filtered, washed with water and then with methanol and dried. The yield of 3-(naphthalene-1-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.613 g (27%). The compound was recrystallized from methanol. It melted at 210 ^{0}C.

Synthesis of 3-(2-Carbamoylphenyl)-1H-1,2,4-triazole-5(4H)-thione

Phthalonitrile (2.65 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were taken in long boiling tube and heated in a wax bath slowly up to ^{o}C for 15 min. The dark brown product was stirred with a little methanol and cooled. The yellow product was filtered, washed with water and then with methanol and dried. The yield of 3-(2-carbamoylphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.808 g (40%). The compound was recrystallized from methanol. It melted at 225 ^{0}C.

4.2 ANTIOXIDANT EVALUATION OF A FEW 3-SUBSTITUTED-1*H*-1,2,4,-TRIAZOLE-5(4*H*)-THIONES

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants are substances that may protect cells from the damage caused by free radicals, which are inevitable byproducts of turning food into energy. These free radicals capture electron from other stable molecules they encounter, there by altering the loser's structure and function. Free radical damage can change the instruction coded in a strand of DNA. It can either caus a circulating low-density lipoprotein (LDL) molecule to get trapped in an artery wall or modify the selective transport of a cell membrane. It has been established that more than sixty human diseases involve free radical damage, including cancer, heart disease and immune system decline.

In 1990s, scientists realized to understand that free radical damage is involved in the early stages of artery-clogging atherosclerosis and thus contribute to cancer, vision loss and most of other chronic conditions.

Free radicals are produced during normal cellular metabolism. Exposure to radiation, whether from the sun or medical X-ray and

environmental pollutants such as tobacco smoke and car exhaust also contribute to the formation of free radicals Free radicals are beneficial to our body since they destroy alien bacteria and help fight off infection. They also help in producing vital hormones and activating certain enzymes[46]. The damage comes when excessive and uncontrolled amounts of free radicals are present in the body. Diet, exposure to toxins and radiation, exercise, illness and certain medications increase oxygen-related reactions in the body and thus the number of free radicals.

In fact, generation of reactive oxygen species (ROS) is unavoidable in an aerobic environment. Cells produce ROS as part of their general metabolic activity. ROS are a family of molecules derived from oxygen and characterized by their high chemical reactivity and ability to act as oxidants. They include free radicals such as superoxide (O^{2-}) and hydroxyl radicals ($OH^{\cdot}$), as well as other molecules such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO), which are not free radicals but can also act as oxidizing agents in biological system[47]. Under physiological conditions there is a balance between ROS generation and the activity of enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (glutathione, alpha-tocopherol, ascorbate, thioredoxin) antioxidant defences that decrease ROS concentrations. However, increased production of ROS or decreased antioxidant defense results in a condition called oxidative stress. Oxidative stress can lead to free radical-induced oxidation and damage to biomolecules such as lipids, DNA and protein. ROS mediated cellular damage has been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age[48,49]. In low concentration, synthetic antioxidants are also in use for many industrial processes such as inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers.

Antioxidants exert their effects by scavenging or preventing the generation of ROS[50], which can protect the formation of free radicals and retard the progress of many chronic diseases[51] including cancer, neurodegenerative, inflammative and cardiovascular diseases[52].

There are two broad classes of antioxidants, which are 'preventive' and 'chain-breaking'. Preventive antioxidants reduce the rate of chain initiation and chain-breaking antioxidants reduce the rate of chain propagation in a free radical reaction.

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant oriented food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk. The commonly available synthetic antioxidants are t-butyl hydroxy toluene (BHT) and t-butyl hydroxy anisole (BHA).

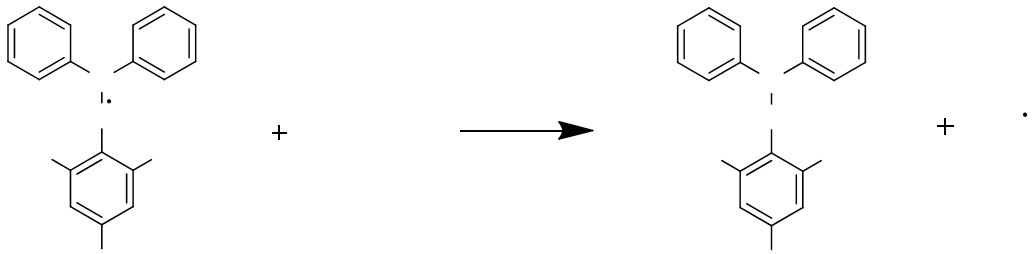
In this perspective, antioxidant evaluation of a few selected 3-Substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones is quite significant.

Present Work

In the recent report[21] on the synthesis and biological evaluation of 4-substituted-5-(2-thienyl)-1,2,4-triazole-3-thiones, the antioxidant activity of 1,2,4- triazoles bearing thienyl moiety has been discussed. In the present study, a few 3-Substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, containing pyridinyl, pyrimidinyl, pyrazinyl and quinolinyl moieties were subjected to their *in vitro* antioxidant activity. Although a number of methods are available, including ORAC, ABTS, DMPD, FRAP, TRAP, TBA, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, xanthine oxidase, cytochrome C, reducing power method, etc. DPPH method is very common and has been shown to be the most appropriate method[53].

The DPPH Method – Principle

A rapid, simple and inexpensive method to measure antioxidant property involves the use of the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods and to quantify antioxidants in complex biological systems. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. With this method it is possible to determine the antiradical power of an antioxidant by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a colour change from purple to yellow the absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule shows an absorbance at 517 nm, which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic spin paired molecule[54]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640, when the odd electron of DPPH radical becomes paired with hydrogen radical from a free radical scavenging antioxidant compound to form the reduced DPPH-H. The resulting decolourization is stoichiometric with respect to the number of electrons captured as shown below **(Scheme 11)**.



Scheme 11

The Parameter EC_{50} or IC_{50}

One of the parameters that has been used to express the antioxidant activity of the compounds using the results from DPPH method is the 'efficient concentration', EC_{50} or 'inhibition coefficient', IC_{50} value. This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour). Antioxidants react at different rates i.e. differing kinetics, and the reaction will often not go to completion in a reasonable assay time. Therefore, sample size that can lower the initial absorbance of DPPH solution by 50% has been selected as the endpoint for measuring the antioxidant activity. This parameter was apparently introduced by Brand-Williams and his colleagues[55,56] and has been used subsequently by several groups of workers for presenting their results.

Result and Discussion

Table 12: Antioxidant Assay of Compound T_1 (3-(Pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.891	-	-	-	**64.9**
2	200	0.739	82.9	17.1	18	
3	400	0.584	65.5	34.5	36	
4	600	0.485	54.4	45.6	54	
5	800	0.426	47.8	52.2	72	
6	1000	0.303	34.0	66.0	90	

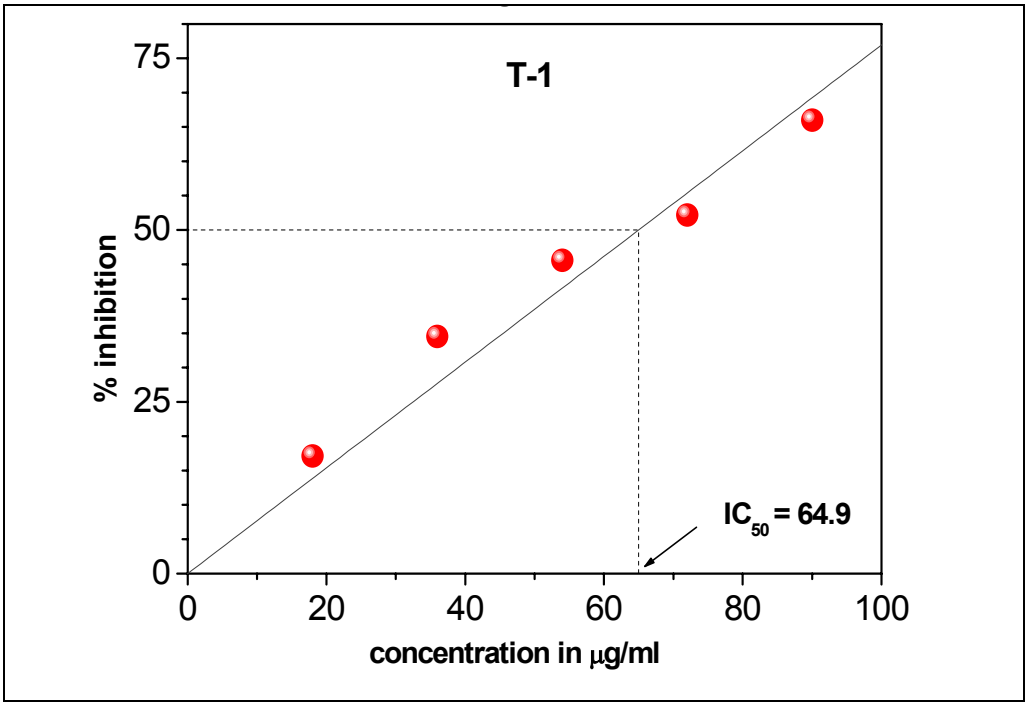


Figure 3

Table 13: Antioxidant Assay of Compound T_2 (3-(Pyridin-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.632	-	-	-	**48.5**
2	200	0.435	68.8	31.2	18	
3	400	0.317	50.2	49.8	36	
4	600	0.261	41.3	58.7	54	
5	800	0.150	23.7	76.3	72	
6	1000	0.114	18.1	81.9	90	

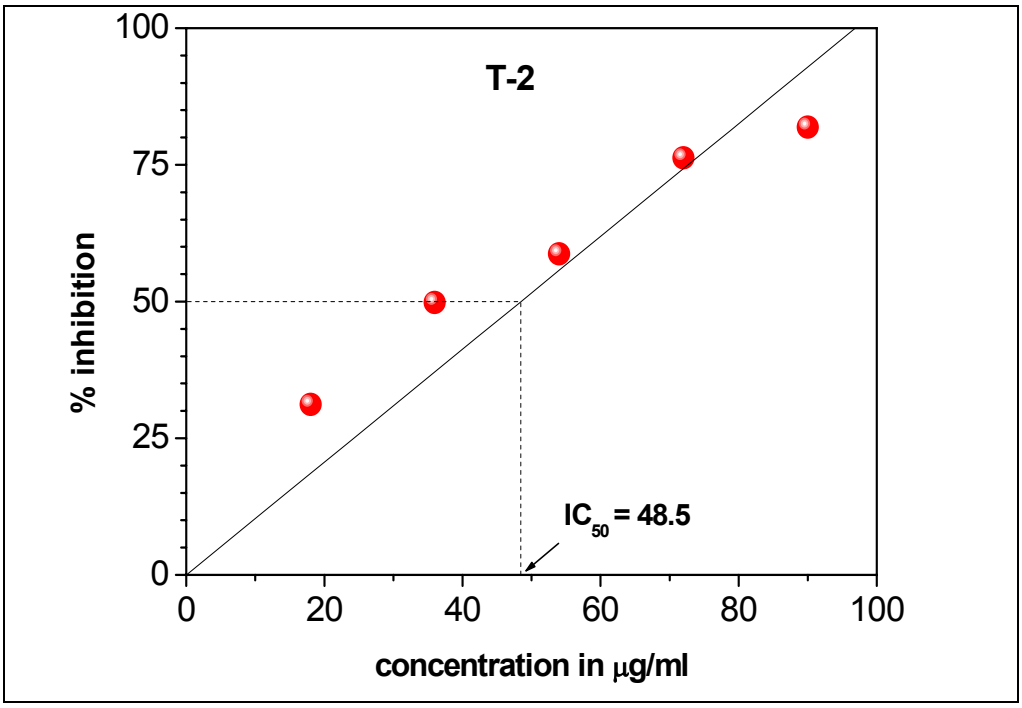


Figure 4

Table 14: Antioxidant Assay of Compound T_3 (3-(Pyridin-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.505	-	-	-	**42.6**
2	200	0.347	82.9	17.1	18	
3	400	0.236	65.5	34.5	36	
4	600	0.150	54.4	45.6	54	
5	800	0.080	47.8	52.2	72	
6	1000	0.024	34.0	66.0	90	

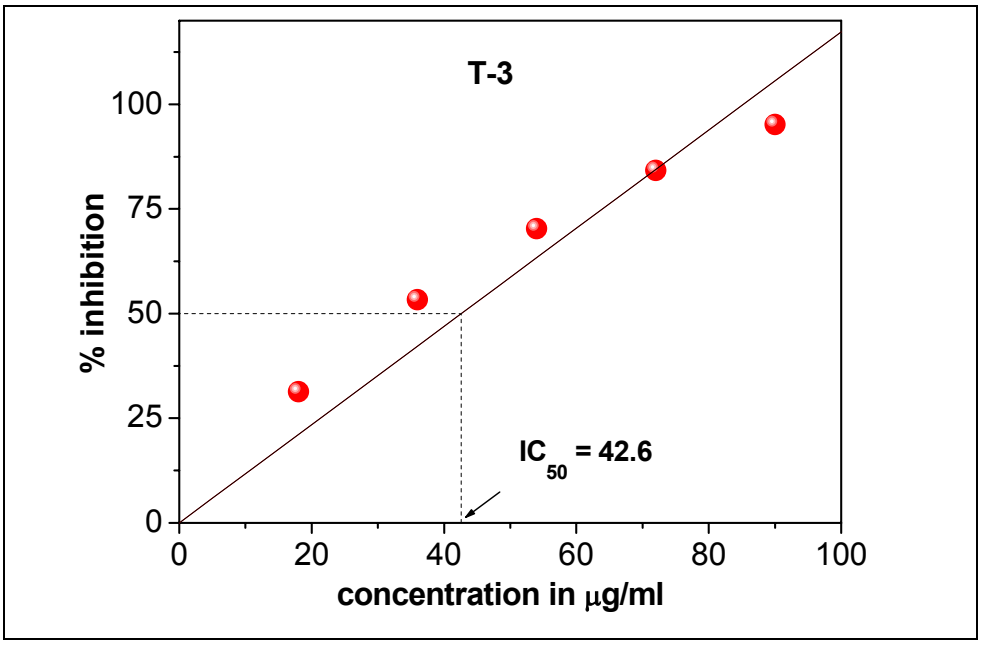


Figure 5

Table 15: Antioxidant Assay of Compound T_4 (3-(Pyrimidin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.724	-	-	-	**85.02**
2	200	0.605	83.6	16.4	18	
3	400	0.530	73.2	26.8	36	
4	600	0.472	65.2	34.8	54	
5	800	0.429	59.3	40.7	72	
6	1000	0.369	51	49	90	

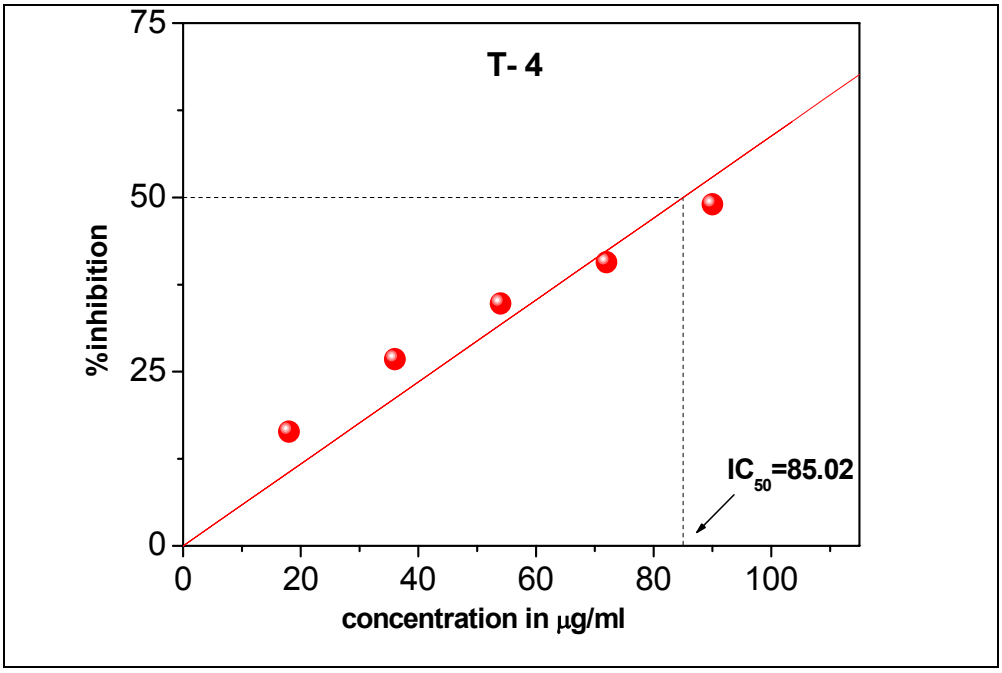


Figure 6

Table 16: Antioxidant Assay of Compound T_5 (3-(Pyrazin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.594	-	-	-	
2	200	0.526	88.6	11.4	18	
3	400	0.489	82.3	17.7	36	
4	600	0.473	79.6	20.4	54	
5	800	0.440	74.1	25.9	72	**134.5**
6	1000	0.408	68.7	31.3	90	

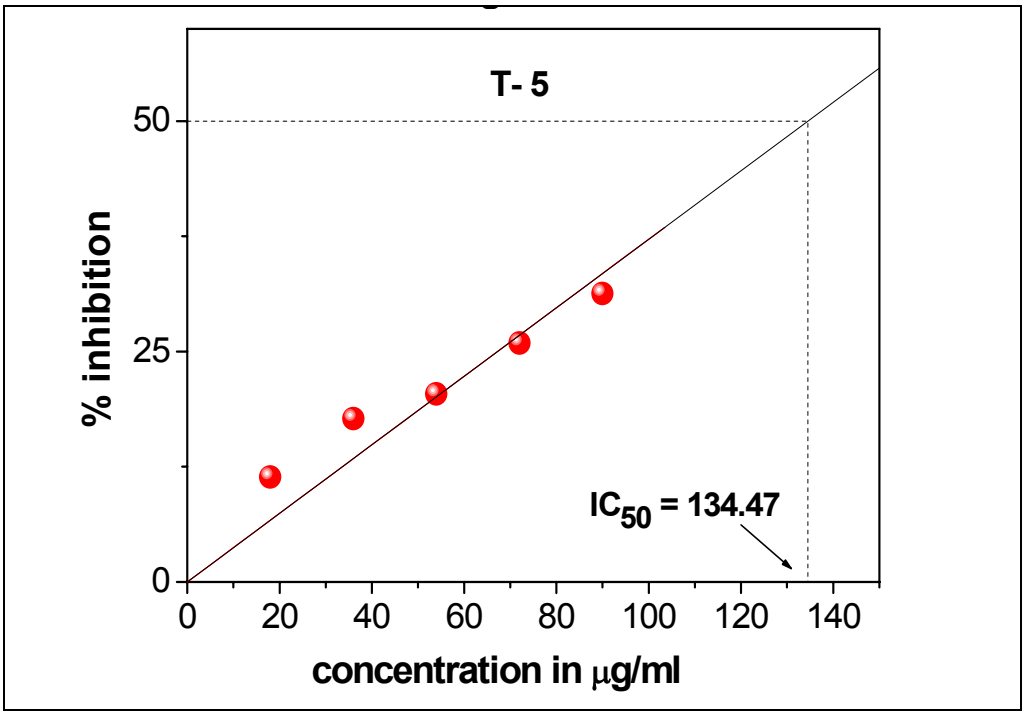


Figure 7

Table 17: Antioxidant Assay of Compound T_6 (3-(Quinolin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

Sl. No.	Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/mL)	IC_{50} (µg/mL)
1	0	0.678	-	-	-	**56.2**
2	200	0.537	79.2	20.8	18	
3	400	0.414	61.1	38.9	36	
4	600	0.329	48.5	51.5	54	
5	800	0.242	35.7	64.3	72	
6	1000	0.175	25.8	74.2	90	

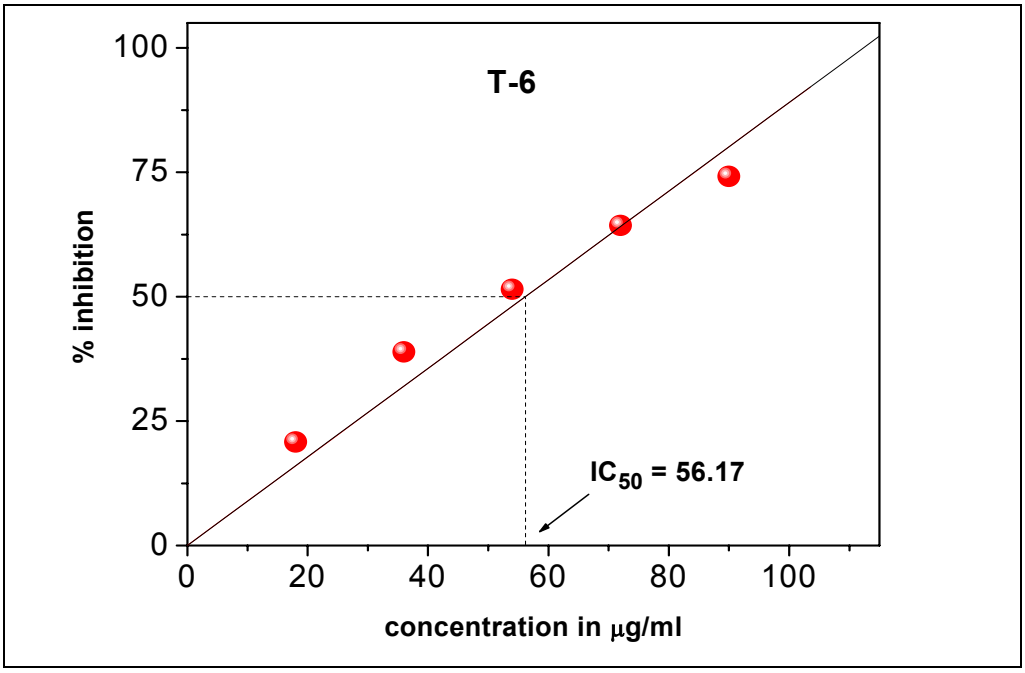


Figure 8

The antioxidant activity of the title compounds are obvious that the scavenging activity increases with increasing sample concentration. The antioxidant activity of any compound depends strongly on its reducing property. Since each of the compounds contains a high percentage of nitrogen, it might have enhanced reducing power to scavenge free radicals[57]. Of all the synthesized compounds, compounds T_1, T_2, T_3 and T_6 showed higher scavenging activity, which can be accounted for the presence of strongly electron withdrawing pyridinyl and quinolinyl groups at 3 position of the triazole ring. Between T_1 and T_6, the higher potential of the latter can be attributed to the additional stabilization of the corresponding radical due to the presence of extended aromatic ring. The unexpected lower activity of T_4 and T_5, despite having stronger electron withdrawing groups need further investigation. Ascorbic acid (reference antioxidant compound) is used as the standard and is known to have an IC_{50} value of 49 μg/mL[57]. From all the synthesized triazoles, compound T_2 and T_3 exhibited the highest radical scavenging activities which were better than the reference compound.

Experimental

A. Synthesis of 3-Substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones from imidic acid ester

The compounds T_4 and T_6 were synthesized as per the procedure described in section A of this chapter.

Synthesis of the compound T_1 (3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-2-carbonitrile (0.95 ml, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml

of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 200 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid (3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione) was filtered, washed with water and then with methanol and dried. The compound was recrystallized from methanol. It melted at 252 ^{0}C.

Synthesis of the compound T_2 (3-(pyridin-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-3-carbonitrile (1.04 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 200 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid (3-(pyridin-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione) was filtered, washed with water and then with methanol and dried. The compound was recrystallized from methanol. It melted at 282 ^{0}C.

Synthesis of the compound T_3 (3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyridine-4-carbonitrile (1.04 g, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added, the boiling tube was plugged with cotton and slowly heated in a wax bath upto 200 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid (3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione) was filtered, washed with water and then with methanol and dried. The compound was recrystallized from methanol. It melted at 288 ^{0}C.

Synthesis of the compound T_5 (3-(pyrazin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione)

A catalytic amount of sodium metal (0.1 g) was dissolved in 10ml absolute methanol in 100 mL RB flask, fitted with calcium chloride guard tube. Pyrazine-2-carbonitrile (0.9 ml, 0.01 mol) was added to it and allowed to stand overnight at room temperature. To the imidic acid ester formed 5 ml of ice cold water and 10 ml of ether were added. The aqueous layer was then saturated with anhydrous potassium carbonate and extracted with ether. To the ether extract anhydrous potassium carbonate was added and kept in refrigerator for 20 minutes and the ether extract was decanted to the long boiling tube. To the solution thiosemicarbazide (0.9 g, 0.01 mol) was added,

the boiling tube was plugged with cotton and slowly heated in a wax bath upto 200 ^{o}C for 15 minutes, so that reddish brown solid formed with evolution of ammonia. It was stirred with a little methanol and cooled. The pale yellow solid (3-(pyrazin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione) was filtered, washed with water and then with methanol and dried. The compound was recrystallized from methanol. It melted at 268 ^{0}C.

B. DPPH radical scavenging assay

The free radical scavenging activity was determined by spectrophotometric measurement of the change in absorbance of $DPPH^{\cdot}$ at 525 nm in DMSO. The absorbance was measured using JASCO V-550 UV-VIS spectrophotometer.

Stock solutions (500 μM) of the tested sample and $DPPH^{\cdot}$ were prepared in DMSO. 1 ml of DPPH solution was added to the sample solution at different concentrations (200, 400, 600, 800 and 1000 μL) and appropriately diluted with DMSO to a constant volume. A control was made by diluting 1 ml of $DPPH^{\cdot}$ solution using DMSO alone. Ascorbic acid was used as a standard (the reference antioxidant) for this test. For the standard, the sample was replaced with the same amount of ascorbic acid. The reaction mixtures were thoroughly shaken and kept in the dark for 30 min. and the absorbance at 525 nm was measured. From the absorbance value the 'Percentage difference' and 'Percentage Inhibition' in each case can be calculated using the equations;

% Difference = [Sample absorbance/DPPH absorbance (blank)] x100

% Inhibition = 100 – (% Difference)

The IC_{50} values were determined from the calibration curve in which the % inhibition is plotted against concentration.

References

1. Padmavathi, V.; Thriveni, P.; Sudhakar Reddy, G.; Deepthi, D. *Eur. J. Med. Chem*. **2008**, 43,5,917-924.
2. Amir, M.; Kumar, H.; Javed, S .A. *Eur. J. Med. Chem*. **2008**, 43, 10, 2056-2066.
3. Sztanke, K.; Tuzimski, T.; Rzymowska, J.; Pasternak, K.; Kandefer-Szerszen, M. *Eur. J.Med. Chem*.**2008**, 43, 2, 404-419.
4. Kuş, C.; Kilcigil, G. A.; Özbey, S.; Kaynak, F. B.; Kaya, M.; Çoban, T.; Eke, B. C.; *Bioorg. Med. Chem*. **2008,** 16, 8, 4294-4303.
5. Isloor, A. M.; Kalluraya, B.; Rao, M.; Rahiman, A. M.; *J. Saudi Chem. Soc*. **2000**, 4, 265-270.
6. Kalluraya, B.; Isloor, A. M.; Shenoy, S. *Indian J. Heterocycl. Chem*. **2001**, 11, 159-162.
7. Kalluraya, B.; Isloor, A. M.; Priya, F. V.; Jagadeesha, R. L. *Indian J. Heterocycl. Chem*. **2004**, 13, 3, 245-248.
8. Venter, Monica M.; Zaharia, Valentin. Studia Universitatis Babes-Bolyai, *Chemia*, **2007**, 52, 103-109.
9. Labanauskas, L.; Kalcas, V.; Udrenaite, E.; Gaidelis, P.; Bruktus, A.; Dauksas, V. *Pharmazil*. **2001**, 56,617.
10. Amir, M.; Kumar, S. *Acta Pharm*. **2007**, 57, 31.
11. Kumamoto, T.; Toyooka, K.; Nishida, M.; Kuwahra, H.; Yoshimura, Y.; Kawada, J.; Kubota, S. *Chemical and Pharmaceutical Bulletin* **1990**, 38, 2595.
12. Ezabadi, I. R.; Camoutsis, C.; Zoumpoulakis, P.; Geronikaki, A.; Sokovic, M.; Glamocilija, J.; Ciric, A. *Bioorg. Med. Chem*. **2008**, 16, 355.
13. Mazzone, G.; Boniana, F.; Arrigo, R.; Blandino, G. *Farmaco.* **1981**, 36, 181.
14. Gokce, M.; Cakir, B.; Erol, K.; Sachin, M. F. *Arch. Pharm*.**2001**, 334, 279.
15. Kucukguzel, I.; Tatar, E.; Kucukguzel, S. G.; Rollas, S.; Declercq, E. *Eur. J. Med. Chem*. **2008**, 43, 381.

16. Kane, J. M.; Dudley, M. K.; Sorensen, S. M.; Miller, P. *J.Med. Chem*. **1988**, 31, 1253.
17. Dutta, S. P.; Acharya, A. K.; Banu, U. P. *J. Ind. Chem. Soc*. **1968**, 45, 338.
18. Diaz, P.; Rafiin, C. PCT Int. Appl. **2006**, Wo 20061033119 A2 20061005.
19. Konuma, T.; Kokai T. K. **2007**, J.P 2007163840 A.20070628.
20. Sugii, A.; Yamazaki, Y.; Nihon Daigaku Yakugakuenkyu Hokoku 1958, 2, 6-9. (Sci-Finder, Database, CAPLUS). *Chemia*, **2007**, 52, 103-109.
21. Koparir, M.; Orek, C.; Parlak, A. E.; Söylemez, A.; Koparir, P.; Karayepe, M.; Dastan, S. D. *Eur. J. Med. Chem***. 2013**, 63, 340-346.
22. Elderfield, R.C. "Heterocyclic Compounds" Vol.7 (Jhon Wiley & Sons. Inc.) New york, **1961**, 425.
23. Hoggarth, E. *J. Chem. Soc*. **1949**, 1160.
24. Willems, J. F.; Vandenberghe, A. *Bulletin des societies chimiques Belges*. **1966**, 75 (5-6), 358-365.
25. Boyle John, T. A.; Saunders, John C. U.S. **1976**, US 3962237 A 19760608.
26. Barnikow, G.; Ebeling, Horst, *Zeitschrift fuer chemie*. **1980**, 20, 55-56.
27. Uher, M.; Bosansky, M.; Kovac, S.; Martvon, A. *Collection of czechosvak chemical communications*. **1980**, 45, 2804-07.
28. Dubina, V. L.; Pedan, V. P.; Dembinskii, I. K. USSR. **1981**, SU 883033 A1 19811123.
29. Malbec, F.; Milcent, R.; Barbier, G.; *J Heterocylclic Chem*. **1984**, 21, 1689.
30. Somarai, T.; Szilagyi, G.; Bozo, Eva, Nagy, Gabor, Hung, *Teljes* **1985**, HU 34457 A2 19850328.
31. Andrae, S.; Schmitz, E. *Ger (East)*. **1985**, DD 229404 A1 19851106.
32. Kalluraya, B.; Shotty, S.; John, N.; Jancy, K. *Chimica Acta Turcica*, **1992**, 20, 173.
33. Huaxue Xuebao. **1992**, 7, 936-39.

34. Modzelewska, B.; Szumilo, H. *Acta Poloniac Pharmaceutica*. **1996**, 53 (3), 213-216.
35. Wang, Z. Y.; Shi, H. J.; Shi, H.; *Youji Huaxue*. **1997**, 17, 271.
36. Wang, Z.; You, Tianpa, *Huaxue Shiji*, **1997**, 19, 321.
37. Foks, H.; Janowil, M.; Zwoiska, Z.; Auguestynowicz-kopiec, Ewa. *Annales Academiae Medicae Gedanensis*. **2002**, 32, 301.
38. Cansiz, A.; Cetin, A.; Kutulay, P.; Koparir, M. *Asian J Chem***. 2009**, 21, 617.
39. Koparir, M.; Orek, C.; Koparir, P.; Sarac, K. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2013**, 105, 522-531.
40. Koparir, M.; Orek, C.; Parlak, A. E.; Söylemez, A.; Koparir, P.; Karayepe, M.; Dastan, S. D. *Eur. J. Med. Chem*. **2013**, 63, 340-346.
41. Pinner, A. *Ber*. **1833**, 61, 1643.
42. Mackenzia, C. A.; Schmidt, G. A.; Wedd, L. R. *J. Am. Chem. Soc*. **1951**, 73, 4990.
43. Holly, F. N.; Stammer, C. H. U. S. Pat.2, 772, 281; C. A. **1957**, Vol.51, 8145.
44. Thomas, P. R.; Tylor, G. T. *J. Chem. Soc*. **1957**, 125, 2197.
45. Fred, C. S.; Grace, A. P., *J. Org. Chem.* **1961**, Vol. 26, 412-418.
46. Remi Cooper "Age-Reversing Free Radicals – Antioxidants" A Woodland Health Series, **1997**.
47. Martial G Bourassa; Jean-Claude Tardif "Antioxidants and Cardiovascular Disease" Springer **2006.**
48. Uchida, K. *Free Rad. Biol. Med*. **2000**, 28, 1685-1696.
49. Cadenas, E; Davies, K. J. A. *Free Rad. Biol. Med*. **2000**, 29, 222-230.
50. Kinsella, J. K.; Frankel, E.; German, B,; Kanner, J. *J. Food technol*. **1993**, 47, 85-89.
51. Singh, N.; Rajini, P. S. *Food Chem*. **2004**, 85, 611-616.
52. Prior, R. L.; Wu, X.; Schaichs, K. *J. Agric. Food Chem*. **2005**, 53, 4290-4302.
53. Bondet, V.; Brand-Williams, W.; Berset, C. *Lebensm.-Wiss. Technol*. **1997**, 30, 609 – 615.

54. Matthaus, B. Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural Food Chemistry* **2002**, 50, 3444-3452.

55. Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Food Science and Technology* **1995**, 28, 25.

56. Bondet, V.; Brand-Williams, W.; Berset, C. *Food Science and Technology* **1997**, 30, 609.

57. Gomes, A.; Fernandes, E.; Garcia, M. B.; Silva, A. M.; Pinto, D. C.; Santo, C. M.; Cavaleiro, J. A.; Limla, J. L. Cyclic voltametric analysis of 2-styrylchromones: relationship with the antioxidant activity. *Bioorg. Med. Chem*. **2008**, 16, 7939-7943.

CHAPTER V

SYNTHESIS, ANTIBACTERIAL AND ANTI FUNGAL STUDIES OF A FEW DERIVATIVES OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONES

5.1. SYNTHESIS OF A FEW DERIVATIVES OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONES

5.1.1 Introduction

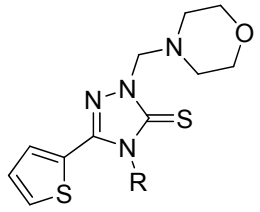
1,2,4-Triazole rings are typically planar 6π-electron aromatic systems, featuring an extensive chemistry[1,2]. Literature survey revealed that triazole derivatives have been the subject of extensive study in the recent past. Diverse biological activities such as antibacterial, antifungal, anti-inflammatory, antihypertensive and antiviral have been associated with 1,2,4-triazole derivatives[3-11].

Mannich reaction is a three-component condensation reaction involving an active hydrogen containing compound, formaldehyde and a secondary amine[12]. The aminomethylation of aromatic substrates by Mannich reaction is of great importance for the synthesis and modification of biologically active compounds[13].

In recent years, Mannich bases have gained importance due to their application in pharmaceutical chemistry. They have been found to possess antibacterial, antifungal, anticancer[13,14], antitubercular[15], analgesic and anti-inflammatory[16] properties. They are also used in polymer industry as paints and surface active agents[17].

Quite recently, Koparir and co-workers[18] reported the synthesis and antimicrobial activities of some novel 4-substituted-5-(2-thienyl)-1,2,4-

triazole-3-thione and their Mannich bases. They found that these compounds exhibited significant antibacterial and antifungal activity.

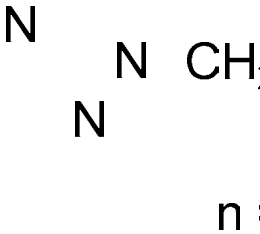


Rezaei and co-workers.[19] synthesized a few N-alkylated-1,2,4-triazoles which showed significant antifungal activity comparable to fluconazole, an antifungal azole with triazole ring.

In 2011, Danilova and co-workers[20] obtained a series of 1-alkyl-3,5-diamino-1,2,4-triazoles containing 1-pentyl, 1-decyl, and 1-dodecyl residues and explored their mesomorphic properties.

A recent report[21] has come this year, about a series of cationic iridium(III) complexes with triazole-pyridine ligands showing interesting piezochromic behavior, which is tunable by varying the length of N-alkyl chain on the 1,2,4-triazole ring.

In view of the above facts, we proposed to synthesize two types of derivatives of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, namely Mannich base and alkylated triazole and explore some of their properties.

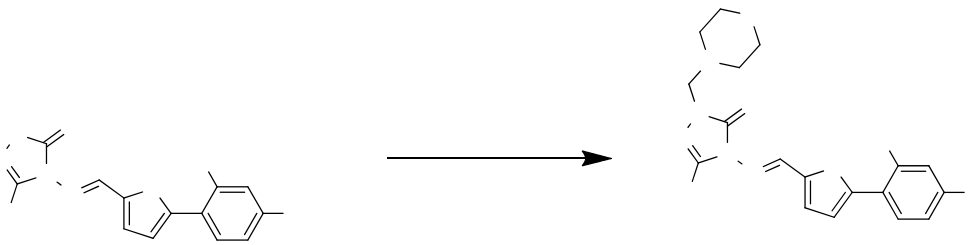


5.1.2 Review

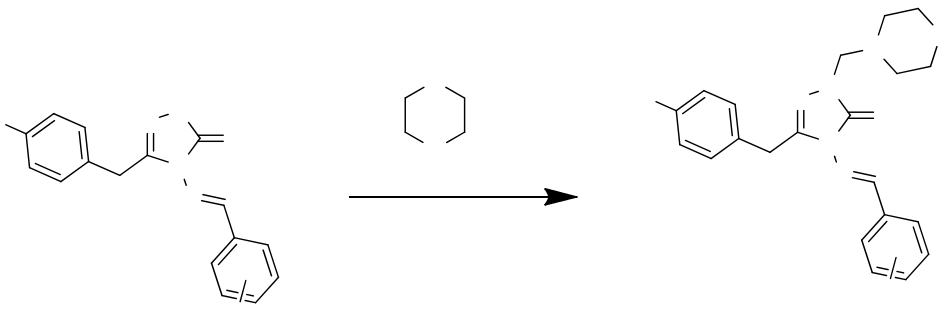
Since this chapter deals with the synthesis and antimicrobial activities of Mannich bases and alkylated derivatives of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, a brief review of various synthetic routes to the formation of these two derivatives would be quite appropriate.

a. Mannich reaction of 1,2,4-Triazoles

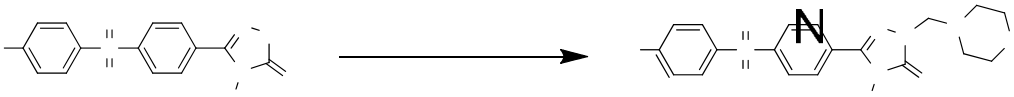
Holla and co-workers [10] synthesized a series of Mannich bases of 3-substituted 4-(5-nitro-2-furfurylidene)amino-1,2,4-triazole-5-thiones, by treating the Schiff's bases with secondary amines (morpholine and N-methylpiperazine) in presence of formaldehyde in ethanol medium.



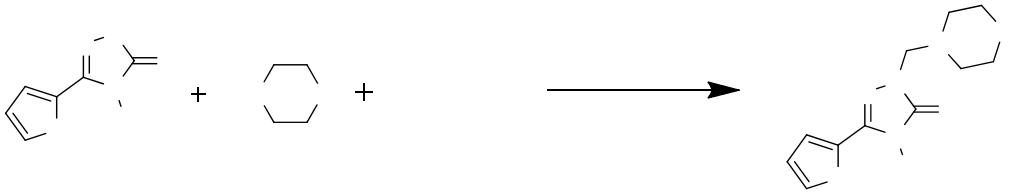
In 2007, Ashok and co-workers[13] reported the synthesis of a new series of Mannich bases namely 1-(morpholino)methyl-3-(4-methylthiobenzyl)-4-(substituted)-4-(substituted arylidine)amino-1,2,4-triazol-5-thiones in a one pot multi-component Mannich reaction involving corresponding triazoles, formaldehyde and morpholine/N-methylpiperazine. They were also screened for their antibacterial and antifungal activities against a variety of microorganism.



Almajan and co-workers [22] obtained a series of Mannich bases of 4-substituted 5-(4-(4-X-phenylsulfonyl) phenyl-2, 4-dihydro-3H-1,2,4-triazole-3-thiones, when the 1,2,4- triazoles dissolved in methanol were refluxed for 2 hours with morpholine and 37% formaldehyde.

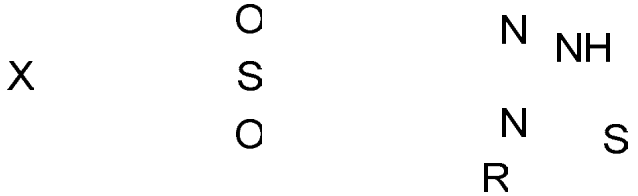


Recently in 2013, Koparir and co-workers[18] synthesized Mannich bases of 4-substituted-5-(2-thienyl)-1,2,4-triazole-3-thione by the reaction between corresponding triazoles with formaldehyde and various secondary amines.

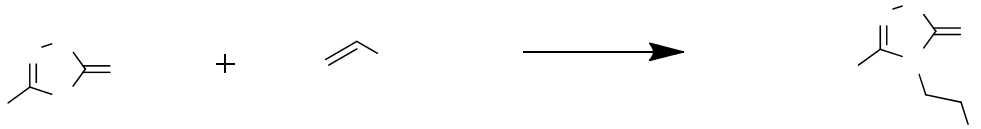


b. Alkylation of 1,2,4-Triazole

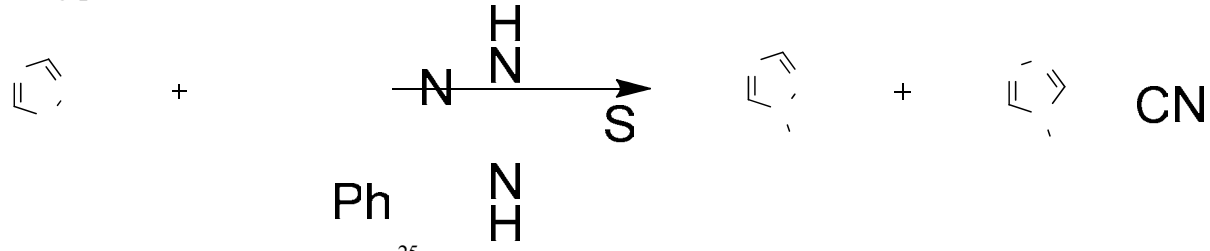
Fahmy and co-workers[23] reported that the reaction of 1,2,4-triazoline-5-thione with acrylonitrile afforded N-substituted adduct.



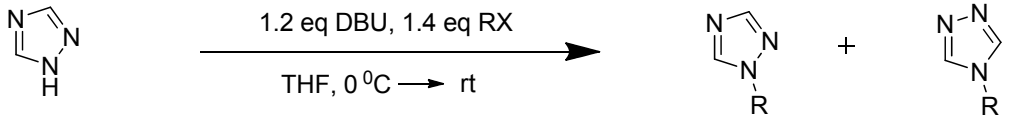
(X = H, Cl, Br)



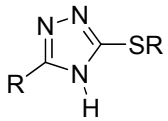
In 1991, Katritzky and co-workers[24] reported a simple and elegant method for the synthesis of N-substituted 1,2,4-triazoles by N-alkylation using powdered NaOH in DMF.



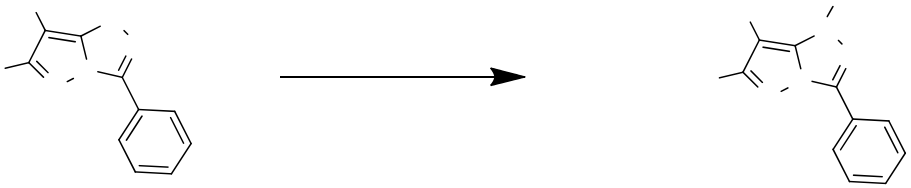
Bulger and co-workers[25] disclosed a mild and convenient method for the alkylation of 1,2,4-triazole, which uses the weakly nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Using DBU as base and THF as solvent, they obtained an 88% yield of products as a 90:10 isomers favouring the 1-isomer.



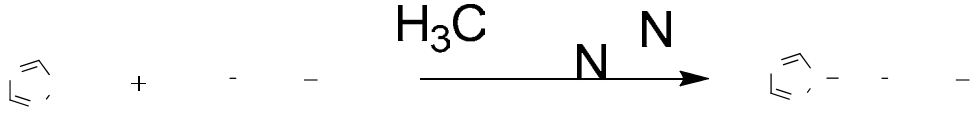
The alkylation[26,27] of triazolethione with alkyl halide in refluxing ethanol gave 32-99% yields of 5-alkylthio-1,2,4-triazole, which had moderate bacteriostatic activity and diuretic activity that increased with the size of R.



In 2006, Badea and co-workers [28] carried out ethylation and methylation of 1H-7-ethoxycarbonyl-6-methyl-3-phenyl-pyrazolo[5,1-c][1,2,4] triazole with ethyl bromide and methyl iodide respectively in DMF and excess NaOH to get 1-N-ethylated and 1-N-methylated products.



In 2008, Rezaei and co-workers[19] synthesized N-alkylated-1,2,4-triazole by refluxing with alkyl halide, potassium carbonate, tetraethylammonium iodide (TEAI) and NaOH in acetonitrile for 24-90 h.



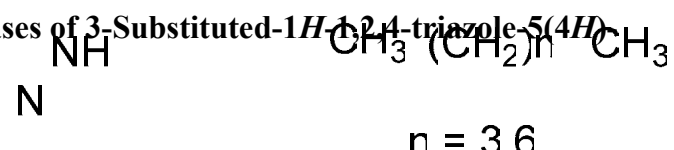
5.1.3 Present work

Mannich bases[18] of 1,2,4-triazole have been found to possess potential antimicrobial properties. Similarly, alkylated triazole derivatives are known for their mesomorphic property as well as piezochromic luminescent behavior, which are tunable by changing the length of alkyl groups.

Prompted by these observations it was proposed to synthesize newer series of such derivatives with a view to investigate their antimicrobial activity.

5.1.4 Result and Discussion

a. Synthesis of Mannich bases of 3-Substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones

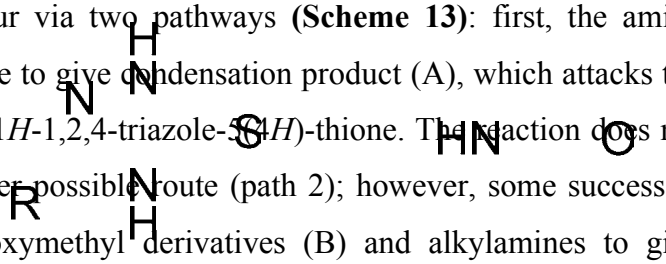


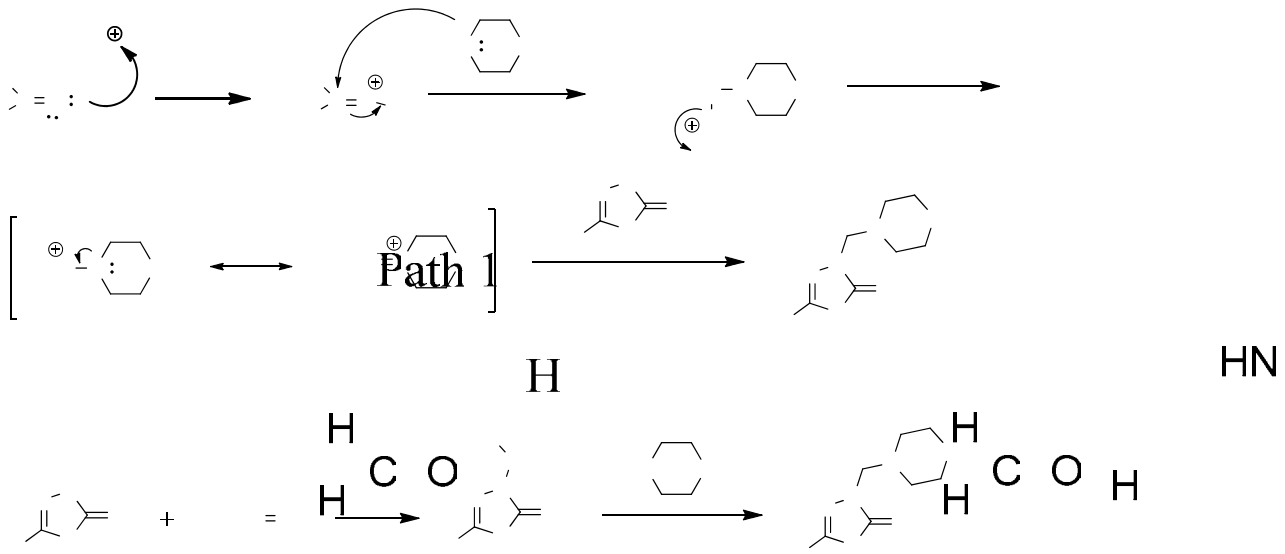
In the present work, we synthesized a few Mannich bases of 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, by stirring the corresponding triazole, 40% formaldehyde and morpholine in ethanol. The fluffy white solid obtained was recrystallized from ethanol. The starting triazoles were prepared

by the method (method A) discussed in chapter IV. The reaction is depicted below **(scheme 12)**.

Scheme 12

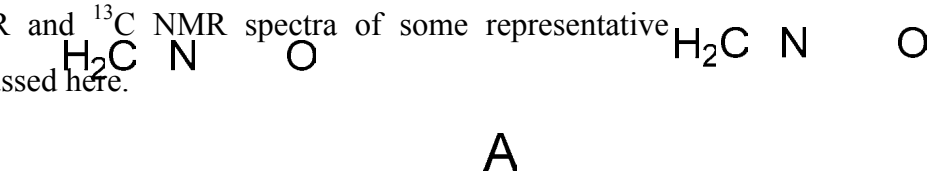
The Mannich reaction consists of the condensation of a substrate (3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione having at least one active hydrogen with formaldehyde and the secondary amine (morpholine). The condensation[22] can occur via two pathways **(Scheme 13)**: first, the amine reacts with formaldehyde to give condensation product (A), which attacks the substrate, 3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione. The reaction does not normally follow the other possible route (path 2); however, some successful reaction between hydroxymethyl derivatives (B) and alkylamines to give Mannich base can take place. If the nucleophilicity of the carbanion derived from the labile hydrogen compound is greater than that of amine, formation of a hydroxymethyl derivative (B) would be favoured over formation of derivative (A).





Scheme 13

All the synthesized compounds **(Table 18)** gave satisfactory analytical data. The mass, ^{1}H NMR and ^{13}C NMR spectra of some representative compounds are being discussed here.



A

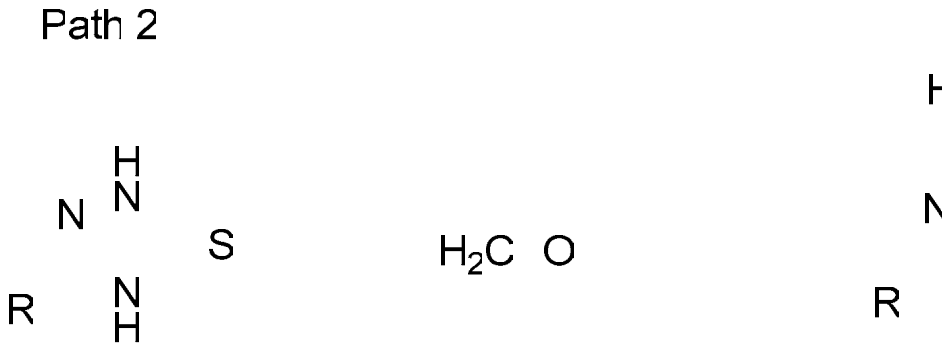
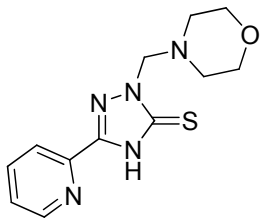


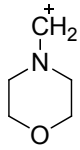
Table 18: Characterisation data of 1-(Morpholinomethyl)-3-(pyridinyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones

Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Elemental analysis found (calcd.)		
					C	H	N
1	1-(morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{12}H_{15}N_5OS$	85	190	51.95 (51.97)	5.41 (5.45)	25.22 (25.25)
2	1-(morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{12}H_{15}N_5OS$	80	225	51.94 (51.97)	5.48 (5.45)	25.23 (25.25)
3	1-(morpholinomethyl)-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{12}H_{15}N_5OS$	78	238	51.99 (51.97)	5.44 (5.45)	25.21 (25.25)

1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



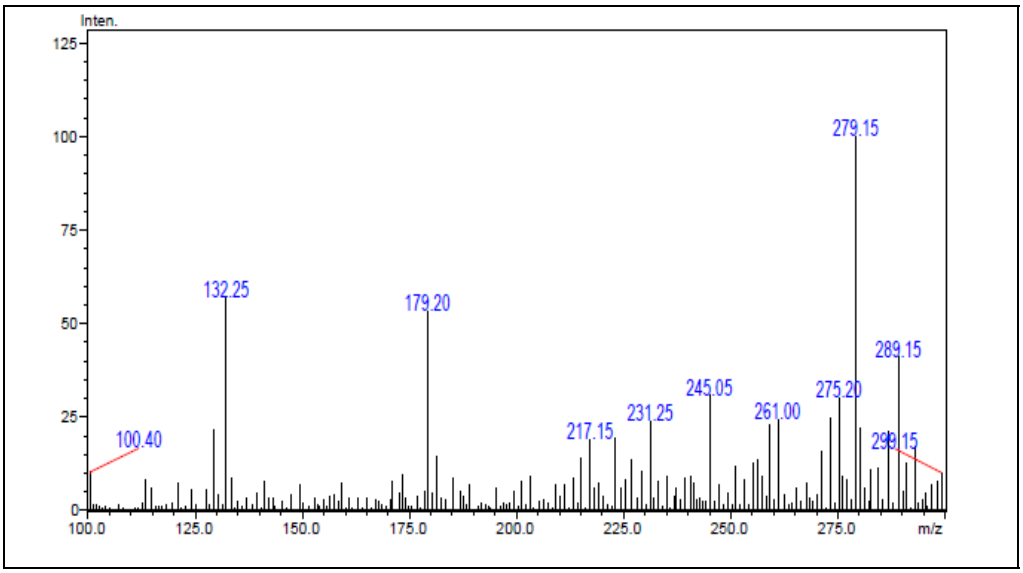
The mass spectrum **(Spectrum 42)** showed a peak at m/z = 279, while the expected molecular mass is 277. This can happen due to the attachment of two hydrogen atoms that occur during this mode of ionization (ESI). The odd molecular is in agreement with nitrogen rule, as the proposed structure has odd number of nitrogen atoms. The peak observed at m/z = 100 is a strong evidence for the formation of Mannich base, which is due to the formation of morpholinomethyl cation.



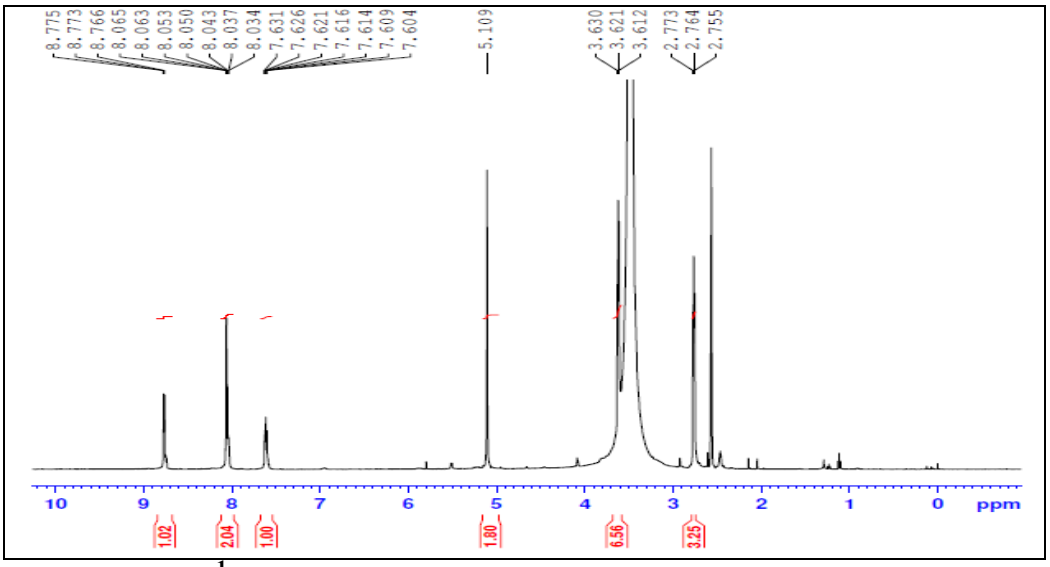
In the ^{1}H NMR spectrum **(Spectrum 43, 44)**, the singlet at δ 5.1 is due to N-CH_2-N link between the triazole and morpholine rings. The pair of triplets at δ 2.7 and 3.6 are characteristic of morpholine ring owing to N-CH_2 and O-CH_2 respectively. The four aromatic protons of pyridine ring were recorded in the region δ 7.6 to 8.7. The low field NH proton of the triazole ring, which absorb at around δ 14, seem to be off the scale in the given spectrum.

The ^{13}C NMR spectrum **(Spectrum 45)** exhibited 10 peaks in the region δ 50.2 to 168.6, supporting the proposed structure.

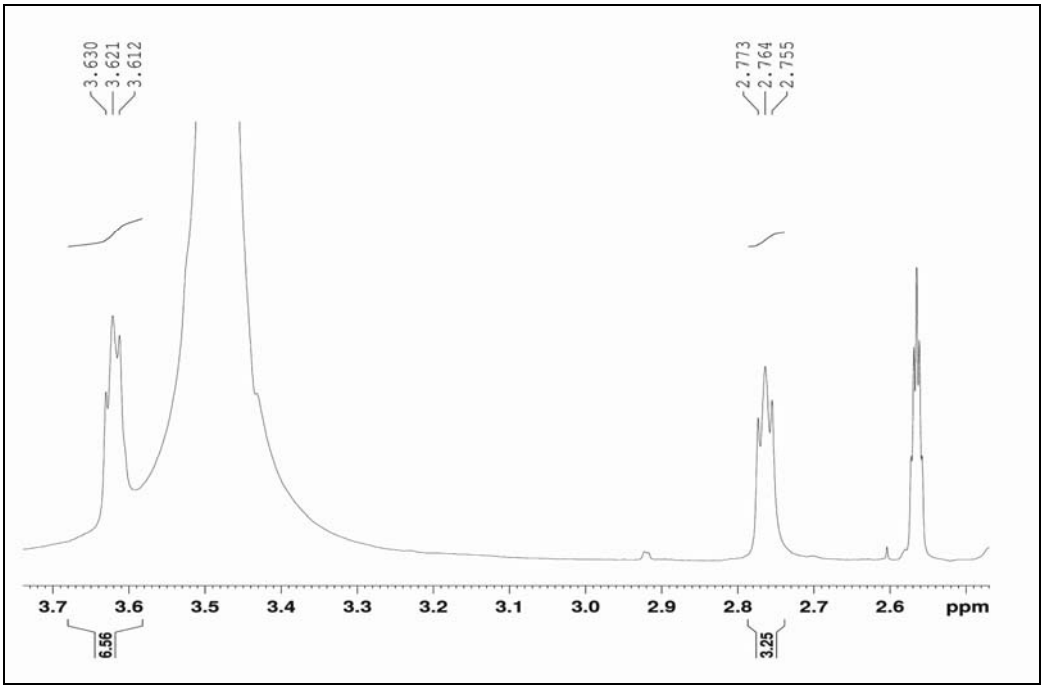
The IR spectrum also supported the above structure. It showed major peaks at 3448 cm^{-1} (N-H), 3055 cm^{-1} (Ar-H), 2848-2967 cm^{-1} (CH str.), 1566 cm^{-1}, 1576 cm^{-1} (C=N), and 1277 cm^{-1} (C=S).



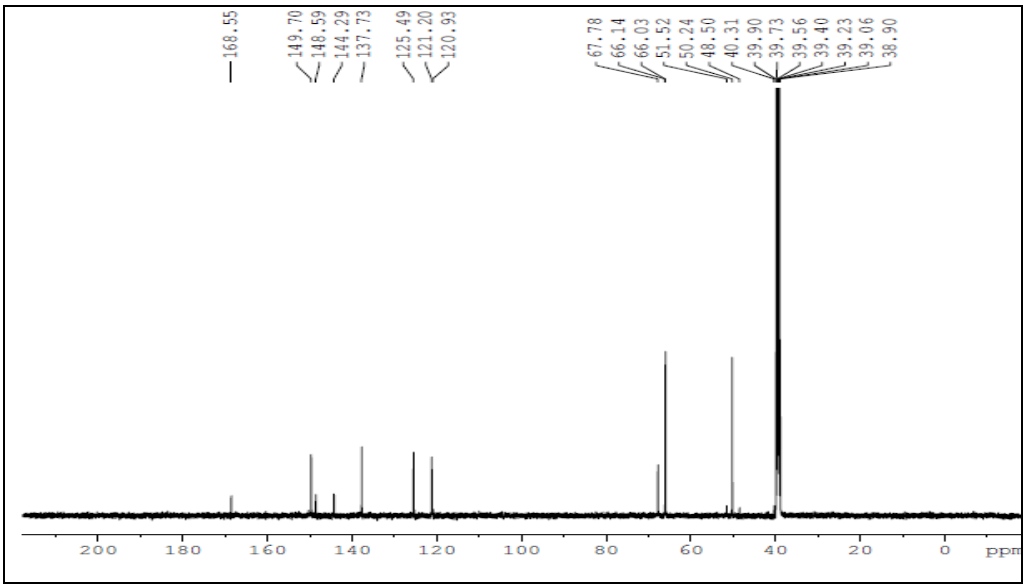
Spectrum 42: Mass spectrum of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



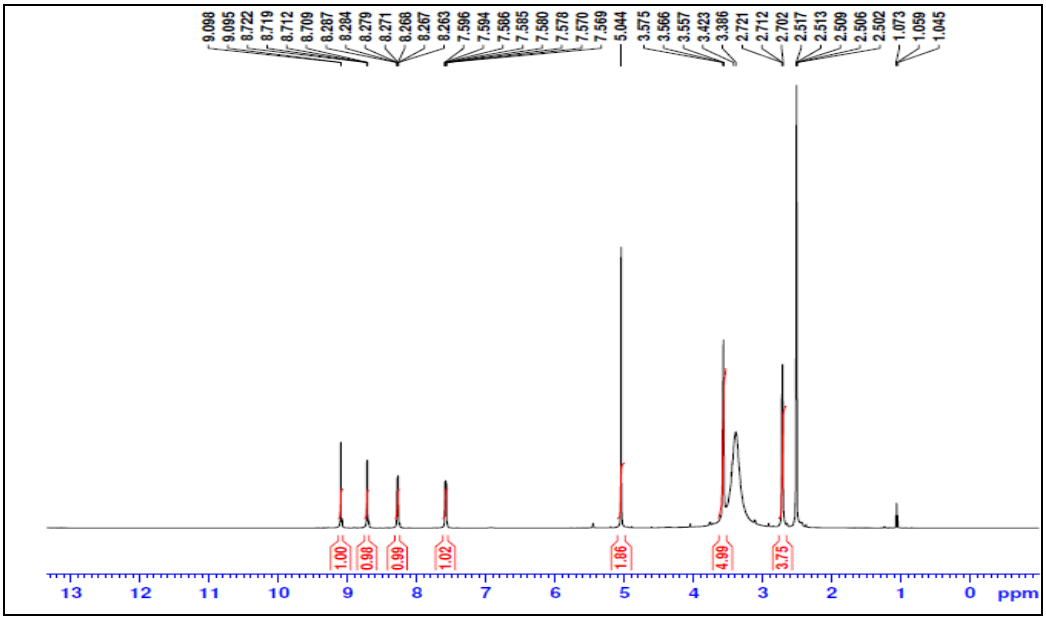
Spectrum 43: ^{1}H NMR spectrum of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



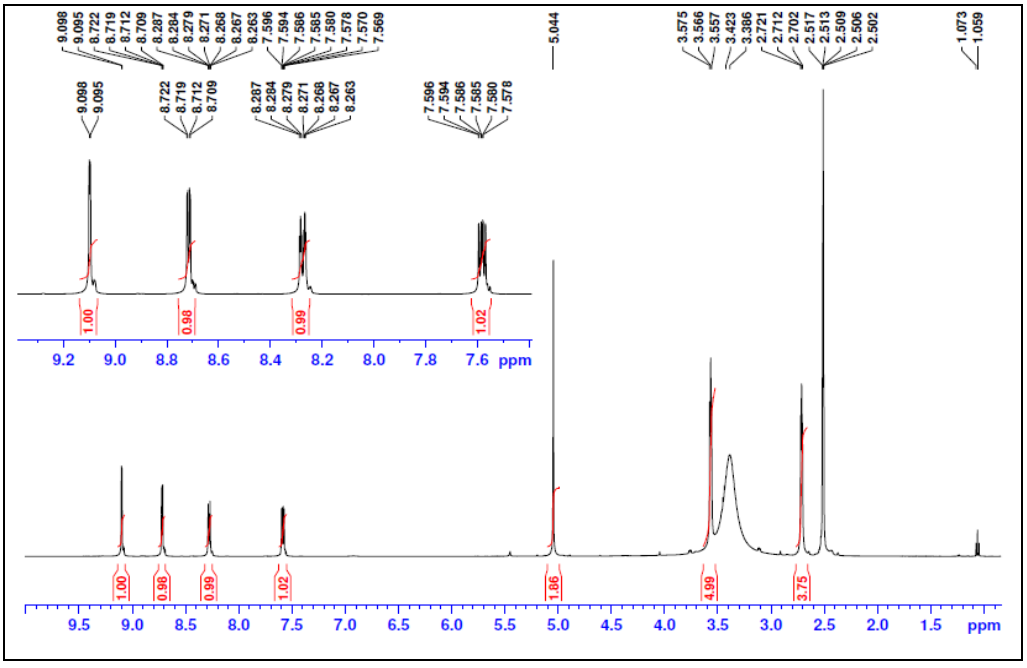
Spectrum 44: ^{1}H NMR spectrum of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 45: ^{13}C NMR spectrum of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 46: ^{1}H NMR spectrum of 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 47: ^{1}H NMR spectrum of 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

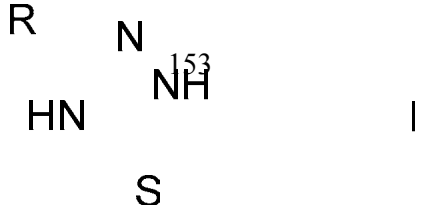
b. Synthesis of alkylated-1,2,4-Triazoles

In the present investigation, we carried out hexylation and octylation of (3-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione, by stirring the corresponding triazoles with 1-iodohexane and 1-iodooctane respectively, in ice-bath in dry DMF using sodium hydride as the base. After the reaction was completed, the mixture was poured into ice-cold water to precipitate the product, which was washed thoroughly with plenty of water, followed by petroleum ether and air dried. The off-white product was fluffy powder. The starting triazoles were prepared by the method (method A) discussed in chapter IV. The reaction can be depicted as follows **(Scheme 14).**

Scheme 14

The formation of the product was signaled by rising of its TLC spot as compared to the parent triazole. It is to be mentioned that the reaction was not successful in protic solvents like methanol and propanol, using bases like K_2CO_3 and sodium methoxide. As expected, on alkylation, the melting point of triazoles got substantially lowered by about 100 degrees and solubility in ethanol got increased so that they could be recrystallized from ethanol only. The melting range of the product indicated somewhat mesomorphic nature of it.

Six alkylated triazoles were synthesized by this method, as given in **(Table 19)**. All the synthesized compounds gave satisfactory analytical data.



The mass, ^{1}H NMR and ^{13}C NMR spectra of some representative compounds are being discussed here.

1-Octyl-3-(pyridine-2-yl)-1H-1,2,4-triazole-5(4H)-thione

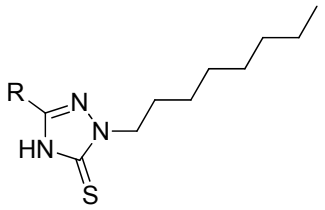


Table 19: Characterisation data of 1-Alkyl-3-(pyridinyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones

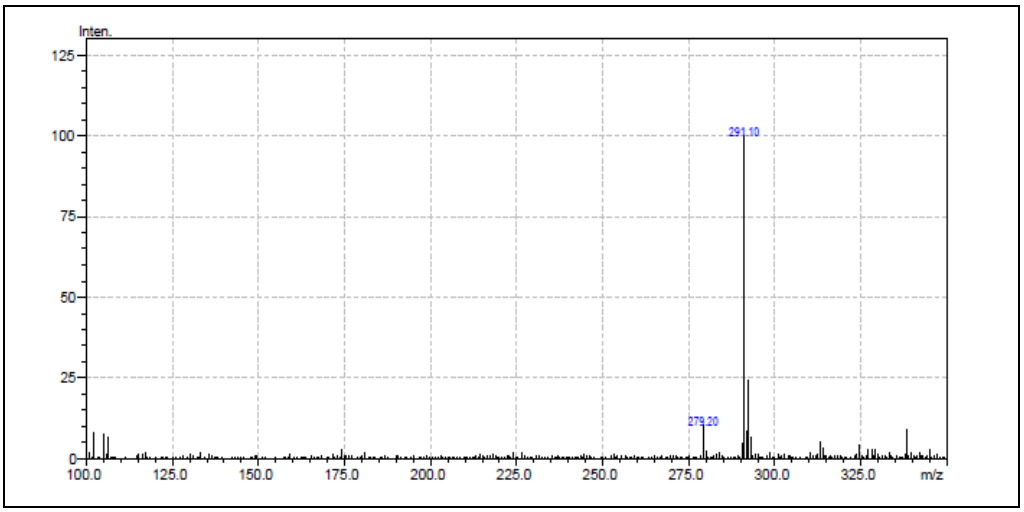
Sl. No.	Name	Molecular Formula	Yield (%)	M. P. (^{0}C)	Elemental analysis found (calcd.)		
					C	H	N
1	1-octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{15}H_{22}N_4S$	80	90-95	62.01 (62.03)	7.61 (7.64)	19.31 (19.29)
2	1-octyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{15}H_{22}N_4S$	55	80-85	62.05 (62.03)	7.62 (7.64)	19.32 (19.29)
3	1-octyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{15}H_{22}N_4S$	78	95-100	62.07 (62.03)	7.60 (7.64)	19.26 (19.29)
4	1-hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{13}H_{18}N_4S$	78	140-45	59.49 (59.51)	6.88 (6.91)	21.33 (21.35)
5	1-hexyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{13}H_{18}N_4S$	57	120-25	59.53 (59.51)	6.93 (6.91)	21.31 (21.35)
6	1-hexyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione	$C_{13}H_{18}N_4S$	75	142-48	59.48 (59.51)	6.89 (6.91)	21.38 (21.35)

The mass spectrum **(Spectrum 48)** of this compound exhibited the base peak at m/z = 291, which corresponds to the quasimolecular ion $[M+1]^+$ peak, suggesting that the molecular mass of the compound is 290. The proposed structure is in agreement with this mass. The even molecular mass is in accordance with the nitrogen rule.

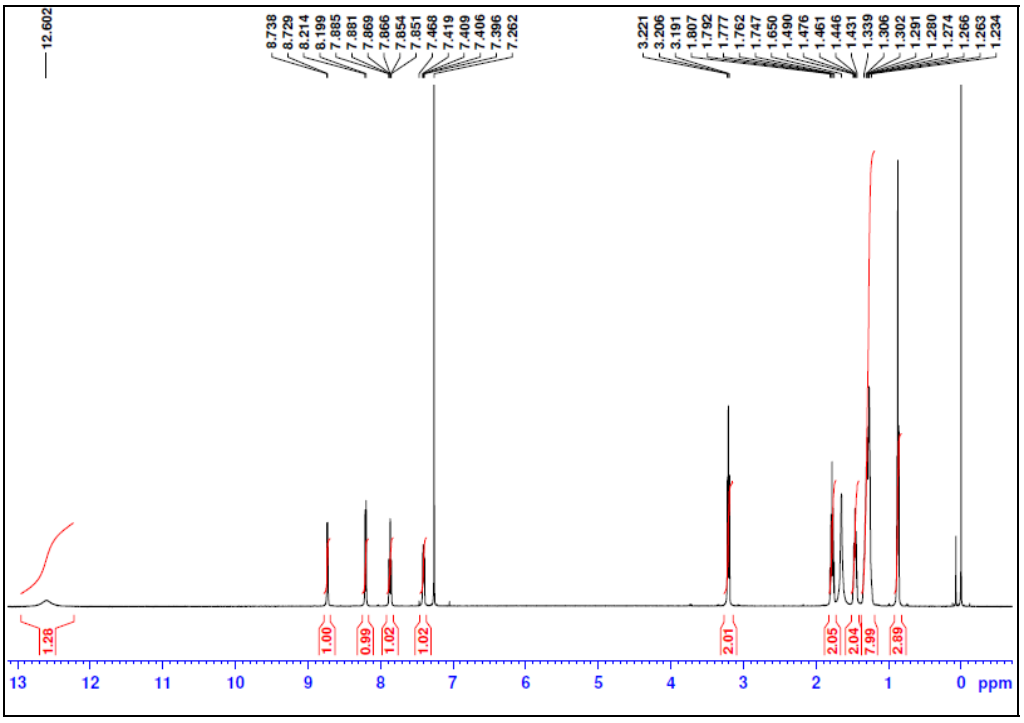
In the ^1H NMR spectrum, **(Spectrum 49, 50)** out of the two NH singlets, characteristic of the parent triazolethione (chapter IV; page), only one singlet was seen at δ 12.6 because the other got disappeared due to octylation. The larger chemical shift of the attached CH_2 (δ 3.2) of the octyl chain confirmed the possibility of N-alkylation over S-alkylation. The remaining aliphatic protons of octyl group marked their absorption between 0.87 and 1.77. The four aromatic protons of the pyridine ring absorbed between δ 7.3 and 8.7.

The ^{13}C NMR spectrum **(Spectrum 51)** exhibited intense peaks for all the eight aliphatic carbons between δ 14.0 and 32.3. Due to poor relative intensity, many aromatic peaks are not observed.

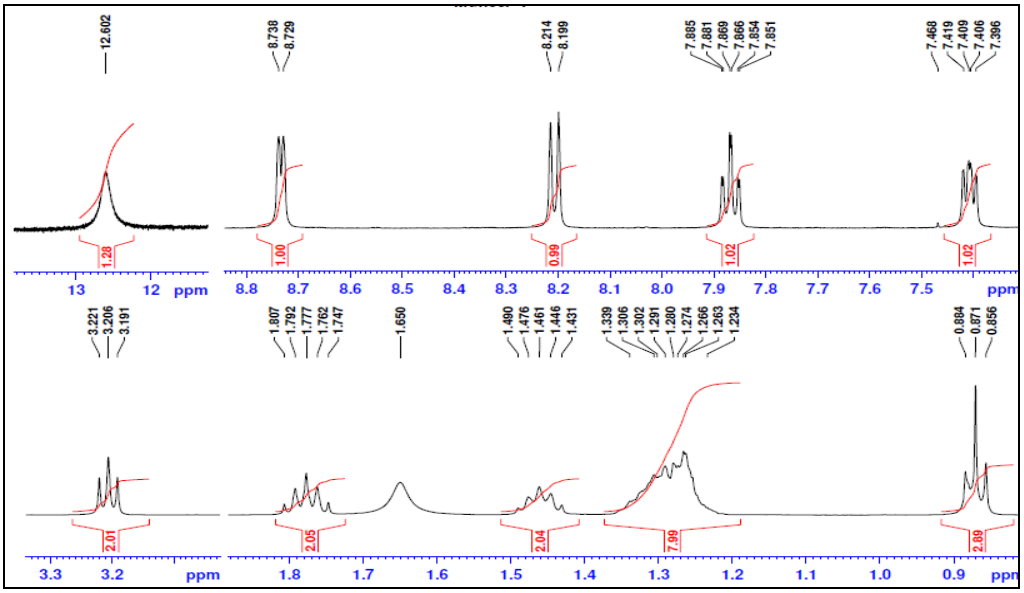
The IR spectrum showed a notable peak at 722 cm^{-1}, which is attributed to the bending (rocking) motion associated with four or more CH_2 groups in an open chain, called long-chain band. The characteristic stretching and bending peaks of methylene and methyl groups are obtained at 2854 – 2955 cm^{-1} and 1333 – 1471 cm^{-1} respectively. The NH absorption was observed at 3418 cm^{-1}.



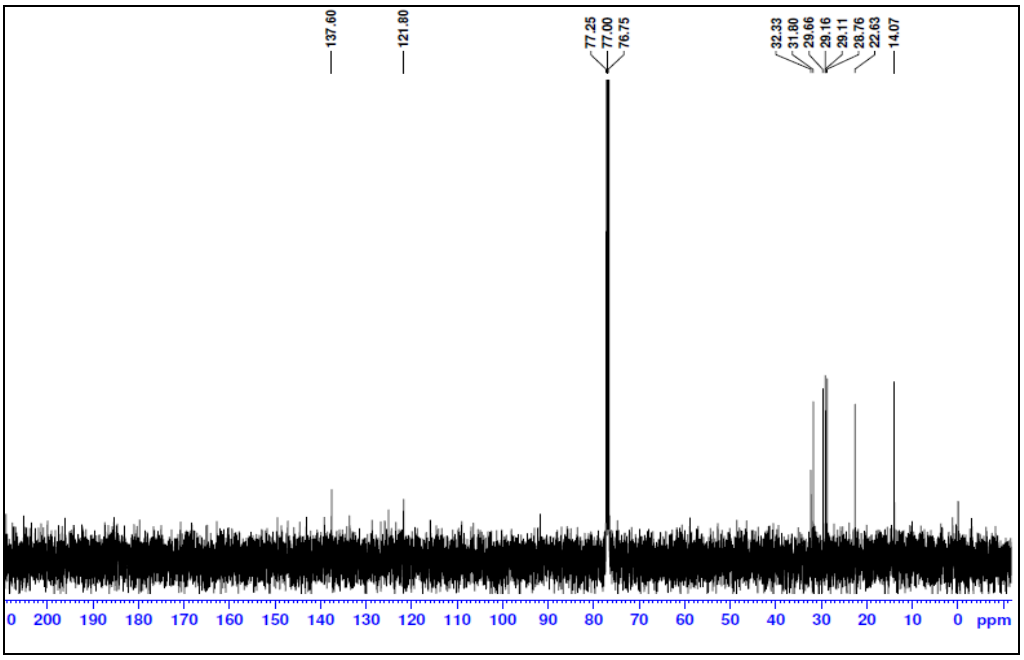
Spectrum 48: Mass spectrum of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 49: ^{1}H NMR spectrum of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

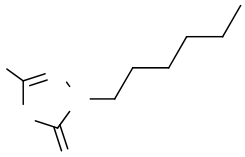


Spectrum 50: ^{1}H NMR spectrum of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

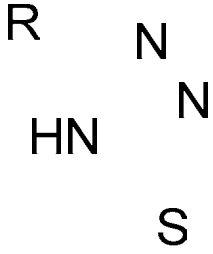


Spectrum 51: ^{13}C NMR spectrum of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



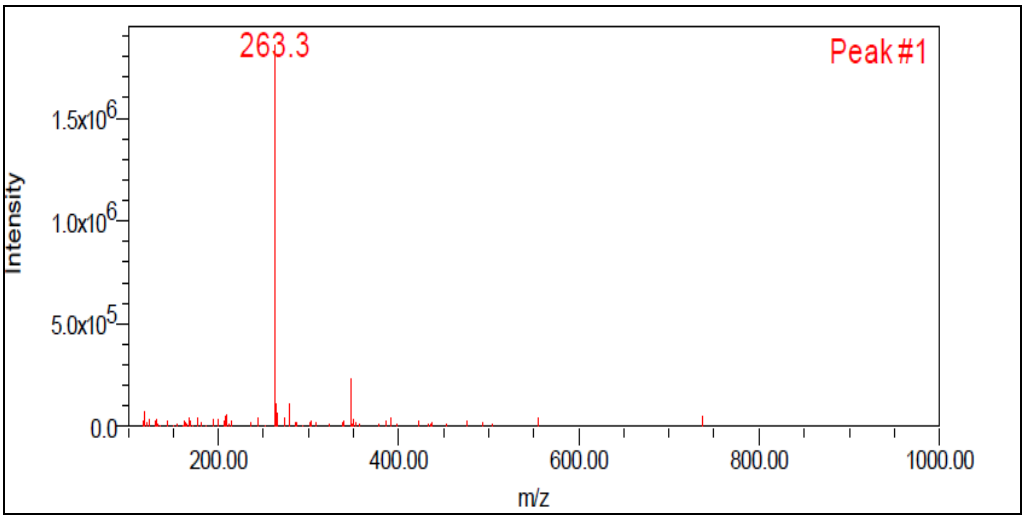
The mass spectrum **(Spectrum 52)** of this compound showed the base peak at m/z = 263, corresponding to the quasimolecular ion $[M+1]^+$ peak, indicating that the molecular mass of the compound is 262, which is in agreement with the proposed structure. The even molecular mass agrees with the even number of nitrogen atoms..



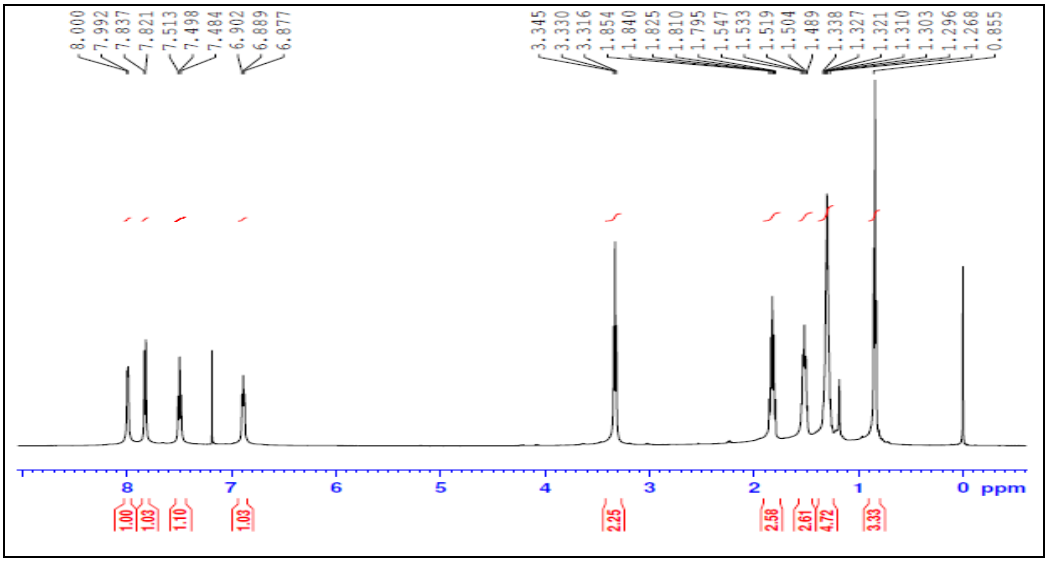
In the ^{1}H NMR spectrum **(Spectrum 53, 54, 55)**, all the aliphatic protons of the hexyl chain absorbed between δ 0.86 and 3.3. The four aromatic protons of the pyridine ring absorbed between δ 6.9 and 8.0.

The ^{13}C NMR spectrum **(Spectrum 56)** exhibited more intense peaks for the aliphatic carbons between δ 13.0 and 32.4, whereas the less intense aromatic protons absorbed between δ 119.4 and 161.5.

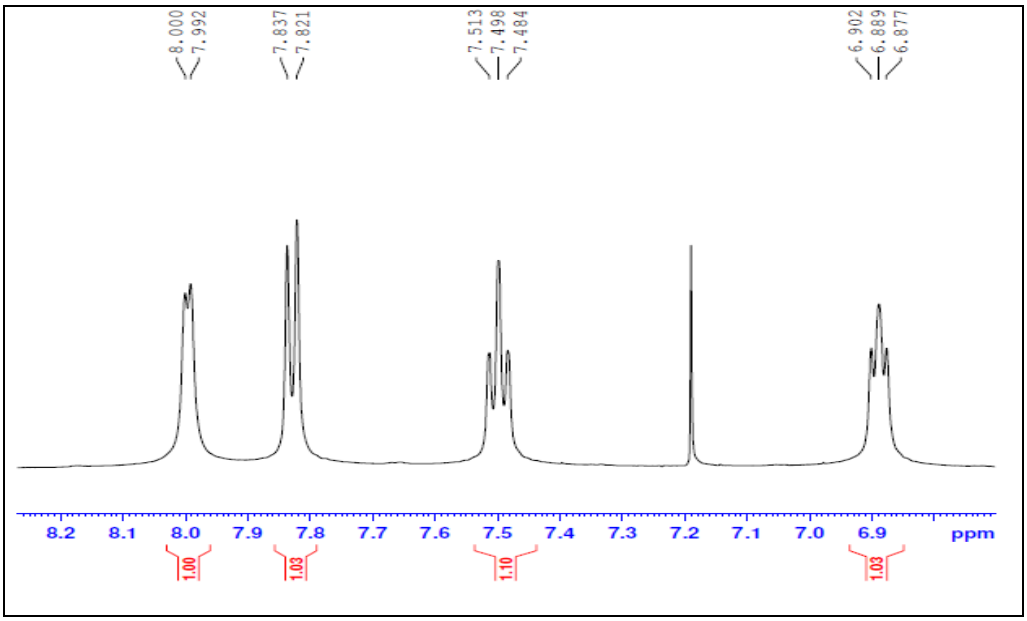
The IR spectrum also showed the characteristic peaks for various stretching and bending vibrations.



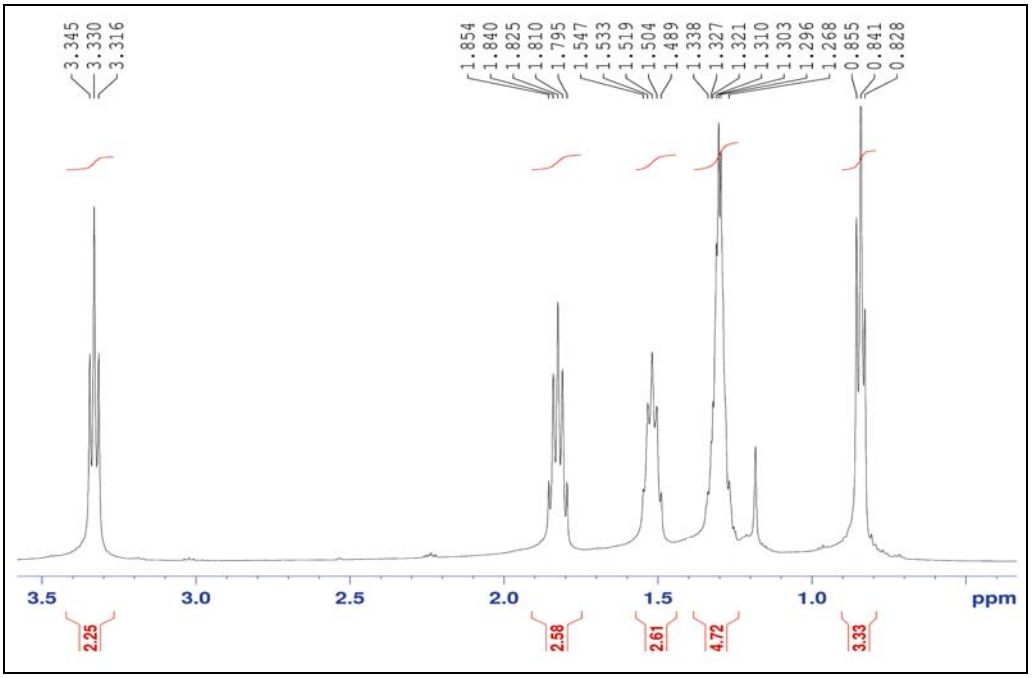
Spectrum 52: Mass spectrum of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



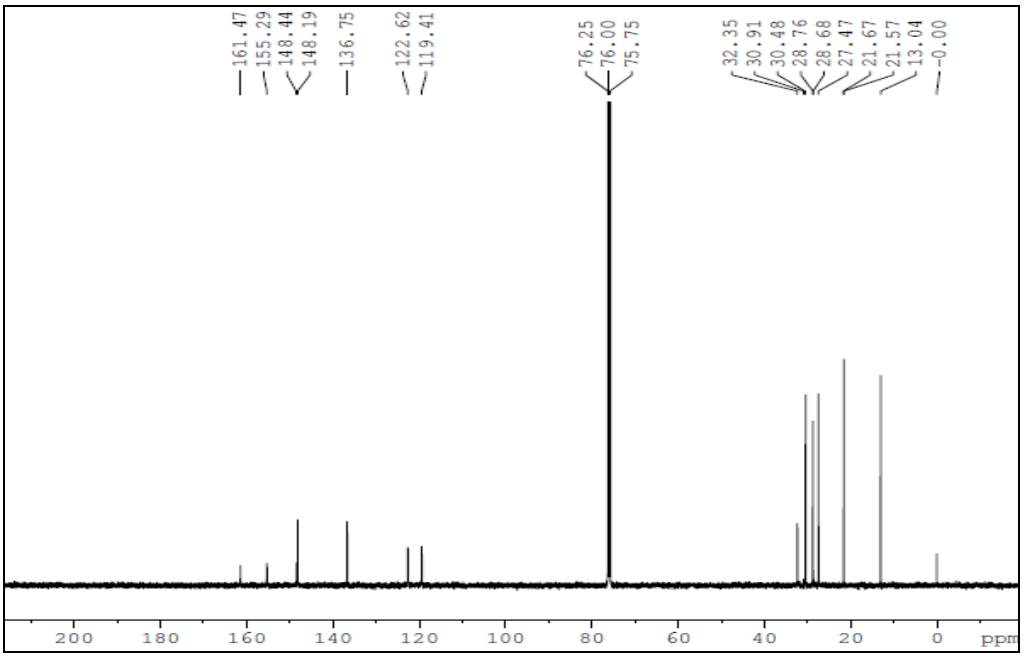
Spectrum 53 ^{1}H NMR spectrum of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 54: ^{1}H NMR spectrum of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione



Spectrum 55: ^{1}H NMR spectrum of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

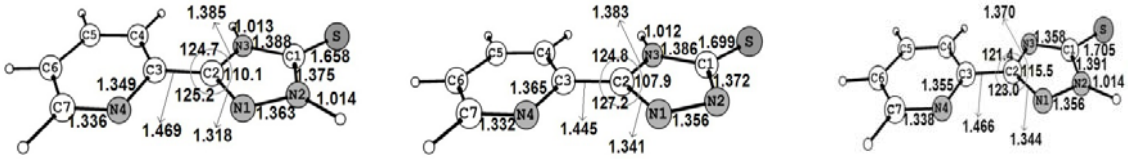


Spectrum 56: ^{13}C NMR spectrum of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

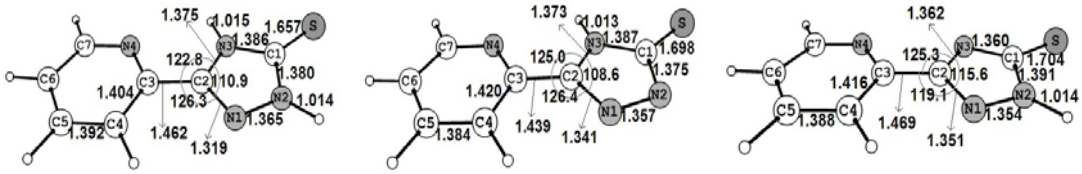
5.1.5 Theoretical Study

To investigate the optimized geometries of the conformers of of 5-(2-pyridyl)-3-thio-1, 2, 4-triazole and to predict the thermodynamic preference for the formation of N-hexylated derivative, computational calculations were carried out using the Gaussian 09 program package as mentioned in chapter 2.

Results and Discussion:

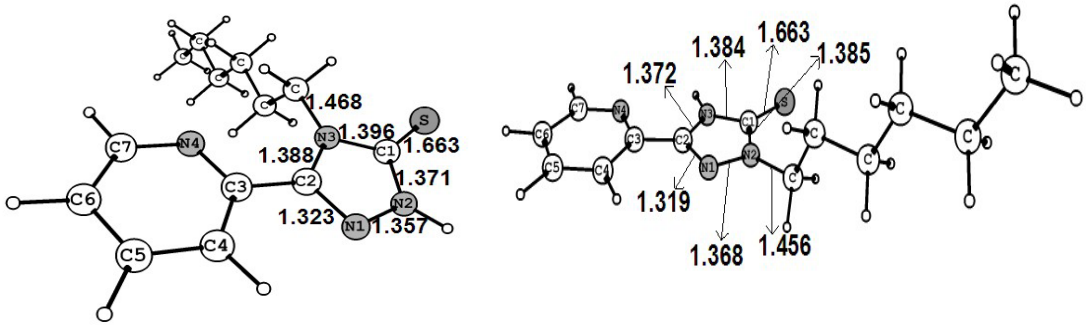


1 (6.8 kcal/mol) **3** **3'**



2 (0.0 kcal/mol) **4** **4'**

Figure 9: Optimized geometries of the different conformers of 3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (1 and 2) and its mono deprotonated (3, 3', 4 and 4') at the BP86/TZVPP level of theory. The relative energy between 1 and 2 at the M06/TZVPP//BP86/TZVPP level of theory is given in the parenthesis.



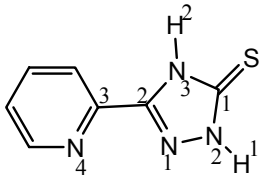
5 (N1─C2─C3─N4 = 0.4°) **5'** (N1─C2─C3─N4 = 5.9°)

Figure 10: Optimized geometries of 5 and 5' at the BP86/TZVPP level of theory. The N1─C2─C3─N4 dihedral angle is given in the parenthesis.

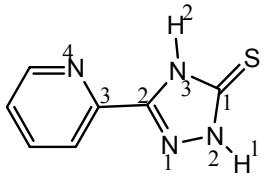
Table 20: Deprotonation energies of geometries 1 and 2 at the M06/TZVPP//BP86/TZVPP level of theory.

Reaction	Energy (kcal/mol)
1 → **3** + H^+	337.5
1 → **3'** + H^+	328.3
2 → **4** + H^+	337.5
2 → **4'** + H^+	337.0

Table 21: NBO charge distribution in **1** and **2** at the M06/TZVPP//BP86/TZVPP level of theory.



1



2

	NBO charge	
Atom	**1**	**2**
N2	-0.38	-0.38
N3	-0.56	-0.55
H1	0.42	0.42
H2	0.43	0.45

Table 22: Energy of the reaction of 1 and 2 with RI, (where R = C_6H_{13}) to form corresponding alkyl substituted products at the M06/TZVPP//BP86/TZVPP level of theory.

Reaction	Energy (kcal/mol)
2 + **RI** → **5** + HI	-4.9
2 + **RI** → **5'** + HI	-1.2

The optimized geometries of two possible conformers of 3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**1** and **2**) are given in the **Figure 9**. The relative energy shows that the conformer in which diazo group trans to the nitrogen atom of the pyridine ring (**2**) is stable by 6.8 kcal/mol than the corresponding cis-conformer (**1**). The two hydrogen atoms attached to the nitrogen atoms, N2 and N3, can undergo substitution reactions. The deprotonation energy of **2** calculated for the removal of H1 and H2 is ≈ 337 kcal/mol (**Figure 9, Table 20**). The reaction energy calculated for the substitution reaction of **2** with 1-iodo-hexane indicates that the reaction is exothermic (**Figure 10 and Table 22**). The optimized geometries of n-hexyl substituted products (**5** and **5'**) of **2** at the BP86/TZVPP level of theory are given in **figure 10**. The substitution at N2 atom is more exothermic than that at N3. The thermodynamic preference for **5** can be explained by the minimum sterric repulsion between ─C6H13 group and pyridine ring, indicated by the N1─C2─C3─N4 dihedral angle (**Figure 10**). From the reaction energetics, it can be concluded that the most favorable product for electrophilic substitution reaction of **2** by C_6H_{13}−I is the N2 substituted product.

5.1.6 Experimental

Melting point recorded on a Toshniwal capillary melting point apparatus are uncorrected. The mass spectrum was recorded on LCMS-2020 Shimadzu machine. The NMR experiments were conducted using Bruker Avance III 500 MHz FT-NMR instrument. IR spectra were recorded as KBr pellets using Shimadzu 8101A FTIR equipment. TLC was performed on the glass-backed silica gel sheets (BSS 350).

Preparation of starting materials

The triazoles employed in the reactions such as 3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione, 3-(pyridin-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione and 3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione were prepared by the method described in chapter IV.

a. Synthesis of Mannich base of Triazoles

Synthesis of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol), 40% formaldehyde (0.75 ml, 0.005 mol) and morpholine (0.5 ml, 0.005 mol) in ethanol (20 ml) was stirred for 2 hours and left overnight at room temperature. The colourless solid thus separated was collected by filtration, washed and dried. The yield of 1-(Morpholinomethyl)-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.18 g (85%). The compound was recrystallized from ethanol to get colourless shining crystals which melted at 190 ^{0}C.

Synthesis of 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol), 40% formaldehyde (0.75 ml, 0.005 mol) and morpholine (0.5 ml, 0.005 mol) in ethanol (20 ml) was stirred for 2 hours and left overnight at room temperature. The colourless solid thus separated was collected by filtration, washed and dried. The yield of 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.1 g (80%). The compound was recrystallized from ethanol to get colourless shining crystals which melted at 225 ^{0}C.

Synthesis of 1-(Morpholinomethyl)-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol), 40% formaldehyde (0.75 ml, 0.005 mol) and morpholine (0.5 ml, 0.005 mol) in ethanol (20 ml) was stirred for 2 hours and left overnight at room temperature. The colourless solid thus separated was collected by filtration, washed and dried. The yield of 1-(Morpholinomethyl)-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.08 g (78%). The compound was recrystallized from ethanol to get colourless shining crystals which melted at 238 ^{0}C.

b. Synthesis of alkylated triazoles

Synthesis of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodooctane (1 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using

ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of water and finally with petroleum ether and air dried. The yield of 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.16 g (80%). The compound was recrystallized from ethanol, which melted at 90-95^{0}C.

1-Octyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodooctane (1 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of water and finally with petroleum ether and air dried. The yield of 1-Octyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.798 g (55%). The compound was recrystallized from ethanol, which melted at 80-85^{0}C.

1-Octyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodooctane (1 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of water and finally with petroleum ether and air dried. The yield of 1-Octyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.13 g (78%). The compound was recrystallized from ethanol, which melted at 95-100^{0}C.

1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodohexane (0.75 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of water and finally with petroleum ether and air dried. The yield of 1-Hexyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 1.02 g (78%). The compound was recrystallized from ethanol, which melted at 140-145^{0}C.

1-Hexyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodohexane (0.75 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of water and finally with petroleum ether and air dried. The yield of 1-Hexyl-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.75 g (78%). The compound was recrystallized from ethanol, which melted at 120-125^{0}C.

1-Hexyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione

A solution of 3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (0.9 g, 0.005 mol) in dry DMF (15 ml) taken in 100 ml RB flask is kept in an ice-bath. Added 60% NaH (200 mg, 0.005 mol) followed by 1-iodohexane (0.75 ml, 0.005 mol) and stirred for 2 hours. The reaction was monitored by TLC using ethyl acetate as eluent. The mixture was poured into crushed ice to precipitate a colourless fluffy solid, which was filtered, washed with plenty of

water and finally with petroleum ether and air dried. The yield of 1-Hexyl-3-(pyridine-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was 0.98 g (78%). The compound was recrystallized from ethanol, which melted at 142-148^{0}C.

5.2. ANTIBACTERIAL STUDY OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONE AND DERIVATIVES

Present Study

Three compounds namely 3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_1), 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_2), and 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_3) were tested for their *in vitro* antibacterial activity against the same bacterial strains as discussed in chapter I.

Experimental

The antibacterial test carried out remained the same Kirby-Bauer disc diffusion method, as discussed in chapter I.

Result and Discussion

The compound M_1 showed activity both against the Gram positive and negative bacteria (**Table-23**). The activity of M_1 against *E. coli and Pseudomonas sp.* was in a dose dependent manner. The compound M_2 also showed activity against all tested bacteria (**Table-24**). But M_2 showed no antibacterial activity. Among the tested compounds M_1 was found to be a better antibacterial agent.

Table 23: Antibacterial Activity of Compound M_1

Compound (µg/mL)	Zone diameter (mm)			
	E.coli	***Pseudomonas***	***Streptococcus***	***Staphylococcus***
DMSO	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	NA*
250	9	10	NA*	NA*
500	12	13	8	9

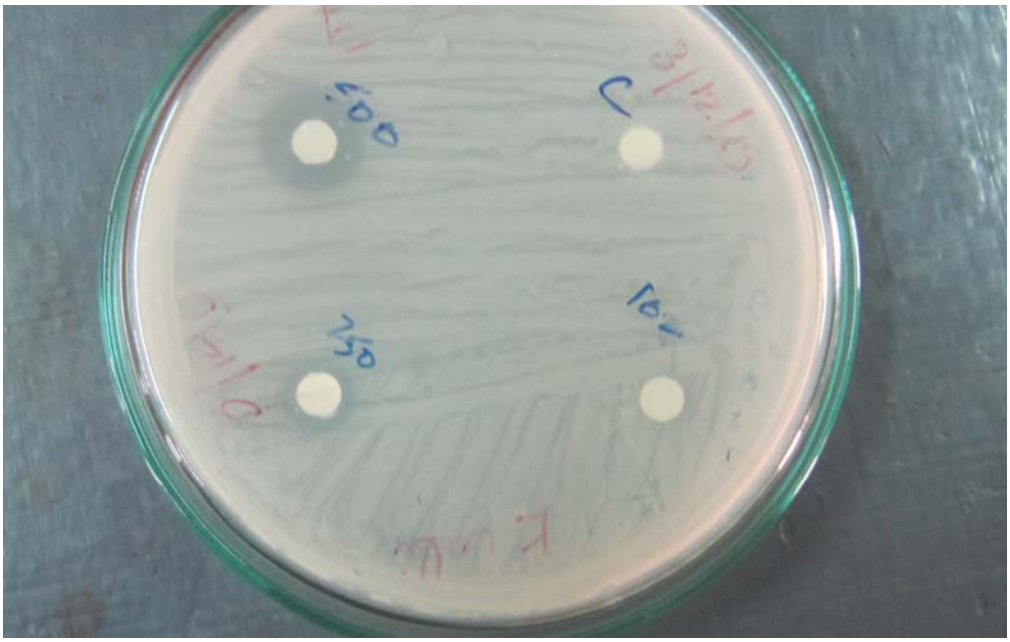
NA*= No activity, n = 2

Table 24: Antibacterial Activity of Compound M_2

Compound (µg/mL)	Zone diameter (mm)			
	E.coli	***Pseudomonas***	***Streptococcus***	***Staphylococcus***
DMSO	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	NA*
250	8	6	NA*	6
500	10	9	9	9

NA*= No activity, n = 2

Compound M_1 (*Pseudomonas*)



Compound M_1 (*E. coli*)

5.3 ANTIFUNGAL STUDY OF 3-SUBSTITUTED-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONE AND DERIVATIVES

Introduction

Fungal infections pose a continuous and serious threat to human health and life. Fungal infections in human can be classified into (a) allergic reactions to fungal proteins (b) toxic reactions to toxins present in certain fungi and (c) infections (mycoses). Many fungal infections are caused by opportunistic pathogens that may be endogenous (candida infection) or acquired from the environment (Cryptococcus, Aspergillus infections)[29].

Individuals with increased vulnerability such as neonates, cancer patients receiving chemotherapy, organ transplant patients, burns patients, and AIDS patients are more prone to fungal infections. Other risk factors include corticosteroid and antibiotic treatments, diabetes, lesions of epidermis and

dermis, malnutrition, neutropenia and surgery[30-33]. In recent years, the incidence and severity of fungal diseases has increased, particularly in patients with impaired immunity.

Aspergillus and Candida spp. account for the majority of infections. Clinically candidiasis and aspergillosis account for between 80% and 90% of systemic fungal infections in immunocompromised patients[34].

Present Study

Three compounds namely 3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_1), 1-(Morpholinomethyl)-3-(pyridine-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_2), and 1-Octyl-3-(pyridine-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (M_3)were tested for their *in vitro* antifungal activity against two fungal strains namely *Aspergillus flavus* and *Aspergillus heteromorphus*.

Experimental

For activity measurement by disc diffusion method, the prepared discs (that had been soaked in the various concentrations of compounds: 100, 50, 25 μgmL^{-1} in DMSO) were placed at different positions on a surface of petri plates covered by Potato Dextrose Agar (PDA) medium which have been fecundated with 10 µL of fungal spore suspension. The plates were incubated at 32 ^{0}C for 2-3 days.

Result and Discussion

Unfortunately all the compounds did not show any significant activity *in vitro* against the tested organisms.

References

1. Benson, F. R. Tetrazoles, tetrazines and purines and related ring systems, in: Elderfield (Ed.), Heterocyclic Compounds, vol. 8, Wiley, New York, **1967**, p. 1-104.
2. Temple, C. Jr. Triazoles 1,2,4, in: Montgomery (Ed.), Chemistry of the Heterocylic Compounds, vol. 37, Wiley, New York, **1981**, pp. 155-162.
3. Malbec, F.; Milcent, R.; Vicart, P.; Bure, A. M. *J. Heterocycl. Chem*. **1984**, 21, 1769-1774.
4. El-Masry, A. H.; Fahmy, H. H.; Ali Abdelwahed, S. H. *Molecules*, **2000**, 5, 1429-1438.
5. Shaker, R. N.; Mahmoud, A. F.; Abdel-Latif F. F. *Phosphorus Sulfur Silicon Relat. Elem.* **2005**, 180, 397-406.
6. Salgin-Gökşen, U.; Gökhan-Kelekçi, N.; Götas, O.; Köysal, Y.; Kiliç,.; Işik, S.; Aktayb, G.; Özalpd, M. *Bioorg. Med. Chem.* **2005**, 15, 5738-5751.
7. Garoufalias, S. S.P.; Todoulou, O. G.; Filippatos, E. C.; Valiraki, A. E. P.; Chitirogiou-Lada, A. *Arzeim. Forsch./Drug Res.* **1998**, 48, 1019-1023.
8. Sadana, K. A.; Mirza, Y.; Aneja, K. R.; Prakash, O. *Eur. J. Med. Chem*. **2003**, 38, 533-536.
9. Mullican, M. D.; Wilson, M. W.; Connor, D. T.; Kostlan, C. R.; Schrier, D. J.; Dyer, R. D. *J. Med. Chem.* **1993**, 36, 1090-1099.
10. Holla, B. S.; Veerendra, B.; Shivananda, M. K.; Poojari, B. *Eur. J. Med. Chem*. **2003**, 58, 759-767.
11. Karthikeyan, M. S.; Prasad, D. J.; Poojari, B.; Bhat, K. S.; Holla, B. S.; Kumari, N. S. *Bioorg. Med. Chem***. 2006**, 14, 7482-7489.
12. Tramontini, M.; Angiolini, L. *Tetrahedron* **1990**, 46, 1791-1837.
13. Ashok, M.; Holla, B. S.; Poojary, B. *Eur. J. Med. Chem.* **2007**, 42, 1095-1101.
14. Holla, B. S.; Poojary, K. N.; Sooryanarayana Rao, B.; Shivananda, M. K. *Eur. J. Med. Chem.* **2002**, 37, 511-517.

15. Walczak, K.; Gondela, A.; Suwiński, J. *Eur. J. Med. Chem*. **2004**, 39, 849-853.
16. Amir, M.; Shikha, K. *Eur. J. Med. Chem*. **2004**, 39, 535-545.
17. Tramontini, M.; Augioline, L.; Ghedeni, N. *Polymer* **1988**, 29, 771.
18. Koparir, M.; Orek, C.; Parlak, A. E.; Söylemez A.; Koparir, P.; Karatepe, M.; Dustan, S. D. *Eur. J. Med. Chem***. 2013**, 63, 340-346.
19. Rezaei, Z.; Khabnadidesh, S.; Pakshir, K.; Hossaini, Z.; Amiri, F. *Eur. J. Med. Chem*. **2009**, 44, 3064-3067.
20. Danilova, E. A.; Ivoline, A. A.; Vorontsova, A. A.; Islyaikin, M. K.; Anan'eva, G. A.; Zharnikova, N. V.; Bykova, V. V.; Usol'tseva, N. V. *Liquid Crystals and Their Application,* **2011**, 3(37), 5-14.
21. Shan, G.-G.; Li, H.-B.; Cao, H.-T.; Sun, H.-Z.; Zhu, D.-X.; Su, Z.-M. *Dyes and Pigments*, **2013**, 99, 1082-1090.
22. Almajan, G. L.; Barbuceann, S-F; Almajan, E-R; Draghici, C.; Saramet, G. *Eur. J. Med. Chem.* **2009**, 44, 3083-3089.
23. Fahmy, S. M.; Kandeel, E. M.; Elsayed E.-F. R.; Elnagdi, M. H. *J. Heterocycl. Chem*. **1978**, 15, 1291.
24. Katritzky, A. R.; Kuzmierkiewicz, W.and Greenhill, J. V. Department of Chemistry, University of Florida, Gainesville, **1991**, Fl 32611-2046, U.S.A.
25. Bulger, P. G.; Cottrell, I. F.; Cowden, C. J.; Davies, A. J.; Dolling, U-H. *Tetrahedron Letters*, **2000**, 41, 1279-1301.
26. Cherkovskaya, L. G.; Knysh, E. G.; Rogul'chenko, G. K.; Drogovoz, S. M.; Sal'nikova, S. I.; Steblyuk, I. N. *Farmatsevtichnii Zhurnal (Kiev)* **1989**, 5, 67; *Chem. Abstr*. 112, 198233.
27. Yang, G. F.; Lu, R. J.; Fie, X. N. *Chin. J. Chem*. **2000**, 18, 435.
28. Badea, V.; Sofei, M. D.; Venter, M. M.; Bercean, V. N. *Tetrahedron* **2007**, 63, 1467-1473.
29. Garibotto, F. M.; Garro, A. D.; Masman, M. F.; Rodriguez, A. M.; Luiten, P. G.; Raimondi, M. M.; Zacchino, S. A.; Somlai, C.; Penke, B.; Enriz, R. D. *Bioorg. Med. Chem***. 2010**, 18, 158.
30. Lv, Z.; Sheng, C.; Zhang, Y.; Wang, T.; Feng, J.; Sun, H.; Zhong, H.; Zhang, M.; Chen, H.; Li, K. *Bioorg. Med. Chem. Lett*. **2010**, 20, 7160.

31. Masman, M. F.; Rodrguez, A. M.; Raimondi, M.; Zacchino, S. A.; Luiten, P. G. M.; Somlai, C.; Kortvelyesi, T.; Penke, B.; Eriz, R. D. *Eur. J. Med*, **2009**, 44, 212.
32. Geogopapadakou, N.; Tkacz, J.; *Trends Microbial*, **1995**, 3, 98.
33. Nagiec, M.; Nagiec, E.; Baltisberger, J.; Wells, G.; Lester, R.; Dickson, R. *Biol. J. Chem*. **1997**, 272, 9809.
34. Onnis, V.; De Logu, A.; Cocco, M. T.; Fadda, R.; Meleddu, R.; Congiu, C. *Eur. J. Med. Chem*. **2009**, 44, 1288.