4–अमीनो–3–मर्केप्टो–5–फिनायल–1,2,4–ट्रायाज़ोल के शिफ़ बेसों का संश्लेषण एंव कवक अविषालुता का मूल्यांकन

Synthesis and Fungitoxicity Evaluation of Schiff Bases of 4-Amino-3-Mercapto-5-Phenyl-1,2,4-Triazole.

ARNAB ROY CHOWDHURY

DIVISION OF AGRICULTURAL CHEMICALS
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI – 110012

2008

4-अमीनो-3-मर्केप्टो-5-फिनायल-1,2,4-ट्रायाज़ोल के शिफ़ बेसों का संश्लेषण एंव कवक अविषालुता का मूल्यांकन

सार

प्रतिरोधिता, पीड़क समस्याओं में परिवर्तन, कई पर्यावरण कारकों एवं नए-नए उत्पादों से प्रतिस्पर्धा के कारण भी पीड़कनाशी लम्बे समय तक प्रचलन में नहीं रह पाते। इसलिए पुराने पीड़कनाशियों के स्थान पर नए पीड़कनाशियों के विकास की आवश्यकता होती है जो अधिक सक्षम होने के साथ-साथ पर्यावरण को भी सुरक्षित रख सकें। कई विषमचक्रीय अर्धांशों वाले शिफ़ बेसों का प्रयोग औषिधियों के रूप में किया जाता है और उनका पीड़कनाशीयों के रूप में प्रयोग भी सूचित किया गया है। कुछ 4-अमीनो-1,2,4-ट्रायाज़ोलस् के शिफ़ बेसों का संश्लेषण किया जा चुका है और उनमें कवक प्रतिरोधिता, जीवाणु प्रतिरोधिता एवं पादप वृद्धि नियामक (पी जी आर) गुण सूचित किए गए है। हाल ही में कुछ 5-एरिल-4-अमीनो-3-मर्केप्टो-1,2,4-ट्रायाज़ोलस् में कवकनाशी होने की क्षमता सूचित ही गई है।

सक्षम कवकनाशियों की खोज के प्रयास में बीस शिफ़ बेसों की एकश्रृखंला से जुड़े एरिल रिंग में भिन्न-भिन्न स्थानों पर प्रतिस्थापन वाले 4-अमीनो-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल के एरीलाइडेनअमीनो-3 मर्केप्टो-5-फिनायल -4एच-1,2,4-ट्रायाज़ोलस् का एक पाँच चरणों वाली संश्लेषण योजना द्वारा संश्लेषण किया गया जो बेंजोइक अम्ल से आरम्भ होती है जो पहले मिथायल एस्टर मे परिवर्तित होता है ⟶ हायड्रेज़ाइड ⟶ डाइथायोकार्बाज़िनिक एसिड साल्ट ⟶ ट्रायाज़ोल एवं अन्ततः ⟶ शिफ़ बेस। इस संश्लेषण के पाँच में से तीन चरण अर्थात मिथायल एस्टर से हायड्रेज़ाइड़: डाइथायोकार्बाज़िनिक एसिड साल्ट से ट्रायाज़ोल एवं ट्रायाज़ोल से शिफ़ बेसों का सूक्ष्मतरंगों का उपयोग कर संश्लेषण किया गया। परम्परागत विधि के बजाय सूक्ष्मतरंगों से संश्लेषण अधिक त्वरित एवं क्षमता पूर्वक होता है। शिफ़ बेस, 4-एरीलाईडेनअमीना-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल, यथेष्ट एल्डिहायड्स के साथ इथेनॉल में 4-अमीनो-3-मर्केप्टो-5-फिनायल-1,2,4-ट्रायाज़ोल की 2-4 घन्टे रिफ्लक्सिंग द्वारा 52-92% उत्पादन के साथ प्राप्त किए गए।

तात्विक विश्लेषण, आई आर एवं एन एम आर स्पेक्ट्रोस्कोपी द्वारा शिफ़ बेसों की संरचना का गुण निर्धारण किया गया।

तीन पादप रोगजनक कवकों, *राइज़ोक्टोनिया सोलेनाइ*, *फ्यूज़ेरियम ऑक्सीस्पोरम* एवं *बाइपोलेरिस सोरोकीनियाना* के विरूद्ध कवक अविषालुता हेतु शिफ़ बेसों का मूल्यांकन किया गया। इस श्रृंखला के सभी यौगिकों में 4-(3-मिथायलबेंज़ायली

डेनअमीनो)-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल (ई डी$_{50}$= 17.34 पीपीएम), 4-(4-क्लोरोबेंजायलीडेनअमीनो)-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल (ई डी$_{50}$= 95.55 पी पी एम) एवं 4-(3-क्लोरोबेंजायलीडेनअमीनो)-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल (ई डी$_{50}$= 181.3 पी पी एम) ने क्रमशः *रा.* सोलेनाइ, *फ्यू. ऑक्सीस्पोरम* एवं *बा. सोरोकीनियाना* के विरूद्ध मूल ट्रायाज़ोल, 4-अमीनो-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोल (ई डी$_{50}$= 76 पी पी एम) की तुलना में सर्वाधिक सक्रियता दर्शायी।

यद्यपि कवक आविषालुता संबंधी आँकड़े, संरचना सक्रियता संबंध के विषय में कुछ महत्वपूर्ण निष्कर्षों तक ले जाने हैं किन्तु कवक आविषालुता हेतु सही संरचनात्मक गुणों की पहचान हेतु कोशिकाओं के बाहर प्रयोगशाला में लिए गए कवक अविषालुता आँकड़ो तथा बेंजायलीडीन रिंग प्रतिस्थापियों के हायड्रोफोबिक, इलेक्ट्रॉनिक एवं स्टीरिक गुणों का मल्टीपल रिग्रैशन विश्लेषण तकनीकों द्वारा विभिन्न भैतिक-रासायनिक प्रचलों का उपयोग कर मात्रात्मक संरचना सक्रियता संबंधों (क्यू एस ए आर) का विश्लेषण किया गया। क्यू एस ए आर मॉउल दर्शाते हैं कि 4 एरिलाइडेनअमीनो-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोलस् की *रा. सोलेनाइ* के विरूद्ध कवकनाशी सक्रियता, हायड्रोफोबिसिटी (π) के साथ प्रतिलोम पेराबोलिक संबंध दर्शाती है जबकि *फ्यू. ऑक्सीस्पोरम* एवं *बा. सोरोकीनियाना* के विरूद्ध कवक आविषालुता, बैंजायलीडीन रिंग में उपस्थित प्रतिस्थापी को इलेक्ट्रॉनिक प्रवृत्ति (σ) पर निर्भर है। *बा. सोरोकीनियाना* के विरूद्ध ऑर्थो-सब्सटीट्यूंटस् की लम्बाई हेतु स्टीरिक प्राचल [$\sum$L(0)] तथा *फ्यू. ऑक्सीस्पोरम* के विरूद्ध ऑर्थो-सब्सटीट्यूंटस् का इलेक्ट्रॉनिक प्रभाव अर्थात 'प्रॉक्सिमिटी पोलर इफैक्ट' (F) भी कवकनाशी सक्रियता को प्रभावित करते पाए गए। तीन कवकों, *रा. सोलेनाइ*, *फ्यू. ऑक्सीस्पोरम* एवं *बा. सोरोकीनियाना* के विरूद्ध 4-एरिलाइडेनअमीनो-3-मर्केप्टो-5-फिनायल-4एच-1,2,4-ट्रायाज़ोलस् की कवक आविषालुता हेतु संरचनात्मक आवश्यकता को मात्रात्मक संरचना सक्रियता संबंध स्पष्ट रूप से दर्शाते हैं।

Synthesis and Fungitoxicity Evaluation of Schiff Bases of 4-Amino-3-Mercapto-5-Phenyl-1,2,4-Triazole

By

Arnab Roy Chowdhury

A Thesis
Submitted to the Faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi
in partial fulfillment of
requirements for the degree of

MASTER OF SCIENCE

IN

AGRICULTURAL CHEMICALS

2008

Approved by:

Chairman : _____
(Dr. R. L. Gupta)

Members **1.** : _____
(Dr. P. Dureja)

2. : _____
(Dr. Rajesh Kumar)

3. : _____
(Dr. S. C. Datta)

4. : _____
(Dr. R. Aggarwal)

Dr. R. L. Gupta
Principal Scientist

Division of Agricultural Chemicals
Indian Agricultural Research Institute
New Delhi-110 012, India

CERTIFICATE

This is to certify that the thesis entitled "**Synthesis and Fungitoxicity Evaluation of Schiff Bases of 4-Amino-3-Mercapto-5-Phenyl-1,2,4-Triazole**." submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of **Master of Science** in Agricultural Chemicals, embodies the results of bona fide research work carried out by **Mr. Arnab Roy Chowdhury**, under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

(R. L. Gupta)
Chairman
Advisory Committee

Place: New Delhi
Date: July 2, 2008

ACKNOWLEDGEMENT

Euripides (484 BC - 406 BC) once told that, "The best and safest thing to keep a balance in your life, acknowledge the great powers around us and in us. If you can do that, and live that way, you are really a wise man." So it is essential that I acknowledge the great powers, who paved the way on which I have walked so far. As a prelude to my thanksgiving, at first I wish to thank the almighty for giving me powers to complete my entire course …after all He is the "greatest".

I wish to express my deepest sense of gratitude and indebtedness to Dr. R. L. Gupta, Principal Scientist, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi and chairperson of my Advisory Committee for his invaluable guidance, constant encouragement, cooperative attitude, immense patience, useful discussion and peerless criticisms during the course of investigation and preparation of the manuscript. He has always been a fountain of inspiration to me. My gratitude is again to my sir.

It is great privilege for me to express my esteem and profound sense of gratitude to Dr. (Ms.) P. Dureja, Co-chairperson of my Advisory Committee and Principal Scientist, Division of Agricultural Chemicals, for her constructive and valuable suggestions. She was always there in all my needs and helped her best whenever I seek for it.

I feel immense pleasure to convey my heartfelt thanks to Dr. Rajesh Kumar, Senior Scientist, Division of Agricultural Chemicals and member of my Advisory Committee for his encouragement and invaluable suggestions endowed during the course of my research work. I am highly indebted Dr. S. C. Datta, Principal Scientist, Division of Soil Science and Agricultural Chemistry and member of my Advisory Committee for his constructive suggestions and generous advice. I am highly grateful to Dr. (Ms.) R. Aggarwal for her valuable suggestions throughout the research. My sincere thanks are to Dr. (Ms.) Prem Dureja, Head and Dr. D.B.Saxena, Professor of the Division of Agricultural Chemicals, for providing me the necessary facilities and encouragement throughout this study.

My hearty gratitude to Dr. S.B.Singh, Dr. J. Kumar, Dr. A.K. Dixit, Dr. S. Walia, Dr. C.. Devakumar, Dr. N.A. Sakil, Dr. S. S. Tomar, Dr. V. T. Gajbhiye, Dr. Irani Mukharjee, Dr. Neera Singh, Dr. Suman Gupta, Dr. Madhuban Gopal, Dr. S. Rengaswamy and Dr. Anupama and all other scientists of Division of Agricultural Chemicals for their encouragement and kind concern throughout the study.

I convey my heartiest thanks to Renuji, Dr. H. K. Taneja, Dr. R. S. Tanwar, and Mr. Yajulu for their help during the entire span of study. My heart felt thanks to Koushikda, Rupakda, Totanda, Achintyada, Aditidi, Rakesh, Ajay, Drubha, Niladri and Abinesh, without whom this thesis may not have seen the light of the day. Their selfless help during the crucial periods of my course work is simply unforgettable.

It gives me immense pleasure to mention names of Bhaskar, Sudip, Ayan, Kali, Bishal and Jayanta whose constant help and collective efforts have been reflected in the completion of this venture. The unceasing affection and support of seniors, Lokesh Bhaiya, Dharmaraj sir, Sabber sir, Praveen sir, Jaydeepda, Asitda, Mendelda, Laluda, Soumenda, Mridulda Prabirda, Randa, Sunayanda, Tanmoyda, Birajda, Subhashda, Jholuda, Kousikda, Subhadeepda, Pradipda, Isoda, Praloyda, Tarunda and Rezada would be in my memory for all the time. I extend my heartfelt thanks to my juniors like Chatta, Nilimesh, Kalyan, Sujit, Hillol, Soham, Kamal, Rajeeb and Sushovit; I feel really proud of them.

The endless love, affection, sacrifice and constant inspiration from Baba and Maa have enabled me to reach the footsteps of my long cherished aspiration. My vocabulary utterly fails in expressing my love to my sisters, Munna and Tutul for their unstinted support during all the years of study. Also a lot of love and respect to Boromama, Chotomama, Boromaima, Chotomaima, Pisimoni and Jethu, who also had great supportive roles to play.

I am grateful to IARI for providing me the Jr. Research Fellowship during my M. Sc. Programme.

Lastly, I bow my head to the Almighty for hearing me always and showering me with all the blessings.

Place: New Delhi *Arnab Roy Chowdhury*

Dated:

CONTENTS

S. No.	CHAPTER	PAGE No.
1.	INTRODUCTION	1-3
2.	REVIEW OF LITERATURE	4-27
3.	MATERIALS AND METHODS	28-51
4.	RESULTS AND DISCUSSION	52-62
5.	SUMMERY AND CONCLUSION	63-68
6.	BIBLIOGRAPHY	i-vi
	APPENDIX I. NMR SPECTRA	
	APPENDIX II. IR SPECTRA	

LIST OF TABLES

Table No.	Title	After page
1.	4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles synthesized	52
2.	Physical data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles	52
3.	Elemental analysis data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles	55
4.	IR spectral data 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles	55
5.	^{1}H NMR data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles	55
6.	Fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*	56
7.	Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *F. oxysporum*	57
8.	Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *B. sorokiniana*	57
9.	Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Rhizoctonia solani*	59
10.	Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Fusarium oxysporum*	60
11.	Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Bipolaris sorokiniana*	61
12.	Correlation matrix for the parameters used in the regression equation	61

LIST OF FIGURES

Figure No.	Title	After page
1.	Fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*	56
2.	Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Fusarium oxysporum*	57
3.	Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Bipolaris sorokiniana*	57
4.	Comparison of fungitoxicity of the 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles	58
5.	Comparison of fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles with the fungitoxicity of 4-amino-3-mercapto-5-phenyl-4H-1,2,4 triazole	58
6.	Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*	61
7.	Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Fusarium oxysporum*	61
8.	Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Bipolaris sorokiniana*	61

LIST OF PLATES

Plate No.	Title	After Page
1.	Apparatus used in microwave synthesis of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole	33
2.	Fungicidal activity of 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA9] against *R. solani*	56

LIST OF ABBREVIATIONS AND SYMBOLS

IR	infra-red spectroscopy
υ	wave number
NMR	nuclear magnetic resonance spectroscopy
δ	measure of chemical shift in NMR
s	singlet (nature of peak in NMR)
m	multiplet (nature of peak in NMR)
J	coupling constant in NMR
TLC	thin layer chromatography
Me	methyl
o	ortho
m	meta
p	para
PDA	potato-dextrose-agar medium
ED_{50}	effective dose required for 50% growth inhibiton of the fungi
QSAR	quantitative structure activity relationship
m.p.	melting point
BOD	biological oxygen demand
PC	personal computer
π	hydrophobicity parameter
σ	Hammett constant
F	Swain-Lupton constant
L	STERIMOL length parameter

ABSTRACT

Thesis title: Synthesis and Fungitoxicity Evaluation of Schiff Bases of 4-Amino-3-Mercapto-5-Phenyl-1,2,4-Triazole.

The pesticides suffer from high rate of obsolescence due to resistance, change in pest problems, various environmental considerations and also competition from new introductions. Therefore, newer pesticides with greater potency and increased safety from environment point of view are required to be developed to replace the older ones. Schiff bases of various heterocyclic moieties have found use as drugs and have also been reported as pesticides. Schiff bases of some 4-amino-1,2,4-triazoles have already been synthesized and their antifungal, antibacterial, PGR activities have been reported. Some 5-aryl-4-amino-3-mercapto-1,2,4-triazoles have been recently reported to posses potential fungicidal activity.

In an attempt to discover potential fungicides, a series of twenty Schiff bases, 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole having different substitution in the aryl ring attached to imino group were synthesized following a five step synthesis scheme starting with benzoic acid, which was first converted to methyl ester $\longrightarrow$ hydrazide $\longrightarrow$ dithiocarbazinic acid salt $\longrightarrow$ triazole and finally to $\longrightarrow$ Schiff base. Three of the five steps of this synthesis i.e. methyl ester to hydrazide; dithiocarbazinic acid salt to triazole and triazole to Schiff base were accomplished by using microwaves. The microwave synthesis has been found much faster and efficient than conventional method. The Schiff bases, 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles were obtained in 52-92% yield by refluxing 4-amino-3-mercapto-5-phenyl-1,2,4-triazole in ethanol with respective aldehydes for 2-4 hrs.

The structure of the Schiff bases were characterized by elemental analysis, IR and NMR spectroscopy.

The Schiff bases were evaluated for fungitoxicity against three phytopathogenic fungi viz. *Rhizoctonia solani*, *Fusarium oxysporum* and *Bipolaris sorokiniana*. Among all the compounds in this series, 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (ED_{50} = 17.34 ppm), 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (ED_{50}=95.55 ppm) and 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (ED_{50}=181.3 ppm) exhibited the highest activity against *R. solani*, *F. oxysporum* and *B. sorokiniana* respectively. The most active compound in this series i.e. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (ED_{50} = 17.34 ppm) exhibited higher activity than the parent triazole, 4-amino-3-mercapto-5-phenyl-1,2,4-triazole (ED_{50} = 76 ppm) against *R. solani*.

The fungitoxicity data although have led to some important generalizations on structure activity relationship but to identify exact structural features favourable for fungitoxicity, quantitative structure activity relationships (QSAR) were analysed by using in vitro fungitoxicity data and various physico-chemical parameters for hydrophobic, electronic and steric properties of the benzylidene ring substituents, by means of multiple regression analysis techniques. The QSAR models have revealed that the fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles

against *R. solani* show an inverse parabolic relationship with the hydrophobicity (π), whereas the fungitoxicity against *F. oxysporum* and *B. sorokiniana* is dependent on the electronic nature (σ) of the substituent present in the benzylidene ring. The steric parameters for length of ortho-substituents [ΣL(o)] against *B. sorokiniana* and the electronic effect of the *ortho*-substituents i.e. 'proximity polar effect' (F) against *F. oxysporum* were also found influencing the respective fungicidal activity. The quantitative structure activity relationship has clearly revealed the structural requirement for the fungitoxicity of the 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against the three fungi viz. *R. solani*, *F. oxysporum* and *B. sorokiniana*.

1. INTRODUCTION

Agriculture, as the largest private enterprise in India, has been and will continue to be the lifeline of the Indian economy at least in the forsible future. Despite great agricultural advances in the past, million still go hungry, live under constant threat of famine. So, we have to raise our production to feed the increasing population. Therefore, we should have concentrated on food production upto 250 million tons by utilizing the untapped potentials. We must ensure that the agriculture, which is the mainstay of our livelihood and ecological security is carried out in such a way that it can produce more but in a sustainable way.

Crop losses due to different pest threaten the sustainability. Insects, diseases and weed causes considerable damage of the agricultural produce. So, we have to control those disease, weeds, and insects to get desired agricultural produce. One of the most effective control measures is the chemical control, where different plant protection chemicals are used to control the pests. Progress in chemical crop protection has been extraordinary over the last 60 years. The crop protection measures have contributed in a decisive manner to the production of food, feed, and fiber. Famine and food polluted with noxious, naturally occurring toxins have become almost unknown in the western world. With the world population still growing and the calorie intake growing even steeper, production of sufficient food will continue to remain a major challenge to humans. Chemical crop protection therefore, will continue to play an important role in agribusiness in spite of the emergence of novel biotechnological solutions. Scientific progress in chemistry, biology, and molecular biology has revolutionized the way of searching for new agrochemicals over the past decade. The search for and the development of novel agrochemicals with novel modes of action, improved safety profiles, and adapted to the changing requirement of the food and feed production chain are more than ever the challenge. Adequate crop protection with green chemicals will contribute in the future to increase the yield per hectare and help to ensure food, feed, and fiber of high quality despite novel biotechnological methods becoming increasingly available.

The plant protection chemicals used in the past have different disadvantages. They were used in large volumes, so the load of the chemicals in the environment was very large. Pest resurgence and pesticide resistance is another threat due to indiscriminate use of agrochemicals. Persistence of the agrochemicals in the environment raising the issue of the residual problem in food commodity. So, to overcome these problems, we have to develop newer molecules which have low persistence in the environment, effective in low doses, selective to the target pests and safe to non-target organisms and have higher potential to work in multidimensional aspect of crop protection. Hence, the development of new pesticide molecules as is a continuous process.

Recent literature is enriched with progressive findings about the synthesis and biological activity of many heterocyclic molecules. These molecules were proven their bioactivity in the field of pharmaceuticals and have found effective in the medical field against diseases caused by different pathogens like fungus, bacteria, virus etc. The heterocyclic nuclei in the research aspects are triazoles, thiadiazoles, oxazoles and fused ring nuclei like benzimidazoles and some others have also shown potential as pesticides and several of them have shown activity against many phytopathogens. The triazoles like triademinol, triademifon, propiconazole, etaconazole, hexaconazole etc. were proven their activity against fungal pathogens and are in great use as fungicides.

Schiff bases of different triazoles were found having broad spectrum of activity. Due to great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviour was studied. Literature survey shows that Schiff bases show bacteriostatic, bactericidal, antifungal, anticancer, antitumuor activityand are used as drugs. Some have also been reported as potent pesticides, however only a little work has been done on their activity on different phytopathogenic fungi. In search for new fungicides and in continuation to earlier investigation on 5-aryl-4-amino-3-mercapto-1,2,4-triazoles (Bijul, 2005) which led to some potential fungicidal molecules, a series of Schiff bases of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole having different substitution in the benzylidene ring attached to

imine group, were designed for the present investigation. The present investigation was therefore, undertaken with the following objectives:

(i) To synthesize new Schiff bases of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole and

(ii) To study their fungicidal activity against some important phytopathogenic fungi.

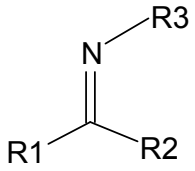
2. REVIEW OF LITERATURE

2.1 Schiff bases

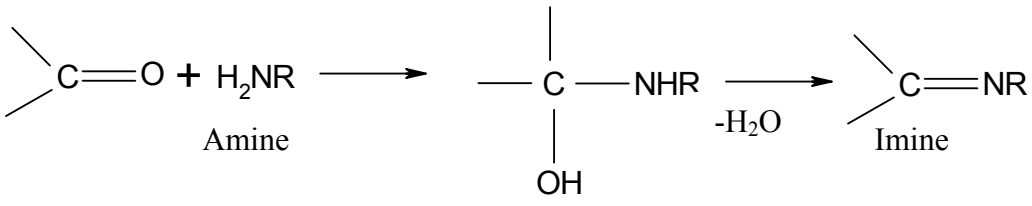
2.1.1 General

The synthesis and biological activity of Schiff bases, the compounds obtained by condensing carbonyl compounds with primary amines (Layer, 1963), have received considerable attention in past decades. Schiff bases are the compounds containing a carbon-nitrogen double bond in the molecule. Schiff bases are so named, after the name of Hugo Schiff who first carried out this condensation reaction. Depending upon their starting carbonyl compound, these are classified into two types - aldimines (resulting from condensation of aldehyde with amines) and ketimines (resulting from condensation of ketone with amines). Due to the presence of carbon-nitrogen double bond in the molecule, the imines possess potential site for both chemical and biological activity (Pacheco *et al*., 1970; Dhar and Taploo, 1982).

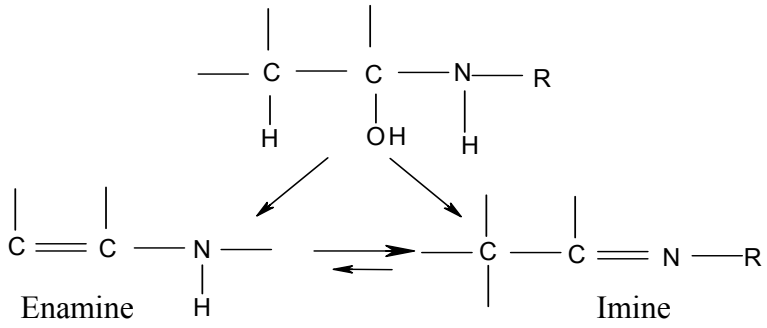
A Schiff base is a functional group or a chemical compound containing, carbon-nitrogen double bond. It is also called imine or azomethine. An imine can be synthesised by nucleophilic addition from a ketone or aldehyde and ammonia or an amine to a hemiaminal –C (OH)(NHR)- followed by elimination of water to the imine.



If the amine is primary, the initial addition product undergoes dehydration to form a compound containing a carbon-nitrogen double bond, an imine.

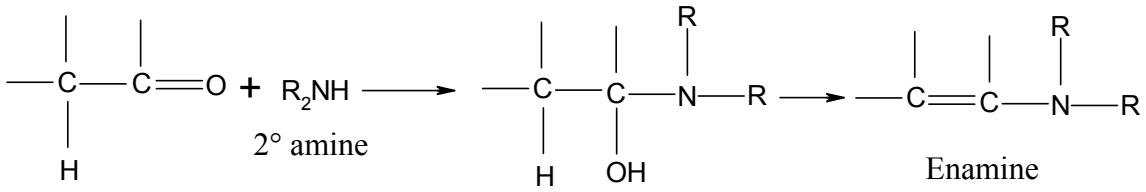


Elimination occurs with this orientation even if the carbonyl compound contains an α-hydrogen, that is, the preferred product is the imine rather than the enamine (group containing carbon – carbon double bond). If some enamine should be formed initially, it rapidly tautomerizes into the more stable imino form.



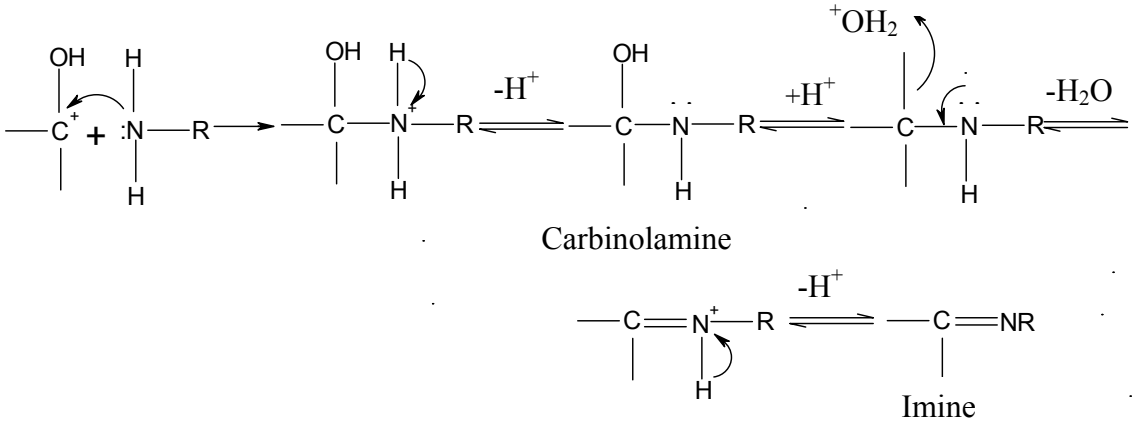
Imine – enamine tautomerism

A secondary amine, too, can react with a carbonyl compound, and yields the same kind of initial product. But there is no hydrogen left on nitrogen; if dehydration is to occur, it must be in the other direction, to form a carbon carbon double bond. In such cases, enamine is the only product.

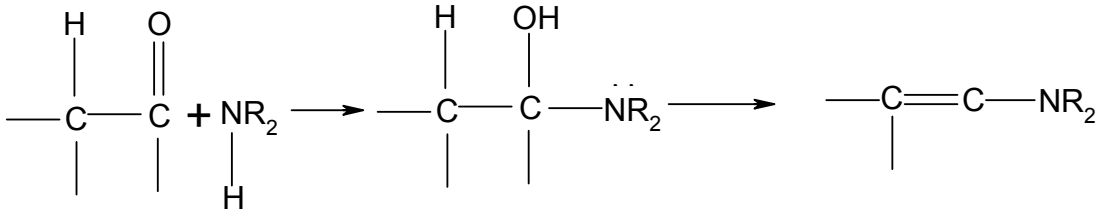


Carbonyl compounds having an α-hydrogen atom react with RNH_2 (1°) give an imine and with R_2NH (2°) give an enamine.

After protonation of the O, the nucleophilic RNH_2 adds to the C and the adduct loses H^+, to give the carbinolamine. Dehydration proceeds by protonation of the O of OH, loss of H_2O, and then loss of H^+, to give the imine.



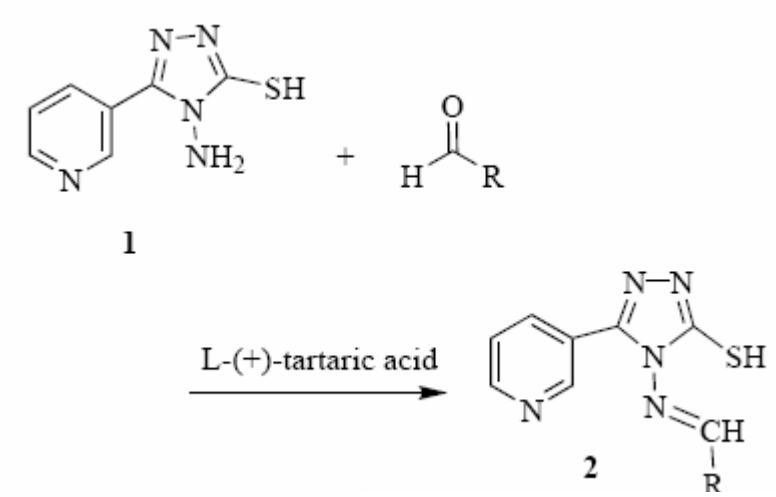
The carbinolamine formed from R_2NH lacks an H on N, and its dehydration involves loss of the acidic α-H to give the resonance-stabilized enamine



2.1.2 Schiff bases of triazoles

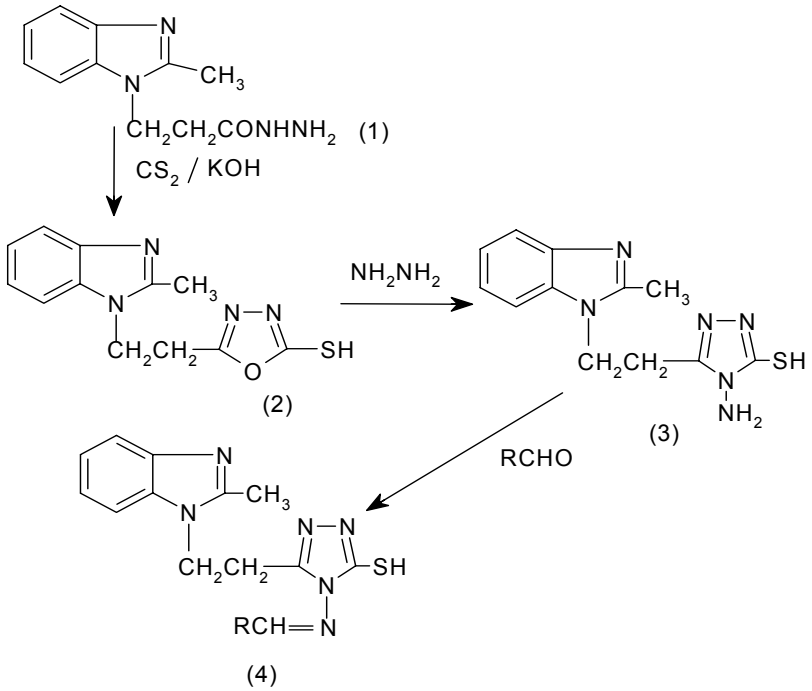
In search for potent bioactive molecules researchers were involved to synthesize and test different chemical groups. Huang *et al.* (1994) synthesized the Schiff bases of pyrazoles with substituted aldehydes and compounds were tested for control of mite, aphid, insect, nematode and protozoa.

Zhongyi *et al*. (1996) synthesized Schiff bases as a reaction intermediate of 3-(β-pyridyl)-4-amino-5-mercapto-1,2,4-triazole with aromatic aldehydes. Reaction was done by heating an aromatic aldehyde with 3-(β-pyridyl)-4-amino-5-mercapto-1,2,4-triazole in the presence of a catalytic amount of L(+)- tartaric acid for 5 h at 45 up to 70 °C leads to condensation products.

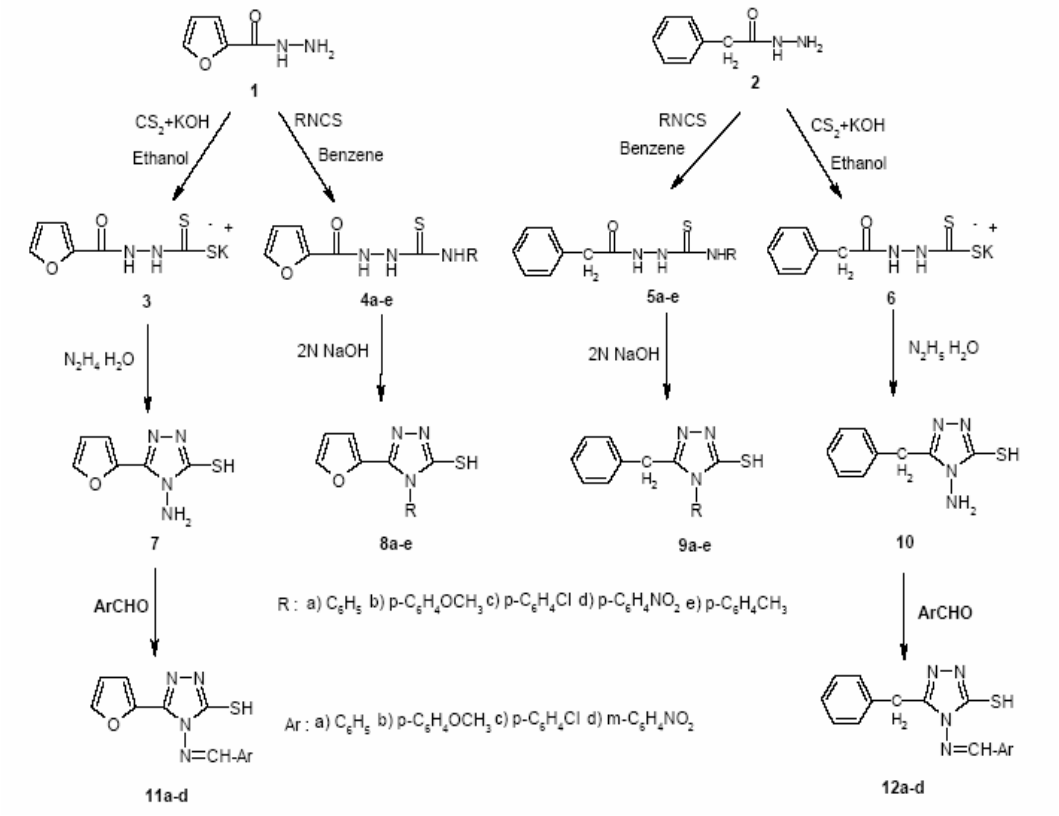


R= *p*-C_6H_5Cl, *p*- $C_6H_5NO_2$, *p*-$C_6H_5OCH_3$, *o*-C_6H_5OH etc.

El-masry *et al*. (2000) developed Schiff bases of benzimidazole derivatives. In this work, 3-(2-methylbenzimidazol-1-yl)propanoic acid hydrazide (1), was used as the key intermediate for further synthesis. Thus, when compound (1) was treated with carbon disulphide and potassium hydroxide 5-[2-(2-methylbenzimidazol-1-yl)ethyl]-[1,3,4] oxadiazole-2- thione (2) was obtained, A benzimidazole incorporated into a triazole moiety was synthesized by the reaction of (2) with hydrazine hydrate (99%) in absolute ethanol afforded 1-[(1-amino-2-mercapto-1,3,4-triazol-5-yl)ethyl]-2 methylbenzimidazole (3). A number of arylidine hydrazones incorporated into the parent benzimidazole were also synthesized. Thus condensation of this compound with aromatic aldehydes, namely, *p*-methoxy benzaldehyde and *o*-chlorobenzaldehyde in absolute ethanol afforded the corresponding Schiff's bases (4). Spectroscopic data of the compound are as follows, IR (KBr, cm-1): 3047 (NH), 2993 (CH), 2580 (SH), 1267 (C=S). ^{1}H-NMR (DMSO-d6, δ ppm): 2.5 (3H, s, CH_3 at C-2 of benzimidazole), 3.2 (2H, t, J = 7 Hz, NCH_2CH_2), 3.85 (3H, s, OCH_3), 4.55 (2H, t, J = 7 Hz, NCH_2CH_2) and 7.1-7.8 (8H, m, aromatic protons), 9.4 (1H, s, CH=N) and 13.8 (1H, s, SH).

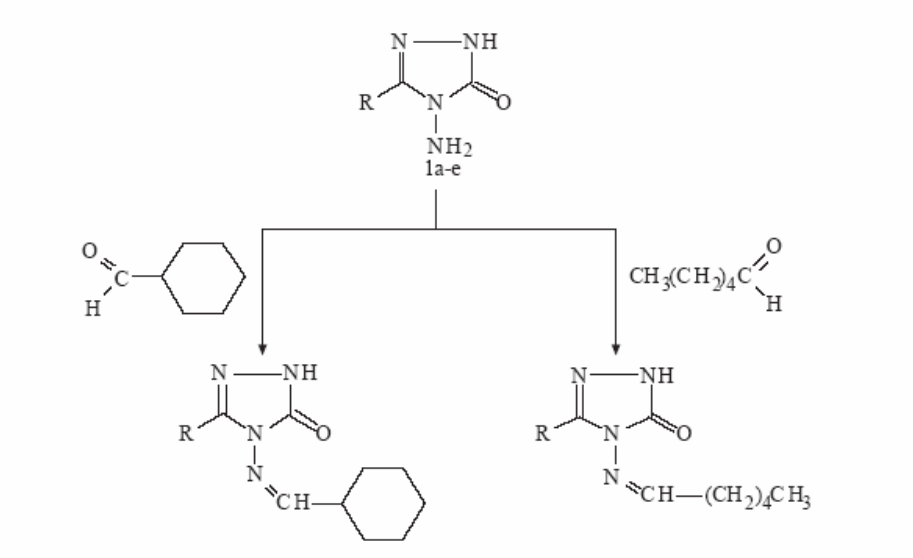


Cansiz *et al.* (2004) synthesized novel series of Schiff bases form the corresponding aryl aldehydes and the 4-amino-3-mercapto-5-(furan-2-yl-or benzyl) -4H- 1,2,4-triazole. The new derivatives were prepared following the reaction sequences depicted in the following Scheme. Initial compounds were prepared from furan-2-carboxylic acid hydrazide (1), and phenylacetic acid hydrazide (2). Potassium 3-(2-furoyl) or (phenylacetyl) dithiocarbazates 3, 6 were prepared by reaction of compounds **1** and **2** with carbon disulfide in ethanolic potassium hydroxide. 1-(2-Furoyl or phenylacetyl)-4 substituted thiosemicarbazides 4a-e and 5a-e were prepared in yields ranging from 88 to 95% by the condensation of (1) and (2) with arylisothiocyanates. Ring closure of arylthiosemicarbazides in an alkaline medium is a well known method for the synthesis of 1,2,4-triazoles, and 5-(furan-2-yl or benzyl)-4-(aryl)-4H-1,2,4-triazole-3-thiols 8a-e and 9a-e were obtained in 62-79% yields from the respective 4a-e, 5a-e by this method. Compounds 7 and 10 were obtained from the reaction of **3** and **6** with hydrazide hydrate under reflux in solution. When an arylaldehyde was added to 7 or 10 in ethanol, the reactions gave 11a-d, 12a-d.

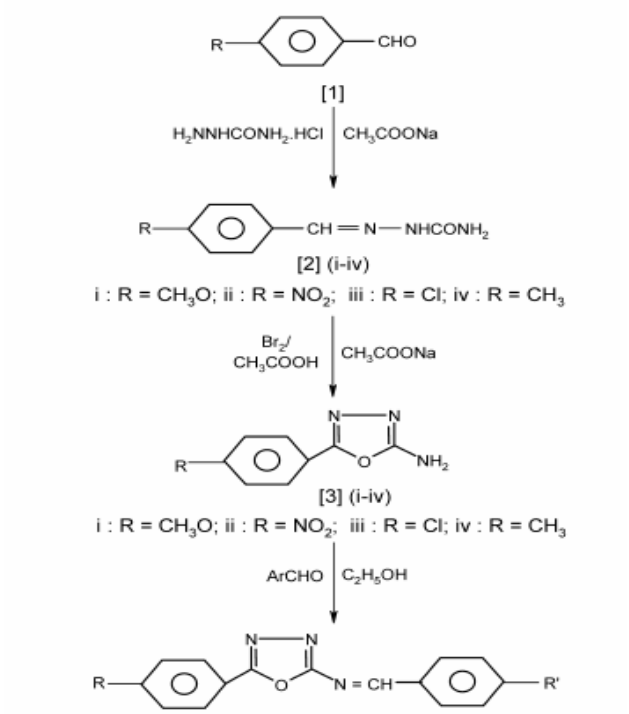


The IR spectra of the 1,4-substituted-thiosemicarbazide derivatives 4a-e, 5a-e have C=O stretching bands at 1687-1672 cm^{-1} and C=S stretching bands at 1290-1250 cm^{-1}. In the ^{1}H NMR NH protons of 4a-e, 5a-e were observed at 9.20-10.15 ppm, (O=C-NH-NH-C=S) and 8.00-8.25 ppm (S=C-NH-Ar). Compounds 7, 8a-e, 9a-e, 10, 11a-d, 12a-d exist as thiol-thione tautomers as indicated by their IR spectra which showed a band due to SH and four bands due to N-C=S I, II, III, IV. The azomethine derivatives 11a-d, 12a-d were charcterized by the presence of the methine protons (N=CH) at 9.18-10.33 ppm.

Demirbus *et al.* (2004) prepared A series of novel 3-alkyl-4-cyclohexylmethylenamino-5-oxo-4,5-dihydro-[1,2,4] triazoles and 3-alkyl-4-hexylidenamino-5-oxo-4,5-dihydro-[1,2,4] triazoles were synthesized by the reaction of corresponding 3-alkyl-4-amino-5-oxo-4,5-dihydro-[1,2,4] triazoles with cyclohexancarboxaldehyde and capronaldehyde.



Mishra *et al.* (2005) synthesized twenty Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles by reaction with different aromatic aldehydes.

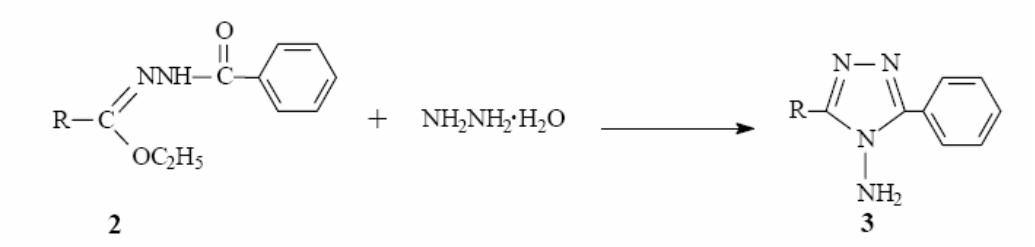


A solution of 2-Amino-5-aryl-1,3,4-oxadiazoles (0.01 M) was prepared in 20 ml alcohol in a round bottomed flask. Required aldehyde (0.01 M) dissolved in 15 ml alcohol, was then added to it. The mixture was refluxed for 5–6 h. The volume of

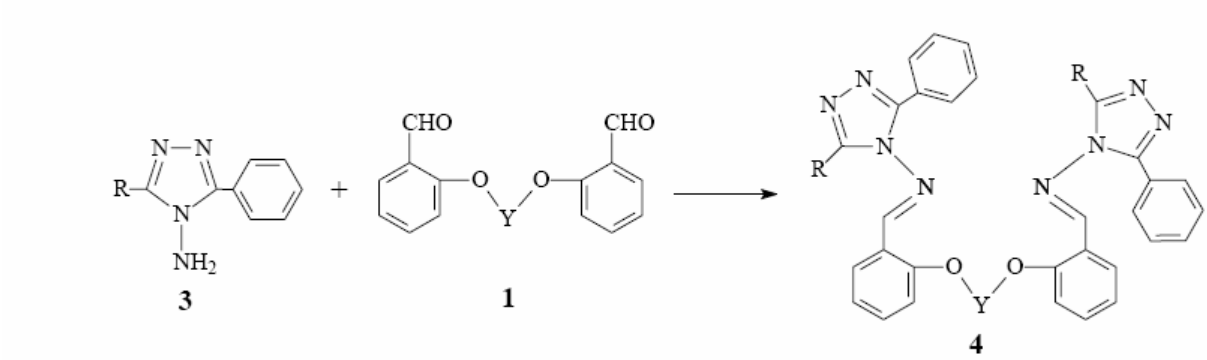
alcohol was reduced to half by distillation under reduced pressure. The resulting solution was poured on crushed ice. The precipitate which got separated was dried and recrystallized from alcohol.

IR spectra of these compounds give peaks for (C=N) stretching at 1532-1547cm^{-1}. ^{13}C NMR shows peaks at δ 163 ppm for C-atom at (C=N).

Bekircan *et al.* (2006) prepared a series of new 1,2/1,3-bis[o-(N-methylidenamino-3-aryl-5-phenyl-4H-1,2,4-triazole-4-yl) phenoxy]ethane/propane derivatives in good yields by treatment of 4-amino-3-aryl-5-phenyl-4H-1,2,4-triazoles with certain bis-aldehydes.



2 3

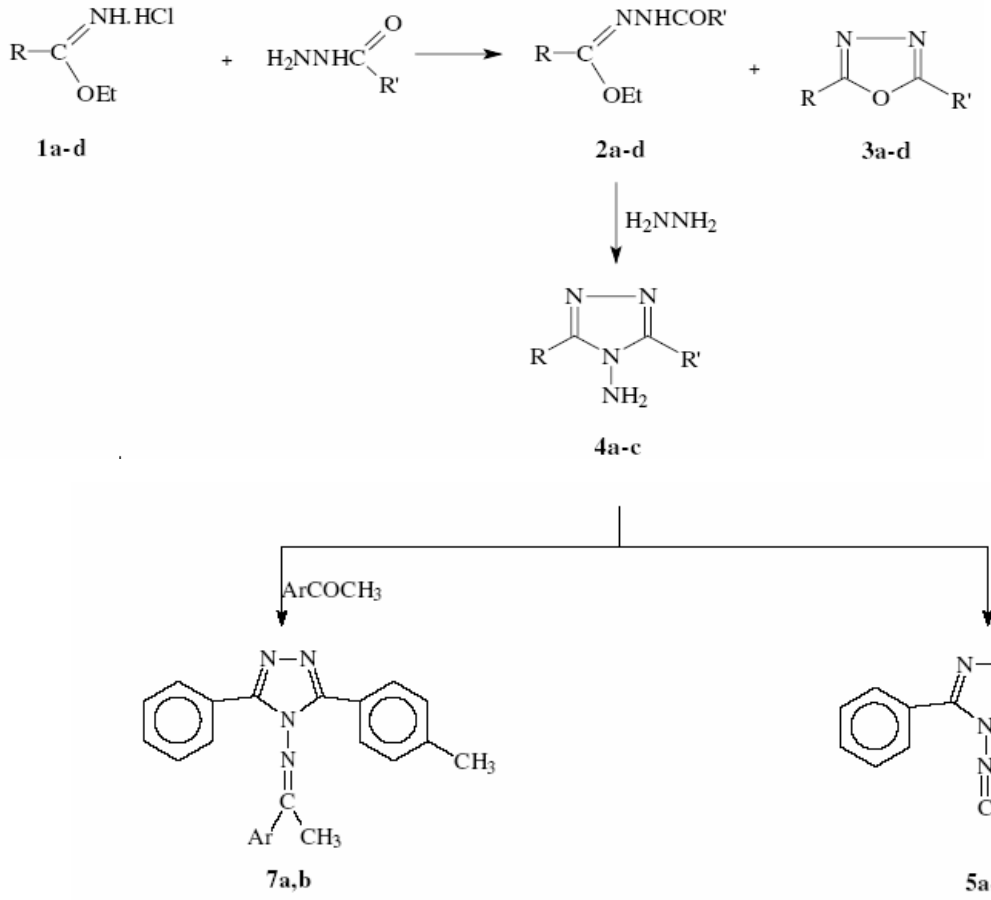


3 1 4

In the IR spectra of compounds **4** the characteristic C=N absorption bands appeared at 1597 cm^{-1}. The^{1}H-NMR signals for the -N=CH group were observed at δ 8.23-8.70 ppm. The ^{13}C-NMR signals for the –N=CH- group were recorded at δ 164 ppm.

Serdar *et al.* (2007) synthesized a series of acylhydrazones from the reactions of iminoester hydrochlorides with acyl hydrazines. 2,5-Dialkyl 1,3,4-oxadiazoles were obtained in the same reaction media. The treatment of acylhydrazones with hydrazine

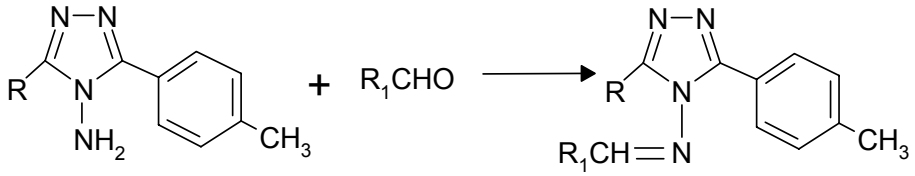
hydrate afforded 4-amino-3,5-dialkyl-1,2,4-triazoles. The treatment of 4-amino-3,5-dialkyl-1,2,4-triazoles with various aromatic aldehydes or acetophenone and 4-nitroacetophenone resulted in the formation of 4-arylidenamino-3,5-dialkyl-4*H*-1,2,4-triazoles.



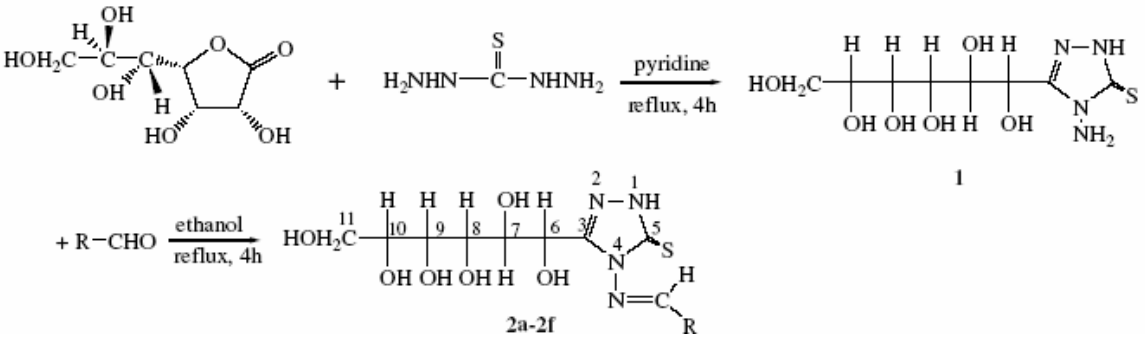
The solution of 4-amino-3-(4-tolyl)-5-phenyl-*4*H-1,2,4-triazole (10 mmol) in acetic acid was refluxed with an aromatic aldehyde or acetophenone and 4- nitroacetophenone for 4 hrs. Then the reaction mixture was poured into ice-water under stirring. The precipitated product was filtered off and washed with water. The obtained white solid was recrystallized from ethanol or ethyl acetate to afford pure compounds.

Gumrukcuoglu *et al.* (2007) synthesized Schiff bases by condensing 4-amino-3,5-dialkyl-1,2,4-triazoles with various aromatic aldehydes and resulted in the

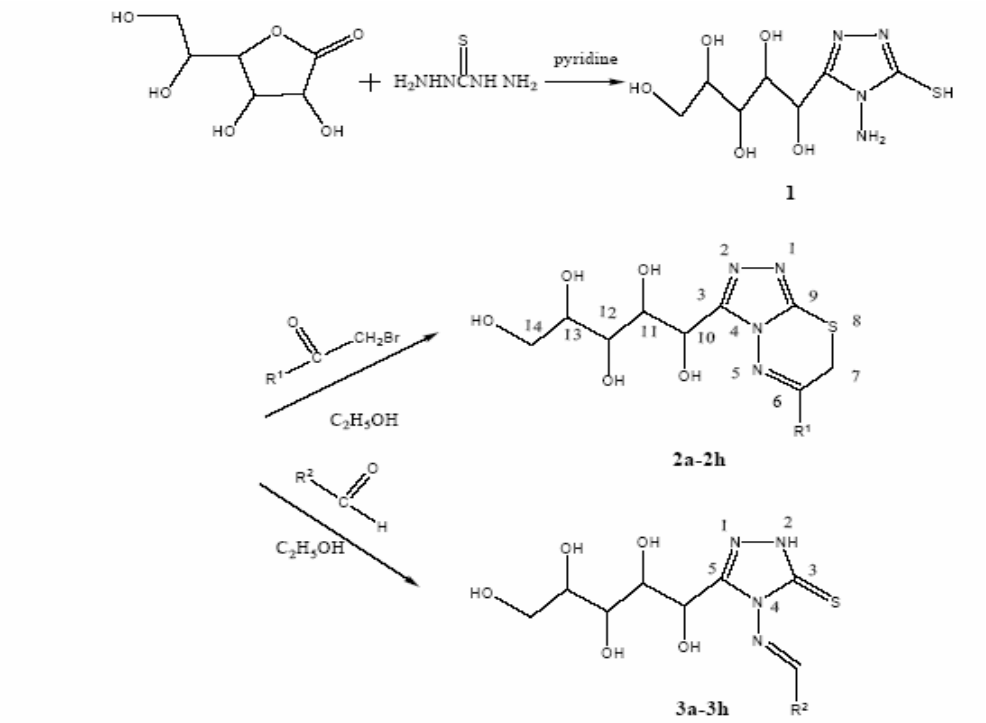
formation of 4-arylidenamino-3,5-dialkyl-1,2,4-triazoles. The solution of 4-amino-3-aryl-5-(4-tolyl)-4H-1,2,4-triazole (10 mmol) in acetic acid was refluxed with an aromatic aldehyde for 4 h. Then, the reaction mixture was poured into ice-water under stirring. The precipitated product was filtered off and washed with water. The white solid obtained was recrystallized from ethanol or ethyl acetate. It was reported that Schiff bases can be obtained as their *E* and *Z*-geometrical isomers about the -C=N double bond. The ratio of *E* isomer is generally higher than that of the other isomer, and polar solvents, such as dimethyl sulfoxide, augment the ratio of *E* isomer. The IR spectra of the Schiff bases show characteristic peak at 1624-1561 cm^{-1}. ^{1}H NMR gives characteristic peak as a singlet at δ 8.3 to 8.6 ppm and ^{13}C NMR shows peak at δ 164-176 ppm for N=CH group.



Ye *et al.* (2007) synthesized a number of novel Schiff's bases from 4-amino-3-(*D*-glucoheptonic-hexitol-1-yl)-1*H*-1,2,4-triazole-5-thione. By attaching *D*-glucoheptonichexitol-1-yl residues to 1,2,4-triazole at the 3-position, the solubility of the title compounds has been improved greatly.



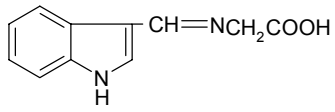
Jian-yu Jin *et al.* (2007) synthesized a series of novel 6-aryl-3-(D-galactopentitol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines and 4-(arylmethylidene)amino-5-(D galactopentitol- 1-yl)-3-mercapto-4*H*-1,2,4-triazoles from 4-amino-3-(D-galactopentitol-1-yl)-5- mercapto-1,2,4-triazole.



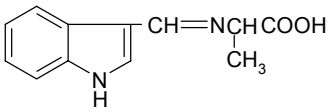
2.1.3 Schiff bases other than triazole

Sari (2003) prepared some new amino acid-Schiff bases were prepared by the condensation of indole-3-carboxaldehyde and DL-glycine, DL-alanine and DL-valine and characterized by elemental analysis, IR, UV-Vis, ^{1}H-NMR spectroscopy and ^{13}C-NMR spectroscopy. A quantity of 5 mmol amino acid was dissolved in 10 mL of water and added with constant stirring to 20 ml MeOH solution containing 5 mmol KOH. The solution was stirred on a water bath at 300^{o}C for two hours and then filtered. The filtrate was added drop wise to 10 mL of MeOH solution of indole-3-carboxaldehyde (5 mmol) with stirring on a water bath at 300^{o}C for four hours, filtered and left to stand. On

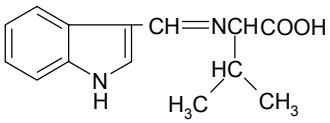
standing for a further 6 h, the yellow solid product that formed was collected by vacuum filtration, washed with a small volume of acetone and dried in vacuum.



Indole 3-carboxylene-DL-glycine

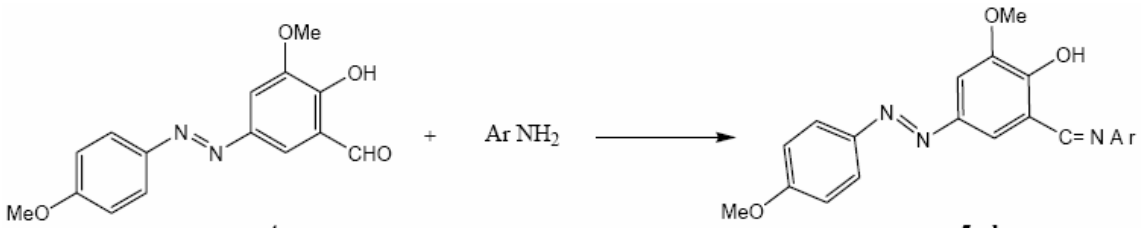


Indole 3-carboxylene-DL-alanine

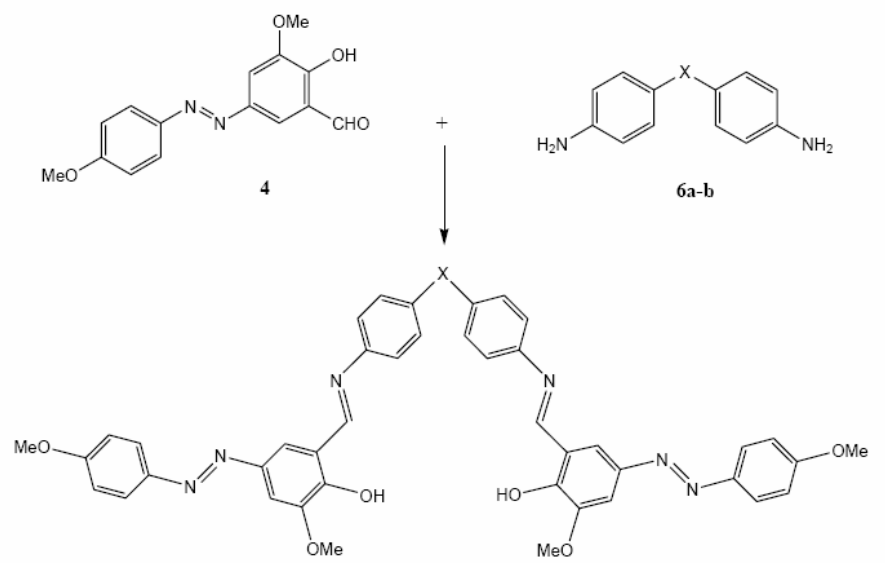


Indole 3-carboxylene-DL-valine

Jarrahpour *et al.* (2004) prepared ten new azo Schiff bases in excellent yields via the condensation of different aromatic amines and a new azoaldehyde, 2-hydroxy-3 methoxy-5-(4-methoxyphenylazo)benzaldehyde by two different methods. Treatment of azoaldehyde with different aromatic amines, either in dry dichloromethane in the presence of anhydrous $MgSO_4$ (method A) yielded the novel azo Schiff bases in excellent yields.



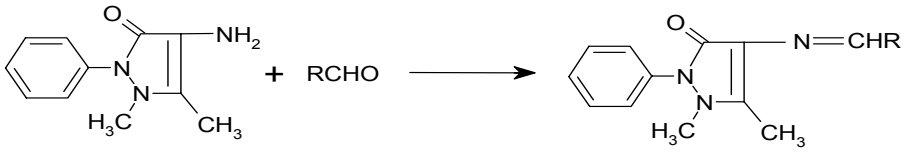
Treatment of two moles of azoaldehyde with one mole of diamine in refluxing absolute ethanol (Method B) gave in excellent yields the novel ompounds bis [5-(4-methoxyphenylazo)-2-hydroxy-3-methoxy benzaldehyde]-4,4′-diimino phenylether and bis [5-(4-methoxyphenylazo)-2-hydroxy-3-methoxy benzaldehyde]-4,4′-diimino phenyl sulfone.



Vaghasiya *et al.* (2004) synthesized Schiff bases from 4-aminoantipyrin and vanillin.

Synthesis of Schiff bases derived from 4-aminoantipyrine:

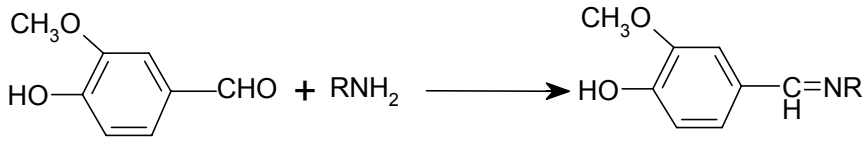
To the required amount of aldehyde dissolved in 200 ml methanol was added 0.1 mol of amine and few drops of glacial acetic acid, which acts as a catalyst. The mixture was refluxed for 10–12 h at 70–80 °C in a water bath. The resulting solution was cooled to room temperature, and then poured onto crushed ice with constant stirring. The precipitate was filtered off and washed with sodium bisulfite solution to remove the excess aldehyde. The product was crystallized from hot methanol and dried.



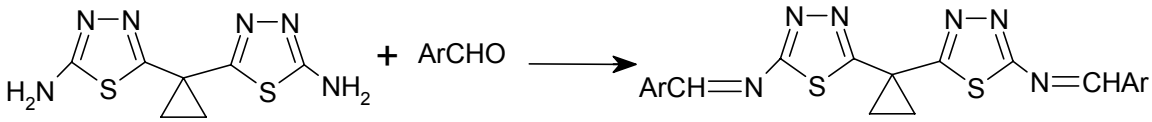
Synthesis of Schiff bases derived from vanillin

To 15.2 g of vanillin dissolved in 200 ml methanol were added 0.1 mol of the required aniline derivative and a few drops of glacial acetic acid. The mixture was refluxed for

10–12 h at 70–80 °C. The mixture was then poured onto crushed ice with constant stirring, filtered and dried.



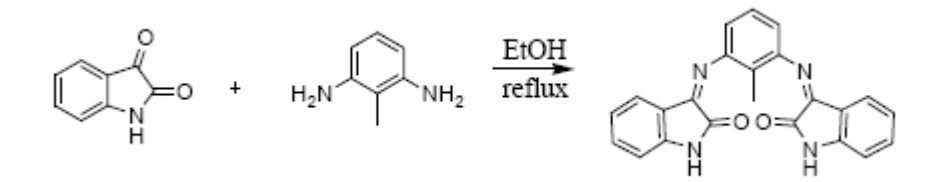
Sharba *et al.* (2005) synthesized bis Schiff bases from 1,1 bis (2-amino-1,3,4-thiadiazole -5-yl) and 1,1 bis (3-thio-4H-1,2,4-triazol-5-yl) cyclopropane condensing with aromatic aldehyde.



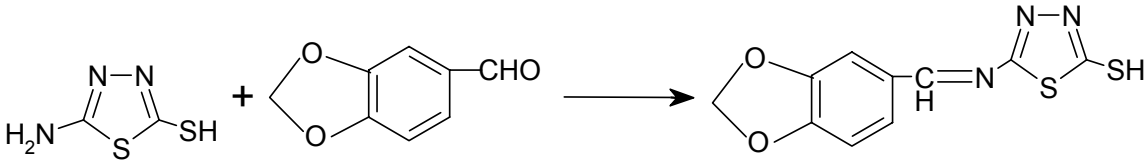
Shrivastava *et al.* (2005) synthesized schiff bases by condensing 2-amino-5-phenyl 1, 3, 4-thiadiazole with different aromatic aldehydes. Schiff bases are then complexed with different transition metals.

Perona *et al.* (2006) prepared new Schiff bases by reacting 3-hydroxy-4-pyridinecarboxaldehyde with various amines. NMR spectroscopic methods provided clear evidence that the Schiff bases exist in the solid state and in solution as hydroxyimino tautomers with the *E*-configuration.

Jarrahpour *et al.* (2007) prepared twelve new bis-Schiff bases of isatin. The desired bis-Schiff bases of isatin and its derivatives were prepared by the reactions of isatin, 5-fluoroisatin and benzylisatin with commercially available aromatic diamines in the presence of catalytic amounts of glacial acetic acid in ethanol under reflux condition.



Salih (2008) prepared Schiff bases of 2-amino-5-mercapto-1,3,4-thiadiazole with piperonal. A mixture of 2-amino-5-mercapto-1,3,4-thiadiazole (0.013 mol) in absolute ethanol (15 ml), and piperonal (0.013 mol) were refluxed for 7-9 h. After cooling to room temperature, the precipitate was filtered, dried, and then recrystallized



from ethanol.

These compounds showed peaks at 1630 cm^{-1}(C=N) and 2550 cm^{-1}(SH) and ^{1}H-NMR (DMSO-d6) δ: 8.56 (s, 1H, N=C-H), 14.43 (s, 1H, S-H) (D_2O exchange, disappear). ^{13}C-NMR (DMSO-d6) δ: 115.1(1C, N=CH-), 164.6, 163.8 (thiadiazole carbons).

2.2 Biological activity

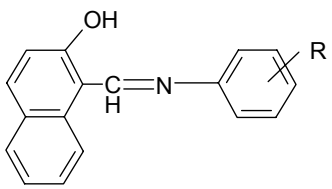
Schiff bases have been reported to possess antifungal (Manrao & Kohli, 1986, Sharma *et al.*, 1994, Pandeya *et al*., 2000, Jarrahpour *et al*.,2004, Mishra *et al*., 2005, Hothi *et al*., 2006, Serdar *et al*., 2007 etc.) herbicidal activity (Holla *et al*., 2000) antibacterial (Zhongyi *et al*., 1996, Holla *et al*., 1998, Pandeya *et al*., 2000, Vaghasiya *et al*., 2004, Gumrukcuoglu *et al*., 2007) anticancer (Holla *et al*., 2003) anti HIV(Pandeya *et al*., 2000) antitumuor activity (Demirbus *et al*., 2004) and antiviral activity (Jarrahpour *et al*., 2007). Some Schiff bases are also reported to act as plant growth regulators (Zhou *et al*.,Ye *et al*., 2007, Jian-yu-jin *et al*., 2007). The synthesis and assaying of biological potential of compounds containing carbon-nitrogen double bond have received considerable attention in recent years. Schiff bases of triazoles and other heterocyclics have a great potential for discovering new potent pesticides.

2.2.1 Fungicidal activity of Schiff bases

Sharma *et al*., (1994) prepared five vanillin Schiff bases containing both hydroxy and methoxy group in the C-phenyl ring. All the Schiff bases were tested against *Colletotricum capsici, Helminthosporium maydis, Alternaria alternata, Fusarium oxysporum* and *Penicillium italicum*. Although all the compounds possessed antifungal activity, but 4-hydroxy-3-methoxybenzal-p-phenetidine was highly effective with ED_{50} value of 69 ppm against *P. italicum*.

Manrao *et al*., (1995) studied the effect of presence of one methoxy group in the C-phenyl nucleus on benzalaniline and fungicidal activity was examined by preparing 4-methoxybenzalaniline and its N-phenyl derivatives by condensing anisaldehyde with aniline, *p*-toluidine, *p*-aminophenol and 2-mercaptoaniline respectively in equimolar ratio in methanol. 4-Methoxybenzal substituted aniline was prepared by condensing *o*-phenylenediamine with 4-anisaldehyde in presence of glacial acetic acid. Of all the imines tested against *Curvularia lunata, Alternaria alternata, Fusarium oxysporum, Stemphylium pori and Helminthosporium maydis*, 4-methoxybenzal-(2-hydroxyaniline) has been found to be most effective against *A. alternata, F. oxysporum* and *S. pori* with ED_{50} value of 34, 47 and 180 ppm respectively. The higher activity of 4-methoxybenzal-(2-hydroxyaniline) is attributed to the presence of hydroxy group in the molecule.

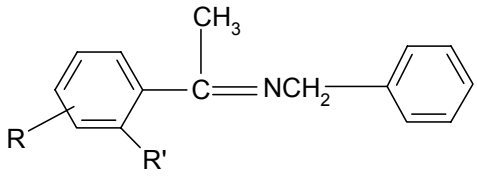
Manrao *et al*., (1996) again attempted to examine the effect of hydroxyl group in C-naphthyl nucleus and synthesized a series of 2-hydroxy-1-naphthalaniline and its N-Phenyl derivatives. The imines were tested against *Alternaria brassicae, Fusarium oxysporum* and *Ustilago tritici*. Among these, 2-hydroxy-1-naphthal-4-hydroxyaniline was found to possess considerable antifungal activity with ED_{50} value of 340 ppm against *A. brassicae* and 2-hydroxy-1-naphthal-4-chloroaniline against *U. tritici* with ED_{50} value of 465 ppm.



Matharu *et al*., (2004) screened methyl-substituted imines of 2-chlorobenzaldehyde against *Alternaria alternata, Fusarium oxysporum, Helminthosporium gramineum, Ustilago tritici* and *Myrothecium roridum*. 2-chlorobenzal-2-toluidine has been found to possess promising activity against all the fungi with ED_{50} value less than 200 ppm.

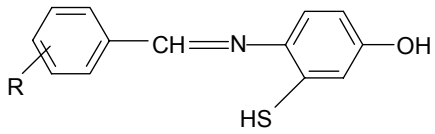
Matharu *et al*., (2005) screened benzalaniline and its N-phenyl derivatives for their antifungal activity against *Alternaria alternata, Fusarium oxysporum, Colletotrichum capsici* and *Helminthosporium tetramera.* Two compounds have shown ED_{50} value less than 1000 ppm against *H. tetramera,* while only one compound has ED_{50} value less than 1000 ppm against *A. alternata* and *C. capsici.* The presence of methyl substituent in the *p*-position of both the C-phenyl and N-phenyl rings increase the fungitoxicity of benzalaniline.

Manrao and Kohli (1986) prepared a series of ketimines having hydroxyphenyl group and all the new compounds were tested against *Alternaria triticina*, *Alternaria tenuis, Puccinia striformis*, *Puccinia recondita* and *Pestalotia psidii* by employing standard method of spore germination inhibition. All the compounds have been found to possess promising antifungal activity against the above fungi and caused 100% spore germination inhibition at 1000 ppm. It was concluded that, the substitution by the second hydroxyl particularly in 6-position, increases the activity of the parent compound.

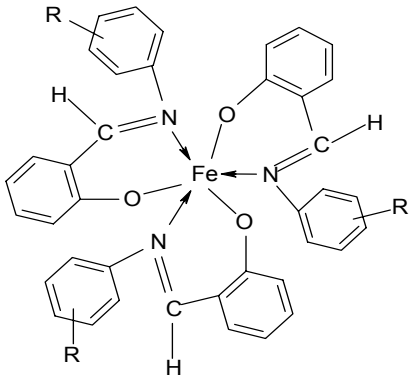


Sharma *et al*., (1991) prepared eight Schiff bases containing sulphur atom and tested their activity *in vitro* by spore germination inhibition method against phytopathogenic fungi, viz. *Curvularia lunata, Alternaria alternata, Helminthosporium maydis, Fusarium oxysporum and Stemphylium* spp. and reported that all the compound possess antifungal activity. However, the compound 4-hydroxy-3-methoxybenzal-2-thioaniline was the most effective of all, ED_{50} values ranging between 8 and 37 ppm

against all the fungi. 4-chlorobenzal-2-thioaniline was also effective against *F. oxysporum* due to presence of chlorine whose presence has also been reported to enhance the activity of the parent compound. Again, Sharma *et al*., (1993) developed seven thio-substituted Schiff bases and all were screened for their fungitoxicity against the same fungi. Out of these Schiff bases 4-hydroxy-3-methoxybenzal-2-thioaniline was found to be highly effective and it also reduced the intensity of powdery mildew disease on wheat seedlings. Higher activity of this compound was attributed to presence of methoxy and hydroxy group.

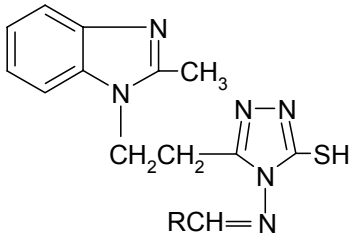


Hothi *et al*. (2006) prepared Fe(III) complexes of chloro substituted Schiff bases by condensing ferric chloride with 2-hydroxybenzalaniline, 2-hydroxybenzal-2-chloroaniline, 2-hydroxybenzal-3-chloroaniline, and 2-hydroxybenzal-4-chloroaniline, respectively, in 1:3 molar ratio . The Schiff bases and their iron complexes were screened *in vitro* against *Alternaria alternata, Fusarium oxysporum*, *Helminthosporium oryzae* and *Myrothecium roridum*. Most of the Schiff bases recorded ED_{50} values more than 1000 ppm except 2-hydroxybenzalaniline, which had ED_{50} value of 740 ppm against *F. oxysporum*. However, iron complexes have been found to be effective against all the test fungi with ED_{50} value ranging from 65-540. Complexation of Schiff bases with iron in general increases their fungitoxicity manifold.



Fungicidal activity of Schiff bases of triazoles and other heterocyclic compounds

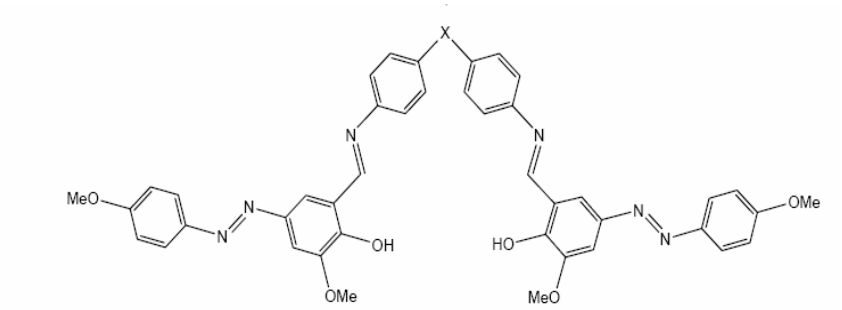
El-masry *et al*. (2000) developed Schiff bases of Benzimidazole derivative. Thus condensation of 1-[(1-amino-2-mercapto-1,3,4-triazol-5-yl)ethyl]-2 methylbenzimidazole with aromatic aldehydes, namely, *p*-methoxy benzaldehyde and *o*-chlorobenzaldehyde in absolute ethanol afforded the corresponding Schiff's bases. All the compound tested against two fungi Yeast (*Saccharomyces cerevisae*) and *Aspergillus niger*. The test was performed according to the disk diffusion method. All the compounds showed moderate activity against these two fungi.



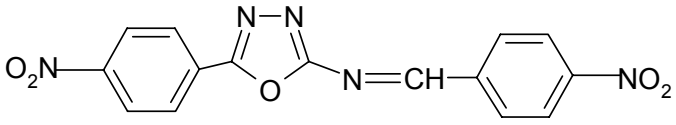
Pandeya *et al*. (2000) synthesized Schiff bases with Isatin (indole 2,3-dione) and its 5-chloro and 5-bromo derivatives have been reacted 3-(4'-pyridyl)-4-amino-5-mercapto-4-(H)-1,2,4-triazole. Investigation of antifungal activity of compounds was done by agar dilution method against 8 pathogenic fungi. Among the compounds tested 1-(piperidinomethyl) 5-bromo 3-[3'-(4''-pyridyl)-5'-mercapto-4'-(H)-1',2',4'-triazol 4'-yl]imino isatin showed the most favourable antifungal activity.

Jarrahpour *et al*. (2004) prepared ten new azo Schiff bases in excellent yields via the condensation of different aromatic amines and a new azoaldehyde, 2-hydroxy-3 methoxy-5-(4-methoxyphenylazo) benzaldehyde. The antifungal activities of ten new azo Schiff base were tested against eight different fungi by the disk-diffusion method. The species of fungi used included: yeasts (*Candida albicans* and *Cryptococus neoformans*), dermatophytes (*Triscophyton mentagrophytes*, *Microsporium canis* and *Epidermophyton floccosum*) and opportunistic filamentous fungi (*Aspergillus*

fumigatus, *Aspergillus niger* and *Alternaria*). Inhibitory zone areas were observed at the end of incubation period and these compounds therefore seem to have antifungal effects for fungi at 200 µg.



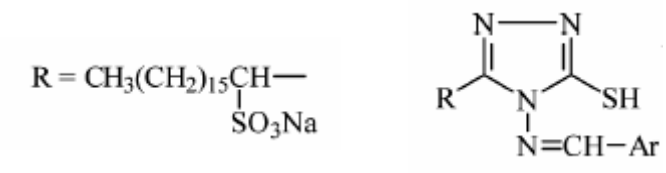
Mishra *et al.* (2005) synthesized twenty Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles with different aromatic aldehydes. Antifungal screenining of all these synthesized compounds was done. The fungal strains selected for antifungal screening were *Aspergillus niger* and *Candida albicans*. Clotrimazole was chosen as the standard drug. The MIC of the most effective compounds were (52 µg/ml against *A. niger*, 58µg/ml against *C. albicans*).The structure of the most effective compound is given below.



Shrivastava et al. (2005) synthesized schiff bases by condensing 2-amino-5-phenyl 1, 3, 4- thiadiazole with different aromatic aldehydes. Schiff bases are then complexed with different transition metals. Antifungal activity of Schiff bases and their corresponding bis(cyclopentadienyl)titanium(IV) complexes were tested The fungicidal activity of the ligands and the complexes were evaluated in DMF against *Aspergillus niger, Aspergillus alternata* and *Helminthosporium oryzae* by the agar plate technique. The compounds show significant toxicity at 1000 ppm conc. against all species of fungi. However, the complexes are more active than ligands, which may be owing to the chelation and the presence of sulphur atom.

El-syed (2006) synthesized the novel Schiff bases of Sodium 1-(4-amino-5-mercapto-4*H*-[1,2,4] triazol-3-yl)heptadecane-1-sulfonate with various aromatic

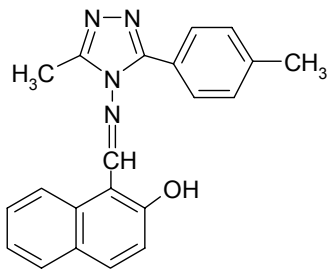
aldehyde. The compound tested against *Aspergillus niger*. These compounds shows moderate activity against the fungi. The MIC of the compounds is 125 μg/ml.



Ar = *p*-C_6H_4Cl, C_6H_5, *p*-$C_6H_4OCH_3$

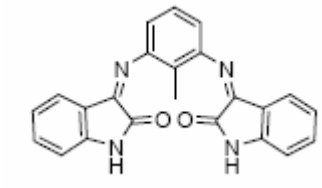
Karthkeyan *et al.* (2006) prepared a series of 2,4-dichloro-5-fluorophenyl bearing Mannich base from triazole Schiff bases by aminomethylation with formaldehyde and secondary/substituted primary amines. All newly synthesized compounds were screened for their antimicrobial activity. The compounds exhibited promising antibacterial and good antifungal activity.

Gumrukcuoglu *et al.* (2007) synthesized Schiff bases by condensing with 4-amino-3,5-dialkyl-1,2,4-triazoles with various aromatic aldehydes and resulted in the formation of 4-arylidenamino-3,5-dialkyl-1,2,4-triazoles. The compounds were screened against yeast like fungi, *Candida albicans* and *Candida tropicalis*. A simple susceptibility screening test using agar-well diffusion was used. For *C. albicans* and *C. tropicalis*, Sabouraud dextrose agar (SDA) was used. Antifungal activity was evaluated by measuring the zone of inhibition against the test organism. Triflucan served as the control fungicide. The most effective compound show moderate antifungal activity. The structure of the most effective compound is given below.



Serdar *et al.* (2007) prepared a novel series of Schiff bases of triazoles. The treatment of compound 4-amino-3-(4-tolyl)-5-phenyl-4*H*-1,2,4-triazole with various aromatic aldehydes or acetophenone and 4-nitroacetophenone resulted in the formation of 4-arylidenamino-3,5-dialkyl-4*H*-1,2,4-triazoles. All newly synthesized compounds were screened for their antifungal activities using agar-well diffusion method. Among these synthesized compounds only one compound 4-[(3-Methoxy-4-hydroxyphenyl)methylamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole showed moderate antifungal activity against *Candida tropicalis*.

Jarrahpour *et al.* (2007) synthesized twelve new bis-Schiff bases of isatin, benzylisatin and 5-fluoroisatin by condensation of isatin, benzylisatin and 5 -fluoroisatin with primary aromatic amines. These newly synthesized bis-Schiff bases were also tested for their antibacterial and antifungal activities. They display moderate activity against *S. cerevisiae* and *C. albicans*.



2.2.2 Biological activity of Schiff bases other than fungicidal activity

Herbicidal activity

Holla *et al.* (2000) synthesized a series of Schiff bases 3-substituted-4-[5-(2,4-dichlorophenyl)-2-furfurylidine]amino-5-mercapto-1,2,4-triazoles. All the newly synthesised compounds are tested for their herbicidal activities. Some of the selected compounds show good herbicidal properties.

Antibacterial activity

Zhongyi *et al.* (1996) prepared Schiff bases with 3-pyridyl-4-amino-5-mercapto-1,2,4-triazole with aromatic aldehydes and furaldehyde in the presence of a catalytic amount of tartaric acid. All of them were screened for antibacterial activity against *S. Aur.* and *E. Coli*. and five of them showed significant activities.

Pandeya *et al*. (2000) synthesized series of Schiff bases with Isatin (indole 2,3-dione) and its 5-chloro and 5-bromo derivatives have been reacted with 3-(4'-pyridyl)-4-amino-5-mercapto-4-(H)-1,2,4-triazole with formaldehyde and several secondary amines. Investigation of antimicrobial activity of compounds was done by agar dilution method against 27 pathogenic bacteria. Among the compounds tested 1-(piperidinomethyl) 5-bromo 3-[3'-(4''-pyridyl)-5'-mercapto-4'-(H)-1',2',4'-triazol 4'-yl]imino isatin showed the most favourable antimicrobial activity.

Vaghasiya *et al.* (2004) prepared Schiff bases derived from 4-aminoantipyrine and vanillin and evaluated for their potential as antibacterial agents against some Gram positive and Gram negative bacterial strains. The antibacterial activity was studied against *P. pseudoalcaligenes*, *P. vulgaris*, *C. freundii*, *E. aerogenes*, *S. subfava* and *B. megaterium*. The determination of the antibacterial activity was done using the Agar dilution method. The Schiff bases derived from vanillin as the central molecule with 2,4-dimethylaniline and sulphamethoxazole as the side chain in DMSO effectively inhibited the investigated bacteria and appear to be promising antimicrobial agents.

Gumrukcuoglu *et al.* (2007) synthesized Schiff bases by condensing with 4-amino-3,5-dialkyl-1,2,4-triazoles with various aromatic aldehydes and resulted in the formation of 4-arylidenamino-3,5-dialkyl-1,2,4-triazoles. Compounds were screened against *Escherichia coli, Pseudomonas aeruginosa, Klepsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus,*and *Bacillus cereus.* Some of the compounds showed activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Antiviral activity

Jarrahpour *et al.* (2007) prepared twelve new bis-Schiff bases of isatin, benzylisatin and 5-fluoroisatin by condensation of isatin, benzylisatin and 5 -fluoroisatin with primary aromatic amines. The compounds were screened for antiviral activity against a panel of DNA and RNA viruses. Minimum cytotoxic and minimum virus inhibitory concentrations of these compounds were determined. Some of the compounds exhibited specific antiviral activity, which means that they inhibit the

replication (induction of viral cytopathogenicity) of the viruses tested at a concentration that was ≥ 5-fold lower than the minimum cytotoxic concentration.

Plant growth regulatory activity

Zhou *et al.* (2007) synthesized 3-substituted-4-amino-5-mercapto-1,2,4-triazoles which are versatile compounds for constructing various biologically active heterocycles. To find more 1,2,4-triazole derivatives that may possess significant biological activities, they synthesized a number of novel Schiff bases 4-(arylmethylidene)amino-5-(4-ethoxyphenyl)-3-mercapto-4H-1,2,4-triazoles. The plant-growth regulating effects of those Schiff bases were examined, and they showed an inhibiting effect on the growth of wheat radicles and radish radicles.

Jin *et al.* (2007) synthesized a series of novel 6-aryl-3-(D-galactopentitol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines and 4-(arylmethylidene)amino-5-(D galactopentitol- 1-yl)-3-mercapto-4*H*-1,2,4-triazoles from 4-amino-3-(D-galactopentitol-1-yl)-5- mercapto-1,2,4-triazole. It was interesting to note that some compounds showed inhibitory activities towards the growth of the dicotyledon (radish) at two concentration levels, but under the same conditions it expressed stimulative activities towards the growth of the monocotyledon (wheat). It has a good level of activity and is worthy of further study to establish a relationship between structure and activity.

Ye *et al.* (2007) synthesized a number of novel Schiff bases from 4-amino-3-(*D*-glucoheptonic-hexitol-1-yl)-1*H*-1,2,4-triazole-5-thione. The plant-growth regulating effects of the synthesized compounds were examined. The newly synthesized Schiff bases containing 3-(*D*-glucoheptonic-hexitol-1-yl)-1*H*-1,2,4-triazole-5-thione and possessing good water-solubility, showed an inhibiting effect on the growth of the stalk and the radicel of the wheat but a promoting effect on the growth of the stalk and the radicle of radish.

3. MATERIALS AND METHODS

This chapter discusses about the experimental procedures under the following sections.

3.1 Synthesis, purification and structure characterization of the compounds

This includes a scheme for synthesis of compounds, related materials and techniques, description of general methods of preparation, purification and characterization of intermediates and desired compounds followed by a detailed description of preparation, purification and characterization of individual compounds

3.2 Fungicidal activity

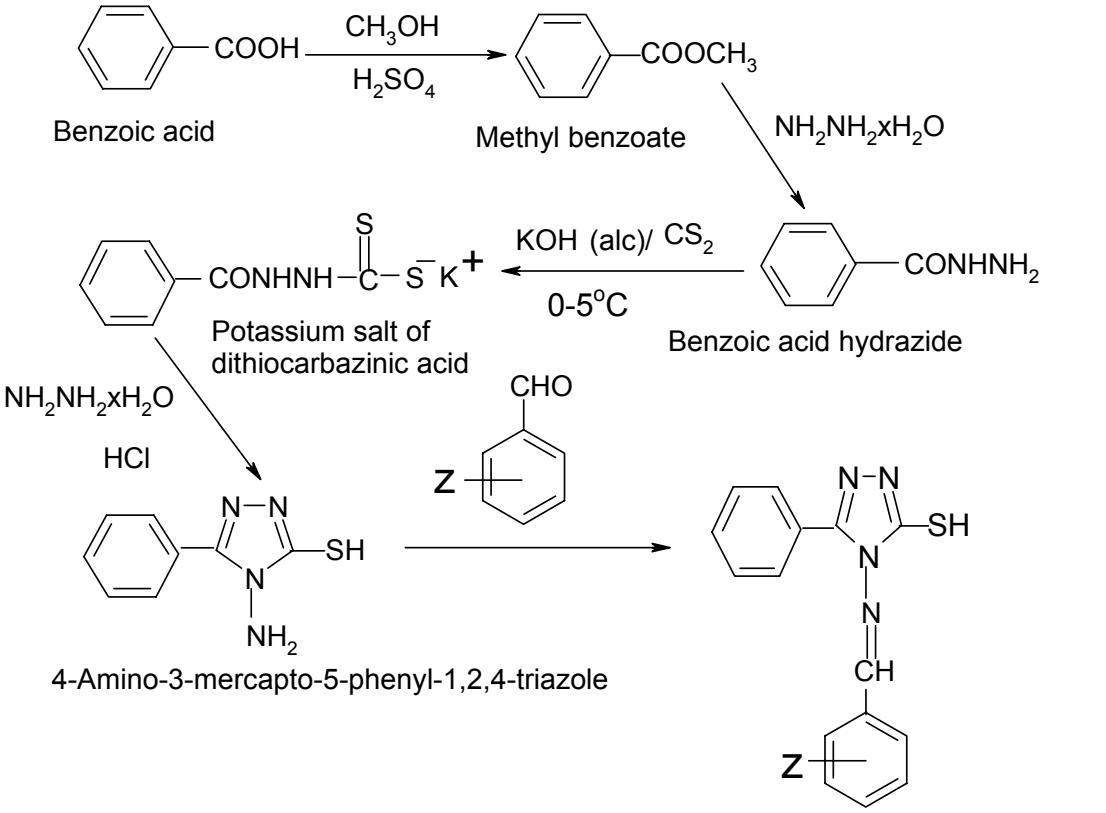
This section describes bioassay techniques used for the evaluation of the compounds for fungitoxicity against three test fungi viz. *Rhizoctonia solani, Fusarium oxysporum, & Bipolaris sorokiniana* and other related aspects.

3.3 Quantitative Structure Activity Relationship study

This deals with the methodology and physicochemical parameters used in the QSAR study.

3.1 Synthesis, purification and structure characterization of the compounds

Scheme: Synthesis of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1, 2, 4-triazoles:



Z=Cl, NO_2, CH_3, OCH_3, OH, Br, F, CN, $N(CH_3)_2$

Materials and Techniques:

Methanol an important reagent/ solvent was dried by treatment with magnesium methoxide followed by distillation, prior to use for the synthesis. Ethanol was dried using the same procedure. Dichloromethane was dried on calcium chloride and then

distilled. The acetone was refluxed with potassium permanganate before distillation. The other solvent used was dimethyl sulphoxide (DMSO) procured as AR grade.

The various reagents used include sulphuric acid (conc.), hydrazine hydrate (99%), carbon disulphide, potassium hydroxide, hydrochloric acid (conc.) and different substituted benzaldehydes. These were used of pure grade as supplied by the companies.

Thin layer chromatography:

TLC was performed using 250 μm thick silica gel G (containing 30% gypsum as binder) plates, coated with oxalic acid (by developing the plates in 1% oxalic acid solution) and activated at 110^{o}C for 1hr. Iodine vapour was used as visualizing agent.

Melting point:

Melting point (^{o}C) of the solid compounds were determined by using sulphuric acid bath and are uncorrected.

Infra-red (IR) spectroscopy:

The infrared spectra were recorded on a Shimadzu Fourier Transform Infra-red spectrometer (Model IR Prestige 21). The samples were dissolved in UV grade chloroform and analyzed in NaCl cell. The absorption bands in IR spectrum were expressed in terms of frequency i.e. ν_{max} in cm^{-1}.

Nuclear Magnetic Resonance (NMR) spectroscopy:

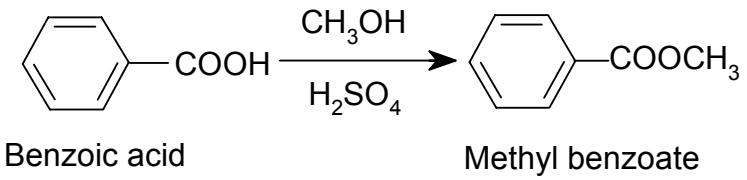
The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer (Model Avance 400) in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. The chemical shift (δ) were expressed in ppm and coupling constant (J) in Hz.

Elemental analysis:

The elemental analysis of the compounds for C, H, N and S was done on EURO-EA elemental analyzer using sulphanilamide as reference standard.

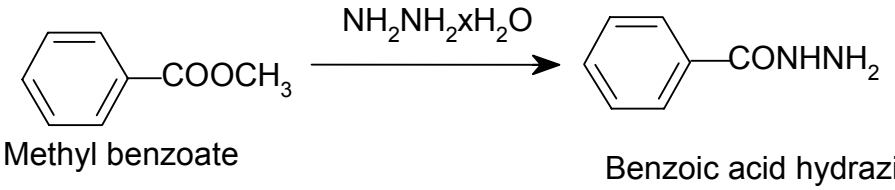
3.1.1 Preparation of methyl benzoate:

The Benzoic acid was reacted with methanol in presence of concentrated sulphuric acid



Benzoic acid (30.5 g, 0.25 mol), MeOH (101 ml, 2.5 mol) and conc. sulphuric acid (2.7 ml, 5 g) were taken in a 500 ml R.B. flask, fitted with a reflux condenser. 1 or 2 pieces Pumice stone were added and the mixture was gently refluxed for 5-6 hrs on a heating mantle. Excess methanol was distilled on a water bath or using rotary evaporator. The residue after cooling was quantitatively transferred with 250 ml of distilled water to a separatory funnel. Because of the less density difference between the lower ester layer and the water layer, it was difficult to obtain a sharp separation; 10-15 ml of CCl_4 was added in the separating funnel to obtain a solution of ester with distinct layer separation. The lower ester layer was taken in a beaker, treated with saturated solution of sodium bicarbonate until all free acid was removed (CO_2 effervescence ceased). This was then washed once with distilled water in the separatory funnel and then dried by pouring into a dry conical flask containing about 5 g of anhydrous sodium sulphate. The flask was allowed to stand for about half an hour with occasional shaking. The methyl ester solution was filtered into a R.B. flask which was fitted with a still head carrying a condenser and a 360^oC thermometer and then distilled over a heating mantle. The temperature was first slowly raised until all the CCl_4 has passed over and then heated more strongly to distill off the ester. The methyl benzoate was obtained as a colourless liquid. (B.p. 198-199 ^oC; Yield 26.3 g, 78.64 %).

3.1.2 Preparation of benzoic acid hydrazide:



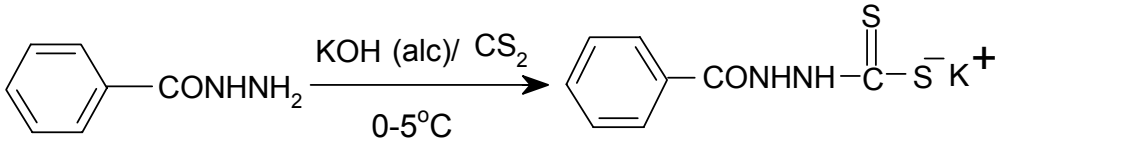
Method A-Conventional

Methyl ester of benzoic acid (13.6 g, 0.1mol), hydrazine hydrate 99% (10 g, 0.2mol) and methanol (100 ml) were taken in a 500 ml R.B. flask fitted with a water condenser. The mixture was refluxed on a heating mantle for 4 hrs. Excess methanol was distilled off on a water bath. The solid residue was washed twice with 15-20 ml of distilled water and dried. The benzoic acid hydrazide was further purified by recrystallization using CCl_4 and obtained as white crystalline solid and the purity was cheked by melting point and t.l.c. using acetone as developing solvent and iodine vapor as visualizing agent. (M.p. 115 ^oC; R_f 0.4; Yield 11.89g, 87.48 %).

Method B- Microwave

Methyl ester of benzoic acid (13.6 g, 0.1mol), hydrazine hydrate 99% (10 g, 0.2mol) and methanol (100 ml) were taken in a 500 ml conical flask. The mixture was irradiated in microwave. The microwave was used at 900 Watt and the microwave irradiation was done for 4 minutes until the solution become yellowish in colour. The reaction mixture became solidified after some time. The solid residue was washed twice with 15-20 ml of distilled water and dried. The benzoic acid hydrazide was further purified by recrystallization using CCl_4 and obtained as white crystalline solid and the purity was cheked by melting point and t.l.c. using acetone as developing solvent and iodine vapor as visualizing agent. (M.p. 115 ^oC; R_f 0.4; Yield 12.5g, 92 %).

3.1.3 Preparation of potassium salt of benzoyl dithiocarbazinic acid:



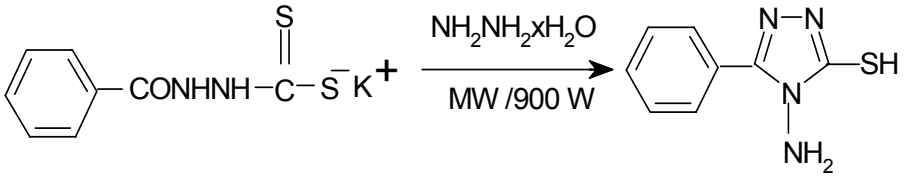
Benzoic acid hydrazide — Potassium salt of benzoyl dithiocarbazinic acid

The benzoic acid hydrazide (13.6 g, 0.1 mol) dissolved in saturated solution of chilled methanolic potassium hydroxide (50 ml) was taken in a 250 ml three necked R.B. flask fitted with mechanical stirrer, water condenser with a guard tube of calcium chloride and a dropping funnel. Carbon disulphide (7.22 ml, 0.12 mol) was taken in a

dropping funnel. The mixture was cooled to 0-5^{o}C in an ice bath. Carbon disulphide was added drop wise with rigorous stirring, in about 15 min., a cream colored solid separated out. The mixture was further stirred for 15-20 min. The solids were filtered and washed with cold distilled acetone. The dried cream coloured solid (Potassium salt of benzoyl dithiocarbazinic acid) was then stored in a desiccator, since the product is highly hygroscopic. (Yield 20 g, 80 %).

3.1.4 Preparation of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole:

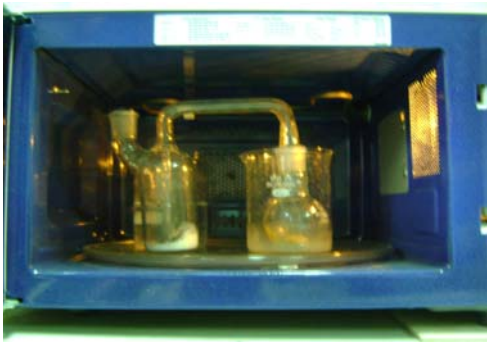
The 4-amino-3-mercapto-5-phenyl-1,2,4 triazole was prepared using microwave.



Potassium salt of benzoyl dithiocarbazinic acid 4-Amino-3-mercapto-5-phenyl-1,2,4-triazole

Potassium salt of benzoyl dithiocarbazinic acid (1 g, 4 mmol) and hydrazine hydrate-99% (0.4 ml, 8 mmol) was taken in a 100 ml R.B. flask. The R.B. flask was then connected with a specially designed apparatus containing lead acetate powder. The lead acetate works as a trap for hydrogen sulphide, which is emitted during the microwave reaction. The microwave was used at 900 Watt and the microwave irradiation was done for 36 seconds till a white solid appeared at the bottom or the hydrogen disulphide emission ceased. The solid was taken in 15-20 ml of water and acidified with concentrated hydrochloric acid. The white precipitate obtained was washed to neutral pH with water and dried. The product was recrystallized using benzene and acetone. The triazole was obtained as white crystalline solid. The purity was checked by melting point and t.l.c. using methanol as developing solvent and iodine vapor as visualizing agent. (M.p. 203-205 ^{o}C; R_f 0.94; Yield 0.74 g, 95.6 %).

Before Reaction

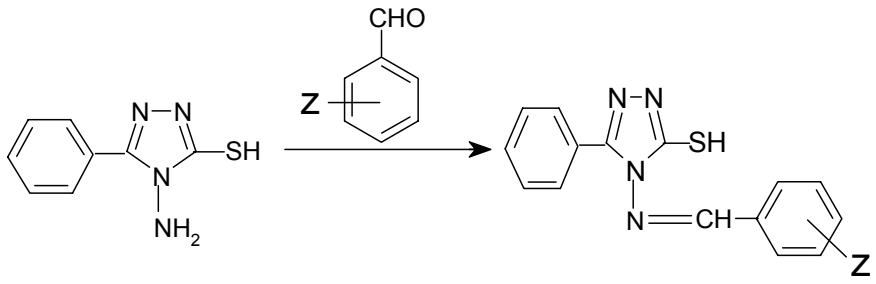


Microwave Irradiation

After reaction

Plate 1: Apparatus used in microwave synthesis of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole

3.1.5 Preparation of 4-Arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles:



4-Amino-3-mercapto-5-phenyl-1,2,4-triazole

4-Aryldenamino-3-mercapto-5-phenyl-1,2,4-triazole

Method A – Conventional:

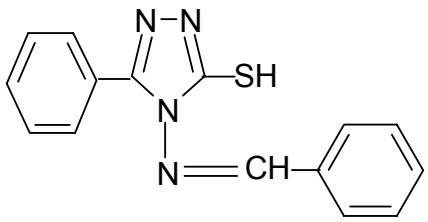
4-amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mole) was taken in a R.B. flask . The compound was dissolved in 35 ml of ethanol and then required amount of aldehyde (0.01 mol) was added to this solution. The mixture was refluxed for 2-4 hrs. The resulting solution was poured into the crushed ice. The precipitate which got separated was dried and recrystallized from benzene- hexane mixture. The purity of the product was checked by melting point and t.l.c. using benzene:acetone (9:1) as developing solvent and iodine vapor as visualizing agent.

Method B - Microwave:

4-amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was taken in a R.B. flask , was dissolved in minimum amount of ethanol (10 ml) and then required amount of benzaldehyde (1.06 g, 0.01 mol) was added to this solution. The microwave was used at 900 Watt and the microwave irradiation was done for 5 minutes until the solution become yellowish in colour. The resulting solution was poured into crushed ice. The precipitate which got separated was dried and recrystallized from benzene-hexane mixture. The purity of the product was cheked by melting point and t.l.c. using benzene + acetone (9:1) as developing solvent iodine vapour as visualizing agent. This method was standardized only for GA1. (M.p. 210^{o}C; R_f 0.73; Yield 2.24 g, 80 %).

Synthesis of individual compound:

3.1.5.1 4-Benzylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA1]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was taken in a R.B. flask, was dissolved in 35 ml of ethanol and then required amount of benzaldehyde (1.06 g, 0.01 mol) was added to this solution. The mixture was refluxed for 2 hrs. The resulting solution was poured into the crushed ice. The precipitate which got separated was dried and recrystallized from benzene- hexane mixture. The purity of the compound was checked by melting point and t.l.c. using benzene:acetone (9:1) as developing solvent and iodine vapor as visualizing agent. (M.p. 210^{o}C; R_f 0.73; Yield 2.1 g, 75.02 %).

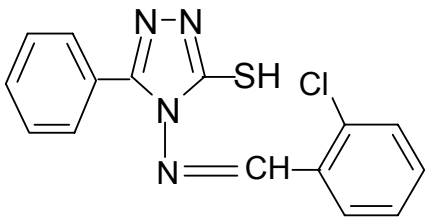
^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 7.34-8.06 (m, 10H, Ar-H), 9.69 (s, 1H, N=CH), 14.65 (s, 1H, SH).

^{13}C NMR $\delta^{DMSO\text{-}d6}$ (ppm): 122.7, 126.4, 128.7, 129.2, 129.9, 132.7 (aromatic carbons), 161 (N=CH), 177.8 (triazole carbons).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{12}N_4S$ (280): C, 64.28; H, 4.28; N, 20; S, 11.42; Found (%): C, 62.69; H, 3.91; N, 18.89; S, 10.72.

3.1.5.2 4-(2-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA2]



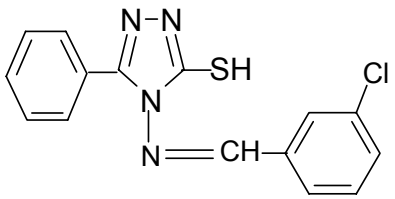
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *o*-chlorobenzaldehyde (1.41g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(2-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellowish white solid. (M.p. 212 ^{o}C; R_f 0.66; Yield 2.89 g, 92.04%).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.28-7.80 (m, 9H, Ar-H), 10.2 (s, 1H, N=CH), 14.27 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1600 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}N_4SCl$ (314.5): C, 57.23; H, 3.49; N, 17.80; S, 10.17; Found (%): C, 58.43; H, 3.44; N, 17.43; S, 10.47.

3.1.5.3 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA3]



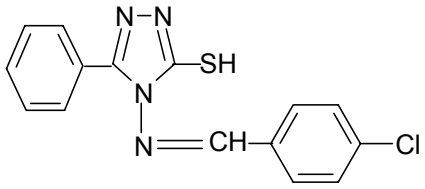
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *m*-chlorobenzaldehyde (1.41g, 0.01 mol) for 2.5 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellow solid. (M.p. 203-205 ^{o}C; R_f 0.63; Yield 2.88 g, 91.72 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.28-7.80 (m, 9H, Ar-H), 9.54 (s, 1H, N=CH), 14.27 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1600 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}N_4SCl$ (314.5): C, 57.23; H, 3.49; N, 17.80; S, 10.17; Found (%): C, 56.62; H, 3.91; N, 17.21; S, 10.95.

3.1.5.4 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA4]



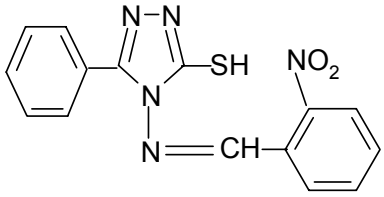
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-chlorobenzaldehyde (1.41g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellow solid. (M.p. 190 ^{o}C; R_f 0.64; Yield 2.50 g, 79.49 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 7.34-7.90 (m, 9H, Ar-H), 9.72 (s, 1H, N=CH), 14.27 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1600 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}N_4SCl$ (314.5): C, 57.23; H, 3.49; N, 17.80; S, 10.17; Found (%): C, 58.12; H, 3.45; N, 18.92; S, 10.65.

3.1.5.5 4-(2-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA5]



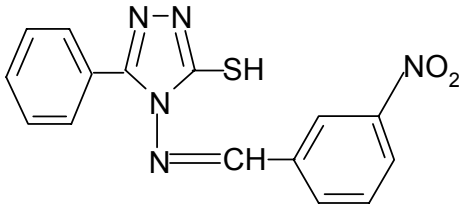
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *o*-nitrobenzaldehyde (1.51 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(2-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellow solid. (M.p. 207-209 ^{o}C; R_f 0.64; Yield 2.16 g, 66.46 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.34-8.19 (m, 9H, Ar-H), 10.49 (s, 1H, N=CH), 14.30 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic) 3051(C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}O_2N_5S$ (325): C, 55.38; H, 3.38; N, 21.53; S, 9.84; Found (%): C, 55.96; H, 2.85; N, 21.86; S, 10.73.

3.1.5.6 4-(3-Nitrobenzyidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA6]



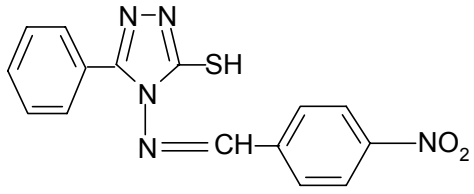
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *m*-nitrobenzaldehyde (1.51 g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(3-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellowish white solid. (M.p. 205-206 ^{o}C; R_f 0.64; Yield 2.38 g, 73.23 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.34-8.64 (m, 9H, Ar-H), 10.04 (s, 1H, N=CH), 14.33 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}O_2N_5S$ (325): C, 55.38; H, 3.38; N, 21.53; S, 9.84; Found (%): C, 57.92; H, 2.93; N, 20.67; S, 11.92.

3.1.5.7 4-(4-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA7]



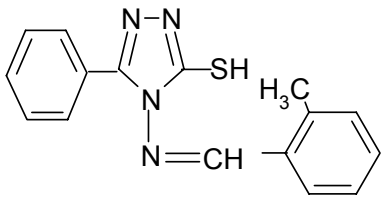
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-nitrobenzaldehyde (1.51 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellow solid. (M.p. 209-210 ^{o}C; R_f 0.66; Yield 2.80 g, 86.15 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.36-8.08 (m, 9H, Ar-H), 10.49 (s, 1H, N=CH), 14.75 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}O_2N_5S$ (325): C, 55.38; H, 3.38; N, 21.53; S, 9.84; Found (%): C, 54.82; H, 3.67; N, 22.45; S, 10.35.

3.1.5.8 4-(2-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA8]



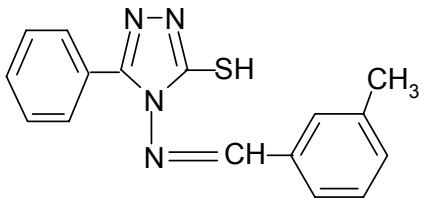
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *o*-methylbenzaldehyde (1.2 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(2-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 160 ^{o}C; R_f 0.69; Yield 1.60 g, 54.42 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 2.4 (s, 3H, Ar-CH_3), 7.48-8.01 (m, 9H, Ar-H), 9.72 (s, 1H, N=CH), 14.27 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{16}H_{14}N_4S$ (294): C, 65.30; H, 4.76; N, 19.04; S, 10.88; Found (%): C, 61.24; H, 4.11; N, 17.89; S, 12.43.

3.1.5.9 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA9]



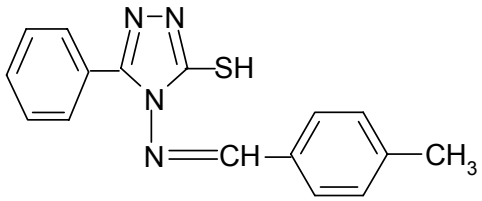
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *m*-methylbenzaldehyde (1.2 g, 0.01 mol) for 2.5 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 155 ^{o}C; R_f 0.70; Yield 2.34 g, 79.59 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 2.4 (s, 3H, Ar-CH_3), 7.11-8.00 (m, 9H, Ar-H), 9.66 (s, 1H, N=CH), 14.25 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd. (%) for $C_{16}H_{14}N_4S$ (294): C, 65.30; H, 4.76; N, 19.04; S, 10.88; Found (%): C, 52.17; H, 2.67; N, 19.83; S, 16.24.

3.1.5.10 4-(4-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA10]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-methylbenzaldehyde (1.2 g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in

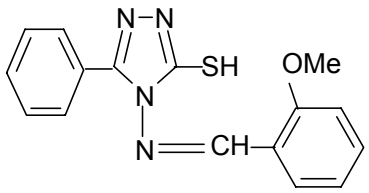
3.1.5.1. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as light yellow solid. (M.p. 158-160 ^{o}C; R_f 0.71; Yield 1.74 g, 59.18 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 2.4 (s, 3H, Ar-CH_3), 7.48-8.01 (m, 9H, Ar-H), 9.72 (s, 1H, N=CH), 14.27(s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd. (%) for $C_{16}H_{14}N_4S$ (294): C, 65.30; H, 4.76; N, 19.04; S, 10.88; Found (%): C, 64.82; H, 4.13; N, 18.93; S, 11.63.

3.1.5.11 4-(2-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA11]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *o*-methoxybenzaldehyde (1.2 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(2-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellowish white solid. (M.p. 172-174 ^{o}C; R_f 0.54; Yield 1.66 g, 53.54 %).

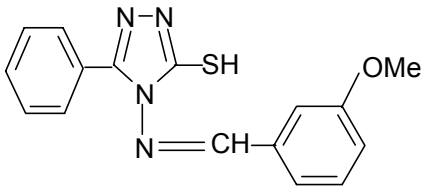
^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 3.35 (s, 3H, Ar-OCH_3), 7.34-8.01 (m, 9H, Ar-H), 9.34 (s, 1H, N=CH), 14.75 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051(C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{16}H_{14}ON_4S$ (310): C, 61.93; H, 4.51; N, 18.06; S, 10.32; Found (%): C, 57.78; H, 4.62; N, 17.75; S, 11.96.

3.1.5.12 4-(3-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole

[GA12]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *m*-methoxybenzaldehyde (1.2 g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(3-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 173-175 ^{o}C; R_f 0.54; Yield 2.20 g, 70.96 %).

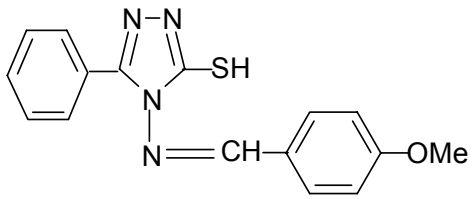
^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 3.35 (s, 3H, Ar-OCH_3), 6.99-7.88 (m, 9H, Ar-H), 9.56 (s, 1H, N=CH), 14.75 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{16}H_{14}ON_4S$ (310): C, 61.93; H, 4.51; N, 18.06; S, 10.32; Found (%): C, 63.41; H, 4.92; N, 18.55; S, 11.43.

3.1.5.13 4-(4-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole

[GA13]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-methoxybenzaldehyde (1.2 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as

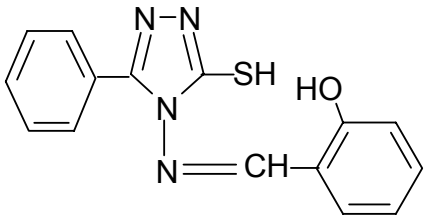
in 3.1.5.1. 4-(4-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as yellow solid. (M.p. 210-211 ^{o}C; R_f 0.55; Yield 1.76 g, 56.77 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 3.35(s, 3H, Ar-OCH_3), 7.34-8.01 (m, 9H, Ar-H), 9.34 (s, 1H, N=CH), 14.75 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051(C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd. (%) for $C_{16}H_{14}ON_4S$ (310): C, 61.93; H, 4.51; N, 18.06; S, 10.32; Found (%): C, 59.73; H, 5.12; N, 19.44; S, 10.72.

3.1.5.14 4-(2-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA14]



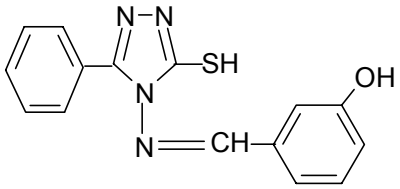
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *o*-hydroxybenzaldehyde (1.22 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(2-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as light yellow solid. (M.p. 189-190 ^{o}C; R_f 0.71; Yield 2.01 g, 67.90 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 3.9 (s, 1H, Ar-OH), 7.4-8.1 (m, 9H, Ar-H), 10.2 (s, 1H, N=CH), 14.27(s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{12}ON_4S$ (296): C, 60.81; H, 4.05; N, 18.92; S, 10.81; Found (%): C, 58.32; H, 4.71; N, 18.64; S, 13.92.

3.1.5.15 4-(3-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA15]



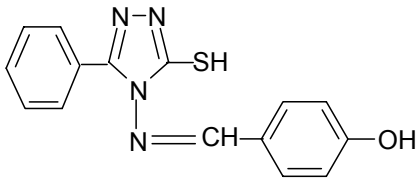
4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *m*-hydroxybenzaldehyde (1.22 g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(3-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 198-200 ^{o}C; R_f 0.74; Yield 1.90 g, 64.19 %).

^{1}H NMR $\delta^{DMSO\text{-}d6}$ (ppm): 3.9 (s, 1H, Ar-OH), 7.1-8.1 (m, 9H, Ar-H), 10.35 (s, 1H, N=CH), 14.25 (s, 1H, SH).

IR spectrum υ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051(C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{12}ON_4S$ (296): C, 60.18; H, 4.05; N, 18.92; S, 10.81; Found (%): C, 59.47; H, 4.81; N, 17.98; S, 11.81.

3.1.5.16 4-(4-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA16]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-hydroxybenzaldehyde (1.22 g, 0.01

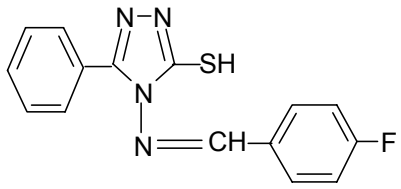
mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 205 ^{o}C; R_f 0.72; Yield 2.10 g, 70.94 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 3.9 (s, 1H, Ar-OH), 7.4-7.8 (m, 9H, Ar-H), 10.2 (s, 1H, N=CH), 14.27(s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{12}ON_4S$ (296): C, 60.81; H, 4.05; N, 18.92; S, 10.81; Found (%): C, 62.81; H, 5.16; N, 19.11; S, 11.21.

3.1.5.17 4-(4-Fluorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA17]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-fluorobenzaldehyde (1.24 g, 0.01 mol) for 3 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Fluorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 205-206 ^{o}C; R_f 0.52; Yield 1.98 g, 66.44 %).

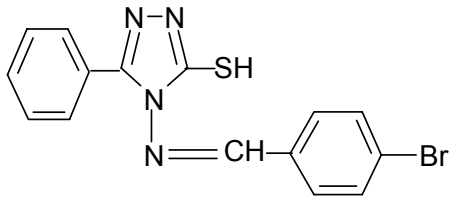
^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 6.9-7.87 (m, 9H, Ar-H), 9.88 (s, 1H, N=CH), 14.76 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051(C-H, aromatic) 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{15}H_{11}N_4SF$ (298): C, 60.40; H, 3.69; N, 18.79; S, 10.74; Found (%): C, 62.64; H, 3.69; N, 19.54; S, 10.52.

3.1.5.18 4-(4-Bromobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole

[GA18]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-bromobenzaldehyde (1.84 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Bromobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as white solid. (M.p. 203-204 ^{o}C; R_f 0.57; Yield 3.05 g, 84.96 %).

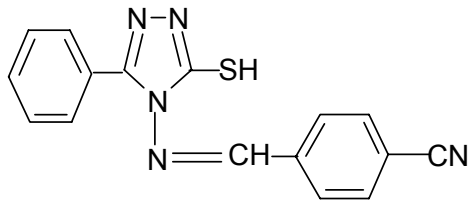
^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.3-8.05 (m, 9H, Ar-H), 9.72 (s, 1H, N=CH), 14.27(s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517(C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd. (%) for $C_{15}H_{11}N_4SBr$ (359): C, 50.14; H, 3.34; N, 15.59; S, 8.91; Found (%): C, 55.68; H, 5.82; N, 16.72; S, 9.51.

3.1.5.19 4-(4-Cyanobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole

[GA19]



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-cyanobenzaldehyde (1.84 g, 0.01 mol) for 4 hrs. The preparation, isolation and purification procedures were the same as in

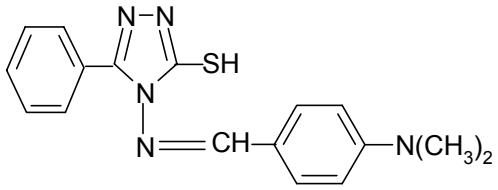
3.1.5.1. 4-(4-Cyanobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as bright yellow solid. (M.p. 181-182 ^{o}C; R_f 0.46; Yield 1.60 g, 52.45 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 7.34-7.91 (m, 9H, Ar-H), 9.16 (s, 1H, N=CH), 14.09 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{16}H_{11}N_5S$ (305): C, 62.95; H, 3.61; N, 22.95; S, 10.49; Found (%): C, 63.22; H, 2.69; N, 21.03; S, 6.02.

3.1.5.20 4-(4-Dimethylaminobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA20)



4-Amino-3-mercapto-5-phenyl-1,2,4 triazole (1.92 g, 0.01 mol) was dissolved in 35 ml of ethanol and refluxed with *p*-dimethylaminobenzaldehyde (1.42 g, 0.01 mol) for 2 hrs. The preparation, isolation and purification procedures were the same as in 3.1.5.1. 4-(4-Dimethylaminobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole was obtained as light brown solid. (M.p. 184-185 ^{o}C; R_f 0.58; Yield 2.33 g, 72.13 %).

^{1}H NMR δ $^{DMSO\text{-}d6}$ (ppm): 3.34(s, 6H, Ar-N $(CH_3)_2$), 7.34-8.11 (m, 9H, Ar-H), 9.95 (s, 1H, N=CH), 14.32 (s, 1H, SH).

IR spectrum υ ^{CHCl_3} (cm^{-1}): 2434 (SH), 1517 (C=N), 2896 (C-H, aliphatic), 3051 (C-H, aromatic), 1476, 1251, 1046, 931 (N-C=S).

Calcd (%) for $C_{16}H_{17}N_5S$ (323): C, 63.16; H, 5.26; N, 21.67; S, 9.90; Found (%) : C, 56.04; H, 3.59; N,17.60; S, 13.25.

3.2 Fungicidal activity

The fungitoxicity testing of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles was carried out by Poisoned food technique. Poisoned food technique using potato-dextrose agar (PDA) medium against *Rhizoctonia solani* Kühn., *Fusarium oxysporum* (Padwik) Snyd. & Hans. and *Bipolaris sorokiniana*.

3.2.1 Cultures of Fungi

Cultures of *Rhizoctonia solani, Fusarium oxysporum, Bipolaris sorokiniana* were obtained from the Division of Plant Pathology, IARI, New Delhi. The cultures were maintained on PDA slants at 25^{o}C and were subcultured in petridishes prior to testing.

3.2.2 Preparation of media

39 g PDA (Potato-dextrose-agar supplied by Titan Biotech Ltd., TM344) was suspended in 1000 ml distilled water. This was boiled to obtain uniform media. The media (65 ml) was transferred to each of the 100 ml conical flasks and the flasks were plugged with surgical grade cotton. The media and petridishes were sterilized in an autoclave at 15 psi for half an hour prior to use.

3.2.3 Preparation of test concentrations

Required quantity (130 mg for a set of 1000, 500 and 250 ppm conc.) of each of the test compounds were weighed and dissolved in DMSO (2 ml). 1 ml, 0.5 ml and 0.25 ml of the above solution were taken in vials and made up to 1 ml by adding DMSO. DMSO (1 ml) was taken in a separate vial for using as control. These solutions were added and mixed thoroughly to the media (65 ml) contained in a conical flask to obtain the desired concentration of the chemical in the media. The media from conical flask (65 ml) was poured into two petridishes (2 replication), under aseptic conditions in a

laminar flow chamber and allowed the media to solidify. The petridishes are suitably labeled.

3.2.4 Inoculation

A 5 mm thick disc of fungus (spores and mycelium) cut from earlier subcultured fungus in a petridish was inoculated asceptically to the centre of the petridishes.

3.2.5 Incubation

Both treatment and control petridishes were kept in B.O.D incubator at 25 ± 1^{o}C till the fungal growth in the control petridishes was almost complete. The incubation periods were:

Fungus	Incubation period (Days)
Rhizoctonia solani	5-6
Fusarium oxysporum	7-8
Bipolaris sorokiniana	9-10

3.2.1.6 Recording of observations

Fungus mycelial growth in both treated (T) as well as in control (C) was measured diametrically and % inhibition of growth (%I) was calculated from the following formula

$$\% \text{ Inhibition of growth } (\%I) = [(C - T)/ C] \times 100$$

3.2.1.7 Calculation of ED_{50} (Effective dose required for 50% inhibition of fungus growth)

The %I was converted to corrected % inhibition (Ic) by using the formula

$$Ic = [(\%I - C.F.)/ (100 - C.F.)] \times 100$$

Where $C.F. = [(9 - C)/ C] \times 100$, is the correction factor

Here, 9 is the diameter of petridish in cm, C is the growth of the fungus in control

The ED_{50} values were determined by using BASIC LD_{50} programme version 1.1 (Trevors, 1986) using a personal computer (PC).

3.3 Quantitative Structure Activity Relationship (QSAR) study

QSAR was attempted using the experimentally determined ED_{50} values and various physicochemical parameters representing hydrophobic, electronic and steric properties of the compounds of the series.

3.3.1 Fungicidal activity parameter [pED_{50} (M)]

QSAR study requires biological activity to be expressed in molar quantity. The ED_{50} value in ppm of each compound was divided by 1000 and then by molecular weight of the compound to obtain molar ED_{50} value. The negative logarithm of this molar ED_{50} value i.e. pED_{50} (M) was used as the the dependent variable in the QSAR study.

3.3.2 Physicochemical parameters

Several physicochemical parameters representing hydrophobic, electronic and steric properties of the molecules were used in the QSAR study as independent variables.

The values for the physico-chemical parameters of benzene ring substituents used in the QSAR analysis were taken from the literature (Hansch & Leo, 1979). Hansch π value were used for hydrophobicity, Hammett constant σ for electronic, molar refractivity (MR) and Verloop STERIMOL parameters L, B1 and B4 for steric. For the electronic effect of *ortho*-substituents, the σ values of the corresponding para-substituents along with F, Swain-Lupton constant for 'proximity polar effect' suggested by Fujita & Nishioka (1976) were used.

The values for the steric parameters was used as summation for each position i.e. *ortho*-, *meta*- or *para*-. Further these were considered separately for each of the three positions corresponding to *ortho*-, *meta*- and *para*-.

The values of physicochemical parameters used in the regression equation have been included in the respective tables.

3.3.3 Methodology of QSAR analysis

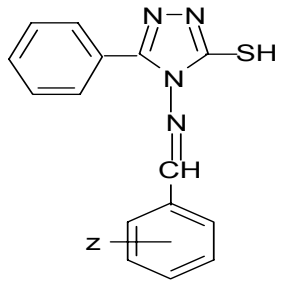
The structure activity correlations were analyzed by the multiple regression analysis technique using experimental pED_{50} (M) values for the fungicidal activity as dependent variable and the physicochemical parameters for the hydrophobic, electronic and the steric properties of each member of the series as independent variables. The significance of the equations and the terms were examined by F and t tests.

4. RESULTS AND DISCUSSION

4.1 Synthesis and structure characterization of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1, 2, 4-triazoles

4.1.1 Synthesis

In continuation to earlier investigations on 5-Aryl-4-amino-3-mercapto-1,2,4-triazoles (Bijul, 2005) for finding potential fungicides, a series of twenty Schiff bases of 4-Amino-3-mercapto-5-phenyl-1,2,4-triazole having different substituents in the aryl ring attached to imine group as represented by the general structure (I) were designed and synthesized. The Schiff bases i.e. 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles synthesized are given in the **Table 1** and their physical data in **Table 2**.



(I)

The synthesis of the Schiff bases has involved five steps starting with benzoic acid which was first converted ⟶ methyl ester ⟶ hydrazide ⟶ dithiocarbazinic salt ⟶ triazole and finally to ⟶ Schiff base.

Methyl benzoate

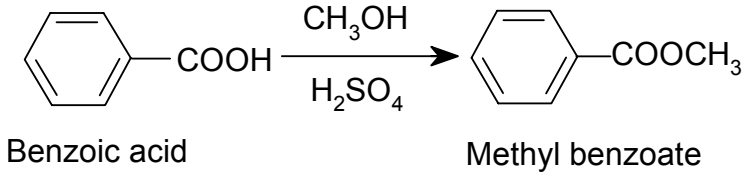
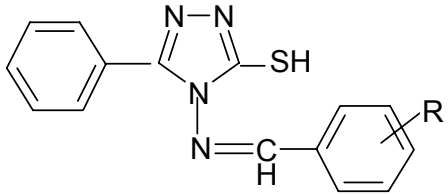
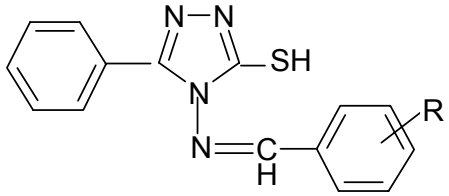


Table 1: 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles synthesized



Compd No	Substituent (R)	Chemical name	Molecular formula	Formula weight
GA1	H	4-Benzylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{12}N_4S$	280
GA2	2-Cl	4-(2-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}N_4SCl$	314.5
GA3	3-Cl	4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}N_4SCl$	314.5
GA4	4-Cl	4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}N_4SCl$	314.5
GA5	2-NO_2	4-(2-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}O_2N_5S$	325
GA6	3-NO_2	4-(3-Nitrobenzyidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}O_2N_5S$	325
GA7	4-NO_2	4-(4-Nitrobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}O_2N_5S$	325
GA8	2-CH_3	4-(2-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{14}N_4S$	294
GA9	3-CH_3	4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{14}N_4S$	294
GA10	4-CH_3	4-(4-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{14}N_4S$	294
GA11	2-OCH_3	4-(2-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{14}ON_4S$	310
GA12	3-OCH_3	4-(3-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{14}ON_4S$	310
GA13	4-OCH_3	4-(4-Methoxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{14}ON_4S$	310
GA14	2-OH	4-(2-hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{12}ON_4S$	296
GA15	3-OH	4-(3-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{12}ON_4S$	296
GA16	4-OH	4-(4-Hydroxybenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{12}ON_4S$	296
GA17	4-F	4-(4-Fluorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}N_4SF$	298
GA18	4-Br	4-(4-Bromobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{15}H_{11}N_4SBr$	359
GA19	4-CN	4-(4-Cyanobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{11}N_5S$	305
GA20	4-$N(CH_3)_2$	4-(4-Dimethylaminobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole	$C_{16}H_{17}N_5S$	323

Table 2: Physical data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles

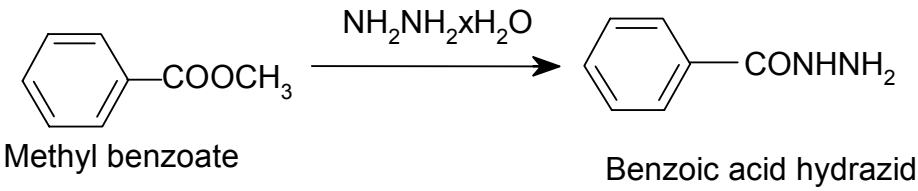


Compd No	Substituent (R)	Quantity of aldehyde (g, 0.01mol) [Reacted with 0.01mol, 1.92g of triazole]	Time of reaction (hr)	Yield		R_f*	M.p. (^oC)
				(g)	(%)		
GA1	H	1.06	2	2.10	75.02	0.73	210
GA2	2-Cl	1.41	4	2.89	92.04	0.66	212
GA3	3-Cl	1.41	2	2.88	91.72	0.63	203-205
GA4	4-Cl	1.41	2.5	2.50	79.49	0.64	190
GA5	2-NO_2	1.51	4	2.16	66.46	0.64	207-209
GA6	3-NO_2	1.51	2	2.38	73.23	0.64	205-206
GA7	4-NO_2	1.51	4	2.80	86.15	0.66	209-210
GA8	2-CH_3	1.20	4	1.60	54.42	0.69	160
GA9	3-CH_3	1.20	2.5	2.34	79.59	0.70	155
GA10	4-CH_3	1.20	2	1.74	59.18	0.71	158-160
GA11	2-OCH_3	1.36	4	1.66	53.54	0.54	172-174
GA12	3-OCH_3	1.36	2	2.20	70.96	0.54	173-175
GA13	4-OCH_3	1.36	4	1.76	56.77	0.55	210-211
GA14	2-OH	1.22	4	2.01	67.90	0.71	189-190
GA15	3-OH	1.22	2	1.90	64.19	0.74	198-200
GA16	4-OH	1.22	2	2.10	70.94	0.72	205
GA17	4-F	1.24	3	1.98	66.44	0.52	205-206
GA18	4-Br	1.84	4	3.05	84.96	0.57	203-204
GA19	4-CN	1.31	4	1.60	52.45	0.46	181-182
GA20	4-$N(CH_3)_2$	1.49	2	2.33	72.13	0.58	184-185

*Solvent: Benzene+Acetone (9:1, by volume)

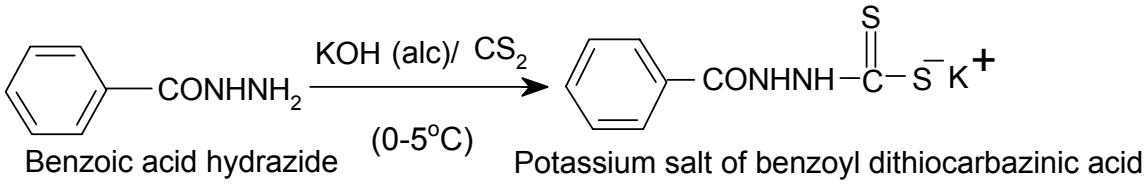
The methyl ester of benzoic acid i.e. methyl benzoate was prepared by refluxing benzoic acid with methanol in the presence of concentrated sulphuric acid. (Vogel, 1989) The yield of methyl benzoate was 78.64 %.

Benzoic acid hydrazide



Benzoic acid hydrazide was prepared by two methods. Method-A involved refluxing of methyl benzoate with hydrazine hydrate in methanol for 4 hrs and method-B involved the reaction of similar quantities of methyl benzoate with hydrazine hydrate in methanol under microwave irradiation at 900 Watt, for 4 minutes. The yield of benzoic acid hydrazide was 87.48 % by method-A and 92 % by method–B. The method-B that involved microwave thus was much faster and efficient than conventional method i.e. method-A. The isolation and purification of the product after the reaction was same by both the methods.

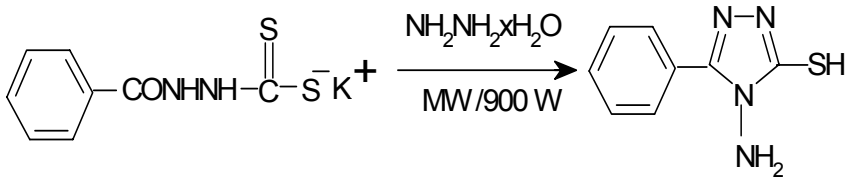
Potssium salt of benzoyl dithiocarbazinic acid



The potassium salt of benzoyl dithiocarbazinic acid was prepared in 80 % yield by reacting benzoic acid hydrazide in methanolic potassium hydroxide with carbon di sulphide under cold condition, 0-5^{o}C. (Kidwai & Bhushan, 1998)

Earlier workers (Reid & Heindel, 1976) have prepared the potassium salt of aroyl dithiocarbazinic acid by refluxing for about 10 hrs. Both these methods have been compared by Bijul (2005) and have found that the method using cold reaction condition was fast and efficient.

4-Amino-3-mercapto-5-phenyl-1,2,4 triazole

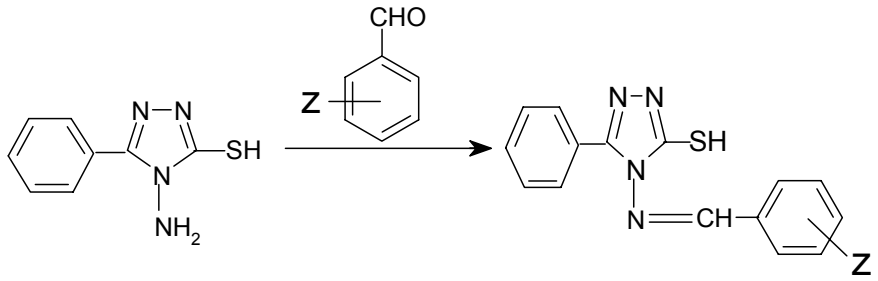


Potassium salt of benzoyl dithiocarbazinic acid 4-Amino-3-mercapto-5-phenyl-1,2,4-triazole

4-Amino-3-mercapto-5-phenyl-1,2,4 triazole was prepared by reacting the potassium salt of benzoyl dithiocarbazinic acid with hydrazine hydrate in a specially designed apparatus containing lead acetate powder by microwave irradiation at 900 Watt for 36 seconds and then acidifying the solid product with concentrated hydrochloric acid. The triazole was obtained as white crystalline solid in 95.6% yield.

Bijul (2005) have prepared 4-Amino-3-mercapto-5-phenyl-1,2,4 triazole by 2 methods, conventional involving 4 hrs refluxing and microwave irradiation and have found that the microwave method was much faster and efficient.

4-Arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles



4-Amino-3-mercapto-5-phenyl-1,2,4-triazole 4-Aryldenamino-3-mercapto-5-phenyl-1,2,4-triazole

4-Arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles were prepared by refluxing 4-amino-3-mercapto-5-phenyl-1,2,4 triazole in ethanol with respective aldehydes for 2-4 hrs, in yield varying from 52-92%.

Similar methods of Schiff bases of some other heterocyclic moieties have been reported in the literature (Cansiz *et al*., 2004; Vaghasiya *et al*., 2004; Sharba *et al*., 2005; Mishra *et al*., 2005; Serdar *et al.*, 2007).

A microwave method was also standardized for the preparation of 4-benzylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazole that involved microwave irradiation of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole in ethanol with benzaldehyde

at 900 Watt for 5 minutes, yield 80%, better than in the conventional method (75%). The method is less time consuming and also efficient in terms of yield of the product and required only 30% ethanol than used in the conventional method.

4.1.2. Structure characterization

Structures of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles were confirmed by elemental analysis, IR and NMR spectroscopy.

Elemental analysis

Elemental analysis of all the 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles were done for C, H, N and S. The found and the corresponding calculated value in respect of these elements are presented in **Table 3** and are very close, thus confirm the structure and purity of compounds.

Infra Red (IR) spectroscopy

The Infra Red (IR) spectra of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles showed characteristic peaks for SH (2434 cm^{-1}), C=N (1517 cm^{-1}), C-H aliphatic (2896 cm^{-1}), C-H aromatic (3051 cm^{-1}) and N-C=S (1476, 1251, 1046, 931 cm^{-1}). The peaks for N-C=S signifies that thiono–thiolo rearrangement takes place because of the presence of SH group at 3-position of the triazole ring.

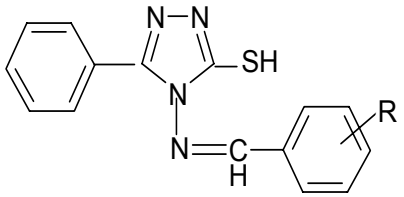
The Infra Red spectral data 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles was given in **Table 4**.

Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR

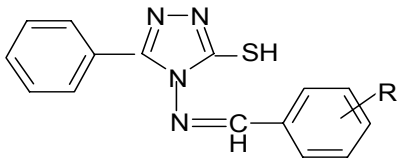
The most characteristic feature of ^{1}H NMR spectra of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles was the presence of singlet at around δ 9.16 to 10.49 for N=CH and another singlet at around δ 14.09 to 14.75 for SH. The aryl protons

Table 3: Elemental analysis data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles



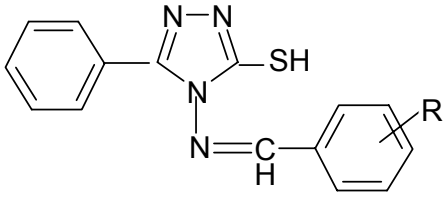
Compd No	Substituent (R)	Analysis (%)							
		C		H		N		S	
		Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
GA1	H	64.28	62.69	4.28	3.91	20	18.89	11.42	10.72
GA2	2-Cl	57.23	58.43	3.49	3.44	17.80	17.43	10.17	10.47
GA3	3-Cl	57.23	56.62	3.49	3.91	17.80	17.21	10.17	10.95
GA4	4-Cl	57.23	58.12	3.49	3.45	17.80	18.92	10.17	10.65
GA5	2-NO_2	55.38	55.96	3.38	2.85	21.53	21.86	9.84	10.73
GA6	3-NO_2	55.38	57.92	3.38	2.93	21.53	20.67	9.84	11.92
GA7	4-NO_2	55.38	54.82	3.38	3.67	21.53	22.45	9.84	10.35
GA8	2-CH_3	65.30	61.24	4.76	4.11	19.04	17.89	10.88	12.43
GA9	3-CH_3	65.30	52.17	4.76	2.67	19.04	19.83	10.88	16.24
GA10	4-CH_3	65.30	64.82	4.76	4.13	19.04	18.93	10.88	11.63
GA11	2-OCH_3	61.93	57.78	4.51	4.62	18.06	17.75	10.32	11.96
GA12	3-OCH_3	61.93	63.41	4.51	4.92	18.06	18.55	10.32	11.43
GA13	4-OCH_3	61.93	59.73	4.51	5.12	18.06	19.44	10.32	10.72
GA14	2-OH	60.81	58.32	4.05	4.71	18.92	18.64	10.81	13.92
GA15	3-OH	60.81	59.47	4.05	4.81	18.92	17.98	10.81	11.81
GA16	4-OH	60.81	62.81	4.05	5.16	18.92	19.11	10.81	11.21
GA17	4-F	60.40	62.64	3.69	5.33	18.79	19.54	10.74	10.52
GA18	4-Br	50.14	55.68	3.34	5.82	15.59	16.72	8.91	9.51
GA19	4-CN	62.95	63.22	3.61	2.69	22.95	21.03	10.49	6.02
GA20	4-$N(CH_3)_2$	63.16	56.04	5.26	3.59	21.67	17.60	9.90	13.25

Table 4: IR spectral data 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles



Compd No	Substituent (R)	υ cm^{-1}				
		SH	C=N	C-H aliphatic	C-H aromatic	N-C=S
GA1	H	2434	1517	2896	3051	1476,1251,1046,931
GA2	2-Cl	2433	1600	2896	3051	1476,1251,1046,931
GA3	3-Cl	2433	1600	2896	3051	1476,1251,1046,931
GA4	4-Cl	2433	1600	2896	3051	1476,1251,1046,931
GA5	2-NO_2	2433	1517	2896	3051	1476,1251,1046,931
GA6	3-NO_2	2433	1517	2896	3051	1476,1251,1046,931
GA7	4-NO_2	2433	1517	2896	3051	1476,1251,1046,931
GA8	2-CH_3	2434	1517	2896	3052	1476,1251,1046,931
GA9	3-CH_3	2434	1517	2896	3052	1476,1251,1046,931
GA10	4-CH_3	2434	1517	2896	3052	1476,1251,1046,931
GA11	2-OCH_3	2434	1517	2896	3051	1476,1251,1046,931
GA12	3-OCH_3	2434	1517	2896	3051	1476,1251,1046,931
GA13	4-OCH_3	2434	1517	2896	3051	1476,1251,1046,931
GA14	2-OH	2434	1517	2896	3051	1476,1251,1046,931
GA15	3-OH	2434	1517	2896	3051	1476,1251,1046,931
GA16	4-OH	2434	1517	2896	3051	1476,1251,1046,931
GA17	4-F	2434	1517	2896	3051	1476,1251,1046,931
GA18	4-Br	2434	1517	2896	3051	1476,1251,1046,931
GA19	4-CN	2434	1517	2896	3051	1476,1251,1046,931
GA20	4-$N(CH_3)_2$	2434	1517	2896	3051	1476,1251,1046,931

Table 5: ^{1}H NMR data of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles



Compd No	Substituent (R)	Chemical shift, δ (ppm)		
		Ar-H (m, 10H/9H)	N=CH (s, 1H)	SH (s, 1H)
GA1	H	7.34-8.06	9.69	14.65
GA2	2-Cl	7.28-7.80	10.2	14.27
GA3	3-Cl	7.28-7.80	9.54	14.27
GA4	4-Cl	7.34-7.90	9.72	14.27
GA5	2-NO_2	7.34-8.19	10.49	14.30
GA6	3-NO_2	7.34-8.64	10.04	14.33
GA7	4-NO_2	7.36-8.08	10.49	14.75
GA8	2-CH_3	7.48-8.01	9.72	14.27
GA9	3-CH_3	7.11-8.00	9.66	14.25
GA10	4-CH_3	7.48-8.01	9.72	14.27
GA11	2-OCH_3	7.34-8.01	9.34	14.75
GA12	3-OCH_3	6.99-7.88	9.56	14.75
GA13	4-OCH_3	7.34-8.01	9.34	14.75
GA14	2-OH	7.4-8.1	10.2	14.27
GA15	3-OH	7.1-8.1	10.35	14.25
GA16	4-OH	7.4-7.8	10.2	14.27
GA17	4-F	6.90-7.87	9.88	14.76
GA18	4-Br	7.3-8.05	9.72	14.27
GA19	4-CN	7.34-7.91	9.16	14.09
GA20	4-$N(CH_3)_2$	7.34-8.11	9.95	14.32

showed multiplets ranging from δ 6.90 to 8.64, besides the usual peaks of some aryl substituents, δ 2.4 (s, CH_3); δ 3.35 (s, OCH_3); δ 3.9 (s, OH) and δ 3.24 (s, $N(CH3)_2$).

The ^{1}H NMR spectral data of 4-Arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles was given in **Table 5**.

^{13}C NMR

^{13}C NMR spectral data of 4-benzylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazole showed peaks at δ 122.7, 126.4, 128.7, 129.2, 129.9, 132.7 (aromatic carbons); 161 (N=CH); 177.8 (triazole carbons).

4.2 Fungitoxicity study

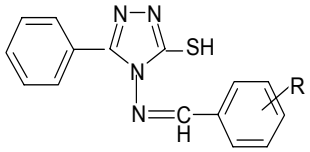
The fungitoxicity evaluation of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles was carried out against three phytopathogenic fungi viz. *Rhizoctonia solani*, *Fusarium oxysporum* and *Bipolaris sorokiniana* by means of poisoned food technique using standard potato-dextrose-agar (PDA) media. The ED_{50} (effective dose for 50% inhibition for fungus growth) values expressed in ppm were determined from the growth inhibition data and are given in **Table 6** for *R. solani,* **Table 7** for *F. oxysporum* and **Table 8** for *B. sorokiniana.*

4.2.1 Fungicidal activity against *Rhizoctonia solani*

The fungitoxicity data of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani* in **Table 6** show that the fungitoxicity of the compounds in these series is not dependent on the electronic nature of the substituents in the benzylidene ring. The position dependent effect of the substituents is also not evident in determining the fungitoxicity against *R. solani*. Out of the twenty compounds seven compounds [GA1,

78.08; GA9, 17.34; GA12, 92.42; GA15, 65.30; GA16, 84.26; GA19, 67.73] showed ED_{50} less than 100 ppm. (**Figure 1**) Among all the compounds 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole exhibited the highest activity (ED_{50}= 17.34 ppm) against *R. solani.*

Table 6: Fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*



Compd No	R	Conc. (ppm)	%Ic	ED_{50} (ppm)	pED_{50} (M)
GA1	H	200	75.45		
		100	53.86	78.08	3.55
		50	40.34		
GA2	2-Cl	500	70.88		
		250	60.50	220.05	3.16
		125	31.50		
GA3	3-Cl	500	74.63		
		250	48.13	240.29	3.12
		125	31.88		
GA4	4-Cl	200	57.50		
		100	28.38	173.32	3.26
		50	15.00		
GA5	2-NO_2	250	58.50		
		125	50.63	165.98	3.29
		62.5	20.00		
GA6	3-NO_2	500	81.75		
		250	55.63	205.15	3.20
		125	33.75		
GA7	4-NO_2	500	77.50		
		250	49.63	224.52	3.16
		125	33.75		
GA8	2-CH_3	250	60.93		
		125	43.62	168.35	3.24
		62.5	25.28		
GA9	3-CH_3	100	80.30		
		50	75.85	17.34	4.23
		25	55.01		
GA10	4-CH_3	520	53.79		
		125	35.86	235.82	3.10
		62.5	29.20		

GA11	2-OCH_3	250 125 62.5	52.53 27.59 21.49	261.65	3.07
GA12	3-OCH_3	250 125 62.5	70.00 61.38 39.89	92.42	3.53
GA13	4-OCH_3	250 125 62.5	60.92 46.78 23.22	164.29	3.28
GA14	2-OH	250 125 62.5	65.96 59.82 44.53	83.96	3.55
GA15	3-OH	250 125 62.5	66.18 59.82 49.89	65.30	3.66
GA16	4-OH	250 125 62.5	65.07 57.59 45.54	84.26	3.55
GA17	4-F	250 125 62.5	69.53 44.64 33.04	130.99	3.36
GA18	4-Br	250 125 62.5	67.52 44.53 35.71	131.92	3.43
GA19	4-CN	250 125 62.5	75.22 61.38 49.22	67.73	3.65
GA20	4-$N(CH_3)_2$	250 125 62.5	53.01 29.35 28.46	263.34	3.09
4-Amino-3-mercapto-5-phenyl-1,2,4-triazole				76	
*Carbendazim				0.65	

% Ic= Corrected % Inhibition * Reference standard

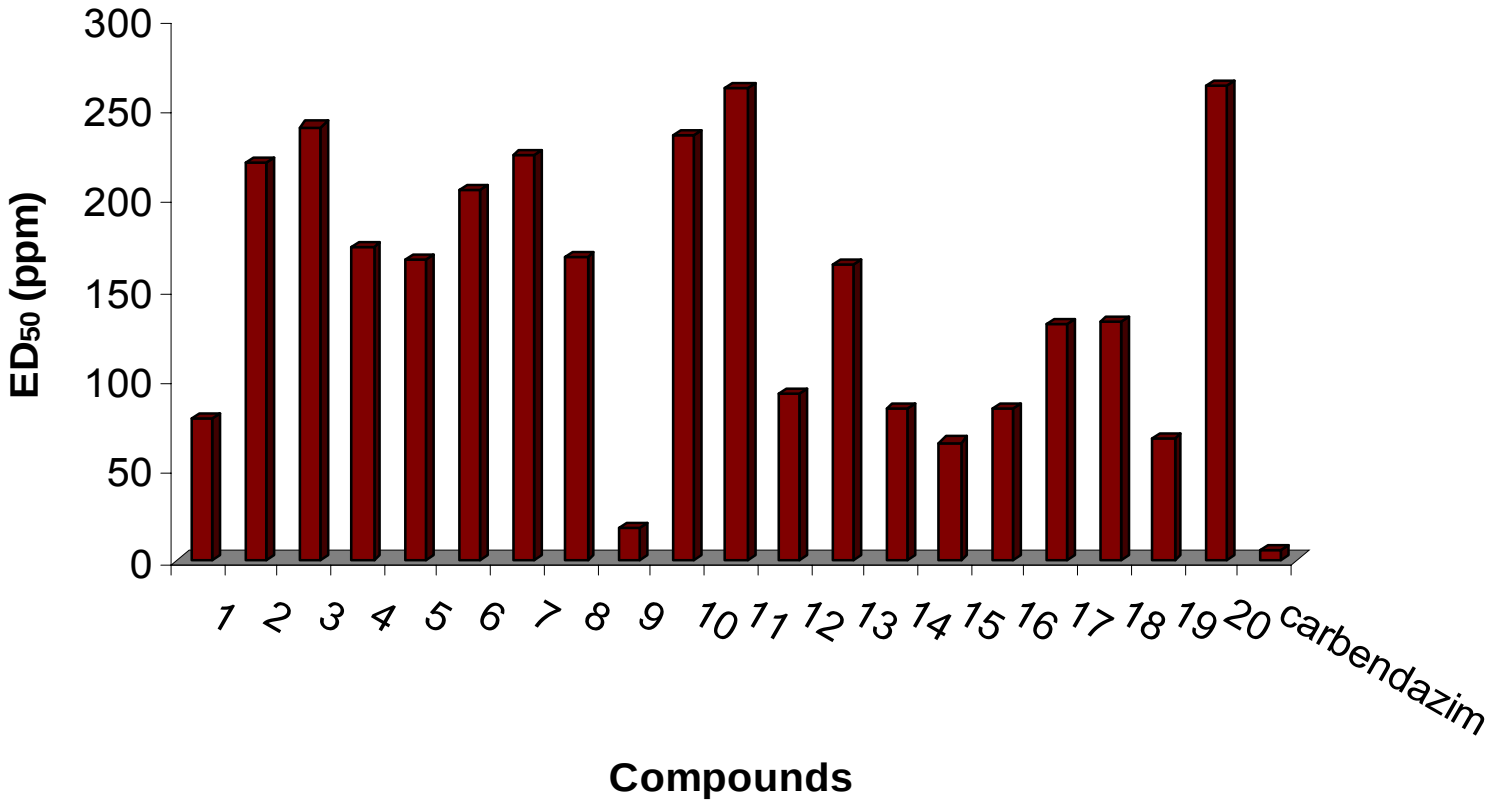


Figure 1: Fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*

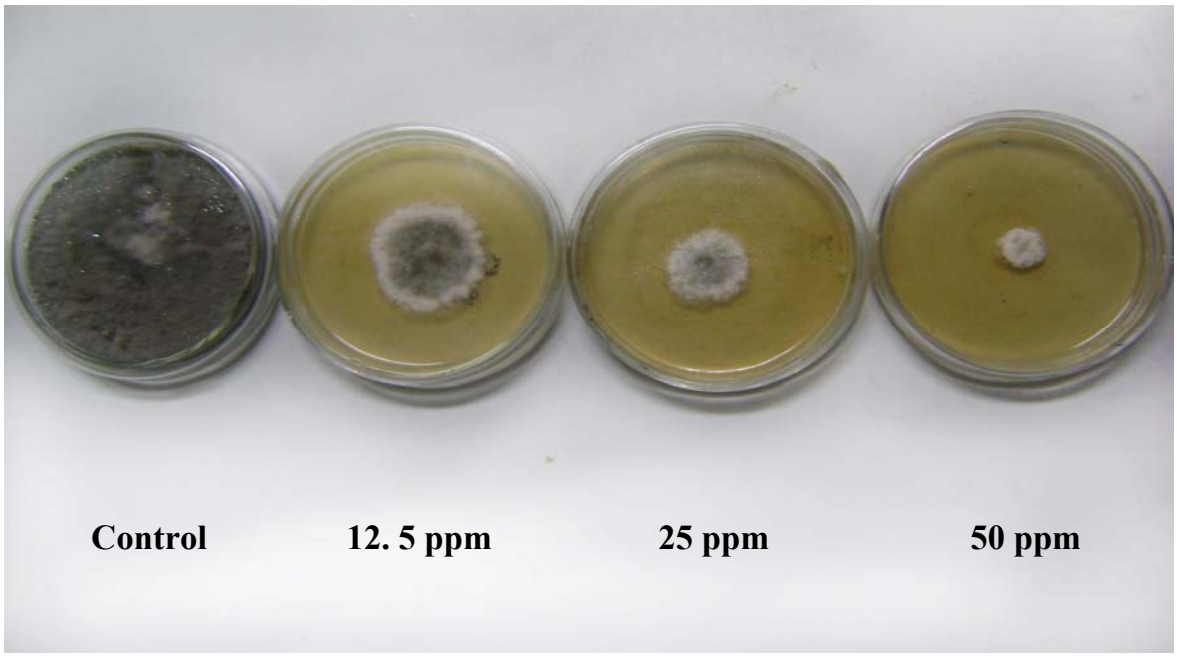


Plate 2: Fungicidal activity of 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA9] against *R. solani*

4.2.2 Fungicidal activity against *Fusarium oxysporum*

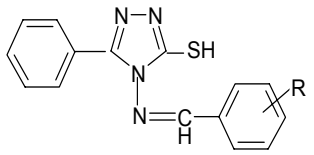
The fungicidal activity data of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *F. oxysporum* in **Table 7** show that the fungitoxicity of the compounds in this series is dependent on the electronic nature of the substituents present in the benzylidene ring. The simple phenyl derivative (GA1) exhibited a moderate fungicidal activity against *F. oxysporum* (ED_{50}=197.29 ppm). The compounds with electron withdrawing substituents, Cl, NO_2, F, Br exhibited higher activity [ED_{50} (ppm) =95.55 to 192.09] than the electron releasing substituents CH_3, OCH_3, OH, $N(CH_3)_2$ [ED_{50} >200 ppm, except for 2-OH (GA14)] except the compound with CN (GA19) substituent (ED_{50} =252.33 ppm).

Among all 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA4) exhibited the highest activity (ED_{50} =95.55 ppm) against *F. oxysporum*, only four [GA2, 138.18; GA4, 95.55; GA17, 127.73 and GA18, 136.35] out of twenty showed ED_{50} less than 150 ppm and only one [GA4, 95.55] out of twenty ED_{50} less than 100 ppm (**Figure 2**).

4.2.3 Fungicidal activity against *Bipolaris sorokiniana*

Fungitoxicity data of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *B. sorokiniana* in **Table 8** show no apparent relations of fungicidal activity with the electronic nature of the benzylidene substituents. The compounds in general showed moderate activity against *B. sorokiniana* [ED_{50} (ppm) 181.31 to 414.70]. Among all 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA3) exhibited the highest activity [ED_{50}=181.3 ppm] against *B. sorokiniana***.** Out of twenty compounds only two exhibited ED_{50} less than 200 ppm [GA3=181.3 and GA5= 197.30] as shown in **Figure 3**.

Table 7: Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *F. oxysporum*



Compd No	R	Conc. (ppm)	%Ic	ED_{50} (ppm)	pED_{50} (M)
GA1	H	500	68.68		
		250	50.43	197.29	3.15
		125	44.25		
GA2	2-Cl	250	59.34		
		125	51.44	138.18	3.36
		62.5	35.63		
GA3	3-Cl	250	59.05		
		125	49.28	156.68	3.30
		62.5	27.87		
GA4	4-Cl	250	62.93		
		125	56.18	95.55	3.52
		62.5	43.53		
GA5	2-NO_2	250	55.17		
		125	48.62	152.5	3.33
		62.5	41.95		
GA6	3-NO_2	500	64.14		
		250	50.57	192.09	3.23
		125	45.75		
GA7	4-NO_2	250	54.60		
		125	49.31	154.74	3.32
		62.5	41.15		
GA8	2-CH_3	250	53.70		
		125	38.90	224.93	3.12
		62.5	31.39		
GA9	3-CH_3	250	52.69		
		125	42.49	221.02	3.12
		62.5	35.54		
GA10	4-CH_3	250	53.25		
		125	40.02	218.73	3.13
		62.5	34.19		

GA11	2-OCH_3	250 125 62.5	53.70 37.78 31.05	229.79	3.13
GA12	3-OCH_3	250 125 62.5	53.48 39.46 34.75	223.98	3.14
GA13	4-OCH_3	250 125 62.5	53.81 39.13 34.42	223.98	3.14
GA14	2-OH	250 125 62.5	64.59 35.27 24.22	160.70	3.27
GA15	3-OH	250 125 62.5	55.10 38.67 20.68	203.63	3.16
GA16	4-OH	250 125 62.5	52.12 37.68 21.81	224.82	3.12
GA17	4-F	250 125 62.5	61.76 51.84 37.25	127.73	3.37
GA18	4-Br	250 125 62.5	77.34 39.80 23.23	136.35	3.42
GA19	4-CN	500 250 125	73.88 43.61 36.32	252.33	3.08
GA20	4-$N(CH_3)_2$	500 250 125	82.86 48.73 29.46	226.59	3.15
4-amino-3-mercapto-5-phenyl -1,2,4-triazole				57	

% Ic= Corrected % Inhibition

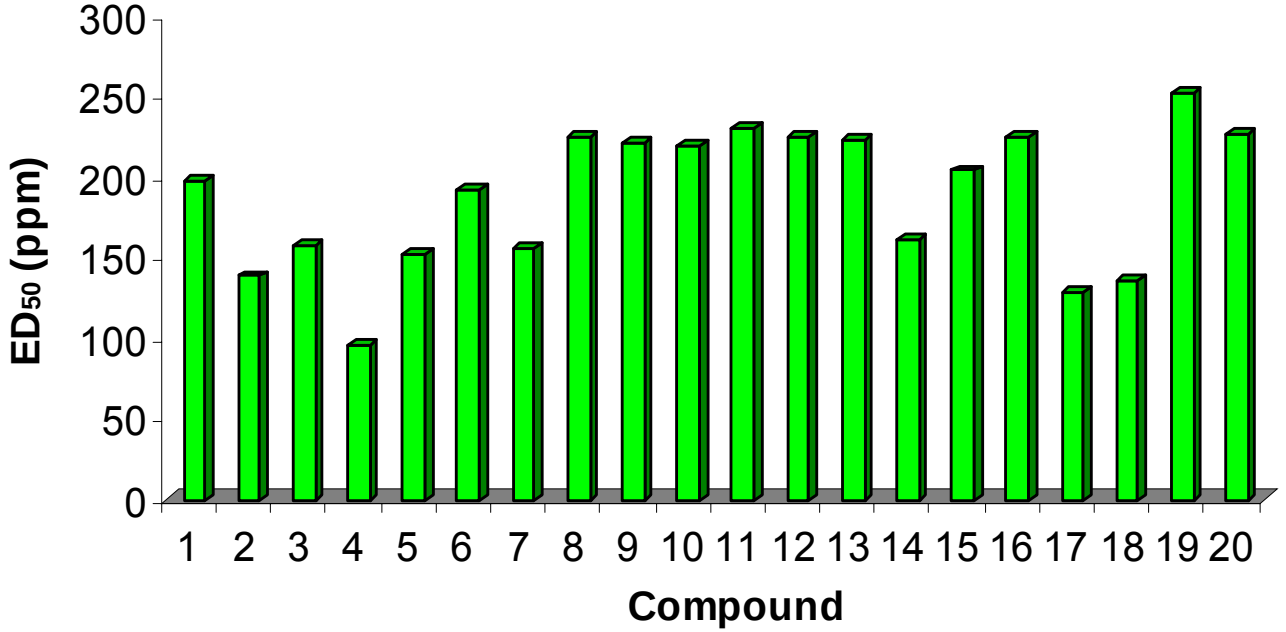
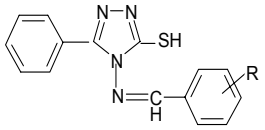


Figure 2: Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *Fusarium oxysporum*

Table 8: Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *B. sorokiniana*



Compd no	R	Conc. (ppm)	%Ic	ED_{50} (ppm)	pED_{50} (M)
GA1	H	250 125 62.5	58.77 27.76 5.84	209.10	3.13
GA2	2-Cl	250 125 62.5	58.28 15.58 4.22	230.51	3.13
GA3	3-Cl	250 125 62.5	61.04 43.02 6.17	180.31	3.24
GA4	4-Cl	250 125 62.5	54.87 18.18 1.62	227.31	3.14
GA5	2-NO_2	250 125 62.5	56.49 39.12 4.71	197.30	3.22
GA6	3-NO_2	250 125 62.5	56.01 26.46 5.84	216.66	3.18
GA7	4-NO_2	250 125 62.5	55.52 37.66 195	201.46	3.21
GA8	2-CH_3	500 250 125	65.68 29.32 18.78	367.42	2.90
GA9	3-CH_3	500 250 125	71.35 30.54 14.46	336.60	2.94
GA10	4-CH_3	500 250 125	66.89 22.97 12.16	390.89	2.88

GA11	2-OCH_3	500 250 125	71.35 40.81 18.24	303.41	3.01
GA12	3-OCH_3	500 250 125	68.24 35.41 14.73	336.78	2.96
GA13	4-OCH_3	500 250 125	63.51 21.89 12.03	414.70	2.87
GA14	2-OH	500 250 125	66.62 32.16 8.38	362.44	2.91
GA15	3-OH	500 250 125	71.89 32.02 11.08	336.21	2.94
GA16	4-OH	500 250 125	72.84 33.51 12.43	327.93	2.96
GA17	4-F	500 250 125	69.59 42.84 18.51	306.44	2.99
GA18	4-Br	500 250 125	66.62 32.16 6.48	364.95	2.99
GA19	4-CN	250 125 62.5	50.77 34.62 17.95	242.25	3.10
GA20	4-$N(CH_3)_2$	500 250 125	58.97 34.10 16.15	398.23	2.91
4-amino-3-mercapto-5-phenyl -1,2,4-triazole					

% Ic= Corrected % Inhibition

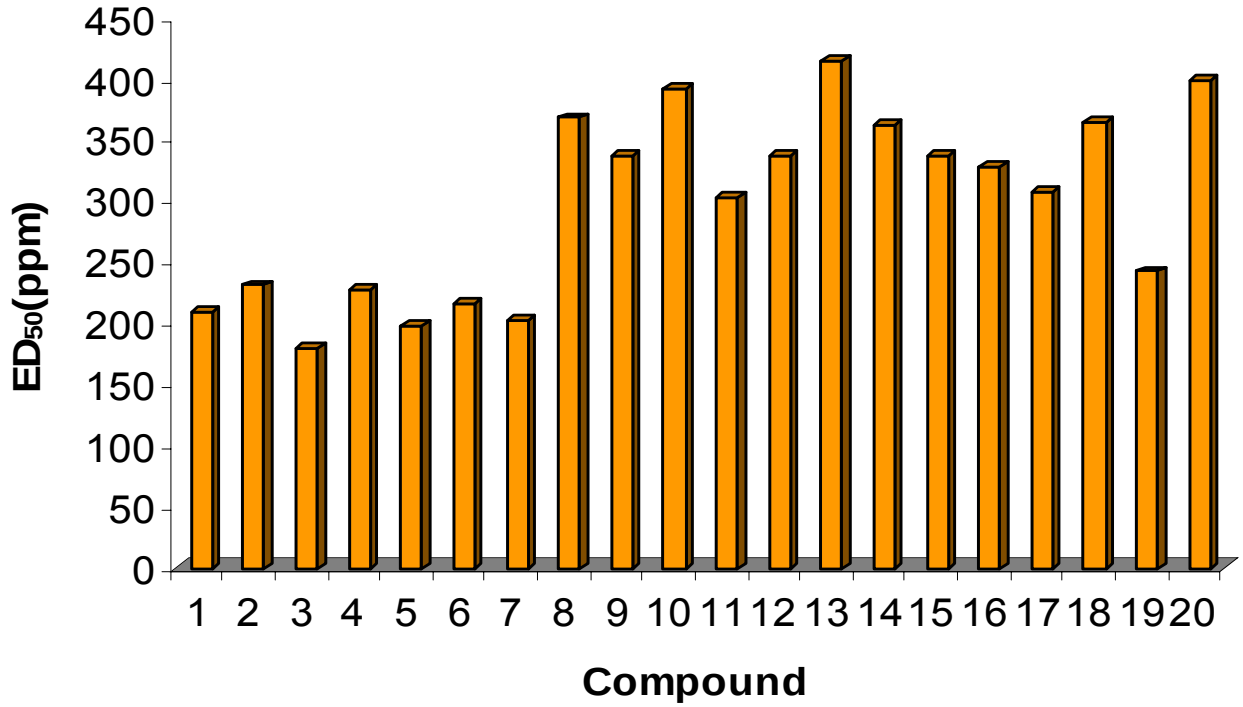


Figure 3: Fungicidal Activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *Bipolaris sorokiniana*

4.2.4 Comparison of fungicidal activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Bipolaris sorokiniana*

The fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*, *F. oxysporum* and *B. sorokiniana* is compared in **Figure 4**.

The compounds in general showed least activity against *B. sorokiniana* whereas some compounds showed higher activity against *R. solani* than *F. oxysporum* and vice versa. The fungitoxicity data against the three fungi in **Table 6, 7 & 8** showed that the nature of the substituent present in the benzylidene ring in different compounds is differently influencing the fungicidal activity against the three fungi viz. *R. solani*, *F. oxysporum* and *B. sorokiniana*.

4.2.5 Comparison of fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles with the fungitoxicity of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole and carbendazim, a reference standard fungicide

A comparison of the fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*, *F. oxysporum* and *B. sorokiniana* with respect to the parent compound, 4-amino-3-mercapto-5-phenyl -1,2,4-triazole is shown in Figure 5. The most active compound in this series against *R. solani* i.e. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA9) exhibited higher activity (ED_{50}=17.34 ppm) than 4-amino-5-phenyl-3-mercapto-1,2,4-triazole (ED_{50}=76 ppm), whereas the most active compound against *F. oxysporum*, 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA4) [ED_{50}=95.55 ppm] and 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA3) against *B. sorokiniana* (ED_{50}=181.31 ppm) exhibited less activity than the 4-amino-3-mercapto-5-phenyl-1,2,4-triazole (ED_{50}=57, 29 ppm respectively).

The activity of the most active compounds in these series i.e. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole (GA9) was also compared with carbendazim, a standard fungicide and its activity (ED_{50}=17.34 ppm, *R. solani*) was found less than carbendazim (ED_{50}=0.65 ppm, *R. solani*).

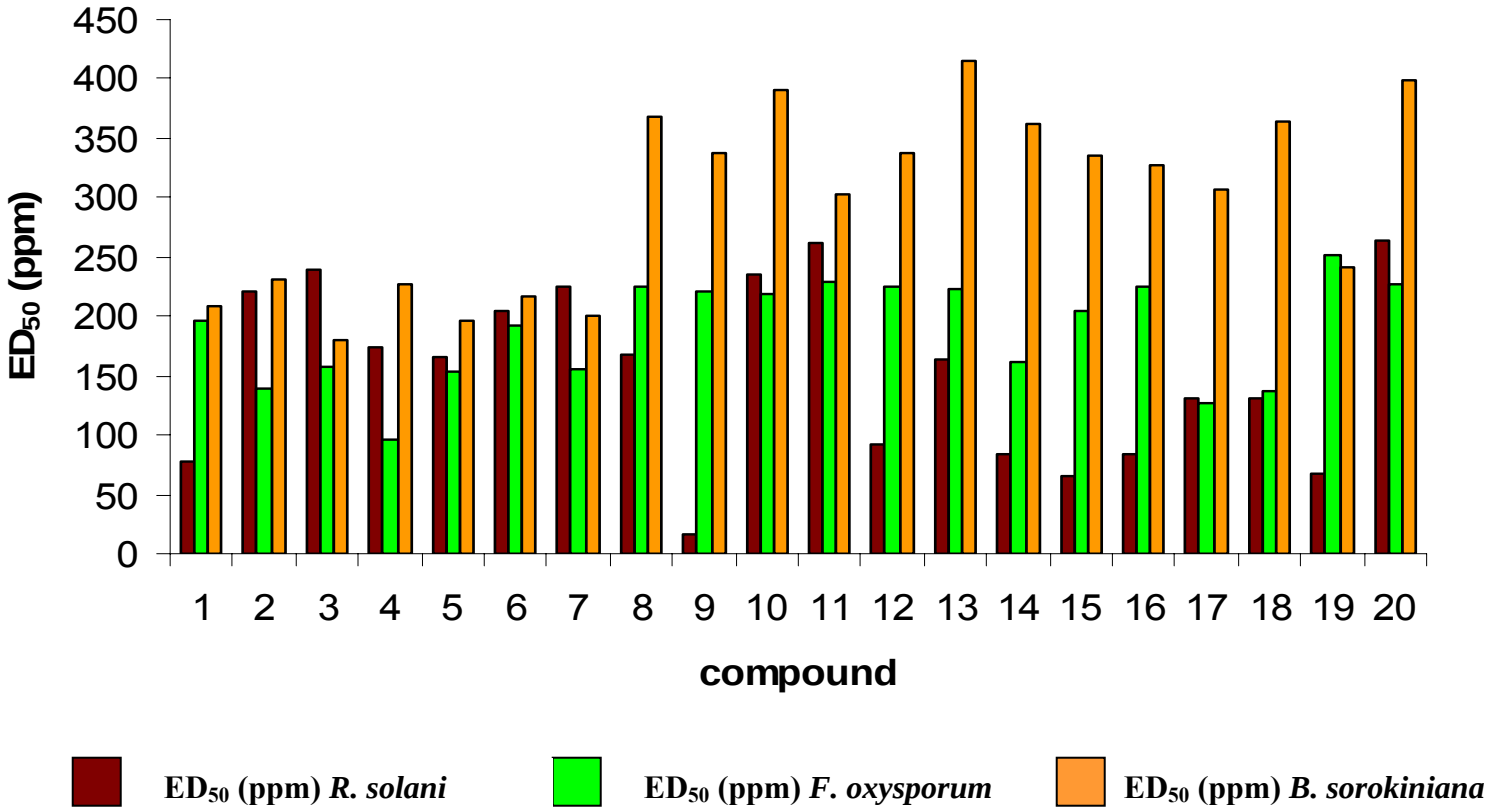
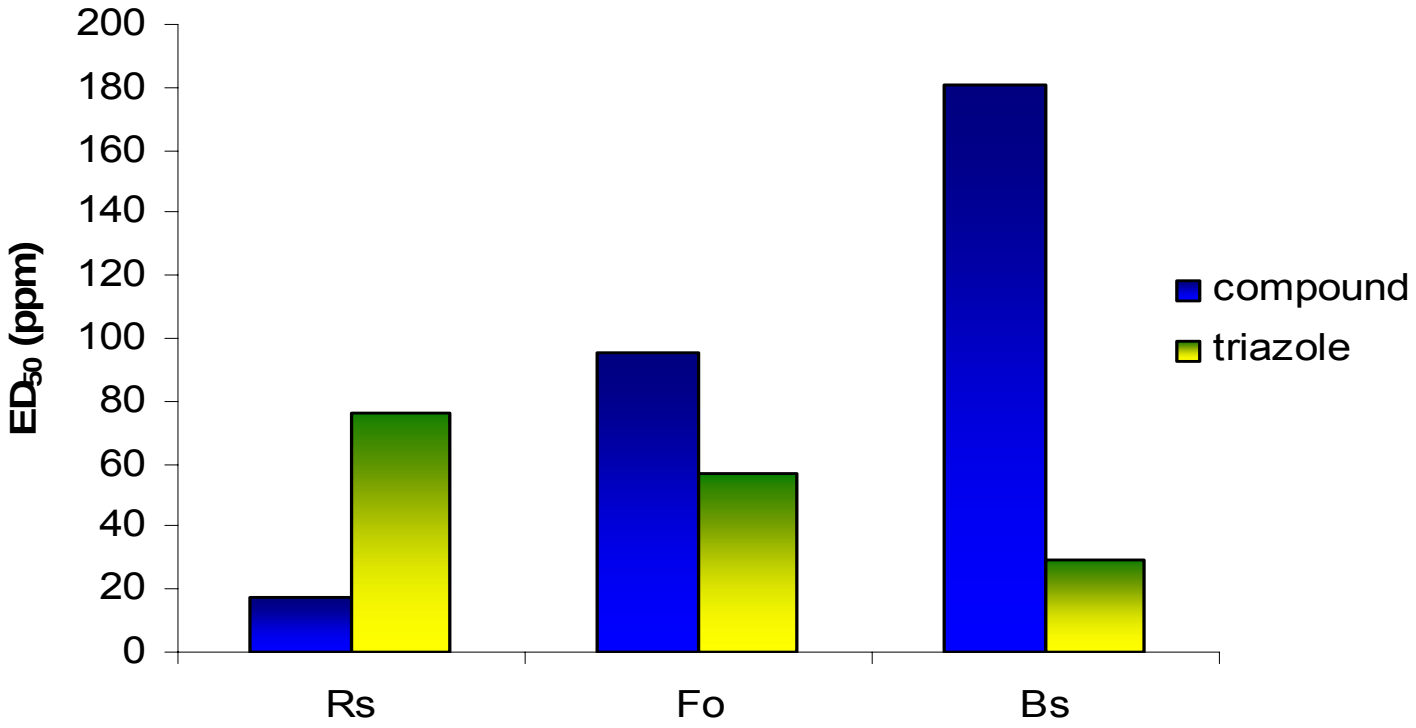


Figure 4: Comparison of fungitoxicity of the 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles



Rs = *R. solani*; Fo = F. *oxysporum*; Bs = *B. sorokiniana*

Figure 5: Comparison of fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles with the fungitoxicity of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole

4.3 Quantative Structure Activity Relationship (QSAR) study

In order to examine the effect on fungitoxicity of the substituents in the benzylidene ring precisely, quantative structure activity relationship were analysed by means of multiple regression analysis using measured pED_{50} (M) [negative logarithm of molar ED_{50}] values of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*, *F. oxysporum* and *B. sorokiniana* (**Table 6, 7 & 8**) as dependent variable and various physico-chemical substituent parameters for hydrophobic, electronic and steric properties of each member of the series as independent variables.

Several regression equations were obtained, significance of which was judged by F and students't' test. Few relevant equations was selected and discussed here [Eq. (1) to Eq. (6)]. A correlation matrix for the parameters used in these regression equation isgiven in **Table 12**. Activity of the compounds have also been predicted on the basis of statistically best fit equations (**Table 9** to **Table 11**).

In the regression equations, n is the number of compounds included in the correlation, s is the standard error of estimate, r is the correlation coefficient and $F_{V1, V2}$ is the F ratio of the correlation, where v_1= m and v_2= n-m-1; m is the number of independent variables used in the correlation. The figures in the parenthesis are the 95% confidence interval for the respective constants. The corresponding tabulated F values at 99% level are also given in the parenthesis along with the F values.

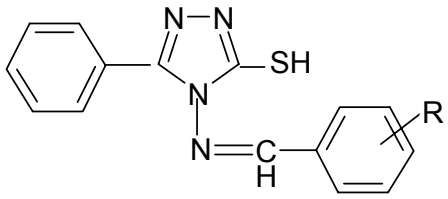
4.3.1 Regression equations for fungitoxicity against *Rhizoctonia solani*

Following regression equations were obtained for the fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*.

$$pED_{50}\ (M) = 3.312 - 0.215\pi \qquad \ldots\ldots (1)$$
$$(\pm\ 0.165)$$
$$n = 16 \quad s = 0.168 \quad r = 0.598 \quad r^2 = 0.358 \quad F_{1,14} = 7.812\ (\ 4.60^*, 8.86)$$

* F value at 95% level

Table 9: Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Rhizoctonia solani*



Compd No	Substituent (R)	Parameters		pED_{50} (M)	
		π	π^2	Observed	Calculated
GA1	H	0.00	0.00	3.55	-
GA2	2-Cl	0.71	0.504	3.16	3.231
GA3	3-Cl	0.71	0.504	3.12	3.231
GA4	4-Cl	0.71	0.504	3.26	3.231
GA5	2-NO_2	-0.28	0.078	3.29	3.258
GA6	3-NO_2	-0.28	0.078	3.20	3.258
GA7	4-NO_2	-0.28	0.078	3.16	3.258
GA8	2-CH_3	0.56	0.314	3.24	3.154
GA9	3-CH_3	0.56	0.314	4.23	-
GA10	4-CH_3	0.56	0.314	3.10	3.154
GA11	2-OCH_3	-0.02	0.0004	3.07	3.129
GA12	3-OCH_3	-0.02	0.0004	3.53	-
GA13	4-OCH_3	-0.02	0.0004	3.28	3.129
GA14	2-OH	-0.67	0.449	3.55	3.616
GA15	3-OH	-0.67	0.449	3.66	3.616
GA16	4-OH	-0.67	0.449	3.55	3.616
GA17	4-F	0.14	0.020	3.36	-
GA18	4-Br	0.86	0.740	3.43	3.327
GA19	4-CN	-0.57	0.325	3.65	3.506
GA20	4-$N(CH_3)_2$	0.18	0.032	3.09	3.088

pED_{50} (M) = 3.122 - 0.305π + 0.642 π^2 ……. (2)
(± 0.097) (± 0.236)

n = 16 s = 0.092 r = 0.908 r^2 = 0.824 $F_{1,13}$ = 30.407 (6.70)*

Regression Eq. (1) with only π term is significant at 95% level and explains 35.8 % (r =0.598) variation in fungicidal activity against *R. solani*. Further addition of a π^2 term in Eq. (1) resulted in Eq. (2) with a considerably improvement in correlation (r = 0.908). The π and π^2 together account for 82.4% variation in the fungitoxicity against *R. solani*. The π and π^2 terms in Eq. (2) are justified at 99% level. Further addition of any other term from Eq. (2) did not improve the correlation. Regression Eq. (2) is thus statistically best fit equation for the fungicidal activity of this series of compounds against *R. solani*. The values of the parameters, π and π^2 and observed pED_{50}(M) and also the predicted pED_{50} (M) obtained by Eq. (2) are given in **Table 9**. The observed pED_{50}(M)and the predicted pED_{50} (M) values of the compounds are also compared in **Figure 6**. Since π and π^2 terms together are present in the statistically best fit Eq. (2), with a negative sign with π and a positive sign with π^2, the fungicidal activity against *R. solani* showed an inverse parabolic relationship with hydrophobicity of the compound with a minima at π = 0.236. This indicates that farther the value of π from 0.236, greater will be the fungicidal activity of the compounds in these series against *R. solani*.

4.3.2 Regression equations for fungitoxicity against *Fusarium oxysporum*

Following regression equations were obtained for the fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *F. oxysporum.*

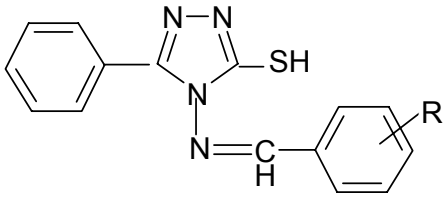
pED_{50} (M) = 3.193 + 0.126σ …… (3)
(± 0.084)

n = 16 s =0.069 r = 0.654 r^2 = 0.428 $F_{1,14}$ = 10.469 (8.86)

pED_{50} (M) = 3.175 + 0.102σ + 0.642 F ……. (4)
(± 0.073) (± 0.166)

n = 16 s = 0.058 r = 0.788 r^2 = 0.621 $F_{1,13}$ = 10.629 (6.70)

Table 10: Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Fusarium oxysporum*



Compd No	Substituent (R)	Parameters		pED_{50} (M)	
		σ	F	Observed	Calculated
GA1	H	0.00	0.00	3.15	3.175
GA2	2-Cl	0.23	0.41	3.36	3.279
GA3	3-Cl	0.37	0.00	3.30	3.212
GA4	4-Cl	0.23	0.00	3.52	-
GA5	2-NO_2	0.78	0.67	3.33	3.387
GA6	3-NO_2	0.71	0.00	3.23	3.247
GA7	4-NO_2	0.78	0.00	3.32	3.254
GA8	2-CH_3	-0.17	-0.04	3.12	3.149
GA9	3-CH_3	-0.07	0.00	3.12	3.168
GA10	4-CH_3	-0.17	0.00	3.13	3.157
GA11	2-OCH_3	-0.27	0.26	3.13	3.199
GA12	3-OCH_3	0.12	0.00	3.14	3.187
GA13	4-OCH_3	-0.27	0.00	3.14	3.147
GA14	2-OH	-0.37	0.29	3.27	3.195
GA15	3-OH	0.12	0.00	3.16	3.187
GA16	4-OH	-0.37	0.00	3.12	3.137
GA17	4-F	0.06	0.00	3.37	-
GA18	4-Br	0.23	0.00	3.42	-
GA19	4-CN	0.66	0.00	3.08	-
GA20	4-$N(CH_3)_2$	-0.83	0.00	3.15	3.090

Regression Eq. (3) with only σ term is significant at 99% level and explained 42.8% (r =0.654) variation in fungicidal activity against *F. oxysporum.* Further introduction of F term in Eq. (3) gave Eq. (4) with an improved correlation (r = 0.788). The σ and F terms together account for 62.1% variation in fungitoxicity against *F. oxysporum.* The σ term in Eq. (4) is justified at 99% level while F term at 95% level. Further addition of any other term to Eq. (4) will not improve the correlation. Eq. (4) is thus statistically best fit equations for the fungicidal activity of the series of compound against *F. oxysporum.* The values of the physico-chemical parameters σ and F and the observed pED_{50} (M) and predicted pED_{50} (M) obtained by Eq. (4) are given in **Table 10**. The observed pED_{50} (M) and predicted pED_{50} (M) values for *F. oxysporum* were also compared in **Figure 7**. The positive sign associated with σ indicate that electron withdrawing substituents in the benzylidene ring will enhanced the fungicidal activity of the compounds of this series against *F. oxysporum.* The presence of F term in the statistically best fit Eq. (4) and the positive sign with it indicate that the electronic effect of the *ortho-* substituent will enhance the fungicidal activity against F. *oxysporum.*

4.3.3 Regression equations for fungitoxicity against *Bipolaris sorokiniana*

Following regression equations were obtained for the fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *B. sorokiniana.*

$$pED_{50}\ (M) = 2.972 + 0.272\ \sigma \qquad \ldots\ldots (5)$$
$$(\pm\ 0.080)$$

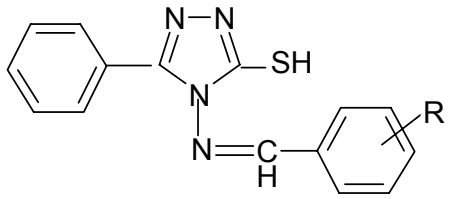
n = 15 s =0.055 r = 0.899 r^2 = 0.808 $F_{1,13}$ = 54.629 (9.07)

$$pED_{50}\ (M) = 3.175 + 0.0.286\sigma + 0.642\ \Sigma\ L(o) \qquad \ldots\ldots. (6)$$
$$(\pm\ 0.050) \quad (\pm\ 0.030)$$

n = 15 s =0.034 r = 0.966 r^2 = 0.933 $F_{2.12}$ = 84.025 (6.93)

Regression Eq. (5) with only σ term gave a very high correlation (r = 0.899), this shows that 80.8% variation in fungicidal activity against *B. sorokiniana* is due to

Table 11: Physicochemical parameters and observed vs predicted fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl- 4H-1,2,4-triazole against *Bipolaris sorokiniana*



Compd No	Substituent (R)	Parameters		pED_{50} (M)	
		σ	ΣL(o)	Observed	Calculated
GA1	H	0.00	4.12	3.13	-
GA2	2-Cl	0.23	5.58	3.13	3.103
GA3	3-Cl	0.37	4.12	3.24	-
GA4	4-Cl	0.23	4.12	3.14	-
GA5	2-NO_2	0.78	5.50	3.22	3.255
GA6	3-NO_2	0.71	4.12	3.18	3.146
GA7	4-NO_2	0.78	4.12	3.21	3.166
GA8	2-CH_3	-0.17	5.06	2.90	2.955
GA9	3-CH_3	-0.07	4.12	2.94	2.923
GA10	4-CH_3	-0.17	4.12	2.88	2.894
GA11	2-OCH_3	-0.27	6.04	3.01	2.989
GA12	3-OCH_3	0.12	4.12	2.96	2.977
GA13	4-OCH_3	-0.27	4.12	2.87	2.865
GA14	2-OH	-0.37	4.80	2.91	2.881
GA15	3-OH	0.12	4.12	2.94	2.977
GA16	4-OH	-0.37	4.12	2.96	-
GA17	4-F	0.06	4.12	2.99	2.960
GA18	4-Br	0.23	4.12	2.99	3.009
GA19	4-CN	0.66	4.12	3.10	3.132
GA20	4-$N(CH_3)_2$	-0.83	4.12	2.91	-

Table 12: Correlation matrix for the parameters used in the regression equation

	π	σ	F	$\Sigma L(o)$	π^2
π	1.000				
σ	-0.073	1.000			
F	-0.132	0.203	1.000		
$\Sigma L(o)$	0.034	-0.016	0.787	1.000	
π^2	0.312	0.049	-0.041	-0.075	1.000

Figure 6: Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Rhizoctonia solani*

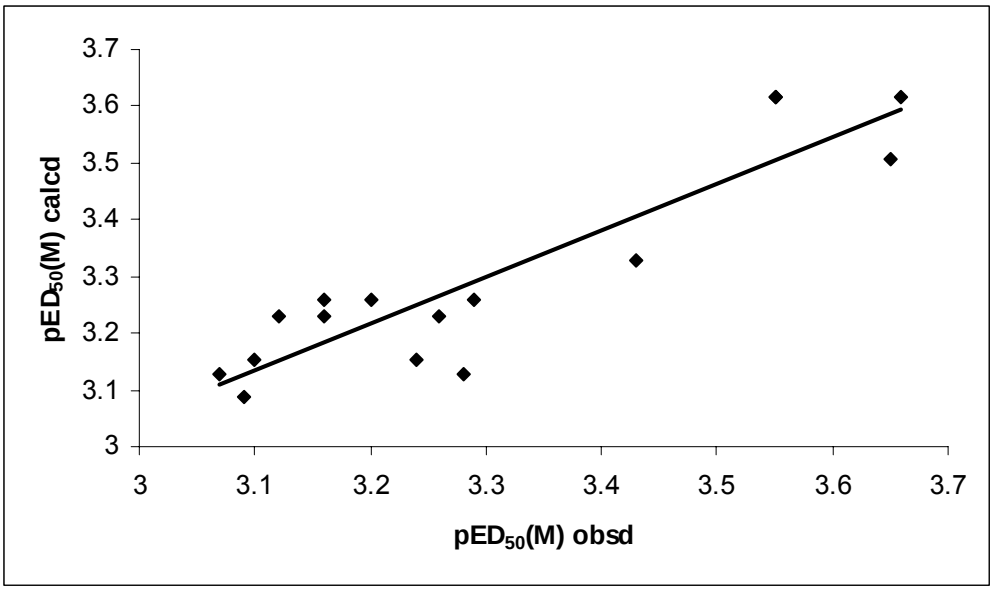


Figure 7: Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Fusarium oxysporum*

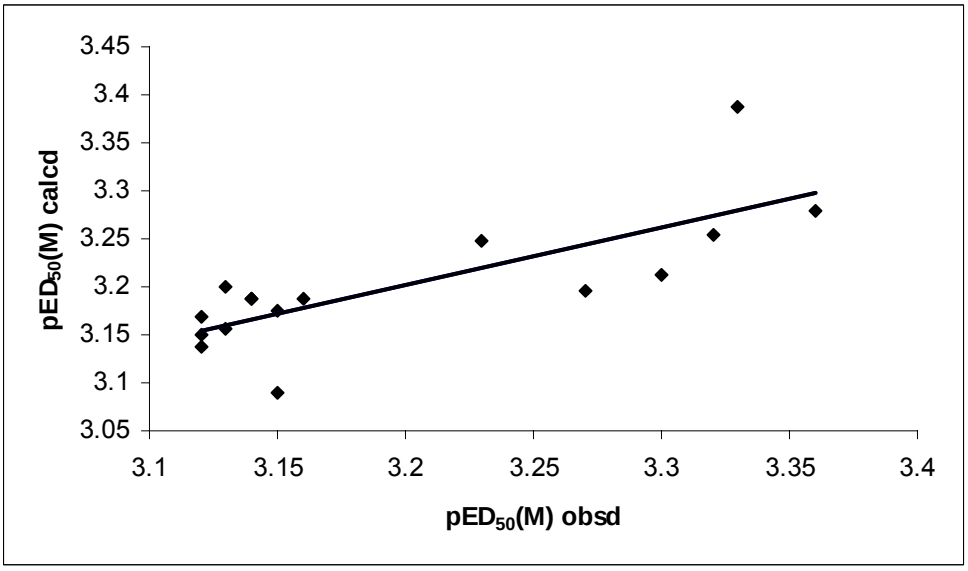
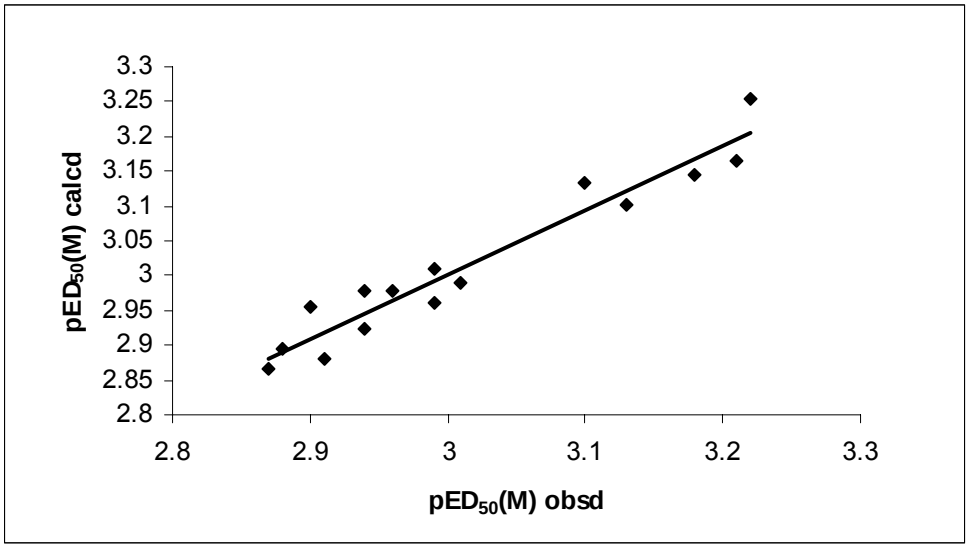


Figure 8: Observed vs Calculated fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles against *Bipolaris sorokiniana*



electronic effect (σ) of the benzylidene ring of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles. The regression equation is highly significant at 99% level. Further introduction of a ΣL(o) term in Eq. (5) gave Eq. (6) with an improved correlation (r = 0.966). The σ and ΣL(o) together account for 93.3% variation in fungicidal activity against *B. sorokiniana.* Both the terms σ and ΣL(o) in Eq. (6) are significant at 99% level. Further addition of any other term into Eq. (6) did not improve the correlation. The Eq. (6) is thus statistically best fit for the fungicidal activity against *B. sorokiniana.* The values of the physico-chemical parameters σ and ΣL(o) and observed pED_{50} (M) and predicted pED_{50} (M) obtained by Eq. (6) given in **Table 11**. The observed pED_{50} (M) and predicted pED_{50} (M) values for *B. sorokiniana* were also compared in **Figure 8**. The positive sign with σ term indicate that the electron withdrawing substituents in the benzylidene ring will enhance the fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *B. sorokiniana.* The positive sign with ΣL(o) indicate that high value of the STERIMOL length parameter of the *ortho*- substituents are favourable for the fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *B. sorokiniana.*

The quantitative structure activity relationship study has clearly revealed the structural requirement for the fungitoxicity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against the three fungi viz. *R. solani*, *F. oxysporum* and *B. sorokiniana.* It is precisely clear from the regression equations that the fungitoxicity against *R. solani* is determined by the hydrophobicity of the substituents in the benzylidene ring, whereas the fungitoxicity against *F. oxysporum* and *B. sorokiniana* is dependent on the electronic nature of the substituent present in the benzylidene ring of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles. The fungitoxicity of the compounds against *B. sorokiniana* is also found dependent upon the steric parameter, STERIMOL parameter for length of *ortho*-substituent [ΣL(o)] and that against *F. oxysporum* the electronic effect of ortho-substituents as expressed by F, Swain–Lupton constant for 'proximity polar effect'. The high values of ΣL(o) and F are favourable for the respective fungicidal activity.

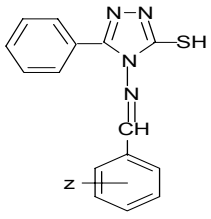
5. SUMMERY AND CONCLUSION

The pesticides of synthetic type suffer from high rate of obsolescence due to resistance, change in pest problems, various environment consideration and also competition from new introductions. Therefore, newer pesticides with greater potency and increased safety from environmental point of view must be developed to replace the older ones. The present investigation was undertaken with the following objectives.

1. Synthesis of a series of 4-Arylidenamino-3-mercapto-5-phenyl-4H-1,2,4-triazoles.
2. Study their fungicidal activity against some phytopathogenic fungi.
3. Study of Structure Activity Relationship to identify structural features essential for fungitoxicity.

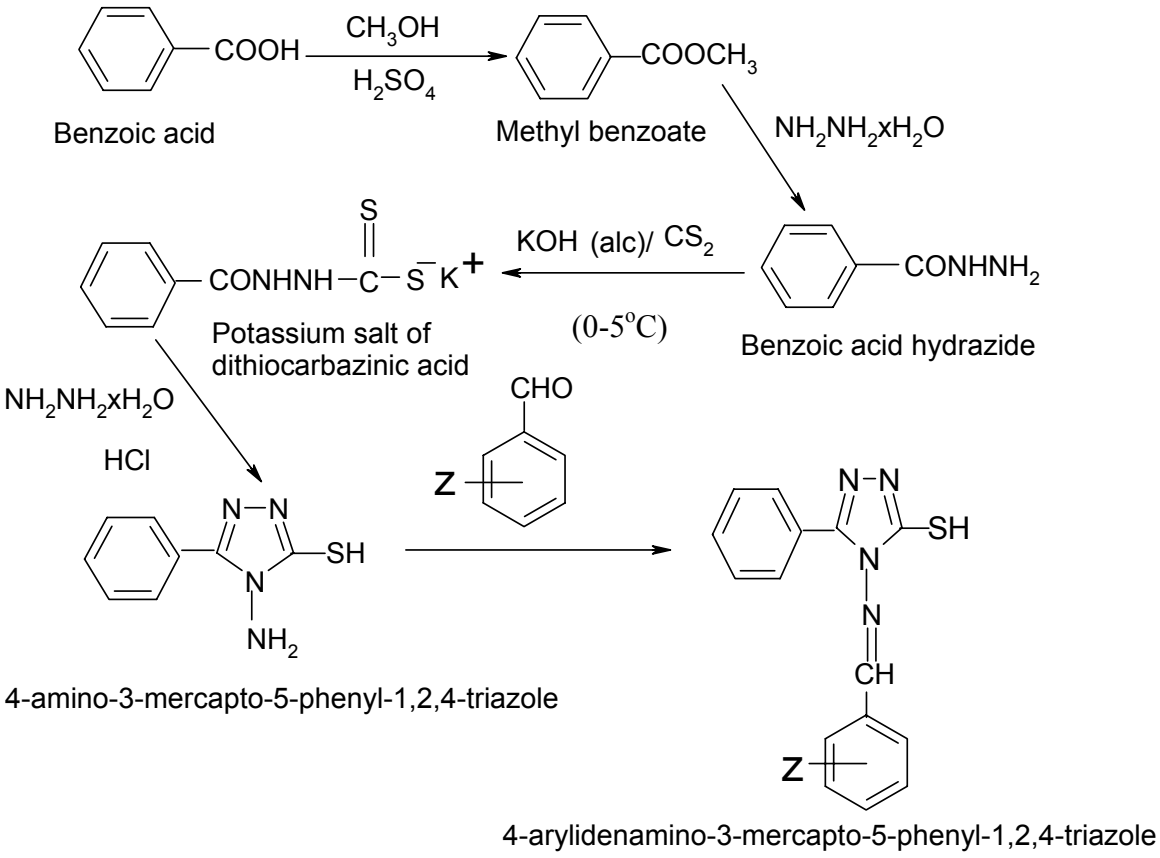
Schiff bases of various heterocyclic moieties have found use as drugs, and have also been reported as pesticides. Review of literature on biological activity of Schiff bases have revealed that Schiff bases of 4-amino-1,2,4-triazoles viz. 4-amino-3-aryl-5-phenyl-4H-1,2,4-triazole; 4-amino-3,5-dialkyl-1,2,4-triazole; 4-amino-3-(4-tolyl)-5-phenyl-4H-1,2,4-triazole and 4-amino3-mercapto-5-substituted-1,2,4-triazole have already been synthesized and their antifungal, antibacterial, PGR activities have been reported [Bekirkan and Bektas, 2006; Serdar *et al*., 2007; Gumrukcuoglu *et al*., 2007].

In continuation to an earlier investigation on 4-amino-5-aryl-3-mercapto-1,2,4-triazole (Bijul, 2005) for finding potential fungicides, a series of twenty Schiff bases of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole having different substitution in the aryl ring attached to the imine goup represented by the general structure (I) were designed and synthesized.



(I)

The synthesis of Schiff bases (Scheme I) has involved five steps starting with benzoic acid which was first converted to ⟶ methyl ester ⟶ hydrazide ⟶ dithocarbazinic salt ⟶ triazole and finally to ⟶ Schiff base.



Scheme I

The methyl ester of benzoic acid i.e. methyl benzoate was prepared by refluxing benzoic acid with methanol in the presence of concentrated sulphuric acid. Benzoic acid hydrazide was prepared by two methods. Method-A involved refluxing of methyl benzoate with hydrazine hydrate in methanol for 4 hrs and Method-B involved the reaction of similar quantities methyl benzoate with hydrazine hydrate in methanol in the microwave irradiation at 900 Watt for 4 minutes. Potassium salt of benzoyl dithiocarbazinic acid was prepared by reacting benzoic acid hydrazide in methanolic potassium hydroxide with carbon disulphide under cold condition (0-5 ^{o}C). The 4-amino-3-mercapto-5-phenyl-1,2,4-triazole was prepared by reacting the potassium salt of benzoyl dithiocarbazinic acid with hydrazine hydrate by microwave irradiation at 900 Watt for 36 seconds. The Schiff bases, 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-

triazoles were prepared following a general procedure that involved refluxing of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole in ethanol with respective aldehydes for 2-4 hrs. A microwave method was also standardized for the preparation of 4-benzylidenamino-3-mercapto-5-phenyl-1,2,4-triazole (GA1) that involved microwave irradiation of 4-amino-3-mercapto-5-phenyl-1,2,4 triazole in ethanol with benzaldehyde at 900 Watt

for 5 minutes. Three of the five steps of this synthesis i.e. methyl ester ⟶ hydrazide; dithiocarbazinic acid salt ⟶ triazole and triazole ⟶ Schiff base were accomplished by using microwaves. The microwave methods have been found much faster and efficient than conventional method as shown below:

Reactions	**Conventional**		**Microwave**	
	Time	**Yield**	**Time**	**Yield**
Methyl benzoate ⟶ Hydrazide of benzoic acid	4-5 hr	87.4%	4 min	92%
Potassium salt of dithiocarbazinic acid ⟶ 4-Amino-3-mercapto-5-phenyl-1,2,4-triazole	4 hr	88.5%	36 sec	95.6%
4-Amino-3-mercapto-5-phenyl-1,2,4-triazole ⟶ 4-Benzylidenamino-3-mercapto-5-phenyl-1,2,4-triazole	2-3 hr	75%	5 min	80%

The structure of the Schiff bases, were confirmed by elemental analysis, for C, H, N and S; IR and NMR spectroscopy. The IR spectra showed characteristic peaks for SH (2434 $cm^{-1)}$; C=N (1517 cm^{-1}); C-H aliphatic (2896 cm^{-1}); C-H aromatic (3051 cm^{-1}) and N-C=S (1476, 1251, 1046, 931 cm^{-1}).

The characteristic feature of the proton magnetic resonance was the presence of a singlet at around δ 9.16 to 10.49 for N=CH and another singlet at around δ 14.09 to 14.75 for SH. The aryl protons showed multiplets ranging from δ 6.90 to 8.64, besides usual peak of some aryl substituents.

The Schiff bases, 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles were evaluated for fungitoxicity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Bipolaris sorokiniana* by the poisoned food technique using potato-dextrose–agar (PDA) media. The ED_{50} (ppm) values for each compound were determined from the the data on inhibition of fungal growth. Among all the compounds in this series, 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA9] (ED_{50} = 17.34 ppm); 4-(4-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA4] (ED_{50}=95.55 ppm) and 4-(3-Chlorobenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA3] (ED_{50}=181.3 ppm) exhibited highest activity against *R. solani*, *F. oxysporum* and *B. sorokiniana* respectively.

The compounds in general showed least activity against *B. sorokiniana,* whereas some compounds showed higher activity against *R. solani* than *F. oxysporum* and vice versa. The fungitoxicity data against the three fungi showed that the nature of the substituent present in the benzylidene ring in different compounds is differently influencing the fungicidal activity against the three fungi viz. *R. solani*, *F. oxysporum* and *B. sorokiniana*.

The most active compound in this series i.e. 4-(3-Methylbenzylidenamino)-3-mercapto-5-phenyl-4H-1,2,4-triazole [GA9] exhibited higher activity (ED_{50} = 17.34 ppm) than the parent triazole, 4-amino-5-phenyl-3-mercapto-1,2,4-triazole (ED_{50}=76 ppm) but was found less active than carbendazim (ED_{50}=0.65 ppm), a standard fungicide against *R. solani*.

Although few important generalization on structure activity relationship have been made based on fungitoxicity data obtained in this investigation to identify exact

structural feature favourable for fungitoxicity, quantitative structure activity relationship (QSAR) have also been analysed by means of multiple regression analysis using pED_{50}(M) [negative logarithm of molar ED_{50}] values of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*, *F. oxysporum* and *B. sorokiniana* as dependent variable and various physico-chemical substituent parameters for hydrophobic, electronic and steric properties of each member of the series as independent variables. The following statistically best fit regression models were obtained.

Rhizoctonia solani

pED_{50} (M) = 3.122 - 0.305π + 0.642 π^2

(±0.097) (± 0.236)

n = 16 s = 0.092 r = 0.908 r^2 = 0.824 $F_{1,13}$ = 30.407 (6.70)

Fusarium oxysporum

pED_{50} (M) = 3.175 + 0.102σ + 0.642 F

(± 0.073) (± 0.166)

n = 16 s = 0.058 r = 0.788 r^2 = 0.621 $F_{1,13}$ = 10.629 (6.70)

Bipolaris sorokiniana

pED_{50} (M) = 3.175 + 0.0.286σ + 0.642 Σ L(o)

(± 0.050) (± 0.030)

n = 15 s =0.034 r = 0.966 r^2 = 0.933 $F_{2.12}$ = 84.025 (6.93)

In these regression equations, n is the number of compounds included in the correlation, s is the standard error of estimate, r is the correlation coefficient and $F_{V1, V2}$ is the F ratio of the correlation, where v_1= m and v_2= n-m-1; m is the number of independent variables used in the correlation. The figures in the parenthesis are the 95% confidence interval for the respective constants. The corresponding tabulated F values at 99% level are also given in the parenthesis along with the F values.

The QSAR models for the fungicidal activity of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against *R. solani*, *F. oxysporum* and *B. sorokiniana* are significant at 99% level and explains 82.4% (r =0.908), 62.1% (r =0.788) and 93.3% (r

=0.966) variation in fungitoxicity respectively. The fungicidal activity of the compounds in this series against *R. solani* show an inverse parabolic relationship with the hydrophobicity of the compounds with a minima at π =0.236, indicates that farther the value of π from 0.236, greater will be fungicidal activity of the compound. The fungitoxicity against *F. oxysporum* and *B. sorokiniana* is dependent on electronic nature of the substituent present in the bezylidene ring of 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles, the electron withdrawing substituents will enhance the fungicidal activity. The fungitoxicity of the compounds against *B. sorokiniana* is also dependent upon the steric parameter, STERIMOL parameter for length of *ortho*-substituent [ΣL(o)] and that against *F. oxysporum* the electronic effect of the *ortho*-substituents as expressed by F, Swain-Lupton constant for 'proximity polar effect', the high value of these parameters favourable for the respective fungicidal activity.

The quantitative structure activity relationship study has thus clearly revealed the structural requirement for the fungitoxicity of the 4-arylidenamino-3-mercapto-5-phenyl-1,2,4-triazoles against the three fungi viz. *R. solani*, *F. oxysporum* and *B. sorokiniana*.

6. BIBLIOGRAPHY

Bekircan, O. and Bektas, H. (2006). Synthesis of new bis-1,2,4-triazole derivatives. *Molecules* **11**: 469-477.

Bijul, L.A. (2005). Synthesis and QSAR aided fungicidal activity optimization of some new 1,2,4-triazole. Ph. D. Thesis. Post Graduate School, IARI. New Delhi.

Cansiz, A., Koparir, M. and Demirdag, A. (2004). Synthesis of some new 4,5-substituted-4H- 1,2,4-triazole-3-thiol derivatives. *Molecules* **9** : 204-212.

Demirbas, N. and Ugurluoglu, R. (2004). Synthesis of novel 4-alkylidene- and 4-alkylamino-5-oxo-4,5-dihydro-[1,2,4]triazole derivatives and investigation of their antitumor activities. *Turk. J. Chem.* **28**: 559-576.

Dhar, D.N. and Taploo, C.L. (1982). Schiff bases and their application. *Journal of Scientific and Industrial Research* **41**: 501-506.

El-masry, A.H., Fahmy, H.H. and Abdelwahed, S.H.A. (2000). Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecules* **5**: 1429-1438.

El-Syed, R. (2006). Synthesis, antibacterial and surface activity of 1,2,4-triazole derivatives. *GRASAS Y ACEITES*. **57** (2): 180-188.

Fujita, T. and Nishioka, T. (1976). The analysis of ortho effect. *Prog. Phys. Org. Chem.* **12**: 49.

Gumrukcuoglu, N., Serdar, M., Celik, E., Sevim, A. and Demirbas, N. (2007). Synthesis and antimicrobial activities of some new 1,2,4-triazole derivatives. *Turk. J. Chem.* **31**: 335-348.

Hansch, C. and Leo, A. (1979). Substituents constants for correlation analysis in chemistry and biology. John Wiley & Sons, New York, USA.

Holla, B.S., Shivananda, M.K., Shenoy, M.S. and Antony, G. (1998) Studies on arylfuran derivatives Part VII. Synthesis and characterization of some Mannich bases carrying halophenylfuryl moieties as promising antibacterial agents. *Farmaco* **53**(8-9): 531-535.

Holla, B.S., Veerendra, B., Shivananda, M.K. and Poojary, B. (2003). Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. *European Journal of Medicinal Chemistry* **38**(7-8): 759-767.

Holla, B.S., Rao, B.S., Sridhar, K. and Akberali, P. M. (2000). Studies on arylfuran derivatives Part XI. Synthesis, characterisation and biological studies on some Mannich bases carrying 2,4-dichlorophenylfurfural moiety. *Farmaco* **55**(5): 338-344.

Hothi, H.S., Debele, D., Sharma, J.R. and Manrao, M.R. (2006). Synthesis and fungitoxicity of Fe(III) complexes of chloro substituted Schiff bases. *Pesticide Research Journal* **18**: 116-118.

Huang, J., Ayad, H. M. and Timmons, P.R. (1994). Pesticidal 1-aryl-5-(substituted alkylideneimino) pyrazoles. US Patent 546/272.

Jarrahpour, A.A., Motamedifar, M., Pakshir, K., Hadi, N. and Zarei, M. (2004). Synthesis of novel azo Schiff bases and their antibacterial and antifungal activities. *Molecules* **9**: 815-824.

Jarrahpour, A., Khalili, D., Cletcq, E.D., Salmi, C. and Brunel, J.M. (2007). Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. *Molecules* **12**: 1720-1730.

Jin, J.Y., Zhang, L.X., Zhang, A.J. Lei, X.X. and Zhu, J.H. (2007). Synthesis and biological activity of some novel derivatives of 4-amino-3-(D-galactopentitol-1-yl)-5-mercapto-1,2,4-triazole. *Molecules* **12**: 1595-1605.

Karthikeyan, M.S., Prasad, D.J., Poojary, B., Bhat, K.S., Holla, B.S. and Kumari, N.S. (2006). Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety. *Bioorganic & Medicinal Chemistry* **14**(22): 7482-7489.

Kidwai, M., and Bhushan, K.R. (1998) A novel synthetic method for fungicidal organomercurials, *Chem. papers.* **53**(2): 114-117.

Layer, R.W. (1963). The chemistry of imines. *Chem. Rev.* **63**: 489-510.

Manrao, M.R. and Kohli, S. (1986). Synthesis and antifungal activity of hydroxyphenyl ethylidenebenzylamines. *Journal of Indian Chemical Society* **63**: 348-350.

Manrao, M.R., Jolly, M., Sharma, J.R. and Kalsi, P.S. (1995). Fungicidal activity of 4-methoxybenzalaniline. *J. Res. Punjab Agric. Univ.* **32** (1): 58-60.

Manrao, M.R., Sethi, R.K., Sharma, R.C. and Kalsi, P.S. (1996). Synthesis and antifungal activity of 2-hydroxy-1-naphthalaniline and its N-phenyl derivatives. *Journal of Indian Chemical Society* **73**: 695.

Matharu, B. K., Sharma, J. R. and Manrao, M. R. (2004). Synthesis and fungitoxicity of Schiff bases of 2-chlorobenzaldehyde. *Indian J. Agric. Chem.* **1**: 45-47.

Matharu, B. K., Sharma, J. R. and Manrao, M. R. (2005). Fungitoxicity of benzalaniline and its N-phenyl derivatives. *Proc. Nat. Symp. on Agroforestry in 21st Century*, Punjab Agricultural University, Ludhiana. 397-399.

Mishra, P., Rajak, H. and Mehta, A. (2005). Synthesis of Schiff bases of 2-amino-1,3,4-oxadiazoles and their evaluation for antimicrobial activities. *J. Gen. Appl. Mirobiol*. **51**: 133-141.

Nene, Y.L. and Thapliyal, P.N. (1997). Fungicides in plant disease control. Oxford and IBH publishing Co., Pvt. Ltd., New Delhi, 531.

Pacheco, H., Correnberger, L., Pillon, D. and Thiolliere, J.T. (1970). Fungicidal Schiff bases. *Chemical Abstract* **72**: 111001-111002.

Pandeya, S.N., Sriram, D., Nath, G., and de Clercq, E. (2000). Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin and its derivatives with triazole. *Arzneimittelforschung* **50**(1): 55-59.

Perona, A., Sanz, D., Claramunt, R.M. and Elguero, J. (2006). Syntheses and structural studies of Schiff bases involving hydrogen bonds. *Molecules* **11**: 453-463.

Reid, R.J. and Heindel, N.D. (1976) Improved synthesis of 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazoles. *J. Heterocyclic Chem*. **13**: 925-926.

Salih, N. A. (2008). Synthesis and characterization of novel azole heterocycles based on 2,5-disubstituted thiadiazole. *Turk. J. Chem.* **32**: 1-7.

Sari, N. (2003). Antibacterial activities of some new amino acid-Schiff Bases. *G. U. Journal of Science* **16**(2): 283-288.

Serdar, M., Gumrukcuoglu, N., Karaoglu, S.A. and Demirbas, N. (2007). Synthesis of some novel 3,5-diaryl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. *Turk. J. Chem*. **31**: 315-326.

Sharba, A.H.K., Al-Bayati, R.H., Aouad, M. and Rezki, N. (2005). Synthesis of oxadiazoles, thiadiazoles and triazoles derived from benzo[b] thiophene. *Molecules* **10**: 1161-1168.

Sharma, J.R. Singh, B., Manrao, M.R. and Kalsi, P.S. (1993). Fungitoxicity of thio substituted Schiff bases. *Pesticide Research Journal* **18**: 119-122.

Sharma, J.R., Manrao, M.R., Kaur, D. and Kalsi, P.S. (1991). Antifungal activity of benzal-2-thioanilines and its C-phenyl substituted derivatives. *Plant Disease Research* **6**(1): 90-91.

Sharma, J.R., Singh, B., Manrao, M.R. and Kalsi, P.S. (1994). Fungitoxicity of vanillin Schiff bases. *Plant Disease Research* **9**(2): 202-203.

Srivastava, A.K., Pandey, O.P. and Sengupta, S.K. (2005). Synthesis, spectral and antimicrobial studies of bis(cyclopentadienyl)titanium(IV) derivatives with Schiff bases derived from 2-amino-5-phenyl-l,3,4-thiadiazole. *Bioinorganic Chemistry and Applications* **3**(3-4): 289-297.

Trevors, J. T. (1986). A BASIC programme for estimating LD_{50} value using IBM-PC, *Bull. Environ. Contam. Toxicol*. **37**: 18.

Vaghasiya, Y. K., Nair, R.; Soni, M., Baluja, S. and Chanda, S. (2004). Synthesis, structural determination and antimicrobial activity of compounds derived from vanillin and 4-aminoantipyrine. *J. Serb. Chem. Soc***. 69**(12): 991-998.

Furniss, B.S., Hannford, A.J., Smith, P.W.G. and Tatchell, A.R. (1996) Vogel's textbook of practical organic chemistry. Fifth Edition. ELBS.

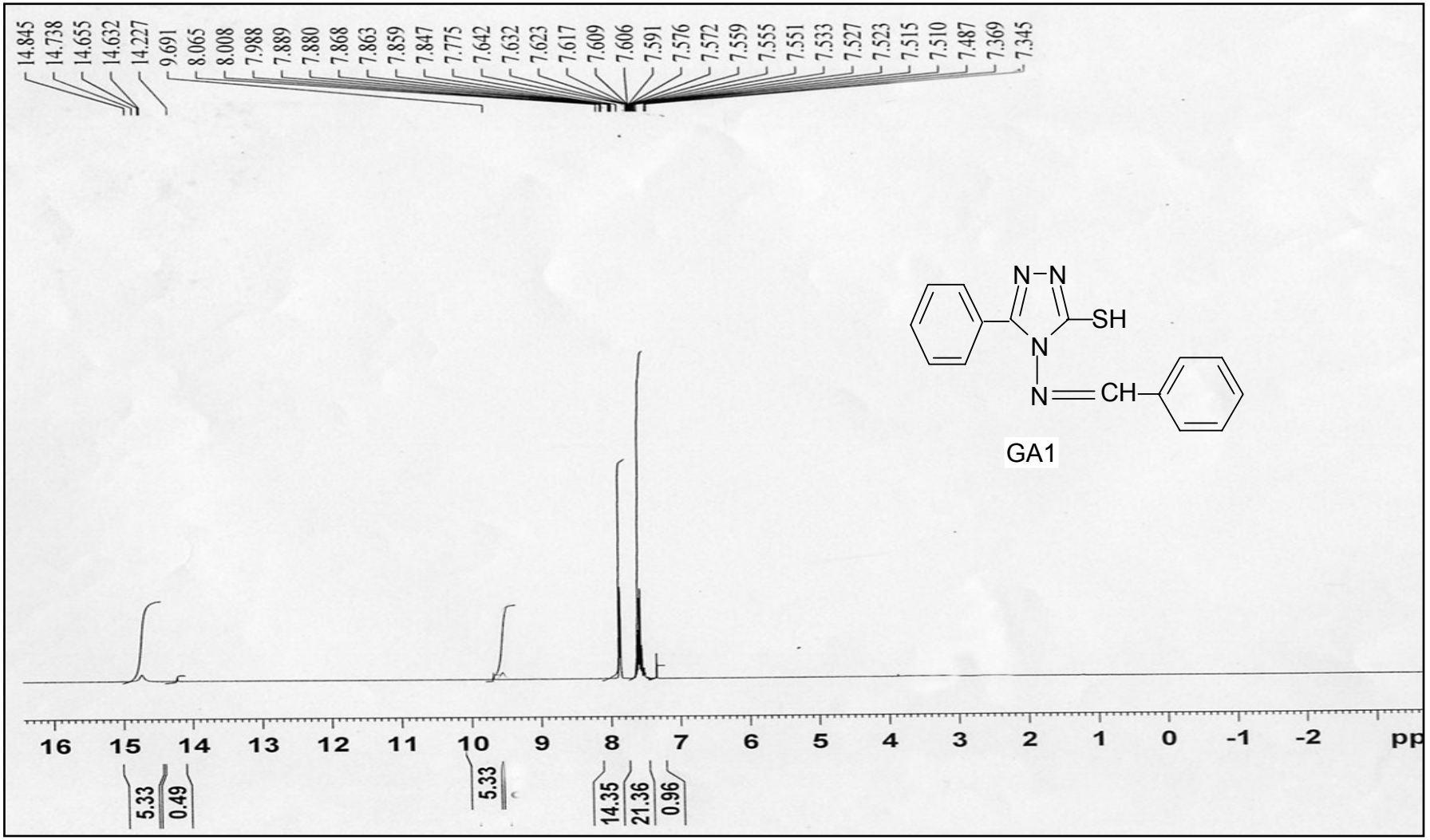
Ye, X.X., Chen, Z.F., Zhang, A.J. and Zhang, L.X. (2007). Synthesis and biological evaluation of some novel Schiff bases from 1,2,4-triazole. *Molecules* **12**:1202-1209.

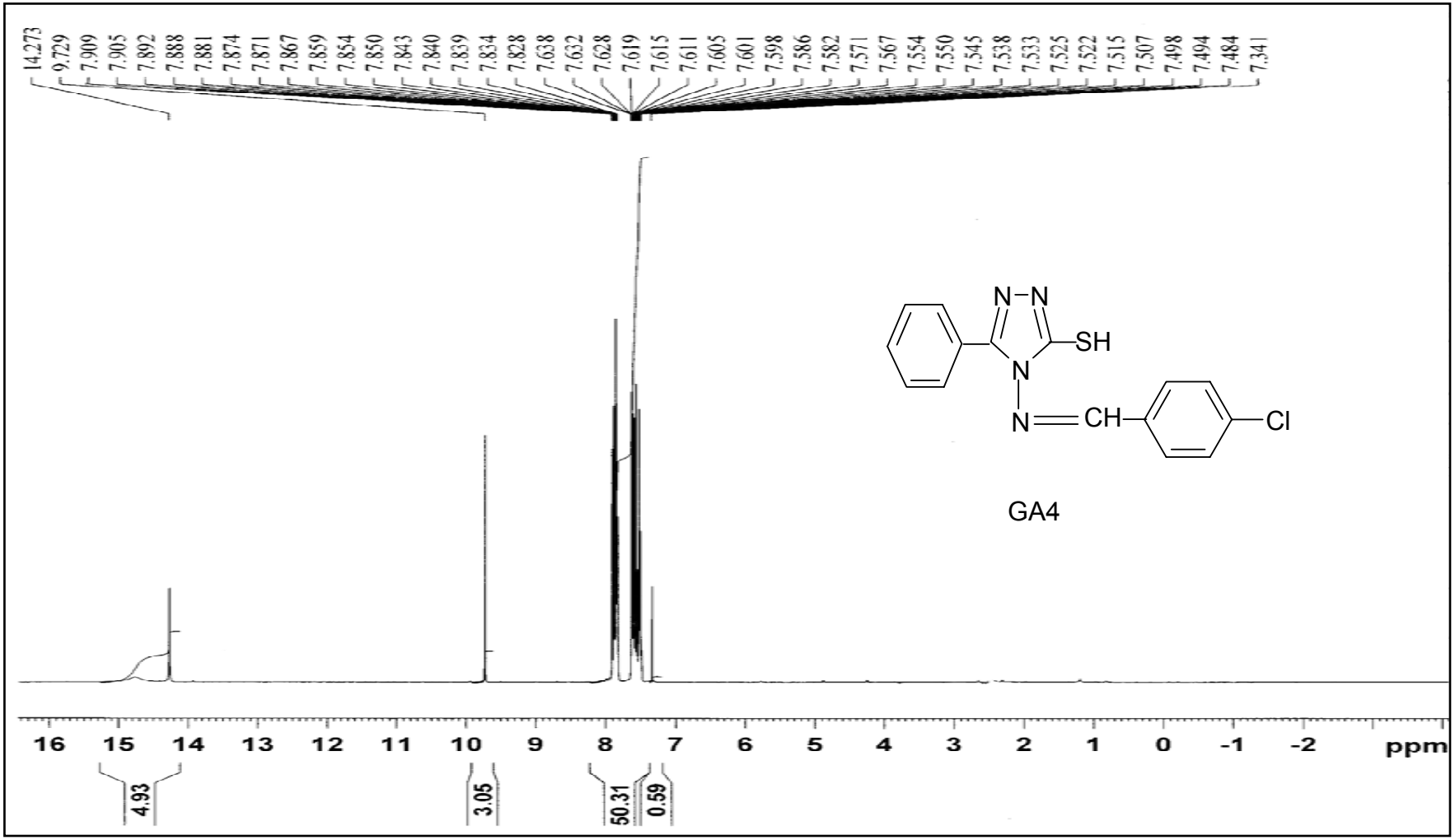
Zhongyi, W., You, T., Xu, Y., Shi, H. and Shi H. (1996). Synthesis and biological activities of 2-substituted- 5-(b-pyridyl)-2,3-dihydro-1,2,4-triazolo[3.4-b]-1,3,4-thiadiazoles. *Molecules* **1**: 68-71.

Zhou, S., Zhang, L., Jin, J., Zhang, A., Lei, X., Lin, J., He, J. and Zhang, H. (2007) Synthesis and biological activities of some novel triazolothiadiazines and Schiff bases derived from 1,2,4-triazole. *Phosphorus, Sulfur, and Silicon and the Related Elements* **182**(2): 419 – 432.

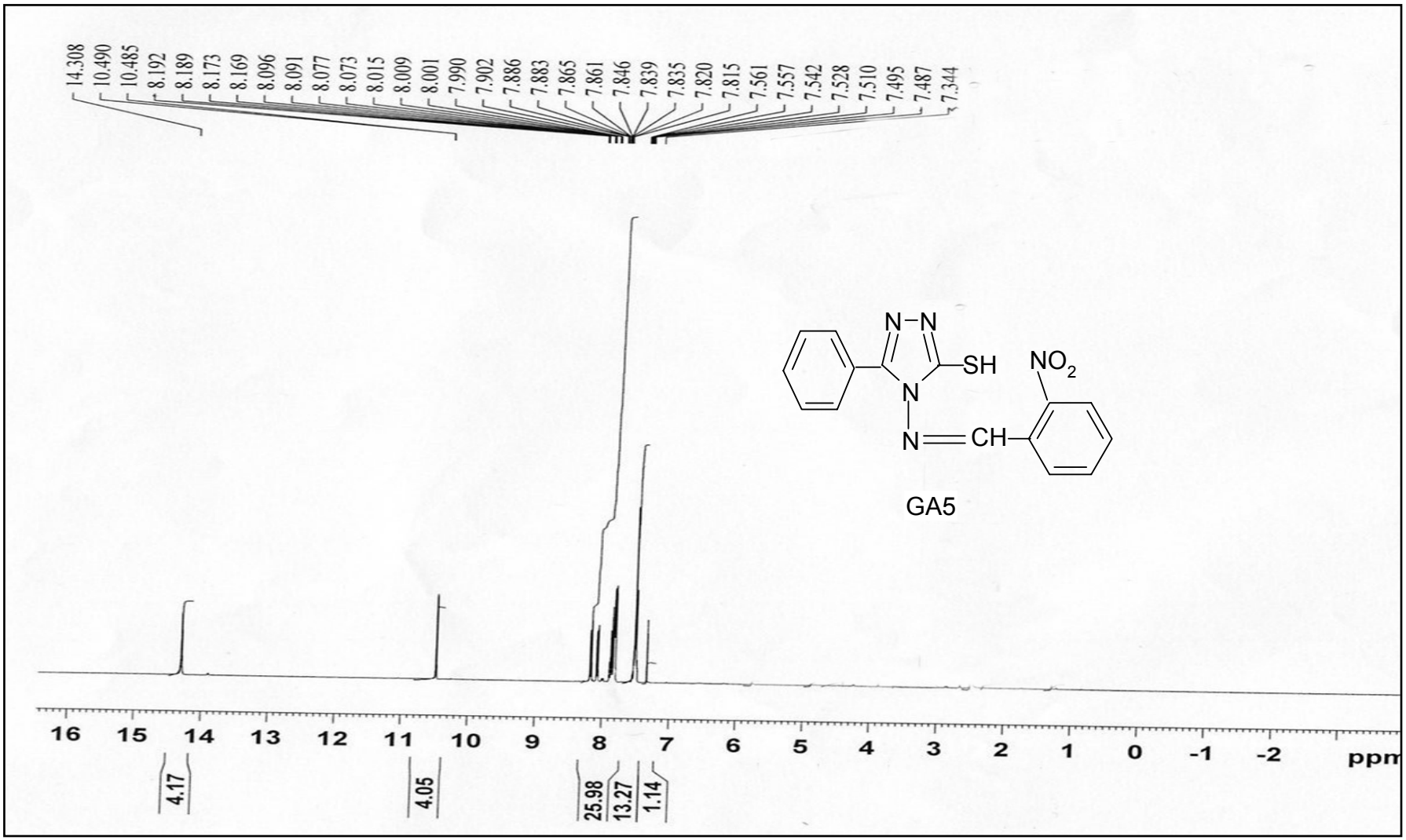
APPENDIX I

NMR Spectra

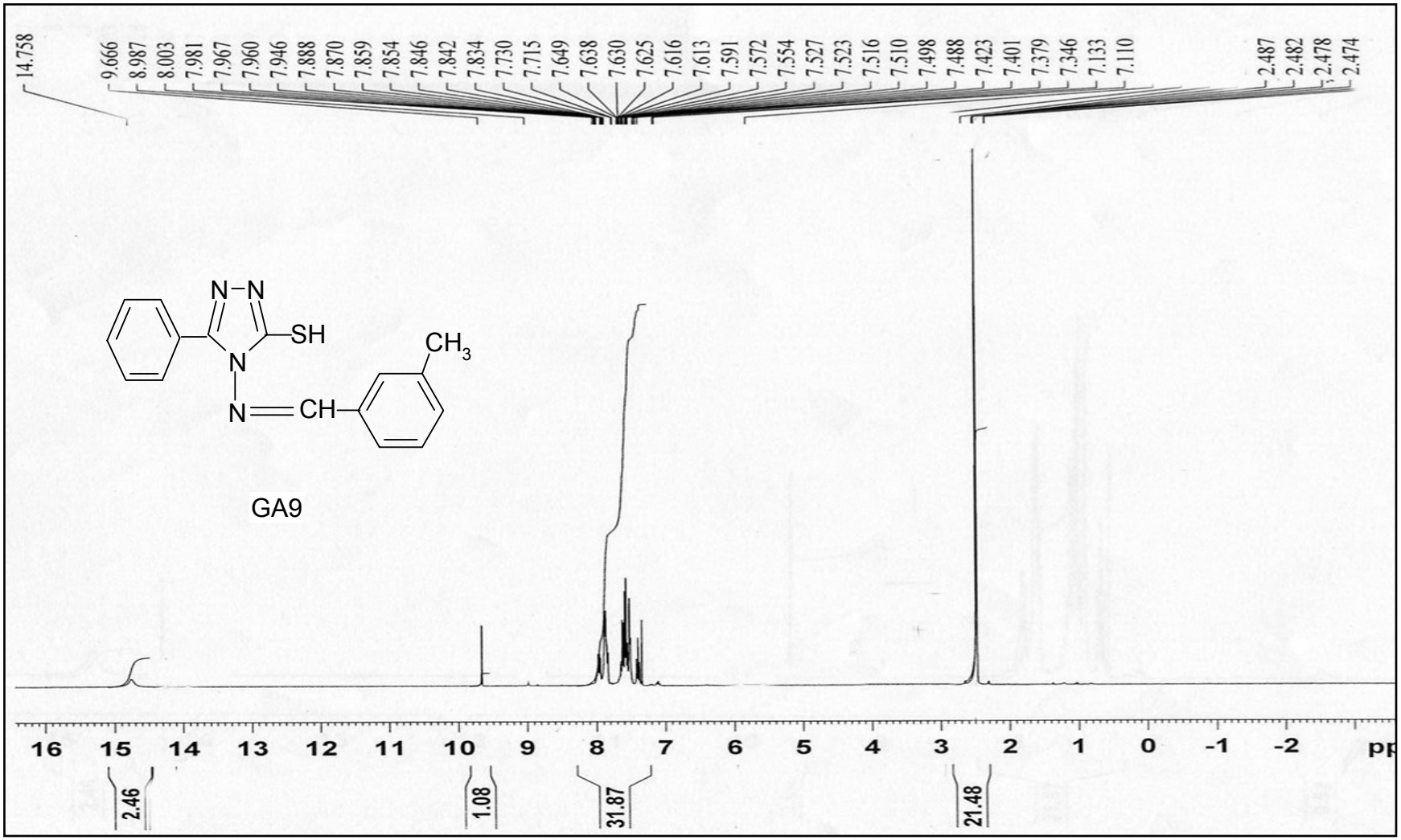

14.845
14.738
14.655
14.632
14.227
9.691
8.065
8.008
7.988
7.889
7.880
7.868
7.863
7.859
7.847
7.775
7.642
7.632
7.623
7.617
7.609
7.606
7.591
7.576
7.572
7.559
7.555
7.551
7.533
7.527
7.523
7.515
7.510
7.487
7.369
7.345
N
N
SH
N
N
CH
GA1
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
pp
5.33
0.49
5.33
14.35
21.36
0.96

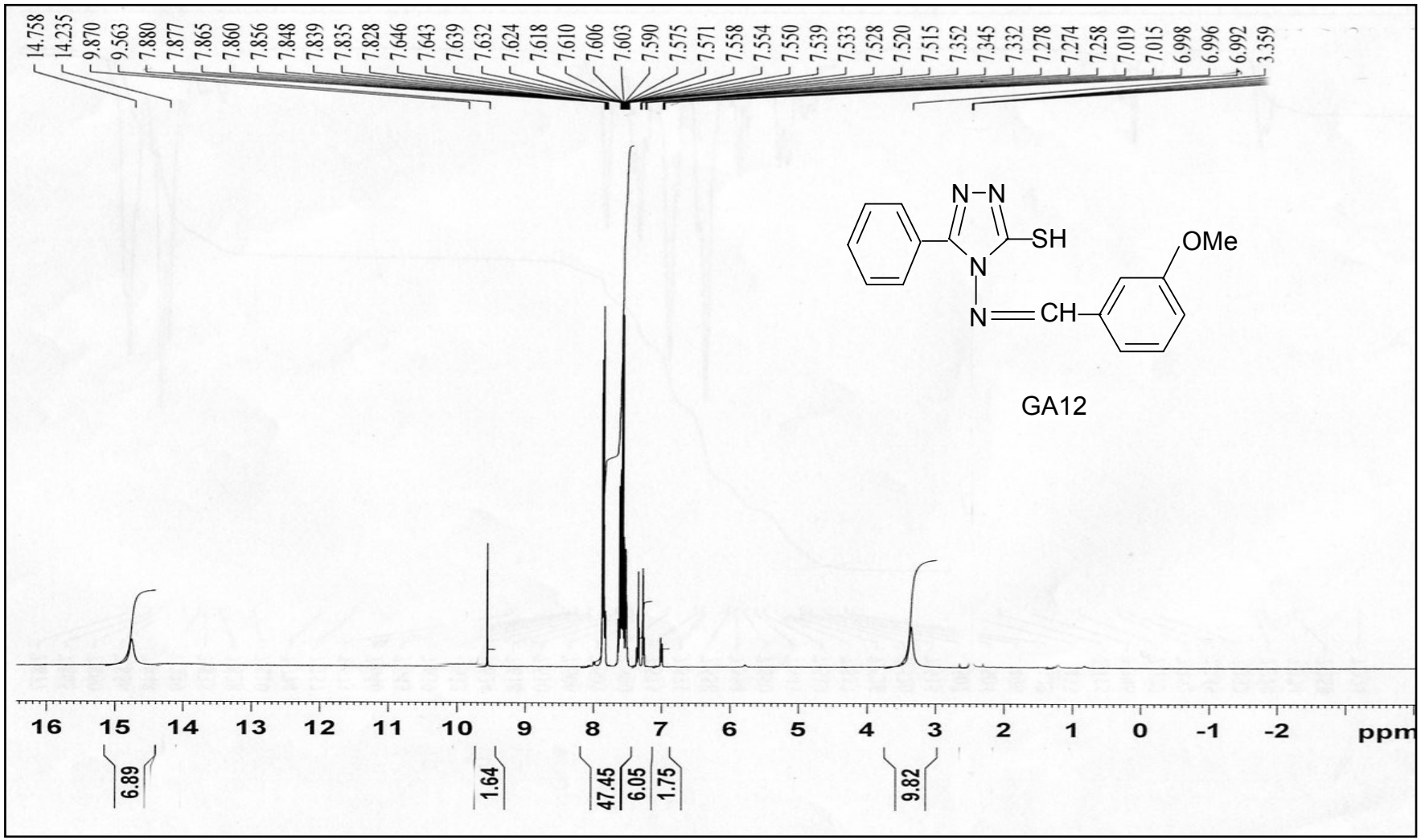

14.273
9.729
7.909
7.905
7.892
7.888
7.881
7.874
7.871
7.867
7.859
7.854
7.850
7.843
7.840
7.839
7.834
7.828
7.638
7.632
7.628
7.619
7.615
7.611
7.605
7.601
7.598
7.586
7.582
7.571
7.567
7.554
7.550
7.545
7.538
7.533
7.525
7.522
7.515
7.507
7.498
7.494
7.484
7.341
N
N
SH
N
N
CH
Cl
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
4,93
3,05
50,31
0,59

GA4

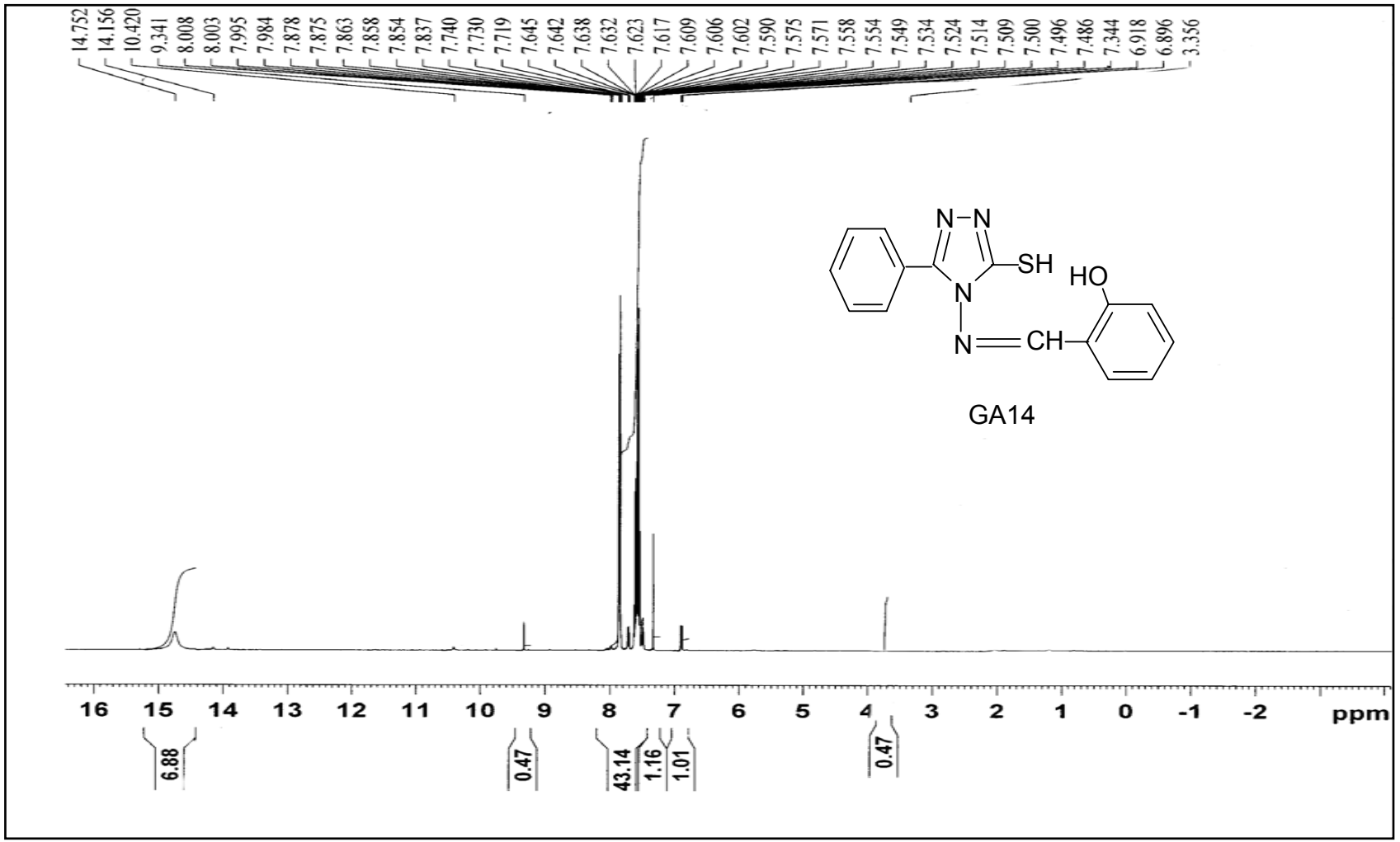

14.308
10.490
10.485
8.192
8.189
8.173
8.169
8.096
8.091
8.077
8.073
8.015
8.009
8.001
7.990
7.902
7.886
7.883
7.865
7.861
7.846
7.839
7.835
7.820
7.815
7.561
7.557
7.542
7.528
7.510
7.495
7.487
7.344
N–N
SH
NO2
N
N=CH
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
4.17
4.05
25.98
13.27
1.14

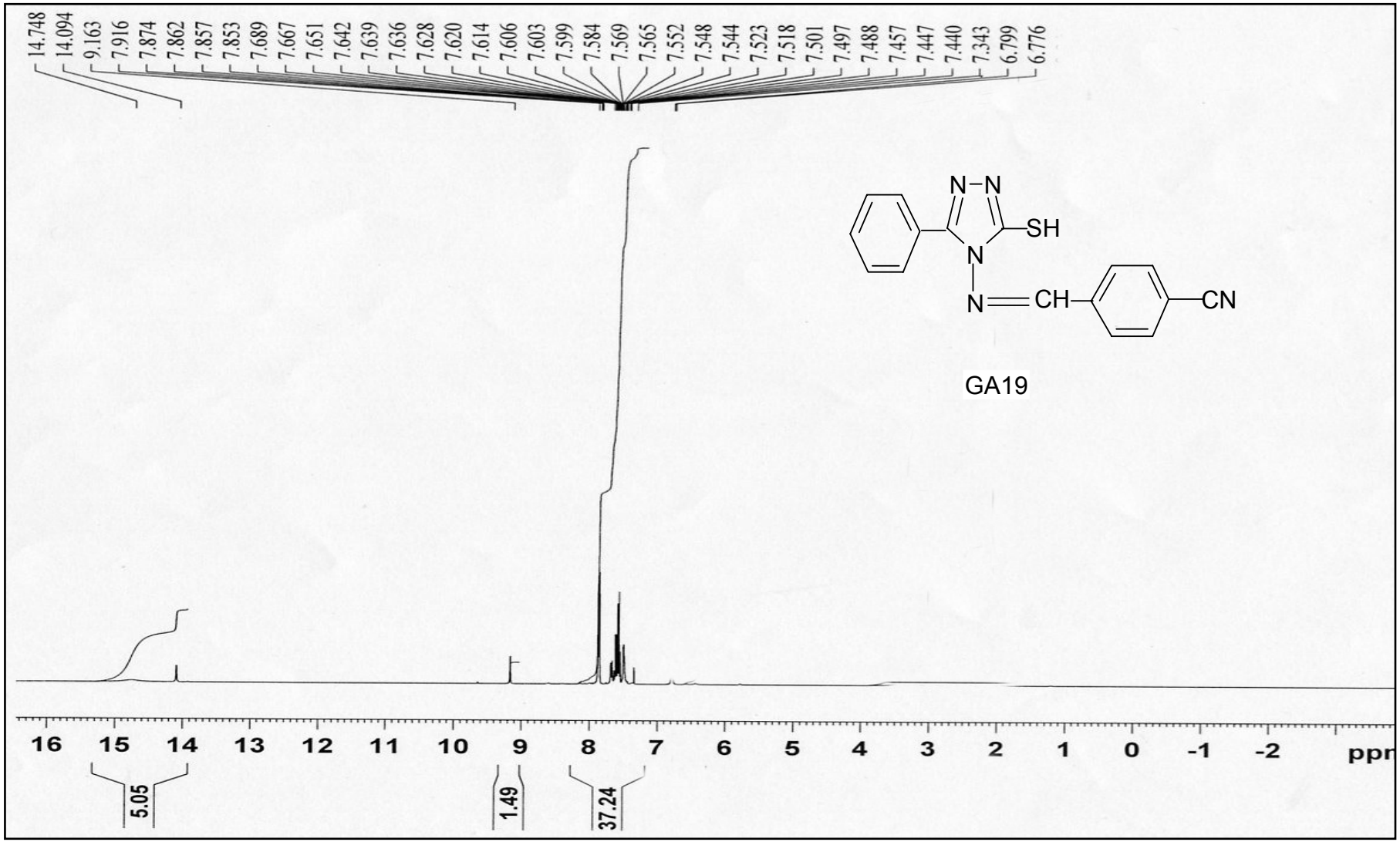
GA5

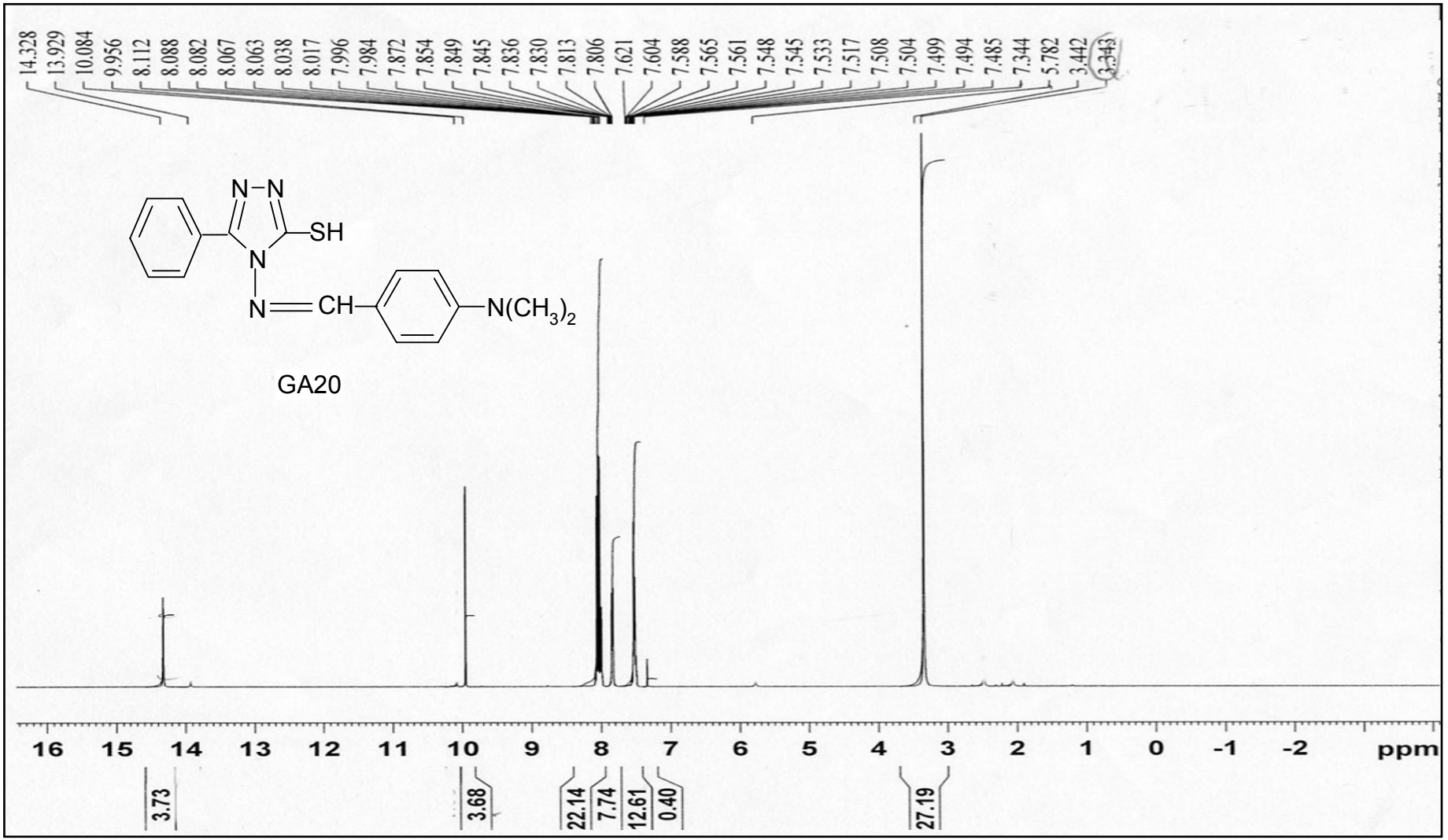

14.758
9.666
8.987
8.003
7.981
7.967
7.960
7.946
7.888
7.870
7.859
7.854
7.846
7.842
7.834
7.730
7.715
7.649
7.638
7.630
7.625
7.616
7.613
7.591
7.572
7.554
7.527
7.523
7.516
7.510
7.498
7.488
7.423
7.401
7.379
7.346
7.133
7.110
2.487
2.482
2.478
2.474
N
N
SH
N
CH3
N
CH
GA9
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
pp
2.46
1.08
31.87
21.48


14.758
14.235
9.870
9.563
7.880
7.877
7.865
7.860
7.856
7.848
7.839
7.835
7.828
7.646
7.643
7.639
7.632
7.624
7.618
7.610
7.606
7.603
7.590
7.575
7.571
7.558
7.554
7.550
7.539
7.533
7.528
7.520
7.515
7.352
7.345
7.332
7.278
7.274
7.258
7.019
7.015
6.998
6.996
6.992
3.359
N–N
SH
OMe
N
N
CH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
6.89
1.64
47.45
6.05
1.75
9.82

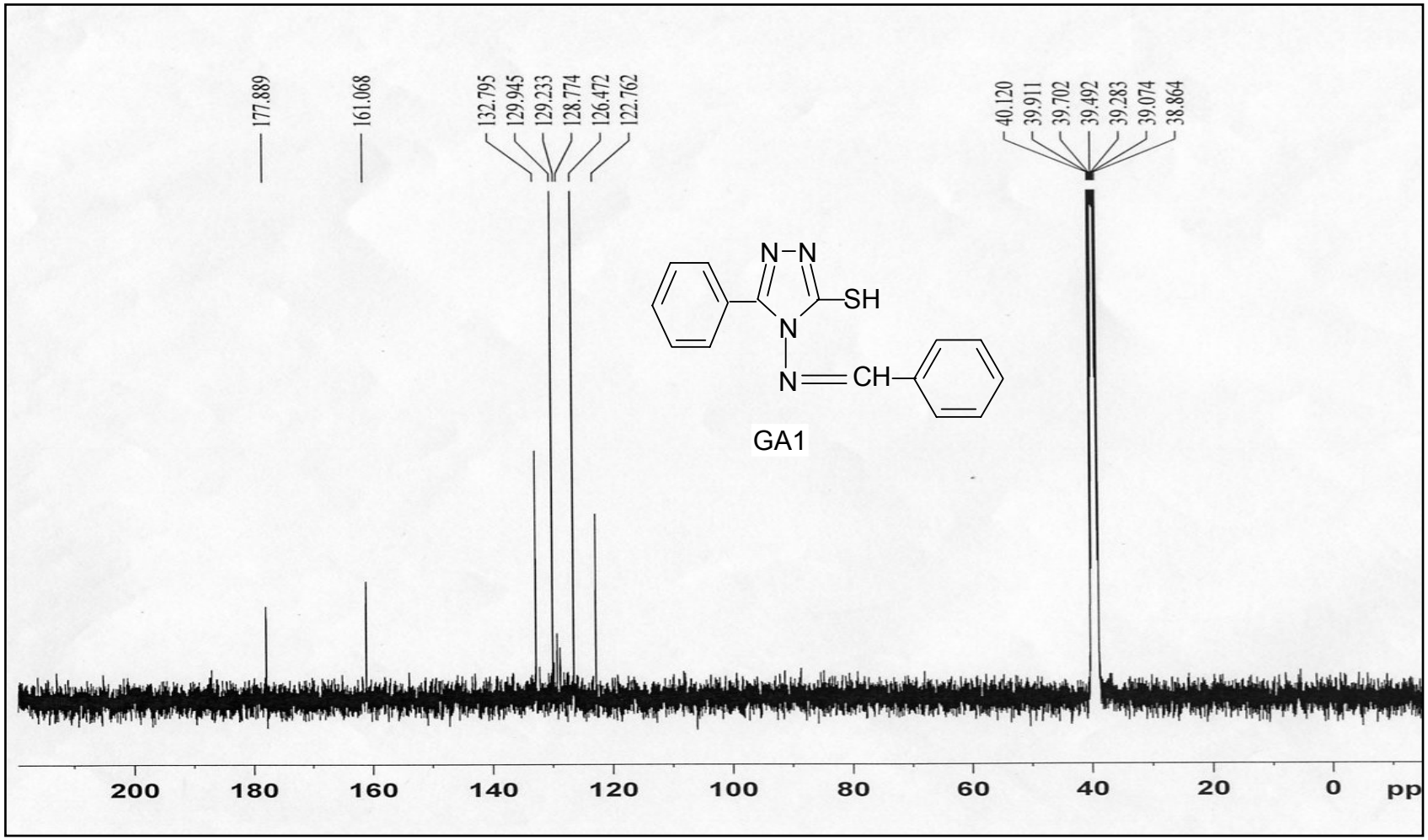
GA12


14.752
14.156
10.420
9.341
8.008
8.003
7.995
7.984
7.878
7.875
7.863
7.858
7.854
7.837
7.740
7.730
7.719
7.645
7.642
7.638
7.632
7.623
7.617
7.609
7.606
7.602
7.590
7.575
7.571
7.558
7.554
7.549
7.534
7.524
7.514
7.509
7.500
7.496
7.486
7.344
6.918
6.896
3.356
N–N
SH
HO
N
N
CH
GA14
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
6.88
0.47
43.14
1.16
1.01
0.47


14.748
14.094
9.163
7.916
7.874
7.862
7.857
7.853
7.689
7.667
7.651
7.642
7.639
7.636
7.628
7.620
7.614
7.606
7.603
7.599
7.584
7.569
7.565
7.552
7.548
7.544
7.523
7.518
7.501
7.497
7.488
7.457
7.447
7.440
7.343
6.799
6.776
N–N
SH
N
N
CH
CN
GA19
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
5.05
1.49
37.24

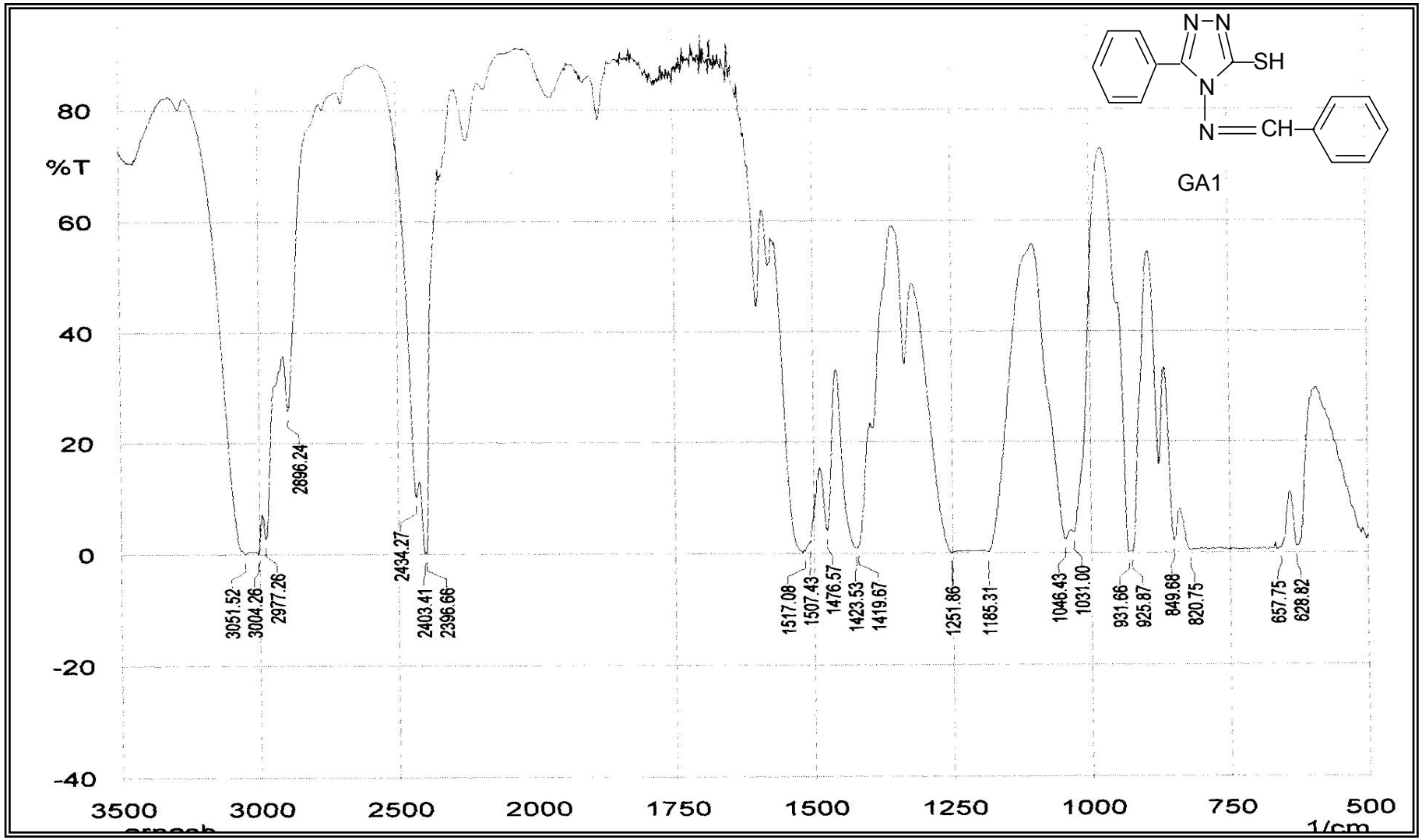

14.328
13.929
10.084
9.956
8.112
8.088
8.082
8.067
8.063
8.038
8.017
7.996
7.984
7.872
7.854
7.849
7.845
7.836
7.830
7.813
7.806
7.621
7.604
7.588
7.565
7.561
7.548
7.545
7.533
7.517
7.508
7.504
7.499
7.494
7.485
7.344
5.782
3.442
3.343
N–N
SH
N
N
CH
N(CH3)2
GA20
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
3.73
3.68
22.14
7.74
12.61
0.40
27.19

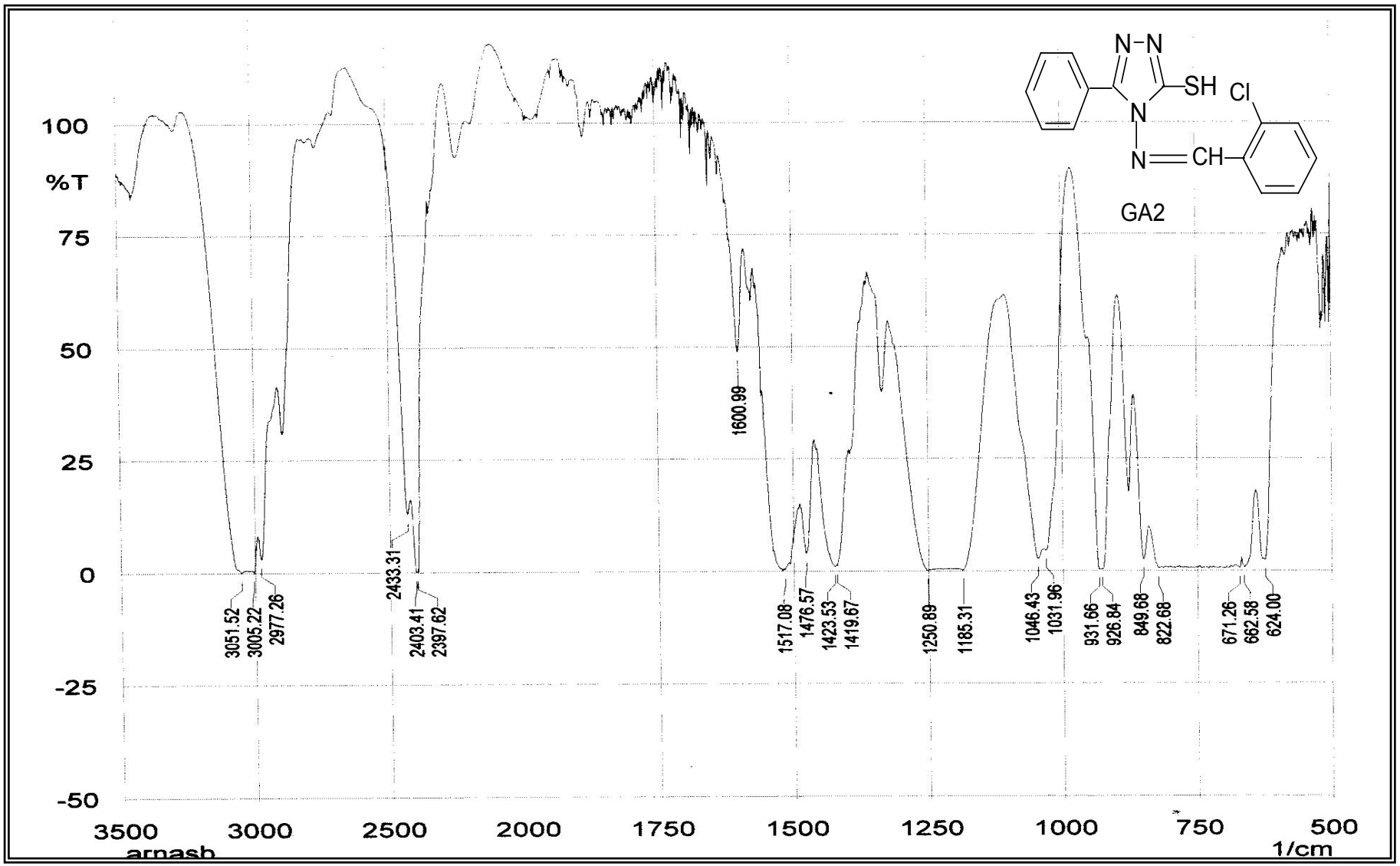
^{13}C NMR Spectra



APPENDIX II

IR Spectra


80
%T
60
40
20
0
-20
-40
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
3051.52
3004.26
2977.26
2896.24
2434.27
2403.41
2396.66
1517.08
1507.43
1476.57
1423.53
1419.67
1251.86
1185.31
1046.43
1031.00
931.66
925.87
849.68
820.75
657.75
628.82
N
N
SH
N
N
CH
GA1


100
%T
75
50
25
0
-25
-50
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
arnasb
3051.52
3005.22
2977.26
2433.31
2403.41
2397.62
1600.99
1517.08
1476.57
1423.53
1419.67
1250.89
1185.31
1046.43
1031.96
931.66
926.84
849.68
822.68
671.26
662.58
624.00
N-N
SH
Cl
N
N
CH
GA2