SYNTHESIS AND SPECTRAL CHARACTERIZATION OF 1,3,4-THIADIAZOLE DERIVATIVES

Thesis submitted in partial fulfillment for the requirement of the

Degree of Master of Pharmacy in Pharmaceutical Chemistry

Faculty of Engineering and Technology

By

SHRABANTI SARKAR

Class Roll No.: 002011402019

Examination Roll No.: M4PHC22005

Reg. No.: 154280 of 2020-2021

Under the Guidance of

PROF. (Dr.) TAPAN KUMAR MAITY

Synthetic and Natural Product Research Laboratory

Division of Pharmaceutical Chemistry

Department of Pharmaceutical Technology

Faculty Council of Engineering & Technology

Jadavpur University

Kolkata-700032

2022

DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700032

Certificate of Approval

This is to certify that **Shrabanti Sarkar** (**Examination Roll No:** M4PHC22005**, Registration No: 154280 of 2020-2021**) has carried out the project work on the subject entitled **"Synthesis and Spectral Characterization of 1,3,4-Thiadiazole Derivatives"** under the supervision of **Prof. (Dr.) Tapan Kumar Maity**, Professor, Department of Pharmaceutical Technology, Jadavpur University. This project work is submitted by her in partial fulfillment of the requirements for the degree of **Master of Pharmacy (Pharmaceutical Chemistry)** of Jadavpur University. She has carried out her work independently and with proper care and attention to our entire satisfaction.

Forwarded by

Prof. (Dr.) Sanmoy Karmakar

Head of the department of pharmaceutical
Technology,
Jadavpur University
Kolkata-700032

Supervised by

Prof. (Dr.) Tapan Kumar Maity

Synthetic and Natural Product
Research Laboratory,
Department of Pharm. Tech
Jadavpur University
Kolkata-700032

(Prof. Chandan Mazumdar)

Dean, Faculty of Engineering and Technology

Jadavpur University, Kolkata

ACKNOWLEDGEMENT

First and foremost. I feel immense pleasure in expressing my sincere and deep sense of gratitude to my supervising guide ***Prof. (Dr.) Tapan Kumar Maity****, Professor, Department of Pharmaceutical Technology, Jadavpur University, for giving me such a progressive and unconventional topic for my thesis work and of course for the patience and support in overcoming numerous obstacles I have been facing through my research. This work would have been impossible without his exemplary guidance, sustained interest, creative suggestions and motivation.*

I would like to express my sincere gratitude to ***Prof. (Dr.) Sanmoy Karmakar,*** *Head of the Department of Pharmaceutical Technology, Jadavpur University, for rendering me valuable help and necessary facilities to carry out this work.*

I would also like to express my thanks to all of my respected teachers, laboratory seniors ***(Mr. Avik Maji*** *and* ***Mr. Abhik Paul****, juniors (****Ms. Sourin Nahar*** *and* ***Mr. Pankaj Nahata)*** *for their help and support in each step of work.*

I would also like to thank ***Mr. Ankur Kumar, Ms. Riya Biswas, Mr. Abhishek Kumar Ms. Richita Monda****l for helping me to perform my research work and special thanks to my friend* ***Ms. Tanushree Sarkar*** *for her constant support throughout the whole M.Pharm journey.*

I also thank my friends and all others who have extended their cooperation and helped me immensely during the entire project work.

Shrabanti Sarkar

Class roll no.: 002011402019

Examination roll no.: M4PHC22005

Reg. No.: 154280 of 2020-2021

DECLARATION OF THE ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research as part of my work on **"Synthesis and Spectral Characterization of 1,3,4-Thiadiazole Derivatives"**.

All the information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that as required by these rules and conduct, I have fully cited and referred all the information and results that are not original to this work.

NAME.: SHRABANTI SARKAR

EXAMINATION ROLL NO.: M4PHC22005

REGISTRATION NUMBER.: 154280 of 2020-2021

CLASS ROLL NO.: 002011402019

THESIS TITLE: **"Synthesis and Spectral Characterization of 1,3,4-Thiadiazole Derivatives."**

SIGNATURE WITH DATE:

PREFACE

Research is carried out to design and develop newer drugs in academic institutions and pharmaceutical industry. The new drug design involves in modifying the existing bioactive drugs to change their therapeutic effects along with developing new bioactive chemical molecules.

Antibacterial resistance is now well documented for many pathogens, and studies with a variety of bacteria indicate that resistance can develop within just a few years. Resistance against many members of fluoroquinolones, particularly older ones, such as ciprofloxacin is increasing. Further advances in quinolone field are likely to provide better compounds capable of dealing with the resistant strains. So, there is ongoing research for the synthesis of less resistance antimicrobial agents with better efficacy.

The heterocyclic compounds display various biological activity. Among the heterocyclic compounds, 1,3,4-Thiadiazole molecule exhibits versatile biological activities including antibacterial and antifungal activity and the compounds with urea functional group were found to have cytotoxic activity.

The present work entitled **"Synthesis and Spectral Characterization of 1,3,4-Thiadiazole Derivatives"** undertaken with an aim to synthesize a suitable lead compound which can be exploited to develop novel antimicrobial agents.

Dedicated to

my guide, family and well wishers

Table of Contents

LIST OF FIGURES:

CHAPTER - 1

INTRODUCTION

1.1. Introduction:

Heterocyclic compounds and their numerous analogues, as well as their uses in the medicinal and chemical area, have attracted chemists' attention over the years. Multiple recent reviews have focused on research on heterocyclic compounds such as pyrazole, tetrahydroquinolines, benzotriazole, 1,2,3,4-tetrazine, thiazole, 2-thiazoline, pyrimidine, and so on. Thiadiazole is a 5-membered ring system containing hydrogen binding domain, Sulphur atom and two-electron donor nitrogen system that exhibit a wide variety of biological activity. Due to the inductive effect of sulfur atom this thiadiazole ring is very weak base. Thiadiazole has four isomeric form which are 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole, among them 1,3,4-thiadiazole is most pharmacologically active [1] (**Figure 1**).

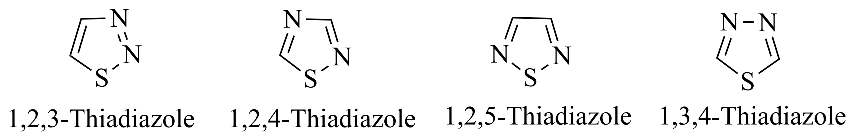


Figure 1: Isomers of Thiadiazole.

Thiadiazoles are nitrogen–sulphur heterocycles that have a wide range of applications as structural units in physiologically active compounds and as valuable intermediates in medicinal chemistry. Because of their broad range of pharmacological characteristics, substituted 1,3,4-thiadiazole derivatives have gotten a lot of attention and have been explored a lot in recent years. Because of the presence of the =N-C-S- moiety, 1,3,4-thiadiazole derivatives are likely to have a variety of biological effects. [2]. Other researchers believe that the biological activities of 1,3,4-thiadiazole derivatives are related to the ring's strong aromaticity, which gives the five-membered ring structure exceptional *in vivo* stability and lesser side effects in higher vertebrates, including humans [3]. Some investigations have demonstrated the significance of isosterism for a compound's pharmacological profile. According to these findings, the bioisostere of pyrimidine is 1,2,4-thiadiazole, while the bioisostere of pyridazine is 1,3,4-thiadiazole due to the displacement of -CH=CH- by -S- [4, 5]. The benzene ring, oxadiazole, oxazole, and thiazole rings are all bioisosteres of the thiadiazole ring [1,4]. Compounds with greater lipophilicity and improved biological characteristics may result from the bioisosteric substitution of one ring with another. Because of the sulphur atom in thiadiazole derivatives which provides high liposolubility, slow oral absorption and cell permeability, resulting in high bioavailability. Furthermore, replacing a homocyclic ring with a heterocyclic ring enables the production of certain other compounds that interact more with receptors.[4, 6, 7]. Given the high prevalence of pyrimidine derivatives in nature and the presence of the pyridazine ring in substances with pharmacological effects (such as the antidepressant minaprine, the GABA-A antagonist gabazine, the nonsteroidal anti-inflammatory drug emorfazone, and the antibacterial cephalosporin cefozopran), it is highly likely that thiadiazole derivatives will demonstrate biological effects [8, 9, 10]. Moreover, 1,3,4-thiadiazole derivatives can produce mesoionic salts (**Figure 2**). Mesoionic system contains a pentatomic heterocyclic ring which possesses a sextet of *p* and π electrons and positive charge counterbalanced by formal negative charge. Despite their internal charges, the mesoionic compounds are neutral and able to cross cellular membranes, and this contributes to the good cell permeability of 1,3,4-thiadiazole derivatives. The mesoionic nature of 1,3,4-thiadiazoles enables these compounds to interact strongly with biomolecules (e.g. DNA and proteins) [4, 11].

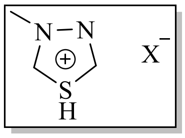


Figure 2: Mesoionic salt derivatives of 1,3,4-thiadiazole.

1,3,4-Thiadiazole ring is an important scaffold known to be associated with several biological activities including antimicrobial, antituberculosis, antiviral, analgesic, antidepressant and anxiolytic, antihypertensive, anticonvulsant, anti-inflammatory, local anesthetic and kinesin inhibitors [12]. The biological importance of 1,3,4-thiadiazole derivatives has been reported following the discovery of heterocyclic sulfonamides as reasonable antimicrobial agents (e.g. sulfathiazole **1**; Winthrop Chemical Company, NY, USA, 1940) [13, 14]. In analogy to sulfathiazole, other sulfonamides showing similar activity such as “sulfamethizole” **2** (4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl) benzene sulfonamide, Rufol [Urgo Laboratories, Chenove, France]) [15,16] or “sulfaethidole” **3** (4-amino-*N*-[5-ethyl-1,3,4-thiadiazol-2-yl] benzene sulfonamide, Globucid [Schering, Berlin, Germany]) [17] were prepared. Except sulfathiazole that is still used in the treatment of *Haemophilus vaginalis* vaginitis [18], sulfamethizole and sulfaethidole currently possess only historical importance. The synthesis of "acetazolamide" **4** (5-acetylamino-1,3,4-thiadiazol-2-sulfonamide) by Roblin and Clapp [19] (Lederle Laboratories, Pearl River, NY, USA) as carbonic anhydrase inhibitor reoriented the researchers to sulfonamides bearing 1,3,4-thiadiazole ring. The synthetic studies concerning the therapy of parasitic infections gave "megazol" **6** (2-amino-5-[1-methyl-5-nitro-1*H*-2-imidazolyl]-1,3,4-thiadiazole, CL 64855) [20]a nitroimidazole extremely active in experimental infections caused by *Trypanosoma cruzi* and *Trypanosoma brucei* as well as drug-resistant forms of trypanosomiasis. 1,3,4-Thiadiazole ring is the constitutive part of some cephalosporins and cephamycins that showed high in vitro activity against both Gram-positive and Gram-negative bacteria. A good example is "cefazolin" **7**, a first-generation cephalosporin which has been used worldwide since the early 1970s (GlaxoSmithKline plc, London, UK; Ancef) [21, 22].

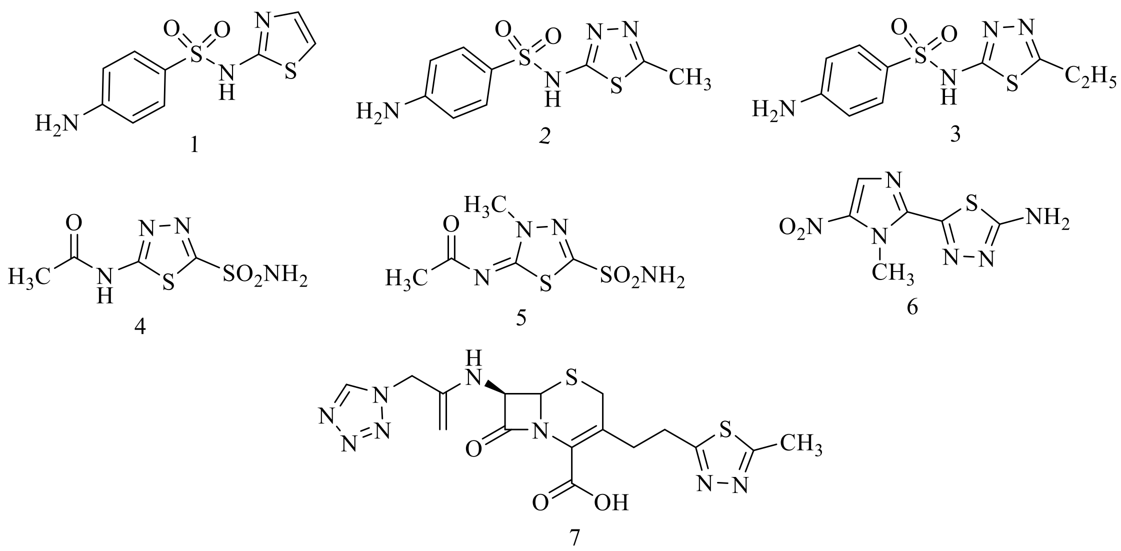


Figure 3: Structure of established molecules bearing 1,3,4-thiadiazole.

1.2. Reference:

1. Hu, Y., Li, C., Wang, X., Yang, Y., & Zhu, H. (2014). 1,3,4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry. *Chemical reviews, 114 10*, 5572-610.
2. Holla, B. S., Poojary, K. N., Rao, B. S., &Shivananda, M. K. (2002). New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents. *European journal of medicinal chemistry*, 37(6), 511–517. https://doi.org/10.1016/s0223-5234(02)01358-2.
3. Yousif, E., Majeed, A., Al-Sammarrae, K., Salih, N., Salimon, J., & Abdullah, B. (2017). Metal complexes of Schiff base: Preparation, characterization and antibacterial activity. *Arabian Journal of Chemistry*, 10, S1639-S1644. https://doi.org/10.1016/j.arabjc.2013.06.006.
4. Li, Y., Geng, J., Liu, Y., Yu, S., & Zhao, G. (2013). Thiadiazole-a promising structure in medicinal chemistry. *ChemMedChem*, 8(1), 27–41. https://doi.org/10.1002/cmdc.201200355.
5. Wermuth CG, Aldous D, Raboisson P, Rognan D. *The Practice of Medicinal Chemistry*. 4th ed. London: Academic Press, Elsevier; 2015:196.
6. Brown N. *Bioisosteres in Medicinal Chemistry*. Weinheim, Germany: Wiley-VCH Verlag and Co., KGaA; 2012.
7. Tripathy, R., Ghose, A., Singh, J., Bacon, E. R., Angeles, T. S., Yang, S. X., Albom, M. S., Aimone, L. D., Herman, J. L., &Mallamo, J. P. (2007). 1,2,3-Thiadiazole substituted pyrazolones as potent KDR/VEGFR-2 kinase inhibitors. *Bioorganic & medicinal chemistry letters*, 17(6), 1793–1798. https://doi.org/10.1016/j.bmcl.2006.12.054.
8. C. G. Wermuth, *Med. Chem. Commun.*, 2011, **2**, 935 **DOI:** 10.1039/C1MD00074H.
9. Bizière, K., Worms, P., Kan, J. P., Mandel, P., Garattini, S., &Roncucci, R. (1985). Minaprine, a new drug with antidepressant properties. *Drugs under experimental and clinical research*, 11(12), 831–840.
10. Ueno, S., Bracamontes, J., Zorumski, C., Weiss, D. S., & Steinbach, J. H. (1997). Bicuculline and gabazine are allosteric inhibitors of channel opening of the GABAA receptor. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 17(2), 625–634.https://doi.org/10.1523/JNEUROSCI.17-02-00625.1997.

11. Senff-Ribeiro, A., Echevarria, A., Silva, E. F., Franco, C. R., Veiga, S. S., & Oliveira, M. B. (2004). Cytotoxic effect of a new 1,3,4-thiadiazolium mesoionic compound (MI-D) on cell lines of human melanoma. *British journal of cancer*, 91(2), 297–304. https://doi.org/10.1038/sj.bjc.6601946.
12. Serban, G., Stanasel, O., Serban, E., & Bota, S. (2018). 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents. *Drug design, development and therapy*, 12, 1545–1566. https://doi.org/10.2147/DDDT.S155958.
13. Fosbinder, R. J., & Walter, L. A. (1939).Sulfanilamido derivatives of heterocyclic amines. *J Am Chem Soc*, 61(8), 2032–2033.
14. Lott, W. A., &Bergeim, F. H. (1939). 2-(*p*-Aminobenzenesulfonamido)-thiazole: a new chemo therapeutic agent. *J Am Chem Soc*, 61, 3593–3594.
15. Tappi, G. (1943). Heterocyclic derivatives of sulfanilamide. *Recl Trav Chim*, 62, 207–209.
16. Masaki, O., Mifune, A., Higashijima, T., & Nagato, S. (1951). 1,3,4-Thiadiazole derivatives. Several reactions of aminothiadiazoles. *J Pharm Soc Jpn*, 71, 481–484.
17. Frisk, A. R. (1943). Sulfanilamide derivatives, chemotherapeutic evaluation of N_1-substituted sulfonamide. *Acta Med ScandSuppl*, 142, 1.
18. Beale, J. M., &Block J. H. (2011).*Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*. 12th ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer, 238.
19. Roblin, R. O., & Clapp, J. W. (1950). Carbonic anhydrase inhibitor. *J Am Chem Soc*, 72, 4890.
20. Berkelhammer, G., &Asato, G. (1968). 2-Amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole: a new antimicrobial agent. *Science*, 162(3858), 1146.
21. Nishida, M., Matsubara, T., Murakawa, T., Mine, Y., Yokota, Y., &Kuwahara, S. (1969). In vitro and in vivo evaluation of cefazolin, a new cephalosporin C derivative. *Antimicrob Agents Chemother (Bethesda)*, 9, 236–243.
22. Nishida, M., Matsubara, T., Murakawa, T., Mine, Y., &Yokota, Y. (1970). Cefazolin, a new semisynthetic cephalosporin antibiotic. II. In vitro and in vivo antimicrobial activity. *J Antibiot (Tokyo)*, 23(3), 137–148.

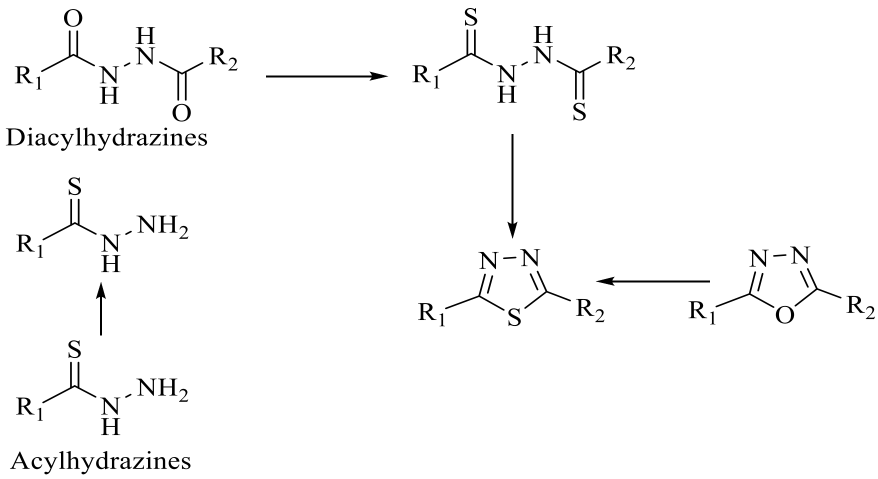
CHAPTER – 2

LITERATURE REVIEW

2.1. Synthetic Procedures of 1,3,4-Thiadiazoles:

Development of 1,3,4-thiadiazole chemistry is linked to the discovery of phenylhydrazines and hydrazine in the late 19th century. Commonly, 1,3,4-thiadiazoles can be available via general routes from cyclization of acylhydrazines including N,N′-diacylhydrazines and monoacylhydrazines or transformation from 1,3,4-oxadiazoles (**Scheme 1**). We can also synthesize 1,3,4-thiadiazoles from thiohydrazines including thiosemicarbazides, thiocarbazides, dithiocarbazates, thioacylhydrazines and bithioureas. Herein, summarized some recent strategies on the synthesis of 1,3,4-thiadiazolederivatives hoping that this classification can aid chemists in their preparation.

Scheme 1. General Preparation of 1,3,4-Thiadiazoles from Acylhydrazines or 1,3,4-Oxadiazoles



2.1.1. From Acylhydrazines:

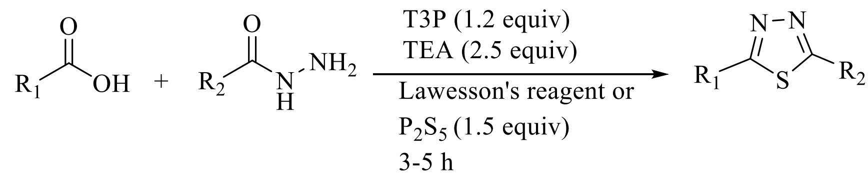
As summarized in Scheme 1, 1,3,4-thiadiazoles can be prepared via sulfuration of the corresponding 1,4-dicarbonyl or acyl precursors using phosphorus sulfide reagents such as P_2S_5 and Lawesson's reagent [1]. However, the common methods reported always suffer from harsh conditions or stoichiometric formation of an intractable byproduct.

2.1.1.1. From Acid Hydrazides:

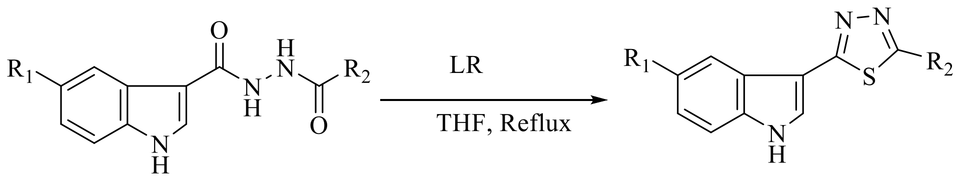
In the past decades, several kinds of one-pot syntheses of 1,3,4-thiadiazoles have been reported, which can avoid the tedious work of multistep syntheses. Among these methods, some are still conducted under harsh conditions whereas others have been improved. Augustine et al. report a one-pot synthesis of 1,3,4-thiadiazoles directly from carboxylic acids using propylphosphonic anhydride (T3P) (**Scheme 2**) [2].

2.1.1.2. From Diacylhydrazines:

Cyclization of N,N′-diacylhydrazines is a very common and convenient way to synthesize 1,3,4-thiadiazoles. This method has been well studied by many chemists employing phosphorus sulphides (i.e. P_2S_5 and Lawesson's reagent) in solvents, such as DMF, CH_2Cl_2, THF, dioxane, and PhMe (**Scheme 3**) [3].



Schme 2



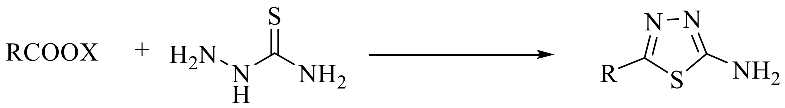
Scheme 3

2.1.2. From Thiohydrazines:

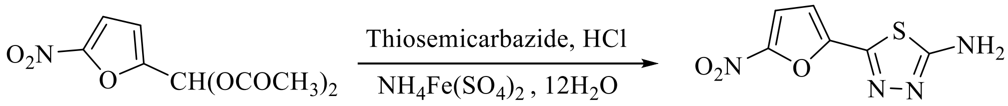
1,3,4-Thiadiazoles can also be prepared by cyclizing thiohydrazines or its equivalents. Each derivative of thiohydrazines can introduce special kinds of substituents to the thiadiazole ring, which allows for 1,3,4-thiadiazoles with a broad spectrum of reactivity and bioactivity. We herein classify thiohydrazines into thiosemicarbazides, thiocarbazides, dithiocarbazates, thioacylhydrazines, bithioureas, and other miscellanea. The strategies on the synthesis of 1,3,4-thiadiazole derivatives have been summarized below.

2.1.2.1. From Thiosemicarbazides:

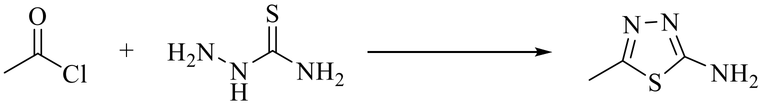
Many syntheses of 1,3,4-thiadiazoles proceed from thiosemicarbazides, substituted thiosemicarbazides, or thiosemicarbazones. Cyclization of thiosemicarbazides or substituted thiosemicarbazides efficiently lead to 2-amino-1,3,4-thiadiazoles, which have been widely studied as crucial intermediates when preparing 1,3,4-thiadiazole derivatives. In this reaction, acylation (Scheme 4) or Schiff base formation on the α-amino group initiates cyclization of thiosemicarbazides and upon the action of a dehydrating agent such as EDCI, DCC, TMSCl, TsCl, PPh_3, $SOCl_2$, PCl_5, and diphenyl chlorophosphate to obtain thiadiazoles. Many common acylating agents such as carboxylic acid [4, 5] acid halides [6], and acid anhydride (**Scheme 5a and 5b**) have been used [7].



Scheme 4



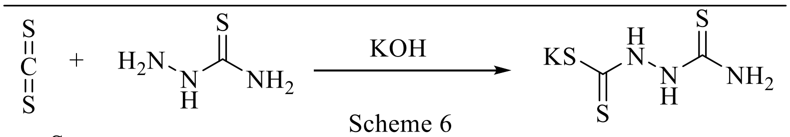
Scheme 5a



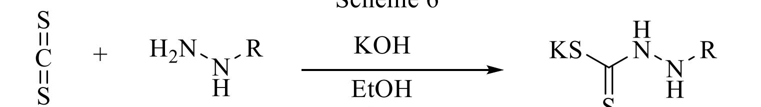
Scheme 5b

2.1.2.2. From Dithiocarbazates :

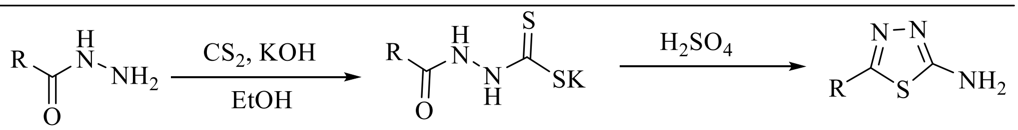
As mentioned above, dithiocarbazates are synthesized by carbon disulfide as the sulfur source reagent reacting with hydrazine, hydrazides, hydrazone, thiosemicarbazide (mentioned in Scheme 6), or thioacylhydrazine usually under basic conditions (Scheme 7). In the same step, dithiocarbazates were always acylated followed by cyclodehydration (concentrated sulfuric acid always being the dehydrant, occasionally CF_3COOH) to generate 2-thiol/thione 1,3,4-thiadiazoles. Wei et al. and Kadi et al. reported a general method to synthesize dithiocarbazate and 1,3,4-thiadiazole with acylhydrazide (**Scheme 8**) [8].



Scheme 6



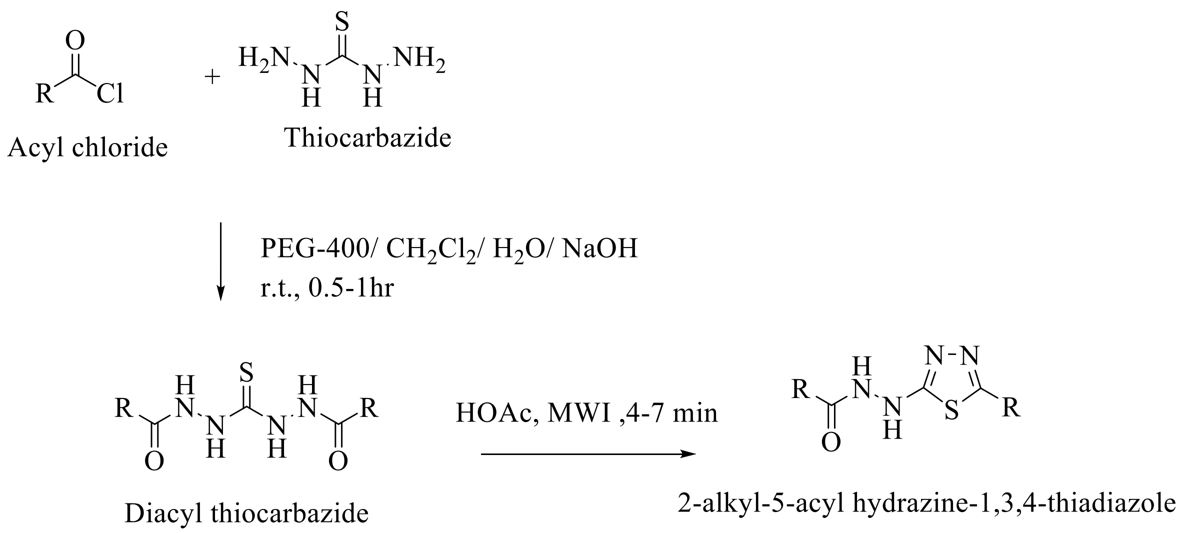
Scheme 7



Scheme 8

2.1.2.3. From Thiocarbazides:

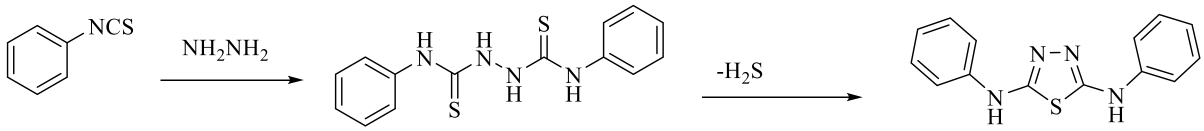
In reaction between Acyl chloride with thiocarbazide in presence of polyethylene glycol 400, dichloromethane, water, sodium hydroxide at room temperature for 5-1 hr, produces diacyl thiocarbazide. This compound then treated with acetic acid in presence of microwave irridation for 4 to 7 min and produces 2-alkyl-5-acyl hydrazine-1,3,4-thiadiazine (**Scheme 9**) [1].



Scheme 9

2.1.2.4. From bithioureas:

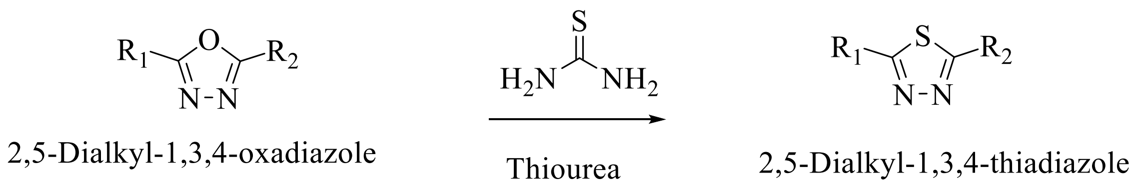
Thiadiazole was prepared by reaction of an alpha amino group on each end of hydrazines or thiosemicarbazides with isothiocyanates and followed by loss of hydrogen sulfide to get 2,5-diamino-1,3,4-thiadiazoles (**Scheme 10**) [9].



Scheme-10

2.1.3. From 1,3,4-oxadiazole:

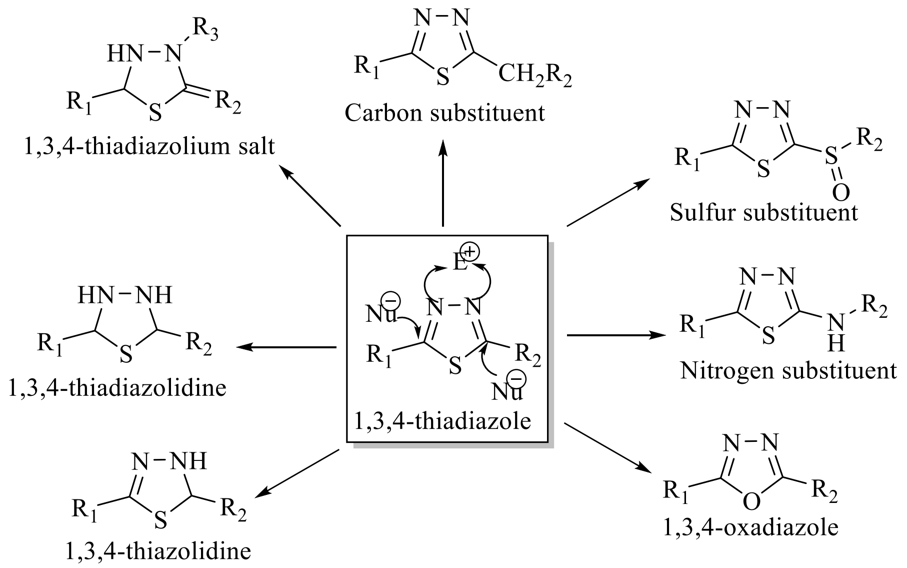
The 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring. When 2,5-dialkyl-1,3,4-oxadiazole treated with thiourea, which produces 2,5-dialkyl-1,3,4-thiadiazole yields of 55–69 %, but the reaction time was long (24-30 h) (**Scheme- 11**) [1].



Scheme-11

2.2. Reactivity of 1,3,4-Thiadiazole:

As with most azoles, thiadiazoles are very weak bases due to the inductive effects of the extra heteroatoms. Unsubstituted 1,3,4-thiadiazole ring (first achieved by Goerdeler and Ohm in 1956) with obvious aromaticity cannot easily be substituted nucleophilic, and electrophilic substitutions on carbon are practically unknown, apart from a few halogenations and mercurations [1], while substituted thiadiazoles are susceptible to nucleophilic attack on a carbon atom with leaving groups generally displaced easily [10]. The ring nitrogen atoms suffer electrophilic attack depending on the tautomerizability of the substituents on the C-2 or C-5 position and 1,3,4-thiadiazolium salts or 1,3,4-thiadiazol-2(3H)-ones that can be prepared. Electrophilic attack on ring sulfur atoms is rarely found; as a result, the reactivity of 1,3,4-thiadiazoles arises from the nucleophilic center localized on the ring nitrogen atoms and from the electrophilic center on the carbon of the C=N bond. A supplementary reactivity can arise from conversions of substituents attached to C2/5. Thus, 1,3,4-thiadiazoles are versatile reagents for synthesis of various compounds (**Scheme 9**). The tautomerism of 1,3,4-thiadiazole is primarily presented by thione−thiol or amino−imino transformation on the C-2 or C-5 position.



Scheme 9: General reactions of 1,3,4-thiadiazoles

2.3. Pharmacological Activity:

After the discovery of the carbonic anhydrases inhibitor acetazolamide AAZ, the synthesis and biological activities of many 1,3,4-thiadiazoles were reported. A large number of these derivatives have been reported to possess diverse pharmacological properties such as herbicidal, antiviral, antiparasitic, antitubercular, anticonvulsant, analgesic, and antisecretory activities. Moreover, much interest has also been focused on the antibiotic (including antibacterial and antifungal), anti-inflammatory, and anticancer activities displayed by compounds incorporating other heterocyclic systems. Owing to the different activities 1,3,4-thiadiazoles possess, we classify them as follows.

2.3.1. Anticancer Activity:

Juszczak M *et al* in their research work reported that a 2-amino-1,3,4-thiadiazole derivative FABT exhibited antiproliferative activity on human non-small lung carcinoma cells (A549). Further mechanistic study involving western blot analysis revealed that FABT inhibit extracellular extracellular-signal regulated kinase (ERK1/2) pathway activity in A549 cells and also promote G0/G1 phase cell cycle arrest [11].

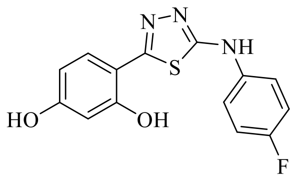
A number of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles was synthesized by Kumar D *et al* and evaluated for cytotoxic activity against various cancer cell lines (prostate; DU145 and LnCaP, breast; MCF-7 and MDA-MB-231, cervical; Hela and ovarian; ovcar-3). The compounds were synthesized by the treatment of some intermediate thiosemicarbazides with acetyl chloride, whereas the reaction between indolyl hydrazides and various aryl isothiocyanates afforded intermediate thiosemicarbazides. All the compounds showed selective cytotoxicity towards cell lines used. Among them the most potential cytotoxic compound is **6f** with IC_{50} value 0.15-1.18 µM [12].

With an aim to develop potential antitumour agents, Altıntop M D *et al* synthesized some novel 1,3,4-thiadiazole derivatives and evaluated for their cytotoxicity on various human cancer cell lines including Bcr-Abl positive myelogenous leukemia (K562) cell line. In vitro study reported that tested cancer cell lines showed sensitivity towards synthesized molecule differently, whereas only compound 2 showed high degree of selectivity against K562 cell line (IC_{50} = 33µM). It also efficiently inhibits Abl protein kinase (IC_{50} = 7.4µM). In silico docking study revealed that nitro thiazole moiety of compound **2** is responsible for hydrogen bonding and hydrophobic interaction with important amino acids of protein kinase (Docking score -9.2 Kcal/mole). Furthermore, the result of the study provides a way to develop potential inhibitor against protein kinase and leukemia [13].

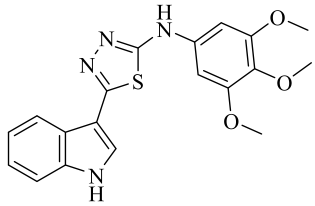
In continuation of research effort to develop anticancer agent Revelant *et al* have synthesized series of 1,3,4-thiadiazole derivatives incorporating thienyl amino group. Synthesis of the targeted compounds was achieved via two step procedure, firstly conversion of thiophene isothiocyanate to thiosemicarbazide intermediate and then cyclization of intermediate thiosemicarbazides to final thiadiazole derivatives. The synthesized thiadiazoles and intermediate thiosemicarbazides subjected to anticancer evaluation on panel of six human cancer cell line. Among the compounds, derivative **3f** and **3i** exhibited better anticancer activity with half inhibitory concentration value lesser than 10 µM [14].

Sun J *et al* synthesized a new series of 1,3,4-thiadiazole containing 1,4-benzodioxan derivatives were prepared by condensing 2,3-dihydrobenzo[b][1,4] dioxine-6-carboxylic acid with thiosemicarbazide . Synthesized compounds were evaluated for antiproliferative activity which acts by inhibiting FAK (Focal adhesion kinase) by using antiproliferative and Western-blot assay. Result of this study revealed that compound **2p** showed most potent activity against HEPG2 cancer cell line (EC_{50} = 10.28 µg/mL for HEPG2 and EC_{50} = 10.79 µM for FAK) and these results were compared with 5-Fluorouracil and Staurosporine using as standard [15].

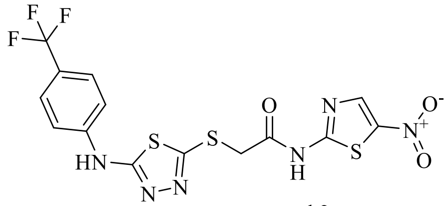
Histone deacetylase (HDAC) inhibitors containing 1,3,4-thiadiazole hydroxamic acid derivatives are some new series of compounds that are synthesized by Guan P *et al* by condensing aromatic acids with thiosemicarbazide. Synthesized compounds were evaluated for anticancer activity by using HDAC enzyme assay and potent growth inhibition in some tumor cell lines. Among the synthesized compound **6i** (IC_{50} = 0.089 µM), exhibited better inhibitory effect compared with Suberoylanilide hydroxamic acid (SAHA, Vorinostat) (IC50 = 0.15 µM) [16].



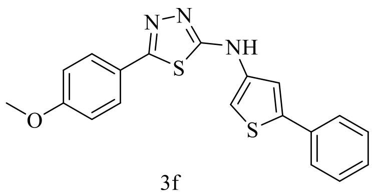
FABT



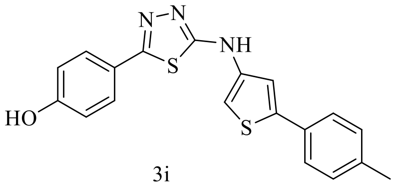
6f (IC_{50} - 0.15-1.18 µM)



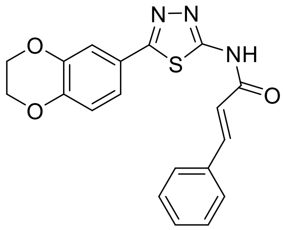
compound 2
IC_{50} - 33 µM , K562



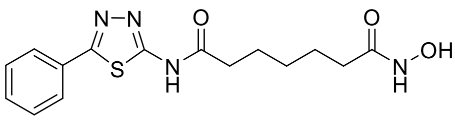
3f



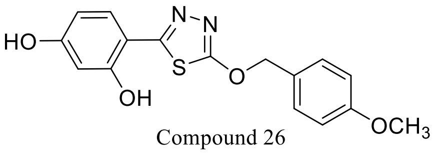
3i



Compund 2p



Compound 6i
IC_{50} = 0.089 µM



Compound 26

New series of 5-substituted-2-(2,4-dihydroxyphenyl)1,3,4-thiadiazole derivatives were synthesized and these compounds were evaluated for anticancer activity against various human cancer cells. Result of this study revealed that compound **26** was most active among them. This compound 26exhibited higher inhibitory activity against T47D cells (human breast cancer cells) than cisplatin revealed by Matysiak J *et al* [17].

2.3.2. Antimicrobial Activity:

2.3.2.1. Antibacterial Activity:

Treatment of infectious diseases like tuberculosis has been becoming a serious issue due to multidrug resistance posed by Mycobacterium tuberculosis strains. The present research work pursued by Oruc *et al* deals with synthesis of 1,3,4-thiadiazole based molecules active against tuberculosis. The structure of the synthesized molecule was confirmed by IR, ^{1}H NMR and MS data. Among the synthesized derivatives, compound **22** was significantly inhibited H37Rv strains of the microbes as reported by BACTEC 460 radiometric assay. Also, a system for the design of new active molecule has been evolved using SAR studies based on Electronic

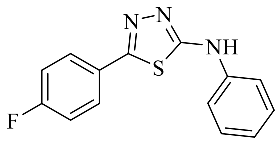
Topological Method (ETM) and application of Artificial Neuronal Network (ANN) method [18].

Jazayeri *et al* aims to design and synthesize some nitroaryl-1,3,4-thiadiazole and quinole hybrids which will be active against bacteria. The compounds were designed by incorporating nitroaryl-1,3,4-thiadiazole moiety in piperazine ring at C-7 position of the gatifloxacin. The synthesized derivatives were tested for antibacterial activity on a range of Gram +ve and Gram –ve bacteria. Result revealed that among the compounds the most active compounds **6a** showed potential growth inhibition of some Gram +ve bacteria. Also in vitro cytotoxicity study on mouse fibroblast demonstrated that the compounds exhibited antibacterial activity without having any cytotoxicity [19].

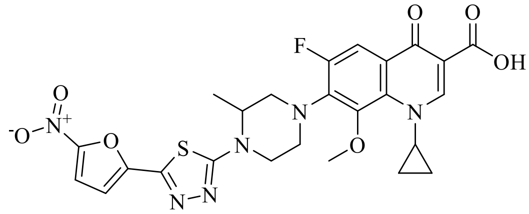
Recently it has been demonstrated that SecA protein might be effective target for antibacterial drugs. Research work performed by Cui *et al* aims to design triazole and thiadiazole hybrid molecules having thiouracil moiety against Sec A proteins, the new molecular were design based on a known inhibitor. The compounds were synthesized and evaluated for antibacterial activity against three Gram negative bacterial strains. Compounds with significantly higher antibacterial activity also tested for Sec A inhibitory potential. It was seen that the compound **7d** (IC_{50}-9.7μg/ml) is a promising antibacterial molecule with SecA inhibition property. In silico docking study also went in agreement with result of the in vitro experiment [20].

A series **(8a-g)** of 1,3,4-thiadiazole derivatives bearing adamantyl moiety were synthesized from corresponding adamantly isothiocyanate via a thiosemicarbazide intermediate by Kadi *et al* for the development of new antibacterial agent. The compounds were screened for antibacterial activity against Gram-positive and Gram-negative bacteria following agar well disffusion procedure. Result revealed that the new thiadiazoles exhibit notable antibacterial activity on tested bacteria, where the compound **8e** showed prominent antibacterial activity compared to standard drug Ampicillin [21].

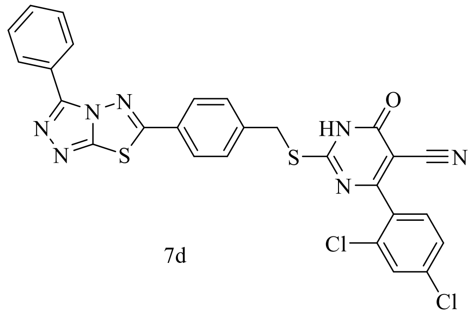
New series of nitroaryl-1,3,4-thiadiazol derivatives were synthesized by condensing 2-chloro,5- nitroaryl-1,3,4-thiadiazol with gatifloxacin and $NaHCO_3$ in DMF by Jazayeri *et al*. Synthesized compounds were evaluated for antimicrobial activity against gram-positive bacteria including staphylococcus epidermidis (MIC = 0.0078 μg/mL), bacillus subtilis (MIC = 0.125 μg/mL), E. Faecalis (MIC = 0.0039 μg/mL) and micrococcus luteus, escherichia coli using agar dilution method and their zone of inhibition was compared with gatifloxacin as standard. Due to the presence of nitrofuran at C-2 of thiadiazole ring caused complete inhibition of DNA gyrase or DNA topoisomerase IV. Result of this study revealed that compound **6a** was most potent compound [22].



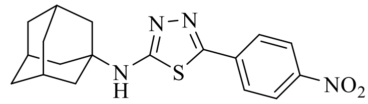
22



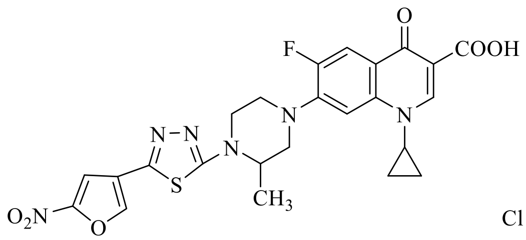
6a



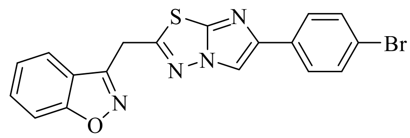
7d



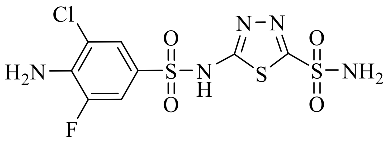
8e



Compound 6a



Compound 3d



Compound 16

Nishimori I. *et al* synthesized some sulfonamide containing 1,3,4-thiadiazole derivatives which acts on β-carbonic anhydrases enzyme isolated from bacterial pathogen (Salmonella enterica, Salmonella typhimurium, Candida albicans etc), involved in hydration of carbon dioxide to bicarbonate and protons, which is essential for many organisms, including bacteria and fungi. These synthesized compounds were evaluated for antimicrobial activity against Salmonella enterica, Salmonella typhimurium and their activity was compared with acetazolamide as standard drug. Result of this study revealed that compound **16** was most potent with inhibition constant of 51nM against stCA 1 and 38 nM against stCA 2, while acetazolamide inhibited stCA 1 and stCA2 with KI of 59 and 84 nM, respectively [23].

A new series of novel methylene bridged benzisoxazolylimidazo[2,1-b][1,3,4]thiadiazole derivatives were synthesized by condensing benzisoxazolyl-3-acetic acid and thiosemicarbazide by Lazmi S. R. *et al*. Synthesized compounds were evaluated for antimicrobial activity against two Gram-positive bacteria, Staphylococcus aureus, Bacillus subtilis and Gram-negative bacteria Pseudomonas aeruginosa, Escherichia coli using

Ampicillin as standard drug and antifungal activity against Candida albicans and Aspergilus fumigatus using Clotrimazole as standard drug. Result of this study revealed that compound **3d** was most potent [24].

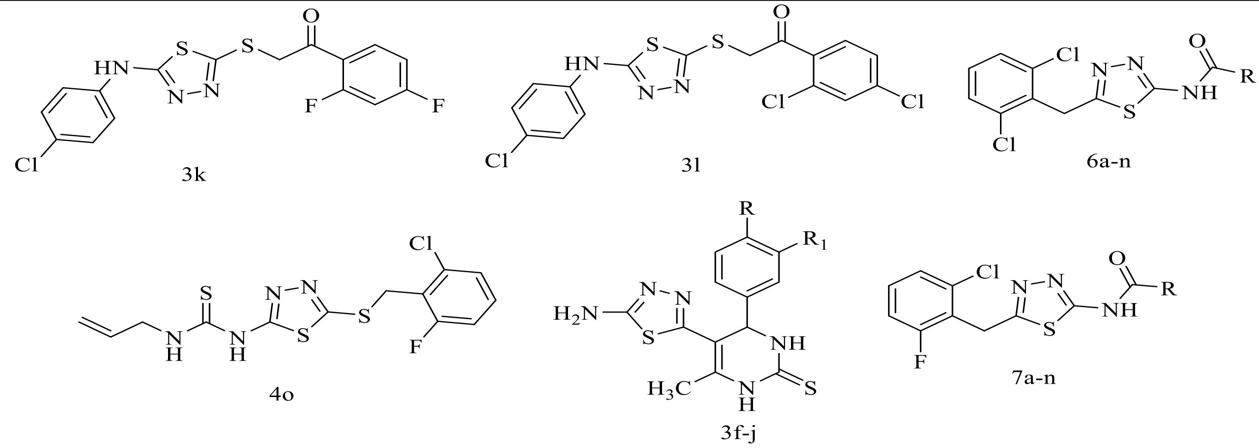
2.3.2.2. Antifungal Activity:

With an aim for the development of new antifungal agent, Karaburun *et al* have synthesized some new 1,3,4-thiadiazoles via three steps and evaluated for their antifungal activity against eight candida strains. Among the synthesized compounds the derivative **3k** and **3l** showed potential antifungal activity. The mechanism of antifungal activity was assessed using in vitro ergosterol quantification assay via LC-MS/MS method and in silico docking study against 14-α-demethylase for investigation of inhibitory potency on ergosterol biosynthesis. Theoretical calculation of ADME profile revealed that the compounds are druggable candidates, which elevated their Pharmacologic importance. From the result of this research work it is concluded that the new thiadiazole compounds are potential antifungal agents with inhibitory activity on ergosterol biosynthesis [25].

In search of novel lead molecule having antifungal potency, Wang *et al* has designed and synthesized a series of 1,3,4-thiadiazole based thiourea entails with thioether functionality. The targeted compounds were synthesized using commercially supplied hydrazine carbothioamide as starting material. Structure of the prepared compounds analysed by experimental data obtained from HRMS, IR, ^{1}H NMR and ^{13}C NMR. Antifungal activity evaluation on four fungal strains (*Curvularialunata, Cotton Fusarium Wilt, P. P. var nicotianae* and *Fusarium spp*) showed that some compounds exhibit significant antifungal activity against tested fungal strain, among them the best molecule is **4o**. The work warrant further development of potent antifungal agent [26].

A series of 5-amino-1,3,4-Thiadiazole tethered with dihydropyrimidine **3f-j,** was synthesised via an easy synthetic procedure by Karthic *et al*. The thiadiazole containing pyrimidine moiety synthesized from intermediate carbothioamide derivatives, and under appropriate reaction condition the pyrimidine ester was converted to carbothioamides. The structure of the synthesized compound assured by analysing the data obtained from IR, ^{1}H NMR, ^{13}C NMR and GC-MS. The result of the antifungal activity evaluation revealed that the compound exhibits notable growth inhibition of fungal strain used [27].

A series of new 2-amino-1,3,4-thiadiazole derivatives (**6a-n and 7a-n**) containing acyl functionality was synthesized by Mustafa *et al* and tested for their antifungal activity on plant pathogenic fungal strains. The result of the antifungal evaluation showed moderate to excellent activity of the compounds against fungal strain employed. Also, an in-silico study via similarity search, homology modelling, molecular dynamics and molecular docking assist in finding potential molecular target of the synthesized compound. The outcome of the study demonstrated a way for further development of antifungal agent [28].

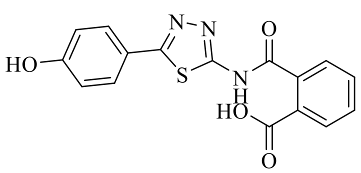


2.3.2.3. Antiviral Activity:

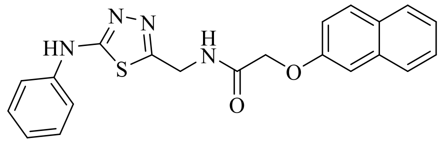
To develop novel antiviral agents, Brai *et al* has designed and synthesized a series of thiadiazole based molecules against viral target human helicase DDX3X. Preliminarily compounds were screened for their enzyme inhibitory activity and then the best active compounds were subjected to antiviral assay on HIV-1 cells. Cytotoxicity of the compounds were also determined on H9 cells. In vitro ADME assays of the compound revealed different metabolic stability of the compounds, however exhibit good membrane permeability and aqueous solubility. In silico computational docking study showed required binding interactions of the compounds towards enzyme. Taking in to account both the biological profile and pharmacokinetic profile it is concluded that the best compound **24** (K_i = 1.9 ± 0.4 μM) is a good antiviral agent, which may be further modified for the development of promising antiviral agents [29].

Some 1,3,4-thiadiazole derivatives synthesized by Hamad et al from amino acid analogues were screened for anti-HIV-1 (strain IIIB) and anti-HIV-2 (strain ROD) activity by the inhibition of the virus-induced cytopathic effect in human MT-4 cells based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. 2-(Naphthalen-2-yloxy)-*N*-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)methyl)acetamide **6a** possessed in vitro inhibitory activity with EC_{50} values of 0.96 μg/mL (HIV-1 strain IIIB) and 2.92 μg/mL (HIV-2 strain ROD), respectively, but low selectivity (SI <1). Structure–activity relationship (SAR) studies have suggested that the substitution of the acetamide moiety with a thiadiazole ring may lead to more active derivatives compared to other compounds bearing different heterocyclic rings. Even though anti-HIV activity and selectivity of derivative **6a** is limited compared to efavirenz (EC_{50} value of 0.003 µg/mL and SI ≈ 13333), it may serve as the basis for future modification in the search for new potent non-nucleoside antiviral agents [30].

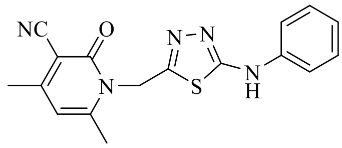
A pyridine containing aryl amino 1,3,4-thiadiazole **12** was synthesized through cyclization of corresponding thiosemicarbazide. Thiadiazole **12** was evaluated for antiviral activity on hepatitis B virus (HBV) using HepG2 cells and cytotoxicity of the compound was also tested for cell safety at antiviral concentration. From the result of the work, it was seen that compound is effective toward inhibition of virus (IC_{50} = 0.3 µM) at non cytotoxic concentration (CC_{50} = 333.3 µM). The outcome of the research work warrants further optimization and development of 1,3,4-thiadiazole based antiviral agents [31].



24



6a



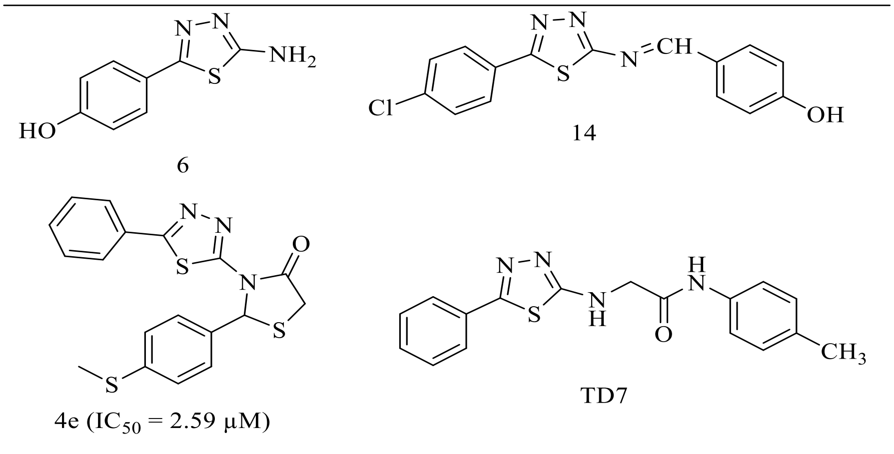
12

2.3.3. Antidiabetic Activity:

Inhibition of α-glucosidase enzyme is an important aspect for the control of type-2 DM, in view of this fact Javid *et al* have designed and synthesized a series of thiadiazole derivatives to develop molecule as a α-glucosidase inhibitor. In this study new series of thiadiazole imine derivatives were synthesized and subjected to α-glucosidase inhibition assay. Biological activity study data suggested that compounds are excellent inhibitor of the enzyme with activity in the micromolar range. The best active compound among the series are **6** and **14** having **IC_{50}** value of **2.30** µM. Docking study of the compounds against enzyme also revealed required binding interaction at the catalytic site and in good agreement with experimental results [32].

Datar *et al* have synthesized some thiadiazole derivatives and evaluated for their in vivo antidiabetic activity on alloxan induced diabetic rat and also subjected to in vitro α-amylase inhibitory assay. The compounds were preliminarily designed based on their docking study against PPAR-γ and synthesized thereafter. Outcome of this work revealed that compounds showed significant lowering of blood glucose level, among them the best active compound was found to be **TD7** in in vivo and in vitro study [33].

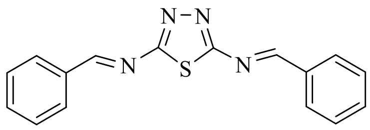
Gummidi *et al* reported the synthesis and antidiabetic activity evaluation of 1,3,4-thiadiazole and thiazolidine hybrid molecules. The compounds were synthesized via multicomponent one pot reaction step and subjected to in vitro α-glucosidase and α-amylase inhibitory assay. Outcome of the biological study suggested that the compounds exhibit excellent enzyme inhibition property in the micromolar range. Among the compounds, derivative **4e** (**IC_{50} = 2.59 μM**) showed best inhibitory potency and its potency is superior than standard drug acarbose. Furthermore, molecular docking experiment directed the significance of para thiomethyl group in phenyl moiety on thiadiazolidine ring towards enzyme inhibition. This research work provides novel lead molecule for the development of compounds active against diabetic complications [34].



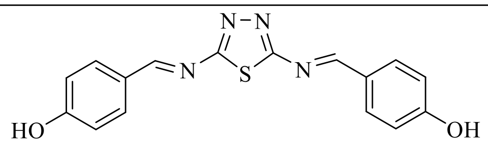
2.3.4. Antihyperlipidemic Activity:

Hamadneh *et al* deals with the synthesis and hypolipidemic potential of three 1,3,4-thiadiazole derivatives. Compound **1**, **2** and **3** were prepared and evaluated for anti hyperlipidemic activity in Triton WR-1339-induced acute hyperlipidaemia of rat model. Biological activity study result revealed that compound **1** and **2** causes significant reduction of TG and TC levels. All three compounds induced significant reduction of LDL level, while only compound **2** exhibited increased level of HDL. In silico docking study involving binding interaction between compounds and PPAR-α at 1KKQ binding site revealed significant bonding interaction, validated from docking score [35].

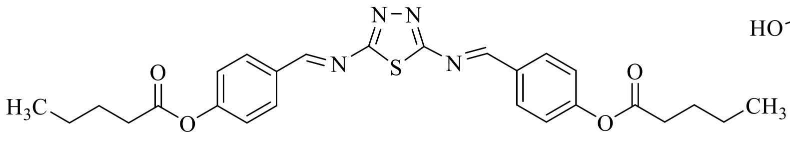
Patel *et al* carried out the synthesis of a series of imidazo-thiadiazole with substitution at 2, 5 and 6 position and evaluated their antihyperlipidemic activity on Triton-X-100 induced hyperlipidaemia in rat model. The compounds were designed based on pharmacophore model, developed from existing compounds and synthesized via several reaction steps. The result of the biological study revealed that after treatment with compounds causes significant lowering of serum lipid profile in hyperlipidemic rat, among the compound the best active molecule is **7e**. This study provides a way for the development of novel lipid lowering agent [36].



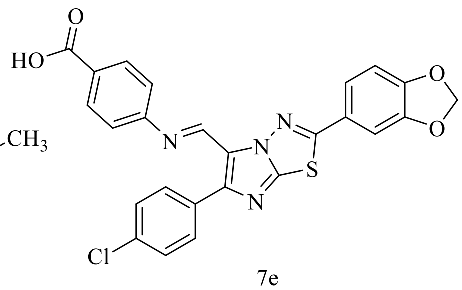
Compound 1



Compound 2



Compound 3



7e

2.3.5. Anticonvulsant Activity:

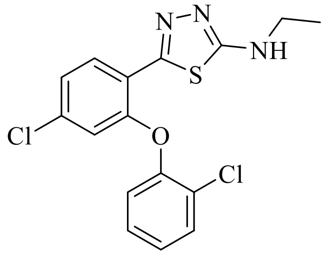
Foroumadi *et al* have reported the synthesis of amino thiadiazole derivatives **4a-d** via simple reaction steps, and assessed their anticonvulsant property on pentylene tetrazole (PTZ) and maximal electroshock (MES) induced convulsion in animal model. Outcome of the experiment demonstrated compound **4c** (MES (ED_{50}=20.11) and PTZ (ED_{50}=35.33)) is the better active compound among the tested derivatives [37].

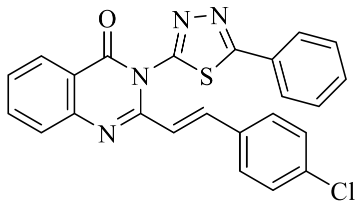
A series of quinazoline containing 1,3,4-thiadiazole derivatives were synthesized by Jatav *et al* and evaluated for anticonvulsant activity in PTZ and MES induced convulsion in mice model. Biological activity data displayed compound **4a**, **4e** and **4p** are only compound exhibit anticonvulsant activity among the derivatives synthesized [38].

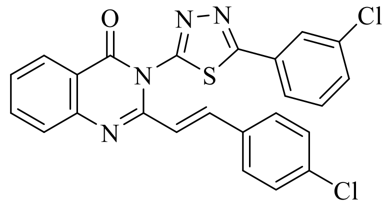
In another research study, Botros et al carried out the synthesis of some 1,3,4-thiadiazole and phenytoid hybrid molecules (**6a-e**) and tested their anticonvulsant activity in maximal electroshock seizure induced mice. A moderate potency towards reduction of convulsion was observed for the prepared derivatives [39].

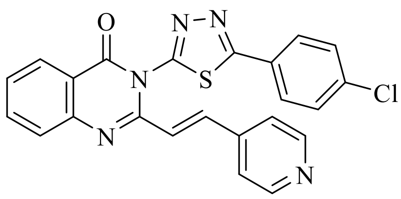
New series of substituted 2-hydrazino-1,3,4-thiadiazole derivatives were prepared by condensing aryl dithio esters with thiocarbonyl hydrazines. Synthesized compounds were evaluated for anticonvulsant activity by using Maximal Electroshock Seizures (MES) and Maximal Metrazol Seizures (MMS) in the Mouse and Rat and results were Compared with Phenytoin, Phenobarbital, and Carbamazepine After Oral Administration. Result of this study revealed that **compound 7** (2-(aminomethyl)-5-(2-biphenylyl)-1,3,4-thiadiazole) was most potent compound among them by Stillings *et al* [40].

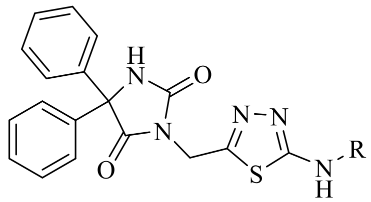
Dogan *et al* synthesized new series of 2,5-disubstituted-1,3,4-thiadiazoles by dehydrative cyclization in acidic medium of the thiosemicarbazides. Synthesized compounds were evaluated for anticonvulsant activity against pentylenetetrazole-induced convulsions and results were compared with sodium valproate (150 mg/kg, ip, 80% protection) as a standard pharmacological drug. Highest protection 90 % was obtained by compound 2a [2-Ethylamino-5-(3-hydroxy-2-naphthyl)-1,3,4-thiadiazole] (ED_{50} 33 mg/kg) [41].

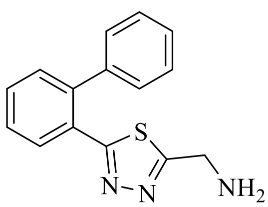

4C

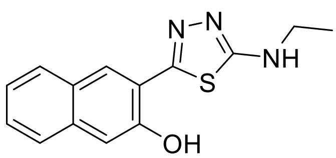

4a


4e


4p


6a-e


Compound 7

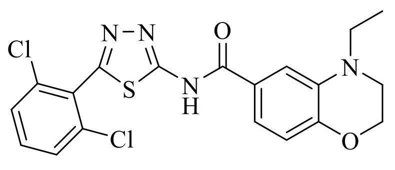

Compound 2a

2.3.6. Analgeasic and Anti-Inflammatory Activity:

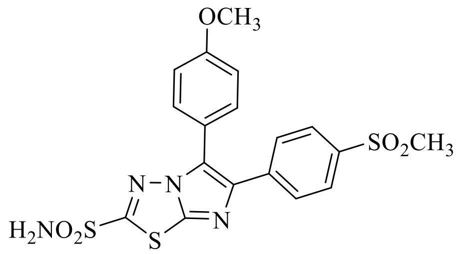
Hilfiker *et al* synthesized new series of aminothiadiazole amide derivative by condensation of semicarbathiazide with desired carboxylic acid and these synthesized compounds were evaluated for anti-inflammatory activity with cyclooxygenase-1 (COX-1), COX-2, and thromboxane synthase inhibitory activity with selective EP3 receptor antagonist. Result of this study revealed that compound **24** shows good antagonist activity for human EP3 and also against other EP subtypes like DP, FP, TP prostenoid receptor [42].

New series of 2-trifluoromethyl/ sulfonamido-5,6-diaryl substituted imidazo[2,1-b]-1,3,4thiadiazoles derivatives were synthesized by condensing 2-amino-5-trifluoromethyl/sulfonamido-1,3,4-thiadiazoles with a-bromo-1,2-(p-substituted)diaryl-1-ethanones by Gadad *et al* . Synthesized compounds were evaluated for anti-inflammatory activity and their activity was compared with celecoxib in the carrageenan-induced rat paw edema method. Among them compound **15j** shown selective inhibitory activity toward COX-2 (80.6%) over COX-1 (30.6%) [43].

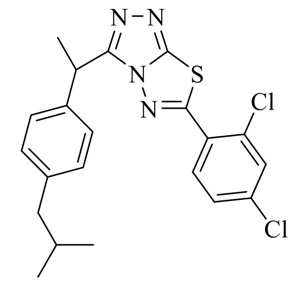
Some 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives were prepared by condensing 4-amino-5-substituted-3-mercapto-(4H)1,2,4-triazoles with aromatic acids through one-pot reaction. Synthesized compounds were evaluated for anti-inflammatory and analgesic activity with reduced ulcerogenic effect with compared to Ibuprofen, Flurbiprofen. Result of this study revealed that **4a** showed most potent activity by Amir M *et al* [44].



Compound 24



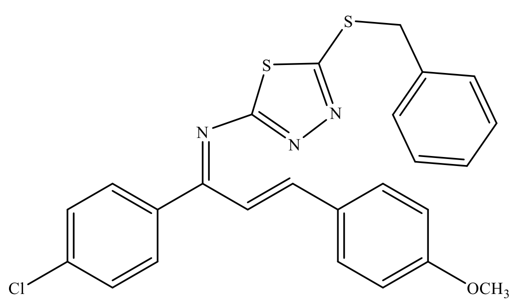
Compound 15j



Compound 4a

2.3.7. Antidepressant Activity:

A number of new imine derivatives of 5-amino-1,3,4-thiadiazole-2-thiol synthesized by Ahmed B *et al* by carbon disulfide addition to thiosemicarbazide under reflux and subsequent addition of different chalcones. Synthesized compounds were evaluated for their anti-depressant activity using imipramine as reference drug. compounds 5-{[1-(4-chlorophenyl)-3-(4-methoxy-phenyl)prop-2-en-1ylidene]-amino}- 5-benzylthio-1, 3,4 –thiadiazole (4ia) have shown significant anti-depressant activity, which decreased immobility time by 77.99% compared to the standard imipramine (82%). These compounds in the series have passed neurotoxicity tests also [45].



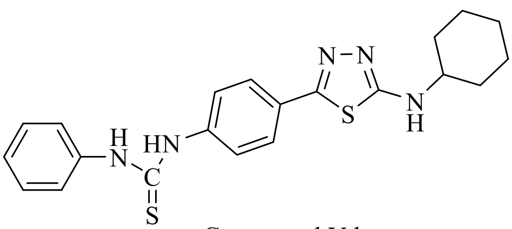
Compound 4ia

2.3.8. Antitubercular Activity:

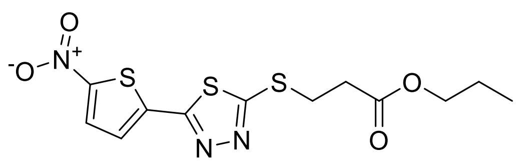
Rollas S *et al* synthesized a new series of N-phenyl-N7-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thiourea derivatives by condensing 4-(benzoylamino) benzoylhydrazine with isothiocyanates. Synthesized compounds were evaluated for antitubercular activity by using in vitro BACTEC 460 Radiometric System against Mycobacterium tuberculosis. Results were compared with Rifampicin using as standard. The highest inhibition observed with the synthesized compound **Vd** is 67% for N-phenyl-N7-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenyl]thiourea (MIC- 6.25 µg/ml) [46] .

Two series of 2- and 3-[5-(nitroaryl)-1,3,4-thiadiazol-2-ylthio, sulfinyl and sulfonyl] propionic acid alkyl esters were synthesized by the reaction of nitroaryl aldehyde with thiosemicarbazide in refluxing ethanol. Synthesized compounds were screened for antituberculosis activity against Mycobacterium tuberculosis using the BACTEC 460 radiometric system. The MIC values for the compounds showing more than 90% inhibition were determined. The result of the study was compared with standard Rifampicin. The compound **7i** was the most active one (MIC = 1.56 µg/ml) revealed by Foroumadi A *et al* [47].

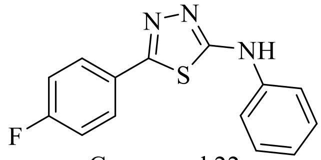
A series of 2,5-disubstituted-1,3,4-thiadiazoles were synthesized by reacting 4-substituted benzoic acid hydrazides with thiosemicarbazides by Rollas S *et al.* Synthesized compounds were elucidated and screened for the antituberculosis activity against Mycobacterium tuberculosis using the BACTEC 460 radiometric system. Among the tested compounds, 2-phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole (**22**) showed the highest inhibitory activity (69% inhibition) [48].



Compound Vd



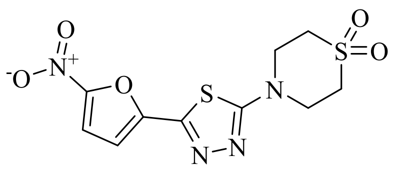
Compound 7i



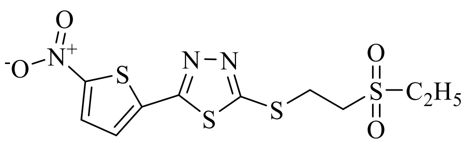
Compound 22

2.3.9. Anti-Helicobacter Pylori Activity:

Foroumadi *et al* synthesized a new series of N-[5-(5-nitro-2-heteroaryl)-1,3,4thiadiazol-2-yl]thiomorpholine derivative by refluxing 5-nitroaryl-2-carboxaldehydes with thiosemicarbazide. Synthesized compounds were evaluated in vitro Anti helicobacter pylori activity and the results were compared with Metronidazole and Amoxicillin. They found that nitrofuran analogue 4-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol2-yl] thiomorpholine 1,1-dioxide (**7b**) containing thiomorpholine-S, S dioxide moiety was the most potent compound tested [49]. A series of 5-(nitroaryl)-1,3,4-thiadiazoles with sulfur containing alkyl side chain similar to tinidazole molecule were synthesized by condensing 5-nitroarylcarboxaldehyde diacetate with thiosemicarbazide by Shafiee A *et al*. synthesized compounds were evaluated against Helicobacter pylori by using disk diffusion method. Both the structure of the nitroaryl unit and the pendent group on 2-position of 1,3,4-thiadiazole ring dramatically impact the anti-H. pylori activity. Among them 2-((2-(ethylsulfonyl) ethyl) thio)-5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazole, compound **7a** was the most potent compound tested against clinical isolates of Helicobacter pylori [50].



Compound 7b



Compound 7a

2.4. Reference:

1. Hu, Y., Li, C., Wang, X., Yang, Y., & Zhu, H. (2014). 1,3,4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry. *Chemical reviews, 114 10*, 5572-610.

2. Augustine, K. J., Vairaperumal, V., Narasimhan, S., Alagarsamy, P., &Radhakrishnan, A. (2009). Propylphosphonic anhydride (T3P®): an efficient reagent for the one-pot synthesis of 1,2,4-oxadiazoles, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles. *Tetrahedron*, 65(48), 9989-9996. https://doi.org/10.1016/j.tet.2009.09.114.

3. Ghozlan, S. A. S., Al-Omar, M. A., Amr, A. E. G. E., Ali, K. A., & El-Wahab, A. A. A. (2011). Synthesis and antimicrobial activity of some heterocyclic 2,6-bis(substituted)-1,3,4-thiadiazolo-, oxadiazolo-, and oxathiazolidino-pyridine derivatives from 2,6-pyridine dicarboxylic acid dihydrazide. *J. Heterocycl. Chem*, 48 (5), 1103.

4. Rzeski, W., Matysiak, J., &Kandefer-Szerszeń, M. (2007). Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound. *Bioorganic & Medicinal Chemistry*, 15(9), 3201-3207. https://doi.org/10.1016/j.bmc.2007.02.041.

5. Tu, G., Li, S., & Huang, H. (2008) Novel aminopeptidase N inhibitors derived from 1,3,4-thiadiazole scaffold. *Bioorganic & Medicinal Chemistry*. 16(14): 6663-6668. DOI: 10.1016/j.bmc.2008.05.081.

6. Schüttelkopf, A. W., Gros, L., Blair, D. E., Frearson, J. A., van Aalten, D. M., & Gilbert, I. H. (2010). Acetazolamide-based fungal chitinase inhibitors. *Bioorganic & medicinal chemistry*, *18*(23), 8334–8340. https://doi.org/10.1016/j.bmc.2010.09.062.

7. Alegaon, S. G., Alagawadi, K. R., &Sonkusare, P.V. (2012). Novel imidazo[2,1-b][1,3,4]thiadiazole carrying rhodanine-3-acetic acid as potential antitubercular agents. *Bioorganic & Medicinal Chemistry Letters*, 22(5), 1917-1921. DOI: 10.1016/j.bmcl.2012.01.052.

8. Wei, M. X., Feng, L., Li, X. Q., Zhou. X. Z., &Shao Z. H. (2009) Synthesis of new chiral 2,5-disubstituted 1,3,4-thiadiazoles possessing gamma-butenolide moiety and preliminary evaluation of in vitro anticancer activity. *European Journal of Medicinal Chemistry*, 44(8), 3340-3344. DOI: 10.1016/j.ejmech.2009.03.023.

9. Hall, M. D., Salam, N. K., Hellawell, J. L., Fales, H. M., Kensler, C. B., Ludwig, J. A., Szakács, G., Hibbs, D. E., & Gottesman, M. M. (2009). Synthesis, activity, and

pharmacophore development for isatin-beta-thiosemicarbazones with selective activity toward multidrug-resistant cells. *Journal of medicinal chemistry*, *52*(10), 3191–3204. https://doi.org/10.1021/jm800861c

10. Joule, J. A., Mills, K. Heterocyclic Chemistry; John Wiley &Sons:New York, 2008.
11. Juszczak, M., Matysiak, J., Szeliga, M., Pożarowski, P., Niewiadomy, A., Albrecht, J., &Rzeski, W. (2012). 2-Amino-1,3,4-thiadiazole derivative (FABT) inhibits the extracellular signal-regulated kinase pathway and induces cell cycle arrest in human non-small lung carcinoma cells. *Bioorganic & medicinal chemistry letters*, *22*(17), 5466–5469. https://doi.org/10.1016/j.bmcl.2012.07.036.
12. Kumar, D., Kumar, MN., Noel, B., Shah, K. (2012). A series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles as potent cytotoxic agents. *European Journal of Medicinal Chemistry*, 55, 432-438. https://doi.org/10.1016/j.ejmech.2012.06.047.
13. Altıntop, M. D., Ciftci, H. I., Radwan, M. O., Sever, B., Kaplancıklı, Z. A., Ali, T., Koga, R., Fujita, M., Otsuka, M., &Özdemir, A. (2017). Design, Synthesis, and Biological Evaluation of Novel 1,3,4-Thiadiazole Derivatives as Potential Antitumor Agents against Chronic Myelogenous Leukemia: Striking Effect of Nitrothiazole Moiety. *Molecules (Basel, Switzerland)*, *23*(1), 59. https://doi.org/10.3390/molecules23010059.
14. Revelant, G., Gadais, C., Mathieu, V., Kirsch, G., Hesse, S. (2014). Synthesis and antiproliferative studies of 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles. *Bioorganic & Medicinal Chemistry Letters*, 24(12), 2724-2727. https://doi.org/10.1016/j.bmcl.2014.04.043.
15. Sun, J., Yang, Y. S., Li, W., Zhang, Y. B., Wang, X. L., Tang, J. F., & Zhu, H. L. (2011). Synthesis, biological evaluation and molecular docking studies of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan as potential antitumor agents. *Bioorganic & medicinal chemistry letters*, *21*(20), 6116–6121. https://doi.org/10.1016/j.bmcl.2011.08.039.
16. Guan, P., Sun, F., Hou, X., Wang, F., Yi, F., Xu, W., & Fang, H. (2012). Design, synthesis and preliminary bioactivity studies of 1,3,4-thiadiazole hydroxamic acid derivatives as novel histone deacetylase inhibitors. Bioorganic & medicinal chemistry, 20(12), 3865–3872. https://doi.org/10.1016/j.bmc.2012.04.032
17. Matysiak J. (2007). Evaluation of electronic, lipophilic and membrane affinity effects on antiproliferative activity of 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-

thiadiazoles against various human cancer cells. European journal of medicinal chemistry, 42(7), 940–947. https://doi.org/10.1016/j.ejmech.2006.12.03

18. Oruç, E. E., Rollas, S., Kandemirli, F., Shvets, N., &Dimoglo, A. S. (2004). 1,3,4-Thiadiazole Derivatives. Synthesis, Structure Elucidation, and Structure−Antituberculosis Activity Relationship Investigation. Journal of Medicinal Chemistry, 47(27), 6760–6767. https://doi.org/10.1021/JM0495632.
19. Foroumadi, A., Mansouri, S., Emami, S., Mirzaei, J., Sorkhi, M., Saeid-Adeli, N., &Shafiee, A. (2006). Synthesis and antibacterial activity of nitroaryl thiadiazole-levofloxacin hybrids. *Archiv der Pharmazie*, *339*(11), 621–624. https://doi.org/10.1002/ardp.200600108.
20. Cui, P., Li, X., Zhu, M., Wang, B., Liu, J., & Chen, H. (2017). Design, synthesis and antimicrobial activities of thiouracil derivatives containing triazolo-thiadiazole as SecA inhibitors. *European journal of medicinal chemistry*, *127*, 159–165. https://doi.org/10.1016/j.ejmech.2016.12.053.
21. Kadi, A. A., El-Brollosy, R. N., Al-Deeb, A. O., Habib, E. E., Ibrahim, M. T., El-Emam, A. A. (2007). Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. *European journal of medicinal chemistry*, 42(2), 235-242. https://doi.org/10.1016/j.ejmech.2006.10.003.
22. Jazayeri S, Moshafi MH, Firoozpour L, et al. Synthesis and antibacterial activity of nitroaryl thiadiazole-gatifloxacin hybrids. European Journal of Medicinal Chemistry. 2009 Mar;44(3):1205-1209. DOI: 10.1016/j.ejmech.2008.09.012.
23. Nishimori, I., Minakuchi, T., Vullo, D., Scozzafava, A., & Supuran, C. T. (2011). Inhibition studies of the β-carbonic anhydrases from the bacterial pathogen Salmonella enterica serovar Typhimurium with sulfonamides and sulfamates. *Bioorganic & medicinal chemistry*, *19*(16), 5023–5030. https://doi.org/10.1016/j.bmc.2011.06.038
24. Lamani, R. S., Shetty, N. S., Kamble, R. R., & Khazi, I. A. (2009). Synthesis and antimicrobial studies of novel methylene bridged benzisoxazolyl imidazo[2,1-b][1,3,4]thiadiazole derivatives. *European journal of medicinal chemistry*, *44*(7), 2828–2833. https://doi.org/10.1016/j.ejmech.2008.12.019
25. Karaburun, A., AcarÇevik, U., Osmaniye, D., Sağlık, B., Kaya Çavuşoğlu, B., Levent, S., Özkay, Y., et al. (2018). Synthesis and Evaluation of New 1,3,4-Thiadiazole Derivatives as Potent Antifungal Agents. *Molecules*, *23*(12), 3129. MDPI AG. http://dx.doi.org/10.3390/molecules23123129.

26. Wang, C., Song, H., Liu, W. *et al.* Design, synthesis and antifungal activity of novel thioureas containing 1,3,4-thiadiazole and thioether skeleton. *Chem. Res. Chin. Univ.* **32,** 615–620 (2016). https://doi.org/10.1007/s40242-016-6023-8.
27. Karthic R, Andrews B, Subramani K. Microwave assisted synthesis and antifungal studies of 5-amino thiadiazole substituted pyrimidine compounds. *Asian J. Research Chem.* 2017; 10(2):119-123. doi: 10.5958/0974-4150.2017.00018.9.
28. Er, M., Abounakhla, A. M., Tahtaci, H., Bawah, A. H., Çınaroğlu, S. S., Onaran, A., &Ece, A. (2018). An integrated approach towards the development of novel antifungal agents containing thiadiazole: synthesis and a combined similarity search, homology modelling, molecular dynamics and molecular docking study. *Chemistry Central journal*, *12*(1), 121. https://doi.org/10.1186/s13065-018-0485-3.
29. Brai, A., Ronzini, S., Riva, V., Botta, L., Zamperini, C., Borgini, M., Trivisani, C. I., Garbelli, A., Pennisi, C., Boccuto, A., Saladini, F., Zazzi, M., Maga, G., &Botta, M. (2019). Synthesis and Antiviral Activity of Novel 1,3,4-Thiadiazole Inhibitors of DDX3X. *Molecules (Basel, Switzerland)*, *24*(21), 3988. https://doi.org/10.3390/molecules24213988.
30. Hamad, N.S., Al-Haidery, N.H., Al-Masoudi, I.A., Sabri, M., Sabri, L. and Al-Masoudi, N.A. (2010). Amino Acid Derivatives, Part 4: Synthesis and Anti-HIV Activity of New Naphthalene Derivatives. Arch. Pharm. Pharm. Med. Chem., 343: 397-403. https://doi.org/10.1002/ardp.200900293.
31. El-Essawy, A., El-Sayed, W. A., El-Kafrawy, S. A., Morshedy, A. S., & Abdel-Rahman, A. H. (2008). Anti-hepatitis B virus activity of new 1,2,4-triazol-2-yl- and 1,3,4-oxadiazol-2-yl-2-pyridinone derivatives. *Zeitschrift fur Naturforschung. C, Journal of biosciences*, *63*(9-10), 667–674. https://doi.org/10.1515/znc-2008-9-1010.
32. Javid, M. T., Rahim, F., Taha, M., Rehman, H. U., Nawaz, M., Wadood, A., Imran, S., Uddin, I., Mosaddik, A., & Khan, K. M. (2018). Synthesis, in vitro α-glucosidase inhibitory potential and molecular docking study of thiadiazoleanalogs. *Bioorganic chemistry*, *78*, 201–209. https://doi.org/10.1016/j.bioorg.2018.03.022.
33. Datar, A. P., &Deokule, A. T. (2014). Design and Synthesis of Thiadiazole Derivatives as Antidiabetic Agents. *Med chem,* 4(4), 390-399. https://doi.org/.10.4172/2161-0444.1000170.
34. Gummidi, L., Kerru, N., Ebenezer, O., Awolade, P., Sanni, O., Islam, S. M., Singh, M. (2021). Multicomponent reaction for the synthesis of new 1,3,4-thiadiazole-thiazolidine-4-one molecular hybrids as promising antidiabetic agents through α-

glucosidase and α-amylase inhibition. *Bioorganic Chemistry,* 115, 105210. https://doi.org/10.1016/j.bioorg.2021.105210.

35. Hamadneh, L. A., Sabbah, D. A., Hikmat, S. J., Al-Samad, L. A., Hasan, M., Al-Qirim, T. M., Hamadneh, I. M., & Al-Dujaili, A. H. (2019). Hypolipidemic effect of novel 2,5-bis(4-hydroxybenzylidenamino)-1,3,4-thiadiazole as potential peroxisome proliferation-activated receptor-α agonist in acute hyperlipidemic rat model. *Molecular and cellular biochemistry*, *458*(1-2), 39–47. https://doi.org/10.1007/s11010-019-03528-5.
36. Patel, H. M., Noolvi, M.N., Goyal, A., Thippeswamy, B. S. (2013). 2,5,6-trisubstituted imidazo[2,1-b][1,3,4]thiadiazoles: search for antihyperlipidemic agents. *European Journal of Medicinal Chemistry*, 65, 119-133. DOI: 10.1016/j.ejmech.2013.04.020.
37. Foroumadi, A., Vahid, S., Sakhteman, A., Abasi, M., Shafiei, A., Rameshk, M., Farazifard, R., & Tabatabai, S.A. (2007). SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NOVEL 2-AMINO-5-[4-CHLORO-2-(2-CHLOROPHENOXY) PHENYL]-1,3,4-THIADIAZOLE DERIVATIVES. *DARU, 15*, 89-93.
38. Jatav, V., Mishra, P., Kashaw, S., Stables, J.P. (2008). CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones, European Journal of Medicinal Chemistry, 43(9), 1945-1954. https://doi.org/10.1016/j.ejmech.2007.12.003.
39. Botros, S., Khalil, N. A., Naguib, B. H., & El-Dash, Y. (2013). Synthesis and anticonvulsant activity of new phenytoin derivatives. *European journal of medicinal chemistry*, *60*, 57–63. https://doi.org/10.1016/j.ejmech.2012.11.025.
40. Stillings, M. R., Welbourn, A. P., & Walter, D. S. (1986). Substituted 1,3,4-thiadiazoles with anticonvulsant activity. 2, Aminoalkyl derivatives. Journal of medicinal chemistry, 29(11), 2280–2284. https://doi.org/10.1021/jm00161a025
41. Dogan, H. N., Duran, A., Rollas, S., Sener, G., Uysal, M. K., & Gülen, D. (2002). Synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. Bioorganic & medicinal chemistry, 10(9), 2893–2898. https://doi.org/10.1016/s0968-0896(02)00143-8
42. Hilfiker, M. A., Wang, N., Hou, X., Du, Z., Pullen, M. A., Nord, M., Nagilla, R., Fries, H. E., Wu, C. W., Sulpizio, A. C., Jaworski, J. P., Morrow, D., Edwards, R. M., & Jin, J. (2009). Discovery of novel aminothiadiazole amides as selective EP(3) receptor

antagonists. *Bioorganic & medicinal chemistry letters*, *19*(15), 4292–4295. https://doi.org/10.1016/j.bmcl.2009.05.074

43. Gadad, A. K., Palkar, M. B., Anand, K., Noolvi, M. N., Boreddy, T. S., & Wagwade, J. (2008). Synthesis and biological evaluation of 2-trifluoromethyl/sulfonamido-5,6-diaryl substituted imidazo[2,1-b]-1,3,4-thiadiazoles: a novel class of cyclooxygenase-2 inhibitors. *Bioorganic & medicinal chemistry*, *16*(1), 276–283. https://doi.org/10.1016/j.bmc.2007.09.038
44. Amir M, Kumar H, Javed SA. Condensed bridgehead nitrogen heterocyclic system: synthesis and pharmacological activities of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid. European Journal of Medicinal Chemistry. 2008 Oct;43(10):2056-2066. DOI: 10.1016/j.ejmech.2007.09.025.
45. Monika Szelig,(2020), Thiadiazole derivatives as anticancer agents, Pharmacological Reports (2020) 72:1079–1100, https://doi.org/10.1007/s43440-020-00154-7
46. Yusuf, M., Khan, R. A., & Ahmed, B. (2008). Syntheses and anti-depressant activity of 5-amino-1, 3, 4-thiadiazole-2-thiol imines and thiobenzyl derivatives. *Bioorganic & medicinal chemistry*, *16*(17), 8029–8034. https://doi.org/10.1016/j.bmc.2008.07.056
47. Alireza Foroumadi, Zahra Kargar, Amirhossein Sakhteman, Zahra Sharifzadeh, Robabeh Feyzmohammadi, Mahnoush Kazemi, Abbas Shafiee, Synthesis and antimycobacterial activity of some alkyl [5-(nitroaryl)-1,3,4-thiadiazol-2-ylthio]propionates, Bioorganic & Medicinal Chemistry Letters,Volume 16, Issue 5, 2006, Pages 1164-1167, ISSN 0960-894X, https://doi.org/10.1016/j.bmcl.2005.11.087.
48. Karakuş, S., & Rollas, S. (2002). Synthesis and antituberculosis activity of new N-phenyl-N'-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thioureas. *Farmaco (Societa chimica italiana : 1989)*, *57*(7), 577–581. https://doi.org/10.1016/s0014-827x(02)01252-1
49. Foroumadi, Alireza & Rineh, Ardeshir & Emami, Saeed & Siavoshi, Farideh & Massarrat, Sadegh & Safari, Fatemeh & Rajabalian, Saeed & Falahati, Mehraban & Lotfali, Ensieh. (2008). Synthesis and anti-Helicobacter pylori activity of 5-(nitroaryl)-1,3,4-thiadiazoles with certain sulfur containing alkyl side chain. Bioorganic & medicinal chemistry letters. 18. 3315-20. 10.1016/j.bmcl.2008.04.033
50. Mirzaei, J., Siavoshi, F., Emami, S., Safari, F., Khoshayand, M. R., Shafiee, A., & Foroumadi, A. (2008). Synthesis and in vitro anti-Helicobacter pylori activity of N-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines and related

compounds. *European journal of medicinal chemistry*, *43*(8), 1575–1580. https://doi.org/10.1016/j.ejmech.2007.11.019

CHAPTER – 3

EXPERIMENTAL WORKS

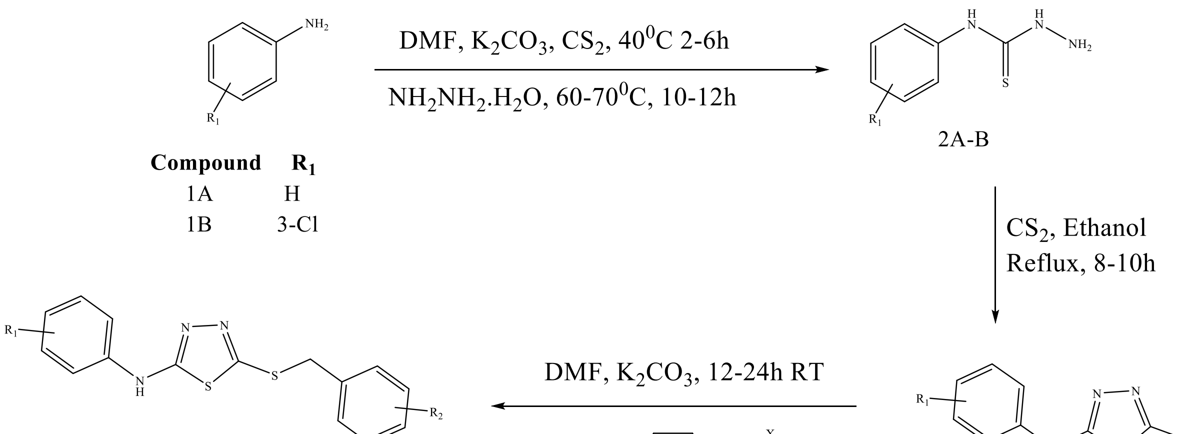
3.1. Plan of Work:

In My M.Pharm work, I am planning for design and synthesis of some [1, 3, 4] Thiadiazole derivatives having suitable functional group, those will be possessed antimicrobial activity. After synthesis the compounds will be characterised by IR Spectroscopy, NMR Spectroscopy (^{1}H NMR, ^{13}C NMR) for structure Elucidation.

3.2. Materials used:

All the chemicals required for this research work were reagent grade and used without further purification. KOH, NaOH, DMF, ethanol, methanol, hydrazine hydrate, DMSO was purchased from Merck, USA, aniline, 3-chloroaniline and CS_2 from Lobe chemicals, benzyl chloride, 4-chloro benzyl chloride, 2,4-dichlorobenzyl chloride, 3-nitro benzyl bromide, 4-methyl benzyl chloride from SpectroChem.

5.2. Scheme for Synthesis of 1,3,4-thiadiazoles



Compound	R_1
1A	H
1B	3-Cl

Compound	R_3	X
2A	H	Cl
2B	4-Cl	Cl
2C	2,4-Cl	Cl
2D	$3-NO_2$	Br
2E	$4-CH_3$	Cl

Compound	R_1	R_2
5A	H	H
5B	H	4-Cl
5C	H	2,4-Cl
5D	H	$3-NO_2$
5E	H	$3-CH_3$
6A	3-Cl	H
6B	3-Cl	4-Cl
6C	3-Cl	2,4-Cl
6D	3-Cl	$3-NO_2$
6E	3-Cl	$3-CH_3$

3.3. Procedure:

3.3.1. Synthesis of N-(substituted phenyl) hydrazine carbothioamides (2A-2B):

Potassium carbonate (0.02 mol) was dissolved in water, then this solution was added with DMF (10ml). After that substituted aniline (0.01 mol) (1A & 1B) and carbon disulphide (0.02 mol) was added in 10 min interval to that mixture and stirred well for 6-7 hour at 20-25^{o}C temperature. Hydrazine hydrate (0.03 mol) was added to the mixture, and stirring was maintained at 60-70^{o}C for 7 hours. TLC was checked whether new product is formed or not using reaction mixture and N-substituted aniline spot comparison. When crushed ice was added, a solid separated, which was recrystallized from 90% ethanol.

3.3.2. Synthesis of N-((4-substituted phenyl) amino)-1,3,4-thiadiazole-2-thiol/thione (3A-3B):

In EtOH (30ml) containing NaOH/KOH (0.02 mol), a combination of N-(substituted phenyl) hydrazine carbothioamide (0.01 mol) (2A & 2B) and carbon disulphide (0.012 mol) was added on 10 minutes of interval and refluxed for 8 hours. After the reaction was completed, the reaction mixture was acidified with 20 percent HCl to pH 4–5. The product was precipitated then filtered and rinsed with water. The residue was then dried and recrystallized using EtOH or methanol as a solvent.

3.3.3. 5-(benzylthio)-N-substituted phenyl-1,3,4-thiadiazol-2-amine (5A-E & 6A-E):

In DMF (15mL), potassium carbonate (0.01mol) was mixed and then N-((4-substituted phenyl) amino)-1,3,4-thiadiazole-2-thiol/thione and different substituted benzyl chloride (0.005 mol) (4A-E) was stirred at room temperature for 7-9hour. After the reaction was finished, crushed ice was added, and the product was recovered through filtering and crystallisation with ethanol or methanol to yield the final pure compound.

CHAPTER – 4

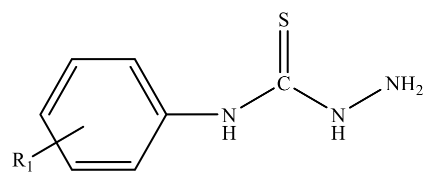
RESULT AND DISCUSSION

4. Results And Discussion:

4.1. Chemistry:

The reaction of aniline derivatives (**1A-B**) on reaction with carbon disulphide (CS_2) followed by addition of hydrazine hydrate afforded 4-(substituted) phenyl amino carbothioamides or 4-substituted phenyl thiosemicarbazides (**2A-B**). The thiosemicarbazides or carbothioamides on reflux with carbonsulfide (CS_2) in presence of base (KOH) produce dithiocarbazates and dithiocarbazades in acidic pH (2-4) addition of HCL undergo cyclization and yielded the 5-substituted phenyl-1,3,4-thiadiazole-2-thiol derivatives (**3A-B**). Reaction of 5-(substituted phenyl amino) 1,3,4-thiadiazole-2-thiol derivatives with benzyl chlorides or bromides in DMF and base (K_2CO_3) furnished final compounds 5-(substituted phenyl amino)-2-(substituted benzyl thio)-1,3,4-thiadiazoles (**5A-E & 6A-E**). The IR (ATR-FTIR) spectrum of some newly synthesized compounds have been recorded in the frequency region 4000-500 cm-1 are listed in the Table-2. The IR spectrum of the compounds (**2A, 2B**) showed absorption bands 3455.12-3446.75 cm-1 (broad str. -NH), 2980.35-2980.29 cm-1 (Aromatic - CH str.), 1487.52-1471.85 (HN-CS-NH) absorption bands in the region 1379.82.0-1379.97 cm^{-1} (-C=S str.) and 1252.41-1252.25 (-C-N str.), stretching vibrations in the region 637.8-674.0 cm^{-1} indicates the mono substitution. These results confirming the structures of compounds (2A,2B). The IR spectrum of the compounds (**3A, 3B**) shows absorption peak at 3247.50-3176.79 cm^{-1} (NH str.), 3100-2900 (Ar C-H), 1600-1550 (C=N), 1481-1480 (N=C-S), 1398-1397 (C=S) etc. Also, ^{1}H NMR spectrum of compound 3A display a sharp resonance peak at δ, 13.63 ppm (-SH) and δ, 10.09 ppm (-NH) confirm the structure of compounds **3A** and **3B**. ^{1}H NMR spectral data of final series (**5A-E and 6A-E**) of compounds displayed chemical shift position at δ, 10.58-10.37 (for NH proton) and δ, 4.56 - 4.38 for methylene proton of benzyl group and also ^{13}C NMR data of the derivatives confirming the final structure of the compounds.

4.2. Structure and Spectral Data of Synthesized Compound:



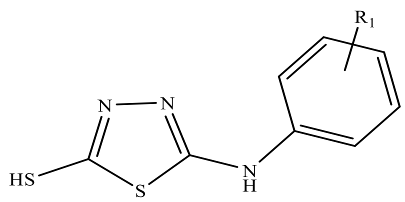
Compound	R_1	Melting Point (ºC)
2A	H	181-183
2B	3-Cl	118-120

N-phenylhydrazinecarbothioamide (2A):

Off white solid; Yield 89%; Mp 181-183 ºC; solubility: DMSO: FTIR (cm^{-1}); 3455.12, 3299.10, 3154.10 (-NH str.), 2980.29, 2971.40, 2888.34 (Ar CH), 1633.69, 1593.04 (NH bend), 1521.05 (C=C), 1487.52, 1473.17, 1461.89,1445.35 (NH-CS-NH), 1379.82 (C=S).

N-(3-chlorophenyl) hydrazinecarbothioamide (2B):

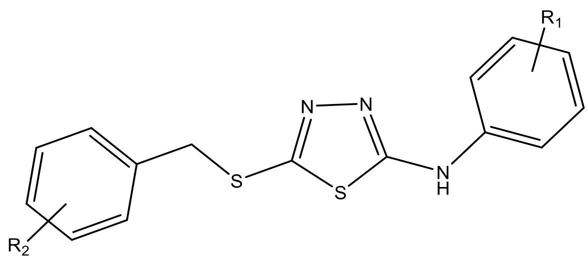
Off white solid; Yield 84%; Mp 118-120 ºC; solubility: DMSO: FTIR (cm^{-1}); 3446.75 (-NH str.), 2980.35, 2971.53, 2887.91 (Ar CH), 1620.84, 1585.18 (NH bend), 1537.79 (C=C), 1471.85, 1461.96 (NH-CS-NH), 1379.97 (C=S).



Compound	R
3A	H
3B	3-Cl

5-(phenylamino)-1,3,4-thiadiazole-2-thiol (3A): Off white solid; Yield 79%; solubility: DMSO: FTIR (cm^{-1}); 3225.18 3178.26 (NH str), 3107.97-2913.04 ((Ar CH str.), 1600.06-1556.53 ((C=N str.), 1480.99 (N=C-S), 1397.83-1316.05 (C=S) etc;

5-((3-chlorophenyl) amino)-1,3,4-thiadiazole-2-thiol (3B): Off white solid; Yield 84%; solubility: DMSO: FTIR (cm^{-1}); 3225.63-3177.62 (NH str), 3108.30-2909.25 (Ar CH str.), 1600.56-1555.96 (C=N str.), 1481.33 (N=C-S), 1398.34 1316.61 (C=S) etc.



Compound	R_1	R_2	Melting Point (ºC)
5A	H	H	133-136
5B	H	4-Cl	165-167

5C	H	2,4-Cl	174-176
5D	H	3-NO_2	129-132
5E	H	4-CH_3	152-155
6A	3-Cl	H	165-167
6B	3-Cl	4-Cl	174-176
6C	3-Cl	2,4-Cl	149-152
6D	3-Cl	3-NO_2	144-146
6E	3-Cl	4-CH_3	180-182

5-(benzylthio)-N-phenyl-1,3,4-thiadiazol-2-amine (5A): Off white solid; Yield 75%; Mp 133-136 °C; Solubility: DMSO, Acetonitrile: FTIR (cm^{-1}); 3240.80, 3192.22, 3140.84 (NH str.), 3047.83, 3027.67, 2909.66 (Ar CH), 1613.70, 1599.84 (NH bend), 1455.22, 1433.15, 1411.73 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d_6) $\delta_{H:}$ 10.37 (s, 1H), 7.62 – 7.53 (m, 2H), 7.40 (dt, *J* = 5.9, 1.6 Hz, 2H), 7.37 – 7.23 (m, 5H), 7.03 – 6.96 (m, 1H), 4.41 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C: 164.95, 152.11, 140.35, 136.92, 129.10, 129.05, 128.53, 127.54, 122.00, 117.38, 38.02.

5-((4-chlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5B): Off white solid; Yield 80%; Mp 165-167 °C; Solubility: DMSO, sparingly soluble in Acetonitrile: FTIR (cm^{-1}); 3238.07 (NH str.), 3012.80 (Ar CH), 1667.87, 1595.92 (NH bend), 1498.38, 1440.97, 1406.46 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d_6) $\delta_{H:}$ 10.38 (s, 1H), 7.60 – 7.52 (m, 2H), 7.46 – 7.28 (m, 6H), 7.03 – 6.95 (m, 1H), 4.40 (s, 2H), ^{13}C NMR (75 MHz, DMSO) δ 165.08, 151.71, 140.31, 136.29, 132.14, 130.93, 129.12, 128.49, 122.04, 117.40, 37.15.

5-((2,4-dichlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5C): Off white solid; Yield 87%; Mp 174-176°C; Solubility: DMSO: FTIR (cm^{-1}); 3200.68 (NH str.), 2917.47 (Ar CH), 1621.54, 1601.39, 1576.06 (NH bend), 1458.18, 1436.08, 1409.57 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 10.42 (s, 1H), 7.67 (d, *J* = 2.2 Hz, 1H), 7.60 – 7.49 (m, 3H), 7.44 – 7.30 (m, 3H), 7.05 – 6.97 (m, 1H), 4.47 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 165.50, 150.83, 140.25, 134.27, 133.76, 133.22, 132.73, 129.13, 129.05, 127.52, 122.12, 117.44,

5-((3-nitrobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5D): Yellow solid; Yield 69%; Mp 129-132 °C; Solubility: DMSO, Ethanol, Acetonitrile: FTIR (cm^{-1}); 3252.47, 3191.16, 3127.47 (NH str.), 3058.27, 3016.43 (Ar CH), 1616.39, 1600.15, 1576.67, 1569.35, 1558.32 (NH bend), 1480.24, 1457.86, 1442.33, 1419.20 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 10.38 (s, 1H), 8.30 (t, *J* = 2.0 Hz, 1H), 8.13 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.86 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.66 – 7.52 (m, 3H), 7.36 – 7.27 (m, 2H), 7.03 – 6.95 (m, 1H), 4.56 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ_C: 165.19, 151.37, 147.72, 140.28, 139.92, 135.79, 130.02, 129.12, 123.68, 122.42, 122.10, 117.43, 36.79.

5-((4-methylbenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5E): Off white solid; Yield 82%; Mp 152-155 ºC; Solubility: DMSO: FTIR (cm^{-1}); 3192.01 (NH str.), 2980.44, 2887.45, 2774.47 (Ar CH), 1614.01, 1596.82, 1569.48 (NH bend), 1497.96, 1461.04, 1436.85, 1415.24 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ_H: 10.36 (s, 1H), 7.61 – 7.50 (m, 2H), 7.31 (dd, *J* = 19.4, 7.8 Hz, 4H), 7.13 (d, *J* = 7.8 Hz, 2H), 6.99 (t, *J* = 7.3 Hz, 1H), 4.36 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 164.90, 152.24, 140.36, 136.80, 133.79, 129.12, 128.99, 122.00, 117.36, 37.85, 20.74.

5-(benzylthio)-N-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine (6A): Off white solid; Yield 80%; Mp 165-167 ºC; Solubility: DMSO: FTIR (cm^{-1}); 2980.34, 2971.43, 2887.62 (Ar CH), 1684.18, 1653.57, 1645.79, 1635.49 (NH bend), 1473.25, 1446.83 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ_H: 10.58 (s, 1H), 7.83 (t, *J* = 2.0 Hz, 1H), 7.44 – 7.25 (m, 7H), 7.04 (dt, *J* = 6.9, 2.0 Hz, 1H), 4.43 (s, 2H);

5-((3-chlorobenzyl) thio)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (6B): Off white solid; Yield 68%; Mp 174-176 ºC; Solubility: FTIR (cm^{-1}); 2980.33, 2971.43, 2887.87 (Ar CH), 1669.62, 1653.51, 1623.16 (NH bend), 1486.72, 1447.75 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d$_6$) $\delta_{H:}$ 10.58 (s, 1H), 7.83 (t, *J* = 1.7 Hz, 1H), 7.47 – 7.30 (m, 6H), 7.07 – 7.00 (m, 1H), 4.42 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ_C: 164.49, 152.83, 141.55, 136.22, 133.51, 132.17, 130.94, 130.70, 128.51, 121.55, 116.78, 115.86, 36.98.

N-(3-chlorophenyl)-5-((2,4-dichlorobenzyl) thio)-1,3,4-thiadiazol-2-amine (6C): Off white solid; Yield 89%; Mp 149-152 ºC; Solubility: DMSO; FTIR (cm^{-1}); 2980.35, 2971.46, 2888.24 (Ar CH), 1619.37, 1594.97, 1566.57 (NH bend), 1484.34, 1472.38, 1448.46 (N=C-S); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H: 10.63 (s, 1H), 7.84 (d, *J* = 2.5 Hz, 1H), 7.67 (q, *J* = 2.0 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.44 – 7.32 (m, 3H), 7.08 – 7.02 (m, 1H), 4.49 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ_C: 164.91, 152.02, 141.50, 134.31, 133.71, 133.52, 133.28, 132.78, 130.77, 129.09, 127.58, 121.65, 116.82, 115.92, 35.40.

N-(3-chlorophenyl)-5-((3-nitrobenzyl) thio)-1,3,4-thiadiazol-2-amine (6D): Off white solid; Yield 79%; Mp 144-146 ºC; Solubility: DMSO, Acetonitrile: FTIR (cm^{-1}); 2980.35, 2971.48, 2888.17 (Ar CH), 1621.79, 1599.85, 1564.33, 1526.40 (NH bend), 1484.91, 1460.94 (N=C-S); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ_H: 165.59, 153.44, 148.33, 141.98, 140.32, 136.42, 134.29, 131.47, 130.75, 124.15, 123.15, 122.58, 117.52, 116.60, 37.57.

N-(3-chlorophenyl)-5-((4-methylbenzyl) thio)-1,3,4-thiadiazol-2-amine (6E): Off white solid; Yield 87%; Mp 180-182 °C; Solubility: DMSO, Chloroform: FTIR (cm^{-1}); 2980.34, 2971.49, 2888.05 (Ar CH), 1649.75, 1621.03, 1592.82, 1563.49 (NH bend), 1484.91, 1460.94 (N=C-S); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ_H: 10.57 (s, 1H), 7.84 (t, J = 2.0 Hz, 1H), 7.41 – 7.25 (m, 4H), 7.14 (d, J = 7.8 Hz, 2H), 7.04 (dt, J = 6.8, 2.0 Hz, 1H), 4.38 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ_C: 164.33, 153.35, 141.60, 136.84, 133.71, 133.51, 130.71, 129.13, 129.00, 121.51, 116.75, 115.85, 37.70, 20.74.

4.3. FT-IR Spectra of the Synthesized Compound:

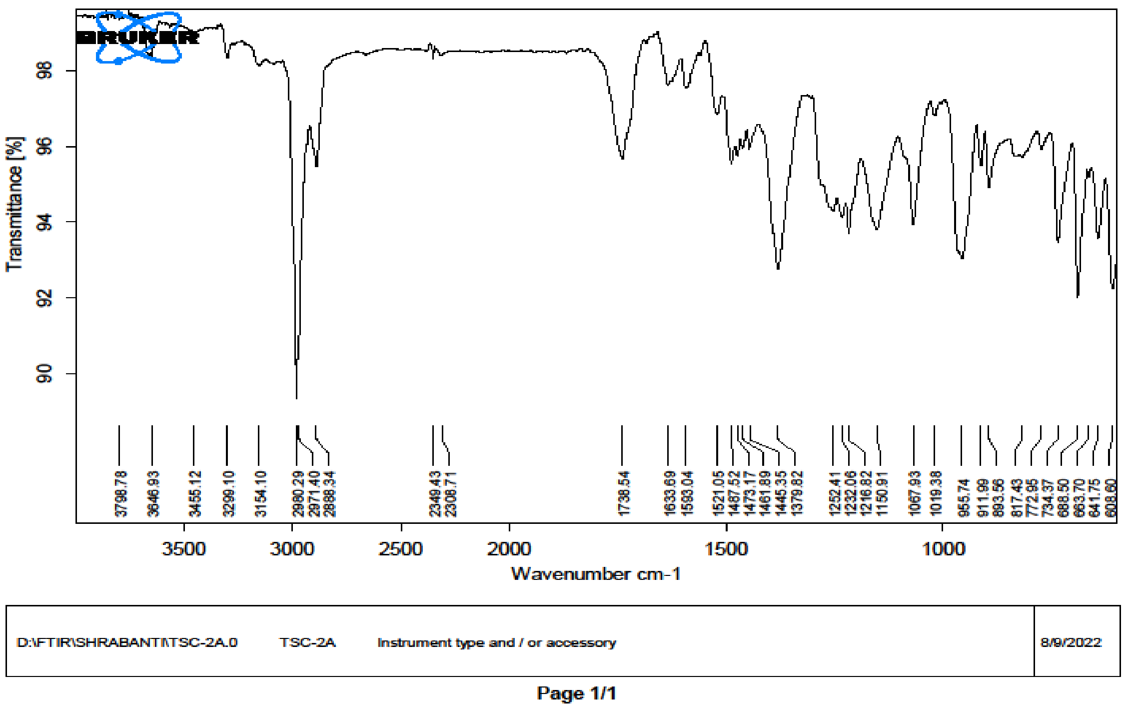


Figure 4: FT-IR spectra of N-phenylhydrazine carbothioamide (2A).

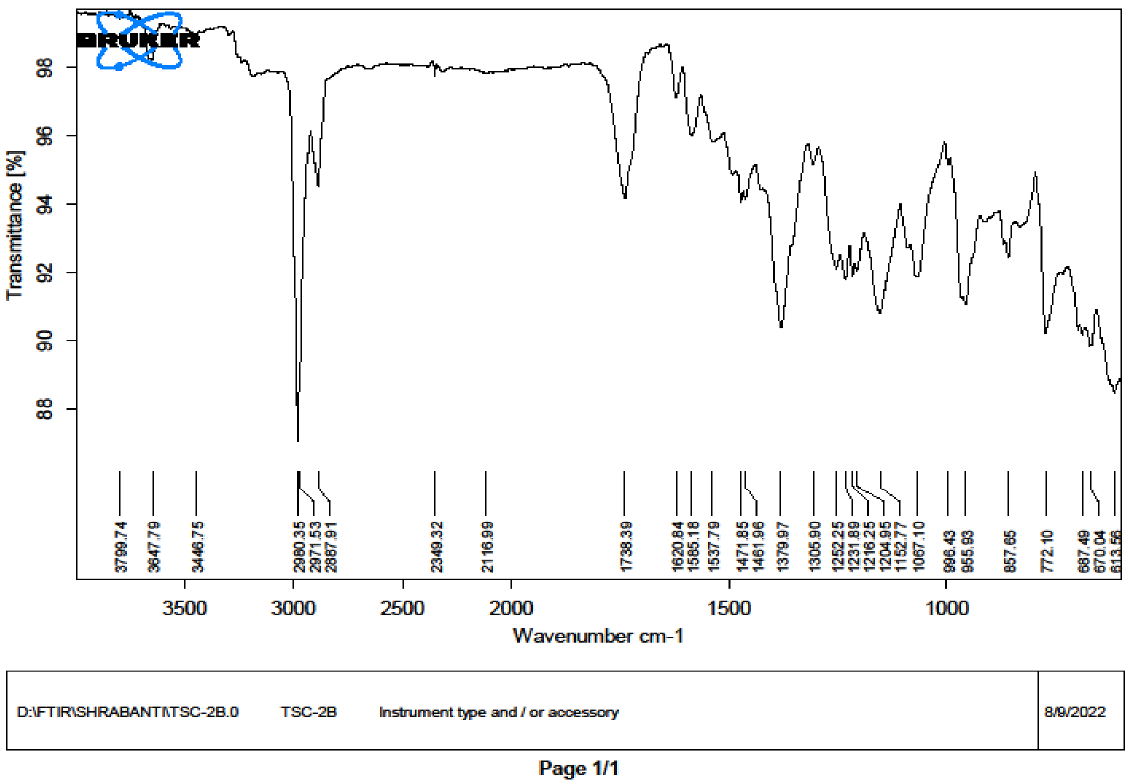


Figure 5: FT-IR spectra of N-(3-chlorophenyl) hydrazinecarbothioamide (2B).

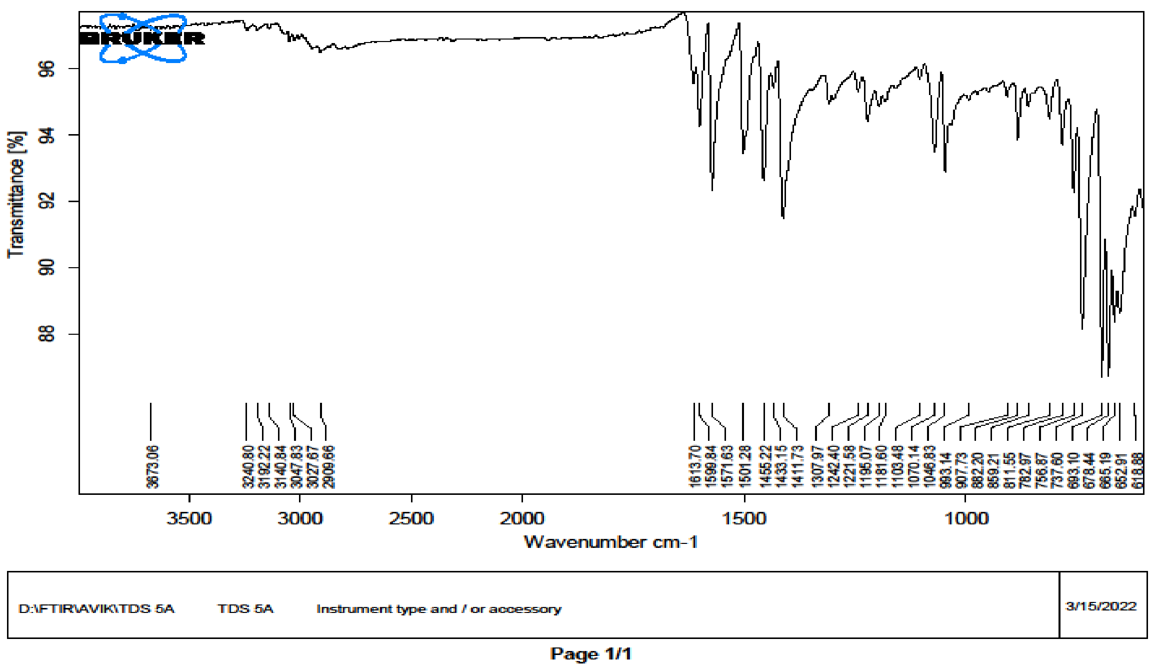


Figure 6: FT-IR spectra of 5-(benzylthio)-N-phenyl-1,3,4-thiadiazol-2-amine (5A).

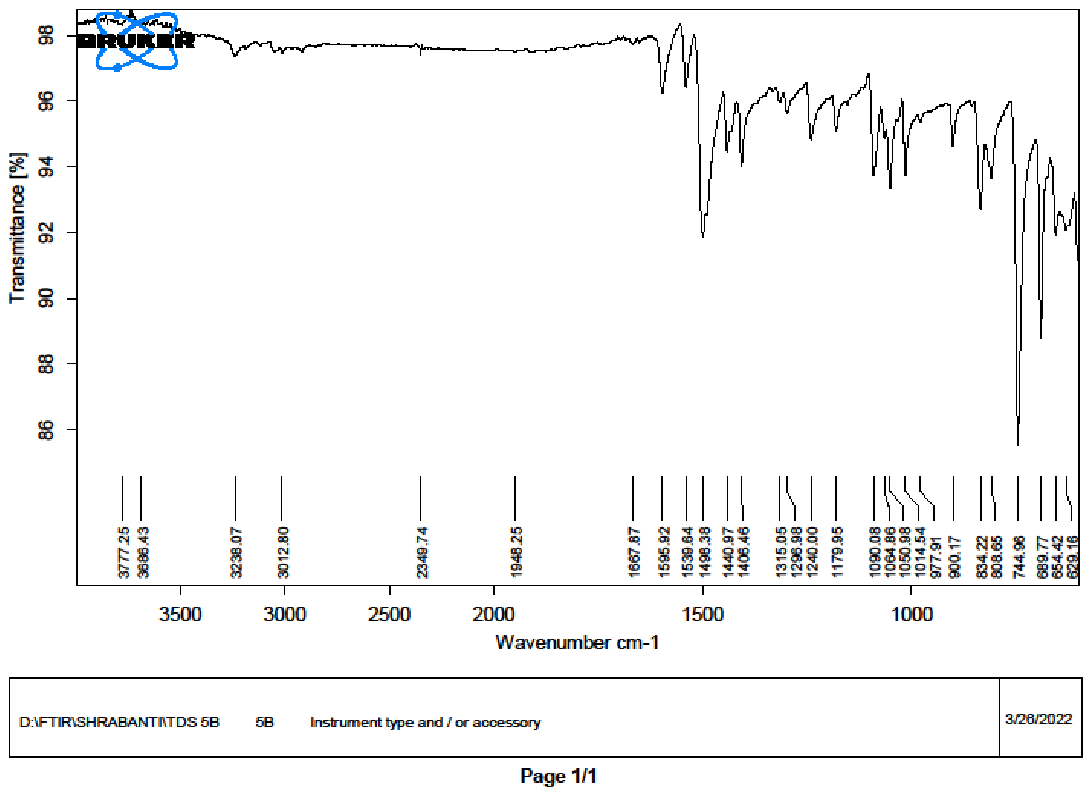


Figure 7: FT-IR spectra of 5-((4-chlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5B).

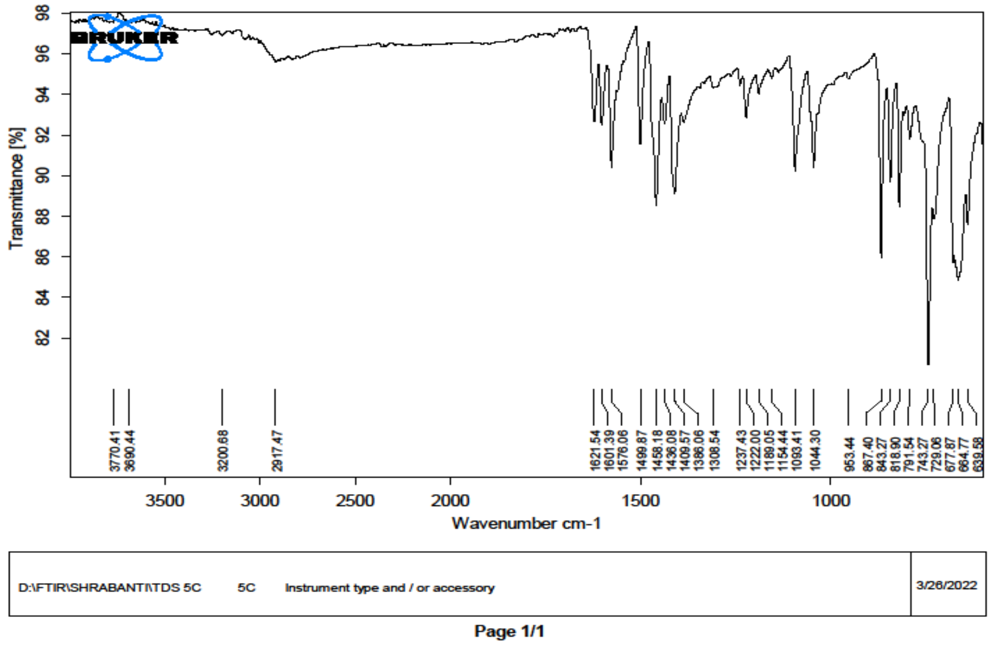


Figure 8: FT-IR spectra of 5-((2,4-dichlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5C).

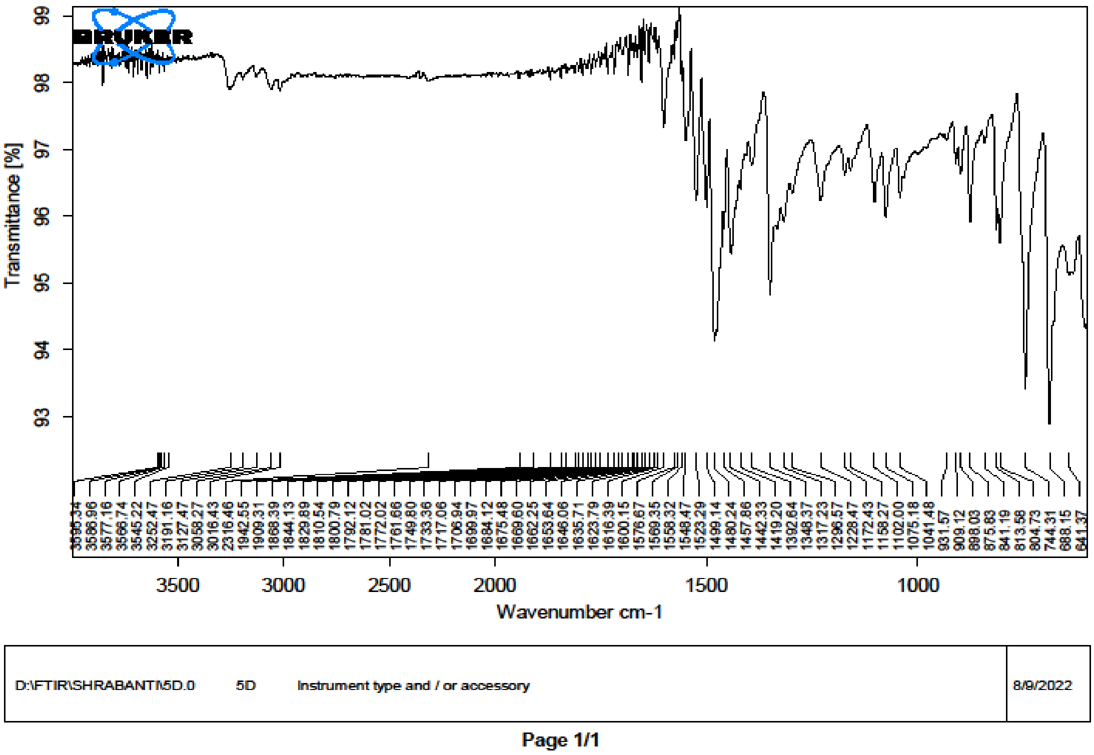


Figure 9: FT-IR spectra of 5-((3-nitrobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5D).

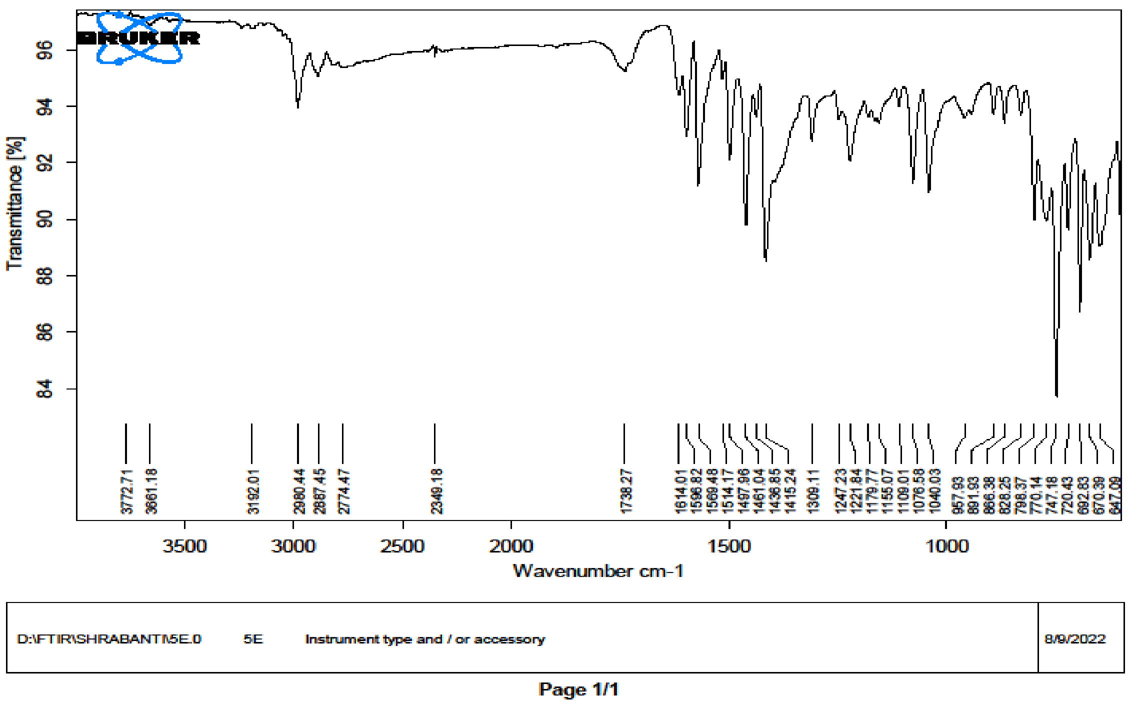


Figure 10: FT-IR spectra of 5-((4-methylbenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5E).

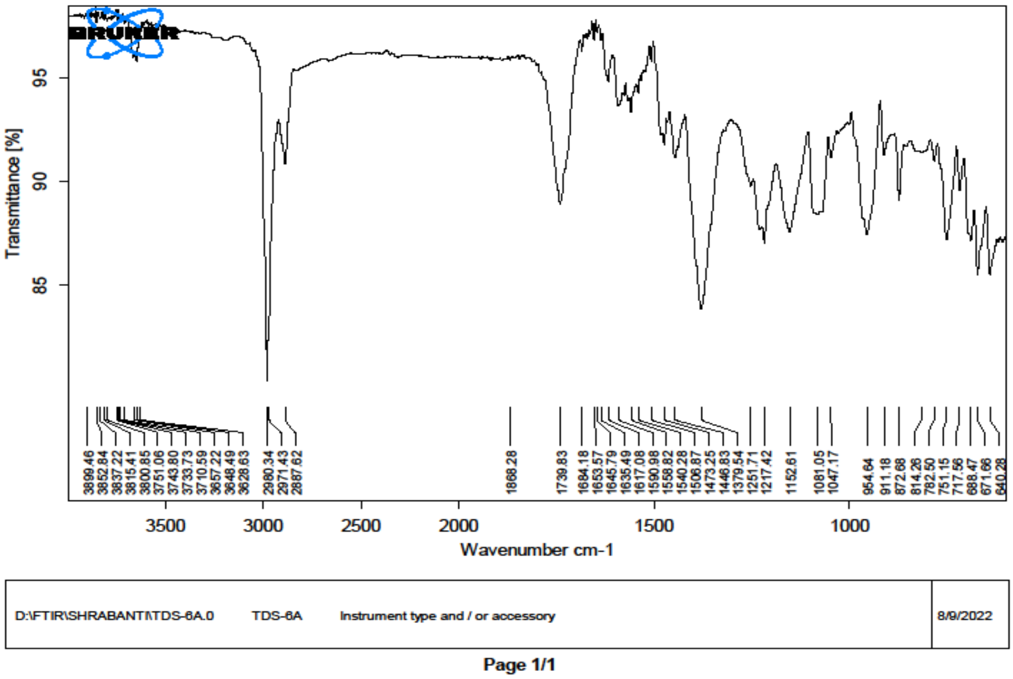


Figure 11: FT-IR spectra of 5-(benzylthio)-N-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine (6A).

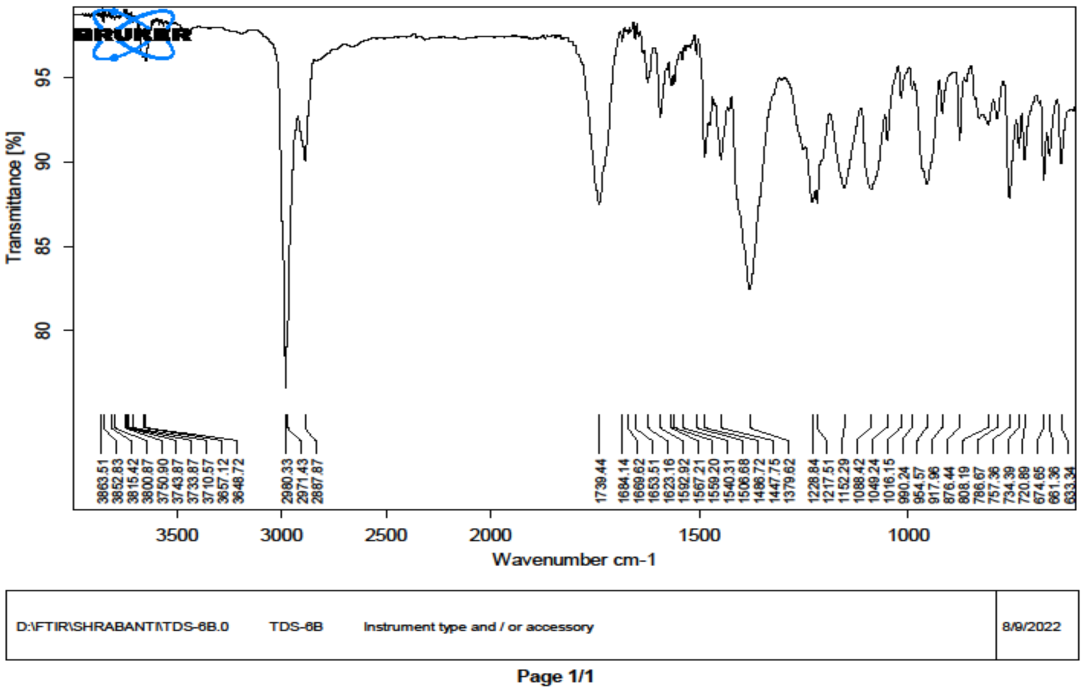


Figure 12: FT-IR spectra of 5-((3-chlorobenzyl) thio)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (6B).

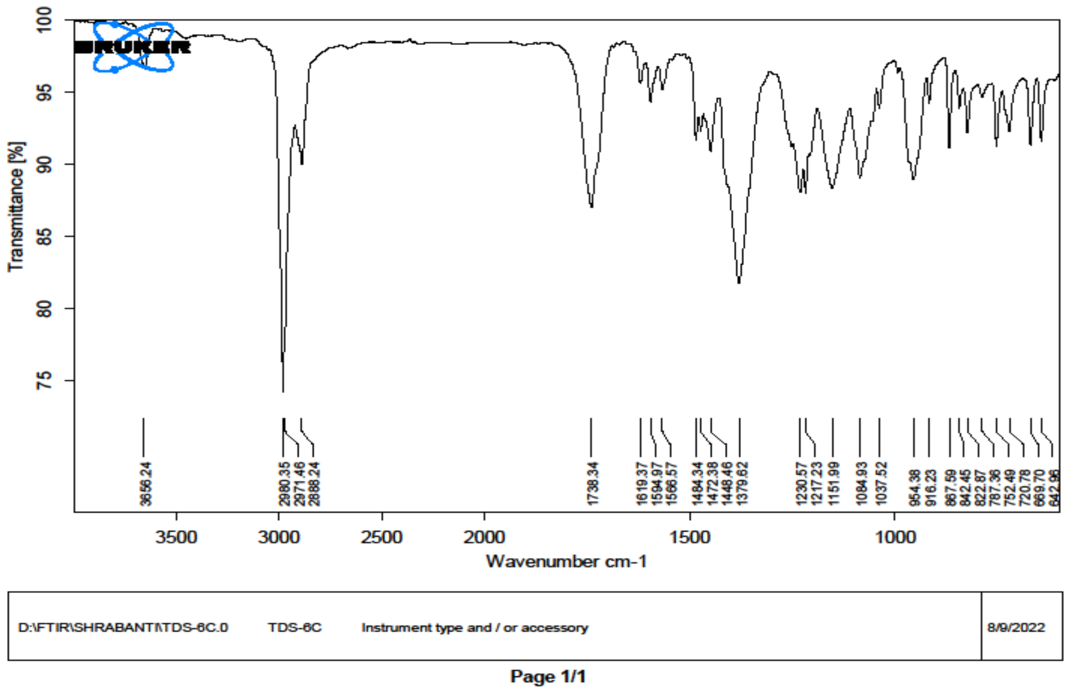


Figure 13: FT-IR spectra of N-(3-chlorophenyl)-5-((2,4-dichlorobenzyl) thio)-1,3,4-thiadiazol-2-amine (6C).

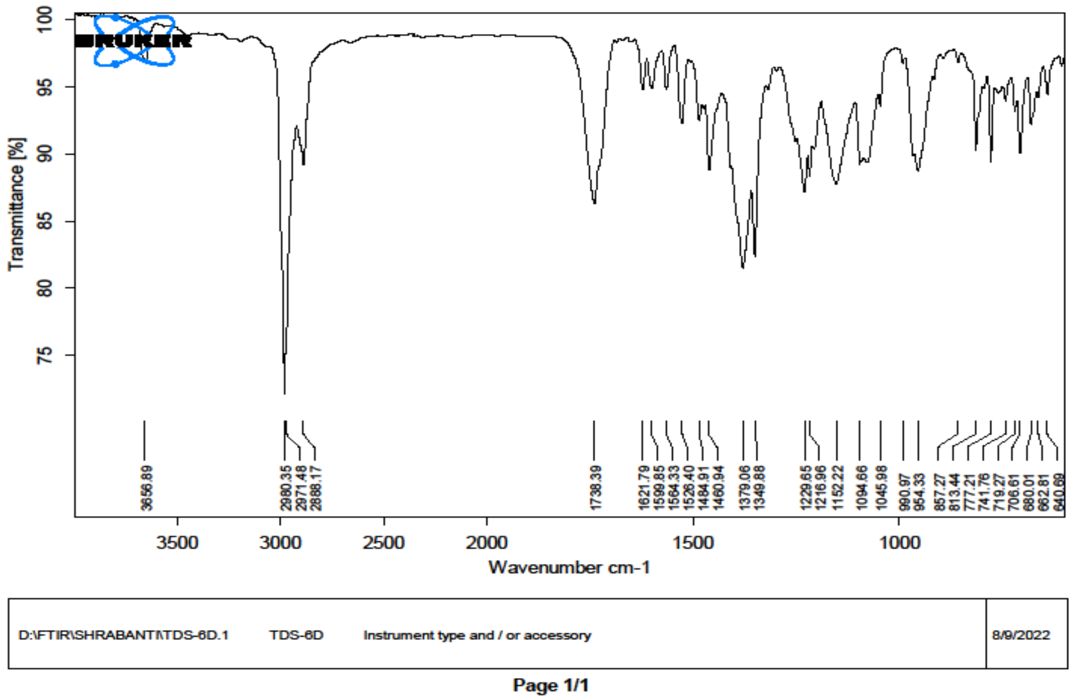


Figure 14: FT-IR spectra of N-(3-chlorophenyl)-5-((3-nitrobenzyl) thio)-1,3,4-thiadiazol-2-amine (6D).

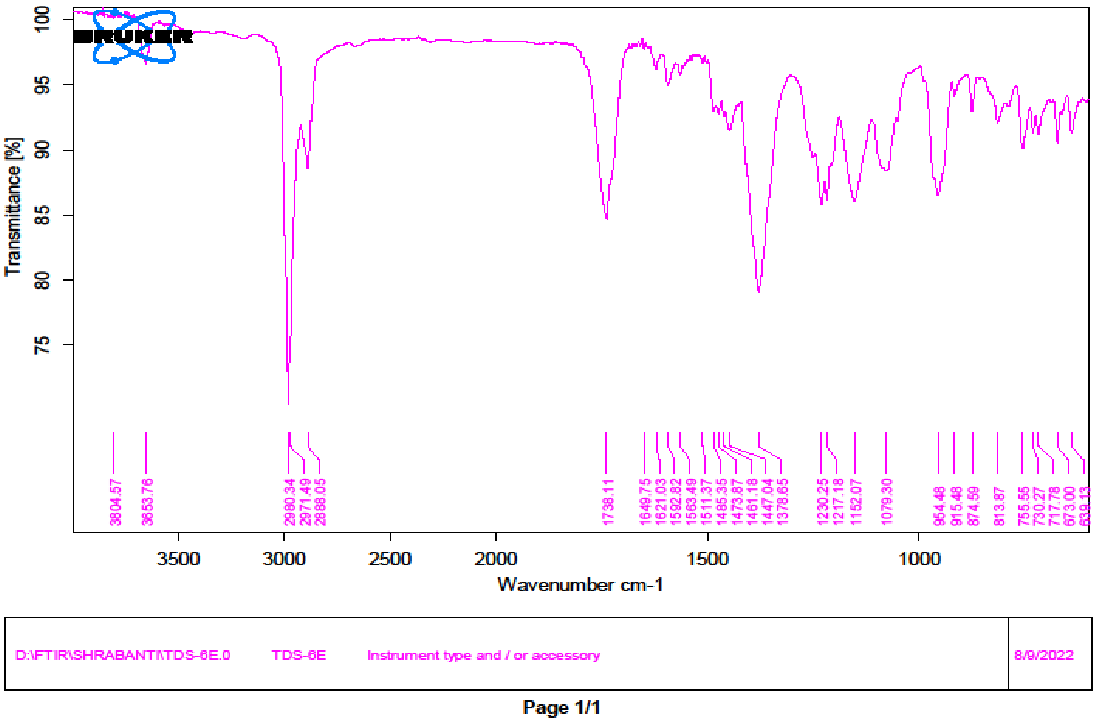


Figure 15: FTIR spectra of N-(3-chlorophenyl)-5-((4-methylbenzyl) thio)-1,3,4-thiadiazol-2-amine (6E).

4.4. ^{1}H NMR Spectra of the Synthesized Compound:

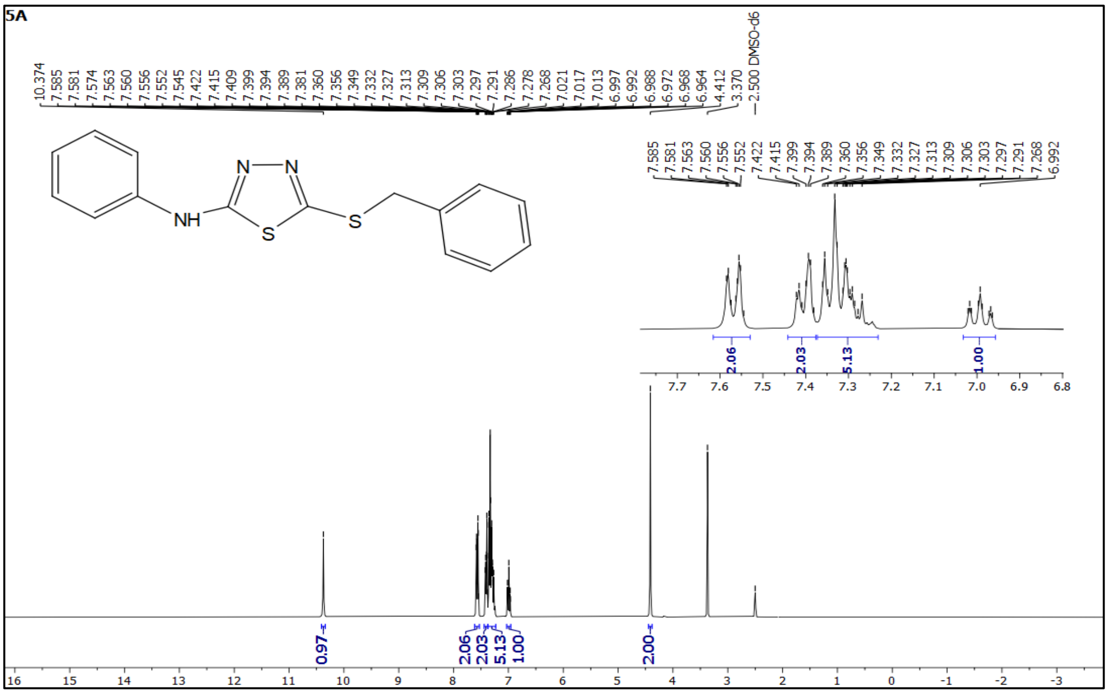


Figure 16: ^{1}H NMR spectra of 5-(benzylthio)-N-phenyl-1,3,4-thiadiazol-2-amine (5A).

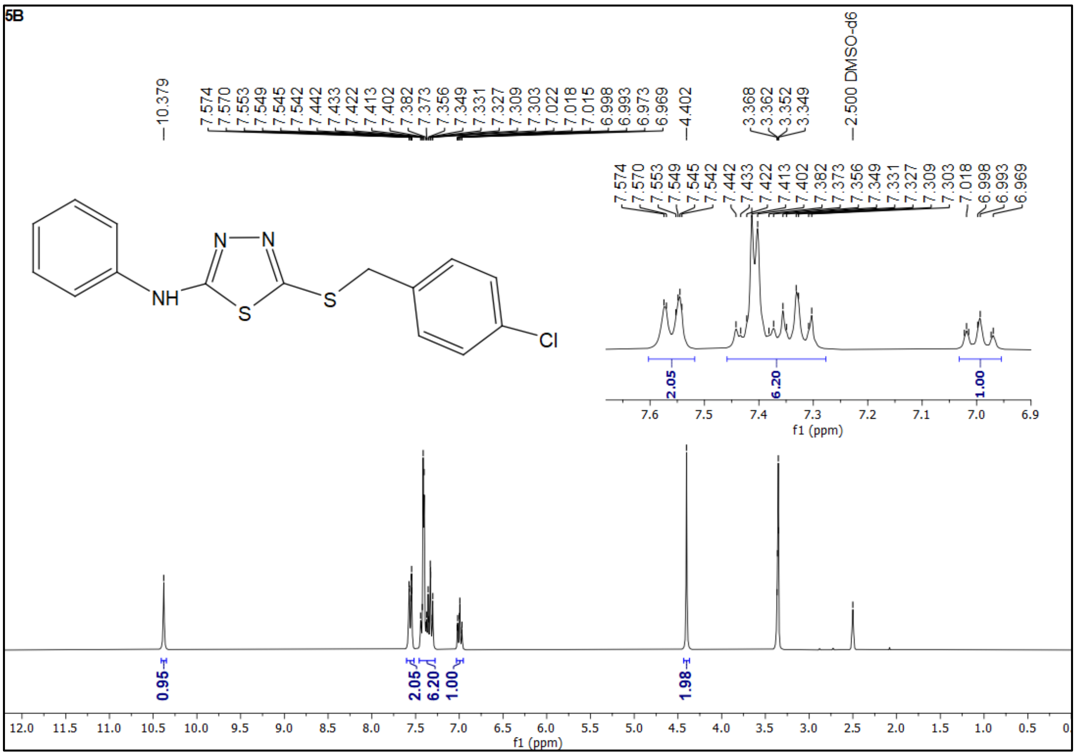


Figure 17: ^{1}H NMR spectra of 5-((4-chlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5B).

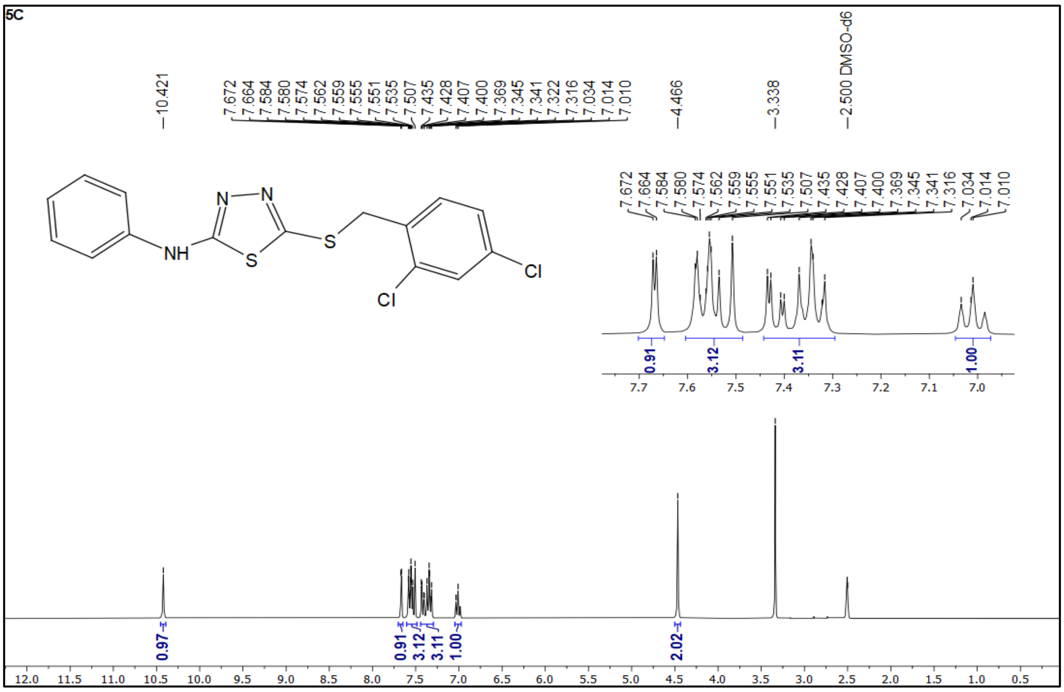


Figure 18: ^{1}H NMR spectra of 5-((2,4-dichlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5C).

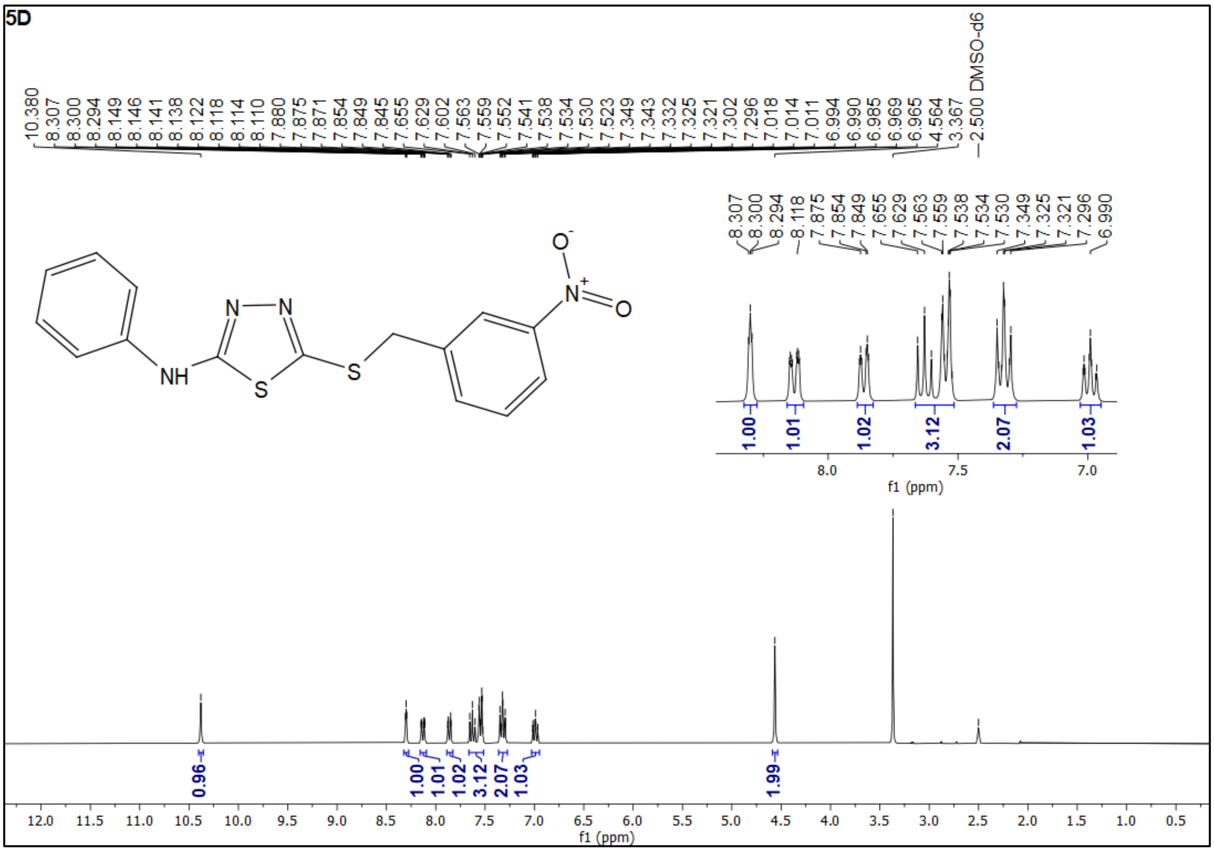


Figure 19: ^{1}H NMR spectra of 5-((3-nitrobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5D).

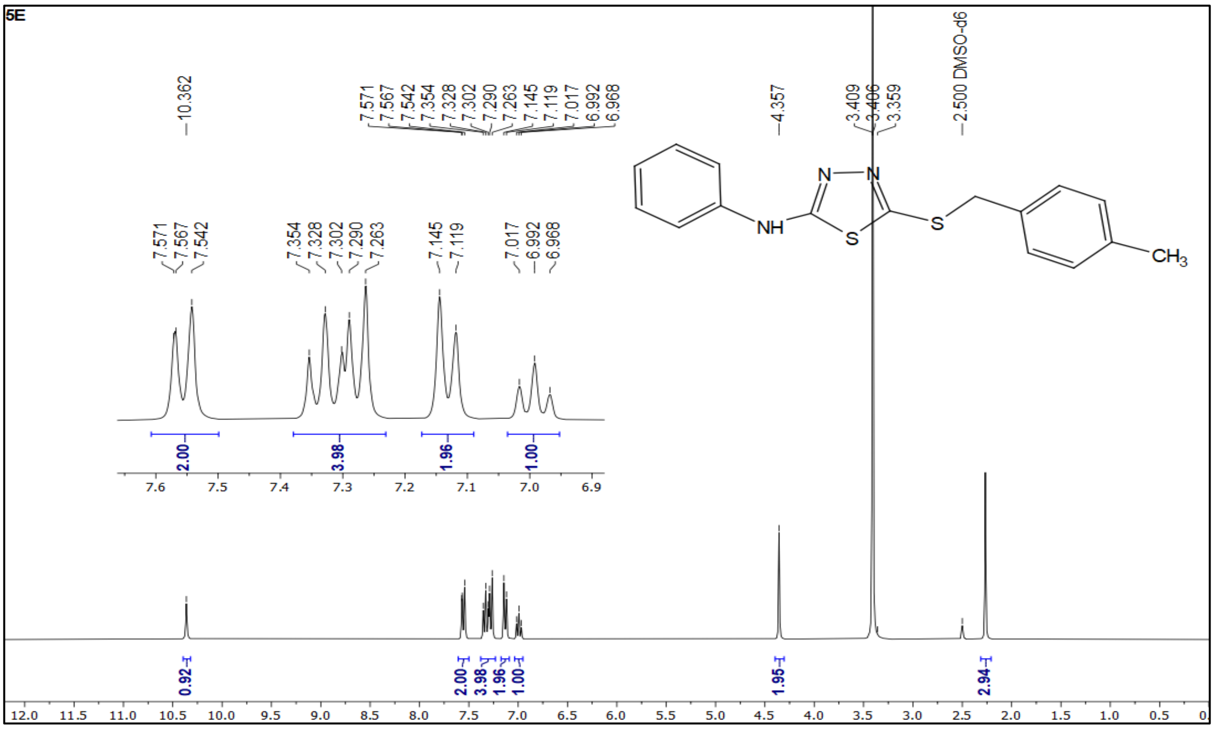


Figure 20: [1]H NMR spectra of 5-((4-methylbenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5E).

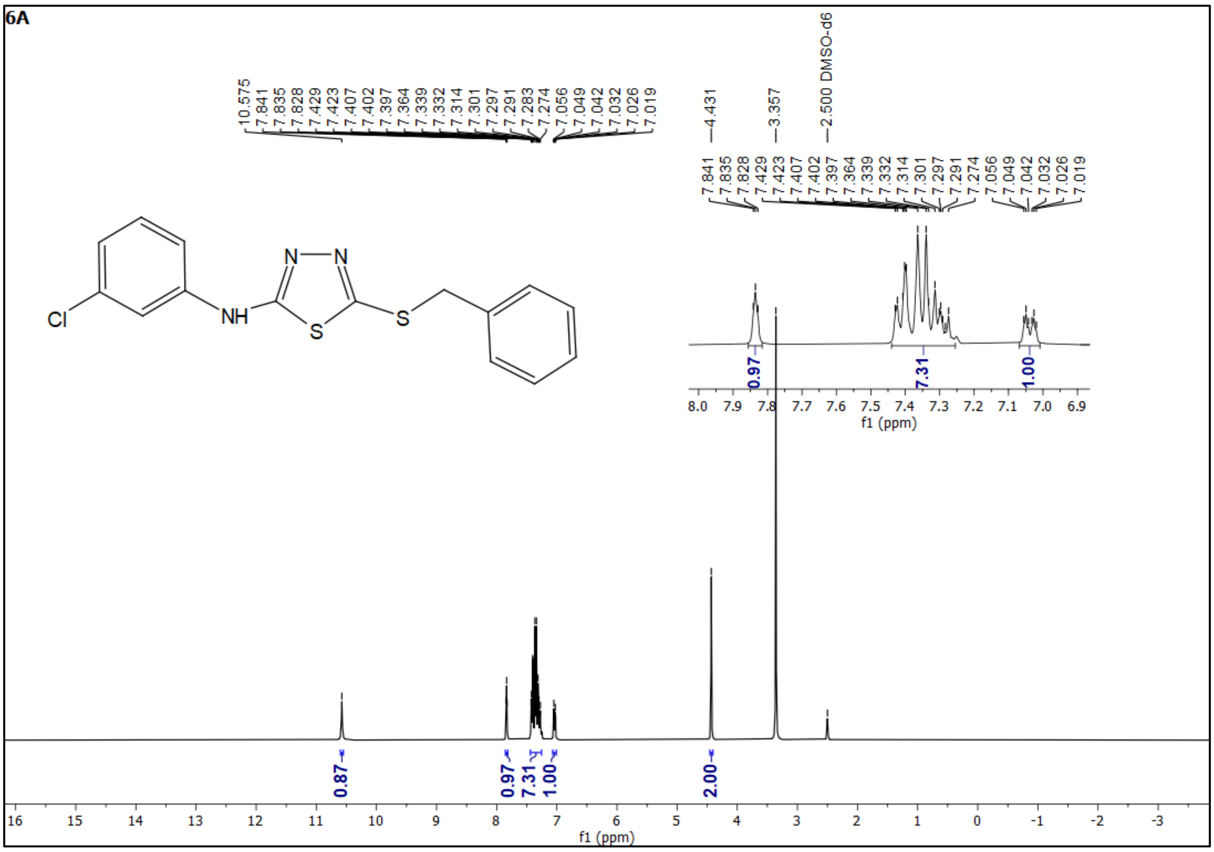


Figure 21: [1]H NMR spectra of 5-(benzylthio)-N-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine (6A).

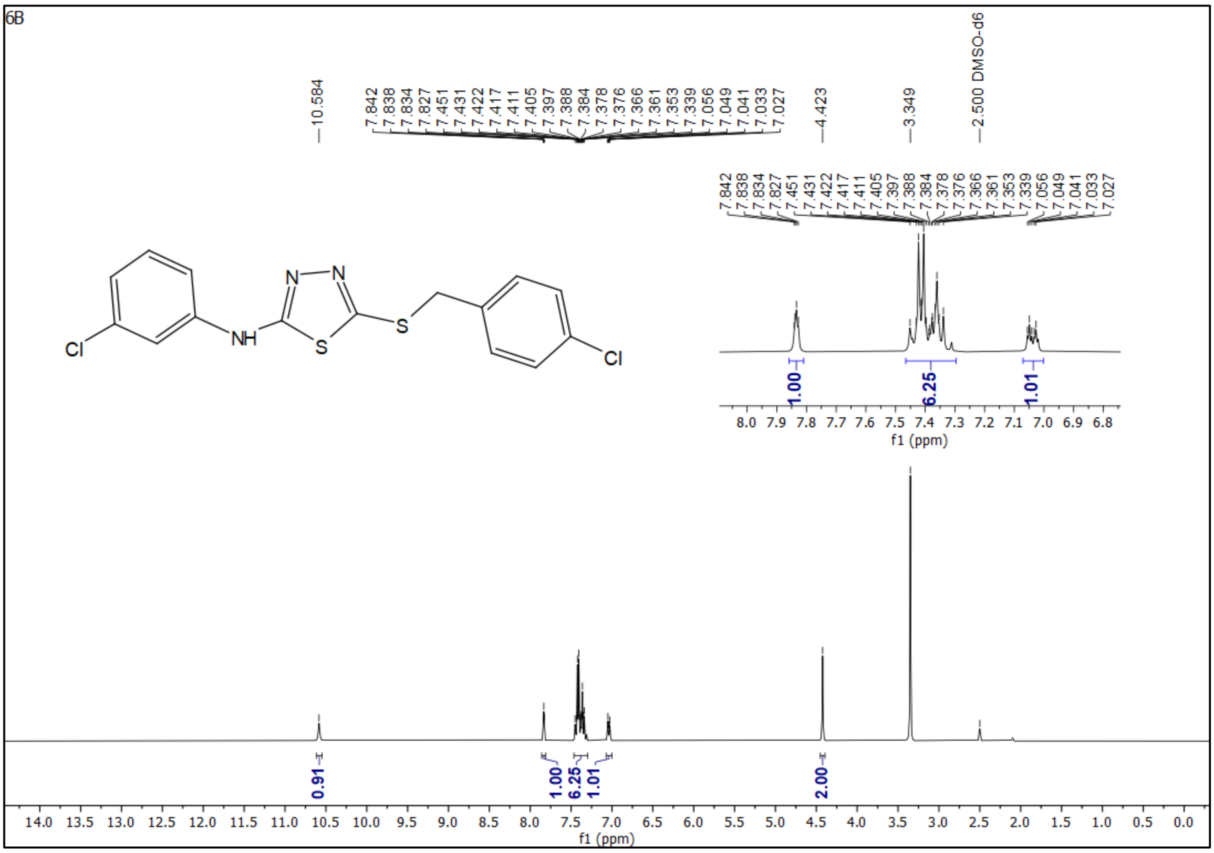


Figure 22: [1]H NMR spectra of 5-((3-chlorobenzyl) thio)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (6B).

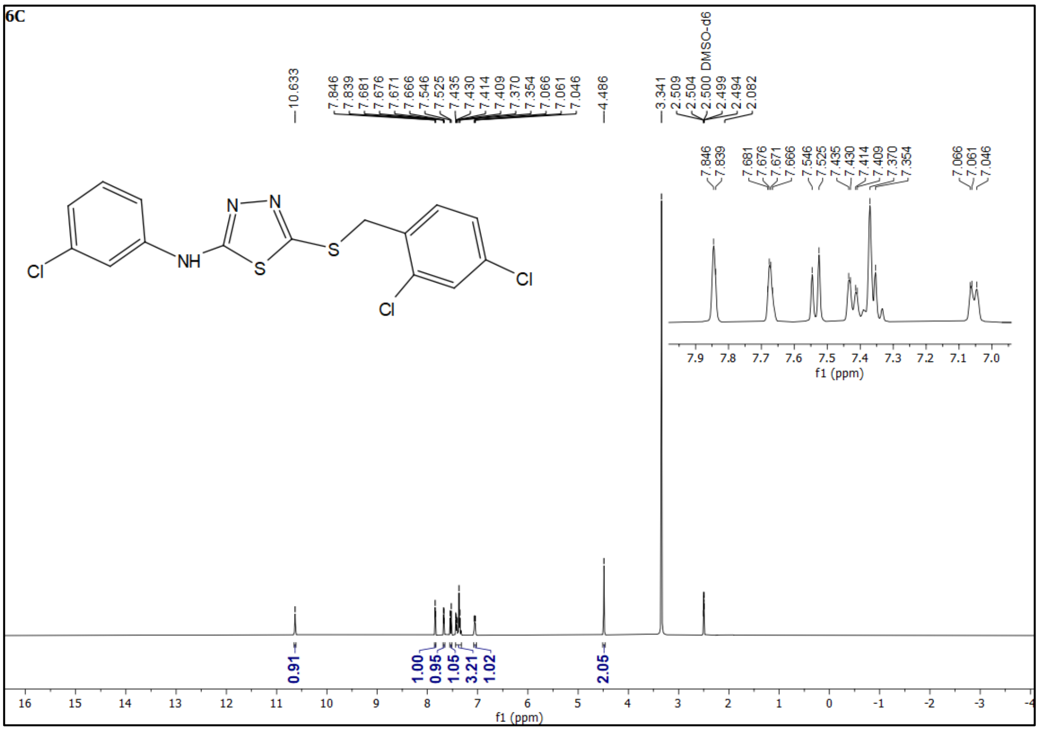


Figure 23: [1]H NMR spectra of N-(3-chlorophenyl)-5-((2,4-dichlorobenzyl) thio)-1,3,4-thiadiazol-2-amine (6C).

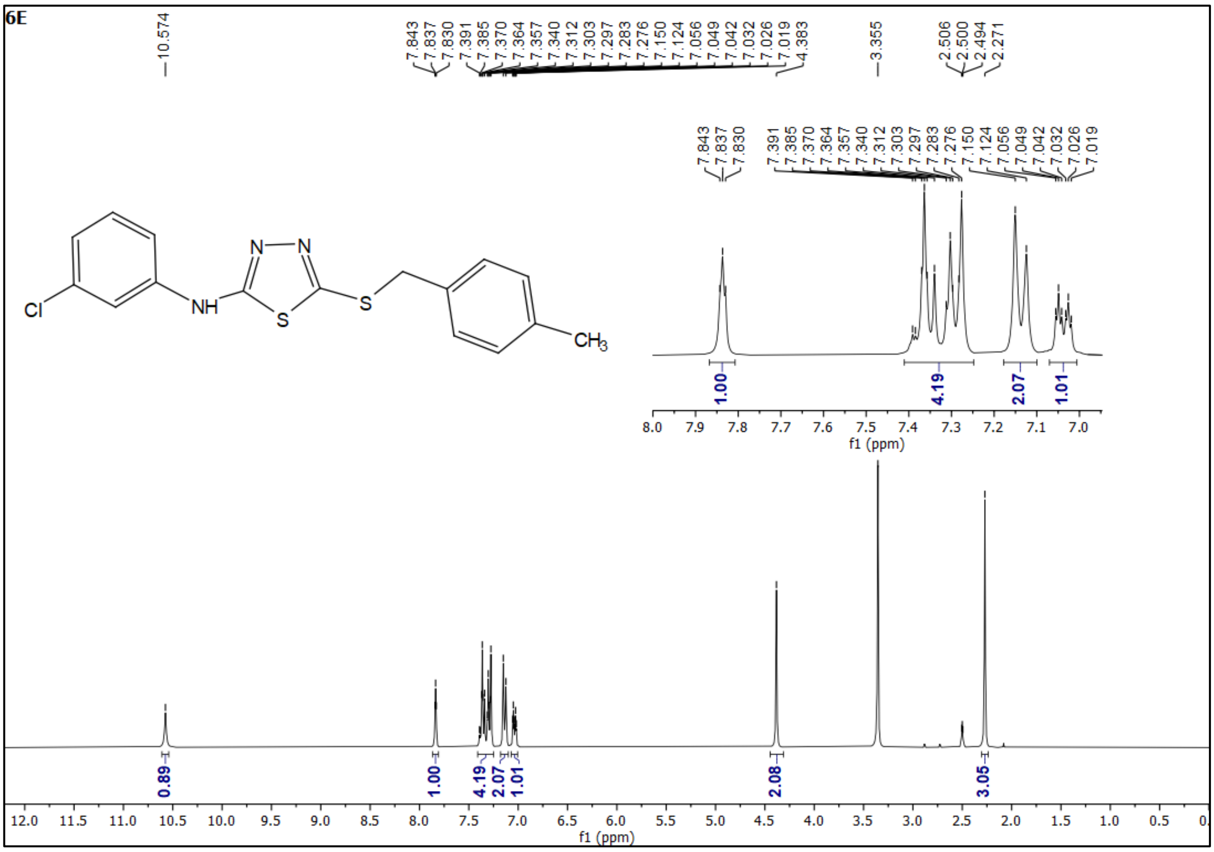


Figure 24: ^{1}H NMR spectra of N-(3-chlorophenyl)-5-((4-methylbenzyl) thio)-1,3,4-thiadiazol-2-amine (6E).

4.5. ^{13}C NMR Spectre of the Synthesized Compound:

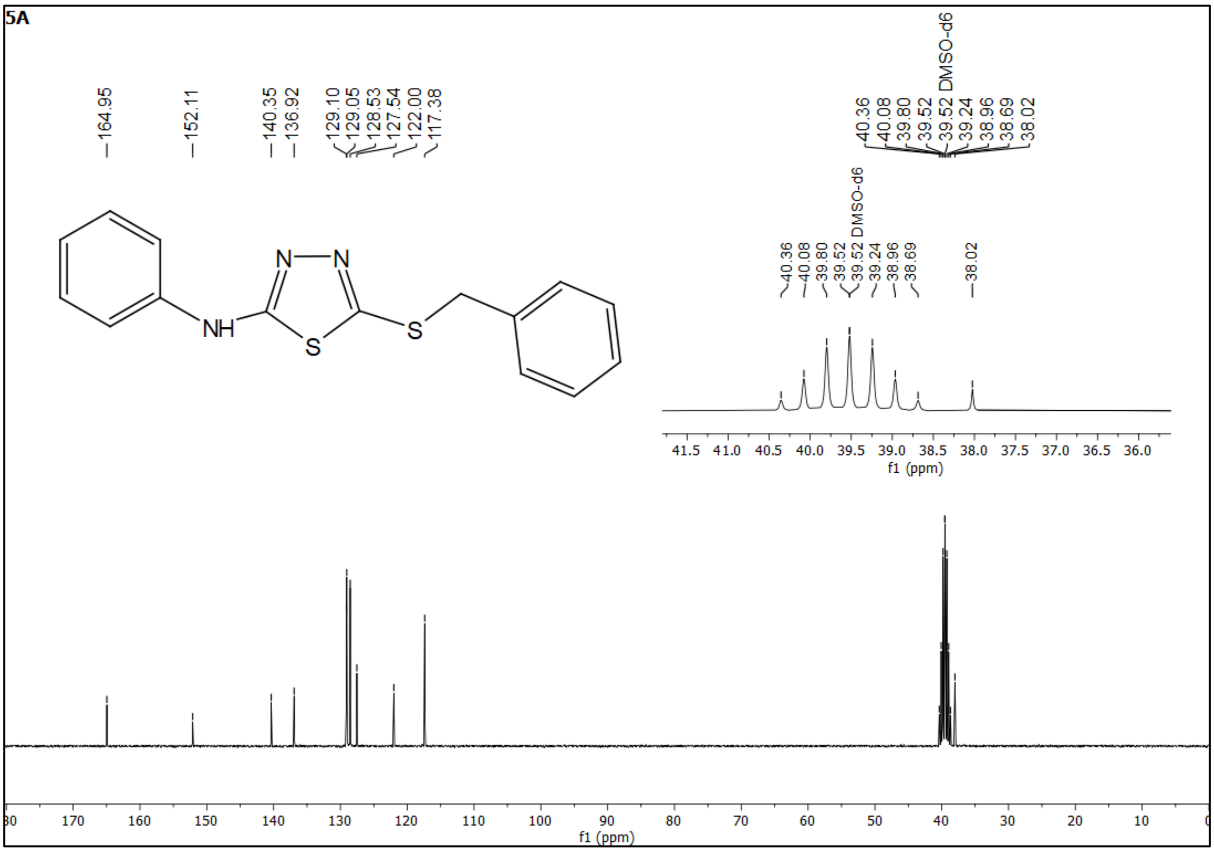


Figure 25: ^{13}C NMR spectra of 5-(benzylthio)-N-phenyl-1,3,4-thiadiazol-2-amine (5A).

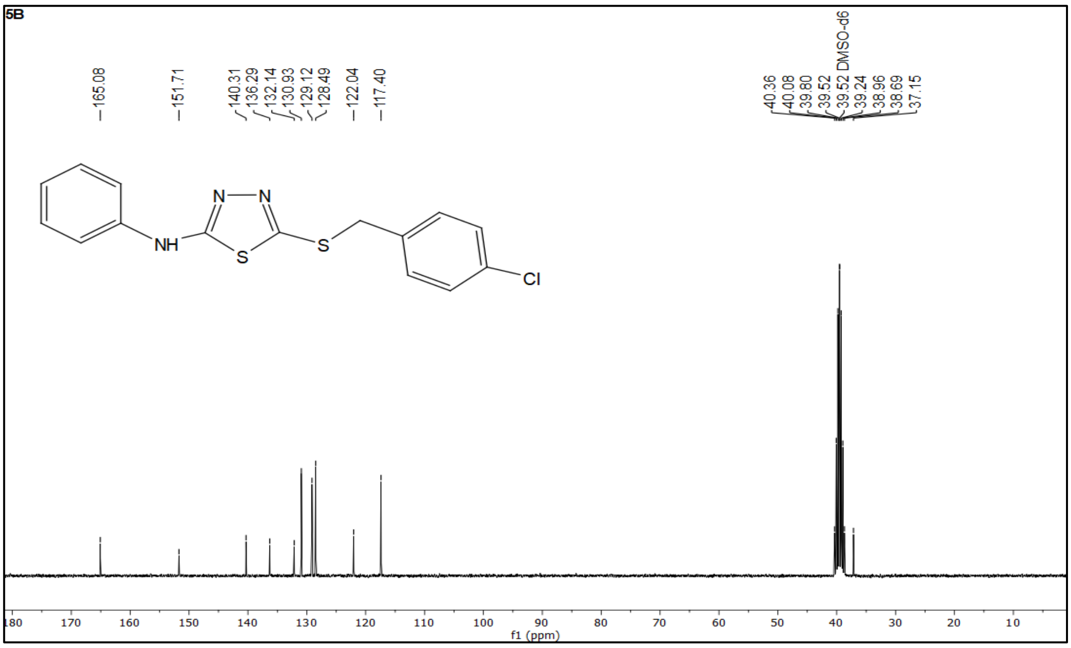


Figure 26: ^{13}C NMR spectra of 5-((4-chlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5B).

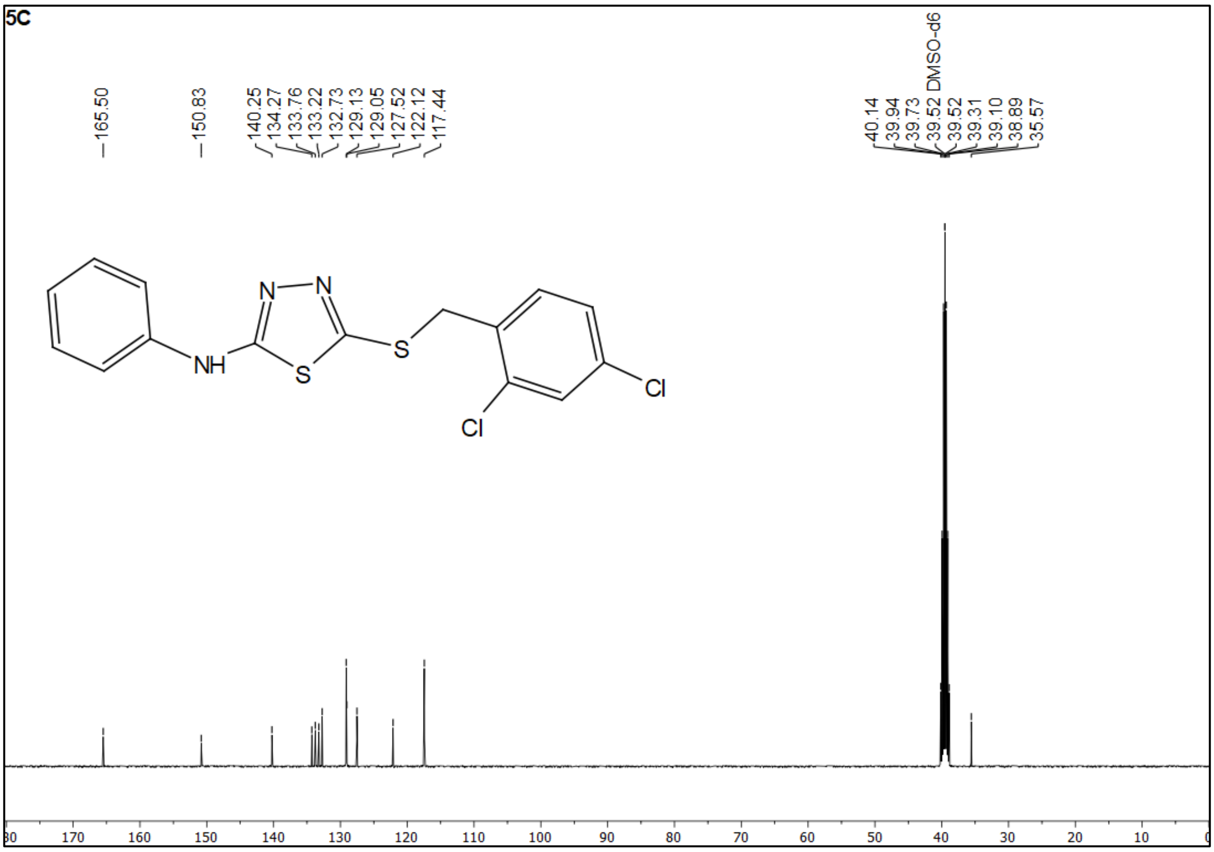


Figure 27: ^{13}C NMR spectra of 5-((2,4-dichlorobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5C).

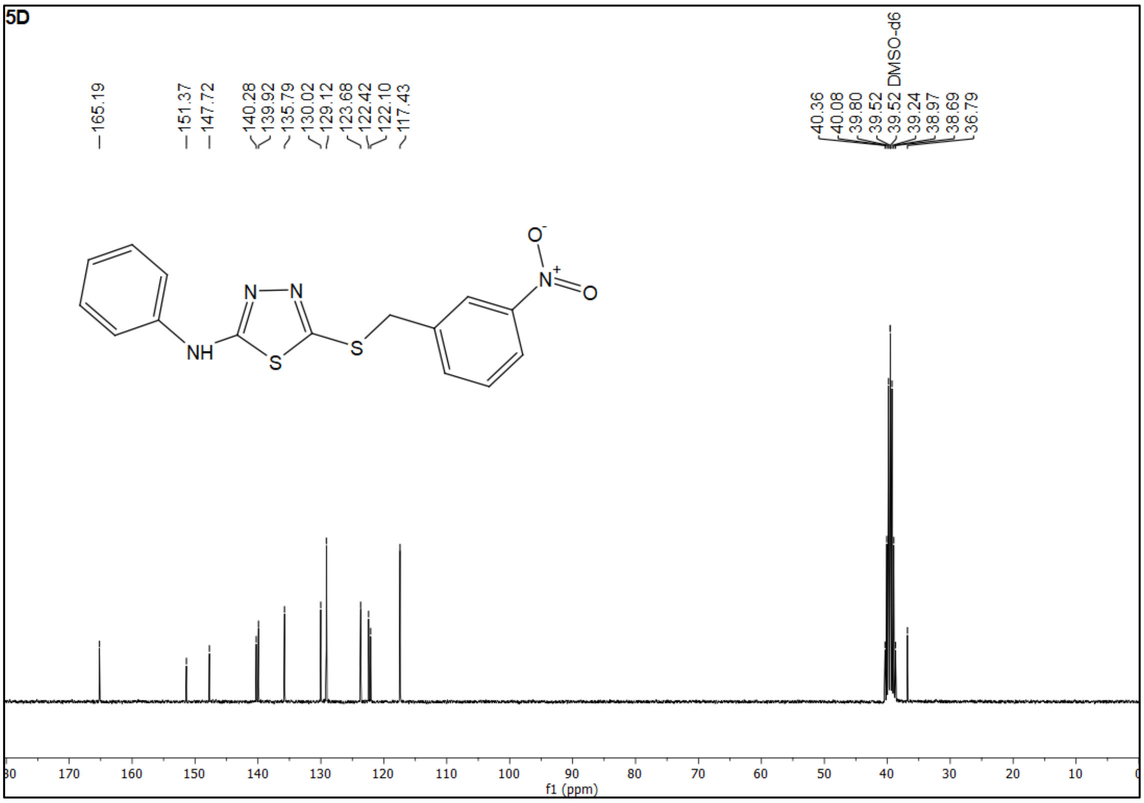


Figure 28: ^{13}C NMR spectra of 5-((3-nitrobenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5D).

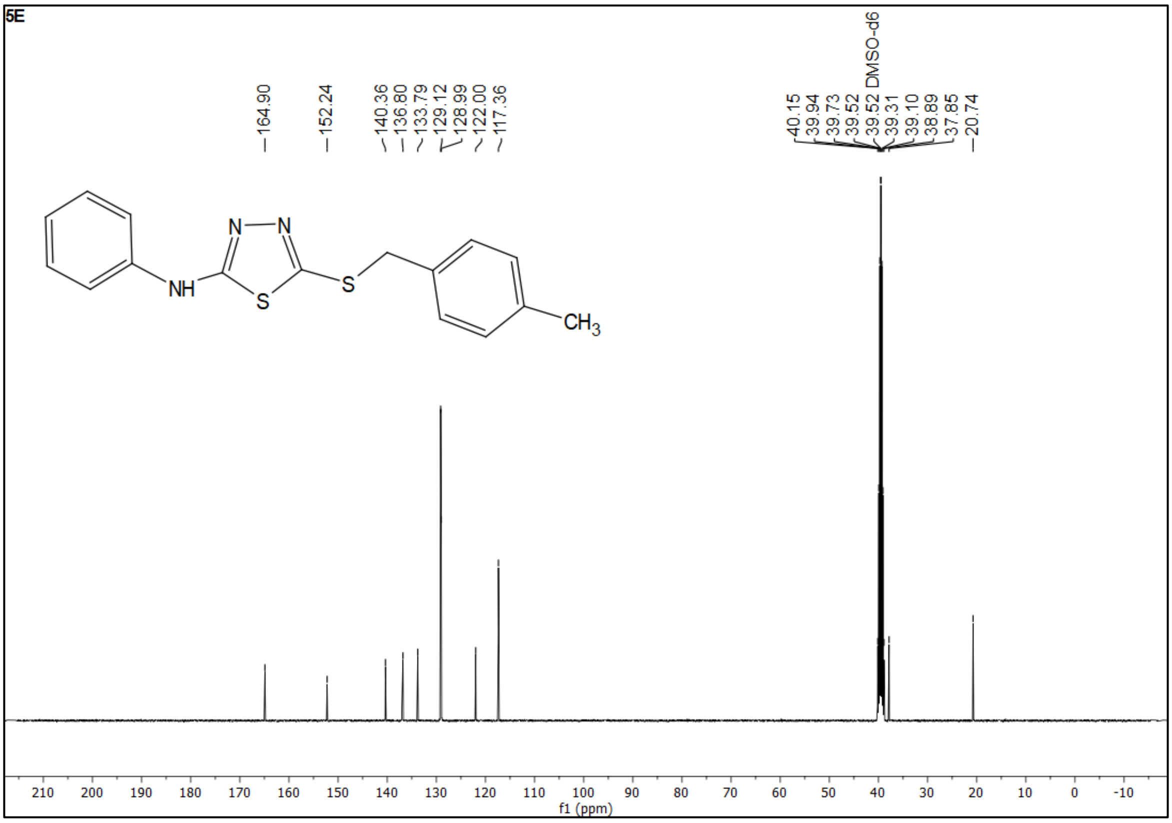


Figure 29: ^{13}C NMR spectra of 5-((4-methylbenzyl) thio)-N-phenyl-1,3,4-thiadiazol-2-amine (5E).

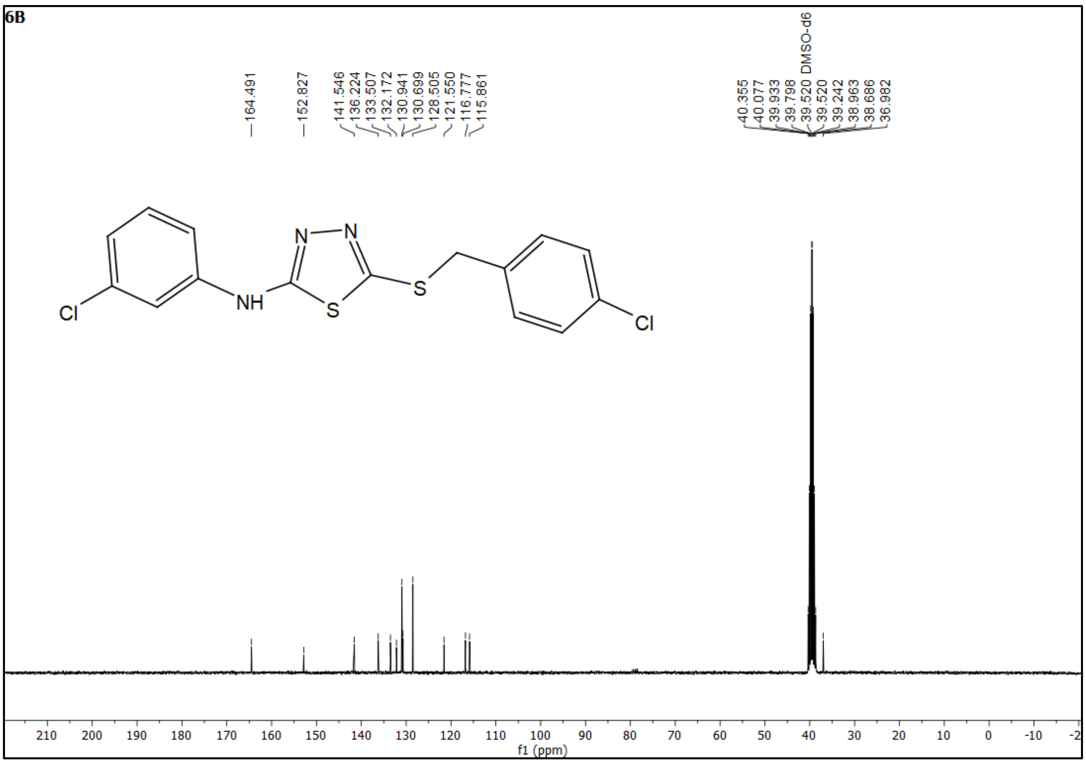


Figure 30: ^{13}C NMR spectra of 5-((3-chlorobenzyl) thio)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (6B).

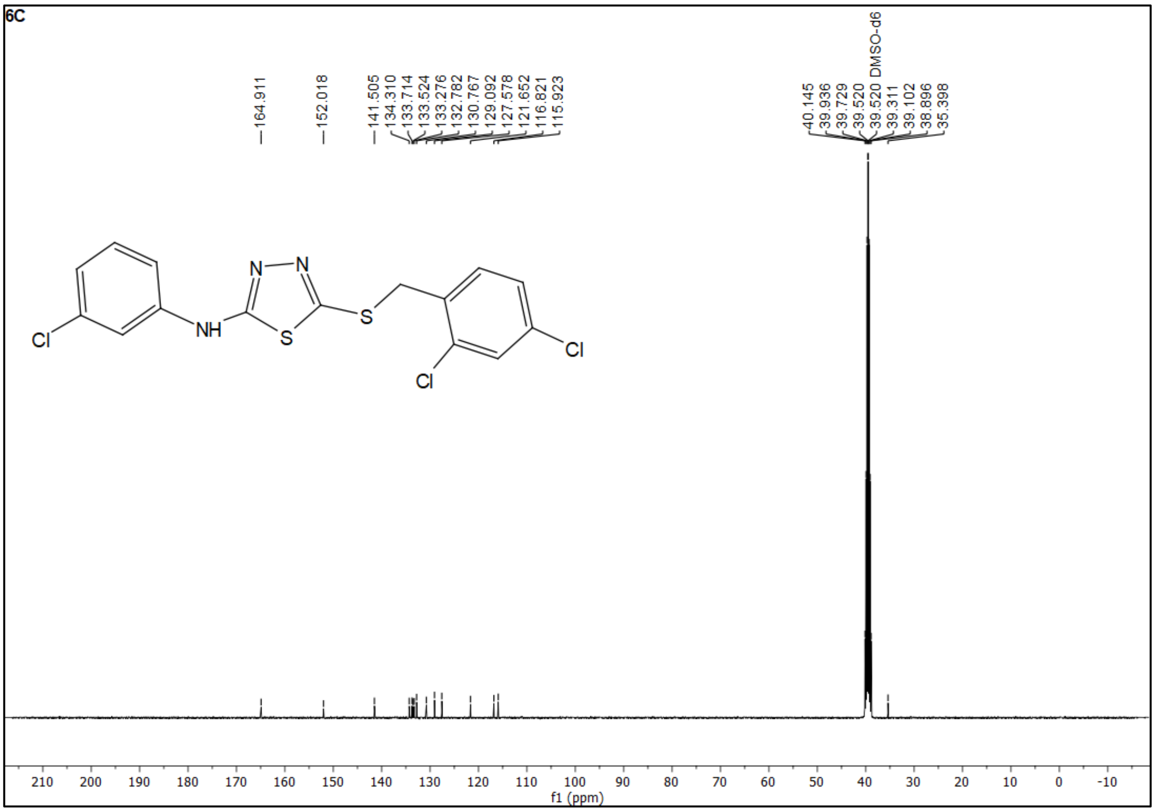


Figure 31: ^{13}C NMR spectra of N-(3-chlorophenyl)-5-((2,4-dichlorobenzyl) thio)-1,3,4-thiadiazol-2-amine (6C).

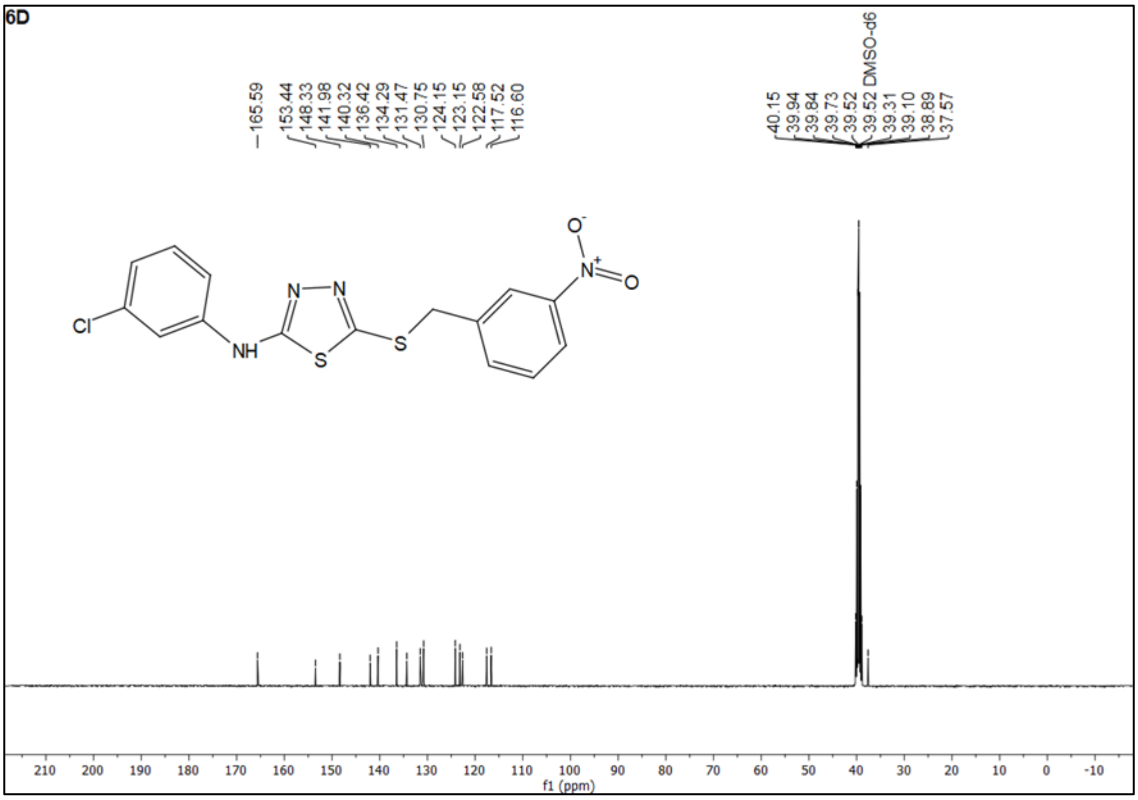


Figure 32: ^{13}C NMR spectra of N-(3-chlorophenyl)-5-((3-nitrobenzyl) thio)-1,3,4-thiadiazol-2-amine (6D).

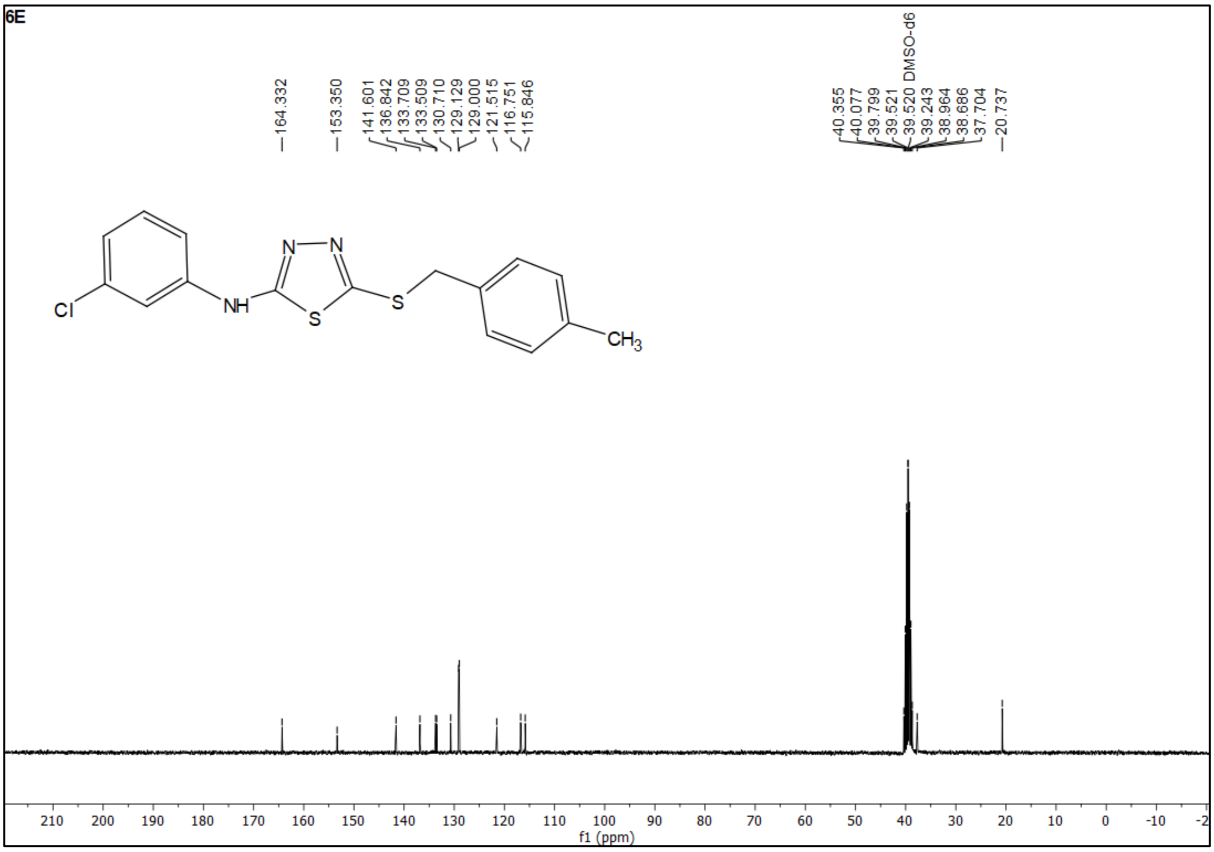


Figure 33: ^{13}C NMR spectra of N-(3-chlorophenyl)-5-((4-methylbenzyl) thio)-1,3,4-thiadiazol-2-amine (6E).

CHAPTER - 5

CONCLUSION

5. Conclusion:

1,3,4-thiadiazole derivatives are found to be pharmacologically more potent due to their wide range of biological activities. Modification of 1,3,4-thiadiazole moiety produces valuable pharmacological activity, mostly due to their N=C-S bond and substitution at different position in heterocycle possess the potential activity with lower adverse effects and toxicity in humans. Mesoionic character of this ring allows thiadiazole-containing compounds to cross cellular membrane and interact strongly with biological targets. Their synthesis process is not so complex. Antibacterial activity will be evaluated in terms of Zone of inhibition, MBC, and MIC value determination of established compounds. Molecular docking study will also be done to identify the drug-antibacterial target (viz. Penicillin-binding protein, DNA gyrase, NAM, NAG and Sortase A, etc.) interaction along with ADME (Lipinski's rule of five) and toxicological calculation via computational approach to determine the drug-likeness property of the molecules.