

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF THIADIAZOLE AND BENZOTHIAZOLE DERIVATIVES

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**FOR THE DEGREE OF
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IN
CHEMISTRY**

**SUBMITTED BY
ASHISH VIJAY SHINDE**

**UNDER THE GUIDANCE OF
DR. ASHISH L. ASRONDKAR**

**HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI**

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF THIADIAZOLE AND BENZOTHAIAZOLE DERIVATIVES

A Thesis submitted
to the
University of Mumbai
for the
**M.Sc. (By Research) Degree
in Chemistry**

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STATEMENT BY THE CANDIDATE

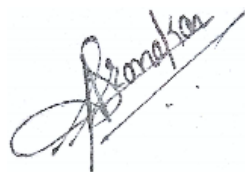
As required by the University Regulation No. R.2190, I wish to state that the work embodied in this thesis titled **“Synthesis and Biological Activities of Thiadiazole and Benzothiazole Derivatives”** forms my own contribution to the research work carried out under the guidance of Dr. Ashish L. Asrondkar at the Haffkine Institute for Training, Research and Testing, Mumbai. This work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.



Signature of candidate

Shinde Ashish Vijay Meena

Certified by



Signature of Guide

Dr. Ashish L. Asrondkar

Dedicated To My

Parents, Teachers

And

Beloved Friends

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LIST OF ABBREVIATION

G	gram
mL	Milliliter
M	Molar
µg	Micro gram
µL	Micro liter
mg	Microgram
%	Percentage
Min	Minute
Hr	Hour
IC50	Inhibitory concentration
g/mol	Gram per mole
µg/mL	Microgram per milliliter
mL/min	Milliliter per minute
µL/min	Micro liter per milliliter
°C	Degree Celsius
m/z	Mass to charge ratio
Mol.Wt	Molecular weight

CHEMICALS AND MATERIALS

POCl ₃	Phosphorus oxychloride
EDC	1,2-Dichloroethane
NaOH	Sodium hydroxide
KOH	Potassium hydroxide
TEA	Triethylamine
DPPH	2,2-Diphenyl-1-picrylhydrazyl
BHT	Butylated hydroxytoluene
DFS	Dichlofenac Sodium
Hr	Hour

INTRODUCTION

The subject of drugs is as previous as disease. Illness has been man's heritage from the beginning from his existence, and therefore the search of remedies combat it is perhaps equally old. The sincere attempt by man to control and cure disease has led to the search of new drugs or appropriate derivatives of existing drugs.

The earlier source of drugs were from plant, animal and mineral source, however due to the lack of potential action and definitive cure and generally additional toxicity, the discovery of a new drugs that are a lot of potential and fewer toxicant is important. The synthesis of derivatives has been a vital half and is geared toward modifying the action of drugs, significantly to scale back the side effect and to heighten the drug action. Nowadays more than 60% of drugs uses in practice are synthesized derivatives and day-by-day the scope of synthetic medicinal chemistry is broadening.

Drugs during this category dissent from all different therein they're designed to inhibit/kill the infectious organism and to possess no/minimum impact on the recipient. This type of therapy is generally called chemotherapy which has come to mean 'treatment of general infection with the specific drugs that selectively suppress the infecting the host. Due to the analogy between the malignant cell and the pathogenic microbes, treatment of neoplastic disease with drugs is called chemotherapy.

Along with globalization several new diseases arising within the world, an infection caused by the micro-organism render a serious contribution to it. Some of the infections are acute and a few are chronic which can be air born, water born or food born. Unsanitary conditions are determined to be the root cause of these infections. In addition to it the development of resistance in micro-organism against current microbial therapy continues to drive the search for a more effective antimicrobial agent.^[1]

Bacteria have lived on earth for several billion years. Throughout now, they're encountered in nature a wide range of naturally occurring antibiotics. To survive, bacteria developed antibiotics resistance mechanism. Therefore, it is not surprising that they have

become resistant to most of the anti-microbial agent that have been developed over the 50 years. This resistance progressively limits the effective antimicrobial drugs. The matter isn't simply antimicrobial resistance but also multidrug resistance. In 2004, quite 70% of pathogenic bacteria were estimated to be resistance to a minimum of one amongst the presently out there antibiotics. The alleged 'superbugs' (an organism that is resistant to most of the clinically used antibiotics) are emerging at a rapid rate. *S. aureus*, which is resistant to methicillin, is responsible for several cases of infections each year.

The incidence of multidrug-resistant pathogenic bacteria is increasing. One further reason for developing new antibiotic is said to their own toxicity. As alternative therapeutic agents, the use of an antibiotic may also cause a side effect in patients. These include mild reaction such as upset stomach, vomiting and diarrhea (cephalosporin, macrolides, and penicillins), rash, other mild and severe allergic reactions (cephalosporins and penicillins), sensitivity to sunlight (tetracyclines), nervousness, tremors and seizures (quinolones). Some side effects are a lot of severe and depending on the antibiotics might disrupt the hearing function (aminoglycosides), kidneys (aminoglycosides and polypeptides) or liver (rifampin).

In order to combat the microbial infections, antimicrobial agents are frequently used concurrently i.e. the combined used of antimicrobial with the following objectives.

- To prevent emergence of resistance
- To lower the adverse or side effect
- To have an synergetic effect
- To broaden the antimicrobial spectrum

Since the microorganism developed rapid resistance there is an ample scope of the development of the antibacterial agents that are active against resistant bacteria.

Another field of greater concern is that the synthesis of a more effective inflammatory agent. Inflammation is a host defense mechanism in response to varied infectious or metabolic stimuli. Inflammation can be defined as the native response of living mammalian tissue to injury due to an agent. It is a body defense mechanism in order to eliminate or limit the unfold of injurious agent as well as take away the ensuing

necrossed cells and tissues. ^[2] Its a necessary protecting and normal response to any noxious stimulus, which may threaten the well-being of the host. The four cardinal signs of inflammations are rubor (redness), tumor (swelling), calor (heat), and dolor (pain). Suppression of inflammation still continues to be a challenge despite the availability of variety of NSAID's. This can be as a result of NSAID's not only exhibit anti-inflammatory and analgesic activity but also cause gastrointestinal complication ranging from dyspepsia to upper GI tract bleeding and perforation. Efforts to enhance the adverse impact profile of current NSAID's have been focused on developing pro-drugs and modification of marketed formulations. ^[3] These efforts have been partially successful. A recent approach is that the development of selective cyclooxygenase inhibitors (COX-2). Presently accessible NSAID's inhibits COX-1 and COX-2. Inhibition of COX-1 reduces production of cytoprotective prostaglandins PGE2 and PGI2 and hence known as ulceration, whereas inhibition of COX-2 inhibits inflammation. Complete inhibition of COX-1 is therefore not preferred and drugs that selectively inhibit COX-2 are better anti-inflammatory agent. ^[4]

Heterocycles bearing thiazole, sulphur and nitrogen moieties represent the core structure of a variety of pharmacologically and biologically active fascinating compounds. The Potency of azoles as chemotherapeutic agent is well established. So the roles of nitrogen and sulphur containing heterocyclic compounds which are endowed with distinctive structure and potent antimicrobial activity have to be over emphasized. We thought of it of interest to synthesized some 1, 3, 4-thiadiazole and benzothiazole derivatives for their antimicrobial and anti-inflammatory properties.

1.1 Chemistry of Thiadiazole ^[5]

Thiadiazole moiety act as “hydrogen binding domain” and “two-electron donar system”. Thiadiazole act as bioisosteric replacement of thiazole moiety. So, it acts as third and fourth generation cephalosporin. Thiadiazole is five membered ring system containing sulphur and nitrogen atom. They occur in four isomeric forms viz., 1, 2, 3-thiadiazole (1), 1, 2, 4-thiadiazole (2), 1, 2, 5-thiadiazole (3), 1, 3, 4-thiadiazole.

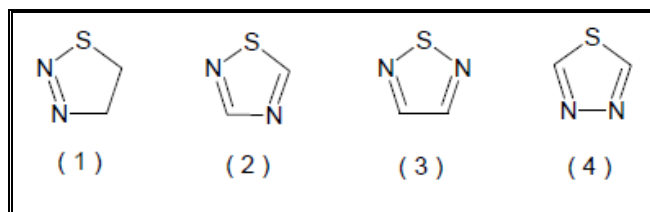


Fig 1.1: Isomeric forms of Thiadiazole

A glance at the standard reference work shows that more work administered on a 1,3,4-thiadiazole that every one alternative isomers are members of a ring system have found their way into diverse application as pharmaceutical application oxidation inhibitors, cyanide dye, and metal complexing agent. The literature showed thiadiazole nuclei exhibit many biological activities and therefore the -azole designates a five membered a ring system with two or more hetero atom. The ending ole used for other five membered heterocyclic rings without nitrogen. The numbering of monocyclic azole system begins with a heteroatom that is in the highest group in the periodic table and with the element of lowest atomic weight in that group. Hence the numbering of the 1, 3, 4-thiadiazole (4) is done in the following manner. This designated on sulphur group is present in the ring.

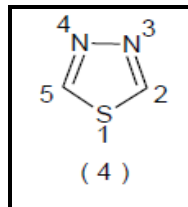


Fig 1.2: 1, 3, 4-thiadiazole ring structure

1.2 Synthesis of 1, 3, 4-Thiadiazole

The recent and advanced increases the wide spectrum of activity due to the development of new and more sensitive screening techniques. Further progress had been added continues synthetic derivatization to those compounds. Several strategies had been reported in the literature.

Thiadiazole can be synthesized from mainly thiosemicarbazide or hydrazide that is thiadiazole can cyclized from thiosemicarbazide or a hydrazide by methods like conventional method, ultrasound or microwave using catalyst like H_2SO_4 , POCl_3 , CS_2 , polyphosphoric acid and HCl .

Methods of synthesis of 2, 5-disubstituted-1, 3, 4-thiadiazoles

Important new general routes of 1, 3, 4-thiadiazole have been reported. The major routes are:

1.2.1. From Thiosemicarbazide

Desai et al ^[6], synthesized 2-(2-(4-chloro phenyl)acetyl)-N-aryl hydrazine carbothioamides were prepared by reacting 4-chloro phenyl acetyl hydrazide and aryl isothiocyanate in the presence of ethanol. Various 5-(4-chloro-benzyl)-4-aryl-4H-1,2,4-triazole-3-thiols, 2,5-(4-chloro benzyl)-N-aryl-1,3,4-thiadiazole-2-amine have been prepared by the cyclization with sodium hydroxide, sulfuric acid and iodine in potassium iodide in presence of sodium hydroxide.

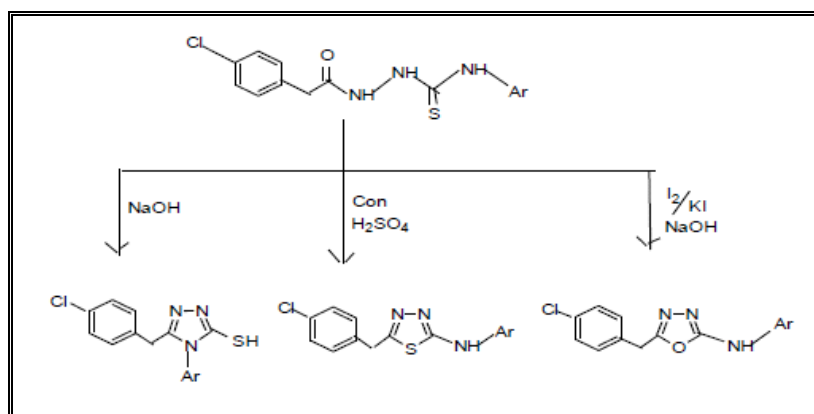


Fig 1.3: Cyclization reaction of 2-(2-(4-chloro phenyl)acetyl)-N-aryl hydrazine carbothioamides

1.2.2 By using phosphorous oxychloride

Pokhodylo et al ^[7], synthesized 4-amino-5-benzyl-4H-1,2,4-triazole-3-thiol with 5-methyl-1-aryl-1H-1,2,3-triazole-4-carboxylic acid in phosphorous oxychloride. It was established reaction performed with closing thiadiazole ring. Thus by the reaction of 4-amino-5-benzyl-4H-1,2,4-triazole-3-thiol with 5-methyl-1-aryl-1H-1,2,3-triazole-4-carboxylic acid new 3-benzyl-6-(5-methyl-1-phenyl-1H-1,2,3-triazole-4-yl)(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazole have been synthesized.

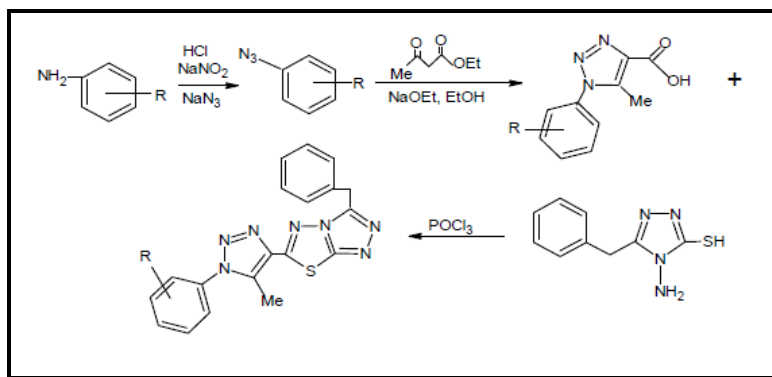


Fig 1.4: 3-benzyl-6-(5-methyl-1-phenyl-1H-1,2,3-triazole-4-yl)(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazole

1.2.3 By using carbon disulphide

Dhiman *et al* ^[8], synthesized thiosemicarbazide with carbon disulphide and DMF under results formation of 5-(3-aryl-1H-pyrazole-5-yl)-2-mercapto-1, 3, 4-thiadiazoles.

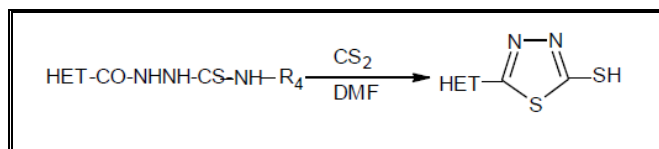


Fig 1.5: 5-(3-aryl-1H-pyrazole-5-yl)-2-mercapto-1, 3, 4-thiadiazoles

1.2.4 By using polyphosphoric acid

Foromaudi *et al* ^[9], synthesized 4-chloro-2-(2-chlorophenoxy) benzoic acid was prepared from 2, 4-dichlorobenzoiuc acid and 2-chloro phenol. Treatment with thionyl chloride yielded 4-chloro-2-(2-chlorophenoxy) benzoyl chloride, which upon the reaction with 4-alkyl-thiosemicarbazide provided 1-(4-chloro-2-(2-chlorophenoxy) benzoyl)-4-alkyl thiosemicarbazide. Cyclization of the latter compound with sulfuric acid gave a final compound, 1-(4-chloro-2-(2-chlorophenoxy) benzoyl)-4-alkyl amino thiadiazole. 1-(4-chloro-2-(2-chlorophenoxy) benzoyl)-4-alkyl amino thiadiazole was prepared from direct reaction of thiosemicarbazide in polyphosphoric acid.

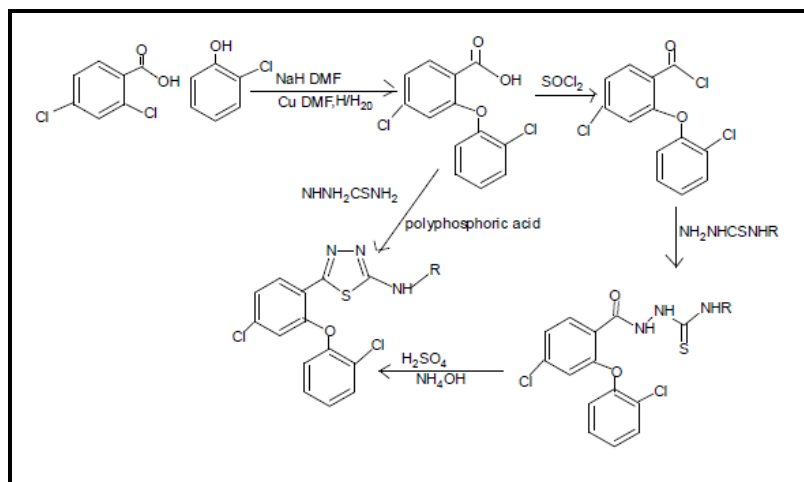


Fig 1.6: 1-(4-chloro-2-(2-chlorophenoxy) benzoyl)-4-alkyl amino thiadiazole

1.2.5 By using hydrochloric acid

Nazar Trotsko *et al*^[10], synthesized starting material were thiosemicarbazide derivative of 5-arylidine-2,4-dioxothiazolidine-3-actic acid were obtained in the reaction of corresponding (5-arylidine-2,4-dioxothiazolidine-3-yl) acetyl chloride with 4-phenyl thiosemicarbazide, cyclization of the obtained new thiosemicarbazide derivative carried out in a acid media of hydrochloric acid and conc. Sulphuric acid to form a 1,3,4-thiadiazole derivative.

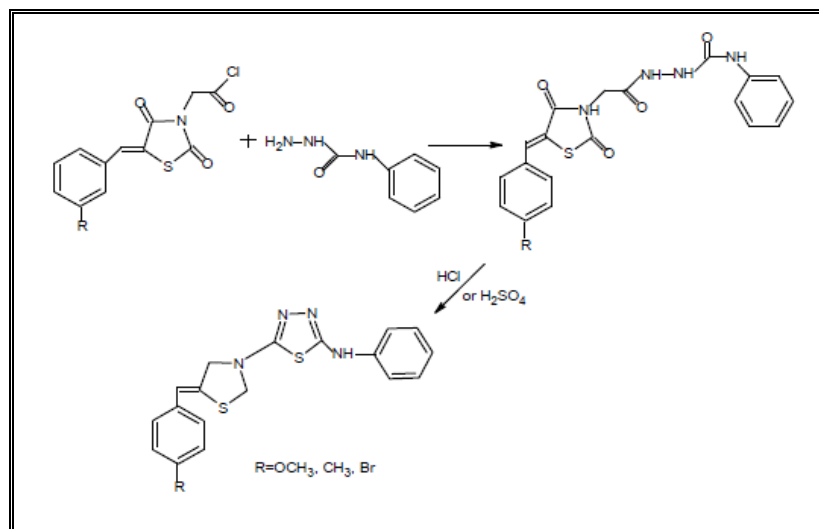


Fig 1.7: 1,3,4-thiadiazole derivative

1.3. Chemistry of 1, 3- benzothiazole

Benzothiazoles are consolidated membered ring that contains the heterocyclic bearing thiazole, sulfur and nitrogen moieties represent the core structure of a variety of pharmacologically and biologically active compounds.

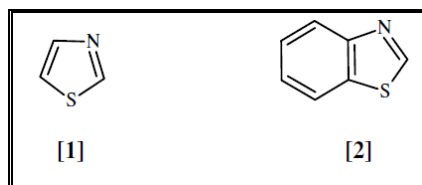


Fig 1.8: 1, 3-thiazole bearing benzothiazole moiety

Thiazole (1) is structurally associated with thiophene and pyridine, however in most of its properties resembles the latter. The Structure (2) is benzothiazole. Thiazole was first described by the Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. The numbering in thiazole starts from the sulfur atom. The basic structure of benzothiazole consists of a benzene ring fused with 4, 5-position of thiazole. The two rings together constitute the basic nucleus 1, 3-benzothiazole. The thiazole ring has been extensively studied and its type of a part of B vitamin, penicillins and antibacterial thiazoles ^[11].

1.2.6 Synthesis of Benzothiazole

a. Thiocyanation of ester of p-amino benzoic acid yields 2-amino-6-carboxy-benzothiazolderivative as reported by **kaye et al** ^[12].,

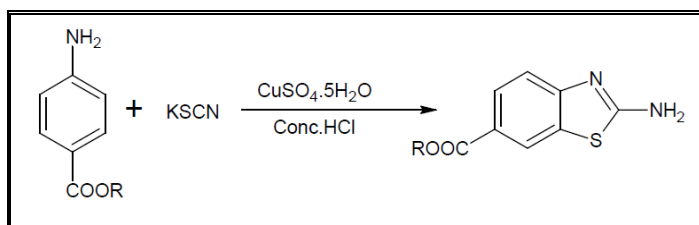


Fig 1.9: 2-amino-6-carboxy-benzothiazolderivative

b. The cyclization of 2-aminothiophenol with malononitrile yields 2-cynomethyl benzothiazole and this work reported by **EI- Kashouti et al** ^[13].,

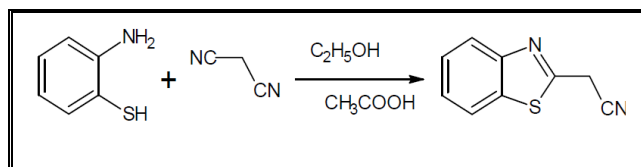


Fig 1.10: 2-cynomethyl benzothiazole

c. **Kini *et al*** ^[14]., synthesis the 2-aryl substituted benzothiazole derivatives by cyclising 2-aminophenol with substituted benzoic acids in presence of polyphosphoric acid at reaction temperature.

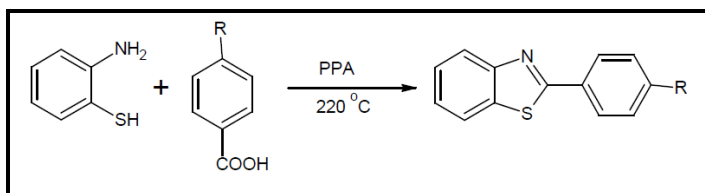


Fig 1.11: 2-aryl substituted benzothiazole derivatives

Review of Literature

The 1, 3, 4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents. Thiadiazole nucleus is present as a core structural component in an array of drug categories. Extensive literature review has been done to understand the contribution of various researchers on this field. The articles from various resources are collected and few of them are summarized in the below stated literatures.

- GuoGang Tu, *et al* have reported the synthesis of novel aminopeptidase N inhibitors derived from 1,3,4-thiadiazole scaffolds.^[15]

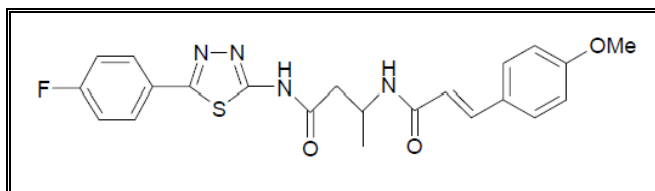


Fig 2.1: The synthesis of novel aminopeptidase N inhibitors from 1,3,4-thiadiazole

- V Padmavati, *et al* have reported the synthesis and bioassay of new class of heterocycles pyrrolyl oxadiazoles/thiadiazoles/triazoles.^[16]

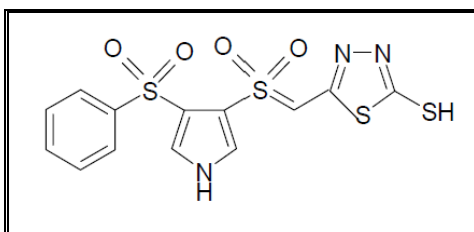


Fig 2.2: Synthesis a new class of heterocycles pyrrolyl thiadiazoles.

- Demirbas N, *et al* have reported the synthesis and characterization of triheterocyclic compounds consisting of 1,2,3-triazole-3-one, 1,3,4-thiadiazole, 1,3,4-oxadiazole rings.^[17]

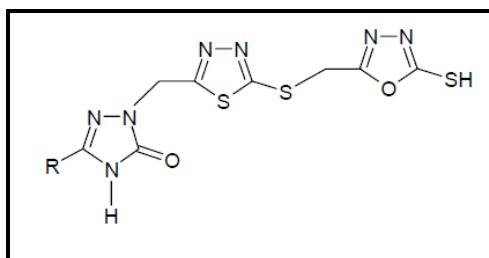


Fig 2.3: synthesis of triheterocyclic 1,3,4-thiadiazole derivative

- Jayamma Y, et.al have reported the synthesis and biological evaluation of 2(3,4-dihydro-3-oxo-2H-1,4-benzoxazin-2-yl)methyl)-5-arylamino-1,3,4-thiadiazol.^[18]

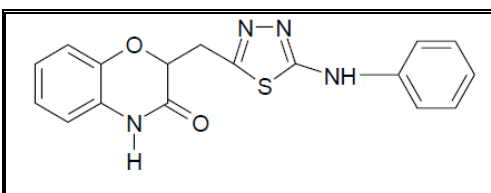


Fig 2.4: Synthesis of (3,4-dihydro-3-oxo-2H-1,4-benzoxazin-2-yl)methyl)-5-arylamino-1,3,4-thiadiazol

- Yin, Da wei et al. reported synthesis and antibacterial activity of ferrocene based thiadiazole. The compounds were found to have increased antibacterial activity against one or more species.^[19]

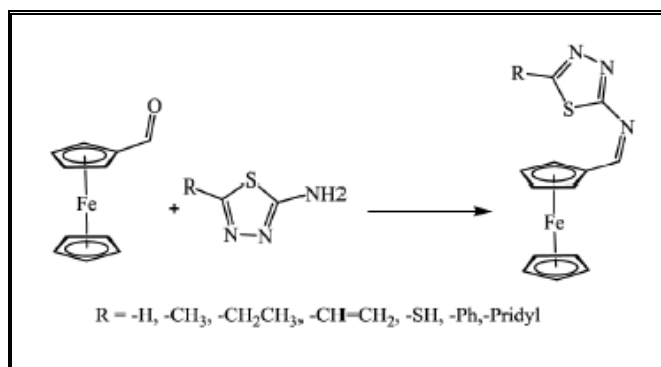


Fig 2.5: ferrocene based thiadiazole

There are several reports in the literature describing the 1,3,4-thiadiazole derivatives for their various biological activities and the most relevant and recent studies have revealed that 1,3,4-thiadiazole derivatives have a broad spectrum of pharmacological activities that can be classified into the following categories.

Antibacterial and Antifungal activity

- He et al. ^[20] synthesized 5-(1-aryl-1H-tetrazol-5-ylsulfanylmethyl)-N-xylopyranosyl-1,3,4-thiadiazole-2-amine derivatives and investigated in vitro antibacterial activity against *S. aureus*.

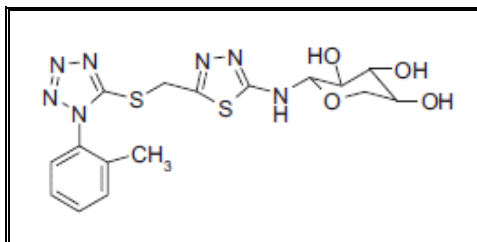


Fig 2.6: 5-(1-aryl-1H-tetrazol-5-ylsulfanylmethyl)-N-xylopyranosyl-1, 3, 4-thiadiazole-2- amine

- Antibacterial activity of C incorporated with 5-(5-nitroheteroaryl)-1, 3, 4-thiadiazol-2-yl groups at C-7 position had been studied by Jazayeri et al. ^[21].

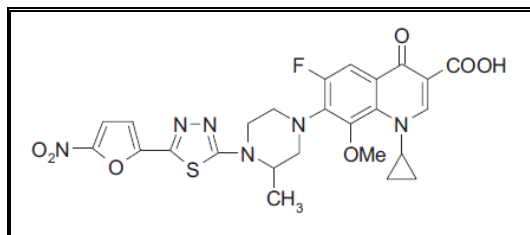


Fig 2.7: Gatifloxacin derivatives

- Chen et al. ^[22] reported, synthesis of 5-(3, 4, 5-trimethoxyphenyl)-2-sulfonyl-1, 3, 4-thiadiazoles and evaluated for antifungal activities against *G. zeae*, *B. cinerea*, and *S. sclerotiorum*.

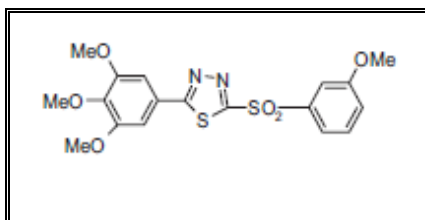


Fig 2.8: 5-(3, 4, 5-trimethoxyphenyl)-2-sulfonyl-1, 3, 4-thiadiazole

Anticancer activity

- Kumar et al. ^[23] reported the synthesis of 5-(3-indolyl)-1, 3, 4-thiadiazoles and evaluated for anticancer activity. Compound 2-(4-(Benzyloxy)-5-(5-bromo-3-indolyl)-3-methoxyphenyl)-1,3,4-thiadiazole with 4-benzyloxy-3-methoxyphenyl at C-2 position and 5-bromoindole at C-5 position was found to be the most potent compound of the series.

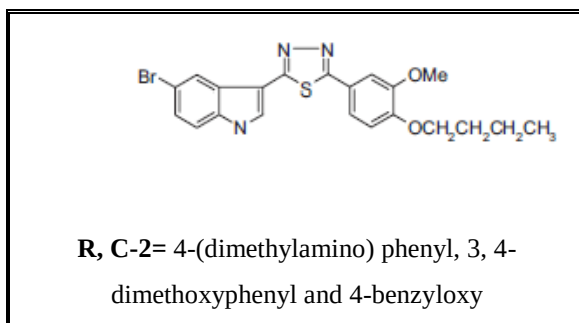


Fig 2.9: 2-(4-(Benzyloxy)-5-(5-bromo-3-indolyl)-3-methoxyphenyl)-1, 3, 4-thiadiazole

- Matysiak et al. ^[24] examined the effect of various substitution at 5-position of 2-(2, 4 dihydroxy-phenyl)-1, 3, 4-thiadiazoles on antiproliferative activity against different human tumor cell lines. 2-(2,4-Dihydroxyphenyl)-5-(4-methoxybenzyloxy)-1,3,4-thiadiazole showed ID₅₀ of 1.1 lg/mL against HCV29T bladder cancer cell line and found to be significantly lower (T47D) than that of cisplatin, used as the reference compound.

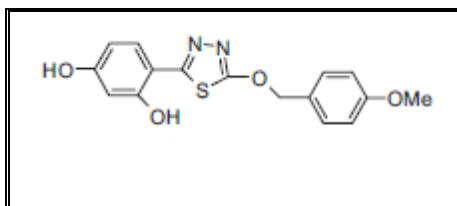


Fig 2.10: 2-(2,4-Dihydroxyphenyl)-5-(4-methoxybenzyloxy)-1,3,4-thiadiazole

- Wei et al ^[25] reported the synthesis of new chiral 2, 5-disubstituted 1, 3, 4-thiadiazoles derivatives possessing γ -butenolide moiety, compound was screened against HeLa cell lines by MTT assay and exhibited IC₅₀ of 0.9 μ M.

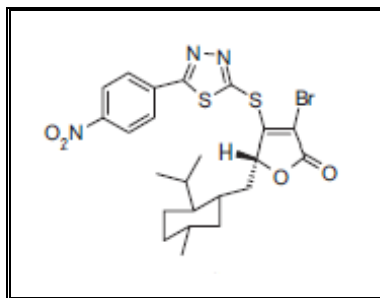


Fig 2.11: 2, 5-disubstituted 1, 3, 4-thiadiazoles derivatives

Anti-Helicobacter pylori

- Moshaf et al. ^[26] synthesized 2-(3, 5dimethylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1, 3, 4-thiadiazole and screened for bactericidal activity against *H. pylori* at 0.5 lg/disk (average of inhibition zone >20 mm) which was superior to that of metronidazole.

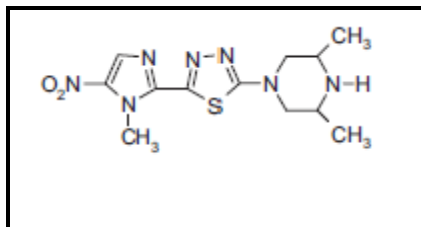


Fig 2.12: 2-(3,5-dimethylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole

Anticonvulsant activity

- Rajak et al. ^[27] synthesized (Z)-N-(5-(4-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl)-2-((4-nitrophenyl(phenyl)methylene)hydrazinecarboxamide and evaluated their potential anticonvulsant activity.

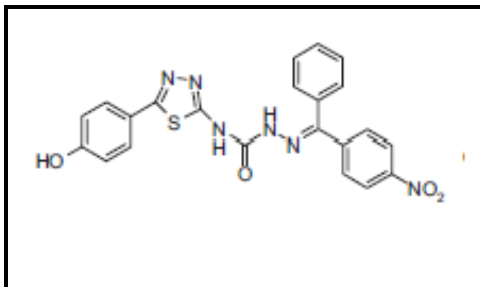


Fig 2.13: (Z)-N-(5-(4-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl)-2-((4-nitrophenyl(phenyl)methylene)hydrazinecarboxamide

Anti-inflammatory activity (COX-inhibitors)

- Anti-inflammatory activity of new series of 5-(1-adamantyl)-1, 3, 4-thiadiazole derivatives was reported by Kadi et al. ^[28]. Substituted with propionic acid at 2nd position of 1, 3, 4-thiadiazoline-2-thiones, showed almost equal anti-inflammatory activity at 20 mg/kg to that of Indomethacin (5 mg/kg).

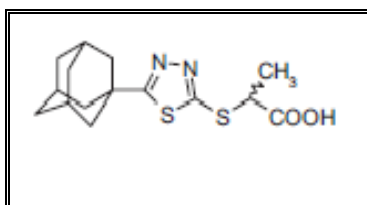


Fig 2.14: 5-(1-adamantyl)-1, 3, 4-thiadiazole derivatives

- Amir et al examined 1,3,4-thiadiazole analogs of naproxen carrying a 4-bromophenyl amino group at second position of the thiadiazole ring showed 78.02% inhibition in rat paw edema ^[29].

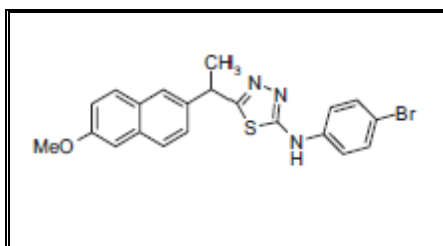


Fig 2.15: 1, 3, 4-thiadiazole analogs of naproxen

Antitubercular activity

- Foroumadi et al. ^[30] synthesized 2-((4-nitrobenzyl)thio)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole and screened for in vitro antimycobacterial activity against Mycobacterium tuberculosis H37Rv using alamar-blue susceptibility test. The data compared with the standard drug rifampin at 0.031 µg/mL concentration which showed 97% inhibition.

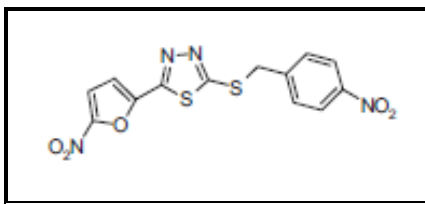


Fig 2.16: 2-((4-nitrobenzyl)thio)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole

Among nitrofuran derivatives, substitutions of the thioester group at C-5 of 1, 3, 4-thiadiazole ring with a benzyl analog was found to be the most active (MIC = 0.78 lg/mL) ^[31].

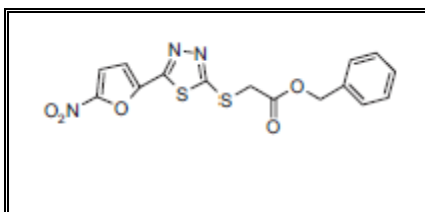


Fig 2.17: Nitrofuran derivatives

Antiviral activity

- Hamad et al. ^[32] synthesized 2-(naphthalen-2-yloxy)-N-((5-(phenylamino) - 1, 3, 4-thiadiazol-2-yl) methyl) acetamide and tested its in vitro anti-HIV-1 (strain IIIB) and anti-HIV-2 (strain ROD) activity by the inhibition of the virus induced cytopathic effect in the human T-lymphocyte (MT-4) cells, based on MTT assay which showed EC₅₀ values of 0.96 µg/ml.

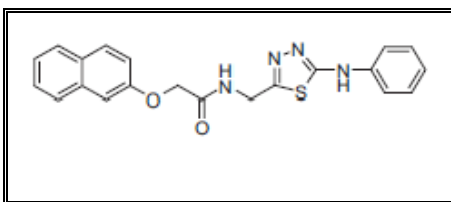


Fig 2.18: 2-(naphthalen-2-yloxy)-N-((5-(phenylamino) - 1, 3, 4-thiadiazol-2-yl) methyl) acetamide

Anti-leishmanial activity

- Foroumadi et al ^[33] synthesized 2-(5-nitrofuran-2-yl)-5-(4-phnylpiperazin-1-yl)-1,3,4-thiadiazole and results showed a high activity level of IC₅₀ = 0.1µM against L. major promastigotes.

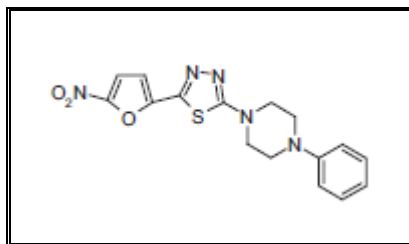


Fig 2.19: 2-(5-nitrofuran-2-yl)-5-(4-phenylpiperazin-1-yl)-1,3,4-thiadiazole

PLAN OF WORK

Pharmaceutical research particular in the field of chemistry provides a basis for the development of new approaches to combat human diseases. The biomedical research can be basic seeking on understanding of biological phenomenon which is unknown or applied by using principles which are known to produce desire new product or effect. In either cases research has the effect of altering the established therapies in favor of new modalities which would be safer and more effective.

Tropical diseases affects hundreds of million people every year causing immense suffering and hindering development. This has been hard fact even though considerable advances have been made in the field of chemotherapy of microbial diseases. The major problem is the fast development of resistance among the micro-organisms and the adverse effects that prevail along with the drug. Thus there is always a need for search of newer compounds with potent activity, good tolerances, and lesser side effects.

The present work consists of three parts:

- Synthesis of heterocyclic molecule
- Characterization of the compound
- Biological evaluation

A] Synthesis of (Z)-N-[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl]-1-substituted phenylmethanimine

The Synthesis of 1, 3, 4-thiadiazole and its derivatives have received significant attention and have been increasingly investigated due to their diverse range of biological properties. The therapeutic importance of these rings prompted us to develop selective molecules in which a substituted could be arranged in a pharmacological activity. The ring moiety of thiadiazole was the most significant nuclei, as well-known whole characteristics of diversity of natural medicinal agents. As essence structural, thiadiazoles are show in configuration of drugs category ^[34].

Al-Amiery *et al* had been synthesized new thiadiazoles that have coumarin moieties [fig.3.1] and tested for their antimicrobial activity. *In vitro* anti-bacterial and anti-fungal investigations various microbes named (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* with *Aspergillus niger* and *Candida albicans*)^[35].

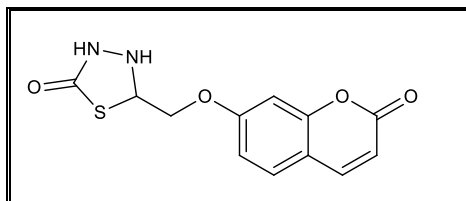


Fig 3.1: 5-(((2-oxo-2H-chromen-7-yl)oxy)methyl)1,3,4-thiadiazole-2-one

Baghel U. S. *et al* had been synthesized a new thiadiazoles and examined the anti-bacterial and antifungal activities for them. The novel prepared thiadiazoles were investigated against Gram positive and gram negative species named *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative species *Pseudomonas aeruginosa*, *Escherichia coli* addition to fungi *Candida albicans* and *A. niger*. Compounds 4-(5-(3-chlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)phenol [a] and 4-(5-(4-chlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)phenol [b] shows potent activities comparing with the examined microbes^[36].

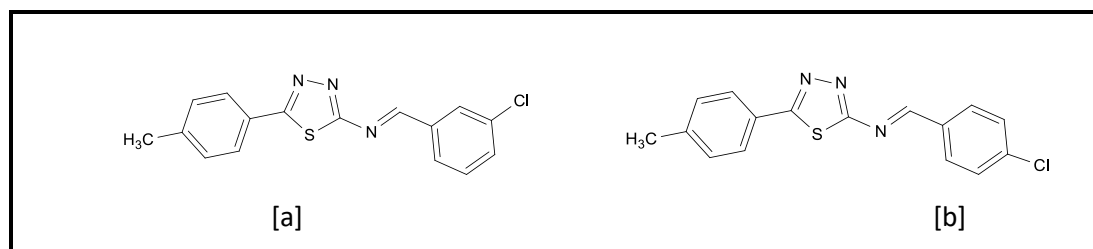


Fig 3.2: New thiadiazole derivatives

Suddasatwa Banerjee^[37] *et al* synthesized thiadiazole derivatives with new amino group by refluxing furan-2-carboxylic acid with thiosemicarbazide in presence of

conc.H₂SO₄ and then different Schiff bases were prepared by reacting with various substituted aldehydes in presence of catalyst. Screening for in vitro anticancer activity was carried out using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl terazolium bromide) assay on HT-29 colorectal cancer cell line. The compounds had shown significant activity at very less concentration.

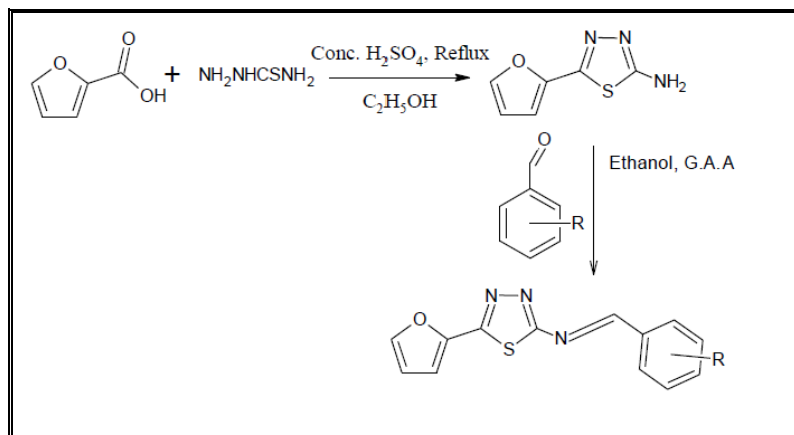


Fig 3.3: thiadiazole derivatives

Hence considering the importance of 1, 3, 4-thiadiazole and Schiff base it was decided to integrate and derive Schiff bases from 1, 3, 4-thiadiazole with a motive to develop a possible anti inflammatory agent.

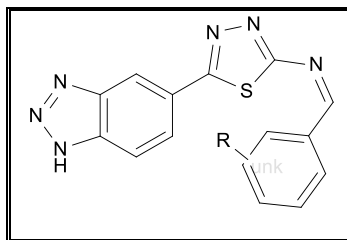


Fig 3.4: (Z)-N-[5-(1H-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl]-1-substituted phenylmethanimine

B] Synthesis of *N*-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino} acetamide

Acetamide have been extensively explored for their chemotherapeutic activities and have been reported to posses broad spectrum of biological activities such as anti-inflammatory [38], anticancer [39], antifungal [40] etc. Acetamide derivatives also found application in solid stat chemistry in the synthesis of amino acids, natural compounds (alkaloids) and their homologs pharmacologically promising substances and biomarkers reagent for polymer modification ion exchange resins for heavy and radioactive metal sorption. Chloroacetamide pesticides and dyes are also well known. Thus, investigation of acetamide chemistry is an actual task both from theoretical and applied view points. Some important acetamide derivatives which deserved to mentioned are as following:

A. R. Bhat *et al.* [41] have synthesized 3-(1,3,4-thiadiazol-2-yl) quinoline derivatives fig. 3.5 from the chloroquinone molecule with an aim to explore their effect on in vitro growth of microorganism causing microbial infection.

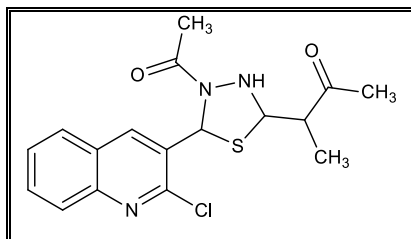


Fig 3.5: 3-(4-acetyl-5-(2-chloroquinolin-3-yl)-1,3,4-thiadiazolidin-2-yl)butan-2-one

D. P. Singh *et al.* have prepared *N*-(benzo[*d*]thizole-2-yl)-2-((3-methyl-3,4-dihydroquinoxalin-2-yl)thio)actamide compound are found to posses significant anti-inflammatory activity [42].

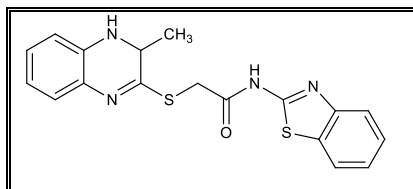


Fig 3.6: *N*-(benzo[*d*]thizole-2-yl)-2-((3-methyl-3,4-dihydroquinoxalin-2-yl)thio)actamide

Consideration of reactivity, compound availability, synthetic economy and simplicity in operation, has lead us to favor of the use of 1, 3, 4-thiadiazole derivatives with substituted N-1, 3-benzothiazol-2-yl-2-chloroacetamide in the synthesis acetamide derivatives.

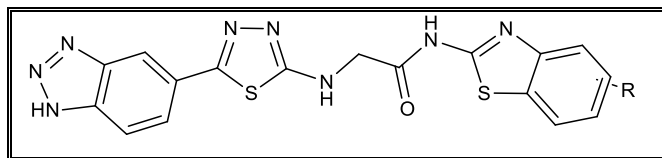


Fig 3.7: *N*-(substituted 1,3-benzothiazol-2-yl)-2-([5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino)acetamide

C] Synthesis of 2-([5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino)-*N*-[5-(substitutedbenzylsulfanyl)-1, 3, 4-thiadiazol-2-yl] acetamide

1, 3, 4-thiadiazoles have become an important class of heterocycles and a great interest of researches because of their broad types of biological activity. 1, 3, 4-thiadiazoles are the most studied class among the other thiadiazole isomers and their 2, 5-disubstituted derivatives have been widely discussed in terms of the interesting biological activities. In particular, such class as 5-amino-1, 3, 4-thiadiazole-2-thiol derivatives are known as antimicrobial, anticonvulsant and anti-diabetic agents.

He et al. ^[20] carried out synthesis of 5-(1-aryl-1*H*-tetrazol-5-ylsulfanylmethyl)-Nxylopyranosyl-

1, 3, 4-thiadiazole-2-amine derivatives and investigated *in-vitro* anti-bacterial activity against *S. aureus*. The compound was found to be the most active against tested strain.

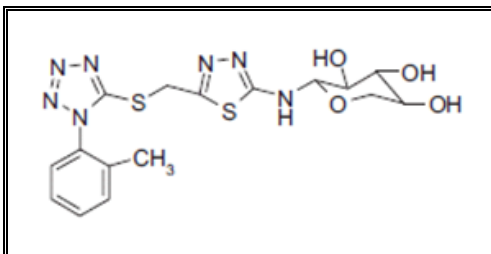


Fig 3.8: 5-(1-aryl-1*H*-tetrazol-5-ylsulfanylmethyl)-Nxylopyranosyl-1, 3, 4-thiadiazole-2-amine

1,3-Bis(5-alkyl-1,3,4-thiadiazol-2-yl)ureas were tested for hypoglycemic activity by Ermili and Cortese ^[43], the compounds were found to be good hypoglycemic agents.

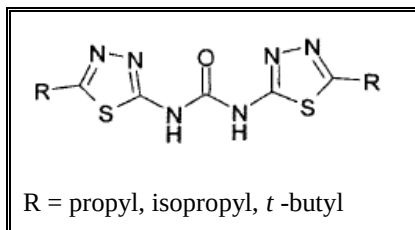


Fig3.9: 1,3-Bis(5-alkyl-1,3,4-thiadiazol-2-yl)

Keeping this in view, we have synthesized 2, 5-disubstituted-1, 3, 4-thiadiazole derivatives club with 1, 3, 4-thiadiazole (Intermediate) for their improved anti-inflammatory agent with combination to antimicrobial agent.

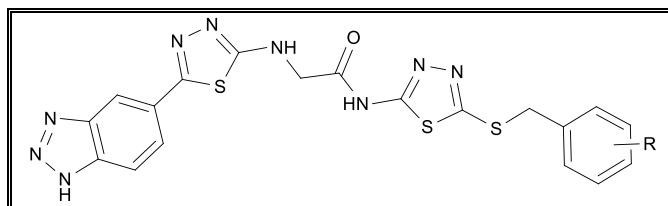


Fig 3.10: 2-[[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino}-N-[5-(substitutedbenzylsulfanyl)-1,3,4-thiadiazol-2-yl] acetamide

D] Synthesis of 2-[[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino}-N-[4-(substitutedphenylamino)-1,3-thiazol-2-yl] acetamide

Thiazole and its derivatives are the most utilized heterocycles. As the heterocyclic compounds are interest for its theoretical implications due to its synthetic procedure and physiological and industrial significance. Thiazole ring system plays an important role in the drug market with a wide spectrum of activity such as antibacterial, antifungal and antiviral ^[44]. Natural products having thiazole ring are known for their antibiotic and antifungal activities ^[45]. Marine products having thiazole ring exhibit antineoplastic, antifungal and inflammatory activity ^[46]. Due to its potent and significant biological activities it has great pharmaceutical importance.

Kouatly *et al.* ^[47] synthesized a series of adamantane derivatives of thiazolyl-N substituted amides and tested for anti-inflammatory activity as well as lipxygenase and cyclooxygenase inhibitory actions.

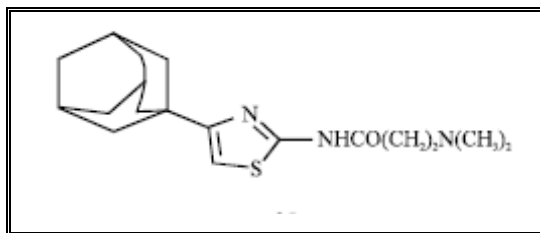


Fig 3.11: Adamantine derivatives of thiazolyl-N substituted amides

Saravanan *et al.* ^[48] synthesized 2-(5-(4-hydroxy-3-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)-N-(4-phenylthiazol-2-yl)acetamide and compound were screened for anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) activity exhibited highest anti-fungal activity.

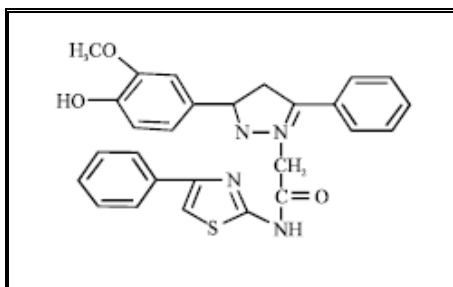


Fig3.12: 2-(5-(4-hydroxy-3-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)-N-(4-phenylthiazol-2-yl)acetamide

Shreenivas *et al.* ^[49] synthesized 4'-(2-n-Butyl-4-chloro-5-hydroxymethyl-imidazol-1-yl)-methyl-biphenyl-2-carboxylic acid- (4-phenyl/ substituted phenyl thiazole)-amide. The Compound was screened for their *in vitro* antibacterial activity against *S. aureus* showed maximum activity.

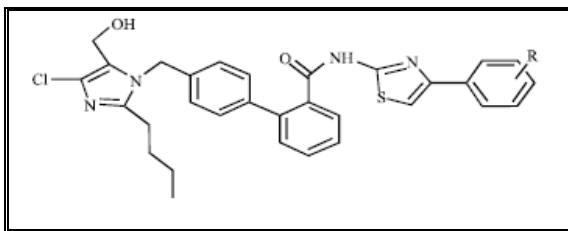


Fig 3.13: 4'-(2-n-Butyl-4-chloro-5-hydroxymethyl-imidazol-1-yl-methyl-biphenyl-2-carboxylicacid-(4-phenyl/ substituted phenyl thiazole)-amide

Here considering the importance of thiazole ring, we have synthesized a new series of 1,3,4-thiadiazole derivatives from substituted thiazole derivatives for their potent anti-fungal and antibacterial agent.

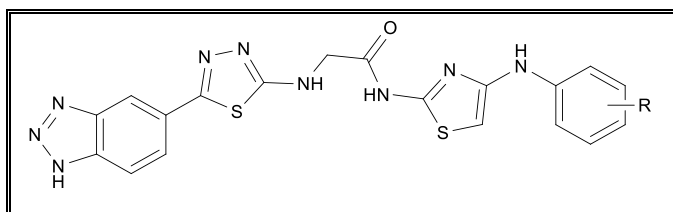


Fig 3.14: 2-{[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-N-[4-(substitutedphenylamino)-1,3-thiazol-2-yl]acetamide

CHEMISTRY

1. CHEMISTRY OF 1,3,4-THIADIAZOLE
2. CHEMISTRY OF SCHIFF BASE
3. CHEMISTRY OF BENZOTHAZOLE
4. CHEMISTRY OF AMIDE
5. CHEMISTRY OF 5-AMINO-1,3,4-THIADIAZOLE-2-THIOL
6. CHEMISTRY OF BENZYL CHLORIDE
7. CHEMISTRY OF 1,3-THIAZOLE

CHEMISTRY OF 1, 3, 4-THIADIAZOLE

Thiadiazole is a heterocyclic organic compound that has a five-member ring having one sulphur and two nitrogen atoms. ^[50] The development of 1, 3, 4-thiadiazole chemistry is linked to the discovery of phenylhydrazines and hydrazine in the late nineteenth century. The first 1,3,4-thiadiazole was described by Fischer in 1882, but the true nature of the ring system was demonstrated first in 1890 by Freund and Kuh. 1,3,4-Thiadiazoles represents one of the foremost biologically active classes of compounds, possessing wide spectrum of activities. Thiadiazoles have become important compounds in medicine, agriculture, and many fields of technology. A large variety of 1, 3, 4-thiadiazoles have been patented within the agricultural field as herbicides and bactericides. ^[51]

There are several isomers of thiadiazole, that is, 1,2,3-thiadiazole **(1)**, 1,2,5-thiadiazole **(2)**, 1,2,4-thiadiazole **(3)**, and 1,3,4-thiadiazole **(4)**. There are four possible structure in thiadiazole.

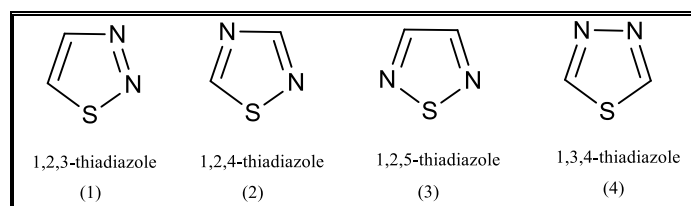


Fig 4.1: Isomers of thiadiazole

Methods of Synthesis of 1, 3, 4-Thiadiazole

(a) From Hydrazine: - 3-Thiocarbamoyl thione methyl carbonate on oxidation with H_2O_2 gave alkoxythiadiazole ^[52].

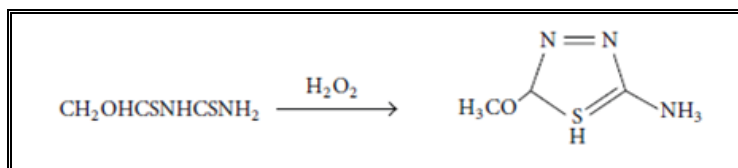


Fig 4.2: Alkoxythiadiazole

(I) Thiobenzoyl hydrazine cyclized to produce 1, 3, 4-thiadiazole-thiobenzoyl hydrazine ^[53].

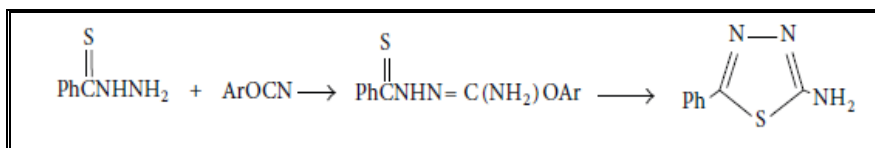


Fig 4.3: 1, 3, 4-thiadiazole-thiobenzoyl hydrazine

(II) Thione carbazate are cyclized by cyanogens chloride or bromide to give 1, 3, 4-thiadiazole ^[54].

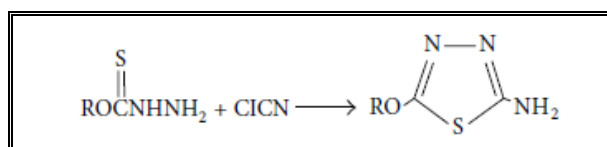


Fig 4.4: 1, 3, 4-thiadiazole derivative

(b) From Semicarbazide: - 4-phenyl-1-(thiobenzole) semicarbazide reacts in the presence of concentrated HCl giving 2-hydroxyl-5-phenyl-1, 3, 4-thiadiazole ^[55].

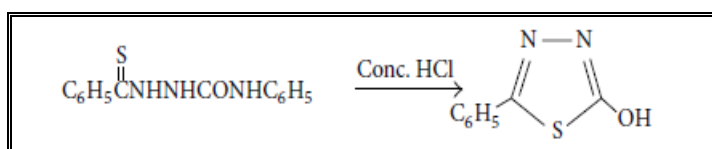


Fig 4.5: 2-hydroxyl-5-phenyl-1, 3, 4-thiadiazole

(c) From Thiosemicarbazide: - Many syntheses of the 1, 3, 4-thiadiazole proceed from thiosemicarbazide or substituted thiosemicarbazide.

Method 1. Gupta et al. have shown that thiosemicarbazide cyclizes directly to 2-amino-5-diazole with acetyl chloride. This simple route to 2-amino 5-substituted-1, 3, 4-thiadiazole seems to be quite general. In the example shown, R maybe methyl, nor hydnocarpyl, benzyl, cyclopropyl ^[56] and many others.

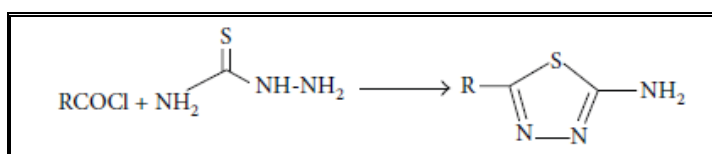


Fig 4.6: 2-amino 5-substituted-1, 3, 4-thiadiazole

(d) From Thiosemicarbazone: - 2-Amino-5-substituted thiadiazoles are prepared by oxidative cyclization of thiosemicarbazones with ferric chloride found 1, 3, 4-thiadiazole ^[57].

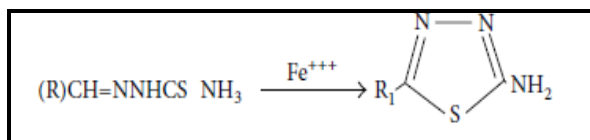


Fig 4.7: 2-Amino-5-substituted thiadiazoles

(e) From Resin: - Resin with TMSCl, MCPBA, and R² R³ NH gave 1, 3, 4-S thiadiazole ^[58].

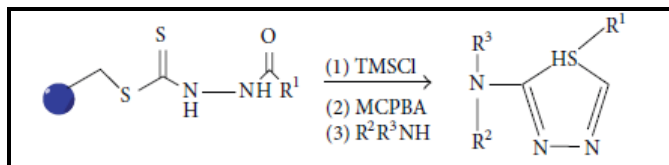


Fig 4.8: 1, 3, 4-S thiadiazoles

(f) From Bithioureas: - Bithiourea and substituted bithioureas have been converted to 1, 3, 4-thiadiazole by several methods.

Method 1. Bithiourea when treated with 3% hydrogen peroxide is cyclized to 2, 5-diamino-1, 3, 4-thiadiazole.

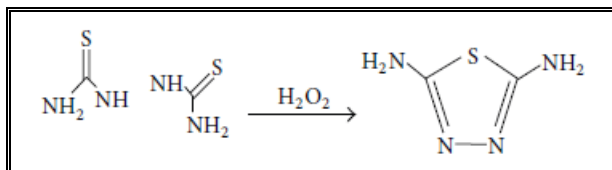


Fig 4.9: 2, 5-diamino-1, 3, 4-thiadiazole

Method 2. Acetic anhydride acts on bithiourea to form a diacetyl derivative of 2, 5-diamino-1, 3, 4-thiadiazole. The acetyl group is easily removed by hydrolysis to give the parent thiazole ^[59].

CHEMISTRY OF SCHIFF BASE

Imines, known even as azomethines or **Schiff bases** (named after Hugo **Schiff**) is a compound with the general formula $R_3R_2C=NR_1$. The substituents R_2 and R_3 may be alkyl, aryl, heteroaryl, hydrogen. The substituent at the *N*-imino ($C=N$) may be alkyl, aryl, heteroaryl, hydrogen or metallo (usually Si, Al, B, Sn).

2. Preparations of Imines

2.1. Preparation of *N*-Aryl or Alkyl Substituted Imines

2.1.1. Reaction of Aldehydes and Ketones with Amines

The most common method for preparing imines is the original reaction discovered by Schiff^[60]. Basically it consists in the reaction of an aldehyde (respectively a ketone) with a primary amine and elimination of one water molecule. This reaction can be accelerated by acid catalysis and is generally carried out by refluxing a mixture of a carbonyl compound **1** and an amine **2**, in a Dean Stark apparatus in order to remove the water. This removal is important as the conversion of aminal **3** into the imine **4** is reversible. As far as the use of acid catalyst is required^[61], mineral acids, like H_2SO_4 or HCl , organic acids such as *p*-toluene sulphonic acids or pyridinium *p*-toluenesulphonate, acid resin, montmorillonite or even Lewis acids like $ZnCl_2$, $TiCl_4$, $SnCl_4$, BF_3Et_2O , $MgSO_4$, $Mg(ClO_4)_2$, etc., have been reported.

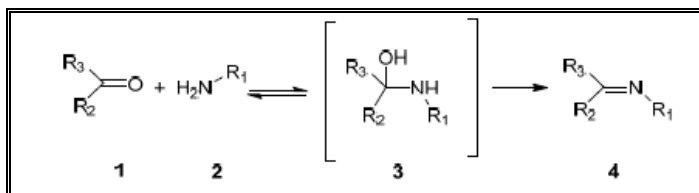


Fig 4.10: Schiff reaction for the preparation of imines.

In the course of the preparation of imines, if aliphatic aldehydes are used, a known competitive reaction, due to the formation of a condensation product arising from an aldol type reaction, can occur as well.

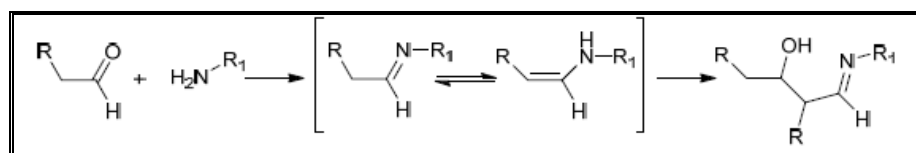


Fig 4.11: Aldol like condensation of aliphatic aldehydes.

2.1.2. Aerobic Oxidative Synthesis in the Preparation of Schiff's Bases

Aldehydes and ketones are mostly obtained from the corresponding alcohols via oxidative process, a straightforward preparation of imines from amines and alcohols, through tandem oxidative processes, have recently been developed ^[62].

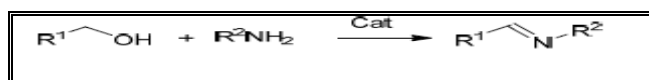


Fig 4.12: Oxidative synthesis of imines from alcohols and amines.

Huang and Largeron has been reported, a mild and efficient method of amine oxidation ^[63].

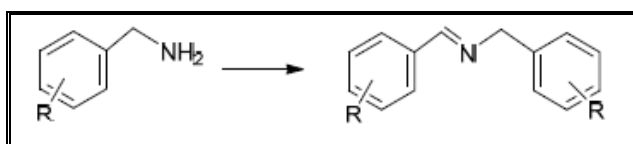


Fig 4.13: Oxidative synthesis of imines from amines.

2.1.3. Reaction of Phenols and Phenol-Ethers with Nitriles

Alkyl and aryl cyanides react smoothly with phenols and their ethers producing ketimines in very good yields in the presence of an acid catalyst ^[64]. The reaction is performed by mixing the nitrile and phenol in ether and saturating the solution with gaseous HCl, whereas, for less reactive phenols, ZnCl₂ must be used.

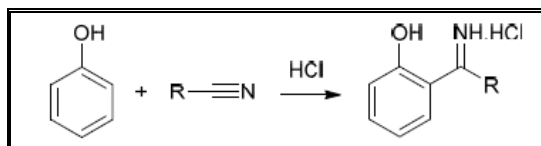


Fig 4.14: Synthesis of ketimines from phenols and nitriles.

2.1.4. Reaction of Metal Amides

Britton et.al., ^[65] has been reported, Ketimine produced by the addition reactions of alkali metal (or calcium amine salts) to aromatic ketones.

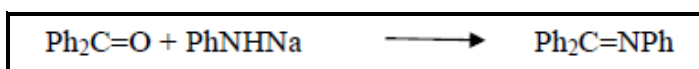


Fig 4.15: Synthesis of Ketimine from alkali metal and aromatic amines

Mosher and Blanz,^[66] has been reported, the oxidation of metalloamines bearing α -hydrogen by 2-bromoanisole to yield imines.

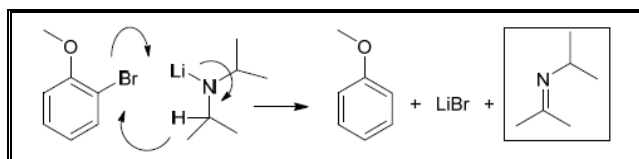


Fig 4.16: Oxidation of metal amines to imines by 2-bromoanisole.

2.1.5. Other Methodologies

Ketimine can be prepared in high yield using aryl ketone diethyl ketals and arylamines, while alkylamines give only low yields^[67].

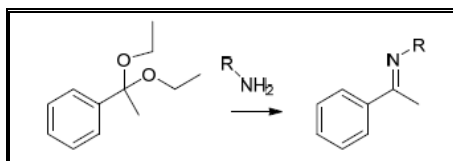


Fig 4.17: Synthesis of ketimines from ketals.

Olefins and tertiary alcohols can be converted into ketimines^[68] by reaction of hydrazoic acid in sulfuric acid.

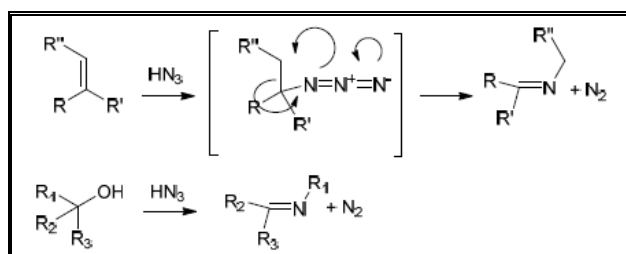


Fig 4.18: Reaction of olefins and tertiary alcohols with hydrazoic acid.

Imines can also be formed by reaction of amino acids with sodium hypochlorite. The first step of this reaction is the formation of a chloramine intermediate that gives rise to the imine via elimination of carbon dioxide and sodium chloride^[69].

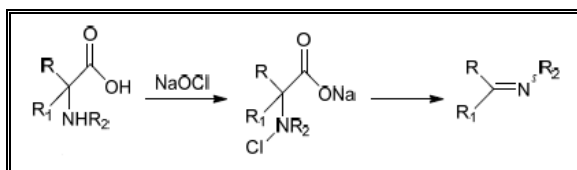


Fig 4.19: Conversion of α -amino acids into imines.

• Schematic Representation III-A

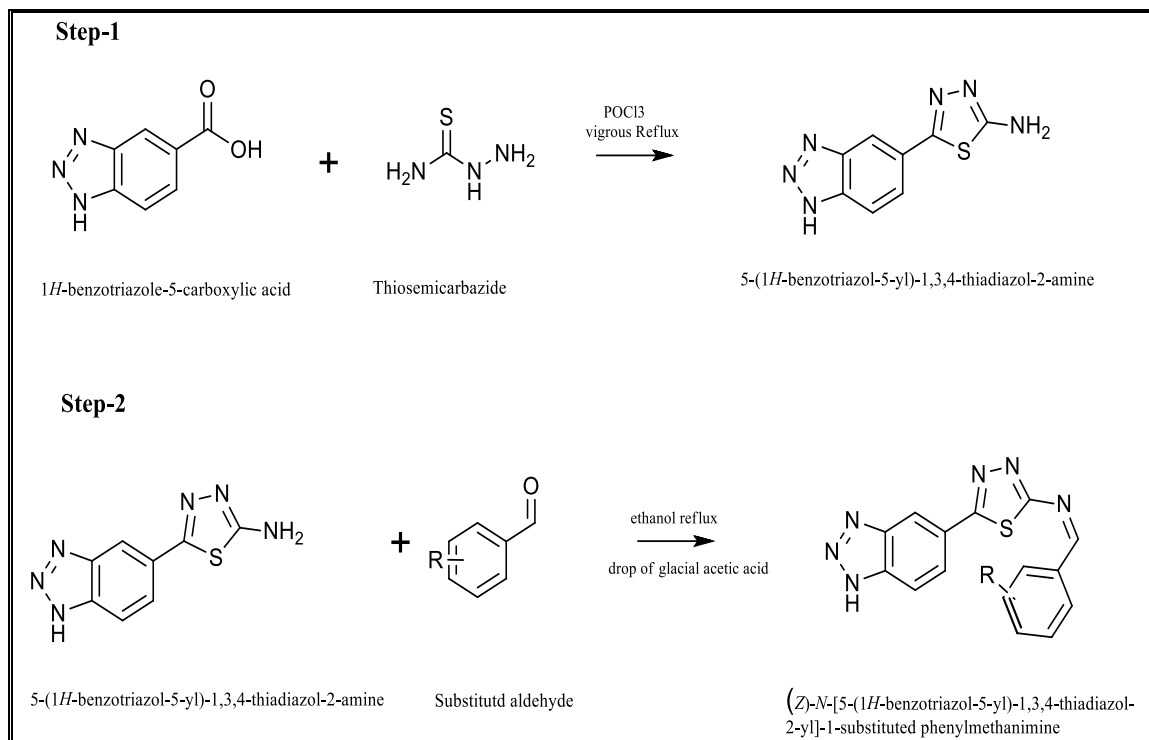


Fig 4.20: Schematic Representation III-A

CHEMISTRY OF BENZOTHAIAZOLE

Benzothiazole is a privileged bicyclic ring system. It contains a benzene ring fused to a thiazole ring. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like antimicrobial, antitubercular, antitumor, antimalarial, anticonvulsant, anthelmintic, analgesic and anti-inflammatory activity. In addition, the benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities. Due to their importance in pharmaceutical utilities, the synthesis of various benzothiazole derivatives is of considerable interests.

Characteristics of nucleus:

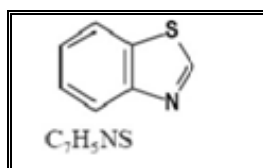


Fig 4.21: Structure of Benzothiazole

Physical and Chemical Properties:

- | | |
|--------------------|---|
| ▪ Chemical Name | :- Benzothiazole |
| ▪ Description | :- Yellow liquid with unpleasant odor |
| ▪ Boiling Point | :- 231°C |
| ▪ Melting Point | :- 2°C |
| ▪ Flash Point | :- >110°C |
| ▪ Density | :- 1.2460 g/cm ³ at 20°C |
| ▪ Solubility | :- Slightly soluble in water; very soluble in Ethanol diethyl ether and carbon soluble in acetone |
| ▪ Refractive Index | :- 1.6379 at 20°C |
| ▪ Vapor Pressure | :- 0.07 mm Hg at 20°C |

Methods of synthesis of Benzothiazole

1. Hofmann method

1-Mercapto-benzothiazol was first obtained by A. W. Hofmann in an attempt to prepare the di sulfyl hydryl derivative of thiocarbanilide by the action of carbon disulfide on o-aminophenol. He obtained the same substance by the action of sodium hydro- sulfide on chlorophenyl mustard oil

(1-chloro-benzothiazole). The product thus obtained, after recrystallization from alcohol, melted at 179°C and was easily oxidized to a disulfide melting at 180°C. Hofmann also noted formation of 2- anilinobenzothiazole from the reaction of 2-aminothiophenol and phenyl isothiocyanate ^[70]

2. Sulfuric acid as a catalyst

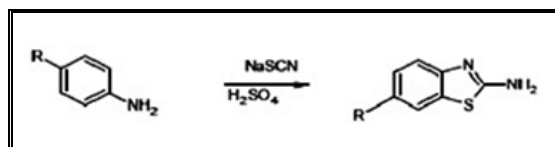


Fig 4.22: Scheme for the synthesis of benzothiazole by using sulfuric acid as a catalyst

In this method p-substituted aniline cyclized by sodium thiocyanate to give 2-amino-6-substituted benzothiazole in the presence of sulfuric acid which act as a catalyst ^[71]

3. Lawesson's reagent

In this method the synthesis of benzothiazole in which o-anisidine was first reacted with p-nitrobenzoyl chloride to form N-2'-methoxyphenyl-4- nitrobenzamide. The amide was then converted to the thiobenzamide using Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane- 2,4 disulphide) which is a useful thiation reagent to replace the carbonyl oxygen atoms of ketones, amides and esters by sulphur. In the presence of potassium ferricyanide, it cyclized to the 2-(4- nitrophenyl)-Benzothiazole ^[72]

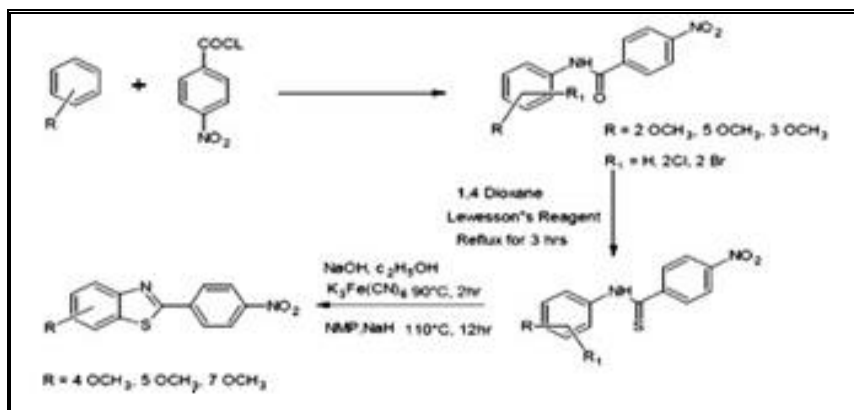


Fig 4.23: Scheme for the synthesis of benzothiazole by using Lawesson's reagent

4. Bromine as a catalyst

Recently several methods reported which utilize bromine as catalyst. Basically cyclization with bromine achieved by oxidation of aniline, substituted aniline and arylthiourea in acid or chloroform with alkali thiocyanate. Hegerschoff in early 1900s synthesized 2-aminobenzothiazole and found that an arylthiourea can be cyclized with liquid bromine in chloroform to form a 2-aminobenzothiazole. (Hegerschoff 1901). Here the 2-amino-6-methylmercaptobenzothiazole was prepared by oxidation of 4-Methylmercaptophenylthiourea with bromine as a catalyst. [73]

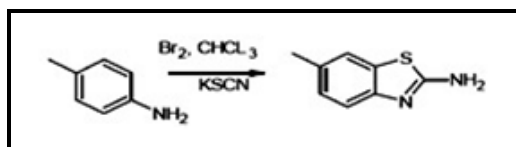


Fig 4.24: Scheme for the synthesis of benzothiazole by using bromine as a catalyst

5. Benzyl trimethylammonium tribromide as catalyst:-

In this method Benzyl trimethylammonium (PhCH₂NMe₃Br₃) is an electrophilic bromine source for the conversion of substituted arylthioureas to 2-aminobenzothiazoles under mild conditions in a variety of solvents with good yields. One of the key benefits for this reagent when compared with molecular bromine in ease of addition and handling, which minimizes the risk of forming brominated side products. They have extended the use of this reagent to a direct, one-pot synthesis of 2-aminobenzothiazoles from either aryl isothiocyanate and anilines or tetrabutylammonium thiocyanate and anilines. [74]

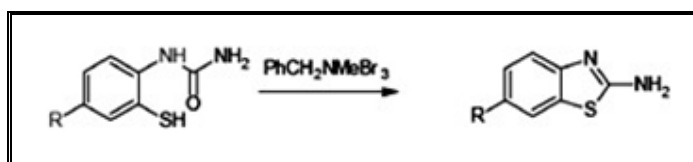


Fig 4.25: Scheme for the synthesis of benzothiazole by using Benzyltrimethylammonium tribromide as catalyst

6. Appel's salt

In this method benzothiazole nucleus was synthesized through cyclization which involve reaction of 2-Bromo-4-nitroaniline with 4,5-dichloro-1,2,3-dithiazol-1-ium chloride, also referred to as Appel's salt, to form (Z)-2-bromo-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-4-nitroanilin. This intermediate could be cyclized to the desired benzothiazole (Chi *et al.*, 2009) [75]

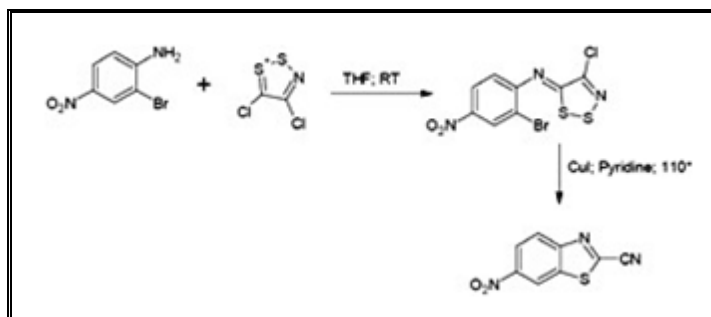


Fig 4.26: Scheme for the synthesis of benzothiazole by using Appel's salt

Method – A

One-pot reaction of appropriate anilines with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to formation of the desired product in good to moderate yields.

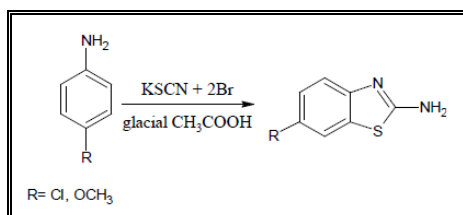


Fig 4.27: Synthesis of benzothiazole using bromine and alkaline thiocyanate in acetic acid medium

Method –B

In this method cyclization of phenylthiourea with bromine in CHCl_3 leads to formation of 2-amino benzothiazole.

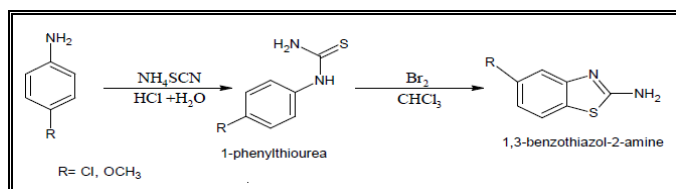


Fig 4.28: Cyclization of phenylthiourea with Bromine

CHEMISTRY OF AMIDE

Amides are derived from carboxylic acids. A carboxylic acid contains the -COOH group, and in an amide the -OH part of that group is replaced by an -NH_2 group. So amides contain the -CONH_2 group. (R-CO-NR'R'')

The amide linkage is one of the most fundamental and widespread chemical bonds in nature ^[76]. Amides are common moieties in naturally occurring substances like proteins and peptides and are also found in various synthetic materials. This makes the amide functionality an important building block in synthetic chemistry and many methods for its synthesis were reported in the literature ^[77-79]

Amide compounds have attracted much attention because of their extensive applications in the pharmaceutical industry; they have been generally used as a protective group in synthetic routes, an amide functional group is contained in many bioactive compounds or drugs, and amide modification is an important optimization methodology for new chemical entities in medicinal chemistry^[80-82] Furthermore, peptide bond, a special kind of amide bond in life science, constitutes the backbone of proteins.

The stable and polar amide functionality is an important unit among the organic molecules present in natural occurring materials. It is also found in many synthetic substances, an intermediate or as active pharmaceutical product or prodrugs ^[83] Due to its interest in organic synthesis the preparation of amides from the corresponding amines is an important and well known transformation. It has been noted recently that around 66% of all preliminary screening reactions in industrial medicinal chemistry laboratories involve amide formation.

Since, amide bond linkage is ubiquitous and important core structure in pharmaceuticals, chemicals, as well as many natural products ^[84] and hence it was thought to synthesize new amide linkage by condensation of formylated pyrazolyl benzothiazole and different substituted benzhydrazide.

• Schematic Representation III-B

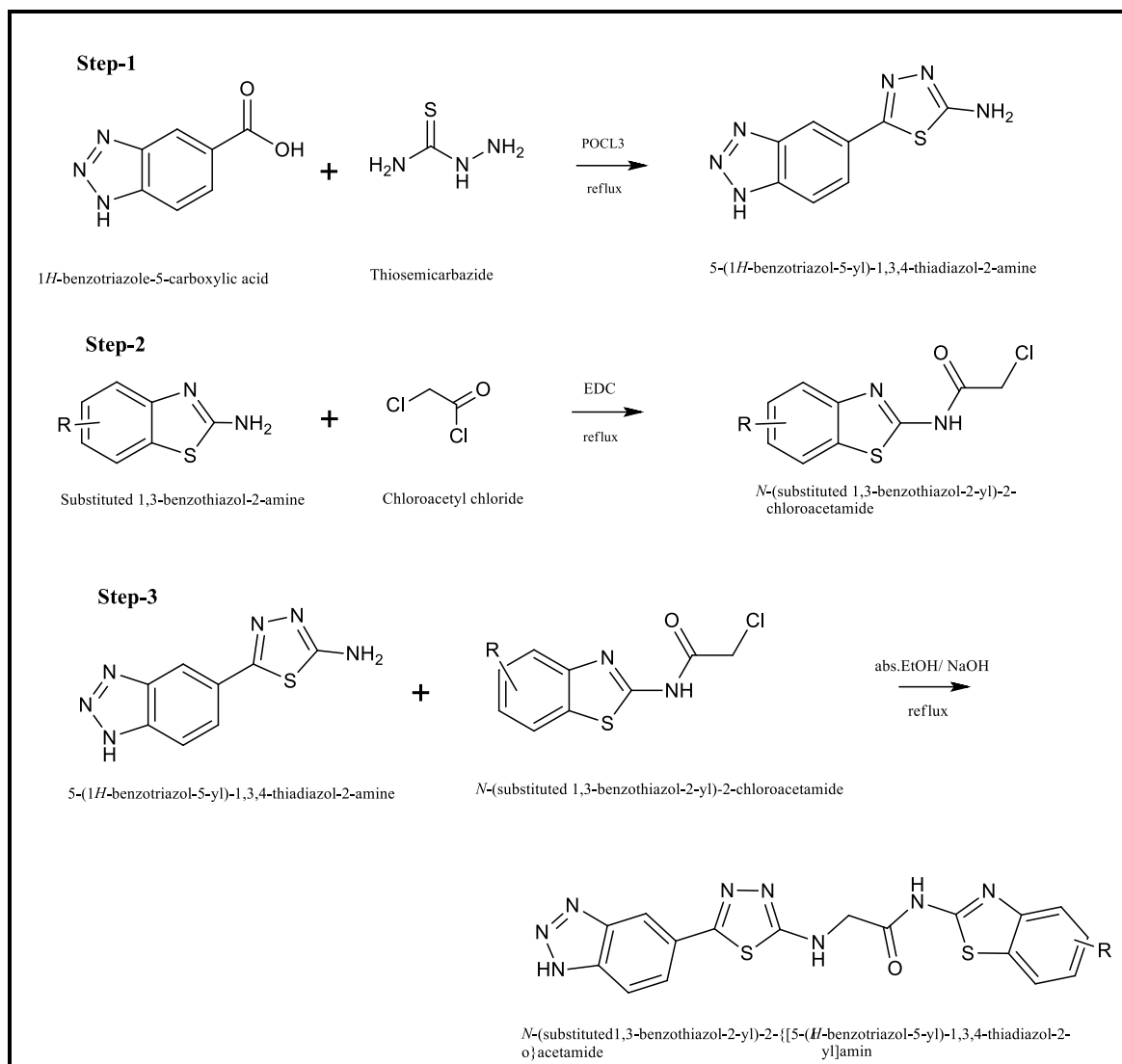


Fig 4.29: Schematic Representation III-B

CHEMISTRY OF 5-AMINO-1, 3, 4-THIADIAZOLE-2-THIOL

Syntheses of small nitrogen containing heterocycles are of a special interest nowadays because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities. Thiadiazoles has attracted wide-spread attention of medicinal chemists due to their chemical potential and high pharmacological efficacy and low side effects.

1, 3, 4-thiadiazoles are the most studied class among the other thiadiazole isomers and their 2, 5-disubstituted derivatives have been widely discussed in terms of the interesting biological activities ^[85]. In particular, such class as 5-amino-1, 3, 4-thiadiazole-2-thiol derivatives are known as antimicrobial ^[86], anticonvulsant ^[87], and anti-diabetic ^[88] agents.

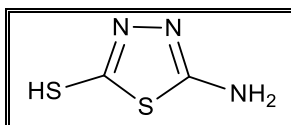


Fig 4.30: 5-amino-1, 3, 4-thiadiazole-2-thiol

The therapeutic importance of 1, 3, 4-thiadiazole ring prompted us to design new molecules in which substituents could be arranged in a pharmacophore pattern to display higher pharmacological activities. The purpose of the work was to obtain new series of derivatives via combination of 1, 3, 4-thiadiazole, well-known heterocyclic pharmacophore, with hydrophobic (hetero) aromatic rings and hydrogen bonding groups such as amide groups.

Method of Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol

Petrow *et al.*, ^[89] have shown that thiosemicarbazide cyclizes directly to 5-amino-1, 3, 4-thiadiazole-2-thiol with sodium bicarbonate and carbon disulfide.

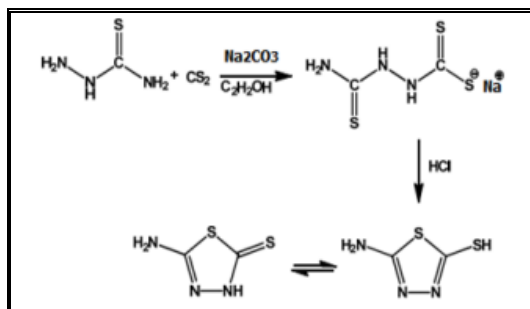


Fig 4.31: Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol

CHEMISTRY OF BENZYL CHLORIDE

Benzyl chloride, or α -chlorotoluene, is an organic compound with the formula $C_6H_5CH_2Cl$. This colorless liquid is a reactive organochlorine compound that is a widely used chemical building block.

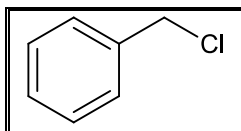


Fig 4.32: Benzyl chloride

Physical and Chemical properties

- Chemical name : Benzyl chloride, or α -Chlorotoluene
- Chemical formula : C_7H_7Cl
- Molar mass : 126.58 g/mol
- Appearance : colorless to slightly yellow liquid
- Odour : pungent, aromatic
- Density : 1.100 g/cm³
- Melting point : $-39\text{ }^{\circ}\text{C}$ ($-38\text{ }^{\circ}\text{F}$; 234 K)
- Boiling point : $179\text{ }^{\circ}\text{C}$ ($354\text{ }^{\circ}\text{F}$; 452 K)
- Solubility : soluble in ethanol, ethyl ether, chloroform, CCl_4
miscible in organic solvents
- Vapor pressure : 1 mmHg (20 $^{\circ}\text{C}$)

Preparation of Benzyl chloride

Benzyl chloride is prepared industrially by the gas-phase photochemical reaction of toluene with chlorine ^[90]. The reaction proceeds by the free radical process, involving the intermediacy of free chlorine atoms.

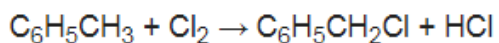


Fig 4.33: synthesis of benzyl chloride

Other method of preparation of benzyl chloride, such as the Blanc chloromethylation of benzene. The Blanc reaction is the chemical reaction of aromatic rings with formaldehyde and hydrogen chloride catalyzed by zinc chloride or other Lewis acid to form chloromethyl arenes. The reaction was discovered by **Gustave Louis Blanc** (1872-1927) in 1923 ^[91]. The reaction is performed with care as, like most chloromethylation reactions, it produces highly carcinogenic bis (chloromethyl) ether as a by-product.

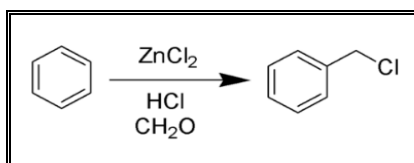


Fig 4.34: Blanc reaction

Mechanism

The reaction is carried out under acidic conditions and with a ZnCl_2 catalyst. These conditions protonate the formaldehyde carbonyl making the carbon much more electrophilic. The aldehyde is then attacked by the aromatic pi-electrons, followed by rearomatization of the aromatic ring. The benzyl alcohol thus formed is quickly converted to the chloride under the reaction conditions.

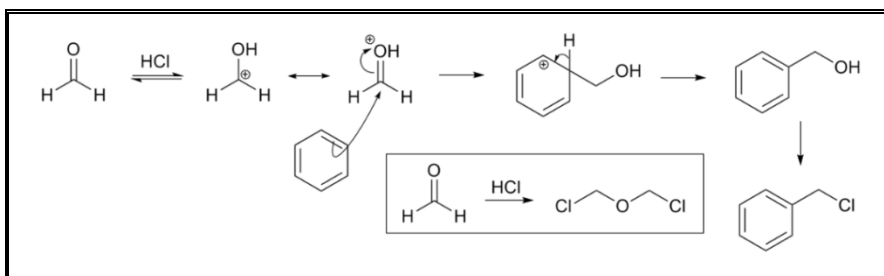


Fig 4.35: Mechanism of Blanc chloromethylation of benzene

• Schematic Representation III-C

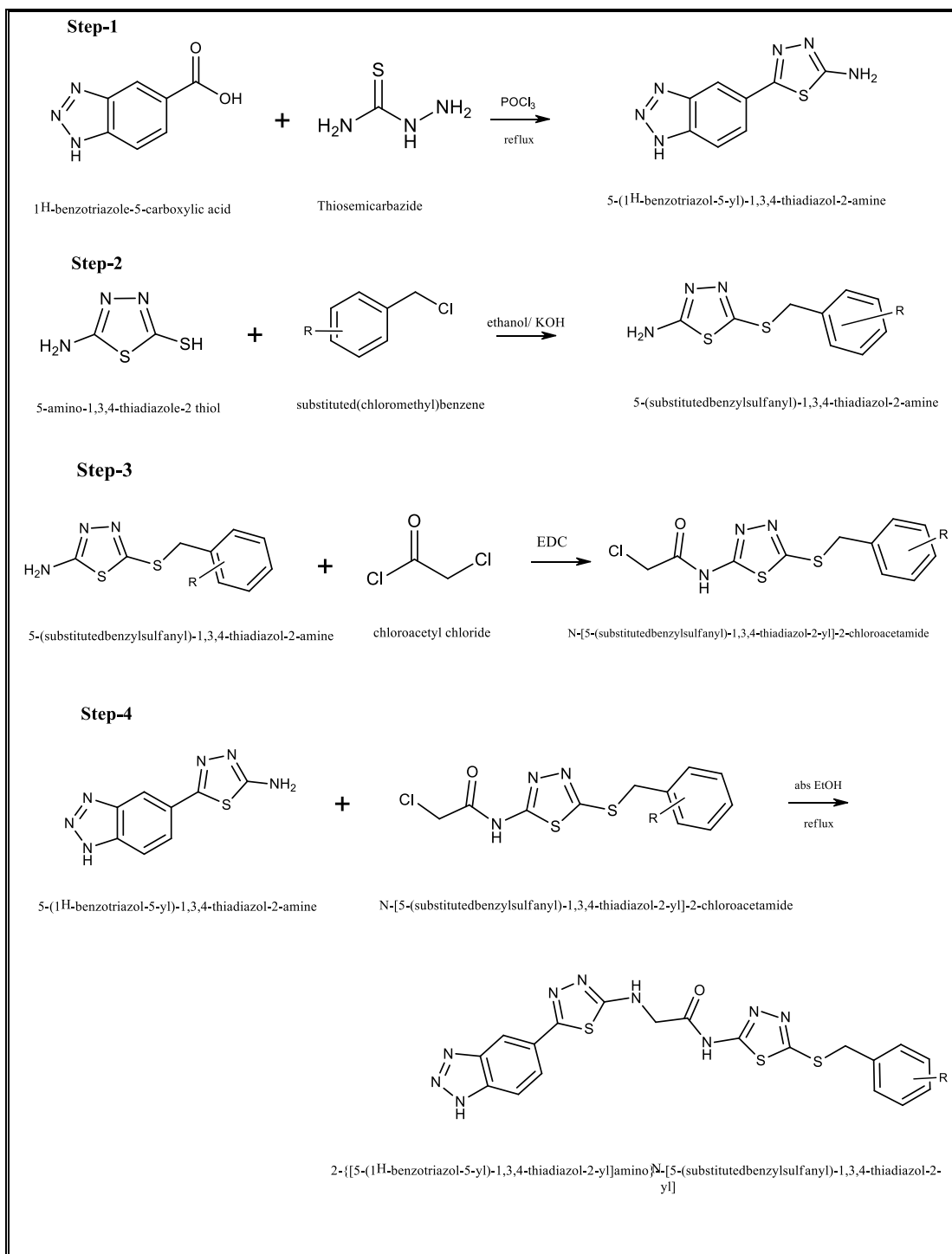


Fig 4.36: Schematic Representation III-C

CHEMISTRY OF 1, 3-THIAZOLE

Thiazole, or 1, 3-thiazole, is a heterocyclic compound that contains both sulfur and nitrogen; the term 'thiazole' also refers to a large family of derivatives. Thiazole itself is a pale yellow liquid with a pyridine-like odor and the molecular formula C_3H_3NS . It possesses both an electron-donating group (-S-) and an electron-accepting group (C=N). It is a stable heterocyclic compound. Thiazole is aromatic on the basis of delocalization of a lone pair of electrons from the sulphur atom completing the desirable 6 p electrons to fulfill Huckel's rule.

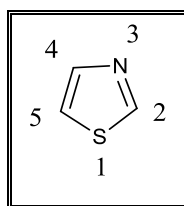


Fig 4.37: 1, 3-thiazole

Properties

- Chemical formula : C_3H_3NS
- Molar mass : $85.12 \text{ g}\cdot\text{mol}^{-1}$
- Boiling point : $116 \text{ to } 118^\circ\text{C}$
- Acidity (pK_a) : 2.5 (of conjugate acid)
- Magnetic susceptibility : $-50.55 \cdot 10^{-6} \text{ cm}^3/\text{mol}$

Molecular and electronic structure

Thiazoles are members of the azoles, heterocycles that include imidazoles and oxazoles. Thiazole can also be considered a functional group. Oxazoles are related compounds, with sulfur replaced by oxygen. Thiazoles are structurally similar to imidazoles, with the thiazole sulfur replaced by nitrogen.

Thiazole rings are planar and aromatic. Thiazoles are characterized by larger pi-electron delocalization than the corresponding oxazoles and have therefore greater aromaticity. This aromaticity is evidenced by the chemical shift of the ring protons in proton NMR spectroscopy (between 7.27 and 8.77 Ppm), clearly indicating a strong diamagnetic ring current. The calculated

pi-electron density marks C5 as the primary site for electrophilic substitution, and C2 as the site for nucleophilic substitution.

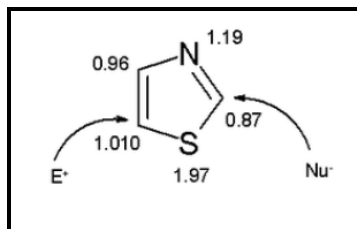


Fig 4.38: electrophilic and nucleophilic substitution site in thiazole ring

The thiazole ring is present in many natural and synthetic products with a broad range of biological activities for example, thiazole containing vitamin B1 (thiamine) helps in the normal functioning of the nervous system by its role in the synthesis of acetylcholine^[92]. Thiazole ring is highly reactive due to the presence of an acidic proton at C-2 and has emerged as an important synthon to generate variety of NCEs (New Chemical Entities). Diverse modifications of the thiazole ring at various positions led to a variety of novel compounds with wide spectrum of pharmacological activities such as antioxidant, antibacterial, antifungal, antitubercular, diuretic, anti-inflammatory and anticancer activities^[92].

Synthesis of thiazoles

The synthesis of thiazole derivatives is of great interest in the field of medicinal chemistry. A number of methods have been employed to synthesize thiazole derivatives such as Hantzsch's synthesis, Gabriel synthesis, and Cook-Heilborn's synthesis^[93].

1. Hantzsch's synthesis

This is the most common method for synthesis of thiazoles which involves cyclization and condensation of haloketones with thioamide. The reaction proceeds with nucleophilic attack of sulphur atom of thioamide on of the α -carbon atom of α -haloketone with formation of intermediate α -thio ketone and following dehydration generates the corresponding thiazoles.

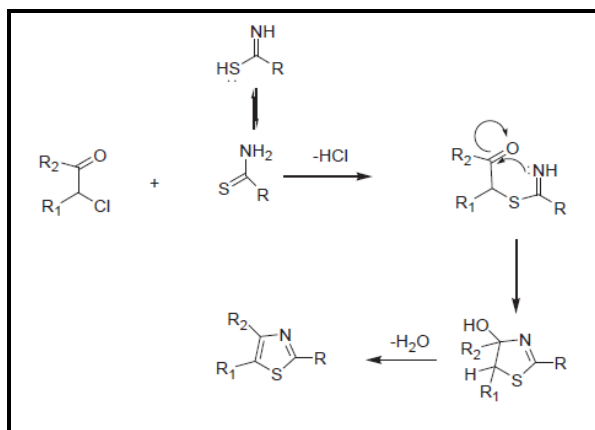


Fig 4.39: Hantzsch's synthetic approach of thiazole

2. Gabriel synthesis

This is another method which is widely used to synthesize alkyl, aryl or alkoxy substituted thiazole at the 2 or 5 or 2, 5 positions involving treatment of α-acylaminoketones with stoichiometric amount of P₂S₅.

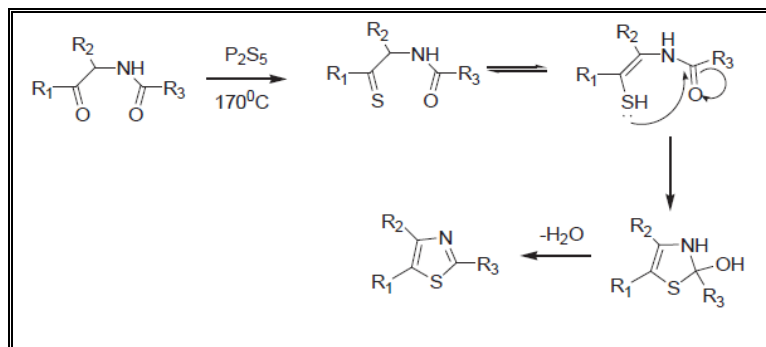


Fig 4.40: Gabriel synthesis of thiazole.

3. Cook-Heilborn's synthesis

This is a versatile method for the synthesis of substituted aminothiazoles. The reaction of α-aminonitriles with dithioacids or esters, carbon disulfide, carbonyl sulfide and isothiocyanate under mild conditions results in the formation of 5-aminothiazoles.

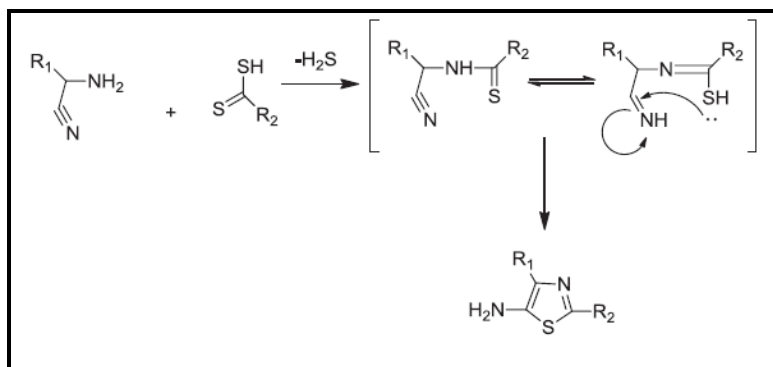


Fig 4.41: Cook-Heilborn's synthetic method of thiazole.

• Schematic Representation III-D

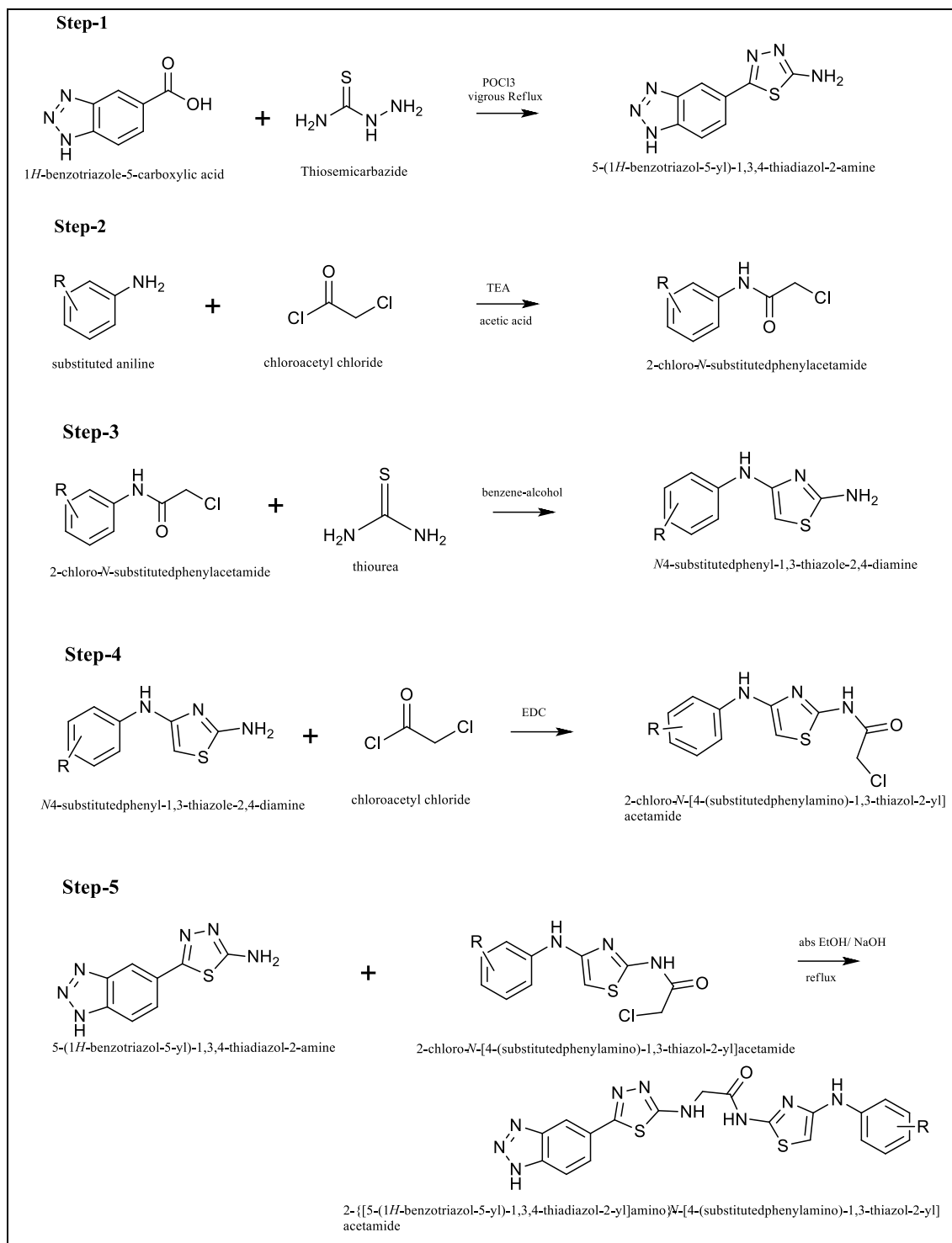


Fig 4.42: Schematic Representation III-D

EXPERIMENTAL & CHARACTERIZATION

Experimental section deals with two parts

- 1. Synthesis**
- 2. Characterization of synthesized derivatives.**

Synthesis: In general, the noun synthesis refers to a combination of two or more entities that together form derivative. In this project synthesis is done through conventional method which includes various types of reaction such as Substitution, Addition, and Condensation etc.

Identification and Characterization of synthesized compounds were carried out by following procedure:

- I. Determination of Melting point
- II. FTIR Spectrophotometer
- III. Nuclear Magnetic Resonance (NMR) spectroscopy
- IV. Gas Chromatography
- V. Bio-chemical synthesis
- VI. Thin Layer Chromatography
- VII. High performance Liquid Chromatography

Melting point Determination

Melting point of the synthesized compounds is carried out by open capillary tube method. Melting point is valuable criteria of purity for as a pure crystals having definite & sharp melting point. The purity should not to be assumed but must be established by observation of any changes in the melting point when the compound is subjected to the purification by recrystallization. The synthesized compound shows minute changes after recrystallization. Melting points were recorded on a Thermo-nik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected.

Infrared (IR) spectroscopy ^[94]

IR spectra were recorded on **IR-Affinity**; Shimadzu using DRS system. IR spectroscopy was carried out for the characterization and the identification of functional groups in the solid samples. Material containing sample must be transparent to the IR radiation. So, the salts like NaCl, KBr were used.

- **Sample preparation:** - A small amount of finely ground solid sample (0.5 or 1 mg) was intimately mixed with about 100 times its weight of powdered potassium bromide (KBr) (100 mg) in mortar-pestle. Then this mixture was filled in the IR solid sample disc and put in the IR cell and mixture was run as such.

Gas Chromatography

Gas Chromatography is a common type of chromatography used for separating and analyzing compounds that can be vaporized without decomposition.

- **Sample preparation:** - 1mg/ml sample concentration used for GC analysis. Methanol used as diluent. The above sample concentration was transferred in the vials and run for the GC analysis.

Chemical Test ^[95]

Chemical tests are generally performed for the confirmation of functional groups in the compounds but these tests are not that much confirmatory compared to IR and GC-MS, so that these tests were performed at initial stage only to confirm the presence of functional groups in the synthesized derivatives.

Thin Layer Chromatography

TLC was performed to confirm the completion of reaction and purity of product formed. TLC was carried out in three steps- spotting, development, and visualization.

- **Identification of development solvent:** - Identification of development solvent was carried out to find an ideal solvent in which all the derivatives are run and progress of reaction checked. Various solvents were tried from which we got

the satisfactory results in ethyl acetate and n-hexane. Ethyl acetate: n-hexane (30:70 %v/v) proportions were used.

- **Procedure:** - The sample to be analyzed was dissolved in a methanol to produce a very dilute (about 1%) solution. spotting of the sample was done using capillary tube and transferred a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet was used. Then the TLC plate was placed into a shallow pool of a development solvent. Ethyl acetate and n-Hexane was used as mobile phase in the ratio of (30:70%v/v). When the solvent has traveled almost to the top of the plate, the plate was removed, and the solvent allowed evaporating. Finally, the visualization of the spots was done under the UV light.

High performance Liquid Chromatography ^[96]

HPLC was carried out to separate the components in a sample, to quantify each component and to determine the purity of the samples.

- **Solubility Studies:** - These studies were carried out to find an ideal solvent in which all the derivatives are completely soluble and stable. Various solvents were tried for checking solubility of derivatives. Derivatives are soluble methanol.
- **Selection of analytical wavelength:** - Accurately weighed and transferred about 10 mg of title compound into a 100 mL volumetric flask, added about 70 mL of mobile phase and shake to dissolve the sample completely. Diluted to volume with mobile phase and mixed well. The standard solution was further diluted with mobile phase to get concentration of 10 μ g/mL. Similarly, the sample solutions were prepared for the other derivatives. The above solutions were scanned between 200 to 400 nm by UV spectroscopy and absorption bands of the sample solutions were recorded. For analytical purposes the wavelength of the photodiode array detector was set at which it presents better reproducibility than the other UV-bands.
- **Sample preparation:-** Accurately weighed and transferred about 10 mg of title compounds into a 100 mL volumetric flask, added about 70 mL of mobile phase

and shake to dissolve the material completely. Diluted to volume with mobile phase and mixed well. The standard solution was further diluted with mobile phase to get concentration of 10µg/ml. Similarly, the sample solutions were prepared for the other derivatives. The above solutions were transferred in the vials and run for the HPLC analysis.

- **Optimization of Chromatographic Conditions:-** Initially Methanol: Acetonitrile combination was tried in different proportions. Then Methanol: Water was tried in different proportions in order to determine the best conditions for the effective separation of title derivatives. The Flow rate was set at 1 and 1.5 ml/min to give best system suitability test parameters.

Recrystallization ^[97]

The most commonly used procedure for the purification of a solid material by recrystallization from a solvent involves the following steps:

- The impure material is dissolved in a suitable solvent, by shaking or vigorous stirring, at or near the boiling point, to form a near-saturated solution.
- The hot solution is filtered to remove any insoluble particles. To prevent crystallization during this filtration, a heated filter funnel can be used, or the solution can be diluted with more of the solvent.
- The solution is then allowed to cool so that the dissolved substance crystallizes out.
- The crystals are separated from the mother liquor, either by centrifuging or by filtering, under suction, through a sintered glass, a Hirsch or a Buchner, funnel. Usually, centrifugation is preferred because of the greater ease and efficiency of separating crystals and mother liquor, and also because of the saving of time and effort, particularly when very small crystals are formed or when there is entrainment of solvent.

Experimental is further divided into sub section based on the number of series

III A] Synthesis of (Z)-N-[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl]-1-substituted phenylmethanimine

III B] Synthesis of N-(substituted1, 3-benzothiazol-2-yl)-2-{[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino} acetamide

III C] Synthesis of 2-{[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino}-N-[5-(substitutedbenzylsulfanyl)-1, 3, 4-thiadiazol-2-yl] acetamide

III D] Synthesis of 2-{[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino}-N-[4-(substitutedphenylamino)-1, 3-thiazol-2-yl] acetamide

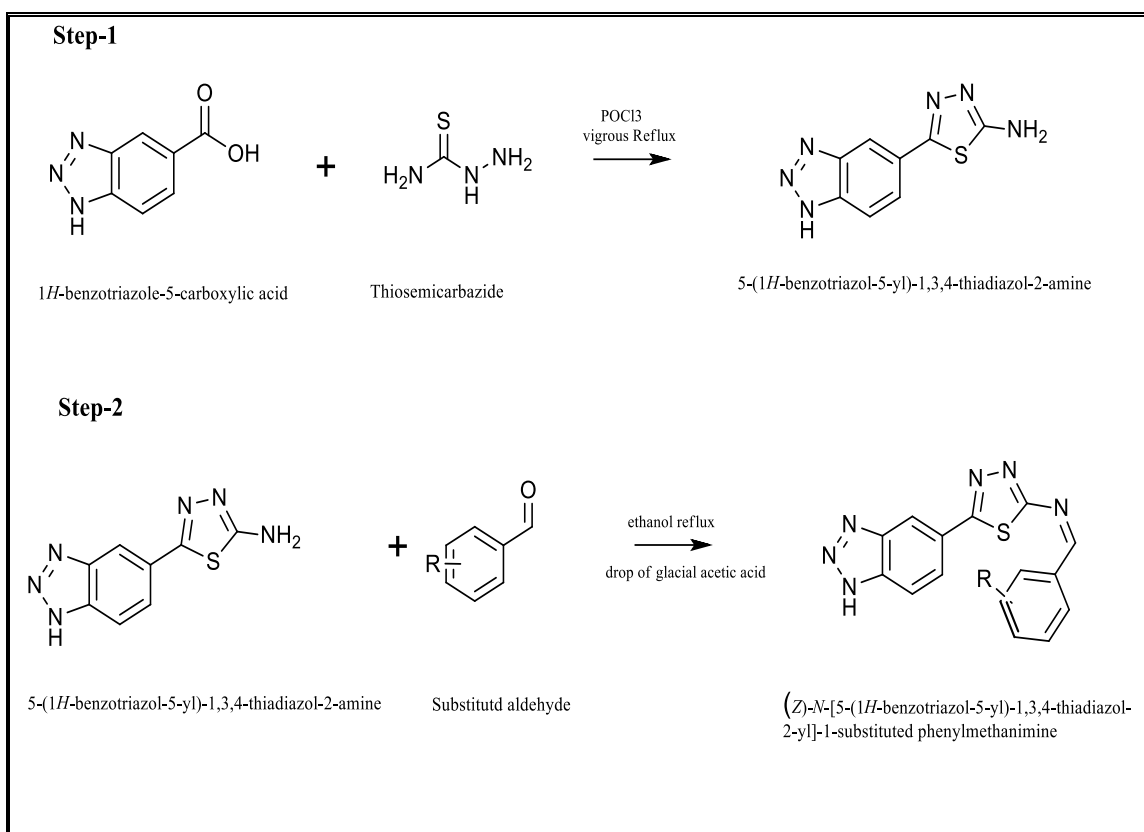
III A] Synthesis of (Z)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-1-substituted phenylmethanimine

This series consists of two step of reaction:

III A.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

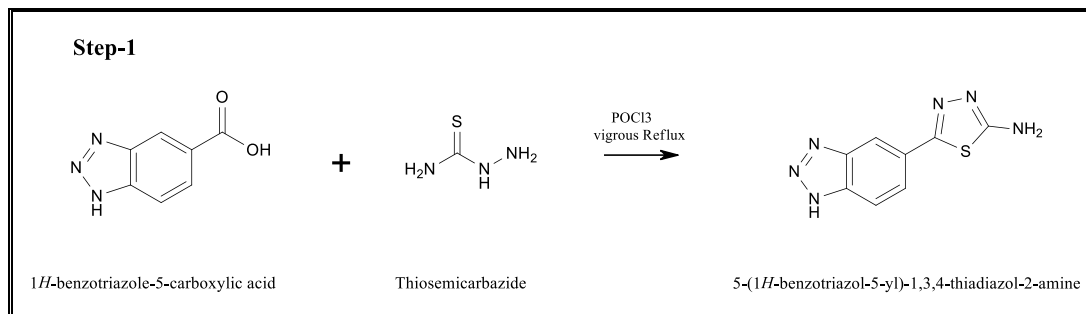
III A.2 Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]1(Substitutedphenyl)Methanimine

- Schematic representation of III-A



III A.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-amine

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol . Wt	Density g/cm ³	Mole ratio
1-H Bnzotriazol-5-carboxylic acid	50mmol	163.13	-	1
Thiosemicarbazide	50mmol	91.13	-	1

- Procedure**

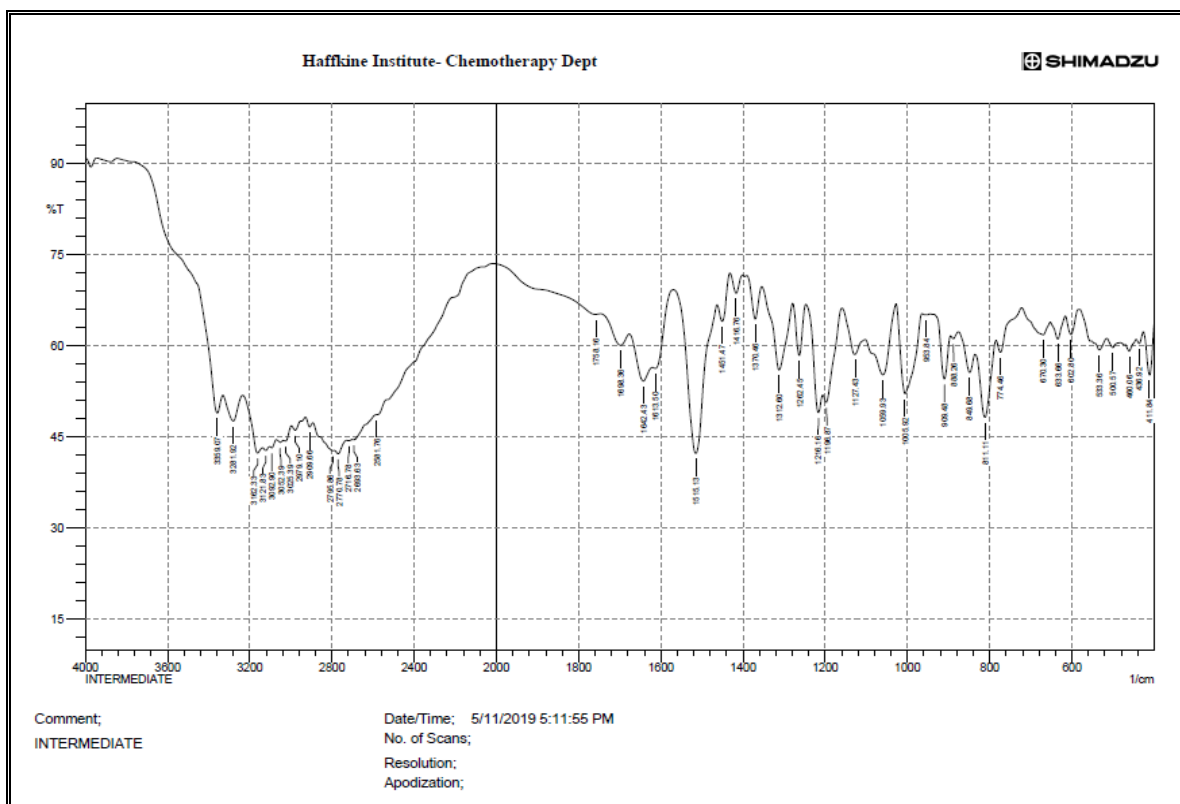
The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1). The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	264°C	Physical Constant

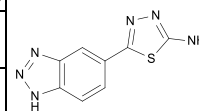
2	Yield	70%	
3	Colour	Light Brown	Physical appearance
4	Nature	Amorphorous	
• Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine

• Spectrum 1 : FT-IR of III A.1



• IR spectral data of III A.1

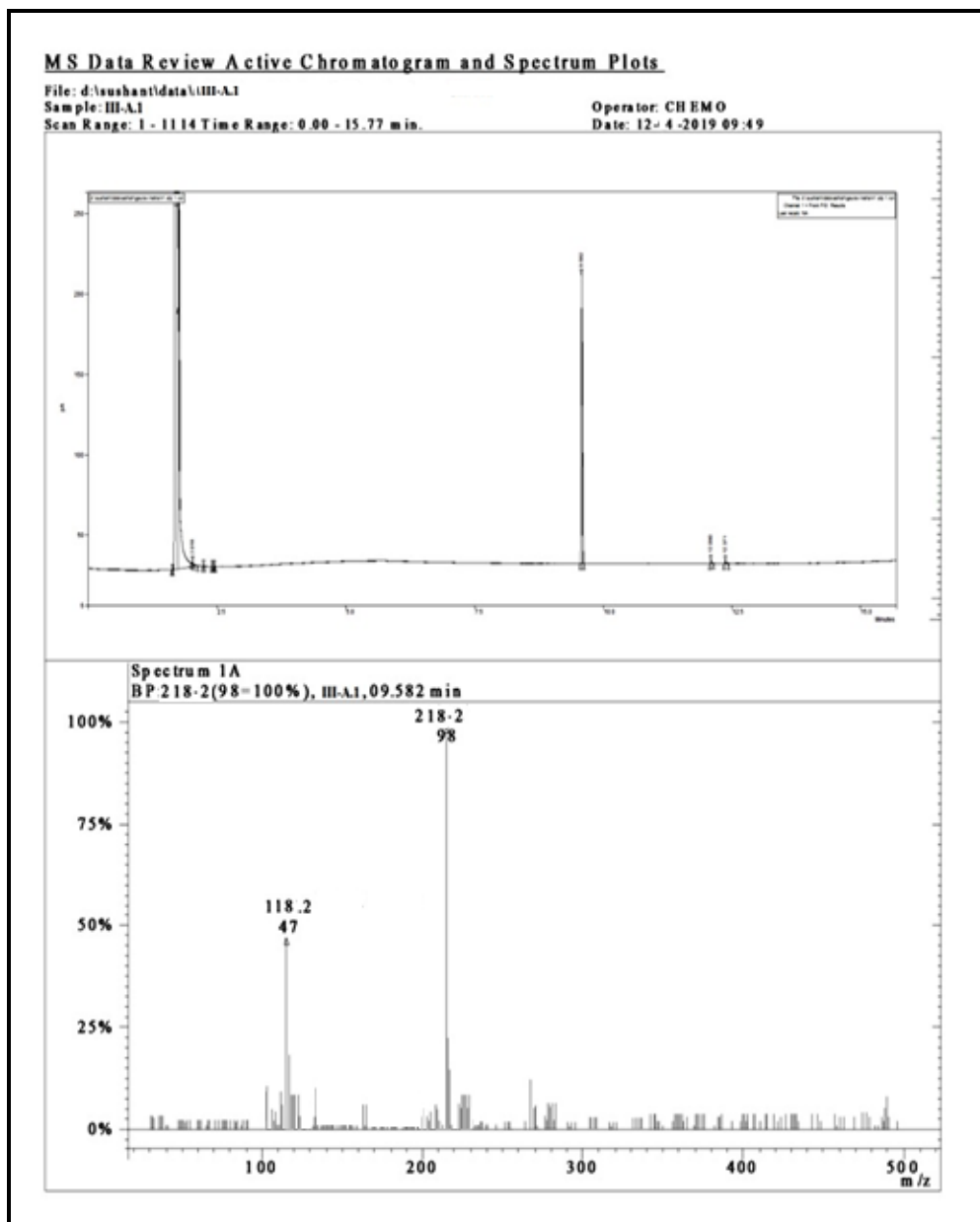
Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	(3359, 3281) cm ⁻¹	3400-3250 cm ⁻¹
N-H bend	1515 cm ⁻¹	1650-1580 cm ⁻¹
C=N	1613 cm ⁻¹	1600 cm ⁻¹



- **GCMS method for III A.1**

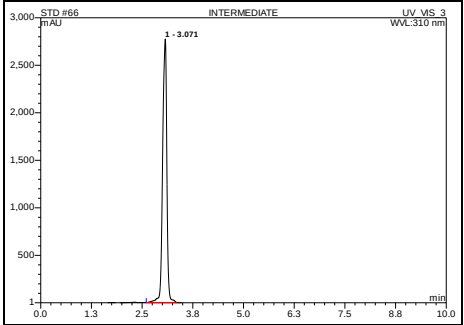
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 2 : Mass spectra of III A.1



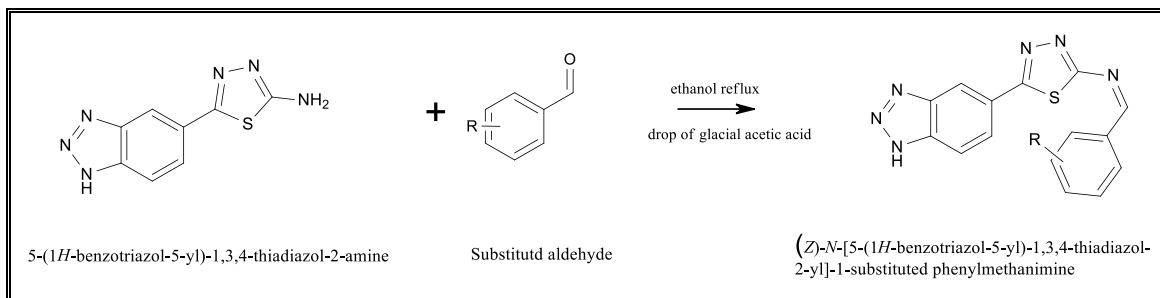
m/z value	Mass signal
218.2	Molecular ion peak
218.2	Base peak

• Chromatogram 1 : HPLC method for III-A.1

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.07	
Theoretical Plates	4267	
Peak Intensity	2774.92	
Asymmetry (EP)	0.87	
Peak Width	0.177	

III A.2 Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]1(Substitutedphenyl)Methanimine

- Reaction



- Reaction Calculation table:

Compound	Mole	Mol. Wt	Mole ratio
III-A.1	0.01 mol	218.238	1
4-benzyloxybenzaldehyde	0.01 mol	212.248	1

- Procedure

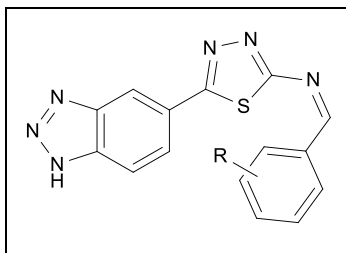
The III-A-1 (0.01 mol) was dissolved in ethanol (50 mL), substituted benzaldehyde (0.01 mol) and drops of glacial acetic acid was added and the reaction mixture was heated under reflux for 16 hr. The excess of solvent was distilled-off under reduced pressure, recrystallized from methanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table

Sr. no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	235°C	Physical Constant
2	Yield	72%	
3	Colour	Light Yellow	Physical appearance
4	Nature	Amorphorous	

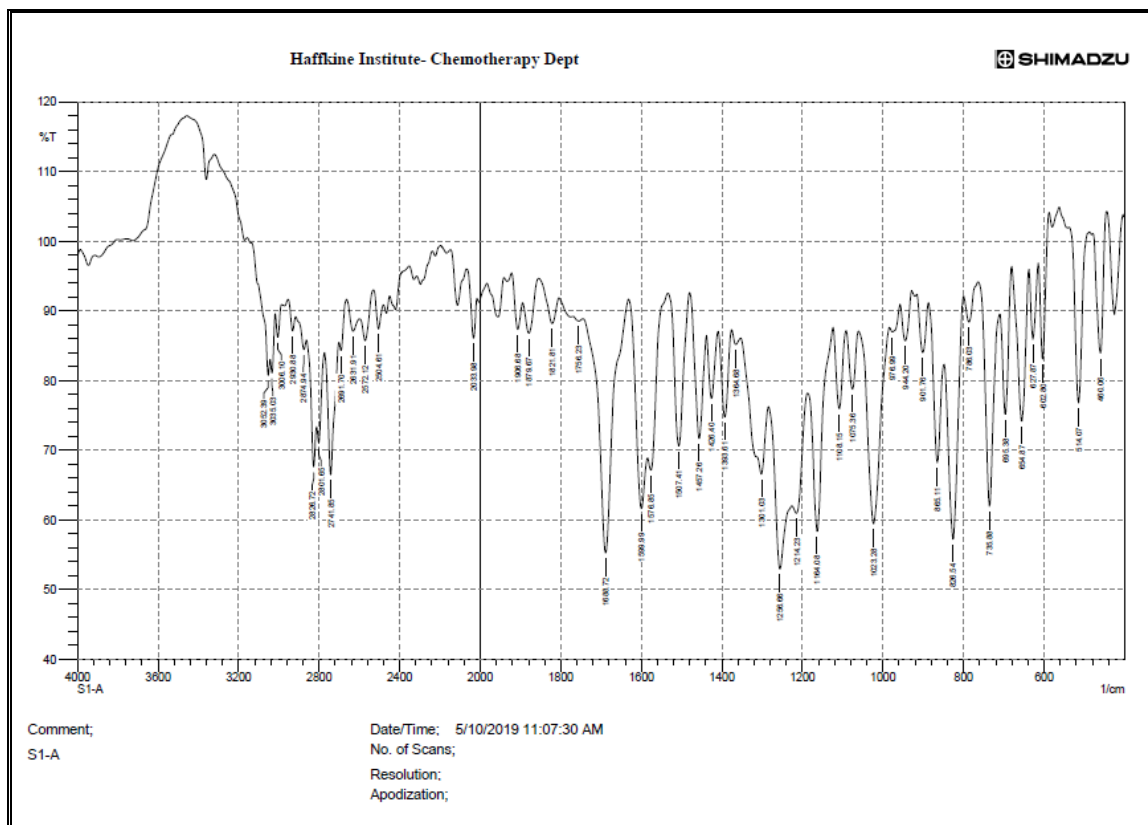
Chemical Test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	No Bubbles of nitrogen gas	Absence of primary aliphatic amine

Physical data of compound III-A.2(a-j)

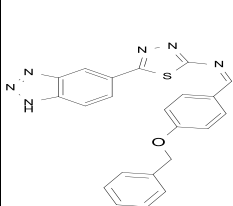


Comp.	R	Mol wt.	Colour	Solubility	MP (°C)	Yield (%)
III-A.2a	4-benzyloxybenzaldehyde	412.467	Light yellow	Methanol	235	63
III-A.2b	4-Dimethylamino benzaldehyde	349.412	Gray	Methanol	224	74
III-A.2c	Furfuraldehyde	296.307	Light brown	Methanol	229	68
III-A.2d	Syringaldehyde	328.396	Yellow	Methanol	225	75
III-A.2e	3,4-dihydroxy-5-NO ₂ benzaldehyde	383.341	Yellow	Methanol	234	65
III-A.2f	3,4,5-trimethoxy benzaldehyde	396.423	Light yellow	Methanol	240	71
III-A.2g	2-Thiophenecarboxaldehyde	312.372	Light brown	Methanol	253	70
III-A.2h	4-Methyl Thiobenzaldehyde	352.436	Brown	Methanol	263	65
III-A.2i	5-Methyl furfural	310.33	Moss green	Methanol	243	63
III-A.2j	4-Trifluoromethyl benzaldehyde	374.343	Light Brown	Methanol	221	68

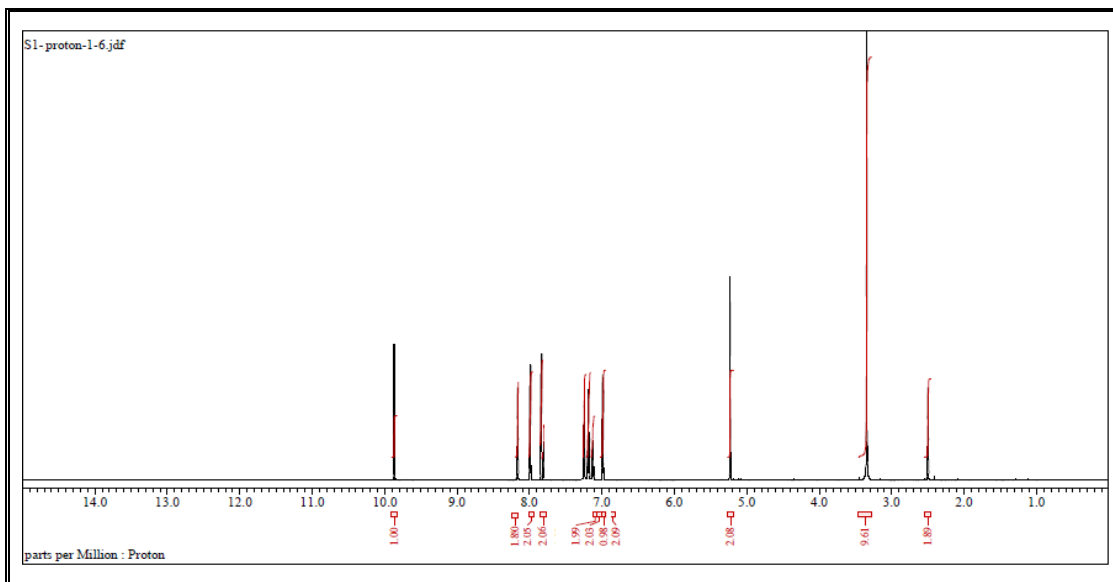
- Spectrum 3 : IR spectra of III-A.2a



- IR spectral data of III-A.2a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
C=N	1688.72cm ⁻¹	1640-1690cm ⁻¹	
C-O	1164.08	1040-1350 cm ⁻¹	

- Spectrum 4: NMR SPECTRA OF III-A.2a**



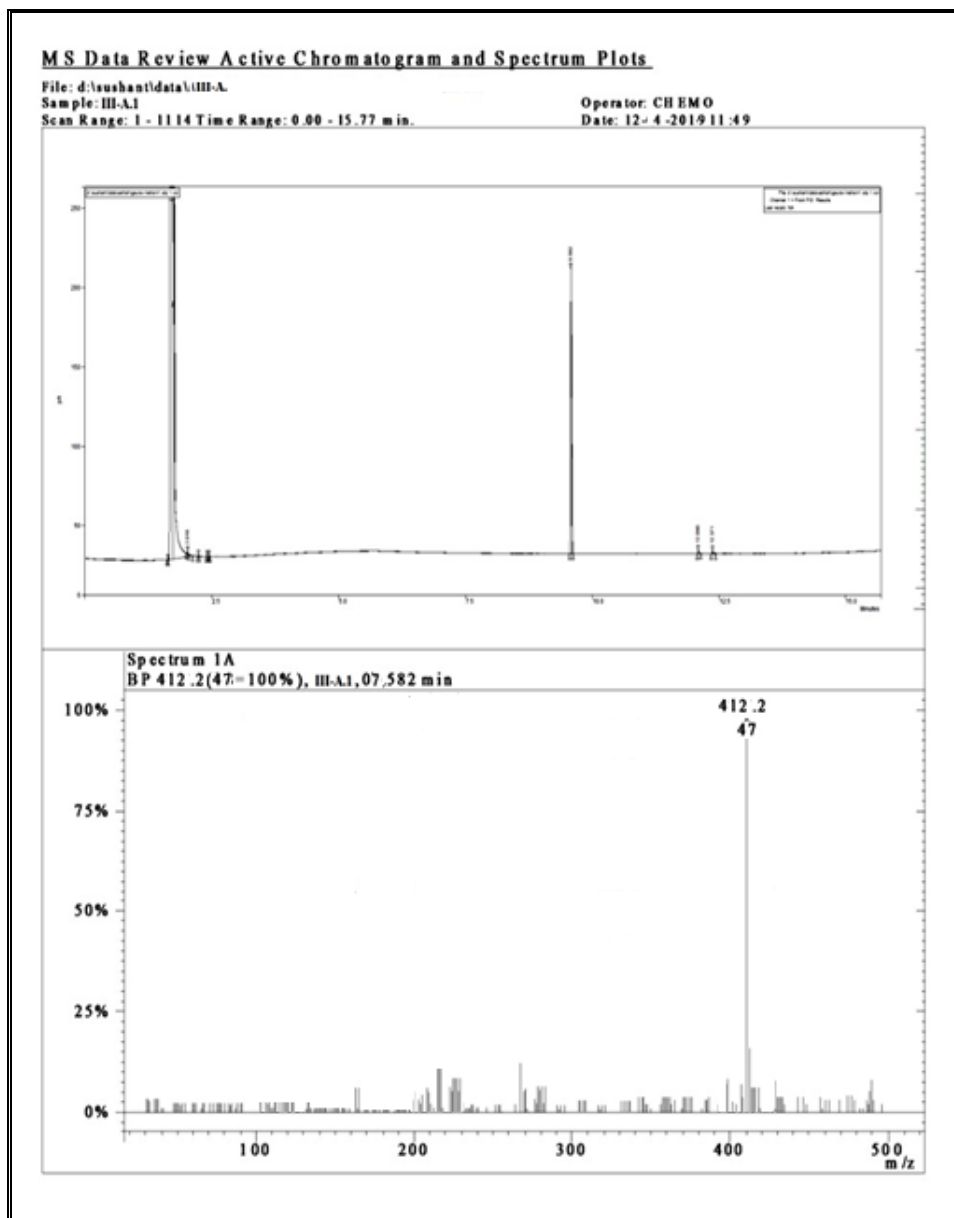
- NMR spectral interpretation of III-A.2a**

Δ values	H ¹ NMR signal
2.5	s, 1H, NH(benzotriazole)
5.2	s, 2H, CH ₂
9.8	s, 1H, CH (aldehyde)
7.0-8.2	m, 11H, Ar-H

• GCMS method for III-A.2a

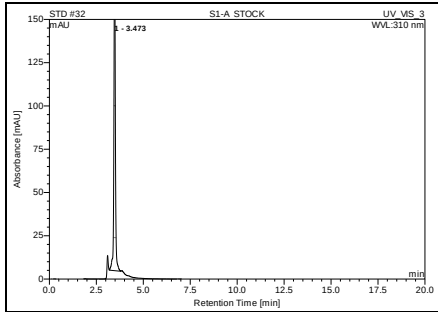
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/min)	Temperature (°C)	Hold time (min)
	0	100	2
	10	120	3
	10	150	2
	10	180	4
	10	260	3
	20	300	3
Injection mode	Split mode; split ratio 90:1		
Injection temperature	300°C		
Injection volume	1.5 µL		
Carrier gas	Helium gas		
Detector temperature	300°C		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		
Analysis time			
Run time	07.582		

• Spectrum 5 : MASS SPECTRA OF III-A.2a



m/z value	Mass signal
412.2	Molecular ion peak
412.2	Base peak

• Chromatogram 2 : HPLC method for III-A.2a

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	2μL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.473	
Theoretical Plates	12042	
Peak Intensity	190.447	
Asymmetry (EP)	1.07	
Peak Width	0.129	

III B] Synthesis of *N*-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino} acetamide

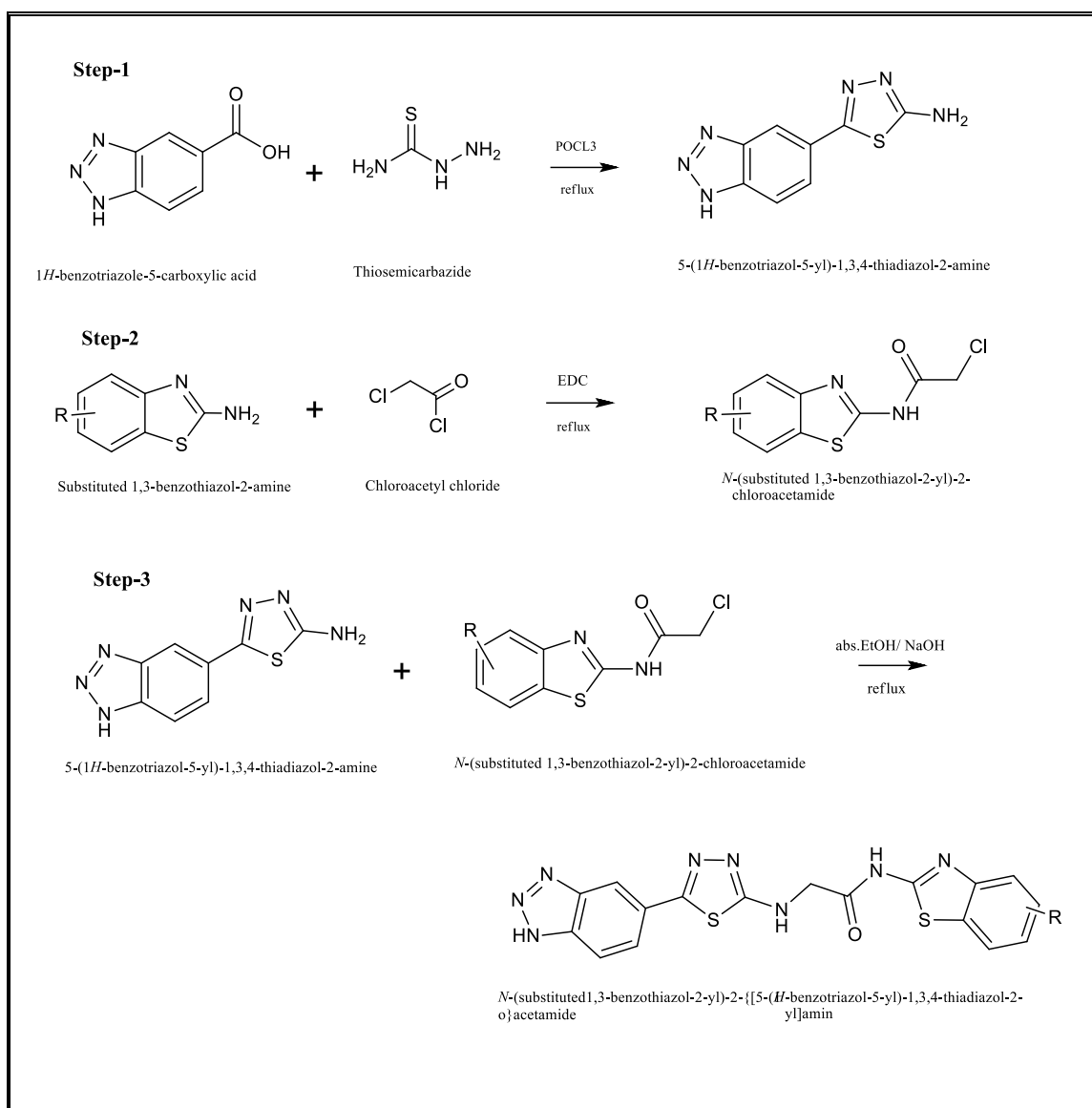
This series consist of three step of reaction

III B.1 Synthesis of 5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

IIIB.2 Synthesis of *N*-(1,3-benzothiazol-2-yl)-2-chloroacetamide

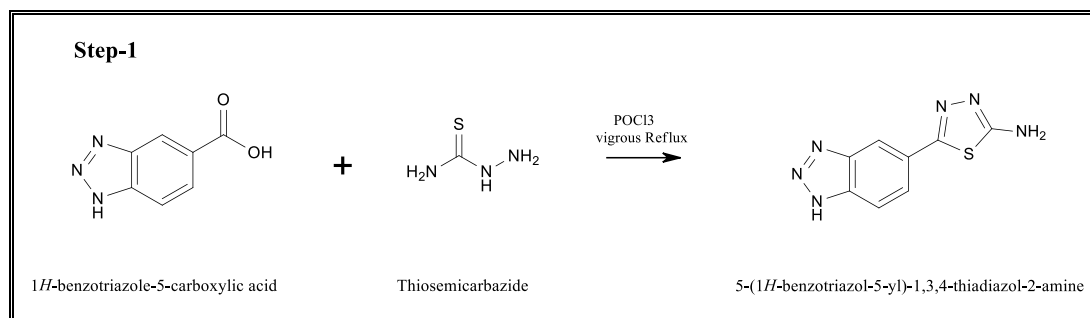
III B.3 Synthesis of *N*-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino}acetamide

• Schematic representation of III-B



III B.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

- Reaction



- Reaction Calculation table:

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
1-H Benzotriazol-5-carboxylic acid	50mmol	163.13	-	1
Thiosemicarbazide	50mmol	91.13	-	1

- Procedure

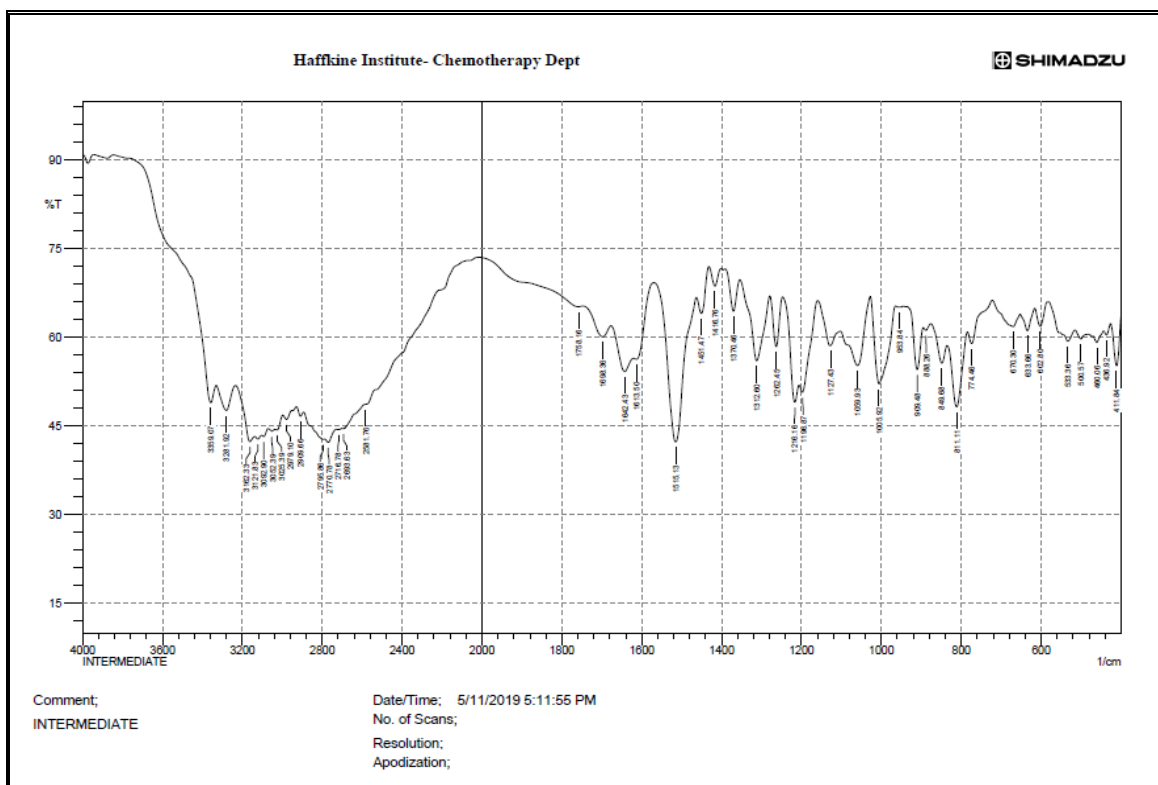
The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1). The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table

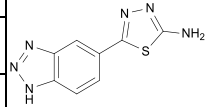
Sr.no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	264°C	Physical Constant

2	Yield	70%	
3	Colour	Light Brown	Physical appearance
4	Nature	Amorphorous	
• Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine

• Spectrum 6 : FT-IR of III B.1



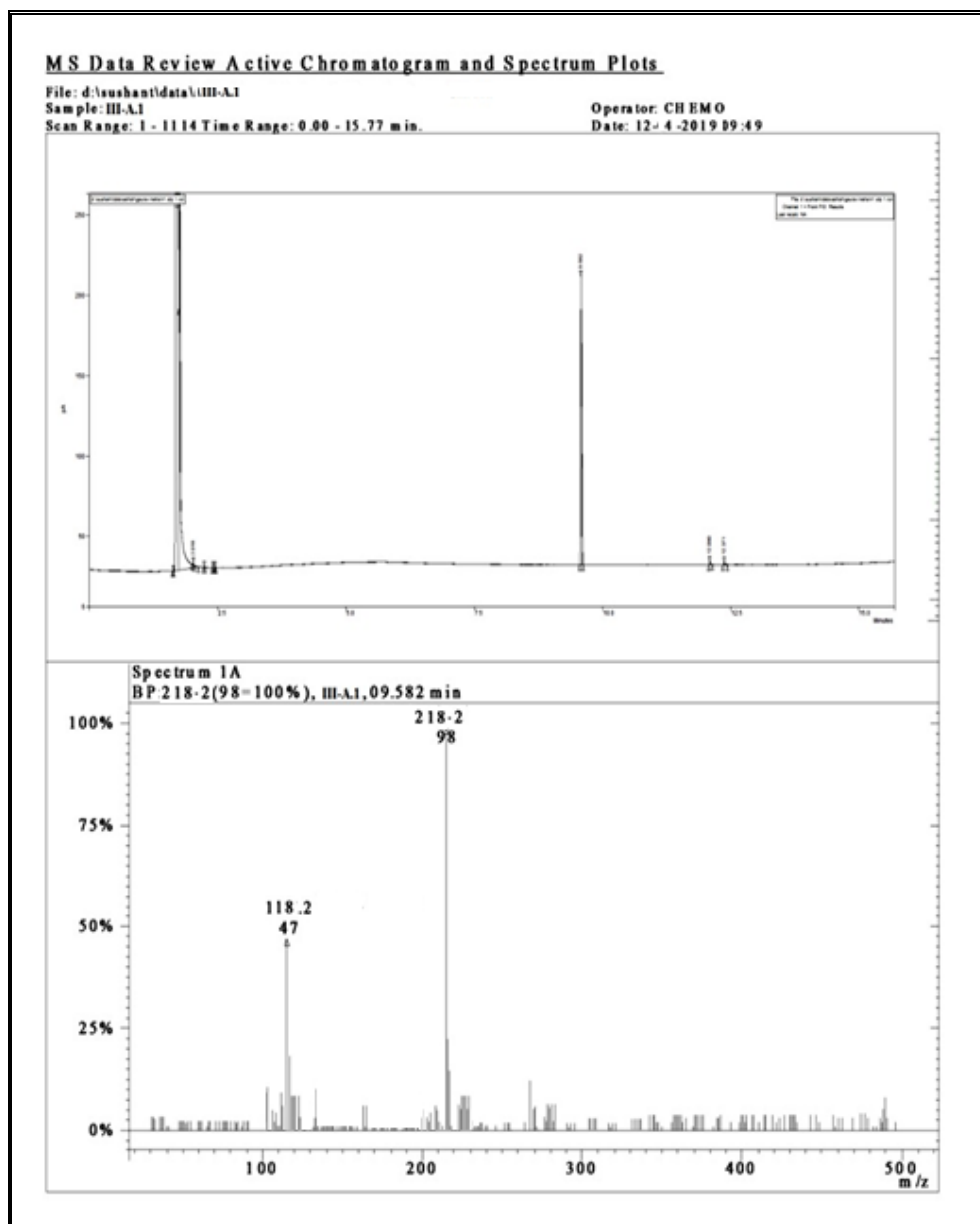
• IR spectral data of III B .1

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
N-H stretch	(3359, 3281) cm ⁻¹	3400-3250 cm ⁻¹	
N-H bend	1515 cm ⁻¹	1650-1580 cm ⁻¹	
C=N	1688.72 cm ⁻¹	1690-1640 cm ⁻¹	

- **GCMS method for III B .1**

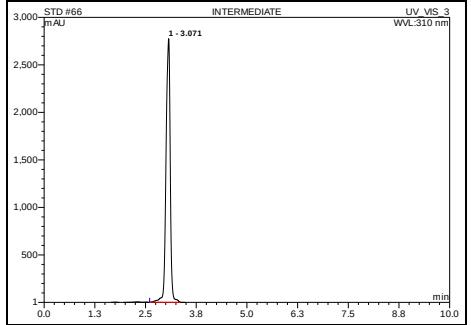
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 7 : Mass spectra of III B .1



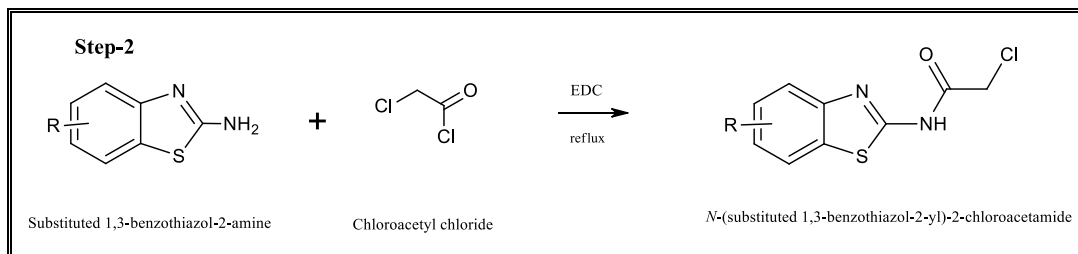
m/z value	Mass signal
218.2	Molecular ion peak
218.2	Base peak

• Chromatogram 3 : HPLC method for III B .1

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.07	
Theoretical Plates	4267	
Peak Intensity	2774.92	
Asymmetry (EP)	0.87	
Peak Width	0.177	

III B.2 Synthesis of *N*-(1,3-benzothiazol-2-yl)-2-chloroacetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
Benzothiazole	0.01mol	150.199	-	1
Chloroacetyl chloride	0.01mol	112.937	1.42g/cm ³	1

- Procedure**

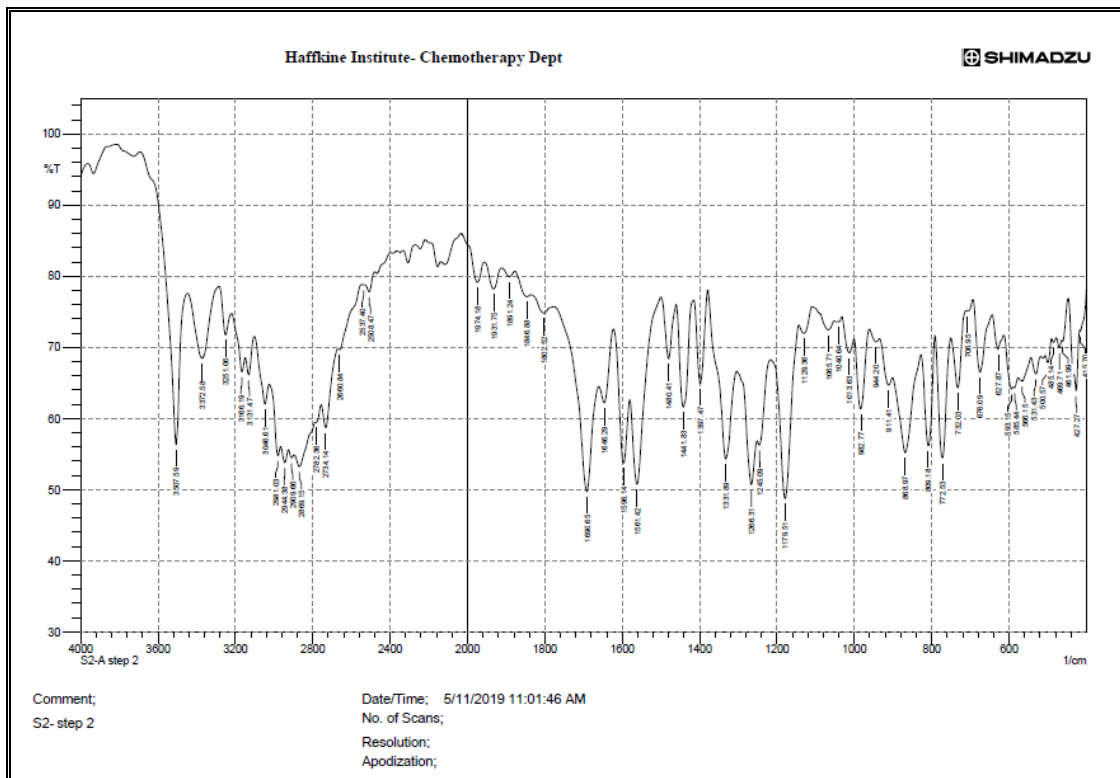
To a solution of chloroacetyl chloride (0.01mol) in (10 ml) ethylene dichloride, substituted amino benzothiazole (0.01mol) was added with constant stirring. The reaction mixture was stirred for 5 hrs. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	195°C	Physical Constant
2	Yield	97	
3	Colour	Light Brown	Physical appearance

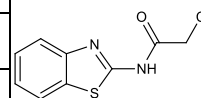
4	Nature	Amorphorous	
• Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	Test for Halogens:- OS+ dil AgNO ₃	Thick White ppt is observed	Halogen may be present
2	1cm ³ of OS+1 drop of dil H ₂ SO ₄ +few drops CHCl ₃ + Cl ₂ water Shake well	Colourless layer is observed	Chlorine present
3	1cm ³ of compound+3cm ³ of 1:1 HCl, shake well. Cool add few drops of NaNO ₂ solution	White emulsion	-NH group present

• Spectrum 8 : FT-IR of III B.2a



• IR spectral data of III B.2a

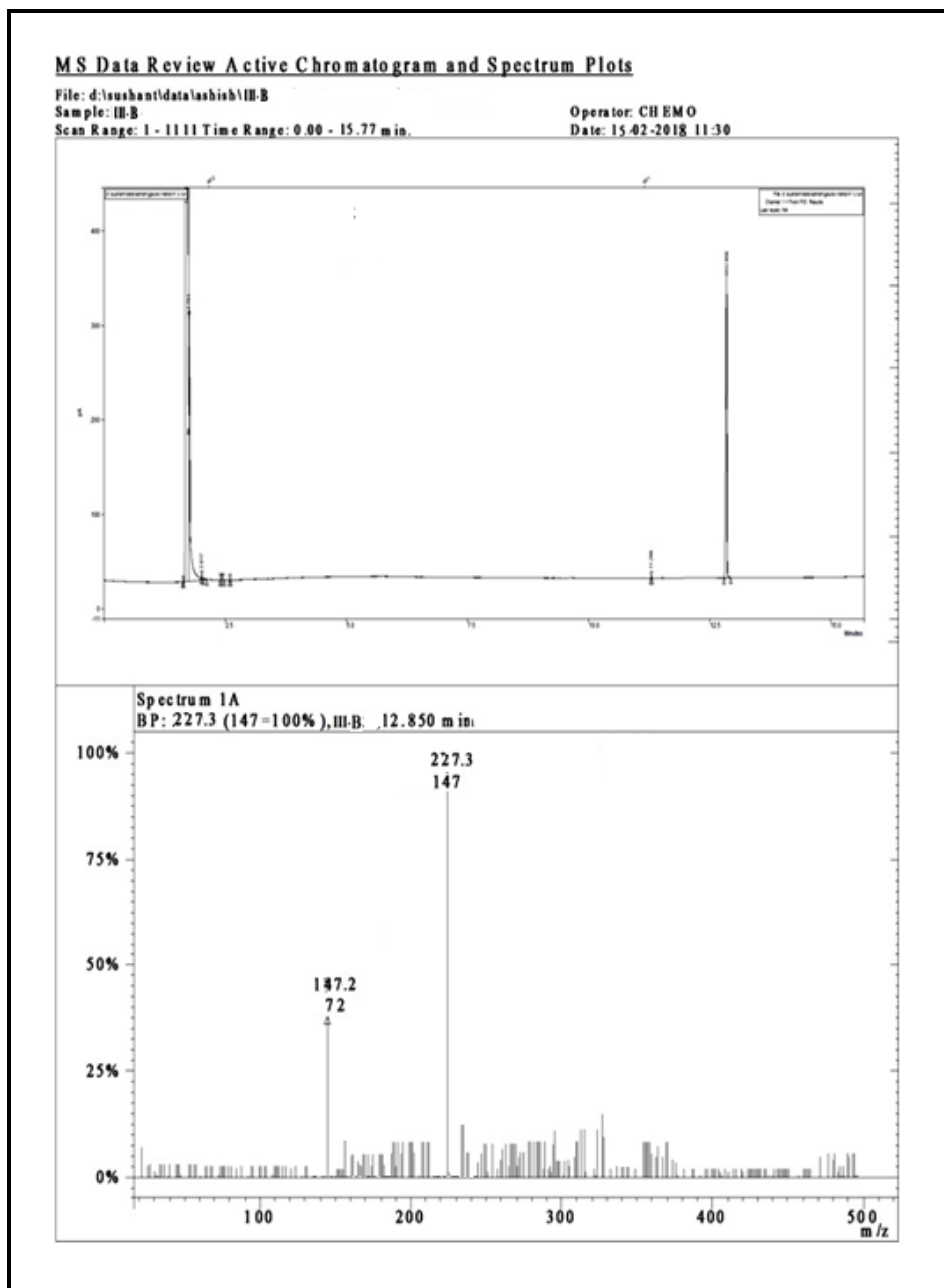
Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	3372.58 cm ⁻¹	3500-3100 cm ⁻¹
N-H bend	1561.52 cm ⁻¹	1550-1640 cm ⁻¹
-CONH	1690.65 cm ⁻¹	1680-1630 cm ⁻¹
C-Cl	676.09 cm ⁻¹	800-600 cm ⁻¹



- **GCMS method for III B.2a**

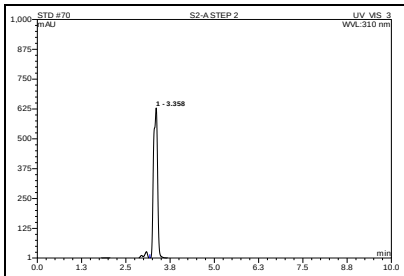
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 9 : Mass spectra of B.2a



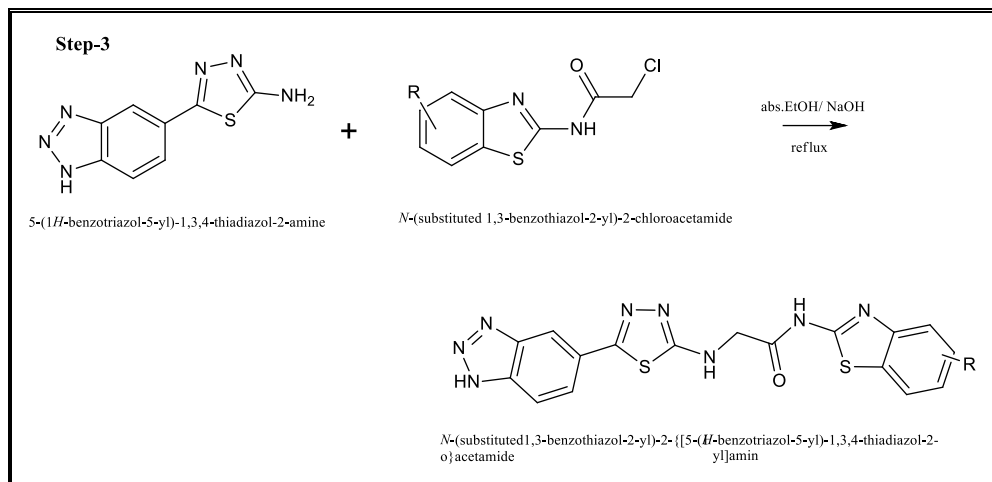
m/z value	Mass signal
147.2	Molecular ion peak
227.3	Base peak

• Chromatogram 4 : HPLC method for III B .2a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.358	
Theoretical Plates	3522	
Peak Intensity	628.775	
Asymmetry (EP)	0.87	
Peak Width	0.303	

III B.3 Synthesis of *N*-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino}acetamide

• Reaction



• Reaction Calculation table:

Compound	Mole	Mol. Wt	Density	Mole ratio
III-B.1	0.03	218.238	-	1
III B.2	0.03	226.628	-	1

• Procedure

Product of step 1 (0.03 mol) in absolute ethanol (30 ml), (0.12 gm) of sodium hydroxide and (0.03mole) substituted *N*-(1,3-benzothiazol-2-yl)-2-chloroacetamide were added, the mixture was refluxed for (4 hr). After cooling precipitate was collected by filtration, dried and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

• Observation Table

Sr. no.	Parameters	Observation	Inference
• Physical Test			

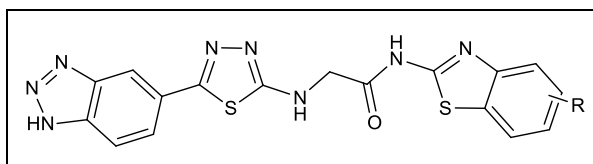
1	Melting Point	245°C	Physical Constant
2	Yield	76%	
3	Colour	Light Brown	Physical appearance
4	Nature	Amorphorous	Crystal absent

• **Chemical Test**

Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS

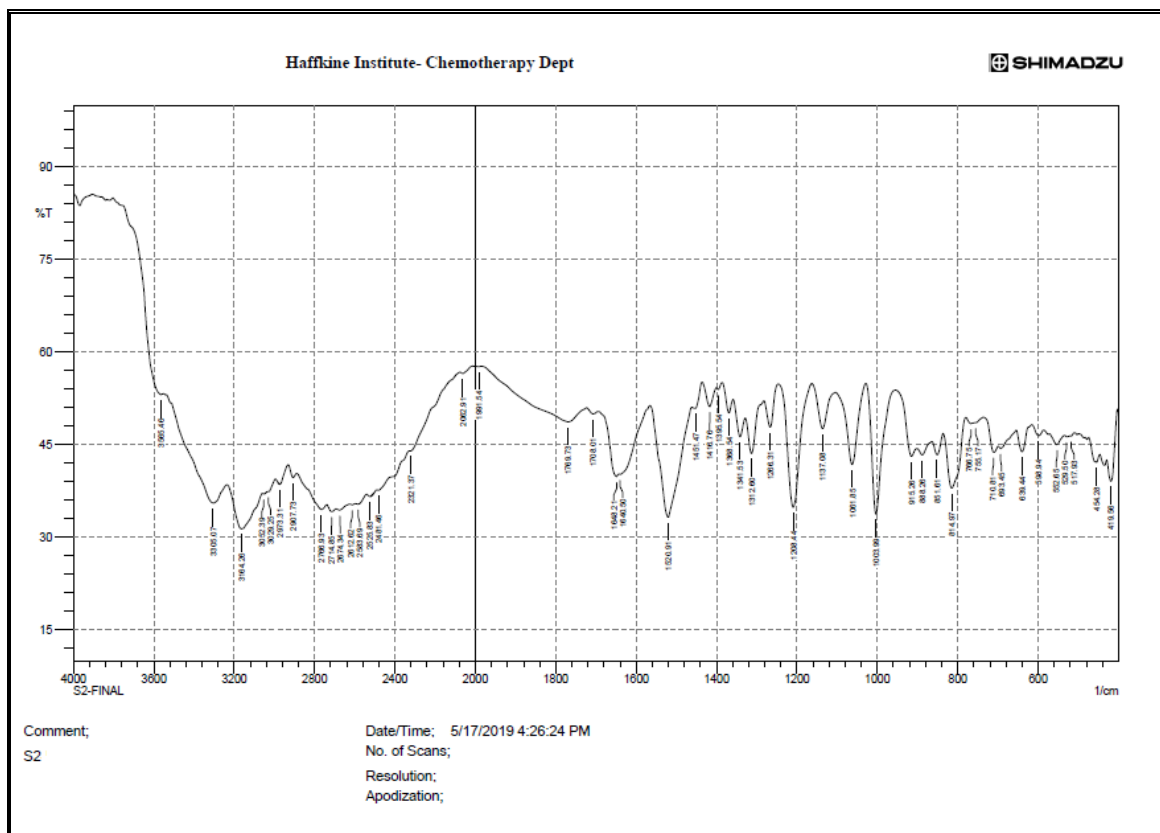
1	2 drop of filtrate + few drops of freshly prepared FeSO ₄ sol., boil for a few minutes, cool and 0.5 cm ³ of dilute H ₂ SO ₄ .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
3	1cm ³ of compound+3cm ³ of 1:1 HCl, shake well. Cool add few drops of NaNO ₂ solution	White emulsion	-NH group present

• **Physical data of compound III-B.3(a-i)**



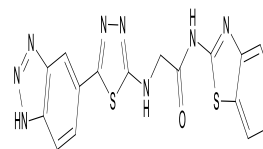
Comp.	R	Mol wt.	Colour	Solubility	MP (°C)	Yield (%)
III-B.3a	2-amino benzothiazole	408.460	Light brown	Methanol	223	63
III-B.3b	2-amino-6- methoxy benzothiazole	438.486	Light brown	Methanol	245	74
III-B.3c	2-amino-6- methyl benzothiazole	422.486	Light brown	Methanol	253	68
III-B.3d	2-amino-4,6- difluorobenz othiazole	440.441	Light brown	Methanol	229	45
III-B.3e	2-amino-5,6- dimethyl benzothiazole	436.513	Mustard	Methanol	263	52
III-B.3f	2-amino-6- NO ₂ benzothiazole	453.457	Mustard	Methanol	270	71
III-B.3g	2-amino-6- bromo benzothiazole	487.356	Light brown	Methanol	256	50
III-B.3h	2-amino-4- methyl benzothiazole	422.486	Light brown	Methanol	266	65
III-B.3i	2-amino-6- ethoxy benzothiazole	438.486	Mud	Methanol	278	68

• Spectrum 10 : IR spectra of III-B.3a

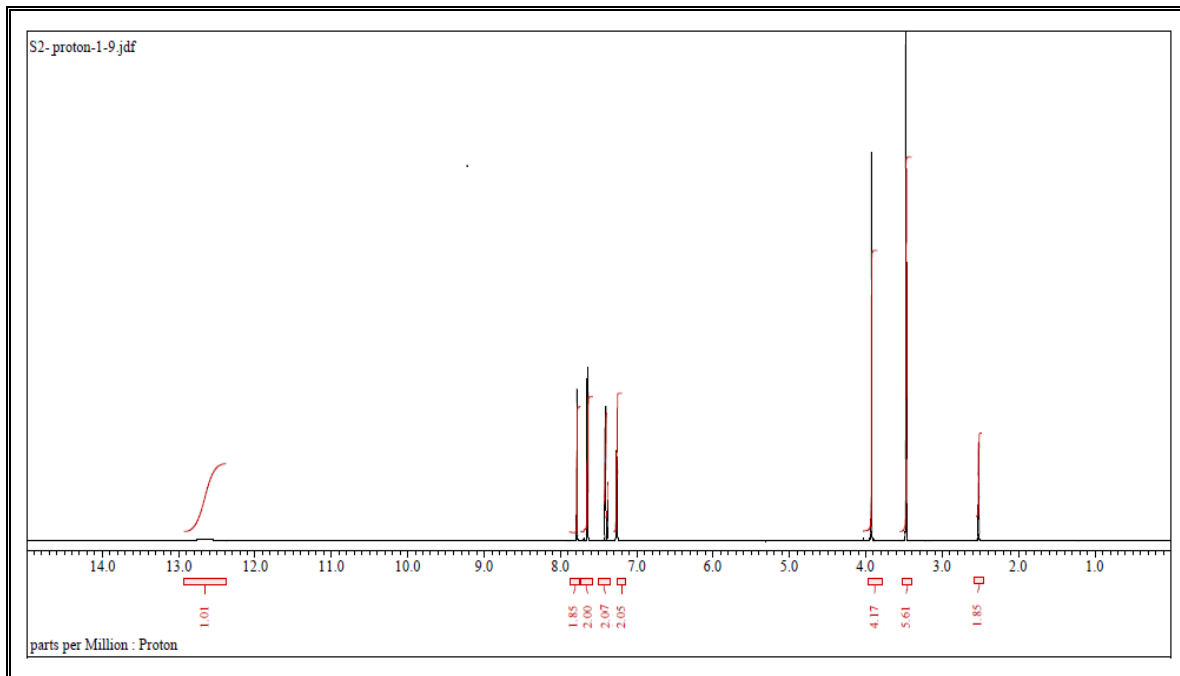


• IR spectral data of III-B.3a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch 2° amide	3164.26 cm ⁻¹	3180-3500
N-H bend 2° amide	1520.91 cm ⁻¹	1550-1640 cm ⁻¹
-CONH	1648.21 cm ⁻¹	1630-1680 cm ⁻¹



- **Spectrum 11 : NMR SPECTRA OF III-B.3a**



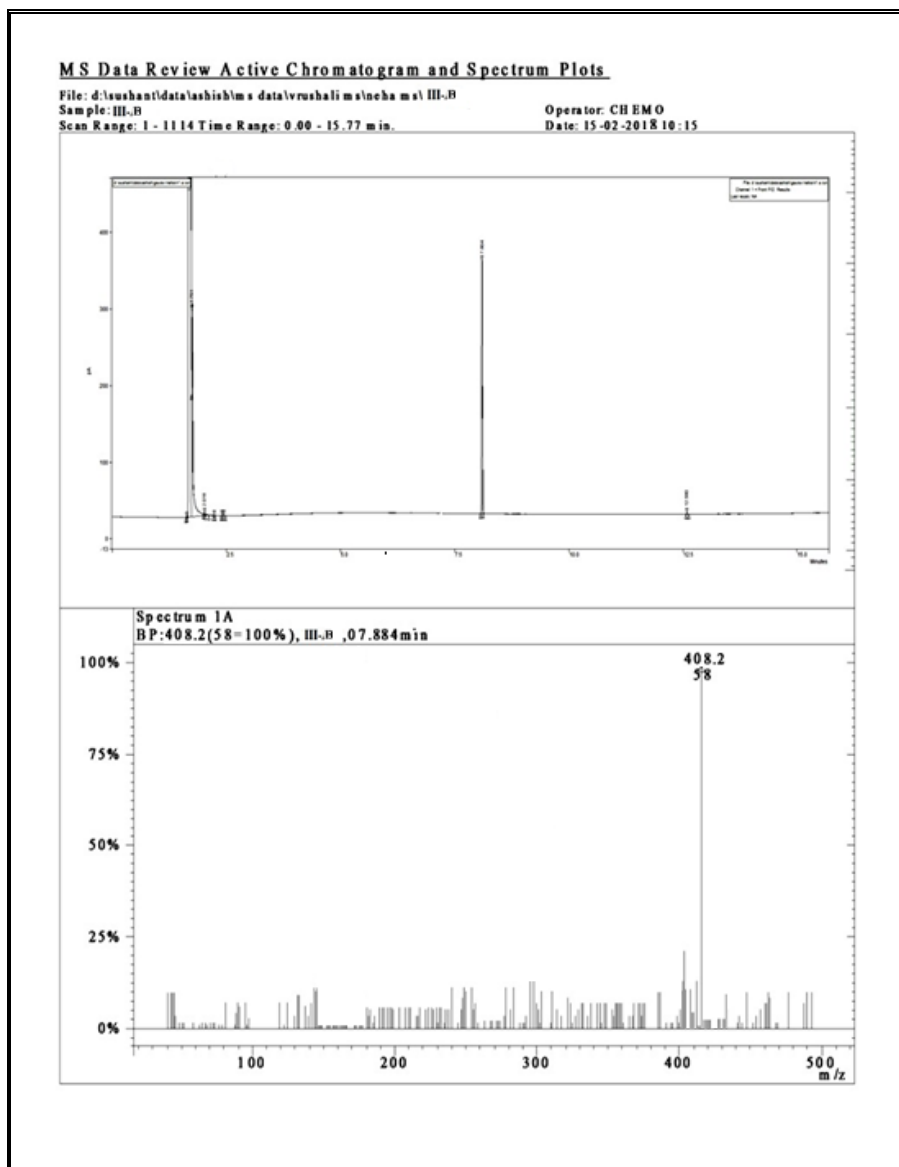
- **NMR spectral interpretation of III-B.3a**

Δ values	H ¹ NMR signal
2.5	s, 1H, NH(benzotriazole)
3.9	s, 2H, CH ₂
12.7	s, 1H, NH (sec.amide)
7.2-7.8	m, 7H, Ar-H

• GCMS method for III-B.3a

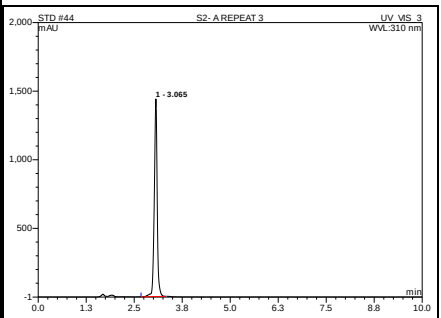
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/min)	Temperature (°C)	Hold time (min)
	0	100	2
	10	120	3
	10	150	2
	10	180	4
	10	260	3
	20	300	3
Injection mode	Split mode; split ratio 90:1		
Injection temperature	300°C		
Injection volume	1.5 µL		
Carrier gas	Helium gas		
Detector temperature	300°C		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		
Analysis time			
Run time	7.884		

• Spectrum 12 : MASS SPECTRA OF III-B.3a



m/z value	Mass signal
408.2	Molecular ion peak
408.2	Base peak

• Chromatogram 5 : HPLC method for III B .3a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.065	
Theoretical Plates	9010	
Peak Intensity	1440.78	
Asymmetry (EP)	0.99	
Peak Width	0.132	

III C] Synthesis of 2-{[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino}-*N*-[5-(substitutedbenzylsulfanyl)-1, 3, 4-thiadiazol-2-yl] acetamide

This series consists of four step of reaction

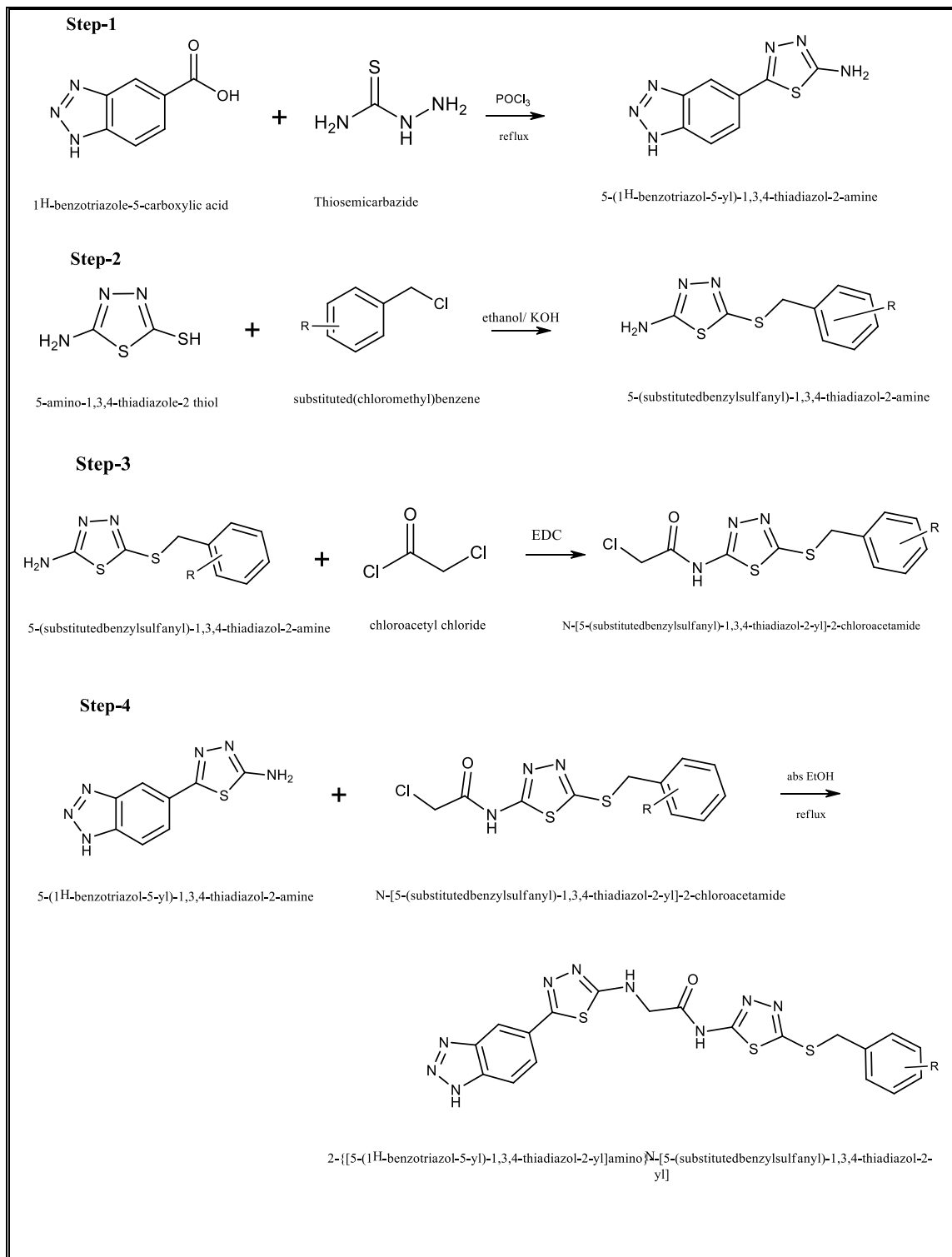
IIIC.1 Synthesis of 5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

III C.2 Synthesis of 5-(benzylsulfanyl)-1,3,4-thiadiazol-2-amine

IIIC.3 Synthesis of *N*-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide

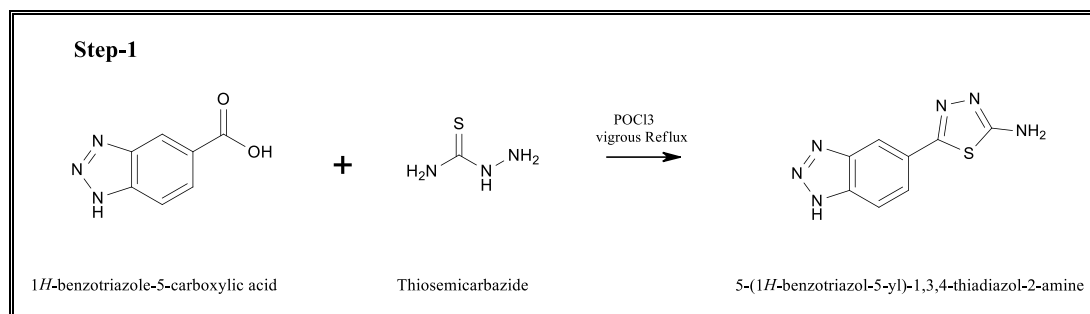
IIIC.4 Synthesis of 2-{[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-*N*-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]acetamide

• Schematic representation of III-C



III C.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
1-H Bnzotriazol-5-carboxylic acid	50mmol	163.13	-	1
Thiosemicarbazide	50mmol	91.13	-	1

- Procedure**

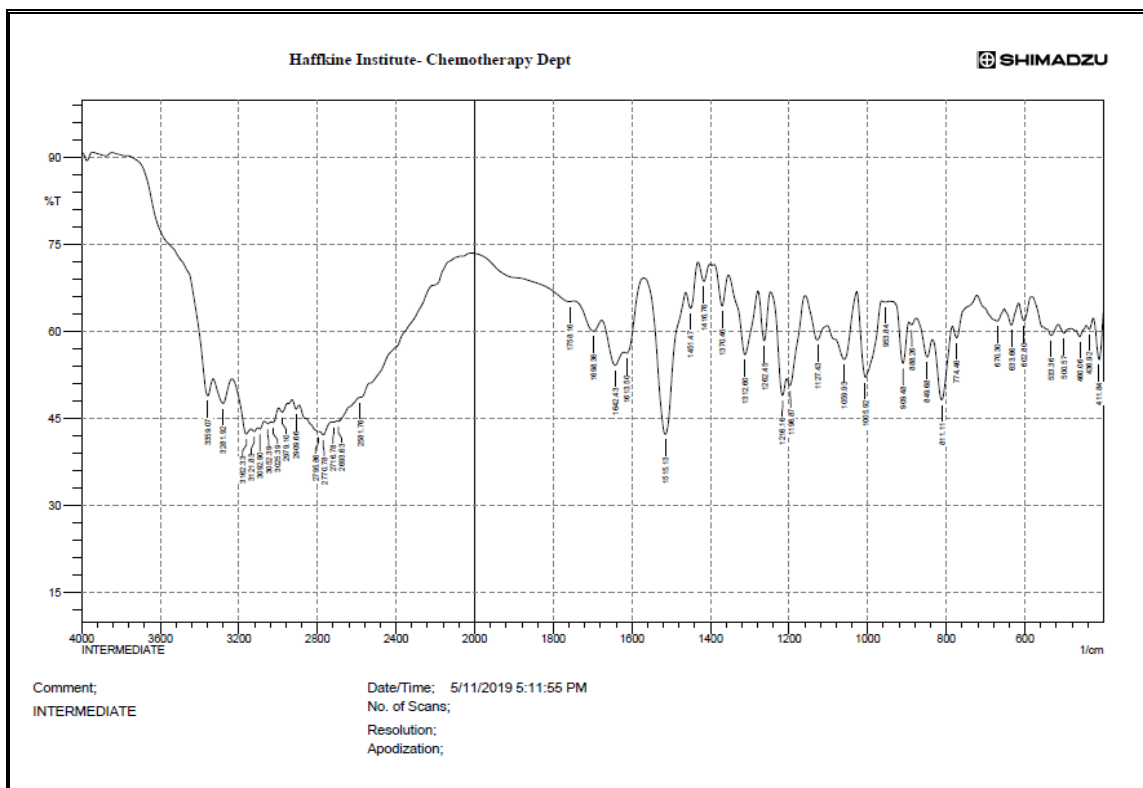
The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1). The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	264°C	Physical Constant
2	Yield	70%	

3	Colour	Light Brown	Physical appearance
4	Nature	Amorphorous	
• Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine

• Spectrum 13 : FT-IR of III C.1



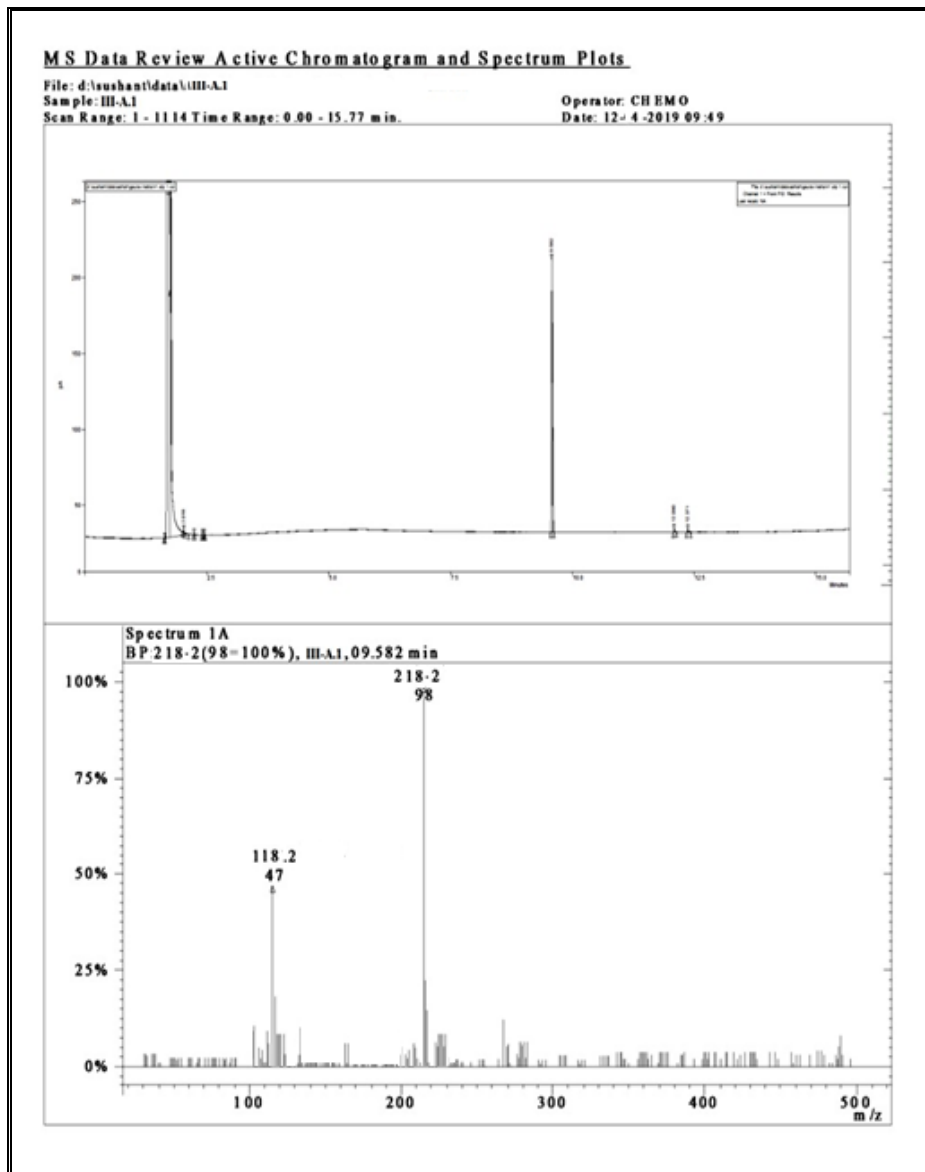
• IR spectral data of III C.1

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
N-H stretch	(3359, 3281)cm ⁻¹	3400-3250 cm ⁻¹	
N-H bend	1515 cm ⁻¹	1650-1580 cm ⁻¹	
C=N	1613 cm ⁻¹	1600 cm ⁻¹	

- **GCMS method for III C.1**

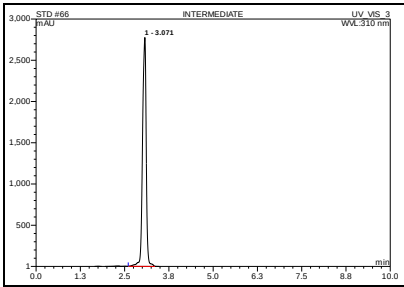
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 14 : Mass spectra of III C.1



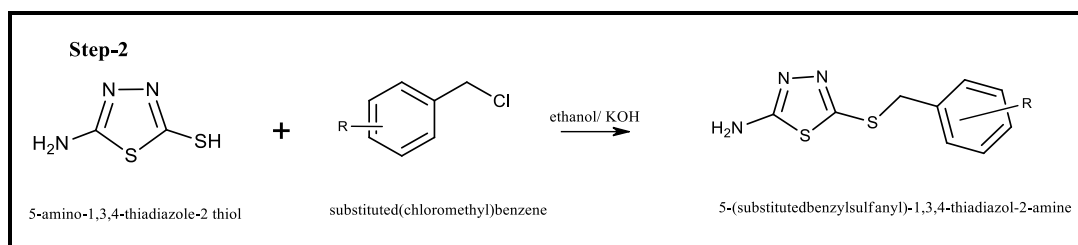
m/z value	Mass signal
218.2	Molecular ion peak
218.2	Base peak

• Chromatogram 6 : HPLC method for III C .1

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.07	
Theoretical Plates	4267	
Peak Intensity	2774.92	
Asymmetry (EP)	0.87	
Peak Width	0.177	

III C.2 Synthesis of 5-(benzylsulfanyl)-1,3,4-thiadiazol-2-amine

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
5-mercapto-2-amino-1,3,4-thiadiazole	0.003 moles	133.195	-	1
4-fluoro benzyl chloride	0.003 moles	144.576	1.207	1

- Procedure**

To a solution of 5-mercapto-2-amino-1,3,4-thiadiazole (0.003 moles) in absolute ethanol (30 ml), (1.12 gm) of potassium hydroxide and (0.03mole) substituted benzyl halides were added, the mixture was refluxed for (2 hr). After cooling precipitate was collected by filtration, dried and recrystallized from acetic acid. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	289	Physical Constant
2	Yield	80	
3	Colour	White	Physical appearance
4	Nature	Amorphorous	
Chemical test			

Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine



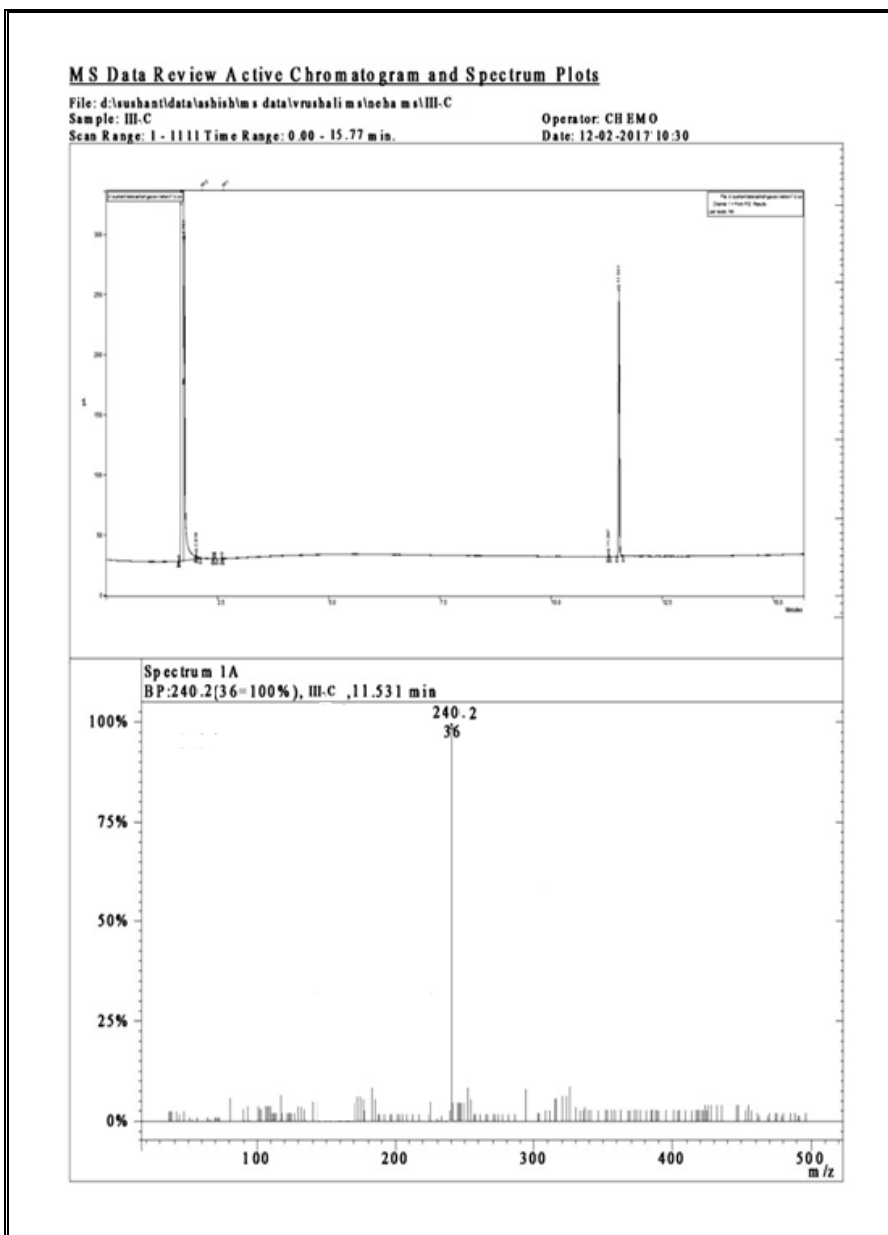
Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	3270.35cm ⁻¹	3400-3250 cm ⁻¹
N-H bend	1505.48cm ⁻¹	1650-1580 cm ⁻¹
C-F	1221.95 cm ⁻¹	1400-1000 cm ⁻¹


Nc1ccc(cc1)SC2=NC=NC=S2

- **GCMS method for III C.2a**

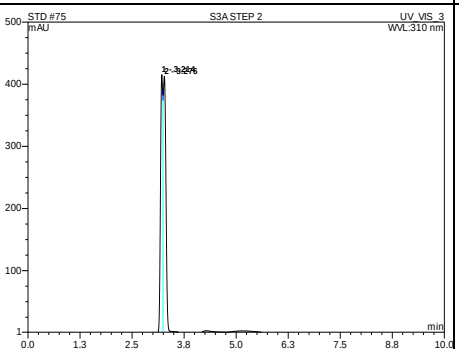
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 16 : Mass spectra of III C.2a



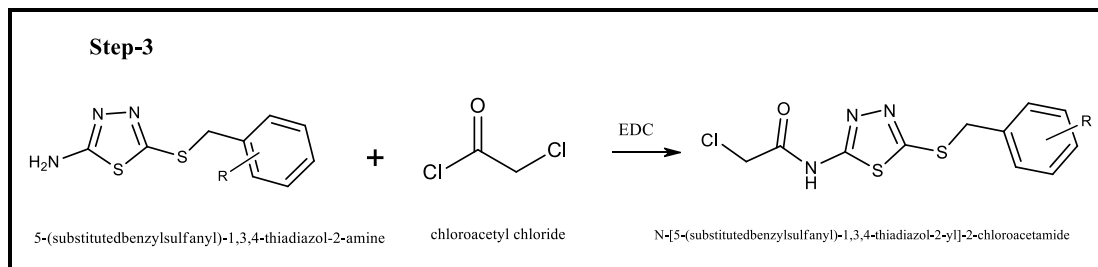
m/z value	Mass signal
240.2	Molecular ion peak
240.2	Base peak

• Chromatogram 7 : HPLC method for III C .2a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.28	
Theoretical Plates	1400	
Peak Intensity	415.37	
Asymmetry (EP)	0.62	
Peak Width	0.294	

III C.3 Synthesis of N-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
III C.2	0.01mol	241.208	-	1
Chloroacetyl chloride	0.01mol	112.94	1.42g/cm ³	1

- Procedure**

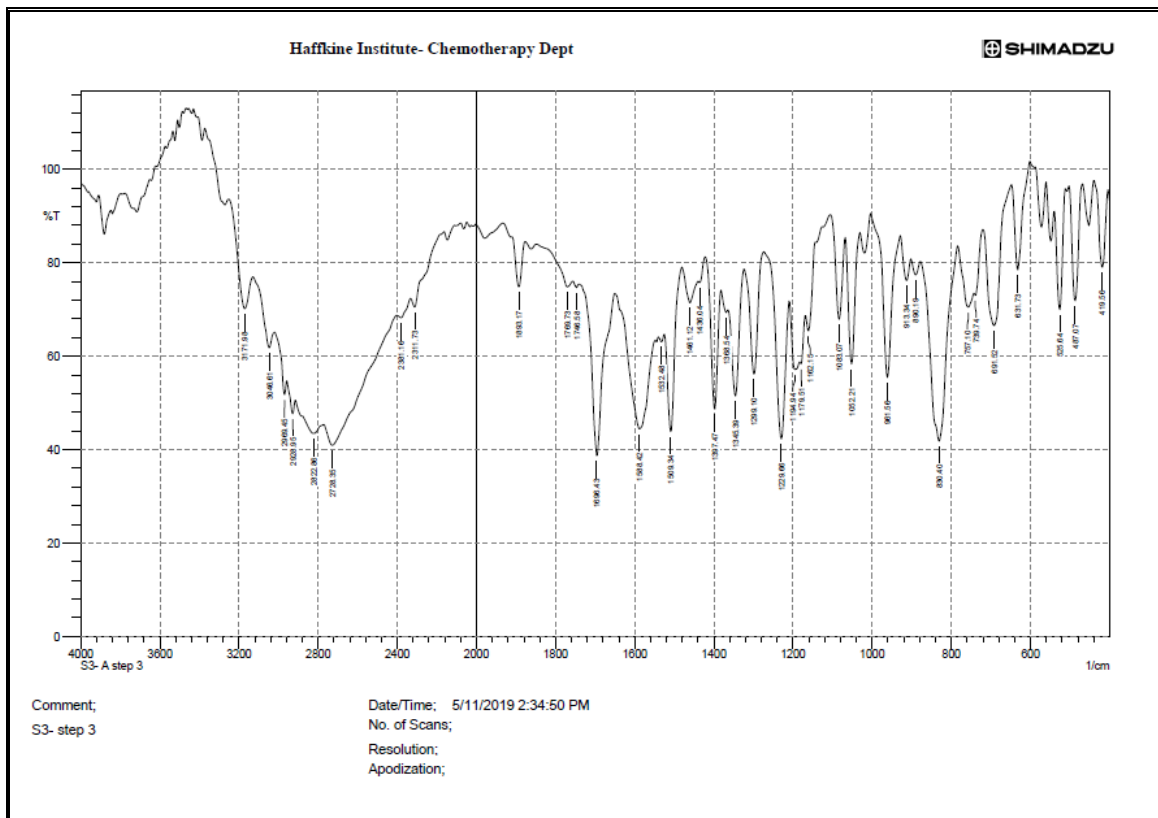
To a solution of chloroacetyl chloride (0.01mol) in (10 ml) ethylene dichloride, substituted step 2 product (0.01mol) was added with constant stirring. The reaction mixture was stirred for 5 hrs. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	210°C	Physical Constant
2	Yield	78%	
3	Colour	White	Physical appearance
4	Nature	Amorphorous	
Chemical test			

Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	Test for Halogens:- OS+ dil AgNO ₃	Thick White ppt is observed	Halogen may be present
2	1cm ³ of OS+1 drop of dil H ₂ SO ₄ +few drops CHCl ₃ + Cl ₂ water Shake well	Colourless layer is observed	Chlorine present
3	1cm ³ of compound+3cm ³ of 1:1 HCl, shake well. Cool add few drops of NaNO ₂ solution	White emulsion	-NH group present

• Spectrum 17 : FT-IR of III C.3a



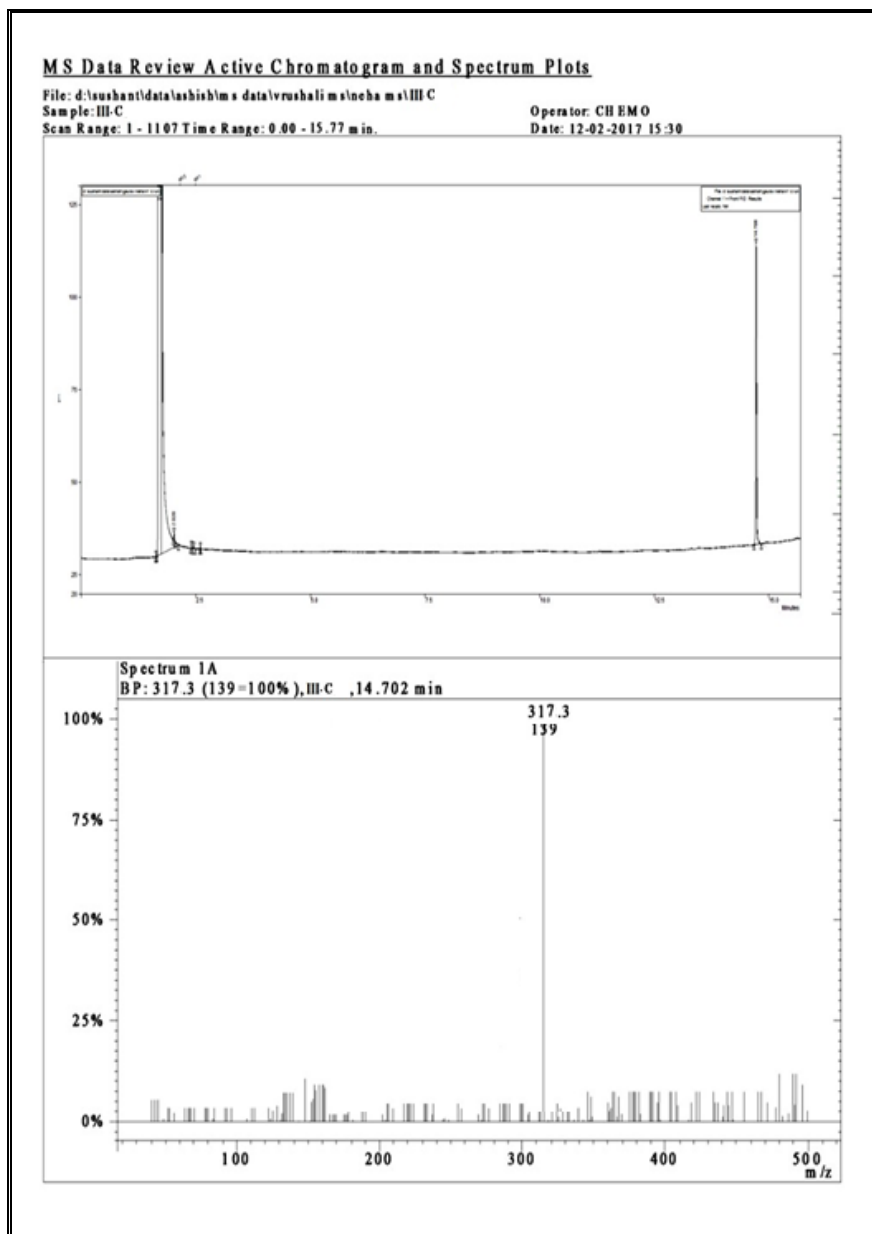
• IR spectral data of III C.3a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	3171.98 cm ⁻¹	3500-3100
N-H bend	1588.42 cm ⁻¹	1640-1550 cm ⁻¹
-CONH	1696.43 cm ⁻¹	1680-1630 cm ⁻¹
C-Cl	691.52 cm ⁻¹	800-600 cm ⁻¹
C-F	1229.66 cm ⁻¹	1400-1000 cm ⁻¹

- **GCMS method for III C.3a**

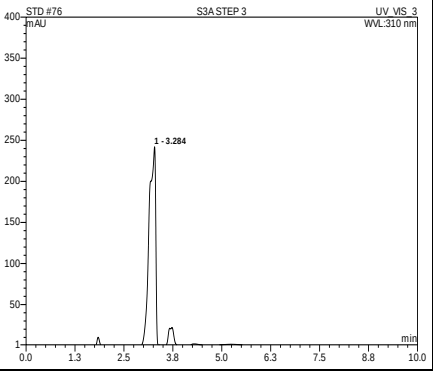
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 18 : Mass spectra of III C.3a



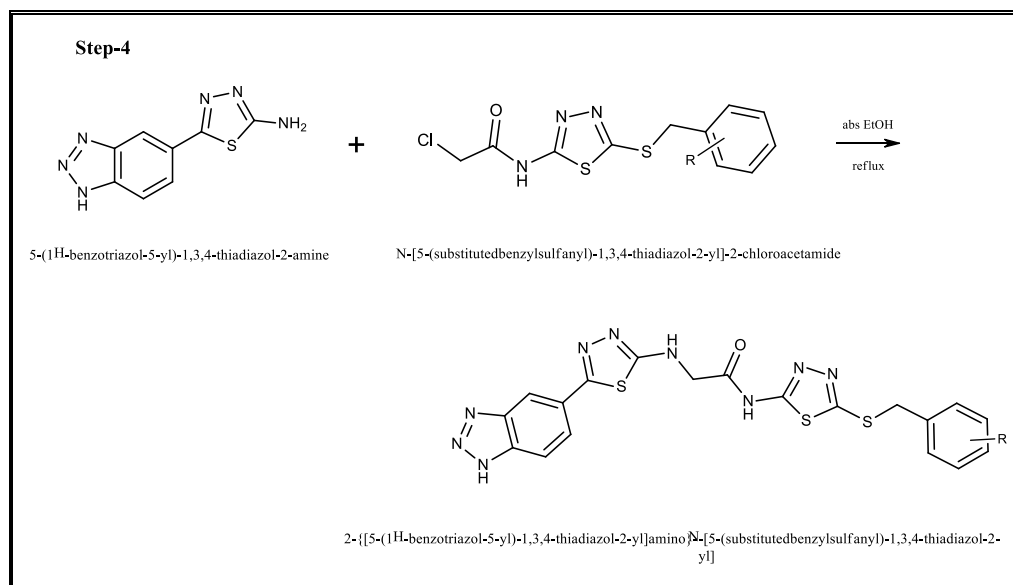
m/z value	Mass signal
317.3	Molecular ion peak
139	Base peak

• Chromatogram 8 : HPLC method for III C .3a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.284	
Theoretical Plates	1590	
Peak Intensity	241.36	
Asymmetry (EP)	0.62	
Peak Width	0.36	

III C.4 Synthesis of 2-[[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino]-N-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]acetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density	Mole ratio
III-C.1	0.003	218.238	-	1
III C.3	0.003	317.790	-	1

- Procedure**

Product of step 1 (0.003 mol) in absolute ethanol (30 ml), (0.12 gm) of sodium hydroxide and (0.03mole) substituted step 3 product were added, the mixture was refluxed for (4 hr). After cooling precipitate was collected by filtration, dried and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

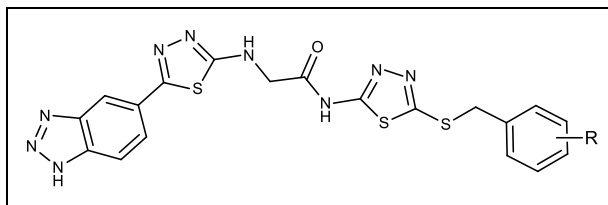
• Observation Table

Sr. no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	255°C	Physical Constant
2	Yield	50%	
3	Colour		Physical appearance
4	Nature	Amorphorous	Crystal absent

• Chemical Test

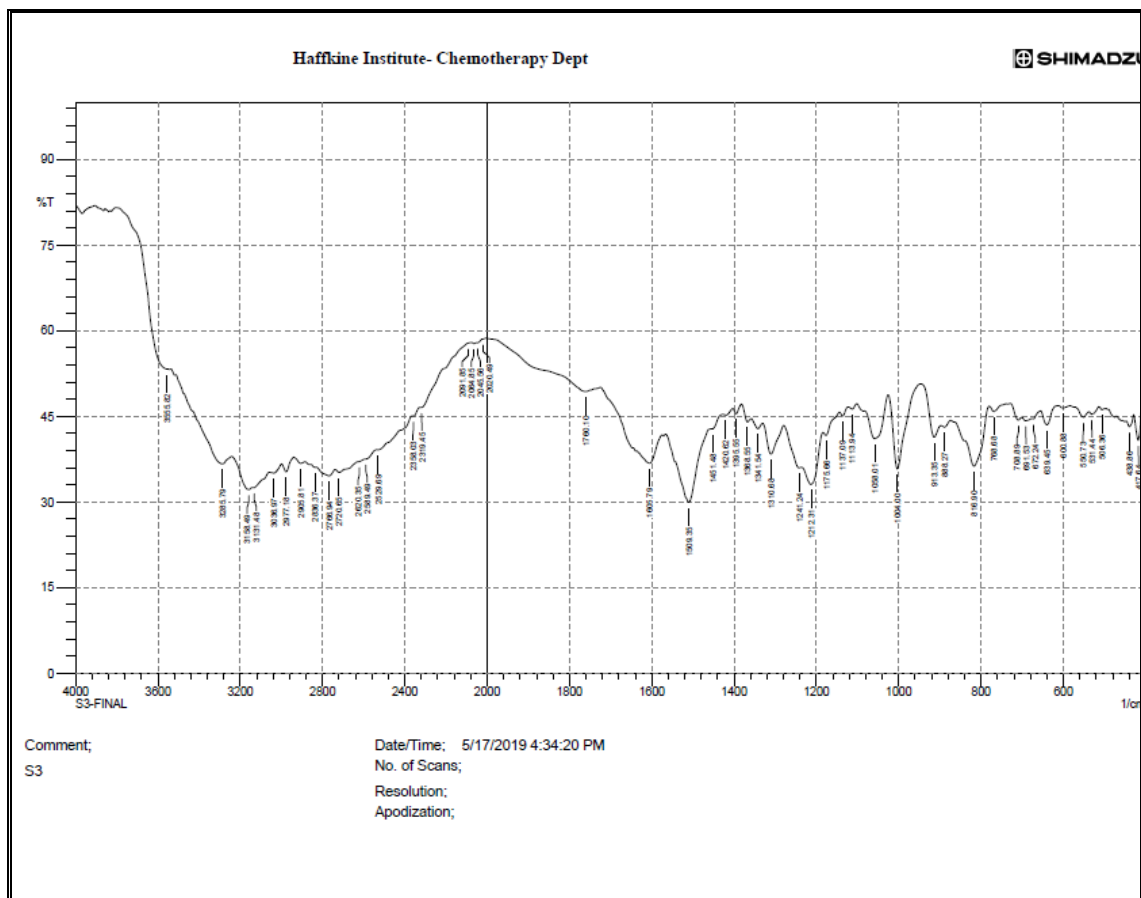
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
3	1 cm^3 of compound+3 cm^3 of 1:1 HCl, shake well. Cool add few drops of NaNO_2 solution	White emulsion	-NH group present

• Physical data of compound III-C 4(a-f)

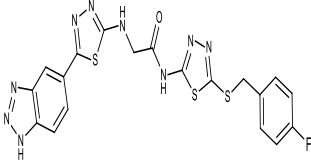


Comp.	R	Mol wt.	Colour	Solubility	MP (°C)	Yield (%)
III-C.4a	4-F benzyl chloride	499.5 6	Gray	Methanol	283	63
III-C.4b	4-Methoxybenzyl chloride	511.6 0	Dark Brown	Methanol	266	74
III-C.4c	Alpha- chloroisodurne	523.6 55	Reddish Brown	Methanol	287	68
III-C.4d	4-Isopropyl benzyl chloride	523.6 56	Light Reddish Brown	Methanol	278	45
III-C.4e	4-NO ₂ benzyl chloride	526.5 74	Dark Brown	Methanol	240	52
III-C.4f	2,4-Dichloro benzyl chloride	550.4 67	Brown	Methanol	259	71

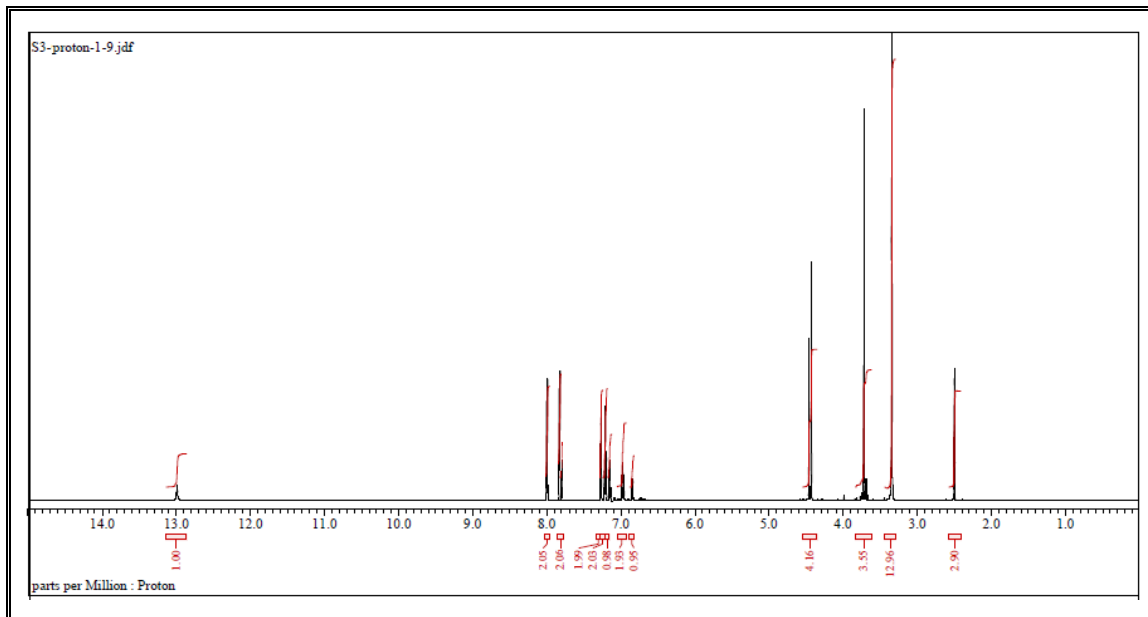
• Spectrum 19 : IR spectra of III-C.4a



• IR spectral data of III-C.4a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
N-H stretch	3285.79 cm ⁻¹	3500-3100	
N-H bend	1509.35 cm ⁻¹	1640-1550 cm ⁻¹	
-CO	1605.79 cm ⁻¹	1680-1630	
C-F	1229.66 cm ⁻¹	1400-1000 cm ⁻¹	

• Spectrum 20 : NMR SPECTRA OF III-C.4a



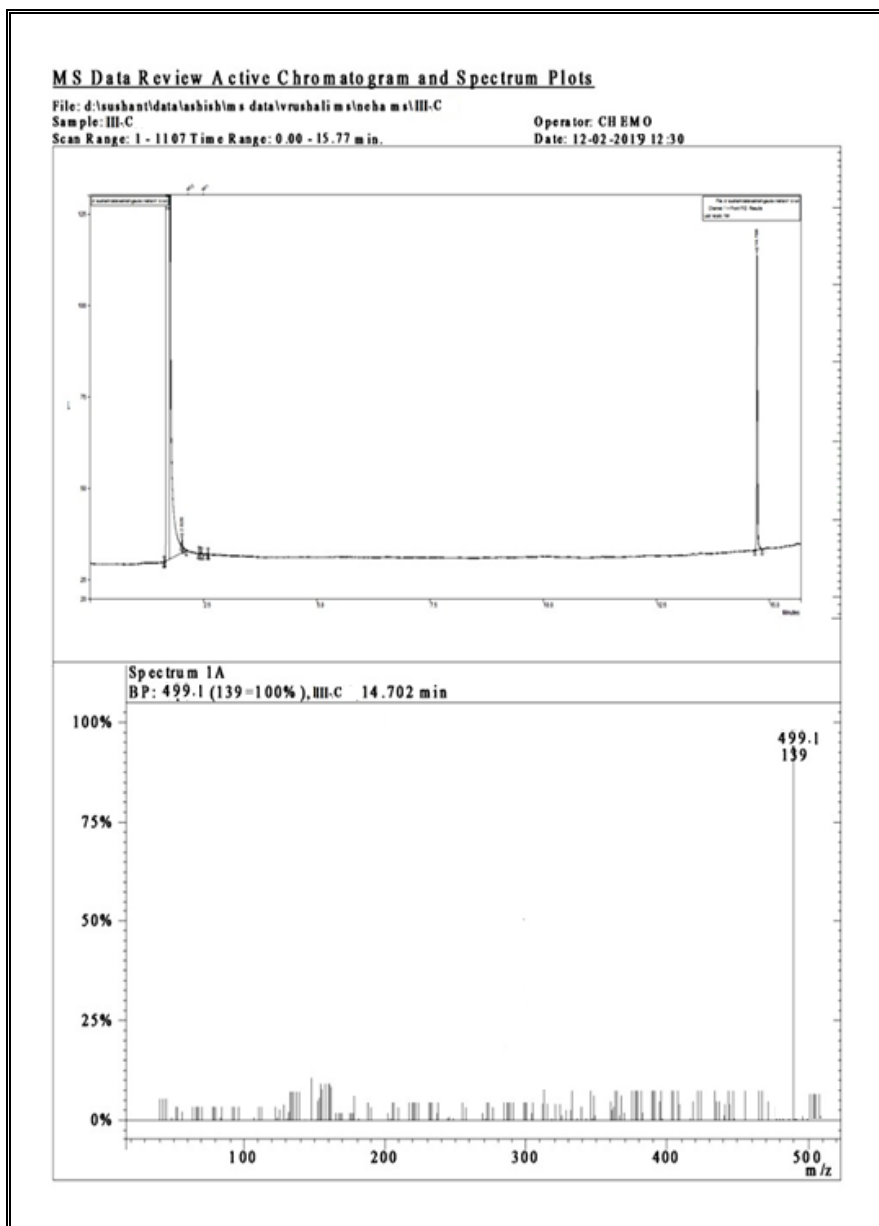
• NMR spectral interpretation of III-C.4a

Δ values	H^1 NMR signal
2.5	s, 1H, NH(benzotriazole)
3.7	s, 2H, CH_2
4.4	s, 2H, CH_2
13.0	s, 1H, NH (sec.amide)
7.2-7.8	m, 7H, Ar-H

• GCMS method for III-C.4a

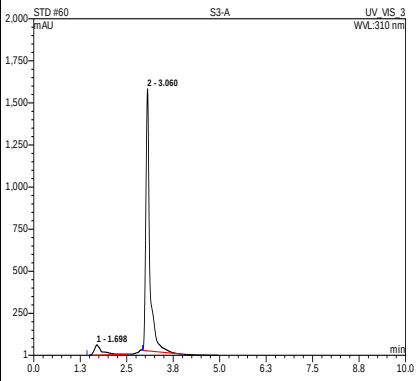
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/min)	Temperature (°C)	Hold time (min)
	0	100	2
	10	120	3
	10	150	2
	10	180	4
	10	260	3
	20	300	3
Injection mode	Split mode; split ratio 90:1		
Injection temperature	300°C		
Injection volume	1.5 µL		
Carrier gas	Helium gas		
Detector temperature	300°C		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		
Analysis time			
Run time	14.027		

• Spectrum 21 : MASS SPECTRA OF III-C.4a



m/z value	Mass signal
499.1	Molecular ion peak
499.1	Base peak

• Chromatogram 9 : HPLC method for III-C.4a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.060	
Theoretical Plates	6196	
Peak Intensity	1556.9	
Asymmetry (EP)	1.73	
Peak Width	0.158	

III D] Synthesis of 2-[[5-(1*H*-benzotriazol-5-yl)-1, 3, 4-thiadiazol-2-yl] amino}-*N*-[4-(substitutedphenylamino)-1, 3-thiazol-2-yl] acetamide

This series consists of four step of reaction:

D.1 Synthesis of 5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

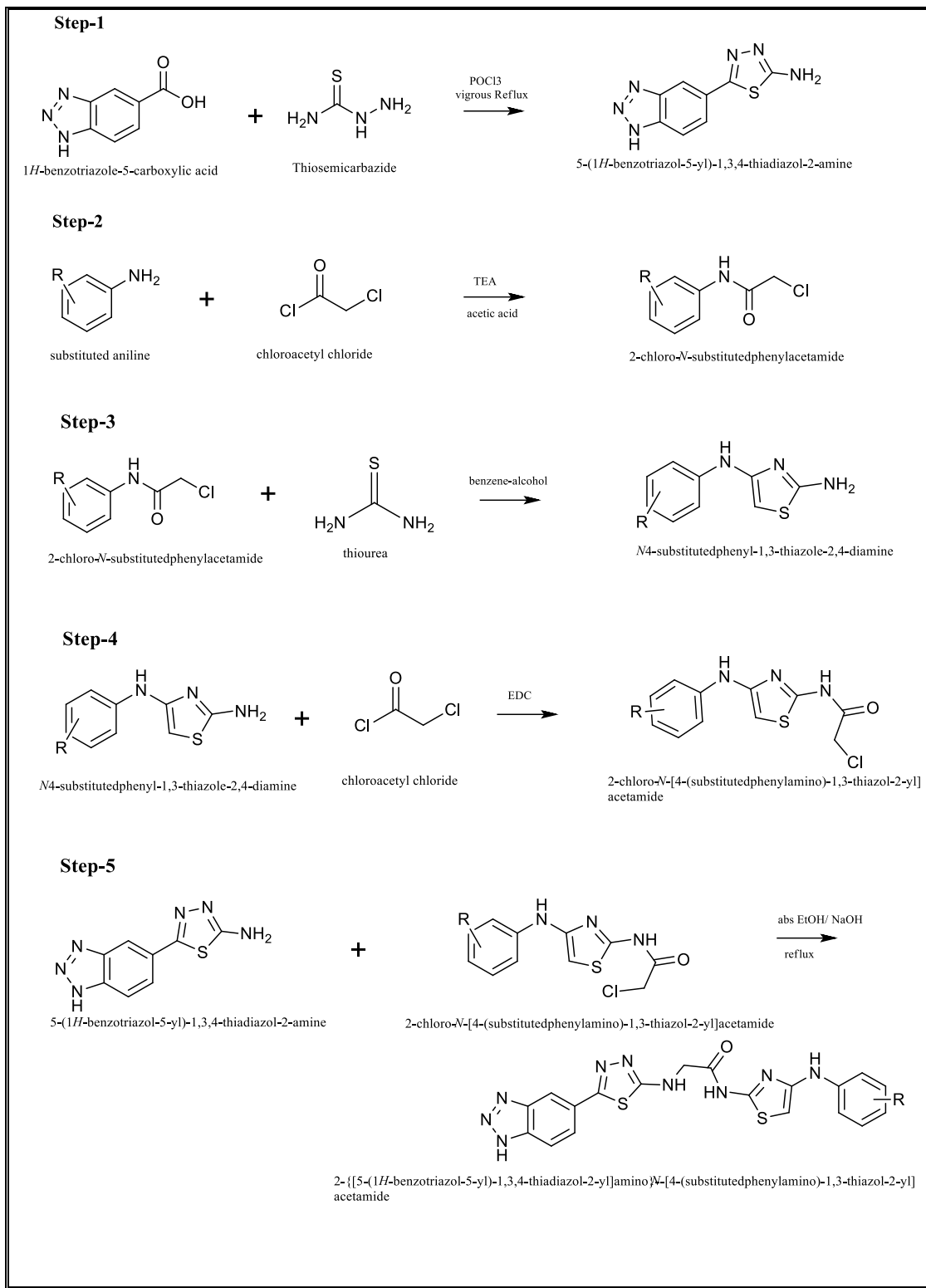
D.2 Synthesis of 2-chloro-*N*-phenylacetamide

D.3 Synthesis of *N*4-phenyl-1,3-thiazole-2,4-diamine

D.4 Synthesis of 2-chloro-*N*-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide

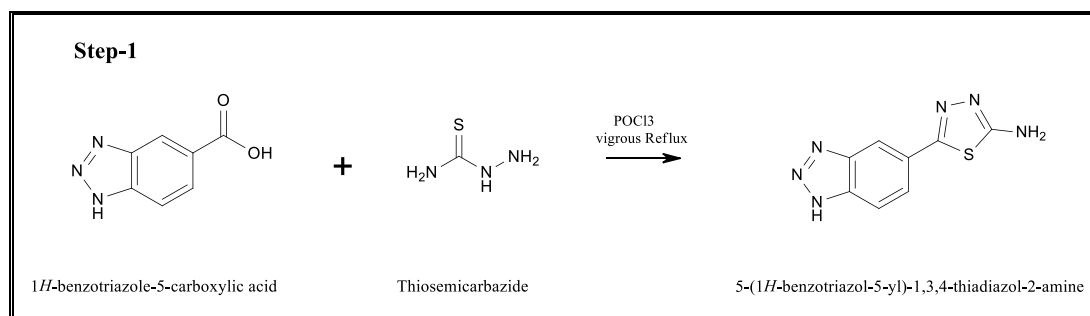
D.5 Synthesis of 2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-*N*-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide

• Schematic representation of III-D



III D.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
1-H Bnzotriazol-5-carboxylic acid	50mmol	163.13	-	1
Thiosemicarbazide	50mmol	91.13	-	1

- Procedure**

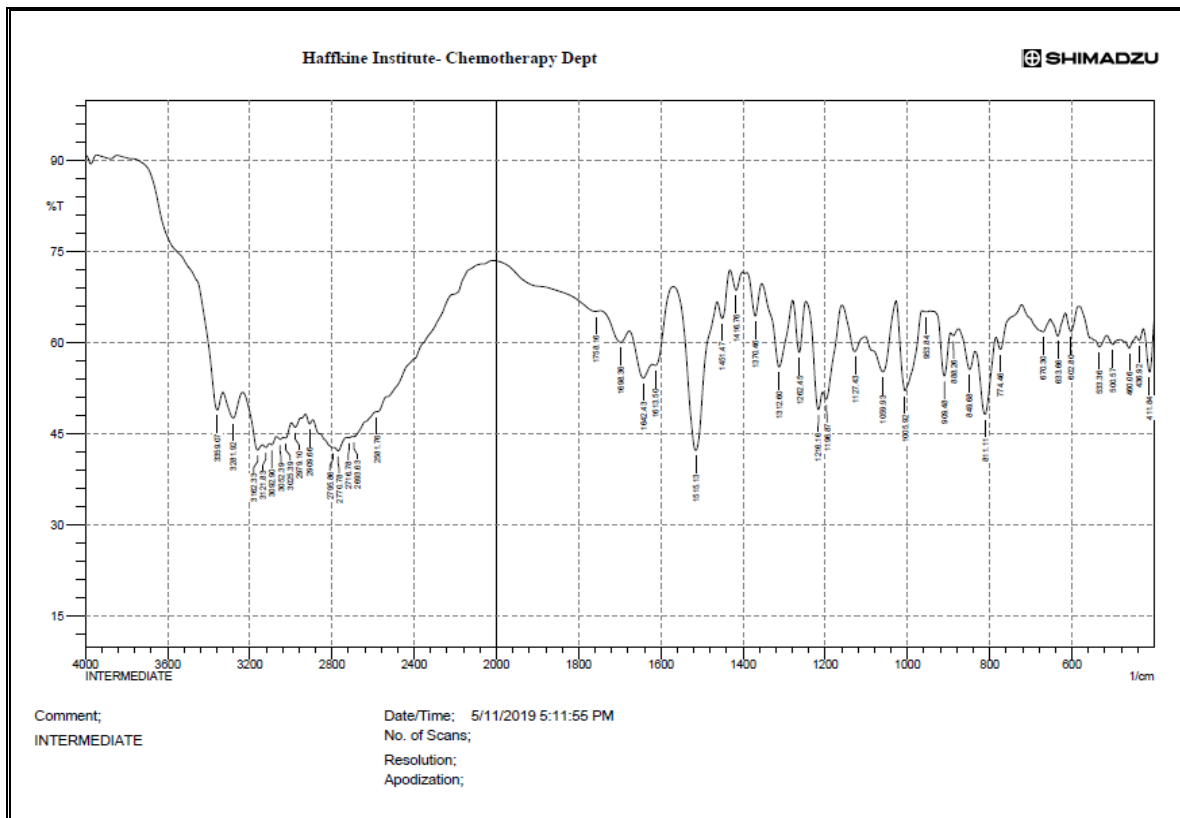
The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1). The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	264°C	Physical Constant
2	Yield	70%	
3	Colour	Light Brown	Physical appearance
4	Nature	Amorphorous	

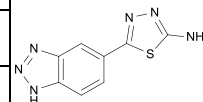
• Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine

• Spectrum 22: FT-IR of I I I D.1



• IR spectral data of III D.1

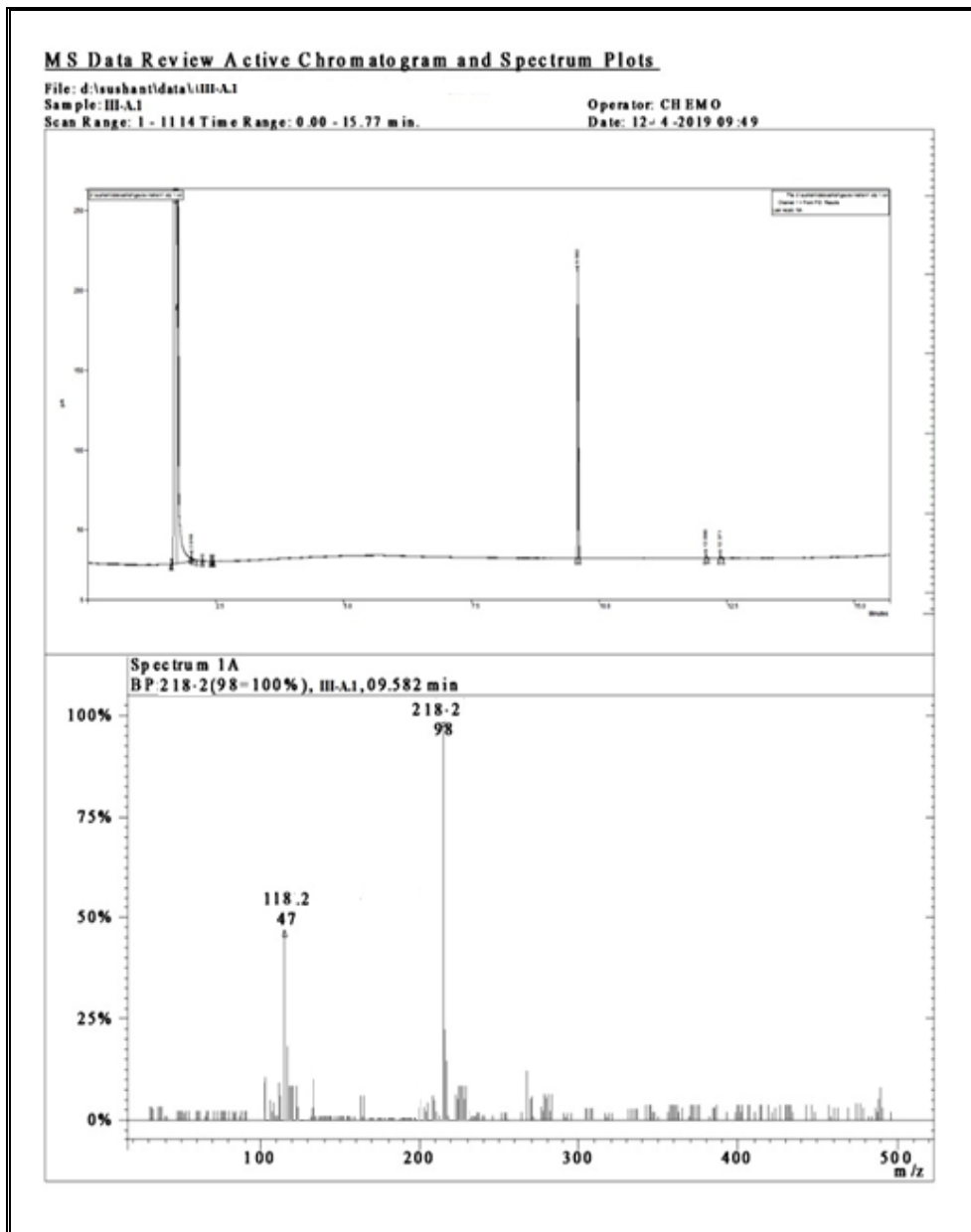
Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	(3359, 3281)cm ⁻¹	3400-3250 cm ⁻¹
N-H bend	1515 cm ⁻¹	1650-1580 cm ⁻¹
C=N	1613 cm ⁻¹	1600 cm ⁻¹



- **GCMS method for III D.1**

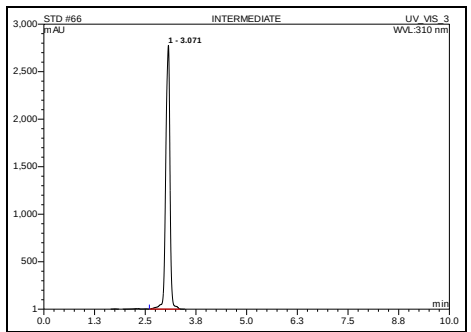
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 23 : Mass spectra of III D.1



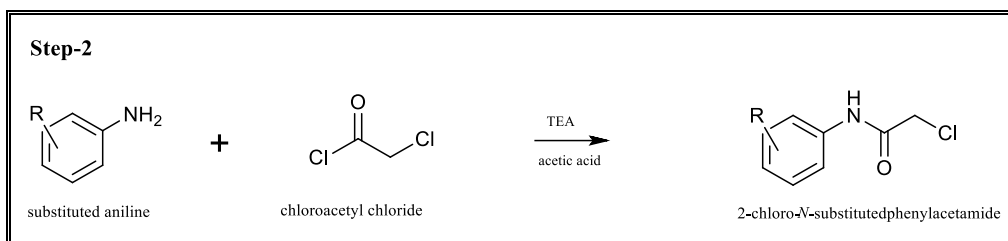
m/z value	Mass signal
218.2	Molecular ion peak
218.2	Base peak

• Chromatogram 10 : HPLC method for III D .1

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.07	
Theoretical Plates	4267	
Peak Intensity	2774.92	
Asymmetry (EP)	0.87	
Peak Width	0.177	

III D.2 Synthesis of 2-chloro-N-phenylacetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
Aniline	0.01	93.13	1.02	1
Chloroacetyl chloride	0.01	112.94	1.42	1

- Procedure**

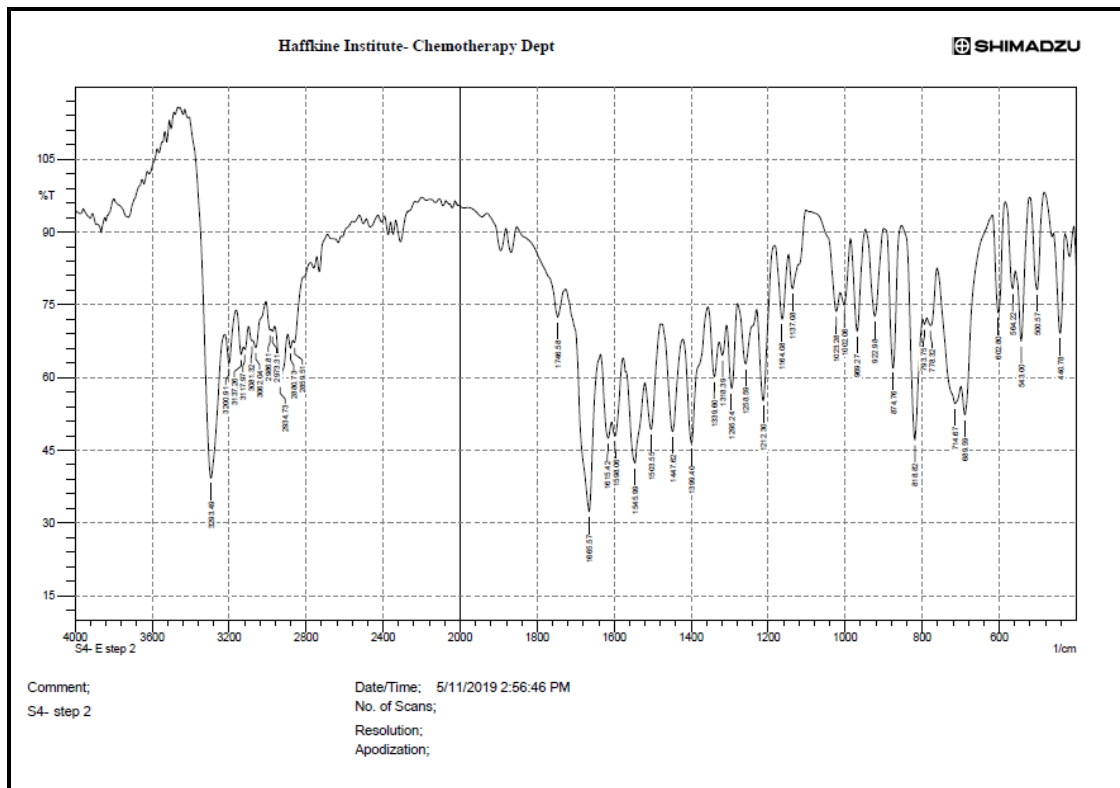
Aniline (0.01mole), chloroacetyl chloride (0.01) and triethylamine (0.01 mole) were dissolved in acetic acid (5ml) was kept for stirring for 3 hrs. The product was isolated as precipitate after pouring reaction mixture to an ice-cold water. Precipitate was filtered, washed with cold water and dried. Recrystallized using ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

- Observation Table**

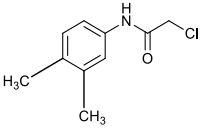
Sr.no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	170°C	Physical Constant
2	Yield	80%	
3	Colour	Yellow	Physical appearance
4	Nature	Amorphorous	
• Chemical test			

Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	Test for Halogens:- OS+ dil AgNO ₃	Thick White ppt is observed	Halogen may be present
2	1cm ³ of OS+1 drop of dil H ₂ SO ₄ +few drops CHCl ₃ + Cl ₂ water Shake well	Colourless layer is observed	Chlorine present
3	1cm ³ of compound+3cm ³ of 1:1 HCl, shake well. Cool add few drops of NaNO ₂ solution	White emulsion	-NH group present

• Spectrum 24 : FT-IR of III D.2a



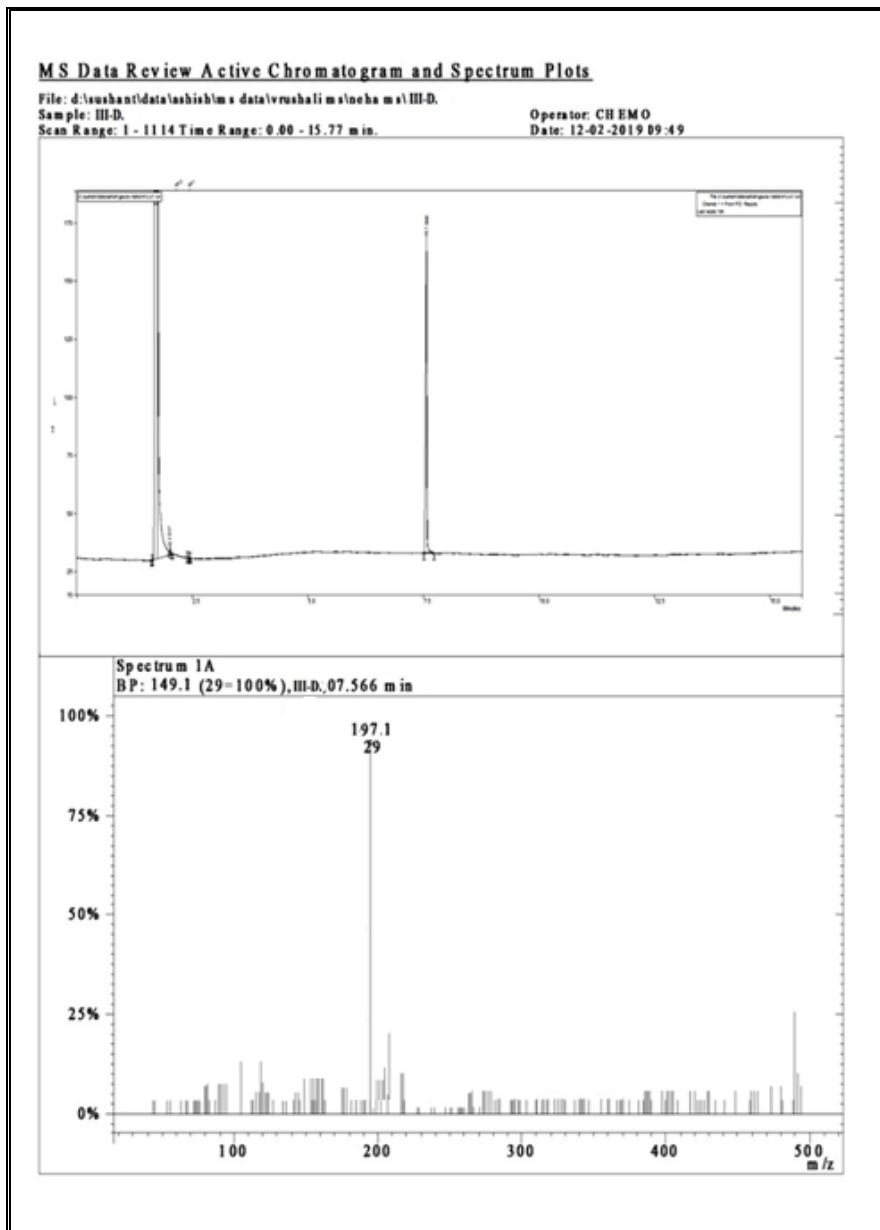
• IR spectral data of III D.2a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
N-H stretch	3293.49 cm ⁻¹	3500-3100	
N-H bend	1545.99cm ⁻¹	1640-1550 cm ⁻¹	
-CONH	1665.57 cm ⁻¹	1680-1630 cm ⁻¹	
C-Cl	689.59 cm ⁻¹	800-600 cm ⁻¹	

- **GCMS method for III D.2a**

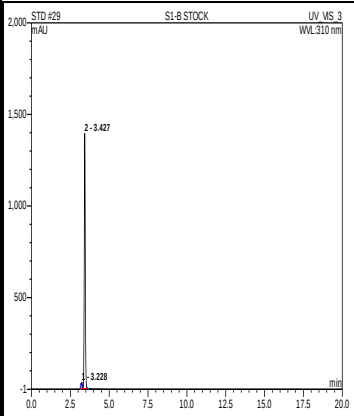
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 25 : Mass spectra of D.2a



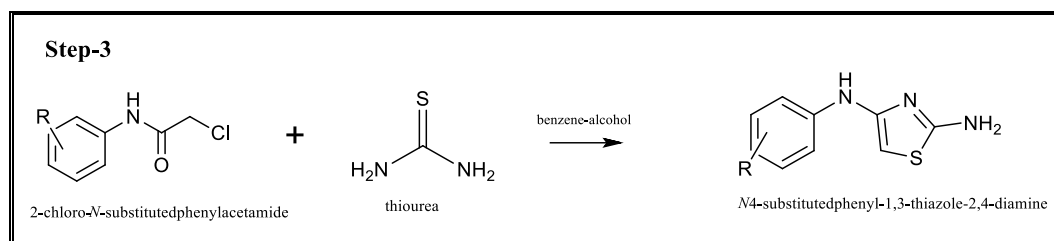
m/z value	Mass signal
197.1	Molecular ion peak
197.1	Base peak

• Chromatogram 11 : HPLC method for III D .2a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.427	
Theoretical Plates	12844	
Peak Intensity	1393.38	
Asymmetry (EP)	1.06	
Peak Width	0.122	

III D.3 Synthesis of N4-phenyl-1,3-thiazole-2,4-diamine

- Reaction



- Reaction Calculation table:

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
III D.2	0.01	197.66	-	1
Thiourea	0.01	76.12	-	1

- Procedure

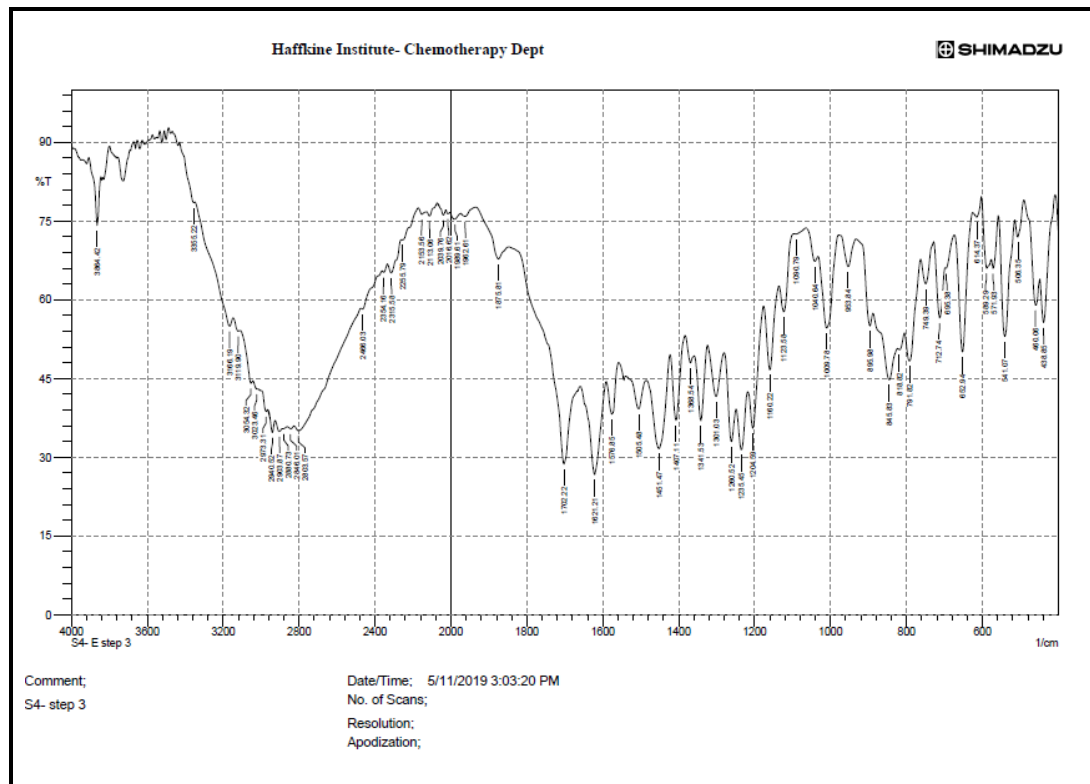
The step 2 product (0.01 moles) and thiourea (0.01 moles) were dissolved in ethanol, the mixture was refluxed for 10 -12 hours and poured in to ice cold water. The mass obtained was filtered and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

- Observation Table

Sr.no.	Parameters	Observation	Inference
Physical Test			
1	Melting Point	234°C	Physical Constant
2	Yield	88%	
3	Colour	Orange	Physical appearance
4	Nature	Amorphorous	
Chemical test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube.			

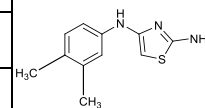
Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO_4 sol., boil for a few minutes, cool and 0.5 cm^3 of dilute H_2SO_4 .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
Nitrous acid test	Make a solution of about 1g of sodium nitrite in about 5ml of distilled water in a test tube and cool it in an ice bath. In a separate test tube, dissolve a small quantity of the given compound in about 1ml of sulphuric acid and cool this also in ice bath. Mix both the solution.	Bubbles of nitrogen gas	Presence of primary aliphatic amine

• Spectrum 26 : FT-IR of III D.3a



• IR spectral data of III D.3a

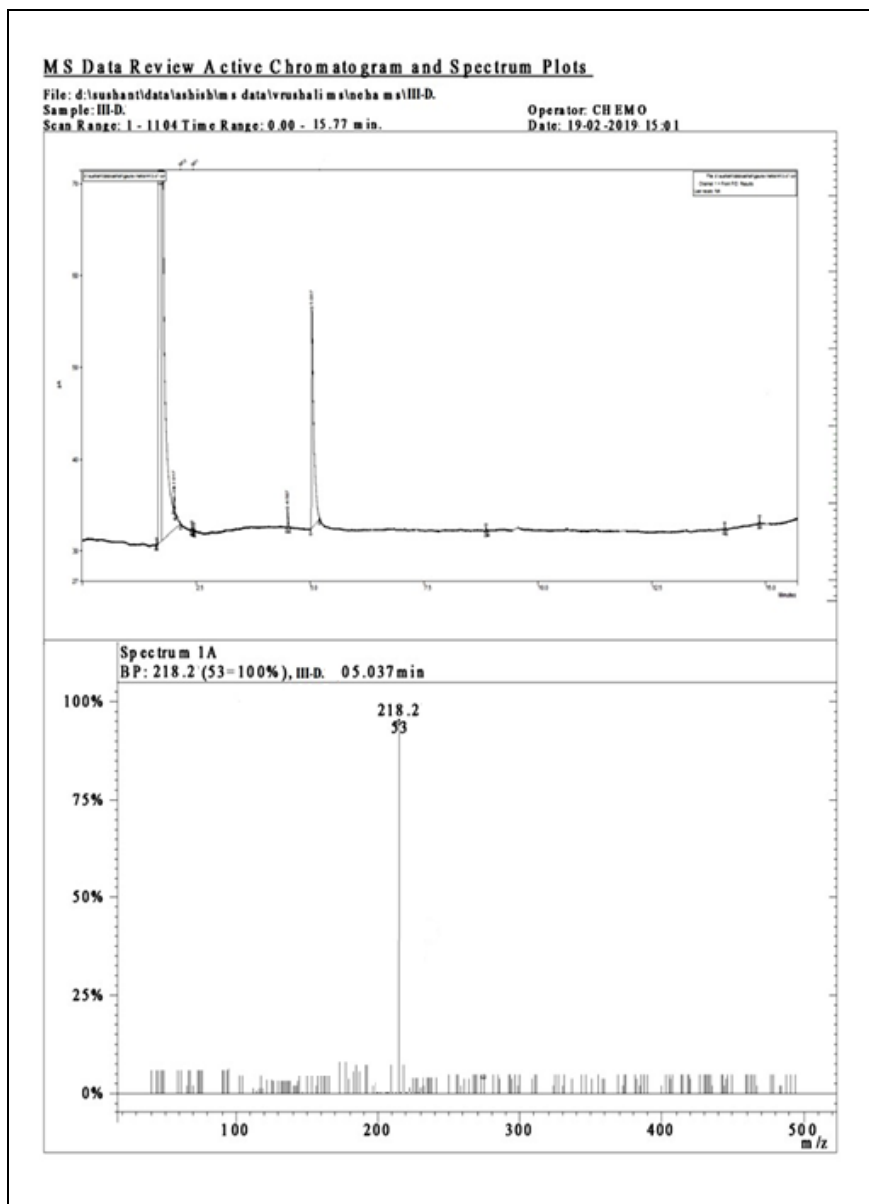
Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
-NH stretch	3355.22 cm ⁻¹	3500-3300 cm ⁻¹
N-H bend	1576.85 cm ⁻¹	1640-1550 cm ⁻¹
C=N	1702.22 cm ⁻¹	1690-1640 cm ⁻¹



- **GCMS method for III D.3a**

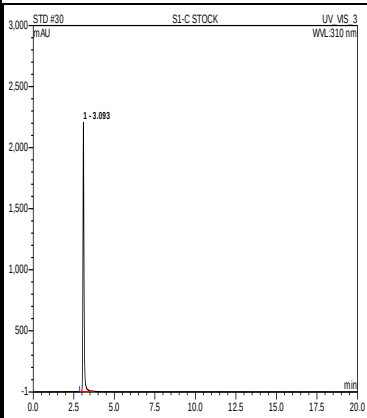
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 27 : Mass spectra of III D.3a



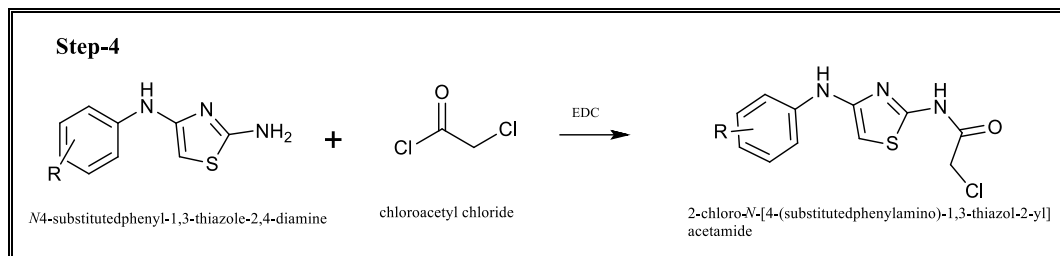
m/z value	Mass signal
218.2	Molecular ion peak
218.2	Base peak

• Chromatogram 12 : HPLC method for III D .3a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol-ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.093	
Theoretical Plates	11927	
Peak Intensity	2205.73	
Asymmetry (EP)	1.28	
Peak Width	0.114	

III D.4 Synthesis of 2-chloro-N-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density g/cm ³	Mole ratio
III D.3a	0.01	219.30	-	1
Chloroacetyl chloride	0.01	112.94	1.42	1

- Procedure**

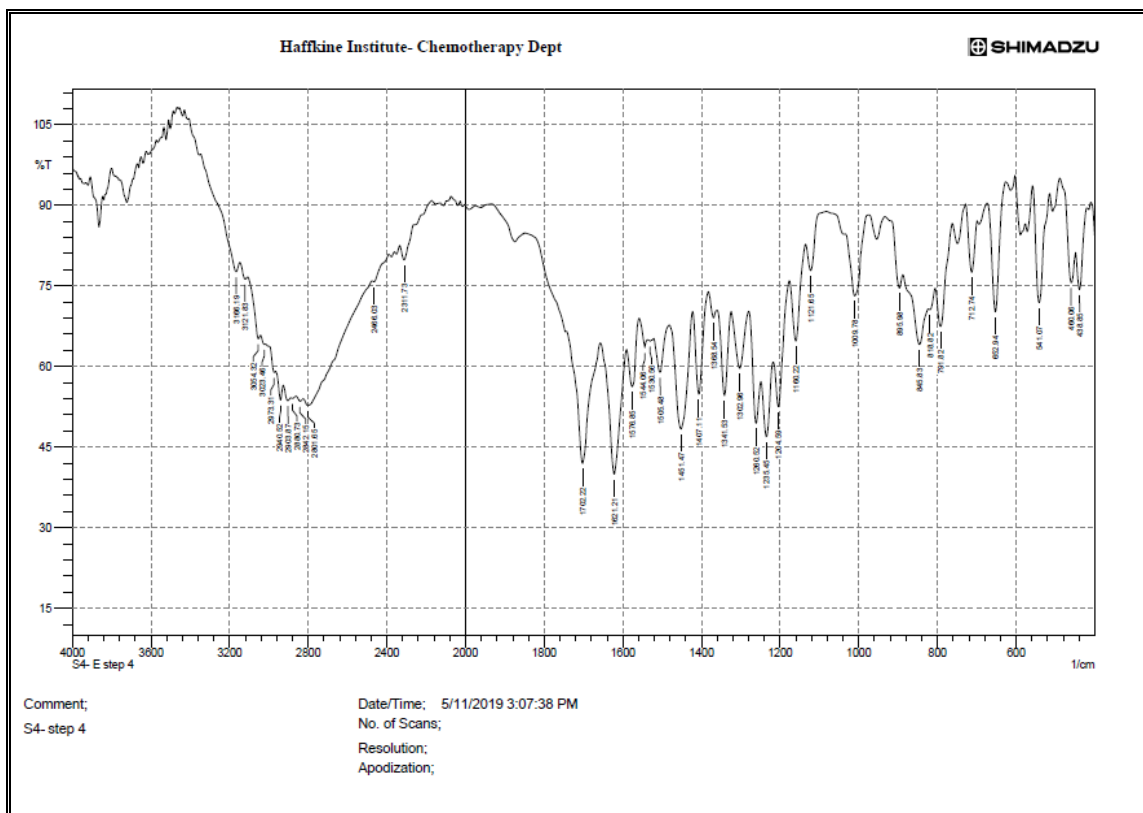
The Step 3 product (0.01 moles) in 30 ml ethylene dichloride, chloroacetyl chloride (0.01moles) was added drop wise with constant stirring .The mixture was then stirred for 1-2 hours. The excess of solvent was distilled-off under reduced pressure, solid mass washed with ice cold water and crystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

- Observation Table**

Sr.no.	Parameters	Observation	Inference
• Physical Test			
1	Melting Point	298°C	Physical Constant
2	Yield	56%	
3	Colour	Yellow	Physical appearance
4	Nature	Amorphorous	
• Chemical test			

Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	Test for Halogens:- OS+ dil AgNO_3	Thick White ppt is observed	Halogen may be present
2	1cm^3 of OS+1 drop of dil H_2SO_4 +few drops CHCl_3 + Cl_2 water Shake well	Colourless layer is observed	Chlorine present
3	1cm^3 of compound+ 3cm^3 of 1:1 HCl , shake well. Cool add few drops of NaNO_2 solution	White emulsion	-NH group present

• Spectrum 28 : FT-IR of III D.4a



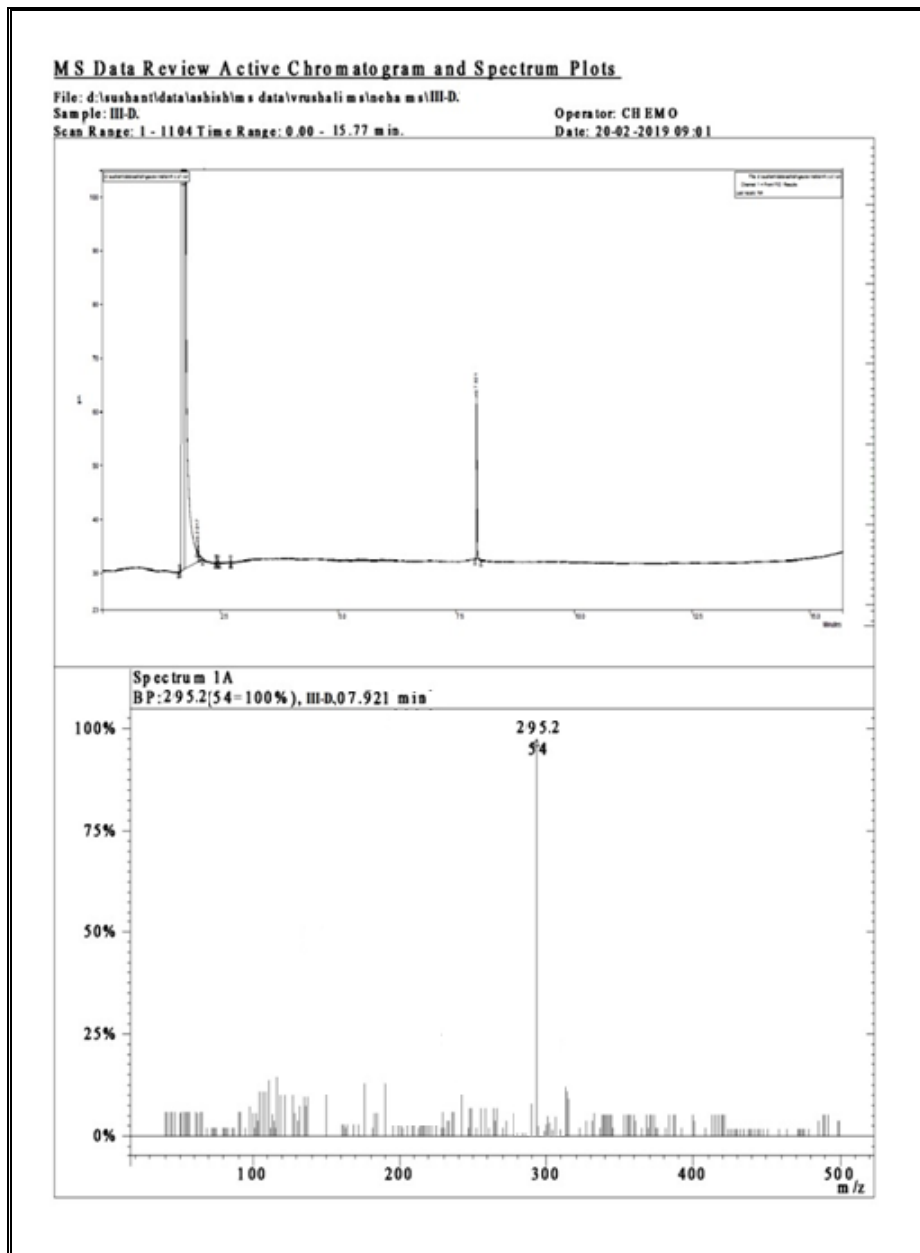
• IR spectral data of III D.4a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)
N-H stretch	3166.19 cm ⁻¹	3500-3100
N-H bend	1576.85 cm ⁻¹	1640-1550 cm ⁻¹
-CONH	1621.21 cm ⁻¹	1680-1630 cm ⁻¹
C-Cl	652.94 cm ⁻¹	800-600 cm ⁻¹

- **GCMS method for III D.4a**

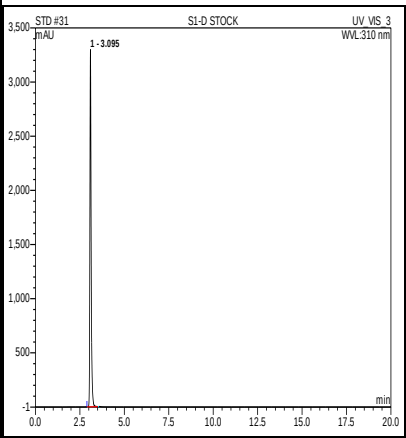
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	3
	20	300	7
Injection mode	Split mode; split ratio 90:1	Carrier gas	Helium gas
Injection temperature	300°C	Detector temp	300°C
MS condition			
Ion source temperature	280°C		

- Spectrum 29 : Mass spectra of III D.4a



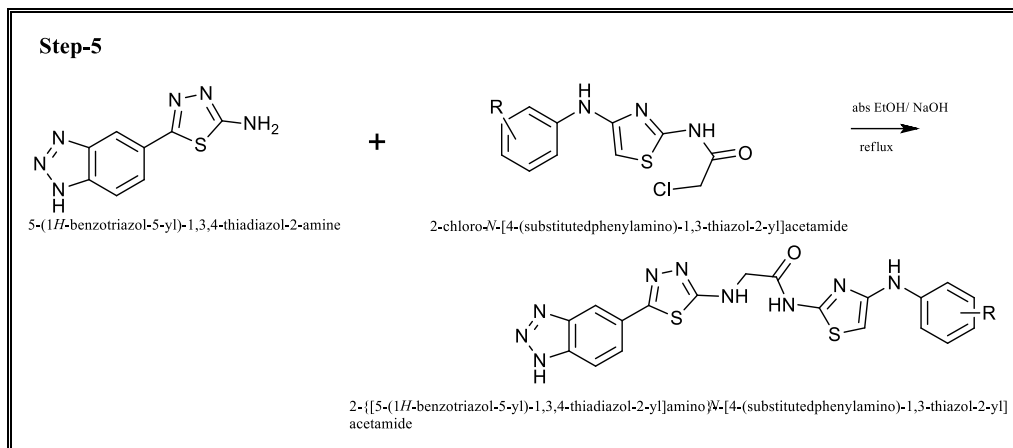
m/z value	Mass signal
295.2	Molecular ion peak
295.2	Base peak

• Chromatogram 13 : HPLC method for III D .4a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol- ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.095	
Theoretical Plates	10.378	
Peak Intensity	32.97.82	
Asymmetry (EP)	1.34	
Peak Width	0.121	

III D.5 Synthesis of 2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-*N*-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide

- Reaction**



- Reaction Calculation table:**

Compound	Mole	Mol. Wt	Density	Mole ratio
III-D.1	0.01	218.238	-	1
III D.4a	0.01	295.787	-	1

- Procedure**

Synthesis of title product is accomplished by condensation of step 1 and step 4 products using alcoholic NaOH. The reaction mixture was heated under refluxed for 10-12 hours. The precipitate formed was filtered, washed with water and recrystallized from ethanol to afford the final product. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

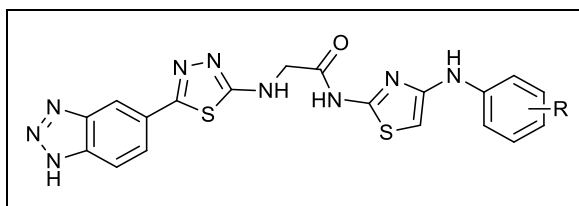
- Observation Table**

Sr. no.	Parameters	Observation	Inference
Physical Test			

1	Melting Point	295°C	Physical Constant
2	Yield	50%	
3	Colour	Mustard	Physical appearance
4	Nature	Amorphorous	Crystal absent

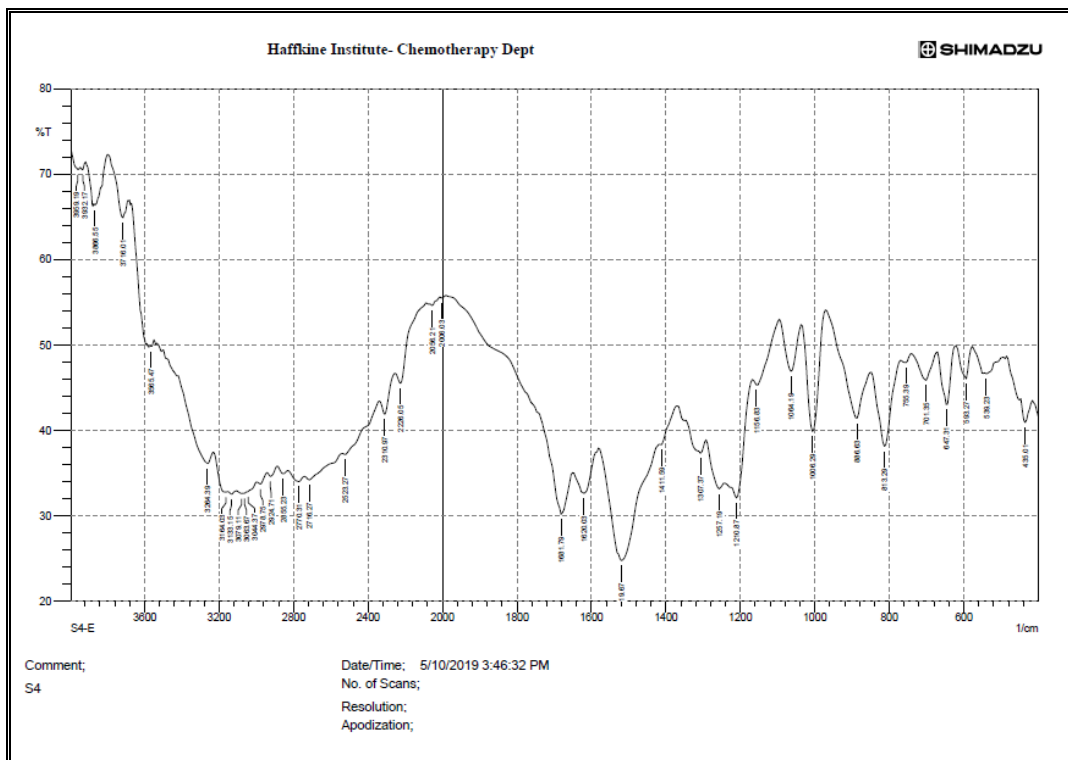
Chemical Test			
Preparation of Original Solution for Lassaingase Test: A small pellet of sodium together with a little of the substance is heated to red hot in an ignition tube. Plunged into about 10ml of distilled water in a china dish. The mixture is boiled well and filtered. The filtrate is used as OS			
1	2 drop of filtrate + few drops of freshly prepared FeSO ₄ sol., boil for a few minutes, cool and 0.5 cm ³ of dilute H ₂ SO ₄ .	Blue colour solution	Nitrogen present.
2	1 drop of filtrate + 1 drop of 2N acetic acid + 1 drop of lead acetate solution.	Black precipitate	Sulphur present
3	1cm ³ of compound+3cm ³ of 1:1 HCl, shake well. Cool add few drops of NaNO ₂ solution	White emulsion	-NH group present

• **Physical data of compound III-A.2(a-f)**

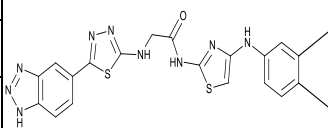


Comp.	R	Mol wt.	Colour	Solubility	MP (°C)	Yield (%)
III-D.5a	3,4-Dimethyl aniline	477. 565	Mustard	Methanol	250	63
III-D.5b	4-methoxy- 2-NO ₂ aniline	524. 535	Dark Brown	Methanol	255	74
III-D.5c	5-chloro-2- NO ₂ aniline	528. 95	Yellow	Methanol	265	68
III-D.5d	4,5- dichloro-2- NO ₂ aniline	563. 399	Yellow	Methanol	275	45
III-D.5e	2,4-Dinitro- aniline	539. 507	Dark Brown	Methanol	258	52
III-D.5f	4-(4- chloropheno xy)aniline	576. 052	Light Brown	Methanol	271	71

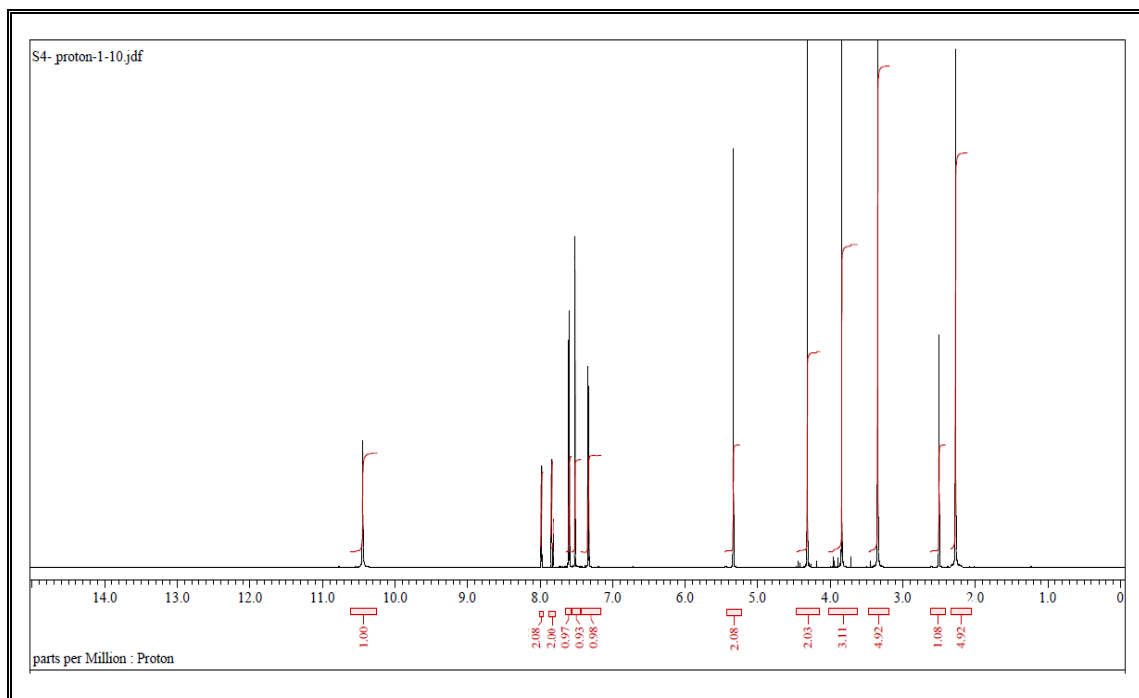
- Spectrum 30 : IR spectra of III-D.5a



- IR spectral data of III-D.5a

Functional Group	Peak (cm ⁻¹)	Standard IR Range(cm ⁻¹)	
N-H stretch	3264.39 cm ⁻¹	3500-3100	
N-H bend	1519.67 cm ⁻¹	1640-1550 cm ⁻¹	
-CONH	1620.03 cm ⁻¹	1680-1630 cm ⁻¹	

• Spectrum 31 : NMR SPECTRA OF III-D.5a



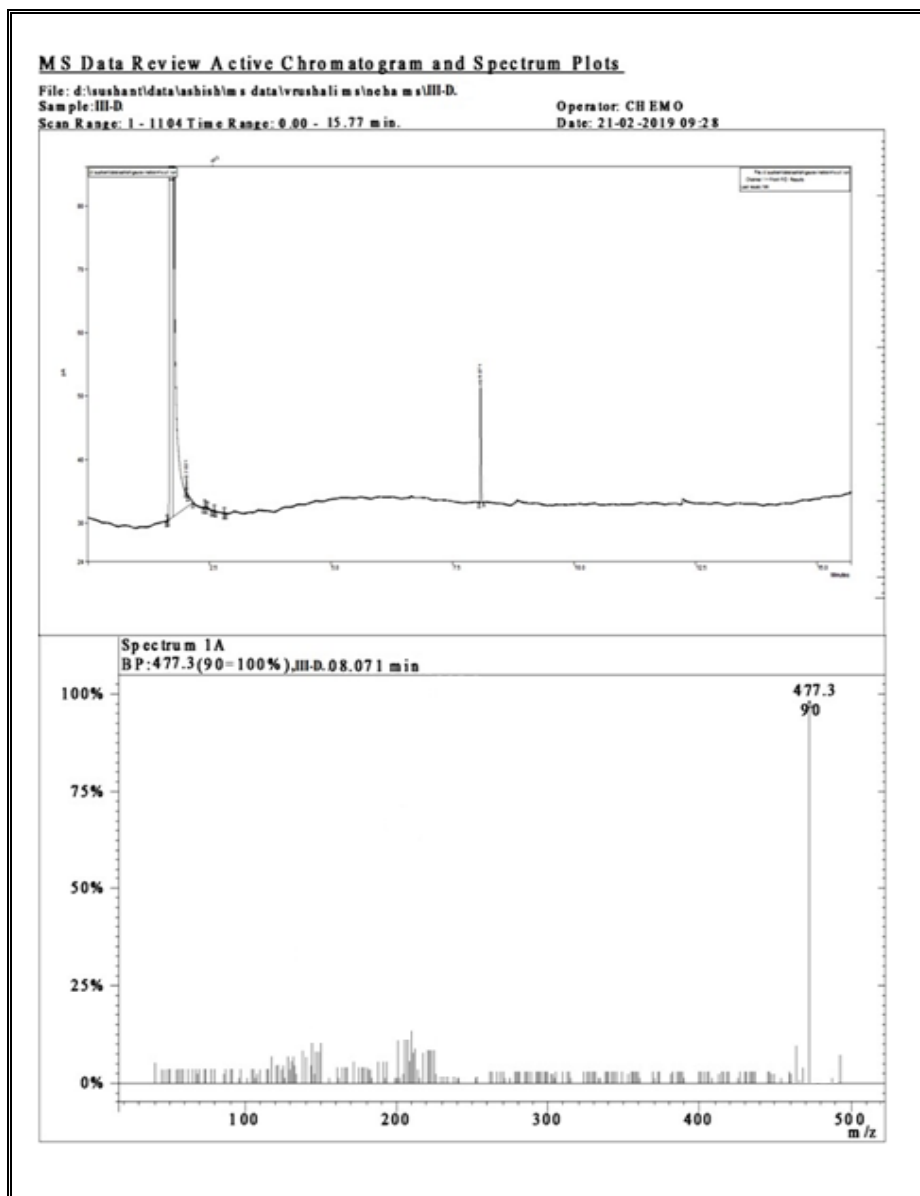
• NMR spectral interpretation of III-D.5a

Δ values	¹ H NMR signal
2.2	s, 3H, CH ₃
2.5	s, 1H, NH(benzotriazole)
3.8	s, 2H, CH ₂
4.3	s, 1H, NH (Ar. C-NH)
5.3	s, 1H, CH(thiazole)
10.5	s, 1H, NH(sec.amide)
7.2-8.0	m, 5H, Ar-H

• GCMS method for III-D.5a

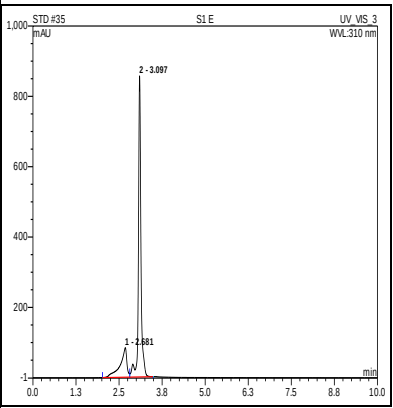
GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/min)	Temperature (°C)	Hold time (min)
	0	100	2
	10	120	3
	10	150	2
	10	180	4
	10	260	3
	20	300	3
Injection mode	Split mode; split ratio 90:1		
Injection temperature	300°C		
Injection volume	1.5 µL		
Carrier gas	Helium gas		
Detector temperature	300°C		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		
Analysis time			
Run time	8.071		

• Spectrum 32 : MASS SPECTRA OF III-D.5a



m/z value	Mass signal
477.3	Molecular ion peak
477.3	Base peak

• Chromatogram 14 : HPLC method for III D.5a

COLUMN	COSMOSIL (4.6 ID x 250mm)	
Mobile phase	Methanol- ACN(80:20)	
Pump mode	Isocratic	
Flow rate	1ml/min	
Injection volume	5µL	
Column temperature	28°C	
Wavelength	310	
Sample concentration	1mg/ml	
Retention Time	3.097	
Theoretical Plates	12258	
Peak Intensity	855.700	
Asymmetry (EP)	1.34	
Peak Width	0.113	

IN VITRO-ANTIOXIDANT ACTIVITY

Antioxidants in food participate as a health protecting factor. Free radicals are trapped by antioxidants. Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. Active antioxidative defense system is required to balance the production of free radicals. The oxidative damage created by free radical generation is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the aging process. In treatment of these diseases antioxidant therapy has gained an enormous importance.

We used two in vitro tests that make use of different principles of red-ox reactions: 1,1-diphenyl-2-picrylhydrazyl scavenging capacity test, i.e. DPPH radical scavenging capacity (or DPPH) assay^[98] and Ferric reducing antioxidant power (FRAP) assay^[99] :

DPPH radical scavenging activity

DPPH radical scavenging activity by antioxidants are very interesting, particularly in terms of prevention of the presumed deleterious effects of free radicals in the human body and in fats or other constituents of food stuffs. There is therefore, a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants. One such method that is currently popular is based on the use of the stable, free radical diphenylpicrylhydrazyl (DPPH). The purpose of this study has been to examine the percentage inhibition of DPPH, compared to the BHT as standard. When a solution of DPPH is mixed with that of the substance that can donate a hydrogen atom, then this gives rise to the reduced form along with the loss of its violet color.

Methodology

DPPH Method:

A solution of 0.002gm of DPPH in 100 ml of methanol was prepared and its absorbance was checked with U.V spectrophotometer.

To test this activity, compounds were first dissolved in the solvent methanol (which was used in all the cases in the present work to keep uniformity). To prepare 1 mM solution of the test compounds in methanol (stock solution) so as to make necessary dilution at 1, 5, 10, 50, 100 and 500 μ M to form the stock solutions of the compounds to be tested in the second phase.

Different medium

1. Control: DPPH

2. Standard: BHT (Butylated hydroxytoluene)

3. Solvent control: Methanol solvent was used to dissolve drugs and control.

DPPH Radical Scavenging Assay

Procedure:

The radical in the 1,1-diphenyl-1-picrylhydrazyl (DPPH) gives a strong absorption maximum at 517 nm and is purple in color. The absorbance of DPPH reduces, when the radical of the DPPH becomes paired with an electron or acceptance of the hydrogen radical from the antioxidant. 1 ml of various concentrations of the test compounds (1, 5, 10, 50, 100 and 500 μ M) in methanol were used to make homogeneous solution. After making desired concentration methanol was added in 1 ml solution of DPPH and it was dropped in each tube by using micro pipette. The room temperature was recorded and the test tube was incubated at 27°C for 30 min. to complete the reaction. The absorbance was read against blank at 517 nm. Decrease in the absorbance of DPPH solution indicated an increase in the radical scavenging activity. The DPPH solution without sample was used as control. BHT was used as standard. The experiments were carried out in duplicate.

$$\% \text{ scavenging} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100$$

The inhibitory effects of different concentrations of synthesized compounds on DPPH radical are presented in Tables.1. The antioxidant activity is expressed in terms of %

inhibition and IC_{50} (effective concentration for scavenging 50% of the initial DPPH) value (μM).

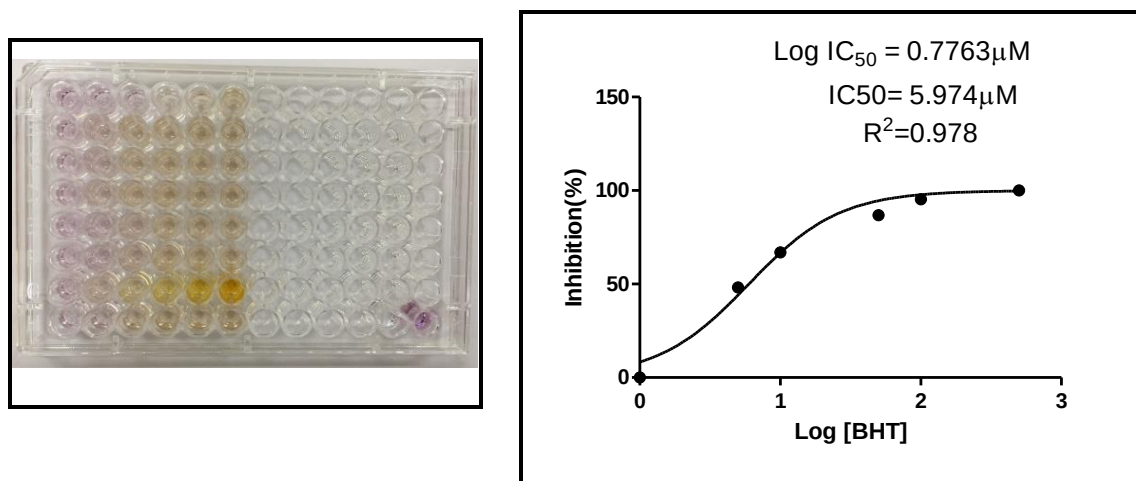


Fig 6.1: Determination of DPPH free radical scavenging activity with standard graph

Table No. 1: Anti-oxidant activity of synthesized compounds

Sr. NO.	Code	IC ₅₀ ($\mu M/ml$)
1	III-A.2a	-
2	III-A.2b	-
3	III-A.2c	-
4	III-A.2d	22.90
5	III-A.2e	-
6	III-A.2f	-
7	III-A.2g	-
8	III-A.2h	-
9	III-A.2i	-
10	III-A.2j	-
	STD	5.974

Sr. NO.	Code	IC ₅₀ ($\mu M/ml$)
1	III-B.3a	20.11
2	III-B.3b	26.86
3	III-B.3c	-
4	III-B.3d	-
5	III-B.3e	-
6	III-B.2f	
7	III-B.3g	32.32
8	III-B.3h	32.78
9	III-B.3i	32.14
	STD	5.974

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-C.4a	-
2	III-C.4b	16.89
3	III-C.4c	-
4	III-C.4d	-
5	III-C.4e	-
6	III-C.4f	8.237
	STD	5.974

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-D.5a	12.11
2	III-D.5b	-
3	III-D.5c	-
4	III-D.5d	-
5	III-D.5e	-
6	III-D.5f	6.281
	STD	5.974

Observation:-

- In-vitro antioxidant activity done by Radical Scavenging method, Standard drug (BHT) shows IC₅₀ at 5.974 μ M/ml.
- Compounds III-C.4f and III-D.5f present highest antioxidant activity having IC₅₀ value at 8.237 μ M/ml and 6.281 μ M/ml.
- Compound III-A.2d, III-B.3a, III-B.3b, III-B.3g, III-B.3h, III-B.3i, III-C.4b and III-D.5a exhibit moderate activity.
- The remaining compounds showed no scavenging effect.

Ferric reducing antioxidant power (FRAP) assay

Principle:

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant, Fe (III). Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine)₂ ie: Ferric (III) [colourless] to Ferrous (II) [blue] can be monitored by measuring absorbance at 593nm. The absorption readings are related to the reducing power of the electron- donating antioxidants present in the test compound. Hence the FRAP assay can rank the reducing power and the antioxidant potential of a wide range of test compounds.

Reagents:

300mM Acetate buffer

- Weigh out 3.10g sodium acetate trihydrate.
- Add 16mL glacial acetic acid.
- Adjust pH to 3.6.
- Make up to a final volume of 1L using distilled deionised H₂O.

40mM HCl

- Measure 3.4mL concentrated HCl.
- Make up to a final volume of 1L using distilled deionised H₂O.

The following reagents must be prepared fresh on the day of the assay:

10mM TPTZ

3.123g TPTZ/1L 40mM HCl.

20mM FeCl₃.6H₂O (make up fresh on the day)

5.406g/L using distilled deionised H₂O.

FRAP reagent

Standard curve: 10:1:1 (acetate buffer: TPTZ: H₂O)

Samples: 10:1:1 (acetate buffer: TPTZ: FeCl₃.6H₂O)

Standard

Method: 0.001M FeSO₄.7 H₂O

- Weigh 2.78 FeSO₄.7H₂O.

- Make up to a final volume of 1L using distilled deionised H₂O.
- Make a 1/10 dilution to obtain a 0.001M concentration

PROCEDURE

STANDARD CURVE

1. Add following reagents into tubes (add standard last):
2. For blank: 2ml FRAP reagent + 1ml H₂O
3. Into a cuvette add 100µL sample + 900µL distilled H₂O + 2mL FRAP reagent.
4. Invert tubes to mix.
5. Leave for 30min in the dark.
6. Transfer the mixture into 96 well plates.
7. Zero spectrophotometer at 593 nm using blank.
8. Read at 593 nm.

	1	2	3	4	5	6	7	8
H ₂ O(µl)	1000	985	970	940	880	820	760	700
1mM Std(µl)	0	15	30	60	120	180	240	300
FRAP(mL)	2	2	2	2	2	2	2	2
Std conc. (µM)	0	5	10	20	40	60	80	100

CALCULATIONS:

The FRAP equation is:

$$\text{FRAP value of sample (}\mu\text{M)} = \text{Abs (sample)} \times \text{FRAP value of Std (}\mu\text{M)} / \text{Abs (std)}$$

1. Standard curve Abs V's Fe²⁺ Conc. (X) and obtain equation for best fit line.
2. The sample absorbance values that have been zeroed (i.e. Abs sample – Abs blank) to express FRAP in molarity.

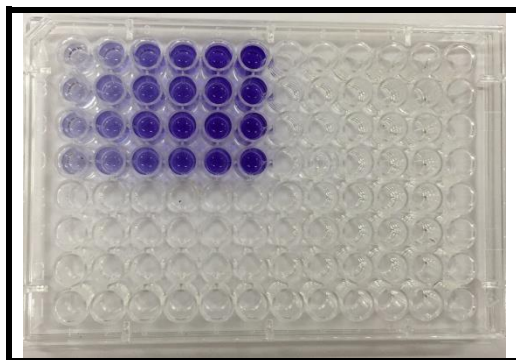


Fig 6.2: Determination of Ferric reducing antioxidant activity

Ascorbic Acid

Stock (10mM): Weigh 0.0176g ascorbic acid and make up to a final volume of 10mL using methanol.

1. Dilute to 5mM by mixing 500 μ l of water and 500 μ l of stock ascorbic acid.
2. Dilute to 250M by mixing 50 μ L ascorbic acid (5mM) and 950 μ L water.

Note. FRAP value of Ascorbic acid is 2.

Table No. 2: Ferric reducing antioxidant activity of synthesized compounds

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-A.2a	-
2	III-A.2b	-
3	III-A.2c	-
4	III-A.2d	38.07
5	III-A.2e	-
6	III-A.2f	-
7	III-A.2g	-
8	III-A.2h	-
9	III-A.2i	-
10	III-A.2j	-

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-B.3a	48.66
2	III-B.3b	33.96
3	III-B.3c	-
4	III-B.3d	-
5	III-B.3e	-
6	III-B.3f	-
7	III-B.3g	48.80
8	III-B.3h	37.81
9	III-B.3i	-

Sr. NO.	Code	IC ₅₀ (μ g/ml)
1	III-C.4a	-
2	III-C.4b	37.45
3	III-C.4c	-
4	III-C.4d	-
5	III-C.4e	-
6	III-C.4f	41.35

Sr. NO.	Code	IC ₅₀ (μ g/ml)
1	III-D.5a	38.07
2	III-D.5b	-
3	III-D.5c	-
4	III-D.5d	-
5	III-D.5e	-
6	III-D.5f	38.46

Observation:

- The compounds III-A.2d, III-B.3a, III-B.3g, III-B.3h III-C.4b, III-C.4f, III-D.5a and III-D.5f exhibit FRAP value of 38.07, 48.66, 48.80, 37.81, 37.45, 41.35, 38.07 and 38.46 μ M/ml.
- The compound III-B.3b showed the lowest FRAP value *i.e*, 33.96 μ M/ml.
- The remaining compounds showed no effect.

IN VITRO-ANTI-INFLAMMATORY ACTIVITY

Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even though most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are search for natural anti-inflammatory drugs without any adverse effects.

The anti-inflammatory activities of various compounds are assessed by in vitro HRBC membrane stabilization method. Blood are collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever's solution (Dextrose 2%, Sodium citrate 0.8%, Citric acid 0.05%, Sodium chloride 0.42% and Distilled water 100 ml) and centrifuged with Isosaline. To 1 ml of HRBC suspension equal volume of test drug in different concentrations are added. All the assay mixtures are incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution is estimated by using spectrophotometer at 560nm. The percentage of haemolysis was calculated.

Principle

The lysosomol enzymes produced during the process of inflammation produces a variety of disorders. The extra cellular activity of these enzymes is said to be related to the acute of chronic inflammation. The non-steroidal drug (DFS in case) act either by inhibiting these lysomol enzymes or by stabilizing the lysosomol membrane (HRBC is similar to the lysosomol membrane).

In this case, the standard used was Diclofenac sodium (DFS) which acts by stabilizing the membrane. The hypo saline used causes the heamolysis of RBC, as the concentration of hypo saline increases this causes the membrane components to leak out. This is then measured spectrophotometrically at 560nm. Thus increases concentration of hypo saline,

increases the O.D (as increase concentration of hypo saline increases the extent of heamolysis).

DFS stabilizes the membrane, thereby reducing the heamolysis .Thus with the increase in the components are prevented from leaking, Thus as the concentration of DFS increases, the O.D decreases thereby decreasing the effect of the hypo tonicity caused by hypo saline.^[100]

$$\% \text{ Haemolysis} = (\text{O.D. of Test Sample} / \text{O.D. of Control}) \times 100$$

The ability of the test compound to stabilize the membrane or to prevent haemolysis is compared with the standard.

Procedure

The Human Red Blood Cell (HRBC) membrane stabilization method:

1. Fresh whole human blood (5mL) are collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting.
2. The tubes are centrifuged at 3000 rpm for 10 min and are washed three times with equal volume of normal saline. The volume of the blood is measured and reconstituted as 10% v/v suspension with normal saline.
3. The reaction mixture consists of 1.0mL of test sample of different concentrations in normal saline and 0.5mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 30 mins.
4. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560 nm.

The percentage of HRBC heamolysis and membrane stabilization or protection was calculated by using the following formula.

Protocol

Step 1- RBC solution

- Alsever's solution (3ml) + Blood (3ml).
- Centrifuge at 3000 rpm for 10 min.
- Discard the supernatant and wash the pellet with iso-saline thrice.
- Dilute 1ml of pellet with 9 ml of iso-saline i.e. 10% v/v.

Step-2- Standard preparation

Concentration	DFS (μl)	Isosaline (μl)	Total volume (μl)
1mM	200	1800	2000
100μM	200	1800	2000
10μM	200	1800	2000
1μM	200	1800	2000
100nM	200	1800	2000
10nM	200	1800	2000
1nM	200	1800	2000

Step-3-Raction set up

Sr.no	Compounds (μl)	RBC(μl)	Incubate at 37° C for 1hour and then add 1ml distilled water to make it hypo saline, incubate 30 min more.
1	50	450	
2	50	450	
3	50	450	
4	50	450	
5	50	450	
6	50	450	
7	50	450	
8	50	450	
9	50	450	
10	50	450	

- Centrifuge at 3000rpm for 20 min
- Measure the O.D. of the supernatant at 560nm

Alsever's solution (50 mL):

- Dextrose - 1gm
- Sodium citrate - 0.4gm
- Citric acid – 0.025gm
- Sodium chloride - 0.21gm

Dissolve in distilled water to make up the volume up to 50mL.

Phosphate buffer (anhydrous):

A. 0.2 M of NaH_2PO_4

Dissolve 4.792gm of NaH_2PO_4 in 200mL of distilled water.

B. 0.2 M of Na_2HPO_4

Dissolve 5.678gm of Na_2HPO_4 in 200mL of distilled water.

C. Mix 32ml of 'A' with 168ml of 'B'.

D. Take 150ml of 'C' adjust the pH to 7.5 and make up the volume to 200mL using distilled water

Hypo saline:

Dissolve 0.9gm of NaCl in 250mL of distilled water.

Iso saline:

Dissolve 2.125gm of NaCl in 250mL of distilled water

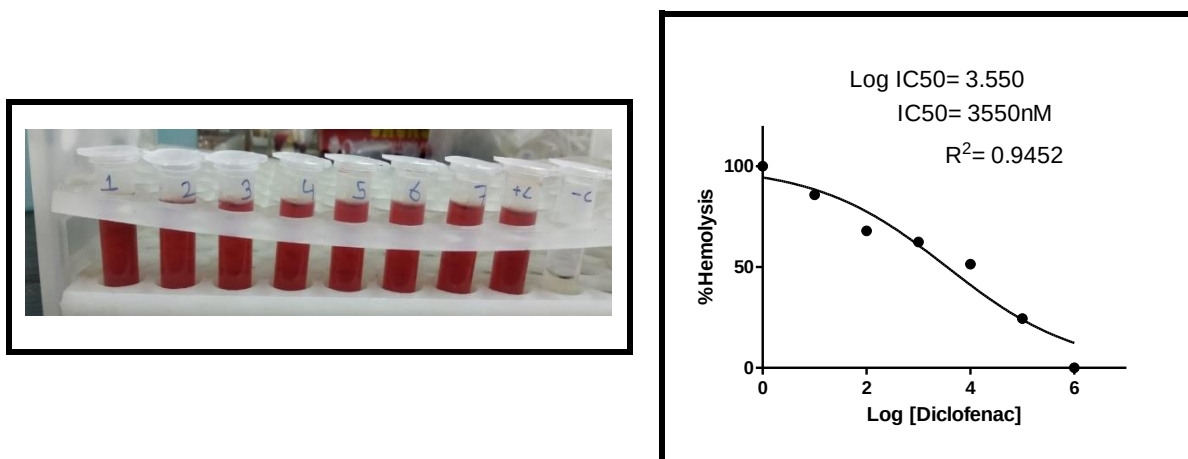


Fig 6.3: In-vitro anti-inflammatory activity of Diclofenac (STD)

Results:

Table No.3: Anti-inflammatory activity of synthesized compounds

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-A.2a	-
2	III-A.2b	-
3	III-A.2c	-
4	III-A.2d	7.198
5	III-A.2e	41.19
6	III-A.2f	-
7	III-A.2g	0.26
8	III-A.2h	-
9	III-A.2i	9.25
10	III-A.2j	3.87
	STD	3.55

Sr. NO.	Code	IC ₅₀ (μ M/ml)
1	III-B.3a	1.05
2	III-B.3b	-
3	III-B.3c	0.93
4	III-B.3d	-
5	III-B.3e	-
6	III-B.3f	0.34
7	III-B.3g	2.09
8	III-B.3h	-
9	III-B.3i	1.83
	STD	3.55

Sr. NO.	Code	IC ₅₀ (μg/ml)
1	III-C.4a	0.88
2	III-C.4b	-
3	III-C.4c	2.49
4	III-C.4d	-
5	III-C.4e	2.68
6	III-C.4f	-
	STD	3.55

Sr. NO.	Code	IC ₅₀ (μg/ml)
1	III-D.5a	-
2	III-D.5b	22.21
3	III-D.5c	-
4	III-D.5d	1.14
5	III-D.5e	0.73
6	III-D.5f	1.11
	STD	3.55

Observation:

- Anti-inflammatory activity done by Human Red blood Cell (HRBC) membrane stabilization method, standard drug shows IC₅₀ at 3.55μM/ml.
- Compounds III-A.2g, III-B.3c, III-B.3f, III-C.4a and III-D.5e showed very good activity IC₅₀ at 0.26, 0.93, 0.34, 0.88 and 0.88 μM/ml.
- Compounds III-A.2j, III-B.3a, III-B.3g, III-B.3i, III-C.4c, III-C.4e, III-D.5d and III-D.5f showed significant activity IC₅₀ at 3.38, 1.05, 2.09, 1.83, 2.49, 2.68, 1.14 and 1.11 μM/ml.
- Compounds III-A.2d, III-A.2e, III-A.2i and III-D.5b showed good to moderate activity.
- The remaining compounds showed no effect.

ANTIMICROBIAL ACTIVITY

The development in chemotherapy of synthetic drugs totally depends upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern for future clinical studies and set the pattern of future drug evaluation.

Taking this into consideration the above fact, in our present work, different heterocyclic derivatives, derived from different biological important moieties, were prepared and were screened for their *in vitro* antimicrobial activity against gram positive and gram negative bacteria viz.

- *E. coli* (ATCC 25922)
- *P. aeruginosa* (Fisher's immuno type IV)
- *S. aureus* (NCTC 3750)
- *E. faecalis* (ATCC 29212)
- *Candida albicans* (ATCC 10231)

Escherichia coli

- Characteristic

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod shaped, and are about 2.0 micrometers (μm) long and 0.25- μm in diameter, with a cell volume of 0.6–0.7 μm^3 . *E.coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Optimal growth of *E. coli* occurs at 37 °C but some laboratory strains can multiply at temperatures of up to 49 °C. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs

- Diseases

Abdominal pain - typically, the first symptom is severe abdominal cramping that comes on suddenly.

Diarrhea - a few hours after the sudden abdominal pain, the patient typically has watery diarrhea. A day later there may be bright red bloody stools, caused by sores in the intestines.

Vomiting - note that many patients who become ill may not vomit.

- Source of infection

Ingesting contaminated water - even though tap water contains chlorine and has undergone ozone or ultraviolet treatment. Ingesting contaminated food - examples include ground beef, unpasteurized milk, or fresh vegetables.

Pseudomonas aeruginosa

- Characteristic

Pseudomonas aeruginosa are shaped, aerobic gram with one or more polar flagella, providing motility. They are non-spore forming showing positive catalase test and positive oxidase test., the absence of gas formation from glucose, glucose is oxidized in oxidation/fermentation test using Hugh and Leifson O/F test, beta hemolytic (on blood agar), indole negative, methyl red negative, Voges-Proskauer test negative, and citrate positive. Other characteristics that tend to be associated with *Pseudomonas* species include secretion of pyoverdine, a fluorescent yellow-green siderophore.

- Diseases

- ◇ Endocarditic: Fever, murmur, and positive blood culture findings; peripheral stigmata such as Roth spots, Janeway lesions, Osler nodes, splinter hemorrhages, and splenomegaly
- ◇ Pneumonia: Rales, rhonchi, fever, cyanosis, retractions, and hypoxia; occasionally shock; with cystic fibrosis, clubbing, increased anteroposterior (AP) diameter, and malnutrition
- ◇ Eye infections: Lid edema, conjunctival erythema and chemosis, and

severe mucopurulent discharge Bacteremia: Fever, tachypnea, and tachycardia; hypotension and shock; jaundice

- Source of infection

Serious *Pseudomonas* infections usually occur in people in the hospital and/or with weakened immune systems. Infections of the blood, pneumonia, and infections following surgery can lead to severe illness and death in these people. However, healthy people can also develop mild illnesses with *Pseudomonas aeruginosa*, especially after exposure to water. Ear infections, especially in children, and more generalized skin rashes may occur after exposure to inadequately chlorinated hot tubs or swimming pools

Staphylococcus aureus

- Characteristic

Staphylococcus aureus is a gram-positive, round shaped bacterium that is a member of Firmicutes, and it is usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.

- Source of infection

S. aureus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, scalded skin syndrome and abscess, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and sepsis. It is still one of the five most common causes of hospital acquired infections and is often cause of wound infection following surgery.

- Diseases

While *S. aureus* usually act as a commensal bacterium,

asymptomatically colonizing about 30% of the human population, it can sometimes cause disease. *S. aureus* is one of the most common causes of Bacteremia and infective endocarditis. Additionally, it can cause various skin and soft tissue infections.

Enterococcus faecalis

- Characteristic

Enterococcus faecalis is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and mammals. *E. faecalis* has been frequently found in reinfected, root canal-treated teeth in prevalence values ranging from 30% to 90% cases. They resist bile salts, detergents, heavy metals, ethanol, azide and desiccation. They can grow in the range of 10 to 45°C and survive at temperatures of 60°C for 30 min.

- Source of infection

Infection commonly caused by enterococci includes urinary tract infection, endocarditis, bacteremia, catheter-related infections, wound infections, and intra-abdominal and pelvic infections.

- Diseases

Enterococci can cause a variety of infections. It can cause urinary tract infection, intra-abdominal, pelvic and soft tissue infections, Bacteremia and endocarditis.

Candida albicans

Candida is a genus of yeasts and is currently the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they

can invade and cause disease. *Candida albicans* is currently the most commonly isolated species, and can cause infections (candidacies or thrush) in humans and other animals.

- Diseases

Candidacies: In adults, oral yeast infections become more common with increased age. Adults also can have yeast infections around dentures, in skin folds under the breast and lower abdomen, nail beds, and beneath other skin folds. Most of these candidal infections are superficial and clear up easily with treatment. Infections of the nail beds often require prolonged therapy. Yeast infections that return may be a sign of more serious diseases such as diabetes, leukemia, or AIDS.

- Source of infection

Candidal infections commonly occur in warm moist body areas, such as underarms. Usually your skin effectively blocks yeast, but any breakdown or cuts in the skin may allow this organism to penetrate.

Materials and methods:

- Sample preparations:

5mg of synthesized drugs sample dissolved in 1ml of Dimethyl Sulphoxide (DMSO) to give stock solution 5mg/ml. Aliquot of 100µl of sample from the stock used for antimicrobial activity.

- Test microorganism:

The compounds were tested against *E. coli* (ATCC 25922), *P. aeruginosa* (Fisher's immuno type IV), *S. aureus* (NCTC 3750), *E. faecalis* (ATCC 29212), and *Candida albicans* (ATCC 10231). Ciprofloxacin and Fluconazol used as standard /positive control.

➤ Microbial inoculums:

A 24hr microbial culture grown in the bacteriological Mueller-Hinton Broth (MHB) at 37°C and in the fungal Sabouraud Dextrose Broth (SDB) at 30°C were adjusted at 2×10^6 colony forming units (CFU mL⁻¹) and 2×10^5 spore mL⁻¹, respectively.

➤ Antimicrobial activity screening tests ^[101]:

The antimicrobial activity of the test organisms to the synthesized drugs was screened by using the agar-well diffusion method. An inoculums suspension was swabbed uniformly to solidified 20 mL Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi and the inoculums was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Aliquot of 100 µL from stock was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37 C for 24 h. The resulting inhibition zones were measured in millimeters (mm). The same procedure was followed for the fungus *C. albicans* but incubated at 30 C. These studies were performed in triplicate.

Results:

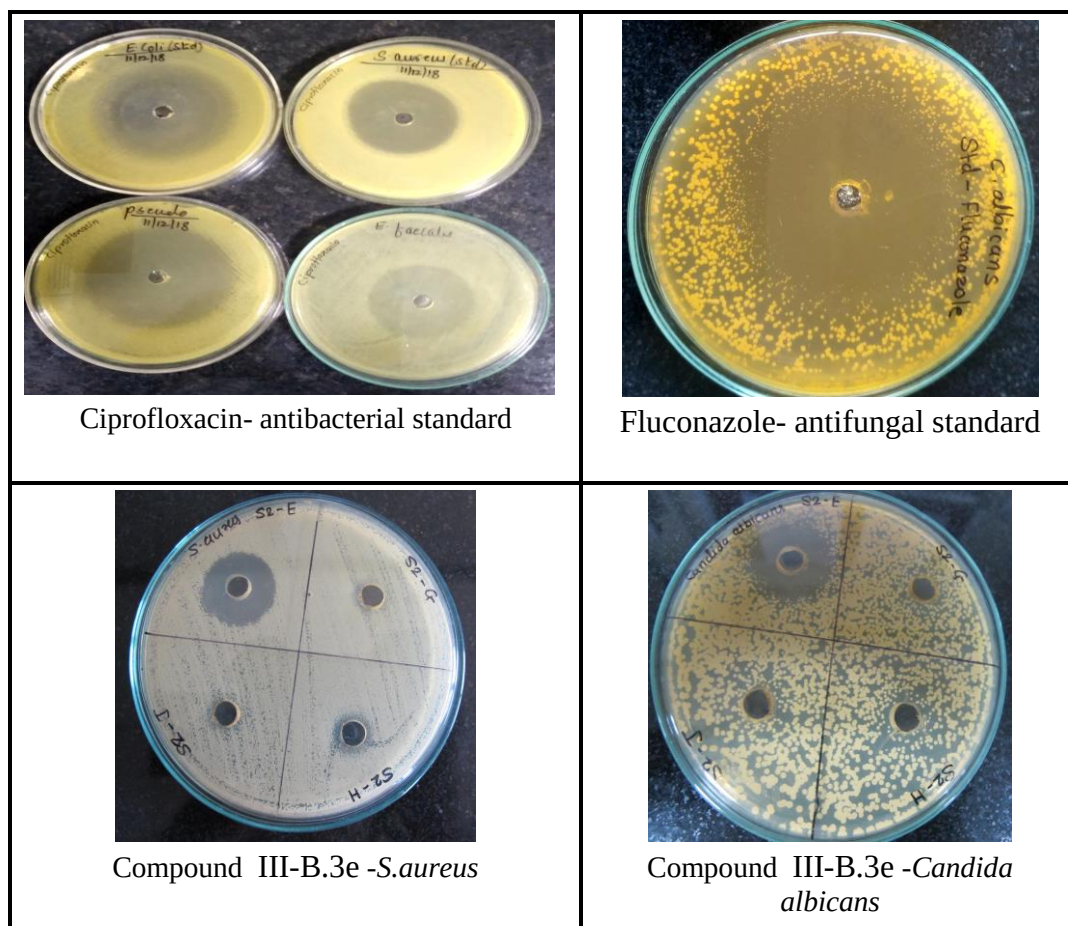


Fig 6.4: Agar plate showing susceptibility of organism through zone of inhibition against compound III-B.3e, ciprofloxacin and Fluconazole control.

Table no. 4: Anti-microbial activity of the synthesized compounds

Sr. no.	Compound code	Zone of Inhibition				
		Bacterial strains				Fungal strain
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>Candida albicans</i>
1	III-A.2a	-	-	-	-	-
2	III-A.2b	-	-	-	-	-
3	III-A.2c	13mm	-	-	-	-
4	III-A.2d	13mm	-	-	-	-

5	III-A.2e	12mm	-	-	-	-
6	III-A.2f	12mm	-	-	-	-
7	III-A.2g	-	-	-	-	-
8	III-A.2h	-	-	-	13mm	-
9	III-A.2i	-	-	-	-	-
10	III-A.2j	-	-	-	-	-
11	III-B.3a	-	-	-	-	-
12	III-B.3b	-	2mm	-	-	-
13	III-B.3c	-	-	-	-	-
14	III-B.3d	-	-	-	-	-
15	III-B.3e	-	2mm	21mm	10mm	18mm
16	III-B.3f	-	-	-	10mm	-
17	III-B.3g	-	2mm	-	10mm	-
18	III-B.3h	-	-	-	-	-
19	III-B.3i	-	-	-	10mm	-
20	III-C.4a	-	2mm	10mm	12mm	
21	III-C.4b	-	2mm	-	12mm	-
22	III-C.4c	-	2mm	-	12mm	-
23	III-C.4d	-	2mm	10mm	14mm	-
24	III-C.4e	-	-	12mm	12mm	-
25	III-C.4f	-	-	14mm	16mm	-
26	III-D.5a	10mm	2mm	-		-
27	III-D.5b	13mm	6mm	12mm	12mm	-
28	III-D.5c	10mm	2mm	-	-	-
29	III-D.5d	-	2mm	13mm	10mm	-
30	III-D.5e	12mm	3mm	14mm	10mm	-
31	III-D.5f	12mm	-	12mm	12mm	-
	Ciprofloxacin	50	50	40	40	-
	Fluconazol	-	-	-	-	52

- ✓ Ciprofloxacin used as standard against bacterial stains possessed significant antibacterial activity.

- ✓ Fluconazol used as standard against fungal strain showed zone of inhibition 52mm.

All compounds showed good to moderate potency against all pathogenic stains shown in the above table

- In series I compounds III-A.2c, III-A.2d, III-A.2e, III-A.2f showed moderate activity against *E. coli* while compound III-A.2h also showed moderate activity against *E. faecalis*.
- In series 2 compound III-B.3e showed good activity against *S. aureus* and *Candida albicans* zone of inhibition 21mm and 18 mm while rest of the compound in the series 2 showed moderate activity against bacterial and fungal stains.
- In series 3 all compound showed remarkable activity against *P.aeruginosa*, *S. aureus* and *E. faecalis*.
- In series compound 4 III-D.5a to III-D.5f showed remarkable activity all reference bacterial stains.

RESULT AND DISCUSSION

The structure of new compounds prepared during present investigation has been authentically established by their IR, mass spectroscopy and ^1H NMR. These compounds were evaluated for its antioxidant, anti-inflammatory and antimicrobial activity.

1. Discussion for compound III-A.2 (a-j)

- ✓ This series includes two step of reaction in which step 1 includes cyclization reaction by using acid catalyst formation of 1, 3, 4-thiadiazole (Intermediate).
- ✓ III A.1 thus formed. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at (3359,3281) cm^{-1} due to N-H stretch, 1515 cm^{-1} due to N-H bend, 1613 cm^{-1} due to C=N,. The mass spectrum of compound III A.1 showed molecular ion peak (M^+) at 218.2. This clearly indicated the formation of III A.1.
- ✓ The second step involves formation of Schiff base product from III A.1 using acid catalyst. The formation of final compound III A.2a has been indicated by preliminary test and its IR spectrum. The IR spectrum showed peak at 1688.72 cm^{-1} due to C=N, 1164.08 cm^{-1} due to C-O. The ^1NMR spectrum of this compound has been showed its characteristic peak at δ 2.5 (s, 1H, NH-benzotriazole), 5.2 (s, 2H, CH_2), 9.8 (s, 1H, CH (aldehyde), 7.0-8.2 (m, 11H, Ar-H). The mass spectrum of compound III A.2a. showed molecular ion peak (M^+) at 412.2. This clearly indicated the formation of III A.2a.
- ✓ The purity of compound III A.2a. was checked by HPLC and GC.
- ✓ Compound III A.2g in this series showed very good anti-inflammatory activity.

2. Discussion for compound III-B.3 (a-i)

- ✓ This series includes two step of reaction in which step 1 includes cyclization reaction by using acid catalyst formation of 1, 3, 4-thiadiazole (Intermediate).
- ✓ III B.1 thus formed. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at (3359,3281) cm^{-1} due to N-H stretch, 1515 cm^{-1} due to N-H bend, 1613 cm^{-1} due to C=N. The mass spectrum of compound III B.1 showed molecular ion peak (M^+) at 218.2. This clearly indicated the formation of III B.1.
- ✓ The second step involves formation Chloroacetamide from substituted benzothiazole and chloroacetyl chloride. III B.2a thus formed. . The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3372.58 cm^{-1} due to N-H stretch, 1561.52 cm^{-1} due to N-H bend, 1690.65 cm^{-1} due to -CONH, 676.09 cm^{-1} due to C-Cl. The mass spectrum of compound III B.1 showed molecular ion peak (M^+) at 227.3. This clearly indicated the formation of III B.2a.
- ✓ The third step involves condensation of III B.1 and III B.2a using base to form final derivative III B.3a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3164.26 cm^{-1} due to N-H stretch, 1520.91 cm^{-1} due to N-H bend, 1648.21 cm^{-1} due to -CONH. The ^1NMR spectrum of this compound has been showed its characteristic peak at δ 2.5 (s, 1H, NH-benzotriazole), 3.9 (s, 2H, CH_2), 12.7 (s, 1H, NH (sec.amide), 7.2-7.8 (m, 7H, Ar-H). The mass spectrum of compound III B.3a showed molecular ion peak (M^+) at 408.2. This clearly indicated the formation of III B.3a.
- ✓ The purity of compound III B.3a. was checked by HPLC and GC.
- ✓ In this series compound III B.3a, III B.3c, III B.3f, III B.3i and III B.3g showed excellent anti-inflammatory activity.

- ✓ Compound III B.3e showed best antimicrobial results against *s.aureus* and *candida albicans*.

3. Discussion for compound III-C.4 (a-f)

- ✓ This series includes four step of reaction in which step 1 includes cyclization reaction by using acid catalyst formation of 1, 3, 4-thiadiazole (Intermediate).
- ✓ III C.1 thus formed. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at (3359,3281) cm^{-1} due to N-H stretch, 1515 cm^{-1} due to N-H bend, 1613 cm^{-1} due to C=N. The mass spectrum of compound III C.1 showed molecular ion peak (M^+) at 218.2. This clearly indicated the formation of III C.1.
- ✓ The second step involves 5-mercapto-2-amino-1, 3, 4-thiadiazole reacts with substituted benzyl chloride using base to form compound III C.2a. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at 3270.35 cm^{-1} due to N-H stretch, 1505.48 cm^{-1} due to N-H bend, 1221.95 cm^{-1} due to C-F. The mass spectrum of compound III C.2a showed molecular ion peak (M^+) at 240.2. This clearly indicated the formation of III C.2a.
- ✓ The third step involves compound III C.2a reacts with chloroacetyl chloride using base as a triethylamine to form compound III C.3a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3171.98 cm^{-1} due to N-H stretch, 1588.42 cm^{-1} due to N-H bend, 1696.43 cm^{-1} due to -CONH, 691.52 cm^{-1} due to C-Cl, 1229.66 cm^{-1} due to C-F. The mass spectrum of compound III C.3a showed molecular ion peak (M^+) at 317.3. This clearly indicated the formation of III C.3a.
- ✓ The step four involves condensation of III C.3a and III C.1 using base to form final derivatives III C.4a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at

3285.79 cm^{-1} due to N-H stretch, 1509.35 cm^{-1} due to N-H bend, 1605.79 cm^{-1} due to -CONH, 1229.66 cm^{-1} C-F. The ^1NMR spectrum of this compound has been showed its characteristic peak at δ 2.5 (s, 1H, NH-benzotriazole), 3.7 (s, 2H, CH_2), 4.4 (s, 2H, CH_2), 13.0 (s, 1H, NH-sec.amide), 7.2-7.8 (m, 7H, Ar-H). The mass spectrum of compound III C.4a showed molecular ion peak (M^+) at 499.1. This clearly indicated the formation of III C.4a.

- ✓ The purity of compound III C.4a. was checked by HPLC and GC.
- ✓ In this series compound III-C.4f showed good antioxidant activity IC_{50} at 8.237 $\mu\text{M}/\text{ml}$.
- ✓ Compound III C.4a, III C.4c, III C.4e showed significant anti-inflammatory activity IC_{50} at 0.88, 2.49 and 2.68 $\mu\text{M}/\text{ml}$.

4. Discussion for compound III-D.5 (a-f)

- ✓ This series includes five step of reaction in which step 1 includes cyclization reaction by using acid catalyst formation of 1, 3, 4-thiadiazole (Intermediate).
- ✓ III D.1 thus formed. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at (3359,3281) cm^{-1} due to N-H stretch, 1515 cm^{-1} due to N-H bend, 1613 cm^{-1} due to $\text{C}=\text{N}$. The mass spectrum of compound III D.1 showed molecular ion peak (M^+) at 218.2. This clearly indicated the formation of III D.1.
- ✓ The step 2nd involves substituted aniline reacts with chloroacetyl chloride in presence of triethylamine to form III D.2a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3293.49 cm^{-1} due to N-H stretch, 1545.99 cm^{-1} due to N-H bend, 1665.57 cm^{-1} due to -CONH, 689.59 cm^{-1} due to C-Cl. The mass spectrum of compound III D.2a showed molecular ion peak (M^+) at 197.1. This clearly indicated the formation of III D.2a.

- ✓ The 3rd involves compound III D.3a synthesized through cyclization which involve reaction of compound III D.2a with Thiourea. III D.3a thus formed. The compound has been indicated by Lassaingase test and nitrous acid test and its IR spectrum and showed its characteristic peak at 3355.22 cm^{-1} due to N-H stretch, 1576.85 cm^{-1} due to N-H bend, 1702.22 cm^{-1} due to C=N. The mass spectrum of compound III D.3a showed molecular ion peak (M^+) at 218.2. This clearly indicated the formation of III D.3a.
- ✓ The fourth step involves III D.3a reacts with chloroacetyl chloride to form compound III D.4a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3166.19 cm^{-1} due to N-H stretch, 1576.85 cm^{-1} due to N-H bend, 1621.21 cm^{-1} due to –CONH, 652.94 cm^{-1} due to C-Cl. The mass spectrum of compound III D.4a showed molecular ion peak (M^+) at 295.2. This clearly indicated the formation of III D.4a.
- ✓ The step five involves compound III D.5a synthesized through condensation reaction which involves reaction of compound III D.1 with compound III D.4a. The compound has been indicated by Lassaingase test and its IR spectrum and showed its characteristic peak at 3264.39 cm^{-1} due to N-H stretch, 1519.67 cm^{-1} due to N-H bend, 1620.03 cm^{-1} due to –CONH. The ^1NMR spectrum of this compound has been showed its characteristic peak at δ 2.2 (s, 3H, CH_3), 2.5 (s, 1H, NH-benzotriazole), 3.8 (s, 2H, CH_2), 4.3 [s, 1H, NH (Ar. C-NH)], 5.3 [s, 1H, CH(thiazole)], 10.5 [s, 1H, NH(sec.amide)], 7.2-8.0 (m, 5H, Ar-H). The mass spectrum of compound III D.5a showed molecular ion peak (M^+) at 477.3 This clearly indicated the formation of III D.5a.
- ✓ The purity of compound III D.5a was checked by HPLC and GC.
- ✓ Compound III-D.5f showed significant antioxidant activity IC_{50} at 6.281 $\mu\text{M/ml}$.
- ✓ Compounds III-D.5d, III-D.5 and III-D.5f showed excellent anti-inflammatory activity IC_{50} at 1.14, 0.73 and 1.11 $\mu\text{M/ml}$.

CONCLUSION

- ❖ The purpose of the present work was to synthesize, characterize and evaluate the biological activity of thiadiazole and benzothiazole derivatives. The synthesized were characterized by melting point, TLC, FT-IR, ¹H NMR, GC-MS.
- ❖ The compounds were studied for antioxidant activity. In which compound III-C.4f and compound III-D.5f has shown better result for DPPH assay. In FRAP assay some compounds shows moderate activity.
- ❖ All synthesized compounds were screened for anti-inflammatory activity. In which compound III-A.2g, III-B.3f, III-C.4a and III-D.5e has shown best results compared to standard.
- ❖ The compounds were screened for antimicrobial activity, among all the compound, compound III-B.3e showed best activity against bacterial and fungal strain.

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APPENDIX

Instruments and equipments:

NAME OF INSTRUMENTS	COMPANY NAME	MODEL NO.
GCMS	AGILENT TECHNOLOGIES	7890 GC and 220 Ion Trap MS
HPLC	THERMO SCIENTIFIC	ULTIMATE 3000 UHPLC (Dionex) System
IR	SHIMADZU	IRAffninity-1
UV	JASCO	JASCO V-500
UV Cabinet	EXPO	UV CABINATE C-19
Digital Balance	KERN	ABT-320-4M
Melting Point	CAMPBELL ELECTRONICS	Pmb-2 THERMONIK
Magnetic stirrer	REMI	1 MLH Magnetic Stirrer

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF MASTER OF SCIENCE (SCIENCE)
IN THE FACULTY OF ORGANIC CHEMISTRY

TITLE OF THE PROPOSAL : Synthesis and Biological activities of
Thiadiazole and Benzothiazole Derivatives.

NAME OF THE CANDIDATE : Shinde Ashish Vijay Meena.

**QUALIFICATION OF THE
CANDIDATE** : B.Sc (Chemistry)

**NAME AND DESIGNATION OF
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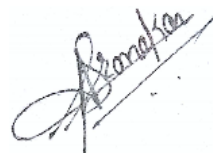
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Signature of Research Guide

Introduction

Chemistry is not merely a science of making observations in order to better understand nature. Chemistry, as the science of matter and its transformation, plays a central role in bridging between physics, material sciences and life sciences. Our science is creative and productive, generating substances and materials of very high value from almost nothing. In view of its significance, chemical synthesis demands the highest level of scientific/technological creativity and insight to explore its limitless possibilities. Chemical synthesis must pursue the goal of practical elegance; it must be logically elegant and at the same time technologically practical. We must manufacture useful compounds in an economical, energy-efficient, resource-preserving, and environmentally benign way ^[1]. To maintain our current standard of living and to improve quality of life, society has come to depend on the products of chemical industry. The last century has been highly productive in pharmaceutical development, water treatment, material science, polymers, agriculture pesticides and fungicides, detergents, petroleum additives and so forth.

The role of organic chemistry in the pharmaceutical industry endures to be one of the main bases in the drug discovery process. However, the defined nature of that role is enduring a visible change, not only because of the new synthetic methods and technologies now available to the synthetic and medicinal chemist, but also due to several key areas, mainly in drug metabolism and chemical toxicology, as chemists deal with the ever more rapid turnaround of testing data that impacts their day-to-day decisions.

Thiadiazole drugs were the first effective chemotherapeutic agents to be employed systematically for the prevention and cure of bacterial infection in human beings. The 1, 3, 4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents. The broad and potent activity of thiadiazole and their derivatives has established them as pharmacologically significant scaffolds ^[2].

Benzothiazole is a privileged bicyclic ring system. It contains a benzene ring fused to a thiazole ring. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities ^[3]. In addition; the benzothiazole ring is present in various marine or terrestrial

natural compounds, which have useful biological activities. Due to their importance in pharmaceutical utilities, the synthesis of various benzothiazole derivatives is of considerable interests.

Benzotriazole is extensively used as a synthetic auxiliary in organic chemistry, and is also a versatile ligand in coordination chemistry. Benzotriazole can be a good leaving group, acts as an ambient anion director, an electron donor, radical or carbanion precursor. It is easy to introduce benzotriazole into molecules by a variety of condensations, additions, benzotriazolyl-alkylation reactions. Benzotriazole derivatives exhibit a good degree of analgesic, anti-inflammatory, diuretic, antiviral and antihypertensive activities^[4].

It is the mission of pharmaceutical research companies to take the path from understanding a disease to bringing a safe and effective new treatment to patients. Scientists work to piece together the elementary causes of disease at the level of genes, proteins and cells. Out of this understanding emerge targets, which potential new drugs might be able to affect. Researchers work to confirm these targets, discover the right molecule (potential drug) to interact with the target chosen, test the new compound in the lab and clinic for safety and effectiveness and gain approval and get the new drug into the hands of doctors and patients.

We planned present objectives of the study will be below:-

1. Synthesis and development of newer synthetic method for title thiadiazole and benzothiazole derivatives.
2. Characterisation of newly synthesised compound by chromatographic and spectroscopic technique.
3. Evaluation of biological activities.

The thesis will consist of the following part:-

Part I :- INTRODUCTION

Part II :- REVIEW OF LITERATURE & PLAN OF WORK

Part III :- EXPERIMENTAL & CHARACTERIZATION**Part IV :- BIOLOGICAL EVALUATION****Part V :- RESULT, DISCUSSION & CONCLUSION****Part VI :- REFERENCES****Part I :- Introduction**

This part will give general Introduction about the brief details of Heterocyclic Moieties & their application in medicinal chemistry field, which is selected for the research work.

Part II :- Review of Literature & Plan of work

This part will cover salient aspects of the research project & highlights the aims & objectives in the synthesis work. It covers Chemistry of the main entities which is selected for the research with their probable mechanism.

Part III :- Experimental & Characterization

This part includes the synthesis work of the research projects with schematic representation of the series & their Chromatographic & spectroscopic characterization. This part will further sub-divided into following sections according to synthesis of titled series of compounds.

A) Synthetic studies**III-A] Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-1-(Substitutedphenyl)Methanimine****III-B] Synthesis of N-(substituted 1,3-benzothiazol-2-yl)-2-[5-(1H-benzotriazo5-yl)-1,3,4-thiadiazol-2-yl]amino}acetamide****IV-C] Synthesis of 2-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-N-[5-(substitutedbenzylsulfanyl)-1,3,4-thiadiazol-2-yl]acetamide**

III-D] Synthesis of N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-2-[4-(Substitutedphenylamino)-1,3-thiazol-2-yl]amino}acetamide

B) Physiochemical studies

1. Physical constant (Melting point/boiling point)
2. Chemical Analysis (functional group determination by chemical assay)
3. Thin layer chromatography (TLC)
4. FT-IR Spectrophotometer.
5. Gas chromatography and High performance liquid chromatography
6. Nuclear Magnetic Spectroscopic technique.
7. Mass Spectrophotometer

C) Biological Evaluation

1. Anti- Oxidant activity
2. Anti- Inflammatory activity
3. Anti- Microbial activity

I-A] Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]1(Substitutedphenyl) Methanimine

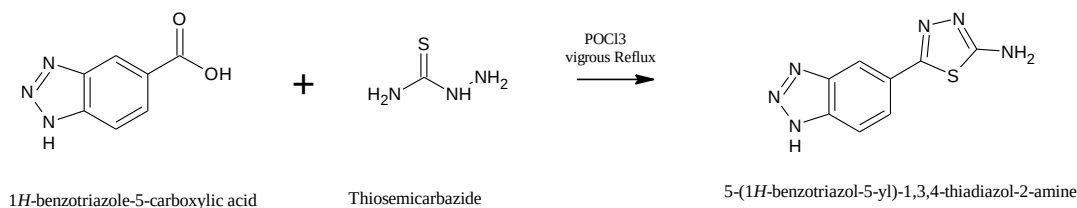
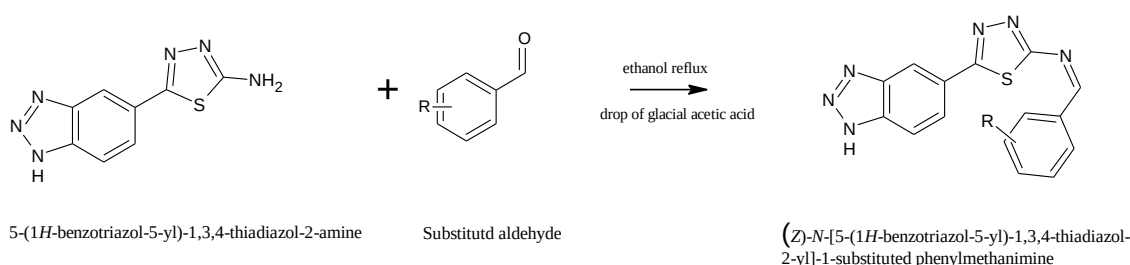
Schiff bases are the compounds carrying imine or azomethine ($-C=N-$) functional group. These are the condensation products of primary amines with carbonyl compounds and were first reported by Hugo Schiff^[5]. Schiff bases have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like antimicrobial^[6], antiparasitic^[7], anti-inflammatory^[8], anticancer^[9] etc. The Schiff bases known as azomethines dyes are used for dyeing acetate and synthetic fibers; they are also used in color photography to reduce the photosensitivity of photographic emulsions. The chemistry of polymer-based macromolecules including Schiff bases has been receiving significant attention as one of the promising immobilization alternatives for biopolymer biosensors in various industry areas.

Methodology**Step 1:- I-A.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine** ^[10]

The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1). The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Step 2:- I-A.2 Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]1(Substitutedphenyl)Methanimine ^[5]

The Intermediate (0.01 mol) was dissolved in ethanol (50 mL), substituted benzaldehyde (0.01 mol) and drops of conc. sulphuric acid was added and the reaction mixture was heated under reflux for 16 hr. The excess of solvent was distilled-off under reduced pressure, recrystallized from methanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Schematic representation: Series -1**Step-1****Step-2****II-B] Synthesis of N-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1H-benzotriazol-5-yl)-1,3,4- thiadiazol-2-yl] amino}acetamide**

Benzothiazole ring made from thiazole ring fused with benzene ring. Thiazole ring is a five-member ring consists of one nitrogen and one sulphur atom in the ring. Benzothiazole derivatives have been studied and found to have various chemical reactivity and biological activity. Benzothiazoles are bicyclic ring system. A number of 2-aminobenzothiazoles have been studied as central muscle relaxants and found to interfere with glutamate neurotransmission in biochemical, electrophysiological and behavioral experiments. It was found to be possessing pharmacological activities such as anti-viral, anti-bacterial, anti-microbial and fungicidal activities. ^[11] Benzothiazole nucleus containing molecules are also reported as anti-allergic, ^[12] anti-diabetic, ^[13] antitumor, ^[14] anti-inflammatory, anti-helminthic, and anti-HIV agents.

Amide derivatives were associated with broad spectrum of biological activities including antituberculosis, ^[15] anticonvulsant, ^[16] anti-inflammatory, ^[17] insecticidal,

antifungal, and Antitumor properties. Amide derivatives find their wide spectrum of antimicrobial activity. Amide derivatives also show anti-platelet activity. When amides are conjugates with other aliphatic, aromatic and heterocyclic ring produces various type of biological activity.

Amide derivatives are synthesized very easily by the reaction of substituted acid group (-COOH) with different substituted amines. A number of aromatic amides of aromatic and heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity. Generally, benzylamides were found to be more active than other amides.

Methodology

Step 1:- III-B.1 Synthesis of 5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine ^[10]

The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1).

The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

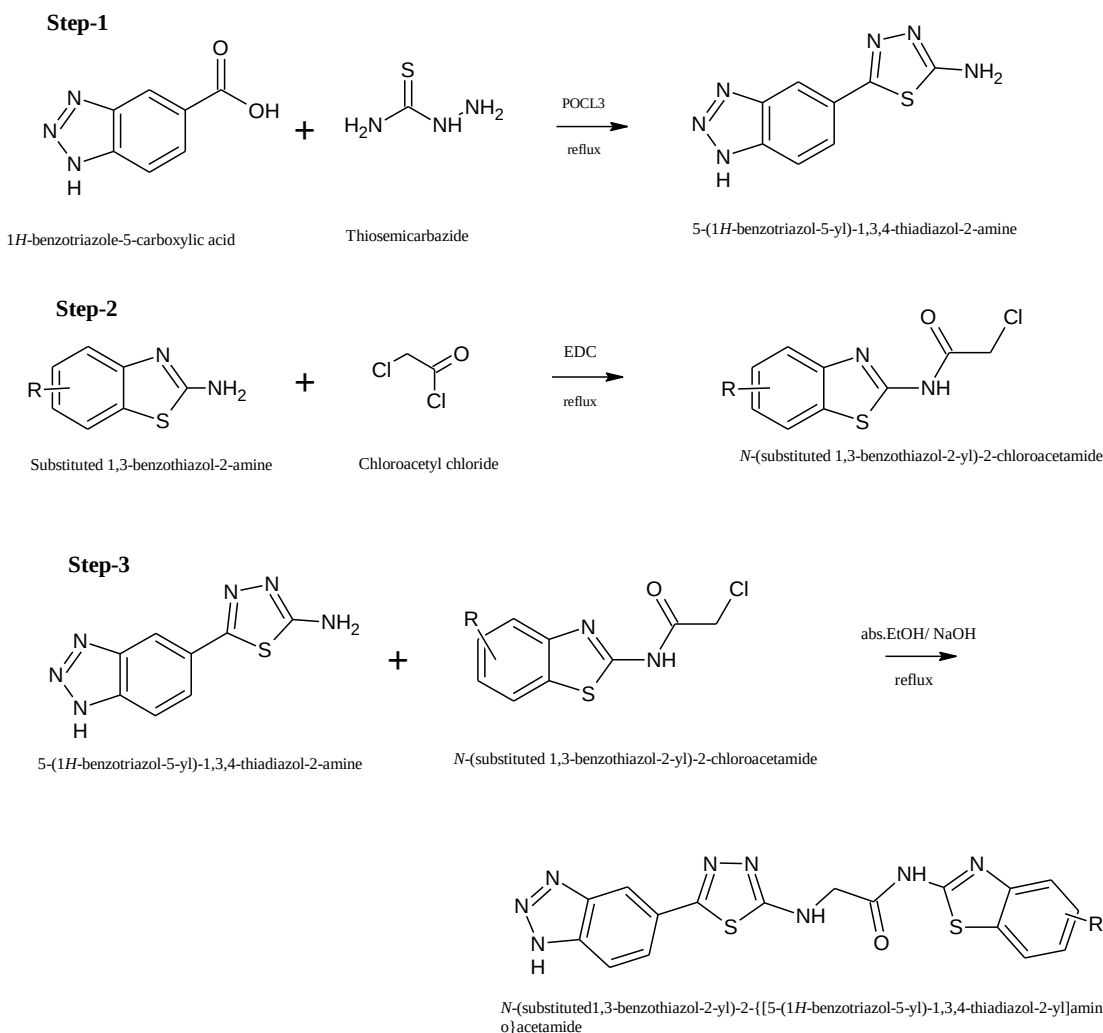
Step 2:- II-B.2 Synthesis of *N*-(1,3-benzothiazol-2-yl)-2-chloroacetamide ^[18]

To a solution of chloroacetyl chloride (0.01mol) in (10 ml) ethylene dichloride, substituted amino benzothiazole (0.01mol) was added with constant stirring. The reaction mixture was stirred for 5 hrs. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

Step 3:- II-B.3 Synthesis of *N*-(substituted 1,3-benzothiazol-2-yl)-2-[[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl] amino}acetamide ^[19]

Product of step 1 (0.03 mol) in absolute ethanol (30 ml), (0.12 gm) of sodium hydroxide and (0.03mole) substituted *N*-(1,3-benzothiazol-2-yl)-2-chloroacetamide were added, the mixture was refluxed for (4 hr). After cooling precipitate was collected by filtration, dried and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Schematic representation: Series -2



III-C] Synthesis of 2-{[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-N-[5-(substitutedbenzylsulfanyl)-1,3,4-thiadiazol-2-yl]acetamide

1,3,4-thiadiazoles are the most studied class among the other thiadiazole isomers and their 2,5- disubstituted derivatives have been widely discussed in terms of the interesting biological activities. In particular, such class as 5-amino-1,3,4-thiadiazole-2-thiol derivatives are known as antimicrobial ^[20], anticonvulsant ^[21], anti-depressant ^[22], anti-anxiety ^[23], anti-diabetic ^[24] agents. The therapeutic importance of 1,3,4-thiadiazole ring prompted us to design new molecules in which substituent's could be arranged in a pharmacophores pattern to display higher pharmacological activities. Thiadiazoles has attracted wide-spread attention of medicinal chemists due to their chemical potential and high pharmacological efficacy and low side effects.

Methodology**Step 1:- III -C.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine** ^[10]

The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1).

The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Step 2:- III -C.2 Synthesis of 5-(benzylsulfanyl)-1,3,4-thiadiazol-2-amine ^[25]

To a solution of 5-mercapto-2-amino-1,3,4-thiadiazole (0.003 moles) in absolute ethanol (30 ml), (1.12 gm) of potassium hydroxide and (0.03mole) substituted benzyl halides were added, the mixture was refluxed for (2 hr). After cooling precipitate was collected by filtration, dried and recrystallized from acetic acid. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Step 3:- III -C.2 Synthesis of N-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide ^[26]

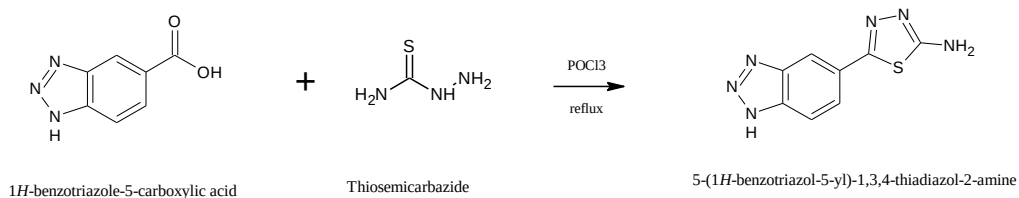
To a solution of chloroacetyl chloride (0.01mol) in (10 ml) ethylene dichloride, substituted step 2 product (0.01mol) was added with constant stirring. The reaction mixture was stirred for 5 hrs. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Step 4 :- III-C.3 Synthesis of 2-{[5-(1*H*-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-N-[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]acetamide ^[19]

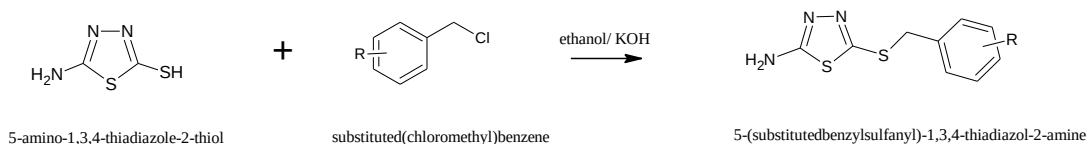
Product of step 1 (0.003 mol) in absolute ethanol (30 ml), (0.12 gm) of sodium hydroxide and (0.03mole) substituted step 3 product were added, the mixture was refluxed for (4 hr). After cooling precipitate was collected by filtration, dried and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Schematic representation: Series -3

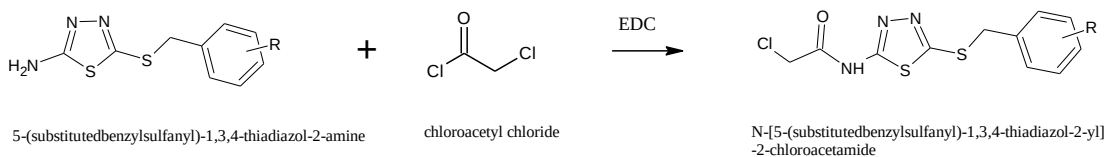
Step-1



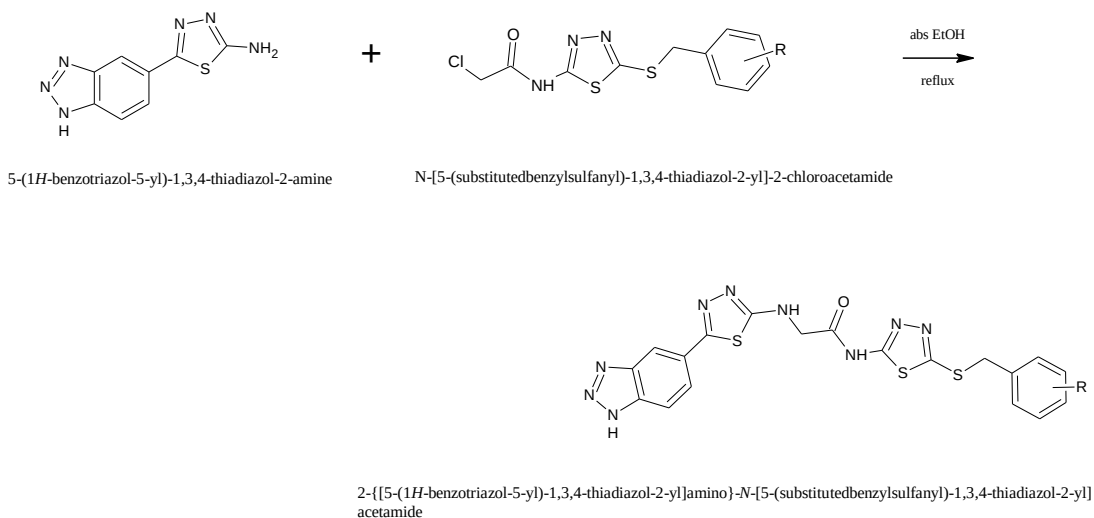
Step-2



Step-3



Step-4



IV-D] Synthesis of N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-2-{[4 (Substitutedphenylamino)-1,3-thiazol-2-yl]amino}acetamide

1,3,4-thiadiazoles have become an important class of heterocycles and a great interest of researches because of their broad types of biological activity. Thiadiazole is a 5-membered ring system containing hydrogen-binding domain, sulfur atom, and two-electron donor nitrogen system that exhibit a wide variety of biological activity. 1,3,4-Thiadiazole derivatives possessed a wide range of therapeutic activities like antimicrobial [27], antifungal [28], antimycobacterial, antileishmanial [29], analgesic, anti-inflammatory antidepressant and anticonvulsant [30]. Substitution of 1,3,4-thiadiazole ring with both amino and phenyl groups resulted in compounds with promising anticancer activity against several cell lines [31]. The broad and potent activity of thiadiazole and their derivatives has established them as pharmacologically significant scaffolds. In this study, an attempt has been made with recent research findings on this nucleus, to review the structural modifications on different thiadiazole derivatives for various pharmacological activities.

Methodology

Step 1:- IV-D.1 Synthesis of 5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-amine [10]

The mixture of benzotriazole-5-carboxylic acid (50mmol), thiosemicarbazide (50mmol) and POCl₃ (5ml) was refluxed for 2 hrs. Cool the reaction mixture to room temperature and cold water was added. The aqueous mixture was again refluxed for 4 hrs. The solid separated out was filtered, washed with cold water and recrystallized 1st step product from ethanol (intermediate 1).

The completion of reaction was monitored by running T.L.C. using solvent system: chloroform: methanol (9: 1) as mobile phase.

Step 2:- IV-D2 Synthesis of 2-chloro-N-phenylacetamide [32]

Aniline (0.01mole), chloroacetyl chloride (0.01) and triethylamine (0.01 mole) were dissolved in acetic acid (5ml) was kept for stirring for 3 hrs. The product was isolated as

precipitate after pouring reaction mixture to an ice-cold water. Precipitate was filtered, washed with cold water and dried. Recrystallized using ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

Step 3:- IV -D.3 Synthesis of N4-phenyl-1,3-thiazole-2,4-diamine ^[33]

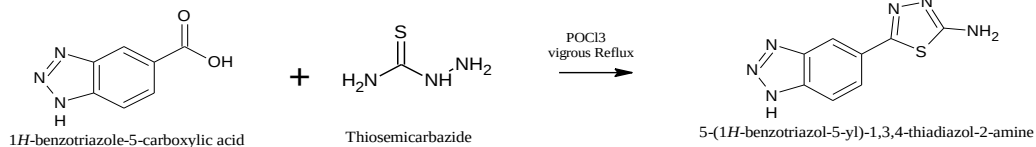
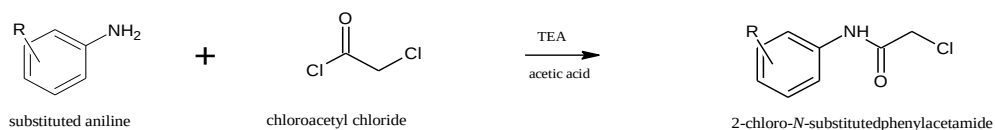
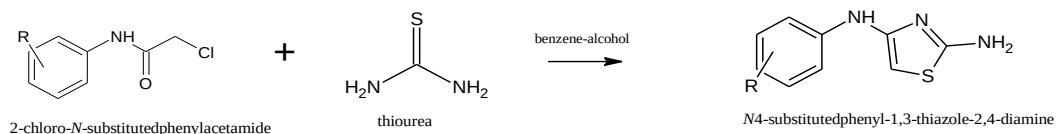
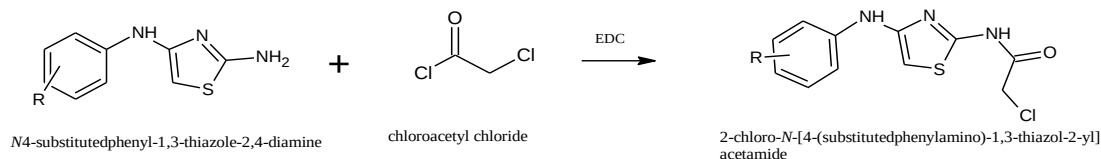
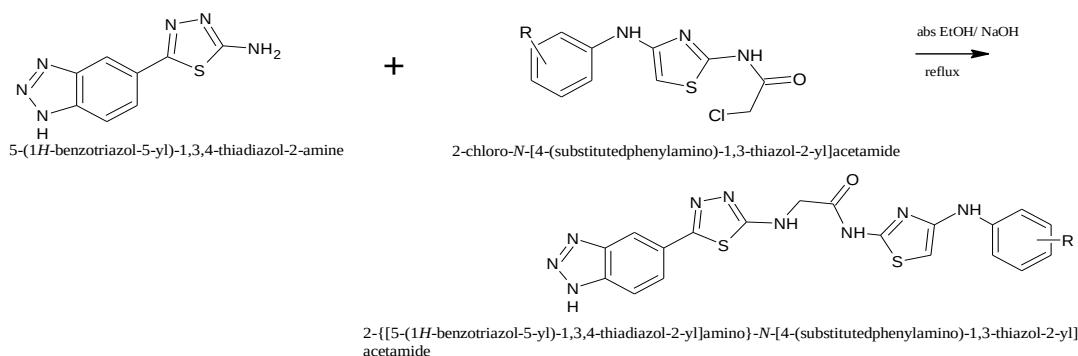
The step 2 product (0.01 moles) and thiourea (0.01 moles) were dissolved in ethanol, the mixture was refluxed for 10 -12 hours and poured in to ice cold water. The mass obtained was filtered and recrystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

Step 4:- IV-D.4 Synthesis of 2-chloro-N-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide ^[26]

The Step 3 product (0.01 moles) in 30 ml ethylene dichloride, chloroacetyl chloride (0.01moles) was added drop wise with constant stirring .The mixture was then stirred for 1-2 hours. The excess of solvent was distilled-off under reduced pressure, solid mass washed with ice cold water and crystallized from ethanol. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

Step 5:- IV -D.5 Synthesis of 2-{{[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]amino}-N-[4-(phenylamino)-1,3-thiazol-2-yl]acetamide ^[19]

Synthesis of title product is accomplished by condensation of step 1 and step 4 products using alcoholic NaOH. The reaction mixture was heated under refluxed for 10-12 hours. The precipitate formed was filtered, washed with water and recrystallized from ethanol to afford the final product. The completion of reaction was monitored by running T.L.C. using solvent system: hexane: ethyl acetate (8: 2) as mobile phase.

Schematic representation: Series -4**Step-1****Step-2****Step-3****Step-4****Step-5**

Part IV: - Biological Evaluation

This part covers the biological evaluation of synthesized compounds by suitable method.

The development in chemotherapy of synthetic drugs totally depends upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern for future clinical studies and set the pattern of future drug evaluation.

Taking this into consideration the above fact, in our present work, different heterocyclic derivatives, derived from different biologically important moieties, were prepared and were screened for following biological evaluations:

Anti-oxidant Screening ³⁴ :-

All the synthesized compounds were screened for antioxidant activity using the DPPH assay method. The DPPH assay method depended on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH, which showed maximum absorption at 517 nm was measured. About 2 ml of various concentrations of the synthesized compounds or standards were added to 2 ml of DPPH solution (0.1 mM, 2 ml). After 20 min of incubation at 37° in the dark, the absorbance was recorded at 517 nm. Butylated hydroxytoluene (BHT) was used as positive controls. The formula used for percent inhibition is as follows; %inhibition=(blank OD– sample OD/blank OD)×100.

Anti-inflammatory Screening ³⁵ :-

All synthesized test compounds were screened for anti-inflammatory activity using HRBC membrane stabilization method. Blood was collected (2ml) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3000 rpm .The packed cells were washed with isosaline solution and a 10% v/v suspension was prepared with normal saline. Different concentrations of synthesized derivatives (25µg/ml, 50µg/ml, 100µg/ml) Diclofenac sodium (25µg/ml,

50µg/ml, 100µg/ml) as standard and control (distilled water instead of hyposaline to produce 100% haemolysis) were separately mixed with 1ml of phosphate buffer, 2ml hyposaline solution and 0.5ml of 10% HRBC suspension was added to prepared reaction mixture. The assay mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The formula used for percentage of HRBC membrane stabilizations is as follows: % Membrane Stabilization= {(Absorbance of control-Absorbance of test)/Absorbance of control} × 100.

Anti Bacterial Screening³⁶ :-

The antibacterial activities of the synthesized compounds were evaluated in vitro at different concentrations. The compounds were tested against gram positive bacteria: *Staphylococcus aureus* ((NCTC-3750)) and gram negative bacteria: *Escherichia coli* ((ATCC-25923)). The inoculated sterilized nutrient agar media was poured into petri dishes and allowed to solidify. 6mm wells were made on the agar surface, into each of these wells, 30µl of the test compound with different concentrations /reference standard/control was added by using a micropipette. Ciprofloxacin was used as standard reference and DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37°C for 24 hours for bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in triplicate and the average values were tabulated.

Anti Fungal Screening³⁶ :-

All the synthesized compounds were evaluated, in vitro for antifungal activity, by using agar well diffusion method against fungal strains viz. *Candida albicans* (ATCC-10231). The plates were incubated at 28°C for 24 hrs and the zone of inhibition was measured in mm. Fluconazol was used as a standard reference and DMSO was used as a solvent (control), which did not possess any inhibition zone.

Part V: - Result, Discussion & Conclusion

This part will cover the part of the project was to synthesize, purify and characterize the new heterocyclic thiadiazole and benzothiazole derivatives as possible anti-microbial agents.

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