SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITIES AND CYTOTOXICITY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES CONTAINING 2-SUBSTITUTED BENZIMIDAZOLE

A DISSERTATION

SUBMITTED FOR

THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

BY

PRATEEK ARYAL

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INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

KATHMANDU, NEPAL

October, 2022

BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled "SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITIES AND CYTOTOXICITY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES CONTAINING 2-SUBSTITUTED BENZIMIDAZOLE", by Mr. Prateek Aryal, under the supervision of Associate Professor Dr. Bhushan Shakya, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, is hereby submitted for the partial fulfillment of the Masters of Science (M.Sc.) degree in Chemistry. This dissertation has not been submitted in any other university or institution previously for the award of a degree.

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ii

RECOMMENDATION LETTER

It is to recommend that this dissertation work entitled "**SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITIES AND CYTOTOXICITY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES CONTAINING 2-SUBSTITUTED BENZIMIDAZOLE**" has been carried out by Mr. Prateek Aryal as a partial fulfillment for the requirements of M.Sc. Degree in Chemistry under my supervision. To the best of my knowledge, this work has not been submitted elsewhere for any other degree.

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iii

DECLARATION

I, Prateek Aryal, hereby declare that the work presented herein is genuine work done originally by me under the supervision of Associate professor Dr. Bhushan Shakya and has not been published or submitted elsewhere for the requirement of a degree program. Any literature, data and works done by others and cited in this dissertation has been given due acknowledgement and listed in the reference section.

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LIST OF ABBREVIATIONS

AMQ	Amino methyl quinazolinone
ATCC	American type culture collection
DMSO	Dimethyl sulphoxide
DPPH	Diphenyl picryl hydrazyl hydrate
GABA	Gamma amino butyric acid
HCT-116	Human colorectal carcinoma cell line
HeLa	Human cervical cancer cell line
HepG2	Human hepatocellular carcinoma cell line
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
IC_{50}	Half maximal inhibitory concentration
FT-IR	Fourier transform-infrared
FCFF	Final compound with furfuraldehyde
FCPC	Final compound with *p*-chlorobenzaldehyde
LC_{50}	Lethality concentration to kill 50% of organisms
MCF-7	Human breast cancer cell line
MGC-803	Human gastric cancer cell line
MHA	Mueller hinton agar
Mia PaCa-2	Human pancreatic cancer cell line
MIC	Minimum inhibitory concentration
MMC	Minimum microcidal concentration
NCI	National cancer institute
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
OPD	*o*-phenylenediamine
PDB	Potato dextrose broth
Ppm	Parts per million
R*f*	Retention factor
RSA	Radical scavenging activity

SBPC	Schiff base with *p*-chlorobenzaldehyde
SBFF	Schiff base with furfuraldehyde
SF	Steroidogenic factor
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultraviolet
WHO	World health organization
XRD	X-ray diffraction
ZOI	Zone of inhibition

ABSTRACT

The development of a potent new drug with high biological activity is a challenge in drug design and is of strategic importance. Due to the increasing resistance of pathogens on the existing drugs there is always a need for the development of a potent drug with high biological activity. The pharmacological effects are seen in compounds with the presence of different moieties of pharmaceutical importance such as triazole, Schiff's base, benzimidazole as described in the chemical literature. By applying different synthetic reactions in a convergent pattern, two new compounds have been prepared with three pharmacophores *viz.* triazole, benzimidazole and Schiff base incorporated in a single compound. The structure of the prepared compounds was confirmed by different spectroscopic techniques like, FT-IR, UV-VIS, ^{1}H-NMR, and ^{13}C-NMR. The progress of the reaction was monitored by TLC and determination of melting point. All compounds exhibited moderate antibacterial activity against *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermidis* (ATCC 1228). Antioxidant activity was carried out by DPPH radical scavenging test and among the tested five compounds, the IC_{50} value of 4-amino-2-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione was found to be 32.364 μgmL^{-1} which is closer to the ascorbic acid sample that was found to be 28.546 μgmL^{-1}. All tested compounds were toxic against brine shrimp where 5-((1*H*-benzo[*d*]imidazol-2-yl)thio)-4-((4-chlorobenzylidene) amino)-4*H*-1,2,4-triazol-3-yl) (2-hydroxyphenyl) methanone was comparatively more toxic (LC_{50}= 26.827 μgmL^{-1})

Keywords: Triazole, Schiff's base, Benzimidazole, Biological activity.

TABLE OF CONTENTS

Page no.

LIST OF TABLES

LIST OF FIGURES

List of Schemes

Page no.

Chapter-1

1. INTRODUCTION

1.1 Heterocyclic chemistry

Heterocyclic compounds that contain nitrogen in their structure are used widely as pharmaceuticals and agrochemicals because they show promising biological activity. They have a variety of applications as herbicides, insecticides, adhesives, flavor and fragrances (Higashio *et al.*, 2004), pesticides (Pavlath, 1986) and other drugs like antitubercular multi drug resistant compounds (Fernandes *et al.*, 2017). Due to the vast area of the biological activity possessed by nitrogenous compounds they are of huge interest to the researchers around the world. The nitrogen containing compounds are also an important part of the natural products that have important pharmacological activity (Blair *et al.*, 2013). Pyridine, pyrroles, pyrans, imidazole, triazoles and benzimidazoles are some of the examples of the heterocyclic compounds containing nitrogen.

1.2 Triazoles and different activities of their derivatives

Triazoles are a class of the heterocyclic organic compounds with molecular formula $C_2H_3N_3$ that contain three nitrogen in their five-membered unsaturated ring with carbon and nitrogen at non-adjacent positions. Triazoles have two isomeric forms that vary on the placement of the nitrogen atom inside the heterocyclic ring *viz.* 1,2,3-triazoles or 1,2,4-triazoles. These structural isomers further have two tautomers as shown in figure 1 (Kaur *et al.*, 2016).

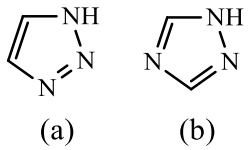


Figure 1.1. Structures of (a) 1,2,3-triazole and (b) 1,2,4- triazole

Figure 1.2. Tautomers of triazole (a) 1*H*-1,2,3-triazole, (b) 2*H*-1,2,3-triazole, (c) 1*H*-1,2,4-triazole and (d) 4*H*-1,2,4-triazole

The nitrogen containing heterocyclic compounds have been tested by many scientists due to their different biological activities, less toxicity and competent selectivity. Triazoles also

belong to these types of the heterocyclic nitrogenous compounds. Triazoles are known for their wide spectrum of biological activities. Different derivatives of triazoles have been reported as potential biologically active agents that act as antimicrobial, antihistaminic, anticonvulsant, antimycobacterial, analgesic, antiprotozoal, antimalarial agents (Kumar & Kavitha, 2013; Sharma *et al.*, 2012) and anticancer properties (Bekircan *et al*., 2006). Triazole nucleus has a prominent antifungal activity. Some examples out of many commercially marketed compounds exhibiting antifungal activity that contain triazole nucleus are Fluconazole, Voriconazole, Posaconazole, Intraconazole, etc. (Zonios *et al.*, 2008).

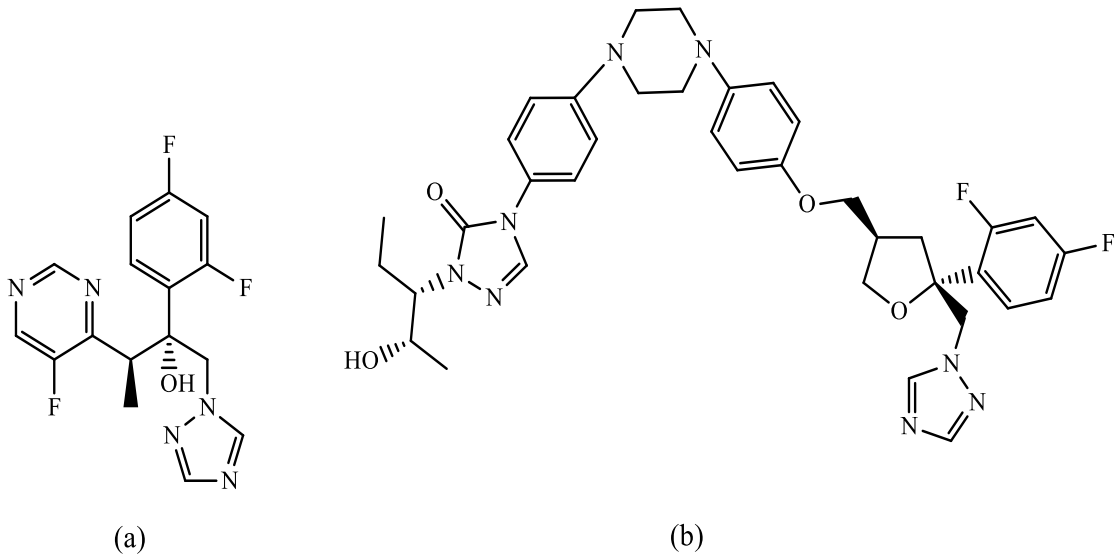


Figure 1.3. Antifungal triazoles: (a) Voriconazole and (b) Posaconazole

Triazoles derivatives are also studied to have antitumor and antiviral properties (Al-Soud *et al.*, 2004).

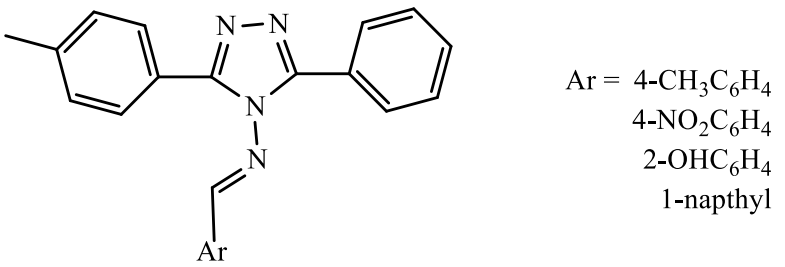


Figure 1.4. Triazole derivative with anticancer properties

Besides the applications as a bioactive agent, one another application of the triazole derivatives is that they can be used as electron transport layer in organic electroluminescent devices (Kido *et al.*, 1993). Some of the triazole derivatives have been effective in the

corrosion inhibition. Some studies show the use of triazoles as good corrosion inhibitors in acidic medium for mild steel (Guo *et al.*, 2014).

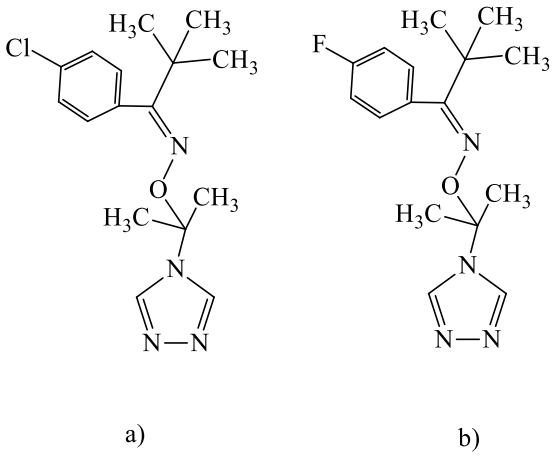


Fig 1.5: Triazole derivatives that are corrosion inhibitors

1.3 Schiff's base and its importance

Schiff's bases are the compounds produced by condensation of primary amine with carbonyl compound and contain an imine or azomethine functional group. They were first reported by Hugo Schiff. They are important group of compounds employed in various fields like analytical chemistry, biology and inorganic chemistry and is a versatile pharmacophore. Schiff's base of different compounds has wide scope of biological activities such as antimicrobial (Malladi *et al*., 2013), anticonvulsant (Bhat & Al-Omar, 2011), antidyslipidemic (Sashidhara *et al.*, 2009) and antioxidant (Yu *et al.*, 2015) properties.

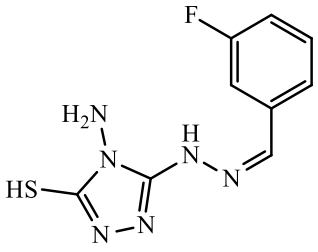


Figure 1.6. Schiff's base of triazole derivative having antioxidant properties

1.4 Benzimidazole and the biological activities of their derivatives

Benzimidazole is a compound containing a bicyclic ring system in which an imidazole ring is fused with benzene at 4 and 5 position. The numbering of atoms in benzimidazole ring is

done by indicating the imino function as number one. The benzimidazoles possess tautomeric forms when free imino hydrogen is present (Reddy, 2010).

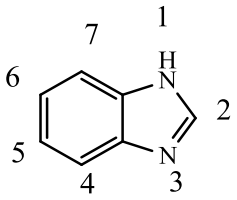
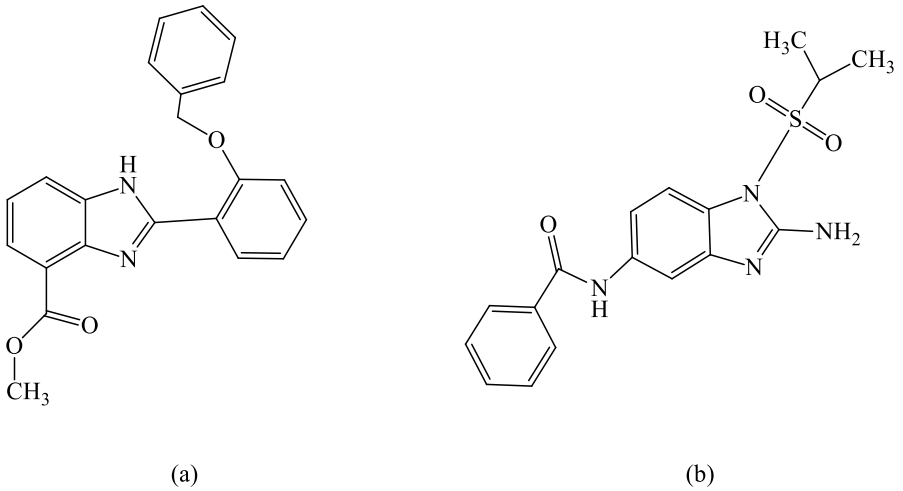


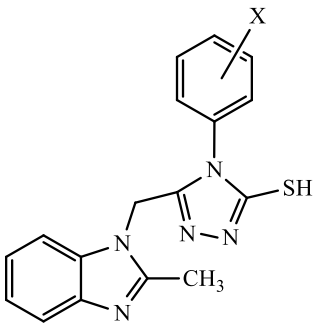
Figure 1.7. Structure of Benzimidazole



(a) (b)

Figure 1.8. Benzimidazole derivatives possessing anticancer(a) and antihepatitic(b) activity

Different derivatives of the benzimidazole have shown anticancer (Kumar et al., 2002), anti-amoebic (Sondhi *et al.*, 2002), antimalarial (Ndakala *et al.*, 2011), antiallergic (Nakano *et al.*, 2000), antihepatitic (Li *et al.*, 2007) and many other biological activities. A series of heterocyclic and phenyl substituted benzimidazoles containing triazoles have shown potent antimicrobial and antifungal properties (Ansari *et al.*, 2011).

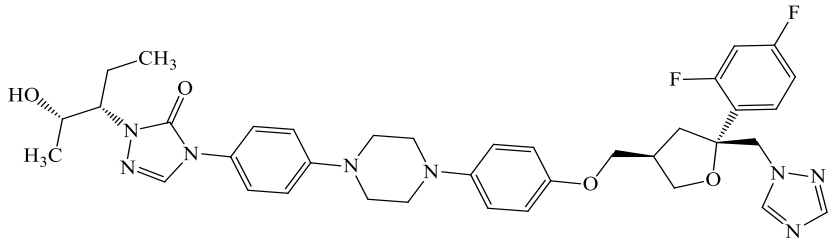


X = 2-CH_3, 2-CH_2CH_3, 2-$CH_2CH_2CH_3$, 2-OH, 4-OH, 2-O-CH_3, 4-OCH_3, 2-Cl, 4-NH_2

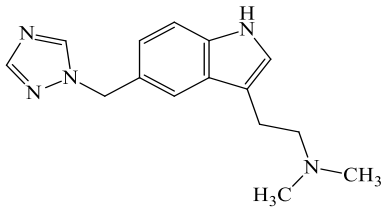
Figure 1.9. Benzimidazole derivatives with triazole nucleus possessing antimicrobial and antifungal activities

1.5 Some marketed drugs that contain 1,2,4-triazole ring

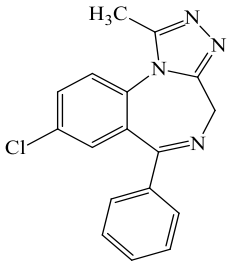
1,2,4-triazole moiety has been an integral part of the pharmacophores that are interesting drug candidates such as central nervous system stimulants, sedatives, anti-anxiety compounds, antivirals, antimicrobial agents, antitumor agents, and anti-inflammatories etc. Some of the marketed drugs containing the 1,2,4-triazole group are rizatriptan (anti-migraine agent), alprazolam (anti-convulsant drugs) and fluconazole, itraconazole and, posaconazole (figure 1.10) (all antimycotic agents) (Mohammed *et al.*, 2021). Ribivarin is also an important compound used as a broad-spectrum antiviral medication that is used for the treatment of HSV, HIV, hepatitis etc. among others (Mohammed *et al*, 2021).



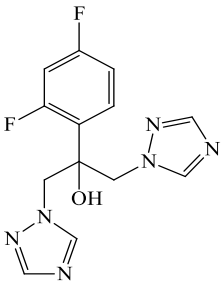
Posaconazole



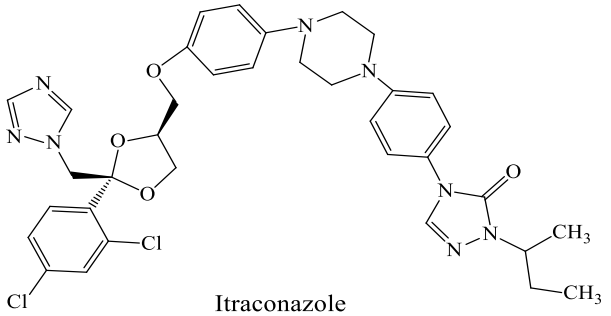
Rizatriptan



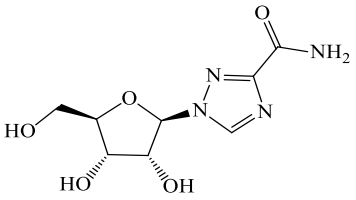
Alprazolam



Fluconazole



Itraconazole



Ribivarin

Figure 1.10: Marketed drugs with triazole ring

Chapter-2

2. OBJECTIVES OF THE STUDY

2.1 General objectives:

Synthesis of benzimidazole derivatives with Schiff's base of 1,2,4-triazole as potential antimicrobial and cytotoxic agents.

2.2 Specific objectives:

Synthesis of Schiff's bases of 1,2,4-triazole-5-thione.

Synthesis of 2-substituted benzimidazole derivative of the Schiff's base.

Characterization of the synthesized compounds by UV-VIS, FT-IR, ^{1}H-NMR and ^{13}C-NMR.

Screening of the synthesized compounds for antimicrobial activities against different strains.

Evaluation of cytotoxicity of the synthesized compounds.

Chapter-3

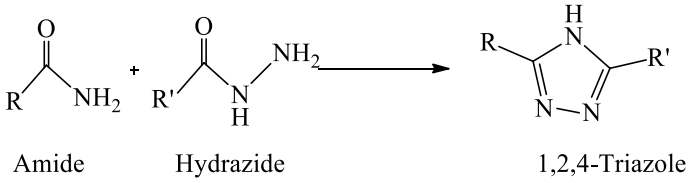
3. LITERATURE REVIEW

Researchers have studied about 1,2,4-triazoles a lot in the last decade and therefore the synthesis scheme of such 1,2,4-triazoles have been in large numbers. The importance of triazoles in the field of pharmaceutical and medicinal sciences can also be understood by their various synthesis schemes.

3.1 Synthesis of 1,2,4-triazole and their derivatives

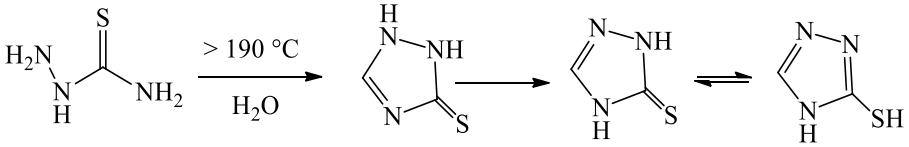
Numerous schemes are available for the synthesis of triazoles and their derivative compounds. They can be prepared by simple heating, cyclization, reflux, one-pot reaction, cyclo-condensation, ring closure, regioselective reaction, reduction cleavage, etc.

Pellizzari (1911) has obtained substituted 1,2,4-triazole by reacting an amide with a hydrazide.



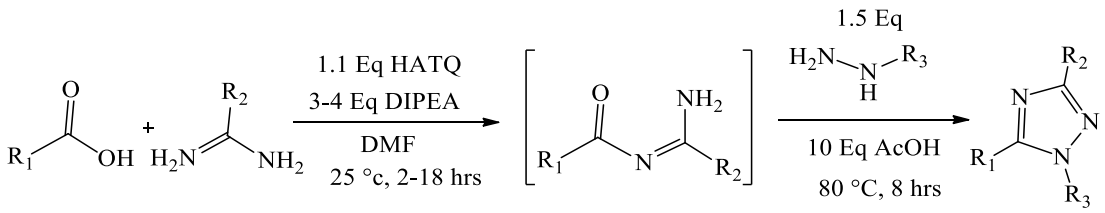
Scheme 3.1. Synthesis of 1,2,4-triazole derivatives *via* amide and hydrazide

Freund (1896) has been able to synthesize 1,2,4-triazole-3-thione by dry heating formyl thiosemicarbazide.



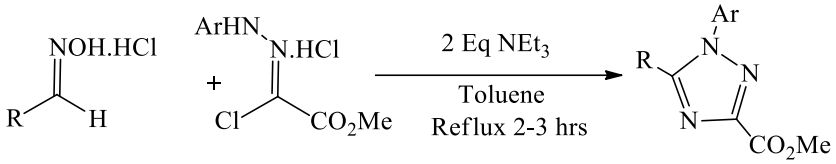
Scheme 3.2. Synthesis of the 1,2,4-triazole 3-thione by heating formyl thiosemicarbazide

Castanedo *et al.,* (2011) have synthesized 1,3,5-substituted 1,2,4-triazoles by highly regioselective one pot synthesis from carboxylic acids, primary amidines and monosubstituted hydrazines.



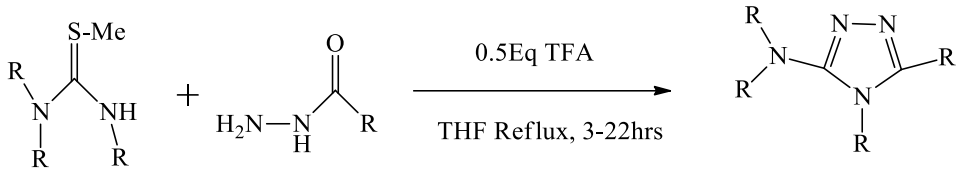
Scheme 3.3. Regioselective one pot synthesis of 1,2,4-triazole derivatives

1,3-dipolar cycloaddition of oximes with hydrazonoyl hydrochlorides in the presence of base triethylamine produced trisubstituted 1,2,4-triazoles in good yields (Wang *et al.*, 2011).



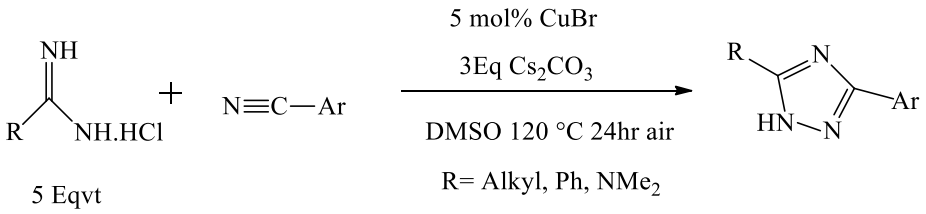
Scheme 3.4. Synthesis of 1,2,4-triazole derivatives *via* cycloaddition reaction

Batchelor *et al.*, (2008) prepared 3-*N*,*N*-dialkylamino-1,2,4-triazoles by reacting *S*-methylisothioureas and acyl hydrazides under relatively mild reaction conditions and with various functional groups.



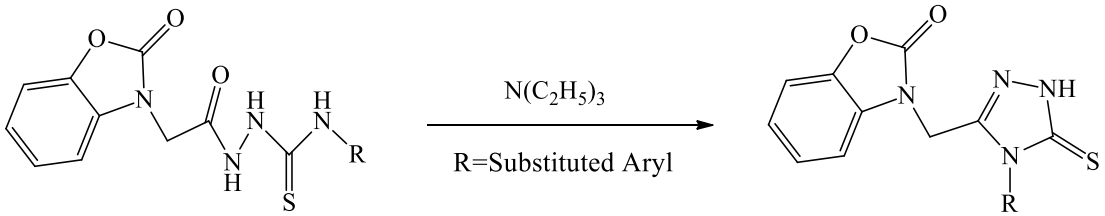
Scheme 3.5. Synthesis of 1,2,4-triazole derivatives from *S*-methylisothioureas

Ueda *et. al.*, (2003) synthesized the triazole nucleus from an oxidative coupling reaction between amidine hydrochloride and benzonitrile using CuBr as catalyst.



Scheme 3.6. Synthesis of 1,2,4-triazole derivatives by substituted amidine

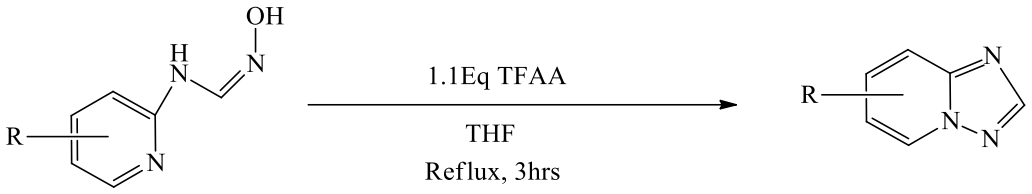
1,2,4-Triazoles have also been prepared by cyclization of thiosemicarbazides in presence of trimethylamine (Umut *et al.*, 2007).



Scheme 3.7. Synthesis of 1,2,4-triazole derivatives by cyclization of thiocarbazides

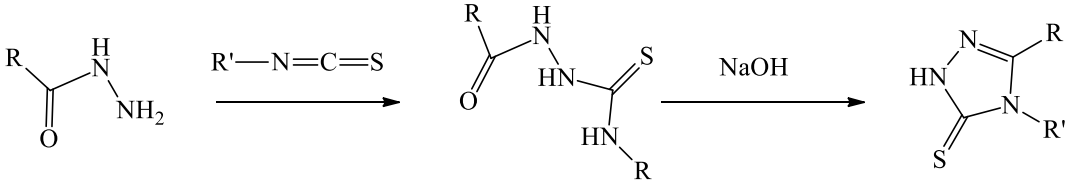
Cyclization of *N*-(pyrid-2-yl) formamidoximes with trifluoroacetic anhydride under milder conditions may also be employed for conversion of 2-aminopyridines into 1,2,4-

triazolo[1,5-*a*]pyridines in good yields (Huntsman & Balsells, 2005).



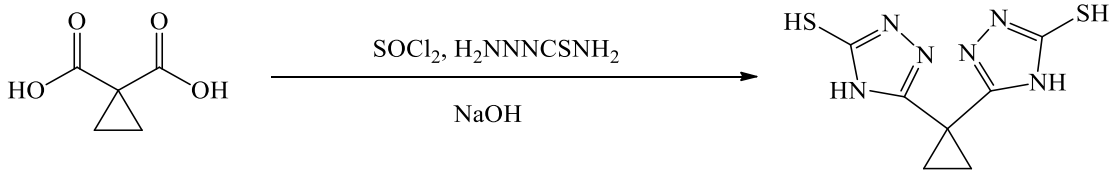
Scheme 3.8. Synthesis of 1,2,4-triazole derivatives by cyclization of *N*-(pyrid-2-yl) formamidoximes

Kucukguzel *et al*., (2015) used classical method for the synthesis of triazoles by heating substituted isothiocyanates in an alkaline medium.



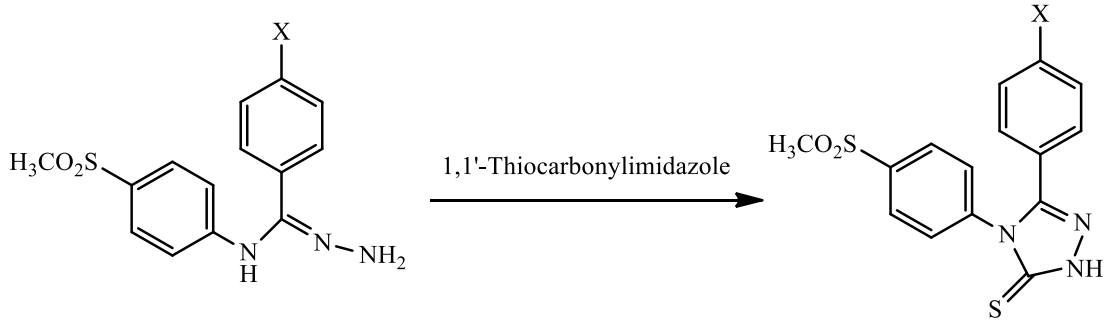
Scheme 3.9. Synthesis of 1,2,4-triazole derivatives from isothiocyanates

Triazole derivatives can also be synthesized by refluxing a mixture of thiosemicarbazide, 1,1-cyclopropane dicarboxylic acid and thionyl chloride (Sharba *et al*., 2005).



Scheme 3.10. Synthesis of 1,2,4-triazole derivatives *via* cyclization of thiosemicarbazide by cyclopropane dicarboxylic acid

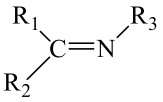
The ring closing reaction of carbohydrazonamides with 1,1'-thiocarbonyldiimidazole followed by alkylation gives 5-(4-halophenyl)-4-(4-(methylsulfonyl)phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (Daniel *et al.*, 2010).



Scheme 3.11. Synthesis of 1,2,4-triazole derivatives by ring closure of carbohydrazonamides using 1,1'-thiocarbonyldiimidazole

3.2 The synthesis of Schiff's base having 1,2,4-triazole skeleton

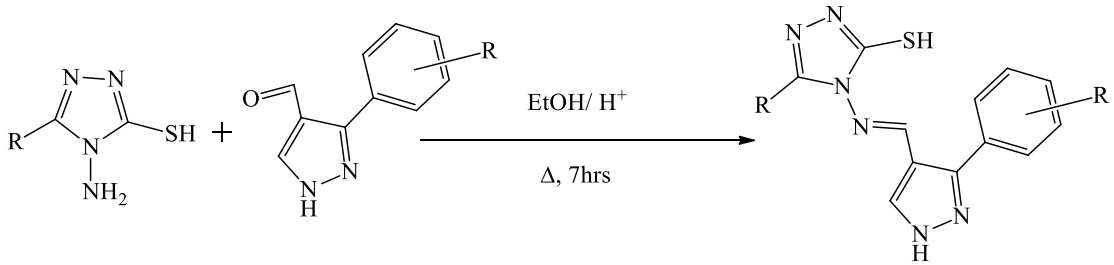
The Schiff's base is a compound similar to aldehyde or ketone where the carbonyl group is replaced by azomethine group. These compounds have been employed in various industries and also found to possess a broad spectrum of biological activities.



$R_1/R_2/R_3$ = Alkyl or Aryl grp

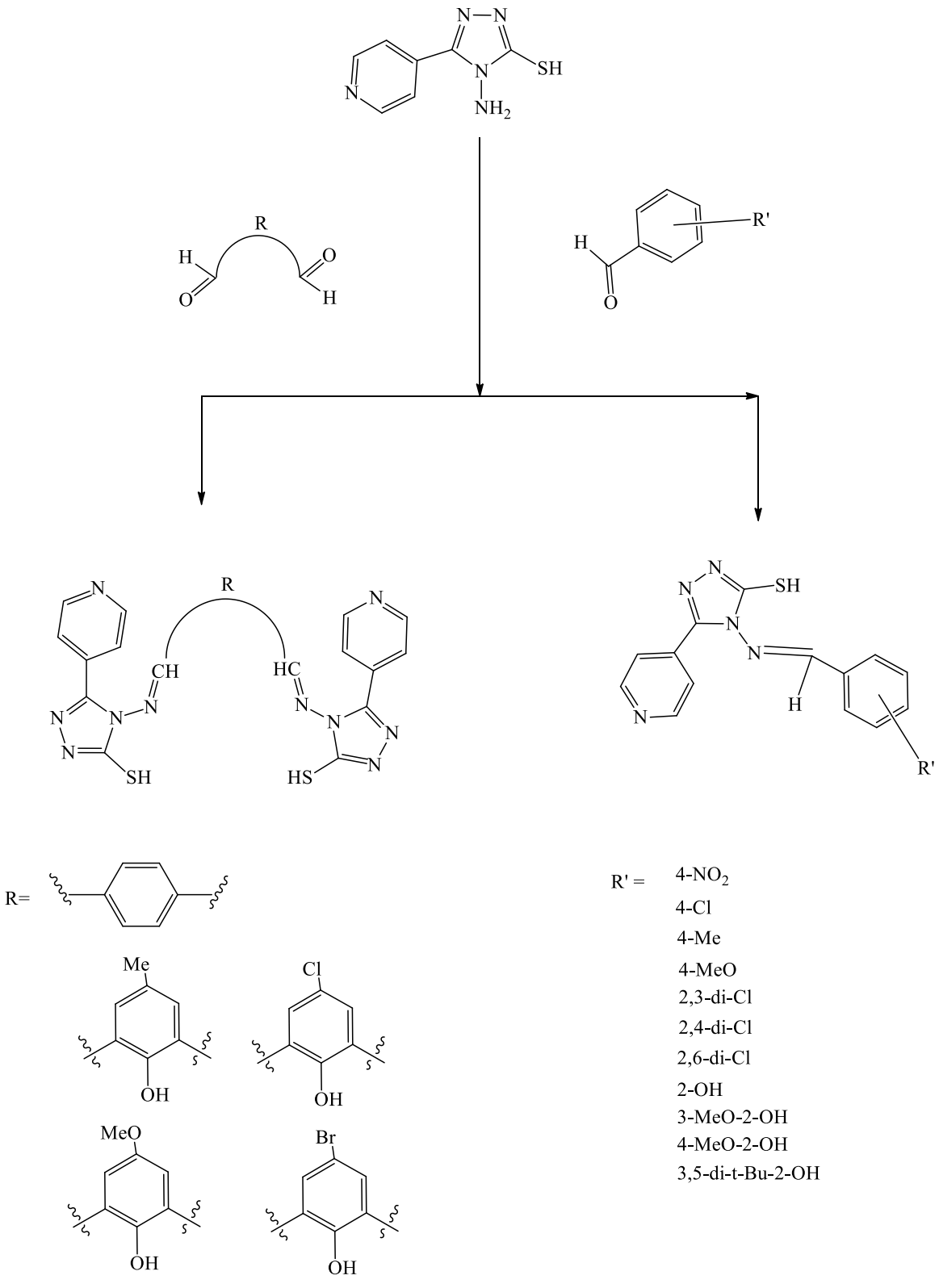
Fig 3.1: General structure of Schiff's base

Malladi *et al.,* (2013) used equimolar mixture of substituted 1,2,4-triazole with substituted pyrazole carbaldehyde and refluxed for 7 hr. in ethanol-dioxane medium in the presence of a concentrated sulphuric acid as a catalyst to produce Schiff's bases of triazole.



Scheme 3.12 Synthesis of Schiff's base by triazole thione and aldehyde

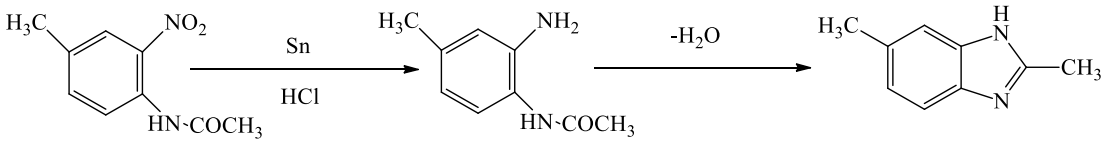
Khanmohammadi *et al.,* (2008) synthesized new Schiff bases by condensing different aldehydes and dialdehydes with 4-amino-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol.



Scheme 3.13 Synthesis of Schiff's base by reacting thione with various aldehydes and dialdehydes

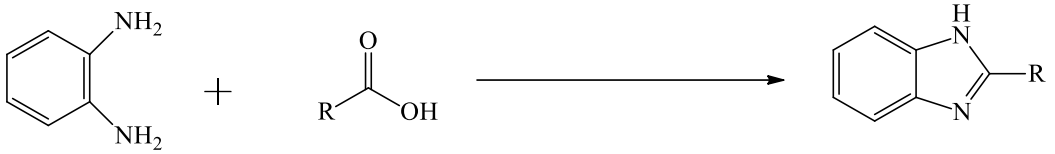
3.3 Synthesis of Benzimidazole derivatives

Hoebrecker in 1872 first synthesized benzimidazole in the form of 2,5-dimethylbenzimidazole by reducing and dehydrating 2-nitro-4-methylacetanilide (Alamgir *et al*., 2007).



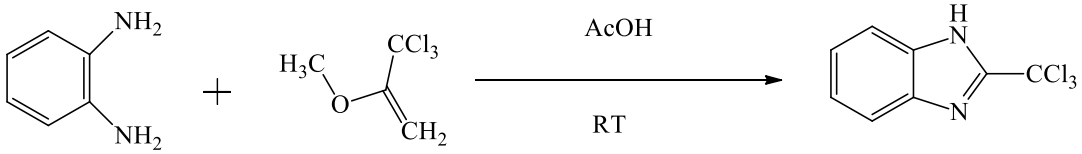
Scheme 3.14. Synthesis of benzimidazole derivative by reduction of acetanilide

The most common method for preparing benzimidazoles is Phillip's method which is the acid catalyzed condensation of *o*-diaminobenzene (OPD) with carboxylic acid (Alaqeel, 2017).



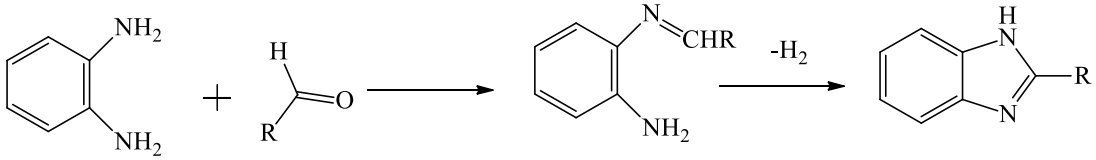
Scheme 3.15. Synthesis of benzimidazole derivative by reaction of *o*-phenylene diamine with carboxylic acid

Condensation reaction between imidate ester and *o*-diaminobenzene or its salt at room temperature had been used to obtain 2-(trichloromethyl)-1*H*-benzo(*d*)imidazole (Alaqeel, 2017).



Scheme 3.16. Synthesis of benzimidazole derivative from imidate ester

Rathod *et al.,* (2013) reported that aldehydes may react with *o*-phenylenediamines under oxidative reaction conditions to yield 2-substituted benzimidazoles (Scheme 3.17). The oxidation can be done by the air or cupric acetate or any other oxidizing agents.



Scheme 3.17. Synthesis of benzimidazole derivative by reacting OPD with Aldehydes

3.4 Biological activity of Schiff's base and triazole compounds

3.4.1 Antibacterial activities

Various antibacterial agents that are currently in use act by suppressing the growth of the bacteria or by killing them. With the increase in the antibacterial resistance, the medicinal chemists are still in search of new antibacterial drugs that are more potent without any side effects.

Biological and computational study of triazole derivatives containing Schiff's base hydrazones (figure 3.2a,b) was carried out that showed good inhibition against *Staphylococcus aureus* (Khanmohhammadi *et al.*, 2008).

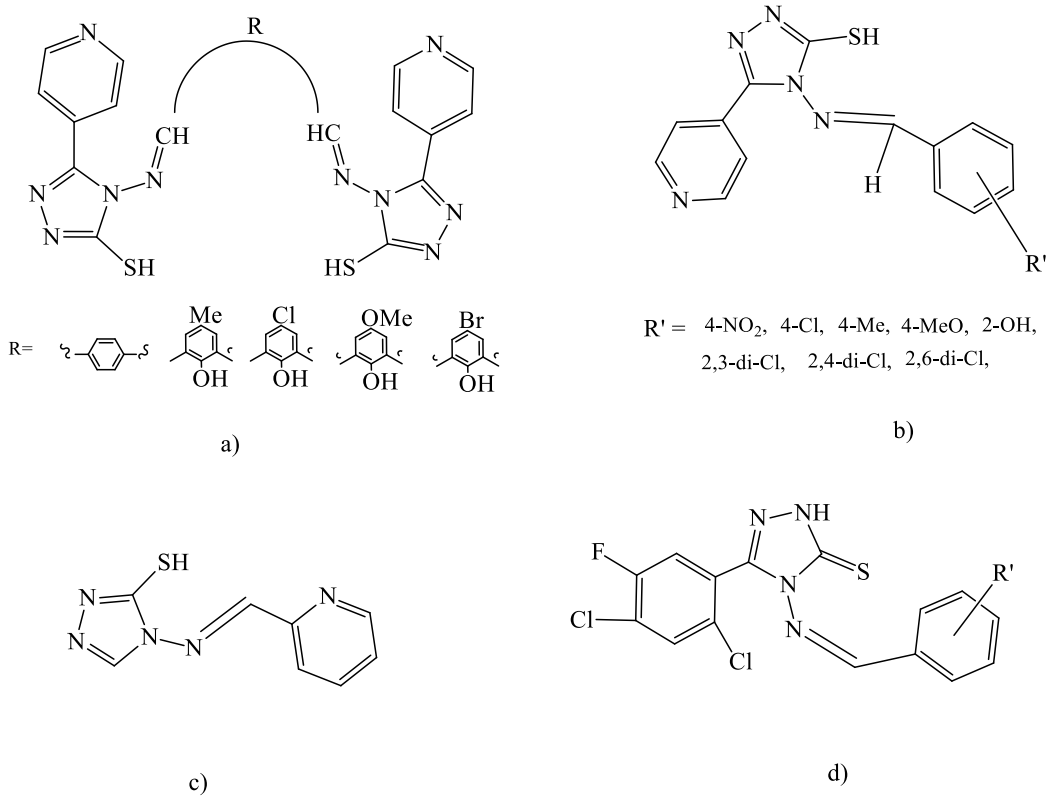


Figure 3.2: Some antibacterial Schiff's bases with triazoles

Alaghaz *et al.*, (2015) synthesized some 4-amino-5-mercapto-*S*-triazole Schiff's bases (figure 3.2c) and their complexes and studied their thermal, potentiometric and antibacterial activities. The study showed that the complexes can be potential photoactive materials and they also exhibited some significant antibacterial activities against gram positive; *B. Subtilis* and *S. aureus* as well as gram negative: *E. coli* and *P. aeruginosa*.

Karthikeyan *et al.*, (2006) have synthesized Schiff's and Mannich bases from the 1,2,4-triazole with 2,4-dichloro-5-fluorophenyl substituent (figure 3.2d) that showed some promising activity against the bacteria; *Escherichia coli* (ATCC- 25922), *Pseudomonas*

aeruginosa (ATCC-27853), *Staphylococcus aureus* (ATCC-25923), and *Klebsiella pneumoniae*. The investigation of antibacterial screening was done by disc diffusion method and the tested compounds exhibited good to moderate inhibition.

Malladi *et al*., (2013) reported significant antibacterial activity of pyrazole-based Schiff's bases against *Escherichia coli, Staphylococcus aureus and Pseudomonas aeuroginosa.*

In an *in vitro* antimicrobial assay some novel Schiff and Mannich base derivatives of isatin were found to exhibit MIC comparable with the standard drug ciprofloxacin. Some of these compounds were found to be more active against tested than bacterial strains than the standard drug (Prakash & Raja, 2013).

3.4.2 Antifungal activities

Wide species of fungi are potential human pathogens. Among them, *Candida* is the most common organism responsible for the fungal infection in humans. It is estimated that over a billion of people are affected by these microscopic fungi each year.

Kumar *et al*., (2010) have reported the antibacterial and antifungal activity of different triazoles and their derivatives. The compound 3-(α-naphthyl)-*S*-triazolo[3,4-*b*]-1,3,4-thiadiazolo[3,2-*b*]imidazo[4,5-*b*]quinoxaline (figure 3.3a) among many showed high antifungal activity against the fungi *Candida albicans* with MIC 2 μg/mL and *Candida tropicalis* with MIC 8 μg/mL.

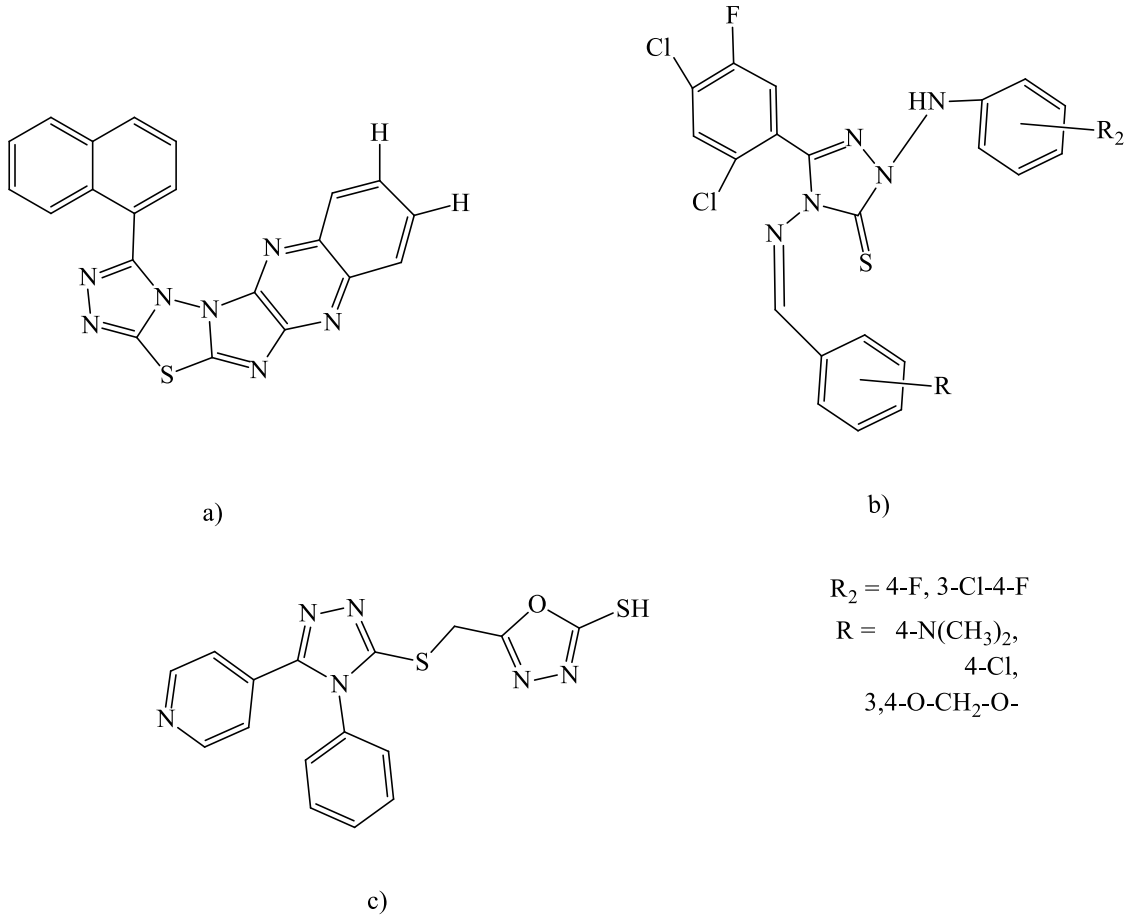


Figure 3.3: Some antifungal Schiff's bases with triazoles

Among the various Schiff bases and Mannich bases containing 2,4-dichloro-5-fluorophenyl substituent, the triazole derivatives (figure 3.3b) were found to exhibit almost equivalent activity as standard drug fluconazole against the fungi *A. flavus, A. flavus, P. marneffeii* and *T. mentagrophytesin* (Karthikeyan *et al*., 2006).

The metal complexes of tetradentate Schiff base ligand (*N*4)-6,7,14,15-tetrahydroxy-1,4,9,12-tetraazacyclohexadecane-5,8,13,16-tetraone complexes synthesized and their *in vitro* antifungal activity against *C. albicans, C. glabrata* and *C. tropicalis.* These complexes were found to be active against tested fungal strains in terms of MIC and ergosterol content assay (Sheikh *et al*., 2016).

Bayrack *et al.* (2009) synthesized the Mannich and Schiff's base of 1,2,4- triazoles and evaluated their antimicrobial activity against various bacteria and fungi. The Schiff's base derivatives (figure 3.3c) showed moderate activity against *C. tropicalis* (ATCC 13803) and *C. albicans* (ATCC 60193).

3.4.3 Anticancer activities

One of the leading causes of death in the world is cancer. In an effort to prevent or treat malignant tissues, numerous drugs and chemical compounds are being produced. The research and manufacturing of novel anticancer drugs are essential because the current anticancer medications are pricy and need to be modified.

Some novel chiral 1,2,4-triazole Schiff bases with a butenolide group were synthesized and were tested for their anticancer properties. The experiment's findings demonstrated that in HeLa cells, each chiral 1,2,4-triazole derivative has anticancer properties (Li *et al*., 2012).

Anticancer properties of some novel 1,2,3-triazole-dithiocarbamate hybrids studied and the findings suggested that majority of synthesized compounds showed moderate to potent activities against human tumor cell lines, MCF-7 and MGC-803 (Duan *et al*., 2013).

Some 4-arylidenamino-4*H*-1,2,4-triazole compounds with anticancer properties were studied. On screening studies with 60 human cancer cell lines, the compound 3-phenyl-substituted 1,2,4-triazole (figure 3.4) showed outstanding anticancer potential with NCI-H460, MCF-7, and SF-268 (Bekircan *et al.*, 2006).

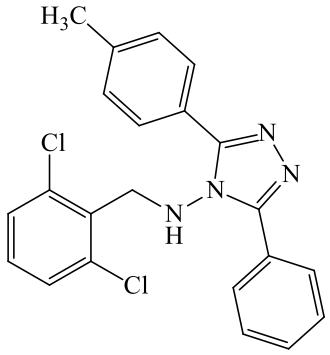


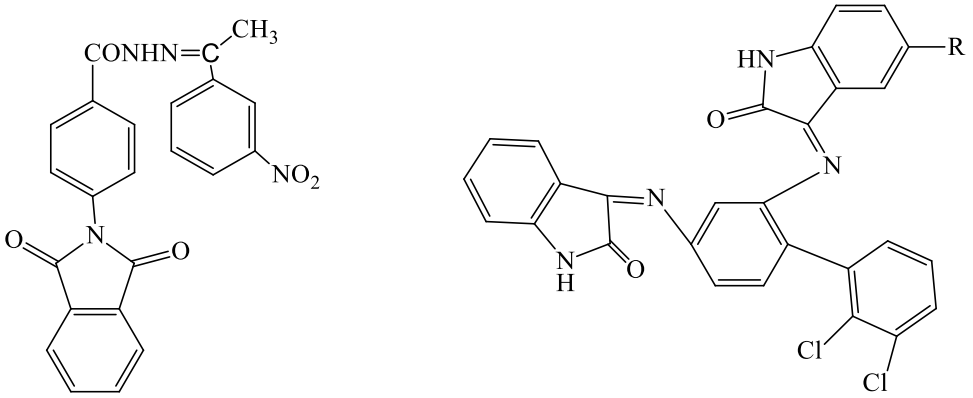
Figure 3.4: Anticancer triazole derivative

Triazole ribonucleosides with arylethynyl substitutions were tested for their antiviral and antiproliferative properties. The substance demonstrated strong apoptosis-induced antiproliferative action against Mia PaCa-2 pancreatic cancer cells both *in vitro* and *in vivo* (Wan *et al.*, 2009).

3.4.4 Anticonvulsant activities

Anticonvulsants are the compounds used in the treatment of epileptic seizures and convulsions. They are crucial in the management of borderline personality disorder and bipolar disorder. They are also widely utilized as neuropathic pain relievers and mood stabilizers.

Some phthalimide based Schiff's bases (figure 3.5a,b) were synthesized and their anticonvulsant and neurotoxic activities were tested. The experiment showed that all the compounds were active and less toxic than standard drug phenytoin. Compound containing nitro group at ortho position of aryl ring showed high anticonvulsant activity (Bhat & Al-Omar, 2011).



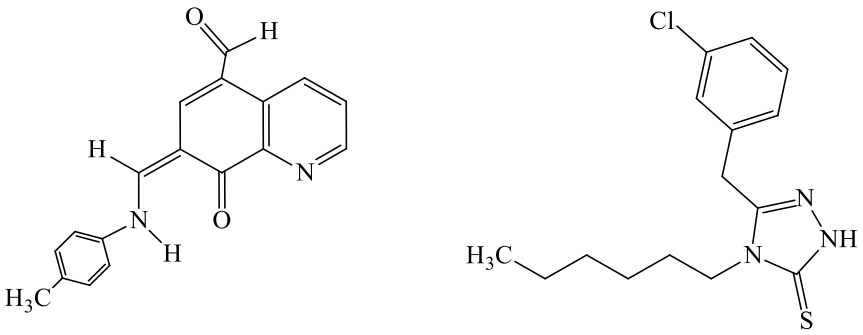
a) b)

Figure 3.5: Some anticonvulsant triazoles

Lamotrigine derivatives were created using isatin and isatin substitutes, and their anticonvulsant activities were evaluated. According to the findings, the synthetic compounds have stronger anticonvulsant properties than the common medication lamotrigine (Kulkarni *et al*., 2017).

3.4.5 Antioxidant properties

8- Hydroxyquinoline based Schiff's base derivatives were synthesized and were subjected to *in vitro* and *in vivo* antioxidant, anti-dyslipidemic and post-heparin lipolytic activities. In particular, the derivative 8-oxo-7-((*p*-tolylamino)methylene)-7,8-dihydroquinoline-5-carbaldehyde (figure 3.6a) among the compounds showed more anti-dyslipidemic and antioxidative activity (Sashidhara *et al*., 2009).



(a) (b)

Fig 3.6: Antioxidant Schiff bases

Several Schiff's bases of triazole worked by reducing the activity of tyrokinase, reduced melanin synthesis and also shown antioxidant effects when compared to standard ascorbic acid. The antioxidant capabilities of the compounds contrasted with their anti-tyrosinase actions (Yu *et al.*, 2015).

Some 1,2,4-triazole-3-thione based compounds and their derivatives were studied for their implications on GABA-ergic neurotransmission. When the substances were tested at levels of 300 mg/kg, all of the test animals were effectively protected. Among the derivatives, the compound 5-(3-chlorobenzyl)-4-hexyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (figure 3.6b) was possible medication (Plech *et al.*, 2014).

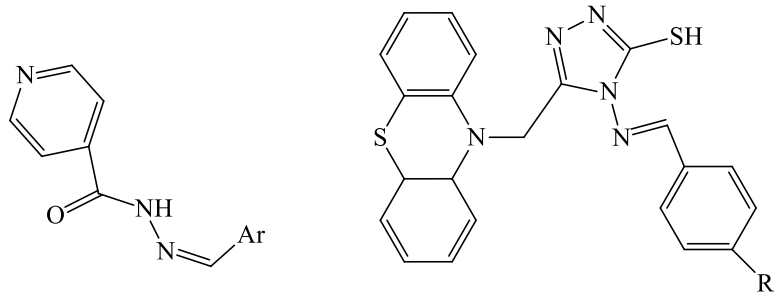
A number of 1,2,4-triazole-based Schiff's bases were created and their various characteristics, along with their antioxidant activity, were assessed. Through the use of the free radical scavenging method, it was discovered that two derivatives have the potential to act as antioxidants. These substances effectively inhibited DPPH by 89.2% and 86.8%, respectively, demonstrating strong radical scavenging action (Aswathanarayanappa *et al*., 2013).

3.4.6 Antidepressant activities

One of the growing issues in today's generation is depression. Antidepressants are used to treat such depressive disorders since they lead to an unhappy life and, in rare instances, life-threatening effects from attempted suicide.

A few phenothiazine derivatives based on triazoles were synthesized starting from 3-(10-phenothiazinyl)propionic acid and tested for their antidepressant potential. According to the study, compounds with (-H, -CH_3, -$N(CH_3)_2$, -Cl, and -OCH_3) substituents showed good

antidepressant effects (Turan *et. al*., 2002).



a) b)

Figure 3.7: Some antidepressant triazoles

Schiff's bases of isonicotinoyl hydrazone, *N*-[(1*Z*)-(substituted aromatic)methylidene]pyridine-4-carbohydrazides was made using microwave synthesis and sonication techniques (figure 3.7b). The synthetic substances were tested for their ability to treat depression *in vivo*. Significant antidepressant effects were seen in the nitro, halogen, and dimethoxy compounds. In terms of antidepressant action, *N*-[(1*Z*)-(2,5-dimethoxyphenyl)methylidene]pyridine-4-carbohydrazide stood up as the most promising contender (Thomas *et al*., 2016).

3.5 Some other activities of triazole containing compounds

Physicochemical studies were conducted on various 1,2,4-triazole based Schiff's bases. The results of the experiment show that the substance 4-(4-hydroxy-3-methoxy benzyledene amino)-4-*H*-1,2,4-triazole-3,5-dimethanol effectively inhibits mild steel corrosion in 0.5 M HCl (Ammal *et al.*, 2018).

Studies on some pyridyl based Schiff's bases of triazole derivatives were done for their corrosion inhibition characteristics for mild steel in HCl solution and found that the compound (3-phenylallylidene) amino-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol displayed best inhibition performance (96.6 g%) at 150 mg/L (Ansari *et al*., 2014).

By condensing 3-amino-2-methyl-4(3*H*)quinazolinone (AMQ) with various aldehydes in methanolic solution, novel Schiff bases containing the quinazoline ring system were produced. The compounds were tested in comparison with piperazine citrate as the standard drug and DMSO as the control to examine the antihelmintic effects on *P. posthuma*. Results

indicated that the most effective anthelmintic agent was a synthetic molecule with a chloro group (Revanasiddappa *et al.*, 2010).

Cyclization between 3-(3-ethylphenyl)-hydrazino-3*H*-quinazolin-4-one and various one carbon donating compounds to produce novel triazole compounds and were tested for *in vivo* H_1-antihistaminic activity on guinea pigs. Every tested substance protected the guinea pigs from histamine induced bronchospasm (Alagarsamy *et al*., 2009).

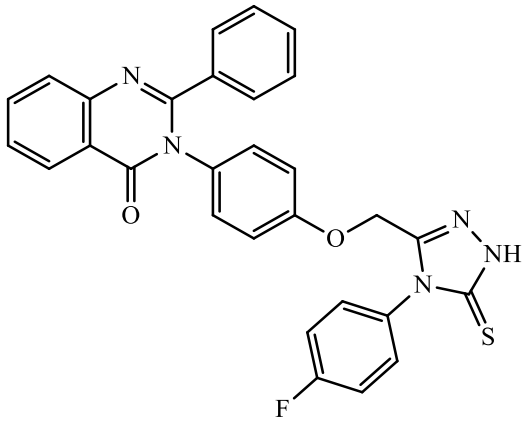


Figure 3.8. Triazole derivative with antimalarial properties

Some *S*-substituted phenacyl 1,3,4-oxadiazoles and their Schiff bases were investigated for their ability to reduce inflammation, provide pain relief, and treat ulcers. Acetic acid-induced writhing test and carrageenan-induced rat paw edema technique were used to measure the anti-inflammatory and analgesic effects respectively. *N*-(4-bromobenzylidene)-[2-(2,6-dichloroaniline)benzyl carbazide] was a powerful anti-inflammatory agent among the tested substances. The molecule had a 68.66% analgesic effect, which was higher than the 64.65% of normal diclofenac sodium (Bhandari *et al.*, 2008).

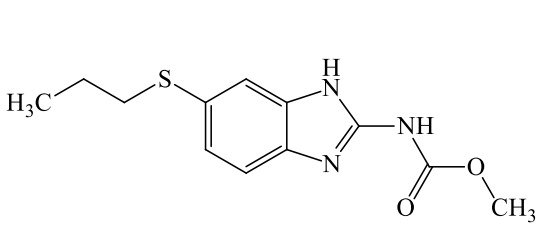
Some novel triazole and thiadiazole derivatives were prepared under different conditions from intermediate thiosemicarbazides and assayed for antiviral characteristics. With an EC_{50} value of 23.9 mg/mL and 9.90 mg/mL, respectively, the molecule with the toluene sulphonyl substituent at the para position demonstrated greater effectiveness against HIV-1 and HIV-2. According to the study, some active substances can operate as non-nucleoside reverse transcriptase inhibitors (NNRTI) (Akhtar *et. al*., 2007).

Other than bioactive agents, triazoles also have numerous applications. Some 1,2,4-triazole derivatives were used to create organic electroluminescent devices with multilayer

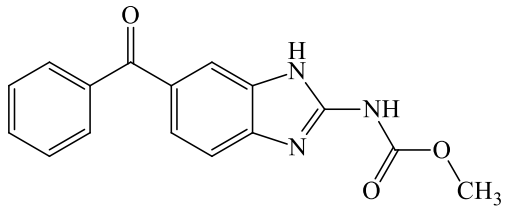
architectures. This layer served as the carrier transport. A cell made of a glass substrate, indium-tin oxide, triphenylamine derivative, 3-(4-biphenylyl)-4-phenyl-5-(4-tert-butylphenyl)-1,2,4-triazole, showed strong blue electroluminescence from the triphenylamine derivative layer (Kido *et. al*., 1993).

3.6 Benzimidazole derivatives and their biological activities

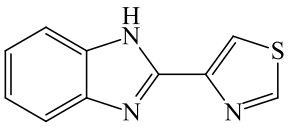
The mechanism of action and resistance of the marketed benzimidazole containing drugs albendazole, mebendazole, thiabendazole (figure 3.9) and other well-known antihelmintic medications were demonstrated (Kohler, 2001).



Albendazole



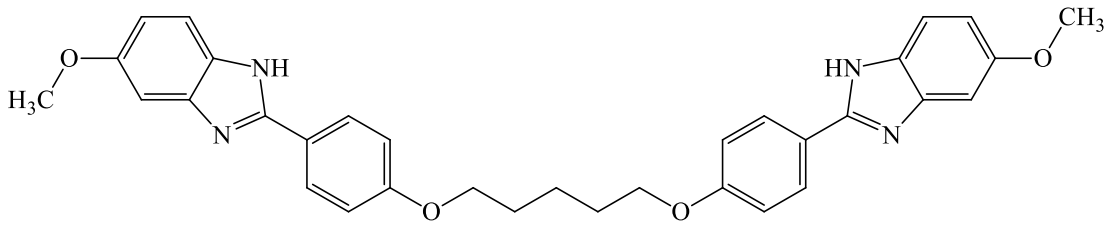
Mebendazole



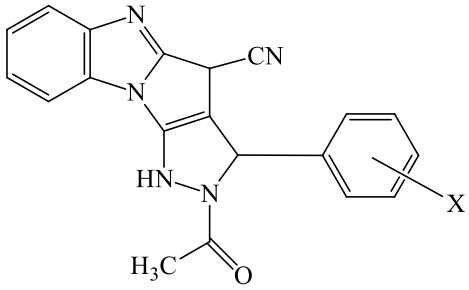
Thiabendazole

Figure 3.9. Some marketed benzimidazole drugs

In vitro antiprotozoal activity of a few novel benzimidazole derivatives were investigated in *Trichomonas virginalis, Entamoeba histolytica, Giardia lamblia, Leishmania mexicana*, and *Plasmodium berghei*. The compounds had strong anti-malarial effects. In comparison to metronidazole and pentamidine, 1,5-bis[4-(5-methoxy-1*H*-benzimidazole-2-yl)phenoxy]pentane (figure 3.10 a) was 3 times more effective against *G. lamblia* and nine times more potent than pentamidine (Torres-Gomez *et al*., 2008).



(a)



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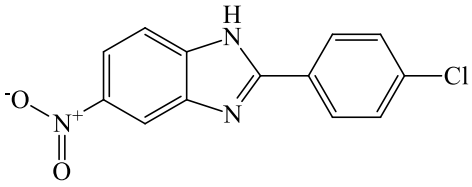
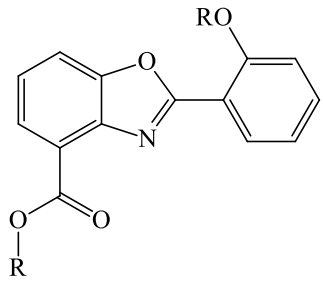


(c)



(d)

Figure 3.10: Benzimidazole derivatives with different biological properties

Various benzimidazole derivatives were synthesized and tested against *Fusarium*, *Alternaria brassicicola*, *Staphylococcus* (Gram +ve), and *E. coli* (Gram -ve) bacteria. The substance containing nitro and chloro substituents (figure 3.10b) demonstrated strong antibacterial activity (Shaharyar *et al*., 2017).

A series of benzimidazole derivatives based on 2-thioalkyl and thioaryl substituted benzimidazole and 5,6- dinitrobenzimidazole were synthesized and the compounds were tested for their antibacterial activity. The compounds were found to exhibit potent

antimicrobial activity against strains of *Stenotrophomonas malthophilia* (Kazimierczuk *et al.*, 2002). Some derivatives of benzimidazole like 7-(arylamidoalkyl)-3,4-diphenyl-isoquinolinyl-[1,5-*c*]-benzimidazoles were created and screened for their *in vivo* antiviral activity against the influenza virus. The maximum antiviral potential among the compounds was found to be of isoquinonyl based derivative with nicotinamido substituent (Pandey and Shukla., 1999). A few novel benzimidazole derivatives were produced and evaluated for their properties as anticonvulsant agent. 2-(4-chloro-phenyl)-5-nitro-1*H*-benzimidazole (figure 3.10c) showed maximum anticonvulsant potential (Jain *et al*., 2010). Many 2-substituted benzimidazoles were produced and tested for *in vitro* anticancer activities. With the IC_{50} values less than 10 mg/mL, all the compounds demonstrated anticancer activity against the human hepatocellular carcinoma (HepG2), human colon carcinoma (HCT 116), and human breast adenocarcinoma (MCF7) cell lines (Refaat, 2010). The natural product obtained from *Streptomyces* was modified to obtain a series of carbomethoxy-substituted benzimidazole derivatives (figure 3.10d). The semi-synthetic compounds exhibited anticancer potential (Kumar *et al.*, 2002). A number of 2,6-dihalophenyl substituted benzimidazoles were created that had anti-enterovirus action against echovirus 9 and 11, as well as several strains of coxsackievirus A9. The compounds' structure-activity relationship showed that the antiviral activity is affected by the presence of substituents at position 6 of the tricyclic system. The 1-(2-chloro-6-fluorophenyl)-6-trifluoromethyl-1*H*,3*H*-thiazolo[3,4-*a*]-benzimidazole derivative among the compounds demonstrated antiviral activity with an EC_{50} value of 0.41 μg/mL (Palma *et al*., 2007).

Chapter-4

4. MATERIALS AND METHODS

4.1 Materials

The precursors chemicals like hydrazine monohydrate, ethanol, *conc*. sulphuric acid, methanol, carbon disulphide, chloroacetic acid, *o*-phenylenediamine, methyl salicylate, *p*-chlorobenzaldehyde, furfuraldehyde of analytical grade were purchased and intermediates are synthesized in successive steps. The triazole derivative is prepared first and its Schiff's bases are synthesized separately. In the final step, 2-substituted benzimidazole is fused with each of the Schiff's bases and desired compounds are synthesized in a convergent synthesis pattern.

The reflux condenser apparatus, magnetic stirrer and other accessory equipment such as digital weighing balance, hot air oven, pH meter, autoclave, incubator, thiele tube, UV lamp (254 nm-visible light) were used from the Amrit campus research laboratory, Kathmandu, Nepal.

Table 4.1: List of Chemicals used

S.N	Name of chemical	Name of company
1	Methyl salicylate	Fisher scientific
2	Hydrazine hydrate	Qualigens
3	Chloroacetic acid	Qualigens
4	Ethanol(absolute)	Changshu Hongsheng Fine Chemical Co. Ltd.
5	Carbon disulphide	Fisher scientific
6	Furfuraldehyde	Fisher scientific
7	*p*-chlorobenzaldehyde	Fisher scientific
8	Methanol	Fisher scientific
9	Potassium hydroxide	Fisher scientific
0	*o*-phenylenediamine	Loba chemie
11	Sulphuric acid	Fisher scientific
12	Hydrochloric acid	Fisher scientific

4.2 Methods

The melting point of the synthesized compounds was determined, chromatographic method was carried out for purity and the compounds were characterized by spectroscopic methods- Ultraviolet spectroscopy, Infrared spectroscopy and Nuclear magnetic resonance spectroscopy.

4.2.1 Thin layer chromatography (TLC)

The TLC of all compounds was performed on silica gel coated aluminum plates with *n*-hexane and ethyl acetate in varying proportions. The spots were visualized by UV light of

short and long wavelength.

4.2.2 Melting point determination

The melting point of the synthesized compounds was determined with the electrothermal apparatus from Optics technology and also checked manually with the thiele tube.

4.2.3 Ultraviolet spectroscopy (UV-VIS)

The UV-VIS spectroscopic measurements of the compounds in methanol were done on double beam UV-VIS spectrometer, (Labtronics, Model LT-2802) in the region of 500-200 nm with 0.2 nm resolution at the department of chemistry, Amrit campus.

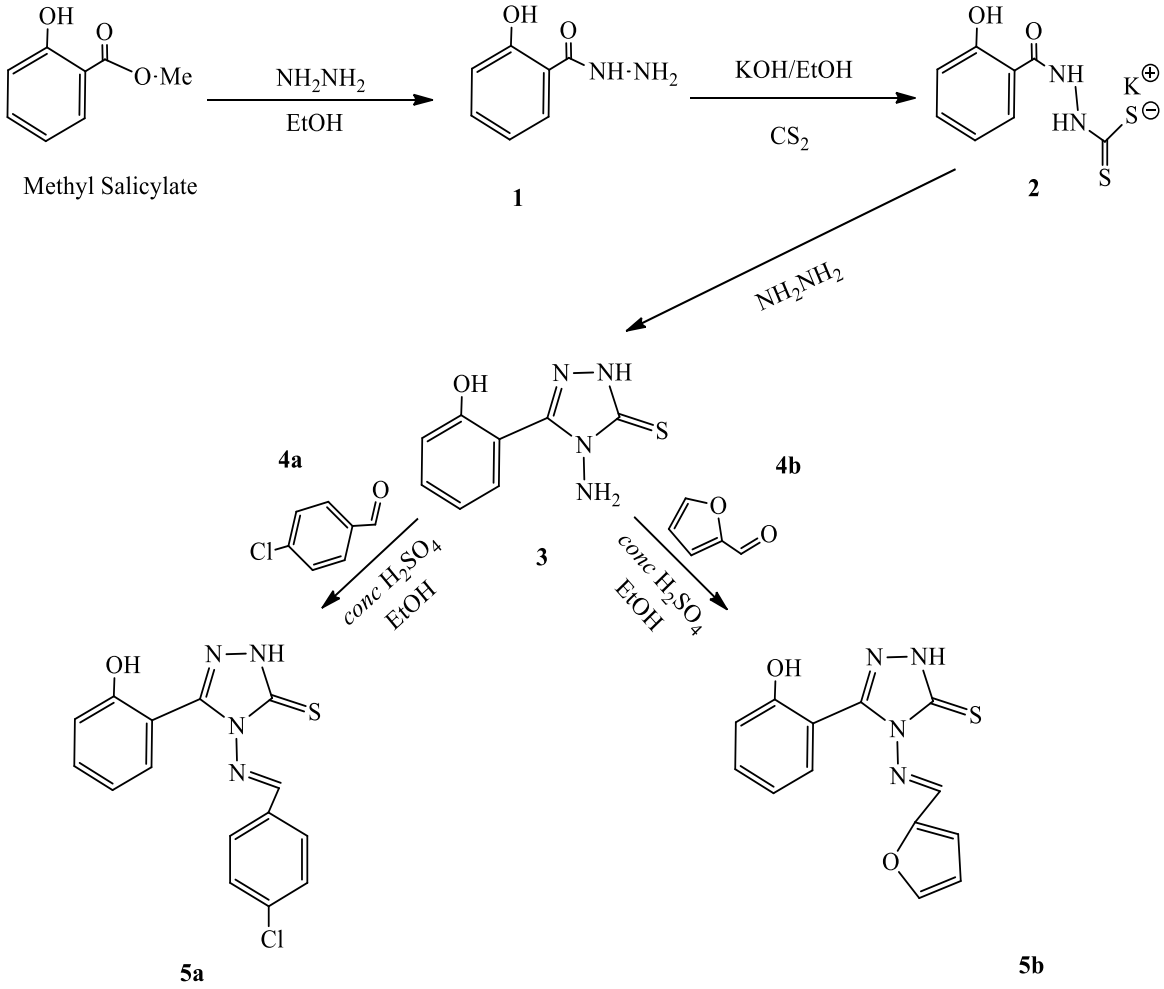
4.2.4 Infrared spectroscopy (FT-IR)

FT-IR spectral data of the compounds were collected on Perkin-Elmer spectrum two spectrometer (version 10.6.2) in the region of 4000-500 cm^{-1}, at department of chemistry, Amrit campus, Kathmandu, Nepal.

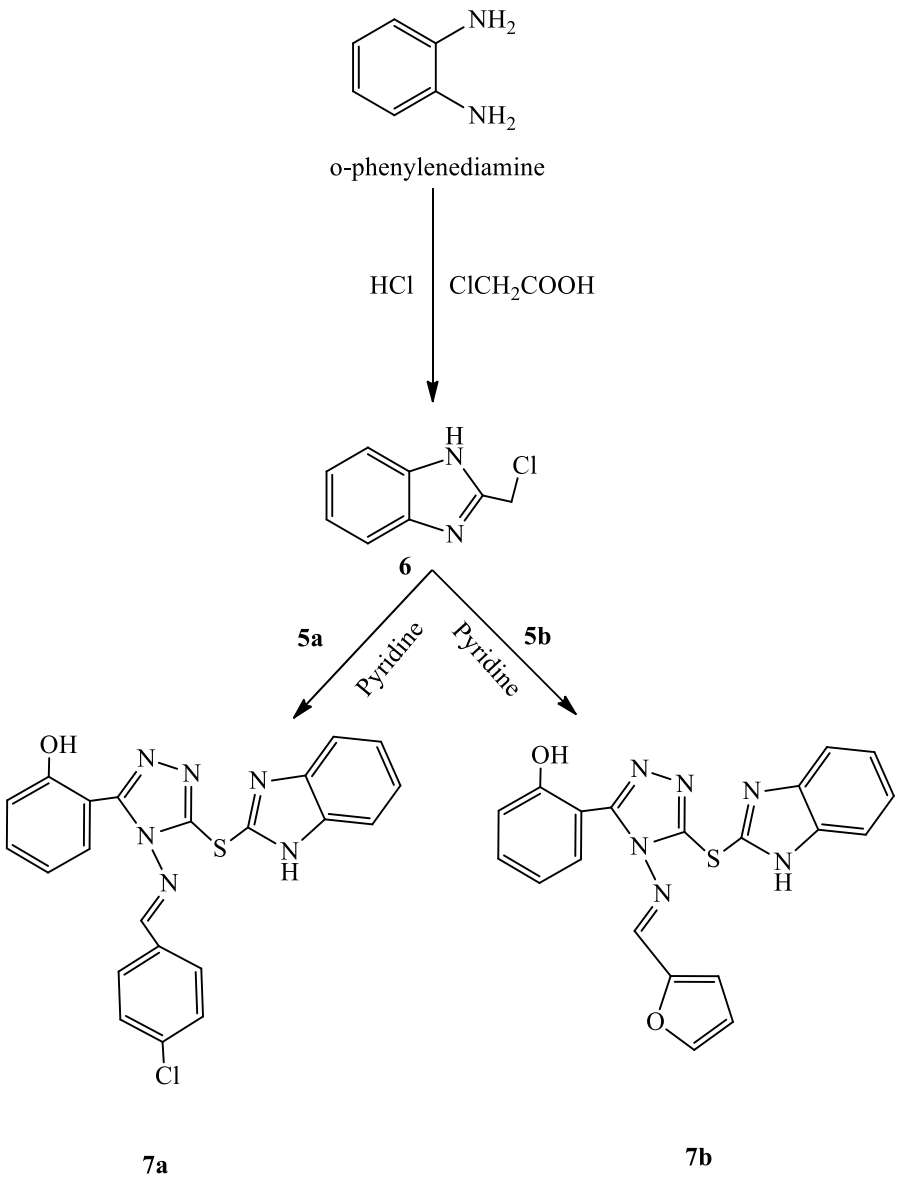
4.2.5 Nuclear magnetic resonance spectroscopy (NMR)

^{1}HNMR and ^{13}CNMR spectroscopic techniques were used for the analysis of the synthesized triazole, Schiff's bases and 2-substituted benzimidazole derivatives of triazoles. NMR spectra of the compounds in DMSO-d_6 were recorded on Bruker AV III 500 MHz NMR spectrometer at SAIF, IIT Madras, Chennai, India.

4.3 Synthesis of Schiff's base of 1,2,4-triazole containing 2 substituted benzimidazole



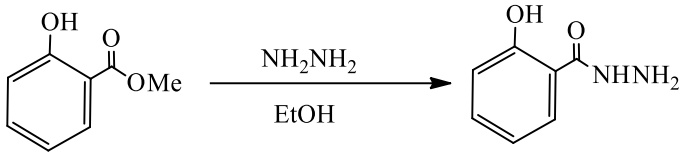
Scheme 4.1: Synthesis of 1,2,4-triazole with Schiff's bases



Scheme 4.2: Synthesis of 1,2,4-triazole with 2-substituted benzimidazole

4.3.1 Synthesis of acid hydrazide (1)

4.45 mL (0.090 mol) of hydrazine monohydrate was added slowly with constant stirring to the round bottom flask containing 9.129 g (0.060 mol) of methyl salicylate and refluxed for six hours. The excess solvent was evaporated on a hot water bath until the volume of solution was half reduced. The solution was cooled when white solid was crystallized out. The solid was separated by filtration, washed with cold ethanol, purified by recrystallization in absolute ethanol and was dried at 50-60 °C. The yield, m.pt, R*f* of synthesized acid hydrazide were recorded.

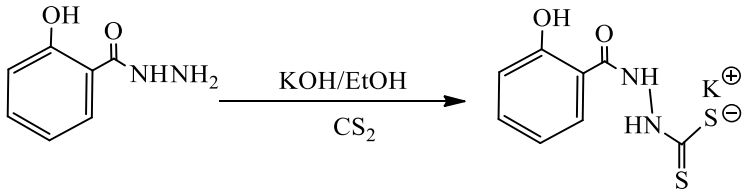


Scheme 4.3: Synthesis of acid hydrazide

Yield: 78%, white shining crystalline solid, m.pt. 140 °C, R*f:* 0.64 (ethyl acetate: *n*-hexane, 2:8)

4.3.2 Synthesis of potassium 2-(2-hydroxybenzoyl)hydrazinecarbodithioate (2)

4.565 g (0.030 mol) of acid hydrazide (1) was added to 1.683 g (0.030 mol) of potassium hydroxide in 20 mL ice cold absolute ethanol. 1.82 mL (0.030 mol) of carbon disulphide was added dropwise to the mixture with constant stirring (not exceeding 30 °C temperature) and was stirred for 21 hours at room temperature in a magnetic stirrer. 20 mL of anhydrous diethyl ether was added. Potassium dithiocarbazinate that was separated out was washed twice with diethyl ether and dried at a temperature lower than 60 °C. The yield, m.pt., R*f* of synthesized dithiocarbazinate was recorded.

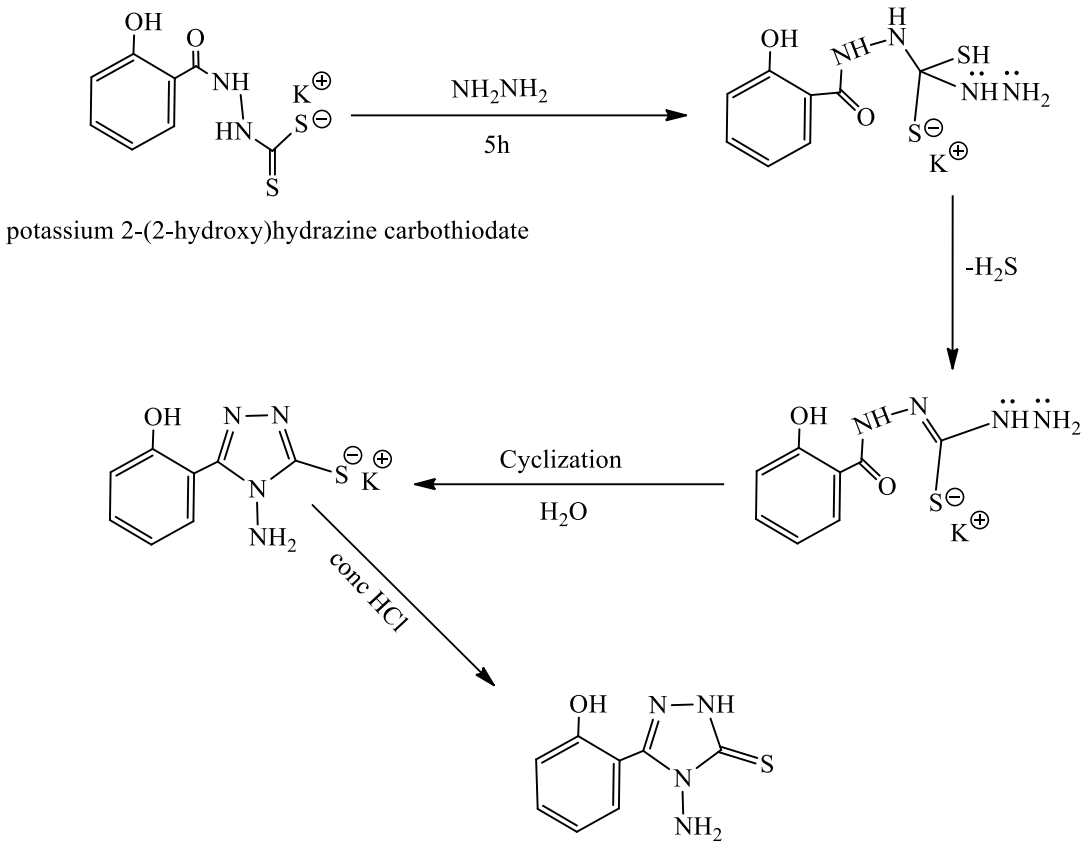


Scheme 4.4: Synthesis of dithiocarbazinate

Yield: 87.35%, White shining crystalline solid, m.pt. 241 °C,
R*f:* 0.58 (ethyl acetate: *n*-hexane, 2:8)

4.3.3 Synthesis of 4-amino-2-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione (3, TZ)

4.680 g (0.018 mol) of potassium dithiocarbazinate (2) and 1.48 mL of the hydrazine monohydrate was suspended in 5 mL of distilled water. The resulting mixture was refluxed for 5 hours till no further evolution of hydrogen sulphide gas (checked with $K_2Cr_2O_7$). After cooling to room temperature, it was diluted with 100 mL of cold water containing some crushed ice (made from distilled water). The reaction mixture was acidified with *conc* HCl. The corresponding triazole precipitated out was filtered, washed with 30 mL cold water twice and purified by recrystallization in absolute ethanol. The yield, m.pt., R*f* value of synthesized triazole was recorded.



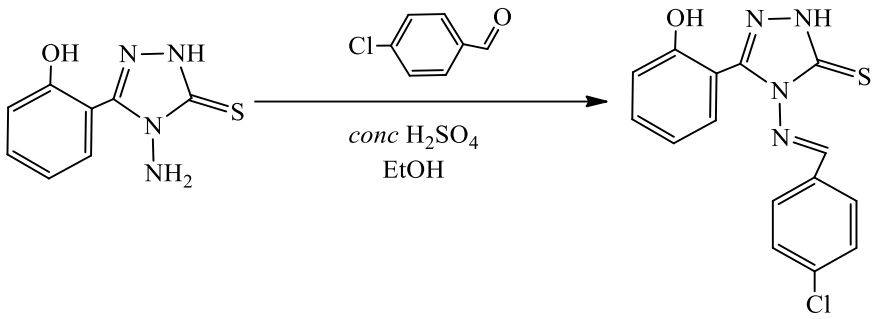
Scheme 4.5: Synthesis of 1,2,4-triazole-5-thione

Yield: 84.05%, White crystalline solid, m.pt. 178 °C, R*f*: 0.43 (ethyl acetate: *n*-hexane, 2:8). UV- Visible (λ_{max}) nm = 302, 309, 331, 340. IR in KBr (selected bands): cm^{-1} = 3287 (O-H str, aromatic), 3055 (-NH, str, aromatic), 3024 (C-H, str, aromatic) 1612 (C=N, str), 1590 (N-H, bend), 1543 (C=C str, aromatic), 1492 (C=C, str, aromatic), 1011 (N-N str, aliphatic), 1240 (C-O, str, aromatic), 946 (C=S, str), 741 (C-H bend, aromatic), 687 (C-H, bend, aromatic). ^{1}H-NMR (500 MHz, DMSO- d_6) ppm = 13.86 (br s, 1H, NH), 10.36 (s, 1H, OH), 7.43-7.31 (m, 2H, Ar-H), 7.00 (d, *J* = 8.20 Hz, 1H, Ar-H), 6.93 (t, *J* = 7.57 Hz, 1H, Ar-H), 5.62 (br s, 2H,). ^{13}C- NMR (100 MHz, DMSO- d_6) = 166.54 (triazole-C5), 156.53 (Ar-C), 149.62 (triazole-C3), 132.6 (Ar-C), 131.32 (Ar-C), 119.5 (Ar-C), 116.67 (Ar-C), 113.52 (Ar-C).

4.3.4 Synthesis of Schiff's base 4-(5-chlorobenzene-2-yl-methyleneamino)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione (5a, SBPC)

2.8 g (0.020 mol) of *p*-chlorobenzaldehyde was dissolved in a minimum quantity of hot ethanol to which 4.165 g (0.020 mol) triazole thiol (3) was added. The suspension was heated until the solution became clear. 5 drops of concentrated sulphuric acid was added to

the mixture and then refluxed for 5 hours and then cooled to room temperature. The solid precipitated was separated by filtration under suction, washed with cold ethanol and purified by recrystallization in hot ethanol. The yield, m.pt., R*f* of synthesized Schiff's base was recorded.

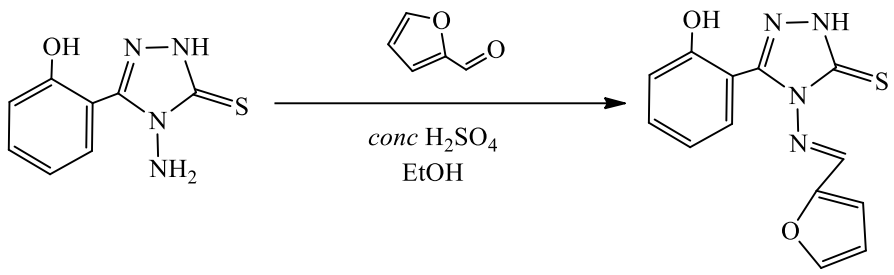


Scheme 4.6: Synthesis of Schiff's base of 1,2,4-triazole-5-thione.

Yield: 72.09%, White cottony solid, m. pt. 215 ˚C, R*f*: 0.78 (ethyl acetate: *n*-hexane, 2:8). UV-Visible (λ_{max}) nm = 302, 309, 331, 340, 370. IR in KBr (selected bands) cm^{-1} = 3482 (O-H, str, aromatic), 3218 (N-H, str, aromatic), 3092 (C-H, str, aromatic), 1620 (C=N, str), 1583 (NH, str), 1528 (C=C, str, aromatic), 1508 (C=C, str, aromatic), 1244 (C-O, str, aromatic), 1015 (N-N, bend, aromatic), 941 (C=S, str), 737 (C-H bend), 687 (C-H, bend). ^{1}H-NMR (500 MHz, DMSO- d_6) ppm. = 14.13 (br s, 1H, SH), 10.09 (s, 1H, OH), 9.64 (s, 1H, N=CH), 7.79 (d, *J* = 8.55, 2H, Ar-H), 7.57 (d, *J* = 7.93 Hz, 2H, Ar-H), 7.33-7.42 (m, 2H, Ar-H), 6.88-6.95 (m, 2H, Ar-H) ^{13}C-NMR (100 MHz, DMSO-d_6) = 164.59 (triazole-C5), 162.24 (Ali-C), 148.88 (triazole-C3), 156.62 (Ar-C), 148.88 (Ar-C), 137.76 (Ar-C), 132.91 (Ar-C), 131.59 (Ar-C), 131.55(Ar-C) , 130.65(Ar-C) , 129.79(Ar-C) , 119.42(Ar-C) , 116.43(Ar-C) , 113.28(Ar-C).

4.3.5 Synthesis of Schiff's base 4-(4-furan-2-yl-methyleneamino)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione (5b, SBFF)

1.8 mL (0.020 mol) of furfuraldehyde was dissolved in a minimum quantity of hot ethanol to which 4.165 g (0.020 mol) triazole thiol (3) was added. The suspension was heated until the solution became clear. 5 drops of concentrated sulphuric acid were added to the mixture and then it was refluxed for 5 hours. The reaction mixture was cooled and precipitated solid was filtered under suction, washed with cold ethanol and purified by recrystallization with hot ethanol.

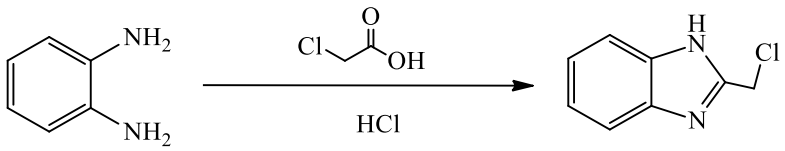


Scheme 4.7: Synthesis of Schiff's base of 1,2,4-triazole-5-thione

Yield: 70.9%, White cottony solid, m. pt. 215 ˚C, R*f*: 0.78 (n- hexane: ethyl acetate, 8:2). UV- Visible (λ_{max}) nm = 302, 309, 331, 340, 370. IR in KBr (selected bands) cm^{-1} = 3443 (O-H, str, aromatic), 3221 (N-H, str, aromatic), 3092 (C-H, str, aromatic), 1615 (C=N, str), 1583 (NH, str), 1551 (C=C, str, aromatic), 1583 (N-H, bend, aromatic), 1542 (C=C, str, aromatic), 1002 (N-N, str, aromatic), 1243 (C-O, str, aromatic), 934 (C=S, str), 756 (C-H bend), 687 (C-H, bend). ^{1}H-NMR (500 MHz, DMSO- d_6) ppm = 14.09 (br s, 1H, SH), 10.06 (s, 1H, OH), 9.41 (s, 1H, N=CH), 7.97 (br, s, 1H, Ar-H), 7.34-7.45 (m, 2H, Ar-H), 7.30 (d, *J* = 3.66, 1H, Ar-H), 6.87-6.96 (m, 2H, Ar-H), 6.73 (dd, *J* = 3.05, 1.83Hz, 1H). ^{13}C- NMR (100 MHz, DMSO-d_6) = 162.16 (triazole-C5), 156.56 (Ali-C), 148.82 (triazole-C3), 154.42 (Ar-C), 148.34 (Ar-C), 147.66 (Ar-C), 132.82 (Ar-C), 131.59 (Ar- C), 120.89 (Ar- C), 119.39 (Ar- C), 116.47 (Ar- C), 115.30 (Ar- C) ,113.39 (Ar- C).

4.3.6 Synthesis of 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (6)

2.16 g (0.020 mol) of *o*-phenylenediamine was dissolved in a 10 mL of HCl and 1.89 g (0.020 mol) chloroacetic acid was added. It was refluxed for 6-10 hrs. with constant stirring. The reaction mixture was then cooled to 5 °C and neutralized with $NaHCO_3$ solution. Solid yellow precipitate was formed which was separated by filtration under suction, washed with cold water and purified by recrystallization from hot ethanol. The yield, m.pt., R*f* of synthesized benzimidazole was recorded.



Scheme 4.8: Synthetic route for the preparation of benzimidazole

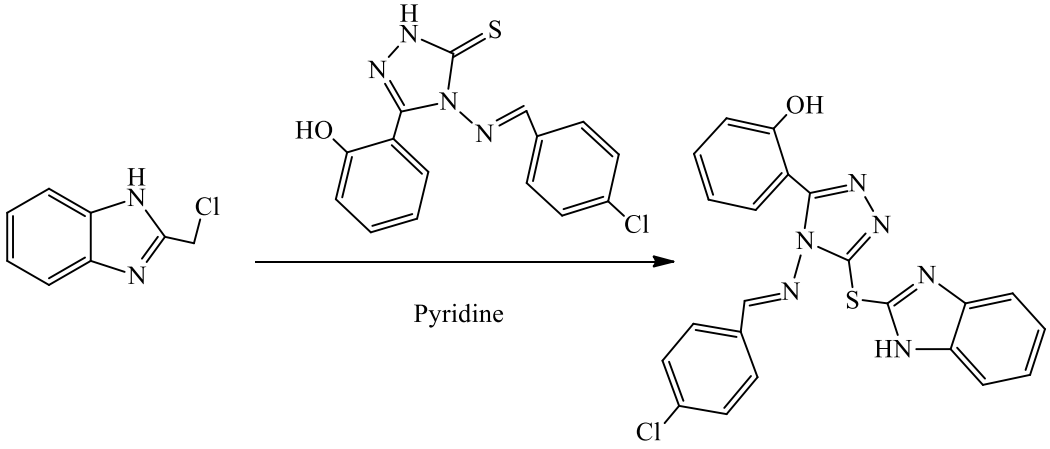
Yield: 79 %, Yellow crystalline solid, m.pt. 154 °C, R*f*: 0.71 (ethyl acetate: *n*-hexane, 2:8). UV-Visible (λ_{max}) nm = 292, 309, 331. IR in KBr (selected bands) cm^{-1} = 3061 (N-H, str, aromatic), 3042 (C-H, str, aromatic), 1622 (C=N, str), 1540 (NH, str), 1448 (C=C, str, aromatic), 1442 (C=C, str, aromatic), 735 (C-H bend), 698 (C-H, bend), 642 (C-Cl, str).

4.3.7 General procedure for the synthesis of benzimidazole derivative

Each of the Schiff's bases were dissolved in a 10 mL of pyridine to which compound 6a was added. The reaction mixture was refluxed for 6-10 hrs. with constant stirring. It was then poured to 100 mL ice cold water. The precipitate formed was separated by filtration, washed with cold ethanol and purified by recrystallization from hot ethanol.

4.3.7.1 Synthesis of benzimidazole derivative 5-((1*H*-benzo[*d*]imidazol-2-yl) thio)-4-((4-chlorobenzylidene) amino)-4*H*-1,2,4-triazol-3-yl) (2-hydroxyphenyl) methanone (7a, FCPC)

2.16 g (0.020 mol) of compound 6 was dissolved in a 10 mL of pyridine to which 3.30 g (0.01 mol) compound 5a was added. After refluxing for 6-10 hr., it was poured to 100 mL ice cold water. Yellow-green precipitate was formed which was separated by filtration, washed with cold ethanol and purified by recrystallization from hot ethanol.



Scheme 4.9: Synthesis of benzimidazole derivative with1,2,4-triazole

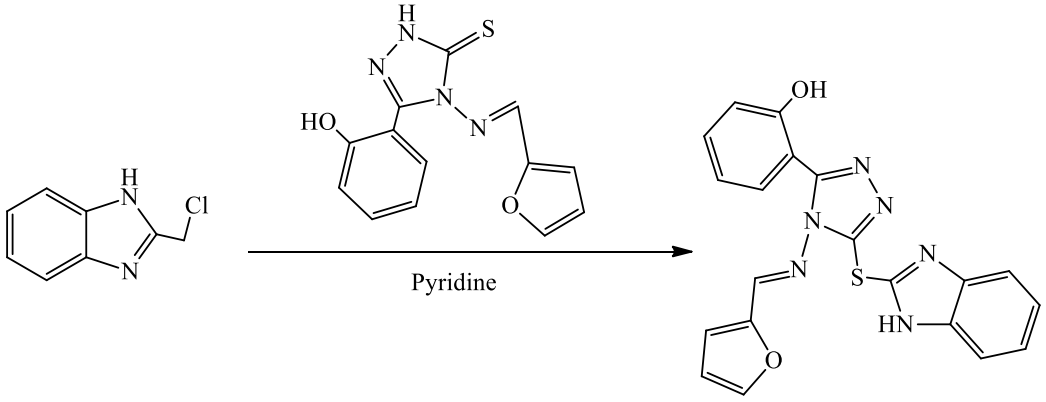
Yield: 78.25%, Dark green solid, m. pt. 175 ˚C, R*f*: 0.77 (ethyl acetate: *n*-hexane, 2:8).

UV- Visible (λ_{max}) nm = 302, 309, 331, 340, 370. IR in KBr (selected bands) cm^{-1} = 3075 (O-H, str, aromatic), 3261 (NH, str), 3134 (C-H, str, aromatic), 1620 (C=N, str), 1584 (NH, str), 1534 (C=C, str, aromatic), 1485 (C=C, str, aromatic), 1015 (C-O, str, aromatic), 744 (C-H bend), 696 (C-H, bend). ^{1}H-NMR (500 MHz, DMSO- d_6) ppm = 12.69 (br s, 1H, NH), 10.09 (br s, 1H, OH), 9.66 (s, 1H, N=CH), 7.77-7.83 (m, 2H, Ar-H), 7.71(d, *J* = 7.32 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.55 Hz, 2H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.34-7.4 (m, 2H, Ar-H), 6.88-7.05 (m, 2H, Ar-H). ^{13}C- NMR (100 MHz, D M S O - d_6) = 164.52 (triazole-C5), 162.25 (Ali-C), 156.75 (benzimidazole-C), 148.89 (triazole-C3), 156.65 (Ar-C), 143.62 (benzimidazole-C), 137.74 (Ar-C), 132.88 (Ar-C), 131.58 (Ar-C), 131.50 (Ar-C), 130.65

(Ar-C), 129.79 (Ar-C), 129.40 (Ar-C), 119.40 (Ar-C), 116.46 (Ar-C), 113.29 (Ar-C).

4.3.7.2 Synthesis of benzimidazole derivative 5-((1*H*-benzo[*d*]imidazol-2-yl) thio)-4-((4-furan) amino)-4*H*-1,2,4-triazol-3-yl) (2-hydroxyphenyl) methanone (7b, FCFF)

1.08 g (0.010 mol) of compound 6 was dissolved in a minimum amount of pyridine to which 2.86 g (0.020 mol) compound 5b was added. The reaction mixture was refluxed for 6-10 hr. with constant stirring. The reaction mixture was then poured to ice cold water. Solid dark brown precipitate was formed which was filtered, washed with cold ethanol and purified by recrystallization from hot ethanol. The yield, m.pt., R*f* of synthesized derivative was recorded.



Scheme 4.10: Synthesis of benzimidazole derivative with1,2,4-triazole.

Yield: 80.42%, Dark brown solid, m. pt. 161˚C, R*f*: 0.74 (ethyl acetate: *n*-hexane, 2:8).

UV- Visible (λ_{max}) nm = 302, 309, 331, 340, 370. IR in KBr (selected bands) cm^{-1} = 3076 (O-H, str, aromatic), 3261 (NH, str), 3101 (C-H, str, aromatic), 1620 (C=N, str), 1585 (NH, str), 1534 (C=C, str, aromatic), 1486 (C=C, str, aromatic), 1015 (C-O, str, aromatic), 740 (C-H bend), 702 (C-H, bend). ^{1}H-NMR (500 MHz, DMSO- d_6) ppm= 12.70 (s, 1H, NH), 10.09 (br s, 1H, OH), 9.41 (s, 1H, N=CH), 7.97 (br s, 1H, Ar-H) 7.72-7.88 (m, 2H, Ar-H), 7.54-7.63 (m, 2H, Ar-H), 7.36-7.40 (m, 2H Ar-H), 7.30 (d, *J* = 3.66, 1H, Ar-H), 6.88-6.95 (m, 2H, Ar-H), 6.73 (dd, *J* = 3.66, 1.83 Hz, 1H). ^{13}C- NMR (100 MHz, DMSO- d_6) = 162.16 (triazole-C5), 156.75 (Ali-C), 156.66 (benzimidazole-C), 151.41 (triazole-C3), 148.83 (Ar-C), 148.33 (benzimidazole-C), 147.68 (Ar-C), 143.62 (Ar-C), 132.82(Ar-C), 131.58 (Ar-C), 129.41 (Ar-C), 120.89 (Ar-C), 119.58 (Ar-C), 119.40 (Ar-C), 116.49 (Ar-C), 115.30 (Ar-C), 113.39 (Ar-C).

4.4 Antimicrobial screening

The antimicrobial activities of the Schiff's bases and newly synthesized benzimidazole derivatives were screened against different bacterial strains such as *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 8739), *Enterococcus faecalis* (ATCC 29212), *Proteus vulgaris* (ATCC 8360), *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aeruginosa (ATCC 9027), Salmonella enterica (Clinical sample), Staphylococcus aureus*(ATCC 6538P), *Shigella dysenteriae* (Clinical sample) *and Staphylococcus epidermidis* (ATCC 1228) and fungal strains such as *Candida albicans* (ATCC 2091), *Saccharomyces cerevisiae* (ATCC 18824), *Fusarium oxysporum., Alternaria alternata* of certain concentrations by using ciprofloxacin and azithromycin as standard (positive control) for bacterial strains and clotrimazole, carbendazim as standard (positive control) for fungal strains.

4.4.1 Preparations of the working solutions

The screw-capped tubes were sterilized, calibrated and marked for 10 mL each. Around 1g of each of the sample was transferred in calibrated screw capped tubes. DMSO was added in the tube up to the line marked by 10 mL label. The mixture was homogenized by vortexing.

4.4.2 Preparations of the standard culture inoculums

Freshly cultured test organisms (within 18–24 hours) in required no of colonies were aseptically inoculated to a tube containing 5 mL of sterilized nutrient broth. The test solution was homogenized by vortexing. The solution was compared with the turbidity of 0.5 McFarland Nephelometer standard recommended by (WHO, 1991) for antimicrobial susceptibility test.

4.4.3 Screening and evaluation of antibacterial and antifungal activity

The samples were screened for antibacterial activity using agar well diffusion methods as described by Perez *et. al.*, 1999.

Antibacterial screening

Bacterial culture drawn from the respective inoculums equivalent to 0.5 McFarland standard turbidity was spread uniformly over Mueller-Hinton Agar (MHA) medium with a sterile swab. Swabbing was repeated twice and the plate was rotated through an angle of 60˚ after each swabbing. After drying for maximum of 15 minutes, four well each of 6 mm diameter were made in the inoculated plates with the help of sterile cork borer. Each of the test solution of the samples and negative control, DMSO were added to each of the four wells

using micropipette. The test solutions were allowed to diffuse into the media by standing the plates in upright condition with lids closed for 30 minutes. After incubation in an inverted position at 35±2 ˚C for 18-24 hours, the plates were examined for the growth of organisms and zone of inhibition (ZOI) was measured. Ciprofloxacin and azithromycin were used as the positive control and the ZOI was compared.

Antifungal screening

Potato dextrose broth (PDB) was prepared to inoculate the pure colonies of the fungal specimens (*Fusarium oxysporum, Alternaria alternata, Candida albicans, Saccharomyces cerevisiae*) and were placed at the incubator at around 28 ˚C for a duration of 72 hours. A sterile cotton swab was used to spread the culture onto the Mueller Hinton Agar (MHA) plates and 6 mm wells were made with the help of a sterile borer. Following this, 0.5 mg, 1 mg, and 2 mg of the compounds were loaded into the well and were allowed to diffuse properly by keeping them for 0.5 hr. at room temperature. All the compounds were tested in triplicates. Clotrimazole (200 mcg) and Carbendazim (0.1 %) positive controls were used to compare the activity and DMSO was used for negative control. The results were observed after the plates were left for incubation at 28 ˚C for 48 - 72 hours.

4.5 Antioxidant activity

Antioxidants are any substances that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. The indication of antioxidant activity is also important in other sciences such as medicine, biology, health and nutrition, epidemiology etc. Antioxidants are important class of chemical compounds and have various health benefits as well as used widely in the food industries as lipid peroxidation inhibitors. Different plants, vegetables, and plant materials like flower, leaf, roots, herbs, roots, seeds, pulp etc. are inspected for source of antioxidants (Aparadh *et.al*, 2012).

1,1-diphenyl-2-picryl hydrazyl (DPPH) is used for the DPPH assay technique to test the antioxidant activity of freshly synthesized compounds and to determine their radical scavenging capacity. The equation below demonstrates that the DPPH radical is a stable radical whose scavenging activity is measured by the drop in absorbance at 517 nm as a result of reduction by the antioxidant or interaction with the radical species.

$$DPPH^{\bullet} + R^{\bullet} \longrightarrow DPPH\text{-}R$$

The radical scavenging activity of any extract or compound can be determined by;

$$\% \text{ RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \ldots\ldots\ldots\ldots (1)$$

After plotting the % RSA vs concentration graph, using a four-parameter logistic regression model graph is plotted and the IC_{50} is calculated using the Quest graph ^{TM} IC_{50} calculator at aatbio.com. The equation used to determine IC_{50} regression is as follows:

$$y = \frac{\text{Max} - \text{Min}}{1 + \left(\frac{x}{\text{IC}_{50}}\right)^{h}} \ldots\ldots\ldots (2)$$

Where y = Final IC_{50} value, Max = maximum value, Min = minimum value, h = Hill coefficient.

4.6 Cytotoxicity assay

4.6.1 Brine shrimp bioassay/ Toxicity assay

Brine shrimp bioassay was carried out according to Bajracharya *et al.* (2011). A brine shrimp is a small crustacean called *Artemia salina*. The eggs of these species are inexpensively accessible and may survive for years in a dry state. In around 48 hours, the eggs molt, producing a large number of larvae (nauplii) in the brine solution. For this assay's biological screening, larvae are employed. It is an efficient, quick, in-house method that is also cheap for showing active synthesized chemicals. Using this procedure, the LC_{50} values (μg/mL) of several synthetic chemicals as well as plant extracts are also determined. Chemically speaking, substances having LC_{50} values under 1000 ppm (μg/mL) are often regarded as active.

4.6.2 Required materials for bioassay

For this technique, eggs of Brine shrimp (*Artemia salina*), artificial sea water, sterilized beakers for hatching, table lamp, sterilized disposable pipette, micropipette, sterilized test tube and test tube stand were required.

4.6.3 General procedure of brine shrimp bioassay

4.6.3.1 Preparation of the artificial sea water

The preparation of artificial sea water was done by dissolving the following chemicals in 1-liter distilled water and the pH was maintained to 8 ± 0.2.

Table 4.2: Chemical composition of sea water

S.N.	Composition	Amount (g/L)
1.	NaCl	23.5000
2.	Na_2SO_4	4.0000
3.	KCl	0.6800
4.	H_3BO_3	0.0270
5.	$MgCl_2.2H_2O$	10.6800
6.	$CaCl_2.2H_2O$	1.4800
7.	$NaHCO_3$	0.1970
8.	Na_2EDTA	0.0003

4.6.3.2 Hatching of the brine shrimp eggs

One full spatula eggs of brine shrimp were sprinkled on the artificial sea water taken in a plastic container. The container was illuminated for 48 hours using bulb (60 Watt) at room temperature. The eggs hatching container was adjusted by passing heat, light and air. After 48 hr., large number of tiny larvae (nauplii) can be seen swimming on the container. The nauplii are then counted and transferred to test tubes for toxicity assay.

4.6.3.3 Sample preparation

Firstly, stock solution of 10000 ppm was prepared in the methanol. Solutions of concentrations 1000 ppm, 100 ppm, 10 ppm were prepared by serial dilution of the prepared stock solution. After that, experiments were performed in the triplicate. Dry sterilized test tubes were labeled properly. In nine different test tubes 2 mL solutions were transferred from each solution (1000 ppm, 100 ppm, 10 ppm), three for each concentration. Similarly, blank solution of 2 mL methanol in three test tubes (as blank) was prepared. The methanol in these test tubes were evaporated in hot air oven for 24 hours.

4.6.3.4 Bioassay procedure

5 mL of the artificially created sea water was added to each test tube once the solvent had fully evaporated, and it was properly mixed. Then, using a Pasteur pipette, 10 mature, living brine shrimp nauplii were removed and placed in 12 test tubes. After 24 hours, the surviving nauplii were counted.

4.6.3.5 Data analysis

LC_{50} value is the lethal concentration dose needed to kill 50% of organisms. It can be determined as follows.

If 'n' is the number of replicates (in this experiment three), 'x' is the log of the concentration of the solution in µg/mL (log10, log100, log1000 here) and 'y' is the probit for average survivors for all replicates, then we have,

$$\alpha = \frac{[\sum y - \beta \sum x]}{n} \dots\dots (1)$$

$$\beta = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}} \dots\dots (2)$$

Now, from the probit regression,

$$y = \alpha + \beta x \dots\dots (3)$$

$$x = \frac{y - \alpha}{\beta} \dots\dots (4)$$

Here, Z= Concentration, *x*= log(Z) & *y* is constant having value 5 for calculating LC_{50} value. Hence,

$$LC_{50} = \text{Antilog}\,(x) \dots\dots (5)$$

The calculation of the data for probit regression, calculation of LC_{50} values and plots were done in MATLAB software and plots were visualized in Orange data mining software Version 3.32.

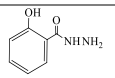
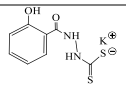
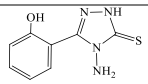
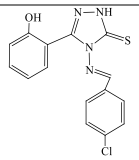
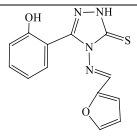
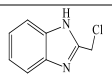
Chapter-5

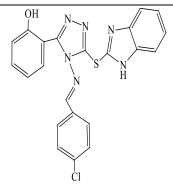
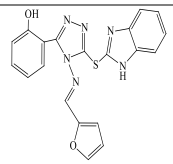
5. RESULTS AND DISCUSSION

5.1 General discussion

The Schiff's bases of 1,2,4 triazole derivatives were prepared from methyl salicylate as a starting material. 2-Chloromethylbenzimidazole was prepared separately and the Schiff's base was reacted with it and final compounds were synthesized in the convergent synthesis manner. Intermediates were prepared throughout the reaction steps in good yield and were purified by recrystallization process. The melting point was determined for each synthesized compound and TLC plates were made for assigning R_f values and assurance of purity of synthesized compounds. The physical properties of synthesized compounds are summarized in the table 5.1 below.

Table 5.1: Physical properties of the synthesized compounds

Compound	Structure	Physical Appearance	Molecular Formula	Molecular Mass	Yield (in %)	M.pt. (in ˚C)	R*f* Value
1		White Solid	$C_7H_8N_2O_2$	152.15	78	137-140	0.64
2		White Solid	$C_8H_7KN_2O_2S_2$	266.38	87.35	241-243	0.58
3		White Solid	$C_8H_8N_4OS$	208.24	84.05	178-180	0.43
5a		White Cottony Solid	$C_{15}H_{11}ClN_4OS$	330.79	72.09	215	0.78
5b		Greyish Cottony Solid	$C_{13}H_{10}N_4O_2S$	286.31	70.69	210	0.76
6		Yellow Solid	$C_8H_7ClN_2$	166.61	79	154	0.71

7a		Dark Green solid	$C_{22}H_{15}ClN_6OS$	446.91	78.25	174-176	0.77
7b		Dark Brown solid	$C_{20}H_{14}N_6O_2S$	402.43	80.42	160-162	0.74

5.2 UV-VIS spectroscopy

The UV Visible spectra of all the synthesized compounds (acid hydrazide, dithiocarbazinate, 1,2,4-triazole-5-thione, Schiff's bases, 2-chloromethyl benzimidazole, and benzimidazole derivatives are listed in table 5.2 below.

Table 5.2 Absorption maxima in the UV Visible spectrum of the synthesized compounds.

Compound	λ_{max}	Inference
3	302	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
	340	$n \rightarrow \pi^*$
5a	302	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
	340	$n \rightarrow \pi^*$
	370	$n \rightarrow \pi^*$
5b	302	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
	340	$n \rightarrow \pi^*$
	370	$n \rightarrow \pi^*$
6	292	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
7a	302	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
	340	$n \rightarrow \pi^*$
	370	$n \rightarrow \pi^*$
7b	302	$\pi \rightarrow \pi^*$
	309	$\pi \rightarrow \pi^*$
	331	$n \rightarrow \pi^*$
	340	$n \rightarrow \pi^*$
	370	$n \rightarrow \pi^*$

For compound 3, four bands at 302 nm, 309 nm, 331 nm, and 340 nm are observed in the UV absorption spectra. The first two peaks are due to presence of π→π* of C=C and C=N groups of aromatic triazole ring. The third band of 331 nm is due to n→π* transitions of C=N group that has the non-bonding electrons. It is also associated with nonbonding electrons of sulphur atom in C=S. The fourth band at 353 nm is attributed to n→π* transitions associated with the *o*-hydroxy group.

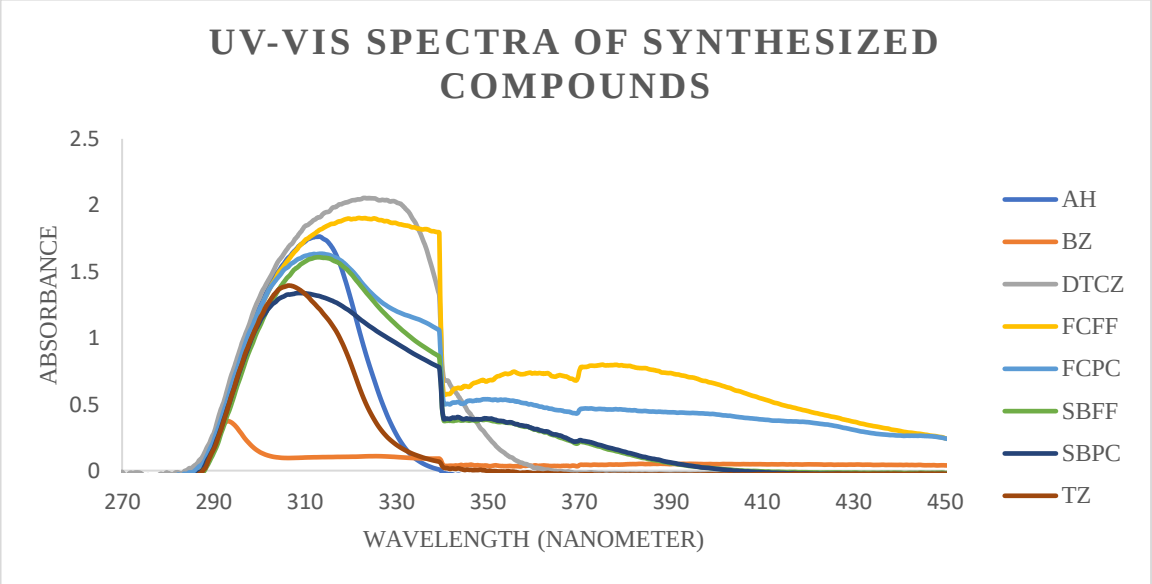


Figure 5.1: UV-VIS spectra of synthesized compounds (AH, BZ, DTCZ, FCFF, FCPC, SBFF, SBPC, TZ)

In an electronic spectrum of Schiff's base 5a(SBPC) and 5b(SBFF), five bands are observed in both compounds. The band at 302 nm is due to C=C and at 309 nm is due to C=N that has π electrons and cause π→π* transitions of the triazole ring. Third band is observed at 331 nm that relates with the transition of non- bonding electrons of nitrogen atom in triazole C=N and sulphur atom in C=S. Fourth band is observed at 340 nm that is associated with n→π* transitions of the non-bonding electrons in *o*-hydroxy group. Fifth band at 370 nm is attributed to n→π* transitions in C=N azomethine group.

For 2-chloromethyl benzimidazole, 3 bands are observed in the absorption spectra. The bands at 292 nm and 309 nm are due to π→π* transitions of aromatic C=C and C=N of the benzimidazole ring. Band at 331 nm is due to the n→π* transition of non-bonding electron in C-N of benzimidazole ring.

Five bands are exhibited in the absorption spectra of compounds 7a(FCPC) and 7b(FCFF). Two bands at 302 nm and 309 nm are due to π→π* transitions of the aromatic C=C and C=N electrons of the triazole ring. Next band at 331 nm is attributed to the non- bonding

electron pair of the nitrogen atom of triazole C=N. Another band at 340 nm is related with $n \rightarrow \pi^*$ transitions of the nonbonding electrons of *o*-hydroxy group. Band at 370 nm can be seen that is attributed to $n \rightarrow \pi^*$ transitions of electrons in C=N azomethine group.

The similarities in the UV-VIS peaks of the different shows the similarities between the compounds. The first two peaks 302 and 309 are due to presence of $\pi \rightarrow \pi^*$ of C=C and C=N groups of aromatic triazole ring and can be seen in compounds 3, 5a, 5b, 7a, and 7b that are derived from triazole. The Schiff' bases show similar peaks in the UV-VIS spectrum confirming the similarity in the compounds. The benzimidazole derivative shows peaks at similar range that further confirms the similarities between the derivatives.

5.3 FT-IR spectroscopy

The FT- IR measurement was done for the identification of possible functional groups present in the synthesized compounds: Triazole (3), Schiff bases (5a and 5b), and benzimidazole derivatives (7a & 7b) which is listed below.

Table 5.3: Diagnostic bands (cm^{-1}) in the FTIR spectra of the synthesized compounds

Wave number (cm^{-1})						
Groups	(3)	(5a)	(5b)	6	(7a)	(7b)
v(Ar O-H)	3287 (m)	3482 (m)	3443 (m)		3315 (m)	3472 (m,b)
v (NH)	3055 (m)	3218 (m)	3221(m)	3061 (s)	3140 (m)	3218 (m)
v (Ar C-H)	3024 (m)	3092 (m)	3092 (w)	3042 (s)	3074 (w)	3076 (s)
v (C=N)	1612 (m)	1620 (m)	1615 (m)	1622 (m)	1620 (s)	1620 (s)
δ(NH)	1590 (m)	1583 (m)	1583 (m)	1540 (s)	1584 (s)	1585 (s)
v (C=C)	1543 (m)	1528 (m)	1551 (m)	1448 (s)	1534 (m)	1534 (m)
	1492 (s)	1508 (m)	1542 (w)	1442 (s)	1485 (s)	1486 (s)
v (N-N)	1011 (m)	1015 (s)	1002 (s)		1015 (s)	1015 (s)
v (Ar C-O)	1240 (s)	1244 (s)	1243 (s)		1246 (s)	1245 (s)
v (C=S)	946 (s)	941 (m)	934 (m)		…	….
ρ(C-H)	741 (s)	737 (s)	756 (m)	735 (s)	744 (s)	740 (s)
	687 (s)	687(s)	687 (m)	698 (s)	696 (s)	702 (s)
v (C-Cl)				642 (s)		

In FT-IR spectrum of compound (3), a medium absorption band can be seen at 3287 cm^{-1} due to presence of phenolic functional group in the compound. The medium absorption band at 3055 cm^{-1} is due to stretching vibration of N-H bond in NH_2. This absorption band consists of two short peaks in which one peak is merged with –OH. The medium band for C-H stretching is seen at 3024 cm^{-1} and bonding peaks are seen at 741 and 687 cm^{-1}. Two bands for C=C is seen, one stretching medium band at 1543 and one strong bending band at 1492 cm^{-1}. Medium band for Aliphatic C=N bond stretching is seen at 1612 cm^{-1}, medium band for N-N bond is seen at 1011 cm^{-1} and N-H bending is seen at 1590 cm^{-1}, for C=S at 946 cm^{-1}. Aromatic C-O bending of medium absorption is seen at 1240 cm^{-1}. The formation of triazole can be confirmed by the medium stretching band at 946 cm^{-1} corresponding to the C=S group present in 1,2,4-triazole-3-thione. Also, we cannot see the absorption band of C=O group at around 1600-1700 cm^{-1} in the compound which indicates that it is cyclized in the triazole ring (Cretu *et al*., 2010).

Schiff's base (SBPC) shows a medium absorption band at 3482 cm^{-1} due to presence of phenolic functional group in the compound. The medium absorption band at 3218 cm^{-1} corresponds to stretching vibration of N-H bond. This absorption band consists of two short peaks in which one peak is merged with –OH. The medium band for aromatic C-H stretching is seen at 3092 cm^{-1} and bonding peaks are seen at 737 and 687 cm^{-1}. Two bands for C=C is seen, one stretching medium band at 1528 and one strong bending band at 1508 cm^{-1}. medium band for aliphatic C=N bond stretching is seen at 1620 cm^{-1}, medium band for N-N bond is seen at 1015 cm^{-1}, N-H bending is seen at 1583 cm^{-1}, for C=S at 941 cm^{-1}. Aromatic C-O bending of medium absorption is seen at 1244 cm^{-1}.

Schiff's base SBFF shows a similar medium absorption band at 3443 cm^{-1} as that of SBPC due to phenolic functional group in the compound. The medium absorption band for stretching vibration of N-H bond for SBFF can be seen at 3221 cm^{-1}. This absorption band consists of two short peaks in which one peak is merged with –OH. The medium band for aromatic C-H stretching is seen at 3042 cm^{-1} and two out of plane bonding peaks are seen at 756 and 687 cm^{-1}. Two bands for C=C is seen, one stretching medium band at 1551 and one strong bending band at 1542 cm^{-1}. Medium band for Aliphatic C=N bond stretching is seen at 1622 cm^{-1}, medium band for N-N bond is seen at 1002 cm^{-1}, N-H bending is seen at 1583 cm^{-1}, for C=S at 934 cm^{-1}. Aromatic C-O bending of medium absorption is seen at 1243 cm^{-1}. The formation of Schiff's bases can be confirmed by the absence of band in the

1700 cm^{-1} region. The absence of bands in 3200-3500 region relating to NH_2 protons also demonstrates the formation of Schiff's bases (Kapri & Shakya, 2018).

For compound (6), a medium absorption band of N-H stretching vibration at 3061 cm^{-1}. The medium band for aromatic C-H stretching is seen at 3042 cm^{-1} and two out of plane bonding peaks are seen at 735 and 698 cm^{-1}. Two bands for C=C is seen, one stretching strong band at 1448 and one strong bending band at 1442 cm^{-1}. Medium band for Aliphatic C=N bond stretching is seen at 1622 cm^{-1}, N-H bending is seen at 1540 cm^{-1}. A strong absorption at 642 cm^{-1} is seen for C-Cl bond stretching vibration. All the above information corresponds to the formation of benzimidazole (Alaqeel, 2017).

Benzimidazole derivative FCPC shows a medium absorption band at 3315 cm^{-1} due to presence of phenolic functional group in the compound. The medium absorption band at 3140 cm^{-1} is due to stretching vibration of N-H bond. This absorption band consists of two short peaks in which one peak is merged with –OH. The medium band for aromatic C-H stretching is seen at 3074 cm^{-1} and bonding peaks are seen at 744 and 696 cm^{-1}. Two bands for C=C is seen, one stretching medium band at 1534 and one strong bending band at 1485 cm^{-1}. Medium band for aliphatic C=N bond stretching is seen at 1620 cm^{-1}, medium band for N-N bond is seen at 1015 cm^{-1}, N-H bending is seen at 1584 cm^{-1} and aromatic C-O bending of medium absorption is seen at 1246 cm^{-1}.

Benzimidazole derivative FCFF shows a medium absorption band at 3472 cm^{-1} due to presence of phenolic functional group in the compound. The medium absorption band at 3218 cm^{-1} is due to stretching vibration of N-H bond. This absorption band consists of two short peaks in which one peak is merged with –OH. The medium band for aromatic C-H stretching is seen at 3076 cm^{-1} and bonding peaks are seen at 740 and 702 cm^{-1}. Two bands for C=C is seen, one stretching medium band at 1534 and one strong bending band at 1486 cm^{-1}. Medium band for aliphatic C=N bond stretching is seen at 1620 cm^{-1}, medium band for N-N bond is seen at 1015 cm^{-1}, N-H bending is seen at 1584 cm^{-1} and aromatic C-O bending of medium absorption is seen at 1245 cm^{-1}. The formation of benzimidazole derivative can be demonstrated by the absence of medium intensity bands for C=S group at around 1200-1300 cm^{-1}. There is also absence of strong intensity C-Cl absorption bands at around 550-800 cm^{-1} region further confirming the formation of benzimidazole derivative (Alaqeel, 2017).

5.4 NMR spectroscopy

5.4.1 ^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra data of the synthesized compounds, Schiff's bases (5a & 5b) and Benzimidazole derivatives (7a &7b) are listed below in Table 5.4.

Table 5.4: ^{1}H-NMR spectral assignments (ppm) of synthesized compounds (500 MHz, DMSO-d_6)

	Chemical Shift (ppm)				
Proton	TZ (4)	SBPC(5a)	FCPC(7a)	SBFF(5b)	FCFF(7b)
SH		14.13 (br s, 1H)		14.09(s, 1H)	
NH	13.86(br s, 1H)		12.69 (br s, 1H)		12.70(s, 1H)
OH	10.36 (br s,1H)	10.09 (s, 1H)	10.09(br s, 1H)	10.06(br s, 1H)	10.09(br s, 1H)
	7.43-7.31(m, 2H)	7.43-7.31 (m, 2H)			
	6.93(t, *J* = 7.57Hz)	7.00(d, *J* = 8.20Hz, 1H)			
NH_2	5.62(br s, 2H)				
N=CH		9.64 (s, 1H)	9.66 (s, 1H)	9.41(s, 1H)	9.41(s, 1H)
16				7.30 (d, *J* = 3.66, 1H)	7.30 (d, *J* = 3.66, 1H)
16 & 20		7.79 (d, *J* = 8.55, 2H)	7.77-7.83 (m, 2H)		
18				7.97 (br s, 1H)	7.97 (br s, 1H)
f & i			7.71 (d, *J* = 7.32 Hz, 2H)		7.72-7.88 (m, 2H)
17 & 19		7.57 (d, *J* = 7.93 Hz, 2H)	7.58 (d, *J* = 8.55 Hz, 2H)		
g & h			7.45 - 7.55 (m, 2H)		7.54-7.63 (m, 2H)
10 & 12		7.33-7.42 (m, 2H)	7.34- 7.4 (m, 2H)	7.34-7.45 (m, 2H)	7.36-7.40 (m, 2H)
9 & 11		6.88-6.95 (m, 2H)	6.88-7.05 (m, 2H)	6.87- 6.96 (m, 2H)	6.88- 6.95 (m, 2H)
17				6.73 (dd, *J* = 3.05, 1.83 Hz,1H)	6.73 (dd, *J* = 3.66, 1.83 Hz, 1H)

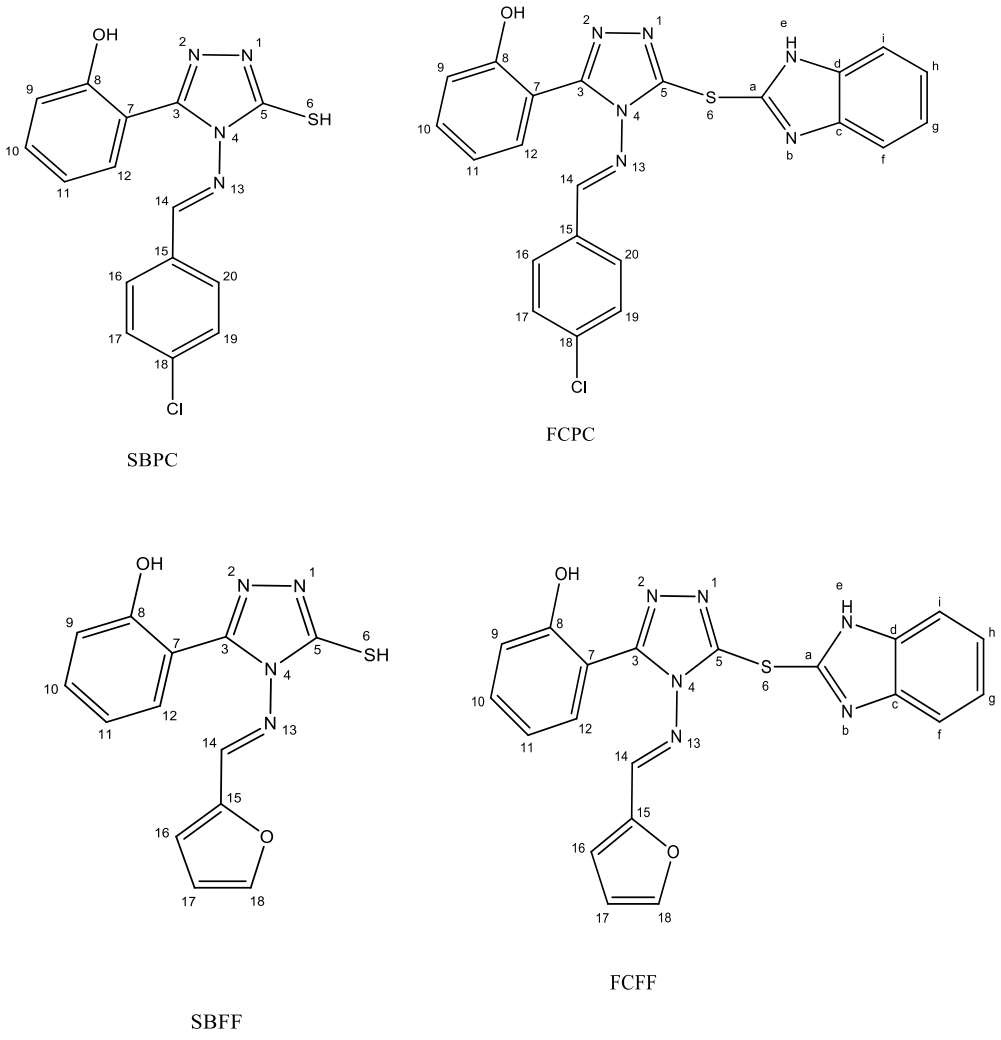


Figure 5.2: The numbering of atoms in the compounds SBPC, SBFF, FCPC and FCFF in NMR spectrum.

In ^{1}H-NMR spectrum of compound (3), the broad singlet at 13.86 ppm is attributed to the hydrogen associated with nitrogen which implies that triazole thione is formed (Silverstein *et. al.,* 1974). The singlet at 10.36 ppm is associated with the hydrogen of -OH group of 2-hydroxyphenyl group. The peak at 5.62 ppm attributes to the shielded hydrogen of NH_2 functional group.

Both of the Schiff's bases have singlet around 14 ppm. Schiff's base (5a) and (5b) contain a singlet at 14.13 ppm and 14.09 ppm due to the presence of SH proton. The singlet each at 10.09 ppm and 10.06 ppm is attributed to the hydrogen of OH group. The aromatic hydrogens due to *p*-chlorobenzyl were found at broad singlet at 7.79 ppm and 7.57 ppm. The aromatic hydrogens due to 2-hydroxyphenyl were found at broad singlet 7.33-7.42 ppm and 6.88-6.95 ppm. Moreover, the ^{1}H – NMR spectrum of Schiff's base showed a singlet at 9.64 due to the presence of –N=CH group. In Schiff base (5b), the peaks for aromatic hydrogens due to *p*-chlorobenzyl were found at broad singlet at 7.30 ppm and

6.73 ppm. The aromatic hydrogens due to 2-hydroxyphenyl were found at broad singlet 6.87-6.96 ppm and 7.34-7.45 ppm. Moreover, the ^{1}H–NMR spectrum of Schiff's base has a singlet at 9.41 from the Hydrogen of –N=CH group and no signals at 5.76 ppm (NH_2) in the molecule implies that Schiff bases are formed. The predominance of thione isomer in DMSO- d_6 can be confirmed by the absence of -SH signals at 4.0 ppm (Ali *et. al*., 2006).

The benzimidazole derivative (7a) shows a singlet at 12.69 ppm attributing to -NH proton. A singlet at 10.09 ppm is related to the hydrogen of OH group. The aromatic hydrogens due to *p*-chlorobenzyl were found at broad singlet at (7.77- 7.83) ppm and 7.58 ppm. The aromatic hydrogens due to 2-hydroxyphenyl were found at broad singlet 7.34-7.4 ppm and 6.88-7.05 ppm. Moreover, the ^{1}H–NMR spectrum of the benzimidazole derivative, a singlet at 9.66 can be seen relating to –N=CH group. The spectrum also shows signals at 7.71 and 7.45-7.4 due to the presence of aromatic hydrogens present in benzimidazole indicating the formation of benzimidazole derivative.

The benzimidazole derivative (7b) contains the singlet at 12.70 ppm due to the presence of NH proton. The singlet at 10.09 ppm is attributed to the hydrogen of -OH group. The aromatic hydrogens due to *p*-chlorobenzyl were found at broad singlet at (6.88- 6.95) ppm and (7.36-7.40) ppm. The aromatic hydrogens due to 2-hydroxyphenyl were found at broad singlet 7.34-7.4 ppm and 6.88-7.05 ppm. Moreover, the ^{1}H – NMR spectrum of the benzimidazole derivative showed a singlet at 9.66 due to the presence of –N=CH group. The spectrum also shows signals at (7.72-7.88) ppm and (7.54-7.63) ppm due to the presence of aromatic hydrogens present in benzimidazole indicating the formation of benzimidazole derivative (Alaqeel, 2017).

5.4.2 ^{13}C-NMR Spectroscopy

The ^{13}C-NMR spectra data of the synthesized compounds (3), Schiff's bases (5a & 5b) and Benzimidazole derivatives (7a &7b) are listed below in table 5.5.

Table 5.5: ^{13}C-NMR spectral assignments (ppm) of synthesized compounds(100MHz, DMSO-*d*6).

Carbon	Chemical Shift (ppm)			Chemical Shift (ppm)	
	Triazole	SBPC	FCPC	SBFF	FCFF
3	149.62	148.88	148.89	148.82	148.83
5	166.54	164.59	164.52	162.16	162.16

8	156.53	156.62	156.65	154.42	154.41
10	132.60	131.55	131.50	131.59	131.58
12	131.32	131.59	131.58	132.82	132.82
	119.53				
(Aromatic	116.67				
due to 2-hydroxyphenyl)	113.52				
14		162.24	162.25	156.56	156.75
15		130.65	130.65	148.34	148.33
16 & 20		132.91	132.88	120.89	120.89
17 & 19		129.79	129.79	120.89	120.89
18		137.76	137.74	147.66	147.68
A			156.75		156.66
c & d			143.62		143.62
g & h			129.40		129.41
f & i			119.59		119.58

The typical carbon resonance at δ 162.57-167.67 was the signal from C5 carbon of triazole i.e.C=S group (Wang *et. al*., 2011). The compounds 3, 5a, 5b 7a & 7b contains the spectrum at 166.54, 164.59, 164.52, 162.16 and 162.16 ppm respectively. This downfield value is due to deshielding in presence of sulphur along with two nitrogen atoms attached to α- position of triazole and thus the predominance of thione tautomer is revealed in TZ, SBPC and SBFF. The peaks 130.65, 132.91, 129.79, and 137.76 ppm attributes to the *p*-chlorobenzyl group in Schiff's base. Similar peaks can be identified such as 130.65, 132.88, 129.79, 137.74 ppm in the benzimidazole derivative. The aromatic carbons associated with the furfuryl group show chemical shift values at 143.62, 129.40, 129.41, 119.59, 119.58 ppm in the Schiff's base as well as the benzimidazole derivative. Different signals can be observed for C=N at 149.62, 148.88, 148.89, 148.82 and 148.83 ppm for compounds 3, 5a, 5b, 7a & 7b respectively. Different peaks can be seen at 156.62, 152.65, 154.42 and 154.41 ppm of 5a, 5b, 7a and 7b respectively relating to carbon in the azomethine group that also confirms Schiff base formation (Awad *et. al*., 2009). Similarly, peaks were found at 143.62, 143.62, 129.40, 129.41, 119.59 and 119.58 ppm representing the carbon of benzimidazole in the derivative. These peaks were not present in the Schiff's bases spectrum further confirming the structure (Alaqeel, 2017).

5.5 Antimicrobial Screening

5.5.1 Antibacterial activity of synthesized compounds

The Schiff's bases (5a and 5b) and Benzimidazole derivatives (7a and 7b) were screened against different bacterial strains and the result is summarized in table 5.6.

Table 5.6: Antibacterial screening of synthesized compounds in DMSO.

Bacterial strains	Compd (5a) SBPC	Compd (5b) SBFF	Compd (7a) FCPC	Compd (7b) FCFF	Positive Control	Conc^{n}	ZOI (mm)
	Conc^{n}: 100 mg/mL, ZOI (mm)					(mcg/mL)	
Bacillus subtilis[a] (ATCC 6051)	0	0	0	0	Ciprofloxacin	5	31.02
Enterococcus faecalis[a] (ATCC 6051)	0	0	0	0	Ciprofloxacin	5	20.03
Escherichia coli[b] (ATCC 29212)	0	0	0	0	Ciprofloxacin	5	28.00
Klebsiella pneumoniae[b] (ATCC 700603)	0	0	0	0	Ciprofloxacin	5	25.90
Proteus vulgaris[b] (ATCC 8360)	0	0	0	0	Ciprofloxacin	5	27.30
Pseudomonas aeruginosa[b] (ATCC 9027)	0	0	0	0	Ciprofloxacin	5	33.86
Salmonella typhii[b]	0	0	0	0	Ciprofloxacin	5	25.00
Shigella dysenteriae[b]	0	0	0	0	Ciprofloxacin	5	27.72
Staphylococcus aureus[a] (ATCC 6538P)	14.20	0	9.74	9.12	Azithromycin	30	30.18
Staphylococcus epidermidis[a] (ATCC 1228)	16.12	15.5	0	0	Azithromycin	30	29.72
a= gram positive bacteria b= gram negative bacteria							

The antibacterial activity of the synthesized compounds were tested against ten organisms and they showed no activity in most of the bacterial strains and moderate activity against some bacterial strains: *S. aureus* and *S. epidermidis* using Ciprofloxacin and Azithromycin as standard (positive controls).

The tested compounds were found active against two gram-positive bacteria. Schiff's bases SBPC and SBFF showed more activity than the Benzimidazole derivatives. For the organism, *S. aureus*, SBPC had a ZOI of 14.20, FCPC with 9.74 mm and FCFF with 9.12 mm whereby the azithromycin had a ZOI of 30.18 mm. For the organism, *S. epidermidis*, SBPC had a ZOI of 16.12 mm, SBFF with 15.5 mm at which azithromycin had a ZOI of 29.72 mm.

The tested compounds were found active against gram-positive bacteria only. The activities against specific type of bacterium can be due to the possibility of the compounds to be of narrow spectrum antibacterial drugs or the compounds maybe active against untested bacterial strains.

5.5.2 Minimum inhibitory concentration (MIC) and Minimum microbicidal concentration (MMC)

MIC refers to minimum inhibitory concentration and is lowest concentration of a chemical where the growth of bacteria is prevented. MMC refers to the Minimum microbicidal concentration and refers to lowest concentration of compound or extract required to kill the specific bacterium. The values of MIC and MMC are summarized in table 5.7.

Table 5.7: MIC, MMC of the synthesized compounds

		Zone of Inhibition (mm)					
Compounds	Conc^n	Bacterial species					
		S. aureus	MIC (mg/mL)	MMC between (mg/mL)	*S. epidermidis*	MIC (mg/mL)	MMC between (mg/mL)
5a, SBPC	100mg/mL	14.2	12.5	12.5-25	16.12	12.5	12.5- 25
5b, SBFF	100mg/mL	0.0	-	-	15.5	12.5	12.5- 25
7a, FCPC	100mg/mL	9.74	12.5	12.5- 25	0.0	-	-
7b, FCFF	100mg/mL	9.12	12.5	12.5-25	0.0	-	-
Azithromycin	30mcg/mL	30.18			29.72		

The Schiff base SBPC had MMC of 12.5-25 mg/mL for the organisms, *S. aureus* and *S. epidermidis*. Compound SBFF had MMC of 12.5-25 mg/mL for *S. epidermidis*. The benzimidazole derivatives FCPC and FCFF had MMC of 12.5-25 mg/mL for bacterium *S. aureus*. The results show that the bacterium *S. aureus* is resistant to the compounds SBPC, FCPC and FCFF with MIC at 12.5 mg/mL (>200 μg/mL) and completely resistant to SBFF.

Similarly, the bacterium *S. epidermidis* is resistant to the Schiff's bases SBPC, SBFF with MIC at 12.5 mg/mL and completely resistant with the benzimidazole derivatives FCPC and FCFF (Khatun *et al*., 2012).

5.5.3 Antifungal activity of synthesized compounds

The Schiff's bases (5a and 5b) and Benzimidazole derivatives (7a and 7b) were screened against different fungal strains and the result is summarized in table 5.8.

Table 5.8: Antifungal screening of synthesized compounds in DMSO.

Fungal strains	Compd (5a) **SBPC**	Compd (5b) **SBFF**	Compd (7a) **FCPC**	Compd (7b) **FCFF**	**Positive Control**	**Conc^{n}**	**ZOI (mm)**
Conc^{n}: 2 mg/mL, ZOI (mm)							
Candida albicans (ATCC 2091)	0	0	0	0	Clotrimazole	200 mcg/mL	30.50
Saccharomyces cerevisiae (ATCC 18824)	0	0	0	0	Clotrimazole	200 mcg/mL	21.00
Alternaria alternata	0	0	0	0	Carbendazim	0.1%	30.00
Fusarium oxysporum	0	0	0	0	Carbendazim	0.1%	30.00

None of the tested compounds (5a, 5b, 7a, 7b) showed any noticeable antifungal activities. The solvent in which the compounds were dissolved might have made the compounds less stable resulting in low activity, so needs to be tested either using new solvent or in the presence of a stabilizer. The bulkier groups present in the compound may have led to steric hindrance in the molecules that decreased the biological activity. The panel of test organisms need to be broadened to ensure the absence of activity for any of the compounds

5.6 Antioxidant activity of synthesized compounds

Antioxidant activity was studied from DPPH assay of synthesized compounds with reference to ascorbic acid. The absorbance values of different concentration of SBPC, SBFF, FCFF, FCPC of 10, 20, 40, 60, 80, and 100 ppm were measured at 517 nm. These values were used to calculate percentage inhibition of DPPH radicals against the sample. The antioxidant activity was estimated by plotting free radical scavenging percentage vs concentration and IC_{50} value of respective synthesized compounds. The IC_{50} values of compounds were calculated from the percentage inhibition. The observed absorbance with the different concentrations in the form of free radical scavenging percentage of ascorbic

acid as well as the compound TZ is plotted in the graph as shown in figure 5.3 and 5.4. The data for percentage radical scavenging activity and IC_{50} values reported for the compounds 5a, 5b, 7a and 7b are given in Table 5.9.

Table 5.9: Radical scavenging percentage and IC_{50} values of 5a,5b, 7a, 7b, TZ and Ascorbic Acid.

λ	Compounds	% Radical Scavenging activity					IC_{50} ($\mu g mL^{-1}$)
		Concentration (ppm)					
		20	40	60	80	100	
517	SBPC(5a)	15.195	27.926	47.228	66.530	77.823	62.137
517	SBFF(5b)	28.131	52.772	66.735	78.645	93.018	38.941
517	FCPC(7a)	2.464	6.571	14.784	17.454	25.462	206.367
517	FCFF(7b)	2.053	28.337	40.246	69.199	78.645	64.110
517	TZ(3)	30.801	58.932	72.485	83.984	87.680	32.364
517	Ascorbic acid	43.326	59.959	74.127	89.938	99.589	28.546

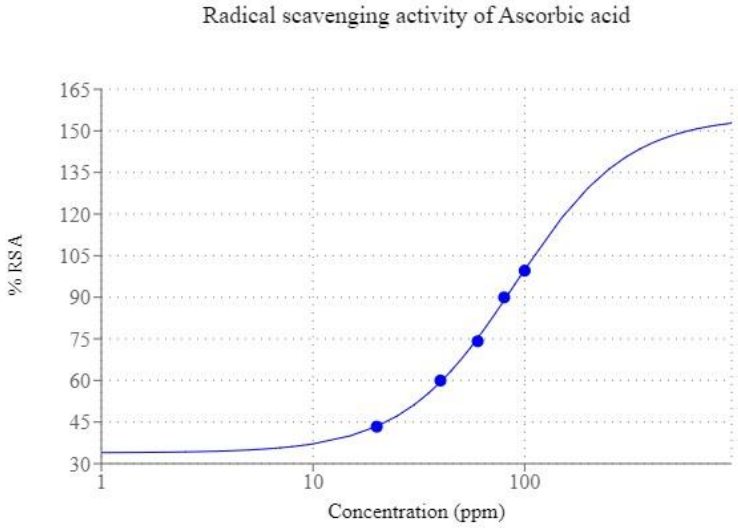


Figure 5.3: Antioxidant activity of ascorbic acid

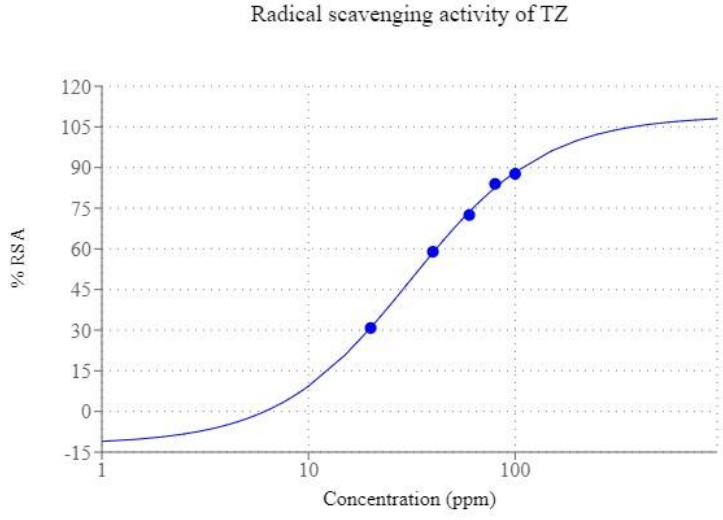


Figure 5.4: Antioxidant activity of triazole-5-thione(3)

The radical scavenging activity is expressed in the form of IC_{50}. The IC_{50} value of ascorbic acid was reported to be 28.546 μgmL^{-1}. The compounds showed moderate radical scavenging activity against DPPH with values SBPC (62.137 μgmL^{-1}), SBFF (38.941 μgmL^{-1}), FCPC (206.367 μgmL^{-1}), FCFF (64.110 μgmL^{-1}). Among the tested five compounds, the IC_{50} value of triazole thione (TZ) was found to be 32.364 μgmL^{-1} which is closer to the ascorbic acid sample.

5.7 Brine Shrimp bioassay/ Cytotoxicity assay

The synthesized compounds (5a, 5b, 7a, 7b) were subjected to brine shrimp bioassay. At different concentrations, the no. of nauplii added, alive, dead, and no. of replicates were collected. The data is summarized in table 5.10.

Table 5.10: Toxicity assay of synthesized compounds.

Compound	Conc^n in µg/mL (Z)	No. of Nauplii Added	Alive (y)	Death	Death %	No of replicates	X= log(Z)	Xy	y^2
	1000	10	4	6	60	3	3	12	9
SBPC(5a)	100	10	8	2	20	3	2	16	4
	10	10	9	1	10	3	1	9	1
	1000	10	4	6	60	3	3	12	9
SBFF(5b)	100	10	7	3	30	3	2	14	4
	10	10	10	0	0	3	1	10	1
	1000	10	1	9	90	3	3	3	9
FCPC(7a)	100	10	6	4	40	3	2	12	4
	10	10	8	2	20	3	1	8	1
	1000	10	4	6	60	3	3	12	9
FCFF(7b)	100	10	6	4	40	3	2	12	4
	10	10	9	1	10	3	1	9	1

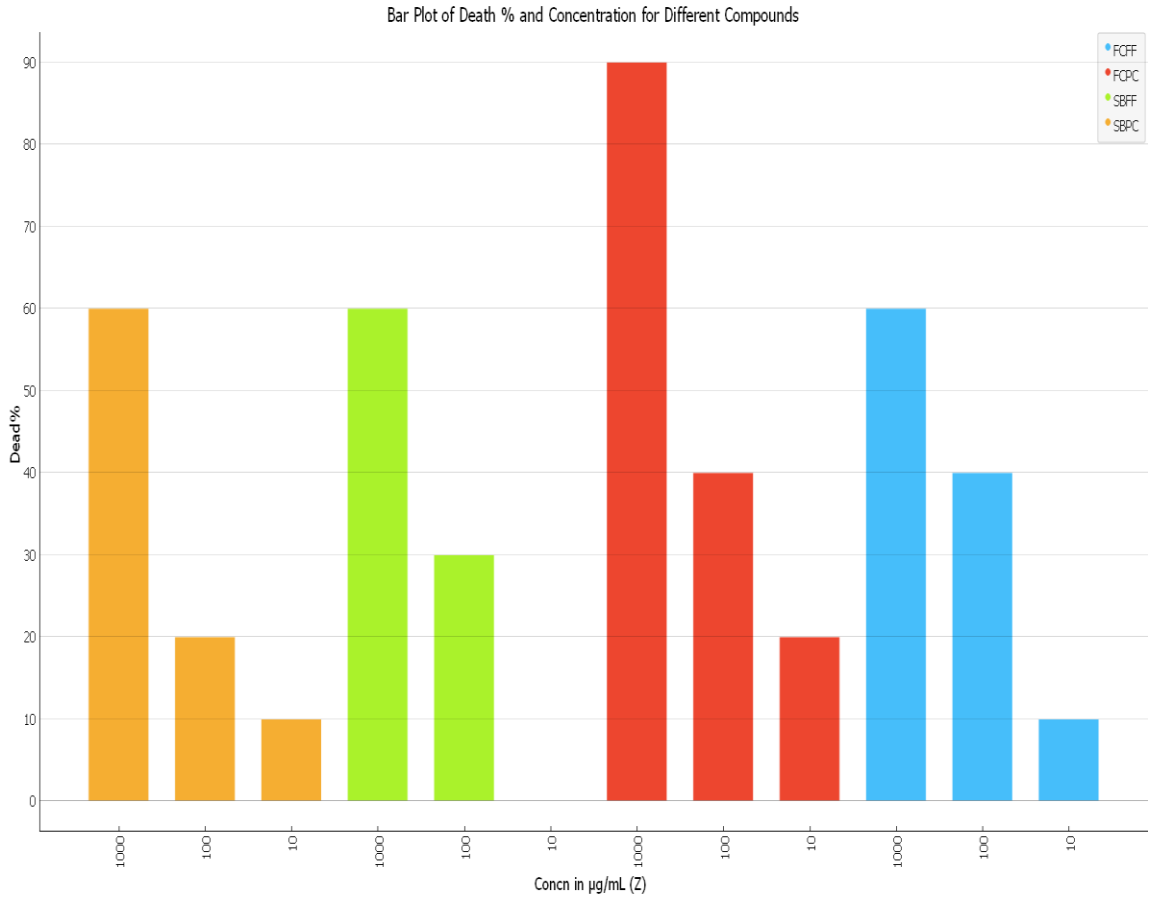


Figure 5.5: Death percentage of nauplii after 24 hr. at different concentrations.

Table 5.11: Calculated toxicity of the synthesized compounds.

Compound	β	α	X	LC_{50} (µgmL^{-1})
SBPC(5a)	-2.5	12	2.800	630.957
SBFF(5b)	-3	12	2.333	215.443
FCPC(7a)	-3	10	1.428	26.87
FCFF(7b)	-2.5	11.33	2.533	341.455

The compounds that have LC_{50} value less than 1000 is counted active and toxic pharmacologically. from the table 5.10, it can be seen that all the synthesized compounds are toxic to the brine shrimp at different concentrations. Based on the obtained data of LC_{50} values compounds SBPC, SBFF and FCFF are seen as moderately toxic with LC_{50} values 630.957 µgmL^{-1}, 215.443 µgmL^{-1} and 341.455 µgmL^{-1} respectively whereas the compound FCPC is comparatively more toxic with LC_{50} value of 26.827µgmL^{-1}. Figure 5.5 shows the

death percentage of nauplii at three different concentrations where the death percentage of nauplii is seen highest for the compound FCPC. Other tested compounds showed similar death percentage of nauplii at the at 1000 μgmL^{-1} concentration.

Chapter-6

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The Schiff's bases and benzimidazole derivatives containing 1,2,4-triazole moiety has centered itself in the field of research in recent times because of their potential utility in medicinal chemistry, agriculture, corrosion science, complex chemistry and chemical synthesis.

The Schiff's bases 4-(5-chlorobenzene-2-yl-methyleneamino)-3-(2- hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione**(5a)** and 4-(4-furan-2-yl-methyleneamino)-3-(2- hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione**(5b)** as well as the benzimidazole derivatives 5-((1*H*-benzo[*d*]imidazol-2-yl) thio)-4-((4-chlorobenzylidene) amino)-4*H*-1,2,4-triazol-3-yl) (2-hydroxyphenyl) methanone **(7a)** and 5-((1*H*-benzo[d]imidazol-2-yl) thio)-4-((4-chlorobenzylidene) amino)-4*H*-1,2,4-triazol-3-yl) (2-hydroxyphenyl)methanone **(7b)** were successfully prepared in lab. They were characterized by spectroscopic techniques such as UV, IR, ^{1}H-NMR and ^{13}C- NMR. The synthesized compounds showed moderate activity against some bacterial strains but were unexpectedly non-effective against the fungal strains. The compound Schiff's base 5a was found to be more effective than compound 5b against bacterial strains whereas both of the derivatives showed no antifungal activities. Despite having chemical moieties/groups with known antifungal as well as antibacterial properties, the tested compounds did not show any such biological activities.

The IC_{50} value of Ascorbic acid was found to be 28.546 μgmL^{-1}. The compound triazole 3-thione showed good antioxidant property with IC_{50} value of 32.364 μgmL^{-1}. Schiff's bases and benzimidazole derivatives showed good to moderate antioxidant properties. This is due to the presence of -OH group in the triazole derivative.

For the toxicity assay, all compounds were found toxic against the brine shrimp. Among them, the compound FCPC is found comparatively more toxic with LC_{50} value of 26.827 μgmL^{-1} and highest death percentage.

6.2 Recommendations

The work that has been done here assures the possibility of synthesized compounds in the field of pharmaceuticals to help with the problem of increased antibiotic resistance. However, the study of various other biological activities is yet to be done which may

unlock their new biological potential. Biological activitieslike antidiabetic, analgesic, antitubercular are the future recommendation of this work.

There is no denying that Schiff's bases containing 1,2,4- triazole moiety has been the area of study and research in recent times due to its exceptional biopotentials. In this paper, the addition of benzimidazole moiety explored new potentials for the variety of compounds that could be synthesized. Hence, this work would like to be going ahead in the same direction.

REFERENCES

Alagarsamy, V., Kavitha, K., Rupeshkumar, M., Solomon, V.R., Kumar, J., Kumar, D.K.S., & Sharma, H. (2009). Synthesis and pharmacological investigation of novel 4-(3-ethylphenyl)-1-substituted-4*H*-(1,2,4) triazolo (4,3-*a*) quinazolin-5-ones as a new class of H1-antihistaminic agents. *Acta Pharmaceutica*, *59*(1), 97-106.

Alaghaz, A. N. M., Zayed, M. E., Alharbi, S. A., Ammar, R. A., & Chinnathambi, A. (2015). Synthesis, spectroscopic identification, thermal, potentiometric and antibacterial activity studies of 4-amino-5-mercapto-*s*-triazole Schiff's base complexes. *Journal of Molecular Structure*, *1087*, 60-67.

Alaqeel, S. I. (2017). Synthetic approaches to benzimidazoles from o-phenylenediamine: A literature review. *Journal of Saudi Chemical Society*, *21*(2), 229-237.

Al-Soud, Y. A., Al-Dweri, M. N., & Al-Masoudi, N. A. (2004). Synthesis, antitumor and antiviral properties of some 1,2,4-triazole derivatives. *Il Farmaco*, *59*(10), 775-783.

Ammal, P. R., Prajila, M., & Joseph, A. (2018). Physicochemical studies on the inhibitive properties of a 1,2,4-triazole Schiff's base, HMATD, on the corrosion of mild steel in hydrochloric acid. *Egyptian Journal of Petroleum*, *27*(3), 307-317.

Ansari, K. F., Lal, C., & Khitoliya, R. K. (2011). Synthesis and biological activity of some triazole-bearing benzimidazole derivatives. *Journal of the Serbian Chemical Society*, *76*(3), 341-352.

Ansari, K. R., Quraishi, M. A., & Singh, A. (2014). Schiff's base of pyridyl substituted triazoles as new and effective corrosion inhibitors for mild steel in hydrochloric acid solution. *Corrosion Science*, *79*, 5-15.

Aswathanarayanappa, C., Bheemappa, E., Bodke, Y. D., Krishnegowda, P. S., Venkata, S. P., & Ningegowda, R. (2013). Synthesis and evaluation of antioxidant properties of novel 1,2,4-triazole-based Schiff base heterocycles. *Archiv der Pharmazie*, *346*(12), 922-930.

Akhtar, T., Hameed, S., Al-Masoudi, N. A., & Khan, K. M. (2007). Synthesis and anti-HIV activity of new chiral 1,2,4-triazoles and 1,3,4-thiadiazoles. *Heteroatom Chemistry: An International Journal of Main Group Elements*, *18*(3), 316-322.

Alamgir, M., Black, D. S. C., & Kumar, N. (2007). Synthesis, reactivity and biological activity of benzimidazoles. *Bioactive heterocycles III*, 87-118.

Ali, M. A., Mirza, A. H., Butcher R. J., and Crouse, K. A., (2006). The preparation, characterization and biological activity of palladium(II)and platinum(II) complexes of tridentate NNS ligands derived from S-methyl- and S-benzyldithiocarbazates and the X-ray crystal structure of the [Pd(mpasme)Cl] complex. *Transition Metal Chemistry*, **31**, 79–87.

Aparadh, V. T., Naik, V. V., & Karadge, B. A. (2012). Antioxidative properties (TPC, DPPH, FRAP, metal chelating ability, reducing power and TAC) within some Cleome species. *Annali di Botanica*, *2*, 49-56.

Awad, M. K., Issa, R. M., & Atlam, F. M. (2009). Theoretical investigation of the inhibition of corrosion by some triazole Schiff bases. *Materials and corrosion*, *60*(10), 813-819.

Bajracharya, G. B., & Tuladhar, S. M. (2011). Brine-shrimp bioassay for assessment of anticancer property of essential oils from spices. *Nepal Journal of Science and Technology*, *12*, 163-170.

Batchelor, D. V., Beal, D. M., Brown, T. B., Ellis, D., Gordon, D. W., Johnson, P. S., Helen, J. M., Ralph, M. J., Underwood, T. J. & Wheeler, S. (2008). A convenient synthesis of highly substituted 3-*N*, *N*-dialkylamino-1, 2, 4-triazoles. *Synlett*, *2008*(16), 2421-2424.

Bayrak, H., Demirbas, A., Karaoglu, S. A., & Demirbas, N. (2009). Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. *European Journal of Medicinal Chemistry*, *44*(3), 1057-1066.

Bekircan, O., Kahveci, B., & Küçük, M. (2006). Synthesis and anticancer evaluation of some new unsymmetrical 3, 5-diaryl-4*H*-1,2,4-triazole derivatives. *Turkish Journal of Chemistry*, *30*(1), 29-40.

Bhandari, S. V., Bothara, K. G., Raut, M. K., Patil, A. A., Sarkate, A. P., & Mokale, V. J. (2008). Design, synthesis and evaluation of antiinflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1, 3, 4-oxadiazole-2-thiol and Schiff bases of diclofenac acid as nonulcerogenic derivatives. *Bioorganic & medicinal chemistry*, *16*(4), 1822-1831.

Bhat, M. A., & Al-Omar, M. A. (2011). Synthesis, characterization and *in vivo* anticonvulsant and neurotoxicity screening of Schiff bases of phthalimide. *Acta Poloniae Pharmaceutica*, *68*(3), 375-380.

Blair, L. M., & Sperry, J. (2013). Natural products containing a nitrogen–nitrogen bond. *Journal of Natural Products*, *76*(4), 794-812.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Cretu, O. D., Barbuceanu, S. F., Saramet G., and Draghici, C., (2010). Synthesis and characterization of some 1,2,4-triazole-3-thiones obtained from intramolecular cyclization of new 1-(4-(4-X-phenylsulfonyl)benzoyl)-4-(4-iodophenyl)-3-thiosemicarbazides. *Journal of Serbian Chemical Society*, *75(11)*, 1463-1471.

Castanedo, G. M., Seng, P. S., Blaquiere, N., Trapp, S., & Staben, S. T. (2011). Rapid synthesis of 1,3,5-substituted 1,2,4-triazoles from carboxylic acids, amidines, and hydrazines. *The Journal of Organic Chemistry*, *76*(4), 1177-1179.

Daniel, L., Jorg, L., and Harald, K., (2010). Highly functionalised 3,4,5-trisubstituted 1,2,4-triazoles for future use as ligands in coordination polymers. *Tetrahedron Letters, 51*, 653-656.

Duan, Y. C., Ma, Y. C., Zhang, E., Shi, X. J., Wang, M. M., Ye, X. W., & Liu, H. M. (2013). Design and synthesis of novel 1,2,3-triazole-dithiocarbamate hybrids as potential anticancer agents. *European Journal of Medicinal Chemistry*, *62*, 11-19.

Fernandes, G. F. D. S., Man Chin, C., & Dos Santos, J. L. (2017). Advances in drug discovery of new antitubercular multidrug-resistant compounds. *Pharmaceuticals*, *10*(2), 51-68.

Freund, M., (1896). A process for the representation of triazole and its homologues. *Berichte der Deutschen Chemischen Gesellschaft, 29*, 2483-2490.

Guo, L., Zhu, S., Zhang, S., He, Q., & Li, W. (2014). Theoretical studies of three triazole derivatives as corrosion inhibitors for mild steel in acidic medium. *Corrosion Science*, *87*, 366-375.

Havaldar, F. H., & Patil, A. R. (2008). Syntheses of 1, 2, 4 triazole derivatives and their biological activity. *Journal of Chemistry*, *5*(2), 347-354.

Higashio, Y., & Shoji, T. (2004). Heterocyclic compounds such as pyrrole, pyridines, pyrrolidine, piperidine, indole, imidazole and pyrazines. *Applied Catalysis A: General*, *260*(2), 251-259.

Huntsman, E., & Balsells (2005). New Method for the General Synthesis of [1,2,4]triazolo [1,5-*a*] pyridines. *Journal of Organic Chemistry,* 3761-3765.

Jain, P., Sharma, P. K., Rajak, H., Pawar, R. S., Patil, U. K., & Singour, P. K. (2010). Design, synthesis and biological evaluation of some novel benzimidazole derivatives for their potential anticonvulsant activity. *Archives of Pharmacal Research*, *33*(7), 971-980.

Kapri, K. P., & Shakya, B. (2018). Synthesis, characterization and antimicrobial evaluation of Schiff bases of 4-amino-5-(4-tert-butylphenyl)-4*H*-1,2,4-triazole-3-thiol. *Journal of Nepal Chemical Society*, *39,* 1-9.

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

Kaur, R., Ranjan Dwivedi, A., Kumar, B., & Kumar, V. (2016). Recent developments on 1, 2, 4-triazole nucleus in anticancer compounds: a review. *Anti-Cancer Agents in Medicinal Chemistry*, *16*(4), 465-489.

Kazimierczuk, Z., Upcroft, J. A., Upcroft, P., Górska, A., Starosciak, B., & Laudy, A. (2002). Synthesis, antiprotozoal and antibacterial activity of nitro-and halogeno-substituted benzimidazole derivatives. *Acta Biochimica Polonica-English Edition*, *49*(1), 185-196.

Khanmohammadi, H., Abnosi, M. H., Hosseinzadeh, A., & Erfantalab, M. (2008). Synthesis, biological and computational study of new Schiff base hydrazones bearing 3-(4-pyridine)-5-mercapto-1,2,4-triazole moiety. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *71*(4), 1474-1480.

Khatun, S., Pervin, F., Karim, M. R., Ashraduzzaman, M., & Rosma, A. (2012). Phytochemical screening and antimicrobial activity of Coccinia cordifolia L. plant. *Pakistan journal of pharmaceutical sciences*, *25*(4). 757-761.

Kido, J., Ohtaki, C., Hongawa, K., Okuyama, K., & Nagai, K. (1993). 1,2,4-Triazole derivative as an electron transport layer in organic electroluminescent devices. *Japanese journal of applied physics*, *32*(7A), L917.

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International Journal of Parasitology*, *31*, 336–345.

Kulkarni, A. A., Wankhede, S. B., Dhawale, N. D., Yadav, P. B., Deore, V. V., & Gonjari, I. D. (2017). Synthesis, characterization and biological behavior of some Schiff's and Mannich base derivatives of Lamotrigine. *Arabian Journal of Chemistry*, *10*, S184-S189.

Kumar, D., Jacob, M. R., Reynolds, M. B., & Kerwin, S. M. (2002). Synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1. *Bioorganic & Medicinal Chemistry*, *10*(12), 3997-4004.

Kumar, P., Kuamr, A., Mohan, L. J., & Makrandi, J. K. (2010). Heterocyclic systems containing bridgehead nitrogen atom: Synthesis and evaluation of biological activity of imidazo[2,1-*b*]-1,3,4-thiadiazolo[2,3-*c*]-*s*-triazoles, *s*-triazolo[3,4-*b*]-1,3,4-thiadiazolo[3,2-*b*]imidazo[4,5-*b*]quinoxaline and bis-(*s*-triazolo[3,4-*b*]-1,3,4-thiadiazolo[3,2-*b*][imidazo[4,5-*b*]-cyclohexane]-5*a*, 6*a*-diene). *Bulletin of the Korean Chemical Society*, *31*(11), 3304-3308.

Kumar, S. S., & P Kavitha, H. (2013). Synthesis and biological applications of triazole derivatives–a review. *Mini-Reviews in Organic Chemistry*, *10*(1), 40-65.

Küçükgüzel, Ş. G., & Çıkla-Süzgün, P. (2015). Recent advances bioactive 1, 2, 4-triazole-3-thiones. *European Journal of Medicinal Chemistry*, *97*, 830-870.

Li, X., Li, X. Q., Liu, H. M., Zhou, X. Z., & Shao, Z. H. (2012). Synthesis and evaluation of antitumor activities of novel chiral 1,2,4-triazole Schiff bases bearing γ-butenolide moiety. *Organic and Medicinal Chemistry Letters*, *2*(1), 1-5

Li, Y. F., Wang, G. F., Luo, Y., Huang, W. G., Tang, W., Feng, C. L., Shi, L.P., Ren,Y.D., Zuo, J.P. & Lu, W. (2007). Identification of 1-isopropylsulfonyl-2-amine benzimidazoles as a new class of inhibitors of hepatitis B virus. *European Journal of Medicinal Chemistry*, *42*(11-12), 1358-1364.

Malladi, S., Isloor, A. M., Isloor, S., Akhila, D. S., & Fun, H. K. (2013). Synthesis, characterization and antibacterial activity of some new pyrazole-based Schiff bases. *Arabian Journal of Chemistry*, *6*(3), 335-340.

Mohamed, A. M., El-Sayed, W. A., Ibrahim, A. A., Abdel-Hafez, N. A., Ali, K. A., & Mohamed, S. F. (2021). Recent trends in the chemistry of [1,2,4]triazole[1,5-*a*] pyrimidines. *Organic Preparations and Procedures International*, *53*(3), 211-239.

Nakano, H., Inoue, T., Kawasaki, N., Miyataka, H., Matsumoto, H., Taguchi, T., Inagaki, N. & Satoh, T. (2000). Synthesis and biological activities of novel antiallergic agents with 5-lipoxygenase inhibiting action. *Bioorganic & Medicinal Chemistry*, *8*(2), 373-380.

Ndakala, A. J., Gessner, R. K., Gitari, P. W., October, N., White, K. L., Hudson, A., Fakorede, F., Shakkleford, D.M., Kaiser, M., Yeates, C., Chibale, K. & Charman, S. A. (2011). Antimalarial pyrido[1,2-*a*]benzimidazoles. *Journal of Medicinal Chemistry*, *54*(13), 4581-4589.

Palma, A. M. D., Heggermont, W., Leyssen, P., Pürstinger, G., Wimmer, E., De Clercq, E., Rao, E., Monforte, A. M., Chimirri, A. & Neyts, J. (2007). Anti-enterovirus activity and structure–activity relationship of a series of 2,6-dihalophenyl-substituted 1*H*, 3*H*-thiazolo[3,4-*a*] benzimidazoles. *Biochemical and biophysical research communications*, *353*(3), 628-632.

Pandey, V. K., & Shukla, A. (1999). Synthesis and biological activity of isoquinolinyl benzimidazoles. *Indian Journal of Chemistry*, *38B* , 1381-1383.

Pavlath, A. E. (1986). Theoretical considerations for the application of aromatic and heterocyclic fluorine compounds as pesticides. *Pesticide Science*, *17*(4), 412-417.

Pérez, C., Agnese, A. M., & Cabrera, J. L. (1999). The essential oil of Senecio graveolens (Compositae): chemical composition and antimicrobial activity tests. *Journal of ethnopharmacology*, *66*(1), 91-96.

Pellizzari, G., (1911). Triazolo ei suoi derivati: *Gazzetta Chimica Italiana*, *41,* 20-28.

Plech, T., Kaproń, B., Łuszczki, J. J., Paneth, A., Siwek, A., Kołaczkowski, M., Zoinierek, M. & Nowak, G. (2014). Studies on the anticonvulsant activity of 4-alkyl-1,2,4-

triazole-3-thiones and their effect on GABAergic system. *European Journal of Medicinal Chemistry*, *86*, 690-699.

Prakash, C. R., & Raja, S. (2013). Synthesis, characterization and in vitro antimicrobial activity of some novel 5-substituted Schiff and Mannich base of isatin derivatives. *Journal of Saudi Chemical Society*, *17*(3), 337-344.

Rathod, C. P., Rajurkar, R. M., & Thonte, S. S. (2013). Benzimidazole synthesis and biological evaluation: A review. *Indo American Journal of Pharmaceutical Research*, *3*(2), 2323-2329.

Reddy, B. A. (2010). Synthesis, characterization and biological evaluation of 1,2-disubstituted benzimidazole derivatives using Mannich bases. *Journal of Chemistry*, *7*(1), 222-226.

Refaat, H. M. (2010). Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. *European Journal of Medicinal Chemistry*, *45*(7), 2949-2956.

Revanasiddappa, H. D., Prasad, K. S., Kumar, L. S., & Jayalakshmi, B. (2010). Synthesis and biological activity of new Schiff bases containing 4-(3*H*)-quinazolinone ring system. *International Journal of ChemTech Research*, *2*(2), 1344-1349.

Sashidhara, K. V., Kumar, A., Bhatia, G., Khan, M. M., Khanna, A. K., & Saxena, J. K. (2009). Antidyslipidemic and antioxidative activities of 8-hydroxyquinoline derived novel keto-enamine Schiffs bases. *European Journal of Medicinal Chemistry*, *44*(4), 1813-1818.

Shaharyar, M., & Mazumder, A. (2017). Benzimidazoles: A biologically active compounds. *Arabian Journal of Chemistry*, *10*, S157-S173.

Sharma, J., Ahmad, S., & Alam, M. S. (2012). Bioactive Triazoles: A potential review. *Journal of Chemical and Pharmaceutical Research*, *4*(12), 5157-5164.

Sheikh, R. A., Wani, M. Y., Shreaz, S., & Hashmi, A. A. (2016). Synthesis, characterization and biological screening of some Schiff base macrocyclic ligand-based transition metal complexes as antifungal agents. *Arabian Journal of Chemistry*, *9*, S743-S751.

Silverstein R. M., and Webster, F. C., (2002). Spectrometric Identification of Organic Compounds, 6th ed., John Wiley, USA, **3**, 106.

Sondhi, S. M., Rajvanshi, S., Johar, M., Bharti, N., Azam, A., & Singh, A. K. (2002). Anti-inflammatory, analgesic and antiamoebic activity evaluation of pyrimido [1,6-a] benzimidazole derivatives synthesized by the reaction of ketoisothiocyanates with mono and diamines. *European Journal of Medicinal Chemistry*, *37*(10), 835-843.

Thomas, A. B., Nanda, R. K., Kothapalli, L. P., & Hamane, S. C. (2016). Synthesis and biological evaluation of Schiff's bases and 2-azetidinones of isonocotinyl hydrazone as potential antidepressant and nootropic agents. *Arabian Journal of Chemistry*, *9*, S79-S90.

Torres-Gómez, H., Hernández-Núñez, E., León-Rivera, I., Guerrero-Alvarez, J., Cedillo-Rivera, R., Moo-Puc, R., Argotte-Ramos, R., Rodriguez-Gutierrez, M. C., Chan-Bacab, M. J. & Navarrete-Vázquez, G. (2008). Design, synthesis and *in vitro* antiprotozoal activity of benzimidazole-pentamidine hybrids. *Bioorganic & Medicinal Chemistry Letters*, *18*(11), 3147-3151.

Turan-Zitouni, G., Kaplancikli, Z. A., Kiliç, F.S., and Erol, K., (2002). The synthesis of some triazolylphenothiazine derivatives and their antidepressant and anxiolytic activities, *Bollettino Chimico Farmaceutico*, 141, 192-196.

Ueda, S., and Nagasawa, H., (2009). Facile synthesis of 1,2,4-triazoles via a copper-catalyzed tandem addition-oxidative cyclization. *Journal of American Chemical Society*, *131*, 15080-15081.

Umut, S. G., Nesrin, G. K. I., Koysal, Y., Ekrem, K., Samil, I., Goknur, A. and Ozalpd, M., (2007). 1-Acylthiosemicarbazides, 1,2,4-triazoles-5(4*H*)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones:Synthesis, analgesic--anti-inflammatory activities. *Bioorganic and Medicinal Chemistry*, *15*, 5738-5751.

Wan, J., Xia, Y., Liu, Y., Wang, M., Rocchi, P., Yao, J., Qu, F., Neyts, J., Iovanna, J. L. & Peng, L. (2009). Discovery of novel arylethynyltriazole ribonucleosides with selective and effective antiviral and antiproliferative activity. *Journal of Medicinal Chemistry*, *52*(4), 1144-1155.

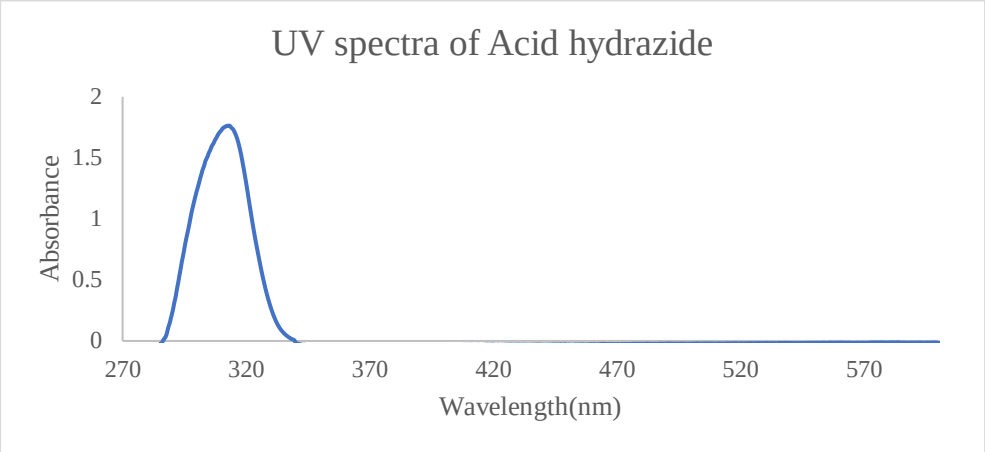
Wang, L. Y., Tseng, W. C., Lin, H. Y., & Wong, F. F. (2011). An effective nitrilimine cycloaddition for the synthesis of 1,3,5-trisubstituted 1,2,4-triazoles from oximes with hydrazonoyl hydrochlorides. *Synlett*, *2011*(10), 1467-1471.

Yu, F., Jia, Y. L., Wang, H. F., Zheng, J., Cui, Y., Fang, X. Y., Zhang, L. F. & Chen, Q. X. (2015). Synthesis of triazole schiff's base derivatives and their inhibitory kinetics on tyrosinase activity. *PloS one, 10*(9), e0131578.

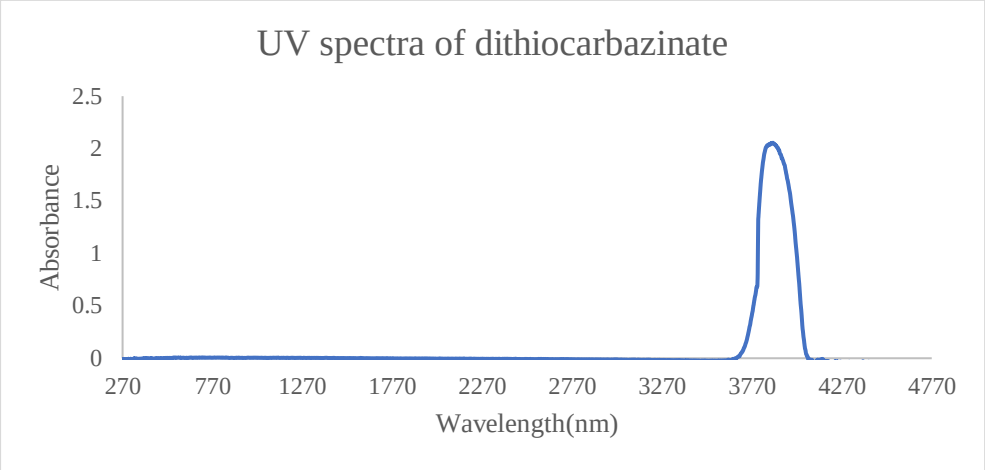
Zonios, D. I., & Bennett, J. E. (2008). Update on azole antifungals. In *Seminars in Respiratory and Critical Care Medicine 29*(2), 198-210.

APPENDICES

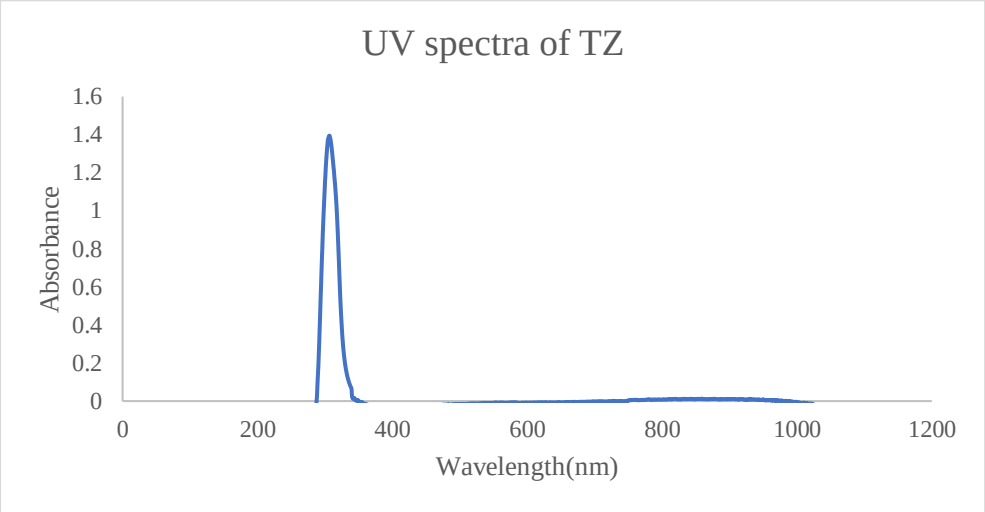
Appendix A: UV-VIS Spectrum of synthesized compounds



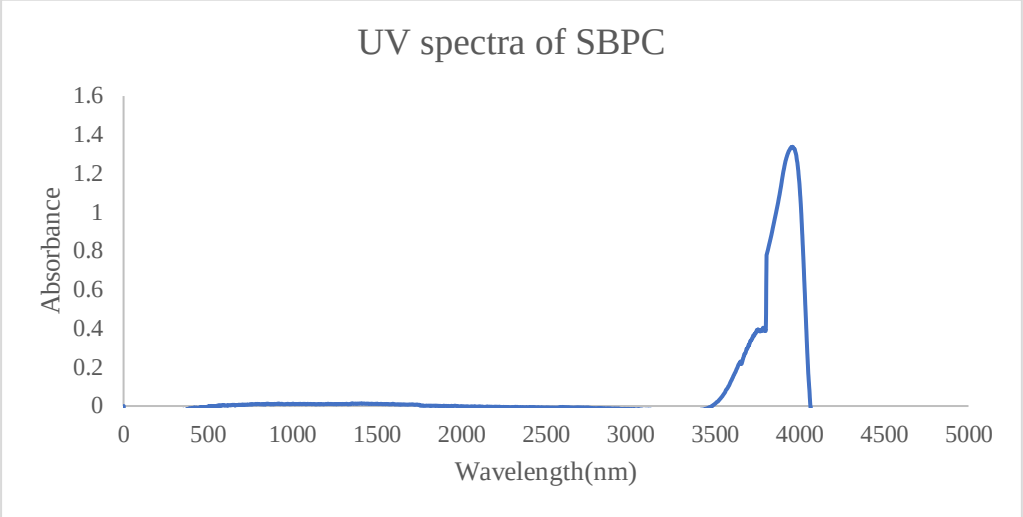
Appendix A1: UV spectra of Acid hydrazide



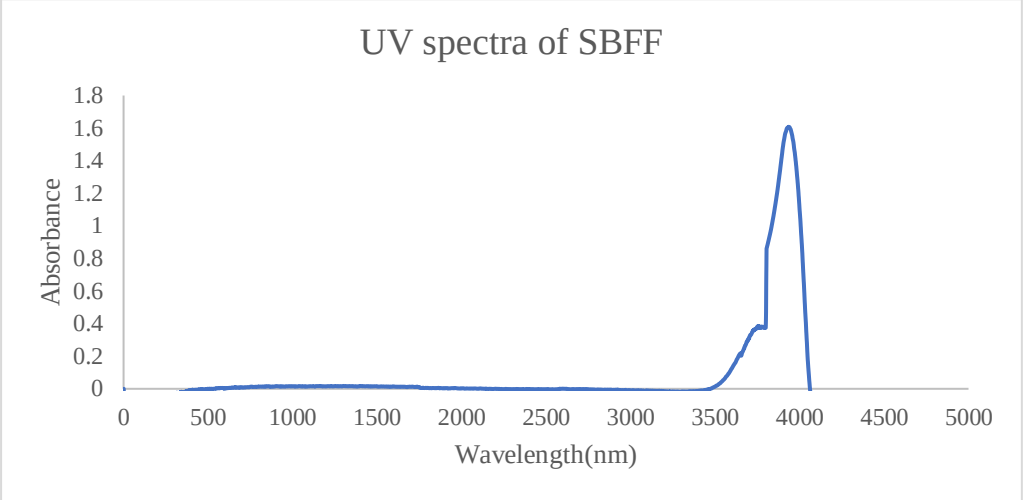
Appendix A2: UV spectra of dithiocarbazinate



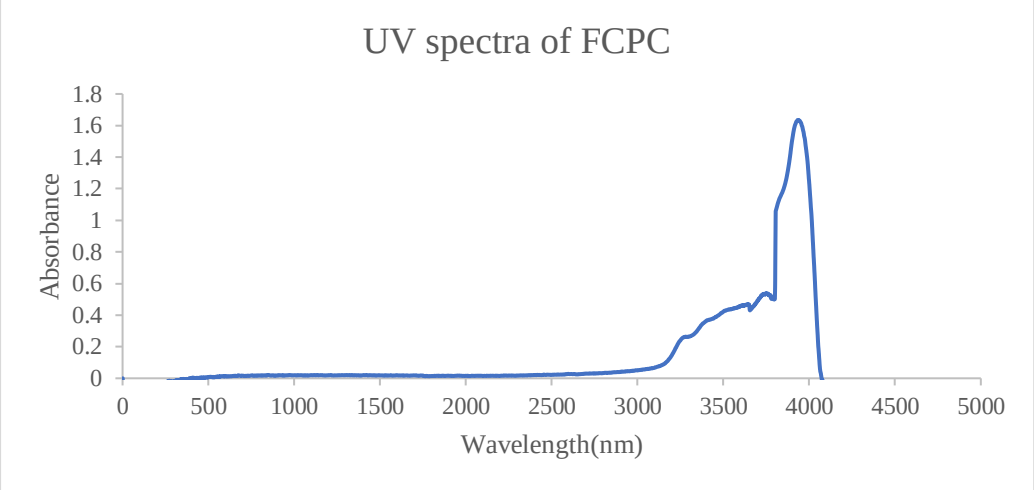
Appendix A3: UV spectra of triazole



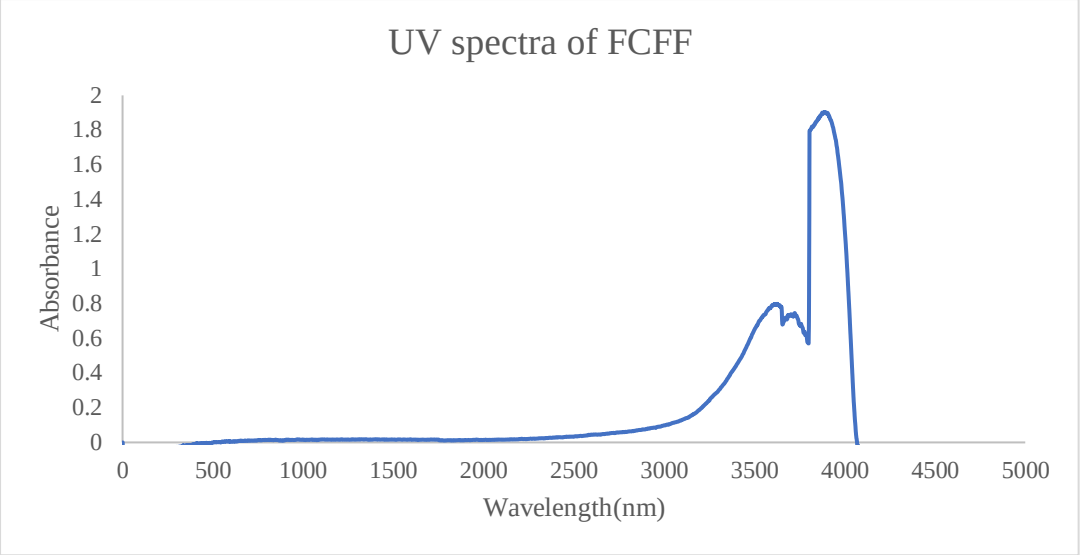
Appendix A4: UV spectra of Schiff's base SBPC



Appendix A5: UV spectra of Schiff's base SBFF

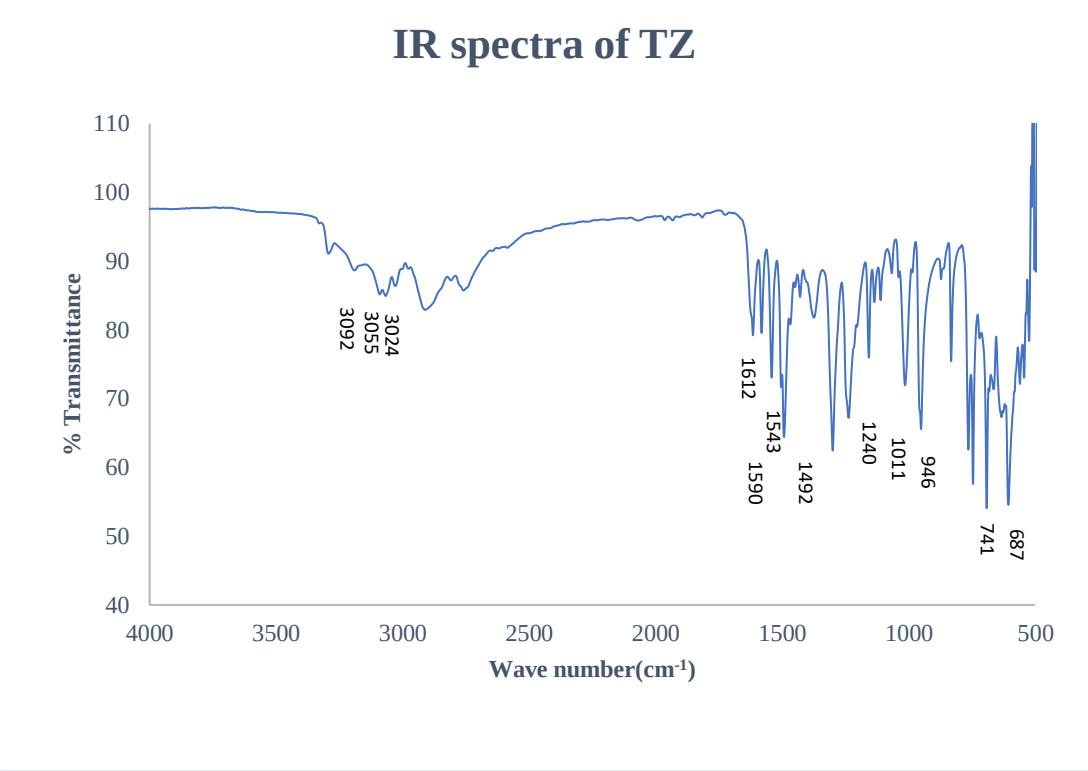


Appendix A6: UV spectra of benzimidazole derivative FCPC

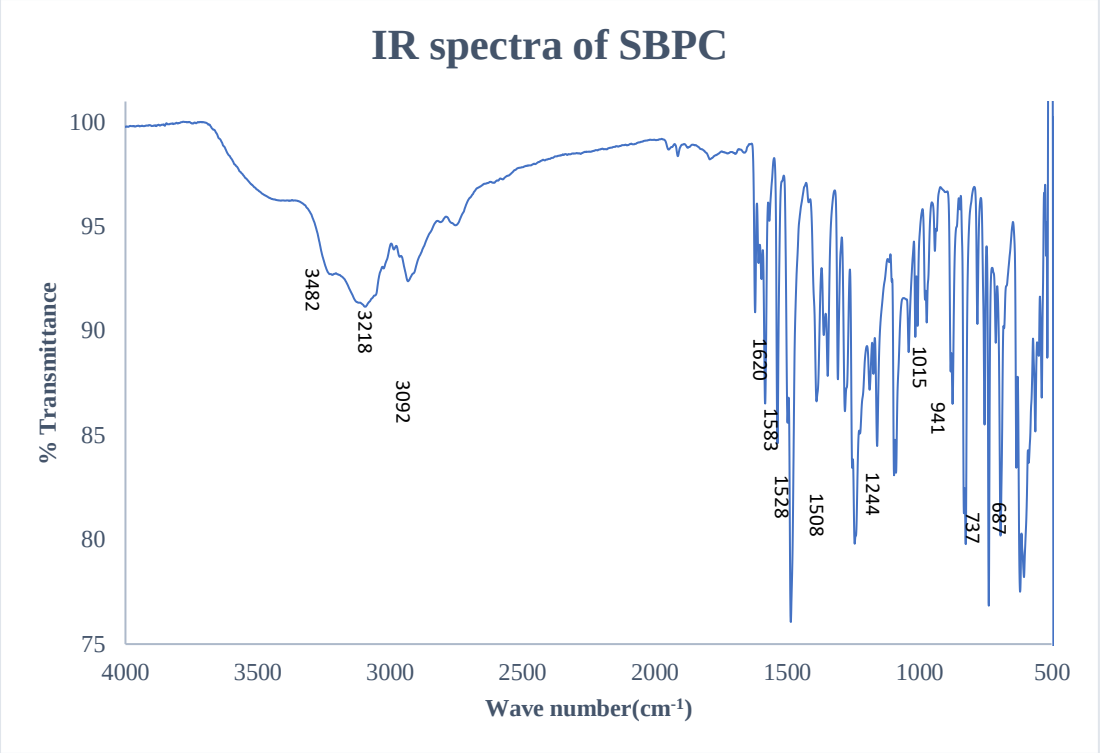


Appendix A7: UV spectra of benzimidazole derivative FCFF

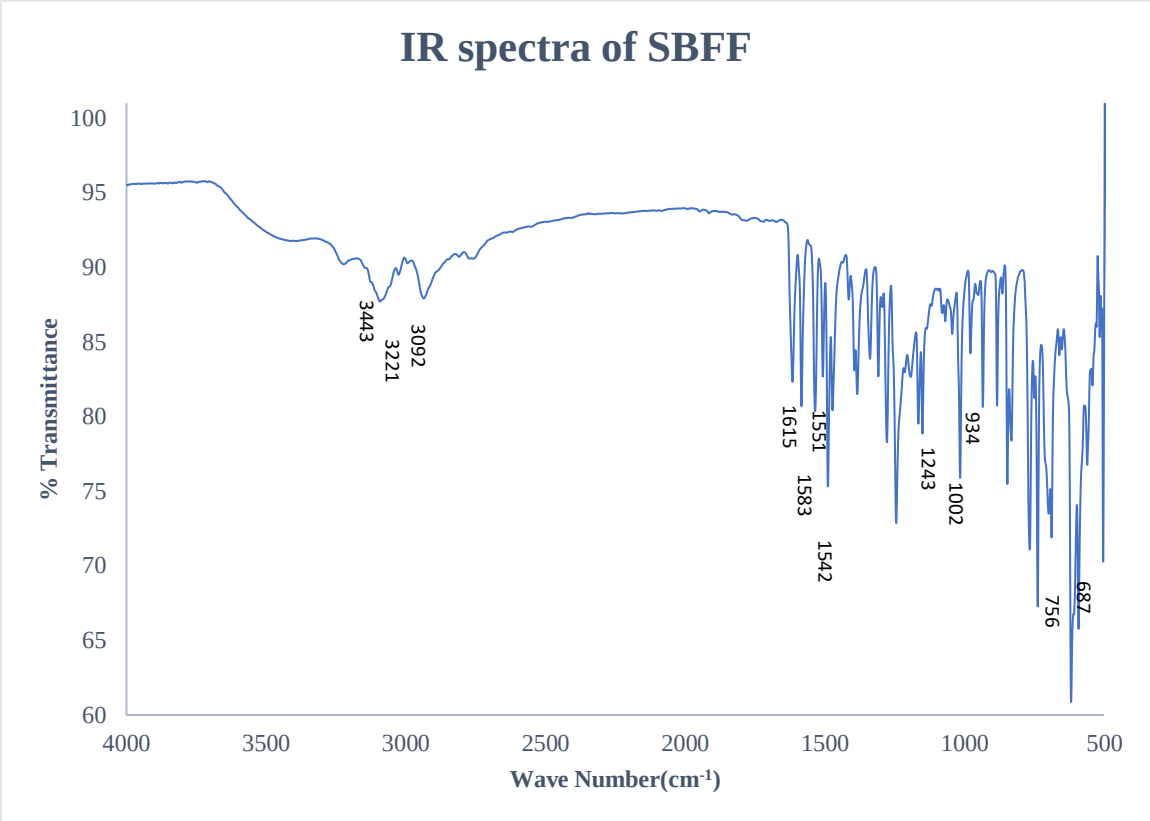
Appendix B: IR spectrum of synthesized compounds



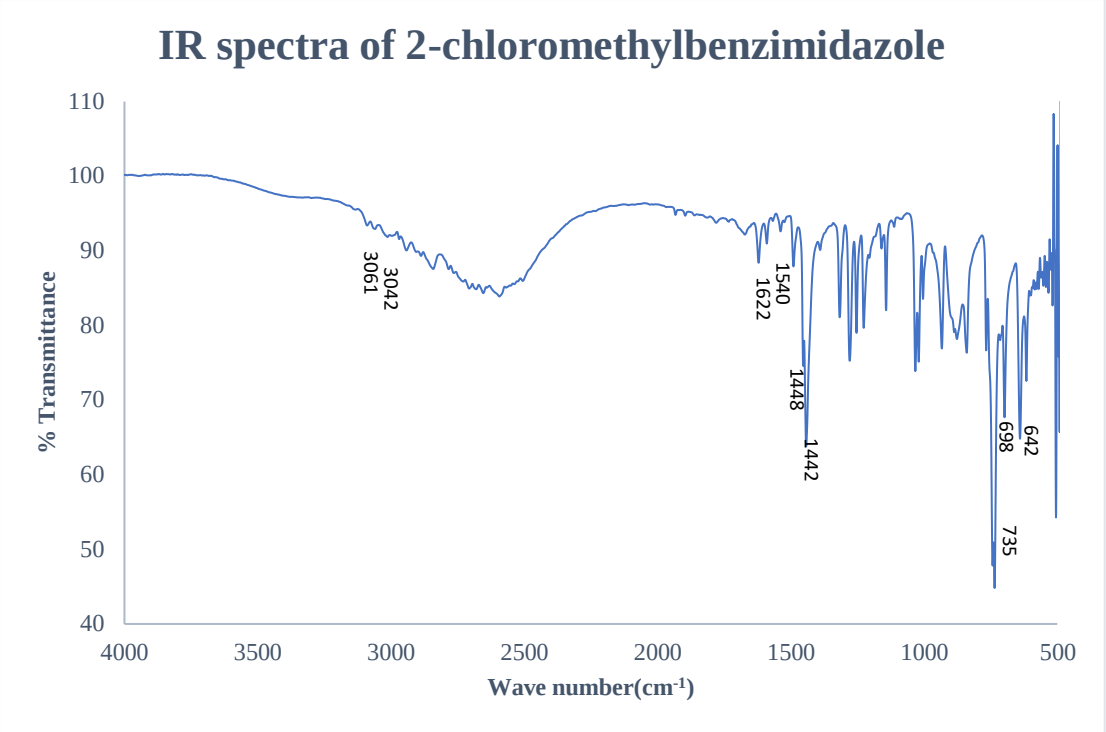
Appendix B1: IR spectra of triazole(3)



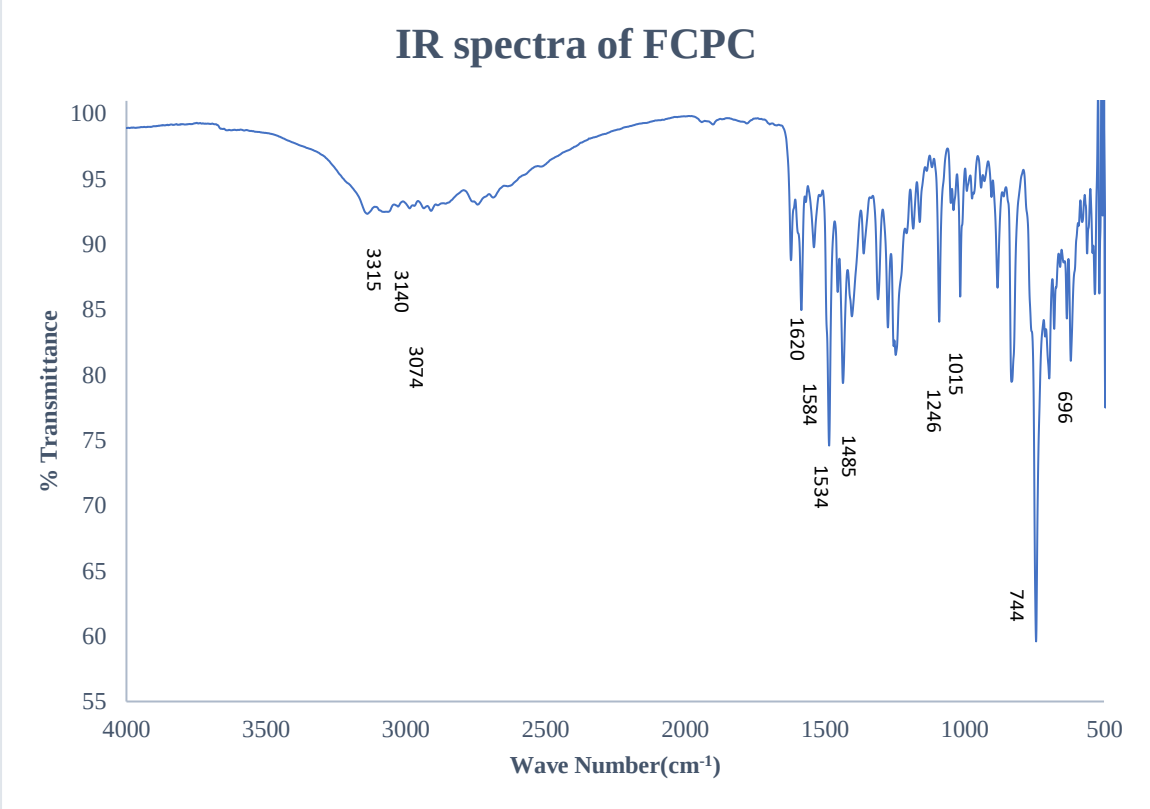
Appendix B2: IR spectra of Schiff's base SBPC(5a)



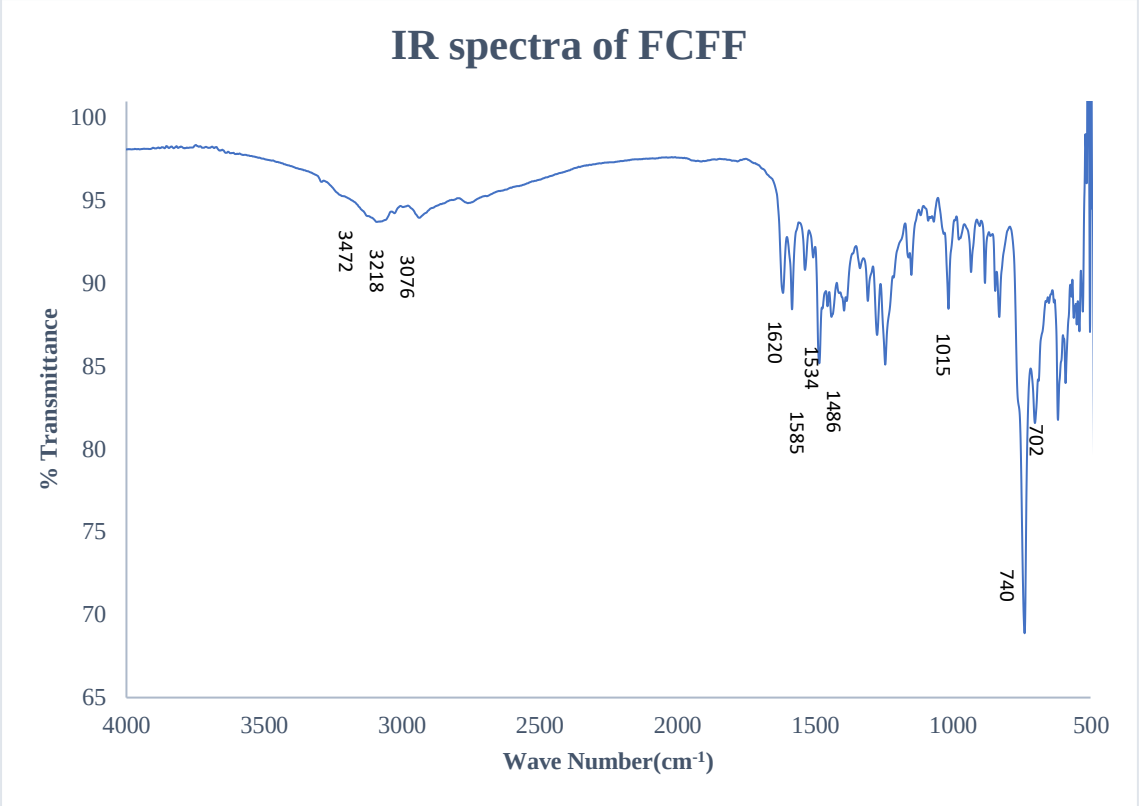
Appendix B3: IR spectra of Schiff's base SBFF(5b)



Appendix B4: IR spectra of 2-chloromethylbenzimidazole(6)

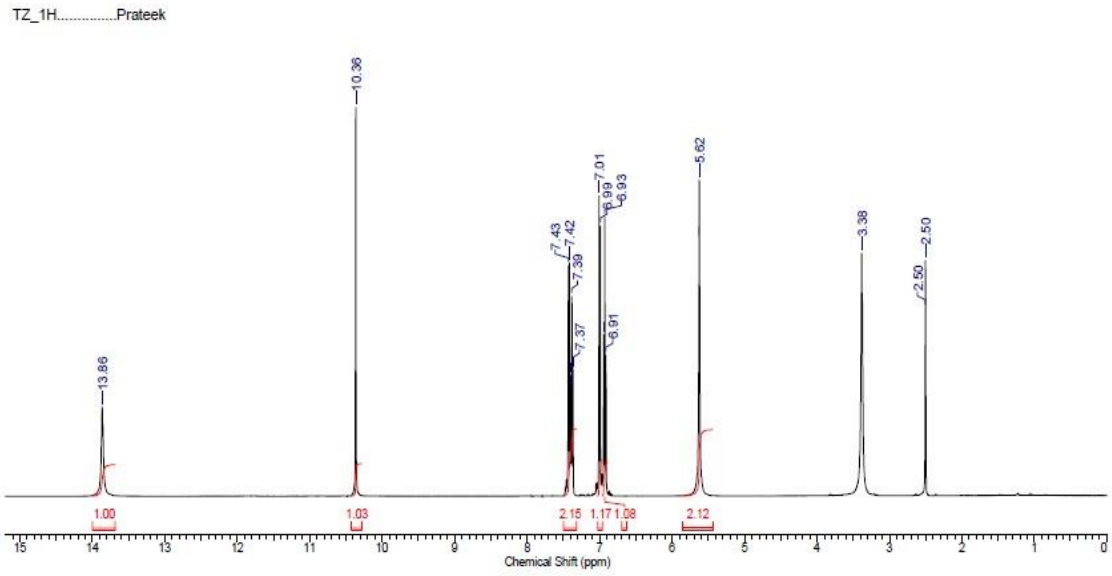


Appendix B5: IR spectra of benzimidazole derivative FCPC(7c)

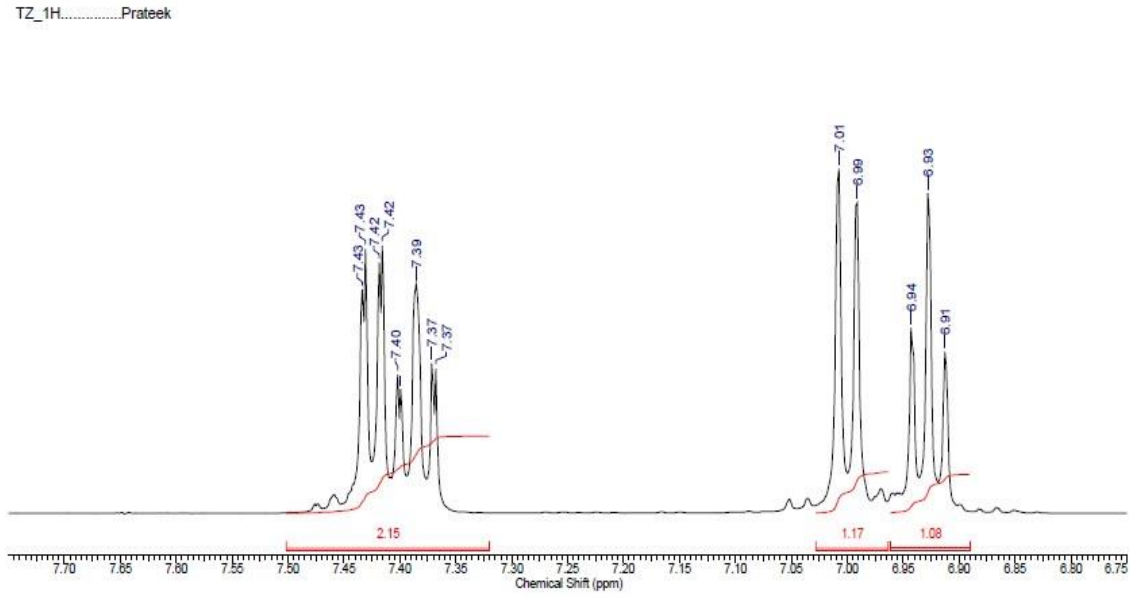


Appendix B6: IR spectra of benzimidazole derivative FCFF(7b)

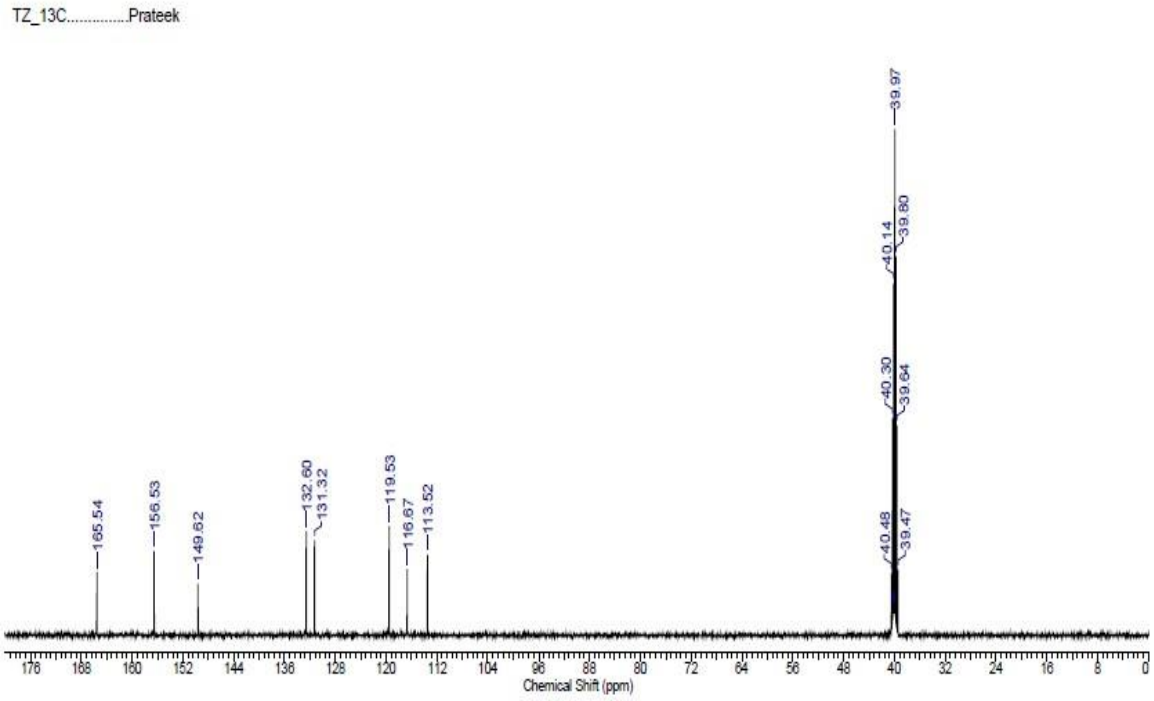
Appendix C: NMR spectra of synthesized compounds: ^{1}H-NMR and ^{13}C-NMR.



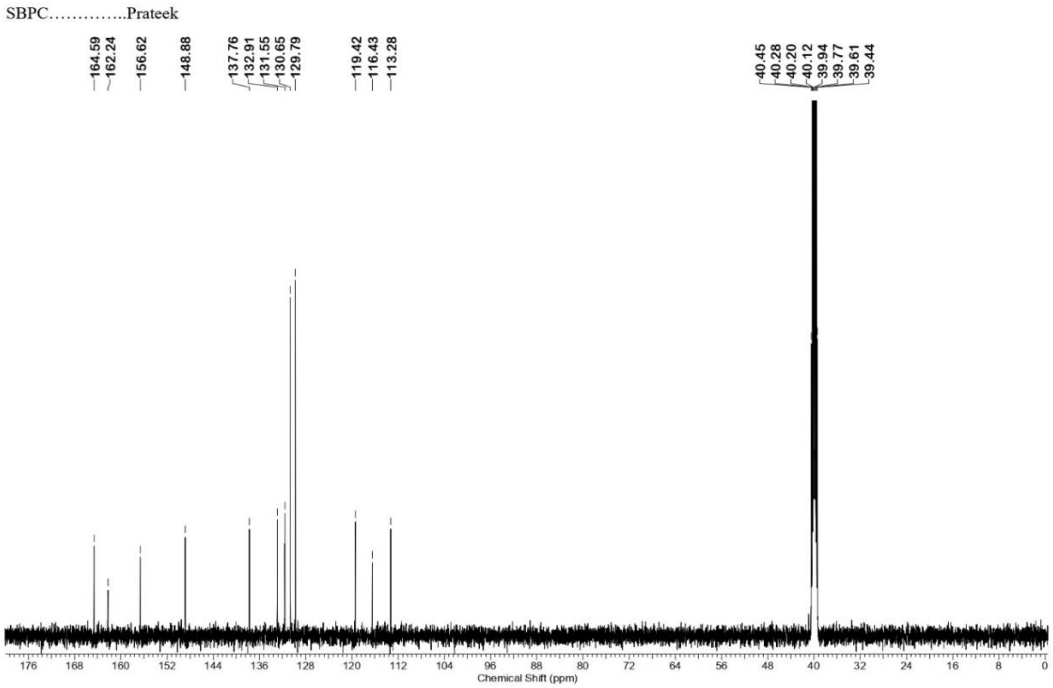
Appendix C1: ^{1}H spectrum of triazole(3)



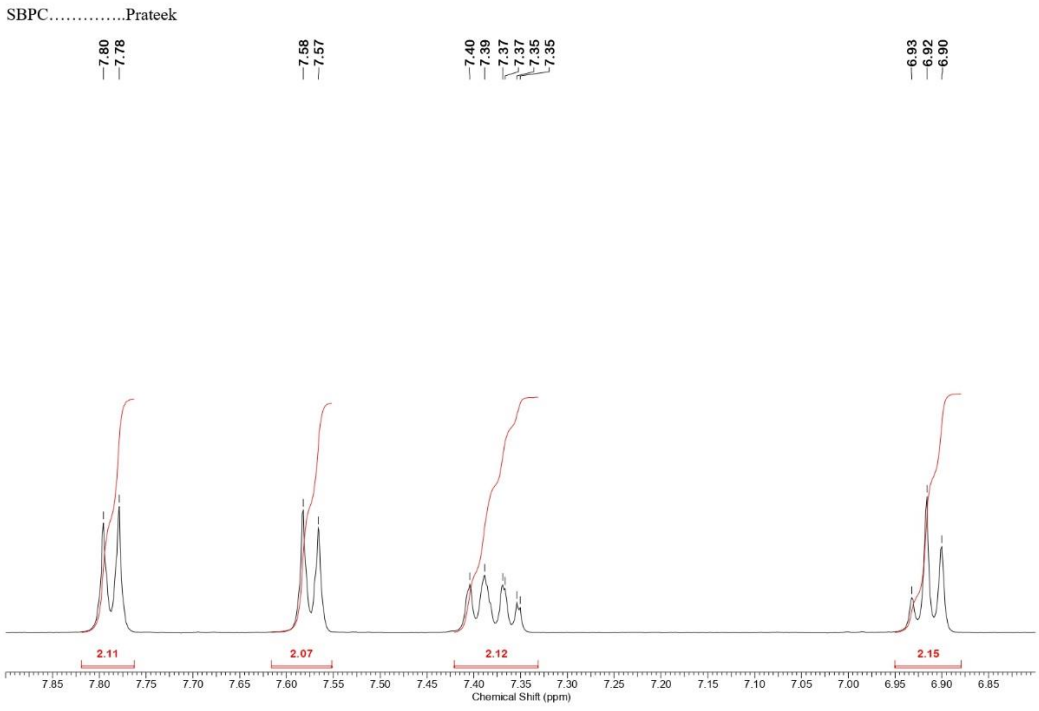
Appendix C2: Expanded ^{1}H spectrum of triazole(3)



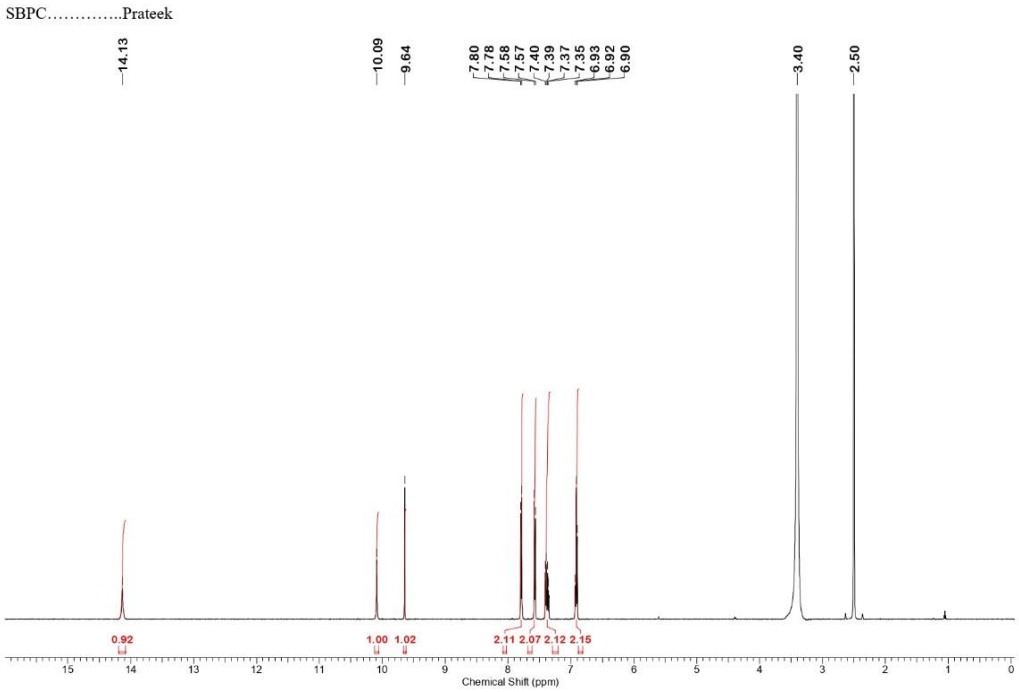
Appendix C3: ^{13}C spectrum of triazole(3)



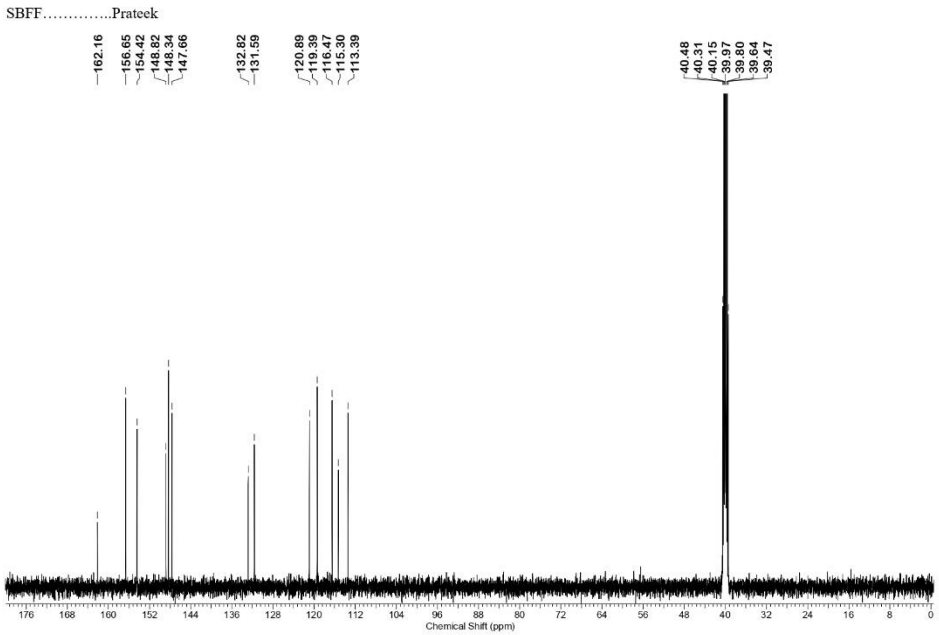
Appendix C4: ^{1}H spectrum of Schiff's base SBPC(5a)



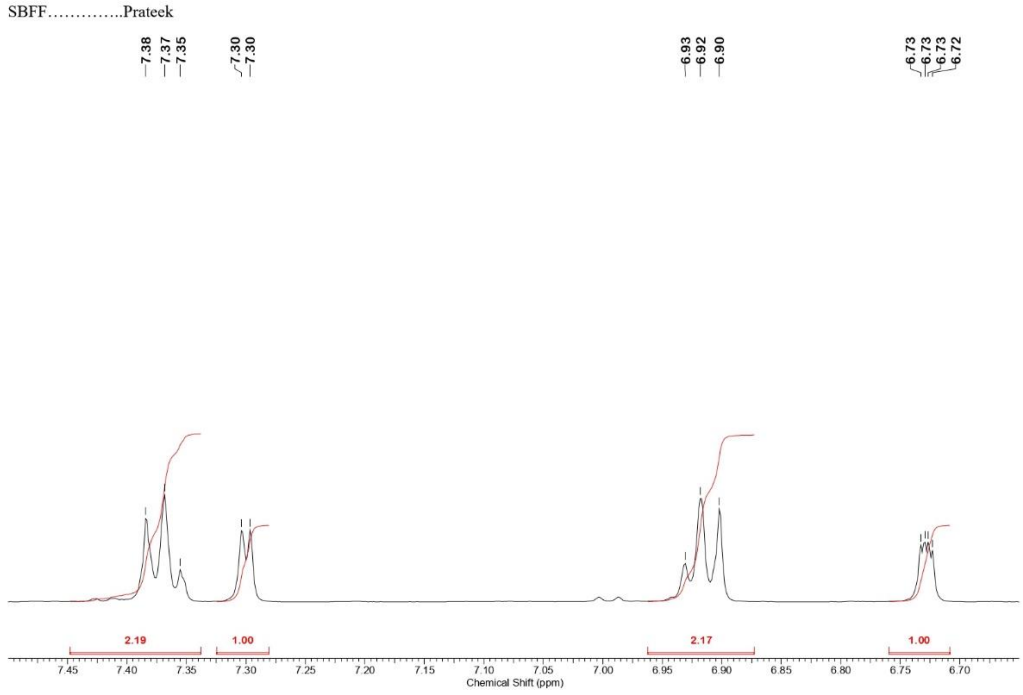
Appendix C5: Expanded ^{1}H spectrum of Schiff's base SBPC(5a)



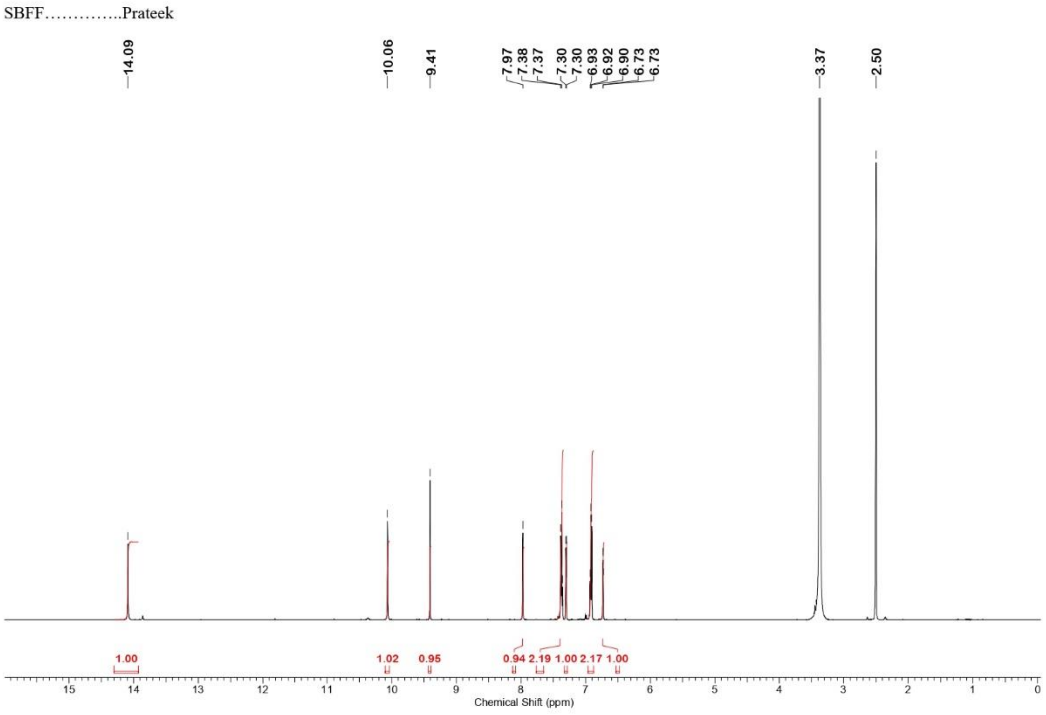
Appendix C6: ^{13}C spectrum of Schiff's base SBPC(5a)



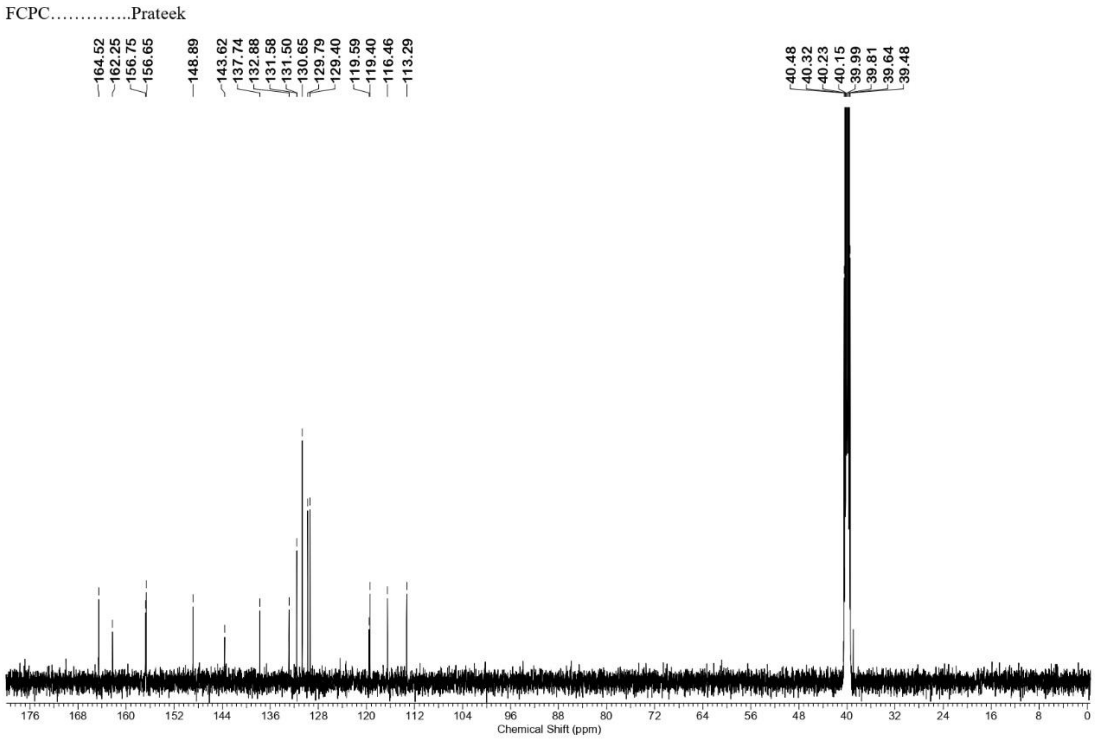
Appendix C7: ^{1}H spectrum of Schiff's base SBFF(5b)



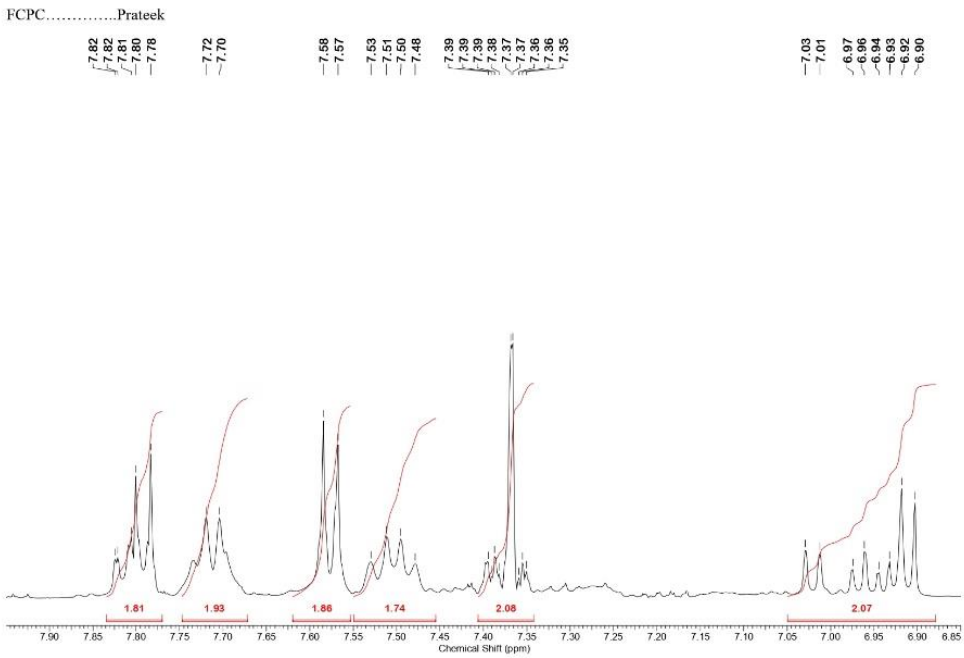
Appendix C8: Expanded ^{1}H spectrum of Schiff's base SBFF(5b)



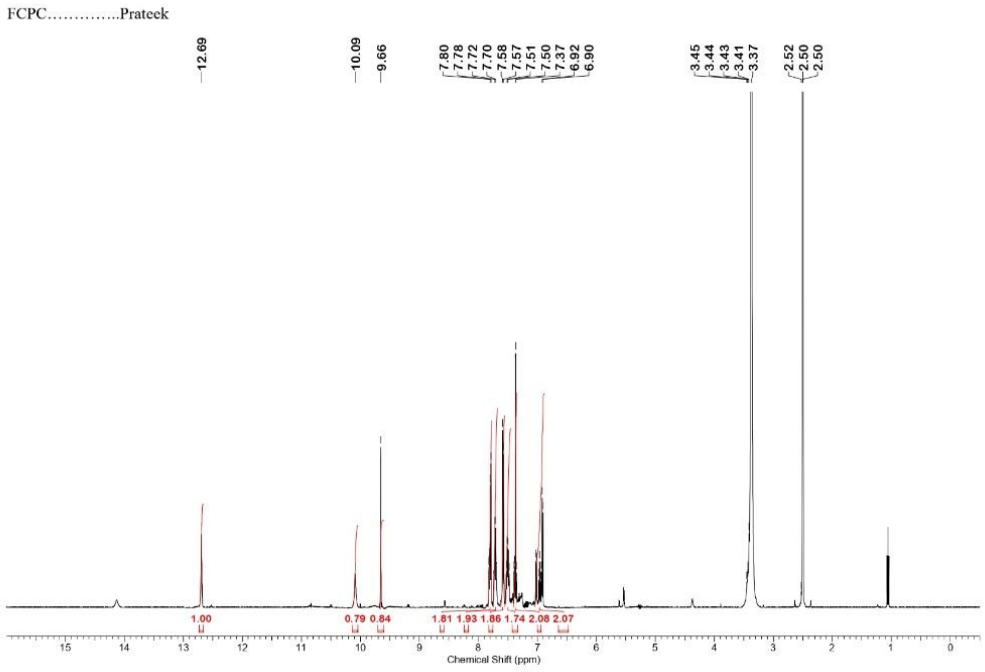
Appendix C9: ^{13}C spectrum of Schiff's base SBFF(5b)



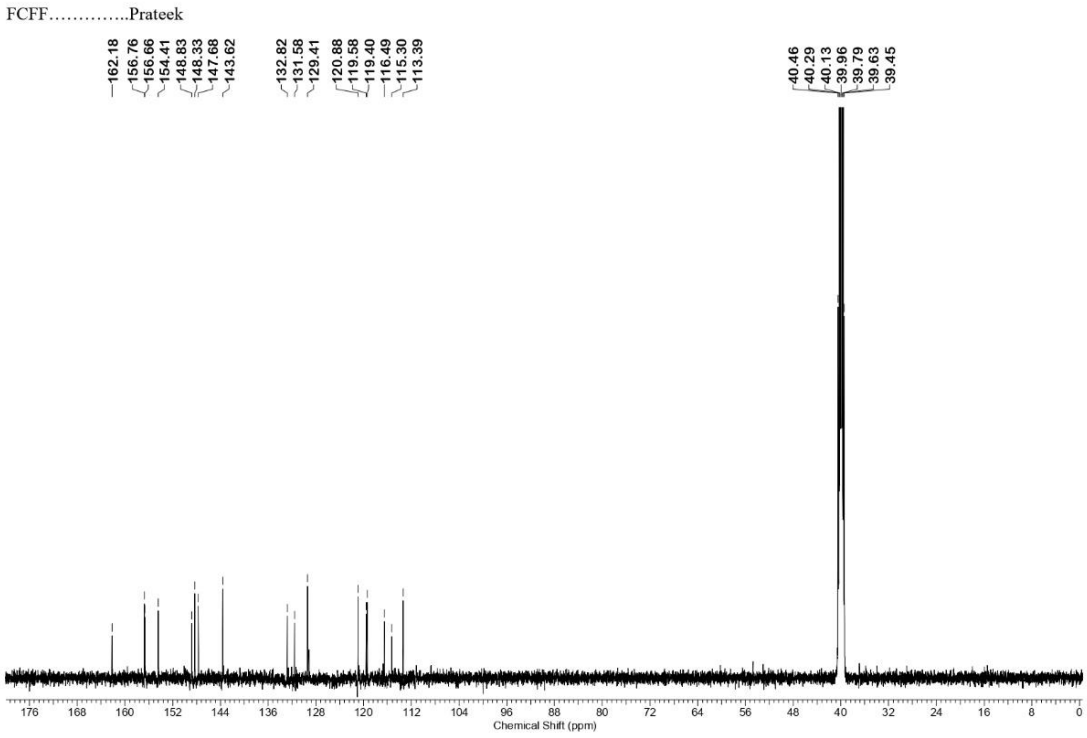
Appendix C10: ^{1}H spectrum of benzimidazole derivative FCPC(7a)



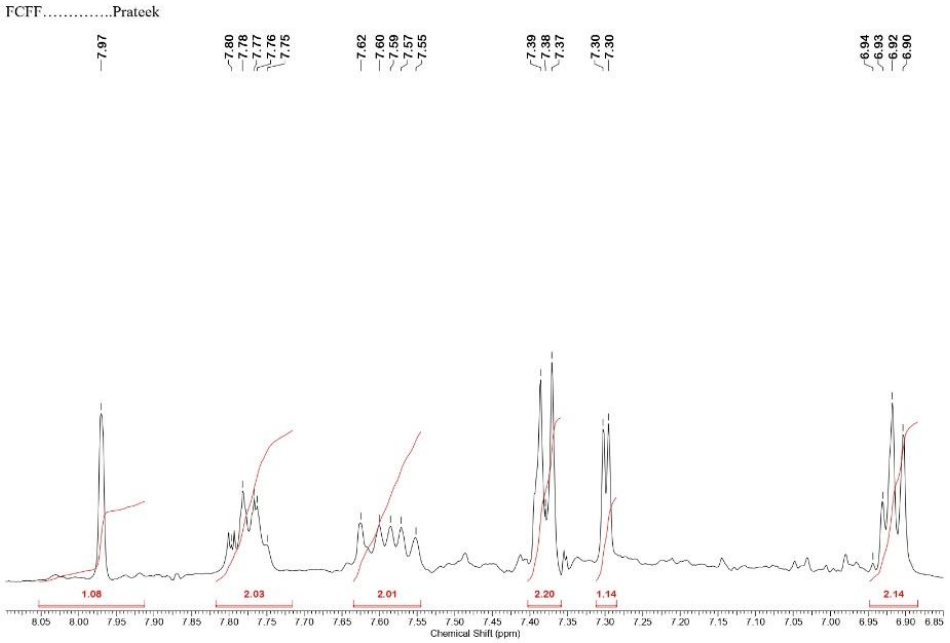
Appendix C11: Expanded ^{1}H spectrum of benzimidazole derivative FCPC(7a)



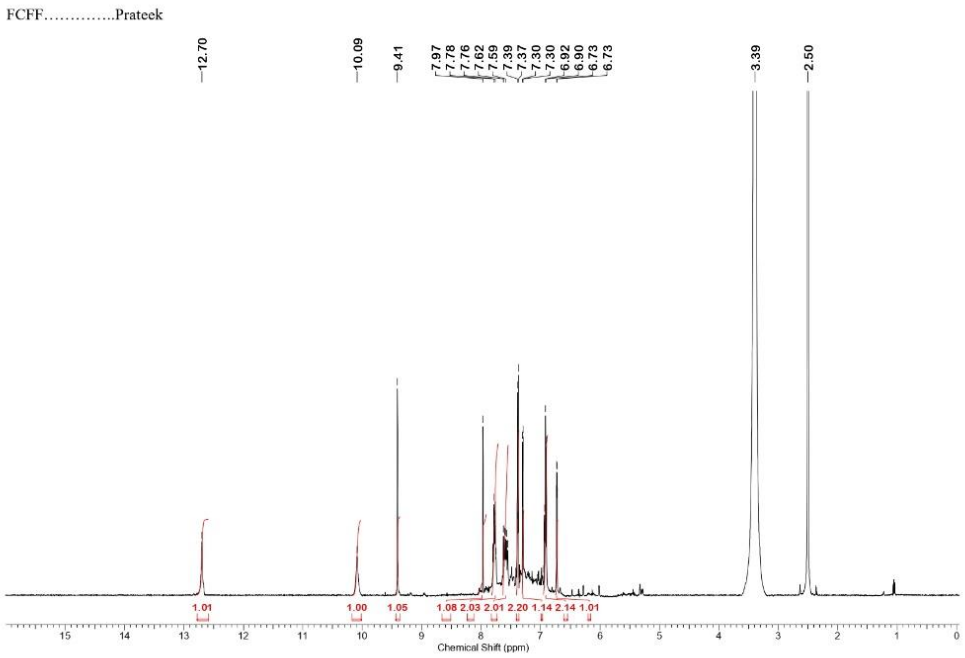
Appendix C12: ^{13}C spectrum of benzimidazole derivative FCPC(7a)



Appendix C13: ^{1}H spectrum of benzimidazole derivative FCFF(7b)

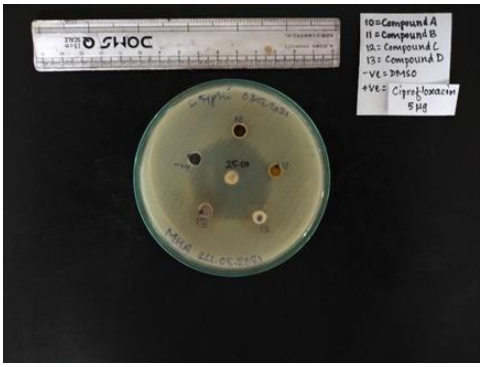


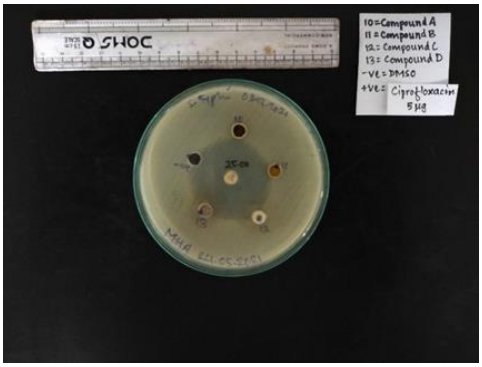
Appendix C14: Expanded ^{1}H spectrum of benzimidazole derivative FCFF(7b)

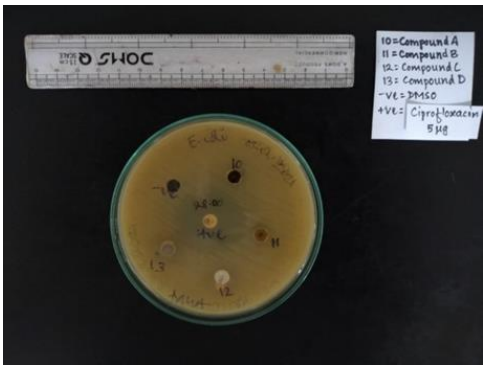


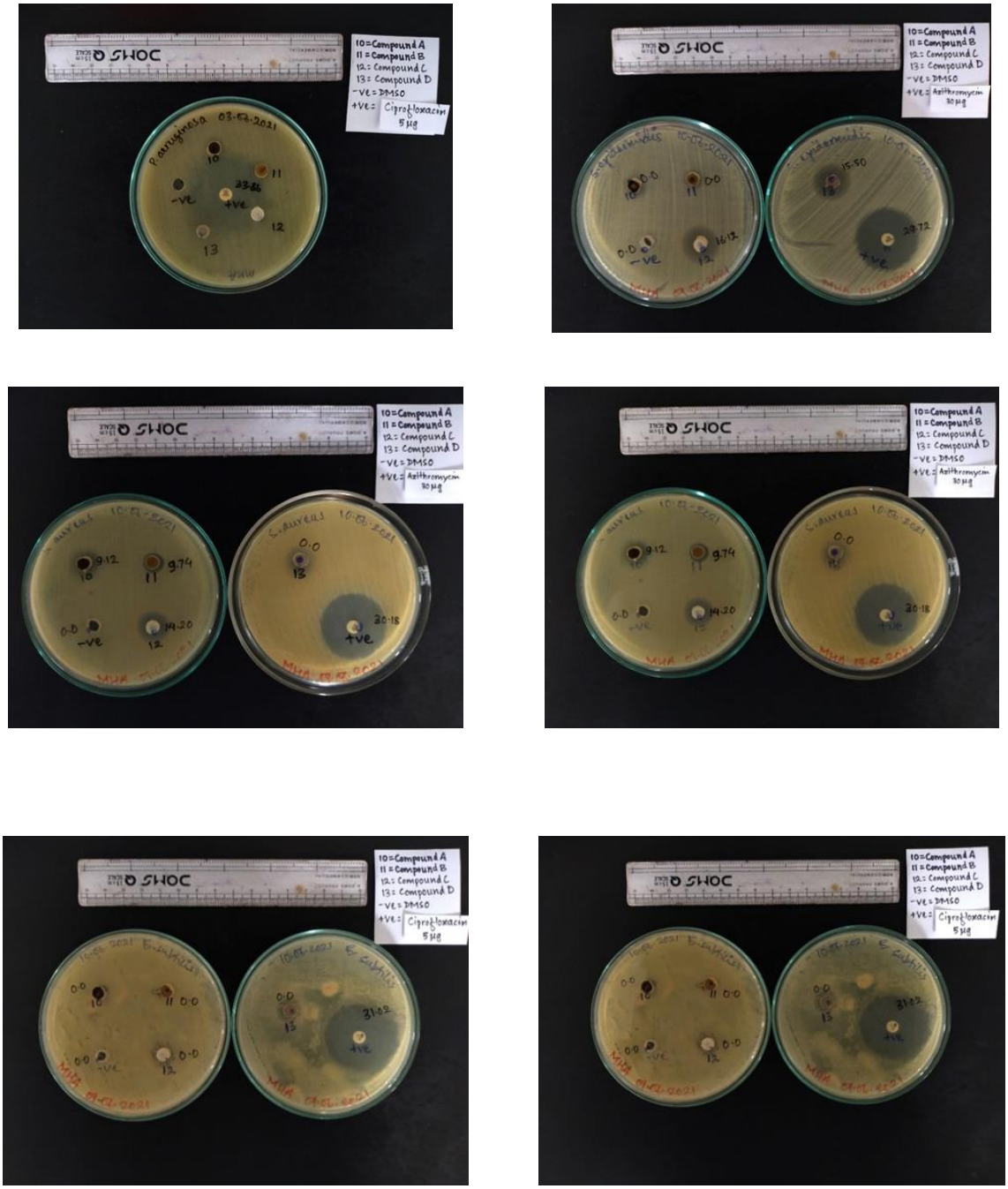
Appendix C15: ^{13}C spectrum of benzimidazole derivative FCFF(7b)

Appendix D: Antimicrobial data of synthesized compounds.



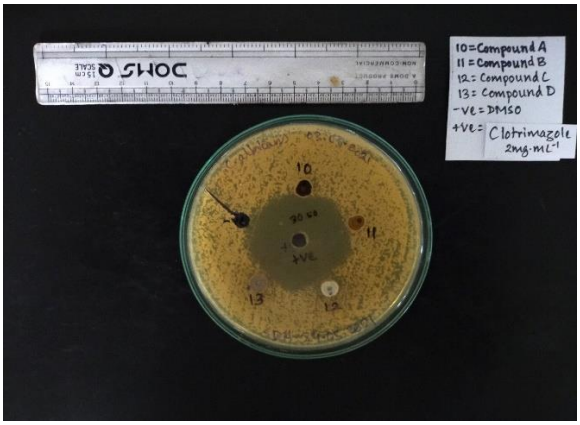




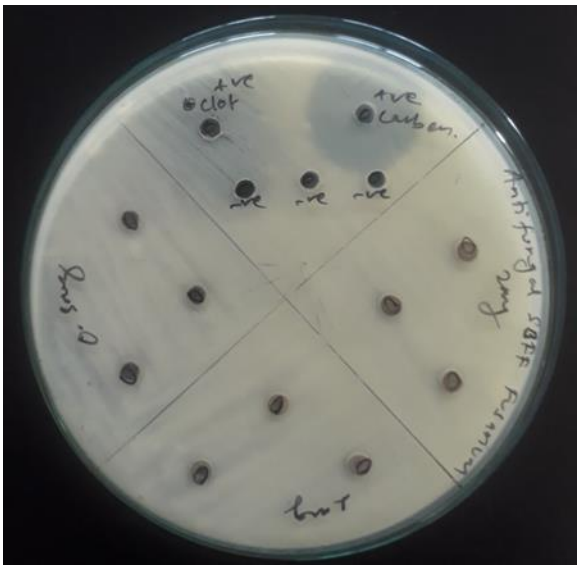


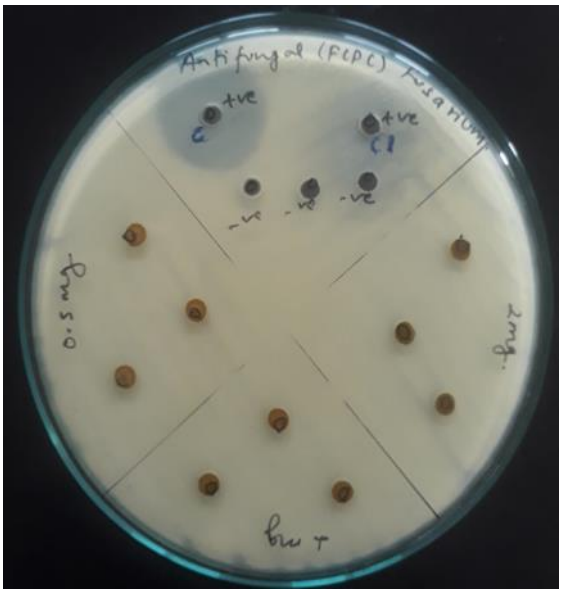
Appendix D1: Antibacterial screening of compounds

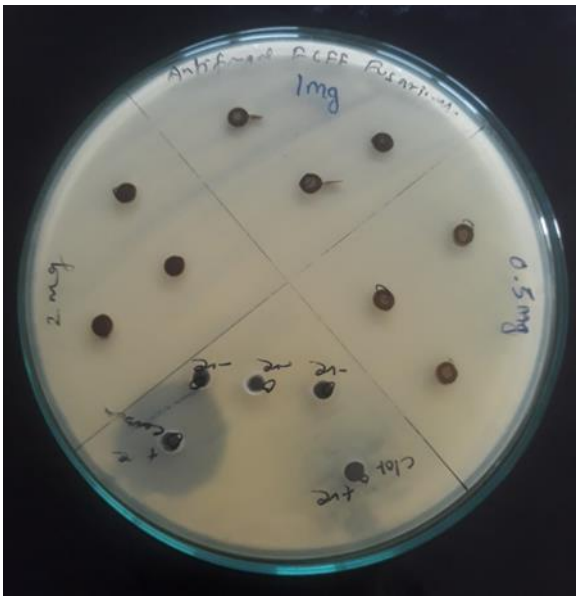

10=Compound A
11=Compound B
12=Compound C
13=Compound D
-ve=DMSO
+ve=Clotrimazole 2mg·mL⁻¹

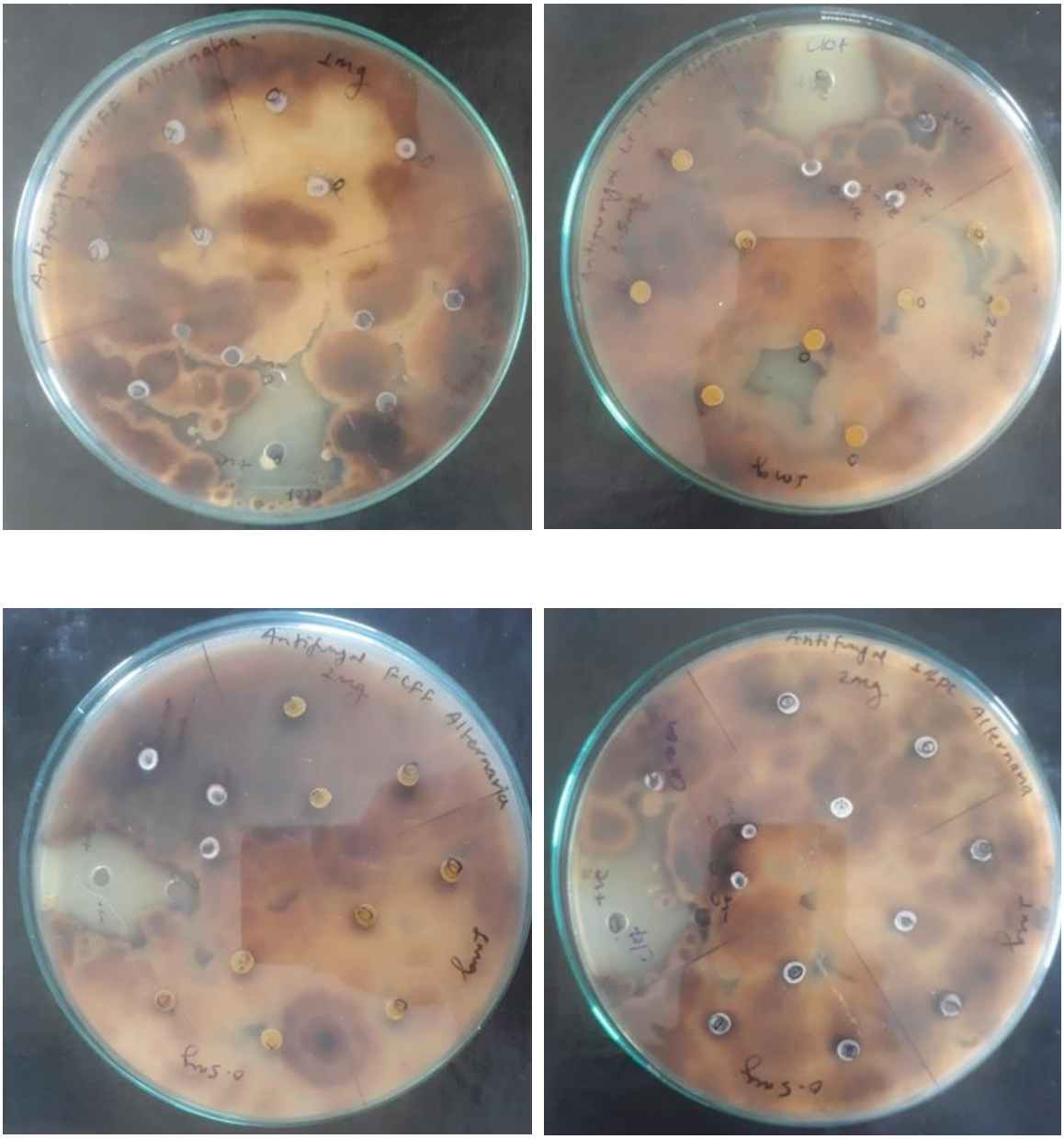

10=Compound A
11=Compound B
12=Compound C
13=Compound D
-ve=DMSO
+ve=Clotrimazole 2mg·mL⁻¹


Antifungal. SBDC Fusarium.
2mg
1mg
0.5mg
+ve
-ve
Clot


+ve Clot
+ve Carbem.
-ve
Antifungal of SBDC Fusarium
1mg
0.5mg

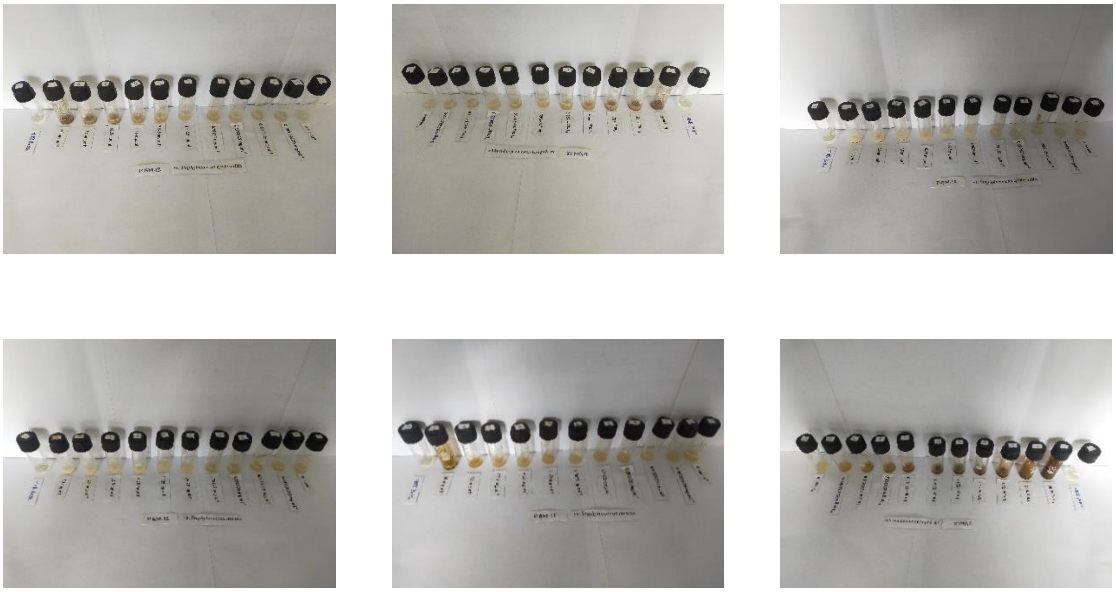

Antifungal (FLDC) Fusarium
+ve
-ve
0.5mg
2mg
1mg


1mg
2mg
0.5mg
+ve
Clot
-ve



Appendix D2: Antifungal screening of synthesized compounds.

Appendix E: MIC and MBC of the synthesized compounds



Appendix E1: MIC of synthesized compounds

Appendix E2: MBC of synthesized compounds

Appendix E: Cytotoxicity (Brine shrimp) assay of synthesized compounds.

Appendix E1: Test solutions (left) and counting of nauplii(right)

Appendix F: Synthesis work and data collection.

Appendix F1: Crystallization in R.B. flask(left) and synthesis work(right)

Appendix F2: UV spectrum data collection in Amrit Campus research lab.

Appendix G: Brine shrimp MATLAB Code

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% program to calculate the LC50 value (bioassay of
chemicals)d
%data1 = importdata('Antioxidant data.xlsx');
data2 = importdata('Brine shrimp assay.xlsx');
brine = data2.data
shrine = log10(brine(:,1))
brine(:,end+1) = log10(brine(:,1))
brine(:,end+1) = brine(:,3).*brine(:,6)
brine(:,end+1) = brine(:,6).*brine(:,6)
rows_13 = brine(1:3,:)
rows_46 = brine(4:6,:)
rows_79 = brine(7:9,:)
rows_1012 = brine(10:12,:)
beta_13 = (sum(brine(1:3,7))-
((sum(brine(1:3,6)))*(sum(brine(1:3,3))))/3)/(sum(brine(
1:3,8))-(((sum(brine(1:3,6)))^2)/3))
beta_46 = (sum(brine(4:6,7))-
((sum(brine(4:6,6)))*(sum(brine(4:6,3))))/3)/(sum(brine(
4:6,8))-(((sum(brine(4:6,6)))^2)/3))
beta_79 = (sum(brine(7:9,7))-
((sum(brine(7:9,6)))*(sum(brine(7:9,3))))/3)/(sum(brine(
7:9,8))-(((sum(brine(7:9,6)))^2)/3))
beta_1012 = (sum(brine(10:12,7))-
((sum(brine(10:12,6)))*(sum(brine(10:12,3))))/3)/(sum(br
ine(10:12,8))-(((sum(brine(10:12,6)))^2)/3))
```

```
alpha_13 = (sum(brine(1:3,3))- beta_13 *
sum(brine(1:3,6)))/3
alpha_46 = (sum(brine(4:6,3))- beta_13 *
sum(brine(4:6,6)))/3
alpha_79 = (sum(brine(7:9,3))- beta_13 *
sum(brine(7:9,6)))/3
alpha_1012 = (sum(brine(10:12,3))- beta_13 *
sum(brine(10:12,6)))/3
Y = 5
X_13 = (Y-alpha_13)/beta_13
X_46 = (Y-alpha_46)/beta_46
X_79 = (Y-alpha_79)/beta_79
X_1012 = (Y-alpha_1012)/beta_1012
LC_13 = 10^X_13
LC_46 = 10^X_46
LC_79 = 10^X_79
LC_1012 = 10^X_1012

%parameters =
```