SYNTHESIS, CHARACTERIZATION AND STUDY OF BIOLOGICAL ACTIVITIES OF MANNICH BASES DERIVED FROM 4-(FURAN-2-YL-METHYLENEAMINO)-3-(2-HYDROXYPHENYL)-1*H*-1,2,4-TRIAZOLE-5-THIONE

A DISSERTATION

SUBMITTED FOR

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

BY

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KATHMANDU, NEPAL

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BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled "Synthesis, Characterization and Study of Biological Activities of Mannich Bases of 4-(Furan-2-yl-Methyleneamino)-3-(2-Hyderoxyphenyl)-1*H*-1,2,4-Triazole-5-thione," prepared by Prem Shankar Deo, under the supervision of Associate Professor Dr. Bhushan Shakya, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, is hereby submitted for the partial fulfillment of Master of Science (M.Sc.) degree in chemistry. This dissertation has not been submitted to any other university or institution previously for the award of a degree.

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LETTER OF RECOMMENDATION

It is to recommend that this dissertation work entitled “Synthesis, Characterization and Study of Biological Activities of Mannich Bases of4-(Furan-2-yl-Methyleneamino)-3-(2-Hyderoxyphenyl)-1*H*-1,2,4-Triazole-5-thione,” has been carried out by Mr. Prem Shankar Deo as 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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DECLARATION

I, **Prem Shankar Deo**, hereby declare that the work presented herein is genuine work done originally by me under the supervision of Assoc. Prof. Dr. Bhushan Shakya and has not been published or submitted elsewhere for the requirements of a degree program. Any literature, data and work done by others and cited in this dissertation has been given due acknowledgement and listed in the reference section.

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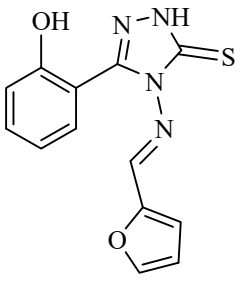
Prem Shankar Deo

ABSTRACT

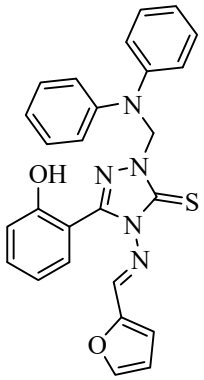
Triazole nucleus has been studied a lot since the last decade because of its various potent biological activities. The pharmacological application of triazoles has been widely recognized and well documented. Allocating various biological activities of triazole derivative in one place has been the milestone for new research towards this moiety.

Triazole derivatives such as Schiff bases have been introduced as the potent bioactive compound. They possess various biological activities and are mostly derived from the triazole nucleus *via* condensation of primary amino compounds with aldehyde or ketone. However, there is not much documentation about the Mannich bases as their study has begun lately. Mannich bases are the β–aminoketone which are generally formed when amine, aldehyde and carbon acid reacts. Mannich bases have shown multiple biological activities such as antibacterial, antifungal, antiviral, anticancer, etc.

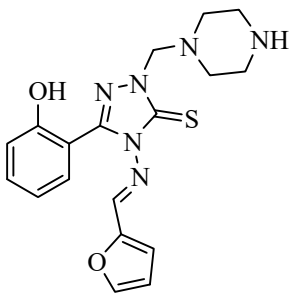
Schiff base (4) was converted into thione based Mannich bases (5a and 5b) by reaction with diphenylamine/piperazine in presence of formaldehyde in ethanol. These newly synthesized thione based Mannich bases were characterized by elemental analysis such as UV, FT-IR, ^{1}H-NMR and ^{13}C-NMR. These Mannich bases were also tested against several bacterial and fungal strains. Mannich bases containing Piperazine moiety (5b) showed better antifungal property than Mannich bases with diphenylamine moiety (5a) whereas 5a showed better antibacterial activity than 5b.



Schiff Base (4)



Mannich base (5a)



Mannich Base (5b)

Keywords: 1,2,4- triazole, Schiff bases, Mannich bases, antibacterial, antifungal activity.

LIST OF ABBREVIATIONS

^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
DIPEA	*N*,*N*-Diisopropylethylamine
DMSO	Dimethyl sulphoxide
EDC	Electronic data capture
FTIR	Fourier-transform infrared
HATU	1-[Bis(dimethylamion)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium)
MHB	Muller-hinton broth
MHz	Megahertz
mol	Mole
NS	Normal saline
PC	Positive control
PDA	Potato dextrose agar
pH	Potential of hydrogen
ppm	Parts per million
sp.	Species
TLC	Thin layer chromatography
TMS	Tetra methyl silane
UV	Ultra-violet
ZOI	Zone of inhibition

LIST OF SYMBOLS

br s	Broad singlet (NMR)
d	Doublet (NMR)
dd	Doublets of doublets
J	Spin-spin coupling constant (NMR)
m	Medium intensity (IR), Multiplet (NMR)
s	Strong intensity (IR), Singlet (NMR)
t	Triplet (NMR)
w	Weak intensity (IR)
δ	Chemical shift (NMR), Bending vibration (IR)
(w/v)	Weight per volume

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CHAPTER 1

1. INTRODUCTION

1.1. General Overview

Life on Earth relies exquisitely on heterocyclic compounds that play key roles in the biochemical reactions involved in fundamental activities such as metabolism, energy delivery, the replication of genetic material, nerve impulse transmission, etc. With their tunable properties available from the variety of structure, heterocyclic compounds allow for the design of new synthetic compounds for specific purposes like drugs, pesticides (Bretschinder *et. al.*, 2014) and detergents (Gourdon *et. al.*, 2011), as well as into the correlated fields such as biochemistry (Yang *et. al.*, 2015), polymers (Fuji *et. al.*, 2013), and material sciences (Thottempudi *et. al.*, 2012). It is also a classical division of medicinal chemistry, mainly involved in the development of chemotherapeutic agents (Kareem, 2018). The nitrogen containing heterocyclic compounds have been utilized by many researchers because of their various potent biological activities, low toxicity and efficient selectivity. Triazoles are examples of such nitrogen containing heterocyclic compounds.

1.2 Triazoles

The presence of three nitrogen hetero-atoms in five-membered ring systems defines an interesting class of compounds, the triazole. Triazoles also known as pyrrodiazole, containing a five membered di-unsaturated ring structure composed of three nitrogen atoms and two carbon atoms at non-adjacent positions having molecular formula $C_2H_3N_3$ (molecular mass 69.06).

The compound triazole was first identified by Fischer in 1878. But, in 1885, Bladin first gave the name of triazole to the carbon nitrogen ring system and described derivatives of triazoles (Potts, 1961). Triazoles exist as two isomers – 1,2,3- triazoles and 1,2,4- triazoles as shown in Figure 1.1 (Balabin, 2009).

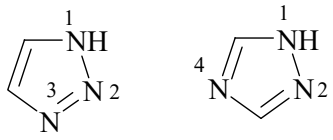


Figure 1.1. Isomers of triazole (a) 1,2,3-triazole and (b) 1,2,4-triazole

The two tautomeric forms of the 1,2,4- triazoles 1*H*- and 4*H*- 1,2,4-triazoles are characterized by the position of hydrogen (Fig.1.2).

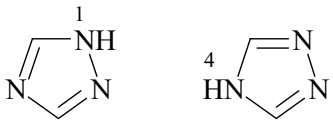


Figure 1.2. Structure of tautomers (a) 1*H*-1,2,4-triazole and (b) 4*H*-1,2,4-triazole

In the substituted 1,2,4-triazoles, 3-mercapto-1,2,4-triazoles exist in two tautomeric forms, because the labile hydrogen may be attached either to the nitrogen or the sulfur atom. It exhibits thione-thiol tautomeric forms (Fig. 1.3). This compound exists predominantly in the thione (a) form (Pinto *et. al*., 2007).

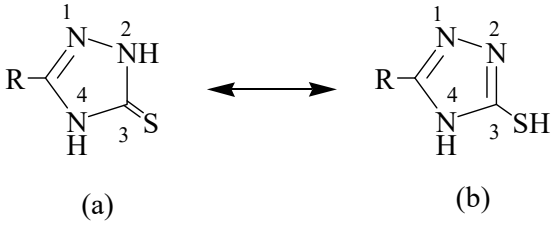


Figure 1.3. Thione (a) and Thiol (b) tautomers of substituted 3-mercapto 1,2,4-triazole.

Triazole is a white to pale yellow crystalline solid with a weak, characteristic odour, it is soluble in water and alcohol, melts at 120 °C and boils at 260 °C (Asif, 2014).

1,2,4 triazoles are amphoteric and can form salts with both acid and bases. Among the tautomers, structure (1.3a) has been found more stable than structure (1.3b), indicated by temperature coalescence studies, x-rays studies, basicity measurements, dipole moment studies, NMR-spectra and theoretical methods (Sathwara, 2016). 1,2,4 triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological applications.

Table 1.1: Properties of triazoles (Asif and al, 2014)

Physical Properties	1,2,3-triazole	1,2,4-triazole
Boiling point	203 °C	260 °C
Melting point	23-25 °C	120-121 °C
Density	1.192gm/cm^{-3}	1.394gm/cm^{-3}
Basicity (pK_b)	9.4	10.3
Acidity (pK_a)	1.2	2.2
Vapour pressure	0.4 mm Hg (25 °C)	0.02 mm Hg (25 °C)
Appearance	Colourless liquid	White solid

1.2.1. Biological Importance of Triazole Derivatives

Triazoles have received significant attention in the field of medicinal chemistry because of their diversified biological properties.

1,2,4- triazoles occupy a distinctive place in the field of medicinal and pharmaceutical chemistry (Maddila *et. al.*, 2013; Sharma *et. al.*, 2012), as well as in industry (Antonijevic & Petrovic, 2008). A large number of 1,2,4-triazole compounds exhibit important structural fragments and are considered as biologically active compounds such as antifungal (Maddila *et. al*., 2013), antibacterial activity (Chandrakantha *et. al*., 2010, Zoumpoulakis *et. al.*, 2012), anticonvulsant (Küçükgüzel, 2004), anti-tubercular (Küçükgüzel *et. al.*, 2001), anti-inflammatory inhibition (Maddila *et. al*., 2013), anticancer (Holla *et. al.*, 2003, Al-Soud *et. al*., 2006; Baviskar *et. al*., 2012), antioxidant activity (Sancak *et. al*., 2012).

The compounds containing a triazole ring have shown versatile and useful biological properties and have been developed as herbicides, fungicides, or plant growth regulators (W. Li *et. al*., 2004). 1,2,4-triazole derivative such as Triadimefon represents the most important category of fungicides to date and have excellent protective, curative, and eradicant activity against a broad spectrum of foliar, root, and seedling diseases caused by many ascomycetes, basidiomycetes, and imperfect fungi (García *et. al*., 2002; Menegola *et. al*., 2001).

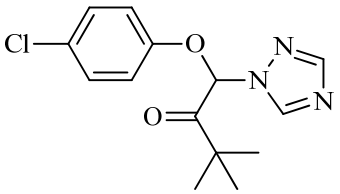
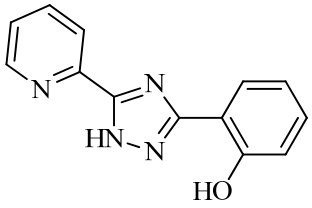


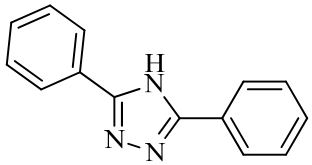
Figure 1.4. Triadimefon

1.2.2. Other Applications of Triazole Derivatives

Some other applications of triazoles derivatives other than bioactive agents are that they can be used as an electron transport layer in organic electroluminescent devices (Kido *et. al*., 1993). Some triazole derivatives have been evaluated as new corrosion inhibitors for the corrosion of Muntz alloy (Cu: Zn 60:40) in acidic and neutral solutions (Allam, 2006). Various other triazole derivatives in acidic medium inhibit steel corrosion (Bentiss *et. al*., 2000, 2003, 2007; Arshad *et. al*., 2017). Some of the triazoles are capable of inhibiting the fog formation in photographic emulsions (Yoshioka *et. al.*, 2013). They are also used in analytical chemistry.



2(5-(2-Pyridyl)-1H-1,2,4-trizol-3-yl)phenol (PPT)



3,5-Diphenyl-4H-1,2,4-triazole (DHT)

Figure 1.5. 1,2,4-triazole derivatives PPT and DHT as Mixed Type Inhibitor.

In the cotton industry, 1,2,4-triazole has been used as a commercial defoliant for several numbers of years (Allen, 1954, Linser & Kiermayer, 1957).

The triazole derivatives have many applications in the textile industry as some of them possess good detergent action and some have useful properties in inhibiting the acid fading of dyestuff (Fig. 1.6, Grimmel *et. al*., 1951).

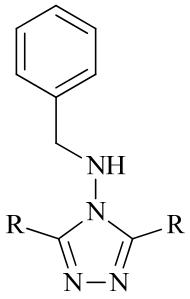


Figure 1.6. *N*-Benzylated aminotriazole inhibiting acid fading of dyestuff.

Triazole derivatives have been considered as environmentally friendly compounds (Wang *et. al*., 2004; Wang, 2006; Musa *et. al*., 2010a, b). The synthetic utility of triazoles is such that they can be subjected to different types of reactions to yield other heterocyclic compounds such as Schiff bases (Küçükgüzel *et. al*., 2004), Mannich bases (Plech *et. al*., 2013), thioureas (Küçükgüzel *et. al*., 2008, 2001, 1994), triazolothiadiazoles (Khan *et. al*., 2013) and many other heterocyclic compounds.

1.3 Schiff and Mannich Bases

Schiff bases are triazole derivatives formed by condensation of primary amino compounds with aldehyde or ketone. These compounds derived from heterocycles show various biological activities such as anticonvulsant (Küçükgüzel *et. al*., 2004), antiproliferative (Vicini *et. al*., 2003), anticancer and antifungal (Pignatello *et. al.,* 1994), cytotoxic (Tarafder *et. al*., 2002).

Mannich bases are the β-aminoketone which are generally formed when amine, aldehyde and carbon acid react. Mannich bases are the end products formed from Mannich reaction, i.e., nucleophilic addition reaction of non-enolizable aldehyde and any primary or secondary amine to produce stabilized imine.

The first Mannich reaction took place accidentally in 1912, when Carl Mannich, a young professor at that time, at the pharmaceutical lab of Gottingen University, mixed Salicyl antipyrine and urotropine (hexamethylenetetramine) in acidic condition to form a crystalline precipitate. Mannich later identified the structure (Fig. 1.7) with the help of W. Krögen Krösche. On his observation he found that the same condensation product was also formed by mixing antipyrine, formaldehyde and ammonium chloride. Regardless of the order of the reaction, he realized great synthetic relevance of the reaction as two different chemical moieties could be brought together by the help of Methylene Bridge (Tramontini & Angiolini, 1990).

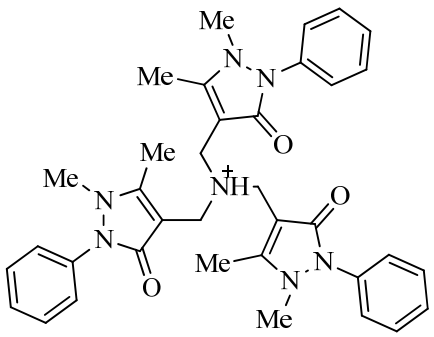


Figure 1.7. Product of First Mannich reaction

Mannich reaction is widely used for the construction of nitrogen containing compounds (Arend *et. al*., 1998). The scope of Mannich reaction includes a wide variety of amines, ammonia equivalents, imines and acetylated imines (Jia *et. al.*, 2012). The amino methylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds (Tramontini *et. al.*, 1990).

Mannich bases are physiologically reactive because of the basic function rendering the molecule soluble in an aqueous solvent when it is transferred into iminium salt. In recent past, the use of Mannich bases has been increased significantly because of their ability to show biological activities as antitubercular (Joshi *et. al*., 2004, Sriram *et. al*., 2009; Mulla *et. al*., 2011), antimalarial (Lopes *et. al*., 2004), vasorelaxing (Ferlin *et. al*., 2002), anticancer (Holla *et. al*., 2003; Ivanova *et. al*., 2007), anti-inflammatory (Kallurya *et. al*., 2005; Koksal *et. al*., 2007), antifilarial (Kallurya *et. al*., 2005), antibacterial (Ashok *et. al*., 2007, Pandeya *et. al*., 2000), antifungal (Pandeya *et. al*., 2000, Singh *et. al*., 2007), anticonvulsant (Vashistha *et. al*., 2004), anthelmintic (Bennet – Jenkins & Bryant, 1996), analgesic (Malinka *et. al*., 2005), anti-HIV (Sriram *et. al*., 2009), antipsychotic (Scott *et. al*., 1992), antiviral (Edwards *et. al*., 1983) activities and so forth.

Piperazine or morpholine ring is significant for antimicrobial activity (Foroumadi *et al*., 2006). Mannich bases of 1,2,4-triazole containing *N*-methylpiperazine moiety are known as antimicrobial agents (Holla *et. al.*, 2003) and *N*-substituted piperazine moiety possesses antifungal activity (Liang *et. al*., 2004; Ye *et. al*., 2005) as well. Some *in vitro* cytotoxic activity of some Mannich bases against lymphocytic leukemia has also been reported (Dimmock *et. al*., 1995).

Mannich bases containing aminoalkyl chain act as important pharmacophores or bioactive leads which are used for the synthesis of various potential agents of high medicinal value. The examples of such Mannich bases are cocaine, fluoxetine, atropine, ethacrynic acid, trihexyphenidyl, procyclidine, ranitidine, biperiden (Racane *et. al*., 2001, Kashiyama *et. al*., 1999, Bhusare *et. al*., 2001), and so forth. Mannich bases and their derivatives are intermediates for the synthesis of bioactive molecules (Ji *et. al*., 2003; Huang *et. al*., 2008) and can be easily converted to other compounds, for example, reduced to form physiologically active amino alcohols (Raman *et. al*.,

2004). Mannich bases are known to play a vital role in the development of synthetic pharmaceutical chemistry.

Along with biological activities Mannich bases are also known for their uses in detergent additives (Karll & Lee, 1983), resins, polymers, surface active agents (Otto, 1972). Mannich bases have gained importance due to their application in antibacterial activity (Holla *et. al*., 1998) and other applications are in agrochemicals such as plant growth regulators. Prodrugs of Mannich bases of various active compounds have been prepared to overcome the limitations (Dimmock & Kumar, 1997).

1.4. Some Marketed Drugs Containing 1,2,4-Triazole Ring

Rivabarin (Fig. 1.8a) is found to be effective against both DNA and RNA viruses. It is used in an aerosol for lower respiratory tract viral disease as well as in the treatment of influenza, Lassa fever, and Hantaan virus (Jenkins *et. al*., 1989). Amidine and guanidine derivatives of Rivabarin (Fig. 1.8b) exhibit a broad spectrum of antiviral activity (Kini *et. al*., 1989).

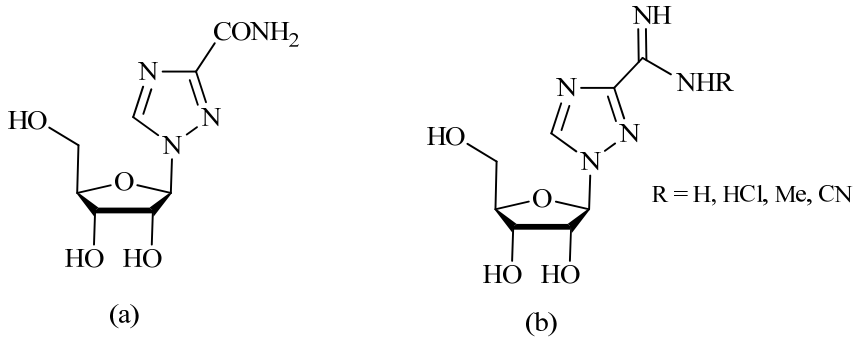
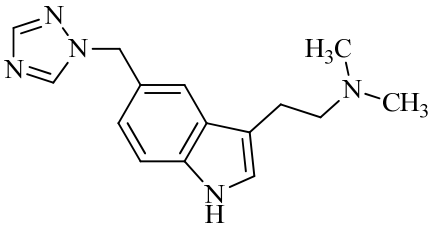
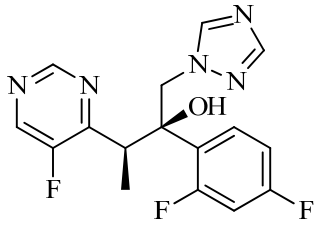


Figure 1.8. Antiviral triazole derivatives (a) Ribavarin and (b) its amidine and guanine derivatives.

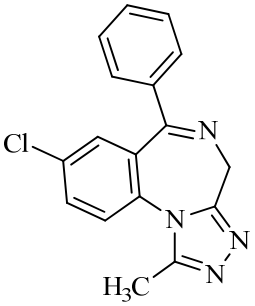
Various drugs have been found with 1,2,4-triazole ring such as Rizaptriptan (antimigraine agent, Karthikeyan *et. al*., 2006), Fluconazole, Itraconazole, (antimycotic agent, Karthikeyan *et. al*., 2006), Triazolam, Alprazolam, Estazolam (anticonvulsant drug, Küçükgüzel& Çıkla-Süzgün, 2015), Letrozole, Anastrozole, Vorozole (aromatase inhibitors, Gross & Strasser- Weippl, 2004; Santen, 2003).



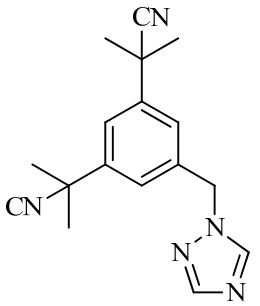
Rizatriptan (antimigraine drug)



Itraconazole (antimycotic drug)



Alprazolam
(anticonvulsant drug)



Anastrozole
(aromatase inhibitor)

Figure 1.9. Drugs containing 1,2,4-triazole moiety.

CHAPTER 2

2. OBJECTIVES OF THE STUDY

2.1. General Objective

- To synthesize the Mannich bases of the 1,2,4-triazole and study their biological activities.

2.2. Specific Objectives

- To synthesize Mannich bases of 4-(furan-2-yl-methyleneamino)-3-(2-Hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione.
- To characterize the synthesized compounds by UV, IR and NMR spectral techniques.
- To evaluate the antibacterial and antifungal activities of the synthesized compounds.

CHAPTER 3

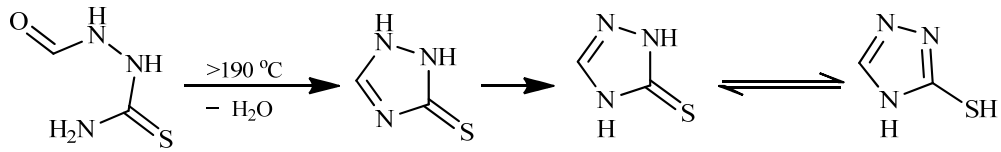
3. LITERATURE SURVEY

1,2,4 triazoles have been studied a lot in the last decade by researchers and hence, the synthesis scheme of such 1,2,4 triazoles have been in large number. Their importance in the field of pharmaceutical and medicine can also be understood by their various synthesis schemes.

3.1. Synthesis of 1,2,4 Triazole and Their Derivatives

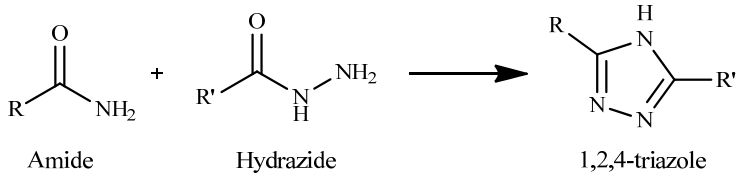
Various schemes are available for the synthesis of triazole and their derivatives. They can be prepared by simple heating, cyclization, reflux, one-pot reaction, cyclo condensation, ring closure, regioselectively, reduction cleavage, etc.

Freund (1896) first synthesized of 1,2,4 triazole-3-thione by dry heating of the formyl thiosemicarbazide.



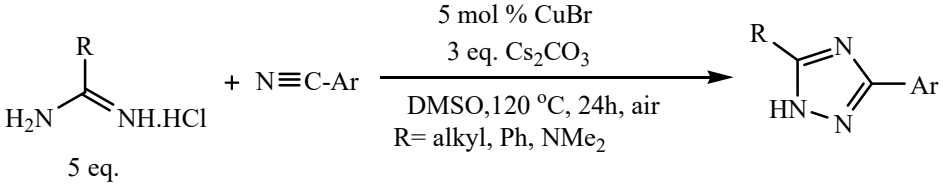
Scheme 3.1. Synthesis of 1,2,4-triazole-3-thione *via* heating of formyl thiosemicarbazide.

Pellizzari (1911) reported the synthesis of substituted 1, 2, 4-triazole by the reaction of an amide and a hydrazide.



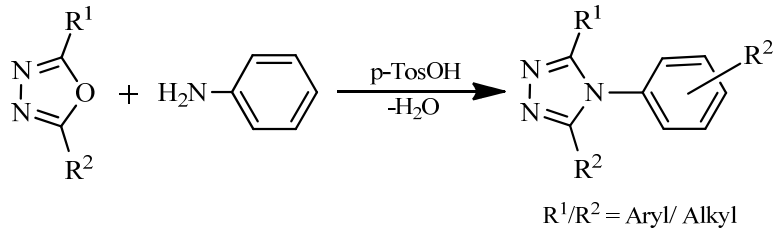
Scheme 3.2. Synthesis of the substituted 1,2,4-triazole by the reaction of an amide with hydrazide.

Ueda *et. al*., (2003) synthesized the triazole nucleus by using a copper catalyst. Substituted amidine and benzonitrile were treated in presence of Cu catalyst to give 1,2,4 triazole nucleus. It is also termed as an oxidative coupling reaction as sequential N-C and N-N bonds were formed.



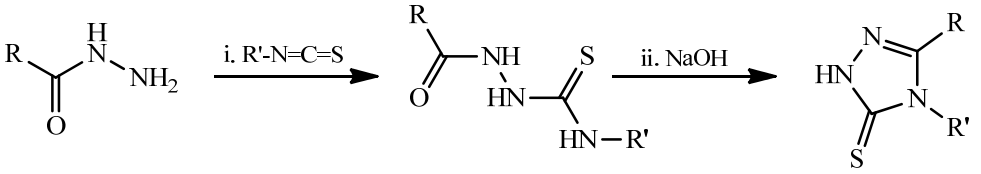
Scheme 3.3. Synthesis of the 1,2,4-triazole nucleus *via* an oxidative coupling.

1,3,4-oxadiazole on reaction with the primary amines gives 3,5-disubstituted 1,2,4-triazole (Levin & Skorobogatova *et. al*, 1967).



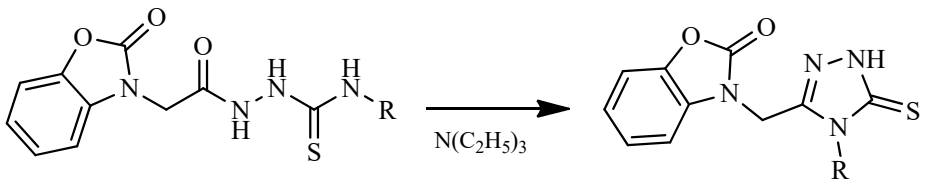
Scheme 3.4. Synthesis of substituted 1,2,4-triazoles by using substituted oxadiazole.

The classical method for synthesis of triazole containing five membered nucleus is synthesized by heating the substituted isothiocyanates in presence of an alkaline medium. (SGüniz *et. al*., 2015)



Scheme 3.5. Synthesis of 1,2,4-triazole derivatives *via* the classical method.

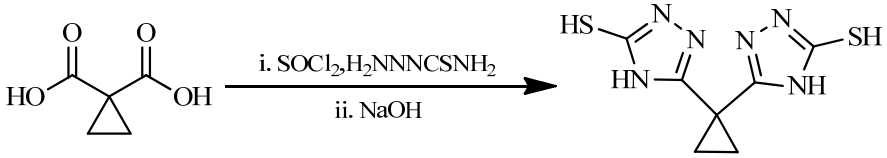
Thiosemicarbazides on heating with trimethylamine in ethanol cyclizes to give 1,2,4 triazoles (Umut *et. al*., 2007).



R = Substitued aryl

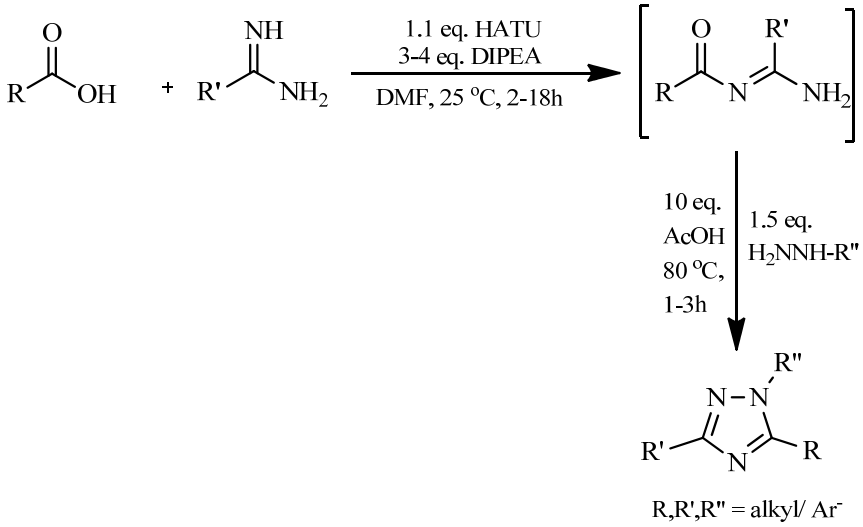
Scheme 3.6. Synthesis of 1,2,4-triazole derivatives *via* cyclization of thiosemicarbazides by trimethylamine.

Similarly, Thiosemicarbazide on refluxing with 1,1 cyclopropane dicarboxylic acid and thionyl chloride gives desired triazole derivatives. (Sharba *et. al*., 2005).



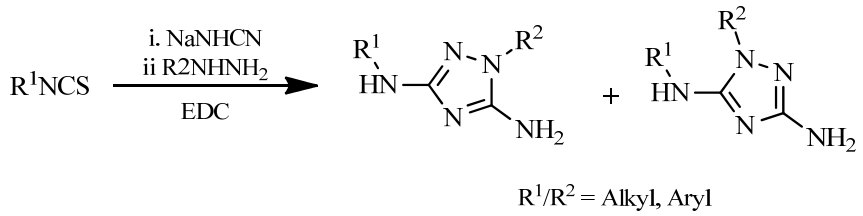
Scheme 3.7. Synthesis of 1,2,4-triazole derivatives *via* cyclization of thiosemicarbazide by cyclopropane dicarboxylic acid.

Castanedo *et. al*., (2011) introduced a regioselective one-pot process for the formation of 1,2,4 triazoles. Carboxylic acid and primary amidines were regiospecifically treated to form the desired triazole rapidly.



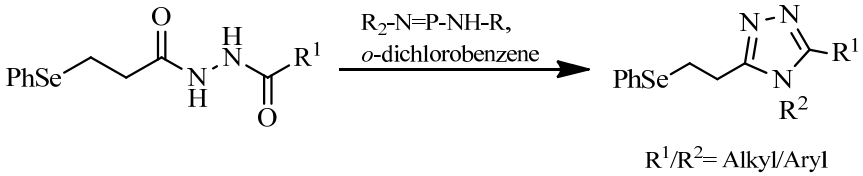
Scheme 3.8. Regioselective one-pot Synthesis of 1,2,4-triazole.

l,2,4-Triazole-3,5-diamine derivatives were synthesized in moderate to high yields in one-pot reaction from the corresponding isothiocyanates, monosubstituted hydrazines, and sodium hydrogen cyanamide in the presence of l-(3-dimethylaminopropyl)-3 ethylcarbodiimide hydrochloride. Typically, two target compounds were obtained, but high regioselectivity to one isomer was observed when aromatic and sterically bulky hydrazines were used (Liu & Iwanowicz, 2003).



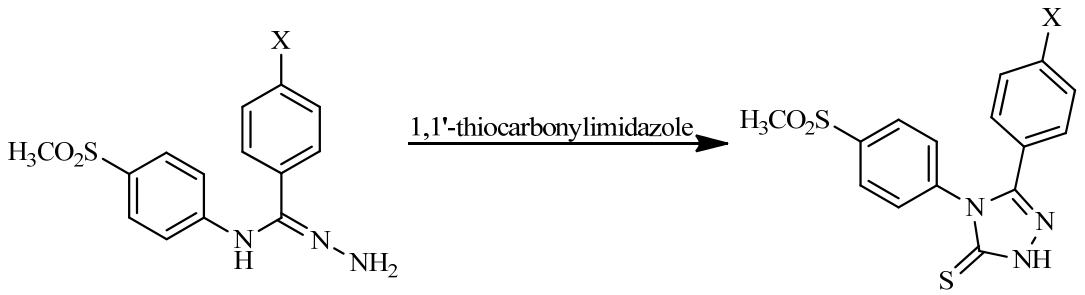
Scheme 3.9. One-pot synthesis of 1,2,4-triazole-3,5-diamine derivatives.

Wang *et. al.* (2007) synthesized 3,4-disubstituted-1,2,4-triazole by cyclo-condensation reactions of acyl phenylselanyl propanehydrazide.



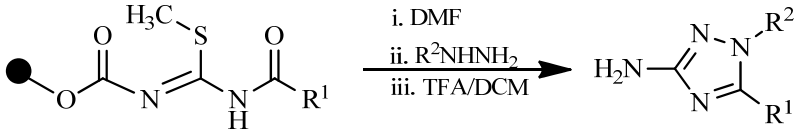
Scheme 3.10. Synthesis of 3,4-disubstituted-1,2,4-triazole by cyclocondensation of acyl phenylselanyl propanehydrazide.

The *N*-(4-methylsulfonylphenyl)-aryl carbohydrazonamides undergoes a ring closure using 1,1'-thiocarbonyldiimidazole and subsequent alkylation to afford 5-(4-halophenyl)-4-(4-(methylsulfonyl)phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (Daniel *et. al.*, 2010).



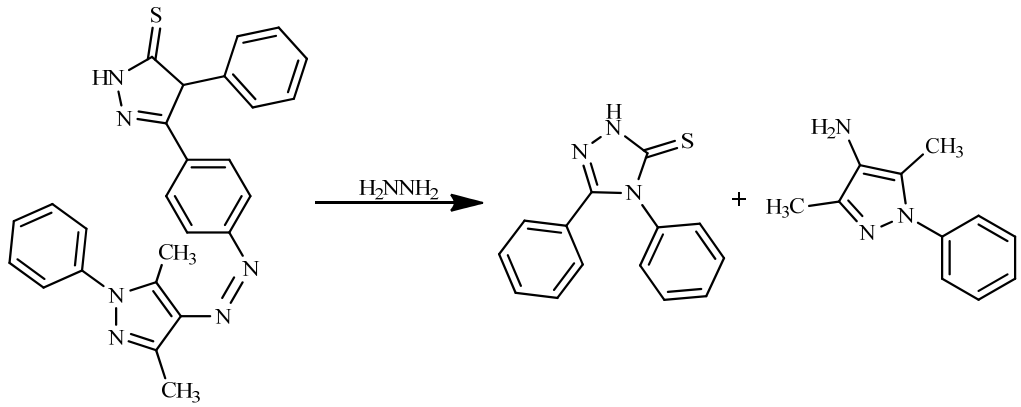
Scheme 3.11. Synthesis of 1,2,4-triazole-3-thione derivatives by ring closure of carbohydrazonamides using 1,1'-thiocarbonyldiimidazole.

The reaction of resin bound *S*-methyl isothiourea with carboxylic acids yielded resin-bound *S*-methyl- acylisothiourea which reacted with hydrazines under mild conditions to afford the corresponding 3-amino-1,2,4-triazoles with regioselectivity (Yongping *et. al.*, 2003).



Scheme 3.12. Solid phase synthesis of 3-amino-1,2,4-triazole derivatives.

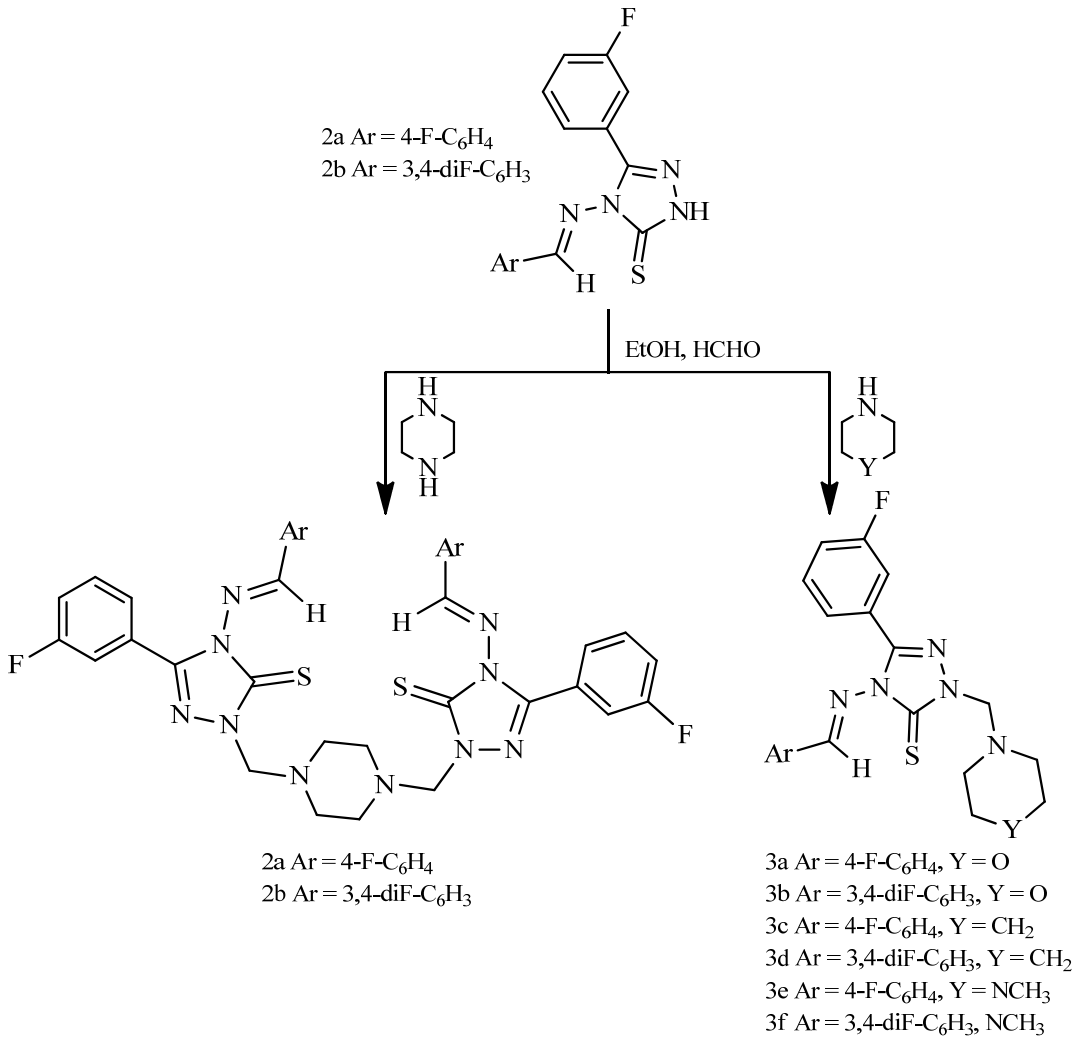
Rollas *et. al.*, synthesized 1,2,4 triazoles by reduction cleavage of azo compounds using hydrazine hydrate.



Scheme 3.13. Synthesis of 1,2,4-triazole derivatives *via* reduction cleavage of azo compounds.

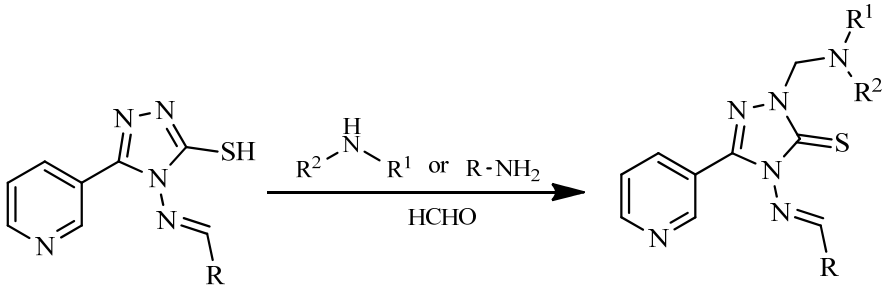
3.2. Synthesis of Mannich bases having 1,2,4 triazole skeleton

Mannich reactions on 3,4,5-trisubstituted-1,2,4-triazole which exist as thiol-thione tautomers gave the new Mannich bases *via* aminomethylation of the endocyclic nitrogen (*N*-2) of the triazole ring with formaldehyde and the appropriate secondary amine in ethanol (Aoud, 2014).



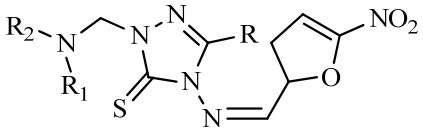
Scheme 3.14. Synthesis of Mannich bases *via* Mannich reaction.

Similarly, Dave *et. al*., (2006), synthesized Mannich base by Mannich reaction. 4-amino-5-(3-pyridyl)-4*H*-1,2,4-triazole-3-thiol in the presence of primary/secondary amines and formaldehyde gave Mannich base 2-(bis-aryl-amino-methyl)-5-pyridin-3'-yl-4-substituted-banzal-amino-2,3-dihydro-1,2,4-triazole-3-thione (Scheme 3.15).



Scheme 3.15. Synthesis of Mannich base from Schiff base.

Bala *et. al*., (2014) synthesized many novel Mannich bases of 3-substituted-4-(5-nitro-2-furfurylidene)amino-5-mercapto-1,2,4-triazoles (Fig. 3.1) and were screened for antimicrobial tests.



	R_1	R_2
c	H	3-Cl, 4-F-aniline
d	Me	morpholine
f	Me	3-Cl, 4-F-aniline
g	Et	morpholine
h	Et	3-Cl, 4-F-aniline
n	Ph	3-Cl, 4-F-aniline

Figure 3.1. 3-substituted-4-(5-nitro-2-furfurylidene)amino-5-mercapto-1,2,4-triazole (Mannich base).

3.3 Biological importance of Mannich bases

3.3.1. Antibacterial Activity

A variety of antibacterials currently in use act suppressing the growth of the bacteria or killing the bacteria. The medicinal chemists are still in search of new antibacterial drugs without any side effects.

Plech *et. al*., (2013), synthesized a series of Mannich bases derived from 4,5 disubstituded 1,2,4-triazole-3-thiones and evaluated the antibacterial activity of synthesized compounds against five gram-positive bacterial strains, *S. aureus*, *S. epidermidis*, *B. cereus*, *B. subtilis* and *M. luteus*. Some of the Mannich bases (Fig. 3.2. a, b. c) was similar or higher than the activity of usually used antibacterial agents such as ampicillin and cefuroxime.

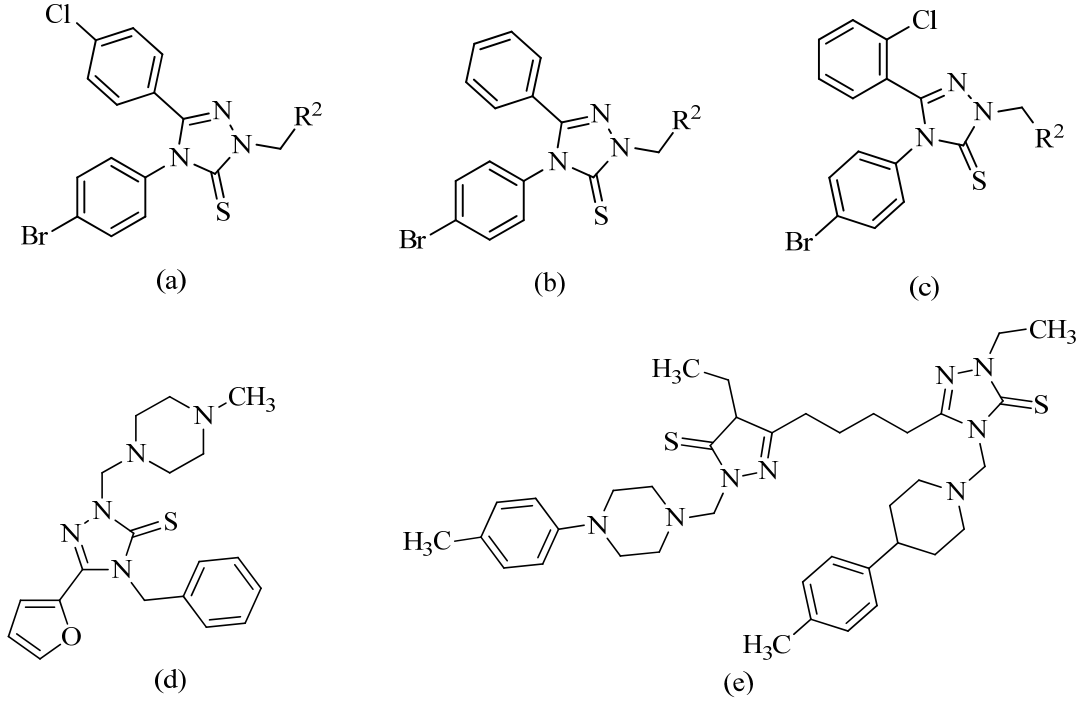


Figure 3.2. Potent antibacterial Mannich bases with 1,2,4-triazole nucleus.

Basoglu *et. al*., (2013), synthesized 1,2,4-triazoles and their Mannich bases and found that 4-benzyl-5-(furan-2-yl)-2-[(4-methylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.2.d) demonstrated relatively good activity against human pathogens such as *E. coli*, *E. aerogenes* and *Y. pseudotuberculosis*. Against *E. aerogenes* compound 3.2 d showed equal activity with the inhibition zone of 10 mm when compared to ampicillin as standard.

Koparir *et. al*., (2013) synthesized 5,5'-butane-1,4-diyl-bis(4-ethyl)-3*H*-1,2,4-triazole-3-thione and their Mannich bases and were tested against all bacterial strains. 5,5'-butane-1,4-diyl-bis{4-ethyl-2-[4-methylpiperidin-1-yl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.2 e) and 5,5'-butane-1,4-diyl-bis[4-ethyl-2-({4-[3-(trifluoromethyl) phenyl]piperazin-1-yl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.2 f) displayed significant antibacterial against all the tested bacterial strains at 3.12-1.56 $\mu g.mL^{-1}$ concentrations.

Murthy *et. al*., (2012), synthesized Schiff and Mannich bases of Isatin derivatives (Fig. 3.2 g) with 4-amino-5-benzyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione and determined their antimicrobial activities compared with ciprofloxacin. Mannich bases showed of Isatin derivatives showed more activity than that of Schiff bases which confirmed the presence of >N-CH_2-N< bond enhances the antimicrobial activity.

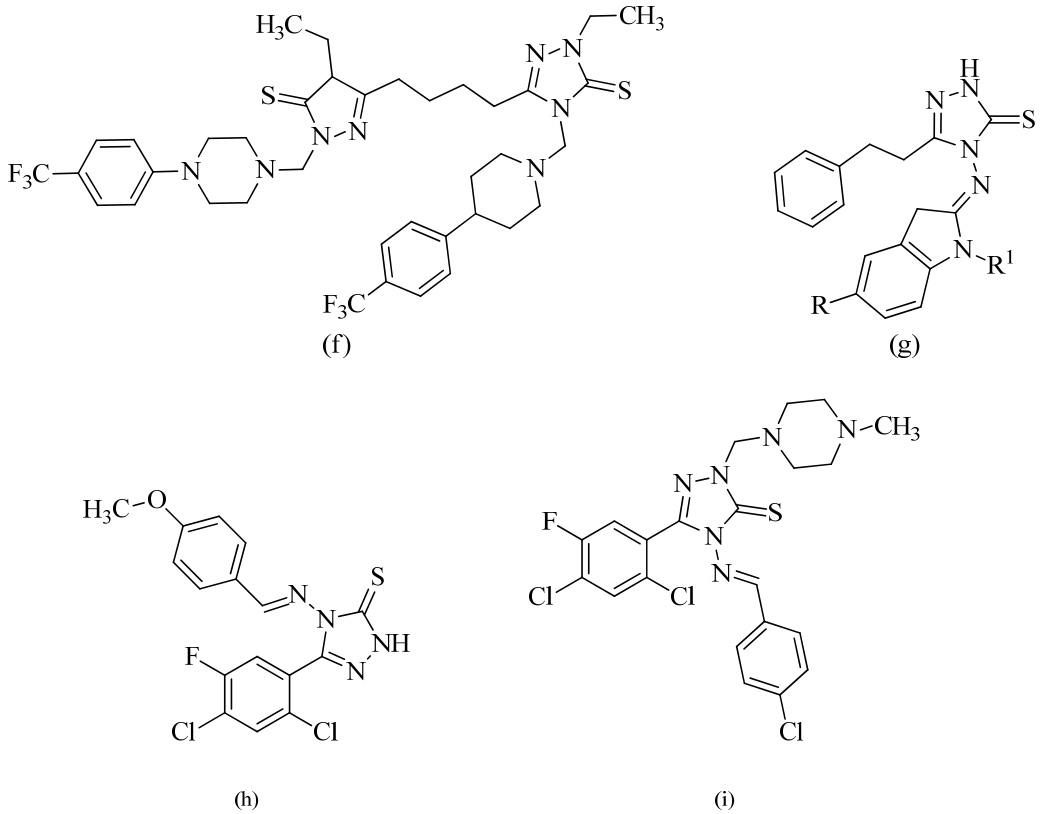
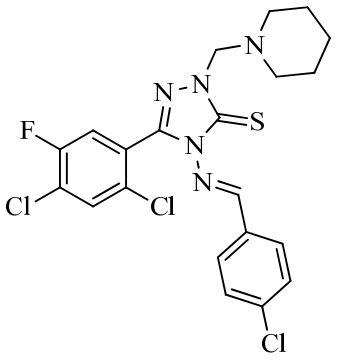


Figure 3.3. Some potent antibacterial Schiff and Mannich bases.

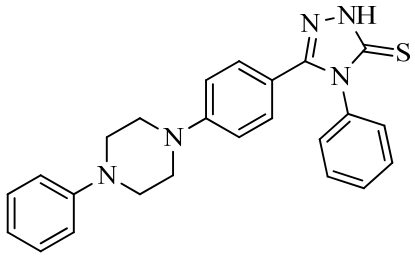
Karthikeyan *et. al*., (2006), prepared Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety and evaluated for their antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* bacterial strains by disc diffusion method by using ciprofloxacin as a standard drug. The results of antibacterial activity displayed that three of the compounds (Fig. 3.3h, i and Fig 3.4k) showed good bacterial inhibition.

Rajaka *et. al*., (2011), designed and synthesized new derivatives of [5-(4- substituted-piperazin-1-yl)-phenyl]-4-substituted-2,4-dihydro1,2,4-triazole-3-thione to evaluate their antimicrobial activity against different bacteria *(P. mirabilis, P. aeruginosa, B. subtilis, and S. aureus*). Their findings revealed that [5-(4-phenyl-piperazin1-yl)-phenyl]-4-phenyl-2,4-dihydro-1,2,4-triazole-3-thione (Fig. 3.4.l) showed promising antimicrobial activity among all the tested compounds.

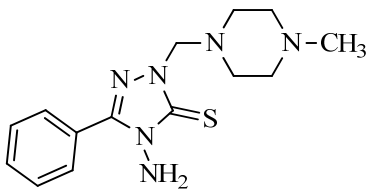
Bayrak *et. al*., (2009), synthesized novel 1,2,4 triazoles starting from isonicotinic acid hydrazide and evaluated their anti-bacterial properties. It was found that 4-amino-2-[9 4-methylpiperazin-1-yl)methyl]-5-pyridin-4-yl-2,4-dihydro-3*H*-1,2,4-triazole-thione active (Fig. 3.4.m) against *E. coli*, *Y. pseudituberculosis*, *P. aeruginosa*, *E. faecalis, S. aureus*, *B. cereus*.



(k)



(l)



(m)

Figure 3.4. Some potent antibacterial Mannich bases.

3.3.2. Antifungal activity

Fungal infections are caused by microscopic organisms that can spread over the epithelial tissues, several species of fungi are potentially pathogenic in humans, *Candida* is the organism responsible for most fungal infections that are estimated to happen over a billion of people each year. The search for new antifungal agents will consequently always remain as an important and challenging task for medicinal chemists.

Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety were synthesized to test for their antifungal properties against *A. flavus*, *A. fumigatus*, *P. marneffei* and *T. mentagrophytesin* by serial plate dilution method. Among the screened compounds, three triazole derivatives (Fig. 3.5. a, b, c) occurred as active against all the fungal strains almost equivalent to that of fluconazole as a standard (Karthikeyan *et. al*., 2006).

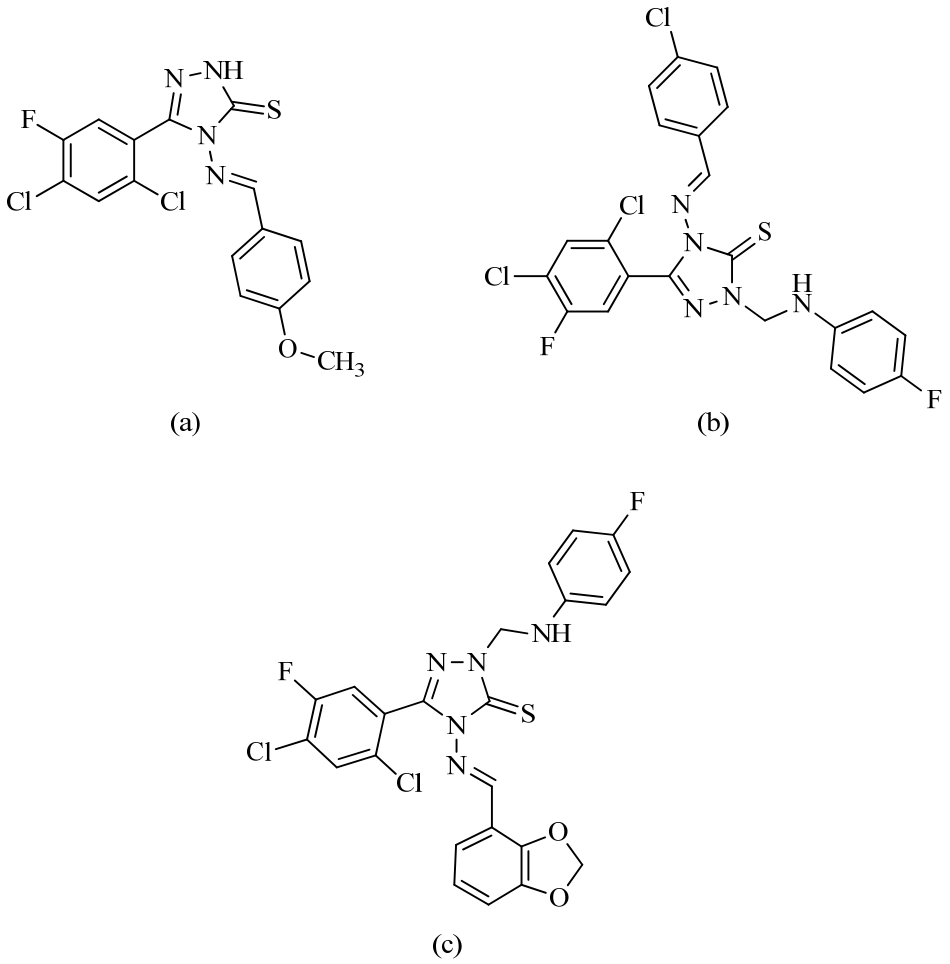


Figure 3.5. Antifungal Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety.

In 2010 Wang *et. al*., synthesized a series of trifluoromethyl-substituted-1,2,4-triazole Mannich base and bis(1,2,4-triazole) Mannich base containing pyrimidinylpiperazine rings *via* the Mannich reaction. 1-[4-(4,6-disubstitued-pyrimidin-2-yl)piperazin-1-yl)methyl]-4-(substituted)benzylideneamino-3-trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione (Fig. 3.6.d) and showed potential fungicidal activity against *C. cassiicola*, *P. syringae pv. lachrymans*, *A. citrallina Smith*, *P. cubensis*, and *S. sclerotiorum* at a concentration of 500 mg.mL^{-1}.

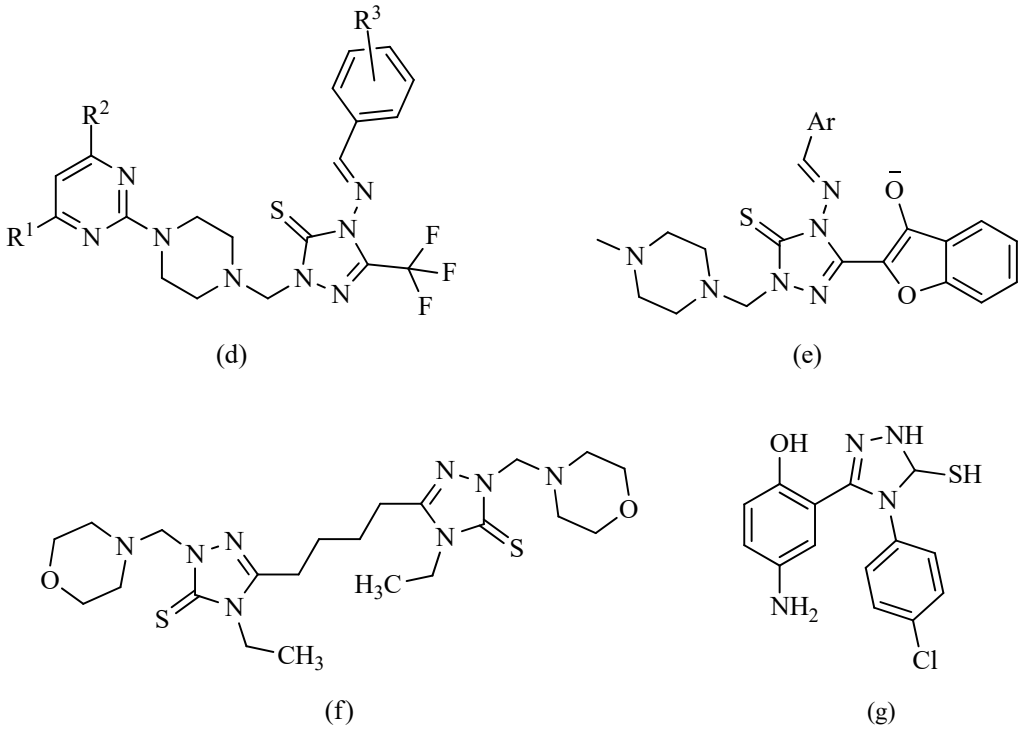


Figure 3.6. Some potent antifungal Mannich bases.

Ramakrishna *et. al*., (2012), reported the synthesis of some novel Mannich bases derived from 1,2,4 triazoles and evaluated their antifungal activity by using ketoconazole as a reference standard. Compounds with *N*-methyl piperazine derivatives were antifungal active against *C. albicans* (Fig. 3.6e).

Koparir *et. al*., (2013) also synthesized new Mannich bases like 5,5' -butane-1,4-diyl bis[4-ethyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.6f) and evaluated that it showed excellent antifungal activity against the fungal strains of *A. flavis* and *C. albicans* 1.56-3.12 µgm/mL.

Hussain *et. al*., (2008), synthesized 4-amino-2-[4-(4-substitutedphenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]phenol and evaluated for their antifungal activity against *A. niger* by the cup plate method using ketoconazole as a standard. The 1,2,4-triazole

derivative, having chloro group at para position of phenyl ring (Fig. 3.6 g) displayed a MIC of 25 mg/mL against *A. niger.*

3.3.3. Antiviral Activity

Antiviral drugs are used against viral infections. However, because of the lack of effectiveness and high cost of some antiviral therapies, there is a need of finding new effective antiviral compounds.

Akhtar *et. al*., (2007) synthesized 4-(4-Chlorophenyl-5-(2-mercapto-1-(*p*-toulenesulfonylamion)propyl)-2*H*-1,2,4-triazole-3-thione (Fig. 3.7a), was found be the most active with an EC_{50} of 23.9 µg/mL against HIV-1 and 9.90 µg/mL against HIV-2 of CC50 of 72.7 ± 1.4 mg/mL, resulting in selectivity index of 3 and 7, respectively. This study was indicated that active compounds could be proposed to act as a nonnucleoside reverse transcriptase inhibitor (NNRTI).

Küçükgüzel *et. al*., (2008), synthesized some novel thiourea derivatives and evaluated their antiviral activities. *N*-alkyl/aryl-*N'*-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy] phenyl}thioureas(Fig. 3.7b) bearing an allyl group at *N*-4 of 1,2,4-triazole ring exhibited moderate protection against Coxackie virus B4 with an MIC value of 16 mg/mL and a selectivity index of 5. This compound was also showed activity against thymidine kinase positive Varicella-zoster virus (TKs¸ VZV, OKA strain) with an EC_{50} value of 9.9 µg/mL.

Cikla *et. al*., (2013, 2015) synthesized etodolac 1,2,4-triazoles and etodolac thiosemicarbazides starting from etodolac thiosemicarbazides and etodolac hydrazide respectively and evaluated their anti–HCV NS5B polymerase activities. 5-[(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)methyl]-4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3- thione (Fig. 3.7c), was found to be the most active with IC_{50} value of 14.8 µM.

Benci *et. al.,* (2011), synthesized and evaluated 1,2,4-triazoleacyclic cyclopropane nucleoside analogues and identified it as an antiviral agent (Fig. 3.7d).

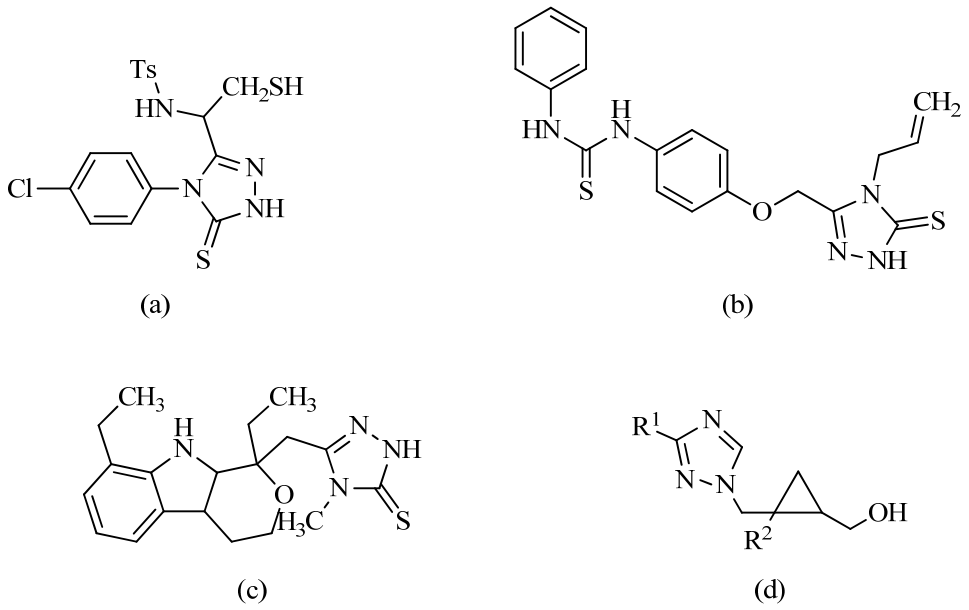


Figure 3.7. Some potent antiviral Mannich bases.

3.3.4. Anticancer Activity

Cancer is a condition of abnormal growth of cells or the presence of a huge amount of abnormal cells, which, indeed require the most effective anticancer drugs. The drugs up to date used are expensive and require modification, hence, the study and synthesis of effectively new anticancer drugs have been experienced.

Holla *et. al*., (2003), synthesized 3-substituted 4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene]amino-5-mercapto-1,2,4-triazoles and Mannich bases with anticancer activity were designed. The synthesized compounds (Fig. 3.8a, b, c) were screened against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia at NIH, Bethesda, Maryland, USA under the Drug Discovery Programmer of NCI.

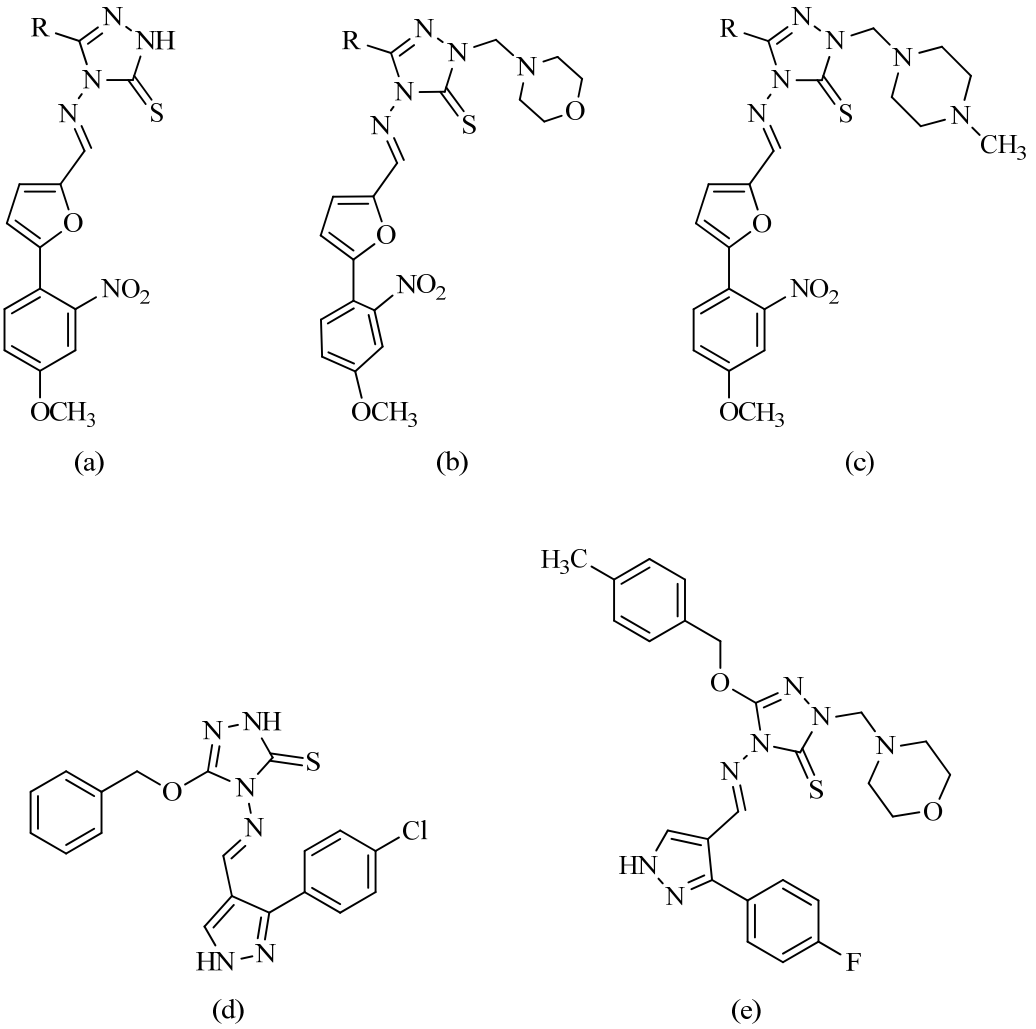


Figure 3.8. Reported anticancer Schiff and Mannich bases.

Sunil *et. al*., (2010), synthesized some Schiff and Mannich bases and were screened for cytotoxic activity against the human hepatocellular liver carcinoma cell line (HepG2) by MTT assay. This study revealed that compound (Fig. 3.8d) was found to be with IC_{50} value of 0.018g/L, comparable to doxorubicin (IC_{50} value of doxorubicin was found to be 0.017 g/L). On the other hand, compound (Fig. 3.8e) showed better activity with IC_{50} value of 0.034 g/L.

Popiołek *et al*., (2014) investigated Mannich bases bearing 1,2,4 triazole system against six cancer cell lines namely A549, Hela, human ovarian cancer cell line (ToV-112D), murine aneuploid fibrosarcoma cell line (L929) and green monkey kidney cell line (GMK). This study revealed that 4-(4-chlorophrnyl)-5-cyclohexyl-2-[(4-phenylpiperazin-1-yl)methyl)]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione and 4-phenyl-2-(pyrrolidin-1yl-methyl)-5-cyclohexyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione were

found to have cytotoxicity activity with 15-25% growth inhibition against T47D (Fig. 3.9 f & g).

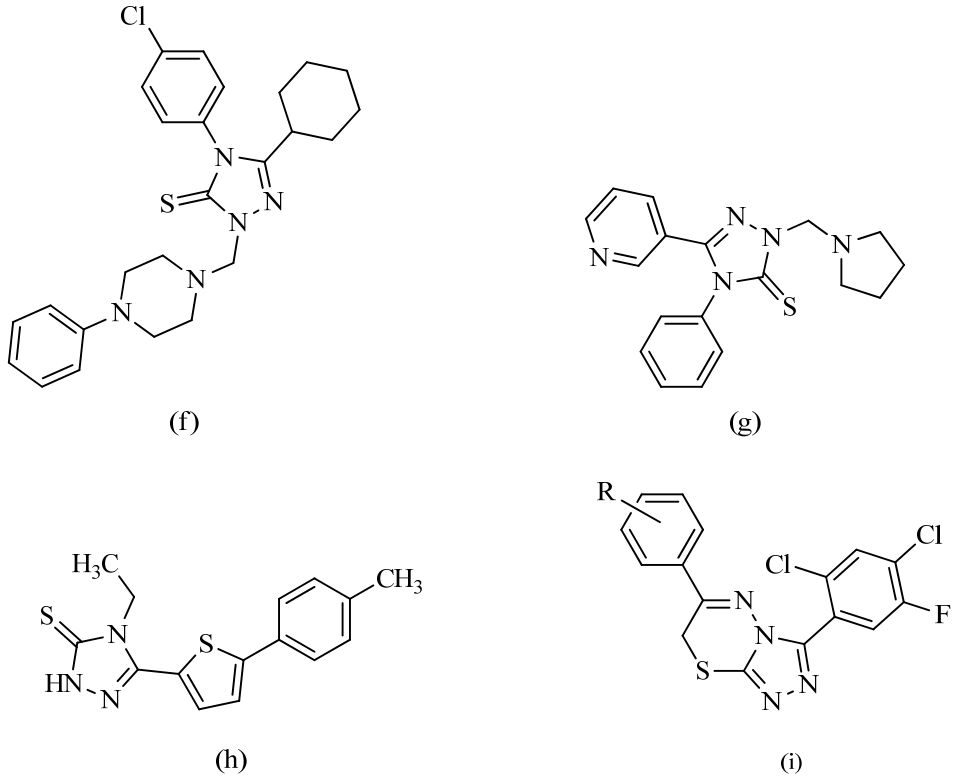


Figure 3.9. Some potent anticancer Schiff and Mannich bases.

Mavrova *et. al*., (2012), investigated various 1,2,4 triazoles derivatives for cytotoxicity activity against tumor cells and immunocompetent cells (spleen lymphocytes) and revealed that 4-ethyl-5-(5-(4-methylphenyl)thione-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.9h) was cytotoxic to affected cells but not to normal cells.

Bhat *et. al*., (2009), synthesized a series of 3-(2,4-dichloro-5-fluorophenyl)-6-(substitutedphenyl)-1,2,4-triazolo[3,4,*b*]-1,3,4-thiadizines and evaluated for their antitumor activity. Some of the compounds exhibited *in vitro* antitumor activity with moderate to excellent growth inhibition against a panel of sixty cancer cell lines (Fig. 3.9i).

3.3.5. Anticonvulsant Activity

Anticonvulsants are used in the treatment of epileptic seizures. Their use is highly increased for the treatment of bipolar disorder and borderline personality disorder. They are also used as mood stabilizers and neuropathic pain relievers.

Sudhir *et. al*., (2011), synthesized various 3-substituted-4-amino-5-Mercapto-4*H*-1,2,4-triazole derivatives (Fig. 3.10a) and evaluated their anticonvulsant activity by Maximum electro seizures (MES)and Minimum electro threshold seizures (METS) methods.

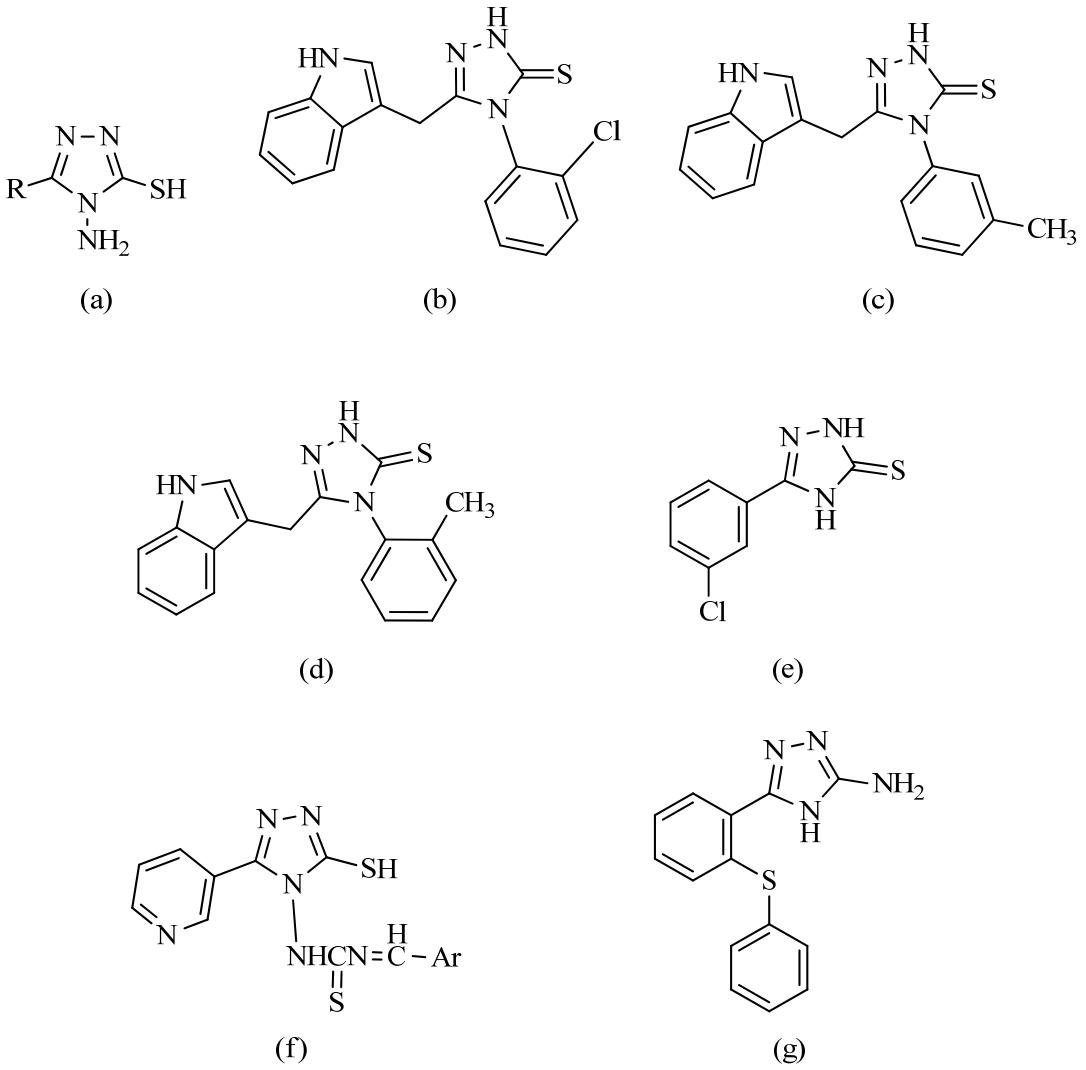


Figure 3.10. Some potent anticonvulsant 1,2,4-triazole derivatives.

Siddiqui *et. al*., (2008), prepared a various 5-(1*H*-indol-3-yl)-methyl-4-(substituted aryl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (Fig. 3.10 b, c, d) and screened for their anticonvulsant activity. Three of the derivatives, -2 Cl, -2 CH_3 and 3 CH_3 substituents showed comparable MES activity to phenytonin and carbamazepine.

Plech *et. al*., (2013), synthesized 5-(3-chlorophenyl)-2,4-dichloro-3*H*-1,2,4-triazolo-3-thione (Fig. 3.10e) and screened for their anticonvulsant activity which showed excellent result.

Kshirsagar *et. al*., (2009), synthesized the substituted *N*-(5-mercapto-3-pyridyl-3-yl-4*H*-1,2,4-triazol-4-yl)-thiosemicarbazone (Fig. 3.10f) from nicotinic acid and evaluated their anticonvulsant activity by Maximum Electroshock (MES) method and found that recovery time and time for hind limb extension recovery compound was less than the standard Phenytonin.

Almasirad *et. al*., (2004), prepared 5-[2-(2-fluorophenoxy)-phenyl[-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig. 3.10g) and it showed mild effects in scPtz and MES tests.

3.3.6. Anti-inflammatory Activity

Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain.

5-(2-Naphthyloxymethyl)-4-methyl-1,2,4-triazole-3-thione (Fig. 3.11a) showed promising activity with the 60.62 ± 8.55% CPE inhibition and 32.43 ± 3.15*10^5 /cm^3 for inhibiting PMNL production. In this study, this compound was found to have a superior anti-inflammatory profile with low gastric ulceration incidence with similar toxic profiles of reference NSAIDs in the liver (Palaska *et. al*., 2002).

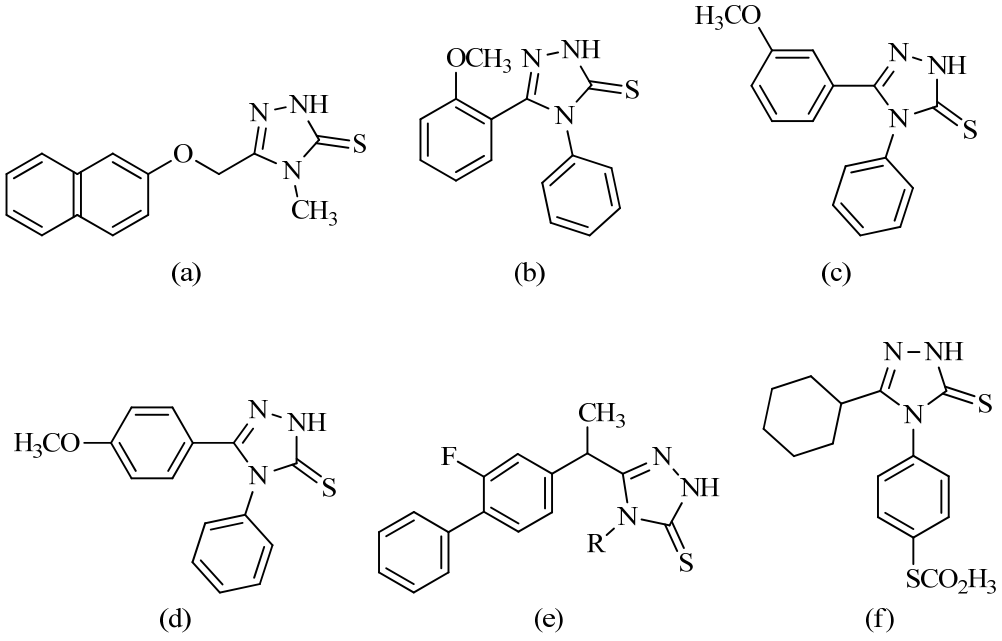


Figure 3.11. Some potent anti-inflammatory Schiff and Mannich bases.

3-(2-,3-and4-methoxyphenyl)-4-phenyl-4,5-dihydro-4*H*-1,2,4-triazole-5-thiones (Fig.3.11b, c, d) were exhibited anti-inflammatory activity. The acute toxicity (LD50) of the most active compounds with 4-methoxy phenyl substituent (Fig.3.11c) was less than acute toxicity of acetylsalicylic acid and significantly less than that of ibuprofen, (Labanausakas *et. al*., 2004).

In 2005, Amir and Kumar prepared 5-substituted-4-alkyl/aryl3-mercapto-4*H*-1,2,4-triazoles derived from flurbiprofen and evaluated for their anti-inflammatory activities. This study indicated that 1,2,4-triazoles derived from flurbiprofen showed anti-inflammatory activity ranging from 66.07% to 90.58%. The triazole derivative having the n-butyl group at 4th position (Fig. 3.11e) was displayed the highest activity with 90.58% inhibition, whereas the standard drug flurbiprofen showed 95.57% inhibition after 4h.

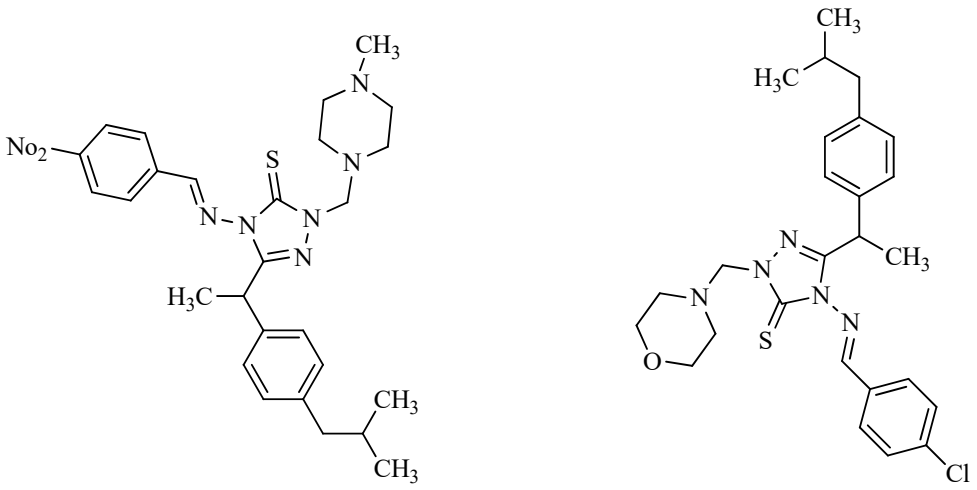


Figure 3.12. Potent anti-inflammatory Mannich bases derived from Ibuprofen (g) and with Morpholine group (h).

Navidpour *et. al*. (2006), noted that compound having cyclohexyl substituent, namely, 4-cyclohexyl-5-(4-methylsulfonylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (Fig. 3.11f) was the most active anti-inflammatory agent (51% and 37% reduction in inflammation at 3 and 5 h postdrug administration, respectively) for 50 mg/kg oral dose. This compound was also displayed potent inhibition of COX-2 and good selectivity index as compared to reference compound celecoxib.

A series of 4-arylideneamino-2-substituted aminomethyl-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-3*H*-1,2,4-triazole-3-thione were synthesized from ibuprofen and evaluated for their anti-inflammatory activity using the CPE method in Wistar albino rat. The compounds having a morpholine group (Fig. 3.12g) and methyl piperazine (Fig. 3.12. h) were found to possess the highest activity (74%, Sujith *et. al*., 2009).

3.3.7. Antioxidant Activity

Antioxidants reduce or neutralize the free radicals therefore, keeping the cells from oxidative injuries. They are used in treatment of lifestyle diseases like diabetes, cancer, ageing, cardiovascular. Hence, researchers are interested in designing some new oxidants *via* a synthetic methodology.

Koparir *et. al*., (2013), reported that 5,5' -butane-1,4-diyl-*bis* [4-allyl-2-(morpholine-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione] (Fig.3.13a) exhibited the highest radical scavenging activities which were better than using ascorbic acid as a reference.

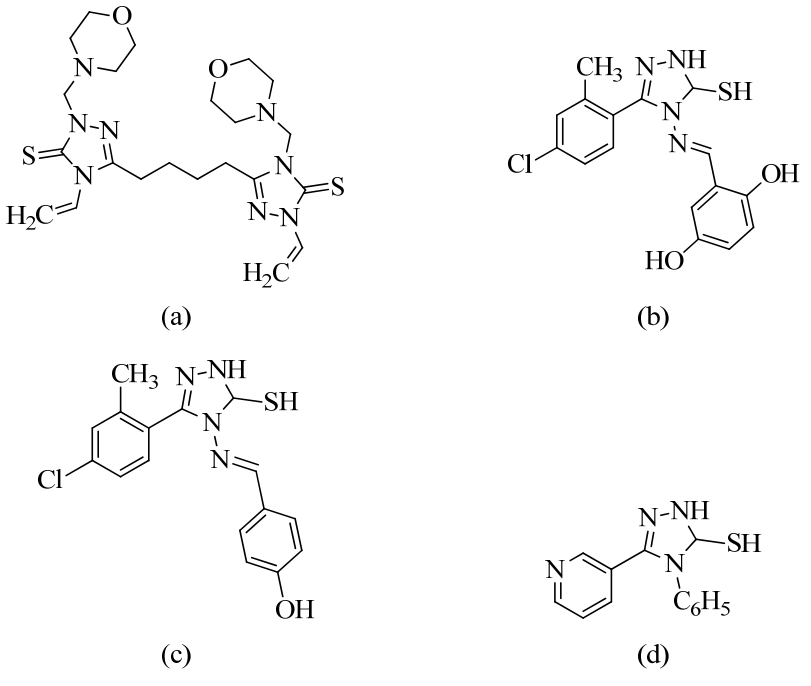


Figure 3.13. Potent antioxidant Schiff and Mannich Bases.

Aswathanarayanappa *et. al*., (2013), prepared Schiff bases of 1,2,4- triazole-based to evaluate for antioxidant properties by free radical scavenging and reported that especially two derivatives (Fig.3.13b, c) were candidate antioxidant agents. These

compounds showed promising DPPH radical scavenging activity in the level of inhibition of 89.2% and 86.8%, respectively.

Synthesis and antioxidant activity evaluation of novel derivatives of 4,5-disubstituted-1,2,4-triazole-3-thione had been performed by Nadeem *et. al*., (2013). 4-Hexyl-2,4-dihydro-5-(3-pyridyl)-3*H*-1,2,4-triazole-3-thione, (Fig.3.13d) (92.5%) and 5-benzyl-4-hexyl-2,4-dihydro-3*H*-1,2,4- triazole-3-thione, (Fig.3.14e) displayed a significant decrease in the concentration of DPPH radical (92.3%) due to the scavenging ability of these compounds.

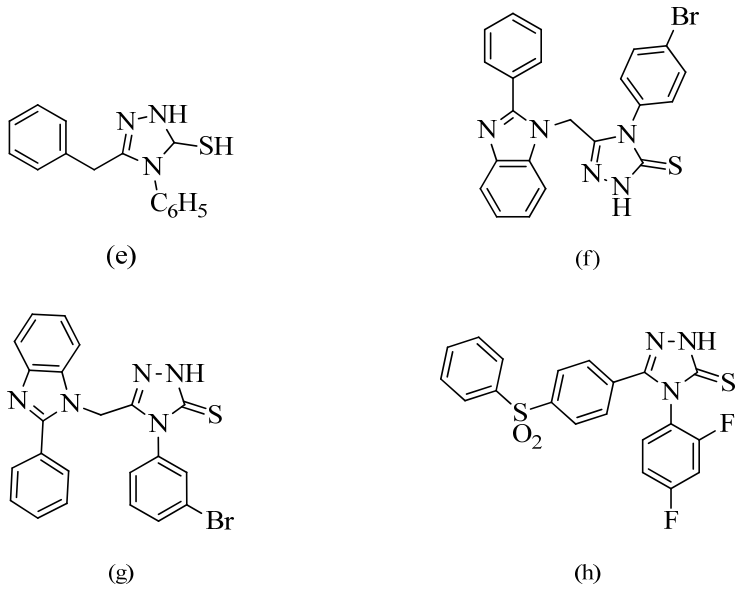


Figure 3.14. Some potent antioxidant Schiff and Mannich bases.

Kus¸ *et. al*., (2004) designed a new analogue of 5-(2-phenylbenzimidazol-l-yl-methyl)-4-substitutedphenyl-2,4-dihydro-1,2,4-triazole-3-thiones and screened for their antioxidant properties by using NADPH-dependent lipid peroxidation assay. This study revealed that compounds (Fig.3.14 f & g) with *p*- and *m*-bromo phenyl groups, as aryl substituents had stronger inhibitory effects on the liver lipid peroxidation levels, by approximately 65% and 62%, respectively.

Synthesis and antioxidant activity evaluation of novel compounds derived from 1,2,4-triazole ring containing diarylsulfone and 2,4-difluorophenyl moieties were reported by Barbuceanue *et. al*., in 2014. The free radical scavenging activity of 5-(4-(4-X-phenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-1,2,4-triazole3(4*H*)-thiones was evaluated by DPPH method using ascorbic acid, tert-butyl-4-hydroxyanisole and 2,6-*bis*-(1,1-dimethylethyl)-4-methylphenol antioxidant agents as positive control. These

results of preliminary screening of antioxidant activity showed that 1,2,4-triazole-3-thione derivatives (Fig.3.14. h) displayed a good antioxidant activity

3.3.8. Analgesic Activity

Analgesics are simply used for killing pain. Triazoles are found to be analgesic.

Amir and Kumar (2005) investigated 5-[(1- (4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl)methyl]-4-nbutyl-3-mercapto-1,2,4-(4*H*)-triazole (Fig.3.15a) derived from indomethacin, thus tested for analgesic activity (73.62% inhibition).

Oruç *et. al*., (2006), synthesized 1,2,4-triazole derivatives and determined for their analgesic activity at a dose of 100 mg/kg i.p. by using Hotplate and tail-immersion tests. Among the tested compounds, 4-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-4-(2phenethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (Fig.3.15b) displayed acceptable results and the analgesic effect of the compound was close to that of morphine at 30 min.

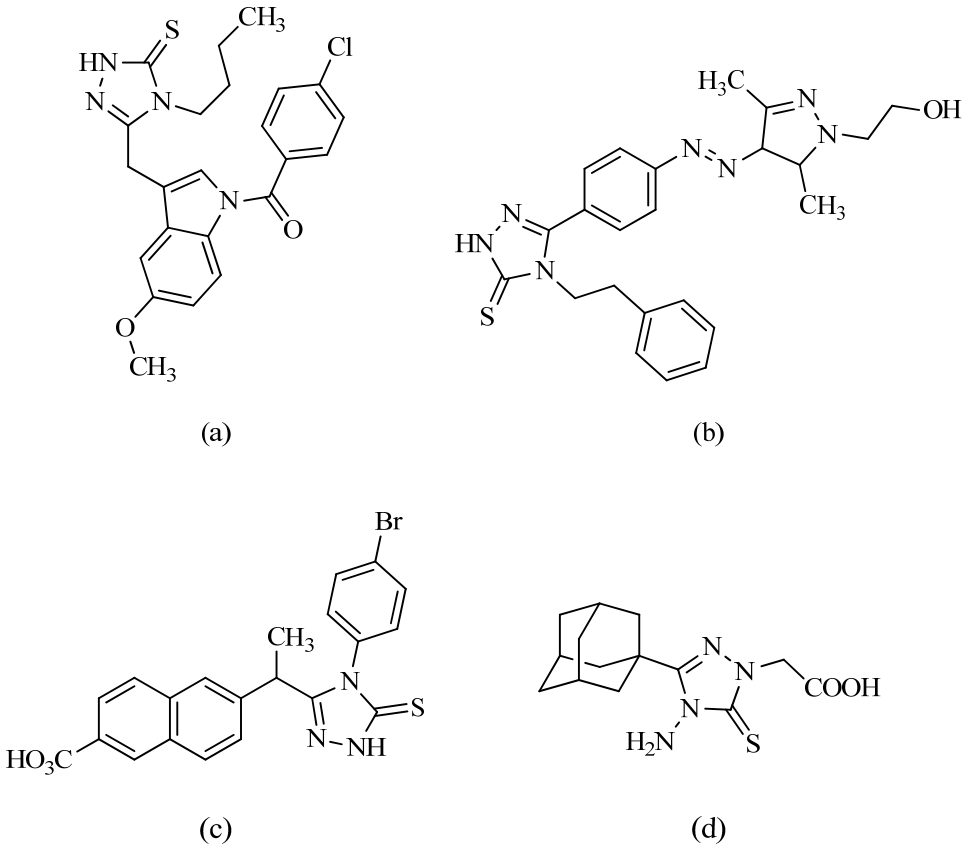


Figure 3.15. Some potent analgesic Schiff and Mannich bases.

Amir *et. al*., ((2007), synthesized 4-alkyl/aryl-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazoles, (Fig.3.15c) derived from naproxen and their findings revealed that this compound was found to have a higher analgesic activity (86.6%) and showed a significant reduction in the severity index, compared to the standard reference drug naproxen with a very low severity index of 0.08 as compared to naproxen.

Goyal *et. al*., (2010), reported a synthesis of 3-substituted-4-(3-disubstituted-1-triazenyl)-4*H*-1,2,4-triazole-5-thiol and their findings revealed these compounds showed excellent analgesic activity. (Fig.3.15d).

3.3.9. Antidepressant Activity

Depression is the major problem of modern generation. This leads to an unhappy life and also suicidal attempts to cost their life, hence antidepressants are used against such depressive disorder.

In 1988, Kane *et. al*., reported several 5-aryl-2,4-dialkyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones as potential antidepressant agents (Fig.3.16a).

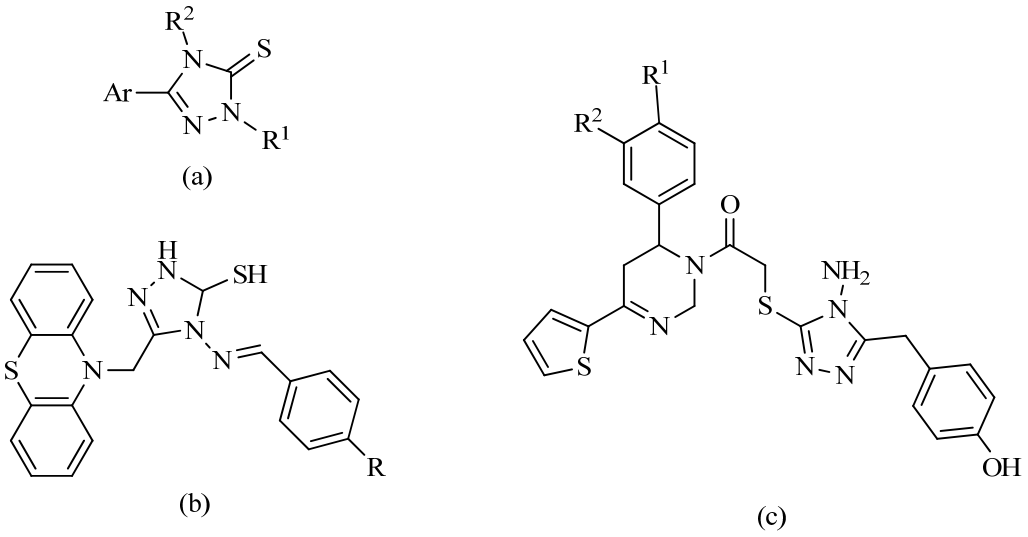


Figure 3.16. Some potent antidepressant triazole derivatives.

In 2002, Turan *et. al*., synthesized some triazolylphenothiazine derivatives and evaluated for their antidepressant activity. This study indicated that screened compounds (Fig.3.16b), (with -H, -Cl, $-CH_3$, $-N(CH_3)_2$ and $-OCH_3$ substituents) showed antidepressant activity.

Zafir *et. al*., (2010), synthesized 1-[[4-amino-3-{2-(4-hydroxyphenylethyl)-4*H*-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-aryl-2-purazoline and found their excellent antidepressant activity (Fig.3.16c).

3.3.10. Antitubercular Activity

The rising prevalence of tuberculosis infections threatens the world population. Antitubercualrs are used against such tuberculosis infections caused by *Mycobacterium tuberculosis*.

Gülerman *et. al*., (2001), synthesized 4-(4-fluorophenyl)-5-(4-pyridinyl)- 2,4-dihydro-3*H*-1,2,4-triazol-3-thione (Fig.3.17a) and 4-(phenethyl)-5- (4-pyridinyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thione (Fig.3.17b) to determine primary antimycobacterial activities. The biological screening indicated that the MIC values of compounds were higher than 6.25 mg mL^{-1}, while the MIC value of reference rifampicin was 0.25 mg mL^{-1}.

Folks *et al*., (2001), synthesized some 2-piperazinmethylene derivatives (Fig.3.17c) 1,2,4-triazole-3-thiones and were tested against *M. tuberculosis* H37Rv strain and found MIC values for most of the compounds within 25-100 μcg.mL^{-1}.

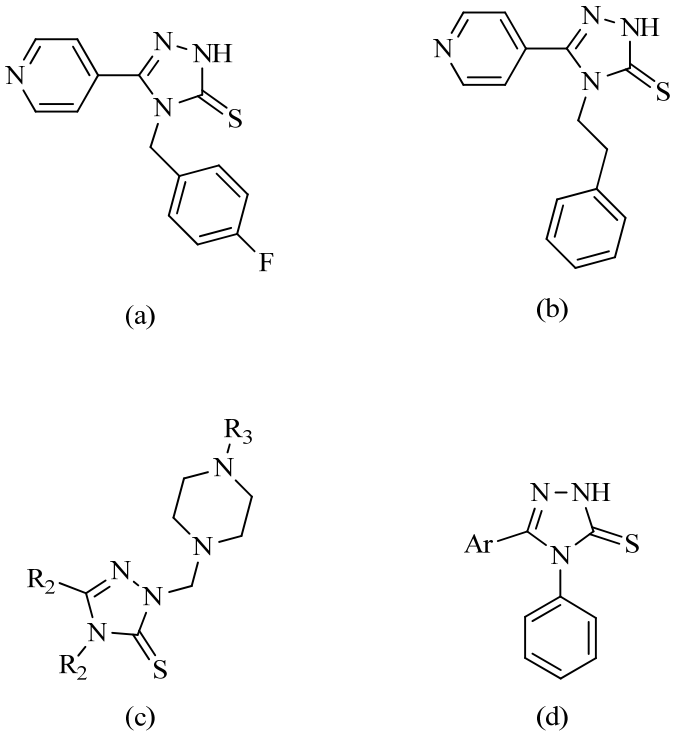


Figure 3.17. Some potent antitubercular Schiff and Mannich bases

Kini *et. al*., (2009), synthesized triazole-thione derivatives (Fig.3.17d) and screened for their activity against *M. tuberculosis* H37Rv strain. All compounds inhibited the growth of the *M. tuberculosis* H37Rv strain at concentrations as low as 1 μg.mL^{-1}.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 Materials

The precursors like hydrazine, ethanol, *conc.* sulphuric acid, methanol, carbon disulphide, diphenylamine, piperazine, etc. were purchased and intermediates were formed in successive steps.

Table 4.1: Name of Chemicals used and their company

S.N.	Name of Chemical	Name of Company
1	Methyl Salicylate	Fischer Scientific
2	Hydrazine Hydrate	Qualigens
3	Ethanol (absolute)	Alpha Chemika
4	Carbon disulphide	Merck
5	Furfuraldehyde	Fischer Scientific
6	Methanol	Fischer Scientific
7	Potassium Hydroxide	Fischer Scientific
8	Diphenyl amine	Merck
9	Piperazine	Loba Chemic
10	Sulphuric acid	Fischer Scientific
11	Hydrochloric acid	Fischer Scientific

4.2. Methods

The melting point of the synthesized compounds was determined, chromatographic methods (TLC) were carried out and the compounds were characterized by spectroscopic methods (UV, IR and NMR).

4.2.1. Thin Layer Chromatography (TLC)

TLC of synthesized compounds was performed by using silica gel coated plates, using *n*-hexane: ethyl acetate solvent system and the spot was visualized by iodine vapors in an iodine chamber.

4.2.2. Melting Point Determination

The melting point of the synthesized compounds was determined with the electrothermal apparatus from Optics technology.

4.2.3. Ultraviolet Spectroscopy (UV)

The UV-visible electronic spectra in DMSO (concentration 5 × 10^{-5} mol.L^{-1}) were recorded on double beam UV-Visible spectrophotometer of Labtronics (Model LT-2802) in the region 1100-200 nm at Department of Chemistry, Amrit Campus.

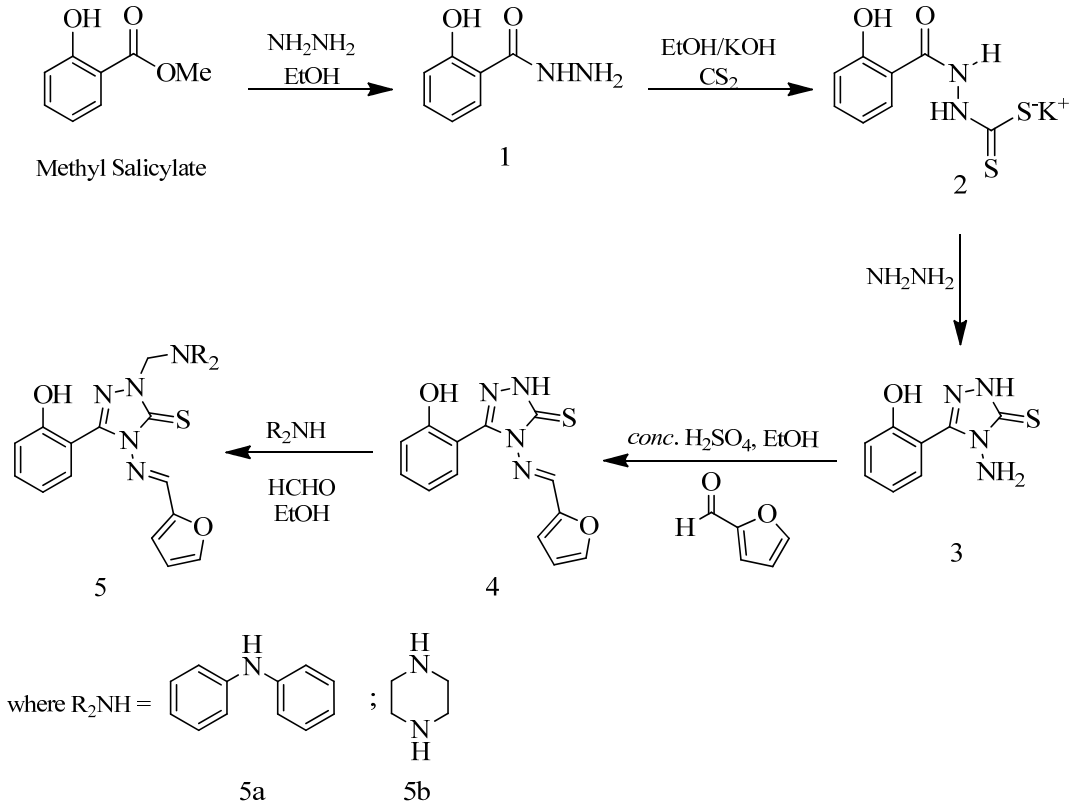
4.2.4. Infrared Spectroscopy (IR)

FT-IR spectra were measured in the range of (4000-400) cm^{-1}using KBr on IR prestige-21, Shimadzu, Japan at Department of Plant Resources, Thapathali, 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 and Mannich bases. NMR spectra were recorded in DMSO-d_6 on a Bruker AV III 500 MHz NMR spectrometer at SAIF, IIT Madras, Chennai, India.

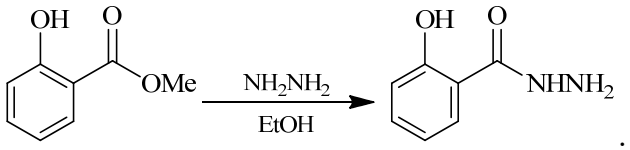
4.3. Synthesis of Mannich bases (1,2,4-triazole derivatives)



Scheme 4.1: Synthetic route for the preparation of Mannich base of 1,2,4-triazole

4.3.1. Synthesis of acid hydrazide (1)

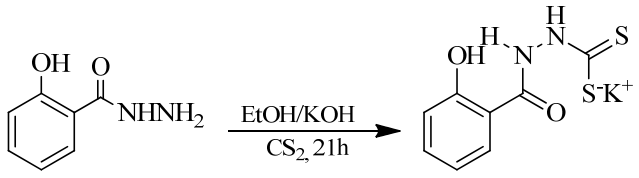
4.45 mL (0.090 mol) of hydrazine monohydrate was added slowly with constant stirring to the round bottom flask containing 9.129 g (0.060mol) of methyl salicylate and refluxed for six hours. The excess solvent was evaporated on a hot water bath until the total volume of the solution was reduced to half and was cooled. White crystalline solid separated out was filtered, washed with cold ethanol and recrystallized with absolute ethanol and was dried in a hot air oven at 50-60 °C. The yield, m.p., R_f of synthesized acid hydrazide were recorded.



Yield: 79% (7.228 g, 0.0475 mol), white shining crystalline solid, m.p. 149°C, R_f : 0.64 (*n*-hexane : ethyl acetate, 8:2).

4.3.2. Synthesis of Dithiocarbazinate (2)

4.565 g (0.030 mol) of acid hydrazide (1) was added to the solution of potassium hydroxide 1.683 g (0.030 mol) and 20 mL absolute ethanol in ice cold condition. 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 and the precipitated potassium dithiocarbazinate was washed with anhydrous diethyl ether twice and dried at a lower temperature (not higher than 60 °C). The yield, m.p., R_f of synthesized dithiocarbazinate were recorded.

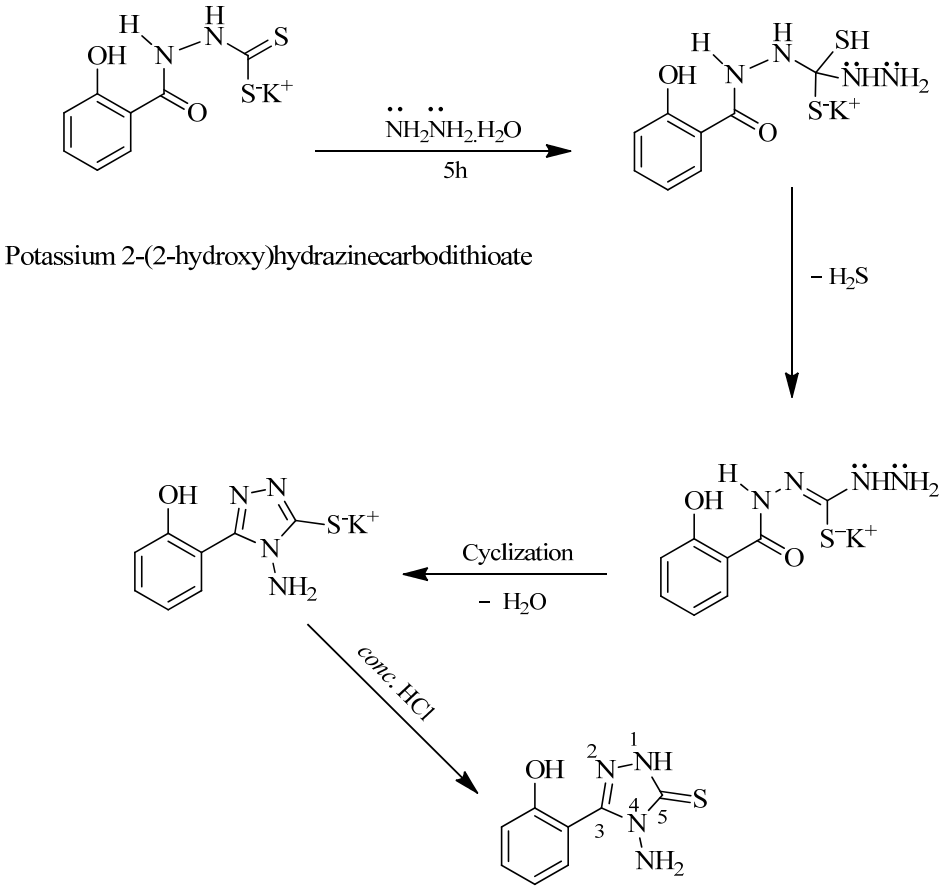


Yield: 62% (4.898 g, 0.018 mol), white crystalline solid, m.p. 240°C, R_f : 0.58 (*n*-hexane : ethyl acetate, 8:2).

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

A suspension of4.680 g (0.018 mol) of potassium dithiocarbazinate (2) and 1.48 mL of hydrazine monohydrate in 5 mL distilled water was refluxed for 5 hours till the

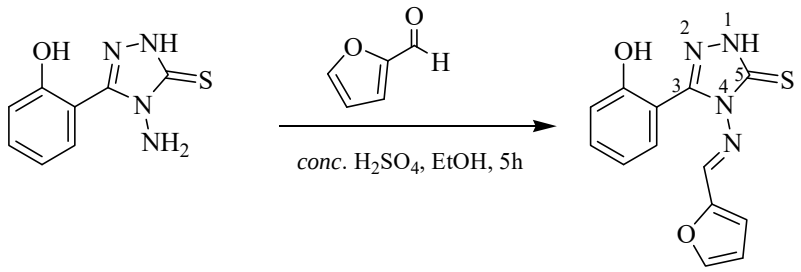
color of the reaction mixture changed to green and the evolution of hydrogen sulphide gas was ceased. The reaction mixture was cooled to room temperature and diluted with 100 mL of cold water containing some crushed ice. The reaction mixture was acidified with concentrated hydrochloric acid. The corresponding triazole was precipitated, filtered, washed with 30 mL cold water twice and recrystallized with absolute ethanol. The yield, m.p., R_f of synthesized triazole thiol were recorded.



Yield: 79 % 2.894 g (0.014 mol), white crystalline solid, m.p. 146 °C, R_f : 0.35 (*n*-hexane : ethyl acetate, 8:2). UV- Visible spectrum (λ_{max}) nm = 302, 309, 331, 353. IR spectrum (selected bands) cm^{-1} = 3287 (m), 3186 (m), 3063 (m), 1612 (m), 1590 (m) 1543 (m), 1489 (s), 1296 (m), 1011 (m), 946 (s), 741 (s), 687 (s). ^{1}H-NMR (500 MHz, DMSO- d_6) δ, 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, NH_2). ^{13}C- NMR (100 MHz, d_6) δ = 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-(furan-2-yl-methyleneamino)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione (4)

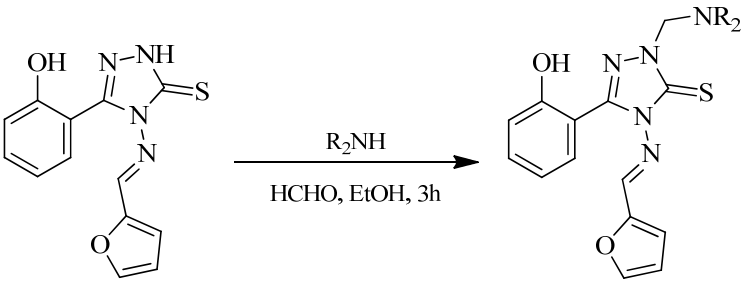
1.8 mL (0.020 mol) of furfuraldehyde was dissolved in a minimum quantity of hot ethanol to which 4.165 g (0.020 mol) triazole thiol (3) was added. The suspension was heated until the solution became clear. 5 drops of concentrated sulphuric acid were added to the mixture and then fluxed for 5 hours. The reaction mixture was cooled and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized with hot ethanol. The yield, m.p., R_f of synthesized Schiff's base was recorded.



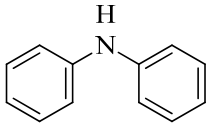
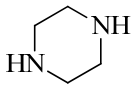
Yield: 65% (3.626 g, 0.013 mol), greyish black crystalline solid, m.p.162 °C, R_f : 0.74 (*n*-hexane: ethyl acetate, 8:2). UV- Visible spectrum (λ_{max}) nm = 302, 309, 331, 353. 394. IR spectrum (selected bands) cm^{-1} = 3356 (m), 3225 (w), 3093 (m), 2931 (m), 1614 (m), 1582 (m) 1540 (m), 1463 (m), 1236 (m), 1009 (s), 948 (m), 761 (m), 698 (s). ^{1}H-NMR (500 MHz, DMSO- d_6) δ, ppm = 14.09 (br s, 1H, triazole NH), 10.06 (s, 1H, OH), 9.42 (s, 1H, N=CH), 7.98 (br s, 1H, Furan-H), 7.39 (br s, 1H, Ar-H), 7.37 (br s, 1H, Ar-H), 7.31 (d, *J* = 3.15 Hz, 1H, Ar-H), 6.93-6.90 (m, 2H, Ar-H & Furan-H), 6.73 (dd, *J* = 1.89, 3.78 Hz, 1H, Furan-H), ^{13}C- NMR (100 MHz, d_6) δ = 162.14 (N=CH), 156.66 (Triazole C5), 154.43 (Ar-C), 148.83 (Triazole C3), 148.35 (Furan-C), 147.67 (Furan-C), 132.82 (Ar-C), 131.61 (Ar-C), 120.93 (Ar-C), 119.37 (Furan-C), 116.46 (Ar-C), 113.42 (Ar-C), 113.39 (Furan-C).

4.3.5. General procedure for the synthesis of Mannich Bases

To the hot ethanol solution of Schiff's base, aldehyde and amine were added. The reaction mixture was stirred for 3 hours at room temperature. The excess amount of distilled water was added and the reaction mixture was left overnight. The precipitate formed was filtered under suction, washed with cold ethanol and recrystallized by ethanol.



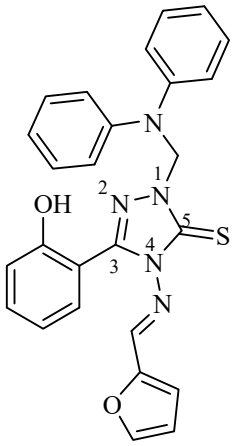
Schiff's base (4) Mannich base (5)

where, R_2NH =  , 

5a 5b

4.3.5.1. Synthesis of Mannich base 1-{(diphenylamino)methyl}-4-(furan-2-ylmethyleneamino)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5-thione (5a)

0.3 mL (0.01 mol) of 40% formaldehyde was added to a hot ethanolic solution 1.432 g (0.005 mol) of Schiff's base. 0.846 g (0.005 mol) of diphenylamine was added to the reaction mixture and refluxed for 3 hours. The excess amount of distilled water was added and the reaction mixture was left overnight. The precipitated was filtered under suction, washed with cold ethanol and recrystallized by absolute ethanol. The yield, m.p., and R_f of synthesized Mannich base were recorded.



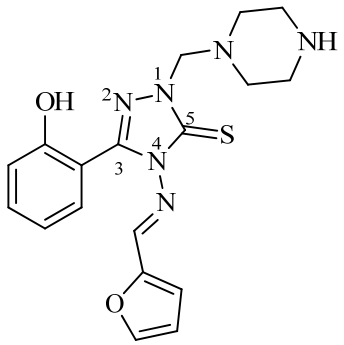
Mannich base (5a)

Yield: 66% (1.540 g, 0.033 mol), brownish black crystalline solid, m.p. 110 °C, R_f : 0.72 (*n*-hexane : ethyl acetate, 8 : 2). UV- Visible spectrum (λ_{max}) nm =302, 309, 339, 353, 393. IR spectrum (selected bands) cm^{-1} = 3217 (m), 2932 (m), 1605 (m), 1589 (m) 1543 (m), 1497 (m), 1234 (m), 1011 (s), 933 (m), 748 (s), 687 (s). ^{1}H-NMR (500 MHz, DMSO- d_6) δ, ppm = 10.93 (s, 1H, O<u>H</u>), 9.32 (s, 1H, N=C<u>H</u>), 8.14 (br s, 1H.

Ar-H), 8.00 (br s, 1H, Furan-H), 7.96-7.94 (dd, *J* = 8.20×(2), 1H, Ar-H), 7.51-7.44 (m, 1H, Ar-H), 7.37-7.29 (m,4H, Diphenylamine-H & Furan-H), 7.27-7.20 (m, 4H, Diphenylamine-H & Furan-H), 7.08 (d, *J*= 8.20, 1H, Ar-H), 6.82 (dd, *J* = 5.7 Hz, 1H, Furan –H), 6.16 (s, 2H, N-C$\underline{H}_2$-N).^{13}C- NMR (100 MHz, d_6) δ= 166.30 (Triazole C5), 159.02 (Ar-C), 148.57 (Triazole C3), 147.54 (Furan-C), 146.65 (2C, Diphenhylamine–C), 143.90 (2C, Furan-C), 134.55 (N=$\underline{C}$H), 129.61 (4C, Diphenyl–C), 121.93 (2C, Diphenhylamine–C), 120.11 (Ar-C), 119.72 (Furan-C), 117.74 (4C, Diphenhylamine–C), 117.19 (Ar-H), 115.43 (2C, Furan-H), 113.44 (Ar-H), 88.61 (N-$\underline{C}$H$_2$-N).

4.3.5.2. Synthesis of Mannich base 4-(furan-2-ylmethylemeamino)-3-(2-hydroxyphenyl)-1-(piperazin-1-ylmethyl)-1*H*-1,2,4-triazole-5-thione(5b)

.3 mL (0.01 mol) of 40% formaldehyde was added to a hot ethanolic solution of 1.432 g (0.005 mol) of Schiff's base. 0.431 g (0.005 mol) of piperazine was added to the reaction mixture and refluxed for 3 hours. The excess amount of distilled water was added and the reaction mixture was left overnight. The precipitated was filtered under suction, washed with cold ethanol and recrystallized by absolute ethanol. The yield, m.p., R_f of synthesized Mannich base was recorded.



Mannich base (5b)

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Yield: 78% (1.501 g, 0.004 mol), brown coloured crystalline solid, m.p. 140°C, R_f: 0.89 (*n*-hexane: ethyl acetate, 8:2). UV- Visible spectrum (λ_{max}) nm =302, 309, 339, 354, 405. IR spectrum (selected bands) cm^{-1} = 3240 (m), 2939 (m), 2831 (m), 1705 (m), 1604 (m), 1551 (m), 1442 (w), 1304 (w), 1002 (s), 934 (m), 756 (m), 687 (m). ^{1}H-NMR (500 MHz, DMSO- d_6) δ, ppm = 10.13 (s, 1H, O$\underline{H}$), 9.39 (s, 1H, N=C$\underline{H}$), 8.12 (br s, 1H, Ar-H), 8.00 (br s, 1H, Furan-H), 7.93 (dd, *J* = 8.20 Hz×(2), 1H, Ar-H),

7.51-7.42 (m, 1H, Ar-H), 7.13 (d, *J* = 8.20, 1H, Ar-H), 5.13 (s, 2H, N-C<u>H</u>$_2$-N), 3.87 (br s, 4H, Piperazine-C), 3.82 (br s, 4H, Piperazine-C), 2.79 (br s, 1H, N<u>H</u>-Piperazine). ^{13}C- NMR (100 MHz, d_6) δ= 166.13 (Triazole C5), 159.05 (Ar-C), 148.57 (Triazole C3), 147.22 (Furan-C), 145.55 (Furan-C), 134.55 (N=<u>C</u>H), 129.27 (Ar-C), 125.60 (Ar-C), 121.58 (Ar-C), 119.63 (Furan-C), 117.73 (Ar-C), 113.15 (Furan-C), 77.33 (N-<u>C</u>H$_2$-N), 53.90 (2C, Piperazine-C), 44.22 (2C, Piperazine-C),

4.4. Antimicrobial Screening

The antimicrobial activities of the newly synthesized Mannich bases were screened against different bacterial strains such as *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella. pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Shigella dysenteriae, Staphylococcus aureus, and Staphylococcus epidermis* and fungal strains such as *Candida albicans, Saccharomyces cerevisiae* of certain concentrations by using Chloramphenicol as standard (positive control) for bacterial strains and Clotrimazole as standard (positive control) for fungal strains.

4.4.1. Preparations of the working solution

Sterilized screw-capped tubes were calibrated and marked for 10 mL. About 1gm of the sample was transferred in calibrated screw capped tubes. Ethanol was added in the tube up to the line marked by 10 mL. The mixture was homogenized by vortexing.

4.4.2. Preparation of Standard Culture Inoculums

Required numbers of colonies of freshly cultured (within 18–24 hours) test organisms were inoculated aseptically 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 Mc Farland 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*., 1990.

A sterile swab was used to evenly distribute bacterial or fungal culture drawn from the respective inoculums equivalent to 0.5 Mc Farland standard of turbidity over the appropriate medium Muller-Hinton Agar (MHA) for bacteria and Muller-Hinton Agar with Glucose and Methylene Blue (MHA, GMB) for fungi. The plate was rotated through an angle of 60º after each swabbing. The swabbing was done three times. The

inoculated plates were allowed to dry for maximum 15 minutes. Four wells were of 6 mm diameter were created in the inoculated plates using the sterile cork borer (three well for test samples and one well for the solvent as negative control). Micropipettes were used to dispense 50 µL of the test solution of the samples and solvent as negative into each of the four wells. The plates were left in the upright condition with lids closed for half an hour so that the test solutions diffused into the media. The inoculated plates were then incubated in an inverted position at a suitable temperature (35±2 °C for bacteria and 25±2 °C for fungi). After proper incubation (18-24 hours for bacteria, 24-48 hrs for fungi) the plates were examined for the zone of inhibition (ZOI) around the well which is suggested by clear area with no growth of organisms. The activity of both compounds was compared with chloramphenicol and clotrimazole as standard (Positive Control) for bacteria and fungi respectively.

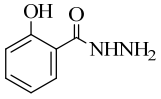
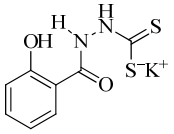
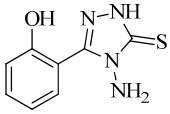
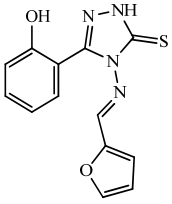
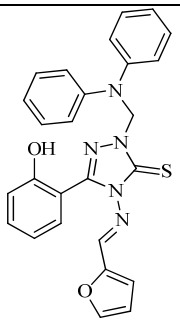
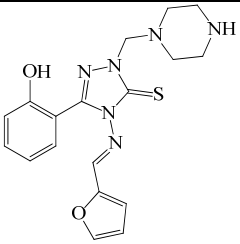
CHAPTER 5

5. RESULT AND DISCUSSION

5.1. General Discussion

The Mannich bases of 1,2,4 triazole derivatives were prepared from methyl salicylate as a starting material. 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 value and assurance of purity of synthesized compounds. Their physical properties are summarized in table 5.1 below,

Table 5.1: Physical properties of the synthesized compound

Compound	Structure	Physical appearance	Molecular formula	Molecular mass	Yield (%)	M.P. (°C)	R_f
1		White solid	$C_7H_8N_2O_2$	152.15	79	147-150	0.64
2		White solid	$C_8H_7KN_2O_2S_2$	266.38	62	240	0.58
3		White solid	$C_8H_8N_4OS$	208.24	79	145-148	0.35
4		Greyish black crystalline solid	$C_{13}H_{10}N_4O_2S$	286.31	65	160-162	0.74
5a		Brownish White crystalline solid	$C_{26}H_{21}N_5O_2S$	467.54	66	108-110	0.72
5b		Brownish white crystalline solid	$C_{18}H_{18}N_6O_2S$	384.46	78	137-140	0.89

5.2. UV Spectroscopy

The UV-visible spectra of synthesized compounds 1,2,4-triazole-5-thione (3), Schiff base (4) and Mannich bases (5a &5b) are listed below in Table 5.2.

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

Compound	λ_{max}(nm)	Inference
3	302	$\pi\rightarrow\pi^*$
	309	$\pi\rightarrow\pi^*$
	331	$n\rightarrow\pi^*$
	353	$n\rightarrow\pi^*$
4	302	$\pi\rightarrow\pi^*$
	309	$\pi\rightarrow\pi^*$
	339	$n\rightarrow\pi^*$
	353	$n\rightarrow\pi^*$
	394	$n\rightarrow\pi^*$
5a	302	$\pi\rightarrow\pi^*$
	309	$\pi\rightarrow\pi^*$
	339	$n\rightarrow\pi^*$
	353	$n\rightarrow\pi^*$
	405	$n\rightarrow\pi^*$
5b	302	$\pi\rightarrow\pi^*$
	309	$\pi\rightarrow\pi^*$
	339	$n\rightarrow\pi^*$
	354	$n\rightarrow\pi^*$
	405	$n\rightarrow\pi^*$

The absorption spectra of compound 3 exhibited four bands around 302 nm, 309 nm, 331 nm, and 353 nm. The first and second peaks are due to aromatic C=C and C=N of triazole ring. The third band at 331 nm is due to $n\rightarrow\pi^*$ transitions associated with the nonbonding electron pair of the nitrogen atom of triazole C=N and sulphur atom of C=S. The fourth band at 353 nm is attributed to $n\rightarrow\pi^*$ transitions associated with the *o*-hydroxy group.

In an electronic spectrum of Schiff's base 4, it exhibited five bands. The first two bands at 302 nm and 309 nm are due to aromatic C=C and C=N ($\pi\rightarrow\pi^*$) of the triazole ring. The third band at 339 nm is due to transitions associated with the non-bonding electron pair of the nitrogen atom of triazole C=N and Sulphur atom of C=S. The fourth band at 353 nm is associated with $n\rightarrow\pi^*$ transitions of the *o*-hydroxy group. The fifth band at 394 nm is due to $n\rightarrow\pi^*$ transitions of azomethine group C=N.

Similarly, Mannich bases (5a & 5b) also showed five absorption spectra. The first two absorption bands at 302 nm and 309 nm were of the electronic transitions caused by the aromatic C=C and C=N ($\pi \rightarrow \pi^*$) of the triazole ring. The third band at 339 nm in both the Mannich bases was found because of the electronic transitions that occurred between non-bonding electron pair of the nitrogen atom of triazole C=N and Sulphur atom of C=S. The fourth band at 353 and 354 in Mannich bases 5a and 5b respectively is associated with $n \rightarrow \pi^*$ transitions of an *o*-hydroxy group. The fifth band at 405 nm in both 5a & 5b was found due to $n \rightarrow \pi^*$ transitions of azomethine group C=N.

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 (4) and Mannich bases (5a & 5b) which is listed below.

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

Group ↓	Wave number (cm^{-1})			
	(3)	(4)	(5a)	(5b)
ν(Ar O-H)	3287 (m)	3356 (w)	3217 (m)	3240 (m)
ν(NH_2)	3186 (m)	……	……	…….
ν (NH)	…….	3225(w)	……..	…….
ν (Ar C-H)	3063 (w)	3093 (m)	2932 (m)	2939 (w)
ν (Ali C-H)	……..…….	2931 (m)	…….	2831 (w)
ν (C=N)	1612 (m)	1614(m)	1605 (m)	1705(m)
δ (NH)	1590 (m)	1582 (m)	1589 (m)	1604 (m)
ν (C=C)	1543 (m)	1540 (m)	1497 (m)	1551 (m)
	1489 (s)	1463 (m)	1420 (m)	1442(w)
ν (N-C=S)	1296 (s)	1236 (m)	1234 (m)	1304 (w0
ν (Ar C-O)	1011 (m)	1009 (s)	1011 (s)	1002 (s)
ν (C=S)	946 (s)	948 (m)	933 (m)	934 (m)
δ (C-H) oop	741 (s)	761 (m)	748 (s)	756 (m)
	687 (s)	698 (m)	687(s)	687 (m)

In FT-IR spectrum of compound (3), there is a medium absorption band at the region of 3287 cm^{-1} due to the presence of phenolic functional group in the compound. The medium absorption band at 3186 cm^{-1} is due to the stretching vibration of NH_2. This absorption band consists of two short peaks in which one peak is merged with –OH.

However, both these small peaks are merged with –OH group in the case of 5a & 5b. The medium absorption band found at 1612 cm^{-1} confirms the presence of –C=N group in the molecule. The medium absorption band at 1543 cm^{-1} and strong absorption band at 1489 cm^{-1}.is due to carbon-carbon (C=C) stretching vibrations. The strong absorption band at 1011 cm^{-1} corresponds to the bending vibration of the aromatic carbon-oxygen bond of phenol. The strong absorption bands at 741 cm^{-1} and 687 cm^{-1} is due to out of plane (oop) bending vibrations of aromatic -C-H group in the molecule. These absorption bands also refer to the mono substitution and hence no band is found in the region of 710-690 cm^{-1}, which confirms the *o*- substitution.

The formation of triazole is confirmed by the presence of the medium band at 1296 cm^{-1} and strong band at 946 cm^{-1} correspondings to N-C=S thioamide II and C=S thioamide IV. Moreover, no absorption bands were detected about 1651-1707 cm^{-1} indicating the absence of C=O group in the compound which is the evidence for the conversion of dithiocarbazinate to triazoles (Cretu *et. al*., 2010).

Schiff base (4) shows a weak intensity absorption band at 3356 cm^{-1} due to –OH and the medium intensity absorption band at 3225 cm^{-1} due to –NH stretching vibrations. The aromatic C-H stretching vibrations were found at the medium absorption band at 3093 $cm^{-1}$and that of aliphatic were found in the region of 2931 $cm^{-1.}$ The medium absorption band at 1614 cm^{-1} refers to the C=N group. The medium absorption bands at 1582 cm^{-1} is due to the bending vibrations of -NH group present in the molecule. The medium absorption bands at 1540 cm^{-1} and 1463 cm^{-1} shows the presence of C=C stretching vibrations. The medium absorption bands due to the presence of N-C=S group show at the region of 1236 cm^{-1} thioamide II and similarly the bending vibrations band at 948 cm^{-1} shows the presence of C=S thioamide IV. The strong absorption band at 1009 cm^{-1} shows the stretching vibration of carbon-oxygen bond of phenol. The out of plane bending vibrations due to –C-H group are found in the region of 761 cm^{-1} and 698 cm^{-1}.

The absence of band at 1700 cm^{-1} indicates the amino condensation and hence the formation of Schiff bases (Sunil *et. al*., 2010). The absence of medium intensity bands in the region of 3500-3200 cm^{-1} attributed to $–NH_2$ protons demonstrates the formation of Schiff bases (Hanif and Chohan, 2013) and absence of an absorption band in the region of 2300-2600 cm^{-1} region cited for the –SH group clearly states

that, in the solid state, the compound exists predominantly in thionic form (Zamani *et. al*., 2004; Baluja *et. al*., 2007).

The medium absorption band at 3217 $cm^{-1}$and at 3240 cm^{-1} refers to –OH group respectively for compound 5a & 5b. In compound 5a, the aliphatic methyl (C-H) group is found at the medium absorption band at 2932 cm^{-1} whereas it is found at a weak absorption band at 2939 $cm^{-1}$and 2831 cm^{-1} in compound 5b. The medium band found at 1605 cm^{-1} and 1705 cm^{-1} supports the presence of –C=N group in the molecule 5a and 5b respectively. The medium absorption bands at 1589 cm^{-1} and 1604 cm^{-1} are due to the bending vibrations of -NH group present in the molecule. The aromatic (C=C) shows at the medium absorption band of 1497 cm^{-1} and 1420 cm^{-1} for 5a and 1551 cm^{-1} and 1442 cm^{-1} for 5b. The medium absorption band found at the region of 1234 cm^{-1} refers to the N-C=S (thioamde II) group in 5a whereas the same group is found in the region of 1304 cm^{-1} in 5b. For thioamide IV group the absorption bands at 933 cm^{-1} and 934 cm^{-1} are respectively found for 5a & 5b. The strong absorption band at 1011 cm^{-1} and 1002 cm^{-1} refers to the carbon-oxygen bond of phenol in both the compound respectively. The out of plane (oop) bending vibrations due to –C-H group in both the compounds represent the *o*-substituion and are found at the region of 748-687 cm^{-1} and 756-687 cm^{-1} respectively for 5a & 5b.

The formation of Mannich bases 5a & 5b is supported by the presence of the medium absorption band at 1234 $cm^{-1}$and weak absorption band at 1304 cm^{-1} respectively due to N-C=S group and also the absence of the absorption band at 2250cm^{-1} due to -SH group, hence, supporting the formation of N- Mannich bases but not S-Mannich bases (Sunil *et. al*., 2010).

5.4 NMR SPECTROSCOPY

5.4.1.^{1}H-NMR Spectroscopy

The ^{1}HNMR spectra data of the synthesized compounds (3), (4), (5a & 5b) are listed below in Table 5.4.

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

Proton ↓	Chemical Shift (ppm)			
	(3)	(4)	(5a)	(5b)
NH	13.86 (br s, 1H)	14.09 (br s, 1H)		
OH	10.36 (s, 1H)	10.06 (br s, 1H)	10.93 (s, 1H)	10.13 (br s, 1H)
N=CH		9.42 (s, 1H)	9.32 (s, 1H)	9.39 (br s, 1H)
Aromatic (due to 2-hydroxyphenyl)	7.43-7.31 (m, 2H) 7.00 (d, *J* = 8.20 Hz, 1H)	7.39 (br s, 1H) 7.37 (br s, 1H)	8.14 (br s, 1H) 7.96 - 7.94 (dd, *J* = 8.20 × (2), 1H)	8.12 (br s, 1H) 7.93 (dd, *J* = 8.20 Hz × (2), 1H)
	6.93 (t, *J* = 7.57 Hz, 1H)	6.93-6.90 (m, 2H)	7.51 - 7.44 (m, 1H) 7.08 (d, *J* = 8.20, 1H)	7.51 - 7.42 (m, 1H) 7.13 (d, *J* = 8.2, 1H)
Aromatic (due to furanyl)		7.98 (br s, 1H) 7.31 (d, *J* = 3.15 Hz, 1H)	8.00 (br s, 1H) 7.05 - 6.98 (m, 3H) (overlap with diphenyl amine) 6.82 (dd, *J* = 5.7 Hz, 1H)	8.00 (br s, 1H) 7.04 - 6.95 (m, 1H)
		6.73 (dd, *J* = 1.89, 3.78 Hz, 1H)		6.95 - 6.88 (m, 1H)
Aromatic (due to diphenyl amine)			7.37 - 7.29 (m, 4H) 7.27 - 7.20 (m, 4H) overlap with furanyl (2H)	
N<u>H</u>$_2$	5.62 (br s, 2H)			
N-C<u>H</u>$_2$-N			6.16 (s, 2H)	5.13 (br s, 2H)
C<u>H</u>$_2$ piperazine				3.87 (br s, 4H) 3.82 (br s, 4H)
N<u>H</u> piperazine				2.79 (br s, 1H)

In ^{1}HNMR spectrum of compound (3), the broad singlet at 13.86 ppm is attributed to the hydrogen attached to the Nitrogen which suggests the formation of thione based triazole (Silverstein *et. al*., 1974) which is equally supported by the absence of IR

absorption band at 2600 cm^{-1} due to thiol group. The singlet at 10.36 ppm is attributed to the hydrogen of OH group. The aromatic hydrogens due to 2-hydroxyphenyl are found at 7.43-7.31 ppm, 7.00 ppm, 6.93 ppm. The peak at 5.62 ppm attributes to the shielded hydrogen of NH_2 group.

The Schiff base (4) also contains the singlet at 14.09 ppm due to the presence of NH proton. The singlet at 10.06 ppm is attributed to the hydrogen of OH group. The aromatic hydrogens due to furanyl were found at broad singlet at 7.98 ppm, 7.31 and at 6.73 ppm. The aromatic hydrogens due to 2-hydroxyphenyl were found at broad singlet 7.3-7.39 ppm and 6.93-6.90 ppm. Moreover, the ^1H – NMR spectrum of Schiff base showed a singlet at 9.42 due to the presence of –N=CH group and absence of signals approximately at 5.76 ppm (NH_2) in the molecule confirming the formation of Schiff bases. The absence of exchangeable -SH signals c.a. 4.0 ppm indicated the predominance of the thione tautomer in DMSO-d_6 (Ali *et. al*., 2006).

In compound 5a & 5b, the peak obtained due to –NH proton is absent. The peak due to –OH group is found to be at 10.93 ppm and 10.13 ppm respectively for both the Mannich bases. The –N=CH proton was found to be singlet at 9.32 and 9.39 ppm for 5a and 5b respectively. In compound 5a, the aromatic hydrogens found due to diphenylamine are found at the peak of 737-7.29 ppm and 7.27-7.20 ppm. The piperazine ring proton (CH_2, 5b) was found to be quartets at 3.87-3.82 ppm (Wang *et. al*., 2015) and –NH piperazine was found to be broad singlet at 2.79 ppm.

The singlets found at 6.16 ppm and 5.13 ppm due to N-CH_2-N group confirm the formation of Mannich bases (5a & 5b) from Schiff base. Moreover, the absence of the peak at 11.5 ppm suggests the absence of thiol group in the structure instead thione based Mannich base is formed (Sunil *et. al*., 2010).

5.4.2 ^{13}C-NMR Spectroscopy

The spectral data of the synthesized compounds are listed in table 5.5.

Table 5.5: ^{13}C-NMR spectral assignments (ppm) of synthesized compounds (100MHz, DMSO-d_6).

Carbon ↓	Chemical Shift (ppm)			
	(3)	(4)	(5a)	(5b)
Triazole C5	166.54	162.14	166.30	166.13
Triazole C3	149.62	148.83	148.57	148.57
N=CH		156.66	134.55	134.45
Aromatic (due to 2-hydroxyphenyl)	156.53 132.60 131.32 119.53 116.67 113.52	154.43 132.82 131.61 120.93 116.46 113.42	159.02 123.04 120.11 117.19 113.44	159.05 129.27 125.60 121.58 117.73 107.15
Aromatic (due to furanyl)		148.35 147.67 119.37 113.39	147.54 143.90 (2C) 119.72 115.43 (2C)	147.22 145.55 119.63 113.15
Aromatic due to diphenyl amine			146.65 (2C) 129.61 (4C) 121.93 (2C) 117.74 (4C)	
N-$\underline{C}H_2$-N			88.61	77.33
$\underline{C}H_2$ piperazine				53.90 (2C) 44.22 (2C)

The typical carbon resonance at δ 162.57-167.67 was indicative of triazole C5 i.e. C=S group (Wang *et. al*., 2015). The compounds 3, 4, 5a & 5b consists of the peak at 166.54, 162.14, 166.30 and 166.13 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. The signal of C=N is observed at 149.62, 148.83, 148.57 and 148.57 ppm for compounds 3, 4, 5a & 5b respectively. The peak found at 156.66 ppm due to the azomethine carbon confirms the formation of the Schiff base (Issa *et. al*., 2009). Similarly, peaks were found at 134.55 and 134.45 ppm adhered to –N=CH group in compound 5a & 5b respectively.

The aromatic carbon associated with the 2-hydroxy phenyl group of the compound 3 was found in the region 156.53-113.52 ppm, 154.43-113.42 ppm for compound 4, 159.02-113.44 ppm for compound 5a, 107.15-159.05 ppm for compound 5b. The aromatic carbon associated with furfuranyl group was found in the region of 148.35-113.39 ppm, 147.54-115.43 ppm, and 147.22-113.15 ppm for compounds 4, 5a & 5b respectively. In compound 5a, the aromatic carbons of the diphenyl group were found in the region of 146.65-117.74 ppm whereas the piperazine carbon (5b) was found in the region of 53.90 & 44.22 ppm (Wang *et. al*., 2015).

The formation of Mannich bases (5a & 5b) were confirmed by the presence of the peak at 88.61 ppm and 77.33 ppm respectively.

5.5. Antimicrobial Screening

5.5.1. Antibacterial Screening

Mannich bases (5a & 5b) were screened against different bacterial strains and the result is summarized as below in Table 5.6.

Table 5.6: Antibacterial screening of the synthesized Mannich Base (5a& 5b)

Bacterial Strain	Diameter of Zone Of Inhibition		
	Compound (5a)	Compound (5b)	Chloramphenicol (PC) Conc^{n} (60 mcg.mL^{-1})
Bacillus subtilis[a]	0	0	26.58
Enterococcus faecalis[a]	7.02	7.99	20.86
Escherichia coli[b]	7.8	7.29	20.86
Klebsiella pneumoniae[b]	7.66	0	12.28
Proteus vulgaris[b]	9.676	12.18	0
Pseudomonas aeruginosa[b]	8.46	0	0
Salmonella typhii[b]	0	0	27.44
Shigella dysenteriae[b]	7	0	28.99
Staphylococcus aureus[a]	0	14.47	28.4
Staphylococcus epidermidis[a]	0	0	31.47

[a]Gram positive bacteria: [b]Gram negative bacteria

The antibacterial activity of the synthesized compounds Mannich bases exhibited moderate activity against the tested bacterial strains using Chloramphenicol as standard (positive control).

Compound 5a with diphenylamine appeared more active against gram –ve bacteria than gram +ve bacteria, while compound 5b with piperazine was found to be more effective against gram +ve bacteria than gram –ve. Compound 5a showed better

activity against the gram –ve bacterial strain of *Proteus vulgaris* than any other bacterial strain whereas 5b showed the greatest activity against gram +ve bacterial strain *Staphylococcus aureus* against which 5a was ineffective. Compound 5a exhibited remarkable activity against the bacterial strains *P. vulgaris* and *P. aeruginosa* towards which the standard antibacterial chloramphenicol showed no activity. Likewise, compound 5b was also found to be effective against *P. vulgaris*. Both Mannich bases were ineffective against *B. subilitis, S. typhii* and *S. epidermidis.* Both compounds 5a and 5b are almost similar in structure, hence, the only difference seen in their antibacterial activities is due to the presence of two phenyl rings in diphenylamine which acts as an electron-withdrawing group to show the broad antibacterial spectrum. It may also suggest that the bulkiness of substituent at N-1 of triazole nucleus enhances the antibacterial activity, however, which is in contrast to antifungal activity. The results are in the agreement with the observation that the electron density at N-1 of Mannich bases mostly determines the antibacterial and antifungal activities.

5.5.2 Antifungal Activity of Mannich bases

The antifungal activity of synthesized compounds Mannich bases against fungal strains using Clotrimazole as standard (positive control) is summarized in Table 5.7.

T**able 5.7:** Antifungal screening of the synthesized Mannich Base (5a & 5b)

Fungal Strain	Diameter of Zone Of Inhibition		
	Compound (5a)	Compound (5b)	Clotrimazole (PC) Conc^{n} (200 mcg.mL^{-1})
Candida albicans	18.11	21.69	32.33
Saccharomyces cerevisiae	12.54	19.42	24.35

Both 5a and 5b exhibited remarkable activity against the fungal strains *C. albicans* and *S. crevisiae* comparable to that of the standard drug clotrimazole. The piperazinyl derivative 5b exhibited more potent antifungal activity than the diphenylamine derivative 5a. Compound 5b is less bulky than compound 5a which results in significant activity against the fungal strain.

CHAPTER 6

6.1. CONCLUSION

The Mannich bases 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 product2-(diphenylamino)methyl-4-furan-2-methylamino-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3-thione (5a) and 4-(furan-2-methyleneamino)-5-(2-hydroxyphenyl)-2-(piperazine-1-ylmethyl)-2*H*-1,2,4-triazole-3-thione (5b) were successfully prepared in lab. They were characterized by spectroscopic techniques like UV, IR, ^{1}H-NMR and ^{13}C- NMR. The synthesized compounds showed moderate activity against bacterial strains but were found to be very effective against the fungal strain. The compound Mannich base 5b was found to be more effective than compound 5a against fungal strains. Whereas compound 5a was more active against bacterial strains than compound 5b. The little difference in the activity of the synthesized compounds suggests that the antimicrobial activity is mainly due to the N-CH_2-N part of the Mannich bases.

CHAPTER 7

7.1. FUTURE RECOMMENDATIONS

The work that has been done here assures the possibility of synthesized compounds in the field of pharmaceuticals. However, the study of various other biological activities is yet to be done which may unlock their new potent biopotential. Biological activities like antioxidant, analgesic, antitubercular are the future recommendation of this work. Cytotoxicity is another activity that could also be done in near future.

There is no denying that Mannich bases containing 1,2,4- triazole moiety has been the area of study and research in recent times due to its exceptional biopotentials. Hence, this work would like to be going ahead in the same direction.

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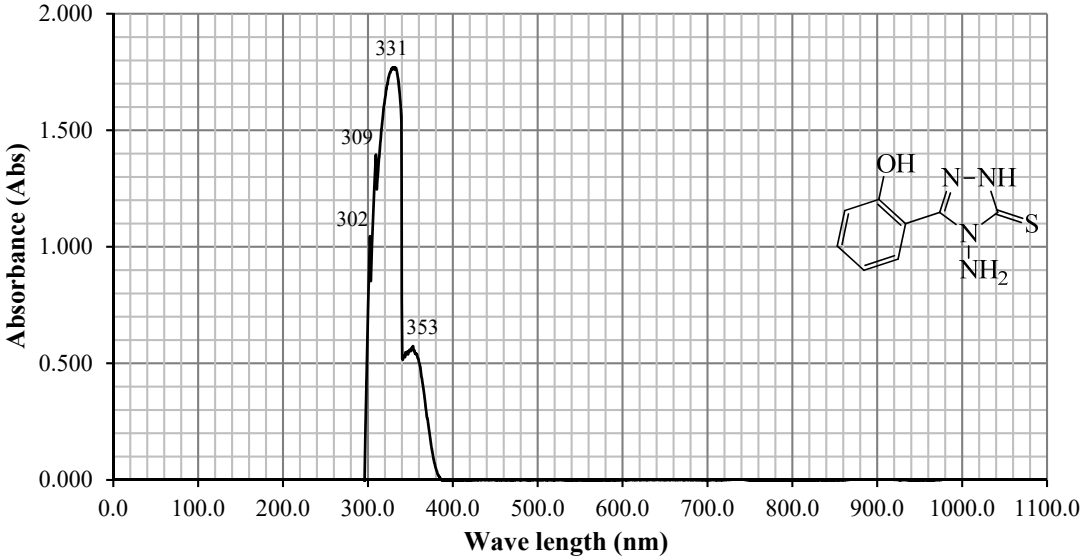
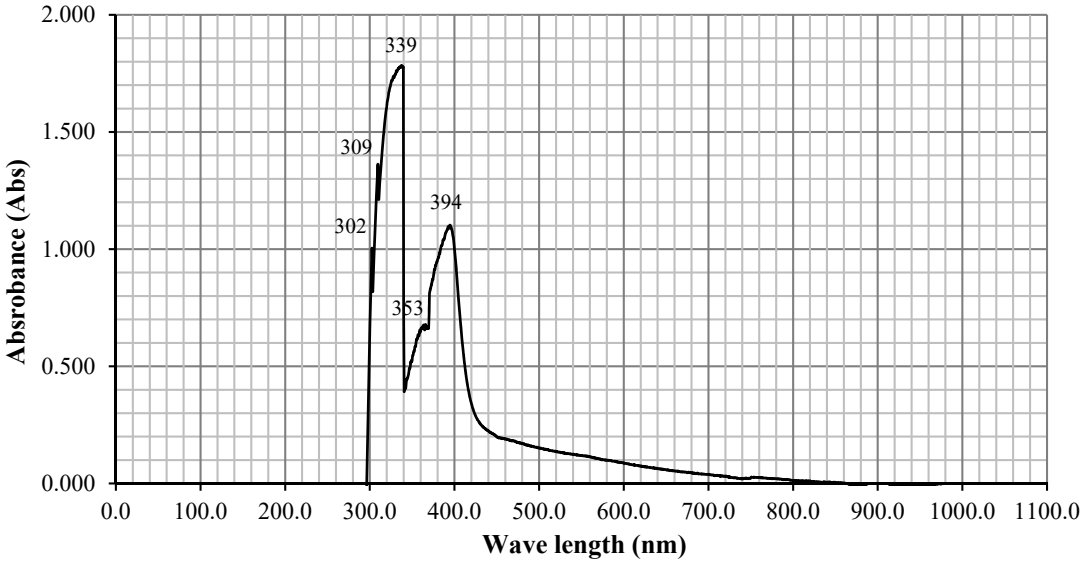
APPENDICES

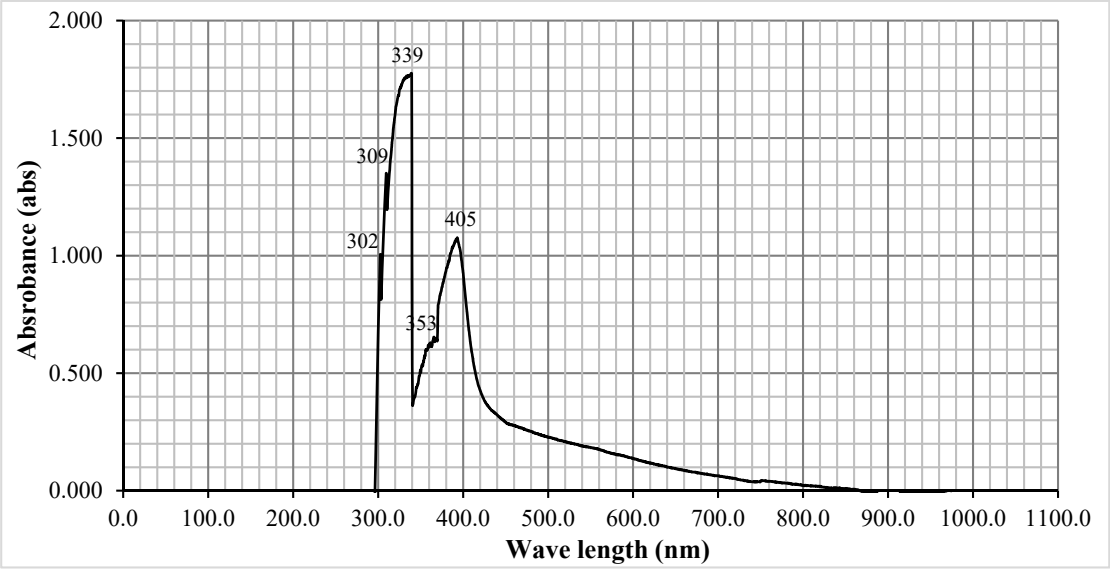
Appendix A UV-Visible spectra of synthesized compounds



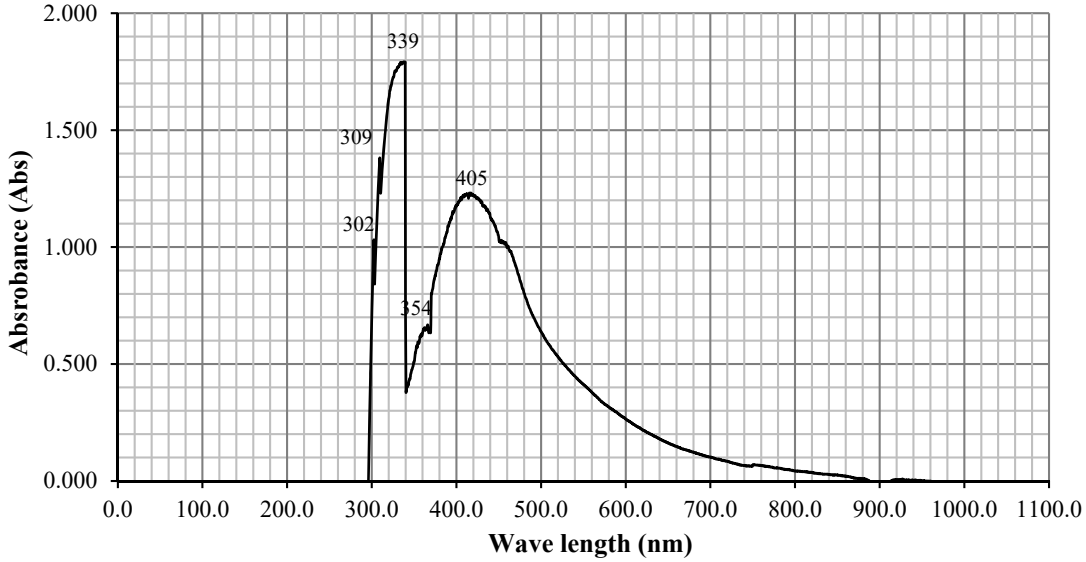
Appendix A1: UV-Visible spectrum of compound **3**



Appendix A2: UV-Visible spectrum of compound **4**

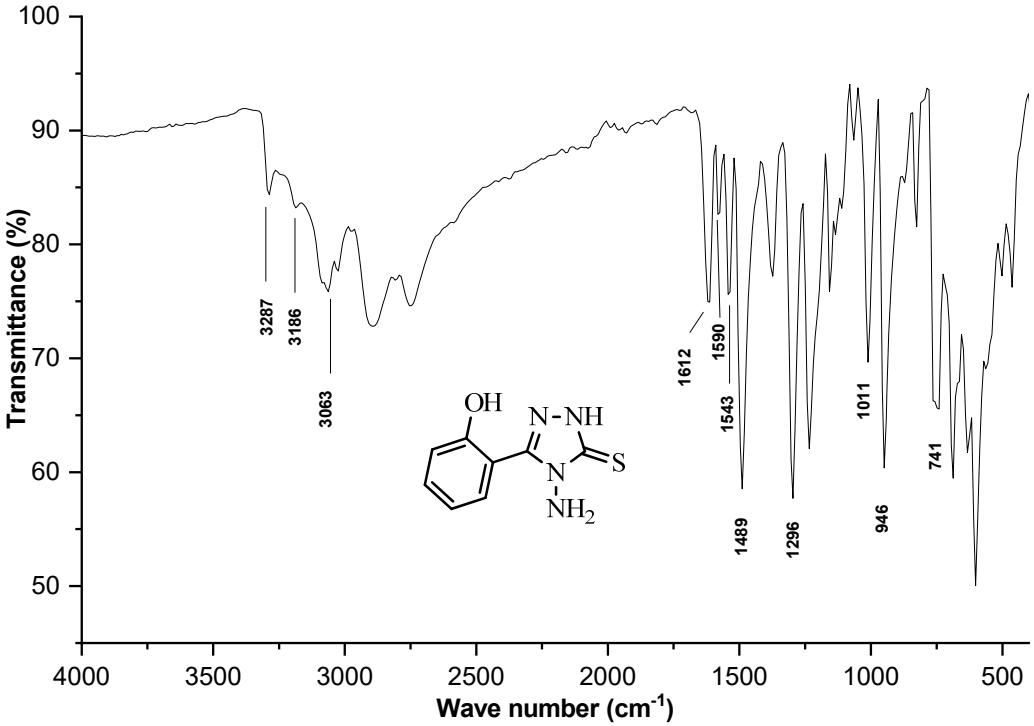


Appendix A2: UV-Visible spectrum of compound **5a**

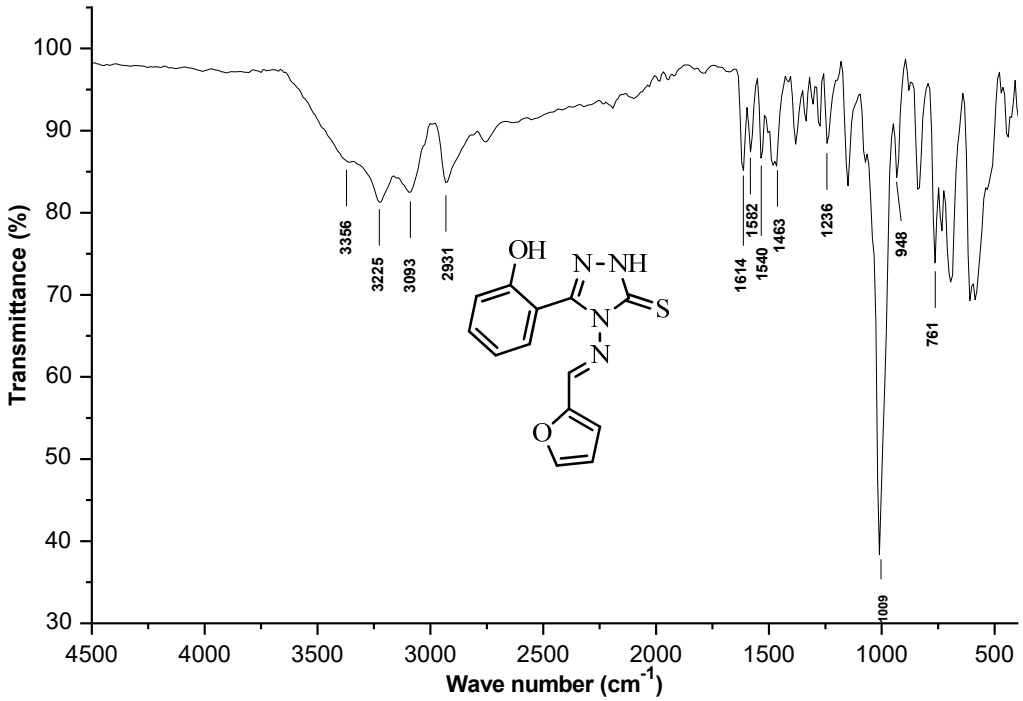


Appendix A4: UV-Visible spectrum of compound **5b**

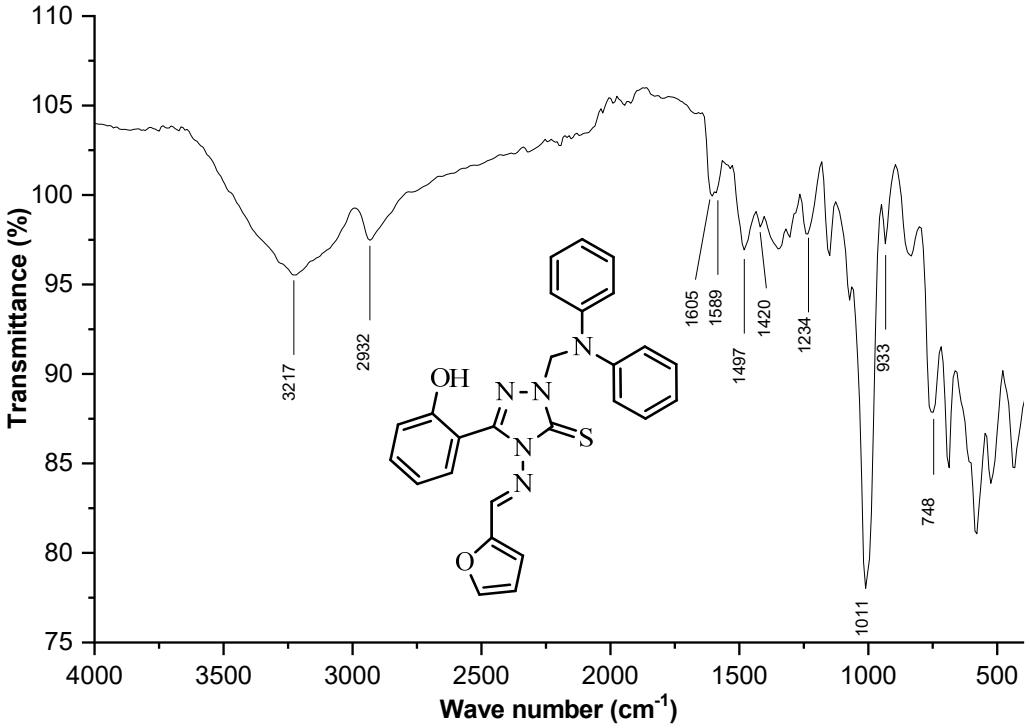
Appendix B IR spectra of synthesized compounds



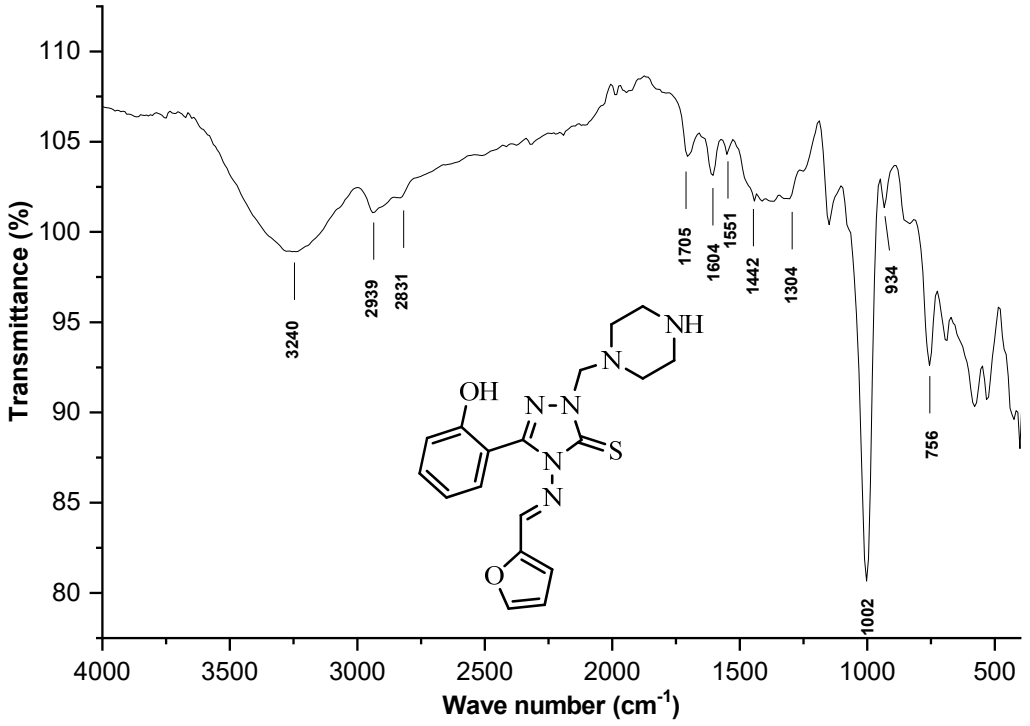
Appendix B1: IR spectrum of compound **3**



Appendix B2: IR spectrum of compound **4**

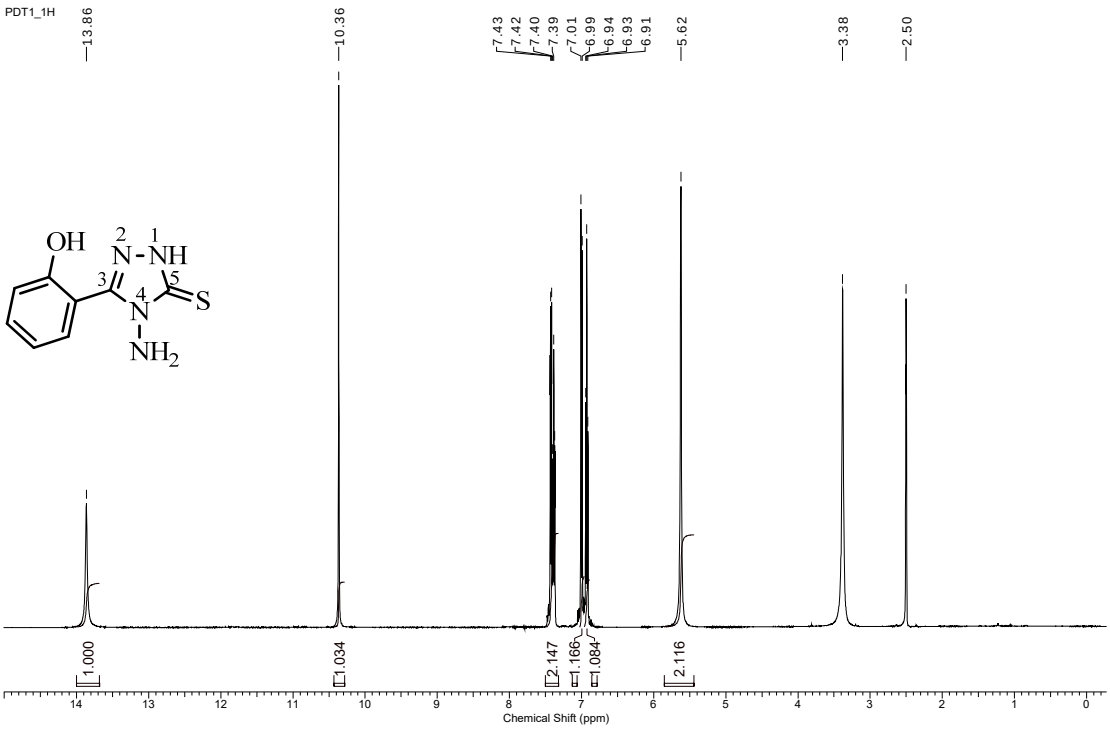


Appendix B3: IR spectrum of compound **5a**

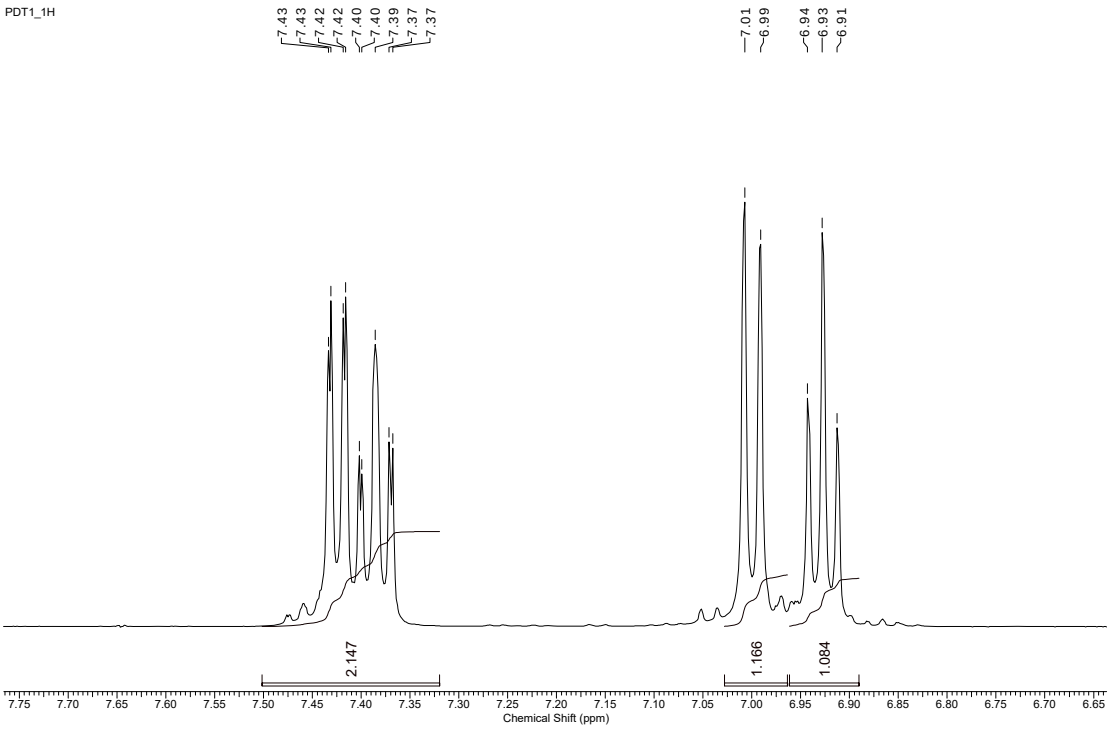


Appendix B4: IR spectrum of compound **5b**

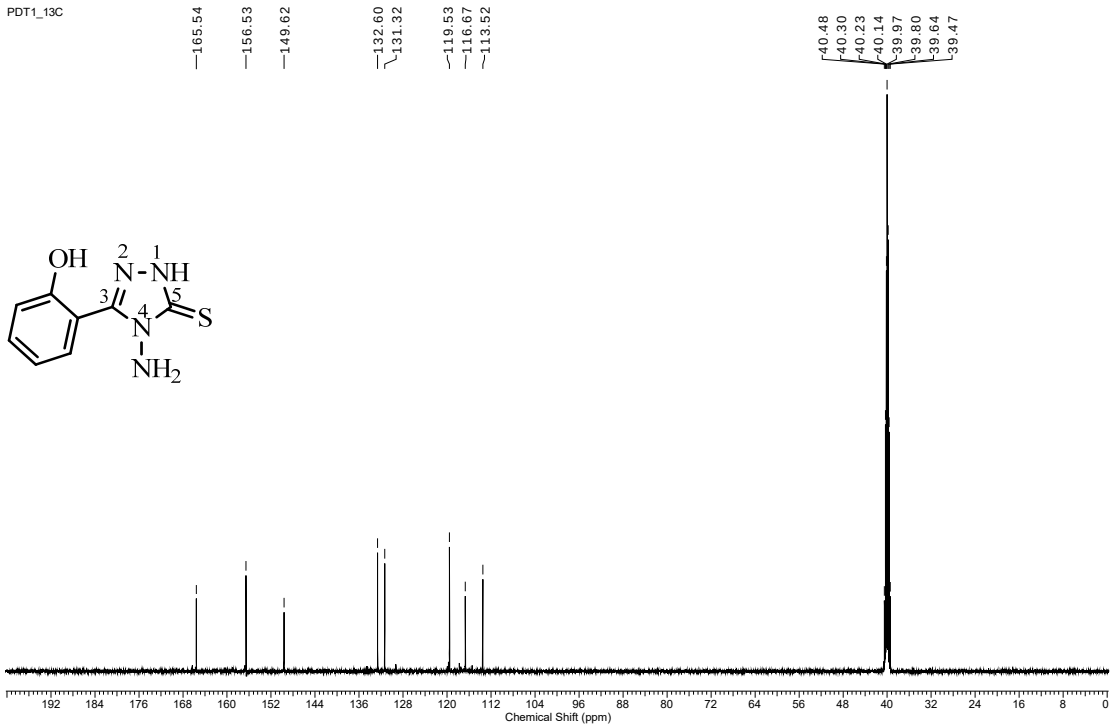
Appendix C NMR spectra of Synthesized Compounds



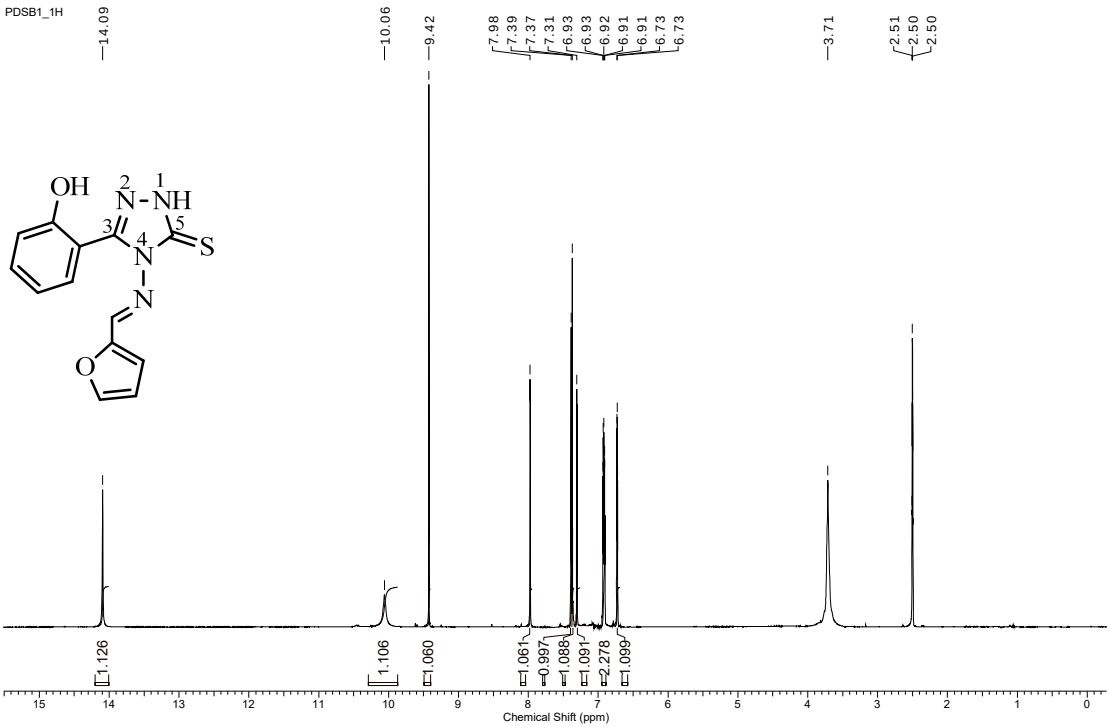
Appendix C1: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **3**



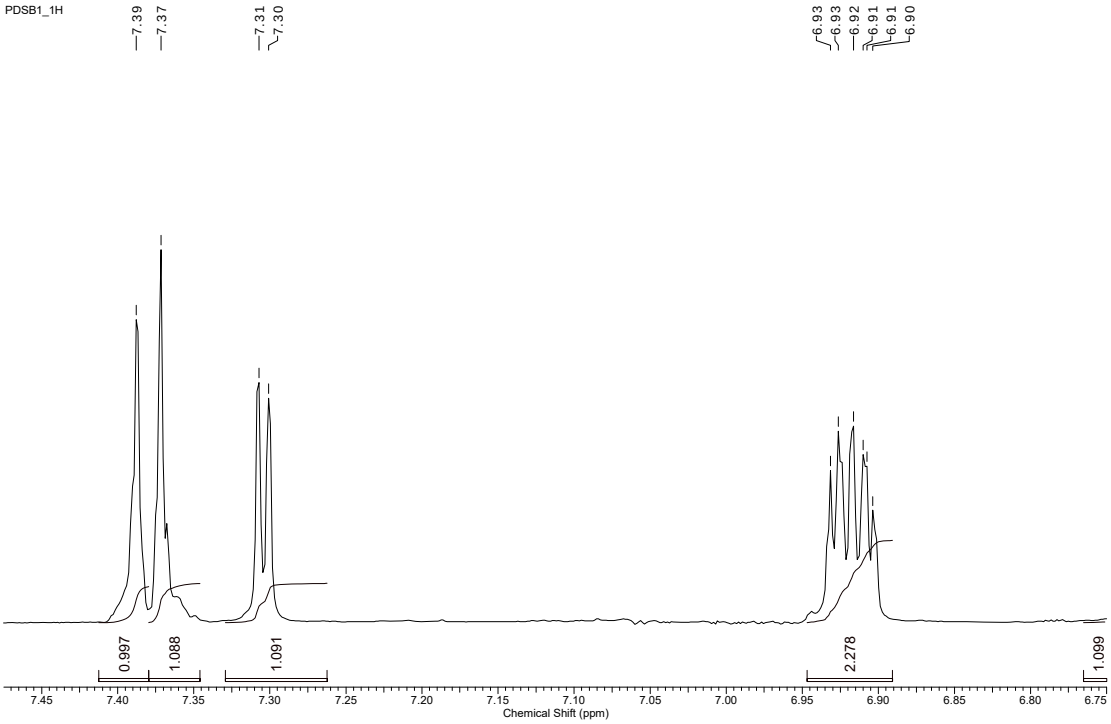
Appendix C2: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **3** Expanded



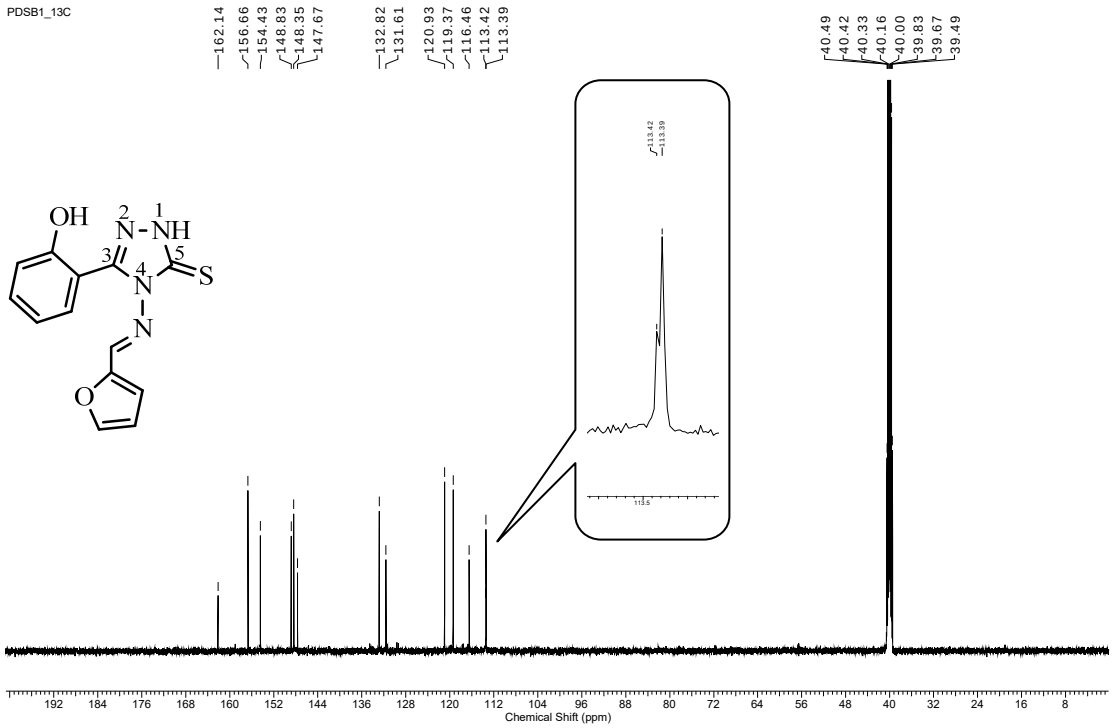
Appendix C3: ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **3**



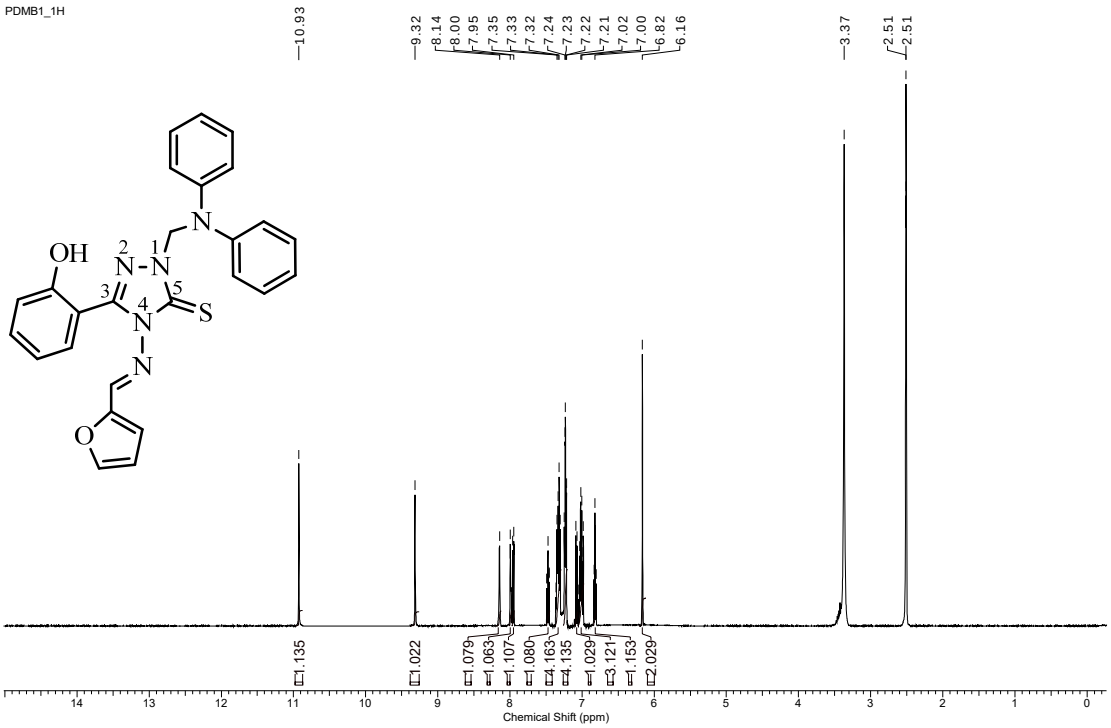
Appendix C4: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4**



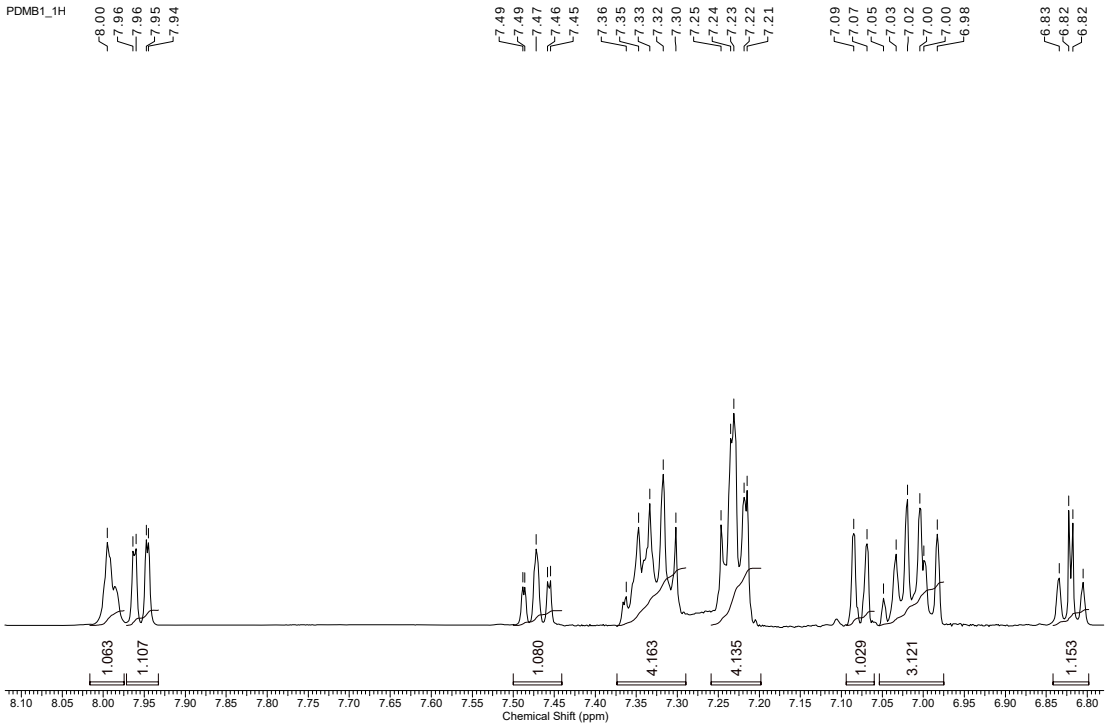
Appendix C5: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4** Expanded



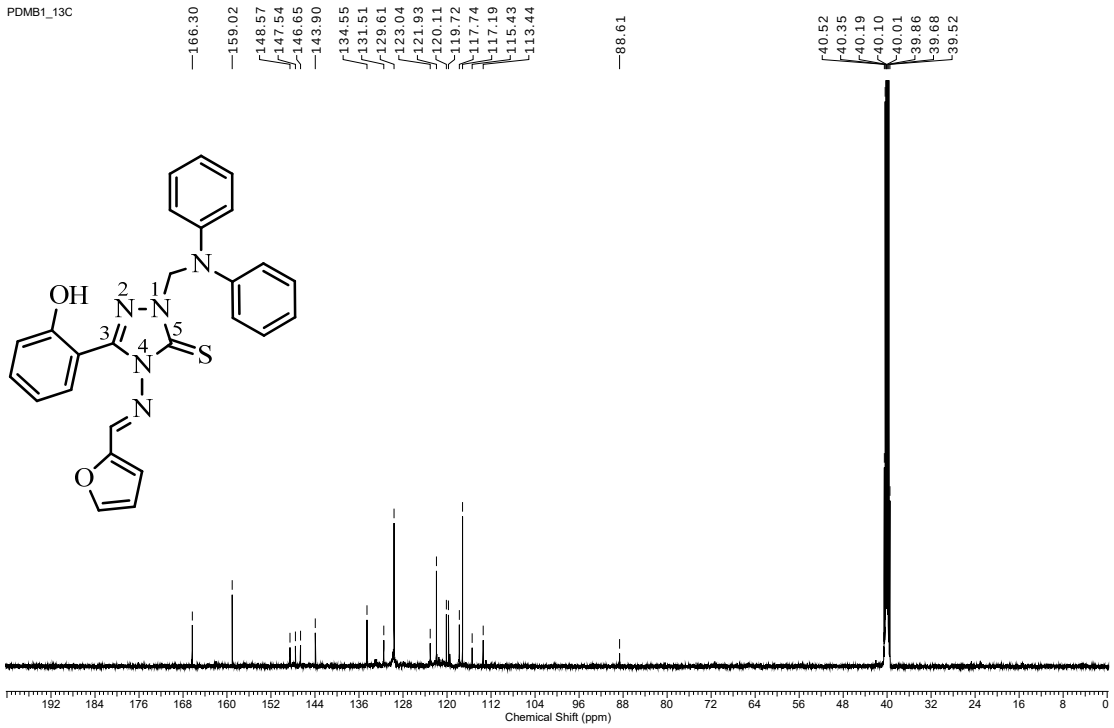
Appendix C6: ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4**



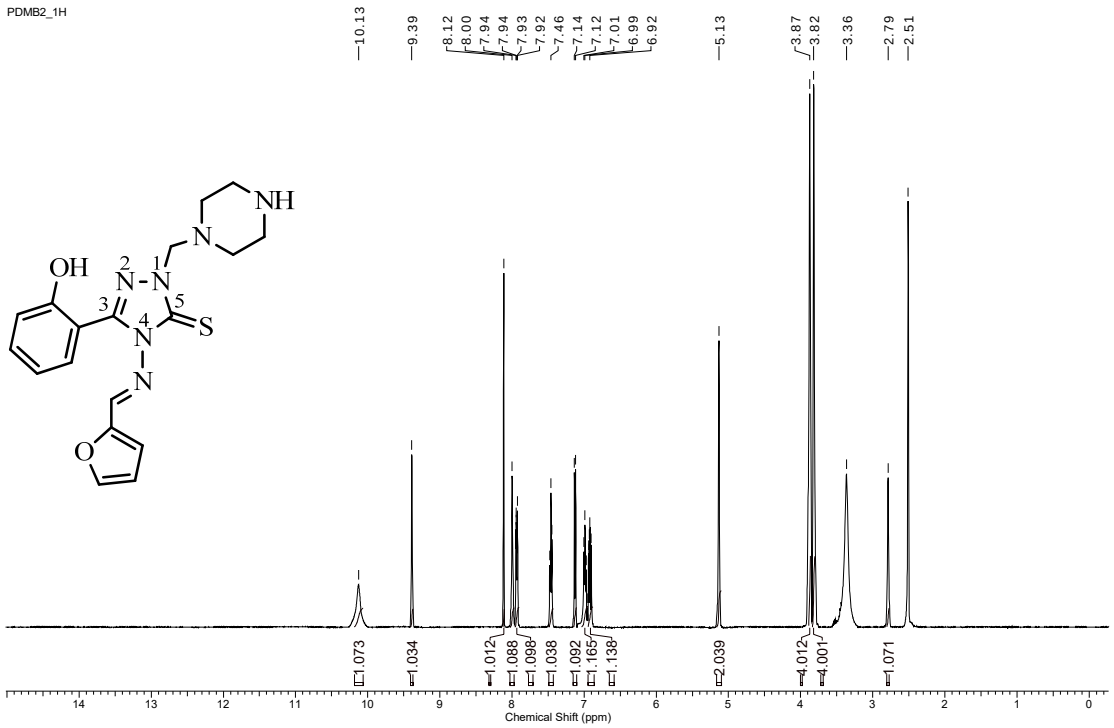
Appendix C7: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **5a**



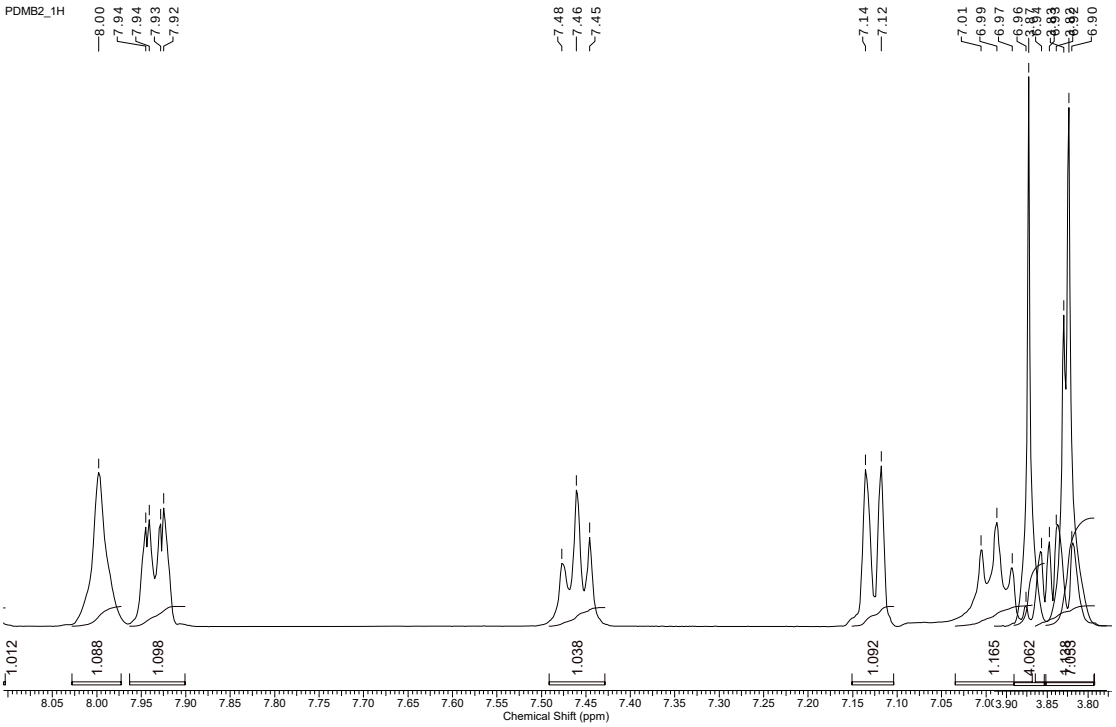
Appendix C8: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **5a** Expanded



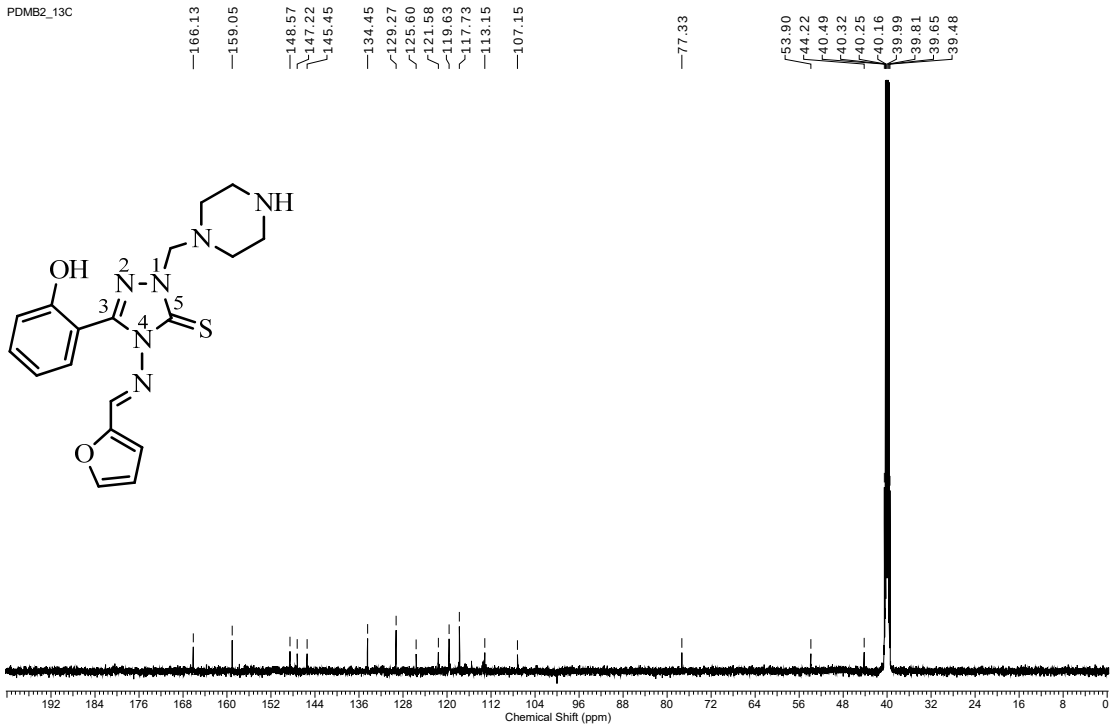
Appendix C9: ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **5a**



Appendix C10: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **5b**

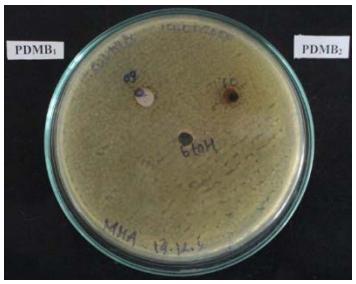


Appendix C11: ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **5b** Expanded

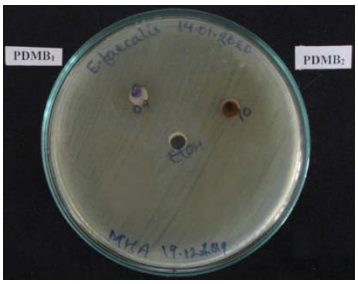


Appendix C9: ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **5b**

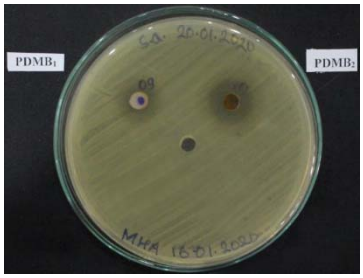
Appendix D Antibacterial Activity of Mannich Bases (**5a**) and (**5b**)



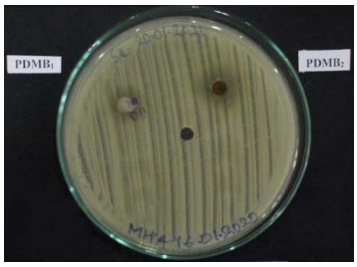
Against *Bacillus subitilis*



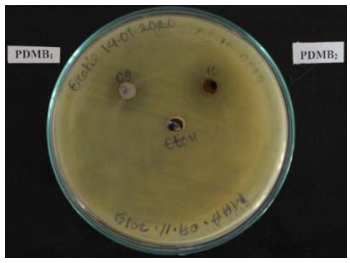
Against *Enterococcus faecalis*



Against *Staphylococcus aureus*



Against *Staphylococcus epdermidis*

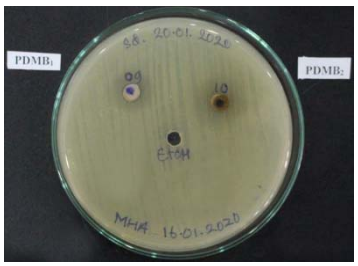


Against *Escherichia coli*

Against *Klebsiella pneumoniae*

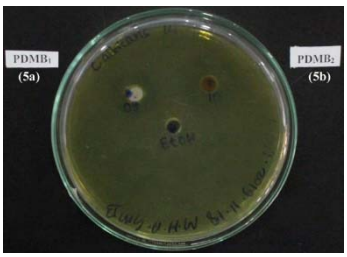
Against *Proteus vulgaris*

Against *Psseudomonas aeruginosa*



Against *Shigella dysentriae*

Appendix E Antifungal Activity of Mannich Bases (**5a**) and (**5b**)



Against *Candida albicans*

Against *Saccharomyces cerevisiae*