SYNTHESIS AND CHARACTERIZATION OF AZO-SCHIFF BASES CONTAINING THIADIAZOLE MOIETY OF BIOLOGICAL INTEREST

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT

FOR THE DEGREE OF MASTER OF SCIENCE (M.Sc) IN CHEMISTRY

SUBMITTED

BY

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DEPARTMENT OF CHEMISTRY

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The thesis entitled "SYNTHESIS AND CHARACTERIZATION OF AZO-SCHIFF BASES CONTAINING THIADIAZOLE MOIETY OF BIOLOGICAL INTEREST " submitted by Rafiqul Alam, Roll No: 1015032604, Session- October / 2015 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Master of Science (M.Sc) in chemistry on July 8, 2018.

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To

My Mother

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Author

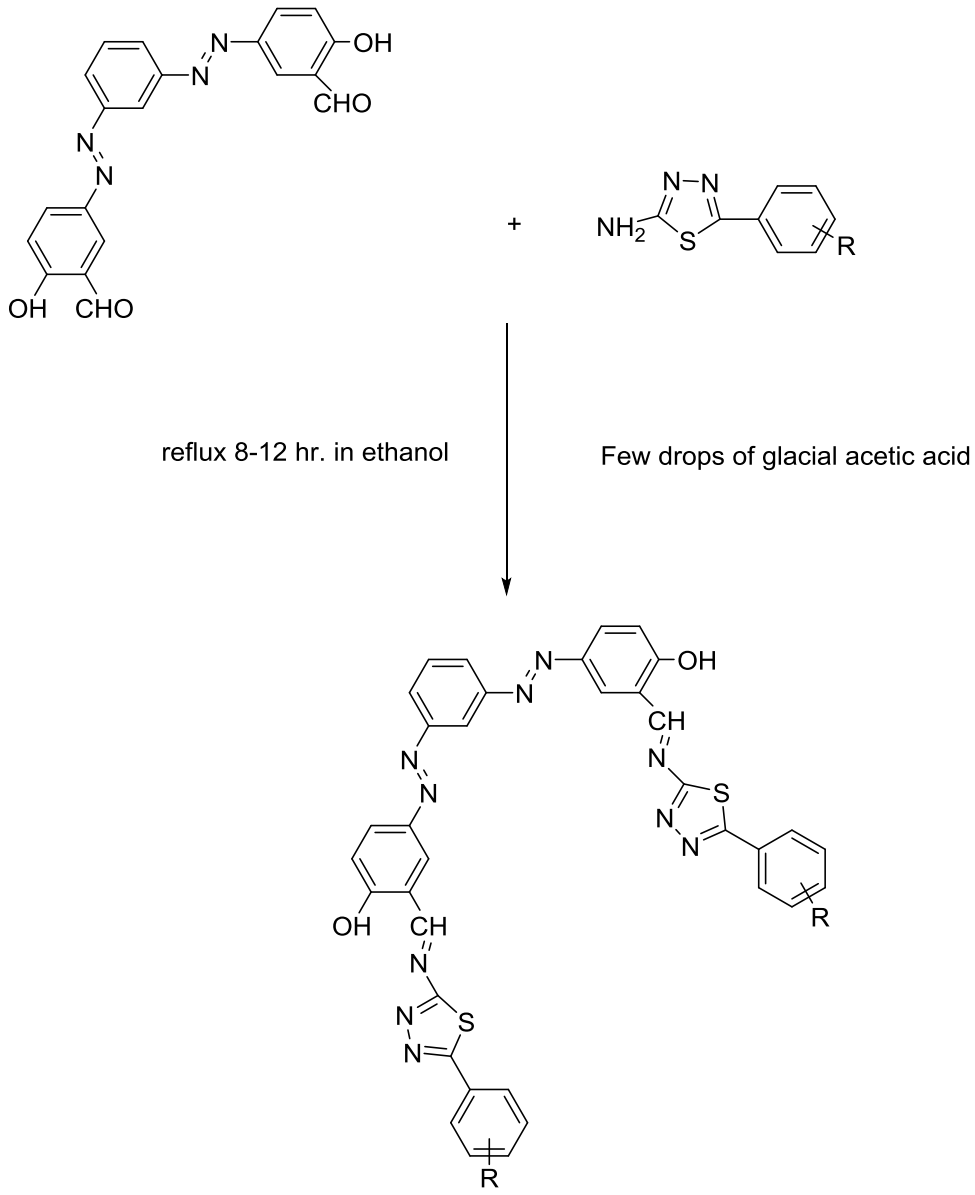
(Rafiqul Alam)

Contents

Abstract

Azo compounds or azo dyes are characterized by the presence of the azo moiety (-N=N-) in their structure conjugated with two distinct or identical mono or polycyclic aromatic or heteroaromatic systems. Because of their specific physico-chemical properties and biological activities, they have a broad spectrum of application in the field of pharmaceutical, textile industry, and in analytical chemistry. However the most typical and popular field of utility remains as their coloring function. The Azo compounds are applicable for biocidal treatment of textile materials because they exhibit biological activity. Azo compound are well known for their medicinal importance and are recognized for their applications as anti-diabetics, antiseptics, anti-neoplastics, antibacterial and anti-tumor. They are also involved in many biological reactions such as inhibitions of DNA and RNA, carcinogenesis, protein synthesis and nitrogen fixation. The azo-imine linkage is responsible for the biological activities are displayed by some Schiff bases. On the other hand, the 1, 3, 4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal compounds. Thiadiazole nucleus is present as a core structural component in an array of drug categories such as anti-microbial, anti-inflammatory, anticancer, antiviral and anti-tubercular agents. The broad and potent activity of thiadiazole and their derivatives have established them as pharmacologically significant scaffolds. Therefore, the combination of bis-azo and thiadiazole has received considerable attention to synthesize bis-azo-schiff bases containing thiadiazole moiety.

The thiadiazole derivatives were synthesized from aromatic aldehydes and thiosemicarbazide. Then azo compounds were prepared by the conventional diazocoupling reaction using diazonium salts and salicyldehyde. Finally the bis-azo-imine compounds were synthesized from thiadiazole and bis-azo compounds. Synthesized compounds were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR. The newly synthesized compounds showed moderate to good antibacterial property against S. aureus, B. cereus and E.coli.



R= (NO_2, Cl, OH, H)

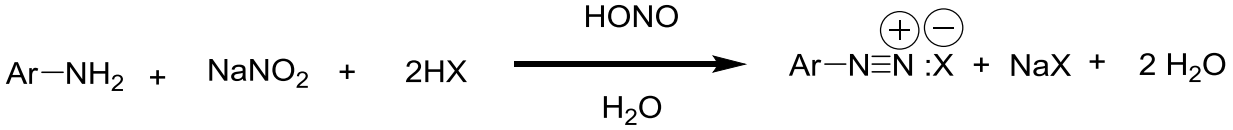
Scheme: Azo-Schiff Bases Containing - 1, 3, 4-thiadiazol moiety

Chapter 1

Introduction

Azo compounds are compounds bearing the functional group R−N=N−R′, in which R and R′ can be either aryl or alkyl. IUPAC defines azo compounds as the derivatives of diazene (diimide), HN=NH, where in both hydrogens are substituted by hydrocarbyl groups, e.g. PhN=NPh azobenzene or diphenyldiazene. The more stable derivatives contain two aryl groups. The N=N group is called an azo group. The name azo comes from the Greek word azote.

General chemical formula of azo compounds

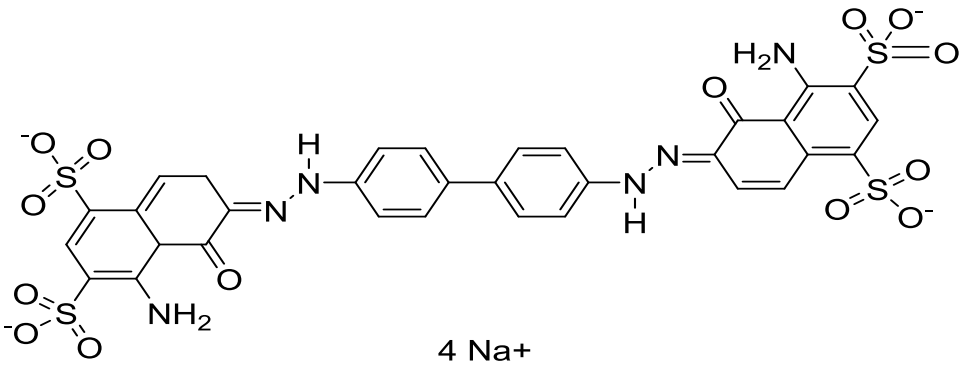


General reaction of diazonium salt formation.

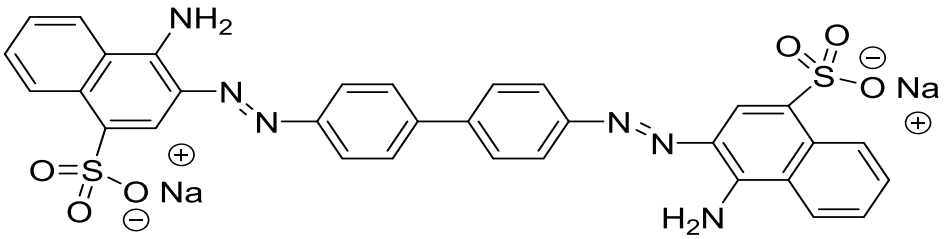
Azo compounds or azo dyes are characterized by the presence of the azo moiety (-N=N-) in their structure conjugated with two distinct or identical mono or polycyclic aromatic or heteroaromatic systems. Because of their specific physico-chemical properties and biological activities, they have a broad spectrum application in the field of pharmaceutical [1], textile industry [2], and in analytical chemistry [3]. However, the most typical and popular field of utility remains as their coloring function. The Azo compounds are applicable for biocidal treatment of textile materials because they exhibit biological activity [4]. Azo compound are well known for their medicinal importance and are recognized for their applications as anti-diabetics [5], antiseptics [6], antineoplastics [7], antibacterial [8] and antitumor [9]. They are also involved in many biological reactions such as inhibition of DNA, RNA, carcinogenesis, protein synthesis and nitrogen fixation [10-11]. Probably the azo-imine linkage is responsible for the biological activities displayed by some Schiff bases [12-13]

The azo compounds viz. Evans blue, **1** and Congo red, **2** are being studied as HIV inhibitors of viral replications [19].The existence of azo moiety show antibacterial and pesticidal activities. Recently, azo group containing compounds as antimicrobial agents has been the subject of study

reported by H. N. Chopde [20], A. H. Shridhari [21] and C. J. Patil [22-24]. Synthesis of most azo compounds involves diazotization of a primary aromatic amine, followed by coupling with one or more nucleophiles. Thus, benzoic, phenolic, salicylic and naphtholic compounds undergoes diazotization reactions. Because of multiple applications of azo compounds, it has been grown interest azo compounds and their derivatives in order to explore the newer potential derivatives to synthesize. Azo compounds are often described as chromogen [25]. The amino and hydroxyl groups are commonly used coupling components [26]. The emergence of diverse classes of synthetic dyes including azo dyes now available due to constant effort to find specific dye for application in diverse materials of industrial importance which include, but not limited to textile fabric [27], ink-jet printer, paper, leather and aluminium sheet [28]. Furthermore, azo compounds also have a many applications in photo industry such as photodynamic therapy, photographic or electro-photographic systems and are dominant organic photo conductives [9, 21].



1. Structure of Evans Blue



2. Structure of Congo Red

Nowadays, synthetic azo compounds are widely used in different applied fields, such as medicines, cosmetics, food, paints, plastics, shipbuilding, automobile industry, cable manufacture, etc [29-47].

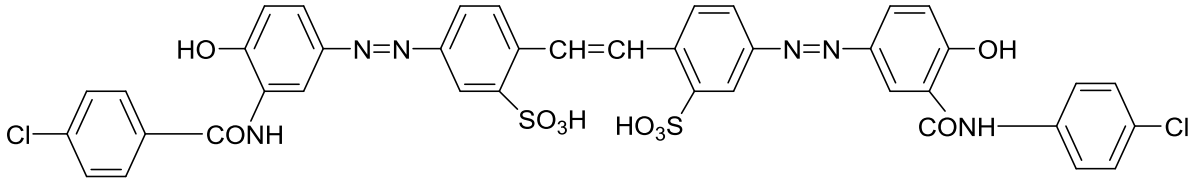
However, the traditional applied field of the synthetic azo dyes still remains the textile industry, and the finishing of fibrous materials in order to impart simultaneously with coloration, antimicrobial properties is of great interest.

This is due to the fact that textile materials undergo biological degradation, and it seems that about 40 % of the damage is due to the effect of microorganisms. The activity of fungi and bacteria results in the reduction of mechanical strength of a material, color change, stains and stale odor. In this regard, the use of materials with antimicrobial properties extends the service life of these materials, and avoids damage caused by biological degradation.

The manufacture of biologically active materials can be performed either by impregnation with antimicrobial compounds, or by chemical reaction (adding antimicrobial compounds by means of chemical bonding to functional groups of the fiber-forming polymers).

Dyes are among the compounds which are suitable for biocidal treatment of textile materials due to the fact that some of them exhibit biological activity, resulting from the presence in their molecule of some antiseptic groups that form a definite type of bonding with the molecules of the fibrous material.

Synthesis of two new diazo compounds with structure **3** (derived from 4, 4′-diaminostilbene 2, 2′-disulfonic acid) and structure **4** (derived from 4, 4′-diaminobenzanilide) have been reported by simu et, al [4].



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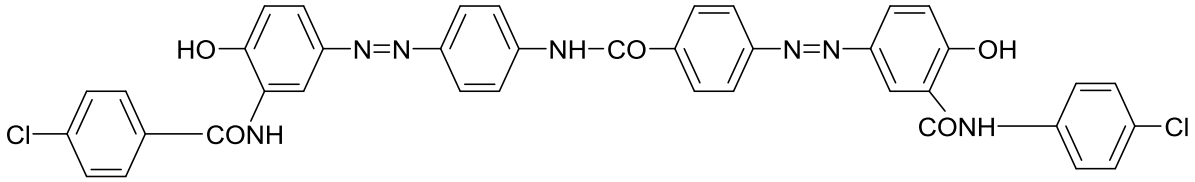

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The synthesis of the azo compounds **3** and **4** were performed by a two step process. The preparation procedure involved the bis-diazotization of the corresponding diamine (4,4'diaminostilbene-2,2'-disulfonic acid and 4,4'-diaminobenzanilide respectively) and there after the coupling reaction (in a 1:2 molar ratio) of the resulting bis-diazonium salt with 4-chlorosalicylanilide.

The bis-diazotization of the two diamines was carried out by the direct method, in an HCl aqueous solution. In the case of 4, 4′-diaminostilbene-2,2′-disulfonic acid, the resulting bis-diazonium salt was separated by filtration for the complete removal of the salts, unlike the classical method. The coupling reactions were performed in an alkaline aqueous medium (pH around 8), in presence of Na_2CO_3 at a temperature around 10ºC, and a 3% excess of coupling component. It was noticed that the direct adding of the alkaline solution of the coupling components to the acid suspension of the bis-diazonium salts of both 4, 4'-diaminostilbene-2,2'-disulphonic acid and 4,4'diaminobenzanilide, was optimum for the synthesis of the disazo compounds **3** and **4.**

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The coloristic and the application properties of the disazo compounds **3** and **4** were determined and compared to those exhibited by a classical direct dye, with quite close chemical structure (e.g. C. I. Direct Orange 1).

In the case of the azo compound **3**, the untreated dyeing on cotton was reddish-orange; the dye had good migration and good leveling properties. The percentage uptake on cotton was found to be 68 %. In the case of **4**, the untreated dyeing on cotton was orange and the migration was good, Compound **4** exhibited fair leveling properties and the percentage uptake on cotton was 75 %. The obtained results are presented in **Table 1.** As it can be seen in **Table 1**, the application properties of the disazo dye [**3**] are quite similar to the reference dye Direct Orange 1. Moreover, the after-treatment of the dyed samples with CuSO4 shows improvements in the case of the wet fastness of dye [**3]**. The obtained results indicate that the synthesized dye can be ranged as a direct dye.

Table 1

Application properties on cotton of dye [**3**] and of C. I. Direct Orange 1 (C. I. 22.250).

	Disazo dye **3**		Disazo dye **4**		C. I Direct Orange 1* (C. I. 22.430)
Fastness:	Direct dyeing	After treatment with CuSO4	Direct dyeing	After treatment with CuSO4	Direct dyeing
Light	2-3	3	3	3	2
Water	3-4	4	3-4	4	3-4
Wash 40 oC	2-3	3	2-3	3	1-2
Perspiration (alkaline)	2-3	3	2-3	3	2
Perspiration (acid)	2-3	3	2-3	3	4
Hot pressing	3	3	3	3	3

Further, the azo compounds **3** and **4** were subjected to *in vivo* imagistic skin evaluation tests. The possibilities of apparition of adverse effect were monitored with an imagistic skin

evaluation, *in vivo,* daily after each application [48-50]. For all the volunteers involved in this study, and for all concentrations applied, any pathological sign were registered. Concerning to skin coloration, no modification was noticed, excepting the transitory color of the studied dyes. As to the skin texture, no peeling effect was registered, and the epidermal desquamative registrations were no more different than the blank skin areas. Moreover, during all the period of skin determination, no case of contact dermatitis was registered. The results of screening antimicrobial activity are given in **Table 2**. The disazo compounds **3** and **4** were subjected to antibacterial activity screening against two grampositive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and three gram-negative bacteria (*Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*) employing the disk diffusion technique. The results showed that the studied compounds showed antimicrobial activity against most of all the tested species, especially against *Staphylococcusaureus* and *Escheria coli*.

Table 2

Antimicrobial activities of compounds

Compound	Diameter of zone of inhibition in mm				
	Staphylococcus aureus	Streptococcus Pyogenes	Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris
3	18	10	17	16	14
4	20	18	20	13	15
Septonex	18	17	14	19	16

***In vivo* skin evaluation**

The evaluation of the possible skin adverse effects of the disazo compounds **3** and **4** was performed on a study group of 45 healthy volunteers, all women, aged between 25 – 51 years. The disazo compounds **3** and **4** were applied as dilute aqueous solution of 5 %, 2.5 %, 1 % and 0.5 %, on 4 areas of forearms, for 30 days, daily, 1 time a day. In the volunteer selection, the subjects presenting skin pathologic signs (seborrhea dermatitis, psoriasis lesions) were eliminated. However, physiological signs, like photo aging signs, dilatation of sebaceous channel

glands, or other physiological signs were admitted. No restrictions regarding to the human skin photo type were considered. For all volunteers, a blank skin area was also chosen, on which we applied distilled water. The possibilities of apparition of adverse effect were monitorized with an imagistic skin evaluation, *in vivo,* daily after the previous application. The tests were performed using a ProDerm II Skin Analyzer. The following parameters were evaluated: the skin coloration (normal or the apparition of a rush); the skin texture (preservation of the normal skin quadrilagde, and eventually irritation signs – a peeling effect), and the possible skin dryness, eventually induced by the disazo compounds (involution of density of sebaceous glands channels).

In vitro antimicrobial investigations

The disazo compounds **3** and **4** were tested for their antimicrobial activity against the micro-organisms: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonasaeruginosa, Proteus vulgaris* and *Escherichia coli*, using the cup plate diffusion method [51]. The bacterial organisms were isolated from human being with characteristic infections and diseases. The disazo compounds **3** and **4** were dissolved in ethanol, yielding solutions of 0.2% concentration. Wells (diameter 6 mm) were loaded with 0, 1 mL solution of the studied disazo compounds. The bacterial suspensions were inoculated on sterile Mueller Hinton agar, in each Petri dish with 9 cm diameter. After 24 hours of incubation, the diameters of the inhibition zones were measured (including the 6 mm diameter of the disc). The results were compared with [1- (ethoxycarbonyl) -pentadecyl] -trimethylammonium bromide (Septonex), a commercial antiseptic agent. Inhibitory effect of the compounds was established at zones greater than 12 mm.

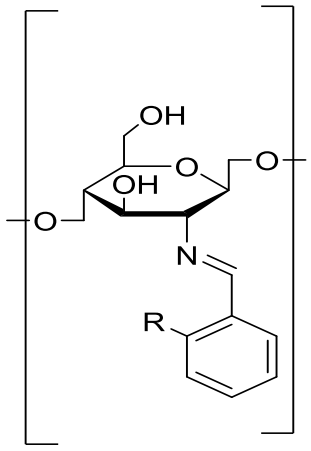
SCHIFF'S BASE

A Condensation product of aromatic amines and aldehydes forming azomethine is called as Schiff's base. They have general formula $R^{I}R^{II}C=NR^{III}$, where R is aryl or alkyl group. Schiff Hugo Josef in 1864 discovered Schiff's base [52].

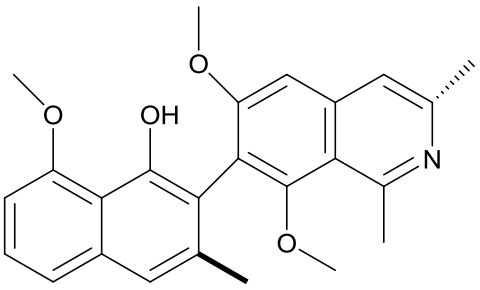
Importance of Schiff's Base

Schiff's bases are some of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediatesin organic synthesis, and as polymer stabilizers [53]. Schiff's

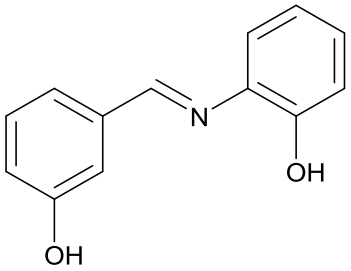
bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [53, 54]. Imine or azomethine groups are present in various natural, natural-derived, and non-natural compounds see (**Fig. 1** for some examples). The imine grouppresent in such compounds has been shown to be critical to their biological activities [55–57].



Chitosan-derived Schiff base
[R = H (**6**) or OH (**7**)
(Antifungal activity)
Natural Product-derived
Compound



Ancistrocladidine (**5**)
(Antimalarial activity)
Natural Product

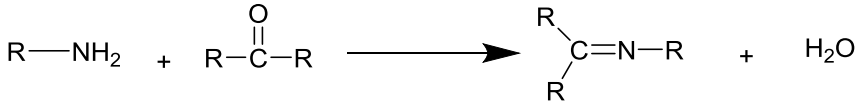


N-(Salicylidene)-2-hydroxyaniline (**8**)
(Antibacterial activity)
Non-natural Compound

Fig: 1-Struture 5,6,7,8 Examples of bioactive Schiff bases. The imine or azomethine group present in each molecular structure is shaded

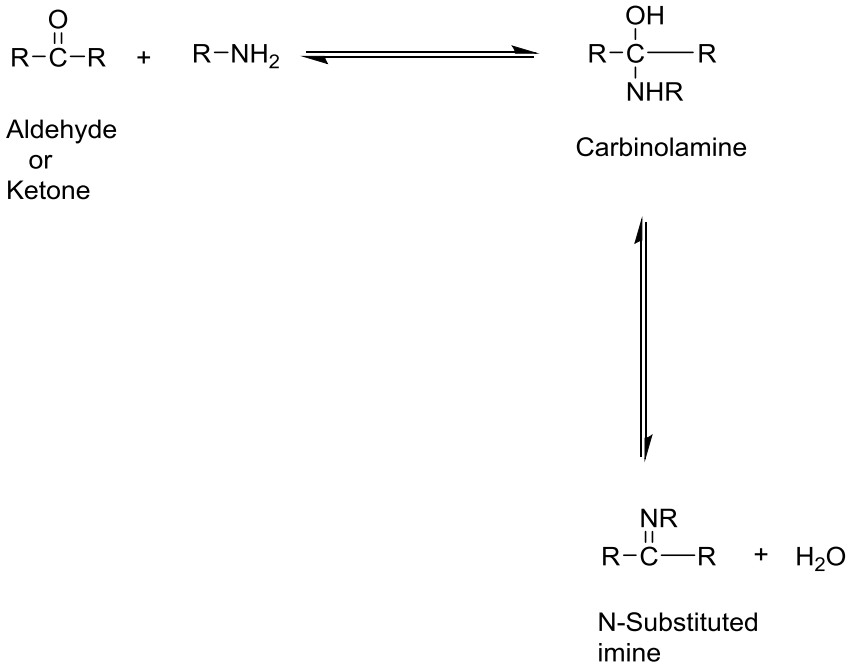
Formation of Schiff's Bases

A Schiff's base or azomethine, is nitrogen analog of an aldehyde or a Ketone in which the C=O group is replaced by a C=N-R group. It is usually formed by condensation of an aldehyde or a ketone with a primary amine according to the following Scheme

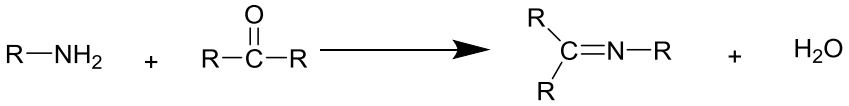


Primary Amine Aldehyde or Ketone Schiff's base

R = H, alkyl or aryl group. Schiff's bases, which contain aryl substituents, are substantially more stable and more readily synthesized. While those, which contain alkyl substituents are relatively unstable and readily polymerizable, the aromatic aldehydes having effective configuration are more stable The formation of the Schiff's bases from an aldehyde or a ketone is a reversible reaction and generally takes place under acid or base catalysis.

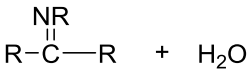


The formation of Schiff's base is generally driven to completion by separation of the product or removal of water, or both. Many Schiff's bases can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base. The mechanism of Schiff's base formation is another variation on the theme of nucleophilc addition to carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound, called carbinolamine and this subsequently loses water by acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.

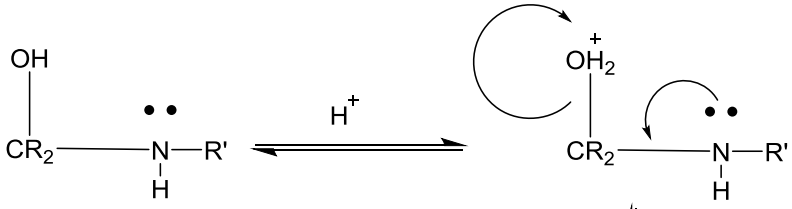


Aldehyde
or
Ketone

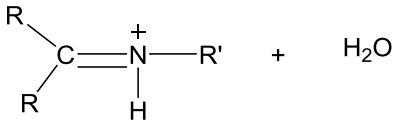
Carbinolamine

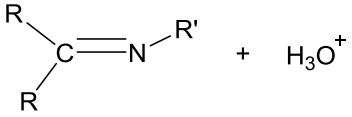


N-Substituted
imine



(acid catalysed dehydration)





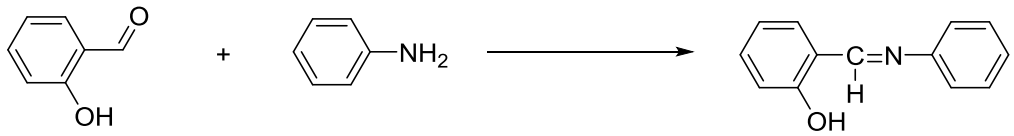
Typically the dehydration of the carbinolamine is the rate determing step of Schiff's base formation and this is why acids catalyze the reaction. Yet the acid concentration cannot be too high, because amines are basic compounds. If the amine is protonated and becomes non nucleophilic, equilibrium is pulled to the left and carbinolamine cannot occur. Therefore many Schiff's base synthesis is best carried out at mildly acidic pH.

The dehydration of carbinolamine is also catalyzed by base. The reaction is somewhat analogous to E2 elimination of alkyl halides, except that, it is not a concerted reaction. It proceeds in two steps through an anionic intermediate. The Schiff's base formation is really a sequence of two types of reactions i.e. addition followed by elimination.

General method for the preparation of Schiff's Bases

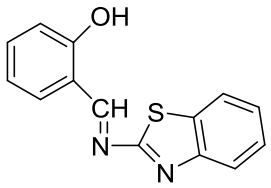
The Schiff's base can be prepared by refluxing equimolar quantities of any primary amine and the aldehyde or ketone on a water bath for 2-5 hrs. Schiff's base thus obtained, is filtered and purified by recrystallisation from the appropriate solvents (Yield 70-80%). A few examples are given below:

Condensation reaction between salicyladehyde and aniline gives Schiff's base namely 2-(phenyl imino methyl) phenol.



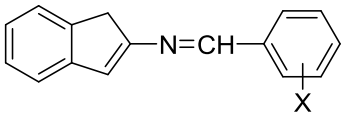
Salicyladehyde Aniline 2-(phenyl imino methyl) Phenol

Condensation reaction between salicyladehyde and 2-aminobenzothiazole yields 2-hydroxybenzylidine-2-aminobenzothiazole Schiff's base.



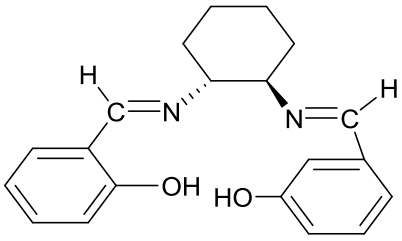
2-Hydroxybenzylidine-2-aminobenzothiazole

Condensation reaction between 2-amino benzothiazole and derivatives of benzaldehyde gives the Schiff's base.

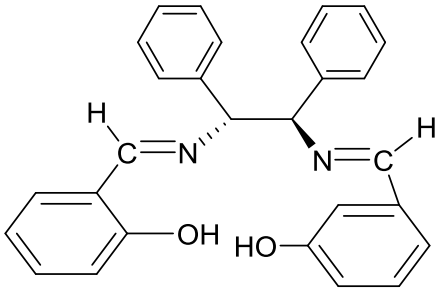


X=H, *p*-N $(CH_3)_2$, *p*-OH, *o*-OH, *p*-Cl, *m*-Cl, *p*-Br, *p*-NO2

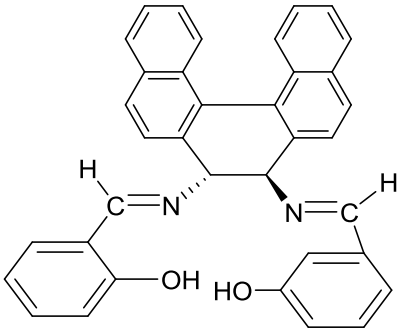
Condensation reaction of 2-hydroxyacetophenone with 1, 2′ diamino cyclohexane, 1,2-diphenyl ethylene diamine and 2,2′-diamino-1,1′ bi naphthalene gives the Schiff's bases of (I), (II) and (III) respectively



(I)

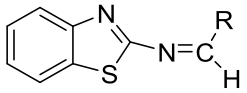


(II)

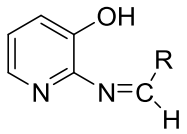


(III)

Condensing 2-amino benzothiazole with 2-hydroxy-1-naphthaldehyde, 2-hydroxy benzaldehyde, 4-methoxy benzaldehyde, 4-hydroxy benzaldehyde, and benzaldehyde (a) and 2-amino-3-hydroxypyridine with 2-hydroxy-1- naphthaldehyde and 2-hydroxy benzaldehyde (b) gives the Schiff's bases.



(a)



(b)

(a) R=2-OH-1-naphthaldehyde, *O*-OH-Ph, *P*-OCH_3-Ph and*P*-$N(CH_3)_2$-Ph.

(b) R=2-OH-1-naphthaldehyde and *O*-OH-Ph

SCHIFF'S BASES AS LIGANDS

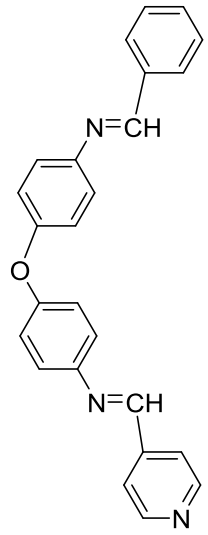
Ligands which posses two or more donating groups may share more than one pair of electrons with a single metal ion by coordinating around the central metal ion. These ligands, generally known as multidentate ligands, are specifically called bidentate, tridentate etc. Multidentate ligands, which coordinate with metal ions to form complexes, are also called chelates. Instead of a linear structure, they have a chelate ring structure.

A common case is where the chelating ligand has at least one acidic group (-CO_2H or -OH) or donor atom, as well as one or more basic atoms like nitrogen. During the chelation, the acidic group loses a proton and becomes anionic donor, thus resulting in charge neutralization. The basic nitrogen donates a pair of electrons to the metal ion. A chelate ligand must possess two acidic or two coordinating groups or one acidic and one coordinating group. Almost all organic compounds contain –OH, -SH or –NH groups in some form. Molecules containing Nitrogen, Oxygen, and Sulphur atoms frequently form coordinate bonds with metal ions in forming chelate rings. These groups must be located in the molecule in such positions that, the metal ion will be involved in the ring formation of five or six atoms. An organic compound containing more than twoDonor groups is capable of forming multiple rings with the metal ion resulting in more stable chelate structure. The Schiff's base act as a bidentate monobasic donor for Cu (II), Co (II), Ni

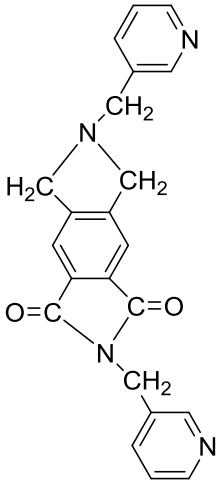
(II), Mn (II) and Fe (II) and prominent sites of coordination are nitrogen ofazomethine group and oxygen of the hydroxyl group.

Monodentate Schiff's Bases as Ligands

Ligands having one atom, which can be an electron donor often, function as monodentate ligands. Schiff's base ligand of bis [4-(4-Pyridyl methylene amino) phenyl] ether (L^1) and N,N′-bis (3-pyridyl methyl)-diphthalic diimide (L^2) act as monodentate ligands. These ligands are used in the preparation of two supramolecular coordination polymers. Ligand L^1 forms an interestingly infinite cross-linked double helical structure, whereas (L^2) forms the one-dimensional zig-zag chains, which are parallel with each other. Each ligand coordinates to two Hg (II) ions and each Hg (II) ion is coordinated by two L^2 to generate the 1D zig-zag chains, which are parallel with each other.



L1



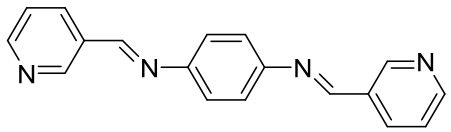
L2

Bis [4-(4-Pyridyl methylene amino) phenyl] ether (L^1)

N, N'-bis(3-Pyridyl methyl)-diphthalic diimide (L^2)

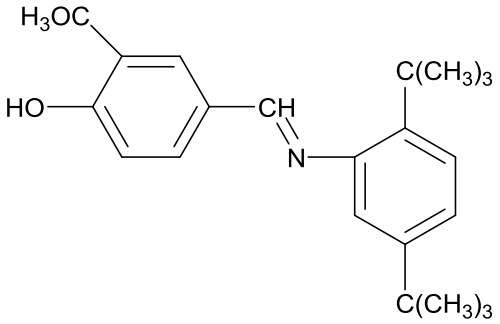
A long, bis (monodentate), linking Schiff's base ligand L (Py-CH=N-C_6H_4-N=CH-Py) (N^1 E, N4 E)-N^1N^4- (Pyridin-3-yl methylene) benzene-1, 4-diamine was prepared from 1, 4-phenylenediamine and 3-pyridine carboxaldehyde by the Schiff's base condensation. Ligand L has two terminal pyridyl groups capable of coordinating to metals through their nitrogen atoms. The Schiff's base was used to prepare Zinc coordination polymers. X-ray crystallographic

studies proved that the Zinc metal chelate is coordinated by two Schiff's base ligands (L) and four aqua ligands. The geometry of the Zinc metal chelate can be described as adistorted octahedron, in which the aqua ligands form an equatorial plane.

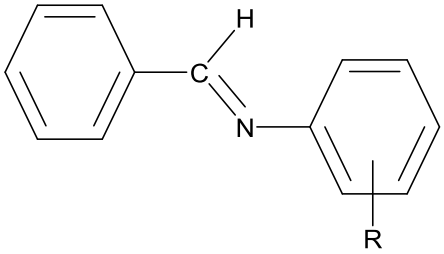


L= (N1 E, N4 E)-N1 N4- (Pyridin-3-yl methylene) benzene-1, 4-diamine**.**

Few examples of monodentate Schiffs bases

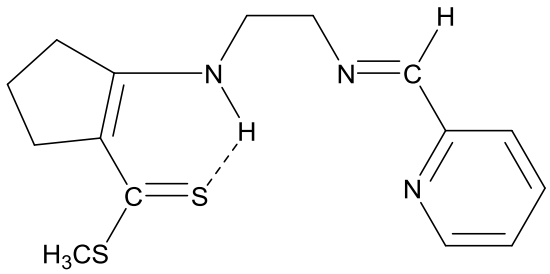


(E)-4-[(2,5-di-tert-butyl phenylimino) methyl]-2-methoxy Phenol



R=H, 2Me, 3Me, 4Me, 2, 3 Me, 2, 4 Me

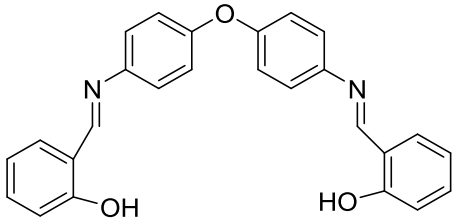
(E)-N-benzylidenebenzenamine, (E)-N-benzylidene-2-methyl benzenamine, (E)-N-benzylidene-3-methyl benzenamine, (E)-N-benzylidene-4-methyl benzenamine, (E)-N-benzylidene-2, 4-dimethylbenzenamine



(E)-Methyl-2- (2-(pyridin-2-yl methyleneamino) cyclopent-1-Enecarbodithioate

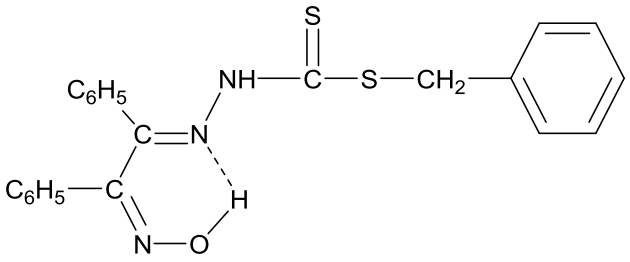
Bidentate Schiff's Bases as Ligands

Ligands having more than one atom, which can be an electron donor often functions as bidentate ligands. The bis-bidentate ligands were prepared by condensation reaction between salicylaldehyde and 4,4'-diaminodiphenyl ether. Complexation of a new bis-bidentate schiff's base, 2-[{{4-[{3-{[(z)-1-(2-hydroxyphenyl) methylidene] amino} phenyl) oxy] phenyl} imino) methyl]-1-benzenol (APOPIB) with some mono, di and trivalent metal ions were investigated by the theoretical calculations and conductance studies. Since APOPIB was able to form a selective complex with Sm(III) ion (Kf =5.23± 0.24), it was applied as a sensing material in a poly vinyl chloride (PVC) membrane sensor for determination of Sm (III) ions.

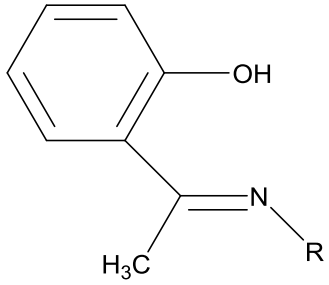


2-[{{4-[{3-{[(Z)-1-(2-hydroxy phenyl) methylidene] amino} phenyl) oxy] phenyl} imino) methyl] -1-benzenol (APOPIB)

Few examples of bidentate Schiff's bases

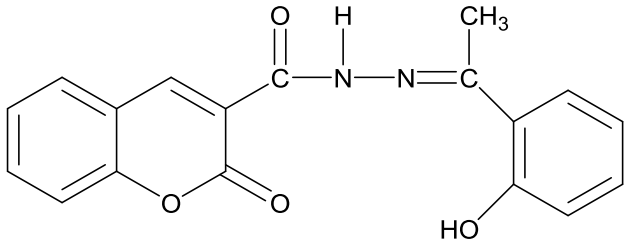


(E)-benzyl-2- [(Z)-2-hydroxyimino)-1, 2-diphenylethylidene] hydrazine carbodithioate

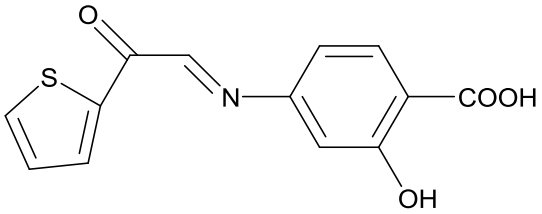


[R=Ph, 2-MeC6H4, 4-MeC6H4]

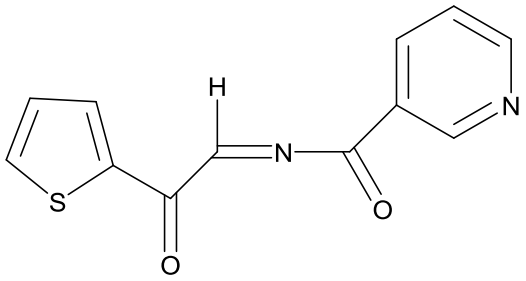
(E)-2-[1-(o-tolyl imino) ethyl] phenol, (E)-2-[1-(phenyl imino) ethyl] phenol, (E)-2-[1-(p-tolyl imino) ethyl] phenol



N′- (1-(2-hydroxy phenyl) ethylidene)-2-oxo-2Hchromene-3-carbohydrazide



2-Hydroxy-4- {[2-oxo-2-(thiophen-2-yl) ethylidene] amino}benzoic acid [TEAB]



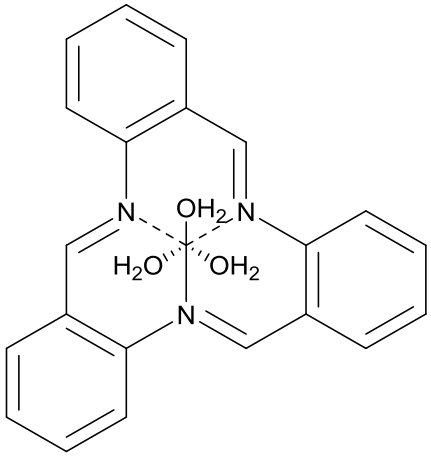
[N- [2-oxo-2- (thiophen-2-yl) ethylidene] pyridine-3-carboxamide] (TEPC)

Multidentate Schiff's Bases as Ligands

Ligands having more than two atoms, which can be electron donors often, function as multidentate ligands. They are specifically called as tridentate, tetradentate, pentadentate, hexadentate, heptadentate ligands.

Tridentate Schiff's Base

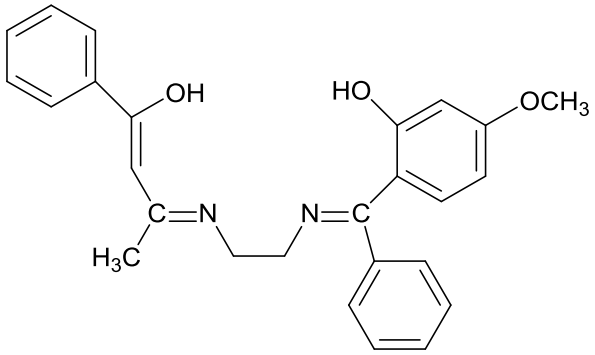
Triaquotribenzo- [b, f, j] [l,5,9]–triazacyclodecine Nickel (II) commonly called (TRI)Ni(OH2)3^{2+} is an example of tridentate Schiff's base complex .



Tridentate Schiff's base Ni (II) complex

Tetradentate Schiff's Base

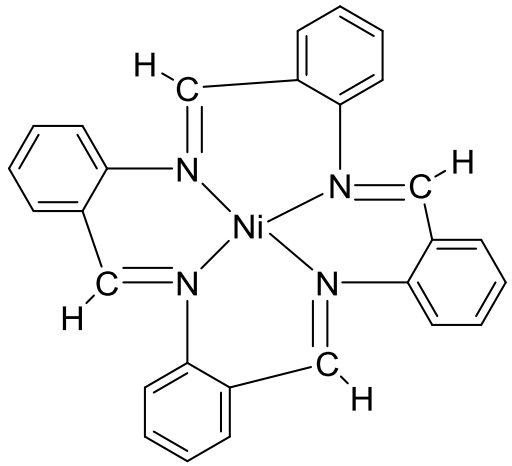
A tetradentate Schiff's base was prepared by the condensation reaction between ethylenediamine and 2-hydroxy-4-methoxybenzophenone. This Schiff's base coordinates *via* the imine nitrogen and enolic oxygen atoms. Their Ni(II) and Cu(II) complexes adopt a four coordinate square planar geometry, the Vo(IV) complex is five coordinate square pyramidal and the heteroleptic complexes are 6-coordinate, octahedral geometry.



Tetradentate Schiff's base

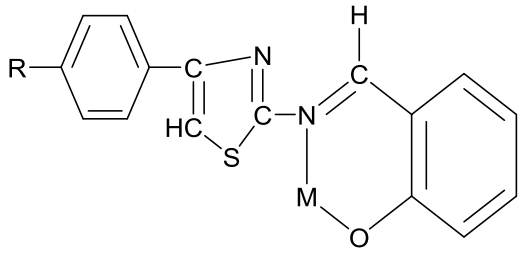
SCHIFF'S BASE METAL COMPLEXES

Schiff's base metal complexes are very important in coordination chemistry due to their facial synthesis, involvement in catalytic processes and discovery that the proteins and enzymes require two or more metal ions for their activity. Schiff's bases have remarkable property of forming complexes. Several reports are available only for the synthesis, characterization of Schiff's bases and their metal complexes. Schiff's base derived from the self-condensation of o-aminobenzaldehyde was used for the preparation of Nickel (II) complex.



Nickel complex of Schiff's base derived from self-condensation of *o*-amino benzaldehyde

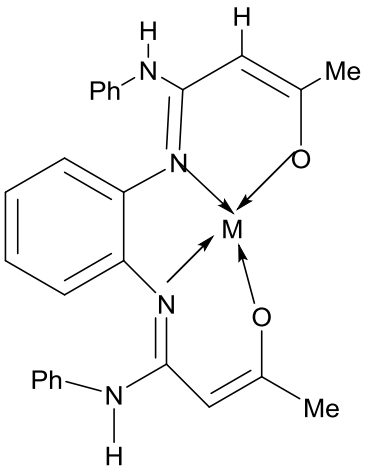
Synthesized and studied the structural features of Co (II), Ni (II), Cu (II) and Zn (II) complexes of Schiff's bases derived from 4-aryl-2-aminothiazoles and salicyladehyde.



M=Co (II), Ni (II), Cu (II), Zn (II), R=H, Cl, CH_3, OCH_3, C_2H_5

Schiff's base metal complexes derived from 4-aryl-2-aminothiazoles and Salicyladehyde

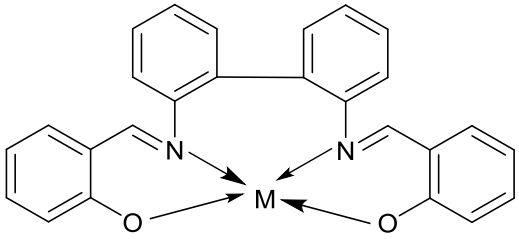
Schiff's bases synthesized by condensation of salicyladehyde and 2-amino-4-phenyl-5-arylazothiazoles and their metal complexes with Pd(II), Rh(III), Ru(III), Fe(III) were characterized and their structural studies were reported. Ligand 5-bromo-2-hydroxy benzylidine-2-amino benzothiazole and its complexes with Co(II), Cu(II) and Ni(II) were synthesized and characterized. It was suggested that, two ligands with water molecules coordinate to each metal atom by hydroxyl oxygen and imino nitrogen to form high spin distorted octahedral complexes with Co (II), Ni (II), and Cu (II).



M=Cu (II), Ni (II), Mn (II), Zn (II)

Schiff's base metal complexes derived from o-phenylendiamine and Acetoaetanilide

Cu(II), Co(II) and Mn(II) complexes of Schiff's base ligand derived from 2,2′-bis(p-methoxyphenylamine) and salicyladehyde were synthesized and spectral properties and electrochemical behaviors investigated.



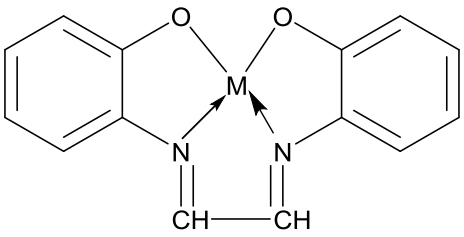
M=Cu (II), Co (II), Mn (II)

Metal complexes of Schiff's base ligand derived from 2-2′-bis (p-methoxy phenylamine) and salicyladehyde

Cu (II), Mn (II), Ni (II) and Zn (II) metal complexes with novel heterocyclic Schiff's bases derived from 5-Phenyl azo-salicyladehyde and o-amino benzoic acid were synthesized, characterized. These Schiff's bases behave as neutral tri dentate ligands forming chelates with (1:1) metal ligand stoichiometry.

SCHIFF'S BASES AS ANALYTICAL REAGENTS

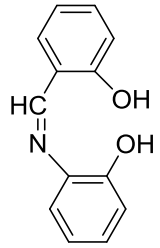
Schiff's bases chelate with many transition metals either in weakly acidic medium or in weakly alkaline medium to form either 1:1 (metal: ligand) or 1:2 (metal: ligand) complexes. Hence Schiff's bases derived from aromatic amines and aromatic aldehydes have wide variety of applications in many fields. Schiff's bases are attractive as analytical reagents because, they enable simple and inexpensive determination of various organic and inorganic substances. In general, there are two principal ways of their analytical applications: first determination of organic compounds bearing an amino or an active carbonyl group by the formation of coloured (Chromophore containing), fluorescent or insoluble Schiff's bases and secondly, the determination of various metal ions as well as amino and carbonyl compounds, using complex formation reactions. The analytical methods based on complex formation are used more frequently. Owing to the relatively simple preparation procedures of Schiff's bases, it is possible to obtain ligands of different design and characteristics by selecting appropriate reactants.Glyoxal bis(2-hydroxyanil) was used as a sensitive reagent for the determination of calcium and also used as metal indicator in the chelatometric titration of Calcium. The reagent behaves as quadridentate ligand, forming a 1:1 chelate.



Glyoxal bis (2-hydroxyanil)

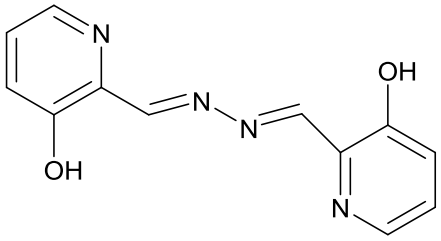
Schiff's base reagents derived from pyridinaldehyde, pyridine -2-aldehyde- 2′-pyridyl hydrazone and pyridine-2-aldehyde-2′-quinolylhydrazone were used as extraction photometric reagents for

the determination of Pd (II) and for Cu (II). The only disadvantage of this reagent in comparison with cuprion reagent was the lack of selectivity. Schiff's bases of o-hydroxy aromatic aldehyde (salicyladehyde) with aliphatic and aromatic mono and diamines (1,2-benzene diamine, aniline, 2-aminophenol and their derivatives) which were used in the determination of Cu (II), Be (II), Mg (II), Ca (II), Al (III), Ga (III), Sc (III), Mn (II), Fe (III), U (VI). These Schiff's bases are used as volumetric, gravimetric, fluorimetric, spectrophotometric reagents. Schiff's base of salicylidene-o-aminophenol and Schiff's base 2-hydroxyaniline-N-salicylidene were used to study the fluorescence properties of some metal complexes of Zinc, Tin, Scandium, Aluminium, and Galium.



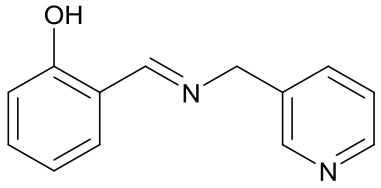
2-Hydroxyaniline-N-salicylidene

Aromatic 2-hydroxy aldehyde was condensed with a number of aliphatic and aromatic amines to form a series of bi, tri and tetra dentate Schiff's bases like bis-salicylidene-o-phenylenediamine, salicylidene ethylimine, N, N′-bis (salicylidene)-2,3-diamino benzofuran and salicylidene anthranilic acid. These were used as highly sensitive extraction photometric reagents for Cu (II) (extraction with chloroform, methyl isobutyl ketone and toluene). 3-Hydroxy picolinaldehyde Azine was used as photometric reagent for Ag (I), Cu (II), Hg (II), Pt (IV), Co (II), Ni (II), Zn (II), Pd (II), Cd (II), Mn (II) and Fe (II, III).

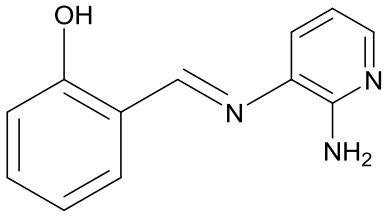


3-Hydroxypicolinaldehyde Azine

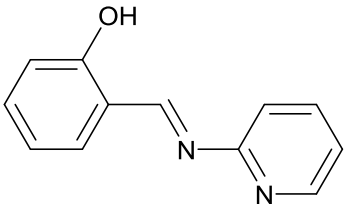
N,N′-bis (3-carboxysalicylidene) trimethylenediamine was used for the determination of trace amounts of Mercury by anode stripping voltammetry method and the method was used for the determination of mercury in natural water samples. Heteroaromatic Schiff's bases, 2-(3-pyridyl methyl iminomethyl) phenol, 2-(2-pyridyl iminomethyl) phenol, 2-(2-amino-3-pyridyl iminomethyl) phenol, N,N′-bis (salicylidene)-2,6-pyridine diamine and 2-(2-amino-4-methoxymethyl-6-methyl-3-pyridyl methyl imino methyl) phenol were used as reagents for the spectrophotometric and spectrofluorimetric determination of Copper by extraction method. The spectrophotometric determination of Cu(I) after extraction was very sensitive and selective with regard to Cd(II) and Pb(II). Highest sensitivity was achieved with compound 2-(2-amino-4-methoxy methyl-6-methyl-3-pyridyl methyl imino methyl) phenol.



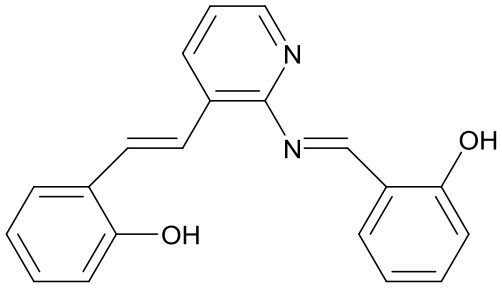
2-(3-pyridyl methyl imino methyl) phenol,



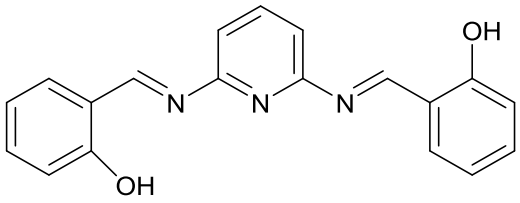
2-(2-amino-3-pyridyl imino methyl) phenol



2-(2-pyridyl imino methyl) phenol,



N, N′-bis (salicylidene)-2, 3-pyridinediamine



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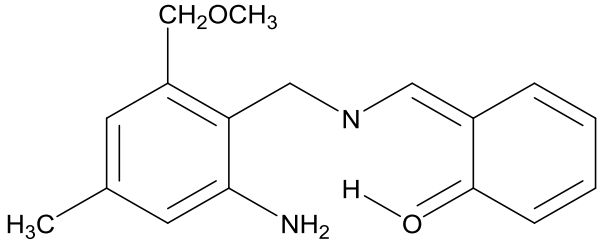
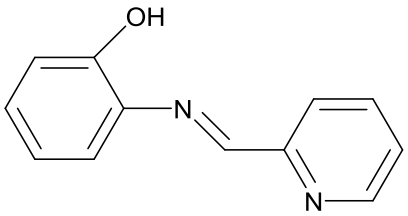


2-(2-amino-4-methoxy methyl-6-methyl-3-pyridyl methyl imino methyl) phenol

Schiff's base of N-furoylphenyl hydroxyl amine forms complexes with Co (II), Cu (II), Zn (II) and Fe (II). The reagent is used for the gravimetric determination of Co (II), Cu (II), Zn (II) and Fe (II) through the precipitation of their complexes.

The Schiff's base 2-(2-pyridyl methylene amino) phenol (PMAP) was investigated as a spectrophotometric reagent for the determination of Iron in caustic soda, cotton yarn and fabric, woolen fabric and industrial water.



2-(2-pyridyl methylen amino) phenol

Nickel was determined by flow injection spectrophotometry at 370 nm after extraction of Nickel (II)bis(acetylacetone)ethylenediaminate chelate into chloroform using phosphate buffer (pH 7). Calibration graph was linear up to 25µg mL-1 Nickel. The system was applied to the determination of Nickel in Nickel-Copper alloys and in synthetic electroplating solutions [Chimpalee N.*et al*., 2000]. Salicylhydrazidone-2′-Hydroxy acetophenone was used as the spectrophotometric reagent for the determination of Al(III) at pH 4.6 and was also used for the determination of microgram quantities of sulfanilamide.

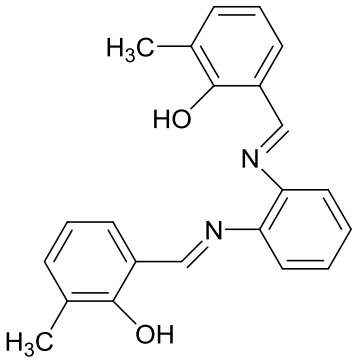
Schiff's base metal complexes of *N*,*N*′-*cis*-1,2-cyclohexylene bis (salicylideneaminato) Cobalt(II),[Co(II)(c-Salcn)] and*N*′-(±)-*trans*-1,2-cyclohexylene bis(salicylideneaminato)Cobalt(II), [Co(II)(t-Salcn)] were synthesized and characterized by elemental analysis, Melting points, IR, electronic, and $_{1}H$ and $_{13}C$ NMR spectra. Their thermo gravimetric studies were also discussed.

Salen-type Schiff's base is obtained by condensing ethyl-o-hydroxybenzene with ethylene diamine and 1-ethyl-salicylidene bis ethylene diamine. This reagent was used as spectrophotometric reagent for the determination of Mn (II) to a concentration range of 10-70 µg/mL. This method was successfully applied for the determination of to Mn (II) present in

pharmaceutical products.

Schiff's bases synthesized by the condensation reaction between 5-[3-(1, 2, 4– triazolyl-azo]-2,4-dihydroxy benzaldehyde with 1,3-diaminopropane and 1,6-diaminohexane were used for the spectrophotometric determination of Cobalt (II).

Sensitive chromogenic reagent N, N′-bis(3-methylsalicylidene)-ortho phenylene diamine was used in the spectrophotometric determination of Nickel. At pH 8 the ligand reacts with Nickel to form 1:1 complex. The method was successfully applied for the determination of trace amounts of Nickel in some natural food samples.



N, N′-bis (3-methyl salicylidene)-ortho phenylene diamine

Schiff's bases derived from 2-thiophene carbaldehyde with 2-aminothiazoleWere used for the spectrophotometric determination of Ni (II). The method was applied for the determination of Ni(II) in various synthetic and natural samples.

2-Ethanolimino-2-pentylidino-4-one Schiff's base derived from monoethanolamine and acetyl acetone was used as spectrophotometric reagent for the determination of Fe (III), Cu (II), and UO_2 (II). Fe (III) complex was detected at λ_{max} 440 nm, (pH 3.5), Cu (II) complex at λ_{max} 340 nm (pH 6) and UO_2 complex at λ_{max} 370 nm (pH 4). Beer Lambert's law obeyed in a concentration range of 0.5 to 3.0 $x10^{-4}$.

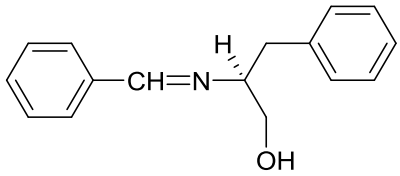
N, N′ bis-salicylidene-o-phenylene di imine was used as analytical reagent to determine trace concentration of metal ions like Fe (II), V (II) and Co (II) by spectrophotometric method.

Two new chemically modified silica gel covalently bonded with 4,4′- diaminodiphenyl ether (DDE), 4,4′-diaminodiphenyl sulfone salicyladehyde Schiff's bases were synthesized and were

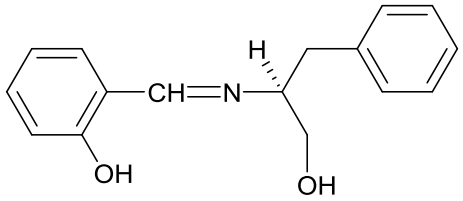
characterized by FTIR and BET surface area measurement techniques. These synthesized chelating materials were tested for the pre concentration of metal ions like Zn (II), Mn (II), Cr (III) in batch and column techniques with variation in parameter in competitive and non competitive conditions. The method was found very useful in recovery of the metal ions from dilute aqueous solutions.

Schiff's bases prepared by the condensation of aromatic mono and diamines derivatives and were used as fluorometric analytical reagents.

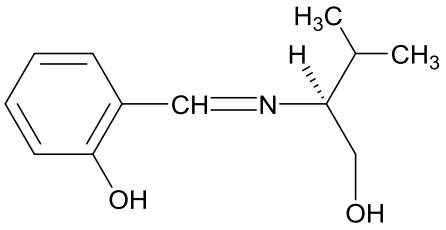
Extractive spectrophotometric determination of Ca (II), Mg (II), Cr (III), Fe (II), Zn (II), Cd (II), Ni (II), Mn (II), and Co (II) were done using some chiral Schiff's bases Benzaldehydene-(S)-2-amino-3-phenyl propanol **(I),** o-hydroxy benzaldehydene-(S)-2-amino-3-phenyl-propanol **(II),** Benzaldehydene-(S)-2- amino-3-methylbutanol **(III)** as complexing agents.



(I)



(II)



(III)

Schiff's bases derived from aromatic aldehydes namely Vanillin and p-dimethyl amino benzaldehyde were used for the spectrophotometric determination of metronidazole in tablets.

Biological activities of schiff bases

Antimalarial activity

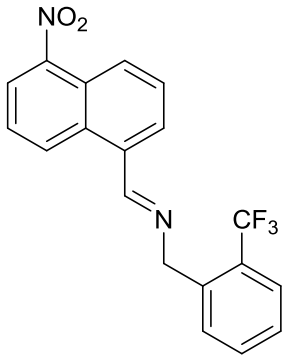
Malaria is a neglected disease that still causes serious public health problems. Every year, approximately 500 million people are afflicted by the disease, of whom around 1–3 million die, 90% of who in sub-Sahara Africa are primarily children [58]. Malaria is currently found in more than 100 countries throughout Africa, Latin America, Asia, and Oceania. Human malaria is mainly caused by four species of Plasmodium (P. falciparum, P. vivax, P. ovale, and P. malariae). The female mosquito of the Anopheles genus is the vector of Plasmodium [59]. The search for new drugs, vaccines, and insecticides to prevent or treat this disease is clearly a priority.

Schiff bases have been shown to be interesting moieties for the design of antimalarial agents. Ancistrocladidine **[5]** is a secondary metabolite produced by plants from the families Ancistrocladaceae and Dioncophyllaceae that present an imine group in its molecular scaffold. Compound **5** has been shown to be active against P. falciparum K1 and 3D7. The minimum inhibitory concentrations (MIC values) of ancistrocladidine necessary to completely abolish P. falciparum K1 and 3D7growth were 0.3 and 1.9 lg/mL, respectively. Interestingly, compound 1 was 90- and 10-fold more selective to P. falciparum K1 and 3D7, respectively than to rat skeletal myoblast L-6 cells [55]. Rathelot et al. [60] described the synthesis of Schiff base-functionalized 5-nitroisoquinolines and investigated the in vitro activity of these compounds against an ACC Niger chloroquine resistant P. falciparum strain. Schiff base **9** was the most effective antimalarial agent among the synthesised 5-nitroisoquinoline derivatives. The concentration of compound 5 necessary to inhibit P. falciparum growth by 50% (IC50) was 0.7l g/mL. Under the same experimental conditions the IC50 value for chloroquine was 0.1 l g/mL [60].

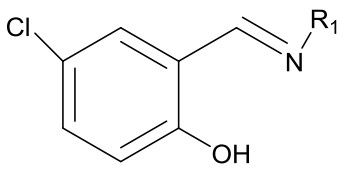
Antibacterial activity

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatments is the main cause of this problem [61, 62]. The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need [63].

Schiff bases have been pointed to as promising antibacterial agents. For example, N-(salicylidene)-2-hydroxyaniline (**8)** is effective against Mycobacterium tuberculosisH37Rv, exhibiting an MIC value of 8 lg/mL [56]. The selectivity of compound **8** was checked by performing experiments with J774 macrophages. No cytotoxic effect on J774 macrophages was observed for compound **8**, even when it was tested at concentrations as high as 1000 lg/mL. More than 80% of macrophage cells were viable at such experimental conditions, demonstrating the high selectivity of compound **8**. The synthesis and antimicrobial activity of a series of Schiff bases derived from the condensation of 5-chloro-salicylaldehyde and primary amines has recently been reported [64]. The 5-chloro-salicylaldehyde-Shiff base derivatives **10–19** were most active against at least one of the evaluated bacterial species. Pseudomonas fluorescence was the strain most sensitive to compounds **10-15** and **17-19**, with MIC values ranging from 2.5 to 5.2 lg/mL. The MIC value for the reference drug kanamycin against the same bacterial strain was 3.9 lg/mL. The Schiff bases **10, 11, 13–15, 18**, and **19** presented MIC values in the range of 1.6–5.7 lg/mL against Escherichia coli, while the MIC value for kanamycin was 3.9 lg/mL. Bacillus subtilis was sensitive to the Schiff base **18** only (MIC= 1.8 lg/mL). The MIC values for compounds **10** and **11** against Staphylococcus aureus were, respectively, 3.1 and 1.6 lg/mL [64].



(**9)**



(10-16)

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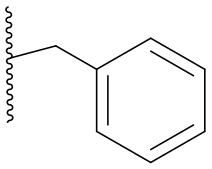

(10)

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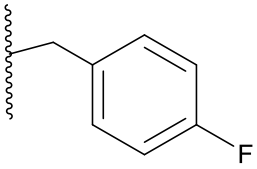
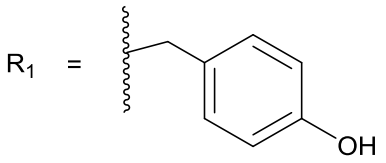


(11)



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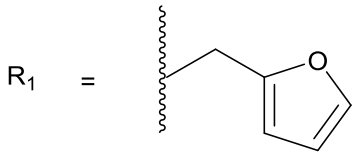

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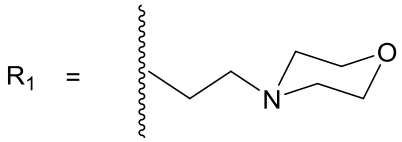

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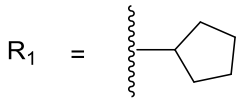

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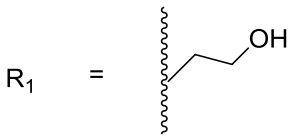
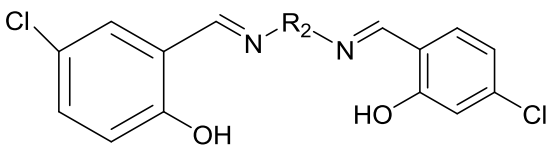
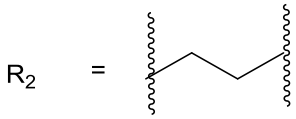


(16)

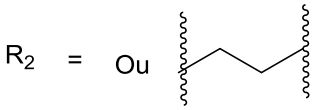
Fig. 2 Chemical structure of some synthetic antibacterial Schiff bases *Compound 5 is an antimalarial agent



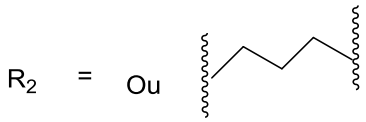
(17-19)



[17]



(18)



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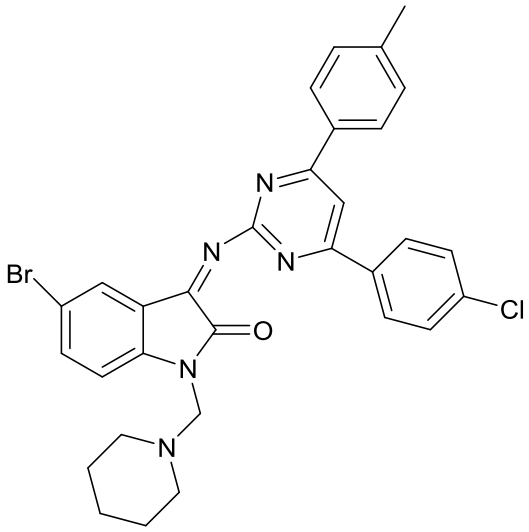

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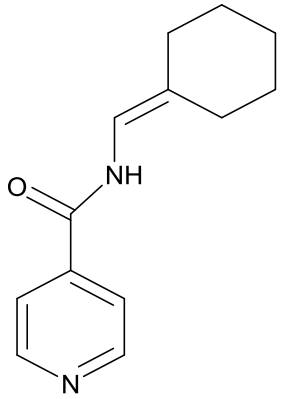

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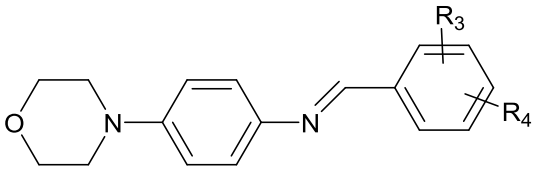
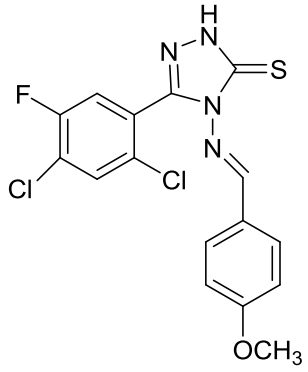


R_3 = *O*-Cl and R_4 = H [22] R_3 =*O*-OH and R_4 = H [23] R_3 = *P*-OH and R_4 = H [24]

Fig. 3 Chemical structure of some synthetic antibacterial Schiff bases



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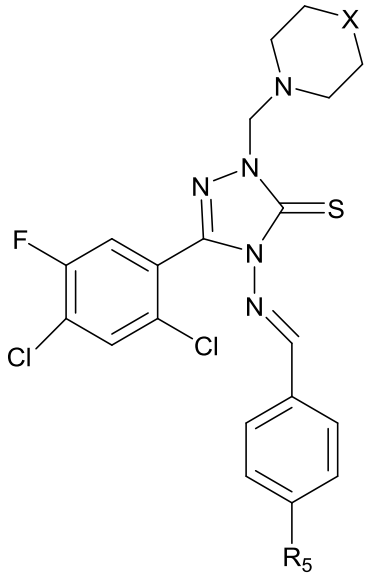


R_5 = 4-$N(CH_3)_2$ and X = CH_2 **(26)**

R_5 = Cl and X = NCH_3 **(27)**

R_5 = Cl and X = CH_2 **(28)**

Fig. 4 Chemical structure of some synthetic antibacterial Schiff bases

Isatin-derived Schiff bases have also been reported to possess antibacterial activity [65]. Twenty-eight bacteria of clinical interest were used in the studies performed by Pandeya and colleagues. The authors disclosed the isatin-derived Schiff base **20** (Fig. 3) as the most potent compound amongst those synthesized against all the pathogenic bacteria studied.

The MIC values for compound **20** against E. coli NCTC 10418, Vibrio cholerae non-01, Enterococcus faecalis, Proteus shigelloides were 2.4, 0.3, 1.2, and 4.9 lg/mL, respectively, while the MIC values for sulfamethoxazole (reference drug) against the same bacterial strains were in the range of 312–5000 lg/mL. Thus compound **20** was notably 1040-, 1040-, 4160-, and 1020-fold more potent than sulphamethoxazole. Other isatinderived Schiff bases have been described in the literature, but with no expressive antibacterial activities [66, 67].

The isoniazid-derived Schiff base **21** was active against M. tuberculosis H37Rv, exhibiting an MIC value of 0.03 mg/L [68]. In this respect, compound **21** was slightly more potent than isoniazid, its immediate synthetic precursor. Additionally, the isoniazid-derived Schiff base **21**

was not toxic against the cell line VERO (epithelial cells from healthy monkey kidney). The IC50 for compound **21** against VERO cells was as high as 1 g/mL, indicating that this isoniazid-derived Schiff base is selective for bacterial cells. The therapeutic safety and effectiveness for compound **21** is higher than 40,000, making this Schiff base an excellent lead for the development of antitubercular agents [68].

In 2005, Panneerselvam et al. [69] described the synthesis and in vitro antibacterial activity of eleven morpholine-derived Schiff bases. Shows the chemical structure of three of them (compounds **22–24**). The authors found that S. aureus and Micrococcus luteus were the bacteria most sensitive to the morpholine-derived Schiff base **22** (MIC= 20 and 32 lg/mL, respectively). Streptococcus epidermidis was more sensitiveto the morpholine-derived Schiff base **23** (MIC =17 lg/mL) and Bacillus cereus and E. coli were more sensitive to compound **24** (MIC= 21 and 16lg/mL, respectively).

Schiff bases with a 2, 4-dichloro-5-fluorophenyl moiety are also effective in the inhibition of bacterial growth. Schiff bases from this class (compounds **25–28** in Fig. 4) completely inhibited the growth of S. aureus, E. coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae [70]. MIC values for these compounds varied from 6.3 to 12.5l g/mL, which are comparable to those obtained for the reference drug ciprofloxacin [70].

Madurahydroxylactone Schiff bases are imines derived from natural products. Madurahydroxylactones are secondary metabolites produced by the plant Actinomadura rubra [71]. The imines **29-34** are examples of Schiff bases belonging to this class. With the exception of compounds **29** and **34**, all madurahydroxylactone-derived compounds were effective in the in vitro inhibition of B. subtilis, Micrococcus flavus, Sarcinalutea, and S. aureus growth, with MIC values varying from 0.2 to 3.1l g/mL [72]. These same compounds (28–33) presented very low activity against Mycobacterium phlei or Proteus vulgaris (MIC values higher than >50.0 lg/mL) [72]. Other molecules of natural or non-natural origin that are platforms for the synthesis of Schiff bases for antibacterial activities include amino acids, coumarins, sulfonamides, or resacetophenones, aminothiazolyl bromocoumarins, crown ethers, O-phthaldehyde, or 2-aminophenol and 1,2,4-triazoles[73-80]. The antibacterial property of compounds representative of these classes was examined. However, they did not exhibit any notable activity.

Antifungal activity

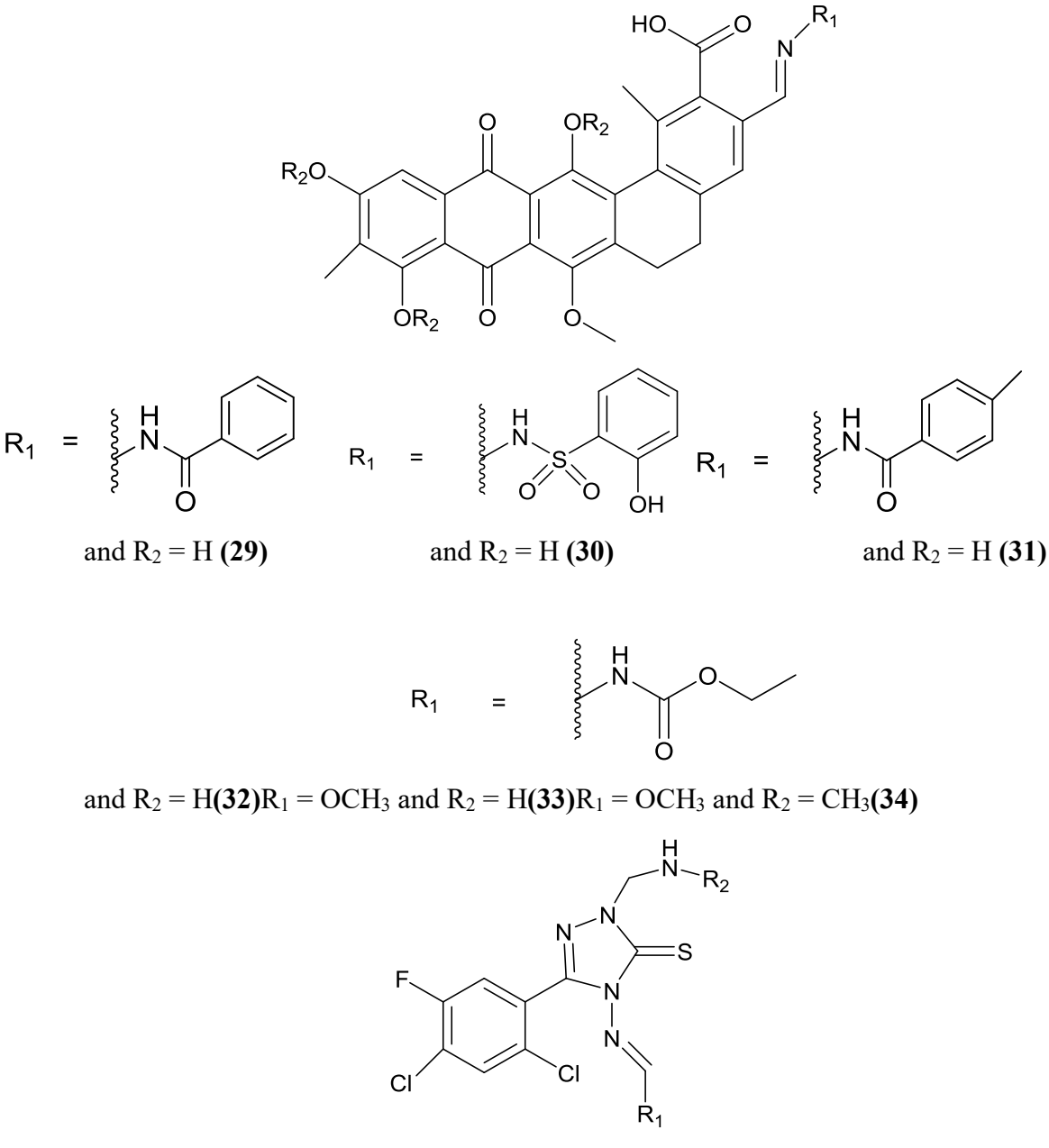
Fungal infections are not usually limited to the superficial tissues; indeed, a significant increase in life threatening systemic fungal infections has been reported [81]. The fundamental reason for this is the increasing number of patients at risk, including those with advanced age, major surgery, immunosuppressive therapy, acquired immunodeficiency syndrome (AIDS), cancer treatment, and solid-organ and hematopoietic stem cell transplantation [82]. The search and development of more effective antifungal agents are mandatory [83, 84] and some Schiff bases are known to be promising antifungal agents.

Alternaria brassicae and Alternaria brassicicola are phytopathogenic fungi that severely affect the production of most cruciferous crops (broccoli, cauliflower, mustard, turnip, cabbage, rape, and radish). N-(Salicylidene)-2-hydroxyaniline **5** at the concentration of 500 ppm inhibited the growth of these fungi by 67–68% [85]. Compounds **6** and **7** are examples of chitosan-derived Schiff bases with antifungal activity. They inhibited the growth of Botrytis cinerea and Colletotrichum lagenarium by 26–33% and 35–38% when used at 1000 ppm, respectively [57]. Overall, studies evaluating the effect of Schiff bases on phytopathogenic fungal growth have been modest and deserve more investigation.

Schiff bases with a 2, 4-dichloro-5-fluorophenyl moiety, such as compounds **20** and **35–38** have been demonstrated to inhibit the growth of fungi of clinical interest, such as Aspergillus fumigatus, Aspergillus flavus, Trichophyton mentagrophytes, and Penicillium marneffei. The MIC values for these compounds were in the range of 6.3–12.5 lg/mL, indicating that they are as potent as the reference fluconazole [70].

Piperonyl-derived Schiff bases **(39-44)** were active against some fungi at micromolar concentrations. They inhibited the growth of Trichophyton rubrum (MIC= 820–980 lM) and Epidermophyton floccosum (MIC =200–930 lM) [86]. The isatin-derived Schiff bases **29** and **45–54** were considerably active against Microsporum audouinii (MIC values ranging from 2.4 to 9.7 lg/mL) and Microsporum gypseum (MIC values ranging from 1.2 to 9.7 lg/mL) [66]. Compounds **20** and **45–54** also inhibited the growth of Candida albicans, Aspergillus niger, Cryptococcus neoformans, T.mentagrophytes, E. floccosum, and Histoplasma capsulatum at

MIC values higher than 10 lg/mL and lower than 79 lg/mL [66]. In another study, Panneerselvam et al. [69] showed that the growth of both C. albicans and A.niger was compromised by treatment with compound **24** at 20 l g/mL or compound **55** at 30l g/mL.



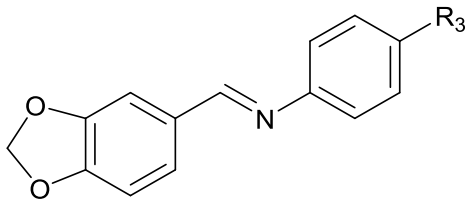
and R_2 = H **(29)** and R_2 = H **(30)** and R_2 = H **(31)**

and R_2 = H**(32)**R_1 = OCH_3 and R_2 = H**(33)**R_1 = OCH_3 and R_2 = CH_3**(34)**

R_1 = 4-F-C_6H_4 and R_2 = 4-Cl-C_6H_4 **(35)** R_1 = 3-Cl-4-F-C_6H_4 and R_2 = 4-Cl-C_6H_4 **(36)**

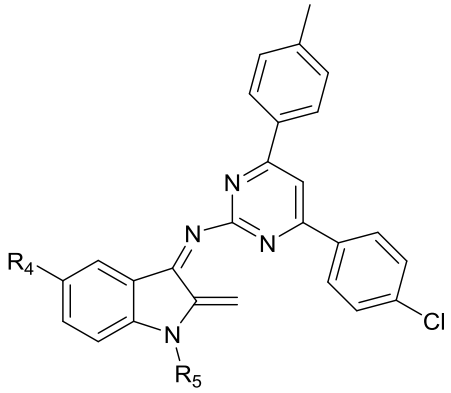
R_1 = 4-F-C_6H_4 and R_2 = Piperonyl **(37)** R_1 = 3-Cl-4-F-C_6H_4 and R_2 = Piperonyl **(38)**

Fig. 5 Examples of antibacterial Schiff bases derived from plant natural products

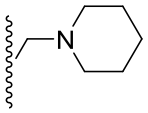


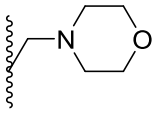
$R_3 = OCH_3$ **(39)** $R_3 = OC_2H_5$ **(40)** $R_3 = C_2H_5$ **(41)** $R_3 = Cl$ **(42)** $R_3 = Br$ **(43)**

$R_3 = I$ **(44)**



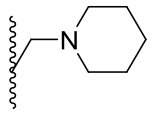
$R_4 = H$ and $R_5 = H$ **(45)**

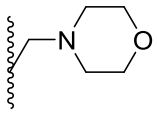
$R_4 = H$ and $R_5 =$  **(46)**

$R_4 = H$ and $R_5 =$ 

(47)

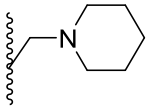
$R_4 = Cl$ and $R_5 = H$ **(48)** $R_4 = Cl$ and $R_5 = CH_2\text{-}N(CH_3)_2$ **(49)**

$R_4 = Cl$ and $R_5 =$  **(50)**

$R_4 = Cl$ and $R_5 =$  **(47)** $R_4 = Br$ and $R_5 = H$

(51)

$R_4 = Br$ and $R_5 = CH_2\text{-}N(CH_3)_2$ (52)

$R_4 = Br$ and $R_5 =$  **(53)**

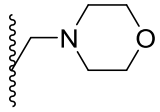
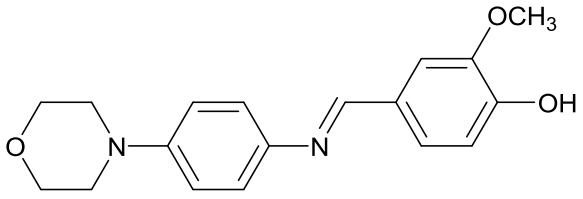
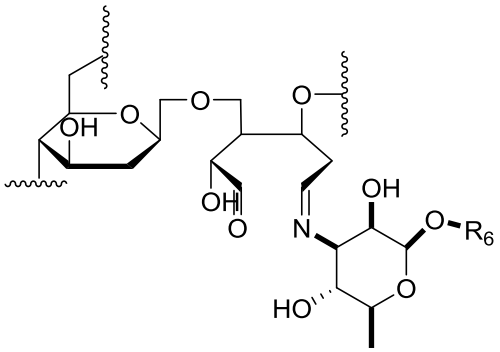
$R_4 = Br$ and $R_5 =$  **(54)**

Fig. 6 Chemical structure of some antifungal Schiff bases derived from natural or non-natural compounds

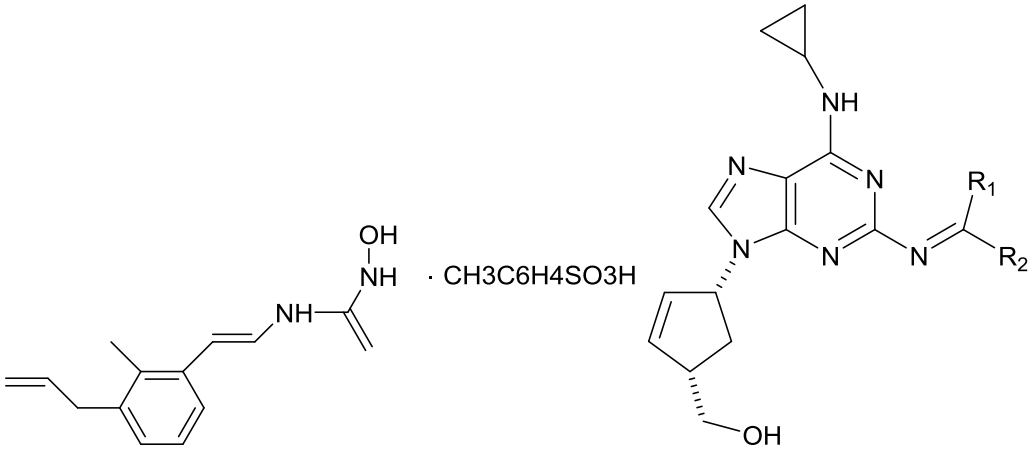


(55)



Nystatin-dextran-derived Schiff base **(56)**

Fig. 7 Chemical structure of some antifungal Schiff bases derived from natural or non-natural compounds



(57)

R_1 = H and R_2 = 2-NO_2-C_6H_4 **(58)**

R_1 = H and R_2 = 4-CH_3-C_6H_4 **(59)**

R_1 = H and R_2 = 4-$(CH_3)_2N$-C_6H_4 **(60)**

R_1 = H and R_2 = 4-NO_2-C_6H_4 **(61)**

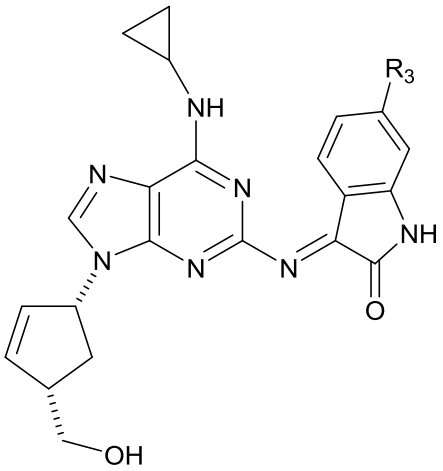
R_1 = H and R_2 = 4-OCH_3-C_6H_4**(62)**

R_1 = H and R_2 = 4-OCH_3-2-OH-C_6H_3 **(63)**

R_1 = CH_3 and R_2 = 4-OH-C_6H_4 **(64)**

R_1 = C_6H_6 and R_2 = 4-Br-C_6H_4 **(65)**

Fig. 8 Examples of antiviral synthetic Schiff bases



R_3 = H **(66)** R_3 = F **(67)** R_3 = CH_3 **(68)**

Fig. 9 Examples of antiviral synthetic Schiff bases

As for antibacterial activity, natural product-derived Schiff bases are also promising for the design of new antifungalagents. Domb and colleagues have described an interesting approach to synthesize a nystatin-dextran-derived Schiff base **(56).** This approach dramatically improved nystatin solubility in water [87]. Compound **56** completely inhibited the growth of C. albicans and C. neoformans at 20 lg/mL, while a concentration of 10 lg/mL was required for free nystatin to have a similar effect. Although the nystatin-dextran-derived Schiff base **56** was less active than nystatin itself, the former was shown to be much less toxic to normal cells [87].

Antiviral activity

The use of vaccines may lead to the eradication of viral pathogens, such as smallpox, polio, and rubella. However, virus-related and hepatitis C human immunodeficiency diseases have been the drawback of vaccine approaches [88]. Viral diseases are life-threatening for immune compromised patients and a prompt treatment is required to overcome this problem. Although there are many therapeutic options for viral infections, currently available antiviral agents are not yet fully effective, probably due to the high rate of virus mutation. They may also present any of a number of side effects.

Salicylaldehyde Schiff bases of 1-amino-3-hydroxyguanidine tosylate are a good platform for the

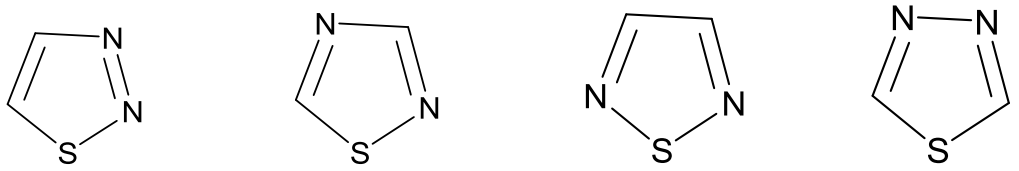
design of new antiviral agents [89, 90]. In fact, from a set of different 1-amino-3-hydroxyguanidine tosylate-derived Schiff bases, compound **57** was shown to be very effective against mouse hepatitis virus (MHV), inhibiting its growth by 50% when employed at concentrations as low as 3.2 lM [90].

Recently, Sriram and colleagues [90] reported the synthesis and antiviral activity of the abacavir-derived Schiff bases **58-68** these compounds are a new series of abacavir prodrugs. Abacavir is a nucleoside analogue capable of inhibiting the activity of reverse transcriptase. It is used to treat human immunodeficiency virus (HIV) and AIDS, and is available under the trade name Ziagen_ (GlaxoSmithKline). Compounds **58-68** were significantly effective against the human immunodeficiency virus-type 1 (HIV-1). The effective concentration (EC50) of these abacavir-derived Schiff bases necessary to achieve 50% protection of human leukemic cells (CEM) against the cytopathic effect of HIV-1 was lower than 6 l M [90]. Notably, compound **60** was the most potent Schiff base, being effective at 50 nM. This compound is only toxic to CEM cells at concentrations higher than 100 lM, indicating its potential as a lead compound for the design of new anti-HIV-1 [90].

Schiff bases have been widely explored for industrial applications. However, the biological activity of this class of compounds deserves further investigation. This becomes clear when plant pathogens are considered. Although the research on this subject is incipient, a number of reports disclosing the effects of the Schiff bases on the pathogens of clinical interest have recently been increasing. Schiff base compounds have been shown to be promising leads for the design of more efficiency

Thiadiazole

Firstly, Fischer introduced 1, 3, 4-thiadiazole in 1882, whereas Freund and Kuh45 described the true nature of the ring. In addition, thiadiazole is a widespread and important five-member heterocyclic system which contains two nitrogen atoms and a sulfur atom. 1,2,3-Thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole are the isomer of thiadiazole.



1 **2** **3** **4**

1, 2, 3-Thiadiazole 1, 2, 4-Thiadiazole 1, 2, 5-Thiadiazole 1, 3, 4-Thiadiazole

Figure 10: Structural formulae of the isomers of thiadiazole.

1, 3, 4-Thiadiazole have been the most promised isomer than the other. Due to the inductive effect of sulfur atom of 1, 3, 4-thiadiazole ring. It shows very weak base property and possesses relatively high aromaticity [91-97].

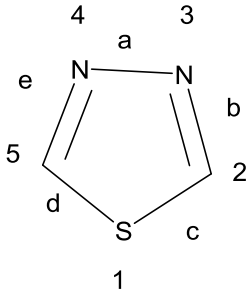
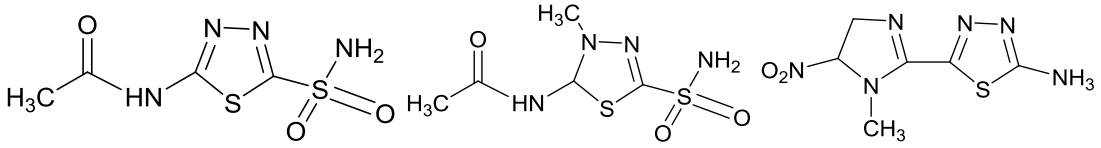


Figure 11: Numbering of the 1, 3, 4-thiadiazole ring system

In addition, the nitrogen atoms of 1, 3, 4-thiadiazole ring is also shown to be very electron underprovided due to the electron-withdrawing effect and comparatively still toward electrophilic substitution, but susceptible to nucleophilic attack. Thus, possessing the substitution into the 2′ or a 5‟ position of this ring and these substitutions involves highly activating reaction. Till date many 1, 3, 4-thiadiazole nucleus containing drugs are available in the market such as

acetazolamide, methazolamide, megazol and whereas 1,2,4-thiadiazole ring containing drug is the antibiotic cefozopran [98,99].



69 **70** **71**

Acetazolamide **Methazolamide** **Megazol**

Figure 12: Structural formula of 1, 3, 4-thiadiazole containing marketed drugs.

Among their isomers, particularly the 1, 3, 4-thiadiazoles possessed a broad spectrum of biological activities including antimicrobial [100] antituberculosis [101] antioxidant [102] anti-inflammatory [103] anticonvulsants [104] antidepressant and anxiolytic [105] antihypertensive [106] anticancer [107] and antifungal activity [108].

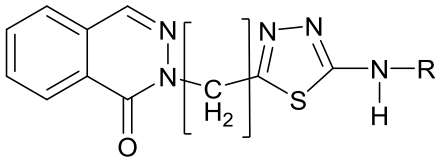
The 1, 3, 4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents. Thiadiazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, and antitubercular agents. The broad and potent activity of thiadiazole and their derivatives has established them as pharmacologically significant scaffolds.

1, 3, 4-Thiadazoles have become an important class of heterocycles and a great interest of researches because of their broad types of biological activity. Thiadiazole is a 5- membered ring system containing hydrogen-binding domain, sulfur atom, and two-electron donor nitrogen system that exhibit a wide variety of biological activity. They occur in four isomeric forms in the nature viz. 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole; and 1,3,4-thiadiazole.

Many drugs containing 1, 3, 4-thiadiazole nucleus such as acetazolamide 1, methazolamide 2, megazol 3 are available in the market, although the only commercial 1, 2, 4-thiadiazole drug is the antibiotic cefozopram [109,110].

Antibacterial and Antifungal activity

1, 3, 4-Thiadiazole has shown a broad spectrum of activity against various pathogens, and extensive research has been performed on the synthesis of new potent antibacterial and antifungal agents. A new series of 2-[[1(2H)-phthalazinone- 2-yl] methyl/ethyl]-5-arylamino-1,3,4-thiadiazole derivatives (**72**) have been evaluated in vitro antimicrobial activity against bacteria and fungal species. The results showed that the tested compounds possessed weak antibacterial and antifungal activity compared with standard drugs chloramphenicol and rifampicin [111]. A number of 5-substituted 2-(2, 4-dihydroxyphenyl)-1, 3, 4-thiadiazole derivatives (**73**) have been evaluated for antifungal activity against several clinical isolates of Candida albicans. The compounds with methyl, phenyl, 4-ethoxyphenyl, and halogenophenyl groups at C-2 of thiadiazole ring showed higher antifungal activity [111].



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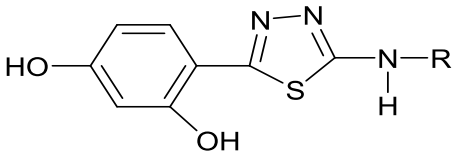
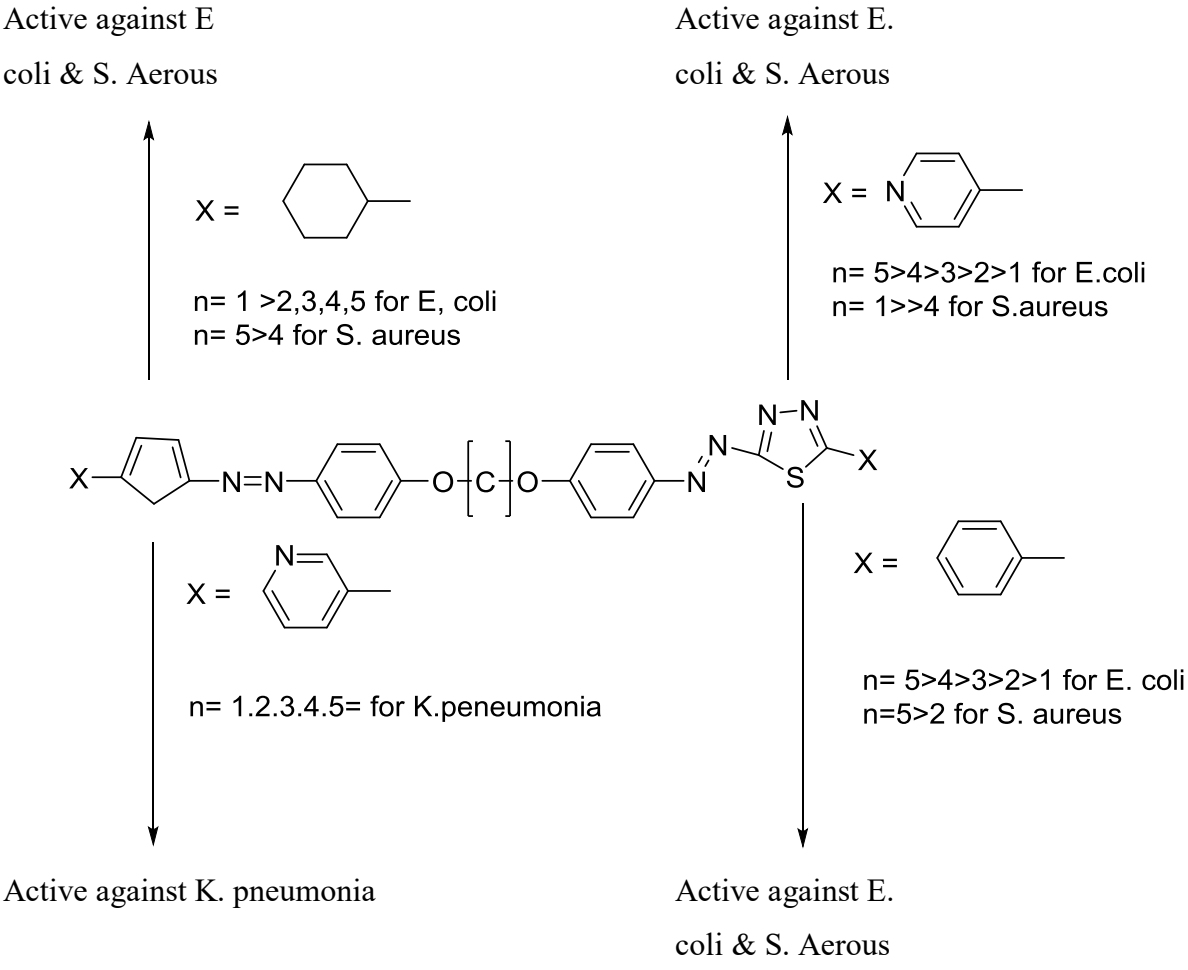


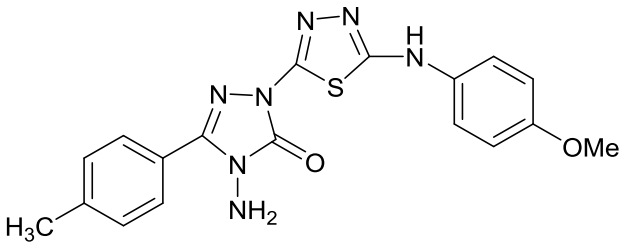
73

Tomi *et al*. [112] reported azo derivatives of aminothiadiazole derived from nicotinic and isonicotinic acid by cyclization, diazotization, and etherification, and evaluated them for in vitro antimicrobial activity against several microbes like: E. coli, K. pneumonia, P. aeruginosa and S. aureus. All the synthesized compounds showed good activity against E. coli. Compounds having 3-pyridyl group C-2 of thiadiazole were found to exhibit good activity against K. pneumonia (**Scheme 1**)



Scheme 1

Demirbas *et al.* [113] synthesized four different derivatives of 4-Amino-2-[(5-arylamino-4,5-dihydro-1,3,4-thiadiazol-2-yl) methyl]-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-ones and investigated its antimicrobial activity. Thiadiazole (**74**) with 2-({5-[(4-methoxyphenyl) amino] group was found to possess highest antibacterial activity, whereas N-alkylation at C-5 of thiadiazole ring did not resulted in improved antibacterial activity. An attempt to prepare active compounds in the series of thiadiazolyl derivatives of antipyrine turned up unsuccessful. All the synthesized derivatives bared weak growth inhibitory activity against the tested Gram-positive bacteria (MIC 100 lg/mL) [114].



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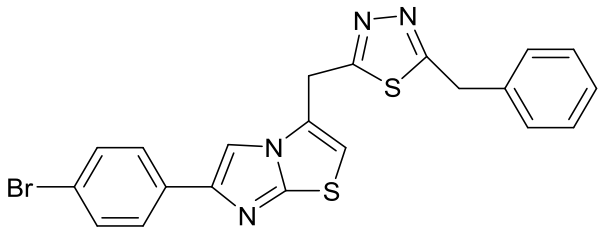
1,3,4-Thiadiazole and 2-azetidinones derivatives of 2-methyl-1H-benzimidazoles were tested for antibacterial, antifungal activity and some of the tested compounds had comparable activity against B. subtilis and E. coli with reference to amphicillin (25 lg/mL). Compounds having *o*-chloro, *o*-methyl, *p*-methoxy, *o*-hydroxy, and *p*-amino group in phenyl ring showed good antibacterial activity. Antifungal activity data indicated that some of the derivatives revealed a broad spectrum of activity against tested fungi; however, none of the derivatives showed a better spectrum of activity when compared to the reference drug [115].

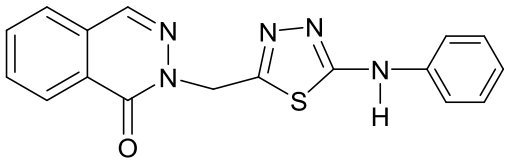
A recently published article reported the antibacterial and antifungal activity of 1, 3, 4 thiadiazoles bearing imidazo [2,1-b]thiazole moiety [**75**] against S. aureus, P. aeruginosa, E. coli, and T. tonsurans with MIC of 64, 32, and 8lg/mL, respectively [116]. Applying QSAR study, it has been observed that positions-2 or position-3 of benzene attached with thiadiazole ring where as electron-donating and bulky group would be favorable for higher antifungal activity. On the basis of CoMFA findings, Liu et al. [117] designed a compound which was found to display a good antifungal activity (79.38%).



75

2-(*N*-acetyl-*N*-*m*-trifluoromethylphenylamino)-5-(3-acetyloxy-2-naphthyl)-1, 3, 4-thiadiazole was found to possess good activity against P. aeruginosa and equally active, comparable with the standard drug penicillin [39]. A novel series of 2, 5-disubstituted-1,3,4-thiadiazoles derivatives

[**76**] have been synthesized and showed good inhibition against both Gram-(+) and Gram-(-) bacterial strains at 6.25 lg/mL concentration.The hydroxyalkenyl chain at 5th position and internal double bond in the long alkenyl substituent of synthesized thiadiazoles [**77**] were found to be more active against S. pyogenes, S. aureus, P. aeruginosa, K. pneumoniae, and E. coli [118].



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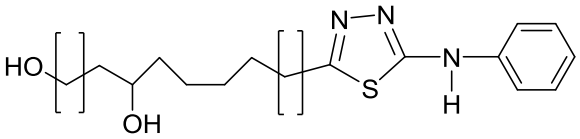

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Antibacterial activity is strongly dependent on the nature of the substituents at 5-arylamino-1,3,4-thiadiazoles in a series of 2-[[1(2H)-phthalazinone-2-yl]methyl/ethyl]-5-arylamino-1,3,4-thiadiazole derivatives. Unsubstituted compound showed 50% inhibition against B. subtilis with respect to ampicillin [119]. Compound having chloro group at para-position of

Arylsulfonylmethane moiety attached with thiadiazole ring exhibited higher antimicrobial activity [120].The antifungal activity of 1, 2, 4-triazolylmercaptomethyl- 1,3,4-thiadiazoles has been tested against M. gypseum (NCPF-580), M. canis, T. mentagrophytes, T. rubrum, and C. albicans. Of the five synthesized compounds screened against fungal strains, four compounds showed measurable activity against T. mentagrophytes [121].

Ranjina et al. [122] synthesized a number of aminothiadiazole derivatives containing 4-pyridyl and oxothiazolidin moieties in the same molecules. All the compounds had good antimicrobial activity but the compounds having a nitro group were present at the *-m* and *-p* position of the aryl ring, respectively, possessed stronger antibacterial activity than others.

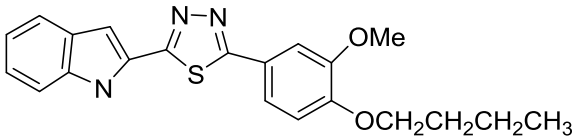
Camoutsis *et al*. [123] synthesized a series of N-{5-[2- (N-substituted sulfamoyl)-4, 5-dimethoxy benzyl]-1, 3, 4-thiadiazole- 2-yl}-N-arylamines. All the newly sulfonamide- 1, 2, 4-thiadiazoles were assayed in vitro for their growth inhibitory activity against panel of selected Gram–positive bacteria, Gram-negative bacteria, and fungi and compared with reference drug ampicillin, streptomycin, bifonazole, and ketoconazole, respectively. The results of antimicrobial screening clearly indicated that the nature of substituents and their position on 1, 3, 4-thiadiazole nucleus affected the in vitro activity. SAR of the compounds revealed that pyrrolidine substituted

compounds were found to be more potent than piperidine-, methylpiperazine-, and dimethylamino- containing compounds. Thus, it can be said that introduction of -CF3 moiety and chloro atom in the para position of the benzene ring of pyrrolidine substituted compound is an essential part for improving antibacterial activity.

Anticancer activity

Kumar *et al*. [124] reported the synthesis of 5-(3-indolyl) - 1, 3, 4-thiadiazoles and evaluated for anticancer activity. Primary screening was performed at a concentration ranging from 100 nM to 1 mM. Change in cell number and cell morphology in 96-well plates was observed at 24 and 48 h had been detected. Compounds that exhibited toxicity to cancer cell lines but not to normal cells were selected for the secondary confirmation assays. For secondary screening, the same concentration which was previously used in the primary screening was used and compounds were screened in triplicate. As a result, eight compounds were identified as potent agents for inducing cytoselective toxicity. It was found that substitution on C-2 position of the 1, 3, 4-thiadiazole ring plays an important role in imparting the cytotoxic activity to the compound.

Replacement of phenyl ring at C-2 position with benzyl, 4-(dimethylamino) phenyl, 3, 4 dimethoxyphenyl and 4-benzyloxy group enhanced the antiproliferative activity, while replacement of the phenyl group with p-chlorophenyl and introduction of third methoxy group reduced the biological activity. Compound 2-(4-(Benzyloxy)-5-(5-bromo-3-indolyl) - 3-methoxyphenyl)-1, 3, 4-thiadiazole (**78**) with 4-benzyloxy- 3-methoxyphenyl at C-2 position and 5-bromoindole at C-5 position was found to be the most potent compound of the series. Compound (4-hydroxyphenyl)[5-(2,6-dichloro)- 2-thioxo-1,3,4-thiadiazol-3-yl]methanone showed broad spectrum of growth inhibition activity against human tumor cells and remarkable cytotoxic activity on nonsmall lung cancer (HOP 92) having log GI50 value at -6.49, colon cancer (HCC-2998) at GI50 value _5.31 and significant cytotoxic activity on prostate cancer (PC-3) having GI50 value _5.48. SAR study revealed that electron withdrawing group at position C-5 of thiadiazol was favorable for activity [125].



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New derivatives of 2-arylamino-5-aryl-1, 3, 4-thiadiazoles were synthesized by refluxing aryl aldehydes, hydrazine hydrate, and aryl isothiocyanates in methanol followed by oxidative cyclization with ferric ammonium sulfate. Study of in vitro cytotoxic activity revealed a cytotoxic effect of individual compounds on cancer cells of prostate (PC3, DU145, and LnCaP), breast (MCF7 and MDA-MB-231), and pancrease (PaCa2). The SAR study showed that the 3, 4, 5-(OCH3)3C6H2 at C-5 position was responsible for binding to the Colchicine siteon tubulin and found to be favorable for activity. Further variation of C-2 arylamino group was associated with lesser degree of effect on the activity of 1, 3, 4-thiadiazoles. Most of the synthesized compounds were moderate in activity and compound displayed a greater potency toward pancreatic (PaCa2) cancer cell lines (IC50 = 4.3 lM) [126]. Marganakop et al. [127] synthesized quinolines derivatized with 1, 3, 4-thiadiazole via cyclization of quinoline thiosemicarbazones in a single step and investigated for their primary cytotoxic activity against cervical cancer cell lines (Hela). Compounds with methoxy at C- 6, 7, 8 of quinoline showed the potent anticancer activity and the cell lyses occurred only at10 lg/mL. Zheng *et al*. [128] prepared several N1-acetylamino- (5-alkyl/ aryl-1, 3, 4-thiadiazole-2-yl)-5-fluorouracil derivatives. These compounds were evaluated for their anticancer activity onA-549 (human lung cancer cell), Bcap-37 (human breast cancer cell) by MTT assay. Compound with electron with-drawing group attached to benzene ring was found to have activity against tested cell lines and possessed more potent antitumor inhibitory activity than 5-fluorouracil. Compound (E, E)-2,5-bis[4-(3-dimethyl-aminopropoxy)styryl]- 1,3,4-thiadiazole was found to be the most potent one by the MTT assay against A549, PC-3, and HA22T [129]. A number of N-substituted 2-amino-5-(2, 4 dihydroxyphenyl)- 1,3,4-thiadiazole derivatives were investigated as antiproliferative agent, their in vitro cytotoxicity against the four human cell lines: SW707 (rectal), HCV29T (bladder), A549 (lung), and T47D (breast) suggested their potential as novel anticancer agents. Compound 2-(2, 4- dichlorophenylamino)- 5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole , with ID50 two times lower (SW707, T47D) than that of cisplatin displayed the highest cytotoxicty. It was noticed that the compounds with electron donating groups at C-terminal of the phenyl ring did not increased its cytoselective toxicity and the compounds with electron withdrawing groups (Cl, F) resulted in an increased activity by inducing cell death [130]. Compound 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole inhibited proliferation of tumor cells derived from cancers of nervous system (medulloblastoma/rhabdosarcoma, neuroblastoma, and glioma) and peripheral

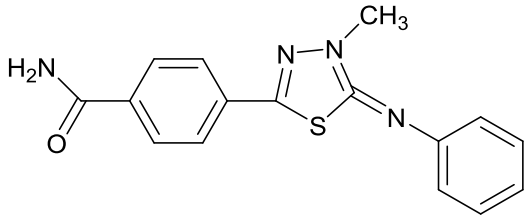
cancers including colon adenocarcinoma and lung carcinoma [131]. Matysiak et al. [132] examined the effect of various substitution at 5-position of 2-(2, 4 dihydroxy-phenyl)-1,3,4-thiadiazoles on antiproliferative activity against different human tumor cell lines. 2-(2,4-Dihydroxyphenyl)-5-(4- methoxybenzyloxy)-1,3,4-thiadiazole showed ID50 of 1.1 lg/mL against HCV29T bladder cancer cell line and found to be significantly lower (T47D) than that of cisplatin, used as the reference compound. In a series of chiral 2, 5- disubstituted 1, 3, 4-thiadiazoles possessing c-butenolide moiety, compound 50 was screened against Hela cell lines by MTT assay and exhibited IC50 of 0.9 lM [133].

Focal adhesion kinase (FAK) is a 125 kDa protein that was involved in multiple cellular functions like cell proliferation, survival, motility, invasion, metastasis, and angiogenesis. The inhibition of FAK plays an important role in cancer therapy through decreased cellular viability, growth inhibition, or apoptosis. Recently, FAK was proposed to be a new potential therapeutic target in cancer. Sun *et al*. [134] had designed a series of novel 1, 3, 4-thiadiazole derivatives containing 1,4-benzodioxan and evaluated their activity as FAK inhibitors. The results of the inhibitory activity of the designed compounds showed that compound 51 possessed high potency against FAK (EC50 = 10.79 lM). EC 50 values of 14.21–32.45l g/mL against HEPG2, HELA, SW1116 and BGC823 cell lines. The SAR study suggested that substitution with different acids led to different antitumor activity, and the potency order was phenylpropinic acid >phenylacetic acid >benzoic acid. Compounds with substituted Cl group on benzene ring showed better antitumor activity than substitution with Br group. Replacement of -Cl with –CH3 group, however, led to decrease in cytotoxic activity against all cell lines [135].

Thiadiazoles as miscellaneous agents

Vergne *et al*. [136] discussed the synthesis and SAR studies of a series of novel small thiadiazoles as inhibitors of PDE7. Out of the synthesized compounds, derivatives with 4-CONH2 on benzene ring (**79**), 4-aminoquinazoline and 2-methyl-4-aminoquinazoline on C-5 of thiadiazole ring exhibited high PDE4 inhibitory activity with an IC50 value of 0.061, 0.027, and 0.0039 lM, respectively. From SAR studies, they concluded that the 4-aminoquinazoline derivatives along with hydrophobic steric bulk attached with nitrogen of C-2 of thiadiazole showed an increase in activity because of its structural similarity with the adenine part of cAMP.

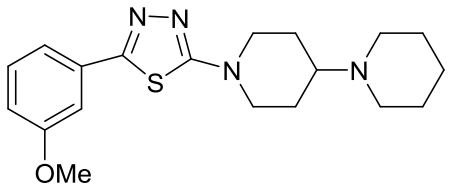
Replacement of the cyclohexyl moiety with smaller ring was not as selective and found to be detrimental to the enzymatic activity.



79

Introduction of an OH group on 3rd position of cyclohexyl group of represented IC50 of 0.088 nM toward PDE7. Modification of 4-CONH2 group with sulfonamide significantly improved the pharmacokinetic profile and binding affinity for PDE7 [137].

De *et al*. [138] reported novel small molecules thiadiazole derivatives as c-Jun N-terminal kinase inhibitors. On the basis of a lead structure from high throughput screening, they identified that substitution on 2nd-position with either 2-methoxyethyl group, sec-butyl group or n-propyl group improved the pepJIP1 displacement (DELFIA) and the kinase activity (LANTHA) assays. Out of the synthesized compounds, an IC50 of 4.8 lM in the kinase assay substrate and it displaced pepJIP1 with an IC50 of 158 nM. Modification on 4-(2,3 dihydrobenzo[b][1,4]dioxin-6-yl)-5-(5-nitrothiazol-2-ylthio)-4H-1,2,4-triazol-3-ol which showed competitive inhibition of the interactions between JNK and pepJIP1 with an IC50 of 280 nM resulted the discovery of which could bind at the JIP site with the nitrothiazol group crossing the ridge close to residues Arg127 and Cys163 of enzyme side with an IC50 of 239 nM [139]. Xiao *et al*. [140] discovered the 2-piperidinopiperidine-5-arylthiadiazoles as H3 antagonists which lead to increase histamine levels by blocking the histaminergic neurons irreversibly and may be useful in treating obesity, diabetes as well as other CNS disorders such as cognitive disorders like Alzheimer‟s and Parkinson‟s disease. SAR investigations revealed that o, m and p substituent such as polar groups OMe, CN, and COCH3 on phenyl ring increases the H3 receptor antagonistic activity. Further replacement of phenyl ring with 2-pyridyl was found to be favorable, while pyrimidine and pyrazole offered less activity. Compound (**80**) with 3-methoxy group at 2-pyridyl ring substituted on C-5 of thiadiazole was found to be the most active.



80

Thiadiazole contains the five-membered diunsaturated ring structure having molecular structure formula C2H3N3S containing a two carbon atom, three hydrogen, three nitrogen and one sulphur. Thiadiazole and its derivatives are used for biological activities such as antiviral, antibacterial, antifungal and antitubercular. It is a clear to yellowish liquid with a pyridine like odor. It is soluble in alcohol and ether and slightly soluble in water. It is parent material for numerous of chemical compounds including sulfur drugs, biocides, fungicides, dyes, chemical reaction accelerators. Thiadiazoles carrying mercapto, hydroxyl and amino substituent‟s can exist in many tautomeric forms and this property is being intensively studied using modern instrumental methods. Two azo-derivatives have been prepared containing the 1, 3, 4-thiadiazole system starting from the heterocyclic 2-amino-5-mercapto-1, 3, 4- thiadiazole system. These two azo compounds form complexes in a powder form with aluminum salts. The synthesis of the azo-derivatives gave an average yield of 50%. Heterocyclic with the molecular formula C2H3S3N2 present various isomers, among which may be cited bismuthiol, xanthan and perthiocyanic acid, which have many industrial applications and surprising chemical properties among which are included their capacity of forming complexes with metals. One derivative which merits special mention is 2-amino-5- mercapto-1, 3, 4-thiodiazole due to its properties in forming well-known azo dyes. These compounds will be used to evaluate their potential for coating metal surfaces in processes of corrosion inhibition. Thiadiazole and its derivatives are used for biological activities such as antiviral, antibacterial, antifungal and antitubercular 10. The antileukemic action and host toxicity of the thiadiazoles were blocked by administration of nicotinamide. 1, 3, 4 - Thiadiazole are diversified biocidal activities probably by virtue of a toxophoric – N=C-S- Grouping. A large number of 4-thiazolidinones have been reported to be antifungal, antibacterial and antileukemic properties. These observations prompted us to synthesis the title compound with a presumption that incorporation thiadizole and thiazolidinones wound produce new compound with significant fungicidal properties. A series of 2-aryl-5-hydrizino-1, 3, 4-thiadiazole exemplified by the structure designed as analogue of the known vasodilator

hydrazine and pyridazinyl hydrazine. Subsequent evaluation of this series showed that some analogue possessed both antihypertensive activity and anticonvulsant activity. Furthermore it found that particular substitution in the 2-position of aromatic ring to produced compound reduced with antihypertensive activity with desirable anticonvulsant activity. It was found that methylation of the α-nitrogen of the hydrazine group in the *o*-tolyl series decrease vasodilator activity without concurrent decrease in anticonvulsant activity Thus a combination of the preferred aromatic substituted units in the 2-position coupled with various alkyl and aryl substitution on the hydrazine moiety was a objective in this work. 1,3,4-thiadiazole confirmed on the corresponding thiadiazole hydrazine the best combination of potency and lack of toxicity. 1,3,4- thiadiazole was started first synthesized by Goerdereler, ohm, and Tegtmeyer who used a four stage process that started from thiosemicarbazide. Jensen and Pedersen obtained the heterocycle from hydrazine and potassium dithiformate.The synthesis which quite large number of thiadiazole can be obtained starts from N,N-dimethylormamide azine dihydrochloide which is found to good yield from dimethylfrmamidoyl and N,N-dimethyhydazine and hydrazine hydrochloride methyl azine. Sodium ethoxide converted into compound into the free dimethylazine this react with H2S in methanol in the presence of sodium methoxide, the hydrochloride give products.

Further developments

Novel substituted 1, 3, 4-thiadiazoles were synthesized under both sonication and classical conditions. Generally, improvements in rates and yield of reactions were observed when reactions were carried out under sonication compared with classical condition.

Diabetes is a group of diseases characterized by high blood glucose level. When a person has diabetes, the body either does not produce enough insulin or is unable to use its own insulin effectively. Glucose builds up in the blood and causes a condition that, if not controlled, Can lead to serious health complications and even death. The risk of death for a person with diabetes is twice the risk of a person of similar age who does not have diabetes. Diabetes mellitus is a heterogeneous clinical disorder with numerous causes. Two main classifications of diabetes mellitus exist, idiopathic and secondary. Idiopathic diabetes is divided into two main types: Insulin dependent and Non-insulin dependent. Non insulin dependent diabetes mellitus (NIDDM

or type 2 diabetes) is characterized by milder hyperglycemia and rarely leads to ketoacidosis. It leads to nephropathy, neuropathy, and retinopathy. Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century. The incidence of the disease currently is estimated to reach 210 million by the year 2010 and 300 million by the year 2025 [141]. Most cases will be of type 2 diabetes, which is strongly associated with a sedentary life style and obesity. Nowadays diabetes is becoming a leading cause of death in most nations. 1,3,4-thiadiazole and its derivatives possesses wide range of therapeutic activities like activities like antimicrobial [142], antifungal, diuretics, antiulcer [143], antimycobacterial [144], antioxidant/radio-protective [145], anti inflammatory, anticonvulsant, antidepressant, anticancer, anti-leshmanial [146-147], carbonic anhydrase inhibitors [148] and antidiabetic [149]. Due to presently known limitations of liver toxicity in the use of thiazolidinediones, it is necessary to find new heterocyclic compounds with equivalent potency as that of thiazolidinedione. Thiadiazole compounds are known to bind peroxisome proliferator activating receptor (PPAR) effectively [50]. Therefore present study involves design of thiadiazole compounds on the basis of docking studies on peroxisome proliferator activating receptor (PPAR) effectively and to find important binding residues. The knowledge of binding residues would help in design of novelthiadiazole molecules as antidiabetic agents.

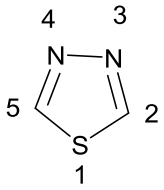
Designing of new molecules was done with help of docking. Virtual screening has emerged as a reliable, cost-effective and time-saving technique for the discovery of lead compounds. The virtual screening approach for docking small molecules into a known protein structure is a powerful tool for drug design that has become an integral part of the drug discovery process in recent years. Theoretically the application of virtual screening is constrained only by the chemical compounds features that can be calculated and the relation between these features and the targeted protein can be established. Practical implementation of virtual screening application requires some considerations. If the site geometry is not known, as is often the case, the design should be based on other ligand molecules that bind well to the site. If the molecules are rigid, the problem becomes one of identifying the substructures or active groups that contribute to the fit. If the site geometry is known, molecules with good affinity for the site can be built by joining the groups with alternative molecular scaffolding. In present study, thiadiazole scaffold was considered as replacement to thiazolidinedione while considering its expected interaction with

PPARgamma receptor as per the previous literature reports [151]. Thiazolidinediones belongs to a chemical class that has a different pharmacological action than the sulfonylureas, biguanides, or the (alpha)-glucosidase inhibitors. Synthesized thiadiazole compounds were tested for alpha-glucosidase inhibition to verify whether these compounds work by mechanism similar to thiazolidinediones or else. In vivo study was carried out on alloxan induced diabetes rat model for all the synthesized molecules using glibenclamide, a second generation sulphonylurea as standard since it was used as a reference standard compound in the study of reference analogous compounds [152,153]. Molecular docking studies were performed by using Glide v5.6 (Schrodinger, LLC) [154]. The X-ray crystal of peroxisome proliferator-activated receptor gamma (PPARgamma) in complex with rosiglitazone (PDB id. 1FM6) was obtained from the RCSB protein Data Bank (PDB) and utilized in order to get the detailed insights of ligand-protein structure in this study.

Protein structure was added with hydrogens and then only hydrogens were energetically optimized. In case of docking poses showing high score, some manual adjustment were done to remove large steric hindrance and final complex structures were subjected to energy minimization using OPLS force field in Schrodinger software. During the energy minimization procedure firstly whole protein structure was fixed and secondly that amino acid residues within 3.5 A from the each ligand were relaxed. Rosiglitazone was removed from the complex and the reported thiadiazole compounds were docked in PPARgamma protein and their binding interaction was validated with previously reported binding residues. These binding residues were used as reference guide. Several thiadiazole compounds have been reported for their antidiabetic effect by acting as agonist at PPAR gamma [155,156]. Docking was performed selectively on reported thiadiazole analogues for which antidiabetic activity is known to be potent and comparable to standard.

A major review which covers the synthetic chemistry of the ring system up to 2002 has also appeared [157]. Since 1991 advances in the chemistry of 1, 3, 4-thiadiazole has been annually reviewed in Progress in Heterocyclic Chemistry [158]. The numbering of the 1, 3, 4-thiadiazole ring is given below. The present chapter is intended to update the previous work on the aromatic 1, 3, 4-thiadiazole (**81**), the nonaromatic 2-thiadiazolines (**82**), 3-thiadiazolines (**83**), the

thiadiazolidines (**84**), the tautomeric forms (**85**) and (**86**), and the mesoionic systems (**87**). Reference is made to earlier chapters of CHEC (1984) and CHEC-II (1996) where appropriate.



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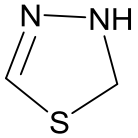

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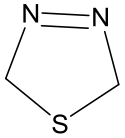

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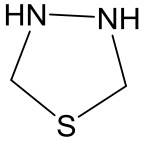

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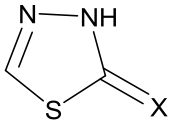

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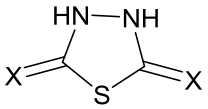

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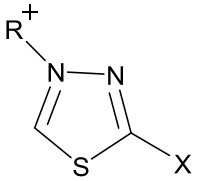

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Chapter 2
Experimental

2.0 a) Purification of solvents and chemicals

All the commercial grade solvents such as n-hexane, ethyl acetate, diethyl ether, acetone, ethanol, and methanol were purified by distillation. All the liquid samples such as benzaldehyde, *p*-hydroxy benzaldehyde, anisaldehyde, p-choloro benzaldehyde were also purified by distillation. The solid samples were purified by recrystallizing in different solvents.

b) Determination of melting points

Melting points of different synthesized compounds were determined on Gallenkamp melting point apparatus are uncorrected.

c) Infra-red (IR) spectra

The infra-red spectra were recorded on KBr pellet for films with a Shimadzu FTIR spectrophotometer from the department of chemistry, BUET, Dhaka, Bangladesh.

d) Nuclear Magnetic Resonance (NMR) spectra

The ^{1}H NMR (400MHz) and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO) and (CDCl3) with a Bruker BPX- 400 spectrophotometer using tetramethylsilane (TMS) as internal standard at the Wazed Miah Science Research Center - Jahangirnagar University, Saver, Dhaka, Bangladesh.

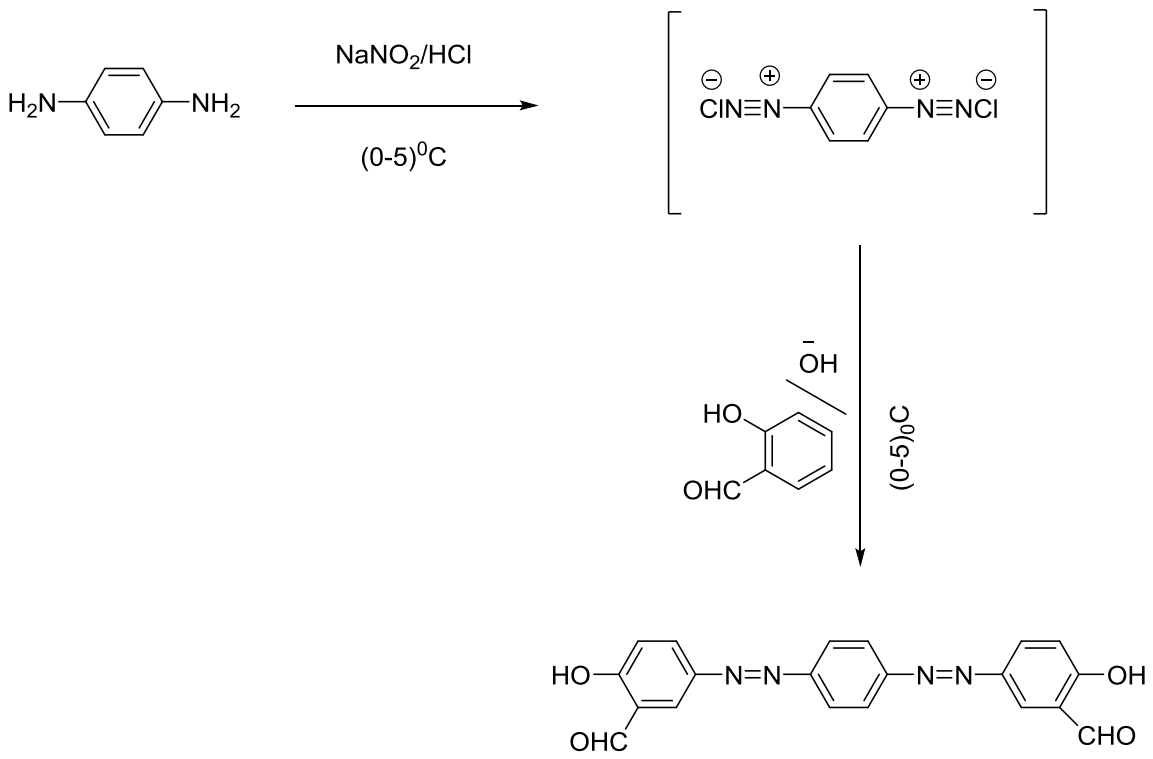
e) Drying

All the synthesized compounds were dried over anhydrous silica gel under vacuum desiccator after and before recrystallization.

f) Evaporation

The solvents were evaporated under reduced pressure in Buchi rotatory evaporator (West Germany) with a bath temperature below 40 ^{0} C.

2.1 Synthesis of 5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde)



1

P-Phenylidendiamine (0.01 mol, 1.08g) was dissolved in 20 ml (1:1) aqueous solution of concentrated hydrochloric acid in a small beaker. The mixture was allowed to cool at 0^0 C in an ice bath. Then a cold saturated aqueous solution of sodium nitrite (0.04 mol, 2.76 g) was added drop wise to the mixture at the same temperature. A solution of 2-hydroxy benzaldehyde (2.44 g, 0.02 mol) in 10% sodium hydroxide (20 ml) was prepared and cold separately to 0^0 C and stirred for 20 minutes at the same temperature. Then the diazonium salt solution was added very slowly to the aldehydic solution and was allowed of stand in an ice bath for at least one hour with occasional stirring. The reaction mixture was then filtered, washed with distilled water and dried. A reddish semi solid (1.50g) was collected.

IR (KBr, cm^{-1}, Fig 1): 3450-3350 (O-H, Phenolic), 3054 (C-H, aromatic), 2980 (C-H, -CHO), 1625 (C=O of H- bonded aldehyde group - CHO), 1573, 1490,1460 (C=C, aromatic),1520 (-N=N-, weak absorption), 1405(O-H in plane), 750 (O-H, out of plane).

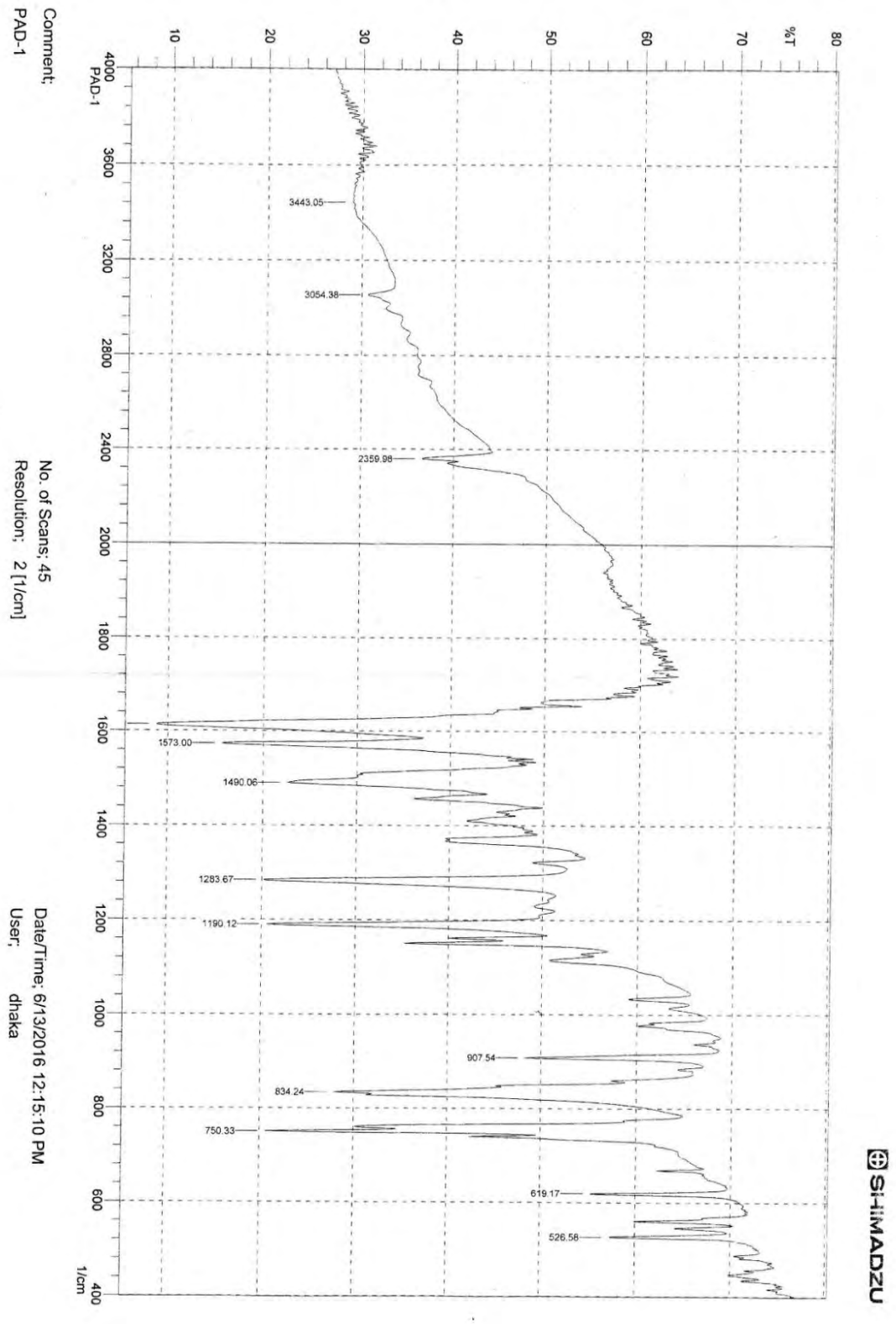
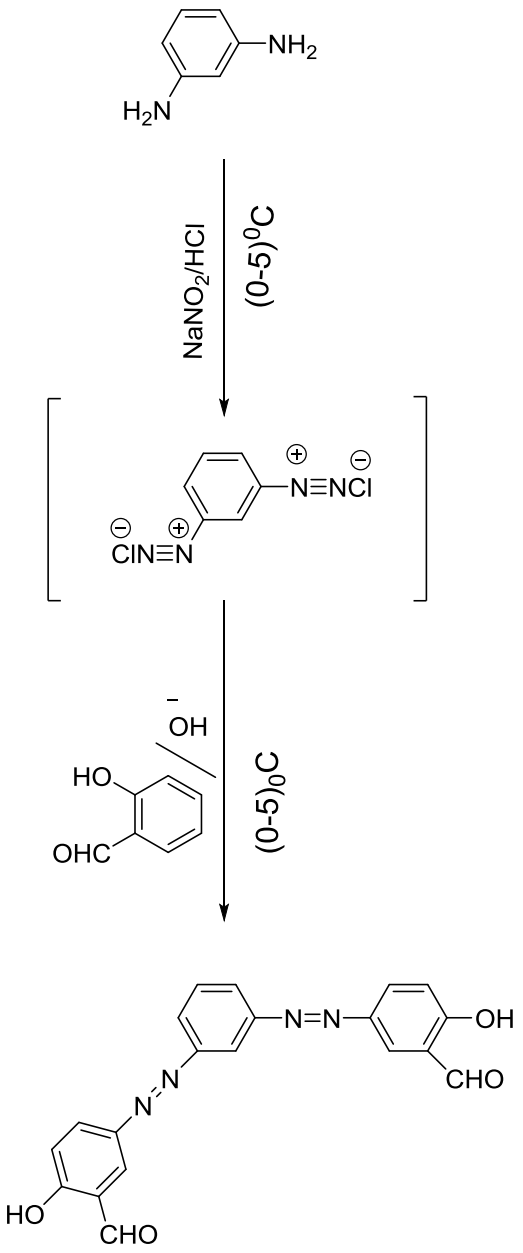


Fig - 1: IR Spectrum of Compound 1

2.2. Synthesis of 5, 5'-(1, 3- Phenylenebis (diazene-2, 1-diyl)) bis (2-hydroxybenzaldehyde)



2

m- Phenylidendiamine (0.01 mol, 1.08 g) was dissolved in 20 ml (1:1) aqueous of concentrated hydrochloric acid in a small beaker. The mixture was allowed to cool at 0^0 C in an ice bath, then a cold saturated aqueous solution of sodium nitrite (0.04 mol, 2.76 g) was added drop wises to the mixture at the same temperature. A solution of 2-hydroxy benzaldehyde (2.44 g, 0.02 mol) in 10% sodium hydroxide (20 ml) was prepared and cold separately to 0^0 C and stirred for 20

minute at the same temperature. Then the diazonium salt solution was added very slowly to the aldehydic solution and was allowed of stand in an ice bath for at least one hour with occasional stirring. The reaction mixture was then filtered, washed with distilled water and dried. A reddish semisolid (1.50g) was collected.

IR (KBr, cm^{-1}, Fig 2): 3400-3305 (O-H, Phenolic), 3192, 3063(C-H, aromatic), 2854 (C-H, aldehyde group -CHO), 1665 (C=O of H- bonded aldehyde group - CHO), 1606, 1578, 1474 (C=C, aromatic), 1381(O-H in plane), 842 (O-H, out of plane).

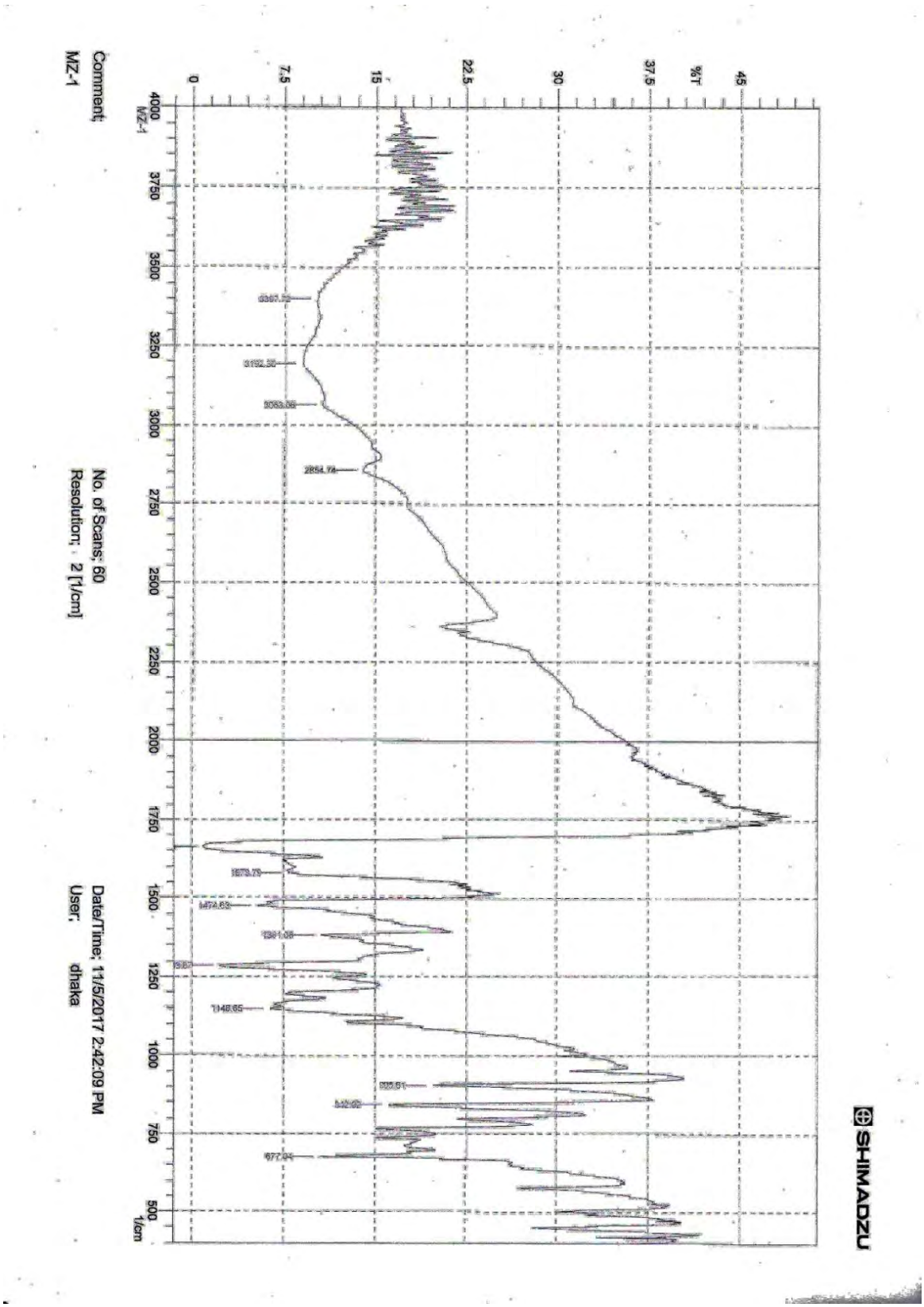
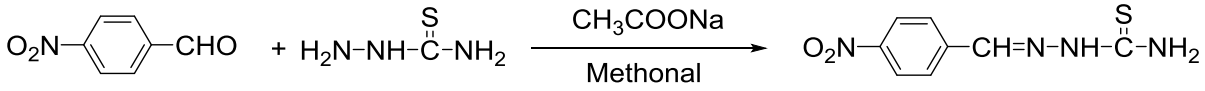


Fig - 1: IR Spectrum of Compound 2

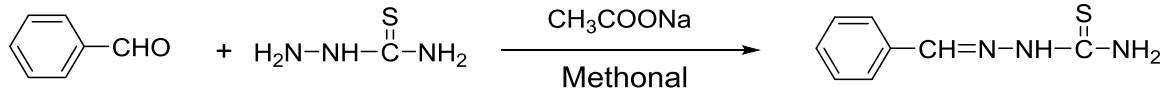
2.3. Synthesis of 2-(4-nitrobenzylidene) hydrazine-1-carbothioamide



3 **4** **5**

Aqueous solution of thiosemicarbazide (0.02 mol, 1.82 g), crystalline sodium acetate (0.04 mol, 3.28 g) and nitro- benzaldehyde (0.02 mol, 3.02 g) were taken in round bottom flask. To this mixture, the solvent methanol was added until clear the turbidity of the mixture. The reaction mixture was then mildly warmed and stirred for three hours. The reaction mixture was then poured into crushed ice. The solid precipitate was filtered, washed and dried. The yellow solid product was then recystallized with ethanol, found 78% yield and the melting point was recorded as 221- 223^0 C.

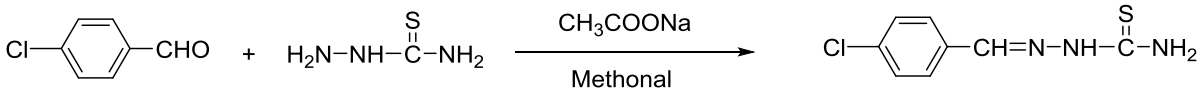
2.4. Synthesis of 2-benzylidenehydrazine-1-carbothioamide



6 **7**

Aqueous solution of thiosemicarbazide (0.02 mol, 1.82g), crystalline sodium acetate (0.04 mol, 3.28g) and benzaldehyde (0.02 mol, 2.12g) were taken in round bottom flask. To this mixture, the solvent methanol was added until clear the turbidity of the mixture. The reaction mixture was then mildly warmed and stirred for three hours. The reaction mixture was then poured into crushed ice. The solid precipitate was filtered, washed and dried. The white solid product was then recystallized with ethanol, found 85% yield and the melting point was recorded as 154 - 156^0 C.

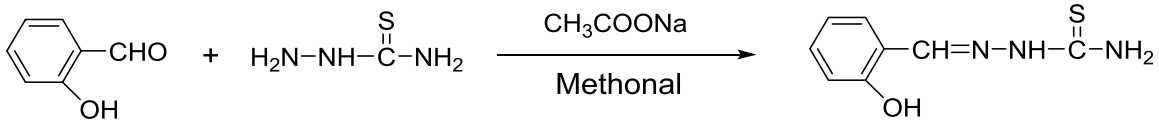
2.5. Synthesis of 2-(4-chlorobenzylidene) hydrazine-1-carbothioamide



8 **9**

Aqueous solution of thiosemicarbazide (0.02 mol, 1.82g), crystalline sodium acetate (0.04 mol, 3.28g) and chloro- benzaldehyde (0.02 mol, 2.82 g) were taken in round bottom flask. To this mixture, the solvent methanol was added until clear the turbidity of the mixture. The reaction mixture was then mildly warmed and stirred for three hours. The reaction mixture was then poured into crushed ice. The solid precipitate was filtered, washed and dried. The white solid product was then recystallized with ethanol, found 67% yield and the melting point was recorded as 202- 204^0 C.

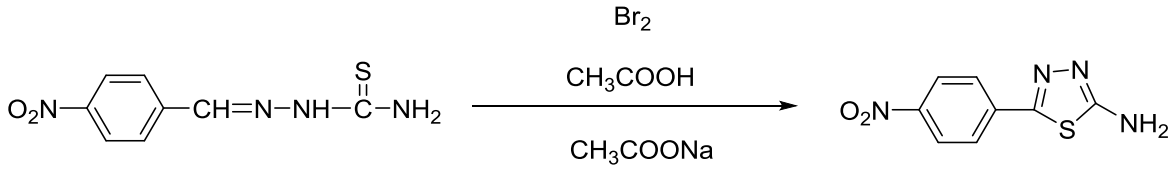
2.6. Synthesis of 2-(2-hydroxybenzylidene) hydrazine-1-carbothioamide



10 **11**

Aqueous solution of thiosemicarbazide (0.02 mol, 1.82g), crystalline sodium acetate (0.04 mol, 3.28g) and 2-hydroxy benzaldehyde (0.02 mol, 2.44 g) were taken in round bottom flask. To this mixture, the solvent methanol was added until clear the turbidity of the mixture. The reaction mixture was then mildly warmed and stirred for three hours. The reaction mixture was then poured into crushed ice. The solid precipitate was filtered, washed and dried. The white solid product was then recystallized with ethanol, found 72% yield and the melting point was recorded as 216- 218 ^{0} C.

2.7. Synthesis of 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine



5 **12**

A mixture of thiosemicarbazone **5** (0.01 mol, 1.92 g), sodium acetate (0.02 mol, 1.64 g) and glacial acetic acid 30 ml was taken in a round bottomed flask equipped with a separating funnel. To this mixture, 5 ml glacial acetic acid containing 0.7 ml bromine was added slowly and stirred continuously for three hours. After completion of the reaction, the reaction mixture was poured into crushed ice. The resulting solid was separated, dried and recrystallized from ethanol. The melting point was recorded as 240-242^{0}C.

IR (KBr, cm^{-1}, Fig.3) 3493 (N-H sym, NH_2), 3365 (N-H asym, NH_2), 3145, 3050 (C-H, aromatic), 1578 (C=N), 1526, 1516, 1453 (C=C, aromatic), 1099 (N-N, inside thiadiazole ring), 820 (C-S-C linkage).

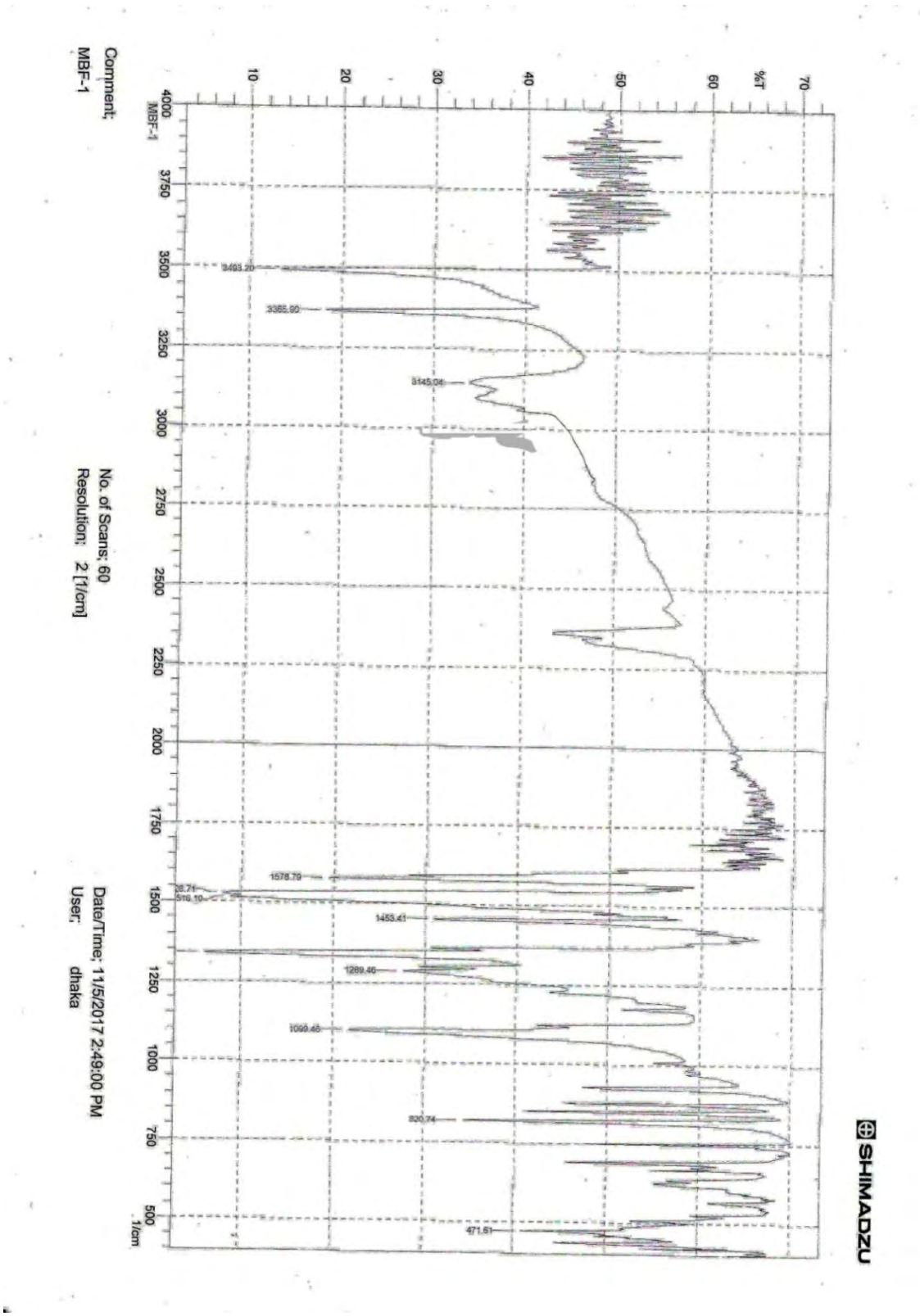
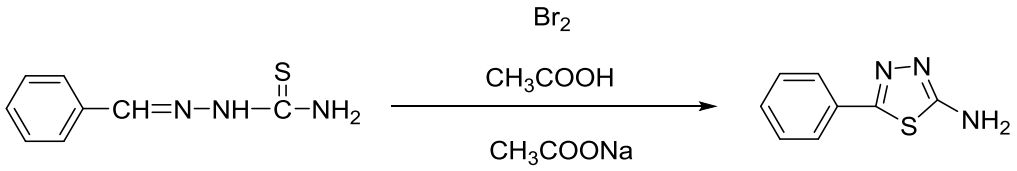


Fig - 3: IR Spectrum of Compound 12

2.8. Synthesis of 5-phenyl-1, 3, 4-thiadiazol-2-amine



7 **13**

A mixture of thiosemicarbazone**7**(0.01 mol, 1.63g), sodium acetate (0.02 mol, 1.64g) and glacial acetic acid 30 ml was taken in a round bottomed flask equipped with a separating funnel. To this mixture, 5 ml glacial acetic acid containing 0.7 ml bromine was added slowly and stirred continuously for three hours. After completion of the reaction, the reaction mixture was poured into crushed ice. The resulting solid was separated, dried and recrystallized from ethanol. The melting point was recorded as 223-225^{0}C.

IR (KBr, cm^{-1}, Fig 4)3281 (N-H, wide, NH_2), 3089 (C-H, aromatic) 1635 (C=N), 1518, 1469 (C=C, aromatic), 1059 (N-N inside thiadiazole ring), 780 (C-S-C, linkage).

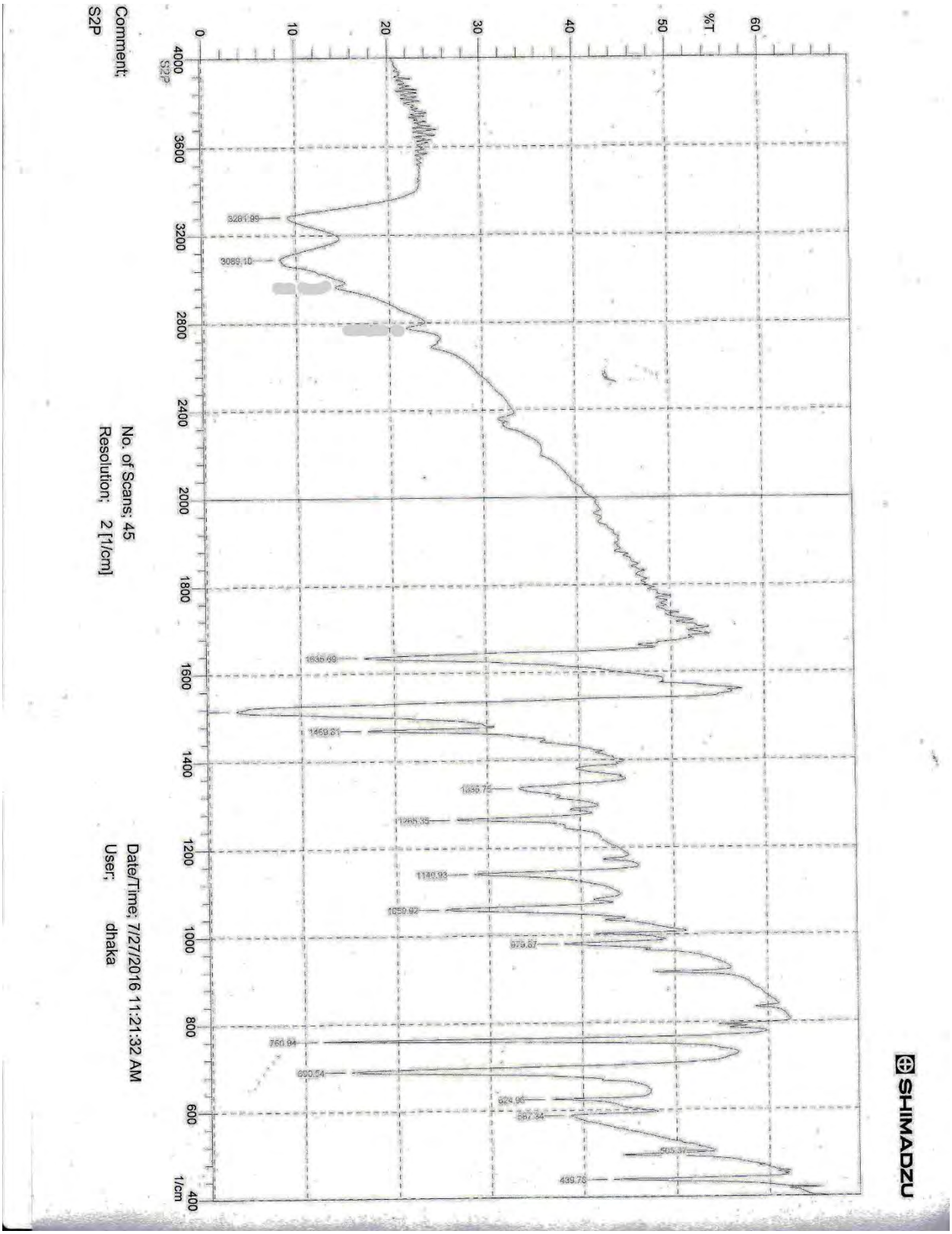
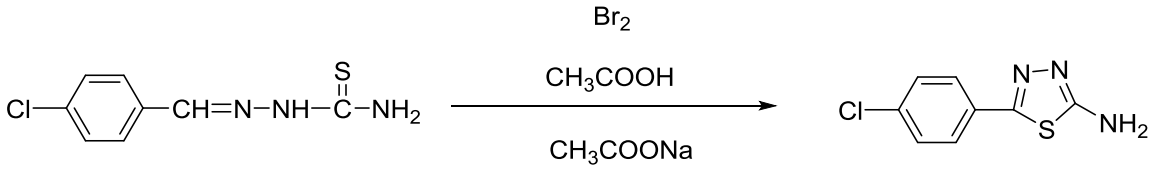


Fig - 4: IR Spectrum of Compound 13

2.9. Synthesis of 5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-amine



9 **14**

A mixture of thiosemicarbazone **9** (0.01 mol, 1.98 g) sodium acetate (0.02 mol, 1.64 g) and glacial acetic acid 30 ml was taken in a round bottomed flask equipped with a separating funnel. To this mixture, 5 ml glacial acetic acid containing 0.7 ml bromine was added slowly and stirred continuously for three hours. After completion of the reaction, the reaction mixture was poured into crushed ice. The resulting solid was separated, dried and recrystallized from ethanol. The melting point was recorded as 230- 232^{0}C.

IR (KBr, cm^{-1}, Fig.5)3440 (N-H, sym, NH_2), 3280 (N-H asym, NH_2), 3170 (C-H, aromatic), 1600 (C=N), 1530, 1490, 1455 (C=C, aromatic), 1091 (N-N, inside thiadiazole ring), 816 (C-S-C linkage).

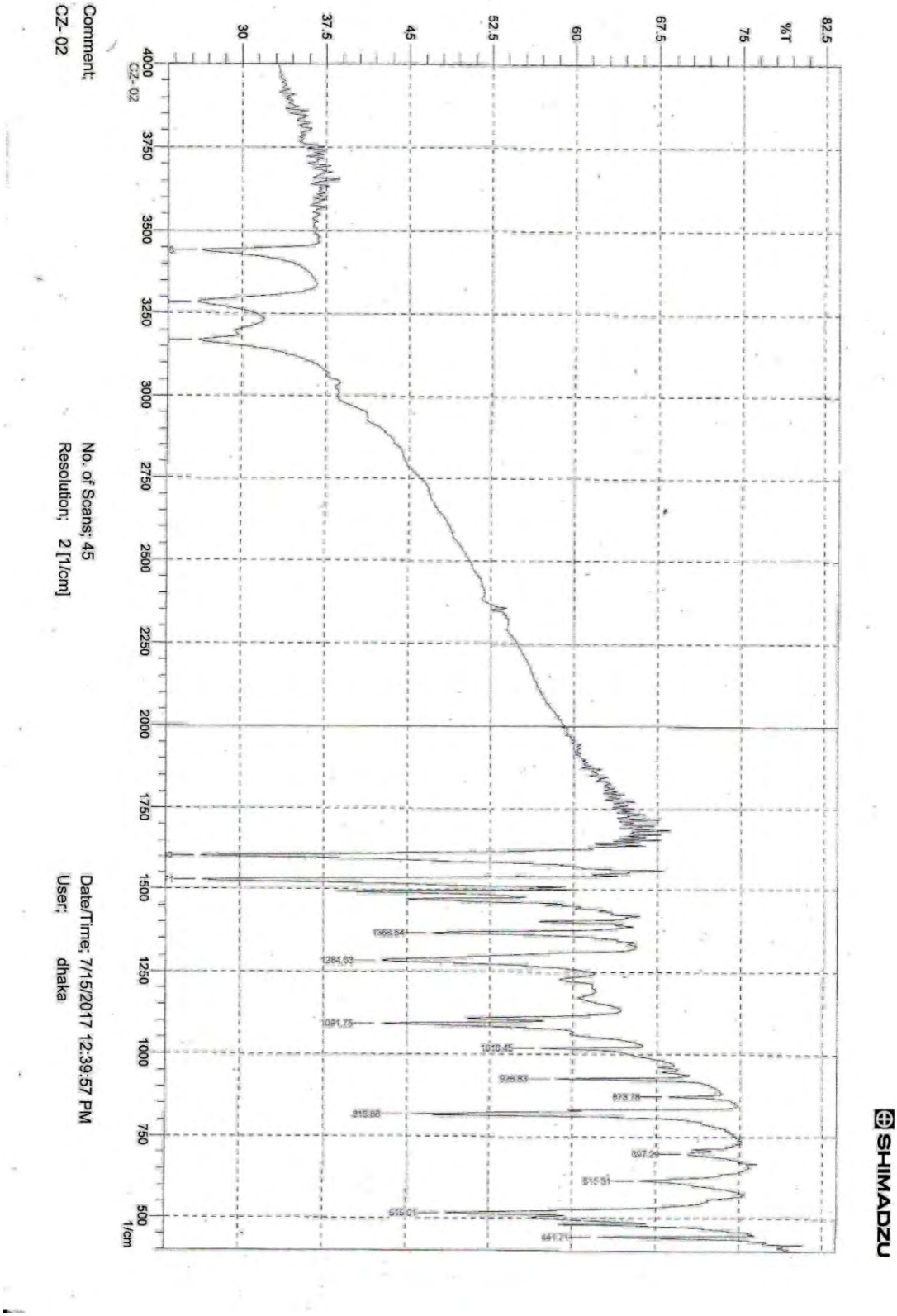
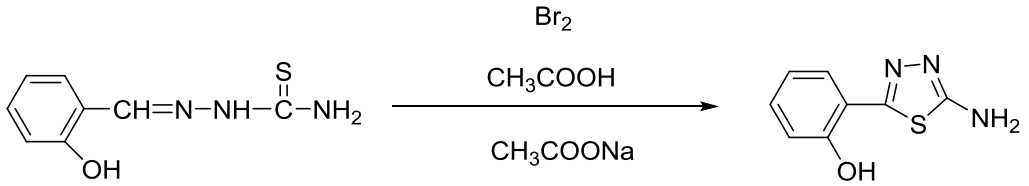


Fig – 5: IR Spectrum of Compound 14

2.10. Synthesis of 5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-amine



11 **15**

A mixture of thiosemicarbazone **11**(0.01 mol, 1.79g) sodium acetate (0.02 mol, 1.64g) and glacial acetic acid 30 ml was taken in a round bottomed flask equipped with a separating funnel. To this mixture, 5 ml glacial acetic acid containing 0.7 ml bromine was added slowly and stirred continuously for three hours. After completion of the reaction, the reaction mixture was poured into crushed ice. The resulting solid was separated dried and recrystallized from ethanol. The melting point was recorded as 186- 188^0C.

IR (KBr, cm^{-1}, Fig 6) 3444 (O-H, phenolic), 3322 (N-H, sym, NH_2), 3176 (N-H, asym), 3037 (C-H, aromatic), 1620 (C=N), 1535, 1491, 1450 (C=C, aromatic), 1111 (N-N str.), 831 (C-S-C, linkage).

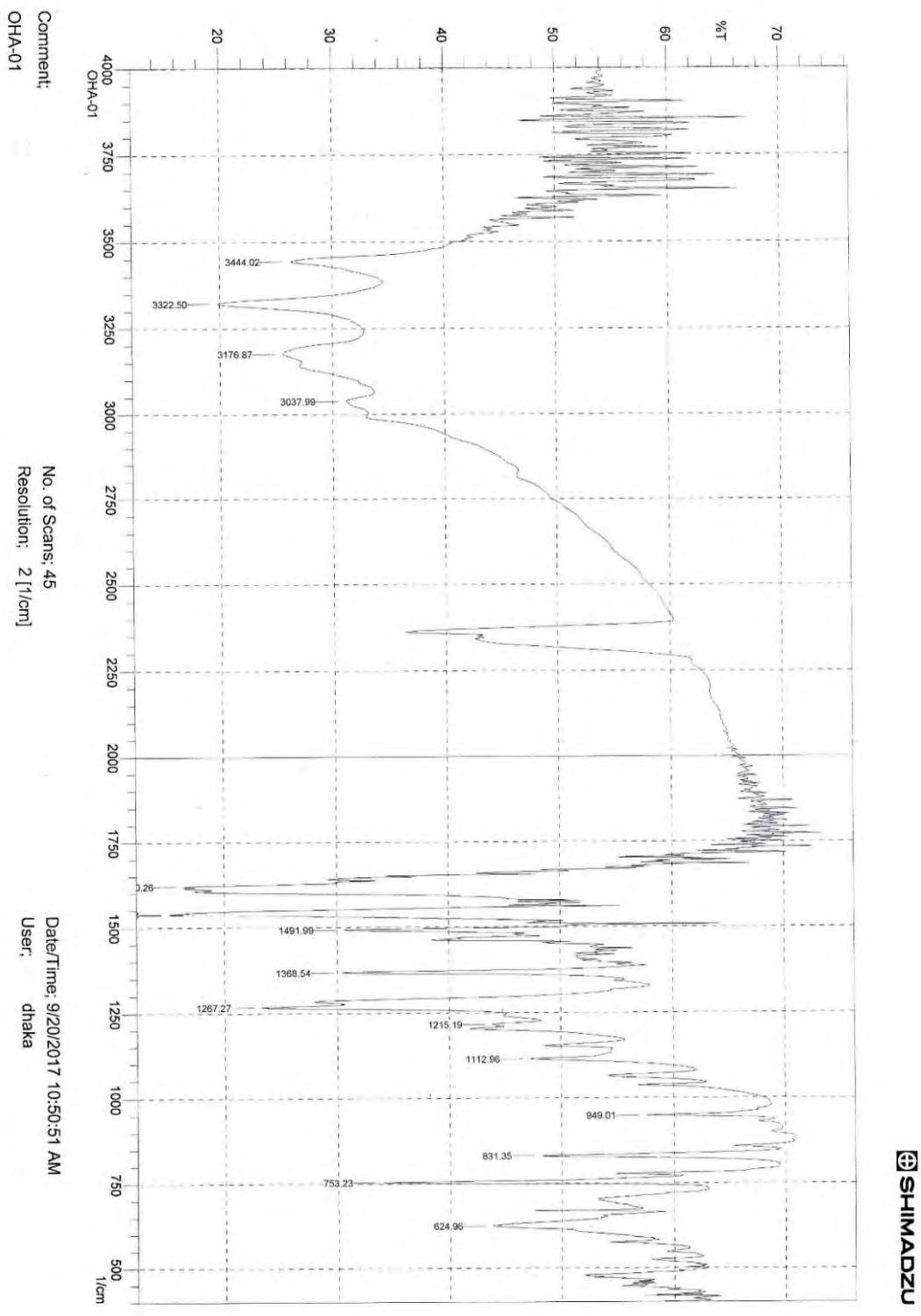
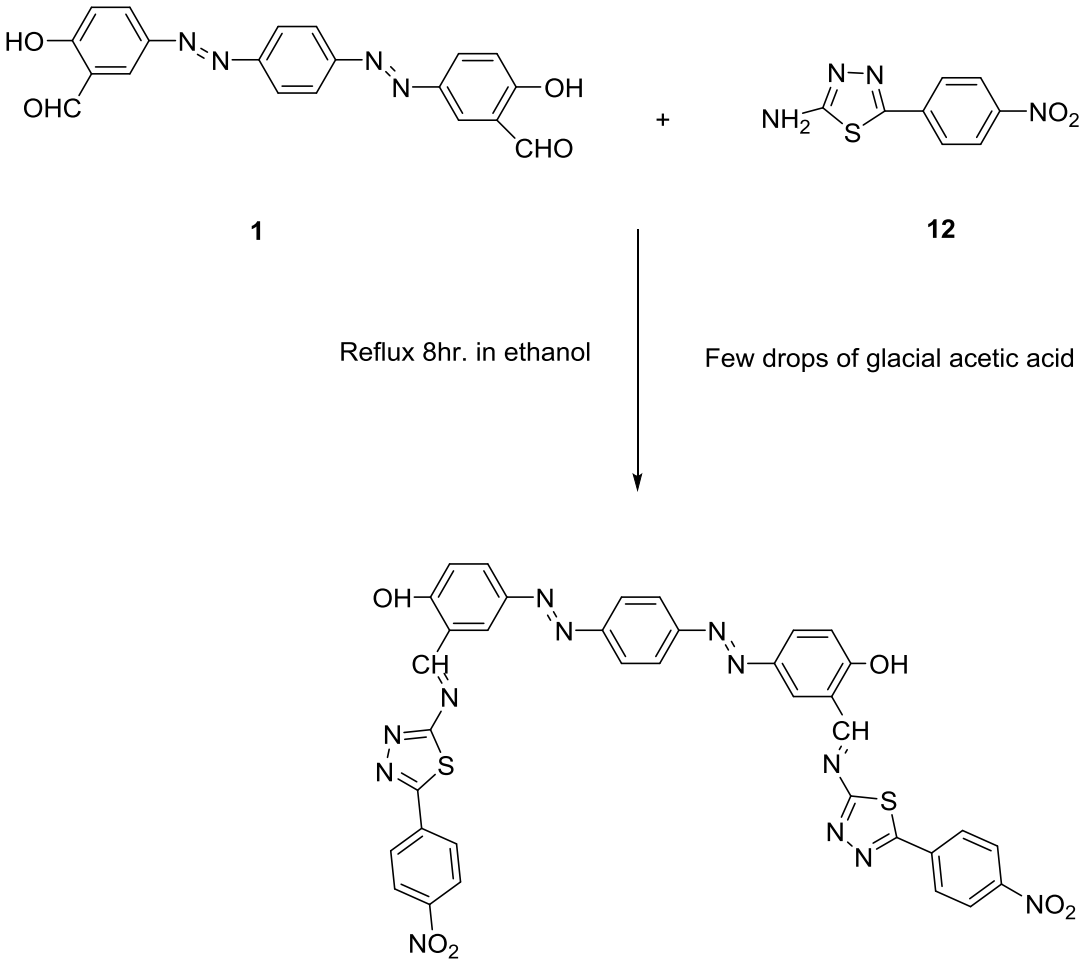


Fig – 6: IR Spectrum of Compound 15

2.11. Synthesis of 4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)



16

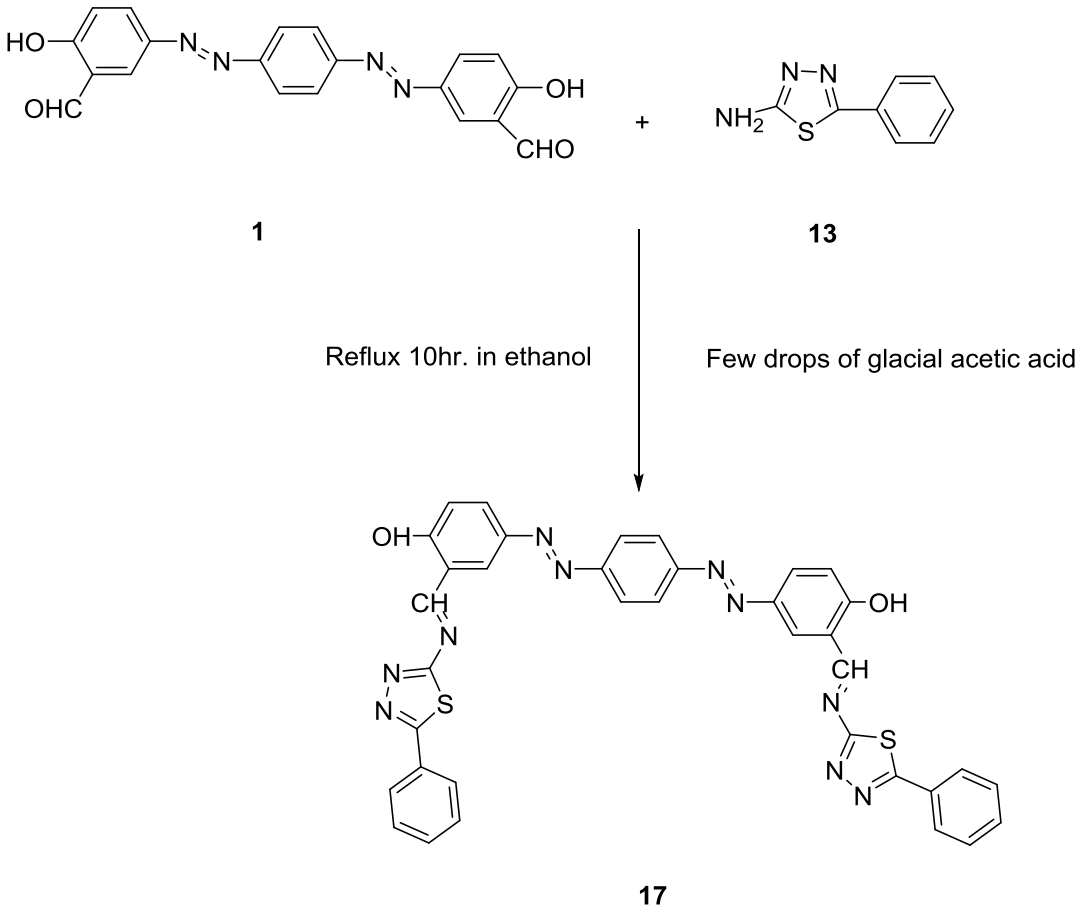
5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **1** (0.001 mol, 0.374 g) and 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine **12** (0.002 mol, 0.412 g) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture, a few drops of glacial acetic acid were added as catalyst. Then the reaction mixture was stirred under reflux condition for eight hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was stored in refrigerator overnight. The reddish precipitate was filtered. The crude product was then purified by column chromatography. A reddish crystalline product with 70% yield was isolated and the melting point was recorded as 188-192^{0}C

IR (KBr, cm^{-1}, Fig.7) 3405(O-H, sym), 3252(O-H, asym), 3151(C-H, aromatic), 1600 (C=N), 1599, 1542, 1489, (C=C, aromatic), 952(C-N, Ar-NO_2), 828 (C-S-C, linkage).

^{1}H NMR (400 MHz, DMSO-d_6, Fig 8-9) δ 6.81 (t,2H,C- 8, C- 8,' J=8.0,J=4.0), 6.87 (d, 2H,C-12,C- 12,' J=8.0),7.21 (t, 2H, C- 9, C- 9′, J=8.0, J=4.0), 7.90 (s, 2H, HC=N), 7.91 (bd,s, 4H, C -17, C- 17′, C-21,C- 21′), 8.10 (bd, s, 4H, C-18,C-18′,C-20,C-20′), 8.39 (s, 4H, C-2,C-3,C-5,C-6), 9.39 and 11.38 (s, 2H, OH)

^{13}C NMR (100 MHz, DMSO-d_6, Fig 10-11) δ 178 (C-13,C-13′),165 (C-15,C-15′), 156 (C-14,C-14′),144 (C-10,C-10′), 144(C-19,C-19′), 140 (C-1,C-4), 131.57 (C-11,C-11′), 131.50 (C-17,C-17′), 130 (C-16,C-16′),129 (C-7,C-7′), 128 (C-8,C-8′), 127 (C-9,C-9′),124 (C-2,C-6), 120 (C-18,C-18′), 119(C-21,C-21′), 116.53(C-20,C20′), 116.15(C-3,C-5).

2.13. Synthesis of 4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)



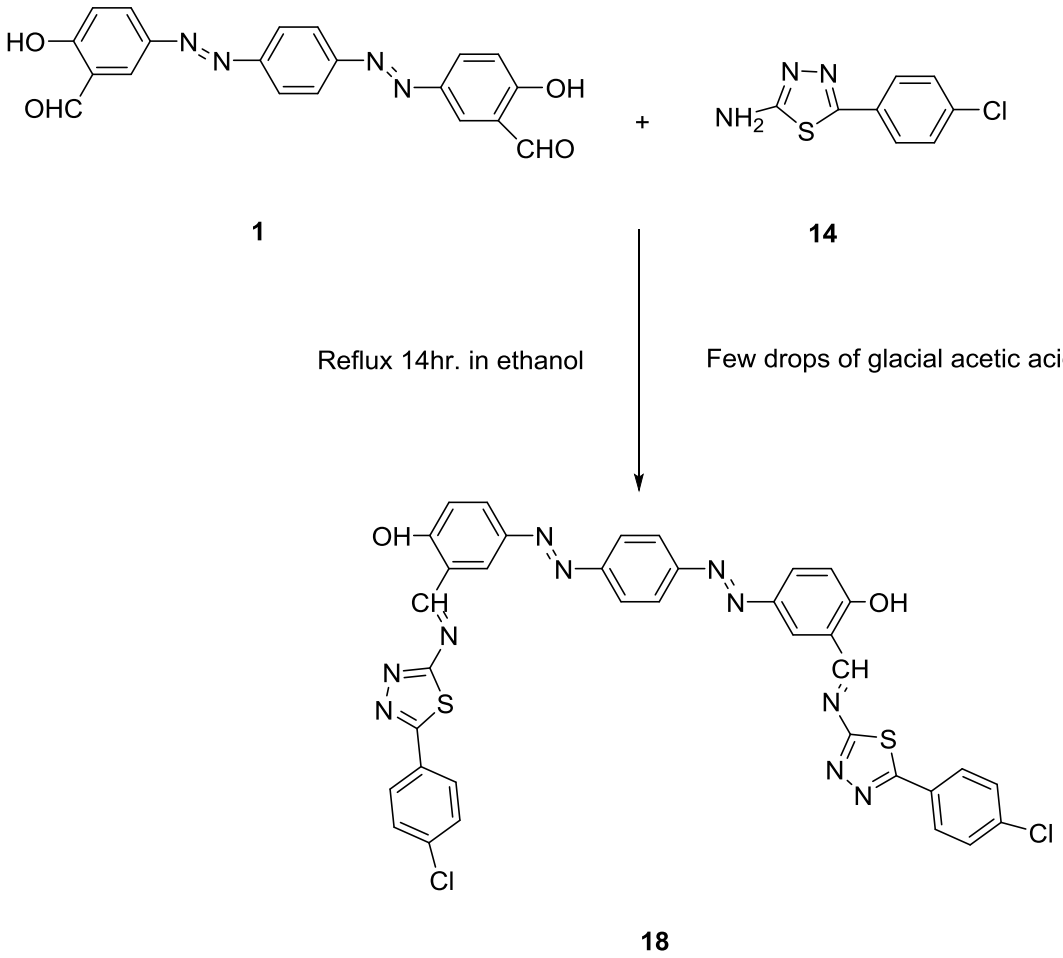
5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **1** (0.374 g, 0.001 mol) and 5-phenyl-1, 3, 4-thiadiazol-2-amine **13** (0.322 g, 0.002 mole) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture, a few drops of glacial acetic acid were added as catalyst. Then the reaction mixture was stirred under reflux condition for ten hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was stored in refrigerator overnight. The reddish precipitate was filtered. The crude product was then purified by column chromatography. A reddish crystalline product with 53% yield was isolated and the melting point was recorded as 168-174^{0}C.

IR (KBr, cm^{-1}, Fig.12)3300-3470 (O-H, str.), 3064 (C-H, aromatic), 1620 (C=N), 1575, 1554, 1418 (C=C, aromatic), 754 (C-S-C linkage).

^{1}H NMR (400 MHz, $CDCl_3$, Fig 13-14) δ 6.85 (bd, s, 4H, C-18, C-18′,C-20,C-20′), 6.90 (bd, s, 4H, C-17, C-17′, C-21, C-21′), 7.20 (bd, s, 2H, C-20, C-20′), 7.92 (s, 2H , C-2, C-6), 7.38 (s, 2H C-3, C-5), 7.59 (bd, s, 4H, C-8, C-8′, C-9, C-9′), 8.02(bd, s, 2H, C-12, C-12′), 8.21 (s, 1H, C-13), 8.32 (s, 1H, C-13′), 9.92 (s, 1H, C-10), 9.75 (s, 1H, C-10′).

^{13}C NMR (100 MHz, $CDCl_3$, Fig 15-16) δ 157.76 (C-13, C-13′), 157.56 (C-15, C-15′),151 (C-14, C-14′), 137 (C-10, C-10′), 133 (C-7, C-7′), 131.57 (C-1, C-4), 131.15 (C-2, C-6), 130 (C-3,C-5), 129.77 (C-8, C-8′), 129.04 (C-9, C-9′), 128.85 (C-11, C-11′), 128.80 (C-12, C-12′), 128.23(C-16, C-16′), 127.59 (C-17, C-17′, C-21, C-21′), 127.18 (C-18, C-18′, C-20, C-20′), 126 (C-19, C-19′).

2.13. Synthesis of 4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)

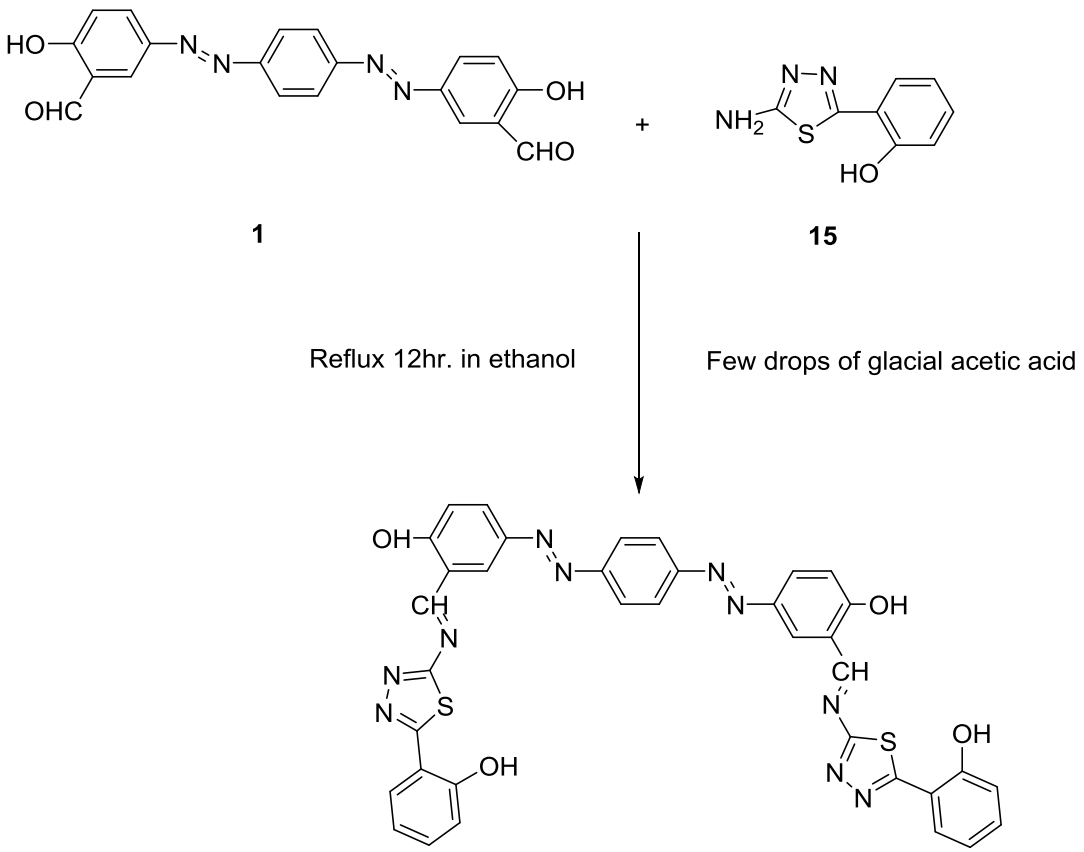


5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **1** (0.374g, 0.001 mol) and 5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-amine **14** (0.391g, 0.002 mole) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture, a few drops of glacial acetic acid were added as catalyst. Then the reaction mixture was stirred under reflux condition for fourteen hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was stored in refrigerator overnight. The pale yellow precipitate was filtered. The crude product was then purified by column chromatography. A yellow crystalline product with 62% yield was isolated and the melting point was recorded as 195-199^{0}C.

IR (KBr, cm^{-1}, Fig17) 3443(O-H, sym), 3175 (O-H, asym), 3114 (C-H aromatic), 1600 (C=N, exo and endo) 1575, 1550, 1480 (C=C, aromatic), 826(C-S-C, linkage), 750 (C-Cl, Ar-Cl).

^{1}H NMR (400 MHz, DMSO-d_6, Fig 18-19) δ6.80 (t, 2H, C-8, C-8′, J = 4.0, J = 8.0), 6.88 (d, 2H, C-12, C-12′, J = 8.0), 7.18 (t, 2H, C- 9, C- 9′, J = 8.0), 7.88 (bd, s, 6H, C- 17, C-17′ C-21, C-21′, C-13, C-13′), 8.06 (bd, s, 4H, C- 18, C-18′, C- 20, C- 20′), 8.38 (s, 4H, C- 2, C- 3, C- 5, C-6), 10.23 (s, 1H, O-H, C-10), 11.36 (s, 1H, O-H, C-10′) .

2.14. Synthesis of 4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)



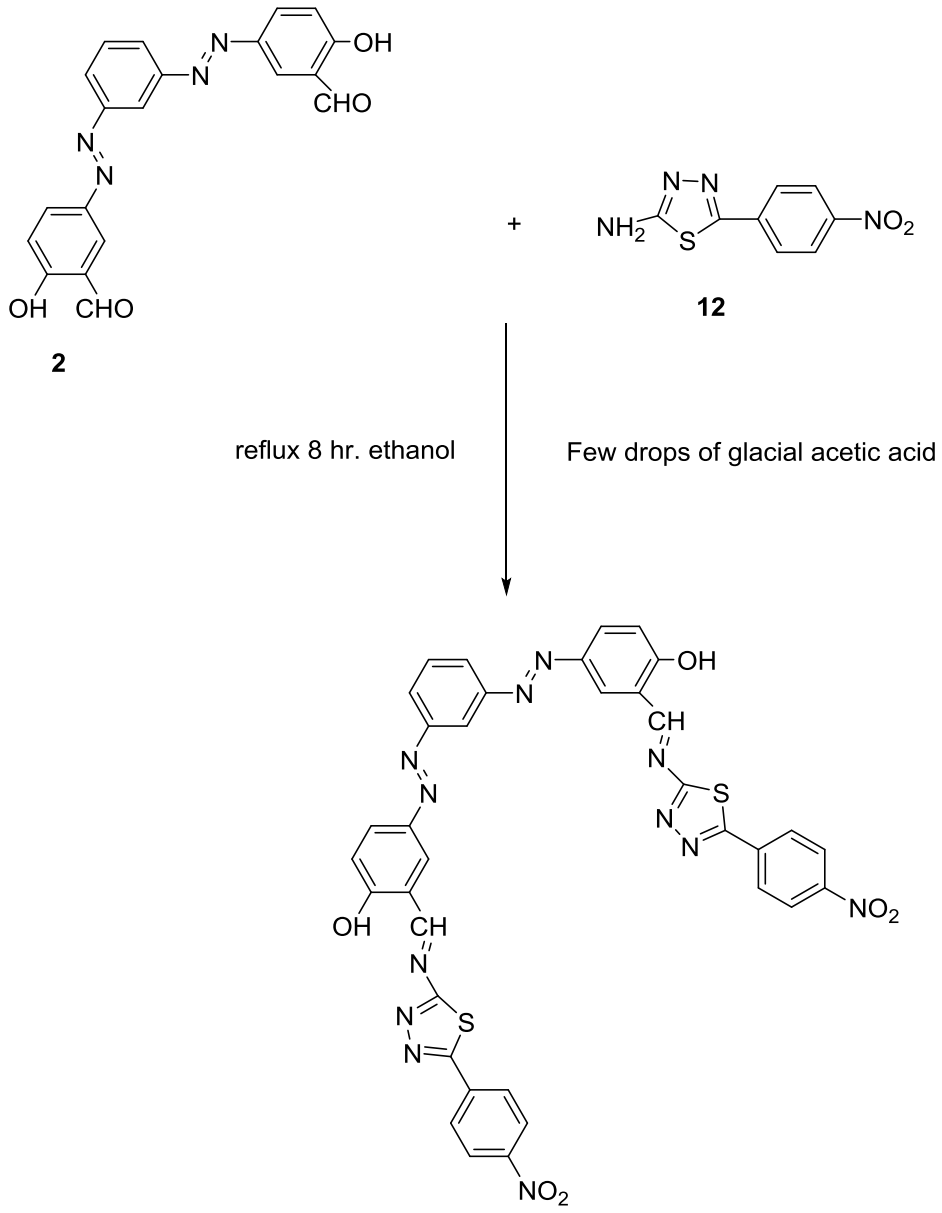
19

5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **1** (0.374g, 0.001 mol) and 5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-amine **15** (0.354 g, 0.002 mole) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture a few drops of glacial acetic acid was added as catalyst. Then the reaction mixture was stirred under reflux condition for twelve hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was stored in refrigerator overnight. The yellow precipitate was filtered. The crude product was then purified by column chromatography. A yellow crystalline product with 75% yield was isolated and the melting point was recorded as 199-202^{0}C.

IR (KBr, cm^{-1}, Fig 20)3450(O-H, sym), 3325(O-H, asym), 3180 (O-H, phenolic), 3125 (O-H, Phenolic), 3036(C-H aromatic), 1615 (C=N),1540, 1490, 1450 (C=C, aromatic),830(C-S-C, linkage).

^{1}H NMR (400 MHz, DMSO-d_6, Fig 21-22) δ6.89 (d, 1H, C-12, J = 8.0), 6.95 (d, 1H, C-12′ , J = 8.0), 7.46 (d, 2H, C-2, C- 6, J = 8.0),7.48 (d, 2H, C- 3, C- 5, J = 4.0), 7.52 (d, 2H, C- 9, C- 9′, J = 8.0),7.60 (d, 2H, C- 8, C-8′, J = 8.0),7.70 (d, 2H, C-17, C - 21, J = 8.0), 7.84 (d, 2H, C- 17′, C- 20′, J = 8.0), ′), 8.02 (d, 2H, C- 18, C- 20, J = 8.0), 8.05 (d, 2H, C- 18′, C- 20′, J = 8.0), 8.21 (bd, s, 1H, C-13), 8.24 (bd, s, 1H, C- 1311.27 (s, 1H, C- 10, O-H), 11.29 (1H, C- 10′, O-H), 11.42 (s, 1H, O-H, C- 21),11.46 (s, 1H, O-H, C - 21′).

2.15. Synthesis of 4,4'-((1E,1'E)-1,3-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)



20

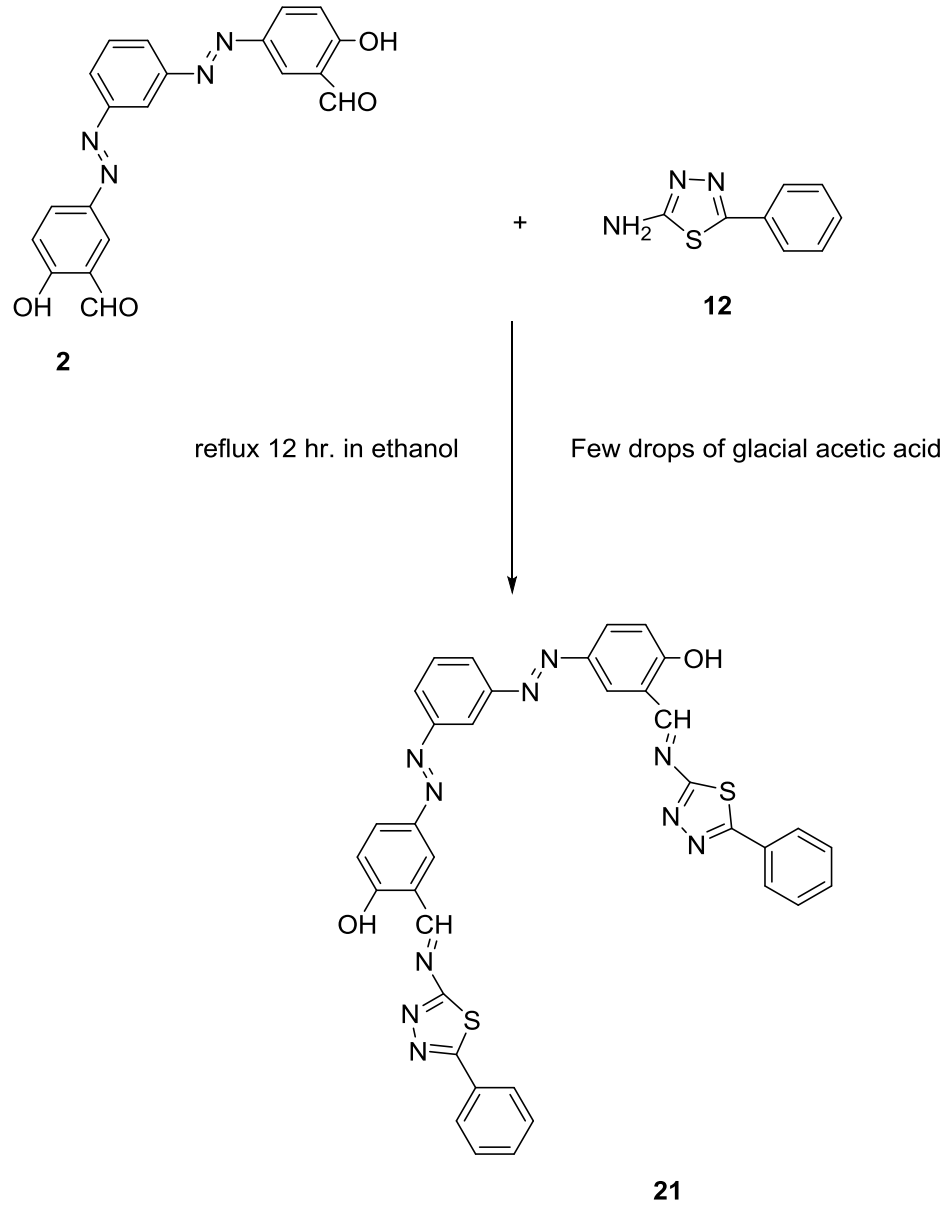
5, 5'-(1, 3- Phenylenebis (diazene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **2** (0.001 mol, 0.374 g) and5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine **12** (0.002 mole, 0.412 g) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture, a few drops of glacial acetic acid were added as catalyst. Then the reaction mixture was stirred under

reflux condition for eight hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was stored in refrigerator overnight. The yellow precipitate was filtered. The crude product was then purified by column chromatography. A yellow crystalline product with 70% yield was isolated and the melting point was recorded as 166-168^{0}C.

IR (KBr, cm^{-1}, Fig 23)3445(O-H, sym), 3325(O-H asym), 3150 (C-H, aromatic), 1614 (C=N), 1590, 1530, 1490 (C=C, aromatic), 830 (C-S-C, linkage), 752 (C-N, NO_2).

^{1}H NMR (400 MHz, DMSO-d$_6$, Fig 24-25) δ 6.81 (d, 4H, C-16, C-17, C-16′, C- 17′, J = 8.0),6.83 (d, 4H, C-18, C-18′, C- 20, C- 20′, J = 8.0), 7.21 (t, 1H, C-3, J = 8.0), 7.42(bd, s, 2H, C- 4, C- 2),7.98 (bd, d, 4H, C-8, C- 8′, C- 9, C- 9′, J = 8.0), 8.05 (bd, s, 2H, C-12, C - 12′),8.08 (bd, s, 1H, C- 6), 8.37 (s, 2H, HC=N), 9.89 (s, H, -OH, C- 10), 11.36 (s, 1H, OH, C- 10′).

2.16. Synthesis of 4,4'-((1E,1'E)-1,3-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol)



5, 5'-(1, 3- Phenylenebis (diazene-2, 1-diyl)) bis (2-hydroxybenzaldehyde) **2** (0.001 mol, 0.374 g) and 5-phenyl-1, 3, 4-thiadiazol-2-amine **13** (0.002 mole, 0.322 g) were separately dissolved in ethanol and combined together in a round bottomed flask. To this mixture, a few drops of glacial acetic acid were added as catalyst. Then the reaction mixture was stirred under reflux condition for eight hours. The progress of the reaction was monitored by TLC. After completion of the

reaction, the mixture was stored in refrigerator overnight. The brown precipitate was filtered. The crude product was then purified by column chromatography. A brown crystalline product with 75% yield was isolated and the melting point was recorded as 172-173^{0}C.

IR (KBr, cm^{-1}, Fig 26) 3313(O-H, sym), 3244 (O-H, asym), 3050 (C-H, aromatic), 1610 (C=N), 1550, 1491, 1449 (C=C, aromatic), 758 (C-S-C, linkage).

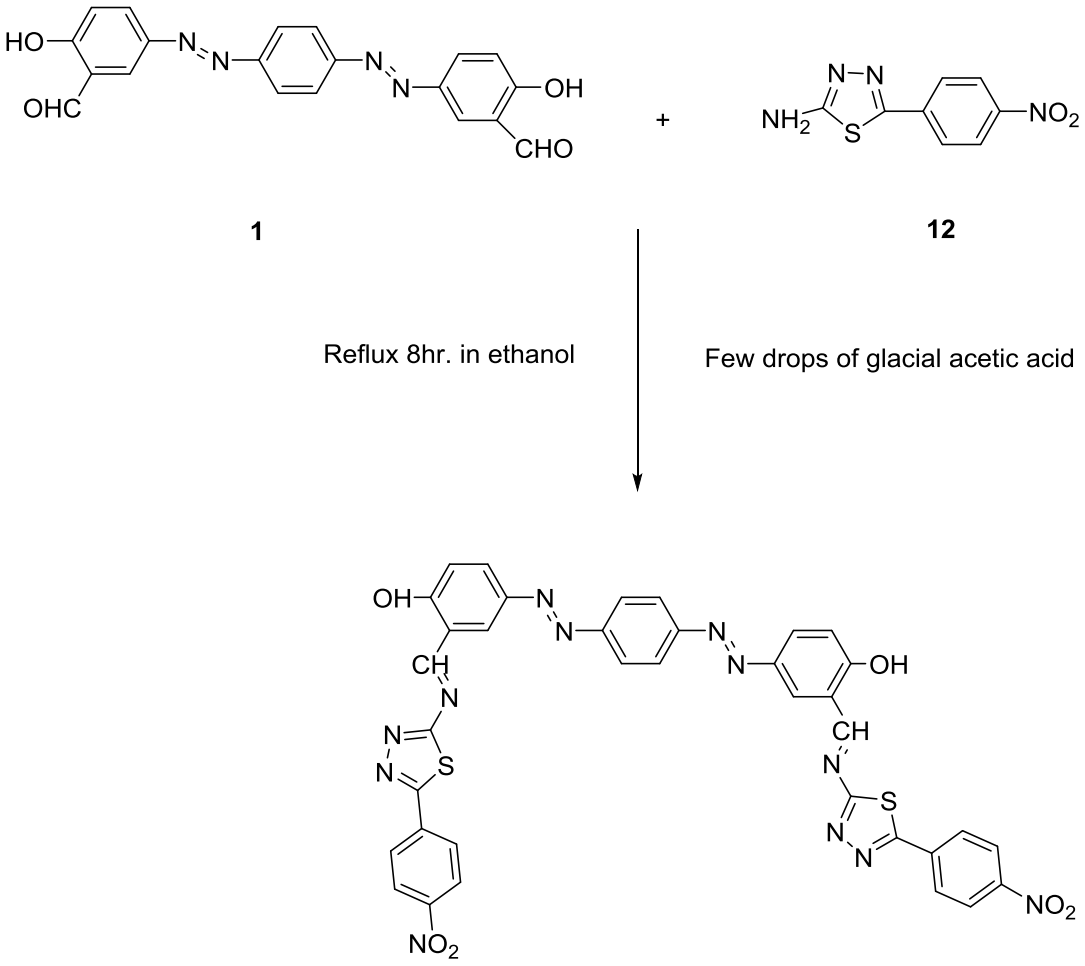
^{1}H NMR (400 MHz, DMSO-d$_6$, Fig 27-28) δ 10.01 (s, 1H, O-H), 9.02 (s, 1H, O-H), 8.52 (bd, s, 4H, C-16, C- 16′, C – 17, C- 17′), 8.45 (bd, s, 4H, C- 18, C- 18′, C- 20, C- 20′), 8.43 (s, 2H, HC=N), 7.95 (m, 4H, aromatic), 7.89 (m, 2H, aromatic),7.5 (m, 6H, aromatic).

Chapter 3

Results and Discussion

3.1. Characterization of the compound 16 (4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **16** was synthesized by refluxing 1: 2 molar ethanolic solution of **1** (5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde)) and **12** (5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine) for eight hours in presence of catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After purification a reddish crystalline product was obtained and found 70% yield. The melting point was recorded as 188-192^0 C.



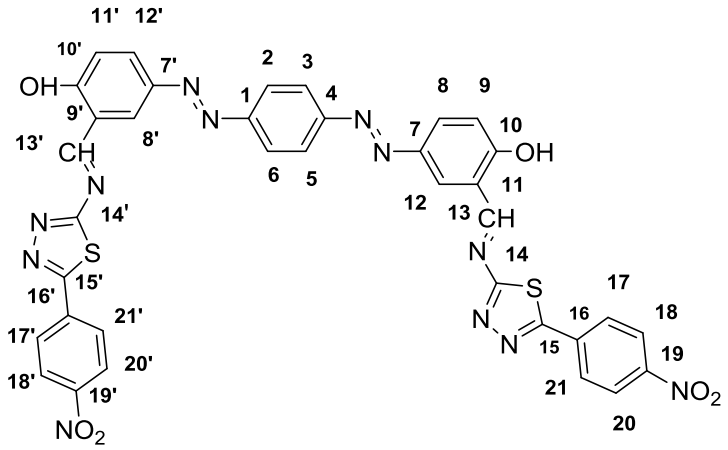
The IR spectra (**Fig. 7**) of the compound **16** showed a broad absorption band at 3405 cm^{-1} for phenolic O-H group. The broad absorption band at 3252 cm^{-1} was attributed for asymmetric stretching vibration of phenolic O-H group. The wide characteristic stretching frequency of aromatic C-H was observed at 3151 cm^{-1}. The sharp peak at 1600 cm^{-1} was detected for C=N moiety. The peak at 1599, 1542 and 1489 cm^{-1} were distinguished for aromatic C=C bonds. The weak C-N absorption frequency of Ar-NO_2 was detected at 952 cm^{-1}. The C-S-C linkage was designated at 828 cm^{-1}.

The ^{1}H NMR spectra (**Fig. 8-9**) of the compound **16** showed triplet at 6.8 for two equivalent protons at C-8 and C-8′ with the coupling constant J = 8.0 and J = 4.0. These coupling constants were indicative for short and long range couplings with the nearby proton and axial proton. The doublet at 6.87 was designated for two equivalent protons at C-12 and C-12′ with the coupling constant J = 8.0. The sharp triplet at 7.21 was attributed for the two equivalent protons of C-9, C-9′ with coupling constant J = 8.0 and J = 4.0. These coupling constants were distinctive for short and long range coupling with the nearby proton and axial proton. The singlet at 7.90 was indicative for two equivalent N=C-H protons. The broad singlet at 7.91 was designated for four protons at C-17, C-17, C-21′, and C- 21. The broad singlet at 8.10 was ascribed for four protons at C-18, C-18′, C-20 and C-20′. The sharp singlet at 8.39 was distinctive for four protons at C-2, C-3, C-5, and C-6. The singletsat 9.39 and 11.38 were assigned for two phenolic-OH protons.

The ^{13}C NMR spectra (**Fig. 10-11**) of the compound **16** showed a signal 178 for two equivalent carbons of N=C-H, outside the ring. The signal at 165 was designated for two carbons of C=N inside the thiadiazole ring. The two equivalent C=N carbons inside the thiadiazole ring was designated at 156. The signal at 144 was ascribed for two carbons of C-10 and C-10′. Two equivalent carbons of C-19 and C-19′ were detectable at 144. The signal at 140 was designated for two equivalent carbons of C-1 and C-4. The signal at 131.57 was distinctive for two carbons of C-11 and C-11′. Another two carbons of C-17 and C-17′were distinguished at 131.50. The signal at 130 was attributable for two identical carbons of C-16 and C-16′. Two carbons of C-7 and C-7′ were identified at 129. The signal at 128 was detectable for two identical carbons of C-8 and C-8′. Two carbons of C-9 and C-9′ were designated at 127. The signal at 124 was assignable

for two equivalent carbons of C-2 and C-6. The two carbons of C-18 and C-18′ were detected at 120. The signal at 119 was identifiable for two identical carbons of C-21 and C-21′. The two carbons of C-20 and C-20′ were indicative at 116.53. The rest two carbons of C-3 and C-5 were assigned at 116.15.

All the spectral evidences expressed harmony with the structure of the compound **16** as



16

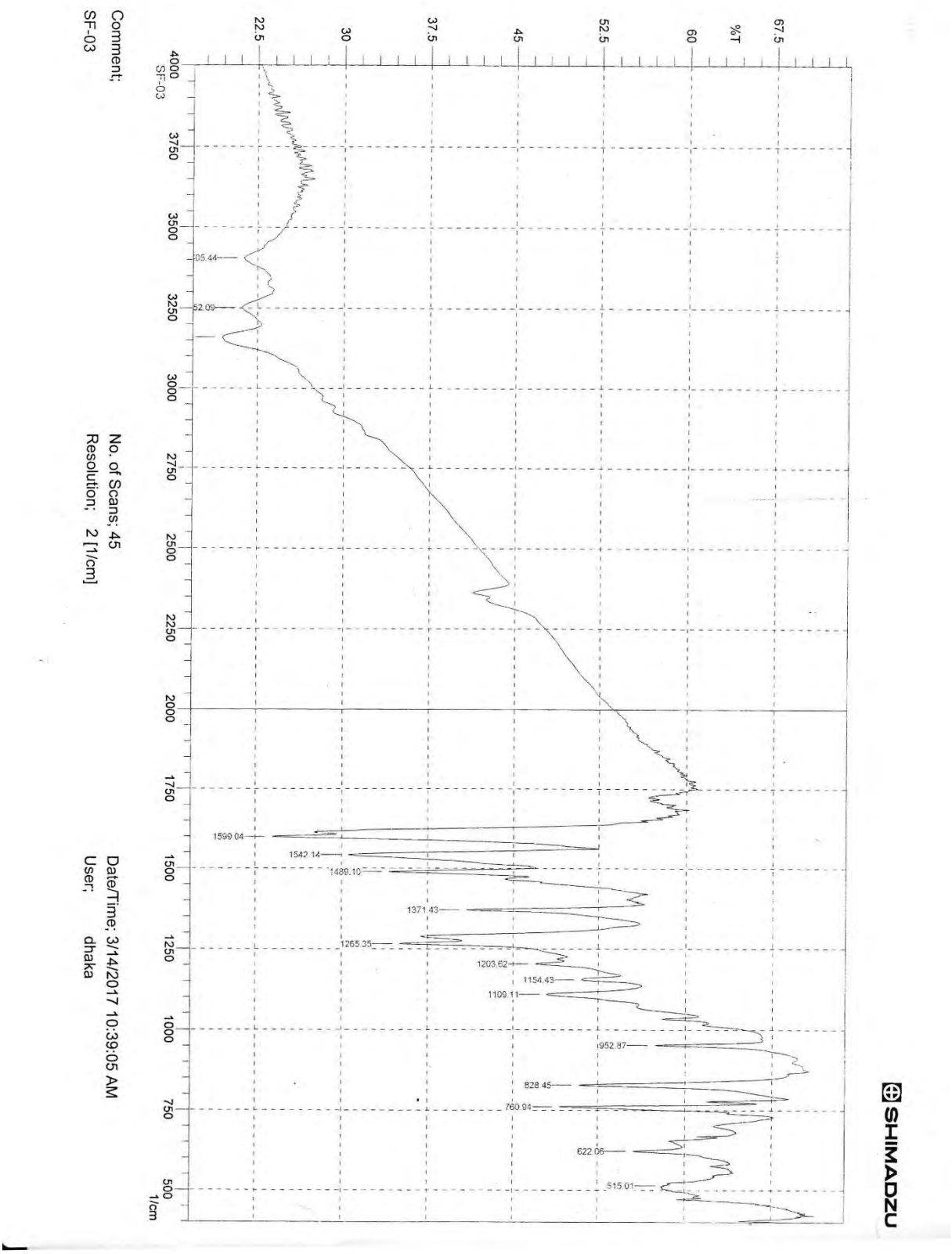


Fig – 7: IR Spectrum of Compound 16

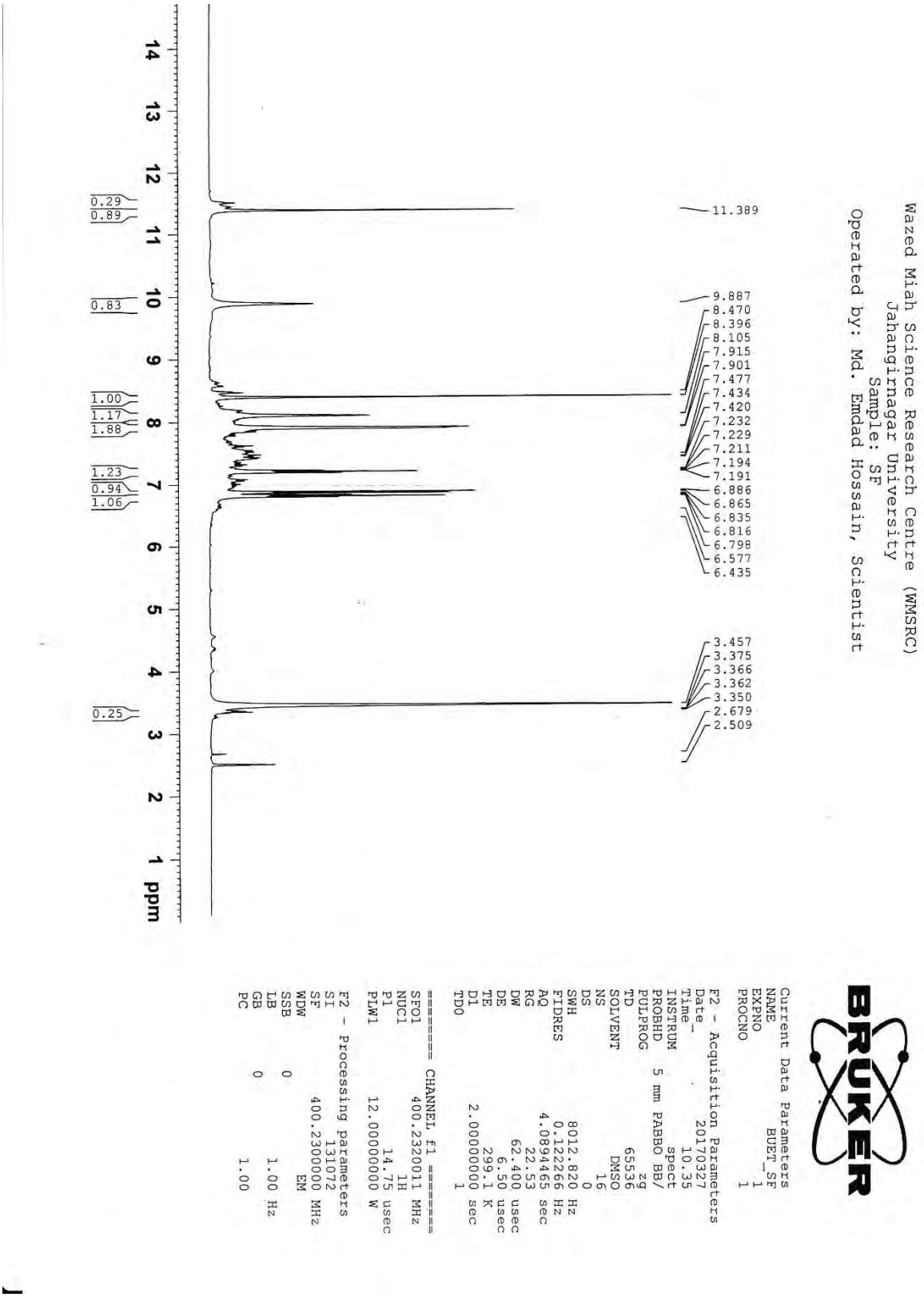


Fig – 8: ^{1}H NMR Spectrum of Compound 16

Fig – 9: Extended ^{1}H NMR Spectrum of Compound 16

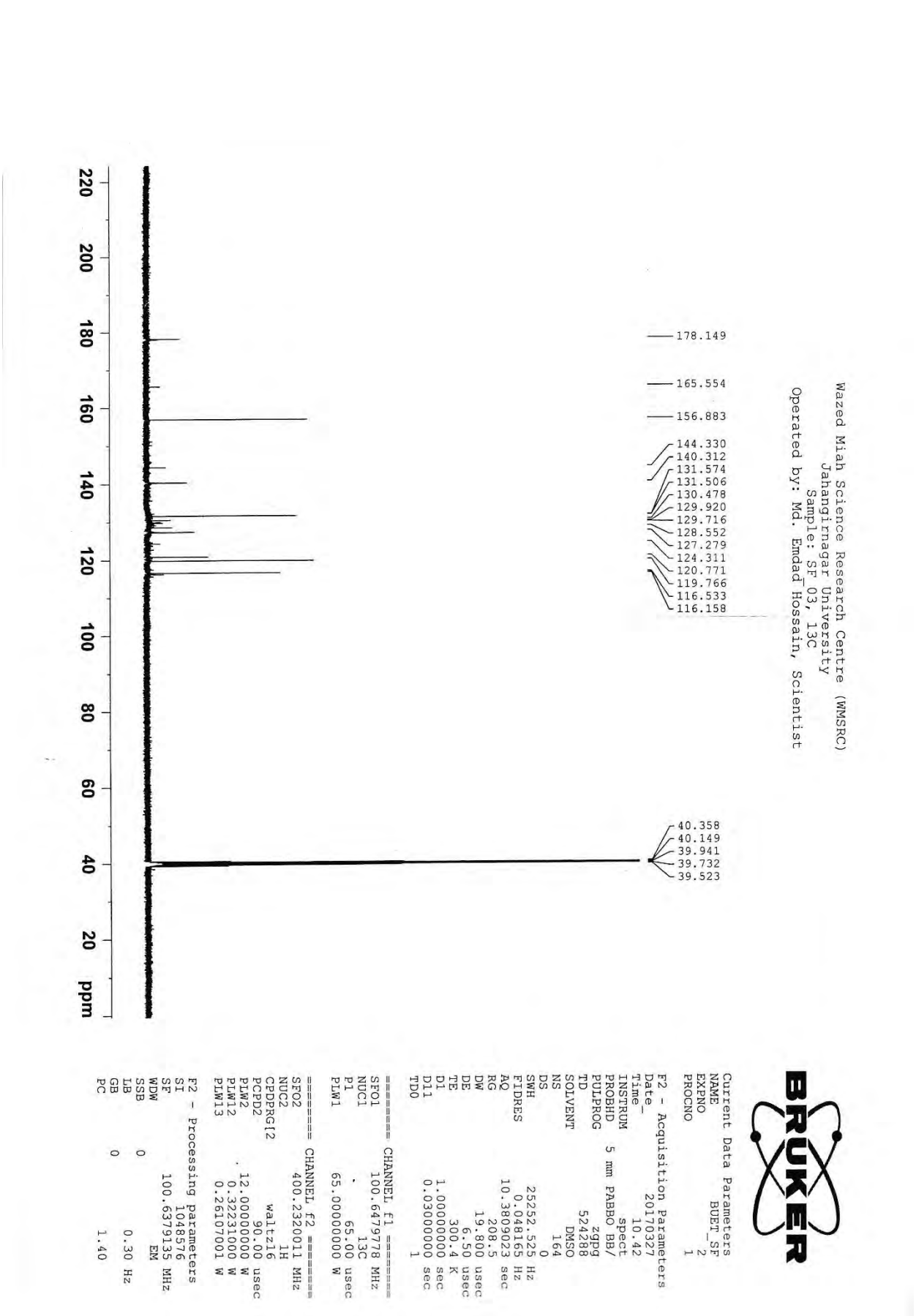
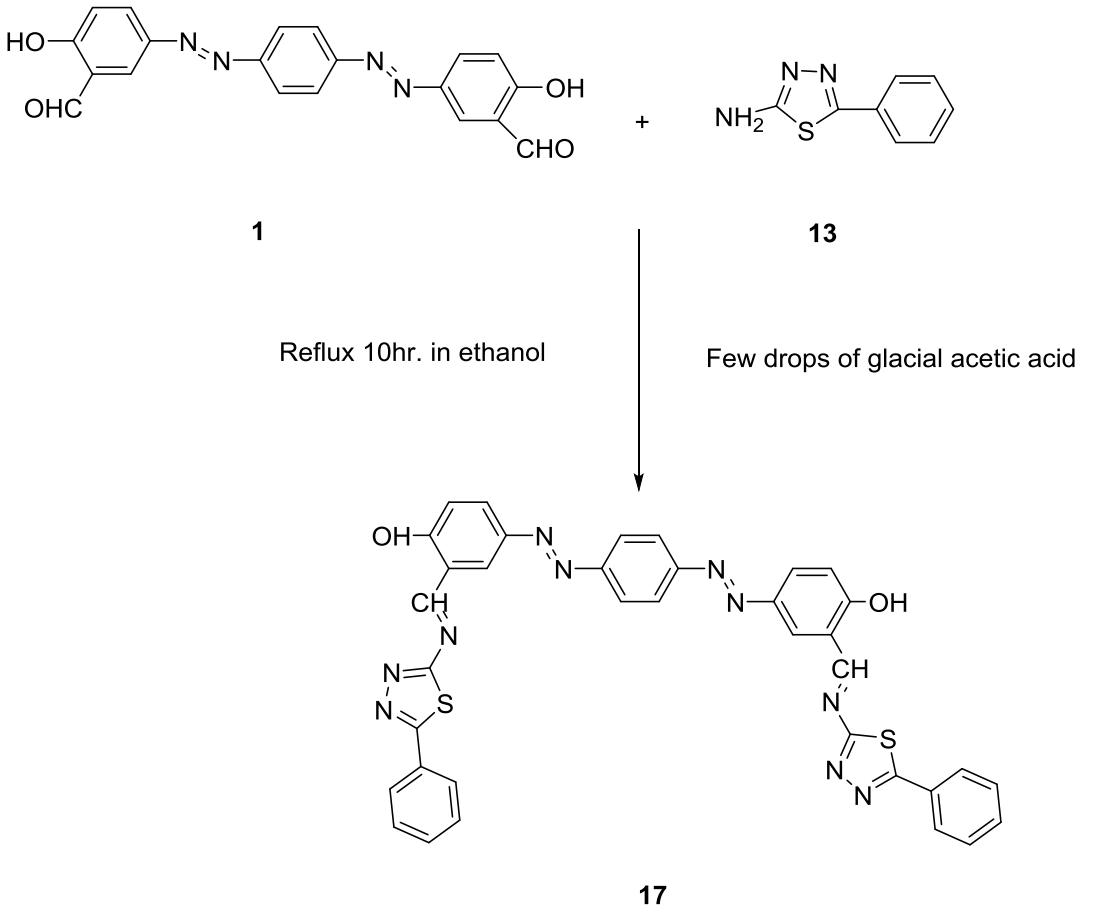


Fig – 10: ^{13}C- NMR Spectrum of Compound 16

Fig – 11: Extended ^{13}C- NMR Spectrum of Compound 16

3.2. Characterization of the compound 17 (4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **17** was synthesized by refluxing 1: 2 molar ethanolic solution of **1** (5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl))bis(2-hydroxybenzaldehyde)) and **13** (5-phenyl-1, 3, 4-thiadiazol-2-amine) for eight hours in presence of catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After purification a reddish crystalline product was obtained and found 53% yield. The melting point was recorded as 168-174^0 C.

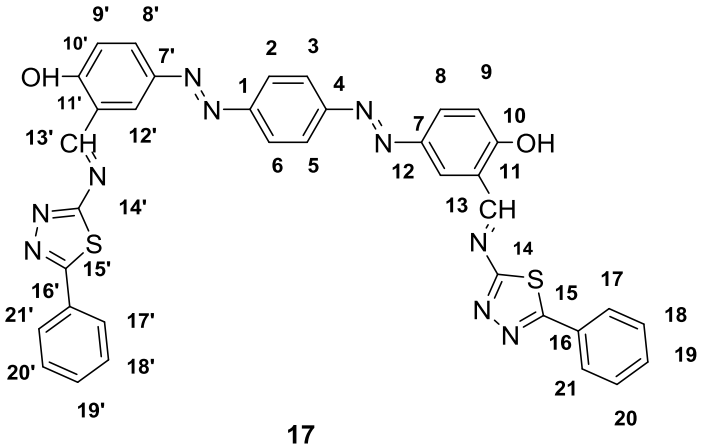


The IR spectra (**Fig. 12**) of the compound **17** showed a broad band at 3300-3470 cm^{-1} for phenolic O-H group. The aromatic C-H stretching frequency was observed at 3064 cm^{-1}. The peak at 1620 cm^{-1} was detected for C=N moiety. The characteristic peaks at 1575, 1554 and 1418 cm^{-1} were distinguished for aromatic C=C bonds. The weak C-S-C linkage peak was assigned at 754 cm^{-1}.

The ^{1}H NMR spectra (**Fig. 13-14**) of the compound **17** showed broad singlet for four identical protons of C-18, C-18′, C-20 and C-20′ at 6.85. The broad singlet at 6.90 was identified for four equivalent protons of C-17, C-17′, C-21 and C-21′. The weak broad signal at 7.20 was attributed for two protons of C-20 and C-20′. The wide singlet at 7.38 was ascribable for two equivalent protons of C-3 and C-5. The broad signal of four protons of C-8, C-8′, C-9 and C-9′ were assigned at 7.59. The weak singlet at 7.92 was ascribed for two protons of C-2 and C-6. The broad singlet for two protons of C-12 and C-12′ was assigned at 8.02. The single protonic singlet was detectable at 8.21 for the proton C-13. Another single protonic singlet was distinctive at 8.32 for C-13′. The characteristic phenolic proton singlet signals were identified at 9.92 and 9.75 for C-10 and C-10′ respectively.

The ^{13}C NMR spectra (**Fig. 15-16**) of the compound **17** showed signal at 157.76 for two equivalent carbons forC-13 and C-13′. The signals were assigned at 157.56for two identical carbons for C-15 and C-15′, two carbons for C-14 and C-14′ at 151, two carbons at 137 for C-10 and C-10′, two identical carbons at 133 for C-7 and C-7′, two equivalent carbons at 131.57 for C-1 and C-4, two carbons at 131.15 for C-2 and C-6, two carbons at 130 for C-3 and C-5, two identical carbons at 129.77 for C-8 and C-8′, two equivalent carbons at 129.04 for C-9 and C-9′, two carbons at 128.85 for C-11 and C-11′, two carbons at 128.80 for C-12 and C-12′, two equivalent at 128.13 for C-16 and C-16′, four identical carbons at 127.549 for C-17, C-17′, C-21 and C-21′, four equivalent carbons 127.18 for C-18, C-18′, C-20 and C-20′ and two carbons at 126 for C-19 and C-19′.

All the spectral evidences supported the structure of the **17** as



17

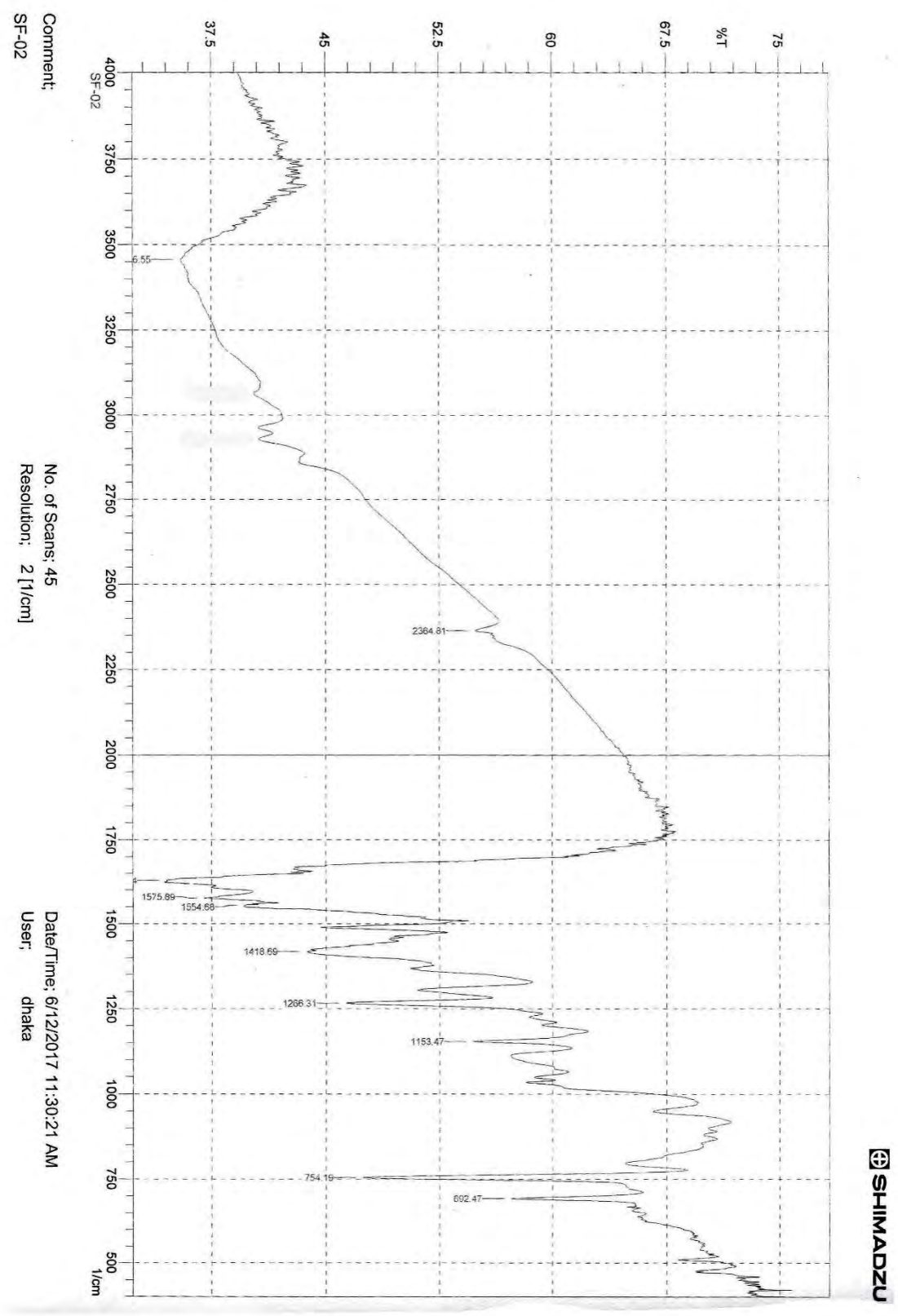


Fig – 12: IR Spectrum of Compound 17

Fig – 13: ^{1}H- NMR Spectrum of Compound 17

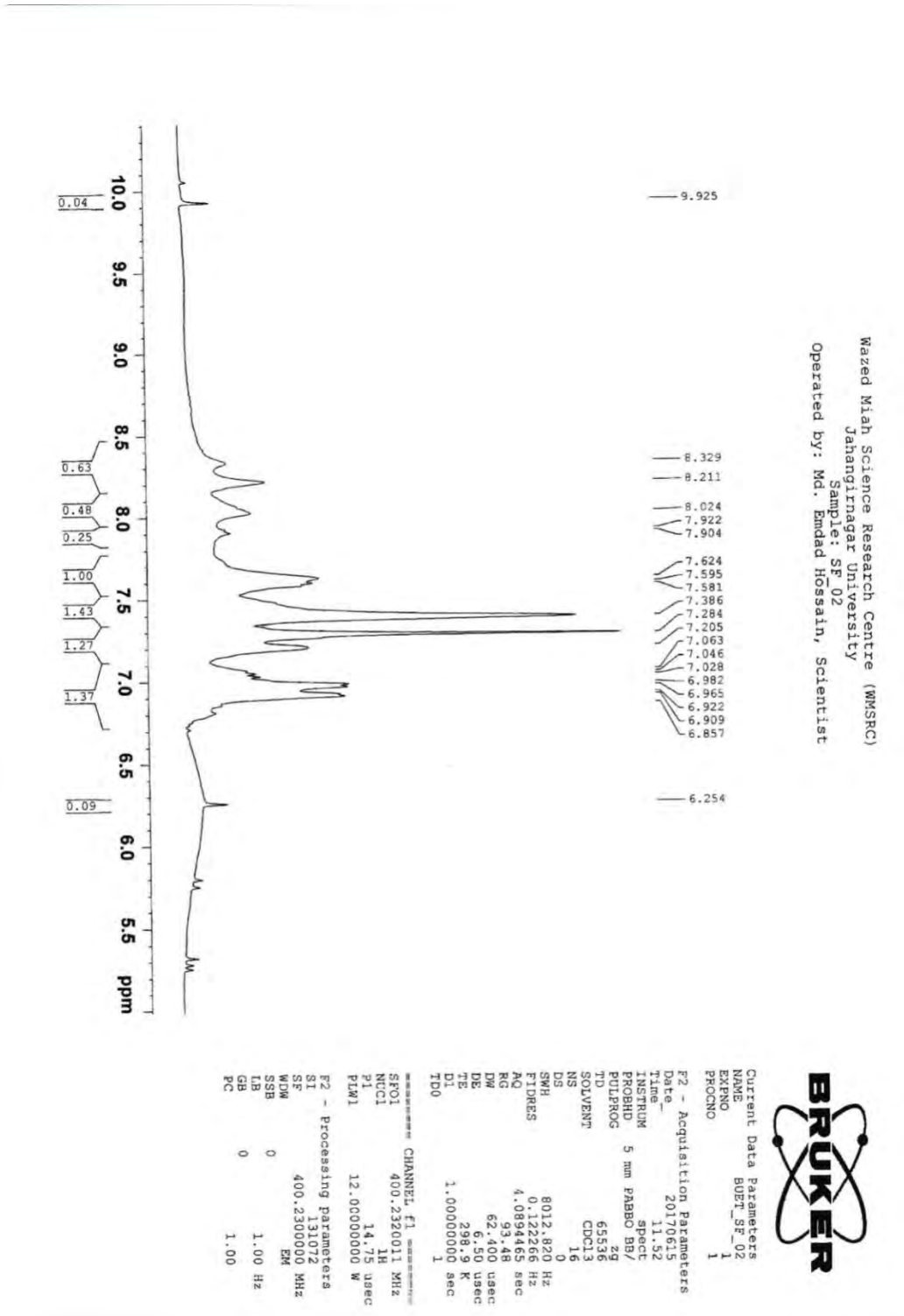


Fig – 14: Extended ^{1}H- NMR Spectrum of Compound 17

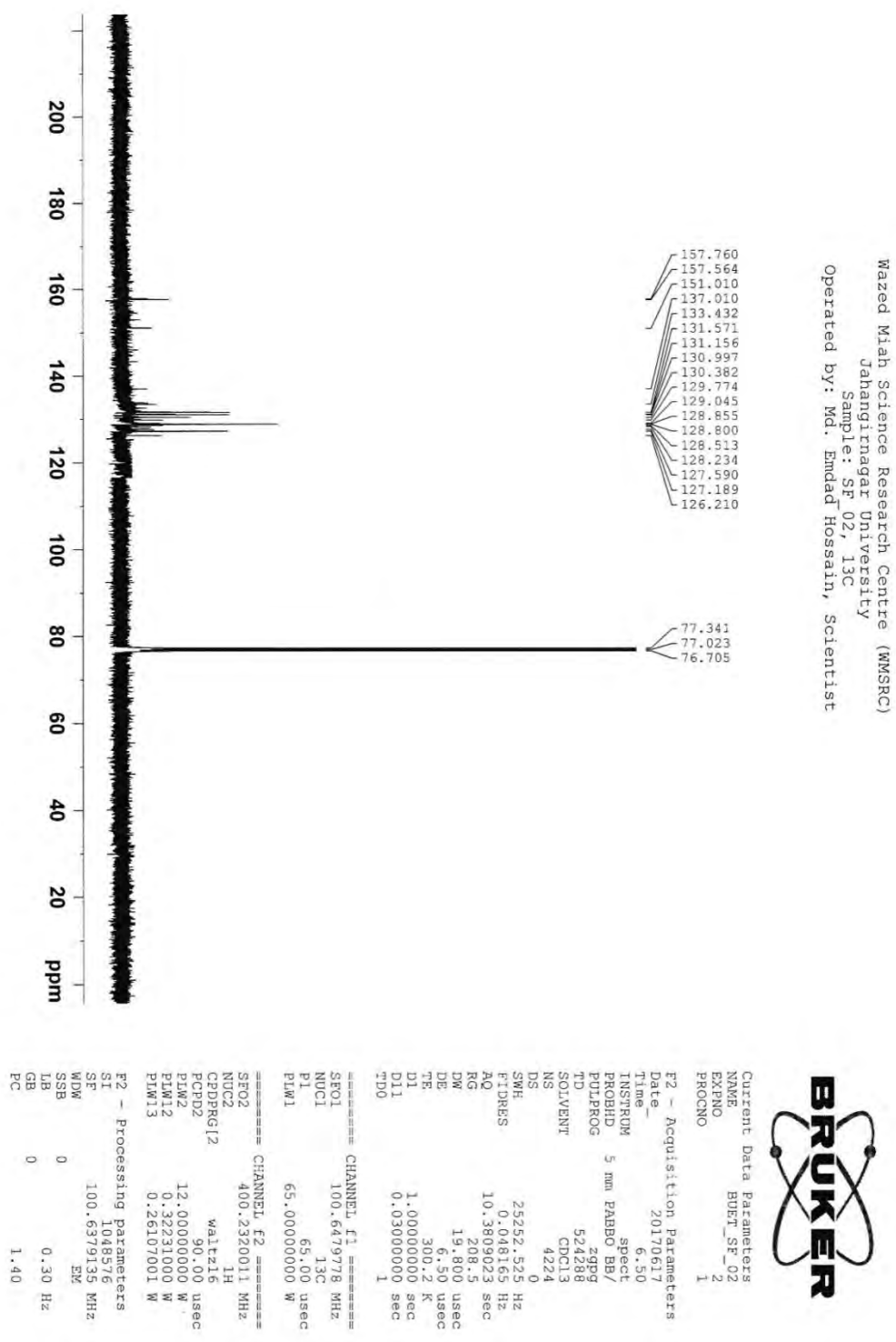
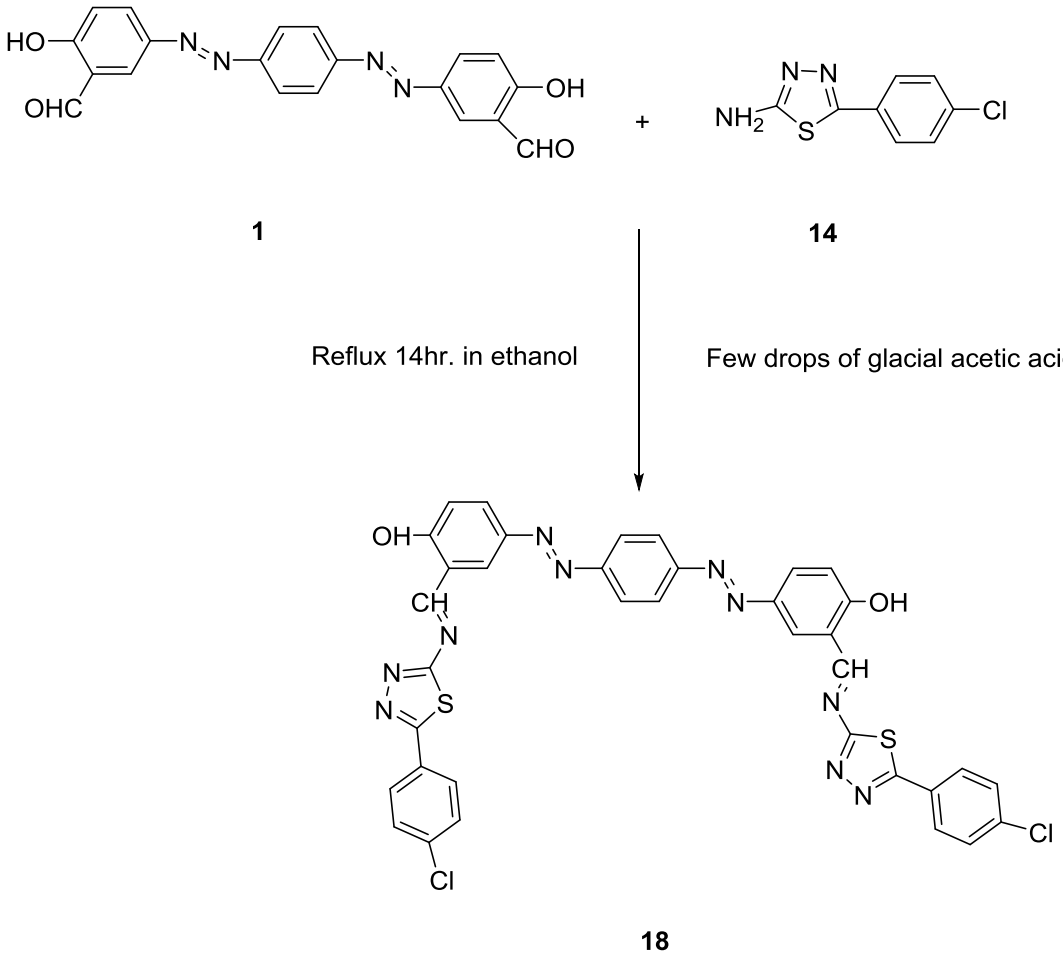


Fig – 15: ^{13}C-NMR Spectrum of Compound 17

Fig – 16: Extended ^{13}C-NMR Spectrum of Compound 17

3.3. Characterization of the compound 18 (4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **18** was synthesized by refluxing 1: 2 molar ethanolic solution of **1** (5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde)) and **14** (5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-amine) for fourteen hours in presence catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After completion of the reaction, a yellow product was obtained and found 62% yield. The melting point was recorded as 195-199^0 C.



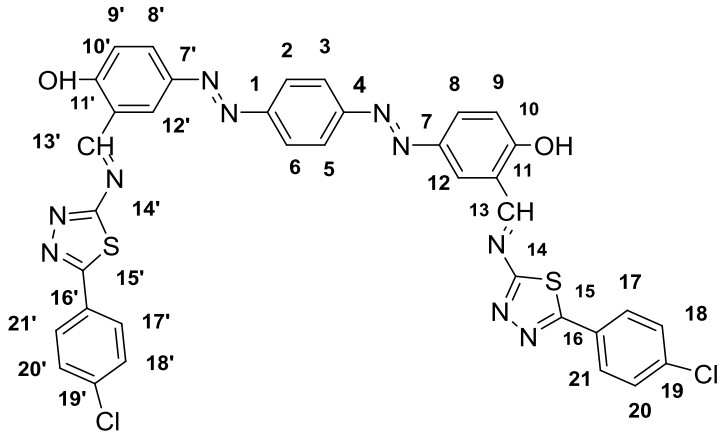
18

The IR spectrum (**Fig. 17**) of the compound **18** showed symmetric absorption band at 3443 cm^{-1} for phenolic O-H group. Theasymmetric O-H vibration was observed at 3175 cm^{-1}. The aromatic

C-H stretching frequency was identified at 3114 cm^{-1}. The band at 1600 cm^{-1} was detectable for C=N moiety. The characteristic peaks at 1575, 1550 and 1480 cm^{-1} were distinguished for aromatic C=C bonds. The C-S-C linkage was assignable at 826 cm^{-1}.

The ^{1}H NMR spectra (**Fig. 18-19**) of the compound **18**showed triplet at 6.80 for two equivalent protons at C-8 and C-8′ with the short and long range coupling constant J = 8.0 at J = 4.0 respectively. The doublet for two protons at C-12 and C-12′ with the coupling constant J = 8.0 was detectable at 6.88. The triplet for the two protons of C-9, C-9′ was assignable at 7.18. The broad singlet at 7.88 was designated for four protons of C-17, C-17, C- 21, C-21′ and two protons of C-13 and C-13′. The other broad singletwas ascribed for four protons of C-18, C-18′, C-20 and C-20′ at 8.06. The sharp singlet at 8.38 was distinctive for four protons of C-2, C-3, C-5, and C-6. The singlet at 10.23 for one proton was distinguishable for phenolic O-H. The other O-H proton was identifiable at 11.36.

All the spectral evidences expressed harmony with the structure of the compound **18** as



18

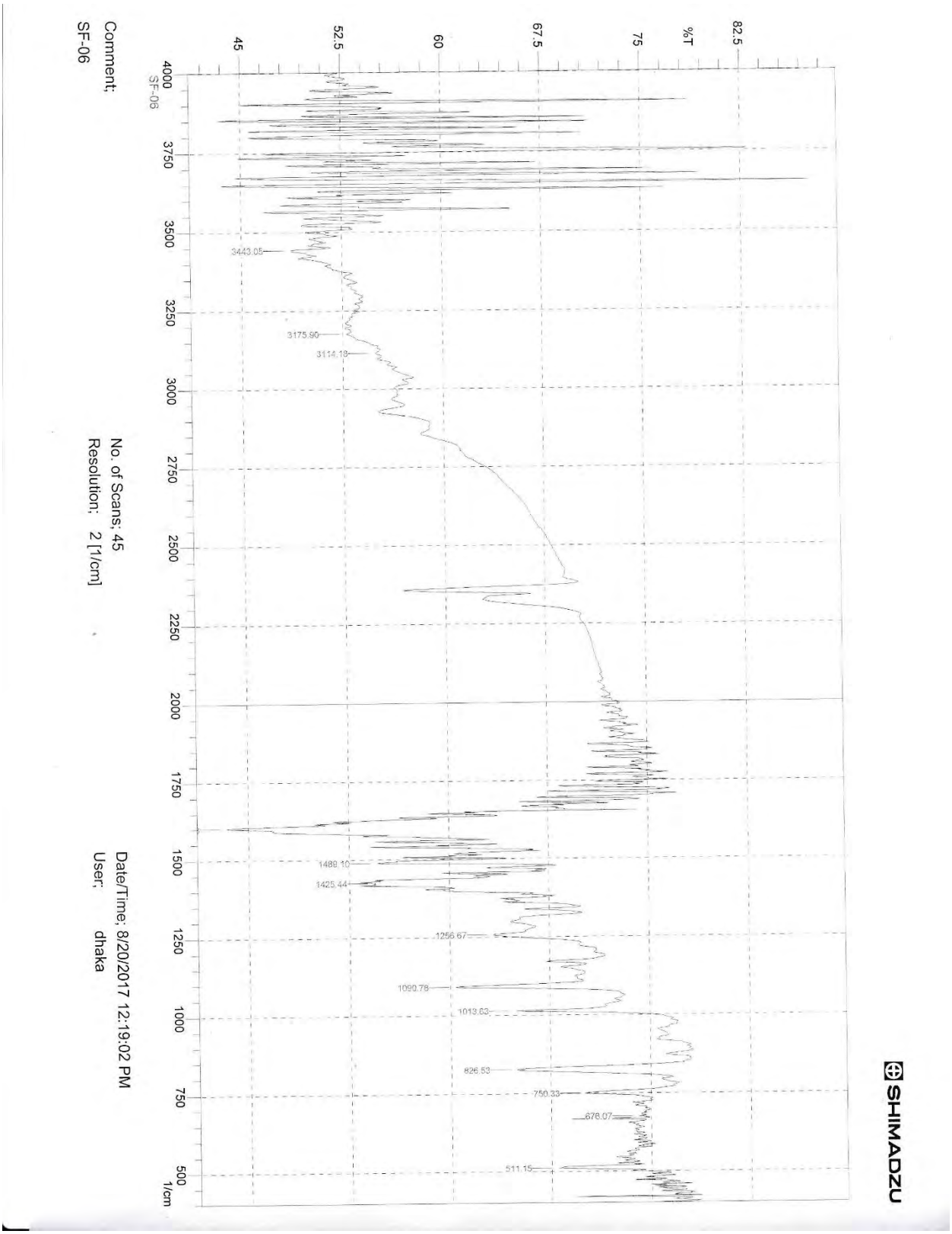


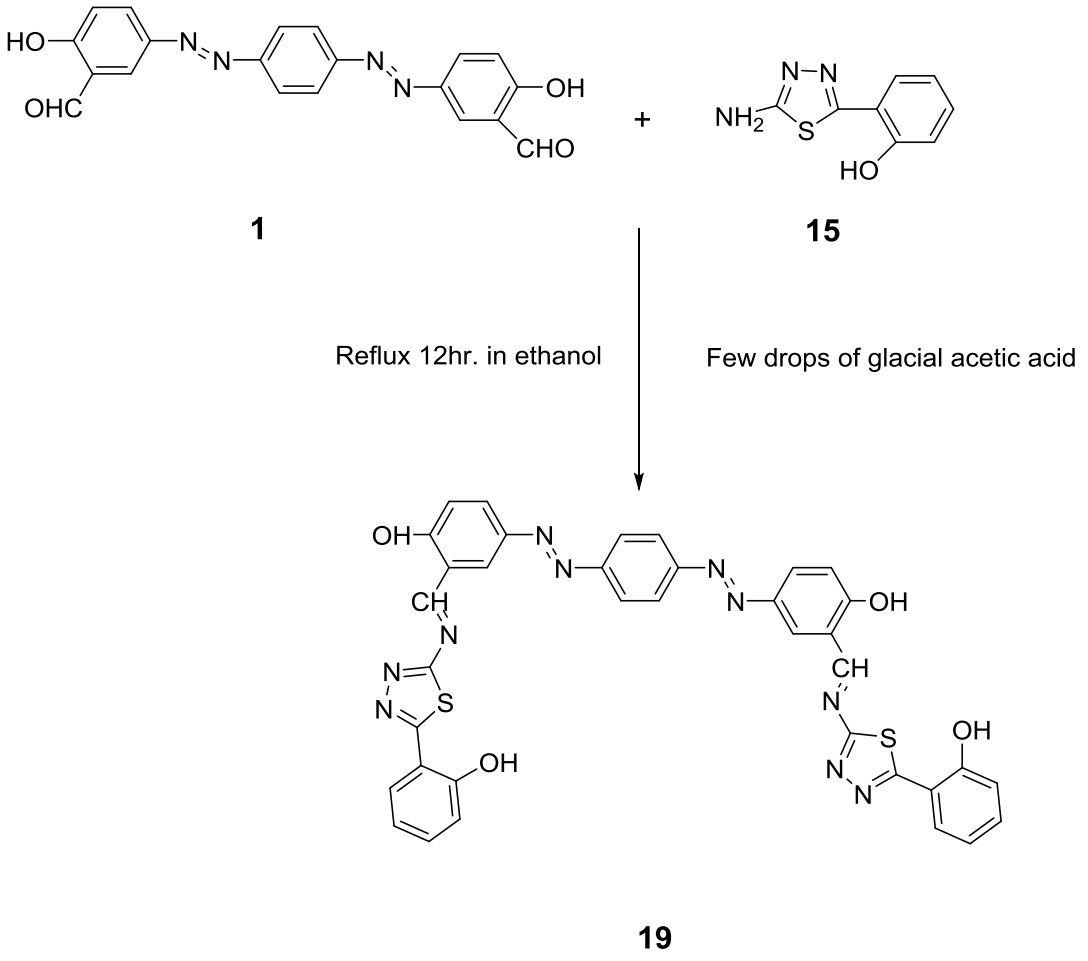
Fig – 17: IR Spectrum of Compound 18

Fig – 18: ^{1}H- NMR Spectrum of Compound 18

Fig – 19: Extended ^{1}H- NMR Spectrum of Compound 18

3.4. Characterization of the compound 19 (4,4'-((1E,1'E)-1,4-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **19** was synthesized by refluxing 1: 2 molar ethanolic solution of **1** (5, 5'-(1, 4-phenylenebis (diasene-2, 1-diyl)) bis (2-hydroxybenzaldehyde)) and **15** (5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-amine) for twelve hours in presence catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After completion of the reaction, a yellow crystalline product was obtained and found 75% yield. The melting point was recorded as 199-202^0 C.

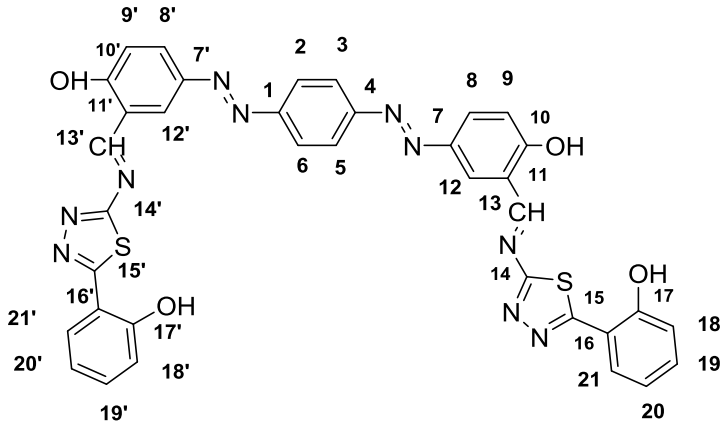


19

The IR spectra (**Fig. 20**) of the compound **19** showed broad absorption band at 3450 cm^{-1} for symmetric stretching of phenolic O-H group. Theasymmetric vibration of O-H band was found at 3325 cm^{-1}. The other phenolic O-H absorption bands were distinctive at 3180 cm^{-1} and 3125 cm^{-1} respectively. The aromatic C-H stretching frequency was assigned at 3036 cm^{-1}. The characteristic band at 1615cm^{-1} was detectable for C=N moiety. The peaks at 1540, 1490 and 1450 cm^{-1} were distinguished for aromatic C=C bonds. The C-S-C linkage was identified at 830 cm^{-1}.

The ^{1}H NMR spectra (**Fig. 21-22& 23**) of the compound **19**showed doublet at 6.89 for one proton of C-12 with the coupling constant J = 8.0. Another doublet at 6.95 was attributable for one proton of C-12′ with coupling constant J = 8.0. The doublet at 7.46 was ascribed for two protons of C-2 and C-6 with coupling constant J = 8.0. The signal at 7.48 showed a doublet for two protons of C-3 and C-5 with the long range coupling constant J = 4.0. The doublet at 7.52 was assigned for two protons of C-9 and C-9′ with coupling constant J = 8.0. The signal at 7.60 was designated as doublet for two protons of C-8 and C-8′ with the coupling constant J = 8.0. The signal at 7.70 showed a doublet for two protons of C-17 and C-21 with the coupling constant J = 8.0. The doublet at 7.84 was distinctive for two protons of C-17′ and C-20′ with the coupling constant J = 8.0. The doublet at 8.02 was indicative for two protons of C-18′ and C-20′ with the coupling constant J = 8.0. The signal at 8.05 showed a doublet for two protons of C-18 and C-20 with the coupling constant J = 8.0. The broad singlet at 8.21 was ascribable for one proton of C-13 (HC=N). Another broad singlet for one proton C-13′ was designated for (HC=N) at 8.24. The downfield singlet signals at 11.27, 11.29, 11.42 and 11.46 were distinguished for four O-H protons of C-10, C-10′, C-21 and C-21′ respectively. The presence of four O-H protons in the 1H NMR spectrum clearly indicates that all the aromatic protons of the two sides of the *P*-Phenylene diamine not equivalent. This may be due to the hydrogen bonding of the O-H group with the sulphur atom of thiadiazole ring.

All the spectral evidences expressed harmony with the structure of the compound **19** as



19

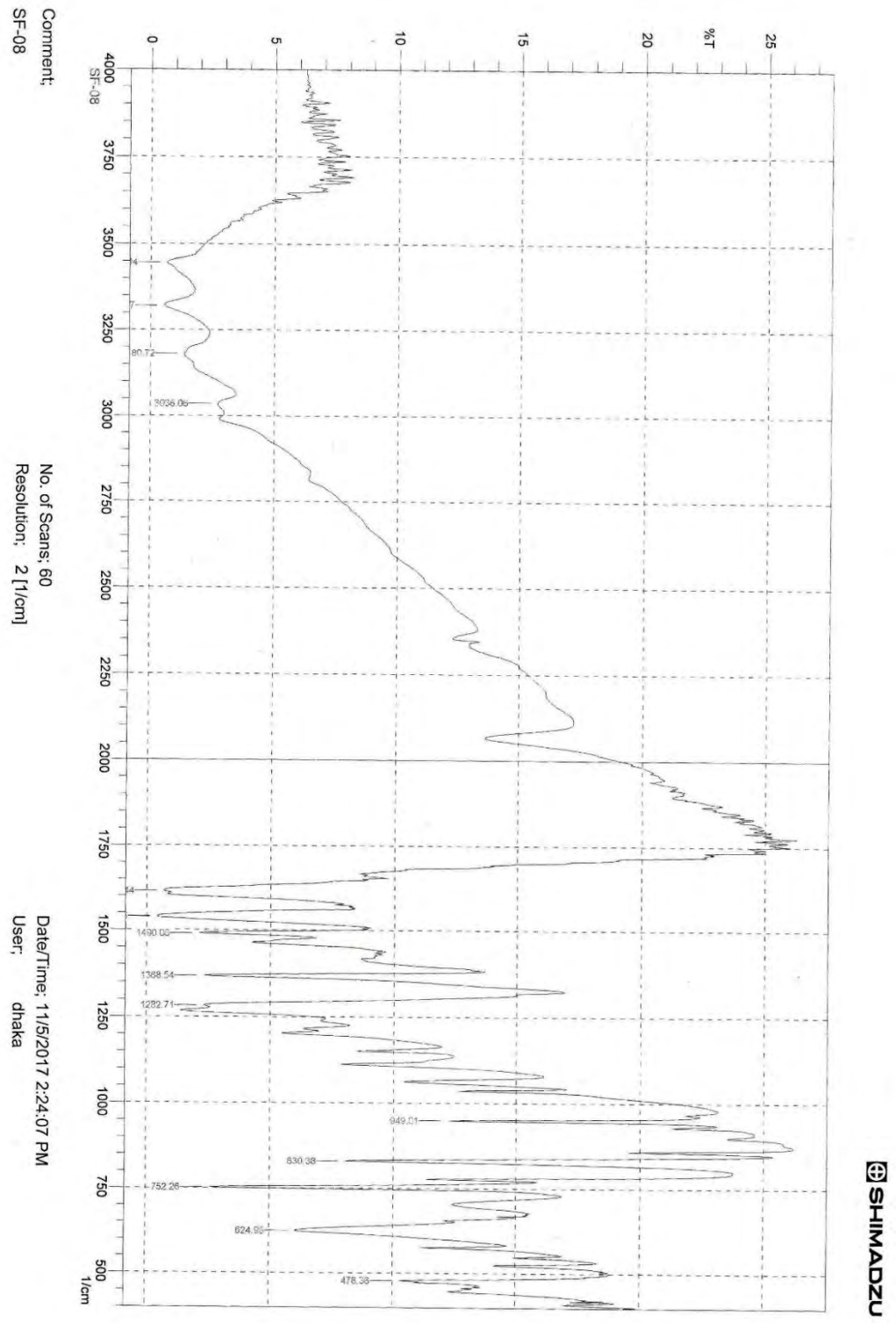


Fig – 20: IR Spectrum of Compound 19

Fig – 21: ^{1}H- NMR Spectrum of Compound 19

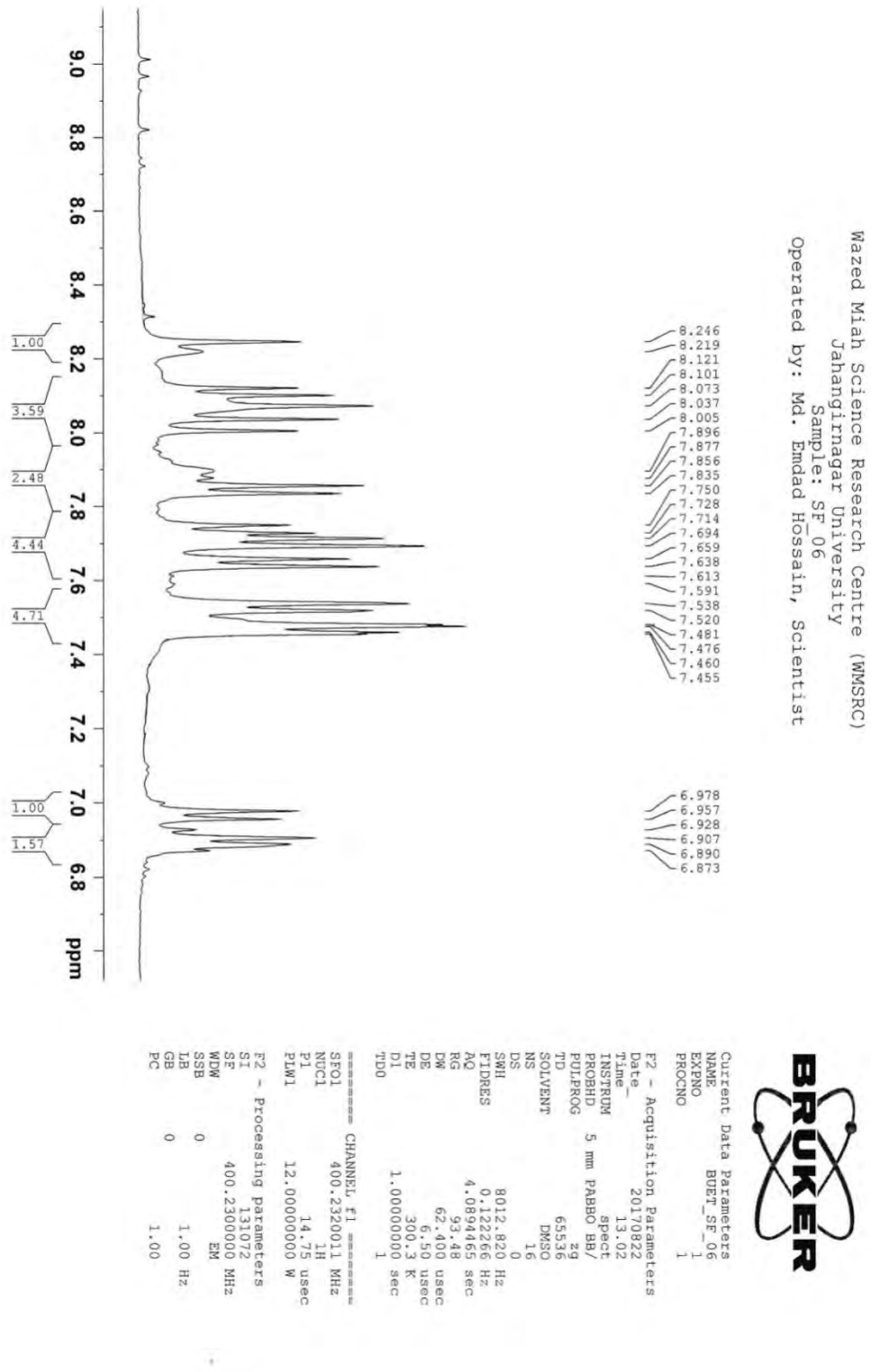
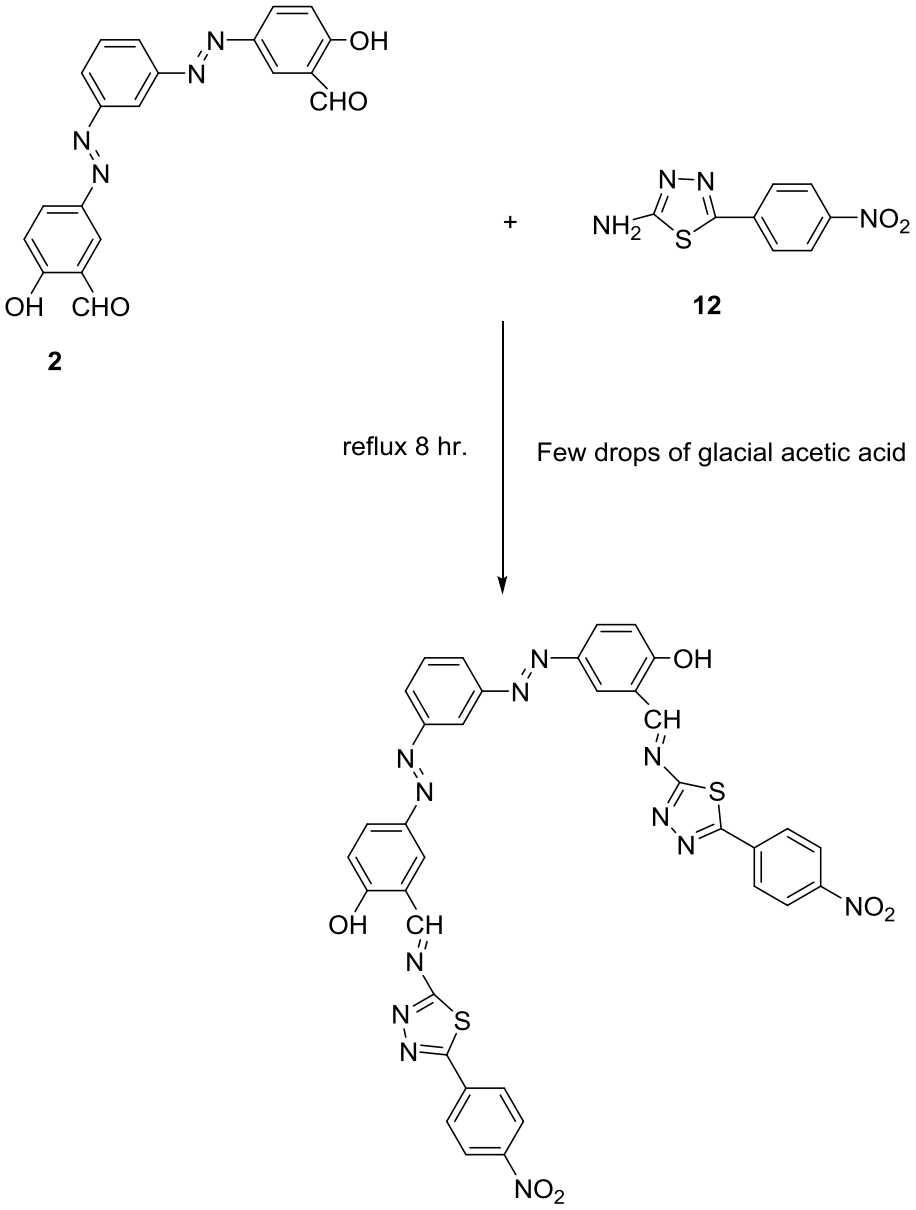


Fig – 22: Extended ^{1}H- NMR Spectrum of Compound 19

Fig – 23: Extended ^{1}H- NMR Spectrum of Compound 19

3.5. Characterization of the compound 20 (4,4'-((1E,1'E)-1,3-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **20** was synthesized by refluxing 1: 2 molar ethanolic solution of **2** (5, 5'-(1, 3-Phenylenebis(diazene-2,1-diyl)) bis (2-hydroxybenzaldehyde)) and **12** (5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine) for eight hours in presence of catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After completion of the reaction, a yellow crystalline product was isolated and found 70% yield. The melting point was recorded as 166-168^0 C.

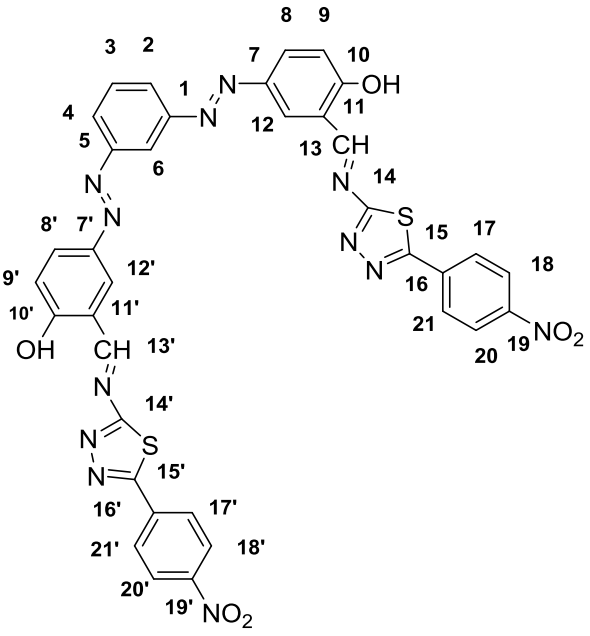


20

The IR spectra (**Fig. 23**) of the compound **20** showed symmetric absorption band at 3445 cm^{-1} for phenolic O-H group. Theasymmetric vibration of the O-H was observed at 3325 cm^{-1}. The aromatic C-H stretching was identified at 3150 cm^{-1}. The absorption peak of C=N moiety was designated at 1614cm^{-1}. The characteristic band at 1590, 1530 and 1490 cm^{-1} were distinguished for aromatic C=C bonds. The weak absorption band at 830 $cm^{-1}$was attributed for C-S-C linkage. The ^{1}H NMR spectra (**Fig. 24-25**) of the compound **20** showed a doublet at 6.81 for four protons

of C-16, C-17, C-16′ and C-17′ with the coupling constant J = 8.0. The doublet at 6.83 was attributed for four protons of C-18, C-18′, C-20 and C-20′, with the coupling constant J = 8.0. The triplet at 7.21 was ascribable for one proton of C-3 with the coupling constant J = 8.0. The broad singlet at 7.42 was distinctive for two protons of C-4 and C-2. The broad doublet at 7.98 was designated for four protons of C-8, C-8′, C-9 and C-9′ with coupling constant J = 8.0. The broad singlet at 8.05 was indicative for two protons of C- 12 and C-12′. The broad singlet for one proton of C-6 was assigned at 8.08. The singlet signal for two protons of HC=N moiety was attributed at 8.37. The two down fielded singlet were characterized for two protons of two O-H groups at 9.89 and 11.36 respectively.

All the spectral evidences supported the structure of the compound **20** as



20

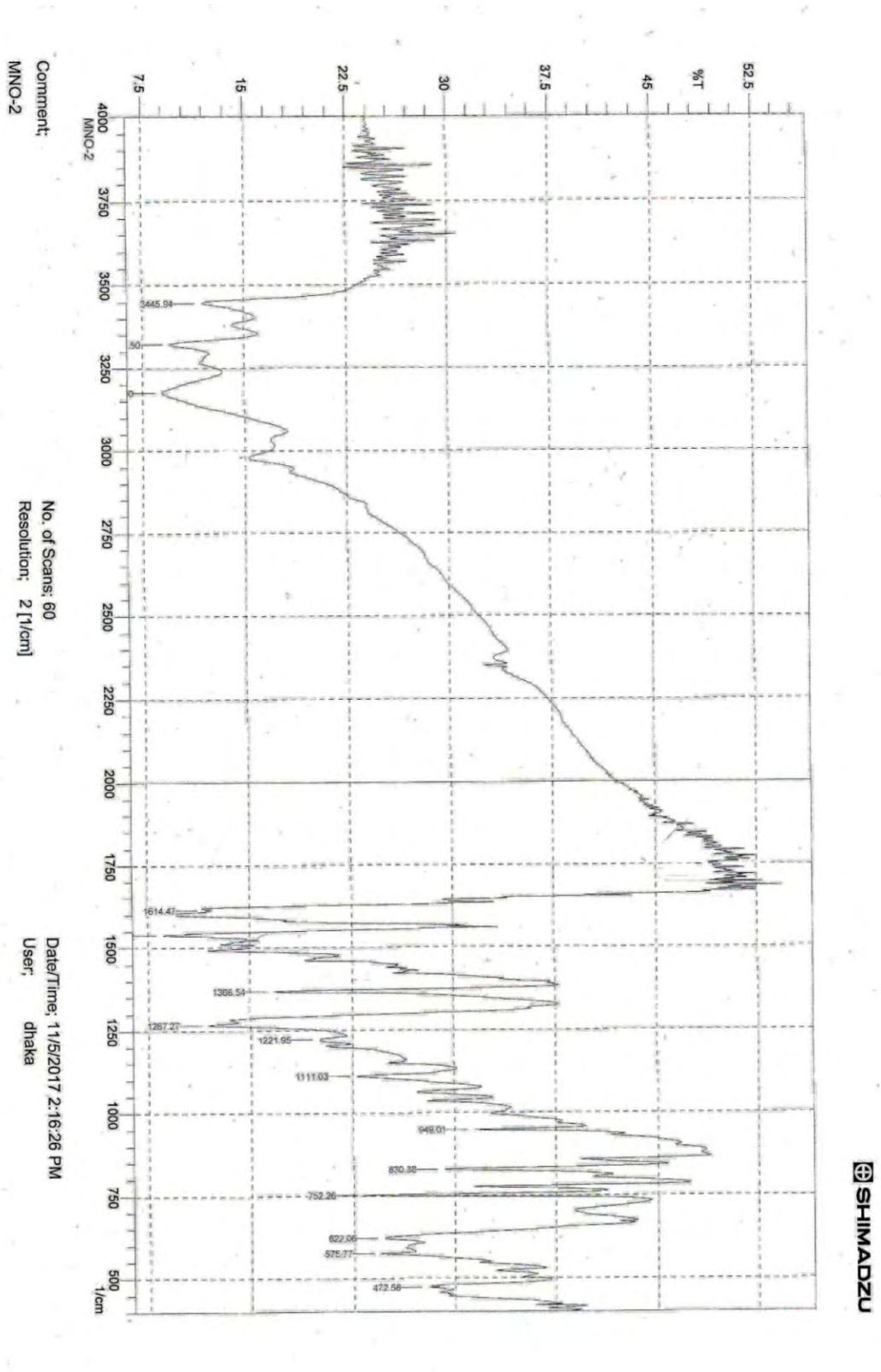


Fig – 24: IR Spectrum of Compound 20

Fig – 25: ^{1}H NMR of Compound 20

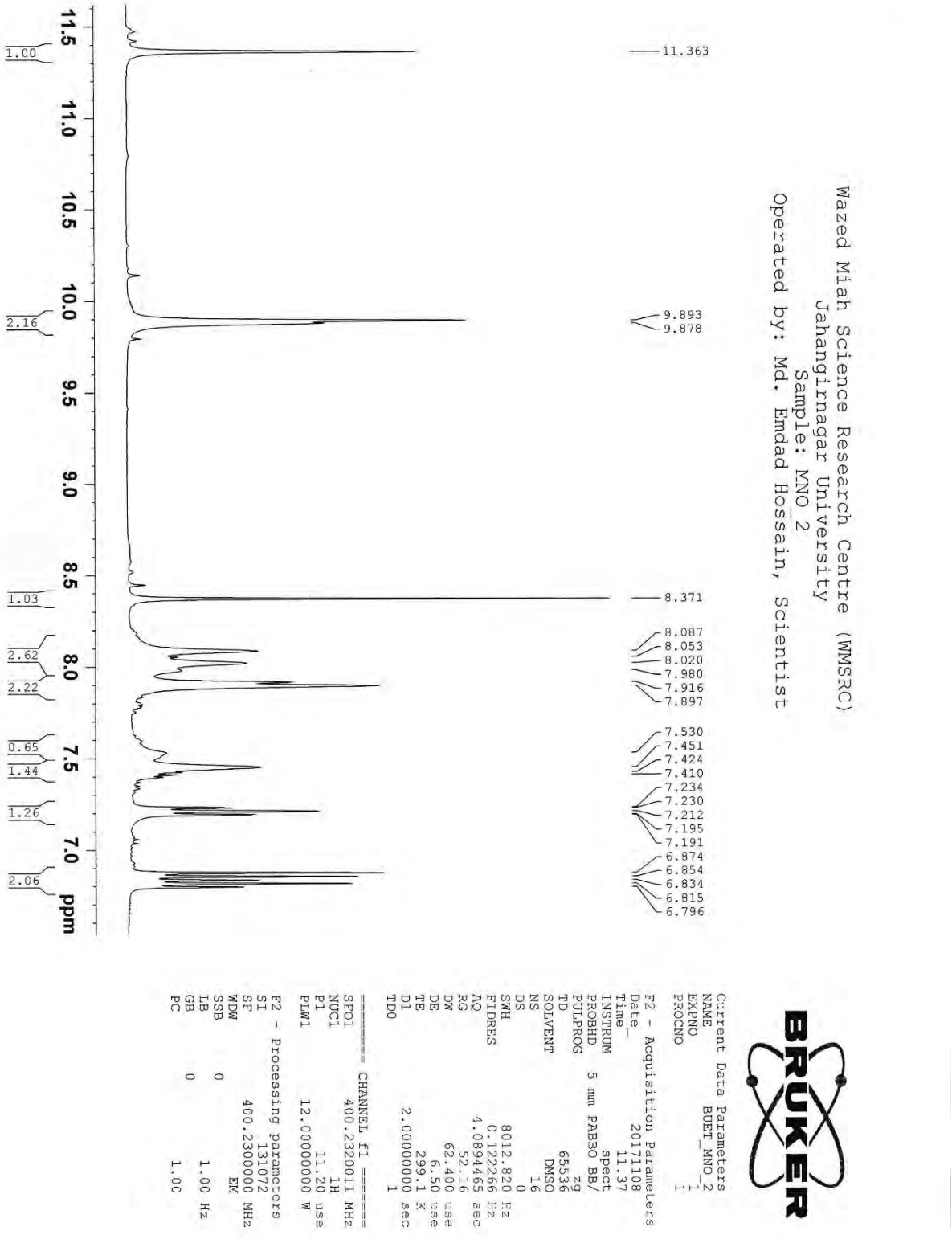
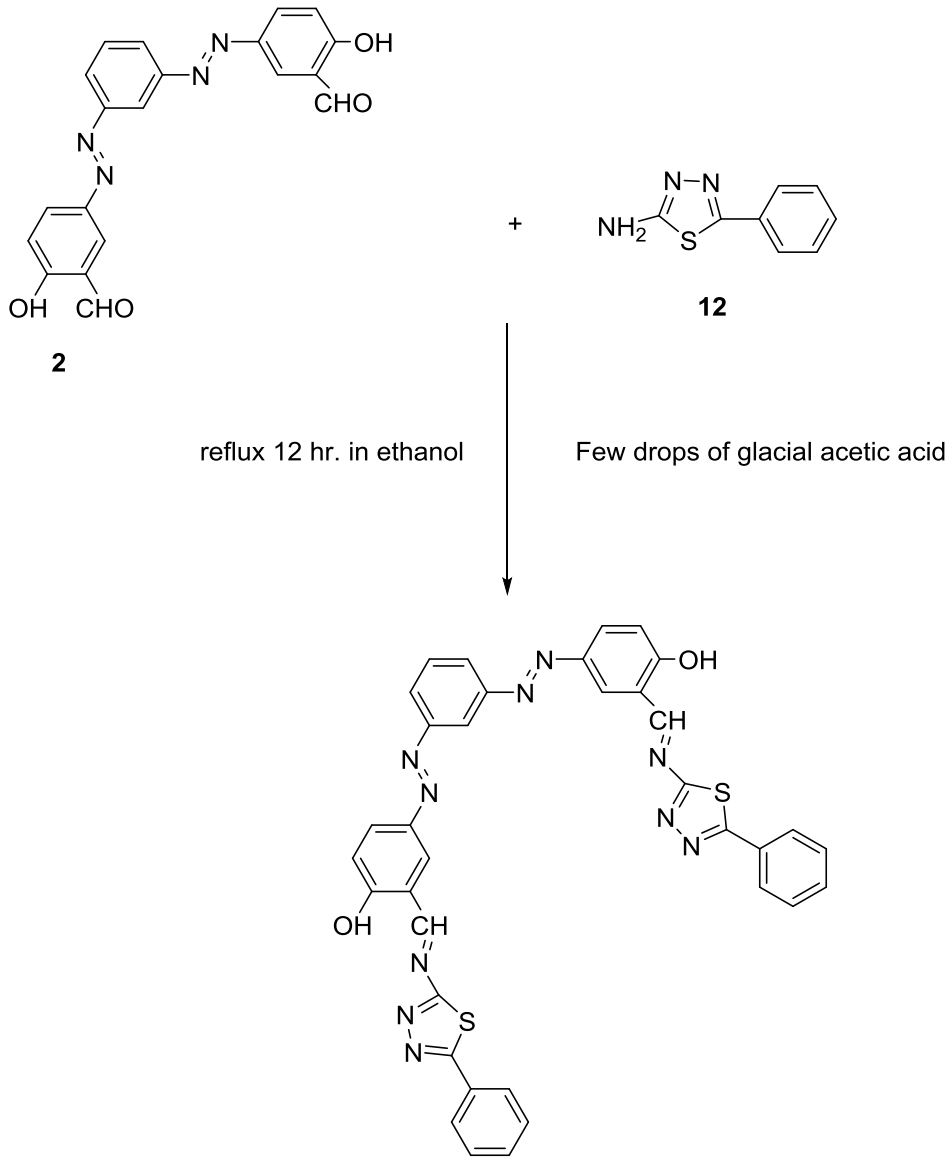


Fig – 26: Extended ^{1}H NMR Spectrum of Compound 20

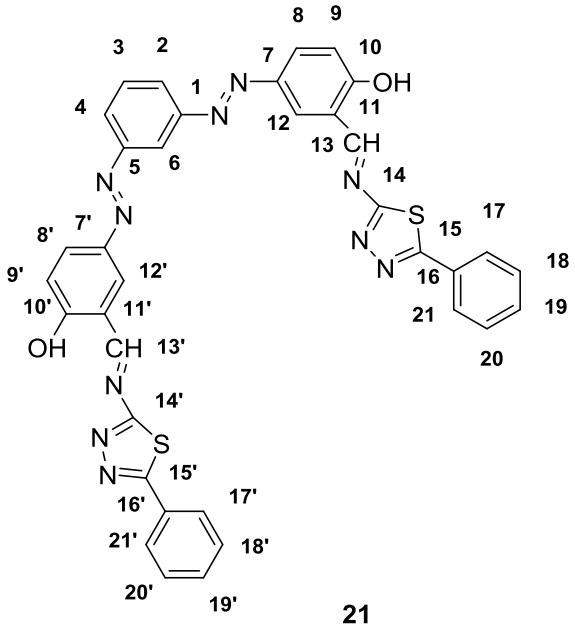
3.6. Characterization of the compound 21 (4,4'-((1E,1'E)-1,3-phenylenebis(diazene-2,1-diyl)bis(2-((E)-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol))

The compound **21** was synthesized by refluxing 1: 2 molar ethanolic solution of **2** (5, 5'-(1, 3-Phenylenebis(diazene-2,1-diyl))bis(2-hydroxybenzaldehyde)) and **13** (5-phenyl-1, 3, 4-thiadiazol-2-amine) for eight hours in presence of catalytic amount of glacial acetic acid. The progress of the reaction was monitored by TLC. After completion of the reaction, the brown solid product was isolated and found 75% yield. The melting point was recorded as 172-173^0 C.



The IR spectra (**Fig. 26**) of the compound **21** showed wide symmetric absorption band at 3313 cm^{-1} for phenolic O-H group. Theasymmetric absorption peak at 3244 cm^{-1} was distinctive for O-H group. The aromatic C-H stretching vibration was detectable at 3050 cm^{-1}. The absorption peak of C=N moiety was assigned at 1610 cm^{-1}. The characteristic bands at of 1550, 1491 and 1449 cm^{-1} were distinguished for aromatic C=C bonds. The peak at 758 cm^{-1} was designated for C-S-C linkage.

The ^{1}H NMR spectra (Fig. 27-28) of the compound **21** showed down fielded singlet at 10.01 and 9.02 for two phenolic protons. The singlet at 8.43 was attributable for two protons of HC=N. All other protons distinguished as aromatic, because the signals were in aromatic protonic range. The structure of the compound**21** was suggested as



21

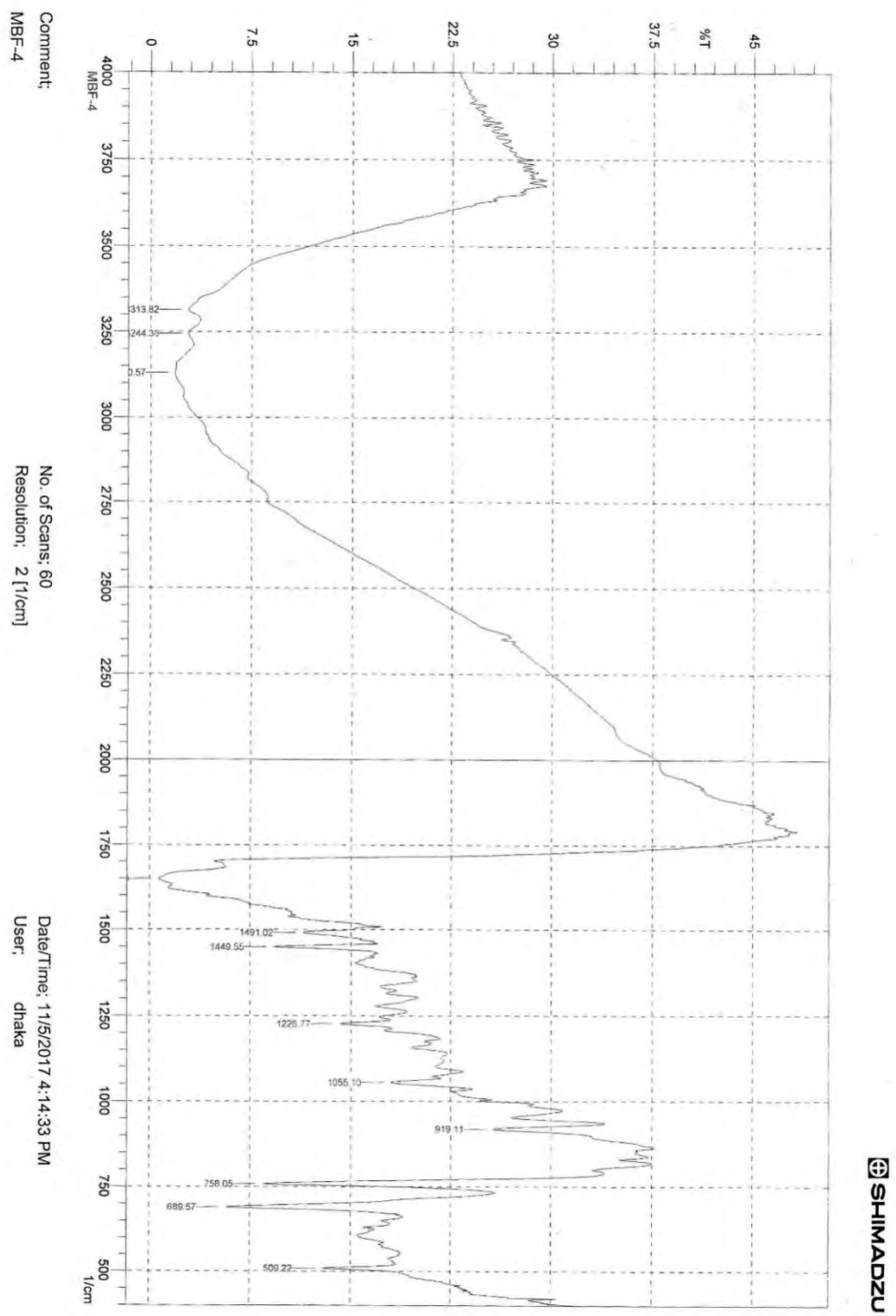


Fig – 26: IR Spectrum of Compound 21

Fig – 27: ^{1}H Spectrum of Compound 21

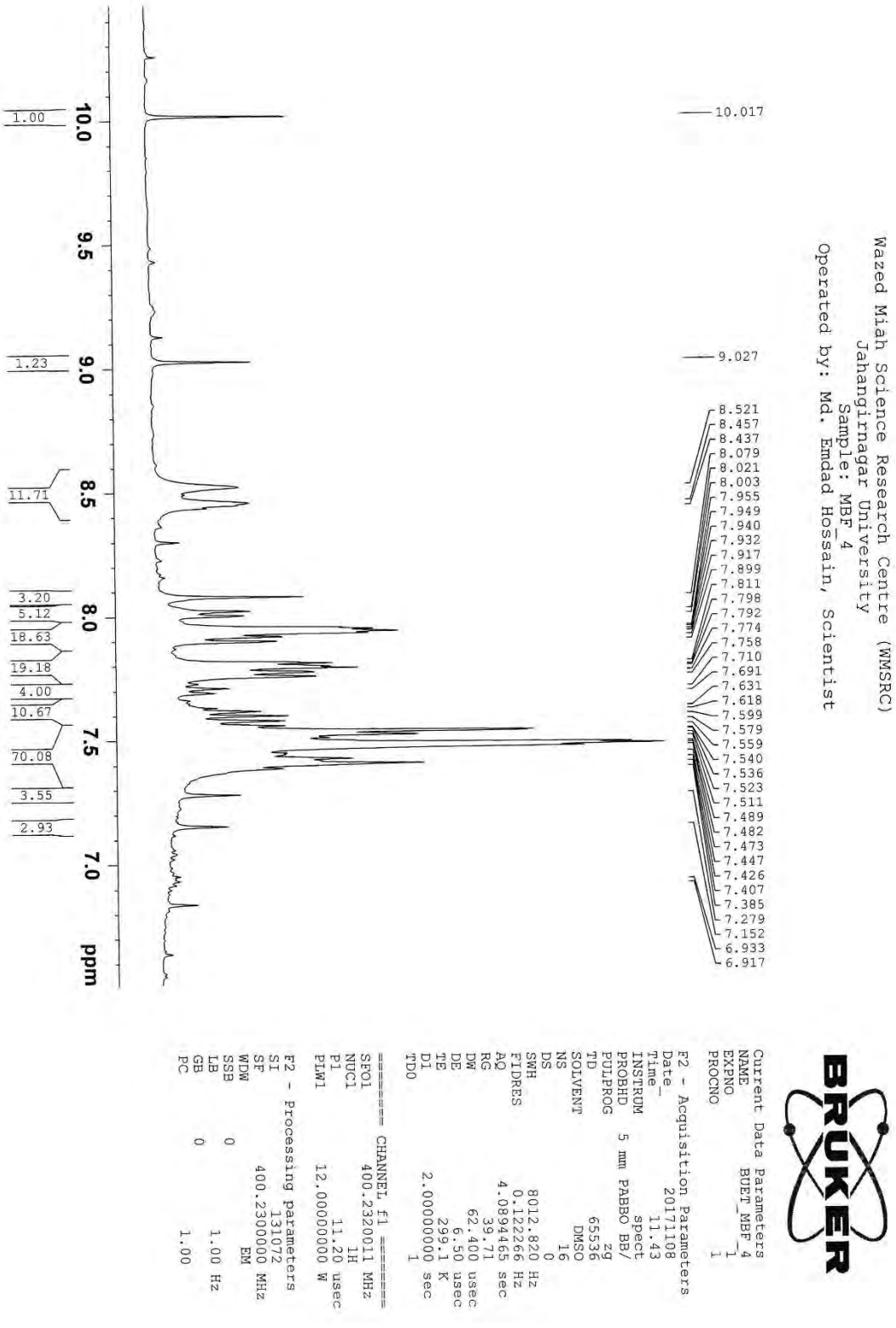


Fig – 28: Extended ^{1}H NMR of Compound 21

Chapter 4

Biological Activity

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Principle

An antibiotic refers to a substance produced by a microorganism or to a similar substance produced wholly or partly by Chemical synthesis, which in low concentration inhibit the growth of other microorganisms. Many antibiotics are used as chemotherapeutic agent but other is too highly toxic for use. In case of chemotherapeutic use, an antibiotic should, ideally be toxic only for pathogenic microorganisms and should be harmless to the host. Assay of antibiotics include certain methods such as microbiological assays, radio enzymatic assays, high performance of high-pressure liquid chromatography (HPLC), immunoassay etc. There are three main methods of microbiological assay by which the potency of an antibiotic can be compared with that of a standard antibiotic.

A. Dilution method
B. Diffusion method
C. Turbidimetric, titrimetric and gravimetric methods.

Among these methods the agar diffusion method is most commonly used to assay the potency of an antibiotic. In agar diffusion assays, the response of a growing population of microorganism to the antimicrobial agent is measured. Potency is a term used to express the strength of chemical. Antibiotics, originated from microorganism, also undergo chemical formulation and thereby need to be evaluated in terms of potency for clinical use as well as for market value. While assaying the potency of an antibiotic, proper calibration is essential to express the result in terms of absolute units. Thus a pure sample of the drug or a sample of known potency is required for the preparation of calibrator solutions.

The agar diffusion assay may be one dimensional, two- dimensional or three-dimensional. Two or three-dimensional assay is the commonest form of microbiological assay. This experiment employs the three-dimensional agar diffusion assay. In this system samples are applied in reservoir (well) to a thin layer of agar seeded with indicator organism. The drug diffuses in to the medium. After incubation a zone of growth inhibition forms (a circle around the reservoir). The diameter of the zone is related to the concentration of antibiotic in the reservoir.

The edge of a zone is formed when the minimum concentration of antibiotic which will inhibit the growth of the organism on plate (Critical Concentration) reaches for the first time (when the population density is too great for the antibiotic to inhibit). The position of the zone edge is thus determined by.

1. The initial population density.
2. Growth rate of the organism.
3. The rate of diffusion of antibiotic.
4. Agar thickness.

A balance design is one in which all controllable variables have been accommodated and such design is called "Latin square design". A Latin square arrangement is acceptable for pharmacokinetic or clinical assays. In situations where the concentration range of tests will lie in anarrow range and where high precision is sought, a Latin square design with tests and calibrators at two or three levels of concentration may be used. As a result this assay ensures maximum precision. Different types of Latin square designs are known. In this experiment, the potency of the supplied antibiotic was determined by three-dimensional agar diffusion assay using 4*4 (2+2) Latin square design.

The 4*4 (2+2) Latin square design is used for the assay of one sample and one standard at two concentrations each and allows four zones for each concentration in a true Latin square design where each treatment appears once in every row and in every column. The plate size was (20*20) inches.

Material and Method

Sample

Supplied 6 synthesized sample

Culture

S.aureus, B.cereus, E.coli

Medium

Nutrient agar

Reagents

Phosphate buffer and 0.1 N HCl

Equipment

Large Petri-dish, micropipette, electric balance, pipette, puller, conical, flask, borer, incubator, inoculating loop, burner and test tubes.

Procedure

1. **Preparation of test sample**: 0.01mg of sample dissolves in 0.9 ml of solvent; prepare total 1ml stock sample. This experiment employs the three- dimensional agar diffusion assay. In this system samples are applied in reservoir (well) to thin layer of ager seeded with indicator organism. The drug diffuses in to the medium. After incubation a zone of growth inhibition forms (a circle around the reservoir). The diameter of the zone is related to the concentration of antibiotic in the reservoir.

2. After finishing all these preparations, 0.75 ml bacterial suspension was added to 150 ml nutrient agar media (in liquid state) in conical flask and by swivel to avoid bubble.
3. The petri-plates were placed on a level bench and precaution was taken to keep the plate sterile. Then media seeded with bacterial suspension was poured in the petri-plate using a circular motion to ensure the even distribution of medium over the plate and allowed to stand for a few minutes for solidification.
4. Then using a sterile borer, well were designed in such a way that every well is apart from another by the same distance.
5. Two drops of each of the standard and the test solutions were dropped in wells.
6. After dropping the plate was kept undisturbed for about half an hour and then incubated at 37^0 c for 24 h.

7. After overnight incubation, formation of any zone of growth inhibition was monitored.
8. The diameter of zone of inhibition was then measured and the potency of drug was calculated as below.

Biological Activity

It was judicious to investigate the synthesis of new Azo Schiff bases and studied their anti-bacterial activity against three strains of bacteria (*S.aureus*, *B.cereus*, and E.coli) **(diagram 1 & 2)**. The volume used for the screened compounds are 50 µl and 100 µl. The sample was prepare by 0.01 mg of sample dissolves in 0.9 ml DMSO solvent; prepare total 1ml stock solution. Azithromycin was used as reference standard while DMSO as control and inhibition zones is measured in mm. The new compounds were tested against one strain each of a gram negative and two gram positive. The test results presence in **Table** (1). All new compounds were active against tested in 100 µl but in 50 µl compounds not shown activity aginist E.coli.

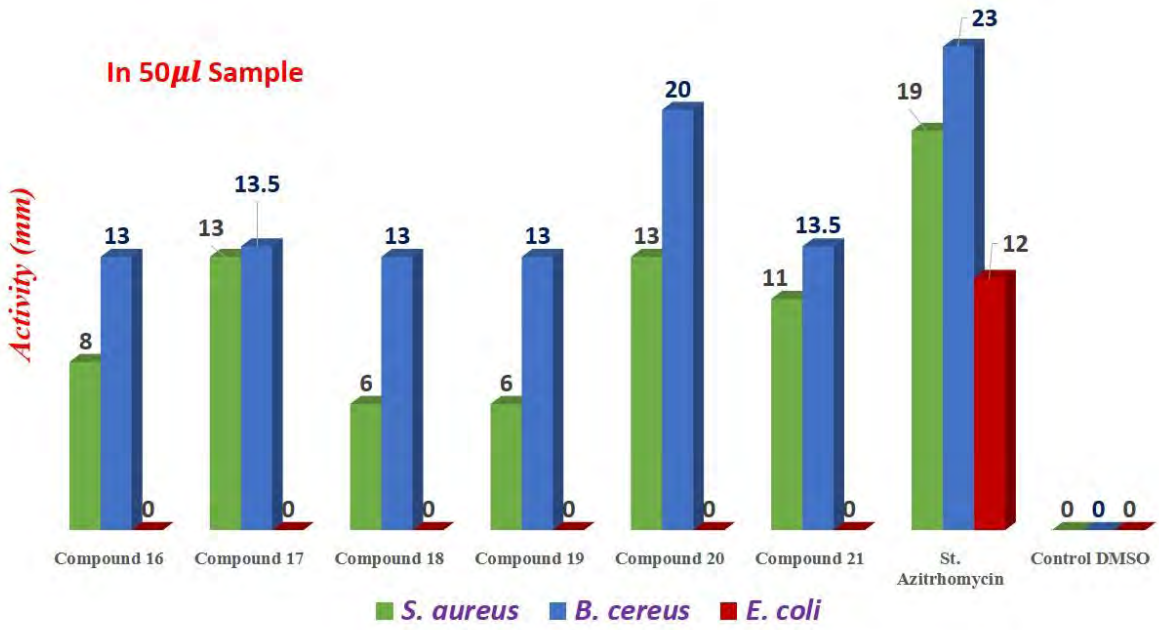


Diagram – 1: Biological activity Chart of 50 µl Sample

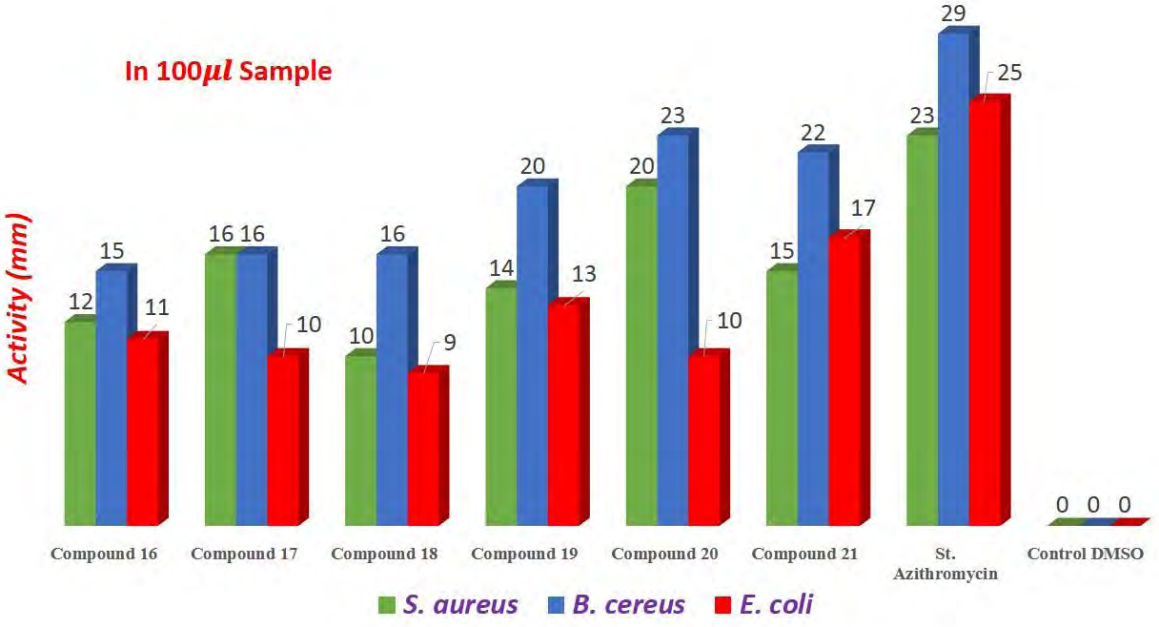
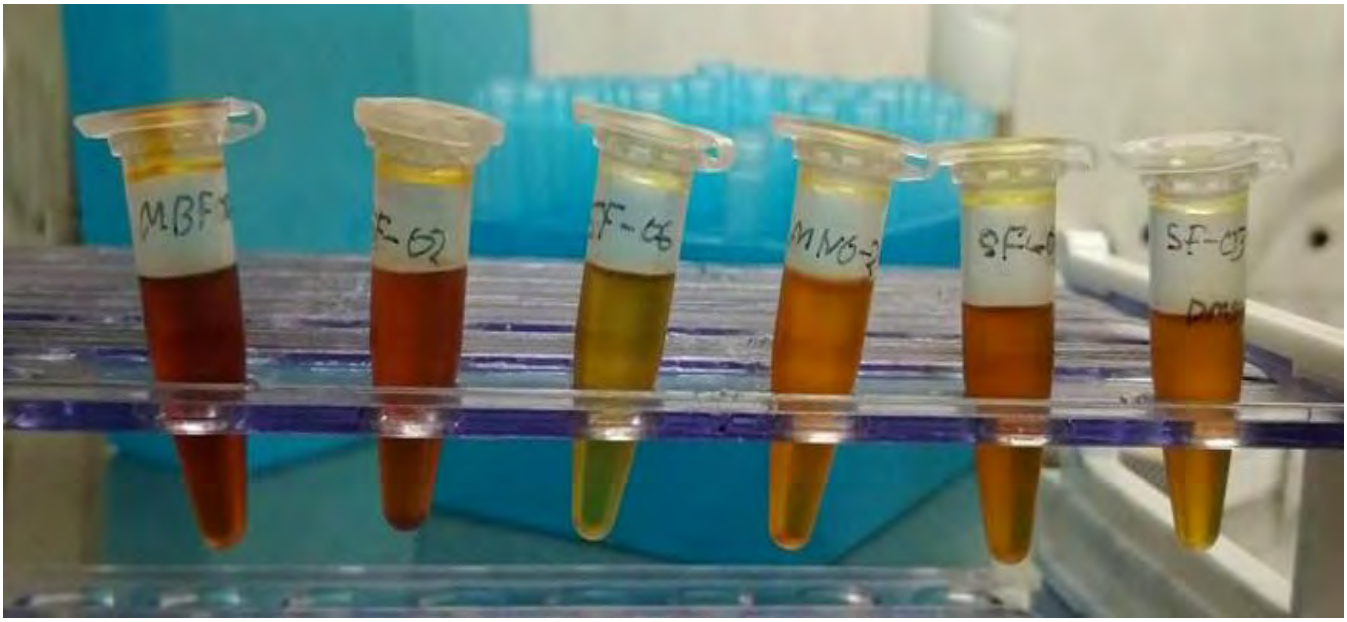


Diagram – 2: Biological activity Chart of 100 µl Sample

Table 1: Effect of new azo Schiff bases on the growth of tested bacteria

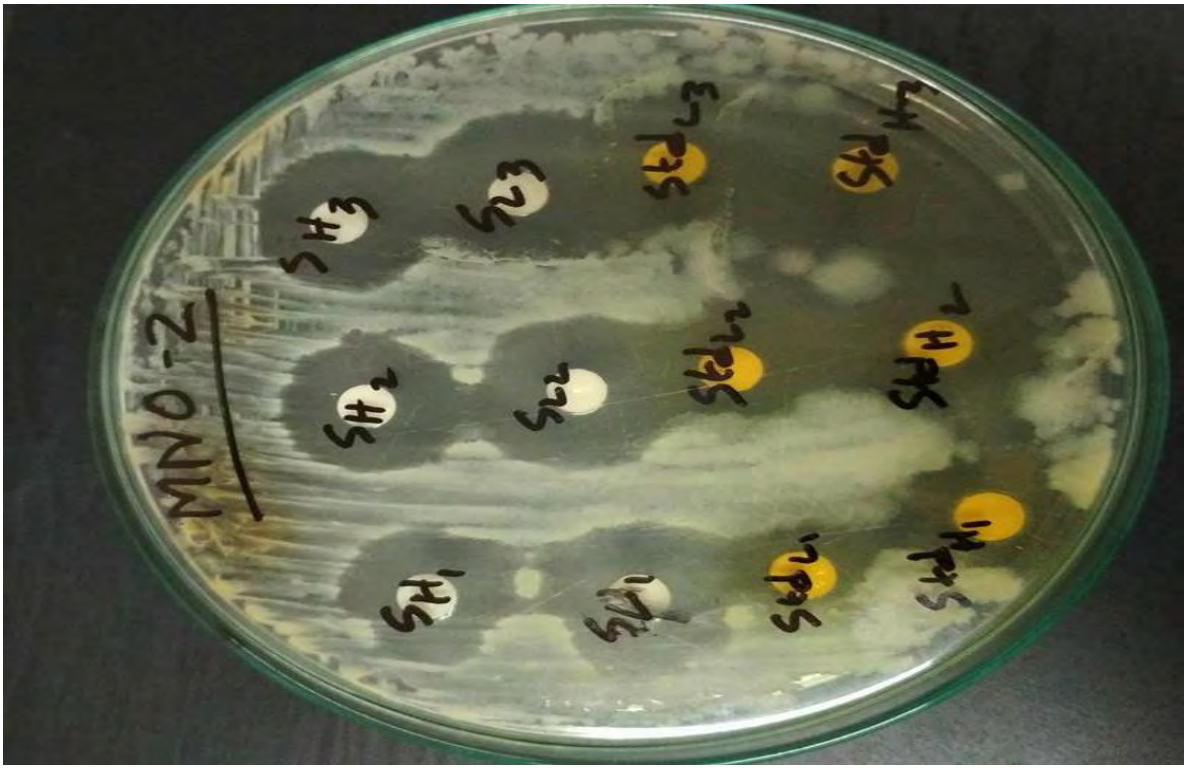
Sample	Gram positive				Gram negative	
	S.aureus		*B.cereus*		*E.coli*	
	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
Compound 16	8 mm	13 mm	13 mm	15 mm	0 mm	11 mm
Compound 17	13 mm	16 mm	13.5 mm	16 mm	0 mm	10 mm
Compound 18	6 mm	10 mm	13 mm	16 mm	0 mm	9 mm
Compound 19	6 mm	14 mm	13 mm	20 mm	0 mm	13 mm
Compound 20	13 mm	20 mm	20 mm	23 mm	0 mm	10 mm
Compound 21	11 mm	15 mm	13.5 mm	22 mm	0 mm	17 mm
Satandard Azithromycin	19 mm	23 mm	20 mm	25 mm	12 mm	22 mm
Control DMSO	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm



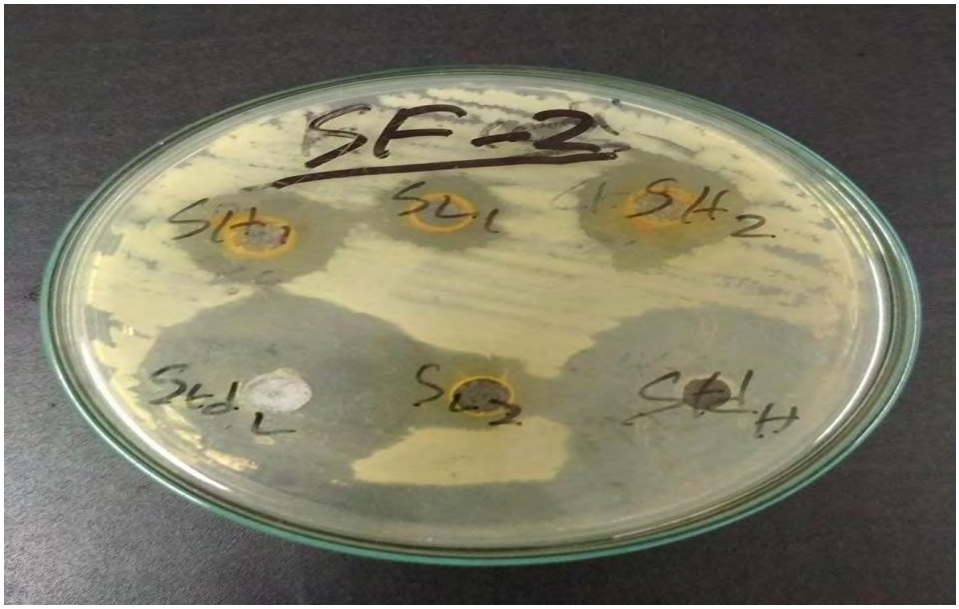
Pic: New Schiff's Base Compounds Dissolve in DMSO Solvent for Biological Activity Test

Pic: Biological activity of 6 Compounds new azo schiff's base against *B.cereus* Becteria in 100 µl sample

Pic: Biological activity of 6 new azo schiff's base Compounds against *B.cereus* Becteria in 50µl sample



Pic: Biological Activity of Standard Azithromycin (white colour) Compare to Compound 20



Pic: Biological Activity of Standard Azithromycin (white colour) Compare to Compound 17

CONCLUSION

During the last two or three decades, attention has been increasingly paid to the synthesis of Azo Schiff bases which exhibits various biological activities including antibacterial, fungicidal, tuberculostatic and plant growth regulative properties. The (16, 17, 18, 19, 20, 21) compounds are new and were prepared for the first time. The new compounds were identified by IR, ^{1}H NMR, ^{13}C NMR spectral methods. Some of the prepared compounds have been biologically screened i.e. studying their effects against two gram-positive, one gram-negative bacteria. The results show that their activities were found to vary from moderate to very strong.

References

Reference:

1. Patil, C. J., Patil Manish, C., Pachpol, N. R., and Waykole, V. S., Coupling Reaction Involving Aryldiazonium salt: part-Ⅱ Chemoselective Condensation with Acetylacetone and Antibacterial Activity, *Der. Chemica. Sinica*; 6 (50), PP. 115-114, **2015**.
2. Chakraborty, A., Saha, P. K., Datta, C., Synthesis and Application of Azo-napthol dyes on wool, silk and naylon fabric, *7th Int. Conf. TEXSCI ,*Liberec, Czech. Rep. 6-8, Sept., **2010**.
3. Gosai, D. R., Nimavat, K. S., Vyas, K. B., Azo dyes based on salicylic acid-formadehyde polymer as a polymeric ligands. *Der. Pharma.Chem*., 3 (4), PP. 491-500, **2011**.
4. Simu, G. M., Dragomirescu A., Grad M. E., Savoiubalint, G., Andoni, M., Bals G., Azo compounds with antimicrobial activity, *14th Int. Electron. Conf. Syn. Org. Chem.* ECSOC-14, PP.1–30, **2010.**
5. Garg, H. G., Prakash, C., Preparation of 4-arylazo-3,5- disubstituted-(2H)-1,2,6-thiadiazine 1,1- dioxides, *J. Med. Chem*., 15 (4), PP. 435-436, **1972.**
6. Browing, C. H., Cohen, J. B., Ellingworth, S., Gulbransen, R., The antiseptic properties of the amino derivatives of styryl and anil quinoline, *Journal Storage*, 100, PP.293-325, **1926**.
7. Child, R. G., Wilkinson, R. G., Tomcu-Fucik. A., Effect of substrate orientation of the adhesion of polymer joints, *Chem. Abstr*., 87, PP. 603,**1977**.
8. Khalid, A., Arshad, M., Crowley, D. E., Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains, *Appl. Microbiol. Biotech*., 78, PP. 361- 369, **2008**.
9. Thoraya, A., Farghaly, Abdallah, Z. A., Synthesis, Azo-hydrazone tautomerism and antitumor screening of N-(3-ehtoxycarbonyl-4,5,6,7-tetrahydro-benzo[b]thien-2-yl)-2-arylhyrazono-3-oxobutanamide derivatives, *Arkivoc,* 17, P. 295, **2008**.
10. Goyal, R. N, Verma, M. S, Singha, N. K, Voltammetric investigations of the reduction of direct orange-31 a bisazo dye, *Croatica Chem. Acta*., 71(3), PP.715-726,**1998**.
11. Park C, Lim J, Lee Y, Lee B, Kim S, Lee J, Kim S, Optimization and morphology for decolorization of reactive black 5 by Funalia trogii, *Enzy. Microb. Tech*., 40,PP. 1758-1764, **2007**.

12. Chandravadivelu, G., Senniappan, P., In-vitro antimicrobial activity of novel derivative of azo dye from cyano ester, *Int.J.Res.Pharm.Chem*, 1 (4), PP. 1082-1086, **2011**.
13. Zaid Hassan Abood., Rahman Tama., Haiwal Hasan., Thamer ghanim., Sajid Maqsad Radhi., Synthesis of Some New Schiff Bases, Tetrazole and 1,3-Oxazepine Derivatives Containing Azo Group and 1,3,4- Thiadiazole Moiety. *Journal of Babylon University/Pure and Applied Sciences*/ No.(1)/ Vol.(21), **2013**.
14. M. Raj1., Hemul, V., Patel2., Lata, M., Raj3., and Naynika, K., Patel4.,synthesis and biological evaluation of some new 1,3,4-thiadiazole derivatives for their antimicrobial activities. *International journal of pharmaceutical, chemical and biological sciences (IJPCBS)*, 3(3), PP. 814-819, **2013**.
15. Kadi, A.A., Al-Abdullah, E.S., Shehata, I.A., Habib, E.E., Ibrahim, T.M., El-Emam, A., Synthesis antimicrobial and anti-inflammatory activities of novel 5-(1- adamantyl)-1,3,4-thiadiazole derivatives. *Eur J Med Chem*, 45, PP. 5006–5010, **2010**.
16. Kumar, D., Kumar, N.M., Chang, K., Shah, K., Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4- thiadiazoles. *Eur J Med Chem*, 45, PP. 4664–4668, **2010**.
17. Chen, Z., Xu, W., Liu,K., Yang, S., Fan, H., Bhadury, P. S., Hu, D., Zhang, Y., Synthesis and antiviral activity of 5-(4-chlorophenyl)-1,3,4-thiadiazole sulfonamides. *Molecules*; 15, PP. 9046–9056, **2010**.
18. Foroumadi, A., Soltani, F., Jabini, R., Moshafi, M.H., Rasnani, F.M., Antituberculosis agents X. synthesis and evaluation of In Vitro antituberculosis activity of 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitrolH-imidazol-2-yl)-1,3,4-thiadiazole derivatives. *Arch Pharm,* Res. 27, PP. 502–506, **2004**
19. Swati, G; Romila, K; Sharma, IK; Verma, PS; Synthesis, characterization and anti-microbial screening of some azo compounds, *Int. J. Appl. Biol. Pharm. Tech.,* 2(2), 332-338, **2011.**
20. Chopde, HN; Meshram, JS; Pagadala, R; Mungole, AJ; Synthesis, characterization and antibacterial activity of some novel azo-azoimine dyes of 6-bromo-2-naphthol, *Int. J. Chem. Tech. Res*., 2(3), PP 1823-830.**2010.**
21. Shridhari, AH; Keshavayya, H; Hoskeri, HJ; RAS, Ali; Synthesisof some novel bis 1,3,4-oxadiazole fused azo dye derivatives as potent antimicrobial agents, *Int. Res. J. Pure Appl. Chem.,* 1(3), PP 119-129,**2011**.

22. Patil, CJ; Patil Pooja, A; Patil, PB; and Patil Manisha, C; Coupling Reactions Involving Aryldiazonium Salt: Part-I. Chemoselective Synthesis of 3-Oxo-(Substituted-2-phenylazo)-butyricacid ethylester derivatives and their Antibacterial Activity, *Der. Chemica. Sinica.,* 6(5), PP 108-114, **2015**
23. Patil, CJ; Patil Manisha, C; Pachpol, NR; and Waykole, VS., Coupling Reactions Involving Aryldiazonium Salt: Part-II. Chemoselective Condensation with Acetylacetone and Antibacterial Activity, *Der. Chemica. Sinica.,*6(5), PP 115-121,**2015.**
24. Patil, C; Patil Manisha, C; Rane Vivek, Mahajan Kunal, and Nehete, CA; Coupling Reactions Involving Reactions of Aryldiazonium Salt: Part-III. Chemoselective Condensation with b-Naphthol to Synthesize Sudan-I, its Nitro Derivatives and Antibacterial Potential, *Int. J. Chem. Biol. Phy. Envir. Sci*., **2015**.
25. Al-Rubaie, LAR; Mhessn, RJ; Synthesis and characterization of azo dye para red and new derivatives, *E-J. Chem.,* 9(1), PP 465-470,**2012.**
26. Heinrich, Z; Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments, *VCH*, **2007**.
27. Elisangela, F; Andrea, Z; Fabio, DG; Cristiano, RM; Regina, DL; Artur, CP;Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic/aerobic process, *Int. Biodeter. Biodegrad*., 63, PP 280-288,**2009.**
28. Chakraborty, A; Saha, PK; Datta, C; Synthesis and application of azo-naphthol dyes on wool, silk and nylon fabric, *7th Int. Conf. TEXSCI*, PP 6–8,.**2010**.
29. Wainwright, M., *J. Antimicrob. Chem*., **47**,p. 1, **2001.**
30. Klink, S.I., Alink, P.O., Grave, L., Peters, F.G.A., Hofstraat, J.W., Geurts, F., Van Veggel., F.C.J.M., *J. Chem. Soc., Perkin Trans*., p.363, **2001**
31. Gong, G., Gao, X., Wang, J., Zhao, D., Freeman, H. S., *Dyes Pig*ments, **53**, p.109, **2002.**
32. Hinks, D., Freeman, H. S., Nakpathom, M., Sokolowska, J., *Dyes Pigments*, **44**, p.199, **2000.**
33. Wojciechowski, K., Wyrebak, A., Gumulak, J., *Dyes Pigments*, **56**, p. 99, **2003**.
34. Wojciechowski, K., Gumulak, J., *Dyes Pigments*, **56**, p. 195,**2003**.
35. . Rudyk, H., Knaggs, M.H., Vasiljevic, S., Hope, J., Birkett, C., Gilbert, I.H., *Eur. J. of Med. Chem.,* **38**, p.567, **2003**.

36. Pielesz, A., Baranowska, I., Rybak, A., Wlochowicz, A., *Ecotoxicology andenvironmental safety*, **53**, p. 42, **2002.**
37. Mittal, A., Kurup, L., Mittal, J., *Journal of Hazardous Materials*, **146**, p. 243,**2007.**
38. Wainwright, M., *Dyes and Pigments,* **76**, 2008, p. 582
39. Ahmed, A.E.I., Hay, J.N., Bushell, M.E., Wardell, J.N., Cavalli, G., *Reactive &Functional Polymers*, **68**, p. 248,**2008 .**
40. Simu, G., Chicu, S. A., Morin, N., Schmidt, W., Sisu, E., *Turk. J. Chem.*, **28**, p. 579,**2008.**
41. Simu, G., Funar-Timofei, S., Hora, S., Schmidt, W., Kurunczi, L., Sisu, E., Morin, N.,*Rev. Chim.-Bucharest*, **53**, p. 826, **2002.**
42. Simu, G. M., Hora, S. G., Grad, M. E., Sisu, E. N. V., *Rev. Roum. Chim*., **50(2)**, p.113, **2005.**
43. Bae, J.-S., Freeman, H. S., El-Shafei, A., *Dyes and Pigments*, **57**, p. 121,**2003**.
44. Combellas, C., Kanoufi, F., Pinson, H., Podyorica, F., *Am. Chem. Soc*., **34**, p. 429, **2008.**
45. Pinson, J., Podyorica, F., *Chem. Soc. Rev*., **34**, p. 429, **2005.**
46. Dabbagh, H. A., Teimouri, A., Chermahini, A. N., *Dyes Pigments*, **73**, p. 239, 2007.
47. Murray, P. S., Ralph, S. F., *Electrochimica Acta*, **51**, p. 2471,**2006.**
48. Simu, G. M., Agrigoroaie, G., Dragomirescu, A., Andoni, M., *Rev. Chim. (Bucharest)*,*60(12)*, pp 1309-1312, **2009**
49. Dragomirescu, A., Andoni, M, Muresan, A. R., *TMJ*, **58**, suppl 2, p. 396, **2008.**
50. Martini M.-C., Introduction à la dermatopharmacie et à la cosmetologie. Ed. Lavoisier, pp. 107-139, **2006.**
51. Kavanagh, F., *"Analytical Microbiology",* Academic Press, New York, pp.125-126, **1963.**
52. Schiff H. Mittheilungen aus dem universita¨ tslaboratorium in Pisa: Eine neue reihe organischer basen. *Justus Liebigs Ann Chem*;131(1): pp 118–9.
53. Dhar, DN., Taploo, CL., Schiff bases and their applications. *J Sci Ind Res* ;41(8), pp 501–6.**1982.**
54. Przybylski, P., Huczynski, A., Pyta, K., Brzezinski, B., Bartl, F., Biological properties of schiff bases and azo derivatives of phenols. *Curr Org Chem*;13(2): pp124–48,**2009.**
55. Bringmann, G., Dreyer, M., Faber, JH., Dalsgaard, PW., Staerk, D., Jaroszewski, JW., et al. Ancistrotanzanine C and related 5,1'-and 7,3'-coupled naphthylisoquinoline alkaloids from Ancistrocladus tanzaniensis. *J Nat Prod*, 67(5): pp. 743–8,**2004.**

56. de Souza, AO., Galetti, FCS., Silva, CL., Bicalho, B., Parma, MM., Fonseca, SF., et al. Antimycobacterial and cytotoxicity activity of synthetic and natural compounds. *Quim Nova*;3.0(7): pp. 1563–6, **2007.**

57. Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., et al. Antifungal properties of Schiff bases of chitosan, N -substituted chitosan and quaternized chitosan. *Carbohydr Res*,342(10): pp. 1329–32, **2007.**

58. Bohach, GA., Fast, DJ., Nelson, RD., Schlievert, PM.. Malaria, In., Rodes, J., Benhamou, JP., Blei, A., Reichen, J., Rizzetto, M., editors. *The textbook of hepatology: from basic science to clinical practice. Oxford (UK): Wiley Blackwell*, pp. 1029–34, **2007.**

59. Kayser, O., Kiderlen, AF., Croft, SL., Natural products as potential antiparasitic drugs. *Parasitol Res*;90 pp. 55–62,**2003.**

60. Rathelot, P., Vanelle, P., Gasquet, M., Delmas, F., Crozet, MP., Timon-David, P., et al. Synthesis of novel functionalized 5-nitroisoquinolines and evaluation of in vitro antimalarial activity. *Eur J Med Chem*;30(6), pp. 503–8, **1995.**

61. Baquero, F., Gram-positive resistance: challenge for the development of new antibiotics. *J Antimicrob Chemother*; 39(Suppl.A), pp.1–6, **1997.**

62. Alekshun, MN., Levy, SB., Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 128(6), pp. 1037–50, **2007.**

63. Rice, LB., Unmet medical needs in antibacterial therapy. *Biochem Pharmacol,* 71(7), pp. 991–5, **2006.**

64. Shi, L., Ge, HM., Tan, SH., Li, HQ., Song, YC., Zhu, HL., et al. Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. *Eur J Med Che*, 42(4):558–64, **2007.**

65. Pandeya, SN., Sriram, D., Nath, G., de, Clercq, E.. Synthesis and antimicrobial activity of Schiff and Mannich bases of isatin and its derivatives with pyrimidine. *IL Farmaco* , 54(9): pp. 624–8, **1999.**

66. Pandeya, SN., Sriram, D., Nath, G., de, Clercq, E., Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(40-chlorophenyl)thiazol-2-yl] thiosemicarbazide. *Eur J Pharm Sci*. 9(1): pp. 25–31,**1999**.

67. Jarrahpour, A., Khalili, D., de, Clercq, E., Salmi, C., Brunel, JM., Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. Molecules;12(8), pp. 1720–30, **2007.**

68. Hearn, MJ., Cynamon, MH., Design and synthesis of antituberculars: preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base. *J Antimicrob Chemother*,53 (2),p.p 185–91,**2004.**

69. Panneerselvam, P., Nair, RR., Vijayalakshmi, G., Subramanian, EH., Sridhar, SK., Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. *Eur J Med Chem,* 40(2), pp 225–9,**2005.**

70. Karthikeyan, MS., Prasad, DJ., Poojary, B., Bhat, KS., Holla, BS., Kumari, NS., Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety. *Bioorg Med Chem,*14(22), pp 7482–9, **2006.**

71. Paulus, EF., Dornberger, K., Werner, W., Fenske, D., Madurahydroxylactone. *Acta Crystallogr,* 50(12), pp. 2064–7, **1994.**

72. Heinisch, L., Roemer, E., Jutten, P., Haas, W., Werner, W., Mollmann, U., Semisynthetic derivatives of madurahydroxylactone and their antibacterial activities. *J Antibiot (Tokyo),* 52(11): pp. 1029-41, **1999.**

73. Kulkarni, A., Patil, SA., Badami, PS., Synthesis, characterization, DNA cleavage and in vitro antimicrobial studies of La(III), Th(IV) and VO(IV) complexes with Schiff bases of coumarin derivatives. *Eur J Med Chem,* 44(7), pp. 2904–12, **2009.**

74. Chohan ZH, Arif M, Sarfraz M. Metal-based antibacterial and antifungal amino acid derived Schiff bases: their synthesis, characterization and in vitro biological activity. *Appl Organomet Chem,* 21(4). pp. 294–302, **2007.**

75. Baluja, S., Solanki, A., Kachhadia, N., Evaluation of biological activities of some Schiff bases and metal complexes. *J Iran Chem Soc,* 3(4), pp. 312–7, **2006.**

76. Venugopala, KN., Jayashree, BS., Microwave-induced synthesis of Schiff bases of aminothiazolyl bromocoumarins as antibacterials. *Indian J Pharm Sci,* 70(1), pp 88–91,**2008.**

77. Yildiz, M., Kiraz, A., Du,¨ lger, B., Synthesis and antimicrobial activity of new crown ethers of Schiff base type. *J Serb Chem Soc,* 72(3), pp. 215–24, **2007.**

78. Abdallah, SM., Mohamed, GG., Zayed, MA., El-Ela, MSA., Spectroscopic study of molecular structures of novel Schiff base derived from O-phthaldehyde and 2-aminophenol

and its coordination compounds together with their biological activity. *Spectrochim Acta Part A: Mol Biomol Spectrosc*,73(5) pp. 833–40, **2009.**

79. T'ang, A., Lien, EJ., Lai, MMC., Optimization of the Schiff bases of N-hydroxy-N0-aminoguanidine as anticancer and antiviral agents. *J Med Chem,* 28(8) pp. 1103–6,**1985.**
80. Bayrak, H., Demirbas, A., Karaoglu, SA., Demirbas, N., Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases andevaluation of their antimicrobial activities. *Eur J Med Chem,* 44(3) pp.1057–66, **2009.**
81. Sundriyal S, Sharma RK, Jain R. Current advances in antifungal targets and drug development. *Curr Med Chem,* 13(11), pp. 1321–35,**2006.**
82. Nucci, M., Marr, KA., Emerging fungal diseases. *Clin Infect Dis,* 41(4), pp. 521–6, **2005.**
83. Martins, CVB., da Silva, DL., Neres, ATM., Magalha˜ es, TFF., Watanabe, GA., Modolo, LV., et al. Curcumin as a promising antifungal of clinical interest. *J Antimicrob Chemother,* 63(2), pp 337–9, **2009.**
84. Martins, CVB., de Resende, MA., da Silva, DL., Magalha˜ es, TFF., Modolo, LV., Pilli, RA., et al. In vitro studies of anticandidal activity of goniothalamin enantiomers. *J Appl Microbiol*,107(4), pp 1279–86, **2009.**
85. Rehman W, Baloch MK, Muhammad B, Badshah A, Khan KM. Characteristic spectral studies and in vitro antifungal activity of some Schiff bases and their organotin (IV) complexes. *Chin Sci Bull,* 49(2), pp 119–22,**2004.**
86. Echevarria A, Nascimento MG, Geroˆ nimo V, Miller J, Giesbrecht A. NMR spectroscopy, hammett correlations and biological activity of some Schiff bases derived from piperonal. *J Braz Chem Soc,*10(1), 60–4, **1999.**
87. Domb AJ, Linden G, Polacheck I, Benita S. Nystatin-dextran conjugates: synthesis and characterization. *J Polym Sci Part A: Polym Chem,* 34(7), pp. 1229–36, **1996.**
88. de Clercq, E., Strategies in the design of antiviral drugs. *Nat Rev Drug Discov*, 1, pp 13–25, **2002.**
89. Wang, PH., Keck, JG., Lien, EJ., Lai, MMC., Design, synthesis, testing and quantitative structure–activity relationship analysis of substituted salicylaldehyde Schiff bases of 1-amino-3- hydroxyguanidine tosylate as new antiviral agents against coronavirus. *J Med Chem,* 33(2), pp. 608–14,**1990.**

90. Sriram D, Yogeeswari P, Myneedu NS, Saraswat V. Abacavir prodrugs: microwave-assisted synthesis and their evaluation of anti-HIV activities. *Bioorg Med Chem Lett,* 16(8), pp. 2127–9, **2006.**

91. March. J; "Advanced Organic Chemistry" *3rd. ed. ; John wiley and Sons, New York*; **1985**.

92. Kalefuda,A; T, Suzuki., T, Tobe., and A, Tahara*., J. of Bio orgamic and Medicinal Chemistry*; 10, pp. 1905-1912, **2002**.

93. Jokeme, J., J, Bery., and T, staley., "Microbiology Dynamic and Diversity"; pp. 880-881,**1999**.

94. Feeny, J., "*Phytochemistry" 8th. ed*. pp. 2116- 2129, **1998**.

95. Buyten., J., B, Francis., and W, Mathew; *"Antibiotic"***2005**.

96. Shankar., P, Saha., *et al*. "A prospective incidence study of epilepsy in a rural community of West-Bengal, India". *Neurology Asia* 13 , pp. 41-48, **2008**.

97. John., Greenfield, Jr., "Molecular mechanisms of antiseizure drug activity at GABAA receptors". *Seizure* 22.8 , pp. 589-600, **2013**.

98. Abhishek., Kumar Jain., *et al*. "1,3,4-Thiadiazole and its Derivatives: A Review on Recent Progress in Biological Activities". *Chemical Biology & Drug Design* 81.5 , pp. 557-576, **2013**.

99. Supuran, CT., and Scozzafava, A., "Carbonic anhydrase inhibitors".*Current Medicinal Chemistry - Immunology, Endocrine & Metabolic Agents* 1 , pp. 61–97, **2001**.

100. Iizawa, Y., *et al*. "Therapeutic effect of cefozopran (SCE-2787), a new parenteral cephalosporin, against experimental infections in mice". *Antimicrobial Agents and Chemotherapy* 37.1 , pp. 100-105, **1993**.

101. EE, Oruc., *et al.* "1,3,4-thiadiazole derivatives. Synthesis, structure elucidation, and structure-antituberculosis activity relation- ship investigation". *Journal of Medicinal Chemistry* 47.27 , pp. 6760-6767, **2004**.

102. Foroumadi A., *et al*. "Antituberculosis agents VIII. Synthesis and in vitro antimycobacterial activity of alkyl alpha-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates". *Farmaco* 58.11 , pp.1073-1076, **2003**.

103. MD Kamal., *et al*. "Synthesis, anticonvulsant, and anti-inflammatory evaluation of some new benzotriazole and benzofuran-based heterocycles". *Bioorganic and Medicinal Chemistry* 14.11 , pp.3672–3680, **2006**.

104. Mullick.P, *et al*. "Thiadiazole derivatives as potential anticonvulsant agents". *Bulletin of the Korean Chemical Society* 32.3, pp. 1011-1016, **2011**.

105. Clerici, F., *et al*. "Synthesis of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxi-olytic activity". *Journal of Medicinal Chemistry* 44.6 ,931-936, **2001**.

106. Hasui,T., *et al*. "Identification of benzoxazin-3-one derivatives as novel, potent, and selective nonsteroidal mineralocorticoid receptor antagonists". *Journal of Medicinal Chemistry* 54.24 ,pp.8616-8631, **2011**.

107. Zheng, K, B., *et al*. "Synthesis and antitumor activity of N1-acetylamino-(5-alkyl/aryl-1,3,4-thiadiazole-2-yl)-5-fluorouracil deriva- tives". *Chinese Chemical Letters* 19.11, pp.1281-1284, **2008**.

108. Chen, CJ., *et al*. "Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5- trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives". *Bioorganic & Medicinal Chemistry* 15.12 , pp.3981-3989, **2007**.

109. Supuran, C.T., Scozzafava, A., Carbonic anhydrase inhibitors. *Curr Med Chem Immunol Endocrinol Metab Agents*;1: pp.61–97, **2001**.

110. Iizawa, Y., Okonogi, K., Hayashi, R., Iwahi, T., Yamazaki, T., Imada, A., Therapeutic effect of cefozopran (SCE-2787), a new parenteral cephalosporin, against experimental infections in mice. *Antimicrob Agents Chemother*;37, pp. 100–105, **1993.**

111. Onkol, T., Doruer, D.S., Uzun, L., Adak, S., Ozkan ,S., Ahin, F.M., Synthesis and antimicrobial activity of new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *J Enz Inhib Med Chem*; 23, pp. 277–284, **2008**.

112. Matysiak, J., Malinski., Z.2-(2,4-dihydroxyphenyl)- 1,3,4 thiadiazole analogues: antifungal activity In Vitro against Candida species. *Russ J Bioorg Chem*; 33, pp. 594–601,**2007**.

113. Demirbas A., Sahin D., Demirbas N., Karaoglu S.A.Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl- 1,2,4-triazole derivatives and investigation of their antimicrobial activities. *Eur J Med Chem*; 44, pp. 2896–2903, **2009**.

114. Rostom, S.A.F., El-Ashmawy, I.M., Abd, El, Razik, H.A., Badr, M.H., Ashour , H.M.A., Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic and antimicrobial agents. *Bioorg Med Chem*; 17 pp. 882–895, **2009**.

115. Ansari, K.F., Lal, C.,Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents. *Eur J Med Chem*; 44, pp.2294–2299,**2009**.

116. Liu, X., Shi, Y., Maa, Y., Zhang, C., Dong, W., Pan, L., Wang, B., Li, B., Li, Z., Synthesis, antifungal activities and 3D-QSAR study of N-(5-substituted- 1,3,4-thiadiazol-2 yl)cyclopropanecarboxamides. *Eur J Med Chem*; 44, pp.2782–2786, **2009**.

117. Guzeldemirci, N.U., Kucukbasmaci, O., Synthesis and antimicrobial activity evaluation of new 1,2,4- triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-b] thiazole moiety. *Eur J Med Chem*; 45, pp. 63–68, **2010**.

118. Dogan, H.N., Duran A., Rollas, S., Sener, G., Uysal, M.K., Gulen ,D., Synthesis of new 2,5-disubstituted- 1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. *Bioorg Med Chem*; 10 pp. 2893–2898, **2002**.

119. Farshori, N.N., Banday, M.R., Ahmad, A., Khan, A.U., Rauf A.Synthesis, characterization, and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl- 2-phenylamine-1,3,4-oxadiazoles and thiadiazoles. *Bioorg Med Chem Lett*; 20, pp.1933–1938, **2010**.

120. Onkol, T., Dogruer, D.S., Uzun, L., Adak, S., Ozkan, S., Sahin ,M.F., Synthesis and antimicrobial activity of new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *J Enz Inhib Med Chem*; 23, pp. 277–284, **2008**.

121. Padmavathi, V., Reddy, S.N., Reddy, G.D.,Padmaja, A., Synthesis and bioassay of aminosulfonyl-1,3,4- oxadiazoles and their interconversion to 1,3,4-thiadiazoles. Eur J Med Chem;45, pp. 4246–4251, **2010**.

122. Klip, N.T., Capan, G., Gursoy, A., Uzun, M., Satana, D.Synthesis, structure, and antifungal evaluation of some novel 1,2,4-triazolylmercaptoacetylthiosemicarbazide and 1,2,4-triazolylmercaptomethyl-1,3,4-thiadiazole analogs. *J Enz Inhib Med Chem*; 25, pp. 126–131, **2010**.

123. Ranjina, S., Devendra, P.N., Ganpat, L.T.Synthesis of various isoniazido thiazolidinones and their imidoxy derivatives of potential biological interest. *Arkivoc*; pp. 1–5, **2006**.

124. Camoutsis, C., Geronikaki, A., Ciric, A., Sokovic, M., Zoumpoulakis, P.,Zervou, M., Sulfonamide-1,2,4- thiadiazole derivatives as antifungal and antibacterial agents: synthesis, biological evaluation, lipophilicity, and conformational studies. ; 58, pp. 160–167, **2010**.

125. Kumar, D., Kumar, N.M., Chang, K., Shah, K., Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4- thiadiazoles. *Eur J Med Chem*; 45, pp. 4664–4668, **2010**.

126. Bhole, R.P., Bhusari, K.P., Synthesis and antitumor activity of (4-hydroxyphenyl)[5-substituted alkyl/ aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone and [(3,4- disubstituted)-1,3-thiazol-2ylidene]-4-hydroxybenzohydrazide. *Med Chem Res*;20, pp.695–704, **2010**.

127. Kumar, D., Vaddula, R., Chang, K., Shah, K., One-pot synthesis and anticancer studies of 2-arylamino- 5-aryl-1,3,4-thiadiazoles. *Bioorg Med Chem Lett*; 21, pp. 2320–2323, **2011**.

128. Marganakop, S.B., Kamble, R.R., Taj T., Kariduraganvar, M.Y., An efficient one-pot cyclization of quinoline thiosemicarbazones to quinolines with 1,3,4-thiadiazole as anticancer and anti-tubercular agents. Med Chem Res;21, pp.185–191, **2010**.

129. Zheng, K.B., He, J., Zhang, J., Synthesis and antitumor activity of N1-acetylamino-(5-alkyl/aryl- 1,3,4-thiadiazole-2-yl)-5-fluorouracil derivatives. *Chin Chem Lett*; 19, 1281–1284, **2008**.

130. Chou, J., Lai, S., Pan, S., Jow, G., Chern, J., Guh, J., Investigation of anticancer mechanism of thiadiazole-based compound in human non-small cell lung cancer A549 cells. *Biochem Pharmacol*; 66, pp. 115–124, **2003**.

131. Matysiak, J., Opolski, A., Synthesis and antiproliferative activity of N-substituted 2-amino-5-(2,4- dihydroxyphenyl)-1,3,4-thiadiazoles. *Bioorg Med Chem*; 14 pp. 4483–4489, **2006**.

132. Rzeski, W., Matysiak, J., Kandefer-Szerszen, M., Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound. *Bioorg Med Chem*;15, pp. 201–3207, **2007**.

133. Matysiak, J., Nasulewicz, A., Pełczynska, M., Switalska, M., Jaroszewicz, I., Opolski, A., Synthesis and antiproliferative activity of some 5-substituted 2-(2,4- dihydroxyphenyl)-1,3,4-thiadiazoles. *Eur J Med Chem*; 41, pp. 475–482, **2006**.

134. Wei, M., Feng, L., Li, X., Zhou, X., Shao, Z., Synthesis of new chiral 2,5-disubstituted 1,3,4-thiadiazoles possessing c-butenolide moiety and preliminary evaluation of in vitro anticancer activity. *Eur J Med Chem*; 44, pp. 3340–3344, **2009**.

135. Sun, S., Yang, Y., Li, W., Zhang, Y., Wang, X., Tang, J., Zhu, H., Synthesis, biological evaluation and molecular docking studies of 1,3,4-thiadiazole derivatives containing 1,4 benzodioxan as potential antitumor agents. *Bioorg Med Chem Lett*; 21, pp. 6116–6121, **2011**.
136. Vergne, F., Bernardelli, P., Lorthiois, E., Pham, N., Proust, E., Oliveira, C., Mafroud, A., et al. Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 1: design, synthesis and structure–activity relationship studies. *Bioorg Med Chem Lett*;14: pp. 4607–4613, **2004**.
137. Vergne, F., Bernardelli, P., Lorthiois, E., Pham, N., Proust, E., Oliveira, C., Mafroud, A., et al. Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 2: metabolism- directed optimization studies towards orally bioavailable derivatives. *Bioorg Med Chem Lett*; 14, pp. 4615–4621, **2004**.
138. De, S.K., Chen, V., Stebbins, J.L., Chen, L., Cellitti, J.F., Machleidt, T., Barile, E., Riel-Mehan, M., Dahl, D., Yang, L., Emdadi, A., Murphy, R., Pellecchia, M., Syn-thesis and optimization of thiadiazole derivatives as a novel class of substrate competitive c-Jun N-terminal kinase inhibitors. *Bioorg Med Chem*; 18, pp. 590–596, **2010**.
139. World Health Organization, *Fact sheet*, **2013**.
140. Kempgowda, S., Dev, Prakash, GP., Tamiz, MT., Thiadiazoles: Progress Report on Biological Activities. *Der Pharma Chem* 3, pp. 330-341, **2011**.
141. Kamal, M., Shakya, AK., Talha, J., 1, 3, 4-thiadiazoles as antimicrobial agent: A review. *Int J Biomed Res,* 2, pp. 41-61, **2011**.
142. Singh, AK., Mishra, G., Jyoti, K., Review on Biological activities of 1, 3, 4-thiadiazole derivatives. *J Appl Pharm Sci* 1, pp. 44-49, **2011**.
143. Jalhan, S., Anil, J., Avneet, G., Hemraj., Synthesis, biological activities and chemistry of thiadiazole derivatives and Schiff bases. *Asian J Pharm and Clinical Res* 5, pp. 199-208, **2012**.
144. Siddiqui N, Ahuja P, Ahsan W, Pandeya SN, Alam MS Thiadaizoles: Progress report on biologiacal activities. *J Chem Pharm Res* 1, pp. 19-30, **2009**.
145. Kalidhar, U., Kaur, A., 1, 3, 4-thiadiazole derivatives and their biological activities: A review. *Research J Pharm Bio Chem Sci* 2, pp. 1091-1106, **2011**.

146. Hemal, B., Dipansu, S., Biren, S., Dixit, CM., Mandev, P., Biological profile of thiadiazole. *Pharmacologyonline* 1, pp. 528-543, **2011**.

147. Shen, L., Zhang, Y., Wang, A., Sieber-McMaster, E., Chen, X., *et al*. Synthesis and structure–activity relationships of thiadiazole-derivatives as potent and orally active peroxisome proliferator-activated receptors a/d dual agonists. *Bioorg & Med Chem* 16, p. 3321, **2008**.

148. Mougenot, P., Namane, C., Fett, E., Camy, F., Dadji-FaÃ¯hun, R., *et al*.Thiadiazoles as new inhibitors of diacylglycerol acyltransferase type 1. *Bioorg Med Chem Lett* 22, 2497-2502, **2012**.

149. Pattan SR, Kekare P, Dighe NS, Nirmal SA, Musmade DS, *et al*. Synthesis and biological evaluation of some 1, 3, 4-thiadiazoles. *Journal of Chemical and Pharmaceutical Research* 1, pp. 191-198, **2009**.

150. Pattan, SR., Kittur, BS., Sastry, BS., Jadav, SG., Thakur, DK., *et al.* Synthesis and evaluation of some novel 1,3,4-thiadiazoles for antidiabetic activity. *Indian Journal of Chemistry* 50, pp. 615-618, **2011**.

151. Gianti , E., Zauhar, RJ., Modeling androgen receptor flexibility: a binding mode hypothesis of CYP17 inhibitors/antiandrogens for prostate cancer therapy. *J Chem Inf Model* 52, pp. 2670-2683, **2012**.

152. Arun, KP., Nag, VL., Panda, CS., Studies on the synthesis and bioactivity of some thiadiazole derivatives. *Ind J Chem* 38B pp. 998-1001, **1999**.

153. Elzahany, E A., K, H, Hegab., S K H, Khalil., and N, S Youssef., Synthesis, characterization and biological activity of some transition metal complexes with Schiff bases derived from 2-formylindole, salicylaldehyde, and N-amino rhodanine. Aust. *J. Basic Appl. Sci*. ,2(2): pp. 210- 220, **2008**.

154. Bambas, .L.L; in „Chemistry of Heterocyclic Compounds", L. L. *Bambas and A.John Wiley, vol*. 4, p. 81, New York, **1952**.

155. Ainsworth ,C., *J. Am. Chem. Soc*., 80, p. 5201, **1958**.

156. Huisgen,R., Sturm, H.J., and Seidel,M., *Chem. Ber*., 94, p. 1555, **1961**.

157. McCarthy, A.R., W. D. Ollis., and C. A. Ramsden., *J. Chem. Soc*., Chem.Commun.,499, **1968**.

158. Ayad, S., Hameed, Nadia, A., Saleh., and Amar, H., AlDujaily, *National Journal of Chemistry*, Vol.5, pp. 121-131, **2002**.