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Synthesis and Characterization of New Benzimidazole Derivatives Containing 1,3,4-Thiadiazole Ring Tethered to Azo Group and Study Their Antibacterial Activity

A thesis

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By

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿ وَقُل رَّبِّ زِدْنِي عِلْمًا ﴾

صَدَقَ اللهُ العَظيم

طه الاية (١١٤)

*I'm dedicating this thesis to the owner of the great creation, the master of humanity and the best of messengers…..**Muhammad** (peace be upon him)*

*Then I'm dedicating this thesis to the two people, who are the reason for my existence in this universe and who raised me for my parents, my pure angel who flooded me with her kindness and her mercy my dear mother **Batool Habib** and my dear father, **Mahmood Hajhooj**, who was patient and sacrificed for my happiness. Although he is no longer in this world. His memories are still in my mind, I will never forget you.*

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Haider

Abstract

The current study includes synthesis, characterization and evaluation of the antibacterial activity of novel benzimidazole derivatives containing 1,3,4-Thaidiazol ring with azo group by the following steps:

First step, 2-(bromomethyl)-1*H*-benzimidazole (1) was synthesized by the reaction of *o*-phenylenediamine with bromoacetic acid using in the presence of 4N HCl.

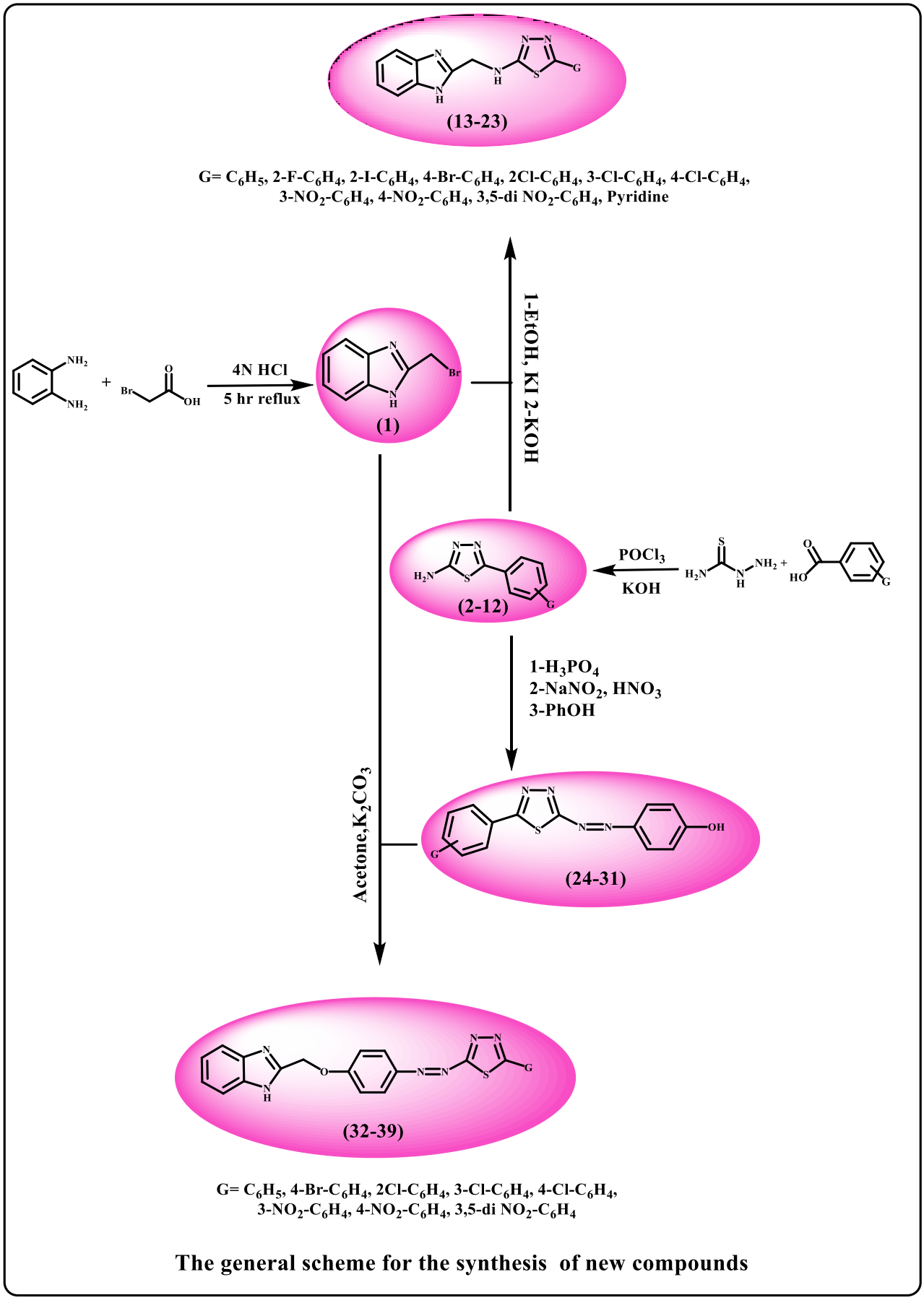
Second step, synthesis of 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole (2-12) were synthesized by the reaction of benzoic acid derivatives with thiosemicarbazide using phosphoroyl chloride ($POCl_3$).

Third step, benzimidazole derivatives (13-23) were synthesized by the reaction of 2-(bromomethyl)-*1H*-benzimidazole (1) with 1,3,4-thiadiazole derivatives (2-12) in the presence of (KI) and (KOH) using ethanol.

Fourth step, 4-((5-substitutedphenyl)-1,3,4-thiadiazol-2-yl)diazenyl) phenol (24-31) were synthesized by the reaction of 1,3,4-thiadiazole derivatives (2 and 5-11) with H_3PO_4, HNO_3, $NaNO_2$ and phenol.

Fifth step, benzimidazole derivatives (32-39) were synthesized by the reaction of 2-(bromomethyl)-*1H*-benzimidazole (1) with 4-((5-substitutedphenyl)-1,3,4-thiadiazol-2-yl)diazenyl) phenol (24-31) in the presence of K_2CO_3 and using acetone.

The synthesized compounds were characterized by using infrared (FT-IR) spectroscopy and nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR), the biological activity was studied of some synthesized compounds against two gram-positive bacteria (Gr^{+ve}) (*Staphylococcus aureus and Staphylococcus epidermidis*) and two gram-negative bacteria (Gr^{-ve}) (*Pseudomonas aeruginosa and Escherichia coli*) using disk diffusion method, some of these compounds showed good activity as antibacterial activity. In the final step, the molecular docking was studied for most active compounds (10, 11, and 39).



The general scheme for the synthesis of new compounds

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List of Abbreviations:

^{13}C-NMR : Carbon Nuclear Magnetic Resonance

^{1}H-NMR : Proton Nuclear Magnetic Resonance

Ar. : Aromatic

Asy. : Asymmetric

B.M. : Ball Milling

DABCO : 1,4-Diazabicyclo[2.2.2]Octane

DCM : Dichloromethane

DMAC : Dimethylacetamide

DMAP : 4-Dimethylaminopyridine

DMF : Dimethylformamide

DMSO : Dimethyl Sulfoxide

EtOH : Ethanol

FT-IR : Fourier Transform-Infrared

HAUT :1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium3-oxidehexafluorophosphate ,Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium

HIV : Human Immunodeficiency Virus

IBD :(diacetoxyiodo)benzene

M.P : Melting Point

MeOH : Methanol

MW : Microwave

NBS : N-Bromosuccinimide

***o*-Q1 : ortho-Quinone**

P.T : Proton Transfer

Ph : phenyl

PPA : Polyphosphoric Acid

Sy. : Symmetric

T.L.C : Thin Layer Chromatography

TBAB : Tetrabutylammonium bromide

TEA : Triethylamine

TEMPO : 2,2,6,6-tetramethyl-1-piperidinyloxyl

THF : Tetrahydrofuran

TosCl : p-tosyl chloride

TsOH : P- Toluenesulfonic Acid

YFV : Minimal yellow fever virus

Chapter one

Introduction

1.1. Heterocyclic chemistry:

Heterocyclic chemistry is one of the wide branches of organic chemistry that deals with heterocyclic compounds. It's has a special significance in nature because of the wide variety of physiological activities and the industrial value[1].

Heterocyclic compounds are compounds with one ring or more that contain at least one atom other than carbon (called heteroatom)[2] such as nitrogen, oxygen or sulfur, for example pyridine, pyrrole, furan and thiophene[3], also more than one heteroatoms with same or different heteroatoms as shown in Figure (1-1) [4,5].

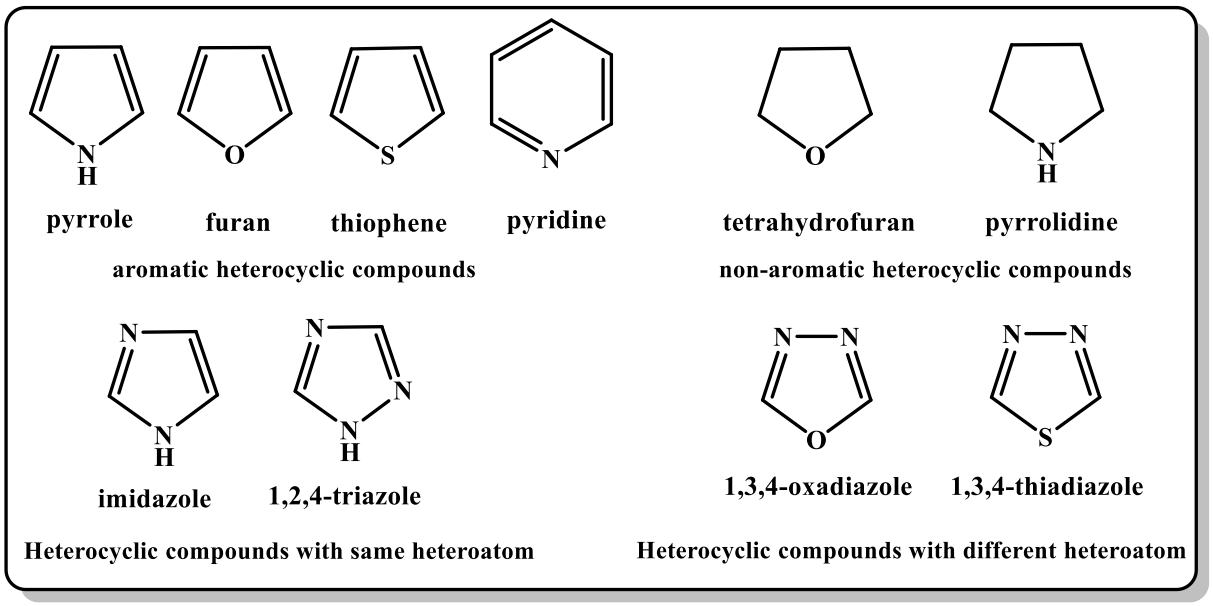


Figure (1-1): Some of heterocyclic ring

Some heterocyclic compounds consist of two fused rings one heterocyclic ring and the other benzene ring such as indole, benzofuran, benzo[*b*]thiophene, benzimidazole, benzoxazole and benzothiazole[6,7] as shown in Figure (1-2).

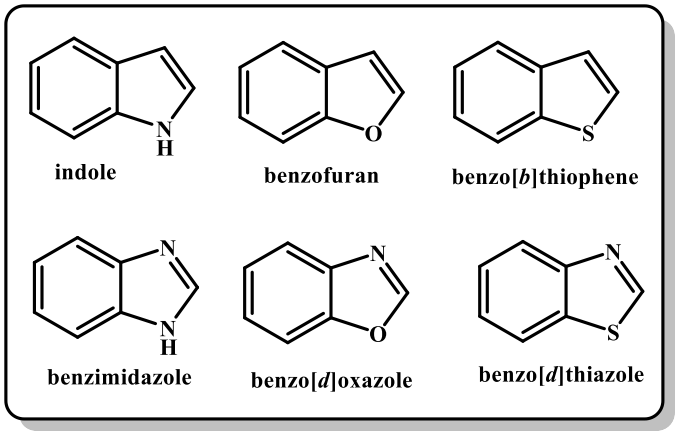


Figure (1-2): Some heterocyclic ring fused with a benzene ring

Heterocyclic compounds have many Fields and very wide applications in chemistry, medicine, industrial and agriculture[8,9].

Heterocyclic compounds have an important role in medicine because most drugs contain these compounds in their structure[10], essentially, the analyses of drugs have shown that 68% of them are heterocyclic and a lot of number of these compounds show biological activities[11], the heterocyclic compounds were used in medicine for development novel activities[12], anti-bacterial[13], anthelmintic[14], anti-inflammatory agents[15], anti-oxidant [16], and anti-cancer [17].

Heterocyclic compounds also have industrial applications such as copolymers[18], accelerators[19], dyes[20] and corrosion inhibitors[21].

Heterocyclic compounds with five or six atoms in ring structure are important in biochemistry because the bioorganic compounds containing heterocyclic compounds within their structures such as enzymes, amino acids, vitamin B complexes (such as vitamin B_{12}) Figure (1-3), drugs and the genetic material (nitrogen bases)[22].

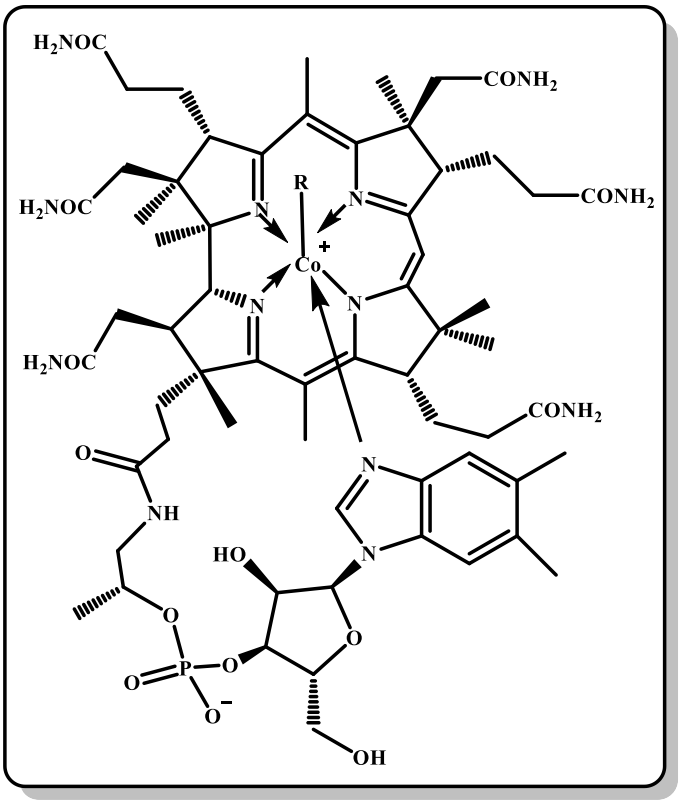


Figure (1-3): Chemical structure of vitamin B_{12}

1.2. Benzimidazole:

Benzimidazole is aromatic heterocyclic compound as shown in Figure (1-4), This compound is bicyclic, consist of the fusion heterocyclic five-membered ring imidazole with benzene ring[23]. Benzimidazole also named as 3-azaindole, azindole, benziminazole, benzoglyoxaline, 3-benzodiazole and 1,3diazaindene [24 25].

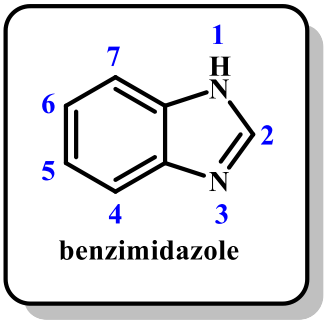
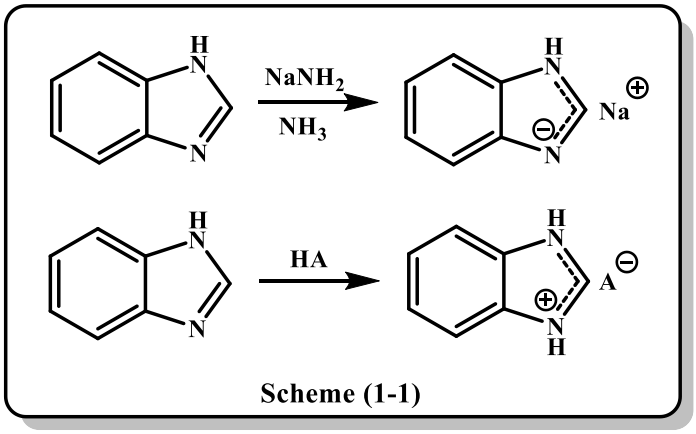


Figure (1-4): Chemical structure of benzimidazole

Benzimidazole ring containing in imidazole ring two nitrogen atoms at 1,3 positions shown an amphoteric nature Scheme (1-1), which have both acidic and

basic properties[26]. Benzimidazoles with pK_a= 5.30 are weak bases and weaker base than imidazoles, but the acidity is like imidazole, but with pK_a= 12.3 are more acidity of N-H[27]. So the hydrogen atom can be found on either 1,3 nitrogen atoms in imidazole ring, therefore, benzimidazole have two equal tautomeric forms [24,28] as shown in Figure (1-5).



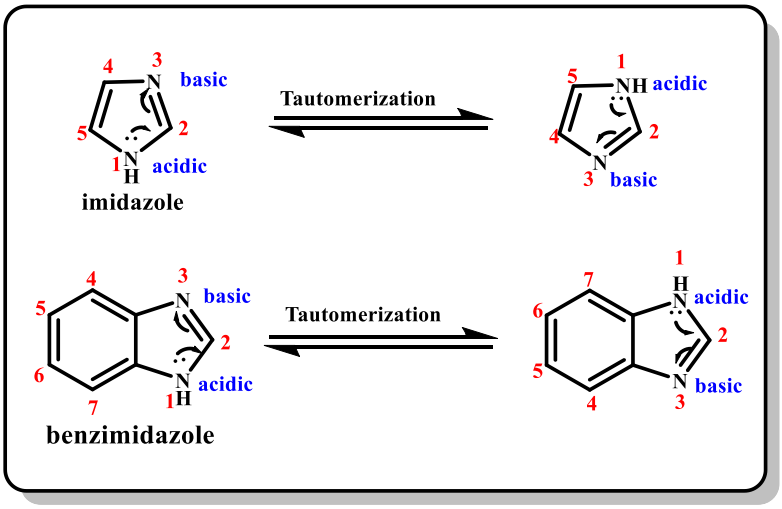


Figure (1-5): Tautomer forms of imidazole and benzimidazole

N-substituted benzimidazole doesn't show any tautomeric forms but unequal chemically two molecules compounds, the substitution occurs on benzene ring then benzimidazole display two equal tautomeric forms as shown in Figure (1-6) [29,26].

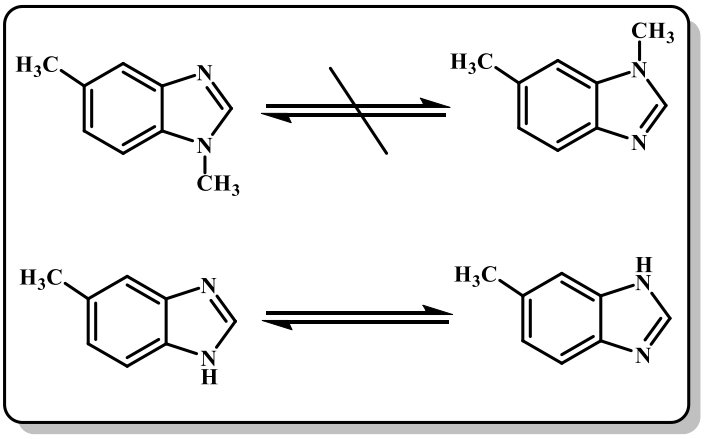


Figure (1-6): Tautomer forms of *N*-substituted benzimidazole

From the resonance of benzimidazole as shown in Figure (1-7), the electrophilic substitution occurs either on 1-position or on benzene ring, while the nucleophilic substitution occurs on 2-position which leads to more stable benzimidazole [27] as shown in Figure (1-8).

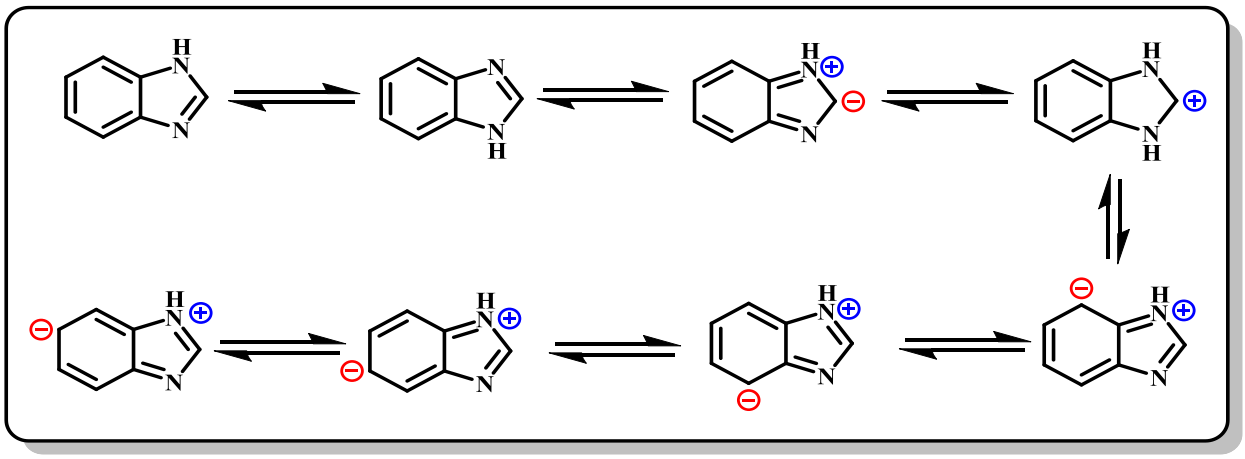


Figure (1-7): Resonance forms of benzimidazole

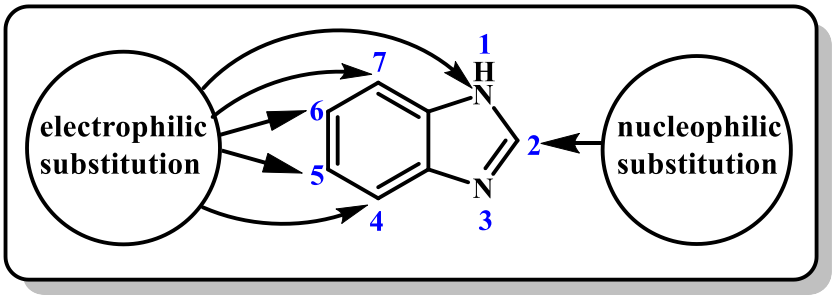


Figure (1-8): Electrophilic and nucleophilic substitution of benzimidazole

Benzimidazole can form hydrogen bond by N-H in imidazole ring[30] as shown in Figure (1-9).

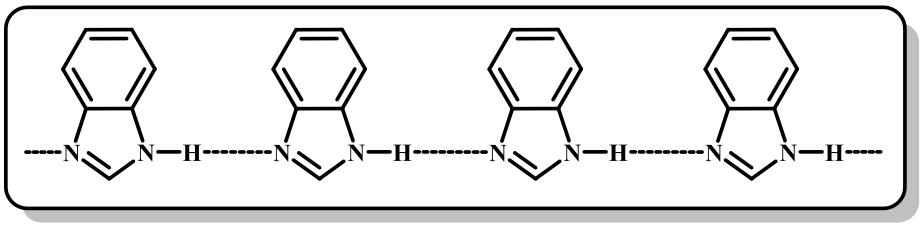


Figure (1-9): Hydrogen bonds between benzimidazole molecules

1.2.1. Physical characteristic of Benzimidazole:

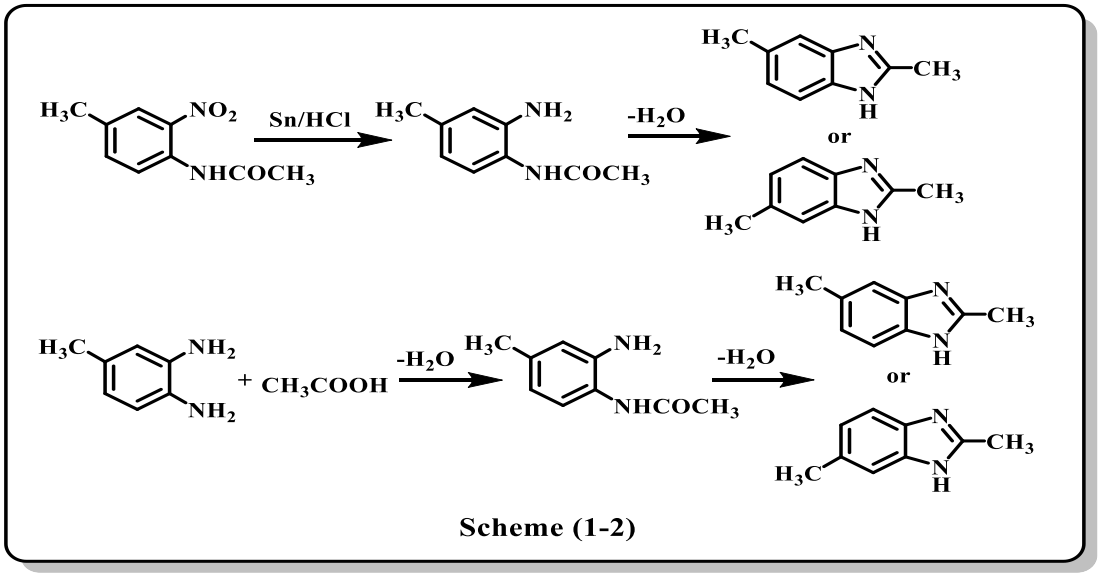
Physical characteristic of Benzimidazole [26,28] as shown in Table (1-1).

Table (1-1): Physical characteristic of benzimidazole

No.	Characteristic	Description
1	Physical state	Tubular crystal
2	Molecular formula	$C_7H_6N_2$
3	Molecular weight	118.053 g/mole
4	color	whitish
5	Odor	Special
6	Melting point	171^{o}C
7	Boiling point	360 ^{o}C
8	Solubility	Moderate soluble in water, good soluble in aqueous alkali and N-metallic compounds, very soluble in ethanol, less soluble in ether, unsoluble in benzene and petroleum ether
9	Isomer	Tautomer

1.2.2. Synthesis of benzimidazole:

The first synthesis of benzimidazole was in 1872 by Hoebrecker, he obtained 2,5(or 2,6)-dimethylbenzimidazole by reduction of 2-nitro-4-methylacetanilide[31]. After many years, Ladenburg was obtained the same product by refluxed of 3,4-diaminotoluene with acetic acid Scheme (1-2)[32].



Scheme (1-2)

There are many methods to the synthesis of benzimidazole and its derivatives, all these methods based on the reaction of *o*-phenylenediammine with many compounds such as aldehydes, ketones, carboxylic acids…etc. as shown in Figure (1-10).

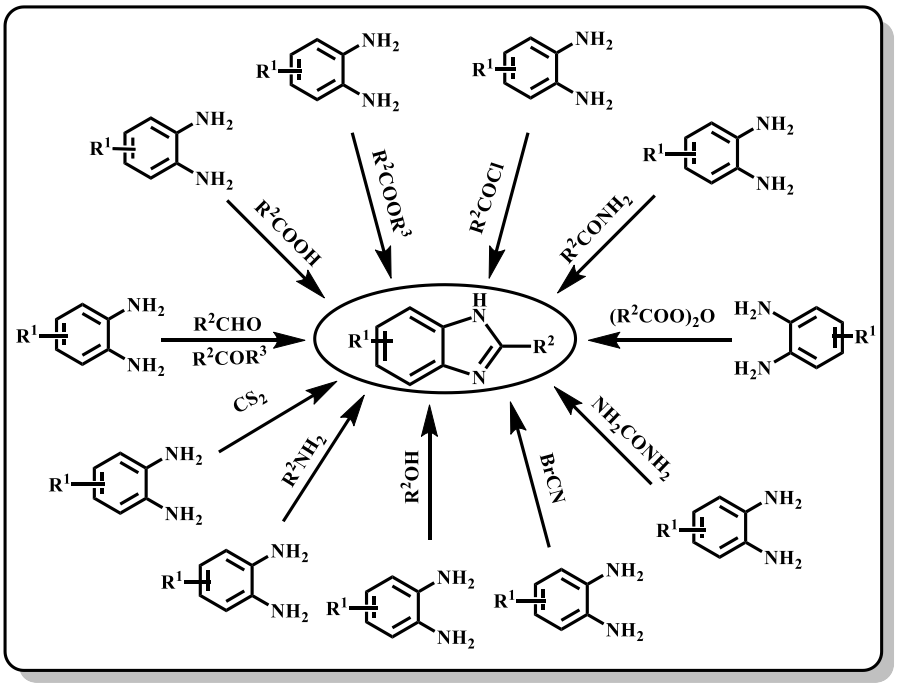
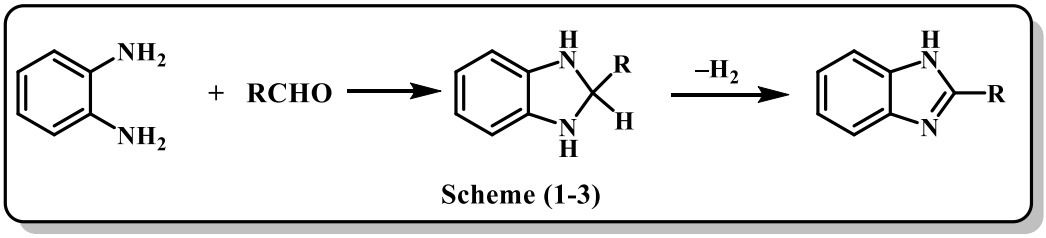


Figure (1-10): Synthetic methods of benzimidazole

The following are reactions that used for the synthesis of benzimidazole and its derivatives:

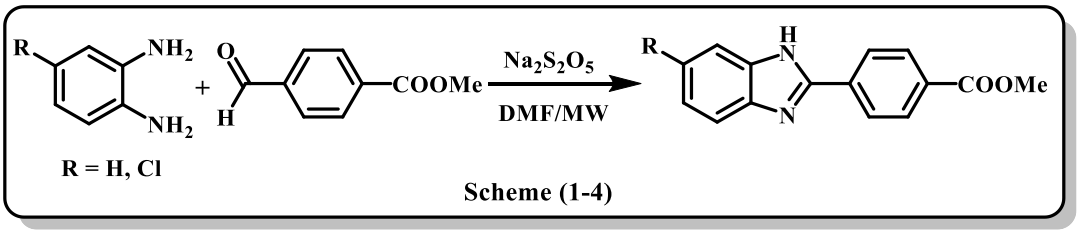
1.2.2.1. Reaction with aldehydes and ketones:

o-phenylenediammine can be reacted with aldehydes and ketones to give 2-substituted benzimidazole. This method is one of the best used methods for the synthesis of benzimidazole, by oxidation of aldehydes either by air (easy) or by using oxidant reagents such as copper acetate Scheme (1-3)[33].



Scheme (1-3)

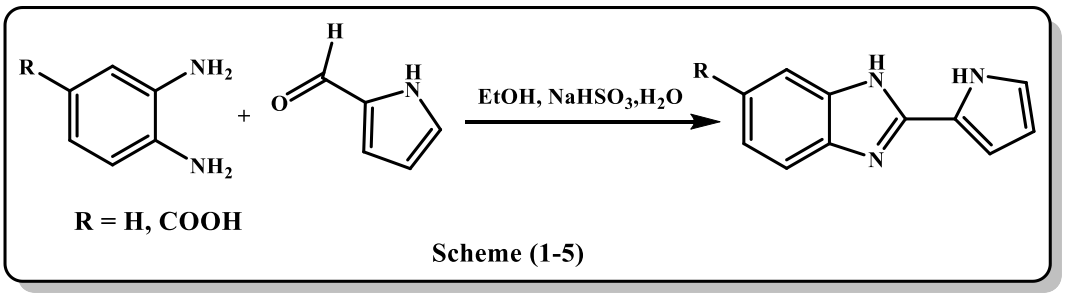
Ulviye Acar Çevik, et al.[34] synthesized benzimidazole by the reaction of *o*-phenylenediammine derivative with methyl4-formylbenzoate using microwave synthesis reactor Scheme (1-4).



R = H, Cl

Scheme (1-4)

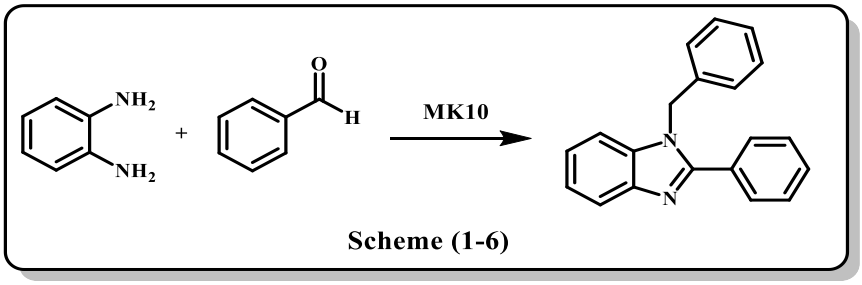
Ernestine, et al.[35] synthesized new benzimidazole derivatives by refluxing *o*-phenylenediammine derivatives with 1H-pyrrole-2-carbaldehyde as shown in Scheme (1-5).



R = H, COOH

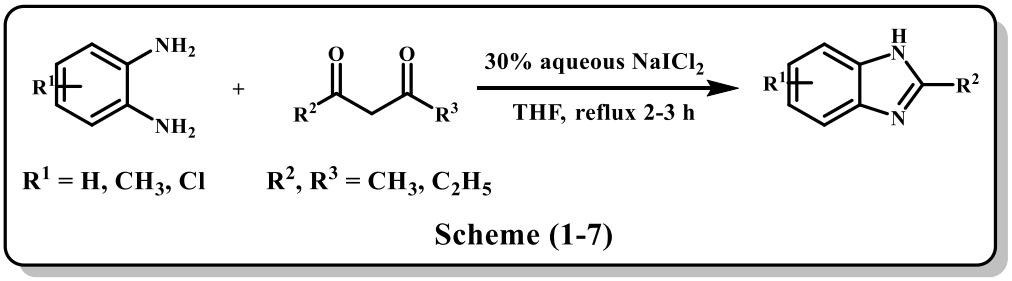
Scheme (1-5)

Sonia Bonacci, et al.[36] synthesized novel benzimidazole derivative from reaction of *o*-phenylenediammine with benzyldehyde as shown in Scheme (1-6).



Scheme (1-6)

Saket, et al.[37] synthesized new benzimidazole by refluxing of β-diketones derivatives with *o*-phenylenediammine as shown in Scheme (1-7).

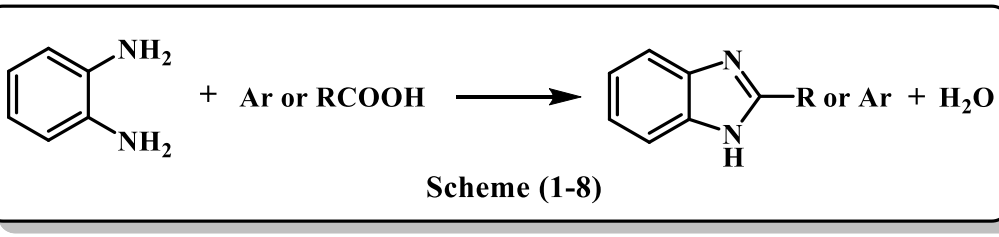


R^1 = H, CH_3, Cl R^2, R^3 = CH_3, C_2H_5

Scheme (1-7)

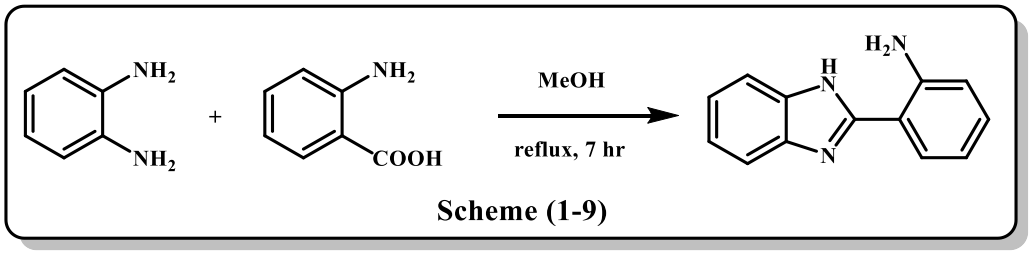
1.2.2.2. Reaction with carboxylic acids:

o-phenylenediammine reacted easily with aliphatic and aromatic carboxylic acids to give good yield of 2-substituted benzimidazole, by reflux under heating in high temperature or heating in Sealed tube as shown in Scheme (1-8)[38].



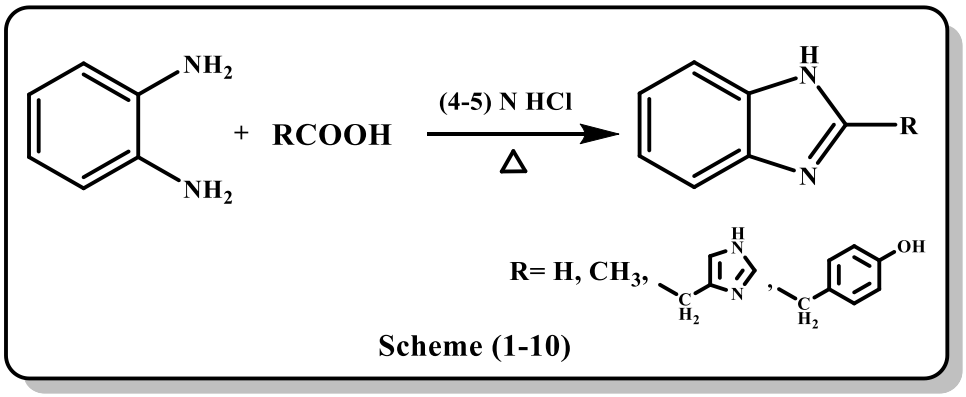
Scheme (1-8)

Lei Yang, et al.[39] synthesized benzimidazole by heating under reflux for 7 h of *o*-phenylenediammine with anthranilic acid to produce 2-(1H-benzo[d] imidazol-2-yl)aniline as shown in Scheme (1-9).



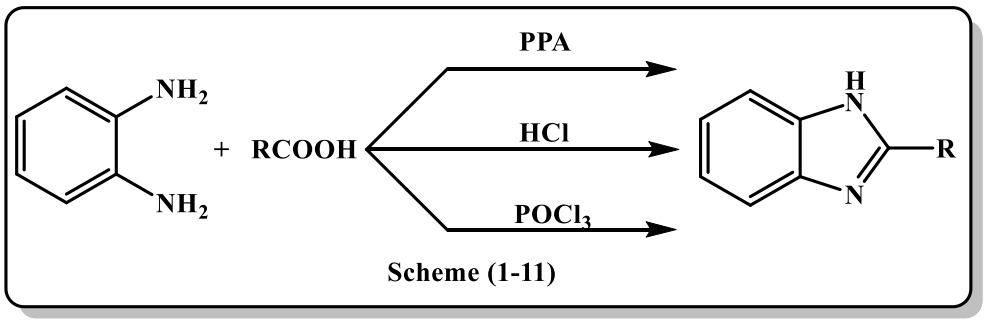
Scheme (1-9)

Mahmut Gozelle, et al.[40] synthesized benzimidazole from reaction of *o*-phenylenediammine with some carboxylic acids in (4-5) N HCl as shown in Scheme (1-10).



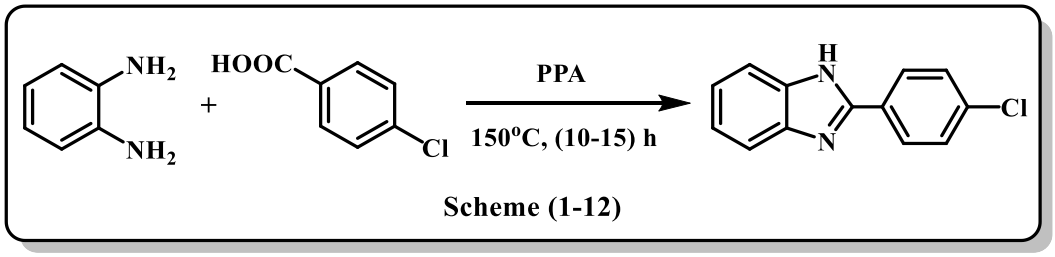
Scheme (1-10)

Also, Özden Tarı, et al.[41] synthesized novel benzimidazole derivatives by reaction of *o*-phenylenediammine with carboxylic acids in presence of PPA, HCl, and POCl3 as shown in Scheme (1-11).



Scheme (1-11)

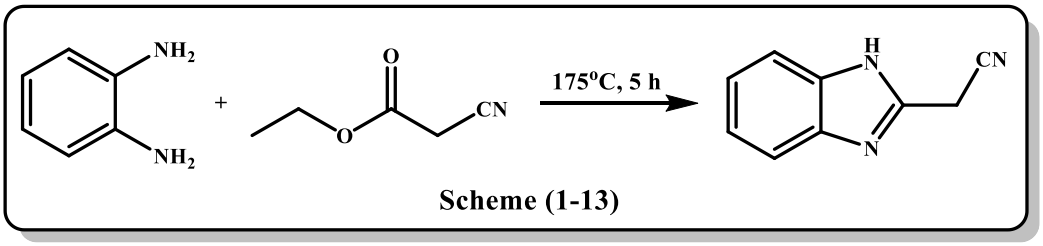
Xu Yuan, et al.[42] synthesized 2-(4-chlorophenyl)-1H-benzimidazole by reflux under150^{o}C for (10-15) h of *o*-phenylenediammine with 4-chlorobenzoic as shown in Scheme (1-12).



Scheme (1-12)

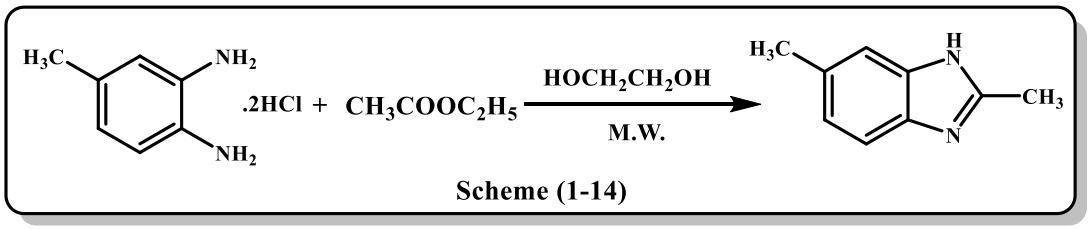
1.2.2.3. Reaction with esters

Mustafa, et al.[43] synthesized 2-(1H-benzo[d]imidazol-2-yl)acetonitrile from refluxed of *o*-phenylenediammine with ethylcyanoacetate in the oil bath as shown in Scheme (1-13).



Scheme (1-13)

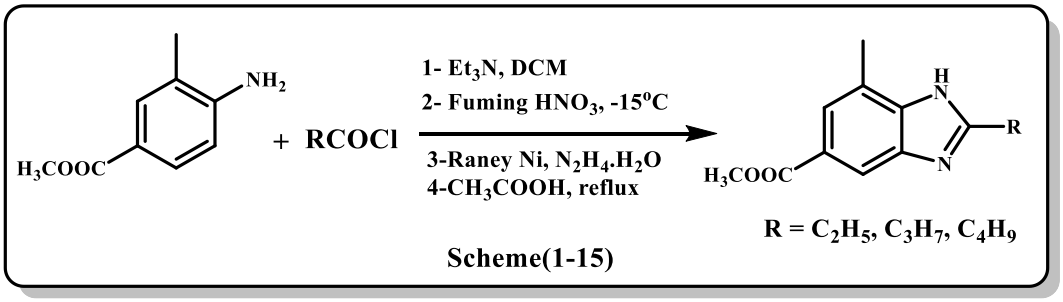
Also, 2-methyl-1H-benzimidazole have been synthesized from the reaction of 3,4-diaminotoluene dihydrochloride with ethylacetate as shown in Scheme (1-14)[44].



Scheme (1-14)

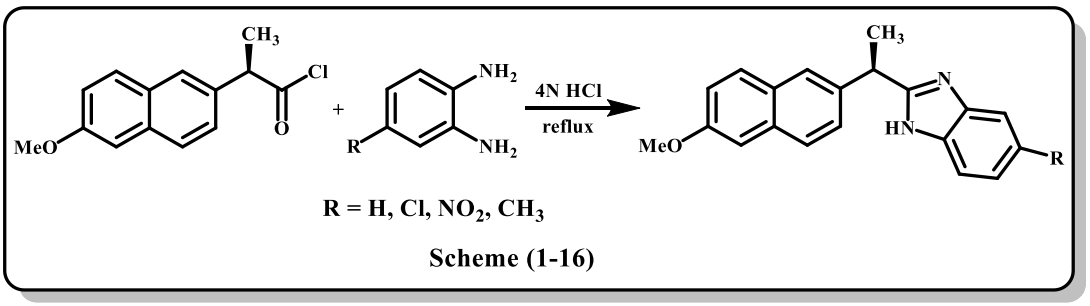
1.2.2.4. Reaction with acid chloride:

Acid chloride can be reacted with *o*-phenylenediammine to produce 2-substituted benzimidazole as shown in Scheme (1-15)[45].



Scheme(1-15)

Hasil Aman, et al.[46] synthesized novel benzimidazole from refluxed of *o*-phenylenediammine derivatives with naproxenoyl chloride as shown in Scheme (1-16).

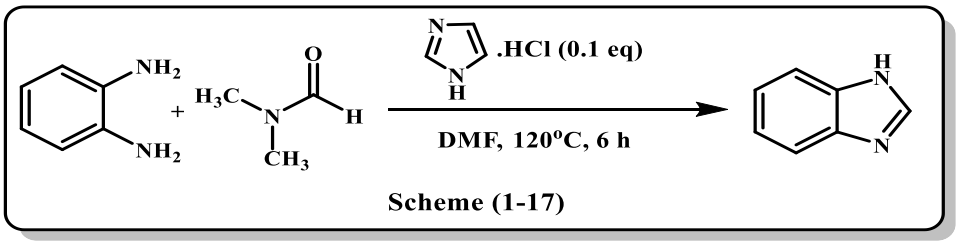


R = H, Cl, NO_2, CH_3

Scheme (1-16)

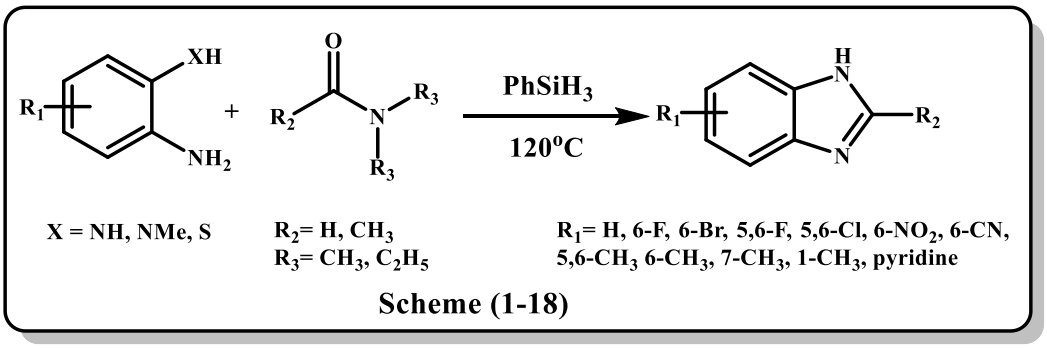
1.2.2.5. Reaction with amides:

In 2018, Zongjie Gan, et al.[47] synthesized 2-substituted benzimidazole from the reaction of *o*-phenylenediammine with DMF as shown in Scheme (1-17).



Scheme (1-17)

Zhu, et al.[48] synthesized novel benzimidazole derivatives from the reaction of *o*-phenylenediammine derivatives with DMF derivatives as shown in Scheme (1-18).



X = NH, NMe, S

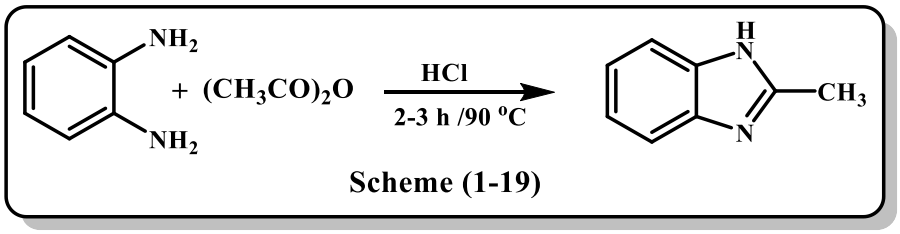
R_2= H, CH_3
R_3= CH_3, C_2H_5

R_1= H, 6-F, 6-Br, 5,6-F, 5,6-Cl, 6-NO_2, 6-CN, 5,6-CH_3 6-CH_3, 7-CH_3, 1-CH_3, pyridine

Scheme (1-18)

1.2.2.6. Reaction with acid anhydride:

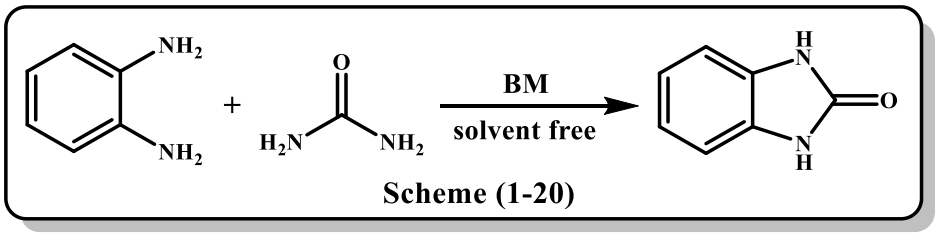
o-phenylenediammine can be reacted with acetic anhydride in presence of dilute HCl to produce 2-methylbenzimidazole as shown in Scheme (1-19)[49].



Scheme (1-19)

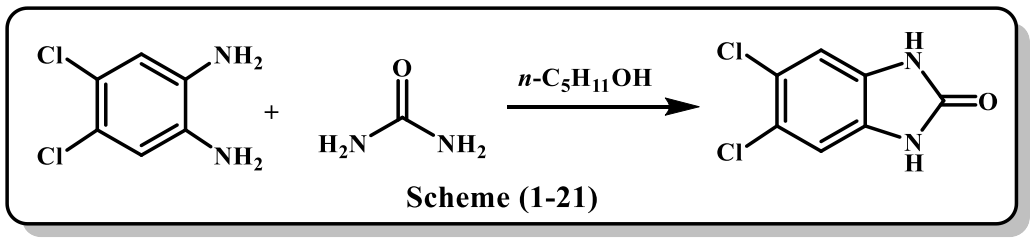
1.2.2.7. Reaction with urea:

Taghreed, et al.[50] synthesized 1,3-dihydro-2H-benzo[d]imidazol-2-one from the reaction of *o*-phenylenediammine with urea as shown in Scheme (1-20).



Scheme (1-20)

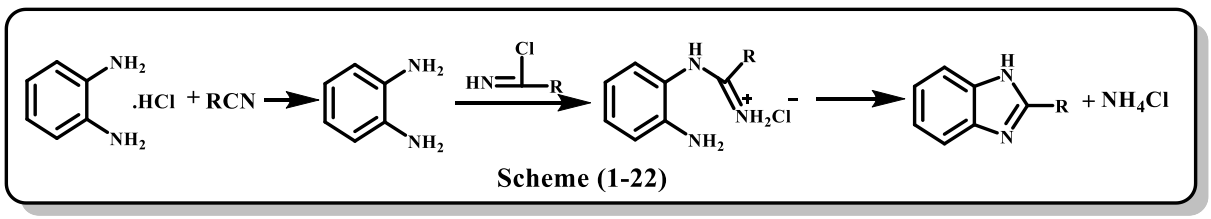
Meral Tunçbilek, et al.[51] synthesized 5,6-dichloro-1,3-dihydro-2H-benzo [d]imidazol-2-one from the reaction between *o*-phenylenediammine and urea as shown in Scheme (1-21).



Scheme (1-21)

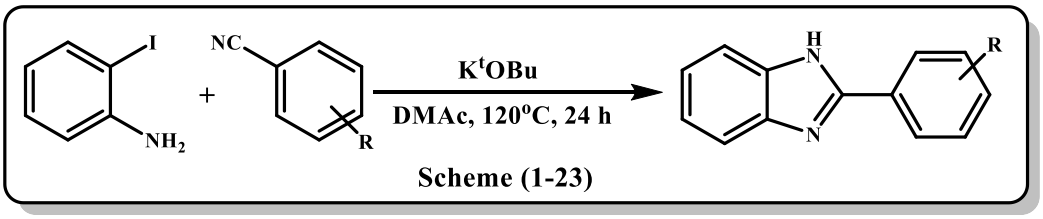
1.2.2.8. Reaction with nitriles:

o-phenylenediammine reacted with nitriles to produce 2-sustituted benzimidazole as shown in Scheme (1-22)[49].



Scheme (1-22)

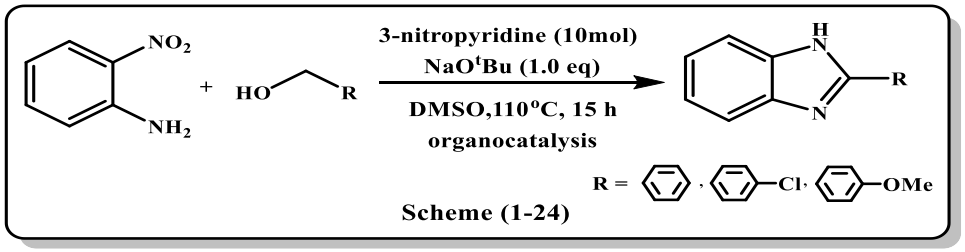
Also, 2-phenyl-1H- benzimidazole derivatives synthesized from the reaction of *o*-iodoaniline as shown in Scheme (1-23)[52].



Scheme (1-23)

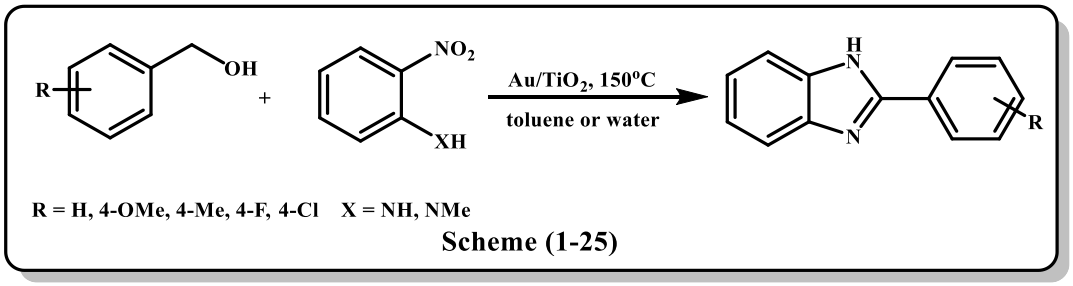
1.2.2.9. Reaction with alcohols:

o-phenylenediammine or *o*-nitroaniline reacted with alcohols to produce 2-sbstitutedbenzimidazole as shown in Scheme (1-24)[53].



Scheme (1-24)

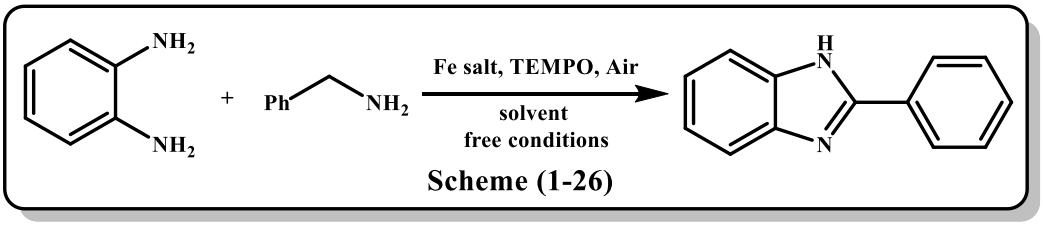
Lin Tang, et al.[54] synthesized benzimidazole from the reaction of *o*-nitroaniline derivatives with benzyl alcohol derivatives as shown in Scheme (1-25).



Scheme (1-25)

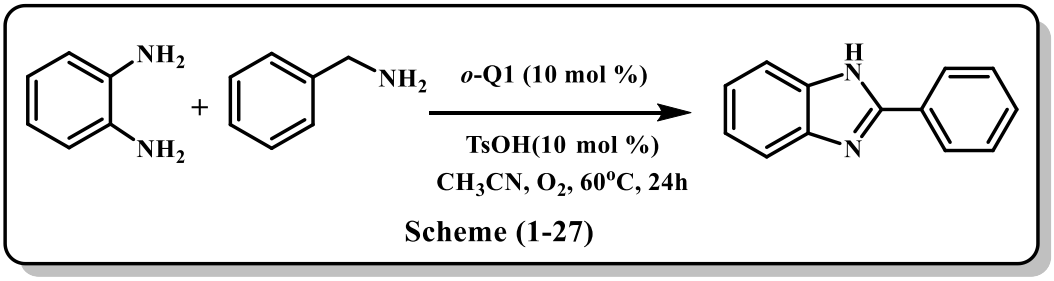
1.2.2.10. Reaction with primary amines:

2-phenyl-1H-benzimidazole can be produced from the reaction of aromatic primary amines with *o*-phenylenediammine as shown in Scheme (1-26)[55].



Scheme (1-26)

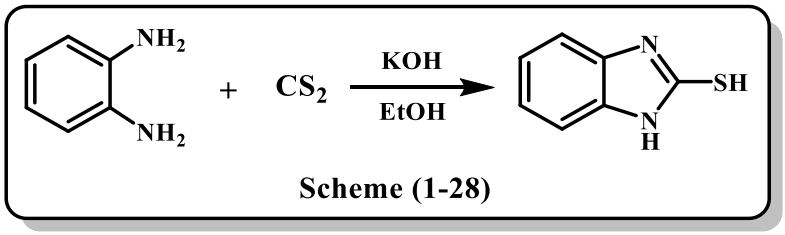
Zhang, et al.[56] synthesized 2-phenyl-1H-benzimidazole from the reaction of *o*-phenylenediammine with benzyl amine in presence of *o*-quinone as shown in Scheme (1-27).



Scheme (1-27)

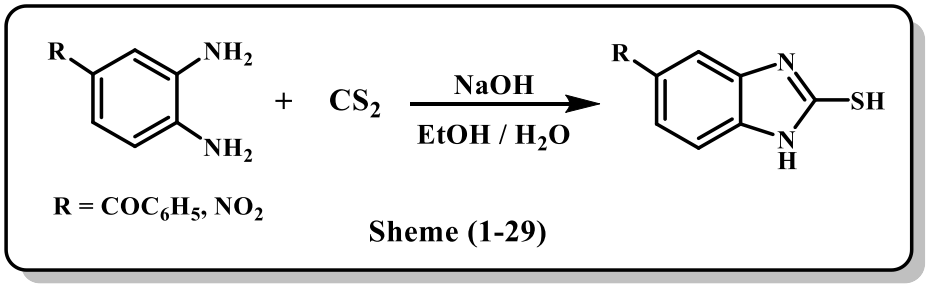
1.2.2.11. Reaction with CS_2:

In the presence of KOH, *o*-phenylenediammine refluxed with CS_2 in ethanol as a solvent produce 2-mercapto-1H-benzimidazole as shown in Scheme (1-28)[57].



Scheme (1-28)

Also, A. T. Mavrova, et al.[58] synthesized 2-mercapto-1H-benzimidazole from the reaction of *o*-phenylenediammine with CS_2 in ethanol and water in presence of NaOH as shown in Scheme (1-29).



Sheme (1-29)

1.2.3. Literature survey of Benzimidazole Compound and Biological Applications:

Benzimidazole and its derivatives have an important various biological activity and wide rang in the pharmaceutical industry such as antibacterial, anti-inflammatory, antiviral activity, etc. Figure (1-10)[25].

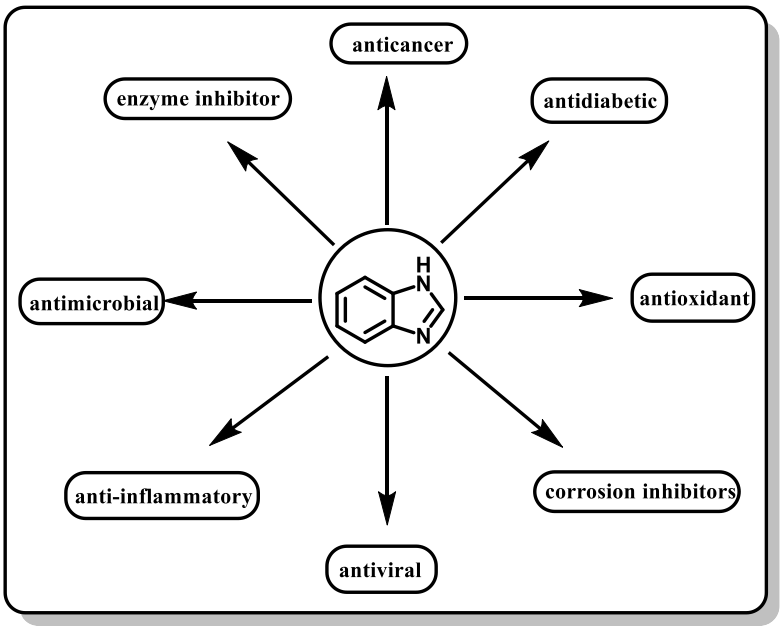
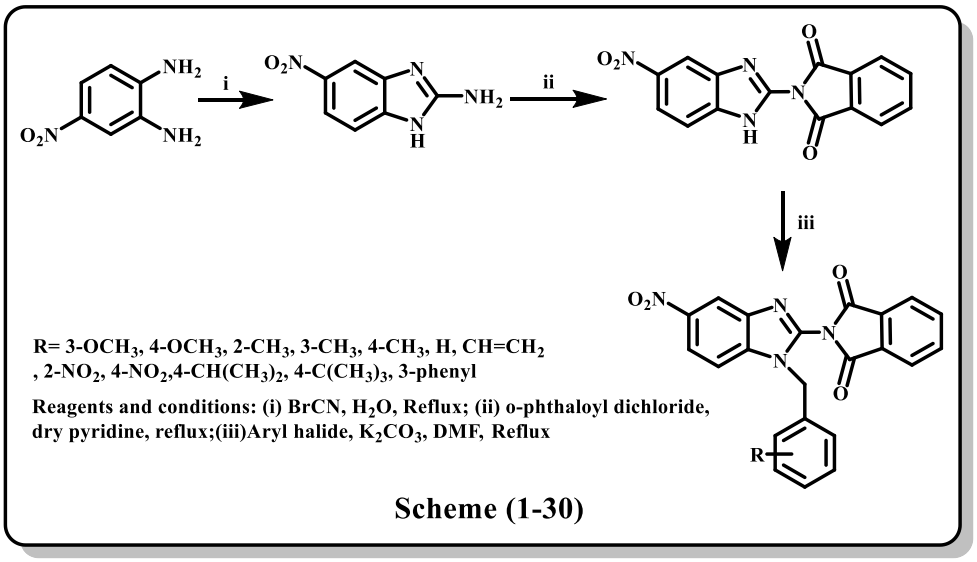


Figure (1-11): Some industrial and pharmaceutical uses of benzimidazole

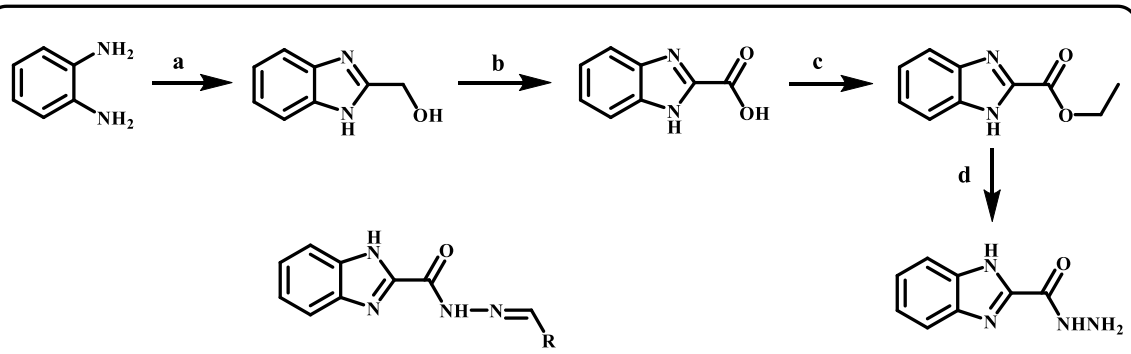
1.2.3.1. Anti-inflammatory agents:

Gaganpreet Kaur, et al.[59] synthesized novel benzimidazole derivatives and gave good activity as anti-inflammatory agents Scheme (1-30).



Scheme (1-30)

Yanguo Shang, et al.[60] synthesized Synthesized 1H-benzo[d]imidazole-2-carbohydrazide derivatives and some of these compounds gave good activity as anti-neuroinflammatory agents Scheme (1-31).

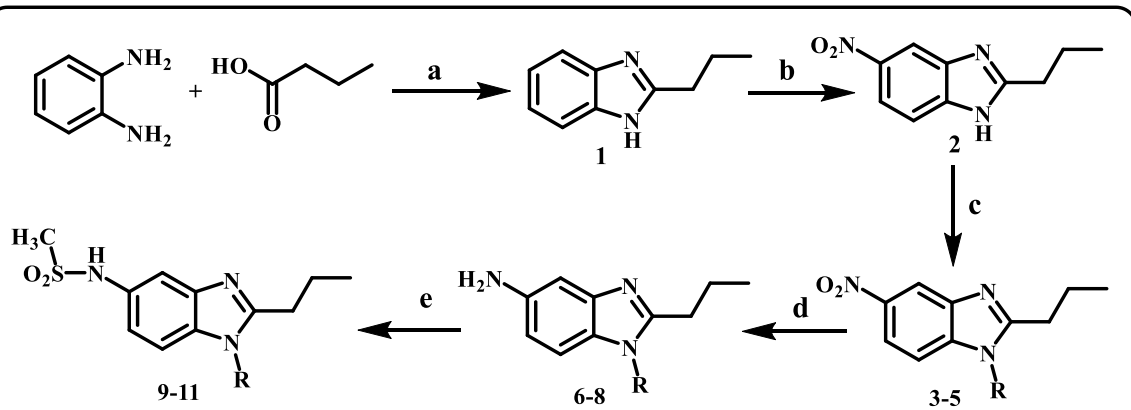


R= cyclohexyl, phenyl, 3-flurophenyl, 4-fluorophenyl, 3-chiorophenyl, 4-chlorophenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-hydrophenyl, 4-morpholinphenyl, 4-biphenyl, 4-phenoxyphenyl, 4-benzyloxyphenyl, 2-naphthyl, 5-indolyl, 4-(3-fluorobenzyloxy)phenyl

Reagents and conditions: (a) Glycolic acid, EtOH, reflux; (b) Potassium permanganate, $NaOH/H_2O$, reflux, 5 h;(c) Ethanol, HATU,THF, rt;(d) Hydrazine hydrate, EtOH, reflux, 3 h

Scheme (1-31)

Ratika Sharma, et al.[61] synthesized a good anti-inflammatory agents of *N*-(2-propyl-1H-benzo[d]imidazol-5-yl)methane sulfonamide derivatives Scheme (1-32).



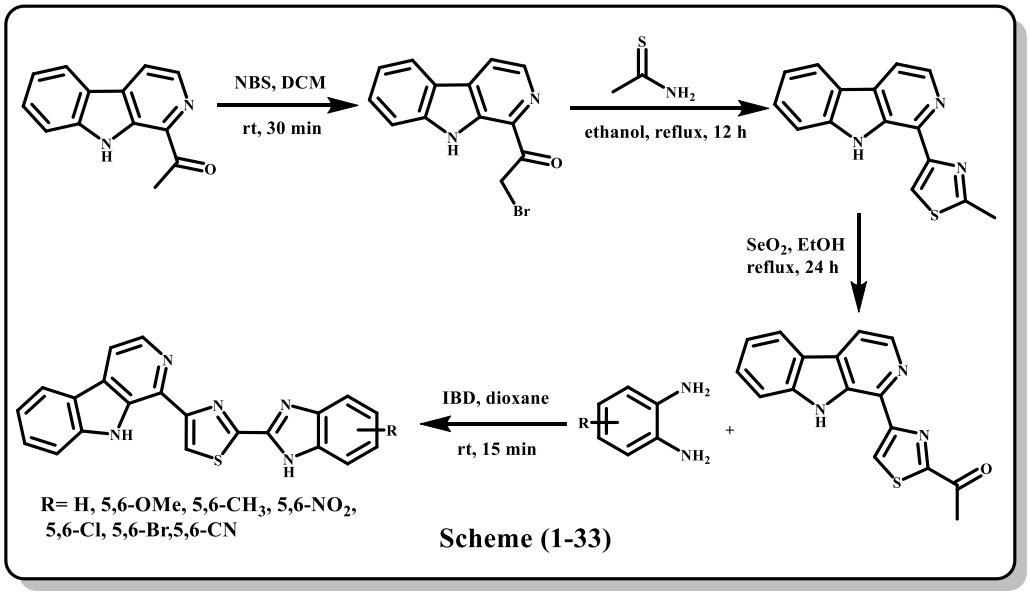
R = n-butyl, n-pentyl, n-hexyl

Reagents and conditions: (a) Reflux, 7 h (b) conc. HNO_3, conc. H_2SO_4, 10 h (c) Alkyl bromide, DMF, anhydrous K_2CO_3, room temp. (d) $SnCl_2.2H_2O$, anh. EtOH, 75^{0}C (e) CH_3SO_2Cl, DCM, room temp.

Scheme (1-32)

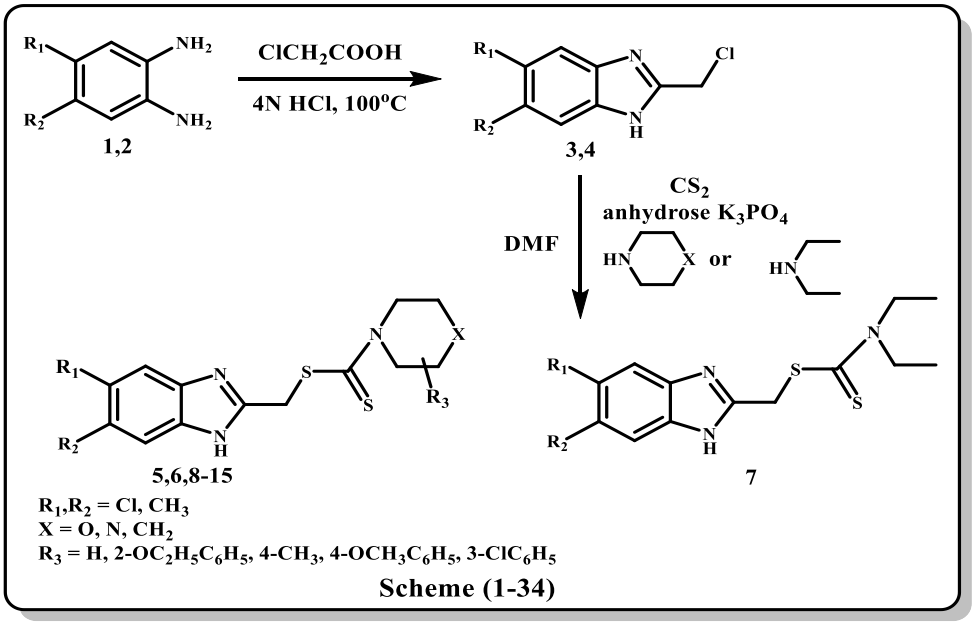
1.2.3.2. Anti-cancer agents:

Reddymasu Sireesha, et al.[62] synthesized novel series of benzimidazole derivatives and some of them show a good anticancer activity Scheme (1-33).



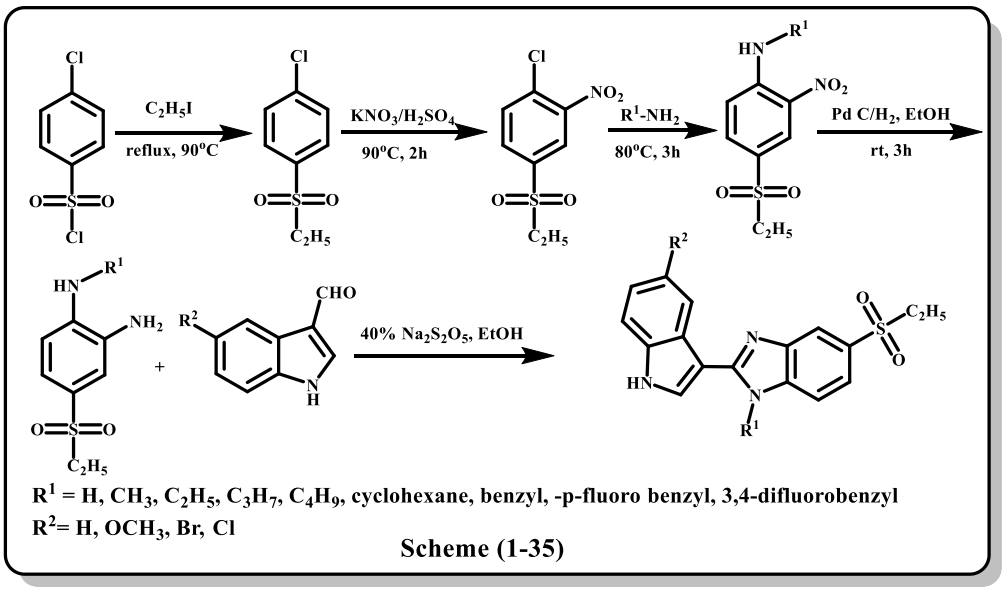
Scheme (1-33)

Samira Nashaat, et al.[63] synthesized new benzimidazole derivatives and some compounds show good activity against breast cancer Scheme (1-34).



Scheme (1-34)

Fikriye Zengin Karadayi, et al.[64] synthesized new indole-benzimidazoles derivatives and some compounds gave a good activity as anticancer /antiestrogenic agents Scheme (1-35).

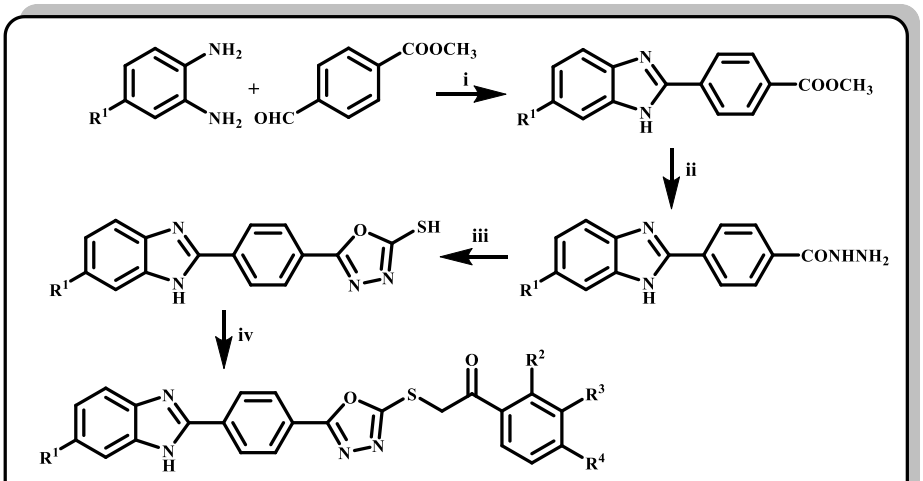


R^1 = H, CH_3, C_2H_5, C_3H_7, C_4H_9, cyclohexane, benzyl, -p-fluoro benzyl, 3,4-difluorobenzyl
R^2= H, OCH_3, Br, Cl

Scheme (1-35)

1.2.3.3. Anti-microbial agents:

Ahmet Karaburun, et al.[65] synthesized novel benzimidazole derivatives as antifungal agents, some compounds show good activity against some fungai Scheme (1-36).

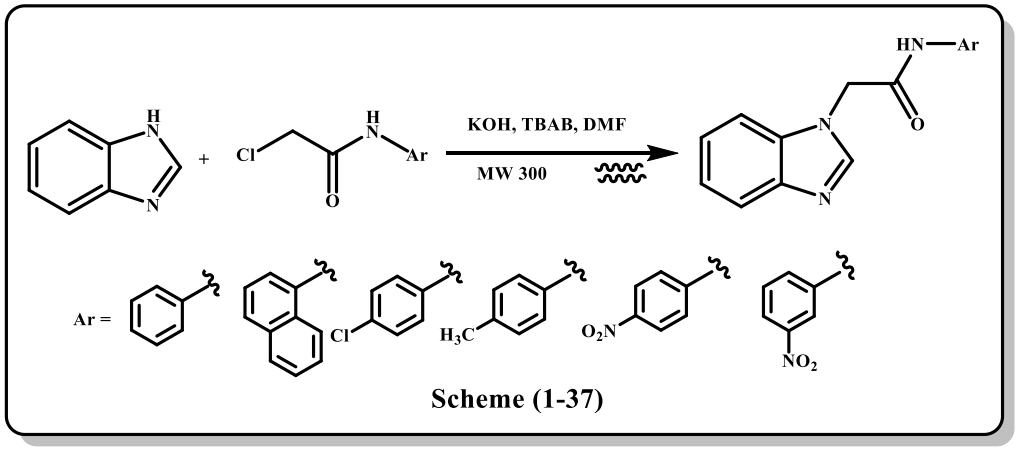


R^1: -F, -Cl; R^2: -H, -Cl; R^3: -H, -OH; R^4: -H, $-CH_3$, -Cl, -Br, $-NO_2$, -CN, -Cl, -OH

Reagents and conditions: (i) $Na_2S_2O_5$, DMF, MWI, 10 min, (ii) $NH_2NH_2xH_2O$, EtOH, MWI, 10 min, (iii) CS_2/NaOH,EtOH, reflux, 8 h, (iv) appropriate phenacyl bromides, K_2CO_3, acetone, rt, 8 h.

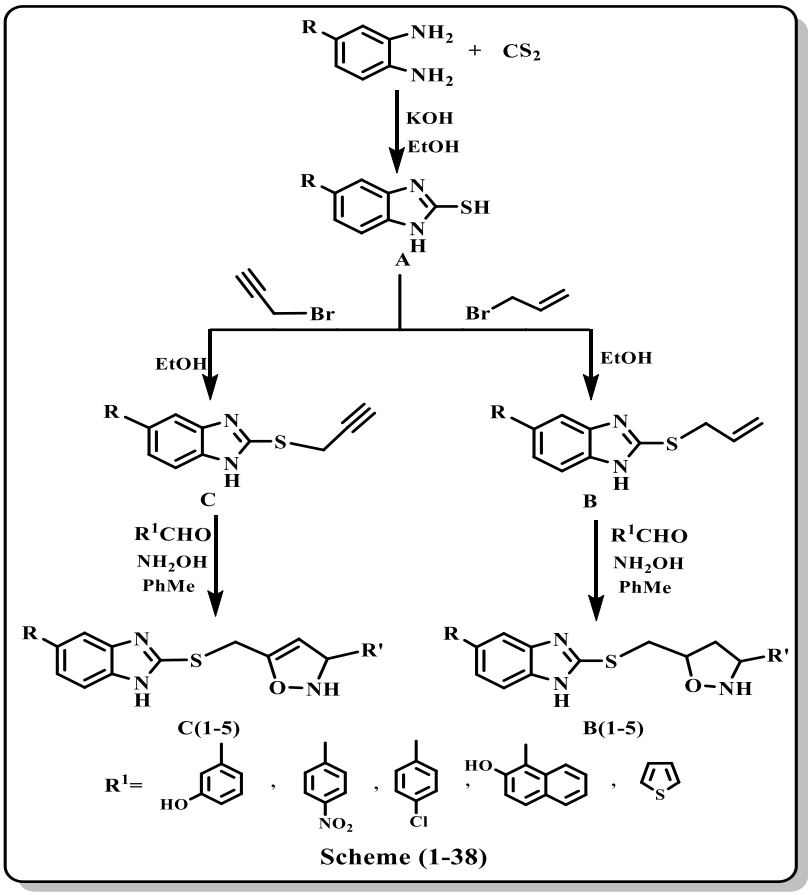
Scheme (1-36)

In 2020, Sanjay R. Chaudhari, et al.[66] synthesized new *N* -substituted benzimidazoles derivatives as *Staphylococcus aureus* inhibitor and some of them gave good antibacterial activity Scheme (1-37).



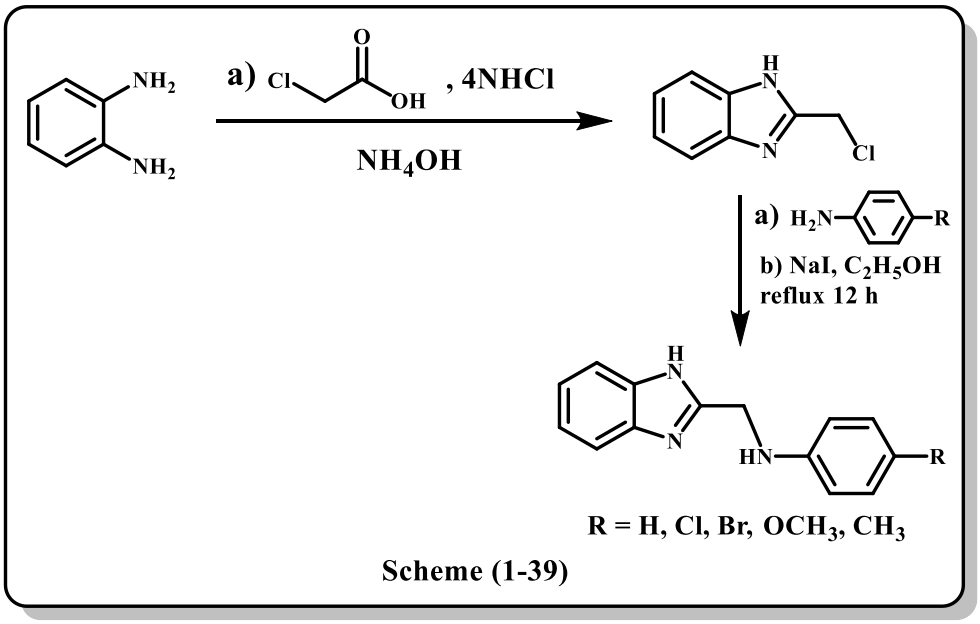
Scheme (1-37)

Muayad A. Rdaiaan, et al.[67] synthesized novel benzimidazole derivatives and some compounds give good activity as antibacterial agents Scheme (1-38).



Scheme (1-38)

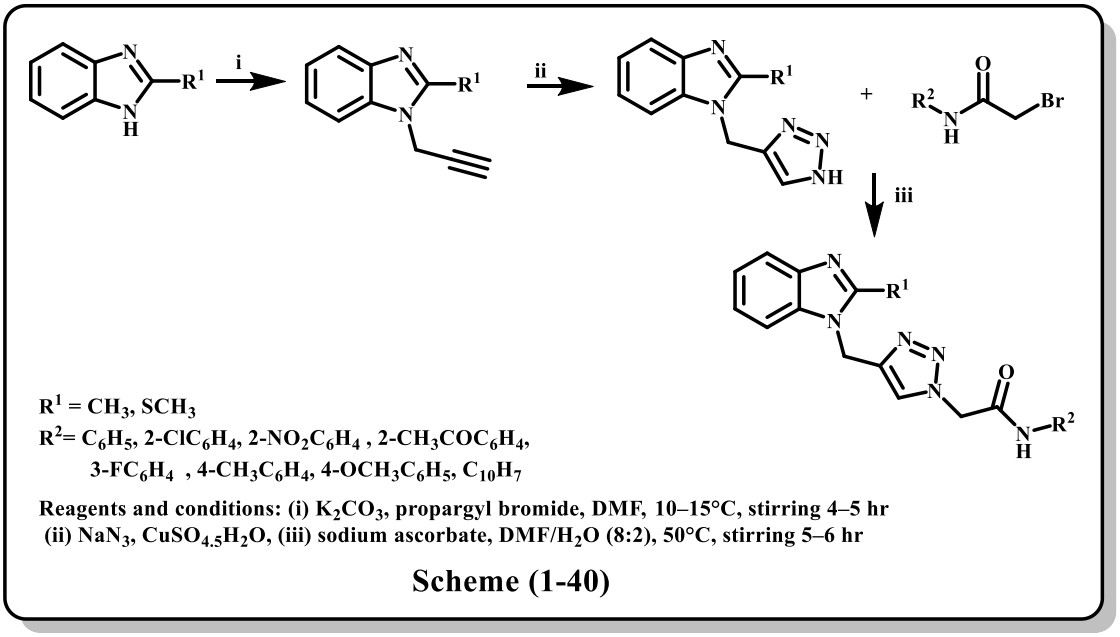
Ola R. Shehab, et al.[68] synthesized some of benzimidazole derivatives, some of these compounds showed good activity as antibacterial agents Scheme (1-39).



Scheme (1-39)

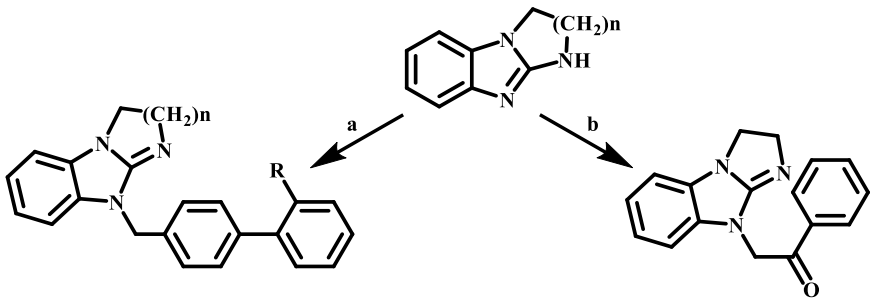
1.2.3.4. Anti-diabetic agents:

In 2020, Laxmi Deswal, et al.[69] synthesized new benzimidazole derivatives and some of these compounds and showed good anti-diabetic activity Scheme (1-40).



Scheme (1-40)

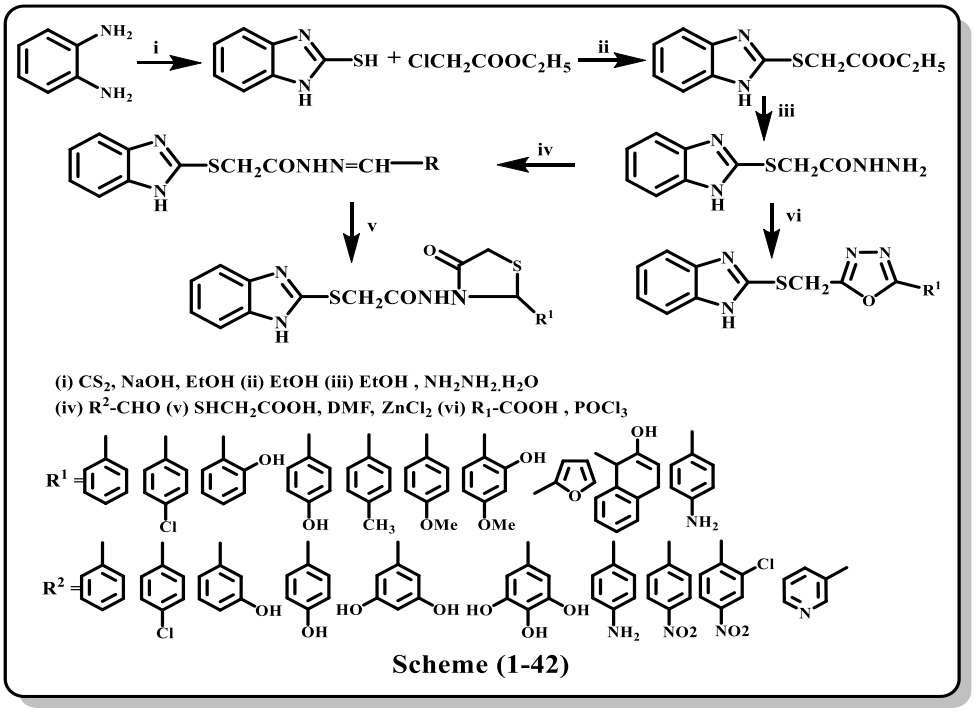
Denis Babkov, et al.[70] synthesized a new series of benzimidazole derivatives as antidiabetic agents and some of them showed good activity Scheme (1-41).



n = 1,2,3
R = H, CN, COOMe, COOEt, COOH
Reagents and conditions: (a) benzylic bromide, $MeNO_2$, reflux; (b) ethyl 4-(bromomethyl) benzoate, MeCN, reflux

Scheme (1-41)

Ramya Shingalapur, et al.[71] synthesized new benzimidazole derivatives pharmacophore and showed good activity as anticonvulsant, antidiabetic and DNA cleavage studies Scheme (1-42).

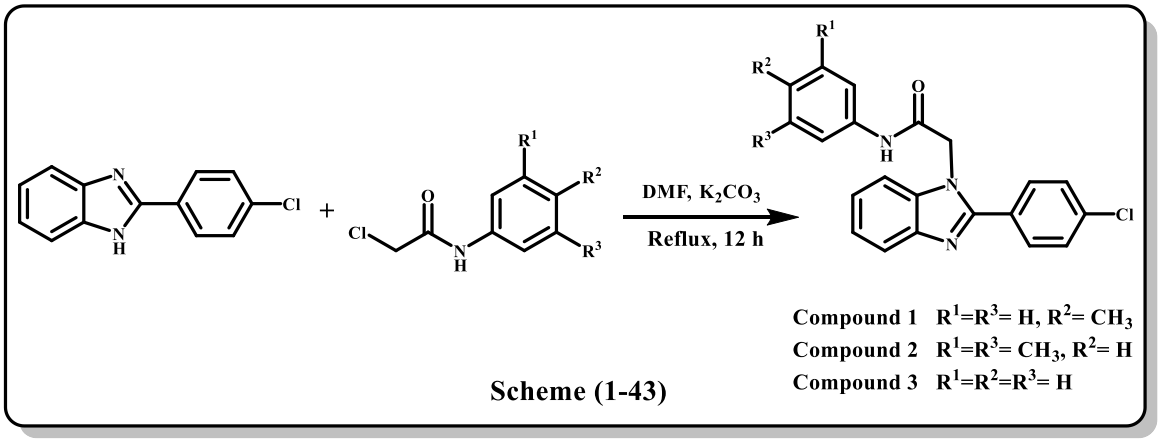


(i) CS_2, NaOH, EtOH (ii) EtOH (iii) EtOH , $NH_2NH_2.H_2O$
(iv) R^2-CHO (v) $SHCH_2COOH$, DMF, $ZnCl_2$ (vi) R_1-COOH , $POCl_3$

Scheme (1-42)

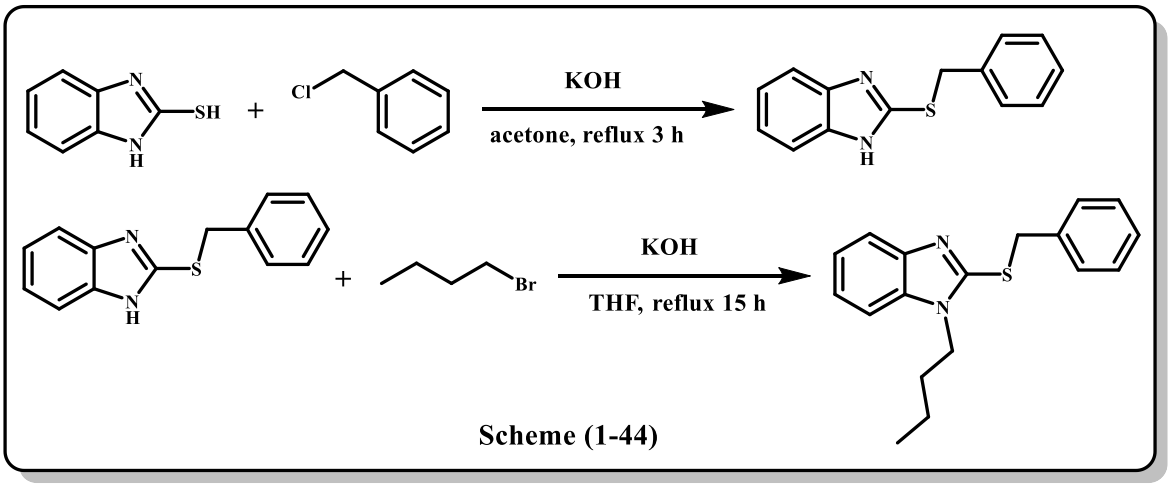
1.2.3.5. Corrosion inhibitors agents:

Z. Rouifi and others, et al.[72] synthesized a newly benzimidazole derivative as corrosion inhibitors and some of them showed Impairs inhibitory efficiency Scheme (1-43).



Scheme (1-43)

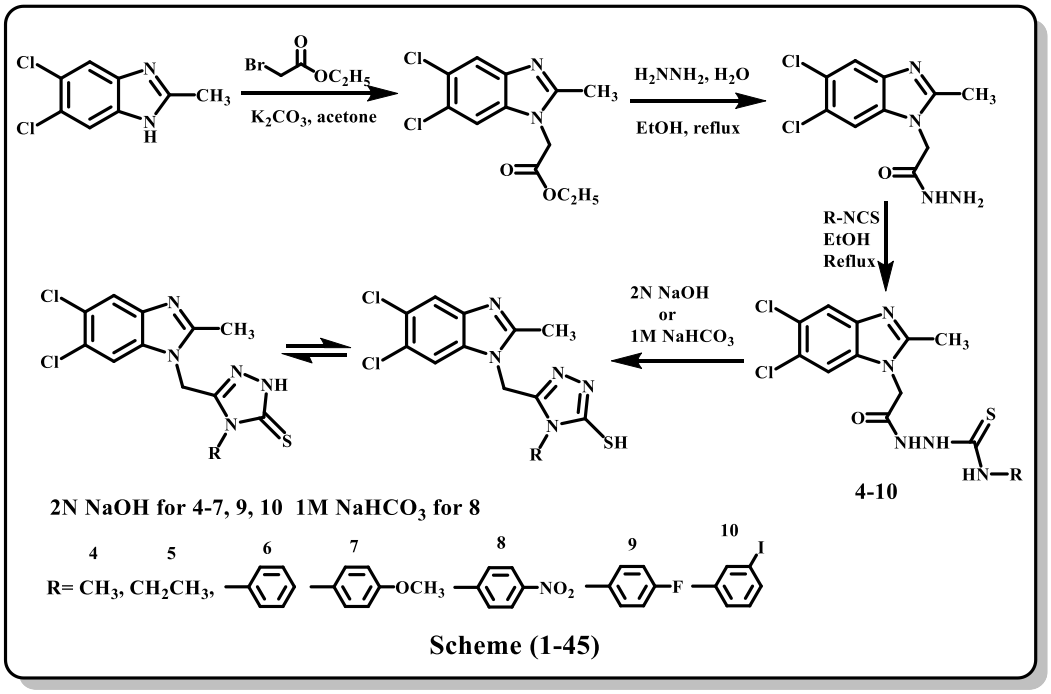
G.A. Zhang, et al.[73] synthesized a novel benzimidazole derivative as corrosion inhibitors and some of them showed good activity for inhibition of corrosion Scheme (1-44).



Scheme (1-44)

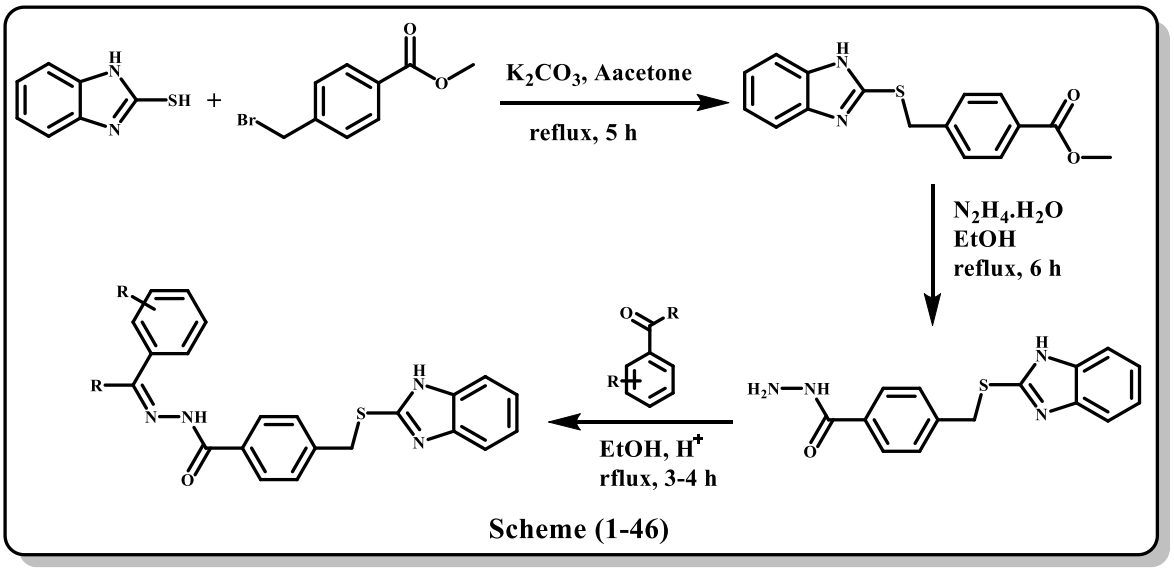
1.2.3.6. Enzymes inhibitors agents:

Emre Menteşe, et al.[74] synthesized new benzimidazole derivatives as potential inhibitors agents of urease enzyme, some of these compounds gave a good inhibition of urease Scheme (1-45).



Scheme (1-45)

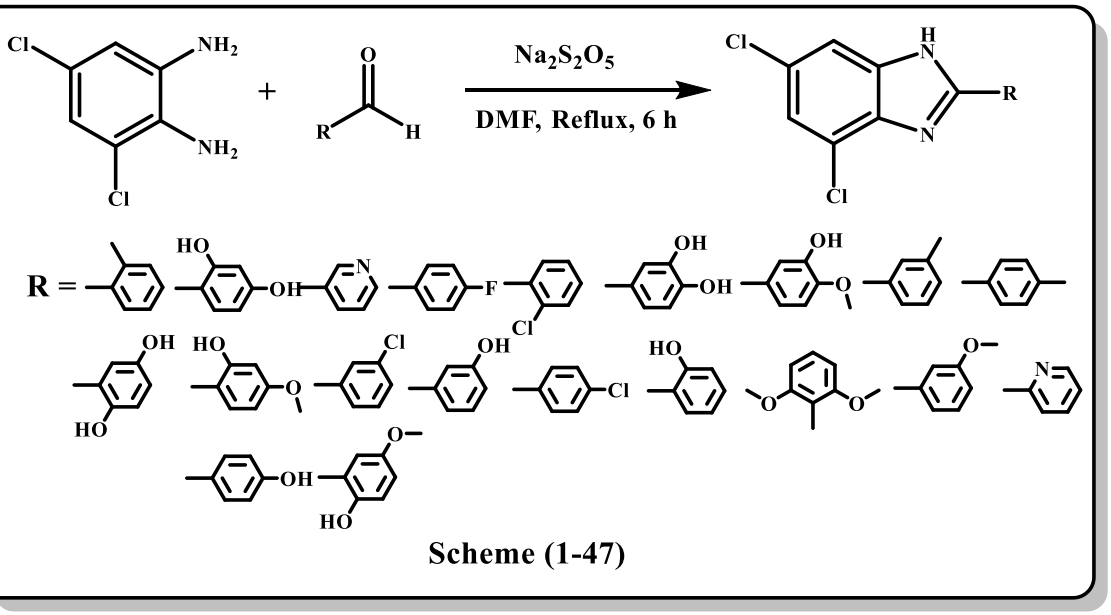
Khalid Zaman, et al.[75] synthesized newly benzimidazole derivatives and some of these compounds showed good activity as urease inhibitors Scheme (1-46).



Scheme (1-46)

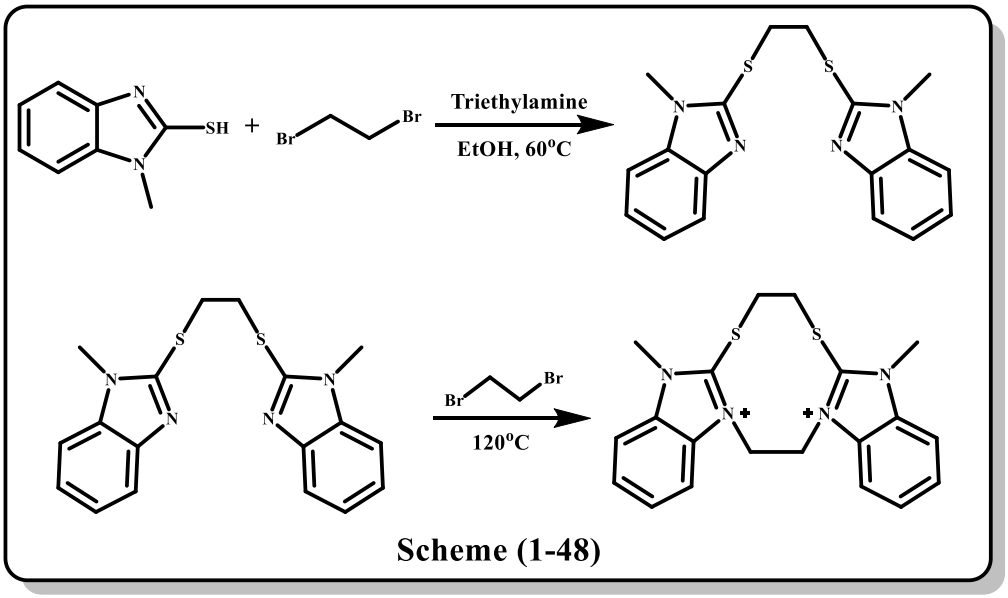
1.2.3.7. Anti-oxidants agents:

Muhammad Taha, et al.[16] synthesized new derivatives of 4,6-dichloro-2-substituted-1H-benzimidazole and some of them showed good activity as antioxidants Scheme (1-47).



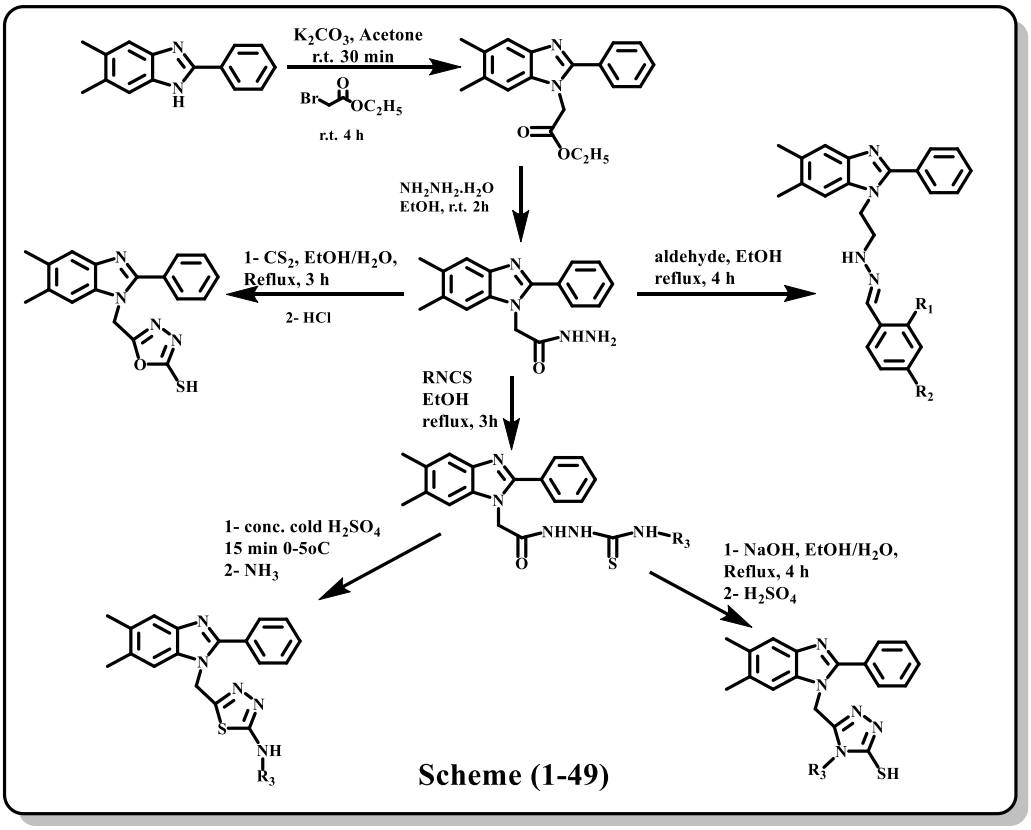
Scheme (1-47)

Ufuk Yildiz [76] synthesized new thiol bridged bis-benzimidazole derivative and showed good activity as antioxidant agents Scheme (1-48).



Scheme (1-48)

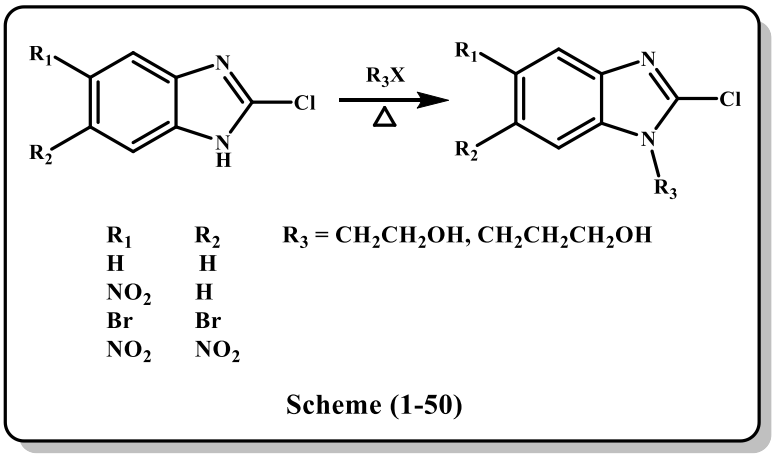
In 2020, Nesrin, et al.[77] synthesized tetra-substituted benzimidazole derivatives and showed good activity as antioxidants and anti-urease activities Scheme (1-49).



Scheme (1-49)

1.2.3.8. Anti-viral agents:

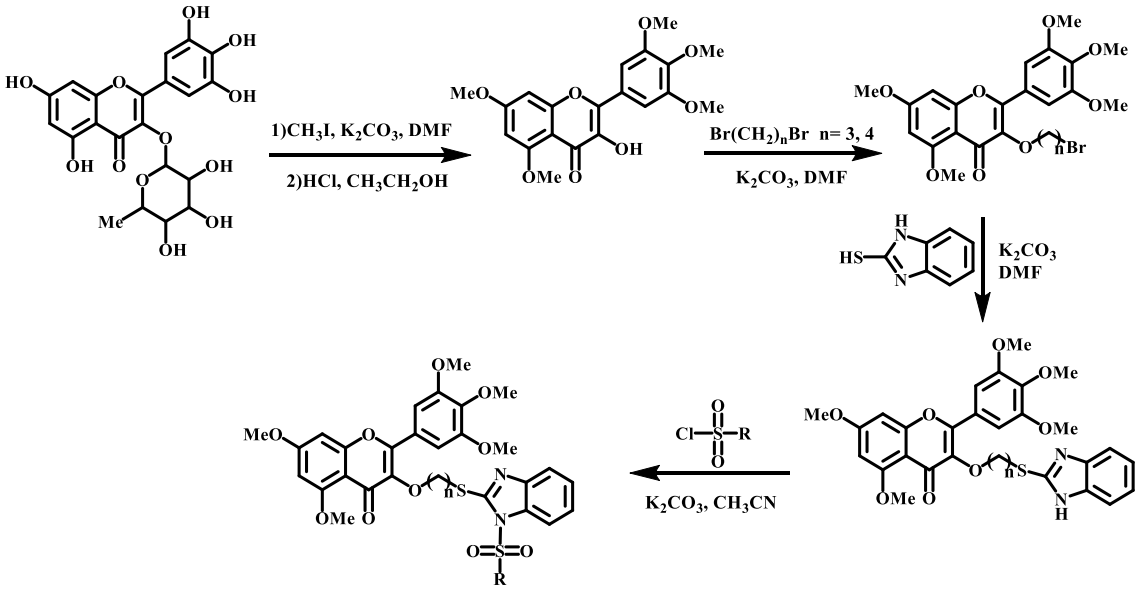
Ritika Srivastava, et al.[78] synthesized 2-chloro-1H-benzimidazole derivatives and tested as antiviral against HIV and YFV and gave a good activity as antiviral agents Scheme (1-50).



R_1	R_2	R_3 = CH_2CH_2OH, $CH_2CH_2CH_2OH$
H	H	
NO_2	H	
Br	Br	
NO_2	NO_2	

Scheme (1-50)

Mei Chen, et al.[79] synthesized novel benzimidazole derivatives as antiviral and some compounds showed good activity as antiviral agents Scheme (1-51).



n=3,4 R= thiophene-2-yl, ph, 2-Br-ph, 2-Cl-ph, 4-Me-ph, 3-Br-ph, 4-F-ph, 4-OMe-ph, 4-NO_2-ph, 2-F-ph

Scheme (1-51)

1.3- Thiadiazole:

Thiadiazole is an aromatic five-membered heterocyclic ring, containing three heteroatoms, one is sulfur atom and two are nitrogen with general formula $C_2H_2SN_2$[80].

The first discovery of 1,3,4-Thidiazole was by Emil Fischer in 1882[81]. Its five-membered heterocyclic ring contains a sulfur atom in **(1)** position and two nitrogen atoms in **(3,4)** positions, also of two carbon atoms in **(2,5)** positions [82].

Thiadiazole have four isomer 1,2,3,-Thiadiazole **(I)**, 1,2,4-Thiadiazole **(II)**, 1,2,5-Thiadiazole **(III)**, 1,3,4-Thiadiazole **(IV)** as shown in Figure (1-12).

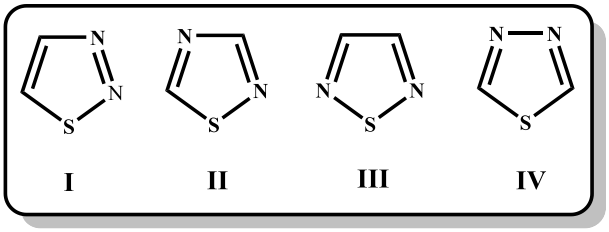


Figure (1-12): Isomeric structure of Thiadiazole

Each of 2-mercapto-1,3,4-thiadiazole, 2-hydroxy-1,3,4-thiadiazole and 2-aminothiadiazole show two tautomeric forms, both 2-mercapto-1,3,4-thiadiazole, 2-hydroxy-1,3,4-thiadiazole mostly exist in ketoform **(I)** but their thiol/ hydroxyl function always elicited through the chemical reactions[83] as shown in Figure (1-13).

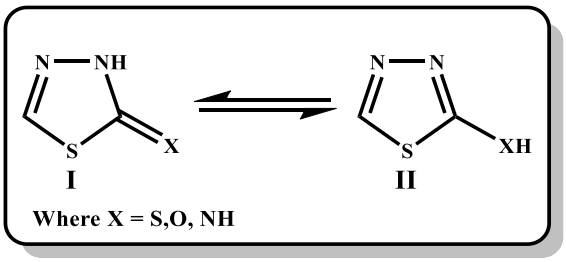
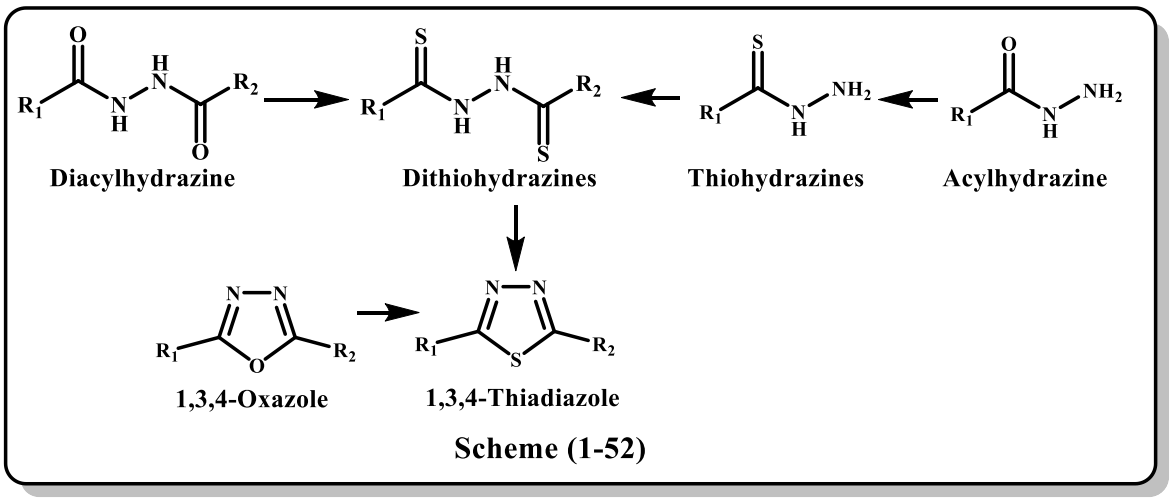


Figure (1-13): Tautomeric forms of Thiadiazole

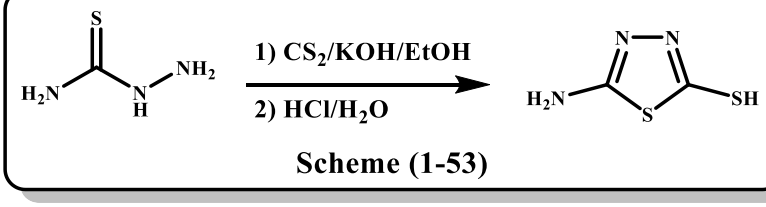
1.3.1. Synthesis of 1,3,4-thiadiazole and its derivatives:

Generally, 1,3,4-Thiadiazole can be obtained by ring closure reaction of acylhydrazine derivatives or by conversion of 1,3,4-Oxadiazole or 1,3,4-Thiadiazole can be obtained from thiohydrazine Scheme (1-52)[84].



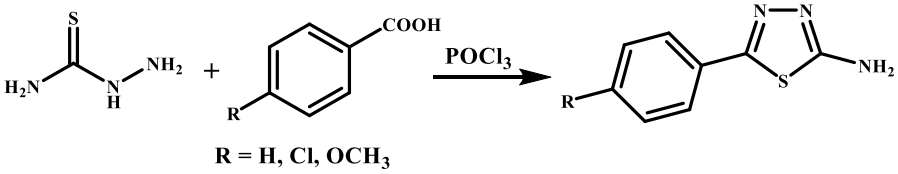
1.3.1.1. From Thiosemicarbazides:

Jixiang Chenand, et al.[85] synthesized 2-thio-5-amino-1,3,4-thidiazole from the reaction of thiosemicarbazide with CS_2 in the presence of KOH Scheme (1-53).



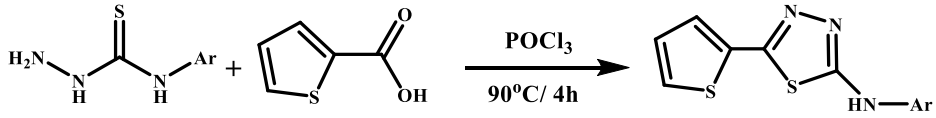
Scheme (1-53)

Cristina A. Mei Chen, et al. [86] synthesized 2-amino-5-phenyl-1,3.4-thiadiazole from the reaction of thiosemicarbazide with benzoic acid derivatives in presence of $POCl_3$ Scheme (1-54).



Scheme (1-54)

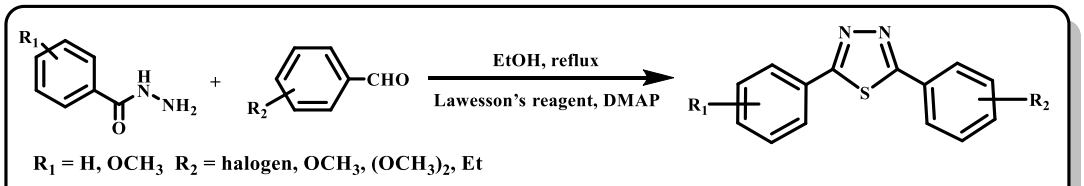
Halit Muğlu Mei Chen, et al.[87] synthesized 2-*N*-aryl-5-thiophene-1,3,4-thiadiazole from the reaction of *N*-arylthiosemicarbazide derivatives with 2-thiophenecarboxylic acid in the presence of $POCl_3$ Scheme (1-55).



Scheme (1-55)

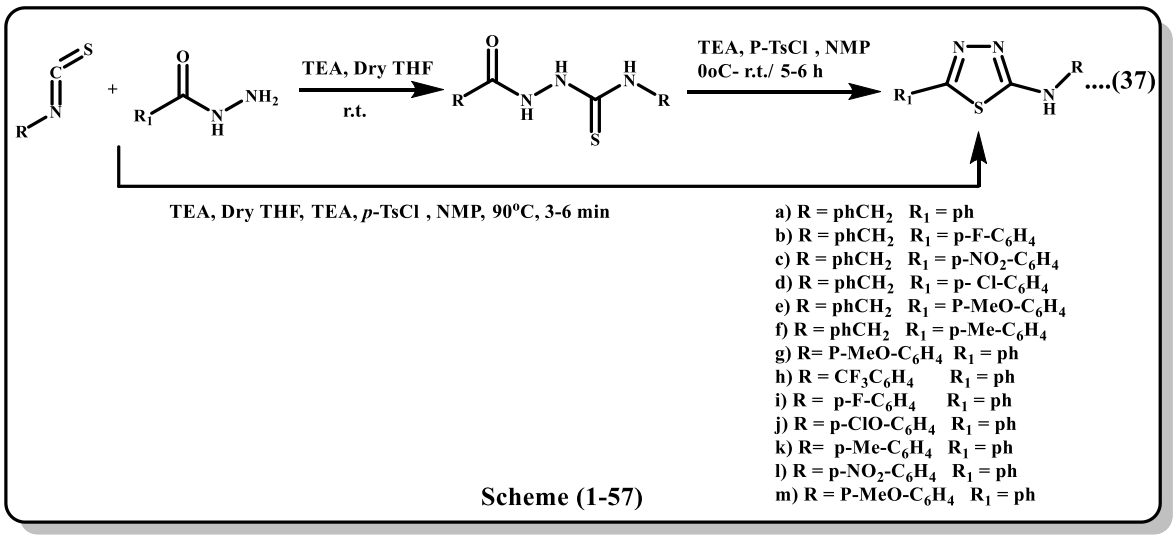
1.3.1.2. From Acid Hydrazides:

Inseok Ko Mei Chen, et al.[88] synthesized 2,5-diphenyl-1,3,4-thiadiazole derivatives from the reaction of aryl acid hydrazine with aryl aldehyde Scheme (1-56).



Scheme (1-56)

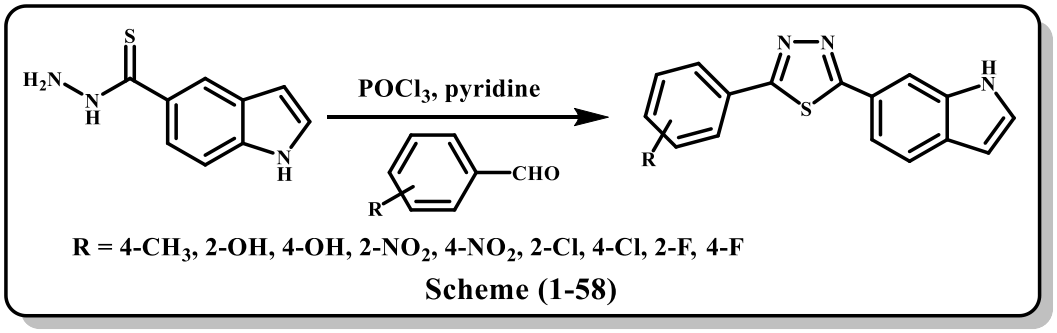
D.C. Sekhar, et al.[89] synthesized 2-amino-1,3,4-thiadiazole form reaction of isocyanates with acid hydrazides in THF with presence of TEA, NMP and P-TsCl Scheme (1-57).



Scheme (1-57)

1.3.1.3. From Thiohydrazides:

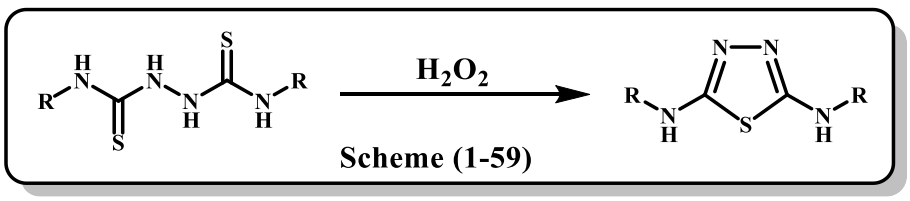
Almandil N. B., et al. [90,91] synthesized from the reaction of 1H-indole-5-carbothiohydrazide with benzyaldehyde derivatives in pyridine and $POCl_3$ Scheme (1-58).



Scheme (1-58)

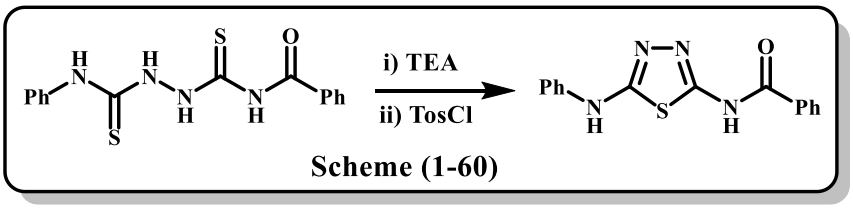
1.3.1.4. From Acylbithioureas:

Papi D., et al.[92] synthesized 2,5-diamino-1,3,4-thiadiazole derivatives from reaction of bisthioureas with 3% H_2O_2 Scheme (1-59).



Scheme (1-59)

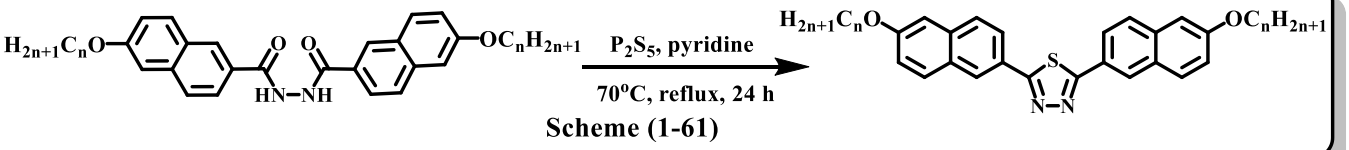
Also, benzoylated thiadiazole was produced by the reaction of acylbithioureas with p-TsCl in presence of TEA via intermediate Scheme (1-60) [93].



Scheme (1-60)

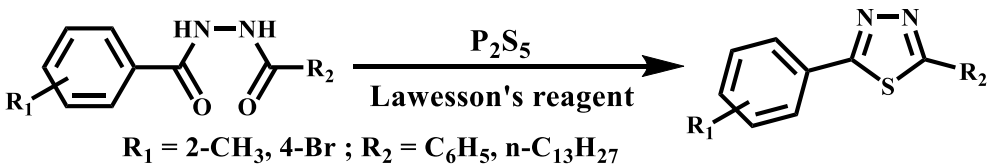
1.3.1.5. From Diacylhydrazines:

Hsiu-Ming Kuo, et al.[94] synthesized novel 2,5-disubstituted thiadiazole from the reaction of diacylhydrazines with P_2S_5 in pyridine Scheme (1-61).



Scheme (1-61)

Also, diacylhydrazines can be reacted with P_2S_5 or Lawesson's reagent to produce 2,5-disubstituted thiadiazole Scheme (1-62) [95].

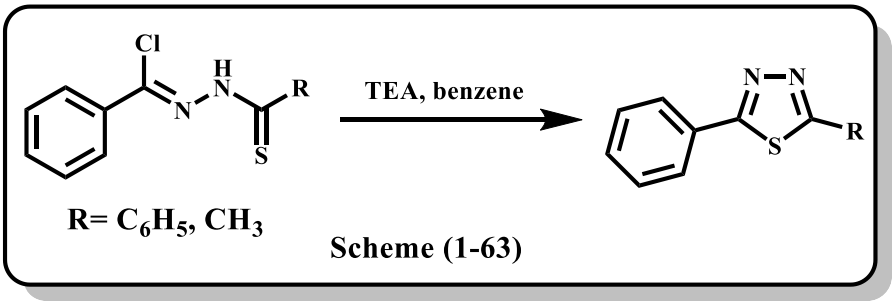


R_1 = 2-CH_3, 4-Br ; R_2 = C_6H_5, n-$C_{13}H_{27}$

Scheme (1-62)

1.3.1.6. From Thioacyl hydrazonoyl chloride:

Ahmad Shawali[96] synthesized 1,3,4-haidiazole by reaction of *N*-thioacety and *N*-Thiobenzoyl hydrazonoyl chlorides with TEA in benzene Scheme (1-63).

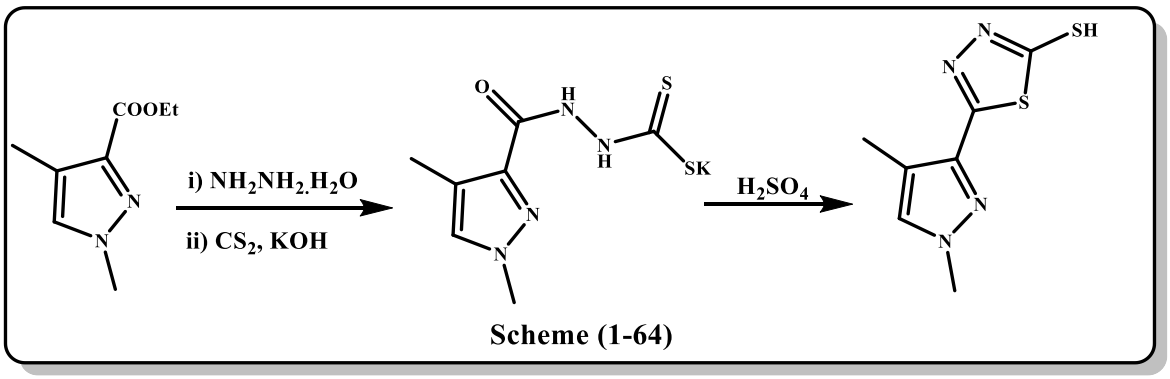


R= C_6H_5, CH_3

Scheme (1-63)

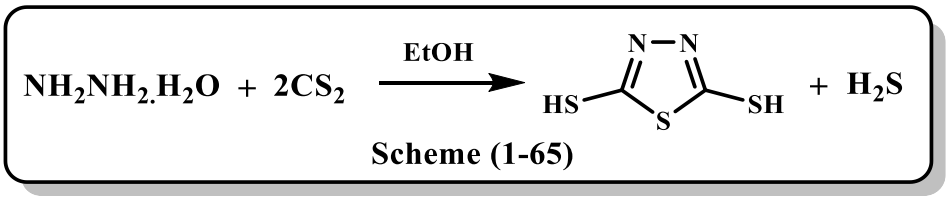
1.3.1.7. From Hydrazine:

Xiaoming Ding, et al.[97] synthesized new 1,3,4-thiadiazol derivatives from the reaction of pyrazole ester with hydrazine in presence of CS_2 and KOH followed by addition of H_2SO_4 Scheme (1-64).



Scheme (1-64)

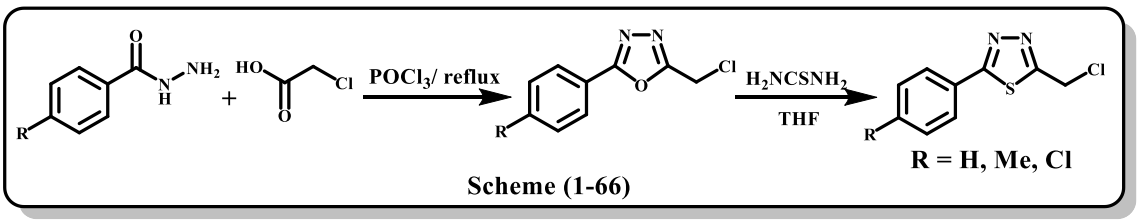
S. F. Al-Zobaydiy Mei Chen, et al.[98] synthesized 2,5-Dimercapto-1,3,4-thiadiazole from the reaction of hydrazine with CS_2 in ethanol Scheme (1-65).



Scheme (1-65)

1.3.1.8. From 1,3,4-Oxadiazoles:

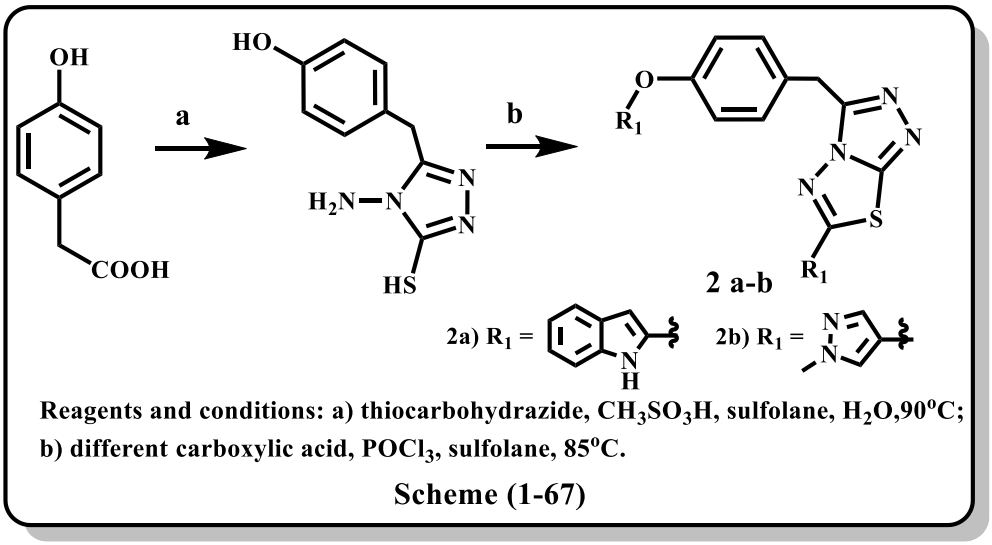
V. Padmavathi, et al.[99] synthesized1,3,4-thiadiazole derivatives from the reaction of 1,3,4-Oxadiazoles with thiourea in THF Scheme (1-66).



Scheme (1-66)

1.3.1.9. From 4-Amino-5-mercapto-1,2,4-triazoles:

Li Zhang, et al.[100] synthesized 1,3,4-thiadiazole derivatives from the reaction of 4-Amino-5-mercapto-1,2,4-triazoles with carboxylic acids Scheme (1-67).



1.3.2. Biological and industrial applications of 1,3,4-thiadiazole derivatives:

1,3,4-Thiadiazole and their derivatives have a wide range of applications such as medicine, agriculture, photographic materials and dyes,...etc. as shown in Figure (1-14) [101].

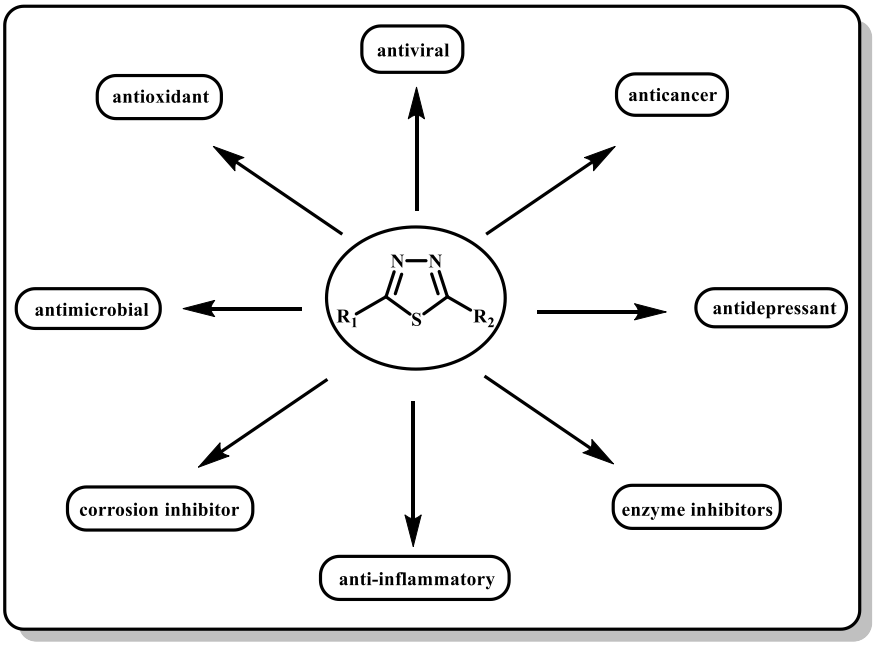
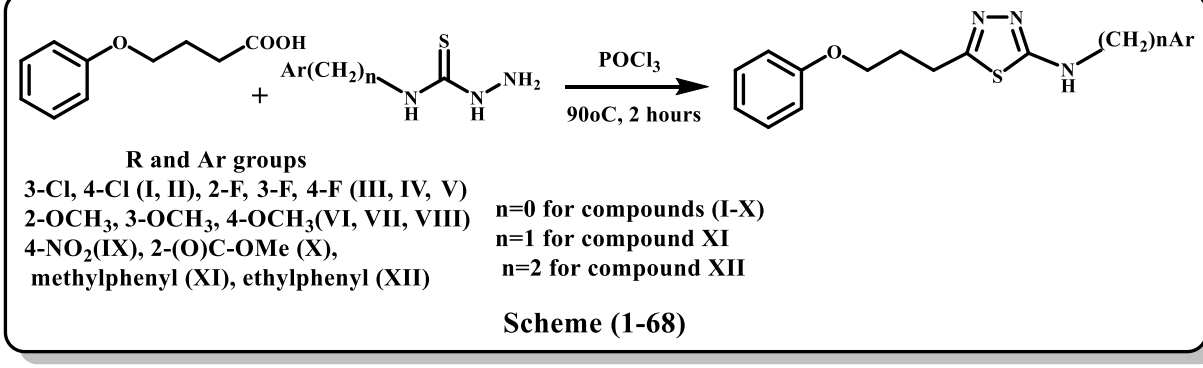


Figure (1-14): some industrial and pharmaceutical uses of 1,3,4-thiadiazole

Halit Muğlu, et al.[102] synthesized new 1,3,4-thiadiazole derivatives as antimicrobial, some of them gave a good activity against some bacteria Scheme (1-68).

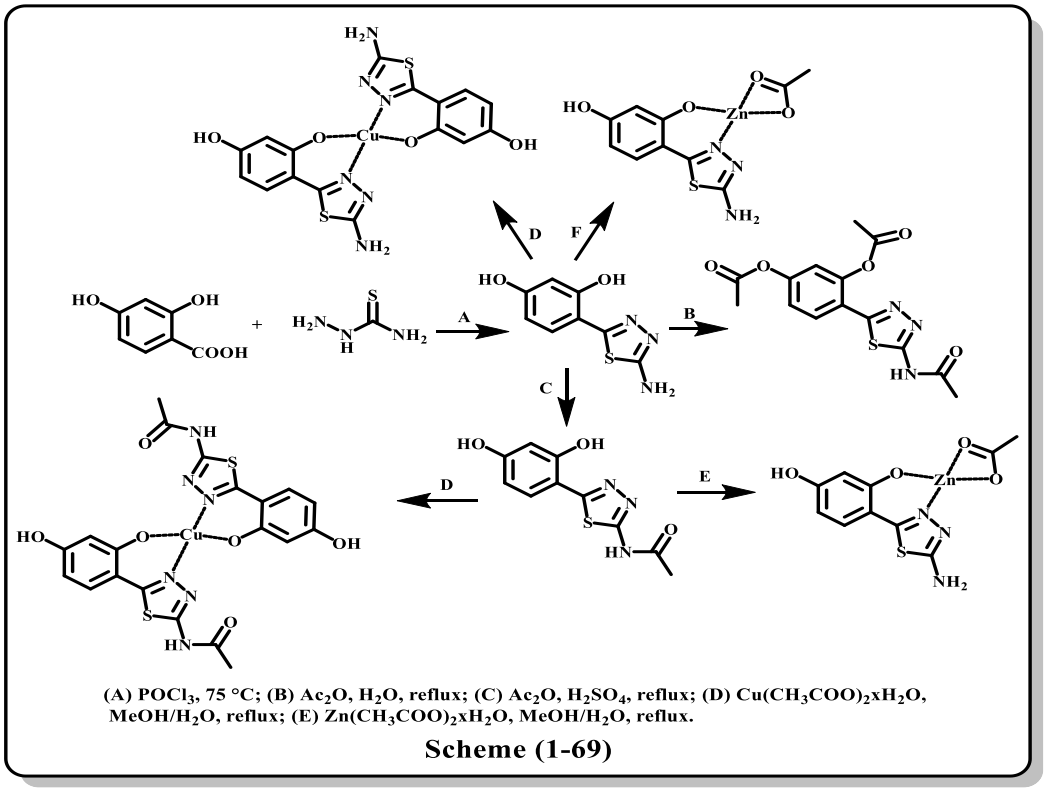


R and Ar groups
3-Cl, 4-Cl (I, II), 2-F, 3-F, 4-F (III, IV, V)
2-OCH_3, 3-OCH_3, 4-OCH_3(VI, VII, VIII)
4-NO_2(IX), 2-(O)C-OMe (X),
methylphenyl (XI), ethylphenyl (XII)

n=0 for compounds (I-X)
n=1 for compound XI
n=2 for compound XII

Scheme (1-68)

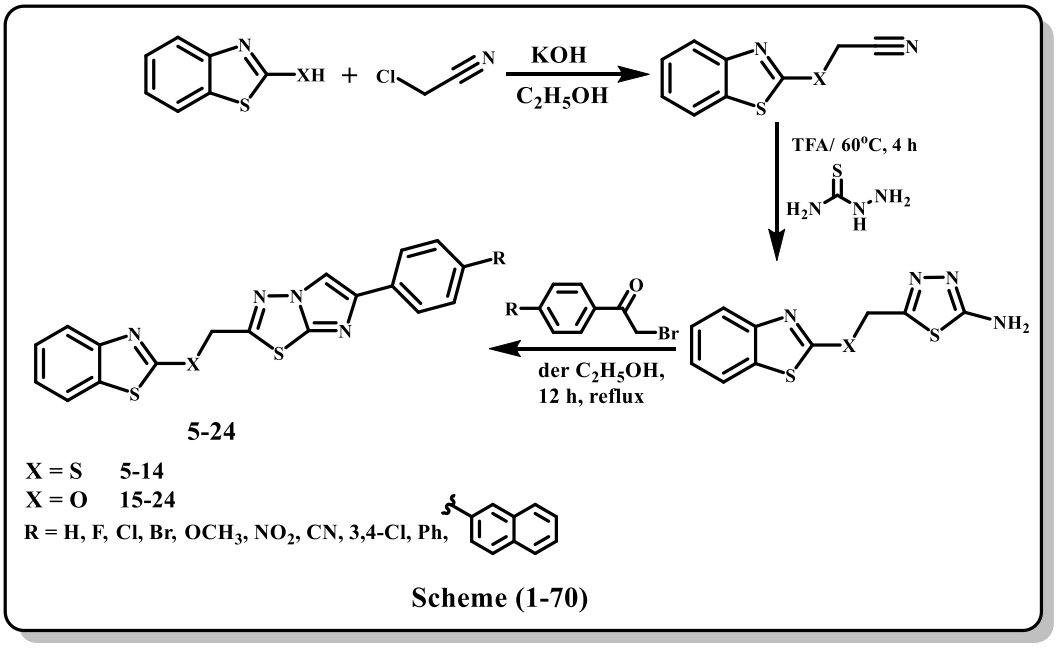
Dariusz, et al.[103] synthesized novel ligand of 1,3,4-thiadiazole to produce complexes of Cu and Zn as antibacterial with good antibacterial agents Scheme (1-69).



(A) $POCl_3$, 75 °C; (B) Ac_2O, H_2O, reflux; (C) Ac_2O, H_2SO_4, reflux; (D) $Cu(CH_3COO)_2xH_2O$, $MeOH/H_2O$, reflux; (E) $Zn(CH_3COO)_2xH_2O$, $MeOH/H_2O$, reflux.

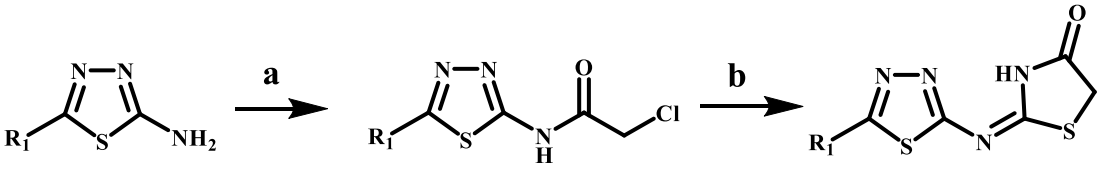
Scheme (1-69)

Mustafa Er, et al.[104] synthesized novel 1,3,4-thiadiazole derivatives as antibacterial and antileishmanial, some of them gave a good activity as antibacterial and anti-leishmanial Scheme (1-70).



Scheme (1-70)

Yasser M.Omar, et al.[105] synthesized novel derivatives of 1,3,4-thiadiazole containing thiazolidinone ring, some of them gave a good activity as anti-inflammatory agents Scheme (1-71).

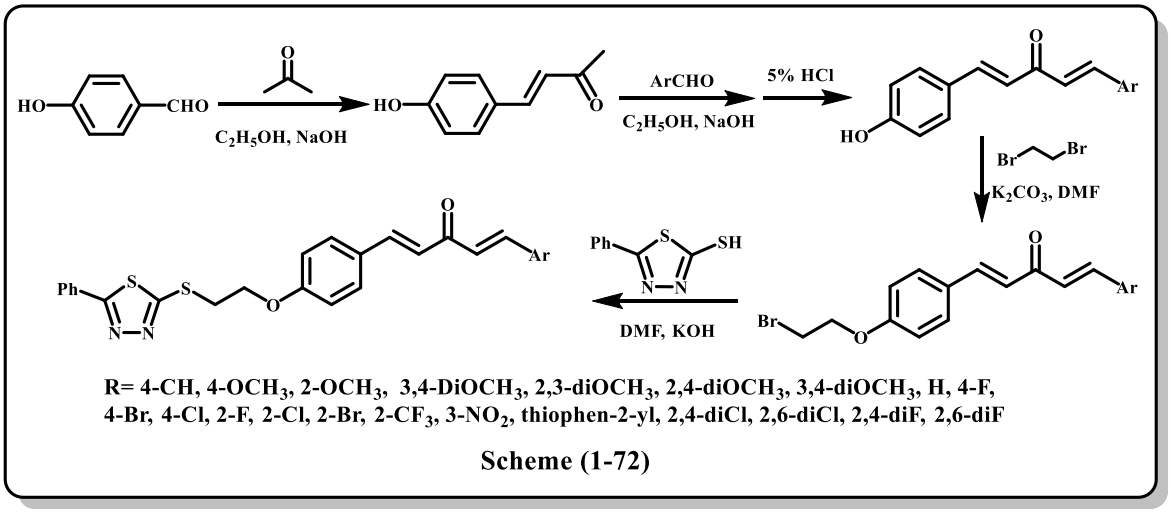


R_1= CH_3, Ph, 2-OH-Ph, 4-OH-Ph, 4-CH_3-Ph, 4-Br-Ph, 4-CH_3O-Ph, 3,4-$(CH_3O)_2$-Ph, 3,4,5-$(CH_3O)_3$-Ph

Reagents and conditions: (a) $ClCH_2COCl$, benzene, reflux, 3–5 h, 76–96% yield; (b) NH_4SCN, EtOH,reflux, 3–5 h, 83–93% yield.

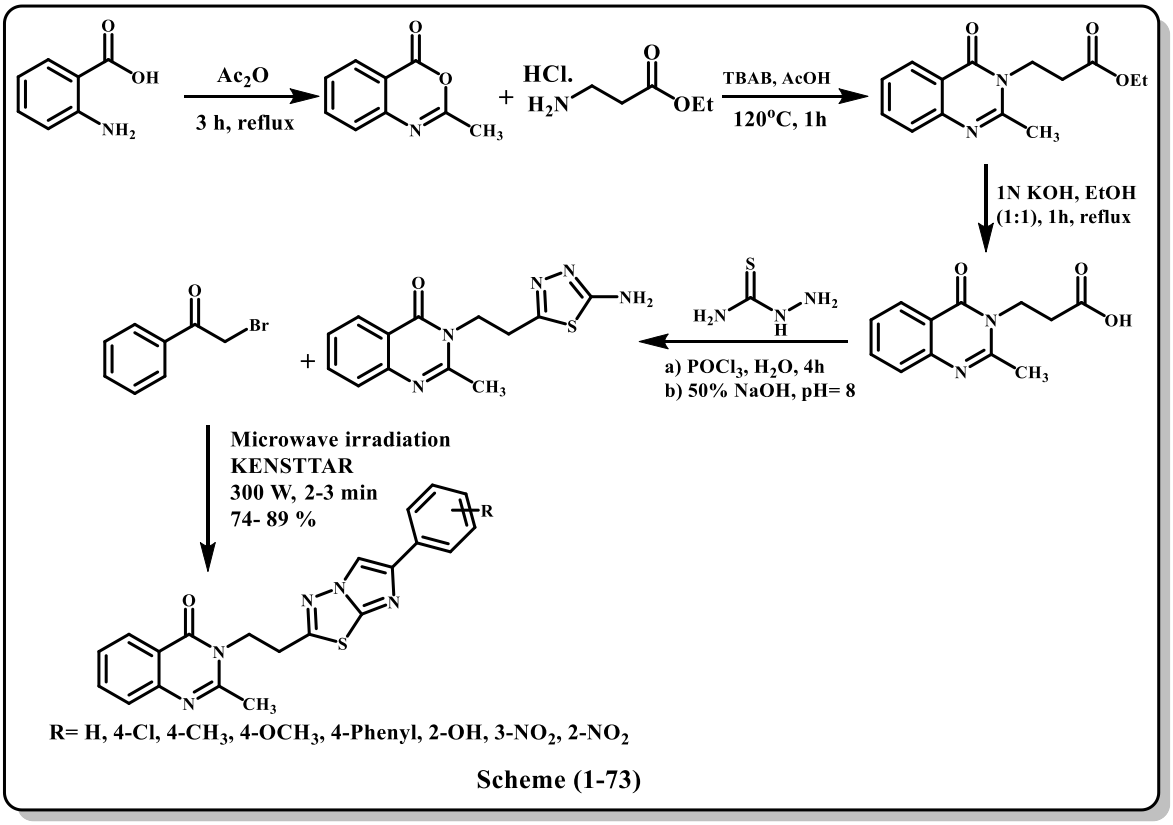
Scheme (1-71)

Lu Yu, et al.[106] successfully prepared a novel derivatives of 1,3,4-thiadiazole as antiviral agents and showed good activity verses CMV and TMV Scheme (1-72).



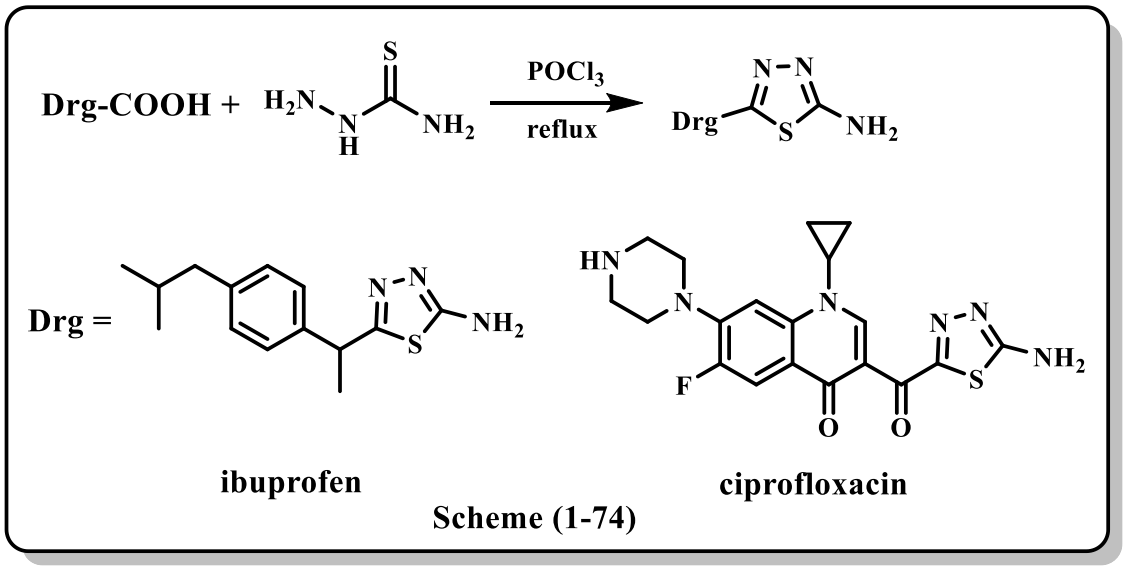
Scheme (1-72)

Alugubelli Gopi, et al.[107] synthesized new 1,3,4-thiadiazole derivatives as anticancer and some compounds show good activity agents against lung carcinoma Scheme (1-73).



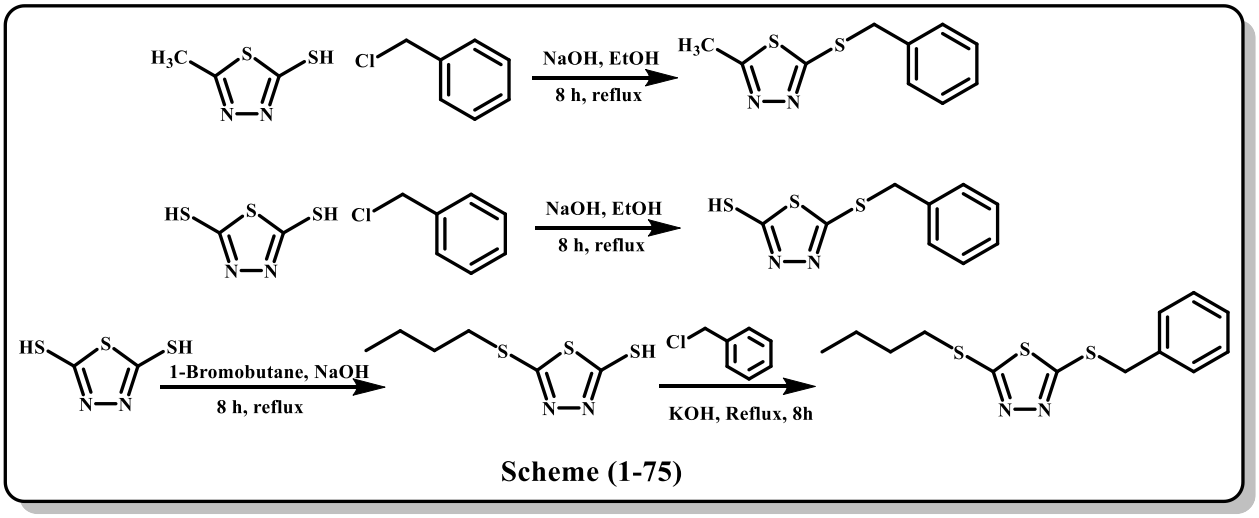
Scheme (1-73)

Shahid Farooqi, et al.[108] synthesized derivatives of 1, 3, 4-thiadiazole of ciprofloxacin and ibuprofen which gave good activity as anticancer agents Scheme (1-74).



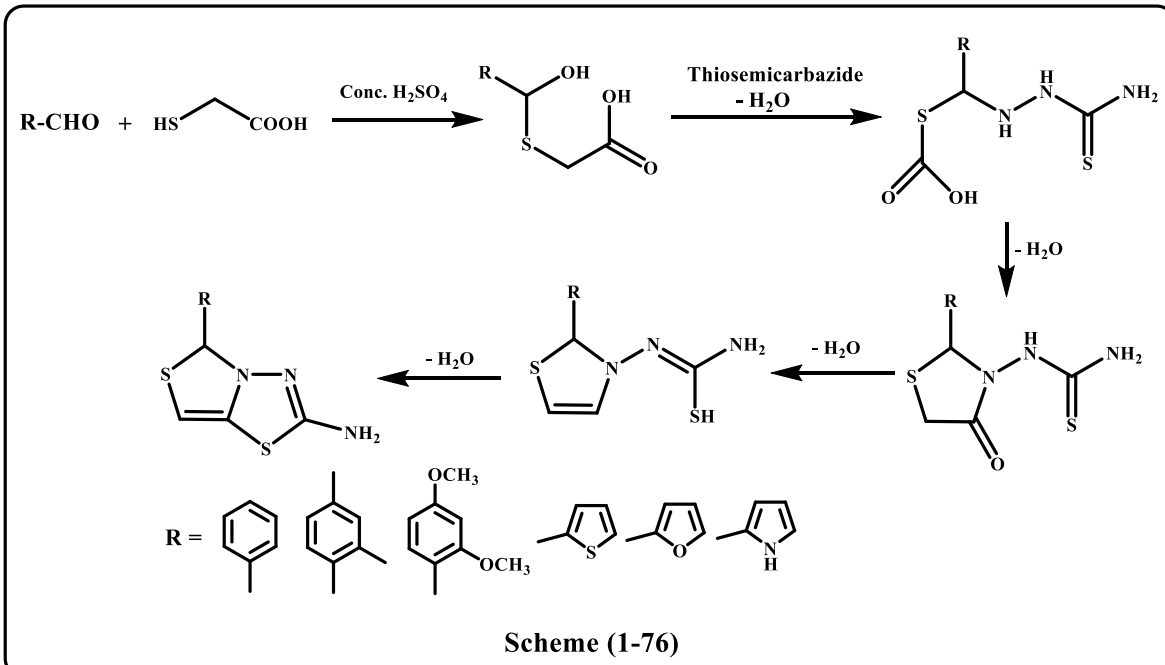
Scheme (1-74)

Q.H. Zhang, et al.[109] synthesized new 1,3,4-thiadiazole derivatives as corrosion inhibitor on carbon steel and showed good activity as corrosion inhibitor agents Scheme (1-75).



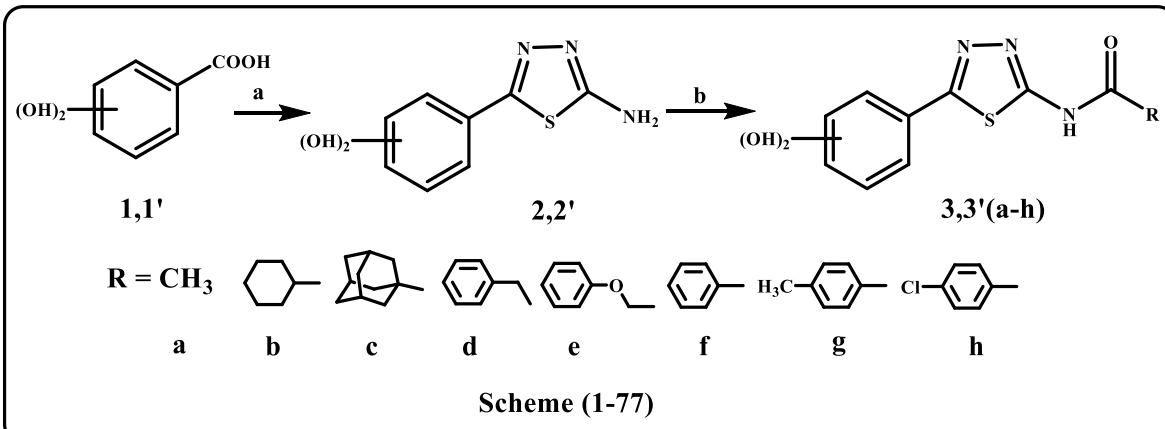
Scheme (1-75)

J. Saranya, et al.[110] synthesized new derivatives of 1,3,4-thiadiazole as anti-corrosion and showed a good activity as carbon steel corrosion in CO_2-saturated oilfield produced water Scheme (1-76).



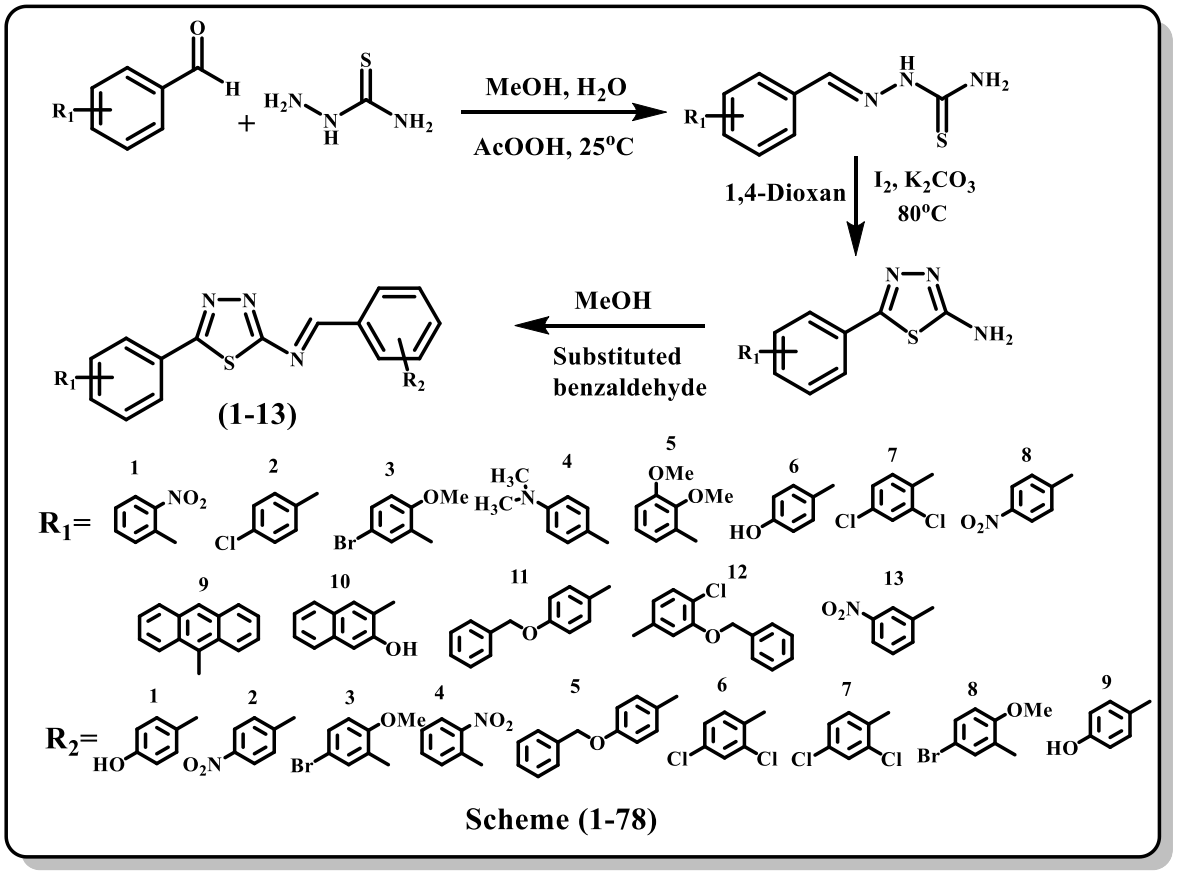
Scheme (1-76)

Katarina Jakovljevic, et al.[111] synthesized new 1,3,4-thiadiazole derivatives which showed good activity as anti-proliferative and antioxidant agents Scheme (1-77).



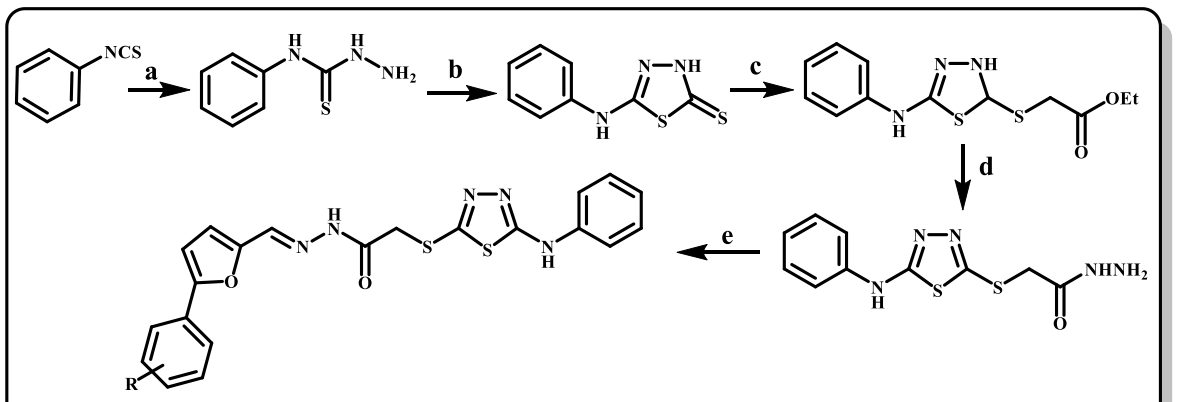
Scheme (1-77)

Muhammad Javid, et al.[112] synthesized new derivatives of 1,3,4-Thiadiazole as enzyme inhibitors agents, some of them gave a good activity as α-glucosidase inhibitors Scheme (1-78).



Scheme (1-78)

Mehlika Altıntop, et al.[113] synthesized new derivatives of 1,3,4-Thiadiazole as enzyme inhibitors agents, some of them gave a good activity as carbonic anhydrase isozymes I and II inhibitors Scheme (1-79).



R= 2-Cl, 3-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2, 4-Br,2,4-DiCl,3,4-DiCl,4-Cl-2-NO_2

Reagents and conditions: (a) NH_2NH_2 H_2O, ethanol, rt, 5 h; (b) (1) CS_2/KOH, ethanol,reflux, 10 h; (2) HCl, pH 4–5; (c) $ClCH_2COOEt$, K_2CO_3, acetone, reflux, 10 h; (d) $NH_2NH_2H_2O$,ethanol,rt,3h; (e) 5-arylfurfural, ethanol, reflux, 6 h.

Scheme (1-79)

1.4. Azo dyes:

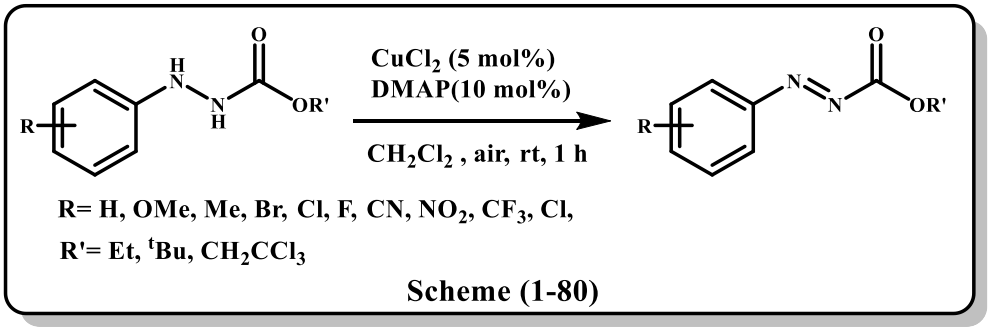
The first discovery of azo dyes by accidently was in 1856 by William Henry when he succeeded to prepare synthetic azo named mauveine [114115], azo dyes can be defined as coloured compounds that give their permanent colour on fibers when applied to them [116]. At the end of the 19th century, more than 10000 types of synthetic dyes were developed and used in industry[117].

Azo compounds are the most common synthetic colourings, they have widely uses such as the leather paper, food, cosmetics, textile and pharmaceutical industries. However, more attention have been focused on the biological activity of azo compounds[118,119].

Azo compounds are characterized by the presence of one or more azo group (-N=N-) [120,121], the azo bond (R-N=N-R') linkaged to R,R' groups such as benzene or naphthaline or both with various substitution groups such as (NH_2), (OH), (CH_3), (Cl) and (NO_2)[122].

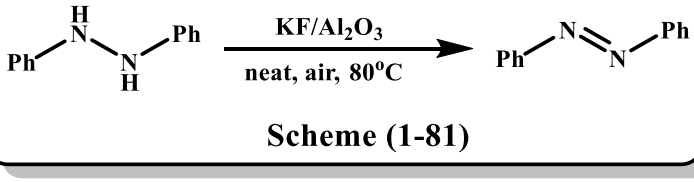
1.4.1. Synthesis of azo dyes:

The general methods of synthesis azo dyes compounds (R-N=N-R') are catalyzed aerobic oxidation of alkyl 2-Phenylhydrazinecarboxylate by $CuCl_2$ and DMAP under moderate conditions Scheme (1-80)[123].



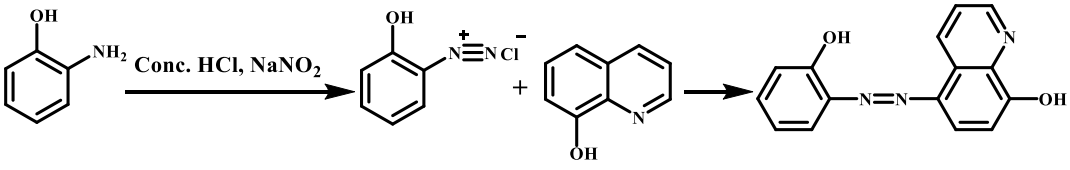
Scheme (1-80)

From dehydrogenation of hydrazobenzene by using basic alumina accelerated by air Scheme (1-81)[124].



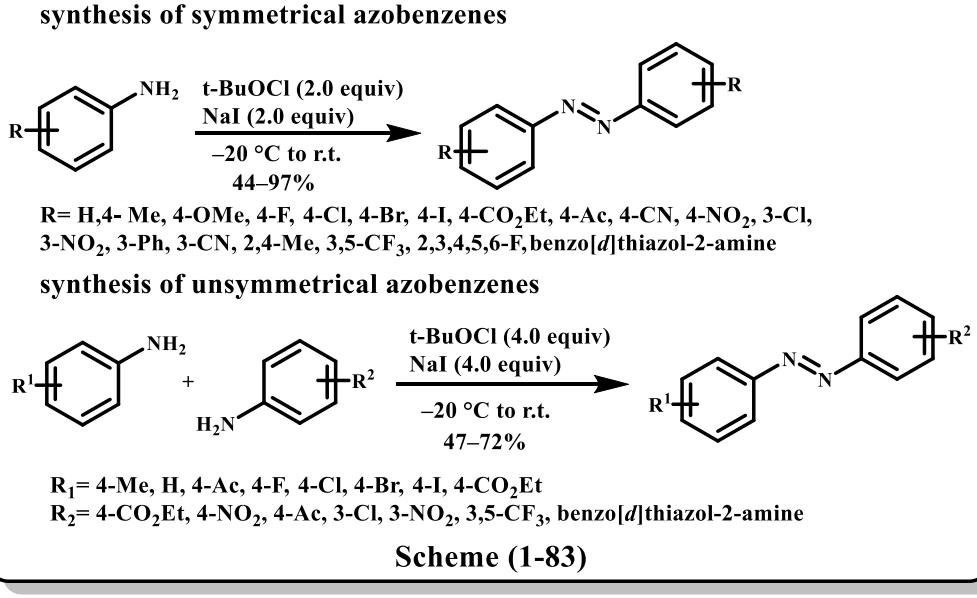
Scheme (1-81)

From coupling of diazonium salt with phenols in presence of $NaNO_2$ /HCl for example, the reaction of 2-aminophenol with 8-hydroxyquinoline Scheme (1-82)[125].



Scheme (1-82)

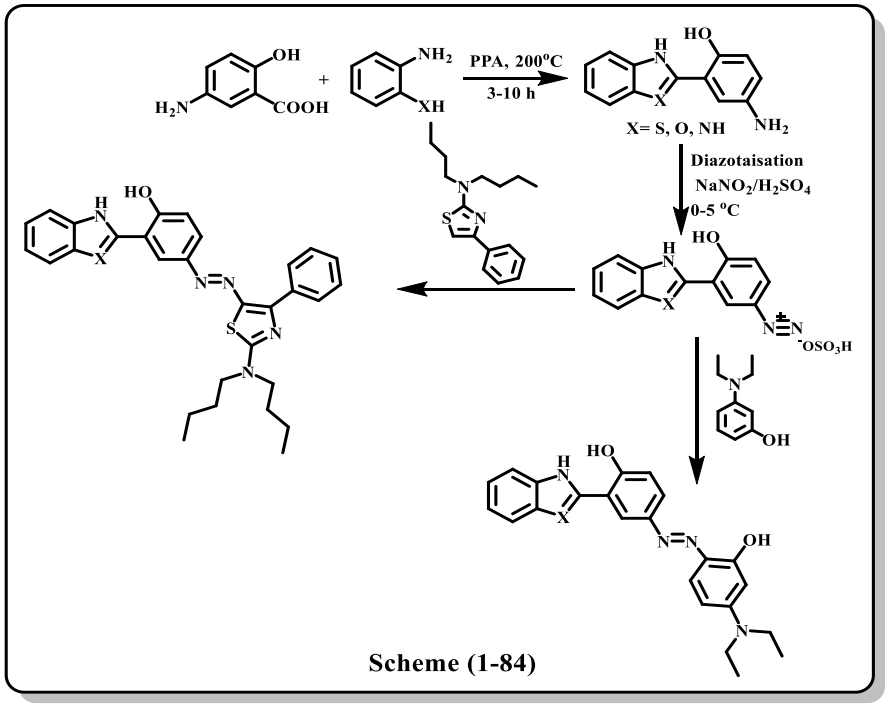
From oxidation of aromatic amines by using tert-Butyl Hypoiodite Scheme (1-83)[126].



Scheme (1-83)

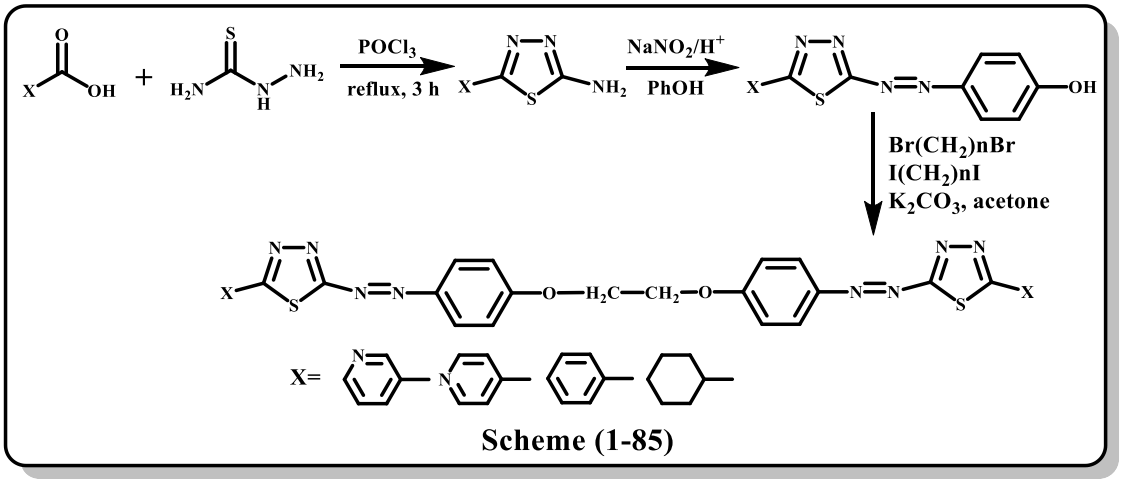
1.4.1. Biological and industrial applications of Azo dyes derivatives

Virendra Mishra, et al.[127] synthesized novel azo derivatives of benzimidazole, benzothiazole and benzoxazole some of them showed good antimicrobial activity Scheme (1-84).



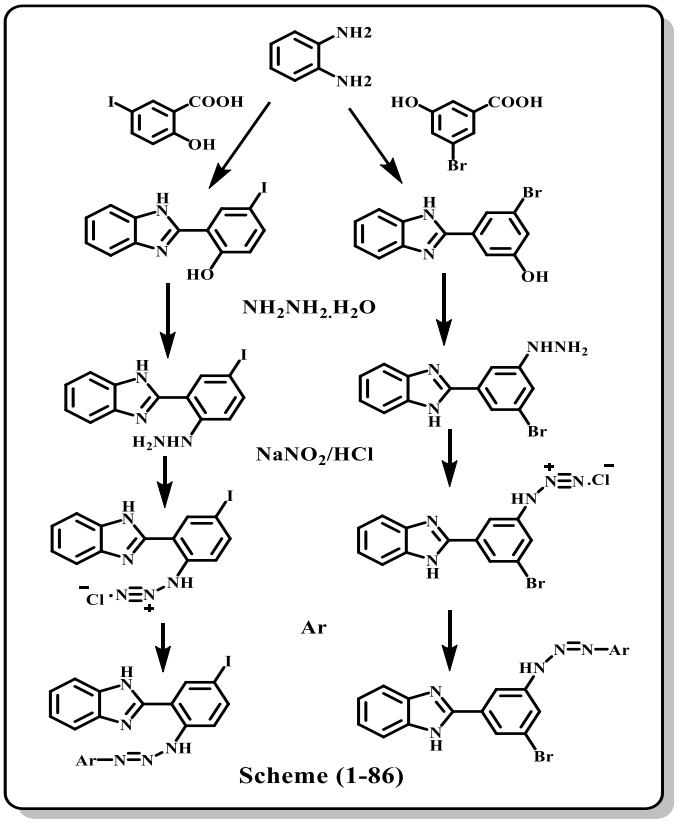
Scheme (1-84)

I.H.R.Tomi, et al.[128] synthesized new derivatives of 1,3,4-Thiadiazole and some of them showed good antibacterial activity Scheme (1-85).



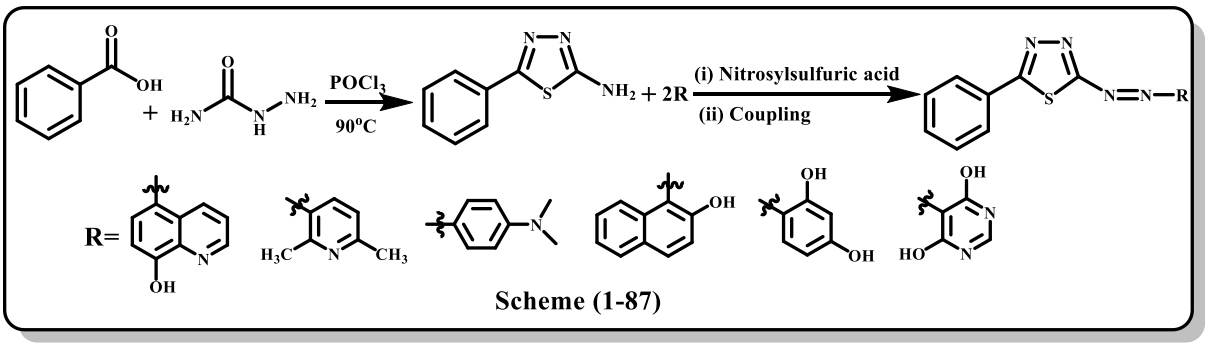
Scheme (1-85)

Sujit Kumar Mohanty, et al.[129] synthesized azo derivatives of and gave good antibacterial and anti-tubercular activity Scheme (1-86).



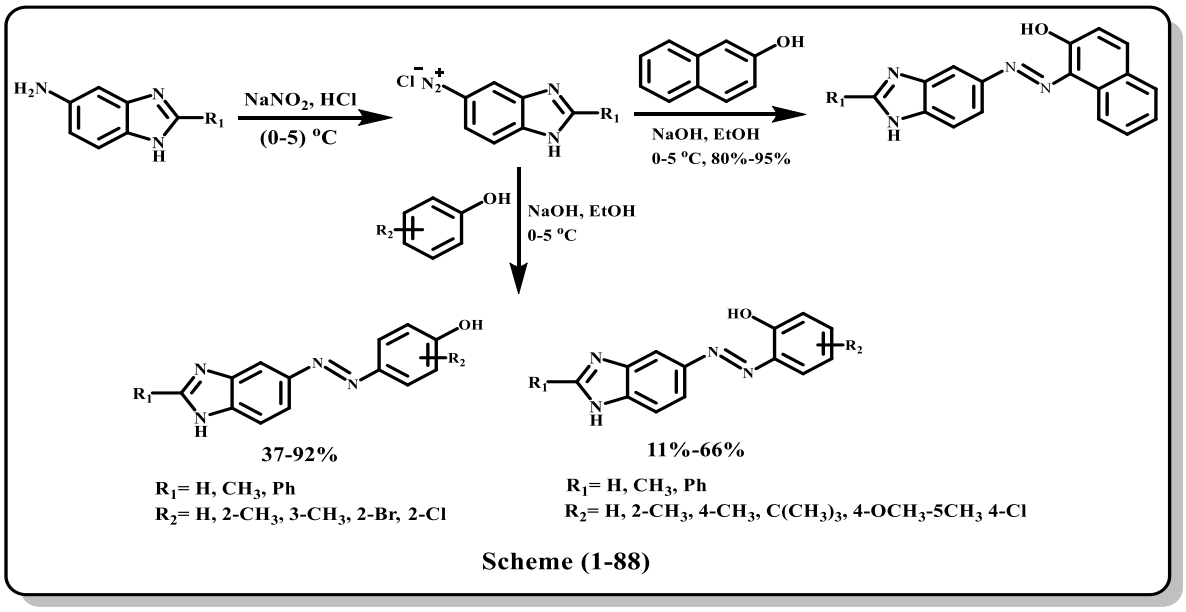
Scheme (1-86)

Keerthi Kumar, et al.[130] synthesized azo-5-Phenyl-1,3,4-thiadiazole-2-amine derivatives some of them gave good antibacterial and antifungal activity Scheme (1-87).



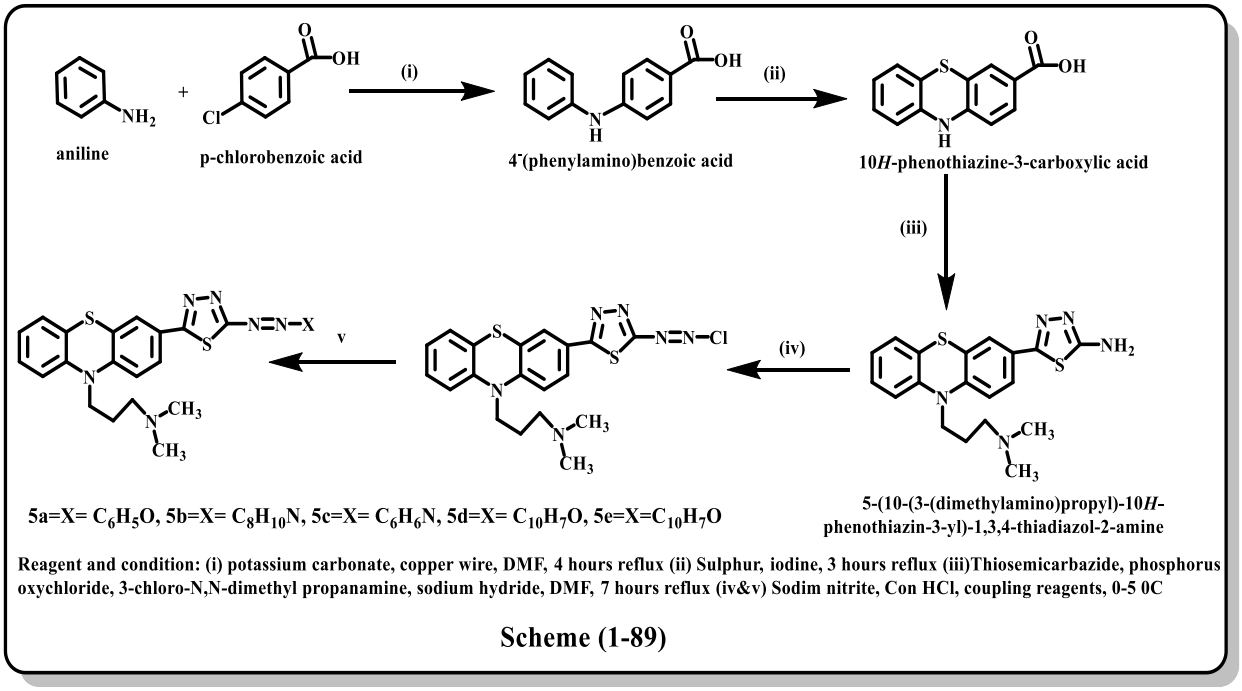
Scheme (1-87)

Yazhen Ke, et al.[131] synthesized azo-benzimidazole derivatives containing phenol and some of them gave a good antifungal activity Scheme (1-88).



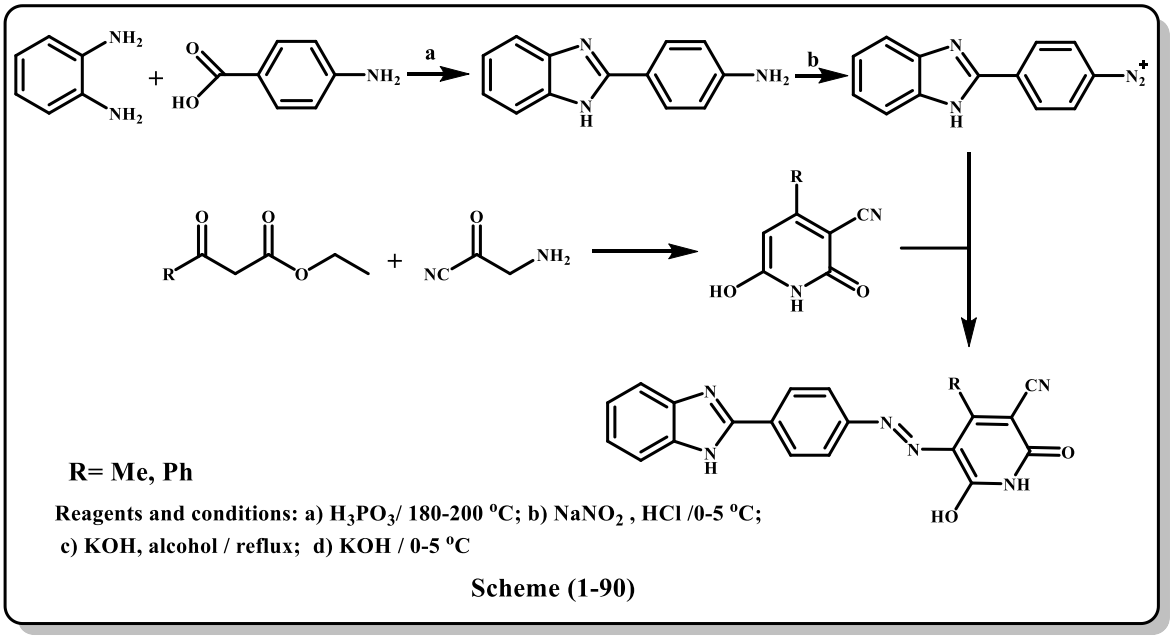
Scheme (1-88)

Chandravadivelu Gopi, et al.[132] synthesized novel azo derivatives of 1,3,4-thiadiazole, these derivatives showed moderate to good antimicrobial activity as shown in Scheme (1-89).



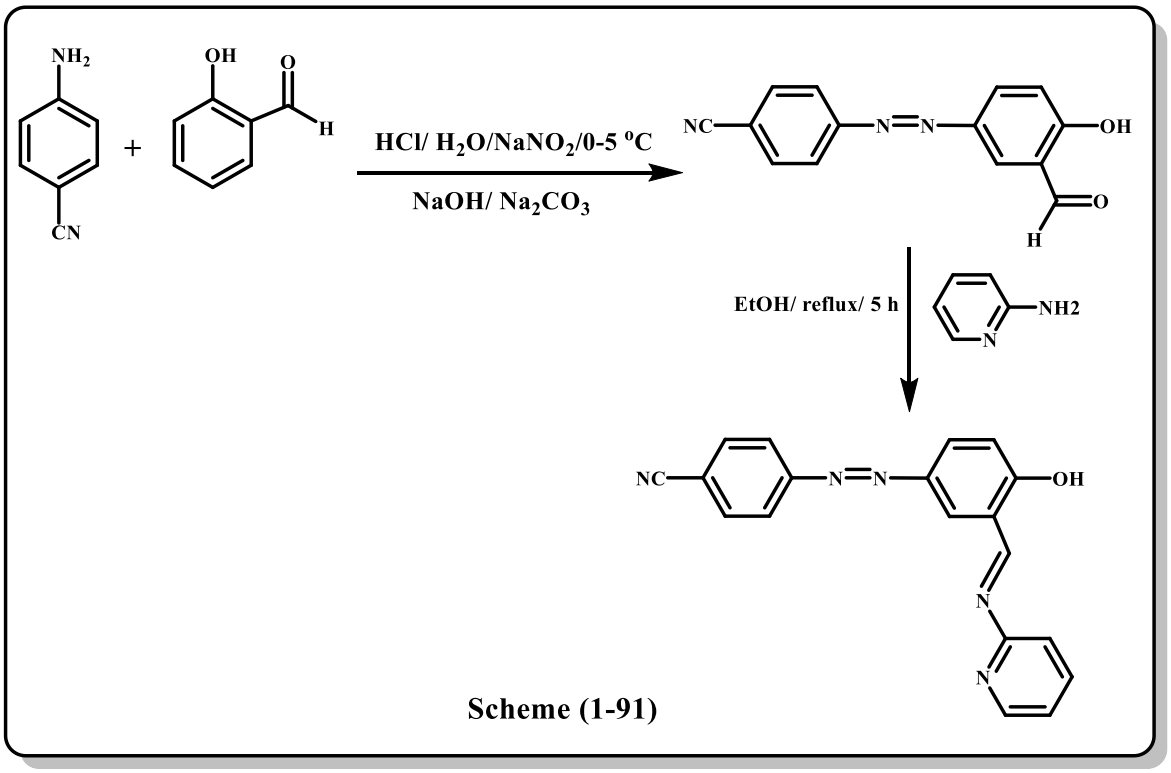
Scheme (1-89)

D.Mijan, et al.[133] synthesized new derivatives of azo-benzimidazole and some of them showed good anticancer activity Scheme (1-90).



Scheme (1-90)

Ali Abdulridha, et al.[134] synthesized new azo derivatives and some of them showed good corrosion inhibitor agents Scheme (1-91).



Scheme (1-91)

1.6. Aim of study:

The goal of this study is to synthesis new benzimidazole derivatives containing 1,3,4-Thiadiazole and azo group and characterization using various spectroscopy methods and evaluating antibacterial activity of some derivatives against four types of bacteria, then studying of molecular docking for most active compounds (21,22, 39).

Chapter Two

Experimental Part

2.1. Chemicals:

The chemicals used for prepared compounds with its purity as shown in table (2-1).

Table (2-1): The chemicals and their manufactures

No.	Chemicals	Company name	Purity%
1	2-Chlorobenzoic acid	CDH	99
2	2-Fluorobenzoic acid	Fluka	99
3	2-Iodobenzoic acid	Sigma aldrich	98
4	3,5-Dinitrobenzoic acid	BDH	99.9
5	3-Chlorobenzoic acid	BDH	99
6	3-Nitrobenzoic acid	CDH	99
7	4-Bromobenzoic acid	Merck	99
8	4-Chlorobenzoic acid	CDH	98
9	4-Nitrobenzoic acid	CDH	98
10	Acetone	Alpha chemika	99
11	Ammonia solution	Scharlau	32
12	Benzoic acid	BDH	99.9
13	Bromoacetic acid	Merck	99.9
14	Dimethyl sulfoxide (DMSO)	CDH	99
15	Ethanol	Scharlau	99.9
16	Ethyl acetate	CDH	99
18	Hexane	BDH	99
17	Hydrochloric acid	Himedia	35
19	Nicotinic acid	Himedia	98
20	Nitric acid	Scharlau	69.5
21	*o*-Phenylenediamine	schuchardt	98
22	Phenol	Alpha chemika	99
23	Phosphoric acid	Labort	85
24	Phosphoroyl Chloride ($POCl_3$)	CDH	99
25	Potassium bromide	BDH	99.9
26	Potassium carbonate	Sigma aldrich	96
27	Potassium hydroxide	CDH	85
28	Potassium iodide	BDH	99
29	Sodium nitrate	Merck	99
30	Thiosemicarbazid	Thomas backer	99

2.2. Instrumentals and Techniques:

1- Melting points prepared compounds were measured using Stuart- United kingdom digital melting point Advanced smp3 at laboratory of Chemistry department / College of education for pure science / University of Diyala.

2- Thin-layer chromatography (TLC) was used to check the completion of the reaction and the purity of compounds by using alumina plates that have dimensioned (20×20 cm) covered with silica gel. The spots were visualized by using UV Cabinet for TLC.

3- (SHIMADZU, FT-IR 8400S, Spectrophotometer) was used for recorded FT-IR spectra at laboratory of Chemistry department / College of education for pure science / University of Diyala/ was done on KBr disc 1/10 percentage.

4- (Bruker, 400MHz) was used for recorded ^{1}H and ^{13}C-NMR spectroscopy for some prepared compounds at laboratory of Jordan University of Science and Technology/ Irbid / Jordan using DMSO-d_6 as a solvent.

2.3. Experimental procedures:

2.3.1. Synthesis of 2-(bromomethyl)-1*H*-benzimidazole(1) [135]:

A mixture of (20 mmol, 2.16 g) of *o*-phenylenediamine and (30 mmol, 4.16 g) of bromoacetic acid was dissolved in 30-40 mL of 4N HCl by heating under reflux, the reaction mixture was refluxed for 5 hrs. The mixture was cooled to room temperature and neutralized with 32% ammonia solution. The precipitate was filtered and washed with distilled water three times. The precipitate was filtered, washed with cold distilled water three times and recrystallized form ethanol. The completion of the reaction was monitored by TLC eluting with (hexane: ethyl acetate) (6:4).

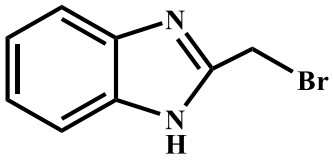


Table (2-2): physical properties of compound (1)

Comp. No.	Molecular formula	M.wt	Color	mp (^{o}C)	Yield %	R_f
1	$C_8H_7BrN_2$	211.06	Beige to dark yellow	148-150	80	0.36

2.3.2. Synthesis of 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole (2-12) [128]:

A mixture of the corresponding benzoic acid derivatives (10 mmol), (10 mmol, 0.91 g) thiosemicarbazide and phosphoroyl Chloride ($POCl_3$) (10 mmol, 5mL) of was gently refluxed for 3 h. After cooling (25 mL) of distilled water was added dropwise with stirring, and refluxed for 4 hrs. The mixture was cooled and then neutralized with 10% potassium hydroxide solution. The precipitate was filtered, washed with cold distilled water three times and recrystallized form ethanol. The completion of the reaction was monitored by TLC eluting with (hexane: ethyl acetate) (7:3).

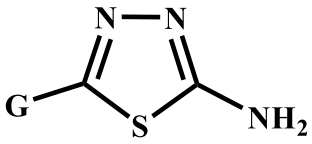


Table (2-3): physical properties of compounds (2-12)

Comp No.	G	Molecular formula	M.wt	Color	mp (^{o}C)	Yield %	R_f
2	C_6H_5	$C_8H_7N_3S$	177.23	Pale yellow	223-225	86	0.46
3	2-F-C_6H_4	$C_8H_6FN_3S$	195.22	Pale yellow	215-217	89	0.52
4	2-I-C_6H_4	$C_8H_6IN_3S$	303.12	Pale yellow	212-214	77	0.60
5	4-Br-C_6H_4	$C_8H_6BrN_3S$	256.13	Pale yellow	174-176	81	0.51
6	2-Cl-C_6H_4	$C_8H_6ClN_3S$	211.68	Pale yellow	213-215	80	0.46
7	3-Cl-C_6H_4	$C_8H_6ClN_3S$	211.68	Pale yellow	204-206	85	0.48
8	4-Cl-C_6H_4	$C_8H_6ClN_3S$	211.68	Pale yellow	226-228	83	0.43
9	3-NO_2-C_6H_4	$C_8H_6N_4O_2S$	222.23	Yellow	234-236	89	0.55
10	4- NO_2-C_6H_4	$C_8H_6N_4O_2S$	222.23	Yellow	244-246	82	0.45
11	3,5-di NO_2-C_6H_3	$C_8H_5N_5O_4S$	267.23	Dark yellow	265-267	85	0.62
12	pyridine	$C_7H_6N_4S$	178.22	Yellow	>300	75	0.45

2.3.3. Synthesis of *N*-((1H-benzimidazol-2-yl)methyl)-5-(substitutedphenyl)-1,3,4-thiadiazol-2-amine (13-23)[136]:

A mixture of (5 mmol, 1.05 g) compound (1), (5mmol) of 1,3,4-thiadiazol-2-amine derivatives (2-13) and (5mmol, 0.83 g) potassium iodide was dissolved in 25 mL of ethanol. After 6 hrs of refluxing a solution of potassium hydroxide (5 mmol, 0.28g in 2.5mL of H_2O) was added to the mixture, and refluxing continuous for 2 hrs. The mixture cooled down and poured onto crushed ice. The precipitate was filtered, washed with cold distilled water three times and recrystallized form ethanol. The

completion of the reaction was monitored by TLC eluting with (hexane: ethyl acetate) (9:1).

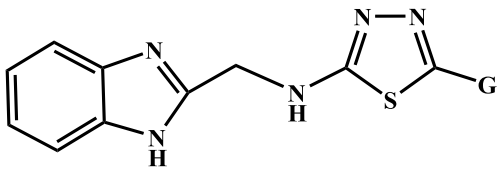


Table (2-4): physical properties of compounds (13-23)

Comp No.	G	Molecular formula	M.wt	Color	mp (^{o}C)	Yield %	R_f
13	C_6H_5	$C_{16}H_{13}N_5S$	307.38	yellow	253-255	55	0.36
14	2-F-C_6H_4	$C_{16}H_{12}FN_5S$	325.37	Dark yellow	195-197	57	0.42
15	2-I-C_6H_4	$C_{16}H_{12}IN_5S$	433.27	Yellow	222-224	64	0.50
16	4-Br-C_6H_4	$C_{16}H_{12}BrN_5S$	386.28	Yellow	214-216	60	0.45
17	2-Cl-C_6H_4	$C_{16}H_{12}ClN_5S$	341.83	Dark yellow	175-177	85	0.40
18	3-Cl-C_6H_4	$C_{16}H_{12}ClN_5S$	341.83	Dark yellow	245-247	65	0.43
19	4-Cl-C_6H_4	$C_{16}H_{12}ClN_5S$	341.83	Dark yellow	202-204	54	0.47
20	3-NO_2-C_6H_4	$C_{16}H_{12}N_6O_2S$	352.38	Yellow	193-195	53	0.46
21	4-NO_2-C_6H_4	$C_{16}H_{12}N_6O_2S$	352.38	Dark yellow	>250	71	0.39
22	3,5-di NO_2-C_6H_3	$C_{16}H_{11}N_7O_4S$	397.38	Yellow	207-209	61	0.55
23	pyridine	$C_{15}H_{12}N_6S$	308.37	Pale yellow	215-217	56	0.62

2.3.4. Synthesis of 4-((5-substitutedphenyl)-1,3,4-thiadiazol-2-yl)diazenyl) phenol (23-31) [128]:

Compounds (2 and 5-11) (1.78 mmol) were dissolved in (8 mL) of 85% H_3PO_4 under heating with stirring, the solution was cooled to 0^{o}C an ice bath. Then a mixture of (1.87 mmol, 0.13 g in 2mL of H_2O) sodium nitrate and 4mL of concentrated HNO_3 was added thoroughly with vigorously stirring and the temperature was kept below 5 ^{o}C over 10 mints. Afterwards a solution of phenol (1.78 mmol, 0.17 g in 0.5 mL H_2O) was added dropwise with stirring. The mixture

was poured into cold-water (100 mL). The precipitate was filtered, washed with cold distilled water three times and recrystallized form ethanol. The completion of the reaction was monitored by TLC eluting with (hexane: ethyl acetate) (6:4).

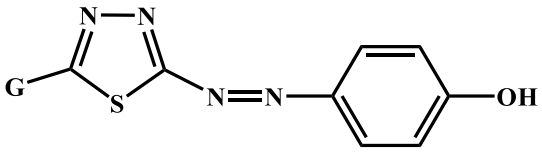


Table (2-5): physical properties of compounds (23-31)

Comp No.	G	Molecular formula	M.wt	Color	mp (^{o}C)	Yield%	R_f
24	C_6H_5	$C_{14}H_{10}N_4OS$	282.33	Brown	197-199	77	0.46
25	4-Br-C_6H_4	$C_{14}H_9BrN_4OS$	361.22	Brown	188-190	86	0.38
26	2-Cl-C_6H_4	$C_{14}H_9ClN_4OS$	316.77	Pale brown	183-185	85	0.50
27	3-Cl-C_6H_4	$C_{14}H_9ClN_4OS$	316.77	Pale brown	293-295	75	0.44
28	4-Cl-C_6H_4	$C_{14}H_9ClN_4OS$	316.77	Pale brown	171-173	87	0.36
29	3-NO_2-C_6H_4	$C_{14}H_9N_5O_3S$	327.32	Brown	186-188	80	0.43
30	4-NO_2-C_6H_4	$C_{14}H_9N_5O_2S$	327.32	Brown	193-195	82	0.60
31	3,5-di NO_2-C_6H_3	$C_{14}H_8N_6O_5S$	372.32	Pale brown	273-275	70	0.33

2.3.5. Synthesis of 2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-substitutedphenyl-1,3,4-thiadiazole(32-39) [128]:

A mixture (1.05 g, 5 mmol) compound (1), (5 mmol) compounds (23-31) and anhydrous potassium carbonate in acetone (10 mL) and refluxed for 24 hrs. The mixture cooled down and poured into cold water (100 mL). The precipitate was filtered, washed with cold distilled water three times and recrystallized form ethanol. The completion of the reaction was monitored by TLC eluting with (hexane: ethyl acetate) (6:4).

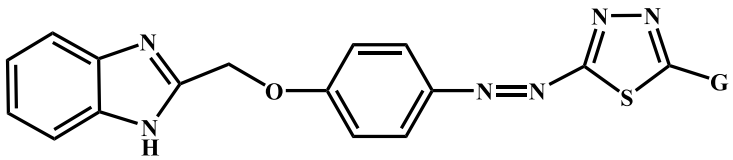


Table (2-6) physical properties of compounds (32-39)

Comp No.	G	Molecular formula	M.wt	Color	mp (^{o}C)	Yield %	R_f
32	C_6H_5	$C_{22}H_{16}N_6OS$	412.48	Dark brown	288-290	65	0.56
33	4-Br-C_6H_4	$C_{22}H_{15}BrN_6OS$	491.37	Dark brown	278-280	88	0.44
34	2-Cl-C_6H_4	$C_{22}H_{15}ClN_6OS$	446.92	Brown	>300	82	0.60
35	3-Cl-C_6H_4	$C_{22}H_{15}ClN_6OS$	446.92	Brown	221-223	57	0.46
36	4-Cl-C_6H_4	$C_{22}H_{15}ClN_6OS$	446.92	Dark brown	>300	75	0.52
37	3-NO_2-C_6H_4	$C_{22}H_{15}N_7O_3S$	457.47	Dark brown	248-250	70	0.68
38	4-NO_2-C_6H_4	$C_{22}H_{15}N_7O_2S$	457.47	Dark brown	248-250	85	0.59
39	3,5-di NO_2-C_6H_3	$C_{22}H_{14}N_8O_5S$	501.46	Dark violet	>300	61	0.48

2.4. Study of biological activity:

The biological activity of synthesized compounds was studied using disk diffusion methods [137,138] against four types of pathogenic identified bacterial isolates in al amine laboratory, two gram-positive bacteria (Gr^{+ve}) (*S. aureus* and *S. epidermidis*) and two gram-negative bacteria (Gr^{-ve}) (*E. coli* and *P. aeruginosa*).

2.4.1. Preparation the culture media:

Muller Hinton agar medium was prepared according to directions of the supplying company by dissolving the agar (38 g) in a conical flask in (1L) of distilled water, sterilized at 121°C under pressure 15 pounds for 15 minutes after that the solution was left to cooled and poured into sterile dishes until use.

2.4.2. Preparation of concentrations

The concentrations were prepared by dissolve synthesized compounds in dimethyl sulfoxide (DMSO). The four target concentrations were (100, 50, 25, and 12.5) mg/mL.

2.4.3. Evaluation of antibacterial activity[139,140]:

1- A number of bacteria colonies were transported by loop to prepare the suspended bacteria and put it in tubes contain brain heart infusion broth to activate the bacteria. The tubes were incubated for (18 - 24) h at 37 °C. The suspended bacteria were compared to the standard MacFarland solution (1.5 x 108) cells/ml. After that the bacteria suspended was spread by Sterile Swab, it was spread on the plates containing Muller Hinton agar and then left the plate for a while to dry.

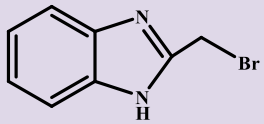
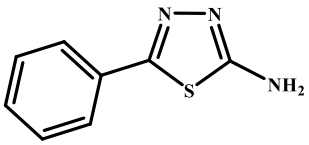
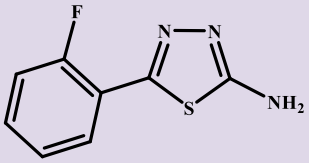
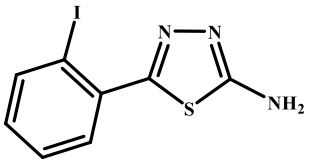
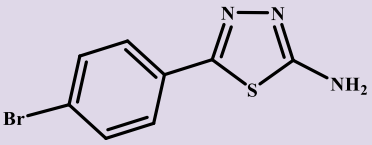
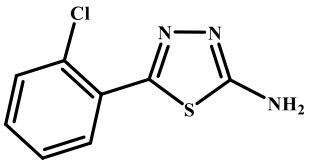
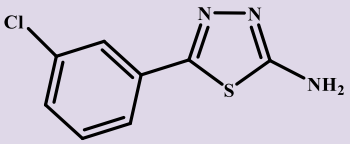
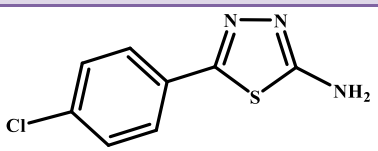
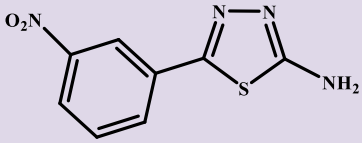
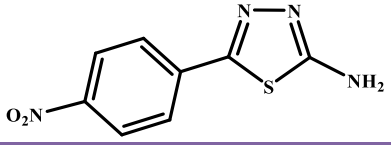
2- A holes were made with a diameter of 5 mm in the culture media by using sterilized a cork borer.

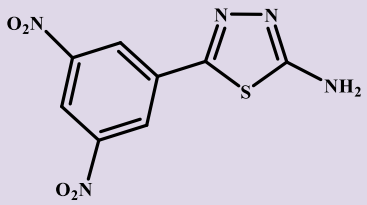
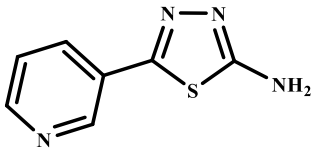
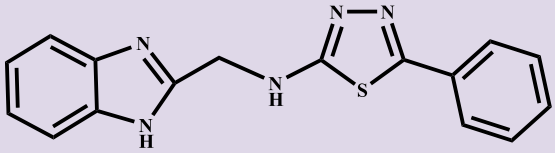
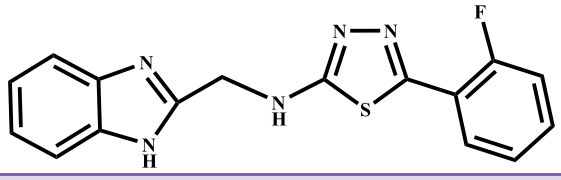
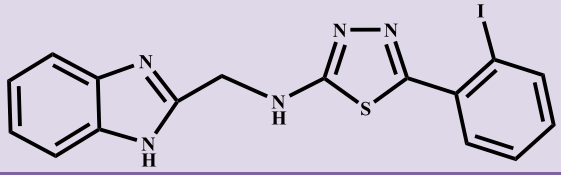
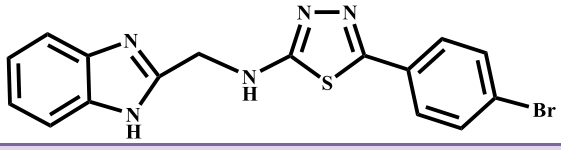
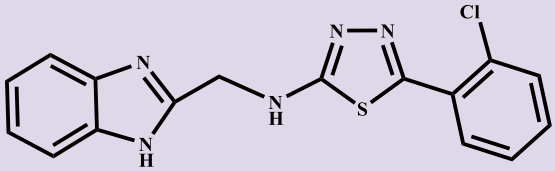
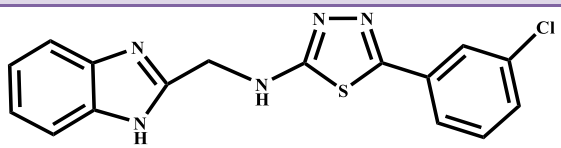
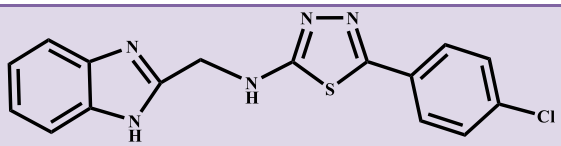
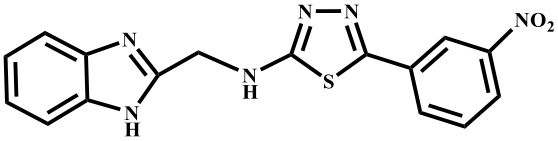
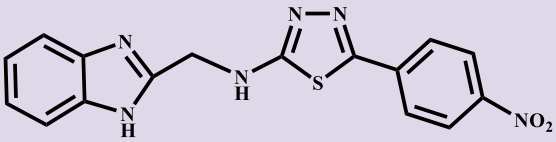
3- 100 µL of the material were added to each hole individually by micropipette. The positive control represented by adding sterile distilled water. After then, incubate the dishes at 37 °C for 24 h.

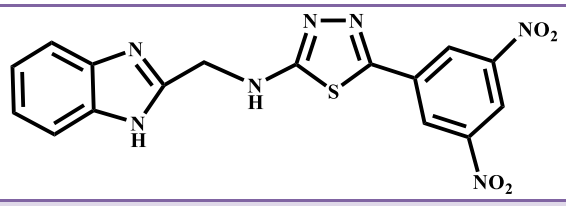
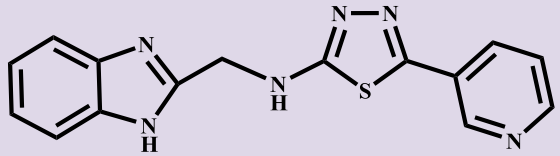
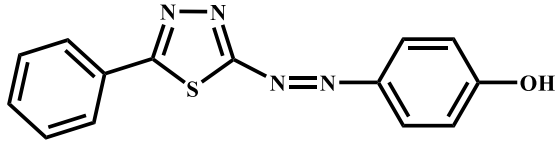
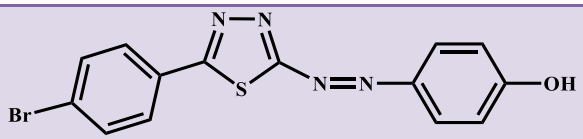
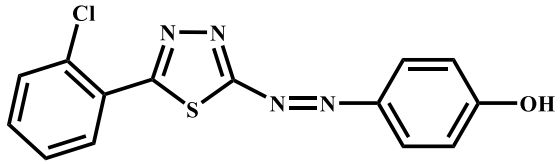
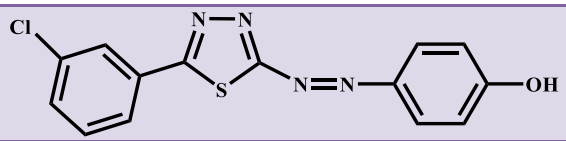
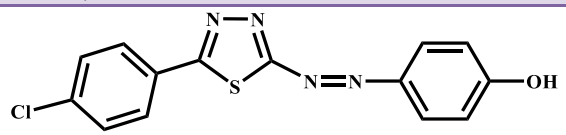
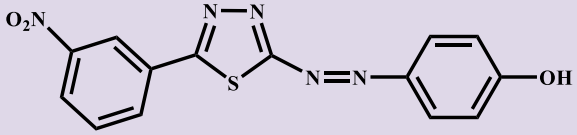
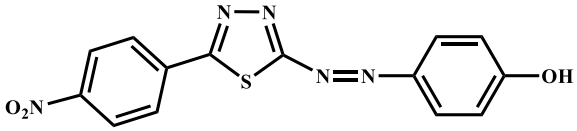
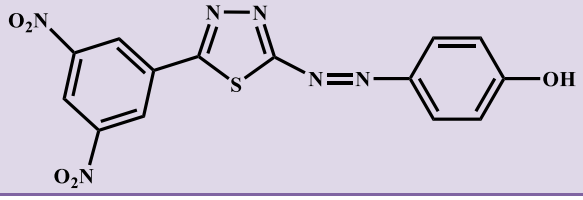
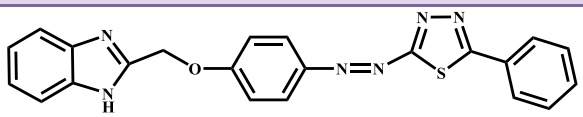
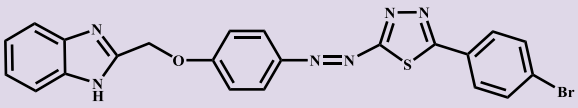
4- A disk of (Ceftazidime and Azetronam) was placed in the center of the plates of *P. aeruginosa*, a disk of (Ceftriaxone and Chloramphenicol) was placed in the center of the plates of *E. coli* and a disk of (Clarithromycin and Amikacin) was placed in the center of the plates of *S. aureus* and *S. epidermidis*. The inhibition zone was compared according to Clinical & Laboratory Standards Institute (CLSI) 2020.

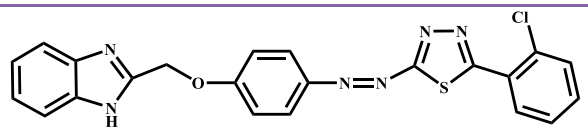
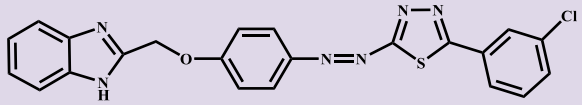
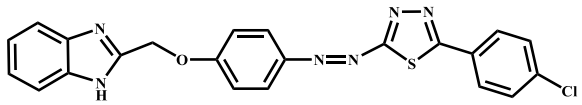
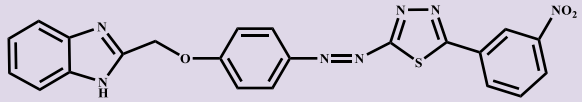
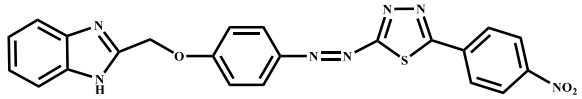
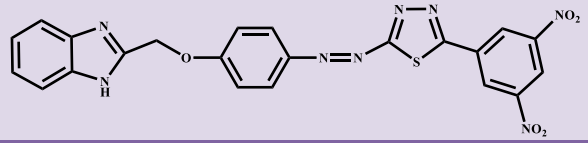
5-The effectiveness of each concentration was determined by measuring the diameter of the inhibition zone around each hole.

Table (2-7): the chemical structure and name of the prepared compounds

Comp No.	Compound structure	Compound name
1		2-(bromomethyl)-1H-benzimidazole
2		5-phenyl-1,3,4-thiadiazol-2-amine
3		5-(2-fluorophenyl)-1,3,4-thiadiazol-2-amine
4		5-(2-iodophenyl)-1,3,4-thiadiazol-2-amine
5		5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine
6		5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine
7		5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine
8		5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine
9		5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine
10		5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine

11		5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine
12		5-(pyridin-3-yl)-1,3,4-thiadiazol-2-amine
13		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-phenyl-1,3,4-thiadiazol-2-amine
14		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(2-fluorophenyl)-1,3,4-thiadiazol-2-amine
15		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(2-iodophenyl)-1,3,4-thiadiazol-2-amine
16		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine
17		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine
18		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine
19		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine
20		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine
21		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine

22		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine
23		*N*-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-3-yl)-1,3,4-thiadiazol-2-amine
24		4-((5-phenyl-1,3,4-thiadiazol-2-yl)diazenyl)phenol
25		4-((5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
26		4-((5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
27		4-((5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
28		4-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
29		4-((5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
30		4-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
31		4-((5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)diazenyl)phenol
32		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-phenyl-1,3,4-thiadiazole
33		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(4-bromophenyl)-1,3,4-thiadiazole

34		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole
35		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(3-chlorophenyl)-1,3,4-thiadiazole
36		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(4-chlorophenyl)-1,3,4-thiadiazole
37		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(3-nitrophenyl)-1,3,4-thiadiazole
38		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(4-nitrophenyl)-1,3,4-thiadiazole
39		2-((4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl)diazenyl)-5-(3,5-dinitrophenyl)-1,3,4-thiadiazole

Chapter Three

Results and discussion

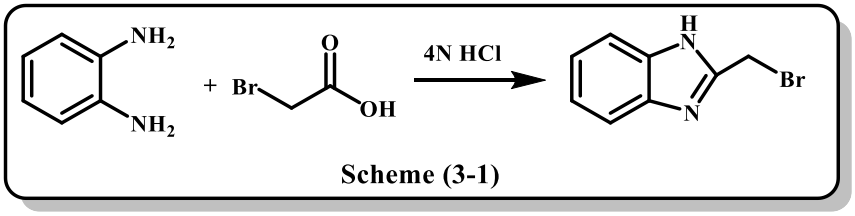
3.1-Introduction

Among of the available several heterocyclic compounds, benzimidazoles still one of the importance antimicrobial agents. The derivatives of 2-bromomethyl-1H-benzimidazoles play a significant role in biological chemistry, they are important intermediates in the synthesis routes of many biologically active compounds[141]. To deal with resistant bacteria, researchers are now focusing on the design of novel antibacterial chemicals. Since harmful bacteria's resistance to commonly used antibiotics has become a big problem around the world[142].

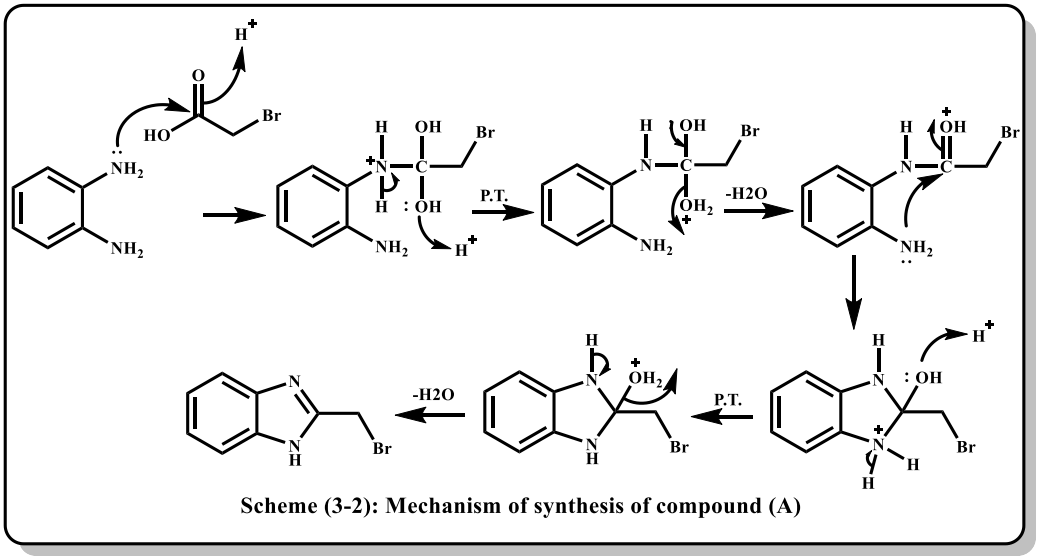
3.2- Synthesis and characterization of compounds:

3.2.1-Synthesis and characterization of 2-(bromomethyl)-1H-benzimidazole (A):

Compound (A) was synthesized by the reaction of *o*-phenylenediamine with bromoacetic acid in the presence of 4N HCl as shown in Scheme (3-1) and mechanism in Scheme (3-2)[143].



Scheme (3-1)



Scheme (3-2): Mechanism of synthesis of compound (A)

This compound was identified by melting points, TLC, (FTIR, ^{1}H and ^{13}C-NMR) spectra. FT-IR spectrum was showed absence the stretching of N-H bond of amine group of *o*-phenylenediamine and showed the stretching of C=N bond in (1624 cm^{-1}) [144] as shown in the following figure:

3.2.1.1 Spectral analysis of Compound (1)

FT-IR spectrum of compound (1) showed the following frequencies, N-H stretching for benzimidazole showed at (3425 cm^{-1}), and one band at (3024 cm^{-1}) and two bands at (2943 and 2848) cm^{-1} related to stretching of C-H aromatic and aliphatic respectively, one band showed at (1624 cm^{-1}) accounted to C=N group and two bands at (1593 and 1440) cm^{-1} accounted for stretching of C=C aromatic and C-Br showed at (636 cm^{-1}) as shown in Figure (3-1) [145].

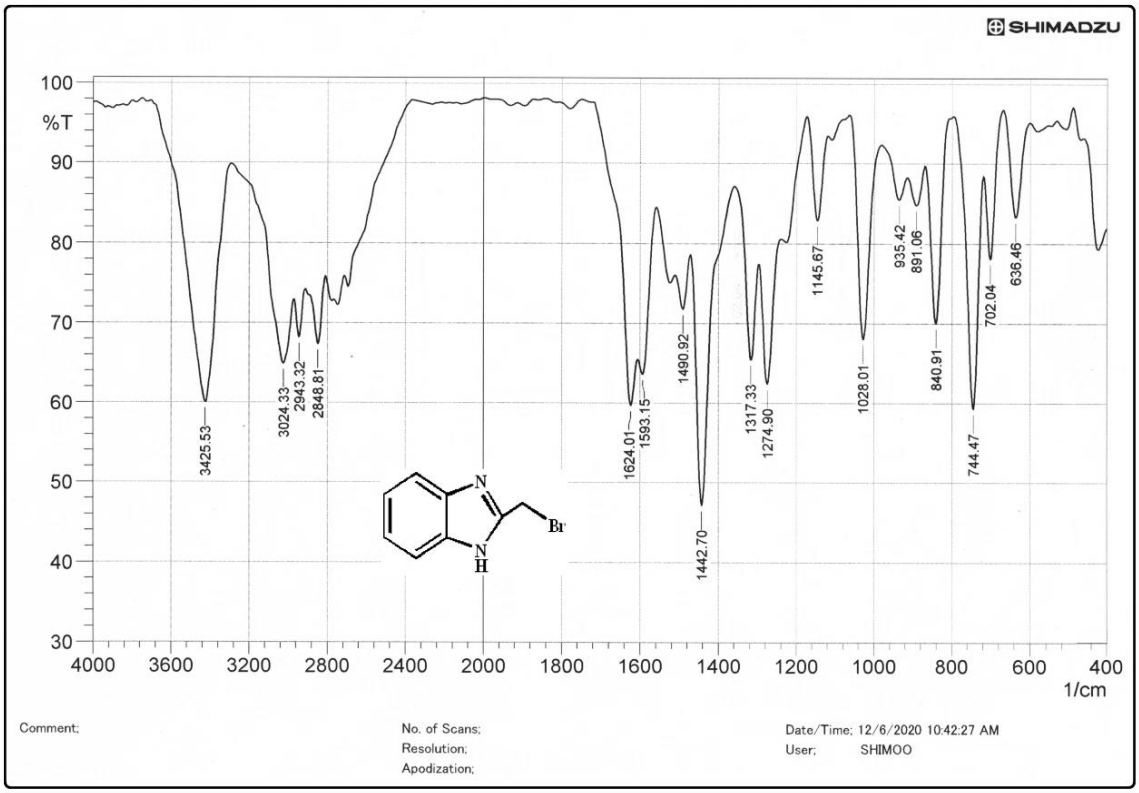


Figure (3-1): FT-IR spectrum of compound (1)

^{1}H-NMR spectrum for compound (1) was showed in Figure (3-2) included strong singlet peak at (4.95 ppm) related to two protons of CH_2 and multiplet peaks at rang

(7.22 –7.57) ppm accounted for (4H) aromatic protons and singlet peak at (10.54 ppm) related to N-H benzimidazole [145] as shown in Figure (3-2).

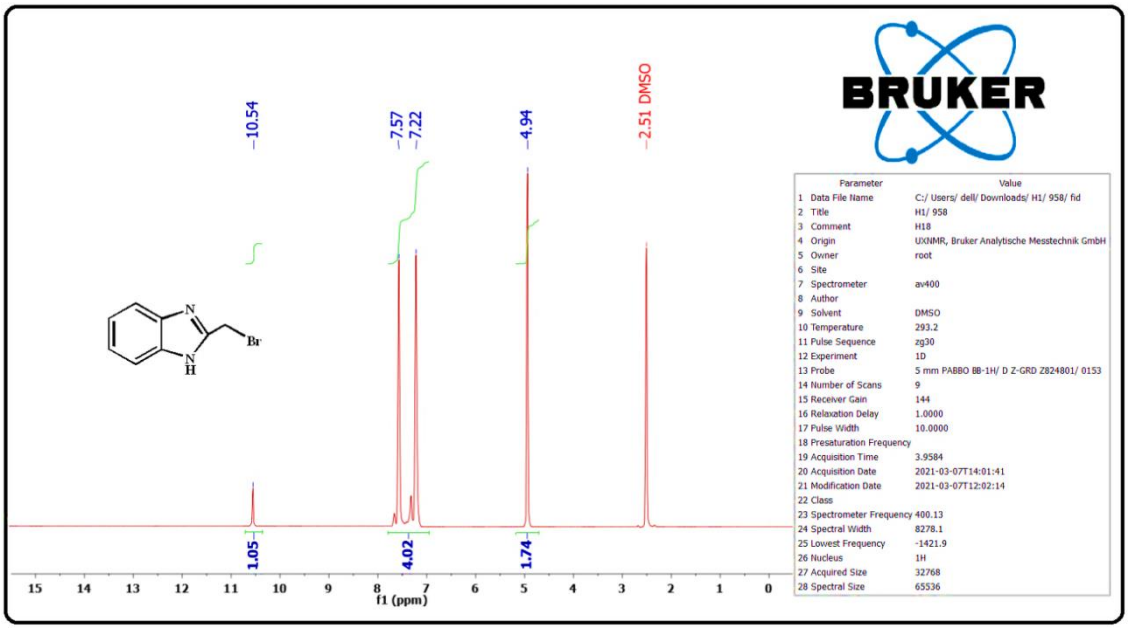


Figure (3-2): ^{1}H-NMR spectrum of compound (1)

^{13}C-NMR spectrum for compound (1) showed peak at (37.97 ppm) related to carbon of CH_2 and peaks at (114.96-138.15) ppm accounted for aromatic carbons and peak at (149.28 ppm) belong to C=N of benzimidazole as shown in Figure (3-3).

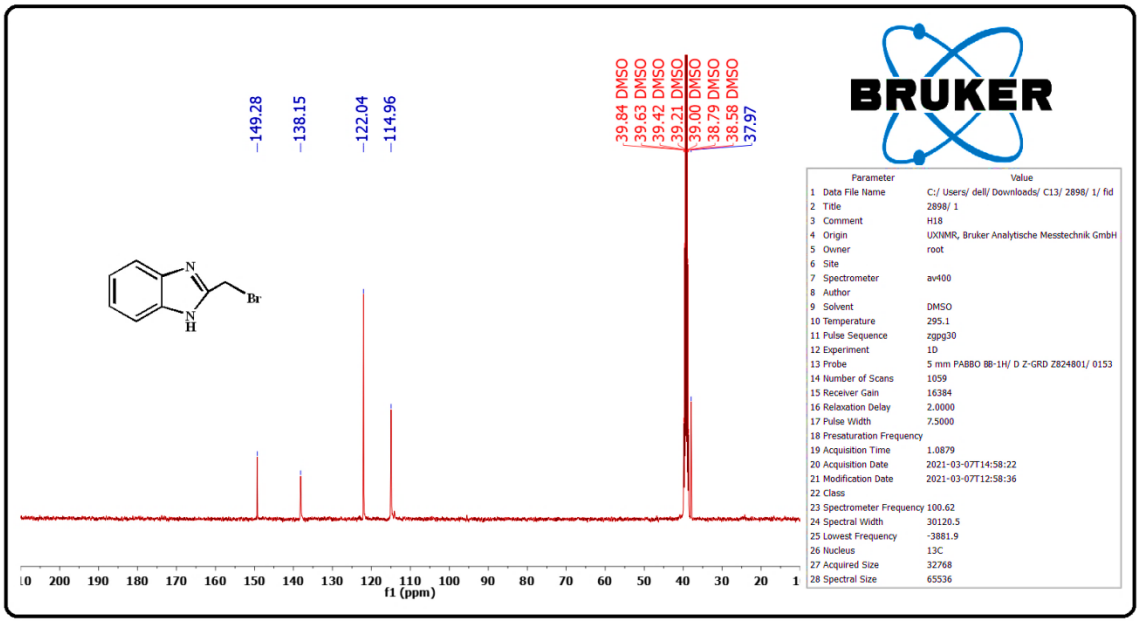
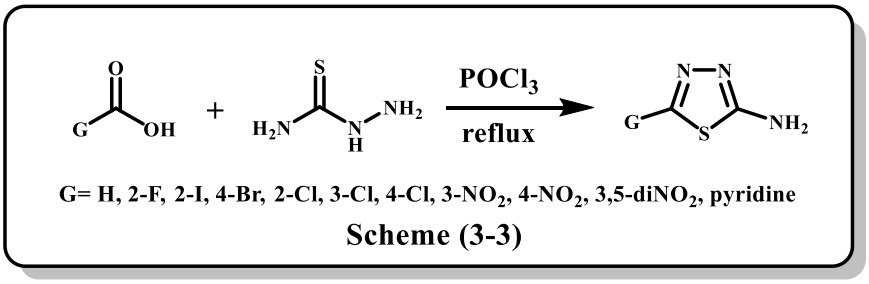


Figure (3-3): ^{13}C-NMR spectrum of compound (1)

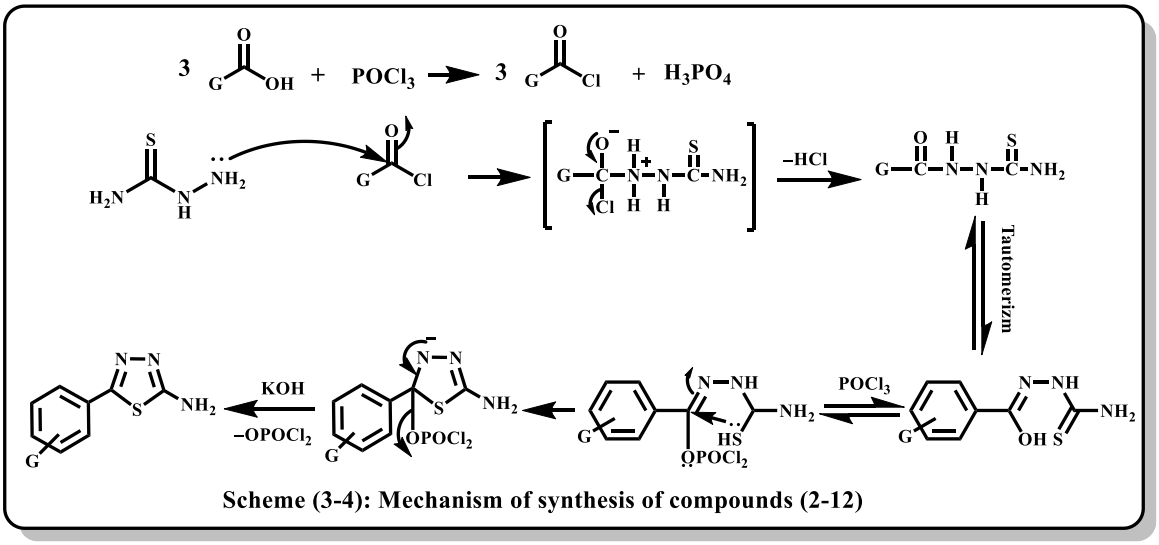
3.2.2- Synthesis and characterization of 2-amino-5-(substitutedphenyl)-1,3,4-thiadiazole (2-12) :

Compounds (2-12) were synthesized by the reaction of thiosemicarbazide with benzoic acid derivatives in the presence of $POCl_3$ as shown in Scheme (3-4) and mechanism in Scheme (3-4)[146].



G= H, 2-F, 2-I, 4-Br, 2-Cl, 3-Cl, 4-Cl, 3-NO_2, 4-NO_2, 3,5-diNO_2, pyridine

Scheme (3-3)



Scheme (3-4): Mechanism of synthesis of compounds (2-12)

These compounds were identified by melting points, TLC and FT-IR spectrum was showed absence the stretching of O-H and C=O of benzoic acid derivatives and showed the stretching of C=N at (1614-1643) cm^{-1} [144] as shown in the following Figures:

3.2.2.1. FT-IR spectral analysis for compound (2):

FT-IR spectrum for compound (1) showed two bands at (3391 and 3270) cm^{-1} accounted for symmetric and asymmetric stretching of NH_2, and band at (3098 cm^{-1}) related to stretching of C-H aromatic. C=N stretch appears at (1630 cm^{-1}) and two

bands at (1586 and 1455) cm^{-1} belong to aromatic C=C stretching as shown in Figure (3-4).

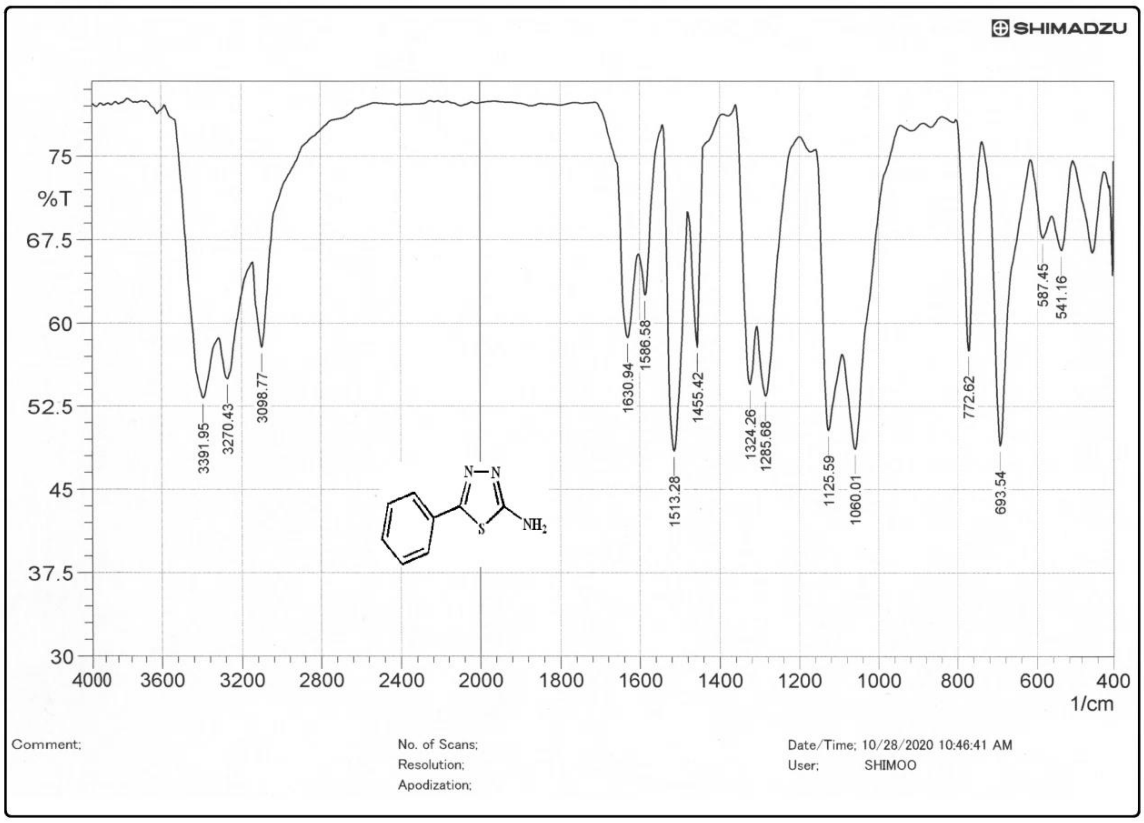


Figure (3-4): FT-IR spectrum of compound (2)

3.2.2.2. FT-IR spectral analysis of compound (3):

FT-IR spectrum for compound (3) showed two bands at (3272 and 3197) cm^{-1} accounted for symmetric and asymmetric stretching of N-H bonds of amine group, and one band at (3089 cm^{-1}) related to stretching of C-H aromatic, and band at (1633 cm^{-1}) for C=N stretch, aromatic C=C stretching showed two bands appears at (1581 and 1475) cm^{-1} as shown in Figure (3-5).

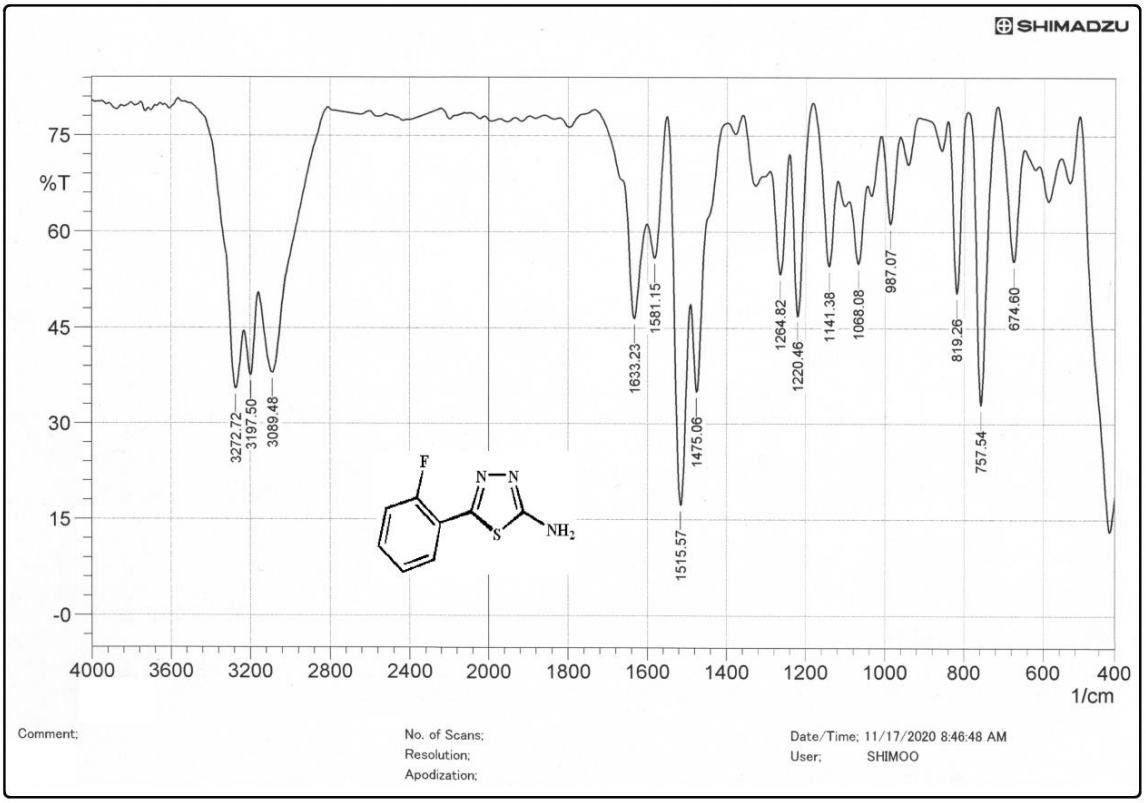


Figure (3-5): FT-IR spectrum of compound (3)

3.2.2.3. FT-IR spectral analysis for compound (4):

FT-IR spectrum for compound (4) showed two bands at (3340 and 3232) cm^{-1} accounted for symmetric and asymmetric stretching of N-H bonds of amine group, and one band at (3084 cm^{-1}) related to stretching of C-H aromatic, and band at (1616 cm^{-1}) accounted for C=N stretching and aromatic C=C stretching showed two bands appears at (1585 and 1494) cm^{-1} as shown in Figure (3-6).

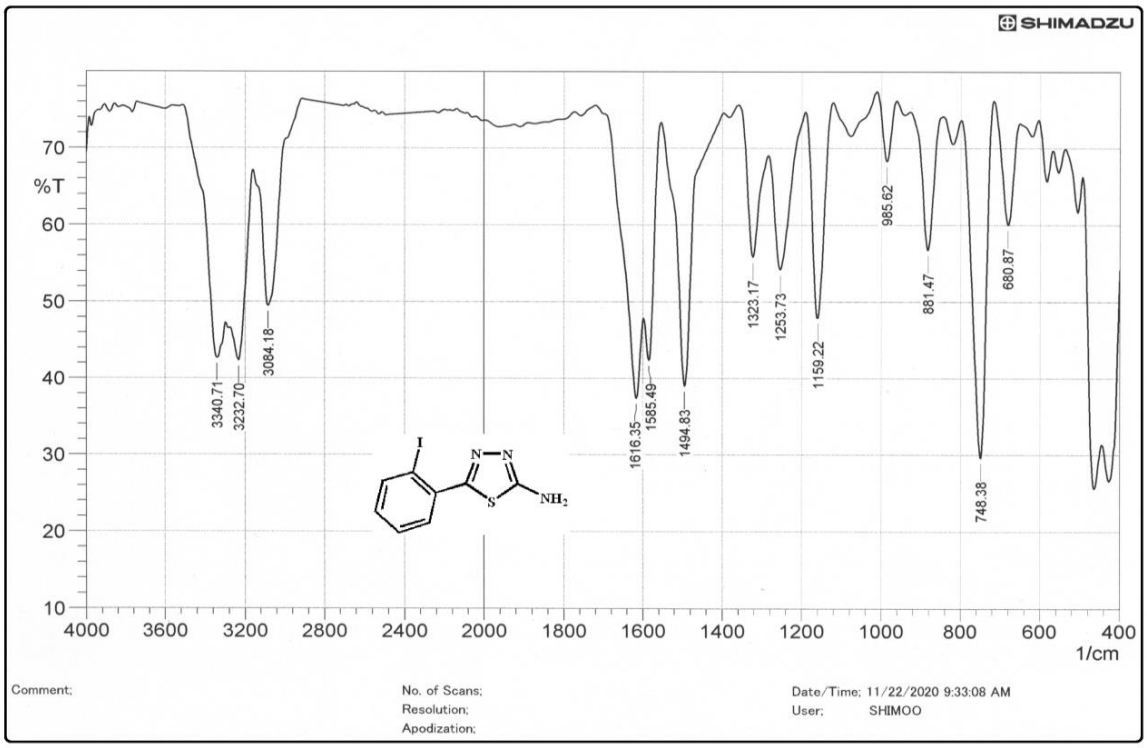


Figure (3-6): FT-IR spectrum of compound (4)

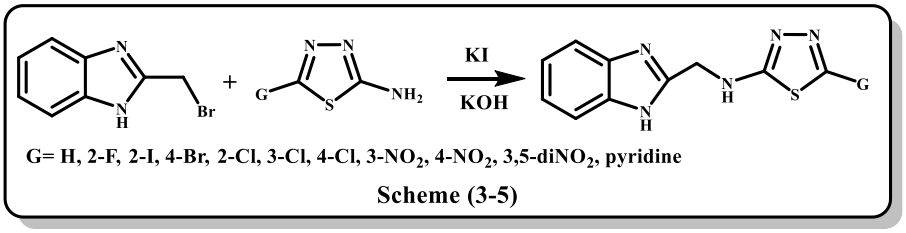
The FT-IR spectral data for the compounds (5-12) were listed in table (3-1) and the spectra were showed in appendix in Figures (3-49) to (3-56).

Table (3-1): FT-IR for the compounds (5-12)

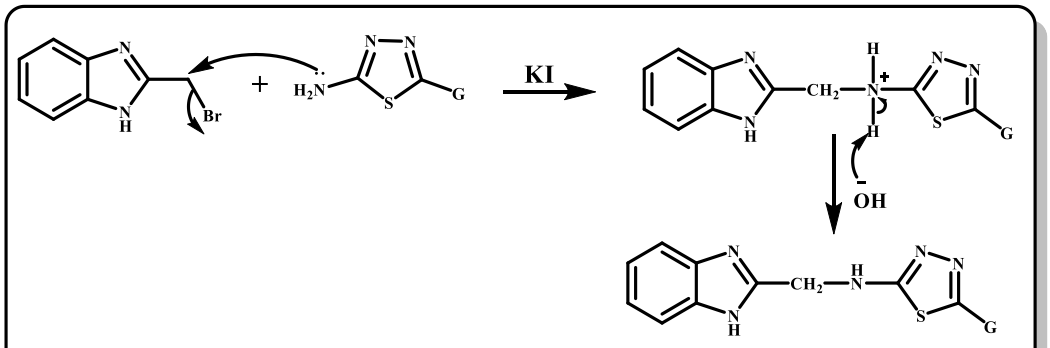
Comp. No.	Characteristic stretching bands of FT-IR (cm^{-1}, KBr disc)						
	N-H (amine)		Ar.C-H	C=N	Ar.C=C		others
	Asy.	Sy					
5	3277	3159	3035	1639	1602	1460	-
6	3290	3203	3086	1639	1596	1458	-
7	3296	3170	3084	1614	1566	1415	-
8	3271	3196	3088	1633	1593	1479	-
9	3379	3280	3074	1620	1579	1423	1531,1348 NO_2
10	3377	3294	3069	1631	1604	1440	1508,1342 NO_2
11	3360	3251	3097	1627	1593	1435	1535,1350 NO_2
12	3369	3284	3087	1643	1597	1421	-

3.2.3. Synthesis and characterization of *N*-((1H-benzimidazol-2-yl)methyl)-5-(substitutedphenyl)-1,3,4-thiadiazol-2-amine (13-23) :

Compounds (13-23) were synthesized by the reaction of 2-(bromomethyl)-1H-benzimidazole (1) with 2-amino-5-(substitutedphenyl)-1,3,4-thiadiazole (2-12) in the presence of KI and KOH in ethanol as shown in Scheme (3-5) and mechanism in Scheme (3-6).



Scheme (3-5)



Scheme (3-6): Mechanism of synthesis of compounds (13-23)

These compounds were identified by TLC, (FTIR, ^{1}H and ^{13}C-NMR) spectra of some compounds, FT-IR spectrum showed absence one stretching band of N-H for NH_2 group of 1,3,4-Thiadiazole and showed the stretching of N-H for benzimidazole at range (3444-3323) cm^{-1} and stretching for N-H of amine at range (3259-3174) cm^{-1} and stretching for C=N at range (1635-1616) cm^{-1} as shown in the following Figures:

3.2.3.1. Spectral analysis of compound (13):

FT-IR spectrum for compound (13) showed one band of stretching N-H for benzimidazole at (3350 cm^{-1}) and one band of N-H of amine at (3178 cm^{-1}) and one band at (306 cm^{-1}) related to C-H aromatic and two bands at (2962 and 2852) cm^{-1} belong to stretching of aliphatic C-H of CH_2 and one band at (1620.21 cm^{-1}) accounted for C=N stretching and two bands at (1589 and 1480) cm^{-1} belong to aromatic C=C stretching as shown in Figure (3-7).

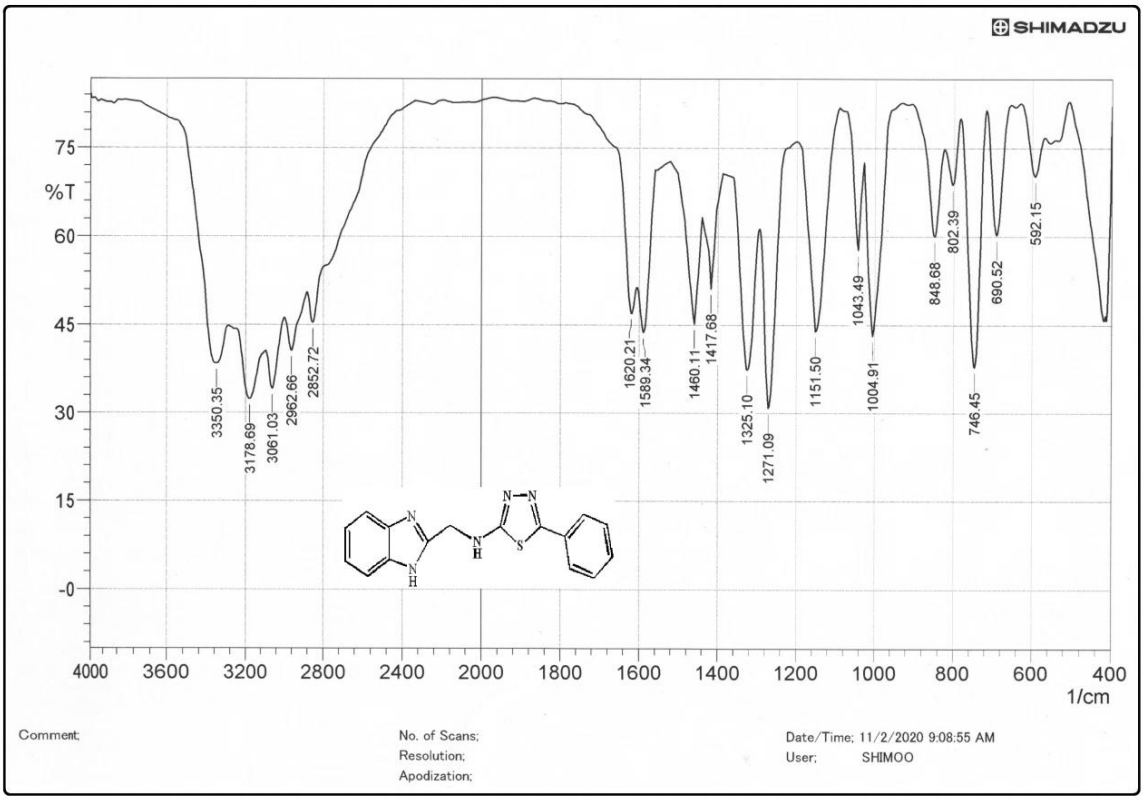


Figure (3-7): FT-IR spectrum of compound (13)

^{1}H-NMR spectrum for compound (13) showed singlet peak at (3.36 ppm) belong to 2H of aliphatic CH_2 and singlet peak at (6.75 ppm) accounted for 1H of N-H of amine and multiplet peaks between (7.13-7.74) ppm related to 9H of aromatic rings and singlet peak at (10.48 ppm) related to 1H of N-H of benzimidazole as shown in Figure (3-8).

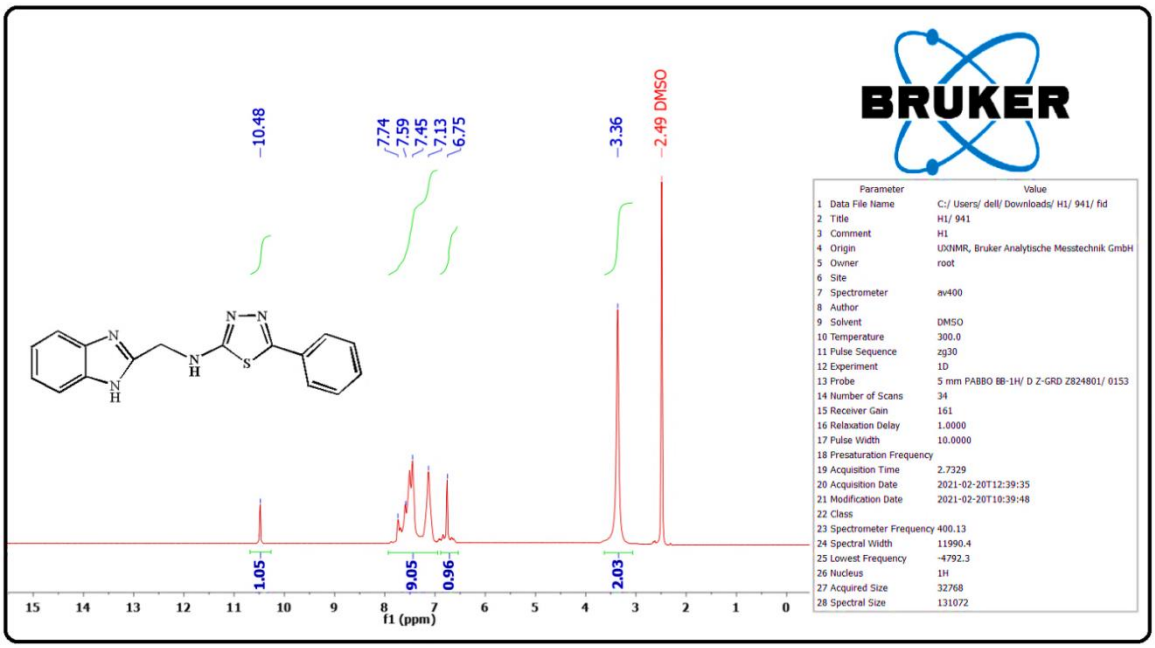


Figure (3-8): ^{1}H-NMR spectrum of compound (13)

^{13}C-NMR spectrum for compound (13) showed peak at (45.04 ppm) related to carbon of aliphatic CH_2 and peaks between (114.91-130.08) ppm related to carbons of aromatic rings and peak at (149.82 ppm) accounted for C=N of benzimidazole and peaks at (165.69 and 168.39) ppm of C=N of 1,3,4-thiadiazole as shown in Figure (3-9).

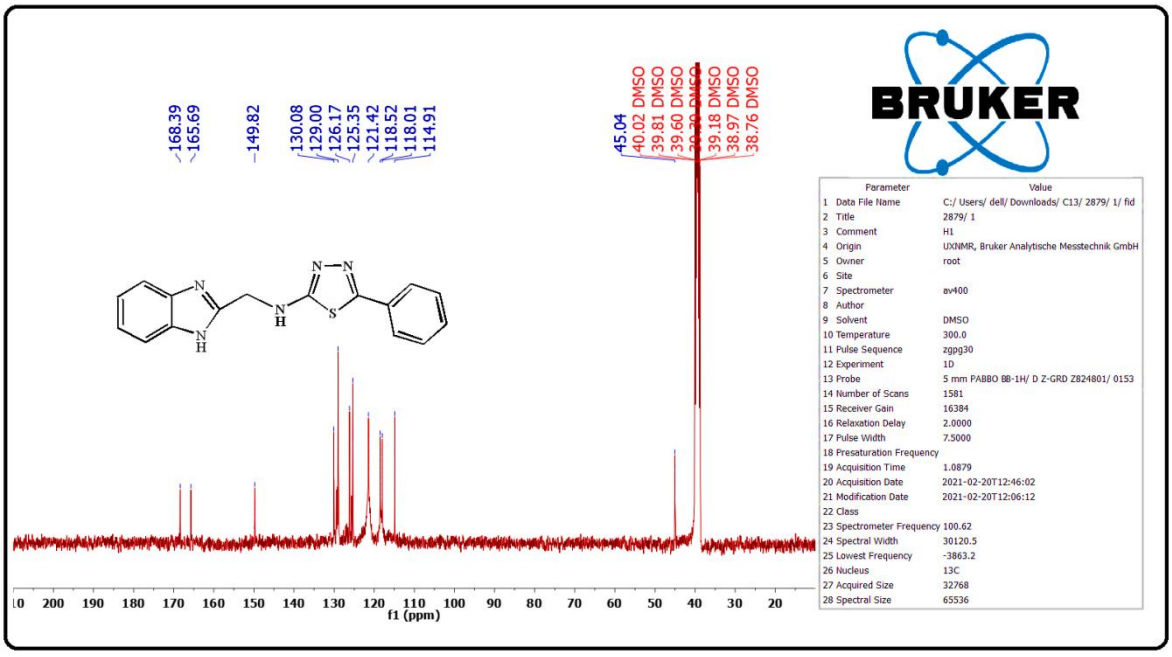


Figure (3-9): ^{13}C-NMR spectrum of compound (13)

3.2.3.2. Spectral analysis of compound (14):

FT-IR spectrum for compound (14) showed one band of stretching N-H for benzimidazole at (3421.67 cm^{-1}) and band for N-H of amine at (3259 cm^{-1}) and one band at (3057 cm^{-1}) related to C-H aromatic and two bands at (2987 and 2889) cm^{-1} related to stretching of aliphatic C-H of CH_2 and one band at (1629 cm^{-1}) accounted for C=N stretching and two bands at (1597 and 1463) cm^{-1} related to aromatic C=C stretching as shown in Figure (3-10).

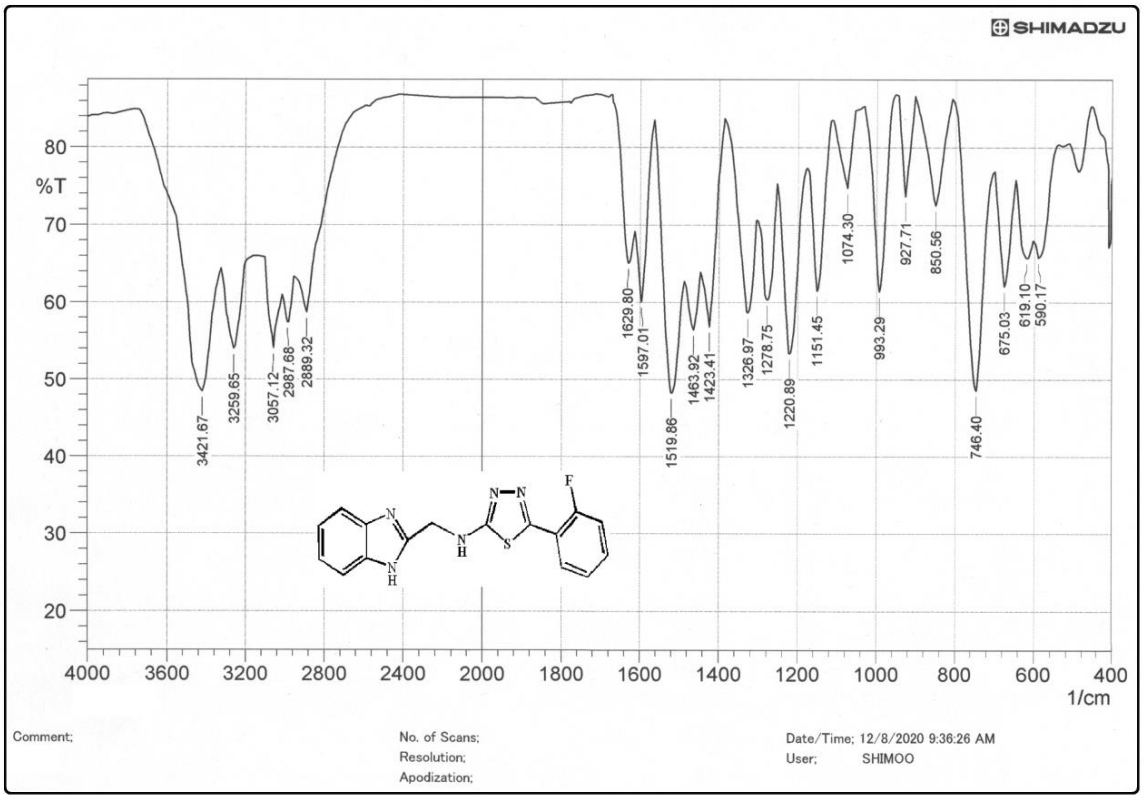


Figure (3-10): FT-IR spectrum of compound (14)

^{1}H-NMR spectrum for compound (14) showed singlet peak at (3.42 ppm) related to 2H of aliphatic CH_2 and singlet peak at (6.52 ppm) accounted for 1H of N-H of amine and multiple peaks between (7.13-8.07 ppm) related to 8H of aromatic rings and singlet peak at (10.47 ppm) related to 1H of N-H of benzimidazole as shown in Figure (3-11).

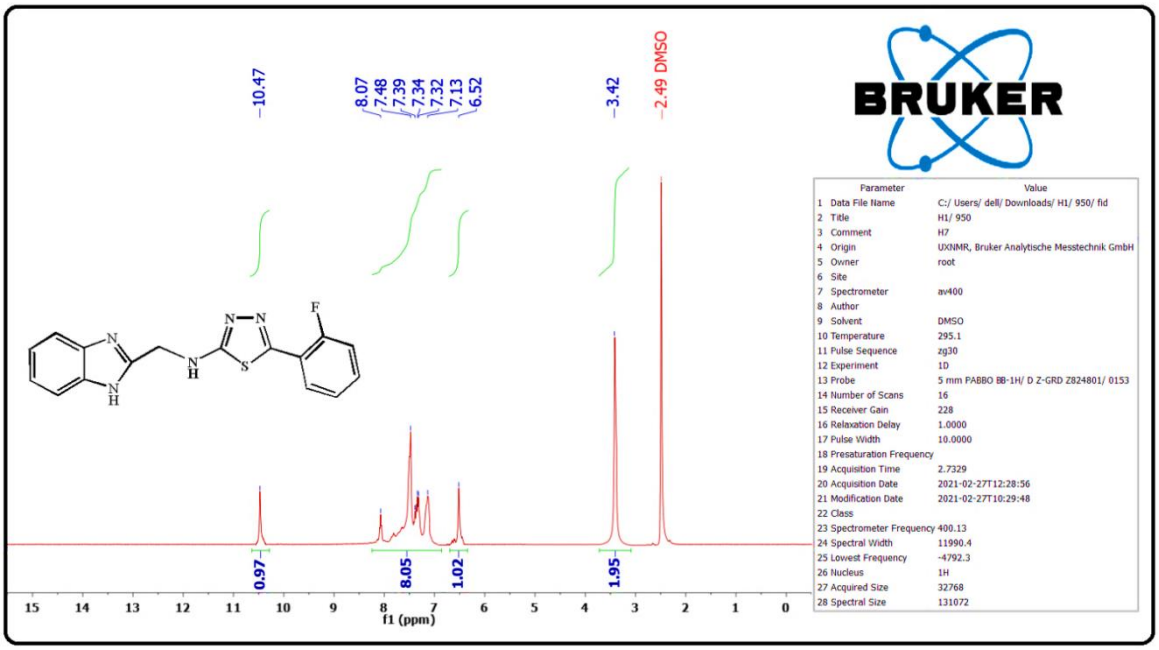


Figure (3-11): ^{1}H-NMR spectrum of compound (14)

^{13}C-NMR spectrum for compound (14) showed peak at (42.06 ppm) related to carbon of aliphatic CH_2 and peaks between (110.27-136.12 ppm) related to carbons of aromatic rings and peak at (148.25 ppm) accounted for C=N of benzimidazole and peaks at (158.84 and 169.84) ppm of C=N of 1,3,4-thiadiazole as shown in Figure (3-12).

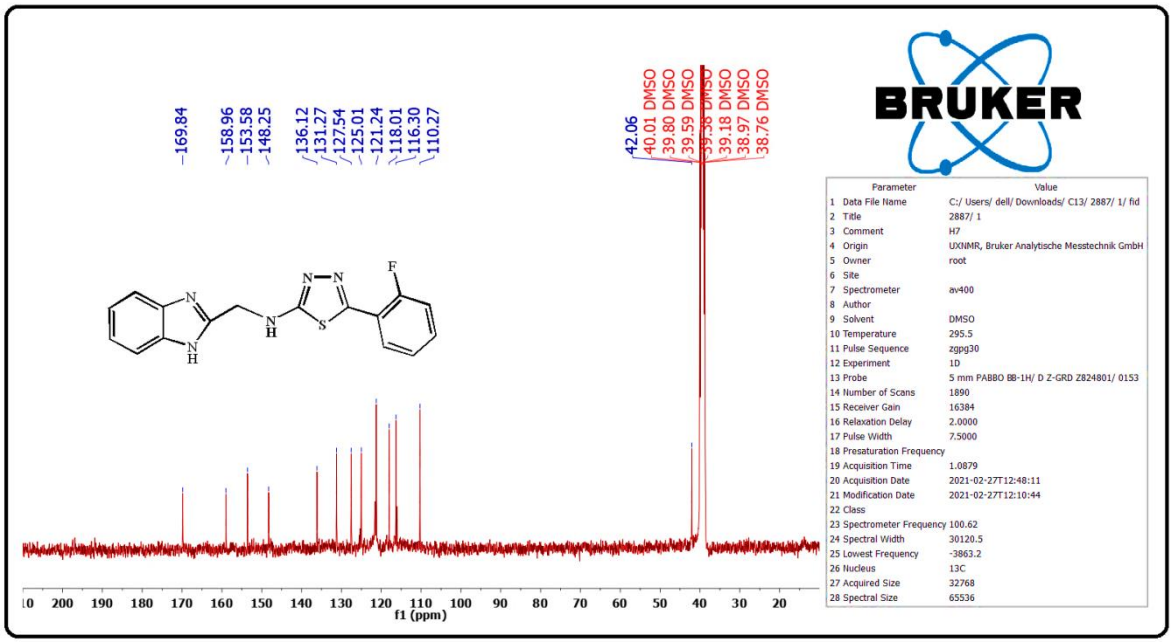


Figure (3-12): ^{13}C-NMR spectrum of compound (14)

3.2.3.3. Spectral analysis of compound (15):

FT-IR spectrum for compound (15) showed one band of stretching of N-H for benzimidazole at (3323 cm^{-1}) and one band N-H for amine at (3178 cm^{-1}) and one band at (3055 cm^{-1}) related to C-H aromatic and two bands at (2964 and 2873 cm^{-1}) related to stretching of aliphatic C-H of CH_2 and one band at (1616 cm^{-1}) accounted for C=N stretching and two bands in (1579 and 1469) cm^{-1} related to aromatic C=C stretching as shown in Figure (3-13).

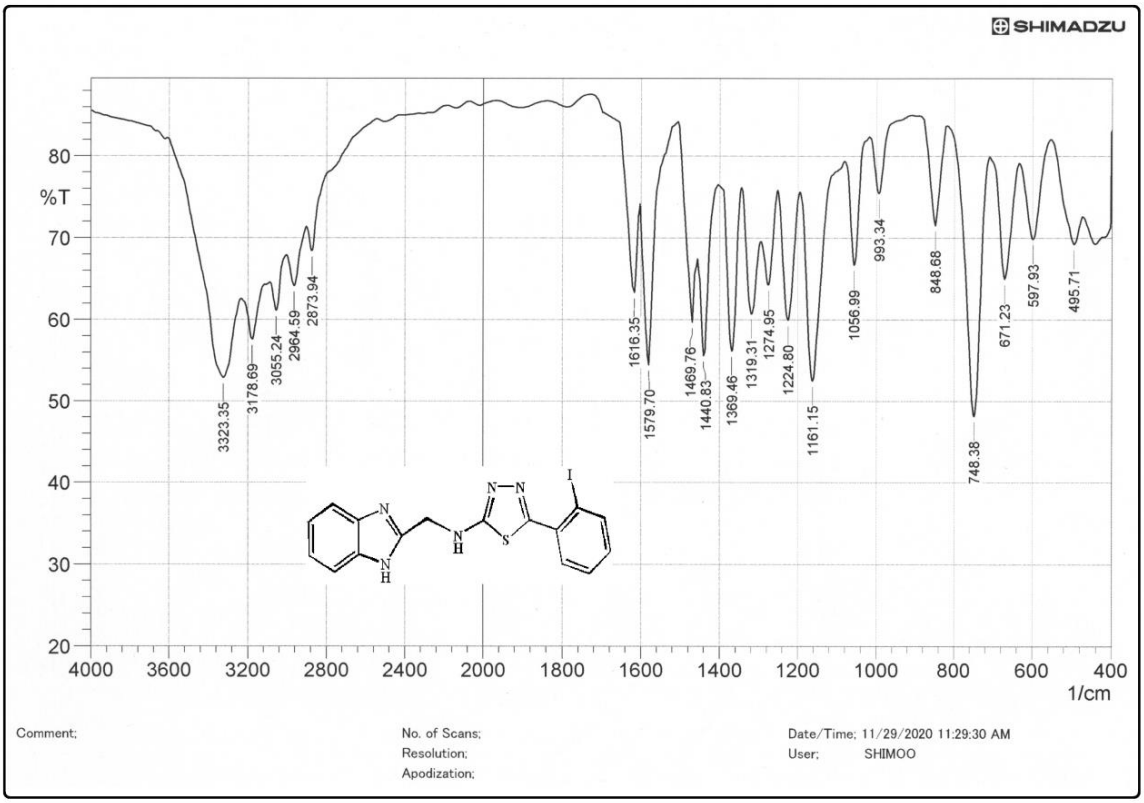


Figure (3-13): FT-IR spectrum of compound (15)

^{1}H-NMR spectrum for compound (15) showed singlet peak in (3.44 ppm) related to 2H of aliphatic CH_2 and singlet peak at (6.79 ppm) accounted for 1H of N-H amine and multiple peaks between (7.13-8.07) ppm related to 8H of aromatic rings and singlet peak at (10.47 ppm) related to 1H of N-H of benzimidazole as shown in Figure (3-14).

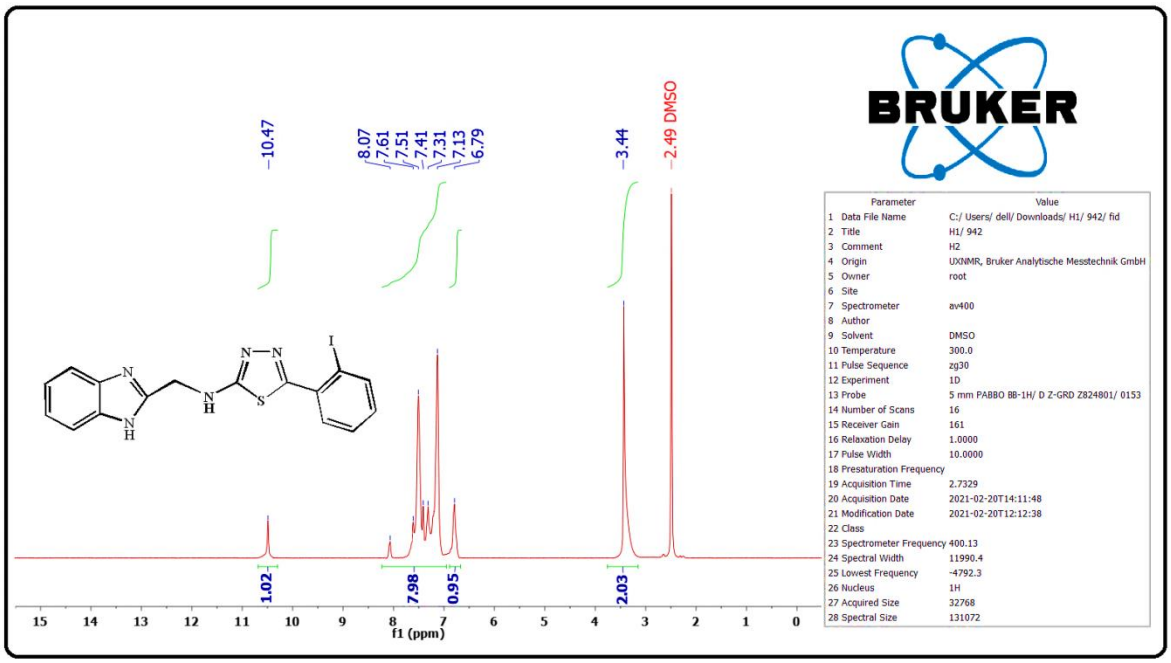


Figure (3-14): ^{1}H-NMR spectrum of compound (15)

^{13}C-NMR spectrum for this compound showed peak at (42.04 ppm) related to carbon of aliphatic CH_2 and peaks between (98.23-130.12 ppm) related to carbons of aromatic rings and peak at (153.63 ppm) accounted for C=N of benzimidazole and peaks at (166.46 and 167.41 ppm) of C=N of 1,3,4-thiadiazole as shown in Figure (3-15).

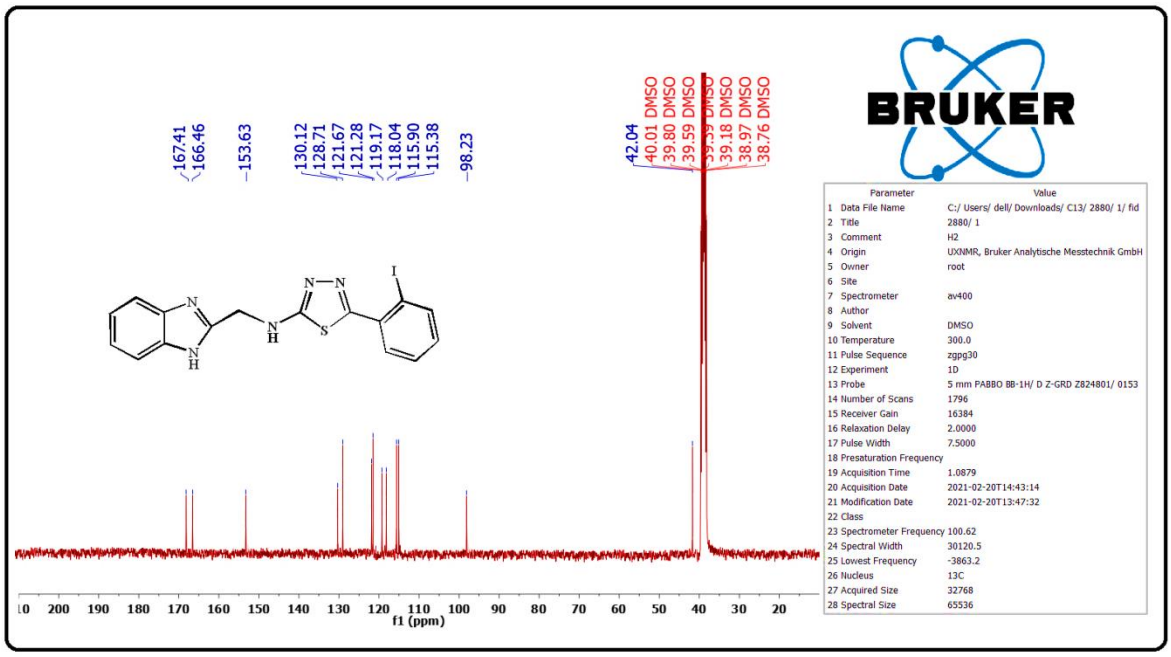


Figure (3-15): ^{13}C-NMR spectrum of compound (15)

The FT-IR spectral data for the compounds (16-23) and ^{1}H and ^{13}C-NMR spectral data of some compounds were listed in table (3-2), (3-3) and (3-4) and the spectra were showed in appendix in Figures (3-57) to (3-72).

Table (3-2): FT-IR spectral data for the compounds (16-23)

Comp. No.	Characteristic stretching bands of FT-IR (Cm^{-1}, KBr disc)								
	N-H benzimidazole	N-H amine	Ar. C-H	C-H aliph.		C=N	Ar. C=C		others
				Asy.	Sy.				
16	3348	3213	3086	2964	2841	1633	1591	1477	-
17	3427	3174	3064	2976	2873	1635	1595	1477	-
18	3417	3230	3068	2968	2881	1631	1591	1479	-
19	3444	3213	3074	2968	2891	1631	1589	1465	-
20	3361	3153	3039	2954	2858	1633	1589	1469	1537,1346 NO_2
21	3410	3201	3033	2931	2860	1635	1591	1463	1525,1346 NO_2
22	3417	3251	3074	2945	2837	1629	1598	1456	1530,1340 NO_2
23	3387	3194	3057	2972	2899	1618	1598	1456	-

Table (3-3): ^{1}H-NMR spectral data for the compounds (17-19 and 21)

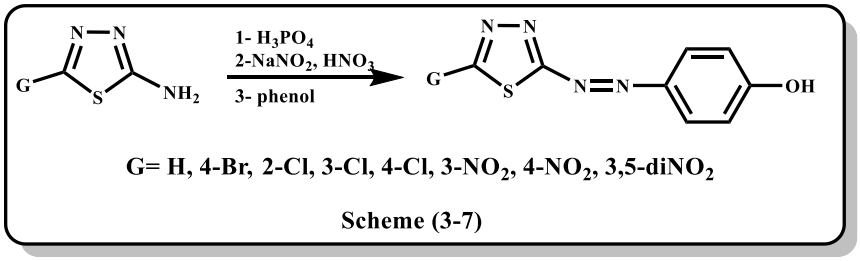
Comp. No.	Characteristic protons of ^{1}H-NMR (ppm)			
	CH_2	N-H (amine)	Ar. protons	N-H (benzimidazole)
17	3.44	6.52	7.17-7.98	10.15
18	3.43	6.51	7.13-7.89	10.49
19	3.41	6.52	7.15-7.92	10.48
21	3.95	6.47	7.08-8.25	10.46

Table (3-4): ^{13}C-NMR spectral data for the compounds (17-19 and 21)

Comp. No.	Characteristic carbons of ^{13}C-NMR (ppm)				
	CH_2	Ar. carbons	C=N (benzimidazole)	C=N (1,3,4-thiadaizole)	
17	44.79	114.97-138.46	151.50	166.08	169.99
18	41.64	114.64-133.67	151.96	163.15	168.94
19	41.84	114.55-133.81	153.62	166.32	168.70
21	41.96	115.56-147.18	153.92	165.86	169.86

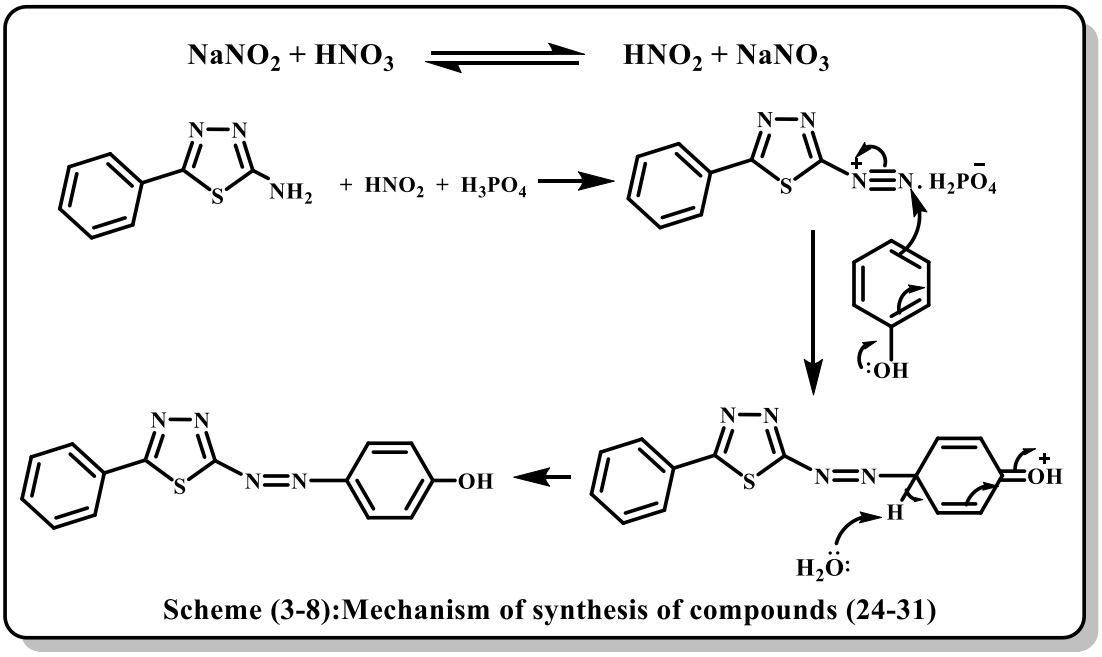
3.2.4. Synthesis and characterization 4-((5-substitutedphenyl)-1,3,4-thiadiazol-2-yl) diazenyl)phenol (24-31) :

Compounds (24-31) were synthesized from the reaction of 2-amino-5-(substitutedphenyl)-1,3,4-thiadiazole (2 and 5-11) with each of H_3PO_4, $NaNO_2$, HNO_3 and phenol in H_2O as shown in Scheme (3-7) and mechanism in Scheme (3-8)[147].



G= H, 4-Br, 2-Cl, 3-Cl, 4-Cl, 3-NO_2, 4-NO_2, 3,5-diNO_2

Scheme (3-7)



Scheme (3-8):Mechanism of synthesis of compounds (24-31)

These compounds were identified by TLC, (FTIR, ^1H and ^{13}C-NMR) spectra of some compounds, FT-IR spectrum was showed absence the stretching of N-H of NH_2 group of 1,3,4-thiadiazole derivatives and showed a broad band related to stretching of O-H and stretching of N=N at rang (1494-1525) cm^{-1} [154,155] as shown in the following Figures:

3.2.4.1. Spectral analysis of compound (26):

FT-IR spectrum for compound (26) showed a broad band at (3417 cm^{-1}) accounted for O-H bond and band at (3088 cm^{-1}) related to stretching of C-H aromatic and band at (1620 cm^{-1}) related to stretching of C=N, and N=N stretching appeared at (1510 cm^{-1}), and aromatic C=C stretching showed was appeared at (1585 and 1469) cm^{-1} as shown in Figure (3-16).

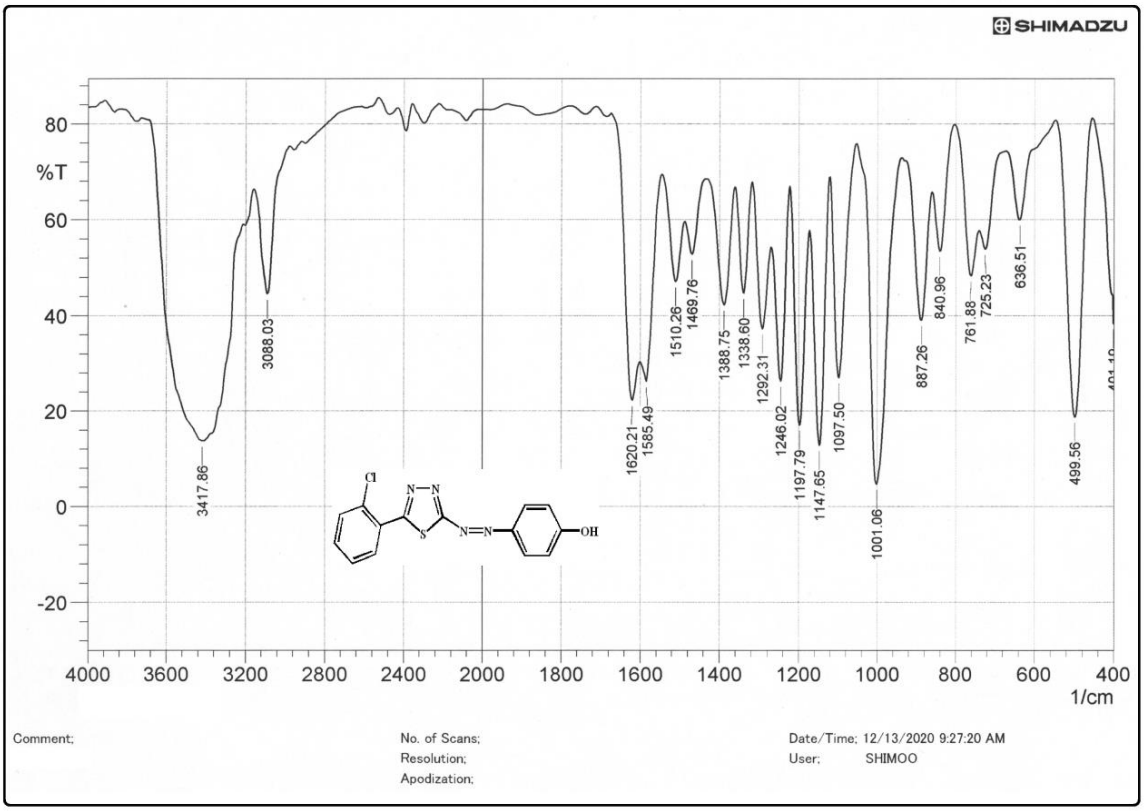


Figure (3-16): FT-IR spectrum of compound (26)

^{1}H-NMR spectrum for compound (26) showed multiplet peaks between (7.60-8.10 ppm) related to 8H of aromatic rings and singlet peak at (8.58 ppm) related to 1H of OH group as shown in Figure (3-17).

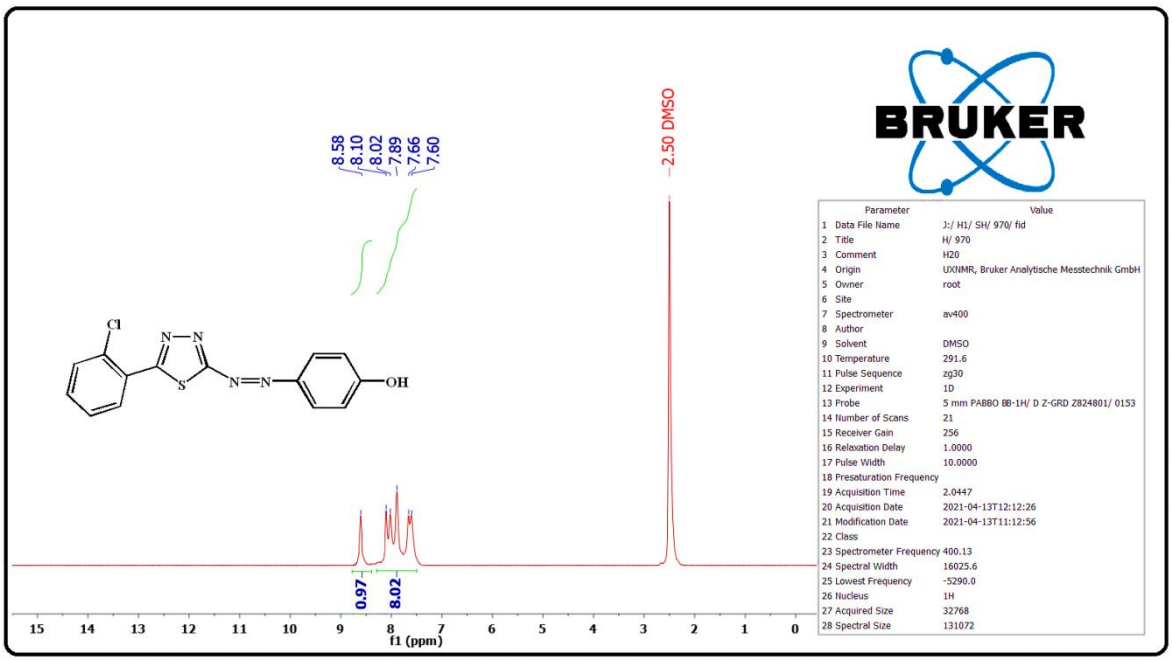


Figure (3-17): ^{1}H-NMR spectrum of compound (26)

^{13}C-NMR spectrum for compound (26) showed peaks between (112.12-154.49ppm) accounted for carbons of aromatic rings and peak at (160.46 ppm) related to aromatic C-OH and peaks at (157.11 and 177.22) ppm of C=N of 1,3,4-thiadiazole as shown in Figure (3-18).

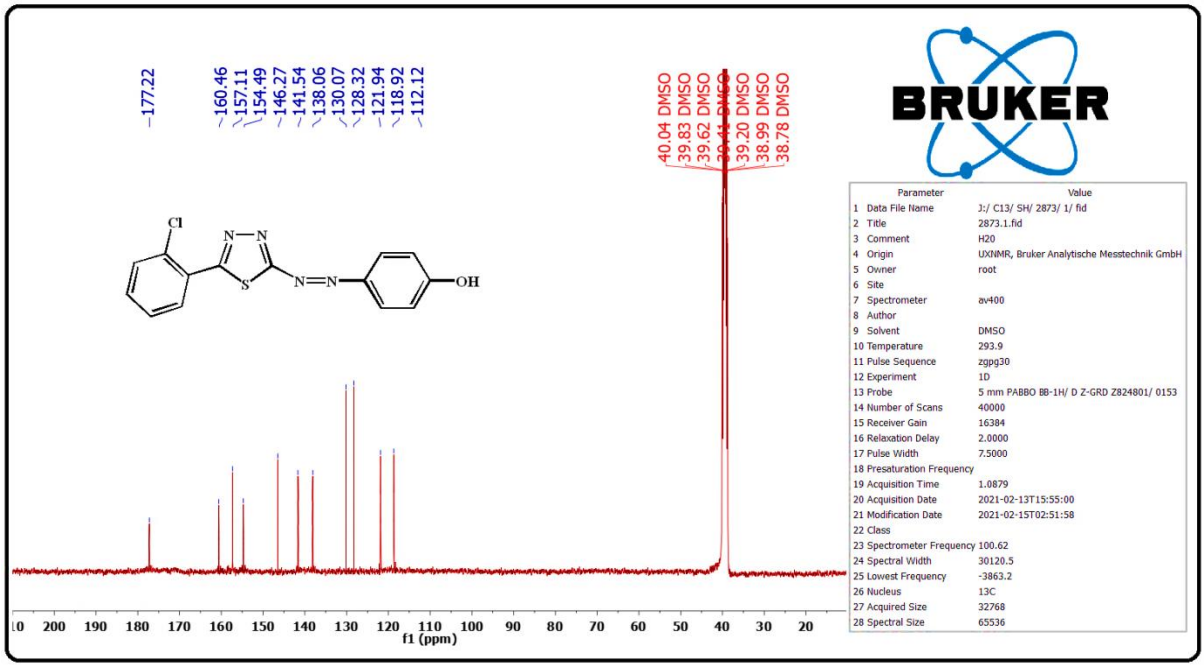


Figure (3-18): ^{13}C-NMR spectrum of compound (26)

3.2.4.2. Spectral analysis of compound (27):

FT-IR spectrum for compound (27) showed broad band at (3439 cm^{-1}) related to O-H bond and band at (3074 cm^{-1}) related to stretching of C-H aromatic, and band at (1639 cm^{-1}) for C=N stretch, N=N stretching showed appeared at (1516 cm^{-1}) and aromatic C=C stretching showed bands was appeared at (1579 and 1473) cm^{-1} as shown in Figure (3-19).

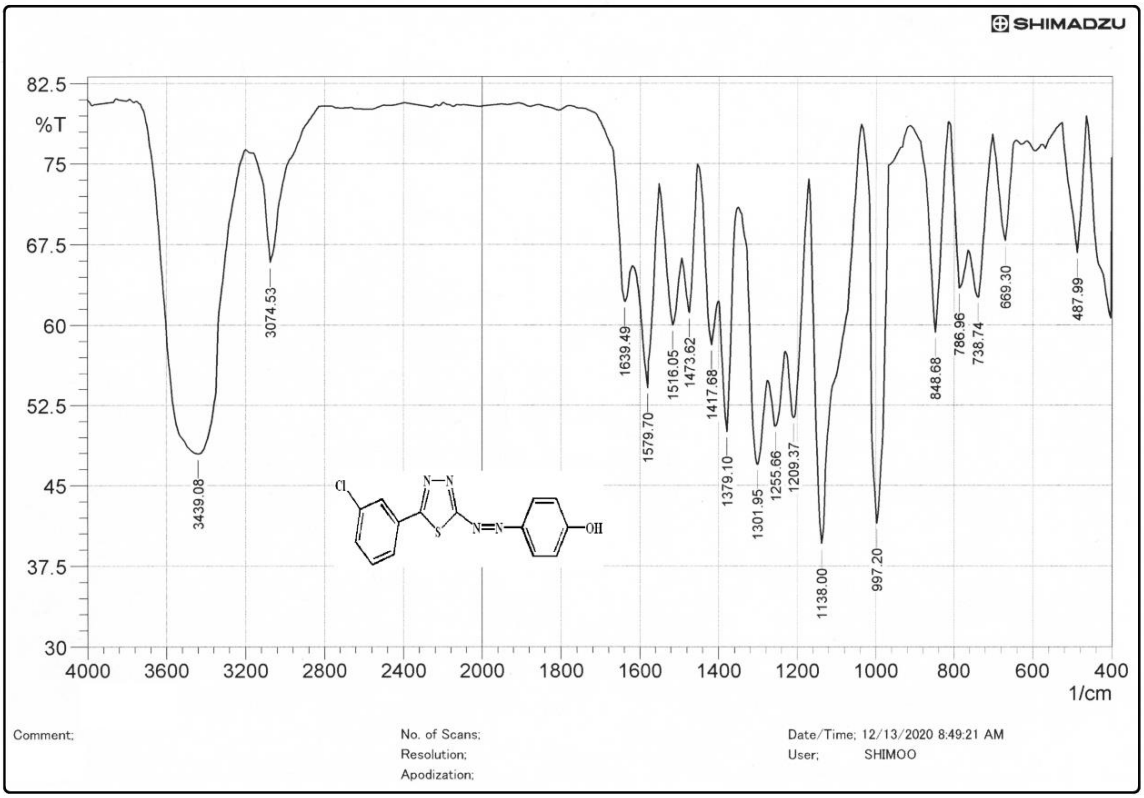


Figure (3-19): FT-IR spectrum of compound (27)

^{1}H-NMR spectrum for compound (27) showed multiplet peaks between (7.01-8.13) ppm related to 8H of aromatic rings and singlet peak at (8.48 ppm) related to 1H of OH group as shown in Figure (3-20).

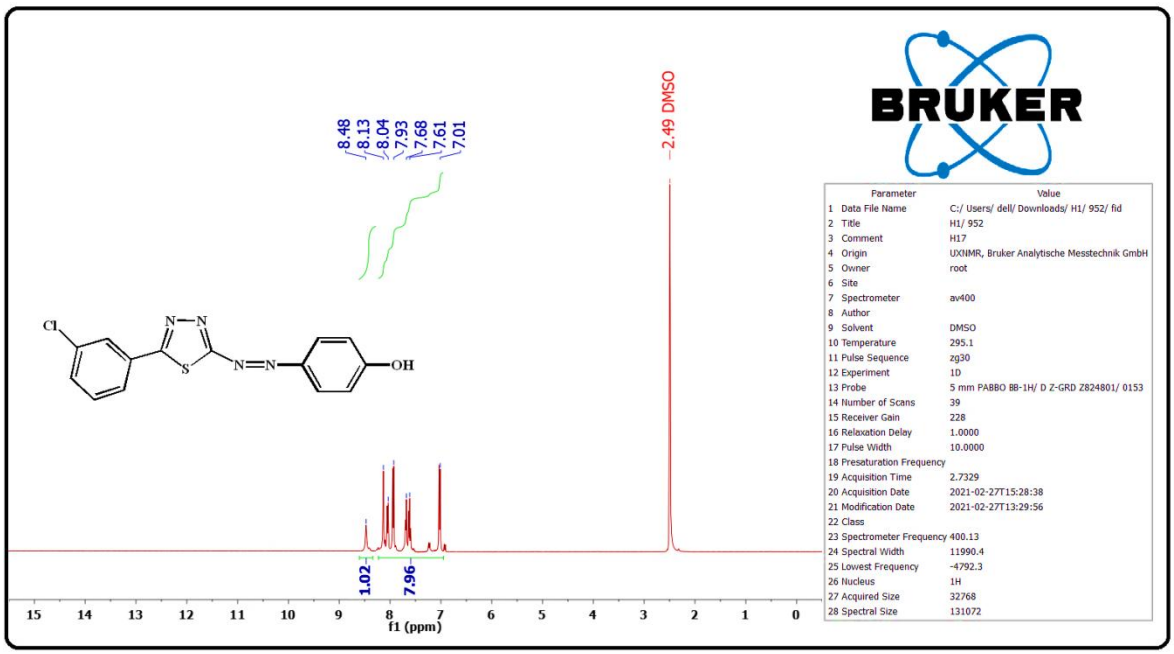


Figure (3-20): ^{1}H-NMR spectrum of compound (27)

^{13}C-NMR spectrum for compound (27) showed peaks between (126.71-139.45) ppm related to carbons of aromatic rings and peak at (164.33 ppm) accounted for aromatic C-OH and peaks at (142.27 and 179.56) ppm of C=N of 1,3,4-thiadiazole as shown in Figure (3-21).

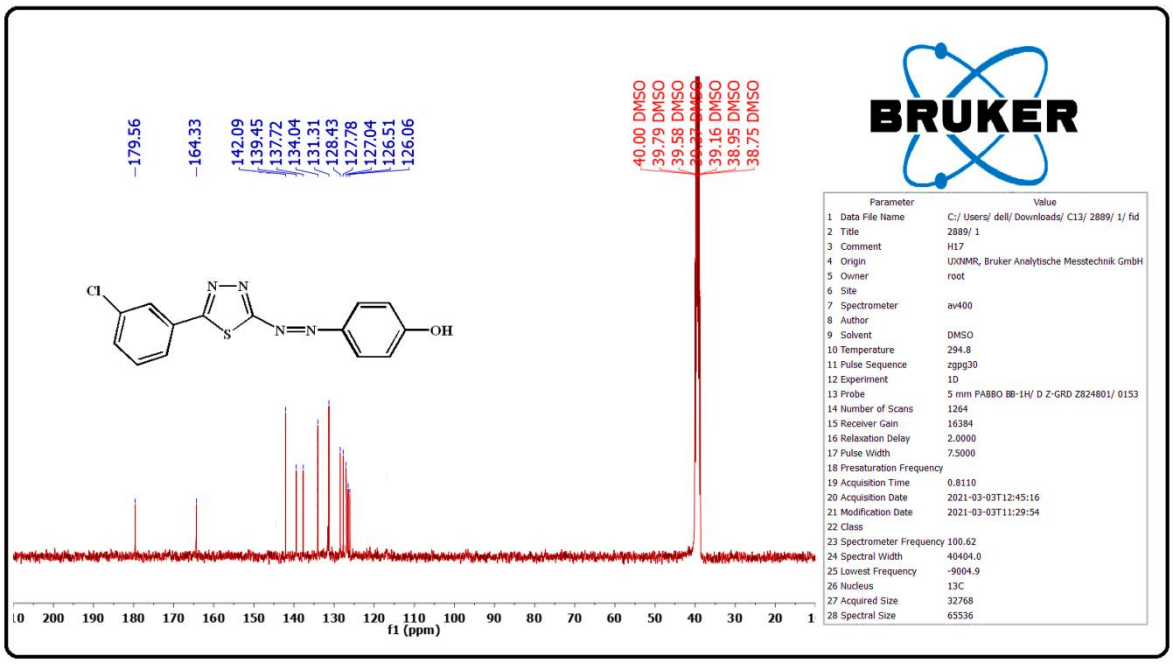


Figure (3-21): ^{13}C-NMR of compound (27)

3.2.4.3. Spectral analysis of compound (28):

FT-IR spectrum for compound (28) showed a broad band at (3371 cm^{-1}) related to O-H bond and band at (3057 cm^{-1}) related to stretching of C-H aromatic and band at (1633 cm^{-1}) for C=N stretch, N=N stretching showed appeared at (1519 cm^{-1}) and aromatic C=C stretching showed bands was appeared at (1589 and 1448) cm^{-1} as shown in Figure (3-22).

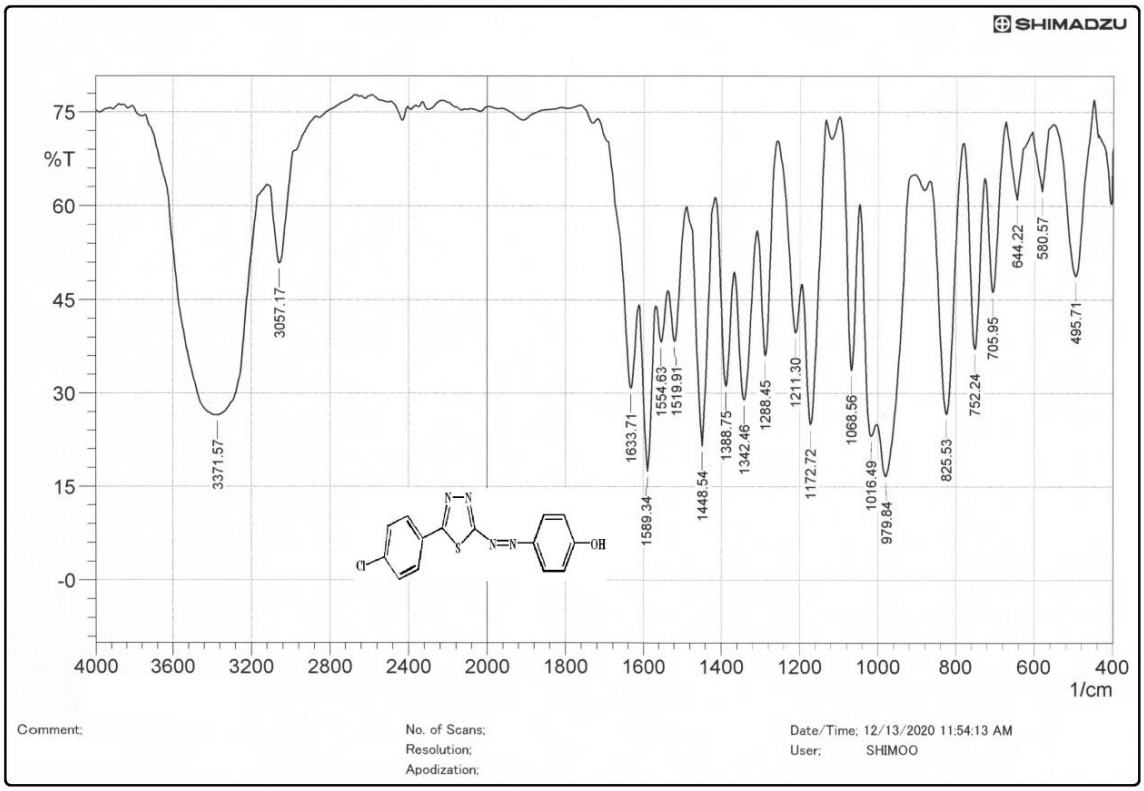


Figure (3-22): FT-IR spectrum of compound (28)

^{1}H-NMR spectrum for compound (28) showed peaks between (7.00-8.07) ppm related to 8H of aromatic rings and peak at (8.47 ppm) related to 1H of OH group as shown in Figure (3-23).

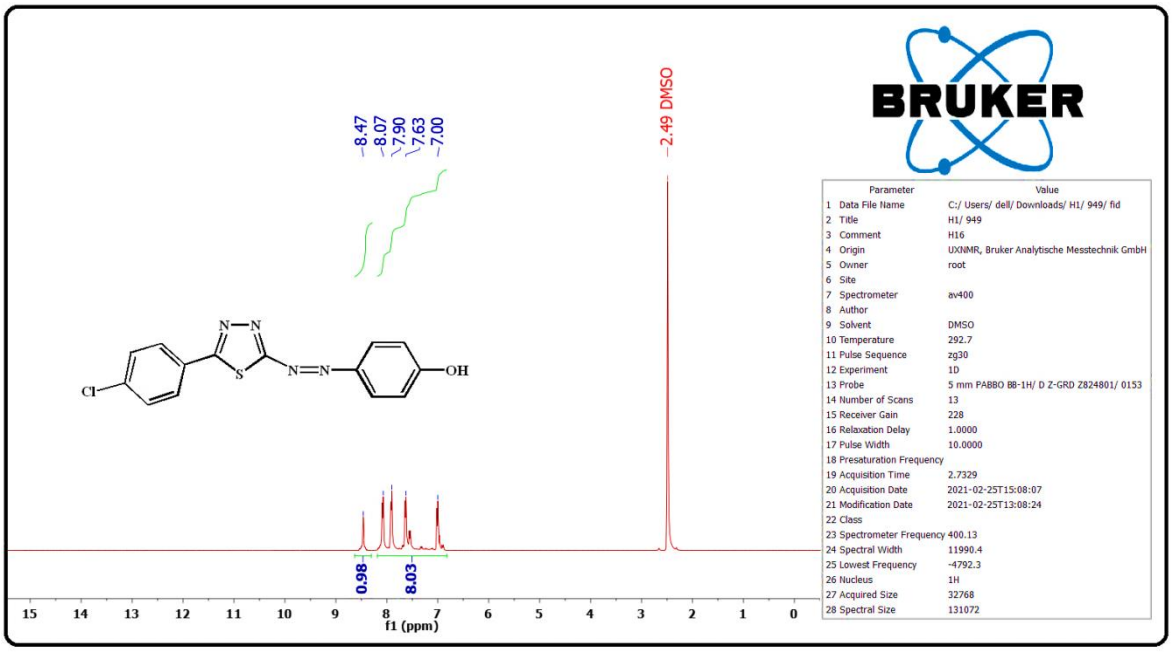


Figure (3-23): ^{1}H-NMR spectrum of compound (28)

^{13}C-NMR spectrum for compound (28) showed peaks between (116.71-144.64) ppm related to carbons of aromatic rings and peak at (166.24 ppm) accounted for aromatic C-OH and peaks at (164.27 and 179.33) ppm of C=N of 1,3,4-thiadiazole as shown in Figure (3-24).

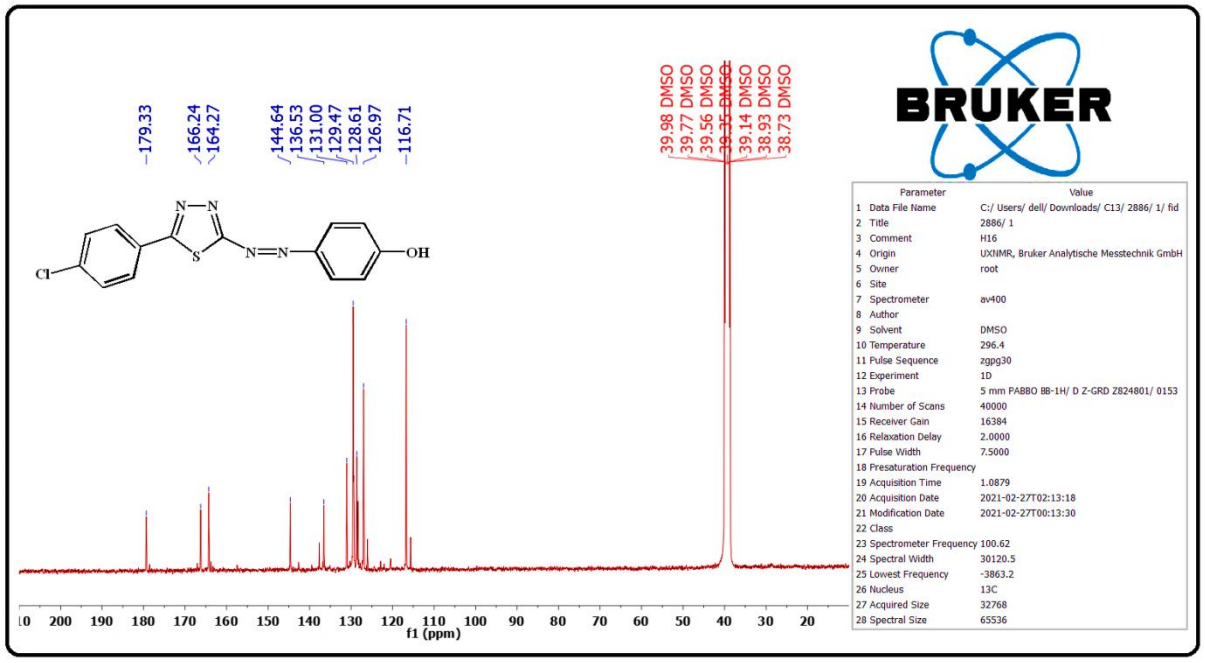


Figure (3-24): ^{13}C-NMR spectrum of compound (28)

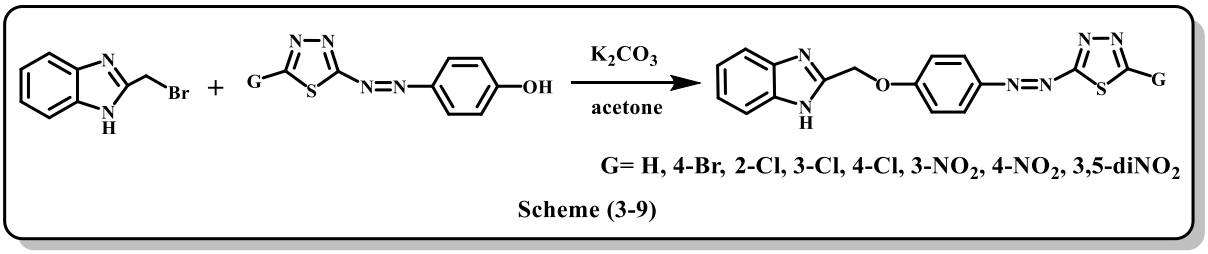
The FT-IR spectral data for the compounds (24-25 and 29-31) were listed in table (3-5) and the spectral were showed in appendix in Figures (3-73) to (3-77).

Table (3-5): FT-IR spectral data for the compounds (24-25 and 29-31)

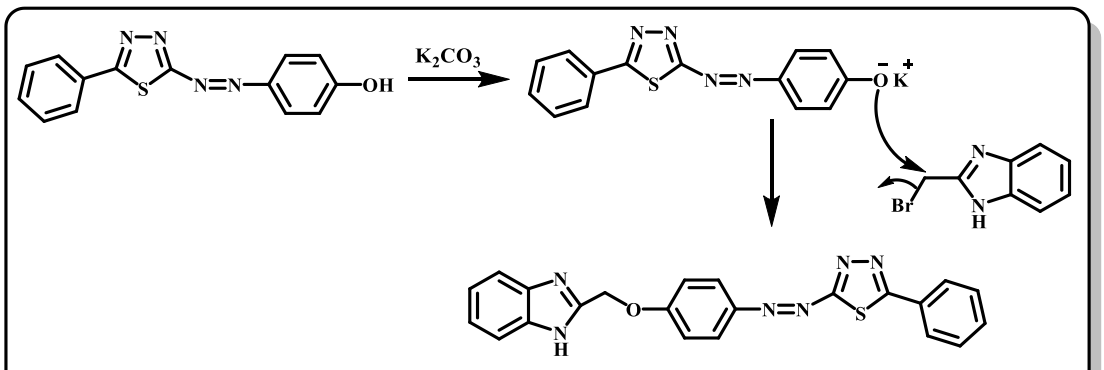
Comp. No.	Characteristic stretching bands of FT-IR (cm^{-1}, KBr disc)						
	O-H	Ar.C-H	C=N	N=N	C=C aromatic		others
24	3441	3066	1649	1512	1593	1469	-
25	3415	3074	1645	1504	1591	1427	-
29	3404	3097	1633	1506	1598	1454	1552,1384 NO_2
30	3452	3095	1643	1494	1600	1467	1531,1386 NO_2
31	3446	3099	1631	1504	1598	1471	1544,1348 NO_2

3.2.5. Synthesis and characterization 2-((4-((1H-benzo[d]imidazol-2-yl) methoxy)phenyl) diazenyl)-5-substitutedphenyl-1,3,4-thiadiazole (32-39):

Compounds (32-39) were synthesized by the reaction of compounds (24-31) with Compound (1) in the presence of K_2CO_3 in acetone as shown in Scheme (3-9) and mechanism in Scheme (3-10).



Scheme (3-9)



Scheme (3-10): Mechanism of synthesis of compoundS (32-39)

The obtained compounds were identified by TLC, (FTIR, ^{1}H and ^{13}C-NMR) spectra of some compounds, FT-IR spectrum of all these compounds showed absence the stretching of O-H and showed sharp band for stretching of N-H benzimidazole and two bands of C-O-C stretching near (1089-1020) cm^{-1} and (1281-1211) cm^{-1144} as shown in the following figures:

3.2.5.1. Spectral analysis of compound (34):

FT-IR spectrum for compound (34) showed a sharp band at (3276 cm^{-1}) accounted for N-H stretching of benzimidazole and band related to C-H aromatic at (3050 cm^{-1}) and bands related to stretching of aliphatic CH_2 at (2932 and 2844) cm^{-1} and band at (1642 cm^{-1}) and N=N stretching was appeared at (1505 cm^{-1}) and bands was appeared at (1584 and 1445) Cm^{-1} related to stretching of C=C aromatic and bands was appeared at (1281 and 1081) cm^{-1} accounted for C-O-C stretching as shown in Figure (3-25).

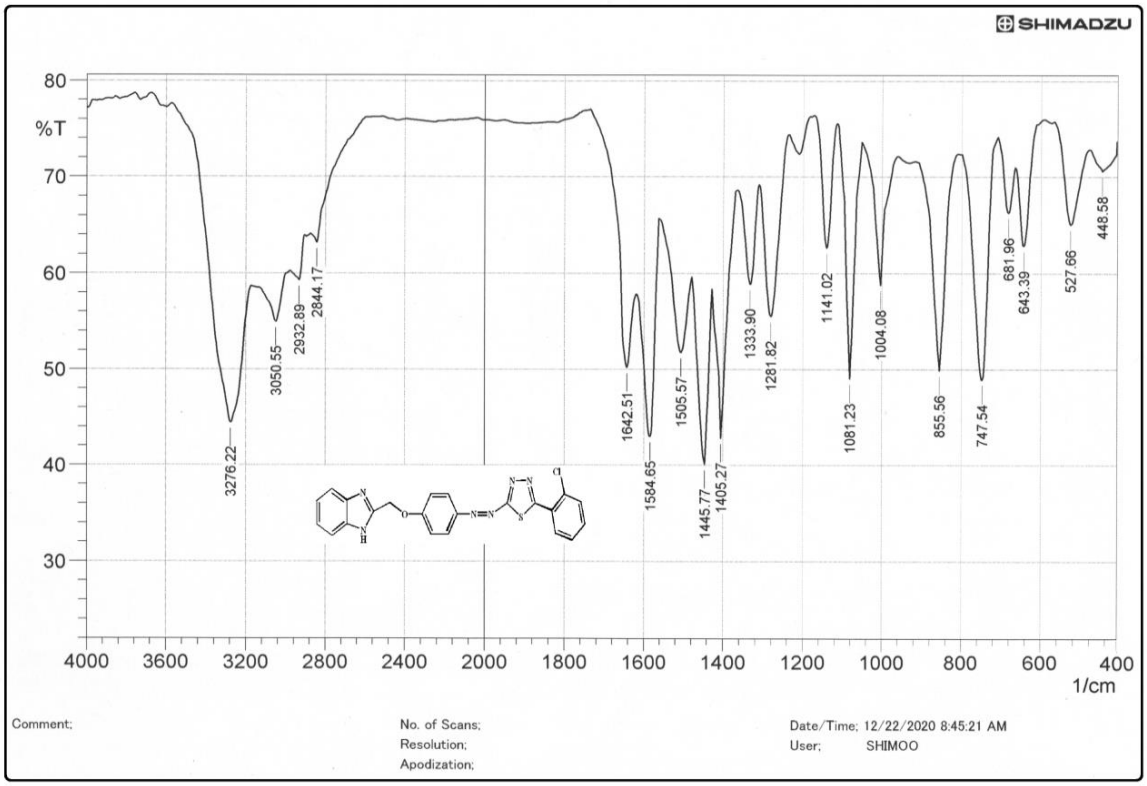


Figure (3-25): FT-IR spectrum of compound (34)

^{1}H-NMR spectrum for compound (34) showed singlet peak at (3.47 ppm) accounted for 2H of aliphatic CH_2 and multiplet between (7.14-8.08 ppm) related to 12H of aromatic rings and singlet peak at (10.49 ppm) related to N-H of benzimidazole as shown in Figure (3-26).

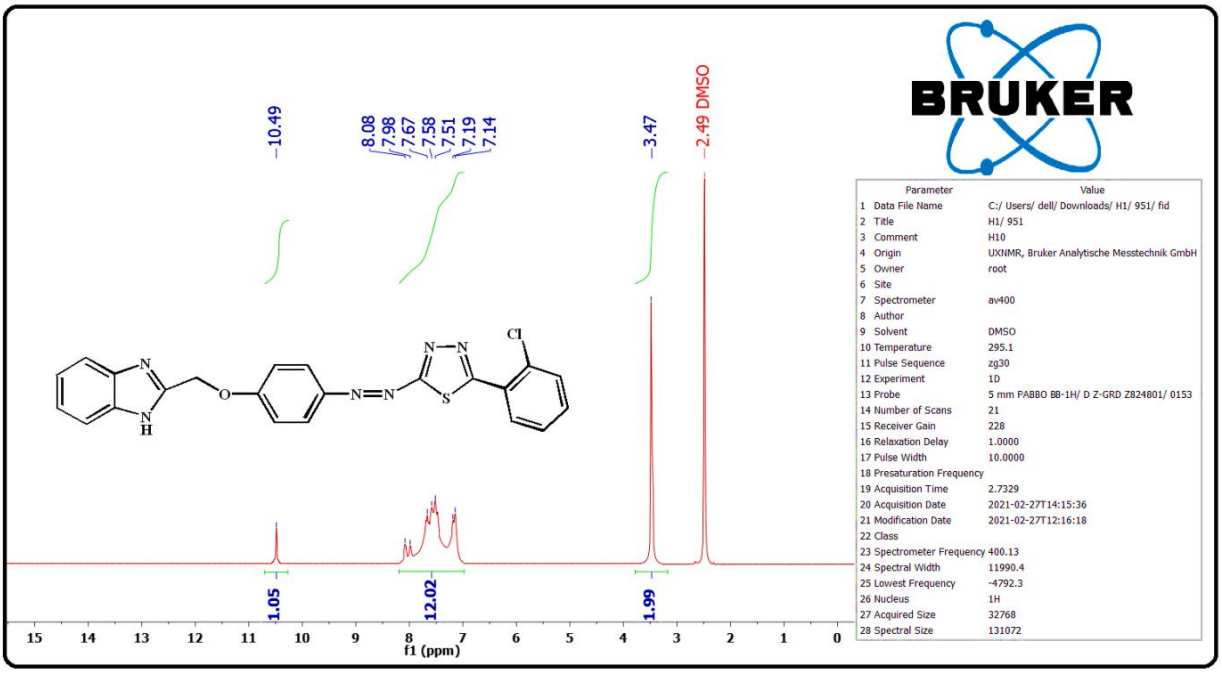
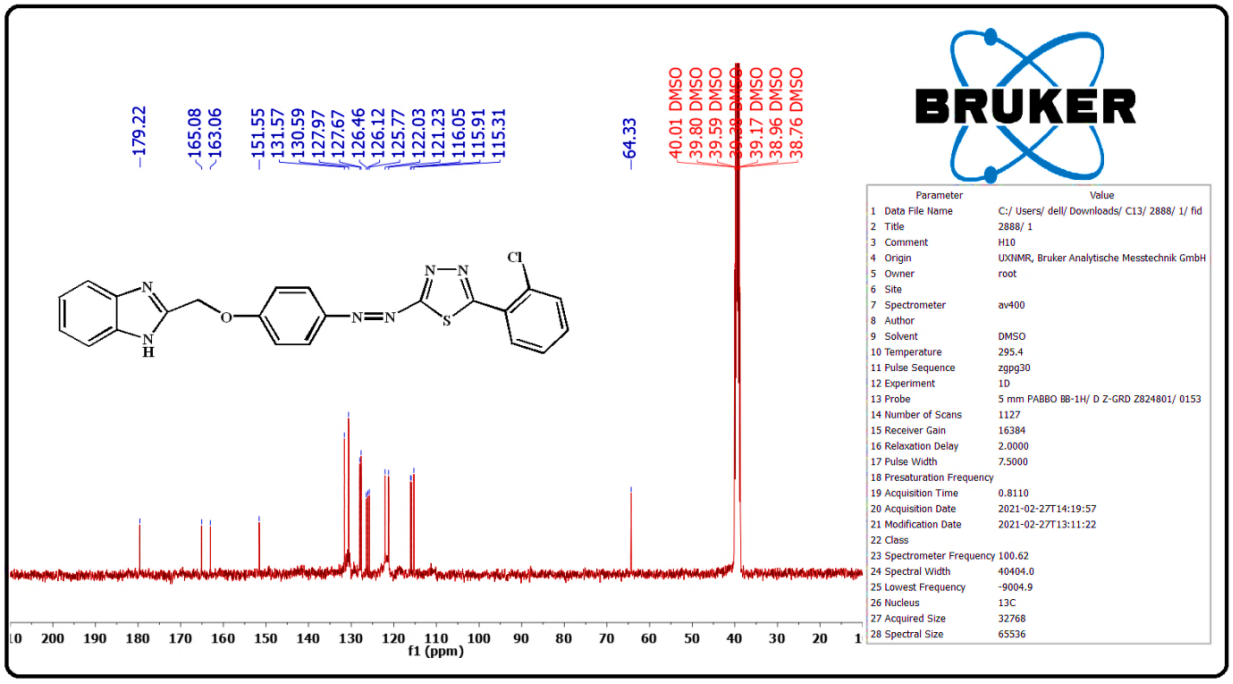


Figure (3-26): ^{1}H-NMR spectrum of compound (34)

^{13}C-NMR spectrum for compound (34) showed peak at (64.33 ppm) related to aliphatic CH_2 and peaks between (115.31-131.57ppm) related to carbons of aromatic rings and peak at (165.08 ppm) accounted for aromatic C-O and singlet peak at (151.55 ppm) related to C=N of benzimidazole and peaks at (163.27 and 179.22 ppm) related to C=N of 1,3,4-thiadiazole as shown in Figure (3-27).



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22 Class	
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Figure (3-27): ^{13}C-NMR spectrum of compound (34)

3.2.5.2. Spectral analysis of compound (35):

FT-IR spectrum for compound (35) showed a sharp band at (3386 cm^{-1}) accounted for N-H stretching of benzimidazole and one band related to C-H aromatic in (3060 cm^{-1}) and two bands related to stretching of aliphatic CH_2 at (2933 and 2850 cm^{-1}) and band at (1635 cm^{-1}) and one band for N=N stretching was appeared at (1506 cm^{-1}) belong to C=N and bands near (1589 and 1452) cm^{-1} related to stretching of C=C aromatic and two bands was appeared at (1218 and 1020) cm^{-1} accounted for C-O-C stretching as shown in Figure (3-28).

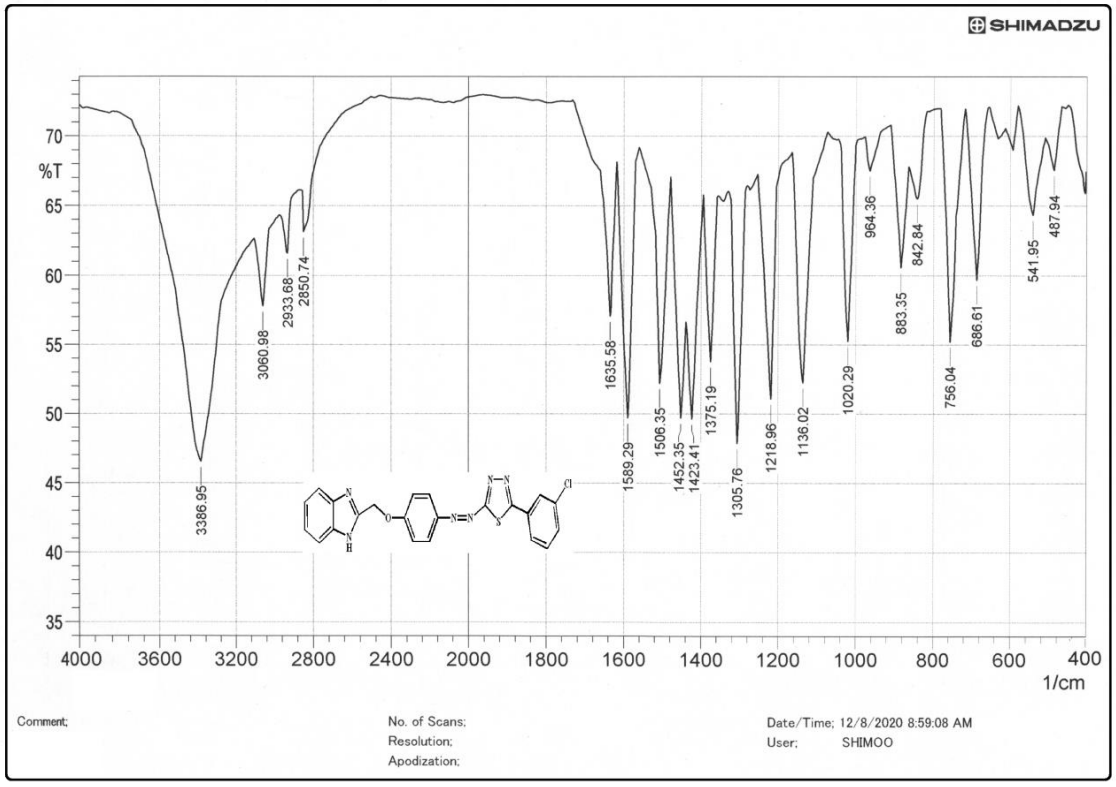


Figure (3-28): FT-IR spectrum of compound (35)

^{1}H-NMR spectrum for compound (35) showed singlet peak at (3.53 ppm) related to 2H of aliphatic CH_2 and multiplet peaks between (6.91-8.13) ppm belong to 12H of aromatic rings and singlet peak at (10.49 ppm) related to N-H of benzimidazole as shown in Figure (3-29).

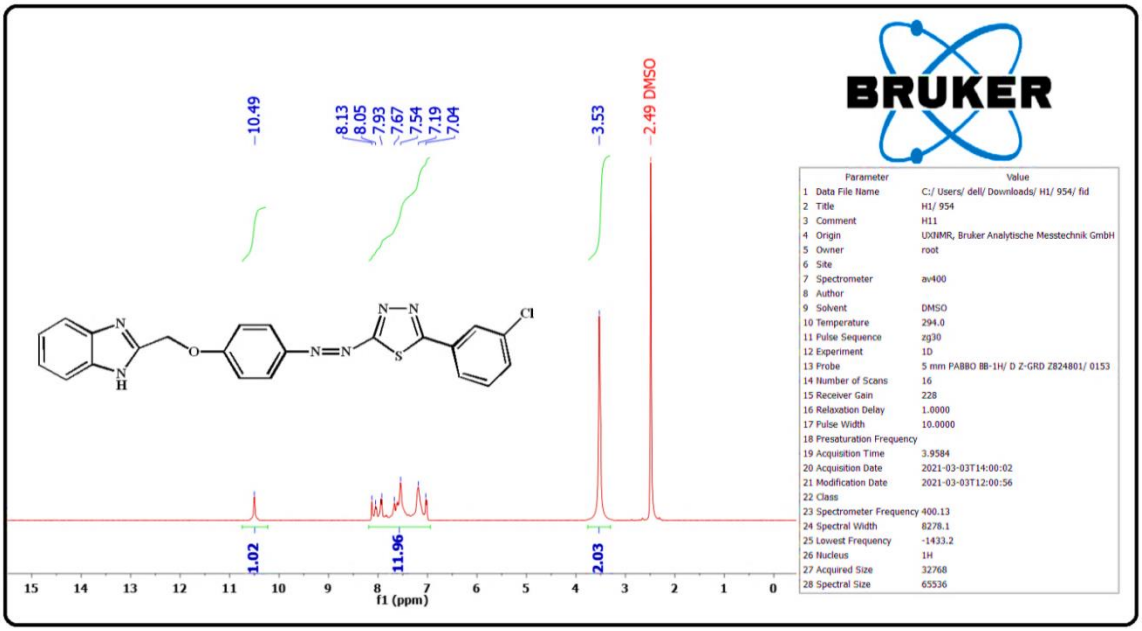
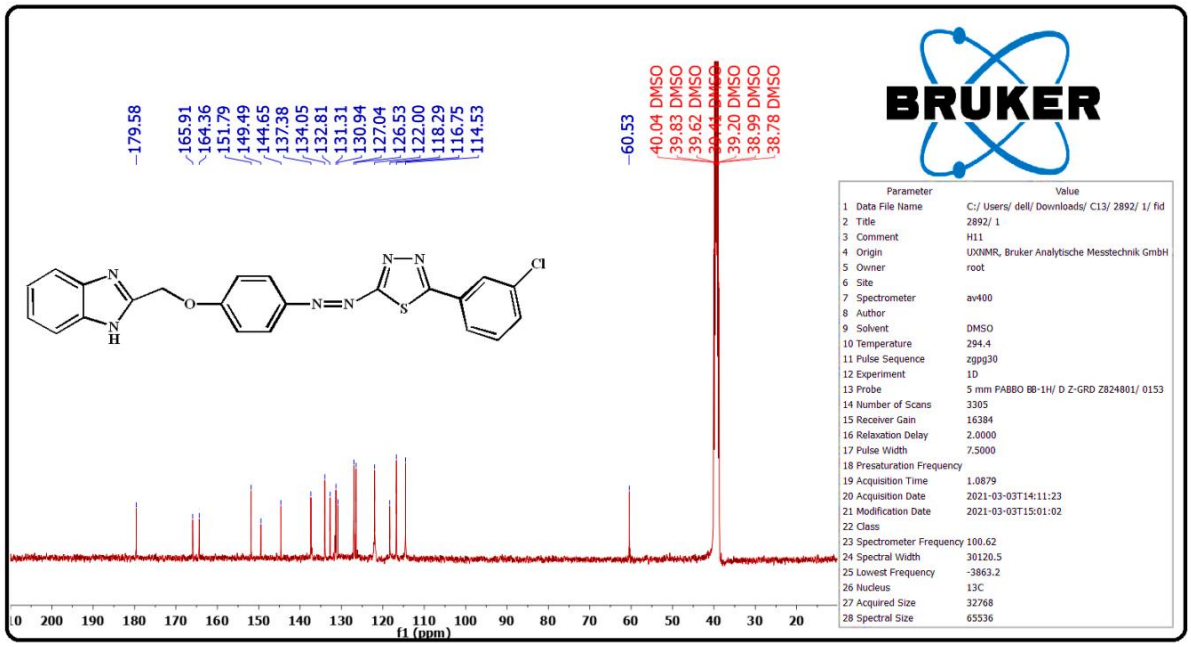


Figure (3-29): ^{1}H-NMR spectrum of compound (35)

^{13}C-NMR spectrum for compound (35) showed peak at (60.53pm) related to aliphatic CH_2 and peaks between (114.53-149.49 ppm) accounted for carbons of aromatic rings and peak at (165.91ppm) accounted for aromatic C-O and peak in (151.79 ppm) accounted for C=N of benzimidazole and peaks at (164.36 and 179.58 ppm) related to C=N of 1,3,4-thiadiazole as shown in Figure (3-30).



Parameter	Value
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2 Title	2892/ 1
3 Comment	H11
4 Origin	UXNMR, Bruker Analytische Messtechnik GmbH
5 Owner	root
6 Site	
7 Spectrometer	av400
8 Author	
9 Solvent	DMSO
10 Temperature	294.4
11 Pulse Sequence	zgpg30
12 Experiment	1D
13 Probe	5 mm PABBO BB-1H/ D Z-GRD Z824801/ 0153
14 Number of Scans	3305
15 Receiver Gain	16384
16 Relaxation Delay	2.0000
17 Pulse Width	7.5000
18 Presaturation Frequency	
19 Acquisition Time	1.0879
20 Acquisition Date	2021-03-03T14:11:23
21 Modification Date	2021-03-03T15:01:02
22 Class	
23 Spectrometer Frequency	100.62
24 Spectral Width	30120.5
25 Lowest Frequency	-3863.2
26 Nucleus	13C
27 Acquired Size	32768
28 Spectral Size	65536

Figure (3-30): ^{13}C-NMR spectrum of compound (35)

3.2.5.3. Spectral analysis of compound (36):

FT-IR spectrum for compound (36) showed a sharp band at (3415 cm^{-1}) accounted for N-H stretching of benzimidazole and band related to C-H aromatic at (3066 cm^{-1}) and two bands related for stretching of aliphatic CH_2 at (2954 and 2835) cm^{-1} and band at (1625 cm^{-1}) belong to C=N and band for N=N stretching was appeared at (1502 cm^{-1}) and two bands near (1562 and 1462) cm^{-1} related to stretching of C=C aromatic and bands was appeared at (1269 and 1087cm^{-1}) accounted for C-O-C stretching as shown in Figure (3-31).

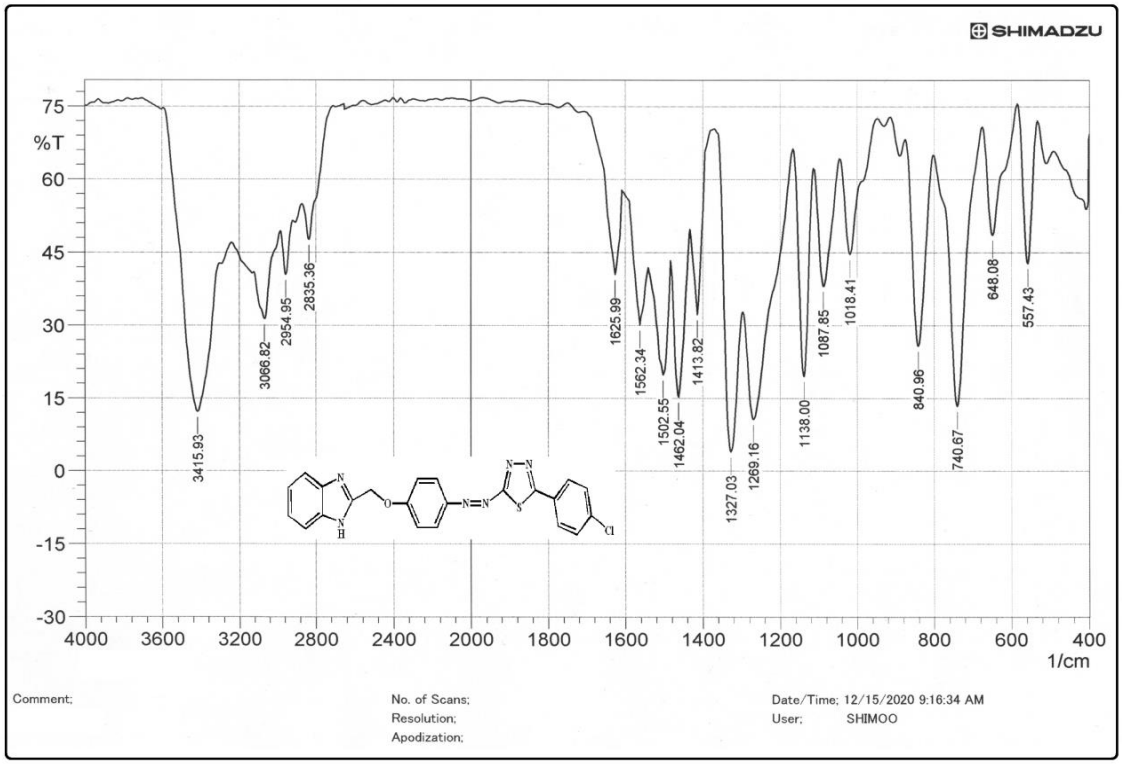


Figure (3-31): FT-IR spectrum of compound (36)

^{1}H-NMR spectrum for compound (36) showed singlet peak at (3.35 ppm) related to 2H of aliphatic CH_2 and multiplet peaks between (7.14-8.05 ppm) related to 12H of aromatic rings and singlet peak at (10.49 ppm) belong to N-H of benzimidazole as shown in Figure (3-32).

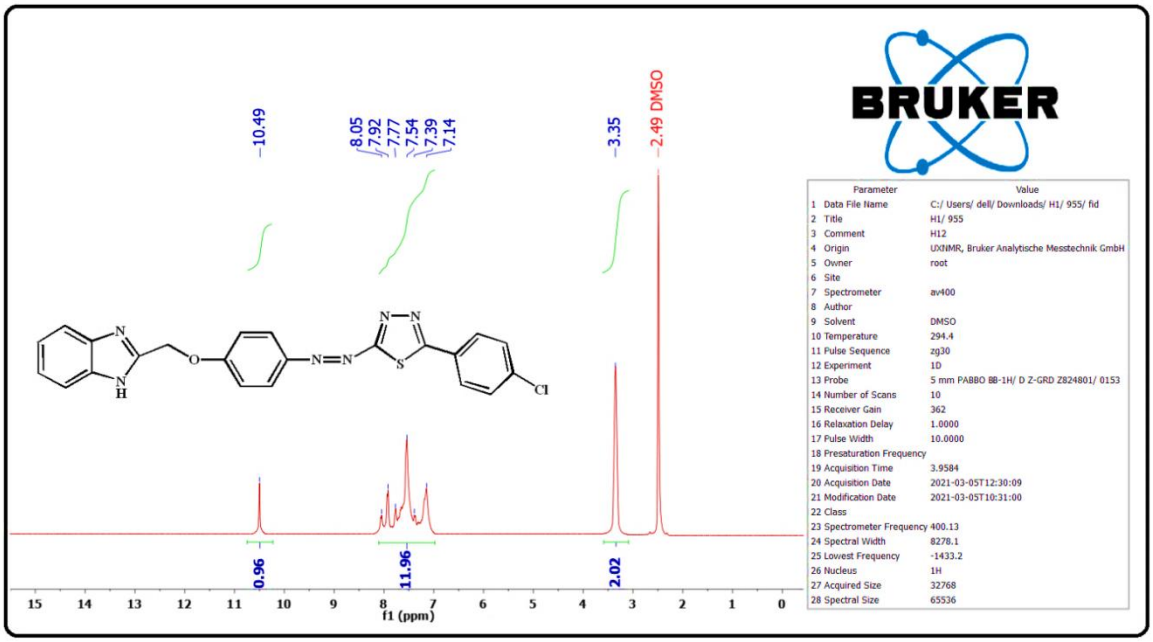


Figure (3-32): ^{1}H-NMR spectrum of compound (36)

^{13}C-NMR spectrum for compound (36) showed singlet peak at (64.28 ppm) related to aliphatic CH_2 and peaks between (115.81-148.11ppm) related to carbons of aromatic rings and peak at (167.39 ppm) accounted for aromatic C-O and singlet peak in (151.27 ppm) related to C=N of benzimidazole and two peaks at (159.47 and 169.32) ppm accounted for C=N of 1,3,4-thiadiazole as shown in Figure (3-33).

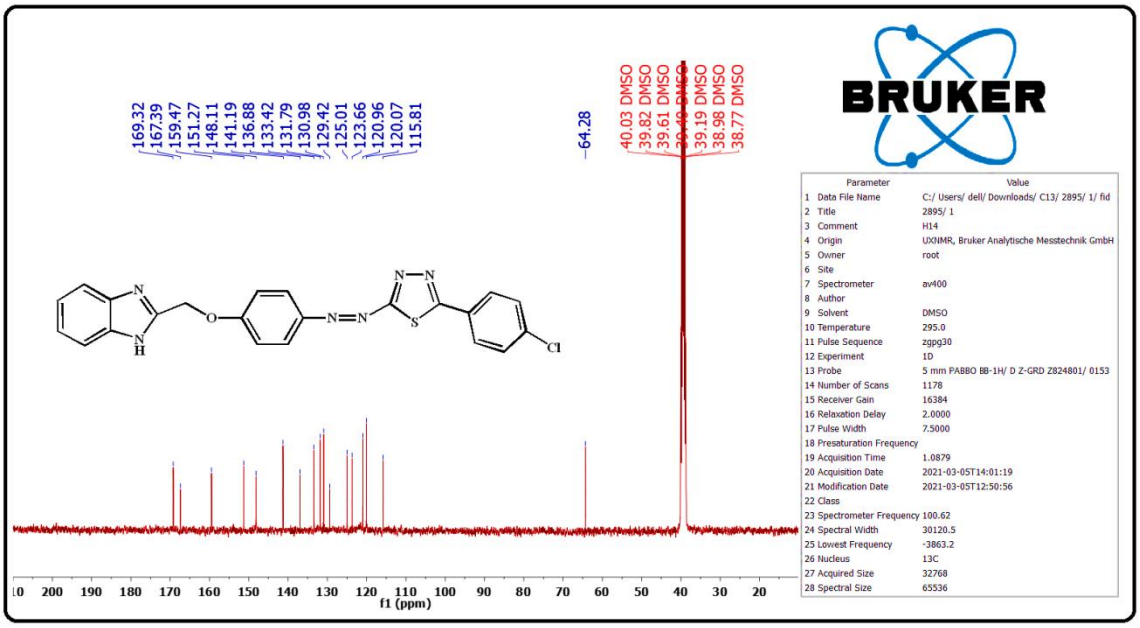


Figure (3-33): ^{13}C-NMR spectrum of compound (36)

The FT-IR, ^{1}H and ^{13}C-NMR spectra data for the compounds (32-33 and 37-39) and of some compounds spectral data were listed in table (3-6), (3-7) and (3-8) and the spectra were showed in appendix in Figures (3-78) to (3-90).

Table (3-6): FT-IR spectral data for the compounds (32-33 and 37-39)

Comp. No.	Characteristic stretching bands of FT-IR (cm^{-1}, KBr disc)										
	N-H (benzimidazole)	Ar. C-H	C-H aliph.		C=N	N=N	Ar.C=C		C-O-C		others
			Asy.	Sy.					Asy.	Sy.	
32	3408	3061	2941	2839	1643	1508	1598	1452	1217	1039	-
33	3197	3068	2943	2839	1635	1525	1597	1463	1269	1072	650 C-Br
37	3429	3078	2966	2843	1625	1502	1598	1460	1274	1012	1546,1340 NO_2
38	3319	3097	2958	2862	1635	1496	1598	1465	1211	1089	1537,1348 NO_2
39	3385	3080	2954	2852	1627	1596	1591	1452	1219	1062	1544,1348 NO_2

Table (3-7): ^{1}H-NMR spectral data for the compounds (37 and 39)

Comp. No.	Characteristic protons of ^{1}H-NMR (ppm)		
	CH_2	Ar. protons	N-H (benzimidazole)
32	3.58	7.17-8.00	10.51
37	3.88	6.64-8.30	10.47
38	3.47	7.15-8.24	10.49
39	3.34	7.12-8.88	10.47

Table (3-8): ^{13}C-NMR spectral data for the compounds (37 and 39)

Comp. No.	Characteristic carbons of ^{13}C-NMR (ppm)					
	CH_2	Ar. carbons	Ar. C-O	C=N (benzimidazole)	C=N (1,3,4-thiadaizole)	
32	69.52	121.25-134.64	162.02	151.13	156.77	173.24
37	60.77	116.05-142.44	173.60	148.14	161.63	181.87
38	64.82	120.07-148.16	169.32	151.27	161.80	181.52
39	64.33	115.31-151.11	169.74	152.83	155.64	184.10

3.3- Biological activity of synthesized compounds:

Biological activity as antibacterial agents of some synthesized compounds were tested against some identified and isolated bacteria, against two gram-positive bacterial isolates (Gr^{+ve}) (*S. aureus and S. epidermidis*) and two gram-negative bacterial isolates (Gr^{-ve}) (*P. aeruginosa and E. coli*), these bacteria were selected because of their importance in the medical field since it causes many diseases and its resistance to many chemical drug and antibiotic.

The results in table (3-1) showed the study of the antibacterial activity of some synthesized compounds in four concentrations (100, 50, 25, 12.5) mg/mL, and showed moderate to good activity as the antibacterial agents compared to standard antibiotic (Ceftazidime, Ceftriaxone, Clarithromycin, Amikacin, Chloramphenicol, Azetronam), the biological activity can be explaining in the following:

1. *Staphylococcus Aureus*:

Compounds (22 and 39) most active on this type of bacteria belong to nitro groups, the compounds (16, 17, 20, 21, 33, 34, 36, 37) gave moderate to good activity against the same bacteria compared to Clarithromycin and Amikacin.

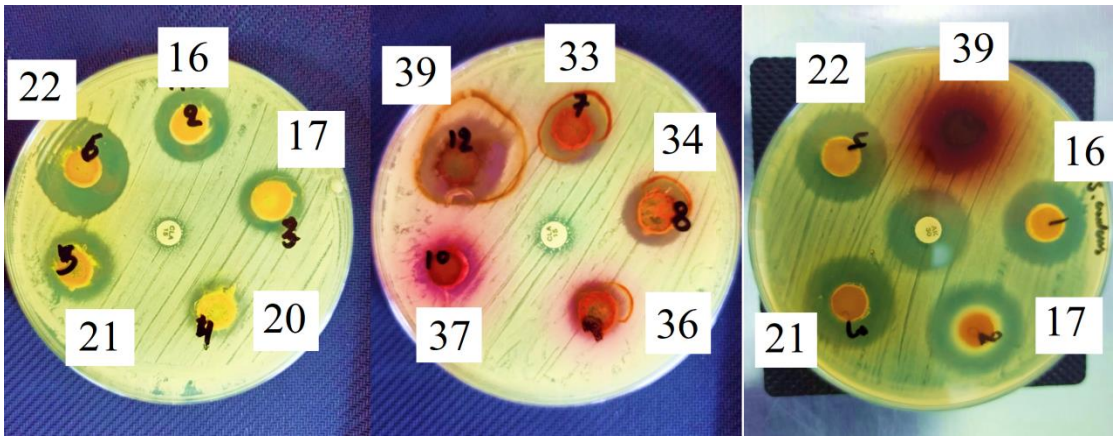


Figure (3-34): antibacterial activity against *S. Aureus* at 12.5 mg/ml

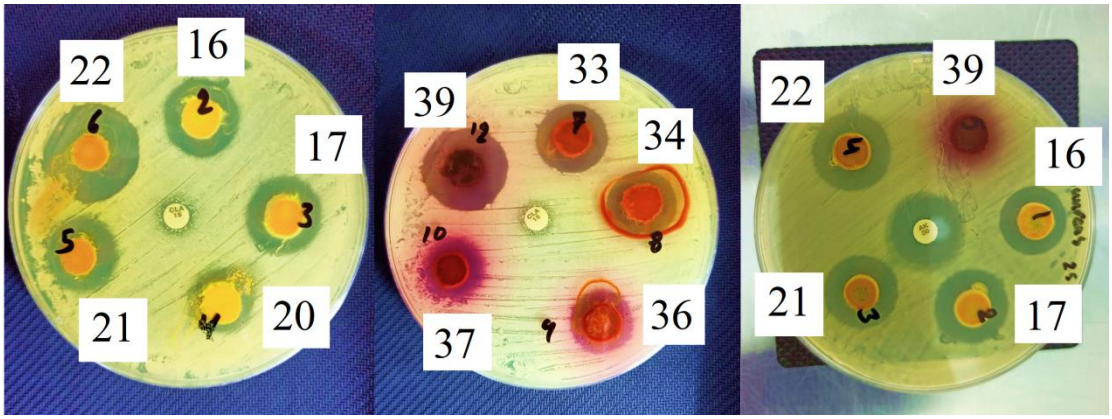


Figure (3-35): antibacterial activity against *S. Aureus* at 25 mg/ml

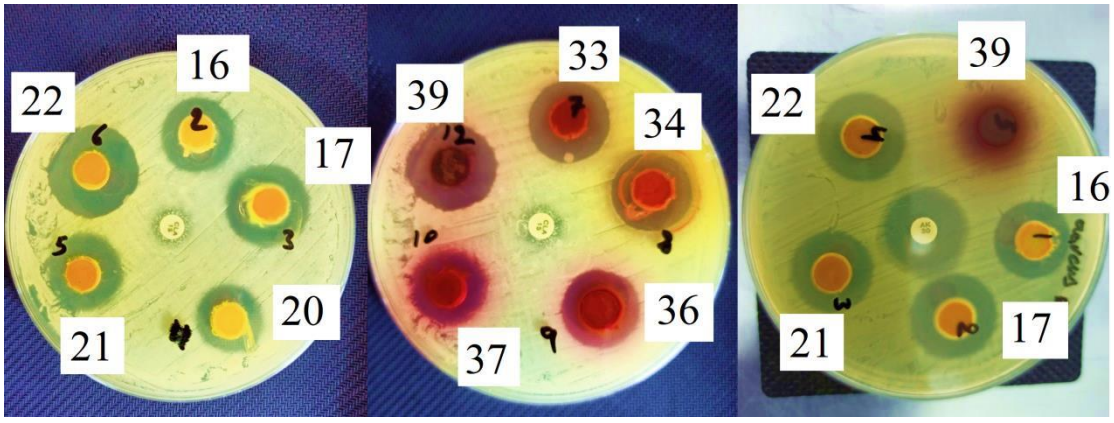


Figure (3-36): antibacterial activity against *S. Aureus* at 50 mg/ml

2- *Staphylococcus epidermidis*:

Compounds (21, 22) most active on this type of bacteria belong to nitro groups, the compounds (16, 17, 20, 33, 34, 36, 37, 39) gave moderate to good activity against the same bacteria compared to Clarithromycin and Amikacin.

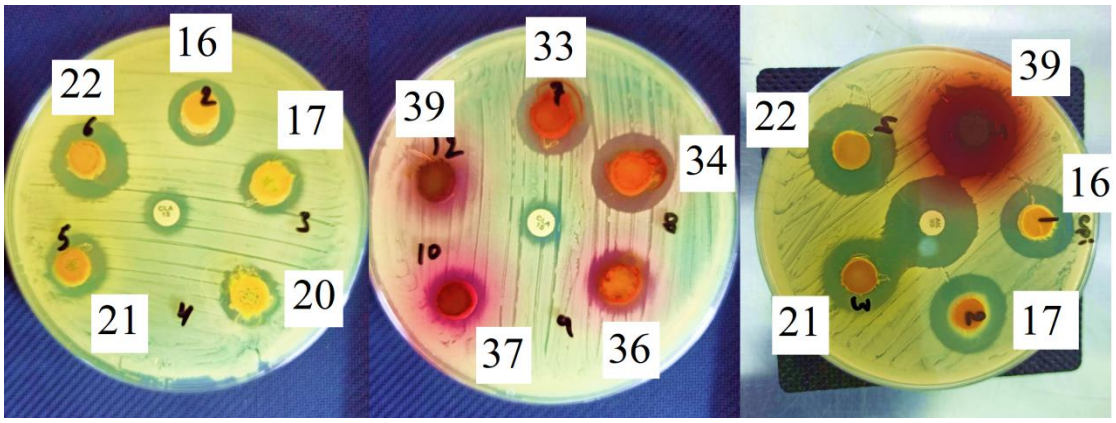


Figure (3-37): antibacterial activity against *S. epidermidis* at 12.5 mg/ml

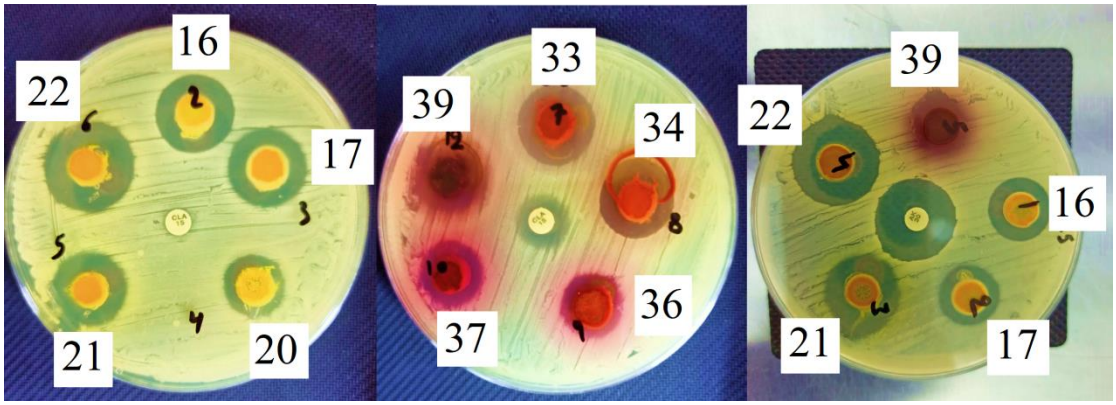


Figure (3-38): antibacterial activity against *S. epidermidis* at 25 mg/ml

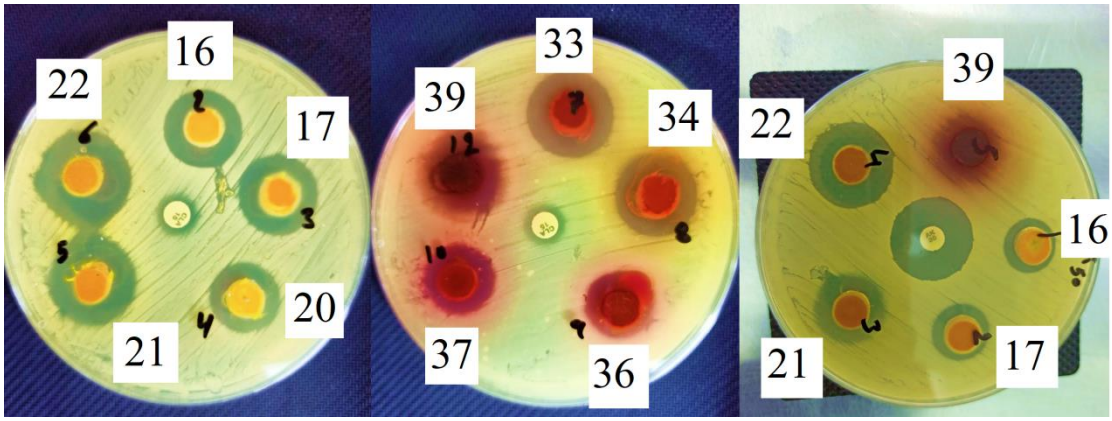


Figure (3-39): antibacterial activity against *S. epidermidis* at 50 mg/ml

3- *Pseudomonas aeruginosa*:

Compounds (16, 17, 20, 21) showed poor activity in 100 mg/mL on this type of bacteria and other concentrations didn't show any activity, the compounds (22, 33, 34, 36, 37, 39) didn't show any activity against the same bacteria due to position and type of substituted group compared to Ceftazidime and Azetronam.

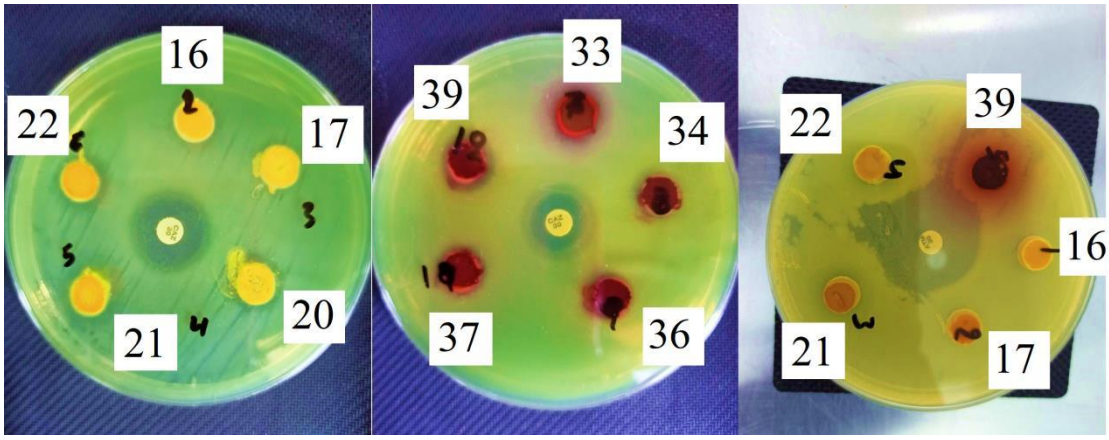


Figure (3-40): antibacterial activity against *P. aeruginosa* at 12.5 mg/ml

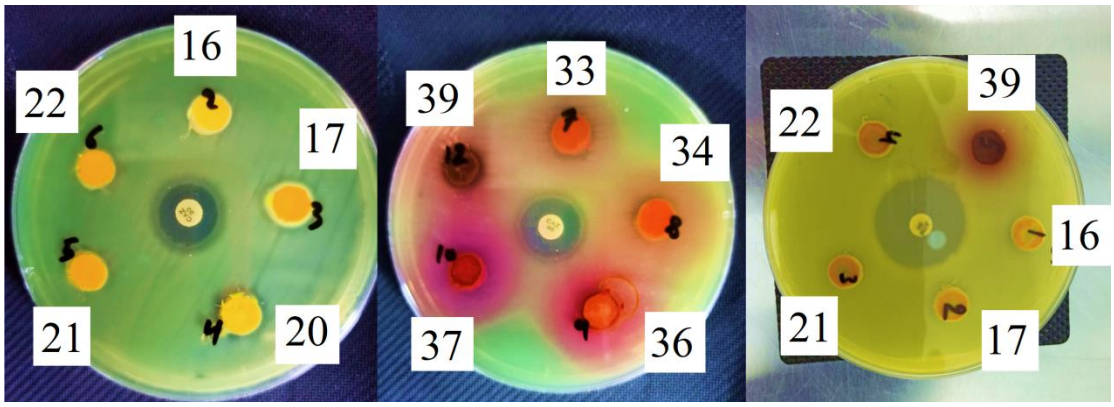


Figure (3-41): antibacterial activity against *P. aeruginosa* at 25 mg/ml

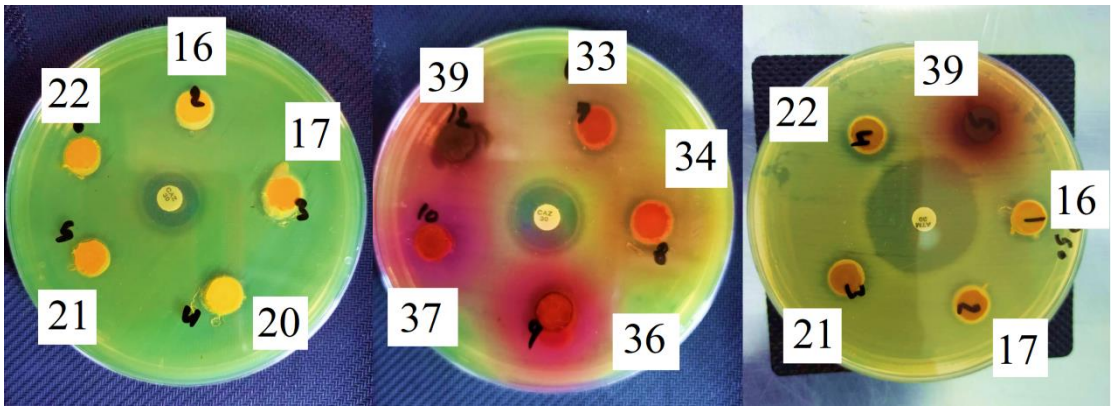


Figure (3-42): antibacterial activity against *P. aeruginosa* at 50 mg/ml

4- *Escherichia coli*:

Compounds (16, 17, 22) most active on this type of bacteria belong to bromide of (16), chloride of (17) and nitro group of (22), (20, 21) showed medium to good activity, the compounds (33, 34, 36, 37, 39) showed good activity in (100) mg/mL and other concentrations didn't show any activity due to position and type of substituted group compared to Ceftriaxone and Chloramphenicol.

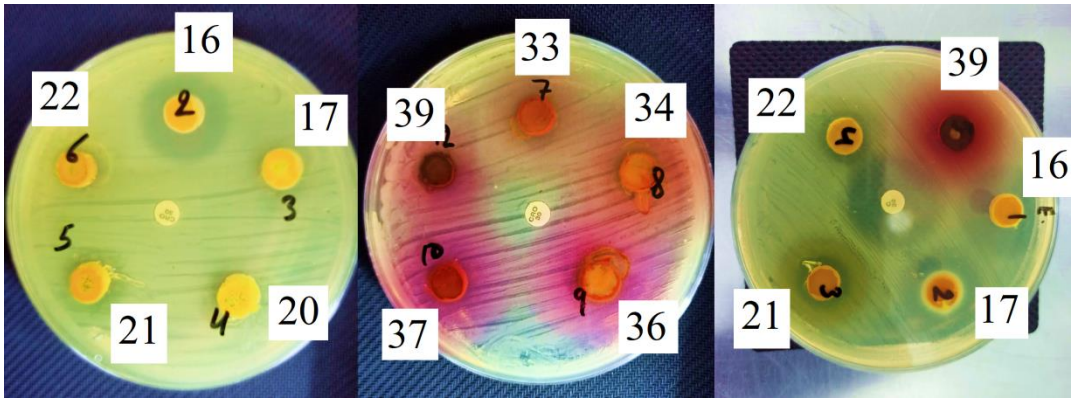


Figure (3-43): antibacterial activity against *E. coli* at 12.5 mg/ml

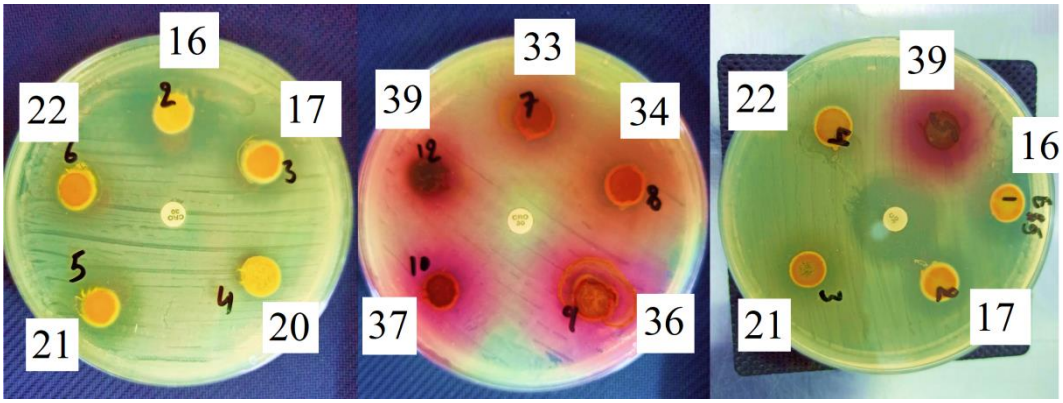


Figure (3-44): antibacterial activity against *E. coli* at 25 mg/ml

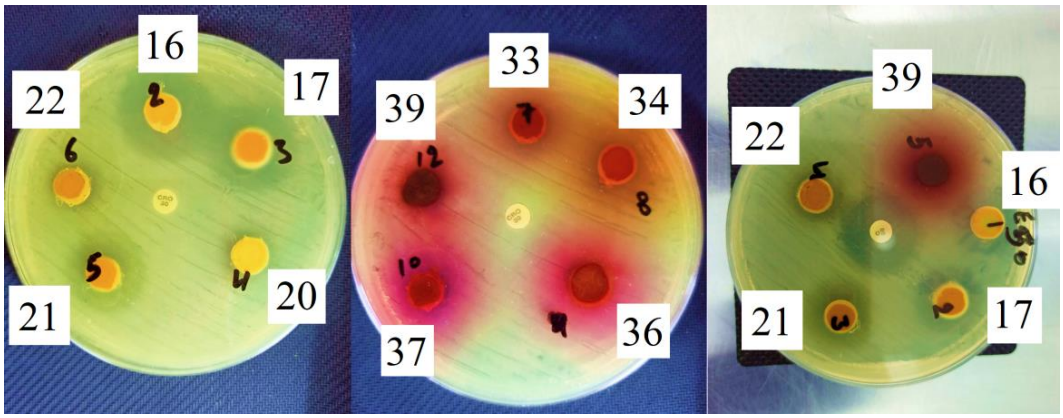
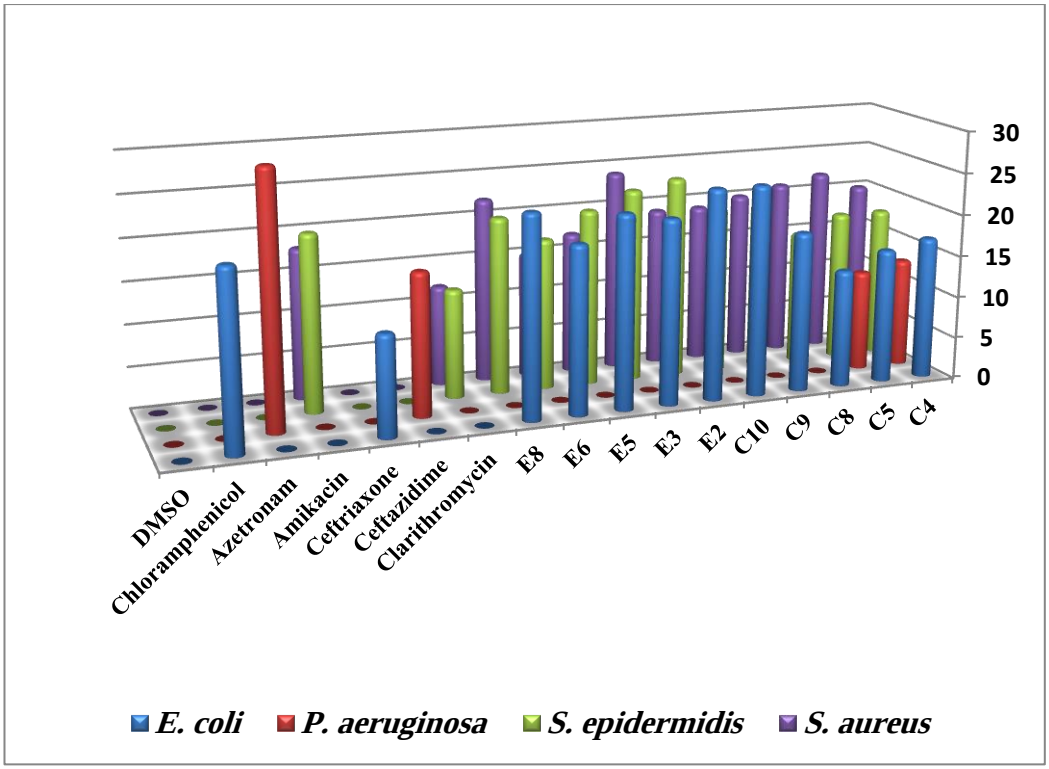


Figure (3-45): antibacterial activity against *E. coli* at 50 mg/ml

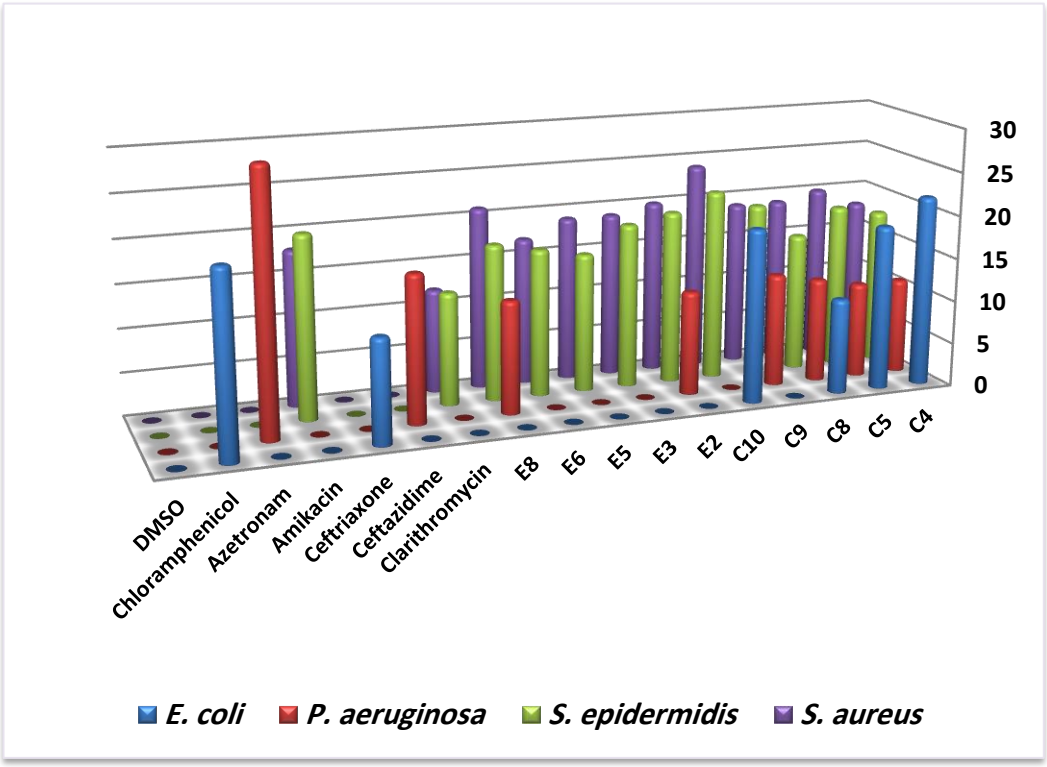
Table (3-9): Biological values of some synthesized compounds against four bacteria

Comp. No.	Concentration (mg/mL)	Zone of inhibition (in mm)			
		Gram-positive		Gram-negative	
		S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. coli*
16	100	20	18	13	17
	50	18	18	11	22
	25	18	19	0	22
	12.5	18	17	11	21
17	100	22	18	12	16
	50	20	19	11	19
	25	20	19	0	18
	12.5	15	15	11	0
20	100	21	16	0	14
	50	19	16	12	11
	25	16	15	11	0
	12.5	14	14	13	0
21	100	20	20	0	19
	50	19	20	13	0
	25	18	18	12	17
	12.5	17	16	15	0
22	100	17	20	0	22
	50	24	22	0	20
	25	24	23	0	15
	12.5	24	20	0	0
33	100	19	24	0	25
	50	20	20	12	0
	25	19	19	0	0
	12.5	16	18	0	0
34	100	24	23	0	22

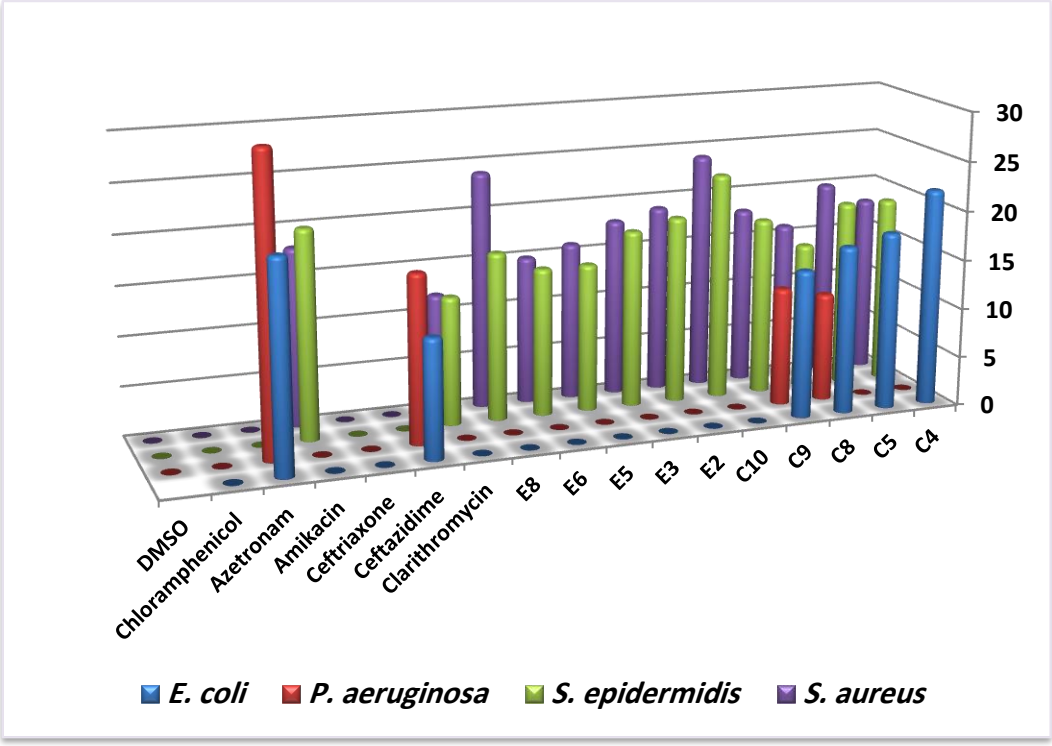
	50	19	19	0	0
	25	18	18	0	0
	12.5	16	19	0	0
36	100	17	21	0	23
	50	19	16	0	0
	25	16	15	0	0
	12.5	13	15	0	0
37	100	15	18	0	20
	50	17	17	0	0
	25	15	15	0	0
	12.5	12	0	0	0
39	100	22	21	0	24
	50	21	18	13	0
	25	24	17	0	0
	12.5	23	0	0	0
Clarithromycin	15	12	13	-	-
Ceftazidime	30	-	-	17	-
Ceftriaxone	30	-	-	-	12
Amikacin	30	18	21	-	-
Azetronam	30	-	-	30	-
Chloramphenicol	30	-	-	-	21
DMSO	-	0	0	0	0



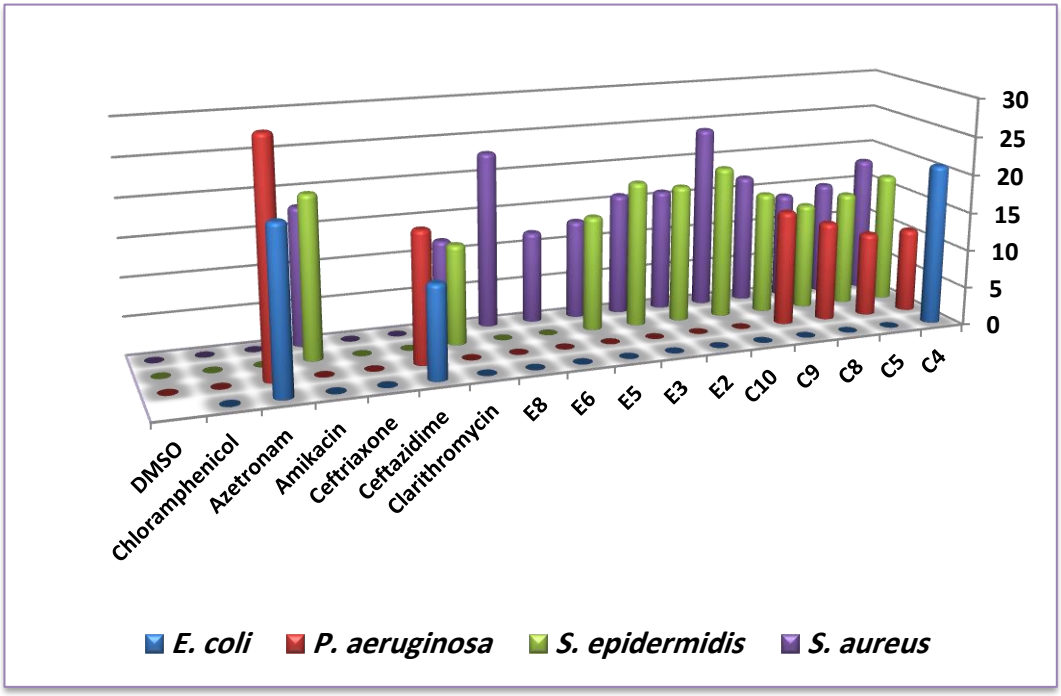
Graph (3-1): antibacterial activity of synthesized compounds at 100 mg/ml



Graph (3-2): antibacterial activity of synthesized compounds at 50 mg/ml



Graph (3-3): antibacterial activity of synthesized compounds at 25 mg/ml



Graph (3-4): antibacterial activity of synthesized compounds at 12.5 mg/ml

3.4. Molecular docking study:

Molecular docking studies have been performed on eubacterial ribosomal decoding A site (*Escherichia coli* 16S rRNA A site) to rationalize the probable mode of action binding affinity, and orientation of the molecules at the active site of receptor[150].

The molecular docking study was carried out using (Auto Dock Vina and PyMOL) on X-ray structure of eubacterial ribosomal decoding A. site (*Escherichia coli* 16S rRNA A. site) receptor domain (1j7t; pdb) was used as starting model for this study. The protein was prepared and docked using standard procedures.

Molecular docking is a powerful tool in drug design, which could predict the best mode by which a given compound fits well into a binding site of a macromolecular target (12). With results *in vitro* antimicrobial in hand, we thought it is worthwhile to perform *in silico* studies to support the results.

Bacterial protein synthesis, one of the several targets, has been the most successful one for developing effective antibacterial agents and has been used for efficient structure-based interventions against antibiotic resistance. Most of the naturally occurring clinically approved antibiotics including macrolides, tetracyclines, chloramphenicol and aminoglycosides target the ribosome[151]. These antibiotics bind to one functional sites in the ribosome and inhibit the process of protein synthesis by blocking the formation of peptide bond[152]. In bacterial protein synthesis, the mutational propensity of ribosome is low because of the redundancy of ribosomal RNA (rRNA) gene in bacterial genome and, therefore, it is an ideal target for antibacterial[153]. The 16S rRNA, a constituent of 30S subunit of bacterial 70S ribosome, is the site for mRNA binding and protein synthesis initiation and the molecules studied have been docked into the binding pocket of this16S rRNA[154]. Docking results were discussed on the parameters such as hydrogen bonds because they help to stabilize non-bonded π-π interactions, the π-π interactions are non-

covalent interactions which have pivotal role on protein-ligand parallel pose to generate hydrophobic contacts.

3.4.1. Molecular docking analysis of compound (21):

From table (3-10), it was clear that the compound (21) formed (5) close interatomic hydrogen bonds. The first, second and third resulted hydrogen bonds from interaction oxygen atoms of nitro group attached to benzene ring of compound (21) with hydrogen respectively, H_{42}, H_{41}, H_{41} atoms of nitrogen bases (Cyt-11), (Gau-33) and (Gau-32) with distance of bond respectively (2.2), (2.4) and (2.4) A^o. The fourth hydrogen bond resulted from interaction between nitrogen atom of 1,3,4-thiadiazole ring with hydrogen H_7 atom of nitrogen base (Ade-29) with distance of bond (2.6) A^o. The fifth hydrogen bond resulted from interaction between nitrogen atom of benzimidazole ring with hydrogen H_{42} atom of nitrogen base (Cyt-28) with distance of bond (2.1) A^o, as shown in Figure (3-46).

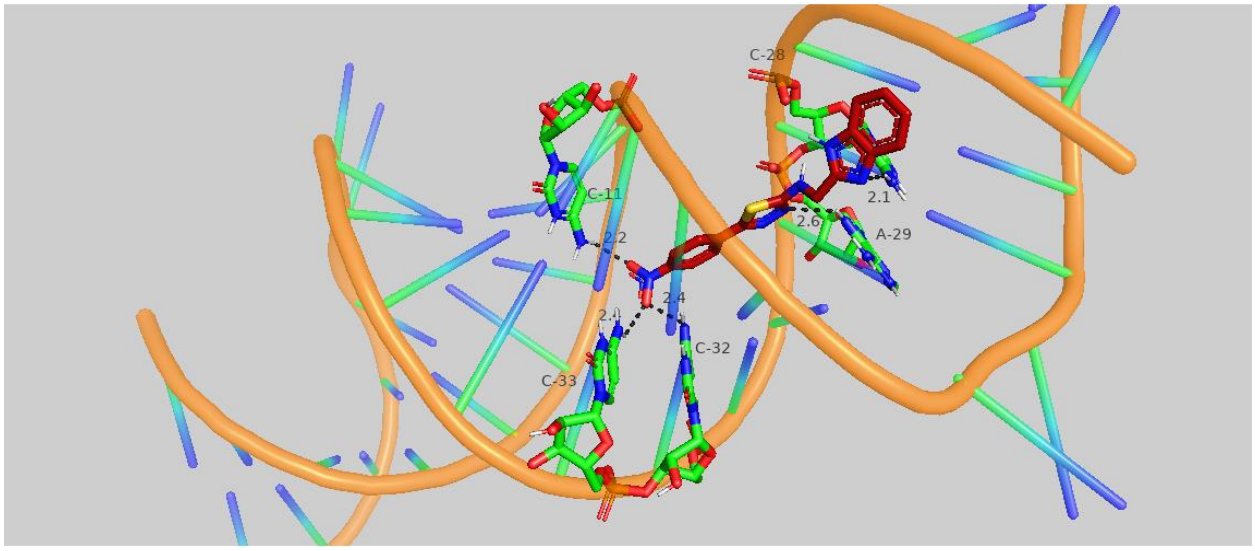


Figure (3-46): Docked conformation of compound (21) (red color) in the binding site of eubacterial ribosomal decoding A site (pdb:1j7t). Hydrogen bonds are shown by black dashed line.

3.4.2. Molecular docking analysis of compound (22):

From table (3-10), it was clear that the compound (22) formed (7) close interatomic hydrogen bonds (O⋯⋯H). The first and second hydrogen bonds resulted from interaction between hydrogen H_7 and H_{62} respectively of nitrogen base (Ade) with oxygen atoms of nitro groups connected to benzene ring of compound (22) with distance of bond (1.9) and (2.2) A^o respectively. The third hydrogen bond resulted from interaction between hydrogen H_{33} of nitrogen base (Cyt-33) with nitrogen atom of benzimidazole ring with distance of bond (2.6) A^o. The fourth and fifth hydrogen bonds resulted from interaction between oxygen atoms O_6 atoms of both nitrogen bases (Gau-12) and (Gau-13) with hydrogen of secondary amine group of compound (22) with distance of bond (1.9) and (2.6) A^o respectively. The sixth and seventh hydrogen bonds resulted from interaction between hydrogen H_7 of both nitrogen bases (Gau-12) and (Gau-13) with nitrogen atom of 1,3,4-thiadiazole ring with distance of bond (2.6) and(1.9) A^o respectively, as shown in Figure (3-47).

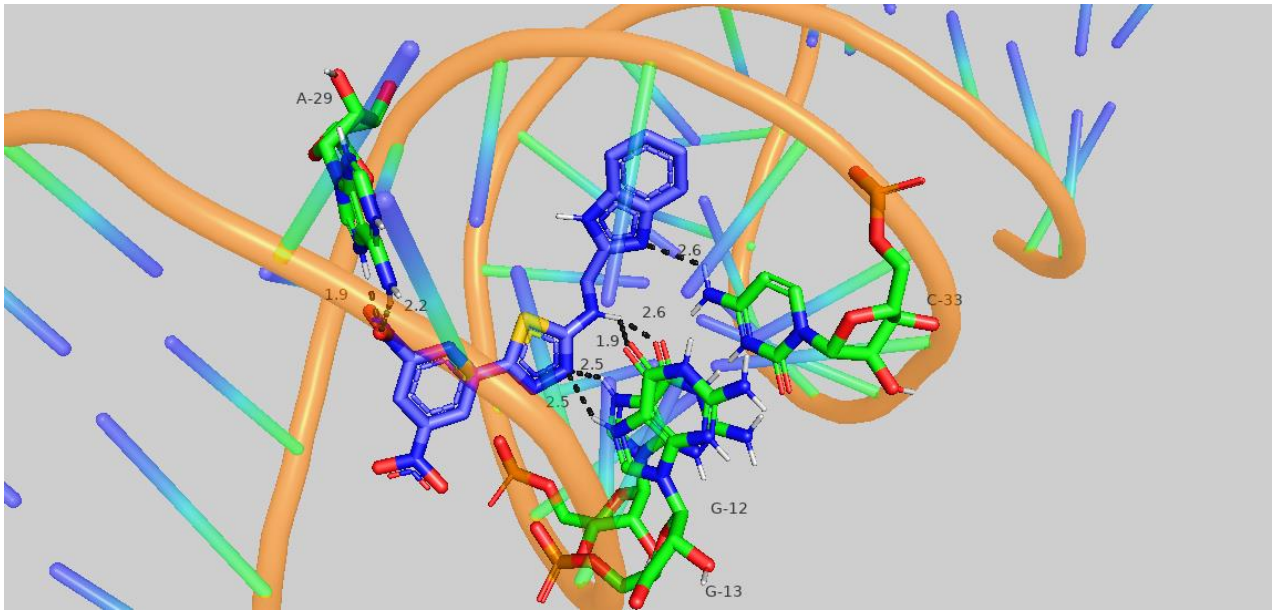


Figure (3-47): Docked conformation of compound (22) (blue color) in the binding site of eubacterial ribosomal decoding A site (pdb:1j7t). Hydrogen bonds are shown by black dashed line.

3.4.3. Molecular docking analysis of compound (39):

From table (3-10), it was clear that the compound (39) formed (6) close interatomic hydrogen bonds. The first hydrogen bond resulted from interaction between hydrogen of benzimidazole ring with OP2 of nitrogen base (Gau-12) with distance of bond (2.2) A^o. The second hydrogen bond resulted from interaction between oxygen of ether with hydrogen H_7 atom of nitrogen base (Gau-13) ring with distance of bond (2.3) A^o.

The third and fourth hydrogen bonds resulted from interaction between nitrogen N_1 atom of azo group with hydrogen H_{41} atom nitrogen base (Cyt-30) with distance of bond respectively (2.5) and (3.2) A^o.

The fifth and sixth hydrogen bonds resulted from interaction between nitrogen atoms of 1,3,4-thidiazole with hydrogen H_7 atom of nitrogen base (Ade-29) with distance of bond respectively (1.9) and (2.4) A^o as shown in Figure (3-48).

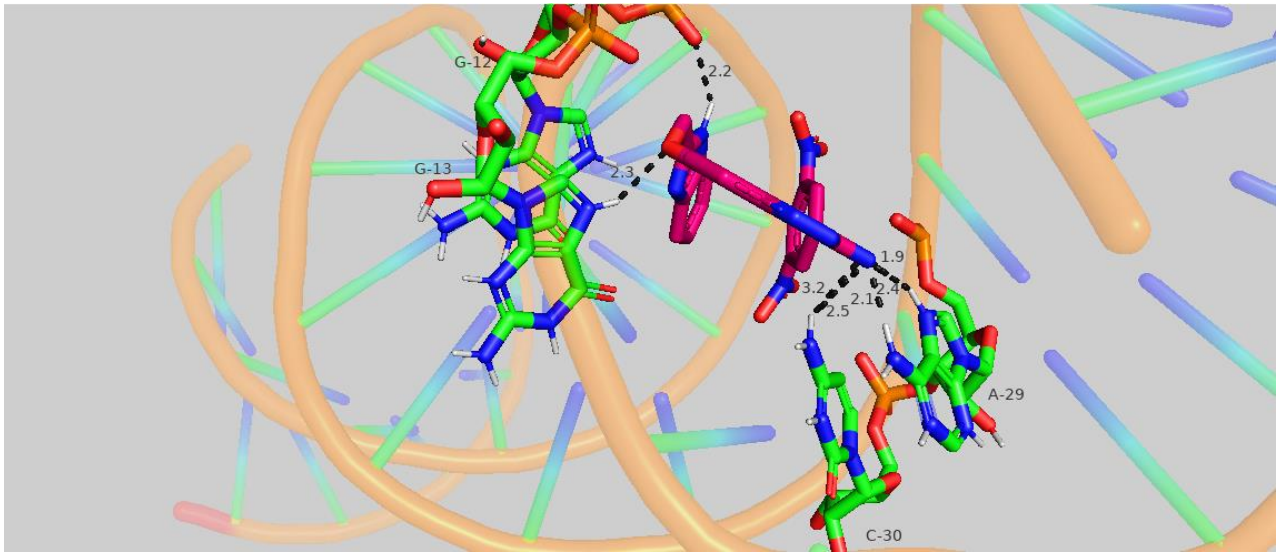


Figure (3-48): Docked conformation of compound (39) (red color) in the binding site of eubacterial ribosomal decoding A site (pdb:1j7t). Hydrogen bonds are shown by black dashed line.

Table (3-10): Molecular docking data of (22, 22 and 39)

Comp. No.	Position of H-B	Distance of bond (A^o)	π-π interactions
21	22: O (NO_2)-B(Cyt-11) : H_{42}	2.2	21-(A-29) ((B)
	21: O (NO_2)-B(Gau-33) H_{41}	2.4	21-(C-30) (B)
	21: O (NO_2)-B(Gau-32) H_{41}	2.4	21-(G-26)(B)
	21: N (1,3,4-thiadiazole)-B(Ade-29) H_7	2.6	21-(G-13) (A)
	21: N (benzimidazole)-B(Cyt-28) H_{42}	2.1	21-(A-31) (B)
			C-(U-27) (B)
22	22: O (NO_2)-B(Ade-29) H_7	1.9	22-C11 (A)
	22: O (NO_2)-B(Ade-29) H_{62}	2.2	22-C32 (B)
	22: N (benzimidazole)-B(Cyt-33) H_{33}	2.6	22-C28 (B)
	22: H (amine)-A(Gau-12): O_6	1.9	22-C31 (B)
	22: H (amine)-A(Gau-13): O_6	2.6	-
	22: N (1,3,4-thiadiazole)-A(Gau-12) H_7	2.5	-
	22: N (1,3,4-thiadiazole)-A(Gau-13): H_7	2.5	-
39	39: H (benzimidazole)-A(Gau-12):OP2	2.2	39-(C-30) (B)
	39: O (ether)-A(Gau-13): H_7	2.3	39-(C-28) (B)
	39:(azo)N_1-B(Cyt-30): H_{41}	2.5	39-(G-14) (A)
	39: N (1,3,4-thiadiazole)-B(Cyt-30): H_{41}	3.2	39-(U-13) (A)
	39: N (1,3,4-thiadiazole)-B(Ade-29):H_7	1.9	39-(C-11) (A)
	39: N_2 (azo)-B(Ade-29): H_7	2.4	-
ref	ref: O_{10}-A: Gua 13: OP2	3.1	-
	B: Cyt 25-OP2 - ref: O_{40}	2.9	-
	ref: N_{42}-B: Gua 26: O_6	2.8	-
	ref: O_{28}-B: Urd 27: OP2	2.7	-

H-B = Hydrogen bond; **ref=** Paromomycin (Antibiotic)

3.5. Conclusion:

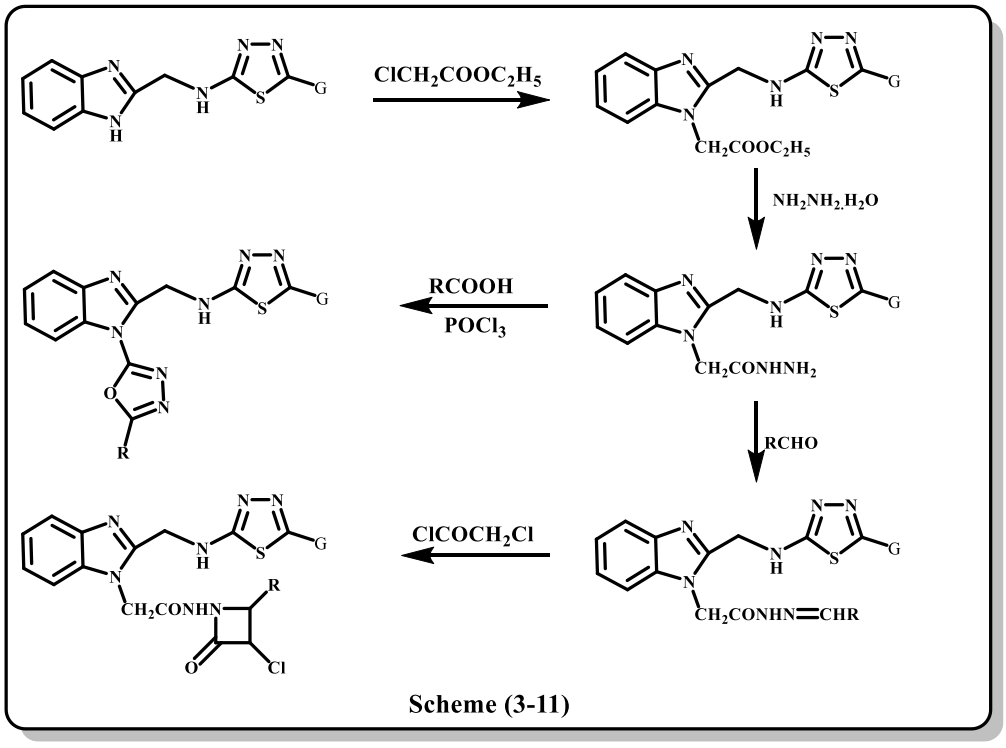
This work focused on the synthesis and characterization of new benzimidazole derivatives and study of their antibacterial activity of some synthesized compounds, conclusion can be explaining by the following:

1. A good yield was obtained of the synthesized compounds.
2. The synthesized compounds were characterized by (FT-IR, ^{1}H and ^{13}C-NMR) spectrums.
3. The antibacterial activity of some synthesized compounds was tested against four types of bacteria, two gram-positive (Ge^{+Ve}) (*Staphylococcus aureus and Staphylococcus epidermidis*) and two gram-negative (Ge^{-Ve}) (*Pseudomonas aeruginosa and Escherichia coli*), some tested compounds showed moderate to good activity, while, some compounds showed poor activity, and some compounds didn't show any activity against same types of bacteria.
4. The molecular docking was studied for most active compounds (21, 22 and 39), these compounds were showed good docking by made hydrogen bonds with nitrogen bases of (*Escherichia coli* 16S rRNA A site).

3.6. Future work:

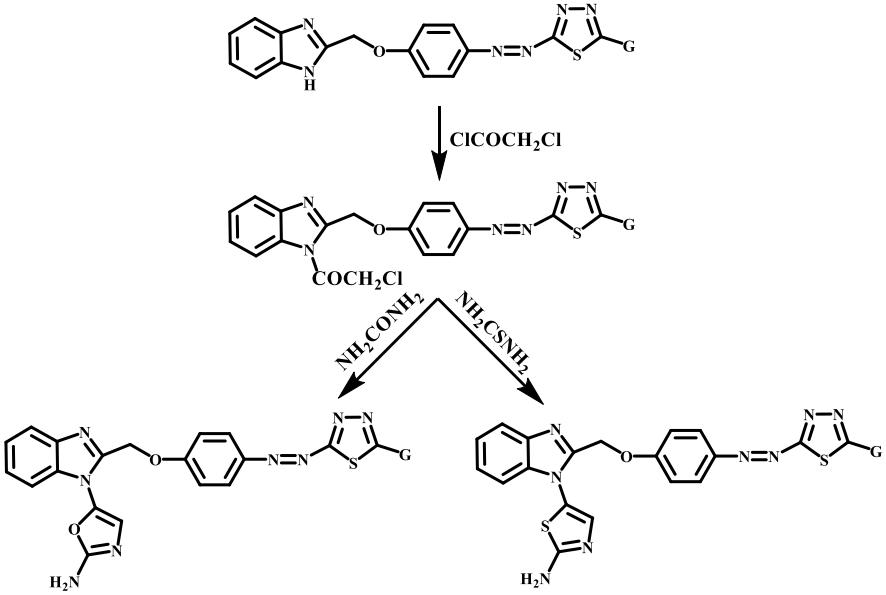
Based on the experience gained from the current study, we suggest the following:

1. Synthesis of novel benzimidazole derivatives by react of compounds (13-23) with ethyl chloroacetate, the next step include reaction of ester group with hydrazine, then react of the product with carboxylic acids to produce new 1,3,4-oxadiazole derivatives or react with aldehydes to produce Schiff bases as shown in Scheme (3-11).



Scheme (3-11)

2. Also new benzimidazole derivatives can prepare from the reaction of the compounds (32-39) with chloroacetyl chloride, and then follows by the reaction of the product with urea or thiourea as shown in Scheme (3-12).



Scheme (3-12)

Appendix

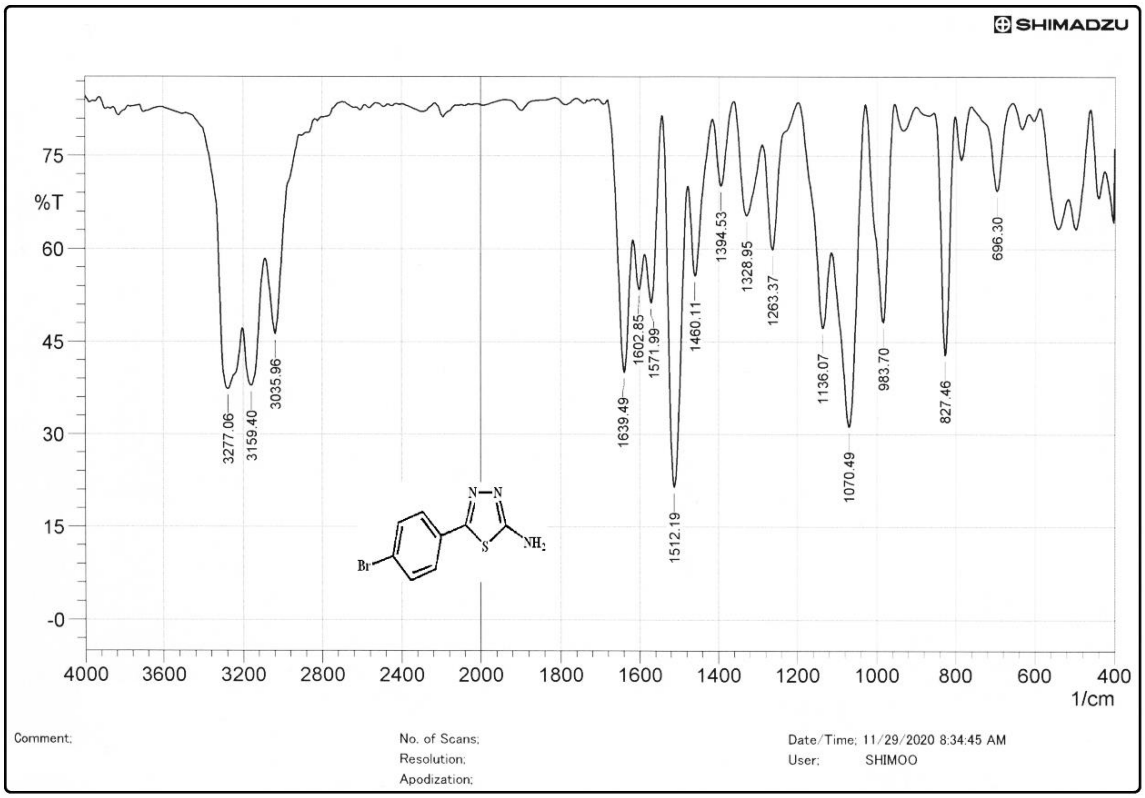


Figure (3-49): FT-IR spectrum of compound (5)

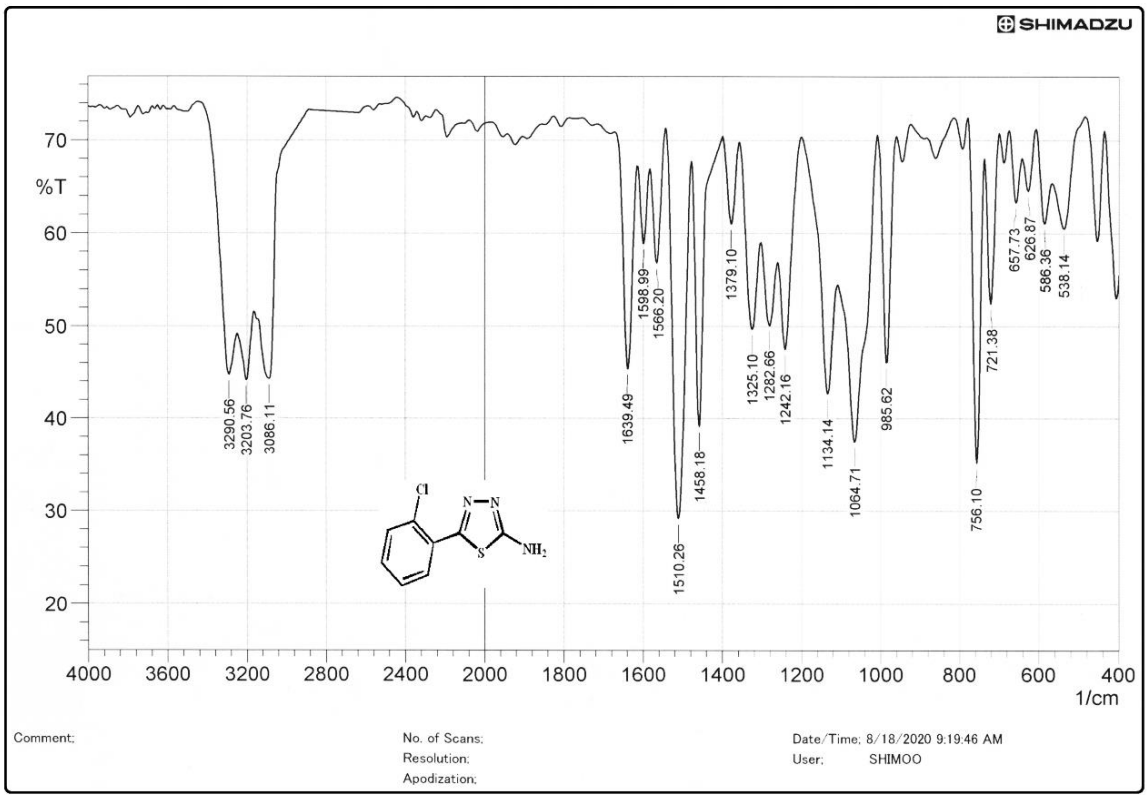


Figure (3-50): FT-IR spectrum of compound (6)

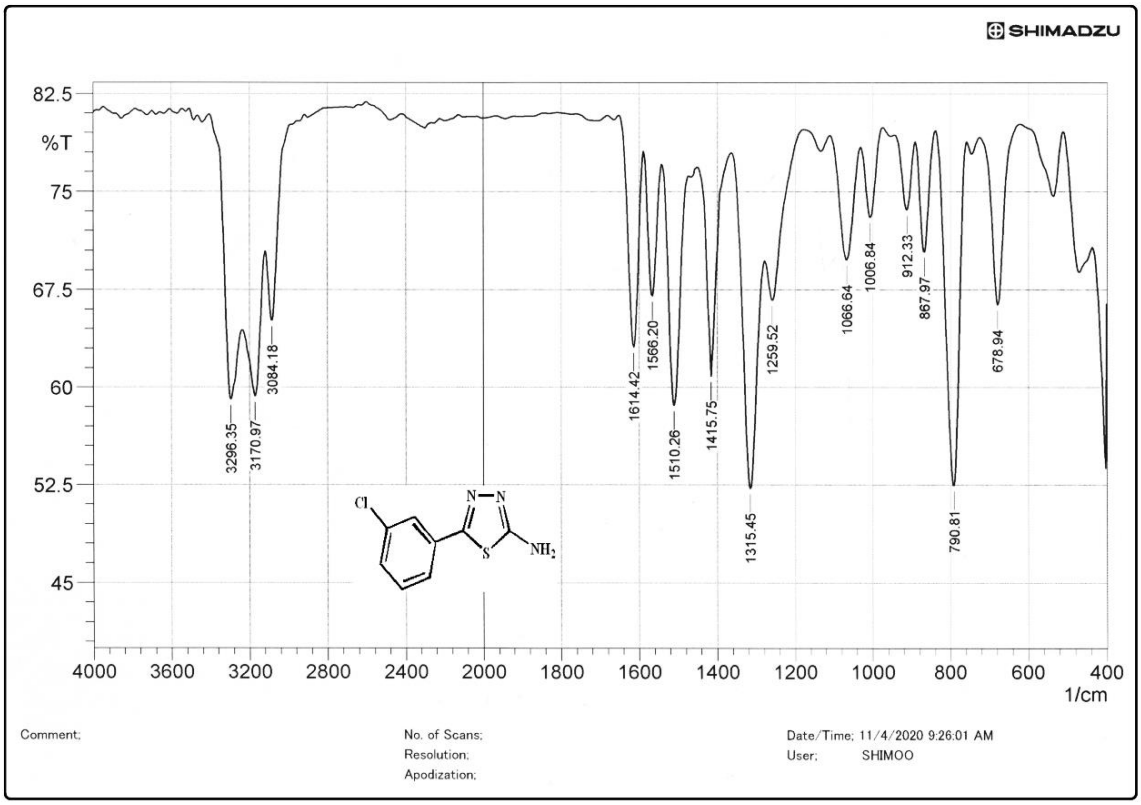


Figure (3-51): FT-IR spectrum of compound (7)

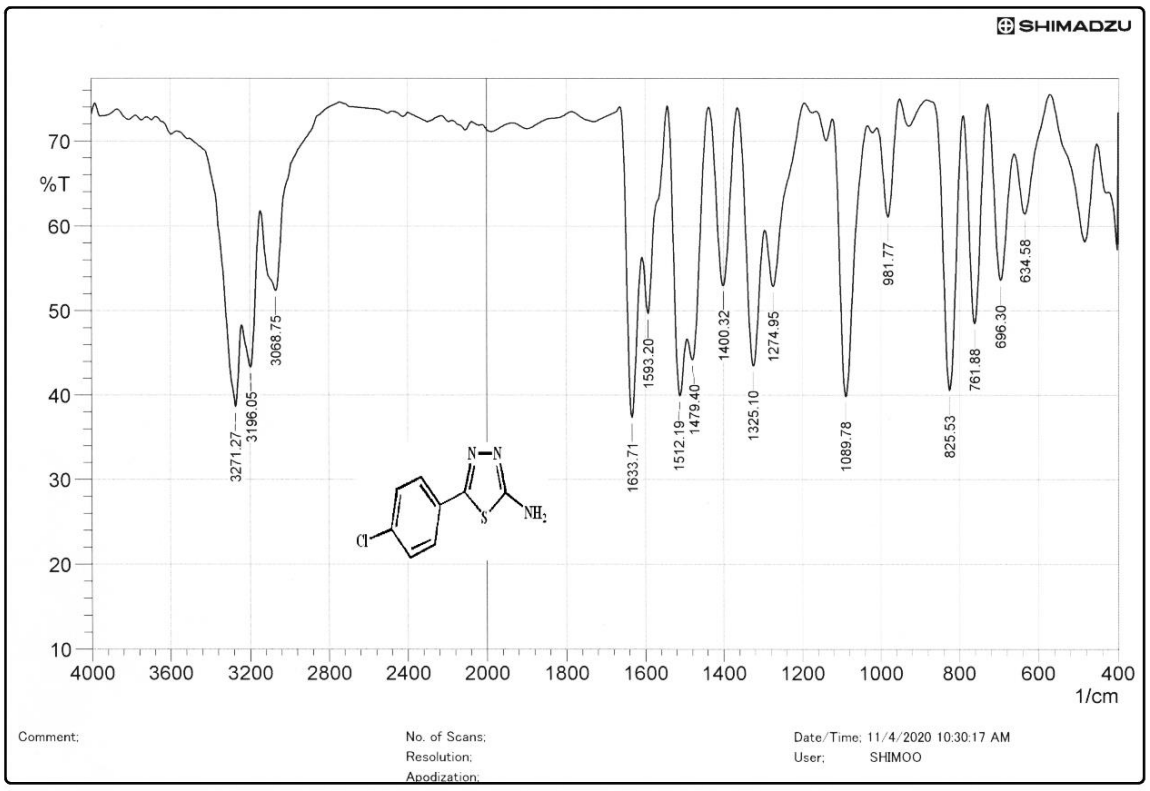


Figure (3-52): FT-IR spectrum of compound (8)

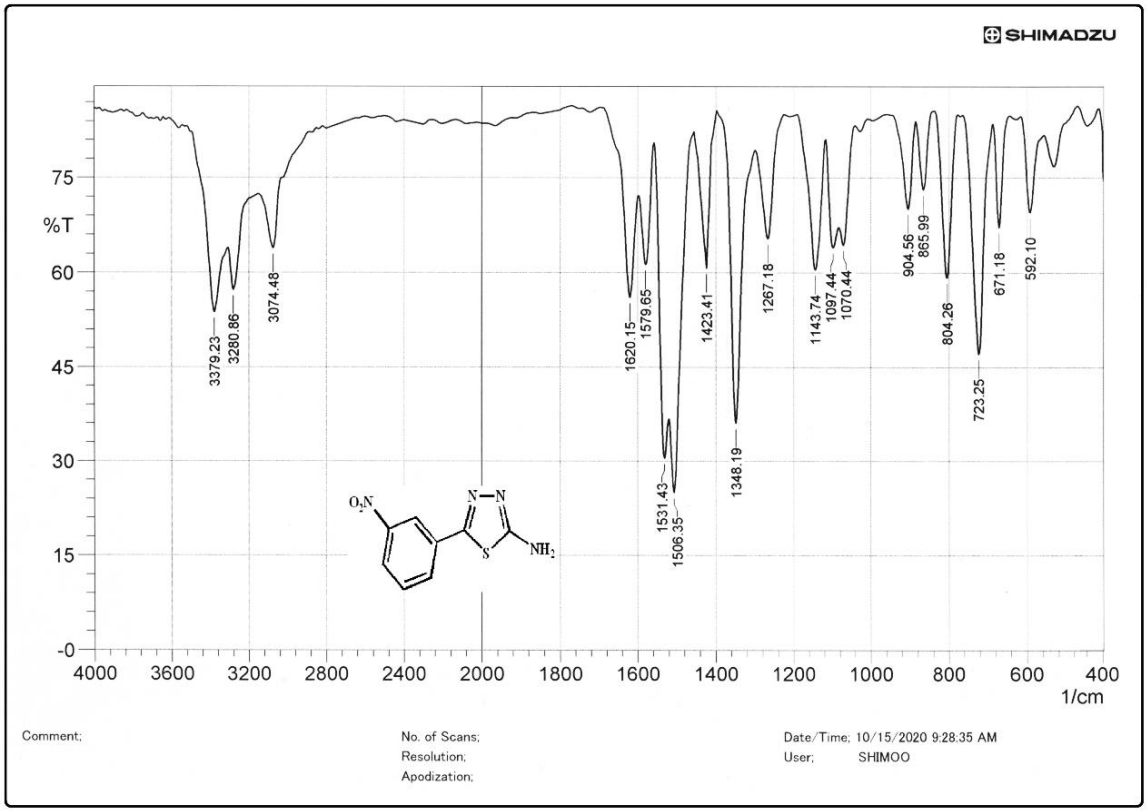


Figure (3-53): FT-IR spectrum of compound (9)

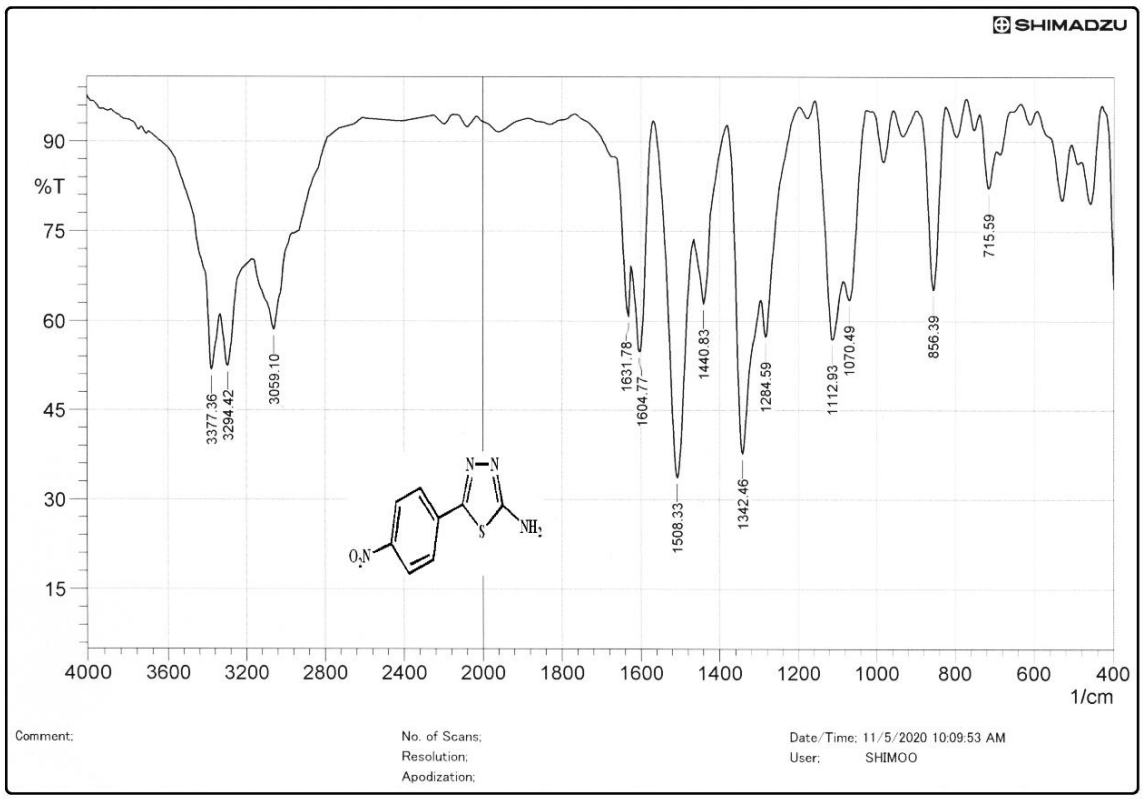


Figure (3-54): FT-IR spectrum of compound (10)

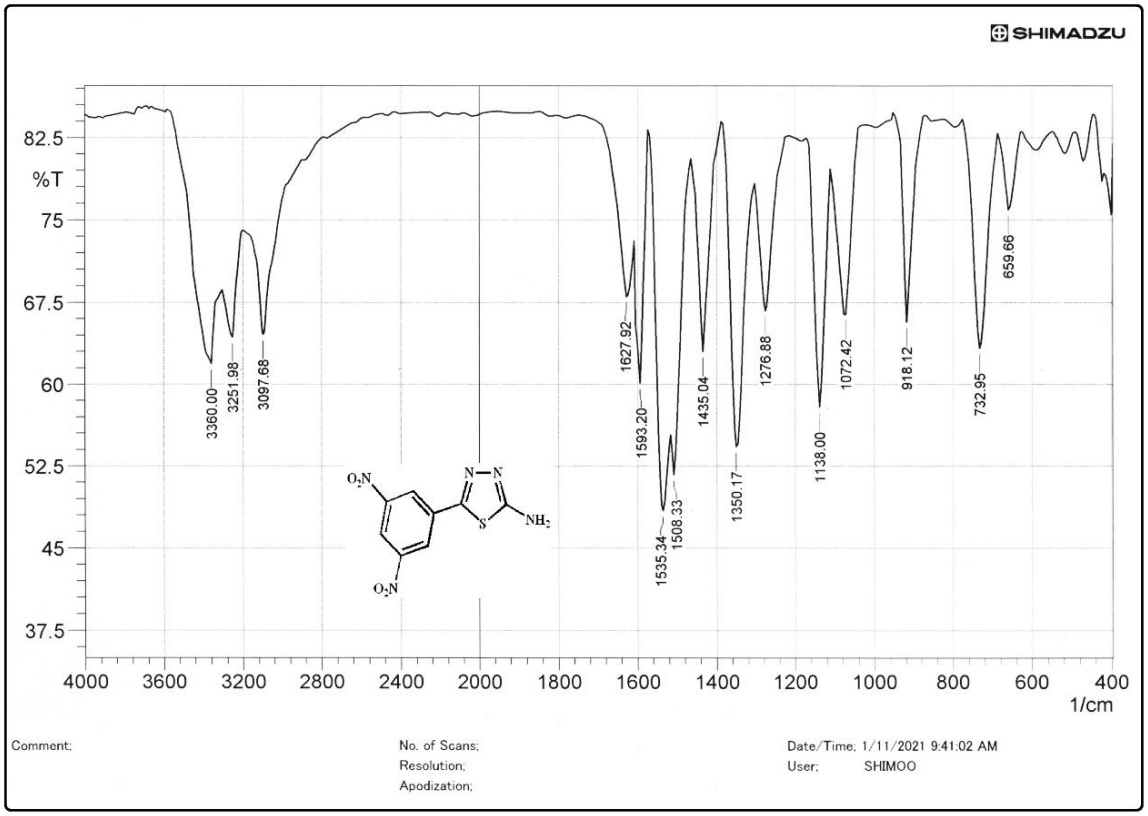


Figure (3-55): FT-IR spectrum of compound (11)

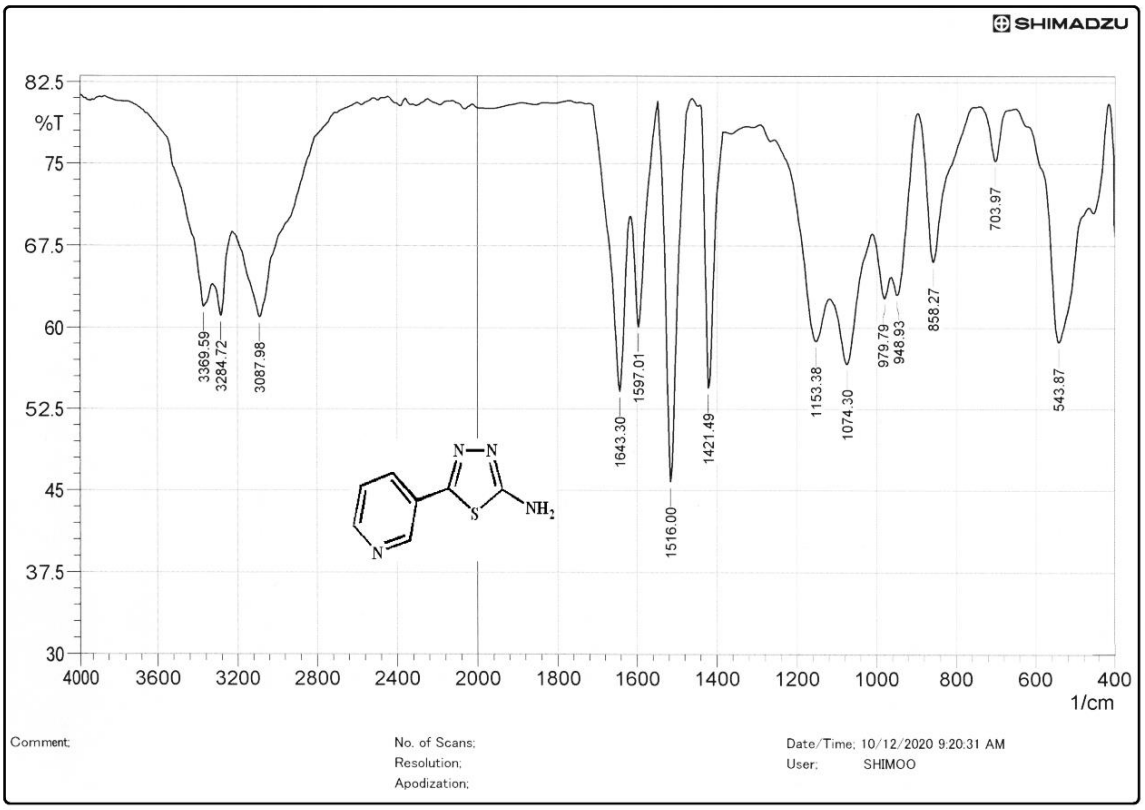


Figure (3-56): FT-IR spectrum of compound (12)

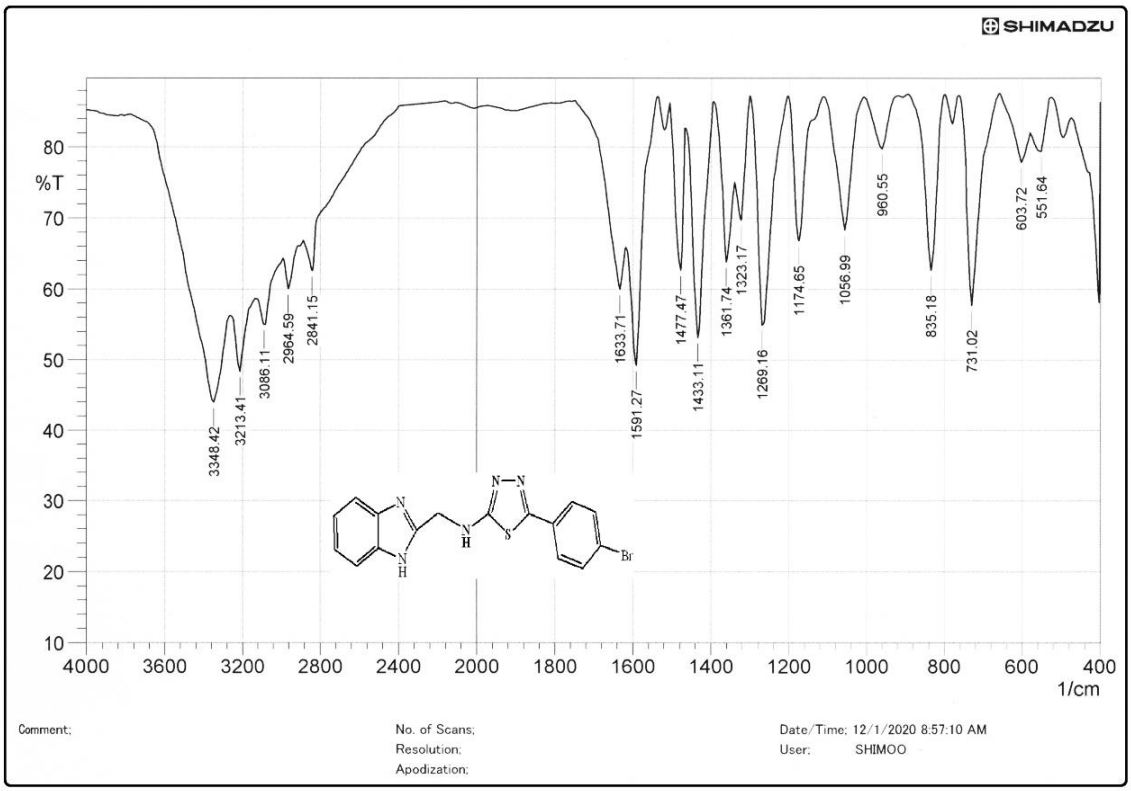


Figure (3-57): FT-IR spectrum of compound (16)

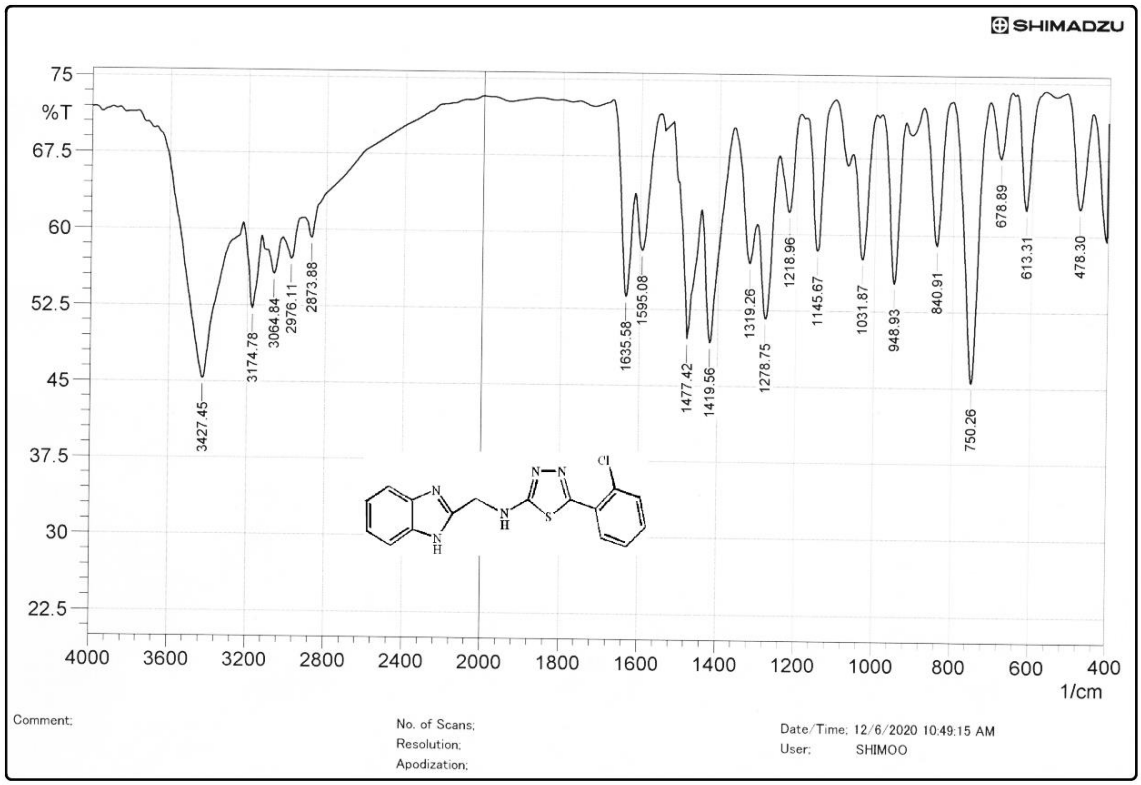


Figure (3-58): FT-IR spectrum of compound (17)

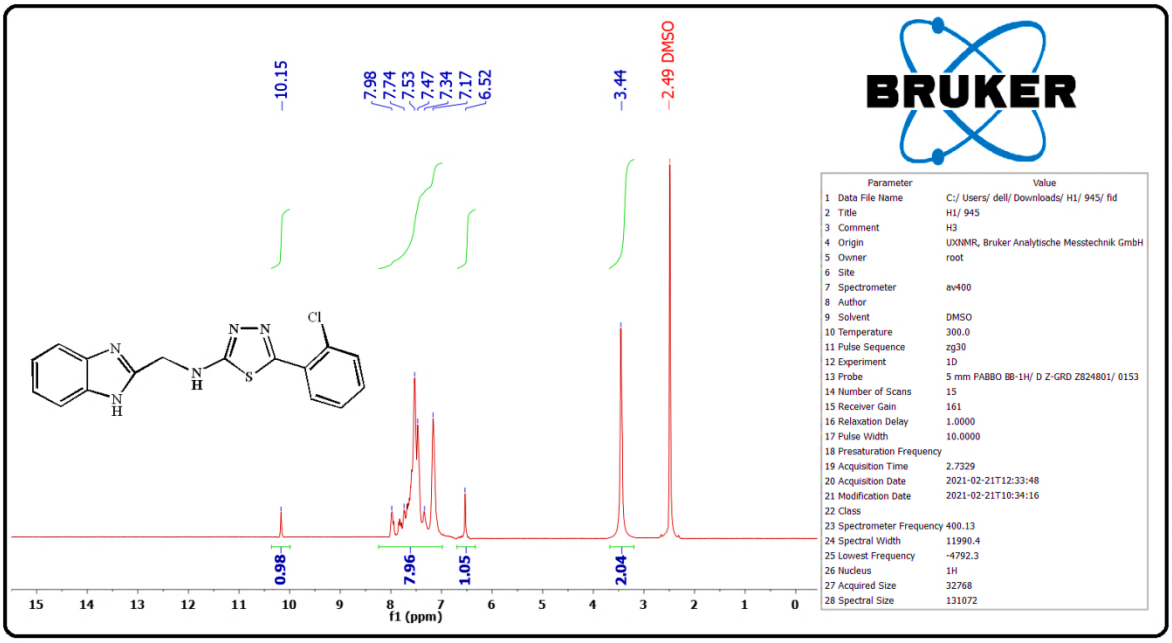


Figure (3-59): ^{1}H-NMR spectrum of compound (17)

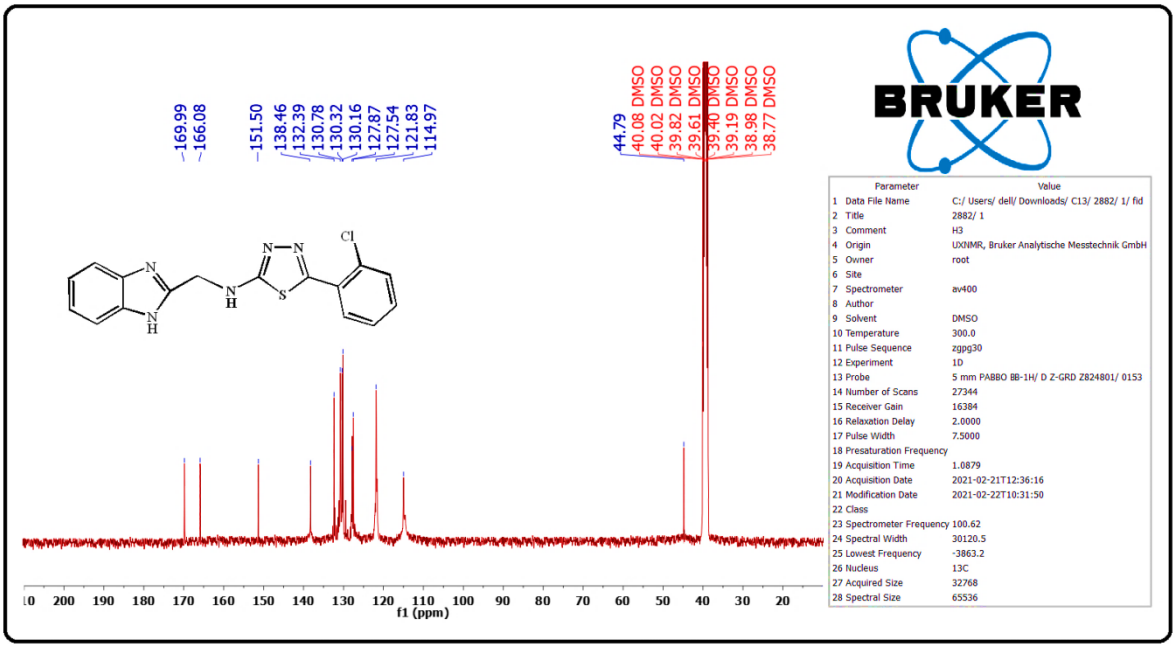


Figure (3-60): ^{13}C-NMR spectrum of compound (17)

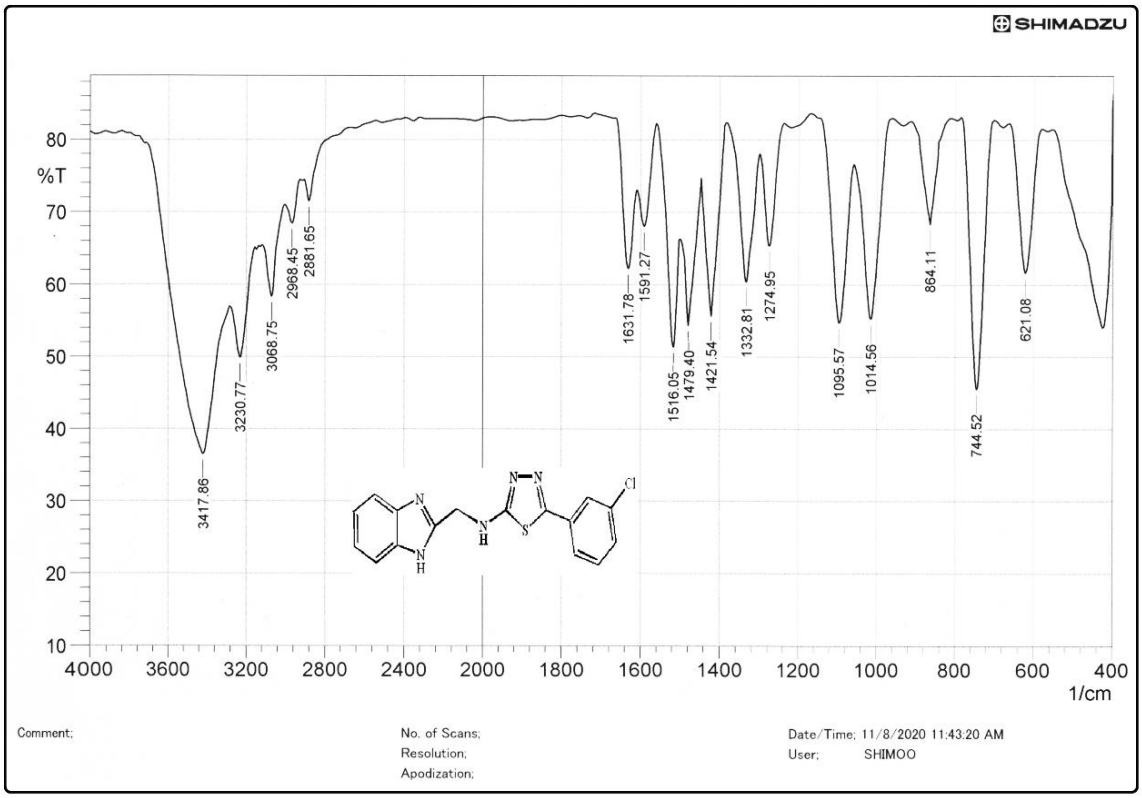


Figure (3-61): FT-IR spectrum of compound (18)

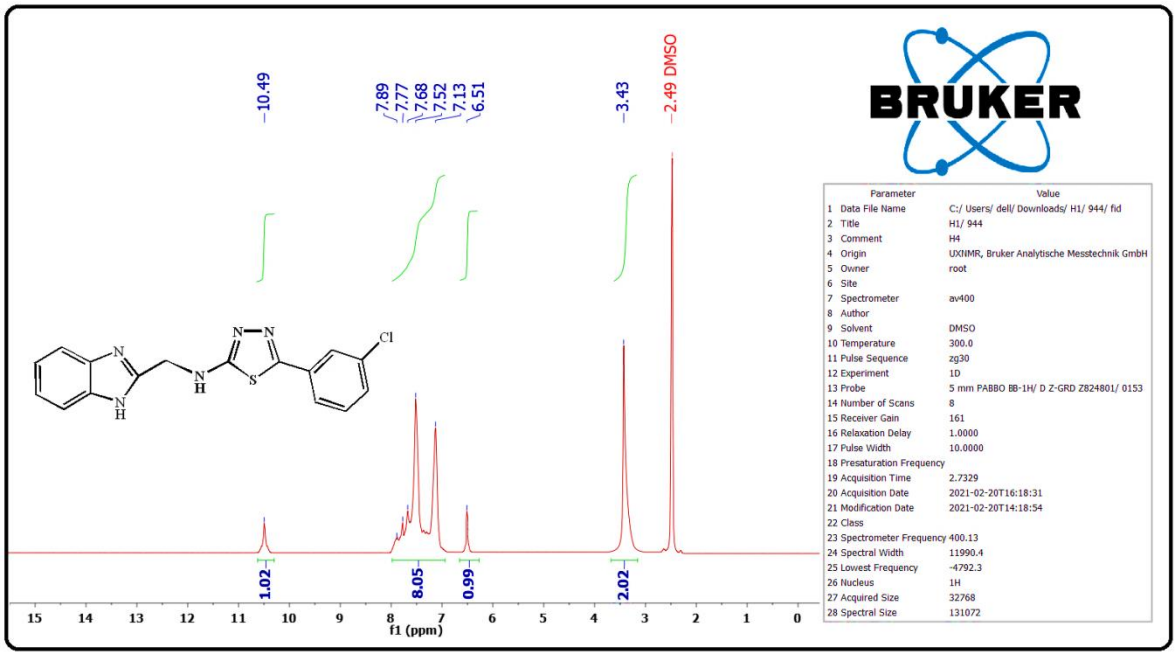


Figure (3-62): ^{1}H-NMR spectrum of compound (18)

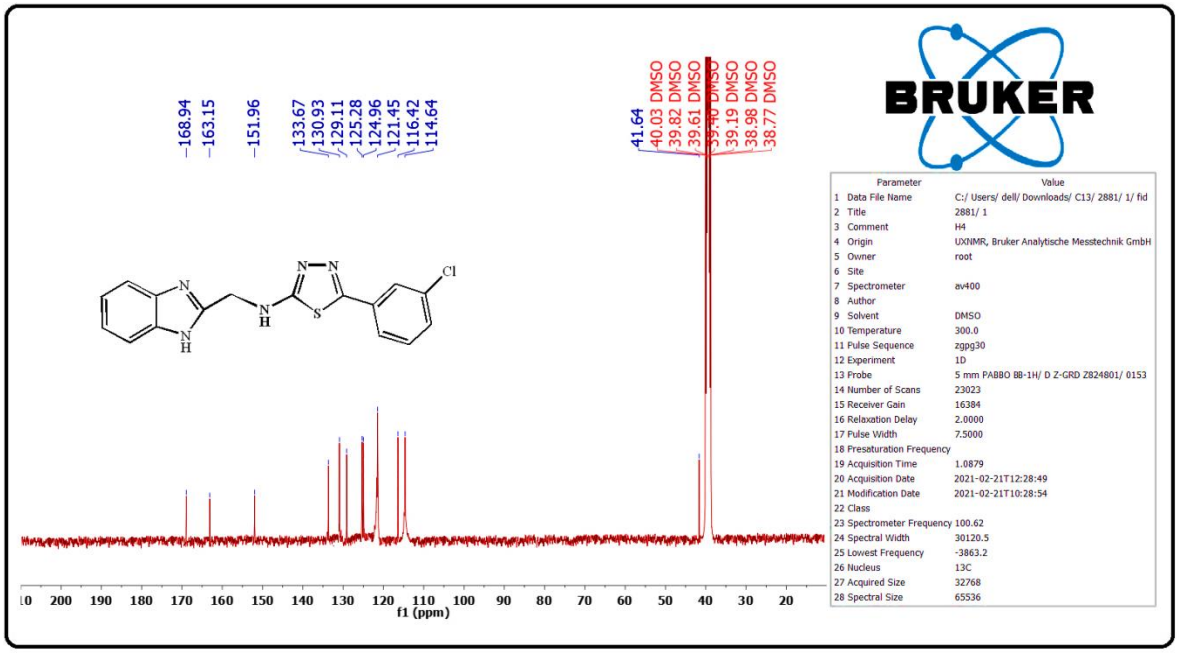


Figure (3-63): ^{13}C-NMR spectrum of compound (18)

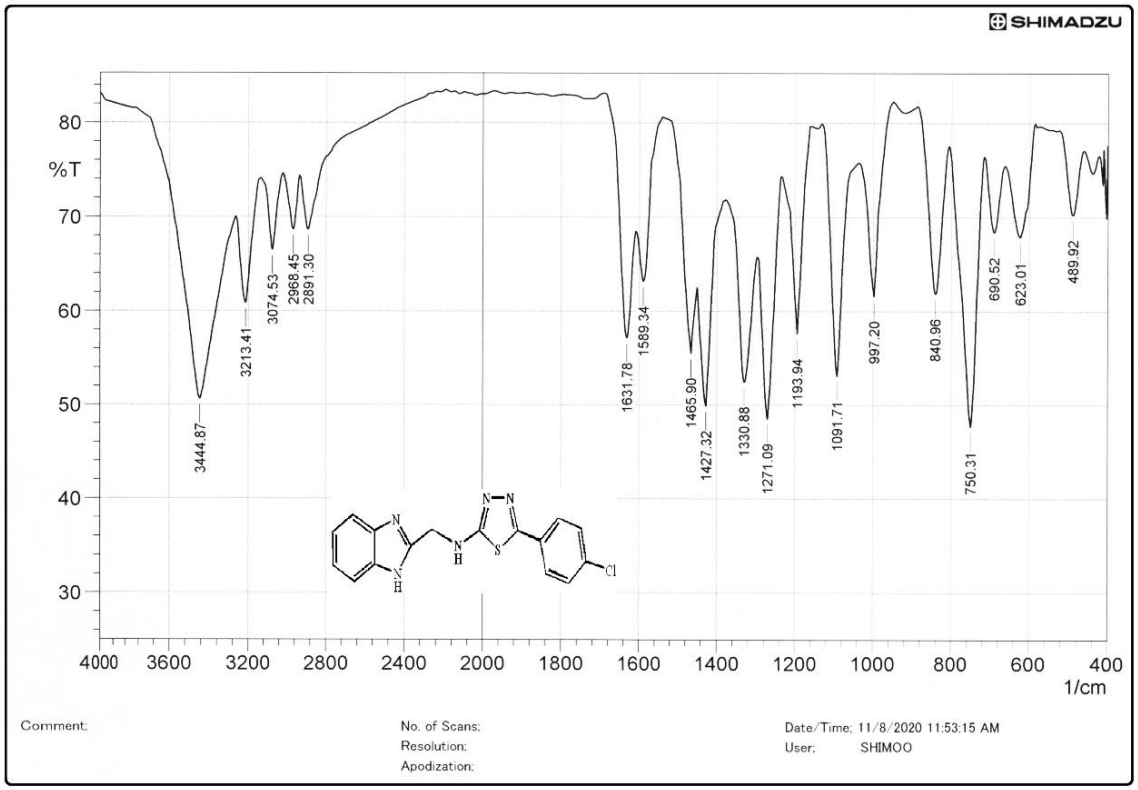


Figure (3-64): FT-IR spectrum of compound (19)

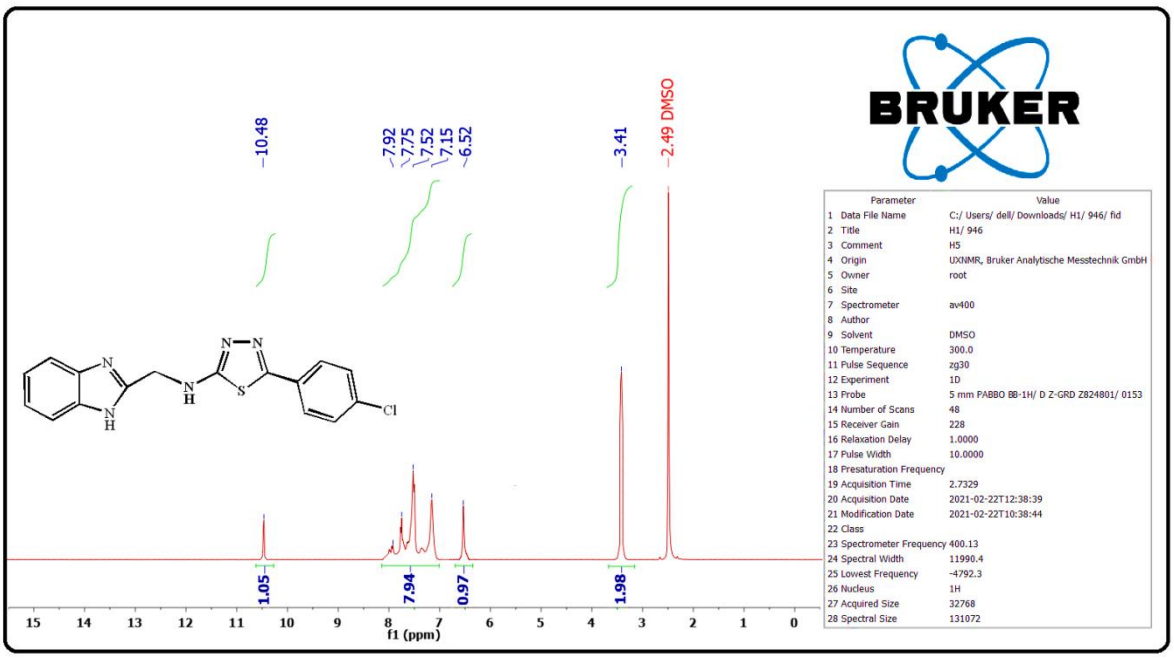


Figure (3-65): ^{1}H-NMR spectrum of compound (19)

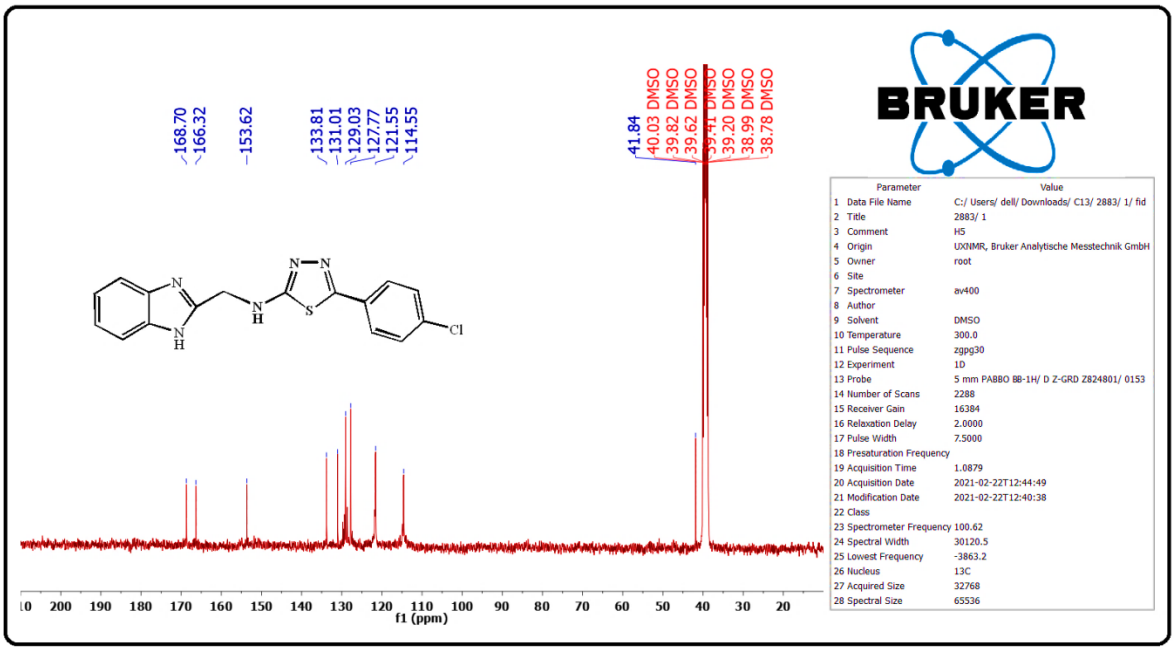


Figure (3-66): ^{13}C-NMR spectrum of compound (19)

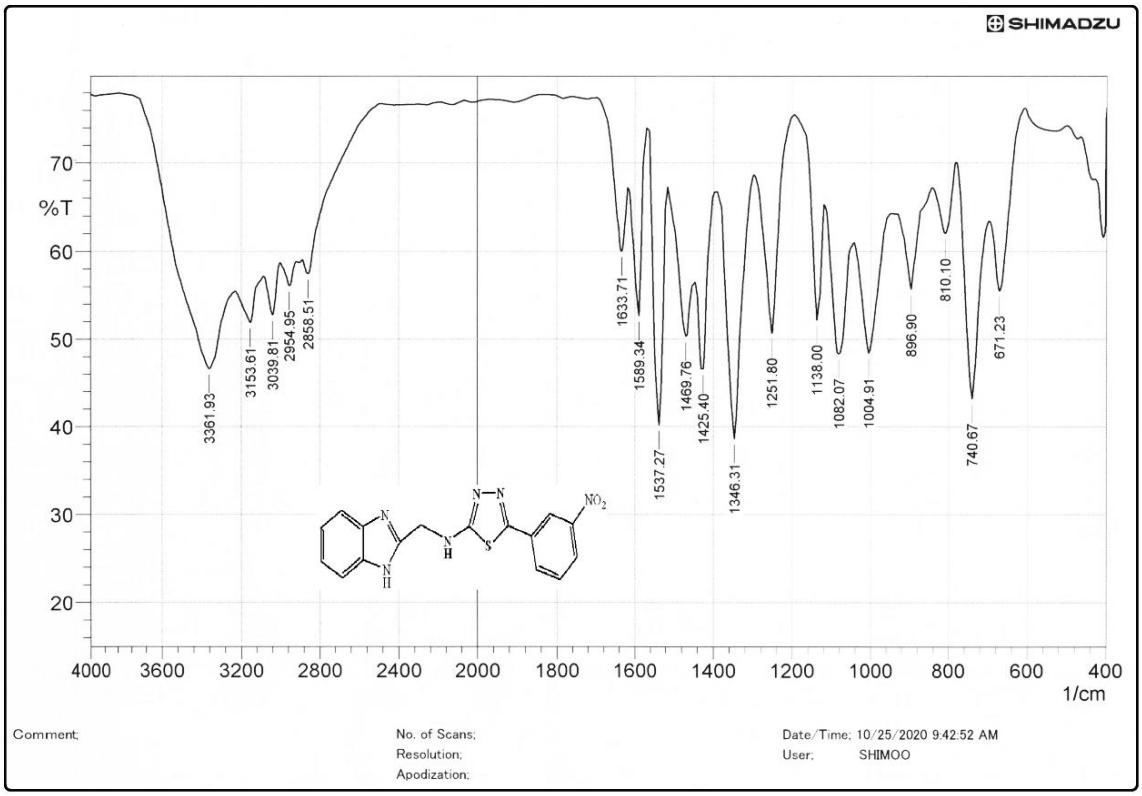


Figure (3-67): FT-IR spectrum of compound (20)

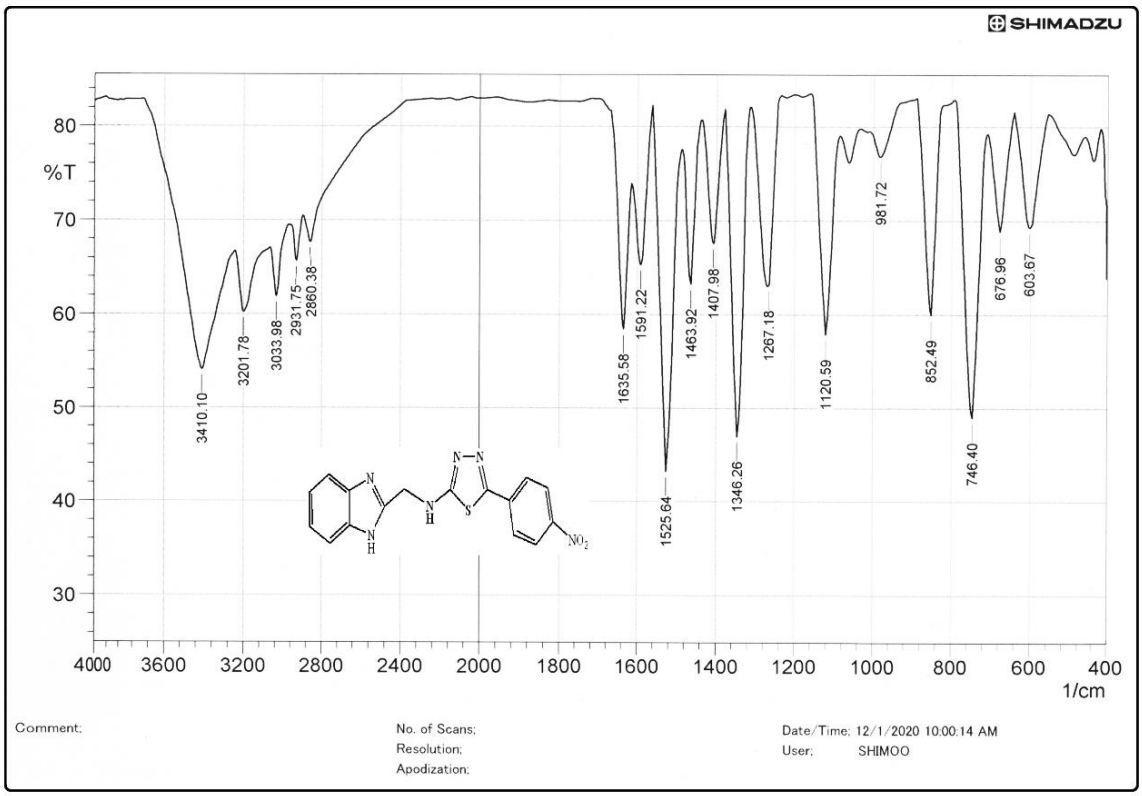


Figure (3-68): FT-IR spectrum of compound (21)

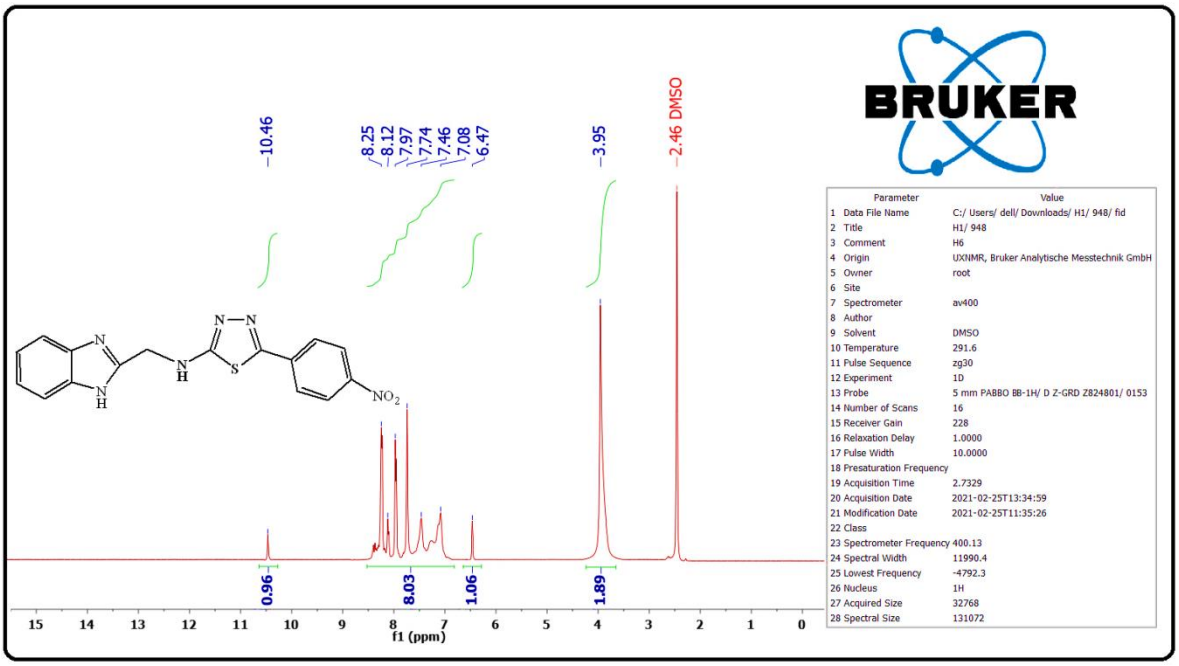


Figure (3-69): ^{1}H-NMR spectrum of compound (21)

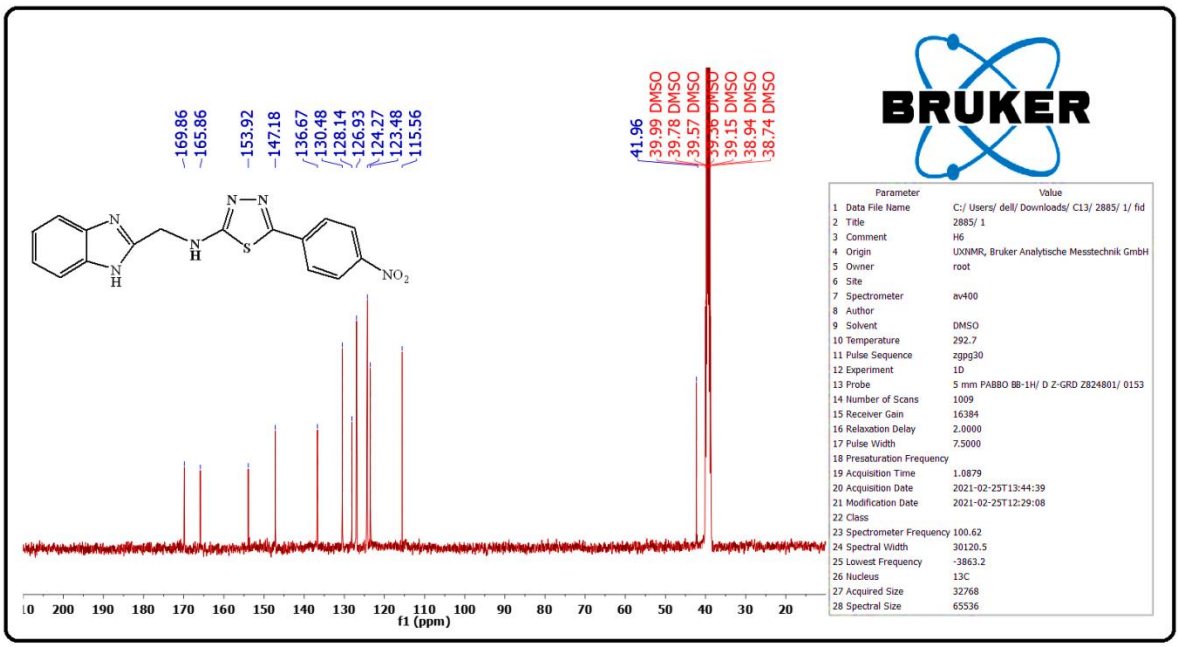


Figure (3-70): ^{13}C-NMR spectrum of compound (21)

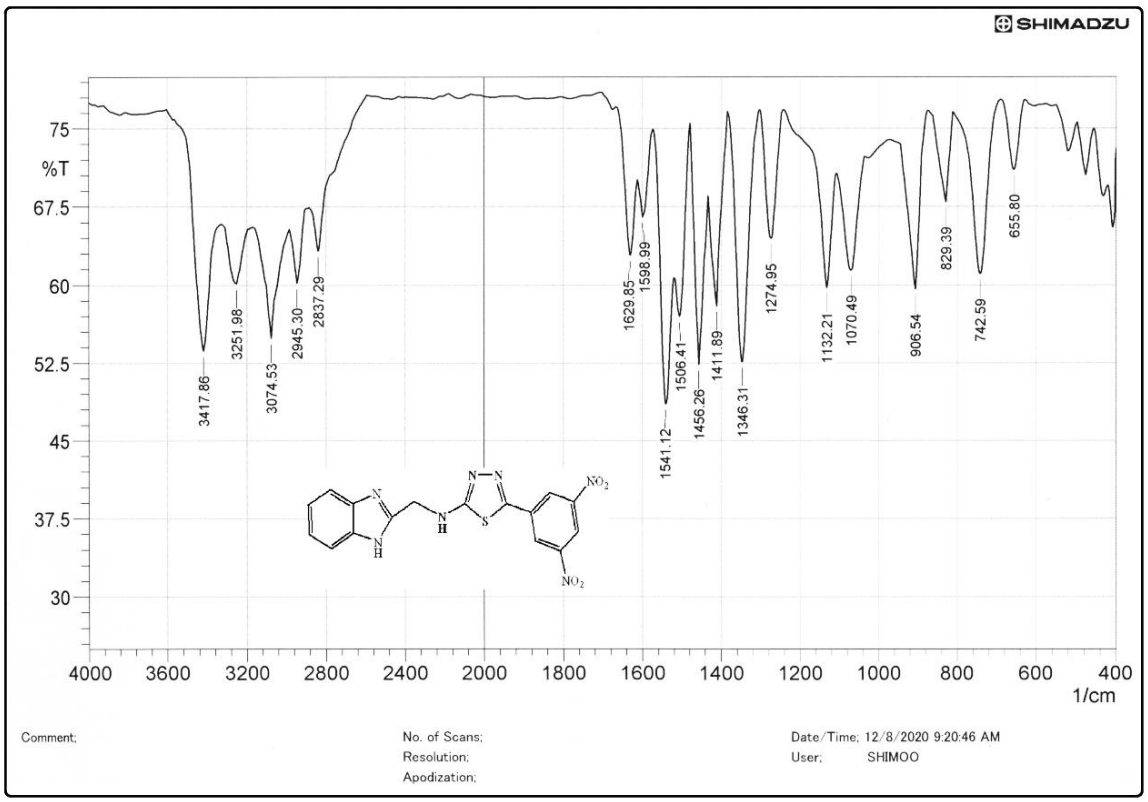


Figure (3-71): FT-IR spectrum of compound (22)

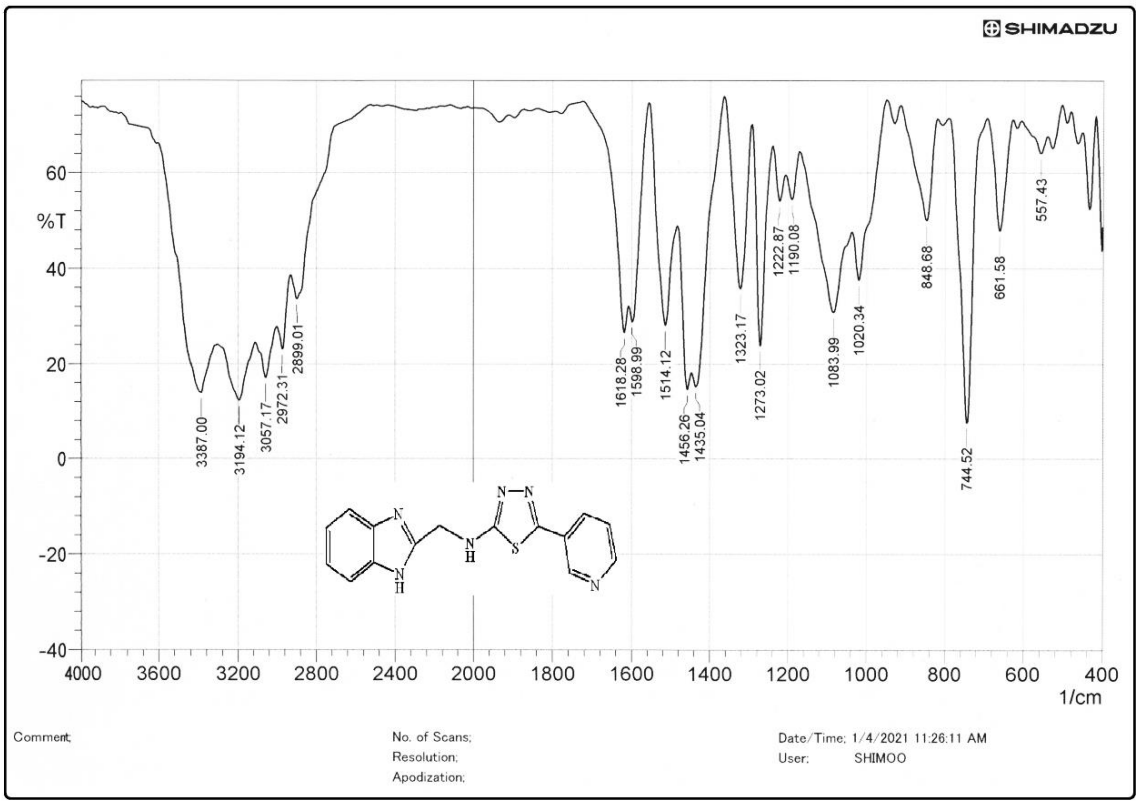


Figure (3-72): FT-IR spectrum of compound (23)

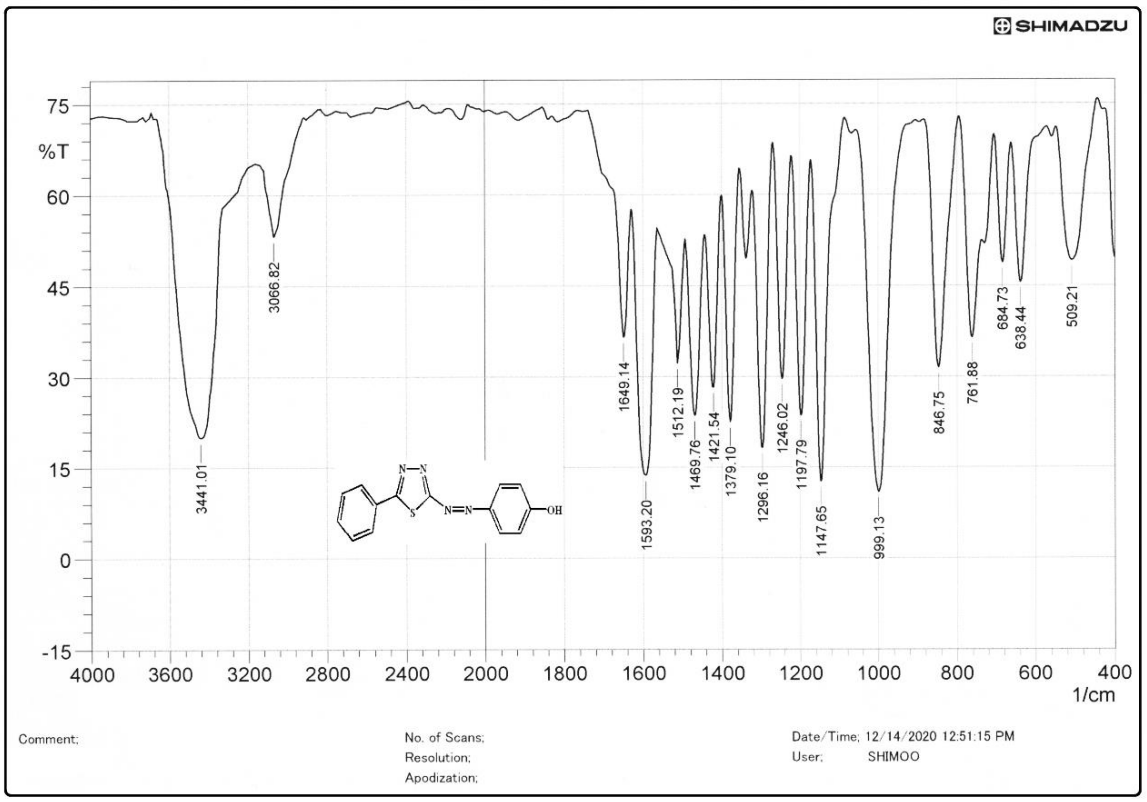


Figure (3-73): FT-IR spectrum of compound (24)

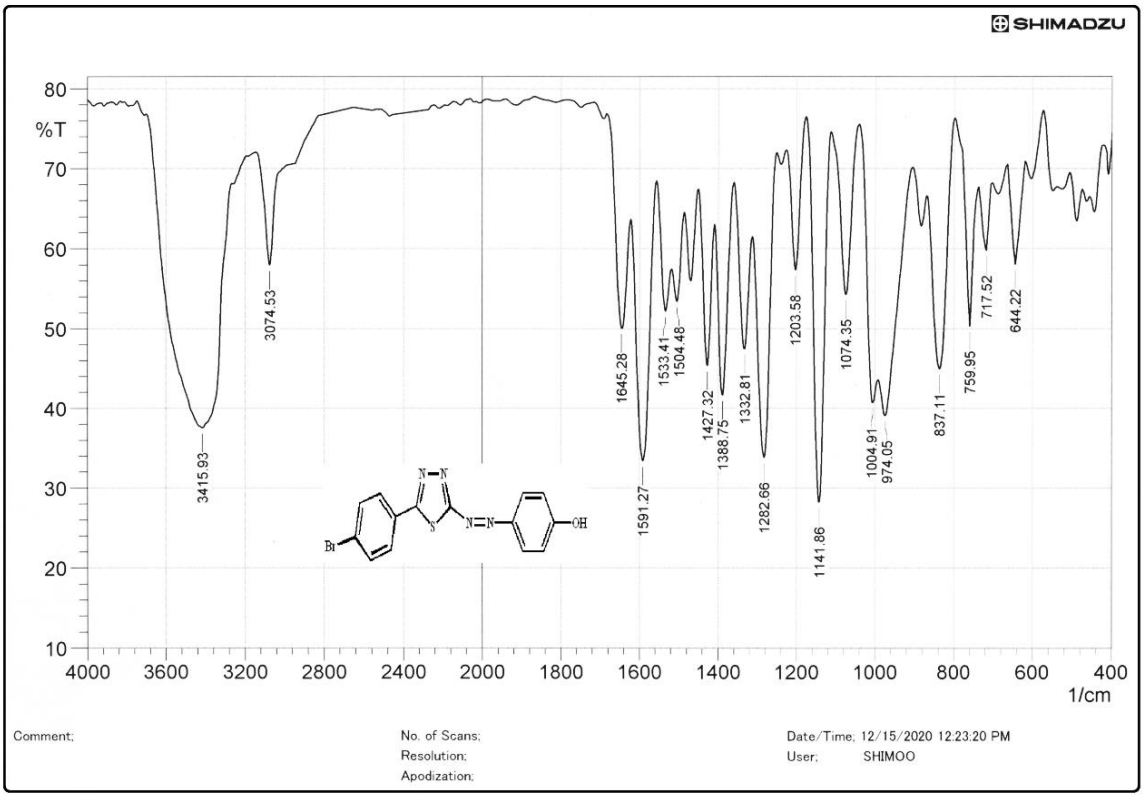


Figure (3-74): FT-IR spectrum of compound (25)

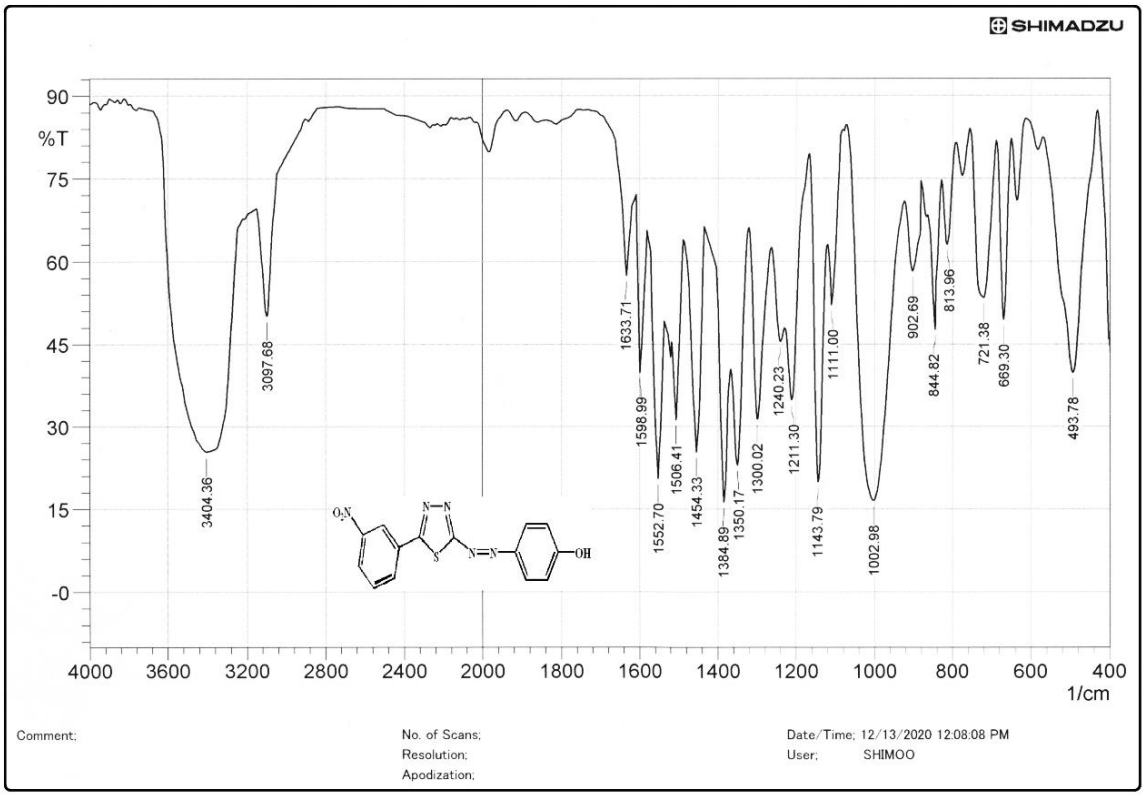


Figure (3-75): FT-IR spectrum of compound (29)

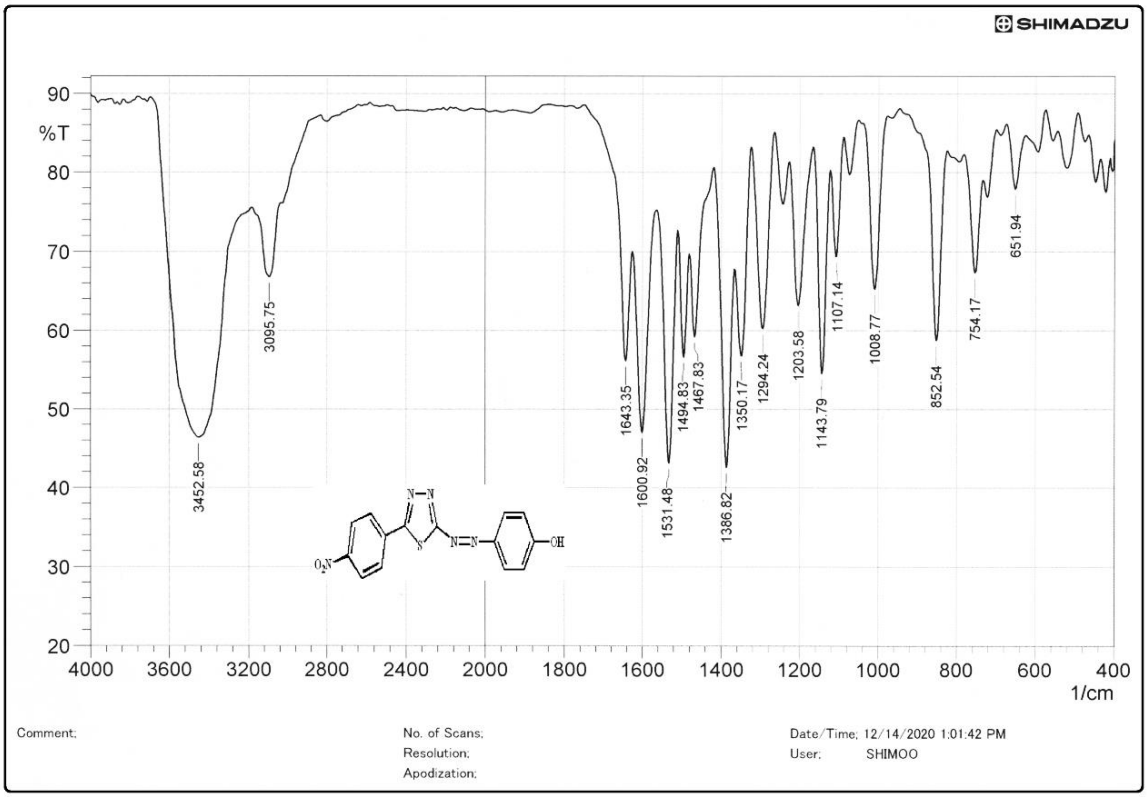


Figure (3-76): FT-IR spectrum of compound (30)

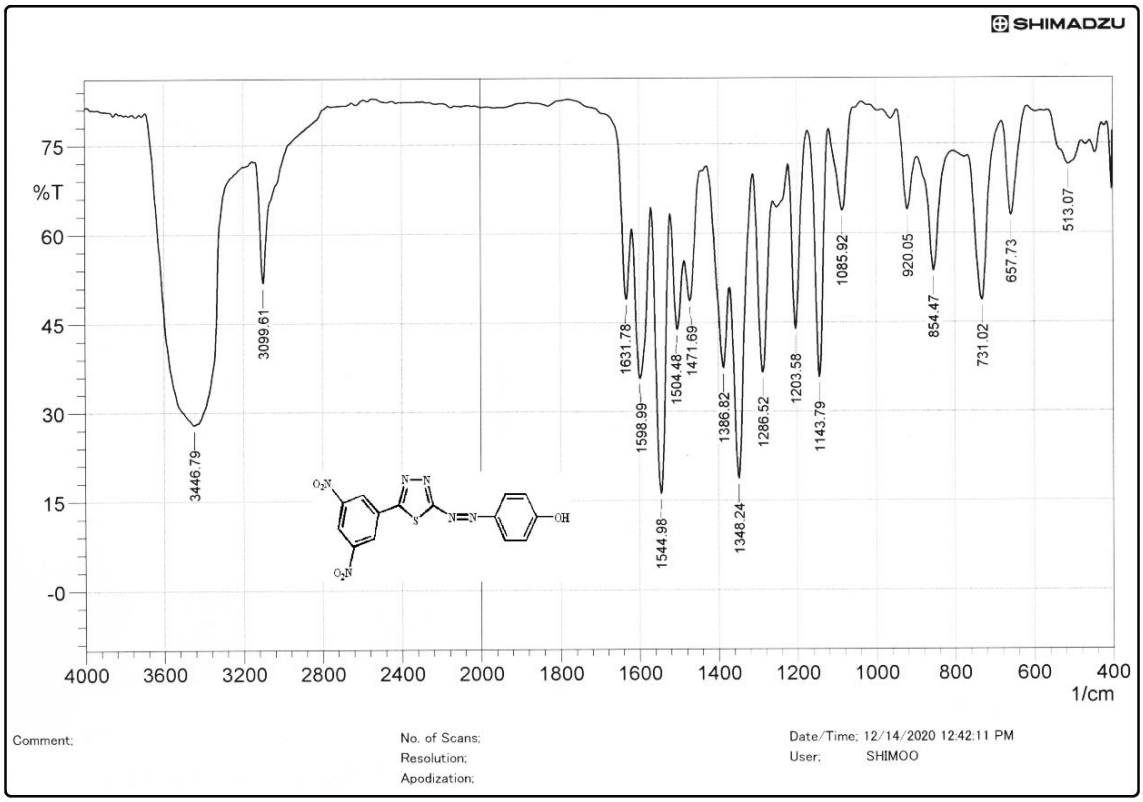


Figure (3-77): FT-IR spectrum of compound (31)

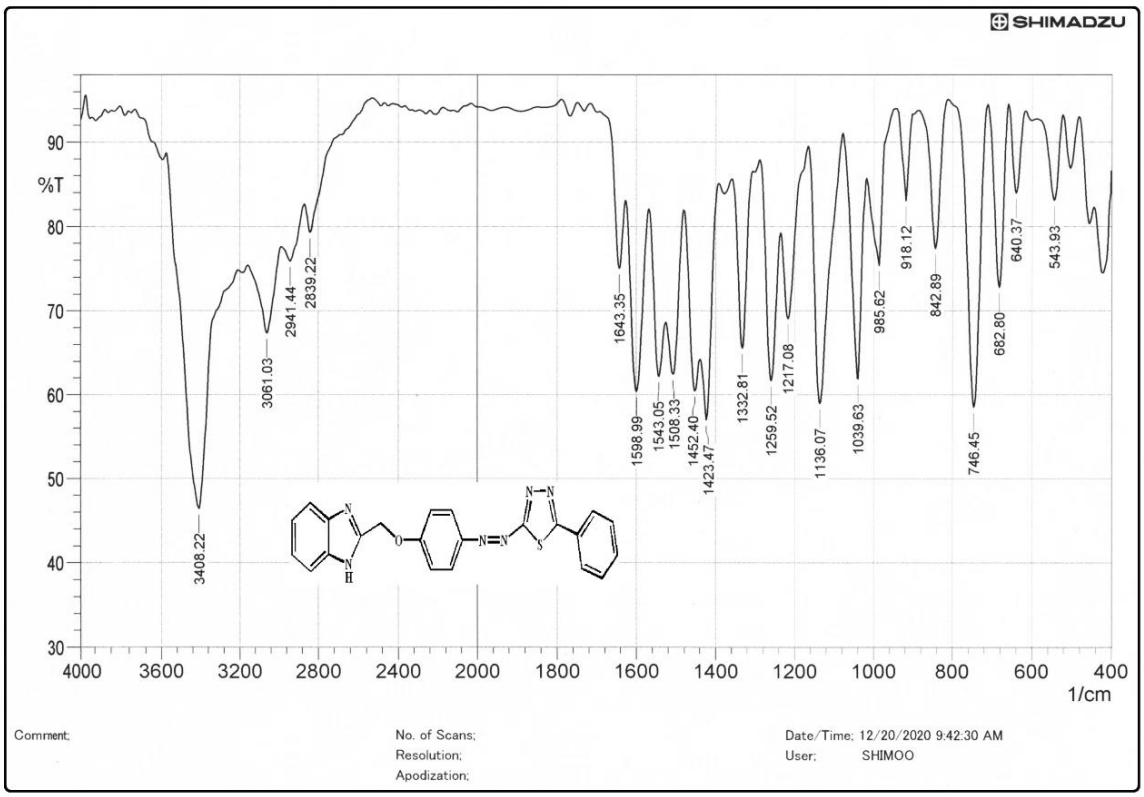


Figure (3-78): FT-IR spectrum of compound (32)

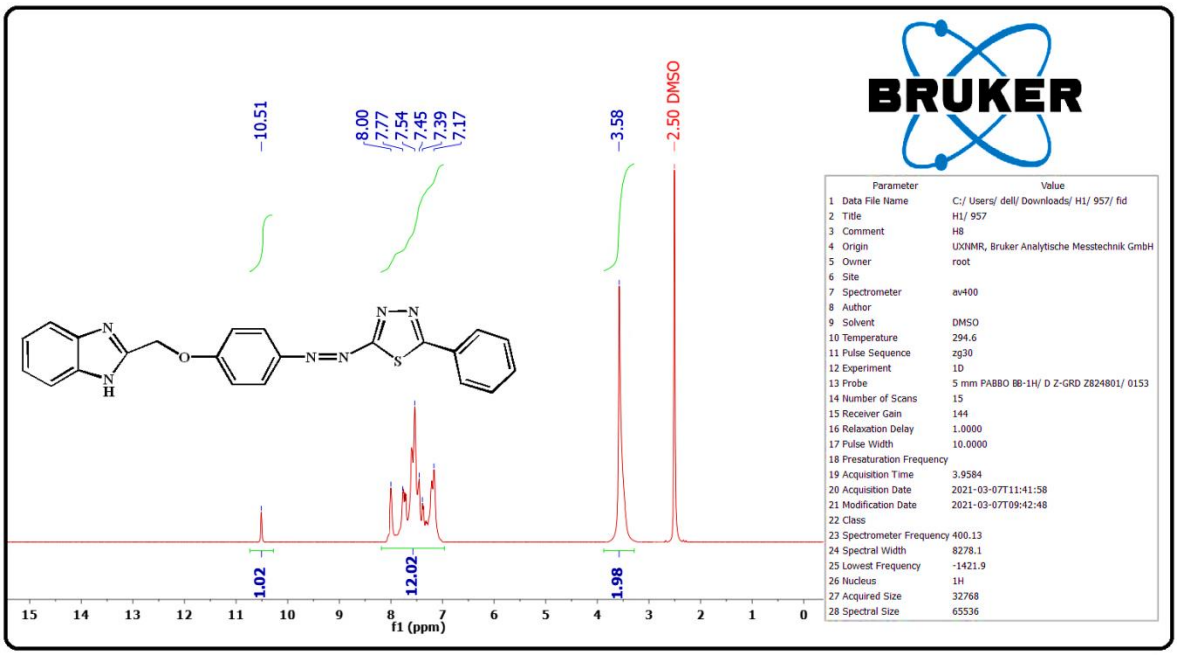


Figure (3-79): ^{1}H-NMR spectrum of compound (32)

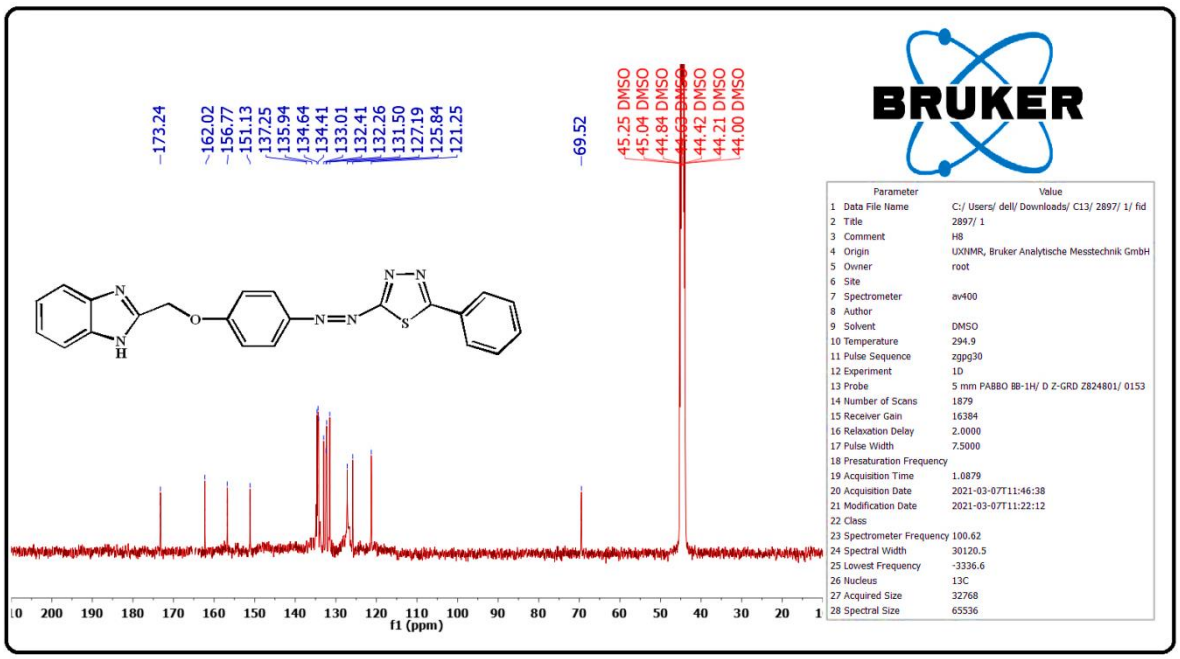


Figure (3-80): ^{13}C-NMR spectrum of compound (32)

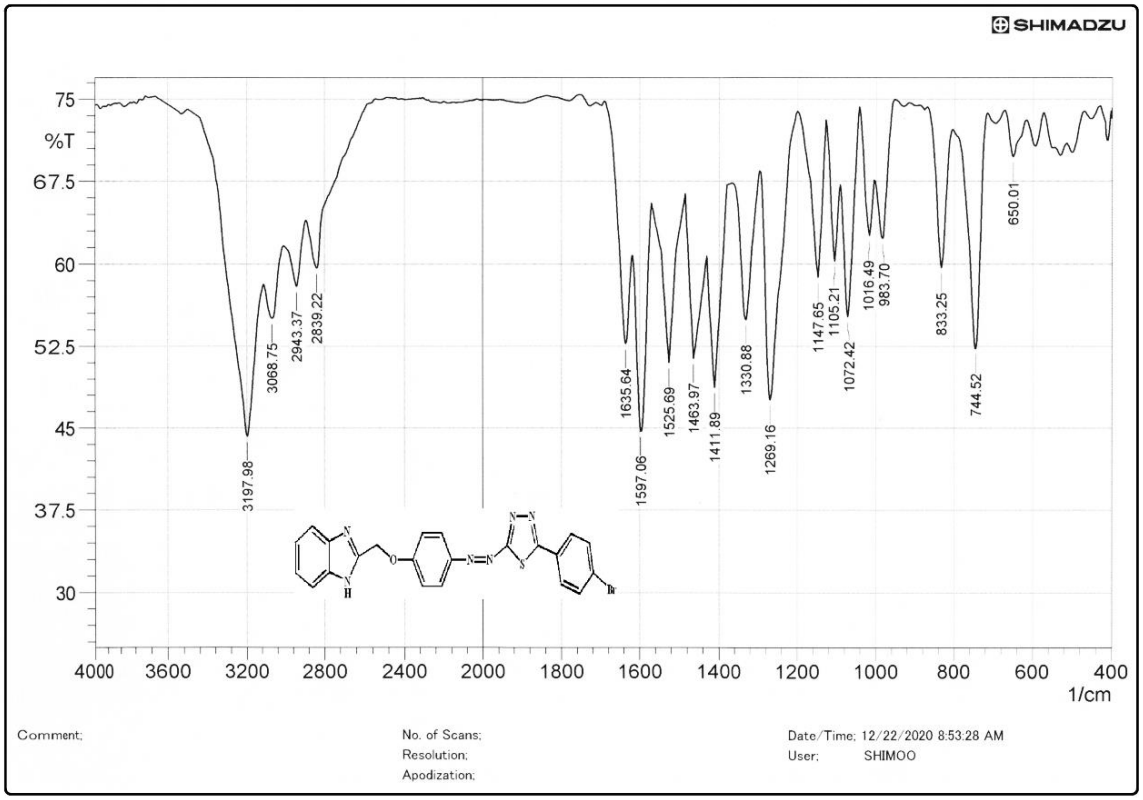


Figure (3-81): FT-IR spectrum of compound (33)

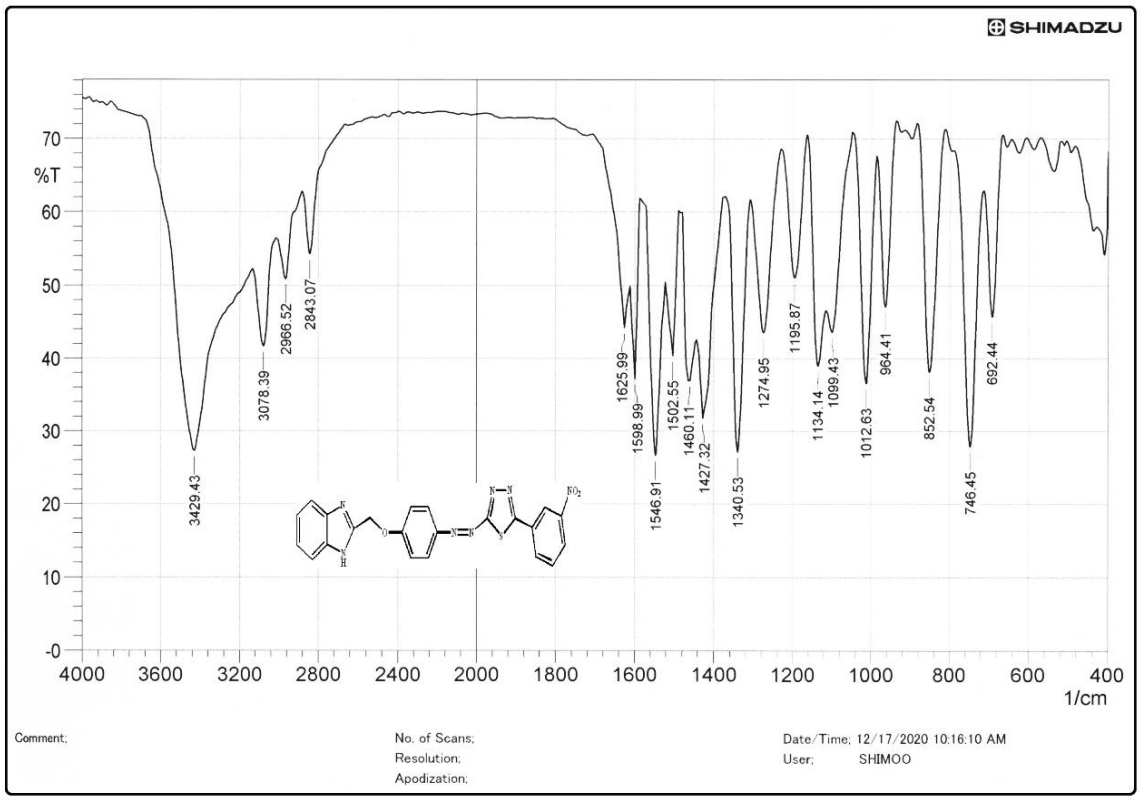


Figure (3-82): FT-IR spectrum of compound (37)

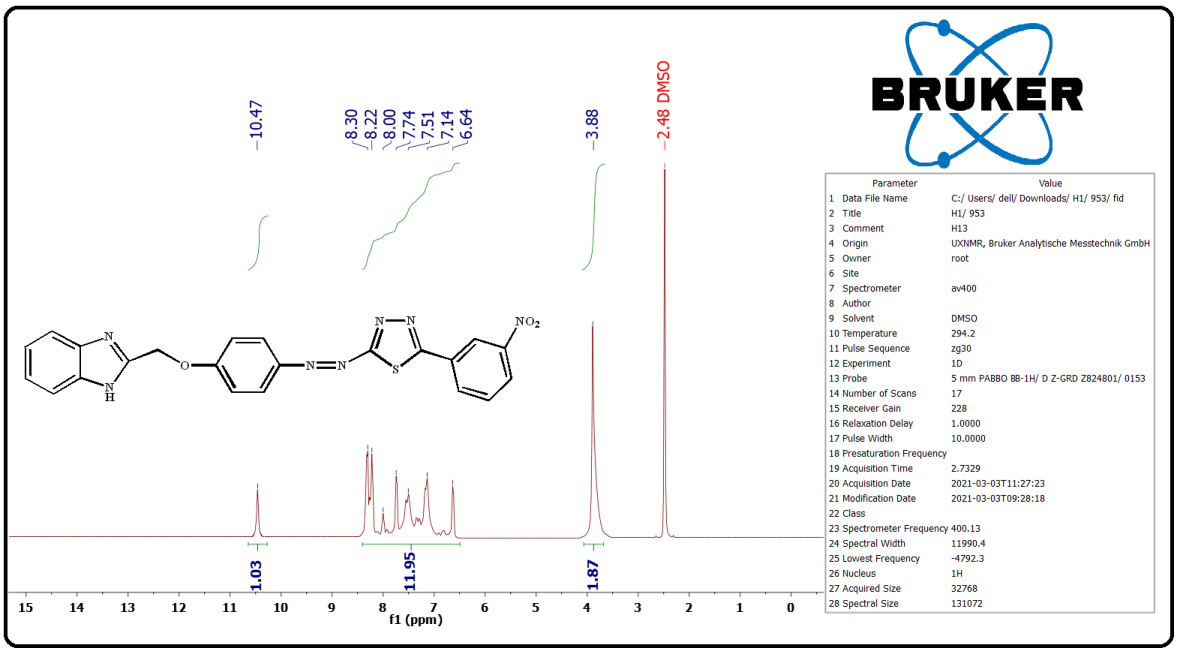


Figure (3-83): ^{1}H-NMR spectrum of compound (37)

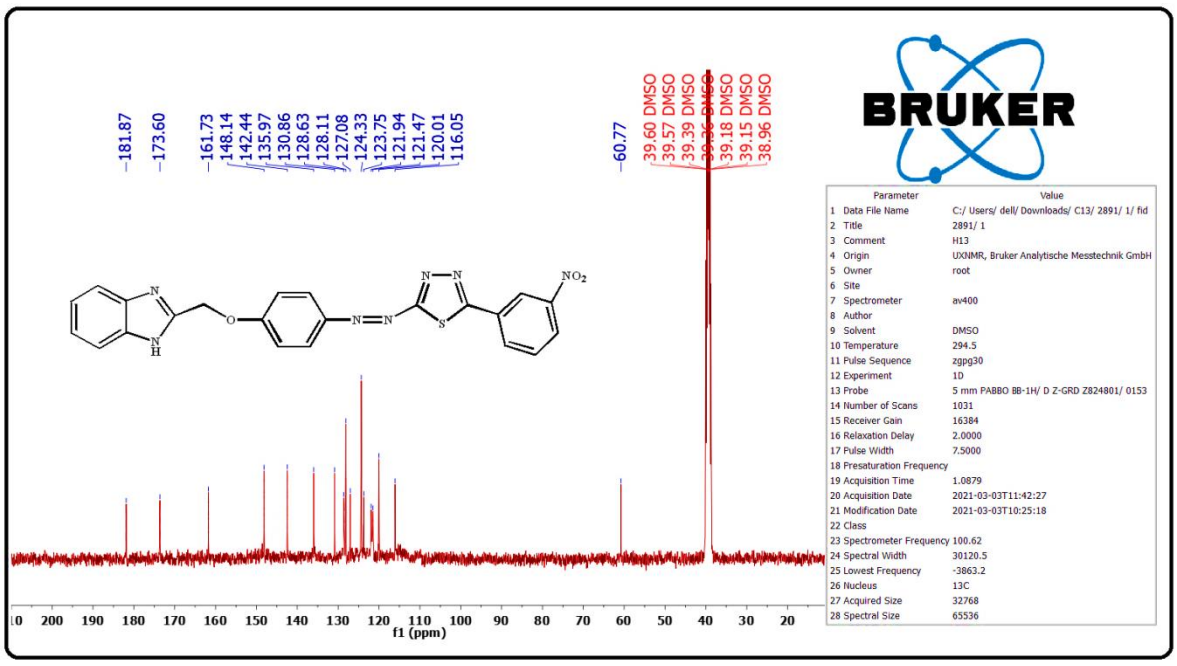


Figure (3-84): ^{13}C-NMR spectrum of compound (37)

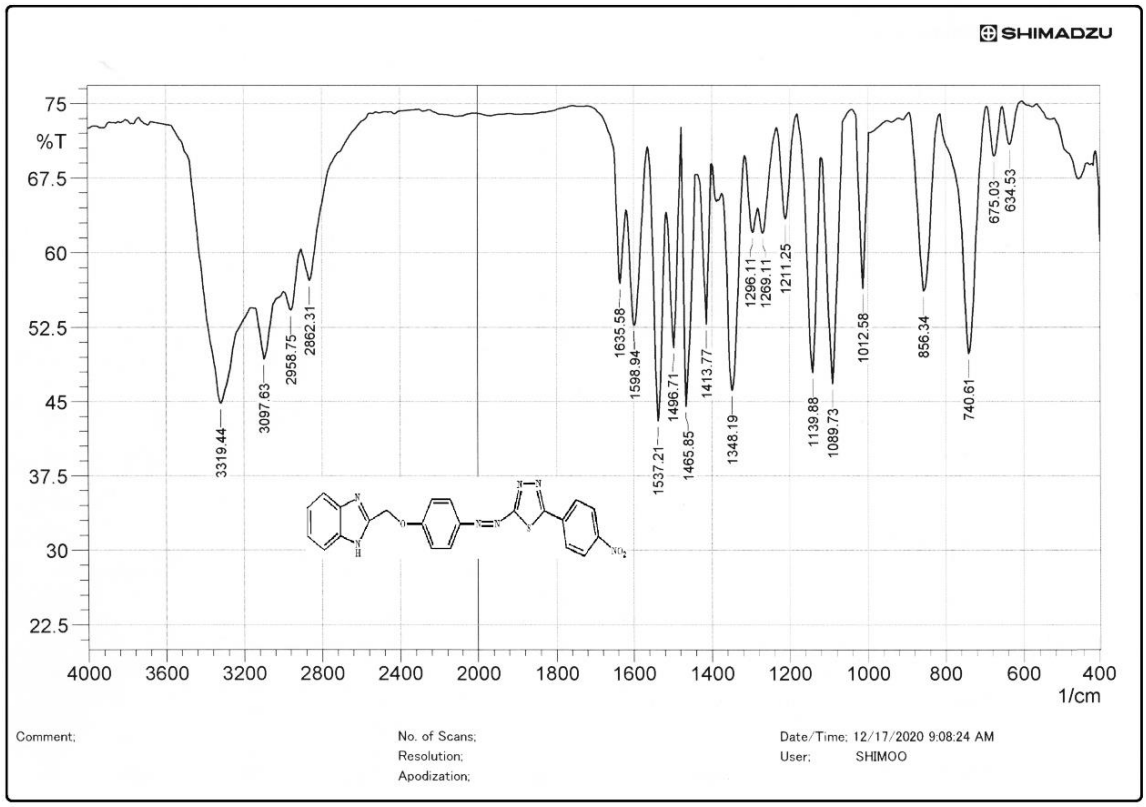


Figure (3-85): FT-IR spectrum of compound (38)

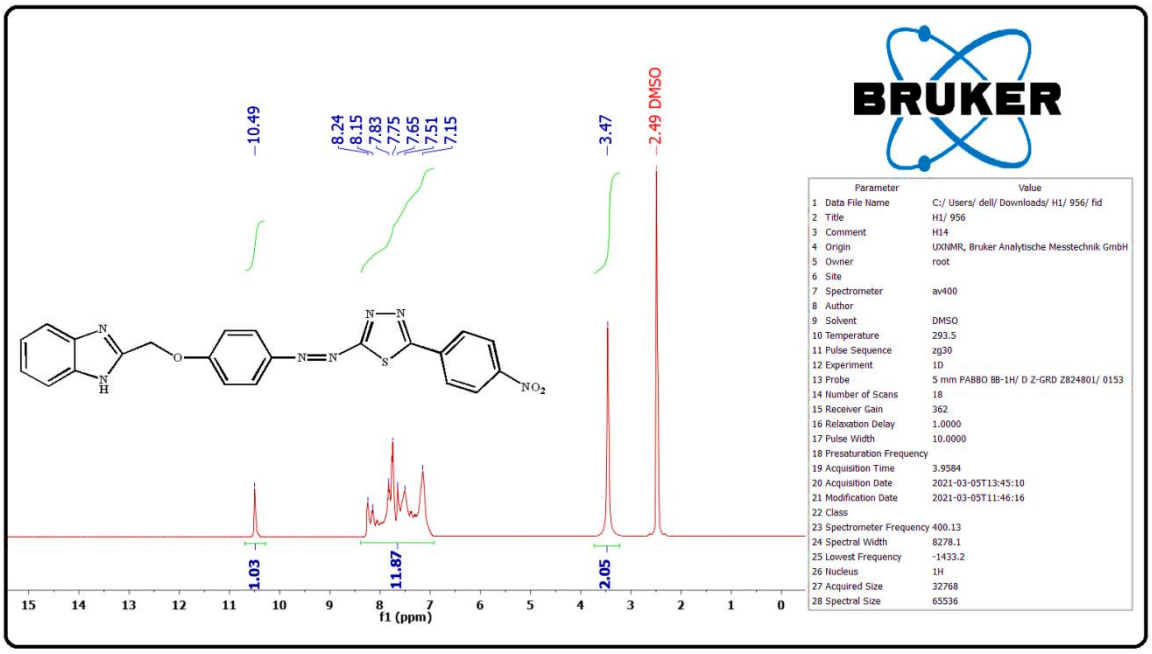
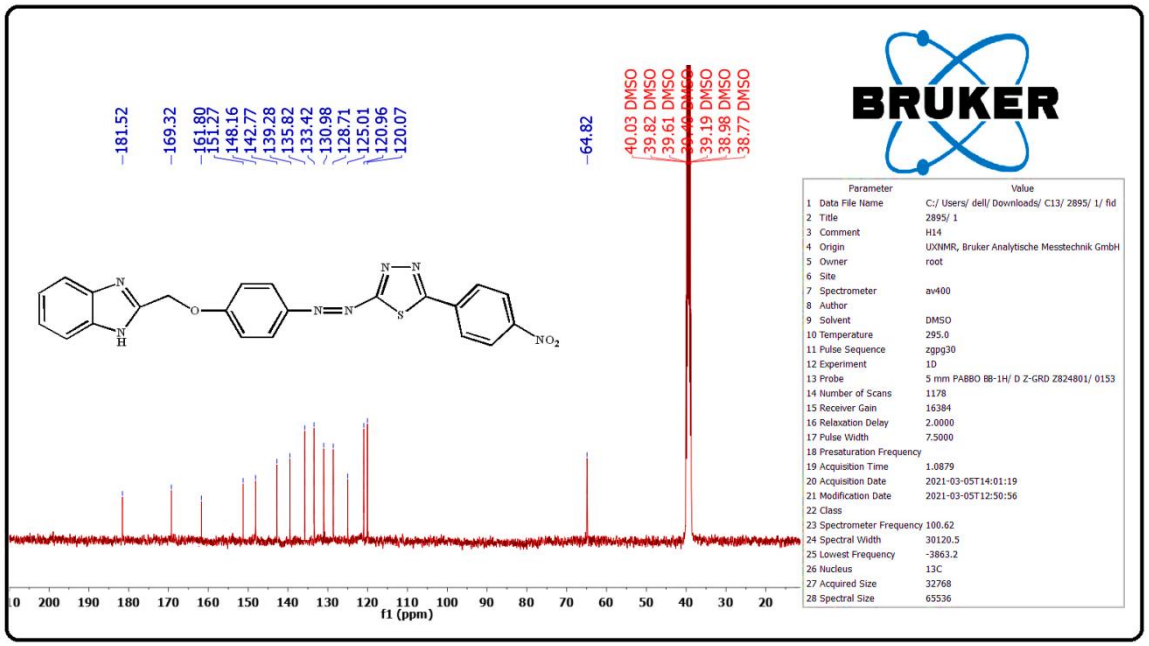


Figure (3-86): ^{1}H-NMR spectrum of compound (38)



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Figure (3-87): ^{13}C-NMR spectrum of compound (38)

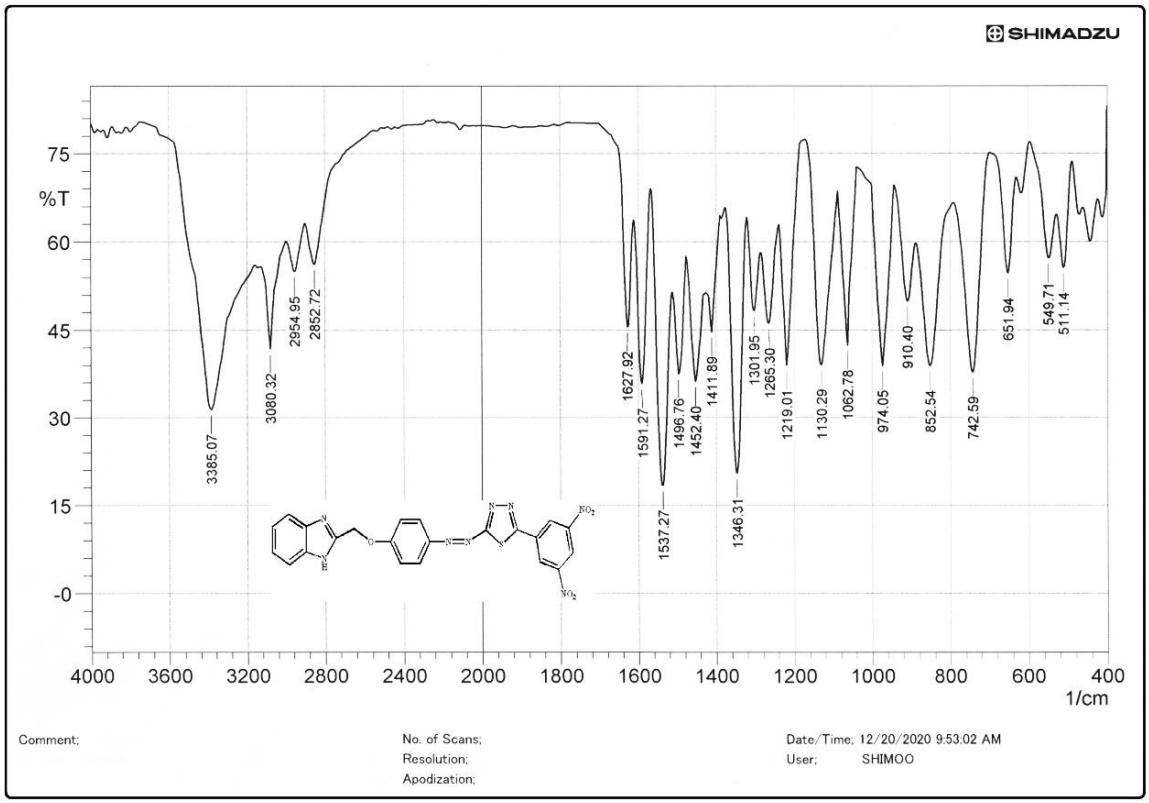


Figure (3-88): FT-IR spectrum of compound (39)

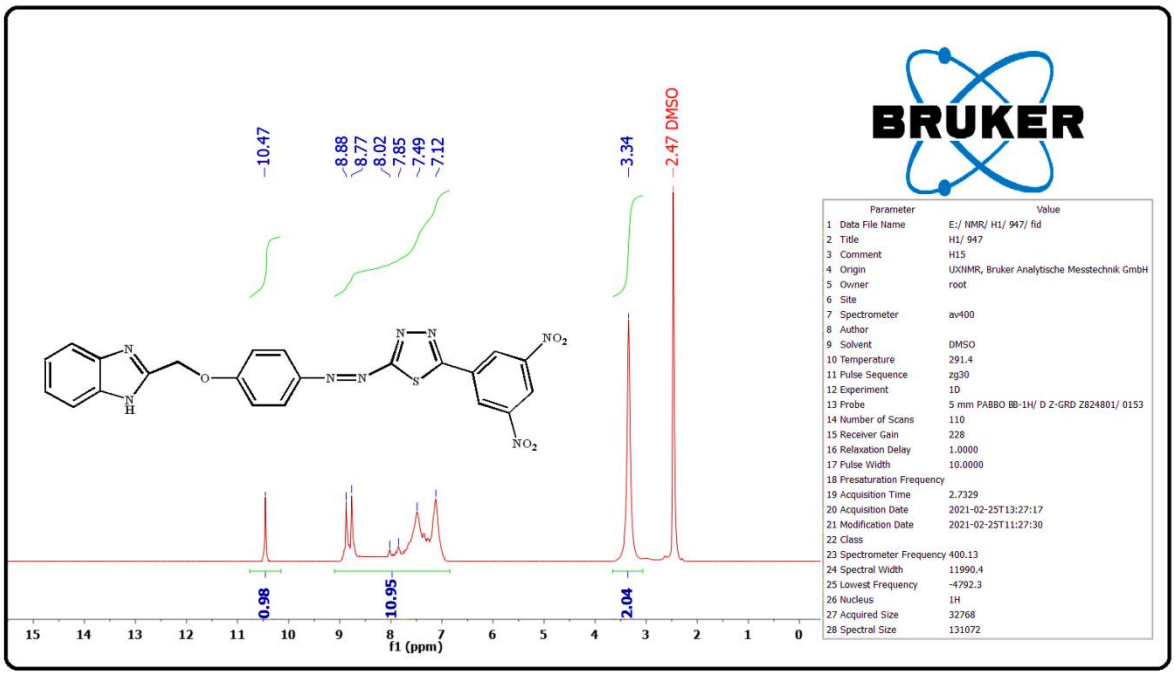


Figure (3-89): ^{1}H-NMR spectrum of compound (39)

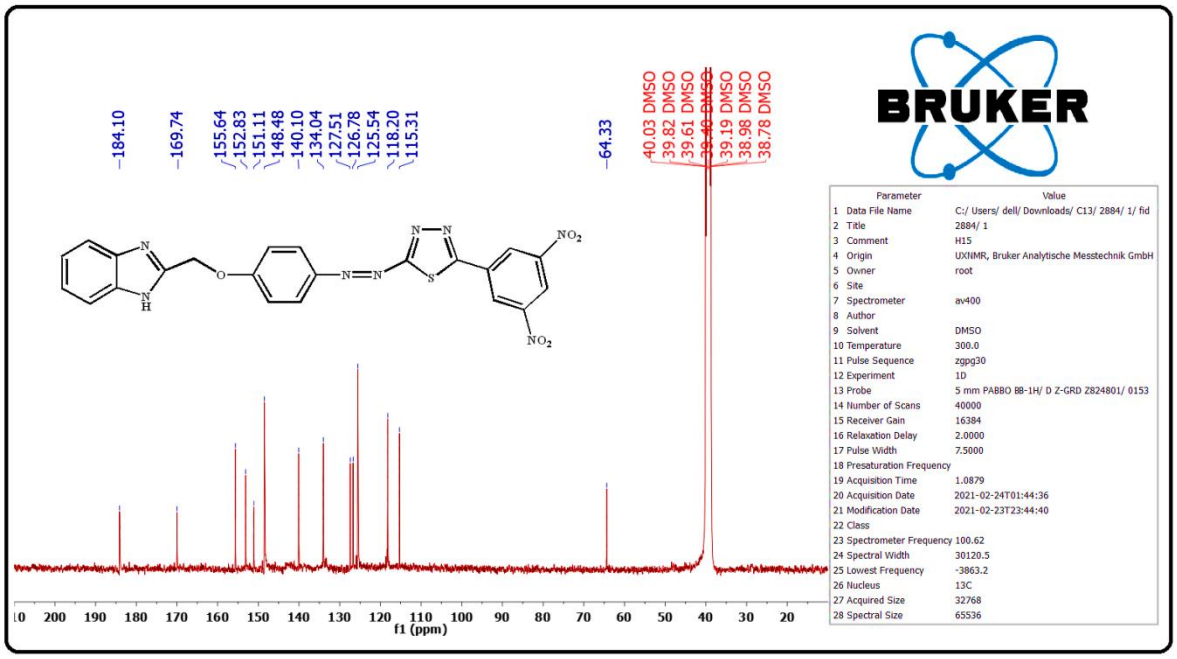


Figure (3-90): ^{13}C-NMR spectrum of compound (39)

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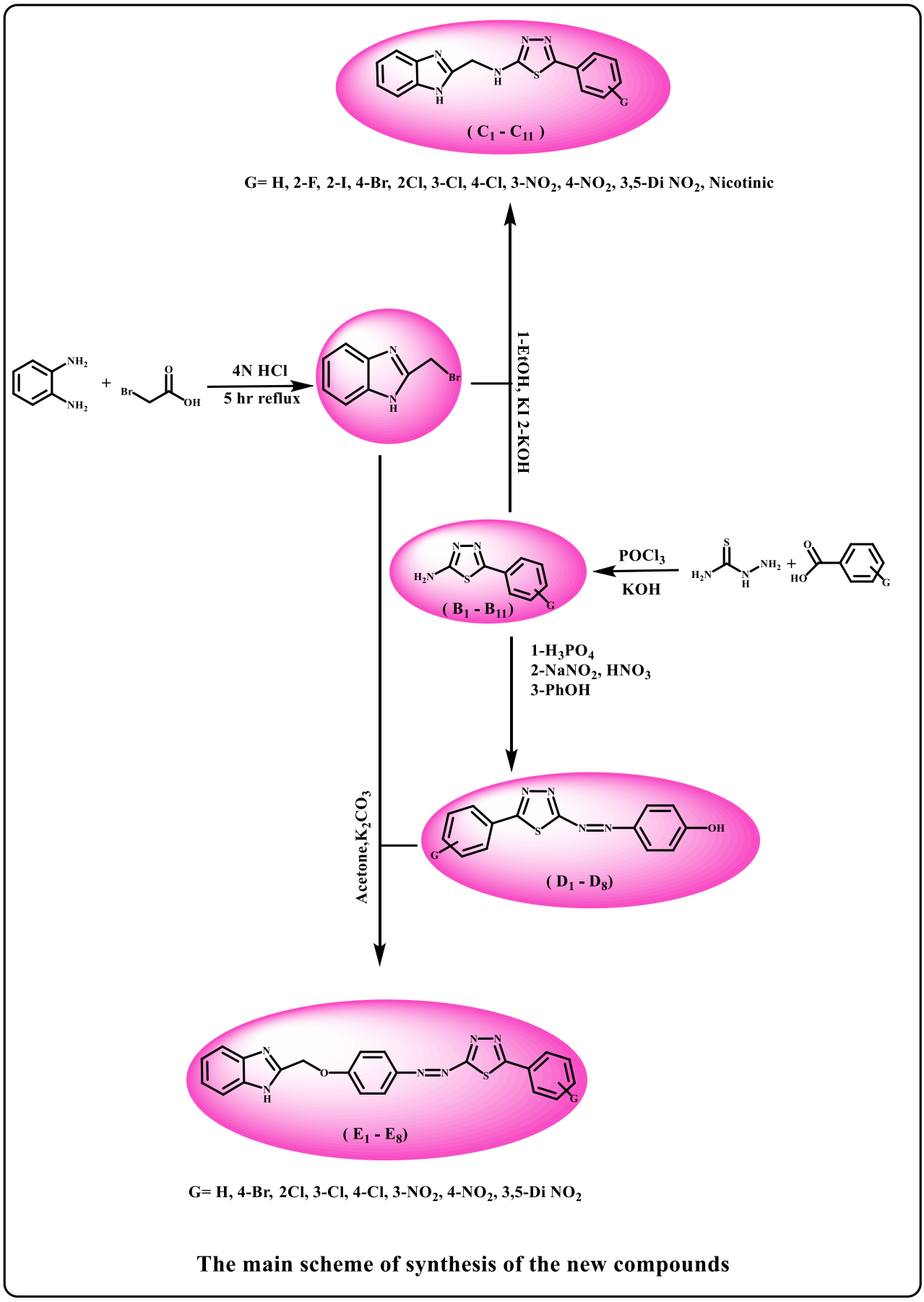
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The main scheme of synthesis of the new compounds

الخلاصة

تضمنت الدراسة الحالية تحضير بعض المشتقات الجديدة للبنزايميدازول تحتوي على حلقة ٤,٣,١- ثاياديازول ومجموعة الازو. يمكن اجمال العمل المنجز من خلال الخطوات التالية:

الخطوة الأولى: تتضمن تحضير المركب ٢- بروموميثيل بنزايميدازول (1) من تفاعل الاورثو فنيلين ثنائي أمين مع برومو حامض الخليك في 4N HCl كعامل مساعد.

الخطوة الثانية: تتضمن تحضير مشتقات ٤,٣,١- ثاياديازول (12-2) من تفاعل معوضات حامض البنزويك مع ثايوسيميكاربازايد وباستخدام $POCl_3$ كعامل مساعد.

الخطوة الثالثة: تتضمن تحضير مشتقات البنزايميدازول (23-13) بواسطة تفاعل ٢- بروموميثيل بنزايميدازول (1) مع المركبات (12-2) وبوجود KI, KOH كعوامل مساعدة وباستخدام الايثانول كمذيب.

الخطوة الرابعة: تتضمن تحضير مشتقات الازو (31-24) من تفاعل مشتقات ٤,٣,١- ثاياديازول (2,5 and 6-11) مع كل من H_3PO_4، $NaNO_2$، HNO_3 والفينول.

الخطوة الخامسة: تتضمن تحضير مشتقات البنزايميدازول (39-32) بواسطة تفاعل ٢- بروموميثيل بنزايميدازول (1) مع المركبات (31-24) وبوجود K_2CO_3 كعامل مساعد وباستخدام الاسيتون الجاف كمذيب.

شخصت هذه المركبات بواسطة اطياف الاشعة تحت الحمراء (FT-IR) وبعضها بواسطة أطياف الرنين النووي المغناطيسي (^1H and ^{13}C-NMR) لبعض المركبات المحضرة، بعد ذلك درست الفعالية البايلوجية لبعض المركبات المحضرة ضد اثنتين من العزلات البكتيرية الموجبة لصبغة جرام (Gr^{+ve}) هي (*Staphylococcus epidermidis*, *Staphylococcus aureus*) واثنتين سالبة لصبغة جرام (Gr^{-ve}) هي (*Pseudomonas aruginosa*, *Escherichia Coli*) بطريقة الانتشار (Disk Diffusion Method) وقد أظهرت بعض المركبات المختبرة فعالية جيدة كمضادات للبكتريا. وفي اخير خطوة تمت دراسة الارتباط الجزيئي (Molecular docking) للمركبات التي الاكثر فعالية (10 و 11 و 39).



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى - كلية التربية للعلوم الصرفة



تحضير وتشخيص مشتقات جديدة للبنزايميدازول تحتوي على حلقة4,3,1-ثاياديازول المرتبطة الى مجموعة الازو ودراسة الفعالية المضادة للبكتيريا لها

رسالة مقدمة الى
مجلس كلية التربية للعلوم الصرفة / جامعة ديالى
وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

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