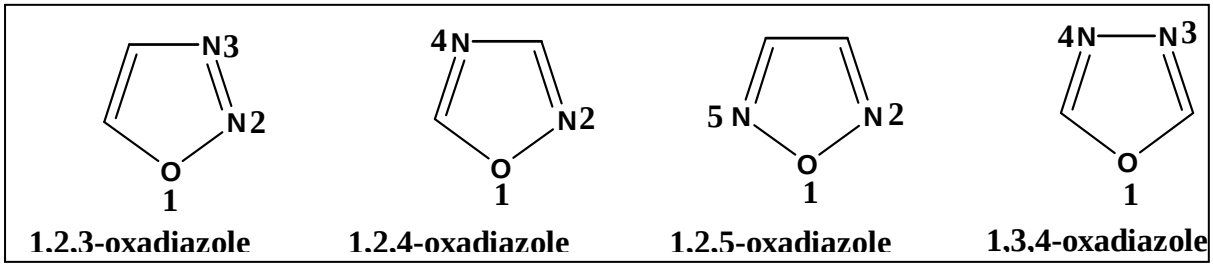
Chapter One

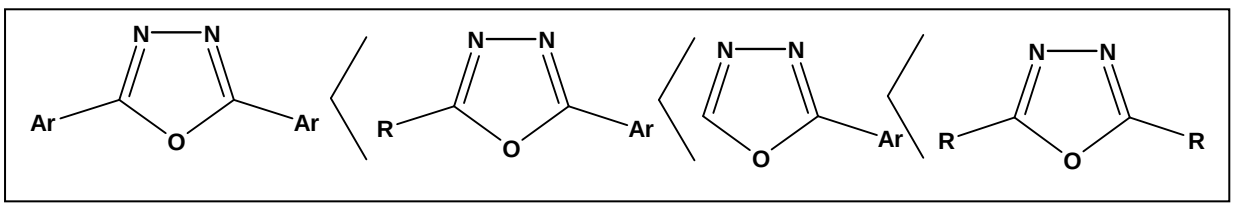
1. Introduction

1.1 Oxadiazoles:

The oxadiazoles are five membered ring aromatic compounds with three heteroatoms one oxygen and two nitrogen atoms , which have four different isomers as shown bellow[1] :



1,3,4-oxadiazole is most thermally stable isomer and its stability is controlled in general by the electron density at the C-2 and C-5 atoms, which is largely dependent on the substitute. The stability of 1,3,4-oxadiazole is especially enhanced by alkyl and aryl substitution at positions 2 and 5[2]. In general, the aryl substituted 1,3,4-oxadiazoles are less sensitive to acid than the alkyl-substituted ones. ***Grekov et al.***[3] have established that the susceptibility to hydrolysis increases with increasing solubility as in the series shown below:



Derivatives of 1,3,4-oxadiazoles represent an important family of heterocyclic compounds, since many of them display a remarkable biological activity like antibacterial[4], antifungal[5], antitumor[6] ,analgesic[7], anti-inflammatory[8] and anticonvulsant[9]. Some material applications of 1,3,4-oxadiazole lie in the field of photography and liquid crystals[10]. Moreover various 1,3,4-oxadiazoles are suitable for uses in dyestuffs industry[11], polymers[12], and corrosion inhibitors[13]. In agriculture, 1,3,4-oxadiazole derivatives are used as insecticides[14], in combating unwanted vegetation[15], and in preventing nitrification of the soil[16].

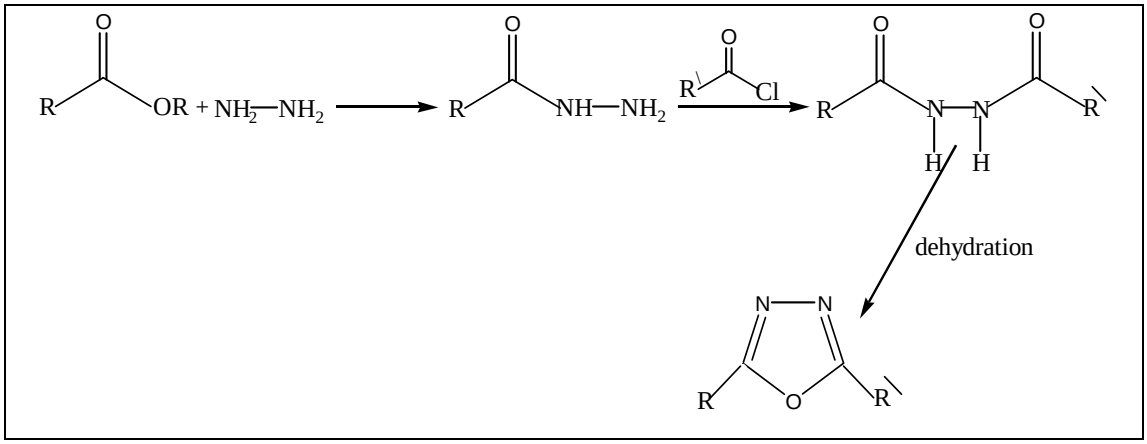
1.2 Methods for Synthesizing 1,3,4 – Oxadiazoles :

Several methods have been used to synthesize 1,3,4–oxadiazoles. Among these, the following are the most important methods:-

1.2.1 Dehydration of Acid Hydrazides :

The common synthetic approach to oxadiazoles involves cyclization of diacid hydrazine. Hydrazides and related compounds have been described as useful building blocks for assembly of various heterocyclic rings[17].

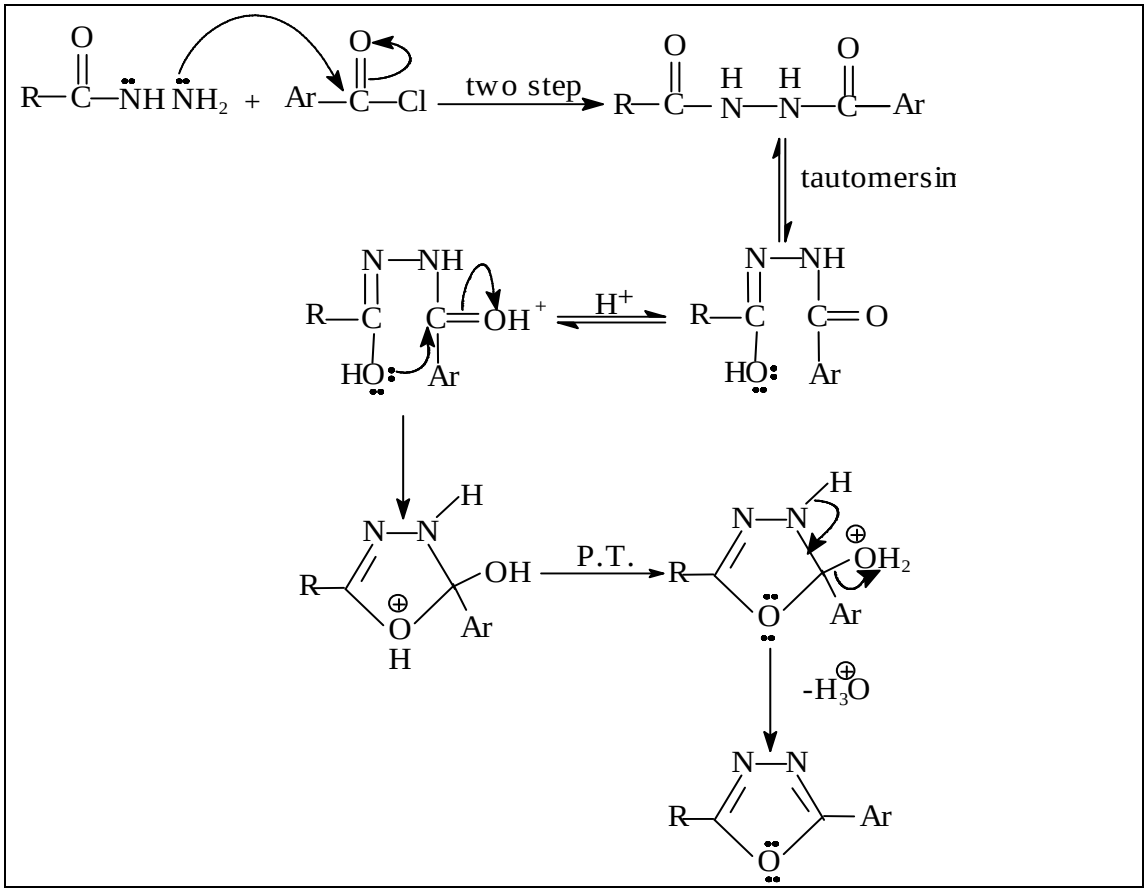
Acid hydrazides are usually prepared from corresponding esters with hydrazine hydrate. These hydrazides are converted to di-acid hydrazides through their reaction with appropriate acid chlorides. The di-acid hydrazides are established to be the most convenient precursors for the synthesis of substituted 1,3,4 – oxadiazoles[18].



Where R`=phenyl

R=methyl

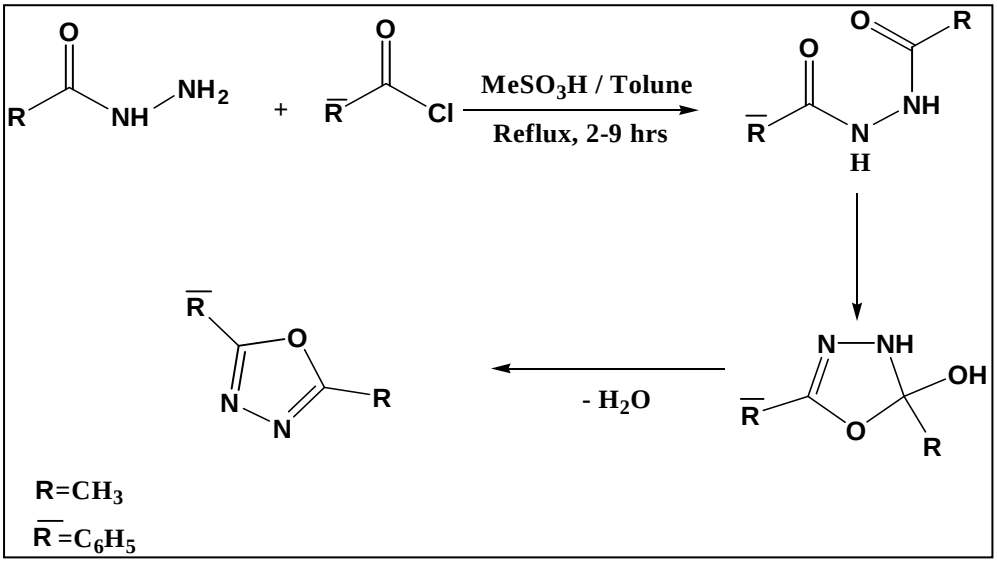
The mechanism of this reaction [19] is depicted in scheme (1.1)



Scheme (1.1) Mechanism of formation of 1,3,4 oxadiazole ring by dehydration of di -acid hydrazide

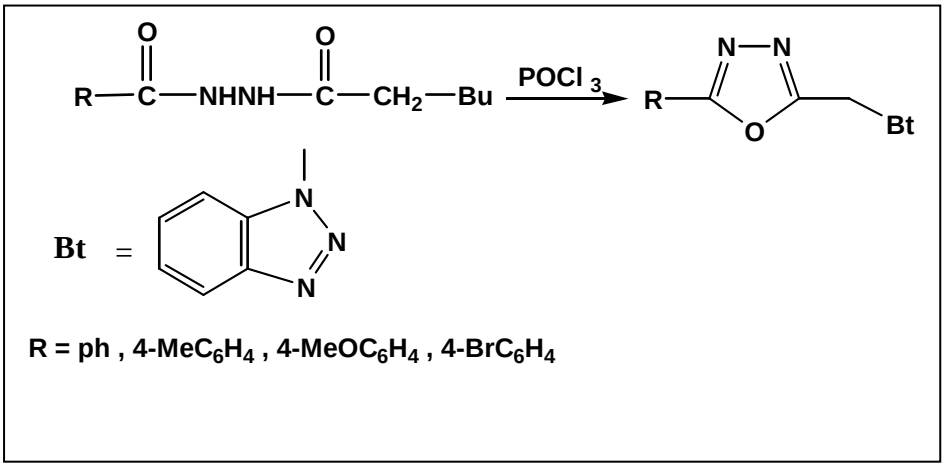
Different dehydrating agents can be used such as phosphorous oxychloride[20] , phosphorous pentaoxide[21] , zinc chloride, thionyl chloride [22], and phosphorus pentachloride[23] .

Recently, 1,3,4 –oxadiazoles derivatives have been synthesize using a new methods by treatment of a suspension of acid hydrazide with acid chloride in the presence of an equimolecular amount of methane sulfonic acid at room temperature , and then heating to reflux temperature gave 1,3,4-oxadiazoles[24] .

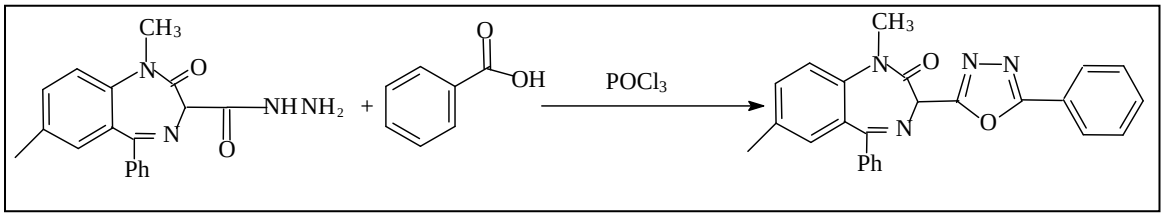


In the last few years, a great number of the 1,3,4-oxadiazole derivatives were synthesized; the following examples include some of these compounds:

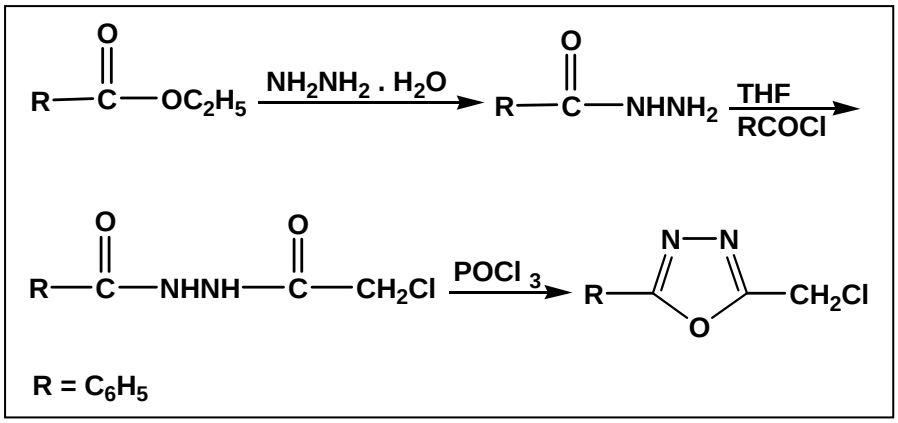
Katritzky el al.[25] synthesized 1-([1,3,4]-oxadiazole -2-yl-methyl)-1-H- benzotriazoles, they used an ester as starting material.



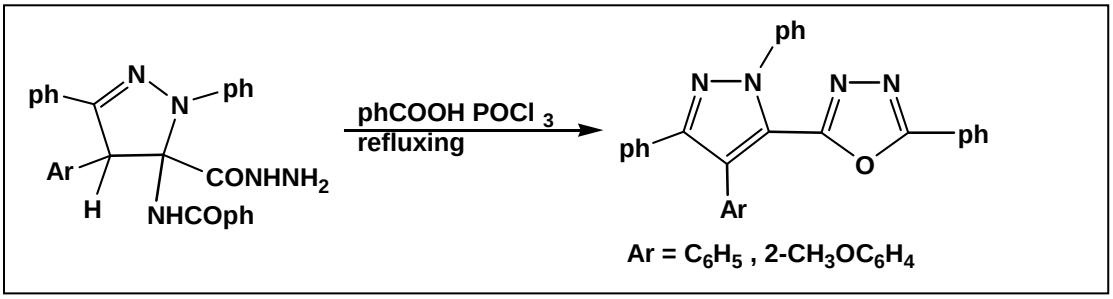
Berghot.[26] synthesized a new 1,3,4-oxadiazole derivatives in one step reaction of an acid hydrazide with benzoic acid in the presence of phosphorusoxychloride.



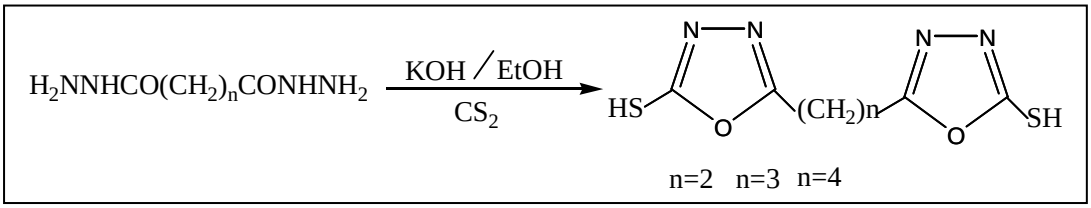
S.Cao[27] synthesized 5-aryl-2-chloromethyl-1,3,4-oxadiazoles by cyclodehydration of N-chloroacetyl-N-arylhydrazines in boiling $POCl_3$



A.K.Mansour[28] synthesized 5-phenyl-2-(1,3,4-triphenyl pyrazol-5-yl)-1,3,4-oxadiazole by treatment of 4-aryl-5-benzoylamino1,3,4-diphenyl-2-pyrazdine-5-carbohydrazides with benzoic acid and phosphorus oxychloride was found to proceed via concurrent cyclocondensation and elimination of a benzamide molecule.

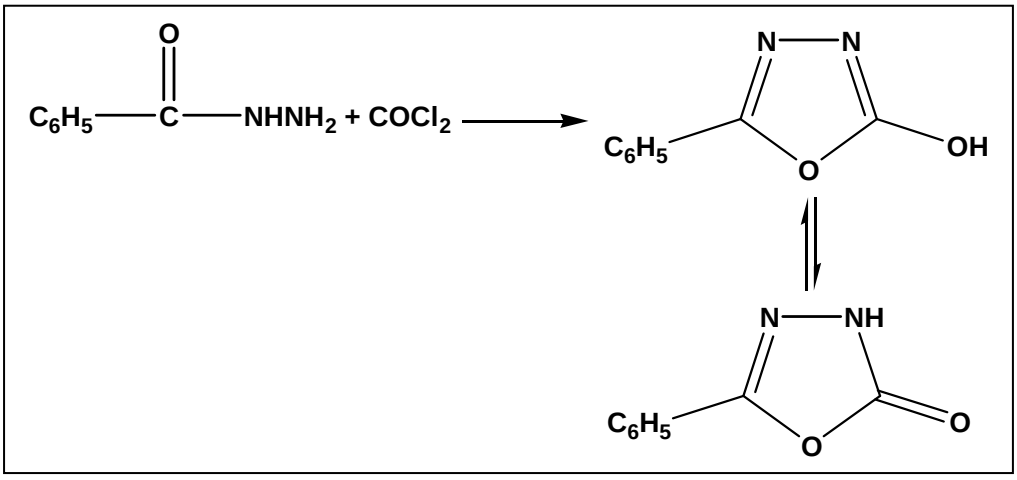


A.O.mostat. et al. [29] reported the preparation of bis [5-mercapto-1,3,4-oxadiazole-2-yl] alkanes from the reaction of alkanedioic dihydrazides with carbon disulfide in alcoholic potassium hydroxide.



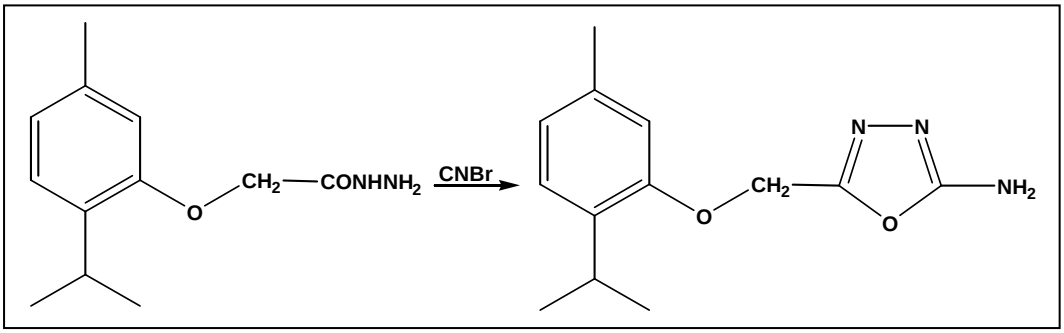
1.2.2 Reaction of acid hydrazide with phosgene:

Hydroxyl-1,3,4-oxadiazoles or 5-substituted-1,3,4-oxadiazole-2-one are obtained from the reaction of the appropriate hydrazide with phosgene in chloroform, for example, benzoyl hydrazide and phosgene gave -2-phenyl-5- Hydroxyl-1,3,4-oxadiazole in quantitative yield[30].



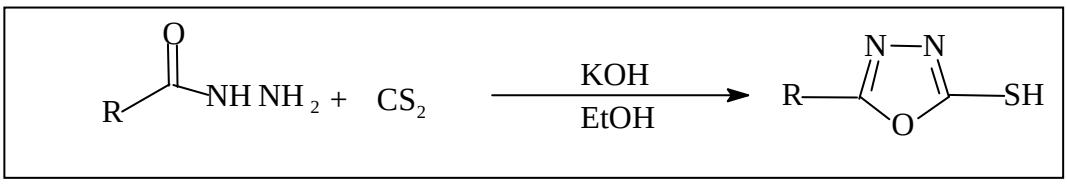
1.2.3 Reaction of acid hydrazide with cyanogenbromide:

Acid hydrazides are reacted with cyanogenbromide to form 1,3,4-oxadiazoles with amino group at position (2). **Roda**[31] synthesized 2-amino-5-(2-isopropyl-5-methylphenoxymethyl)-1,3,4-oxadiazole according to this method.



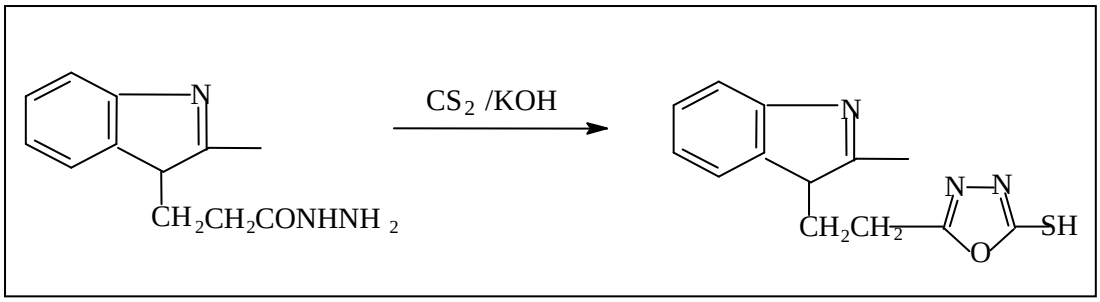
1.2.4 Reaction of acid hydrazide with carbon disulfide:

2-mercapto-1,3,4-oxadiazole ring is synthesized by following the general method of Young and **Wood** i.e. by refluxing ethanolic mixture of the appropriate acid hydrazide , carbon disulfide and potassium hydroxide[32].



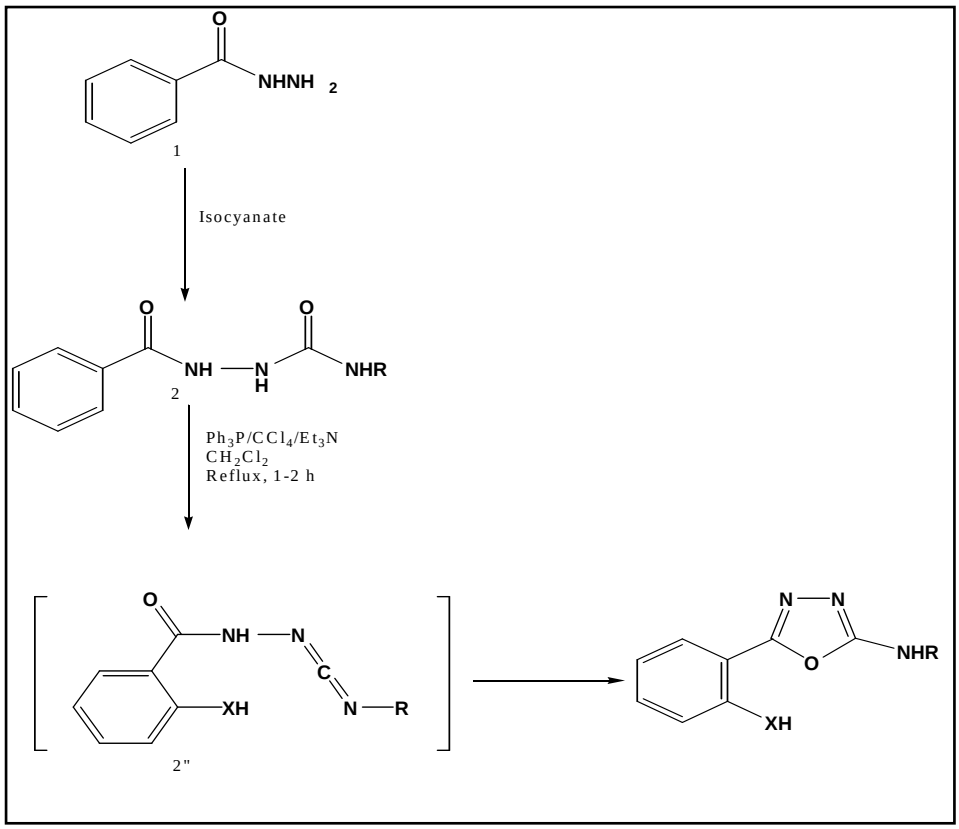
Where R=phenyl group

El–Masry et al.[33] Synthesized 5-[2-(2-methylbenzimidazol-1-yl)ethyl]-[1,3,4]-oxadiazole-2(3H)-thione from the reaction of 3-(2-methylbenzimidazol-1-yl) propinoic acid hydrazide with carbon disulfide and potassium hydroxide.



1.2.4 Reaction of acid hydrazide with isocyanates (Appel's Method)[34]*:*

The preparation of 2- substituted amino – 1,3,4-oxadiazoles from acid semicarbazide (2) was conducted. Thus, treatment of acid semicarbazide (2), Which were readily obtainable by the reaction of acid hydrazide with isocyanate , under Appel′s dehydration condition (Ph_3P/CCl_4 / Et_3N)[35] smoothly afforded 1,3,4-oxadiazoles via carbdiimide intermediate ($2'''$).

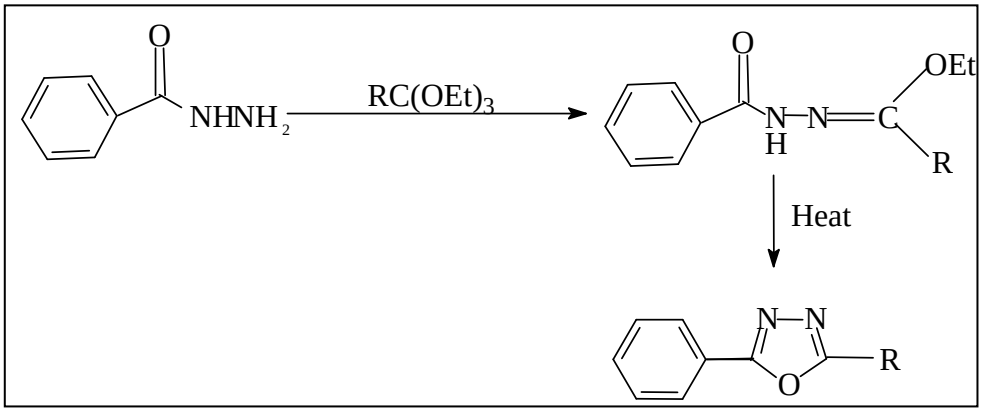


Where R = amino group

The advantage of the present method is cheap, non toxic, stable, and easy to handle.

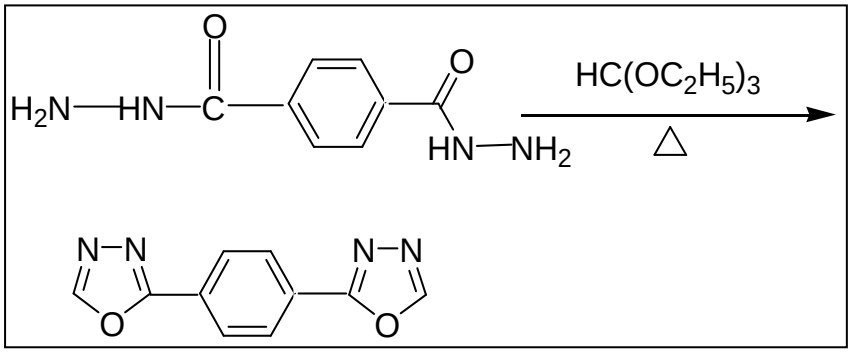
1.2.6 Reaction of acid Hydrazides with Orthoesters:

Treatment of the acid hydrazides with orthoesters $RC(OEt)_3$ gave N-aroylhydrazoic esters, which are cyclized upon heating to 1,3,4 – oxadiazoles.



Where R= phenyl group

Also, bis(1,3,4-oxadiazol-2yl)-*p*-phenylene, was prepared by heating under reflux terphthalic acid dihydrazide in triethylorthoformate [36]

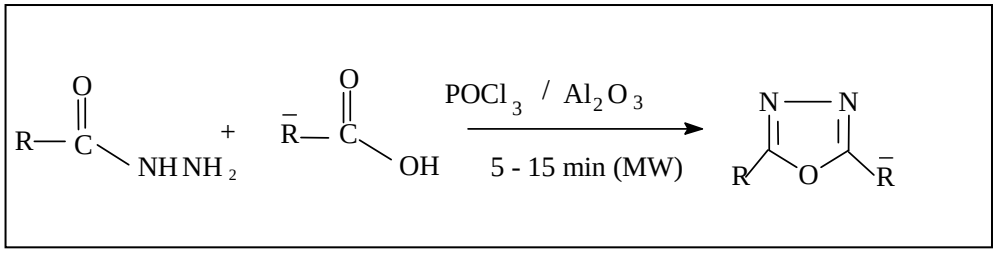


1.2.7 Microwave method [37-40] *:*

Recent advances in technology have now made microwave energy a more efficient means of heating reactions. Chemical transformations that took hours, or even days, to complete can now be accomplished in minutes. Microwave energy offers numerous benefits for forming synthesis including increased reaction rates, yield enhancements, and cleaner chemistries.

The microwave technology has been applied to a number of useful research and development processes such as polymer technology, organic synthesis, application to waste treatment, drug release / targeting; ceramic and alkane decomposition.

Khalid M.Kham et. al.[41] synthesized a number of 2,5-disubstituted 1,3,4 – oxadiazoles under microwave irradiation through the reaction of commercially available hydrazides with different carboxylic acids in the presence of phosphorous oxychloride.



Where R=methyl

R`=phenyl

Finally, this method provides an excellent approach for the safe, rapid, inexpensive and simple synthesis of medicinally important 2,5-disubstituted – 1,3,4 – oxadiazoles . Table (1-1) shows further examples of using these methods

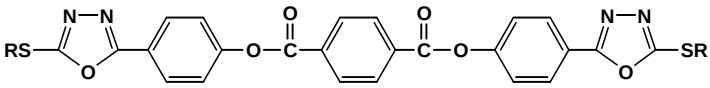
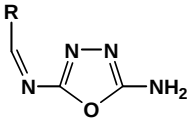
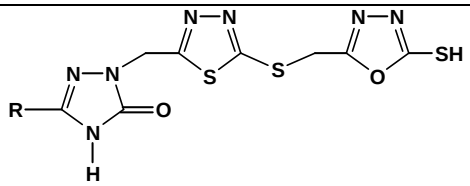
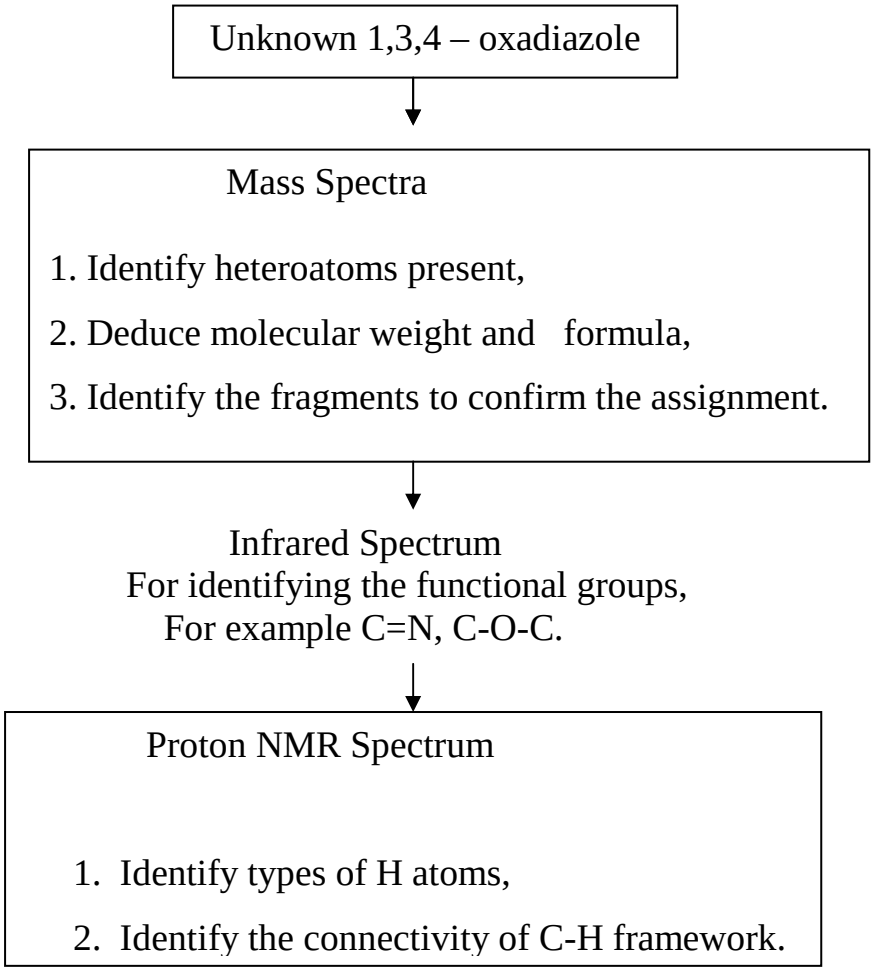
No.	Structure	Method	Ref.
1	 R= ethyl	**Reaction of acid hydrazide with carbon disulfide**	**42**
2	 R=4-MeC_6H_4	**Reaction of acid hydrazide with cyanogen bromide**	**43**
3	 R=phenyl	**Reaction of acid hydrazide with carbon disulfide**	**44**

Table (1-1) Methods for synthesizing 1,3,4-oxadiazole

1.3 Spectroscopic Identification of 1,3,4 – oxadiazoles:

Molecular, electronic, and nuclear resonance spectra have been used in the elucidation and identification of different 1,3,4 – oxadiazole derivatives. A suggested strategy for the identification process is outlined in scheme (1.2).

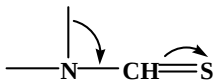


Scheme (1.2) A flowchart summarizing a strategy for the identification of 1,3,4-oxadiazole derivatives.

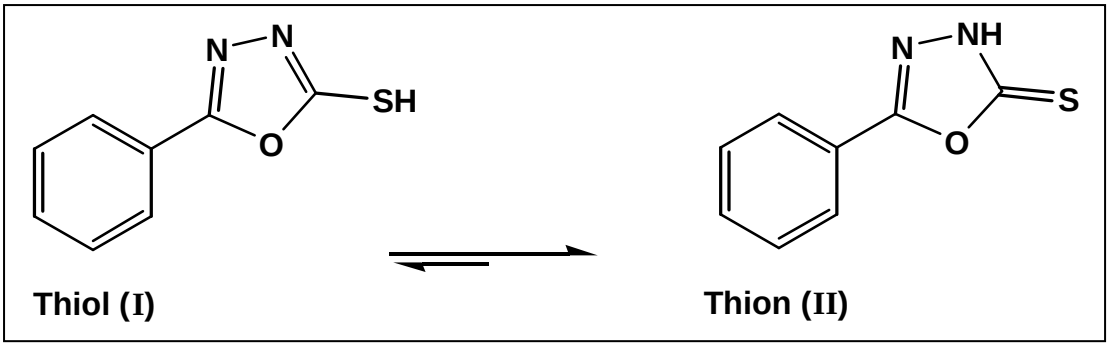
The analysis begins with the mass spectrum, which is used to find the molecular weight and the molecular formula of the 1,3,4-oxadiazoles.The mass spectrum is used to identify whether atoms, N,O,...etc are present. After interpretation of the mass spectrum comes step(2), in which the IR spectrum is interpreted to identify which major function groups are present (C=N, C-O-C, C=O, etc). Once functional groups have been identified, step (3) is undertaken, in which the proton NMR spectrum is used to categorize the different C-H fragment.

1.3.1 Infrared spectroscopy

In the IR field, the oxadiazole ring has been characterized primary by the bands at about 1020-1050cm^{-1}, 1245-1255cm^{-1}, due to the symmetrical and asymmetrical C-O-C stretching[45], and about at 1600-1615 cm^{-1} due to the C=N stretching frequency[46], besides this, amino-oxadiazoles are characterized by a doublet in the region 3440-3330cm^{-1} due to the symmetric and asymmetric stretching vibration of $–NH_2$ group[47]. Mercapto-oxadiazoles, displayed absorption bands in the regions (2600-2550 cm^{-1}) and (1380-1310cm^{-1}), due to the (S-H) and (C=S) stretching vibration[48] respectively, the absence of peaks in the region (2600-2550cm^{-1}) established the existence of the thione form in the soild states, because 2-mercapto-1,3,4-oxadiazoles, like all other compounds containing

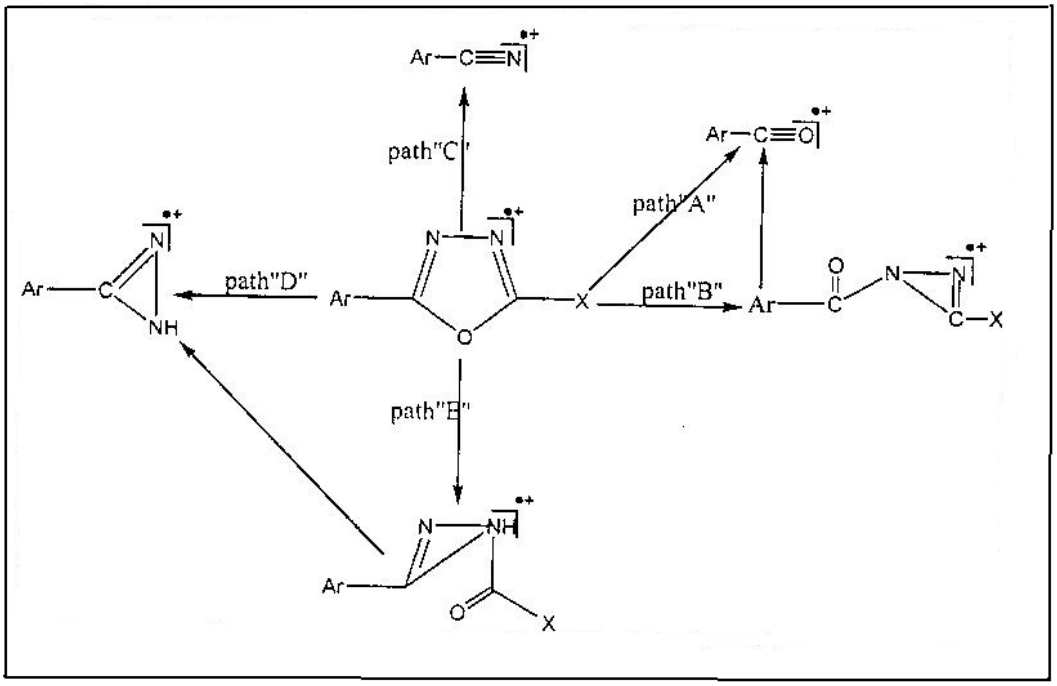


moiety could exist in two equilibrium tautomeric forms enol form (I) and keto form (II). Despite the fact, that keto form is comparatively more stable [49].



1.3.2 Mass Spectral Studies of 1,3,4-oxadiazoles:

The structure of 1,3,4-oxadiazoles have been confirmed by mass spectral studies. The fragmentation of 2-substituted-5-aryl-1,3,4-oxadiazole is initiated mainly by five routes indicated by path “A”, path “B”, path “C”, path “D” and path “E”, scheme (1.3)



Where Ar= $HO\text{-}C_6H_4$, $H_2N\text{-}C_6H_4$

X= SH

Scheme (1.3) fragmentation pattern of 1,3,4-oxadiazole ring

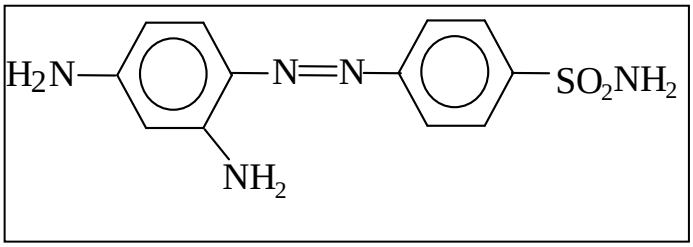
This can be shown in the mass spectrum of 5-aryl-1,3,4-oxadiazole-2-thiol (X=SH), (Ar=HO-C_6H_4, H_2N-C_6H_4-) which shows interesting ions at m/z 121 (HO-C_6H_4-C$\equiv$$O^+$) and m/z 120 (H_2N-C_6H_4-C$\equiv$$O^+$) respectively, these ions may be formed by direct fragmentation, path "A", or via a diazirine intermediate, path "B",. The formation of number of significant peaks in these spectra, such as $M^{\cdot+}$ -75, path "C", and $M^{\cdot+}$ -60, path "D" and path "E" also is explained in terms of the diazirine intermediates or by direct fragmentation[50].

1.4 Biological Activityof 1,3,4-oxadiazole:

Antimicrobials:

Antimicrobial chemotherapeutic agents are chemical agents that have the ability to destroy the pathogenic microorganisms or inhibit their growth at concentrations low enough to avoid undesirable damage to the host[51]. Most of these agents are antibiotic microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth. The modern era of chemotherapy began with the work of the German physician ***paul Ehrlich*** (1854-1915) who began experimenting with dyes, ***Ehrlich's*** successes in the chemotherapy of sleeping sickness and syphilis established his concept of selective toxicity and led to the testing of hundreds of compounds for their therapeutic potential. In 1927, the German chemical industry began along-term search for chemotherapeutic agents under the direction of ***Cerhard Domagk*** who discovered that prontosil red [I] a new dye for staining leather, was nontoxic for animals and completely protected mice against pathogenic bacteria ***Streptococci*** and ***Staphylococci*** . In 1929 the scottish physician ***Alexander Fleming*** found that broth from ***penicillium*** culture contained penicillin, an antibiotic that could destroy a number of pathogenic

bacteria. The discovery of penicillin stimulated the search for other antibiotics [51]. ***Selman waksman*** and his associates lead to isolation of streptomycin in 1944 from ***streptomyces griseus*** [52]. The development of the broad spectrum antibiotics such as chloroamphenicol in1947which isolated from ***Streptomyces venzuelae*** [53]. Chlorotetracycline in 1948 from ***Streptomyces aureofaciens***[54] and isolation of antifungal nystatin in 1951 from ***Streptomyces noursei***[55], this was led to broad screening programs that were set up to find agents which would be effective in the treatment of infections that had been resistant to chemotherapeutic agent, as well as to provide safer and more rapid therapy for infections. As a result large numbers of antibiotics were discovered such as erythromycin [56] , Lincomycin[57] , gentamycin[58] and the researches were continued in order to discover new antibiotics.



(I)

2,4-diamino azobenzene-4-sulfonamide
(prontosil red.)

1.4.1 Resistance to Antimicrobial agents:

Sooner or later bacteria develop resistance to virtually any antibiotic agents. Resistance has many consequences and also requires the use of more toxic or more expensive alternative drugs [59]. Resistance thus affects the antibiotic options available to every practitioner and is no less a problem in the developing world [60].

Despite the versatility of antimicrobial agents and the evolution of new ones, bacteria have a limited number of mechanisms of antimicrobial resistance; including: The production of detoxifying enzyme like β-lactamase which destroy β-lactam antibiotics, or the production of chloramphenicol acetyl-transferase in gram-negative chloramphenicol resistant bacteria[61-62]. In some cases the alteration in the target for the drug; which includes both reduction of receptor affinity and the substitution of an alternative pathway [61]. For example, resistance to some penicillins and cephalosporins may be a function of the loss or alternation of the penicillin binding proteins (PBP_S) of bacterial ribosome [63].

Resistance may arise by a mutation that reduces target affinity or allows the over production of a drug modifying enzyme, sometimes the insertion of foreign DNA by recombination accomplishes the same end [61]. Also decreased antibiotic uptake; which occurs through either diminished permeability or an active efflux system [61]. In ***Pseudomonas aeruginosa,*** 50 to 85% of resistant strains produce chloramphenicol acetyl transferase [64] (CAT). In the remainder, resistance is caused by decreased outer membrane permeability[65-66] reported that the active efflux of tetracycline is mediated by new membrane transport system ***Pseudomonas aeruginosa*** can also become resistant specifically to imipenem through the loss of an outer membrane protein (porin) that provides a channel for the entry of imipenem[67].

In general the activities of antibacterial agents are related to:

a) Inhibition of cell wall synthesis such as cycloserine[68] , penicillin Binding Protein[69] and bacitracin[70]
b) Alteration of cell membrane permeability or inhibition of active transport across cell membrane such as surfactants[71]**.**
c) Inhibition of protein synthesis i.e. “Inhibition of translation and

transcription of genetic material"., such as Erythromycin[72]. Streptomycin [73] and tetracycline [74].

d) Inhibition of nucleic acid synthesis such as sulfonamide [75] and nalidixic acid [76].

1.4.2 Antimicrobial Activity of 1,3,4-Oxadiazole Compounds:

Microorganism causes different kinds of diseases to human and animals. Discovery of antimicrobial agents played a very important role against infections caused by bacteria. For this reason searching for new antimicrobial agents is a continuous process and great efforts have been employed to find new antibiotics or new chemical compounds to show a high degree of selective toxicity toward microorganism. 1,3,4-oxadiazoles constitute an important class of compounds having a wide spectrum of biological activity.

In the past years considerable evidence has been accumulated to demonstrate the efficacy of substituted 1,3,4-oxadiazole as antibacterial[77], antifungal[78], antimalarial[79], anticonversant[80] and anti-inflammatory[81] compounds. When properly substituted in 2-and 5-positions. 2-amino-5-substituted-l,3,4-oxadiazoles were used as antimitotic[82] muscle relaxant[83] and tranquilizing agents[84]. Moreover, some schiff bases of 2-amino -1,3,4-Oxadiazole have also shown remarkable antibacterial and antifungal activities, 2-hydroxymethyl-5-aryl-1,3,4-Oxadiazole exhibit anti-inflammatory and anticonvulsive activity. Further, it was suggested that the (-SH) group attached to a heterocyclic nucleus may induce fungicidal activity [85]. Table (1-2) Summarize structures and biological activity of some these compounds

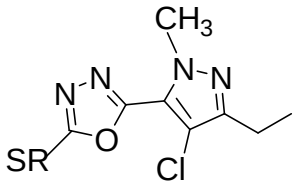
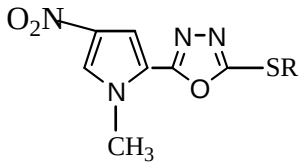
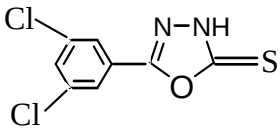
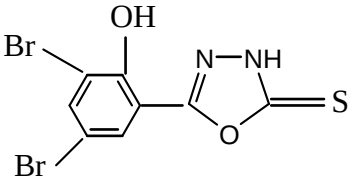
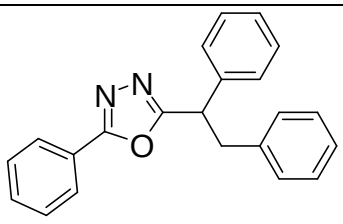
No.	Compounds name	Structure	Biological activity	Ref.
1.	2-alkyl (alkylthio)-5-(4-chloro-3-ethyl-1-methyl-1H-pyrazole-5-yl)-1,3,4-oxadiazole	 **R=CH_3**	Potential fungicides	86
2.	2-(1-methyl-4-nitro pyrroyl)-5-alkylthio-1,3,4-oxadiazole	 **R= -CH_3 , CH_3CH_2-**	Effective drugs against tropical Diseases	87
3.	5 (2,4dichlorophenyl)-1,3,4oxadiazol-2-thione		Fungi toxic activity	88
4.	5-(2-hydroxy-3,5-dibromophenyl)1,3,4-oxadiazol-2-thione		Monoamine oxidase and succinate dehydrogenase inhibitory	89
5.	2-phenyl-5-(1,2-diphenyl-ethyl)-1,3,4-oxadiazole		Anti Inflammatory	90

Table (1-2) summarization structures and biological activity of some 1,3,4-oxadiazoles derivatives

1.4 Aim of the study:

1,3,4-oxadiazole compounds play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells are based on aromatic heterocyclic.

This work was designed to reach the following targets:

1. Synthesize some new 5-(phenyl)-2-substituted-1,3,4-oxadiazoles [3-15] derived from 3-nitrobenzoic acid.

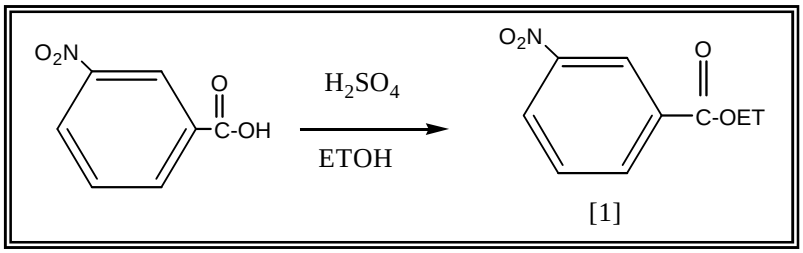
2. Elucidate the biological activity of these synthesized compounds.

CHAPTER THREE

3. Results and Discussion

3.1.1 Synthesis of ethyl- 3-nitrobenzoate [1]:

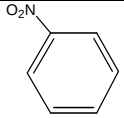
3-nitrobenzoic acid with H_2SO_4 in absolute ethanol was refluxed overnight to afford the ester. The structure of the ester was confirmed from it's melting point and FTIR spectrum.

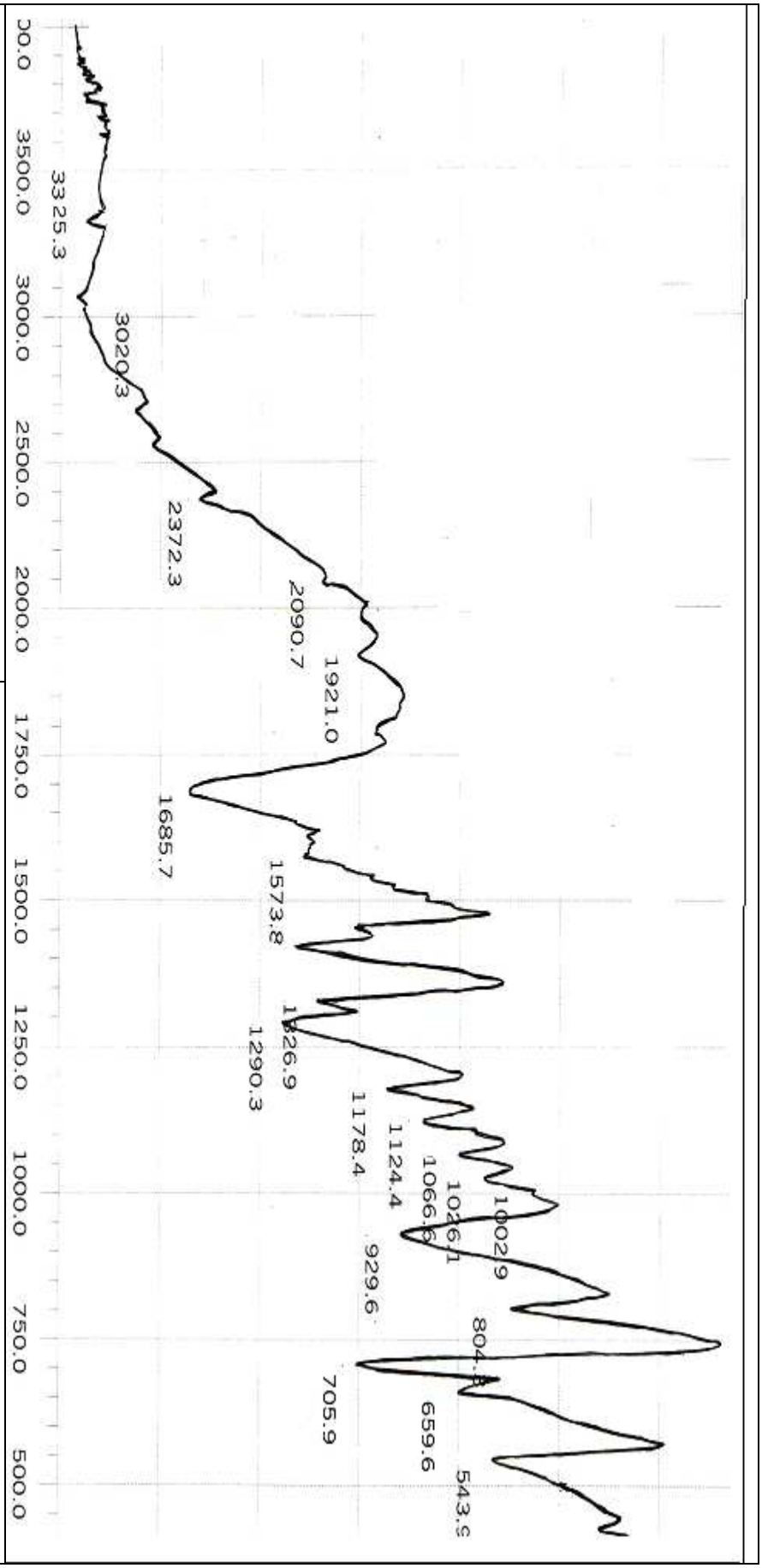


[1]

The FTIR spectrum showed the disappearance of the carbonyl band of 3-nitrobenzoic acid at (1685.7 cm^{-1}) and appearance of the ester carbonyl at (1716.5 cm^{-1}). Band of C-H aromatic appeared at (3097.5 cm^{-1}) and C-H aliphatic appeared at (2927.7cm^{-1}). Band at (1608.5 cm^{-1}) represented stretching of C=C. Figures (3-1a) and (3-1b) show the F.T.IR spectrum of the acid and ester. Table (3-1b) shows characteristic bands of compound [1].

Table (3-1): Characteristic bands of compound [1]

Comp. No.	Ar	ν(C-H) arom. cm^{-1}	ν(C-H) aliph. cm^{-1}	ν(C=O) cm^{-1}	ν(C=C) cm^{-1}	ν(C-NO_2) cm^{-1}	
						Asym.	Sym.
1		3097.5	2927.7	1716.5	1608.5	1531.4	1350.1



M.P.:140-142 °C

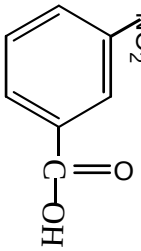
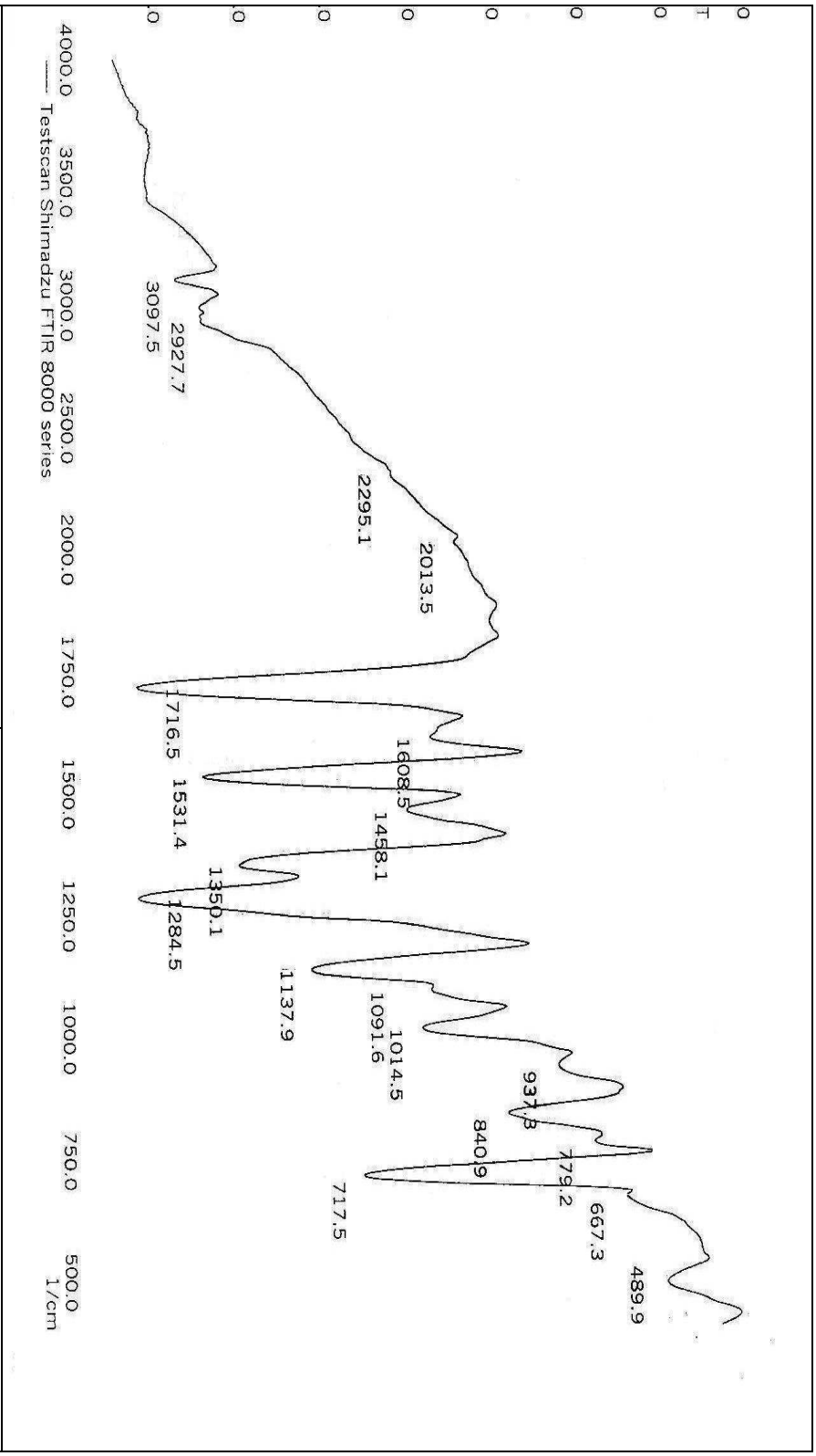


Figure (3-1a): FTIR- spectrum of 3-nitrbenzoic acid.



M.P.:45-47 °C

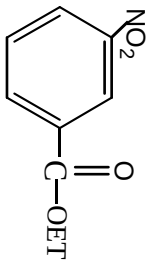
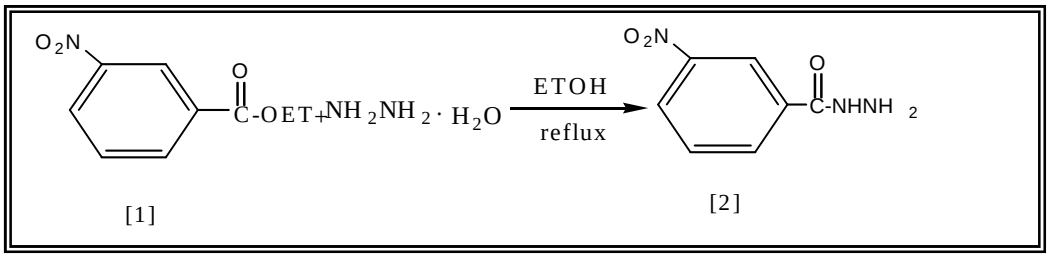


Figure (3-1b):FTIR- spectrum of Ethyl-3-nitrbenzoate.

3.1.2 Synthesis of 3-nitro benzoic hydrazide [2]:

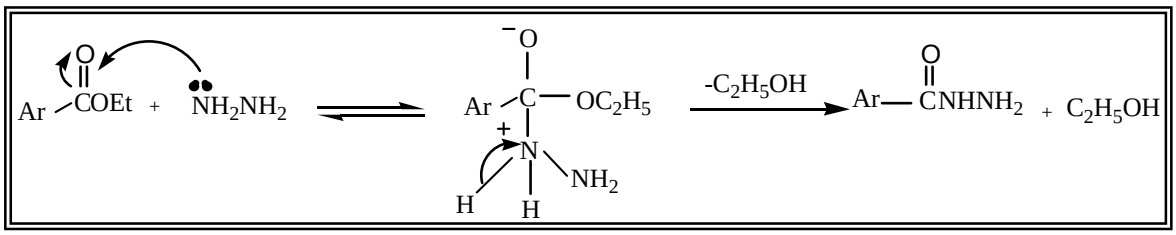
The acid hydrazide was synthesized by reacting [1] with hydrazine hydrate in absolute ethanol. The compound was confirmed from it's Lit. melting point and FTIR spectrum.



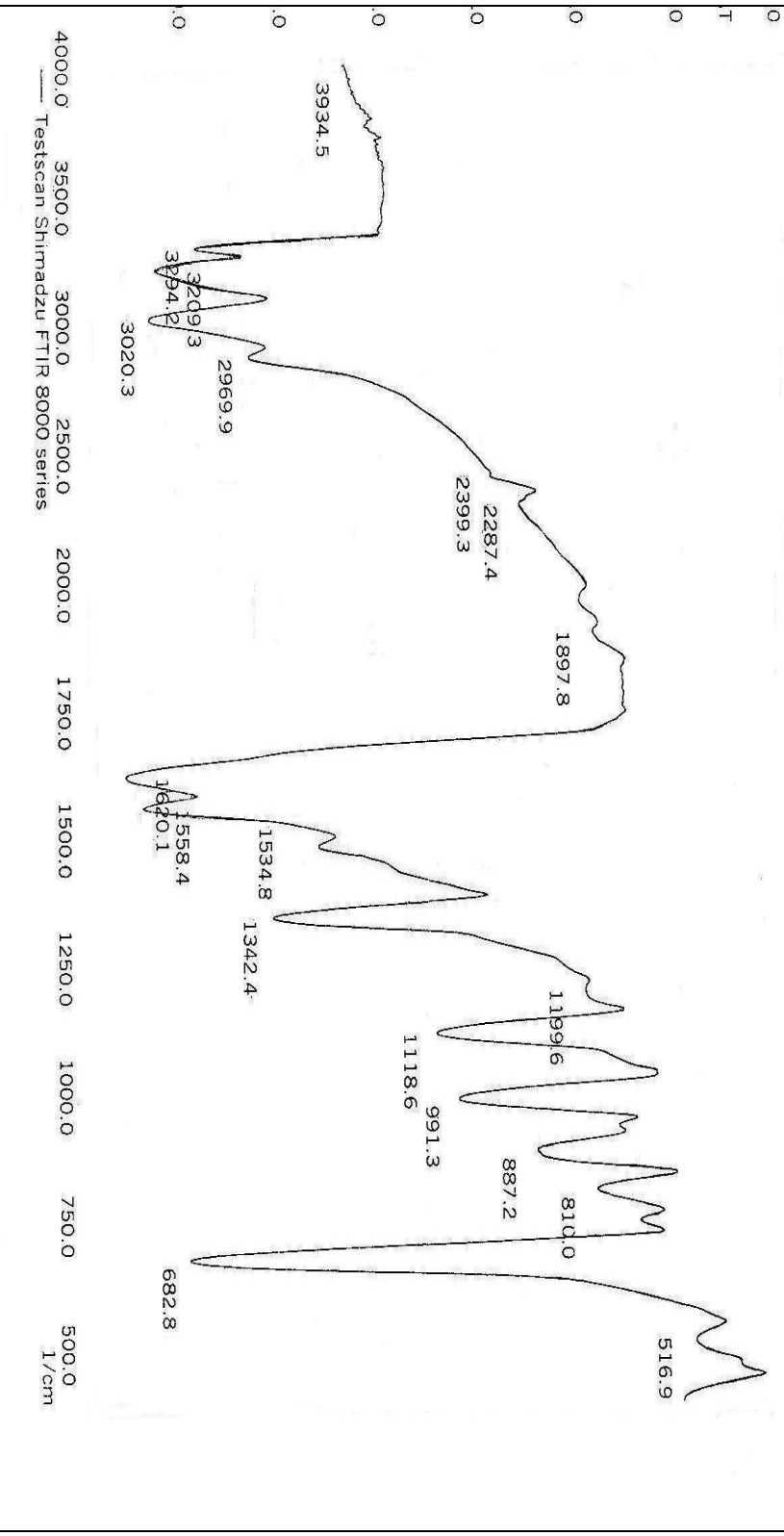
The FTIR spectrum of compound [2] indicated the disappearance of the ester carbonyl band at (1716.5 cm^{-1}) and appearance of two stretching bands of NH_2 asymmetric and symmetric at (3294.2 and 3209.3 cm^{-1}). The carbonyl amide (Amide I band) appeared at (1620.1 cm^{-1}). At (1558.4 cm^{-1}) NH δ bending (Amide II). Figure (3-2) shows the F.T.IR spectrum of compound [2]. The mechanism of the reaction [101] is as shown in scheme (3-1). Table (3-2) shows characteristic bands of compound [2].

Table (3-2): Characteristic bands of compound [2]

Comp. No.	ν(-NH-NH_2) cm^{-1}	ν(C-H) arom.cm^{-1}	ν(C=O) cm^{-1}	ν(C=C) cm^{-1}	δ(-NH) cm^{-1}	ν(C-NO_2) cm^{-1}	
2	3294.2 3209.3	3020.3	1620.1	1620.1	1558.4	Asym. ١٥٣٤.٨	Sym. 1342.4



Scheme (3-1): Mechanism steps for the preparation of compound [2].



M.P.:135-137 °C

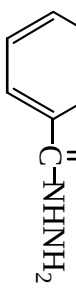
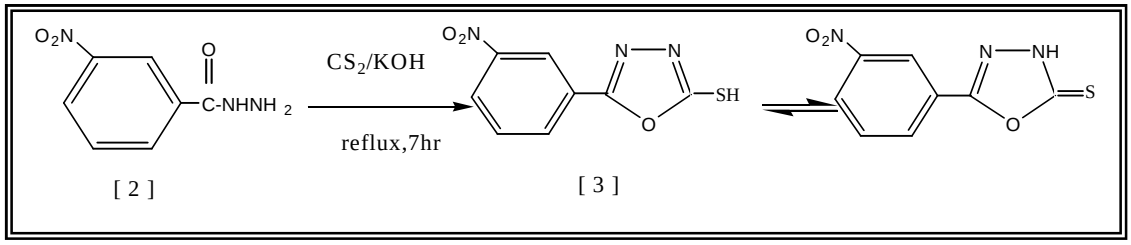


Figure (3-2): FTIR- spectrum) of 3-nitrbenzoic hydrazide.

3.1.3 Synthesis of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole [3]:

Reaction of compound [2] with CS_2, KOH in absolute ethanol afforded compound [3]. The compound was characterize by it's melting point and FTIR spectrum.

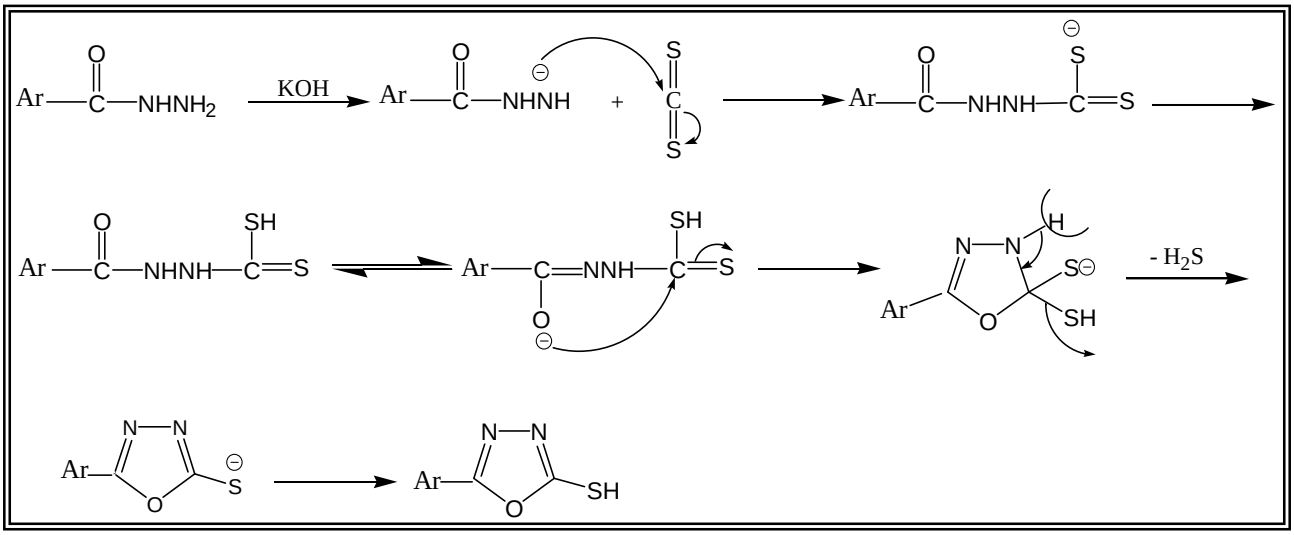


The FTIR spectrum of compound [3] indicated the disappearance of NH_2 asymmetric and symmetric stretching bands at (3294.2 cm^{-1}, 3209.3 cm^{-1}) respectively, and appearance of sulfohydryl absorption band S-H at (2521.9 cm^{-1}) and absorption of (C=S) band at (1350.1cm^{-1}). Also F.T.IR show a typical absorptions of oxadiazole ring endo cyclic C-O-C asymmetrical and symmetrical at (1292.2 and 1068.5 cm^{-1}) and absorption band of (C=N) at (1632.1 cm^{-1}). Figure (3-3) shows the F.T.IR spectrum of compound [3].Table (3-3) shows characteristic bands of compound [3].

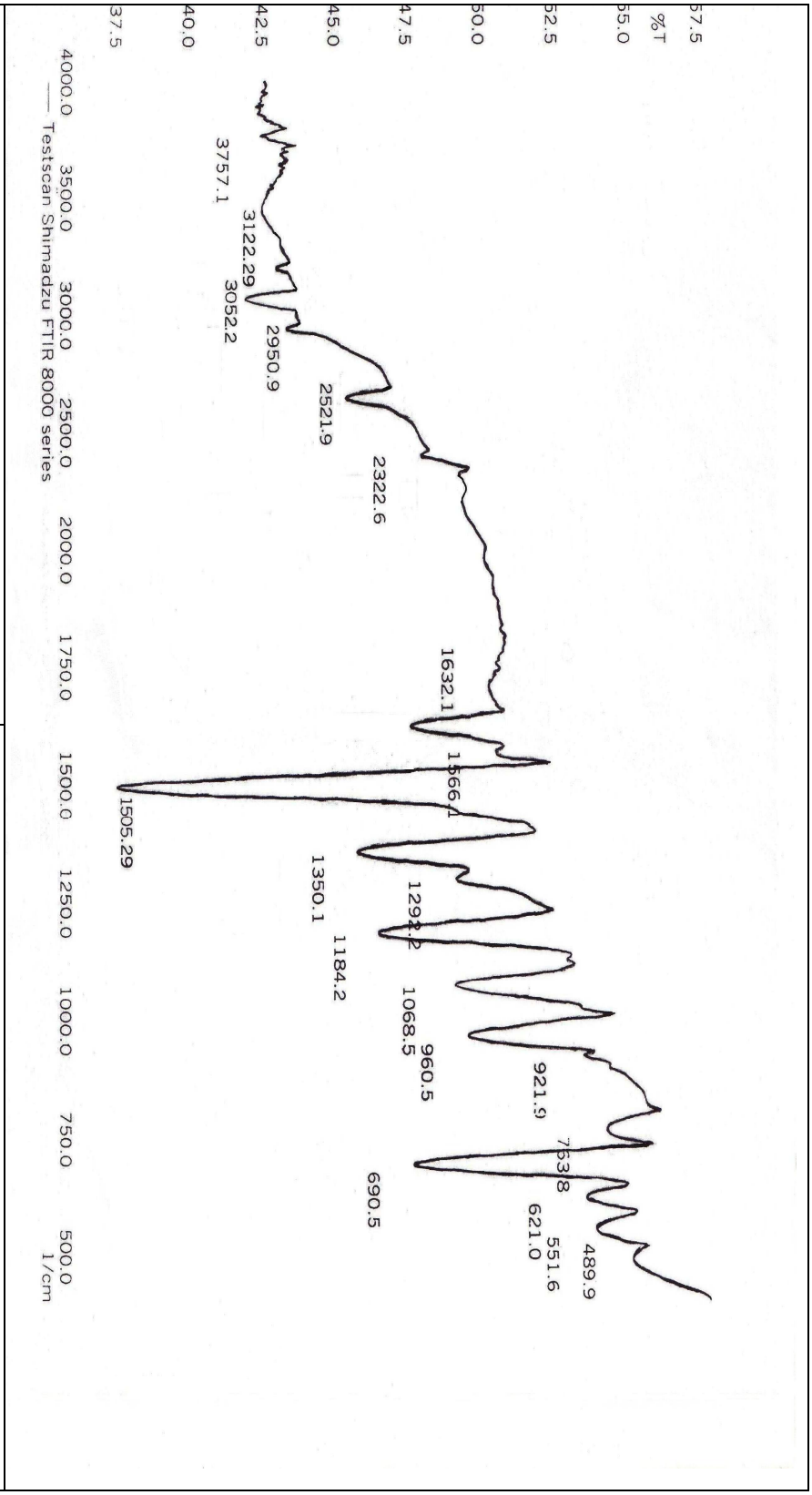
Table (3-3): Characteristic bands of compound [3].

Comp. No.	ν(C-H) arom.cm^{-1}	ν (-SH) cm^{-1}	ν(C=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C-NO$_2$) cm^{-1}		ν(C-O-C) cm^{-1}	
					Asym.	Sym.	Asym.	Sym.
3	3052.2	2521.9	1632.1	1566.1	1505.29	1350.1	1292.2	1068.5

The mechanism of the reaction [102] is as shown in scheme (3-2):



Scheme (3-2): Mechanism steps for the preparation of compound [3].



M.P. : ١٥٣ -١٥٥°C

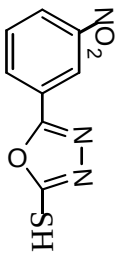
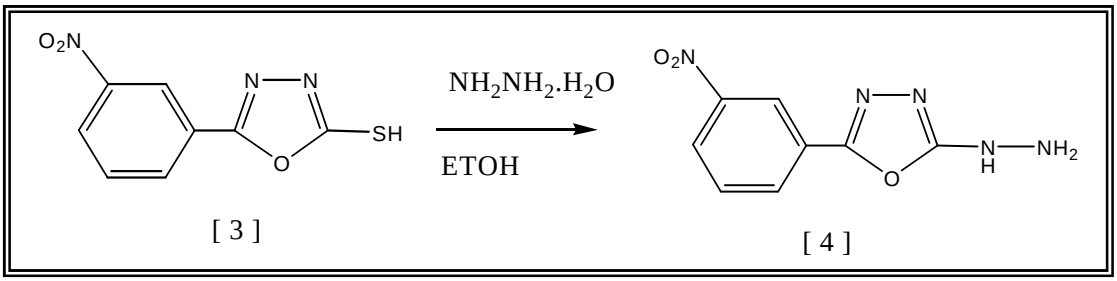


Figure (3-3): FTIR- spectrum of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole.

3.1.4 Synthesis of 2-hydrazido-5-(3-nitrophenyl)-1,3,4-oxadiazole [4]:

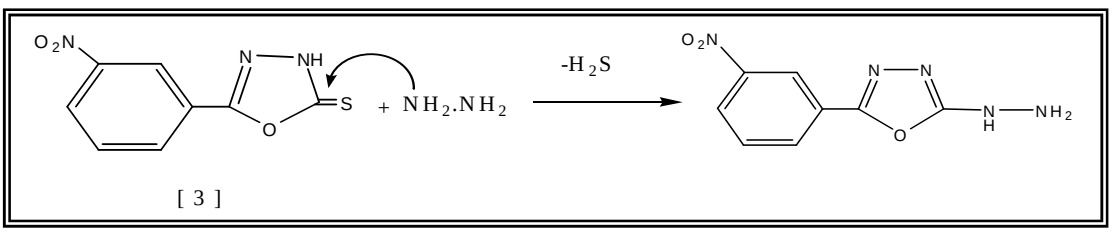
Compound [4] was synthesized by reacting compound [3] with hydrazine hydrate in absolute ethanol. The compound was characterized by it's melting point and FTIR spectrum.



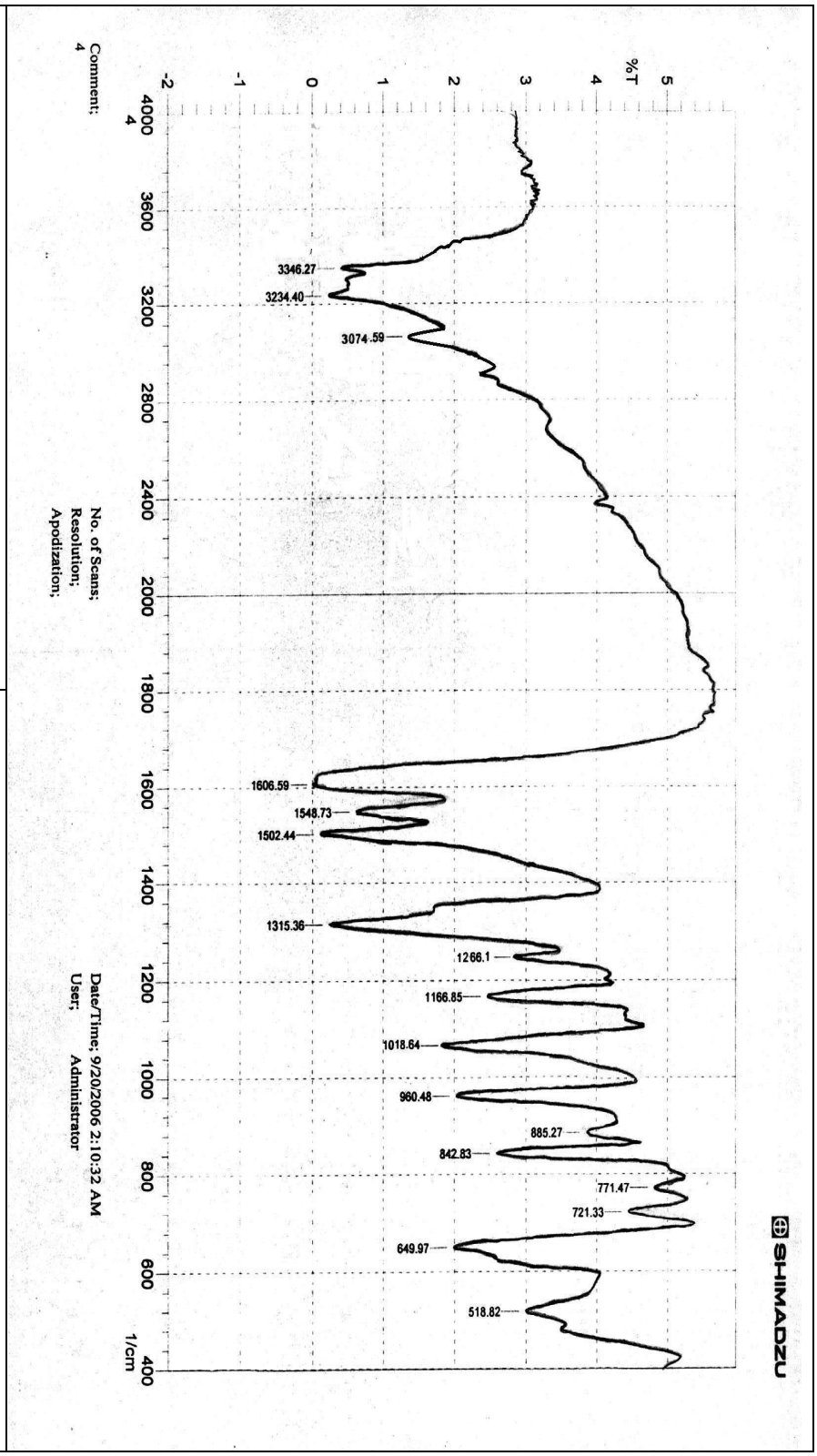
The FTIR spectrum of compound [4] showed the appearance of NH_2 asymmetric and symmetric stretching bands at (3346.27cm^{-1}), (3234.40 cm^{-1}) respectively, *v*(C=N) absorption band at (1606.59 cm^{-1}), *v*(C-O-C) asymmetric and symmetric appeared at (1266.1 cm^{-1}), (1018.64 cm^{-1}). Figure (3-4) shows the F.T.IR spectrum of compound [4]. The suggested mechanism of the reaction is as shown in scheme (3-3). Table (3-4) shows characteristic bands of compound [4].

Table (3-4): Characteristic bands of compound [4]

Comp. No.	ν(-NH-NH_2) cm^{-1}	ν(C-H) arom.cm^{-1}	ν(C=C) cm^{-1}	ν(C=N) cm^{-1}	ν(C-NO_2) cm^{-1}		ν(C-O-C) cm^{-1}	
4	3346.27 3234.40	٣٠74.59	1548.73	1606.59	Asym. 1502.44	Asym. 1315.36	Sym. 1266.1	Sym. 1018.64



Scheme (3-3): Mechanism steps for the preparation of compound [4].



M.P. : 228-230°C

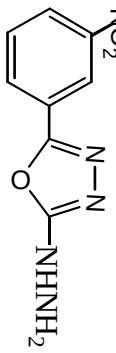
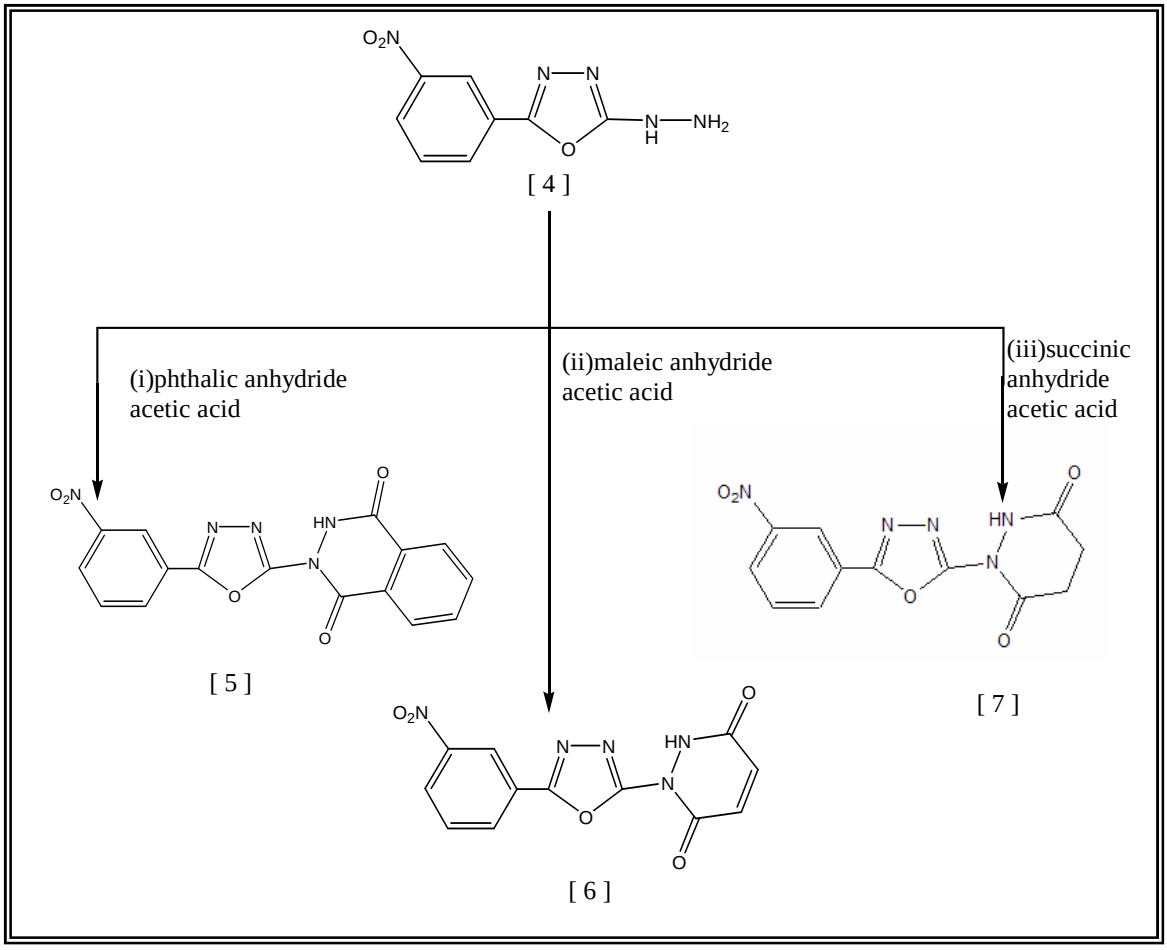


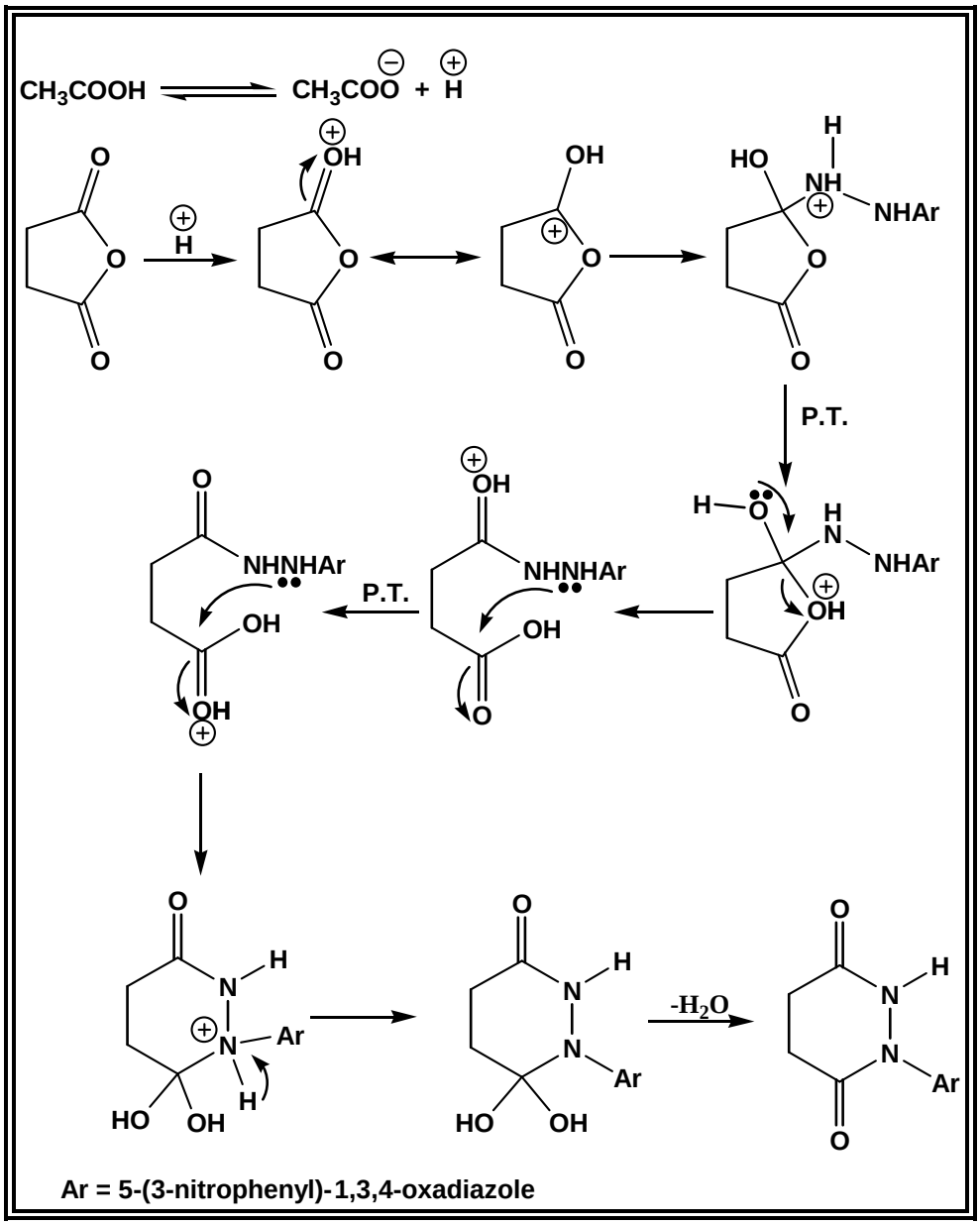
Figure (3-4): FTIR- spectrum of 2-hydrazido-5-(3-nitrophenyl)- 1,3,4-oxadiazole.



3.1.5 Synthesis of phthalazine and pyridazin-dione derivatives [5], [6] and [7]:

Compound [5], [6] and [7] were synthesized from the reaction of compound [4] with phthalic anhydride, maleic anhydride and succinic anhydride respectively in the presence of acetic acid as solvent and catalyst. The suggested mechanism for the synthesis of the titled derivatives can be explained as follows in the general mechanism. In the first step of the reaction, a protonation process takes place by the acid, followed by a nucleophilic attack by the hydrazide on the carbon atom bearing the positive charge. Losing a proton and rearrangement lead to

cyclization with losing a water molecule. The suggested mechanism of the reaction is shown in Scheme (3-4).



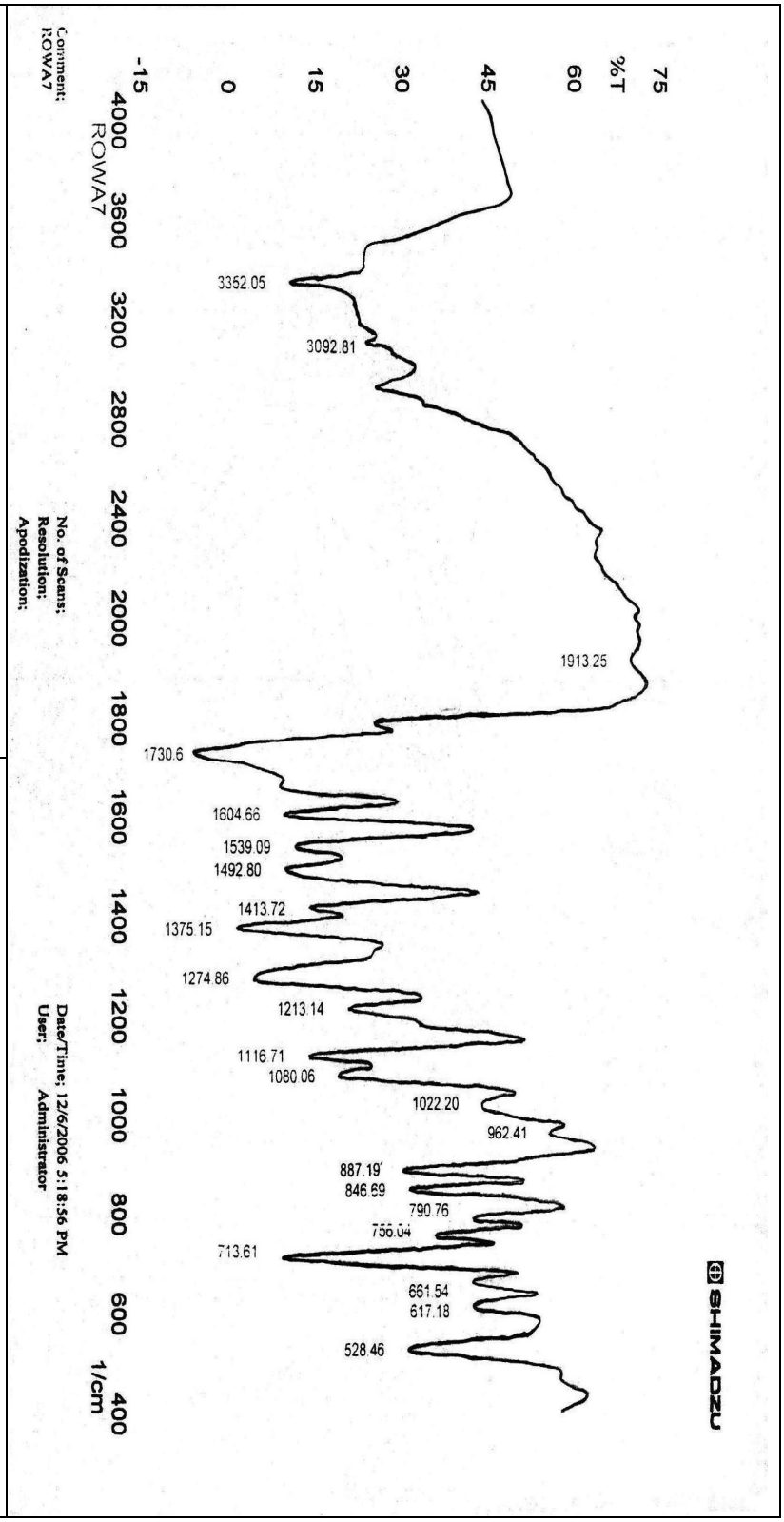
Scheme (3-4): Mechanism steps for the preparation of compounds [5, 6 and 7].

The F.T.IR spectrum of compounds [5], [6] and [7] indicated the disappearance of NH_2 band (3346.27 and 3234.40 cm^{-1}) of the starting material [4] together with the of bands at (3360-3130 cm^{-1}) and at (1740-1720 cm^{-1}) which assignable to stretching vibration of (N-H) and (C=O), respectively, of new heterocyclic ring. These represent the most characteristic evidence for the success of this step of the reaction.

Figures (3-5), (3-6) and (3-7) show the F.T.IR spectrum of compounds [5], [6] and [7].Table (3-5) shows the characteristic bands of compounds (5, 6, 7).

Table (3-5): Characteristic bands of compounds (5,6,7).

Comp. No.	ν(-NH) cm^{-1}	ν(C-H) arom. cm^{-1}	ν2(C=O) hetrocyclic cm^{-1}	ν(C=C) arom. cm^{-1}	ν(C=N) cm^{-1}	ν(C-O-C) cm^{-1}	
						Asym.	Sym.
5	3352.05	٣٠٩2.81	1730.6	1539.09	1604.66	1274.86	1080.06
6	3282.62	3058.29	1726.17	1604.66	1627.48	1278.72	1016.42
7	3138.0	3087.5	1726.2	1604.7	1652.9	1296.1	1093.6



M.P. : 290-292 °C

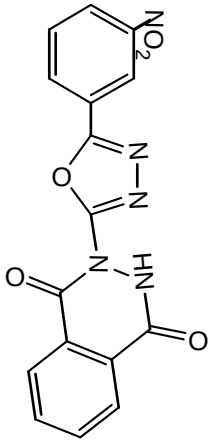
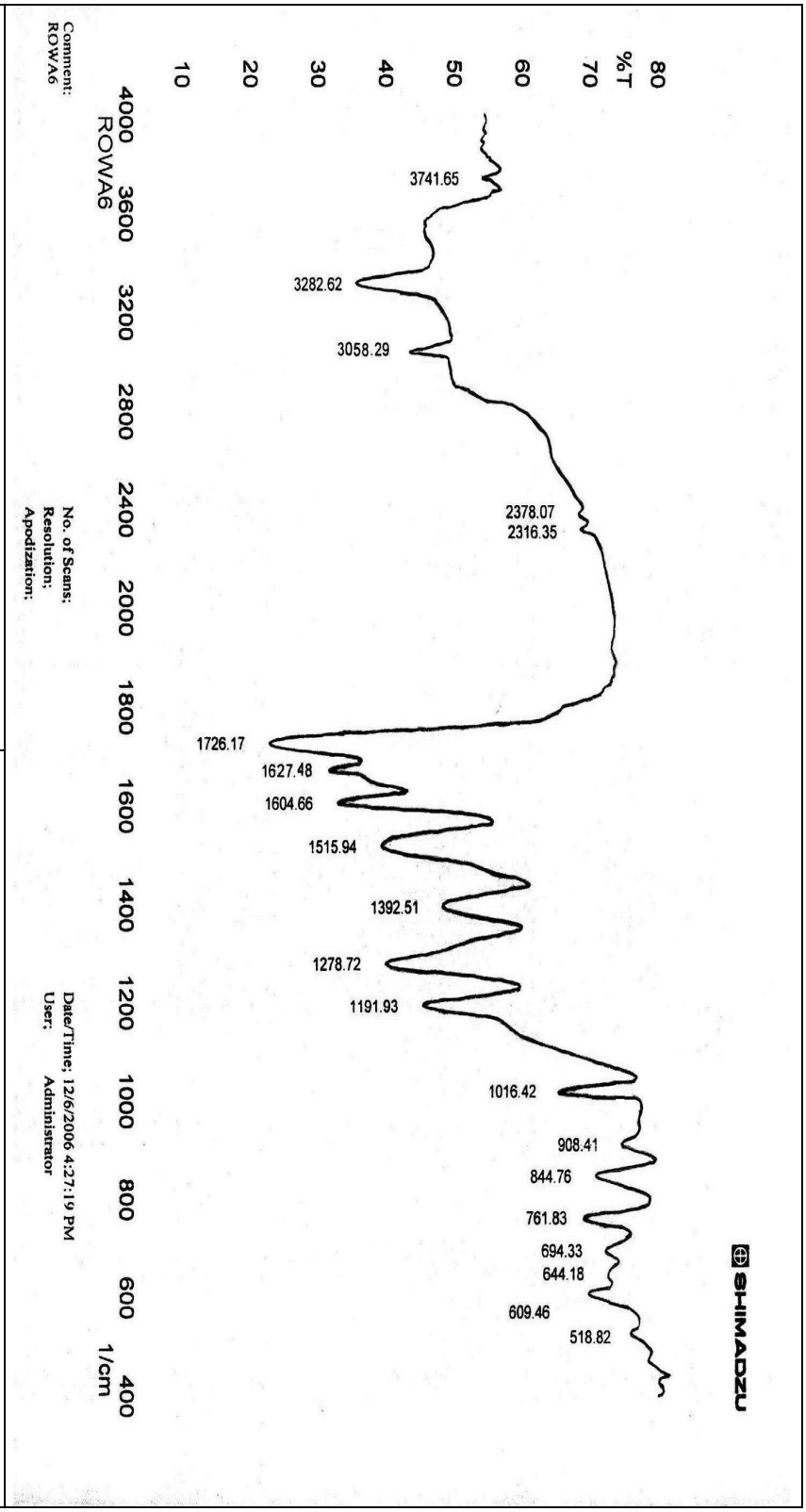


Figure (3-5): FTIR- spectrum of 5-(3-nitrophenyl)-2-(1,2-dione-1H-2-yl phthalazine)-1,3,4-oxadiazole.



M.P. : 185-187 °C

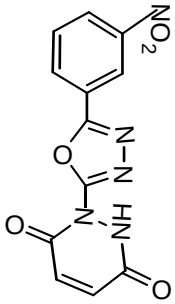
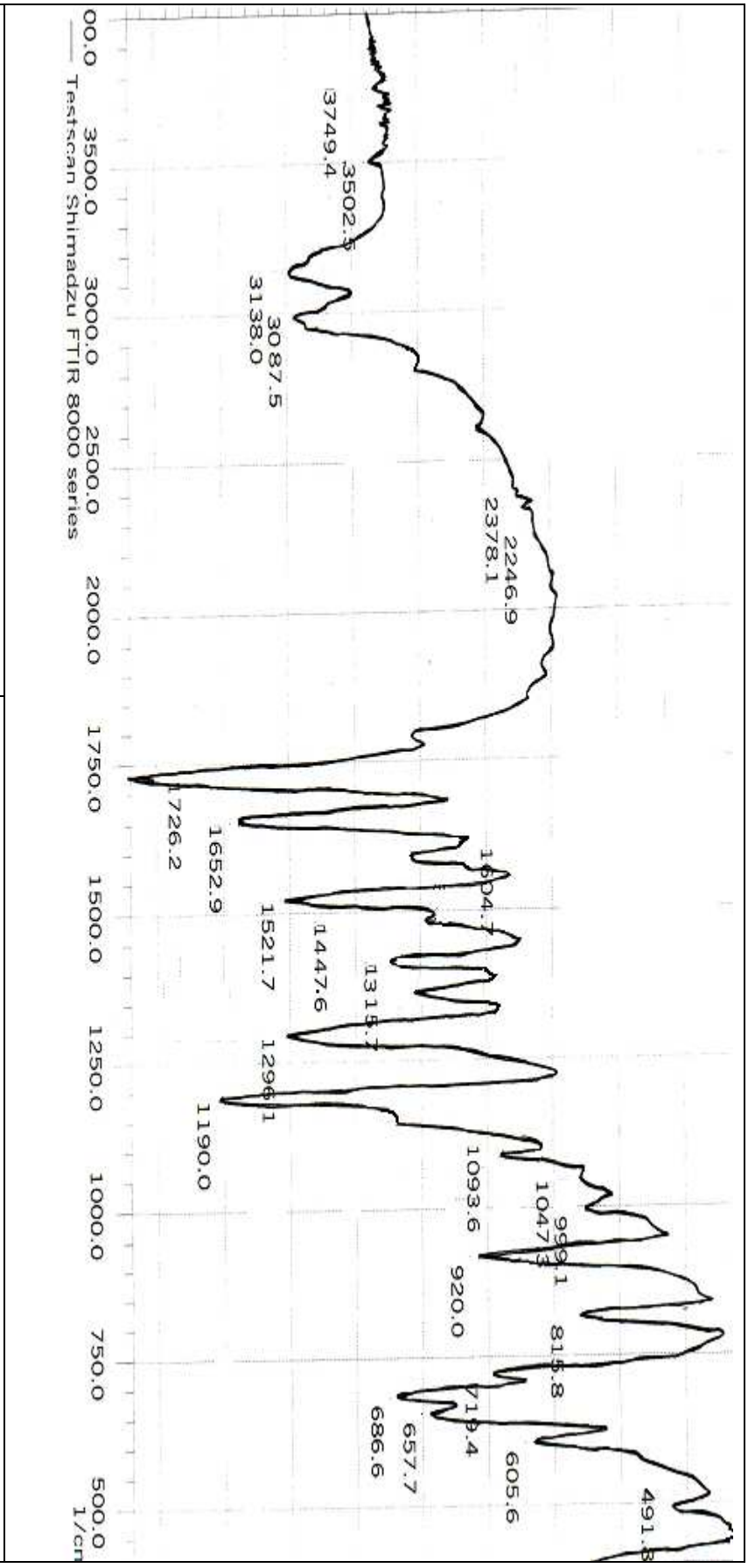


Figure (3-6): FTIR- spectrum of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-1,2-dihydro- pyridazine-3,6-dione.



M.P. : 166-168 °C

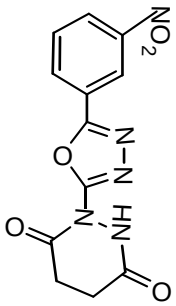
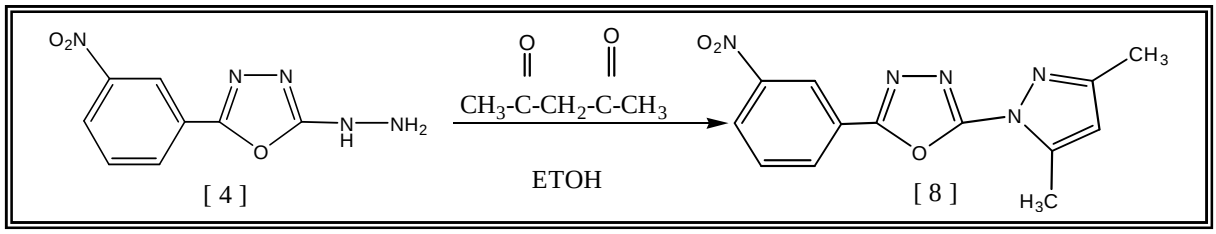


Figure (3-7): FTIR- spectrum of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-1,2-tetrahydro- pyridazine-3,6-dione.

3.1.6 Synthesis of 2-(3,5-Dimethyl-pyrazol-1-yl)-5-(3-nitrophenyl)- 1,3,4-oxadiazole [8]:

Compound [8] was synthesized from the reaction of compound [4] with acetyl acetone and acetic acid in absolute ethanol. The compound was characterized by it's melting point and F.T.IR spectroscopy.

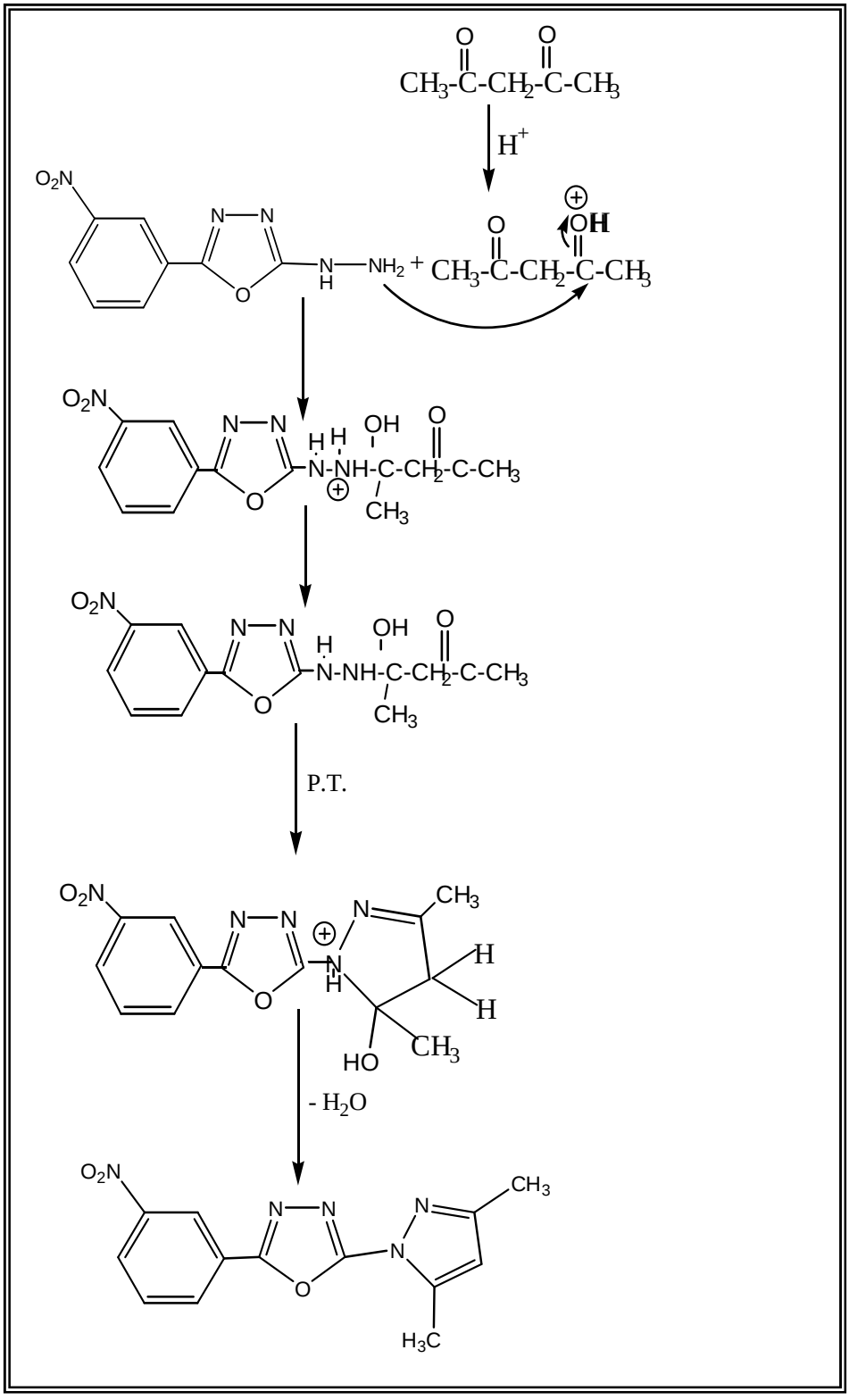


The F.T.IR spectrum of compound [8] indicated the disappearance of NH_2 bands (3346.27 and 3234.40 cm^{-1}) of the starting material and the appearance of the C=N band at (1634.5 cm^{-1}) and C=C at (1589.6 cm^{-1}) and C-H aliphatic (2891.8 cm^{-1}). Figure (3-8) shows the F.T.IR spectrum of compound [8]. Table (3-6) shows the characteristic bands of compound [8].

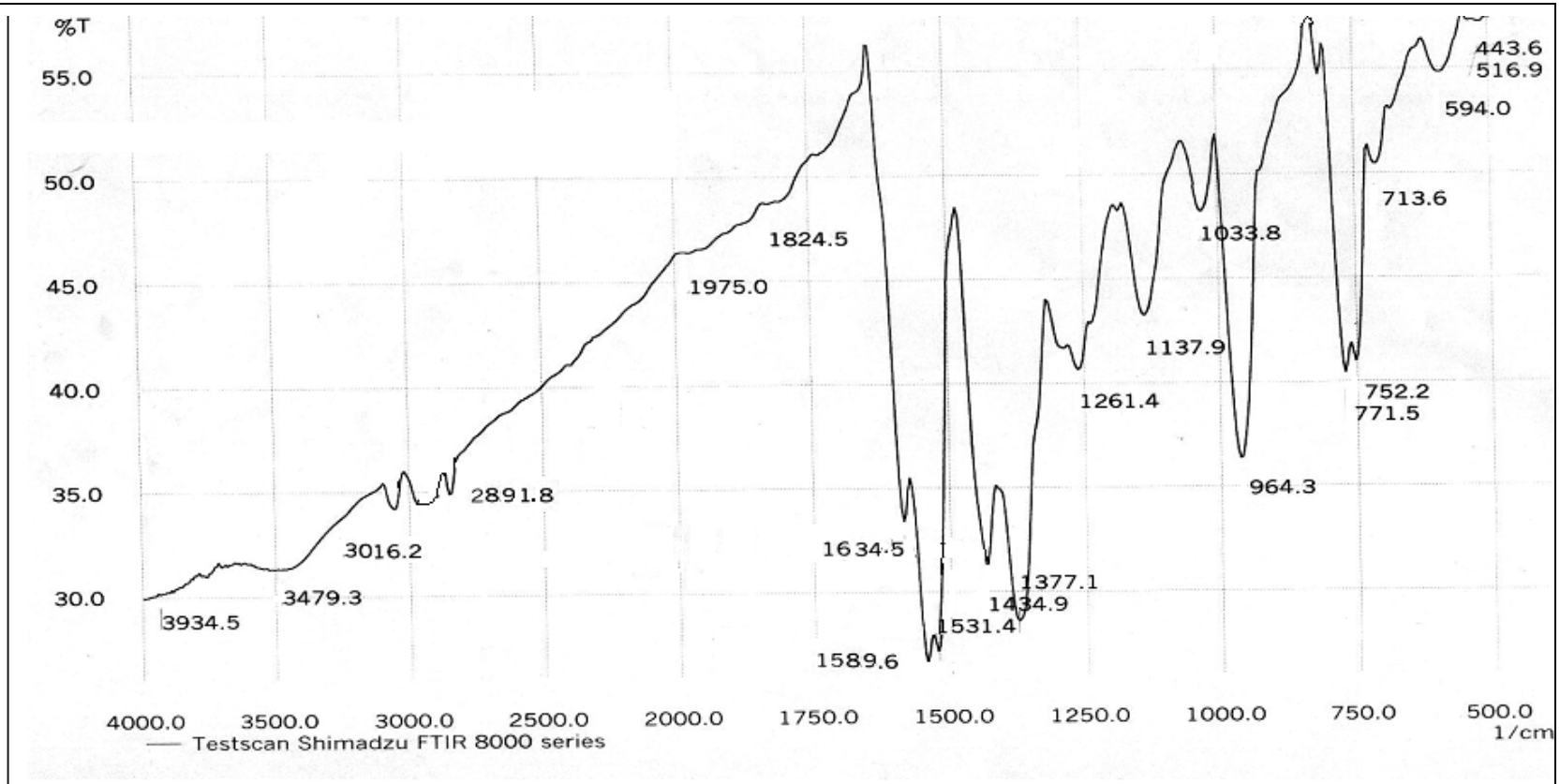
Table (3-6): Characteristic bands of compound [8].

Comp. No.	ν(C-H) arom.cm^{1}	ν(C-H) aliph.cm^{-1}	ν(C=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C-NO_2) cm^{-1}		ν(C-O-C) cm^{-1}	
					Asym.	Sym.	Asym.	Sym.
8	3016.2	2891.8	1634.5	1589.6	1531.4	1377.1	1261.4	1033.8

The suggested mechanism of the reaction is shown in Scheme (3-5).



Scheme (3-5): Mechanism steps for the preparation of compound [8].



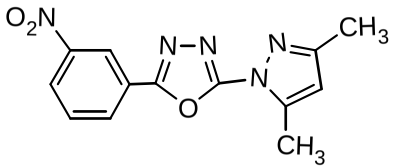
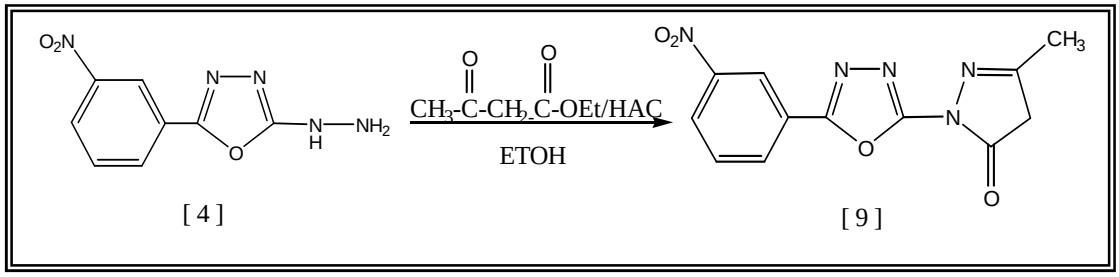
M.P. : 115-117 °C	

Figure (3-8): FTIR- spectrum of 2-(3,5-Dimethyl-pyrazol-1-yl)-5-(3-nitrophenyl)-1,3,4-oxadiazole .

3.1.7 Synthesis of 5-(3-nitrophenyl)-2-(3-methyl-5-one-1-yl pyrazole)-1,3,4-oxadiazole [9]:

Compound [9] was synthesized from the reaction of compound [4] with ethyl aceto acetate and acetic acid in absolute ethanol. The compound was characterized by it's melting point and F.T.IR spectroscopy.

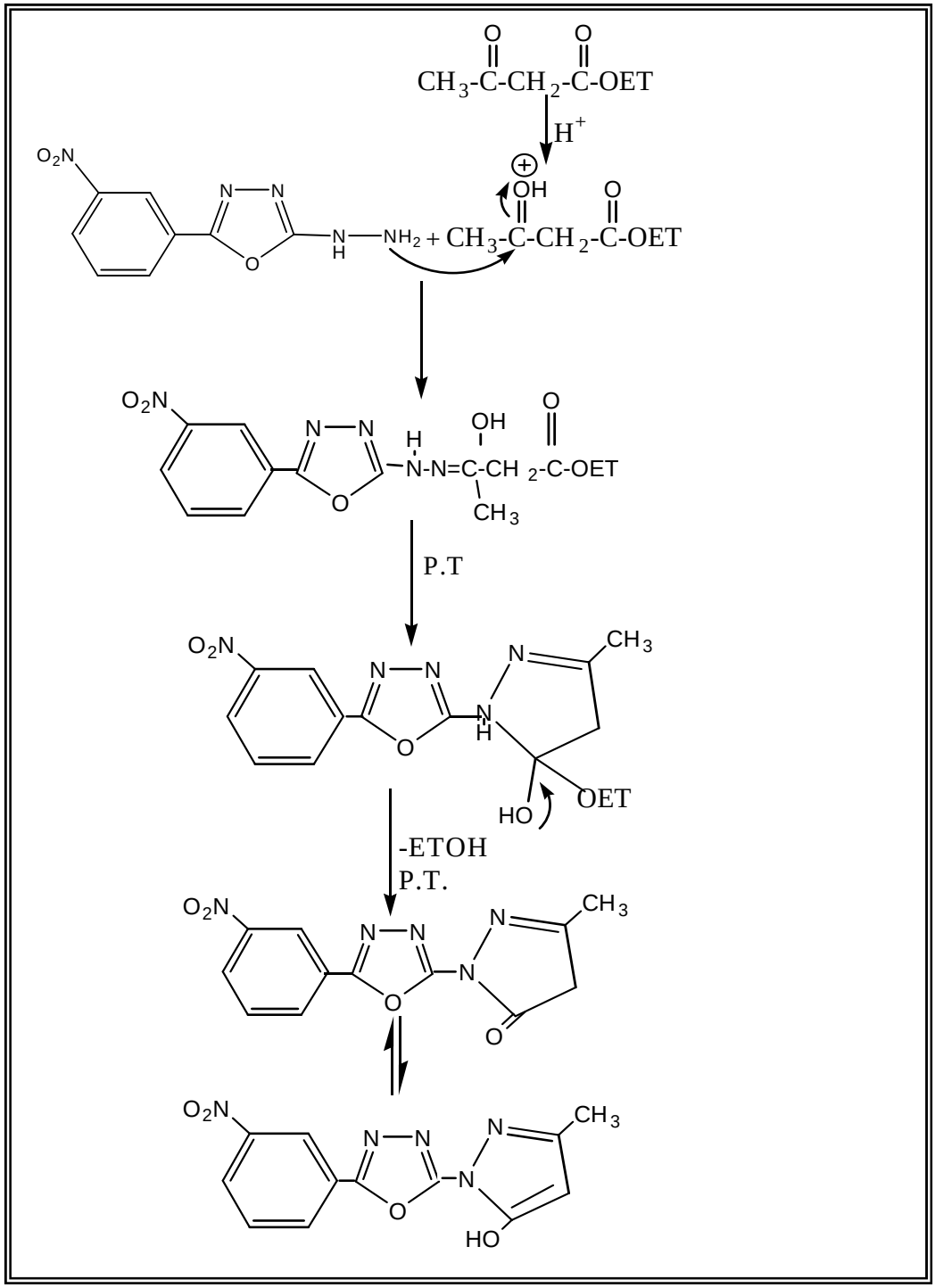


The F.T.IR spectrum of compound [9] indicated the disappearance of NH_2 bands (3346.27 and 3234.40 cm^{-1}) of the starting material and the appearance of carbonyl group band at (1720.6 cm^{-1}) and the C-H aliphatic band at (2927.74 cm^{-1}). Figure (3-9) shows the F.T.IR spectrum of compound [9]. Table (3-7) shows the characteristic bands of compound [9].

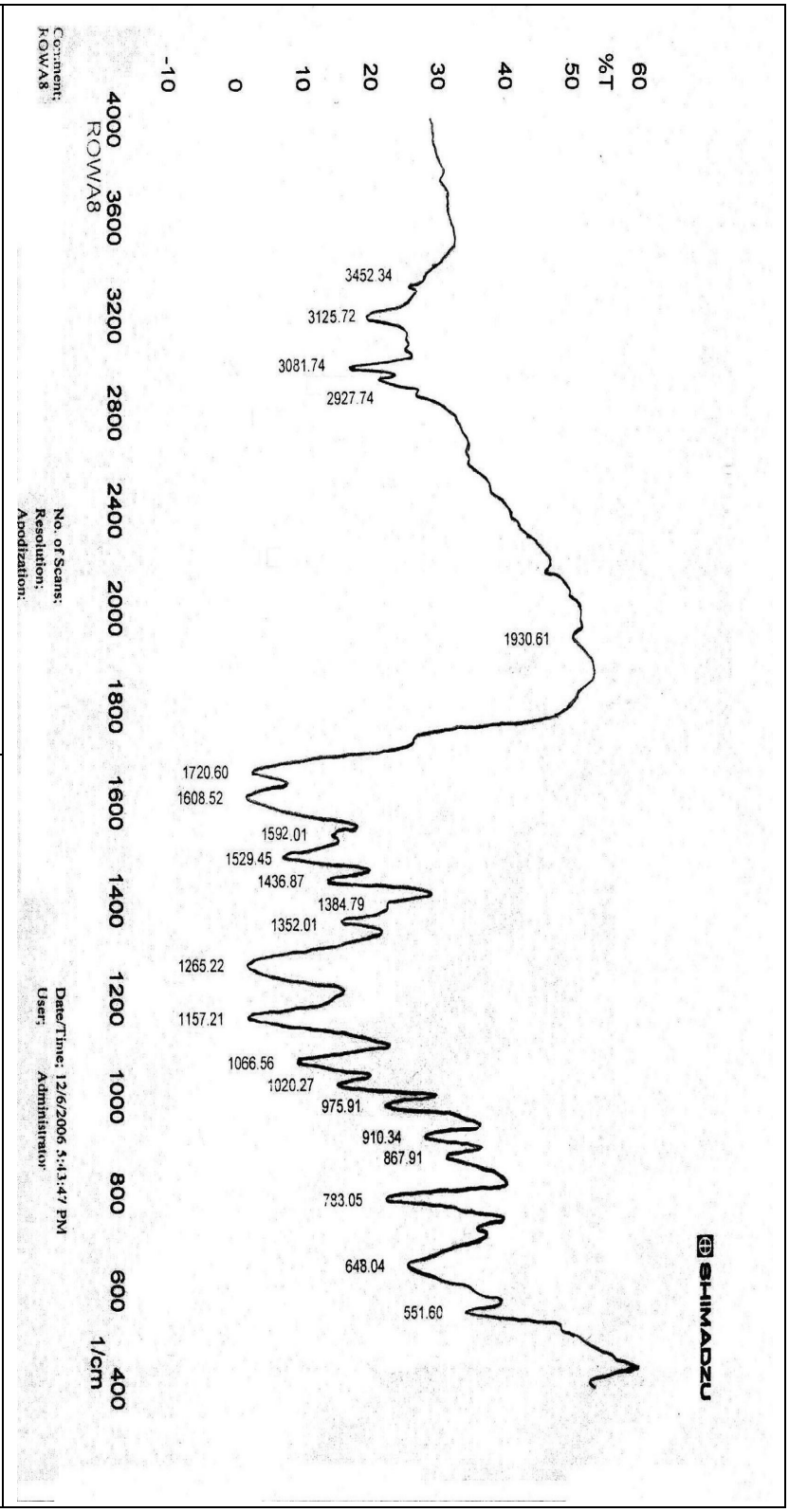
Table (3-7): Characteristic bands of compound [9].

Comp. No.	ν(C-H) arom.cm^{-1}	ν(C-H) aliph.cm^{-1}	ν(C=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C=O) cm^{-1}	ν(C-O-C) cm^{-1}	
9	3081.74	2927.74	1608.52	1592.01	1720.6	Asym. 1265.22	Sym. 1066.56

The suggested mechanism of the reaction is shown in Scheme (3-6).



Scheme (3-6): Mechanism steps for t of compound [9].



M.P. : 270-272 °C

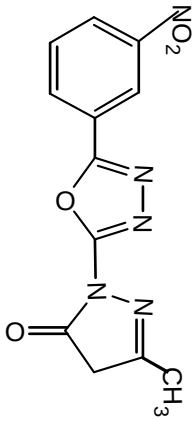
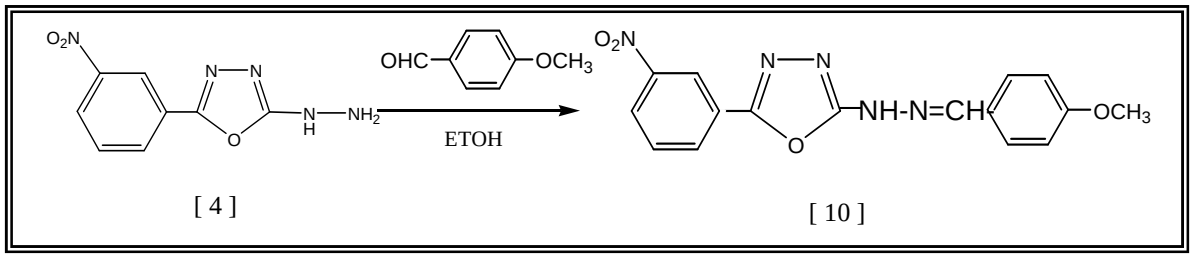


Figure (3-9): FTIR- spectrum of 5-(3-nitrophenyl)-2-(3-methyl-5-one-1-yl pyrazole)-1,3,4-oxadiazole .

3.1.8 Synthesis of 4- [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl-hydrazino]-4-amino benzylidine [10]:

Compound [10] was synthesized from the reaction of compound [4] with 4-methoxy benzaldehyde in absolute ethanol afforded the imine. The compound was characterized by its melting point and F.T.IR spectroscopy.



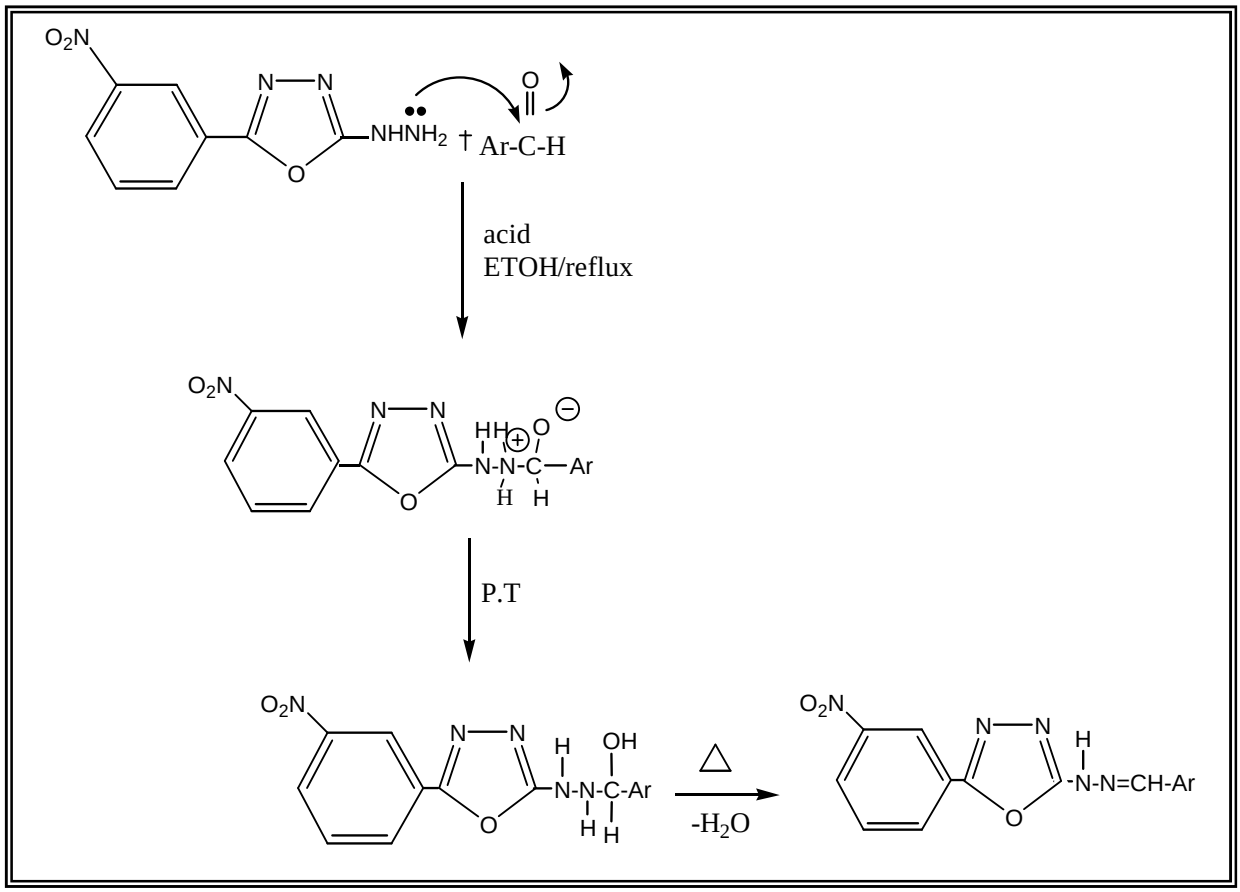
[4] [10]

The F.T.IR spectrum of compound [10] indicated the disappearance of NH_2 bands at (3346.27 and 3234.40 cm^{-1}) of the starting material and the appearance of N-H band at (3174.3 cm^{-1}) and the (δC-H) bands at (898.8 cm^{-1}) and the C=N band of the imine appeared at (1616. 2 cm^{-1}) and the C-H aliphatic (2831.3 cm^{-1}). Figure (3-10) shows the F.T.IR spectrum of compound [10]. Table (3-8) shows the characteristic bands of compound [10].

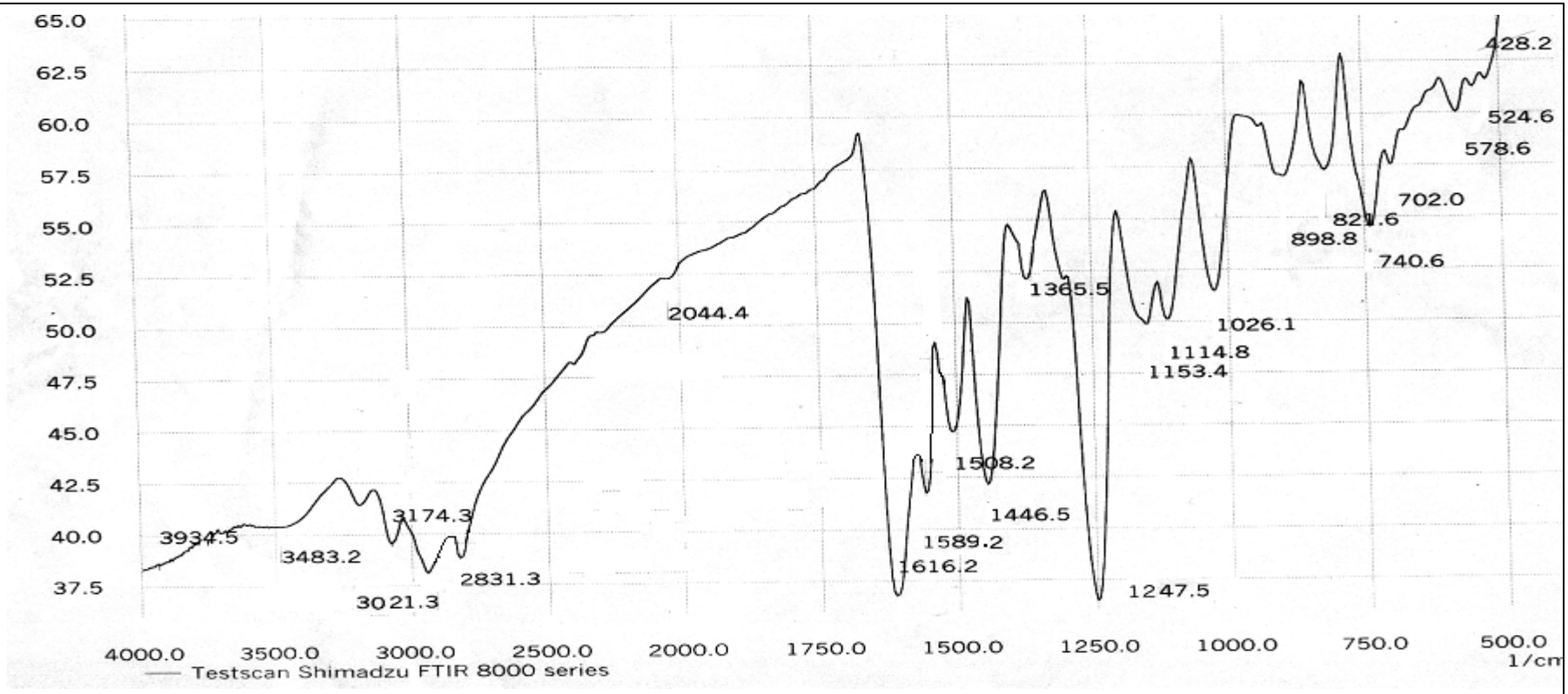
Table (3-8): Characteristic bands of compound [10].

Comp. No.	ν(C-H) arom.cm^{1}	ν(C-H) aliph.cm^{-1}	ν(C=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C-NO_2) cm^{-1}		ν(C-O-C) cm^{-1}	
					Asym.	Sym.	Asym.	Sym.
10	3021.3	2831.3	1616.2	1589.2	1508.2	1365.5	1247.5	1026.1

The mechanism of the reaction [103] is shown in Scheme (3-7).



Scheme (3-7): Mechanism steps for the preparation of compound [10].



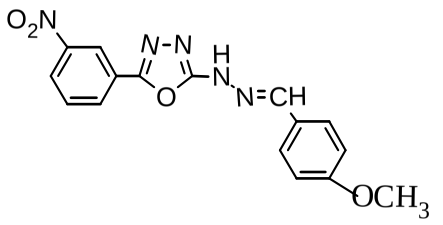
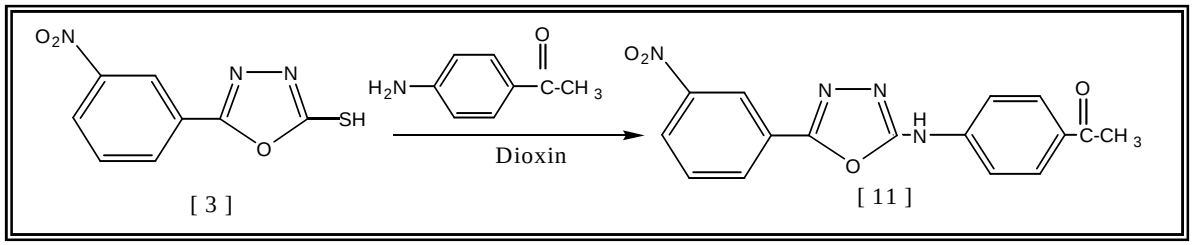
M.P. : 167-169 °C	

Figure (3-10): FTIR- spectrum of 4- [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl-hydrazino]-4-amino benzylidine .

3.1.9 Synthesis of 5-(3-nitrophenyl)-2-(4-ylacetophenone) amino-1,3,4-oxadiazole [11]:

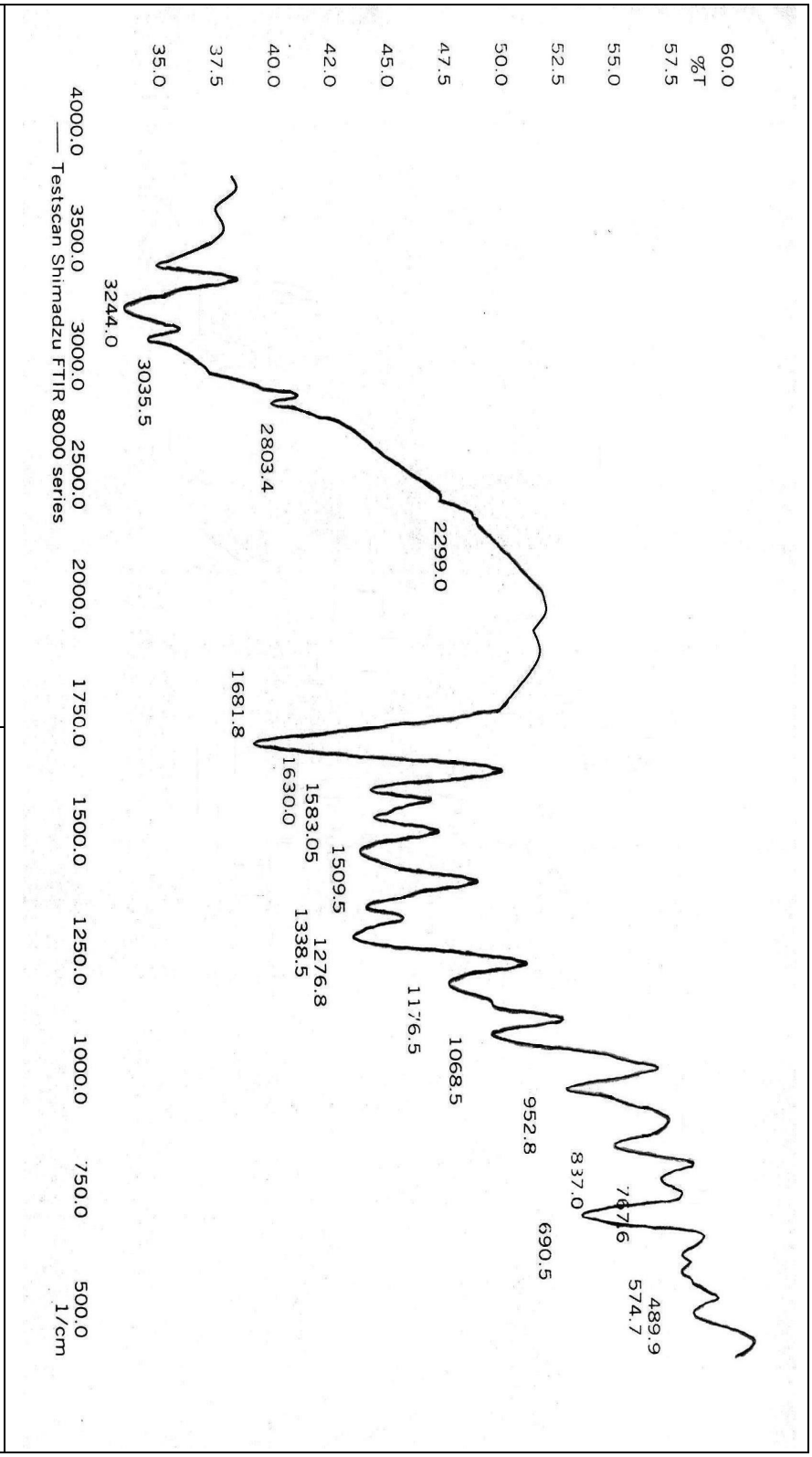
Compound [11] was synthesized from the reaction of compound [3] with 4-aminoacetophenone in dioxin. The compound was characterized by it's melting point and F.T.IR spectroscopy.



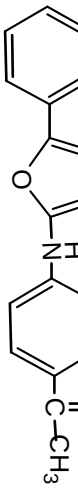
The F.T.IR spectrum of compound [11] indicated the disappearance of S-H band at (2521.9) of the starting material and the appearance of N-H band at (3244.0cm^{-1}) and carbonyl group at (1681.8 cm^{-1}). Band of C-H aromatic appeared at (3035.5 cm^{-1}) and aliphatic at (2803.4cm^{-1}). Figure (3-11) shows the F.T.IR spectrum of compound [11].Table (3-9) shows the characteristic bands of compound [11].

Table (3-9): Characteristic bands of compound [11].

Comp. No.	ν(NH) cm^{-1}	ν(C-H) arom.cm^{1}	ν(C-H) aliph.cm^{-1}	ν(C=C) cm^{-1}	ν(C=N) cm^{-1}	ν(C-O-C) cm^{-1}	
11	3244.0	3035.5	2803.4	1583.05	1630.0	Asym. 1276.8	Sym. 1068.5



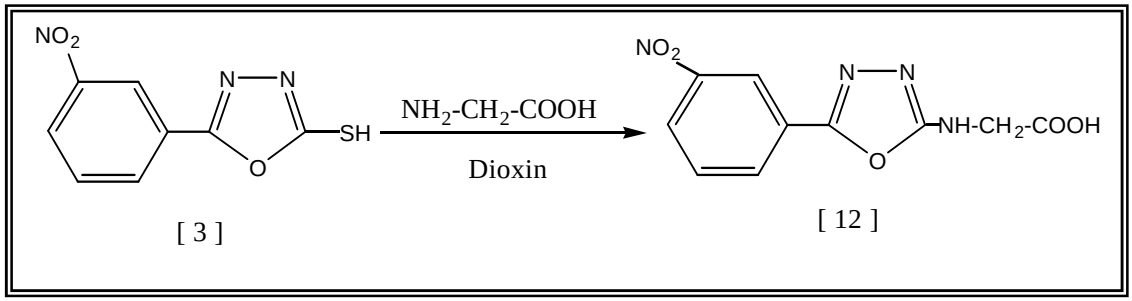
M.P. : 173-175 °C



Figure(3-11): FTIR- spectrum of 5-(3-nitrophenyl)-2-(4-ylacetophenone) amino-1,3,4-oxadiazole.

3.1.10 Synthesis of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid [12]:

Compound [12] was synthesized from the reaction of compound [3] with glycine in dioxin. The compound was characterized by it's melting point and F.T.IR spectroscopy.

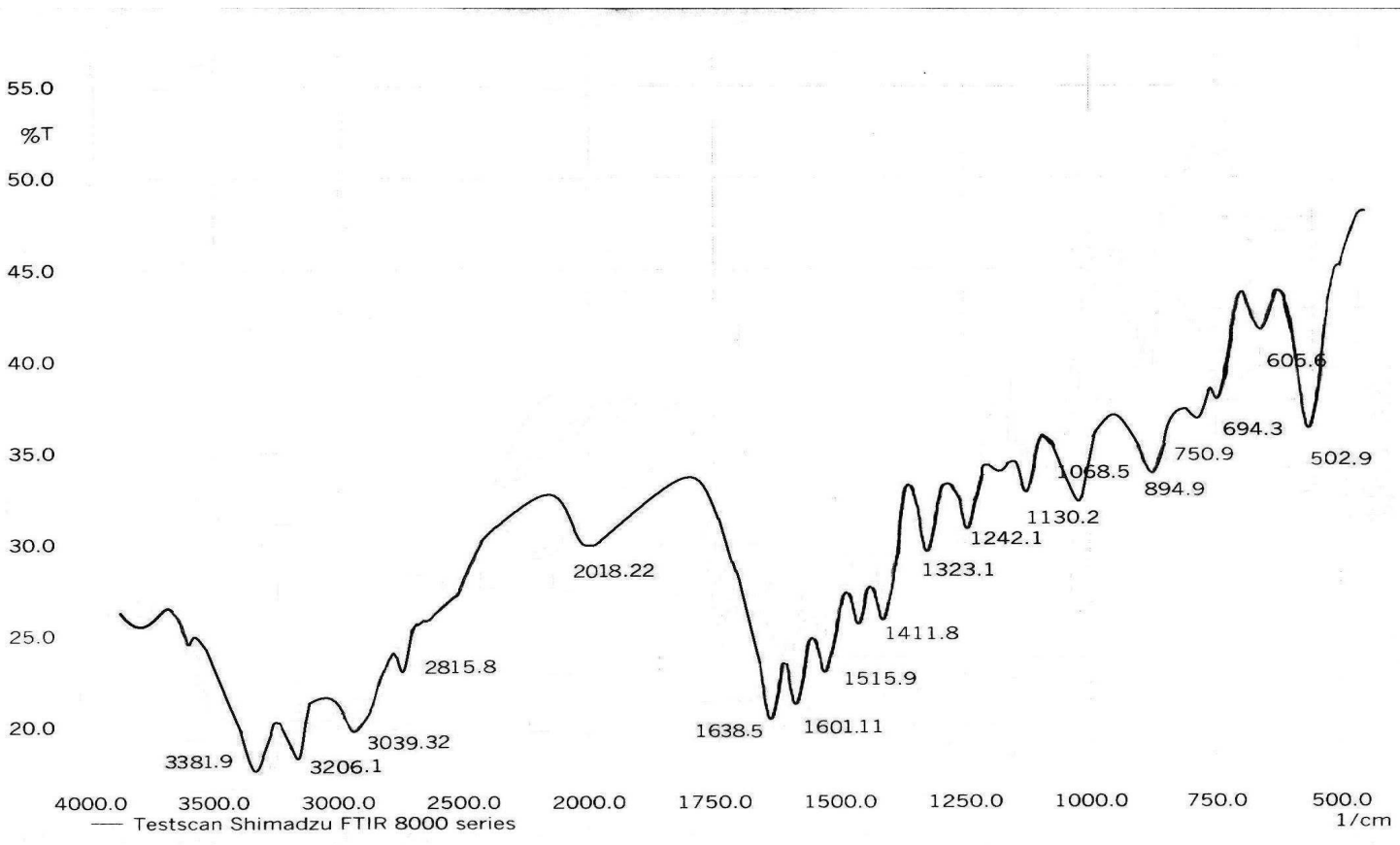


[3] [12]

The F.T.IR spectrum of compound [12] indicated the disappearance of S-H band at (2521.9 cm^{-1}) of the starting material and the appearance of N-H band at (3206.1 cm^{-1}) and carbonyl group at (1638.5cm^{-1}), the O-H band appear near (3381.9 cm^{-1}). Figure (3-12) shows the F.T.IR spectrum of compound [12]. Table (3-10) shows the characteristic bands of compound [12].

Table (3-10): Characteristic bands of compound [12].

Comp. No.	ν(-NH) cm^{-1}	ν(C-H) arom.cm^{1}	ν(C-H) aliph.cm^{-1}	ν(C=C) cm^{-1}	ν(C=N) cm^{-1}	ν(C-O-C) cm^{-1}	
12	3206.1	3082.3	2815.8	1601.11	1638.5	Asym. 1242.1	Sym. 1068.5



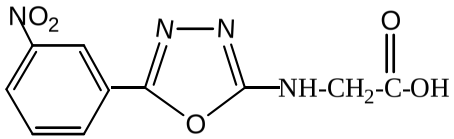
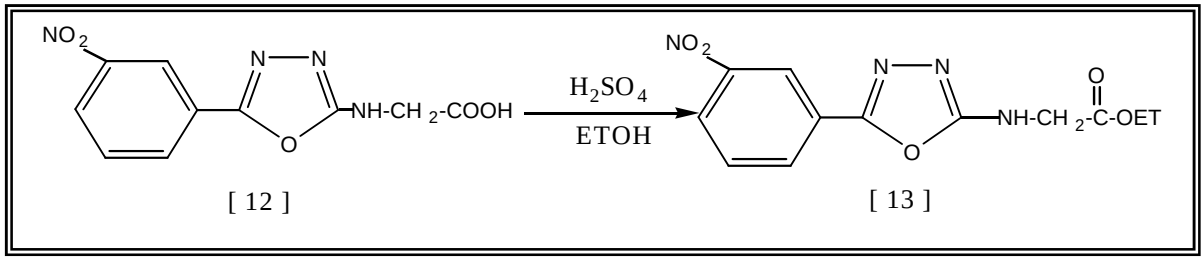
M.P. : 181-183 °C	

Figure (3-12): FTIR- spectrum of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid.

3.1.10 Synthesis of 5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole [13]:

[5-(3-nitro-phenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid with H_2SO_4 in absolute ethanol was refluxed overnight to afford the ester. The structure of the ester was confirmed from it's melting point and FTIR spectrum.

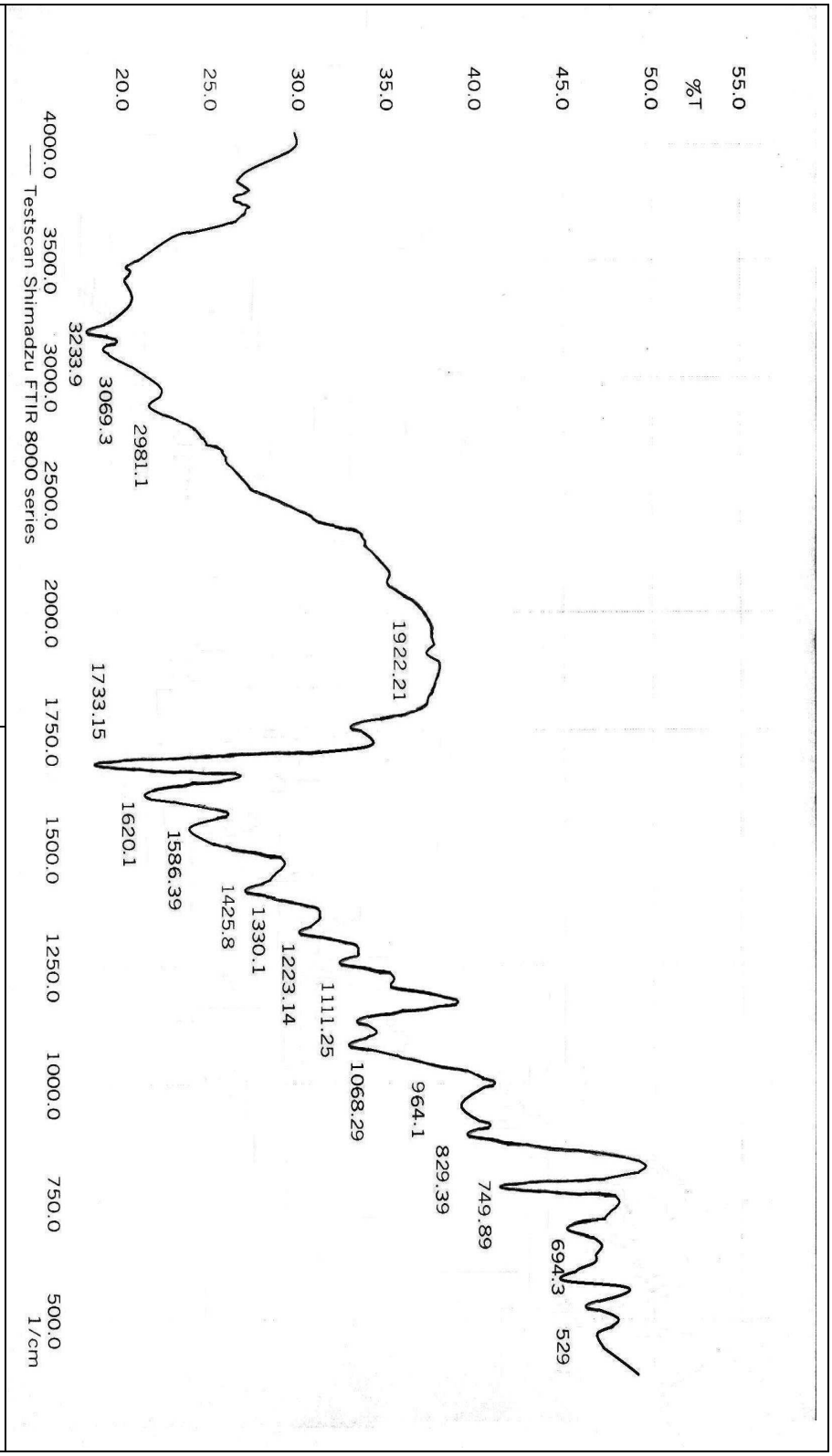


[12] [13]

The FTIR spectrum showed the disappearance of the carbonyl band of [5-(3-nitro-phenyl)-[1,3,4] oxadiazol-2-ylamino]-acetic acid at (1638.5 cm^{-1}) and appearance of the ester carbonyl at (1733.15 cm^{-1}). Band of C-H aromatic appeared at (3069.3 cm^{-1}) and aliphatic at (2981.1 cm^{-1}). Band at (1586.39 cm^{-1}) represented stretching of C=C. Figure (3-13) shows the F.T.IR spectrum of compound [13]. Table (3-11) shows characteristic bands of compound [13].

Table (3-11): Characteristic bands of compound [13]

Comp. No.	ν(C-H) arom.cm^{-1}	ν(C-H) aliph. cm^{-1}	ν(C=O) cm^{-1}	ν(C=C) cm^{-1}	ν(C=N) cm^{-1}	ν(C-O-C) cm^{-1}	
13	3069.3	2981.1	1733.15	1586.39	1620.1	Asym. 1223.14	Sym. 1086.29



M.P. : 202-204 °C

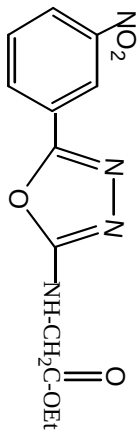
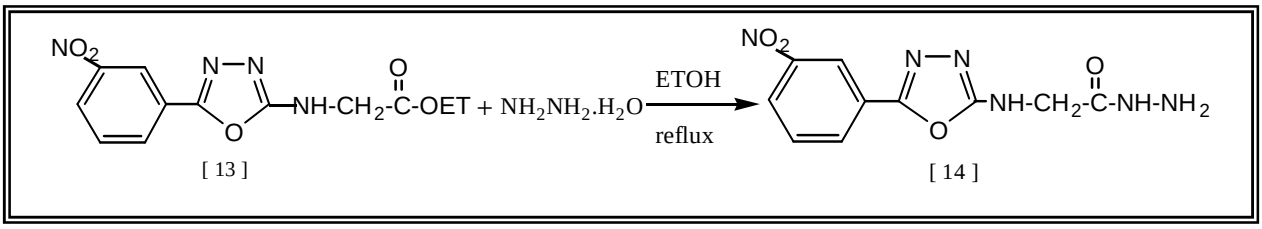


Figure (3-13): FTIR- spectrum of 5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole.

3.1.11 Synthesis 0f 5-(3-nitrophenyl)-2-aminoacetic acid hydrazide-1,3,4-oxadiazole [14]:

The acid hydrazide was synthesized by reacting [13] with hydrazine hydrate in absolute ethanol. The compound was confirmed from it's melting point and FTIR spectrum.

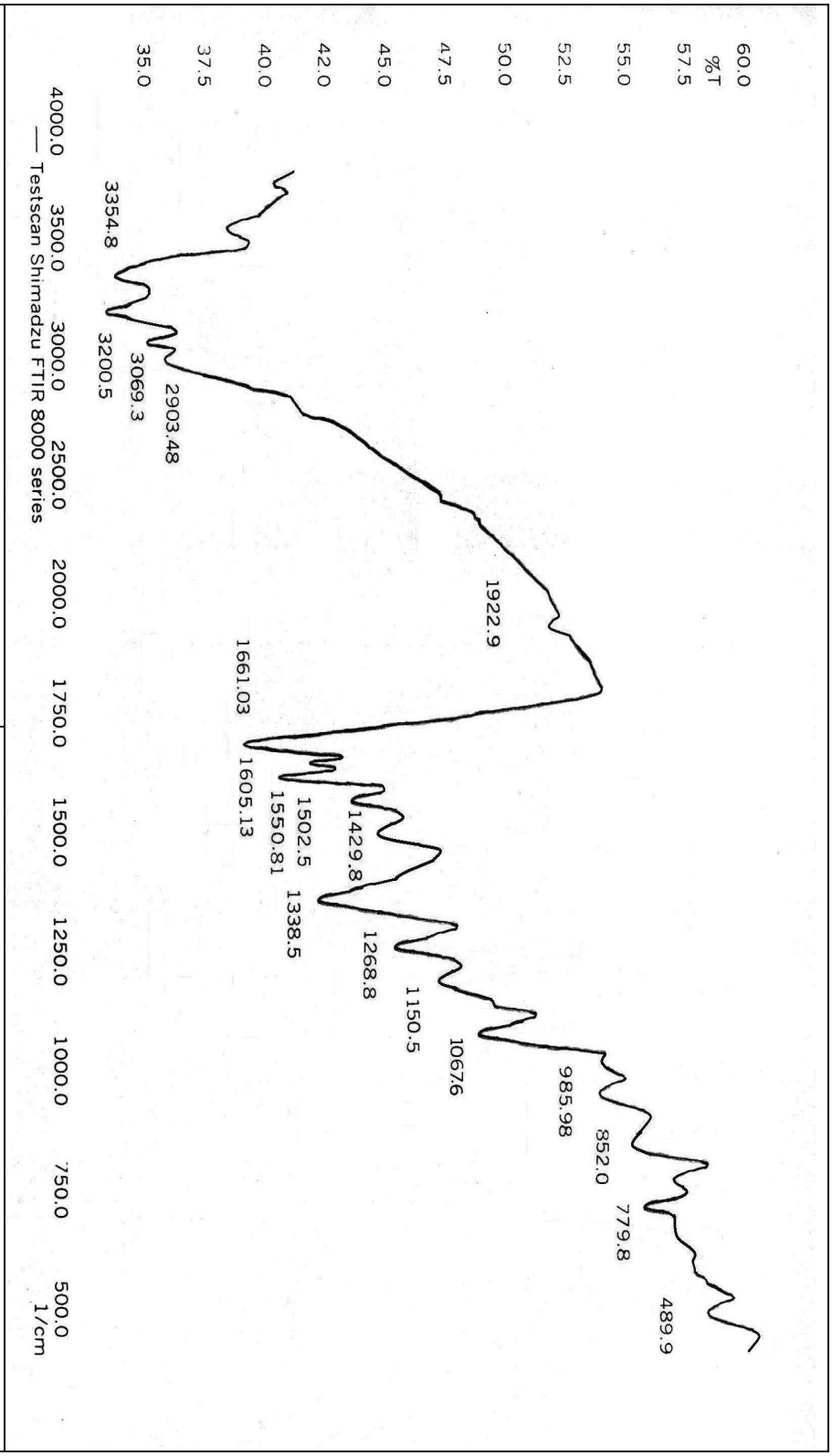


The FTIR spectrum of compound [14] indicated the disappearance of the ester carbonyl band at (1733.15 cm^{-1}) and appearance of two stretching bands of NH_2 asymmetric and symmetric at (3354.8 and 3200.5 cm^{-1}). The carbonyl amide (Amide I band) appeared at (1661.03 cm^{-1}). At (1550.81 cm^{-1}) NH δ bending (Amide II). Figure (3-14) shows the F.T.IR spectrum of compound [13]. Table (3-12) shows characteristic bands of compound [14].

Table (3-12): Characteristic bands of compound [14]

Comp. No.	ν(-NH-NH_2) cm^{-1}	ν(C-H) arom.cm^{-1}	ν(C-H) aliph.cm^{-1}	ν(C=O) cm^{-1}	δ(-NH) cm	ν(C=N) cm^{-1}	ν(C-O-C) cm^{-1}	
14	3354.8 3200.5	3069.3	2903.48	1661.03	1550.81	1605.13	Asym. 1268.8	Sym. 1067.6

The mechanism of the reaction [101] is as shown in scheme (3-1)



M.P. : 262-264 °C

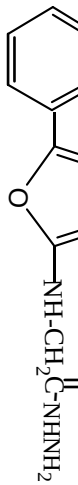
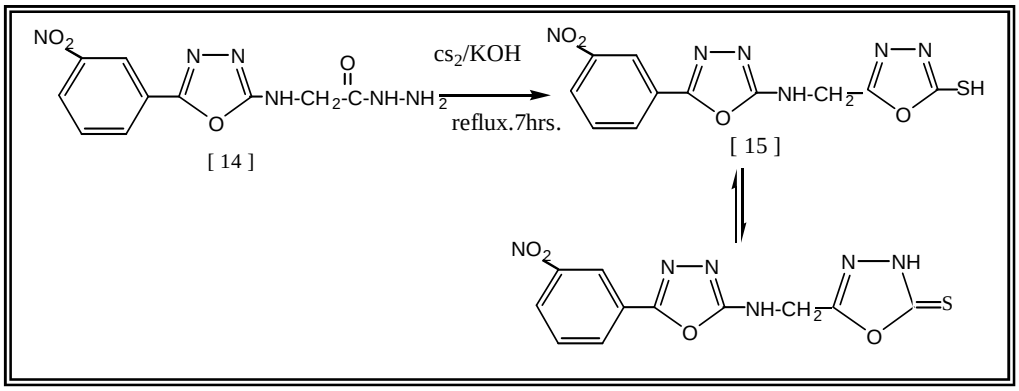


Figure (3-14): FTIR- spectrum of 5-(3-nitrophenyl)-2-aminoacetic acid hydrazide-1,3,4-*oxadiazole*.

3.1.12 Synthesis 0f 5-(3-nitrophenyl)-2-[2`-mercapto-5`-methyl-1`,3`,4`-oxadiazole]-2-ylamino-1,3,4-oxadiazole) [15]:

Reaction of compound [14] with CS_2, KOH in absolute ethanol afforded compound [15]. The compound was characterize by it's melting point and FTIR spectrum.

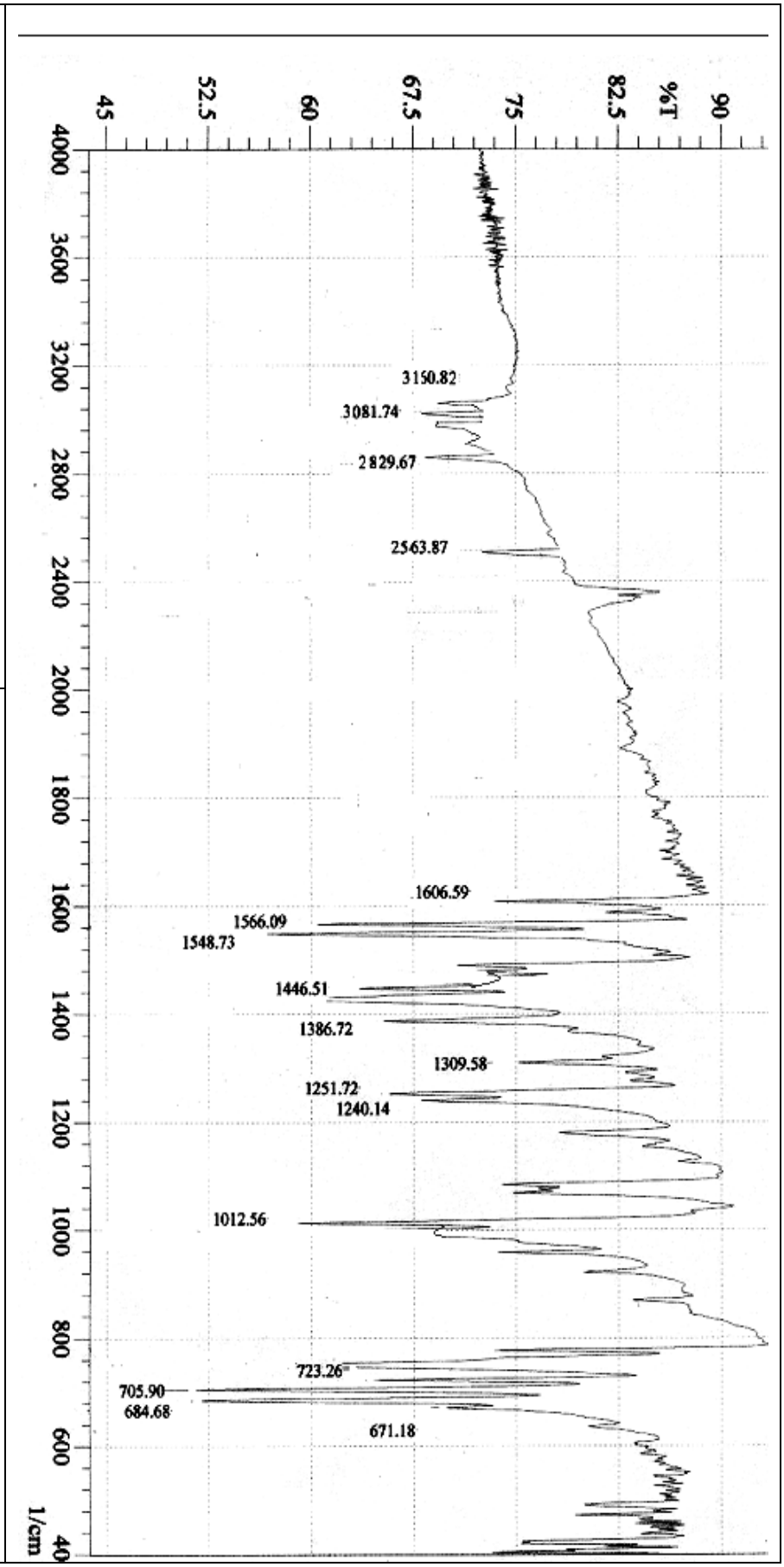


The FTIR spectrum of compound [15] indicated the disappearance of NH_2 asymmetric and symmetric stretching bands at (3294.2 cm^{-1}, 3209.3 cm^{-1}) respectively, and appearance of sulfohydryl absorption band S-H at (2563.87cm^{-1}) and absorption of (C=S) band at (1386.72cm^{-1}). Also F.T.IR show a typical absorptions of oxadiazole ring endo cyclic C-O-C asymmetrical and symmetrical at (1251.72 and 1012.56 cm^{-1}) and absorption band of (C=N) near (1606.59 cm^{-1}). Figure (3-15) shows the F.T.IR spectrum of compound [15]. Table (3-13) shows characteristic bands of compound [15].

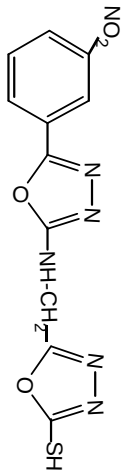
Table (3-13): Characteristic bands of compound [15].

Comp. No.	ν(C-H) arom.cm^{-1}	ν (-SH) cm^{-1}	ν(C=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C-NO_2) cm^{-1}		ν(C-O-C) cm^{-1}	
					Asym.	Sym.	Asym.	Sym.
15	3081.74	2563.87	1606.59	1566.09	1548.73	1309.58	1251.72	1012.56

The mechanism of the reaction [102] as shown in scheme (3-2)



M.P. : 298-300 °C

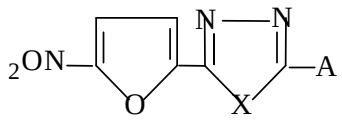


Figure(3-15):FTIR-spectrum)of5-(3-nitrophenyl)-2-[2`-mercapto-5`-methyl-1`,3`,4`-oxadiazole]-2-ylamino-1,3,4-oxadiazole).

3.2 Antibacterial Activity:

In agreement with 1944, ***Dodd and Stiliman***[104] who published their finding that furans with a nitro group in the 5- position possessed antibacterial activity. The 2- position of the 5-furan must be substituted, by a group of the general type -C=N-N=C-A (A=N, O, and S).

Scherman[105] found that the —C=N—N=C— system described. By (Dodd et al.) may be incorporated in heterocyclic systems, such compound is described in general terms by structure [101]



(X, A)= O, N or S

Applying the agar plate diffusion technique some of the synthesized compounds were screened *in vitro* for antibacterial activity against Gram positive *S. aureus* and Gram negative *E. coli*. The zone of inhibition of bacterial growth around the disc was observed the screening results given in table (3.1) and shown in figures (3.16), (3.17), (3.18), (3.19), (3.20), (3.21) indicated that most of the synthesized compounds exhibited antibacterial activities against at least one of the two types of the tested bacteria. Almost all the compounds have an inhibitory action against *S. aureus* more than *E .coli*

Table (3.14) Antibacterial activity of synthesized compounds in conc. 0.005/5ml of DMSO

Compound No.	*S. aureus* (G +ve)	*E. coli* (G –ve)
[1] [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-1,2-dihydro-pyridazine-3,6-dione	++	+++
[2] [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-tetrahydro pyridazine-3,6-dione	++	++
[3] 5-(3-nitrophenyl)-2-(3-methyl-5-one-1-yl pyrazole)-1,3,4-oxadiazole	+++	++
[4] 5-(3-nitrophenyl)-2-(1,2-dione-1H-2-ylphthazine)-1,3,4-oxadiazole	++	++
[5] 4- [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl-hydrazino]-4-amino benzylidine	+++	+++
[6] 2-(3,5-Dimethyl-pyrazol-1-yl)-5-(3-nitrophenyl)-1,3,4-oxadiazole	++	++
[7] 5-(3-nitrophenyl)-2-(4-ylacetophenone) amino-1,3,4-oxadiazole	+++	++
[8] 5-(3-nitrophenyl-2-aminoacetic acid hydrazide-1,3,4-oxadiazole	+++	+++
[9] [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid	++	++
[10]5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole	++	++
[11] 5-(3-nitrophenyl)-2-[2`-mercapto-5`-methyl-1`,3`,4`-oxadiazole]-2-ylamino-1,3,4-oxadiazole	+++	+++
Control (DMSO)	-	-

Note :

(-) = No inhibition
+ = (5-10) mm.
++ = (11-20) mm.
+++ = More than (20) mm

Thus, when screening for the antimicrobial activity of the synthesized substituted oxadiazole compounds against *S. aureus* and *E. coli* bacteria (Table 3.14), it has been found that the drug was more active against *S. aureus* bacteria. The difference in effectiveness of the drug between the two types of bacteria might be attributed to the difference in the cell wall structure. Bacterial cell wall consists of a branched chains of polysaccharide containing alternating units of N-acetylglucose amine and N-acetyl muramic acid connected by polypeptide cross linkage, this layers is called the peptidoglycan[106] In Gram positive bacteria, this basic layer is covered with teichoic acid which is ribitol phosphate, N-acetyl glucose amine polymer, and glycine, making up to 20% of cell weight. While in Gram nagative bacteria, lipopolysaccharides with lipoproteins were external to glycopeptides, which makes about 80% of the cell wall weight [107] this lipid containing layer of Gram negative bacteria cell wall keeps various small molecules from reaching the membrane[106] In addition to the fact that Gram positive bacteria cell wall is more permeable to molecules than Gram negative bacteria cell wall[106].

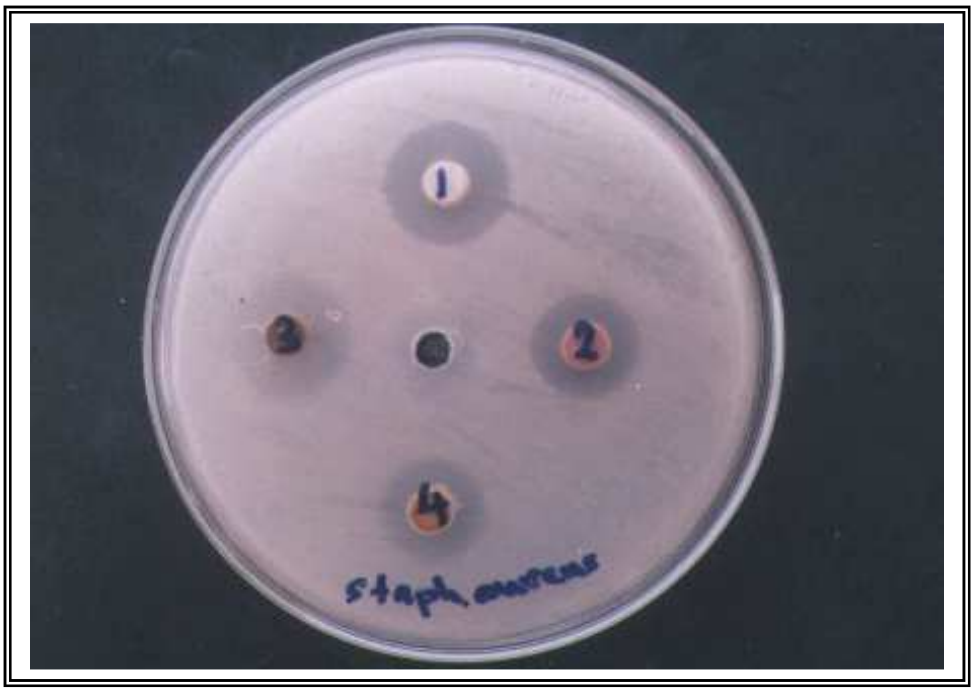


Figure (3.16) Effect of compounds [1], [2], [3], [4] on *S. aureus* in conc. (0.005g/5ml) of DMSO at 37 °C for 24 hrs of incubation.

Figure (3.17) Effect of compounds [5], [6], [7], [8] on *S. aureus* in conc. (0.005g/5ml) of DMSO at 37 °C for 24 hrs of incubation.

Figure (3.18) Effect of compounds [9], [10], [11] on *S. aureus* in conc. (0.005g/5ml) of DMSO at 37 ˚C for 24 hrs of incubation.

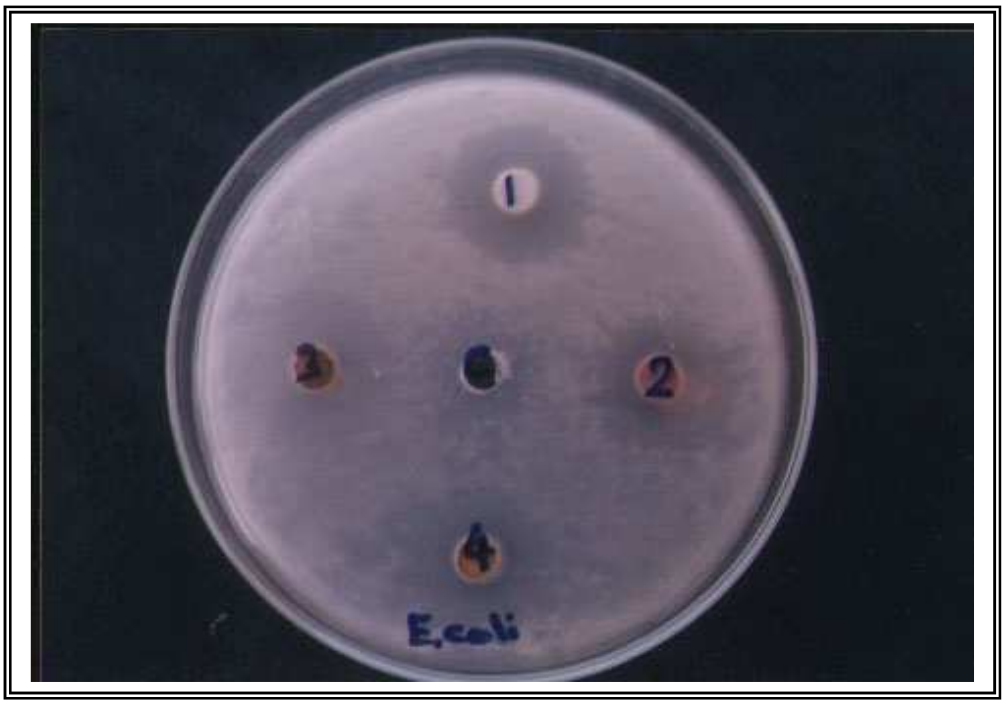


Figure (3.19) Effect of compounds [1], [2], [3], [4] on *E. coli* in conc. (0.005g/5ml) of DMSO at 37 ˚C for 24 hrs of incubation.

Figure (3.20) Effect of compounds [5], [6], [7], [8] on *E. coli* in conc. (0.005g /5ml) of DMSO at 37 °C for 24 hrs of incubation

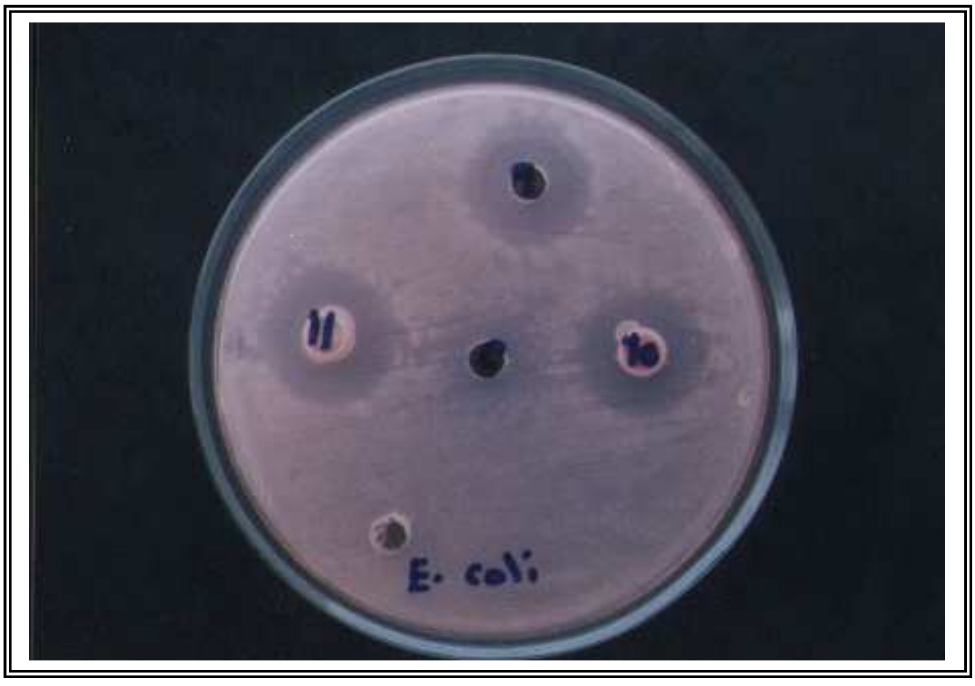


Figure (3.21) Effect of compounds [9], [10], [11] on *E. coli* in conc. (0.005g /5ml) of DMSO at 37 °C for 24 hrs of incubation

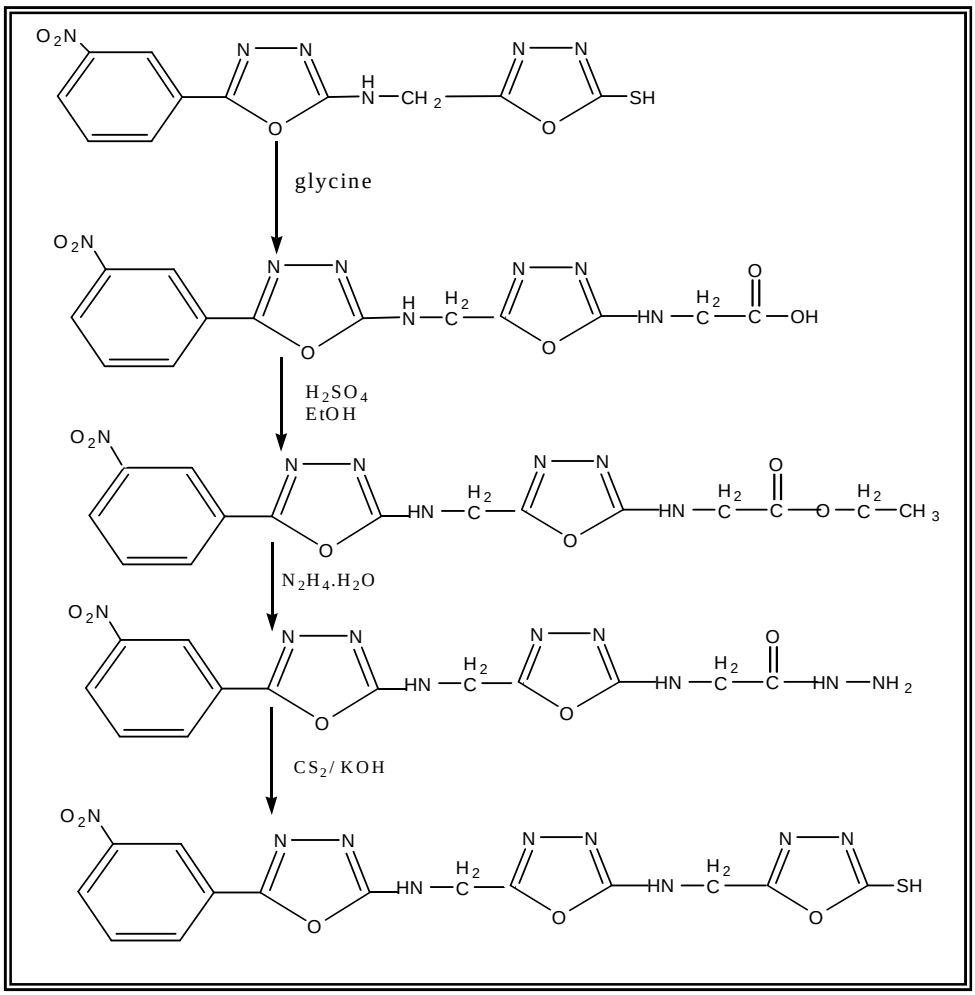
3.3 Conclusion:

In the present work synthesis and study the effect of some 1,3,4-oxadiazole compounds on two type of bacteria *S. aureus (G+)* and *E. coli*. *(G-)* have been described. Some of these derivatives have shown high activity at (0.005g/5ml).

It has been found that the drug was more active against *S. aureus* bacteria. These results confirm the fact that compounds with NH, SH and with heterocyclic have great potential as antibacterial agents.

3.4 Suggestion for further work

We hope to continue our extensive program directed toward the synthesis of novel heterocyclic compounds of potential biological applications, a variety of modification will be done in order to synthesize the following compounds:



This work involve adding of glycine to 5-(3-nitrophenyl)-2-[2`-mercapto-5`-methyl-1`,3`,4`-oxadiazole]-2-ylamino-1,3,4-oxadiazole) converting it to acid which is changed to ester derivative in the presence of ethanol and acid, then after adding excess of hydrazine hydrate we can obtain a new derivative of acid hydrazide, at last after adding carbon disulfide in the presence of potassium hydroxide a tris 1,3,4-oxadiazole derivative be available.

Chapter Two

EXPERIMENTAL PART

2-1 Chemicals

The chemicals used and the manufacturers are listed in Table (2-1).

Table (2-1): The chemicals and manufactures used through the project.

Chemicals	Supplied from
Acetic acid	BDH
Acetyl acetone	BDH
4-aminoacetophenone	Merck
Carbon disulphide	Merck
Diethyl ether	BDH
Dry benzene	Fluka
Dioxin	BDH
Ethanol (96%)	BDH
Ethanol (100%)	BDH
Etheyl acetoacetate	BDH
Glycine	Merck
Hydrazine hydrate (99%)	BDH
Hydrochloric acid	BDH
Magensium sulfate	BDH
Maliec acid	Fluka
4-methoxy benzalehyde	BDH
3-nitrobenzoic acid	BDH
Phthalic anhydride	BDH
Potassium hydroxide	BDH
Sodium bicarbonate	BDH
Succinic anhydride	Hopkin and Williams
Sulphuric acid	BDH

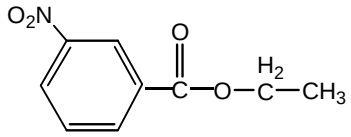
2.2 Instruments:

1- Melting points were recorded using hot stage ***Gallen Kamp*** melting point apparatus and were approximate.

2- The F.T.IR spectra in the range (4000-500) cm^{-1} were recorded using KBr disk on a ***SHIMADZU*** F. T. IR 8300 spectrophotometer Japan.

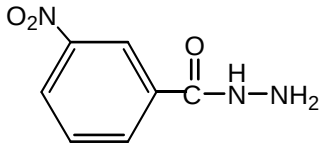
3- Thin Layer Chromatoghraphy (TLC) was carried out using Fertigfollen precoated sheets type PolyGram silg, and the plates were developed with iodine vapor.

2.3.1 Synthesis of ethyl- 3-nitrobenzoate [91] [1]:



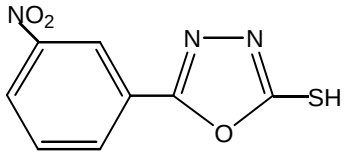
In a 250ml round bottom flask a mixture of (21g) of 3-nitrobenzoic acid, (14.5ml) of absolute ethyl alcohol, (2ml) of concentrated sulphuric acid was refluxed overnight, after that excess of solvent was removed by distillation. 10% sodium bicarbonate was added to the solution until litmus paper turned blue. The ester layer was separated by adding the mixture to a separating funnel and extracting it using (50 ml) of ether. The ether layer was separated, dried using unhydrous $MgSO_4$, filtered and evaporated on a water bath to remove the ether. M.p. (45-47 °C), yield (75 %).

2.3.2 Synthesis 0f 3-nitro benzoic hydrazide[92][2]:



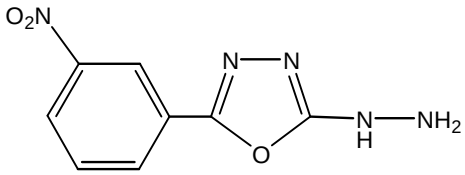
A mixture of ethyl 3-nitrobenzoate [1] (0.05 mol) and excess of hydrazine hydrate (10 ml) were refluxed for (2 hrs), ethanol (15 ml) was added and refluxed for (5-7 hrs). The precipitate which separated on cooling was filtered and washed with cold water. M.p. (135-137 °C), yield (65%), recrystilization solvent: toluene.

2.3.3 Synthesis of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole[93] [3]:



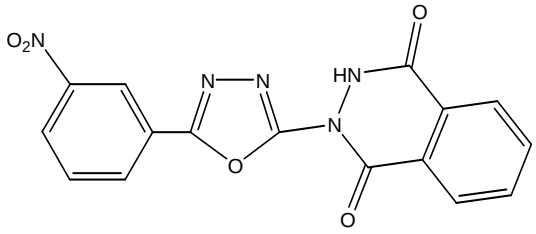
A mixture of 3-nitrobenzoic hydrazide [2] (0.01 mol, 1.81g) with (0.015mol, 0.5 ml) carbon disulfide and potassium hydroxide (0.015 mol, 0.82g) was refluxed for 7 hours. Then the solvent was evaporated and the residue was dissolved in water and acidified by dilute hydrochloric acid, the precipitate was filtered. M.p. (153-155 °C), yield (70%), recrystilization solvent :(ethanol-water).

2.3.4 Synthesis of 2-hydrazido-5-(3-nitrophenyl)- 1,3,4-oxadiazole [94][4]:



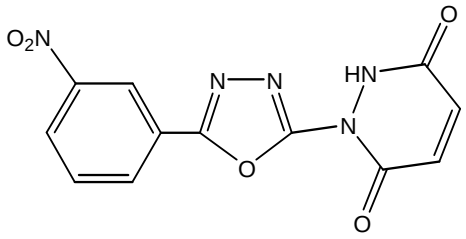
A mixture of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole [3] (0.003mol,0.7g) with hydrazine hydrate (0.003mol,0.07g,0.2ml) and 20ml absolute ethanol was refluxed for 5hours. After that the solvent was removed and the formed precipitated was filtered and dried. M.p. (228-230 °C), yield (65%), recrystilization solvent: ethanol.

2.3.5 Synthesis of 5-(3-nitrophenyl)-2-(1,2-dione-1H-2-yl phthalazine)-1,3,4-oxadiazole [95][5]:



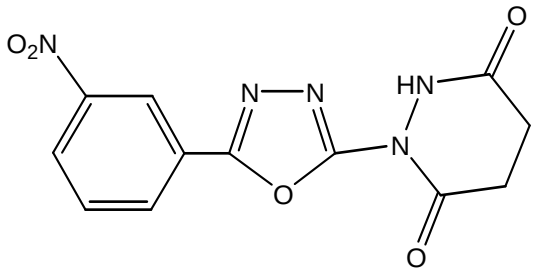
A mixture of 2-hydrazido-5-(3-nitrophenyl)-1,3,4-oxadiazole [4] (0.7g, 0.003 mol) was mixed with phthalic anhydride (0.5 g, 0.003 mol) in acetic acid (30 ml), the mixture was refluxed for 7 hours, and then cooled and added to crushed ice. The precipitate was filtered off, washed with water and recrystallized to the final product, m.p. (290-292 °C), and yield (79%).

2.3.6 Synthesis of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-1,2-dihydro-pyridazine-3,6-dione[95][6]:



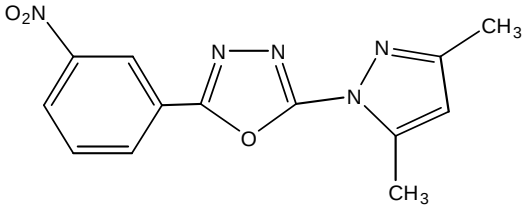
A mixture of 2-hydrazido-5-(3-nitrophenyl)- 1,3,4-oxadiazole [4] (0.7 g, 0.003 mol) was mixed with maleic anhydride (0.35 g, 0.003 mol) in acetic acid (30ml) the mixture was refluxed for 7 hours, then cooled on crushed ice, the precipitate was filtered off, washed with water, recrystallized to give the final product, m.p. (185-187 °C), yield(71%).

2.3.7 Synthesis of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl]-tetrahydro pyridazine-3,6-dione[95][7]:



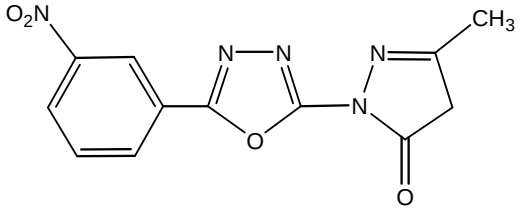
A mixture of 2-hydrazido-5-(3-nitrophenyl)- 1,3,4-oxadiazole [4] (0.7g, 0.003 mol) was mixed with succinic anhydride (0.4 g, 0.003 mol) in acetic acid (30ml). The mixture was refluxed for 7 hours then cooled and added on to crushed ice, the precipitate was filtered off, washed with water, recrystallized to give the final product m.p. (166-168 °C), yield (67%).

2.3.8 Synthesis of 2-(3,5-Dimethyl-pyrazol-1-yl)-5-(3-nitro-phenyl)- 1,3,4-oxadiazole[96][8]:



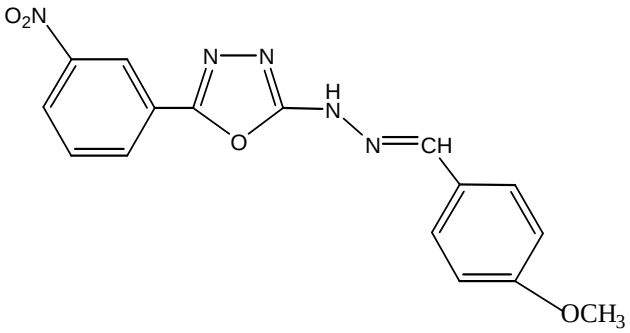
A mixture of 2-hydrazido-5-(3-nitrophenyl) - 1,3,4-oxadiazole [4] (0.7g,0.003 mol) and acetyl acetone (0.3 g, 0.003 mol) and (0.1 ml) of acetic acid in absolute ethanol (30ml) was refluxed for 7 hours. After concentration and cooling, the soild product that was formed filtered off and recrystallized from ethanol m.p. (115-117 °C), yield (87%).

2.3.9 Synthesis of 5-(3-nitrophenyl)-2-(3-methyl-5-one-1-yl pyrazole)-1,3,4-oxadiazole [96] [9]:



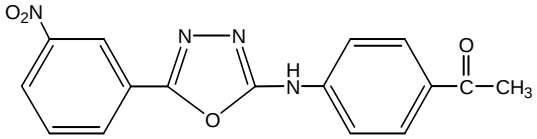
Compound [9] was synthesized by the same method described by the preparation of compound [8]. The precipitate was obtained m.p. (270-272 °C), yield (72%).

2.3.10 Synthesis of 4- [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-yl-hydrazino]-4-amino benzylidine [97] [10]:



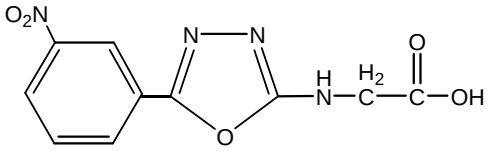
A mixture of 2-hydrazido-5-(3-nitrophenyl)- 1,3,4-oxadiazole [4] (0.7g, 0.003 mol) with 4-methoxy benzaldehyde (0.4g, 0.003mol) in absolute ethanol (15ml) and dropes of glacial acetic acid was refluxed for 8 hours cooling the mixture produced which was collected by filteration m.p. (167-169 °C), yield (88%), recrystallization solvent: ether.

2.3.11 Synthesis of 5-(3-nitrophenyl)-2-(4-ylacetophenone) amino-1,3,4-oxadiazole [98][[11]:



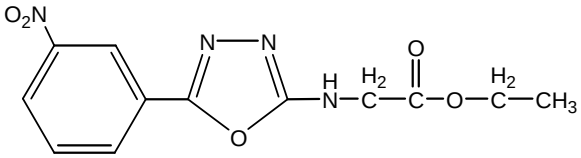
A mixture of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole [3] (0.7g, 0.003 mol) with 4-aminoacetophenone (0.4 g, 0.003 mol) in dry dioxin (5ml) was refluxed for 3 hours, then the mixture was allowed to cool and the solid product was obtained by filtration, m.p. (173-175 °C), yield (83%).

2.3.12 Synthesis of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid [98] [12]:



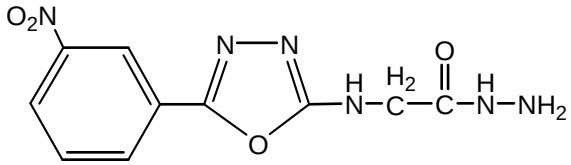
A mixture of 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole [3] (0.7g, 0.003 mol) with glycine (0.23g, 0.003 mol) in dry dioxin (5ml) was refluxed for 3 hours, then the mixture was allowed to cool and the solid product was obtained by filtration, m.p. (181-183 °C), yield (79%).

2.3.13 Synthesis of 5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole [91] [13]:



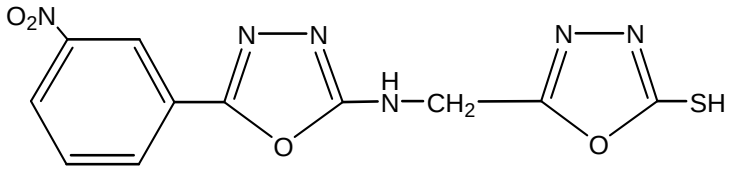
In a 50ml round bottom flask a mixture of (4.2g, 0.016mol) of [5-(3-nitrophenyl)-[1,3,4]oxadiazol-2-ylamino]-acetic acid [12], (2.5ml) of absolute ethyl alcohol, (0.41ml) of concentrated sulphuric acid was refluxed overnight, after that excess of solvent was removed by distillation.10% sodium bicarbonate was added to the solution until litmus paper turned blue. The ester layer was separated by adding the mixture to a separating funnel and extracting it using (10 ml) of ether. The ether layer was separated, dried using unhydrous $MgSO_4$, filtered and evaporated on a water bath to remove the ether. M.p. (202-204 °C), yield (71%)

2.3.14 Synthesis 0f 5-(3-nitrophenyl-2-aminoacetic acid hydrazide-1,3,4-oxadiazole [92] [14]:



A mixture of [5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole [13] (0.01 mol) and excess of hydrazine hydrate (5 ml) were refluxed for (2 hrs), ethanol (10 ml) was added and refluxed for (5-7 hrs). The precipitate which separated on cooling was filtered and washed with cold water. M.p. (262-264 °C), yield 60%, recrystilization solvent: toluene.

2.3.15 Synthesis of 5-(3-nitrophenyl)-2-[2`-mercapto-5`-methyl-1`,3`,4`-oxadiazole]-2-ylamino-1,3,4-oxadiazole [(93)][15]:



A mixture 0f 5-(3-nitrophenyl-2-aminoacetic acid hydrazide-1,3,4-oxadiazole [14] (0.01 mol, 2.78g) with (0.015mol, 0.5 ml) carbon disulfide and potassium hydroxide (0.015 mol, 0.82g) was refluxed for 7 hours. Then the solvent was evaporated and the residue was dissolved in water and acidified by dilute hydrochloric acid, the precipitate was filtered. M.p. (298-300 °C), yield 75%, recrystilization solvent :(ethanol-water).

2.4 BIOLOGICAL ACTIVITY DETERMANTION

2.4.1 Microbiological Method

In this work the antimicrobial test was performed according to agar well diffusion method [(99)]. The prepared compounds were tested against two pathogenic microorganism, Gram positive *S. aureus* and Gram negative *E. coli*. On the solidified media (Nutrient agar), suitable spaced apart holes were made (6 mm in diameter) these holes were filled with (0.1 ml) of prepared compound concentration that dissolve in DMSO (Di Methyl Sulfoxid) after spread the bacteria on agar. These plate were incubated at 37 ^{o}C for 24 hour, the zone of inhibition of bacteria growth around the hole was observed and measured in mm and are represented by (+), (++) and (+++) depending upon the diameter and clarity[(100)].

Contents

Chapter One : Introducation

Chapter Two : Experimental

Chapter Three : Results and Discussion

الأسم: وئام عبدالواحد عبدالنبي.

التحصيل الدراسي: ماجستير- علوم كيمياء.

سنة التخرج: بكالوريوس ٢٠٠٤

الجامعه:جامعة النهرين

المواليد: ١٩٨٣

العنوان: بغداد.

الايميل : chemwiaam@yahoo.com.

رقم الهاتف:لايوجد.

عنوان السكن: محله : ٤٣٤ ، الزقاق:٢٩، الدار:١٠

تاريخ المناقشه : ٣٠-٥-٢٠٠٧

اسم المشرف:د.سلمان علي احمد.

عنوان الاطروحه: **تحضير بعض مركبات ١ ، ٣ ، ٤ -اوكسادايازول الجديدة وتأثيرها المضاد على *S. AUREUS* و*E.COLI***

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Chemistry

SYNTHESIS OF SOME NEW 1,3,4-OXADIAZOLE COMPOUNDS AND ITS INHIBITORY EFFECT ON *S. AUREUS* AND *E.COLI*

A THESIS
SUBMITTED TO THE COLLEGE OF SCIENCE AL-NAHRAIN UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

By
Wiaam Abdal-wahed Abdal-nabi
(B.Sc 2004)

March 2007 *Safar 1428*

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

تحضير بعض مركبات
١ ، ٣ ، ٤ - اوكسادايازول الجديدة وتأثيرها المضاد على *S. AUREUS* و*E.COLI*

رسالة
مقدمة إلى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
وئام عبدالواحد عبدالنبي
بكالوريوس ٢٠٠٤ (جامعة النهرين)

صفر ١٤٢٨ اذار ٢٠٠٧

Supervisor certification

I certify that this thesis was prepared under our supervision at the Department of Chemistry, College of Science, Al-Nahrain University as a partial requirements for the Degree of Master of Science in Chemistry.

Assistant professor

Dr. Salman A. Ahmed

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Assistant Professor

Dr. Salman A. Ahmed

Head of the Department

of Chemistry College of

Science Al-Nahrain University

Examining Committee's Certification

We, the Examining Committee, certify that we read this thesis and have examined the student ***Wiaam Abdal-wahed Abdal-nabi***, in its contents and that, in our opinion; it is adequate as a thesis for the Degree of Master of Science, in Chemistry.

Signature:

Name:
(Chairman).

Signature:

Name:
(Member)

Signature:

Name:
(Member)

Signature:

Name: Assistant Professor Dr. Salman A. Ahmed
(Member\advisor)

Approved for the College of Graduate Studies

Assistant Professor

Dr.L◌◌◌AITH ABDUL AZIZ AL-ANI

Dean of College of

Science Al-Nahrain

University

بِسِم الله الرحمن الرحيم

الرَحمنْ (١) عَلَمَ القُرآنْ (٢) خَلَقَ الأنسانْ (٣) عَلَمَهُ البيانْ

صدق الله العظيم
الآية (١-٣)

الإهداء

إلى نور الهدى... ومقلة العيون

...الحبيب المصطفى (ص)

إلى من ضحى لأجل أن أكون

... والدي العزيز

إلى طيب الجنّة... وشذاها

... أُمي الغالية

الى من أشد بهم أزري في الحياة

... اخوتي

إلى كل من مد يد العون والمساعدة لي

... زملائي

أهدي ثمرة جهدي المتواضع

وئام

Acknowledgment

"O Allah to you belongs all praise, you are the light of the heavens and the earth and all that is within them. To you belongs all praise, you are the lord of the heavens and the earth and all that is within them. To you belongs all praise, and the kingdom of the heavens and the earth and all that is within them, and the prayer and peace upon the master of the mankind our beloved messenger of Allah "Muhammad" and his pure progeny: companions and all those who follow his way in charity to Judgment Day".

I wish to express my sincere gratitude and great appreciation to my respected supervisor Dr. Salman A. Ahmed for his guidance and his encouragement through this work.

I am especially grateful to Miss Hanin Saadun , Miss Israa A. Mohamed and all the staff in the Department of Biotechnology, College of Science, Al-Nahrain University for their great help in biological study.

I would like to thank my nearest friends especially Ruaa, Sarah, Hadeel

Many thanks are due to the Department of Chemistry, College of Science, Al-Nahrain University for providing the facility which helped in accomplishing this research.Finally to all my friends, I present my thanks.

Wiaam 2007

SUMMARY

The scheme of this work involves synthesis and evaluation the biological activity of some new 1,3,4-oxadiazole compounds derived from 3-nitrobenzoic acid as starting material. This work is divided into four parts:

First part: This part involved synthesizing 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole [3] which derived from the reaction of compounds 3-nitrobenzoic acid [0], ethyl-3-nitrobenzoate [1],3-nitro benzoic hydrazide [2], with different organic compounds.

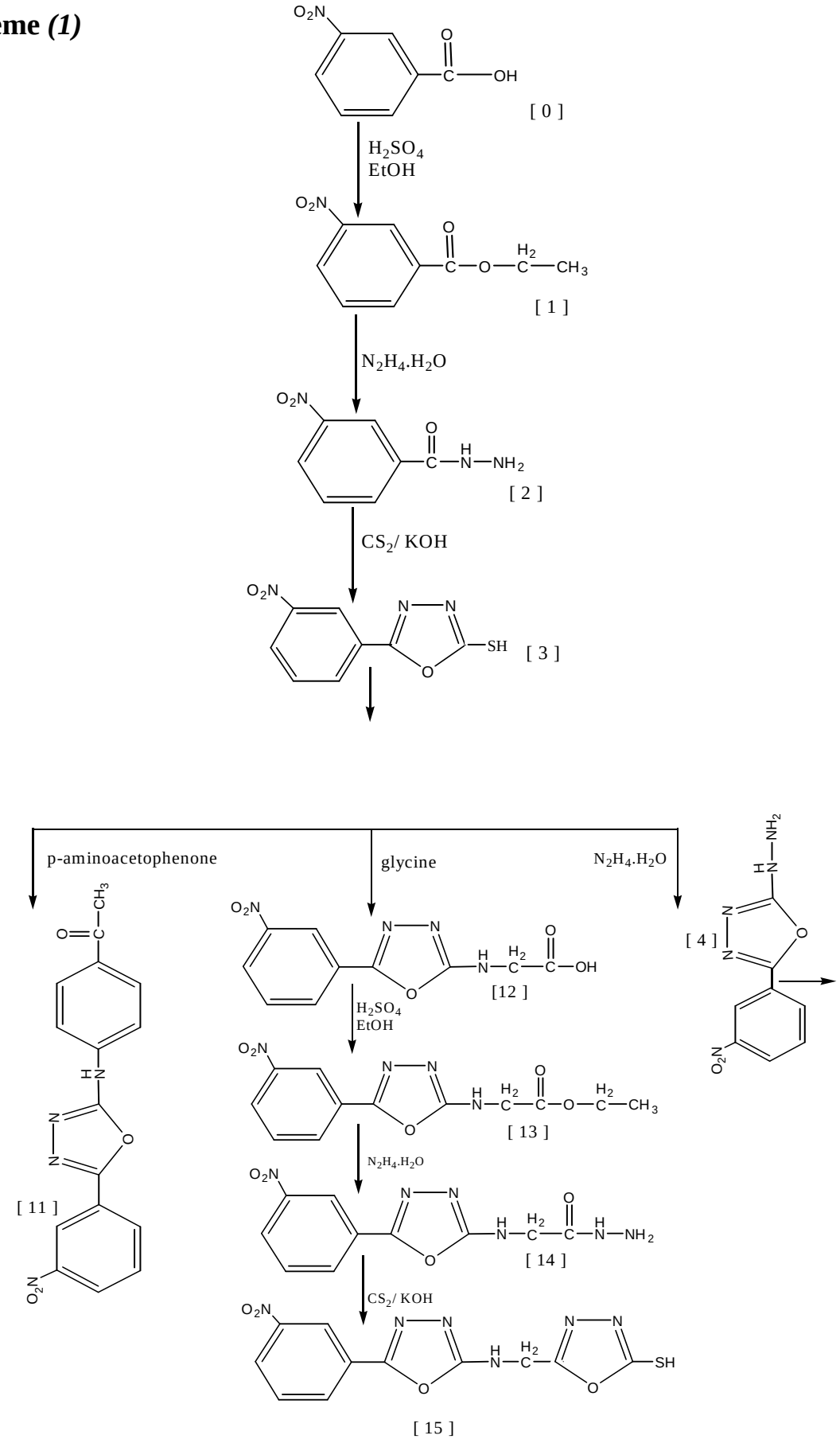
Second part: This part involved synthesizing of phthalazin,pyridazin-dione derivatives and Schiff base, from the reaction of material 2-hydrazido-5-(3-nitrophenyl)-1,3,4-oxadiazole [4] with different organic compounds.

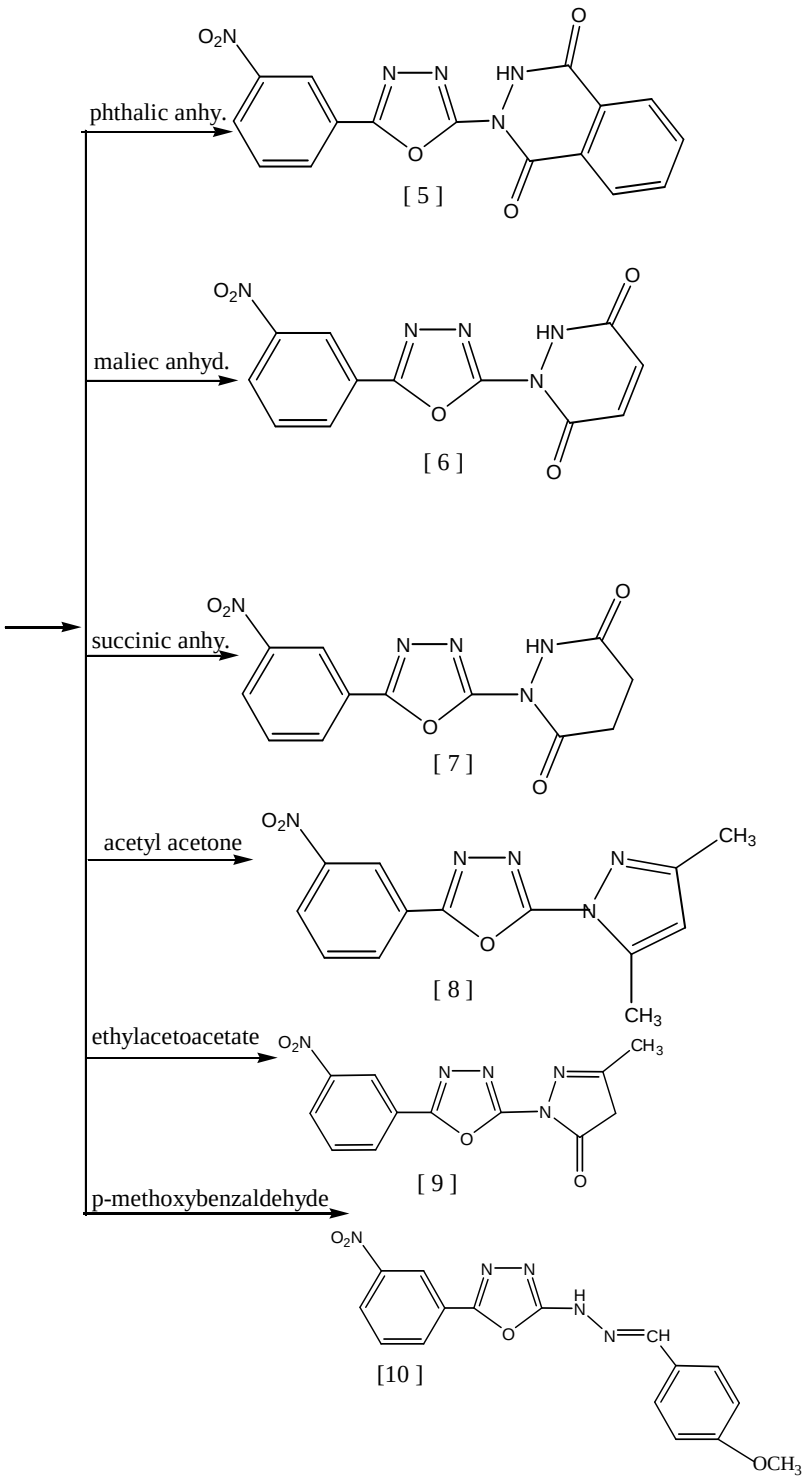
Third part: This part involved connecting p-aminoacetophenone, glycine with compound [3] 2-mercapto-5-(3-nitrophenyl)-1,3,4-oxadiazole through elimination reaction. Then synthesizing bis 1,3,4-oxadiazole compound which derived from the reaction of compounds [5-(3-nitrophenyl)-[1,3,4-oxadiazole]-2-ylamino]-acetic acid [12], 5-(3-nitrophenyl)-2-aminoethylacetate-1,3,4-oxadiazole [13], 5-(3-nitrophenyl)-2-aminoacetic acetic acid hydrazide-1,3,4-oxadiazole [14] with different organic compounds.

Forth part: This part involved evaluate the biological activity of some synthesized compounds for their antimicrobial activity against two strains of pathogenic microorganism Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli*.

The following scheme shows the synthesized compounds

Scheme *(1)*





Scheme (1) shows the synthesezied compounds

ABBREVIATIONS

No.	Symbol	Description
1	CAT	Chloramphenicol Acetyl-Transferase
2	DMSO	DiMethyl Sulfoxid
3	FTIR	Fourir Trans InfraRed
4	NMR	Nuclear Magnatic Resonance
5	THF	Tetrahydrofuran
6	TLC	Thin Layer Chromatoghraphy

الخلاصة

يتضمن موضوع ألرسالة تحضير وتقييم الفعالية البايولوجية لبعض مركبات ١ ، ٣ ، ٤ - أوكسادايازول الجديدة والمشتقة من ٣-نايتروبنزويك اسيد كمادة اساسية وقد تم تقسيم العمل الى اربعة اجزاء :

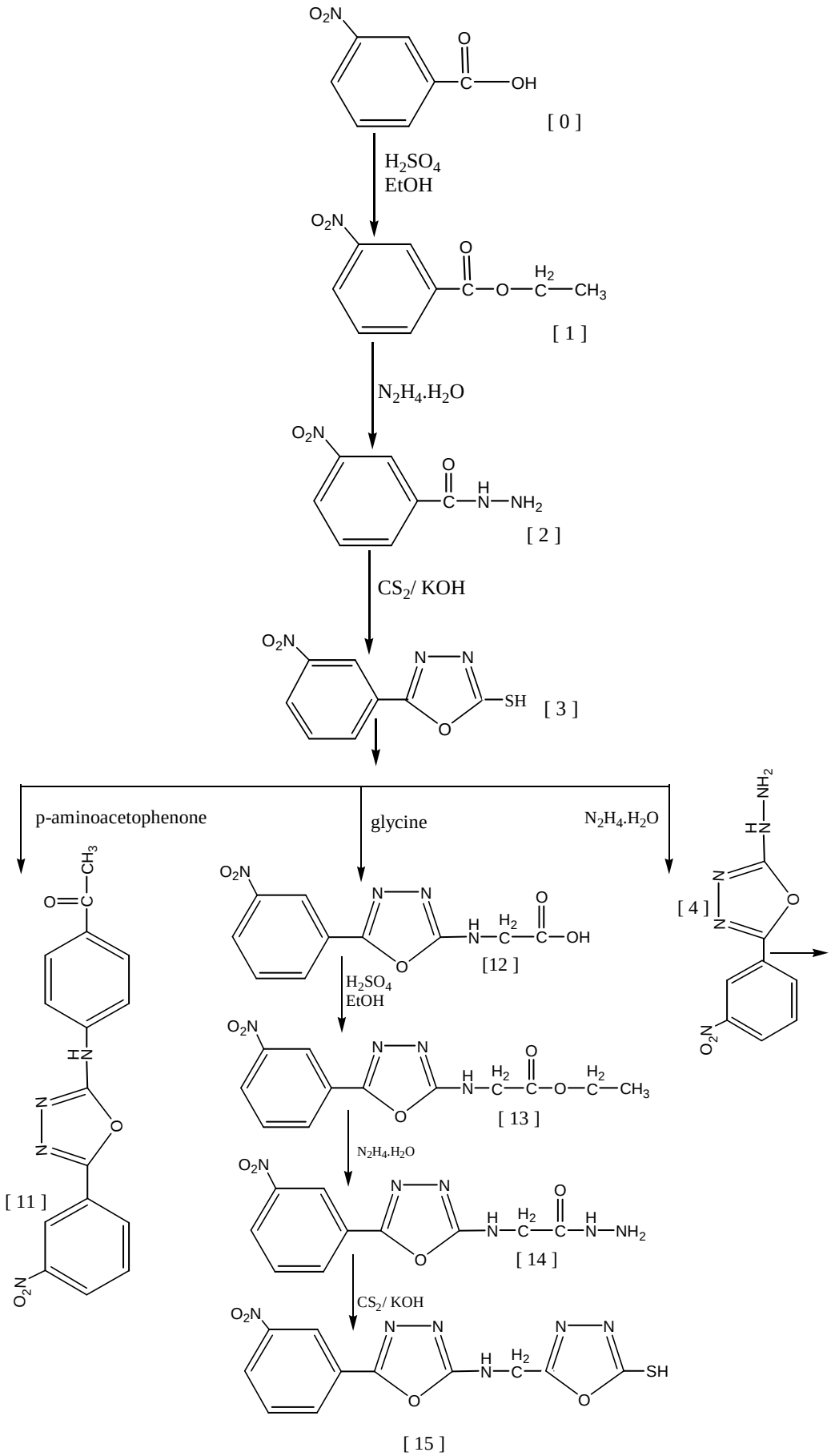
ألجزء الاول. يتضمن هذا الجزء تحضير مركب ٢-مركيبتو-٥-(٣-نايتروفنيل)- ١ ، ٣ ، ٤ -اوكسادايازول [3] الذي اشتق من تفاعل المواد ٣-نايتروبنزويك اسيد[0], اثيل -٣-نايتروبنزويت [1]،٣-نايتروبنزويك هايدرازايد[2] مع مواد عضوية مختلفة.

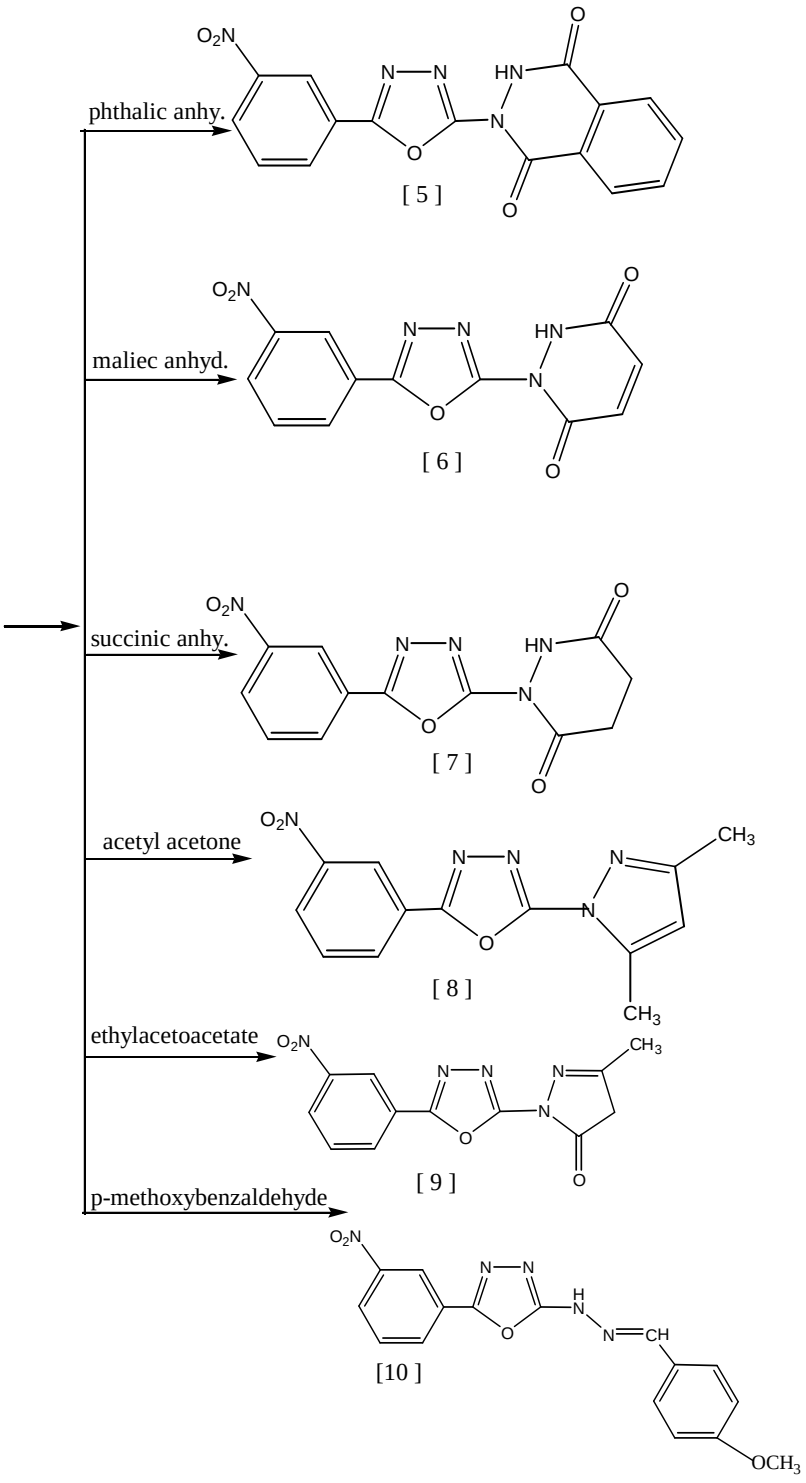
ألجزء الثاني : يتضمن هذا الجزء تحضير مركبات الفثالازين، البريدازين-دايون وقواعد شيف، من تفاعل المادة ٢-هايدرازيدو-٥-(٣-نايتروفنيل) - ١ ، ٣ ، ٤ -اوكسادايازول [٤] مع مواد عضوية مختلفة.

ألجزء الثالث: يتضمن هذا الجزء ربط، بارا امينواسيتوفينون، الكلايسين مع مركب ٢-مركيبتو-٥-(٣-نايتروفنيل)- ١ ، ٣ ، ٤ -اوكسادايازول [3] عن طريق تفاعلات الحذف. ثم تحضير مركب ثنائي ١ ، ٣ ، ٤ - اوكسادايازول الذي اشتق من تفاعل المواد [٥ –(٣-نايتروفنيل) [1,3,4] اوكسادايازول-٢-يال امينو]-اسيتك اسيد [12]، ٥ –(٣-نايتروفنيل)-٢-امينواثيل اسيتيت ١ ، ٣ ، ٤ -اوكسادايازول [١٣], ٥ –(٣-نايتروفنيل)-٢-امينواسيتك اسيدهايدرازايد ١ ، ٣ ، ٤ - اوكسادايازول [14] مع مواد عضوية مختلفة.

ألجزء الرابع: يتضمن هذا الجزء تقييم الفعالية البايولوجية لبعض المركبات المحضرة ضد أنواع منتخبة من البكتريا وهما البكتريا الموجبة للصبغة(*Staphlococcus aureus*) و البكتريا السالبة للصبغة (*Escherichia. coli*) وقد دلت النتائج المستحصلة بأن بعض المركبات أظهرت فعالية بايولوجية ضد البكتريا المستخدمة.

المخطط (١):


[0]
H_2SO_4
EtOH
[1]
$N_2H_4.H_2O$
[2]
CS_2/ KOH
[3]
p-aminoacetophenone
glycine
$N_2H_4.H_2O$
[4]
[11]
[12]
H_2SO_4
EtOH
[13]
$N_2H_4.H_2O$
[14]
CS_2/ KOH
[15]


phthalic anhy.
[5]
maliec anhyd.
[6]
succinic anhy.
[7]
acetyl acetone
[8]
ethylacetoacetate
[9]
p-methoxybenzaldehyde
[10]