Synthesis and Studies The Biological Activity of New Azo Compounds

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الخلاصة

تم في هذا البحث ادخال مركب السايكلوهكسانون الى تفاعل تحضير Hantzsch بواسطة معاملته مع اليود و الثايويوريا ليعطي المركب الوسطي 2-amino-4,5,6,7- tetrahydrobenzothiazole . هذا المركب تم تحويله الى ملح الديازونيوم وثم الازدواج مع مركبات مختلفة من مشتقات الفينول و النفثول ليعطي مشتقات الازو وهذه المشتقات شخصت بواسطة اطياف HNMR , FT-IR, UV-VIS. [1] .

تم فحص هذه المشتقات ضد ثلاث انواع من الكائنات الحية المجهرية وهي Klebsiella, Pseudomonas aeuroginosa و Escherichia Coli وقد اظهرت بعض المركبات تأثير بسيط وبعضها لم يظهر أي تأثير وكان مشتق 2-naphthol الاكثر فعالية ضد جميع انواع البكتريا .

Abstract

The cyclohexanone was subjected to Hantzsch synthesis by treating with iodine and thiourea to give an intermediate 2-amino-4,5,6,7- tetrahydrobenzothiazole. This was then diazotized and coupled with various phenols and naphtholes derivatives to give a series of new disperse dyes. These azo derivatives were characterized by spectral methods [1]HNMR , FT-IR, UV-VIS..

These derivatives were tested against three microorganisms Klebsiella, Pseudomonas aeuroginosa and Escherichia Coli. Some of azo compounds was slightly and non active while azo-2-naphthol was highly acive against all the bacteria species tested.

Introduction

Before the middle-nineteenth century, all of the dyes used to dye fabric were extracted from natural sources. In 1856, William Perkin, synthesized the first dye that was used commercially. This dye is known as Perkin's mauve or mauveine. The first commercially important azo dye was Bismarck Brown, first manufactured in 1865. By the 1970s, over 60% of the synthetic dyes were azo dyes[(1)].

Azo compounds constitute one of the largest classes of industrially synthesized organic compounds. They are important in dye, drugs and cosmetics[(2)]. and show a variety of interesting biological activities including antibacterial and pesticidal activities. Compounds of naphthalene have been reported to show a variety of biological activities including antimicrobial activities, HIV-1 integrase inhibitory effect.

Heteocyclic amines have been used extensively in the preparation of disperse dyes. These dyes show outstanding discharge-ability on polyester. Disperse dyes before 1950 were mostly amino anthraqunone derivatives. Through these dyes are bright in color they have limitations of poor discharge-ability and are sensitive to the oxides of nitrogen. Dyes contain heterocyclic ring as 2-amino -5-nitrothiazole have been reported to have extinction coefficient , this type of azo dyes are classified as donor – acceptor chromogen[(3)].

Compounds containing one or more azo groups (-N=N- linked to two carbon atoms) have a variety of uses. Aliphatic azo compounds, like azo*bis*isobutyronitrile (AIBN), can be as radical initiators in polymerization of alkenes to make plastics. Aromatic azo compounds are used as acid-base indicators, biological stains, and commercial colorants for clothing, plastics, cosmetics, and food beverages. Many azo-dyes, such as methyl red, methyl orange, and congo red, can be used as

acid-base indicators due to their ability to function as weak acids or bases. Color changes are caused by changes in extent of delocalization of electrons: more delocalization shifts the maximum absorption to longer wavelengths and makes the light absorbed redder, while less delocalization shifts the maximum absorption to shorter wavelengths. Color changes can also be due to geometrical isomerism of the azo group. UV radiation can cause a *trans* azo group to become *cis*. This can lead to photochromism, a light-induced reversible color change. Some azo dyes with this property (and which can revert slowly to the *trans* isomer in the dark) are used in sunglasses and car sunroofs.

Many azo dyes, like Sudan red and scarlet red, can be used as biological dyes because they are fatsoluble and can be absorbed into fat cell tissues on microscope slides. Azo dyes form 60-70% of all synthetic dyes used as commercial colorants. Azo dyes have several advantages over other commercial dyes including their wide color range, good color fastness and ability to absorb light.

They can also be synthesized cheaply because the starting materials are readily available, inexpensive compounds; most of the chemistry is completed at or below room temperature; and the environmental impact is low due to the use of water as a solvent in all of the reactions. Cost advantages tend to compensate for the lower resistance to bleaching and lower brilliance of azo dyes compared to anthraquinones, the second most used dye class [4-8].

In this paper, we synthesized azo compounds derived from 2-Amino-4,5,6,7-tetrahydrobenzothiazole and used them to investigate their inhibitory effects on the biological activity of some bacteria. The contribution of the azo moiety toward the biological activity is also evaluated .

Experimental

Melting points were determined in open capillary tubes on a Gallen kamp melting point apparatus and uncorrected.

The FTIR - 8400S spectra (KBr disc were recorded with Shimadzu-2N in college of science , university of al-mustamsiryah, UV Spectra were recorded on Varian, UV-Vis spectrophotometer using absolute ethanol as solvent in college of science , university of al-mustamsiryah. ^{1}H-NMR spectra were recorded on Bruker spectrometer model ultra shield at (300MHz) in Al-Albait University, Jordan. Tetramethylsilane (TMS) was used as an internal reference and DMSO-d6 as solvent.

Preparation 2-Amino-4,5,6,7-tetrahydrobenzothiazol (1)

Cyclohexanone (4.5 mL ,0.05mole,) was dissolved in (35 mL) absolute ethanol. Thiourea (6gm ,0.1mole) and iodine (12.7gm ,0.05 mole,) were added and the reaction mixture was heated with stirring under reflux for 5 hrs., the reaction mixture was then cooled to 20 $^{\circ}$C and quenched in (100 mL) of distilled water. The quenched mass was basified with liquor ammonia solution and extracted in (150 mL) ethyl acetate,(3×50mL). The ethyl acetate layer was washed with (100 mL) water and then filtered to remove insoluble solids. The ethyl acetate extract was then concentrated and the crude residue was purified by column chromatography using chloroform as eluent and silica gel (70-230 mesh) as solid phase. The pure fraction on concentration gave red product as syrup , yield (80 %).

General method for the prepare of derivatives (2-6) .

Mixture of concentrated HCl (1mL) and compound (1) (0.001 mole) was kept at 0 – 5 °C using an ice-water bath. A solution of sodium nitrite 0.20 g in distilled water 0.5 mL was prepared. The sodium nitrite solution was added to the solution of compound (1) with stirring by using a glass rod. The temperature was controlled below 10 °C through out the addition. Phenolic or naphtholic derivatives (0.001 mol) was dissolved in 10% NaOH solution (10mL) and then put an ice-water bath to cool to 5 °C. Then ,diazonium salt solution was added occasionally stirring very slowly to the Phenolic or naphtholic derivatives solution. The color of the solution was changed , then the reaction mixture was left to complete for about 15 min with occasional stirring , then the formed

precipitate was filtered and dried in air and then recrystallized from absolute ethanol to give azo compounds (2-6), the physical properties were tabulated in table (1) .

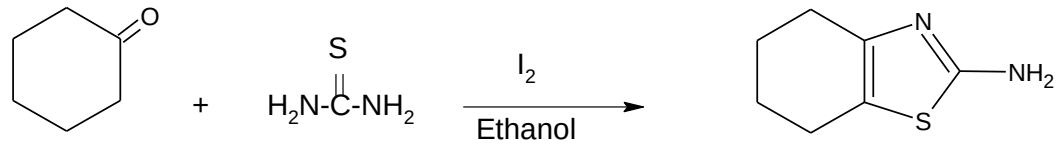
Study the biological activity for compounds

The biological activity of the new compounds was studied against selected types of bacteria which included *Eschericha coli* , *Klebsiella* and *Pseudomonas aeruginosa* were cultivated in nutrient agar medium .

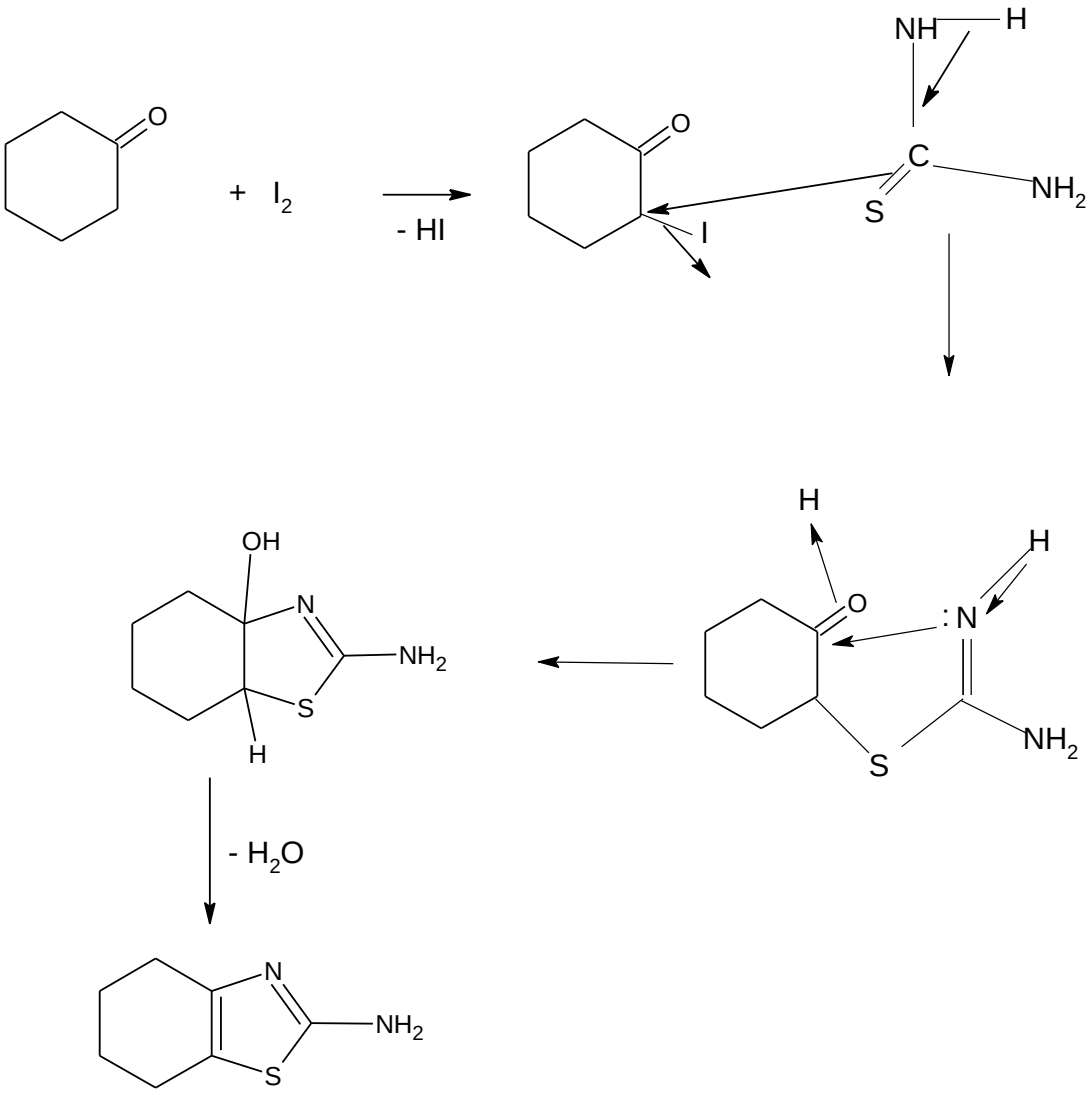
Two ***in vitro*** techniques were proceeded for studying antibacterial activity against the two strains , DMSO was used as a solvent and as a control , for both techniques the concentaction of the compounds in this solvent were (10^{-3} M) . The first technique was the Disc Sensitivity Test[9], this method involves the exposure of the zone of inhibition towards the diffusion of micro-organism on agar plate. The plates were incubated for 24hr. at 37 ^{o}C, the zone of inhibition of bacterial growth around the disc was observed.

Results and Discussion

In the present study we report the synthesis of the azo compounds derived from a 2-Amino-4,5,6,7-tetrahydrobenzothiazol (1) **.** This compound was synthesized from cyclohexanone using a variation of Hantzsch aminothiazole synthesis[10]. The cyclohexanone was treated with iodine and thiourea in alcoholic medium, wherein α – iodination occurs at the 6th position in cyclohexanone followed by insitu condensation with thiourea. Then cylices with the elimination of HI to give 2-amino-4,5,6,7-tetrahydrobenzothiazol (1) , the following scheme shows the reaction and mechanisms for preparation of compound (1).



Cyclohexanone **thiourea** **(1)**



Scheme (1) : The reaction and mechanisms for preparation of compound (1).

The compound (1) was characterized by spectral analysis , the FT-IR spectrum of compound (1) (figure 1) showed the present of a peak at 3296 and 3153 cm^{-1} corresponding to the asymmetric and symmetric stretching vibrations of amino group (υ_{N-H}) respectively , FT.IR spectrum also conformed the absence of a peak at 1720 cm^{-1} (keto group), which was present in the starting material. Compound (1) was diazotized using sodium nitrate and concentrated hydrochloric acid in aqueous medium. The resulting diazonium salt intermediate was coupled with various phenolic and naphtholic derivatives to obtain new azo compounds , the physical properties of these new compounds were shown in table (1). The low yield of compounds (4-6) due to these compounds have aromatic ring contain releasing groups .

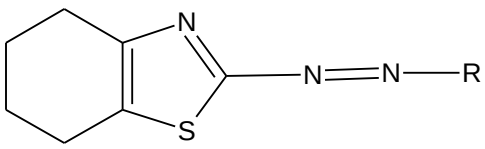


Table (1) : physical properties of prepared compounds

No.of comp.	R	Name of comp.	M.P ^{o}C	Yield %	Color
2	2-naphthol	1-[4,5,6,7-tetrahydro-1,3-benzothiazol-(2-yldiazenyl)]-2-naphthol	60-62	90	Palle yellow
3	1-naphthol		55-57	66	violet
4	Phenol	2-[4,5,6,7-tetrahydro-1,3-benzothiazol-(2-yldiazenyl)]-1-naphthol	170-172	4	yellow
5	m-resorcinol		Syrup	11	Orange
6	p-methoxy phenol	4-[4,5,6,7-tetrahydro-1,3-benzothiazol-(2-yldiazenyl)]phenol	Syrup	12	Orange
		4-[4,5,6,7-tetrahydro-1,3-benzothiazol-(2-yldiazenyl)]benzene-1,3-diol			
		4-methoxy-3-[4,5,6,7-tetrahydro-1,3-benzothiazol-(2-yldiazenyl)]phenol			

All new derivatives dyes were characterized by spectral analysis as UV-Vis., FTIR and ^{1}HNMR spectra, FT.IR spectra of (1-azo naphthole derivative (2), figure 2) showed =C-H stretching vibrations of the aromatic ring appearing at 3100 cm^{-1} and C-H bending vibrations of the aromatic ring appearing at 700 – 820 cm^{-1}, the azo group stretching vibrations band appeared at 1512 cm^{-1} and stretching vibration of hydroxyl group at 3280 cm^{-1} and disappearance the peaks of amino group. The U.V –Visible spectra for azo phenol ((4),figure 3) shows 224 nm for $\pi \rightarrow \pi^{*}$, and 277 nm for $n \rightarrow \pi^{*}$, while azo naphthole showed peaks at 233 nm for $\pi \rightarrow \pi^{*}$, and 325 nm for $n \rightarrow \pi^{*}$ this due to conjugated bonds.

The ^{1}HNMR spectra of 1-azo naphthol derivative ((2),figure 4) showed singlet at 10.2 ppm for proton of (OH) group , signal multiplied at 6.8-8.2 ppm for protons of aromatic ring , singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle . The all data of spectra for prepared compounds showed in table (2).

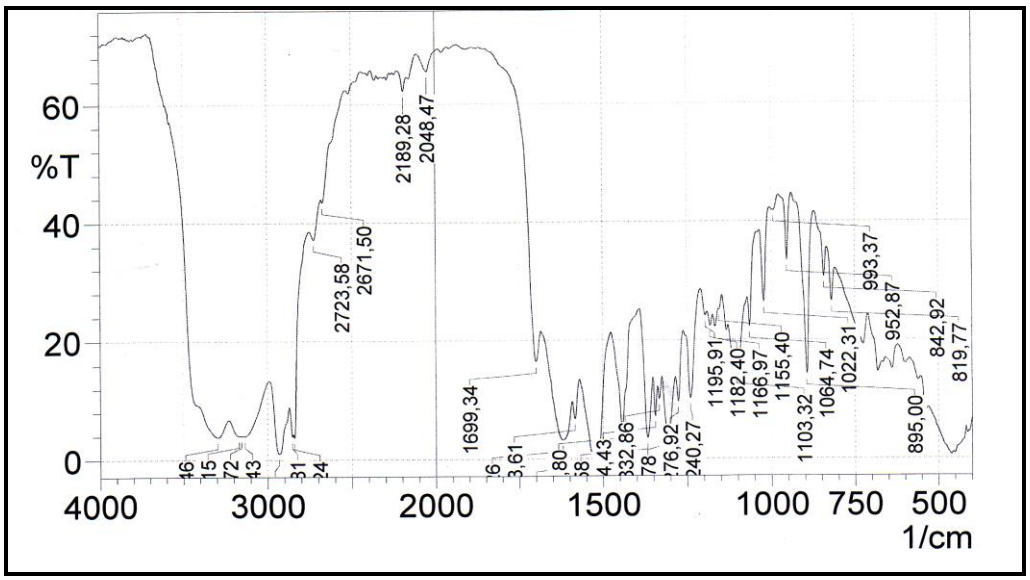


Figure (1) : The FTIR spectrum of compound (1) , amino derivative

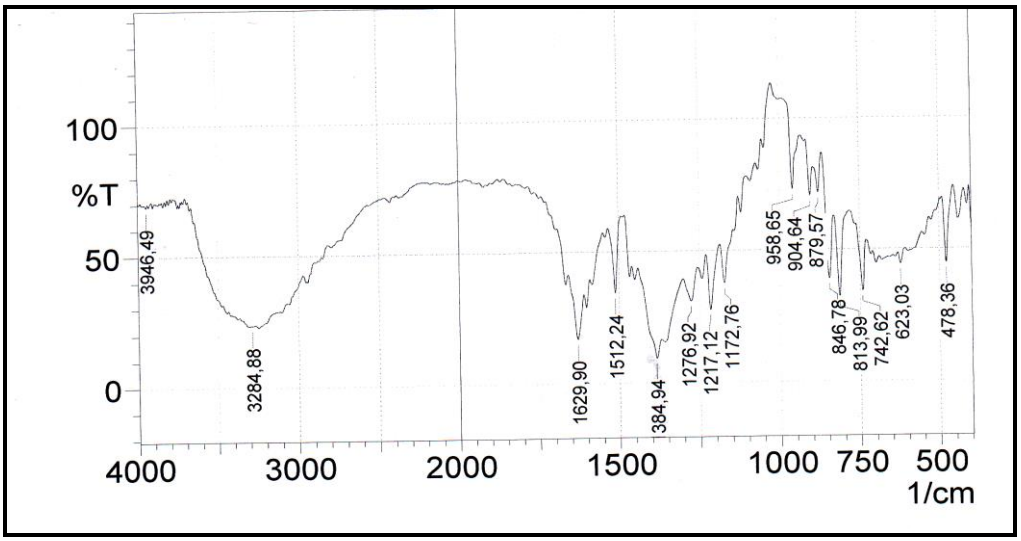


Figure (2) : The FTIR spectrum of 1-azo naphthol (2)

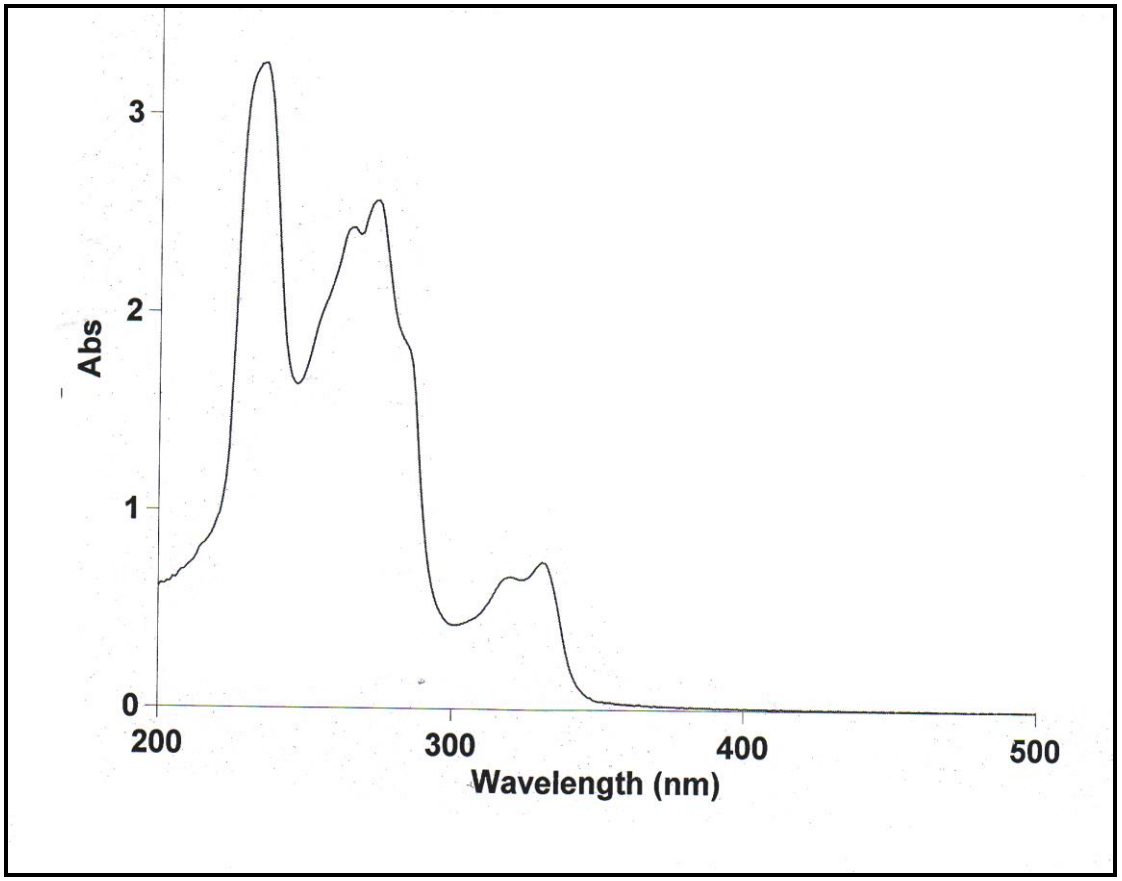


Figure (3) : The UV-Vis. spectrum of 1-azo naphthol (2)

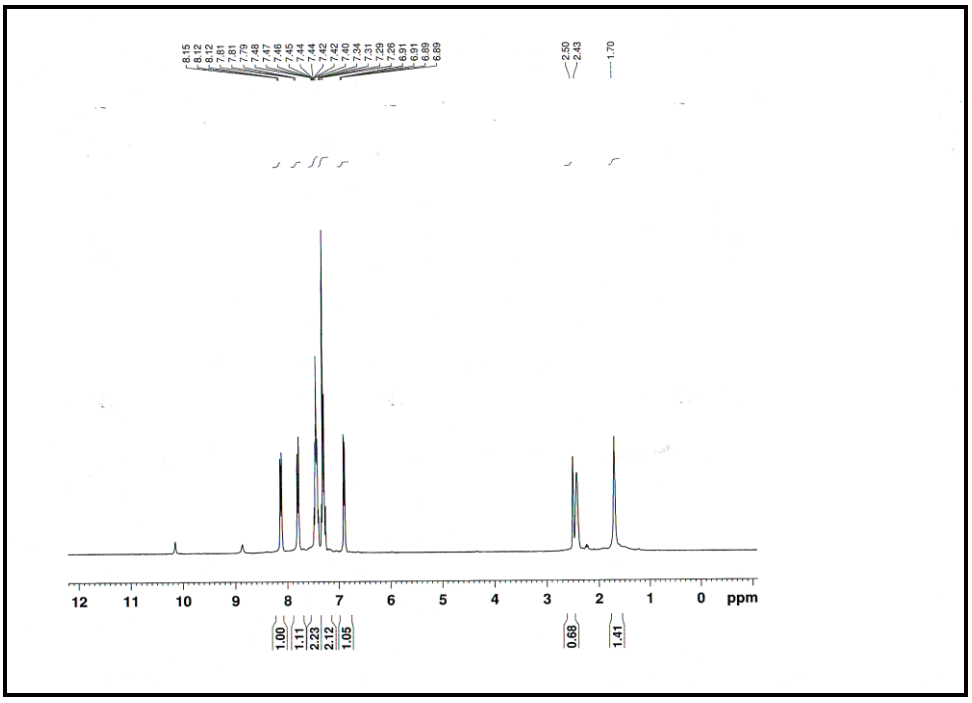


Figure (4) : The ^{1}HNMR spectrum of 1-azo naphthol (2)

Table (2) : The data of spectra for prepared compounds

No.of comp.	U.V-Vis. spectra	FTIR spectra	^{1}HNMR spectra
2	233 nm for $\pi \rightarrow \pi^*$, and 325 nm for $n \rightarrow \pi^*$	3284 cm^{-1} for (OH) , 1629 cm^{-1} for (C=C), 1640 cm^{-1} for (C=N),	10.2 ppm for (OH) group , multiplied at 6.8-8.2 ppm for aromatic protons , singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle .
3			
4	235 nm for $\pi \rightarrow \pi^*$, and 330 nm for $n \rightarrow \pi^*$	1512 cm^{-1} for (N=N)	10 ppm for (OH) group , multiplied at 6.8-8.2 ppm for aromatic protons , singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle .
5		3280 cm^{-1} for (OH) , 1625 cm^{-1} for (C=C),	
6	224 nm for $\pi \rightarrow \pi^*$, and 277 nm for $n \rightarrow \pi^*$	1645 cm^{-1} for (C=N), 1512 cm^{-1} for (N=N)	singlet at 9.2 ppm for (OH) group , multiplied at 6.6-7.3 ppm for aromatic protons , singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle .
	230 nm for $\pi \rightarrow \pi^*$, and 280 nm for $n \rightarrow \pi^*$	3278 cm^{-1} for (OH) , 1624 cm^{-1} for (C=C), 1639 cm^{-1} for (C=N), 1512 cm^{-1} for (N=N)	singlet at 9.4 ppm for (OH) group , multiplied at 6.6-7.3 ppm for aromatic protons , singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle .
	227 nm for $\pi \rightarrow \pi^*$, and 279 nm for $n \rightarrow \pi^*$	3280 cm^{-1} for (OH) , 1635 cm^{-1} for (C=C), 1637 cm^{-1} for (C=N), 1512 cm^{-1} for (N=N)	singlet at 9.1 ppm for (OH) group , multiplied at 6.6-7.3 ppm for aromatic protons , singlet at 5 pmm for protons of (OCH_3), singlet at 1.7 ppm and doublet at 2.5 ppm for protons of aliphatic cycle .
		3286 cm^{-1} for (OH) , 1633 cm^{-1} for (C=C), 1640 cm^{-1} for (C=N), 1512 cm^{-1} for (N=N)	

Biological activity

Biological Screening: Antimicrobial Activity Tests.

The biological activity of some of the prepared compounds was tested against one strain of Gram +ve bacteria (*Klebsiella*), Gram –ve bacteria (*Eschericha coli, Pseudomonas aeruginosa*.

Disc sensitivity test[11]was employed for the *in vitro* study for anti bacterial studies. This method involves the exposure of the zone of inhibition towards the diffusion of microorganism on agar plate. The plates were incubated for 24 hrs. at 37 °C, the zone of inhibition of bacterial growth around the disc was measured.

The resulted are presented in table (2), Compound (2) showed higher biological activity than other compounds against all bacteria , this agreement with literatures[12] and compound (5) showed no effect against all bacteria. ,while other compounds showed different effects on bacteria this due to type of phenol compound.

Table (3): Results of antimicrobial activities of the compounds (10^{-3} mg. mL^{-1})

Compound	*Klebsiella*	*E. Coli*	*Pseudomonas aeruginosa*
Control (DMSO)	-	-	-
2	++	++++	++
3	+	++	-
4	+	-	-
5	-	-	-
6	+	-	+

Where:

(-) : no effect, (+) : 4-5 mm , (++) : 8-10 mm , (++++) : 16-18 mm

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