Ministry of Higher Education and Scientific Research
University of Basrah
College of Pharmacy
Department of pharmaceutical chemistry

Synthesis of new Azo Compounds of p-Hydroxybenzaldehyde and Determination of Their Activity

A Project Submitted to The College of Pharmacy/Basrah University in Partial Fulfillment of the requirement for the B.Sc. in pharmacy sciences.

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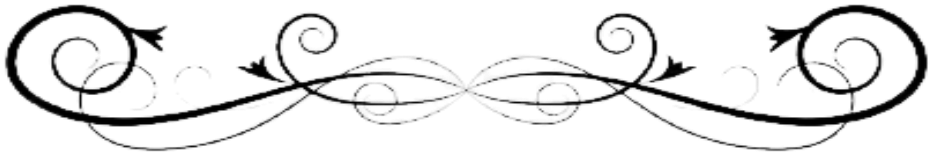
بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

صدق الله العظيم



(سورة المجادلة/ الآية 11)

Dedication

To those who paved the way of knowledge for us, to everyone
who taught us a letter, to our esteemed teachers…

To those who taught us the alphabet of life, to our resilient mothers…

To the magnificent men behind the curtain of our successes,
to our great fathers…

To those who stood by us with their spirits and hearts,
to the companions of our life's journey…

We are grateful.

ABSTRACT

This study aims to prepare some azo compounds by diazo coupling reaction of aromatic amines with p-hydroxybenzaldehyde.

Azo dyes are Compounds containing in their structure a group or more of the AZO groups (–N=N–) called azo compounds [1], in which the nitrogen atom hybridization is sp^2 . azo's properties entirely depend on the structure of the compound, the number of azo linkages, and both groups on each end of the -N=N- linkage.

The prepared compounds then identified using FT-IR spectroscopy .

The purity of the dye was checked by thin layer chromatography(TLC) using solvent system and melting point.

The results supported the structure of concerned compounds.

Finally The synthesized azo compounds were screened in vitro for their biological activity as antibacterial agents against: Staphylococcus aureus, Esherichia Coli, Klebsiella pneumonia and Pseudomonas aerug inosa.

List of Contents

List of tables

List of Figures

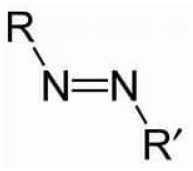
List of Schemes

Chapter One

Introduction

1.1 Introduction

Compounds containing in their structure a group or more of the AZO groups (–N=N–) called azo compounds [1], in which the nitrogen atom hybridization is sp^2 .as shown in figure(1-1);



Figure(1-1)

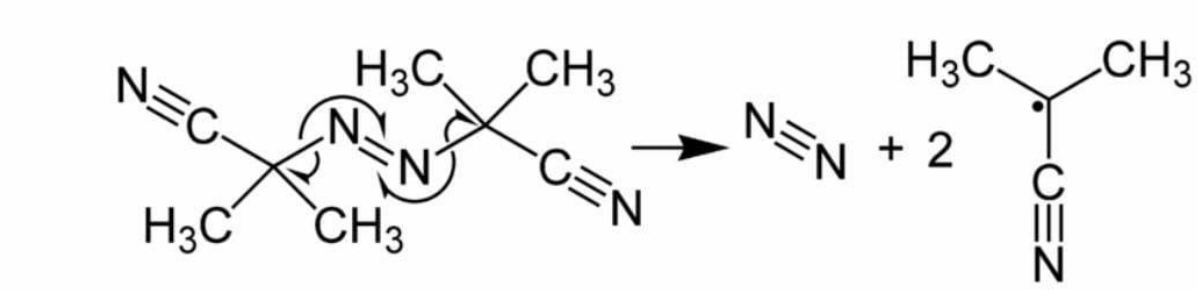
IUPAC defines azo compounds as "derivatives of diazene (diimide), HN=NH, wherein both hydrogens are substituted by hydrocarbyl groups, e.g. PhN=NPh azobenzene or diphenyldiazene." [1] The more stable derivatives contain two aryl groups. The N=N group is called an azo group.

As mentioned earlier, azo's properties entirely depend on the structure of the compound, the number of azo linkages, and both groups on each end of the -N=N- linking unit. For instance, an aromatic azo compound commonly contains two or more aromatic rings. Consequently, this structure has a rigid core due to its ability to absorb light. By altering the number of azo linkages, and the type of substituent on both sides of the linking unit, it is possible to synthesize an infinite number of aromatic azo nucleus structures with different characteristics for diverse applications [2]. Azo compounds are the largest class of compounds ever to be industrially synthesized for their wide range of applications, especially organic azo dye. Initially, dyes were extracted through flora and fauna, and synthetic azo dye was used as a substitute to preserve the natural habitat [3]. The estimated percentage of azo dyes used in the industry is up to 70% [4, 5]. Even the simplest of azo dye has its enactment in various fields. Azo dyes are used in the textile industry [6], colorants in the food industry [7], and in cosmetics due to the low production cost and highly stable compound [8,9]. On the other hand, the presence of an azo compound as a linking unit boosts the chromogenic activity of an azo-based compound, making it easy to detect heavy metals [10]. Azo compounds are quite durable and chemically stable, so another application of azo dyes is in the pharmaceutical industry [8]. The azo compound has excellent antimicrobial properties [11], and it varies in targeted properties such as antibacterial, anticancer, antifungal, antioxidant, and anti-inflammatory properties of azo compound [12-14].

1.2 Classification of azo compounds

1.2.1 Alkyl azo compounds

Aliphatic azo compounds (R and/or R′ = aliphatic) are less commonly encountered than the aryl azo compounds. A commercially important alkyl azo compound is azo bis *iso*-butyronitrile (AIBN), which is widely used as an initiator in free-radical polymerizations and other radical-induced reactions. It achieves this initiation by decomposition, eliminating a molecule of nitrogen gas to form two 2-cyanoprop-2-yl radicals as shown in **Scheme 1-1**:



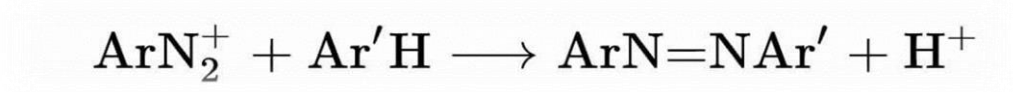
For instance a mixture of styrene and maleic anhydride in toluene will react if heated, forming the copolymer upon addition of AIBN.

A simple dialkyl diazo compound is diethyldiazene, EtN=NEt. Because of their instability, aliphatic azo compounds pose the risk of explosion.

1.2.2 Aryl AZO Compounds

Aryl azo compounds are usually stable, crystalline species. Azobenzene is the prototypical aromatic azo compound. It exists mainly as the *trans* isomer, but upon illumination, converts to the *cis* isomer.

Aromatic azo compounds can be synthesized by azo coupling, which entails an electrophilic substitution reaction where an aryl diazonium cation is attacked by another aryl ring, especially those substituted with electron-donating groups as shown in Scheme 1-2:



Scheme(1-2)

Since diazonium salts are often unstable near room temperature, the azo coupling reactions are typically conducted near 0 °C. The oxidation of hydrazines (R−NH−NH−R′) also gives azo compounds. Azo dyes are also prepared by the

condensation of nitro aromatics with anilines (Scheme 1-3) followed by reduction of the resulting azoxy intermediate:

$$ArNO_2 + Ar'NH_2 \longrightarrow ArN(O)=NAr'$$

Scheme (1-3)

For textile dying, a typical nitro coupling partner would be disodium 4,4′-dinitrostilbene-2,2′-disulfonate. Typical aniline partners are shown below Figure (1-2).

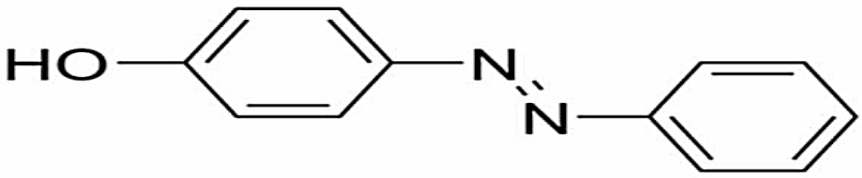


Figure (1-2)

Because of π-delocalization, aryl azo compounds have vivid colors, especially reds, oranges, and yellows. Therefore, they are used as dyes, and are commonly known as azo dyes, an example of which is Disperse Orange 1. Some azo compounds, e.g., methyl orange, are used as acid-base indicators due to the different colors of their acid and salt forms. Most DVD-R/+R and some CD-R discs use blue azo dye as the recording layer. The commercial success of azo dyes motivated the development of azo compounds in general.

1.3 The aim of the study :

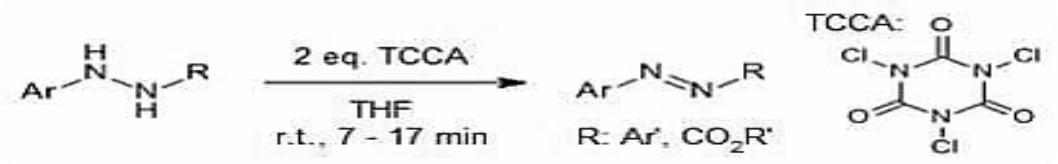
1- Synthesis of some azo compounds by the diazo coupling reaction

2- Spectroscopic study and characterization of the prepared dyes.

3- Determine their activity as antibacterial agents againt several types of microorganisms.

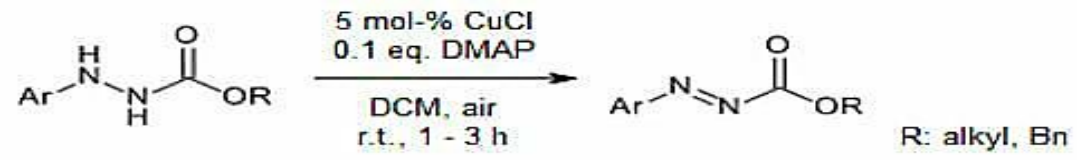
1.4 Synthesis of azo compounds

A highly efficient, metal-free, chemical oxidation of hydrazines using environmentally friendly TCCA as oxidant provides a broad range of azo compounds in THF in excellent yield. This step-economical process offers mild reaction conditions, operational simplicity, high reaction efficiency, and easy scale-up. [15]



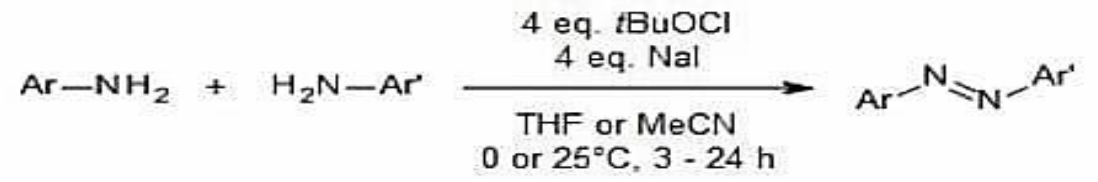
Scheme (1-4)

The synthesis of alkyl 2-phenylazocarboxylates largely depended on the stoichiometric use of toxic oxidants. The use of CuCl and DMAP (4-dimethylaminopyridine) as catalysts enables an environment-friendly aerobic oxidation of alkyl 2-phenylhydrazinecarboxylates to alkyl 2-phenylazocarboxylates under mild conditions (Scheme 1-5). [16]



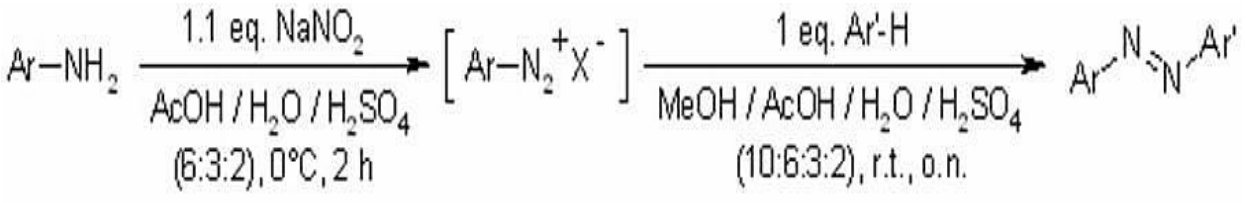
Scheme(1-5)

Straight forward, convenient, and efficient oxidative dimerization of aromatic amines enables an easy access to symmetrical and unsymmetrical azobenzenes under extremely mild conditions using a unique and cost-effective iodinating reagent



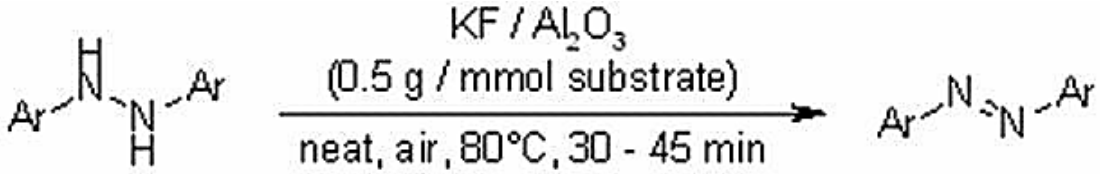
(Scheme 1-6)

Treatment of anilines with N-chlorosuccinimide and 1,8-diazabicyclo[5.4.0]undec-7-ene enables (Scheme 1-7) a convenient one-step procedure for the synthesis of symmetrical azobenzenes in good yields in minutes. [17]

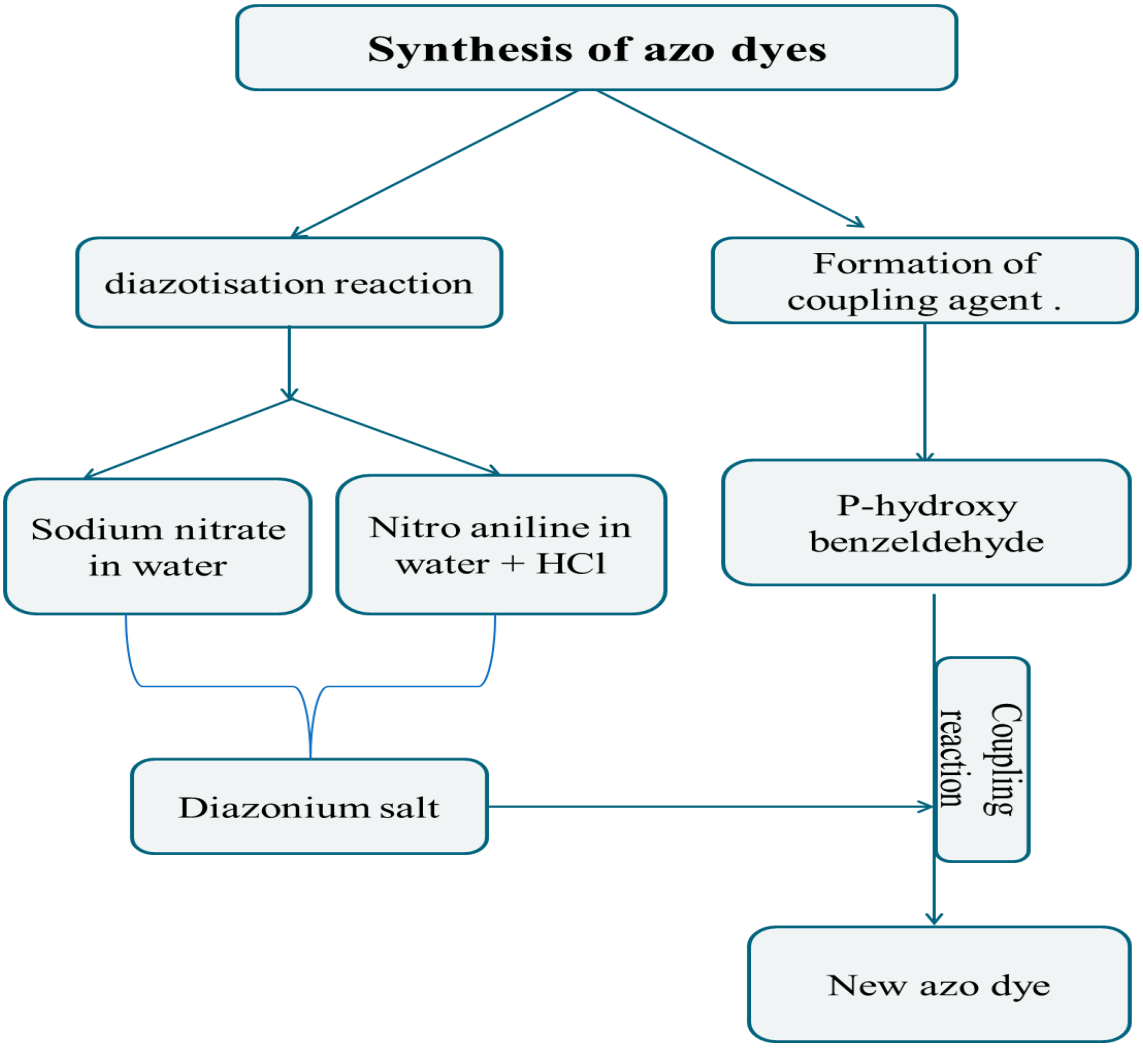


Scheme: (1-7)

Green dehydrogenation of hydrazo compounds using basic alumina or KF/alumina under solvent-free conditions afforded azo compounds (Scheme 1-8) in good to excellent yields. [18]



Scheme (1-8)



Scheme(1-9): general scheme of azo dyes synthesis

1.5 Biological activity

1.5.1 Antibacterial & antifungal

The antimicrobial activity of azo compounds and phenol derivatives was performed against bacterial and fungal species.
The antimicrobial/ antifungal activities of compound 5a are 100% (12/12). [19]

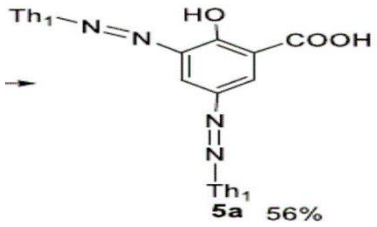
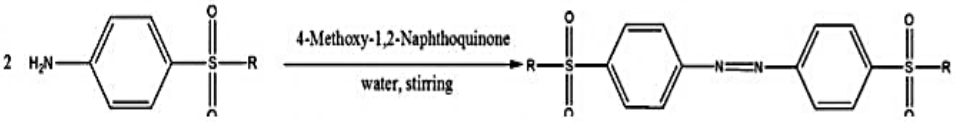


Figure 1-3 chemical structure of the agent

1.5.2 Antioxidant

Azo-sulfonamides compounds synthesized, characterized, and evaluated for their antioxidant test. [20]



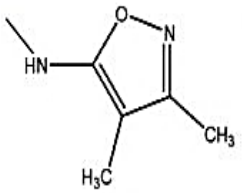
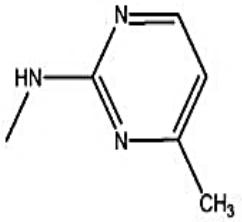
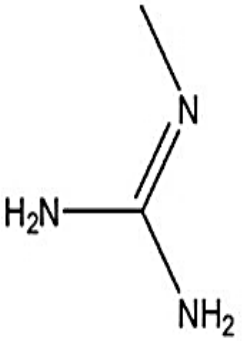
Compound	R	Name
SF		N-(3,4-dimethylisoxazol-5-yl)-4-((4-(N-(3,4-dimethylisoxazol-5-yl) sulfamoyl) phenyl) diazenyl) benzenesulfonamide
SM		4,4'-(diazene-1,2-diyl) bis (N-(4-methylpyrimidin-2-yl) benzenesulfonamide)
SQ		N-(diaminomethylene)-4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) diazenyl) benzenesulfonamide

Figure (1-4). Chemical structure of agent

1.5.3 Anticancer

Azo dye derivatives containing a pyranoquinolinone moiety were designed, synthesized and biologically evaluated. The most potent, compound **7b**, exhibited remarkable inhibitory activity against HepG-2, MCF-7 and HCT-116 tumor cell lines. Therefore, this compound merits further investigation as a drug candidate for cancer therapy. [21]

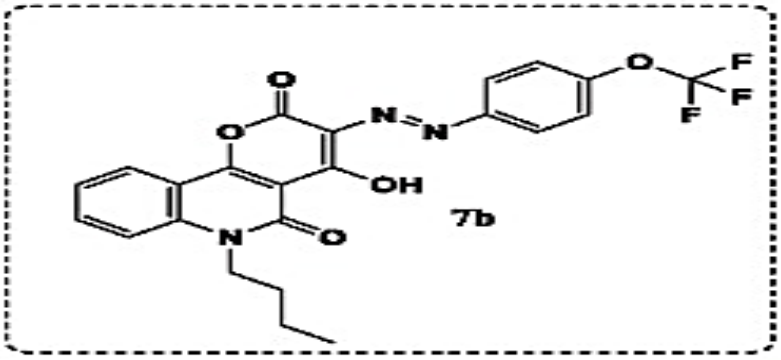
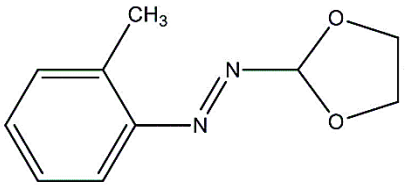


Figure 1-5. Chemical structure of anticancer agent

1.5.4 Antiviral

The structures of newly synthesized molecules were elucidated by spectroscopic techniques (EI-MS and FT-IR). In ovo screening of compounds against avian influenza virus (AIV) H9N2 strain and Newcastle Disease virus (NDV) Lasota strain was done. The evaluation data suggested that azo compound (A5) exhibited the highest anti-AIV and anti-NDV activity (100% inhibition at 0.1 mg/100 µL) compared to the other azo compounds which showed less activity at given concentrations. [22]

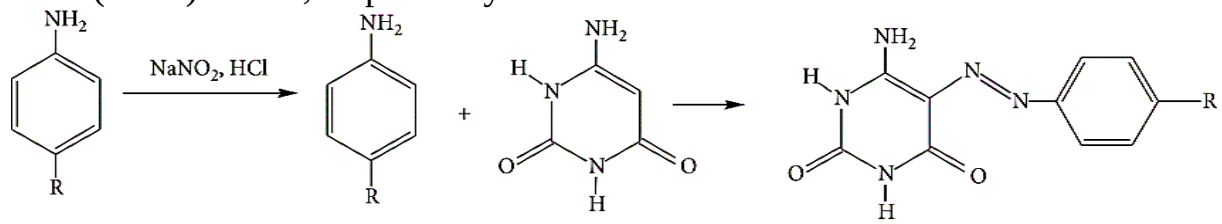


A5

Figure 1-6 chemical structure of agent

1.5.5 Antiprotozoal

The antiparasitic properties of dyes I and II were determined by testing their antileishmanial and trichomonacidal activities against L. infantum, L. tropica, L. major promastigotes, and T. vaginalis trophozoites, respectively. The study was carried out in vitro using microdilution broth assay. The results of antileishmanial activity against Leishmania promastigotes and trichomonacidal activity against T. vaginalis were evaluated by taking into account minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values, respectively. [24]



Scheme 1-10 **Synthesis path of azo dyes containing uracil: dye I (R=NO_2) and dye II (R=Br).**

Chapter Two

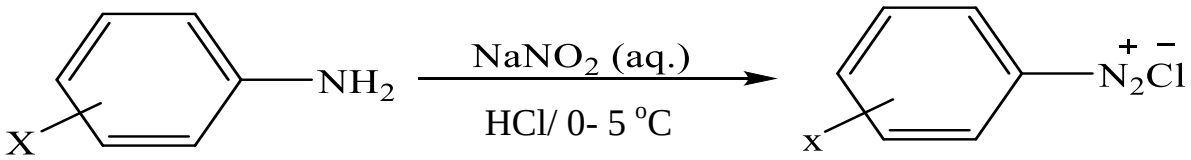
Experimental part

2.1 Experimental Materials

3-Nitroaniline, 4-Nitro aniline, 4-hydroxybenzaldehyde, water, concentrated HCl, $NaNO_2$, NaOH. . The purity of prepared compounds was checked by thin layer chromatography (TLC). Melting points recorded by using Gallenkamp apparatus in college of pharmacy/ university of Basrah. FT- IR spectra (KBr) of prepared compounds determined on Shimadzu spectrometer (400-4000 cm^{-1}) in college of education/ university of Basra.

2.2 Method for synthesis of diazonium salts

In a conical flask (100 ml), a solution of an aromatic amine (5 mmol), 1.5 ml of water and 1.5 ml concentrated HCl kept cooled in an ice-salt bath (0^oC). A solution of sodium nitrite (5.5 mmol) in 1.5 ml of water added slowly with stirring. The mixture kept at 0 ^oC for the next step [24, 25]. The other diazonium salt synthesized in a similar procedure as shown in Scheme (2-1).

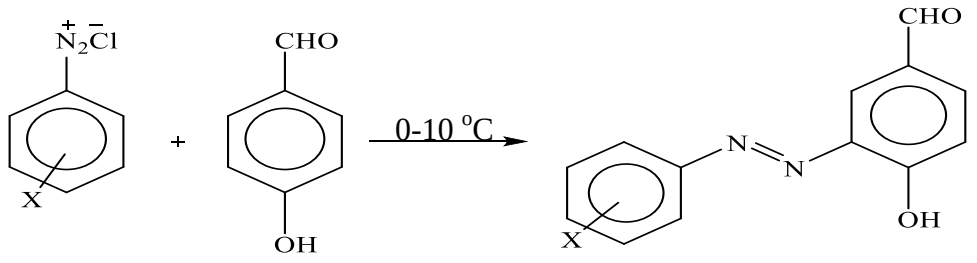


X= 3-NO_2 and 4-NO_2

Scheme 2-1: synthesis of diazonium salt

2.3Method for synthesis of azo compounds (coupling reaction)

The prepared solution of diazonium salt was added portion wise to a solution prepared from p-hydroxybenzaldehyde (5.4 mmol) and 10 ml of 2.5 M aq. Sodium hydroxide. The mixture kept with stirring at (0-5oC) for 3-5 hours. The mixture then acidified with conc. HCl (1.5 ml) up to pH ≈ 3. The precipitated compound separated and washed with H2O. The desired product dried and recrystallized with glacial acetic acid [16, 25].



X= 3-NO_2 and 4-NO_2 X= 3-NO_2 (Z1) and 4-NO_2 (Z2)

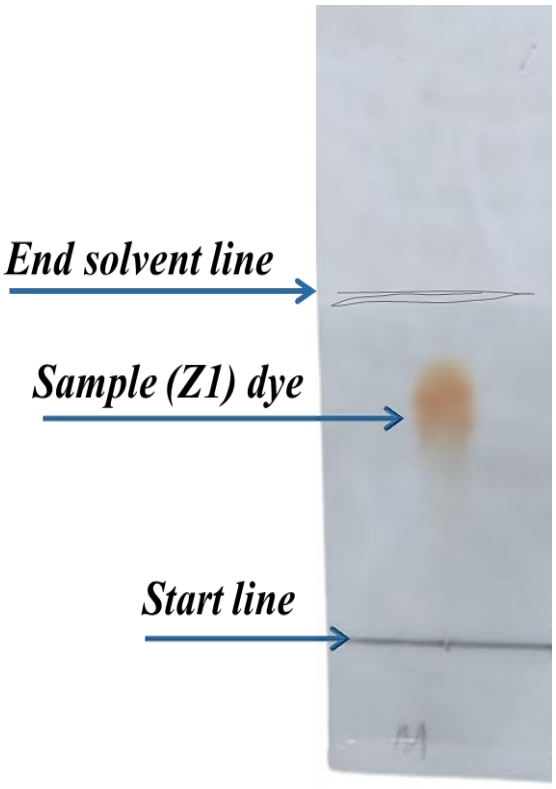
Scheme 2-2 Shows synthesis of azo compounds.

2.4 determination of azo dye purity:

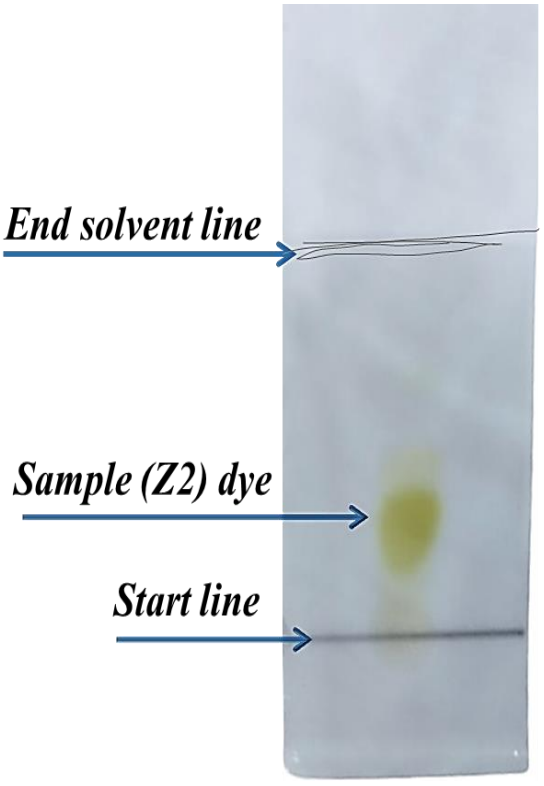
The purity of the dye was checked by thin layer chromatography(TLC) using solvent system(sec.Butanol-water-acetic acid) (2:2:1).The melting point of the purified dye was measured in an open capillary tube.The combined use of melting point analysis and thin liquid chromatography provides valuable insights into the purity of azo dyes

Compound	Mol. For.	The Name	M. P.(^{o}C)	Color
Z1	$C_{13}H_{19}N_3O_4$	(E)-4-hydroxy-3-((3-nitrocyclohex-1-en-1-yl)diazenyl)cyclohexane-1-carbaldehyde	142-146	brown
Z2	$C_{13}H_{19}N_3O_4$	(E)-4-hydroxy-3-((4-nitrocyclohex-1-en-1-yl)diazenyl)cyclohexane-1-carbaldehyde	160-164	brown

Table 2-1: shows the physical properties and molecular formula of the synthesized AZO compounds



Figure(2-1) :thin layer chromatography of prepared (Z1) azo dye compound.



Figure(2-2) :thin layer chromatography of prepared (Z2) azo dye compound.

2.5 Study of anti bacterial activity of azo dyes :

Schematic representation **(2-3):**of agar diffusion test to determine susceptibility of four bacterial strains that are commonly associated with infections. Staphylococcus aureus ,Escherichia Coli, Pseudomonas aeruginosa and Klebsiella pneumoniae are used in the study were obtained from the culture collection of Microbiology Department in College of pharmacy ,University of Basrah;

Susceptibility of the bacterial strains to the azo dyes was investigated using the disc diffusion method

spreading bacteria of specimen onto solidified Mueller Hinton agar medium were punched in the

Cultivate the bacterial strains in appropriate growth media until they reach the desired growth phase.

prepared azo dyes was added to the wells.

the plates were then incubated at 37C˚ for 24h.

After incubation the antimicrobial activity was evaluated by measuring the zone of inhibition

Chapter Three

Results & Discussion

3.1 FT-IR Spectroscopy

FT-IR spectroscopic characterization of synthesized compounds were recorded on Shimadzu's Fourier transform infrared spectrometer (Japan) with frequency range of 4000-400 cm^{-1}.

The FT-IR spectra of prepared (Figures 3-1 and 3-1) compounds show a strong absorption band at 1688.37 $cm^{-1}$and 1692.23 cm^{-1} for carbonyl aldehyde group [1,26] . Both compounds exhibit absorption bands at (1603 cm^{-1} & 1600 cm^{-1}),(1520 cm^{-1} &1528 cm^{-1}) and (1437.67-1528.31 cm^{-1}) for the stretching vibrations of -N=N- and C=C groups because are superimposed in the same ranges [1,13]. The spectra of the azo compounds show strong absorption bands at ranges 1188.9 cm^{-1} and (1341 cm^{-1} & 1348 cm^{-1}) due to the stretching vibrations for, (C-O, phenolic), and (NO_2) respectively.

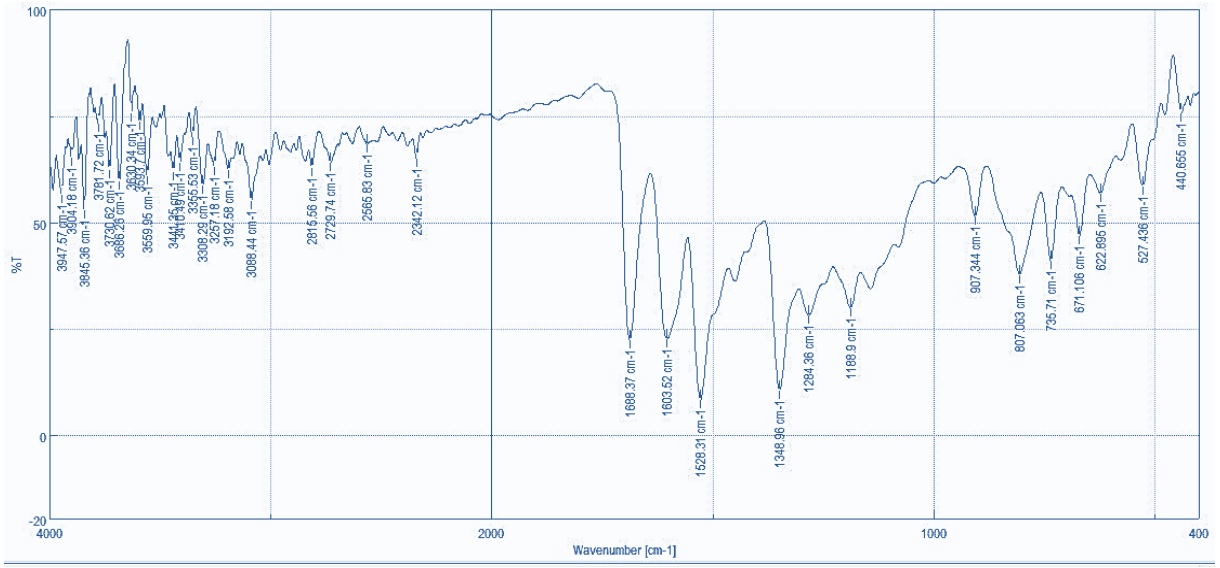


Figure 3-1: The FT-IR spectrum of compound Z1

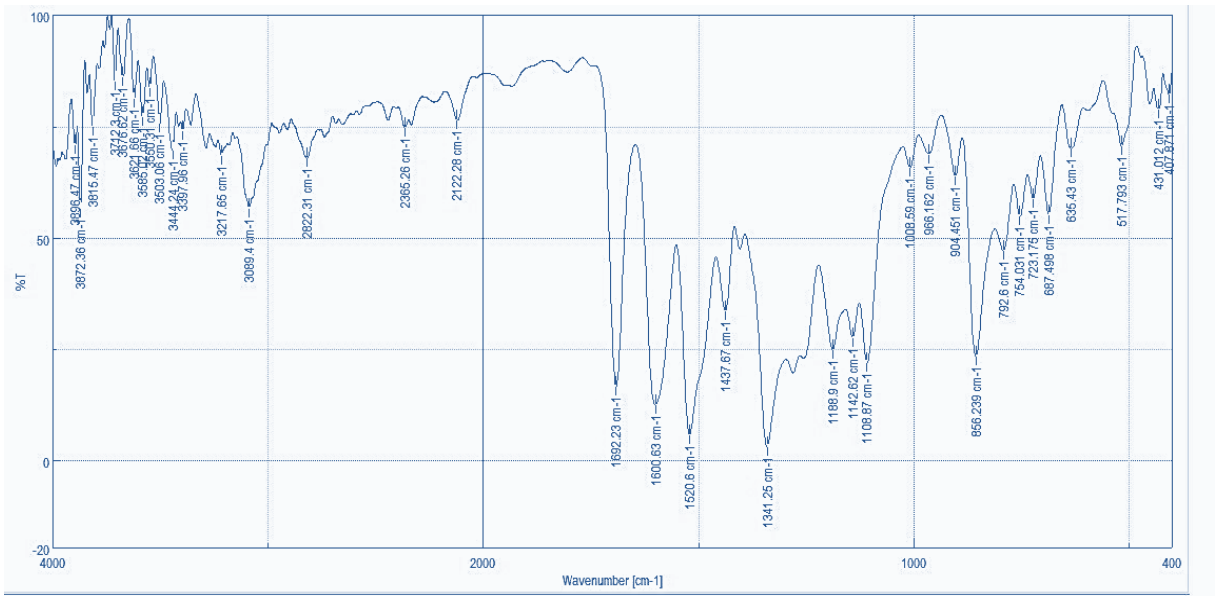


Figure 3-2: The FT-IR spectrum of compound Z2

3.2 Study of anti bacterial activity of dye :

Qualitative screening for antimicrobial activities was performed preliminarily using the disc diffusion assay, in vitro microbial activities were measured from the diameter of clear inhibition zones caused by samples against the same bacteria.

Bacteria	Inhibition zone diameter (mm)					
	Concentration of azo dye (mg/ml)					
	100	50	25	12.5	6.25	3.125
S. aureus	30	28	23	19	18	16
P. Aeruginosa	17	17	13	12	12	11
E. coli	35	32	28	24	22	19
K.pneumoniae	8	6	0	0	0	0

Table (3-1): the Diameters (mm) of suppression for antibacterial activity of (Z1) compound at follow concentration ; 100mg/ml, 50 mg/ml, 25mg/ml, 12.5mg/ml, 6.25 mg/ml and 3.125 mg/ml.

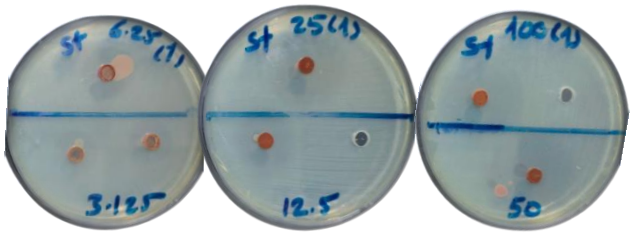


Figure (3-3) : antibacterial activity of azo compound (Z1) against Staphylococcus aureus.

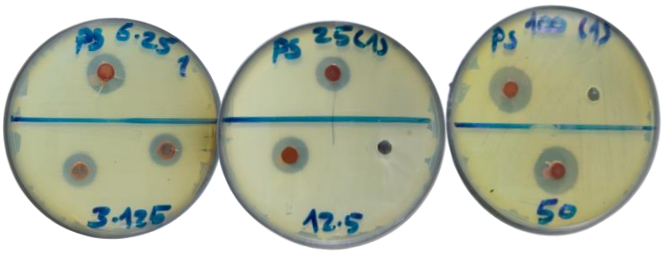


Figure (3-4) : antibacterial activity of azo compound (Z1) against Pseudomonas

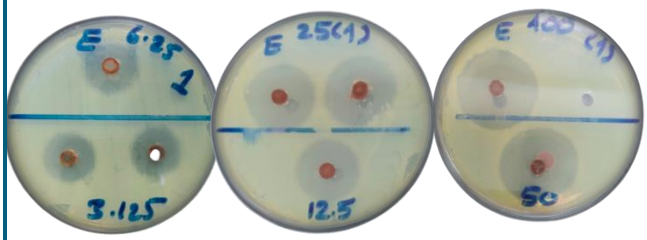


Figure (3-5) : antibacterial activity of azo compound (Z1) against Escherichia coli.

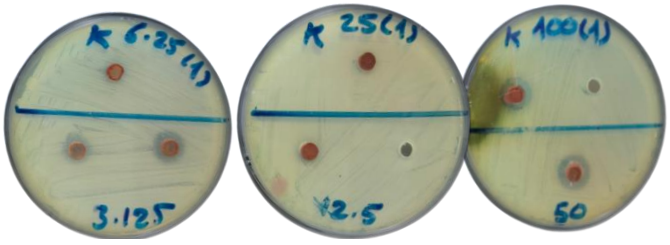


Figure (3-6) : antibacterial activity of azo compound (Z1) against Klebsiella pneumoniae.

Bacteria	Inhibition zone diameter (mm)					
	Concentration of azo dye (mg/ml)					
	100	50	25	12.5	6.25	3.125
S. aureus	4	0	0	0	0	0
P. Aeruginosa	14	11	10	8	11	11
E. coli	0	0	0	0	0	0
K.pneumoniae	0	0	0	0	0	0

Table (3-2):the Diameters (mm) of suppression for antibacterial activity of (Z2) compound at follow concentration ; 100mg/ml, 50 mg/ml, 25mg/ml, 12.5mg/ml, 6.25 mg/ml and 3.125 mg/ml.

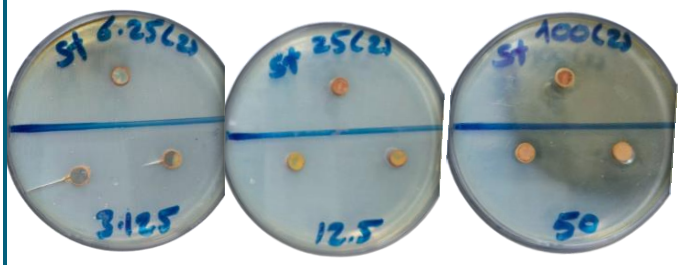


Figure (3-7) : antibacterial activity of azo compound (Z2) against Staphylococcus aureus.

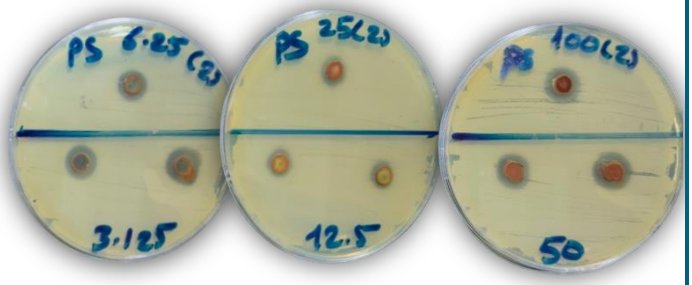


Figure (3-8) : antibacterial activity of azo compound (Z2) against Pseudomonas aeruginosa.

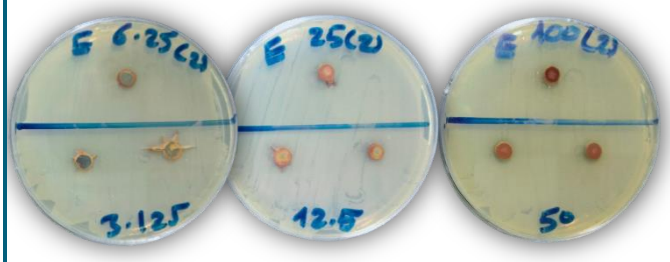


Figure (3-9) : antibacterial activity of azo compound (Z2) against Escherichia coli.

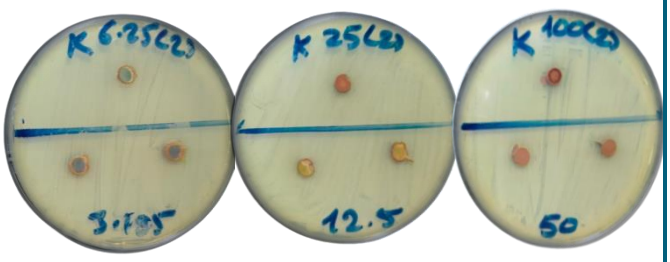


Figure (3-10) : antibacterial activity of azo compound (Z2) against Klebsiella pneumoniae.

Conclusion

the azo compounds were successfully synthesized under reproducible conditions and were obtained in good yields.

The prepared dye was spectroscopically characterized using infrared spectroscopy .

The purity of the dye was checked by thin layer chromatography(TLC) using solvent system(sec.Butanol-water-acetic acid) and melting point.

The biological activity for the compound (z1) showed highest activity against gram positive (Staphylo coccus aureus) and gram negative strains (Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli) compared with compound (Z2) which was active against Pseudomonas aeruginosa (gram negative) only

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