Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2023: 85.868
Volume 14, Issue 2; 2025, 66-77

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Review Article

A Review on Synthesis of new Azo Compounds of Para Hydroxy Benzaldehyde and Determination of their Activity

Shashi Saxena, Pallvi Pandey, Anuj Kumar, Auranjeb Kumar, Amit Kumar, Raushan Kumar

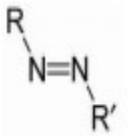
Mahadeva Lal Schroff College of Pharmacy Aurangabad, Bihar

Article Info: Received: 17-02-2025 / Revised: 11-03-2025 / Accepted: 28-03-2025
Corresponding Author: Shashi Saxena
DOI: https://doi.org/10.32553/jbpr.v14i2.1275
Conflict of interest statement: No conflict of interest

Abstract:
This study aims to prepare some azo compounds by diazo coupling reaction of aromatic amines with Para hydroxy benzaldehyde. Azo dyes are Compounds containing in their structure a group or more of the AZO groups (–N=N–) called azo compounds, 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
Key Words: Benzaldehyde, chromatography, Esherichia Coli, Hybridization, Staphylococcus aureus

Introduction

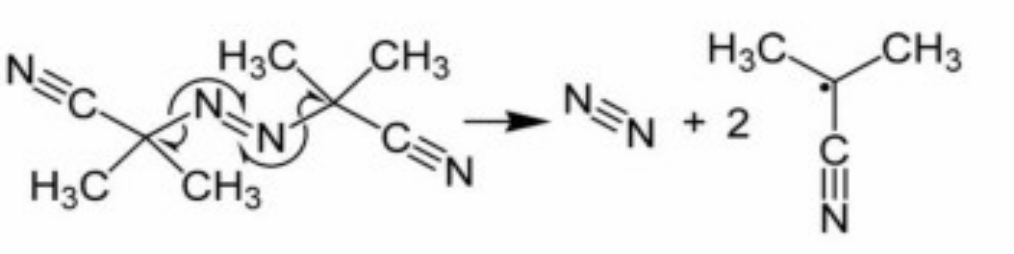
Compounds containing in their structure a group or more of the AZO groups (–N=N–) called azo compounds [1], in which the nitrogen atom hybridization is sp2 .as shown in



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].

Classification of azo compounds

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.

Aryl AZO Compounds

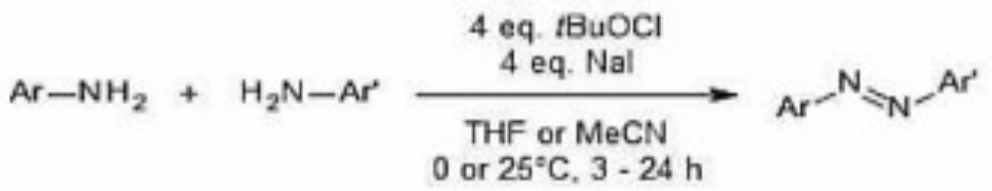
Aryl azo compounds are usually stable, crystalline species. Azobenzene is the prototypical aromatic azo compound. It exists mainly as the trans isomer, but upon illumination, converts to the cis isomer.

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:

$$\mathrm{ArN_2^+ + Ar'H \longrightarrow ArN{=}NAr' + H^+}$$

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 Scheme(1-2) 2 condensation of nitro aromatics with anilines (Scheme 1-3) followed by reduction of the resulting azoxy intermediate:



(Scheme 1-6)

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).

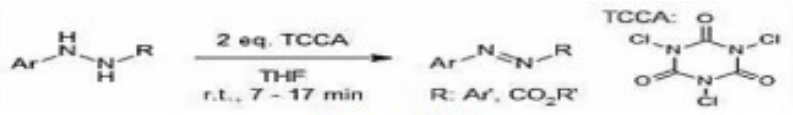
Because of π-delocalization, aryl azo compounds have vivid colors, especially reds, oranges, and yellows. Therefore, they are used as dyes, and are commonly known as azo dyes, an example of which is Disperse Orange 1. Some azo compounds, e.g., methyl orange, are used as acid-base indicators due to the 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.

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.

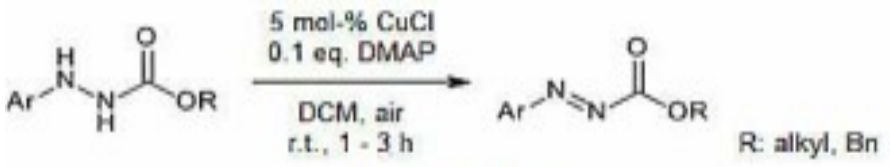
Synthesis of azo compounds

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]



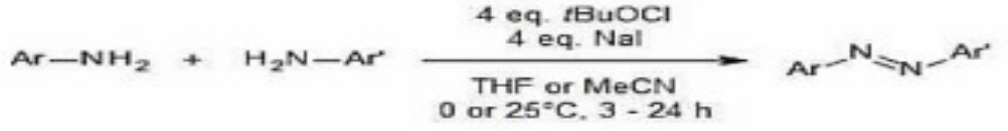
Scheme (1-4)

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]



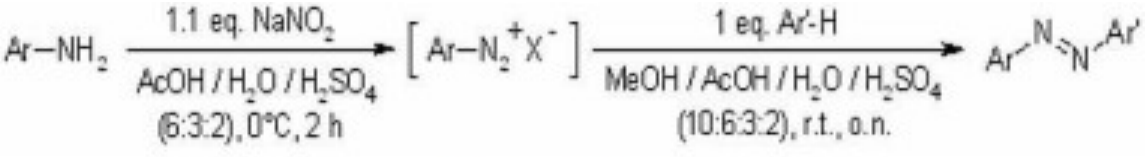
Scheme(1-5)

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.



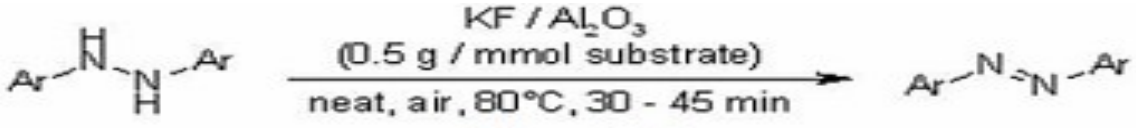
(Scheme 1-6)

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].

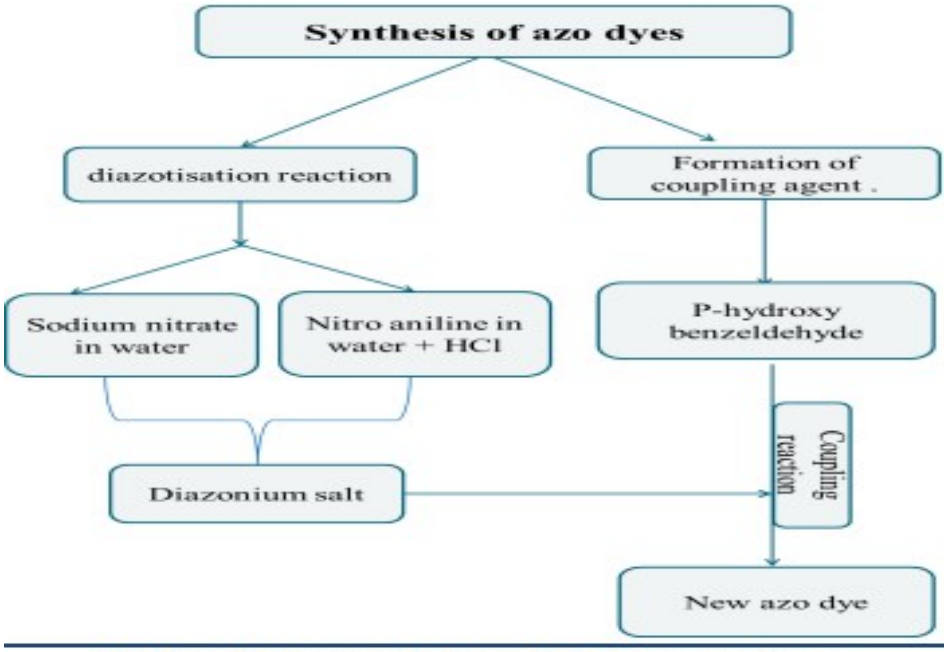


Scheme: (1-7)

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

Biological activity

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].

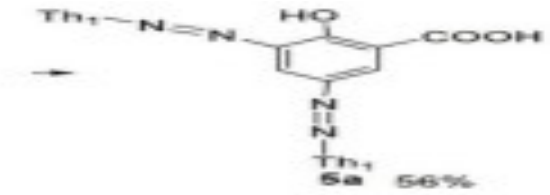


Figure 1-3 chemical structure of the agent

Antioxidant

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

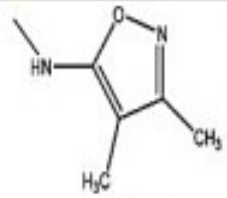
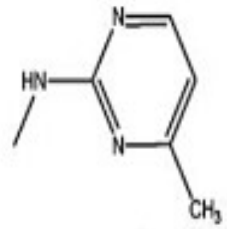
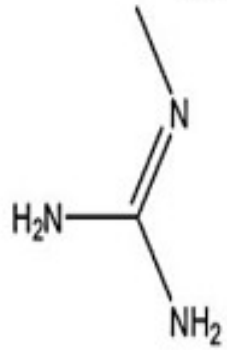
Compound	R	Name
SF		N-(3,4-dimethylisoxazol-5-yl)-4-((4-(N-(3,4-dimethylisoxazol-5-yl) sulfamoyl) phenyl) diazenyl) benzenesulfonamide
SM		4,4'-(diazene-1,2-diyl) bis (N-(4-methylpyrimidin-2-yl) benzenesulfonamide)
SQ		N-(diaminomethylene)-4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) diazenyl) benzenesulfonamide

Figure (1-4). Chemical structure of agent

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]

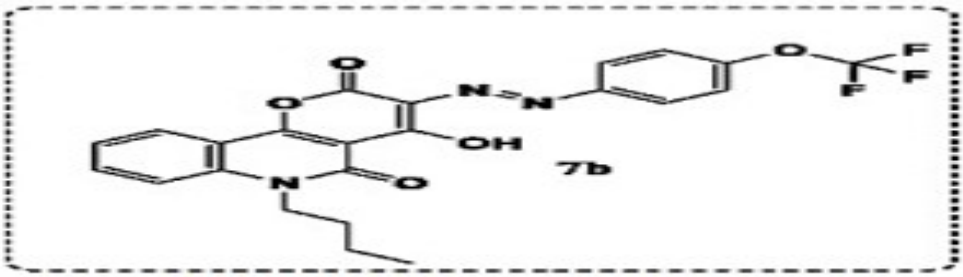
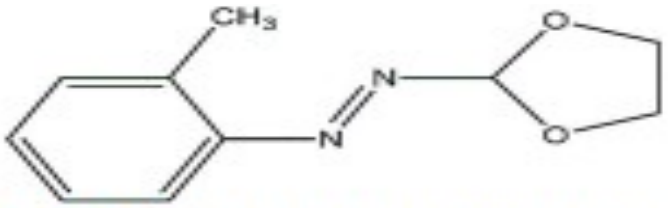


Figure 1-5. Chemical structure of anticancer agent

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]

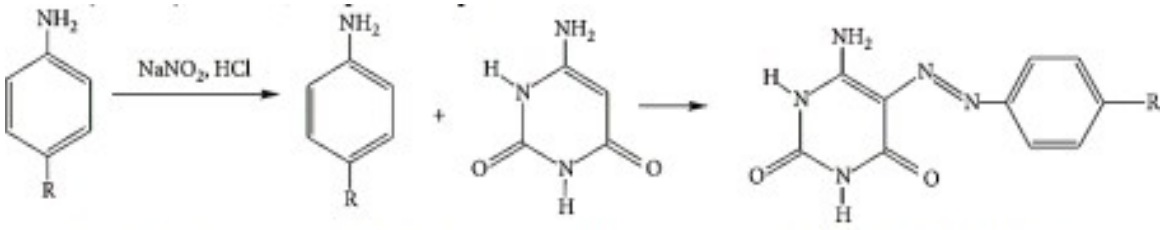


A5

Figure 1-6 chemical structure of agent

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. [2



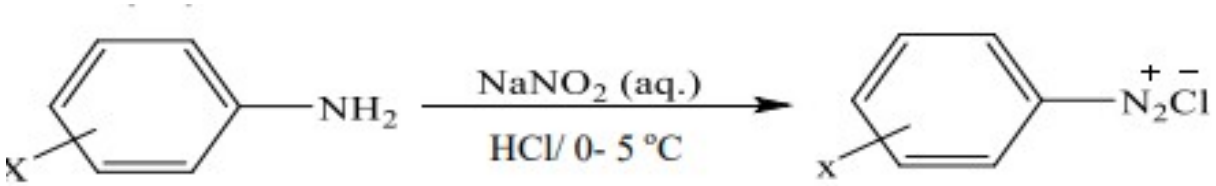
Scheme 1-10 **Synthesis path of azo dyes containing uracil: dye I (R=NO_2) and dye II (R=Br).**

Experimental Materials

3- Nitroaniline, 4-Nitro aniline, 4-hydroxybenzaldehyde, water, concentrated HCl, NaNO2, 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 cm1) in college of education/ university of Basra.

Method for synthesis of diazonium salts

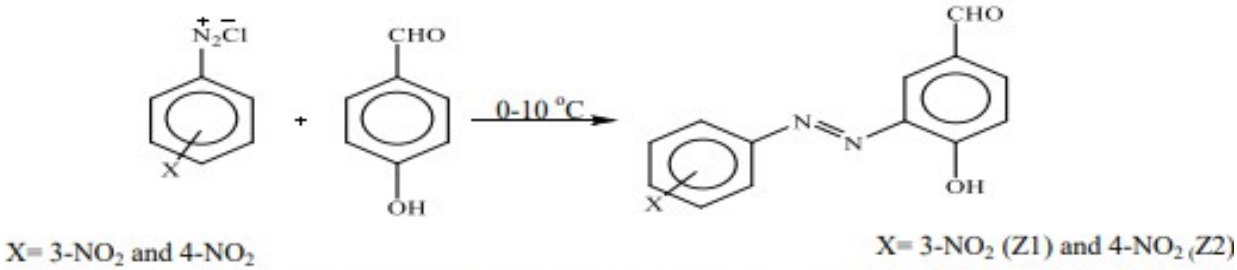
In a conical flask (100 ml), a solution of an aromatic amine (5 mmol), 1.5 ml of water and 1.5 ml concentrated HCl kept cooled in an ice-salt bath (0oC). A solution of sodium nitrite (5.5 mmol) in 1.5 ml of water added slowly with stirring. The mixture kept at 0 oC for the next step [24, 25] . The other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



X= 3-NO_2 and 4-NO_2

Method 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)

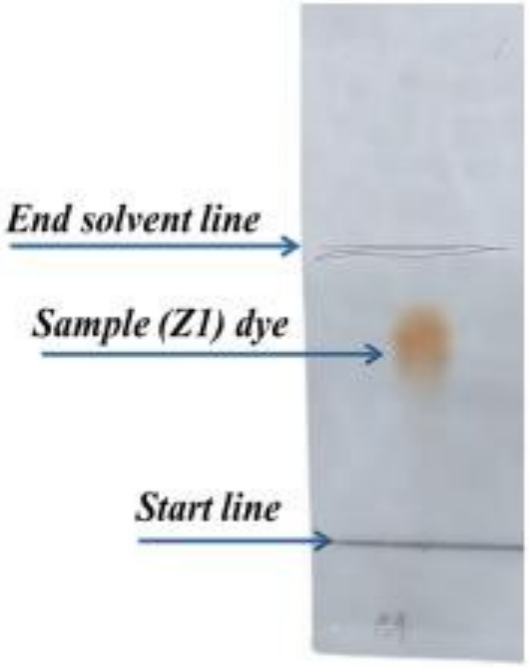
Scheme 2-2 Shows synthesis of azo compounds.

determination of azo dye purity:

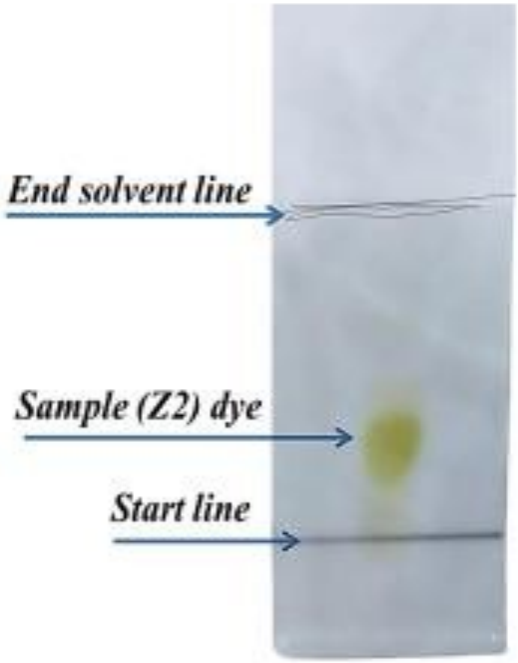
The purity of the dye was checked by thin layer chromatography(TLC) using solvent system(sec.Butanol-water-acetic acid) (2:2:1).The melting point of the purified dye was measured in an open capillary tube.The combined use of melting point analysis and thin liquid chromatography provides valuable insights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

Compound	Mol. For.	The Name	M. P.(°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



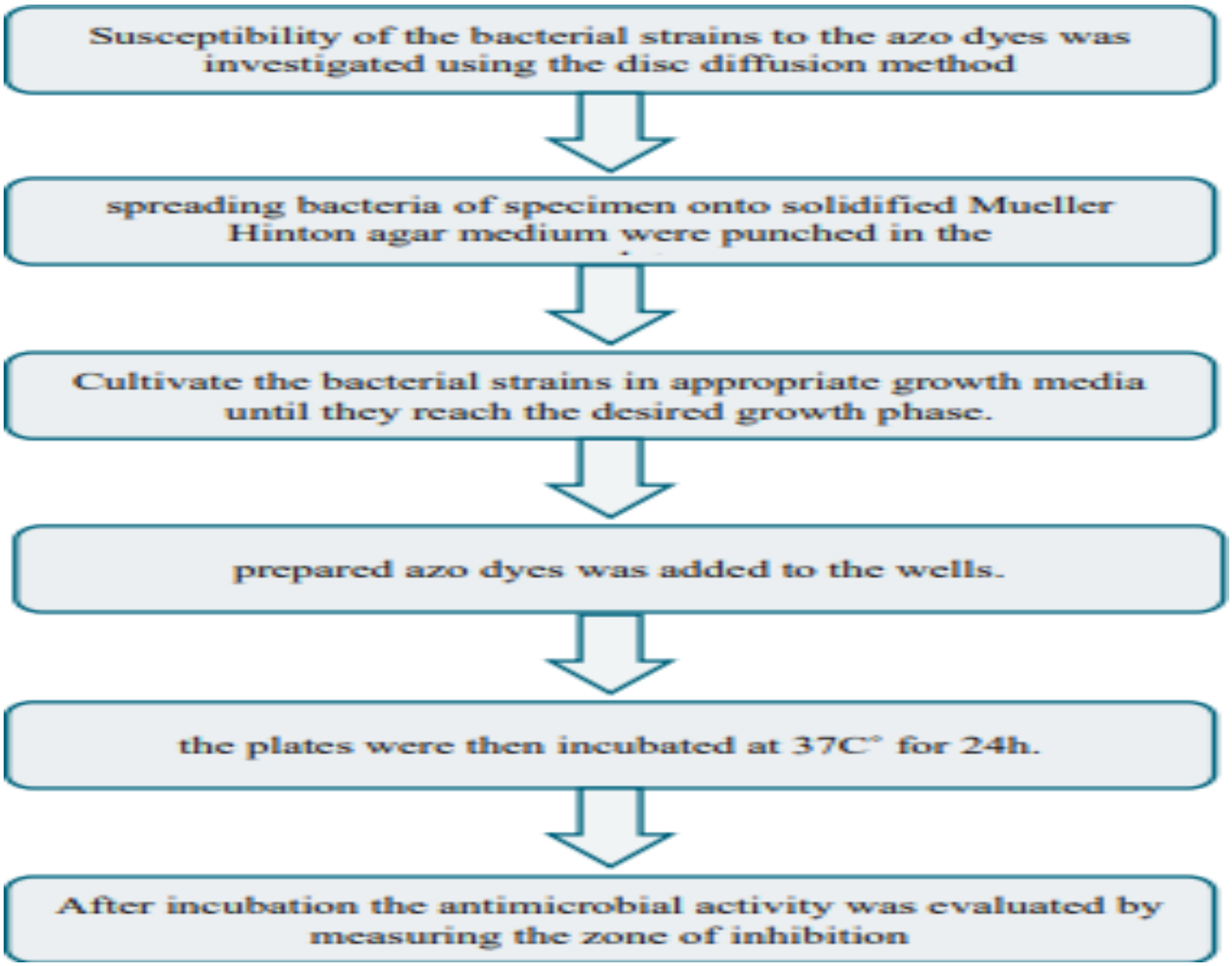
Figure(2-1) :thin layer chromatography of prepared (Z1) azo dye compound.



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

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;



FT-IR Spectroscopy

FT-IR spectroscopic characterization of synthesized compounds was 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 (NO2) respectively.

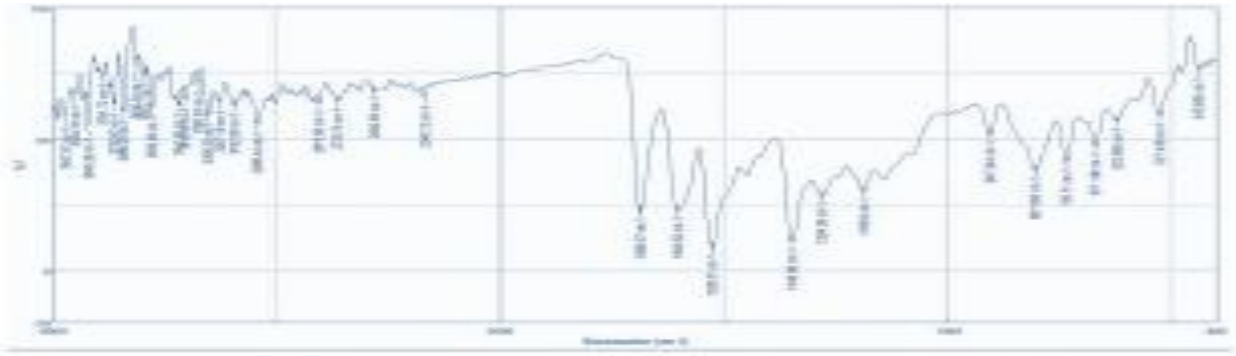


Figure 3-1: The FT-IR spectrum of compound Z1

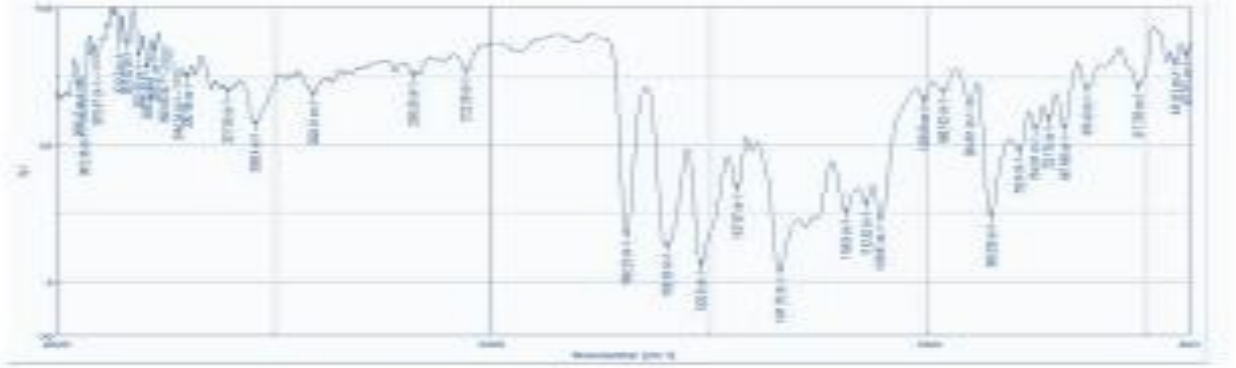


Figure 3-2: The FT-IR spectrum of compound Z2

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.

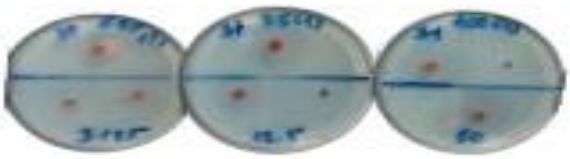


Figure (3-3) : antibacterial activity of azo compound (Z1) against Staphylococcus aureus.

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

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

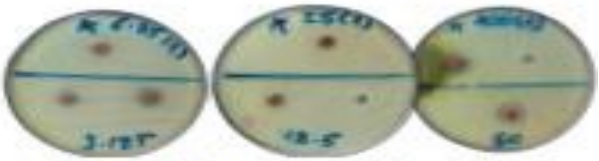


Figure (3-6) : antibacterial activity of azo compound (Z1) against Klebsiella pneumoniae.

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 az 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

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

References:

1. Potey, L. C.; Urade, P.; Aate J.; Kosalge, S., G. Oros et al. "Separation of the strength and selectivity of the microbiological effect of synthetic dyes by spectral mapping technique Chemosphere" Int. J. ChemTech. Res., 2017, 10, 552-556 (2003)"
2. S. Qamar et al. "Synthesis, structural characterization, DNA binding and antioxidant studies of 4, 4′-nitrophenoxyaniline derived azo dyes (2019)"
3. M.L. di Gioia et al. "A simple synthesis of anilines by LiAlH4/TiCl4 reduction of aromatic nitro compounds" Tetrahedron Lett. (2015)
4. K. Abouzid et al. "Pyridazinone Derivatives: Design, Synthesis, and in Vitro Vasorelaxant Activity Bioorg" Med. Chem. (2008)
5. 11.O.I. Lipskikh et al. "Sensors for voltammetric determination of food azo dyes - a critical review Electrochim. Acta" (2018).
6. S. Kinali-Demirci et al. "Synthesis, structure characterization and antimicrobial evaluation of 4-(substituted phenylazo)-3,5-diacetamido-1H- pyrazoles Spectrochim. Acta A Mol. Biomol. Spectrosc." (2013)
7. D.H. Song et al. "Synthesis of stilbene-based azo dyes and application for dichroic materials in poly (vinyl alcohol) polarizing films Dyes Pigm." (2007)
8. G. Kurtoglu et al." A novel azo-azomethine based fluorescent dye and its Co(II) and Cu(II) metal chelatesJ. Mol. Liq." (2014)
9. Synthesis, characterization, antimicrobial activities and electrochemical behavior of new azo dyes from the reactions of two thienocoumarin derivatives with three phenolic compounds January 2020 ARKIVOC 2019
10. J.F. Bunnett "Azo and diazo chemistry. Aliphatic and aromatic compounds J. Am. Chem. Soc." (1962)