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STUDY OF AZO COMPOUNDS DERIVED FROM P-CRESOL LIGAND: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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Staphylococcus aureus

Pseudomonas aeruginosa

ABSTRACT

Azo compounds contain two aromatic rings separated by an azo (-N=N-) link. These are versatile molecules that have garnered a lot of interest in both basic and applied research. In this work, synthesis of some Azo dyes derivatives by coupling P-cresol with diazonium salts obtained from p-chloroaniline and p-methoxyaniline. The structure of the synthesized compound has been characterized by using techniques like UV-Vis Spectroscopy, and Fourier-transform infrared spectroscopy (FTIR). In addition, the antimicrobial activity of these synthesized dyes compounds was examined by using the disc diffusion method against gram negative and gram positive bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* which have been isolated from the patients of wound infection. Further, synthesized compounds showed moderate to significant inhibitory effect at the selected concentrations against the tested microorganisms. The results of antibacterial activities demonstrated that compound p-chloroaniline had good antibacterial activity against *S. aureus* and *P. aeruginosa* at the concentration of 300 mg/ml with 8.079 mm and 6.16 mm inhibition zone, diameter respectively. This study synthesized two new compounds viz., p-chloroaniline and p-methoxyaniline, and among these two p-chloroaniline has good anti-bacterial activity against *S. aureus.* Therefore, this can serve as a new compound for the manufacture of ointment to treat wound infections.

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1 Introduction

Aromatic rings divided by an Azo (N=N-) bonds have received considerable research attention in the fundamental and applied sciences. The high electrical absorption limit can be moved anywhere between the ultraviolet and red visible ranges by replacing the ring, allowing chemical color fine-tuning. This, along with the fact that Azo dyes are unexpectedly solid and chemically resistant, has sparked many dyes and colorant development (Sarigul et al., 2017). These dyes have been widely used in the clothing, fabric, leather, and paint sectors, biological-medical research, and advanced organic synthesis applications for more than a century due to their capacity to absorb visible light and ease of synthesis (Malinovi et al., 2017). The types of dyes that are commonly made in high-volume are thought to be rather recalcitrant. Textiles are the main consumer of these colorants (Mehta & Patel, 2016; Al-Zinkee & Jarad, 2019). Azo dyes are commonly synthesized; their initial primary aromatic amine has been attached to an activated carboxylamine, so it would be coupled with nucleophiles to form a dye (Kyei et al., 2020). Azo compounds are considered the most basic industrial dyes and are employed as coloring agents and pigments (Feng et al., 2012; Shama et al., 2014; Ekennia et al., 2015; Fu et al., 2017). Azo dyes are also well-known for their antibacterial, antifungal neoplastic, anticancer, anti-inflammatory, and other pharmacological (Alkazily & Alasedi, 2013; Kassa, 2015). A recent study explained the synthesis and characterization of 2[(4-Subithen-hydrazinyl)phenol) hydrazide] (HL), a phenolic function group with a phenol substituent. A photophysical study of the chemical and electronic composition has been done, as well as through spectroscopy. The aim of this study is the synthesis of new derivatives compounds from p-cresol ligand and characterization of the synthesized compounds along with biological activity against two aerobic pathogenic gram-positive (*S. aureus*) and gram-negative (*P. aeruginosa*) bacteria isolated from the patients suffering from wound infection.

2 Materials and Methods

2.1 Chemicals

The purest grade chemicals have been used in its preparation of azoic Azo dyes (Merck Company, Germany) uses only the purest form of azoic dyes in the formulation of Azo pigments un-conducting is done by using Stuart model 9300 melting point process by means, which included the analysis of elemental C, H, and N using a C.H.N.S (Carbon Hydrogen and Nitrogen analyzer device, Germany). EA-3000 multi-mm tool. The KBr samples showed strong infrared absorption bands in the range of 400 cm^{-1} to 7,000 cm^{-1} on the Shimadzu spectrophotometer (not an IR line). These techniques were used to obtain an electronic spectrum on a Shimadzu 1700 UV spectrophotometer with flame ionization via a Shimadzu GC2014 with a flame detection element that used to detect substance (Kyei et al., 2020).

2.2 Synthesis of Azo ligand

Preparation of Azo bisubstirenone before adding the 4-substit phenol Azo connection extracts prepared from glycerol was used as substrates to produce p-chlorbenzene diamine ligands. According to the literature, a solution of 302g of p-chloricotrityl chloride and 289g of pindanedion is combined with 25 ml of concentration (Adam et al., 2019). HCl then 2.876g of piperazine, was stirred, and the mixture was applied to a solution of 25g of Na_2O which dissolved in 100 ml of water at room temperature with constant stirring and undercooling (this was followed by the mixing of 4.18 ml of p-cresol with 0.3g of NaOH and cooled to keep the solution until it clear). PHCl was diluted with water until the mixture had a pH of 8 this was followed by filtration and recrystallized twice with the hot ethanol, and allowed to dry for many hours, and then washed and purified with water (Al-Labban, 2017). Figure 1 and Table 1 listed the properties and FTIR spectra of the compounds.

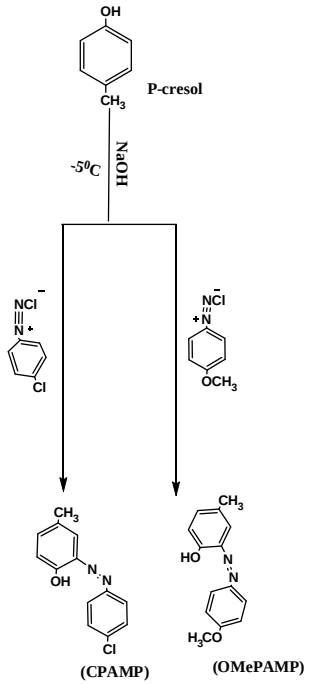


Figure1 Synthesis of Azo ligand

2.3 Examination of biological activity

Two derivatives compounds have been examined to inhibit two kinds of pathogenic bacteria (gram-positive *S. aureus* and gram-negative *P. aeruginosa*) isolated from the patients suffering from wound infection. Collection and maintenance of the selected bacterial pathogens were carried out by the Microbiology

Laboratory, Faculty of Science, University of Kufa. The antibacterial properties of the synthesized Azo dye were measured using the agar well diffusion method (Aljanaby & Aljanaby, 2018; Al-labban & Aljanaby, 2020). For this, selected pathogenic bacteria were swabbed on the Muller-Hinton agar surface as per the 0.5 McFarland turbidity (Aljanaby, 2013; Aljanaby, 2018, Hayder & Aljanaby, 2019). Each derivative compound was made in three concentrations viz., 100, 200, and 300 mg/ml. Crock-poorer was used to make three wells in Muller-Hinton agar surface. Every well-received 50µl of each dilution, which was left at 20^{o}C for two hours before being incubated at 37^{o}C for 24 hours. Each concentration was repeated three times. The inhibition zone for every well's was calculated in millimeters (Majeed & Aljanaby, 2019).

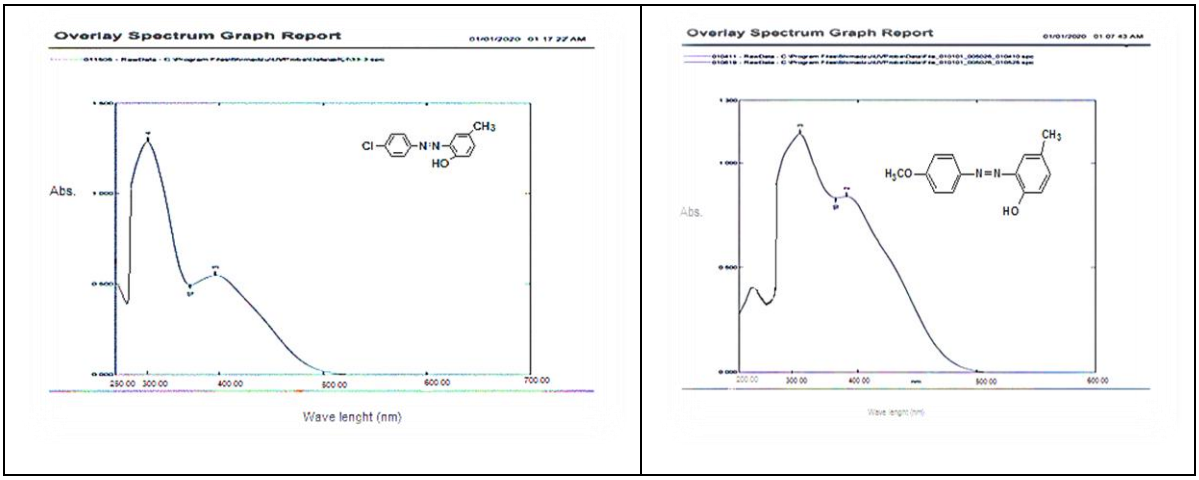
UV-VIS and IR spectra were used to identify these molecules as represented in Figures 2 and 3.

3.1 Biological activity

The result of the study revealed that 300 mg/ml concentration of p-chloroaniline molecule had strong anti-bacterial activity against gram-positive bacteria *S. aureus* and gram-negative *P. aeruginosa* (Figure 4), with inhibition zones of 8.079 mm and 6.164 mm, respectively (Table 2). This concentration is significantly different than the rest two concentrations. Furthermore, used concentrations 100mg/ml and 200 mg/ml are not significantly different from each other.

Table 1 demonstrates the fundamental scientific findings as well as physical features of organic compounds

Compound	M. Wt.	Yield (%)	M.P (ºC)	Color	Rf	Found (calc.) % C H N
CPAMP	246.06	70	154	Yellow	0.82	63.29 4.49 11.36 64.06 4.29 11.64
OMePAMP	242.27	80	95	Red brown	0.85	69.41 5.82 11.56 70.01 5.75 11.91



A B

Figure 2 Organic reagents in ethanol solvent have a UV-Vis spectrum (A: p-chloroaniline, B: p-methoxyaniline)

2.4 Statistical Analysis

T-test comparisons between inhibition zone diameters are made using Graph-pad prism V.5 windows tools in statistical analysis (Al-Labban, 2017; Mohammed & Aljanaby, 2020).

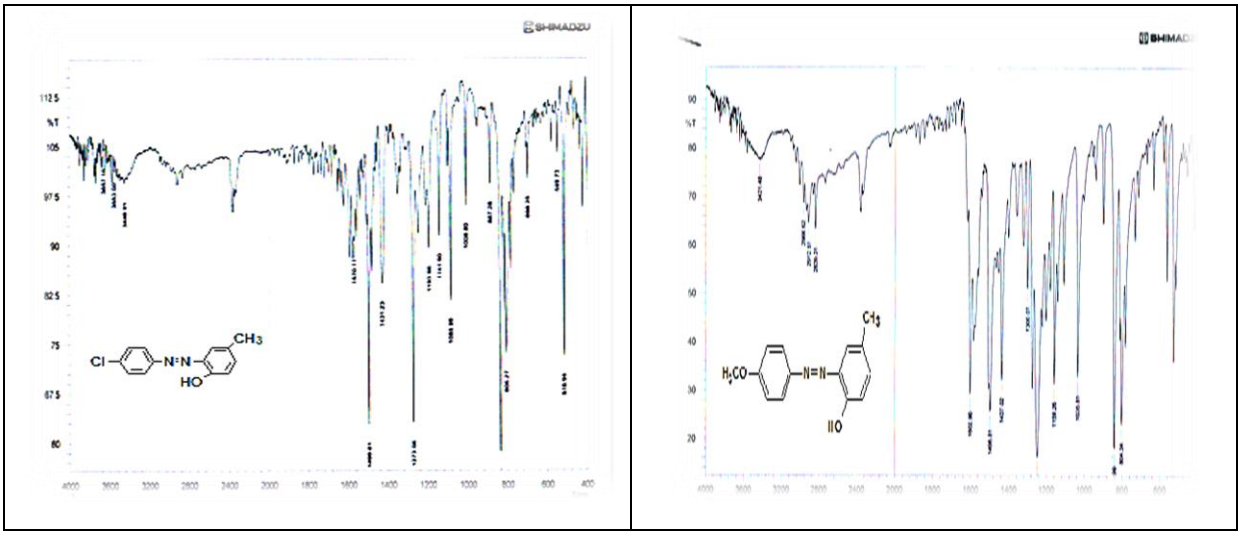
3 Results

The first step of this research involves the development of two Azo ligands that are P-cresol derivatives.

4 Discussions

Results presented in Figure 2 and Figure 3 revealed the final configurations of the synthesized ligands by C, H, and N using a C.H.N.S (Carbon Hydrogen and Nitrogen analyzer device, Germany), and the properties of the synthesized compounds are represented in Table 1 by finding a match between the theoretical measurement and experimental estimates (Al-Labban, 2017). In the case of anti-bacterial properties, the derivative compound p-chloroaniline (300mg/ml) has the highest activity against *S.aureus*

(Table 2; Figure 3). Azo compounds are very important organic materials that have attracted much attention in both applied and basic research (Abdallah, 2012; Kyei et al., 2020). They are an important class of molecules used in many requirements such as pigments, indicators, textiles, dyes, food additives, pharmaceuticals, cosmetics, therapeutic agents, and antibacterial (Jarad et al., 2018). These compounds containing bis-1,3,4-thiadiazole ring derived from nicotinic and isonicotinic acids have been synthesized via diazotization, etherification and cyclization have antimicrobials activity against many gram-positive and gram negative bacteria (Tomi et al., 2014).



A B

Figure 3 FTIR spectra for organic reagents (A: p-chloroaniline, B: p-methoxyaniline)

Table 2 Effect of two derivative compounds' against two multidrug-resistant pathogenic bacteria

Derivative compound	Multi-drug resistance pathogenic bacteria			
	Gram positive *S. aureus*		Gram negative *P. aeruginosa*	
	Concentration (mg/ml)	M±SE mm (R=3)	Concentration (mg/ml)	M±SE mm(R=3)
p-chloroaniline	100	5.880 ± 0.2782	100	3.954 ± 0.1629
	200	6.896 ± 0.3465	200	4.605 ± 0.3052
	300	8.079 ± 0.0700	300	6.164 ± 0.2770
p-methoxyaniline	100	3.2610 ± 0.1591	100	3.947 ± 0.04914
	200	3.3530 ± 0.2494	200	3.967 ± 0.1532
	300	3.750 ± 0.3821	300	5.586 ± 0.2050

M±SE: Mean± Standard error, mm: Millimeter (diameters of inhibition zone), R: number of replicates

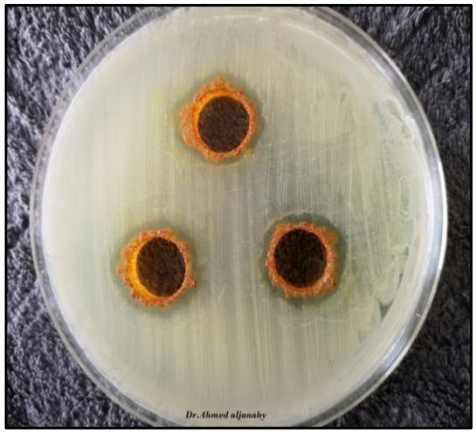


Figure 4 Antibacterial activity test of derivative compound p-chloroaniline (300mg/ml) against *S.aureus*

Conclusions

In this study, two new derivatives compounds p-chloroaniline and p-methoxyaniline have been synthesized from the p-cresol ligand and among these synthesized compounds p-chloroaniline exhibited significant antibacterial activity against two pathogenic bacteria *S. aureus* and *P. aeruginosa* isolated from wound infection patients. In this manner, p-chloroaniline can serve as a raw material for the development of new drugs in the treatment of wound infection.

Conflict of Interest

This study does not have any conflict of interest.

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