SYNTHESIS AND CHARACTERIZATION OF NEW AZO DYE (1-(4-SULFONYL PHENYL AZO)-2-(7-CHLORO-4-[(4-(DIETHYL AMINO)-1-METHYL BUTYL]AMINO]QUINDINE FROM CHLOROQUINE DIPHOSPHATE AND STUDY ANTIBACTERIAL ACTIVITY

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ABSTRACT

New azo dye was synthesized by reaction dizonium salt of sulfanilic acid with antimalaria drug (chloro quin diphosphate). This product was characterized by FTIR (Fourier Transform Infrared) and UV-Visible spectrophotometer. The antibacterial activities of the compound was studied and evaluated using gram positive and negative gram stain. 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.

We have been concluded that the prepared azo dye showed antibacterial activity against this micro organism.

INTRODUCTION

Azo dyes contain at least one nitrogen-nitrogen double bond (N=N); however many different structures are possible (1). Mono azo dyes only (N=N) double bond while diazo and triazo dyes contain two and three (N=N) double bonds, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic hetro cycles such as chloroquine (2). The side groups are necessary for imparting the color of the dye. Synthesis of most azo dyes involve diazotization of a primary aromatic amine followed by coupling with one or more nucleophiles. Amino and hydroxyl groups are commonly used coupling components (3).

Azo dyes acquired wide interest in application to biological system and indicator in complex ometric titratin of analytical chemistry (4), (5). Aromatic azo compounds especially are used as acid-base indicators, also used in biological strains and commercial colorants for clothing, plastics (6). Color changes are caused by change in extent of delocalization of electrons. More delocalization shifts the absorption max to longer wave lengths and makes the light absorbed redder, while less delocalization shifts the absorption max to shorter wave lengths (7). The azo dyes sulfon amide antibacterial drugs were the first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infection in humans. A series of azo dyes containing the sulfonamide functional group were synthesized as potential antimicrobial agents (8). Sulfonamide was classified into three different types; antibacterials that are aniline-substituted sulfonamide, prodrug that react to generate active sulfanilamide and non aniline sulfonamide. There are also other commonly used drugs that are azo dyes sulfonamides or sulfanilamide, the diuretic chlorthlidone and the oral hypoglycemic drug (tolbutamide). Today, there are few sulfonamides and especially sulfonamide-trimethoprim combination that are used extensively for opportunistic infection in the patients with AIDS (9).
MATERIAL AND METHODS

Material: Chemicals used in the present studies some were sourced from Merck (India) and other were sourced from Fulka (swiss)

Method

1- Preparation and characterization of azo dye
   a- Preparation of dizonium salt According to (10)

   1- Place 1g of the sulfanilic acid (0.0058 moles) in 5 ml of water and 2.5 ml of Conc HCl in a beaker and shake. Keep the beaker in Ice bath (5°C).

   2- Dissolve 1g NaNO2 in 5ml of water and put it in Ice too. Add this solution dropwise to solution of sulfanilic acid keeping the temperature (0-5°C).

   b- Coupling reaction

   1- Dissolve 3g of drug (chloroquindiphosphate)(0.005 mole) in 10 ml of 10% NaOH. Keep this solution also in Ice.

   2- Now add the diazonium salt slowly to the cold solution of chloroquine raddish-orange dye is produced yield 2.4 g.

   3- Filter on a Buchner funnel. The raddish-orange product was characterized by FTIR and UV. Visible spectrophotometer. The purity of the dye was checked by TLC using the solvent system (Sec. Butanol-water-acetic acid) (2:2:1). The melting point of the purified dye was measured in an open capillary tube.

2- Study of anti bacterial activity of dye.

   a- Bacterial strains used

   Pure cultures of two bacterial strains *Staphylos coccus aureas*, *Echerichia Coli* used in the study were obtained from the culture collection of Microbiology Department in College of Veterinary Medicinal, University of Basrah.

   b- Anti bacterial activity of dye. Sample was determined by the agar-well diffusion method (11). The test organism was swabbed on to the solidified Muller hinton agar medium there after 6mm dimeter well were punched in the agar plates prepare dye was added to the wells, the plates were then incubated at 37°C for 24h. After incubation the antimicrobial activity was evaluated by measuring the zone of inhibition.

RESULT AND DISCUSSION

The synthesis of an azo dye requires two organic compounds dizonium salt and coupling component (fluoro quine drug). The general synthesis of azo dye is shown below:
The dizonium salt reacts as an electrophile with an electron–rich coupling component, like B-naphthol and quindine derivative through an electrophilic aromatic substitution mechanism. The hydroxyl group (such as B-naphthol or chloride) group (such as chloro quine), direct the aryl diazonium ion to the para site unless that position is occupied, in which case the ion attaches ortho.

The prepared dye [1-(4-sulfonyl phenyl azo)-2-(7-chloro-4-[4-(di ethyl amino)-1-methyl butyl] amino]quidine] was obtained as amorphous powder reddish-orange color yield 2.4g. The melting point of dye was estimated at 253-254°C. The TLC results figure(1) showed that only single spot was observed that have rate flow (Rf) value 0.4.

The prepared product as azo dye was characterized by various available techniques. The infrared spectroscopy is one of the efficient techniques used in the characterization of organic compounds. IR spectrophotometers as FTIR which was used in the present study to confirm the presence of functional groups as following distinguish stretching vibration band of azo group (N=N) at 1523 cm⁻¹ (13) as shown in figure(2). The stretching vibration of NH group appear at 3441.01 cm⁻¹. The asymmetry stretching vibration of S-O(SO3-H) group appearance at 1197.79 cm⁻¹ position, while symmetry at 1049.28 cm⁻¹ position. Other peaks of the principle bonds are shown in Table(1) and figure(2).
Table(1) Major stretching vibration of absorption bonds by FTIR spectroscopy

<table>
<thead>
<tr>
<th>Compound</th>
<th>V C=C aromatic</th>
<th>V S-O sym</th>
<th>V S-O asy</th>
<th>V.s CH alephatic</th>
<th>V.s N=N</th>
<th>V.s C-C aromatic</th>
<th>V.s C-N</th>
<th>V.s C-H aromatic</th>
<th>V.b NH</th>
<th>V.s NH</th>
<th>V.b C-H aromatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azo dye</td>
<td>1581.63</td>
<td>1049</td>
<td>1197.79</td>
<td>2916.37</td>
<td>1523</td>
<td>1382</td>
<td>1228.66</td>
<td>3061.63</td>
<td>623</td>
<td>3441</td>
<td>734</td>
</tr>
</tbody>
</table>

V= vibration ,s=stretching, sym=symmetric, asy=asymmetric, b=bending

Figure(2): FTIR spectroscopy for azo dye compound

The U.V-Visible spectrophotometer study was showed position of transition in 430 cm⁻¹
Figure(3). The broader transition may be due to hyperconjugation system of the molecule:

Scheme 2: hyperconjugation system of azo dye

Figure(3): UV-Visible curves of azo dye

Anti bacterial activity of prepared dye.

The biological activity against two types of both gram positive (*Staphylo coccus aureus*) and gram negative (*E.Coli*) micro organism were studied. *Staphylo Coccus aureus* shows maximum zone of inhibition (14mm) followed by *E.Coli* (13mm) that are shown in figures (4),(5) and table (2). In the study of Saranya Devi, 2014 (14), showed the anti microbial activity of azo dye with inhibition zone (20mm) against gram positive which have highest antibacterial activity than gram negative (18mm).
Table (2) – Effect the azo dye against two types of bacteria

<table>
<thead>
<tr>
<th>bacteria</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Concentration of azo dye (mg/ml)</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
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</table>

Figure (3) plate showing antibacterial activity by agar well diffusion method of dye against *Staph. aureus* at follow concentration: 1 = 300 mg/ml, 2 = 200 mg/ml, 3 = 100 mg/ml, 4 = 50 mg/ml

Figure (4) plate showing antibacterial activity by agar well diffusion method of dye against *E. coli* at follow concentration: 1 = 300 mg/ml, 2 = 200 mg/ml, 3 = 100 mg/ml, 4 = 50 mg/ml
تحضير وتشخيص صيغة أزوية جديدة (1-(4-azo سلفونات البنزيل)-2-(7-كلورو-4-[(ثنائي أثيل أمين)-1-بوتيل المثل) أمين] كوبينديين من كلوروكلورين ثنائي اللفوسفيت ودراسة فعاليتها ضد بكتيرية

الخلاصة

تم تحضير صيغة أزوية جديدة عن طريق مفاعل أحماض الأمينات السلفانيك مع عقار كلوروكلورين ثنائي الفوسفيت المستخدم

لعلاج مرض الملاريا. تم التشخيص الطيفي للصيغة المحضرية بمعالجة الأمينات تحت الحمراء وطبية الأشعة المرئية فوق البنفسجية. تم دراسة

الفعالية المحسدة للجراثيم الصيغة المحضرية باستخدام الجراثيم الموجبة لصيغة عرقية والسلفانية لصيغة عرقية

تم التأكد من فائقة الصيغة المحضرية بواسطة كروموتوغرافيا الطيفية المرئية و باستخدام نظام التصوير (البيوتول الثانوي-الماء-حمض الخليك)

وأيضاً (2:1:2). تم قياس درجة الاستهلاك للصيغة بفترة شعري من طرف واحد. نستنتج من الدراسة أن الصيغة المحضرية أبدت فعالية بالفيويولوجية كجزء للجراثيم في الدراسة.

REFERENCES