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Analytical, theoretical and evaluation of biological activities of a new azo dye derived from the drugs procaine and kojic acid

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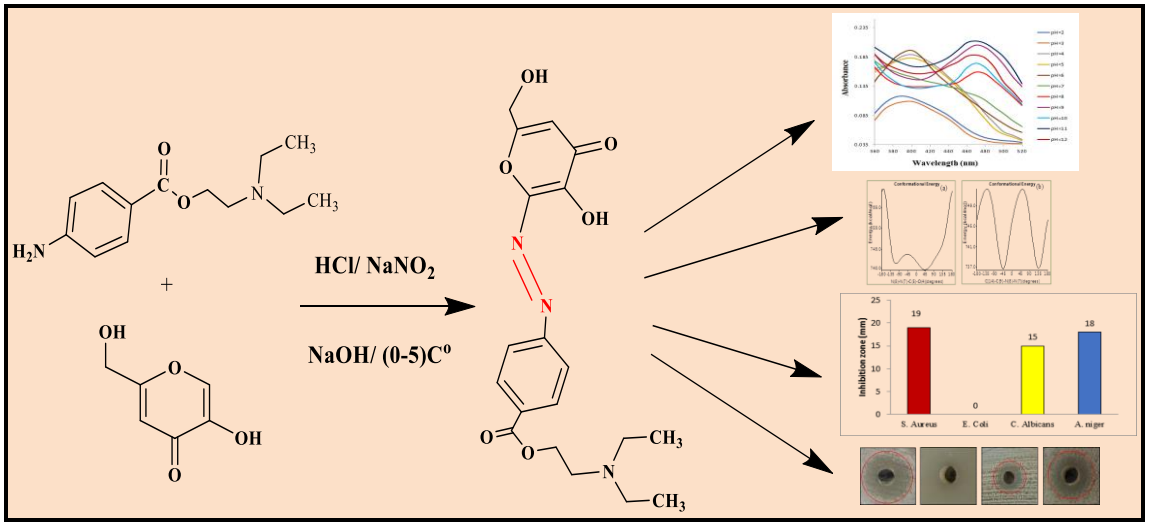
ABSTRACT A new azo dye, (2-(diethylamino)ethyl (E)-4-((3-hydroxy-6-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl)diazenyl)benzoate), was synthesized by diazotization of procaine followed by coupling with kojic acid in alkaline condition. The results showed a stable ionization approach since it works well in liquids with varying pH levels. The theoretical study on azo dye includes atomic close-contact calculations, (-N=N-) group and its related atoms, and analysis utilizing internal coordinate mechanics (ICM). The chemical azo dye showed a potential antibacterial activity against *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*, and negative against *Escherichia coli*.

KEY WORDS Azo dye, Internal Coordinate Mechanics, Conformational Analysis, Sensitizer.



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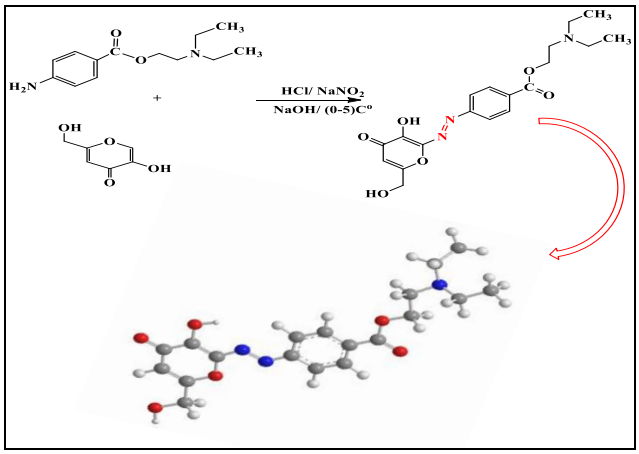
INTRODUCTION

Azo dyes get their name from the azo group (-N=N-) that is Azo dyes get their name from the fact that they have an azo group (-N=N-) in their structure.[1–3] There is a complicated conjugated network that connects each member of this group to every other member.[4] The capacity of these compounds to absorb visible light and produce colored dyes has intrigued analytical chemists and experimentalists.[5] Azo dyes have several effects on living things, such as being anti-diabetic, anti-cancer, and anti-microbial.[6–8] Even though animal trials have shown inconsistent results, azo dyes have been looked at as a novel type of Antibiotic that could treat a wide range of illnesses. demonstrating their effectiveness in inhibiting the growth of Staphylococcus aureus and Candida albicans in a controlled laboratory environment.[9,10] Research has demonstrated that azo dyes can exterminate specific bacteria, such as Staphylococcus aureus, Escherichia coli, and Candida albicans, but the precise effects may vary depending on the dye's structure.[11] Numerous theoretical investigations have concentrated on the molecular composition of azo dyes. Conformational energies can change the properties of dyes, which can then change how they react in tests and in living things.[12,13] To understand these changes, we used internal coordinate mechanics (ICM) and conformational analysis. This work aimed to develop a new azo dye for applications in analytical chemistry and biology. The aim of synthesis this azo dye is to study its biological activity against specific types of bacteria and fungi that have pathogenic effects and which may be used as an antibiotic in the future.

RESULTS AND DISCUSSION

Synthesis and Characterization

Diazotizing procaine and combining it with kojic acid resulted in the appropriately produced target azo dye (**Scheme 1**).



Scheme 1. Synthesis and chemical structure of azo dye.

Mass spectrometry, proton nuclear magnetic, ultraviolet-visible spectroscopy and Fourier transform infrared spectroscopy were used to confirm the structure of azo aye. The stretching of the (O-H) bond caused the broad band at 3311 cm^{-1}, and the stretching vibration of the carbonyl ester group (C=O) is clearly within the spectral of 1699 cm^{-1} in the FT-IR spectrum, as shown in **Figure 1**. The stretching vibration at 1462 cm^{-1} suggested the presence of the azo group (N=N).[14] The bands in the range of 1562 cm^{-1} to 1514 cm^{-1} are due to aromatic (C=C) stretching.[15]

The mass spectrum of the compound (chemical formula $C_{19}H_{23}N_3O_6$)[16] exhibited a molecular ion peak $[C_{19}H_{23}N_3O_6]^{+}$ at m/z 389 and base peak $[C_2H_5N]^{+}$ at m/z 43, as shown in **Figure 2**.

The chemical shifts of azo dye were determined in ^{1}H NMR spectrum of azo dye as shown in **Figure 3**, by using DMSO-d6 as a solvent.[17]

According to reference,[18] the electronic transition (n→π*) in **Figure 4** of the visible absorption of azo dye in ethanol correspond to the two absorption bands at (360 and 450 nm), respectively.

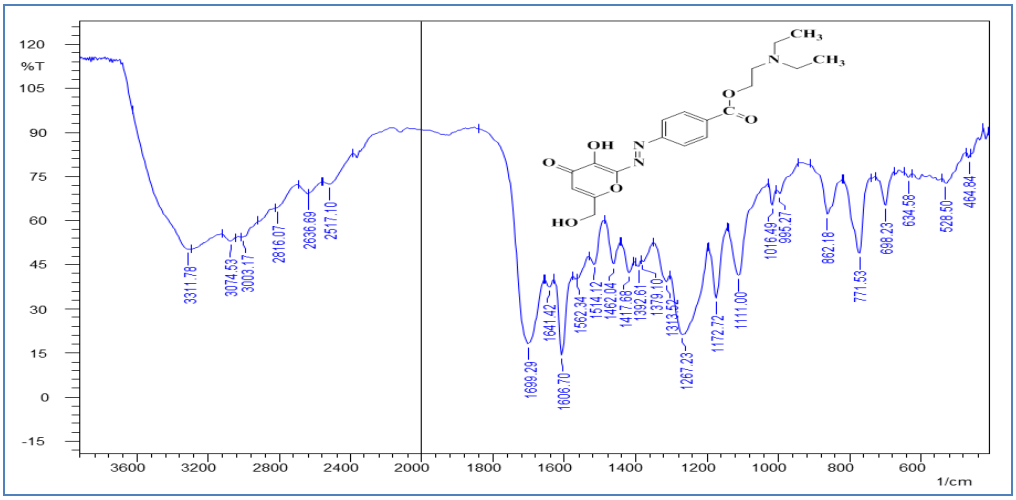


Figure 1. The FT-IR spectrum of azo dye.

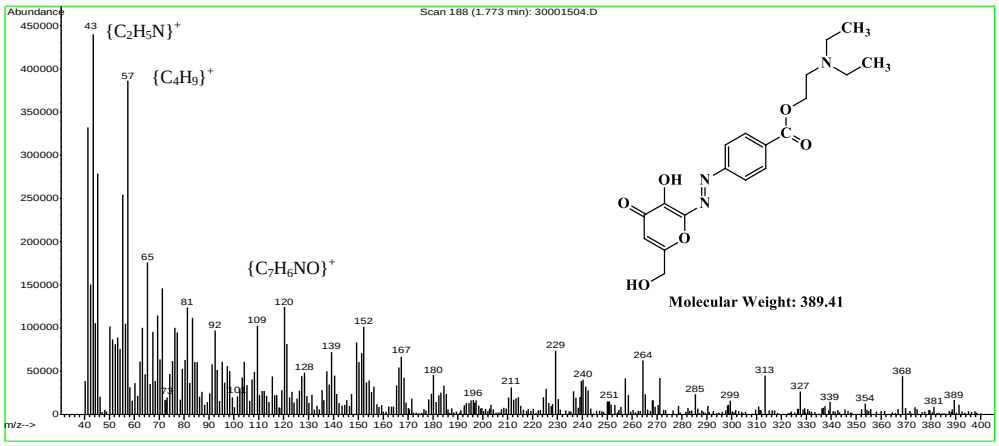


Figure 2. The Mass spectrum of azo dye.

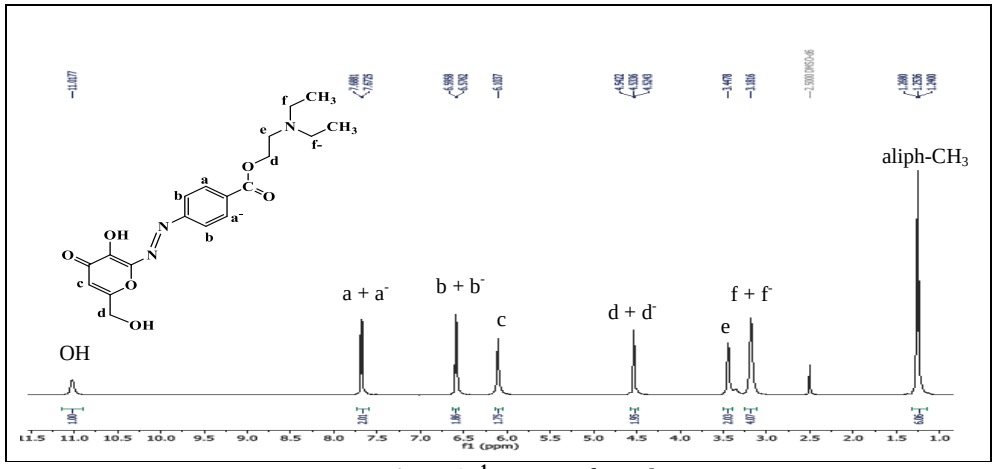


Figure 3. ^{1}H NMR of azo dye.

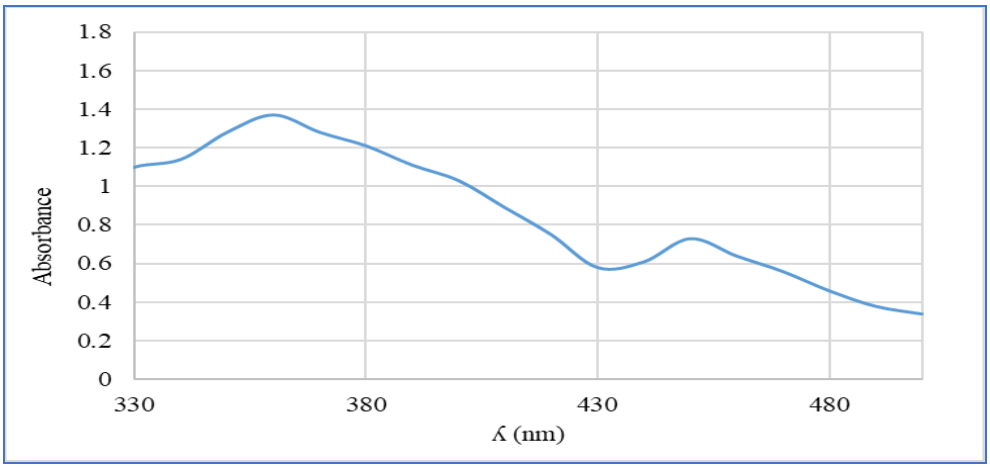


Figure 4. Visible absorption spectra of azo dye in ethanol.

Solvent Effects

We employed a battery of polarity-testing solutions, such as water, ethanol, chloroform, DMSO, and n-hexane, to see how the azo dye changed color in different solvents.[19–21] **Figure 5** shows the visible spectrum. It seems that chloroform is the best solvent for azo dye. The polarity of the solvent influenced the interactions between the solute and solvent, which subsequently determined the positions of the absorption maxima (λ_{max}). We used a basic solvatochromic equation to look at the link between the transition energy and the solvent dielectric constant ***(D):***

$$\Delta V = [(a-b)((n^2 - 1)/(2n^2 + 1))] + b[(D - 1)/(D + 1)] \ldots (1)$$

The results indicated a correlation between the spectrum changes and fluctuations in the solvent's dielectric constant and solvation energy. **Figure 6** shows a straight line that connects λ_{max} and the solvent polarity function (D-1) /(D+1).

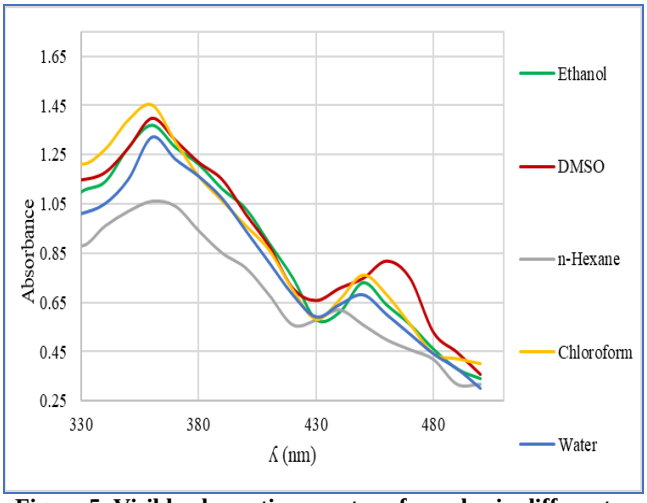


Figure 5. Visible absorption spectra of azo dye in different solvents.

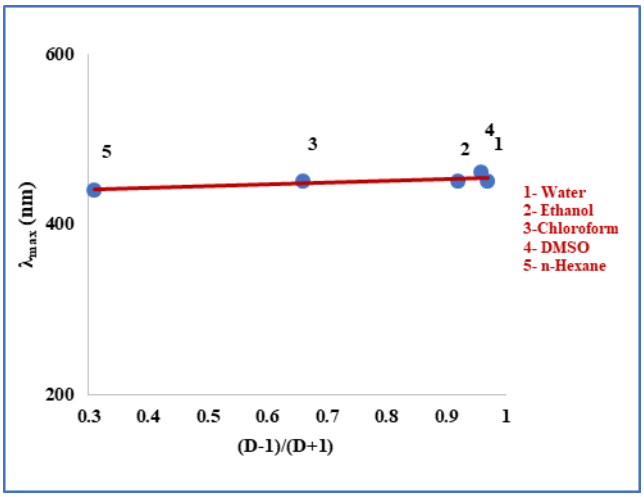


Figure 6. Plot of λ_{max} versus the solvent polarity function (D-1)/(D+1).

pH Effects

Figure 7 shows how the absorption spectra of azo dye change with pH Four isosbestic spots appeared in the spectra between 430 and 450 nm, showing that there were clear changes. [22] This suggests that a number of species may be in a condition of balance. The half-height method (Equations 2 and 3)[19,23] is used to find the pK_a values for the nitrogen atom and hydroxyl group when they are protonated and deprotonated (**Figure 8**).

pK = pH (at $A_{1/2}$) ... (2)

$A_{1/2} = (A_l + A_{min}) / 2$ ... (3)

You may find the pK values of an azo dye under both acidic and basic environments by looking at the pH-absorption curve. These results lead to the suggestion of an ionization-protonation method for azo dye (**Scheme 2**).

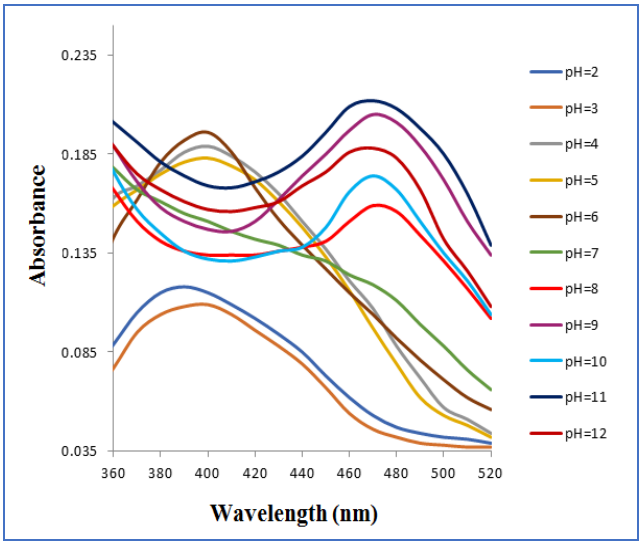


Figure 7. Visible absorption spectra of azo dye in different pH values.

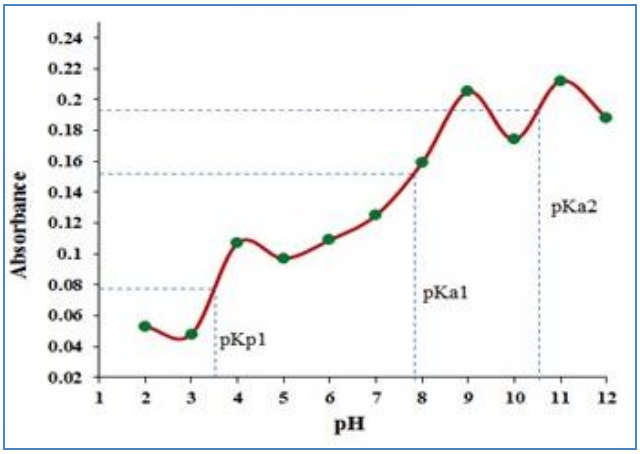
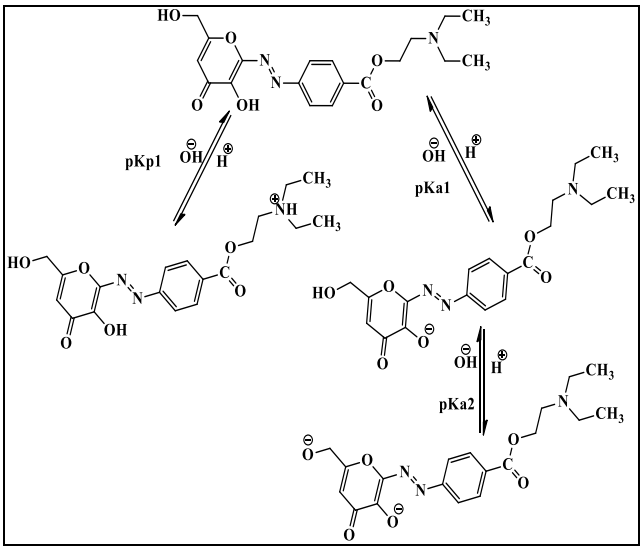


Figure 8. The relation between the absorbance of azo dye and different pH values at 470 nm.



Scheme 2. Proposed ionization-protonation scheme for azo dye.

Cytotoxicity

The cytotoxicity test on red blood cells showed that no amount of azo dye tested (50–1000 µg/ml) produced hemolysis. [24] This demonstrated that it is entirely safe for further biological research. A report utilized theoretical frameworks to enhance their comprehension of the structure-activity relationship. Atomic close-contact simulations are used to find probable interactions between molecules.[25] Assuming we know the compound's stereochemistry, we can designate the structures surrounding the C(2)-C(3), C(5)-C(6), and N(7)-N(8) bonds as (Z), (E), and (E), respectively. The results of the Internal Coordinate Mechanics (ICM) calculations for the important atoms. The distance between nitrogen atoms 8 and 7 was found to be 1.2480 Å**. Figures 9a** and **9b** indicate that the conformational research involves changing the single bonds C5-C7 and C8-O9. The results showed that these rotations had a limited amount of energy, which might change the molecule's internal coordinates and how well it binds to biological targets. **Figures 10c** and **10d** also illustrate the findings of the double-angle plots for the C5-N7=N8 and N7=N8-C9 torsion angles, respectively. The figure shows that the (N=N) group changes the conformational energy landscape of the azo dye, which affects its biological activity. The color of each group shows how much energy it has.

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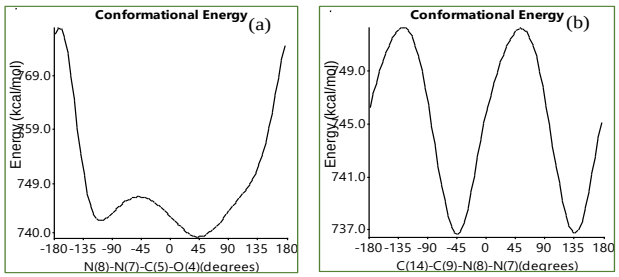

Figure 9. Conformational energy profiles for rotation around (a) C5-C7 and (b) C8-O9 bonds.

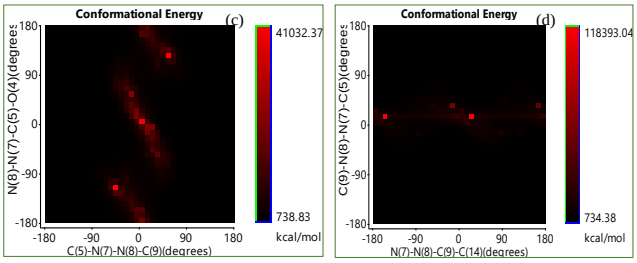

Figure 10. Two-dimensional double-angle plots of conformational energy for (c) C5-N7=N8 and (d) N7=N8-C9 torsion angles.

Antimicrobial Activity

Figure 11 shows the results of studies that looked at how well azo dye worked against two types of bacteria and two types of fungi. The findings indicates that azo dye suppresses the proliferation of Staphylococcus aureus, Aspergillus niger, and Candida albicans to a lesser degree, while exerting no significant effect on Escherichia coli (**Figure 12**).[26,27]

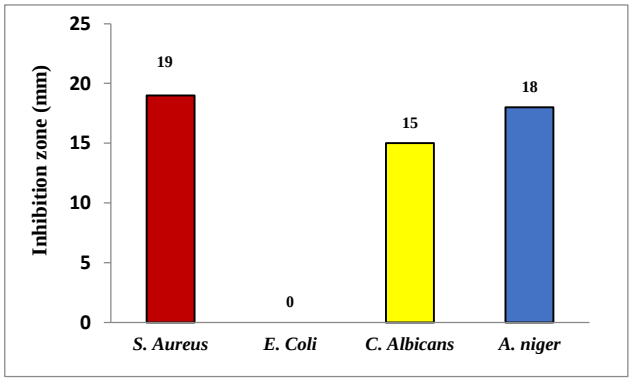

Figure 11. Histogram showing the inhibition zone diameters (mm) for azo dye against different microorganisms.

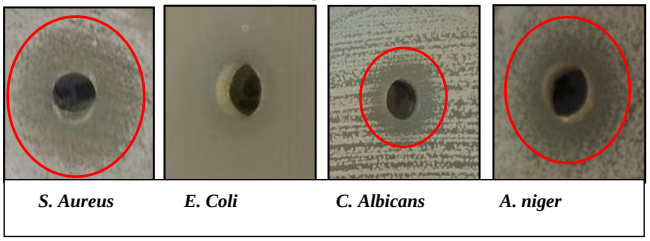

Figure 12. Photographs of the antimicrobial assay plates showing inhibition zones for azo dye.

EXPERIMENTAL SECTION

Chemicals and Reagents

The chemicals and solvents used in this work were purchased from well-known brands like Sigma-Aldrich and Merck. Chemsavers Inc. sold the main ingredients, which are (diethylamino)ethyl 4-aminobenzoate (procaine) and 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (Kojic acid). They were at least 98% pure.

Instrumentation

The researchers use a SHIMADZU FT-IR-8400S spectrometer for Fourier-transform infrared spectroscopy and add KBr disks at a concentration of 1% w/w. Melting points and temperatures (9100). Agilent Technologies makes the mass spectrometer that is used for the test. It uses electron ionization (EI) at 70 eV. We use a JENWAY (6305) ultraviolet-visible absorption spectrophotometer to gather the data. They employed a L Puls Munchen pH meter, which was built in China, to keep an eye on the pH levels.

Synthesis of (2-(diethylamino)ethyl (E)-4-((3-hydroxy-6-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl)diazenyl)benzoate

(0.006 mol, 1.4179 g) of (diethylamino)ethyl 4-aminobenzoate (procaine) was dissolved in solution of 2.1 ml of conc. HCl and 7.9 ml of deionized distilled water. An $NaNO_2$ solution prepared as the first solution by dissolving

0.456 g in 5 ml of distilled water, solution of $NaNO_2$ as the second solution was added gradually to the first solution keeping the mixture temperature in the range 0–5 °C. The resulting diazonium salt was gradually added to a solution (0.006 mol, 0.8527 g) of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (Kojic acid) dissolved in 10 ml of sodium hydroxide solution 25%. The final mixture was stirred for one hour in an ice bath at a temperature not more than 5 °C. The resulting red crudes were recrystallized in hexane and ethanol to yield (83%), m.p: 186–189 °C. Elemental analysis: O=24.65, N=10.79, H=5.95, and C=58.60. FT-IR (KBr, *v,* cm^{-1}): 3311 (O–H), 2816 (C–H aliph.), 1699 (C=O), 1562 (C=C), 1462 (N=N), 1313 (C–N), 1267 (C-O). ^1H NMR (DMSO, 500 MHz, δ ppm): 11.01 (s, 1H, OH), 7.68 (d, 2H, $H_{a, a'}$), 6.57 (d, 2H, $H_{b, b'}$), 6.10 (s, 2H, H_c), 4.53 (s, $2H_d$, O-CH_2), 3.44 (t, $2H_e$, O-CH_2), 3.18 (t, $4H_{f, f'}$, CH_2). 1.25 (t, 6H, aliph-CH_3). UV–vis. in ethanol, λ_{max} (nm): 360, 450.[28]

Preparation of Solutions for Analytical Studies

Solvent Effect

To make solutions of azo dye (1×10^{-4} M), we can use a number of solvents, including water, ethanol, DMSO, chloroform, and n-hexane. These solvents had a range of polarities.[29] Azo dye solutions are prepared with a pH range of 2 to 12 and a concentration of 1×10^{-4} M.[30]

Cytotoxicity Assay

An *in vitro* hemolytic assay is used to check how hazardous the azo dye is to cells. To find out which concentrations of azo dye are safe, we look at different amounts, such as 50, 100, 250, 500, and 1000 µg/ml.[31]

Antimicrobial Activity Assay

The antimicrobial activity of azo dye is investigated using the Well Diffusion Agar method against the bacterial strains Escherichia coli and Staphylococcus aureus, and the fungal strains Candida albicans and Aspergillus niger.[32,33] The microbial cultures are incubated on Mueller Hinton Agar (MHA) at 36 ±1 °C for 24 hours. Wells are bored into the agar, and 0.1 ml of the microbial suspension (adjusted to 0.5 McFarland standard) is spread onto the surface. Subsequently, 0.1 ml of a azo dye solution (5 µg/ml in DMSO) is added to each well. The inoculated plates are incubated at 36 ±1 °C for 24 hours under appropriate conditions. The antimicrobial activity is determined by measuring the diameter of the inhibition zone (in millimeters) around each well.

CONCLUSION

A new azo dye, (2-(diethylamino)ethyl (E)-4-((3-hydroxy-6-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl)diazenyl)benzoate), was synthesized and fully characterized. The polarity and pH of the solvent changed the analytical properties. An ionization approach is proposed to elucidate its behavior in buffer solutions. The molecular structure, which is made clear by theoretical simulations, helps us comprehend how reactive it is. This structure has atomic tight connections, ICM characteristics, and the energy of the (-N=N-) group in its conformation. The compound demonstrated robust and specific antibacterial properties, exhibiting moderate effectiveness against Aspergillus niger and Candida albicans, and high effectiveness against Staphylococcus aureus. This study offers further evidence that azo dye might serve as a valuable foundation for the identification of innovative chemical sanitizers or therapeutic agents targeting these specific illnesses.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

The authors worked together on the concept, the implementation, and the formal analysis of the research. Ghufran A. Mirdan and Jassim H. Al-Waeli are in charge of the project's planning and execution. Hasanain A. Abdullmajed is in charge of the work and wrote the first draft. All of the writers read and revised the article. All of the authors agreed with the final version of the article.

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