REASEARCH ARTICLE

Synthesis and Biological Studies of Some Diazo Dyes As New Drugs

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ABSTRACT

The novel azo dye (1) and diazo dye (2) was synthesized from two different drugs (paracetamol and cefpodoxime proxetil), followed by the purification and characterization of each. Then, the antimicrobial activities of (1) and (2) were screened in vitro towered *Klebsiella spp.*, *Aspergillus terreus* and *Candida glabrata* infections by using different concentrations (0.008, 0.016 and 0.2 mg/mL) from each. Azo dye (1) is given high antimicrobial activity against gram-negative bacteria *Klebsiella spp.*, good activity against *Candida glabrata*, and no activity against *Aspergillus terreus*. These activities were seemed to be better than that received by the diazo dye (2) using the same concentrations. The results were also showed that the synthetic dyes provided a non-toxic effect and didn't show any hemolysis effect in the cells. Further, the results were indicated that each synthetic dye was treated the dull infection with *Candida glabrata* and *Klebsiella spp*. with good results, but the results of (1) still better than (2). Due to recommend these dyes as new drugs for disseminated infections with the two microorganisms.

Keywords: Antimicrobial activity, *Aspergillus terreus,* Azo dyes, *Candida glabrata*, Infection, *Klebsiella spp.*

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INTRODUCTION

Azo dyes were receiving high attention in scientific research,[1-4] and they have great importance in chemical analysis. A strongly colored compound can be yellow, red, orange, blue or even green, depending on the exact structure of the molecule due to make azo dyes as extremely importance as dyes and also as pigments for a long time.[5] The structural features in the organic compounds, that usually produce color are C=C, N=O, N=N, aromatic rings, C=O and NO_2. Though the groups that invariably confer color are the azo (–N=N–) and nitroso (–N=O), while the other groups do so under certain circumstances. Azo dyes contain one or more azo groups (–N=N–), which are linked to SP_2 hybridized carbon atoms, based on the number of such groups These compounds contain more than one active group, which can formulate chelatic coordinational complexes with metal ions distinguished by their color and ability to dissolve in different solvents.[6] Further, the azo is reactive compound,[5] that was reported for its pharmaceutical importance as antidiabetic,[8] antineoplastic,[9] antibacterial,[5,10] And anticancer agent Azo dyes are widely applied in cosmetics,[12] tattooing, food and drinks, pharmaceuticals, printing inks, plastics, leather, as well as paper industries. It was reported that bacteria living on human skin have the ability to reduce some azo dyes to aromatic amines, which raises potential safety concerns regarding human dermal exposure to azo dyes such as those in tattoo ink and cosmetic colorant formulations. To comprehensively investigate azo dye induced toxicity by skin bacteria activation, it is very critical to understand the mechanism of metabolism of the azo dyes at the systems biology level. An LC/MS-based metabolomics approach was employed to globally investigate the metabolism of azo dyes by *Staphylococcus aureus* as well as their effects on the metabolome of the bacterium. This study provided novel information regarding azo dye metabolism by the skin bacterium, the effects of azo dyes on the bacterial cells and the important role of the toxicity and/or inactivation of these compounds due to microbial metabolism.

METHODS

Preparing of the Azo dyes

Azo dyes (1) and (2) were synthesized by a method similar to that described by Fox[13] as following:

The cefpodoxime proxetil (2.788 g, 0.005 mole) was dissolved in 2.1 mL of conc. HCl followed by adding 5 mL of distilled water. The mixture was stirrer and was kept in the ice bath. Then, the $NaNO_2$ (0.400 g) was dissolved in about 10 mL of distilled water and the resulting solution was also kept in the ice bath. The solution was then added to the first mixture resulting diazonium salt. This step was repeated two times. The couplers were then prepared by dissolving the paracetamol

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(756. 0 g, 0.006 mole) and the 1-((isopropoxy carbonyl)oxy) ethyl (1*S*,8*R*)-8-((*Z*)-2-(2-((*Z*)-(5-acetamido-2-hydroxyphenyl) diazenyl)-4,5-dihydrothiazol-4-yl)-2-(methoxyimino) acetamido)-4-(methoxy methyl)-7-oxo-2-thiabicyclo oct-4-ene-5-carboxylate in 25% sodium hydroxide solution in separate beaker. Each diazonium salt was added drop wisely to each coupler with constant stirring and keeping the temperature around 0.5ºC, the two resulting azo dyes were neutralized with dilute hydrochloric acid solution to give red and red brownish from (1) and (2) respectively, which then recrystallized from hexane to yield: (2.550 g, 71%) and (6.463 g, 82%) respectively; M.P= 148-150ºC and 262-258 ºC. λ_{max}= 350 nm and 530 nm (1) and 340 nm and 490 nm (2), ν_{max}= 3446.79, 2920.23, 1612.49 and 1442.75 (1) and 3415.93, 2906.73, 1637.56 and 1419.61 (2).

Preparing of the solutions of (1) and (2)

The solution of each azo dye (50 mL) was prepared in $5*10^{-3}$ concentration using DMSO as a solvent.

Antimicrobial investigations in vitro

- The biological activity of the synthetic azo dyes (1) and (2) were studied towered two kinds of fungi and one gram-negative bacteria: Candida glabrata, Aspergillus terreus and Klebsiella spp. as following:
- Sabouraud dextrose agar (SDA) and general nutrient agar cultures media were prepared and added to the petri dish followed by reactivate the isolates and then by developed of the agricultural sector, which then incubated at 30ºC for 1-3 days for Candida Krusei sector, but at 37ºC for 24 hours for Staphylococcus aureus.
- When the time of incubation was finished, A fungal suspension of each isolate was obtained by taking a small fraction of the fungal colony and bacteria and adding it to a test tube containing 2 mL sterile sterilized water with shaking a solution using an electrode (Vortex) until a fungal suspension become rude, the density was measured by using Malgeland device No. 0.5 to obtain 1 x 106 cell mL concentration.
- The antimicrobial activity in vitro using agar solid diffusion assay by adding (0.2 mL) of each of the fungal suspension on the surface of the agricultural medium SDA and the bacterial suspension on the surface of the agricultural medium (glucose nutrient agar GNA) followed by diffusing each equally on media. Finally, by using a glass rod in a letter L, which was then the dishes were left for 10 minutes. The suspension was absorbed by the medium, and all the names of the isolates and the chemical compounds tested were then labelled. Each hole was applied using a cork hole to create a circular hole of 0.9 mm diameter. Add 1 mL/100 µL per chemical compound in the agricultural middle pit of each of the isolates and in a double repeat of each isolation.

Cellular toxicity

The Xian-guo and Ursola method, was applied to measure the toxity of azo dyes under study using hemolytic red blood cells as following: A stock solution of 200 mg /mL was prepared and followed by preparing a series of diluted (0.2, 0.3 and 0.4 mg/mL) solutions. 0.8 mL of each diluted solution was added to Eppendorf tubes. 0.2 mL of red blood cells was also added to each tube. In addition, two Eppendorf tubes were equipped. In the first tube, 0.8 mL of Ringer solution was added as a negative control, but tap water as a positive control was added to the second tube. Then 0.2 mL of red blood cells was added to each tube. The results were recorded after the incubation of these tubes for 37 minutes in a special incubator and the changes in the solutions were followed checked.

RESULT AND DISSECTION

The two novel dyes that names, 1-((isopropoxy carbonyl)oxy) ethyl (1*S*,8*R*)-8-((*Z*)-2-(2 -((*Z*)-(5-acetamido-2-hydroxyphenyl) diazenyl)-4,5-dihydrothiazol-4-yl)-2-(methoxyimino) acetamido)-4-(methoxymethyl)-7-oxo-2-thiabicyclo [4.2.0] oct-4-ene-5-carboxylate (1) and 1-((isopropoxy carbonyl) oxy)ethyl (1*S*,8*R*)-8-((*Z*)-2-(2-((*Z*)-(5-acetamido-2-hydroxy-3-((*Z*)-(4-((*Z*)-2-(((1*S*)-1-((2*R*)-4-((1-((isopropoxycarbonyl) oxy)ethoxy)carbonyl)-5-(methoxymethyl)-3,6-dihydro-2*H*-thiopyran-2-yl)-2-oxoethyl)amino) -1-(methoxyimino)-2-oxoethyl)-4,5-di hydrothiazol-2-yl) diazenyl)phenyl) diazenyl)-4,5-dihydrothiazol-4-yl)-2-(methoxyimino) acetamido)-4-(methoxymethyl)-7-oxo-2-thiabicyclooct-4-ene-5-carboxylate (2) were synthesized, (Figures 1 and 2).

The synthetic azo dye (1) and diazo dye (2) were derived from two different drugs (paracetamol and cefpodoxime proxetil) using a method similar to that designated by Fox with optimizes the stoichiometry and the conditions of the reactions. Cefpodoxime proxetil is a broad spectrum third-generation cephalosporin,[15] which reveals potent antibacterial activity

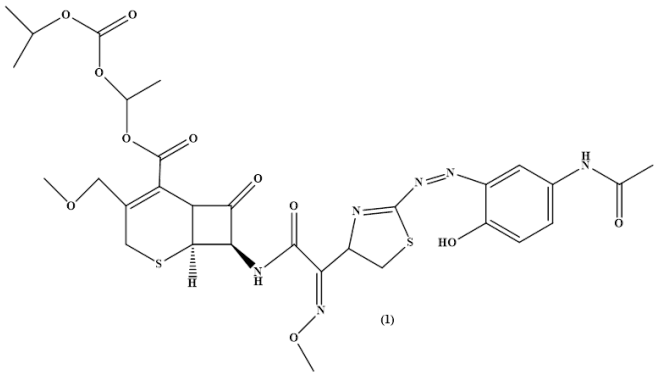

Figure 1: The structure of (1).

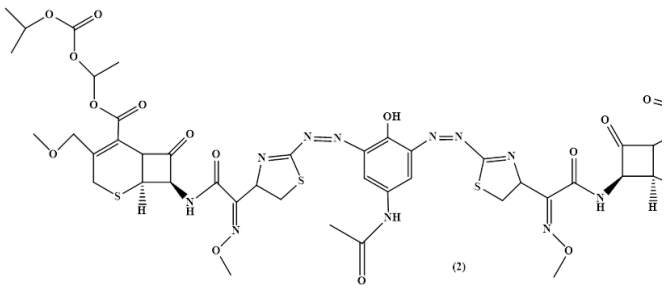

Figure 2: The structure of (2).

against both gram-positive and gram-negative bacteria, and high stability in the presence of beta-lactamases. However, the paracetamol/acetaminophen is one of the most popular and most commonly used analgesic and antipyretic drugs around the world, available without a prescription, both in mono- and multi-component preparations. It is the drug of choice in patients that cannot be treated with non-steroidal anti-inflammatory drugs (NSAID), such as people with bronchial asthma, peptic ulcer disease, hemophilia, salicylate-sensitized people, and children under 12 years of age, pregnant or breastfeeding women. It is recommended as a first-line treatment of pain associated with osteoarthritis. The current study was focused in combination between the cefpodoxime proxetil (1) and paracetamol (2) drugs by a synthesis of new drugs, which used in the dual treatment of the disseminated infections.

The synthetic azo dyes were then characterized using m.p., UV-visible, and IR spectrum. The UV-visible spectrum was documented at the range (250-450) nm. The results were showed that the maximum wavelength of (1) and (2) was equal to (350 nm and 520 nm) and (340 nm and 490 nm) respectively related to (π -π*) and (n-π*) as seen in the Figure 3 below.

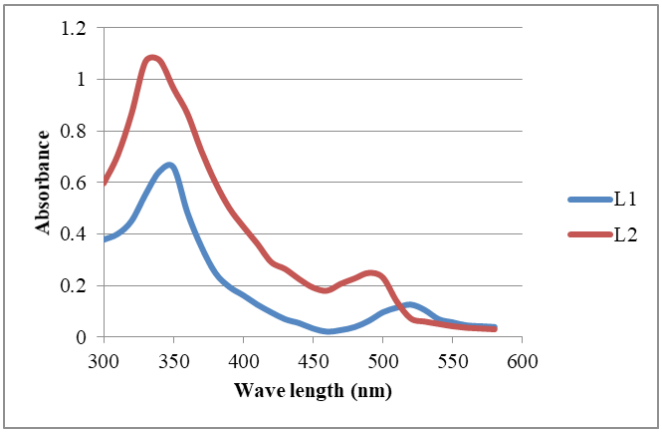

Figure 3: The UV-vis spectrum of (1 = L1) and (2 = L2).

The IR spectrums of the synthetic azo dyes (1) and (2) as seen in Figures 4 and 5 below were also studied.

The results were showed that the stretching vibration of the ν (OH) groups in (1) and (2) appeared in the regions 3446.79 cm^{-1} and 3415.93 cm^{-1}, respectively. But, the ν (N=N) stretching vibration bands seemed in the regions 1442.75 cm^{-1} and 1419.61 cm^{-1}, respectively.[2,3] Other bands with these regions can be considered as skeletal vibrations in (1) and (2), the(C=C) stretching vibration of the aromatic ring shows a strong brand in the region 1612.49 and 1637.56 cm^{-1} respectively.[2,3]And the aromatic CH band were appeared in the regions 2920.23 cm^{-1} and 2906.73 cm^{-1} respectively.[3]

The method of Xian-guo and Ursola[13] was then applied to measure the toxicity of the synthetic azo dyes using hemolytic red blood cells in vitro. The results were showed that the two azo dyes were provided non-toxic effect and didn't show any hemolysis effect in the cells, using different concentrations from each. Antimicrobial activities of (1) and (2) were screened in vitro. These activities were achieved towered *Klebsiella spp.*, *Aspergillus terreus*, and *Candida glabrata* infections by using different concentrations (0.008, 0.016, and 0.2 mg/mL) from each in DMSO, (Table 1).

Azo dye (1) as realized in Figure (1) above is given high antimicrobial activity against gram-negative bacteria *Klebsiella spp.*, good activity against *Candida glabrata*, and no activity against *Aspergillus terreus*, using 0.008 mg/mL, 0.016 mg/mL and 0.2 mg/mL concentrations. These activities were better than that received by azo dye (2) using the same

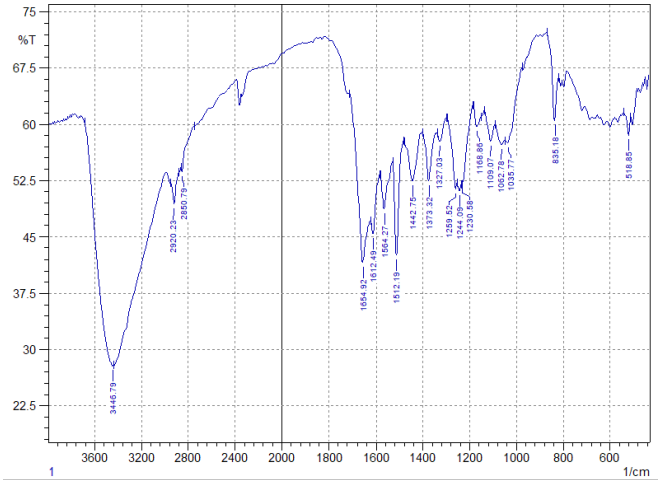

Figure 4: The IR spectrum of (1).

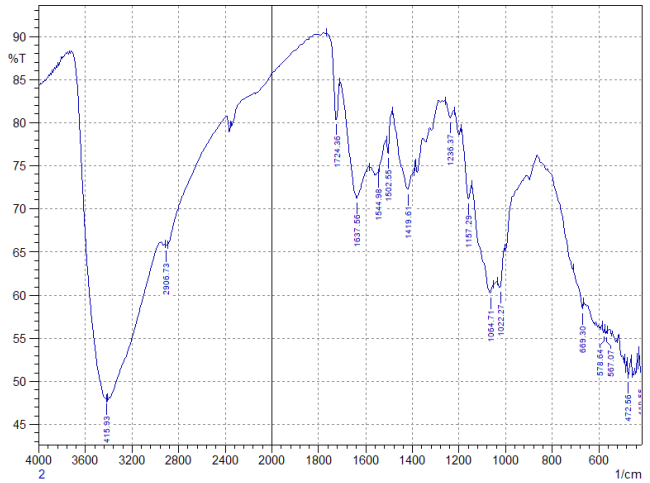

Figure 5: The IR spectrum of (2).

Table 1: The diameter of inhibition zones of (1) and (2) against bacterial and fungal infections

Id	*L1*			*L2*		
Conc.	*Candida glabrata*	*Klebsiella spp.*	*Aspergillus terreus*	*Candida glabrata*	*Klebsiella spp.*	*Aspergillus terreus*
0.008	3.1	4.1	-	2.7	3.3	-
0.016	4.1	4.5	-	3.1	4.1	-
0.2	4.3	5.5	-	3.7	4.7	-

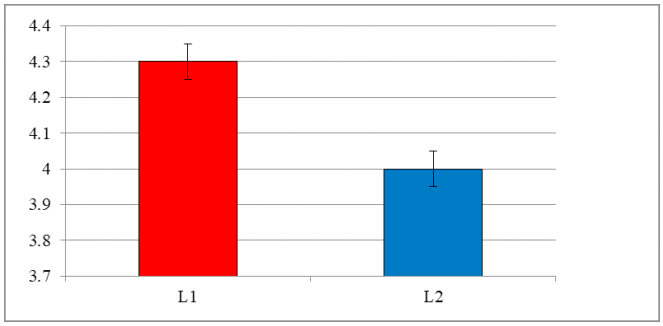

Figure 6: The effect of (1) and (2) in the dual treatment of the *Candida glabrata* and *Klebsiella spp.* disseminated infections.

concentrations. Therefore, these activities were made the synthetic azo dyes as new candidates against the dual infections with *Candida glabrata* and *Klebsiella spp.* as seen in Figure 6 below.

The results were indicated that the synthetic azo dyes (1) and (2) were treated the dull infection with the *Candida glabrata* and *Klebsiella spp*. with good results, due to recommend these azo dyes as a new drug for the disseminated infections. Numerous antimicrobial agents' activity they can be toxic to human beings. Therefore, the antimicrobials have to be non-toxic, non-allergenic, effective and selective, chemically stable, active against possibly more than one bacterium, and inexpensive.[17]

CONCLUSION

The synthetic azo dyes (1) and (2) were prepared cheaply because the starting materials are readily available, and most of the chemistry was done below the room temperature. Also, the synthetic dyes were delivered from two drugs, which all are receiving non-toxic effects and didn't show any hemolysis effect in the cells. Further, the synthetic azo dyes were gained a good color and displayed well activity towered *Candida glabrata* and *Klebsiella spp*., especially in the treatment of the disseminated infection. Due to recommend these azo dyes as novel antibiotics for *Candida glabrata* and *Klebsiella spp.* disseminated infections.

REFERENCES

1. Kirkan, B. and Gup, R. ''Synthesis of New Azo Dyes and Copper (II) Complexes Derived from Barbituric Acid and 4-Aminobenzoylhydrazone'', *Turk. J. Chem*., 2008, 32, 9-17.
2. H. Majeed, Synthesis, Characterization and study of the spectral and electronic properties of a New Azo Dyes Compounds, *J. Thi-Qar Sci*, 2013, 4, 91 – 101.
3. H. Ali, H. Majeed, A. Hussain, Synthesis, Analytical and Theoretical studies of (Z)-4amino-3-hydroxy-2-((4-(N-(5-methyl isoxazol-3yl) sulfamoyl) phenyl) diazenyl) naphthalene-1-Sulfonic Acid, Journal of Natural Sciences Research, 2017, 7, 81 – 88.
4. H. Ali, H. Majeed, I. Al-Asadi, A. Abdulredha, A. Hussain, Structures effect of two azo dyes associated with their antimicrobial activity, *Journal of Chemical, Biological, and Physical Sciences*, 2018, 8, 171-185.
5. Otutu, J. O., '' Synthesis and application of azo dyes derived from 2-amino-1, 3,4-thiadiazole-2-thiol on polyester fibre'', *J. IJRRAS 15*, 2013, pp. 292 – 296.
6. Fayadh, R. H. F., Ali, A. A., and Al –Jabri, F. M., Synthesis and Identification Symmetrically Azo Dyes Derived from Sulfa Compounds and Spectrophotometric study of Nickel (II) Complexes with Prepared Dyes, International Journal of Engineering and Technical Research (IJETR), 2015, 3,25-28.
7. Zollinger, H., ''Color chemistry; synthesis, properties and Application of organic Dyes and Pigments'', VCH,(1991).
8. Garg H. G. and Praksh C., "Preparation of 4-arylazo-3,5-disubstituted-(2H)-1,2,6-thiadiazine1,1-dioxides,"*JournalMedicinalChemistry*,1972, 15,.435–439.
9. Child R. G., Wilkinson R. G., and Tomcu-Fucik A., "Effect of substrate orientation of the adhesion of polymer joints," *Journal of Chemical Society*, 1977, 87, 6031–6038.
10. Ali H., Majeed H., Al-Asadi I., Abdulredha A. and Hussain A., Structures effect of two azo dyes associated with their antimicrobial activity, *Journal of Chemical, Biological and Physical Sciences* 2018, 8, 171-185.
11. Farghaly T. A. and Abdallah Z. A., "Synthesis, azo-hydrazone tautomerism and antitumor screening of *N*-(3-ethoxycarbonyl4,5,6,7-tetrahydro-benzo[b]thien-2-yl)-2-arylhydrazono-3-oxobutanamide derivatives," *Archieve for Organic Chemistry*, 2008, 17, 295–305.
12. Sun J., Jin J., Beger R. D., Carl E. Cerniglia C. E. and Chen H., Evaluation of metabolism of azo dyes and their effects on Staphylococcus aureus metabolome, *J Ind Microbiol Biotechnol*., 2017, 44, 1471–1481.
13. J. Fox, 1910; 97: 1339 (b) H. Majeed, A. Al-Ahmad, and K. Hussain, 2011; 37: 64 –73.
14. H. Xian-guo and M. Ursula, Antifungal compound from *Solanum nigrescens*, *J. Enthopharm*,1994; 43: 173–177.
15. Pahwa R., Rana A. S., Dhiman S., Negi P. and Singha I., Cefpodoxime proxetil: An update on Analytical, Clinical and Pharmacological Aspects, *J. Curr. Chem. Pharm. Sc*., 2015, 5, 56-66.
16. Marta B. and Jerzy N., Paracetamol: mechanism of action, applications and safety concern, *Acta Poloniae Pharmaceutica ñ Drug Research*, 2014, 71, 11–23.
17. Katke S., Amrutkar S., Bhor R., and Khairnar M., Synthesis of Biologically active 2-chloro-N-alkyl/aryl acetamide derivatives, International Journal of Pharma Sciences and Research (IJPSR), 2011; 2: 148–156.