ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Two Azo Dyes well Binding Human DNA as a new Antibiotics.

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ABSTRACT

Antimicrobial activitiesof4-acetaminophenol-[2-(4-azo)]-*N*-2-pyrimidinyl-benzenesulfonamide (1) and 4-acetaminophenol-[2-(4-azo)]-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide (2), were screened in vitro towered *Staphylococcus aureus* NCTC 6571 and *Candida Krusei*. The results were revealed that the azo dyes (1) and (2) were gave well activities towered *Candida Krusei*; these activities were increased in concentrated solutions. But, the achievement of (1) was better than (2) against *Staphylococcus aureus*. These results were then compared with that obtained by antibiotics (erythromycin capsules, amoxicillin capsules and metheprim tablet).It was found that the act of different concentrations of each azo dye were better than antibiotics in the treatment of the disseminated infection. Add to which, the two azodyes were showed non-toxic effect towards of the hemolytic red blood cells and didn't show any hemolysis effect in the cells. Further, the azo dyes (1) and (2) were right binding with human DNA. Due to recommend these azo dyes as new antibiotics that essential for disseminated infections with two diseases. The results were also showed that the azo dye (1) can bind *Staphylococcus aureus* and DNA better than (2). Our observation, the syntheticazo dyes can use as new drugs for disseminated infection.

Keywords: Antimicrobial activity, Azodyes, *Staphylococcus aureus*, *Candida Krusei*, Human DNA, Erythromycin, Amoxicillin, Metheprim

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ISSN: 0975-8585

INTRODUCTION

Azo dyes were received high attention in scientific research [1], and they have great importance in chemical analysis. Azo compounds contain one or more azo groups (–N=N–) which are linked to SP2 hybridized carbon atoms, based on the number of such groups [2]. A strongly coloured compounds extremely importance as dyes and also as pigments for a long time. [3]

The 4-acet aminophenol-[2-(4-azo)]-*N*-2-pyrimidinyl-benzene sulfonamide (1),[4] and 4-acetamino phenol -[2-(4-azo)]-*N*-(5-methyl-3-isoxazolyl benzene sulfonamide (2),[4] were prepared as a new azo dyes using Fox method.[5] These azo dyes were identified by IR, UV-visible and elemental analysis (CHN)4 The optimized structures of (1) and (2) were obtained by molecular mechanics (MM+), followed by further geometry optimization by the semi-empirical molecular orbital theory at the level of AM1.[4]

Antimicrobial activity of azo dyes (1) and (2), that contain 2-pyrimidinyl and 5-methyl-3-isoxazolyl were carried out against two bacterial species; *Staphylococcus aureus*(NCTC 6571), *Escherichia coli* (ATCC 25922) and fungus *Candida albicans*, using Agar-well diffusion method.[6] The results were showed that the minimum concentration of each azo dye was inhibited *Candida albicans* and *Staphylococcus aureus* reasonably. But, the *Escherichia coli* were resistant against azo dye containing 2-pyrimidinyl.

Theoretical studies were approved the structures of azo dyes and their fold a way same to that presents in biomolecules,[6] and showed that the staggered and the eclipsed conformations are interconverted by the rotation about the C-C single bond with an energy variance. Therefore, the conformational analysis of each azo dye is affecting their internal coordinate mechanics (ICM). Add to which, the molecular mechanics (MM2) and molecular mechanics force field (MMFF94) were displayed that the molecular structures of these azo dyes are affecting their properties, binding and antimicrobial activities.

Add to which, the MM2 minimization was achieved efficiently with azo dye containing 2-pyrimidinyl better than that contain 5-methyl-3-isoxazolyl; the later had high steric effect. But, the MMFF94 minimization and MMFF94 minimization/ sampling were attended successfully of each dye [6].

METHODS

Antimicrobial investigations in-vitro

The biological activity of azodyes towered fungi and gram positive bacteria:*Candida Krusei* and *Staphylococcus aureus* NCTC 6571 were studied as:

1. 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 agricultural sector which then incubated at 30^{o}Cfor 1-3 days for *Candida Krusei* sector, but at 37^{o}C for 24 hours for *Staphylococcus aureus*.
2. 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 deviceNo. 0.5 to obtain 1 x 10^{6} cell mL concentration.
3. The antimicrobial activity invitro 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 mediumGNA followed by diffusing each equally on media.
4. 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.

ISSN: 0975-8585

Cellular toxicity

The Xian-guo and Ursola method,[7] was applied to measure the toxity of azodyes 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 the tap water as a positive control was added to 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.

Human Genomic DNA extractions

Nucleic acids from each 200 µL of EDTA-whole blood sample were extracted. After cell lysis and protein denaturation, according to the procedure of Sambrook *et al*.[8] and stored frozen until use.

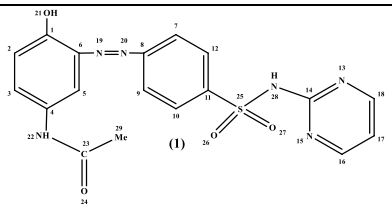
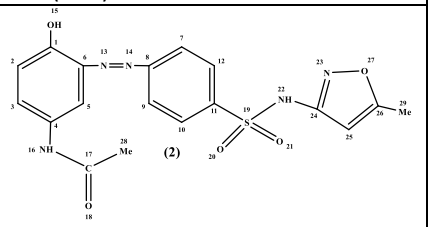
Effect of azodyes (1) and (2) on human DNA

To study the effect of azodyes (1) and (2) on genomic human DNA, 8 µl. of each was mixed with 8 µl of human genomic DNA, the mixture incubated at 37 ºC, and then subjected to 0.8% agarose gel electrophoresis at 60V.

RESULT AND DISSECTION

Antimicrobial activities of 4-acetaminophenol-[2-(4-azo)]-*N*-2-pyrimidinyl-benzene sulfon amide (1) [4,5,6] and 4-acetaminophenol-[2-(4-azo)]-*N*-(5-methyl-3-isoxazolylbezene sulfon amide (2) [4,5,6] were screened in vitro. These activities were obtained towered *Staphylococcus aureus* NCTC 6571 and *Candida Krusei* infections by using different concentrations (0.2, 0.3 and 0.4 mg/ mL) from each in DMSO, (Table 1).

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

Id	Inhibition zones (mm)					
	 (1)			 (2)		
Conc. mg/ mL	0.2	0.3	0.4	0.2	0.3	0.4
Staphylococcus aureus	35	36	38	20	25	30
Candida Krusei	31	40	42	35	41	43

The results from table (1) above were showed that the azo dye (1) more reactive than (2) towered *Staphylococcus aureus* using 0.3 mg/ mL and 0.4 mg/ mL concentrations. But, the two azo dyes were displayed better reactivity towered *Candida Krusei* using same concentrations, (Figure 1).

ISSN: 0975-8585

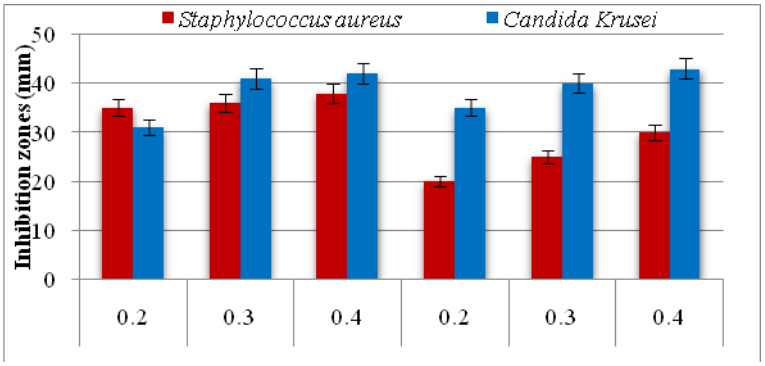


Figure 1: Antimicrobial activity of different concentrations from (1) and (2).

Azodyes (1) and (2) as seen in figure (1) aboveare given good antimicrobial activities against fungal infection and reasonable activities against gram positive bacteria. These results then compared with that attended by using different antibiotics (erythromycin capsules, amoxicillin capsules and metheprim tablet) using same concentrations, (Table 2).

Table 2: The diameter of inhibition zone of drugs against bacterial and fungal infections

Id	Inhibition zones (mm)					
	Antibiotics					
Conc. mg/ mL	Erythromycin capsules		Amoxicillin capsules		Metheprim tablet	
	Staphylococcus aureus	*Candida Krusei*	*Staphylococcus aureus*	*Candida Krusei*	*Staphylococcus aureus*	*Candida Krusei*
0.2	25	21	-	-	41	-
0.3	31	24	31	2	43	-
0.4	32	28	37	2.5	45	-

Table (2) above shows that the 0.2 mg/ mL of erythromycin capsule was gave low reactivity toward *Staphylococcus aureus* and *Candida Krusei*, but the other (0.3 and 0.4 mg/ mL) concentrations were showed better reactivity, (Figure 2). Though, the two microorganisms were showed resistant with amoxicillin capsule in 0.2 mg/ mL. The later still showed low activity in 0.3 mg/ mL and 0.4 mg/ mL concentrations. However, the metheprim was displayed greatest reactivity towered *Staphylococcus aureus* with resistant effect towered *Candida Krusei*.

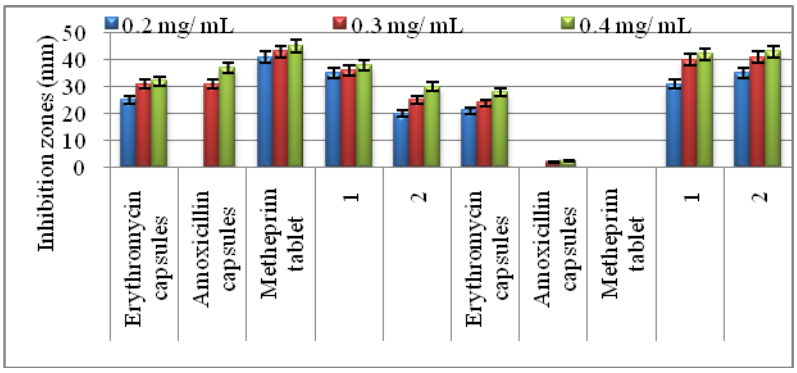


Figure 2: Antimicrobial activity of (1) and (2) in contrast with antibioticsin different concentrations.

The figure shows high reactivity of metheprim tablet against *Staphylococcus aureus*, but no reactivity toward *Candida Krusei*. However, the diameters of inhibition zones of (1) and (2) against *Candida Krusei* in three (0.2, 0.3 and 0.4 mg/ mL) concentrations were seemed to be higher than that achieved by antibiotics. Further, the reactivity of two azodyes was seemed to be better thanerythromycin and amoxicillin drugs. Thus, the average of data from table (1) and (2) were calculated as realised in Figure (3) below.

ISSN: 0975-8585

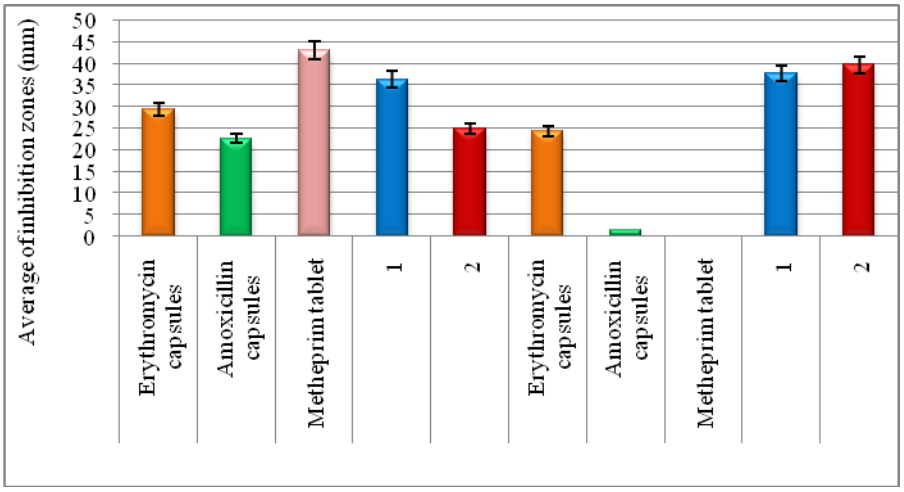


Figure 3: The average of the results of antibiotics and azodyes.

The results were showed that the best average was obtained by metheprim against *Staphylococcus aureus.* In contrast, the two azodyes were showed better results than antibiotics in treatment of the two infections.

Further, the method of Xian-guo and Ursola[7] was then applied to measure the toxity of (1) and (2) using hemolytic red blood cells in vitro. The results were showed that the two azodyes were provided non-toxic effects using different concentrations of each, and didn't show any hemolysis effect in cells. Due to recommend these azo dyes as a new drug for dull infection with two microorganisms. Numerous antimicrobial agents' activity they can be toxic to human beings. Antimicrobials have to be non-toxic, non-allergenic, effective and selective, chemically stable, active against possibly more than one bacterium and inexpensive.[9]

There are certain drugs that are shown to effect the very vital functions of living organisms, such as protein biosynthesis, nucleic acid replication and gene expression, collectively called antibiotics.[10]These non-protein molecules that are known to bind DNA molecules includes natural products such as antitumor, antibiotics and other secondary metabolites from bacteria and fungi and plants, synthetic compounds, and also heterocyclic and multi ring heterocyclic aromatic compounds and homo pyrimidine oligonucleotides. The interaction of relatively small molecules of this type with DNA may lead to a "useful" results such as antibacterial and anticancer activity, but may also involve undesirable biological responses, such as carcinogenesis or mutagenesis.[10]Thus, the DNA binding of each dye was studied as seen in Figure (4) below.

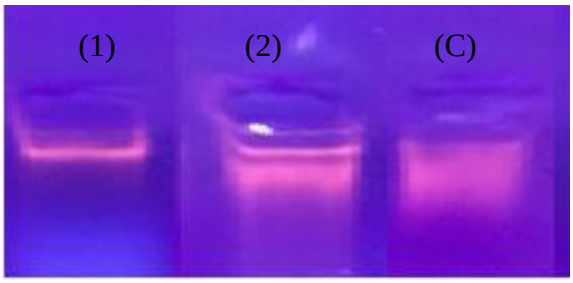


Figure 4: The DNA binding of (1), (2) and the control (C).

The figure displays that the two azodyes were bonded with human DNA. But, the azo dye (1) better bonded DNA than (2). By compared these results with that obtained by the biological activity, we find that the azo dye (1), which gave well reactivity toward *Staphylococcus aureus,* was better bind human DNA. The DNA in which two helical chains of nucleotides are held together by the hydrogen bonds that occur in a selective fashion between a purine and a pyrimidine nucleic base giving rise to the Watson-Crick pairs adenine-thymine

(AT) and guanine-cytosine (GC).[11]The hydrogen bonds were conceived as predominantly electrostatic phenomena that in the case of DNA base pairs are reinforced by polarization of the π-electron system (Resonance Assisted Hydrogen Bonding, RAHB).[11]the analyses of the bonding mechanism that donor-acceptor orbital interactions between the DNA bases in the Watson-Crick pairs are of comparable strength as electrostatic interactions. The donor acceptor or charge-transfer term is provided by the interactions of lone-pair orbitals on O or N of one base with N-H σ* acceptor orbitals of the other base. Our observation, that the two azo dyes can bind DNA in same manner. Further, the azo dye (1) can bind *Staphylococcus aureus* and DNA better than (2).

SUMMARY

The azo dyes (1) and (2) can be synthesized cheaply because the starting materials are readily available, and most of the chemistry is completed below room temperature. The two synthetic dyes were gained good color and delivered non-toxic effects using different concentrations from each. Also, the synthetic azo dyes didn't show any hemolysis effect in cells and well bind human DNA. They were gained better reactivity towered *Staphylococcus aureus* and *Candida Krusei* than antibiotics, spicily in the treatment of disseminated infection. Due to recommend these azodyes as novel antibiotics for *Staphylococcus aureus* and *Candida Krusei* disseminated infection.

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