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Synthesis and Biological Activity of Three Novel Azo Dyes

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Abstract
The azo dyes that named, (*E*)-4-((2-nitrophenyl) diazenyl) benzene-1,3-diol (1), (*E*)-4- ((3-nitrophenyl) diazenyl) benzene-1,3-diol (2) and (*E*)-4-((4-nitrophenyl) diazenyl) benzene-1,3-diol (3) were synthesized and then characterized using IR, UV-visible spectrum. These results were compared with that obtained by ChemBio 3D Ultra - [Chem3D XML] Gaussian Interface and were seems to be identical. Then, the antimicrobial activity of each azo dye was carried out against two bacterial strains: *Staphylococcus aureus* NCTC 6571, and *Escherichia coli* ATCC 25922, and fungal strains of *Candida albicans* using Agar-well diffusion method. The results were showed that the three azo dyes were biologically active and the best reactivity was observed in (2). Though, the biological activity of (1) with NO_2 group in ortho- position remained reasonable against *Candida albicans*. But, the effect of (1) was resisted by *Staphylococcus aureus* and *Escherichia coli*. However, the (2) and (3) with substituted NO_2 group in meta- and para- positions respectively were showed better reactivity's than (1) towered *Candida albicans* and *Staphylococcus aureus*. Further, the Gaussian interface properties and the conformational analysis of (1), (2) and (3) were intended. The results were indicated that the variations in the properties of each azo dye and their conformational energies of generated conformers can affect their biological activity afterward.
Keywords: key words, Azo dyes, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, Agar-well diffusion method, Antimicrobial activity

1. Introduction

Azo compounds were received high attention in scientific research (Kirkan & Gup 2008), and they have great importance in chemical analysis. Azo compounds contain one or more azo groups (–N=N–) which are linked to SP_2 hybridized carbon atoms, based on the number of such groups (Zollinger 1991). A strongly coloured compounds extremely importance as dyes and also as pigments for a long time (Otutu 2013).

The 4-acet aminophenol-[2-(4-azo)]-*N*-2-pyrimidinyl-benzene sulfonamide (Majeed 2013), and 4-acetamino phenol -[2-(4-azo)]-*N*-(5-methyl-3-isoxazolyl benzene sulfonamide (Majeed 2013), were prepared as new azo dyes using Fox method (Fox 1910) & (Majeed H., Al-Ahmad A. & Hussain K., 2011). These azo dyes were identified by IR, UV-visible and elemental analysis (CHN)4 The optimized structures of these azo dyes were obtained by molecular mechanics (MM+), followed by further geometry optimization through the semi-empirical molecular orbital theory at the level of AM1 (Majeed 2013). The antimicrobial activity of each azo dye (Ali *et al.* 2018), which contain 2-pyrimidinyl and 5-methyl-3-isoxazolyl respectively in their structures, were studied (Ali *et al.* 2018). These activities were carried out against two bacterial species; *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (ATCC 25922) and fungus *Candida albicans*, using Agar-well diffusion method (Perez *et al.* 1990). 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 (Ali *et al.* 2018).

2. Methods

2.1 Synthesis of azo dyes

The azo dyes (1), (2) and (3) were prepared by a method similar to that described by Fox. Using x-nitroaniline (0.006 mol., 0.828 g), (x = o, m and p aniline) and $NaNO_2$ (0.468 g) in 2.1 mL of diluted HCl and resorcinol (0.006 mol., 0.661 g) in 25% sodium hydroxide solution to yield (1.469 g, 95%), (1.45 g, 95%) and (1.463 g, 94%). The resulting crudes were recrystallized in hexane to yield light to dark orange brownish compounds; M.P.= (184-185)^oC, (178-179) ^oC, (182-183) ^oC; ν_{max} (3415, 3040, 1604, 1485-1444,1338) cm^{-1}, (3415, 3039, 1618, 1525-1479 and 1352) cm^{-1} and (3406, 3030, 1593, 1506-1479, 1338) cm^{-1} respectively; λ_{max}= 400 nm, (310, 380) nm, 432 nm respectively.

2.2 Solution of azo dyes in ethanol

The solutions of azo dyes were prepared by dissolved in ethanol to give (1x10-4 M) concentration.

2.2.1 Culture media

Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) were used to culture the bacterial and fungal strains, whereas the antimicrobial activity was carried out using Mueller Hinton Agar (MHA) and Sabouraud Dextrose

Agar (SDA).

2.2.2 Procedure

The Agar - Well diffusion method (Perez *et al.* 1990), was applied by pouring 20 mL of MHA (medium pathogenic bacteria) or SDA (pathogenic fungi) for each petri dish 90 mm) and then irrigate the medium with 0.1 mL of bacterial suspension or 0.1 µm optical suspension at a wavelength of 540 nm using a spectrophotometer using a spreader glass diffuser. Leave the dishes for 30 - 15 minutes until drying. (0.5 mm) was drilled in each dish using a sterile metal well. 100 µL of chemical solutions were added to each well while 100 µL of the DMSO was placed as a control. The dishes were heated at 37 ° C for 24 hours in the incubator and a diameter gauge Inhibition zone.

3. Result and discussion

The azo dyes (*E*)-4-((2-nitrophenyl)diazenyl)benzene-1,3-diol (1), (*E*)-4-((3-nitrophenyl) diazenyl)benzene-1,3-diol (2) and (*E*)-4-((4-nitrophenyl)diazenyl)benzene-1,3-diol (3), (Figure 1) were synthesized.

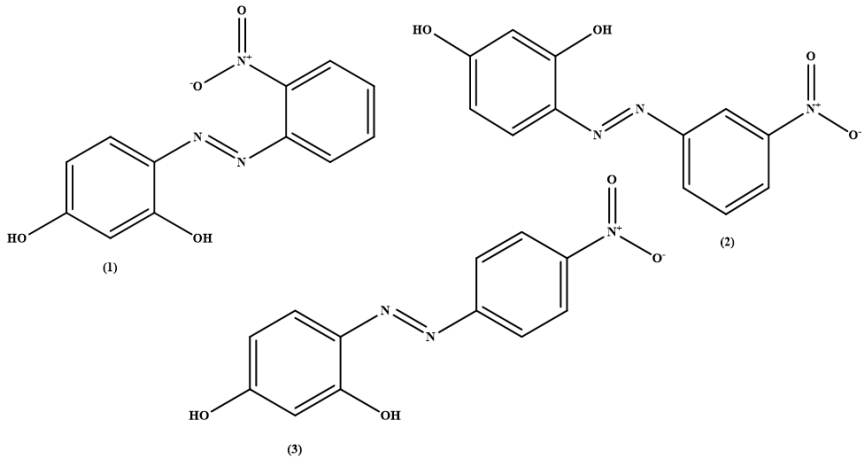


Figure 1. The structures of azo dyes (1), (2) and (3).

The synthetic azo dyes were then characterized using IR and UV-visible spectrum. The results of UV-visible spectrum were documented at the range (250-500) nm as seen in Figure (2) below.

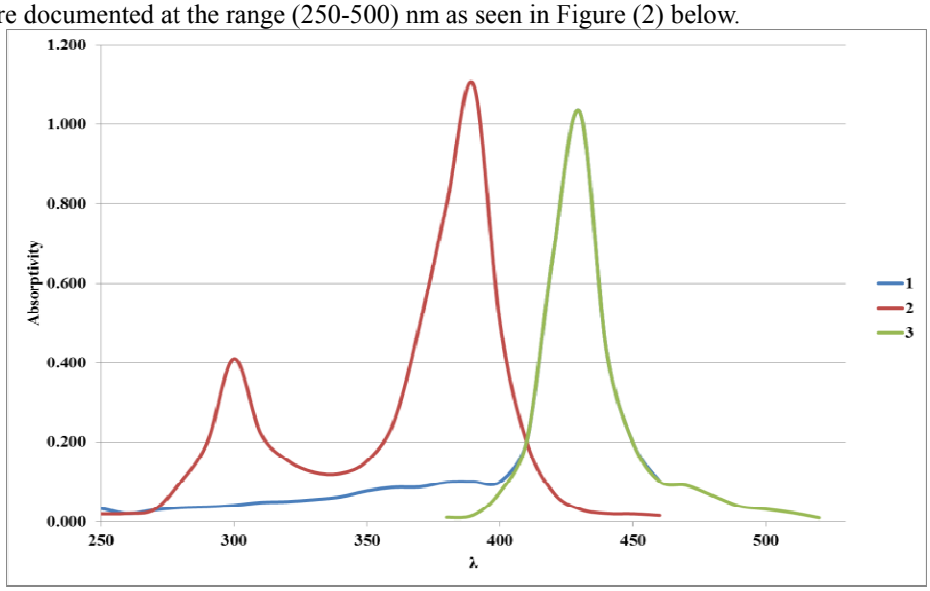
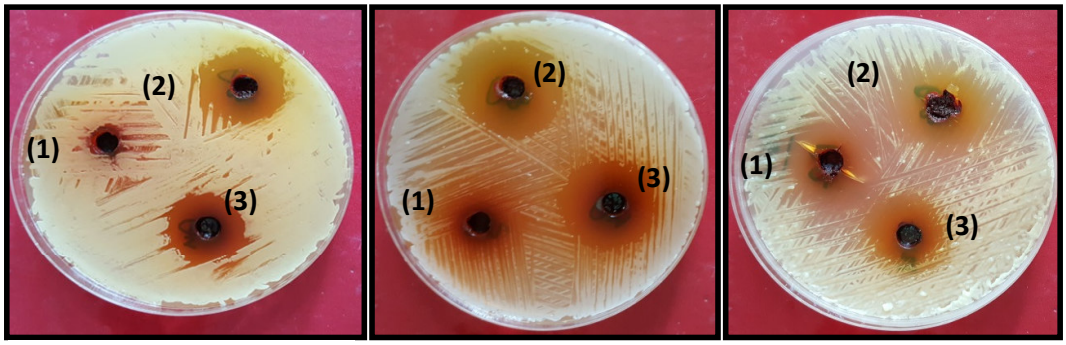


Figure 2. UV-visible spectrum of the three azo dye.

The figure above displays that the maximum wave length of azo dyes (1), (2) and (3) were equal to 430 nm, (300, 390) nm and 430 nm respectively. These results were compared with that obtained by ChemBio 3D Ultra - [Chem3D XML] Gaussian Interface and were seems to be identical. Add to which, the IR spectrum of the prepared azo dyes were showed that the stretching vibration of the ν (OH) groups were in the region (3406-3415) cm^{-1}. But, the ν (N=N) in the region (1338-1352) cm^{-1}, other bands with this region can be considered as skeletal vibrations, the (C=C) stretching vibration of the aromatic ring shows a strong band in the region (1593-1618) cm^{-1} and the aromatic CH bands were appeared in the region (3030-3040) cm^{-1}.

The antimicrobial activity, (Figure 3) of each synthetic azo dye was carried out against bacterial strains: *Staphylococcus aureus* NCTC 6571, and *Escherichia coli* ATCC 25922, and fungal strains of *Candida albicans*

using Agar-well diffusion method. The results were showed that the three azo dyes were biologically active and the best reactivity was observed in (2).



Staphylococcus aureus NCTC 6571 | *Escherichia Coli* ATCC 25922 | *Candida albicans*

Figure 3. The antimicrobial activities of azo dyes (1), (2) and (3).

The results of antimicrobial activity of (1), (2) and (3) were also gave the inhibition zones (mm) of each dye against the bacterial and fungal infections in vitro, (Table 1).

Table 1. The diameter of inhibition zones of azo dyes against bacterial and fungal infections in vitro

azodyes	Inhibition zones (mm)		
	Candida albicans	*Staphylococcus aureus*	*Escherichia coli*
(1)	22	-	-
(2)	20	23	15
(3)	20	20	15

Table (1) above demonstrates that the values of diameter of inhibition zones of (1), which has NO_2 group in ortho- position were reasonable against *Candida albicans*, but, the *Staphylococcus aureus* and *Escherichia coli* were resisted the effect of (1). Though, the antimicrobial activities of (2) and (3) with substituted NO_2 group in meta- and para- positions respectively were seemed to be higher than (1), towered *Candida albicans* and *Staphylococcus aureus*. These actions were also appeared to be better towered *Escherichia coli.* Further, the properties of (1), (2) and (3) were attended using ChemBioDraw Ultra - [Microsoft word], ChemBio3D Ultra - [Chem3D XML], Gaussian interface. The results were revealed that the Gaussian Interface Dipole, Molecular Volume, RMS Force and SCF Energy of (1), (2) and (3) were equal to (0.5616, 4.1076, 0.0001) 4.1458 Debye, (4.5190, 1.7233, 0.0009) 4.8364 Debye and (9.1826, -3.9253, 0.0000) 9.9864 Debye, 0.000 bohr**3/mol, 0.000 bohr**3/mol and 0.000 bohr**3/mol, 27.7830 Kcal/Mol, 30.4856 Kcal/Mol and 28.5863 Kcal/Mol, and -578381.81 Kcal/Mol, -578391.36 Kcal/Mol and -578394.38 Kcal/Mol respectively. The charges (Electron Density) of each azo dye were also obtained as seen in Table (2) below.

Table 2. The charges (Electron Density) of synthetic (1), (2) and (3) azo dyes

Id	Charges (Electron Density)		
Atoms	(1)	(2)	(3)
C(1)	4.682915	4.68544	4.67934
C(2)	5.190633	5.17856	5.19263
C(3)	4.768918	4.77335	4.76695
C(4)	5.562412	5.52956	5.56116
C(5)	5.12242	5.11795	5.11428
C(6)	5.356298	5.32784	5.33575
O(7)	8.423922	8.43456	8.42127
O(8)	8.424482	8.43389	8.41824
N(9)	7.242927	7.28747	7.23335
N(10)	7.328993	7.21023	7.24881
C(11)	5.175815	5.15755	5.00479
C(12)	6.090778	4.97256	5.10135
C(13)	4.959863	5.72407	4.92911
C(14)	5.076882	4.91917	5.71753
C(15)	5.012277	5.09093	4.95911
C(16)	5.349532	5.15452	5.27327
N(17)	7.303906	7.19371	7.15911
O(18)	8.536504	8.51325	8.53087
O(19)	8.597684	8.51137	8.53393
H(20)	0.417399	0.419947	0.411916
H(21)	0.309685	0.372925	0.364181
H(22)	0.445462	0.459059	0.452199
H(23)	0.329249	0.334108	0.330511
H(24)	0.305631	0.310492	0.303277
H(25)	0.372261	0.366525	0.433331
H(26)	0.4413	0.370596	0.371858
H(27)	0.436262	0.429826	0.372018
H(28)	0.415939	0.402447	0.420699

The results were displayed that the position of NO_2 group was affecting their properties, and the variations in these properties were affecting their biological activities subsequently. Thus, the conformational analysis of (1), (2) and (3) were studied. The energy of C(12)-C(11)-N(10)-N(9) and N(10)-N(9)-C(4)-C(3) conformers in (1), (2) and (3) respectively were calculated by rotation around each side of -N=N- single bond, (Figure 4(a-F)).

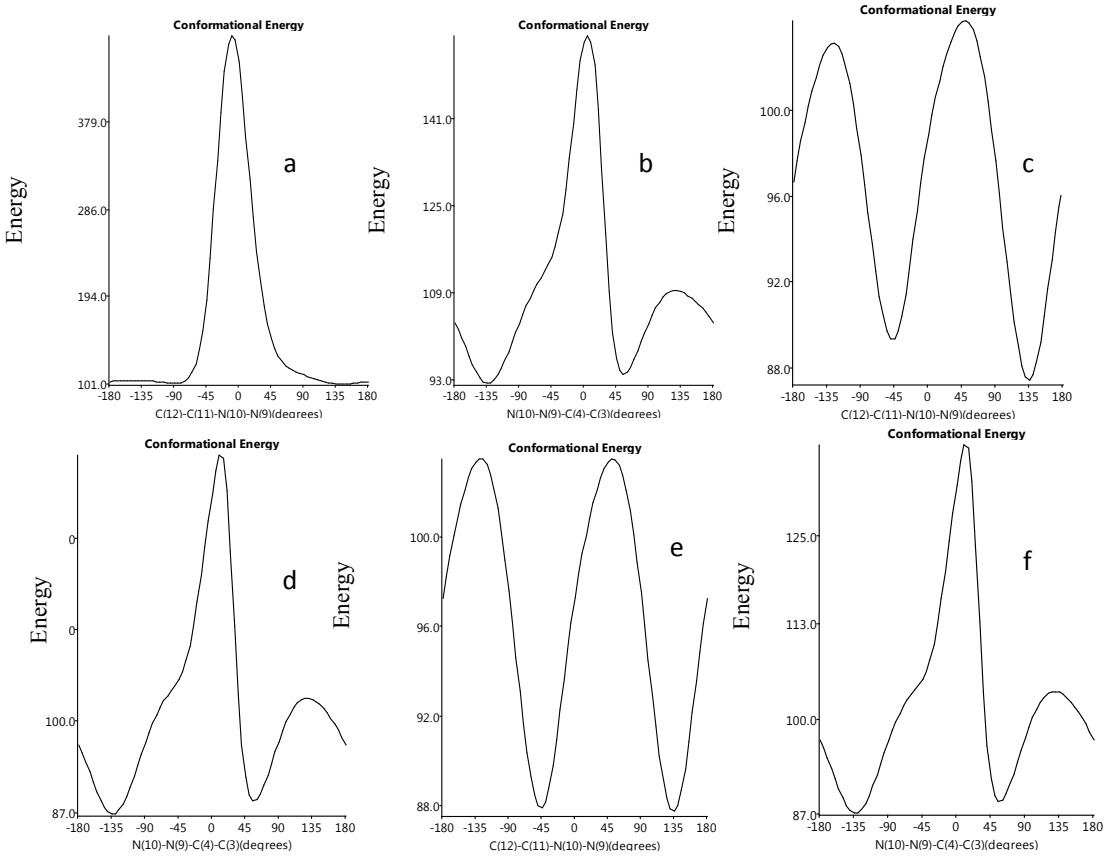


Figure 4. The conformational analysis of C(12)-C(11)-N(10)-N(9) and N(10)-N(9)-C(4)-C(3) conformers in (1), (2) and (3) respectively.

The results were showed that each rotation can generate eclipsed and staggered conformers as seen in figure (3) above. The results of figure 3a, 3c and 3e were showed that the eclipsed E for E(-10o), (E(-125o) and E(50o)) and (E(-125o) and E(50o)) in (1), (2) and (3) were equal to 471.40 kcal/ mole, (103.15 and 104.22) kcal/ mole and (103.50 and 103.49) kcal/ mole respectively. But, the staggered conformers for (E(-90o), E(135o)), (E(-50o), E(135o)) and (E(-45o), E(135o)) were equal to (101.62 and 100.94) kcal/ mole, (89.34 and 87.44) kcal/ mole and (87.87 and 87.73) kcal/ mole respectively. But, the results of figure 3b, 3d and 3f were showed that the eclipsed E for (E(5o) and E(125o)), (E(10o) and E(125o)) and E(10o) in (1), (2) and (3) were equal to (156.43 and 109.50) kcal/ mole, (137.92 and 103.42) kcal/ mole and 137.52 kcal/ mole respectively. But, the staggered conformers for (E(-135o) and E(55o)), (E(-135o)) and (E(55o)) and (E(-135o), E(55o)) were equal to (92.31 and 94.03) kcal/ mole, (86.73 and 88.67) kcal/ mole and (87.18 and 88.71) kcal/ mole respectively. This differ in the energies of generated conformers in each azo dye can explain the variation in their properties and their biological activity consequently, which indicated the previous results.

Summary

The structures of synthetic azo dyes (1), (2) and (3), which have a substituted NO_2 group in ortho-, meta- and para positions respectively; can affect their properties, conformational analysis and their biological activites subsequently.

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