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SYNTHESIS AND MOLECULAR DOCKING OF SOME NEW AZO DYES DERIVED FROM BENZIDINE AND STUDY SOME OF THEIR ANTIMICROBIAL POTENTIAL

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ABSTRACT*:*
This research includes preparing some new heterocyclic compounds derived from benzidine used as an azo dye such as thiopyrimidine , from the reaction between 2, 2'-((*1E, 1'E*)-[1, 1'-biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) dimalononitrile **3** and thiourea in absoulte ethanol to 5, 5'-(*1E, 1'E*)-[1, 1'-biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) bis (4, 6-diaminopyrimidine-2(*5H*)-thione **4** and another way four compounds were synthesized from diazonium salt of benzildine **2** with 4-hydroxy-6-methoxyquinolin-2(*1H*)-one (**7)**, 4-hydroxy-6-nitroquinolin-2 (*1H*)-one (**9)** and 1, 2-dihydro-4-hydroxy-2-oxoquinoline-6-sulfonic acid **(11)** to give (*3E*)-3, 3'-([1, 1'-biphenyl]-4, 4'-diylbis (hydrazin-2-yl-1-ylidene)) bis (6-methoxyquinoline-2,4 (*1H,3H*)-dione) **8,** (*3E*)-3,3'-([1,1'-biphenyl]-4,4'-diylbis (hydrazin-2-yl-1-ylidene)) bis (6-nitroquinoline-2,4 (*1H,3H*)-dione) **10** and (*3E*)-3,3'-([1,1'-biphenyl]-4,4'-diylbis (hydrazin-2-yl-1-ylidene)) bis (2,4-dioxo-1,2,3,4-tetrahydroquinoline-6-sulfonic acid) **12** respectively. The spectral characteristics (IR, ^{1}HNMR) of the obtained dyes are reported.

KEYWORDS: Synthesis; benzidine; heterocyclic compounds; azo dyes

INTRODUCTION

Azo dyes are used as starting materials for the manufacture of heterocyclic compounds [i], and they are a very important class of chemical compounds that are gaining research interest. They are vibrantly colored and have been used as dyes and pigments for a long time [ii, iii]. Furthermore, they have been extensively researched in applications such as optical recording medium [iv-vi], toner [vii, viii], oil-soluble lightfast dyes [xi] and azo dyes, which are described in the current paper. Three compounds, including 4- printing [ix, x] due to their superior studied thermal and optical properties. The synthesis of new heterocyclic hydroxy-6-methoxyquinolin-2(*1H*)-one (5), 4-hydroxy-6-nitroquinolin-2 (*1H*)-one (6) and 1, 2-dihydro-4-hydroxy-2-oxoquinoline-6-sulfonic acid (**7**) were employed as coupling compounds with diazonium salt of benzildine as the diazo component to make azo dyes for comparison purposes.

EXPERIMENTAL

CHEMISTRY

Chemicals were purchased from Sigma-Aldrich and utilized without further purification. At 600 MHz, the ^{1}HNMR spectrum was recorded Chemical shifts, δ, are presented in ppm relative to the internal standard TMS for ^{1}H NMR. Multiplicities are abbreviated as follows: s singlet, d doublet, t triplet, and m multiplet signals. A *Perkin Elmer* model 1430 spectrophotometer was used to *record* IR spectra.

2, 2'-((*1E, 1'E*)-[1, 1'-Biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) dimalononitrile (3)

A solution of benzidine **1** (0.02 mol, 3.68 g) and concentrated hydrochloric acid (10 ml) was cooled and stirred for 1 hr at 0-5 ^{o}C. Then, a solution of sodium nitrite (0.04 mol, 2.76 g) was added dropwise and stirred for (30 min) at 0-5 ^{o}C. The diazo compound was added slowly to a solution of malononitrile (3 ml) in ethanol (30 ml) and 10% sodium hydroxide with stirring. The mixture was allowed to stand stirred for 4 hr., at 0-5 ^{o}C and then kept in the refrigerator overnight. The residue was filtered, washed with water, dried and recrystallized with ethanol to afford the desired compound as dark brown solid crystals. Yield 65 %, m.p 175 – 177 ^{o}C. IR (KBr): 3050 (arom-H), 2106 (CN). ^{1}HNMR (600 MHz, DMSO, δ): 7.65–7.69 (dd, 4H, *J* = 7.3 Hz, Ar–H), 7.36-7.41 (dd, 4H, *J* = 7.3 Hz), 3.65 (s, 2H, CH). Anal. Calcd for: ($C_{18}H_{10}N_8$; 338.33): C, 63.90; H, 2.98; N, 33.12. Found: C, 66.82; H, 5.33; N, 12.96; S, 9.89.

Synthesis of 5, 5'-((*1E, 1'E)*-[1, 1'-biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) bis (4, 6-diaminopyrimidine-2-thiol) (4)

Compound **3** (0.1 mol, 0.33 g.) was dissolved in of absolute ethanol (15ml). To this mixture a solution of thiourea (0.1 mol, 0.7g) in absolute ethanol (20 ml) was added dropsies .The reaction mixture was stirred for (40 min.) then refluxed for 15 hr, on water bath. The solvent evaporated and the formed brown crystals was crystallized from ethanol. Yield 69 %. M.p 143-145 ^{o}C. IR (KBr): 3325 (NH_2), 3005 (arom-H), 1452 (N = N). ^{1}HNMR (600 MHz, DMSO): δ 13.03 (s, 2H, SH), 9.87 (brs, 8H, NH_2), 7.42–7.46 (dd, 4H, *J* = 7.6 Hz, Ar–H), 7.26–7.31 (dd, 4H, *J* = 7.6 Hz). Anal. Calcd for: ($C_{20}H_{18}N_{12}S_2$; 490.57): C, 48.97; H, 3.70; N, 34.26; S, 13.07. Found: C, 48.90; H, 3.67; N, 34.31; S, 13.14

6,6'-((*1E,1'E*)-[1,1'-biphenyl]-4,4'-diylbis(diazene-2,1-diyl)) bis (pyrimidine-2,4 (*1H,3H*)-dione) (6)

A mixture of diazonium salt of benzildine **2** (0.01 mol) and of uracil (0.01 mol) was dissolving in sodium hydroxide solution then cooled to 0-5 ^{o}C. The solid dye was filtered off and recrystallized from ethanol. The purity of dye was checked by TLC. Yield: 71 %. IR (KBr, νmax, cm^{-1}): 1432 (N=N), 1669 (C=O). ^{1}HNMR (600 MHz, DMSO): δ 10.93 (s, 2H, NH), 10.68 (br, 2H, NH), 7.44–7.49 (dd, 4H, *J* = 7.1 Hz, Ar–H), 7.52–7.58 (dd, 4H, *J* = 7.1 Hz), 6.96 (s, 2H, pyrimidine). Anal. Calcd for: ($C_{20}H_{14}N_8O_4$; 430.38): C, 55.82; H, 3.28; N, 26.04. Found: C, 55.79; H, 3.08; N, 2616

GENERAL PROCEDURE FOR AZO DYE

Benzildine **1** (0.3 mol) was dissolved in con hydrochloric acid (5 ml), and sodium nitrite (0.4 mol) was dissolved in 20 ml of H_2O and added drop wise with stirring. The reaction mixture was immersed in an ice-salt bath and cooled to 0-5 °C until the reaction was complete. 4-hydroxy-quinolin-2(*1H*)-one derivatives (0.3 mol) were dissolved in glacial acetic acid and 10% KOH. The solution was cooled to 0-5 °C in an ice-salt bath and then the prepared cold diazonium salt from above was stirred and added very slowly by the wall of the beaker with vigorous stirring. After the addition of the whole diazonium salt, the reaction mixture was allowed to stand in the ice bath for a further 14 h with stirring, washed with cold water and dried.

(*3E*)-3,3`-([1,1`-Biphenyl]-4, 4'-diylbis (43ydrazine-2-yl-1-ylidene)) bis (6-methoxyquinoline-2, 4 (*1H, 3H*)-dione) (8)

Red crystals. IR (KBr): 3125 (NH), 3096 (arom-H), 1683 (C=O). ^{1}HNMR (600 MHz, DMSO): 15.33–14.21 (br, hydrazone, NH), 10.14 (br, amide, NH), 7.66–7.42 (dd, 4H, *J* = 7.1 Hz, Ar–H), 7.32–7.13(dd, 4H, *J* = 7.1 Hz), 8.96 (m, 8H, Ar–H), 2.41 (s, 6H, 2(OCH_3). Anal. Calcd for: $C_{32}H_{24}N_6O_6$ (588.18): C, 65.30; H, 4.11; N, 14.28. Found: C, 65.26; H, 4.09; N, 14.03. Yield: 57%, Mp: 263–265 °C.

(*3E*)-3, 3'-([1,1'-biphenyl]-4,4'-diylbis(43ydrazine-2-yl-1-ylidene)) bis (6-nitroquinoline-2,4(*1H,3H*)-dione) (10)

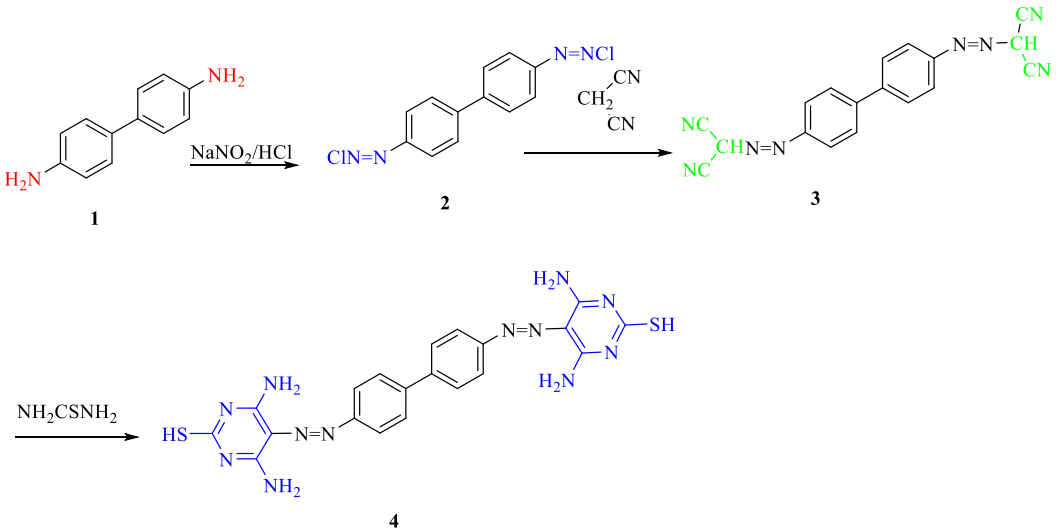
Red crystals. IR (KBr): 3160 (NH), 3012 (arom- H), 1683 (C=O). ^{1}HNMR (600 MHz, DMSO): 14.98 (br, hydrazone, NH), 10.23 (br, amide, NH), 6.83–6.62 (dd, 4H, *J* = 6.8 Hz, Ar–H), 7.46 (dd, 2H, *J* = 6.8 Hz, Ar–H), 8.76 (m, 8H, Ar–H). Anal. Calcd for: $C_{30}H_{18}N_8O_8$ (618.12): C, 58.26; H, 2.93; N, 18.12; O, 20.69. Found: C, 58.07; H, 18.07; N, 20.65. Yield: 65%, Mp: 293-295°C.

(*3E*)-3, 3'-([1,1'-biphenyl]-4,4'-diylbis (43ydrazine-2-yl-1-ylidene)) bis (2,4-dioxo-1,2,3,4-tetrahydroquinoline-6-sulfonic acid) (12)

Dark red crystals. IR (KBr): 3410 (OH), 3216 (NH), 3012 (arom-H), 1683 (C = O). ^{1}H-NMR (600 MHz, DMSO): 15.23–14.21 (br, 1H, hydrazone, NH), 13.06 (s, 1H, SO_3H), 10.22 (br, 1H, amide, NH), 7.82–7.89 (dd, 4H, *J* = 7.8 Hz, Ar–H), 7.50–7.53 (dd, 4H, *J* = 7.8 Hz, Ar–H), 7.32-7.56 (m, 8H, Ar–H). Anal. Calcd for $C_{30}H_{20}N_6O_{10}S_2$ (688.07): C, 52.32; H, 2.93; N, 12.20; O, 23.23; S, 9.31. Found: C, 52.26; H, 2.87; N, 12.05. Yield: 60%, Mp: 250–252 °C.

RESULTS AND DISCUSSION

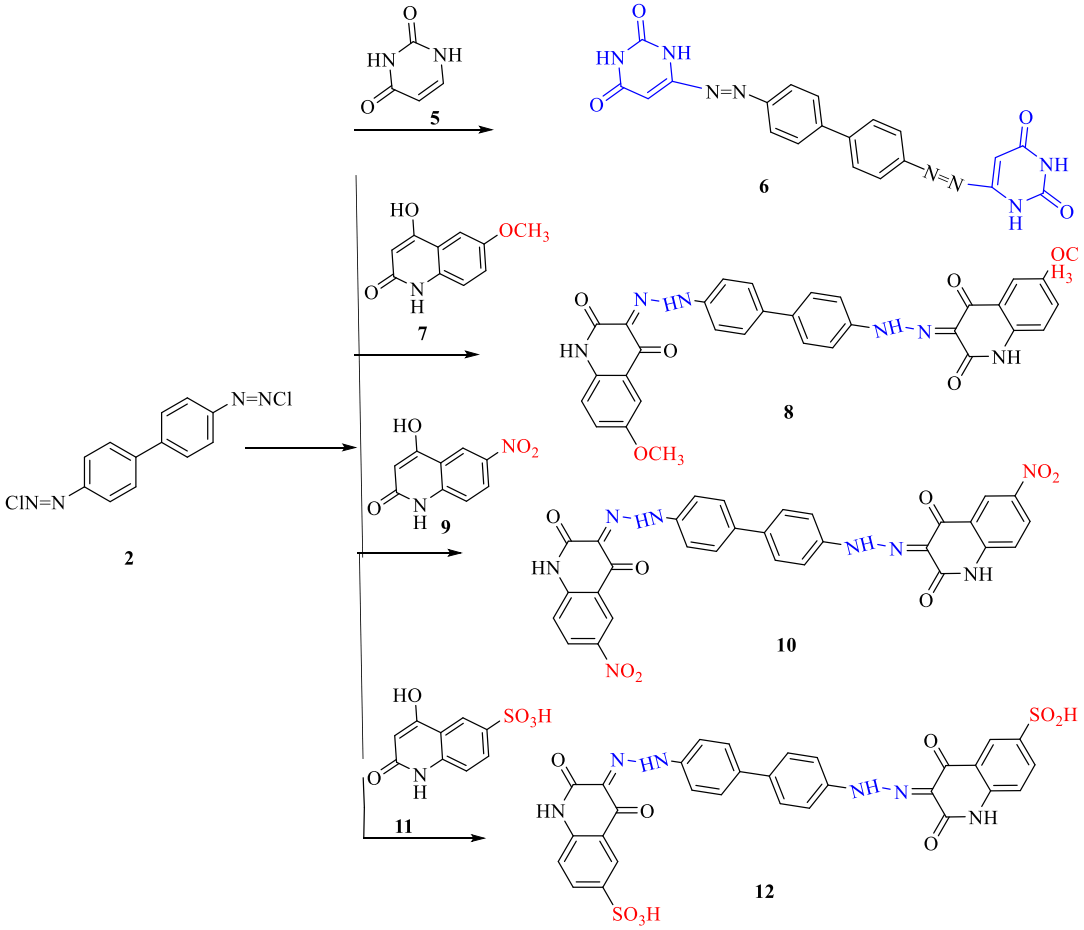
Diazotization of benzidine (**1**) was carried out by reaction with sodium nitrite in an ice bath containing methanol at 0-5 ^{o}C to form diazo compound (**2**) [xi], as shown in scheme 1. 2, 2'-((*1E, 1'E*)-[1, 1'-biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) dimalononitrile **3** was synthesized by coupling diazonium salt **2** with malononitrile in the presence of 10% sodium hydroxide as shown in scheme 1. The IR spectrum of compound (**3**), showed the disappearance of the stretching band of the (NH_2) group at (3356-3502) cm^{-1} and the appearance of stretching bands of the (CN) group at 2106 cm^{-1} and the (C-H aliph) group at 2869-2976 cm^{-1}. The ^{1}H-NMR spectrum of compound (**3**) shows the following characteristic chemical shifts: protons of (CH) groups at δ 3.35 ppm and protons of aromatic rings appeared in the range of δ 7.36-7.69 ppm.



Scheme 1. Synthesis of compound **4**

5, 5'-(*1E, 1'E*)-[1, 1'-biphenyl]-4, 4'-diylbis (diazene-2, 1-diyl)) bis (4, 6-diaminopyrimidine-2(5*H*)-thione **4** was prepared from the reaction of compound **3** with thiourea in absolute ethanol as shown in scheme 1. Compound **4** was confirmed by the IR spectrum through the disappearance of absorption bands of the (CN) group at (2106) cm^{-1} and the (C-H aliphatic) group at (2869-2976) cm^{-1} , and the appearance of an asymmetry band of the (NH_2) group at (3325) cm^{-1}, (C=N) group at (1612-1631) cm^{-1}, (C=S) group at (1329) cm^{-1}. ^{1}H-NMR spectrum of compound **4** shows the characteristic shifts at δ 13.03 ppm due to proton of the (SH) and at δ 9.87 ppm due to protons of (NH_2).

Diazonium salt of benzildine **2** coupled with uracil in the presence of sodium hydroxide at 0-5 ^oC to give azo dye **6**. The diazonium salt of benzildine **2** coupled with various substitutes such as 4-hydroxy-6-methoxyquinolin-2(*1H*)-one (**7**), 4-hydroxy-6-nitroquinolin-2 (*1H*)-one (**9**) and 1, 2-dihydro-4-hydroxy-2-oxoquinoline-6-sulfonic acid **(11)** [xii] in an alkaline medium of potassium hydroxide (10%) to synthesize azo dye compounds. (*3E*)-3, 3'-([1, 1'-biphenyl]-4, 4'-diylbis (44ydrazine-2-yl-1-ylidene)) bis (6-methoxyquinoline-2,4 (*1H,3H*)-dione) **8,** (*3E*)-3,3'-([1,1'-biphenyl]-4,4'-diylbis (44ydrazine-2-yl-1-ylidene)) bis (6-nitroquinoline-2,4 (*1H,3H*)-dione) **10** and (*3E*)-3,3'-([1,1'-biphenyl]-4,4'-diylbis (44ydrazine-2-yl-1-ylidene)) bis (2,4-dioxo-1,2,3,4-tetrahydroquinoline-6-sulfonic acid) **12** respectively, scheme 2. The IR spectrum of all dyes in KBr showed a broad band at 3500-3275 cm^{-1} which confirms the presence of a hydroxyl group (OH) and another band at 1418-1438 cm^{-1} due to the (N=N) group.



Scheme 2. Synthesis of compounds **6-12**

Antibacterial potential

In this research, the antibacterial study was determined according to the disc diffusion method. The antimicrobial activity of compounds (4, 6, 8, 10 and 12) against gram-negative bacteria

(*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive bacteria (*staphylococcus aurous* and *Bacillus cereus*) was tested in vitro. Prepared agar and Petri dishes were sterilized by autoclaving for 15 min. at 121 ^{o}C. The agar plates were surface inoculated uniformly with the broth culture of the tested microorganisms. In the solidified medium, appropriately spaced apart holes of 6 mm diameter were made, filled with 0.1 ml of the synthesized compounds (20 mg of the compound dissolved in 1mL of DMSO), DMSO was used as a solvent, and Gentamicin was used as standard antibacterial agent. For bacteria, these plates were incubated at 37 ^{o}C for 24 hours. The inhibition zones (IZ) caused by the prepared compounds were examined. The results of the preliminary screening tests are listed in (table- 1) and figure 1.

Table (1). Screening of the antibacterial activity of synthesized compounds

Compounds	**Gram-positive bacteria**		**Gram-negative bacteria**	
	S.aureus	*B.cereus*	*E.coli*	*P.aeruginosa*
	IZ	IZ	IZ	IZ
4	20	21	17	18
6	19	18	18	17
8	15	21	13	16
10	23	16	24	20
12	14	15	13	19
Gentamicin	26	25	23	25

The results are listed in Table 1 which revealed that all the synthesized compounds exhibited antibacterial potential with inhibition zone diameter ranged from 13 to 25 mm towards all tested bacterial strains. Concerning antimicrobial potential against *S.aureus*, the compound **10** (IZ = 23 mm) and compound **4** (IZ=20 mm) recorded comparable antimicrobial potential to that recorded by gentamicin (IZ=26 mm). Moreover, compound **8** (IZ=21 mm) displayed higher antimicrobial potential towards *B.cereus* than compound **10** and (IZ=16 mm), and also, compound **4** (IZ=21 mm) recorded higher antibacterial effect. For *E.coli* bacteria, compounds' activity was arranged in the following order **10** > **6** > **4**> **8, 12.** For *P.aeruginosa* compound **10** has displayed higher antimicrobial potential.

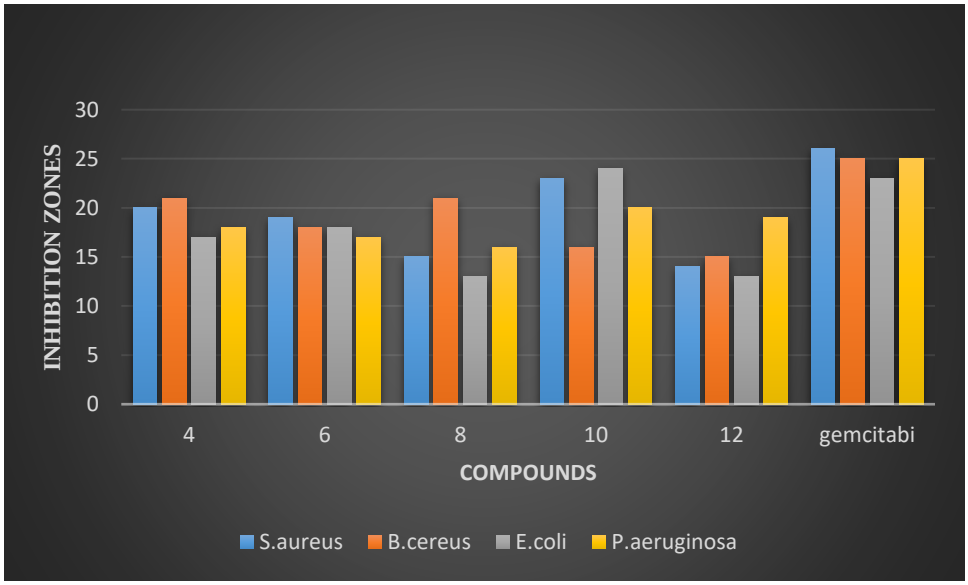
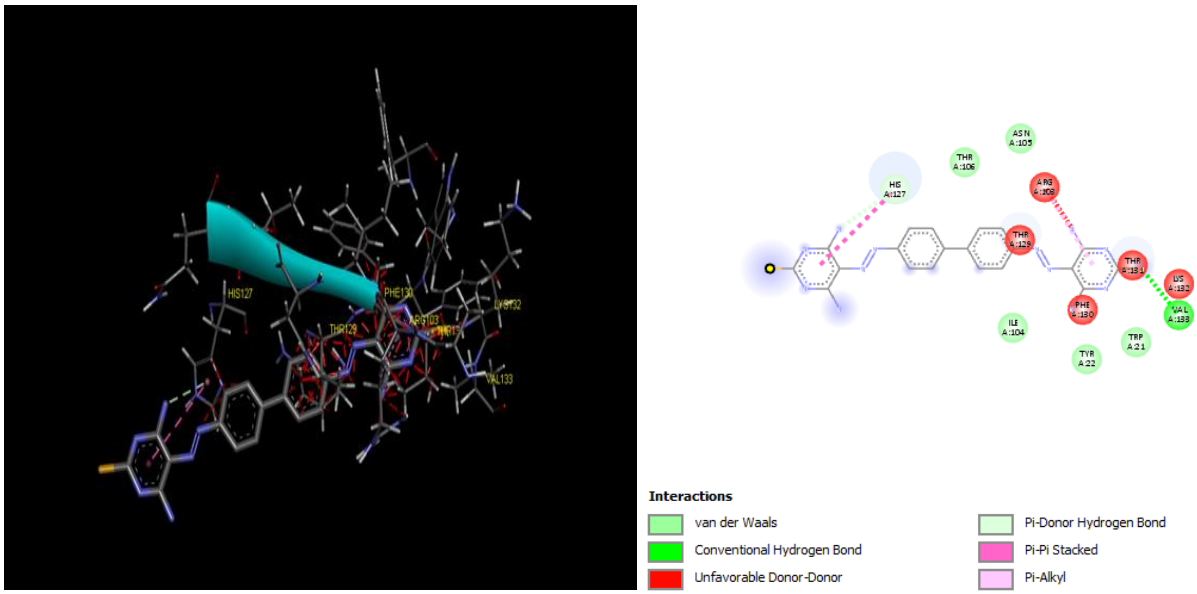


Figure (1).
Auto docking

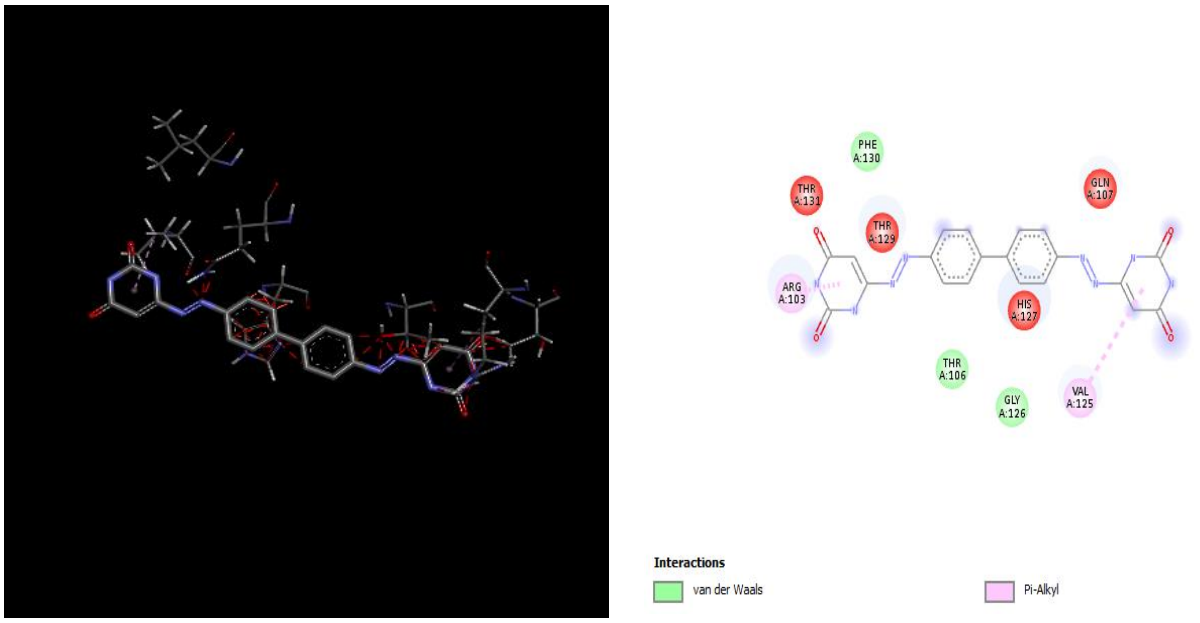
Molecular docking (MD) or docking studies are the computational strategies to understand the nature of synthesized compounds inside protein receptors and to distinguish the way by which they induced fit within the pocket.

Figure **1** indicated that one conventional hydrogen bond was involved in interacting the ligand to protein. This bond appeared with VAL A: 2.12 Å. To make this bond stronger, one pi-alkyl linkage appeared between one ligand ring phenyl and one amino acid ARG A: 103 having bond measurement of 5.16 Å.



A **B**

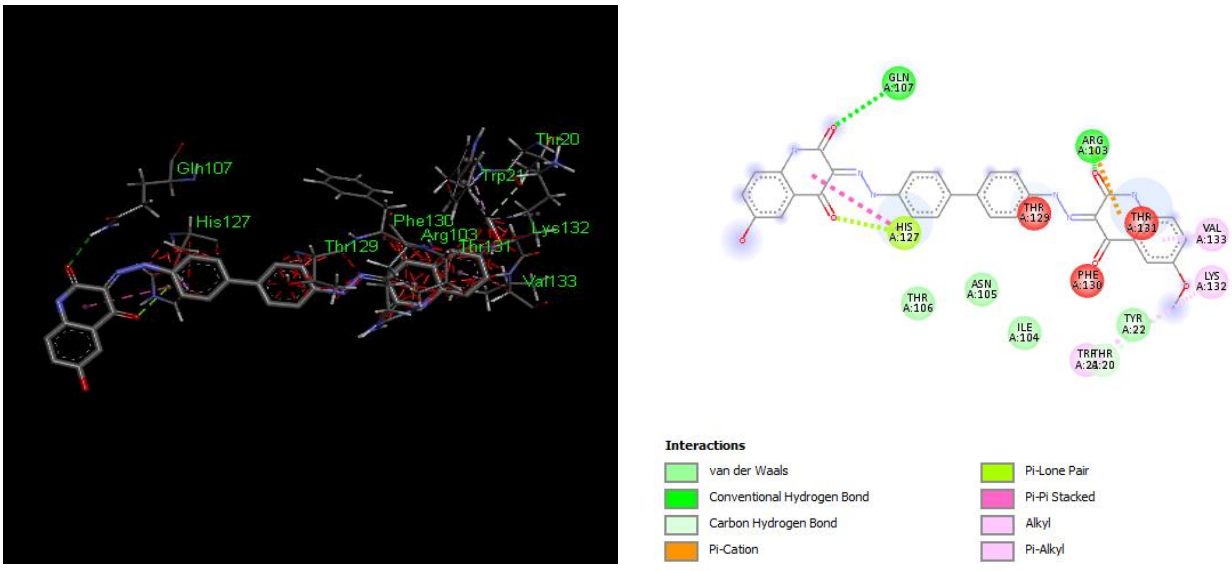
Figure 1: A. Three dimensional image of compound **4** and specific amino acids with bond distances **B**. Two dimensional picture inside binding pocket



A **B**

Figure 2: A. Three dimensional image of compound **6 B**. Two dimensional picture inside binding pocket

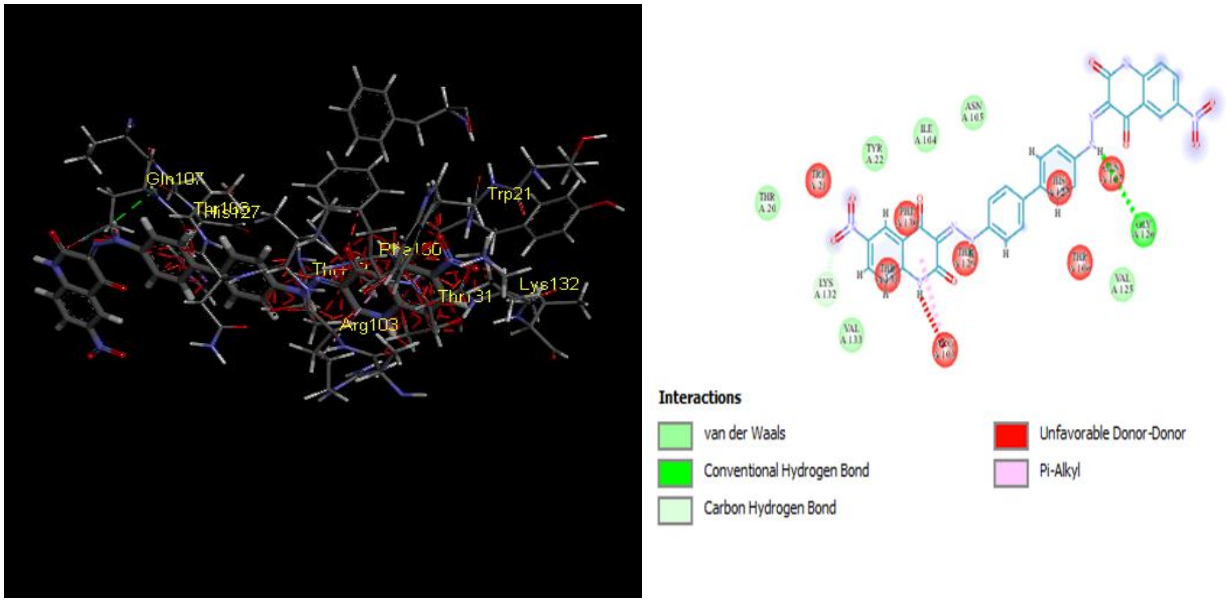
Figure **3** indicated that two conventional hydrogen bonds were involved in interacting the ligand to protein. These bonds appeared with GLN A: 107 and ARG A: 103 having bond lengths of 2.73 Å and 1.78 Å respectively. To make this bond stronger, one pi-cation linkage appeared between one ligand rings phenyl and one amino acid ARG A: 103 having bond measurement of 2.98 Å and another important building residue that formed pi-alkyl at TRP A: 22 linkage with bond length of 5.06 Å and also found pi-lone pair appeared at HIT A: 107 having bond length of 2.92 Å.



A **B**

Figure 3: A. Three dimensional image of compound **8** and specific amino acids with bond distances **B**. Two dimensional picture inside binding pocket

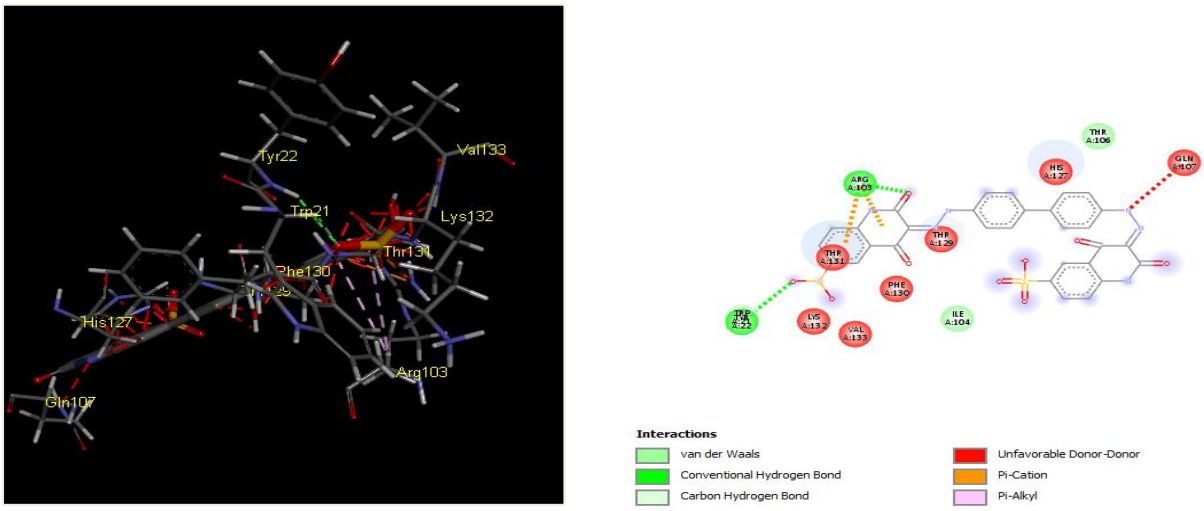
Figure **4** indicated that one conventional hydrogen bond was involved in interacting the ligand to protein. This bond appeared with Gly A: 126 having bond length of 3.07, and another important building residue that formed pi-alkyl at ARG A: 103 having bond measurement of 4.86 Å.



A **B**

Figure 4: A. Three dimensional image of compound **10** and specific amino acids with bond distances **B**. Two dimensional picture inside binding pocket

Figure **5** indicated that three conventional hydrogen bonds were involved in interacting the ligand to protein. These bonds appeared with TRY A: 22, TRP A: 21 and ARG A: 103 having bond lengths of 2.35 Å, 2.44 Å and 4.89 Å respectively. To make this bond stronger, two pi-cation linkage appeared between two ligand rings phenyl, heterocyclic and one amino acid ARG A: 109 having bond measurement of 4.89 Å and 5.15 Å. ARG A: 103 was another important building residue that formed pi-alkyl linkage with bond length of 4.26 Å.



A **B**

Figure 5: A. Three dimensional image of compound **12** and specific amino acids with bond distances **B**. Two dimensional picture inside binding pocket

Conclusion

This research is very efficient in preparation of a novel class of Azo-dyes derivatives which were established by IR, ^{1}H-NMR spectra. Most synthesized compounds were found exhibited significant activity as antibacterial agents.

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