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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES, AND *IN SILICO* STUDY OF NEW AZO DISPERSE DYES CONTAINING PYRAZOLE AND PYRAZOLO[1,5-*A*]PYRIMIDINE RINGS

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ABSTRACT. New *N,N*-dimethyl-*N'*-(5-oxo-4-(aryldiazenyl)-2,5-dihydro-1*H*-pyrazol-3-yl)formimidamide **5a-c** are formed by reaction of 5-amino-1*H*-pyrazol-3(2*H*)-one derivatives **4a-c** with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA). Compounds **5a-c** serve as excellent precursors for the synthesis of new disazo pyrazole disperse dyes, When refluxed with hydrazine hydrate or active methylene reagents **8a,b**, leading to *N*"-(3-hydroxy-4-(arylaza)-1H-pyrazol-5-yl)formimidohydrazide **7a-c** or pyrazolo[1,5-*a*]. pyrimidines **11a**–**e**. *In vitro* studies were carried out to evaluate antioxidant properties of the produced compounds in comparison to standard, BHT. The results indicated that all the compounds exhibited varying levels of antioxidant activity at different doses. Compound (**7b**) exhibited an IC50 value comparable to that of BHT, while the other compounds demonstrated varying levels of activity and IC50 values in comparison to BHT. Regarding antimicrobial activity, compounds **7a**, **7b** and **11c** exhibited antibacterial efficiency against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* with inhibition zone diameters of 14.33, 12.33, and 12.33 mm, respectively. *In silico* study of eleven pyrazole compounds with *S. aureus* 2XCT demonstrated strong hydrogen bonds, short distance interactions, and the highest binding energies were displayed by compounds **5b** and **11a** with binding energy of -6.6 and -6.2 kcal/mol, respectively.

KEY WORDS: Pyrazoles, DMFDMA, Antimicrobial, Antioxidant, Molecular docking, Disperse dyes, Pyrazolo[1,5-a]pyrimidines

INTRODUCTION

Pyrazoles, five-membered heterocycles, are highly valuable in organic synthesis. They are among the most extensively studied compounds within the azole family. Over the years, numerous synthesis methods and synthetic analogues have been developed and documented. The incorporation of the pyrazole nucleus into various structures results in a wide range of applications across technology, medicine, and agriculture. Specifically, medical researchers are interested in the pyrazole structure because of its diverse variety of pharmacological effects; as antitubercular [1, 2], analgesic [3], anti-inflammatory [4, 5], antimicrobial [6-9], covid-19 main protease and papain-like protease inhibitors [10], antiviral [11,12], antidiabetic [13,14], carbonic anhydrase inhibitors [15] and glucosidase inhibitory activity [16]. Additionally, a variety of FDA-approved drugs contain the pyrazole pharmacophore, which is widely used in the pharmaceutical industry such as rimonabant [17], phenylbutazone [18], COX-II inhibitors [19], and anticancer drugs [20], anaesthetic effects, antibacterial, and fungicidal properties [21, 22], and against the Cotton Leafworm [23]. Pyrazolo [1,5–a]pyrimidines are analogues of purines with a wide range of biological applications, including inhibitors of acetylcholinesterase and amylase [24], sedative, antitrypanosomal and antimalarial agents [25], and selective inhibitors of COX-1 and COX-2

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[26]. Additionally, they are utilized in the therapy of a number of illnesses, including antifungal and antimalarial [27], antimicrobial [28], HIV reverse transcriptase [29], HCV inhibitors [30] and as KDR kinase inhibitors [31]. Commercially accessible medications containing pyrazolo[1,5-a]pyrimidine nucleus are included in [32-34].

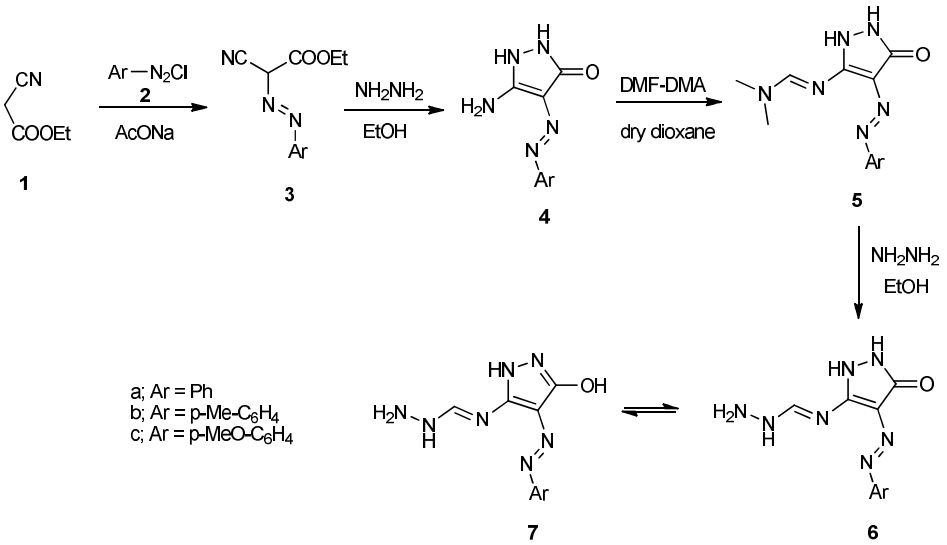
Evolution of chemical analytical techniques and computational tools has enabled the manufacture of antimicrobial compounds in drug discovery research [35] where structure-based drug design represents a crucial role [36]. Molecular docking provides a treasured tool for optimization and identification of drug candidates [37]. Molecular docking simulation is carried out to inspect the compounds' reliability of in protein-binding sites [37]. Integration of computational techniques enables rapid and cost-effective drug progress and suggesting new promises for diseases treating [38].

RESULTS AND DISCUSSION

Chemistry

The starting materials 5-amino-4-(aryldiazenyl)-1*H*-pyrazol-3(2*H*)-one **4a–c**, are shown in (Scheme 1) and were synthesized in accordance with previously published procedures [43].

Azo-aminopyrazole derivatives **4a-c** reacted with DMF-DMA (1:1) in refluxing dioxane to afford new imines **5a-c** (Scheme 1). Compound **5c** was selected as a sample example of the series produced to illustrate how the chemical structures of new compounds were confirmed *via* spectral data. The amino group band's disappearance from its IR spectrum and appearance of new signals at δ_H 3.09, 8.31, 10.54 and 11.04 ppm corresponding to $N(CH_3)_2$, (imine-H) and (exchangeable-2NH), respectively in its ^{1}H NMR spectrum, in an addition of signals at δ_C 41.1, 55.9, 115.4, 117.3, 125.5, 148.8, 153.1, 156.8, 157.3, and 163.5 in its ^{13}C NMR spectrum proved its structure.

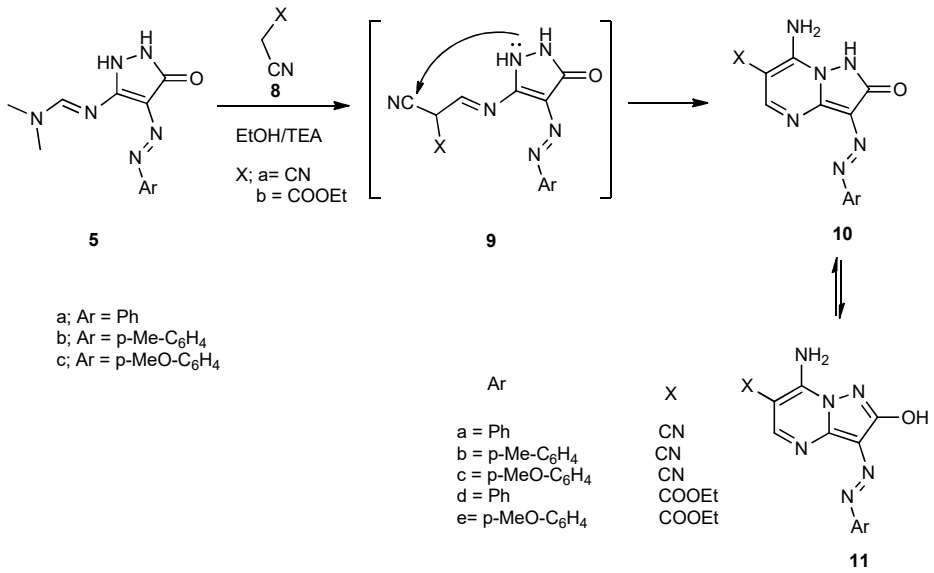


Scheme 1. Synthesis of imines **4a-c** - **7a-c**.

Imines **5a-c** are excellent starting materials to synthesize novel heterocyclic compounds through their reactions with nucleophilic reagents. Imines **5a-c** were allowed to react with hydrazine hydrate in refluxing ethanol to produce *N*"-(3-hydroxy-1*H*-pyrazol-5-

yl)formimidohydrazide **7a-c** (Scheme 1). IR spectrum of compound **7b** showed bands at 3396 and 3310 corresponding to hydroxyl and amino groups, while, its ^{1}H NMR spectrum indicated signals at δ_H 5.77, 8.67, 9.08, 10.5 and 11.08 corresponding to (exchangeable-NH_2), (exchangeable-NH), (CH), (exchangeable-OH), and (exchangeable NH-pyrazole), respectively, While, its signals at δ_C 20.8, 120.7, 121.6, 128.5, 138.3, 146.0, 146.4, 148.6, and 160.0 were observed in the ^{13}C NMR spectrum.

Finally, imines **5a–c** were reacted with active methylene compounds (malononitrile or ethyl cyanoacetate) **8a,b** in presence of triethylamine in ethanol to form new azo pyrazo[1,5–a]pyrimidines **11a–e** (scheme 2). IR spectrum of compound **11c** showed absorption bands at 3405, 3312, and 2221 cm^{-1} corresponding to hydroxyl, amino, and cyano groups, respectively. Its ^{1}H NMR spectrum, appeared signals at δ_H 3.6, 8.49 and 8.93 corresponding to (exchangeable-NH_2), (pyrimidine-H) and (exchangeable-OH). Additionally, signals were detected in its ^{13}C NMR spectrum at δ_C 56.0, 78.0, 115.0, 115.4, 116.0, 123.4, 144.7, 146.0, 149.1, 154.5, 160.5, and 161.1.



Scheme 2. Synthesis of azo pyrazolo[1,5-*a*]pyrimidines **5a-c , 10a-e , 11a-e**.

Antimicrobial activity

The antibacterial activity of test samples and reference standards (positive controls) was evaluated in vitro against a range of Gram-positive and Gram-negative bacterial strains, while *C. albicans* was subjected to antifungal assay. According to the results (Table, 1), compounds **7a,b** and (**11c**) showed antibacterial efficiency against *S. aureus*, *B. subtilis*, and *P. aeruginosa* with IZ diameters of 12.33, 12.33, and 14.33 mm, respectively. In general, the investigated compounds presented antibacterial activity showed fluctuated results in comparison to positive control. *C. albicans*, *K. pneumonia*, and *E. coli* had no susceptibility to any of the investigated compounds.

Antioxidant activity

Using the DPPH assay, the compounds' *in vitro* antioxidant properties were assessed by comparing them to the standard BHT (Table 2). All tested compounds exhibited varied antioxidant capacities at different concentrations as compared to standard. The IC_{50} of compound (**7b**) showed homogeneity with BHT, whereas other examined compounds exhibited varying levels of activity and IC_{50} in relation to BHT. Compounds **11b** and **11c** were not detected as their results were not applicable (Figure 1).

Table 1. Antimicrobial activity of the tested samples (5% w/v) against tested bacteria and *Candida* expressed using the well diffusion method as inhibitory diameter zones in mm.

Sample No.	*B. subtilis*	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*C. albicans*
5a	ND	ND	ND	ND	ND	ND
5b	ND	ND	ND	ND	ND	ND
5c	ND	ND	ND	ND	ND	ND
7a	12.33±0.58^{b}	ND	ND	ND	ND	ND
7b	ND	ND	ND	ND	14.33±0.58^{a}	ND
7c	ND	ND	ND	ND	ND	ND
11a	ND	ND	ND	ND	ND	ND
11b	ND	ND	ND	ND	ND	ND
11c	ND	12.33±0.58^{b}	ND	ND	ND	ND
11d	ND	ND	ND	ND	ND	ND
11e	ND	ND	ND	ND	ND	ND
Chloramphenicol	16.33±0.58^{a}	15.67±0.58^{a}	14.33±0.58	14.67±0.58	14.67±0.58^{a}	-
Clotrimazole	-	-	-	-	-	18.67±0.58

The means of the data were displayed in triplicate (Mean±SD). The values represented by the different letters differ significantly at $p < 0.05$. - : Not tested; ND: Not detected.

Table 2. Antioxidant capacity and IC_{50} value of the tested samples using DPPH assay.

Compound	Antioxidant capacity (%)					IC_{50} value (mg extract/mL DPPH radicals)
	Conc. (mg/mL)					
	1	0.7	0.5	0.3	0.1	
5a	27.38±0.18	25.32±0.28	20.18±1.34	15.09±1.01	3.29±1.55	1.75
5b	36.27±0.59	29.94±0.89	25.21±0.45	22.21±0.34	3.41±0.53	1.33
5c	46.79±1.03	41.66±0.31	25.46±1.11	16.97±1.02	4.18±0.89	0.99
7a	63.57±0.32	59.73±0.05	51.20±0.23	45.31±0.96	41.40±0.68	0.44
7b	71.88±0.18	70.15±0.31	67.29±0.37	63.06±0.05	53.88±1.76	0.28
7c	64.25±1.12	58.87±0.77	52.25±1.16	47.23±0.34	35.44±0.51	0.47
11a	63.24±1.32	61.51±0.15	59.99±0.41	51.67±2.85	26.33±1.07	0.45
11b	ND	ND	ND	ND	ND	ND
11c	ND	ND	ND	ND	ND	ND
11d	19.60±0.18	17.29±0.65	13.71±0.49	11.25±0.72	6.63±0.72	3.06
11e	61.04±0.18	57.93±0.09	50.23±1.16	44.66±1.11	40.39±0.22	0.49
BHT	74.56±0.27	72.97±0.34	61.58±0.95	50.33±0.76	43.61±1.07	0.24

ND: not detected. Data were presented as means of triplicates (Mean±SD).

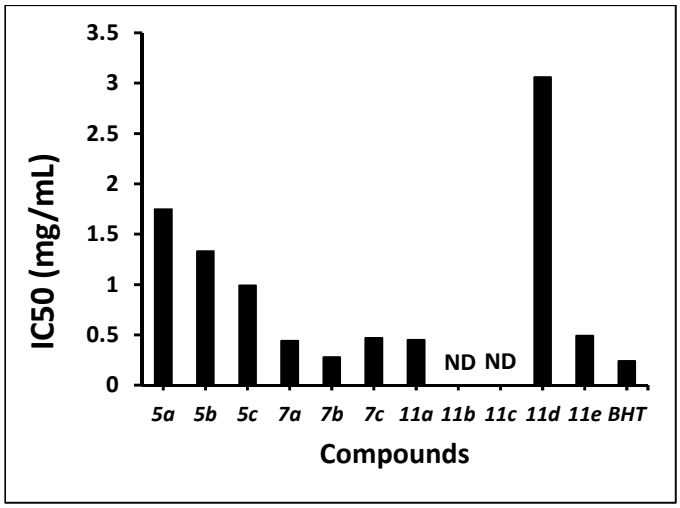


Figure 1. Antioxidant IC_{50} values (mg/mL) of tested compounds.

Molecular docking simulation

The results of molecular docking simulations for 11 chemical compounds, testing their potential as antibacterial agents by interacting with the PDB structure of 2XCT was demonstrated. Strong hydrogen bonds, short distance interactions, and the highest binding energies were displayed by compounds **5b**, **11a**, and **11d**, while moderate binding energies and interactions were exhibited by compounds **7b**, **11c**, and **7c**. On the other hand, due to their weaker interactions and higher RMSD values, compounds **5a**, **7a**, and **11e** were less desirable.

Table 3. Docking pose score results of compounds interactions with targeted *S. aureus* topoisomerase IIA (2XRT).

Compound	S	RMSD (Å)	Ligand	Receptor	Interaction	Distance	Score (kcal/mol)
5a	-4.5296679	1.4984283	5-ring	CA LYS 581	pi-H	4.50	-1.0
5b	-5.0926318	1.7455839	N 12	NH2 ARG 1033	H-acceptor	2.75	-6.6
5c	-4.8278594	1.673272	O 8 N 9 C 31	NH1 ARG 1048 NH2 ARG 1033 5-ring HIS 1081	H-acceptor H-acceptor H-pi	2.65 2.79 3.74	-3.1 -1.3 -0.7
7a	-4.3773732	1.1774772	N 17	OD2 ASP 512	H-donor	3.40	-0.8
7b	-4.5751324	1.5075153	5-ring	CA ASP 1151	pi-H	4.57	-0.6
7c	-4.6434951	1.3758744	N 17	OD2 ASP 510	H-donor	3.24	-2.6
11a	-4.7583709	1.3688647	N 30	NH2 ARG 1048	H-acceptor	3.06	-6.2
11b	-4.7731805	1.677272	O 6	OD2 ASP 510	H-donor	2.94	-2.5
11c	-4.8030953	1.3484924	N 17 6-ring	OD2 ASP 512 CA HIS 1081	H-donor pi-H	3.39 4.37	-2.3 -0.7
11d	-5.525208	1.4983406	O 6 6-ring	OD2 ASP 510 NE2 HIS 1081	H-donor pi-H	2.97 3.63	-0.9 -1.6
11e	-4.9943485	1.8016832	6-ring	CB PRO 1080	pi-H	3.61	-0.6

Compound **5b** provided the strongest binding energy of -6.6 kcal/mol, thus indicated a potentially high affinity for the receptor 2XCT, making it a promising candidate for antibacterial activity. In addition, compound **11a** exhibited strong binding energy of -6.2 kcal/mol. In contrast, compound **7b** had a binding energy of -0.6 kcal/mol, indicating weaker binding, meanwhile compound **7a** displayed a binding energy of -0.8 kcal/mol, indicating weaker interaction with the receptor (Table 3).

Regarding types of interactions, several compounds formed hydrogen bonds with amino acid residues like ARG (e.g., **5b**, **5c**, and **11a**), ASP (e.g., **7a** and **11b**), and HIS (e.g., **11c** and **11d**). These interactions were typically strong and contributed significantly to the stability of the ligand-receptor complex. Many compounds (e.g., **5a**, **7b**, and **7c**) engaged in pi-H interactions, which could be crucial for the stability of the complex, especially in aromatic ring-based compounds. The distances between ligand atoms and receptor atoms ranged from 2.65 to 4.57 Å. Shorter distances (closer to 2.65 Å) generally indicated stronger interactions, with higher potential for binding. For instance, **5c** had interactions with ARG 1048 at a distance of 2.65 Å, suggesting a strong interaction (Figures 2-5). With their substantial binding energies and stable positions, compounds **5b** and **11d** are the best overall and are therefore excellent candidates for additional further research.

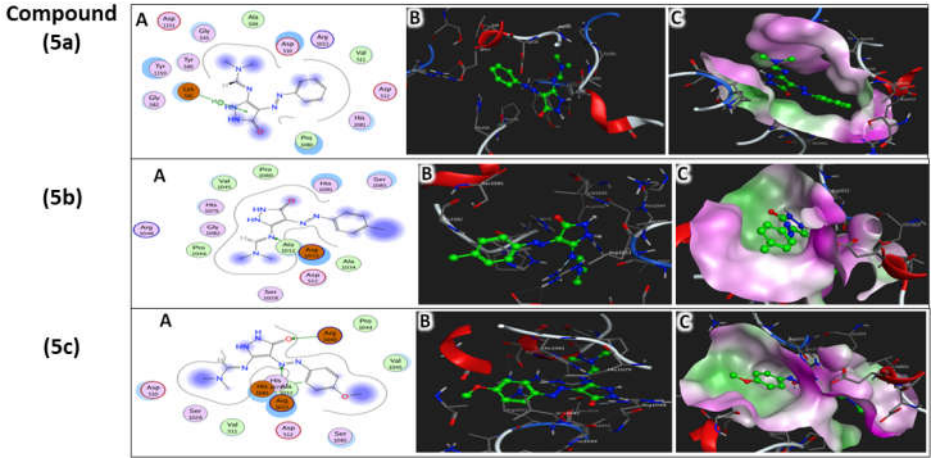


Figure 2. Two-D (A) and three-D (B and C) interactions diagrams of compounds (**5a**, **5b**, and **5c**) with 2XRT active site.

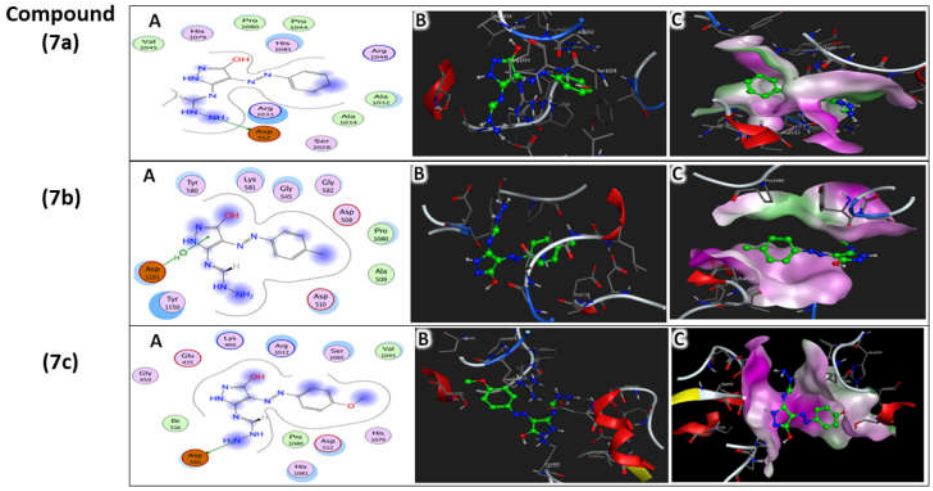


Figure 3. Two-D (A) and three-D (B and C) interactions diagrams of compounds (**7a**, **7b**, and **7c**) with 2XRT active site.

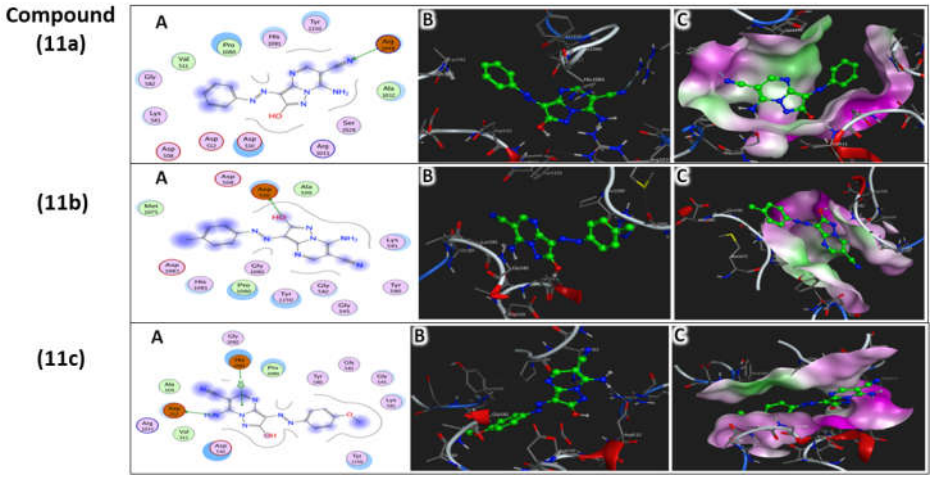


Figure 4. Two-D (A) and three-D (B and C) interactions diagrams of compounds (**11a**, **11b**, and **11c**) with 2XRT active site.

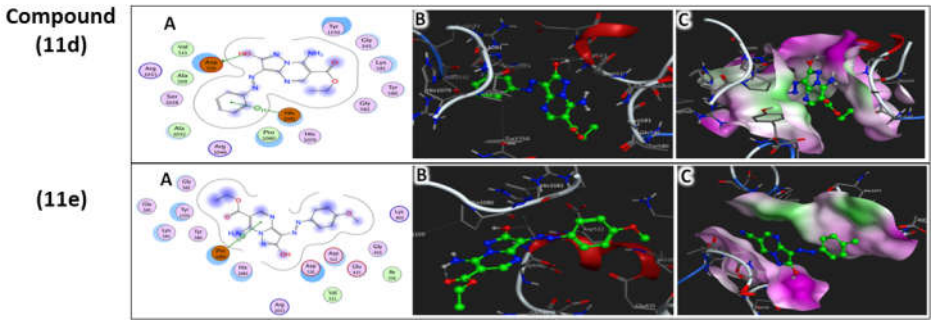


Figure 5. Two-D (A) and three-D (B and C) interactions diagrams of compounds (**11d** and **11e**) with 2XRT active site.

EXPERIMENTAL

All melting points were measured on Kofler melting points equipment and are uncorrected. The IR spectra was obtained using a Nicolet 710 FT-IR spectrometer. On a Bruker A Vance III-400 MHz device, ^{1}H NMR spectra are recorded at 400 MHz using DMSO-d_6 as a solvent, while ^{13}C NMR spectra are recorded at 100 MHz. Elemental microanalysis was performed using a Perkin-Elmer CHN-2400 elemental analyzer.

*General method for synthesis of imines (**5a-c**)*

A mixture of azo-aminopyrazole **4a-c** (0.01 mol) and DMF-DMA (1.32 mL, 0.01 mol) in dry dioxane (30 mL) was refluxed for 3 hours. The resultant solid was filtered off and recrystallized from ethanol.

N,N-Dimethyl-N′-[5-oxo-4-(-phenyldiazenyl)-2,5-dihydro-1H-pyrazol-3-yl]formimidamide ***5a***. Brown crystals; yield 77.5%; m.p. 162-164 °C; IR (υ, cm^{-1}): 3440, 3196 (2NH) and 1696 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 3.11 (s, 6H, N(CH$_3$)$_2$, 7.16-7.45 (m, 5H, aromatic-H), 8.34 (2s, 1H, imine-H), 10.62 and 11.12 (2s, 2H, exchangeable-2NH); ^{13}C NMR: 40.6, 116.2, 123.7, 129.0, 129.1, 145.0, 149.6, 157.0 and 159.2. Anal. calc. for $C_{12}H_{14}N_6O$: C, 55.80; H, 5.46; N, 32.54. Found: C, 55.62; H, 5.34; N, 32.47.

N,N-Dimethyl-N′-5-oxo-4-(-p-tolyldiazenyl)-2,5-dihydro-1H-pyrazol-3-yl formimidamide ***5b***. Deep brown crystals; 81 % yield; m.p. 196-198 °C; IR (υ, cm^{-1}): 3450, 3185 (2NH) and 1688(C=O); ^{1}H NMR (DMSO-d_6, ppm): 2.3 (s, 3H, CH$_3$), 2.98, 3.08 [2s, 6H, N(CH$_3$)$_2$], 7.22, 7.34 (dd, 4H, aromatic-H), 8.39 (2s, 1H, imine-H), 10.58, 11.07 (2s, 2H, exchangeable-2NH); ^{13}C NMR: 34.3, 41.4, 115.5, 116.4, 124.4, 148.3, 155.4, 156.4, 160.2 and 163.5. Anal. calc. for $C_{13}H_{16}N_6O$: C, 57.34; H, 5.92; N, 30.86. Found: C, 57.18; H, 5.72; N, 30.67.

N′-4-(-p-Methoxyphenyl)diazenyl)-5-oxo-2,5-dihydro-1H-pyrazol-3-yl-N,N-dimethylformimidamide ***5c***. Deep brown crystals; 85.7% yield; m.p. 182-184°C; IR (υ, cm^{-1}): 3338, 3183 (2NH) and 1669 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 2.98, 3.09 [2s, 6H, N(CH$_3$)$_2$], 3.78 (s, 3H, OCH$_3$), 7.26, 7.42 (dd, 4H, aromatic-H), 8.31(2s, 1H, imine-H), 10.54 and 11.04 (2s, 2H, exchangeable-2NH); ^{13}C NMR: 41.1, 55.9, 115.4, 117.3, 125.5, 148.8, 153.1, 156.8, 157.3, and 163.5. Anal. calc. for $C_{13}H_{16}N_6O_2$: C, 54.16; H, 5.59; N, 29.15. Found: C, 53.95; H, 5.39; N, 28.94.

*General method for synthesis of compounds **7a-c***

A mixture of imines **5a–c** (0.01 mol) and hydrazine hydrate (0.015 mol) in ethanol was refluxed for 4 hours. The resultant solid was filtered off and recrystallized from ethanol.

*N''-(3-Hydroxy-4-(-phenyldiazenyl)-1H-pyrazol-5-yl)formimidohydrazide **7a***. Brownish yellow crystals; 73.4% yield; m.p. 215-217 °C; IR (υ, cm^{-1}): 3404 (OH), 3334 (NH_2), 3205 and 3151 (2NH); ^{1}H NMR (DMSO-d_6, ppm): 5.86 (d, 2H, exchangeable-NH_2), 7.19-7.68 (m, 5H, aromatic-H), 8.7(d, 1H, exchangeable-NH), 9.13(d, 1H, imine-H), 10.56(s, 1H, exchangeable-OH), 11.14(s, 1H, NH); ^{13}C NMR: 120.8, 122.2, 128.9, 129.0, 146.4, 148.7, 161.4. Anal. calc. for $C_{10}H_{11}N_7O$: C, 48.98; H, 4.52; N, 39.98. Found: C, 48.79; H, 4.46; N, 39.82.

*N''-(3-Hydroxy-4-(p-tolyldiazenyl)-1H-pyrazol-5-yl)formimidohydrazide **7b***. Orange crystals; 77.2% yield; m.p. 230-232 °C; IR (υ, cm^{-1}): 3396 (OH), 3310 (NH_2), 3246 and 3222 (2NH); ^{1}H NMR (DMSO-d_6, ppm): 2.33 (s, 1H, CH_3), 5.77 (d, 2H, exchangeable-NH_2), 7.26, 7.43(dd, 4H, aromatic-H), 8.67 (d, 1H, exchangeable-NH), 9.08 (d, 1H, imine-H), 10.5 (s, 1H, exchangeable-OH), 11.08 (s, 1H, NH); ^{13}C NMR: 20.8, 120.7, 121.6, 128.5, 138.3, 146.0, 146.4, 148.6 and 160.0. Anal. calc. for $C_{11}H_{13}N_7O$: C, 50.96; H, 5.05; N, 37.82. Found: C, 50.82; H, 5.14; N, 37.72.

*N''-(3-Hydroxy-4-(-(4-methoxyphenyl)diazenyl)-1H-pyrazol-5-yl)formimidohydrazide **7c***. Orange crystals; 76.4% yield; m.p. 200-202 °C; IR (υ, cm^{-1}): 3423 (OH), 3332 (NH_2), 3211 and 3178 (2NH); ^{1}H NMR (DMSO-d_6, ppm): 3.79 (s, 1H, OCH_3), 5.77 (d, 2H, exchangeable-NH_2), 7.03, 7.63(dd, 4H, aromatic-H), 8.61 (d, 1H, exchangeable-NH), 9.05 (d, 1H, imine-H), 10.49 (s, 1H, exchangeable-OH), 11.06 (s, 1H, NH); ^{13}C NMR: 55.4, 114.2, 121.0, 121.8, 146.0, 146.4, 147.8, 158.3 and 159.9. Anal. calc. for $C_{11}H_{13}N_7O_2$: C, 48.00; H, 4.76; N, 35.62. Found: C, 47.2; H, 4.65; N, 35.49.

*General method for synthesis of pyrazolo[1,5-a]pyrimidines **11a-e***

A mixture of imines **5a–c** (0.01 mol) and active methylene compounds **8a,b** (0.01 mol) in ethanol with a few drops of triethylamine was refluxed for 6 hours. The resultant solid was filtered off and recrystallized from DMF and ethanol mixture (1:3).

*7-Amino-2-hydroxy-3-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile **11a***. Brown crystals; 75.2% yield; m.p. 285-287 °C; IR (υ, cm^{-1}): 3382 (OH), 3324 (NH_2) and 2223 (C≡N); ^{1}H NMR (DMSO-d_6, ppm): 5.86 (s, 2H, exchangeable-NH_2), 7.11-7.84(m, 5H, aromatic-H), 8.53(s, 1H, pyrimidine-H) and 10.56 (s, 1H, exchangeable-OH); ^{13}C NMR: 108.6, 115.0, 121.6, 122.4, 129.2, 129.6, 145.6, 147.0, 150.2, 154.1 and 157.0. Anal. calc. for $C_{13}H_9N_7O$: C, 55.91; H, 3.25; N, 35.11. Found: C, 55.79; H, 3.09; N, 35.15.

*7-Amino-2-hydroxy-3-(p-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile **11b***. Red brown crystals; 78.5% yield; m.p. 298-300 °C; IR (υ, cm^{-1}): 3357 (OH), 3313 (NH_2) and 2225 (C≡N); ^{1}H NMR (DMSO-d_6, ppm): 2.38(s,3H, CH_3), 4.24 (br, 2H, exchangeable-NH_2), 7.33, 7.73(2d, 4H, aromatic-H), 8.51(s, 1H, pyrimidine-H) and 8.89 (br, 1H, exchangeable-OH); ^{13}C NMR: 21.1, 108.6, 115.0, 119.8, 121.7, 129.0, 138.9, 145.7, 146.8, 148.4, 154.0 and 157.4. Anal. calc. for $C_{14}H_{11}N_7O$: C, 57.33; H, 3.78; N, 33.43. Found: C, 57.24; H, 3.64; N, 33.12.

*7-Amino-3-(4-methoxyphenyl)diazenyl-2-oxo-1,2-dihydropyrazolo[1,5-a]pyrimidine-6-carbonitrile **11c***. Brown crystals; 77.6% yield; m.p. over 300 °C; IR (υ, cm^{-1}): 3405 (OH), 3312 (NH_2) and 2221 (C≡N); ^{1}H NMR (DMSO-d_6, ppm): δ 3.6(br, 2H, exchangeable-NH_2), 3.85 (s, 3H, OCH_3), 7.09, 7.85 (dd, 4H, aromatic-H), 8.49(s, 1H, pyrimidine-H) and 8.93 (br, 1H,

exchangeable-OH); ^{13}C NMR: 56.0, 78.0, 115.0, 115.4, 116.0, 123.4, 144.7, 146.0, 149.1, 154.5, 160.5 and 161.1. Anal. calc. for $C_{14}H_{11}N_7O_2$: C, 54.37; H, 3.58; N, 31.70. Found: C, 54.21; H, 3.42; N, 31.56.

Ethyl-7-amino-2-hydroxy-3-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate ***11d***. Brownish yellow crystals; 76.7% yield; m.p. 278-280 °C; IR (υ, cm^{-1}): 3399 (OH), 3324 (NH_2) and 1697 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 1.37 (t, 3H, CH_3), 4.39 (q, 2H, CH_2), 5.86(s, 2H, exchangeable-NH_2), 7.15-7.84(m, 5H, aromatic-H), 8.33(s, 1H, pyrimidine-H) and 10.62 (s, 1H, exchangeable-OH); ^{13}C NMR: 14.4, 59.6, 109.3, 122.6, 125.0, 129.1, 130.0, 145.9, 146.7, 149.5, 152.0, 157.5 and 164.3. Anal. clc. for $C_{15}H_{14}N_6O_3$: C, 55.21; H, 4.32; N, 25.75. Found: C, 55.12; H, 4.17; N, 25.59.

Ethyl-7-amino-2-hydroxy-3-(4-methoxyphenyl)diazenylpyrazolo[1,5-a]pyrimidine-6-carboxylate ***11e***. Red brown crystals; 73 % yield; m.p. 260-262 °C; IR (υ, cm^{-1}): 3403 (OH), 3328 (NH_2) and 1685 (C=O); ^{1}H NMR (DMSO-d_6, ppm): δ 1.37 (t, 3H, CH_3), 4.36 (q, 2H, CH_2), 5.77 (s, 2H, exchangeable-NH_2), 7.03, 7.52 (dd, 4H, aromatic), 8.32 (s, 1H, pyrimidine-H) and 11.03 (s, 1H, exchangeable-OH); ^{13}C NMR: 14.7, 55.5, 59.8, 109.3, 114.3, 123.3, 125.1, 144.4, 146.7, 150.2, 153.0, 154.8, 159.0 and 164.5. Anal. calc. for $C_{16}H_{16}N_6O_4$: C, 53.93; H, 4.53; N, 23.58. Found: C, 53.78; H, 4.39; N, 23.42.

Evaluating antimicrobial activities

Pathogenic strains of *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 43816, *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 6538, in addition to one fungal strain, *Candida albicans* ATCC 10231, were used previously by [40] as test microbes for biological activities assessment. Muller-Hinton and Sabouraud dextrose media, inoculated previously with cultures of the test strains, were used for assessing antibacterial and antifungal activities, respectively. Agar well diffusion method was performed as described by [41] with 8 mm diameter hole that is filled with 100 µL of 5% (w/v) investigated compounds dispersed by sonication in dimethyl sulfoxide. Chloramphenicol and clotrimazole, at concentrations of 1 mg/mL, were utilized as positive controls for bacteria and *C. albicans*, respectively. Positive bioactivity was indicated by measuring the inhibitory zone's diameter (IZ) in millimetres.

DPPH radical scavenging activity

The antioxidant capacity of test compounds was evaluated by DPPH radical scavenging assay [42]. 1.8 mL of 0.1 mM DPPH solution was added to 0.2 ml of the test sample at different concentrations (1, 0.7, 0.5, 0.3 and 0.1 mg/mL), After being left at room temperature for half an hour, the absorbance at 517 nm was measured using a spectrophotometer. Butylated hydroxytoluene (BHT) was utilized as the positive control, while negative control was represented by positive control. The following formula was used to determine the DPPH radical's scavenging capacity:

$$\%\ \text{DPPH radical scavenging} = \frac{\mathrm{A} - B}{\mathrm{A}} \times 100 \quad (1)$$

where A represents the absorbance of the negative control and B denotes the sample's absorbance. The regression equation (y = ax + b) was used to determine the IC_{50}, and the resulting estimate was $IC_{50} = (0.5 - b)/a$.

Statistical analysis

Every test was run three times; averages were established by analysis of variance (ANOVA) using SPSS, program version 16, and statistical analysis was conducted (at $p < 0.05$).

Molecular docking simulation

The docking analysis was performed by assessing the inhibition of S. aureus topoisomerase IIA (pdb code 2XRT) using Autodock package [43]. The docked ligands of S. aureus topoisomerase IIA was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb (accessed on 20 June 2024). The protein active target was selected and as the complex inhibitor ligand site. Further, 11 tested compounds were vindicated, and generation of a virtual ligand database was carried out. The docking scores were documented by a firm receptor–flexible ligand-docking method, while 2D and 3D interaction figures were constructed using MOE 2015.

CONCLUSION

Utility of azo aminopyrazole as precursors to synthesize new imines, N"-(3-hydroxy-1*H*-pyrazol-5-yl)formimidohydrazide and azo pyrazolo[1,5-a] pyrimidines by refluxed with (DMF-DMA), hydrazine hydrate and active methylene reagents. The antibacterial and antioxidant properties of recently synthesized substances are assessed. To identify potential bacterial targets, an in-silico study was initiated. Molecular docking simulations for eleven chemical compounds with the PDB structure of 2XCT demonstrated strong hydrogen bonds, short distance interactions, and the highest binding energies were displayed by compounds **5b** and **11a** representing valuable candidates for further research.

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