doi.org/10.1002/cbdv.202201209

RESEARCH ARTICLE

CHEMISTRY & BIODIVERSITY

Design, Synthesis, Docking Study of Pyrazolohydrazinopyrimidin-4-one Derivatives and Their Application as Antimicrobials

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New series of pyrazoles **4a**–**c** and pyrazolopyrimidines **5a**–**f** had been constructed. The newly synthesized compounds were assessed for their antimicrobial activity towards *E. coli* and *P. aeruginosa* (gram –ve bacteria), *B. subtilis* and *S. aureus* (gram +ve bacteria) and *A. flavus* and *C. albicans* (representative of fungi). The pyrazolylpyrimidine-2,4-dione derivative **5b** is the most active candidate against *B. subtilis* (MIC = 60 μg/mL) and *P. aeruginosa* (MIC = 45 μg/mL). Regarding antifungal potential, compound **5f** was the most effective against *A. flavus* (MIC = 33 μg/mL). Similarly, compound **5c** displayed strong antifungal activity towards *C. Albicans* (MIC = 36 μg/mL) in reference to amphotericin B (MIC = 60 μg/mL). Finally, the novel compounds had been docked inside dihydropteroate synthase (DHPS) to suggest the binding mode of these compounds.

Keywords: antimicrobial, pyrazole derivatives, pyrimidines, molecular docking, synthesis.

Introduction

Antimicrobial resistance had remained the most significant hindrance towards infectious diseases caused by pathogens.[1,2] Antimicrobial resistance has become a global issue as a result of variety of factors such as unregulated antibiotic use, unauthorized antimicrobial sales, non-human antimicrobial use as in animal production and food chain contamination.[3–5] Because of the antimicrobial resistance, there is a necessary need for developing the strategies for discovery novel medications that can target various sites to combat resistance to current antibiotics.[6–8] Fragment-based drug design strategy is important in the development of novel candidates with high efficacy towards drug sensitive pathogens.[9,10]

Pyrazole ring is well-known for its diverse range of biological potential that include antimicrobial potential.[11–21] In 2021, Mukhtar *et al.*[22] constructed novel pyrazole candidates exhibiting antimicrobial effect, for example the bis-pyrazole derivative **I** (*Figure 1*) displayed antibacterial activity against *S. faecalis* (ZI = 13 mm), *S. typhimurium* (ZI = 9 mm), *E. coli* (ZI = 11 mm) and antifungal potential against *A. flavus* (ZI = 10 mm). In addition, 5-aminopyrazole derivative **II** was reported in 2021 to display antibacterial potential against *B. subtilis* (ZI = 20 mm) and *B. cereus* (ZI = 19 mm) higher than that recorded by the standard drug chloramphenicol (ZI = 16 and 17 mm, respectively).[23] Furthermore, the pyrazole derivative **III** was found to show higher antifungal activity (MIC = 0.03 μg/ml) against *A. fumigates* than amphotericin B (MIC = 0.12 μg/ml).[24] The antifungal potential of compound **III** was attributed to inhibiting dihydropteroate synthase (DHPS) enzyme through binding between the pyrazole moiety of compound **III** with Asp101. Furthermore, ceftolozane **IV** is a semi-synthetic pyrazole derivative belonging to 5th generation cephalosporins approved for treating of many infections as UTI and intra-abdominal infections.[25]

Pyrimidines are heteroaromatic molecules and were considered to be the core of an essential class of pharmaceutically active analogs, so, their synthesis is of high value in the subject of medicinal and pharmaceutical chemistry and was extensively used as a central moiety for the synthesis of a wide variety of

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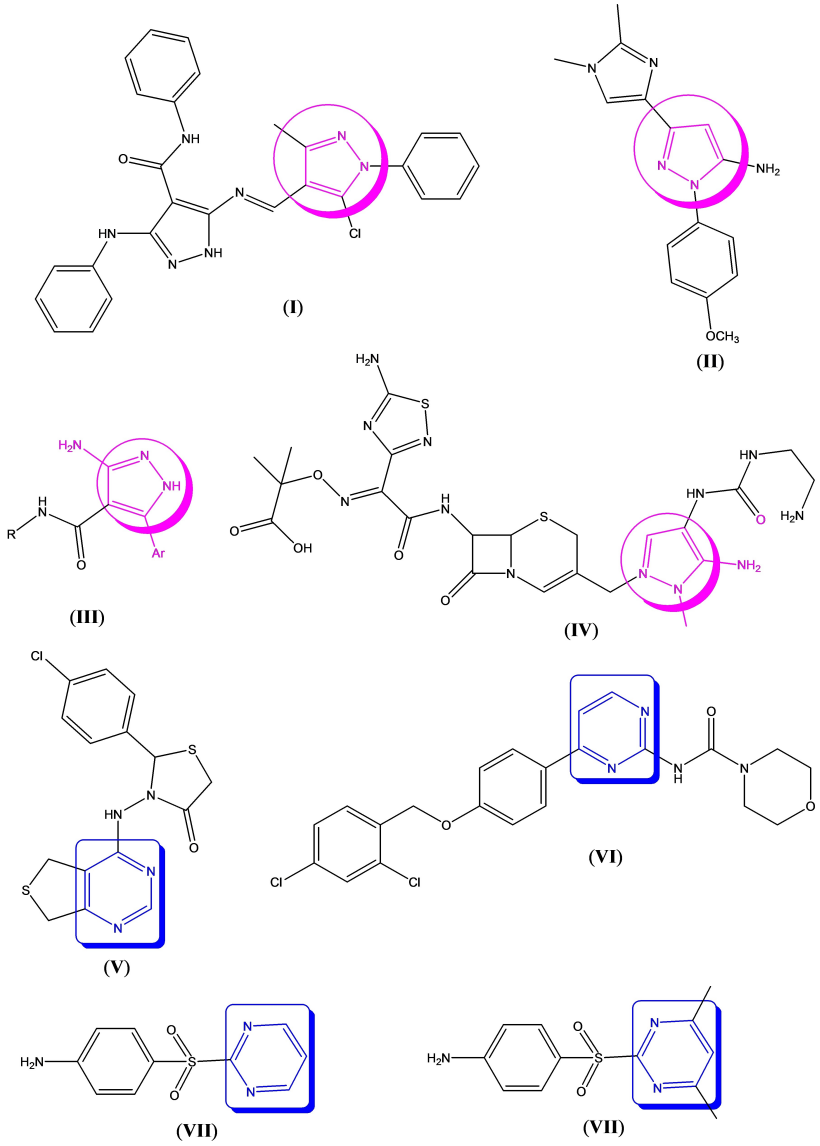


Figure 1. Some documented pyrazoles (**I**–**IV**) and pyrimidines (**V**–**VIII**) with antimicrobial activity.

new bioactive molecules.[26–29] Its derivatives have occupied a marked position in medicinal chemistry due to their broad applications as drugs and drug intermediates. It was reported that the compounds encompassing the pyrimidine ring exhibit a wide range of pharmacological activities. Furthermore, diverse analogs of pyrimidines have been found to possess anticancer,[30,31] antioxidants,[32] anti-inflammatory,[33–36] antimicrobial.[37–39] In 2020, Arshad *et al*[40] synthesized novel antimicrobial set with excellent antimicrobial potential. From this set, compound **V** (*Figure 1*) displayed comparable antibacterial activity towards *S. aureus* (ZI = 21.64 mm), *S. epidermidis* (ZI = 23.18 mm), *E. coli* (ZI = 23.77 mm) and *P. mirabilis* (ZI = 22.28 mm) to ciprofloxacin (ZI = 21.39, 22.87, 23.69 and 22.34 mm, respectively). In addition, the dichlorobenzyloxyphenylpyrimidine derivative **VI** was more potent antibacterial towards *E. coli* (MIC = 2.4 μmol/L) than trimethoprim (MIC = 13.8 μmol/L).[41] Some sulfa drugs incorporating pyrimidine nucleus as sulfadiazine **VII** and sulfadiamidine **VIII** are used in acute bacterial infections. These drugs act through suppressing dihydropteroate synthase DHPS enzyme.[42,43] In the light of medicinal attributes of pyrazole and pyrimidine heterocycles and our work related to the discovery of bioactive candidates,[44–56] we introduce herein the design, construction and antimicrobial screening of novel pyrazole-pyrimidine

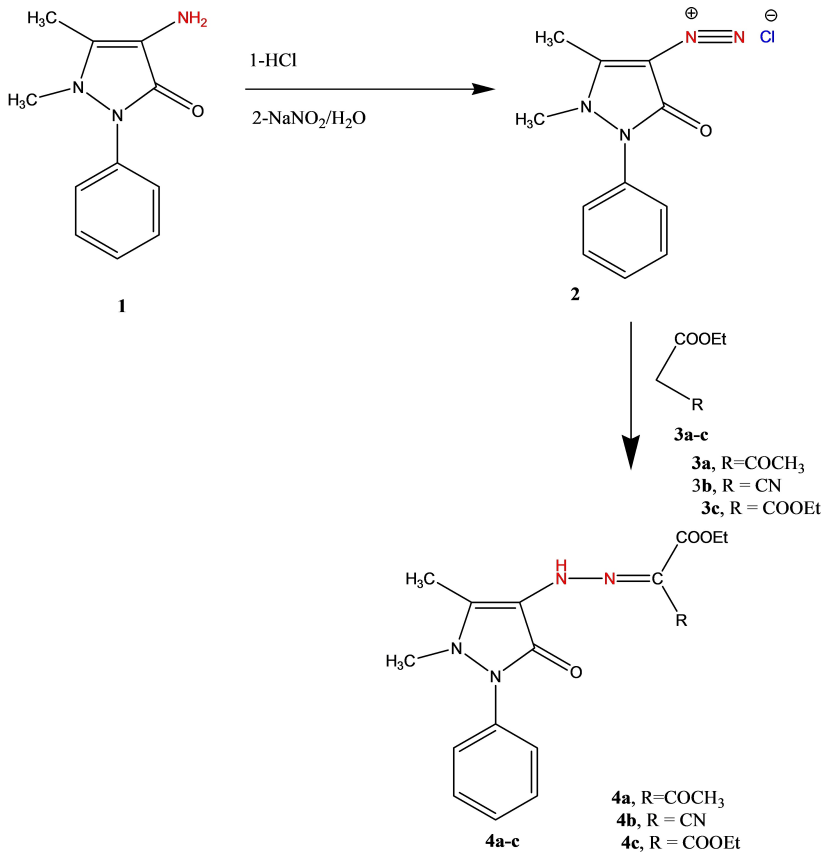
hybrids utilizing fragment-based drug design approach. Our scope in this study was to construct novel pyrazole-pyrimidine derivatives *via* the merge between pyrimidine core and pyrazole nucleus which are important scaffolds as antimicrobial agents with dihydropteroate synthase inhibitory effect. The potential of the novel pyrazole-pyrimidine candidates was also supported by in silico docking study within dihydropteroate synthase.

Results and Discussion

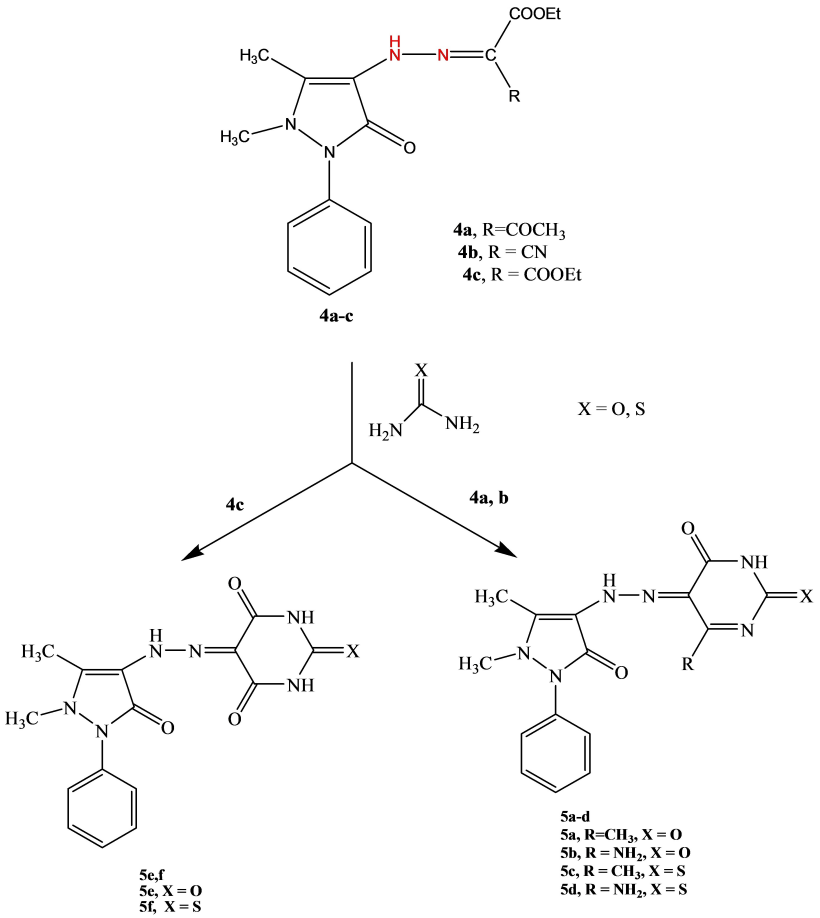
Chemistry

The synthetic methodologies utilized to construct the pyrazole derivatives **4a**–**c** and pyrimidines **5a**–**f** are depicted in *Schemes 1* and *2*. The primary amino group 4-amino antipyrine (**1**) was converted *via* diazotization to its respective diazonium salt (**2**) after treatment with HNO_2 generated in situ from $NaNO_2$/HCl at 0–5 °C applying the reported methodology. The reactions of salt (**2**) with ethyl acetoacetate, ethyl cyanoacetate and diethylmalonate in mixtures of sodium acetate and ethanol solutions afforded ethyl 2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)-3-oxobutanoate (**4a**), ethyl 2-cyano-2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)acetate (**4b**) and diethyl 2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)malonate (**4c**) as crystal powders with shades ranging from bright, light to orange yellow. The infrared spectrum of compound (**4b**) showed a characteristic band appearing at 2223 cm^{-1} corresponding to C≡N stretching of the nitrile group. Besides, IR spectrum of compound (**4c**) showed the characteristic of band at 1720 cm^{-1} corresponding to C=O stretching. Moreover, ^{1}H-NMR spectrum of compound **4a** displayed the presence of two singlet signals at 2.12 and 11.59 ppm attributed to CH_3 and NH_2 protons. In addition, ^{1}H-NMR spectrum of compound **4c** displayed the two ethyl protons of ester moieties at 1.37 and 4.18 ppm which appeared as triplet and quartet peaks.

The synthesis of the pyrazolopyrimidine derivatives (**5a**–**f**) was realized *via* reaction of compounds (**4a**–**c**) with urea and/or thiourea in a mixture of ethanol and HCl is presented in *Scheme 2*. The disappearance of



Scheme 1. Construction of the new pyrazole derivatives (**4a**–**c**).



Scheme 2. Synthesis of pyrazolylhydrazonopyrimidine derivatives **5a**–**f**.

ethyl protons in region of 1.32–1.37 (CH_3 protons) and 4.15–4.18 (CH_2 protons) in the ^{1}H-NMR spectra of compounds **5a**–**f** proved their structures. Moreover, 1H-NMR of compounds **5b** and **5d** displayed the appearance of additional two single peaks at 3.62–3.78 and 10.31–10.59 attributed to NH_2 and NH protons, respectively. In addition, compounds **5e** and **5f** displayed their molecular ion peaks at *m/z* = 342 and 358, sequentially.

Antimicrobial Screening

The nine novel pyrazoles **4a**–**c** and pyrimidines **5a**–**f** were tested *in vitro* for their antimicrobial potential against *S. aureus* and *B. subtilis* (gram positive bacteria), *P. aeruginosa* and *E. coli* (gram negative bacteria) and *C. albicans* and *A. flavus* (fungi) using the microdilution method.[57] The *in vitro* antimicrobial activities of the synthesized compounds were determined from their minimum inhibitory concentration (MIC) values. The MIC is the lowest concentration of a sample that inhibits the visible growth of a microorganism. From the results shown in *Table 1*, it is clear that the 6-Amino-5-pyrazolylhydrazonopyrimidin-2,4-dione derivative **5b** is the most active candidate against *B. subtilis* (MIC = 60 μg/mL) and *P. aeruginosa* (MIC = 45 μg/mL). It is worth noting that the compounds (**5b**–**f**), which comprise the pyrazole ring in a one hybrid structure with a pyrimidine scaffold showed higher antibacterial activity against the tested strains compared to the compounds **4a**–**c**, which have only one pyrazole moiety (*B. subtilis* MIC = 126–130 μg/mL, *S. aureus* = 120–128 μg/mL, *P. aeruginosa* = 125–129 μg/mL and *E. coli* = 126–128 μg/mL). Regarding antifungal activity against *A. flavus* and *C. albicans*, compounds **4a**–**c** had little or no bioactivity against any fungi (most of them > 100 μg/mL). The most potent antifungal activity against *A. flavus* was exerted by the pyrazolylpyrimidine derivatives **5c**, **5d**, **5e**, and **5f** with an MIC between 33 and 62 μg/mL. Among the compounds synthesized, **5f** is the most effective against *A. flavus* (MIC = 33 μg/mL). Similarly,

Table 1. Antibacterial and antifungal activity of pyrazole derivatives **4a**–**c**, pyrazol-4-ylpyrimidines **5a**–**f**, ampicillin and amphotericin B.

Compound	MIC in μg/mL Gram-positive bacteria		Gram-negative bacteria		Fungi	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*A. flavus*	*C. albicans*
Ampicillin	100	100	250	100	–	–
Amphotericin B	–	–	–	–	60	60
4a	126.0	128.0	129.0	127.0	100.0	200.0
4b	127.0	125.0	125.0	126.0	160.0	130.0
4c	129.0	120.0	125.0	128.0	110.0	120.0
5a	130.0	125.0	130.0	129.0	128.0	129.0
5b	60.0	126.0	45.0	63.0	100	32.0
5c	73.0	125.0	126.0	75.0	55.0	36.0
5d	125.0	125.0	125.0	65.0	62.0	125.0
5e	80.0	125.0	77.0	60.0	60.0	125.0
5f	70.0	125.0	128.0	66.0	33.0	125

compound **5c** displayed strong antifungal activity towards *C. Albicans* (MIC = 36 μg/mL) in reference to amphotericin B (MIC = 60 μg/mL).

In silico *Docking Study*

Pyrazole and pyrimidine rings are essential nitrogen containing heterocycles with interesting biological potential.[24,58–65] They are reported to show antimicrobial activity through inhibiting dihydropteroate synthase (DHPS) enzyme.[14,24,66,67]

To suggest the action mode of the novel prepared compounds, the most active candidates **5b** and **5c** were docked within dihydropteroate synthase (DHPS) which was downloaded from protein data bank (PDB: 4DAI). The obtained results were recorded in *Table 2*.

The most active antibacterial derivative **5b** assigned four hydrogen bonding interactions with binding energy score −6.28 kcal/mol as follows: i) ASN207 with pyrimidine NH, ii) ASN207 with pyrazole C=O, iii) GLU251 with NH_2, iv) GLY249 with NH_2. In addition to formation of two Pi-Anion interactions with GLU204 and LYS2 amino acid residues, one Pi-cation interaction with GLU247 amino acid and Pi-alkyl binding with LYS248 amino acid (*Figure 2*).

On the other hand, pyrazolylthioxopyrimidine derivative **5c** exerted 4 hydrogen bondings with GLYA 36, GLYB 36, SERA 34 and SERB 34 amino acid residues.

Table 2. Docking simulation data for compounds **5b** and **5c** within DHPS active site.

Compound	Docking score kcal/mol	Number of bonds	Type of interactions	Amino acids	Function group
5b	−6.28	8	H-bond	ASN207	C=O
			H-bond	ASN207	NH
			H-bond	GLY249	NH
			H-bond	GLU 251	NH
			Pi-Anion	LYS 2	Phenyl
			Pi-Anion	GLU 204	Phenyl
			Pi-cation	GLU 247	Pyrazole N
			Pi-Alkyl	LYS 248	Phenyl
5c	−5.98	7	H-bond	GLYB 36	N=N
			H-bond	GLYA 36	NH
			H-bond	SERA 34	C=O
			H-bond	SERA 38	C=O
			Amide-Pi stacked	ILEB 80	Phenyl
			Pi-Alkyl	VALB 84	Phenyl
			Pi-Alkyl	PROB 85	Phenyl

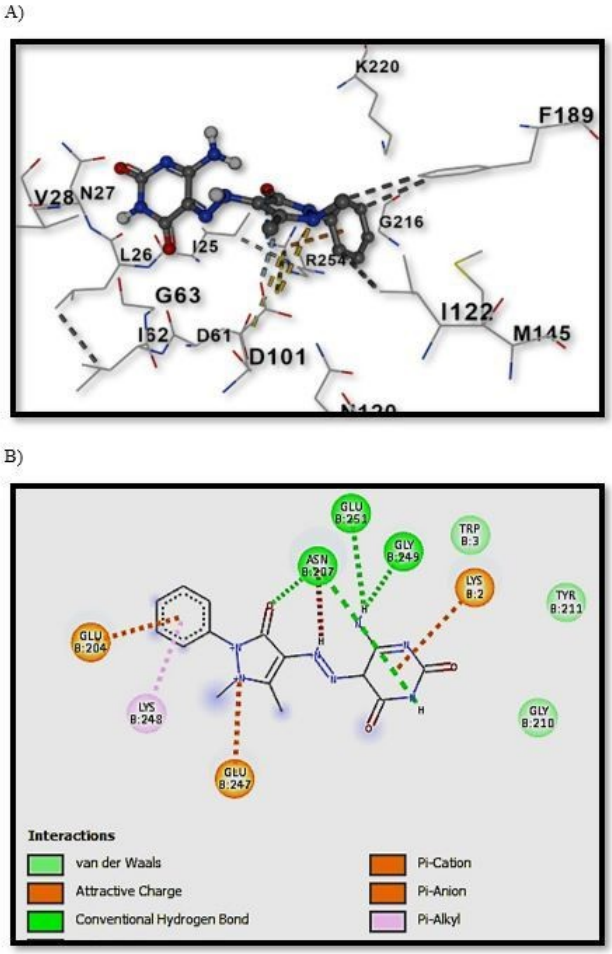


Figure 2. A) 3D fitting of compound **5b** within DHPS active site, B) 2D fitting mode of **5b** inside the active site.

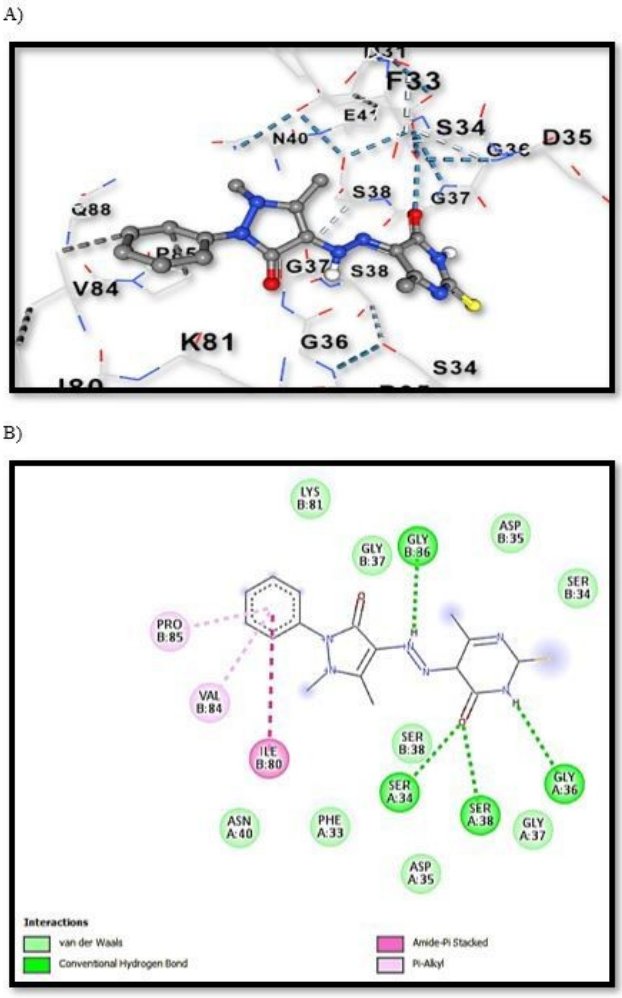


Figure 3. A) 3D fitting of compound **5c** within DHPS active site, B) 2D fitting mode of **5c** inside the active site.

Moreover, this compound **5c** interacted with the phenyl moiety through Amide-Pi stacked and Pi-alkyl interactions with ILEB80, VALB 84 and PROB 85 amino acid residues as depicted in *Figure 3*.

Conclusion

New pyrazoles (**4a**–**c**) and pyrazolopyrimidines (**5a**–**f**) had been synthesized and assessed for their antimicrobial activity. 6-Amino-5-pyrazolylhydrazonopyrimidin-2,4-dione derivative **5b** is the most active candidate against *B. subtilis* (MIC = 60 μg/mL) and *P. aeruginosa* (MIC = 45 μg/mL). In this study, compounds (**5b**–**f**), which comprise the pyrazole ring in a one hybrid structure with a pyrimidine scaffold showed higher antibacterial activity against the tested strains compared to the compounds **4a**–**c**, which have only one pyrazole moiety (*B. subtilis* MIC = 126–130 μg/mL, *S. aureus* MIC = 120–128 μg/mL, *P. aeruginosa* MIC = 125–129 μg/mL and *E. coli* MIC = 126–128 μg/mL). Finally, molecular docking study of the most active novel compounds **5b** and **5c** within dihydropteroate synthase enzyme displayed the ability of these novel candidates to fit within the active region showing 8 and 7 binding mode interactions.

Experimental Section

Chemicals

Reagents were purchased from Sigma Aldrich (Somatco Trading Co. Ltd., Sakaka, Aljouf, Saudi Arabia) and used without further purification. Reaction progress was monitored by thin-layer chromatography on silica

gel pre-coated F254Merck plates (Darmstadt, Germany). Spots were visualized by ultraviolet irradiation. Melting points were determined on a Gallenkamp electro thermal melting point apparatus and are uncorrected, IR spectra were recorded as potassium bromide disks using Shimadzo infrared spectrophotometer central research laboratory, Jouf University

Synthesis of Antipyrine Derivatives (**4a**–**c**)

A cold mixture of ethyl acetoacetate, ethyl cyanoacetate and/or diethylmalonate (**3a**–**c**) (0.01 mol) and sodium acetate (0.01 mol) in abs. ethanol (20 ml) was added dropwise with stirring to a solution of diazonium salt of 4-amino antipyrine (**2**) (0.01 mol) over 15 min, the stirring lasted for 1h. The reaction mixture was left over night at refrigerator; the colored solid product formed was then collected, and recrystallized from water to afford the corresponding compounds (**4a**–**c**).

Ethyl 2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)-3-oxobutanoate (**4a**). 77% yield as orange crystals; m.p. 166–168 °C; U.V (λ_{max}) 407 nm; IR (KBr): ν (cm^{-1}), 2923–3072 (CH_2, CH_3, CH), 1697 (Pyrazole C=O), 1772 (C=O), 1591–1652 (C=N) pyrazolo ring, 3370 (NH); ^{1}H-NMR: 1.11 (t, *J*= 6.27, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.38 (s, 3H, $COCH_3$), 2.54 (s, 3H, CH_3), 3.52 (q, *J*=6.27, 2H, CH_2), 7.26–7.47 (m, 5H, ArH), 11.59 (s, 1H, NH); ^{13}C-NMR: 11.4, 14.3, 27.1, 36.5, 60.5, 114.8, 123.8, 124.0, 129.3, 134.3, 142.3, 158.8, 159.1, 162.9, 195.9; MS: *m/z* 344 (M^+, 32%); Anal calc. for $C_{17}H_{20}N_4O_4$ (344.37): C, 59.29; H, 5.85; N, 16.27; Found: C, 59.01; H, 6.00; N, 16.49.

Ethyl 2-cyano-2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)acetate (**4b**). 70% yield as pale yellow crystals; m.p. 150–152 °C; U.V (λ_{max}) 391 nm; IR (KBr): ν (cm^{-1}), 2870–3154 (Aliphatic CH), 2208 (CN), 1721 (Pyrimidine C=O), 1633 (pyrazole C=O), 1652–1674 (C=N), 1531 (N=N); ^{1}H-NMR: 1.31 (t, *J*=6.15, 3H, CH_3); 2.30 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.20 (q, *J*=6.15, 2H, CH_2), 6.89- 7.66 (m, 5H, ArH), 10.95 (s, 1H, NH); ^{13}C-NMR: 13.6, 23.2, 63.9, 89.7,123.9, 126.3, 128.8, 129.5, 130.2, 131.2, 145.8, 155.8, 157.6, 158.9, DEPT135; 13.6, 23.2, 63.9, 89.7(CH_3,CH_3,CH_2,CH_3); Chemical Formula: $C_{16}H_{17}N_5O_3$ (327.34). MS: *m/z* 327 (M^+, 48%); Calculated: C, 58.71; H, 5.23; N, 21.39; O, 14.66; Found: C, 59.01, H; 4.98; N, 21.48; O, 14.51.

Diethyl 2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)malonate (**4c**). 66% yield as deep yellow Crystals; m.p. 135–137 °C; U.V (λ_{max}) 375 nm; IR (KBr): ν (cm^{-1}), 2870–3154 (CH), 1799 (Pyrimidine C=O), 1662 (pyrazole C=O), 3322 (NH); ^{1}H-NMR: 0.9 (t, *J*=6.92, 6H, $2CH_3$), 1.24 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.18 (q, *J*=6.92, 4H, $2CH_2$), 6.83–7.64 (m, 6H, 5ArH,1NH); ^{13}C-NMR: 23.4, 25.2, 35.3, 48.7, 126.9, 127.4, 129.1, 129.8, 130.5, 140.8, 141.4, 165.7, 166.7; MS: *m/z* 374 (M^+, 43%); Anal calc. for $C_{18}H_{22}N_4O_5$: C, 57.75; H, 5.92; N, 14.96; O, 21.37; Found: C, 58.00; H, 6.01; N, 15.17; O, 21.66.

Synthesis of Pyrazolohydrazinopyrimidin-4-one Derivatives (**5a**–**f**)

A mixture of compound (**4a**–**c**) (0.01 mol), urea and/or thiourea (0.01 mol) in ethanol 20 mL and HCl (0.5 mL) was stirred at 100 °C under reflux for 4 h. the solid product was formed, the extra solvent was removed and crystallized from methanol to afford the compounds (**5a**–**f**).

5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]-6-methyl-5*H*-pyrimidine-2,4-dione (**5a**). 71% Yield; yellow crystals; m.p. 188–200 °C; IR *v* (cm^{-1}): 2347 (CH), 1701 (pyrimidine 2 C=O), 1655 (pyrazole C=O), 1650 (C=N); ^{1}H-NMR: 1.32 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.01–8.02 (m, 6H, 5ArH, 1NH), 9.10 (s, 1H, NH); ^{13}C-NMR: 12.5, 15.5, 35.6, 112.9, 117.4, 119.4, 139.2, 131.9, 145.3, 155.4, 161.5, 163.7, 164.0; 35.7, MS: *m/z* 340 (M^+, 52%); Anal calc. for $C_{16}H_{16}N_6O_3$: C, 56.46; H, 4.74; N, 24.69; Found: C, 56.50; H, 4.95; N, 24.83.

6-Amino-5-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]-5*H*-pyrimidine-2,4-dione (**5b**). 73% Yield; yellow crystals; m.p. 178–180 °C; IR *v* (cm^{-1}): 3348 (NH_2), 2341 (CH), 1707 (pyrimidine 2 C=O), 1654 (pyrazole C=O); ^{1}H-NMR: 1.46 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 3.47 (s, 2H, NH_2), 7.01–8.02 (m, 6H, 5ArH,1NH), 12.11 (s, 1H, NH); ^{13}C-NMR: 15.2, 36.2, 112.7, 117.3, 119.4, 129.3, 131.6, 143.8, 154.7, 161.3, 162.9, 163.1, 163.3; MS: *m/z* 341 (M^+, 73%); Anal calc. for $C_{15}H_{15}N_7O_3$ (341.32): C, 52.78; H, 4.43; N, 28.73; Found: C, 52.51; H, 4.72; N, 28.94.

5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]-6-methyl-2-thioxo-2,3-dihydro-3*H*-pyrimidin-4-one (**5c**). 60% Yield as orange crystals; m.p. 185–187 °C; U.V: (λ) 355 nm; IR: ν (cm^{-1}), 2358(CH), 1698 (pyrimidine C=O), 1654 (C=O) pyrazole, 1650 (C=N); ^{1}H-NMR: 2.31 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 7.01–7.51 (m, 5H, ArH), 9.51

(s, 1H, NH), 10.11 (s, 1H, NH); ^{13}C-NMR: 11.2, 15.2, 35.8, 112.5, 117.2, 119.4, 130.4, 131.9, 143.8, 154.7, 160.9, 164.3,166.2, 189.1; MS: *m/z* 356 (M^+, 41%); Anal calc. for $C_{16}H_{16}N_6O_2S$ (356.40): C, 53.92; H, 4.52; N, 23.58; O, 8.98; S, 9.00; Found: C, 54.09; H, 4.31; N, 23.49; O, 9.00; S, 9.21.

6-Amino-5-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]-2-thioxo-2,5-dihydro-3*H*-pyrimidin-4-one (**5d**). 63% Yield as pale orange crystals; m.p. 189–190 °C; IR ν (cm^{-1}): 2357 (CH), 1701 (pyrimidine C=O), 1656 (C=O) pyrazole, 1649 (C=N); ^{1}H-NMR: 1.89 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.62 (s, 2H, NH_2), 7.01–8.02 (m, 6H,5 ArH,NH), 11.67 (s, 1H, NH); ^{13}C-NMR: 15.4, 35.6, 112.7, 117.5, 119.2, 130.7, 131.8, 143.5, 154.2, 160.8, 164.2,166.7, 187.9; MS: *m/z* 356 (M^+, 41%); Anal calc. for $C_{15}H_{15}N_7O_2S$ (357.39): C, 50.41; H, 4.23; N, 27.43; O, 8.95; S, 8.97; Found: C, 50.44; H, 4.35; N, 27.60; O, 9.02; S, 9.11.

5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]pyrimidine-2,4,6-trione (**5e**). 66% Yield as pale brown crystals; m.p. 172–173 °C; IR *v* (cm^{-1}): 2353 (CH), 1734–1650 (4 C=O), 1647 (C=N); ^{1}H-NMR: 2.30 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 7.01–7. 51(m, 5H, ArH), 9.58 (s, 1H, NH), 10.27 (s, 1H, NH), 10.45 (s, 1H, NH); ^{13}C-NMR: 14.2, 36.1, 112.8, 117.5, 119.1, 130.2, 131.6, 143.4, 154.3, 157.2, 160.7, 164.2,164.3; MS: *m/z* 342 (M^+, 56%); Anal calc. for $C_{15}H_{14}N_6O_4$ (342.31): C, 52.63; H, 4.12; N, 24.55; O, 18.70; Found: C, 52.55; H, 4.15; N, 24.70; O, 18.55.

5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono]2-thioxodihydropyrimidine-4,6-dione (**5f**). 68% Yield as reddish brown crystals; m.p. 175–176 °C; IR *v* (cm^{-1}): 2354 (CH), 1728–1653 (3 C=O), 1645 (C=N); ^{1}H-NMR: 2.10 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 7.01–7. 51 (m, 5H, ArH), 9.51 (s, 1H, NH), 10.11 (s, 2H, 2NH); ^{13}C-NMR: 14.7, 19.2, 121.6, 127.2, 129.0, 130.3, 133.2, 143.7, 155.0, 157.2, 163.2, 165.8, 175.3,188.2; MS: *m/z* 358 (M^+, 60%); Anal calc. for $C_{15}H_{14}N_6O_3S$ (358.38): C, 50.27; H, 3.94; N, 23.45; O, 13.39; S, 8.95; Found: C, 50.05; H, 4.00; N, 23.29; O, 13.55; S, 9.06.

Antimicrobial Activity

MIC Methods for Antibacterial

Four bacterial strains, viz. *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*, were utilized within the examinations for the antimicrobial test. Ampicillin was utilized as a standard drug for antibacterial activity, and minimum inhibitory concentration (MIC) of all compounds was estimated by the microdilution method using sequentially diluted compounds.[56]

MIC Methods for Antifungal Activity

MIC of the compounds was determined by used different concentrations. A series concentration of the compounds in μg/mL showed in *Table 2* was sequentially diluted in microliter plate. Particularly, 0.1 mL of standardized inoculums (1–2×107 cfu/mL) was added in each test tube of microliter plate.[68] The plates were hatched vigorously at 37 °C for 18–24 h. The highest dilution (the lowest concentration) of the compounds demonstrated no turbidity in the result when it might have been compared with the control might have been viewed as this MIC.[69] We used *A. flavus* and *C. albicans* as antifungal activity of synthesized compounds. The synthesized compounds were investigated at various concentrations. Amphotericin B is used as a standard control drug for antifungal activity as shown in *Table 2*.

In silico *Docking Study*

Crystal structure of dihydropteroate synthase was obtained from protein data bank (code: 4DAI). Preparation of the downloaded enzyme and the target compounds were performed according to reported method.[70] Blind docking was done by the use of CB-DOCK2. CB-Dock predicts protein cavities and measures the centers and sizes of top N (n = 5 by default) cavities. The profiles of interaction and visualization were performed for the best-docked complexes using Discovery Studio software.

Acknowledgements

The authors are grateful to the Department of Chemistry, College of science, Jouf University.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Received December 17, 2022
Accepted March 27, 2023

