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Synthesis, Molecular Docking Studies and ADME Properties of Some New Pyrazolo[1,5-a]pyrimidines as Antimicrobial, and Anticancer Agents

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Abstract

New antibacterial and anticancer drugs are needed to protect global health. Purine anti-cancer analogs are structurally similar but act differently. In this study, a series of new pyrazolo[1,5-a]pyrimidines **5a-g**, **9a-c** and pyrazolo[3,4-*b*]pyridines **15a, b** were synthesized to evaluate their *in vitro* antibacterial efficacy against various microbial species. Pyrazolo[1,5-a]pyrimidine derivatives **5a** and **5g** showed moderate antibacterial efficacy against *Staphylococcus aureus* and *Bacillus subtilis*. *In vitro* antitumor activity of **5a-g**, **9a-c** and **15a, b** against Lung carcinoma (A-549), promyelocytic Leukemia (HL-60) and breast cancer MCF-7 showed that **5d** exhibited significant anticancer activity towards A-549 with IC_{50} value = 7.19±0.34 µM when compared to cisplatin (IC_{50}=7.48±0.56 µM). The molecular docking study of **5d** showed good binding scores in the active site of CK2 and CDK9. The molecule's physicochemical, pharmacokinetic, and drug-like properties were assessed for the synthesized compounds using the SwissADME database. The molecular properties data showed that all compounds obey Veber rule with zero violations, indicating drug-likeliness.

Keywords: Pyrazolo[1,5-a]pyrimidine; Antimicrobial activity; Anticancer activity; A-549 cell line: Molecular docking.

1. Introduction

Since cancer treatment increases susceptibility to bacterial infections, a compound with anticancer and antibacterial properties can be beneficial. However, few studies have examined the simultaneous engagement of these two activities or attempted to improve their anti-infection and anti-tumor effects [1]. In particular, Staphylococcus aureus significantly impacts malignancy patients' clinical outcomes [2].

Pyrazoles exhibited various biological activities, including antimicrobial [3, 4] and anticancer activity [5, 6]. Additionally, pyrimidine-based compounds exhibit diverse pharmacological properties, such as antimicrobial and anticancer effects. Recent studies indicate that pyrimidine derivatives TG02 and Meriolin 3, showed excellent cyclin dependent kinase inhibitions against CDK2 and CDK9 and anticancer effects towards several tumor cells [7, 8]. Additionally, CYC-116 is a 2-anilinopyrimidine derivative, inhibits CDK2 and CDK9 with anticancer potency against MCF7, Hela, and HCT-116 [9]. On the other hand, pyridine-based compounds have been reported as antimicrobial agents [10] and acting as dihydrofolate reductase (DHFR) inhibitors [11] in addition to their potent anticancer activity [12, 13].

Fusion between pyrazole moiety and pyrimidine or pyridine ring to give the purine analogues pyrazolo[1,5-a]pyrimidines and pyrazolo[3,4-b]pyridines, respectively is promising strategy to explore new biologically active systems specially as antimicrobial [14, 15] and anticancer agents [16, 17].

The pyrazolo[1,5-a]pyrimidine [18, 19] and pyrazolo[3,4-b]pyridine [20, 21] scaffolds are essential core in several active CDK inhibitors. For example, dinaciclib, pyrazo[1,5-a]pyrimidine antitumor drug, inhibits CDK1, CDK5, and CDK9 with activity against leukemia cell growth [22, 23].

In continuation of our interest in the anticancer activity of pyrazolo[1,5-a]pyrimidines [24, 25] and pyrazolo[3,4-b]pyridines [26, 27], we aimed herein, to synthesize a novel series of pyrazolo[1,5-a]pyrimidines **5a-g**, **9a-c** and pyrazolo[3,4-b]pyridines **15a, b** to evaluate their antimicrobial activity against six microbial species and also to investigate their anticancer potency towards three types of cancer. Furthermore, molecular docking studies of the most active compounds in the active site of CDK2 and CDK9 are performed, in addition to the ADME properties of the newly synthesized compounds.

2. Experimental

2.1. Chemistry

2.1.1. General

Melting points (°C, uncorrected) were determined using a Stuart melting point apparatus. The IR spectra were recorded on a SHIMADZU FT/IR spectrometer. The NMR spectra recorded by BRUKER 400 MHz NMR spectrometers. ^{1}H and ^{13}C spectra were run at 400 and 100 MHz, respectively. Mass spectroscopy analysis was recorded on GCMS-QP 1000EX Shimadzu Gas Chromatography MS Spectrometer. Elemental analyses were performed at the Regional Center for Microbiology and Biotechnology, Al-Azhar University. 3-Phenyl-4-(4-tolyldiazenyl)-1*H*-pyrazol-5-amine (**1**) 28], 3-(dimethylamino)-1-arylprop-2-en-1-ones **2a-f** [29], 3-oxo-3-phenylpropanenitrile (**6**) [30], 3-oxo-3-phenylpropanenitrile (**10**) [31] and 3-phenyl-1*H*-pyrazol-5-amine (**12**) [32] were prepared following the reported method.

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*2.1.2. General procedure for the synthesis of pyrazolo[1,5-a]pyrimidines **5a-g***

A mixture of 3-phenyl-4-(4-tolyldiazenyl)-1*H*-pyrazol-5-amine (**1**) (0.277 g, 1 mmol) and the appropriate 3-(dimethylamino)-1-arylprop-2-en-1-ones **2a-g** (1 mmol) in glacial acetic acid (15 mL) was heated under reflux for 3 h. The formed precipitate was filtered, washed with ethanol, and recrystallized from EtOH/DMF to furnish pyrazolo[1,5-*a*]pyrimidines **5a-g**, respectively.

*2.1.2.1. 2,7-Diphenyl-3-(p-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5a**)*

Orange powder, 83% yield; mp 142-144ºC; IR (KBr) ν_{max}/cm^{-1} 1603, 1539 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 7.37 (d, *J* = 7.9 Hz, 2H, ArH), 7.45 – 7.60 (m, 4H, ArH), 7.67 (t, *J* = 3.3 Hz, 3H, ArH), 7.73 (d, *J* = 7.9 Hz, 2H, ArH), 8.14 – 8.26 (m, 4H, ArH), 8.85 – 8.91 (m, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.52 (CH_3), 111.17, 122.15 (2C), 125.11, 129.00 (2C), 129.07 (2C), 129.82 (2C), 129.91, 130.34 (3C), 130.46, 131.86, 132.24, 140.03, 140.42, 146.45, 152.23, 153.58, 153.99. MS *m/z* (%) 389.53, (M$^+$, 29.05), 373.51 (100). Anal. Calcd. For: $C_{25}H_{19}N_5$ (389.46): C, 77.10; H, 4.92; N, 17.98; found: C, 77.34; H, 5.13; N, 18.23.

*2.1.2.2. 2-Phenyl-7-(4-tolyl)-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5b**)*

Orange powder, 71% yield; mp 188-190ºC; IR (KBr) ν_{max}/cm^{-1} 1709, 1597, 1535, 1505 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.46 (s, 3H, Me), 7.39 (d, *J* = 8.1 Hz, 2H, ArH), 7.45 – 7.62 (m, 6H, ArH), 7.73 (d, *J* = 8.2 Hz, 2H, ArH), 8.12 – 8.23 (m, 4H, ArH), 8.87 (d, J = 4.5 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.43 (CH_3), 21.60 (CH_3), 110.83, 122.15 (2C), 125.05, 127.55, 129.03 (2C), 129.67 (2C), 129.81 (2C), 129.94, 130.31 (2C), 130.36 (2C), 132.27, 140.10, 140.42, 142.10, 146.53, 152.24, 153.54, 153.97. MS *m/z* (%) 403.00 (M$^+$, 22.74), 97.67 (100). Anal. Calcd. For: $C_{26}H_{21}N_5$ (403.49): C, 77.40; H, 5.25; N, 17.3; found: C, 77.58; H, 5.43; N, 17.54.

*2.1.2.3. 7-(4-Methoxyphenyl)-2-phenyl-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5c**)*

Orange powder, 74% yield; mp 196-198ºC; IR (KBr) ν_{max}/cm^{-1} 1601, 1535, 1501 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.24 (d, *J* = 8.6 Hz, 2H, ArH), 7.39 (d, *J* = 8.0 Hz, 2H, ArH), 7.49 – 7.62 (m, 4H, ArH), 7.73 (d, *J* = 8.0 Hz, 2H, ArH), 8.21 (d, *J* = 7.5 Hz, 2H, ArH), 8.31 (d, *J* = 8.5 Hz, 2H, ArH), 8.85 (d, *J* = 4.6 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.43 (CH_3), 56.01 (OCH_3), 110.31, 114.58 (2C), 122.13 (2C), 122.34, 124.96, 129.02 (2C), 129.83 (2C), 129.93, 130.35 (2C), 132.27 (2C), 132.31, 140.21, 140.36, 146.17, 152.26, 153.51, 153.80, 162.25. MS *m/z* (%) 419.32 (M$^+$, 19.02), 117.18 (100). Anal. Calcd. For: $C_{26}H_{21}N_5O$ (419.49): C, 74.44; H, 5.05; N, 16.70; found: C, 74.19; H, 5.13; N, 16.91.

*2.1.2.4. 7-(4-Bromophenyl)-2-phenyl-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5d**)*

Orange powder, 79% yield; mp 187-189ºC; IR (KBr) ν_{max}/cm^{-1} 1586, 1535 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 7.39 (d, *J* = 8.1 Hz, 2H, ArH), 7.48 – 7.62 (m, 4H, ArH), 7.74 (d, *J* = 8.0 Hz, 2H, ArH), 7.90 (d, *J* = 8.5 Hz, 2H, ArH), 8.15 – 8.22 (m, 4H, ArH), 8.90 (d, *J* = 4.5 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.44 (CH_3), 111.11, 122.16 (2C), 125.14, 125.55, 129.00 (2C), 129.57, 129.82 (2C), 129.96, 130.34 (2C), 132.10 (2C), 132.15, 132.36 (2C), 139.95, 140.49, 145.31, 152.19, 153.52, 153.96. MS *m/z* (%) 470.05 (M$^+$+2, 47.19), 468.83 (M$^+$, 42.55), 67.90 (100). Anal. Calcd. For: $C_{25}H_{18}BrN_5$ (468.36): C, 64.11; H, 3.87; N, 14.95; found: C, 63.98; H, 3.95; N, 15.12.

*2.1.2.5. 7-(4-Chlorophenyl)-2-phenyl-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5e**)*

Orange powder, 68% yield; mp 220-222ºC; IR (KBr) ν_{max}/cm^{-1} 1593, 1539, 1485 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 7.38 (d, *J* = 7.9 Hz, 2H, ArH), 7.50-7.58 (m, 4H, ArH), 7.74 (t, *J* = 7.2 Hz, 4H), 8.18 (d, *J* = 7.4 Hz, 2H), 8.26 (d, *J* = 8.2 Hz, 2H), 8.89 (d, *J* = 4.5 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 21.44 (CH_3), 111.18, 122.17 (2C), 125.15, 129.02 (2C), 129.18 (2C), 129.23, 129.83 (2C), 129.97, 130.35 (2C), 132.17, 132.24 (2C), 136.66, 139.98, 140.50, 145.26, 152.21, 153.53, 153.99. MS *m/z* (%) 423.86 (M$^+$, 14.97), 149.24 (100). Anal. Calcd. For: $C_{25}H_{18}ClN_5$ (423.90): C, 70.84; H, 4.28; N, 16.52; found: C, 70.75; H, 4.42; N, 16.59.

*2.1.2.6. 7-(4-Fluorophenyl)-2-phenyl-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5f**)*

Orange powder, 75% yield; mp 215-217ºC; IR (KBr) ν_{max}/cm^{-1} 1601, 1501 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 7.39 (d, *J* = 7.9 Hz, 2H, ArH), 7.51-7.59 (m, 6H, ArH), 7.74 (d, *J* = 7.9 Hz, 2H, ArH), 8.19 (d, *J* = 7.4 Hz, 2H, ArH), 8.33 (dd, *J* = 8.4, 5.4 Hz, 2H, ArH), 8.90 (d, *J* = 4.5 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.44 (CH_3), 111.12, 116.11, 116.33, 122.16 (2C), 125.12, 126.88, 129.03 (2C), 129.83 (2C), 129.97, 130.36 (2C), 132.20, 133.06, 133.14, 140.03, 140.48, 145.46, 152.22, 153.57, 154.00, 162.96. MS *m/z* (%) 407.00 (M$^+$, 23.04), 235.31 (100). Anal. Calcd. For: $C_{25}H_{18}FN_5$ (407.45): C, 73.70; H, 4.45; N, 17.19; found: C, 73.81; H, 4.51; N, 17.40.

*2.1.2.7. 7-(4-Nitrophenyl)-2-phenyl-3-(4-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine (**5g**)*

Orange powder, 74% yield; mp 221-223ºC; IR (KBr) ν_{max}/cm^{-1} 1589, 1529, 1530 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 7.37 (d, *J* = 7.9 Hz, 2H, ArH), 7.49 – 7.60 (m, 4H, ArH), 7.72 (d, *J* = 7.9 Hz, 2H, ArH), 8.17 (d, *J* = 7.3 Hz, 2H, ArH), 8.46 (s, 4H, ArH), 8.92 (d, *J* = 4.6 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6) δ 21.44 (CH_3), 111.86, 122.20 (2C), 123.99 (2C), 125.25, 129.02 (2C), 129.82 (2C), 130.02, 130.35 (2C), 131.89 (2C), 132.03, 136.48, 139.85, 140.62, 144.24, 149.20, 152.15, 153.54, 154.03. MS *m/z* (%) 434.35 (M$^+$, 22.54), 353.16 (100). Anal. Calcd. For: $C_{25}H_{18}N_6O_2$ (434.46): C, 69.11; H, 4.18; N, 19.34; found: C, 69.32; H, 4.40; N, 19.62.

*2.1.3. General procedure for the synthesis of pyrazolo[1,5-a]pyrimidines **9a-c***

A mixture of 3-phenyl-1*H*-pyrazol-5-amine (**1**) (0.16 g, 1 mmol) and the appropriate 3-(dimethylamino)-1-arylprop-2-en-1-ones **2a, d, g** (1 mmol) in glacial acetic acid (15 mL) was heated under reflux for 3 h and then left to cool. The formed precipitate was filtered, washed with ethanol, and recrystallized from EtOH to furnish pyrazolo[1,5-a]pyrimidines **9a-c**, respectively.

*2.1.3.1. 2-Phenyl-7-(4-tolyl)pyrazolo[1,5-a]pyrimidine (**9a**)*

White powder, 68% yield; mp 112-114ºC; IR (KBr) ν_{max}/cm^{-1} 1609, 1544, 1508, 1466 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 7.21 (d, *J* = 4.4 Hz, 1H, H3 pyrimidine), 7.32 (s, 1H, H4 of pyrazole), 7.40 – 7.52 (m, 5H, ArH), 8.01 – 8.08 (m, 2H, ArH), 8.15 (d, *J* = 8.1 Hz, 2H, ArH), 8.57 (d, *J* = 4.4 Hz, 1H, H2 pyrimidine). ^{13}C NMR (DMSO-d_6) δ 21.56 (CH_3), 93.74, 107.89, 126.70 (2C), 128.19, 129.31 (2C), 129.50, 129.58 (2C), 129.83 (2C), 132.99, 141.61, 145.78, 150.11, 151.21, 155.06. MS *m/z* (%) 285.21 (M$^+$, 26.46), 247.75 (100). Anal. Calcd. For: $C_{19}H_{15}N_3$ (285.35): C, 79.98; H, 5.30; N, 14.73; found: C, 79.70; H, 5.41; N, 14.59.

*2.1.3.2. 7-(4-Bromophenyl)-2-phenylpyrazolo[1,5-a]pyrimidine(**9b**)*

White powder, 75% yield; mp 165-167ºC; IR (KBr) ν_{max}/cm^{-1} 1609, 1539 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 7.27 (d, *J* = 4.4 Hz, 1H, H3 pyrimidine), 7.35 (s, 1H, H4 of pyrazole), 7.39 – 7.46 (m, 1H), 7.46 – 7.54 (m, 2H), 7.86 (d, *J* = 8.6, 2H), 8.04 (d, *J* = 7.0, 2H), 8.20 (d, *J* = 8.6 Hz, 2H), 8.61 (d, *J* = 4.4 Hz, 1H, H2 pyrimidine). ^{13}C NMR (DMSO-d_6) δ 93.99, 108.33, 125.13, 126.75 (2C), 129.32 (2C), 129.59, 130.20, 131.96 (2C), 132.06 (2C), 132.85, 144.62, 150.19, 151.11, 155.18. MS *m/z* (%) 352.15 (M$^+$+1, 12.53), 350.64 (M$^+$, 13.07), 40.77 (100). Anal. Calcd. For: $C_{18}H_{12}BrN_3$ (350.22): C, 61.73; H, 3.45; N, 12.00; found: C, 61.89; H, 3.59; N, 12.16.

*2.1.3.3. 7-(4-Nitrophenyl)-2-phenylpyrazolo[1,5-a]pyrimidine (**9c**)*

Yellow powder, 82% yield; mp 208-210ºC; IR (KBr) ν_{max}/cm^{-1} 1578, 1520 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 7.36 (d, *J* = 4.4 Hz, 1H, H3 pyrimidine), 7.39 (s, 1H, H4 of pyrazole), 7.44 (d, *J* = 7.1 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 2H, ArH), 8.05 (d, *J* = 7.5 Hz, 2H, ArH), 8.48 (q, *J* = 8.7 Hz, 4H, ArH), 8.66 (d, *J* = 4.4 Hz, 1H, H2 pyrimidine). ^{13}C NMR (DMSO-d_6) δ 94.28, 109.22, 123.99 (2C), 126.78 (2C), 129.32 (2C), 129.66, 131.44 (2C), 132.71, 137.10, 143.53, 149.02, 150.23, 151.04, 155.34. MS *m/z* (%) 316.30 (M$^+$, 29.05), 71.74 (100). Anal. Calcd. For: $C_{18}H_{12}N_4O_2$ (316.32): C, 68.35; H, 3.82; N, 17.71; found: C, 68.59; H, 4.03; N, 17.94.

*2.1.4. General procedure for the synthesis of pyrazolo[3,4-b]pyridines **15a, b***

A mixture of 3-oxo-3-arylpropanenitrile (**10**) (0.145 g, 1 mmol) with an equimolar amount of aldehydes **11a, b** and 1,3-diphenyl-1*H*-pyrazol-5-amine (**12**) (0.235 g, 1 mmol) in absolute ethanol (20 mL) was refluxed for 12 h. The formed precipitate was filtered, dried and recrystallized from EtOH to give pyrazolo[3,4-b]pyridines **15a, b**, respectively.

*2.1.4.1. 4-(4-Bromophenyl)-1,3,6-triphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**15a**)*

White powder, 61% yield; mp 248-250ºC; IR (KBr) ν_{max}/cm^{-1} 2222 (C≡N), 1589, 1551, 1493 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 7.11 – 7.21 (m, 4H, ArH), 7.30-7.37 (m, 3H, ArH), 7.4 2-7.50 (m, 4H, ArH), 7.62-7.66 (m, 5H, ArH), 7.99 – 8.06 (m, 2H, ArH), 8.30 (d, *J* = 8.0 Hz, 2H, ArH).^{13}C NMR (DMSO-d_6) δ 102.46, 112.46, 117.87, 122.23 (2C), 123.92, 127.60, 128.10 (2C), 128.84, 129.06 (2C), 129.44 (2C), 129.91 (2C), 129.95 (2C), 130.80, 131.41 (2C), 131.54, 131.94 (2C), 133.06, 138.02, 138.60, 147.26, 150.27, 151.77, 160.87. MS *m/z* (%) 528.35 (M$^+$ +1, 15.19), 527.58 (M$^+$, 43.91), 52.03 (100). Anal. Calcd. For: $C_{31}H_{19}BrN_4$ (527.43): C, 70.60; H, 3.63; N, 10.62; found: C, 70.78; H, 3.80; N, 10.81

*2.1.4.2. 4-(4-(Dimethylamino)phenyl)-1,3,6-triphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**15b**)*

White powder, 73% yield; mp 263-265ºC; IR (KBr) ν_{max}/cm^{-1} 2218 (C≡N), 1605, 1524, 1497 (C=N & C=C). ^{1}H NMR (DMSO-d_6) δ 2.91 (s, 6H, 2CH$_3$), 6.52 (d, *J* = 8.4 Hz, 2H, ArH), 7.13-7.20 (m, 7H, ArH), 7.23 – 7.31 (m, 1H, ArH), 7.40-7.44 (m, 1H, ArH), 7.59-7.64 (m, 6H, ArH), 7.97 – 8.03 (m, 2H, ArH), 8.29 (d, *J* = 8.1 Hz, 2H, ArH). ^{13}CNMR (DMSO-d_6) δ 101.98, 111.54 (2C), 112.18, 118.68, 120.54, 122.17 (2C), 127.37, 128.07 (2C), 128.48, 128.92 (2C), 129.38 (2C), 129.80 (2C), 129.97 (2C), 130.56, 131.26 (2C), 132.10, 138.39, 138.76, 147.54, 150.52, 151.74, 153.61, 161.19. MS *m/z* (%) 491.33 (M$^+$, 28.65), 40.67 (100). Anal. Calcd. For: $C_{33}H_{25}N_5$ (491.60): C, 80.63; H, 5.13; N, 14.25; found: C, 80.38; H, 4.19; N, 14.43.

2.2. Biological activity

2.2.1. Antimicrobial activity

Antimicrobial potential of tested compounds was determined by using Agar well-diffusion method [33].

2.2.2. In vitro cytotoxicity screening

Anti-cancer activities were done using MTT colorimetric assay [34].

2.3. Molecular docking studies

All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} with MMFF94X forcefield and the partial charges were automatically calculated.

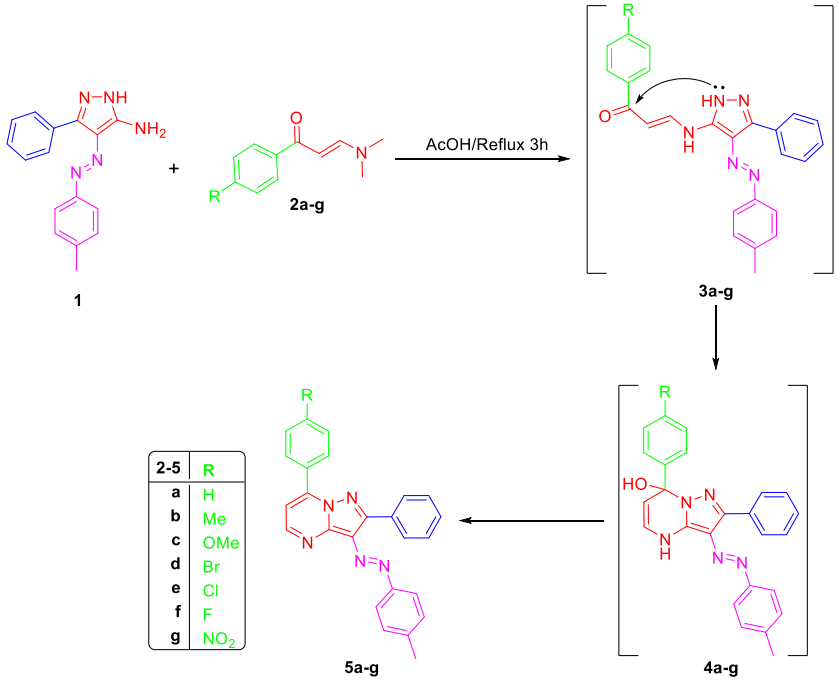
2.4. In-Silico SwissADME predictions

SwissADME is an online utility and availability for free [35, 36].

3. Results and discussion

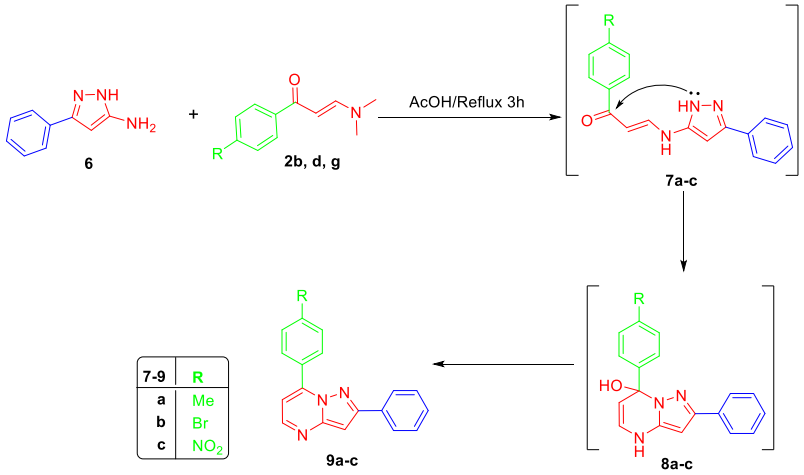
3.1. Chemistry

The synthesis of novel pyrazolo[1,5-*a*]pyrimidines **5a-g, 9a-c** and pyrazolo[3,4-*b*]pyridines **15, b** are showed in **Schemes 1-3**. In **Scheme 1**, the key compound 5-amino-4-(4-tolyl)azo-3-phenylpyrazole (**1**) [28] was reacted with enaminones **2a-g** [29] to yield the non-isolable intermediates **3a-g,** afterwards they were cyclized to produce the hudroxy intermediate **4a-g** that dehydrated rapidly to give the final targeted pyrazolo[1,5-*a*]pyrimidines **5a-g**. The ^{1}HNMR data of compounds **5a-g** revealed the presence of distinct signal of methyl proton of diazinyl side around 2.41 *ppm*. On the other hand, compounds **5a**, **b** characterized with distinct signal of methyl and methoxy protons of phenyl side at 2.46 *ppm* and 3.92 *ppm*, respectively. The ^{13}CNMR of **5a-g** exhibited the existence of a peak of methyl proton of diazinyl substitution around 21.40 *ppm*. In addition, compounds **5a**, **b**, revealed the signals of methyl and methoxy protons at 21.60 and 65.01 *ppm*, respectively.



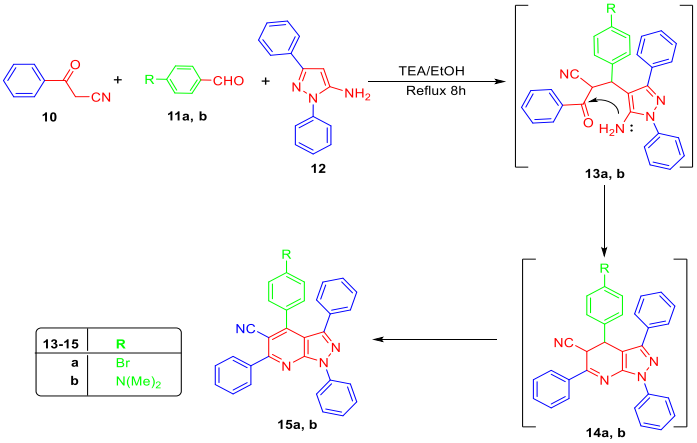
Scheme 1: Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives **5a-g**.

Furthermore, the treatment of 5-amino-1*H*-pyrazole (**6**) [30] with different enaminones (**2b, d, g**) in acetic acid afforded [1,5-*a*]pyrimidines **9a-c** (**Scheme 2**);. ^{1}HNMR data of compounds **9a-c** revealed the existence of three signals around 7.30 ppm, 7.36 ppm and 8.60 ppm due to H2 pyrimidine, H4 pyrazole and H3 pyrimidine, respectively, concerning compound **9a**, it characterized with one distinct signal of methyl proton at 2.44 ppm. Compound **9a** also distinguished by a unique signal of the same proton at 21.56 ppm regarding the spectra of ^{13}CNMR.



Scheme 2: Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives **9a-c**

Finally, cyclizing benzoylacetonitrile (**10**) [31] with phenyl hydrazine gave *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)amine (**12**) [32] (**Scheme 3**) which refluxed with different aldehydes **11a**, **b** and 3-oxo-3-phenylpropanenitrile **(10)** in ethanol/TEA for 8 hrs *via* one pot reaction to yield **13a**, **b**, which then converted to **14a**, **b** *via* cyclization and then dehydration to give the final pyrazolo[3,4-b]pyridines **15a, b**. The ^{1}HNMR of **15a**, **b**, characterized with distinct signal at 2.91 ppm resembling N(CH_3). Regarding the spectra of ^{13}CNMR, compound **5b** reflects the disappearance of dimethyl protons under the signal of DMSO.



Scheme 3: Synthesis of pyrazolo[3,4-b]pyridines **15a, b**

3.2. Biological activity

3.2.1. In vitro antimicrobial activity

The tested compounds displayed no or moderate antimicrobial activity towards six pathogenic bacteria (**Table 1**). Derivatives **5a**, **5b**, **5d**, **5f**, **5e** and **5g** displayed moderate antimicrobial activity against *Staphylococcus aureus* with inhibition zone (IZ) = 13, 11, 10, 11, 12 & 13 mm, respectively, when compared with *Gentamicin* (IZ = 24 mm). Derivatives **5a**, **5b**, **5c**, **5f**, **5g** and **9c** they revealed moderate inhibition activity against *Bacillus subtilis* with IZ = 12, 13, 13, 10, 11 and 9 mm, respectively related to *Gentamicin* (IZ = 26 mm). Compounds **9a**, **9b**, **9c**, **15a** and **15b** showed weak activity against *Escherichia coli ATCC 25922* with IZ = 12, 11, 10, 9 and 9 mm, respectively, when compared with *Gentamicin* (IZ = 30 mm). Moreover, they also revealed moderate inhibition activity against *Proteus vulgaris* with IZ = 11, 12, 11, 10 and 10 mm, respectively whereas *Gentamicin* revealed IZ = 25 mm (**Table 1**). The tested compounds not recorded any activity towards tested fungi *Aspergillus fumigatus* and *Candida albicans* (**Table 1)**.

3.2.2. In vitro anticancer activity

The anticancer activity of the newly synthesized compounds against human breast cancer cell line MCF-7, Lung carcinoma A-549 and promyelocytic Leukemia HL-60 showed a range of anti-tumor activity when compared with cisplatin (**Table 2**). From IC_{50} values of **5a-g**, we can deduce that compound **5d** gave IC_{50}=7.19±0.34 µM activity against Lung carcinoma cells almost the same of Cisplatin (IC_{50}=7.48±0.56 µM). From the SAR point of view, considering the substitutions around pyrazolo[1,5-*a*]pyrimidine system, we found that the potency of **5d** may be attributed to the substitution 4-bromophenyl in position 7. Th comparison between the structure of **5d** and **9b** confirmed the importance of 4-tolyldiazenyl in position 3 of **5d** for its anticancer potency.

3.3. Molecular docking studies

*3.3.1. Docking of compound **5d** on CDK2 and CDK9 active site*

The 3D co-ordinates of CDK2 and CDK9 (PDB IDs 3tnw and 3tn8) have excellent resolution of 2 Å and 2.95 Å for 3tnw and 3tn8, respectively. Pose retrieval step of the co-crystallized ligands = 0.265 and 0.4337 Å between the docked and the co-crystallized poses for CAN508, with energy score = -11.7195 and -10.045 kcal/mol, for CAN508 in CDK2 and CDK9, respectively.

*3.3.1.1. Docking of **5d** on CDK2 active site*

The binding visual inspection of CAN508 with CDK2 active site showed the formation of two H-bonds with residues Asp145 and Leu83, and two pi H-bonds with Val18 and Leu134 (**Figure 1**).

Table 1: Antimicrobial activity by well diffusion method for the synthesized compounds against pathogenic microbes.

Compounds	*Staphylococcus aureus ATCC 25923*	*Bacillus subtilis (1) NRRL B-543 RCMB 015*	*Escherichia coli ATCC 25922*	*Proteus vulgaris (1) ATCC 13315 RCMB 004*	*Aspergillus fumigatus (RCMB 002008)*	*Candida albicans RCMB 005003 (1) ATCC 10231*
5a	13	12	NA	NA	NA	NA
5b	11	13				
5c	NA	13				
5d	10	NA				
5e	11	NA				
5f	12	10				
5g	13	11				
9a	NA	NA	12	11		
9b		NA	11	12		
9c		9	10	11		
15a		NA	9	10		
15b		NA	9	10		
Gentamicin	24	26	30	25	-	-
Ketoconazole	-	-	-	-	17	20

Table 2. Anticancer activity of the tested compounds

Compound	Lung carcinoma A-549	Breast carcinoma MCF-7	promyelocytic Leukemia- HL-60
5a	64.75±4.91	108.87±5.85	123.86±7.06
5b	116.76±6.84	122.45±6.09	183.86±9.48
5c	30.61±1.53	61.24±3.27	75.99±5.72
5d	7.19±0.34	50.26±3.48	56.04±0.32
5e	46.03±3.87	84.12±6.27	56.04±3.41
5f	46.30±3.69	79.90±6.18	97.15± 6.93
5g	31.05±2.07	90.81±6.85	45.31± 3.04
9a	143.88±7.84	202.09±9.42	223.07±10.43
9b	133.65±7.69	161.02±7.04	205.49±9.93
9c	173.11±8.52	207.21±8.97	225.39±8.49
15a	60.31±4.73	70.50±6.49	105.85±7.23
15b	196.75±9.47	225.71±9.73	224.99±9.43
Cisplatin	7.48±0.56	5.67±0.45	16.75±0.91

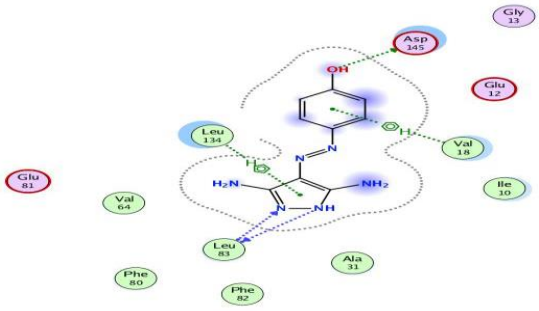
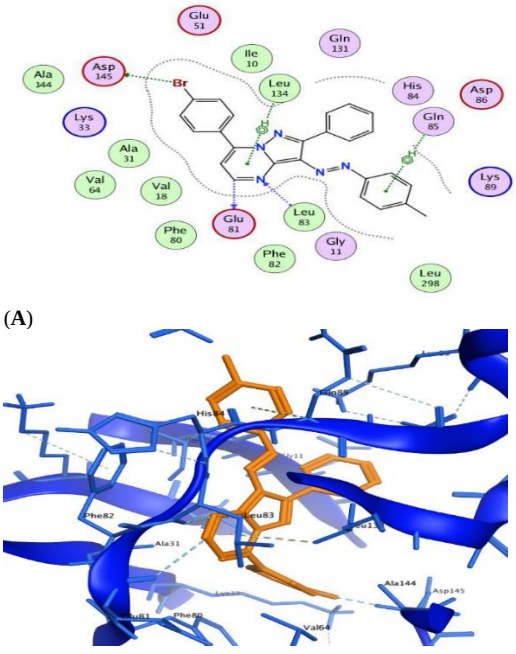


Figure 1: The 2D binding diagram of CAN508 in the active site of CDK2 (PDB: 3tnw).

Next, **5d** showed good docking scores (-12.7053 kcal/mol) comparing CAN508 (-11.7195 kcal/mol). Compound **5d** interact with the binding site through 3 H-bonds with Leu83, Asp145 and Glu81 and 2 pi-hydrogen interactions with Leu134 and Gln85 which indicate good fitting for **5d** in CDK2 binding site (**Figure 2**).



(**B**)

Figure 2. Binding interactions diagrams, 2D (**A**) and 3D (**B**), for **5d**

in the active site of CDK2 (PDB: 3tnw).

*3.3.1.2. Docking of **5d** on CDK9 active site*

Compound **5d** was illustrated good docking scores (-10.2998 kcal/mol) when compared with CAN508 (-10.045 kcal/mol). CAN508 exerts its CDK9 inhibitions activity through formation of H-bond with Lys48 and pi-hydrogen interaction with Val33 (**Figure 3**).

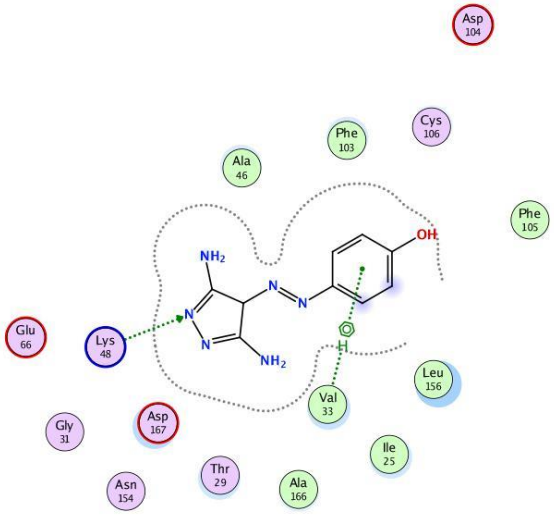
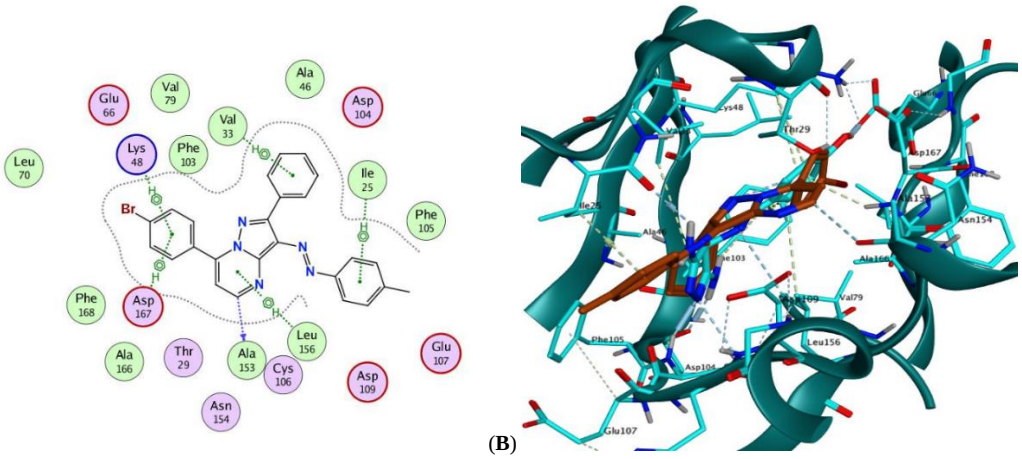


Figure 3. The binding 2D diagram of CAN508 in the active site of CDK9 (PDB: 3tn8).

Docking of compound **5d** showed good fitting for **5d** in CDK9 binding site where **5d** interact with the binding site through a hydrogen bond with Ala153 residue and 5 pi-hydrogen interactions Lys48, Asp167, Ile25, Val33 and Leu156 (**Figure 4**).



(**A**) (**B**)

Figure 4: Binding interactions diagrams, 2D (**A**) and 3D (**B**), for **5d** in the CDK9 active site (PDB: 3tn8).

*3.3.2. Docking of **5a** and **5g** on DHFR active site*

The 3D structure of 2W9S contains DHFR (resolution = 1.80 Å) in complex with trimethoprim as antibacterial agent against *staphylococcus aureus* is used for the docking studies. Pose retrieval of the co-crystallized ligand = 0.5054 Å between the docked and the co-crystallized poses for trimethoprim, indicating a valid docking protocol. This step gave an energy score of -16.0764 kcal/mol, for trimethoprim in DHFR. The trimethoprim binding with active site of DHFR, showed the formation of H-bond with Phe92, and two pi-hydrogen bonds with Ile5 and Asp27 (**Figure 5**).

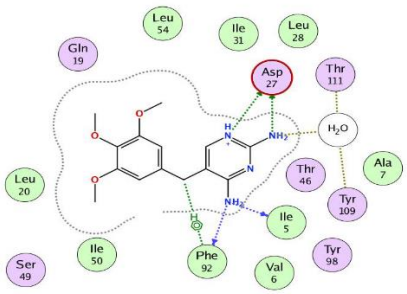


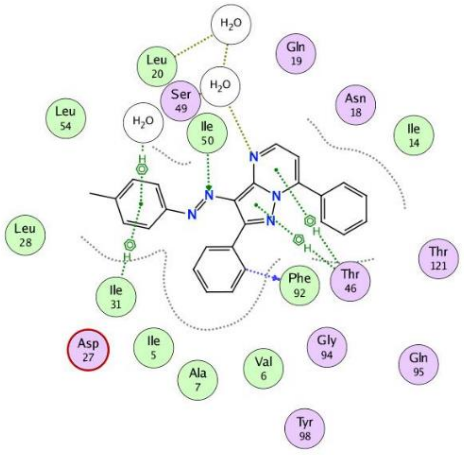
Figure 5. The binding 2D diagram for trimethoprim in the active site of DHFR (PDB: 2W9S).

Docking of **5a** and **5g** in the active site of DHFR showed good docking scores (-16.5266 and -16.5150 kcal/mol, respectively) when compared with trimethoprim (-16.0764 kcal/mol). Compound **5a** interact with the binding site through 2 hydrogen bonds with Phe92 and Ile50 residues, water bridges with Leu20 and Ser49 and pi-hydrogen interactions Ile31and Thr46 (**Figure 6**). Compound **5g** interact with the binding site through 4 hydrogen bonds with Phe92, Ile32 and Ser49 and 3 pi-hydrogen interactions with Leu20 and Thr46; which indicate good fitting for both compounds in DHFR binding site (**Figure 7**).

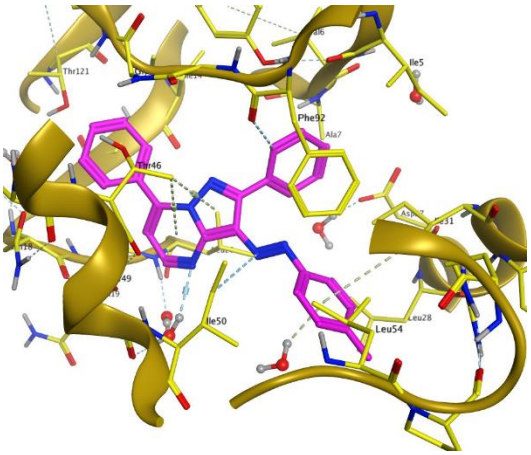
*3.4. In-Silico SwissADME predictions for physiological and pharmacokinetic properties of **5a-g**, **9a-c** and **15a, b**.*

SwissADME model for **5a-g**, **9a, b** and **15a-c** suggested that all compounds obey Veber rule (**Table 3**) with no violations. Derivatives **5c**, **5g** and **9a-c** have no violations in Lipinski's rule of 5 while the rest compounds have 1 violation except **6a** which have 2. All compounds represented acceptable range of clogP values around 2.00-6.50. All compounds have poor solubility (p. s.) except compounds **9a-c** have moderate solubility (m. s.). None of the molecules were blood brain barrier (BBB) penetrant except **9a-c**. Compounds **5a** and **9a-c** have good GI absorption while the rest compounds have low GI absorption. **6a-b**, **9a** and **9b** have zero pain and brenk alerts while **5g** have one pain and three Brenk alerts and the rest of compounds have one pain and brenk alert except **9c** which has no Pain alert but has two brenk alerts (**Table 3**).

The spread of the tested compounds on BOILED-Egg (**Figure 8**) showed that all compounds are non-BBB penetrant except **9a-9c**. All compounds indicate poor GI absorption except **5a** and **5c**. All compounds were predicted to be not substrate for P-gp (PGP-) (red dot) except **9a** was subjected to the active efflux P-gp pump (PGP+) (blue dot).

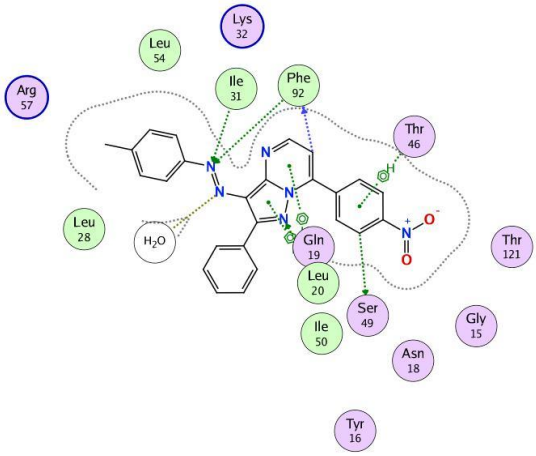


(**A**)

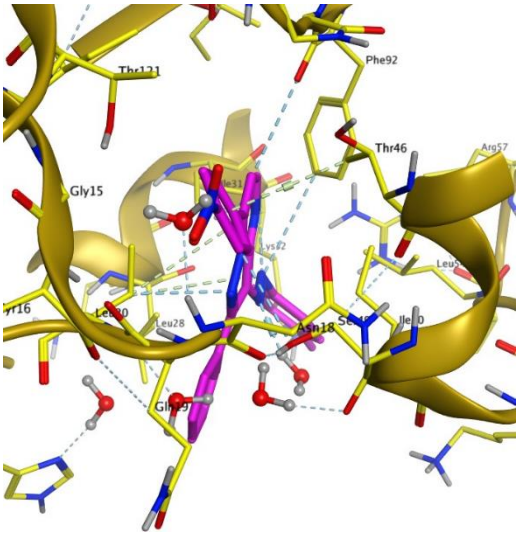


(**B**)

Figure 6. Diagrams, 2D (**A**) and 3D (**B**), for the binding interactions of **5a** in the active site of DHFR (PDB: 2W9S).



(**A**)



(**B**)

Figure 7. Diagrams, 2D (**A**) and 3D (**B**), for the binding interactions of **5g** in the active site of DHFR (PDB: 2W9S).

Table 3. SwissADME predictions for the synthesized compounds.

Compound	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA	Consensus Log P	ESOL Class	GI absorption	BBB permeant	Pgp substrate	Lipinski #violations	Veber #violations	Bioavailability Score	PAINS #alerts	Brenk #alerts
5a	0.04	4	4	0	119.44	54.91	5.33	Poorly soluble	High	No	No	1	0	0.55	1	1
5b	0.08	4	4		124.41	54.91	5.67		Low			1			1	1
5c	0.08	5	5		125.93	64.14	5.27					0			1	1
5d	0.04	4	4		127.14	54.91	5.94					1			1	1
5e	0.04	4	4		124.45	54.91	5.86					1			1	1
5f	0.04	4	5		119.4	54.91	5.64					1			1	1
5g	0.04	5	6		128.26	100.73	4.58					0			1	3
5h	0	4	4		127.19	54.91	6.14					1			1	1
5i	0	5	6		128.31	100.73	4.93					1			1	3
9a	0.05	2	2		88.82	30.19	3.84	m. s.	High	Yes	Yes	0			0	0
9b	0	2	2		91.56	30.19	4.13	m. s.	High	Yes	No	0			0	0
9c	0	3	4		92.68	76.01	2.91	m. s.	High	Yes	No	0			0	2
15a	0	4	3		147.59	54.5	6.65	p. s.	Low	No	No	2			0	0
15b	0.06	5	3		154.09	57.74	6.03	p. s.	Low	No	No	1			0	0

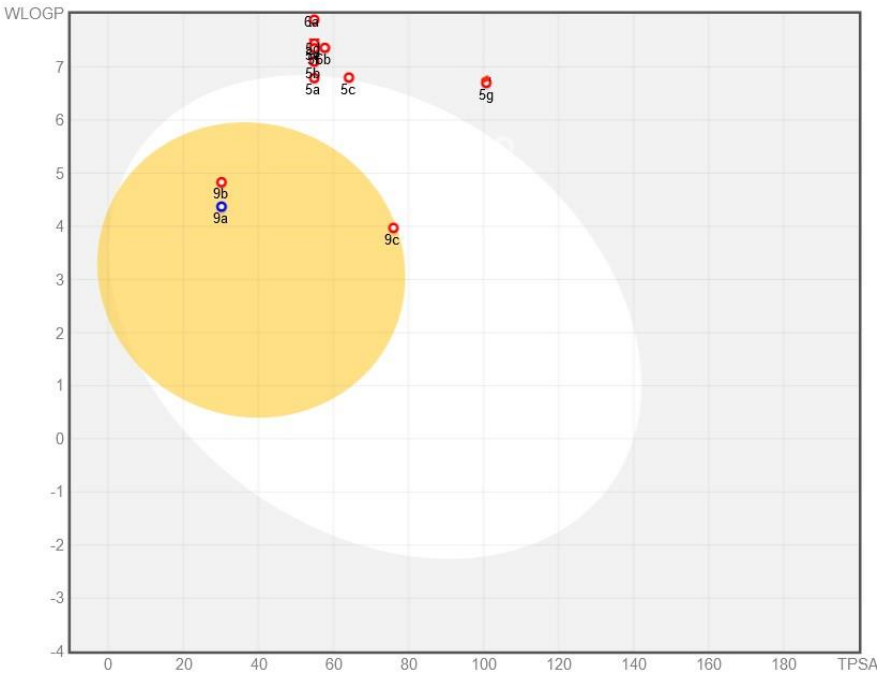


Figure 8. BOILED-Egg plot for the synthesized compounds.

4. Conclusion

A new set of pyrazolo[1,5-a]pyrimidine compounds **5a-g**, **9a-c** and pyrazolo[3,4-*b*]pyridines **15a, b** was synthesized. The newly synthesized compounds were tested *in vitro* for their antimicrobial and anticancer activities. Pyrazolo[1,5-*a*]pyrimidine derivatives **5a** and **5g** revealed moderate antibacterial activity. Pyrazolo[1,5-*a*]pyrimidine **5d** produced significant anticancer activity toward A-549 lung carcinoma cell lines. Docking studies were done to predict their anti-cancer potentials. Based on the results of this study, the synthesized pyrazolo[1,5-*a*]pyrimidines need further research in the future to discover their potentiality.

5. Conflict of Interest

The authors declare that they have no conflict of interest.

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