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Syntheses and in silico pharmacokinetic predictions of glycosylhydrazinyl-pyrazolo[1,5-c]pyrimidines and pyrazolo[1,5-c]triazolo[4,3-a]pyrimidines as anti-proliferative agents

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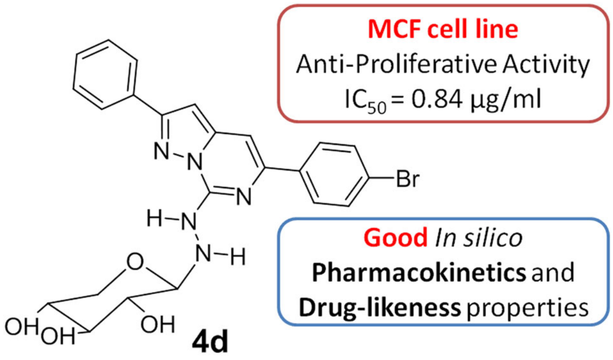
Abstract

New glycosylhydrazinyl-pyrazolo[1,5-c]pyrimidines were synthesized by the reaction of respective 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidines (**1a-d**) with glucose, galactose, and xylose in ethanol. Their glycopyranosyl structures were reasoned to be in chair conformations and each have hydrazine moiety in the β-configuration. Also, pyrazolo[1,5-c]triazolo[4,3-a]pyrimidines derivatives were synthesized by the reaction of **1a-d** with benzoic acid in the presence of phosphorousoxy chloride or by the reaction with benzaldehyde derivatives followed by cyclization in the presence of bromine. All structures of the compounds were confirmed from their IR, ^{1}H, ^{13}C, DPET-135°, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{13}C-^{1}H HMBC spectra and microanalysis. The synthesized compounds showed inhibition of proliferation of MCF-7 human breast cancer cells with IC_{50} values ranging from 0.56 to 8.86 μg/ml. Some of the most active compounds showed acceptable predicted pharmacokinetics and drug-likeness properties.

Graphical Abstract



N²-(β-D-Xylopyranosyl)-N¹-7-(5-(4-bromophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl

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Keywords Glycosylhydrazine · yrazolo[1,5-c]triazolo[4,3-a]pyrimidines · 5-aryl-2-phenyl-7-(2-benzylidenehydrazinyl) pyrazolo[1,5-c]pyrimidines · Cytotoxicity

Introduction

Derivatives of Pyrazolopyrimidines received considerable attention because of their importance in various pharmaceutical chemistry applications, especially for their similarities with natural purines (Demirbas et al. 2004; Romeiro et al. 2009; Rahman et al. 2005; Abdel-Rahman and Farghaly 2004). For instance, they have been reported to show a diversity of biological activity such as antitumor (Dimmock et al. 2000; Tapia et al. 1967; Sava et al. 1985; Xia et al. 2008; Melnyk et al. 2006), antibacterial (Ajani et al. 2010), antimicrobial (Zheng et al. 2009; Bhagavan 2002; Saulnier et al. 2005; Short 1962; Holdiness 1987), hypnotic (Foroumadi et al. 2003), insecticidal (Mohareb et al. 2010), antileukemic (Melnyk et al. 2006), and inhibitors of kinases (Mamolo et al. 2001; Ulusoy et al. 2001; Ergenç et al. 1998; Xia et al. 1997). Moreover some pyrazolopyrimidines have growth inhibitory activity for several human cancer cell lines besides being active against various molecular targets (Cheng and Robins 1956; Markwalder et al. 2004; Krystof et al. 2006; Peat et al. 2004a; Peat et al. 2004b; Di Grandi et al. 2009; Wang et al. 2009).

We focus in this study to demonstrate chemical aspects for glycosylhydrazinyl-pyrazolo[1,5-c]pyrimidine derivatives and pyrazolo[1,5-c]triazolo[4,3-a]pyrimidine derivatives. This complies the applied strategies for their synthesis and their structural elucidation via IR, ^{1}H, ^{13}C, DPET-135°, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{13}C-^{1}H HMBC spectra, and microanalysis. To test their antiproliferative power, they were screened against MCF-7 human breast cancer cells. In addition, the most active compounds were subjected for in silico predictions for their pharmacokinetics and drug-likeness properties.

Materials and methods

Chemistry

Melting points were determined on a Kofler Block and are uncorrected. Elemental analyses were carried out in the micro analytical laboratory of the faculty of science, Cairo University. The infrared (IR) spectra of compounds were recorded on a Tensor 37 Bruker infrared spectrophotometer as potassium bromide pellets and frequencies are reported in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM ECA 500 MHz spectrometer and Bruker AC (500 MHz) spectrometer. Chemical shifts δ are in ppm and Hz relative to tetramethylsilane as internal standard. Reactions were routinely followed by thin-layer chromatography using Merck Kiesel gel; 60-F254-recoated plastic plates. The spots were detected by UV lamp.

N^{2}-(β-D-glycopyranosyl)-N^{1}-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (2a-d), (3a-d), and (4a-d)

General procedure A mixture of 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidine (**1a-d**) (1mmol) and aldoses (3mmol) in ethanol (20 ml) was heated under reflux for 2 h. The solid products were washed with water and ethanol, dried, and recrystallized from a mixture of methanol and chloroform as colorless needles.

N^{2}-(β-D-Glucopyranosyl)-N^{1}-7-(2,5-diphenylpyrazolo[1,5-c]pyrimidin-7-yl) (2a)

Reaction time 2 h (0.54 g, 39% yield); mp 217–219 °C; R$_f$ 0.3 (9:1 CHCl$_3$-MeOH); IR: 1624 (pyrazolo ring C=N), 1590 (pyrimidine C=N), and 1455 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO; dimethyl sulphoxide): $\delta = 3.02$–3.09 (m,2H, H-3' and H-2'), 3.17 (pt, 1H, H-5'), 3.21–3.25 (m, 1H, H-4'), 3.44–3.49 (m, 1H, H-6'), 3.68–3.70 (m, 1H, H-6''), 4.07 (dd, 1H, $J_{1',2'} = 8.4$ Hz, $J_{1',NH} = 2.3$ Hz, H-1'), 4.47 (t, 1H, $J_{OH-6',6'} = 5.4$ Hz, $J_{OH-6',6''} = 6.2$ Hz, OH-6'), 4.92 (d, 1H, $J_{OH-3'} = 5.4$ Hz, OH-3'), 4.94 (d, 1H, $J_{OH-4'} = 3.6$ Hz, OH-4'), 5.45 (d, 1H, $J_{OH-2'} = 3.1$ Hz, OH-2'), 6.23 (s, 1H, HN-sugar), 7.05 (s, 1H, pyrazole-H), 7.37–7.49 (m, 6H, aromatic-H), 7.57 (s, 1H, pyrimidine-H), 8.06 (d, 2H, aromatic-H), 8.11 (d, 2H, aromatic-H), and 8.80 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta_C = 61.8$ (C-6′), 70.9 (C-3′), 71.0 (C-2′), 76.7 (C-4′), 78.3 (C-5′), 90.6 (C-1′), 95.3 (C-pyrazole), 98.1 (C-pyrimidine), 126.8, 127.0, 127.2, 129.0, 129.2, 129.3, 129.5, 132.50 and 138.0 (C-Ar), 143.9 (C-3a pyrazole), 146.9 (C-2), 146.9 (C-5), and 155.2 (C-7). Anal. Calcd for $C_{24}H_{25}N_5O_5$: C, 67.84; H, 4.92; N, 10.79. Found: C, 67.74; H, 5.02; N, 10.69.

N^{2}-(β-D-Glucopyranosyl)-N^{1}-7-(2-phenyl-5-(p-tolyl)pyrazolo[1,5-c]pyrimidin-7-yl) (2b)

Reaction time 2 h (1.3 g, 85% yield); mp 212–214 °C; R$_f$ 0.23 (9:1 CHCl$_3$-MeOH)); IR: 1621 (pyrazolo ring C=N), 1597 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 2.32$ (s, 3H, CH$_3$), 3.00–3.08 (m,2H, H-3' and H-2'), 3.15–3.18 (m, 1H, H-5'), 3.21–3.25 (m, 1H, H-4'), 3.44–3.49 (m, 1H, H-6'), 3.68-3.71 (m, 1H, H-6''), 4.07 (d,1H, $J_{1'.2'} = 8.4$ Hz, H-1'), 4.49 (t, 1H, $J_{OH\text{-}6'.6'} = 4.6$ Hz, $J_{OH\text{-}6'.6''} = 6.1$ Hz, OH-6'), 4.94 (d, 2H, OH-3' and OH-4'), 5.47 (d, 1H, OH-2'), 6.23 (s, 1H, HN-sugar), 7.01 (s, 1H, pyrazole-H), 7.24 (d, 2H, aromatic-H), 7.41 (t, 1H, aromatic-H), 7.47 (t, 2H, aromatic-H), 7.51 (s, 1H, pyrimidine-H), 7.98 (d, 2H, aromatic-H), 8.05 (d, 2H, aromatic-H), and 8.76 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta_C = 21.3$ (C-CH$_3$), 61.6 (C-6′), 70.8 (C-3′), 71.1 (C-2′), 76.6 (C-4′), 78.3 (C-5′), 90.7 (C-1′), 95.0 (C-pyrazole), 97.8 (C-pyrimidine), 126.7, 126.9, 126.9, 127.1, 129.5, 130.2, 132.5,135.2, 139.0 (C-Ar), 144.0 (C-3a pyrazole), 146.4 (C-2), 146.9 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{25}H_{27}N_5O_5$: C, 62.88; H, 5.70; N, 14.67. Found: C, 62.98; H, 5.80; N, 14.37.

N^2-(β-D-Glucopyranosyl)-N^1-7-(5-(4-chlorophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (2c)

Reaction time 2 h (0.95 g, 63% yield); mp 206 °C; R_f 0.34 (9:1 CHCl$_3$-MeOH); IR: 1621 (pyrazolo ring C=N), 1597 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 3.05$–3.14 (m,2H, H-3' and H-2'), 3.18–3.22 (m, 1H, H-5'), 3.25–3.28 (m, 1H, H-4'), 3.47–3.52 (m, 1H, H-6'), 3.71–3.75 (m, 1H, H-6''), 4.11 (d,1H, $J_{1'.2'} = 8.4$ Hz, $J_{1',HN} = 2.8$ Hz, H-1'), 4.48 (t, 1H, $J_{OH\text{-}6'.6'} = 5.7$ Hz, $J_{OH\text{-}6'.6''} = 5.7$ Hz, OH-6'), 4.94 (d, 1H, $J_{OH\text{-}3'} = 5.4$ Hz, OH-3'), 4.95 (d, 1H, $J_{OH\text{-}4'} = 5$ Hz, OH-4'), 5.40 (d, 1H, $J_{OH\text{-}2'} = 3.3$ Hz, OH-2'), 6.23 (d, 1H, $J_{HN,1'} = 2.7$ Hz, HN-sugar), 7.09 (s, 1H, pyrazole-H), 7.24 (d, 2H, aromatic-H), 7.41 (t, 1H, aromatic-H), 7.47 (t, 2H, aromatic-H), 7.52–7.54 (m, 4H, aromatic-H), 7.64 (s, 1H, pyrimidine-H), 8.10 (d, 2H, aromatic-H), 8.19 (d, 2H, aromatic-H), and 8.89 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta_C = 61.7$ (C-6′), 70.7 (C-3′), 71.2 (C-2′), 77.0 (C-4′), 78.5 (C-5′), 90.9 (C-1′), 95.8 (C-pyrazole), 98.3 (C-pyrimidine), 127.0, 127.8, 126.9, 129.0, 129.2, 129.6, 132.4, 134.0, 136.8 (C-Ar), 143.7 (C-3a pyrazole), 145.0 (C-2), 146.7 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{24}H_{24}ClN_5O_5$: C, 57.89; H, 4.86; N, 14.06. Found: C, 57.74; H, 4.86; N, 13.81.

N^2-(β-D-Glucopyranosyl)-N^1-7-(5-(4-bromophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl (2d)

Reaction time 2 h (1.15 g, 80% yield); mp 201–202 °C; R_f 0.37 (9:1 CHCl$_3$-MeOH)); IR: 1621 (pyrazolo ring C=N), 1596 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 3.08$–3.15 (m,2H, H-3' and H-2'), 3.19–3.22 (m, 1H, H-5'), 3.25–3.28 (m, 1H, H-4'), 3.50–3.52 (m, 1H, H-6'), 3.72–3.75 (m, 1H, H-6''), 4.12 (d,1H, $J_{1'.2'} = 8.8$ Hz, $J_{1',HN} = 2.6$ Hz, H-1'), 4.49 (t, 1H, $J_{OH\text{-}6'.6'} = 5.7$ Hz, $J_{OH\text{-}6'.6''} = 5.7$ Hz, OH-6'), 4.94 (d, 1H, $J_{OH\text{-}3'} = 5.4$ Hz, OH-3'), 4.96 (d, 1H, $J_{OH\text{-}4'} = 5$ Hz, OH-4'), 5.40 (d, 1H, $J_{OH\text{-}2'} = 3.2$ Hz, OH-2'), 6.23 (d, 1H, $J_{HN,1'} = 2.5$ Hz, HN-sugar), 7.09 (s, 1H, pyrazole-H), 7.45 (t, 1H, aromatic-H), 7.51 (t, 2H, aromatic-H), 7.63 (s, 1H, pyrimidine-H), 7.66 (d, 2H, aromatic-H), 8.11 (t, 4H, aromatic-H), and 8.89 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta_C = 61.7$ (C-6′), 70.7 (C-3′), 71.2 (C-2′), 77.0 (C-4′), 78.6 (C-5′), 90.9 (C-1′), 95.8 (C-pyrazole), 98.3 (C-pyrimidine), 122.7, 127.0, 128.9, 129.2, 129.6, 132.0, 132.4, 137.2, (C-Ar), 143.7 (C-3a pyrazole), 145.1 (C-2), 146.7 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{24}H_{24}BrN_5O_5$: C, 53.15; H, 4.46; N, 12.91. Found: C, 53.15; H, 4.61; N, 12.73.

N^2-(β-D-Galacopyranosyl)-N^1-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (3a-d)

N^2-(β-D-Galactopyranosyl)-N^1-7-(2,5-diphenylpyrazolo[1,5-c]pyrimidin-7-yl) (3a)

Reaction time 2 h (1 g, 72% yield); mp 198–119 °C; R_f 0.27 (9:1 CHCl$_3$-MeOH)); IR: 1623 (pyrazolo ring C=N), 1566 (pyrimidine C=N), and 1455 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 3.37$–3.46 (m, 3H, H-2', H-3′ and H-5′), 3.53–3.55 (m, 2H, H-6' and H-6′′), 3.62–3.64 (m, 1H, H-4′), 4.01 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.40 (d, 1H, $J_{OH\text{-}4'} = 3.8$ Hz, OH-4′), 4.70 (t, 1H, $J_{OH\text{-}6',6'} = 4.6$ Hz, $J_{OH\text{-}6',6''} = 6.1$ Hz, OH-6′), 4.79 (d, 1H, $J_{OH\text{-}3'} = 5.4$ Hz, OH-3′), 5.40 (s, 1H, OH-2′), 6.16 (s, 1H, HN-sugar), 7.03 (s, 1H, pyrazole-H), 7.37–7.48 (m, 6H, aromatic-H), 7.53 (s, 1H, pyrimidine-H), 8.03 (d, 2H, aromatic-H), 8.07 (d, 2H, aromatic-H), and 8.81 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 61.1$ (C-6′), 68.8 (C-2′), 69.0 (C-4′), 73.6 (C-3′), 77.2 (C-5′), 91.6 (C-1′), 95.3 (C-pyrazole), 98.1 (C-pyrimidine), 126.7, 126.9, 127.0, 127.1, 129.1, 129.3, 129.5, 132.4, 138.0 (C-Ar), 143.9 (C-3a pyrazole), 146.4 (C-2), 147.0 (C-5), and 155.2 (C-7). Anal. Calcd for $C_{24}H_{25}N_5O_5$: C, 67.84; H, 4.92; N, 10.79. Found: C, 67.65; H, 4.99; N, 10.56.

N^2-(β-D-Galactopyranosyl)-N^1-7-(2-phenyl-5-(p-tolyl)pyrazolo[1,5-c]pyrimidin-7-yl) (3b)

Reaction time 2 h (1.3 g, 85% yield); mp 202–204 °C; R_f 0.33 (9:1 CHCl$_3$-MeOH)); IR: 1621 (pyrazolo ring C=N), 1597 (pyrimidine C=N), and 1456 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 2.32$ (s, 1H, CH$_3$), 3.38–3.45 (m, 3H, H-2', H-3′ and H-5′), 3.50–3.58 (m, 2H, H-6' and H-6′′), 3.63 (t, 1H, $J_{4',3'}$ 3.8 Hz, $J_{OH\text{-}4'} = 3.8$ Hz, H-4′), 4.01 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.33 (d, 1H, $J_{OH\text{-}4'} = 4.6$ Hz, OH-4′), 4.56 (t, 1H, $J_{OH\text{-}3',6'} = 5.4$ Hz, $J_{OH\text{-}6',6''} = 6.1$ Hz, OH-6′), 4.75 (d, 1H, $J_{OH\text{-}2'} = 5.4$ Hz, OH-3′), 5.35 (d, 1H, $J_{OH\text{-}2'} = 3.1$ Hz, OH-2′), 6.17 (s, 1H, HN-sugar), 7.01 (s, 1H, pyrazole-H), 7.24 (d, 2H, aromatic-H), 7.42 (t, 1H, aromatic-H), 7.48 (t, 2H, aromatic-H), 7.50 (s, 1H, pyrimidine), 7.98 (d, 2H, aromatic-H), 8.05 (d, 2H, aromatic-H), and 8.73 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 21.3$ (C-CH_3), 61.0 (C-6′), 68.6 (C-2′), 69.0 (C-4′), 73.7 (C-3′), 77.2 (C-5′), 91.6 (C-1′), 95.0 (C-pyrazole), 97.6 (C-pyrimidine), 126.7, 126.9, 127.1, 129.4, 129.6, 130.1, 132.6, 135.3, 138.9 (C-Ar), 143.9 (C-3a pyrazole), 146.5 (C-2), 146.9 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{25}H_{27}N_5O_5$: C, 62.88; H, 5.70; N, 14.67. Found: C, 62.71; H, 5.90; N, 14.45.

N^2-(β-D-Galatcopyranosyl)-N^1-7-(5-(4-chlorophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (3c)

Reaction time 2 h (0.97 g, 70% yield); mp 210–212 °C; R_f 0.41 (9:1 $CHCl_3$-MeOH); IR: 1620 (pyrazolo ring C=N), 1597 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 3.41$–3.52 (m, 3H, H-2', H-3′ and H-5′), 3.55–3.63 (m, 2H, H-6' and H-6′′), 3.69 (t, 1H, $J_{4',3'}$ 3.9 Hz, $J_{OH\text{-}4'} = 3.1$ Hz, H-4′), 4.07 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.36 (d, 1H, $J_{OH\text{-}4'} = 4.3$ Hz, OH-4′), 4.56 (t, 1H, $J_{OH\text{-}3',6'} = 5.5$ Hz, $J_{OH\text{-}6',6''} = 5.5$ Hz, OH-6′), 4.76 (d, 1H, $J_{OH\text{-}2'} = 5.6$ Hz, OH-3′), 5.28 (d, 1H, $J_{OH\text{-}2'} = 2.9$ Hz, OH-2′), 6.18 (d, 1H, $J_{HN\text{-}1'} = 2.1$ Hz, HN-sugar), 7.08 (s, 1H, pyrazole-H), 7.37–7.53 (m, 5H, aromatic-H), 7.61 (s, 1H, pyrimidine), 8.09 (d, 2H, aromatic-H), 8.18 (d, 2H, aromatic-H), and 8.87 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 61.0$ (C-6′), 68.5 (C-2′), 68.8 (C-4′), 73.8 (C-3′), 77.1 (C-5′), 91.7 (C-1′), 95.7 (C-pyrazole), 98.2 (C-pyrimidine), 127.0, 128.6, 128.7, 129.0, 129.2, 129.6, 132.4, 133.9, 136.9 (C-Ar), 143.7 (C-3a pyrazole), 145.0 (C-2), 146.7 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{24}H_{24}ClN_5O_5$: C, 57.89; H, 4.86; N, 14.06. Found: C, 57.59; H, 4.70; N, 13.77.

N^2-(β-D-Galatcopyranosyl)-N^1-7-(5-(4-bromophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl (3d)

Reaction time 2 h (1.3 g, 96% yield); mp 209–211 °C; R_f 0.44 (9:1 $CHCl_3$-MeOH); IR: 1620 (pyrazolo ring C=N), 1596 (pyrimidine C=N), and 1451 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 3.41$–3.49 (m, 3H, H-2', H-3′ and H-5′), 3.55–3.63 (m, 2H, H-6' and H-6′′), 3.69 (t, 1H, $J_{4',3'}$ 3.9 Hz, $J_{OH\text{-}4'} = 3.1$ Hz, H-4′), 4.06 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.37 (d, 1H, $J_{OH\text{-}4'} = 4.3$ Hz, OH-4′), 4.57 (t, 1H, $J_{OH\text{-}3',6'} = 5.5$ Hz, $J_{OH\text{-}6',6''} = 5.5$ Hz, OH-6′), 4.77 (d, 1H, $J_{OH\text{-}2'} = 5.5$ Hz, OH-3′), 5.29 (s, 1H, OH-2′), 6.18 (d, 1H, HN-sugar), 7.08 (s, 1H, pyrazole-H), 7.45 (t, 1H, aromatic-H), 7.51 (t, 2H, aromatic-H), 7.61 (s, 1H, pyrimidine), 8.09 (d, 2H, aromatic-H), 8.11 (d, 2H, aromatic-H), and 8.88 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 61.0$ (C-6′), 68.5 (C-2′), 68.8 (C-4′), 73.8 (C-3′), 77.1 (C-5′), 91.7 (C-1′), 95.8 (C-pyrazole), 98.2 (C-pyrimidine), 122.7, 127.0, 128.7, 128.9, 129.2, 129.6, 131.9, 132.4, 137.2 (C-Ar), 143.7 (C-3a pyrazole), 145.1 (C-2), 146.7 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{24}H_{24}BrN_5O_5$: C, 53.15; H, 4.46; N, 12.91. Found: C, 52.87; H, 4.28; N, 12.61.

N^2-(β-D-Xylopyranosyl)-N^1-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (4a-d)

N^2-(β-D-Xylopyranosyl)-N^1-7-(2,5-diphenylpyrazolo[1,5-c]pyrimidin-7-yl) (4a)

Reaction time 2 h (1.28 g, 88% yield); mp 199–200 °C; R_f 0.54 (9:1 $CHCl_3$-MeOH); IR: 1623 (pyrazolo ring C=N), 1597 (pyrimidine C=N), and 1456 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 3.11$–3.17 (m, 2H, H-5', and H-2′), 3.24 (ddd, 1H, $J_{3',4'} = 8.6$ Hz, $J_{3',2'} = 8.6$ Hz, $J_{OH\text{-}3'} = 4.1$ Hz, H-3′), 3.28–3.34 (m, 1H, H-4′), 3.78 (dd, 1H, $J_{5'',4'} = 5.2$ Hz, $J_{5'',5'} = 11.2$ Hz, H-5"), 4.08 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{1',HN} = 2.7$ Hz, H-1′), 4.99 (d, 1H, $J_{OH\text{-}4'} = 4.8$ Hz, OH-4′), 5.02 (d, 1H, $J_{OH\text{-}3'} = 4.3$ Hz, OH-3′), 5.49 (d, 1H, $J_{OH\text{-}2'} = 3.1$ Hz, OH-2′), 6.22 (d, 1H, $J_{HN,1'} = 2.4$ Hz, HN-sugar), 7.07 (s, 1H, pyrazole-H), 7.41–7.53 (m, 6H, aromatic-H), 7.58 (s, 1H, pyrimidine-H), 8.11 (d, 2H, aromatic-H), 8.15 (d, 2H, aromatic-H), and 9.01 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 67.5$ (C-5′), 70.2 (C-4′), 71.2 (C-2′), 76.9 (C-3′), 91.8 (C-1′), 95.4 (C-pyrazole), 98.0 (C-pyrimidine), 126.8, 126.9, 129.1, 129.2, 129.3, 129.6, 132.5, 137.9, (C-Ar), 143.8 (C-3a pyrazole), 146.3 (C-2), 146.7 (C-5), and 155.0 (C-7). Anal. Calcd for $C_{23}H_{23}N_5O_4$: C, 64.42; H, 5.63; N, 15.65. Found: C, 64.31; H, 5.63; N, 15.65.

N^2-(β-D-Xylopyranosyl)-N^1-7-(2-phenyl-5-(p-tolyl)pyrazolo[1,5-c]pyrimidin-7-yl) (4b)

Reaction time 2 h (1.3 g, 91% yield); mp 202–204 °C; R_f 0.52 (9:1 $CHCl_3$-MeOH)); IR: 1623 (pyrazolo ring C=N), 1595 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^{1}H NMR (DMSO): $\delta = 2.32$ (C-CH_3), 3.06–3.10 (m, 2H, H-5' and H-2′), 3.18–3.19 (m, 1H, H-3′), 3.24–3.37 (m, 1H, H-4′), 3.73 (dd, 1H, $J_{5'',4'} = 5.4$ Hz, $J_{5'',5'} = 10.7$ Hz, H-5"), 4.03 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1′), 4.98 (d, 1H, $J_{OH\text{-}4'} = 1.8$ Hz, OH-4′), 5.00 (d, 1H, $J_{OH\text{-}3'} = 4.6$ Hz, OH-3′), 5.49 (d, 1H, $J_{OH\text{-}2',2'} = 3.1$ Hz, $J_{OH\text{-}2',3'} = 2.3$ Hz, OH-2′), 6.18 (d, 1H, HN-sugar), 6.99 (s, 1H, pyrazole-H), 7.24 (d, 2H, aromatic-H), 7.40 (t, 1H, aromatic-H), 7.45-7.48 (m, 3H, aromatic-H and pyrimidine-H), 7.98 (d, 2H, aromatic-H), 8.06 (d, 2H, aromatic-H), and 8.96 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 21.32$ (C-CH_3), 67.5 (C-5′), 70.0 (C-4′), 70.8 (C-2′), 76.7 (C-3′), 91.7 (C-1′), 94.9 (C-pyrazole), 97.6 (C-pyrimidine), 126.7, 126.9, 127.1, 129.3, 130.1, 132.6, 135.2, 138.9, (C-Ar), 143.8 (C-3a pyrazole), 146.3 (C-2), 146.7 (C-5), and 155.0 (C-7). Anal. Calcd for $C_{23}H_{22}ClN_5O_4$: C, 59.04; H, 4.74; N, 14.97. Found: C, 59.00; H, 4.64; N, 14.97.

N^2-(β-D-Xylopyranosyl)-N^1-7-(5-(4-chlorophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (4c)

Reaction time 2 h (1.3 g, 93% yield); mp 202–204 °C; R_f 0.52 (9:1 $CHCl_3$-MeOH)); IR: 1623 (pyrazolo ring C=N), 1595 (pyrimidine C=N), and 1453 cm^{-1} (C=C); ^1H NMR (DMSO): $\delta = 3.05–3.12$ (m, 2H, H-5' and H-2′), 3.18 (ddd, 1H, $J_{3',4'} = 8.4$ Hz, $J_{3',2'} = 8.4$ Hz, $J_{OH\text{-}3'} = 4.6$ Hz, H-3′), 3.23-3.29 (m, 1H, H-4′), 3.73 (dd, 1H, $J_{5'',4'} = 5.2$ Hz, $J_{5'',5'} = 11.5$ Hz, H-5"), 4.03 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.96 (d, 1H, $J_{OH\text{-}4'} = 4.6$ Hz, OH-4′), 4.99 (d, 1H, $J_{OH\text{-}3'} = 4.6$ Hz, OH-3′), 5.41 (d, 1H, $J_{OH\text{-}2'} = 3.1$ Hz, OH-2′), 6.16 (s, 1H, HN-sugar), 7.04 (s, 1H, pyrazole-H), 7.40 (t, 1H, aromatic-H), 7.47 (q, 4H, aromatic-H), 7.58 (s, 1H, pyrimidine-H), 8.06 (d, 2H, aromatic-H), 8.15 (d, 2H, aromatic-H), and 9.06 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 67.7$ (C-5′), 70.5 (C-4′), 71.0 (C-2′), 77.3 (C-3′), 91.9 (C-1′), 96.1 (C-pyrazole), 98.3 (C-pyrimidine), 126.9, 127.1, 128.5, 128.7, 129.0, 129.2, 129.4, 132.5, 134.5, 136.9, (C-Ar), 143.8 (C-3a pyrazole), 145.0 (C-2), 146.7 (C-5), and 155.1 (C-7). Anal. Calcd for $C_{23}H_{22}ClN_5O_4$: C, 59.04; H, 4.74; N, 14.97. Found: C, 57.85; H, 4.60; N, 14.73.

N^2-(β-D-Xylopyranosyl)-N^1-7-(5-(4-bromophenyl)-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl (4d)

Reaction time 2 h (0.95 g, 70% yield); mp 205–206 °C; R_f 0.57 (9:1 $CHCl_3$-MeOH); IR: 1623 (pyrazolo ring C=N), 1595 (pyrimidine C=N), and 1452 cm^{-1} (C=C); ^1H NMR (DMSO): $\delta = 3.09–3.17$ (m, 2H, H-5' and H-2′), 3.21–3.25 (m, 1H, H-3′), 3.28–3.35 (m, 1H, H-4′), 3.77 (dd, 1H, $J_{5'',4'} = 5.1$ Hz, $J_{5'',5'} = 11.2$ Hz, H-5"), 4.08 (dd, 1H, $J_{1',2'} = 8.4$ Hz, $J_{1',HN} = 2.3$ Hz, H-1′), 4.98 (d, 1H, $J_{OH\text{-}4'} = 4.9$ Hz, OH-4′), 5.01 (d, 1H, $J_{OH\text{-}3'} = 4.7$ Hz, OH-3′), 5.43 (d, 1H, $J_{OH\text{-}2',2'} = 3.2$ Hz, OH-2′), 6.19 (d, 1H, HN-sugar), 7.08 (s, 1H, pyrazole-H), 7.44 (t, 1H, aromatic-H), 7.51 (t, 2H, aromatic-H), 7.61 (s, 1H, pyrimidine-H), 7.66 (d, 2H, aromatic-H), 8.11 (t, 4H, aromatic-H), and 9.08 (s, 1H, HN-ring pyrimidine); ^{13}C NMR (DMSO): $\delta = 67.5$ (C-5′), 70.2 (C-4′), 71.3 (C-2′), 77.0 (C-3′), 91.8 (C-1′), 95.6 (C-pyrazole), 98.1 (C-pyrimidine), 122.7, 126.9, 128.8, 129.2, 129.6, 131.9, 132.4, 137.2, (C-Ar), 143.7 (C-3a pyrazole), 145.0 (C-2), 146.6 (C-5), and 155.0 (C-7). Anal. Calcd for $C_{23}H_{22}BrN_5O_4$: C, 53.92; H, 4.33; N, 13.67. Found: C, 53.73; H, 4.48; N, 13.44.

5-Aryl-2-phenyl-7-(2-benzylidenehydrazinyl)pyrazolo[1,5-c]pyrimidines (5a-d)

General procedure A mixture of 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidine **1a-d** (1 mmol) in ethanol (30 ml), benzaldehyde (1.1 mmol), and drop of acetic acid was heated under reflux for 4h then allowed to cool. The product was filtered, washed with EtOH, dried and recrystallized from methanol in white powder.

7-(2-Benzylidenehydrazinyl)-2,5-diphenylpyrazolo[1,5-c]pyrimidine 5a

Reaction time 2 h (0.54 g, 91% yield); mp 195–196 °C; R_f 0.55 (5:1 hexane-EtOAc); IR: 3455 (NH), 1626 (C=NH), 1571 (pyrimidine C=N), and 1453 cm^{-1} (C=C); ^1H NMR (500 MHz DMSO): $\delta = 7.07$ (s, 1H, pyrazole-H), 7.39–7.52 (m, 9H, aromatic-H), 7.69 (s, 1H, pyrimidine-H), 7.79 (d, 2H, aromatic-H), 8.13 (d, 2H, aromatic-H), 8.20 (d, 2H, aromatic-H), 8.74 (s, 1H, -N=CH), and 11.57 (s, 1H, -NH); ^{13}C NMR (500 MHz DMSO): δ_C 95.0 (C-3), 99.1 (C-4), 126.7, 126.9, 127.0, 127.2, 127.4, 127.4, 129.0, 129.1, 129.2, 129.3, 129.5, 129.6, 132.7, 135.2, 138.1 (C-aromatic), 142.8 (C-3a-pyrazole), 144.1 (C-2), 146.5 (C-5), 147.5 (C-N=CH), and 154.9 (C-7). Anal. Calcd for $C_{25}H_{19}N_5$: C, 77.10; H, 4.92; N, 17.98. Found: C, 76.96; H, 4.90; N, 17.78.

7-(2-Benzylidenehydrazinyl)-2-phenyl-5-(p-tolyl)pyrazolo[1,5-c]pyrimidine 5b

Reaction time 2 h (0.54 g, 88% yield); mp 200–202 °C; R_f 0.47 (5:1 hexane-EtOAc); IR: 3309 (NH), 1626 (C=NH), 1578 (pyrimidine C=N), and 1451 cm^{-1} (C=C); ^1H NMR (500 MHz DMSO): $\delta = 2.33$ (s,3H, CH_3), 7.02 (s, 1H, pyrazole-H), 7.28 (d, 2H, aromatic-H), 7.42–7.49 (m, 6H, aromatic-H), 7.61 (s, 1H, pyrimidine-H), 7.79 (d, 2H, aromatic-H), 8.09 (d, 2H, aromatic-H), 8.12 (d, 2H, aromatic-H), 8.73 (s, 1H, -N=CH), and 11.56 (s, 1H, -NH); ^{13}C NMR (500 MHz DMSO): δ_C 94.9 (C-3), 98.5 (C-4), 126.6, 126.8, 127.0, 127.2, 127.3, 127.4, 129.1, 129.3, 129.4, 129.4, 130.1, 132.7, 135.2, 135.3, 138.9 (C-aromatic), 142.7 (C-3a-pyrazole), 144.2 (C-2), 146.6 (C-5), 147.4 (C-N=CH), and 154.9 (C-7). Anal. Calcd for $C_{26}H_{21}N_5$: C, 77.40; H, 5.25; N, 17.36. Found: C, 77.26; H, 5.05; N, 17.17.

7-(2-Benzylidenehydrazinyl)-5-(4-chlorophenyl)-2-phenylpyrazolo[1,5-c]pyrimidine 5c

Reaction time 2 h (0.54 g, 82% yield); mp 246–248 °C; R_f 0.50 (5:1 hexane-EtOAc); IR: 3458 (NH), 1626 (C=NH), 1575 (pyrimidine C=N), and 1451 cm^{-1} (C=C); ^{1}H NMR (500 MHz CDCl$_3$): $\delta = 6.82$ (s, 1H, pyrazole-H), 7.45 (s, 1H, pyrimidine-H), 7.46–7.54 (m, 7H, aromatic-H), 7.89 (d, 2H, aromatic-H), 8.04 (d, 2H, aromatic-H), 8.13 (d, 2H, aromatic-H), 8.37 (s, 1H, -N=CH), and 9.82 (s, 1H, -NH); ^{13}C NMR (500 MHz CDCl$_3$): δ_C 95.2 (C-3), 99.1 (C-4), 126.7, 127.5, 127.9, 128.3, 128.7, 128.8, 129.1, 129.2, 130.2, 132.2, 133.8, 134.8, 136.5 (C-aromatic), 141.8 (C-3a-pyrazole), 143.4 (C-2), 146.3 (C-N=CH), 146.5 (C-5), and 155.4 (C-7). Anal. Calcd for $C_{25}H_{18}ClN_5$: C, 70.84; H, 4.28; N, 16.526. Found: C, 70.94; H, 4.28; N, 16.35.

7-(2-Benzylidenehydrazinyl)-5-(4-bromophenyl)-2-phenylpyrazolo[1,5-c]pyrimidine 5d

Reaction time 2 h (0.54 g, 86% yield); mp 279–281 °C; R$_f$ 0.39 (5:1 hexane-EtOAc); IR: 3447 (NH), 1623 (C=NH), 1561 (pyrimidine C=N), and 1450 cm^{-1} (C=C); ^{1}H NMR (500 MHz CDCl$_3$): $\delta = 6.85$ (s, 1H, pyrazole-H), 7.34–7.50 (m, 8H, aromatic-H and pyrimidine-H), 7.80 (s, 1H, -N=CH), 8.03 (d, 2H, aromatic-H), 8.10 (d, 2H, aromatic-H), 8.38 (d, 2H, aromatic-H), and 12.9 (s, 1H, -NH); ^{13}C NMR (500 MHz CDCl$_3$): δ_C 95.7 (C-3), 100.1 (C-4), 119.2, 127.3, 128.1, 128.3, 128.7, 128.9, 129.0, 129.1, 129.7, 132.7, 134.8, 136.3, 136.6, 139.1 (C-aromatic), 142.0 (C-3a-pyrazole), 142.7 (C-2), 143.7 (C-5), 146.6 (C-N=CH), and 156.2 (C-7). Anal. Calcd for $C_{25}H_{18}BrN_5$: C, 64.11; H, 3.87; N, 14.95. Found: C, 63.94; H, 4.09; N, 14.65.

6,7-Dibromo-3,8-diphenyl-5-(p-tolyl)pyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine 6b

To a mixture of the 7-(2-benzylidenehydrazinyl)-2-phenyl-5-(p-tolyl)pyrazolo[1,5-c]pyrimidine **5b** (1 mmol) and sodium acetate (3 mmol) in methanol (50 ml), bromine (1 mmol) in of methanol (20 ml) was added drop wise (10 min). The reaction mixture was stirred for 3 days. The methanol was then removed on a rotary evaporator until ~ 3–5 ml of the methanol and then dry acetone (20 ml) was added. The solution was evaporated the solvent by using a rotary evaporator and the product was recrystallized from methanol/chloroform (3:1). (0.54 g, 75% yield); mp 217–219 °C; R$_f$ 0.41 (1:1 hexane-EtOAc); IR: 3447 (NH), 1623 (C=NH), 1517 (pyrimidine C=N), and 1489 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 2.17$ (s, 3H, CH$_3$), 6.87 (d, 2H, aromatic-H), 7.02–7.09 (m, 6H, aromatic-H), 7.20–7.23 (m, 1H, aromatic-H), 7.56–7.63 (m, 3H, aromatic-H), and 7.91–7.93 (m, 2H, aromatic-H); ^{13}C NMR (500 MHz DMSO): δ_C 21.3 (C-CH$_3$), 91.0 (C-7), 99.3 (C-6), 127.3, 127.6, 128.8, 129.1, 129.2, 130.0, 130,2 130.9, 133.4, (C-aromatic), 135.5 (C-6a-pyrazole), 140.0 (C-8), 144.9 (C-3), 149.2 (C-10), and 154.3 (C-5). Anal. Calcd for $C_{26}H_{17}Br_2N_5$: C, 55.84; H, 3.06; N, 12.52. Found: C, 55.84; H, 3.06; N, 12.52.

5-Aryl-3,8-diphenyl-pyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine (7a,7c-d)

General procedure The mixture of 5-aryl-7-Hydrazinyl-2-phenylpyrazolo[1,5-c]pyrimidine (**1a-d**) (1 mmol) and benzoic acid (1 mmol) in the presence of phosphorus oxachloride (10 ml) were refluxed in water bath for 1 h. The reaction mixture was cooled and quenched with cold water (30 ml). The resulting solution was refluxed for additional 6 h. The separated solid was filtered and basified with potassium hydroxide. The solid was filtered, washed with water, dried and recrystallized from methanol/chloroform (3:1) or N, N-dimethylformamide in cooler less crystal.

3,5,8-Triphenylpyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine 7a

Reaction time 7 h (0.54 g, 55% yield); mp 258–260 °C; R$_f$ 0.57 (1:1 hexane-EtOAc); IR: 1643 (pyrazolo ring C=N), 1581 (pyrimidine C=N), and 1449 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 7.00$–7.04 (q, 4H, aromatic-H), 7.12–7.16 (q, 4H, aromatic-H), 7.17 (s, 1H, pyrazole-H), 7.23 (d, 2H, aromatic-H), 7.31 (s, 1H, pyrimidine-H), 7.43 (t, 1H, aromatic-H), 7.51 (d, 2H, aromatic-H), and 8.07 (d, 2H, aromatic-H); ^{13}C NMR (500 MHz DMSO): δ_C 99.4 (C-6), 105.8 (C-7), 126.7, 126.9, 127.5, 127.6, 128.2, 129.4, 129.7, 129.9, 130.1, 130,4 131.7, 132.2, (C-aromatic), 135.7 (C-6a-pyrazole), 139.0 (C-8), 145.8 (C-3), 149.2 (C-10), and 155.5 (C-5). Anal. Calcd for $C_{25}H_{17}N_5$: C, 77.50; H, 4.42; N, 18.08. Found: C, 77.30; H, 4.52; N, 17.96.

5-(4-Chlorophenyl)-3,8-diphenylpyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine 7c

Reaction time 7 h (0.54 g, 70% yield); mp 336–338 °C; R$_f$ 0.56 (1:1 hexane-EtOAc); IR: 1643 ((pyrazole ring C=N), 1586 (triazole ring C=N), and 1446 cm^{-1} (C=C); ^{1}H NMR (500 MHz DMSO): $\delta = 7.13$–7.31 (m, 10H, aromatic-H and pyrazole-H), 7.37 (s, 1H, pyrimidine-H), 7.49 (t, 1H, aromatic-H), 7.57 (t, 2H, aromatic-H), and 8.12 (d, 2H, aromatic-H). Anal. Calcd for $C_{25}H_{16}ClN_5$: C, 71.17; H, 3.82; N, 16.60. Found: C, 70.94; H, 3.92; N, 16.46.

5-(4-Bromophenyl)-3,8-diphenylpyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine 7d

Reaction time 7 h (0.54 g, 62% yield); mp 217–219 °C; R$_f$ 0.55 (1:1 hexane-EtOAc); IR: 1643 ((pyrazole ring C=N), 1586 (triazole ring C=N), and 1446 cm^{-1} (C=C); ^{1}H NMR

Table 1 In vitro cytotoxic activity of some of the synthesized compounds against the human breast cancer cell line (MCF-7)

Compound no.	IC_{50}[a,b] (µg/ml)	LogP[c]
2a	1.62 ± 0.12	1.98
2b	2.71 ± 0.18	2.35
2c	1.24 ± 0.14	2.61
2d	1.47 ± 0.22	2.67
3a	1.02 ± 0.06	1.98
3b	0.56 ± 0.04	2.35
3c	1.45 ± 0.20	2.61
3d	0.66 ± 0.08	2.67
4a	2.32 ± 0.12	2.05
4b	2.36 ± 0.18	2.41
4c	1.98 ± 0.14	2.68
4d	0.84 ± 0.02	2.74
5a	8.62 ± 0.32	5.92
5b	8.86 ± 0.28	6.28
5c	8.24 ± 0.34	6.55
5d	0.82 ± 0.04	6.61
6b	6.28 ± 0.36	8.06
7a	2.88 ± 0.32	6.31
7c	1.11 ± 0.14	6.94
7d	0.96 ± 0.06	7
8a	0.86 ± 0.06	7
Doxorubicin	0.419 ± 0.02	---

[a] IC_{50}: dose of the compound that inhibits tumor cell proliferation by 50%

[b] Values are means of three experiments

[c] logP is logarithm of compound partition coefficient between n-octanol and water, calculated by XLOGP program (Cheng et al. 2007) via SwissADME (Daina et al. 2017)

(500 MHz DMSO): $\delta = 7.12–7.30$ (m, 10H, aromatic-H and pyrazole-H), 7.38 (s, 1H, pyrimidine-H), 7.50 (t, 1H, aromatic-H), 7.57 (t, 2H, aromatic-H), and 8.12 (d, 2H, aromatic-H). Anal. Calcd for $C_{25}H_{16}BrN_5$: C, 64.39; H, 3.46; N, 15.02. Found: C, 64.19; H, 3.36; N, 14.90.

6-Bromo-3,5,8-triphenylpyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine (8a)

To a mixture of 3,5,8-triphenylpyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine **7a** (1 mmol) and sodium acetate (3 mmol) in methanol (50 ml), bromine (1 mmol) in methanol (20 ml) was added drop wise during 10 min. The reaction mixture was stirred for 7 h. The solution was then concentrated under reduced pressure until ~3–5 ml of the methanol was left then dry acetone (20 ml) was added. The product separated was filtered, dried and recrystallized from ethanol/benzene (1:2) in colorless crystal. (0.15 g, 65% yield); mp 258–260 °C; R_f 0.57 (1:1 hexane-EtOAc); IR: 1642, 1585, 1445 cm^{-1} (C=N, C=C); ^1H NMR (500 MHz DMSO-d_6): $\delta = 6.97$ (s, 1H, pyrazole-H), 7.01–7.05 (q, 4H, aromatic-H), 7.11–7.19 (m, 4H, aromatic-H), 7.24 (d, 2H, aromatic-H), 7.52 (t, 1H, aromatic-H), 7.57 (t, 2H, aromatic-H), 8.01 (d, 2H, aromatic-H). Anal. Calcd for $C_{25}H_{16}BrN_5$ C, 64.39; H, 3.46; N, 15.02 Found: C, 64.29; H, 3.46; N, 14.97.

Measurement of cytotoxicity by SRB assay

The cytotoxic activity of the newly synthesized compounds was measured in vitro using the SRB assay according to the method of Skehan et al. (1990). Cells were plated in 96-multiwell microtiter plates (104 cells /well) for 24 h before treatment with the compound(s) to allow attachment of cells to the walls of the plates. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 1, 2.5, 5, and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris ethylenediaminetetraacetic acid buffer. Color intensity was measured in an ELISA reader. The relationship between surviving fraction and drug concentration was plotted to get the survival curve. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated. The results are given in Table 1.

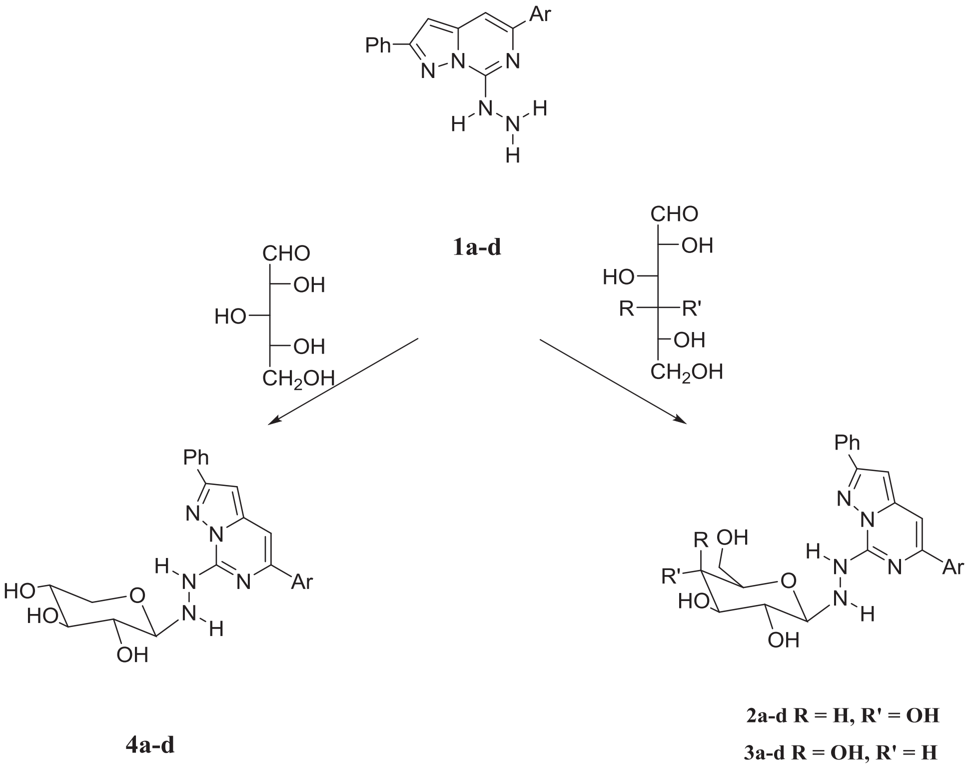
Physicochemical properties and pharmacokinetics profile predictions

Some of the drug-likeness and pharmacokinetic properties of most active compounds **3b**, **3d**, **4d**, **5d**, **7d**, and **8a** were predicted using SwissADME (Daina et al. 2017) with its underlying methodologies.

Results and discussion

Chemistry

The starting material 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidines (**1a-d**) were prepared from the respective pent-4-yne-1,3-diones as described earlier (Gaber et al. 1992). Their reaction with monosaccharides has been investigated in ethanol to give products. The characterization and assignment of the acyclic-cyclic nature of the newly prepared sugar hydrazones were investigated based on the study of their ^1H, ^{13}C NMR and DEPT-135° spectra,

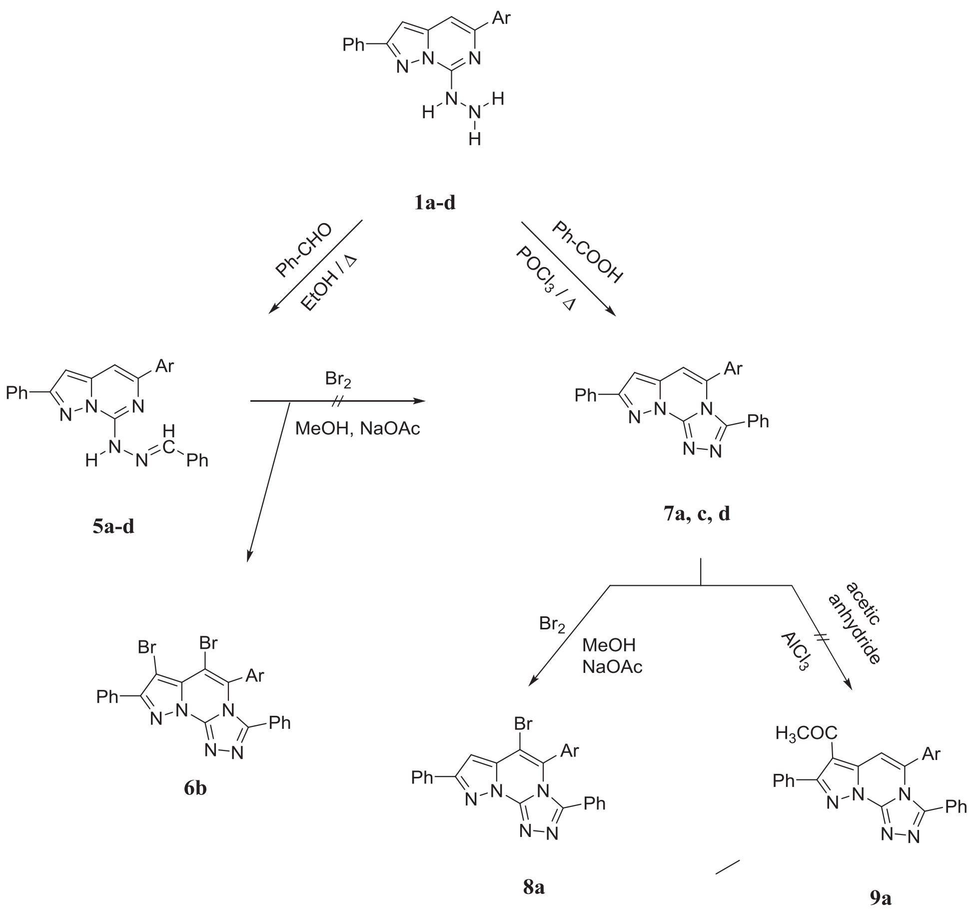


a, Ar = C_6H_5- ; b, Ar = p-Me-C_6H_4- ; c, Ar = p-Cl-C_6H_4- ; d, Ar = p-Br-C_6H_4.

Scheme 1 Reaction of sugars with 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidines

whereas ^{1}H-^{1}H DQFCOSY, ^{1}H-^{13}C HMQC NMR, and ^{13}C-^{1}H HMBC NMR experiments helped in the assignment of both proton and carbon signals. Sugar hydrazones can exist in solution as acyclic Schiff's base or as cyclic glycosylhydrazine and are often present as equilibrium mixtures that are strongly dependent on the pH of the solution as well as on the nature of the sugar and the basicity of the hydrazine derivative (Housaad et al. 1994; Rashed et al. 1994a; Rashed et al. 1994b; Hamed et al. 1998; Khodair et al. 1998; Rashed et al. 1998; Abdel-Aal et al. 2003; El Ashry and Abdul-Ghani 2004; Itano and Robinson 1961; Simon and Kraus 1970; Itano and Matteson 1982; Ashry et al. 2005).

Thus, reaction of D-glucose with **1a-d** afforded the respective cyclic glycosylhydrazine derivatives **2a-d** (Scheme 1). The compounds **2a-d** were deduced from the respective spectral data. Their IR spectra showed characteristic absorption bands owing to the pyrazolopyrimidine moiety at 1452–1455, 1590–1597, and 1621–1624 cm^{-1}. Their ^{1}H NMR spectra indicted the presence of only anomer by assignment of a signal at the downfield region corresponding to H-1' (δ 4.07–4.12), which appeared as dd with, coupling constant, $J_{1',2'} = 8.4$–8.8 Hz for the β-anomer compatible with a trans relationship for H-1', H-2', and $J_{1',NH} = 2.3$–2.8 Hz. The other protons of the sugar moiety appeared at δ_H 3.00–3.28, 3.44–3.52, 3.68–3.75, and 4.47–5.45 ppm region for H-2'-H-6'' and four hydroxyl groups. Whereas the HN-sugar, HN-ring heterocycle, pyrazole, and pyrimidine protons appeared as four signals at 6.23–6.26, 8.76–8.89, 7.01–7.09, and 7.51–7.64 ppm, respectively. The ^{1}H-^{1}H COSY technique facilitated the correlation of protons with each other, thus it showed for **2b** group of spots on the diagonal opposite with each other. It showed that H-1' was correlated with H-2' and HN-sugar. Also found a correlation between H-6' with H-6'' and OH-6' with H-6' and H-6''. The ^{13}C NMR spectra of **2a-d** showed a signal at δ_C 90.6–90.9 ppm in agreement with the expected cyclic pyranosyl structure, as well as five signals at δ_C 61.6–61.8, 70.3–70.9, 71.0–71.2, 76.6–77.0, and 78.3–78.9 ppm, which can be assigned to C-6', C-3', C-2', C-4, and C-5', respectively, whereas the carbon atoms of heterocyclic and aromatic rings appeared six and eight to nine signals, respectively. The DEBT-135° spectra of the **2c** and **2d** showed the presence of 13 signals (12 methines and 1 methylene). The ^{1}H NMR and ^{13}C NMR or DEBT-135° signals are correlated in the ^{1}H-^{13}C HMQC experiment of **2a-d**, which helped in identifying the carbon atoms. The above data confirmed that the structures of the glucose derivatives exist in the glucopyranosyl structure with β-anomeric configuration. Moreover, the hydrazine residues exist in the conformer shown in the Scheme 1 rather than those other conformers.



Scheme 2 Synthesis of pyrazolo [1,5-c]-1,2,4-triazolo[4,3-a] pyrimidines

a, Ar = C_6H_5- ; b, Ar = *p*-Me-C_6H_4- ; c, Ar = *p*-Cl-C_6H_4- ; d, Ar = *p*-Br-C_6H_4-

Similarly, the reaction of **1a-d** with D-galactose in ethanol yielded the corresponding N^{2}-(β-D-galacopyranosyl)-N^{1}-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (**3a-d**). Their spectra exhibited signals confirming their β-cyclic structure in the pyranose form.

The reaction of **1a-d** with D-xylose afforded the corresponding N^{2}-(β-D-xylopyranosyl)-N^{1}-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (**4a-d**). Whose spectra confirmed their presence in the β-anomeric configuration and similar confirmation for the hydrazine residue.

Condensation of **1a-d** with benzaldehyde afforded the respective **5**-aryl-2-phenyl-7-(2-benzylidenehydrazinyl)pyrazolo[1,5-c]pyrimidines **5a-d** (Scheme 2). The structures **5a-d** were deduced by ^{1}H NMR. Their spectra showed the presence of three signals at the downfield region at δ 6.82–7.07, 7.45–7.69, and 7.80–8.74 that correlated with H-3 (pyrazole), H-4 (pyrimidine), and HC=N-, respectively. The exchangeable NH resonance appeared in a downfiled region at δ 9.82–12.9. The ^{13}C NMR spectra of **5a-d** showed a signal at δ_C 146.3–147.5 ppm for carbon of HC=N-, as well as six signals for carbon atoms of the heterocyclic ring at δ_C 95.0–95.7, 98.5–100.1, 141.8–142.8, 142.7–144.2, 143.7–146.6, and 154.9–156.2 ppm, which were assigned to C-3, C-4, C-2, C-3a, C-2, and C-7, respectively, in addition to the of aromatic carbons.

Oxidative cyclization of **5b** was occurred using bromine in methanol in the presence of three equivalents of sodium acetate to give 6,7-dibromo-3,8-diphenyl-5-(p-tolyl)pyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine (**6b**) via cyclization and electrophilic substitution reactions. The reaction occurred through the involvement of the pyrimidine nitrogen-6 and carbon atom of HC=N-, generating a fused tricyclic system with substitution of pyrazole and pyrimidine protons by bromine. Its ^{1}H NMR spectra showed only aromatic protons, whereas both NH, -N=CH, pyrazole, and pyrimidine protons disappeared. Whereas ^{13}C NMR spectra did not show a signal for the carbon of HC=N-, but showed new signal to other carbon atom at δ_C 144.9 for C-3 in the 1,2,4-triazole ring. The DEBT-135° spectra of the **6b** showed the presence of eight signals (seven methines in the aromatic region and one methyl). The ^{1}H NMR and ^{13}C NMR or DEBT-135° signals are correlated in the ^{1}H-^{13}C HMQC experiment of **6b**, which helped to identify the structure of the compound **6b**.

Accordingly, fused tricyclic systems without a substitution reaction were prepared through the reaction of **1a**, **c**, **d**

Table 2 In silico predictions of the pharmacokinetics properties for the most active compounds

Cpd #	GI absorption	BBB	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
3b	Low	No	No	No	No	No	No	No
3d	Low	No	No	No	No	No	No	Yes
4d	High	No	No	No	No	No	No	No
5d	High	No	No	No	Yes	Yes	No	Yes
7d	High	No	No	No	Yes	Yes	No	No
8a	High	No	No	No	Yes	Yes	No	No

GIA human gastrointestinal absorption, *BBB* blood–brain barrier permeation, *P-gp* permeability glycoprotein, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are the five major isoforms of cytochromes P450 (CYP)

with benzoic acid in the presence phosphorous oxychloride. This afforded 5-Aryl-3,8-diphenyl-pyrazolo[1,5-c][1,2,4]triazolo[4,3-a]pyrimidine **7a**, **7c-d**. Their ^{1}H NMR spectra showed single signals for pyrimidine protons at the region δ 7.31–7.38. Pyrazole protons showed two types of signals, the first is a singlet at δ 7.17 ppm for **7a**, and the second signal showed overlap with signals of aromatic protons at δ 7.12–7.31 for **7c-d**. In addition, ^{13}C NMR spectra demonstrated signal to C-3 in triazole ring at δ_C 145.8 in addition to carbon atom signals for aromatic and fused ring.

Electrophilic substitution reaction of **7a** was attempted using bromine in the presence of sodium acetate in methanol to give 6-bromo-3,5,8-triphenylpyrazolo[1,5-*c*][1,2,4]triazolo[4,3-*a*]pyrimidine (**8a**) (Scheme 2). The ^{1}H NMR spectra of **8a** showed only single signal for pyrazole proton at $\delta = 6.97$ with disappearance of signal corresponding to pyrimidine proton. Aromatic protons were assigned at the appropriate positions. On the other hand, reaction of **7a** with acetic anhydride in the presence aluminum chloride did not afford compound **9a**.

Cytotoxic activity against human breast adenocarcinoma cell line (MCF-7)

(Skehan et al. 1990) The cytotoxicity of compounds **2a-d, 3a-d, 4a-d, 5a-d, 6b, 7a, 7c, 7d**, and **8a** was evaluated against a human breast adenocarcinoma cell line (MCF-7). For comparison purposes, the cytotoxicity of doxorubicin, a standard antitumor drug, was evaluated under the same conditions. The IC_{50} (the concentration required for 50% inhibition of cell viability) was calculated for each compound and the results are given in Table 1. Our results revealed that the title compounds showed inhibition of proliferation of MCF-7 human breast cancer cells with IC_{50} range from 0.56–8.86 µg/ml (Table 1). The most active compounds displaying IC_{50} with sub-micromolar range of activity are **3b**, **3d**, **4d**, **5d**, **7d**, and **8a**, as shown in Table 1.

The cytotoxic activity showed a correlation with the calculated lipophilicity (e.g., LogP) (Cheng et al. 2007; Daina et al. 2017) within each scaffold series. For instance, bromo-substituted compounds, e.g., **2d**, **3d**, **4d**, **5d**, and **7d**, displayed the highest lipophilicity and cytotoxic activity among their congeneric analogues. This observation can be attributed to the fact that increased lipophilicity would enhance penetration through the biological membranes facilitating the reach to molecular targets and hence the cytotoxic activity.

Physicochemical properties and pharmacokinetics profile predictions

The aim of this part is to test the drug-likeness and to predict the pharmacokinetic profile of the most active compounds **3b**, **3d**, **4d**, **5d**, **7d**, and **8a**. Generally, such predictions do not provide an absolute guarantee that a compound would be an optimum drug for an illness. Nevertheless, compounds that do not succeed the drug-likeness criteria often do not thrive to be an efficient clinical candidate due to poor bioavailability, adverse effects or other concerns. We used the automated SwissADME (Daina et al. 2017) server for such predictions.

Pharmacokinetics properties (Table 2) employing specialized models assess individual ADME behaviors of the most active compounds. The predictions for passive human gastrointestinal absorption and blood–brain barrier (BBB) permeation both extracted from the readout of the BOILED-Egg model (Daina and Zoete 2016; Daina et al. 2017). Compounds **4d**, **5d**, **7d**,and **8a** showed high gastrointestinal absorption, whereas **3b** and **3d** showed low gastrointestinal absorption. This is correlated by drug-likeness properties (Table 3) as **4d**, **5d**, **7d** and **8a** showed only one violation for Lipinski rule and no violations for Veber rule, whereas **3b** and **3d** demonstrated one violation for Veber rule (Veber et al. 2002) and at least one violation for Lipinski rule. Therefore, **3d** displayed the lowest bioavailability score among all compounds. Fortunately, all these compounds do not qualify to be permeable through BBB, and hence, they expected to be with low incidence for central nervous system (CNS) adverse effects.

Table 3 In silico predictions of the drug-likeness properties for the most active compounds

Cpd #	Lipinski #violations	Veber #violations	PAINS #alerts	Bioavailability Score
3b	1	1	0	0.55
3d	2	1	0	0.17
4d	1	0	0	0.55
5d	1	0	0	0.55
7d	1	0	0	0.55
8a	1	0	0	0.55

Lipinski #violations counts the number of violations of Lipinski rule summarized as: lipophilicity (logP) ≤ 5, molecular weight ≤ 500, number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10. Veber #violations counts the number of violations of Veber rule summarized as: NRB ≤ 10 and TPSA ≤ 140 Å^2. PAINS #alerts: counts the number of pan-assay interference compounds/substrucutres. All calculations were performed using SwissADME (Daina et al. 2017)

P-gp substrate parameter gives information about a compound being substrate or non-substrate of the permeability glycoprotein (P-gp) (Daina et al. 2017). P-gp is suggested to be the most important member among ATP-binding cassette transporters or ABC-transporters. P-gp is a key to judge active efflux through biological membranes, for instance, to protect the CNS from xenobiotics, and included in resistance mechanism of drugs (Szakacs et al. 2008; Daina et al. 2017). All compounds predicted (Table 2) to show no substrate activity towards P-gp, which indicates low resistance incidence via active efflux by cancerous biological membranes.

Having information about interaction of compounds with the cytochromes P450 (CYP) superfamily is of great importance. It has been estimated that 50–90% of therapeutic molecules are substrate of five major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) (Farghaly 2004; Di 2014; Daina et al. 2017). Inhibition of these isoenzymes is undoubtedly one main source of pharmacokinetics-related drug–drug interactions (Hollenberg 2002; Huang et al. 2008) leading to toxic or adverse effects. As seen from Table 2, compounds **3b** and **4d** did not show inhibition of any CYP five major isoforms, indicating low incidence of drug–drug interactions. However, **3d** showed inhibition of one isoform (CYP3A4), whereas **5d**, **7d**, and **8a** showed inhibition of at least two CYP isoforms.

All compounds showed no alert to be a possible PAINS (pan-assay interference compounds) (Baell and Holloway 2010), as seen in Table 3. This means that the compounds chemical structure do not possess an interfering substructure that can have a potent response in protein assays irrespective of the protein target.

Conclusion

Reaction of 5-aryl-7-hydrazino-2-phenylpyrazolo[1,5-c]pyrimidines (**1a-d**) with glucose, galactose, and xylose in ethanol gave N^2-(β-D-glycopyranosyl)-N^1-7-(5-aryl-2-phenylpyrazolo[1,5-c]pyrimidin-7-yl) (**2a-d**, **3a-d**, and **4a-d**). Their structures were deduced to exist in the glycopyranosyl chair structures and each have hydrazine moiety in the β-configuration. Pyrazolo[1,5-c]triazolo[4,3-a]pyrimidine derivatives were synthesized from reaction of **1a-d** with benzoic acid in the presence of phosphorusoxy chloride or by reaction with benzaldehyde derivatives followed by cyclization in the presence of bromine. The structures of the compounds were confirmed from their IR, ^{1}H, ^{13}C, DPET-135°, 1H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{13}C-^{1}H HMBC spectra, and microanalysis. The synthesized compounds showed inhibition of proliferation of MCF-7 human breast cancer cells with IC_{50} range from 0.56 to 8.86 µg/ml. Majority of the most active compounds are bromo-substituted derivatives (**2d**, **3d**, **4d**, **5d**, and **7d**) with IC_{50} values of 1.47 ± 0.22, 0.66 ± 0.08, 0.84 ± 0.02, 0.82 ± 0.04, and 0.96 ± 0.06 µg/ml, respectively. Their high activity is presumed to be due to their high lipophilic character among their congeneric analogues. The predicted in silico pharmacokinetics and drug-likeness properties were acceptable for many of the most active compounds.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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