Design, synthesis and cytotoxic activity of some novel compounds containing pyrazolo[3,4-*d*]pyrimidines nucleus

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Abstract. Novel pyrazolo[3,4-*d*]pyrimidines were designed and synthesized as antitumour agents against human breast cancer adenoma (MCF-7). Molecular modelling and pharmacological screening were performed against breast cancer cell line and also certain synthetic pathways were developed in order to introduce functionality onto C6 and N5 positions of pyrimidine moiety. Surprisingly, all the test compounds showed IC₅₀ lower than that of the standard olomoucine **I**, especially compounds **4b**, **8a**, **10b**, **11a** and **b**, which showed IC₅₀ between 0.009 and 0.004 μM.

Keywords. Pyrazolo[3,4-*d*]pyrimidine derivatives; cytotoxic activity; MCF-7; cyclin-dependent kinase inhibitors (CDKI).

1. Introduction

Cyclin-dependent kinases (CDK) are enzymes of Ser/Thr kinase type. This family of enzymes plays an important role in cell cycle regulation and proliferation.¹ CDK enzymatic regulation requires the binding of catalytic subunits (CDK1–CDK8) with regulatory subunits (cyclin A–cyclin H) to give a fully active complex (CDK–cyclin), which is necessary for phosphorylation of key proteins that regulate the progression through the cell cycle.²

Several types of CDK inhibitors (staurosporine, flavopiridole, butyrolactone-1 and purine derivatives) have so far been described.²

Insight into different biological activities of pyrazolo [3,4-*d*]pyrimidines as antimetabolites in purine biochemical reactions, were gained and several mechanisms were described for their cytotoxic activities as EGFR inhibitors,³ GSK-3 inhibitors,^{4,5} xanthine oxidase inhibitors,⁶ Mer receptor tyrosine kinase inhibitors,⁷ tyrosine kinase c-Src inhibitors,^{8–11} adenosine receptor antagonist^{12,13} and CDK inhibitors.^{14,15}

CDKs are involved in the control of the cell cycle, but are overexpressed or overactive in many cancer cells. Since these enzymes are inactive in normal resting cells, drugs that target them should have fewer and less toxic side effects than conventional cytotoxic drugs, CDKs

are activated by cyclins via binding of a cyclin with its associated kinase, which activates the enzyme and serves to move the cell from one phase of the cell cycle to another, and inhibited by CDKIs. So, particular attention has been focused on CDKIs that offer selective and tolerable treatment for cancer.¹⁶

Moreover, pyrazolo [3, 4-*d*] pyrimidines were known to possess a broad spectrum of biological activities including antimicrobial^{17–19} antiviral^{20,21} and antitumour activities.^{22–25}

Herein, we have described the design, synthesis and pharmacological profile for a new class of compounds containing pyrazolo [3, 4-*d*] pyrimidine scaffold and having structure similar to olomoucine **I**, the known potent CDK inhibitor.²⁶

2. Experimental

2.1 Docking studies

Molecular modelling was performed using the X-ray crystal structure of olomoucine **I** bound to CDK using the 'Molecular Operating Environment (MOE) version 2008.10'.

Hydrogen atoms were added to amino acids of the protein and free energy calculation was assigned. Ligand for docking was prepared, atom types and protonation states and formal charges were assigned

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using default settings. The root mean square deviation (RMSD) of the docked conformation of olomoucine **1** was 0.52 Å and the binding energy score was -19.41 Kcal/mol. Test compounds were prepared as above and the top 10 poses were retained for each molecule from every docking run.

2.2 General

Melting points were determined on a Graffin apparatus and were uncorrected. Element analyses (C, H, and N) were carried out on a Perkin-Elmer 2400 Analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt. All compounds were δ within $\pm 0.4\%$ of theoretical values. IR spectra were determined as KBr discs on Shimadzu IR 435 Spectrophotometer and values were represented in cm^{-1} . $^1\text{H-NMR}$ spectra were carried out on a Bruker 300 MHz NMR Spectrophotometer (Bruker, Munich, Germany) in Cairo University, Egypt, using DMSO- d_6 as a solvent, TMS as internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on Hewlett Packard 5988 Spectrometer, Micro Analytical Center, Cairo University, Egypt. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that were visualized by UV lamp.

2.3 6-Chloromethyl-3-methyl-1-phenyl-1, 5-dihydro-pyrazolo [3, 4-*d*] pyrimidin-4-one (**3**)

A mixture of compound **1** (1.06 g, 4.9 mmol) and chloroacetyl chloride (0.65 g, 5.8 mmol) was heated at 80°C for 6 h. The mixture was cooled and neutralized with sodium carbonate solution (10%). The precipitated solid was collected and crystallized from acetone as white crystals (69% yield); m.p.: 246–248°C; IR (KBr, cm^{-1}): 3276, 2934, 2822, 1675; $^1\text{H-NMR}$ (DMSO- d_6) δ = 2.50 (s, 3H, CH_3), 4.59 (s, 2H, CH_2), 7.34–7.38 (t, 1H, $\text{C}_4\text{-H}$), 7.39–7.56 (t, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 8.00–8.03 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), and 12.61 (s, 1H, NH, D_2O exchangeable); MS (m/z): 274 (M^+ , 85.80%), 77 (100%); Anal. Found: C, 56.84%; H, 4.04%; N, 20.40%; $\text{C}_{13}\text{H}_{11}\text{ClN}_4\text{O}$ Calcd. C, 56.88%; H, 4.10%; N, 20.37%.

2.4 General procedure for the synthesis of **4 a–d**

A mixture of compound **3** (0.52 g, 1.9 mmol), the appropriate primary aromatic amine (10 mmol) and absolute ethanol (20 ml) was heated under reflux for

8 h. The solid that formed on hot, was filtered and crystallized from dioxane as white crystals.

2.4a 3-Methyl-1-phenyl-6-phenylaminomethyl-1, 5-dihydro-pyrazolo [3, 4-*d*] pyrimidin-4-one (4a**):** White colour solids; yield: 58%; m.p. 223–225°C; IR (KBr, cm^{-1}): 3428, 2930, 2871, 1685, $^1\text{H-NMR}$ (DMSO- d_6) δ = 2.49 (s, 3H, CH_3), 4.30–4.32 (d, 2H, CH_2), 6.03–6.08 (t, 1H, NH, D_2O exchangeable), 6.57–6.59 (t, 1H, $\text{C}_4\text{-H}$), 6.60–6.62 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 7.08–7.13 (t, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.29–7.32 (t, 1H, $\text{C}_4\text{-H}$), 7.40–7.45 (t, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.92–7.95 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 12.15 (s, 1H, NH, D_2O exchangeable); MS (m/z): 331 (M^+ , 58.24%), 234 (100%); Anal. Found: C, 68.87%; H, 5.01%; N, 21.09%; $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}$ Calcd. C, 68.87%; H, 5.17%; N, 21.13%.

2.4b 3-Methyl-1-phenyl-6-(*p*-tolylphenyl) aminomethyl-1, 5-dihydro-pyrazolo [3, 4-*d*] pyrimidin-4-one (4b**):** White colour solids; yield: 63%; m.p. 217–219°C; IR (KBr, cm^{-1}): 3427, 2999, 2924, 1684; $^1\text{H-NMR}$ (DMSO- d_6) δ = 2.13 (s, 3H, $\text{CH}_3\text{-ph}$), 2.49 (s, 3H, CH_3), 4.25–4.29 (d, 2H, CH_2), 5.84–5.86 (t, 1H, NH, D_2O exchangeable), 6.56–6.60 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 6.89–6.93 (d, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.28–7.34 (t, 1H, $\text{C}_4\text{-H}$), 7.41–7.44 (t, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.93–7.97 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 12.10 (s, 1H, NH, D_2O exchangeable); MS (m/z): 345 (M^+ , 79.33%), 120 (100%); Anal. Found: C, 69.58%; H, 5.54%; N, 20.32%; $\text{C}_{20}\text{H}_{19}\text{N}_5\text{O}$ Calcd. C, 69.55%; H, 5.54%; N, 20.28%.

2.4c 3-Methyl-1-phenyl-6-(*p*-hydroxyphenyl) amino-methyl-1, 5-dihydro-pyrazolo [3, 4-*d*] pyrimidin-4-one (4c**):** White colour solids; yield: 60%; m.p. 298–300°C; IR (KBr, cm^{-1}): 3439, 3249, 2979, 2924, 1675; $^1\text{H-NMR}$ (DMSO- d_6) δ = 2.48 (s, 3H, CH_3), 4.21–4.22 (d, 2H, CH_2), 5.49–5.51 (t, 1H, NH, D_2O exchangeable), 6.51–6.52 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 6.55–6.58 (d, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.29–7.34 (t, 1H, $\text{C}_4\text{-H}$), 7.44–7.49 (t, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$), 7.95–7.98 (d, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$), 8.48 (s, 1H, OH, D_2O exchangeable), 12.10 (s, 1H, NH, D_2O exchangeable); MS (m/z): 347 (M^+ , 39.30%), 77 (100%); Anal. Found: C, 65.57%; H, 5.20%; N, 19.99%; $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2$ Calcd. C, 65.69%; H, 4.93%; N, 20.16%.

2.4d 3-Methyl-1-phenyl-6-(*p*-carboxyphenyl) amino-methyl-1, 5-dihydro-pyrazolo [3, 4-*d*] pyrimidin-4-one (4d**):** White colour solids; yield: 66%; m.p. 281–283°C; IR (KBr, cm^{-1}): 3404, 3263, 1664; $^1\text{H-NMR}$ (DMSO- d_6) δ = 2.49 (s, 3H, CH_3), 4.39–4.41 (d, 2H,

CH₂), 6.70–6.82 (d, 2H, C_{2'}-H, C_{6'}-H), 6.81–6.83 (t, 1H, NH, D₂O exchangeable), 7.24–7.29 (d, 2H, C_{3'}-H, C_{5'}-H), 7.32–7.37 (t, 1H, C₄-H), 7.70–7.73 (t, 2H, C₃-H, C₅-H), 7.86–7.88 (d, 2H, C₂-H, C₆-H), 12.05 (s, 1H, NH, D₂O exchangeable), 12.27 (s, 1H, OH, D₂O exchangeable); MS (*m/z*): 375 (M⁺, 92.70%), 77 (100%); Anal. Found: C, 63.86%; H, 4.51%; N, 18.66%; C₂₀H₁₇N₅O₃ Calcd. C, 63.99%; H, 4.56%; N, 18.66%.

2.5 General procedure for the synthesis of 5 a–d

To a solution of the appropriate compound **4a–d** (0.6 mmol) in propanol (10 ml), formaldehyde solution (3 ml, 0.10 mol) was added while stirring for 10 min. The solution was stirred continuously for 2 h. The formed precipitate was filtered and crystallized from propanol as white crystals.

2.5a 1,3-Methyl-1-phenyl-7-phenyl-1, 4, 5, 6, 7,8 hexahydropyrazolo [3, 4-d] imidazo [3, 4-a] pyrimidin-4-one (5a): White colour solids; yield: 57%; m.p. 246–248°C; IR (KBr, cm⁻¹): 2990–2857, 1687; ¹H-NMR (DMSO-*d*₆) δ = 2.54 (s, 3H, CH₃), 4.70 (s, 2H, C-CH₂-N), 5.37 (s, 2H, N-CH₂-N) and 6.75–6.79 (t, 1H, C_{4'}-H), 6.82–6.84 (d, 2H, C_{2'}-H, C_{6'}-H), 7.26–7.31 (t, 2H, C_{3'}-H, C_{5'}-H), 7.34–7.40 (t, 1H, C₄-H), 7.52–7.57 (t, 2H, C₃-H, C₅-H), 7.99–8.03 (d, 2H, C₂-H, C₆-H); MS (*m/z*): 343 (M⁺, 44.69%), 77 (100%); Anal. Found: C, 69.99%; H, 4.71%; N, 20.56%; C₂₀H₁₇N₅O Calcd. C, 69.96%; H, 4.99%; N, 20.40%.

2.5b 3-Methyl-1-phenyl-7-(p-tolylphenyl)-1, 4, 5, 6, 7,8 hexahydropyrazolo [3, 4-d] imidazo [3, 4-a] pyrimidin-4-one (5b): White colour solids; yield: 56%; m.p. 284–286°C; IR (KBr, cm⁻¹): 2921–2858, 1704; ¹H-NMR (DMSO-*d*₆) δ = 2.22 (s, 3H, CH₃-ph), 2.48 (s, 3H, CH₃), 4.66 (s, 2H, C-CH₂-N), 5.34 (s, 2H, N-CH₂-N) and 6.67–6.70 (d, 2H, C_{2'}-H, C_{6'}-H), 7.08–7.11 (d, 2H, C_{3'}-H, C_{5'}-H), 7.30–7.37 (t, 1H, C₄-H), 7.52–7.58 (t, 2H, C₃-H, C₅-H), 7.99–8.02 (d, 2H, C₂-H, C₆-H); MS (*m/z*): 357 (M⁺, 63.96%), 240 (100%); Anal. Found: C, 70.40%; H, 5.49%; N, 19.41%; C₂₁H₁₉N₅O Calcd. C, 70.57%; H, 5.36%; N, 19.59%.

2.5c 3-Methyl-1-phenyl-7-(p-hydroxyphenyl)-1, 4, 5, 6, 7, 8 hexahydropyrazolo[3,4-d]imidazo[3,4-a]pyrimidin-4-one (5c): White colour solids; yield: 71%; m.p. 253–255°C; IR (KBr, cm⁻¹): 3267, 2927–2873, 1688; ¹H-NMR (DMSO-*d*₆) δ = 2.54 (s, 3H, CH₃), 4.60 (s,

2H, C-CH₂-N), 5.29 (s, 2H, N-CH₂-N), 6.66–6.72 (d, 2H, C_{2'}-H, C_{6'}-H), 6.95–6.99 (d, 2H, C_{3'}-H, C_{5'}-H), 7.32–7.41 (t, 1H, C₄-H), 7.52–7.60 (t, 2H, C₃-H, C₅-H), 7.99–8.02 (d, 2H, C₂-H, C₆-H), 8.80 (s, 1H, OH, D₂O exchangeable); MS (*m/z*): 359 (M⁺, 0.27%), 240 (100%); Anal. Found: C, 66.79%; H, 4.59%; N, 19.39%; C₂₀H₁₇N₅O₂ Calcd. C, 66.84%; H, 4.77%; N, 19.49%.

2.5d 3-Methyl-1-phenyl-7-(carboxyphenyl)-1, 4, 5, 6, 7,8 hexahydropyrazolo [3, 4-d] imidazo [3, 4-a] pyrimidin-4-one (5d): White colour solids; yield: 59%; m.p. 346–348°C; IR (KBr, cm⁻¹): 3404, 1664; ¹H-NMR (DMSO-*d*₆) δ = 2.49 (s, 3H, CH₃), 4.39–4.41 (d, 2H, CH₂), 6.70–6.82 (d, 2H, C_{2'}-H, C_{6'}-H), 6.81–6.83 (t, 1H, NH, D₂O exchangeable), 7.24–7.29 (d, 2H, C_{3'}-H, C_{5'}-H), 7.32–7.37 (t, 1H, C₄-H), 7.70–7.73 (t, 2H, C₃-H, C₅-H), 7.86–7.88 (d, 2H, C₂-H, C₆-H), 12.05 (s, 1H, NH, D₂O exchangeable), 12.27 (s, 1H, OH, D₂O exchangeable); MS (*m/z*): 375 (M⁺, 92.70%), 77 (100%); Anal. Found: C, 65.24%; H, 4.50%; N, 18.20%; C₂₁H₁₇N₅O₃ Calcd. C, 65.11%; H, 4.42%; N, 18.08%.

2.6 General procedure for the synthesis of 6 a–c

A mixture of compound **3** (2.75 g, 0.01 mol) and the appropriate secondary amine (0.02 mol) in absolute ethanol (20 mL) was heated under reflux for 3 h. The separated solid on hot, was filtered and crystallized from aqueous ethanol as off-white crystals.

2.6a 6-Diethylaminomethyl-3-methyl-1-phenyl-1, 5-dihydro-pyrazolo [3,4-d]pyrimidin-4-one (6a): White off-colour crystals; yield: 47%; m.p. 158–160°C; IR (KBr, cm⁻¹): 3424, 2966–2864, 1674; ¹H-NMR (DMSO-*d*₆) δ = 0.97–1.02 (t, 6H, *J* = 7.2 Hz, 2 CH₂-CH₃), 2.50 (s, 3H, CH₃), 2.58–2.65 (q, 4H, *J* = 7.2 Hz, 2 CH₂-CH₃), 3.60 (s, 2H, CH₂), 7.31–7.36 (t, 1H, C₄-H), 7.45–7.54 (t, 2H, C₃-H, C₅-H), 8.02–8.06 (d, 2H, C₂-H, C₆-H), NH not visible; MS (*m/z*): 311 (M⁺, 0.34%), 72 (100%); Anal. Found: C, 65.85%; H, 6.40%; N, 22.51%; C₁₇H₂₁N₅O Calcd. C, 65.57%; H, 6.80%; N, 22.49%.

2.6b 3-Methyl-1-phenyl-6-piperidin-1-ylmethyl-1,5-dihydro-pyrazolo [3,4-d]pyrimidin-4-one (6b): White off-colour crystals; yield: 62%; m.p. 195–197°C; IR (KBr, cm⁻¹): 3439, 2995–2852, 1673; ¹H-NMR (DMSO-*d*₆) δ = 1.59 (m, 4H, 2 CH₂), 1.83 (m, 2H,

CH₂), 2.64 (s, 3H, CH₃), 2.86 (m, 4H, 2 N-CH₂), 7.32–7.35 (t, 1H, C₄-H), 7.48–7.59 (t, 2H, C₃-H, C₅-H), 7.99–8.01 (d, 2H, C₂-H, C₆-H), NH not visible; MS (*m/z*): 323 (M⁺, 0.5%), 84 (100%); Anal. Found: C, 66.61%; H, 6.29%; N, 21.55%; C₁₈H₂₁N₅O Calcd. C, 66.85%; H, 6.55%; N, 21.66%.

2.6c 3-Methyl-6-morpholin-4-ylmethyl-1-phenyl-1, 5-dihydro-pyrazolo [3, 4-d] pyrimidin-4-one (6c): White off-colour crystals; yield: 62%; m.p. 219–221°C; IR (KBr, cm⁻¹): 3467, 2990–2811, 1670; ¹H-NMR (DMSO-*d*₆) δ = 2.48–2.59 (m, 7H, CH₃ and 2CH₂-O), 3.50 (s, 2H, CH₂), 3.58–3.61 (m, 4H, 2CH₂-N), 7.32–7.34 (t, 1H, C₄-H), 7.49–7.56 (t, 2H, C₃-H, C₅-H), 8.01–8.05 (d, 2H, C₂-H, C₆-H); MS (*m/z*): 325 (M⁺, 0.64%), 206 (100%); Anal. Found: C, 62.91%; H, 5.66%; N, 21.32%; C₁₇H₁₉N₅O₂ Calcd. C, 62.75%; H, 5.89%; N, 21.52%.

2.7 General procedure for the synthesis of 7 a–c

A solution of compound **3** (0.82 g, 3 mmol) in sodium (methoxide, ethoxide and/or propoxide) solution (0.46 g sodium in 30 ml absolute methanol, ethanol or propanol) was heated under reflux for 2 h, the solvent was concentrated to half its volume and acidified with (0.01 N) hydrochloric acid until just acidic to litmus paper. The separated solid was filtered and crystallized from aqueous ethanol as white crystals.

2.7a 6-Methoxymethyl-3-methyl-1-phenyl-1,5-dihydro-pyrazolo [3,4-d]pyrimidin-4-one (7a): Yellow colour solid; yield: 46%; m.p. 199–201°C; IR (KBr, cm⁻¹): 3454, 2995–2928, 1676; ¹H-NMR (DMSO-*d*₆) δ = 2.47 (s, 3H, CH₃), 3.38 (s, 3H, OCH₃), 4.37 (s, 2H, CH₂), 7.33–7.38 (t, 1H, C₄-H), 7.51–7.56 (t, 2H, C₃-H, C₅-H), 8.01–8.05 (d, 2H, C₂-H, C₆-H), 12.20 (s, 1H, NH, D₂O exchangeable); MS (*m/z*): 270 (M⁺, 85.33%), 240 (100%); Anal. Found: C, 61.99%; H, 5.25%; N, 20.94%; C₁₄H₁₄N₄O₂ Calcd. C, 62.21%; H, 5.22%; N, 20.73%.

2.7b 6-Ethoxymethyl-3-methyl-1-phenyl-1, 5-dihydro-pyrazolo [3, 4-d] pyrimidin-4-one (7b): Yellow colour solid; yield: 58%; m.p. 164–166°C; IR (KBr, cm⁻¹): 3436, 2977–2929, 1677; ¹H-NMR (DMSO-*d*₆) δ = 1.14–1.19 (t, 3H, CH₂CH₃), 2.49 (s, 3H, CH₃), 3.55–3.62 (q, 2H, CH₂CH₃), 4.39 (s, 3H, CH₂), 7.33–7.36 (t, 1H, C₄-H), 7.49–7.56 (t, 2H, C₃-H, C₅-H), 8.04–8.06 (d, 2H, C₂-H, C₆-H) and NH not visible; MS (*m/z*): 284 (M⁺, 36.66%), 240 (100%); Anal. Found:

C, 63.12%; H, 5.48%; N, 19.84%; C₁₅H₁₆N₄O₂ Calcd. C, 63.37%; H, 5.67%; N, 19.71%.

2.7c 3-Methyl-1-phenyl-6-propoxymethyl-1, 5-dihydro-pyrazolo [3,4-d]pyrimidin-4-one (7c): Yellow colour solid; yield: 30%. m.p. 158–160°C; IR (KBr, cm⁻¹): 3424, 2957–2871, 1686; ¹H-NMR (DMSO-*d*₆) δ = 0.97–1.02 (t, 3H, CH₂-CH₃), 1.66–1.78 (m, 2H, CH₂-CH₂-CH₃), 2.65 (s, 3H, CH₃), 3.57–3.62 (t, 2H, O-CH₂-), 4.53 (s, 2H, CH₂-O), 7.27–7.35 (t, 1H, C₄-H), 7.45–7.51 (t, 2H, C₃-H, C₅-H), 7.99–8.02 (d, 2H, C₂-H, C₆-H), 9.46 (s, 1H, NH, D₂O exchangeable); MS (*m/z*): 298 (M⁺, 82.94%), 240 (100%); Anal. Found: C, 64.20%; H, 5.89%; N, 18.37%; C₁₆H₁₈N₄O₂ Calcd. C, 64.41%; H, 6.08%; N, 18.78%.

2.8 General procedure for the synthesis of 8 a and b

A mixture of compound **3** (2.75 g, 0.01 mol), appropriate chloroacetanilide derivative (0.01 mol) and potassium hydroxide (1.12 g, 0.02 mol) in absolute ethanol (25 ml) was heated under reflux for 24 h. The separated solid on hot, was filtered, washed with water and crystallized from dioxane as off-white crystals.

2.8a 3-Methyl-1-phenyl-7oxo-8-phenyl-4, 5, 6, 7, 8, 9-hexahydro-pyrazolo [3, 4-d] pyrazino [4, 3-a] pyrimidin-4-one (8a): White colour solid; yield: 18%; m.p. 245–247°C; IR (KBr, cm⁻¹): 2916–2863, 1704; ¹H-NMR (DMSO-*d*₆) δ = 2.54 (s, 3H, CH₃), 4.77 (s, 2H, CH₂-N), 5.01 (s, 2H, CH₂-CO), 7.27–8.04 (m, 10H, Ar-H); MS (*m/z*): 371 (M⁺, 0.33%), 240 (100%); Anal. Found: C, 67.80%; H, 4.71%; N, 18.70%; C₂₁H₁₇N₅O₂ Calcd. C, 67.91%; H, 4.61%; N, 18.86%.

2.8b 3-Methyl-1-phenyl-7oxo-8-(*p*-tolylphenyl)-4, 5, 6, 7, 8, 9-hexahydro-pyrazolo[3,4-d]pyrazino[4,3-a]pyrimidin-4-one (8b): White colour solid; yield: 15%; m.p. 252–254°C; IR (KBr, cm⁻¹): 2924–2850, 1705, 1583; ¹H-NMR (DMSO-*d*₆) δ = 2.22 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 4.66 (s, 2H, CH₂-N), 5.34 (s, 2H, CH₂-CO), 6.67–6.70 (d, 2H, C₂-H, C₆-H), 7.08–7.11 (d, 2H, C₃-H, C₅-H), 7.35–7.40 (t, 1H, C₄-H), 7.52–7.58 (t, 2H, C₃-H, C₅-H), 7.99–8.02 (d, 2H, C₂-H, C₆-H); MS (*m/z*): 385 (M⁺, 55.56%), 206 (100%); Anal. Found: C, 68.30%; H, 4.91%; N, 18.30%; C₂₂H₁₉N₅O₂ Calcd. C, 68.56%; H, 4.97%; N, 18.17%.

2.9 6- Mercaptomethyl-3-methyl-1-phenyl-1*H*-pyrazolo [3, 4-*d*] pyrimidin-4-one (**9**)

A mixture of compound **3** (1.58 g, 5.75 mmol) and thiourea (0.76 g, 0.01 mol) in absolute ethanol (25 ml) was heated under reflux for 4 h. The product which was obtained on hot, was filtered, dissolved in sodium hydroxide (20 ml, 5%) and acidified with (0.01N) hydrochloric acid until just acidic. The solid separated was collected and crystallized from dimethyl

formamide as yellow colour crystals; yield: 50%; m.p. 293–295°C; IR (KBr, cm⁻¹): 3426, 2733, 1675; ¹H-NMR (DMSO-*d*₆) δ = 2.48 (s, 3H, CH₃), 3.97 (s, 2H, CH₂), 7.28–7.31 (t, 1H, C₄-H), 7.42–7.47 (t, 3H, C₃-H, C₅-H and SH (D₂O exchangeable)), 7.99–8.01 (d, 2H, C₂-H, C₆-H), 12.35 (s, 1H, NH, D₂O exchangeable); MS (*m/z*): 272 (M⁺, 45.30%), 198 (100%); Anal. Found: C, 57.11%; H, 4.55%; N, 20.60%; C₁₃H₁₂N₄OS Calcd. C, 57.34%; H, 4.44%; N, 20.57%.

Table 1. Docking study data and cytotoxic activity (IC₅₀) for olomoucine **I** and some newly synthesized compounds on CDK enzyme.

Compound No.	Number of H-bonds	Atoms of compound forming H-bonds	Amino acid residues forming H-bonds	Binding energy score (Kcal/mol)	IC ₅₀ (µg/ml)	IC ₅₀ (µM)
4b		C=O,	Leu 83			
	2	NH		–23.1725	2.7	0.008
4d		C=O,	Leu 83			
	3	NH, COO ⁻	Leu 83 His 84	–17.6897	11.6	0.030
5a	-	-	-	–23.8349	9.6	0.027
5c	1	OH	Lys 128	–18.2302	8.84	0.013
5d		C=O of (COOH),	Thr 14			
	2			–11.7559	17.6	0.045
		C–O ⁻	Lys 129			
6a	1	NH	Asp 145	–19.1579	5.55	0.017
6b		C=O,	Leu 83			
	3	NH, N piperidino group	Leu 83 Lys 89	–23.0477	4.2	0.012
6c		C=O,	Leu 83			
	3	NH, N morpholino group	Leu 83 Lys 89	–23.2674	17.7	0.054
7a		C=O,				
	1		Gln 131	–18.1891	5.85	0.021
7b		C=O,	Leu 83			
	2	NH	Leu 83	–21.2642	10.2	0.035
7c		C=O,	Leu 83			
	2			–20.1295	13.2	0.044
		NH	Leu 83			
8a	1	C=O	Gln 131	–24.2298	3.45	0.009
8b	1	C=O	Gln 131	–25.0835	6.6	0.080
10a		C=O,	Leu 83			
	2			–21.0402	8.4	0.023
		NH	Leu 83			
10b		C=O,	Leu 83			
	2			–24.4210	2.85	0.007
		NH	Leu 83			
11a	1	C=O	Lys 89	–16.2786	4.2	0.007
11b		C=O,	His 84			
	2	NH of (–SCH ₂ CONH–)	Asp 56	–21.6464	2.85	0.004
Olomoucine		N6,	Leu 83,		–	
	2			–19.4146		7
		NH	Leu 83			

Bold numbers refer to the most potent compounds (lower IC₅₀) relative to the standard

2.10 General procedure for the synthesis of **10a** and **b**

A mixture of compound **9** (1.04 g, 3.87 mmol), the appropriate halo compound (3.87 mmol) and sodium acetate (0.7 g, 8.50 mmol) in absolute ethanol was heated under reflux for 5 h, and then allowed to cool. The separated solid was filtered, washed with water and crystallized from acetone as yellow crystals.

2.10a (3-Methyl-4-oxo-1-phenyl-4, 5-dihydro-1H-pyrazolo [3, 4-d] pyrimidin-6-ylmethylsulphonyl)-acetic acid ethyl ester (**10a**): Yellow colour solid; yield: 60%; m.p. 160–162°C; IR (KBr, cm^{-1}): 3445, 2982–2851, 1734, 1674; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ = 1.02–1.06 (t,

3H, CH_2CH_3), 2.49 (s, 3H, CH_3), 3.54 (s, 2H, $-\text{CH}_2-\text{S}$), 3.73 (s, 2H, $\text{S}-\text{CH}_2$), 3.88–3.95 (q, 2H, CH_2CH_3), 7.34–7.37 (t, 1H, C_4-H), 7.49–7.54 (t, 2H, C_3-H , C_5-H), 8.03–8.06 (d, 2H, C_2-H , C_6-H), 12.33 (s, 1H, NH, D_2O exchangeable); MS (m/z): 358 (M^+ , 17.90%), 240 (100%). Anal. Found: C, 57.19%; H, 5.25%; N, 15.89%; $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_3\text{S}$ Calcd. C, 56.97%; H, 5.06%; N, 15.63%.

2.10b 6-Benzylsulphanylmethyl-3-methyl-1-phenyl-1, 5-dihydro-pyrazolo[3,4-d]pyrimidin-4-one (**10b**): Yellow colour solid; yield: 65%; m.p. 217–219°C; IR (KBr, cm^{-1}): 3439, 2990–2857, 1687; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$)

(a)

(b)

Figure 1. (a) Proposed binding mode of olomoucine **I** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. It forms two H-bonds with Leu 83 through its pyrimidine C6-NH and N7 of imidazol ring, and one H-bond with C2 side chain-OH group. (b) 2D interaction of olomoucine **I** with Leu 83 and Gln 131 amino acid.

Figure 2. (a) Structure of the lead compound olomoucine. (b) Common features for the newly synthesized compounds.

δ = 2.38 (s, 3H, CH₃), 3.53 (s, 2H, -CH₂-S), 3.97 (s, 2H, S-CH₂-Ph), 7.18–8.09 (m, 10H, Ar-H), 12.31 (s, 1H, NH, D₂O exchangeable); MS (m/z): 362 (M⁺, 21.10%), 91 (100%). Anal. Found: C, 66.20%; H, 5.05%; N, 15.32%; C₂₀H₁₈N₄OS Calcd. C, 66.28%; H, 5.01%; N, 15.46%.

2.11 General procedure for synthesis of **11 a** and **b**

A mixture of compound **9** (2.72 g, 0.01 mol), appropriate anilide compound (0.02 mol) and potassium carbonate (2.76 g, 0.02 mol) in acetone (20 ml), was heated under reflux for 10 h, then allowed to cool.

(a)

(b)

Figure 3. (a) Proposed binding mode of compound **4b** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. Compound **4b** forms two H-bonds with Leu 83 through its pyrimidine NH and carbonyl group. (b) 2D interaction of compound **7b** with Leu 83 amino acid.

The separated solid was filtered and crystallized from dioxane as yellow crystals.

2.11a 2-(3-Methyl-4-oxo-1-phenyl-5-phenylcarbamoyl methyl-4, 5-dihydro-1H- pyrazolo [3, 4-d] pyrimidin-6-ylmethylsulphanyl)-N-phenyl-acetamide (**11a**): Yellow colour solid; yield: 58%; m.p. 280–282°C; IR (KBr,

cm⁻¹): 3437–289, 2978.52–926, 1691, 1662; ¹H-NMR (DMSO-*d*₆) δ = 2.49 (s, 3H, CH₃), 3.54 (s, 2H, –H₂–S), 4.09 (s, 2H, S–CH₂), 5.08 (s, 2H, N–CH₂–CO), 6.99–8.03 (m, 15H, Ar-H), 9.94 (s, 1H, NH, D₂O exchangeable), 10.45 (s, 1H, NH, D₂O exchangeable); MS (*m/z*): 538 (M⁺, 20.26%), 281 (100%); Anal. Found: C, 64.59%; H, 4.29%; N, 16.62%; C₂₉H₂₆N₆O₃S Calcd. C, 64.67%; H, 4.87%; N, 15.60%.

(a)

(b)

Figure 4. (a) Proposed binding mode of compound **8a** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. Compound **8a** forms a H-bonds with Gln 131 through its pyrimidine C=O. (b) 2D interaction of compound **8a** with Gln 131 amino acid.

2.11b 2-[3-Methyl-4-oxo-1-phenyl-5-(*p*-tolylcarbamoylmethyl)-4, 5-dihydro-1*H*-pyrazolo [3, 4-*d*] pyrimidin-6-ylmethylsulphanyl]-*N*-*p*-tolyl-acetamide (**11b**): White colour solid; yield: 69%. m.p.; 289–291°C; IR (KBr, cm^{-1}) 3437–3304, 3035–2921, 1671 (3C=O); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ = 2.19 (s, 6H, 2CH₃), 2.49 (s, 3H, CH₃), 3.49 (s, 2H, –CH₂–S), 4.08 (s, 2H, S–CH₂), 5.05 (s, 2H, N–CH₂–CO), 6.97–8.15 (m, 13H, Ar-H), 9.86 (s, 1H, NH, D₂O exchangeable) and 10.36 (s, 1H, NH, D₂O exchangeable); MS (m/z): 566 (M^+ , 10.40%), 55 (100%); Anal. Found: C, 65.44%; H, 5.56%; N, 14.62 %; C₃₁H₃₀N₆O₃S Calcd. C, 65.70%; H, 5.34%; N, 14.83%.

2.12 Antitumour bioassay

The effects of compounds on the growth of tumour cell lines (MCF-7), were evaluated according to the

procedure adopted by the National Cancer Institute, Cairo, Egypt for the *in vitro* anticancer drug screening that use the protein-binding dye sulphorhodamine B (SRB) to assess growth inhibition.²⁷ Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in humidified atmosphere containing 5% CO₂.

The cell line was regularly subcultured to be maintained in the exponential growth phase. Cells were exposed for 48 h to different concentrations (2.5–50 $\mu\text{g/ml}$) of test compounds. Test compounds were prepared in dimethylsulphoxide (DMSO), were freshly diluted with cell culture medium just prior to the assays. Olomoucine **I** was used as positive control. For each test compound and cell line, a dose-response curve was generated and growth inhibition of 50% (IC₅₀), corresponding to the concentration of compound that inhibits

Figure 5. (a) The proposed binding mode of compound **10b** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. Compound **10b** forms two H-bonds with Leu 83 through its pyrimidine NH and carbonyl group. (b) 2D interaction of compound **13b** with Leu 83 amino acid.

50% of the net cell growth was determined. Results of *in vitro* cytotoxic activity experiments are presented in table 1.

3. Results and discussion

3.1 Molecular modelling

The lead compound olomoucine **I** (PDB ID: 1W0X),²⁶ which was reported to have CDK inhibitory activity,

was used to generate common feature hypothesis of CDK inhibitor as antitumour agents by introducing pyrazole ring instead of imidazole ring on the purine analogue. Both N7 and NH on C6 of imidazole ring of olomoucine **I** was found to form a H-bond with Leu 83 which is important for the activity. The C2 side chain is bound within the ATP ribose binding pocket, with the methyl group which interacts hydrophobically with glycine-rich loop and val 18. The hydroxyl group of the side chain can form a H-bond with the backbone carbonyl of Gln 131. Purine N9 of methyl group shows a

Figure 6. (a) Proposed binding mode of compound **11a** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. Compound **11a** forms two H-bonds with His 84 through its NH group and with Gln 131 through its pyrimidine C=O group, the other interaction is arene cation interaction with His 84 amino acid. (b) 2D interaction of compound **11a** with His 84 and Gln 131 amino acids.

strong hydrophobic interaction with hydrophobic side chains of Val 18, Ala 31, Phe 80, Leu 134 and Ala 144 (figure 1).¹

Using the common features of the lead compound **I**, we designed new pyrazolo[3,4-*d*]pyrimidine derivatives with the following modifications:

N1 Pyrazole nitrogen of pyrazolopyrimidine ring was substituted with phenyl group instead of the methyl group at N9 of the lead compound **I**. At the C6 side chain, we added additional carbon before NH group to act as a spacer that showed good fitting to the receptor. We explored the usage of alkyl or aromatic side chain at C6 in order to increase hydrophobic interaction towards the receptor. Additional modification was added at N5

of the pyrimidine ring to certain new compounds aiming to increase hydrogen bonding interaction with the receptor binding site (figure 2).

These modifications and docking of new compounds with CDK enzyme has given rise to a new common feature hypothesis for our compounds (figure 2b). This hypothesis showed that carbonyl group at C4 and N5H from a hydrogen bond with Leu 83 and similar interaction was also stated for the lead compound **I**. Alteration of methyl group in **I** with phenyl group in **II** resulted in better hydrophobic interaction, hence phenyl is more deeply socked into ATP ribose binding pocket. By induction of different pharmacophores to C6 side chain, a variety of hydrogen bonding was estimated in

Figure 7. (a) Proposed binding mode of compound **11b** inside the active site of CDK resulting from docking, the most important amino acids are shown together with their respective numbers. Compound **11b** forms two H-bonds with Asp 86 and His 84 through its 2NH groups of the two (SCH₂CONH) groups, the other interaction is arene cation interaction with Lys 20 amino acid. (b) 2D interaction of compound **14b** with Asp 86 and His 84 amino acids.

Scheme 1. Reagents: (a) chloroacetyl chloride; (b) primary aliphatic amines; (c) formaldehyde; (d) secondary aliphatic amines; (e) different alkoxides and (f) chloroacetanilide derivatives.

certain compounds and this hydrogen bonding occurred with Gln 8, 131, His 84, Lys 89, 128, 129, Thr 14 and Asp 56, 145 amino acids (figures 3–7). However, most of our compounds interact with the receptor with minimum binding energy scores as shown in table 1.

3.2 Experimental section

Reaction of 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**1**),²⁸ with chloroacetyl chloride yielded 6-chloromethyl-3-methyl-1-phenyl pyrazolo [3, 4-*d*] pyrimidin-4-one (**3**), which was used as a starting material for synthesis of novel pyrazolopyrimidines. Cyclization of **1** was preceded through chloroacetylation of amino group forming chloroacetyl aminopyrazole

carboxamide **2** as non-isolatable intermediate followed by dehydration to afford **3**. Structure of compound **3** was established on the basis of spectral analyses. IR spectrum showed absorption band at 3276 and 75 cm⁻¹ attributed to the presence of (NH) and (C=O) groups, respectively. Moreover, ¹H-NMR spectrum of compound **3** revealed a single signal at δ 4.45 assigned to the two protons of (CH₂–Cl) group, and a singlet at δ 12.61, D₂O exchangeable attributed to the (NH) proton. Also, its mass spectrum exhibited a molecular ion peak at m/z 274 which agreed with the expected structure.

The key intermediate **3** was used as an alkylating agent to be reacted with different primary aliphatic amines, secondary aliphatic amines and some alkoxides

10, a, R⁵ = CH₂COOC₂H₅,

10, b, R⁵ = CH₂C₆H₅

11, a, R⁶ = H,

11, b, R⁶ = CH₃

Scheme 2. Reagents: a, thiourea; b, a) ethyl chloroacetate, b) benzyl chloride; c, a) anilide, b) *p*-tolyl anilide.

giving series **4a–d**, **6a–c** and **7a–c**, respectively. All the prepared compounds were confirmed by element analysis and spectral data. Mannich reaction of aryl-aminomethylpyrazolopyrimidines **4a–d** with formalin (30%) solution was preceded via hydroxyl methylation of the NH group of aryl amino group which spontaneously underwent elimination of water to yield **5a–d**. Reaction of formaldehyde carbonyl group occurred at the amino NH rather than the pyrimidine NH which is present in tautomerism with the adjacent carbonyl group. The structure of **5a–d** was confirmed using spectral analyses. IR spectra of compounds **5a–d** showed disappearance of bands characteristic of NH groups in the precursor compound. Also showed absorption bands at the range 1704–1664 cm^{−1} for (C=O) group. Their ¹H-NMR showed the disappearance of D₂O exchangeable signals due to 2 NH protons and appearance of two singlet at δ 4.60–4.70 and at δ 5.29–5.37 characteristic of 2 CH₂ groups.

Before adding alkyl pharmacophore to N5H of pyrimidine moiety, compound **3** was reacted with chloroacetanilide derivatives which resulted in the tricyclic products **8a** and **b**. This reaction was suggested to be proceeded via double alkylation; one of them is of the amidic NH with methylene chloride group of pyrimidine **3** and the other for N5H of pyrimidine **3** and −CH₂Cl of the anilide. Structure of compounds **8a** and **b** was confirmed by its IR, which revealed two (C=O) groups at the range of 1705–1704 cm^{−1} and disappearance of NH group. ¹H-NMR spectra showed two singlet signals at δ 4.66–4.77 and at δ 5.01–5.34 characteristic of 2 CH₂ groups. Also, mass spectrum of compounds **8a** and **b** exhibited a molecular ion peak at *m/z* 371 and *m/z* 385, respectively (scheme 1).

On the other hand, 6-chloromethyl-3-methyl-1-phenyl pyrazolo [3, 4-*d*] pyrimidin-4-one (**3**) was converted into the corresponding mercapto derivative **9** by refluxing with thiourea in ethanol resulting in the

formation of the isothiuronium salt which was then treated with sodium hydroxide followed by acidification with hydrochloric acid to give compound **9**. Structure of compound **9** was elucidated from its element and spectral analyses. IR spectrum showed absorption band at 2733 cm^{-1} for SH group. Its $^1\text{H-NMR}$ showed a single signal at δ 3.97 for $-\text{CH}_2$. Mass spectrum of compound **9** revealed a peak at m/z 272 corresponding to molecular ion peak.

As an extension of this synthetic route, compound **9** was alkylated using halogenated compounds such as ethyl chloroacetate and/or benzyl chloride to give S-alkylated compounds **10a** and **b**. Structure of **10a** and **b** was elucidated using element and spectral analyses. IR spectra showed disappearance of SH peak and appearance of absorption bands in the range of $3445\text{--}3439\text{ cm}^{-1}$ for (NH) group, $1674\text{--}1687\text{ cm}^{-1}$ for (C=O), and 1734 cm^{-1} for (C=O) of ester moiety in compound **10a**. The $^1\text{H-NMR}$ showed the appearance of new signals characteristic for $(-\text{S}-\text{CH}_2-)$ at δ 3.73, 3.97, also, mass spectra for compounds **10a** and **b** showed molecular ion peaks at m/z 358 and 362 respectively which is in agreement with the suggested structure.

In addition to S-alkylation, chloroacetanilide derivatives were used for S-alkylation, while the resulting structures **11a** and **b** were the doubly alkylated products on S- and N- atoms. This was attributed to the effect

of $(-\text{CONH})$ group of the chloroacetanilide derivatives which increased the positive charge at (CH_2) and facilitated the acceptance of lone pairs on S and N atoms leading to alkylation on both S and N. The structures were confirmed by micro analytical and spectral data, IR spectra showed absorption bands in the range of $3437\text{--}3289\text{ cm}^{-1}$ for two amidic NH groups, $1691\text{--}1662\text{ cm}^{-1}$ for (C=O) of pyrimidine ring and (2C=O) of amidic bond. $^1\text{H-NMR}$ showed the appearance of single signals characteristic for $(-\text{CH}_2\text{S}-)$ at δ 3.49, 3.54, singlet signals at δ 4.08, 4.09 for $(-\text{S}-\text{CH}_2-)$, at δ 5.05–5.08 for $(-\text{N}-\text{CH}_2-\text{CO}-)$, also appearance of two D_2O exchangeable singlet signals at δ 9.86–9.94 and 10.36–10.45 referred to the two (NH) groups. Mass spectra for compounds **11a** and **b** showed molecular ion peak at m/z 538 and 566, respectively which confirmed the suggested structure (scheme 2).

3.3 Antitumour activity

Olomoucine **I** was used as the reference drug in this study. Some of the synthesized compounds were selected and screened for their anticancer activity. Each compound was tested at different concentrations against human breast cancer cell line. The relationship between survival fraction and drug concentration was plotted to obtain the survival curve of human breast cancer cell line (MCF-7). The response parameters calculated

Table 2. *In vitro* cytotoxic activity of some newly synthesized compounds and the standard olomoucine **I**.

Compound	Survival fraction (%)					IC ₅₀ ^a (μg/ml)	IC ₅₀ (μM)
	Concentration (μg/mL)						
	50	25	12.5	5	2.5		
4b	0.135	0.132	0.101	0.111	0.149	2.7	0.008
4d	0.163	0.105	0.473	0.918	0.987	11.6	0.030
5a	0.124	0.233	0.405	0.652	0.804	9.6	0.027
5c	0.289	0.201	0.271	0.472	0.631	8.84	0.013
5d	0.113	0.371	0.583	0.725	0.901	17.6	0.045
6a	0.188	0.181	0.297	0.523	0.634	5.55	0.017
6b	0.255	0.139	0.152	0.418	0.620	4.2	0.012
6c	0.173	0.391	0.581	0.762	0.940	17.7	0.054
7a	0.233	0.140	0.239	0.521	0.761	5.85	0.021
7b	0.214	0.297	0.392	0.754	0.939	10.2	0.035
7c	0.133	0.235	0.515	0.727	0.913	13.2	0.044
8a	0.201	0.113	0.186	0.307	0.524	3.45	0.009
8b	0.198	0.080	0.154	0.614	0.810	6.6	0.080
10a	0.234	0.161	0.377	0.599	0.771	8.4	0.023
10b	0.048	0.076	0.086	0.103	0.311	2.85	0.007
11a	0.123	0.077	0.080	0.404	0.612	4.2	0.007
11b	0.236	0.209	0.129	0.202	0.483	2.85	0.004
Olomoucine	—	—	—	—	—	—	7

^aThe values given are means of three experiments

Bold numbers refer to the most potent compounds (lower IC_{50}) relative to the standard

Chart 1. Docking scores against IC₅₀.

was the IC₅₀ value, which corresponds to the compound concentration causing 50% mortality in net cells (table 2).

4. Conclusions

The present data showed that compounds **4b**, **8a**, **10b**, **11a** and **b** exhibited promising *in vitro* cytotoxic activity against (MCF-7) giving the highest cytotoxic activity between 0.009 and 0.004 μ M when compared to the other test compounds and olomoucine **I** (7 μ M) as a reference drug. All the other test compounds showed higher IC₅₀ values than that of the reference drug, ranging from 0.012 to 0.080 μ M. Docking studies confirmed this result as compounds **4b**, **8a**, **10b**, **11a** and **b** showed low binding energy scores from -24.2298 to -16.2786 Kcal/mol, also showed binding mode similar to olomoucine **I** which was reported to possess CDK inhibitory effects. IC₅₀ values were plotted against binding energy scores which revealed that there was some sort of consistency between the docking studies and *in vitro* screening (chart 1).

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