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Original article

Design, synthesis, and biological evaluation of novel pyrimidine derivatives as CDK2 inhibitors

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ABSTRACT

Novel derivatives of 2,4,5,6-tetrasubstituted pyrimidine cyclin-dependent kinase (CDK2) inhibitors was designed and synthesized. We built a library of proposed pyrimidine derivatives and by using pharmacophore and docking techniques we made our selections. We modified the proposed compounds due to the interaction of docked structures with the protein to achieve the best fit. The newly synthesized compounds showed potent and selective CDK2 inhibitory activities and inhibited in-vitro cellular proliferation in cultured human tumor cells. The design, synthesis and biological evaluation of these 2,4,5,6-tetrasubstituted pyrimidine derivatives are reported.

1. Introduction

Cyclin-dependent kinases (CDKs) are a family of serine–threonine kinases that play important roles as regulators of cell progression through consecutive phases of the cell cycle [1]. CDKs are regulated by phosphorylation and activated by their association with cyclins [2]. The precise regulation of CDK activity is essential for the stepwise execution of the many processes required for cell growth and division, including DNA replication and chromosome separation [3]. Abnormal CDK control of the cell cycle has been strongly linked to the molecular pathology of cancer [4]. Such revelations have led to the investigation of small molecule CDK inhibitors (CDKIs) as possible cancer therapeutics [5,6]. All CDK inhibitors, identified so far, function by competing with ATP for binding to the catalytic site. The design of inhibitors specific to a particular protein kinase was originally thought of as an impossible task owing to the high degree of homology shared by the ATP binding domains of these enzymes. Actually, several CDKIs have entered clinical evaluation for the treatment of cancer. These include flavopiridol [7], 7-hydroxystaurosporine (UCN-01) [8], roscovitine (CYC202) [9], and the aminothiazole compound (BMS-387032) [10]. Nowadays, the synthesis of novel highly selective CDKs as candidates for CDK-target therapy in cancer treatment is in high demand [11,12].

In our program to develop CDK2 inhibitors as anti-cancer agents, we recently reported that pyrazolo[3,4-*d*]pyrimidines, 3,6-disubstituted [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole and pyrimido[4,5-*d*]pyrimidines analogs are novel anti-cancer inhibitors and anti-proliferative agents [13,14]. To discover new different CDK2 inhibitors with improved activity and selectivity, we have designed, synthesized, and evaluated pyrimidine derivatives as inhibitors of CDK2.

2. Rational and design

2.1. Structure preparation

The coordinate for the protein structure was obtained from the RCSB Protein Data Bank (PDB) (2C6I) [15]. Protein Structure was prepared using Discovery Studio (DS 2.0) software package [16]. The invalid or missing residues were added and the structures were aligned using the protein structure alignment module. Hydrogen atoms were added and the structure was minimized using CHARMm force field to relax the backbone and to remove the clashes. The protein was inspected visually for accuracy in the X2 dihedral angle of Asn and His residues and the X3 angle of Gln, and rotated by 180° when needed to maximize hydrogen bonding. The proper His tautomer was also manually selected to maximize hydrogen bonding.

The proposed compounds were optimized by semiemperical method (AM1) using Chem3D to eliminate bond length and bond

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angle biases and saved to be used in the pharmacophore mapping and docking steps.

2.2. Common features pharmacophore

The pharmacophore model was generated derived from 11 potent inhibitors (Fig. 1) of CDK2 by calculating the common features. This model was made up of hydrophobic center, acceptor atom associated to its protein donor site and donor atom (Fig. 2). The interfeature distances were considered to 7.696, 8.684 and 8.24 A° for the distances between the acceptor and the hydrophobe, the hydrophobe and the donor and the donor to the acceptor, respectively. The association of the acceptor atom to the donor site in the protein ensured the overall orientation of the molecules with respect to the kinase. Only one angle constraint was used for the hydrophobic and the acceptor atom features, thus allowing the hydrophobic and the aromatic centers to cover the large domain in the kinase active site, from the hydrophobic to the sugar pocket.

Using this pharmacophore model, we mapped our proposed compounds which contain pyrimidine moiety in order to find the promising compounds that are capable of binding to CDK2 with a similar set of interactions (Fig. 3). Finally, we selected the proposed compounds with high fit value for the docking step Table 1.

2.3. Docking

However, since we were interested in finding CDK2 inhibitors representing novel chemotypes or at least chemotypes free of intellectual property (IP) constrains, we opted to keep our docking strategy unbiased. The newly proposed compounds which selected from mapping to the pharmacophore models were docked into the active site of CDK2 with Gold docking program [17] using Gold-score function based on protein–ligand complexes. Ligand docking poses were represented using a conventional chromosome representation of translation, rotation and rotatable bond dihedral angles. The overall orientation and internal conformation of the compounds were searched with the GA, while the receptor site was kept fixed. We considered a binding mode to be reasonable if the

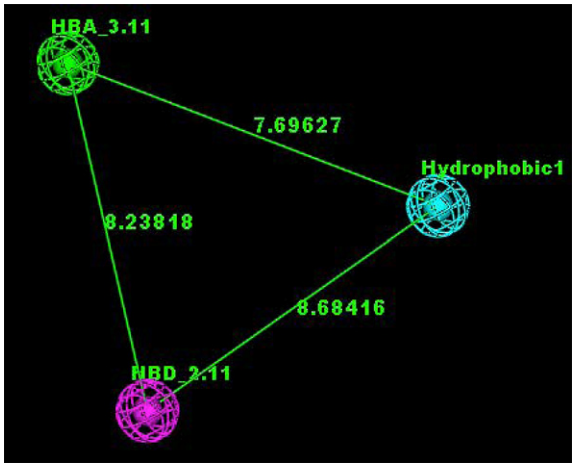


Fig. 2. Pharmacophore model, which derived from potential CDK2 inhibitors and used in the pre-selection of the proposed compounds (hydrophobic center; HBA; HBD).

key hydrogen bond (with respect to the bi-dentate hydrogen bonding of purine moiety) was formed in any of the top 10 solutions and the final interaction could be described by either the Olomoucine- or ATP-type binding model (Fig. 4).

3. Chemistry

We reported an improving method [18] for the synthesis of 6-(2, 4-dihydroxyphenyl) – 2 – mercapto – 4 – methyl-*N*-aryl- l,6-dihydropyrimidine-5-carboxamide (**1a**,**b**), as a key intermediate for the synthesis of the proposed compounds, starting from acetoacetanilids, 2,4-dihydroxybenzaldehyde and thiourea.

Pyrimidine derivatives (**1a**,**b**) were readily alkylated with benzyl chloride in basic medium to afford the 2-benzylthio derivatives (**2a**,**b**) in good yield which in turn reacted with different amines in acetic acid to give the 2-aminoderivatives (**3a**–**g**) in good yields. Also, compounds (**2a**,**b**) were oxidized with hydrogen peroxide to give the sulfanyl derivatives (**4a**,**b**) as shown in Scheme 1.

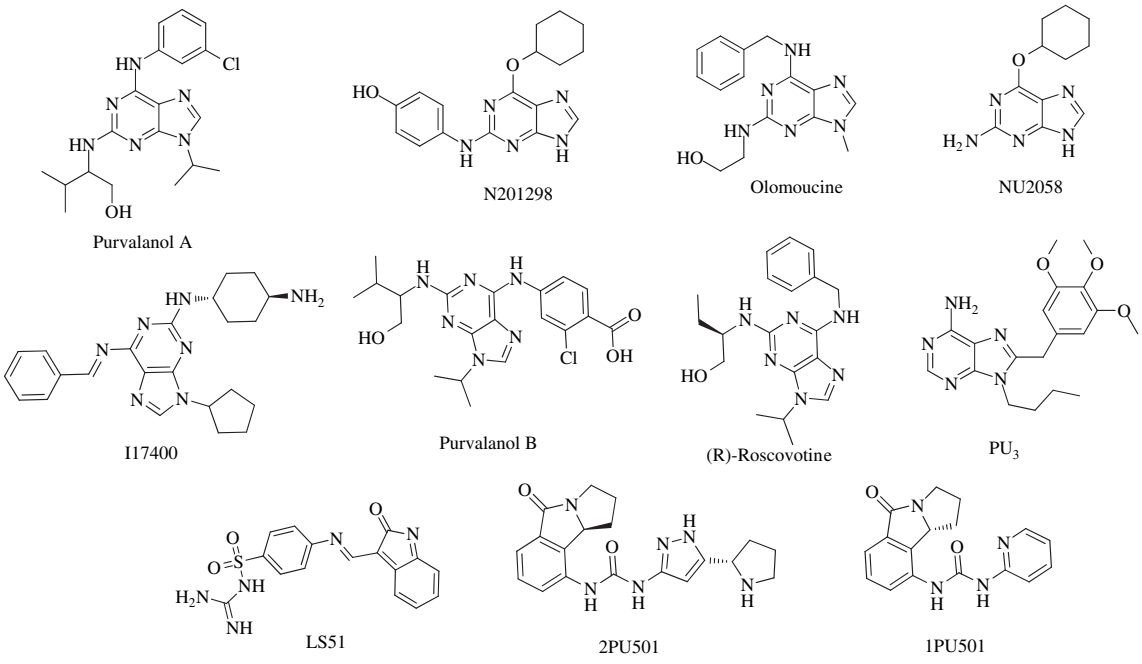


Fig. 1. CDK2 inhibitors which used for building pharmacophore model.

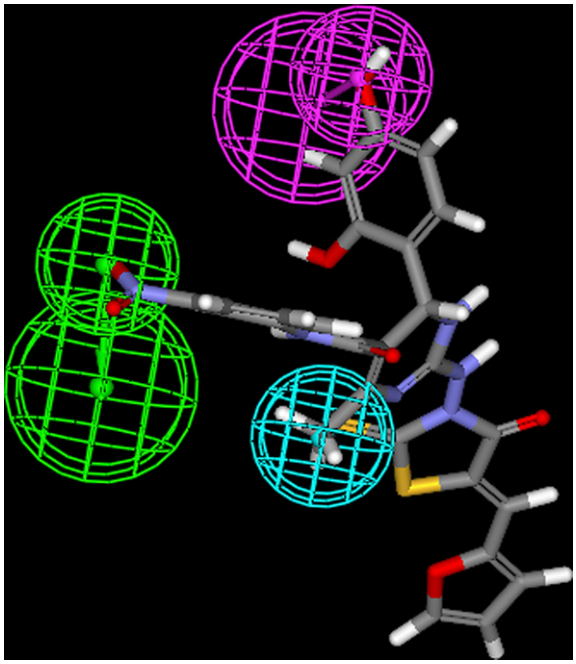


Fig. 3. Compound **8b** mapped to the generated pharmacophore (Fit Value = 2.993).

2-benzylthio derivative (**2a**,**b**) was reacted with hydrazine hydrate to afford 2-hydrazinyl derivative (**5**), which reacted with different aldehydes at room temperature to give hydrazone derivatives (**6a**,**b**). We adopted three one-pot synthesis by the reaction of compound (**5**) with ethyl acetoacetate and different aromatic aldehydes to give the pyrazole derivatives (**7a**,**b**) in high yields. Finally, we carried out another successful one-pot synthesis by reaction of hydrazinyl derivative (**5**) with carbon disulphide, monochloroacetic acid and aryl aldehydes in alcoholic potassium hydroxide to form arylidene thiazolidinone derivatives (**8a–c**) in moderate yields as depicted in Scheme 2.

4. Biological results and discussion

CDK2 and EGFR activities of pyrimidine derivatives prepared above were shown in Table 2, together with those of olomoucine, roscovitine and PYK2104 as reference compounds. CDK2 is one of CDK family protein which has a proline directed serine/threonine kinase activity, phosphorylating serine or threonine residue ahead of proline. Compounds were further evaluated for their cell division inhibitory activities against two human tumor cell lines. These tumor cells divide at least twice for 2-day period of the cell division inhibition test in the absence of inhibitory compounds and the kinase activity of CDK2/cyclinA is indispensable for the division of these cells [19,20].

Table 1
Docking Scores and Fit Values of the proposed pyrimidine derivatives.

Compounds	Docking Score[a]	Fit value
2a	66.292	2.96
2b	67.159	2.99
3a	48.82	2.954
3b	58.272	2.939
3c	48.34	2.995
3d	62.085	2.987
3e	58.76	2.989
3f	63.773	2.993
3g	67.646	2.99
4a	51.273	2.896
4b	74.409	2.995
5	57.265	2.972
6a	67.502	2.994
6b	56.413	2.994
7a	62.366	2.992
7b	67.143	2.99
8a	78.655	2.991
8b	73.552	2.993
8c	53.585	2.98
Ligand-2C6I	61.121	2.696

[a] The RMSD of the re-docking the native ligand is 0.91.

In general, compounds **8a**,**b** and **4a** showed better CDK2 and cell division inhibitory activities than the other compounds. Compounds **8a**,**b** were much more active than the reference compounds (roscovitine and olomoucine) with IC_{50} = 0.4 and 0.3 respectively, whereas compound **8a** had no inhibitory activity on cell division. Compounds **4b** and **3g** exhibited potent CDK2 inhibitory activity and reasonable cell division inhibitory activity. The other compounds showed good to moderate CDK2 inhibitory activities except compounds **3a** and 5 which had no CDK2 inhibitory activities. We study the correlation between docking scores and CDK2 inhibitor activities and we found that there is a good correlation between them (Fig. 5). Finally, most compounds did not show any significant EGFR inhibitory activity, indicating good selectivity in the situation of protein kinase inhibitors.

It seems that there is not a good correlationship between in-vitro CDK2 inhibitory activity and in-vivo cell growth inhibition activity among some tested compounds. For example, comparing compound **8a** with **7a** is more potent than **2a** and **2b** for CDK2 inhibition. However, the degree of in-vivo cell growth inhibition is reversed between them. Currently it is not clear for the reason. It may be due to different abilities to internalize inside cells, or it may be resulted from different inhibitory activities against some other cellular targets within cells besides CDK2 that might be critical for cell growth. The results demonstrate that structural variations at C-2 and C-5 of pyrimidine derivatives might be led to potent inhibitors of CDK2, and these information will be helpful for designing new inhibitors.

5. Experimental

All melting points were uncorrected and determined by the open capillary method using Gallenkamp melting point apparatus. IR spectra ware recorded (KBr) on a Pye-Unicam SP-883 Perkin Elmer spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM 400–600 MHZ spectrometer using DMSO-d_6 as a solvent and TMS as an internal reference, chemical shifts are expressed in δ units (ppm). Mass spectra were recorded with a mass spectrometer MS9 (AEI) 70 eV. All the analytical data were obtained from Microanalytical Data Unit at Cairo University and Toledo University and all the results were in an acceptable range.

5.1. Chemistry

5.1.1. 6-(2,4-Dihydroxyphenyl)-2-mercapto-4-methyl-N-aryl-1,6-dihydropyrimidine-5-carboxamide ***1a,b***

A mixture of acetoacetanilide (60 mmol), 2,4-dihydroxy benzaldehyde (50 mmol, 6.9 g), thiourea (50 mmol, 3.8 g), absolute ethanol (20 ml) and conc. HCl (100 μL, Ca. 4 drops) was placed in a 100 ml three-necked flask and heated at reflux (80 °C inner temp.) with stirring for 3 h. After solid Sodium bicarbonate (100 mg) was added the reaction mixture was allowed to stand at 4 °C for 5 h. The resulting precipitate was filtered off, washed with water and recrystallized from alcohol.

5.1.2. 6-(2,4-Dihydroxyphenyl)-2-mercapto-4-methyl-N-(4-methylphenyl)-1,6-dihydropyrimidine-5-carboxamide ***1a***

6-(2,4-Dihydroxyphenyl)-2-mercapto-4-methyl-*N*-(4-methylphenyl)-1,6-dihydropyrimidine-5-carboxamide **1a** was obtained as orange crystals (86% yield); mp (°C) 180–183; MS *m*/*z* 370 $(M+H)^+$;

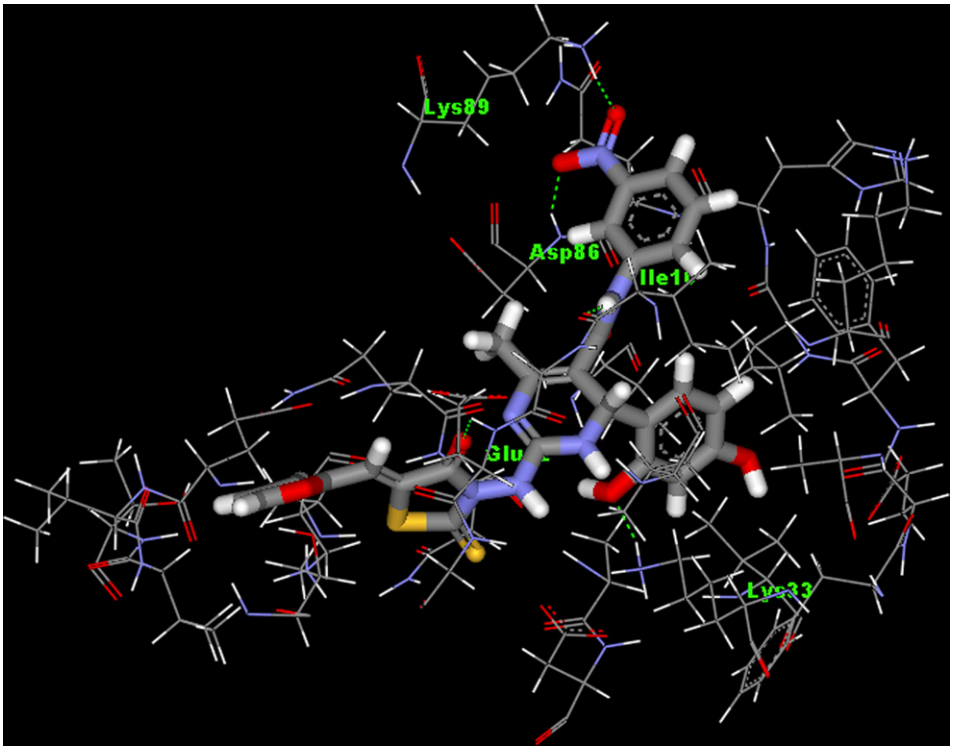


Fig. 4. Hydrogen bonding Interactions of compound **8b** with the active site.

^{13}C NMR (DMSO-d_6) (δ ppm) 18.8, 22.5, 45.3, 103.6, 110.3, 115.2, 120.7, 121.5, 130.2, 133.3, 135.6, 140.1, 147.4, 155.5, 157.8, 160.1, 170.1; ^{1}H NMR (DMSO-d_6) 2.2 (s, 3H, CH_3-phenyl), 2.7 (s, 3H, CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3-dihydroxyphenyl), 6.2–6.8 (d, 2H, j = 8.1 Hz, H5,6; dihydroxyphenyl), 7.1–7.4 (dd, 4H, j = 8.4 Hz), 9.2 (s, lH, NH-Pyrimidine), 9.8 (s, 1H, NH-amide), 10.1–102 (br, 2H, 2OH), 12.1 (s, 1H, SH). Anal. Calcd for $C_{19}H_{19}N_3O_3S$: C, 61.77; H, 5.18; N, 11.37. Found: C, 60.9; H, 4.8; N, 10.9.

5.1.3. 6-(2,4-Dihydroxyphenyl)-2-mercapto-4-methyl-N-(3-nitrophe nyl)-1,6-dihydropyrimidine-5-carboxamide ***1b***

6-(2,4-Dihydroxyphenyl)-2-mercapto-4-methyl-*N*-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **1b** was obtained as yellow crystals (88% yield); mp (°C) 168–170; MS *m*/*z* 402 (M^{+2}); ^{1}H NMR (DMSO-d_6) (δ ppm) 2.5 (s, 3H, CH_3-Pyrimidine), 5.1 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3-dihydroxyphenyl), 6.2–6.8 (d, 2H, j = 7.8 Hz, H5,6; dihydroxyphenyl), 7.8–8.0 (m, 3H, Ar), 8.3 (s, 1H, Ar), 9.5 (s, lH, NH-Pyrimidine), 9.6 (s, 1H, NH-amide), 10.5–10.6 (br, 2H, 2OH), 12.5 (s, 1H, SH). Anal. Calcd for $C_{18}H_{16}N_4O_5S$: C, 53.99; H, 4.03; N, 13.99. Found: C, 54.5; H, 4.6; N, 14.6.

5.1.4. 2-(Benzylthio)-6-(2,4-dihydroxyphenyl)-4-methyl-N-aryl-l,6-dihydro-pyrimidine-5-carboxamide ***2a,b***

Sodium hydroxide (0.024 mol) and compound 1 (0.024 mol) were dissolved in 100 ml of water (pH of solution 7–8), and a solution of benzyl chloride (0.024 mol) and alkali (0.024 mol) in 100 ml of water was added dropwise. The reaction mixture was stirred for 3 h at 60 °C. After cooling, the mixture was acidified with 10% HCl to pH 1–2. The resulting orange precipitate was filtered off, washed with cold water, and dried.

5.1.5. 2-(Benzylthio)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(4-methylphenyl)-l,6-dihydro-pyrimidine-5-carboxamide ***2a***

2-(Benzylthio)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(4-methylphenyl)-l,6-dihydro-pyrimidine-5-carboxamide **2a** was obtained as orange crystals (90% yield); mp (°C) 123–125; MS *m*/*z* 460 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.1 (s, 3H, CH_3-phenyl), 2.7 (s, 3H, CH_3-Pyrimidine), 4.7 (s, 1H, Pyrimidine-H6),4.6 (s, 2H, SCH_2), 6.1 (s, 1H, H3-dihydroxy-phenyl), 6.2–6.8 (d, 2H,, j = 7.9 Hz, H5,6; dihydroxyphenyl), 7.2–7.5 (m, 9H, Ar), 9.2 (s, lH, NH-Pyrimidine), 9.8 (s, 1H, NH-amide), 10.1–10.3 (br, 2H, 2 OH). Anal. Calcd for $C_{26}H_{23}N_3O_3S$: C, 67.95; H, 5.48; N, 9.14. Found: C, 67.1; H, 4.9; N, 8.9.

5.1.6. 2-(Benzylthio)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-l,6-dihydro-pyrimidine-5-carboxamide ***2b***

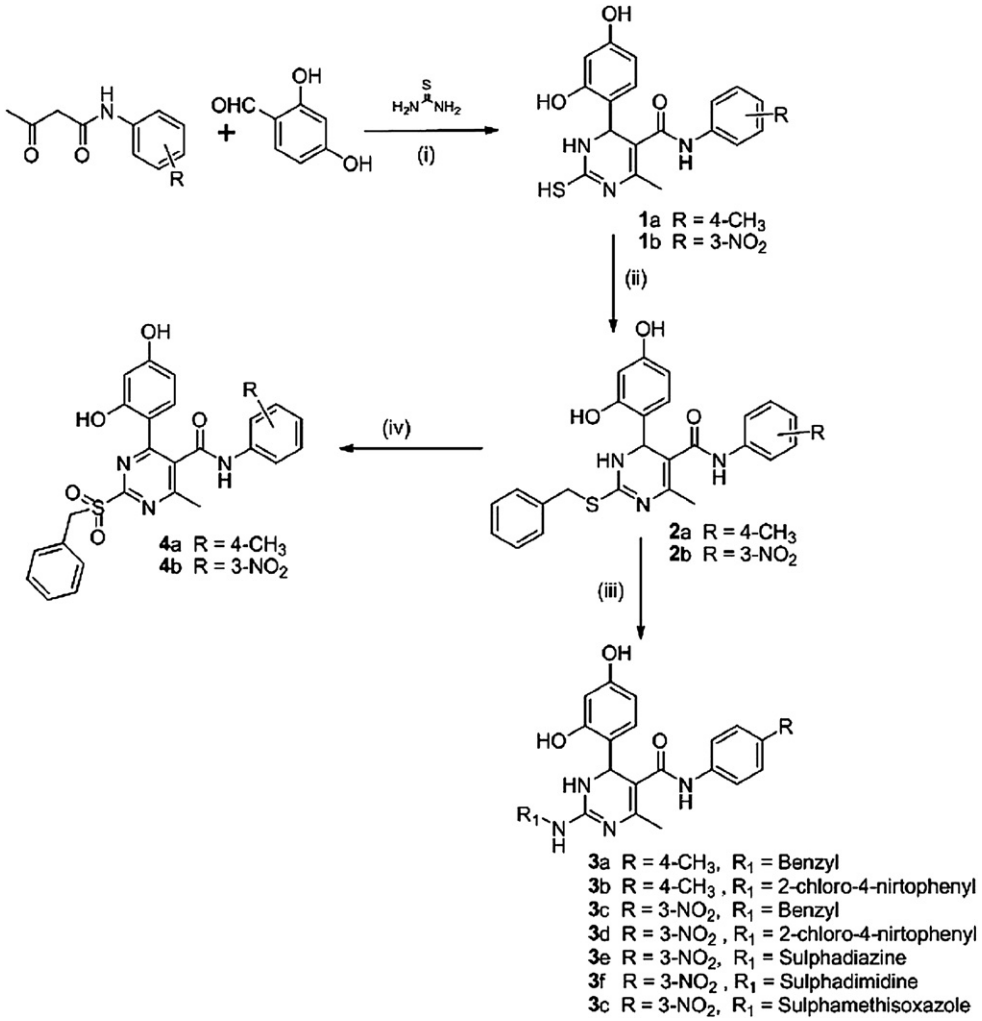
2-(Benzylthio)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-l,6-dihydro-pyrimidine-5-carboxamide **2b** was obtained as orange crystals (95% yield); mp(°C)140–142; MS *m*/*z* 490 $(M + H)^+$; ^{13}C NMR (DMSO-d_6) (δ ppm) 22.5, 35.1, 45.3, 101.6, 103.2, 110.3, 115.2, 118.7, 122.5, 129.2, 132.3, 134.6, 135.9, 140.1, 143.4, 147.1, 150.5, 151.1, 158.3, 160.1, 163.2, 166.4, 171.1; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.9 (s, 3H, CH_3-Pyrimidine) 4.8 (s, 1H, Pyrimidine-H6),4.5 (s, 2H, SCH_2), 6.1 (s, 1H, H3; dihydroxyphenyl), 6.2–6.8 (d, 2H, j = 8.1 Hz, H5,6; dihydroxyphenyl), 7.2–7.5 (m, 5H, j = 7.2 Hz), 7.7–8.1 (m, 3H, H4,5,6; 3-nitrophenyl), 8.5 (s, 1H, H2; 3-nitrophenyl),, 9.1 (s, lH, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.0–10.2 (br, 2H, 2 OH). Anal. Calcd for $C_{25}H_{20}N_4O_5S$: C, 61.21; H, 4.52; N, 11.42. Found: C, 60.5; H, 4.9; N, 11.9.

5.1.7. 2-(Arylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-aryl-l,6-dihydropyrimi-dine-5-carboxamide ***3a–g***

A mixture of compound 2 (0.01 mol) and the appropriate amine (0.015 mol) in glacial acetic acid (25 ml) was refluxed for 6–8 h. After cooling, the reaction mixture was poured onto ice-water (200 ml). The resulting precipitate was filtered off, washed well with water and recrystallized from DMF–H_2O mixture or alcohol.

5.1.8. 2-(Benzylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(4-methylphenyl)-l,6-dihydropyrimi-dine-5-carboxamide ***3a***

2-(Benzylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(4-methylphenyl)-l,6-dihydropyrimi-dine-5-carboxamide **3a** was obtained as



Scheme 1. Reagent and condition: (i) EtOH, HCL, reflux; (ii) NaOH, EtOH, PhzCl; (iii) RNH_2, ACOH, reflux; (iv) H_2O_2, ACOH.

yellow crystals (80% yield); mp (°C) 155–157; MS *m*/*z* 443 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.3 (s, 3H, CH_3-phenyl), 2.8 (s, 3H, CH_3-Pyrimidine), 3.8 (s, 2H, N–CH_2), 4.9 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3, dihydroxyphenyl), 6.3–6.8 (d, 2H, j = 7.8 Hz, H5,6; dihydroxyphenyl), 7.2–7.5 (m, 9H, Ar), 9.2 (s, lH, NH-Pyrimidine), 9.7 (s, 1H, NH-benzyl), 9.9 (s, 1H, NH-amide), 10.0–10.2 (br, 2H, 2 OH). Anal. Calcd for $C_{26}H_{24}N_4O_3$: C, 70.57; H, 5.92; N, 12.66. Found: C, 69.8; H, 4.9; N, 12.9.

5.1.9. 2-(2-Chloro-4-nitrophenylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(4-methylphenyl)-l,6-dihydropyrimidine-5-carboxamide ***3b***

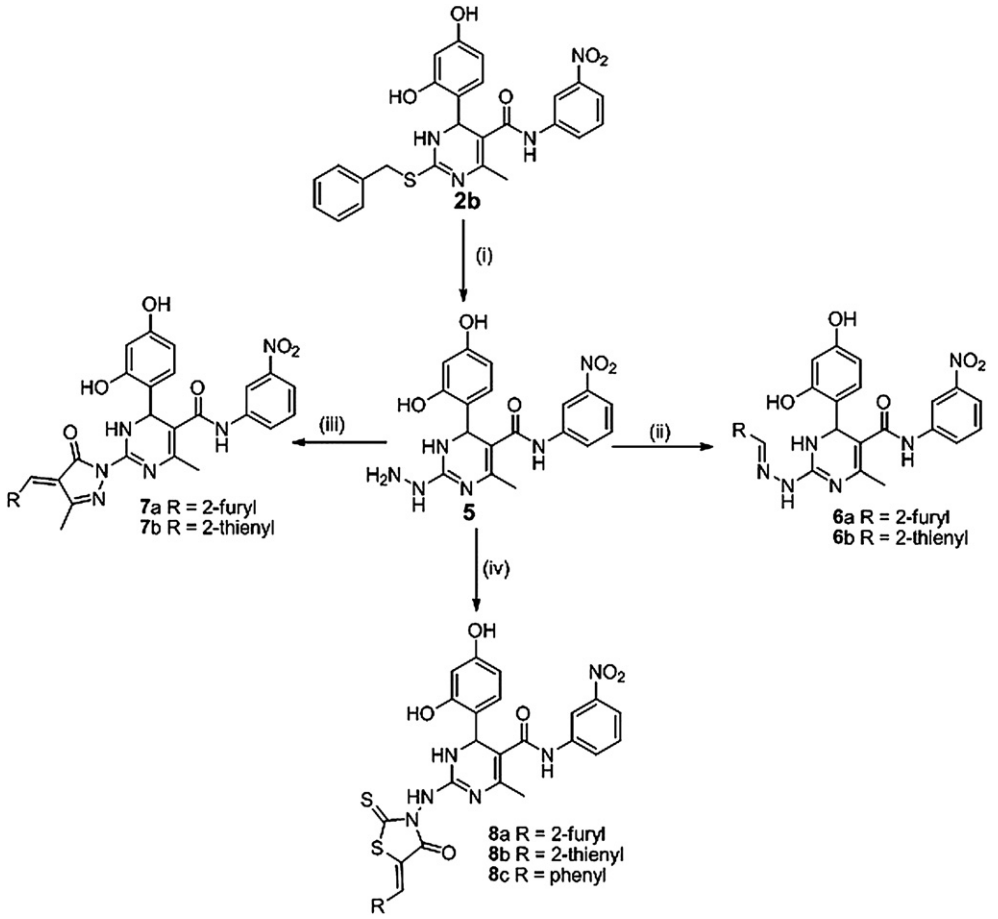
2-(2-Chloro-4-nitrophenylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(4-methylphenyl)-l,6-dihydropyrimidine-5-carboxamide **3b** was obtained as yellow crystals (77% yield); mp (°C) 188–190; MS *m*/*z* 507.5 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.1 (s, 3H, CH_3-phenyl), 2.4 (s, 3H, CH_3-Pyrimidine), 5.0 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3-dihydroxyphenyl), 6.3–6.5 (d, 2H, j = 8.1 Hz, H5,6; dihydroxyphenyl), 7–7.2 (d, 2H, j = 9.5 Hz, H2,3,5,6; 4-methylphenylamino), 7.1–7.4 (m, 4H, Ar), 7.5–7.6 (d, 1H, j = 9.5 Hz, H6; 2-chloro-4-nitrophenyl), 7.9–8 (d, 1H, j = 9.5 Hz, H5; 2-chloro-4-nitrophenyl ring) 8.2 (s, 1H, H3, 2-chloro-4-nitrophenyl ring), 9.0 (s, 1H, NH-Pyrimidine), 9.5 (s, 1H, NH, 2-chloro-4-nitrophenyl ring), 9.9 (s, 1H, NH-amide), 10.2–10.4 (br, 2H, 2 OH). Anal. Calcd for $C_{25}H_{20}ClN_5O_5$: C, 59.12; H, 4.37; N, 13.79. Found: C, 59.8; H, 4.1; N, 12.9.

5.1.10. 2-(Benzylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-l,6-dihydro-pyrimi-dine-5-carboxamide ***3c***

2-(Benzylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-l,6-dihydro-pyrimi-dine-5-carboxamide **3c** was obtained as orange crystals (75% yield); mp (°C) 180–183; MS *m*/*z* 474 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.6 (s,3H,CH_3-Pyrimidine), 3.6(s, 2H, N–CH_2), 4.9 (s, 1H, Pyrimidine-H6), 6.5 (s, 1H, H3; dihydroxyphenyl), 6.6–6.7 (d, 2H,, j = 8.3 Hz, H5,6; dihydroxyphenyl), 7.2–7.4 (m, 5H, Ar), 7.9–8.1 (m, 3H, H4,5,6; m-nitrophenyl), 8.6 (s, 1H, H2; m-nitrophenyl), 10.2 (s, 1H, NH-amide), 10.3–10.5 (br, 2H, 2 OH). Anal. Calcd for $C_{25}H_{21}N_5O_5$: C, 63.42; H, 4.90; N, 14.79. Found: C, 63.9; H, 4.1; N, 13.9.

5.1.11. 2-(2-Chloro-4-nitrophenylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-l,6-dihydropyrimidine-5-carboxamide ***3d***

2-(2-Chloro-4-nitrophenylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-l,6-dihydropyrimidine-5-carboxamide **3d** was obtained as yellow crystals (69% yield); mp(°C) 207–209; MS *m*/*z* 539.5 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.4 (s, 3H, CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.3 (s, 1H, H3, dihydroxyphenyl), 6.2–6.4 (d, 2H, j = 8.0 Hz, H5,6; dihydroxyphenyl), 7.3–7.4 (d, 1H, H6, j = 9.6 Hz, H6; 2-chloro-4-nitrophenyl), 7.6–7.9 (m, 3H, H4,5,6; m-nitrophenyl), 7.8–7.9 (d, 1H, H5, j = 9.6 Hz, H5; 2-chloro-4-nitrophenyl), 8.2 (s, 1H, H3, 2-chloro-4-nitrophenyl ring), 8.5 (s, 1H, H2; m-nitrophenyl), 9.0 (s, lH, NH-Pyrimidine), 9.9 (s, 1H,



Scheme 2. Reagent and condition: (i) NH_2NH_2, reflux; (ii) R_1CHO_1, DMF, RT; (iii) EAA, R_1CHO_1KOH, reflux; (iv) CS_2, $ClCH_2COOH$, R_1CHO, KOH.

NH, 2-chloro-4-nitrophenyl ring), 10.5 (s, 1H, NH-amide), 10.1–10.3 (br, 2H, 2 OH). Anal. Calcd for $C_{24}H_{17}ClN_6O_7$: C, 53.49; H, 3.55; N, 15.59. Found: C, 53.8; H, 4.0; N, 15.9.

Table 2
CDK2 and cell division inhibitory activities of the newly synthesized pyrimidine derivatives.

Compounds	IC_{50} (μM)		GI_{50} (μM)	
	CDK2	EGFR	A431[a]	HCT116[b]
2a	25.4	93.5	20.77	14.5
2b	24.6	55.6	>100	28.98
3a	>100	23.5	42.67	66.71
3b	44.9	60.9	31.86	28.24
3c	46.8	>100	35.60	>100
3d	8.1	>100	3.94	10.88
3e	57.5	88.3	69.22	77.55
3f	11.5	79.8	3.94	8.99
3g	0.9	>100	2.94	16.6
4a	48.3	>100	32.02	23.45
4b	0.9	>100	28.85	10.88
5	>100	47.9	24.28	47.68
6a	3.8	>100	2.91	8.89
6b	22.9	>100	19.14	38.19
7a	10.3	>100	15.84	>100
7b	3.1	>100	37.08	26.46
8a	0.4	99.3	28.88	>100
8b	0.3	80.6	2.78	4.50
8c	28.2	>100	24.28	16.61
Olomoucine	7.0	–	–	–
Rescovitine	0.5	–	–	–
PYK2104	–	0.0008	–	–
Doxorubicine	–	–	0.18	0.13

[a] A341, human vulvar epidermoid carcinoma cell line.
[b] HCT116,human colon epitherial carcinoma cell line.

5.1.12. 6-(2,4-Dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-2-(4-(N-pyrimidin-2-yl-sulfamoyl)-phenylamino)-l,6-dihydropyrimidine-5-carboxamide ***3e***

6-(2,4-Dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-2-(4-(*N*-pyrimidin-2-yl-sulfamoyl)-phenylamino)-l,6-dihydropyrimidine-5-carboxamide **3e** was obtained as yellowish white crystals (55% yield); mp (°C) 162–164; MS *m*/*z* 617 $(M + H)^+$; ^1H NMR (DMSO-d_6) (δ ppm) 2.5(s,3H,CH_3 Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.5 (s, 1H, H3; dihydroxyphenyl), 6.6–6.8 (d, 2H, j = 7.8 Hz, H5,6;

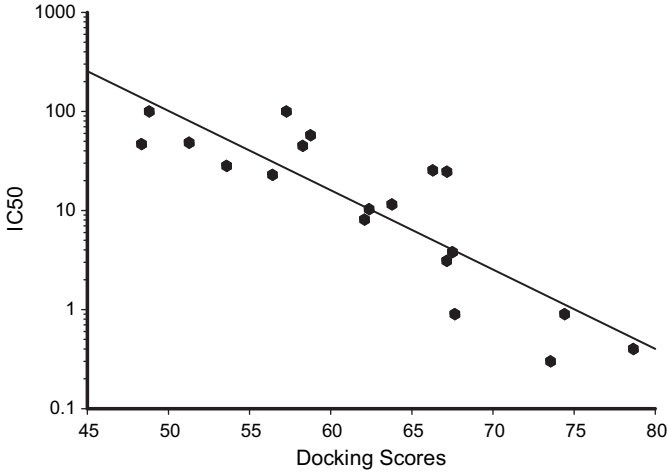


Fig. 5. Correlation between Docking scores and IC50 of the newly synthesized compounds.

dihydroxyphenyl), 6.9 (t, 1H, $j = 5.0$ Hz), 7.0–7.4 (dd, 4H, $j = 7.7$ Hz, H2,3,5,6; Ar), 7.9–8.1 (m, 3H, H4,5,6; m- nitrophenyl), 8.4 (d, 2H, $j = 5.0$ Hz, H4,6 pyrimidine), 8.6 (s, 1H, H2; m-nitrophenyl), 9.6 (s, 1H, NH-Pyrimidine), 10.2 (s, 1H, NH-amide), 10.3–10.5 (br, 2H, 2 OH), 12.3 (s,1H, NH-sulpha). Anal. Calcd for $C_{28}H_{22}N_8O_7S$: C, 54.54; H, 3.92; N, 18.17. Found: C, 53.9; H, 4.1; N, 17.9.

5.1.13. 6-(2,4-Dihydroxyphenyl)-2-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenylamino)-4-methyl-N-(3-nitrophenyl)-l,6-dihydropyrimidine-5-carboxamide **3f**

6-(2,4-Dihydroxyphenyl)-2-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenylamino)-4-methyl-*N*-(3-nitrophenyl)-l,6-dihydropyrimidine-5-carboxamide **3f** was obtained as yellowish white crystals (50% yield); mp (°C) 132–134; MS *m/z* 644 (M^+); ^{13}C NMR (DMSO-d_6) (δ ppm) 19.8, 20.4, 40.6, 100.3, 106.1, 110.6, 115.2, 117.2, 120.1, 122.3, 125.2, 129.3, 132.1, 135.5, 140.2, 142.6, 145.1, 148.6, 150.1, 153.2, 155.8, 159.6, 160.2, 162.4, 166.3, 170.7; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.1 (s, 6H, 2CH_3-pyrimidine), 2.4 (s, 3H, CH_3-Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.4 (s, 1H, H3; dihydroxyphenyl), 6.5–6.7 (d, 2H, $j = 8.0$ Hz, H5,6; dihydroxyphenyl), 7.0 (s, 1H, H5 pyrimidine), 7.0–7.4 (dd, 4H, $j = 7.8$ Hz, H2,3,5,6; Ar), 7.9–8.1 (m,3H, H4,5,6; m-nitrophenyl), 8.6 (s, 1H, H2; m-nitrophenyl), 9.5 (s, 1H, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.1–10.3 (br, 2H, 2 OH), 12.1 (s, 1H, NH-sulpha). Anal. Calcd for $C_{30}H_{26}N_8O_7S$: C, 55.89; H, 4.38; N, 17.38. Found: C, 54.9; H, 4.1; N, 17.9.

5.1.14. 6-(2,4-Dihydroxyphenyl)-4-methyl-2-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenylamino)-N-(3-nitrophenyl)-1,2,5,6-tetrahydropyrimidine-5-carboxamide **3g**

6-(2,4-Dihydroxyphenyl)-4-methyl-2-(4-(*N*-(5-methylisoxazol-3-yl)sulfamoyl)phenylamino)-*N*-(3-nitrophenyl)-1,2,5,6-tetrahydropyrimidine-5-carboxamide **3g** was obtained as yellow crystals (65% yield); mp (°C) 132–134; MS *m/z* 621 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.2 (s, 3H, CH_3-isoxazole), 2.3 (s, 3H, CH_3-Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H4-isoxazole), 6.2 (s, 1H, H3; dihydroxyphenyl), 6.6–6.8 (d, 2H, $j = 8.0$ Hz, H5,6; dihydroxyphenyl),7.0–7.4 (dd, 4H, $j = 7.7$ Hz, H2,3,5,6; Ar), 7.9–8.1 (m,3H, H4,5,6; m-nitrophenyl), 8.6 (s, 1H, H2; m-nitrophenyl), 9.7 (s, 1H, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.1–10.2 (br, 2H, 2 OH), 11.6 (s, 1H, NH-sulpha). Anal. Calcd for $C_{28}H_{25}N_7O_8S$: C, 54.28; H, 4.07; N, 15.82. Found: C, 54.9; H, 3.2; N, 15.9.

5.1.15. 2-(Benzylsulfonyl)-4-(2,4-dihydroxyphenyl)-6-methyl-N-arylpyrimidine-5-carboxamide **4a**,**b**

A mixture of compound 2 (5.49 mmol), hydrogen peroxide 30% (1 ml) and glacial acetic acid (30 ml) was stirred at room temperature for 1 h and stirring was continued for another 3 h at 70 °C. After removal of acetic acid and water under reduced pressure, the residue was washed well with water, filtered off, recrystallized from alcohol and dried.

5.1.16. 2-(Benzylsulfonyl)-4-(2,4-dihydroxyphenyl)-6-methyl-N(4-methylphenyl)pyrimidine-5-carboxamide **4a**

2-(Benzylsulfonyl)-4-(2,4-dihydroxyphenyl)-6-methyl-*N*(4-methylphenyl)pyrimidine-5-carboxamide **4a** was obtained as yellow crystals (91% yield); mp (°C) 160–162; MS *m/z* 490 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.2 (s, 3H, CH_3-phenyl), 2.4 (s,3H,CH_3-Pyrimidine), 5.3 (s,2H,SCH_2), 6.4 (s, 1H, H3-dihydroxyphenyl), 6.5–7.0 (d, 2H, $j = 8.0$ Hz, H5,6; dihydroxyphenyl), 7.2–7.7 (m, 9H, Ar), 10.3 (s, 1H, NH-amide), 10.3–10.5 (br, 2H, 2 OH). Anal. Calcd for $C_{26}H_{23}N_3O_5S$: C, 63.79; H, 4.74; N, 8.58. Found: C, 64.1; H, 4.1; N, 7.9.

5.1.17. 2-(Benzylsulfonyl)-4-(2,4-dihydroxyphenyl)-6-methyl-N-(3-nitrophenyl)pyrimidine-5-carboxamide **4b**

2-(Benzylsulfonyl)-4-(2,4-dihydroxyphenyl)-6-methyl-*N*-(3-nitrophenyl)pyrimidine-5-carboxamide **4b** was obtained as yellow crystals (88% yield); mp (°C) 170–173; MS *m/z* 520 (M^+); ^{1}H NMR (DMSO-d_6) (δ ppm) 2.4 (s, 3H, CH_3-Pyrimidine), 5.1 (s, 2H, SCH_2), 6.4 (s, 1H, H3; dihydroxyphenyl), 6.6–7.0 (d, 2H, $j = 7.9$ Hz, H5,6; dihydroxyphenyl), 7.2–7.4 (m, 5H, Ar), 7.8–8.0 (m, 3H, H4,5,6; 3-nitrophenyl), 8.8 (s, 1H, H2; 3-nitrophenyl), 10.1 (s, 1H, NH-amide), 10.4–10.5 (br, 2H, 2 OH). Anal. Calcd for $C_{25}H_{20}N_4O_7S$: C, 57.69; H, 3.87; N, 10.76. Found: C, 56.9; H, 3.1; N, 10.9.

5.1.18. 6-(2,4-Dihydroxyphenyl)-2-hydrazinyl-4-methyl-N-(3-nitrophenyl)-l,6-dihydro-pyrimidine-5-carboxamide **5**

A mixture of thioester 2 (0.01 mol, 4.9 g) and hydrazine hydrate 85% (0.03 mol, 1.5 g) in 2-propanol (50 ml) was refluxed for 3 h. After cooling, the precipitate was filtered off, recrystallized from alcohol, and dried in a vacuum over P_2O_5 to give the target compound as green crystals. Yield (85%); mp(°C)157–160; MS *m/z* 400 $(M+2H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.7 (s,3H,CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.2 (s, 1H, H3, dihydroxyphenyl), 6.3–6.6 (d, 2H, $j = 7.9$ Hz, H5,6; dihydroxyphenyl), 7.4–7.9 (m, 3H, H4,5,6; m-nitrophenyl), 8–8.2 (t, 1H, NH-hydrazinyl), 8.2–8.3 (d, 2H, NH2-hydrazinyl), 8.5 (s, 1H, H2; m-nitrophenyl), 9.2 (s, lH, NH-Pyrimidine), 10.0 (s, 1H, NH-amide), 10.2–10.4 (br, 2H, 2 OH). Anal. Calcd for $C_{18}H_{16}N_6O_5$: C, 54.27; H, 4.55; N, 21.10. Found: C, 53.8; H, 3.8; N, 20.9.

5.1.19. 6-(2,4-Dihydroxyphenyl)-2-(2-(arylmethylene)hydrazinyl)-4-methyl-N-(3-nitrophenyl)-l,6-dihydropyrimidine-5-carboxamide **6a**,**b**

A mixture of hydrazinyl compound 5 (3.33 mmol, 1.33 g) and the appropriate aldehyde (9.99 mmol) in DMF (20 ml) was stirred at room temperature for 10 h. After the reaction was completed, the solution was evaporated under reduced pressure and the residue was triturated with water to give crystals, which were collected by filtration and recrystallized from alcohol.

5.1.20. 6-(2,4-Dihydroxyphenyl)-2-(2-(furan-2-ylmethylene)hydrazinyl)-4-methyl-N-(3-nitro-phenyl)-l,6-dihydropyrimidine-5-carboxamide **6a**

6-(2,4-Dihydroxyphenyl)-2-(2-(furan-2-ylmethylene)hydrazinyl)-4-methyl-*N*-(3-nitro-phenyl)-l,6-dihydropyrimidine-5-carboxamide **6a** was obtained as greenish yellow crystals (86% yield); mp (°C) 180–182; MS *m/z* 477 $(M + H)^+$; ^{13}C NMR (DMSO-d_6) (δ ppm) 20.1, 40.3, 96.9, 104.1, 110.6, 112.2, 115.4, 120.3, 122.7, 125.1, 129.5, 130.2, 133.3, 135.9, 140.2, 145.1, 150.2, 153.1, 155.4, 159.6, 162.2, 165.1, 170.1; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.4 (s, 3H, CH_3-Pyrimidine), 5.1 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3, dihydroxyphenyl), 6.2–6.4 (d, 2H, $j = 8.0$ Hz, H5,6; dihydroxyphenyl), 6.5–7.3 (m, 3H, furan ring), 7.5–8.0 (m, 3H, H4.5.6; m-nitrophenyl), 8.1 (s, 1H, N=CH), 8.5 (s, 1H, H2; m-nitrophenyl), 9.6 (s, lH, NH-Pyrimidine), 10.1 (s, 1H, NH-amide), 10.4–10.6 (br, 2H, 2 OH), 11.1 (s, 1H, NH-hydrazinyl). Anal. Calcd for $C_{23}H_{18}N_6O_6$: C, 57.98; H, 4.23; N, 17.64. Found: C, 58.1; H, 4.8; N, 18.5.

5.1.21. 6-(2,4-Dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-2-(2-(thiophen-2-ylmethylene)-hydrazinyl)-l,6-dihydropyrimidine-5-carboxamide **6b**

6-(2,4-Dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-2-(2-(thiophen-2-ylmethylene)-hydrazinyl)-l,6-dihydropyrimidine-5-carboxamide **6b** was obtained as greenish yellow crystals (83% yield); mp (°C) 172–175; MS *m/z* 492 (M^+); ^{1}H NMR (DMSO-d_6) (δ ppm) 2.7 (s, 3H, CH_3-Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.3 (s, 1H, H3, dihydroxyphenyl), 6.4–6.6 (d, 2H, $j = 8.0$ Hz, H5,6; dihydroxyphenyl), 7.2–7.5 (m, 3H, thiophen ring), 7.6–8.1 (m, 3H, H4,5,6; m-nitrophenyl), 8.5 (s, 1H, N=CH), 8.7 (s, 1H, H2; m-nitrophenyl), 9.8 (s, lH, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.1–10.3 (br, 2H, 2 OH), 11.3 (s, 1H, NH-hydrazinyl). Anal. Calcd for $C_{23}H_{18}N_6O_5S$: C, 56.09; H, 4.09; N, 17.06. Found: C, 57.3; H, 4.8; N, 17.5.

*5.1.22. 6-(2,4-Dihydroxyphenyl)-2-(4-(arylmethylene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl) -4-methyl-N-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **7a**,**b***

A mixture of hydrazinyl compound 5 (3.33 mmol, 1.33 g) and ethyl acetoacetate (4 mmol, 0.5 g) was heated on a boiling water bath for 20 min. After cooling a mixture of appropriate aldehyde (3.4 mmol) and KOH (8 mmol, 0.45 g) in absolute ethanol (30 ml) was added and the reaction mixture was refluxed for 4 h. The reaction mixture was neutralized with glacial acetic acid, cooled to 0 °C, filtered off and recrystallized from alcohol.

*5.1.23. 6-(2,4-Dihydroxyphenyl)-2-(4-(furan-2-ylmethylene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-methyl-N-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **7a***

6-(2,4-Dihydroxyphenyl)-2-(4-(furan-2-ylmethylene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-methyl-*N*-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **7a** was obtained as yellow crystals (55% yield); mp (°C) 208–210; MS *m*/*z* 543 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.1 (s, 3H, CH_3-pyrazole), 2.4 (s, 3H, CH_3-Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.2 (s, 1H, H3, dihydroxyphenyl), 6.3–6.5 (d, 2H, j = 8.1 Hz, H5,6; dihydroxyphenyl), 6.9 (s, 1H, = CH methylene), 7.1–7.8 (m, 3H, furan ring), 7.8–8.0 (m, 3H, H4,5,6; m-nitrophenyl), 8.5 (s, 1H, H2; m-nitrophenyl), 9.5 (s, lH, NH-Pyrimidine), 10.1 (s, 1H, NH-amide), 10.3–10.5 (br, 2H, 2 OH). Anal. Calcd for $C_{27}H_{22}N_6O_7$: C, 59.78; H, 4.09; N, 15.49. Found: C, 58.9; H, 4.6; N, 15.5.

*5.1.24. 6-(2,4-Dihydroxyphenyl)-4-methyl-2-(3-methyl-5-oxo-4-(thiophen-2-ylmethylene)-4,5-dihydro-1H-pyrazol-1-yl)-N-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **7b***

6-(2,4-Dihydroxyphenyl)-4-methyl-2-(3-methyl-5-oxo-4-(thiophen-2-ylmethylene)-4,5-dihydro-1H-pyrazol-1-yl)-*N*-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **7b** was obtained as yellow crystals (52% yield); mp(°C) 220–223; MS *m*/*z* 558 (M^+); ^{13}C NMR (DMSO-d_6) (δ ppm) 15.1, 22.3, 46.3, 105.1, 110.1, 114.2, 116.9, 120.8, 124.1, 125.6, 130.1, 134.9, 136.1, 138.2, 139.8, 140.1, 142.2, 145.2, 148.6, 150.4, 152.7, 155.8, 158.7, 160.2, 162.1, 165.3, 169.2; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.3 (s, 3H, CH_3-pyrazole), 2.6 (s, 3H, CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3, dihydroxyphenyl), 6.2–6.5 (d, 2H, j = 8.0 Hz, H5,6; dihydroxyphenyl), 6.8 (s, 1H, = CH methylene), 7.2–7.7 (m, 3H, thiophene ring), 7.8–8.2 (m, 3H, H4,5,6; m-nitrophenyl), 8.6 (s, 1H, H2; m-nitrophenyl), 9.8 (s, lH, NH-Pyrimidine), 10.2 (s, 1H, NH-amide), 10.4–10.6 (br, 2H, 2 OH). Anal. Calcd for $C_{27}H_{22}N_6O_6S$: C, 58.06; H, 3.97; N, 15.05. Found: C, 57.9; H, 3.6; N, 15.5.

*5.1.25. 4-(2,4-Dihydroxyphenyl)-2-(arylmethylene)-4-oxo-2-thioxothiazolidin-3-ylamino-6-methyl-N-(3-nitrophenyl)-1,4-dihydropyrimidine-5-carboxamide **8a**–**c***

To a stirred solution of KOH (6.7 mmol, 0.4 g) in absolute ethanol (30 ml) was added the hydrazinyl derivative 5 (3.33 mmol. 1.33 g) and carbon disulphide (4 mmol, 0.3 g) and the reaction mixture was stirred 4h to dissolve carbon disulphide. Chloroacetic acid (3.33 mmol, 0.31 g) and the appropriate aldehyde (4 mmol) were added and the stirring was continued for another 2 h then the reaction mixture was refluxed for 4 h. The reaction mixture was cooled, acidified to pH 2–3 and the resulting precipitate was filtered off, dried and recrystallized.

*5.1.26. 4-(2,4-Dihydroxyphenyl)-2-(5-(furan-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-ylamino)-6-methyl-N-(3-nitrophenyl)-1,4-dihydropyrimidine-5-carboxamide **8a***

4-(2,4-Dihydroxyphenyl)-2-(5-(furan-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-ylamino)-6-methyl-*N*-(3-nitrophenyl)-1,4-dihydropyrimidine-5-carboxamide **8a** was obtained as yellow crystals (56% yield); mp(°C)228–230; MS *m*/*z* 594 $(M+2H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.4 (s, 3H, CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.0 (s, 1H, H3, dihydroxyphenyl), 6.2–6.6 (d, 2H, j = 8.1 Hz, H5,6; dihydroxyphenyl), 7.0 (s, 1H, = CH methylene), 7.4–7.8 (m, 3H, furan ring), 7.9–8.2 (m, 3H, H4,5,6; m-nitrophenyl), 8.6 (s, 1H, H2; m-nitrophenyl), 9.8 (s, lH, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.0–10.2 (br, 2H, 2 OH), 11.2 (s, 1H, NH 3-aminothiazolidine). Anal. Calcd for $C_{26}H_{20}N_6O_7S_2$: C, 52.70; H, 3.40; N, 14.18. Found: C, 53.5; H, 4.1; N, 15.0.

*5.1.27. 6-(2,4-Dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-2-(4-oxo-5-(thiophen-2-ylmethylene)-2-thioxothiazolidin-3-ylamino)-1,6-dihydropyrimidine-5-carboxamide **8b***

6-(2,4-Dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-2-(4-oxo-5-(thiophen-2-ylmethylene)-2-thioxothiazolidin-3-ylamino)-1,6-dihydropyrimidine-5-carboxamide **8b** was obtained as yellow crystals (48% yield); mp (°C) 255–257; MS *m*/*z* 609 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.3 (s, 3H, CH_3-Pyrimidine), 4.9 (s, 1H, Pyrimidine-H6), 6.1 (s, 1H, H3, dihydroxyphenyl), 6.3–6.6 (d, 2H, j = 8.0 Hz, H5,6; dihydroxyphenyl), 7.3 (s, 1H, = CH methylene), 7.5–8.0 (m, 3H, thiophene ring), 8.1–8.3 (m, 3H, H4,5,6; m-nitrophenyl), 8.5 (s, 1H, H2; m-nitrophenyl), 9.7 (s, lH, NH-Pyrimidine), 10.2 (s, 1H, NH-amide), 10.4–10.6 (br, 2H, 2 OH), 11.3 (s, 1H, NH 3-aminothiazolidine). Anal. Calcd for $C_{26}H_{20}N_6O_6S_3$: C, 51.31; H, 3.31; N, 13.81. Found: C, 51.5; H, 4.0; N, 14.0.

*5.1.28. 2-(5-Benzylidene-4-oxo-2-thioxothiazolidin-3-ylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-N-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **8c***

2-(5-Benzylidene-4-oxo-2-thioxothiazolidin-3-ylamino)-6-(2,4-dihydroxyphenyl)-4-methyl-*N*-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxamide **8c** was obtained as pale yellow crystals (51% yield); mp (°C) 270–272; MS *m*/*z* 602 (M^+); ^{13}C NMR (DMSO-d_6) (δ ppm) 20.6, 40.2, 100.6, 102.3, 105.6, 110.1, 115.3, 120.2, 124.5, 126.3, 129.8, 131.5, 135.3, 136.9, 139.2, 141.1, 143.3, 145.6, 147.5, 149.7, 155.1, 157.3, 159.6, 161.3, 165.4, 175.6; ^{1}H NMR (DMSO-d_6) (δ ppm) 2.5 (s, 3H, CH_3-Pyrimidine), 4.8 (s, 1H, Pyrimidine-H6), 6.2 (s, 1H, H3, dihydroxyphenyl), 6.4–6.7 (d, 2H, j = 8.0 Hz, H5,6; dihydroxyphenyl), 7.3–7.6 (m, 5H, Ar), 7.7 (s, 1H,=CH methylene), 8.0–8.3 (m, 3H, H4,5,6; m-nitrophenyl), 8.4 (s, 1H, H2; m-nitrophenyl), 9.6 (s, lH, NH-Pyrimidine), 9.9 (s, 1H, NH-amide), 10.0–10.2 (br, 2H, 2 OH), 12.1 (s, 1H, NH 3-aminothiazolidine). Anal. Calcd for $C_{28}H_{22}N_6O_6S_2$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.1; H, 4.0; N, 14.3.

5.2. Biology

5.2.1. Enzymatic activity inhibition assay

The inhibition studies of cell cycle dependent kinase 2 were performed for the synthesized compounds along with olomoucine, roscovitine and PYK2104 as reference compounds. Olomoucine was purchased from Sigma and we synthesized PYK 2104 and roscovitine [21]. CDK2/cyclinA enzyme was purified from infected sf21 insect cells. For baculoviral overexpressions of proteins, we subcloned human CDK2 c-DNA tagged by hexa-histidine on its N-terminal and human cyclinA c-DNA into pBacPak 8 expression vector, respectively, and baculovirus which carries each gene was generated using baculovirus generating kit. CDK2/cyclinA enzyme was purified using Ni^{+2}-affinity resin from sf21 insect cell culture into which CDK2 and cyclinA carrying baculoviruses were cotransfected. Enzyme assays were done in 20 mL of 50 mMTris–HCl containing 10 μM ATP, 0.2 μCi of gamma-P^{32} ATP, 10 mM $MgCl_2$, 5 mM DTT and 4 μg of histone H1 was used as a substrate. The reaction was continued for 10 min in the presence of inhibitors and stopped by adding 10 mL of 30% phosphoric acid. The stopped mixtures were spotted onto P81 paper and were washed with 10 mM Tris–HCl (pH 8.0) containing 0.1 M NaCl five times. The radioactivity of each spot

was quantified with BAS imager. The concentration of inhibitor that gives 50% inhibition was designated as IC_{50} value.

5.2.2. Cell growth inhibition assay

Human cancer cell lines, A431 (cervical cancer cell line) and HCT116 (colon cancer cell line) were obtained from Cell Bank in National Cancer Institute. Cells were grown in RPMI 1640 medium containing 10% fetal bovine serum at 37 °C and 5% CO_2. For cell division inhibition assay, 1000 cells were plated on 96 well plate and next day, test compounds as well as doxorubicin as reference compounds were treated to the cells at various concentrations. Cells were allowed to grow further for two days in the presence of compounds, then fixed by adding equal volume of 4% formalin for 30 min. Fixed cells were washed with tap water five times and stained in 0.1% Sulphorhodamine B for 30 min. Subsequently cells were washed with 1% acetic acid for four times and the dyes attached to cells were eluted by adding 100 μL of 0.1 M Tris–HCl (pH 8.0) and shaking for 10 min. The absorbance was measured at 520 nm wavelength using microplate reader (Molecular Dynamics). The absorbance is proportional to cell number in each well. Measurements were done triplicate and averaged. The absorbance from cells at the time of compound treatment was designated as 0% and the absorbance from cells after two days growth with no compound treatment was assigned as 100%. The GI_{50} value was defined as the inhibitor concentration which gives 50% cell growth inhibition during 2 days period of compound treatment.

References

[1] J.W. Harper, P.D. Adams, Chem. Rev. 101 (2001) 2511.
[2] M. Mihara, S. Shintani, A. Kiyota, T. Matsumura, D.T.W. Wong, Int. J. Oncol. 21 (2002) 95.
[3] D.M. Gitig, A. Koff, Mol. Biotechnol. 19 (2001) 179;
A. Kamb, N.A. Gruis, J. Weaver-Feldhaus, Q. Liu, K. Harshman, S.V. Tavtigian, E. Stockert, R.S. Day, B.E. Johnson, M.H. Skolnik, Science 264 (1994) 436.
[4] M. Knockaert, P. Greengard, L. Meijer, Trends Pharmacol. Sci. 23 (2002) 417.
[5] N. Kong, N. Fotouhi, P.M. Wovkulich, J. Roberts, Drugs Future 28 (2003) 881.
[6] A.M. Senderowicz, D. Headlee, S.F. Stinson, R.M. Lush, N. Kalil, L. Villalba, K. Hill, S.M. Steinberg, W.D. Figg, A. Tompkins, S.G. Arbuck, E.A. Sausville, J. Clin. Oncol. 16 (1998) 2986.
[7] E. Fuse, T. Kuwabara, A. Sparreboom, E.A. Sausville, W.D. Figg, J. Clin. Pharmacol. 45 (2005) 394.
[8] L. Meijer, A. Borgne, O. Mulner, J.P.J. Chong, J.J. Blow, N. Inagaki, M. Inagaki, J.G. Delcros, J.P. Moulinoux, Eur. J. Biochem. 243 (1997) 527.
[9] R.N. Misra, H. Xiao, K.S. Kim, S. Lu, W. Han, S.A. Barbosa, J.T. Hunt, D.B. Rawlins, W. Shan, S.J. Ahmed, L. Qian, B. Chen, R. Zhao, M.S. Bednarz, K.A. Kellar, J.G. Mulheron, R. Batorsky, U. Roongta, A. Kamath, P. Marathe, S.A. Ranadive, J.S. Sack, J.S. Tokarski, N.P. Pavletich, F.Y.F. Lee, K.R. Webster, S.D.J. Kimball, Med. Chem. 47 (2004) 1719.
[10] L. Meijer, S. Leclerc, M. Leost, Pharmacol. Ther. 82 (1999) 279.
[11] Y.S. Sanghvi, S.B. Larson, S.S. Matsumoto, L.D. Nord, D.F. Smee, R.C. Willis, T.H. Avery, R.K. Robins, G.R. Revankar, J. Med. Chem. 32 (1989) 3629.
[12] C.J. Sherr, The pezcoller lecture: cancer cell cycles revisted. Cancer Res. 60 (2000) 3698.
[13] D.A. Ibrahim, A.M. El- metwaly, E.E. Alarab, Arkivoc vii (2009) 12.
[14] D.A. Ibrahim, Eur. J. Med. Chem. 44 (2009) 2776.
[15] Website: http://www.rcsb.org/pdb/explore.do?structureId=2C6I.
[16] Discovery Studio 2.0. Accelrys, Inc., San Diego, CA, 2003.
[17] Gold version 3.0: http://www.ccdc.cam.ac.uk/products/life_sciences/gold/.
[18] P. Biginelli, Gazz. Chim. Ital. 23 (1893) 360.
[19] D.C. Kim, Y.R. Lee, B. Yang, K.J. Shin, D.J. Kim, B.Y. Chung, K.H. Yoo, Eur. J. Med. Chem. 38 (2003) 525.
[20] U. Kronenwett, J. Castro, U.J. Roblick, K. Fujioka, C. Östring, F. Faridmoghaddam, N. Laytragoon-Lewin, B. Tribukait, G. Auer, BMC Cell Biol. 4 (2003) 1.
[21] C.H. Oh, H.K. Kim, S.C. Lee, C. Oh, B.S. Yang, H.J. Rhee, J.H. Cho, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 345.