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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel 2-thiopyrimidine derivatives as CDK2 inhibitors: molecular modeling, synthesis, and anti-tumor activity evaluation

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Abstract A novel series of pyrimidine-benzenesulfonamide derivatives as potential cyclin-dependent kinase 2 inhibitors were designed depending upon the molecular docking simulation study. This study was preceded by modification and optimization of the lead compound 4-(2-amino-4-methylthiazol-5-yl)-*N*-(3-nitrophenyl) pyrimidin-2-amine. The target proposed compounds were synthesized using the derivative 6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) as a key starting compound. Some of the synthesized derivatives were selected as representative examples to evaluate their anti-proliferative activity against cultured human Hela cell line using doxorubicin as a reference drug and the results obtained were correlated with the data of molecular modeling simulation study. The structures of the novel derivatives were confirmed on the bases of micro-analytical and spectral data.

Keywords Docking · Pyrimidine-benzenesulfonamides · CDK2 · Anti-proliferative activity · Hela cell line

Introduction

Protein kinases are generally divided into two classes: tyrosine kinases which have been identified as the upstream players in mitogenic signal transduction pathways encompassing both receptor and non-receptor tyrosine types and serine/threonine kinases which are players more downstream in proliferative signaling and involved in the likes of MAP kinase signaling cascades as well as cell cycle progression and cell survival pathways (Blume-Jensen and Hunter, 2001; Levitzki and Gazit, 1995).

Serine/threonine kinases, termed cyclin-dependent kinases (CDKs), are activated by phosphorylation and function in complexes with their activating cyclins (proteins that are present at specific stages of the cell cycle) to drive progression through the different phases of the cell division cycle (Draetta, 1990; Sherr, 1993; Coleman *et al.*, 1997). CDK4, CDK6, coupled with their respective cyclin D partners are responsible for progression through G1, whereas CDK2 in combination with cyclin E is required for normal progress from G1 into S-phase, where DNA replication takes place. CDK1 and CDK2 (in association with cyclins A and B) are essential for cells to pass through S-phase into G2 and mitosis (Jhonson *et al.*, 2002; Wang *et al.*, 2004). CDK/cyclin complexes are kept under control by nuclear proteins termed CDK inhibitors and exemplified by p15, p16, p21, and p27, whose loss or inactivation participates in the development of cancer. CDKs phosphorylate and regulate the activity of different cellular proteins that include tumor suppressors (e.g., p^{Rb}, p^{53}), transcription factors (e.g., E2F-DPI, RNA pol II), replication factors (e.g., DNA Pol α, replication protein A), and organizational factors, which influence cellular and chromatin structures (e.g., histone H1, lamin A, MAP4) (Morgan, 1997; Grana and Reddy, 1995; Pines, 1995).

The recent understanding of the role of CDKs in the cell cycle regulations and the discovery that high rates of neoplasias are the result of CDK hyperactivation provide the main impetus to search for their pharmacological inhibitors.

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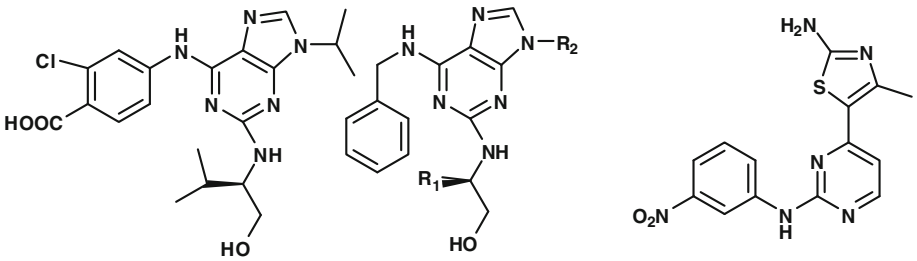
In this study, we aimed to design and synthesize a number of novel compounds as CDK2 inhibitors having potential anti-cancer activity. It has been reported that many pyrimidine-bearing skeletons showed anti-cancer activity (Arris *et al.*, 2000; Barvian *et al.*, 2000) and different drugs such as purvalanol B (I), olumoucine (IIa), and its analogue roscovitine (IIb) that contain pyrimidine ring are potent CDK2 inhibitors (Gray *et al.*, 1998; Schulze-Gahmen *et al.*, 1995; De Azevedo *et al.*, 1997).

Different efforts had resulted in the discovery of a number of 2-anilino-4-(thiazol-5-yl) pyrimidine derivatives that were developed as ATP antagonistic CDK2 inhibitors having anti-cancer activity (Wang *et al.*, 2004), specially 4-(2-amino-4-methylthiazol-5-yl)-*N*-(3-nitrophenyl) pyrimidin-2-amine (III) which showed the best anti-cancer activity in this series against Hela cell lines.



Thus, according to the above-mentioned points and to achieve our aim, pyrimidine nucleus was selected as the main skeleton of the proposed derivatives and the derivative (III) was selected as our lead compound. Design of the new CDK2 inhibitors was performed depending upon the molecular docking simulation study which was preceded by modification and optimization of the **lead compound** (**III**). This could be illustrated in Fig. 1 according to the following:

- Maintenance of the pyrimidine nucleus since it forms hydrophobic and hydrogen bond interactions with the hinge region residues at ATP binding site of CDK2 (Ibrahim and El-Metwally, 2010).
- Replacement of *m*-nitro group with a sulfamoyl moiety (where the presence of electron-withdrawing group preserves or enhances the activity) (Wang *et al.*, 2004) aiming to form hydrogen bonds and electrostatic interactions with the residues of ATP-binding site of CDK2. Also, maintaining the NH group is essential as it is a hydrogen bond donor.
- Replacement of the thiazolyl moiety which overlaps roughly with the space occupied by ribose in ATP-binding site with other 4-(substituted pyrazolyl) moieties to study their effects on the binding modes.
- Modification of the core thiazolylpyrimidine ring with fused imidazo, tetrazolo, and triazolopyrimidines was also performed aiming to study the effect of such fused ring systems on the binding modes and their effects on anti-proliferative activity.
- Introduction of a nitrile moiety at C_3 of the pyrimidine ring as this moiety enhances CDK2 inhibition potency (Wang *et al.*, 2004). Such modification aims to increase the interaction with amino acids residues in the ribose and phosphate binding site through the hydrogen bonds.
- Introduction of 3,4-dimethoxyphenyl moiety at C_6 of the pyrimidine nucleus to investigate the probability of enhancing the pharmacokinetic properties and the interactions of the new derivatives at the binding sites.

It was an important part in our study to evaluate the anti-tumor activity of the derivatives, thus some of the newly synthesized compounds that exhibited promising molecular docking results were examined as anti-cancer agents against human cervical carcinoma cell lines.

Materials and methods

Molecular docking simulation study

Aim

The aim was to study the crystal structure of CDK2 and explain the possible interactions that might take place between the proposed tested derivatives and the CDK2 enzyme in comparing to 4-(2-amino-4-methylthiazol-5-yl)-*N*-(3-nitrophenyl) pyrimidin-2-amine as a lead compound. Table 1 demonstrates the data obtained through the molecular modeling simulation study on all the prepared compounds.

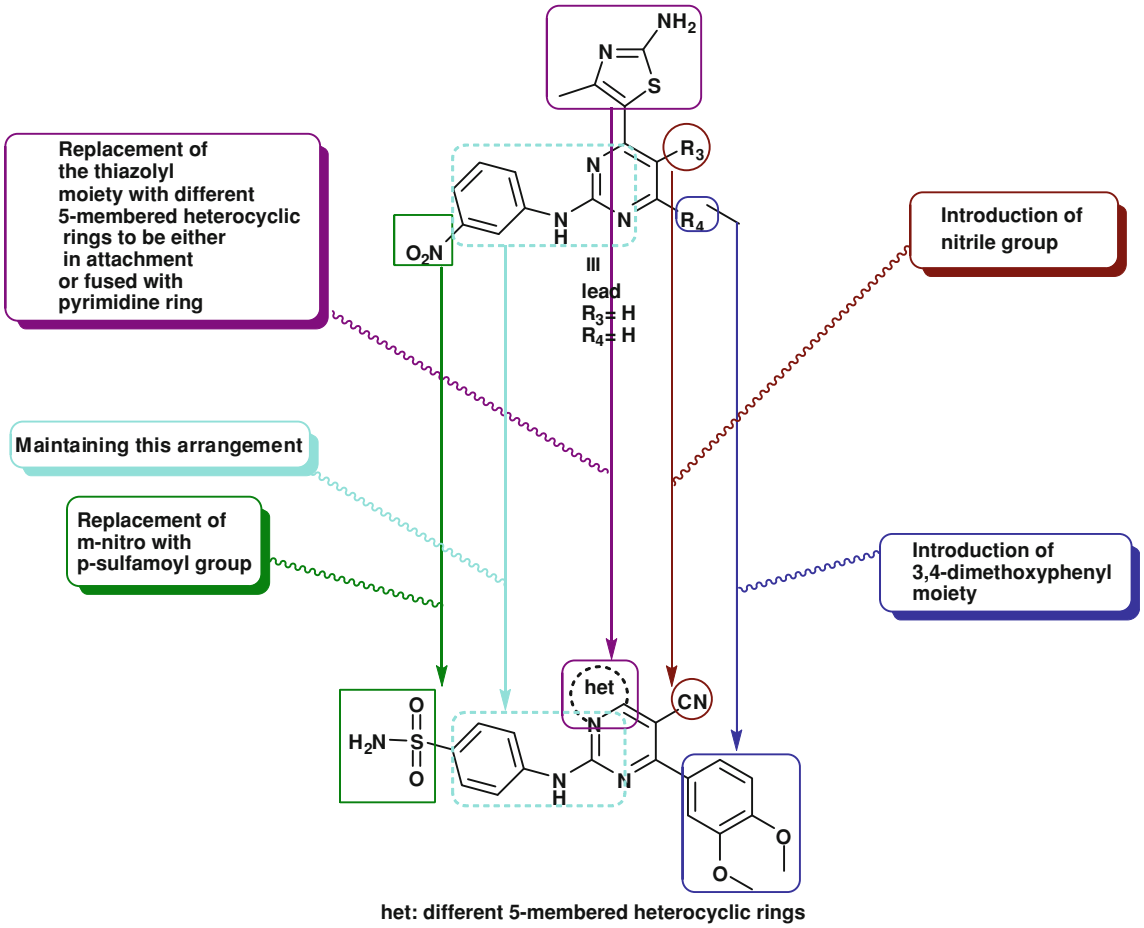


Fig. 1 The design of new CDK2 inhibitors through the modification of the **lead compound** (**III**)

Docking studies of the tested derivatives using Discovery Studio Client V2-5-0-9164

The newly proposed compounds including the intermediate ones were docked into the active site of CDK2 using CDOCKER protocol, which was carried out by downloading the 1PXO PDB file that images the bioactive conformer of the lead compound co-crystallized with the CDK2. The lead compound and the tested molecules were drawn, and underwent energy minimization using the prepared ligand protocol. The test set was docked into the active site of the enzyme using CDOCKER protocol. Then the resulting poses of each molecule were examined. Finally the retained poses were sorted by CHARMm energy (CDOCKER interaction energy) and the top scoring (most negative, thus favorable to binding) poses were selected and arranged in Table 2 which exhibits the tested molecules CDOCKER interaction energy indicating their affinities to the binding site and the hydrogen bonding between the indicated amino acids and features of the tested compounds in comparison with the lead compound.

Chemistry

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Micro-analytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre (NRC), Cairo, Egypt. The found values were within ±0.4 % of the theoretical values. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, NRC. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference, Faculty of science, Cairo University, Egypt. Mass spectra were recorded at 70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan), NRC. TLC was performed on silica gel 60 254F plates (Merck) using a

Table 1 Molecular modeling results of the newly designed derivatives

Compounds	−CDOCKER_interaction_energy (negative values)
Lead (III)	45.81
2	38.72
3	42.83
4	76.86
5	45.64
6	72.73
7	41.69
8	42.56
9	68.22
10	44.40
11	74.49
12a	49.64
13a	85.07
12b	45.80
13b	76.12
14	43.23
15	70.25
16	45.25
17	67.67

mixture of chloroform and ethanol (15:1, v/v) as an eluent. UV light at λ 254 and iodine accomplished visualization.

*4-Chloro-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydro-pyrimidine-5-carbonitrile (**2**)*

A mixture of compound **1** (10 g, 30 mmol), phosphorous oxychloride (40 mL) and phosphorous pentachloride (5 g) was heated on boiling water bath for 8 h. After the reaction was completed, the mixture was cooled and poured gradually onto crushed ice. The obtained precipitate was filtered off and dried. Yield: 75 %; mp (°C): 180–182. IR (KBr, cm^{-1}): 3373 (NH), 2934 (CH_3 alicyclic), 2219 (CN), 1142 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 3.75, 3.80 (2s, 6H, $2OCH_3$), 6.98–7.20 (m, 3H, aromatic-H), 8.20 (s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 308, 310 (25, 9 %), 61 (100 %). Anal. calcd for $C_{13}H_{10}ClN_3O_2S$ (307.77): C, 50.73; H, 3.27; N, 13.65; S, 10.42. Found: C, 50.50; H, 3.11; N, 13.32; S, 10.22.

*7-(3,4-Dimethoxyphenyl)-5-thioxo-5,6-dihydrotetrazolo[1,5-c]pyrimidine-8-carbo nitrile (**3**)*

A mixture of compound **2** (3 g, 10 mmol) and sodium azide (0.65 g, 10 mmol) in glacial acetic acid (30 mL) was refluxed for 8 h. Then, the mixture was cooled and the formed precipitate was filtered off, dried and re-crystallized from DMF/water. Yield: 65 %; mp (°C): 89–91. IR (KBr, cm^{-1}): 3343 (NH), 3005 (CH, aromatic), 2930 (CH, alicyclic), 2210 (CN), 1139 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 3.69, 3.71 (2s, 6H, $2OCH_3$), 6.9–7.2 (m, 3H, aromatic-H) and 8.20 (s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 314 (20 %), 274 (32 %), 177 (20 %), 111 (100 %). Anal. calcd for $C_{13}H_{10}N_6O_2S$ (314.34): C, 49.67; H, 3.20; N, 26.73; S, 10.20. Found: C, 49.31; H, 3.00; N, 26.41; S, 10.42.

*4-(8-Cyano-7-(3,4-dimethoxyphenyl) tetrazolo[1,5-c]pyrimidin-5-ylamino)benzene sulfonamide (**4**)*

A mixture of compound **3** (0.5 g, 1.6 mmol) and sulfanilamide (0.27 g, 1.6 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then the mixture was cooled and poured gradually onto ice/water. The obtained precipitate was filtered off, dried and re-crystallized from DMF/water. Yield: 65 %; mp (°C): 199–210. IR (KBr, cm^{-1}): 3420, 3235 (NH, NH_2), 2229 (CN), 1327, 1155 (SO_2NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 3.70, 3.84 (2s, 6H, $2OCH_3$), 5.20 (1s, 2H, NH_2 exchangeable with D_2O), 7.01–7.99 (m, 7H, aromatic-H) and 7.99 (s, 1H, NH exchangeable with D_2O). MS *m/z*: $(M + 1)^+$ 453 (30 %), 436 (10 %),79 (100 %). Anal. calcd for $C_{19}H_{16}N_8O_4S$ (452.46): C, 50.43; H, 3.56; N, 24.76; S, 7.08. Found: C, 50.05; H, 3.32; N, 24.43; S, 7.42.

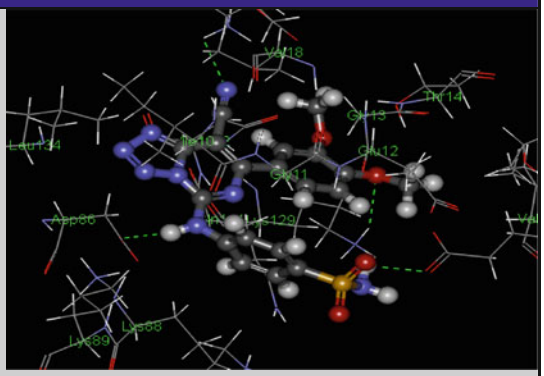
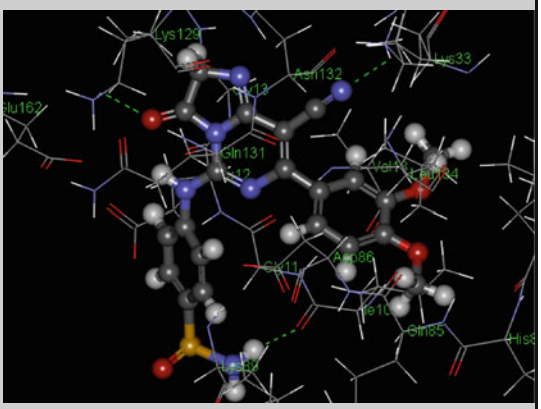
*7-(3,4-Dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro imidazo[1,2-c]pyrimidine-8-carbonitrile (**5**)*

A mixture of compound **2** (3 g, 10 mmol) and glycine (0.75 g, 10 mmol) in DMF (20 mL) was heated under reflux for 9 h. Then the mixture was poured onto ice/water. The solid obtained was filtered off and dried. Then the separated solid was refluxed with acetic anhydride (20 mL) for 3 h. The product obtained after cooling was filtered off, dried and crystallized from DMF/water. Yield: 65 %; mp (°C): 220–222. IR (KBr, cm^{-1}): 3374 (NH), 2212 (CN), 1652 (CO), 1140 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.29 (s, 2H, CH_2, imidazoline), 3.78, 3.82 (2s, 6H, $2OCH_3$), 7.01–7.40 (m, 3H, aromatic-H), 7.92 (1s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 328 (30 %), 327 (20 %), 228 (100 %), 217 (20 %). Anal. calcd for $C_{15}H_{12}N_4O_3S$ (328.36): C, 54.86; H, 3.68; N, 17.06; S, 9.76. Found: C, 54.43; H, 3.51; N, 17.32; S, 9.90.

*4-(8-Cyano-7-(3,4-dimethoxyphenyl)-3-oxo-2,3-dihydroimidazo [1,2-c] pyrimidin-5-ylamino)benzenesulfonamide (**6**)*

A mixture of imidazolo derivative **5** (0.5 g, 1.5 mmol) and sulfanilamide (0.26 g, 1.5 mmol) in DMF (5 mL)

Table 2 Results summary of the molecular modeling studies of compounds **4**, **6**, **9**, **11**, **13a**, **b**, **15** and **17**

Compd.	*- CDOCKER INTERACTION*	*H-Bonds*	*3D-Docking Results*
4	76.86	Asp86 {2.20 A°} Lys33 {2.49 A°} Glu162 {2.38 A°} Lys129 {2.30 A°}	
6	72.73	Lys129 {2.31 A°} Lys33 {2.26 A°} Ile10 {2.41 A°}	

containing few drops of TEA was refluxed for 15 h. Then the mixture was cooled and poured gradually onto ice/water. The formed precipitate was filtered off, dried and recrystallized from DMF/water. Yield: 66 %; mp (°C): 260–262. IR (KBr, cm^{-1}): 3372, 3278 (NH, NH_2), 2209 (CN), 1655 (C=O) and 1332, 1146 (SO_2NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (s, 2H, CH_2, imidazoline ring), 3.75, 3.85 (2s, 6H, $2OCH_3$), 6.80–7.70 (m, 7H, aromatic-H) and 5.62, 7.91 (2s, 3H, NH_2, NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 56.98 ($2OCH_3$), 63.43, 159.23, 173 (imidazoline-C), 113.92 (CN), 98.21, 162.31, 172.00 (pyrimidine-C), 111.12, 122.21, 127.41, 129.34, 131.10, 142.12, 149.11 (aromatic-C). MS *m/z*: M^+ 466 (20 %), 311 (20 %), 295 (30 %), 79 (100 %). Anal. calcd for $C_{21}H_{18}N_6O_5S$ (466.48): C, 54.07; H, 3.88; N, 18.01; S, 6.87. Found: C, 54.15; H, 4.23; N, 17.68; S, 6.53.

Table 2 continued

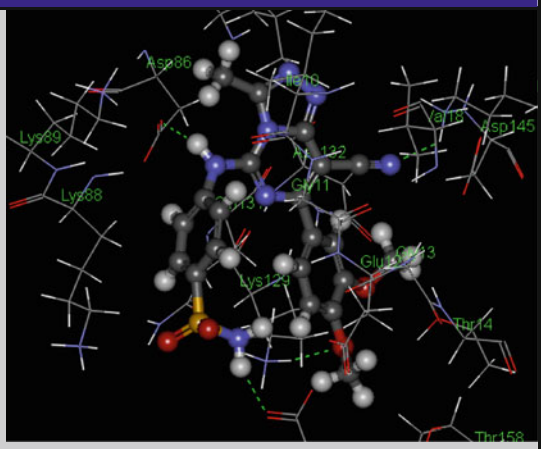
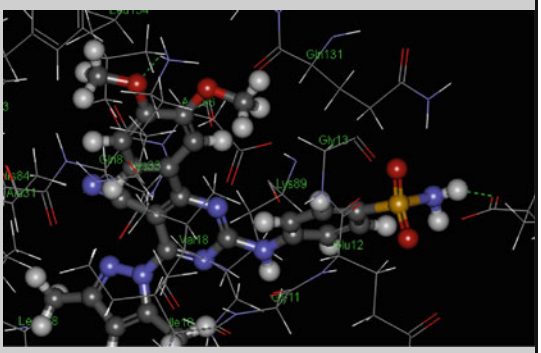
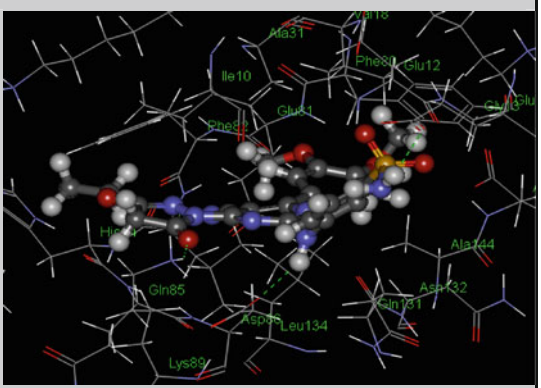
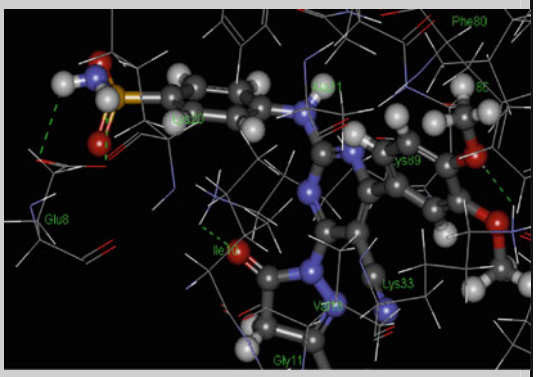
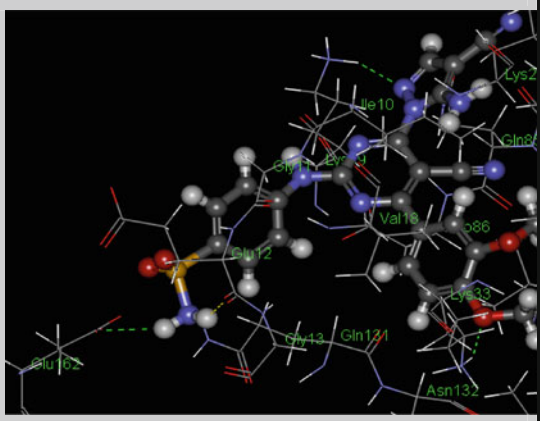
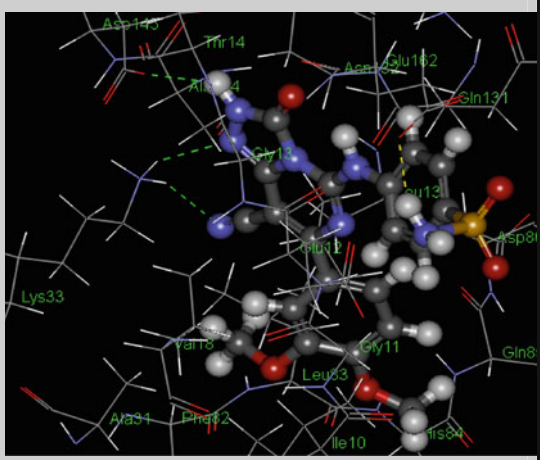
Compd.	*- CDOCKER INTERACTION*	*H-Bonds*	*3D-Docking Results*
9	68.22	Lys129 {2.00 A°} Glu162 {2.01 A°} Lys33 {2.09 A°} Asp86 {2.00 A°}	
11	74.49	Lys33 {1.88 A°} Glu162 {1.91 A°}	
13a	85.07	Asp86 {2.40 A°} Lys89 {1.71 A°} Glu162 {2.05 A°}	

Table 2 continued

Compd.	*- CDOCKER INTERACTION*	*H-Bonds*	*3D-Docking Results*
13b	76.12	Lys33 {1.92 A°} Lys89 {2.43 A°} Glu8 {1.99 A°} Glu8 {2.40 A°}	
15	70.25	Lys33 {1.80 A°} Lys89 {1.86 A°} Glu162 {2.29 A°} Glu12 {1.89 A°}	
17	65.67	Lys33 {2.19 A°} Lys129 {2.17 A°} Asp145 {2.04 A°} Glu162 {2.02 A°}	

*6-(3,4-Dimethoxyphenyl)-4-hydrazinyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**7**)*

A mixture of chloro-derivative **2** (3 g, 10 mmol) and hydrazine hydrate 99 % (1.6 mL, 50 mmol) in methanol (10 mL) was stirred for 8 h at r.t. The formed precipitate was filtered off, dried, and re-crystallized from DMF/water. Yield: 70 %; mp (°C): 220–222. IR (KBr, cm^{-1}): 3420, 3390, 3190 (2NH, NH_2), 3004 (CH, aromatic), 2220 (CN), 1140 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 3.75, 3.80 (2s, 6H, $2OCH_3$), 6.50 (s, 2H, NH_2 exchangeable with D_2O), 6.90–7.30 (m, 3H, aromatic-H), 8.21, 9.82 (2s, 2H, 2NH exchangeable with D_2O). MS *m/z*: $(M - 2)^+$ 301 (30 %), 270 (25 %), 182 (30 %), 78 (100 %). Anal. calcd for $C_{13}H_{13}N_5O_2S$ (303.34): C, 51.47; H, 4.31; N, 23.08; S, 10.57. Found: C, 51.61; H, 4.02; N, 23.32; S, 10.21.

*7-(3,4-Dimethoxyphenyl)-3-methyl-5-thioxo-5,6-dihydro-[1,2,4] triazolo[4,3-c] pyrimidine-8-carbonitrile (**8**)*

A mixture of hydrazinyl derivative **7** (1 g, 3 mmol) and acetic anhydride (10 mL) was heated under reflux for 4 h. Then the reaction mixture was cooled and poured onto ice/water. The formed precipitate was filtered off, dried and re-crystallized from methanol. Yield: 70 %; mp (°C): 268–270. IR (KBr, cm^{-1}): 3429 (NH), 2220 (CN), 1141 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.13 (s, 3H, CH_3), 3.75, 3.85 (2s, 6H, $2OCH_3$), 6.92–7.03 (m, 3H, aromatic-H) and 8.00 (s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 327 (40 %), 247 (20 %), 202 (40 %), 78 (100 %). Anal. calcd for $C_{15}H_{13}N_5O_2S$ (327.37): C, 55.03; H, 4.00; N, 21.39; S, 9.79. Found: C, 55.33; H, 4.34; N, 21.25; S, 9.61.

*4-(8-Cyano-7-(3,4-dimethoxyphenyl)-3-methyl-[1,2,4]triazolo[4,3-c] pyrimidin-5-ylamino)benzenesulfonamide (**9**)*

A mixture of triazolo derivative **8** (0.5 g, 1.5 mmol) and sulfanilamide (0.26 g, 1.5 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then the mixture was cooled and poured gradually onto ice/water. The formed precipitate was filtered off, dried and re-crystallized from DMF/water. Yield: 70 %; mp (°C): 268–270. IR (KBr, cm^{-1}): 3430, 3270 (NH, NH_2), 2224 (CN), 1360, 1160 (SO_2NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.13 (s, 2H, CH_3), 3.70, 3.88 (2s, 6H, $2OCH_3$), 5.92 (s, 2H, NH_2 exchangeable with D_2O), 6.70–7.80 (m, 7H, aromatic-H) and 9.80 (s, H, NH exchangeable with D_2O). MS *m/z*: $(M - 1)^+$ 464 (20 %), 296 (40 %), 78 (100 %). Anal. calcd for $C_{21}H_{19}N_7O_4S$ (465.50): C, 54.18; H, 4.11; N, 21.06; S, 6.88. Found: C, 54.42; H, 4.23; N, 21.26; S, 6.51.

*6-(3,4-Dimethoxyphenyl)-4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-thioxo-1,2-dihydro-pyrimidine-5-carbonitrile (**10**)*

A mixture of hydrazinyl compound **7** (1 g, 3 mmol) and acetylacetone (10 mL, 15 mmol) was heated under reflux for 4 h. The solid obtained was filtered off and crystallized from DMF/water. Yield: 73 %; mp (°C): 220–222. IR (KBr, cm^{-1}): 3340 (NH), 2224 (CN) and 1143 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.15, 2.16 (2s, 6H, $2CH_3$), 3.79, 3.84 (2s, 6H, $2OCH_3$), 6.83–7.40 (m, 3H, aromatic-H and 1H, pyrazole ring) and 8.20 (s, 1H, NH exchangeable with D_2O). MS *m/z*: $(M + 2)^+$ 369 (40 %), 310 (20 %), 217 (10 %), 151 (60 %), 79 (100 %). Anal. calcd for $C_{18}H_{17}N_5O_2S$ (367.43): C, 58.84; H, 4.66; N, 19.05; S, 8.72. Found: C, 54.69; H, 4.45; N, 21.00; S, 6.21.

*4-(5-Cyano-(3,4-dimethoxyphenyl)-4-(3,4-dimethyl-1H-pyrazol-1-yl) pyrimidin-2-ylamino)benzenesulfonamide (**11**)*

A mixture of pyrazolo derivative **10** (0.5 g, 1.3 mmol) and sulfanilamide (0.23 g, 1.3 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then the mixture was cooled and poured gradually onto ice/water. The precipitated material was filtered off, dried and re-crystallized from DMF/water. Yield: 73 %; mp (°C): 220–222. IR (KBr, cm^{-1}): 3440, 3240 (NH, NH_2), 2220 (CN), 1327, 1160 (SO_2NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.20, 2.21 (2s, 6H, $2CH_3$), 3.75, 3.81 (2s, 6H, $2OCH_3$), 5.81 (s, 2H, NH_2 exchangeable with D_2O), 6.81–7.90 (m, 7H, aromatic-H and 1H of pyrazole ring), 8.50 (s, 1H, NH exchangeable with D_2O). MS *m/z*: $(M + 2)^+$ 507 (20 %), 350 (20 %), 156 (40 %), 80 (100 %). Anal. calcd for $C_{24}H_{23}N_7O_4S$ (505.56): C, 57.01; H, 4.58; N, 19.39; S, 6.34. Found: C, 57.31; H, 4.34; N, 19.30; S, 6.11.

*6-(3,4-Dimethoxyphenyl)-4-(3-ethoxy/methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**12a, b**)*

A mixture of hydrazinyl derivative **7** (1 g, 3 mmol) and diethylmalonate (0.55 mL, 3 mmol) or ethylacetoacetate (0.42 mL, 3 mmol) in DMF (10 mL) was heated under reflux for 4 h. Then, the reaction mixture was cooled and poured gradually onto ice/water. The formed precipitate was filtered off, dried, and recrystallized from DMF/water.

12a Yield: 66 %; mp (°C): 258–260. IR (KBr, cm^{-1}): 3399 (NH), 2210 (CN), 1653 (CO), 1138 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.13 (t, 3H, $J = 6.7$ Hz, CH_3 of ethyl group), 2.40 (s, 2H, CH_2, pyrazoline ring), 3.86, 3.89 (2s, 6H, $2OCH_3$), 4.03 (q, 2H, $J = 7.1$ Hz, CH_2 of ethyl group), 6.90–7.21 (m, 3H, aromatic-H) and 8.00 (s, 1H, NH

exchangeable with D_2O). MS *m/z*: M^+ 399 (20 %), 163 (10 %), 137 (20 %), 78 (100 %). Anal. calcd for $C_{18}H_{17}N_5O_4S$: C, 54.12; H, 4.28; N, 17.53; S, 8.02. Found: C, 54.43; H, 4.40; N, 17.32; S, 8.11.

12b Yield: 72 %; mp (°C): 238–240. IR (KBr, cm^{-1}): 3418 (NH), 2208 (CN), 1660 (CO), 1139 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 2.21 (s, 3H, CH_3), 2.46 (s, 2H, CH_2 pyrazoline ring), 3.74, 3.80 (2s, 6H, $2OCH_3$), 6.91–7.20 (m, 3H, aromatic-H) and 8.00 (s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 369 (30 %), 355 (50 %), 201 (80 %), 78 (100 %). Anal. calcd for $C_{17}H_{15}N_5O_3S$ (369.41): C, 55.27; H, 4.09; N, 18.95; S, 8.68. Found: C, 55.01; H, 4.21; N, 18.82; S, 8.40.

*4-(5-Cyano-4-(3,4-dimethoxyphenyl)-6-(3-ethoxy/methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)pyrimidin-2-ylamino)benzenesulfonamide (**13a, b**)*

A mixture of **12a**, **b** (1.2 mmol) and sulfanilamide (1.2 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then, the reaction mixture was cooled and poured gradually onto ice/water. The precipitated product was filtered off, dried, and recrystallized from DMF/water.

13a Yield: 65 %; mp (°C): 279–281. IR (KBr, cm^{-1}): 3420, 3193 (NH, NH_2), 2210 (CN), 1656 (CO). ^1H NMR (DMSO-d_6, δ ppm): 1.21 (t, 3H, $J = 6.8$ Hz, CH_3 of ethyl group), 2.25 (s, 2H, CH_2 pyrazoline ring), 3.78, 3.80 (2s, 6H, $2OCH_3$), 4.10 (q, 2H, $J = 7.2$ Hz CH_2 of ethyl group), 5.61 (s, 2H, NH_2 exchangeable with D_2O), 6.71–7.91 (m, 7H, aromatic-H) and 8.51 (s, 1H, NH exchangeable with D_2O). ^{13}C NMR (DMSO, δ ppm): 15.29, 60.11 (OCH_2CH_3), 55.12 ($2OCH_3$), 36.34, 158.34, 172.10 (pyrazoline-C), 113.11 (CN), 98.29, 159.54, 164.31, 172.90 (pyrimidine-C), 111.02, 122.21, 127.61, 129.34, 131.90, 142.12, 149.11 (aromatic-C). MS *m/z*: M^+ 537 (30 %), 381 (15 %), 156 (20 %), 137 (20 %), 78 (100 %). Anal. calcd for $C_{24}H_{23}N_7O_6S$ (537.56): C, 53.62; H, 4.31; N, 18.23; S, 5.96. Found: C, 53.81; H, 4.10; N, 18.49; S, 5.96.

13b Yield: 65 %; mp (°C): 270–272. IR (KBr, cm^{-1}): 3343, 3190 (NH, NH_2), 2208 (CN), 1657 (CO), 1330, 1148 (SO_2NH_2). ^1H NMR (DMSO-d_6, δ ppm): 2.10 (s, 3H, CH_3), 2.40 (s, 2H, CH_2 pyrazoline ring), 3.75, 3.81 (2s, 6H, $2OCH_3$), 5.61 (s, 2H, NH_2 exchangeable with D_2O), 6.71–7.91 (m, 7H, aromatic-H) and 8.61 (s, 1H, NH exchangeable with D_2O). ^{13}C NMR (DMSO, δ ppm): 16.31 (CH_3), 55.98 ($2OCH_3$), 40.91, 152.41, 165.00 (pyrazoline-C), 113.32 (CN), 98.21, 159.11, 162.31, 172.00 (pyrimidine-C), 111.12, 122.21, 127.41, 129.34, 131.10, 142.12, 149.11 (aromatic-C). MS *m/z*: $(M - 2)^+$ 505 (40 %), 426 (10 %), 172 (10 %), 78 (100 %). Anal. calcd for $C_{23}H_{21}N_7O_5S$ (507.54): C, 54.42; H, 4.17; N, 19.31; S, 6.31. Found: C, 54.12; H, 4.38; N, 19.57; S, 6.31.

*4-(5-Amino-4-cyano-1H-pyrazol-1-yl)-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**14**)*

A mixture of hydrazinyl derivative **7** (1 g, 3 mmol) and 2-ethoxy methylene malononitrile (0.40 g, 3 mmol) in DMF (10 mL) was heated under reflux for 6 h. Then, the mixture was cooled and poured gradually onto ice/water. The formed precipitate was filtered off, dried, and recrystallized from DMF/water. Yield: 66 %; mp (°C): 230–232. IR (KBr, cm^{-1}): 3314, 3180 (NH, NH_2), 2210 (2CN), 1137 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 3.75, 3.80 (2s, 6H, $2OCH_3$), 5.23 (s, 2H, NH_2 exchangeable with D_2O), 6.90–7.53 (m, 3H, aromatic-H and 1H of pyrazole ring), 8.00 (s, 1H, NH exchangeable with D_2O). MS *m/z*: M^+ 379 (50 %), 105 (30 %), 78 (100 %). Anal. calcd for $C_{17}H_{13}N_7O_2S$: (379.41): C, 53.81; H, 3.45; N, 25.84; S, 8.45. Found: C, 53.61; H, 3.23; N, 26.01; S, 8.19.

*4-(4-(5-Amino-4-cyano-1H-pyrazol-1-yl)-5-cyano-6-(3,4-dimethoxyphenyl) pyrimidin-2-ylamino) benzenesulfonamide (**15**)*

A mixture of pyrazole compound **14** (0.5 g, 1 mmol) and sulfanilamide (0.22 g, 1 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then, the mixture was cooled and poured gradually onto ice/water. The precipitated product was filtered off, dried, and recrystallized from DMF/water. Yield: 70 %; mp (°C): 228–230. IR (KBr, cm^{-1}): 3319, 3195, 3120 (NH, $2NH_2$), 2209 (2CN), 1330, 1143 (SO_2NH_2). ^1H NMR (DMSO-d_6, δ ppm): 3.75, 3.80 (2s, 6H, $2OCH_3$), 5.20, 6.50 (2s, 4H, $2NH_2$), 6.90–7.80 (m, 7H, aromatic-H and 1H of pyrazole ring), 10.20 (1s, 1H, NH exchangeable with D_2O). MS *m/z*: $(M - 1)^+$ 516 (20 %), 501 (10 %), 365 (20 %), 78 (100 %). Anal. calcd for $C_{23}H_{19}N_9O_4S$ (517.53): C, 53.37; H, 3.70; N, 24.35; S, 6.20. Found: C, 53.51; H, 3.52; N, 24.54; S, 6.53.

*7-(3,4-Dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro-[1,2,4]triazolo [4,3-c]pyrimidine-8-carbonitrile (**16**)*

A mixture of hydrazinyl compound **7** (1 g, 3 mmol) and ethylchloroformate (0.31 mL, 3 mmol) in pyridine (10 mL) was heated under reflux for 6 h. Then, the mixture was cooled, poured gradually onto ice/water and neutralized with HCl. The formed precipitate was filtered off, dried, and recrystallized from DMF/water. Yield: 70 %;

mp (°C): 250–252. IR (KBr, cm^{-1}): 3320, 3211 (2NH), 2211 (CN), 1657 (CO), 1130 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 3.75, 3.80 (2s, 6H, $2OCH_3$), 6.80–7.80 (m, 3H, aromatic-H), 8.00, 10.11 (2s, 2H, 2NH exchangeable with D_2O). MS *m/z*: $(M-1)^+$ 328 (20 %), 301 (10 %), 191 (40 %), 137 (10 %), 78 (100 %). Anal. calcd for $C_{14}H_{11}N_5O_3S$ (329.34): C, 51.05; H, 3.36; N, 21.26; S, 9.73. Found: C, 51.35; H, 3.52; N, 21.01; S, 9.92.

*4-(8-Cyano-7-(3,4-dimethoxyphenyl)-3-oxo-2,3-dihydro-[1,2,4]triazolo[4,3-c] pyrimidin-5-ylamino) benzenesulfonamide (**17**)*

A mixture of triazolo derivative **16** (0.5 g, 1 mmol) and sulfanilamide (0.26 g, 1 mmol) in DMF (5 mL) containing few drops of TEA was refluxed for 15 h. Then, the reaction mixture was cooled and poured gradually onto ice/water. The obtained precipitate was filtered off, dried, and re-crystallized from DMF/water. Yield: 66 %; mp (°C): 289–291. IR (KBr, cm^{-1}): 3334, 3230, 3130 (2NH, NH_2), 2208 (CN), 1657 (CO), 1337, 1144 (SO_2NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 3.75, 3.82 (2s, 6H, $2OCH_3$), 5.82 (s, 2H, NH_2 exchangeable with D_2O), 6.72–7.92 (m, 7H, aromatic-H), and 8.00, 9.82 (2s, 2H, 2NH exchangeable with D_2O). MS *m/z*: M^+ 467 (30 %), 450 (12 %), 389 (20 %), 78 (100 %). Anal. calcd for $C_{20}H_{17}N_7O_5S$ (467.47): C, 51.38; H, 3.66; N, 20.97; S, 6.86. Found: C, 51.62; H, 3.49; N, 20.57; S, 6.63.

Anti-tumor screening

Cell growth inhibition assay

Cervical cancer cell lines (Hela cell lines) were obtained from Cell Bank in National Cancer Institute, Cairo, Egypt. The potential toxicity of the selected newly synthesized derivatives was done by SRB using the method Skehan *et al.* (1990) as follows: cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (1, 2.5, 5 and 10 g/mL) were added to the cell monolayer triplicate wells which were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. Measurements were done six times ($n = 6$) and averaged. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound. The variation correlation of the obtained data was presented in Table 3, using the following equation to obtain the standard deviation results:

$$S = \sqrt{\frac{\sum (X-M)^2}{n-1}}$$

while V (variance of standard deviation) $= S^2$, Σ = sum of, X = individual score, M = mean of all scores, n = sample size (number of scores).

Table 3 Variation correlation of the anti-cancer evaluation data against human cervix carcinoma cell line (Hela)

Compound no.	Concentration (Ug/mL)	Mean	*V*	SD	SEM	Mean
4	0	1	0.000	0.000	0.000	1.000
	5	1.079	0.007	0.088	0.036	1.079
	12.5	0.676	0.033	0.181	0.074	0.676
	25	0.244	0.002	0.049	0.020	0.244
	50	0.177	0.0003	0.019	0.008	0.177
6	0	1	0.000	0.000	0.000	1.000
	5	1.265	0.025	0.158	0.065	1.265
	12.5	0.819	0.004	0.067	0.028	0.819
	25	0.236	0.001	0.038	0.016	0.236
	50	0.16	0.001	0.031	0.013	0.160
9	0	0	0.000	0.000	0.000	0.000
	5	1.378	0.024	0.156	0.064	1.378
	12.5	0.82	0.007	0.084	0.035	0.820
	25	0.412	0.003	0.061	0.025	0.412
	50	0.316	0.0005	0.023	0.010	0.316
11	0	0	0.000	0.000	1.000	0.000
	5	1.513	0.023	0.154	0.062	1.513
	12.5	0.897	0.047	0.218	0.089	0.897
	25	0.349	0.013	0.117	0.049	0.349
	50	0.195	0.0003	0.018	0.008	0.195
13a	0	1	0.000	0.000	0.000	1.000
	5	1.062	0.194	0.441	0.180	1.062
	12.5	0.983	0.011	0.105	0.043	0.983
	25	0.407	0.002	0.044	0.018	0.407
	50	0.319	0.0003	0.018	0.007	0.319
13b	0	1	0.000	0.000	0.000	1.000
	5	1.438	0.013	0.116	0.048	1.438
	12.5	0.943	0.004	0.064	0.026	0.943
	25	0.422	0.002	0.051	0.021	0.422
	50	0.238	0.0003	0.018	0.007	0.238
17	0	1	0.000	0.000	0.000	1.000
	5	1.199	0.015	0.122	0.050	1.199
	12.5	0.769	0.039	0.197	0.081	0.769
	25	0.42	0.011	0.009	0.045	0.420
	50	0.315	0.002	0.052	0.021	0.315

SEM standard error mean, *SD* standard deviation, *V* variance of standard deviation

$n = 6$

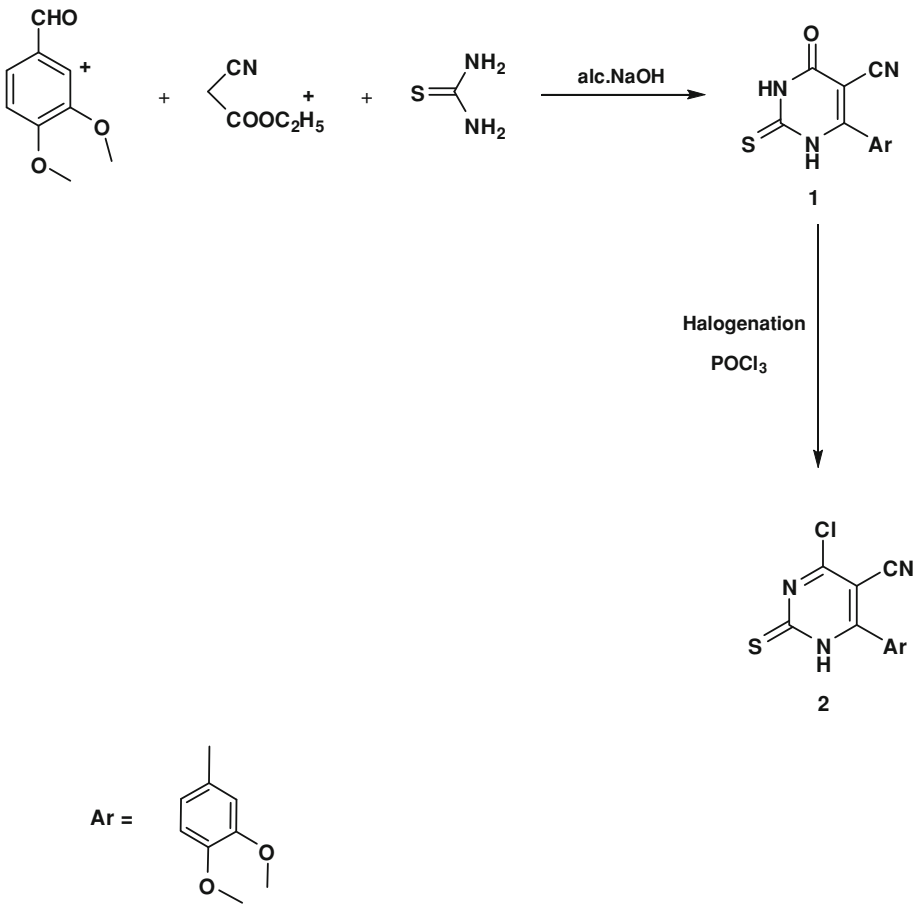
Results and discussion

Molecular docking study

According to Table 1, molecular modeling simulation study demonstrates that the designed derivatives could be promising active hits as CDK2 inhibitors. Best affinities to the active site of CDK2 were gained by the molecules containing pyrimidine-benzenesulfonamide ring system which are: **4**, **6**, **9**, **11**, **13a**, **13b**, **15**, and **17**. All of them showed CDOCKER interaction energy less than that of the lead compound. According to Table 2, showing the 3D docking of the tested compounds in the binding site, the presence of 3-ethoxy-5-pyrazolone (**13a**) exhibited the highest affinity, while the replacement of the ethoxy group of the pyrazolone ring with a methyl group (**13b**) or exchange the pyrazolone moiety with tetrazole ring (**4**) led to remarkable reduction in the affinity. Further affinity decrease was observed by the molecules bearing imidazo-pyrimidine and 5-amino-4-cyanopyrazole ring systems (**6**, **15**). Least affinity was obtained by the molecules carrying the triazole nucleus (**9**, **17**).

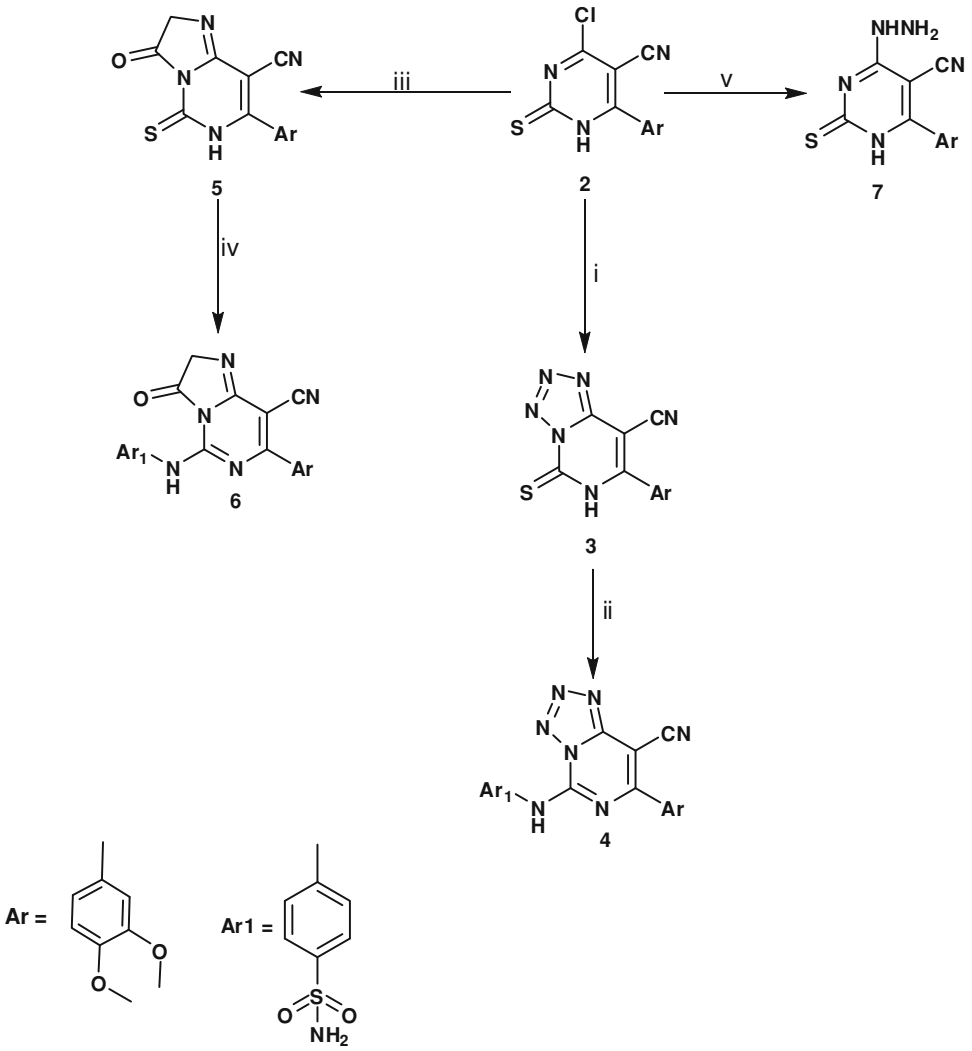
Chemistry

The key starting compound 6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) was synthesized by one-pot reaction of thiourea, ethyl-cyanoacetate and 3,4-dimethoxybenzaldehyde in sodium ethoxide solution with continuous stirring at room temperature according to the reported method (Fathalla *et al.*, 2009). Chlorination of **1** was carried out by its refluxing with $POCl_3/PCl_5$ for 8 h to obtain the chloro-derivative **2** in 75 % yield (Scheme 1). IR spectrum revealed the disappearance of CO group at 1,730 cm^{-1}. Mass spectrum showed the two isotopic molecular ion peaks at 308, 310 in a ratio (3:1). Compound **2** was considered as a precursor for the synthesis of different heterocyclic rings fused with 2-thiopyrimidine ring. Thus, the treatment of **2** with sodium azide in refluxing glacial acetic acid (Ali *et al.*, 2000) led to gain the desired tetrazolo derivative **3**. Mass spectrum of the derivative showed the molecular ion peak at 314 (15 %). Upon reaction of **3** with an equimolar amounts of sulfanilamide in refluxing DMF containing few drops of TEA (Amin *et al.*, 2009) afforded the benzene-



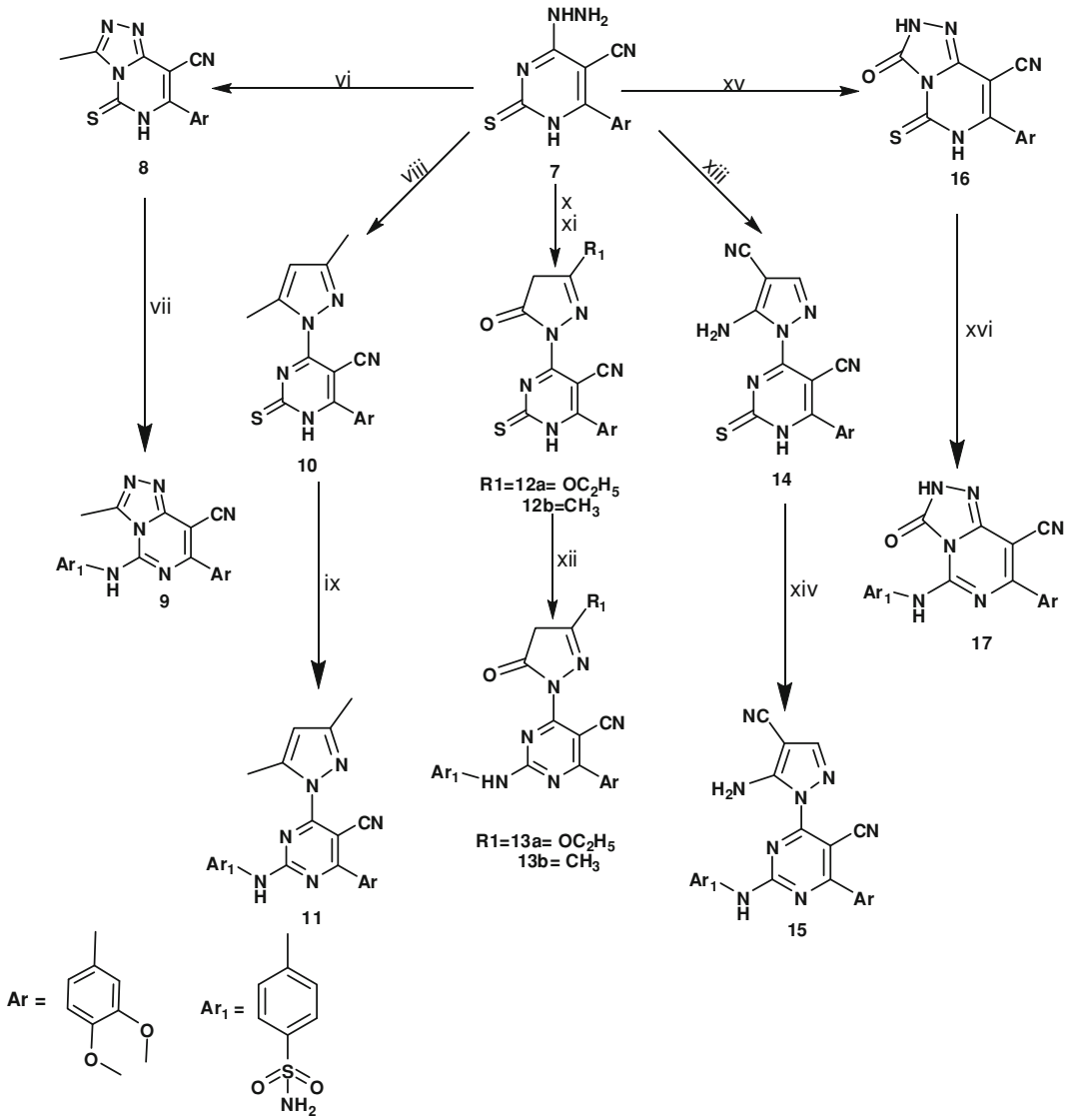
Scheme 1 Synthetic protocols of the starting 4-oxothiopyrimidine **1** and the intermediate 4-chlorothiopyrimidine derivative **2**

sulfonamide derivative **4** in moderate yield. ^{1}H NMR spectrum of **4** demonstrated the presence of multiplet signals at 7.01–7.99 ppm assigned for the seven aromatic protons and a singlet signal at 5.20 ppm attributed to the two protons of NH_2, in addition to the presence of two singlets at 3.69, 3.71 ppm due to 6H of $2OCH_3$ groups and at 7.99 ppm corresponding to 1H of NH group. Further treatment of the chloro-derivative **2** with the amino acid glycine in refluxing DMF gave the imidazopyrimidine analogue **5** in 66 % yield. IR spectrum of the compound showed the appearance of a new absorption band at 1,652 cm^{-1} assigned for the lactamic CO group. Also, ^{1}H NMR spectrum exhibited a singlet signal at 2.29 ppm indicating the two protons of methylene group of imidazoline ring. Upon condensation of **5** with equimolar amounts of sulfanilamide in refluxing DMF afforded the benzenesulfonamide derivative **6** in moderate yield. ^{1}H NMR spectrum of **6** represented three singlets at 2.30, 3.75, 3.85 ppm referring to 2H of CH_2 of imidazoline ring and 6H of $2OCH_3$ groups. The seven aromatic protons appeared multiplet signals at 6.80–7.70 ppm, while NH_2 and NH protons appeared as two singlet signals exchangeable with D_2O at 5.62, 7.91 ppm. Synthesis of hydrazinopyrimidine **7** as a key starting material for many nitrogen bridge head compounds was performed by the reaction of the chloro-derivative **2** with excess hydrazine hydrate 99 % (Fathalla *et al.*, 2009) in methanol with continuous stirring at room temperature (Scheme 2). ^{1}H NMR spectrum exhibited the presence of two singlet signals at 8.21 and 9.82 ppm representing the two protons of 2NH and at 6.50 ppm due to the two protons of NH_2 group.



Scheme 2 Synthetic protocols of the compounds tetrazolo[1,5-*c*]pyrimidine **3**, imidazo[1,2-*c*]pyrimidine **5** and their respective benzenesulfonamide derivatives **4**, **6** and synthesis of 4-hydrazinylthiopyrimidine derivative **7**

Upon refluxing **7** with acetic anhydride for 4 h the desired triazolo[4,3-*c*]pyrimidine derivative **8** was obtained in 70 % yield. ^{1}H NMR spectrum showed the presence of a singlet signal at 2.31 ppm corresponding to the three protons of CH_3 of the triazole ring. The other protons of the molecule appeared at their expected regions. The derivative **8** was in turn condensed with equimolar amounts of sulfanilamide in refluxing DMF containing few drops of TEA to give the benzenesulfonamide derivative **9** in moderate yield. ^{1}H NMR spectrum of the compound **9** exhibited a singlet signal at 2.13 ppm due to CH_3, two singlet signals at 3.70, 3.88 ppm due to $2OCH_3$, multiplet signals at 6.70–7.80 ppm due to 7H aromatic protons, in addition to two singlets at 5.92 and 9.80 ppm due to NH_2 and NH protons. Cyclocondensation of the hydrazinyl derivative **7** with bielectrophilic species such as acetyl



Scheme 3 Synthetic protocols of the compounds triazolo[4,3-*c*] pyrimidine **8**, pyrazolopyrimidines **10**, **12a**, **b**, **14**, triazolo[4,3-*c*]pyrimidine **16** their respective benzenesulfonamide derivatives **9**, **11**, **13a**, **b**, **15**, **17**

acetone, diethylmalonate, and ethylacetoacetate in refluxing DMF furnished the pyrazolo derivatives **10**, **12a**, **b**, respectively. ^{1}H NMR spectrum of **10** represented two singlet signals at 2.15 and 2.16 ppm accomplished for the six protons of the two CH_3 groups. ^{1}H NMR spectrum of **12a** represented the characteristic triplet-quartet pattern of the ethyl group at 1.13 and 4.03 ppm and CH_2 protons of pyrazoline ring appeared as a singlet signal at 2.40 ppm, while ^{1}H NMR spectrum of **12b** showed a singlet signal at 2.21 ppm referring to the 3H of CH_3 group and the methylene protons of the pyrazoline ring appeared as a singlet signal at 2.46 ppm. Further treatment of **10**, **12a**, **b** with equimolar amounts of sulfanilamide was carried out to get the benzenesulfonamide analogues **11**, **13a**, **b**, respectively. Mass spectra of the new derivatives exhibited their molecular ion peaks. Nucleophilic reaction of the hydrazinyl derivative **7** with 2-(ethoxymethylene) malononitrile in refluxing DMF or with ethylchloroformate in refluxing pyridine afforded the desired 4-cyano-5-amino pyrazolo and/or triazolopyrimidine derivatives **14**, **16** respectively. IR spectrum of the compound **14** exhibited a strong stretching band at 2,210 cm^{-1} assigned for 2CN groups, while its ^{1}H NMR spectrum revealed a singlet signal at 5.23 ppm due to the two protons of NH_2 group. The other protons of the molecule were present at their expected regions; 6H of $2OCH_3$ appeared at 3.75, 3.80 ppm as two singlets, 4H aromatic protons appeared at 6.90–7.53 ppm as a multiplet signal and NH proton appeared as an exchangeable signal at 8.00 ppm. IR spectrum of the derivative **16** exhibited the lactamic CO group at 1,657 cm^{-1}. The formation of the respective benzenesulfonamide analogues **15**, **17** was carried out by the reaction of equimolar amounts of **14** and/or **16** with sulfanilamide (Scheme 3). ^{1}H NMR spectra of the two derivatives showed the seven aromatic protons as multiplets at 6.72–7.92 ppm in addition to the other expected protons at their correct regions.

Anti-tumor activity

In order to correlate between the obtained molecular modeling simulation results of the tested compounds and their anti-proliferative activity, the analogues that showed the best molecular docking results (**4**, **6**, **9**, **11**, **13a**, **13b**, **17**) were selected as representative examples to evaluate their in vitro inhibitory effects against cellular proliferation in human cultured Hela cell lines using Doxorubicin as a reference drug. Depending upon the data of Table 4, there is a good correlation between both studies since all the evaluated derivatives appeared to be approximately equipotent cytotoxic agents with IC_{50} values (0.039–0.047 μM) lower than that of the **lead derivative** (**III**) (IC_{50} 0.5 μM). Best result was obtained by tetrazolopyrimidine derivative **4** (IC_{50} 0.039 μM). The obtained data insure that the modifications which were carried out for the lead thiazolyl pyrimidine compound furnished novel derivatives of higher potency as anti-cancer agents but still less active than the reference Doxorubicin. This means that further modifications are required for getting new anti-cancer agents of higher efficacy and selectivity.

Table 4 The effect of some newly synthesized compounds against human cervix carcinoma cell line (Hela)

Compounds	IC_{50} (μM)
4	0.039
6	0.042
9	0.048
11	0.041
13a	0.042
13b	0.046
17	0.047
Doxorubicin (**Dox**)	0.016

Conclusion

In this study design, molecular modeling simulation study and synthesis were carried out for a new series of pyrimidine-benzenesulfonamide derivatives as CDK2 inhibitors taking the derivative 4-(2-amino-4-methylthiazol-5-yl)-*N*-(3-nitrophenyl) pyrimidin-2-amine as a lead compound. Docking results indicated that the novel agents exhibited better affinities to the active site of CDK2 than the lead compound. In vitro anti-proliferative activity evaluation against Hela cell lines indicated that although the compounds (**4**, **6**, **9**, **11**, **13a**, **13b**, **17**) are more potent cytotoxic agents than the lead derivative but they are still less active than the reference drug Doxorubicin and further optimization is required to get more active anti-cancer compounds.

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