### **Synthesis of potent anticancer thieno**[2,3-*d*]**pyrimidine derivatives** M.M. Kandeel, Ashraf A. Mounir, Hanan M. Refaat\* and Asmaa E. Kassab

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As part of our program to identify novel cytotoxic agents, various series of hexahydrocycloocta[4,5]thieno[2,3-*d*] pyrimidines and pyrimidin-4-ones substituted by aryl at the C-2 position together with phenylethylamino, substituted amino, hydrazinyl or arylidenhydrazinyl substituents at the C-4 position were synthesised. These compounds were prepared as bioisosteres of gefitinib, an antitumour drug used for the treatment of gastrointestinal stromal tumours. All compounds exhibited antitumour activity against (HCT 116) cell line *in vitro*. Eight compounds (IC<sub>50</sub>: 3.89, 4.65, 6.63, 6.94, 7.89, 9.53, 12.00 and 12.30  $\mu$ g mL<sup>-1</sup>, respectively) exhibited 4.3 to 1.3 fold more potent antitumour activity than imatinib (IC<sub>50</sub>: 16.93  $\mu$ g mL<sup>-1</sup>). Also, a docking study of the newly synthesised compounds with the active site of CDK2 was described.

Keywords: hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidines, pyrimidin-4-ones, antitumour activity, docking study

The thieno[2,3-d]pyrimidine system is considered today a relevant pharmacophore in a wide range of biological activities, and particularly it was found to be active against different cancer types.<sup>1-6</sup> Certain thieno[2,3-d] pyrimidines were originally prepared as bioisosters of erlotinib (Tarceva®)7 and gefitinib (Iressa®)8 (Fig. 1), a 4-substituted pyrimidine and a 4-amino quinazoline derivatives respectively, that had been approved for the treatment of gastrointestinal stromal tumours and lung cancer. Several studies indicated that the mechanism by which the thieno[2,3-d]pyrimidines exert their cytotoxic activities is strongly related to their inhibitory effect on many enzymes and mediators, for example, receptor tyrosine kinases (RTKs)<sup>9-11</sup> and cyclin dependent kinases (CDKs).<sup>2,3,12,13</sup> Recently, certain thieno[2,3-d]pyrimidines as ATPase inhibitors of heat shock protein 90 (Hsp 90)<sup>14,15</sup> were also identified. Consequently, the thieno[2,3-d]pyrimidine ring system constitutes an attractive target for the design of new anticancer agents. In this context, a wide structure variation of thieno [2,3-d]pyrimidines possessing an interesting biological profile in terms of anticancer activity has been recently synthesised. The structure variation includes substituted thieno1,10 or cycloalkyl-fused thieno moiety,4,16,17 aromatic or aliphatic substitution on pyrimidine C-2,<sup>2,3</sup> 4-pyrimidone,<sup>2,3</sup> 4- substituted amino<sup>9,11,17</sup> or 4-hydrazinyl<sup>5,12,13</sup> pyrimidine ring systems. For example, the thieno[2,3-d]pyrimidine I (Fig. 2) showed cytotoxic effect on leukaemia cell lines,1 whereas the lead compounds II,<sup>2</sup> III<sup>5</sup> and IV<sup>11</sup> (Fig. 2) exhibited inhibitory activity against human colon tumour cell.

As part of our program to identify novel cytotoxic agents, we decided to prepare and evaluate hexahydrocycloocta [4,5]thieno[2,3-*d*]pyrimidine derivatives that could be considered as analogues of the previously reported potent anticancer thieno[2,3-*d*]pyrimidines (Fig. 2). In order to explore the role of the cycloalkyl moiety in the potency of the anticancer activity, the hexahydrocycloocta [4,5]thieno[2,3-*d*]pyrimidine was

not previously screened as anticancer. We also introduced certain aryl substituents at the C-2 position and phenylethylamino, substituted amino, hydrazine or arylidenhydrazinyl substituents at the C-4 position to substantiate the possible influence of such substitution on the *in vitro* antitumour activities against human colon carcinoma (HCT 116) cell line. Furthermore, we also described a docking study of the newly synthesised compounds with the active site of CDK2.

### **Results and discussion**

The synthetic route to the target compounds is outlined in Schemes 1 and 2. 2-Amino-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (3) was selected as our primary starting material for this series of reactions, and was prepared via two steps procedure which involved the reaction of cyclooctanone with cyanoacetamide 1 to give  $\alpha$ -cyano- $\alpha$ -cyclooctylideneacetamide 2 which was then reacted with sulfur and diethylamine.<sup>18</sup> One of the objectives of this study was the preparation of compound 6, a structure analogue of compound I (Fig. 2). Accordingly, compound **3** was cyclised with triethyl orthoformate and acetic anhydride,<sup>18</sup> followed by chlorination with phosphorus oxychloride,<sup>18</sup> to give compound 5, inevitable for the preparation of the target compound 6. Preparation of 4-(2-phenylethylamino)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d] pyrimidine (6) was accomplished via the reaction of 5 with 2-phenylethylamine. The <sup>1</sup>H NMR spectrum showed two triplet signals at  $\delta$  2.70–2.75 and 3.74–3.80 corresponding to  $CH_2C_6H_5$  and NHCH<sub>2</sub> protons respectively. In addition, NH proton appeared as an exchangeable singlet signal at 8 6.49.

Condensation of the amino amide **3** with aromatic aldehydes in ethanol in the presence of concentrated hydrochloric acid afforded **7a,b**, which were oxidised by heating in nitrobenzene to yield the 2-substituted hexahydrocyclooctathieno[2,3-*d*] pyrimidines **8a–c**. Another pathway for the preparation of **8a– c** directly from the amino amide **3** involved the condensation





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Fig. 2 Strategies for structural modifications of leads I, II, III and IV.



**7,8,9 a** Ar:  $4-(CH_3)_2NC_6H_4$ , **7,8,9 b:**  $2-NO_2C_6H_4$ , **8,9 c:** 2-thienyl Scheme 1 Synthesis of compounds 1–9.



Scheme 2 Synthesis of compounds 10–12.

of **3** with the selected aromatic aldehyde in dry dimethylformamide in the presence of concentrated hydrochloric acid. The IR spectra of **7a,b** showed two absorption bands at 3390, 3383 and 3188, 3182 cm<sup>-1</sup> corresponding to two NH groups. Whereas the C=O group appeared as an absorption band at 1635, 1641 cm<sup>-1</sup>, respectively. Further evidence was obtained from the <sup>1</sup>H NMR spectra of **7a,b** that showed two exchangeable singlet signals at  $\delta$  5.70, 9.08 and  $\delta$  5.72, 8.16 ppm corresponding to two NH protons while C-2 proton appeared as a singlet signal at  $\delta$  8.29 and  $\delta$  8.93 for compounds **7a** and **7b**, respectively. Whereas the <sup>1</sup>H NMR spectra of compounds **8a–c** lacked the signals corresponding to C-2 proton and showed an exchangeable singlet signal at  $\delta$  12.10–12.84 corresponding to NH proton which confirmed the structure.

On refluxing 2-aryl-5,6,7,8,9,10-hexahydrocycloocta[4,5] thieno[2,3-d]pyrimidin-4(3H)-ones (**8a–c**), with phosphorus oxychloride, the corresponding 4-chloro derivatives **9a–c** was produced. The IR spectra of compounds **9a–c** proved as useful in tracing the disappearance of the C=O and NH stretching absorption bands of the parent compounds **8a–c**.

The 4-substituted aminothieno[2,3-*d*]pyrimidine derivatives **10a–e** were obtained through the reaction of **9a–c** with the appropriate primary amine in ethanol in the presence of catalytic amount of triethylamine. The <sup>1</sup>H NMR spectra of the products **10a–e** revealed the presence of NH proton exchangeable signals resonating at the range of  $\delta$  3.45–6.78, in addition to the expected signals corresponding to the different N-substituted groups which were indicative for the success of the amination.

Hydrazinolysis of **9a–c** afforded the 4-hydrazinyl derivatives **11a–c**, which upon reaction with aromatic aldeyhdes in ethanol in the presence of acetic acid gave the corresponding 4-arylidenehydrazinyl derivatives **12a–c**. The <sup>1</sup>H NMR spectra of compounds **11a–c** showed NH<sub>2</sub> and NH peaks at around  $\delta$ 4.63–4.78 and 7.86–8.09, respectively. Whereas the <sup>1</sup>H NMR spectra of **12a–c** revealed the presence of singlet signals at  $\delta$ 8.62–8.76 corresponding to N=CH protons.

#### Cytotoxic activity.

The in vitro growth inhibitory activity of all the prepared compounds was evaluated using colon carcinoma cell line (HCT 116). For comparison purposes, the cytotoxicity of imatinib (Gleevec®) (Fig. 1), a standard antitumour drug used for the treatment of gasterointestinal tract tumours,19,20 was evaluated under the same conditions. The IC<sub>50</sub> (dose of the compound which caused a 50% reduction of survival values) are shown in Table 1. The results are represented graphically in Fig. 3. From the analysis of Table 1, it was found that all compounds showed significant antitumour activities. Interestingly, compounds 11b, 7a, 12b, 8c, 9c, 8b, 11c and 9a (IC<sub>50</sub>: 3.89, 4.65, 6.63, 6.94, 7.89, 9.53, 12.00 and 12.30 µg mL<sup>-1</sup>, respectively) exhibited 4.3 to 1.3 fold more potent antitumour activity than imatinib (IC<sub>50</sub>: 16.93  $\mu$ g mL<sup>-1</sup>) and were the most active among their analogues. Further, compounds 6, 7b, 8a, 9b, 10a, b, c, e, 11a and 12c (IC<sub>50</sub>: 14.70, 16.80, 17.90, 17.50, 16.40, 19.00, 19.20, 19.30, 18.10 and 15.90 µg mL-1, respectively) showed comparable cytotoxicity to imatinib. Moreover, compound 12a (IC<sub>50</sub>: 24.20  $\mu$ g mL<sup>-1</sup>) was less active than imatinib and compound **10d** was the least active of all compounds.

Structural changes at the C-2 phenyl group appear to have a considerable effect on the anticancer activity. Compound **7a**,

Table 1 $IC_{50}$  values° of compounds 6–12c and imatinib againstcolon carcinoma cell line (HCT 116)

Compound	<sup>a</sup> <b>IC</b> <sub>50</sub> (μg mL <sup>-1</sup> )
6	14.70
7a	4.65
7b	16.80
8a	17.90
8b	9.53
8c	6.94
9a	12.30
9b	17.50
9c	7.89
10a	16.40
10b	19.00
10c	19.20
10d	50.00
10e	19.30
11a	18.10
11b	3.89
11c	12.00
12a	24.20
12b	6.63
12c	15.90
Imatinib	16.93

 $^{a}$  IC\_{\rm 50} (µg mL^1): dose of the compound which caused 50% reduction of survival.

Values were calculated from dose-response curves done in triplicates for each compound.



Fig. 3 Cytotoxicity of 6-12c and imatinib against (HCT 116) cell line.

one of the most potent test compounds, included a 4-dimethylaminophenyl group. This observation was in accordance with the previously reported studies.<sup>12,13</sup> Among the 2-aryl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidin-4(3H)-ones (**8a–c**) series, interestingly, **8c** with C-2 thienyl substituent was the most active. Our interest was then focused on introducing substituents on C-4 to detect their effect on activity. Compounds **9a–c**, bearing chloro on C-4 maintained relatively potent anticancer activity. Introduction of a hydrazinyl moiety at the 4-position, compounds **11a–c**, improved the anticancer activity over their 4-oxo analogues. Compound **11b**, the most potent derivative of all the synthesised compounds, had a 2-nitrophenyl substituent.

Unexpectedly, the 4-arylidenhydrazinyl derivatives **12a–c**, exhibited lower potency than their parent compounds **11a–c** (in contrast to reported studies<sup>12,13</sup>) however **12b**, a 2-nitrophenyl derivative was the most potent of its analogues. Further, compounds **6** and **10a–e** were designed taking in consideration the previously reported works that signified the 4-phenethylamino and 4-aminopropionic acid, with two carbon spacer, as potent anticancer agents.<sup>1</sup> Also, the 4-aminoethyl derivative

was also prepared keeping the optimum two carbon spacer.<sup>1</sup> Compound **6**, devoid of 2-aryl, was slightly more potent than **10a**, **10d** and **10e**, with 2-aryl substituent.

#### Docking study

CDK2 is a target enzyme for a wide range of antitumour drugs due to its important role in the control of cell cycle by binding with its associated cyclin that moves the cell from one phase of the cell cycle to another one.<sup>21</sup> Several thieno [2,3-d]pyrimidines were reported as CDK2 and CDK4 inhibitors.12,13 Therefore we investigated the binding affinities of the newly synthesised hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidines using Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) as the computational software, into the target CDK2. The validation of the docking accuracy was done by docking of the native ligand 4-(3-hydroxyanilino)-6,7-dimethoxyquinazoline into its binding site of CDK2. The docked results of the above mentioned ligand were compared to the crystal structure of the ligand protein complex where both of them seemed exactly superimposed with binding free energy ( $\Delta G_{\rm b}$ ) of -14.07 kcal mol<sup>-1</sup>. The ligand exhibited four hydrogen bonds between the pyrimidine N-1 and the OH of 4-(3-hydroxyanilino) along with Asp145, Leu 83 and Lys33 (Fig. 4). These results indicated the high accuracy of the MOE simulation in comparison with the biological methods. The vicinity where 4-anilinoquinazoline was situated was considered as the active site of CDK2.

In the present work, all the prepared new compounds were docked using a rigid receptor / flexible ligand approach where the binding affinity was evaluated by the binding free energy  $(\Delta G_b, \text{ kcal mol}^{-1})$  and hydrogen bond interactions. The compounds which revealed the highest binding affinities (in other words, lowest binding free energies), together with hydrogen bond interactions within CDK2 are represented in Table 2. Regarding the hydrogen bond interactions, the more linear hydrogen bond is likely to be stronger, therefore the hydrogen bond angle more than 120° is considered to be of a reasonable strength. Also the formation of more and/or tighter hydrogen bonds provides higher binding affinities.<sup>22,23</sup>

The pyrimidone derivatives **7a,b** and **8a–c** were deeply embedded into CDK2 binding site displayed  $\Delta G_b$  between -8.31 and -10.81 kcal/mol. Compounds **7a,b** and **8c** bound to the active site of CDK2 through 2 hydrogen bonds with Leu 83, whereas compound **8b** showed 2 hydrogen bonds with Asp145 and Asn132. Compound **8a** showed one hydrogen bond with Asn132.

Docking study showed that the chloro derivatives **9a–c** displayed good  $\Delta G_b$  between -10.08 and -12.77 kcal mol<sup>-1</sup> but did not bind to the active site of CDK2 through hydrogen bonds.

The docking for compounds **11a–c** exhibited high binding affinities ( $\Delta G_b$  between -10.63 and -12.02 kcal mol<sup>-1</sup>) due to more proper fitting into the target site by three hydrogen bonds (with Leu 83 for **11a** and with Asp145 and Lys33 for **11b**) and two hydrogen bonds (with Asp145 and Lys33 for **11c**). Compounds **12a–c** exhibited almost similar binding affinities ( $\Delta G_b$  between -9.46 and -12.37 kcal mol<sup>-1</sup>) as compounds **11a–c**. Compound **12a** exhibited two hydrogen bonds with Asp145 and Lys33, while compounds **12b,c** showed one hydrogen bond Asp86.

The 4-substituted amino compounds **6**, **10a–e** had good docking scores between -10.39 and -14.33 kcal mol<sup>-1</sup> and bound to CDK2 active site through one hydrogen bond (compound **6**) or two hydrogen bonds (compound **10c**) with Lys33. On the other hand, compounds **10a,b,d,e** displayed one hydrogen bond with Asp86.

The comparative docking modes of the synthesised compounds showed that compounds 6, 7a,b and 8a-c formed



Fig. 4 2D and 3D structure of CDK2 complexed with 4-(3-hydroxyanilino)-6,7-dimethoxyquinazoline.

Table 2	The best docking	results based	on the bindi	ng free energ	$(\Delta G_b)$ o	f compounds	docked i	into binding	site o	of CDK2,	the
distances	s and angles of hyd	drogen bonds h	etween com	pounds and a	nino acio	ds involved in	CDK2				

Hydrogen	bonds between atom	$\Delta G_{b}^{a}$	Compound		
Angle (°)	ngle (°) Distance (A°) Nucleotides		Atoms of compound	(kcal mol <sup>-</sup> ')	
103.3	1.53	Asp145(C=O),	OH(H-donor)	-14.07	Ligand
169.5	2.29	Asp145(C=O),	OH(H-donor)		-
164.6	2.80	Leu83(NH),	Pyrimidine N-1		
153.8	2.83	Lys33(NH <sub>2</sub> )	OH(H-acceptor)		
163.0	2.94	Lys33(NH <sub>2</sub> )	Pyrimidine N-1	-10.73	6
160.9	1.68	Leu83(NH),	C=O	-10.34	7a
139.4	2.78	Leu83(C=O)	N-3H		
154.5	1.42	Leu83(NH),	C=O	-10.81	7b
131.3	2.21	Leu83(C=O)	N-3H		
156.1	2.78	Asn132(NH)	C=O	-8.31	8a
169.7	1.44	Asp145(C=O),	N-3H	-10.17	8b
146.3	2.80	Asn132(NH <sub>2</sub> )	C=O		
176.5	2.02	Leu83(NH),	C=O	-10.48	8c
146.4	2.76	Leu83(C=O)	N-3H		
-	-	-	-	-10.08	9a
-	-	-	-	-12.77	9b
-	-	-	-	-10.62	9c
167.2	2.16	Asp86(C=O)	NH	-11.03	10a
154.5	1.74	Asp86(C=O)	NH	-11.10	10b
141.0	2.60	Lys33(NH <sub>2</sub> ),	C=O	-14.33	10c
133.7	2.27	Lys33(C=O)	ОН		
132.7	2.04	Asp86(C=O)	NH	-11.65	10d
149.2	2.01	Asp86(C=O)	NH	-10.39	10e
109.2	2.36	Leu83(C=O),	NH	-10.63	11a
147.4	2.81	Leu83(NH),	NH <sub>2</sub> (H-acceptor)		
108.8	3.16	Leu83(C=O)	NH <sub>2</sub> (H-donor)		
153.4	1.39	Asp145(OH),	NH	-12.02	11b
162.5	1.77	Asp145(C=O),	NH₂(H-donor)		
159.5	2.66	Lys33(NH <sub>2</sub> )	NH <sub>2</sub> (H-acceptor)		
139.4	1.60	Asp145(C=0),	NH <sub>2</sub> (H-donor)	-10.93	11c
138.1	2.90	Lys33(NH <sub>2</sub> )	NH <sub>2</sub> (H-acceptor)		
164.3	1.38	Asp145(C=0),	OH(H-donor)	-9.46	12a
145.0	3.07	Lys33(NH <sub>2</sub> )	OH(H-donor)		
149.3	1.54	Asp86(C=0)	NH	-12.37	12b
164.0	1.53	Asp86(C=O)	NH	-10.97	12c

<sup>a</sup>Binding free energy.

hydrogen bond via pyrimidine N-1 or N-3 (similar to the ligand) in addition to other hydrogen bond via the electronegative C-4 oxo group (compounds 7a,b and 8a-c). Compounds 10a-e, 11a-c and 8a-c displayed hydrogen bond between different nucleotides and the electronegative C-4 substituent only; on the contrary, C-2 substituent exhibited no hydrogen bond with different nucleotides. These different binding modes intensify the role of the C-4 substituent to enhance the binding affinities into CDK2 binding site and consequently improve the anticancer activity of the thieno [2,3-d] pyrimidines. The overall correlation between the growth inhibitory activities (IC<sub>50</sub>,  $\mu g \text{ mL}^{-1}$ ) of the synthesised thieno[2,3-d]pyrimidines against colon carcinoma cell line, cited in Table 1 and the binding affinities predicted by docking study was excellent for most of the compounds. However, compound 10d showed low antitumour activity in spite of the high binding affinity. Figures 4–7 illustrate docking of ligand and compounds 7a, 11b and 12b respectively.

### Experimental

Melting points were obtained on a Griffin apparatus and are uncorrected. Microanalyses for C, H and N were carried out at the microanalytical center, Cairo University. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr discs. <sup>1</sup>H NMR spectra were performed on a joel NMR FXQ-200 MHz spectrometer, using TMS as the internal standard. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer. Progress of the reactions were monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and was visualised by UV lamp.

4-(2-Phenylethylamino)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno [2,3-d]pyrimidine (6): A mixture of the chloro derivative 5 (0.25 g, 0.001 mol) and 2-phenylethylamine (0.12 g, 0.001 mol) and triethylamine (0.36 mL, 0.003 mol) in absolute ethanol (12 mL) was heated under reflux for 15 h. The reaction mixture was then cooled; the separated solid was filtered, dried and crystallised from ethanol. M.p. 90–92 °C; yield 60%; IR (KBr)  $v_{max}$ : 3400 (NH), 1640 (C=N), 1560 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.05–1.20 (m, 2H, CH<sub>2</sub>), 1.35–1.46 (m, 4H, 2CH<sub>2</sub>), 1.50–1.60 (m, 2H, CH<sub>2</sub>), 2.70–2.75 (t, 2H, J = 7.5 Hz, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 2.82–2.89 (t, 2H, J = 5.7 Hz, CH<sub>2</sub>), 2.91–2.96 (t, 2H, J = 6.9 Hz, CH<sub>2</sub>), 3.74–3.80 (t, 2H, J = 7.5 Hz, CH<sub>2</sub>NH), 6.49 (s, 1H, NH, D<sub>2</sub>O exchangeable), 7.17–7.28 (m, 5H, ArH) and 8.31 (s, 1H, C<sub>2</sub>-H) ppm; MS [m/z, %]: 339 [M+2, 0.95], 338 [M+1, 1.74], 337 [M+, 4.58] and 57 [C<sub>4</sub>H<sub>9</sub>, 100]. Anal. Calcd for C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>S (337.46): C, 71.17; H, 6.86; N, 12.45. Found: C, 70.99; H, 6.50; N, 12.43%.

# Synthesis of 2-aryl-1,2,3,5,6,7,8,9,10-octahydrocycloocta[4,5]thieno [2,3-d]pyrimidin-4(1H)-ones (**7a,b**); general procedure

The appropriate aldehyde (0.007 mol) was added to a solution of aminoamide **3** (1.12 g, 0.005 mol) in absolute ethanol (5 mL) containing one drop of concentrated hydrochloric acid. The mixture was then refluxed for 36 h and set aside to cool at room temperature. The separated solid was filtered, washed with ethanol, dried and crystallised from ethanol.

2-(4-Dimethylaminophenyl)-1,2,3,5,6,7,8,9,10-octahydrocycloocta [4,5]-thieno[2,3-d]pyrimidin-4(1H)-one (**7a**): M.p. >300 °C; yield 76%; IR (KBr)  $v_{max}$ : 3390, 3182 (2NH), 1635 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.25–1.35 (m, 2H, CH<sub>2</sub>), 1.40–1.55 (m, 2H, CH<sub>2</sub>), 1.60– 1.80 (m, 4H, 2CH<sub>2</sub>), 2.75–2.85 (m, 2H, CH<sub>2</sub>), 3.05–3.15 (m, 2H, CH<sub>2</sub>), 3.09 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 5.70 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.72 (d, 2H, *J* = 8.0 Hz, ArH), 7.66 (d, 2H, *J* = 8.1 Hz, ArH), 8.29 (s, 1H, C2-H) and 9.08 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm; MS [*m*/z, %]: 358 [M+3, 0.97], 357 [M+2, 2.08], 355 [M<sup>+</sup>, 0.43] and 79 [C<sub>6</sub>H<sub>7</sub>, 100]. Anal. Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>OS (355.48): C, 67.56; H, 7.08; N, 11.82. Found: C, 67.94; H, 6.99; N, 11.44%.

2-(2-Nitrophenyl)-1,2,3,5,6,7,8,9,10-octahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(1H)-one (**7b**): M.p. 168–170 °C; yield 63%; IR (KBr) ν<sub>max</sub>: 3383, 3188 (2NH), 1641 (C=O), 1517, 1336 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.24–1.40 (m, 2H, CH<sub>2</sub>), 1.45–1.55 (m, 2H,



Fig. 5 The proposed binding mode of compound 7a in the binding site of CDK2, created with Molsoft ICM-Pro software.



Fig. 6 The proposed binding mode of compound 11b in the binding site of CDK2, created with Molsoft ICM-Pro software.

CH<sub>2</sub>), 1.60–1.74 (m, 4H, 2CH<sub>2</sub>), 2.81–2.86 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 3.02–3.08 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 5.72 (s, 1H, NH, D<sub>2</sub>O exchangeable), 7.59–7.77 (m, 3H, ArH), 8.09 (d, 1H, J = 7.2 Hz, ArH); 8.16 (s, 1H, NH, D<sub>2</sub>O exchangeable) and 8.93 (s, 1H, C2-H) ppm; MS [*m*/*z*, %]: 359 [M+2, 1.55], 358 [M+1, 4.53], 357 [M<sup>+</sup>, 13.54] and 222 [C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>OS, 100]. Anal. Calcd for C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>S (357.41): C, 60.48; H, 5.35; N, 11.75. Found: C, 60.80; H, 5.28; N, 11.55%.

#### Synthesis of 2-aryl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3d]pyrimidin-4(3H)-ones (8a-c); general procedure

*Method A*: A mixture of aminoamide **3** (2.25 g, 0.01 mol) and the appropriate aromatic aldehyde (0.03 mol) in dry dimethylformamide (25 mL) containing concentrated hydrochloric acid (0.2 mL) was refluxed for 24 h. The mixture was cooled, filtered and the precipitate was crystallised from the appropriate solvent.

Method B: A mixture of either **7a** or 7b (0.001 mol) and nitrobenzene (5 mL) was refluxed for 5 h. The reaction mixture was subjected to steam distillation to remove nitrobenzene and the solid obtained was collected, washed with petroleum ether, dried and crystallised from the appropriate solvent.

2-(4-Dimethylaminophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**8a**): M.p. >300 °C (*n*-butanol); yield (method A) 51%, (method B) 70%; IR (KBr)  $v_{max}$ : 3160 (NH), 1651 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 1.25–1.35 (m, 2H, CH<sub>2</sub>), 1.36–1.45 (m, 2H, CH<sub>2</sub>), 1.55–1.70 (m, 4H, 2CH<sub>2</sub>), 2.80–2.90 (m, 2H, CH<sub>2</sub>), 2.95–3.10 (m, 2H, CH<sub>2</sub>), 3.00 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 6.74 (d, 2H, J = 7.2 Hz, ArH), 8.00 (d, 2H, J = 7.2 Hz, ArH) and 12.10 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm. Anal. Calcd for C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>OS (353.46): C, 67.95; H, 6.55; N, 11.88. Found: C, 68.19; H, 6.37; N, 11.68%.

2-(2-Nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**8b**): M.p. 170–172 °C (ethanol); yield (method A) 70%, (method B) 70%; IR (KBr)  $v_{max}$ : 3120 (NH), 1651(C=O), 1533, 1346 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.25–1.35 (m, 2H, CH<sub>2</sub>), 1.37–1.45 (m, 2H, CH<sub>2</sub>), 1.60–1.70 (m, 4H, 2CH<sub>2</sub>), 2.86–2.90 (t, 2H, *J* = 6.0 Hz, CH<sub>2</sub>), 3.07–3.10 (t, 2H, *J* = 6.0 Hz, CH<sub>2</sub>), 7.80– 7.89 (m, 3H, ArH), 8.18 (d, 1H, *J* = 8.1 Hz, ArH) and 12.84 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm; MS [*m*/*z*, %]: 356 [M+1, 11.24], 355 [M<sup>+</sup>, 21.89] and 55 [C<sub>4</sub>H<sub>7</sub>, 100]. Anal. Calcd for C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>S (355.40): C, 60.82; H, 4.82; N, 11.82. Found: C, 61.00; H, 5.00; N, 11.59%.

2-(2-Thienyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (8c): M.p. >300 °C (*n*-butanol); yield (method A) 45%; IR (KBr)  $v_{max}$ : 3167 (NH), 1651 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.25–1.35 (m, 2H, CH<sub>2</sub>), 1.38–1.50 (m, 2H, CH<sub>2</sub>), 1.55– 1.70 (m, 4H, 2CH<sub>2</sub>), 2.80–2.90 (m, 2H, CH<sub>2</sub>), 3.00–3.10 (m, 2H, CH<sub>2</sub>), 7.19, 7.24 (dd, 1H, J = 5.2, 3.6 Hz, thiophene H), 7.84 (d, 1 H, J = 5.2 Hz, thiophene H), 8.19 (d, 1H, J = 3.6 Hz, thiophene H) and 12.64 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm. Anal. Calcd for C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>OS<sub>2</sub> (316.43): C, 60.72; H, 5.09; N, 8.85. Found: C, 61.05; H, 5.06; N, 8.98%.



Fig. 7 The proposed binding mode of compound 12b in the binding site of CDK2, created with Molsoft ICM-Pro software.

*Synthesis of 2-aryl-4-chloro-5,6,7,8,9,10-hexahydrocycloocta*[4,5] *thieno*[2,3-d]*pyrimidines* (**9a–c**); *general procedure* 

Phosphorus oxychloride (15 mL) was added to the corresponding thienopyrimidone **8a–c** (0.003 mol) and the mixture was refluxed for 1 h. The reaction mixture was concentrated under reduced pressure then poured into ice cold water (100 mL). The precipitated product was filtered, washed with water (2×10 mL), dried and crystallised from ethanol.

4-Chloro-2-(4-dimethylaminophenyl)-5,6,7,8,9,10-hexahydrocycloocta-[4,5]thieno[2,3-d]pyrimidine (**9a**): M.p. 190–192 °C; yield 60%; IR (KBr)  $v_{max}$ : 1606 (C=N), 1556 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.25–1.40 (m, 2H, CH<sub>2</sub>), 1.45–1.55 (m, 2H, CH<sub>2</sub>), 1.70–1.90 (m, 4H, 2CH<sub>2</sub>), 2.92–2.98 (t, 2H, J = 5.6 Hz, CH<sub>2</sub>), 3.06 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 3.14–3.20 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 6.83 (d, 2H, J = 9.0 Hz, ArH) and 8.37 (d, 2H, J = 9.0 Hz, ArH) ppm. Anal. Calcd for C<sub>20</sub>H<sub>22</sub>ClN<sub>3</sub>S (371.91): C, 64.58; H, 5.96; N, 11.29. Found: C, 64.45; H, 5.68; N, 11.00%.

4-Chloro-2-(2-nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5] thieno-[2,3-d]pyrimidine (**9b**): M.p. 138–140 °C; yield 62%; IR (KBr)  $v_{\text{max}}$ : 1530, 1358 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.25–1.33 (m, 2H, CH<sub>2</sub>), 1.45–1.53 (m, 2H, CH<sub>2</sub>), 1.66–1.80 (m, 4H, 2CH<sub>2</sub>), 3.03–3.06 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 3.14–3.18 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 7.74, 7.79 (dd, 1H, J = 7.8 Hz, ArH), 7.80, 7.82 (dd, 1H, J = 7.8 Hz, ArH), 8.00 (d, 1H, J = 7.8 Hz, ArH) and 8.10 (d, 1H, J = 7.8 Hz, ArH) ppm; MS [m/z, %]: 376 [M+3, 11.20], 375 [M+2, 43.00], 374 [M+1, 36.70], 373 [M<sup>+</sup>, 95.50] and 134 [C<sub>7</sub>H<sub>4</sub>NO<sub>2</sub>, 100]. Anal. Calcd for C<sub>18</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>2</sub>S (373.84): C, 57.82; H, 4.31; N, 11.23. Found: C, 57.61; H, 4.39; N, 10.88%.

4-*Chloro-2-(2-thienyl)-5,6,7,8,9,10-hexahydrocycloocta*[4,5]*thieno-[2,3-d]pyrimidine* (**9c**): M.p. 148–150 °C; yield 30%; IR (KBr)  $v_{max}$ : 1556 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.20–1.35 (m, 4H, 2CH<sub>2</sub>), 1.50–1.60 (m, 2H, CH<sub>2</sub>), 1.70–1.85 (m, 2H, CH<sub>2</sub>), 2.93–2.96 (t, 2H, J = 6.2 Hz, CH<sub>2</sub>), 3.14–3.20 (t, 2H, J = 6.2 Hz, CH<sub>2</sub>), 7.12, 7.16 (dd, 1H, J = 5.2, 3.6 Hz, thiophene H), 7.48 (d, 1H, J = 5.2 Hz, thiophene H) and 8.03 (d, 1H, J = 3.6 Hz, thiophene H) ppm. Anal. Calcd for C<sub>16</sub>H<sub>15</sub>ClN<sub>2</sub>S<sub>2</sub> (334.87): C, 57.38; H, 4.51; N, 8.36. Found: C, 57.60; H, 4.51; N, 8.20%.

Synthesis of 2-aryl-4-substituted amino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d] pyrimidines (**10a–e**); general procedure A mixture of **9a–c** (0.001 mol), the selected primary amine (0.001 mol) and triethylamine (0.36 mL, 0.003 mol) in absolute ethanol (12 mL) was heated under reflux for 15 h. The separated solid after cooling was filtered, dried and crystallised from ethanol.

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2-(4-Dimethylaminophenyl)-4-(2-phenylethylamino)-5,6,7,8,9,10hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**10a**): M.p. 160– 162 °C; yield 70%; IR (KBr)  $v_{max}$ : 3456 (NH), 1607(C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.10–1.20 (m, 2H, CH<sub>2</sub>), 1.40–1.70 (m, 6H, 3CH<sub>2</sub>), 2.80–2.90 (m, 2H, CH<sub>2</sub>), 2.95–3.10 (m, 4H, CH<sub>2</sub> and CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 3.00 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 3.60–3.80 (m, 2H, NHCH<sub>2</sub>), 6.51 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.82 (d, 2H, J = 8.2 Hz, ArH), 7.15–7.40 (m, 5H, ArH) and 8.23 (d, 2H, J = 8.2 Hz, ArH) ppm. Anal. Calcd for C<sub>28</sub>H<sub>32</sub>N<sub>4</sub>S (456.63): C, 73.64; H, 7.06; N, 12.27. Found: C, 73.64; H, 7.00; N, 12.21%.

4-(*Ethylamino*)-2-(2-*nitrophenyl*)-5,6,7,8,9,10-*hexahydrocycloocta-[4,5]thieno[2,3-d]pyrimidine* (**10b**): M.p. 110–112 °C; yield 97%; IR (KBr)  $v_{max}$ : 3385 (NH), 1531,1348 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.13–1.17 (t, 3H, J = 6.9 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.20–1.25 (m, 2H, CH<sub>2</sub>), 1.45–1.50 (m, 2H, CH<sub>2</sub>), 1.55–1.70 (m, 4H, 2CH<sub>2</sub>), 2.87–2.90 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 3.01–3.05 (t, 2H, J = 6.1 Hz, CH<sub>2</sub>), 3.47–3.51(m, 2H, J = 6.9 Hz, CH<sub>2</sub>CH<sub>3</sub>), 6.78 (t, 1H, J = 6.0 Hz, NH, D<sub>2</sub>O exchangeable), 7.64, 7.70 (dd, 1H, J = 7.8 Hz, ArH), 7.73, 7.76 (dd, 1H, J = 7.8 Hz, ArH) pm; MS [*m*/*z*, %]: 384 [M+2, 21.79], 383 [M+1, 82.60], 382 [M<sup>+</sup>, 100] and 365 [M-OH, 35.25]. Anal. Calcd for C<sub>20</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>S (382.47): C, 62.80; H, 5.79; N, 14.64. Found: C, 62.90; H, 5.80; N, 14.52%.

3-[(2-Nitrophenyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-pyrimidin)-4-yl]aminopropionic acid (**10c**): M.p. 140–142 °C; yield 50%; IR (KBr)  $v_{max}$ : 3200–2501 (NH and OH), 1650 (C=O), 1531, 1361 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.25–1.33 (m, 2H, CH<sub>2</sub>), 1.45–1.55 (m, 2H, CH<sub>2</sub>), 1.65–1.80 (m, 4H, 2CH<sub>2</sub>), 2.22 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>COOH), 2.86 (t, 2H, J = 6.3 Hz, NHCH<sub>2</sub>), 3.04–3.08 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 3.16–3.20 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 3.45 (br s, 2H, OH and NH, D<sub>2</sub>O exchangeable), 7.75, 7.79 (dd, 1H, J = 7.8 Hz, ArH), 7.83, 7.88 (dd, 1H, J = 7.8 Hz, ArH) 8.00 (d, 1H, J = 7.8 Hz, ArH) and 8.11 (d, 1H, J = 7.8 Hz, ArH) ppm; MS [m/z, %]: 426 [M<sup>+</sup>, 5.17] and 89 [C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>,100]. Anal. Calcd for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>S (426.48): C, 59.13; H, 5.20; N, 13.13. Found: C, 59.10; H, 5.17; N, 12.85%.

2-(2-Nitrophenyl)-4-(2-phenylethylamino)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**10d**): M.p. 92–94 °C; yield 60%; IR (KBr)  $v_{max}$ : 3457 (NH), 1533, 1367 (NO<sub>2</sub>), 1566 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.20–1.40 (m, 4H, 2CH<sub>2</sub>), 1.42–1.75 (m, 4H, 2CH<sub>2</sub>), 2.60–2.66 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 2.82–2.88 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 2.96–3.02 (t, 2H, J = 6.4 Hz, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 3.80–3.89 (q, 2H, J = 6.4 Hz, NHCH<sub>2</sub>), 5.28(t, 1H, J = 6.2 Hz, NH, D<sub>2</sub>O exchangeable), 7.23–7.32 (m, 5H, ArH), 7.50–7.67 (m, 3H, ArH) and 8.19 (d, 1H, J = 7.6 Hz, ArH) ppm. Anal. Calcd for C<sub>26</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>S (458.56): C, 68.09; H, 5.71; N, 12.21. Found: C, 67.99; H, 5.45; N, 12.50%.

4-(2-Phenylethylamino)-2-(2-thienyl)-5,6,7,8,9,10-hexahydrocycloocta-[4,5]thieno[2,3-d]pyrimidine (**10e**): M.p. 194–196 °C; yield 80%; IR (KBr)  $v_{max}$ : 3464 (NH), 1568(C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.15–1.25 (m, 2H, CH<sub>2</sub>), 1.45–1.60 (m, 4H, 2CH<sub>2</sub>), 1.65–1.70 (m, 2H, CH<sub>2</sub>), 2.70–2.85 (m, 2H, CH<sub>2</sub>), 2.86–2.95 (m, 2H, CH<sub>2</sub>), 2.96– 3.10 (m, 2H, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.15–4.50 (m, 2H, NHCH<sub>2</sub>), 6.71 (s, 1H, NH, D<sub>2</sub>O exchangeable), 7.20–7.40 (m, 6H, ArH), 7.67 (d, 1H, ArH) and 7.90 (d, 1H, ArH) ppm. Anal. Calcd for C<sub>24</sub>H<sub>25</sub>N<sub>3</sub>S<sub>2</sub> (419.58): C, 68.69; H, 6.00; N, 10.01. Found: C, 68.66; H, 5.71; N, 9.71%.

## Synthesis of 2-aryl-4-hydrazinyl-5,6,7,8,9,10-hexahydrocycloocta[4, 5]thieno[2,3-d]pyrimidines (**11a–c**); general procedure

A mixture of chloro derivative 9a-c (0.002 mol) and hydrazine hydrate (99%, 0.62 g, 0.012 mol) in absolute ethanol (20 mL) was refluxed for 6 h. The reaction mixture was then cooled and the precipitate was filtered, dried and crystallised from *n*-butanol.

4-Hydrazinyl-2-(4-dimethylaminophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**11a**): M.p. 218–220 °C; yield 50%; IR (KBr)  $v_{max}$ : 3327, 3300 (NH / NH<sub>2</sub>), 1608 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.16–1.20 (m, 2H, CH<sub>2</sub>), 1.40–1.50 (m, 2H, CH<sub>2</sub>), 1.55–1.66 (m, 4H, 2CH<sub>2</sub>), 2.85–2.90 (m, 2H, CH<sub>2</sub>), 2.95–3.05 (m, 2H, CH<sub>2</sub>), 2.99 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 4.73 (s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 6.76 (d, 2H, J = 8.8 Hz, ArH), 7.86 (s, 1H, NH, D<sub>2</sub>O exchangeable) and 8.28 (d, 2H, J = 8.8 Hz, ArH) ppm; MS [m/z, %]: 370 [M+3, 2.19], 369 [M+2, 7.41], 368 [M+1, 23.73], 367 [M<sup>+</sup>, 100], 352 [M-NH, 22.89], 351[M-NH<sub>2</sub>, 56.36], 323[M-CH<sub>3</sub>N=CH<sub>2</sub>-H, 14.36], and 77 [C<sub>6</sub>H<sub>5</sub>, 4.14]. Anal. Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>5</sub>S (367.50): C, 65.35; H, 6.85; N, 19.05. Found: C, 65.55; H, 6.55; N, 18.86%. 4-Hydrazinyl-2-(2-nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (11b): M.p. 236–238 °C; yield 60%; IR (KBr)  $v_{max}$ : 3304, 3280 (NH / NH<sub>2</sub>), 1627 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.20–1.30 (m, 2H, CH<sub>2</sub>), 1.45–1.55 (m, 2H, CH<sub>2</sub>), 1.60– 1.75 (m, 4H, 2CH<sub>2</sub>), 2.90–2.95 (t, 2H, CH<sub>2</sub>), 3.00–3.10 (t, 2H, CH<sub>2</sub>), 4.63 (s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.69–7.82 (m, 3H, 2ArH and NH), 7.89 (d, 1H, *J* = 7.2 Hz, ArH) and 8.25 (d, 1H, *J* = 7.2 Hz, ArH) ppm. Anal. Calcd for C<sub>18</sub>H<sub>19</sub>N<sub>5</sub>O<sub>2</sub>S (369.43): C, 58.51; H, 5.18; N, 18.95. Found: C, 58.79; H, 4.99; N, 18.79%.

4-Hydrazinyl-2-(2-thienyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno-[2,3-d]pyrimidine (**11c**): M.p. 218–220 °C; yield 50%; IR (KBr)  $v_{max}$ : 3363, 3292 (NH / NH<sub>2</sub>), 1630 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.10–1.25 (m, 2H, CH<sub>2</sub>), 1.40–1.50 (m, 2H, CH<sub>2</sub>), 1.55–1.70 (m, 4H, 2CH<sub>2</sub>), 2.85–2.90 (m, 2H, CH<sub>2</sub>), 2.95–3.10 (m, 2H, CH<sub>2</sub>), 4.78 (s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.15 (dd, 1H, thiophene H), 7.65 (d, 1H, thiophene H), 7.96 (d, 1H, thiophene H) and 8.09 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm. Anal. Calcd for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>S<sub>2</sub> (330.45): C, 58.14; H, 5.48; N, 16.95. Found: C, 58.34; H, 5.30; N, 16.90%.

# Synthesis of 2-aryl-4-arylidenhydrazinyl-5,6,7,8,9,10-hexahydrocyclo octa[4,5]thieno[2,3-d]pyrimidines (**12a–c**); general procedure

A mixture of hydrazino derivative 11a-c (0.003 mol), the selected aromatic aldehyde (0.003 mol) and few drops of glacial acetic acid in absolute ethanol (17 mL) was heated under reflux for 6 h. The reaction mixture was cooled, the separated solid was filtered, dried and crystallised from the appropriate solvent.

4-(2-Hydroxybenzylidenhydrazinyl)-2-(4-dimethylaminophenyl)-5,6,7,8, 9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**12a**): M.p. 234–236 °C (*n*-butanol); yield 90%; IR (KBr)  $v_{max}$ : 3420 (OH), 3340 (NH), 1608 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.20–1.30 (m, 2H, CH<sub>2</sub>), 1.50–1.60 (m, 2H, CH<sub>2</sub>), 1.65–1.85 (m, 4H, 2CH<sub>2</sub>), 2.90–3.10 (m, 2H, CH<sub>2</sub>), 3.05 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 3.20–3.30 (m, 2H, CH<sub>2</sub>), 6.83 (d, 2H, J = 7.4 Hz, ArH), 6.99–7.09 (m, 2H, J = 8.6 Hz, ArH), 7.35 (d, 1H, ArH), 7.50 (d, 1H, ArH), 8.36 (d, 2H, J = 7.4 Hz, ArH), 8.76 (s, 1H, N=CH), 10.37 (s, 1H, NH, D<sub>2</sub>O exchangeable) and 12.41 (s, 1H, OH, D<sub>2</sub>O exchangeable) ppm. Anal. Calcd for C<sub>27</sub>H<sub>29</sub>N<sub>5</sub>OS (471.57): C, 68.76; H, 6.19; N, 14.85. Found: C, 68.92; H, 5.94; N, 14.67%.

2-(2-Nitrophenyl)-4-(2-thienylidenhydrazinyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**12b**): M.p. 128–130 °C (ethanol); yield 50%; IR (KBr)  $v_{max}$ : 3380 (NH), 1618 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  1.10–1.20 (m, 2H, CH<sub>2</sub>), 1.35–1.50 (m, 2H, CH<sub>2</sub>), 1.55– 1.70 (m, 4H, 2CH<sub>2</sub>), 2.87–2.91 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 3.01–3.05 (t, 2H, J = 6.0 Hz, CH<sub>2</sub>), 7.12, 7.15 (dd, 1H, J = 5.0, 3.6 Hz, thiophene H), 7.44 (d, 1H, ArH), 7.63–7.91 (m, 3H, ArH), 8.10 (d, 1H, J =7.8 Hz, ArH), 8.23 (d, 1H, J = 7.5 Hz, ArH), 8.66 (s, 1H, N=CH) and 10.25 (s, 1H, NH, D<sub>2</sub>O exchangeable) ppm. Anal. Calcd for C<sub>23</sub>H<sub>21</sub>N<sub>5</sub>O<sub>2</sub>S<sub>2</sub> (463.57): C, 59.58; H, 4.56; N, 15.10. Found: C, 59.89; H, 4.68; N, 15.06%.

2-(2-Thienyl)-4-(2-thienylidenhydrazinyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**12c**): M.p. 128–130 °C (ethanol); yield 90%; IR (KBr)  $\nu_{max}$ : 3342 (NH), 1608 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.10–1.25 (m, 2H, CH<sub>2</sub>), 1.30–1.45 (m, 2H, CH<sub>2</sub>), 1.50– 1.75 (m, 4H, 2CH<sub>2</sub>), 2.70–2.80 (m, 2H, CH<sub>2</sub>), 3.05–3.15 (m, 2H, CH<sub>2</sub>), 6.90–7.10 (m, 2H, thiophene H), 7.26–7.31(m, 2H, thiophene H), 7.99–8.04 (m, 2H, thiophene H) and 8.62 (s, 1H, N=CH) ppm. Anal. Calcd for C<sub>21</sub>H<sub>20</sub>N<sub>4</sub>S<sub>3</sub> (424.59): C, 59.40; H, 4.74; N, 13.19. Found: C, 59.18; H, 4.70; N, 12.94%.

*Cytotoxic activity studies* Anticancer activity studies were done at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

Compounds **6–12c** were tested at concentrations between 1 and 10  $\mu$ g mL<sup>-1</sup> using SulfoRhodamine-B (SRB) assay for cytotoxic activity against human colon tumour cell line (HCT116). Imatinib which is 2-substituted aminopyrimidine derivative was chosen as a reference standard anticancer drug because it showed potency against gasterointestinal tract tumours.<sup>19,20</sup>

*Measurement of potential cytotoxicity by SRB assay* Potential cytotoxicity of the compounds was tested using the method of Skehan *et al*<sup>24</sup> as follows. Cells were plated in 96 multiwell plate (104 cells/ well) for 24 h before treatment with the compound(s) to allow attachment to the wall of the plate. Different concentrations of the compounds (0, 1, 2.5, 5 and 10 µg mL<sup>-1</sup>) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C in atmosphere of 5% CO<sub>2</sub>. After 48 h, cells were fixed, washed and stained with SulfoRhodamine-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. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumour cell line after the specified compound.

*Docking steps* All the docking studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) as the computational software. All the minimisations were performed with MOE until a RMSD gradient of 0.05 kcal mol<sup>-1</sup> A°<sup>-1</sup> with MMFF94x force-field and the partial charges were automatically calculated.

In order to perform docking, some preliminary steps were done. <sup>25</sup>Enzyme structures were checked for missing atoms, bonds and contacts. Water of crystallisation was manually deleted. Hydrogens and partial charges were added to the system using Protonate 3D application. The active site was generated using the residues close to the 4-anilino- quinazoline atoms. The interactions of the ligand with the amino acids of the active site were studied (Fig. 7).

*Docking of compounds* The compounds were constructed using the builder module and were energy minimised using the MMFF94x forcefield. Hydrogens and partial charges were added to the system using Protonate 3D application. All antagonist structures were docked into the active site by using the MOE Dock tool.

*Conformational analysis of compounds* The algorithm generated conformations from a single 3D conformation by conducting a systematic search. In this way, all combinations of angles were created for each compound. A collection of poses was generated from the pool of ligand conformations using Triangle Matcher placement method. Poses were generated by superposition of ligand atom triplets and triplets of points in the receptor binding site in a systematic way. Poses generated by the placement methodology were scored using an available method implemented in MOE, the London dG scoring function which estimates the free energy of binding of the ligand from a given pose. The top 30 poses for each ligand were output in a MOE database. Each resulting ligand pose was then subjected to MMFF94x energy minimisation. The minimised docking conformations were then rescored using London dG scoring method.<sup>25</sup>

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