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Research Article

SYNTHESIS OF EFFECTIVE ANTICANCER THIENO [2,3-*d*] PYRIMIDINE-4-ONES AND THIENO [3,2-e]TRIAZOLO[4,3-c]PYRIMIDINES

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ABSTRACT

In continuation to our research program concerned with structural modification of thieno[2,3-*d*]pyrimidines with the purpose of enhancing their anticancer activity, various series of hexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidin-4-ones, hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine-3(2H)-thiones and 4-substituted hydrazinylhexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidines were synthesized. Most of the synthesized compounds exhibited antitumor activity against human colon carcinoma (HCT 116) cell line *in vitro*. Compounds **4a, 4b**, **3, 12b** and **5c** (IC_{50}: 11.90, 12.43, 15.91, 25.80 and 32.11 µM, respectively) exhibited 2.89 to 1.07 fold more potent antitumor activity than imatinib (IC_{50}: 34.40 µM). Also, a docking study of the newly synthesized compounds with the active site of CDK2 was described to explore their affinity and binding mode to CDK2.

Keywords: Hexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidin-4-ones, Hexahydrocycloocta[4,5]thieno[3,2-e]triazolo[4,3-c]pyrimidine-3(2H)-thiones, 4- Substituted hydrazinylhexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidines, Antitumor activity.

INTRODUCTION

Design and synthesis of thieno[2,3-*d*]pyrimidines as potential cancer chemotherapeutic agents have been extensively studied[1-11]. The thieno[2,3-*d*] pyrimidines were reported to exhibit antitumor activity via inhibition of receptor tyrosine kinases (RTKs) [3-5], cyclin dependent kinases (CDKs)[6-9] or check point kinases[10,11]. Since the development of gefitinib (Iressa®)[12], and erlotinib (Tarceva®) [13] (Figure 1), that had been approved for the treatment of gastrointestinal stromal tumors and lung cancer, several works had been reported and reviewed targeting structural modification of gefitinib with its bioisostere thieno[2,3-*d*]pyrimidine and meantime focusing on the C-4 substitution. For example, 4-oxo[6,7], 4-substituted amino [3,5] and 4-hydrazinyl [8,9,14] thieno[2,3-*d*]pyrimidines, with different groups at C-2 demonstrated potent antitumor activity.

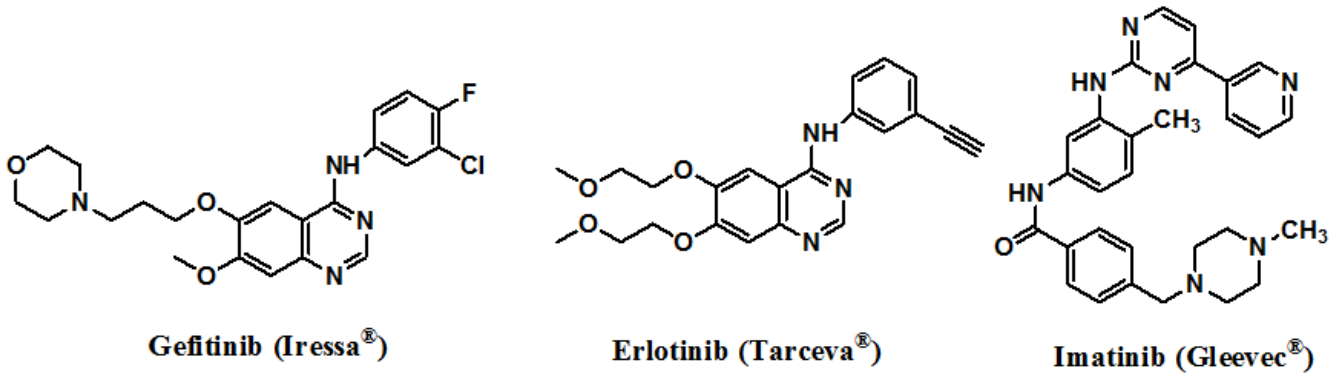


Fig. 1: Structures of clinically potent anticancer pyrimidines

Subsequent research aimed at further exploration of the SAR of this novel template has led to discovery of highly selective compounds with different groups at C-2 that proved useful antitumor activity. Among them, aromatic or aliphatic substitution at pyrimidine C-2[6,7], 2-amino[10,15], 2-thione [2] and 2,4-diones [16,17] derivatives were prepared. In 2010, triazolothienopyrimidines [18,19] were reported to exhibit very potent anticancer activity against different human tumor cell lines including human colon carcinoma (HCT 116) cell line.

Recently in a previous work [20] we synthesized various hexahydrocycloocta [4,5]thieno[2,3-*d*]pyrimidines and hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4-one derivatives. All the synthesized compounds exhibited antitumor activity against human colon carcinoma (HCT 116) cell line *in vitro*. Of particular interest, 2-(4-dimethylaminophenyl)-5,6,7,8,9,10-octahydrocycloocta[4,5]-thieno[2,3-d]pyrimidin-4(3H)-one **(Ia),** 4-hydrazinyl-2-(2-nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]-thieno[2,3-d]pyrimidine **(8b)** and its 4- thienylidenhydrazinyl derivative (Figure 2) were the most potent (IC_{50}: 13.08, 10.52 and 14.30 µM, respectively) exhibiting 4.3 to 1.3 fold more potent antitumor activity than imatinib (IC_{50}: 34.40 µM).

In the same direction, and in continuing effort to find more potent selective lead compound, herein, we describe the design and synthesis of 2-substituted thieno[2,3-d]pyrimidin-4-ones, thienotriazolopyrimidines and 4-substituted hydrazinylhexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidines as possible antitumor agents. Our strategy is thus directed toward designing a variety of ligands with diverse chemical properties hypothesizing that the potency of these molecules might be enhanced by adding alternative binding group such as oxo, thioxo, substituted amino or arylaminomethyl at C-2 position, keeping 4-oxo group unchanged (Scheme 1). Moreover, the previously prepared potent anticancer compounds **8a-c** were used as starting building blocks for the preparation of thienotriazolopyrimidines and other N-substituted hydrazinyl derivatives (Scheme 2). The newly synthesized compounds were tested *in vitro* on human colon carcinoma (HCT 116) cell line. Furthermore, a docking study of the newly synthesized compounds with the active site of CDK2 was described.

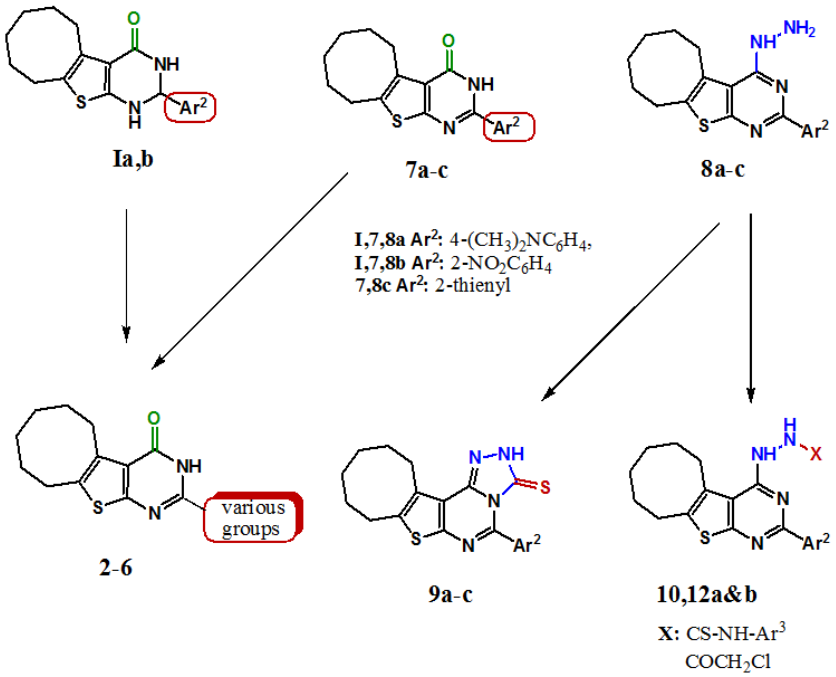
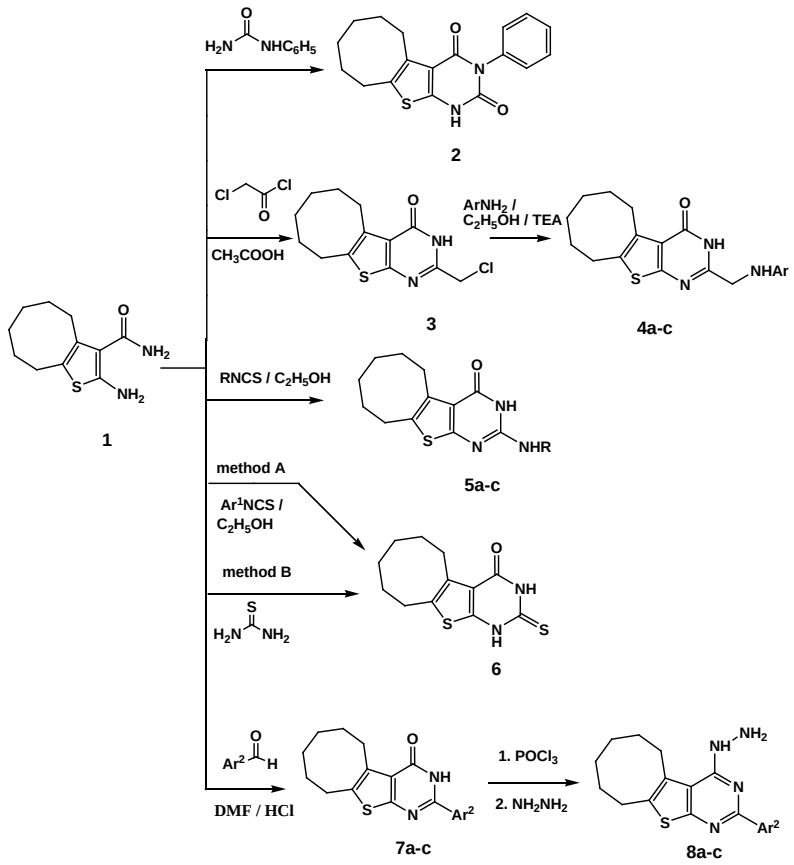


Fig. 2: Strategies for structural modification of leads Ia,b, 7a-c and 8a-c



4a Ar=C_6H_5, **4b** Ar= 4-ClC_6H_4, **4c** Ar=2-$CH_3C_6H_4$
5a R=CH_3, **5b** R=CH_2CH_3, **5c** R=$CH_2C_6H_5$
Ar^1=C_6H_5, 4-ClC_6H_4, 3-$CH_3C_6H_4$
7,8a Ar^2= 4-$(CH_3)_2NC_6H_4$, **7,8b** Ar^2= 2-$NO_2C_6H_4$, **7,8c** Ar^2= 2-thienyl

Scheme 1 Synthesis of compounds **2-8**

Scheme 1: Synthesis of compounds 2-8

MATERIALS AND METHODS

Chemistry

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. ^{1}HNMR 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 visualized by UV lamp.

3-Phenyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine-2,4 (1H,3H)-dione (**2**)

o-Amino amide **1** (0.5 g, 0.002 mol) was added to monophenylurea (0.6 g, 0.004 mol) and the mixture was heated in an oil bath at 200^{o}C for about 2 h. The solid mixture was melted then resolidfied and boiled with water (20 ml), filtered while hot, dried and crystallized from ethanol. M.p. >300 ^{o}C; yield 50 %; IR (KBr) ν_{max}: 3180 (NH) and 1713, 1690 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6) δ: 1.20-1.30 (m, 2H, CH_2), 1.33-1.45 (m, 2H, CH_2), 1.50-1.60 (m, 4H, $2CH_2$), 2.70-2.75 (m, 2H, CH_2), 2.86-2.93 (m, 2H, CH_2), 7.24-7.46 (m, 5H, ArH) and 10.92 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 327 [M+H$^{\cdot+}$, 20], 300 [M-$C_2H_2$$^{\cdot+}$, 18.46], 250 [M-$C_6H_4$$^{\cdot+}$, 56.92] and 222 [M-$C_6H_4$-CO$^{\cdot+}$, 100]. Anal. Calcd for $C_{18}H_{18}N_2O_2S$ (326.40): C, 66.23; H, 5.55; N, 8.58. Found: C, 65.90; H, 5.00; N, 8.89.

2-Chloromethyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**3**)

A mixture of o-amino amide **1** (1 g, 0.004 mol) and chloroacetyl chloride (0.5 g, 0.004 mol) in glacial acetic acid (20 ml) was heated under reflux for 10 h. The reaction mixture was cooled; the formed solid was filtered, dried and crystallized from ethanol. M.p. 258-260 ^{o}C; yield 60 %; IR (KBr) ν_{max}: 3120 (NH) and 1666 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.20-1.30 (m, 2H, CH_2), 1.35-1.46 (m, 2H, CH_2), 1.55-1.70 (m, 4H, $2CH_2$), 2.80-2.90 (m, 2H, CH_2), 2.95-3.10 (m, 2H, CH_2), 4.53 (s, 2H, CH_2Cl) and 12.59 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{13}H_{15}ClN_2OS$ (282.78): C, 55.21; H, 5.34; N, 9.90. Found: C, 55.31; H, 5.20; N, 9.75.

General procedure for the preparation of 2-arylaminomethyl-5,6,7,8,9,10 hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-ones **4a-c**

A mixture of the 2-chloromethyl derivative **3** (0.28 g, 0.001 mol), the appropriate aromatic amine (0.001 mol) and triethylamine (0.53 ml) in absolute ethanol (18 ml) was heated under reflux for 15 h. The reaction mixture was then cooled, the separated solid was filtered, dried and crystallized from suitable solvent.

2-Phenylaminomethyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**4a**)

M.p. 218-220 ^{o}C (ethanol); yield 40 %; IR (KBr) ν_{max}: 3399, 3160 (2 NH), 1655 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.20-1.30 (m, 2H, CH_2), 1.30-1.40 (m, 2H, CH_2), 1.50-1.63 (m, 4H, $2CH_2$), 2.75-2.85 (m, 2H, CH_2), 2.95-3.00 (m, 2H, CH_2), 4.21 (s, 2H, C***H*****$_2$**NH), 6.03 (s, 1H, NH, D_2O exchangeable), 6.54-6.63 (m, 3H, J=7.8 Hz, ArH), 7.05-7.09 (m, 2H, J=7.8 Hz, ArH) and 12.12 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 340 [M+1$^{\cdot+}$, 4.09], 339 [M$^{\cdot+}$, 3.89], 338 [M-H$^+$, 10.48], 283 [M-$C_4H_8$$^{\cdot+}$, 29.79], 92 [$C_6H_6N^+$, 16.79], 70 [$C_5H_{10}$$^{\cdot+}$, 100], 66 [$C_5H_6$$^{\cdot+}$, 54.69] and 58 [$C_4H_{10}$$^{\cdot+}$, 46.75]. Anal. Calcd for $C_{19}H_{21}N_3OS$ (339.44): C, 67.22; H, 6.23; N, 12.37. Found: C, 67.19; H, 5.98; N, 12.06.

2-(4-Chlorophenyl)aminomethyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**4b**)

M.p. 226-228 ^{o}C (ethanol); yield 47 %; IR (KBr) ν_{max}: 3437, 3160 (2 NH), 1661 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.25-1.35 (m, 2H, CH_2), 1.40-1.50 (m, 2H, CH_2), 1.60-1.70 (m, 4H, $2CH_2$), 2.80-2.95 (m, 2H, CH_2), 3.00-3.10 (m, 2H, CH_2), 4.26 (s, 2H, C***H*****$_2$**NH), 6.33 (s, 1H, NH, D_2O exchangeable), 6.70 (d, 2H, ArH), 7.14 (d, 2H, ArH) and 12.25 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{19}H_{20}ClN_3OS$ (373.88): C, 61.03; H, 5.39; N, 11.23. Found: C, 61.33; H, 5.58; N, 10.91.

2-(2-Methylphenyl)5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**4c**)

M.p. 220-222 ^{o}C (n-butanol); yield 50 %; IR (KBr) ν_{max}: 3416, 3170 (2 NH), 1655 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.25-1.30 (m, 2H, CH_2), 1.35-1.45 (m, 2H, CH_2), 1.55-1.65 (m, 4H, $2CH_2$), 2.16 (s, 3H, CH_3), 2.83 (t, 2H, J=6.0 Hz, CH_2), 3.01 (t, 2H, J=6.0 Hz, CH_2), 4.28 (d, 2H, J=6.6 Hz, C***H*****$_2$**NH), 5.47 (t, 1H, J=6.6 Hz, CH_2N***H***, D_2O exchangeable), 6.49 (d, 1H, J=7.5 Hz, ArH), 6.55 (t, 1H, J=7.5 Hz, ArH), 6.90 (t, 1H, J=7.5 Hz, ArH), 6.98 (d, 1H, J=7.5 Hz, ArH) and 12.09 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{20}H_{23}N_3OS$ (353.46): C, 67.95; H, 6.55; N, 11.88. Found: C, 67.75; H, 6.60; N, 12.03.

General procedure for the preparation of 2-substituted amino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-ones **5a-c**

An equimolar mixture of the amino amide **1** and the selected isothiocyanate (0.002 mol) in absolute ethanol (5 ml) was heated under reflux for 3 h, then left to cool. The separated solid was filtered, dried and crystallized from ethanol.

2-Methylamino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**5a**)

M.p. 144-146 ^{o}C; yield 60 %; IR (KBr) ν_{max}: 3387, 3282 (2NH) and 1670 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.20-1.30 (m, 2H, CH_2), 1.35-1.45 (m, 2H, CH_2), 1.50-1.55 (m, 2H, CH_2), 1.56-1.65 (m, 2H, CH_2), 2.78 (t, 2H, J=6.0 Hz, CH_2), 2.96 (t, 2H, J=6.0 Hz, CH_2), 3.59 (s, 3H, CH_3), 6.45 (s, 1H, NH, D_2O exchangeable) and 6.68 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{13}H_{17}N_3OS$ (263.35): C, 59.28; H, 6.50; N, 15.95. Found: C, 59.30; H, 6.60; N, 15.07.

2-Ethylamino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**5b**)

M.p. 146-148 ^{o}C; yield 90 %; IR (KBr) ν_{max}: 3221, 3179 (2NH) and 1647 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.13 (t, 3H, J=7.2 Hz, CH_2C***H*****$_3$**), 1.21-1.23 (m, 2H, CH_2), 1.35-1.45 (m, 2H, CH_2), 1.50-1.60 (m, 4H, $2CH_2$), 2.67-2.75 (m, 4H, J=6.0 Hz, $2CH_2$), 3.35-3.45 (m, 2H, C***H*****$_2$**CH_3), 7.34 (s, 1H, NH, D_2O exchangeable) and 8.59 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 278 [M+H$^+$, 32.77]. Anal. Calcd for $C_{14}H_{19}N_3OS$ (277.37): C, 60.61; H, 6.90; N, 15.14. Found: C, 60.70; H, 6.80; N, 14.92.

2-Benzylamino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**5c**)

M.p. 200-202 ^{o}C; yield 60 %; IR (KBr) ν_{max}: 3360, 3129 (2NH) and 1694(C=O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 1.30-1.40 (m, 2H, CH_2), 1.45-1.50 (m, 2H, CH_2), 1.65-1.75 (m, 4H, $2CH_2$), 2.80 (t, 2H, J=6.0 Hz, CH_2), 3.04 (t, 2H, J=6.0 Hz, CH_2), 5.71 (s, 2H, C***H*****$_2$**C_6H_5), 7.26-7.34 (m, 5H, 3ArH and 2NH) and 7.52 (d, 2H, ArH) ppm. Anal. Calcd for $C_{19}H_{21}N_3OS$ (339.44): C, 67.22; H, 6.23; N, 12.37. Found: C, 66.98; H, 6.02; N, 11.74.

2-Thioxo-2,3,5,6,7,8,9,10-octahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(1H)-one **(6)**

This compound was prepared using two different methods:

Method A

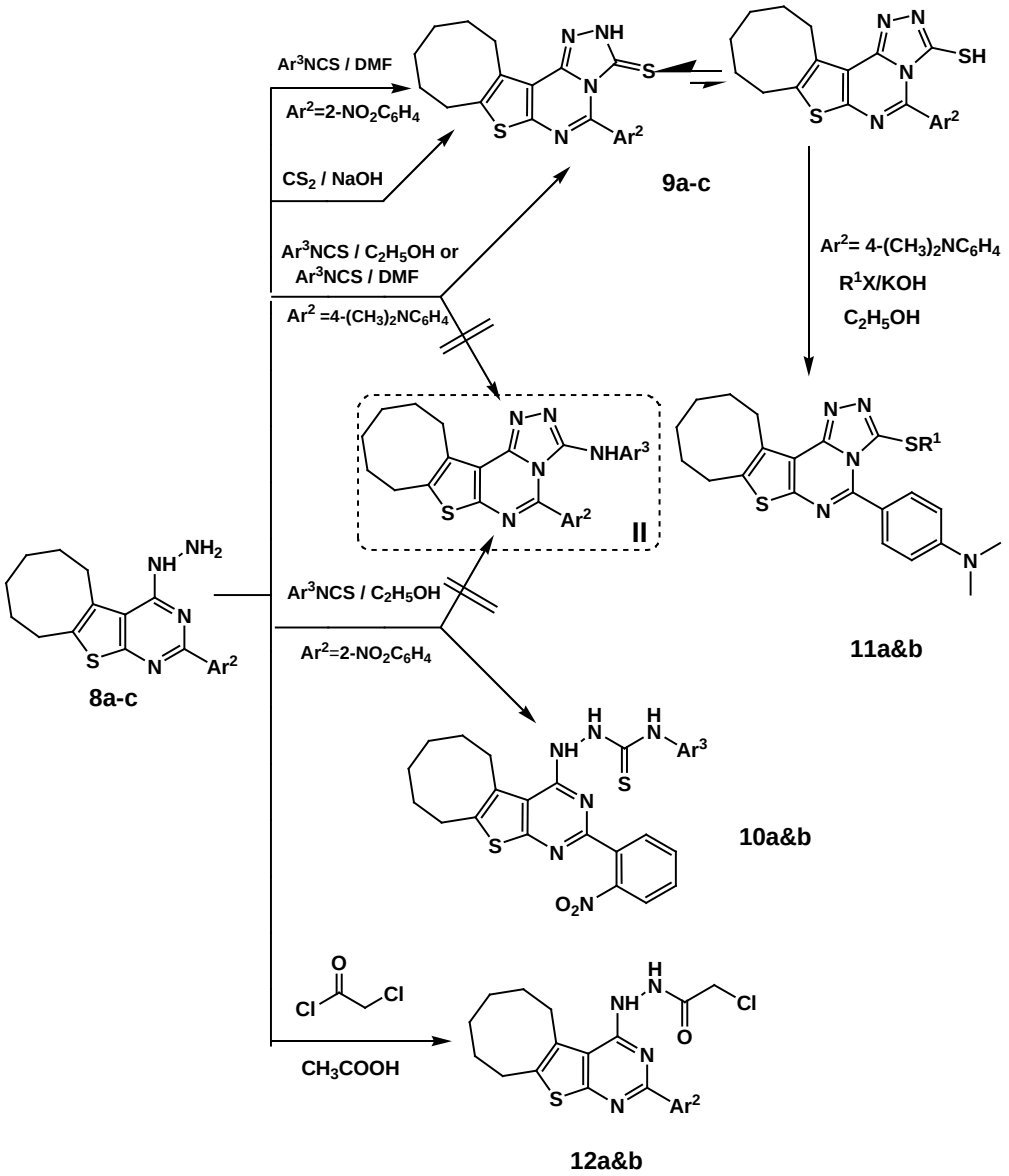
A mixture of amino amide **1** (0.45 g, 0.002 mol) and the aromatic isothiocyanate (phenyl, 4-chlorophenyl or m-tolyl isothiocyanate) (0.002 mol) in absolute ethanol (5 ml) was heated under reflux for 3 h, and then left to cool. The separated solid was filtered, dried and crystallized from ethanol.

Method B

A mixture of amino amide **1** (0.45 g, 0.002 mol) and thiourea (0.3 g, 0.004 mol) was heated in an oil bath at 200^{o}C for about 2 h where the

solid melted then resolidfied, then the mixture was boiled with water (20 ml), filtered while hot, dried and crystallized from ethanol.

M.p. 228-230 ^{o}C; yield 50 % (A), 70% (B); IR (KBr) ν_{max}: 3155, 3128 (2NH), 1670 (C=O) and 1203 (C=S) cm^{-1}; ^{1}HNMR (DMSO-d$_6$): δ 1.25-1.30 (m, 2H, CH_2), 1.35-1.45 (m, 2H, CH_2), 1.50-1.60 (m, 4H, $2CH_2$), 2.77 (t, 2H, J=6.0 Hz, CH_2), 2.92 (t, 2H, J=6.0 Hz, CH_2), 12.24 (s, 1H, NH, D_2O exchangeable) and 13.30 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 268 [M+H+1$^+$, 4.64], 267 [M+H$^+$, 7.13], 266 [M$^{\cdot+}$, 3.89], 238 [M-CO$^{\cdot+}$, 18.49], 222 [M-CS$^{\cdot+}$, 32.47] and 43 [$C_3H_7^+$ & or HNCO$^{\cdot+}$, 100]. Anal. Calcd for $C_{12}H_{14}N_2OS_2$ (266.37): C, 54.10; H, 5.29; N, 10.51. Found: C, 53.96; H, 5.28; N, 10.49.



9a, 12a Ar^2 = 4-$(CH_3)_2NC_6H_4$, **10a** Ar^3 = C_6H_5, **11a** R^1 = CH_2CH_3,
9b,12b Ar^2 = 2-$NO_2C_6H_4$, **10b** Ar^3 = 3-$CH_3C_6H_4$ **11b** R^1 = $CH_2C_6H_5$
9c Ar^2 = 2-C_4H_3S

Scheme 2 Synthesis of compounds **9-12**

Scheme 2: Synthesis of compounds 9-12

General procedure for the preparation of 5-aryl-8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine-3(2H)-thiones Or *5-Aryl-3-sulfanyl-8,9,10,11,12,13-hexahydrocycloocta [4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidines* **9a-c**

These compounds were prepared using three methods:

Method A

A mixture of the hydrazinyl derivative **8a** (0.5 g, 0.001 mol) and phenyl or m-tolyl isothiocyanate (0.001 mol) in absolute ethanol (5 ml) was heated under reflux for 6 h. After cooling, the separated solid was filtered, dried and crystallized from n-butanol to afford **9a**.

Method B

Phenyl or m-tolyl isothiocyanate (0.001mol) was added to a suspension of compound **8b** (0.37 g, 0.001 mol) in dry dimethylformamide (5 ml). The reaction mixture was then heated under reflux for 6 h. The separated solid after cooling was filtered, dried and crystallized from ethanol to afford **9b**.

Method C

To a warmed ethanolic sodium hydroxide solution, prepared by dissolving sodium hydroxide (0.08 g, 0.002 mol) in absolute ethanol (10 ml) compound **8a-c** (0.002 mol) and excess carbon disulphide (2 ml) were added. The reaction mixture was refluxed in a water bath at 80°C for 10 h, and then cooled, poured into ice cold water (20 ml), neutralized by dilute acetic acid. The precipitate was filtered, dried and crystallized from the suitable solvent.

5-(4-Dimethylaminophenyl)-8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine-3(2H)-thione **(9a)**

M.p. 260-262 °C (n-butanol); yield 55 % (A), 70 % (C); IR (KBr) ν_{max}: 3418 (NH), 3067 (SH) and 1184 (C=S) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.35 (m, 2H, CH_2), 1.40-1.50 (m, 2H, CH_2), 1.65-1.75 (m, 4H, $2CH_2$), 2.94-2.96 (m, 2H, CH_2), 3.00 (s, 6H, $N(CH_3)_2$), 3.10-3.16 (m, 2H, CH_2), 6.68 (d, 2H, J=9.0 Hz, ArH), 7.46 (d, 2H, J=9.0 Hz, ArH) and 14.38 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 409 [$M^{\cdot+}$, 0.65], 408 [M-H$^+$, 1.82] and 364 [M-NH$(CH_3)_2$$^{\cdot+}$ & or M-HCS$^+$, 100]. Anal. Calcd for $C_{21}H_{23}N_5S_2$ (409.55): C, 61.58; H, 5.65; N, 17.10. Found: C, 61.82; H, 5.40; N, 17.28.

5-(2-Nitrophenyl)-8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine-3(2H)-thione **(9b)**

M.p. 202-204 °C (ethanol); yield 62 % (B), 74 % (C); IR (KBr) ν_{max}: 3440 (NH), 3080 (SH) and 1198 (C=S) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.40 (m, 2H, CH_2), 1.45-1.50 (m, 2H, CH_2), 1.60-1.75 (m, 4H, $2CH_2$), 2.90-2.95 (m, 2H, CH_2), 3.10-3.18 (m, 2H, CH_2), 7.36 (t, 1H,J=7.8 Hz ArH), 7.41-7.53 (m, 3H, 2ArH and NH, D_2O exchangeable) and 8.26 (d, 1H, J=7.8 Hz, ArH) ppm. Anal. Calcd for $C_{19}H_{17}N_5O_2S_2$ (411.49): C, 55.45; H, 4.16; N, 17.02. Found: C, 55.72; H, 4.24; N, 16.32.

5-(2-Thienyl)-8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine-3(2H)-thione **(9c)**

M.p. 154-156 °C (ethanol); yield 90 % (C); IR (KBr) ν_{max}: 3175 (NH), 3075 (SH) and 1184 (C=S) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 1.35-1.45 (m, 2H, CH_2), 1.47-1.55 (m, 2H, CH_2), 1.75-1.85 (m, 4H, $2CH_2$), 2.99 (t, 2H, J=6.1 Hz, CH_2), 3.20 (t, 2H, J=6.2 Hz, CH_2), 7.15,7.17 (dd, 1H, J=5.4,3.6 Hz, thiophene H), 7.23 (s, 1H, NH, D_2O exchangeable), 7.55 (d, 1H, J=3.6 Hz, thiophene H) and 7.59 (d, 1H, J=5.4 Hz, thiophene H) ppm. Anal. Calcd for $C_{17}H_{16}N_4S_3$ (372.52): C, 54.80; H, 4.32; N, 15.04. Found: C, 54.50; H, 4.58; N, 15.26.

General procedure for the preparation of 4-[2-(N-arylcarbamothioyl)hydrazin-1-yl]-2-(2-nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidines **10a&b**

A mixture of hydrazinyl derivative **8b** (0.37 g, 0.001 mol) and phenyl or m-tolyl isothiocyanate (0.001 mol) in absolute ethanol (5 ml) was heated under reflux for 6 h. After cooling, the separated solid was filtered, dried and crystallized from ethanol.

2-(2-Nitrophenyl)-4-[2-(N-phenylcarbamothioyl)hydrazin-1-yl] -5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine **(10a)**

M.p. 178-180 °C; yield 60 %; IR (KBr) ν_{max}: 3229, 3186, 3126 (3NH) and 1256 (C=S) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.39 (m, 2H, CH_2), 1.40-1.50 (m, 2H, CH_2), 1.60-1.75 (m, 4H, $2CH_2$), 2.90-2.95 (m, 2H, CH_2), 3.10-3.15 (m, 2H, CH_2), 6.90-6.94 (m, 3H, ArH), 7.26-7.31 (m, 3H, 2ArH and NH), 7.48-7.55 (m, 4H, ArH), 8.28 (d, 1H, NH, D_2O exchangeable) and 9.81 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 507 [M+3$^{\cdot+}$, 0.04], 148 [$C_7H_4N_2O_2$$^{\cdot+}$, 0.06] and 76 [$C_6H_4$$^{\cdot+}$, 100]. Anal. Calcd for $C_{25}H_{24}N_6O_2S_2$ (504.61): C, 59.50; H, 4.79; N, 16.65. Found: C, 59.53; H, 4.80; N, 17.00.

4-[2-(N-3-Methylphenylcarbamothioyl)hydrazin-1-yl]-2-(2-nitrophenyl)- 5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine **(10b)**

M.p. 175-177 °C; yield 65 %; IR (KBr) ν_{max}: 3354, 3219, 3200 (3NH) and 1230 (C=S) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.40 (m, 2H, CH_2), 1.41-1.50 (m, 2H, CH_2), 1.65-1.75 (m, 4H, $2CH_2$), 2.49 (s, 3H, CH_3), 2.90-2.95 (m, 2H, CH_2), 3.10-3.15 (m, 2H, CH_2), 6.74 (d, 2H, ArH), 7.16 (t, 3H, ArH), 7.30 (d, 2H, ArH), 7.42 (s, 1H, ArH), 7.52 (d, 1H, NH, D_2O exchangeable), 8.30 (d, 1H, NH, D_2O exchangeable) and 9.73 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{26}H_{26}N_6O_2S_2$ (518.64): C, 60.20; H, 5.05; N, 16.20. Found: C, 60.55; H, 5.35; N, 16.10.

General procedure for the preparation of 5-(4-dimethylaminophenyl)-3-substituted sulfanyl-8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidines **11a&b**

The appropriate alkyl halide (0.0025 mol) was added to **9a** (1 g, 0.0025 mol), dissolved in a solution of potassium hydroxide (0.14 g, 0.0025 mol) in absolute ethanol (6 ml). The reaction mixture was heated under reflux for 10h, cooled, diluted with ice cold water (50 ml) and the separated solid was filtered, dried and crystallized from ethanol.

3-Ethylsulfanyl-5-(4-dimethylaminophenyl)- 8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine **(11a)**

M.p. 148-150 °C; yield 53 %; IR (KBr) ν_{max}: 1605 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.25-1.35 (m, 2H, CH_2), 1.40-1.50 (m, 2H, CH_2), 1.46 (t, 3H, J=7.2 Hz, $CH_2C\boldsymbol{H_3}$), 1.60-1.75 (m, 4H, $2CH_2$), 2.94 (t, 2H, CH_2), 3.02 (s, 6H, $N(CH_3)_2$), 3.19 (t, 2H, CH_2), 3.30 (q, 2H, J=7.2 Hz, $C\boldsymbol{H_2}CH_3$), 6.81 (d, 2H, J=9.3 Hz, ArH) and 8.48 (d, 2H, J=9.3 Hz, ArH) ppm; MS [m/z, %]: 437 [$M^{\cdot+}$, 20.66]. Anal. Calcd for $C_{23}H_{27}N_5S_2$ (437.61): C, 63.12; H, 6.22; N, 16.00. Found: C, 62.91; H, 5.92; N, 16.04.

3-Benzylsulfanyl-5-(4-dimethylaminophenyl)- 8,9,10,11,12,13-hexahydrocycloocta[4,5]thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidine **(11b)**

M.p. 154-156 °C; yield 50%; IR (KBr) ν_{max}: 1612 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.33 (m, 2H, CH_2), 1.40-1.46 (m, 2H, CH_2), 1.65-1.75 (m, 4H, $2CH_2$), 2.93-2.96 (m, 2H, CH_2), 3.04 (s, 6H, $N(CH_3)_2$), 3.20-3.26 (m, 2H, CH_2), 4.55 (s, 2H, $C\boldsymbol{H_2}C_6H_5$), 6.83 (d, 2H, J=9.3 Hz, ArH), 7.21-7.35 (m, 3H, ArH), 7.53 (d, 2H, J=7.8 Hz, ArH) and 8.45 (d, 2H, J=9.3 Hz, ArH) ppm. Anal. Calcd for $C_{28}H_{29}N_5S_2$

(499.67): C, 67.30; H, 5.84; N, 14.01. Found: C, 67.28; H, 5.60; N, 13.82.

General procedure for the preparation of 2-aryl-4-[2-(chloromethylcarbonyl) hydrazin-1-yl]-5,6,7,8,9,10 hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidines **12a&b**

A mixture of either of the hydrazinyl derivative **8a** or **8b** (0.001 mol) and chloroacetyl chloride (0.11 g, 0.001 mol) in glacial acetic acid (5 ml) was heated under reflux for 10 h. The reaction mixture was then cooled and the separated solid was filtered, dried and crystallized from a suitable solvent.

4-[2-(Chloromethylcarbonyl)hydrazin-1-yl]-2-(4dimethylaminophenyl)-5,6,7,8,9,10hexahydrocycloocta[4,5]thieno [2,3-d]pyrimidine **(12a)**

M.p. 230-232 °C (DMF); yield 30 %; IR (KBr) ν_{max}: 3233, 3179 (2NH) and 1716 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.15-1.25 (m, 2H, CH_2), 1.45-1.50 (m, 2H, CH_2), 1.60-1.70 (m, 4H, $2CH_2$), 2.90-2.95 (m, 2H, CH_2), 3.00 (s, 6H, $N(CH_3)_2$), 3.05-3.10 (m, 2H, CH_2), 4.30 (s, 2H, CH_2Cl), 6.76 (d, 2H, J=9.0 Hz, ArH), 8.22 (d, 2H, J=9.0 Hz, ArH), 10.41 (s, 1H, NH, D_2O exchangeable) and 10.46 (s, 1H, NH, D_2O exchangeable) ppm; MS [m/z, %]: 448 [M+H+4$^+$, 3.88], 447

[M+4]$^{+\cdot}$, 9.63], 446 [M+H+2]$^{+}$, 31.49] and 444 [M+H]$^{+}$, 100]. Anal. Calcd for $C_{22}H_{26}ClN_5OS$ (443.98): C, 59.51; H, 5.90; N, 15.77. Found: C, 59.05; H, 5.54; N, 15.77.

4-[2-(Chloromethylcarbonyl)hydrazin-1-yl]- 2-(2-nitrophenyl)-5,6,7,8,9,10 hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine **(12b)**

M.p. 178-180 ^{o}C (ethanol); yield 25 %; IR (KBr) ν_{max}: 3360, 3250 (2NH) and 1694 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.30-1.40 (m, 2H, CH_2), 1.42-1.50 (m, 2H, CH_2), 1.60-1.75(m, 2H, CH_2), 1.78-1.80 (m, 2H, CH_2), 2.90-2.95 (m, 2H, CH_2), 3.00-3.10 (m, 2H, CH_2), 4.24 (s, 2H, CH_2Cl), 7.66-8.47 (m, 4H, ArH), 8.75 (s, 1H, NH, D_2O exchangeable) and 10.34 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{20}H_{20}ClN_5O_3S$ (445.73): C, 53.84; H, 4.52; N, 15.71. Found: C, 53.70; H, 4.40; N, 15.32.

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 µg/mL using SulfoRhodamine-B (SRB) assay for cytotoxic activity against human colon tumor 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 tumors[21,22].

Potential cytotoxicity of the compounds was tested using the method of Skehan *et al* [23] 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) 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 ºc in atmosphere of 5% CO_2. 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 tumor cell line after the specified compound.

Docking steps

Preparation for docking:

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 minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol-1A° -1 with MMFF94x force-field and the partial charges were automatically calculated.

In order to perform docking some preliminary steps were done [24]:

Enzyme structures were checked for missing atoms, bonds and contacts. Water of crystallization 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.

Docking of compounds:

The compounds were constructed using the builder module and were energy minimized 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 minimization. The minimized docking conformations were then rescored using London dG scoring method[24].

RESULTS AND DISCUSSION

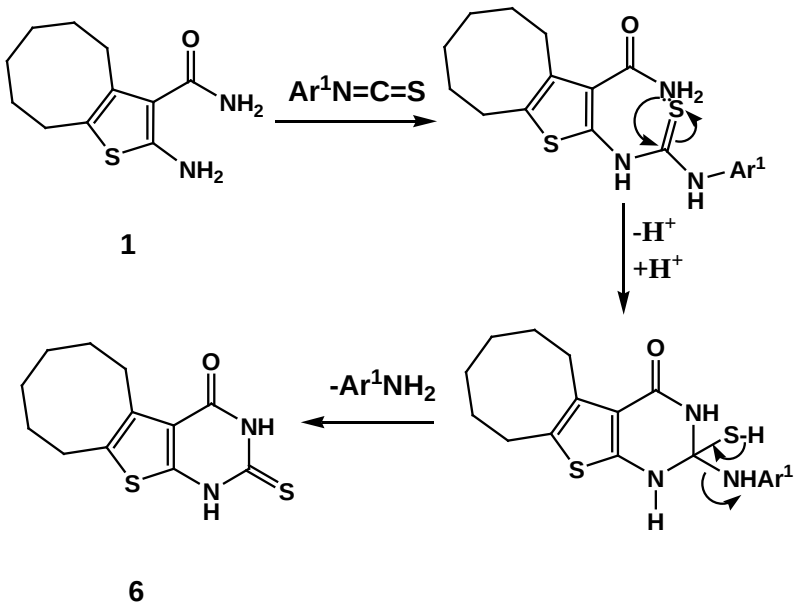
Chemistry

The synthetic route to the target compounds was outlined in Schemes 1and 2. 2-Amino-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**1**) was selected as our primary starting material for this series of reactions, and was prepared via reported procedure[25]. One of the objectives of this study was the preparation of thieno[2,3-d]pyrimidine-4-ones **2-6**, structure analogs of compounds **Ia,b** and **7a-c** (Figure 2). Accordingly, compound **1** was cyclized with monophenylurea to give 3-phenyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidine-2,4 (1H,3H)-dione (**2**). The IR spectrum revealed the appearance of an absorption band at 3180 cm^{-1} corresponding to NH group. In addition the two C=O groups appeared at 1713 and 1690 cm^{-1}. On the other hand, ^{1}HNMR spectrum showed an exchangeable singlet signal at δ 10.92 due to NH proton.

Condensation of the amino amide **1** with chloroacetyl chloride in glacial acetic acid afforded 2-chloromethyl hexahydrocyclooctathieno[2,3-*d*]pyrimidin-4-one **3**, which was reacted with the appropriate primary aromatic amine in ethanol in the presence of catalytic amount of triethylamine to yield the 2-arylaminomethyl derivatives **4a-c**. The ^{1}HNMR spectrum of compound **3** showed a single signal at δ 4.53 corresponding to CH_2Cl protons as well as an exchangeable singlet signal at δ 12.59 corresponding to NH proton. Whereas the ^{1}HNMR spectra of **4a-c** showed additional exchangeable signals corresponding to the 2-arylaminomethyl groups at δ 5.47-6.33 due to the NH protons and at δ 6.49-7.14 corresponding to aromatic protons which were not present in the parent chloromethyl derivative **3.**

The 2-substituted aminohexahydrocyclooctathieno[2,3-d]pyrimidin-4(3H)-ones **5a-c** were obtained through the reaction of **3** with the appropriate isothiocyanate in ethanol applying reported reaction conditions[26]. The IR spectra of **5a-c** showed absorption bands at 3387-3129 cm^{-1} corresponding to two NH groups. Whereas the C=O group appeared as an absorption band at 1694-1647 cm^{-1}. The ^{1}HNMR spectra showed two exchangeable singlet signals at δ 6.45-8.59 corresponding to two NH protons as well as the signals corresponding to the different NHR groups.

On the contrary, reaction of the amino amide **1** with the appropriate aromatic isothiocyanate did not afford the expected 2-arylaminothienopyrimidines analogous to **5** but the 2-thioxo derivative **6** was obtained. A suggested mechanism for the formation of **6** from reacting amino amide **1** with aromatic isothiocyanates is illustrated in figure 3. In this mechanism Ar^1NH_2 is the good leaving group rather than H_2S. Confirmation of the structure of the product **6** was through unambiguous synthesis involving the fusion of **1** with thiourea. The products of both methods were the same; this was confirmed by similarities in mps, TLC and spectral data. The IR spectrum of **6** showed two absorption bands at 3155, 3128 cm^{-1} due to two NH groups, an absorption band at 1670 cm^{-1} corresponding to C=O group and C=S group appeared as an absorption band at 1203 cm^{-1}. The ^{1}HNMR spectrum showed two exchangeable singlet signals at δ 12.24, 13.30 indicating the presence of two NH protons. It is worth mentioning that absence of signals at δ 6-9 of aromatic protons is also indicative for the structure. Moreover the mass spectrum showed three molecular ion peaks at m/z 266 (M^+), 267 (M+1) and 268 (M+2) in addition to the presence of another two peaks at m/z 238 (M-CO) and 222 (M-CS).



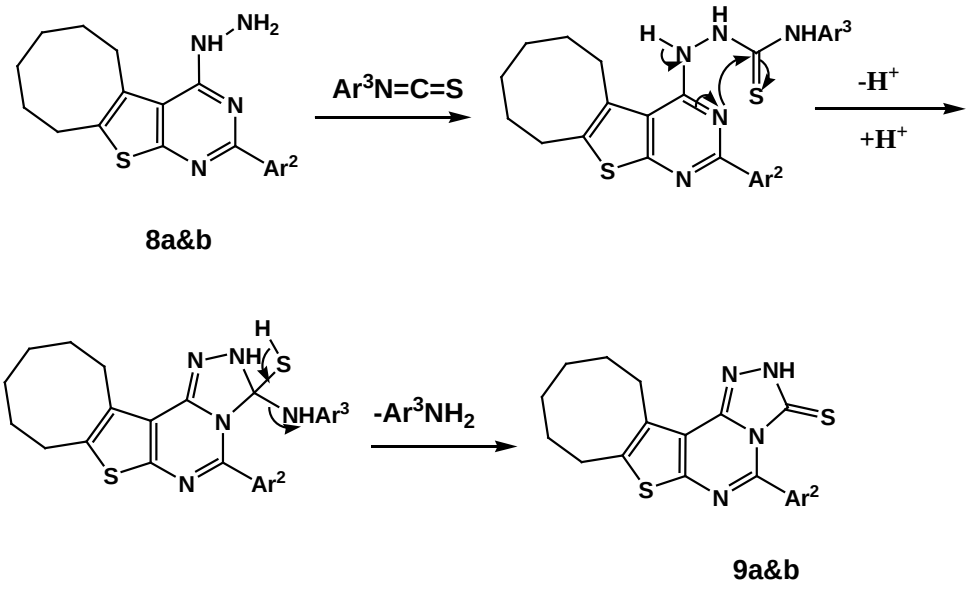
Ar^1= C_6H_5, 4-Cl C_6H_4, 3-$CH_3C_6H_4$

Fig. 3: Formation of compound 6

An attempted synthesis of 3-substituted amino triazolothienopyrimidines **II** via reacting **8a&b** with certain aryl isothiocyanates, namely phenyl and m-tolyl isothiocyanates, was futile due to the formation of unexpected products.

Reaction of the hydrazino derivative **8a** with phenyl and m-tolyl isothiocyanates either in ethanol or in dimethylformamide afforded the corresponding triazolothienopyrimidine **9a**, the dimethylamino group has -I and +M effects so increase the nucleophilicity of pyrimidine N-3 and facilitate the attack of thioxo group with the elimination of Ar^2NH_2 affording **9a.** Elimination of Ar^2NH_2 instead of H_2S may be attributed to steric hindrance between the bulky aromatic group at C-5 and the Ar^2NH as illustrated in figure 4.

Similarly, on reacting **8b** with the same isothiocyanates in dimethylformamide, a high boiling point solvent, furnished **9b**. On the other hand, when the 4-hydrazinyl derivative **8b** was allowed to react with the aforementioned isothiocyantes in ethanol, the unexpected thiosemicarbazides **10a&b** were obtained. The nitro group in ortho position has –I and –M effects so decrease the nucleophilicity of pyrimidine N-3 which became unable to attack the thioxo group resulting in the formation of **10a&b** rather than the cyclized **9b**.



Ar^2= 4-$(CH_3)_2NC_6H_4$, 2-$NO_2C_6H_4$
Ar^3= C_6H_5, 3-$CH_3C_6H_4$

Fig. 4: Formation of compounds 9a,b

Moreover compounds **9a&b** were identical to those obtained by the reaction of 4-hydrazinyl derivatives **8a-c** with carbon disulphide in ethanolic sodium hydroxide – a chemical confirmation – as detected by mps, TLC, IR, ^{1}HNMR and mass spectral data. The IR spectra of **9a-c** showed an absorption band at 3440-3175 cm^{-1} corresponding to NH group. Besides, an absorption band at 1198-1184 cm^{-1} due to C=S group.

The IR spectra of compounds **10a&b** indicated the presence of three absorption bands at the range 3354-3126 cm^{-1} corresponding to three NH groups. In addition, C=S group appeared as an absorption band at 1256 and 1230 cm^{-1}. On the other hand, the ^{1}HNMR spectra displayed three exchangeable signals at δ 7.30-9.81 assigned to three NH protons. Further structural evidence stemmed from the mass spectrum of compound **10a** which corroborates the spectral data and the proposed structure , giving a molecular ion peak with m/z 507 (M+3).

Alkylation of 5-(4-dimethylaminophenyl)-3-sulfanyl-8,9,10,11,12,13-hexahydrocycloocta [4,5] thieno [3,2-*e*] -1,2,4-triazolo [4,3-*c*]pyrimidine (**9a**) with the appropriate alkyl halide in ethanolic potassium hydroxide gave compounds **11a&b** that were identified on the basis of their element analysis, IR and ^{1}HNMR spectroscopy.

Finally, the hydrazinyl derivatives **8a&b** was reacted with equimolar amounts of chloroacetyl chloride in glacial acetic acid to afford the chloroacetylhydrazinyl derivatives **12a&b** instead of the supposed triazolopyrimidine derivatives. The IR spectra of **12a&b** showed two absorption bands at the range 3360-3179 cm^{-1} indicating the presence of two NH groups, in addition to C=O absorption band appeared at 1716, 1694 cm^{-1}, respectively. ^{1}HNMR spectra of **12a&b** showed the presence of $COCH_2Cl$ protons as singlet signals at δ 4.30 and 4.24, respectively, while two NH protons were assigned as two exchangeable singlet signals at δ 10.41, 10.46 and 8.75, 10.34 in the ^{1}HNMR spectrum of **12a** and **12b**, respectively. In addition, the mass spectrum of **12a** displayed molecular ion peaks at 444 (M+1 & M+H), 446 (M+3) in the ratio of 3:1 (Cl pattern).

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®) (Figure 1), a standard antitumor drug used for the treatment of gasterointestinal tract tumors[21,22], was evaluated under the same conditions. The IC_{50} (dose of the compound which caused a 50% reduction of survival values) are shown in Table 1. The results are represented graphically in figure 5. From the analysis of Table 1, it was found that most of the compounds showed significant antitumor activities. Interestingly, compounds **4a, 4b**, **3, 12b** and **5c** (IC_{50}: 11.90, 12.43, 15.91, 25.80 and 32.11 µM, respectively) exhibited 2.89 to 1.07 fold more potent antitumor activity than imatinib (IC_{50}: 34.40 µM) and were the most active among their analogues. Further, compounds **2**, **10a**, **11b**, **10b**, **9c** and **11a** (IC_{50}: 37.68, 41.61, 44.02, 47.23, 47.24 and 47.30 µM, respectively) showed comparable cytotoxicity to imatinib. Moreover, compounds **12a**, **6** and **5b** (IC_{50}: 68.92, 73.58 and 86.16 µM) were less active than imatinib and compounds **4c**, **5a** and **9a,b** were inactive.

Table 1: IC_{50} values[a] of compounds 2-12b and Imatinib against colon carcinoma cell line (HCT 116)

Compound	[a] IC_{50} (µM)
2	37.68
3	15.91
4a	11.90
4b	12.43
4c	------
5a	------
5b	86.16
5c	32.11
6	73.58
9a	------
9b	------
9c	47.24
10a	41.61
10b	47.23
11a	47.30
11b	44.02
12a	68.92
12b	25.80
Imatinib	34.40

[a] **IC_{50}** (µM): dose of the compound which caused 50% reduction of survival.

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

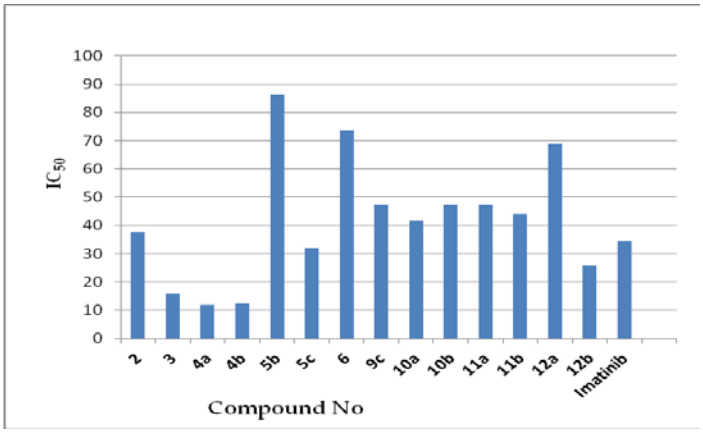


Fig. 5: Cytotoxicity of 2-12b and imatinib against (HCT 116) cell line

The cytotoxic activity of the resulting derivatives appeared to be related to the nature of the substituent group. Generally, compounds **2-6** (Scheme 1) showed better anticancer activity than compounds **9-12** (Scheme 2). Compounds **2-6** had C-4 oxo group in common, also it was clear that the structural changes at the C-2 had a considerable effect on the anticancer activity. Introduction of a CH_2 bridge between pyrimidin-4-one ring and electronegative moieties, Cl and arylamino, in **3** and **4a,b**, respectively, resulted in higher activity than compounds **5b,c**. Compound **2**, with a C-2 oxo group showed more potent anticancer activity than its 4-thioxo analog **6**. Compounds **8a-c** [20] possessed significant anticancer activity (IC_{50}: 49.25, 10.52 and 36.31 µM, respectively) and were thus used in the design of compounds **9-12**. Unexpectedly, compounds **9a,b** showed significant loss of activity compared to their parents **8a,b** where as **9c** maintained relatively potent anticancer activity compared to its parent **8c**. However, introduction of ethyl or benzyl substituents led to the recovery of the anticancer activity for compounds **11a,b**. On the other hand, the anticancer activity did not alter significantly by the nature of the arylcarbothioyl group in **10a** and **10b**, while compound **12b**, a 2-nitrophenyl derivative, exhibited 2.67 fold more potent antitumor activity than **12a**, 2-(4-dimethylaminophenyl) analog.

Docking study

CDK2 is a target enzyme for a wide range of antitumor 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 [27]. Therefore we investigated the binding affinities of the newly synthesized thieno[2,3-*d*]pyrimidines using Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) as the computational software, into the target CDK2.

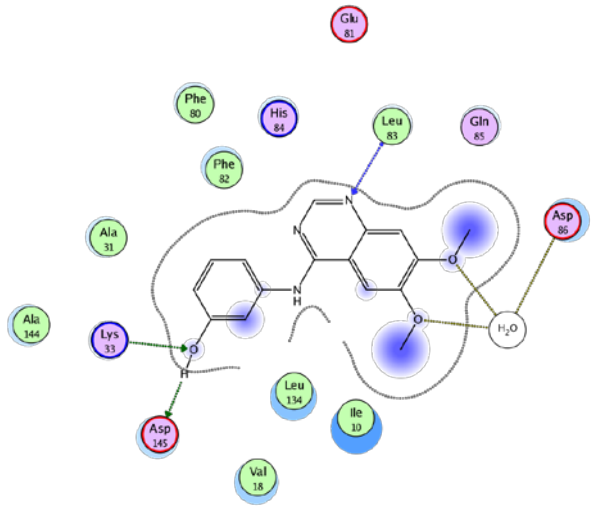


Fig. 6: 3D structure of CDK2 complexed with 4-(3-hydroxyanilino)-6,7-dimethoxyquinazoline.

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 (ΔGb) of -14.07 Kcal/mol. The ligand exhibited four hydrogen bonds between the pyrimidine N-1 along with the OH of 3-hydroxyanilino at C-4 and Asp145, Leu 83 and Lys33 (Figure 6). These results indicated the high accuracy of the MOE stimulation 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 (ΔGb, Kcal/mol) 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[28,29].

The docking for the pyrimidine-4-one derivatives **2-6** exhibited high binding affinities (ΔGb between -8.80 and -11.94) due to more proper fitting into the target site by four hydrogen bonds (with Thr14, Lys129 and Asp145 for **5c**) and three hydrogen bonds (with Asp145, Lys33 and Lys129 for **4a**). Compounds **5a,b** and **6** bound to the active site of CDK2 through 2 hydrogen bonds with Asp145 and Lys33. Similarly, compound **3** showed two hydrogen bonds with Leu 83 and compound **4b** showed two hydrogen bonds with Asp145 and Thr14. Compounds **2** and **4c** exhibited one hydrogen bond with Leu 83 and Asp145, respectively.

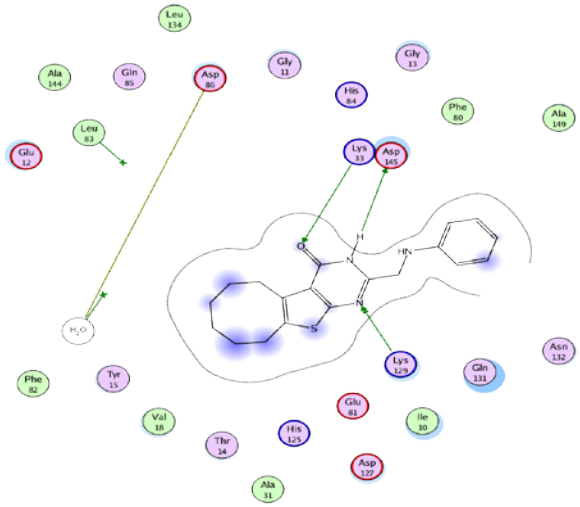


Fig. 7: The proposed binding mode of compound 4a in the binding site of CDK2, created with Molsoft ICM-Pro software

The thienotriazolopyrimidines **9a-c** and **11a,b** as well as the hydrazinylthienopyrimidines **10a,b** and **11a,b** had good docking scores between -6.93 and -13.55 Kcal/mol and bound to CDK2 active site through one hydrogen bond with Asp145 (for **9a**, **9c, 10a,b** and **12a**). While compounds **9b**, **11b** and **12b** displayed one hydrogen bond with Gln131, Lys20 and Lys33, respectively. On the other hand, **11a** displayed good ΔGb -11.89 Kcal/mol but did not bind to the active site of CDK2 through hydrogen bonds.

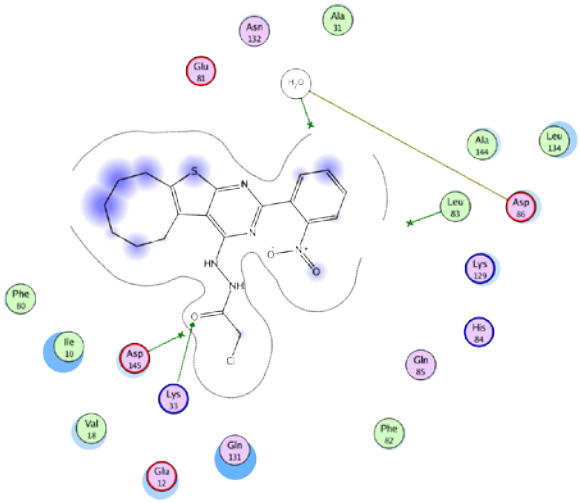


Fig. 8: The proposed binding mode of compound 12b in the binding site of CDK2, created with Molsoft ICM-Pro software.

Table 2:

Compound	ΔG_b [a] (Kcal/mol)	Hydrogen bonds between atoms of compounds and nucleotides			
		Atoms of compound	Nucleotides	Distance (A°)	Angle (°)
Ligand	-14.07	OH (H-donor)	Asp145 (C=O), Asp145 (C=O), Leu83 (NH), Lys33	1.53	103.3
		OH (H-donor)	(NH_2)	2.29	169.5
		Pyrimidine N-1		2.80	164.6
		OH (H-acceptor)		2.83	153.8
2	-11.48	C-2-oxo	Leu83(NH)	2.44	164.6
3	-9.69	C=O	Leu83(NH), Leu83(C=O)	2.66	177.0
		N-3H		2.14	138.0
4a	-11.42	N-3H	Asp145 (OH), Lys33 (NH_2),	1.40	129.9
		C=O	Lys129 (NH_2)	3.05	131.4
		Pyrimidine N-1		2.95	166.0
4b	-10.17	4-ClC_6H_4NH	Asp145 (OH),	1.40	158.5
		C=O	Thr14 (NH)	2.64	149.5
4c	-11.94	2-$CH_3C_6H_4NH$	Asp145 (OH)	1.42	157.1
5a	-8.80	Pyrimidine N-1	Asp145 (NH), Lys33 (C=O)	1.71	161.7
		CH_3NH		2.87	153.0
5b	-9.15	N-3H	Asp145 (OH),	1.55	136.5
		C=O	Lys33 (NH_2)	2.57	178.7
5c	-10.24	N-3H	Asp145 (OH),	1.80	143.6
		$C_6H_5CH_2NH$	Asp145 (OH),	1.68	151.3
		C=O	Thr14 (NH)	3.05	160.0
		C=O	Lys129 (NH_2)	2.62	162.0
6	-9.86	N-3H	Asp145 (C=O),	1.73	156.0
		C=O	Lys33 (NH_2)	2.54	161.2
9a	-8.87	Triazole NH	Asp145 (OH)	1.75	164.7
9b	-13.55	Triazole NH	Gln131 (C=O)	1.60	142.5
9c	-9.03	Triazole NH	Asp145 (OH)	1.42	165.5
10a	-12.78	C_6H_5NH	Asp145 (OH)	1.54	165.2
10b	-13.30	3-$CH_3C_6H_4NH$	Asp145 (OH)	1.54	148.1
11a	-11.89	---	---	---	---
11b	-6.93	Pyrimidine N-1	Lys20(NH_2)	2.77	160.0
12a	-12.19	4-NH	Asp145 (OH)	1.47	175.9
12b	-10.58	CO-CH_2-Cl	Lys33(NH_2)	2.50	158.7

Table 2 The best docking results based on the binding free energy (ΔG_b) of compounds docked into binding site of CDK2, the distances and angles of hydrogen bonds between compounds and amino acids involved in CDK2[a] binding free energy.

The comparative docking modes of the synthesized compounds showed that compounds **3**, **4a**, **5a-c**, **6** and **11b** formed hydrogen bond via pyrimidine N-1 or N-3 (similar to the ligand) in addition to other hydrogen bond via the electronegative C4-oxo group (compounds **3**, **4a,b**, **5b,c** and **6**) or the electronegative C4-NH of hydrazinyl or substituted hydrazinyl groups (compounds **10a,b** and **12a,b**).

Compounds **9a-c** displayed hydrogen bond between different nucleotides and the electronegative triazole NH only. These different binding modes intensify the role of the C-4 substituents 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 (IC50, μM) of the synthesized 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 antitumor activity in spite of the high binding affinity. Figures 6-8 illustrate docking of ligand and compounds **4a** and **12b** respectively.

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