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Original article

Synthesis and anticancer activity of novel 2-pyridyl hexahyrocyclooctathieno [2,3-d]pyrimidine derivatives

Asmaa E. Kassab*, Ehab M. Gedawy

Pharmaceutical Organic Chemistry Department, Cairo University, 33 Kasr El-Aini Street, Cairo 11562, Egypt

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ABSTRACT

A series of new 2-pyridyl hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidines with different substituents as C-4 position was synthesized. The anticancer activity of the newly synthesized compounds was tested *in vitro* using a two-stage process utilizing 60 different human tumor cell lines representing leukemia, melanoma and cancers of lung, colon, central nervous system, ovary, kidney, prostate as well as breast. Compounds **4a**, **6a**, **7a**, **7d** and **7g** showed potent anticancer activity at low concentrations against most of the used human tumor cell lines comparable with doxorubicin as standard potent anticancer drug (average $\log_{10} GI_{50}$ over all cell lines = −6.85). Also, compound **4b** was selective against SNB-75 (CNS cancer) $\log_{10} GI_{50}$ = −5.57. Interestingly, compound **7e** exhibited promising selectivity against 13 tumor cell lines showing growth inhibition percentages between 54.05 and 89.23.

1. Introduction

Cancer remains as a major cause of death worldwide so there is an ongoing need for discovery and development of effective anticancer agents. Recently, the thieno[2,3-d]pyrimidine core was evaluated as bioisostere of 4-anilinoquinazoline core which includes potent marketed anticancer drugs for example gefitinib (Iressa™) [1], erlotinib (Tarceva™) [2] and tandutinib (MLN518) (phase II clinical trials) [3] (Fig. 1). A large number of thieno[2,3-d] pyrimidine derivatives were found to be active against different cancer types exerting their antitumor activities via different mechanisms [4–23]. Consequently, the thieno[2,3-d]pyrimidine ring system constitutes an attractive target for the design of new anticancer drugs through wide structure variations. A research group reported that cycloalkyl ring fused to thiophene was essential for the anticancer activity of thieno[2,3-d]pyrimidines [7]. Then several studies support the anticancer activity of thieno[2,3-d]pyrimidines fused with five-, six- or seven-membered cycloalkyl lipophilic moieties [10,15–18]. Because 8-membered cycloalkyl ring was not incorporated in the synthesis of thienopyrimidine core to be screened as anticancer agent, we reported in previous works the synthesis of new hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidines with different substituents at C-2 and C-4 positions which showed potent *in vitro* anticancer activity against human colon carcinoma (HCT-116) cell line [21–23]. In the same direction to further explore the potential of thieno[2,3-d]pyrimidines fused to 8-membered cycloalkyl ring as anticancer compounds we decided in this work to prepare new hexahydrocycloocta [4,5]thieno[2,3-d] pyrimidines by introducing different heteroaryl groups at C-2 position [2-pyridyl or 4-pyridyl] and different substituents at C-4 position to substantiate the effect of such substitutions on the anticancer activity against a panel of 60 human tumor cell lines provided by US National Cancer Institute.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds is outlined in Scheme 1. 2-amino-4,5,6,7,8,9-hexahydrocycloocta [4,5]thiophene-3-carboxamide (**3**) our primary starting compound was prepared via two steps procedure which involved reacting cyclooctanone with cyanoacetamide **1** to afford α-cyano-α-cyclooctylideneacetamide (**2**) which was then reacted with sulphur and diethylamine [24]. Reacting compound **3** with the appropriate pyridine carboxyaldehyde in dry

* Corresponding author. Tel.: +20 23639307; fax: +20 23635140.
E-mail address: asmaa_kassab2001@yahoo.com (A.E. Kassab).

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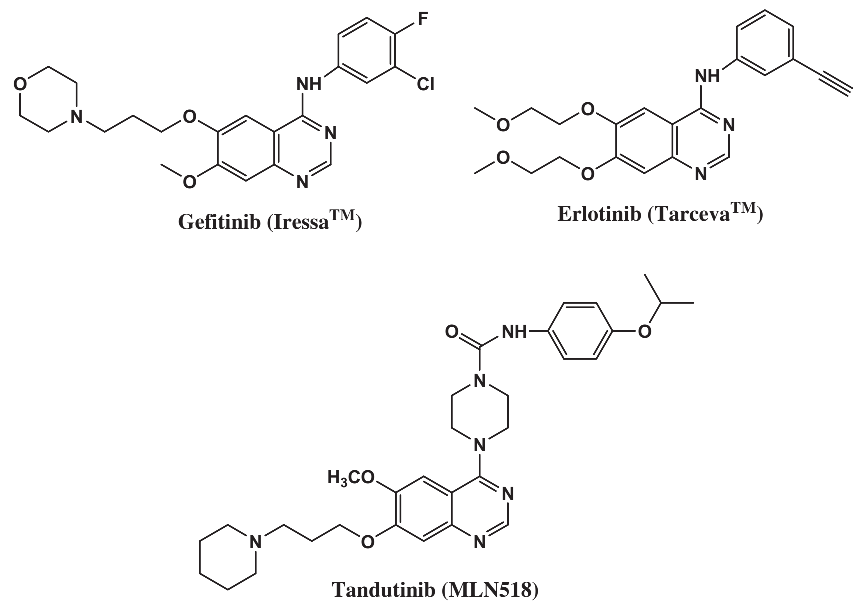


Fig. 1. Examples of 4-anilinoquinazoline compounds are potent anticancer drugs.

dimethylformamide in the presence of concentrated hydrochloric acid resulted in 2-pyridyl hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidin-4-(3H)-ones **4a**,**b**. The IR spectra of **4a**,**b** showed the presence of an absorption band at 3097 and 3163 cm^{-1} corresponding to NH group. Whereas, the C=O group appeared as an absorption band at 1678 and 1658 cm^{-1} respectively. Further evidence was obtained from ^{1}H NMR spectra that showed an exchangeable singlet signals at δ 11.63 and 12.73 ppm corresponding to NH protons.

On refluxing **4a**,**b** with phosphorus oxychloride, the corresponding 4-chloro derivatives **5a**,**b** were obtained. The IR spectra of compounds **5a**,**b** lacked the presence of NH and C=O absorption bands, where as the ^{1}H NMR spectra of these compounds revealed the disappearance of the signal due to NH proton which confirmed the success of chlorination.

Reacting compounds **5a**,**b** with hydrazine hydrate in ethanol afforded the 4-hydrazinyl derivatives **6a**,**b**. The ^{1}H NMR spectra of **6a** and **6b** showed NH_2 and NH peaks as exchangeable singlet signals at δ 4.64, 8.31 and δ 4.85, 8.20 ppm respectively.

The 4-substitutedaminothieno[2,3-d]pyrimidine derivatives **7a**–**h** were obtained through reacting compounds **5a**,**b** with the appropriate secondary amine in ethanol in the presence of catalytic amount of triethylamine. The ^{1}H NMR spectra of the products **7a**–**h** revealed the presence of expected signals corresponding to the different *N*-substituted groups which were indicative for the success of amination.

2.2. Anticancer activity

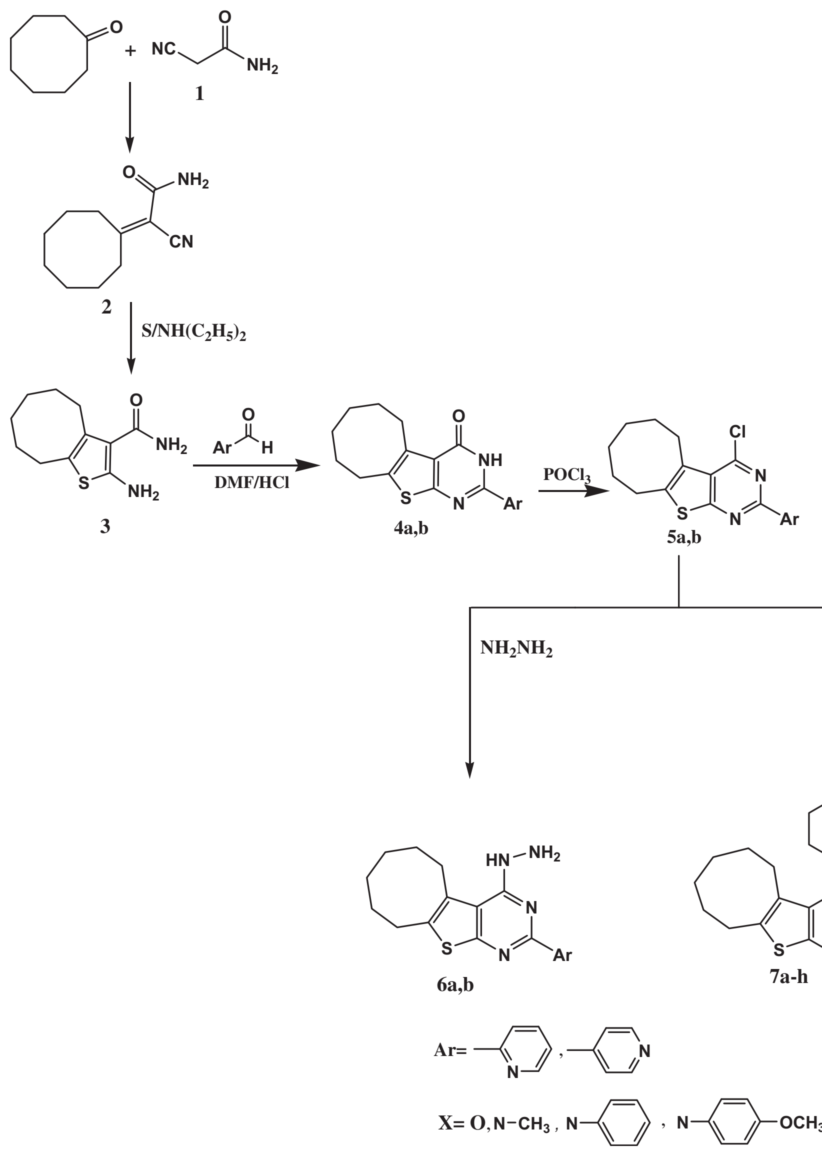
The *in vitro* anticancer activity of all the newly synthesized compounds were evaluated using a two-stage process utilizing 60 different human tumor cell lines, representing leukemia, melanoma and cancers of lung, colon, central nervous system, ovary, kidney, prostate as well as breast. The first stage involved the screening of all compounds against 49 cell lines at a single dose (10^{-5} M). The growth inhibition percentages obtained from the single dose test for compounds **5a**, **5b**, **6b**, **7b**, **7c**, **7e**, **7f** and **7h** are shown in Table 1. Six compounds **4a**, **4b**, **6a**, **7a**, **7d** and **7g** showed significant growth inhibition so they were evaluated against 56 cell lines at 5 concentration levels. The relationship between percentage growth and $\log_{10}$ of sample concentration was plotted to obtain $\log_{10}$ GI_{50} (concentration required for 50% inhibition of cell growth). The $\log_{10}$ GI_{50} of these six compounds are shown in Table 2. From the analysis of the *in vitro* observed data, it was found that compounds **4a**, **6a**, **7a**, **7d** and **7g** showed potent anticancer activity at low concentrations against most of the used human tumor cell lines comparable with doxorubicin as standard potent anticancer drug (average $\log_{10}$ GI_{50} over all cell lines = −6.85). Compound **4b** was selective against SNB-75 (CNS cancer) $\log_{10}$ GI_{50} = −5.57. It worth mentioning that compound **5a** was selective against UO-3, DU-145 and T-47D cell lines (renal, prostate and breast cancers, respectively) showing growth inhibition percentages 83.57, 77.45 and 57.81 at a single dose test. Interestingly, compound **7e** exhibited promising selectivity against 13 cell lines representing leukemia and cancers of lung, colon, central nervous system, ovary, kidney, prostate as well as breast showing growth inhibition percentages between 54.05 and 89.23.

It was found that compounds (**4a**,**b**) with carbonyl group at C-4 position exhibited potent anticancer activity and **4a** was more potent than **4b**. Introduction of a chloro group at C-4 position in compounds (**5a**,**b**) resulted in a marked decrease in the activity, on the other hand, compound **6a** bearing a hydrazinyl moiety at position 4 was one of the most potent test compounds (these observations were in accordance with the previously reported work [22]). Among the 4-substitutedaminothieno[2,3-d]pyrimidines **7a**–**h** compound **7a** with C-2 2-pyridyl and morpholinyl moiety at C-4 position showed the most potent anticancer activity. Also compounds **7d** and **7g** showed significant anticancer activity.

In the present work, we can conclude:

1 The pyridyl group at C-2 position appeared to have a considerable effect on the anticancer activity since it was found that the best activity was obtained by compounds bearing 2-pyridyl group at the 2 position.
2 The introduction of different substituents at 4 position showed a remarkable effect on the anticancer activity. Compounds with carbonyl, hydrazinyl or substitutedamino group exhibited potent anticancer activity, while those with chloro group at C-4 position were inactive.
3 The bulkiness of substituted amino group at C-4 position appeared to have an effect on the anticancer activity.

Further studies are required to discover the mechanism of action and the effect of varying the substitutions at position 2 and position 4 on optimization of the anticancer activity.



Scheme 1. The synthetic path and reagents for the preparation of the target compounds.

3. Conclusion

Hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidines represent novel and promising class of anticancer agents. Compounds **4a**, **6a**, **7a**, **7d** and **7g** showed potent anticancer activity at low concentrations against most of the used human tumor cell lines when compared to doxorubicin as potent anticancer drug. The effect of substitutions at C-2 and C-4 positions on the anticancer activity was shown.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were obtained on a Griffin apparatus and were 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}H NMR and ^{13}C NMR spectra were performed on JOEL NMR FXQ-300 MHz and JOEL NMR FXQ-500 MHz spectrometers, 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 60 F254 and was visualized by UV lamp.

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

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

*4.1.2.1. 2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno [2,3-d]pyrimidin-4(3H)-one (**4a**).* mp 188–190 °C (ethanol); yield 50%; IR (KBr) ν_{max}: 3097 (NH), 1678 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.25–1.35 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.60–1.75 (m, 4H, $2CH_2$), 2.89–2.92 (t, 2H, J = 5.4 Hz, CH_2), 3.09–3.11 (t, 2H,

Table 1
Growth inhibition percentages obtained from the single dose (10^{-5} M) test.

Panel/Cell line	Compound							
	5a	**5b**	**6b**	**7b**	**7c**	**7e**	**7f**	**7h**
Leukemia								
CCRF-CEM	27.62	23.77	25.88	2.07	5.41	30.98	–	–
HL-60(TB)	3.98	3.12	16.41	24.65	4.37	–	2.53	15.19
K-562	20.23	13.63	24.74	29.28	19.98	79.22	7.55	23.23
MOLT-4	32.28	15.59	9.09	24.54	7.6	47.89	4.82	16.12
RPMI-8226	4.46	0.89	12.05	32.16	12.38	57.54	3.42	28.76
SR	33.68	21.42	15.67	24.06	–	–	5.99	–
Non-small cell lung cancer								
A549/ATCC	0.18	−3.56	−1.81	24.92	22.62	68.23	−0.56	14.1
HOP-62	13.37	−9.05	4.03	1.14	−3.13	36.23	−5.44	0.29
HOP-92	–	–	–	–	–	26.78	33.33	–
NCI-H23	17.63	3.14	7.74	8.29	15.99	36.97	1.33	17.28
NCI-H322M	15.47	3.46	3.42	−14.33	1.03	17.72	−2.96	−7.78
NCI-H460	9.1	−2.59	−4.36	8.02	2.79	46.03	−6.17	5.35
Colon cancer								
COLO 205	−14.58	−16.56	2.31	−0.84	0.37	26.28	−1.65	5.76
HCC-2998	36.56	−12.27	−15.99	−8.59	4.02	13.66	−13.96	−11.5
HCT-116	15.54	2.69	4.13	23.24	14.13	80.46	−3.45	23.3
HCT-15	15.77	0.89	7.32	20.79	2.35	41.35	2.1	17.68
HT29	0.67	−1.58	13.17	17.65	5.9	42.02	12.8	23.44
KM12	8.45	0.47	−0.57	19.62	8.11	55.05	4.95	22.39
SW-620	−2.34	−3.44	−8.49	−9.82	−1.34	24	−9.11	1.53
CNS cancer								
SF-268	5.35	0.85	−17.76	4.06	−1.9	20.42	−1.33	2.5
SF-295	–	–	6.22	19.48	13.81	54.8	6.51	11.47
SNB-75	15.03	9.59	18.8	0.97	14.41	9.19	−23.78	−13.66
SF-539	2.75	6.69	−14.85	15.83	37.37	12.6	1.14	7.87
U251	2.82	−0.32	6.15	21.94	8.31	37.1	0.24	15.85
Melanoma								
LOX IMVI	32.24	5.4	5.88	11.6	4.93	42.2	0	8.33
MALME-3M	5.75	7.42	2.19	−1.71	−0.36	7.96	−6.19	−7.69
M14	13.22	−7.52	−11.92	−15.42	6.12	27.09	−8.43	−0.58
MDA-MB-435	8.64	3.14	1.01	0.03	4.89	34.17	2.52	4.86
SK-MEL-28	10.69	−4.12	4.94	−7.03	−5.08	7.85	−15.02	−5.27
UACC-62	4.04	6.14	10.83	15.5	9.41	41.19	5.59	36.85
Ovarian cancer								
IGROV1	10.21	7.04	−16.49	1.53	6.64	62.89	0.86	3.43
OVCAR-3	−7.89	−5.97	−0.84	15.43	−5.6	27.02	7.59	9.75
OVCAR-4	14.27	14.85	18.72	22.72	15.99	43.97	0.34	11.14
OVCAR-5	−7.94	−5.24	−0.54	−1.32	2.04	36.83	1.59	2.26
OVCAR-8	12.86	−1.87	2.51	15.33	−1.5	44.51	8.55	5.9
NCI/ADR-RES	10.65	−6.46	2.44	18.67	22.71	57.49	5.34	9.28
SK−OV-3	−5.16	−7.46	9.44	0.61	−3.22	12.58	1.97	0.84
Renal cancer								
786-0	1.61	0.46	18.15	32.88	10.04	10.69	1.77	11.09
A498	−18.36	37.24	18.55	33.72	34.81	38.39	4.79	6.97
ACHN	120.39	−0.47	8.69	17.12	4.94	56.07	7.05	19.95
CAKI-1	165.16	−4.99	7.27	15.76	19.99	45.88	8.66	28.83
RXF 393	–	–	–	–	–	32.24	0.87	17.21
SN12C	15.14	4.53	4.3	19.31	3.33	28.9	−12.22	31.76
UO-31	83.57	13.95	20.41	38.71	32.83	55.76	16.13	11.09
Prostate cancer								
PC-3	7.81	0.63	7.42	35.43	15.29	54.05	5.9	44.54
DU-145	77.45	8.02	−30.41	1.4	−11.64	21.09	−4.95	2.73
Breast cancer								
MCF7	45.99	3.18	5.87	28.53	50.4	89.23	2.49	18.15
MDA-MB-231/ATCC	−16.91	−6.99	1.46	28.1	6.82	22.41	2.4	12.06
HS 578T	8.7	6.64	−13.67	−13.29	6.23	6.17	−12.86	−5.59
BT-549	14.87	−2.76	1.1	−20.36	10.28	2.77	−14.13	−17.07
T-47D	57.81	6.79	0.16	19.19	24.87	57.56	11.7	26.97
MDA-MB-468	184.32	39.65	20.15	11.82	5.4	30.07	3.79	0.83

$J = 5.4$ Hz, CH_2), 7.60–7.64 (t, 1H, $J = 6$ Hz, ArH), 8.01–8.06 (t, 1H, $J = 7.8$ Hz, ArH), 8.34 (d, 1H, $J = 7.8$ Hz, ArH), 8.74 (d, 1H, $J = 6$ Hz, ArH) and 11.63 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 24.33, 25.24, 25.37, 26.18, 29.80, 31.45, 121.84, 126.29, 134.16, 136.70, 137.96, 148.05, 149.06, 149.41, 157.26, 162.20, 162.75 ppm; MS [m/z, %]: 311 [M^+, 83.66] and 78 [$C_5H_4N^+$, 100]. Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.39): C, 65.56; H, 5.50; N, 13.49. Found: C, 65.59; H, 5.48; N, 13.45.

*4.1.2.2. 2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno [2,3-d]pyrimidin-4(3H)-one (**4b**).* mp > 300 °C (n-butanol); yield 80%; IR (KBr) ν_{max}: 3163 (NH), 1658 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.20–1.30 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.55–1.65 (m, 4H, $2CH_2$), 2.82–2.90 (m, 2H, CH_2), 3.00–3.10 (m, 2H, CH_2), 8.01 (d, 2H, $J = 5$ Hz, ArH), 8.71 (d, 2H, $J = 5$ Hz, ArH), and 12.73 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 24.93, 25.96, 26.77, 30.43, 31.94, 32.02, 115.81, 121.90, 123.12, 131.02, 148.50, 150.76,

Table 2
Concentrations required for 50% inhibition of cell growth ($\log_{10} GI_{50}$).

Panel/Cell line	Compound					
	4a	**4b**	**6a**	**7a**	**7d**	**7g**
Leukemia						
CCRF-CEM	−6.04	>−4.00	−5.35	−5.45	−5.09	−5.36
HL-60(TB)	−5.47	>−4.00	−5.35	−5.35	−4.90	−5.39
K-562	−6.00	>−4.00	−5.42	−5.46	−5.45	−5.38
MOLT-4	−5.62	>−4.00	−5.44	−5.42	−5.42	−5.49
RPMI-8226	−5.44	>−4.00	−5.49	−5.67	−5.08	−5.43
SR	−6.06	>−4.00	−5.40	–	−5.55	−5.50
Non-small cell lung cancer						
A549/ATCC	−5.59	>−4.00	−5.50	−5.45	−4.94	5.49
HOP-62	−5.20	>−4.00	−5.07	−5.02	−5.19	−4.94
HOP-92	−5.41	>−4.00	−5.45	−6.21	−6.18	−5.49
NCI-H226	>−4.00	>−4.00	−4.99	−5.30	−4.98	−5.19
NCI-H23	−4.96	>−4.00	−5.05	−5.22	−4.94	−4.77
NCI-H322M	−4.07	>−4.00	−5.27	−5.26	−4.88	−5.26
NCI-H460	−5.72	>−4.00	−5.38	−5.23	−4.87	−5.33
NCI-H522	>−4.00	>−4.00	−4.92	−5.23	−4.92	−4.73
Colon Cancer						
COLO 205	−5.23	>−4.00	−5.29	−5.03	−5.31	−5.25
HCC-2998	−4.58	>−4.00	−5.08	−5.16	−5.41	−4.99
HCT-116	−5.86	–	−5.45	−5.43	−5.29	−5.49
HCT-15	−5.63	>−4.00	−5.95	−5.47	−4.95	−5.37
HT29	−5.11	>−4.00	−5.26	−5.22	−5.32	−5.30
KM12	−5.39	>−4.00	−5.19	−5.37	−4.87	−5.29
SW-620	−5.31	>−4.00	−5.34	−5.05	−4.92	−5.29
CNS cancer						
SF-268	−5.06	>−4.00	−5.14	−5.05	−5.27	>−4.00
SF-295	–	–	–	−5.47	–	–
SNB-19	–	–	–	−5.08	–	–
SNB-75	−4.07	−5.57	−4.90	−5.15	−5.86	−4.58
SF-539	−5.11	>−4.00	−4.96	−4.89	−5.08	−4.17
U251	−5.67	>−4.00	−5.35	−5.36	−5.03	−5.37
Melanoma						
LOX IMVI	−5.96	>−4.00	−5.21	−5.28	−5.08	−5.20
MALME-3M	–	>−4.00	–	−5.48	–	–
M14	−5.39	>−4.00	−5.26	−5.38	−5.27	−5.27
MDA-MB-435	>−4.00	>−4.00	−5.29	−5.32	−4.93	−5.26
SK-MEL-2	>−4.00	>−4.00	−4.84	−5.10	−4.84	−4.74
SK-MEL-28	−5.22	>−4.00	−4.83	−4.96	−4.90	>−4.00
SK-MEL-5	−5.53	>−4.00	−5.50	−5.70	−5.10	−5.55
UACC-257	>−4.00	>−4.00	−5.18	−4.96	−4.97	−5.14
UACC-62	−5.26	>−4.00	−5.23	−5.46	−4.96	−5.16
Ovarian Cancer						
IGROV1	−5.40	>−4.00	−5.38	−5.50	−5.09	−5.27
OVCAR-3	−5.07	>−4.00	−5.27	−5.34	−4.87	−5.06
OVCAR-4	−5.23	–	−4.98	−5.31	−4.90	−5.07
OVCAR-5	4.04	>−4.00	−5.06	−4.99	−4.97	−4.29
OVCAR-8	−5.36	>−4.00	−5.36	−5.29	−4.91	−5.28
NCI/ADR-RES	−6.02	–	−6.34	−6.08	−5.09	−5.32
SK−OV-3	>−4.00	–	−4.51	−5.03	−4.92	>−4.00
Renal cancer						
786-0	−5.25	>−4.00	−5.25	−4.93	−5.32	−5.07
A498	−5.88	>−4.00	−5.73	−5.78	−5.98	−5.48
ACHN	−5.28	>−4.00	−5.31	−5.35	−5.10	−5.29
CAKI-1	−5.48	>−4.00	−6.24	−5.64	−5.46	−5.27
RXF 393	−5.13	>−4.00	−5.17	−5.08	−5.39	>−4.00
SN12C	−5.20	>−4.00	−5.20	−5.30	−5.25	−5.22
TK-10	>−4.00	>−4.00	−5.04	−5.03	−5.17	>−4.00
UO-31	−5.23	>−4.00	−5.99	−5.93	−5.80	−5.48
Prostate cancer						
PC-3	−5.39	>−4.00	−5.40	−5.57	−5.33	−5.48
DU-145	−5.17	>−4.00	−5.02	−5.16	−4.90	−4.55
Breast cancer						
MCF7	5.61	>−4.00	−5.42	−5.52	−5.18	−5.50
MDA-MB-231/ATCC	−5.06	>−4.00	−4.90	−5.13	−5.37	−4.30
HS 578T	−5.19	>−4.00	−4.96	−5.47	−5.64	−4.24
BT-549	−5.25	>−4.00	−5.01	−5.36	−5.20	−5.16
T-47D	−5.26	–	−5.30	−5.35	−4.87	−5.20
MDA-MB-468	−5.78	>−4.00	−5.44	−5.38	−5.28	−5.39

159.08, 162.21, 168.49 ppm; MS [m/z, %]: 311 [M^+, 100]. Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.39): C, 65.56; H, 5.50; N, 13.49. Found: C, 65.65; H, 5.51; N, 13.51.

*4.1.3. General procedure for the preparation of 4-chloro-2-pyridyl-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidines (**5a**,**b**)*

A mixture of thienopyrimidone **4a**,**b** (0.003 mol) and phosphorus oxychloride (15 mL) was refluxed for 1 h. The reaction mixture was concentrated under reduced pressure then poured into ice cold water (100 mL). The precipitated solid was filtered, washed with water (2 × 10 mL), dried and crystallized from ethanol.

*4.1.3.1. 4-chloro-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5] thieno[2,3-d]pyrimidine (**5a**).* mp 118–120 °C; yield 60%; IR (KBr) ν_{max}: 1581 (C═N), 1550 (C═C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.15–1.25 (m, 2H, CH_2), 1.40–1.47 (m, 2H, CH_2), 1.60–1.68 (m, 2H, CH_2), 1.69–1.75 (m, 2H, CH_2), 2.99–3.01 (t, 2H, J = 5.4 Hz, CH_2), 3.11–3.13 (t, 2H, J = 5.4 Hz, CH_2), 7.50–7.52 (t, 1H, J = 3.85 Hz, ArH), 7.94–7.96 (t, 1H, J = 8.35 Hz, ArH), 8.36 (d, 1H, J = 8.35 Hz, ArH) and 8.73 (d, 1H, J = 3.85 Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.93, 25.48, 26.20, 27.89, 30.43, 31.74, 124.11, 125.74, 127.13, 129.73, 137.74, 144.21, 150.34, 152.99, 153.45, 157.18, 169.24 ppm; MS [m/z, %]: 331 [$(M+2)^+$, 9.95], 329 [M^+, 26.19] and 78 [$C_5H_4N^+$, 100]. Anal. Calcd for $C_{17}H_{16}ClN_3S$ (329.83): C, 61.90; H, 4.89; N, 12.73. Found: C, 62.40; H, 4.93; N, 12.84.

*4.1.3.2. 4-chloro-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5] thieno[2,3-d]pyrimidine (**5b**).* mp 194–196 °C; yield 65%; IR (KBr) ν_{max}: 1631 (C═N), 1550(C═C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.15–1.25 (m, 2H, CH_2), 1.37–1.50 (m, 2H, CH_2), 1.65–1.75 (m, 4H, $2CH_2$), 3.03–3.06 (t, 2H, J = 6.1 Hz, CH_2), 3.12–3.13 (t, 2H, J = 6.1 Hz, CH_2), 8.63 (d, 2H, J = 5 Hz, ArH) and 8.97 (d, 2H, J = 5 Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.40, 24.92, 25.58, 27.38, 29.89, 31.21, 122.57, 127.37, 129.67, 145.16, 145.67, 147.67, 152.64, 154.14, 168.62 ppm; MS [m/z, %]: 331 [$(M+2)^+$, 60.31], 329 [M^+, 100]. Anal. Calcd for $C_{17}H_{16}ClN_3S$ (329.83): C, 61.90; H, 4.89; N, 12.73. Found: C, 61.88; H, 4.88; N, 12.73.

*4.1.4. General procedure for the preparation of 4-hydrazinyl-2-pyridyl-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d] pyrimidines (**6a**,**b**)*

A mixture of chloro derivative **5a**,**b** (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 separated solid was filtered, dried and crystallized from ethanol.

*4.1.4.1. 4-hydrazinyl-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**6a**).* mp 172–174 °C; yield 76%; IR (KBr) ν_{max}: 3363, 3300 (NH/NH_2), 1593 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.30–1.37 (m, 2H, CH_2), 1.40–1.45 (m, 2H, CH_2), 1.68–1.78 (m, 4H, $2CH_2$), 2.88–3.01 (m, 4H, $2CH_2$), 4.64 (s, 2H, NH_2, D_2O exchangeable), 7.51–7.55 (m, 1H, ArH), 7.90–8.00 (t, 1H, ArH), 8.31 (s, 1H, NH, D_2O exchangeable), 8.40–8.50 (m, 1H, ArH) and 8.65–8.80 (m, 1H, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 23.19, 25.18, 25.46, 26.48, 29.50, 30.91, 117.02, 119.20, 119.83, 125.06, 129.88, 137.19, 141.31, 150.15, 156.80, 157.11, 168.43 ppm; MS [m/z, %]: 325 [M^+, 4.11], 57 [100]. Anal. Calcd for $C_{17}H_{19}N_5S$ (325.42): C, 62.73; H, 5.88; N, 21.52. Found: C, 62.63; H, 5.87; N, 21.48.

*4.1.4.2. 4-hydrazinyl-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**6b**).* mp 212–214 °C; yield 80%; IR (KBr) ν_{max}: 3305, 3300 (NH/NH_2), 1593 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.10–1.15 (m, 2H, CH_2), 1.37–1.45 (m, 2H, CH_2), 1.65–1.75 (m, 4H, $2CH_2$), 2.85–2.90 (t, 2H, J = 6 Hz, CH_2), 3.03–3.07 (t, 2H, $J = 6$ Hz, CH_2), 4.85 (s, 2H, NH_2, D_2O exchangeable), 8.20 (s, 1H, NH, D_2O exchangeable), 8.31 (d, 2H, $J = 5$ Hz, ArH) and 8.67 (d, 2H, $J = 5$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.32, 24.88, 25.57, 27.06, 29.99, 31.55, 114.22, 121.62, 129.36, 136.51, 144.99, 149.98, 155.29, 157.87, 164.49 ppm; MS [*m/z*, %]: 325 [M^+, 0.94] and 295 [$C_{17}H_{17}N_3S^+$, 100]. Anal. Calcd for $C_{17}H_{19}N_5S$ (325.42): C, 62.73; H, 5.88; N, 21.52. Found: C, 62.47; H, 5.85; N, 21.43.

*4.1.5. General procedure for the preparation of 2-pyridyl-4-substitutedamino-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d] pyrimidines (**7a–h**)*

A mixture of **5a**,**b** (0.001 mol), the selected secondary 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 crystallized from ethanol.

*4.1.5.1. 4-(morpholin-4-yl)-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7a**).* mp 120–122 °C; yield 79%; IR (KBr) ν_{max}: 1581 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.10 (m, 2H, CH_2), 1.35–1.40 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 1.65–1.70 (m, 2H, CH_2), 2.90–2.95 (m, 2H, CH_2), 3.00–3.05 (m, 2H, CH_2), 3.35–3.45 (m, 4H, –CH_2–N–CH_2–), 3.70–3.80 (m, 4H, –CH_2–O–CH_2–), 7.42–7.50 (t, 1H, $J = 6$ Hz, ArH), 7.90–7.92 (t, 1H, $J = 7.65$ Hz, ArH), 8.38 (d, 1H, $J = 7.65$ Hz, ArH) and 8.70 (d, 1H, $J = 6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.50, 24.94, 25.51, 26.99, 30.72, 31.24, 50.92, 65.77, 118.95, 123.39, 124.46, 129.56, 136.80, 139.65, 149.44, 154.66, 156.15, 162.18, 168.20; MS [*m/z*, %]: 380 [M^+, 59.33], 337 [100]. Anal. Calcd for $C_{21}H_{24}N_4OS$ (380.49): C, 66.28; H, 6.35; N, 14.72. Found: C, 66.04; H, 6.33; N, 14.67.

*4.1.5.2. 4-(morpholin-4-yl)-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7b**).* mp 145–147 °C; yield 67%; IR (KBr) ν_{max}: 1597 (C=N), 1546 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.15 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 1.65–1.70 (m, 2H, CH_2), 2.90–3.05 (m, 4H, 2CH_2), 3.35–3.45 (m, 4H, –CH_2–N–CH_2–), 3.75–3.85 (m, 4H, –CH_2–O–CH_2–), 8.23 (d, 2H, $J = 6$ Hz, ArH) and 8.71 (d, 2H, $J = 6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.51, 24.91, 25.51, 27.01, 30.72, 31.25, 50.83, 65.74, 119.16, 121.41, 129.74, 140.05, 144.41, 150.25, 154.42, 162.09, 167.98 ppm; MS [*m/z*, %]: 380 [M^+, 100]. Anal. Calcd for $C_{21}H_{24}N_4OS$ (380.49): C, 66.28; H, 6.35; N, 14.72. Found: C, 66.22; H, 6.35; N, 14.71.

*4.1.5.3. 4-(4-methylpiperazin-1-yl)-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7c**).* mp 118–120 °C; yield 64%; IR (KBr) ν_{max}: 1630 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.15 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 1.65–1.75 (m, 2H, CH_2), 2.63 (s, 3H, NCH_3), 2.90–2.95 (m, 2H, CH_2), 3.00–3.10 (m, 6H, CH_2 and –CH_2–N(CH_3)–CH_2–), 3.30–3.50 (m, 4H, –CH_2–N–CH_2–), 7.46–7.50 (t, 1H, $J = 4.5$ Hz, ArH), 7.92–7.97 (t, 1H, $J = 7.8$ Hz, ArH), 8.40 (d, 1H, $J = 7.8$ Hz, ArH) and 8.71 (d, 1H, $J = 3.9$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.42, 24.93, 25.53, 26.99, 30.73, 31.23, 42.69, 49.83, 53.87, 118.82, 123.36, 124.41, 129.62, 136.79, 139.44, 149.42, 154.69, 156.06, 162.05, 168.13 ppm; MS [*m/z*, %]: 393 [M^+, 5.83], 323 [$C_{18}H_{19}N_4S^+$, 100]. Anal. Calcd for $C_{22}H_{27}N_5S$ (393.54): C, 67.13; H, 6.91; N, 17.79. Found: C, 66.98; H, 6.90; N, 17.75.

*4.1.5.4. 4-(4-methylpiperazin-1-yl)-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7d**).* mp 264–266 °C; yield 68%; IR (KBr) ν_{max}: 1597 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.10–1.15 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.55–1.65 (m, 2H, CH_2), 1.70–1.75 (m, 2H, CH_2), 2.77 (s, 3H, NCH_3), 2.95–3.05 (m, 2H, CH_2), 3.20–3.40 (m, 6H, CH_2 and –CH_2–N(CH_3)–CH_2–), 3.70–3.90 (m, 4H, –CH_2–N–CH_2–), 8.27 (d, 2H, $J = 6$ Hz, ArH) and 8.74 (d, 2H, $J = 6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.55, 24.94, 25.47, 26.99, 30.79, 31.21, 42.29, 47.46, 51.90, 119.14, 121.45, 129.66, 140.44, 144.27, 150.28, 154.31, 161.06, 169.02 ppm; MS [*m/z*, %]: 393 [M^+, 6.08] and 70 [$C_4H_8N^+$, 100]. Anal. Calcd for $C_{22}H_{27}N_5S$ (393.54): C, 67.13; H, 6.91; N, 17.79. Found: C, 67.03; H, 6.90; N, 17.76.

*4.1.5.5. 4-(4-phenylpiperazin-1-yl)-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7e**).* mp 128–130 °C; yield 80%; IR (KBr) ν_{max}: 1597 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.15 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.53–1.60 (m, 2H, CH_2), 1.65–1.75 (m, 2H, CH_2), 2.93–3.00 (m, 2H, CH_2), 3.05–3.10 (m, 2H, CH_2), 3.15–3.25 (t, 4H, $J = 5.4$ Hz, –CH_2–N(C_6H_5)–CH_2–), 3.50–3.60 (m, 4H, –CH_2–N–CH_2–), 6.79–6.83 (t, 1H, $J = 7.2$ Hz, ArH), 7.00 (d, 2H, $J = 8.4$ Hz, ArH), 7.21–7.26 (t, 2H, $J = 7.2$ Hz, ArH), 7.46–7.50 (t, 1H, $J = 6$ Hz, ArH), 7.92–7.97 (t, 1H, $J = 7.8$ Hz, ArH), 8.43 (d, 1H, $J = 7.8$ Hz, ArH) and 8.73 (d, 1H, $J = 6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.47, 24.92, 25.50, 26.98, 30.66, 31.21, 42.40, 45.32, 47.99, 50.34, 115.59, 115.90, 119.16, 119.80, 120.51, 123.34, 124.42, 128.86, 128.97, 129.57, 136.78, 139.55, 149.94, 150.76, 156.07, 162.17, 168.15 ppm; MS [*m/z*, %]: 455 [M^+, 8.05], 323 [$C_{18}H_{19}N_4S^+$, 100]. Anal. Calcd for $C_{27}H_{29}N_5S$ (455.60): C, 71.17; H, 6.41; N, 15.37. Found: C, 71.24; H, 6.42; N, 15.38.

*4.1.5.6. 4-(4-phenylpiperazin-1-yl)-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7f**).* mp 198–200 °C; yield 91%; IR (KBr) ν_{max}: 1597 (C=N), 1543 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.10 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.55–1.60 (m, 2H, CH_2), 1.65–1.70 (m, 2H, CH_2), 2.95–3.00 (m, 2H, CH_2), 3.01–3.05 (m, 2H, CH_2), 3.15–3.17 (t, 4H, $J = 5.4$ Hz, –CH_2–N(C_6H_5)–CH_2–), 3.54–3.60 (m, 4H, –CH_2–N–CH_2–), 6.80–6.83 (t, 1H, $J = 8.4$ Hz, ArH), 6.94 (d, 2H, $J = 8.4$ Hz, ArH), 7.20–7.23 (t, 2H, $J = 8.4$ Hz, ArH), 8.23 (d, 2H, $J = 6.1$ Hz, ArH) and 8.70 (d, 2H, $J = 6.1$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.50, 24.93, 25.30, 27.03, 30.70, 31.25, 42.48, 45.38, 48.02, 50.29, 115.69, 115.95, 119.25, 119.87, 121.42, 128.93, 129.02, 129.84, 139.99, 149.98, 150.25, 154.42, 162.20 ppm; MS [*m/z*, %]: 455 [M^+, 9.75], 69 [100]. Anal. Calcd for $C_{27}H_{29}N_5S$ (455.60): C, 71.17; H, 6.41; N, 15.37. Found: C, 71.10; H, 6.40; N, 15.35.

*4.1.5.7. 4-[4-(4-methoxyphenyl)-piperazin-1-yl]-2-(2-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7g**).* mp 158–160 °C; yield 93%; IR (KBr) ν_{max}: 1590 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.15 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 1.62–1.70 (m, 2H, CH_2), 2.90–3.00 (m, 2H, CH_2), 3.02–3.10 (m, 2H, CH_2), 3.15–3.25 (m, 4H, –CH_2–N(4-$CH_3OC_6H_4$)–CH_2–), 3.45–3.55 (m, 4H, –CH_2–N–CH_2–), 3.66 (s, 3H, CH_3O), 6.81 (d, 2H, $J = 8.4$ Hz, ArH), 6.93 (d, 2H, $J = 8.4$ Hz, ArH), 7.44–7.46 (t, 1H, $J = 4.6$ Hz, ArH), 7.90–7.93 (t, 1H, $J = 7.65$ Hz, ArH), 8.39 (d, 1H, $J = 7.65$ Hz, ArH) and 8.69 (d, 1H, $J = 4.6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.51, 24.95, 25.52, 27.00, 30.70, 31.24, 42.63, 45.36, 50.37, 114.33, 115.64, 115.94, 117.64, 118.03, 119.20, 119.86, 124.47, 128.92, 129.02, 129.64, 139.61, 149.99, 150.82, 162.24 ppm; MS [*m/z*, %]: 485 [M^+, 12.53] and 323 [$C_{18}H_{19}N_4S^+$, 100]. Anal. Calcd for $C_{28}H_{31}N_5OS$ (485.63): C, 69.24; H, 6.43; N, 14.42. Found: C, 69.09; H, 6.42; N, 14.39.

*4.1.5.8. 4-[4-(4-methoxyphenyl)-piperazin-1-yl]-2-(4-pyridyl)-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine (**7h**).* mp 162–164 °C; yield 81%; IR (KBr) ν_{max}: 1597 (C=N), 1550 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.05–1.15 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.55–1.60 (m, 2H, CH_2), 1.65–1.75 (m, 2H, CH_2), 2.95–3.04 (m, 2H, CH_2), 3.05–3.10 (m, 2H, CH_2), 3.20–3.30 (m, 4H, –CH_2–N(4-

$CH_3OC_6H_4$)–CH_2–), 3.55–3.60 (m, 4H, –CH_2–N–CH_2–), 3.68 (s, 3H, CH_3O), 6.84 (d, 2H, $J = 9.3$ Hz, ArH), 6.84 (d, 2H, $J = 9.3$ Hz, ArH), 8.28 (d, 2H, $J = 6$ Hz, ArH) and 8.74 (d, 2H, $J = 6$ Hz, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 24.50, 24.93, 25.52, 27.04, 30.71, 31.25, 49.42, 50.41, 55.16, 114.29, 117.70, 119.23, 121.42, 129.84, 139.94, 144.45, 145.13, 150.26, 153.20, 154.41, 162.16, 167.96 ppm; MS [*m/z*, %]: 485 [M^+, 12.46], 323 [$C_{18}H_{19}N_4S^+$, 100]. Anal. Calcd for $C_{28}H_{31}N_5OS$ (485.63): C, 69.24; H, 6.43; N, 14.42. Found: C, 69.18; H, 6.42; N, 14.40.

4.2. Measurement of anticancer activity

Anticancer activity screening of the newly synthesized compounds was measured *in vitro* utilizing 60 different human tumor cell lines provided by US National Cancer Institute according to previously reported standard procedure [25–27] as follows:

Cells are inoculated into 96-well microtiter plates in 100 μL. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (*Tz*). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four-, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (*Tz*), control growth, (*C*), and test growth in the presence of drug at the five concentration levels (*Ti*)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$

$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz.$$

For each experimental agent, growth inhibition of 50% (GI_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation, however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.02.011. These data include MOL files and InChiKeys of the most important compounds described in this article.

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