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Design, synthesis and *in vitro* anticancer activity of novel *S*-alkyl thieno[2,3-*d*] pyrimidinone derivatives

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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, a series of S-alkyl thieno[2,3-d] pyrimidinone was designed and synthesized to investigate the effect of varying the linker and aliphatic or aromatic amine on the anticancer activity. The structure of the synthesized compounds has been elucidated on the basis of elemental analyses and spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-NMR and MS). The in vitro cytotoxic activity of the newly synthesized compounds was evaluated against two human cell lines: prostate cancer (PC-3) and colon cancer (HCT-116). Compounds **4a**, **4b**, **11b** and **11c** exhibited potent anticancer activity against PC-3 cell line while **11b** and **11c** displayed promising anticancer activity against HCT-116. Compound **4a** exhibited 5.78 fold more potent activity against PC-3 while compound **11b** displayed 3.62 fold higher activity against HCT-116 compared to Imatinib.*

Keywords: Synthesis, Thieno[2,3-*d*]pyrimidines, Anticancer activity, Prostate cancer, Colon cancer.

INTRODUCTION

The discovery of new leads with anticancer potential is still a great interest of medicinal chemists, hoping that they may design more selective and safer anticancer agents. In recent years, thieno[2,3-*d*] pyrimidin-4-one derivatives, which are analogs of quinazoline alkaloids, have frequently attracted the interest of medicinal chemistry researchers due to their promising anticancer properties [1]. Wang *et al.* [2] identified the thieno[2,3-*d*] pyrimidine **I** (Fig. 1) as potent anticancer lead where the cycloalkyl ring fused to thiophene was a major component for the antitumor activity that was affected by changing the size of the cycloalkyl ring.

Moreover, 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. These mechanisms include the inhibition of; cyclin dependent kinases (CDKs) [3-5]; epidermal growth factor receptor (EGFR) [6]; methionine synthase [7]; *17β*-HSD1 [8]; matrix metalloproteinases (MMPs) [9-11] and P-glycoprotein (P-gp) modulation [12] (Fig.2). Consequently, the thieno[2,3-*d*]pyrimidine ring system continues to constitute an attractive target for the design of new anticancer drugs through wide structure variations.

We were encourged by the potent anticancer activity of thieno[2,3-*d*] pyrimidine derivatives (Fig.2) together with the fact that many functions like amide linkages [13], thioether [14-16] and piperazine scaffolds [17] are known to contribute to the enhancement of the antitumor activity. Therefore, various cycloalkyl thieno[2,3-*d*] pyrimidine derivatives combing theses bioactive moieties were designed and synthesized. Structural variations at C2 and N3 of

the pyrimidine ring of lead compound **I** were performed to study the effect of these variations on the cytotoxic activity.

In the present study, a series of 3-benzyl cycloalkyl thieno[2,3-*d*]pyrimidines bearing various S-(substituted amino alkyl) moieties at position 2 (compounds **4a–d**, **6a-c** and **8a–c**) was synthesized. In this series, different alkyl linkers and different aliphatic and aromatic amines were used to study the effect of these variations on the cytotoxic activity.

On the other hand, a novel series of 2-Alkyl-3-acylamino-5,6,7,8-tetrahydo[1]benzothieno[2,3-*d*] pyrimidin-4(*3H*)-one was prepared (compounds **11a-d**) to investigate the effect of such structure variation on the anticancer activity. All compounds were tested for possible anti-cancer activity on two cell lines (PC-3 and HCT-116).

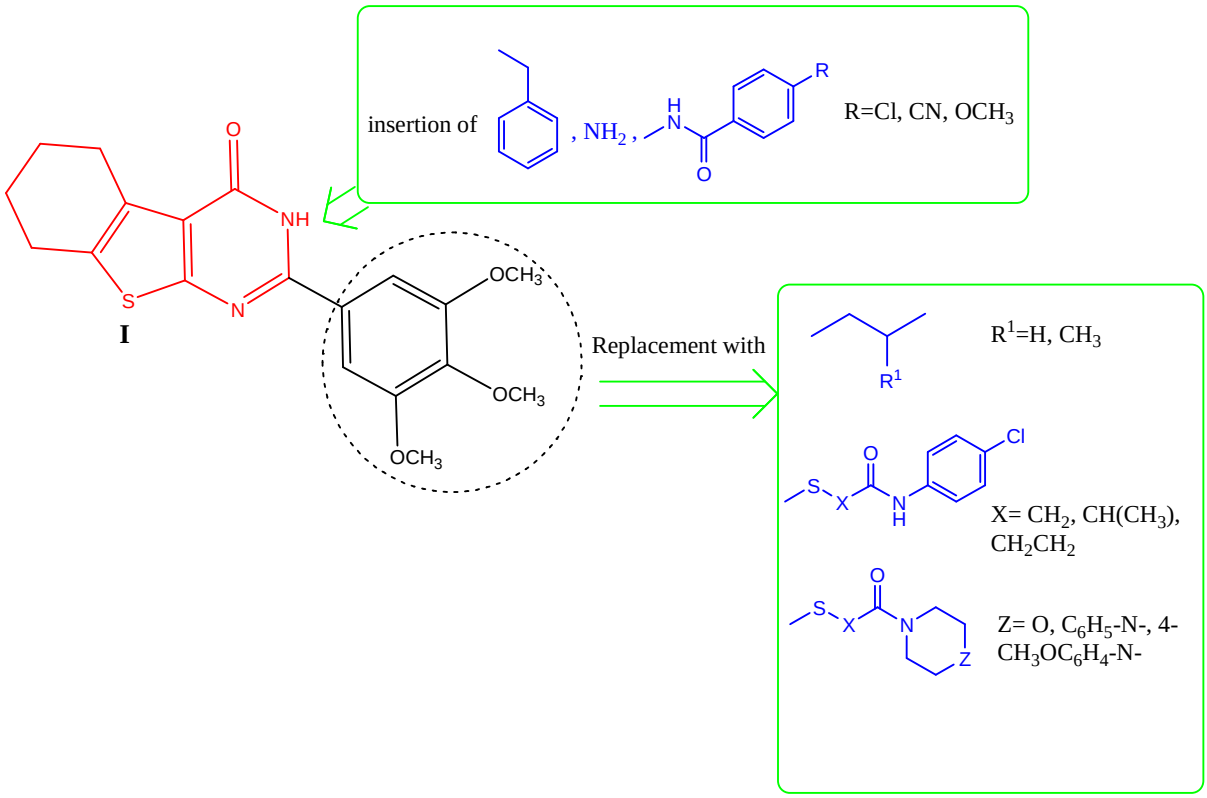
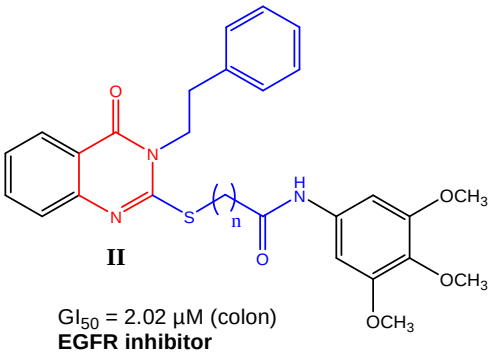
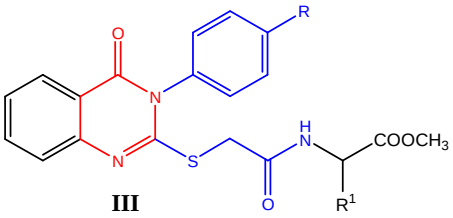


Fig. 1 The potent anticancer lead (I) and the suggested strategies for structural modifications.



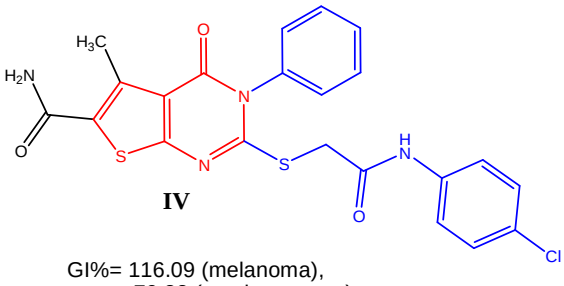
II

GI_{50} = 2.02 µM (colon)
EGFR inhibitor
n=1,2



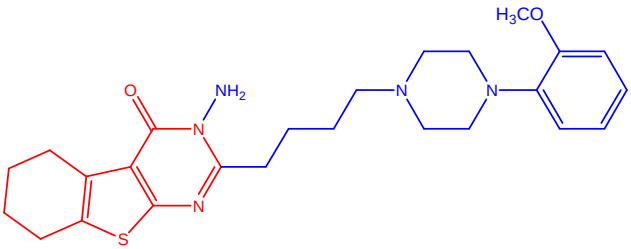
III

IC_{50}=20.6-34 µg/mL (PC-3)
Methionine Synthase inhibitors
R= H, CH_3, OCH_3, OCH_3
R_1= H, CH_2OH



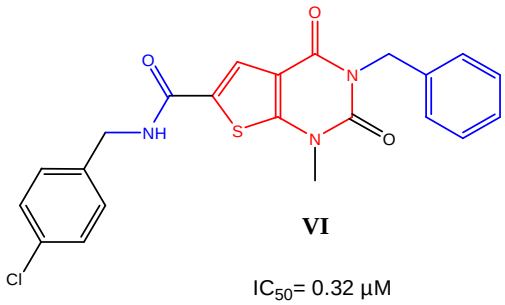
IV

GI%= 116.09 (melanoma),
79.38 (ovarian cancer),
47.22 (Renal cancer)



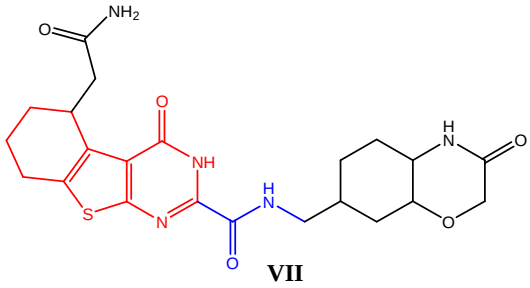
V

IC_{50}= 2.01 µg/ml (HCT-116),
1.07 (HELA)
1.61 (HEP2)



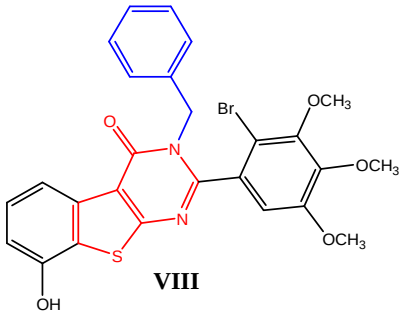
VI

IC_{50}= 0.32 µM
MMP-13 inhibitor



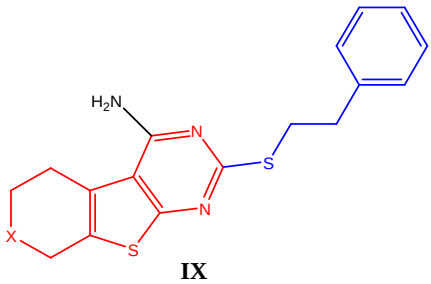
VII

IC_{50}= 7.4 nM
MMP-8 inhibitor



VIII

IC_{50}= 5nM
17ßHSD1inhibitor



IX

EC_{50}=0.56-2.24 µM
P-glycoprotein modulator
X= CH_2, O, N-Bn

Fig. 2 Structures of Thieno[2,3-*d*]pyrimidnes with cytotoxic activity showing the possible chemical optimization.

MATERIALS AND METHODS

Chemistry

All Melting points were determined with Stuart SMP10 apparatus and the values given are uncorrected. IR spectra (KBr, cm^{-1}) were determined on Shimadzu IR 8400s spectrophotometer (Faculty of Pharmacy, Cairo University, Egypt). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Mercury 300-BB 300 MHz (Microanalytical Center, Cairo University, Egypt) and Bruker 400-BB 400 MHz spectrometers (Microanalytical Unit, Faculty of Pharmacy, Cairo University, Egypt) using TMS as an internal standard. Chemical shift values are recorded in ppm on δ scale. Mass spectra were recorded on Hewlett Packard 5988 spectrometer (Microanalytical Center, Cairo University, Egypt). Elemental analyses were carried out at the Regional center for Mycology and Biotechnology, Faculty of Pharmacy, Al Azhar University, Egypt; found values were within ± 0.35% of the theoretical ones. Progress of the reactions was monitored by TLC using aluminium sheets precoated with UV fluorescent silica gel (Merck 60F 254) and visualized using UV lamp. The solvent system used was chloroform: benzene: methanol [9:5:2]. The starting compounds, Ethyl 2-amino-4,5,6,7-tetrahydro [1] benzothiophene-3-carboxylate **(1)** [18], 3-Benzyl-2-sulphanyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*] pyrimidin-4(*3H*)-one **(2)** [19] and the α- or β-chloroamides (**3a-d**, **5a-c** and **7a-c**) [20-26] were prepared according to reported methods.

General procedure for the preparation of 3-benzy-2-[ω (substituted aminocarbonyl alkyl)sulphanyl]-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (4a-d, 6a-d, 8a-d)

Method A:

A mixture of **2** (0.002 mol), the appropriate α- or β-chloroamides (0.002 mol) and potassium hydroxide (0.22 g, 0.004 mol) in dry DMF (20 ml) was heated under reflux for 20-23 h. After cooling, the reaction mixture was concentrated under reduced pressure, cooled and the residue was triturated with cold water (20 ml). The separated solid was collected by filtration, washed with 5% NaOH (10 ml) and crystallized from the appropriate solvent, (Table 1).

Method B: **(4d** and **6c)**

To a well stirred solution of **2** (0.001 mol) in aqueous potassium hydroxide solution (10%, 20 ml), the appropriate α-chloroamides (**3d, 5c**) (0.001 mol) was added and the reaction mixture was stirred at room temperature for 25 h. The separated product was collected by filtration and crystallized from the appropriate solvent to afford the title compounds, (Table 1).

Method C: **(4a)**

To a solution of **2** (0.001 mol) in alcoholic solution of sodium hydroxide (2%, 30 ml), the appropriate α-chloroamide (**3a**) (0.002 mol) was added and the suspension was stirred at room temperature for 25 h. The white precipitate was filtered off and crystallized from ethyl acetate, (Table 1).

Method D: (**4b, 6a** and **8a**)

To a mixture of **2** (0.001 mol) and anhydrous potassium carbonate (0.34 g, 0.0025 mol) in dry acetone (30 ml), the appropriate α- or β-chloroamides (**3b, 5a, 7a**) (0.0015 mol) was added and the reaction mixture was heated under reflux for 20 h. The mixture was filtered off while hot and the filterate after concentration under reduced pressure was poured onto ice cold water (20 ml) and the precipitate formed was collected by filtration, dried and crystallized from the appropriate solvent to afford the title compounds, (Table 1).

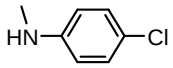
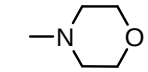
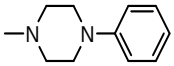
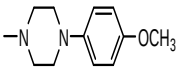
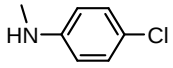
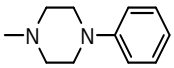
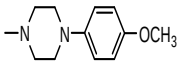
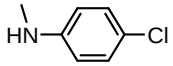
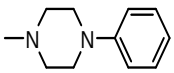
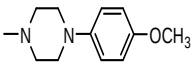
N-(4-chlorophenyl)-2-[(3-benzyl-4-oxo-3,4-dihydro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-2-yl)sulfanyl] acetamide (4a)

IR (KBr, cm^{-1}): 3340 (NH), 2935, 2820 (CH aliphatic), 1681, 1664 (2 C=O), 1595 1535, 1510 (C=C aromatic);^{1}H-NMR (DMSO-d_6) δ: 1.77-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.70-2.72 (m, 2H, CH_2 at C-5), 2.84-2.86 (m, 2H, CH_2 at C-8), 4.14 (s, 2H, S$\underline{CH_2}$), 5.31 (s, 2H, N$\underline{CH_2}C_6H_5$), 7.24-7.59 (m, 9H, Ar-H), 10.45 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 21.67, 22.38, 24.38, 25.11, 37.22, 46.44, 117.94, 120.67, 126.74, 127.00, 127.37, 127.525, 127.527, 128.52, 128.61, 130.66, 131.29, 135.43, 137.76, 156.47, 157.32, 165.39; EIMS (% rel. abundance): 497 (M+2, 1.66), 495 (M^+, 7.56), 91 ($C_7H_7^{1+}$, 100).

3-Benzyl-2-{[2-(morpholino)-2-oxoethyl]sulfanyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d] pyrimidin-4(3H)-one (4b)

IR (KBr) cm^{-1}: 3040, 3020 (CH aromatic), 2981, 2848 (CH aliphatic), 1681, 1654 (2 C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.78-1.83 (m, 4H, 2 CH_2 at C-6, C-7), 2.72-2.79 (m, 2H, CH_2 at C-5), 2.85-2.88 (m, 2H, CH_2 at C-8), 3.43-3.46 (m, 4H, $\underline{CH_2}$-N), 3.56-3.62 (m, 4H, $\underline{CH_2}$-O), 4.22 (s, 2H, S$\underline{CH_2}$), 5.30 (s, 2H, N$\underline{CH_2}C_6H_5$), 7.23-7.36 (m, 5H, Ar-H); EIMS (% rel. abundance): 455 (M^+, 16.14), 91($C_7H_7^{1+}$, 100).

Table 1: Physical and Analytical data of compounds 4a-d, 6a-c, 8a-c

Compound	NR	mp (°C)	Yield %	Crystalization solvent	Molecular formula (M.wt)	Analysis %	
						Calcd	Found
4a		220-222 Method C	50	Ethyl acetate	$C_{25}H_{22}ClN_3O_2S_2$ 496.05	C 60.53 H 4.47 N 8.47	60.67 4.49 8.65
4b		158-160 Method D	55	Acetonitrile	$C_{23}H_{25}N_3O_3S_2$ 455.59	C 60.63 H 5.53 N 9.22	60.85 5.61 9.39
4c		194-196 Method A	25	Absolute ethanol	$C_{29}H_{30}N_4O_2S_2$ 530.71	C 65.63 H 5.70 N 10.56	65.74 5.73 10.67
4d		150-152 Method B	37	Absolute ethanol	$C_{30}H_{32}N_4O_3S_2$ 560.73	C 64.26 H 5.75 N 9.99	64.38 5.79 10.13
6a		261-263 Method D	55	Acetonitrile	$C_{26}H_{24}ClN_3O_2S_2$ 510.05	C 61.22 H 4.74 N 8.24	61.36 4.80 8.37
6b		164-166 Method A	35	Absolute ethanol	$C_{30}H_{32}N_4O_2S_2$ 544.73	C 66.15 H 5.92 N 10.29	66.29 5.97 10.45
6c		170-172 Method B	51	Absolute ethanol	$C_{31}H_{34}N_4O_3S_2$ 574.76	C 64.78 H 5.96 N 9.75	64.89 5.99 9.87
8a		194-196 Method D	61	Hexane/ethanol	$C_{26}H_{24}ClN_3O_2S_2$ 510.05	C 61.22 H 4.74 N 8.24	61.39 4.72 8.46
8b		154-156 Method A	72	Absolute ethanol	$C_{30}H_{32}N_4O_2S_2$ 544.73	C 66.15 H 5.92 N 10.29	66.32 5.90 10.38
8c		140-142 Method A	67	DMF/H_2O	$C_{31}H_{34}N_4O_3S_2$ 574.76	C 64.78 H 5.96 N 9.75	64.86 6.01 9.87

3-Benzyl-2-{[2-(4-phenylpiperazin-1-yl)-2-oxoethyl]sulfanyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (4c)

IR (KBr) cm^{-1}: 2922, 2852 (CH aliphatic), 1681, 1653 (2 C=O), 1600, 1508 (C=C aromatic); ^{1}H-NMR (DMSO-d_6) δ: 1.76-1.83 (m, 4H, 2 CH_2 at C-6, C-7), 2.72-2.79 (m, 2H, CH_2 at C-5), 2.85-2.88 (m, 2H, CH_2 at C-8), 3.11-3.23 (m, 4H, 2 CH_2 piperazine), 3.59-3.71 (m, 4H, 2 CH_2 piperazine), 4.28 (s, 2H, S$\underline{CH_2}$), 5.31 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.82-7.33 (m, 10H, Ar-H); EIMS (% rel. abundance): 530 (M^+, 10.90), 91($C_7H_7^{1+}$, 100).

3-Benzyl-2-({2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}sulfanyl)-5,6,7,8-tetrahydro[1]benzo thieno[2,3-d]pyrimidin-4(3H)-one (4d)

IR (KBr) cm^{-1}: 2920, 2837 (CH aliphatic), 1683, 1654 (2 C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.76-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.69-2.75 (m, 2H, CH_2 at C-5), 2.84-2.87 (m, 2H, CH_2 at C-8), 2.97-3.08 (m, 4H, 2 CH_2 piperazine), 3.58-3.63 (m, 4H, 2 CH_2 piperazine), 3.69 (s, 3H, OCH_3), 4.27 (s, 2H, S$\underline{CH_2}$), 5.31 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.82-7.36 (m, 9H, Ar-H); EIMS (% rel. abundance): 560 (M^+, 36.62), 91 ($C_7H_7^{1+}$, 100).

N-(4-chlorophenyl)-2-[(3-benzyl-4-oxo-3,4-dihydro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d] pyrimidin-2-yl)sulfanyl]propanamide (6a)

IR (KBr) cm^{-1}: 3302 (NH), 2931, 2850 (CH aliphatic), 1678, 1662 (2 C=O), 1597 (C=C aromatic); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.55 (d, J=6.9 Hz, 3H, CH-$\underline{CH_3}$), 1.77-1.80 (m, 4H, 2 CH_2 at C-6, C-7), 2.71-2.79 (m, 2H, CH_2 at C-5), 2.84-2.86 (m, 2H, CH_2 at C-8), 3.44 (q, J=6.9 Hz, 1H, $\underline{CH}$-CH_3), 5.27 (s, 2H, N$\underline{CH_2}C_6H_5$), 7.21-7.61 (m, 9H, Ar-H), 10.50 (s, 1H, NH, D_2O exchangeable); EIMS % rel. abundance): 511 (M+2, 10.39), 509 (M^+, 10.27).

3-Benzyl-2-{[1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl]sulfanyl}-5,6,7,8-tetrahydro[1]benzo thieno[2,3-d]pyrimidin-4(3H)-one (6b)

IR (KBr) cm^{-1}: 2922, 2863 (CH aliphatic), 1672, 1662 (2 C=O), 1600, 1508 (C=C aromatic); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.49 (d, J=6.9 Hz, 3H, CH-$\underline{CH_3}$), 1.77-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.68-2.77 (m, 2H, CH_2 at C-5), 2.84-2.87 (m, 2H, CH_2 at C-8), 3.07-3.18 (m, 4H, 2 CH_2 piperazine), 3.53-3.71 (m, 4H, 2 CH_2 piperazine), 5.03 (q, J=6.9 Hz, 1H, $\underline{CH}$-CH_3), 5.25 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.82-7.34 (m, 10H, Ar-H); ^{13}C-NMR (DMSO-d_6 ppm) δ: 17.13, 21.66, 22.36, 24.35, 25.12, 41.65, 42.50, 46.35, 48.15, 115.83, 117.98, 119.28, 126.63, 127.33, 128.49, 128.91, 130.69, 131.21, 135.38, 150.57, 156.30, 157.26, 160.99, 168.86; EIMS (% rel. abundance): 544 (M^+, 7.67), 91 ($C_7H_7^{1+}$, 100).

3-Benzyl-2-{[1-oxo-1-(4-(4-methoxyphenyl)piperazin-1-yl)propan-2-yl]sulfanyl}-5,6,7,8-tetrahydro [1]benzothieno[2,3-d]pyrimidin-4(3H)-one (6c)

IR (KBr) cm^{-1}: 2929, 2833 (CH aliphatic), 1678, 1651 (2 C=O), 1585, 1552, 1508 (C=C aromatic); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.44 (d, J=6.9 Hz, 3H, CH-$\underline{CH_3}$), 1.75-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.69-2.78 (m, 2H, CH_2 at C-5), 2.84-2.86 (m, 2H, CH_2 at C-8), 2.95-3.06 (m, 4H, 2 CH_2 piperazine), 3.45-3.60 (m, 4H, 2 CH_2 piperazine), 3.69 (s, 3H, OCH_3), 5.0 (q, J=6.9 Hz, 1H, $\underline{CH}$-CH_3), 5.26 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.83-7.32 (m, 9H, Ar-H); EIMS (% rel. abundance): 575 (M+1, 30.91), 574 (M^+, 61.82).

N-(4-chlorophenyl)-3-[(3-benzyl-4-oxo-3,4-dihydro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d] pyrimidin-2-yl)sulfanyl]propanamide (8a)

IR (KBr) cm^{-1}: 3271 (NH), 3113, 3032 (CH aromatic), 2924, 2850 (CH aliphatic), 1685, 1662 (2 C=O), 1593, 1508 (C=C aromatic); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.78-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.72-2.77 (m, 2H, CH_2 at C-5), 2.80 (t, 2H, J=6.3Hz, $\underline{CH_2}$CO), 2.85-2.90 (m, 2H, CH_2 at C-8), 3.42 (t, 2H, J=6.3Hz, S$\underline{CH_2}$), 5.24 (s, 2H, N$\underline{CH_2}C_6H_5$), 7.17-7.36 (m, 5H, Ar-H), 7.55-7.70 (m, 4H, Ar-H), 10.10 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 511 (M+2, 8.17), 509 (M^+, 10.38), 91 ($C_7H_7^{1+}$, 56.68).

3-Benzyl-2-{[3-(4-phenylpiperazin-1-yl)3-oxopropyl]sulfanyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (8b)

IR (KBr) cm^{-1}: 2927, 2806 (CH aliphatic), 1683, 1660 (2 C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.78-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.72-2.76 (m, 2H, CH_2 at C-5), 2.81 (t, 2H, J=15 Hz, $\underline{CH_2}$CO), 2.85-2.89 (m, 2H, CH_2 at C-8), 3.09-3.20 (m, 4H, 2 CH_2 piperazine), 3.38 (t, 2H, J=15 Hz, S$\underline{CH_2}$), 3.56-3.62 (m, 4H, 2 CH_2 piperazine), 5.26 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.78-7.33 (m, 10H, Ar-H); EIMS (% rel. abundance): 544 (M^+, 4.93), 91 ($C_7H_7^{1+}$, 100).

3-Benzyl-2-{[3-(4-(4-methoxyphenyl)piperazin-1-yl)-3-oxopropyl]sulfanyl}-5,6,7,8-tetrahydro[1] benzothieno[2,3-d]pyrimidin-4(3H)-one (8c)

IR (KBr) cm^{-1}: 2945, 2806 (CH aliphatic), 1681, 1654 (2 C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6 ppm) δ: 1.77-1.82 (m, 4H, 2 CH_2 at C-6, C-7), 2.73-2.76 (m, 2H, CH_2 at C-5), 2.80 (t, 2H, J=6.9 Hz, $\underline{CH_2}$CO), 2.85-2.89 (m, 2H, CH_2 at C-8), 2.90-2.95 (m, 4H, 2 CH_2 piperazine), 3.38 (t, 2H, J=6.9 Hz, S$\underline{CH_2}$), 3.56-3.60 (m, 4H, 2 CH_2 piperazine), 3.68 (s, 3H, OCH_3), 5.26 (s, 2H, N$\underline{CH_2}C_6H_5$), 6.82-7.31 (m, 9H, Ar-H); EIMS (% rel. abundance): 575 (M+1, 1.79), 574 (M^+, 1.16), 91 ($C_7H_7^{1+}$, 100).

General procedure for the preparation of ethyl 2-(alkanamido)-4,5,6,7-tetrahydro[1]benzothiophene-3-carboxylate (9a,b)

To a solution of **1** (2.25 g, 0.01 mol) in dry DMF (6 ml), butanoyl chloride (1.42 ml, 0.014 mol) or 3-methylbutanoyl chloride (2 ml, 0.016 mol) was added dropwise with cooling and stirring. After complete addition, the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured onto ice cold water (25 ml) and stirred for 1 h. The yellow precipitate formed was filtered off, washed with water several times, dried and crystallized from benzene/ ethanol (1:1).

Ethyl 2-butanamido-4,5,6,7-tetrahydro[1]benzothiophene-3-carboxylate (9a)[4]

Yield: 81%; mp: 56-58°C; IR (KBr, cm^{-1}): 3242 (NH), 2958, 2839 (CH aliphatic), 1685, 1654 (2 C=O), 1560, 1541 (C=C aromatic).

Ethyl 2-(3-methylbutanamido)-4,5,6,7-tetrahydro[1]benzothiophene-3-carboxylate (9b)

Yield: 73%; mp: 76-78°C; IR (KBr, cm^{-1}): 3251 (NH), 2931, 2870 (CH aliphatic), 1678, 1651 (2 C=O), 1562, 1543 (C=C aromatic). ^{1}H-NMR (DMSO-d_6 ppm) δ: 0.93 (d, 6H, J=6.4 Hz, 2 CH_3), 1.31 (t, 3H, J=14.2 Hz, CH_2-$\underline{CH_3}$), 1.71-1.77 (m, 4H, 2 CH_2 at C-6, C-7), 2.09-2.04 (m, 1H, $\underline{CH}(CH_3)_2$), 2.37 (d, 2H, J=7.16 Hz, $\underline{CH_2}$-$CH(CH_3)_2$), 2.58-2.61 (m, 2H, CH_2 at C-5), 2.70-2.73 (m, 2H, CH_2 at C-8), 4.27 (q, J=14.2 Hz, $\underline{CH_2}$-CH_3), 10.98 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 309 (M^+, 3.78), 236 ($C_{13}H_{18}N_3OS^{1+}$, 7.29), 557 ($C_2H_5^{1+}$, 100). Anal. Calcd for $C_{16}H_{23}NO_3S$ (309.4): C, 62.11; H, 7.49; N, 4.53; Found: C, 62.37; H, 7.56; N, 4.68.

General procedure for the preparation of 3-Amino-2-alkyl-5,6,7,8-tetrahydo[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (10a,b)

A mixture of **9a** or **9b** (3.54 g, 0.012 mol), hydrazine hydrate (2.35 ml, 0.048 mol, 99-100%) in absolute ethanol (15 ml) was heated under reflux for 18 h. After cooling, the crystals separated were filtered off, washed with water and crystallized from absolute ethanol.

3-Amino-2-propyl-5,6,7,8-tetrahydo[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (10a)

Yield: 54%; mp: 140-142°C; IR (KBr, cm^{-1}): 3296-3197 (NH_2), 2956, 2852 (CH aliphatic), 1660 (C=O), 1546 (C=C aromatic); ^{1}H-NMR (DMSO-d_6) δ: 0.87 (t, 3H, J=14.7 Hz, CH_3), 1.54-1.61 (m, 2H, CH_2-$\underline{CH_2}$-CH_3), 1.69-1.80 (m, 4H, 2 CH_2 at C-6, C-7), 2.34 (t, 2H, J=14.7 Hz, $\underline{CH_2}$-CH_2-CH_3), 2.56-2.59 (m, 2H, CH_2 at C-5), 2.62-2.64 (m, 2H, CH_2 at C-8), 7.25 (s, 2H, NH_2, D_2O exchangeable).

Table 2: Physical and Analytical data of compounds 11a-d

Compound	R	R^1	mp (°C)	Yield (%)	Molecular formula (M.wt)	Analysis %	
						Calcd	Found
11a	H	Cl	220-222	61	$C_{20}H_{20}ClN_3O_2S$ 401.91	C 59.77 H 5.02 N 10.46	59.94 5.11 10.72
11b	H	CN	236-238	70	$C_{21}H_{20}N_4O_2S$ 392.48	C 64.27 H 5.14 N 14.28	64.41 5.12 14.45
11c	H	OCH_3	196-198	59	$C_{21}H_{23}N_3O_3S$ 397.49	C 63.45 H 5.83 N 10.57	63.69 5.97 10.78
11d	CH_3	Cl	218-220	67	$C_{21}H_{22}ClN_3O_2S$ 415.94	C 60.64 H 5.33 N 10.10	60.87 5.40 10.21

3-Amino-2-isobutyl-5,6,7,8-tetrahydo[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (10b)

Yield: 64%; mp: 150-152°C; IR (KBr, cm^{-1}): 3298-3199 (NH_2), 2947, 2845 (CH aliphatic), 1658 (C=O), 1541 (C=C aromatic); ^{1}H-NMR (DMSO-d_6) δ: 0.98 (d, 6H, J=6.6 Hz, 2CH_3), 1.79-1.86 (m, 4H, 2 CH_2 at C-6, C-7), 2.29-2.33 (m, 1H, $\underline{CH}(CH_3)_2$), 2.74-2.76 (m, 2H, CH_2 at C-5), 2.83 (d, 2H, J=6.6 Hz, CH_2), 2.90-2.92 (m, 2H, CH_2 at C-8), 5.67 (s, 2H, NH_2, D_2O exchangeable).

General procedure for the preparation of N-(2-alkyl-4-oxo-4,5,6,7-tetrahydro[1]benzothiophene[2,3-d]pyrimidin-3-yl) substituted benzamide (11a-d)

A mixture of the amino derivatives **10a** or **10b** (0.002 mol), anhydrous K_2CO_3 (0.2 g, 0.0014 mol) and the appropriate benzoyl chloride (0.002 mol) in dry benzene (10 ml) was heated under reflux for 6 h. The separated solid was filtered, washed with water, dried and crystallized from methanol to yield **11a-d,** (Table 2).

4-Chloro-N-(2-propyl-4-oxo-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-3-yl)benzamide (11a)

IR (KBr) cm^{-1}: 3209-3174 (NH), 3120, 3040 (CH aromatic), 2939, 2860 (CH aliphatic), 1701, 1664 (2 C=O), 1595, 1552, 1517 (C=N); ^{1}H-NMR ($CDCl_3$-d_6 ppm) δ: 0.97 (t, 3H, *J*=14.7 Hz, CH_3), 1.58-1.60 (m, 4H, 2 CH_2 at C-6, C-7), 1.76-1.88 (m, 2H, CH_2-$\underline{CH_2}$-CH_3), 2.74 (t, 2H, *J*=14.7 Hz, $\underline{CH_2}$-CH_2-CH_3), 2.80-2.83 (m, 2H, CH_2 at C-5), 2.97-3.08 (m, 2H, CH_2 at C-8), 7.29 (d, 2H, *J*=11.1 Hz, Ar-H), 7.82 (d, 2H, *J*=11.1 Hz, Ar-H), 10.12 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 403 (M+2, 7.35), 401 (M^+, 18.08), 139 ($C_7H_4OCl^{1+}$, 100).

4-Cyano-N-(2-propyl-4-oxo-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-3-yl)benzamide (11b)

IR (KBr) cm^{-1}: 3207-3180 (NH), 3089, 3039 (CH aromatic), 2968, 2856 (CH aliphatic), 2233 (CN), 1699, 1666 (2 C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$-d_6 ppm) δ: 0.97 (t, 3H, *J*=7.5 Hz, CH_3), 1.62-1.70 (m, 4H, 2 CH_2 at C-6, C-7), 1.79-1.87 (m, 2H, CH_2-$\underline{CH_2}$-CH_3), 2.69 (t, 2H, *J*=7.5, $\underline{CH_2}$-CH_2-CH_3), 2.75-2.78 (m, 2H, CH_2 at C-5), 2.79-3.2 (m, 2H, CH_2 at C-8), 7.64 (d, 2H, *J*=8.7 Hz, Ar-H), 8.01 (d, 2H, *J*=8.7 Hz, Ar-H), 10.47 (s, 1H, NH, D_2O exchangeable). EIMS (% rel. abundance): 392 (M^+, 30.44), 130 ($C_8H_4NO^{1+}$, 100).

4-Methoxy-N-(2-propyl-4-oxo-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-3-yl)benzamide (11c)

IR (KBr) cm^{-1}: 3259 (NH), 3040, 3000 (CH aromatic), 2960, 2870 (CH aliphatic), 1699, 1660 (2 C=O), 1606 (C=N), 1575, 1552, 1531 (C=C aromatic); ^{1}H-NMR ($CDCl_3$-d_6 ppm) δ: 0.98 (t, 3H, *J*=14.7 Hz, CH_3), 1.60-1.77 (m, 4H, 2 CH_2 at C-6, C-7), 1.79-1.83 (m, 2H, CH_2-$\underline{CH_2}$-CH_3), 2.73-2.30 (m, 6H, $\underline{CH_2}$-CH_2-CH_3& 2 CH_2 at C-5, C-8), 6.79 (d, 2H, *J*=8.7 Hz, Ar-H), 7.89 (d, 2H, *J*=8.7 Hz, Ar-H), 9.77 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$-d_6 ppm) δ: 13.69, 19.98, 22.06, 22.78, 25.01, 25.28, 35.52, 55.25, 113.65, 120.57, 122.56, 129.64, 131.12, 133.73, 157.77, 158.05, 162.36, 162.94, 168.86; EIMS (% rel. abundance): 397 (M^+, 7.91), 135 ($C_8H_7O_2^{1+}$, 100).

4-Chloro-N-(2-isobutyl-4-oxo-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-3-yl)benzamide (11d)

IR (KBr) cm^{-1}: 3240 (NH), 3089, 3055 (CH aromatic), 2939, 2843 (CH aliphatic), 1701, 1666 (2 C=O), 1593 (C=N); ^{1}H-NMR ($CDCl_3$-d_6 ppm) δ: 0.97 (d, 6H, *J*=15.3 Hz, $2CH_3$), 1.80-1.86 (m, 4H, 2 CH_2 at C-6, C-7), 2.26-2.28 (m, 1H, $\underline{CH}(CH_3)_2$), 2.74 (d, 2H, *J*=15.3 Hz, CH_2), 2.83-2.98 (m, 4H, CH_2 at C-5, C-8), 7.27 (d, 2H, *J*=8.4 Hz, Ar-H), 7.81 (d, 2H, *J*=8.4 Hz, Ar-H), 10.60 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 417 (M+2, 6.81), 415 (M^+, 17.89), 139 ($C_7H_4OCl^{1+}$, 100).

Anticancer Screening

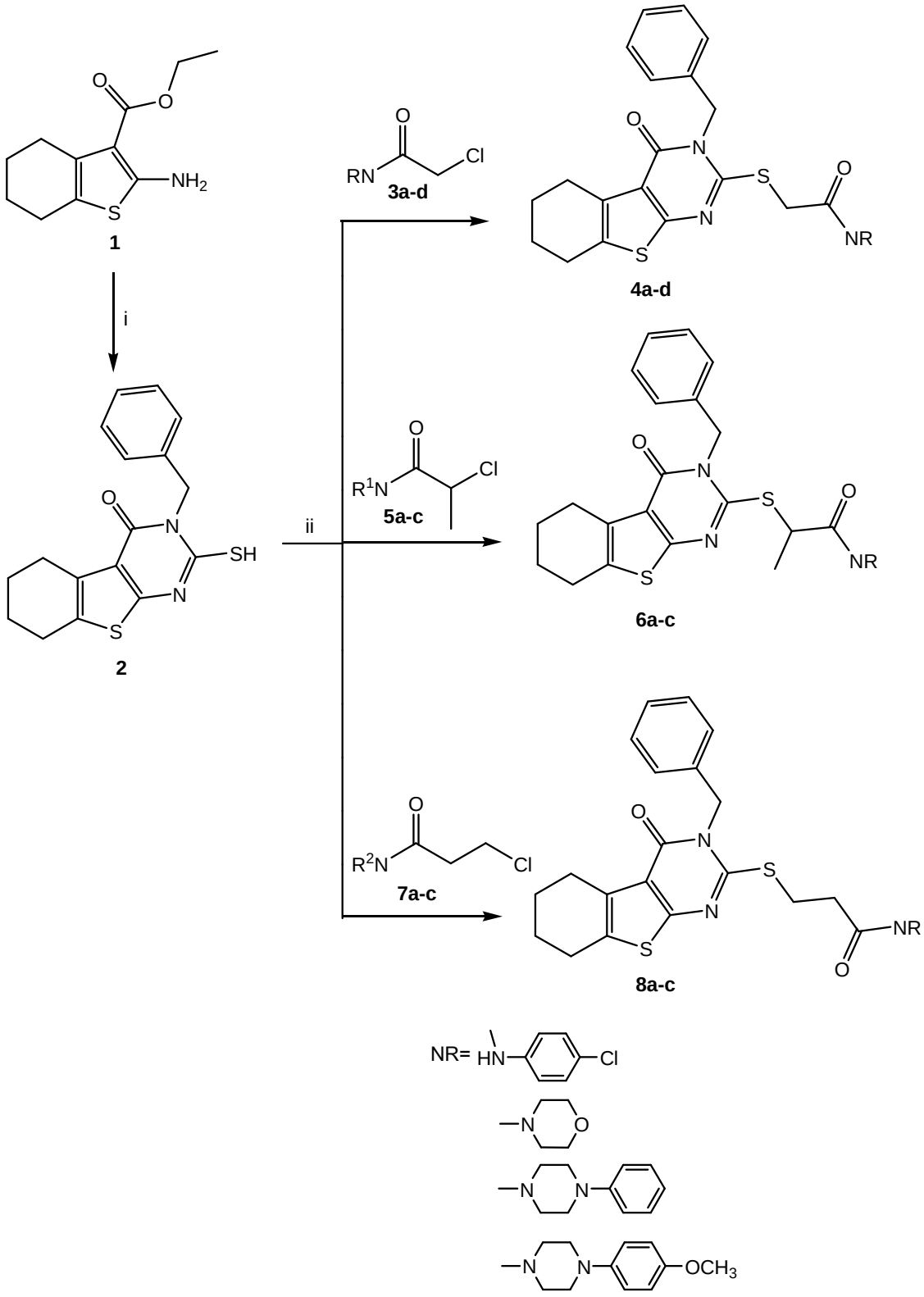
Cell culture

Cancer cells from different cancer cell lines; human prostate carcinoma (PC-3) and human colon adenocarcinoma (HCT-116), were purchased from American type Cell Culture collection (ATCC, Manassas, USA) and grown on the appropriate growth medium Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 mg/ mL of streptomycin, 100 units/ mL of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37 ºC.

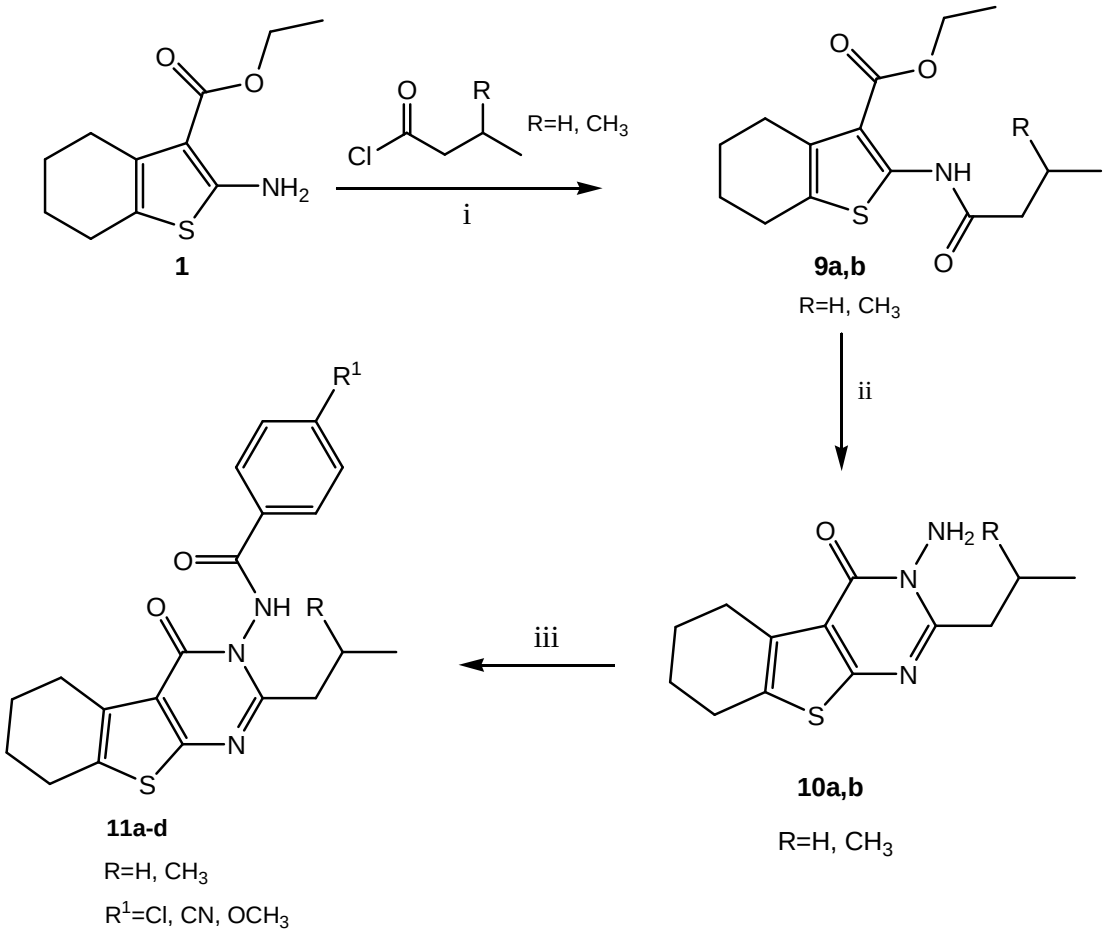
Cytotoxicity assay by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT):

The cytotoxic activity of the newly synthesized compounds was measured *in vitro* against human prostate cancer cell line (PC-3) and colon cancer cell line (HCT-116) using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [27, 28] as follows:

Exponentially growing cells from the two cancer cell lines were trypsinized, counted and seeded at the appropriate densities (2000-1000 cells/0.33 cm^2 well) into 96-well microtiter plates. Cells then were incubated in a humidified atmosphere at 37˚C for 24 hours. Then, cells were exposed to different concentrations of compounds (0.1, 10, 100, 1000 µM) for 72 hours. Then the viability of treated cells was determined using MTT technique as follow; Media were removed; cells were incubated with 200 µl of 5% MTT solution/well (Sigma Aldrich, MO) and were allowed to metabolize the dye into colored-insoluble formazan crystals for 2 h.



Scheme 1: i) $PhCH_2NCS$ / K_2CO_3 / acetonitrile, ii) Method A: DMF/KOH/reflux 20-23 h;
Method B: 10% KOH/stir 25 h; Method C: 2% alcoholic KOH/stir 25 h; Method D: Dry acetone/K_2CO_3/ reflux 20 h.



Scheme 2: i) Dry DMF/ stir at R.T 3 h ii) $NH_2NH_2.H_2O$/ EtOH/ reflux, 18 h iii) Substituted benzoyl chloride/ anhydrous K_2CO_3/ dry benzene/ reflux 6 h.

The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance was measured at 570 nm using a Stat Fax^R 4200 plate reader (Awareness Technology, Inc., FL). The cell viability were expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) were determined using Graph Pad Prism version 5 software (Graph Pad software Inc, CA).

RESULTS AND DISCUSSION

Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and final compounds are illustrated in **Schemes 1 and 2**. In **Scheme 1**, the starting compound Ethyl 2-amino-4,5,6,7-tetrahydro[1]benzothiophene-3-carboxylate **(1)** was prepared according to the well-known Gewald procedure [18]. Reacting **1** with benzyl isothiocyanate in acetonitrile afforded the corresponding 3-Benzyl-2-sulfanylthienopyrimidine derivative **2** [19]. Various aromatic amines were reacted with chloroacetyl chloride, 2-chloropropionyl chloride or 3-chloropropionyl chloride to give compounds **3a-d, 5a-c** and **7a-c** respectively according to the reported procedures [20-26]. The latter compounds were then reacted with **2** to obtain the target compounds **4a-d**, **6a-c** and **8a-c** using different alkylation conditions. Literature reports that *S*-alkylation of thiols using different alkyl halides could be achieved in different base/solvent systems like KOH/DMF [29, 30], KOH/ethanol [31, 32], K_2CO_3/acetone [6, 33-35], K_2CO_3/DMF [29], K_2CO_3/acetonitrile [36], K_2CO_3/THF [37], NaOH/water [34], sodium/ethanol [38], sodium hydride/DMF [39], TEA/THF [40], K_2CO_3/ethanol [41] and Cs_2CO_3/ tetrabutyl ammonium iodide/DMF [42]. Alkylation of the 2-

sulfanylthienopyrimidine **2** was carried out using different solvent/ base systems. Compounds **4c, 6b, 8b** and **8c** were prepared in variable yields from 25-72 % by alkylation of **2** with the respective chloro amide in DMF/KOH. However preparation of the remaining compounds in Table 1 using the DMF/KOH system proved unsatisfactory due to low yields, impure products and tedious work up. However alkylation of **2** was carried out using K_2CO_3/dry acetone under reflux. Under these conditions, compounds **4b**, **6a** and **8a** were obtained in pure state and moderate yields after simple work up procedure. Compounds **4d** and **6c** were obtained in 37 and 51 % yield respectively using a simple green procedure via alkylation by stirring in 10% aqueous NaOH at room temperature. Compound **4a** was obtained in 50% yield by altering the reaction conditions to 2% alcoholic NaOH solution and 2 molar of the chloroamide intermediate **3a**.

The structure of the target S-alkylated derivatives was supported by elemental analyses, IR, NMR and mass spectral data. IR spectra of **4a-d, 6a-c** and **8a-c** confirmed the presence of two C=O moieties in the range of υ 1672-1685 cm^{-1} (C=O pyrimidinone) and 1651-1664 cm^{-1} (C=O amide linkage). The ^{1}H-NMR spectra of the prepared compounds showed the disappearance of the SH signal at δ 5.6 ppm indicating the success of alkylation. ^{1}H-NMR spectra of **4a-d** showed a characteristic singlet signal in the range of δ 4.14-4.28 ppm assigned for SCH_2 protons while the spectra of **6a-c** revealed doublet signals in the range of δ 1.44-1.55 ppm assignable to CH_3 moiety and quartet signals assignable to CH protons in the range of δ 3.44-5.03 ppm. The presence of ethylene moiety (CH_2-CH_2) in compounds **8a-c** was revealed by two triplet signals in the range of δ 2.80-2.81 ppm and δ 3.38-3.40 ppm in ^{1}H-NMR spectra. In addition, ^{1}H-NMR spectra of all the target products **4a-d**, **6a-c** and **8a-c** displayed the expected signals of the morpholino, 4-chloroanilino and substituted phenyl piperazine moieties where:

- ^{1}H-NMR spectra in case of compounds **4a**, **6a** and **8a** showed an exchangeable singlet signal at δ 10.10-10.50 ppm corresponding to 4-chloroanilino NH protons.
- Compound **4b** displayed two multiple signals assignable to CH_2-N and CH_2-O of morpholino moiety at δ 3.43-3.46 ppm and δ 3.56-3.62 ppm respectively.
- In case of compounds **4c**, **6b**, **8b** substituted with 4-phenylpiperazine, two multiple signals assignable to the piperazinyl protons appeared in the range of δ 3.07-3.23 ppm and δ 3.36-3.71 ppm.
- In case of compounds **4d**, **6c**, **8c** substituted with 4-methoxy phenyl piperazine, two multiple signals assignable to the piperazinyl protons appeared in the range of δ 2.90-3.08 ppm and δ 3.45-3.63 ppm in addition to singlet signal of OCH_3 at δ 3.68 ppm.

On the other hand, ^{13}C-NMR spectrum of compound **4a** revealed the presence of (CH_2-C=O) carbon at δ 37.22 ppm in addition to two C=O signals at δ 157.32 ppm and δ 165.39 ppm corresponding to ring C=O and SCH_2-C=O, respectively. Moreover, ^{13}C-NMR spectrum of compound **6b** confirmed the presence of CH_3-CH carbons at δ 17.13 and 41.65 ppm respectively and the piperazinyl carbons at δ 42.50 ppm and δ 48.15 ppm. Carbonyl signal at δ 168.86 ppm indicates the C=O in the linker in addition to the ring C=O at δ 160.99 ppm.

In **Scheme 2**, reacting the ester **1** with n-butyryl chloride or 3-methylbutyryl chloride by stirring in dry DMF yielded the title compounds **9a** [43] and **9b** respectively. Cyclization of **9a,b** to the key intermediates **10a** and **10b** was induced by heating with excess hydrazine hydrate 99-100% under reflux. IR spectra of **10a** and **10b** confirmed the cyclization through the presence of one carbonyl signal at υ 1658 and1660 cm^{-1} respectively instead of two carbonyl moieties in **9a,b**. In addition, ^{1}H-NMR spectra **10a** and **10b** indicated the presence of two exchangeable protons assignable to NH_2 at δ 7.25 and 5.67 ppm. Heating the intermediates **10a,b** with various substituted benzoyl chlorides in dry benzene afforded the target products **11a-d**. IR spectra of **11a-d** showed additional amidic C=O absorption in the range of υ 1660-1666 cm^{-1}. In addition, IR spectrum of compound **11b** showed a characteristic peak at υ 2233 cm^{-1} due to the CN moiety. Furthermore, the ^{1}H-NMR spectra of **11a-d** displayed a D_2O exchangeable signal in the range of δ 9.77-10.60 ppm corresponding to the NH proton. Besides, two doublets in the range of δ 6.77-7.64 ppm and δ 7.78-8.01 ppm were assignable to the p-disubstituted phenyl ring in compounds **11a-d**. ^{13}C-NMR spectrum of **11c** showed a signal at δ 168.86 ppm confirming the presence of an additional amidic C=O group. In addition, the presence of OCH_3 group was confirmed by the presence of a singlet signal at δ 55.25 ppm.

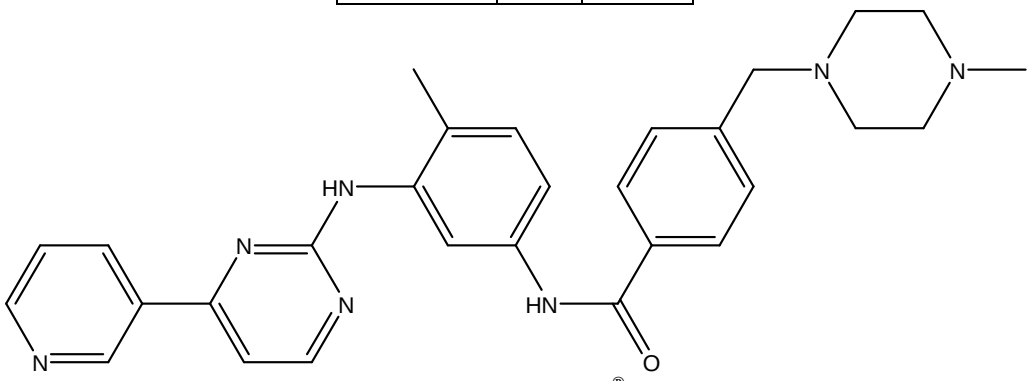
In vitro cytotoxicity

The *in vitro* cytotoxic activity of the newly synthesized compounds was evaluated against two human cancer cell lines including cells derived from human prostate cancer (PC-3) and human colon cancer (HCT-116) according to the standard protocol for IC_{50} determination. Imatinib, being one of the most effective anticancer agents, was chosen

as a reference standard anticancer drug [44]. The IC_{50} values in µM/ml are listed in (Table 3) and the results are represented graphically in (Fig. 3).

Table 3 Results of *in vitro* cytotoxic activity of the synthesized compounds against prostate tumor cell line (PC-3) and colon tumor cell line (HCT-116)

Compound no.	IC_{50} (µM/ml)	
	PC-3	HCT-116
4a	**0.0069**	0.087
4b	**0.0085**	>0.1
4c	>0.1	>0.1
4d	>0.1	>0.1
6a	>0.1	>0.1
6b	>0.1	>0.1
6c	0.081	>0.1
8a	>0.1	>0.1
8b	>0.1	0.044
8c	0.095	0.067
11a	>0.1	>0.1
11b	**0.0102**	**0.0095**
11c	**0.0124**	**0.0120**
11d	0.048	0.057.5
Imatinib	0.04	0.0344



Imatinib (GLIVEC®)

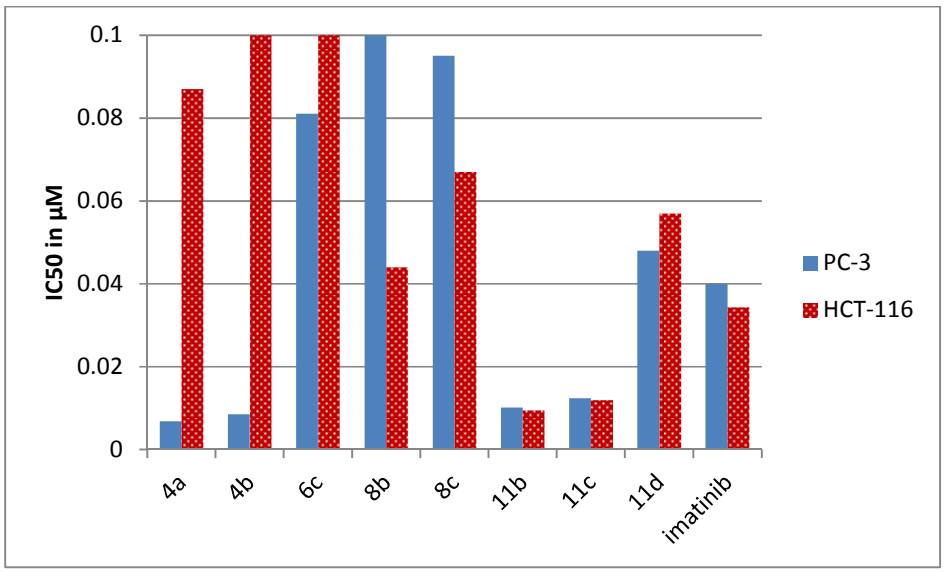


Fig. 3 IC_{50} in µM/ml of compounds 4a, 4b, 6c, 8b, 8d and 11b-d against prostate cell line (PC-3) and colon tumor cell line (HCT-116)

Analyzing the IC_{50} determination results for **Scheme 1** compounds in **Table 3** and SAR analysis of the effect of mercapto alkylation at position 2 of the pyrimidine ring with various aliphatic and aromatic amines together with varying the linker chain length against both cell lines indicated the following:

- Compound **4a** displayed the most potent anticancer activity against PC-3where it exhibited 5.78 fold higher activity than Imatinib.
- Among compounds **4a-d** with acetamide linkage, only compounds **4a** and **4b** substituted with 4-Cl aniline and morpholine moieties respectively displayed potent cytotoxic activity against PC-3 cell line compared to the standard drug Imatinib while **4a** showed only weak activity against HCT-116. On the other hand, the phenyl piperazine substituted derivatives **4c** and **4d** were devoid of activity against both cell lines.
- Branching the alkyl linker in compounds **6a-c** abolished activity against both cell lines.
- Among compounds **8a-c**, extending the alkyl chain in the 4-Cl aniline derivative **8a** decreased the activity against HCT-116 and abolished activity against PC-3 cell line compared to **4a** derivative with one carbon linker. However, **8b** showed moderate activity against HCT-116 with IC_{50} 44 μM (Imatinib 34.40 μM). The 4-methoxy phenyl piperazine derivative **8c** showed only weak activity against both cell lines.

Regarding **Scheme 2** compounds **11a-d** where the amino group at position 3 of the pyrimidine ring was acylated with various substituted benzoyl chlorides, the following was observed:

- Compound **11b** was the most active against both cell lines where it displayed 3.88 folds higher activity against PC-3 and 3.62 folds more potent activity against HCT-116 compared to Imatinib. This activity may be attributed to the presence of cyano group.
- Further, the methoxy derivative **11c** showed good activity against both cell lines with 3.21 folds more potent activity against PC-3 and 2.85 folds more potent activity against HCT-116 compared to Imatinib.
- Among the chloro substituted derivatives **11a** and **11d**; **11a** with propyl chain at position 2 was inactive against both cell lines while **11d** with branched alkyl chain at position 2 displayed equipotent activity to imatinib against PC-3.

CONCLUSION

In summary, a series of *S*-alkyl thieno[2,3-*d*]pyrimidinone was designed and synthesized where a variety of aliphatic and aromatic amines were hooked to a thioether moiety at C2 of the pyrimidine ring through different linkers to investigate the influence of such assemblies on the anticancer activity. It was found, interestingly, that compounds **4a** and **4b** with acetamide linkage were the most active derivatives against PC-3 cell line where **4a** linked to 4-chloroaniline moiety displayed 5.78 fold more potent antitumor activity than Imatinib while the morpholino derivative **4b** showed 4.6 fold more potent activity than Imatinib. In **Scheme 2**, variation at N3 of the pyrimidine ring was carried out while substituting C2 with propyl or isobutyl aliphatic chain to investigate the influence of replacing the benzyl moiety at N3 of **Scheme 1** with various substituted benzoyl functions in compounds **11a-d**. The 4-cyano benzamide derivative **11b** was the most active against both PC-3 and HCT-116 cell lines followed by the 4-methoxy benzamide derivative **11c** which exhibited 3.21 fold more potent cytotoxic activity against PC-3 and 2.85 folds more potent activity against HCT-116 cell line.

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