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Original research paper

Synthesis and evaluation of antitumor activity of new 4-substituted thieno[3,2-*d*]pyrimidine and thienotriazolopyrimidine derivatives

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3-Methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4(1*H*)-one (**2**), on treatment with phosphorous oxychoride, affored 4-chloro-3-methyl-6-phenyl -thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**3**). A series of novel 6-phenyl-thieno[3,2-*d*]pyrimidine derivatives **4**-**9** bearing different functional groups were synthesized *via* treatment of compound **3** with different reagents. On the other hand, compound **2** was used to synthesize ethyl-[(3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4-yl)-oxy]acetate (**10**), 2-hydrazinyl--3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**11**), 3-methyl-2-(methyl-sulfanyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**12**) and *N*-(phenyl)/4-chlorophenyl or methoxy-phenyl)-2-[(3-methyl-4-oxo-6-phenyl-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)-sulfanyl]-acetamide (**13a-c**). In addition, compound **12** was used to synthesize thieno[1,2,4]triazolopyrimidine derivatives **14** and **15** and 3-methyl--2-(methyl-sulfonyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)--one (**16**) through the reaction with the respective reagents. Moreover, the reaction of **16** with 4-phenylenediamine gave 2-[(4-aminophenyl)-amino]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**17**), which reacted with methanesulfonyl chloride to afford *N*-{4-[(3-methyl-4-oxo-6-phenyl-3*H*,4*H*--thieno[3,2-*d*]pyrimidin-2-yl)-amino]phenyl}-methanesulfonamide (**18**). The majority of the newly synthesized compounds displayed potent anticancer activity, comparable to that of doxorubicin, on three human cancer cell lines, including the human breast adenocarcinoma cell line (MCF-7), cervical carcinoma cell line (HeLa) and colonic carcinoma cell line (HCT-116). Compounds **18**, **13b** and **10** were nearly as active as doxorubicin whereas compounds **6**, **7b** and **15** exhibited marked growth inhibition, but still lower than doxorubicin.

Keywords: thieno[3,2-*d*]pyrimidines, thienotriazolopyrimidine derivatives, antitumor activity

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Chemical study of pyrimidine nucleus plays an important role in the synthesis of a variety of fused heterocyclic compounds possessing a wide range of pharmacological activity. Fused pyrimidines such as thienopyrimidines are a promising class of synthetic heterocyclic compounds with numerous biological activities (1–3), in particular antimicrobial (4, 5), antitumor (6, 7) and mitotic arrest of breast cancer (8).

Thieno[3,2-*d*]pyrimidine derivatives display a wide range of activities acting as anticonvulsants (9), antivirals (10), antibiotics (11), antiglaucoma agents (12) and platelet aggregation inhibitors (13). On the other hand, some 2-substituted-thieno[3,2-*d*]pyrimidine--4-ones are considered to exert antihyperlipidemic (14) and anti-inflammatory activity (15). Also, 2-(phenylsulfonyl)methyl-thieno[3,2-*d*]pyrimidine derivatives act as novel HIV-1 replication inhibitors (16). Further, benzo-thieno[3,2-*d*]pyrimidin-4-one sulphonamide thio-derivatives act as inhibitors of COX-2, iNOS and ICAM-1 (17). Thieno[3,2-*d*] pyrimidine derivatives have recently become an important class of chemotherapeutic drugs, notably for the treatment of cancers, such as PI3K inhibitor (18), PI3K and mTOR dual inhibitor (19). Therefore, thieno[3,2-*d*]pyrimidines and their derivatives have consistently attracted scientific interest and prompted us to develop and identify new molecules, in order to investigate the effect of structural modifications on their antitumor activities.

EXPERIMENTAL

Chemicals and equipment

All melting points were measured on an Electrothermal 9100 series digital melting point apparatus (Shimadzu, Japan). Microanalytical data were gathered with a Vario Elementar apparatus (Shimadzu). Elemental analyses of all compounds were within ± 0.4 % of the theoretical values. IR spectra (KBr) were recorded on a Perkin Elmer 1650 spectrometer (USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL EX-300 and a JEOL ECA-500 (Jeol, Japan). Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standard in DMSO-d_6 as a solvent. Mass spectra were recorded on a 70 eV Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). Purity of the compounds was checked on aluminum plates coated with silica gel (Merck, Germany). Chemicals and solvents (Analar ≥ 99 %) were purchased from Sigma-Aldrich (USA). 3-Amino--5-phenyl-thiophene-2-methylcarboxylate was purchased from Sigma-Aldrich. Doxorubicin disks were supplied by the Pasteur Laboratory (Egypt).

Syntheses

*3-Methyl-6-phenyl-2-thioxo-*2H,3H-*thieno[3,2-*d*]pyrimidin-4(1*H*)-one* (**2**). – A mixture of 3-amino-5-phenyl-thiophene-2-methylcarboxylate (0.1 mol) and methyl isothiocyanate (0.15 mol) was refluxed in dry dioxane (30 mL) under dry conditions for 8 h. The reaction mixture was then cooled to room temperature. The resulting mixture was poured onto water, stirred for 10 min and the aqueous part was filtered off. The solid product, methyl 3-[(methylcarbamothioyl)amino]-5-phenylthiophene-2-carboxylate (**1**), was dissolved in NaOMe solution (30 mL, 20 %), stirred in a water bath for 2 h, allowed to cool to room temperature and neutralized with HCl. The solid formed was filtered off and crystallized from dioxane to give compound **2** as white powder.

*4-Chloro-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**3**).* – A mixture of **2** (0.005 mol), phosphorous oxychloride (0.1 mol) and *N*,*N*-dimethylamine (0.005 mol) was refluxed at 110 °C for 24 h. The reaction mixture was then cooled and the excess phosphoryl chloride was removed under reduced pressure. The resulting red oil was poured slowly onto ice water (30 mL), stirred for 10 min and the aqueous part was extracted with EtOAc (10–25 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to give an oily residue, which was purified by column chromatography (hexane/ethyl acetate, 1:1) (100 mL) to obtain a pale yellow solid.

*3-Methyl-4-(substituted secondary amino/aminophenyl)-6-phenyl-thieno[3,2-*d*] pyrimidine-2(3*H*)-thione (**4a-c**, **6** and **7a-c**). General procedure*. – To a warm solution of **3** (0.01 mol) in glacial acetic acid (40 mL), the freshly distilled secondary amine (namely morpholine, piperazine, *N*-methylpiperazine) and aliphatic or aryl-amine (namely, ethanolamine, 4-anisidine, 4-chloroaniline and 4-nitroaniline) (0.01 mol) were added. The reaction mixture was stirred under reflux for 3 h, then allowed to cool to 0 °C for 4–6 h, and the solid obtained was filtered, washed with water (100 mL), dried and recrystallized from an appropriate solvent to produce **4a-c**, **6** and **7a-c**, resp. The following compounds were prepared: 3-methyl-4-(morpholin-4-yl)-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**4a**), 3-methyl-4-(piperazin-1-yl)-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**4b**), 3-methyl-4-(*N*-methyl-piperazin-1-yl)-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**4c**), 4-[(2-hydroxy-ethyl)-amino]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3H)-thione (**6**), 4-[(4-methoxy-phenylamino)]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**7a**), 4-[(4-chloro-phenylamino)]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**7b**) and 3-methyl-4-[(4-nitro-phenylamino)]-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**7c**).

*4-(3,6-Dihydro-2*H*-pyran-4-yl)-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**5**).* – A mixture of compound **3** (10 mmol), tributyl-(3,6-dihydro-2*H*-pyran-4-yl)stannane (10 mmol), *bis*-(triphenylphosphene) palladium(II) chloride (1.40 g, 2 mmol), triphenylphosphine (10 mmol), lithium chloride (5 g, 117 mmol) and 2,6-di-*tert*-butyl-*p*-cresol (0.20 g, 1 mmol) in dimethylformamide (DMF, 30 mL) was stirred under heating at 120 °C for 6 h. The reaction mixture was allowed to cool to room temperature and to concentrate. Flash chromatography (heptane/ethyl acetate, 9:1, then ethyl acetate) afforded compound **5** as pale yellow powder.

*4-Hydrazinyl-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**8**).* – Compound **3** (0.01 mol) and hydrazine hydrate (99 %) (25 mL) were stirred under gentle reflux (70–80 °C) for 8 h; the reaction mixture was allowed to cool to room temperature. The solid formed was filtered off, washed with ethanol and dried. Yellow powder crystals obtained were recrystallized from DMF.

*4-(Alkoxyphenoxy)-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**9a**,**b**). General method*. – Freshly prepared dry sodium 3-methoxyphenolate and/or sodium 4-methoxyphenolate (1.75 g, 12 mmol) was added to a solution of **3** (5 mmol) in dry DMF (3 mL). The reaction mixture was stirred at room temperature for 4 h. Water was added to stop the reaction. The organic layer was washed with 2 mol L^{-1} NaOH, dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The precipitate that was

formed was triturated with a mixture of petroleum ether/diethyl ether and filtered off. The following compounds were prepared: 4-(4-methoxyphenoxy)-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**9a**) and 4-(3-methoxyphenoxy)-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**9b**).

*Ethyl[(3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4-yl)-oxy]-acetate (**10**).* – A mixture of **2** (0.01 mol) and ethyl chloroacetate (1.3 mL, 0.012 mol) in sodium ethoxide solution (0.46 g of sodium metal in 20 mL of absolute ethanol) was heated under reflux for 2 h. The solvent was acidified with cold diluted HCl (2 mol L^{-1}, 20 mL). The obtained oily residue was extracted with ethyl acetate (3 × 50 mL). The solid obtained after evaporation of the solvent was filtered off, dried and recrystallized from dioxane.

*2-Hydrazinyl-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**11**).* – A mixture of **2** (0.01 mol) and hydrazine hydrate (99 %) (25 mL) was stirred under gentle reflux. After 30 min when the solid product started separating out, heating was continued for 12 h and the reaction mixture was allowed to cool to room temperature. The solid that separated was filtered, washed with ethanol and dried. The obtained yellow powder crystallized from DMF.

*3-Methyl-2-(methylsulfanyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**12**),* N-*(phenyl/substituted-phenyl)-2-[(3-methyl-4-oxo-6-phenyle-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl) sulfanyl]-acetamides (**13a-c**). General procedure.* – Compound **2** (0.01 mol) was added to warm ethanolic KOH solution (10 %, 30 mL), the heating was continued for 30 min and the mixture was allowed to cool to room temperature. The respective halo-compound (0.012 mol), namely, methyl iodide, 2-chloro-*N*-phenylacetamide, 2-chloro-*N*-(4-chlorophenyl)-acetamide or 2-chloro-*N*-(4-methoxyphenyl)-acetamide, was added. The reaction mixture was stirred under reflux for 7 h, then cooled to room temperature and poured into cold water (100 mL). The solid product which precipitated was filtered off, washed with 100 mL water, dried and crystallized to produce **12** and **13a-c**. The following compounds were prepared: 3-methyl-2-(methylsulfanyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**12**), 2-[(3-methyl--4-oxo-6-phenyl-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)-sulfanyl]-*N*-phenyl-acetamide (**13a**), *N*-(4-chlorophenyl)-2-[(3-methyl-4-oxo-6-phenyle-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)sulfanyl]-acetamide (**13b**) and *N*-(4-methoxyphenyl)-2-[(3-methyl-4-oxo-6-phenyl-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)sulfanyl]-acetamide (**13c**).

*4-Methyl-1,7-diphenyl-thieno[2,3-e][1,2,4]triazolo[4,3-a]pyrimidin-5(4H)-one (**14**).* – To a mixture of **12** (0.01 mol) and benzohydrazide (0.01 mol) in dioxane (30 mL), a catalytic amount of piperidine was added. The reaction mixture was stirred under reflux for 12 h, allowed to cool to room temperature and poured into water (100 mL). The deposited precipitate was filtered off, dried and crystallized from dioxane. The compound was obtained as green powder.

*1-Amino-4-methyl-7-phenyl-thieno[2,3-e][1,2,4]triazolo[4,3-a]pyrimidin-5(4H)-one (**15**).* – To a mixture of **12** (0.01 mol) and thiosemicarbazide (0.01 mol) in dioxane (30 mL), a catalytic amount of piperidine was added. The reaction mixture was stirred under reflux for 12 h, allowed to cool to room temperature and poured into water (100 mL). The solid product was filtered off, dried and crystallized from dioxane. Compound **15** was obtained as yellow powder.

*3-Methyl-2-(methylsulfonyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**16**).* – A mixture of **12** (0.01 mol) and an excess amount of hydrogen peroxide (5 mL) in acetic acid (30 mL) was heated gently under stirring for 10 h. The reaction mixture was allowed to cool to 0 °C. The deposited precipitate was filtered off and crystallized from dioxane.

*2-[(4-Aminophenyl)-amino]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**17**).* – To a warm solution of **16** (0.01 mol) in glacial acetic acid (40 mL), 4-phenylenediamine (0.01 mol) was added. The reaction mixture was stirred under reflux for 4 h, then allowed to cool to 0 °C for 4-6 h, and the solid obtained was filtered, washed with water (100 mL), dried and recrystallized from DMF to produce **17** as yellow crystals.

*N-{4-[(3-methyl-4-oxo-6-phenyl-3*H*,4*H*-thieno[3,2-*d*]pyrimidin-2-yl)-amino]phenyl}-methanesulfonamide (**18**).* – To a solution of amino compound **17** (1.0 mmol) in pyridine (5 mL), methanesulfonyl chloride (1.50 mmol) was added and the mixture was heated under reflux for 12 h. Under reduced pressure, the solvent was removed and the crude residue was extracted with EtOAC and washed with diluted HCl. The organic layer was dried over $MgSO_4$. The crude residue was purified by flash chromatography on silica gel, dried and recrystallized from ethanol to produce **18** as brown crystals.

Biological screening

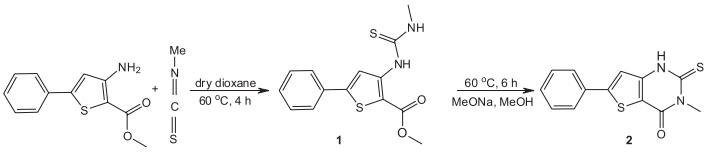
In vitro *anticancer activity.* – We evaluated the activity of compounds **2-11**, **13-16** and **18** against the human breast adenocarcinoma cell line (MCF-7), human cervical carcinoma cell line (HeLa) and human colonic carcinoma cell line (HCT-116) using the sulforhodamine B assay (20). The three human cancer cell lines were provided by the National Cancer Institute (NCI, Cairo, Egypt). Continuous drug exposure for 48 h was the method used. All the cell lines were cultured in Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum (FBS) (in a humidified atmosphere with 5 % CO_2 at 37 °C). Their growth as a monolayer was maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS, 2 mmol L^{-1} glutamine and antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}). The effect of compounds on the *in vitro* growth of human tumor cell lines was evaluated using sulforhodamine B (SRB) as protein binding dye to assess cell growth. Cells growing in 96-well plates were obtained by plating 1.5×10^5 cells mL^{-1}. The microtiter plates were incubated at 37 °C for 24 h prior to addition of experimental drugs and were incubated for 48 h with five different concentrations of each compound (0.01, 0.1, 1, 10, 100 μg mL^{-1}), which were dissolved in DMSO and diluted with saline to the mentioned concentration, starting from a maximum concentration of 100 μg mL^{-1}. After 48 h, the cell monolayers were fixed by addition of 10 % (*m*/*V*) trichloroacetic acid and incubated at 4 °C for 1 h, and then stained for 30 min with 0.4 % (*m*/*V*) sulforhodamine B in 1 % acetic acid. Excess of unbound dye was removed by four washes with 1 % acetic acid and the attached stain was recovered with Tris-EDTA buffer. Absorbance was measured and growth inhibition of 50 % (GI_{50}) was calculated (21). Table III displays GI_{50} values of each compound for the above listed three cell lines. Doxorubicin was used as a reference compound.

The influence of solvent, DMSO, on the growth of cell lines was evaluated in all experiments (negative control). This was performed by exposing untreated control cells to the maximum concentration of DMSO used in each assay (0.5 %).

RESULTS AND DISCUSSION

Chemistry

The reaction sequence employed for the synthesis of 3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4(1*H*)-one (**2**) is outlined in Scheme 1. Initially, the reaction



Scheme 1.

of 3-amino-5-phenyl-thiophene-2-methylcarboxylate with methyl isothiocyanate afforded methyl-3-[(methylcarbamothioyl)amino]-5-phenylthiophene-2-carboxylate (**1**) as an intermediate, which underwent cyclization under basic conditions (22) to form the target compound **2**. The structure of **2** was confirmed by spectral data.

Introduction of halogen on fused pyrimidine nucleus is primarily used to generate 4-chloro-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**3**), which is the key intermediate for the formation of a variety of thieno[3,2-*d*]pyrimidine derivatives (**4**–**7**, **9**, **10**). 4-Chloro-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**3**) was prepared by the treatment of 3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4(1*H*)-one (**2**) with phosphorous oxychloride under dry conditions (dioxane), which was then converted to the corresponding 4-amino derivatives (**4a-c**) with morpholine, piperazine and *N*-methyl piperazine. Besides correct values of elemental analyses, IR, NMR and mass spectra of compounds **4a-c** are in agreement with the assigned structures (Tables I and II). IR spectrum of **4b** shows C=N at 1600 cm^{-1} and the broad absorption band at 3365 cm^{-1} for NH. ^1H NMR spectra showed singlet NCH_3 at δ 3.13 ppm, multiplet signals for 4 CH_2 at δ 2.65 to 3.38 ppm, singlet signal for the thiophene proton at δ 8.01 ppm and multiplet signals at δ 6.96-7.05 (m, 2H, Ar-H) and 7.45-7.53 (m, 3H, Ar-H) for phenyl. Moreover, the broad absorption signal for the NH group at δ 9.60 ppm was observed. The mass spectrum showed *m*/*z* 342 corresponding to the molecular ion peak $[M]^+$ and *m*/*z* 343 $[M+1]^+$.

Reactivity of chlorine in the pyrimidine moiety of compound **3** was responsible for getting the coupling product **5** in the presence of tributyl(3,6-dihydro-2*H*-pyran-4-yl) stannane.

4-[(2-Hydroxyethyl)amino- and 4-methoxy/4-chloro- and/or 4-nitro-phenylamino]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**6**, **7a-c**) were prepared by a substitution reaction of compound **3** with amine derivatives, namely, ethanolamine, 4-methoxy-aniline, 4-chloroaniline and 4-nitroaniline in absolute ethanol. Treatment of compound **3** with hydrazine hydrate gave the 4-hydrazino derivative (**8**), and treatment of 4-chloro-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (**3**) with 3-methoxyphenol and 4-methoxyphenol in the presence of K_2CO_3 in DMF at 25 °C for 6 h afforded 4-(alkoxyphenoxy)-3-methyl-6-phenyl-thieno[3,2-d]pyrimidine-2(3*H*)-thione derivatives (**9a**,**b**). Moreover, treatment of 3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimi-

Table I. Physical and analytical data of newly synthesized compounds

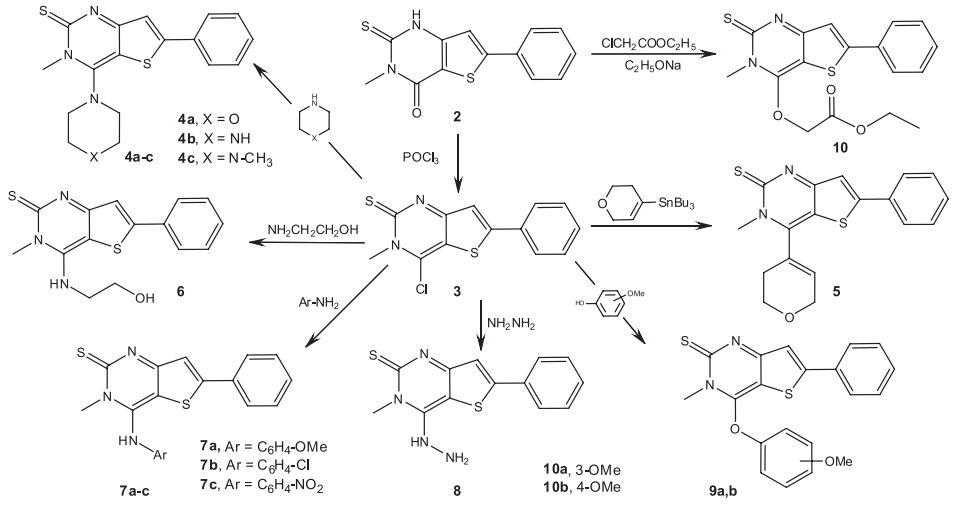
Compd.	Mol. formula (M_r)	M. p. (°C)	Yield (%)	Analysis (calcd./found, %)		
				C	H	N
2	$C_{13}H_{10}N_2OS_2$ (274.3)	199–210	65	56.91 56.86	3.67 3.64	10.21 10.18
3	$C_{13}H_9ClN_2S_2$ (292.80)	156–158	70	53.32 53.29	3.10 3.11	9.57 9.52
4a	$C_{17}H_{17}N_3OS_2$ (342.48)	208–210	83	59.45 59.46	4.99 5.01	12.23 12.19
4b	$C_{17}H_{18}N_4S_2$ (342.48)	215-217	79	59.62 59.59	5.30 5.29	16.36 16.32
4c	$C_{18}H_{20}N_4S_2$ (356.51)	202–204	81	60.64 60.62	5.65 5.63	15.72 15.73
5	$C_{18}H_{16}N_2OS_2$ (340.4)	192–194	69	63.50 63.49	4.74 4.71	8.23 8.20
6	$C_{15}H_{15}N_3OS_2$ (340.4)	222–224	87	56.76 56.74	4.76 4.74	13.24 13.27
7a	$C_{20}H_{17}N_3OS_2$ (379.4)	204–206	76	63.30 63.28	4.52 4.54	11.07 11.10
7b	$C_{19}H_{14}ClN_3S_2$ (383.9)	189–191	79	59.44 59.45	3.68 3.69	10.95 10.98
7c	$C_{19}H_{14}N_4O_2S_2$ (394.4)	210–212	73	57.85 57.87	3.58 3.59	14.20 14.19
8	$C_{13}H_{12}N_4S_2$(288.4)	200–202	73	54.14 54.12	4.19 4.20	19.43 19.39
9a	$C_{20}H_{16}N_2O_2S_2$ (380.4)	250–252	72	63.13 63.11	4.24 4.22	7.36 7.31
9b	$C_{20}H_{16}N_2O_2S_2$ (380.4)	223–225	78	63.13 63.15	4.24 4.21	7.36 7.38
10	$C_{17}H_{16}N_2O_3S_2$ (360.4)	230–232	66	56.65 56.61	4.47 4.51	7.77 7.74
11	$C_{13}H_{12}N_4OS$ (272.3)	235–237	60	57.34 57.31	4.44 4.42	20.57 20.53
12	$C_{14}H_{12}N_2OS_2$ (288.3)	175–177	75	58.31 58.33	4.19 4.21	9.71 9.69
13a	$C_{21}H_{17}N_3O_2S_2$ (407.5)	225–227	75	61.89 61.91	4.20 4.22	10.31 10.28
13b	$C_{21}H_{16}ClN_3O_2S_2$ (441.9)	254–256	76	57.07 57.09	3.65 3.66	9.51 9.49
13c	$C_{22}H_{19}N_3O_3S_2$ (437.5)	240–242	78	60.39 60.41	4.38 4.37	9.60 9.57
14	$C_{20}H_{14}N_4OS$ (358.4)	238-–240	75	67.02 67.04	3.94 3.97	15.63 15.61
15	$C_{14}H_{11}N_5OS$ (297.3)	259–261	75	56.55 56.58	3.73 3.74	23.55 23.49
16	$C_{14}H_{12}N_2O_3S_2$ (320.3)	209–211	70	52.48 52.50	3.78 3.80	8.74 8.73
17	$C_{19}H_{16}N_4OS$ (348.4)	242–244	65	65.50 65.48	4.63 4.67	16.08 16.11
18	$C_{20}H_{18}N_4O_3S_2$ (426.5)	215–217	76	56.32 56.29	4.25 4.27	13.14 13.12

Table II. Spectral data of newly synthesized compounds

Compd.	IR (KBr) (ν_{max}, cm^{-1})	MS (*m/z*)	^1H and ^{13}C NMR (DMSO-d_6) (δ, ppm)
2	3380 (brs, NH), 2951 (CH alkyl), 1689 (C=O), 1580 (C=N)	274 (M^+, 78 %) 275 (M^++1, 29 %)	3.08 (s, 3H, CH_3), 6.96-7.01 (m, 2H, phenyl), 7.39-7.42 (m, 3H, phenyl), 7.90 (s, CH thiophene), 9.01 (br, NH, D_2O exchangeable)
3	3023 (CH aryl), 2918 (CH alkyl), 1615 (C=N)	292 (M^+, 65 %) 293(M^++1, 31 %)	3.21 (s, 3H, CH_3), 6.93-7.02 (m, 2H, phenyl), 7.39-7.43 (m, 3H, phenyl), 7.84 (s, CH thiophene)
4a	3067 (CH aryl), 2951 (CH alkyl), 1630 (C=N)	343 (M^+, 61 %) 344(M^++1, 12 %)	3.11 (s, 3H, NCH_3), 3.26-3.31 (m, 2H, CH_2), 3.39-3.43 (m, 2H, CH_2), 3.82-3.90 (m, 4H, $2CH_2$), 6.96-7.04 (m, 2H, phenyl), 7.39-7.42 (m, 3H, phenyl), 7.92 (s, 1H, CH thiophene); ^{13}C NMR: δ 21.28 (CH_3), 22.76, 23.21 (2 CH_2), 25.58, 25.82 ($2CH_2$), 118.54-158.23 (11 sp^2 carbons), 164.70 (CS)
4b	3365 (brs, NH), 3061 (CH aryl), 2931 (CH alkyl), 1600 (C=N)	342 (M^+, 70 %) 343(M^++1, 21 %)	2.65-2.70 (m, 2H, CH_2), 3.13 (s, 3H, NCH_3), 3.29-3.33 (m, 2H, CH_2), 3.35-3.38 (m, 4H, $2CH_2$), 6.96-7.05 (m, 2H, phenyl), 7.45-7.53 (m, 3H, phenyl), 8.01 (s, CH thiophene), 9.60 (br, NH, D_2O exchangeable)
4c	3061 (CH aryl), 2931 (CH alkyl), 1600 (C=N)	356 (M^+, 59 %) 357 (M^++1, 16 %)	2.65-2.70 (m, 2H, CH_2), 3.10 (s, 3H, NCH_3), 2.97 (s, 3H, NCH_3), 3.29-3.33 (m, 2H, CH_2), 3.35-3.38 (m, 4H, $2CH_2$), 7.00-7.05 (m, 2H, phenyl), 7.45-7.53 (m, 3H, phenyl), 7.89 (s, CH thiophene); ^{13}C NMR: δ 22.30, 23.23 ($2CH_3$), 24.81, 25.09 (2 CH_2), 25.58, 25.89 ($2CH_2$), 118.69-157.90 (11 sp^2 carbons), 165.29 (CS)
5	3032 (CH aryl), 2919 (CH alkyl), 1609 (C=N)	340 (M^+, 73 %) 341(M^++1, 21 %)	2.38 (t, 2H, CH_2), 3.01 (s, 3H, NCH_3), 3.82 (t, 2H, CH_2), 3.98 (d, 2H, CH_2), 6.08 (t, 1H, CH), 6.95-7.08 (m, 2H, phenyl), 7.37-7.44 (m, 3H, phenyl), 8.07 (s, CH thiophene)
6	3415-3375 (brs, OH, NH), 3067 (CH alkyl), 1630 (C=N)	317 (M^+, 65 %) 318 (M^++1, 31 %)	2.28 (t, 2H, NCH_2), 2.95 (s, 3H, NCH_3), 3.26-3.31 (t, 2H, OCH_2), 5.05 (s, 1H, OH), 6.96-7.04 (m, 2H, phenyl), 7.47-7.57 (m, 3H, phenyl), 8.06 (s, CH thiophene), 9.80 (br, NH, D_2O exchangeable)

7a	3420 (brs, NH), 3054 (CH aryl), 2926 (CH alkyl), 1610 (C=N)	379 (M^+, 76 %) 380 (M^++1, 19 %)	2.83 (s, 3H, NCH_3), 3.29 (s, 3H, OCH_3), 6.99-7.06 (m, 2H, phenyl), 7.25 (d, 2H, *J* = 8.38 Hz, phenyl), 7.36-7.45 (m, 3H, phenyl), 7.88 (s, CH thiophene), 8.12 (d, 2H, *J* = 8.40 Hz, phenyl), 10.00 (br, NH, D_2O exchangeable)
7b	3395 (brs, NH), 3049 (CH aryl), 2926 (CH alkyl), 1617 (C=N)	383 (M^+, 58 %) 384 (M^++1, 17 %)	2.98 (s, 3H, NCH_3), 6.98-7.11 (m, 2H, phenyl), 7.23 (d, 2H, *J* = 8.44 *Hz*, phenyl), 7.34-7.43 (m, 3H, phenyl), 7.95 (d, 2H, *J* = 8.42 Hz, phenyl), 8.12 (s, CH thiophene), 9.50 (br, NH, D_2O exchangeable); ^{13}C NMR: δ 25.82 (CH_3), 117.68-159.87 (17 sp^2 carbons), 166.32 (CS)
7c	3410 (brs, NH), 3032 (CH aryl), 2919 (CH alkyl), 1609 (C=N)	394 (M^+, 73 %) 395 (M^++1, 18 %) 385 (M^++2, 10 %)	3.01 (s, 3H, NCH_3), 6.98-7.12 (m, 2H, phenyl), 7.29 (d, 2H, *J* = 8.03 Hz, phenyl), 7.39-7.46 (m, 3H, phenyl), 8.02 (d, 2H, *J* = 8.11 *Hz*, phenyl), 8.17 (s, CH thiophene), 9.35 (br, NH, D_2O exchangeable)
8	3198 (brs, NH), 2934 (CH alkyl), 1649 (C=N)	288 (M^+, 78 %) 289 (M^++1, 21 %)	2.97 (s, 3H, NCH_3), 7.02-7.09 (m, 2H, phenyl), 7.43-7.47 (m, 3H, phenyl), 8.13 (s, CH thiophene), 9.50, 11.00 (2 brs, 2NH, D_2O exchangeable)
9a	3032 (CH aryl), 2919 (CH alkyl), 1609 (C=N)	379 (M^+, 66 %) 380 (M^++1, 13 %)	2.88 (s, 3H, NCH_3), 4.11 (s, 3H, OCH_3), 6.92-7.01 (m, 2H phenyl), 7.09 (s, 1H phenyl), 7.38-7.43 (m, 3H, phenyl), 7.61 (d, 1H, phenyl), 7.89 (t, 1H, phenyl), 8.00 (d, 1H, phenyl), 8.16 (s, CH thiophene)
9b	3028 (CH aryl), 2908 (CH alkyl), 1600 (C=N)	379 (M^+, 72 %) 380 (M^++1, 17 %)	2.92 (s, 3H, NCH_3), 4.13 (s, 3H, OCH_3), 6.95-7,01 (m, 2H phenyl), 7.11 (d, *J* = 8.0 *Hz*, 2H phenyl), 7.37-7,49 (m, 3H, phenyl), 7.91 (d, *J* = 8.2 Hz, 2H phenyl), 8.21 (s, CH thiophene)
10	3032 (CH aryl), 2919 (CH alkyl), 1609 (C=N)	360 (M^+, 53 %) 361 (M^++1, 16 %)	1.42 (t, 3H, CH_3), 3.01 (s, 3H, NCH_3), 4.18 (s, 2H, CH_2), 4,33 (q, 2H, CH_2), 7.01-7.12 (m, 2H, phenyl), 7.41-7.49 (m, 3H, phenyl), 8.09 (s, CH thiophene); ^{13}C NMR: δ 20.11 (CH_3), 25.82 (CH_3), 44.31, 46.27 ($2CH_2$), 118.29-158.40 (11 sp^2 carbons), 164.70 (CS), 173.41 (CO)
11	3320 (brs, NH), 2931 (CH alkyl), 1689 (C=O), 1665 (C=N)	272 (M^+, 82 %) 273 (M^++1, 16 %)	2.89 (s, 3H, NCH_3), 7.00-7.09 (m, 2H, phenyl), 7.31-7.39 (m, 3H, phenyl), 8.03 (s, CH thiophene), 9.25, 10.00 (2 brs, 2NH, D_2O exchangeable); ^{13}C NMR: δ 24.56 (CH_3), 118.34-158.87 (10 sp^2 carbons), 168.87 (CO)
12	2925 (CH alkyl), 1687 (CO), 1650 (C=N)	288 (M^+, 56 %) 289 (M^++1, 16 %)	2.78 (s, 3H, SCH_3), 2.96 (s, 3H, NCH_3), 7.06-7.13 (m, 2H, phenyl), 7.43-7.48 (m, 3H, phenyl), 8.19 (s, CH thiophene)

13a	3410 (brs, NH), 3056 (CH aryl), 2918 (CH alkyl), 1679, 1689, (2CO), 1658 (C=N)	407 (M^+, 54 %) 408 (M^++1, 46 %)	2.29 (s, 2H, SCH_2), 3.01 (s, 3H, NCH_3), 6.94-7.17 (m, 4H, phenyl), 7.27-7.52 (m, 6H, phenyl), 8.11 (s, CH thiophene), 9.80 (br, NH, D_2O exchangeable)
13b	3435 (br, NH), 2924 (CH alkyl), 1689, 1672, (2CO), 1624 (C=N)	441 (M^+, 65 %) 442 (M^++1, 16 %)	2.29 (s, 2H, SCH_2), 2.97 (s, 3H, NCH_3), 6.94-7.03 (m, 2H, phenyl), 7.17 (d, 2H, *J* = 8.40 *Hz*, phenyl), 7.39-7.45 (m, 3H, phenyl), 7.88 (d, 2H, *J* = 8.41 *Hz*, phenyl), 8.12 (s, CH thiophene), 9.80 (br, NH, D_2O exchangeable); ^{13}C NMR: δ 25.31 (CH_3), 31.23 (CH_3), 118.51-158.76 (17 sp^2 carbons), 167.76, 169.23 (2CO)
13c	3410 (brs, NH), 2918 (CH alkyl), 1685, 1676, (2CO), 1651 (C=N)	437 (M^+, 71 %) 438 (M^++1, 13 %)	2.29 (s, 2H, SCH_2), 3.02 (s, 3H, NCH_3), 4.14 (s, 3H, OCH_3), 6.97-7.09 (m, 2H, phenyl), 7.21 (d, 2H, *J* = 8.40 Hz, phenyl), 7.37-7.43 (m, 3H, phenyl), 7.78 (d, 2H, *J* = 8.41 Hz, phenyl), 8.15 (s, CH thiophene), 9.45 (br, NH, D_2O exchangeable)
14	3028 (CH aryl), 2926 (CH alkyl), 1686, (CO), 1615 (C=N)	358 (M^+, 71 %) 359 (M^++1, 21 %)	2.96 (s, 3H, NCH_3), 7.05-7.19 (m, 4H, phenyl), 7.41-7.57 (m, 6H, phenyl), 8.11 (s, CH thiophene)
15	3360 (brs, NH), 2918 (CH alkyl), 1689, (CO), 1625 (C=N)	297 (M^+, 62 %) 298 (M^++1, 17 %)	2.87 (s, 3H, NCH_3), 7.01-7.11 (m, 2H, phenyl), 7.39-7.55 (m, 3H, phenyl), 8.17 (s, CH thiophene), 9.20 (brs, NH_2, D_2O exchangeable); ^{13}C NMR: δ 27.50 (CH_3), 118.58-158.72 (12 sp^2 carbons), 167.82 (CO)
16	2909 (CH alkyl), 1685 (C=O), 1635 (C=N), 1165, 1345 (SO_2)	320 (M^+, 89 %) 321(M^++1, 13 %)	2.86 (s, 3H, NCH_3), 3.31 (s, 3H, SO_2CH_3), 7.08-7.18 (m, 2H, phenyl), 7.38-7.50 (m, 3H, phenyl), 8.00 (s, CH thiophene)
17	3435 (brs, NH), 2920 (CH alkyl), 1689 (C=O), 1626 (C=N)	320 (M^+, 76 %) 349 (M^++1, 19 %)	2.92 (s, 3H, NCH_3), 6.92-7.11 (m, 2H, phenyl), 7.25 (d, 2H, *J* = 8.38 Hz, phenyl), 7.37-7.45 (m, 3H, phenyl), 7.85 (d, 2H, *J* = 8.40 Hz, phenyl), 8.13 (s, CH thiophene), 9.35, 9.85 (2br, 2NH, D_2O exchangeable)
18	3420 (brs, NH), 2926 (CH alkyl), 1686 (C=O), 1610 (C=N), 1165, 1345 (SO_2)	426 (M^+, 71 %) 427 (M^++1, 17 %)	2.89 (s, 3H, NCH_3), 3.28 (s, 3H, SO_2CH_3), 6.96-7.03 (m, 2H, phenyl), 7.25 (d, 2H, *J* = 8.38 Hz, phenyl), 7.35-7.43 (m, 3H, phenyl), 7.88 (d, 2H, *J* = 8.40 Hz, phenyl), 8.18 (s, CH thiophene), 9.40, 10.00 (2brs, 2NH, D_2O exchangeable); ^{13}C NMR: δ 25.56 (CH_3), 31.79 (CH_3), 118.45-158.90 (17 sp^2 carbons), 169.23 (CO)



Scheme 2.

din-4(1*H*)-one (**2**) with ethyl chloroacetate yielded ethyl[(3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4-yl)-oxy]acetate (**10**) in good yield (Scheme 2).

On the other hand, the reaction of compound **2** with hydrazine hydrate in refluxing ethanol afforded the 2-hydrazino derivative **11**. Formation of 2-hydrazino derivative **11** proceeded *via* loss of 1 mol of H_2S. 3-Methyl-2-(methylsulfanyl)-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**12**) and *N*-(phenyl/substituted-phenyl)-2-[(3-methyl-4-oxo-6-phenyle-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)sulfanyl]-acetamides (**13a-c**) were prepared in good yield *via* the one pot reaction of 3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4(1*H*)-one (**2**) with methyl-iodide and/or 2-chloro-*N*-substituted phenyl-(4-chloropenyl/4-methoxyphenyl)-acetamide derivatives. Also, the reaction of compound **12** with benzoylhydrazine and/or thiosemicarbazide afforded 4-methyl-(1-phenyl/ or amino)-7-phenyl-thieno[2,3-*e*][1,2,4]-triazolo[4,3-*a*]pyrimidin-5(4*H*)-one derivatives (**14**, **15**) (Scheme 3). The ^{1}H NMR spectra of compounds **14** and **15** revealed the absence of SCH_3 and the appearance of the phenyl ring as multiplet absorption signals around δ 7.41-7.57 ppm for compound **14**. Moreover, a broad absorption signal for the NH_2 group at δ 9.20 ppm was recorded for compound **15** (Table II).

Further, treatment of compound **12** with hydrogen peroxide in acetic acid afforded sulfonyl derivative **16**, which is a key intermediate for the synthesis of 2-[(4-amino-phenyl)-amino]-3-methyl-6-phenyl-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**17**), in good yield by the treatment of a warm solution of compound **16** and 4-phenylenediamine in glacial acetic acid. Finally, 2-arylamino-thieno[3,2-*d*]-pyrimidine derivative **17** was refluxed with methanesulfonyl chloride in pyridine to afford *N*-{4-[(3-methyl-4-oxo-6-phenyl-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)amino]-phenyl}-methane-sulfonamide (**18**).

Postulated structures of the newly synthesized compounds **16**, **17** and **18** are in agreement with their IR, NMR spectral and elemental analysis data (Tables I, II). The ^{1}H NMR

spectrum of **17**, for example, showed a singlet signal at δ 2.92 ppm corresponding to NCH_3. It also showed the aromatic protons at δ 6.92-7.11 (m, 2H, phenyl), 7.25 (d, 2H, J = 8.38 Hz, phenyl), 7.37-7.45 (m, 3H, phenyl), 7.85 (d, 2H, J = 8.40 Hz, phenyl), 8.13 (s, CH thiophene) and two broad bands corresponding to 2 NH at δ 9.35, 9.85 ppm, which were D_2O exchangeable.

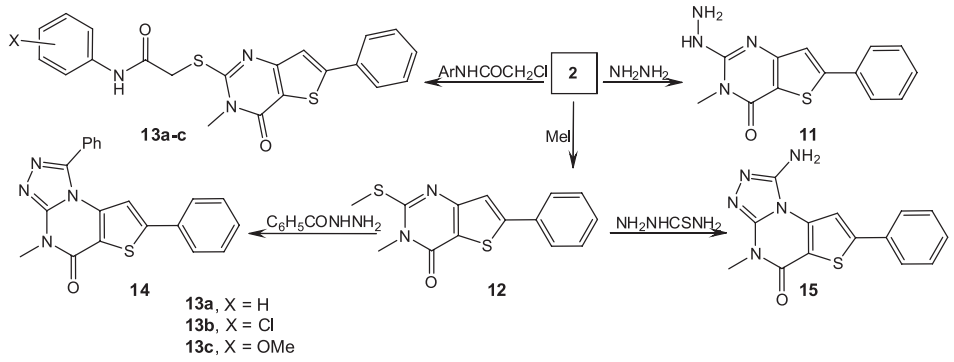
Antitumor activity and structure activity relationship

Most of the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against the human breast adenocarcinoma cell line (MCF-7), cervical carcinoma

Table III. Effects of synthesized compounds on the growth of the human breast adenocarcinoma cell line (MCF-7), cervical carcinoma cell line (HeLa) and colonic carcinoma cell line (HCT-116)

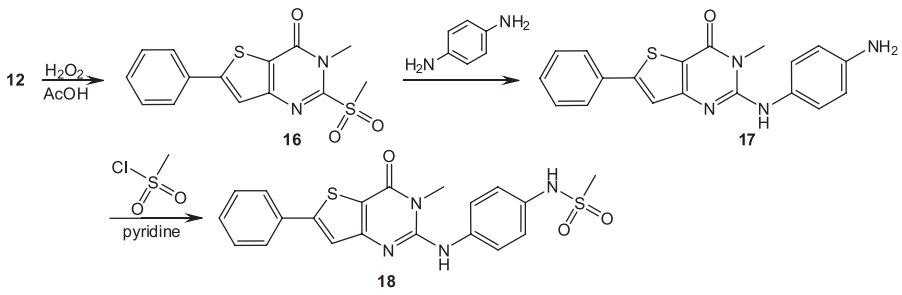
Compd.	GI_{50} (μmol L^{-1})		
	MCF-7	HeLa	HCT-116
2	29.13 ± 5.97	23.44 ± 7.58	20.57 ± 1.89
3	12.86 ± 1.51	15.09 ± 3.71	17.09 ± 2.3
4a	9.26 ± 3.27	12.23 ± 1.10	15.09 ± 3.71
4b	7.24 ± 2.34	10.20 ± 2.05	12.07 ± 1.89
4c	10.86 ± 1.53	12.80 ± 2.51	15.25 ± 2.78
5	18.54 ± 1.35	20.16 ± 1.34	20.60 ± 4.20
6	2.26 ± 0.94	2.95 ± 0.74	4.36 ± 0.82
7a	15.87 ± 1.70	20.16 ± 1.20	20.56 ± 1.72
7b	2.48 ± 0.93	1.49 ± 0.81	2.67 ± 0.38
8	17.09 ± 2.4	15.22 ± 1.43	12.87 ± 1.31
9a	25.44 ± 8.67	42.28 ± 2.98	88.96 ± 12.06
9b	8.58 ± 3.90	4.70 ± 0.49	20.68 ± 3.09
10	0.81 ± 0.26	0.55 ± 0.03	1.01 ± 0.52
11	10.80 ±1.50	9.80 ± 1.08	8.50 ± 2.01
13a	21.48 ± 0.74	44.72 ± 2.19	16.54 ± 1.02
13b	0.70 ± 0.47	0.46 ± 0.42	88.62 ± 10.06
13c	4.90 ± 0.68	5.92 ± 2.01	22.18 ± 4.63
14	7.36 ± 1.63	10.53 ± 1.08	8.05 ± 2.04
15	1.06 ± 0.51	1.80 ± 0.59	5.37 ± 2.40
16	6.27 ± 0.72	23.45 ± 5.85	15.75 ± 1.82
18	$0.09 \pm 0.00_5$	0.38 ± 0.02	1.07 ± 0.67
Doxorubicin	$0.05 \pm 0.00_9$	0.35 ± 0.03	0.65 ± 0.05

GI_{50} – concentrations that cause 50 % cell growth inhibition after continuous exposure for 48 h.
Mean ± SEM of three independent experiments performed in duplicate.



Scheme 3.

cell line (HeLa) and colonic carcinoma cell line (HCT-116). As shown in Table III, it was found that thieno[3,2-*d*]pyrimidine bearing benzene methyl sulphonamide (**18**) was nearly as active as doxorubicin with respective GI_{50} values of 0.09, 0.38 and 1.07 μmol L^{-1} *vs.* 0.05, 0.35 and 0.65 μmol L^{-1}, resp. Dihydrothieno[3,2-*d*]pyrimidinyl)-sulfanyl]-acetamide (**13b**) with GI_{50} values of 0.70 and 0.46 μmol L^{-1} was fairly active against the human breast adenocarcinoma cell line (MCF-7) and active as doxorubicin against the cervical carcinoma cell line (HeLa), resp., but had low inhibition activity against the colonic carcinoma cell line (HCT-116). Also, ethyl[(3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4-yl)-oxy]acetate (**10**), with GI_{50} values of 0.81, 0.55 and 1.01 μmol L^{-1}, was of comparable activity to doxorubicin against HeLa; this may be attributed to the presence of ether linkage. However, compound **15** with GI_{50} values of 1.06, 1.80 and 5.37 μmol L^{-1} also exhibited reasonable activities against the breast adenocarcinoma cell line (MCF-7) and cervical carcinoma cell line (HeLa) but modest activity against the colonic carcinoma cell line (HCT-116), which was probably due to the presence of aminotriazolo attached to the pyrimidine moiety. However, with a phenyl-triazolo moiety in compound **14**, it retained some inhibition activity. Compound **7** with GI_{50} values of 2.26, 2.95 and 4.36 μmol L^{-1} exhibited moderate growth inhibition, probably due to the presence of ethanolamine. Also, replacement of



Scheme 4.

4-Cl in compound **3** by the phenylamine moiety increased the growth inhibitory effect in compound **7b** but decreased growth inhibition in compound **7a**. Increase in the activity of **7b** may be attributed to the presence of the 4-Cl substituent in the phenyl ring (electron withdrawing group), while the presence of the 4-OCH_3 substituent in the phenyl ring (electron donating group) decreased the growth inhibition activity of **7a**. When comparing 4-chlorothieno[3,2-*d*] pyrimidine derivatives, it was found that compound **3** was more effective than compound **2** due to the presence of chlorine instead of carbonyl group. On the other hand, replacement of 4-Cl in compound **3** by six-member heterocyclic groups containing oxygen and nitrogen (namely, morpholine, piperazine and methyl piperazine) led to increased growth inhibition in the case of **4a-c**. Nevertheless, **4a-c** still exhibited modest growth inhibition activity in comparison with doxorubicin. However, replacement of chlorine in compound **3** by a pyrane ring decreased the growth inhibition, like in the case of compound **5**. Compounds **9b**, **11** and **13c** showed modest inhibition activities towards the human breast adenocarcinoma cell line (MCF-7) and cervical carcinoma cell line (HeLa), while compound **16** had modest activity towards MCF-7 but low inhibition towards HeLa and HCT-116. Finally, compounds **2**, **9a**, **13a** exerted low inhibition activities towards all the three tumor cell lines.

CONCLUSIONS

Novel thieno[3,2-*d*]pyrimidine derivatives were synthesized and evaluated for their anticancer activity. *N*-{4-[(3-methyl-4-oxo-6-phenyl-3*H*,4*H*-thieno[3,2-*d*]pyrimidin-2-yl)-amino]phenyl}-methanesulfonamide (**18**), *N*-(4-chlorophenyl)-2-[(3-methyl-4-oxo-6-phenyl-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl)sulfanyl]-acetamide (**13b**) and ethyl[(3-methyl-6-phenyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4-yl)-oxy]acetate (**10**) were nearly as active as doxorubicin against the three tumor cell lines, MCF-7, HeLa and HCT-116.

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