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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel series of thieno[2,3-d] pyrimidine derivatives as anticancer and antimicrobial agents

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Abstract The present study is concerned with the synthesis, anticancer and antimicrobial screening of several new 3-substituted or 2,3-disubstituted-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivatives. Three compounds (**4b**, **8c**, and **11b**) were selected by the National Cancer Institute and were first evaluated at a single-dose primary anticancer assay against 60 human cancer cell lines for their in vitro anticancer activity. Compound **8c** which passed the criteria for activity in this assay was evaluated against the full panel of 60 human cancer cell lines at five concentrations at tenfold dilutions where it showed non-selective broad-spectrum activity against all cancer cell lines. Furthermore, compounds **4b**, **6**, **8c**, **8d**, and **16** showed pronounced antibacterial activities comparable to ampicillin against *P. aeruginosa*. Therefore, it was concluded that compound **8c** may serve as a useful lead compound in search for powerful dual anticancer-antimicrobial agents.

Keywords Thieno[2,3-d]pyrimidine · Synthesis · Anticancer · Antimicrobial

Introduction

Thienopyrimidines, being bioisostere and structural analogs of the natural purines, occupy a special position among condensed pyrimidines. Thienopyrimidine derivatives are characterized by a very broad spectrum of biological activities which includes antimicrobial (Eissa and Moneer, 2004; Omar *et al.,* 2005), antiviral (Nasr and Gineinah, 2002; Shehata *et al.,* 1996), and anticancer (Shehata *et al.,* 1996; Jennings *et al.,* 2005) activities. Some thienopyrimidine derivatives have been reported to display good activity as phosphodiesterase (Chakraborti *et al.,* 2003), dihydrofolate reductase (DHFR) (Donkor *et al.,* 2003), in addition to vascular endothelial growth factor (VEGF) kinase inhibitors (Munchhof *et al.,* 2004).

The increasing number of neoplastic diseases together with the accompanied high mortality rates (Eckhardt, 2002) has stimulated the search for new structure leads that might be of use in designing novel anticancer drugs.

Chemotherapy medicines usually target cells that quickly divide. Therefore, the most common side effects of chemotherapy are linked to their effects on other fast growing cells including those found in the bone marrow, hair follicles and the lining of the gastrointestinal tract.

Patients suffering from neoplastic disorders and receiving chemotherapeutic treatment are more likely to get microbial infections due to subsequent depression of the immune system (Roche, 2008).

Multi-drug treatment of neoplastic conditions associated with microbial infections might inflect some added health problems especially in patients with impaired liver and/or kidney functions (Williams, 2008; Ritter *et al.,* 2008).

Hence, mono-therapy by a single drug having both anticancer and antimicrobial activities with minimum adverse effects and high safety margin might be advantageous from both therapeutic and cost-effective standpoints.

Interest in this field has been intensified after the discovery of the natural pyrazole *C*-glycoside pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1*H*-pyrazole-5 carboxamide. This antibiotic was reported to possess a broad spectrum of antimicrobial and antiviral activities in addition to

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being active against several tumor cell lines (Comber *et al.,* 1991).

Since the combination of two pharmacophores on the same scaffold is a well-established approach to the synthesis of more potent drugs (Pillai *et al.,* 2003; Venkatachalam *et al.,* 2006), and based on the above observations in addition to our new finding on tetrahydrobenzothieno[2,3-d]pyrimidines as antimicrobial agents (Soliman *et al.,* 2009) and in continuation of our research program on the synthesis of novel bioactive heterocyclic compounds (El-Hawash *et al.,* 1999; Habib and El-Hawash, 2005; Soliman *et al.,* 2003; Habib *et al.,* 2003; Bekhit *et al.,* 2004; El-Hawash *et al.,* 2006), the present work is concerned with the synthesis of several 3-substituted or 2,3-disubstituted-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivatives hoping to obtain compounds with dual anticancer and antimicrobial activities. Substituents used in these compounds include alkyl, aralkyl, phenyl, amino, sulfanyl, thioether such as phenyl or 4-substituted phenyl carbamoylmethylthio, 2-oxopropylthio, ethyloxycarbonylmethylthio, alkyl/aralkylthio, 2,3-epoxypropylthio, 3-substituted-2-hydroxypropylthio, in addition to sulfones and thiouredo moieties. It is well documented that these pharmacophoric groups impart various electronic and lipophilic properties to the original compounds, thus may contribute to the enhancement of the anticancer and/or antimicrobial activities of the thienopyrimidine nucleus. In particular, incorporation of thioether (Zhao *et al.,* 2009; Patil *et al.,* 2010), 2,3-epoxypropylthio (Raic-Malic *et al.,* 1999; Al-Smadi and Al-Momani, 2008), 3-substituted-2-hydroxypropylthio (Raic-Malic *et al.,* 1999; Du *et al.,* 2005), sulfones (Patil *et al.,* 2010; Haruna *et al.,* 2006) and thiouredo (Hallur *et al.,* 2006; Cunha *et al.,* 2007) moieties into different heterocyclic templates have been reported to produce compounds with anticancer and antimicrobial activities. Furthermore, various thiazoles and thiazolidinones possess anticancer (Kutschy *et al.,* 2009; Ottana *et al.,* 2005) and antimicrobial activities (Omar *et al.,* 2005; Bondock *et al.,* 2007). This encouraged us to incorporate dihydrothiazole and thiazolidinone moieties at the 3-position of thienopyrimidine nucleus hoping to go a step forward in the field of anticancer and antimicrobial agents.

Materials and methods

Chemistry

Melting points were determined in open glass capillaries on a Gallen-Kamp melting point apparatus and were uncorrected. IR spectra were recorded, for potassium bromide discs, υ (cm^{-1}), on Perkin Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were determined either on a Bruker Avance spectrometer (300 MHz) at the microanalytical unit, Faculty of Science, Cairo University, or on Jeol (500 MHz) at the microanalytical unit, Faculty of Science, Alexandria University, using DMSO-d_6 as a solvent and TMS as internal standard. The chemical shifts are given in ppm δ values (s, singlet; d, doublet; t, triplet and m, multiplet). ^{13}C-NMR spectra were determined on Jeol (125 MHz), Faculty of Science, Alexandria University, using TMS as internal standard. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV), Faculty of Science, Cairo University. Microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University and the microanalytical unit, Faculty of Pharmacy, Alexandria University. The results of the microanalyses were within ± 0.4 % of the calculated values. Follow-up of the reactions and checking the homogeneity of the compounds were made by ascending TLC run on silica gel G (Merck 60) coated glass plates. The spots were visualized by exposure to iodine vapor or UV lamp at λ 254 nm for few seconds.

Compound **1** was prepared according to the reported method (Gewald *et al.,* 1966) using cyanoacetamide, ethylcyanoacetate in the presence of sulfur and morpholine.

*5-Methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**2**)*

A mixture of **1** (2.28 g, 10 mmol) and formamide (10 ml) was refluxed for 3 h. The reaction mixture was set aside overnight at room temperature. The separated solid was filtered, washed with water, and crystallized from ethanol/water to yield 1.6 g (76 %) as a white solid, mp 298–299 °C; IR (KBr, υ, cm^{-1}): 3419, 3200, 3108 (NH), 1630 (C=O), 1575, 1494 (C=N, C=C, δ NH), 1400, 1342 (C–N lactam), 1242,1027 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.80 (s, 3H, CH_3), 7.60 (s, 2H, NH_2, D_2O exchangeable), 8.13 (s, 1H, thienopyrimidine C_2–H), 12.47 (s, 1H, NH, D_2O exchangeable); EI-MS *m/z* (relative abundance %): 211 [M^++2] (5), 210 [M^++1] (13), 209 [M^+] (84), 208 (94), 195 (5), 194 (11), 193 (87), 192 (100), 191 (33), 181 (6), 165 (21), 164 (29), 163 (23), 138 (10), 137 (12), 136 (10), 111 (10), 110 (20), 109 (23), 108 (15), 94 (20), 93 (19), 92 (10), 84 (7), 83 (15), 82 (20), 81 (16), 71 (13), 70 (16), 69 (22), 68 (24). Anal. Calcd. for $C_8H_7N_3O_2S$ (209.23): C, 45.92; H, 3.37; N, 20.08. Found: C, 45.77; H, 3.36; N, 20.04.

*5-Methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide potassium salt (**3**)*

Compound **2** (2.09 g, 10 mmol) was suspended in absolute ethanol (30 ml) and treated with alcoholic potassium hydroxide (0.56 g, 10 mmol in absolute ethanol (10 ml)). The reaction mixture was stirred for 24 h at room temperature. The potassium salt formed was filtered, washed

with absolute ethanol, and dried to yield 2.09 g (85 %) as a yellowish-white solid, mp >300 °C. The obtained product was used directly in the next reaction.

*3-Benzyl-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**4a**)*

Method A: A suspension of the potassium salt **3** (2.48 g, 10 mmol) in dry DMF (10 ml) was treated dropwise with stirring for 15 min with benzyl chloride (10 mmol). The reaction mixture was refluxed for 3 h, poured on crushed ice. The obtained solid was filtered, dried, and crystallized from ethanol to give pale yellow clusters in 65 % yield, mp 216–218 °C; IR (KBr, υ, cm^{-1}): 3330, 3174 (NH), 1685 (C=O), 1635 (C=N, C=C), 1571 (δ NH), 1407, 1369 (C–N lactam), 1259, 1072 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.67 (s, 3H, CH_3), 5.14 (s, 2H, CH_2), 7.24–7.26 (m, 2H, C_6H_5 $C_{2,6}$-H), 7.27–7.32 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.65 (s, 2H, NH_2, D_2O exchangeable), 8.65 (s, 1H, thienopyrimidine C_2-H). Anal. Calcd. for $C_{15}H_{13}N_3O_2S$ (299.35): C, 60.19; H, 4.38; N, 14.04. Found: C, 60.15; H, 4.38; N, 13.99.

Method B: A mixture of **1** (0.23 g, 1 mmol) and triethylorthoformate (5 ml) was heated for 3 h under reflux. The reaction mixture was concentrated under vacuum. Benzyl amine (2 ml) was added, and the mixture was stirred for 3 h at 40 °C. Aqueous ethanol (1:1) was added to the reaction mixture, and the precipitated crystals were filtered, dried and recrystallized from ethanol to give pale yellow clusters in 83 % yield, mp 216–219 °C.

The products obtained from both methods were identical as revealed by mp, mixed mp and superimposability of IR and ^{1}H NMR spectra. However, method B was superior to method A concerning the yield of the prepared compound.

*General procedure for the synthesis of 3-substituted-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**4b–e**)*

Compounds **4b–e** were synthesized following the above procedure under method (B) for preparation of **4a**, by heating a mixture of **1** (0.23 g, 1 mmol) and triethylorthoformate (5 ml) under reflux for 3 h. The reaction mixture was concentrated under vacuum. The appropriate amine (2 ml) was added, and the mixture was stirred for 3 h at 40 °C. Aqueous ethanol (1:1) was added to the reaction mixture and the precipitated crystals were filtered, dried, and recrystallized from the appropriate solvent.

*3-(2-Hydroxyethyl)-5-methyl-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxamide (**4b**)*

White clusters, yield 85 % (ethanol), mp 233–234 °C; IR (KBr, υ, cm^{-1}): 3427, 3379 (OH, NH), 1670 (C=O), 1618 (C=N), 1600, 1552 (C=C), 1573 (δ NH), 1436, 1357 (C–N lactam), 1265, 1043 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.73 (s, 3H, CH_3), 3.64 (q, 2H, $J = 5.4$ Hz, CH_2CH_2OH), 4.02 (t, 2H, $J = 5.4$ Hz, CH_2CH_2OH), 4.93 (t, 1H, $J = 5.4$ Hz, OH, D_2O exchangeable), 7.62 (s, 2H, NH_2, D_2O exchangeable), 8.31 (s, 1H, thienopyrimidine C_2-H). Anal. Calcd. for $C_{10}H_{11}N_3O_3S$ (253.28): C, 47.42; H, 4.37; N, 16.59. Found: C, 47.22; H, 4.36; N, 16.53.

*5-Methyl-4-oxo-3-propyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**4c**)*

Yellowish white crystals, yield 82 % (ethanol), mp 237–239 °C; IR (KBr, υ, cm^{-1}): 3427, 3379 (NH), 1676 (C=O), 1645 (C=N), 1573 (δ NH), 1500 (C=C), 1406, 1367 (C–N lactam), 1265, 1099 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 0.84 (t, 3H, $J = 7.6$ Hz, $CH_2CH_2CH_3$), 1.64 (sextet, 2H, $J = 7.6$ Hz, $CH_2CH_2CH_3$), 2.69 (s, 3H, C_5–CH_3), 3.88 (t, 2H, $J = 7.6$ Hz, $CH_2CH_2CH_3$), 7.64 (s, 2H, NH_2, D_2O exchangeable), 8.44 (s, 1H, thienopyrimidine C_2-H). Anal. Calcd. for $C_{11}H_{13}N_3O_2S$ (251.30): C, 52.57; H, 5.21; N, 16.72. Found: C, 52.39; H, 5.21; N, 16.68.

*3-Cyclohexyl-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**4d**)*

White needles, yield 79 % (ethanol), mp 260–262 °C; IR (KBr, υ, cm^{-1}): 3434, 3275 (NH), 1644 (C=O), 1583, 1480 (C=N, C=C), 1531 (δ NH), 1407, 1325 (C–N lactam), 1295, 1088 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.01–1.38 (m, 6H, cyclohexyl $C_{3,4,5}$-H), 1.61–1.81 (m, 4H, cyclohexyl $C_{2,6}$-H), 2.68 (s, 3H, C_5-CH_3), 4.56–4.61 (m, 1H, cyclohexyl C_1-H), 7.65 (s, 2H, NH_2, D_2O exchangeable), 8.49 (s, 1H, thienopyrimidine C_2-H); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.47 (+ve, CH_3), 25.17 (−ve, cyclohexyl C_4), 26.14 (−ve, cyclohexyl $C_{3,5}$), 32.05 (−ve, cyclohexyl $C_{2,6}$), 53.22 (+ve, cyclohexyl C_1), 122.83 (ab, C_{4a}), 129.34 (ab, C_6), 136.97 (ab, C_5), 147.93 (+ve, C_2), 157.80 (ab, C_4), 163.05 (ab, $CONH_2$), 164.14 (ab, C_{7a}); EI-MS *m/z* (relative abundance %): 292 [M^++1] (23), 291 [M^+] (40), 290 [M^+−1] (11), 211 (16), 210 (66), 209 (99), 208 (18), 195 (8), 194 (17), 193 (100), 192 (60), 191 (11), 166 (8), 165 (12), 164 (13), 153 (7), 137 (8), 136 (7), 110 (7), 109 (12), 83 (8), 81 (11), 79 (11), 69 (8), 58 (17), 55 (53), 54 (27), 53 (16). Anal. Calcd. for $C_{14}H_{17}N_3O_2S$ (291.37): C, 57.71; H, 5.88; N, 14.42. Found: C, 57.72; H, 5.89; N, 14.47.

*5-Methyl-4-oxo-3-(pyridin-2-yl)-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**4e**)*

Yellowish white crystals, yield 80 % (DMF/ethanol), mp 240–241 °C; IR (KBr, υ, cm^{-1}): 3433, 3159 (NH), 1691

(C=O), 1611, 1517 (C=N, C=C), 1562 (δ NH), 1438, 1347 (C–N lactam), 1282, 1096 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.70 (s, 3H, CH_3), 7.56 (t, 1H, $J = 6.85$ Hz, pyridine C_5-H), 7.72 (s, 2H, NH_2, D_2O exchangeable), 7.75 (d, 1H, $J = 8.4$ Hz, pyridine C_3-H), 8.03 (t, 1H, $J = 7.6$ Hz, pyridine C_4-H), 8.57 (s, 1H, thienopyrimidine C_2-H), 8.62 (d, 1H, $J = 4.6$ Hz, pyridine C_6-H). Anal. Calcd. for $C_{13}H_{10}N_4O_2S$ (286.31): C, 54.53; H, 3.52; N, 19.56. Found: C, 54.36; H, 3.51; N, 19.51.

*Ethyl 2-acetamido-5-carbamoyl-4-methylthiophene-3-carboxylate (**5**)*

A mixture of **1** (0.23 g, 1 mmol) and acetic anhydride (5 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured onto ice, and the separated solid was filtered and crystallized from ethanol to yield 0.21 g (77 %) as a white needles, mp 196–198 °C; IR (KBr, υ, cm^{-1}): 3253 (NH), 1683 br (C=O), 1544, 1505 (C=N, C=C, δ NH), 1410, 1305 (C–N lactam), 1244, 1110, 1028 (C–S–C) and (C–O–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.41 (t, 3H, $J = 6.9$ Hz, CH_2–CH_3), 1.6 (s, 1H, NH, D_2O exchangeable), 2.31 (s, 3H, CO–CH_3), 2.56 (s, 3H, CH_3), 4.39 (q, 2H, $J = 6.9$ Hz, CH_2–CH_3), 11.57 (s, 2H, NH_2, D_2O exchangeable); EI-MS *m/z* (relative abundance %): 270 [M^+] (3), 252 (19), 251 (23), 229 (8), 228 (10), 211 (9), 210 (56), 209 (60), 184 (5), 183 (15), 182 (16), 181 (5), 166 (8), 165 (18), 164 (81), 163 (100), 154 (5), 153 (5), 139 (8), 138 (9), 137 (11), 136 (13), 110 (9), 109 (16), 108 (6), 94 (5), 82 (6), 71 (5), 70 (15), 69 (14), 68 (7), 67 (13), 66 (43), 65 (40), 64 (40), 63 (5), 59 (5), 58 (5). Anal. Calcd. for $C_{11}H_{14}N_2O_4S$ (270.30): C, 48.88; H, 5.22; N, 10.36. Found: C, 48.69; H, 5.22; N, 10.33.

*3-Amino-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**6**)*

A mixture of **5** (2.7 g, 10 mmol) and hydrazine hydrate 99 % (15 ml, 30 mmol) was dissolved in absolute ethanol (5 ml). The mixture was refluxed for 6-8 h. The product obtained after cooling was filtered, washed with water, and crystallized from ethanol to yield 1.9 g (79 %) as a yellow needles, mp 172–174 °C; IR (KBr, υ, cm^{-1}): 3393, 3293, 3259 (NH), 1674 (C=O), 1599, 1480 (C=N, C=C), 1526 (δ NH), 1428, 1328 (C–N lactam), 1262, 1179, 1097 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 6H, 2 × CH_3), 2.5 (s, 2H, 3-NH_2, D_2O exchangeable), 8.11 (s, 2H, $CONH_2$, D_2O exchangeable). Anal. Calcd. for $C_9H_{10}N_4O_2S$ (238.27): C, 45.37; H, 4.23; N, 23.51. Found: C, 45.21; H, 4.22, N, 23.42.

*General procedure for the synthesis of 2,5-dimethyl-3-N-substituted thiocarbamoylamino-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**7a, b**)*

A solution of the aminopyrimidine derivative **6** (2.38 g, 10 mmol) in absolute ethanol (25 ml) was treated with the selected isothiocyanate derivative (10 mmol) and heated under reflux for 7–10 h. The reaction mixture was concentrated under vacuum and left to cool to room temperature. The deposited product was filtered and crystallized from ethanol.

*2,5-Dimethyl-3-N-phenylthiocarbamoylamino-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**7a**)*

Yellowish-brown microcrystalline powder, yield 83 %, mp 190–192 °C; IR (KBr, υ, cm^{-1}): 3393, 3294, 3259 (NH), 1674 (C=O), 1599, 1481 (C=N, C=C), 1527 (δ NH), 1428, 1328 (C–N lactam), 1599, 1263, 1180, 1023 (NCS amide I, II, III and IV mixed vibrational bands, respectively), 1066 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H, thienopyrimidine C_2-CH_3), 2.47 (s, 3H, thienopyrimidine C_5-CH_3), 6.96 (t, 1H, $J = 7.6$ Hz, C_6H_5 C_4-H), 7.39 (t, 2H, $J = 7.6$ Hz, C_6H_5 $C_{3,5}$-H), 7.56 (d, 2H, $J = 7.6$ Hz, C_6H_5 $C_{2,6}$-H), 8.11 (s, 2H, NH_2, D_2O exchangeable), 10.5 (s, 1H, *NH*-C_6H_5, D_2O exchangeable), 10.75 (s, ½ H, thienopyrimidine-*NH*, D_2O exchangeable), 11.04 (s, ½ H, SH, D_2O exchangeable); EI-MS *m/z* (relative abundance %): 373 [M^+] (absent), 232 (88), 121 (49), 111 (29), 110 (46), 105 (21), 103 (9), 102 (36), 92 (31), 77 (100), 76 (49), 75 (22), 70 (32), 69 (35), 66 (48), 65 (48). Anal. Calcd. for $C_{16}H_{15}N_5O_2S_2$ (373.45): C, 51.46; H, 4.05; N, 18.75. Found: C, 51.37; H, 4.04; N, 18.69.

*3-N-Benzylthiocarbamoylamino-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**7b**)*

Yellow needles, yield 82 %, mp 220–222 °C; IR (KBr, υ, cm^{-1}): 3273 br (NH), 1640 (C=O), 1586, 1470 br (C=N, C=C, δ NH), 1526, 1222, 1171, and 961 (NCS amide I, II, III, and IV mixed vibrational bands, respectively), 1335 (C–N lactam), 1070 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.50 (s, 3H, thienopyrimidine C_2-CH_3), 2.51 (s, 3H, thienopyrimidine C_5-CH_3), 4.73 (d, 2H, $J = 5.7$ Hz, CH_2), 7.20–7.23 (m, 5H, CH_2-C_6H_5), 7.77 (s, 2H, NH_2, D_2O exchangeable), 8.44 (s, 1H, *NH*-CH_2-C_6H_5, D_2O exchangeable), 9.27 (s, 1H, thienopyrimidine-*NH*, D_2O exchangeable). Anal. Calcd. for $C_{17}H_{17}N_5O_2S_2$ (387.48) : C, 52.69; H, 4.42; N, 18.07. Found: C, 52.47; H, 4.42; N, 18.03.

*General procedure for the synthesis of 3-(3-aryl/aralkyl-4-substituted phenylthiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**8a–e**)*

A solution of the appropriate thiourea derivative **7a** or **b** (10 mmol) in absolute ethanol (20 ml) was treated with equivalent amount of the selected phenacyl bromide. The reaction mixture was heated under reflux for 6–8 h and left to cool to room temperature. The deposited product was filtered, washed with aqueous ethanol, and crystallized from ethanol.

*3-(4-(4-Chlorophenyl)-3-phenylthiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxamide (**8a**)*

Dark yellow microcrystalline powder, yield 78 %, mp 216–218 °C; IR (KBr, υ, cm^{-1}): 3435, 3278 (NH), 1640 (C=O), 1583, 1480 (C=N, C=C), 1550 (δ NH), 1326 (C–N lactam), 1295, 1088 (C–S–C), 829 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.11 (s, 3H, thienopyrimidine C_2-CH_3), 2.46 (s, 3H, thienopyrimidine C_5-CH_3), 6.55 (s, 1H, thiazoline C_5-H), 6.95 (d, 2H, $J = 7.6$ Hz, N-C_6H_5 $C_{2,6}$-H), 7.04 (t, 1H, $J = 7.6$ Hz, N-C_6H_5 C_4-H), 7.14 (d, 2H, $J = 8.4$ Hz, 4-Cl-C_6H_4 $C_{2,6}$-H), 7.28 (d, 2H, $J = 8.4$ Hz, 4-Cl-C_6H_4 $C_{3,5}$-H), 7.32 (t, 2H, $J = 7.6$ Hz, N-C_6H_5 $C_{3,5}$-H), 7.87 (s, 2H, NH_2, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.83 (+ve, C_2-CH_3), 16.86 (+ve, C_5-CH_3), 95.60 (+ve, thiazoline C_5), 115.94 (ab, C_{4a}), 117.38 (+ve, C_6H_5 $C_{2,6}$), 121.38 (+ve, 4-Cl-C_6H_4 $C_{2,6}$), 124.06 (+ve, C_6H_5 C_4), 129.09 (+ve, 4-Cl-C_6H_4 $C_{3,5}$), 130.17 (+ve, C_6H_5 $C_{3,5}$), 134.42 (ab, 4-Cl-C_6H_4 C_1), 138.77 (ab, 4-Cl-C_6H_4 C_4), 139.22 (ab, C_6), 148.39 (ab, C_6H_5 C_1), 149.27 (ab, C_5), 149.92 (ab, thiazoline C_4), 155.65 (ab, thiazoline C_2), 158.13 (ab, C_4), 161.38 (ab, $CONH_2$), 163.89 (ab, C_2), 164.16 (ab, C_{7a}); EI-MS *m/z* (relative abundance %): 510 [M^++2], 508 [M^+] (absent), 467 (17), 466 (10), 465 (33), 373 (13), 303 (19), 302 (11), 301 (51), 300 (10), 289 (14), 288 (40), 287 (38), 286 (100), 285 (48), 250 (7), 210 (10), 179 (33), 170 (17), 169 (10), 168 (29), 165 (39), 151 (18), 150 (25), 149 (18), 140 (9), 138 (19), 137 (14), 136 (18), 135 (11), 134 (19), 133 (27), 132 (10), 125 (19), 124 (14), 123 (10), 113 (10), 111 (14), 110 (21), 91 (10), 89 (33), 77 (52). Anal. Calcd. for $C_{24}H_{18}ClN_5O_2S_2.H_2O$ (526.03): C, 54.79; H, 3.83. Found: C, 54.77; H, 3.82.

*3-(4-(4-Bromophenyl)-3-phenylthiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno [2,3-d]pyrimidine-6-carboxamide (**8b**)*

Pale yellow crystals, yield 71 %, mp 146–148 °C; IR (KBr, υ, cm^{-1}): 3330, 3289, 3107 (NH), 1640 (C=O), 1581, 1481 (C=N, C=C), 1529 (δ NH), 1405 (C–N lactam), 1254, 1175, 1072 (C–S–C), 761 (C–Br); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.15 (s, 3H, thienopyrimidine C_2-CH_3), 2.50 (s, 3H, thienopyrimidine C_5-CH_3), 6.53 (s, 1H, thiazoline C_5-H), 7.00 (d, 2H, $J = 8.1$ Hz, 4-Br-C_6H_4 $C_{2,6}$-H), 7.12 (t, 1H, $J = 8.4$ Hz, N-C_6H_5 C_4-H), 7.38 (t, 2H, $J = 8.4$ Hz, N-C_6H_5 $C_{3,5}$-H), 7.48 (d, 2H, $J = 8.4$ Hz, N-C_6H_5 $C_{2,6}$-H), 7.67 (d, 2H, $J = 8.1$ Hz, 4-Br-C_6H_4 $C_{3,5}$-H), 7.81 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{24}H_{18}BrN_5O_2S_2$ (552.47): N, 12.68. Found: N, 12.65.

*3-(3-Benzyl-4-phenylthiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**8c**)*

White needles, yield 80 %, mp 164–165 °C; IR (KBr, υ, cm^{-1}): 3325, 3280 (NH), 1645 (C=O), 1583, 1418 (C=N, C=C), 1530 (δ NH), 1403, 1326 (C–N lactam), 1245, 1081 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.44 (s, 3H, thienopyrimidine C_2-CH_3), 2.51 (s, 3H, thienopyrimidine C_5-CH_3), 5.00 (s, 2H, CH_2-C_6H_5), 6.30 (s, 1H, thiazoline C_5-H), 7.04 (d, 2H, $J = 7.2$ Hz, CH_2-C_6H_5 $C_{2,6}$-H), 7.22–7.42 (m, 8H, Ar–H), 9.8 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{25}H_{21}N_5O_2S_2$ (487.60): C, 61.58; H, 4.34; N, 14.36. Found: C, 61.44; H, 4.33; N, 14.41.

*3-(3-Benzyl-4-(4-chlorophenyl)thiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxamide (**8d**)*

Yellow clusters, yield 76 %, mp 148–150 °C; IR (KBr, υ, cm^{-1}): 3289, 3132 (NH), 1630 (C=O), 1558 br (C=N, C=C, δ NH), 1404, 1321 (C–N lactam), 1260, 1088 (C–S–C), 819 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.04 (s, 3H, thienopyrimidine C_2-CH_3), 2.46 (s, 3H, thienopyrimidine C_5-CH_3), 4.97 (s, 2H, $CH_2C_6H_5$), 6.36 (s, 1H, thiazoline C_5-H), 7.01 (d, 2H, $J = 6.9$ Hz, CH_2-C_6H_5 $C_{2,6}$-H), 7.17 (t, 1H, $J = 6.9$ Hz, CH_2-C_6H_5 C_4-H), 7.22 (t, 2H, $J = 6.9$ Hz, CH_2-C_6H_5 $C_{3,5}$-H), 7.29 (d, 2H, $J = 8.4$ Hz, 4-Cl-C_6H_4 $C_{2,6}$-H), 7.42 (d, 2H, $J = 8.4$ Hz, 4-Cl-C_6H_4 $C_{3,5}$-H), 7.49 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{25}H_{20}ClN_5O_2S_2$ (522.04): C, 57.52; H, 3.86; N, 13.42. Found: C, 57.46; H, 3.86; N, 13.37.

*3-(3-Benzyl-4-(4-bromophenyl)thiazol-2(3H)-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxamide (**8e**)*

Yellow microcrystalline powder, yield 70 %, mp 172–174 °C; IR (KBr, υ, cm^{-1}): 3419, 3284 (NH), 1648 (C=O), 1560, 1504 (C=N, C=C, δ NH), 1455, 1316 (C–N lactam), 1247, 1063 (C–S–C), 726 (C–Br); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H,

thienopyrimidine C_2-CH_3), 2.46 (s, 3H, thienopyrimidine C_5-CH_3), 4.97 (s, 2H, *CH_2*-C_6H_5), 6.35 (s, 1H, thiazoline C_5-H), 7.01 (d. dist., 2H, CH_2-C_6H_5 $C_{2,6}$-H), 7.17 (t, 1H, $J = 6.8$ Hz, CH_2-C_6H_5 C_4-H), 7.21–7.24 (m, 4H, CH_2-C_6H_5 $C_{3,5}$-H + 4-Br-C_6H_4 $C_{2,6}$-H), 7.56 (d, 2H, $J = 8.4$ Hz, 4-Br-C_6H_4 $C_{3,5}$-H), 7.49 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{25}H_{20}BrN_5O_2S_2$ (566.49): C, 53.01; H, 3.56; N, 12.36. Found: C, 53.20; H, 3.56; N, 12.38.

*General procedure for the synthesis of 3-(4-oxo-3-substituted thiazolidin-2-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**9a, b**)*

To a solution of the appropriate thiourea derivative **7a** or **b** (10 mmol) in absolute ethanol (20 ml), ethyl bromoacetate (1.8 g, 1.1 ml, 10 mmol) was added. The reaction mixture was heated under reflux for 6 h and left to cool to deposit the product which was filtered and crystallized from ethanol.

*3-(4-Oxo-3-phenylthiazolidin-2-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**9a**)*

Brownish clusters yield 89 %, mp 260–262 °C; IR (KBr, υ, cm^{-1}): 3388, 3281 (NH), 1740, 1637 (C=O), 1601, 1481 (C=N, C=C), 1559 (δ NH), 1445, 1356 (C–N lactam), 1245, 1033 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.44 (s, 3H, thienopyrimidine C_2-CH_3), 2.46 (s, 3H, thienopyrimidine C_5-CH_3), 4.09 (s, 2H, thiazolidinone C_5-H), 4.21 (s, 1 H, thiazolidinone C_5-H, enol form), 6.96 (t, 1H, $J = 7.6$ Hz, C_6H_5 C_4-H), 7.32 (t, 2H, $J = 7.6$ Hz, C_6H_5 $C_{3,5}$-H), 7.55 (d, 2H, $J = 7.6$ Hz, C_6H_5 $C_{2,6}$-H), 8.01 (s, 2H, NH_2, D_2O exchangeable), 10.53 (s, 1 H, enolic OH, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 16.78 (+ve, C_2-*CH_3*), 16.84 (+ve, C_5-*CH_3*), 60.10 (−ve, thiazolidinone C_5), 85.64 (+ve, thiazolidinone C_5, enol form), 99.72 (ab, thiazolidinone C_4, enol form), 116.07 (ab, C_{4a}), 117.38 (+ve, C_6H_5 $C_{2,6}$), 122.30 (+ve, C_6H_5 C_4), 129.67 (+ve, C_6H_5 $C_{3,5}$), 139.20 (ab, C_6), 148.40 (ab, C_6H_5 C_1), 153.11 (ab, C_5), 155.65 (ab, thiazolidinone C_2), 157.37 (ab, $CONH_2$), 158.37 (ab, C_4), 161.39 (ab, C_2), 163.10 (ab, C_{7a}), 173.02 (ab, thiazolidinone C_4). Anal. Calcd. for $C_{18}H_{15}N_5O_3S_2$ (413.47): C, 52.29; H, 3.66. Found: C, 52.15; H, 3.65.

*3-(3-Benzyl-4-oxothiazolidin-2-ylideneamino)-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**9b**)*

Pale yellow microcrystalline powder, yield 85 %, mp 244–245 °C; IR (KBr, υ, cm^{-1}): 3400, 3350 (NH), 1707, 1680 (C=O), 1620 (C=N), 1585 (δ NH), 1500 (C=C), 1323 (C–N lactam), 1263, 1074 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.39 (s, 3H, thienopyrimidine C_2-CH_3), 2.51 (s, 3H, thienopyrimidine C_5-CH_3), 4.14 (s, 2H, *CH_2*-C_6H_5), 4.87 (s, 2H, thiazolidinone C_5-H), 7.23–7.39 (m, 6H, Ar–H and thiazolidinone C_5-H enolic), 7.56 (s, 2H, NH_2, D_2O exchangeable), 10.00 (s, 1H, enolic OH, D_2O exchangeable). Anal. Calcd. for $C_{19}H_{17}N_5O_3S_2$ (427.50): C, 53.38; H, 4.01. Found: C, 53.19; H, 4.00.

*2-Mercapto-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**10**)*

A mixture of **1** (2.28 g, 10 mmol) and phenylisothiocyanate (1.35 g, 1.19 ml, 10 mmol) in acetonitrile (30 ml) was heated under reflux for 15 h in the presence of anhydrous potassium carbonate (1.4 g, 10 mmol). The reaction mixture was filtered. The residue obtained was dissolved in water, and neutralized with acetic acid; the obtained crude product was filtered, washed with water, dried, and crystallized from ethanol to yield 2.5 g (78 %) as a yellow amorphous powder, mp >300 °C; IR (KBr, υ, cm^{-1}): 3421, 3347, 3323, 3141 (NH), 2700 (SH), 1698, 1653 (C=O), 1569, 1496 (C=N, C=C), 1537 (δ NH), 1412 (C–N lactam), 1224, 1090 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.55 (s, 3H, CH_3), 7.19 (d, 2H, $J = 7.6$ Hz, C_6H_5 $C_{2,6}$-H), 7.36 (t, 1H, $J = 7.6$ Hz, C_6H_5 C_4-H), 7.43 (t, 2H, $J = 7.6$ Hz, C_6H_5 $C_{3,5}$-H), 7.60 (br s, 2H, NH_2, D_2O exchangeable), 7.90 (s, 1H, OH, imidol, D_2O exchangeable), 13.00 (s, 1H, SH, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 14.83 (+ve, CH_3), 117.69 (ab, C_{4a}), 125.66 (ab, C_6H_5 C_1), 128.68 (+ve, C_6H_5 C_4), 129.48 (+ve, C_6H_5 $C_{2,6}$), 129.52 (+ve, C_6H_5 $C_{3,5}$), 137.67 (ab, C_6), 139.67 (ab, C_5), 152.01 (ab, C_4), 157.98 (ab, $CONH_2$), 163.95 (ab, C_2), 175.97 (ab, C_{7a}). Anal. Calcd. for $C_{14}H_{11}N_3O_2S_2$ (317.39): C, 52.98; H, 3.49. Found: C, 53.01; H, 3.50.

*General procedure for the synthesis of 2-(phenyl or 4-substituted phenylcarbamoylmethylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**11a–c**)*

A mixture of **10** (0.32 g, 1 mmol) and the appropriate chloroacetanilide (1 mmol) was refluxed in dry acetone (20 ml) for 3 h in the presence of anhydrous potassium carbonate (0.14 g, 1 mmol). The mixture was cooled, poured into ice-cold water, filtered, and the solid obtained was dried and crystallized from ethanol.

*2-(Phenylcarbamoylmethylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**11a**)*

Yellow clusters, yield 83 %, mp 284–285 °C; IR (KBr, υ, cm^{-1}): 3404, 3274, 3205 (NH), 1662 (C=O), 1600, 1510 (C=N, C=C), 1544 (δ NH), 1433 (C–N lactam), 1228, 1078 (C–S–C);

^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.68 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 7.05 (t, 1H, $J = 7.5$ Hz, NH–C_6H_5 C_4-H), 7.33 (t, 2H, $J = 7.5$ Hz, NH–C_6H_5 $C_{3,5}$-H), 7.30–7.64 (m, 7H, Ar–H), 7.54 (s, 2H, NH_2, D_2O exchangeable), 10.3 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{22}H_{18}N_4O_3S_2$ (450.53): C, 58.65; H, 4.02; N, 12.43. Found: C, 58.48; H, 4.02; N, 12.38.

*2-(4-Chlorophenylcarbamoylmethylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**11b**)*

White needles, yield 78 %, mp 292–293 °C; IR (KBr, υ, cm^{-1}): 3364, 3322, 3174 (NH), 1694, 1650 (C=O), 1596, 1500 (C=N, C=C), 1536 (δ NH), 1400, 1322 (C–N lactam), 1220, 1158, 1091 (C–S–C), 829 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.62 (s, 3H, C_5-CH_3), 4.04 (s, 2H, S–CH_2), 7.33 (d, 2H, $J = 9.2$ Hz, 4-Cl-C_6H_4 $C_{2,6}$-H), 7.42–7.44 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.54 (d, 2H, $J = 8.4$ Hz, C_6H_5 $C_{2,6}$-H), 7.58 (d, 2H, $J = 9.2$ Hz, 4-Cl-C_6H_4 $C_{3,5}$-H), 7.60 (s, 2H, NH_2, D_2O exchangeable), 10.46 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{22}H_{17}ClN_4O_3S_2$ (484.98): C, 54.48; H, 3.53; N, 11.55. Found: C, 54.51; H, 3.53; N, 11.58.

*2-(p-Tolylcarbamoylmethylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**11c**)*

Pale yellow needles, yield 85 %, mp 242–244 °C; IR (KBr, υ, cm^{-1}): 3325, 3181 (NH), 1694, 1650 (C=O), 1599, 1507 (C=N, C=C, δ NH), 1410, 1327 (C–N lactam), 1224, 1160, 1091 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.20 (s, 3H, 4-*CH_3*-C_6H_4), 2.63 (s, 3H, C_5-CH_3), 4.04 (s, 2H, S–CH_2), 7.06 (d, 2H, $J = 7.6$ Hz, 4-CH_3-C_6H_4 $C_{3,5}$-H), 7.39 (t, 1H, $J = 8.4$ Hz, C_6H_5 C_4-H), 7.43 (d, 2H, $J = 7.6$ Hz, 4-CH_3-C_6H_4 $C_{2,6}$-H), 7.53–7.59 (m, 4H, C_6H_5 $C_{2,3,5,6}$-H), 7.61 (s, 2H, NH_2, D_2O exchangeable), 10.23 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.27 (+ve, C_5-*CH_3*), 20.99 (+ve, 4-*CH_3*-C_6H_4), 38.15 (−ve, S–CH_2), 119.65 (+ve, 4-CH_3-C_6H_5 $C_{2,6}$), 120.18 (ab, C_{4a}), 127.72 (ab, C_6H_5 C_1), 129.72 (+ve, C_6H_5 $C_{2,6}$), 129.84 (+ve, C_6H_5 C_4), 130.20 (+ve, C_6H_5 $C_{3,5}$), 130.67 (+ve, 4-CH_3-C_6H_5 $C_{3,5}$), 132.99 (ab, 4-CH_3-C_6H_5 C_4), 135.99 (ab, 4-CH_3-C_6H_5 C_1), 136.82 (ab, C_6), 137.19 (ab, C_5), 158.50 (ab, C_4), 160.84 (ab, H_2N-CO), 163.26 (ab, C_2), 164.16 (ab, C_{7a}), 165.51 (ab, CO–NH). Anal. Calcd. for $C_{23}H_{20}N_4O_3S_2$ (464.56): C, 59.46; H, 4.34; N, 12.06. Found: C, 59.41; H, 4.33; N, 12.04.

*General procedure for the synthesis of 2-substituted thio-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxamides (**12, 13**)*

A mixture of **10** (0.32 g, 1 mmol) and either chloroacetone or ethyl bromoacetate (1 mmol) and anhydrous potassium carbonate (0.21 g, 1.5 mmol) in dry acetone (10 ml) was heated under reflux for 10 h. The reaction mixture was cooled, poured into ice-cold water; the precipitate formed was dried and crystallized from ethanol.

*2-(2-Oxopropylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**12**)*

Pale yellow crystals, yield 78 %, mp 240–242 °C; IR (KBr, υ, cm^{-1}): 3458, 3312, 3165 (NH), 1732, 1693, 1646 (C=O), 1592, 1509 (C=N, C=C, δ NH), 1455, 1304 (C–N lactam), 1229, 1171, 1023 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.25 (s, 3H, $COCH_3$), 2.66 (s, 3H, thienopyrimidine C_5-CH_3), 4.07 (s, 2H, S–CH_2), 7.43-7.45 (m, 2H, C_6H_5 $C_{2,6}$-H), 7.58–7.61 (m, 3H, C_6H_5 $C_{3,4,5}$-H). Anal. Calcd. for $C_{17}H_{15}N_3O_3S_2$ (373.45): C, 54.67; H, 4.05; N, 11.25. Found: C, 54.45; H, 4.05; N, 11.22.

*Ethyl 2-(6-carbamoyl-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidin- 2-ylthio)acetate (**13**)*

Yellow clusters, yield 87 %, mp 194–196 °C; IR (KBr, υ, cm^{-1}): 3450, 3314, 3163 (NH), 1700, 1692, 1649 (C=O), 1594, 1504 (C=N, C=C, δ NH), 1455 (C–N lactam), 1295, 1228, 1152 (C–S–C), 1228, 1046 (C–O–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.18 (t, 3H, $J = 6.85$ Hz, CH_2–*CH_3*), 2.64 (s, 3H, thienopyrimidine C_5-CH_3), 4.08 (s, 2H, S–CH_2), 4.10 (q, 2H, $J = 6.85$ Hz, *CH_2*–CH_3), 7.41 (d, 2H, $J = 7.65$ Hz, C_6H_5 $C_{2,6}$-H), 7.55–7.62 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.62 (s, 2H, NH_2, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 14.74 (+ve, C_5-*CH_3*), 15.24 (+ve, CH_2*CH_3*) 35.06 (−ve, S–CH_2), 61.68 (−ve, *CH_2*CH_3), 120.15 (ab, C_{4a}), 127.77 (ab, C_6H_5 C_1), 129.79 (+ve, C_6H_5 $C_{2,6}$), 130.25 (+ve, C_6H_5 $C_{3,5}$), 130.76 (+ve, C_6H_5 C_4), 135.86 (ab, C_6), 137.24 (ab, C_5), 158.40 (ab, C_4), 160.41 (ab, H_2N-CO), 163.07 (ab, C_2), 164.10 (ab, C_{7a}), 168.64 (ab, O–C=O). Anal. Calcd. for $C_{18}H_{17}N_3O_4S_2$ (403.48): C, 53.58; H, 4.25; N, 10.41. Found: C, 53.74; H, 4.26; N, 10.38.

*General procedure for the synthesis of 2-substituted thio-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno-[2,3-d] pyrimidine-6-carboxamides (**14a–c**)*

A solution of **10** (0.32 g, 1 mmol) in 10 % sodium hydroxide solution (10 ml) was treated gradually with alkyl or aralkyl halide (CH_3I, C_2H_5I or C_6H_5–CH_2Cl) (1.5 mmol) over 15 min. The reaction mixture was stirred for 5 h at ambient temperature. The precipitate formed was filtered, washed with water, dried, and crystallized from ethanol.

*5-Methyl-2-(methylthio)-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**14a**)*

White needles, yield 85 %, mp 230–232 °C; IR (KBr, υ, cm^{-1}): 3394 (NH), 1680 (C=O), 1635 (C=N), 1564 (δ NH), 1509 (C=C), 1402, 1300 (C–N lactam), 1227, 1159, 1080 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.05 (s, 3H, S–CH_3), 2.65 (s, 3H, C_5-CH_3), 7.40 (dd, 2H, $J = 7.2$, 2.3 Hz, C_6H_5 $C_{2,6}$-H), 7.53–7.54 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.62 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{15}H_{13}N_3O_2S_2$ (331.41): C, 54.36; H, 3.95; N, 12.68. Found: C, 54.13; H, 3.94; N, 12.66.

*2-(Ethylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**14b**)*

Pale yellow needles, yield 72 %, mp 216–217 °C; IR (KBr, υ, cm^{-1}): 3327, 3267 (NH), 1687 (C=O), 1647 (C=N), 1591, 1504 (C=C, δ NH), 1460, 1301 (C–N lactam), 1222, 1159, 1095 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.19 (t, 3H, $J = 7.6$ Hz, CH_2CH_3), 2.63 (s, 3H, C_5-CH_3), 3.02 (q, 2H, $J = 7.6$ Hz, CH_2CH_3), 7.38 (dd, 2H, $J = 7.6$, 1.5 Hz, C_6H_5 $C_{2,6}$-H), 7.48-7.55 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.61 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{16}H_{15}N_3O_2S_2$ (345.44): C, 55.63; H, 4.38; N, 12.16. Found: C, 55.46; H, 4.37; N, 12.13.

*2-(Benzylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**14c**)*

White shiny crystals, yield 79 %, mp 218–220 °C; IR (KBr, υ, cm^{-1}): 3440, 3247, 3166 (NH), 1693 (C=O), 1606 (C=N), 1566, 1550 (δ NH), 1506 (C=C), 1423, 1371 (C–N lactam), 1271, 1045 (C–S–C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.68 (s, 3H, C_5-CH_3), 4.35 (s, 2H, CH_2), 7.23-7.55 (m, 10H, Ar–H), 7.61 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{21}H_{17}N_3O_2S_2$ (407.51): C, 61.89; H, 4.20; N, 10.31. Found: C, 61.73; H, 4.10; N, 10.28.

*General procedure for the synthesis of 2-substituted sulfonyl-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**15a–c**)*

An aqueous solution of 4 % potassium permanganate was added dropwise to a stirred solution of the appropriate **14a–c** (0.5 mmol) in glacial acetic acid (5 ml). The addition of potassium permanganate solution was continued until the purple color persisted for 30 min. Stirring was maintained at room temperature for further 2 h. The reaction mixture was left overnight, then cooled to 5 °C, and saturated sodium sulfite solution was gradually added with stirring until the brown color disappeared. The white precipitate formed was filtered, washed with water, air dried, and crystallized from ethanol.

*5-Methyl-2-(methylsulfonyl)-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**15a**)*

Pale yellow needles, yield 63 %, mp 256–258 °C; IR (KBr, υ, cm^{-1}): 3440, 3275, 3187 (NH), 1696, 1655 (C=O), 1598 (C=N, C=C), 1543 (δ NH), 1402, 1319 (C–N lactam), 1359, 1149 (SO_2), 1269, 1081 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.46 (s, 3H, SO_2-CH_3), 2.65 (s, 3H, C_5-CH_3), 7.40 (t, 1H, $J = 7.6$ Hz, C_6H_5 C_4), 7.47 (t, 2H, $J = 7.6$ Hz, C_6H_5 $C_{3,5}$-H), 7.52 (d, 2H, $J = 7.6$ Hz, C_6H_5 $C_{2,6}$-H), 7.83 (s, 2H, NH_2, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.20 (+ve, C_5-CH_3), 41.82 (+ve, SO_2-CH_3), 124.64 (ab, C_{4a}), 129.01 (+ve, C_6H_5 $C_{2,6}$), 130.33 (+ve, C_6H_5 C_4), 130.55 (+ve, C_6H_5 $C_{3,5}$), 132.99 (ab, C_6H_5 C_1), 133.59 (ab, C_6), 137.09 (ab, C_5), 153.35 (ab, C_4), 158.27 (ab, H_2N-CO), 159.58 (ab, C_2), 163.64 (ab, C_{7a}). Anal. Calcd. for $C_{15}H_{13}N_3O_4S_2$ (363.41): C, 49.57; H, 3.61; N, 11.56. Found: C, 49.72; H, 3.62; N, 11.61.

*2-(Ethylsulfonyl)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**15b**)*

White needles, yield 65 %, mp 254–255 °C; IR (KBr, υ, cm^{-1}): 3465, 3369 (NH), 1670 (C=O), 1605, 1493 (C=N, C=C), 1539 (δ NH), 1388, 1165 (SO_2), 1321 (C–N lactam), 1272, 1080 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.22 (t, 3H, $J = 7.6$ Hz, CH_2CH_3), 2.66 (s, 3H, C_5-CH_3), 3.59 (q, 2H, $J = 7.6$ Hz, CH_2CH_3), 7.40–7.42 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.47 (dd, 2H, $J = 6.8$, 1.5 Hz, C_6H_5 $C_{2,6}$-H), 7.82 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. for $C_{16}H_{15}N_3O_4S_2$ (377.44): C, 50.91; H, 4.01; N, 11.13. Found: C, 50.97; H, 4.01; N, 11.09.

*2-(Benzylsulfonyl)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**15c**)*

White crystals, yield 60 %, mp 204–206 °C; IR (KBr, υ, cm^{-1}): 3433, 3266, 3197 (NH), 1685, 1664 (C=O), 1595, 1499 (C=N, C=C), 1539 (δ NH), 1411, 1334 (C–N lactam), 1370, 1136 (SO_2), 1270, 1078 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.57 (s, 3H, C_5-CH_3), 4.44 (s, 2H, S–CH_2), 7.22 (d, 2H, $J = 7.6$ Hz, CH_2-C_6H_5 $C_{2,6}$-H), 7.26–7.44 (m, 8H, Ar–H), 7.50 (s, 2H, NH_2, D_2O exchangeable); EI-MS *m/z* (relative abundance %): 440 [M^++1] (4), 439 [M^+] (9), 376 (9), 375 (21), 374 (58), 301 (18), 298 (8), 286 (7), 285 (20), 284 (81), 283 (5), 212 (5), 182 (12), 167 (36), 119 (5), 110 (18), 109 (6), 103 (7), 92 (15), 91 (100), 90 (10), 89 (5), 82 (5), 77 (20), 66 (16), 65 (27), 64 (9), 63 (7), 52 (6), 51 (15), 50 (8). Anal. Calcd. for

$C_{21}H_{17}N_3O_4S_2$ (439.51): C, 57.39; H, 3.90; N, 9.56. Found: C, 57.17; H, 3.89; N, 9.53.

*2-[(Oxiran-2-yl)methylthio]-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**16**)*

To a stirred solution of the thiol **10** (0.64 g, 2 mmol) and potassium hydroxide (0.22 g, 4 mmol) in water (50 ml), epichlorohydrin (0.19 g, 0.16 ml, 2 mmol) was added dropwise over 10 min. The reaction mixture was stirred at room temperature for 10 h and the precipitated product was filtered, washed with water and crystallized from ethanol to yield 0.6 g (81 %) as a pale yellow amorphous powder, mp 146–148 °C; IR (KBr, υ, cm^{-1}): 3369, 3220 (NH), 1681, 1674 (C=O), 1633 (C=N), 1558, 1506, 1488 (C=C), 1539 (δ NH), 1434, 1311 (C–N lactam), 1224, 1045, 1026, 771 (C–O–C), 1172, 1072 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.76 (dd, 1H, $J = 15.25$, 6.1 Hz, epoxy CH_2-cis H), 2.63 (s, 3H, CH_3), 3.51-3.58 (m, 1H, epoxy CH_2-trans H), 4.04–4.09 (m, 2H, S–CH_2), 5.09 (dist. quintet, 1H, epoxy CH), 7.25 (t, 1H, $J = 7.65$ Hz, C_6H_5 C_4-H), 7.38 (t, 2H, $J = 7.65$ Hz, C_6H_5 $C_{3,5}$-H), 7.52 (s, 2H, NH_2, D_2O exchangeable), 7.61 (d, 2H, $J = 7.65$ Hz, C_6H_5 $C_{2,6}$-H); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.22 (+ve, C_5-*CH_3*), 33.13 (−ve, S–CH_2), 33.80 (–ve, epoxy CH_2), 42.87 (+ve, epoxy CH), 120.37 (ab, C_{4a}), 128.81 (+ve, C_6H_5 C_4), 129.50 (+ve, C_6H_5 C_2), 129.76 (+ve, C_6H_5 C_6), 130.18 (+ve, C_6H_5 C_3), 130.58 (+ve, C_6H_5 C_5), 130.64 (ab, C_6H_5 C_1), 135.97 (ab, C_6), 137.25 (ab, C_5), 158.62 (ab, C_4), 159.81 (ab, H_2N-CO), 163.20 (ab, C_2), 164.16 (ab, C_{7a}). Anal. Calcd. for $C_{17}H_{15}N_3O_3S_2.1^1/_2H_2O$ (400.47): C, 50.98; H, 4.53; N, 10.49. Found: C, 50.79; H, 4.51; N, 10.46.

*General procedure for the synthesis of 2-(3-substituted 2-hydroxypropylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (**17a, b**)*

A mixture of **16** (0.37 g, 1 mmol) and the appropriate amine (1 mmol) in absolute ethanol (25 ml) was heated under reflux for 10 h, concentrated and left to cool in ice bath. The separated product was filtered and crystallized from the appropriate solvent.

*2-(2-Hydroxy-3-morpholinopropylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**17a**)*

Pale yellow crystals, yield 52 % (ethanol/water), mp 220–222 °C; IR (KBr, υ, cm^{-1}): 3413 (OH), 3265, 3130 (NH), 1681,1674 (C=O), 1635 (C=N), 1507, 1485 (C=C), 1535 (δ NH), 1432, 1315 (C–N lactam), 1275, 1172, 1075 (C–S–C), 1233, 1027 (C–O–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.59 (s, 3H, CH_3), 2.69, 2.85 (two singlets, each 1H, SCH_2), 3.78 (t, 4H, $J = 8.4$ Hz, morpholine $C_{3,5}$-H), 3.79 (t, 4H, $J = 8.4$ Hz, morpholine $C_{2,6}$-H), 4.13 (dd, 2H, $J = 5.8$, 5 Hz, N–CH_2), 4.41–4.43 (m, 1H, CH), 5.76 (d, 1H, $J = 5.35$ Hz, OH, D_2O exchangeable), 7.21 (d, 2H, $J = 7.60$ Hz, C_6H_5 $C_{2,6}$-H), 7.39 (t, 1H, $J = 7.60$ Hz, C_6H_5 C_4-H), 7.44 (t, 2H, $J = 7.60$ Hz, C_6H_5 $C_{3,5}$-H), 7.59 (br s, 2H, NH_2, D_2O exchangeable); EI-MS *m/z* (relative abundance %): 460 [M^+] (absent), 450 (9), 357 (34), 344 (20), 343 (13), 341 (45), 340 (21), 317 (10), 315 (17), 303 (22), 302 (24), 301 (78), 300 (22), 299 (10), 296 (12), 286 (10), 285 (21), 284 (13), 283 (21), 258 (13), 240 (10), 222 (15), 221 (52), 215 (12), 209 (26), 197 (11), 196 (19), 195 (37), 192 (15), 183 (11), 182 (100), 181 (19), 177 (24), 166 (40), 165 (31), 164 (44), 152 (20), 150 (16), 146 (13), 139 (32), 137 (26), 129 (13), 126 (14), 117 (22), 113 (25), 112 (10), 111 (20), 109 (8), 97 (12), 91 (24), 85 (2), 83 (11), 77 (29). Anal. Calcd. for $C_{21}H_{24}N_4O_4S_2.H_2O$ (478.58): C, 52.70; H, 5.48; N, 11.71. Found: C, 52.57; H, 5.48; N, 11.76.

*2-(2-Hydroxy-3-(piperidin-1-yl)propylthio)-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**17b**)*

Yellow microcrystalline powder, yield 56 % (DMF/water), mp 202–204 °C; IR (KBr, υ, cm^{-1}): 3480 (OH), 3263, 3165 (NH), 1706, 1654 (C=O), 1591, 1575, 1508, 1473 (C=N, C=C), 1558 (δ NH), 1419, 1334 (C–N lactam), 1271, 1176, 1091 (C–S–C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 1.22-1.60 (m, 6H, piperidine $C_{3,4,5}$-H), 2.21–2.30 (m, 4H, piperidine $C_{2,6}$-H), 2.63 (s, 3H, CH_3), 3.83 (dd, 2H, $J = 14.5$, 8.3 Hz, SCH_2), 4.16 (dd, 2H, $J = 14.5$, 4.05 Hz, N–CH_2), 4.44–4.47 (m, 1H, CH), 5.77 (d, 1H, $J = 5.1$ Hz, OH, D_2O exchangeable), 7.23–7.26 (m, 2H, C_6H_5 $C_{2,6}$-H), 7.38– 7.50 (m, 3H, C_6H_5 $C_{3,4,5}$-H), 7.54 (s, 2H, NH_2, D_2O exchangeable); ^{13}C-NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 15.19 (+ve, C_5-*CH_3*), 53.53 (−ve, S–CH_2 and piperidine $C_{3,4,5}$), 54.85 (−ve, piperidine-*CH_2* and piperidine $C_{2,6}$), 65.90 (+ve, *CH*-OH), 114.76 (ab, C_{4a}), 124.86 (+ve, C_6H_5 C_4), 128.80 (ab, C_6H_5 C_1), 129.46 (+ve, C_6H_5 $C_{2,3,5,6}$), 136.38 (ab, C_6), 138.20 (ab, C_5), 150.52 (ab, C_4), 156.53 (H_2N-CO), 159.31 (ab, C_2), 163.93 (ab, C_{7a}); EI-MS *m/z* (relative abundance %): 458 [M^+] (absent), 448 (2), 413 (3), 374 (5), 373 (6), 357 (5), 344 (5), 342 (13), 341 (18), 340 (7), 325 (5), 318 (12), 317 (42), 316 (12), 302 (16), 301 (65), 300 (12), 299 (13), 285 (9), 284 (29), 283 (11), 258 (9), 221 (11), 209 (18), 195 (21), 193 (15), 183 (13), 182 (100), 165 (22), 164 (23), 154 (10), 139 (15), 137 (16), 119 (11), 113 (12), 111 (11), 110 (16), 109 (11), 95 (6), 84 (6), 77 (29), 72 (11), 70 (11). Anal. Calcd. for $C_{22}H_{26}N_4O_3S_2$ (458.60): N, 12.22; Found: N, 12.18.

Biological evaluation

In vitro anticancer screening

Out of the newly synthesized thieno[2,3-d]pyrimidine derivatives in the present work, compounds **4b** (NSC 746752), **8c** (NSC 746754), and **11b** (NSC 746751) were selected by the National Cancer Institute (NCI) Development Therapeutic Program (DTP) (www.dtp.nci.nih.gov) for the in vitro disease-oriented human cells screening panel assay to investigate their in vitro anticancer activity.

In vitro one-dose anticancer assay

A primary in vitro one-dose anticancer assay was performed using the full panel of about 60 human tumor cell lines derived from nine neoplastic diseases including leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers, in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda, MD, USA (Monks *et al.,* 1991; Boyd, 1997; Shoemaker, 2006).

In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Tested compounds were added to the culture at a single concentration (10^{-4} mol) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

Results for each tested compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells (Table 1). This allows detection of both growth inhibition (GI %) (values between 0 and 100) and lethality (values less than 0).

In vitro 5-dose anticancer assay

The cytotoxic and/or growth inhibitory effects of the most active selected compounds, which satisfy pre-determined threshold inhibition criteria designed to efficiently capture compounds with antiproliferative activity and based on careful analysis of historical DTP screening data, were tested in vitro against the full NCI 60 cell panel at tenfold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} mol. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth.

Three dose response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line (Table 2). The GI_{50} corresponds for the compounds' concentration causing 50 % decrease in net cell growth which signifies the growth inhibitory power of the test agent. The TGI value which is the drug concentration resulting in total growth inhibition which signifies the cytostatic effect of the test agent. LC_{50} value which is the compounds' concentration leading to 50 % net cell death at the end of the incubation period (48 h) and signifies the cytotoxic effect of the test agent.

Selectivity ratio

Subpanel and full panel mean-graph midpoint values (MG-MID GI_{50}) are the average of individual real and default GI_{50} values of all cell lines in the subpanel or the full panel. The ratio obtained by dividing the compound full panel mean-graph midpoint (MG-MID GI_{50}) (μM) by its individual subpanel MG-MID GI_{50} (μM) is considered as a measure of compound selectivity (Boyd and Paull, 1995). Ratio between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective (Table 3).

In vitro antimicrobial screening

The newly synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphyloccus aureus* (ATCC6538P) as a representative of gram-positive bacteria, *Escherichia coli* (ATCC8739), and *Pseudomonas aeruginosa* (ATCC9027) as representatives of gram-negative bacteria and for their in vitro antifungal activity against *Candida albicans* (ATCC2091) using the cup diffusion technique (Jain and Kar, 1971). Compounds showing reasonable inhibition zones (≥13 mm) were subjected to determine their minimal inhibitory concentration (MIC) values using the two fold serial dilution method (Scott, 1989). Ampicillin was used as standard antibacterial agent, while Clotrimazole was used as standard antifungal agent. Dimethylformamide (DMF) was used as a blank showed no inhibition zones.

Inhibition zone measurement

The agar cup diffusion technique (Jain and Kar, 1971) was adopted to determine the growth inhibition zones. Sterile nutrient agar was inoculated with the test organisms (each 100 ml of molten agar (at 45° C) received 1 ml of 6 h broth culture), and then seeded agar was poured into sterile petri dishes. Cups (8 mm in diameter) were cut in the agar, and each cup received 0.1 ml of the test compound solution in DMF (1 mg/ml). The plates were prepared in triplicate and then incubated at 37 °C for 24 h for bacteria and 48 h for fungi. The resulting inhibition zones were measured in mm diameter (Table 4).

Table 1 In vitro mean growth percentage, delta values, and the percentage growth inhibition (GI %) caused by the test compounds against the most sensitive tumor cell lines at the single-dose assay

Comp. no.	NSC-no.	Mean growth (%)	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
4b	746752	116.73	Renal cancer	TK-10 (47.22)
			CNS cancer	SF-295 (43.78)
8c	746754	19.24	Non-small cell lung cancer	A549/ATCC (100.00), EKVX (106.97), HOP-62 (75.81), HOP-92 (119.09), NCI-H226 (69.12), NCI-H23 (79.54), NCI-H322 M (64.60), NCI-H460 (92.54), NCI-H522 (75.09)
			Colon cancer	HCC-2998 (42.89), HCT-116 (70.87), HCT-15 (85.10), HT 29 (20.50), KM12 (86.43), SW-620 (87.48)
			Breast cancer	BT-549 (106.70), HS578T (114.57), MCF7 (82.68), MDA-MB-231/ATCC (56.97), MDM-MB-435 (87.80), NCI/ADR-RES (81.12), T-47D (65.33)
			Ovarian cancer	IGROV1 (89.09), OVCAR-3 (95.68), OVCAR-4 (66.53), OVCAR-5 (52.15), OVCAR-8 (85.29)
			Leukemia	CCRF-CEM (112.17), HL-60(TB) (135.80), K-562 (91.58), MOLT-4(87.91), RPMI-8226 (110.32), SR (87.20)
			Renal cancer	786-0 (49.31), A498 (99.29), ACHN (92.73), CAKI-1 (140.65), RXF393 (105.46), SN12C (52.27), TK-10 (97.11), UO-31 (95.87)
			Melanoma	LOX IMVI (79.44), M 14 (63.64), SK-MEL-2 (89.96), SK-MEL-28 (51.79), SK-MEL-5 (92.56), UACC-257 (95.15)
			Prostate cancer	DU-145 (57.85), PC-3 (98.03)
			CNS cancer	SF-268 (78.68), SF-539 (77.83), SNB-19 (70.56), SNB-75 (45.25), U251 (92.24)
11b	746751	109.13	Ovarian cancer	IGROV1 (79.38)
			Renal cancer	TK-10 (47.22)
			Melanoma	SK-MEL-2 (116.09)

The data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM conc

Minimal inhibitory concentration measurement

Using the two fold serial dilution method (Scott, 1989), the test organisms were grown in their suitable broth at 37 °C for 24 h for bacteria and 48 h for fungi. Two fold serial dilutions of the test compounds and the proper reference drugs solutions were prepared using the proper nutrient broth to obtain concentrations 500, 250, 125, 62.5, 31.25, 15.63, 7.82, and 3.91 μg/ml with the concentration of DMF not exceeding 2.5 %. The tubes were then inoculated with the test organisms (each 5 ml received 0.1 ml of the above inoculum) and were incubated at 37 °C for 48 h. Subsequently, the tubes were observed for the presence or absence of microbial growth. The lowest concentration showing no growth was taken as the MIC. The MIC values of the prepared compounds are listed in Table 4.

Results and discussion

Chemistry

The reaction sequences employed for synthesis of target compounds are depicted in Schemes 1 and 2. The starting compound ethyl 2-amino-5-carbamoyl-4-methylthiophene-3-carboxylate **1** was prepared by reacting acetoacetamide and ethyl cyanoacetate in the presence of sulfur and morpholine, according to the procedure previously reported (Gewald *et al.,* 1966). The preparation of 3-Benzyl-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivative **4a** (Scheme 1) was achieved through a three steps procedure by adopting the previously established reaction conditions that involved refluxing the amino ester **1** with excess formamide (El-Baih *et al.,* 2006) to give 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide **2**. The ^{1}H-NMR spectrum of **2** showed two singlets at 2.80 and 8.13 ppm assigned for thienopyrimidine C_5-CH_3 protons and thienopyrimidine C_2-proton, respectively; in addition to two deuterium exchangeable singlets at 7.60 and 12.47 ppm assigned for NH_2 and NH protons, respectively. Compound **2** was converted to the corresponding potassium salt **3** by treatment with alcoholic potassium hydroxide followed by heating with benzyl chloride in dry DMF under reflux to afford the required thienopyrimidine derivative **4a** in 65 % yield. Alternatively, compound **4a** was prepared in 83 % yield by reacting the amino ester **1** with triethylorthoformate and benzyl amine in accordance with the previously reported reaction conditions (Nomoto *et al.,* 1991). Accordingly, compounds **4b–e** were synthesized via the one pot reaction conditions in 79–85 % yields by treating the amino ester **1** with triethylorthoformate

Table 2 In vitro cytotoxic activity of the compound **8c** (NSC-No.: 746754) in the five-dose screen (10^{-4} to 10^{-8} M) toward 60 cancer cell lines

Panel/cell line	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
Leukemia			
CCRF-CEM	34.2	>100	>100
HL-60(TB)	6.68	>100	>100
K-562	8.89	>100	>100
MOLT-4	24.6	>100	>100
RPMI-8226	13.9	>100	>100
SR	5.74	>100	>100
Non-small cell lung cancer			
A549/ATCC	3.79	>100	>100
HOP-62	2.82	9.47	>100
HOP-92	1.34	5.33	>100
NCI-H226	3.72	67.9	>100
NCI-H23	3.12	>100	>100
NCI-H322M	3.71	>100	>100
NCI-H460	2.65	7.34	>100
NCI-H522	1.73	7.23	>100
Colon cancer			
COLO205	2.39	7.32	>100
HCC-2998	3.05	>100	>100
HCT-116	2.13	10.03	>100
HCT-15	2.64	>100	>100
HT29	3.04	>100	>100
KM12	3.37	>100	>100
SW-620	3.16	>100	>100
CNS cancer			
SF-268	4.00	>100	>100
SF-295	2.93	>100	>100
SF-539	4.02	>100	>100
SNB-19	4.80	>100	>100
SNB-75	2.80	>100	>100
U251	3.31	>100	>100
Melanoma			
LOX IMVI	3.54	>100	>100
MALME-3M	2.18	>100	>100
M14	3.88	84.5	>100
SK-MEL-2	3.89	86.6	>100
SK-MEL-28	3.56	>100	>100
SK-MEL-5	1.81	3.75	7.75
UACC-257	3.56	24.3	>100
UACC-62	4.26	83.2	>100
Ovarian cancer			
IGROV1	1.43	6.31	>100
OVCAR-3	2.28	8.58	>100
OVCAR-4	2.50	>100	>100
OVCAR-5	4.33	>100	>100
OVCAR-8	3.49	>100	>100
SK-OV-3	4.50	>100	>100
Renal cancer			
786-0	3.82	>100	>100
A498	2.53	8.97	>100
ACHN	2.86	>100	>100
CAKI-1	3.35	>100	>100
RXF393	3.43	>100	>100
SN12C	3.84	>100	>100
TK-10	3.78	>100	>100
UO-31	1.66	7.00	>100
Prostate cancer			
PC-3	2.15	8.31	>100
DU-145	3.90	>100	>100
Breast cancer			
MCF7	2.92	>100	>100
NCI/ADR-RES	2.90	29.2	>100
MDA-MB-231/ATCC	3.28	8.41	>100
HS578T	3.85	>100	>100
MDA-MB-435	2.89	12.5	>100
BT-549	2.41	8.89	>100
T-47D	3.25	>100	>100
MDA-MB-468	2.65	>100	>100

The data obtained from NCI's in vitro disease-oriented human tumor cell screen

Table 3 Median growth inhibitory concentrations (GI_{50}, μM) of in vitro subpanel tumor cell lines, GI_{50} (μM) full panel mean-graph mid-points (MG-MID) and selectivity ratio of compound **8c** toward the nine tumor cell lines

Subpanel tumor cell lines MG-MID	GI_{50} (μM)	Selectivity ratios
Leukemia	15.67	0.28
Non-small cell lung cancer	2.86	1.53
Colon cancer	2.83	1.55
CNS cancer	3.64	1.21
Melanoma	3.34	1.31
Ovarian cancer	3.09	1.42
Renal cancer	3.15	1.39
Prostate cancer	3.03	1.45
Breast cancer	3.02	1.45
Full panel MG-MID	4.39[a]	–

[a] GI_{50} (μM) full panel mean-graph mid-point (MG-MID) = the average sensitivity of all cell lines toward the test agent (**8c**)

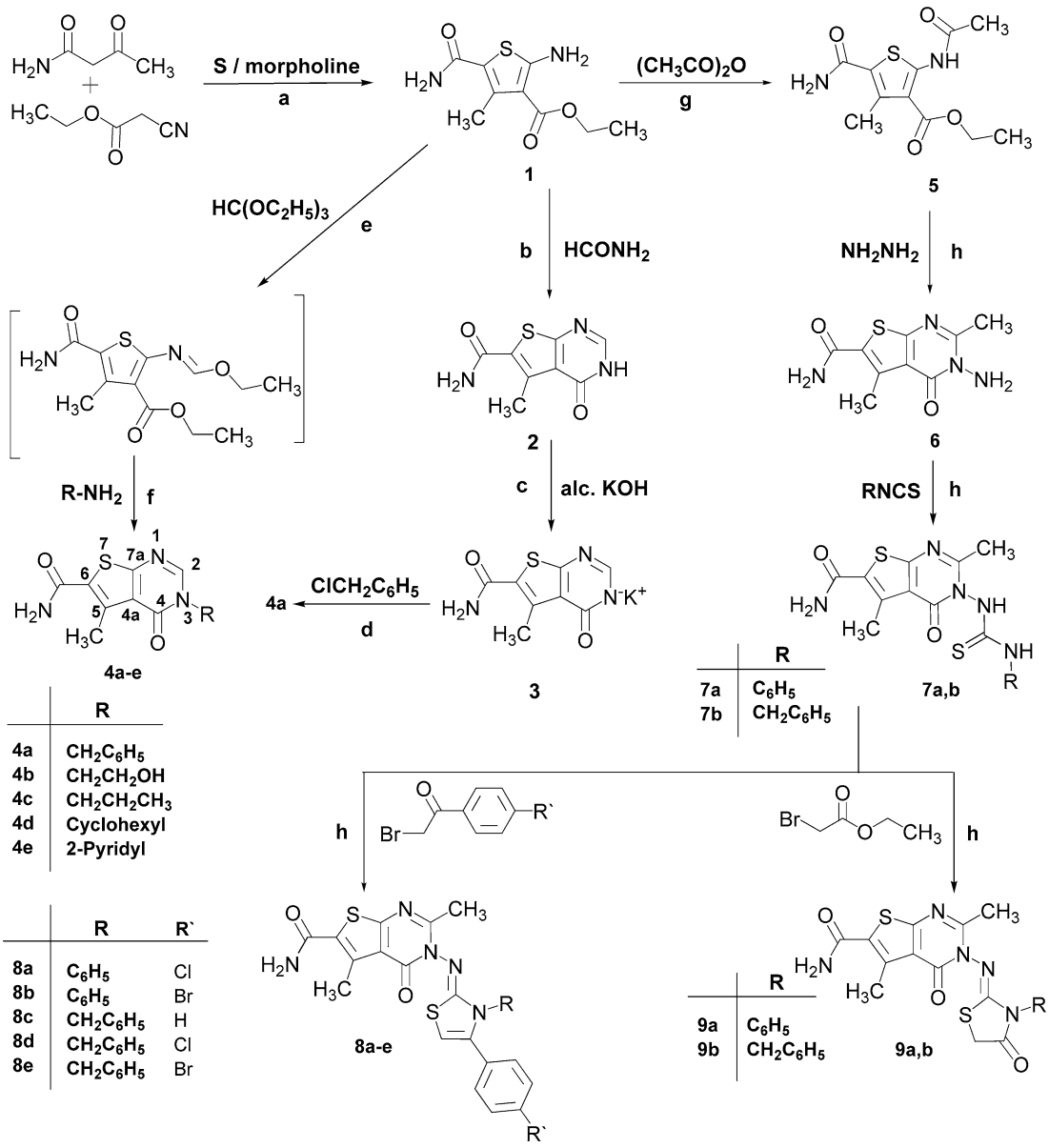
and the appropriate amine. The ^{1}H-NMR spectra of compounds **4a–e** lacked the deuterium exchangeable singlet for the NH proton at position 3 in the precursor **2** and showed signals for the protons of the alkyl and aralkyl substituents at the 3-position of the thienopyrimidine nucleus beside signals characteristic for the other protons at their expected chemical

Table 4 In vitro antimicrobial activity of the synthesized compounds. Inhibition zones (IZ) in mm diameter and MIC in μg/ml

Comp. no.	*S. aureus* (ATCC6538P)		*E. coli* (ATCC8739)		*P. aeruginosa* (ATCC9027)		*C. albicans* (ATCC2091)	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
2	10	–	13	–	12	–	15	–
4a	10	–	15	–	16	–	15	–
4b	10	–	15	–	17	125	15	–
4c	10	–	17	125	12	–	15	–
4d	10	–	17	125	12	–	15	–
4e	10	–	13	–	12	–	15	–
5	10	–	16	125	12	–	15	–
6	10	–	15	–	17	125	15	–
7a	10	–	15	–	12	–	15	–
7b	12	–	13	–	12	–	15	–
8a	10	–	16	125	12	–	15	–
8b	10	–	15	–	12	–	15	–
8c	10	–	15	–	17	125	17	125
8d	10	–	15	–	17	125	15	–
8e	11	–	15	–	12	–	15	–
9a	14	250	15	–	12	–	16	–
9b	14	250	15	–	12	–	16	–
10	12	–	15	–	12	–	15	–
11a	10	–	15	–	12	–	15	–
11b	10	–	15	–	12	–	15	–
11c	10	–	15	–	12	–	15	–
12	13	250	15	–	12	–	15	–
13	10	–	13	–	12	–	15	–
14a	10	–	13	–	12	–	15	–
14b	10	–	16	–	12	–	15	–
14c	10	–	15	–	12	–	15	–
15a	11	–	16	–	12	–	15	–
15b	10	–	13	–	12	–	15	–
15c	10	–	15	–	12	–	15	–
16	10	–	15	–	17	125	15	–
17a	10	–	13	–	12	–	15	–
17b	10	–	15	–	12	–	15	–
Ampicillin	30	3.91	26	7.81	18	125	–	–
Clotrimazole	–	–	–	–	–	–	28	7.81

shifts. ^{13}C-NMR spectrum of **4d** displayed five highly shielded signals due to thienopyrimidine-C_5CH_3 at 15.47 ppm, the five methylene carbons resonated as three signals at 25.17, 26.14, and 32.05 ppm corresponding to cyclohexyl C_4, cyclohexyl C_3 and C_5, and cyclohexyl C_2 and C_6, respectively, and the methine carbon of the cyclohexyl C_1 at 53.22 ppm. In addition, the spectrum showed another deshielded methine carbon of thienopyrimidine C_2 at 147.93 ppm and six deshielded signals at 122.83, 129.34, 136.97, 157.80, 163.05, and 164.14 ppm corresponding to six quaternary carbons of the thienopyrimidine C_{4a}, C_6, C_5, C_4, $CONH_2$, and C_{7a}, respectively. The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation.

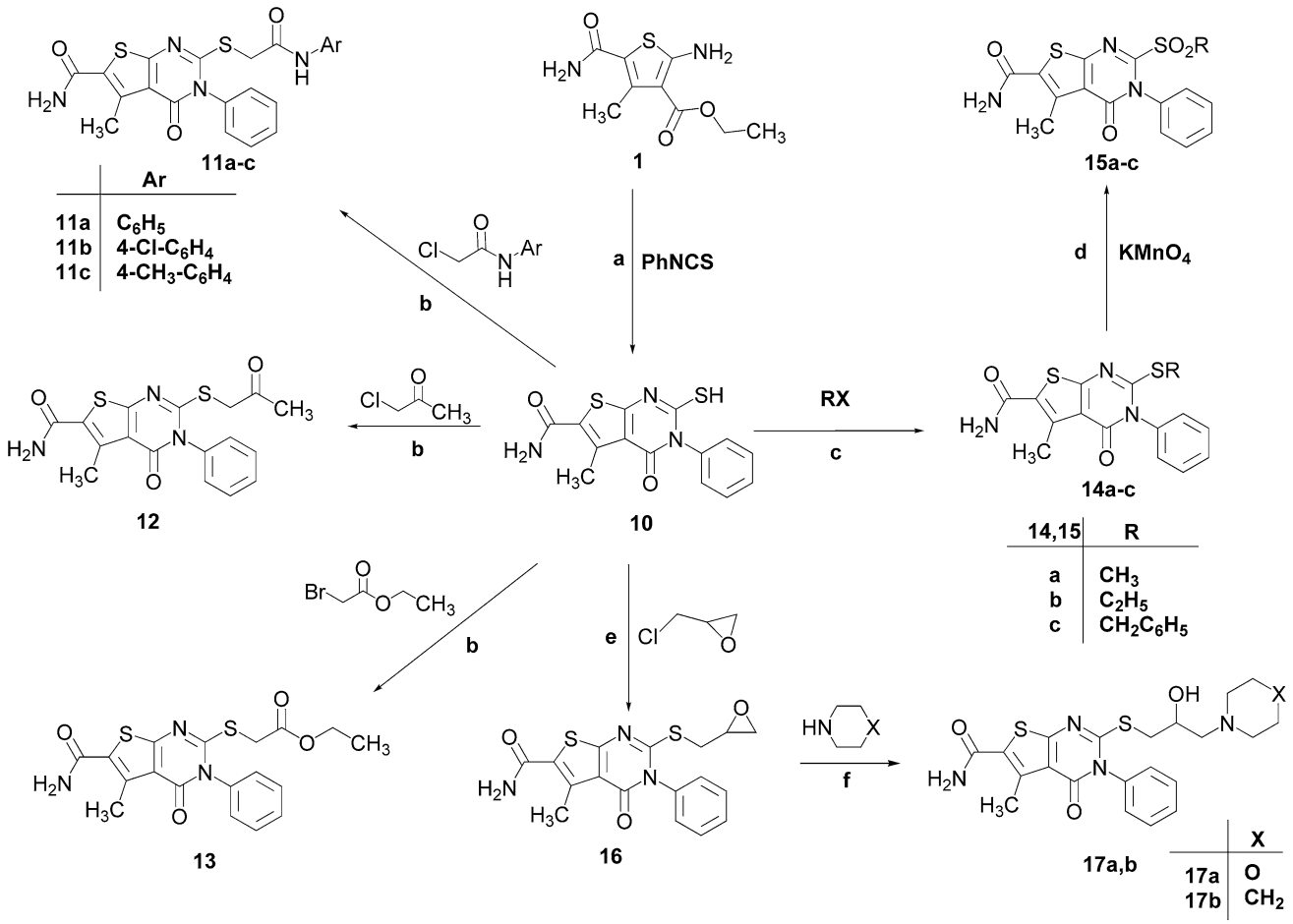
Furthermore, refluxing the amino ester **1** in acetic anhydride gave the N-acetylated derivative **5**. The ^{1}H-NMR spectrum of compound **5** displayed a triplet and a quartet corresponding to the ethyl ester moiety and two deuterium exchangeable singlets at 1.60 and 11.57 ppm assigned for the NH and C_5-carbamoyl NH_2 protons, respectively. It also showed the *N*-acetyl and C_4-methyl protons as two singlets at 2.31 and 2.56 ppm, respectively.



Reagents and conditions: (a): EtOH, stirr at 45 °C, 5h; **(b)**: reflux, 3h, left at r.t., 24 h; **(c)**: absolute EtOH, stirr, r.t., 24h; **(d)**: DMF, reflux, 3h; **(e)**:reflux, 3h, concentrated under vacuum; **(f)**: stirr at 40°C, 3h; **(g)**: reflux, 3h; **(h)**: absolute EtOH, reflux 6-10h.

Scheme 1 Synthetic route for the preparation of target compounds **2–9**

Cyclization of the N-acetylated amino ester **5** following the previously reported reaction conditions (El-Ansary and Omar, 2001) by heating under reflux with hydrazine hydrate in ethanol gave 3-Amino-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]-pyrimidine-6-carboxamide **6**. ^{1}H-NMR spectrum of **6** revealed a singlet at 2.37 ppm integrated for six protons assigned for the two methyl groups in addition to two deuterium exchangeable singlets at 2.5 and 8.11 ppm due to two NH_2 groups. Treatment of **6** with equimolar amounts of the selected aryl or aralkyl isothiocyanate derivative in boiling ethanol afforded 3-N-substituted thiocarbamoylamino derivatives **7a**, **b**. IR spectra of compounds **7a**, **b** were characterized by the appearance of absorption bands at 1599–1526, 1263–1222, 1180–1171, and 1023–961 cm^{-1} attributed to N–C=S function (Omar and AboulWafa, 1984). ^{1}H-NMR spectra of **7a**, **b** showed two deuterium exchangeable singlets due to the protons of the amide-NH_2 and the phenyl or benzyl-*NH* at 8.11 and 10.50 ppm in case of compound **7a** and at 7.77 and 8.44 ppm in case of compound **7b**, respectively.



Reagents and conditions: (a): acetonitrile, anhyd. K_2CO_3, reflux, 15h; **(b)**: dry acetone, anhyd. K_2CO_3, reflux, 3-10h; **(c)**: 10% NaOH, stirr at ambient temp., 5h; **(d)**: glacial AcOH, stirr at r.t., 2h, left overnight, cooled to 5°C,saturated with Na_2SO_3; **(e)**: KOH, H_2O, stirr at r.t., 10h; **(f)**: absolute EtOH, reflux, 10h.

Scheme 2 Synthetic route for the preparation of target compounds **8–17**

The spectra also showed another D_2O exchangeable singlet integrated either for one proton resonated at 9.27 ppm in case of compound **7b** or half proton resonated at 10.75 ppm in case of compound **7a** assigned for thieno-pyrimidine-*NH* proton. Compound **7a** showed as well a D_2O exchangeable singlet at 11.04 ppm integrated for half proton assigned for SH (thiol form) besides other signals appeared at their expected chemical shifts.

Cyclocondensation of the thiourea derivatives **7a, b** with equimolar amount of the appropriate phenacyl bromide or ethyl bromoacetate in boiling absolute ethanol, as reported for the preparation of related compounds (El-Feky, 1993), afforded the corresponding 3-(3-aryl/aralkyl-4-substituted phenylthiazol-2(3*H*)-ylideneamino) derivatives **8a–e** or 3-(4-oxo-3-substituted thiazolidin-2-ylideneamino) derivatives **9a**, **b**, respectively in good yields (Scheme 1). IR spectra of **8a–e** and **9a, b** lacked the mixed vibrational bands due to N–C=S function present in their precursors **7a, b**. ^{1}H-NMR spectra of the products **8a–e** and **9a, b** lacked the NH protons present in the starting material and showed the singlet for the thiazoline C_5-proton (**8a–e**) at 6.30–6.55 ppm and that for the thiazolidinone C_5-protons (**9a, b**) at 4.09–4.87 ppm. The spectra also showed other signals at their expected chemical shifts. Furthermore, the ^{1}H-NMR spectra of the thiazolidinone derivative **9a** showed a singlet at 4.21 ppm integrated for one proton assigned for the enol form of thiazolidinone C_5-H while the corresponding signal in case of compound **9b** was included within the five aromatic protons between 7.23 and 7.39 ppm. In addition, the spectra showed a D_2O exchangeable singlet between 10.00 and 10.53 ppm integrated for one proton assigned for enolic OH pointing out that these two compounds exhibited a typical keto-enol tautomerism. ^{13}C-NMR spectrum of the thiazoline **8a** revealed two high field signals at 15.83 and 16.86 ppm for the two methyl carbons at 2- and 5-positions of the

thienopyrimidine nucleus, respectively. It also showed six signals due to ten methine carbons of the thiazole C_5, the equivalent phenyl C_2 and C_6, *p*-chlorophenyl C_2 and C_6, phenyl C_4, *p*-chlorophenyl C_3 and C_5, and phenyl C_3 and C_5 at 95.60, 117.38, 121.38, 124.06, 129.09, and 130.17 ppm, respectively, and the signals characteristic for the five deshielded quaternary carbons, *p*-chlorophenyl C_1, *p*-chlorophenyl C_4, phenyl C_1, thiazole C_4, and thiazole C_2 at 134.42, 138.77, 148.39, 149.92, and 155.65 ppm, respectively. In addition, the spectrum showed the signals due to thienopyrimidine and $CONH_2$ carbons at their expected chemical shifts. ^{13}C-NMR spectrum of **9a** showed in addition to the signals corresponding to 2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide carbons present almost at the same chemical shifts as in compound **8a**, a highly shielded signal due to the methylene carbon of thiazolidinone C_5 at 60.10 ppm, four signals due to six methine carbons of the thiazolidinone C_5 enol form, the equivalent phenyl C_2 and C_6, phenyl C_4 and the equivalent phenyl C_3 and C_5 at 85.64, 117.38, 122.30, and 129.67 ppm, respectively and the signals characteristic for the quaternary carbons, thiazolidinone C_4 enol form, phenyl C_1, and thiazolidinone C_2 at 99.72, 148.40, and 155.65 ppm, respectively.

On the other hand, heating the amino ester **1** under reflux with equimolar amount of phenyl isothiocyanate in acetonitrile in the presence of anhydrous potassium carbonate in accordance to the previously reported reaction conditions (Badawey *et al.,* 1993) gave 2-mercapto-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide **10** (Scheme 2). IR spectrum of **10** displayed a stretching absorption band due to SH group at 2,700 cm^{-1}. ^{1}H-NMR spectra of **10** displayed a D_2O exchangeable singlet at 13.00 ppm assigned for SH proton in addition to the other protons appeared at their expected chemical shifts. ^{13}C-NMR spectrum of **10** revealed one shielded signal due to CH_3 at 14.83 ppm and three signals at 128.68, 129.48, and 129.52 ppm due to five methine carbons of the phenyl C_4, the equivalent phenyl C_2 and C_6, and phenyl C_3 and C_5, respectively. In addition, the spectrum showed the eight quaternary carbons C_{4a}, phenyl C_1, C_6, C_5, C_4, $CONH_2$, C_2, and C_{7a} resonated at their expected chemical shifts.

S-alkylation of **10** with chloroacetanilides, chloroacetone, or ethyl bromoacetate by heating under reflux in the presence of anhydrous potassium carbonate in dry acetone gave the corresponding 2-(phenyl or 4-substituted phenylcarbamoylmethylthio) derivatives **11a–c**, 2-(2-oxopropylthio) derivative **12**, or 2-(ethoxycarbonylmethylthio) derivative **13**, respectively. The IR spectra of **11a–c**, **12**, and **13** lacked the absorption band due to SH function present in the precursor. The IR spectrum of **12** showed an additional absorption band at 1732 cm^{-1} due to CH_3-C=O moiety. The IR spectrum of **13** showed additional absorption bands at 1700 cm^{-1} due to C=O and at 1228 and 1046 cm^{-1} due to C–O-C of the ester moiety. The ^{1}H-NMR spectra of **11a–c**, **12** and **13** displayed a singlet between 4.04 and 4.10 ppm corresponding to S–CH_2 protons. The ^{1}H-NMR spectra of **11a–c** showed a deuterium exchangeable singlet between 10.23 and 10.46 ppm assigned for NH proton. The ^{1}H-NMR spectrum of **12** showed a singlet at 2.25 ppm due to $COCH_3$ protons while the ^{1}H-NMR spectrum of **13** showed a triplet and a quartet assigned for the ethyl ester group in addition to the other signals at their expected chemical shifts. ^{13}C-NMR spectrum of **11c** showed two high field signals for 4-tolyl-CH_3 and S–CH_2 at 20.99 and 38.15 ppm, respectively and two signals at 119.65 and 130.67 ppm due to four methine carbons of the 4-tolyl moiety. The spectrum also showed the two 4-tolyl quaternary carbons C_4 and C_1 signals at 132.99 and 135.99 ppm, respectively while the most deshielded signal at 165.51 ppm was characterized for CO–NH, besides other signals at their expected chemical shifts. ^{13}C-NMR spectrum of **13** showed two high field signals for the ethyl group at 15.24 and 61.68 ppm and a signal at 35.06 ppm due to S–CH_2. The most deshielded signal at 168.64 ppm was characterized for the quaternary O–C=O carbon. The spectrum also showed the signals corresponding to other carbons at their expected chemical shifts.

Furthermore, reacting the 2-mercapto derivative **10** with methyl iodide, ethyl iodide, or benzyl chloride in aqueous sodium hydroxide solution gave the corresponding S-alkylated derivatives **14a–c**. The ^{1}H-NMR spectra of **14a, 14b, and 14c** displayed a singlet at 2.05 ppm due to S–CH_3 protons, a triplet and a quartet at 1.19 and 3.02 ppm due to S–CH_2CH_3 protons and a singlet at 4.35 ppm due to benzyl-CH_2 protons, respectively. In addition, the spectra showed the signals for other protons at their expected chemical shifts.

Oxidation of the alkylthio compounds **14a–c** with potassium permanganate following the previously reported reaction conditions (El-Emam and Ibrahim, 1991) afforded their corresponding sulfones **15a–c**. IR spectra of compounds **15a–c** characterized by the appearance of absorption bands at 1388–1359 and 1165–1136 cm^{-1} attributed to SO_2. The ^{1}H-NMR spectra of compounds **15a**, **15b,** and **15c** showed the singlet at 2.46 ppm due to SO_2-CH_3, the triplet and quartet at 1.22 and 3.59 ppm characteristic for SO_2-CH_2CH_3 and the singlet at 4.44 ppm due to benzyl-CH_2 protons, respectively, in addition to the signals for other protons at their expected chemical shifts. The ^{13}C-NMR spectrum of **15a** showed a highly shielded signal at 41.82 ppm due to SO_2-CH_3 carbon, besides other signals at their expected chemical shifts.

Treatment of an aqueous solution of the potassium salt of **10** with equimolar amount of epichlorohydrin at room temperature according to the reported procedure (El-Meligie *et al.,* 2001) gave 2-[(oxiran-2-yl)methylthio] derivative **16**. IR spectrum of **16** showed the characteristic

absorption bands of the epoxy C–O–C function at 1224, 1045, 1026, and 771 cm^{-1}. The ^{1}H-NMR spectrum of **16** showed a doublet of doublet at 2.76 ppm assigned for one cis hydrogen of the epoxy CH_2, a multiplet at 3.51–3.58 ppm assigned for the trans proton of the epoxy CH_2, a multiplet at 4.04–4.09 ppm assigned for the S–CH_2 protons and a quintet at 5.09 ppm assigned for the CH epoxy proton in addition to the signals for other protons at their expected chemical shifts. ^{13}C-NMR spectrum of **16** displayed four high field signals at 15.22, 33.13, 33.80, and 42.87 ppm due to the methyl carbon at C_5, S–CH_2, epoxy CH_2 and the epoxy CH methine carbon, respectively. The signals characteristic for 4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide carbons resonated at their expected chemical shifts.

Refluxing the epoxide **16** with the appropriate amine in the presence of absolute ethanol according to the reported procedure (El-Meligie *et al.,* 2001), yielded the corresponding 2-(3-substituted 2-hydroxypropylthio) derivatives **17a**, **b**. The ^{1}H-NMR spectrum of compound **17a** is characterized by the presence of two singlets at 2.69 and 2.85 ppm attributed to S–CH_2 protons, two triplets at 3.78 and 3.79 ppm corresponding to the morpholine $C_{3,5}$ and $C_{2,6}$ protons, respectively, a doublet of doublet at 4.13 ppm attributed to N–CH_2 protons while the ^{1}H-NMR spectrum of compound **17b** showed two multiplets at 1.22–1.60 and 2.21–2.30 ppm assigned for the piperidine $C_{3,4,5}$ and $C_{2,6}$ protons, respectively, and two doublets of doublets at 3.83 and 4.16 ppm assigned for the S–CH_2 protons and N–CH_2 protons, respectively. In addition, the ^{1}H-NMR spectra of **17a** and **17b** showed a multiplet between 4.41 and 4.47 ppm attributed to C–H proton and a deuterium exchangeable doublet at 5.76 and 5.77 ppm corresponding to OH proton besides the signals for other protons at their expected chemical shifts. The ^{13}C-NMR spectrum of **17b** showed seven methylene carbons resonated as two signals at 53.53 ppm due to S–CH_2 and piperidine $C_{3,4,5}$ and at 54.85 ppm due to piperidine-*CH_2* and piperidine $C_{2,6}$. It also showed six methine carbons appeared as three signals at 65.90, 124.86, and 129.46 ppm due to CH–OH, phenyl C_4, and phenyl $C_{2,3,5,6}$, respectively, in addition to the signal for the phenyl C_1 quaternary carbon at 128.80 ppm, besides other signals at their expected chemical shifts.

Biological evaluation

In vitro anticancer screening

The data obtained from the primary one-dose anticancer assay (Table 1) revealed that compound **8c** exhibited the highest anticancer activity among the tested compounds with mean growth of 19.24 % while compounds **4b** and **11b** showed mean growth of 116.73 and 109.13 %, respectively.

Compound **8c**, satisfied the pre-determined threshold inhibition criteria, has been further evaluated at five concentrations ranging from 10^{-4} to 10^{-8} mol against the 60 cell lines of above-mentioned types of human cancers. Three dose response parameters, GI_{50}, TGI, and LC_{50} were calculated for each cell line (Table 2).

From Table 2, compound **8c** showed broad-spectrum antitumor activity against the 60 human tumor cell line tested with GI_{50} values ranging from 1.34 to 8.89 μM with the exception of leukemia cell lines CCRF-CEM, MOLT-4, and RPMI-8226 with GI_{50} values 34.2, 24.6, and 13.9 μM, respectively. **8c** showed the most promising activity toward non-small cell lung cancer HOP-92 and NCI-H522, melanoma SK-MEL-5, ovarian cancer IGROV1, and renal cancer UO-31 with GI_{50} values 1.34, 1.73, 1.81, 1.43, and 1.66 μM, respectively, and TGI values 5.33, 7.23, 3.75, 6.31, and 7.00 μM, respectively.

In addition, it showed moderate activity against colon cancer COLO205, HCT-116, melanoma MALME-3 M, ovarian cancer OVCAR-3, OVCAR-4, renal cancer A498, prostate cancer PC-3 and breast cancer BT-549 with GI_{50} values 2.39, 2.13, 2.18, 2.28, 2.50, 2.53, 2.15, and 2.41 μM, respectively while its TGI value against colon cancer COLO205, HCT-116, ovarian cancer OVCAR-3, renal cancer A498, prostate cancer PC-3, and breast cancer BT-549 was 7.32, 10.03, 8.58, 8.97, 8.31, and 8.89 μM, respectively.

Furthermore, compound **8c** showed minimal cytotoxicity against almost all the cell lines tested with LC_{50} values being generally more than 100 μM.

However, although compound **8c** showed broad-spectrum antitumor activity against the nine tumor subpanels tested, and demonstrated significant activity in the in vitro antitumor screening expressed by the full panel MG-MID GI_{50} value of 4.39 μM, it was non-selective toward any specific subpanel as indicated from Table 3 where its selectivity ratio occurred between 0.28 and 1.55.

In vitro antimicrobial screening

In general, investigation of antimicrobial screening data (Table 4) revealed that all the newly synthesized compounds displayed variable activities against the four tested microorganisms. Compounds **9a**, **9b**, and **12** showed weak antimicrobial activity against *S. aureus* (IZ = 13–14 mm), while compounds **4c**, **4d**, **5, 8a, 14b, and 15a**, showed mild antimicrobial activity against *E. coli* (IZ = 16–17 mm). On the other hand, compounds **4b**, **6**, **8c, 8d, and 16** were equipotent and exhibited good antimicrobial activity against *P. aeruginosa* (IZ = 17 mm; MIC = 125 μg/ml) comparable to that of ampicillin (IZ = 18 mm;

MIC = 125 μg/ml). In addition, compound **8c** showed mild antifungal activity against *C. albicans* (IZ = 17 mm).

SAR studies indicated that, the monocyclic thiophene derivative **5** showed mild antibacterial activity against *E. coli* (IZ = 16 mm; MIC = 125 μg/ml) and weak antifungal activity against *C. albicans* (IZ = 15 mm). Cyclization of **5** into the 3-Amino-2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide **6** improved the antimicrobial activity that was equipotent to ampicillin against *P. aeruginosa* (IZ = 17 mm; MIC = 125 μg/ml) without any activity against the other microorganisms.

Converting the 3-NH_2 group in **6** into the open chain N-substituted thiocarbamoylamino derivatives **7a** and **7b** having N-phenyl and N-benzyl functions, respectively abolished the antimicrobial activity. Cyclization of these thiourea into a rigid 5-membered heterocyclic ring structures linked through an imino function afforded **8a–e** and **9a, b** derivatives. Among the thiazoline derivatives **8a–e**, compound **8c** (3-benzyl, 4-phenyl) and compound **8d** (3-benzyl, 4-*p*-chlorophenyl) were equipotent and displayed the same antimicrobial activity as ampicillin against *P. aeruginosa* (IZ = 17 mm; MIC = 125 μg/ml). Furthermore, compound **8c** showed mild activity against *C. albicans* (IZ = 17 mm; MIC = 125 μg/ml). On the other hand, the thiazolidinone derivatives **9a** (3-phenyl) and **9b** (3-benzyl) displayed weak antimicrobial activity against *S. aureus* (IZ = 14 mm; MIC = 250 μg/ml) and *C. albicans* (IZ = 16 mm).

Among the 5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivatives **10-17** having substituted thio or substituted sulfonyl side chain at 2-position, compound **16** having 2-[(oxiran-2-yl)methylthio] side chain was equipotent with ampicillin against *P. aeruginosa* (IZ = 17 mm; MIC = 125 μg/ml). Among the 3-Substituted-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivatives **4b–d** having free 2-position, compound **4b** having 2-hydroxyethyl moiety at 3-position showed good antibacterial activity comparable to that of ampicillin against *P. aeruginosa* (IZ = 17 mm; MIC = 125 μg/ml), while compounds **4c** and **4d** displayed mild activity against *E. coli*.

Conclusion

We reported here the synthesis and screening for anticancer and antimicrobial activities of a series of 3-substituted or 2,3-disubstituted-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide derivatives. Compound **8c** was selected for a full 60 human cancer cell lines screen at five concentration levels where it showed non-selective broad-spectrum activity against all the cancer cell lines. However, further studies are in progress to explore the possible mechanism of anticancer activity of this class of compounds. Furthermore, compound **8c** showed pronounced antibacterial activities comparable to ampicillin against *P. aeruginosa* with mild antifungal activity against *C. albicans*. Therefore, compound **8c** could be considered as useful template for future development of a new class of dual anticancer-antimicrobial agents. Moreover, further derivatization or modification might help to obtain more potent and selective agents.

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Conflict of interest The authors have declared no conflict of interest.

References

Al-Smadi M, Al-Momani F (2008) Synthesis, characterization and antimicrobial activity of new 1,2,3-selenadiazoles. Molecules 13:2740–2749

Badawey E, Rida SM, Hazzaa AA, Fahmy HTY, Gohar YM (1993) Potential anti-microbials. I. Synthesis and structure-activity studies of some new thiazolo[4,5-d]pyrimidine derivatives. Eur J Med Chem 28:91–96

Bekhit AA, Habib NS, Park JY (2004) Synthesis of some thiazolyl and thiadiazolyl derivatives of 4(3*H*)-quinazolinone as anti-inflammatory-antimicrobial agents. Boll Chim Farm 143:34–39

Bondock S, Khalifa W, Fadda AA (2007) Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydronaphthalene-2-carboxaldehyde. Eur J Med Chem 42(7):948–954

Boyd MR (1997) The NCI in vitro anticancer drug discovery screen; concept, implementation and operation 1985–1995, in Cancer drug discovery and development. In: Teicher BA (ed) Drug development: Preclinical screening, clinical trial and approval, vol 2. Humana, Totowa, pp 23–42

Boyd MR, Paull KD (1995) Some practical considerations and applications of the national cancer institute In vitro anticancer drug discovery screen. Drug Dev Res 34:91–109

Chakraborti AK, Gopalakrishnan B, Sobhia ME, Malde A (2003) 3D-QSAR Studies on thieno[3,2-d]pyrimidines as Phosphodiesterase IV Inhibitors. Bioorg Med Chem Lett 13(8):1403–1408

Comber RN, Gray RJ, Secrist JA (1991) Acyclic analogues of pyrazofurin: syntheses and antiviral evaluation. Carbohydr Res 216:441–452

Cunha S, Macedo FC Jr, Costa GAN, Rodrigues MT Jr, Verde RBV, de Souza Neta LC, Vencato I, Lariucci C, Sa FP (2007) Antimicrobial activity and structural study of disubstituted thiourea derivatives. Monatsh Chem 138:511–516

Donkor IO, Li H, Queener SF (2003) Synthesis and DHFR inhibitory activity of a series of 6-substituted-2,4-diaminothieno[2,3-d]pyrimidines. Eur J Med Chem 38(6):605–611

Du AY, Zhao BX, Yin DL, Zhang SL, Miao JY (2005) Discovery of a novel small molecule, 1-ethoxy-3-(3,4-methylenedioxyphenyl)-2-propanol, that induces apoptosis in A549 human lung cancer cells. Bioorg Med Chem 13(13):4176–4183

Eckhardt S (2002) Recent progress in the development of anticancer agents. Curr Med Chem Anticancer Agents 2(3):419–439

Eissa AAHM, Moneer AA (2004) Synthesis and antimicrobial activity of novel tetrahydrobenzothienopyrimidines. Arch Pharm Res 27:885–892

El-Ansary AK, Omar AH (2001) Synthesis and anti-inflammatory activity of some thieno[2,3-d]pyrimidinone derivatives. Bull Fac Pharm Cairo Univ 39(1):17–25

El-Baih FEM, Al-Blowy HAS, Al-Hazimi HM (2006) Synthesis of Some Thienopyrimidine Derivatives. Molecules 11:498–513

El-Emam AA, Ibrahim TM (1991) Synthesis and anti-inflammatory and analgesic activity of some 3-(1-adamantyl)-4-substituted-5-mercapto-1,2,4-triazoles. Arzneim-Forsch Drug Res 41:1260–1264

El-Feky SAH (1993) Synthesis and anticonvulsant properties of some novel quinazolinone thiazolidine and 4-thiazolidone derivatives. Pharmazie 48:894–896

El-Hawash SA, Habib NS, Fanaki NH (1999) Quinoxaline derivatives part II: synthesis and antimicrobial testing of 1,2,4-triazolo[4,3-a]quinoxalines,1,2,4-triazino[4,3-a]quinoxalines and 2-pyrazolylquinoxalines. Pharmazie 54:808–813

El-Hawash SA, Habib NS, Kassem MA (2006) Synthesis of some new quinoxalines and 1,2,4-triazolo[4,3-a]quinoxalines for evaluation of in vitro antitumor and antimicrobial activities. Arch Pharm 339:564–571

El-Meligie S, El-Ansary AK, Said MM, Hussien MMM (2001) Synthesis and antimicrobial activity of 2-(2-aryl vinyl)-7-substituted-quinazolin-4(3*H*)-ones. Indian J Chem 40B:62–69

Gewald K, Schinke E, Bottcher H (1966) Heterocycles from CH-acidic nitriles. VIII. 2-Aminothiophenes from methylene-active nitriles, carbonyl compounds, and sulfur. Chem Ber 99(1):94–100

Habib NS, El-Hawash SA (2005) Quinoxalines part III: anticancer activity of some quinoxaline derivatives. Boll Chim Farm 144:1–17

Habib NS, Soliman R, Ismail K, Hassan AM, Sarg MT (2003) Pyrimidines part II: synthesis of novel pyrimidines, 1,2,4-triazolo[4,3-a]pyrimidin-7-ones and pyrimidino[2,1-c][1,2,4]triazin-8-ones for their antimicrobial and anticancer activities. Boll Chim Farm 142:396–405

Hallur G, Jimeno A, Dalrymple S, Zhu T, Jung MK, Hidalgo M, Isaacs JT, Sukumar S, Hamel E, Khan SR (2006) Benzoylphenylurea sulfur analogues with potent antitumor activity. J Med Chem 49(7):2357–2360

Haruna K, Kanezaki H, Tanabe K, Dai WM, Nishimoto S (2006) Effects of structural modification on the DNA binding properties and photo-induced cleavage reactivity of propargylic sulfones conjugated with an anthraquinone structure. Bioorg Med Chem 14(13):4427–4432

Jain SR, Kar A (1971) The antibacterial activity of some essential oils and their combinations. Planta Med 20:118–123

Jennings LD, Kincaid SL, Wang YD, Krishnamurthy G, Beyer CF, McGinnis JP, Miranda M, Discafani CM, Rabindran SK (2005) Parallel synthesis and biological evaluation of 5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidin-4(3*H*)-one cytotoxic agents selective for p21-deficient cells. Bioorg Med Chem Lett 15:4731–4735

Kutschy P, Salayova A, Curillova Z, Kozar T, Mezencev R, Mojzis J, Pilatova M, Balentova E, Pazdera P, Sabol M, Zburova M (2009) 2-(Substituted phenyl)amino analogs of 1-methoxyspirobrassinol methyl ether: synthesis and anticancer activity. Bioorg Med Chem 17(10):3698–3712

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 83(11):757–766

Munchhof MJ, Beebe JS, Casavant JM, Cooper BA, Doty JL, Higdon RC, Hillerman SM, Soderstrom CI, Knauth EA, Marx MA, Rossi AMK, Sobolov SB, Sun J (2004) Design and SAR of thienopyrimidine and thienopyridine inhibitors of VEGFR-2 kinase activity. Bioorg Med Chem Lett 14(1):21–24

Nasr MN, Gineinah MM (2002) Pyrido[2,3-d]pyrimidines and Pyrimido[5′,4′:5,6]pyrido[2,3-d]pyrimidines as new antiviral agents: synthesis and biological activity. Arch Pharm Pharm Med Chem 335(6):289–295

Nomoto Y, Takai H, Ohno T, Kubo K (1991) Studies on cardiotonic agents. VI. Synthesis of novel 4,5-dihydro-3(2*H*)-pyridazinone derivatives carrying some benzoheterocycles at the 6-position. Chem Pharm Bull 39(2):352–357

Omar AMME, AboulWafa OM (1984) Novel estradiol-17α-1,2,4-triazoline derivatives. Synthesis and in vitro anabolic-catabolic properties and binding affinity to steroid receptors. J Heterocycl Chem 21:1419–1423

Omar AMME, AboulWafa OM, Hazzaa AAB, El-Samahy SA (2005) Novel triazole and triazoline derivatives fused to thieno[2,3-d]pyrimidin-5(4*H*)-one as potential antimicrobial agents. Alex J Pharm Sci 19:97–105

Ottana R, Carotti S, Maccari R, Landini I, Chiricosta G, Caciagli B, Vigorita MG, Mini E (2005) In vitro antiproliferative activity against human colon cancer cell lines of representative 4-thiazolidinones. Part I. Bioorg Med Chem Lett 15(17):3930–3933

Patil BS, Krishnamurthy G, Naik HSB, Latthe PR, Ghate M (2010) Synthesis, characterization and antimicrobial studies of 2-(4-methoxy-phenyl)-5-methyl-4-(2-arylsulfanyl-ethyl)-2,4-dihydro [1,2,4]triazolo-3-ones and their corresponding sulfones. Eur J Med Chem 45(8):3329–3334

Pillai AD, Rathod PD, Franklin PX, Patel M, Nivsarkar M, Vasu KK, Padh H, Sudarsanam V (2003) Novel drug designing approach for dual inhibitors as anti-inflammatory agents: implication of pyridine template. Biochem Biophys Res Commun 301:183–187

Raic-Malic S, Grdisa M, Pavelic K, Mintas M (1999) Synthesis and biological evaluation of the novel purine and pyrimidine nucleoside analogues containing 2,3-epoxypropyl, 3-amino-2-hydroxypropyl or 2,3-epoxypropyl ether moieties. Eur J Med Chem 34(5):405–413

Ritter JM, Lewis LD, Mant TG, Ferro A (eds) (2008) Drugs in the elderly. In: A textbook of clinical pharmacology and therapeutics, 5th edn. Hodder Arnold, Hachette Livre, London, pp 56–61

Roche VF (2008) Cancer and Chemotherapy. In: Lemke TL, Roche VF, Williams DA, Zito SW (eds) Foye's principles of medicinal chemistry, 6th edn. Lippincott Williams & Wilkins, Philadelphia, p 1153

Scott AC (1989) Laboratory control of antimicrobial therapy. In: Collee JG, Duguid JP, Fraser AG, Marmion BP (eds) Mackie and McCartney practical medical microbiology, vol 2, 13th edn. Churchill Livingstone, Edinburgh, pp 161–181

Shehata IA, El-Subbagh HI, Abdelal AM, El-Sherbeny MA, Al-Obaid AA (1996) Synthesis, antitumor and anti-HIV-1 testing of certain thieno[2,3-d]pyrimidine, thieno[2,3-d]imidazo[1,2-c] pyrimidine and thieno[2,3-d][1,3]thiazine derivatives. Med Chem Res 6:148–163

Shoemaker RH (2006) The NCI 60 human tumour cell line anticancer drug screen. Nat Rev Cancer 6(10):813–823

Soliman R, Habib NS, Ismail K, Moustafa A, Sarg MT, Fanaki NH (2003) Synthesis of novel pyrimidine-2,4-diones and 2-thioxo-pyrimidine-4-ones as potential anticancer and antimicrobial agents. Boll Chim Farm 142:167–174

Soliman R, Habib NS, El-Tombary AA, El-Hawash SAM, Shaaban OG (2009) Synthesis of tetrahydrobenzothieno[2,3-d]pyrimidine and tetrahydrobenzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine derivatives as potential antimicrobial agents. Sci Pharm 77:755–773

Venkatachalam SR, Salaskar A, Chattopadhyay A, Barik A, Mishra B, Gangabhagirathic R, Priyadarsini KI (2006) Synthesis, pulse radiolysis, and in vitro radioprotection studies of melatoninolipoamide, a novel conjugate of melatonin and α-lipoic acid. Bioorg Med Chem 14:6414–6419

Williams DA (2008) Drug metabolism. In: Lemke TL, Roche VF, Williams DA, Zito SW (eds) Foye's principles of medicinal chemistry, 6th edn. Lippincott Williams & Wilkins, Philadelphia, pp 295–296

Zhao LM, Xie TP, He YQ, Xu DF, Li SS (2009) Synthesis and antitumor activity of 6- and 2-(1-substituted-thio-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-diones. Eur J Med Chem 44(4):1410–1414