

Synthesis, cytotoxicity and toxicity of thieno[2,3-*d*]pyrimidine derivatives derived from 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene

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Received: 22 August 2014 / Accepted: 17 August 2015 / Published online: 29 August 2015
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Abstract The 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivative **1** reacted with benzoylthiocyanate to give *N*-benzoylthiourea derivative **3**. The latter underwent ready cyclization to give the tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivative **4** which was used as the key starting compound for a series of heterocyclization reactions to produce thiophene, pyridine, pyrimidine and pyran derivatives. The cytotoxicity of the newly synthesized products was evaluated using six cancer and one normal cell lines. The toxicity of compounds with the optimal cytotoxicity was measured using shrimp larvae.

Keywords Tetrahydrobenzo[*b*]thiophene · Thieno[2,3-*d*]pyrimidine · Cyanomethylene · Cytotoxicity

Introduction

Several types of pyrimidines and thienopyrimidines have attracted much interest due to their valuable pharmacological properties such as antiviral (Hafez *et al.* 2010), antioxidant (Kotaiah *et al.* 2012) and antimalarial (Seerat

et al. 2012; Ashalatha *et al.* 2007). The antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative diseases such as autoimmune diseases, cardiovascular diseases, neurovascular diseases (Kamat *et al.* 2013; Giordano *et al.* 2014) and neurodegenerative changes associated with aging (Esposito *et al.* 2002). The homeostatic balance between the reactive oxygen species (ROS) and endogenous antioxidants is important in maintaining healthy tissues. Excessive ROS states are important in diseases such as acute respiratory distress syndrome and idiopathic pulmonary fibrosis (Vianello *et al.* 2014). Most living organisms possess enzymatic and nonenzymatic defense systems against excessive production of the reactive oxygen species. However, different external factors (smoke, diet, alcohol and some drugs) and aging decrease the efficiency of such protecting systems, resulting in disturbances of the redox equilibrium established under healthy conditions (Ogunro *et al.* 2013). In addition, the pyrimidine-based derivatives such as thieno[2,3-*d*]pyrimidines have extreme importance in medicinal chemistry, exhibiting pharmacological and therapeutic properties such as antidepressant (Kotaiah *et al.* 2012), antibacterial (Aly *et al.* 2011; Dewal *et al.* 2012; Abbas *et al.* 2013), antifungal (Leung *et al.* 2013), anti-inflammatory (Rizk *et al.* 2012; Ashour *et al.* 2013), antiplatelet (Bach *et al.* 2013), antihypertensive (Press *et al.* 1989), herbicidal (El-Sherbeny *et al.* 1995) and plant growth regulatory (Kotaiah *et al.* 2012) properties.

Overview of the literature survey showed the importance of thieno[2,3-*d*]pyrimidines individually in the biological systems and led to assimilate that this moiety may show synergistic effect. In the present work, we report the details of the synthesis of novel thieno[2,3-*d*]pyrimidine derivatives and in vitro antioxidant properties of them.

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Results and discussion

Herein, in order to extend our research on anticancer heterocyclic derivatives with high inhibitory effects toward some cancer cell lines, we report the synthesis of new heterocyclic compounds derived from thieno[2,3-*d*]pyridazine. Moreover, some of the newly synthesized products were good candidates as anticancer drugs through their screening toward cancer and normal cell lines. Thus, 4,5,6,7-tetrahydrobenzo[*b*]thiophene reacted with benzoylthiocyanate to give the thiourea derivative **3**. The structure elucidation of compound **3** was based on analytical and spectral data. Thus, the ^1H NMR spectrum showed the presence of the four CH_2 groups at δ 1.80–1.84 and 2.24–2.26 ppm, a multiplet at δ 7.2–7.35 ppm equivalent to the phenyl protons and two singlets at δ 8.28 and 8.32 (D_2O exchangeable) equivalent to the two NH groups. In addition, the ^{13}C NMR spectrum showed δ 19.2, 22.1, 25.7, 28.6 (4 CH_2), 119.2, 124.1, 124.9, 128.7, 130.4, 133.8, 138.0, 144.2 (thiophene, C_6H_5), 165.3 (CO), 179.5 (C=S). Compound **3** underwent cyclization when heated under reflux in 1,4-dioxane, containing a catalytic amount of triethylamine, to give the (4-amino-2-thioxo-5,6,7-tetrahydro[4,5]thieno[2,3-dpyrimidin-3(2H)-yl)(phenyl)methanone (**4**). Compound **4** was previously synthesized using another reaction routes (Amr *et al.* 2010; Hacker *et al.* 2009; Leistner *et al.* 1989). Compound **4** underwent different heterocyclization reactions. Thus, it reacted with hydrazine hydrate to give the tetrahydrobenzo[4,5]thieno[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine derivative **5** (Scheme 1). The analytical and spectral data of compound **5** are consistent with its proposed structure.

Compound **4** was reacted with either ethyl cyanoacetate (**6a**) or ethyl acetoacetate (**6b**) to give the amide derivatives **7a** or **7b** (Scheme 1). The high yield of compounds **7a**, **b** encouraged us to use them for further work. In order to expand the scope of the present work, compounds **7a**, **b** underwent ready cyclization when heated in sodium ethoxide solution using a boiling water bath to give the benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidine derivatives **8a** and **8b**, respectively. The mass spectrum of compound **8a** showed molecular ion peak at m/e 390 equivalent to the molecular formula $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_2\text{S}_2$. The IR spectrum showed the presence of CN group stretching at ν 2220 cm^{-1} . The ^1H NMR spectrum revealed beside the expected signals, a singlet at δ 8.41 indicating the presence of the NH group. The ^{13}C NMR displayed δ 19.0, 22.4, 25.5, 28.8 corresponding to the four CH_2 groups of the cyclohexene ring, δ 116.2 indicating the CN group, δ 120.6, 123.6, 124.8, 129.3, 134.2, 136.8, 139.0, 143.7, 148.9, 151.6 (Bz, pyrimidine, thiophene C), δ 166.4 equivalent to the CO group of the pyrimidine C-3, δ 170.4 (C=N, C-7) and δ 180.6 (C=S, C-6).

Next, we studied the reactivity of compounds **7a**, **b** toward thiophene formation via the Gewald's thiophene synthesis (Scrowston *et al.* 1981; Mohareb *et al.*, 2012; Hala *et al.*, 2011; Patel *et al.* 2003). Thus, the reaction of either compounds **7a** or **7b** with elemental sulfur and either malononitrile (**9**) or ethyl cyanoacetate (**6a**) gave the thiophene derivatives **10a–d**, respectively. On the other hand, the reaction of either **7a** or **7b** with either benzaldehyde (**11a**), 4-chlorobenzaldehyde (**11b**) or 4-methoxybenzaldehyde (**11c**) gave the benzylidene derivatives **12a–f**, respectively (Scheme 2). The analytical and spectral data of the latter products are consistent with their respective structures (see “Experimental” section).

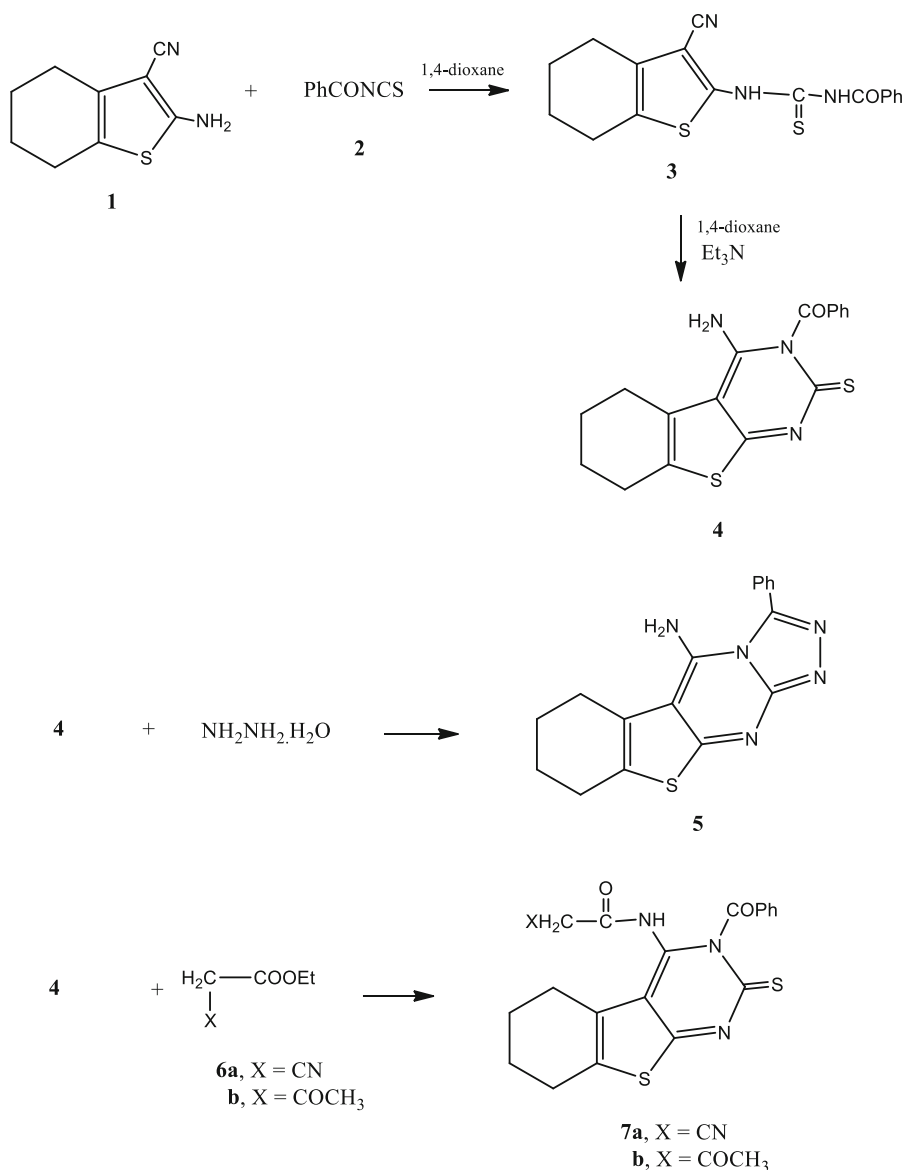
Compounds **7a** or **7b** reacted with the aromatic diazonium salts namely benzenediazonium chloride (**13a**), 4-chlorobenzenediazonium chloride (**13b**) or 4-methylbenzenediazonium chloride (**13c**) to give the arylhydrazo derivatives **14a–f**, respectively. Reaction of compound **7b** with cyanomethylene reagents using different conditions was studied. Thus, compound **7b** reacted with either malononitrile (**9**) or ethyl cyanoacetate (**6b**) in the presence of ammonium acetate at 120 °C to give the condensation products **15a** and **15b**, respectively. On the other hand, carrying out the reaction of compound **7b** with either malononitrile (**9**) or ethyl cyanoacetate (**6a**) in sodium ethoxide solution gave the pyridine-1-yl derivatives **16a** and **16b**, respectively (Scheme 3). The reaction took place at first through condensation followed by the Michael addition of the NH to the nitrile group. The analytical and spectral data of **16a** and **16b** were in agreement with their respective structures. Further confirmation for the structures of compounds **16a** and **16b** was obtained through their synthesis using another reaction route. Thus, either **15a** or **15b** when heated in sodium ethoxide solution in a boiling water bath gave the same two products **16a** and **16b**, respectively (same m.p. and mixed m.p. and fingerprint IR spectra).

Compounds **16a**, **b** reacted with phenyl isothiocyanate (**17**) to give the pyrido[2,3-*d*]pyrimidine derivatives **18a** and **18b**, respectively. Structure of the latter products was confirmed on the basis of analytical and spectral data. Finally, **7a** or **7b** was reacted with either benzaldehyde (**11a**), 4-chlorobenzaldehyde (**11b**) or 4-methoxybenzaldehyde (**11c**) and malononitrile to afford the pyran derivatives **19a–f**, respectively. The analytical and spectral data of **19a–f** were consistent with their respective structures (Scheme 4).

In vitro cytotoxic assay

Chemicals

Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640

Scheme 1 Synthesis of compounds **3**, **4**, **5** and **7a, b**

medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures

The cell cultures were obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK), and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5 % heat

inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 Iu/mL), at 37 °C in a humidified atmosphere containing 5 % CO₂. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for the seven human cancer cell lines including cells derived from 0.75×10^4 cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5 %) of DMSO used in each assay.

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity (Combes *et al.* 2012; Roemer *et al.* 2008; Li *et al.* 2008) against six human cancer cell lines including cells derived from human gastric cancer

(NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). All of IC_{50} values are listed in Table 1. Twelve compounds **4**, **7a**, **8a**, **8b**, **10c**, **10d**, **12c**, **12d**, **12e**, **12f**, **14e**, **15b**, **18b**, **19b** and **19e** displayed significant cytotoxicity against most of the cancer cell lines tested (IC_{50} = 10–1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC_{50} > 10,000 nM). The reference compound used is the CHS-828 which is a pyridyl cyanoguanidine antitumor agent.

Structure activity relationship

From Table 1, the newly synthesized compounds were tested against the six cancer cell lines the human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). The heterocyclic compounds **4**, **7a**, **8a**, **8b**, **10c**, **10d**, **12c**, **12d**, **12e**, **12f**, **14e**, **15b**, **18b**, **19b** and **19e** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50} in the nM range. Comparing the cytotoxicity of the tetrahydrobenzo[*b*]thiophene derivative **3** and the tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-derivatives **4** exhibited clearly that cyclization of compound **3** to the tricyclic product **4** increases the cytotoxicity of the latter compound. On the other hand, the reaction of compound **4** with hydrazine hydrate gave the 3-phenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-amine **5** through which the cytotoxicity decreases against the tested cancer cell lines. Such decrease in cytotoxicity is attributed to the replacement of the thione moiety by the nitrogen of the hydrazine. On the other hand, the reaction of compound **4** with either ethyl cyanoacetate or ethyl acetoacetate produced compounds **7a** and **7b**, respectively. The amide derivative **7a** displayed remarkable cytotoxicity which is more than that of compound **4** against the tested cancer cell lines. Concerning the hexahydro-1H-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidine derivatives **8a** and **8b**, it is clear that both of the two compounds showed high cytotoxicity against NUGC, DLDI, HA22T, HEPG2 and HONE1 cell lines.

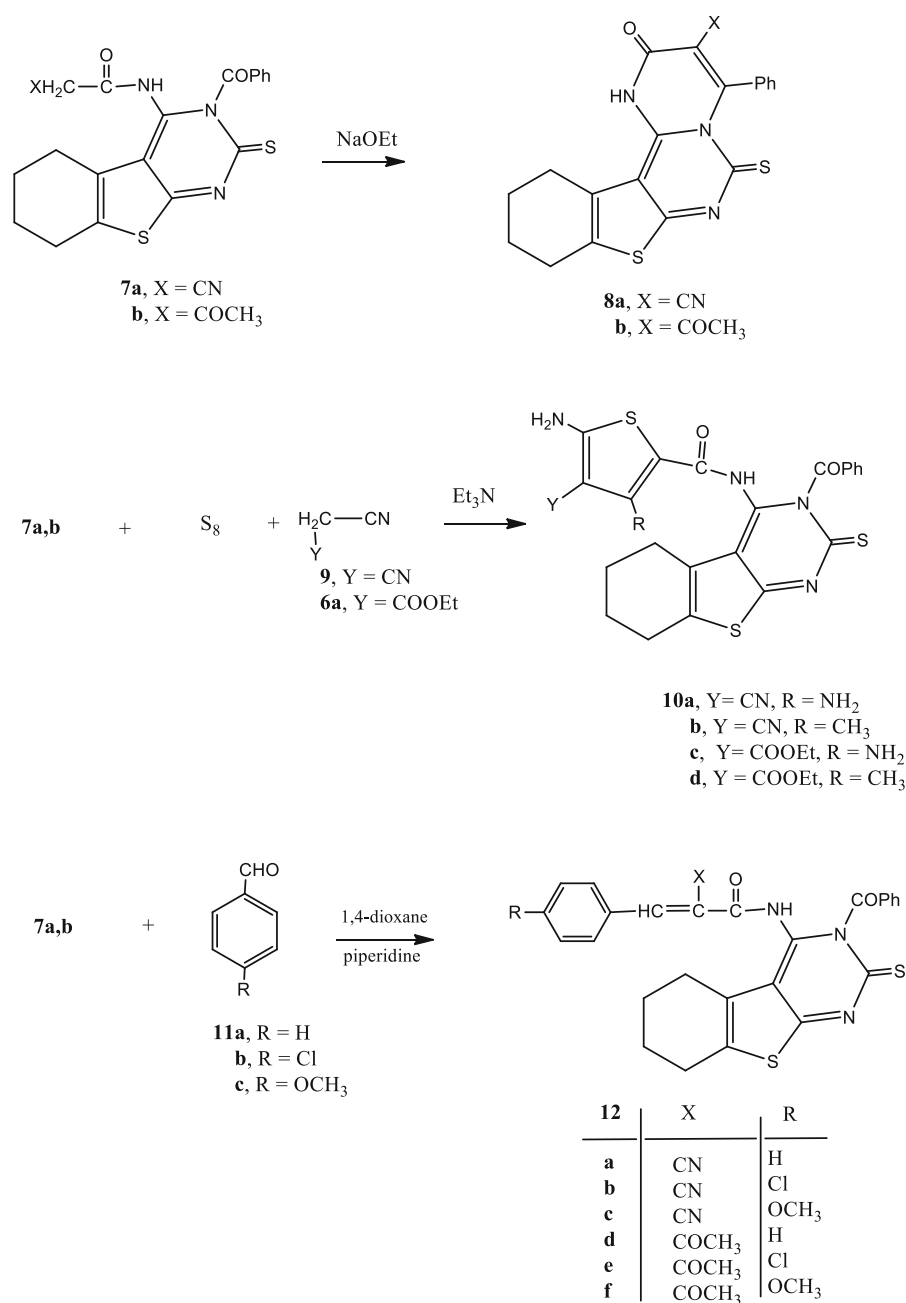
Compound **8b** with the $COCH_3$ moiety is more potent against the five cell lines than compound **8a** with the CN moiety. For the thiophene derivatives **10a–d**, compounds **10c** and **10d** with the $COOEt$ moiety are more potent than **10a** and **10b**. Moreover, compound **10a** showed potency against NUGC, DLDI, HA22T, HEPG2 and HONE1 cell lines, but compound **10b** showed potency against the six cell lines.

From Table 1, it is clear that for the benzylidene derivatives **12a–f**, compounds **12a** and **12b** with the cyano moiety are less potent than compounds **12c–f**, which might be due to the presence of either the OCH_3 in **12c** or the $COCH_3$ moiety as in **12d–f**. It is of great value to note that among the compounds **12a–f**, compound **12f** with the $COCH_3$ and the Cl moieties is responsible for its maximum potency. Similarly concerning the arylhydrazo derivatives **14a–f**, it is noticed that compound **14e** with the $COCH_3$ and Cl moieties showed the highest potency among the five compounds. On the other hand, compound **14b** with the CN and Cl moieties showed high potency toward NUGC, DLDI, HA22T, HEPG2 and HONE1 cell lines, but its potency is less than that of **14e**. Compounds **15a**, **b** and **16a**, **b** showed low potency against the six cancer cell lines. Concerning the 2,3-dihydropyrido[2,3-*d*]pyrimidin-7(8H)-one derivatives **18a** and **18b**, it is clear that compound **18b** with the 4-hydroxy group showed more potency against the six cancer cell lines than compound **18a** with the 4-amino moiety. Finally, regarding the pyran derivatives **19a–f**, it is obvious that compound **19b** with electronegative groups the CN and Cl and compound **19e** with $COCH_3$ and the Cl groups showed the highest cytotoxicity through such series of compounds.

Toxicity

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals in vivo lethality to shrimp larvae (*Artemiasalina*), Brine-Shrimp Lethality Assay (Choudhary and Thomsen 2001) was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95 % confidence intervals (Brayn *et al.* 1993). Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines, respectively, the following twelve compounds: **4**, **7a**, **8a**, **8b**, **10c**, **10d**, **12c**, **12d**, **12e**, **12f**, **14e**, **15b**, **18b**, **19b** and **19e**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials (Carballo *et al.* 2002), natural and synthetic organic compounds (Choudhary and Thomsen 2001). It has also been shown that *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds (Calleja and Persoone 1992).

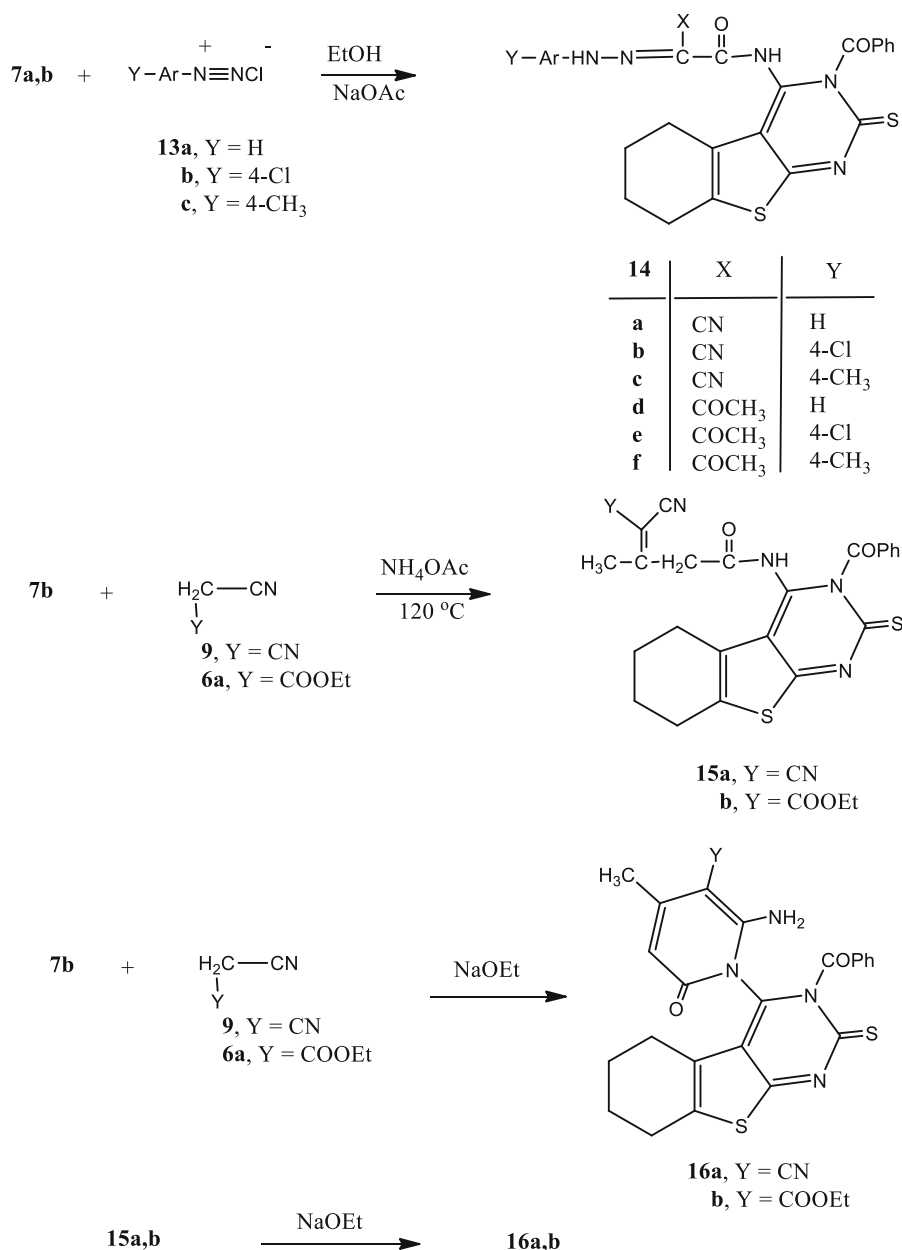
Scheme 2 Synthesis of compounds **8a, b**; **10a–d**; and **12a–f**



In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, solutions of the test compounds were prepared in the suggested DMSO volume ranges. It is clear from Table 2 that the *N*-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-cyano-3-(4-methoxyphenyl)acrylamide (**12c**) and the *N*-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-3-oxo-2-(2-(4-chlorophenyl)hydrazono)-butanamide (**14e**) showed nontoxicity against the tested organisms.

Experimental

¹³C NMR and ¹H NMR spectra were recorded on Bruker DPX400 instrument in DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit at Cairo University. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel

Scheme 3 Synthesis of compounds **14a–f**; **15a, b**; and **16a, b**

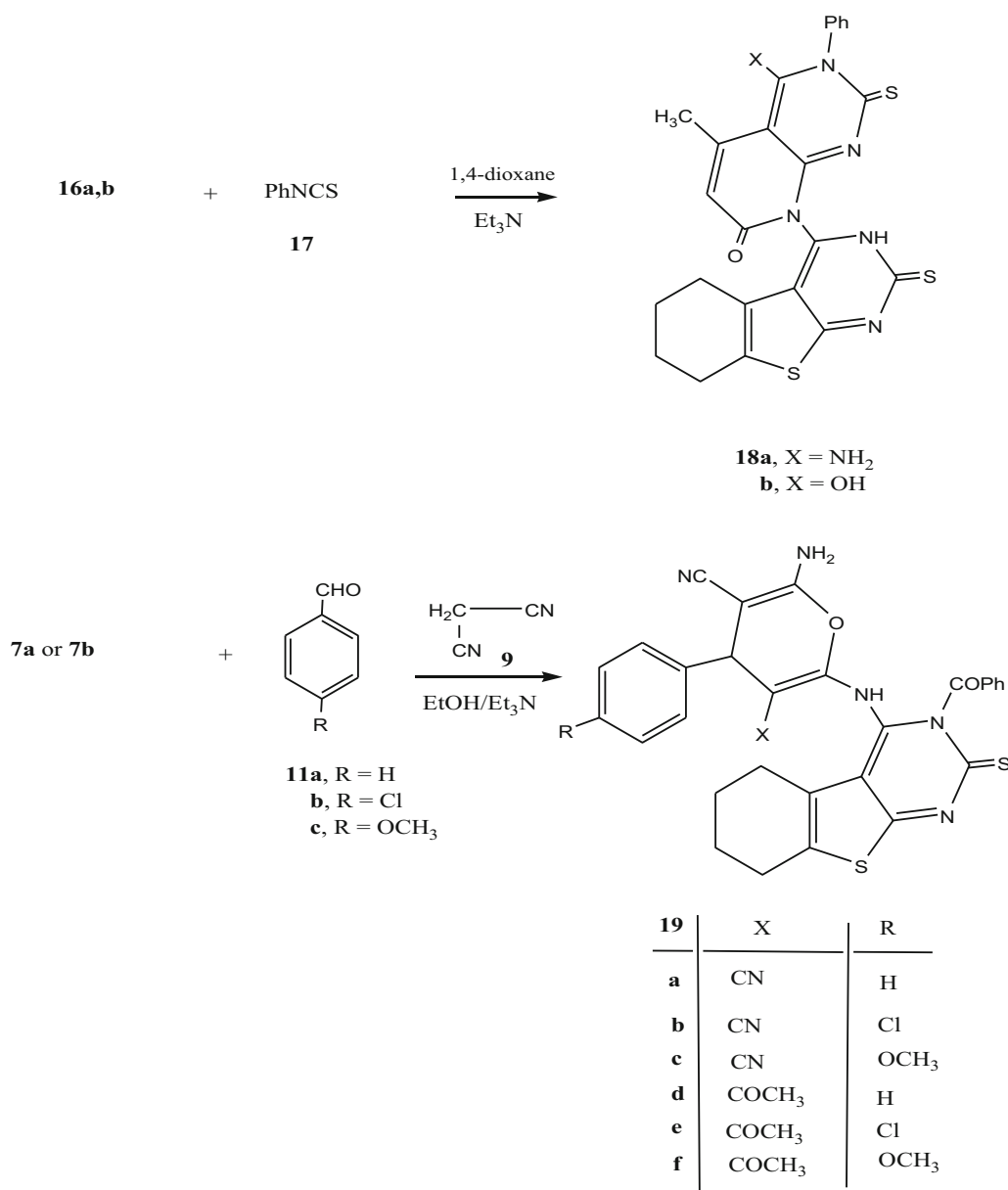
60 F254 plates of thickness of 0.25 mm (Merck). Compound **1** was synthesized through Gewald's thiophene synthesis as reported (Gewald *et al.* 1966). Compound **4** was synthesized earlier according to reported literature (Amr *et al.* 2010; Hacker *et al.* 2009; Leistner *et al.* 1989).

N-((3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamothioyl)benzamide (**3**)

To a solution of benzoyl isothiocyanate [prepared by adding benzoylchloride (0.01 mol) (1.40 g, 0.01 mol) to a solution of ammonium thiocyanate (0.76 g, 0.01 mol) in 1,4-dioxane and heating for 10 min] compound **1** (1.78 g,

0.01 mol) was added. The whole reaction mixture was heated under reflux for 2 h and then poured onto ice/water, and the formed solid product was collected by filtration.

Compound **3**: Yellow crystals (EtOH), yield 80% (2.73 g), mp 180–183 °C. IR (KBr) cm^{-1} : 3460, 3325, 3057, 2220, 1688, 1662, 1625 cm^{-1} . ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.28, 8.32 (2s, 2H, D₂O exchangeable, 2NH), 7.28–7.35 (*m*, 5H, Bz), 2.24–2.26 (*m*, 4H, 2H-5, 2H-6), 1.80–1.84 (*m*, 4H, 2H-4, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 179.5 (C=S, C-2), 165.3 (CO-Bz), 116.7 (CN), 144.2, 138.0, 133.8, 130.4, 128.7, 124.9, 124.1, 119.2, (C-2, C-3, C-4', C-5', Bz), 28.6, 25.7, 22.1, 19.2 (C-4, C-5, C-6, C-7), EIMS m/z 341 [M]⁺ (20); Analysis



Scheme 4 Synthesis of compounds **18a, b** and **19a–f**

Calcd for $\text{C}_{17}\text{H}_{15}\text{N}_3\text{OS}_2$ (341.45): C, 59.80; H, 4.43; N, 12.31; S, 18.78. Found: C, 59.66; H, 4.31; N, 12.46; S, 18.66.

(4-Amino-2-thioxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3(2H)-yl)(phenyl)methanone (4)

A solution of compound **3** (3.41 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) was heated under reflux for 6 h and then left to cool. The solution was evaporated under vacuum and the remaining

product was triturated with ethanol and the formed solid product was collected by filtration.

3-Phenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5-amine (5)

To a solution of compound **4** (3.41 g, 0.01 mol) in 1,4-dioxane (40 mL) hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h and then poured onto ice/water containing few drops of hydrochloric acid, and the formed solid product was collected by filtration.

Table 1 Cytotoxicity of the newly synthesized products against a variety of cancer cell lines [IC₅₀^b (nM)]

Compoundd	Cytotoxicity (IC ₅₀ in nM) ^a						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38 ^c
3	2213	2074	1302	1802	2172	1029	180
4	380	220	480	1099	629	336	770
5	1288	2187	2530	1180	2135	1729	650
7a	48	59	122	1277	3289	480	620
7b	1622	396	274	1028	670	2169	490
8a	356	280	1652	1529	490	2253	210
8b	36	55	270	341	528	2540	680
10a	2210	2433	1650	1850	1544	1893	450
10b	1128	1892	2377	2138	1290	1655	280
10c	40	68	312	328	824	1243	384
10d	86	42	203	253	210	318	482
12a	2101	1380	2258	2166	2180	2330	512
12b	1135	1240	1278	2359	1266	2555	128
12c	38	46	120	337	441	180	320
12d	48	33	28	260	348	38	662
12e	222	234	210	65	252	226	690
12f	122	30	59	270	1140	1160	260
14a	3138	1232	2228	3328	1584	2270	380
14b	232	115	225	370	326	1220	466
14c	2265	122	2257	322	2250	222	589
14d	1180	3268	2560	2128	3330	1180	280
14e	35	60	61	22	66	623	390
14f	1120	1246	1128	2334	2340	2155	520
15a	1270	1290	1355	2120	2440	2457	360
15b	2212	3832	1148	1220	2333	2673	544
16a	1355	160	290	221	2229	2332	631
16b	2283	2458	1844	2560	2313	2148	428
18a	1480	1150	1140	1328	1260	1140	429
18b	148	163	63	232	480	860	860
19a	1620	2255	1760	2520	2088	1264	634
19b	128	447	264	350	470	328	532
19c	1145	3210	3218	2276	2672	2711	493
19d	3320	2366	2781	3744	1589	1130	650
19e	48	66	123	125	386	326	190
19f	1253	2139	2470	1216	2205	1286	262
CHS 828	25	2315	2067	1245	15	18	378

^a NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONE1, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells

^b The sample concentration produces a 50 % reduction in cell growth and CHS-828 is a pyridyl cyanoguanidine antitumor agent

^c IC₅₀ against the normal fibroblast cells (WI38) are indicated as multiples of 10⁴ nM

Compound **5**: Yellow crystals (1,4-dioxane), yield 75 % (2.41 g), mp 177–179 °C. IR (KBr) cm⁻¹: 3477, 3326, 3053, 1636. ¹H NMR (DMSO-d₆, 400 MHz): δ = 7.31–7.38 (*m*, 5H, Bz), 5.01 (*s*, 2H, D₂O exchangeable, NH₂), 2.24–2.29 (*m*, 4H, 2H-6, 2H-9), 1.79–1.84 (*m*,

4H, 2H-7, 2H-8). ¹³C NMR (DMSO-d₆, 75 MHz): δ = 170.3, 172.8, 173.6 (3 C=N), 145.0, 141.1, 138.8, 132.6, 129.0, 124.6, 123.8, 120.3 (thiophene, pyrimidine, Bz), 28.8, 25.7, 22.3, 19.2 (C-6, C-7, C-8, C-9), EIMS *m/z* 321 [M]⁺ (18); Analysis Calcd for C₁₇H₁₅N₅S (321.41): C,

Table 2 Toxicity of compounds **4**, **7a**, **8a**, **8b**, **10c**, **10d**, **12c**, **12d**, **12e**, **12f**, **14e**, **15b**, **18b**, **19b** and **19e**

Compound no.	Cons. ($\mu\text{g/ml}$)	Mortality ^a	Toxicity	LC ₅₀	Upper 95 % lim.	Lower 95 % lim
4	10	5	Harmful	314.03	252.25	110.40
	100	8				
	1000	10				
7a	10	0	Harmful	18.28	660.32	182.32
	100	4				
	1000	8				
8a	10	0	Harmful	251.19	60.28	4.32
	100	5				
	1000	10				
8b	10	5	Very toxic	18.25	20.22	32.51
	100	9				
	1000	10				
10c	10	5	Toxic	102.00	1002.2	140.62
	100	8				
	1000	10				
10d	10	0	Harmful	16.55	–	–
	100	3				
	1000	8				
12c	10	0	Nontoxic	20.56	–	–
	100	0				
	1000	5				
12d	10	4	Toxic	10.00	293.03	74.23
	100	8				
	1000	10				
12e	10	2	Very toxic	254.16	653.32	159.66
	100	5				
	1000	10				
12f	10	4	Harmful	22.45	–	–
	100	5				
	1000	8				
14e	10	0	Nontoxic	18.92	–	–
	100	0				
	1000	5				
15b	10	0	Harmful	14.68	622.17	148.60
	100	4				
	1000	8				
18b	10	0	Harmful	12.28	325.40	150.65
	100	5				
	1000	10				
19b	10	0	Harmful	12.48	350.27	160.20
	100	5				
	1000	10				
19e	10	0	Harmful	14.68	650.37	166.20
	100	5				
	1000	9				

^a Ten organisms (*A. salina*) tested for each concentration

63.53; H, 4.70; N, 21.79; S, 9.98. Found: C, 63.82; H, 4.85; N, 21.84; S, 10.24.

N-(3-benzoyl-2-thioxo-2,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-cyanoacetamide (7a) and N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-3-oxobutanamide (7b)

General procedure: To a solution of compound **4** (3.41 g, 0.01 mol) in dimethylformamide (40 mL), either ethyl cyanoacetate (1.13 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 3 h and then poured onto ice/water, and the formed solid product was collected by filtration.

Compound **7a**: Yellow crystals (1,4-dioxane), yield 80 % (3.27 g), mp 233–235 °C. IR (KBr) cm^{-1} : 3463, 3324, 3058, 2988, 2220, 1687, 1679, 1633. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.39 (*s*, 1H, D_2O exchangeable NH), 7.32–7.39 (*m*, 5H, Bz), 5.20 (*s*, 2H, CH_2), 2.26–2.30 (*m*, 4H, 2H-4, 2H-7), 1.82–1.86 (*m*, 4H, 2H-5, 2H-6). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.4 (C=S, C-2), 170.2 (C=N, C-1), 163.6, 166.8 (CO-Bz, CO-NH), 144.8, 140.9, 136.8, 143.2, 128.5, 124.8, 124.3, 120.6 (Bz, pyrimidine, thiophene C), 116.8 (CN), 28.6, 25.4, 22.6, 19.2 (C-4, C-5, C-6, C-7), EIMS m/z 408 $[\text{M}]^+$ (31); Analysis Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2\text{S}_2$ (408.51): C, 58.80; H, 3.95; N, 13.72; S, 15.70. Found: C, 58.72; H, 4.21; N, 13.49; S, 15.63.

Compound **7b**: Yellow crystals (1,4-dioxane), yield 80 % (3.40 g), mp 170–173 °C. IR (KBr) cm^{-1} : 3476, 3364, 3055, 2983, 1710, 1681, 1633, 1200. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.22 (*s*, 1H, D_2O exchangeable NH), 7.29–7.36 (*m*, 5H, Bz), 4.87 (*s*, 2H, CH_2), 2.63 (*s*, 3H, CH_3), 2.27–2.32 (*m*, 4H, 2H-4, 2H-7), 1.81–1.86 (*m*, 4H, 2H-5, 2H-6). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.2 (C=S, C-2), 170.2 (C=N, C-1), 163.2, 164.8, 166.0 (CO-Bz, CO-NH, COCH_3), 152.3, 146.7, 138.6, 133.2, 128.5, 124.8, 124.3, 121.9 (Bz, pyrimidine, thiophene C), 116.3 (CN), 60.4 (*s*, 2H, CH_2), 28.6, 25.4, 22.8, 19.4 (C-4, C-5, C-6, C-7), 18.3 (CH_3). EIMS m/z 425 $[\text{M}]^+$ (40); Analysis Calcd for $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_3\text{S}_2$ (425.52): C, 59.28; H, 4.50; N, 9.88; S, 15.07. Found: C, 59.40; H, 4.43; N, 9.75; S, 15.23.

2-Oxo-4-phenyl-6-thioxo-2,6,9,10,11,12-hexahydro-1H-benzo[4,5]thieno[3,2-e]pyrimido[1,2-c]pyrimidine-3-carbonitrile (8a) and 3-acetyl-4-phenyl-6-thioxo-9,10,11,12-tetrahydro-1H-benzo[4,5]thieno[3,2-e]pyrimido-2(6H)-one (8b)

General procedure: A suspension of either compound **7a** (4.08 g, 0.01 mol) or **7b** (4.25 g, 0.01 mol) in sodium

ethoxide solution [prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (40 mL)] was heated in a boiling water for 4 h. The reaction mixture was poured onto ice/water containing few drops of hydrochloric acid (till pH 6), and the formed solid product was collected by filtration.

Compound **8a**: Pale yellow crystals (1,4-dioxane), yield 66 % (2.58 g), mp 193–196 °C. IR (KBr) cm^{-1} : 3474, 3334, 3056, 2980, 2220, 1690, 1632. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.41 (*s*, 1H, D_2O exchangeable NH), 7.29–7.38 (*m*, 5H, Bz), 2.24–2.29 (*m*, 4H, 2H-9, 2H-12), 1.81–1.87 (*m*, 4H, 2H-10, 2H-11), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-6), 170.4 (C=N, C-7), 166.4 (CO-pyrimidine C-3), 151.6, 148.9, 143.7, 139.0, 136.8, 134.2, 129.3, 124.8, 123.6, 120.6 (Bz, pyrimidine, thiophene C), 116.2 (CN), 28.8, 25.5, 22.4, 19.0 (C-9, C-10, C-11, C-12), EIMS m/z 390 $[\text{M}]^+$ (26); Analysis Calcd for $\text{C}_{20}\text{H}_{14}\text{N}_4\text{OS}_2$ (390.48): C, 61.52; H, 3.61; N, 14.35; S, 16.42. Found: C, 61.38; H, 3.59; N, 14.49; S, 16.26.

Compound **8b**: Yellow crystals (1,4-dioxane), yield 76 % (3.10 g), mp 153–155 °C. IR (KBr) cm^{-1} : 3456, 3341, 3053, 2986, 1715, 1684, 1632, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.26 (*s*, 1H, D_2O exchangeable NH), 7.26–7.39 (*m*, 5H, Bz), 2.71 (*s*, 3H, CH_3), 2.24–2.36 (*m*, 4H, 2H-9, 2H-12), 1.80–1.85 (*m*, 4H, 2H-10, 2H-11). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-6), 170.6 (C=N, C-7), 166.0, 163.4 (CO-pyrimidine C-3, COCH_3), 152.3, 148.0, 142.7, 136.9, 133.6, 126.8, 124.9, 124.6, 123.8, 122.3 (Bz, pyrimidine, thiophene C), 28.9, 25.4, 22.5, 19.6, (C-9, C-10, C-11, C-12), 18.4 (CH_3). EIMS m/z 407 $[\text{M}]^+$ (32); Analysis Calcd for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_2\text{S}_2$ (407.51): C, 61.90; H, 4.21; N, 10.31; S, 15.74. Found: C, 62.08; H, 4.13; N, 10.29; S, 15.52.

3,5-Diamino-N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-cyanothiophene-2-carboxamide (10a), 5-amino-N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-cyano-3-methylthiophene-2-carboxamide (10b), ethyl 2,4-diamino-5-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)carbamoyl)thiophene-3-carboxylate (10c) and ethyl 2-amino-5-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)carbamoyl)-4-methylthiophene-3-carboxylate (10d)

General procedure: To a solution of either compound **7a** (4.08 g, 0.01 mol) or **7b** (4.25 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), both of elemental sulfur (0.32 g, 0.01 mol) and of either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The whole reaction mixture, in each case, was

heated under reflux for 1 h and then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Compound 10a: Orange crystals (1,4-dioxane), yield 73 % (3.70 g), mp 231–233 °C. IR (KBr) cm^{-1} : 3469, 3324, 3059, 2987, 2220, 1688, 1684, 1630, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.38 (s, 1H, D_2O exchangeable NH), 7.33–7.42 (m, 5H, Bz), 4.21, 4.48 (2s, 4H, D_2O exchangeable, 2 NH_2), 2.25–2.28 (m, 4H, 2H-5, 2H-8), 1.82–1.89 (m, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.3 (C=S, C-2), 170.6 (C=N, C-1), 162.8, 166.8 (CO-Bz, CO-NH), 152.8, 149.0, 148.2, 146.5, 143.8, 139.0, 135.4, 134.2, 129.2, 124.9, 122.8, 120.8 (Bz, pyrimidine, two thiophene C), 116.4 (CN), 28.8, 25.4, 22.7, 19.3, (C-5, C-6, C-7, C-8), EIMS m/z 506 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_6\text{O}_2\text{S}_3$ (506.62): C, 54.53; H, 3.58; N, 16.59; S, 18.99. Found: C, 54.80; H, 3.41; N, 16.33; S, 19.06.

Compound 10b: Yellow crystals (1,4-dioxane), yield 67 % (3.39 g), mp 210–212 °C. IR (KBr) cm^{-1} : 3476, 3332, 3060, 2989, 2220, 1705, 1686, 1631, 1215. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.28 (s, 1H, D_2O exchangeable NH), 7.28–7.37 (m, 5H, Bz), 4.47 (s, 2H, D_2O exchangeable, NH_2), 2.69 (s, 3H, CH_3), 2.22–2.35 (m, 4H, 2H-5, 2H-8), 1.82–1.86 (m, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.4 (C=S, C-2), 170.5 (C=N, C-1), 163.6, 166.3 (CO-Bz, CO-NH), 154.0, 152.7, 146.2, 142.3, 136.9, 132.8, 126.6, 124.5, 124.3, 123.0, 122.6, 121.8 (Bz, pyrimidine, two thiophene C), 117.0 (CN), 28.9, 25.5, 22.7, 19.4 (C-5, C-6, C-7, C-8), 18.7 (CH_3). EIMS m/z 505 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{24}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_3$ (505.64): C, 57.01; H, 3.79; N, 13.85; S, 19.02. Found: C, 56.87; H, 4.09; N, 13.69; S, 18.89.

Compound 10c: Orange crystals (1,4-dioxane), yield 65 % (3.70 g), mp 180–184 °C. IR (KBr) cm^{-1} : 3478, 3320, 3062, 2984, 2887, 1690, 1686, 1630, 1212. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.33 (s, 1H, D_2O exchangeable NH), 7.32–7.40 (m, 5H, Bz), 4.23, 4.45 (2s, 4H, D_2O exchangeable, 2 NH_2), 4.21 (q, 2H, J = 7.02 Hz, CH_2), 2.22–2.29 (m, 4H, 2H-5, 2H-8), 1.83–1.89 (m, 4H, 2H-6, 2H-7), 1.14 (t, 3H, J = 7.02 Hz, CH_3). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-2), 170.4 (C=N, C-1), 162.6, 163.8, 166.2 (CO-Bz, CO-NH, CO-OEt), 152.9, 150.3, 148.6, 146.5, 142.6, 139.0, 134.6, 132.9, 129.0, 124.9, 122.6, 120.3 (Bz, pyrimidine, two thiophene C), 28.7, 25.2, 22.9, 19.6, (C-5, C-6, C-7, C-8), 49.2 (CH_2), 16.0 (CH_3), EIMS m/z 553 $[\text{M}]^+$ (28); Analysis Calcd for $\text{C}_{25}\text{H}_{23}\text{N}_5\text{O}_4\text{S}_3$ (553.67): C, 54.23; H, 4.19; N, 12.65; S, 17.37. Found: C, 54.59; H, 4.52; N, 12.49; S, 17.22.

Compound 10d: Yellow crystals (1,4-dioxane), yield 71 % (3.92 g), mp 166–168 °C. IR (KBr) cm^{-1} : 3483, 3328, 3063, 2984, 2887, 1702, 1689, 1633, 1213. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.31 (s, 1H, D_2O exchangeable NH), 7.29–7.36 (m, 5H, Bz), 4.48 (s, 2H, D_2O exchangeable, NH_2), 4.21 (q, 2H, J = 7.30 Hz, CH_2), 2.65 (s, 3H, CH_3),

2.23–2.38 (m, 4H, 2H-5, 2H-8), 1.83–1.89 (m, 4H, 2H-6, 2H-7), 1.14 (t, 3H, J = 7.30 Hz, CH_3). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.0 (C=S, C-2), 170.3 (C=N, C-1), 166.8, 164.4, 163.2 (CO-Bz, CO-NH, CO-OEt), 154.2, 152.4, 146.2, 142.3, 136.9, 133.6, 126.3, 124.7, 124.6, 123.2, 122.8, 121.4 (Bz, pyrimidine, two thiophene C), 49.2 (CH_2), 28.9, 25.8, 22.7, 19.7 (C-5, C-6, C-7, C-8), 18.5 (CH_3), 16.3 (CH_3 , OCH_2CH_3). EIMS m/z 552 $[\text{M}]^+$ (32); Analysis Calcd for $\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_3$ (552.68): C, 56.50; H, 4.38; N, 10.14; S, 17.41. Found: C, 56.69; H, 4.26; N, 10.09; S, 17.66.

N-(3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-cyano-3-phenylacrylamide (12a), N-(3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-cyano-3-(4-chlorophenyl)acrylamide (12b), N-(3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-cyano-3-(4-methoxyphenyl)acrylamide (12c), N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-benzylidene-3-oxobutanamide (12d), N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-(4-chlorobenzylidene)-3-oxobutanamide (12e) and N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2-(4-methoxybenzylidene)-3-oxobutanamide (12f)

General procedure: To a solution of either compound **7a** (4.08 g, 0.01 mol) or **7b** (4.25 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), either benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 4 h and then evaporated under vacuum. The remaining product was triturated with diethyl ether, and the formed solid product was collected by filtration.

Compound 12a: Yellow crystals (1,4-dioxane), yield 70 % (3.48 g), mp 170–172 °C. IR (KBr) cm^{-1} : 3455, 3331, 3056, 2984, 2222, 1689, 1680, 1632, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.32 (s, 1H, D_2O exchangeable NH), 7.28–7.40 (m, 10H, 2Bz), 6.01 (s, 1H, $\text{CH}=\text{C}$), 2.26–2.29 (m, 4H, 2H-5, 2H-8), 1.80–1.88 (m, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.2 (C=S, C-2), 170.4 (C=N, C-1), 163.2, 166.4 (CO-Bz, CO-NH), 152.6, 149.2, 148.2, 146.5, 143.8, 139.0, 135.4, 128.6, 124.2, 123.9, 120.4, 119.6 (2Bz, pyrimidine, thiophene C), 116.7 (CN), 109.6, 114.3 ($\text{CH}=\text{C}$), 28.8, 25.6, 22.4, 19.1 (C-5, C-6, C-7, C-8). EIMS m/z 496 $[\text{M}]^+$ (21); Analysis Calcd for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{O}_2\text{S}_2$ (496.60): C, 65.30; H, 4.06; N, 11.28; S, 12.91. Found: C, 65.29; H, 3.81; N, 11.08; S, 12.76.

Compound **12b**: Yellow crystals (1,4-dioxane), yield 70 % (3.72 g), mp 188–190 °C. IR (KBr) cm^{-1} : 3483, 3312, 3058, 2984, 2220, 1710, 1688, 1630, 1211. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.03 (*s*, 1H, D_2O exchangeable NH), 7.26–7.39 (*m*, 9H, C_6H_5 , C_6H_4), 5.99 (*s*, 1H, $\text{CH}=\text{C}$), 2.24–2.38 (*m*, 4H, 2H-5, 2H-8), 1.80–1.87 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-1), 170.3 (C=N, C-2), 166.1, 164.8 ($\text{CO}-\text{Bz}$, $\text{CO}-\text{NH}$), 154.2, 153.4, 146.2, 142.6, 136.9, 133.4, 129.6, 126.6, 124.5, 123.8, 122.8, 120.9 (2Bz, thiophene, pyrimidine C), 116.7 (CN), 28.7, 25.5, 22.4, 19.6 (C-5, C-6, C-7, C-8), EIMS m/z 531 $[\text{M}]^+$ (22); Analysis Calcd for $\text{C}_{27}\text{H}_{19}\text{ClN}_4\text{O}_2\text{S}_2$ (531.05): C, 61.07; H, 3.61; N, 10.55; S, 12.08. Found: C, 60.91; H, 3.80; N, 10.76; S, 11.89.

Compound **12c**: Orange crystals (1,4-dioxane), yield 58 % (3.05 g), mp 220–222 °C. IR (KBr) cm^{-1} : 3445, 3328, 3055, 2989, 2884, 2222, 1695, 1688, 1632, 1218. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.09 (*s*, 1H, D_2O exchangeable NH), 7.31–7.38 (*m*, 9H, C_6H_5 , C_6H_4), 6.11 (*s*, 1H, $\text{CH}=\text{C}$), 2.88 (*s*, 3H, CH_3), 2.23–2.26 (*m*, 4H, 2H-5, 2H-8), 1.81–1.87 (*m*, 4H, 2H-6, 2H-7), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.3 (C=S, C-2), 170.9 (C=N, C-1), 164.2, 162.9 ($\text{CO}-\text{Bz}$, $\text{CO}-\text{NH}$), 153.3, 150.3, 148.6, 145.9, 142.8, 138.2, 133.6, 136.3, 129.0, 124.9, 122.8, 120.1 (2Bz, pyrimidine, thiophene C), 116.8 (CN), 28.9, 25.5, 22.9, 19.8 (C-5, C-6, C-7, C-8), 18.3 (CH_3). EIMS m/z 526 $[\text{M}]^+$ (20); Analysis Calcd for $\text{C}_{28}\text{H}_{22}\text{N}_4\text{O}_3\text{S}_2$ (526.62): C, 63.86; H, 4.21; N, 10.64; S, 12.18. Found: C, 63.93; H, 4.30; N, 10.44; S, 12.09.

Compound **12d**: Yellow crystals (1,4-dioxane), yield 68 % (3.49 g), mp 213–215 °C. IR (KBr) cm^{-1} : 3456, 3330, 3060, 2986, 2880, 1710–1687, 1638, 1218. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.28 (*s*, 1H, D_2O exchangeable NH), 7.31–7.42 (*m*, 10H, 2Bz), 6.02 (*s*, 1H, $\text{CH}=\text{C}$), 2.68 (*s*, 3H, CH_3), 2.24–2.38 (*m*, 4H, 2H-5, 2H-8), 1.85–1.89 (*m*, 4H, 2H-6, 2H-7), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.3 (C=S, C-2), 170.6 (C=N, C-1), 166.4, 164.2, 163.6 ($\text{CO}-\text{Bz}$, $\text{CO}-\text{NH}$, COCH_3), 154.3, 152.4, 146.2, 144.2, 136.9, 133.6, 126.3, 124.7, 124.6, 123.9, 122.8, 121.6 (2Bz, pyrimidine, thiophene C), 28.5, 25.8, 22.9, 19.4 (C-5, C-6, C-7, C-8), 18.9 (CH_3), EIMS m/z 513 $[\text{M}]^+$ (21); Analysis Calcd for $\text{C}_{28}\text{H}_{23}\text{N}_3\text{O}_3\text{S}_2$ (513.63): C, 65.48; H, 4.51; N, 8.18; S, 12.49. Found: C, 65.72; H, 4.49; N, 8.09; S, 12.66.

Compound **12e**: Pale yellow crystals (1,4-dioxane), yield 87 % (4.77 g), mp > 300 °C. IR (KBr) cm^{-1} : 3476, 3326, 3060, 2988, 2883, 1708–1688, 1634, 1209. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.28 (*s*, 1H, D_2O exchangeable NH), 7.31–7.42 (*m*, 9H, C_6H_4 , C_6H_5), 6.04 (*s*, 1H, $\text{CH}=\text{C}$), 2.73 (*s*, 3H, CH_3), 2.26–2.36 (*m*, 4H, 2H-5, 2H-8), 1.86–1.90 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.1 (C=S, C-2), 170.7 (C=N, C-1), 163.4, 164.8, 166.2 ($\text{CO}-\text{Bz}$, $\text{CO}-\text{NH}$, COCH_3), 154.8, 152.4,

146.2, 144.2, 136.9, 133.6, 126.3, 124.9, 124.2, 123.9, 123.0, 121.7, (2Bz, pyrimidine, thiophene C), 28.5, 25.8, 22.9, 19.6 (C-5, C-6, C-7, C-8), 19.3 (CH_3). EIMS m/z 548 $[\text{M}]^+$ (21); Analysis Calcd for $\text{C}_{28}\text{H}_{22}\text{ClN}_3\text{O}_3\text{S}_2$ (548.07): C, 61.36; H, 4.05; N, 7.67; S, 11.70. Found: C, 61.49; H, 4.25; N, 6.83; S, 11.92.

Compound **12f**: Pale yellow crystals (1,4-dioxane), yield 67 % (3.64 g), mp 277–279 °C. IR (KBr) cm^{-1} : 3484, 3318, 3058, 2988, 2887, 1720–1684, 1636, 1202. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.34 (*s*, 1H, D_2O exchangeable NH), 7.30–7.46 (*m*, 9H, C_6H_5 , C_6H_4), 6.06 (*s*, 1H, $\text{CH}=\text{C}$), 2.78, 3.01 (2s, 6H, 2 CH_3), 2.22–2.34 (*m*, 4H, 2H-5, 2H-8), 1.88–1.93 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.5 (C=S, C-2), 170.2 (C=N, C-1), 163.2, 164.5, 166.7 ($\text{CO}-\text{Bz}$, $\text{CO}-\text{NH}$, COCH_3), 153.6, 151.9, 146.2, 143.6, 136.9, 133.8, 126.3, 125.3, 124.6, 123.9, 122.8, 121.3 (2Bz, pyrimidine, thiophene C), 24.24 (OCH_3), 28.6, 25.8, 22.9, 19.4 (C-5, C-6, C-7, C-8), 19.8 (CH_3). EIMS m/z 543 $[\text{M}]^+$ (12); Analysis Calcd for $\text{C}_{29}\text{H}_{25}\text{N}_3\text{O}_4\text{S}_2$ (543.65): C, 64.07; H, 4.64; N, 7.73; S, 11.80. Found: C, 63.86; H, 4.43; N, 7.49; S, 11.66.

2-((3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-2-oxo-N'phenylacetohydrazonoyl cyanide (14a), 2-((3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-2-oxo-N'(4-chlorophenyl)acetohydrazonoyl cyanide (14b), 2-((3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-2-oxo-N'(4-methylphenyl)aceto-hydrazonoyl cyanide (14c), N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-3-oxo-2-(2-phenylhydrazono)butanamide (14d), N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-3-oxo-2-(2-(4-chlorophenyl)hydrazono)butanamide (14e) and N-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-3-oxo-2-(2-(4-methylphenyl)hydrazono)-butanamide (14f)

General procedure: To a solution of either compound **7a** (4.08 g, 0.01 mol) or **7b** (4.25 g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (5 mL, 10 %), a cold solution of either benzenediazoniumchloride (0.01 mol), 4-chlorobenzenediazonium chloride (0.01 mol) or 4-methylbenzenediazonium chloride (0.01 mol) [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution (0–5 °C) of the aniline (0.94 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methylaniline (1.08 g, 0.01 mol) in concentrated hydrochloric

acid (10 mL, 18 M) was added with continuous stirring] was added with continuous stirring. The whole reaction mixture was kept with stirring for 2 h, and the formed solid product was collected by filtration.

Compound 14a: Yellow crystals (1,4-dioxane), yield 86 % (4.41 g), mp 144–146 °C. IR (KBr) cm^{-1} : 3478, 3321, 3053, 2986, 2220, 1688, 1683, 1634, 1212. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.26, 3.11 (2s, 2H, D_2O exchangeable, 2NH), 7.32–7.39 (*m*, 10H, 2Bz), 2.21–2.28 (*m*, 4H, 2H-5, 2H-8), 1.82–1.84 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.4 (C=S, C-2), 170.3, 172.3 (2C=N), 166.6, 163.8 (CO-Bz, CONH), 153.8, 149.2, 148.7, 146.5, 143.8, 139.0, 136.3, 134.2, 129.3, 124.2, 121.5, 120.8 (2Bz, pyrimidine, thiophene C), 116.9 (CN), 29.0, 25.3, 22.6, 19.4, (C-5, C-6, C-7, C-8), EIMS m/z 512 $[\text{M}]^+$ (28); Analysis Calcd for $\text{C}_{26}\text{H}_{20}\text{N}_6\text{O}_2\text{S}_2$ (512.61): C, 60.92; H, 3.93; N, 16.39; S, 12.51. Found: C, 61.28; H, 3.68; N, 16.59; S, 12.66.

Compound 14b: Yellow crystals (1,4-dioxane), yield 56 % (3.06 g), mp 193–195 °C. IR (KBr) cm^{-1} : 3480, 3310, 3055, 2986, 2221, 1690, 1687, 1632, 1214. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.32, 8.12 (2s, 2H, D_2O exchangeable, 2NH), 7.41–7.28 (*m*, 9H, C_6H_5 , C_6H_4), 2.26–2.37 (*m*, 4H, 2H-5, 2H-8), 1.82–1.87 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-2), 170.8, 172.2 (2C=N), 164.6, 166.4 (CO-Bz, CO-NH), 154.0, 153.6, 146.2, 142.6, 136.6, 133.7, 129.6, 125.3, 124.8, 122.9, 122.5, 120.4 (2Bz, thiophene, pyrimidine C), 116.9 (CN), 28.9, 25.3, 22.6, 19.5 (C-5, C-6, C-7, C-8), EIMS m/z 547 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{26}\text{H}_{19}\text{ClN}_6\text{O}_2\text{S}_2$ (547.05): C, 57.09; H, 3.50; N, 15.36; S, 11.72. Found: C, 56.93; H, 3.70; N, 15.49; S, 11.93.

Compound 14c: Orange crystals (1,4-dioxane), yield 62 % (3.27 g), mp 132–135 °C. IR (KBr) cm^{-1} : 3458, 3319, 3053, 2988, 2882, 2220, 1705, 1686, 1630, 1220. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.12, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 7.30–7.36 (*m*, 9H, C_6H_5 , C_6H_4), 2.82 (*s*, 3H, CH_3), 2.24–2.28 (*m*, 4H, 2H-5, 2H-8), 1.83–1.89 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.1 (C=S, C-2), 172.3, 170.6 (C=N), 164.2, 162.9 (CO-Bz, CO-NH), 153.8, 150.3, 148.6, 144.2, 143.2, 138.2, 135.8, 133.6, 129.0, 124.7, 123.6, 120.3 (2Bz, pyrimidine, thiophene C), 116.6 (CN), 19.5 (CH_3), 28.6, 25.5, 23.4, 19.4 (C-5, C-6, C-7, C-8), EIMS m/z 526 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{27}\text{H}_{22}\text{N}_6\text{O}_2\text{S}_2$ (526.63): C, 61.58; H, 4.21; N, 15.96; S, 12.18. Found: C, 61.79; H, 4.44; N, 16.27; S, 12.34.

Compound 14d: Yellow crystals (1,4-dioxane), yield 81 % (4.29 g), mp 120–123 °C. IR (KBr) cm^{-1} : 3449, 3323, 3055, 2988, 2880, 1703–1689, 1638, 1220. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.24, 8.32 (2s, 2H, D_2O exchangeable, 2NH), 7.26–7.40 (*m*, 10H, 2Bz), 2.66 (*s*, 3H, CH_3), 2.22–2.37 (*m*, 4H, 2H-5, 2H-8), 1.82–1.87 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.1

(C=S, C-1), 172.4, 170.1 (2C=N), 166.3, 164.2, 162.4 (CO-Bz, CO-NH, COCH₃), 154.3, 151.9, 146.8, 144.2, 136.9, 133.8, 126.6, 124.7, 124.6, 123.9, 122.8, 120.8 (2Bz, pyrimidine, thiophene C), 19.0 (CH_3), 28.7, 26.0, 23.6, 19.6 (C-5, C-6, C-7, C-8). EIMS m/z 529 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{27}\text{H}_{23}\text{N}_5\text{O}_3\text{S}_2$ (529.63): C, 61.23; H, 4.38; N, 13.22; S, 12.11. Found: C, 61.63; H, 4.61; N, 13.47; S, 12.36.

Compound 14e: Yellow crystals (1,4-dioxane), yield 79 % (4.46 g), mp 294–297 °C. IR (KBr) cm^{-1} : 3477, 3340, 3060, 2989, 2885, 1705–1686, 1632, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.24, 8.32 (2s, 2H, D_2O exchangeable, 2NH), 7.28–7.40 (*m*, 9H, C_6H_4 , C_6H_5), 2.68 (*s*, 3H, CH_3), 2.23–2.38 (*m*, 4H, 2H-5, 2H-8), 1.84–1.88 (*m*, 4H, 2H-6, 2H-8). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.3 (C=S, C-2), 172.3, 170.4 (2C=N), 163.2, 164.6, 166.4 (CO-Bz, CO-NH, COCH₃), 153.6, 152.4, 120.9, 123.3, 124.1, 124.3, 124.9, 126.8, 132.4, 135.2, 144.2, 146.2, (2Bz, pyrimidine, thiophene C), 19.6 (CH_3), 28.7, 25.9, 22.6, 19.9 (C-5, C-6, C-7, C-8), EIMS m/z 564 $[\text{M}]^+$ (16); Analysis Calcd for $\text{C}_{27}\text{H}_{22}\text{ClN}_5\text{O}_3\text{S}_2$ (564.07): C, 57.49; H, 3.93; N, 12.42; S, 11.37. Found: C, 57.74; H, 4.02; N, 12.42; S, 11.88.

Compound 14f: Yellow crystals (1,4-dioxane), yield 77 % (4.19 g), mp > 300 °C. IR (KBr) cm^{-1} : 3474, 3339, 3056, 2980, 2881, 1706–1686, 1633, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.20, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 7.28–7.43 (*m*, 9H, C_6H_5 , C_6H_4), 2.64, 3.11 (2s, 6H, 2CH₃), 2.25–2.38 (*m*, 4H, 2H-5, 2H-8), 1.84–1.91 (*m*, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.6 (C=S, C-2), 172.8, 170.11 (C=N), 163.0, 164.6, 166.9 (CO-Bz, CO-NH, COCH₃), 153.8, 151.6, 146.4, 143.9, 136.9, 133.8, 126.8, 125.0, 123.9, 122.7, 121.6, 120.2, 28.9, 25.7, 22.8, 19.8 (C-5, C-6, C-7, C-8), (2Bz, pyrimidine, thiophene C), 19.6, 19.8 (2CH₃), EIMS m/z 543 $[\text{M}]^+$ (8); Analysis Calcd for $\text{C}_{28}\text{H}_{25}\text{N}_5\text{O}_3\text{S}_2$ (543.66): C, 61.86; H, 4.64; N, 12.88; S, 11.80. Found: C, 61.96; H, 4.84; N, 12.70; S, 11.73.

N-(3-Benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4,4-dicyano-3-methylbut-3-enamide (15a) and ethyl 5-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-2-cyano-3-methyl-5-oxopent-2-enoate (15b)

General procedure: To a dry solid of **7b** (4.25 g, 0.01 mol) containing ammonium acetate (0.50 g), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture was heated in an oil bath at 120 °C for 1 h and the formed solid product upon cooling was triturated with ethanol and collected by filtration.

Compound 15a: Pale orange crystals (acetic acid), yield 74 % (3.50 g), mp 210–212 °C. IR (KBr) cm^{-1} : 3483, 3324,

3058, 2986, 2223, 2220, 1690, 1688, 1630. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.38 (*s*, 1H, D_2O exchangeable NH), 7.31–7.39 (*m*, 5H, Bz), 5.25 (*s*, 2H, CH_2), 3.01 (*s*, 3H, CH_3), 2.24–2.29 (*m*, 4H, 2H-5, 2H-8), 1.80–1.87 (*m*, 4H, 2H-6, 2H-7), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 19.9 (CH_3), 20.3, 22.4, 25.2, 28.9 (4 CH_2), 117.2, 116.4 (2CN), 152.8, 151.8, 148.9, 143.7, 139.0, 135.9, 134.8, 129.1, 125.3, 123.9, 120.8 (C_6H_5 pyrimidine, thiophene C), 166.4, 164.2 (CO–Bz, CO–NH), 170.2 (C=N, C-1), 180.3 (C=S, C-2). EIMS m/z 473 $[\text{M}]^+$ (28); Analysis Calcd for $\text{C}_{24}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2$ (473.57): C, 60.88; H, 4.04; N, 14.79; S, 13.54. Found: C, 60.72; H, 3.89; N, 14.53; S, 13.66.

Compound **15b**: Yellow crystals (acetic acid), yield 90 % (4.69 g), mp 254–258 °C. IR (KBr) cm^{-1} : 3456, 3341, 3055, 2986, 2220, 1688, 1683, 1630, 1212. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.28 (*s*, 1H, D_2O exchangeable NH), 7.28–7.39 (*m*, 5H, Bz), 5.21 (*s*, 2H, CH_2), 4.23 (*q*, 2H, J = 7.22 Hz, CH_2 , OCH_2CH_3), 2.89 (*s*, 3H, CH_3), 2.22–2.38 (*m*, 4H, 2H-5, 2H-8), 1.80–1.87 (*m*, 4H, 2H-6, 2H-7), 1.14 (*t*, 3H, J = 7.22 Hz, CH_3 , OCH_2CH_3), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.3 (C=S, C-2), 170.8 (C=N, C-1), 164.8, 163.4, 162.6 (CO–Bz, CO–NH, COCH_3), 154.1, 148.0, 142.7, 136.9, 126.8, 124.9, 123.8, 122.2 (Bz, pyrimidine, thiophene C), 116.6 (CN), 36.8 (OCH_2CH_3), 28.9, 25.4, 22.6, 19.8 (C-5, C-6, C-7, C-8), 18.8 (CH_3), 16.2 (OCH_2CH_3), EIMS m/z 520 $[\text{M}]^+$ (18); Analysis Calcd for $\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_2$ (520.61): C, 59.98; H, 4.65; N, 10.76; S, 12.32. Found: C, 60.09; H, 4.44; N, 10.63; S, 12.52.

2-Amino-1-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (16a) and ethyl 2-amino-1-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-methyl-6-oxo-1,6,5-dihydropyridine-3-carboxylate (16b)

Method (A): General procedure: To a suspension of **7b** (4.25 g, 0.01 mol) in sodium ethoxide [prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (40 ml)], either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture was heated in a boiling water bath for 6 h and then poured onto ice/water containing few drops of hydrochloric acid (till pH 6), and the formed solid product was collected by filtration.

Method (B): To a suspension of either compound **15a** (4.73 g, 0.01 mol) or **15b** (5.20 g, 0.01 mol) in sodium ethoxide [prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (40 mL)] was heated under reflux in a boiling water bath for 4 h. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Compound **16a**: Yellow crystals (1,4-dioxane), yield 77 % (3.65 g), mp 190–192 °C. IR (KBr) cm^{-1} : 3473, 3331, 3056, 2983, 2873, 2220, 1688, 1684, 1630. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 7.34–7.36 (*m*, 5H, C_6H_5), 6.03 (*s*, 1H, pyridine H-5), 4.30 (*s*, 2H, D_2O exchangeable, NH_2), 3.11 (*s*, 3H, CH_3), 2.24–2.30 (*m*, 4H, 2H-5, 2H-8), 1.82–1.88 (*m*, 4H, 2H-6, 2H-7), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.1 (C=S, C-2), 170.8 (C=N, C-1), 164.1, 162.3 (CO–Bz, CO, pyridine C-6), 154.2, 152.4, 148.9, 143.7, 140.0, 135.3, 134.5, 129.0, 124.3, 123.9, 122.4, 120.3 (Bz, pyridine, pyrimidine, thiophene C), 116.6 (CN), 28.9, 25.0, 22.8, 20.1 (C-5, C-6, C-7, C-8), 19.8 (CH_3), EIMS m/z 473 $[\text{M}]^+$ (20); Analysis Calcd for $\text{C}_{24}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2$ (473.57): C, 60.87; H, 4.04; N, 14.79; S, 13.54. Found: C, 60.68; H, 3.99; N, 14.61; S, 13.72..

2-Amino-1-(3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile

Compound **16b**: Yellow crystals (1,4-dioxane), yield 70 % (3.64 g), mp 176–178 °C. IR (KBr) cm^{-1} : 3444, 3337, 3054, 2986, 1688, 1683, 1630, 1212. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 7.25–7.37 (*m*, 5H, Bz), 6.22 (*s*, 1H, pyridine H-5), 4.63 (*s*, 2H, D_2O exchangeable, NH_2), 4.23 (*q*, 2H, J = 9.93 Hz, OCH_2CH_3), 2.76 (*s*, 3H, CH_3), 2.20–2.39 (*m*, 4H, 2H-5, 2H-8), 1.82–1.87 (*m*, 4H, 2H-6, 2H-7), 1.16 (*t*, 3H, J = 6.93 Hz, OCH_2CH_3). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.1 (C=S, C-2), 170.5 (C=N, C-1), 162.8, 163.2, 164.8 (CO–Bz, CO–OEt, CO–pyridine C-6), 154.1, 150.4, 147.2, 142.9, 136.9, 133.6, 126.2, 124.9, 123.3, 122.1, 121.5, 120.3 (Bz, pyridine, pyrimidine, thiophene C), 36.5 (OCH_2CH_3), 28.3, 25.8, 22.4, 19.9 (C-5, C-6, C-7, C-8), 18.6 (CH_3), 16.3 (OCH_2CH_3). EIMS m/z 520 $[\text{M}]^+$ (22); Analysis Calcd for $\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_2$ (520.61): C, 59.98; H, 4.65; N, 10.76; S, 12.32. Found: C, 60.21; H, 4.59; N, 10.49; S, 12.48.

4-Amino-5-methyl-3-phenyl-2-thioxo—(2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-7(8H)-one (18a) and 4-hydroxy-5-methyl-3-phenyl-2-thioxo—(2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-7(8H)-one (18b)

General procedure: To a solution of either compound **16a** (4.73 g, 0.01 mol) or **16b** (5.20 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) phenylisothiocyanate (1.30 g, 0.01 mol), the whole reaction mixture was heated under reflux for 4 h and then poured onto ice/water containing few drops of hydrochloric acid, and the formed solid product was collected by filtration.

Compound **18a**: Yellow crystals (1,4-dioxane), yield 80 % (4.04 g), mp > 300 °C. IR (KBr) cm^{-1} : 3481, 3348, 3054, 2987, 2878, 1689, 1638. ^1H NMR (DMSO- d_6 , 400 MHz):

$\delta = 8.29$ (*s*, 1H, NH), 7.26–7.38 (*m*, 5H, Bz), 6.06 (*s*, 1H, pyridine H-5), 4.30 (*s*, 2H, D₂O exchangeable, NH₂), 2.69 (*s*, 3H, CH₃), 2.22–2.33 (*m*, 4H, 2H-5, 2H-8), 1.80–1.87 (*m*, 4H, 2H-6, 2H-7), ¹³C NMR (DMSO-d₆, 75 MHz): $\delta = 183.2$, 180.0 (C=S, C-2, C-2'), 172.4, 170.3 (C=N, C-1, C-1'), 163.8 (CO, pyridine C-6), 28.7, 25.3, 22.4, 20.4 (C-5, C-6, C-7, C-8), 154.0, 153.6, 150.3, 148.9, 143.9, 140.0, 135.63, 128.4, 123.7, 122.6, 121.4, 119.4 (Bz, pyridine, two pyrimidine, thiophene C), 19.6 (CH₃). EIMS *m/z* 504 [M]⁺ (20); Analysis Calcd for C₂₄H₂₀N₆O₃ (504.66): C, 57.12; H, 3.99; N, 16.65; S, 19.06. Found: C, 57.04; H, 3.68; N, 16.82; S, 18.79.

Compound **18b**: Yellow crystals (1,4-dioxane), yield 65 % (3.29 g), mp 190–192 °C. IR (KBr) cm⁻¹: 3489, 3321, 3056, 2988, 1680, 1633, 1200. ¹H NMR (DMSO-d₆, 400 MHz): $\delta = 10.20$ (*s*, 1H, D₂O exchangeable, OH), 8.22 (*s*, 1H, D₂O exchangeable, NH), 7.23–7.38 (*m*, 5H, Bz), 6.21 (*s*, 1H, pyridine H-5), 2.22–2.37 (*m*, 4H, 2H-5, 2H-8), 2.77 (*s*, 3H, CH₃), 1.80–1.87 (*m*, 4H, 2H-6, 2H-7). ¹³C NMR (DMSO-d₆, 75 MHz): $\delta = 180.2$, 182.1 (C=S, C-2, C-2'), 170.3, 171.3 (C=N, C-1, C-1'), 163.8 (CO, pyridine C-6), 19.8, 22.2, 25.9, 28.6 (C-5, C-6, C-7, C-8), 154.3, 150.6, 147.6, 142.9, 140.3, 138.6, 136.9, 133.6, 126.2, 125.9, 124.9, 123.8, 122.1, 121.8, 120.1 (Bz, pyridine, two pyrimidine, thiophene C), 19.4 (CH₃). EIMS *m/z* 505 [M]⁺ (28); Analysis Calcd for C₂₄H₁₉N₅O₂S₃ (505.64): C, 57.01; H, 3.79; N, 13.85; S, 19.02. Found: C, 56.88; H, 3.84; N, 14.06; S, 19.29

2-Amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-phenyl-4H-pyran-3,5-dicarbonitrile (19a), **2-amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-(4-chlorophenyl)-4H-pyran-3,5-dicarbonitrile (19b)**, **2-amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-(4-methoxyphenyl)-4H-pyran-3,5-dicarbonitrile (19c)**, **5-acetyl-2-amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-phenyl-4H-pyran-3-carbonitrile (19d)**, **5-acetyl-2-amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-(4-chlorophenyl)-4H-pyran-3-carbonitrile (19e)** and **5-acetyl-2-amino-6-((3-benzoyl-2-thioxo-2,3,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)-4-(4-methoxyphenyl)-4H-pyran-3-carbonitrile (19f)**

General procedure: To a solution of either compound **7a** (4.08 g, 0.01 mol) or **7b** (4.25 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL) malononitrile

(0.66 g, 0.01 mol), either benzaldehyde (1.08 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The whole reaction mixture, in each case, was heated under reflux for 6 h and then poured onto ice/water containing few drops of hydrochloric acid, and the formed solid product was collected by filtration.

Compound **19a**: Orange crystals (ethanol), yield 93 % (5.23 g), mp 183–185 °C. IR (KBr) cm⁻¹: 3493–3332, 3056, 2989, 2228, 2226, 2222, 1686, 1630, 1218. ¹H NMR (DMSO-d₆, 400 MHz): $\delta = 8.28$ (*s*, 1H, D₂O exchangeable, NH), 7.25–7.40 (*m*, 10H, 2Bz), 6.13 (*s*, 1H, pyran H-4), 4.26 (*s*, 2H, D₂O, NH₂), 2.21–2.34 (*m*, 4H, 2H-5, 2H-8), 1.81–1.88 (*m*, 4H, 2H-6, 2H-7), ¹³C NMR (DMSO-d₆, 75 MHz): $\delta = 180.2$ (C=S, C-2), 170.8 (C=N, C-1), 164.4 (CO-Bz), 154.0, 149.2, 148.7, 146.8, 144.3, 142.5, 140.2, 139.0, 136.3, 134.2, 129.3, 125.4, 124.8, 124.0, 120.6, 120.3 (2Bz, pyran, pyrimidine, thiophene C), 117.0, 116.8 (2CN), 42.3 (pyran C-4), 29.3, 25.5, 22.8, 19.8 (C-5, C-6, C-7, C-8), EIMS *m/z* 562 [M]⁺ (21); Analysis Calcd for C₃₀H₂₂N₆O₂S₂ (562.67): C, 64.04; H, 3.94; N, 14.94; S, 11.40. Found: C, 64.29; H, 3.88; N, 14.73; S, 11.62.

Compound **19b**: Orange crystals (ethanol), yield 63 % (3.76 g), mp 166–168 °C. IR (KBr) cm⁻¹: 3483, 3318, 3054, 2986, 227, 2223, 1690, 1688, 1210. ¹H NMR (DMSO-d₆, 400 MHz): $\delta = 8.18$ (*s*, 1H, D₂O exchangeable, NH), 7.26–7.40 (*m*, 9H, C₆H₅, C₆H₄), 6.37 (*s*, 1H, pyran H-4), 4.40 (*s*, 2H, D₂O exchangeable, NH₂), 2.22–2.38 (*m*, 4H, 2H-5, 2H-8), 1.84–1.89 (*m*, 4H, 2H-6, 2H-7), ¹³C NMR (DMSO-d₆, 75 MHz): $\delta = 180.4$ (C=S, C-2), 170.2 (C=N, C-1), 164.8 (CO-Bz), 154.2, 153.2, 150.1, 148.3, 146.2, 142.6, 136.3, 134.9, 128.4, 125.8, 124.3, 123.3, 122.9, 121.9, 120.3, 119.8, (2Bz, pyran, thiophene, pyrimidine C), 117.0, 116.6 (2CN), 42.6 (pyran C-4), 28.9, 25.6, 22.7, 19.4 (C-5, C-6, C-7, C-8), EIMS *m/z* 597 [M]⁺ (21); Analysis Calcd for C₃₀H₂₁ClN₆O₂S₂ (597.11): C, 60.35; H, 5.55; N, 14.07; S, 10.74. Found: C, 60.59; H, 3.62; N, 13.4; S, 10.88.

Compound **19c**: Orange crystals (1,4-dioxane), yield 79 % (4.68 g), mp 184–186 °C. IR (KBr) cm⁻¹: 3442, 3337, 3055, 2984, 2867, 2227, 2224, 1690, 1633, 1222. ¹H NMR (DMSO-d₆, 400 MHz): $\delta = 8.28$ (*s*, 1H, D₂O exchangeable, NH), 7.32–7.38 (*m*, 9H, C₆H₅, C₆H₄), 6.20 (*s*, 1H, pyran H-4), 4.38 (*s*, 2H, D₂O exchangeable, NH₂), 2.90 (*s*, 3H, CH₃), 2.23–2.29 (*m*, 4H, 2H-5, 2H-8), 1.81–1.88 (*m*, 4H, 2H-6, 2H-7). ¹³C NMR (DMSO-d₆, 75 MHz): $\delta = 180.3$ (C=S, C-2), 170.9, (C=N, C-1), 163.4 (CO-Bz), 154.2, 153.2, 150.3, 148.6, 144.5, 143.8, 140.5, 138.8, 135.5, 133.9, 129.3, 128.6, 124.7, 123.8, 122.4, 120.1 (2Bz, pyran, pyrimidine, thiophene C), 116.8, 117.2 (2CN), 42.4 (pyran C-4), 19.8 (CH₃), 28.4, 25.8, 23.7, 19.3 (C-5, C-6, C-7, C-8), EIMS *m/z* 592 [M]⁺ (22); Analysis Calcd for C₃₁H₂₄N₆O₃S₂ (592.69): C, 62.82; H, 4.08; N,

14.18; S, 10.82. Found: C, 62.68; H, 4.19; N, 14.35; S, 10.44.

Compound **19d**: Pale orange crystals (1,4-dioxane), yield 74 % (4.29 g), mp 222–225 °C. IR (KBr) cm^{-1} : 3438, 3303, 3058, 2980, 2893, 2220, 1688, 1634, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.26 (s, 1H, D₂O exchangeable, NH), 7.27–7.43 (m, 10H, 2Bz), 6.38 (s, 1H, pyran H-4), 4.40 (s, 2H, D₂O exchangeable, NH₂), 2.72 (s, 3H, CH₃), 2.24–2.39 (m, 4H, 2H-7, 2H-8), 1.83–1.88 (m, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.5 (C=S, C-2) 170.3 (C=N, C-1), 164.2, 166.3 (CO–Bz, COCH₃), 154.3, 151.9, 146.8, 144.2, 143.2, 136.9, 133.8, 129.7, 126.8, 125.2, 124.8, 123.6, 122.5, 121.3, 120.5, 119.3 (2Bz, pyran, pyrimidine, thiophene C), 116.8 (CN), 42.5 (pyran C-4), 19.4 (CH₃), 28.9, 26.6, 23.8, 19.4(C-5, C-6, C-7, C-8), EIMS m/z 579 [M]⁺ (36); Analysis Calcd for C₃₁H₂₅N₅O₃S₂ (579.69): C, 64.23; H, 4.35; N, 12.08; S, 11.06. Found: C, 64.07; H, 4.44; N, 11.95; S, 11.30.

Compound **19e**: Yellow crystals (1,4-dioxane), yield 55 % (3.38 g), mp 190–193 °C. IR (KBr) cm^{-1} : 3488, 3319, 3058, 2980, 2880, 2220, 1720–1688, 1631, 1212. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.30 (s, 1H, D₂O exchangeable, NH), 7.27–7.41 (m, 9H, C₆H₄, C₆H₅), 6.12 (s, 1H, pyran H-4), 4.30 (s, 2H, D₂O exchangeable NH₂), 3.13 (s, 3H, CH₃), 2.23–2.36 (m, 4H, 2H-5, 2H-8), 1.82–1.89 (m, 4H, 2H-6, 2H-7), ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.4 (C=S, C-2), 170.9 (C=N, C-1), 164.3, 165.4 (CO–Bz, COCH₃), 120.5, 121.8, 122.7, 123.9, 125.3, 126.0, 127.3, 129.0, 132.5, 134.0, 137.4, 140.1, 143.9, 146.4, 152.3, 153.4 (2Bz, pyran, pyrimidine, thiophene C), 116.6 (CN), 42.4 (pyran C-4), 19.9, 22.4, 25.6, 28.7 (4 CH₂), 19.7 (CH₃). EIMS m/z 614 [M]⁺ (18); Analysis Calcd for C₃₁H₂₄ClN₅O₃S₂ (614.13): C, 60.63; H, 3.94; N, 11.40; S, 10.44. Found: C, 60.82; H, 3.69; N, 11.28; S, 10.68.

Compound **19f**: Orange crystals (1,4-dioxane), yield 73 % (4.45 g), mp 180–183 °C. IR (KBr) cm^{-1} : 3480, 3320, 3056, 2987, 2882, 2218, 1686, 1630, 1210. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.30 (s, 1H, D₂O exchangeable, NH), 7.29–7.38 (m, 9H, C₆H₅, C₆H₄), 6.12 (s, 1H, pyran H-4), 4.37 (s, 2H, D₂O exchangeable, NH), 2.78, 2.89 (2s, 6H, 2CH₃), 2.21–2.34 (m, 4H, 2H-5, 2H-8), 1.81–1.86 (m, 4H, 2H-6, 2H-7). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ = 180.2 (C=S, C-2), 170.6 (C=N, C-1), 164.8, 165.2 (CO–Bz, COCH₃), 154.6, 153.2, 152.4, 147.7, 146.2, 144.2, 135.2, 133.6, 132.4, 130.4, 126.9, 125.0, 124.9, 124.4, 123.9, 123.3, 120.2, 119.8 (2Bz, pyran, pyrimidine, thiophene C), 116.3 (CN), 42.6 (pyran C-4), 19.8, 19.9 (OCH₃, COCH₃), 28.9, 25.8, 22.3, 19.8 (C-5, C-6, C-7, C-8), EIMS m/z 609 [M]⁺ (28); Analysis Calcd for C₃₂H₂₇N₅O₄S₂ (609.71): C, 63.04; H, 4.46; N, 11.49; S, 10.52. Found: C, 63.32; H, 4.69; N, 11.82; S, 11.63.

Conclusions

In summary, we have shown herein that our strategy is compatible with the synthesis of a wide range of tetrahydrobenzo[*b*]thiophene derivatives and particularly when being incorporated into heterocyclic and fused derivatives. The cytotoxicity of the newly synthesized products was evaluated against human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38). The results showed that compounds **4**, **7a**, **8a**, **8b**, **10c**, **10d**, **12c**, **12d**, **12e**, **12f**, **14e**, **15b**, **18b**, **19b** and **19e** exhibited optimal cytotoxic effect against cancer cell lines with IC₅₀ in the nM range. In addition, compounds **12c** and **14e** showed no toxicity when tested in vivo lethality to shrimp larvae (*Artemiasalina*).

Acknowledgments The authors would like to acknowledge financial support for this work from the Deanship of Scientific Research (DSR), University of Tabuk, Saudi Arabia, under grant no. S-0083-1436.

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