Regular Article

Synthesis of Novel Heterocyclic Compounds Incorporate 4,5,6,7-Tetrahydrobenzo[*b*]thiophene Together with Their Cytotoxic Evaluations

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The 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene was the key starting compound used to synthesize new thiazole, pyrimidine, pyran, pyridine and thiazine derivatives. The cytotoxicity of the synthesized compounds was studied towards the three cancer cell lines namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (central nervous system (CNS) cancer) in addition to the normal cell line (WI-38) using doxorubicin as the reference drug. The study showed that compounds 5, 9a, 15b, 17c, 18 and 21b were the most potent compounds.

Key words tetrahydrobenzo[*b*]thiophene; cytotoxic evaluation; multicompounant reaction; pyrimidine; thiazole

The reactions of the 2-amino-tetrahydrobenzo[*b*]thiophene derivatives with different chemical organic reagents were found to be of great interest in heterocyclic chemistry leading to the formation of this ring system incorporated in many heterocyclic compounds including thiazole,[1)] pyrimidine,[2)] thiazine,[3)] pyridine[4)] and pyran.[5)] On the other side, the derivatives of tetrahydrobenzo[*b*]thiophene exist in many pharmaceutical applications such as anti-tumor,[6)] antimicrobial,[7–9)] antiviral,[10)] anti-leishmanial agents,[11)] antioxidant activity,[12)] anti-arrhythmic, serotonin antagonist and anti-anxiety activities.[13)]

Recently we were involved through the synthesis of polyfunctional heterocyclic compounds, where the 2-cyanomethylbenzo[*c*]imidazole was used as the key starting compound.[14)] In addition, we were involved through the reaction of ethyl acetoacetate with elemental sulphur and either malononitrile or ethyl cyanoacetate gave thiophene derivatives.[15)] In the present work we were concerned through exploring the reactivity of the amino group present in the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene[16)] towards different chemical reagents to afford a vast number of heterocyclic compounds involving tetrahydrobenzo[*b*]-thiophene moiety and evaluating their cytotoxicity.

Results and Discussion

Chemistry The reaction of the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene with triethyl orthoformate in acetic acid gave the ethyl *N*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-2-yl)formimidate **1**. The analytical and spectral data of compound **1** were the tools of its structural elucidation. Compound **1** reacted with aniline to give the *N*-phenyl formamidine derivative **2**. The elemental and spectral data of compound **2** were consistent with its proposed structure. Thus, ^{1}H-NMR spectrum of compound **2** showed, beside the expected signals, a singlet at 6.95 ppm δ for CH moiety, a muliplet at δ 7.27–7.90 ppm for phenyl ring and a singlet at δ 8.36 ppm for NH group. On the other hand, the reaction of compound **1** with malononitrile gave the imino-methyl malononitrile derivative **3**. The analytical and spectral data of compound **3** were in agreement with its structure (see Experimental section). The reaction of compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene with phenylisothiocyanate and chloroacetone gave the thiazole derivative **5**. Formation of the latter product took place through the intermediate formation of **4** followed by water elimination (Chart 1).

Next we studied the possibility of compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene to form thiourea derivative. Thus, compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene reacted with phenyl

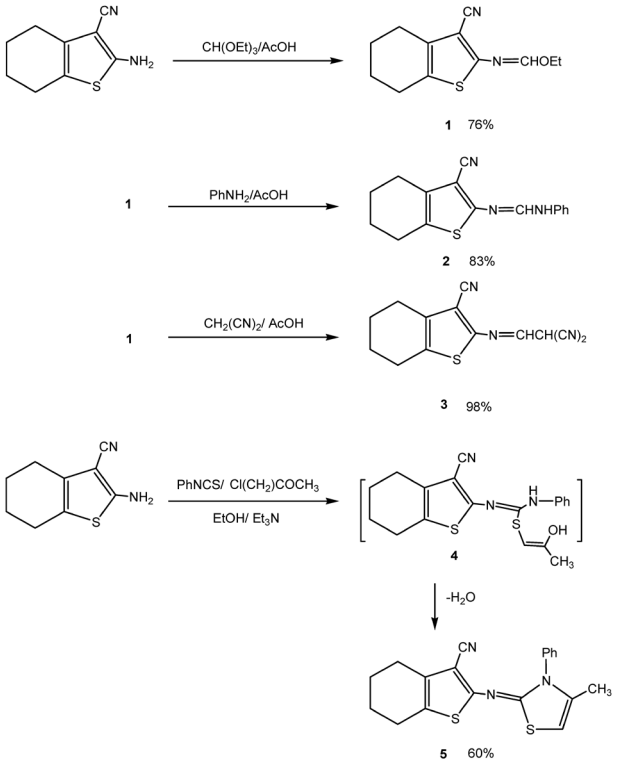

Chart 1.

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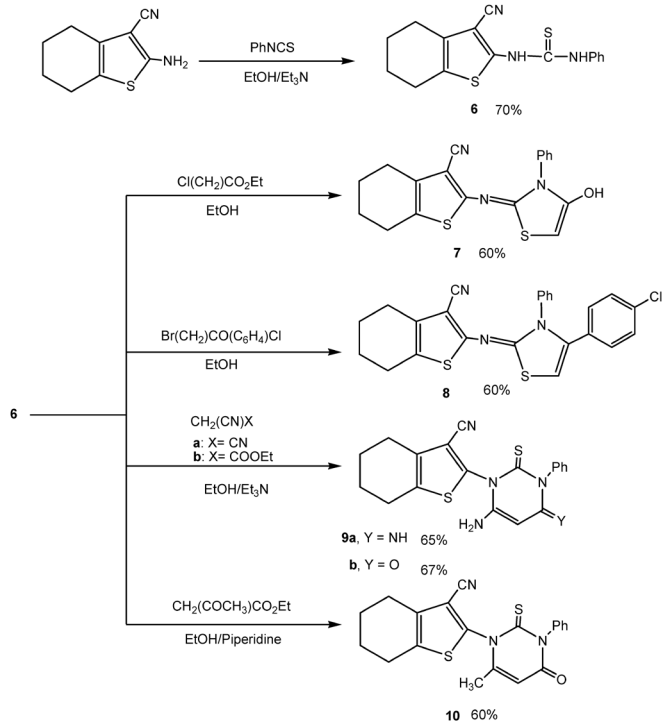

Chart 2.

isothiocyanayte in ethanol containing a catalytic amount of triethylamine to give the *N*-phenylthioura derivative **6**. The latter reacted with ethyl 2-chloroacetate in ethanol to give the thiazole derivative **7**. The structure of compound **7** was confirmed on the basis of analytical and spectral data. Thus, the ^{1}H-NMR showed, beside the expected signals, a singlet at δ 4.50 ppm for CH thiazole ring, a multiplet at δ 6.92–7.70 ppm indicating phenyl group and a singlet at δ 11.10 ppm (D_2O exchangeable) for OH group. In addition, the ^{13}C-NMR spectrum showed beside the expected signals, a signal at δ 180.0 corresponding to the excocyclic C=N group and a signal at δ 60.1 corresponding to the thiazole C-5. Similarly, the reaction of compound **6** with 2-bromo-1-(4-chlorophenyl)ethanone gave the thiazole derivative **8** the structure of which structure was based on its analytical and spectral data (see Experimental section).

Compound **6** was used to synthesize pyrimidine derivatives with potential biological activities. Thus, the reaction of compound **6** with either malononitrile or ethyl cyanoacetate gave the 4,6-diaminodihydropyrimidine derivatives **9a** and **9b**, respectively. On the other hand, the reaction of compound **6** with ethyl acetoacetate gave the 4-methyl-6-oxodihydropyrimidine derivative **10**. The reaction took place through ethanol and water elimination. The analytical and spectral data of compounds **9a**, **9b** and **10** are consistent with their respective structures (Chart 2).

However, the reaction of compound **6** with acetylacetone in 1,4-dioxane containing a catalytic amount of piperidine gave the 4,6-dimethyl-6-hydroxydihydropyrimidine derivative **11**. The latter compound, due to the presence of the acidic pyrimidine *H*-5 coupled with benzenediazonium chloride gave the phenyl azo derivative **12**. It is worthy to note that through the products **11** and **12** the OH group being at the carbon adjacent to the *N*-ph not at the carbon adjacent to the tetahydrobenzo[*b*]thiophene moiety this

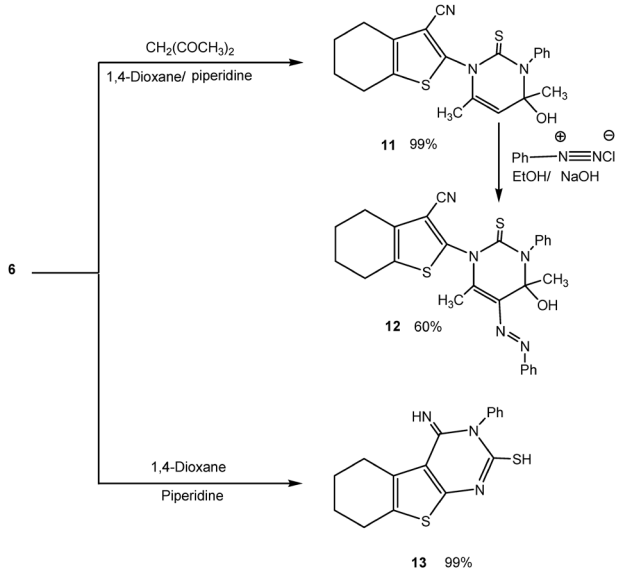

Chart 3.

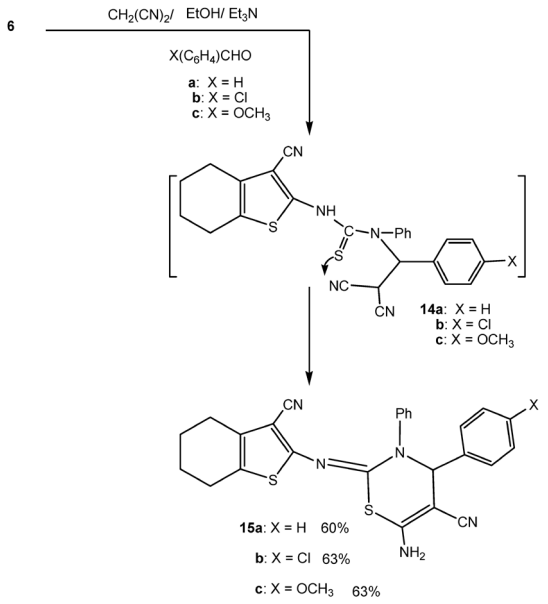

Chart 4.

was based on the reported literature which showed that this structure is the favored one.[17] Compound **6** underwent ready cyclization in 1,4-dioxane and piperidine to afford the 4-imino-3-phenyl-3,4,5,6,7,8-hexa-hydrobenzo[4,5]thieno[2,3-*d*]pyrimidine-2-thiol **13** (Chart 3). The analytical and spectral data of compound **13** were the basis of its structural elucidation (see Experimental section).

In addition compound **6** underwent multi-component reactions through its reaction with malononitrile and any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxy-benzaldehyde to produce the thiazine derivatives **15a**–**c**. The reaction took place through the intermediates formation of **14a**–**c** followed by cyclization (Chart 4).

The compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophene underwent a multi-component reaction when reacted with phenyl isothiocyanate, malononitrile and any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenz-

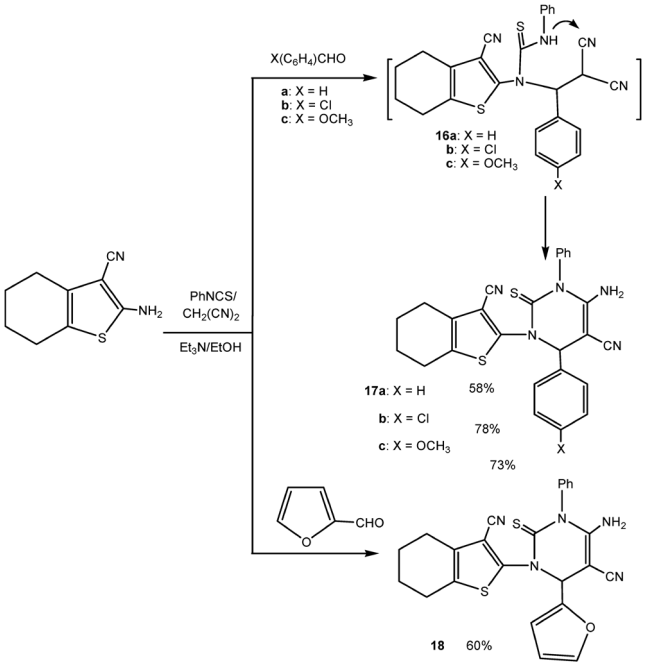

Chart 5.

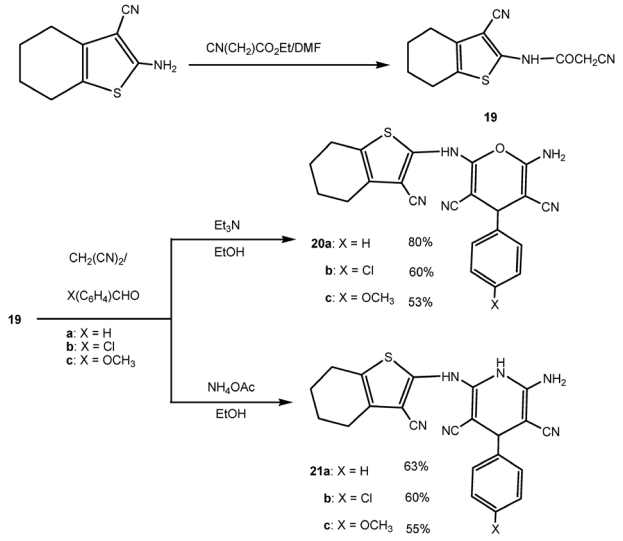

Chart 6.

aldehyde to give the tetrahydropyrimidine derivatives **17a**–**c**, respectively. The reaction took place through the intermediates formation of **16a**–**c**. The analytical and spectral data of **17a**–**c** was in agreement with their respective structures. Thus ^{1}H-NMR spectrum of **17a** (as an example) showed beside the expected signals, a singlet at δ 7.52 ppm indicating the pyrimidine H-6, multiplet at δ 7.53–7.99 ppm for two phenyl moieties and singlet at δ 8.66 ppm (D_2O exchangeable) for NH_2 group. Similarly, the multi-component reaction of compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene with phenyl isothiocyanate, malononitrile and furfural in ethanol and triethylamine produced the tetrahydropyrimidine derivative **18** (Chart 5).

The analytical and spectral data of the latter compound were consistent with its proposed structure (see Experimental section).

It has been reported that the compound 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene reacted with ethyl cyanoacetate in dimethylformamide gave the *N*-cyanoacetamido derivative **19**.[18,19] It has been reported that multi-component reactions (MCRs) can combine three or more components together in a single reaction vessel and produce final products with a minimum of synthetic time and effort[20] because they are no need to separate any reaction intermediate.[21] Such MCRs often result in high atomeconomy and high selectivity products.[22] They are also applicable to the synthesis of heterocyclic systems.[23] It is quite remarkable that many top-selling pharmaceuticals containing 4-*H* pyran derivatives[24–28] which encouraged us to synthesize the 4-*H* pyran and 4-*H* pyridine derivatives through the multi-component reactions of compound **19**. Thus, compound **19** underwent the MCR of malononitrile and any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde in ethanolic triethylamine solution to give the pyran derivatives **20a**–**c**, respectively. It should be useful to mention that similar reaction was carried before by our research group[18] but it was in two steps reaction, firstly the benzaldehyde react with compound **19** in 1,4-dioxane/*N*,*N*-dimethylformamide (DMF) solvent to produce first the benzylidine derivative this was followed by its reaction of malononitrile in the second step to produce the 2-pyridone derivatives. However, in this work the reaction is considered as a multi-component reaction occurred in one step where similar multi-component reactions producing pyran derivatives were reported before.[29–31] The produced pyran derivatives **20a**–**c** were found to be stable especially when heated in 1,4-dioxane/DMF solvent during our trials to convert them to the 2-pyridone derivatives. On carrying the same multi-component reaction but in the presence of ammonium acetate instead of triethylamine gave the pyridine derivatives **21a**–**c**, respectively (Chart 6). Thus, the ^{1}H-NMR and mass spectrum of compound **21c** (as an example) showed that singlet at δ 1.30 ppm for CH_3 group, multiplet at δ 1.69–2.68 ppm for the four CH_2 groups of cyclohexene ring, singlet at 6.95 ppm for pyridine ring, multiplet for [C_6H_4] moiety at δ 7.09–7.99 ppm, singlet at δ 8.30 ppm for NH_2 group and two singlets at δ 8.39 and 8.54 ppm for two 2NH moieties. Also, the mass spectrum of **21c** exhibited a molecular ion peak [M^+] (*m*/*z* 428) corresponding to molecular formula $C_{23}H_{20}N_6OS$.

Biological Activity Evaluations

In Vitro Cytotoxicity Evaluations of the Newly Synthesized Compounds

The cytotoxicity of the newly synthesized compounds was evaluated in the presence of three cancer cell lines and the results were demonstrated (Table 1). The three cancer cell lines were [MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (central nervous system (CNS) cancer)] and normal fibroblasts cells (WI38). The data showed that some compounds showed promising results, and the results were compared to the anti-proliferative affects of the reference control doxorubicin.[32] All the compounds were dissolved in dimethyl sulfoxide (DMSO) at 1 mg/mL immediately before use and diluted just before addition to the cell culture.

The data (Table 1) represents the mean±standard error of the mean (S.E.M.) of three independent experiments performed in duplicate. The results indicated that most compounds demonstrated substantial growth inhibitory effects

against the human tumor cells at the concentrations tested.

Structure Activity Relationship

It is of great value to notice that the newly synthesized products **5**, **9a**, **15b**, **17c**, **18** and **21b** exhibited optimal cytotoxic effect against the three cancer cell lines than the reference control doxorubicin, with GI_{50}'s (50% of growth inhibition) in the μM range. The cytotoxic effect of compound **5** showed that high potency due to the presence of the cyclohexene moiety together with the 3-cyano group of the thiophene moiety. Considering the dihydropyrimidine derivatives **9a** and **9b** it is obvious that compound **9a** with the 6-imino group showed more potency with GI_{50}'s 0.01, 0.04 and 0.70 μM against the three cancer cell lines MCF-7, NCI-H460 and SF-268, respectively than compound **9b** with the 6-oxo moiety. The hexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivative **13** showed high potency against the three cancer cell lines and this was attributed due to its annulated structure. Comparing the cytotoxicity of the thiazine derivatives **15a**–**c**, it is obvious that the cytotoxicity of **15b** the highest cytotoxicity among the three compounds. The presence of the chlorine (Cl) and the thiophene groups were responsible for its high potency.

On the other hand, for the tetrahydropyrimidine derivatives

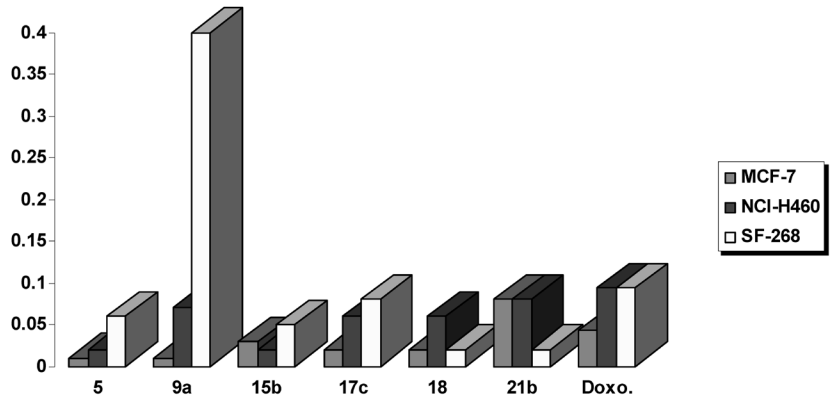

Fig. 1. The Optimal Cytotoxic Effect of the Six Newly Synthesized Compounds against the Three Cancer Cell Lines

Table 1. Cytotoxic Evaluations of the Newly Synthesized Compounds in GI_{50} (μM) on the Growth of Three Human Tumor Cell Lines and Normal Human Cell Line

Compound	GI_{50} (μM)[a]			
	MCF-7	NCI-H460	SF-268	WI-38
2-Amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene	11.20±2.23	16.29±2.26	8.01±2.39	>100
1	30.8±7.21	22.8±3.70	12.31±1.89	>100
2	16.29±4.06	20.81±8.29	18.29±6.39	>100
3	30.02±3.38	28.19±8.24	18.21±6.29	58.70±10.20
5	0.01±0.001	0.02±0.004	0.06±0.002	>100
7	30.0±3.22	20.81±4.21	21.30±2.82	>100
8	28.22±6.15	44.19±4.67	16.20±2.93	>100
9a	0.01±0.003	0.07±0.008	0.40±0.09	>100
9b	22.8±6.18	21.05±5.02	20.39±2.29	>100
10	18.29±3.61	14.11±3.36	10.18±3.53	>100
11	12.80±2.39	18.02±4.11	30.30±4.12	>100
12	28.75±6.16	24.58±16.07	18.41±4.22	>100
13	0.60±0.01	0.4±0.02	0.02±0.006	>100
15a	2.84±2.04	3.62±1.04	6.08±2.16	>100
15b	0.03±0.002	0.02±0.003	0.05±0.002	>100
15c	1.29±0.04	5.29±1.84	3.14±1.06	>100
17a	36.20±6.24	26.19±6.22	22.40±3.21	>100
17b	0.91±0.29	0.80±0.41	0.38±0.06	>100
17c	0.02±0.004	0.06±0.003	0.08±0.005	>100
18	0.02±0.005	0.06±0.004	0.02±0.003	>100
20a	2.84±0.14	4.62±1.04	6.08±1.16	8.21±1.61
20b	31.41±2.83	20.80±4.33	18.21±3.70	28.2±6.2
20c	0.60±0.07	0.80±0.14	0.018±0.002	>100
21a	18.31±8.22	32.21±6.30	10.39±4.23	>100
21b	0.08±0.002	0.08±0.003	0.02±0.002	>100
21c	18.61±6.22	20.22±10.11	17.21±4.51	>100
*Doxorubicin	0.0428±0.0082	0.0940±0.0087	0.0940±0.0070	>100

a) Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h; data are expressed as means±S.E.M. of three independent experiments performed in duplicates; *Doxorubicin was used as positive control.

17a–**c**, it is clear that both of compounds **17b** and **17c** were more cytotoxic than compound **17a** this was attributed to the presence of the Cl and OCH_3 groups in both compounds, respectively. However, compound **17c** showed higher potency than **17b**. It is clear from Table 1 that compound **18** indicated high cytotoxicity and this is attributed to the presence of the pyrimidine, furan and the thione moieties. Considering compounds **21a**–**c** where compound **21b** showed higher cytotoxicity than **21a** and **21c** due to the presence of Cl moiety (Fig. 1).

It is very clear from our present finding that the newly synthesized products with the electronegative moieties such as Cl, cyano (CN) and methoxy (OCH_3) groups in the pyrimidine and thiazine derivatives show greater cytotoxic property and might play a very important role in enhancing the cytotoxic effect. It is important to mention that our previous reports showed the cytotoxicity of other unsaturated benzothiophene, pyran and other *N*-containing heterocyclic derivatives.[33–35]

Conclusion

The objective for the present study was to synthesize a series of tetrahydrobenzo[*b*]thiophene derivatives based on 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile. Study the reactivity of the latter towards different chemical reagents and establish the structure of the newly synthesized compounds based on elemental analysis and spectral data. On the other extreme, the cytotoxic activity of some of the newly synthesized compounds (twenty six compounds) was evaluated on three human cancer cell lines and normal human cell line. Moreover, the results showed that compounds **5**, **9a**, **15b**, **17c**, **18** and **21b** revealed higher effect than the reference doxorubicin when screened *in vitro* against the three human cancer cell lines tested such as MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) and normal fibroblasts human cell line (WI-38).

Experimental

General All melting points were determined on an Electro-thermal digital meting point apparatus and are uncorrected. IR Spectra (KBr discs) were recorded on a FITR plus 460 or Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR spectra were recorded with Varian Gemini-200 (200 MHZ) (Cairo University) and Jeol AS 500 MHz (National Research Center) instruments in DMSO-d_6 as solvent using tetramethylsilane (TMS) as internal standard and chemical shifts are expressed as δ ppm. The mass spectra were recorded with Hewlett Packard 5988 AGC/MS system and GCMS-QP1000 Ex shimadzu instruments. Analytical data were obtained from the Micro analytical data unit at Cairo University and were performed on Vario El III Elemental CHNS analyzer.

Chemistry

Preparation of the 2-Amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene[16] To a solution of cyclohexanone (9.30 g, 0.10 mol) in ethanol (50 mL) containing triethylamine (1.50 mL) both of elemental sulfur (3.20 g, 0.10 mol) and malononitrile (6.60 g, 0.10 mol) were added. The whole reaction mixture was heated under reflux for 30 min. then left to cool. The formed crystals were collected by filtration.

Preparation of Ethyl *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo-[*b*]thiophen-2-yl)formimidate (1) To a solution of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (1.78 g, 0.01 mol) in acetic acid (20 mL), triethyl orthoformate (1.48 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h, then cooled and neutralized by pouring onto ice/water mixture. The solid product formed was collected by filtration and crystallized from acetic acid.

Brown crystals, mp 102–105°C, IR (KBr) cm^{-1}: 2995, 2928, 2214, 1621, 1435, 1554. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (t, 3H, CH_3), 1.70–1.99 (m, 4H, $2CH_2$), 2.13–2.99 (m, 4H, $2CH_2$), 4.10 (q, 2H, CH_2), 6.96 (s, 1H, CH). ^{13}C-NMR (DMSO-d_6) δ: 162.7, 158.9, 146.6, 145.0, 114.2, 101.0, 62.0, 24.8, 24.0, 23.4, 21.7, 21.6. MS *m*/*z* (%): 236 [M^++2] (0.36), 235 [M^++1] (0.12), 234 [M^+] (0.11), 233 [M^+−1] (0.07), 232 [M^+−2] (0.05), 150 (100.00). *Anal.* Calcd for $C_{12}H_{14}N_2OS$ (234.32): C, 61.51; H, 6.02; N, 11.96; S, 13.68. Found: C, 61.73; H, 6.30; N, 12.20; S, 13.32.

Preparation of *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-2-yl)-*N*-phenyl Formamidine (2) To a mixture of equimolar amount of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (2.34 g, 0.01 mol) in acetic acid (20 mL), aniline (0.93 g, 0.01 mol) was added. The reaction mixture were heated under reflux for 2 h, and then cooled by pouring onto ice/water mixture. The solid product formed collected by filtration and crystallized from acetic acid.

Off white crystals, mp 179–182°C, IR (KBr) cm^{-1}: 3426, 3210, 3080, 2997, 2840, 2217, 1622, 1438, 1555. ^{1}H-NMR (DMSO-d_6) δ: 1.70–1.91 (m, 4H, $2CH_2$), 2.04–2.77 (m, 4H, $2CH_2$), 6.95 (s, 1H, CH), 7.27–7.90 (m, 5H, C_6H_5), 8.36 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 162.7, 159.0, 146.6, 146.0, 145.0, 126.9, 126.9, 120.5, 116.8, 116.1, 114.2, 101.0, 24.6, 24.0, 23.7, 21.7. MS *m*/*z* (%): 282 [M^++1] (8.51), 281 [M^+] (43.35), 280 [M^+−1] (4.26), 80 (100.00), 77 [C_6H_5]$^+$ (38.30). *Anal.* Calcd for $C_{16}H_{15}N_3$ (281.38): C, 68.30, H, 5.37, N, 14.93, S, 11.40. Found: C, 67.90; H, 5.02; N, 14.69; S, 11.13.

Preparation of 2-(2,2-Dicyanoethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (3) This compound was prepared according to the same procedure as that applied for **2** using malononitrile (0.66 g, 0.01 mol).

Brown crystals, mp 152–155°C, IR (KBr) cm^{-1}: 2996, 2841, 2260, 2212, 2195, 1623, 1436, 1555. ^{1}H-NMR (DMSO-d_6) δ: 1.70–1.91 (m, 4H, $2CH_2$), 2.17–2.57 (m, 4H, $2CH_2$), 6.80, 6.95 (2s, 2H, 2CH). ^{13}C-NMR (DMSO-d_6) δ: 162.7, 158.1, 146.6, 144.1, 116.1, 114.2, 114.2, 101.1, 24.4, 23.9, 23.4, 22.2, 21.7. MS *m*/*z* (%): 256 [M^++2] (1.13), 255 [M^++1] (1.23), 254 [M^+] (0.30), 253 [M^+−1] (0.34), 252 [M^+−2] (0.38), 69 (100.00). *Anal.* Calcd for $C_{13}H_{10}N_4S$ (254.31): C, 61.40; H, 3.96; N, 22.03; S, 12.61. Found: C, 61.10; H, 3.69; N, 21.80; S, 9.64.

Preparation of (2*Z*)-2-(4-Methyl-3-phenylthiazole-2(3*H*)-ylideneamino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (5) Equimolar amount of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (1.78 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL), α-chloroacetone (0.92 g, 0.01 mol) and phenyl isothiocyanates (1.35 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 3 h and cooled by pouring onto acidified ice/water mixture the solid product formed in each case was collected by filtration, washed with water and crystallized from absolute ethanol.

Off white crystals, mp 142–145°C, IR (KBr) cm^{-1}: 3100, 2954, 2836, 2195, 1521, 1619, 1431. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (s, 3H, CH_3), 1.69–1.90 (m, 4H, $2CH_2$), 2.32–2.51 (m, 4H, $2CH_2$), 6.94 (s, 1H, thiazole C5), 6.94–7.80 (m, 5H, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 162.7, 158.1, 149.1, 144.0,

141.3, 141.1, 120.7, 120.7, 116.8, 116.1, 116.1, 115.0, 101.1, 83.2, 24.5, 24.0, 23.4, 21.8, 18.5. MS *m/z* (%): 353 [M+2] (2.16), 352 [M^++1] (0.43), 351 [M^+] (32.03), 55 (100.00). *Anal.* Calcd for $C_{19}H_{17}N_3S_2$ (351.49): C, 64.92; H, 4.87; N, 11.95; S, 18.25. Found: C, 64.58; H, 4.98; N, 11.63; S, 17.95.

Preparation of 1-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-yl)-3-phenylthiourea (6) Equimolar amount of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (1.78 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL) and phenyl isothiocyanate (1.35 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h and cooled by pouring onto acidified ice/water mixture the solid product formed in each case was collected by filtration, washed with water and crystallized from absolute ethanol.

Brown crystals, mp 97–100°C, IR (KBr) cm^{-1}: 3445, 3204, 3022, 2932–2835, 2195, 1617, 1499, 1332, 1281. ^{1}H-NMR (DMSO-d_6) δ: 1.32–1.79 (m, 4H, $2CH_2$), 2.33–2.50 (m, 4H, $2CH_2$), 6.91 −7.57 (m, 5H, C_6H_5), 9.80, 11.08 (2s, 2H, 2NH). ^{13}C-NMR (DMSO-d_6) δ: 178.0, 154.3, 141.1, 138.5, 132.0, 128.1, 128.1, 126.3, 126.3, 124.4, 114.5, 83.3, 24.3, 24.0, 23.4, 21.8. MS *m/z* (%): 315 [M^++2] (10.75), 314 [M^++ 1] (24.45), 313 [M^+] (92.59), 312 [M^+−1] (45.27), 311 [M^+−2] (2.02), 77 $[C_6H_5]^+$ (100.00). *Anal.* Calcd for $C_{16}H_{15}N_3S_2$ (313.44): C, 61.31; H, 4.82; N, 13.41; S, 20.46. Found: C, 61.28; H, 5.07; N, 13.06; S, 20.18.

Preparation of (2*E*)-2-(4-Hydroxy-3-phenylthiazol-2(3*H*)-ylideneamino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (7) Equimolar amount of **6** (3.13 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethyleamine (0.50 mL), ethyl chloroacetate (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. The formed solid products, in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid were collected by filtration and crystallized from absolute ethanol.

Pale brown crystals, mp 127–130°C, IR (KBr) cm^{-1}: 3442, 3203, 3019, 2931, 2839, 2192, 1618, 1439, 1521. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.80 (m, 4H, $2CH_2$), 2.33–2.50 (m, 4H, $2CH_2$), 4.50 (s, 1H, thiazole C5), 6.92–7.70 (m, 5H, C_6H_5), 11.10 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ: 180.0, 162.7, 155.1, 145.0, 141.0, 131.1, 129.6, 129.6, 118.1, 116.8, 116.1, 115.0, 101.0, 60.1, 24.4, 24.0, 23.4, 21.8. MS *m/z* (%): 354 [M^++1] (29.32), 353 [M^+] (23.56), 352 [M^+−1] (41.36), 351 [M^+−2] (2.62), 127 (100.00). *Anal.* Calcd for $C_{18}H_{15}N_3OS_2$ (353.46): C, 61.16; H, 4.28; N, 11.89; S, 18.14. Found: C, 61.46; H, 4.63; N, 11.54; S, 17.89.

Preparation of 2-((4-(4-Chlorophenyl)-3-phenylthiazol-2(3*H*)-ylidene)amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (8) Prepared according to the method applied for **7**, using *p*-chlorobromo acetate (2.33 g, 0.01 mol).

Pale brown crystals, mp 97–100°C, IR (KBr) cm^{-1}: 3050, 2931–2840, 2194, 1620, 1436, 1518. ^{1}H-NMR (DMSO-d_6) δ: 1.70–1.80 (m, 4H, $2CH_2$), 2.33–2.50 (m, 4H, $2CH_2$), 4.76 (s, 1H, thiazole C3), 6.93–8.03 (m, 9H, C_6H_4, C_6H_5). MS *m/z* (%): 448 [M^+] (0.46), 447 [M^+−1] (3.60), 76 $[C_6H_4]^+$ (10.11), 150 (100.00). *Anal.* Calcd for $C_{24}H_{18}N_3S_2Cl$ (448.00): C, 64.34; H, 4.05; N, 9.38; S, 14.31. Found: C, 64.43; H, 4.34; N, 9.60; S, 14.61.

General Procedure for Preparation of 2-(6-Amino-3,4-dihydro-3-phenyl-2-thioxopyrimidin-1(2*H*)-yl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (9a and b) Derivatives To a mixture of equimolar amounts of **6** (3.13 g, 0.01 mol), in absolute ethanol (25 mL) containing triethylamine (0.50 mL), malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto a beaker containing acidified ice/water mixture. The formed solid products were collected by filtration and crystallized from absolute ethanol.

2-(6-Amino-3,4-dihydro-4-amino-3-phenyl-2-thioxo-pyrimidin-1(2*H*)-yl)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (**9a**)

Off white crystals; mp 97–100°C, IR (KBr) cm^{-1}: 3438, 3209, 3100, 2934, 2836, 2195, 1619, 1435, 1334, 1282. ^{1}H-NMR (DMSO-d_6) δ: 1.50–1.71 (m, 4H, $2CH_2$), 2.33–2.51 (m, 4H, $2CH_2$), 6.93 (s, 1H, pyrimidine C5), 7.20–7.70 (m, 5H, C_6H_5), 8.80 (s, 2H, NH_2), 11.10 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 180.2, 164.0, 162.8, 141.0, 135.2, 134.1, 131.1, 129.1, 129.1, 126.5, 126.5, 124.2, 116.1, 83.3, 67.0, 24.5, 24.0, 23.4, 21.8. MS *m/z* (%): 380 [M^++1] (18.87), 379 [M^+] (21.85), 378 [M^+−1] (20.53), 77 $[C_6H_4]^+$ (100.00). *Anal.* Calcd for $C_{19}H_{17}N_5S_2$ (379.50): C, 60.13; H, 4.52; N, 18.45; S, 16.90. Found: C, 59.89; H, 4.31; N, 18.31; S, 16.54.

2-(6-Amino-3,4-dihydro-4-oxo-3-phenyl-2-thioxopyrimidin-1(2*H*)-yl)-4,5,6,7-tetrahydro-benzo[*b*]-thiophene-3-carbonitrile (**9b**)

Off white crystals, mp 127–130°C, IR (KBr) cm^{-1}: 3429, 3333, 3100, 2938–2835, 2196, 1660, 1620, 1435, 1334, 1282. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.70 (m, 4H, $2CH_2$), 2.32–251 (m, 4H, $2CH_2$), 6.95 (s, 1H, pyrimidine C5), 7.18–7.60 (m, 5H, C_6H_5), 8.20 (s, 2H, NH_2). *Anal.* Calcd for $C_{19}H_{16}N_4OS_2$ (380.49): C, 59.98; H, 4.24; N, 14.73; S, 16.85. Found: C, 59.58; H, 3.97; N, 14.35; S, 16.45.

Preparation of 4,5,6,7-Tetrahydro-2-(3,4-dihydro-6-methyl-4-oxo-3-phenyl-2-thioxopyrimidin-1-(6*H*)-yl)-benzo[*b*]thiophene-3-carbonitrile (10) To a mixture of equimolar amounts of **6** (3.13 g. 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.50 mL), ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto acidified ice/water mixture. The solid products were collected by filtration and crystallized from absolute ethanol.

Pale brown crystals, mp 267–270°C, IR (KBr) cm^{-1}: 3219, 2935, 2836, 2196, 1617, 1555, 1438, 1329, 1280. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (s, 3H, CH_3), 1.70–1.80 (m, 4H, $2CH_2$), 2.40–2.79 (m, 4H, CH_2), 6.74 (s, 1H, pyrimidine C5), 6.93–7.73 (m, 5H, C_6H_5). MS *m/z* (%): 379 [M^+] (25.45), 378 [M^+−1] (19.71), 92 (100.00), 77 $[C_6H_5]^+$ (16.85). *Anal.* Calcd for $C_{20}H_{17}N_3OS_2$ (379.50): C, 63.30; H, 4.52; N, 11.07; S, 16.90. Found: C, 62.93; H, 4.34; N, 11.47; S, 17.29.

Preparation of 4,5,6,7-Tetrahydro-2-(2,3-dihydro-4-hydroxy-4,6-dimethyl-3-phenyl-2-thioxo-pyrimidine-1(2*H*-yl)benzo[*b*]thiophene-3-carbonitrile (11) To a mixture of equimolar amounts of **6** (3.13 g. 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.50 mL), acetylacetone (1.00 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto acidified ice/water mixture. The solid products were collected by filtration and crystallized from absolute ethanol.

Off white crystals, mp 147–150°C, IR (KBr) cm^{-1}: 3443, 3198, 3026, 2929, 2844, 2195, 1586, 1440, 1335, 1290.

^{1}H-NMR (DMSO-d_6) δ: 1.20 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.71–1.79 (m, 4H, $2CH_2$), 2.33–2.50 (m, 4H, $2CH_2$), 6.92 (s, 1H, pyrimidine C5), 7.32–7.57 (m, 5H, C_6H_5), 11.10 (s, 1H, OH). MS *m/z* (%): 397 [M^++2] (0.51), 396 [M^++1] (0.46), 395 [M^+] (0.68), 394 [M^+−1] (0.63), 206 (100.00), 77 [C_6H_5]$^+$ (43.79). *Anal.* Calcd for $C_{21}H_{21}N_3OS_2$ (395.54): C, 63.77; H, 5.35; N, 10.62; S, 16.21. Found: 63.48; H, 4.99; N, 10.92; S, 16.59.

Preparation of 2-(5*E*)-5-(2-Phenyldiazenyl)-3,4-dihydro-4-hydroxy-4,6-dimethyl-3-phenyl-2-thioxo-pyrimidin-1-(2*H*)-yl)-5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (12) To a cold solution (0–5°C) of **11** (3.95 g, 0.01 mol) containing sodium hydroxide (1.00 g), an equimolar amount of diazotized aniline (0.93 mL, 0.01 mol) was gradually added while stirring. The solid product formed upon cooling in an ice-path was collected by filtration, washed with water and crystallized from absolute ethanol.

Dark brown crystals, mp 92–95°C, IR (KBr) cm^{-1}: 3428, 3058, 2933, 2206, 1595, 1445, 1494, 1329, 1250. ^{1}H-NMR (DMSO-d_6) δ: 1.06 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.51–1.99 (m, 4H, $2CH_2$), 2.17–2.65 (m, 4H, $2CH_2$), 6.75–8.02 (m, 10H, $2C_6H_5$), 11.10 (s, 1H, OH). MS *m/z* (%): 500 [M^+] (0.06), 499 [M^+−1] (0.09), 498 [M^+−2] (0.07), 80 (100.00), 77 [C_6H_5]$^+$ (5.62). *Anal.* Calcd for $C_{27}H_{25}N_5OS_2$ (499.65): C, 64.90; H, 5.04; N, 14.02; S, 12.83. Found: C, 64.69; H, 4.89; N, 14.08; S, 12.89.

Preparation of 4-Imino-3-phenyl-3,4,5,6,7,8-hexa-hydrobenzo[4,5]thieno[2,3-*d*]pyrimidine-2-thiol (13) To a solution of compound **6** (3.13 g, 0.01) in 1,4-dioxane (25 mL) a catalytic amount of piperidine (0.50 mL) was added the reaction mixture was heated under reflux for 3 h and the formed solid product pouring onto acidified ice/water mixture was collected by filtration and crystallized from 1,4-dioxane.

Pale yellow crystals, mp 117–120°C, IR (KBr) cm^{-1}: 3444, 3206, 3100, 2933–2835, 1618, 1432, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.91 (m, 4H, $2CH_2$), 2.31–2.79 (m, 4H, $2CH_2$), 6.95–7.58 (m, 5H, C_6H_5), 8.60 (s, 1H, SH), 9.00 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 162.7, 161.0, 143.4, 141.0, 131.1, 129.6, 129.6, 129.0, 127.2, 118.8, 116.8, 116.8, 25.0, 24.0, 23.4, 21.8. MS *m/z* (%): 315 [M^++2] (19.16), 314 [M^++1] (8.35), 313 [M^+] (7.62), 121 (100.00). *Anal.* Calcd for $C_{16}H_{15}N_3S_2$ (313.44): C, 61.31; H, 4.82; N, 13.41; S, 20.46. Found: C, 60.93; H, 4.49; N; 13.06; S, 20.06.

General Procedure for Preparation of (2*E*)-2-(3-Cyano-4,5,6,7-tetrahydro[*b*]thiophen-2-ylimino)-6-amino-3,4-dihydro-3-phenyl-2*H*-1,3-thiazine-5-carbonitrile Derivatives (15a–c) To a solution of compound **6** (3.13 g, 0.01 mol) in absolute ethanol (25 mL), benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.12 g, 0.01 mol) or 4-methoxybenzaldehyde (1.08 g, 0.01 mol) was added with malononitrile (0.66 g, 0.01 mol) in the presence of a catalytic amount of triethylamine (0.50 mL). The reaction mixture was heated under reflux for 3 h. The solid products formed, in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid were collected by filtration and crystallized from absolute ethanol.

(2*E*)-2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylimino)-6-amino-3,4-dihydro-3,4-diphenyl-2*H*-1,3-thiazine-5-carbonitrile (**15a**)

Dark brown crystals, mp 87–90°C, IR (KBr) cm^{-1}: 3423, 3249, 3057, 2927, 2835, 2213, 2195, 1598, 1447, 1556. ^{1}H-NMR (DMSO-d_6) δ: 1.70–1.91 (m, 4H, $2CH_2$), 2.32–2.79 (m, 4H, $2CH_2$), 6.93 (s, 1H, thiazine C4), 7.07–7.98 (m, 10H, $2C_6H_5$), 8.64 (s, 2H, NH_2). MS *m/z* (%): 467 [M^+−1] (21.98), 466 [M^+−2] (14.66), 55 (100.00). *Anal.* Calcd for $C_{26}H_{21}N_5S_2$ (467.61): C, 66.78; H, 4.53; N, 14.98; S, 13.71. Found: C, 66.41; H, 4.28; N, 15.09; S, 13.88.

(2*E*)-2-(3-Cyano-4,5,6,7-tetrahydro[*b*]thiophen-2-ylimino)-6-amino-4-(4-chloro-phenyl)-3,4-dihydro-3-phenyl-2*H*-1,3-thiazine-5-carbonitrile (**15b**)

Brown crystals, mp 67–70°C, IR (KBr) cm^{-1}: 3445, 3327, 3206, 2932, 2837, 2260, 2196, 1624, 1491, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.79 (m, 4H, $2CH_2$), 2.33–2.79 (m, 4H, $2CH_2$), 6.76 (s, 1H, thiazine C4), 6.79–7.99 (m, 9H, C_6H_4, C_6H_5), 8.64 (s, 2H, NH_2). MS *m/z* (%): 502 [M^+] (6.98), 501 [M^+−1] (8.90), 500 [M^+−2] (7.09), 76 [C_6H_4]$^+$ (2.82), 64 (100.00). *Anal.* Calcd for $C_{26}H_{20}N_5S_2Cl$ (502.05): C, 62.20; H, 4.02; N, 13.95; S, 12.77. Found: C, 62.18; H, 4.09; N, 14.35; S, 12.49.

(2*E*)-2-(3-Cyano-4,5,6,7-tetrahydro[*b*]thiophen-2-ylimino)-6-amin-3,4-dihydro-4-(4-methoxyphenyl)-3-phenyl-2*H*-1,3-thiazine-5-carbonitrile (**15c**)

Yellowish brown crystals, mp 72–75°C, IR (KBr) cm^{-1}: 3429, 3332, 3026, 2930, 2837, 2217, 2195, 1602, 1439, 1562. ^{1}H-NMR (DMSO-d_6) δ: 1.31 (s, 3H, CH_3), 1.70–191 (m, 4H, $2CH_2$), 2.16–2.68 (m, 4H, $2CH_2$), 6.94 (s, 1H, thiazine C4), 7.08–7.98 (m, 9H, C_6H_4, C_6H_5), 8.54 (s, 2H, NH_2). MS *m/z* (%): 497 [M^+−1] (13.17), 496 [M^+−2] (2.83), 268 (100.00), 76 [C_6H_4]$^+$ (7.00). *Anal.* Calcd for $C_{27}H_{23}N_5OS_2$ (497.63): C, 65.17; H, 4.66; N, 14.07; S, 12.89. Found: C, 65.07; H, 5.06; N, 14.39; S, 12.68.

General Procedure for Preparation of 6-Amino-3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-1,2,3,4-tetrahydro-1-phenyl-2-thioxopyrimidine-5-carbonitrile Derivatives (17a–c) Equimolar amounts of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (1.78 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL), benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.12 g, 0.01 mol) or 4-methoxybenzaldehyde (1.08 g, 0.01 mol) was added with malononitrile (0.66 g, 0.01 mol) and phenyl isothiocynate (1.35 g, 0.01 mol). The reaction mixture, in each case, was heated under reflux for 3 h, the formed solid products pouring onto ice/water mixture containing few drops of hydrochloric acid were collected by filtration and crystallized from absolute ethanol.

6-Amino-3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-1,2,3,4-tetrahydro-1,4-diphenyl-2-thioxopyrimidine-5-carbonitrile (**17a**)

Yellow crystals, mp over 300°C, IR (KBr) cm^{-1}: 3421, 3251, 3067, 2926–2840, 2210, 2195, 1603, 1441, 1328, 1249. ^{1}H-NMR (DMSO-d_6) δ: 1.80–1.81 (m, 4H, $2CH_2$), 2.50–2.72 (m, 4H, $2CH_2$), 7.52 (s, 1H, pyrimidine C6), 7.53–7.99 (m, 10H, $2C_6H_5$), 8.66 (s, 2H, NH_2). MS *m/z* (%): 467 [M^+−1] (25.83), 466 [M^+−2] (17.22), 124 (100.00), 77 [C_6H_5]$^+$ (31.79). *Anal.* Calcd for $C_{26}H_{21}N_5S_2$ (467.61): C, 66.78; H, 4.53; N, 14.98; S, 13.71. Found: C, 66.38; H, 4.19; N, 15.07; S, 13.46.

6-Amino-4(4-chlorophenyl)-3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-1,2,3,4-tetrahydro-1-phenyl-2-thioxopyrimidine-5-carbonitrile (**17b**)

Pale brown crystals, mp 82–85°C, IR (KBr) cm^{-1}: 3444, 3326, 3027, 2931, 2843, 2220, 2195, 1619, 1440, 1335, 1289. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.80 (m, 4H, $2CH_2$), 2.32–2.72

(m, 4H, $2CH_2$), 6.90 (s, 1H, pyrimidine C6), 7.34–8.00 (m, 9H, C_6H_4, C_6H_5), 8.55 (s, 2H, NH_2). MS *m/z* (%): 502 [M^+] (10.01), 501 [M^+-1] (13.45), 77 [C_6H_5]$^+$ (36.48), 69 (100.00). *Anal.* Calcd for $C_{26}H_{20}N_5S_2Cl$ (502.05): C, 62.20; H, 4.02; N, 13.95; S, 12.77. Found: C, 61.95; H, 4.11; N, 13.79; S, 12.63.

6-Amino-3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-1-phenyl-2-thioxopyrimidine-5-carbonitrile (**17c**)

Reddish brown crystals, mp 67–70°CC, IR (KBr) cm^{-1}: 3415, 3181, 3063, 3021, 2936, 2843, 2216, 2146, 1599, 1445, 1318, 1271. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (s, 3H, CH_3), 1.79–1.80 (m, 4H, $2CH_2$), 2.50–2.68 (m, 4H, $2CH_2$), 7.09 (s, 1H, pyrimidine C6), 7.11–7.99 (m, 9H, C_6H_4, C_6H_5), 8.53 (s, 2H, NH_2). MS *m/z* (%): 498 [M^+] (7.95), 497 [M^+-1] (10.18), 496 [M^+-2] (7.95), 63 (100.00). *Anal.* Calcd for $C_{27}H_{23}N_5OS_2$ (497.63): C, 65.17; H, 4.66; N, 14.07; S, 12.89. Found: C, 65.28; H, 4.29; N, 13.79; S, 12.59.

Preparation of 6-Amino-3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(furan-3-yl)-1,2,3,4-tetrahydro-1-phenyl-2-thioxopyrimidine-5-carbonitrile (18) To a solution of compound 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (1.78 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL), malononitrile (0.66 g, 0.01 mol), phenyl isothiocynate (1.35 g, 0.01 mol) and furfural (0.96 g, 0.01 mol) were added the reaction mixture was heated under reflux for 2 h. The solid products formed upon pouring onto acidified ice/water mixture were collected by filtrations and crystallized from absolute ethanol.

Dark brown crystals, mp 132–135°CC, IR (KBr) cm^{-1}: 3426, 3300, 3113, 2936, 2861, 2218, 2195, 1608, 1486, 1333, 1287. ^{1}H-NMR (DMSO-d_6) δ: 1.78–19.1 (m, 4H, $2CH_2$), 2.50–2.68 (m, 4H, $2CH_2$), 6.76 (s, 1H, pyrimidine C6), 6.77–8.06 (m, 8H, C_6H_5, furan C), 8.40 (s, 2H, NH_2). MS *m/z* (%): 458 [M^+] (16.14), 457 [M^+-1] (18.92), 64 (100.00). *Anal.* Calcd for $C_{24}H_{19}N_5OS_2$ (457.57): C, 63.00; H, 4.19; N, 15.31; S, 14.02. Found: C, 62.72; H, 3.89; N, 15.29; S, 13.79.

Preparation of 2-Cyano-*N*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)acetamide (19) The data for compound **19** has been published earlier.[18)]

General Procedure for Preparation of 2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-4*H*-pyran-3,5-dicarbonitrile Derivatives (20a–c) Equimolar amount of **19** (2.45 g, 0.01 mol) in absolute ethanol (25 mL), containing a catalytic amount of triethylamine (0.50 mL), benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.12 g, 0.01 mol) or 4-methoxybenzaldehyde (1.08 g, 0.01 mol) was added with malononitrile (0.66 g, 0.01 mol). The reaction mixture, in each case, was heated under reflux for 3 h. The solid products formed, in each case, upon pouring onto acidified ice/water mixture were collected by filtration and crystallized from absolute ethanol.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-4-phenyl-4*H*-pyran-3,5-dicarbonitrile (**20a**)

Brown crystals, mp over 300°C, IR (KBr) cm^{-1}: 3462, 3225, 3100, 2933, 2861, 2220, 2210, 2195, 1628, 1450. ^{1}H-NMR (DMSO-d_6) δ: 1.82–1.91 (m, 4H, $2CH_2$), 2.50–2.96 (m, 4H, $2CH_2$), 6.90 (s, 1H, pyran C4), 7.58–7.72 (m, 5H, C_6H_5), 8.10 (s, 2H, NH_2), 9.21 (s, 1H, NH). MS *m/z* (%): 400 [M^++1] (55.74), 399 [M^+] (72.95), 398 [M^+-1] (15.57), 397 [M^+-2] (58.20), 225 (100.00), 77 [C_6H_5]$^+$ (60.66). *Anal.* Calcd for $C_{22}H_{17}N_5OS$ (399.47): C, 66.15; H, 4.29; N, 17.53; S, 8.03. Found: C, 65.79; H, 4.62; N, 17.13; S, 7.98.

2-(3-Cyano–4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-4-(4-chlorophenyl)-4*H*-pyran-3,5-dicarbonitrile (**20b**)

Brown crystals, mp over 300°C, IR (KBr) cm^{-1}: 3462, 3225, 3100, 2936, 2861, 2260, 2214, 2195, 1598, 1482. ^{1}H-NMR (DMSO-d_6) δ: 1.82–1.91 (m, 4H, $2CH_2$), 2.50–2.96 (m, 4H, $2CH_2$), 6.90 (s, 1H, pyran C4), 7.59–7.75 (m, 4H, C_6H_4), 8.10 (s, 2H, NH_2), 9.24 (s, 1H, NH). MS *m/z* (%): 433 [M^+-1] (21.39), 432 [M^+-2] (16.76), 118 (100.00), 76 [C_6H_4]$^+$ (10.12). *Anal.* Calcd for $C_{22}H_{16}N_5OSCl$ (433.91): C, 60.90; H, 3.72; N, 16.14; S, 7.39. Found: C, 60.58; H, 4.09; N, 16.20; S, 7.39.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl-amino)-6-amino-4-(4-methoxy-phenyl)-4*H*-pyran-3,5-dicarbonitrile (**20c**)

Pale brown crystals, mp over 300°C, IR (KBr) cm^{-1}: 3465, 3220, 3065, 2936, 2836, 2220, 2210, 2195, 1611, 1440. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (s, 3H, CH_3), 1.60–1.82 (m, 4H, $2CH_2$), 2.50–2.96 (m, 4H, $2CH_2$), 6.90 (s, 1H, pyran C4), 7.12–7.69 (m, 4H, C_6H_4), 8.10 (s, 2H, NH_2), 9.18 (s, 1H, NH). MS *m/z* (%): 429 [M^+] (86.41), 428 [M^+-1] (53.40), 87(100.00). *Anal.* Calcd for $C_{23}H_{19}N_5O_2S$ (429.49): C, 64.32; H, 4.46; N, 16.31; S, 7.47. Found: C, 63.93; H, 4.06; N, 15.93; S, 7.09.

General Procedure for Preparation of 2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-1,4-dihydro-pyridine-3,5-dicarbonitrile Derivatives (21a–c) Equimolar amount of **19** (2.45 g, 0.01 mol) in absolute ethanol (25 mL), containing a catalytic amount of ammonium acetate, benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.12 g, 0.01 mol) or 4-methoxybenzaldehyde (1.08 g, 0.01 mol) was added with malononitrile (0.66 g, 0.01 mol). The reaction mixture, in each case, was heated under reflux for 3 h. The solid products formed, in each case, upon pouring onto ice/water mixture were collected by filtration and crystallized from absolute ethanol.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-1,4-dihydro-4-phenylpyridine-3,5-dicarbonitrile (**21a**)

Yellowish brown crystals, mp over 300°C, IR (KBr) cm^{-1}: 3445, 3326, 3205, 3032, 2933, 2836, 2240, 2223, 2195, 1620, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.79 (m, 4H, $2CH_2$), 2.32–2.66 (m, 4H, $2CH_2$), 6.95 (s, 1H, pyridine C4), 7.09–7.99 (m, 5H, C_6H_5), 8.10 (s, 2H, NH_2), 8.39, 8.54 (2s, 2H, 2NH). MS *m/z* (%): 399 [M^++1] (13.08), 398 [M^+] (11.32), 396 [M^+-2] (6.86), 177 (100.00), 77 [C_6H_5]$^+$ (24.56). *Anal.* Calcd for $C_{22}H_{18}N_6S$ (398.48): C, 66.31; H, 4.55; N, 21.09; S, 8.05. Found: C, 66.66; H, 3.99; N, 21.48; S, 8.45.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino,4-(4-chloro-phenyl)-1,4-dihydro-pyridine-3,5-dicarbonitrile (**21b**)

Pale brown crystals, mp 97–100°C, IR (KBr) cm^{-1}: 3444, 3327, 3205, 3033, 2932, 2836, 2240, 2225, 2196, 1622, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.80 (m, 4H, $2CH_2$), 2.33–2.72 (m, 4H, $2CH_2$), 6.95 (s, 1H, pyridine C4), 7.27–7.98 (m, 4H, C_6H_5), 8.01 (s, 2H, NH_2), 8.56, 8.67 (2s, 2H, 2NH). MS *m/z* (%): 433 [M^+] (2.09), 432 [M^+-1] (2.86), 431 [M^+-2] (2.44), 153 (100.00), 76 [C_6H_4]$^+$ (34.93). *Anal.* Calcd for $C_{22}H_{17}N_6SCl$ (432.93): C, 61.03; H, 3.96; N, 19.41; S, 7.41. Found: C, 60.66; H, 4.36; N, 19.02; S, 7.81.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)-6-amino-1,4-dihydro-4-(4-methoxyphenyl)pyridine-3,5-dicarbonitrile (**21c**)

Yellow crystals, mp 72–75°C, IR (KBr) cm^{-1}: 3444, 3326,

3206, 3026, 2931, 2840, 2260, 2217, 2193, 1610, 1515. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (s, 3H, CH_3), 1.69–1.79 (m, 4H, $2CH_2$), 2.32–2.68 (m, 4H, $2CH_2$), 6.95 (s, 1H, pyridine C4), 7.09–7.99 (m, 4H, C_6H_4), 8.30 (s, 2H, NH_2), 8.39, 8.54 (2s, 2H, 2NH). MS *m*/*z* (%): 429 [M^++1] (3.33), 428 [M^+] (3.72), 107 (100.00). *Anal*. Calcd for $C_{23}H_{20}N_6OS$ (428.51): C, 64.47; H, 4.70; N, 19.61; S, 7.48. Found: C, 64.17; H, 4.38; N, 19.32; S, 7.84.

Biology

Materials and Methods Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, U.K.). RPMI-1640 medium was provided from Cambrex (NJ, U.S.A.). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were obtained from Sigma Chemical Company. (Saint Louis, MO, U.S.A.).

Samples Stock solutions of the newly compounds were prepared in DMSO and kept at −20°C. Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

Cell Cultures Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, U.K.) and NCI-H460 and SF-268 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 μg/mL), at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, 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.

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Conflict of Interest The authors declare no conflict of interest.

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