SYNTHESIS AND CYTOTOXIC ACTIVITY OF NOVEL TETRAHYDROBENZO[*b*]THIOPHENE-DERIVED HETEROCYCLES

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Series of thiophene, thiazole, and pyrimidine derivatives based on the ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate starting material were synthesized, and cytotoxic activity of synthesized compounds was evaluated. Results showed that three compounds, *viz.* (2)-2-(4-methyl-3-phenylthiazole-2(3H)-ylidenea-mino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile, ethyl 2-(6-amino-4-imino-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate, and 4-imino-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5]-thieno[2,3-d]pyrimidine-2-thiol, were the most active towards MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer), but they were not active towards normal fibroblasts human cell line (WI-38). The toxicity of selected compounds against shrimp larvae was also studied.

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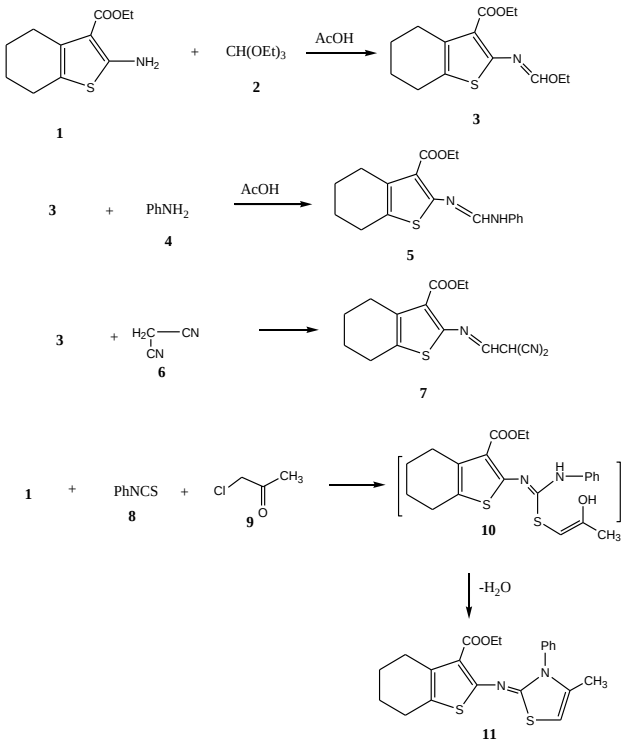
Introduction

Organic compounds containing aromatic heterocyclic rings, like thiophene, are widely distributed in nature and play an important role in various biochemical processes. Therefore, they are often incorporated into new chemical entities by medicinal chemists.[1-3] Numerous thiophene derivatives of important therapeutic compounds containing benzene ring[10,11] were prepared for obtaining better biological activity than the parent compound. For example, a lot of compounds were found to have nematocidal,[4] insecticidal,[5] antibacterial,[6] antifungal,[7] antiviral,[8] and antioxidant activity.[9] Based on this, the aim in this work was to synthesize novel heterocyclic compounds from ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1**) and to evaluate their antitumor activity.

Results and discussion

Synthesis of investigated compounds is summarized in Schemes 1-3. The reaction of the ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (**1**) with ethyl-orthoformate in acetic acid gave the ethyl 2-((ethoxy-methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (**3**). Compound **3** reacted with aniline to give the ethyl 2-((phenylamino)methylene)amino-4,5,6,7-tetra-hydrobenzo[*b*]thiophene-3-carboxylate derivative **5**. The elemental and spectral data of compound **5** were consistent with its proposed structure. Namely, ^{1}H-NMR spectrum of compound **5** showed the presence of a triplet at δ 1.12 ppm corresponding to the CH_3 group, a multiplet at δ 1.66-1.95 ppm corresponding to four CH_2 groups, a quartet at δ 4.22 ppm indicating the ethyl CH_2 group, a singlet at δ 6.65 ppm for CH group, a multiplet at δ 7.30-7.46 ppm corresponding to the C_6H_5 group, and a singlet at δ 8.32 ppm for NH group. On the other hand, the reaction of compound **3** with malononitrile (**6**) gave the iminomethyl malononitrile derivative **7**. The analytical and spectral data of compound **7** were in agreement with its structure.

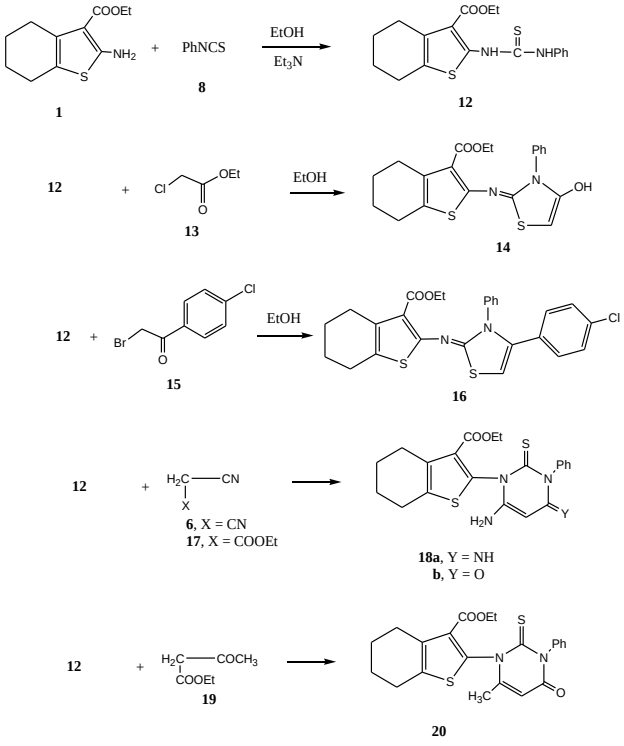
The reaction of compound **1** with phenylisothiocyanate (**8**) and chloroacetone (**9**) gave the thiazole derivative **11**. Formation of the latter product took place through the intermediate formation of **10** followed by water elimination.



Scheme 1. Synthesis of compounds **3**, **5**, **7** and **11**

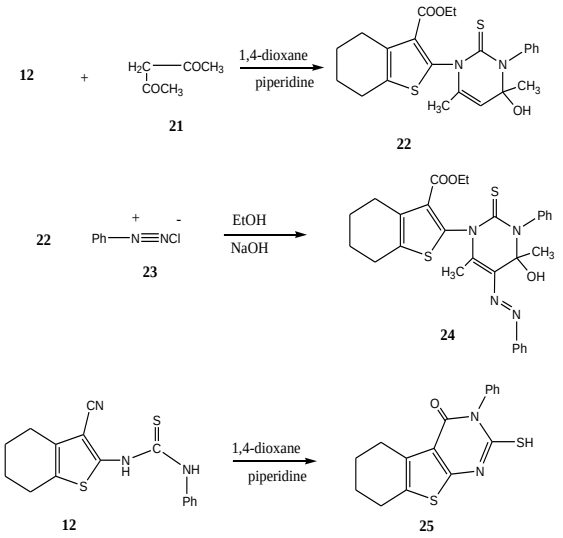
Next we studied the possibility of compound **1** to form thiourea derivative. Therefore, compound **1** was reacted with phenylisothiocyanate (**8**) in ethanol solution to give the *N*-phenylthioura derivative **12**. The latter reacted with ethyl chloroacetate (**13**) in ethanol to give the thiazole derivative **14**. The structure of compound **14** was confirmed on the basis of analytical and spectral data. Thus the ^{1}H-NMR showed a triplet at δ 1.16 ppm for CH_3 group, a multiplet at δ 1.48-1.73 ppm indicating four $4CH_2$, a quartet at δ 4.23 ppm for ethyl CH_2 group, a singlet at δ 6.14 ppm corresponding to H-2 thiazole, a multiplet at δ 7.25–7.38 ppm for C_6H_5 group, and a singlet at δ 10.31 ppm for OH. Similarly, the reaction of compound **12** with α-bromo-4-chloroacetophenone (**15**) gave the thiazole derivative **16**.

Compound **12** was also used to synthesize pyrimidine substituted derivatives with potential biological activities. Therefore, the reaction of compound **12** with malononitrile (**6**) or ethyl cyanoacetate (**17**) gave the 4,6-diamino-pyrimidine derivatives **18a** and **18b**, respectively. On the other hand the reaction of compound **12** with ethyl acetoacetate (**19**) gave the 4-methyl-6-oxopyrimidine derivative **20**. The reaction took place through ethanol and water elimination. The analytical and spectral data of compounds **18a,b** and **20** were consistent with their respective structures.



Scheme 2. Synthesis of compounds **12**, **14**, **16**, **18a,b** and **20**

The reaction of compound **12** with acetylacetone (**21**), however, gave 4,6-dimethyl-6-hydroxypyrimidine derivative **22**. The latter compound, due to the presence of the acidic pyrimidine H-5, was coupled with benzenediazonium chloride to form the phenylazo derivative **24**. Compound **12** underwent ready cyclization in 1,4-dioxane and piperidine to give the hexahydrobenzo[4,5]-thieno[2,3-*d*]pyrimidine derivative **25**. The ^{1}H-NMR spectrum of compound **25** showed a multiplet at δ 1.60-1.94 ppm corresponding to four CH_2 groups, a multiplet at δ 7.21-7.38 ppm for C_6H_5 group and a singlet at δ 10.62 ppm for SH group.



Scheme 3. Synthesis of compounds **22**, **24** and **25**

In vitro cytotoxicity evaluation of synthesized compounds.

The newly synthesized compounds were evaluated towards three cancer cell lines, namely [MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (CNS cancer)] and normal fibroblasts cells (WI38), and results obtained were presented in Table 1. The data obtained showed that some of the compounds proved to be promising candidate for antitumor applications.

The results were compared to the anti-proliferative effects of the reference control doxorubicin. All the compounds were dissolved in DMSO at 1.0 mg mL^{-1} concentration immediately before using and diluted just before addition to the cell culture. The data (Table 1) represents means ± SEM of three independent experiments performed in duplicate. The results indicated that most compounds showed substantial growth inhibitory effects against the human tumor cells at the concentrations tested.

Structure activity relationship

It is clear from Table 1 that compound **1** and **3** showed low potency against the three cancer cell lines investigated. The reaction of compound **3** with aniline gave compound **5** which showed high cytotoxicity against the three cancer cell lines. Compound **7** resulted from the reaction of compound **5** with malononitrile showed very low potency. The thiazole derivative **11** showed high potency and its cytotoxicity was higher than that of the thiazole derivatives **14** and **16**. Considering the pyrimidine derivatives **18a** and **18b** it is clear that compound **18a** with the C=NH moiety showed higher potency than **18b** with the C=O moiety. For the pyrimidine derivatives **22**-**25**, it is obvious that the hexahydrobenzo[4,5]-thieno[2,3-*d*]pyrimidine derivative **25** with the SH moiety showed the highest cytotoxicity among these compounds.

Table 1. Effect of synthesized compounds in GI_{50} (μM) on the growth of three human tumor cell lines and normal human cell line

Compound No..	GI_{50} (μM)[a]			
	MCF-7	NCI-H460	SF-268	WI-38
1	18.20 ± 2.23	14.29 ± 2.26	8.01 ± 2.39	>100
3	22.8 ± 7.21	20.8 ± 3.70	18.31 ± 1.89	> 100
5	8.29 ± 4.06	6.81 ± 2.29	15.29 ± 6.39	>100
7	72.02 ± 3.38	70.19 ± 8.24	68.21 ± 6.29	68.70± 10.2
11	0.01 ± 0.001	0.02 ± 0.006	0.06 ± 0.004	> 100
12	34.52 ± 2.24	28.67 ± 2.68	18.38 ± 8.65	>100
14	38.0 ± 3.92	30.81 ± 4.61	41.30 ± 2.92	>100
16	21.28 ± 6.18	20.19 ± 4.67	17.20 ± 4.63	>100
18a	0.01 ± 0.003	0.06 ± 0.008	0.03 ± 0.009	>100
18b	28.4 ± 6.18	26.05 ± 5.02	24.37 ± 2.49	>100
20	18.29 ± 3.51	14.11 ± 3.36	10.18 ± 3.53	>100
22	10.80 ± 2.39	12.02 ± 4.11	14.30 ± 2.12	>100
24	11.75 ± 1.16	14.58 ± 2.07	19.40 ± 24.22	>100
25	0.86± 0.01	0.53 ± 0.02	0.08 ± 0.006	>100
Doxorubicin	0.04 ±0.008	0.09 ± 0.008	0.09 ± 0.007	>100

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

Table 2. Toxicity of the most potent compounds against the cancer cell lines

Compound No.	Concentration, μg mL^{-1}	Mortality[a]	Toxicity	LC_{50}	Upper 95% lim.	Lower 95% lim.
5	10	6	Very toxic	16.06	-	-
	100	5				
	1000	10				
11	10	3	Very toxic	16.30	-	-
	100	5				
	1000	10				
18a	10	0	Non toxic	977.18	-	-
	100	0				
	1000	3				
22	10	0	Harmful	512.30	114.29	80.32
	100	7				
	1000	10				
24	10	0	Non toxic	904.29	-	-
	100	1				
	1000	4				
25	10	0	Non toxic	922.30	-	-
	100	0				
	1000	3				

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

Toxicity

Bioactive compounds are often toxic to shrimp larvae. Therefore, in order to monitor these chemicals, *in vivo* lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay[12] was used. Results were analyzed with LC_{50} program to determine LC_{50}values and 95 % confidence intervals.[13] Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines; the six compounds are **5**, **11, 18a, 22**, **24** and **25.** 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,[14] natural and synthetic organic compounds.[12] 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.[15]

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 2 that compounds **18a, 24**, and **25** showed no toxicity against the tested organisms. On the other hand, compound **5** and **11** were very toxic, and in addition, compounds **22** is harmful.

Experimentals

All melting points were determined using an Electro thermal digital melting point apparatus and are uncorrected. IR spectra (KBr disks) were recorded on a FTIR plus 460 or Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Varian Gemini-200 (200 MHz) (Cairo University) and Jeol AS 500 MHz (National Research center) instruments in DMSO-d6 as solvent using TMS as internal standard. Chemical shifts are expressed as δ ppm. The mass spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 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.

Ethyl 2-((ethoxymethylene)amino)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate (3).

To a solution of **1** (2.25 g, 0.01 mol) in acetic acid (20 mL), ethylorthoformate (1.06 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.

Compound **3:** Yellow crystals, yield: 80 % (2.20 g); m.p: 180-184 °C. IR (KBr): ν/cm^{-1} = 2990-2938 (CH_2, CH_3), 1689 (CO), 1622 (C=N). ^{1}H-NMR (DMSO-d_6): δ = 1.20, 1.16 (2t, 6H, $2CH_3$), 1.73-2.54 (m, 8H, $4CH_2$), 4.12, 4.22 (2q, 4H, $2CH_2$), 6.56 (s, 1H, CH). MS (relative intensity) m/z: 281 (M^+, 20 %). Analysis for $C_{14}H_{19}NO_3S$ Calcd: 59.76; H, 6.81; N; 4.98; S, 11.40. Found: C, 60.01; H, 6.98; N, 5.33; S, 11.64 %.

Ethyl 2-(((phenylamino)methylene)amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylate (5) and ethyl 2-(2,2-dicyano-ethylidene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-car-boxylate (7)

General procedure: To a mixture of equimolar amount of compound **3** (2.81 g, 0.01 mol) in acetic acid (20 mL), either aniline (0.93 g, 0.01 mol) or malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 hours, and then cooled by pouring onto ice/water mixture. The solid product formed collected by filtration and crystallized from acetic acid.

Compound **5**: Yellow crystals, yield: 65 % (2.10 g); m.p. 220-224 °C. IR (KBr): ν/ cm^{-1} = 3480-3320 (NH), 3050 (CH-aromatic), 2990, 2960 (CH_3, CH_2), 2217 (CN), 1689 (CO), 1622 (C=C). ^{1}H-NMR (DMSO-d_6): δ = 1.12 (t, 3H, CH_3), 1.66-1.95 (m, 8H, $4CH_2$), 4.22 (q, 2H, CH_2), 6.65 (s, 1H, CH), 7.30-7.46 (m, 5H, C_6H_5), 8.32 (s, 1H, NH). MS (relative intensity) m/z: 328 (M^+, 22%). Analysis for $C_{18}H_{20}N_2O_2S$ Calcd: C, 65.83; H, 6.14; N; 8.53; S, 9.75. Found: C, 65.66; H, 5.92; N, 8.72; S, 9.80 %.

Compound **7:** Brown crystals, yield: 77 % (2.30 g); m.p. 198-202 °C. IR (KBr): ν/cm^{-1} = 2988, 2971 (CH_3, CH_2), 1692 (CO). ^{1}H-NMR (DMSO-d_6): δ = 1.16 (t, 3H, CH_3), 1.65-2.68 (m, 8H, $4CH_2$), 4.25 (q, 2H, CH_2), 6.11, 6.35 (2d, 2H, 2CH). MS (relative intensity) m/z: 301(M^+, 28%). Analysis for $C_{15}H_{15}N_3O_2S$ Calcd: C, 59.78; H, 5.02; N; 13.94; S, 10.64. Found: C, 59.93; H, 4.92; N, 14.11; S, 10.80 %.

Synthesis of (2)-2-(4-methyl-3-phenylthiazole-2(3H)-ylidenea-mino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (11).

To an equimolar amount of **1** (2.25 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL), α-chloroacetone **9** (0.92 g, 0.01 mol) and phenylisothiocyanate **8** (1.35 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 3 hours 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.

Compound **11:** Yellow crystals, yield: 68 % (2.70 g); m.p. 180-184 °C. IR (KBr): ν/ cm^{-1} = 3066 (CH aromatic), 2974, 2983 (CH_3, CH_2), 1688 (CO), 1648 (exocyclic C=N), 1580 (C=C). ^{1}H-NMR (DMSO-d_6): δ = 1.13 (t, 3H, CH_3), 2.29 (s, 3H, CH_3), 1.54-2.30 (m, 8H, $4CH_2$), 4.21 (q, 2H, CH_2), 6.32 (s, 1H, thiazole), 7.27-7.38 (m, 5H, C_6H_5). MS (relative intensity) m/z: 398 (M^+, 34 %). Analysis for $C_{21}H_{22}N_2O_2S_2$ Calcd: C, 63.29, H, 5.56; N; 7.03; S, 16.09. Found: C, 63.08; H, 5.48; N, 6.88; S, 16.27 %.

Ethyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thio-phene-3-carboxylate (12).

To an equimolar amount of compound **1** (2.25 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL) phenylisothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 hours and cooled by pouring onto acidified ice/water mixture. The solid product formed was collected by filtration, washed with water, and crystallized from absolute ethanol.

Compound **12:** Orange crystals, yield: 60 % (2.30 g); m.p. 130-132 °C. IR (KBr): ν/ cm^{-1} = 3487, 3349 (2 NH), 3045 (CH-aromatic), 1690 (CO), 1580 (C=C), 1253 (C=S). ^{1}H-NMR (DMSO-d_6) : δ = 1.13 (t, 3H, CH_3), 1.54-2.65 (m, 8H, $4CH_2$), 4.22 (q, 2H, CH_2), 7.28 –7.39 (m, 5H, C_6H_5), 8.23, 8.41(2s, 2H, 2NH). MS (relative intensity) m/z: 360 (M^+, 22). Analysis for $C_{18}H_{20}N_2O_2S_2$ Calcd: C, 59.97; H, 5.59; N, 7.77; S, 17.79. Found: C, 60.12; H, 5.39; N, 7.93; S, 18.80 %.

Ethyl 2-((4-hydroxy-3-phenylthiazol-2(3H)-ylidene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (14) and ethyl 2-(4-(4-chlorophenyl)-3-phenylthiazol-2(3H)-ylidene)ami-no)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (16)

General procedure: To a solution of **12** (3.60 g, 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethyleamine (0.50 mL), either ethyl chloroacetate (1.22 g, 0.01 mol) or α-bromo-4-chloroacetophenone (2.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 hours. 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.

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Compound **14**: brown crystals, yield: 80 % (3.20 g); m.p. 190-193 ºC. IR (KBr): ν/ cm^{-1} = 3045 (CH aromatic), 2980-2873 (CH_3, CH_2), 1706 (C=O), 1618 (C=N). ^{1}H-NMR (DMSO-d_6) : δ = 1.16 (t, 3H, CH_3), 1.48-1.73 (m, 8H, $4CH_2$), 4.23 (q, 2H, CH_2), 6.14 (s, 1H, thiazole H-2), 7.25–7.38 (m, 5H, C_6H_5), 10.31 (s, 1H, OH). MS (relative intensity) m/z: 400 (M^+, 38%). Analysis for $C_{20}H_{20}N_2O_3S_2$ Calcd: C, 59.98; H, 5.03; N; 6.99; S, 16.01. Found: C, 60.21; H, 4.92; N, 7.23; S, 15.85 %.

Compound **16:** Orange crystals, yield: 69 % (3.40 g); m.p. 67-69 ºC. IR (KBr): ν/cm^{-1} = 3050 (CH aromatic), 2977-2870 (CH_3, CH_2), 1677 (CO), 1629 (exocyclic C=N), 1436 (C=C). ^{1}H-NMR (DMSO-d_6): δ=1.l6 (t, 3H, CH_3), 1.49-2.80 (m, 8H, $4CH_2$), 4.24 (q, 2H, CH_2), 6.16 (s, 1H, thiazole H-2), 7.23–7.93 (m, 9H, C_6H_4, C_6H_5). MS (relative intensity) m/z: 495 (M^+, 62%). Analysis for $C_{26}H_{23}ClN_2O_2S_2$ Calcd: C, 63. 08; H, 4.68; N, 5.66; S, 12.95. Found: C, 62.83; H, 4.92; N, 5.80; S, 13.08 %.

Ethyl 2-(6-amino-4-imino-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (18a) and ethyl 2-(6-amino-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate (18b)

General procedure: To a solution of compound **12** (3.60 g, 0.01 mol), in absolute ethanol (25 mL) containing triethylamine (0.50 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 hours then poured into a beaker containing acidified ice/water mixture. The formed solid products were collected by filtration and crystallized from absolute ethanol.

Compound **18a:** Pale yellow crystals, yield: 77 % (3.20 g); m.p. >300 ºC. IR (KBr): ν/ cm^{-1} = 3469-3318 (NH_2, NH), 3070 (CH aromatic), 2994, 2840 (CH_3, CH_2), 1702 (CO), 1620 (exocyclic C=N), 1238 (C=S). ^{1}H-NMR (DMSO-d_6): δ = 1.14 (t, 3H, CH_3), 1.50-2.69 (m, 8H, $4CH_2$), 4.21 (q, 2H, CH_2), 4.53 (s, 2H, NH_2), 6.90 (s, 1H, pyrimidine H-5), 7.26-7.44 (m, 5H, C_6H_5), 8.22 (s,1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 426 (M^+, 66 %). Analysis for $C_{21}H_{22}N_4O_2S_2$ Calcd: C, 59.13; H, 5.20; N, 13.13; S, 15.03. Found: C, 59.30; H, 4.39; N, 13.29; S, 14.92 %.

Compound **18b:** Pale yellow crystals, yield: 80 % (3.40 g); m.p. 233-236 ºC. IR (KBr): ν/ cm^{-1} = 3490, 3348 (NH_2), 3070 (CH aromatic), 2994, 2840 (CH_3, CH_2), 1702, 1688 (2CO), 1242 (C=S).^{1}H-NMR (DMSO-d_6): δ = 1.12 (t, 3H, CH_3), 1.44-2.71 (m, 8H, $4CH_2$), 4.23 (q, 2H, CH_2), 4.59 (s, 2H, NH_2), 6.93 (s, 1H, pyrimidine H-5), 7.29-7.40 (m, 5H, C_6H_5). MS (relative intensity) m/z: 427(M^+, 40%). Analysis for $C_{21}H_{21}N_3O_3S_2$ Calcd: C, 58.99; H, 4.95; N, 9.83; S, 15.00. Found: C, 59.14; H, 4.83; N, 9.64; S, 14.84%.

Ethyl 2-(6-methyl-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (20).

To a solution of compound **12** (3.60 g. 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.05 mL), ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 hours then poured onto acidified ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol.

Compound **20:** Pale yellow crystals, yield: 77 % (3.20 g); m.p. 288-292 ºC, IR (KBr): ν/ cm^{-1} = 3060 (CH-aromatic), 2980, 2878 (CH_3, CH_2), 1708, 1693 (2CO), 1539 (C=C), 1237 (C=S). ^{1}H-NMR (DMSO-d_6): δ = 1.13 (t, 3H, CH_3), 1.66-1.80 (m, 8H, $4CH_2$), 2.23 (s, 3H, CH_3), 4.20 (q, 2H, CH_2), 6.39 (s, 1H, pyrimidine H-5), 7.27-7.42 (m, 5H, C_6H_5). MS (relative intensity) m/z: 426 (M^+, 62 %). Analysis for $C_{22}H_{22}N_2O_3S_2$ Calcd: C, 61.95, H, 5.20; N, 6.57; S, 15.03. Found: C, 61.90; H, 5.43; N, 6.70; S, 15.28 %.

Synthesis of 4,5,6,7-tetrohydro-2-(2,3dihydro-4-hydroxy-4,6-dimethyl-3-phenyl-2-thioxopyrimidine-1(2H-yl) benzo[b]thiophene-3-carbonitrile (22).

To a solution of compound **12** (3.60 g. 0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.05 mL), acetylacetone (1.00 g, 0.01mol) was added. The reaction mixture was heated under reflux for 3 hours then poured onto acidified ice/water mixture. The solid products were collected by filtration and crystallized from absolute ethanol.

Compound **22:** Pale yellow crystals, yield: 65 % (2.80 g); m.p. 166-168 ºC, IR (KBr): ν/cm^{-1} = 3055 (CH-aromatic), 2967, 2873 (CH_3, CH_2), 1704, 1690 (2CO), 1539 (C=C), 1232 (C=S). ^{1}H-NMR (DMSO-d_6): δ=1.12 (t, 3H, CH_3), 1.61-1.84 (m, 8H, $4CH_2$), 2.26, 2.44 (2s, 6H, $2CH_3$), 4.22 (q, 2H, CH_2), 6.36 (s, 1H, pyrimidine H-5), 7.29-7.40 (m, 5H, C_6H_5), 10.09 (s, 1H, OH). m/z: 442 (M^+, 73 %). Analysis for $C_{23}H_{26}N_2O_3S_2$ Calcd: C, 62.42; H, 5.92; N, 6.33; S, 14.49. Found: C, 62.32; H, 5.88; N, 6.42; S, 14.38 %.

Ethyl 2-(4-hydroxy-4,6-dimethyl-3-phenyl-5-(phenyldiazenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (24)

To a cold solution (0-5 ºC) of **22** (4.42 g, 0.01 mol) containing sodium hydroxide (1.00 g), an equimolar amount of benzenediazonium chloride (0.01 mol) [prepared by adding sodium nitrite solution (0.70 g, 0.01 mol) in water (10 mL) to a cold solution (0-5 ºC) of aniline (0.93 g, 0.01 mol) in concentrated hydrochloric acid (30 mL)] was gradually added with continuous stirring. The solid product formed upon cooling in an ice-path was collected by filtration, washed with water, and crystallized from absolute ethanol.

Compound **24:** Pale yellow crystals, yield: 88 % (4.80 g); m.p. 210-212 ºC. IR (KBr): ν/ cm^{-1} = 3052 (CH-aromatic), 2997, 2870 (CH_3, CH_2), 1704, 1690 (2CO), 1539 (C=C), 1232 (C=S). ^{1}H-NMR (DMSO-d_6): δ = 1.12 (t, 3H, CH_3), 1.64-1.99 (m, 8H, $4CH_2$), 2.24, 2.46 (2s, 6H, $2CH_3$), 4.23 (q, 2H, CH_2), 7.22-7.43 (m, 10H, $2C_6H_5$), 10.22 (s, 1H, OH). MS (relative intensity) m/z: 546 (M^+, 55%). Analysis for $C_{29}H_{30}N_4O_3S_2$ Calcd: C, 63.71; H, 5.53; N, 10.25; S, 11.73. Found: C, 63.84; H, 5.70; N, 10.49; S, 11.93 %.

Synthesis of 4-imino-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5]-thieno[2,3-d]pyrimidine-2-thiol (25).

To a solution of compound **12** (3.60 g, 0.01) in 1,4-dioxane (25 mL) a catalytic amount of piperidine (0.5 mL) was added and the reaction mixture was heated under reflux for 3 hours. The formed solid product after pouring the solution into acidified ice/water mixture was collected by filtration and crystallized from 1,4-dioxane.

Compound **25:** Yellow crystals, yield: 69 % (2. 10 g); m.p. 177-180 ^{o}C. IR (KBr): ν/ cm^{-1} = 3056 (CH-aromatic), 2992, 2883 (CH_3, CH_2), 1701 (CO), 1542 (C=C), 1239 (C=S). ^{1}H-NMR (DMSO-d_6): δ = 1.60-1.94 (m, 8H, 4CH_2), 7.21-7.38 (m, 5H, C_6H_5), 10.62 (s, 1H, SH). MS (relative intensity) m/z: 314 (M^+, 40%). Analysis for $C_{16}H_{14}N_2OS_2$ Calcd: C, 61.12, H, 4.49; N, 8.91; S, 20.40. Found: C, 61.29; H, 4.38; N, 9.17; S, 20. 28 %.

Conclusions

In this work, we used ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate for the synthesis of various N-alkylated heterocyclic derivatives. The cytotoxicity of the synthesized products showed that compound **11**, **18a** and **25** were the most potent compounds towards the three cancer cell lines investigated. In addition, compound **18a** and **25** showed no toxicity against shrimp larvae.

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