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SYNTHESIS AND CYTOTOXICITY OF NOVEL THIOPHENE, PYRAN AND PYRIDINE DERIVATIVES

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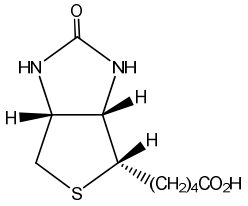
ABSTRACT. The reaction of 2-amino-3-cyano-tetrahydrobenzothiophene **3** with ethyl acetoacetate gave the amide derivatives **5**. The reactivity of **5** toward a variety of chemical reagents was studied to give pyrans, pyridines, thiophenes and the thiazoles and their fused derivatives. The structures of the newly synthesized products were confirmed on the basis of their respective analytical and spectral data. The antitumor evaluations of the synthesized compounds against the three cancer cell lines MCF-7, NCI-H460 and SF-268 showed that compounds **9a**, **9c**, **12a** and **14** were of the highest potencies against the three cancer cell lines among the tested compounds.

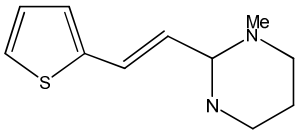
KEY WORDS: Tetrahydrobenz[*b*]thiophene, Pyran, Pyridine, Thiophene, Thiazole, Cytotoxicity

INTRODUCTION

Aromatic thiophenes play on part in animal metabolism; for examples, Biotin, one of the vitamins (Vitamin H) (Figure 1), is a tetrahydrothiophene, however aromatic thiophenes do occur in some plants, in association with polyacetylenes with which they are biogenetically linked and Banminth (pyrantel), available anthelmintic used in animal husbandry, is one of the thiophene compounds in chemotherapy. Thiophenes with a wide spectrum of biological activities are known, several of these derivatives possess potent analgesic [1, 2], anticonvulsant, anti-inflammatory and antibacterial [3-6], antipyretics [7], antitumor [8, 9], antiparasitic [10], antimicrobial [11], antihistaminic (H1) [12], antianexiety test in mice [13], antiarrhythmic [14] and serotonin antagonist [15]. In previous work we have found that certain substituted thiophenes and their heterocyclic derivatives show antitumor activities [16-18]. In the present work we were aiming the synthesis of new heterocyclic derivatives derived from the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene followed by studying the cytotoxicity of the newly synthesized compounds.



(+)-Biotin (Vitamin H)



Banminth

Figure 1. Chemical structures of (+)-Biotin and Banminth.

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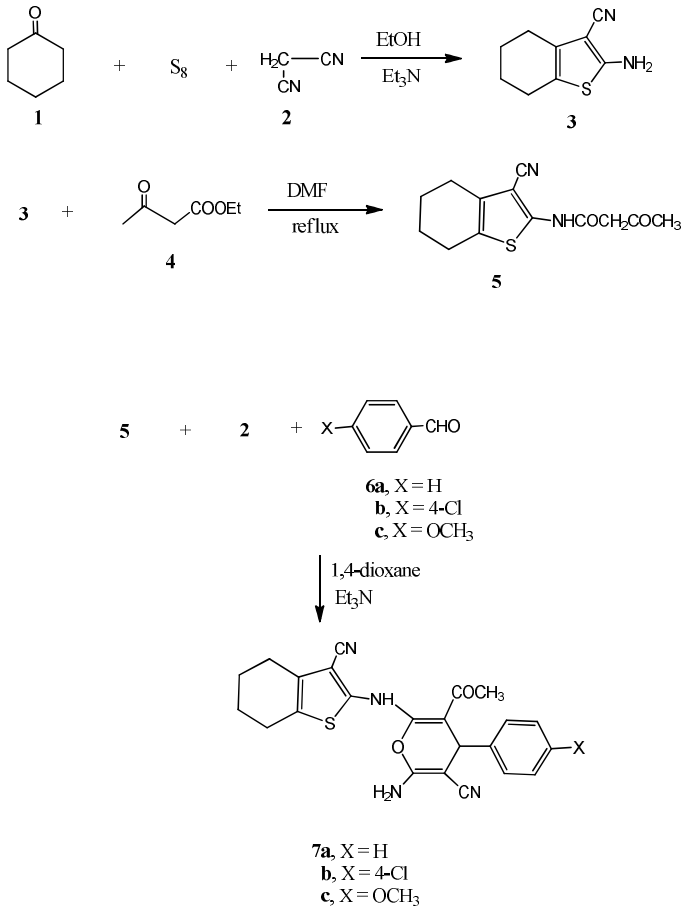
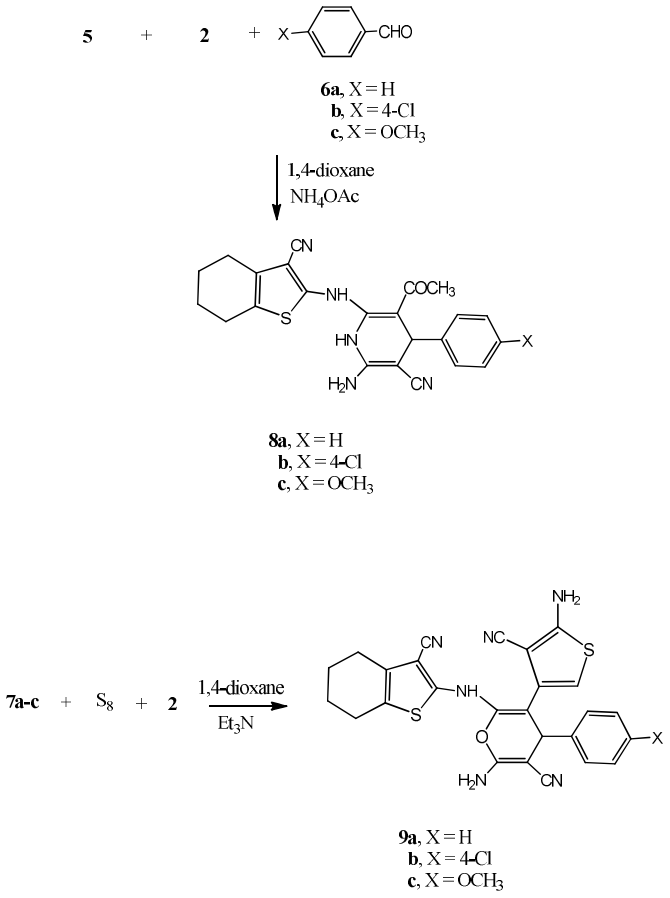
RESULTS AND DISCUSSION

Chemistry

In the present work we synthesised a series of heterocyclic rings incorporated tetrahydrobenzo[*b*]thiophene moiety through the use of 2-amino-3-cyano-4,5,6,7-tetrahydro-benzo[*b*]thiophene (**3**). Compound **3** was synthesized via the reaction of cyclohexanone (**1**) with malononitrile (**2**) and elemental sulfur [19, 20]. Compound **3** reacted with ethyl acetoacetate (**4**) in dimethylformamide under reflux to give the amide derivative (**5)**. The multi-component reaction of compound **5** and any of the aromatic aldehydes namely benzaldehyde (**6a**), 4-chlorobenzaldehyde (**6b**) or 4-methoxybenzaldehyde (**6c**) in 1,4-dioxane containing triethyl-amine with malononitrile gave the pyran derivatives **7a-c**, respectively (Scheme 1). The analytical and spectral data of compounds **7a-c** were the basis of their structural elucidation. Thus, the ^{1}H NMR spectrum of **7a** (as an example) revealed beside the expected signals a singlet at δ 3.19 ppm corresponding to the CH_3 group, a singlet at δ 6.01 ppm corresponding to the pyran H-4 a multiplet at δ 7.28-7.39 ppm equivalent to the C_6H_5 group and a singlet at δ 8.63 ppm, D_2O exchangeable, for the NH group.



Scheme 1. Synthesis of compounds **5**, **6a-c** and **7a-c**.



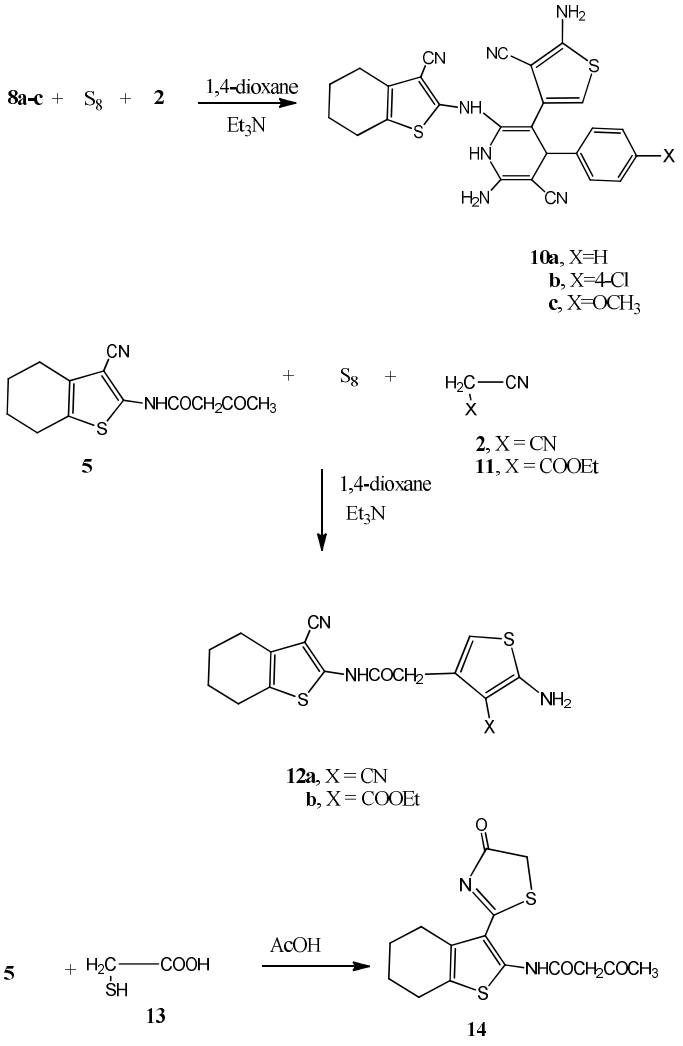
Scheme 2. Synthesis of compounds **8a-c** and **9a-c.**

Scheme 2. Synthesis of compounds **8a-c** and **9a-c**.

In addition the ^{13}C NMR spectrum showed, beside the expected signals, 22.8 (CH_3), 88.3 (pyran C-4), 116.2, 118.3 (2CN), 120.3, 121.3, 121.8, 122.4, 126.0, 128.4, 124.2, 130.8, 133.0, 134.2, 137.3, 139.2 (benzene, pyran, thiophene C), 165.8 (CO). Moreover, the multi-component reaction of compound **5** with any of the aromatic aldehydes namely benzaldehyde (**6a**), 4-chlorobenzaldehyde (**6b**) or 4-methoxybenzaldehyde (**6c**) in 1,4-dioxane containing ammonium acetate gave the pyridine derivatives **8a-c**. Compounds **7a-c** each with the acetyl group at the pyran ring found to be capable for the Gewald's thiophene synthesis. Thus, any of compounds **7a-c** reacted with malononitrile and elemental sulfur to give the thiophene derivatives **9a-c**, respectively. The analytical and spectral data of **9a-c** were consistent with their respective structures as depicted in Scheme 2. Thus, the ^{1}H NMR spectrum of **9a** revealed, beside the expected signals, the presence of two singlets at δ 4.38, 4.70 ppm (D_2O exchangeable) equivalent to the two NH_2 groups, a singlet at δ 5.96 ppm corresponding to the thiophene H-5, a singlet at δ 6.04 ppm for the pyran H-4 and a singlet at δ 8.39 ppm (D_2O exchangeable) equivalent for the NH group. The ^{13}C NMR spectrum showed δ 88.6 (pyran C-4), 115.7, 116.0,

116.3 (3CN), 119.5, 120.8, 121.3, 122.9, 123.3, 126.9, 127.5, 130.8, 132.6, 133.5, 137.7, 138.5, 139.7, 140.6, 142.9, 145.2 (benzene, pyran, two thiophene C).

Similarly, the pyridine derivatives **8a-c** reacted with malononitrile and elemental sulfur to give the thiophene derivatives **10a-c**, respectively. The reaction of compound **5** with either of malononitrile (**2**) or ethyl cyanoacetate (**11**) and elemental sulfur in 1,4-dioxane containing a catalytic amount of triethylamine gave the thiophene derivatives **12a** and **12b**, respectively. Analytical and spectral data were the basis of their respective structure elucidation. Finally, the reaction of compound **5** with thioglycollic acid (**13**) gave the thiazole derivative **14** (Scheme 3).



Scheme 3. Synthesis of compounds **10a-c**, **12a,b** and **14**.

Antitumor and normal cell line activity tests

Chemicals. Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

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, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) 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 μM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 105 cells/mL for MCF-7 and SF-268 and 0.75 x 104 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.

Tumor cell growth assay. The effects of the newly synthesized products on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

Structure activity relationship

It is clear from Table 1 that the 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**3**) showed high cytotoxicity against MCF-7 with GI_{50} 0.30 μmol L^{-1} but it showed low cytotoxicity against NCI-H460. The reaction of compound **3** with ethyl acetoacetate gave the amide derivative **5** which showed moderate cytotoxicity against NCI-H460 and SF-268 with GI_{50}'s 3.1 and 1.96 μmol L^{-1}. For the multi-component products **7a-c** it is obvious that compound **7c** (X = OCH_3) showed the highest cytotoxicity among the three compounds. On the other hand, the pyridine derivatives **8a-c** were of low potency towards the three cancer lines. The reaction of any of compounds **7a-c** with elemental sulfur and malononitrile to yield the thiophene derivatives **9a-c** where compounds **9a** (X = H) and **9c** (X = OCH_3) showed the highest cytotoxicity among the three compounds. On the other hand, compounds **10a-c** showed low potencies. Considering the dithiophene derivatives **12a,b**, it is clear that compound **12a** with X = CN showed the higher potency than **12b**, X = COOEt. It is clear that the presence of the CN group is responsible for its reactivity. Finally, the thiazole derivative **14** showed high potency against the three cancer cell lines.

Table 1. Effect of newly synthesized compounds on the growth of three human tumor cell lines.

Compound	GI_{50} (μmol L^{-1})			
	MCF-7	NCI- H460	SF-268	WI 38
3	0.30±0.02	1.80±0.09	4.20±1.04	>100
5	22.3±0.2	3.1±0.22	1.96±0.09	>100
7a	22.4±2.10	10.42±3.01	8.63±2.83	>100
7b	23.55±4.06	34.6±12.06	45.41±2.16	>100
7c	1.2±0.4	0.3±0.16	2.8±0.06	>100
8a	26.6±8.5	29.3±12.3	18.4±2.8	68.2 ± 2.0
8b	22.4±5.8	26.7±8.2	31.4±2.4	>100
8c	20.8±8.30	22.8±4.32	22.8±6.23	>100
9a	0.01±0.001	0.02±0.006	0.06±0.002	>100
9b	24.1±10.4	30.8±10.8	26.1±2.8	25.2 ± 0.8
9c	0.08±0.004	0.05±0.002	0.06±0.001	>100
10a	36.6±10.2	33.0±8.6	38.6±8.0	>100
10b	38.2±3.6	36.3±12.5	40.6±8.8	>100
10c	30.0±1.4	20.8±4.3	20.3±2.8	>100
12a	0.01±0.002	0.01±0.004	0.04±0.01	-
12b	60.06±8.33	58.03±12.3	33.1±10.82	-
14	0.03±0.002	0.02±0.003	0.05±0.002	-
Doxorubicin	0.04±0.008	0.09±0.008	0.09±0.007	>100

EXPERIMENTAL

Chemistry

All melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or Pyeunicam SP-1000 spectrophotometer. ^{1}H NMR spectra were recorded with Mercury-300BB (300 MHz) (Cairo University) instrument in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. ^{13}C-NMR spectra were recorded in DMSO. Analytical data were obtained from the micro analytical data unit at Cairo University and were performed on Vario El III Elemental CHNS analyzer.

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

Ethyl acetoacetate (13.0 g, 0.1 mol) was heated till 140 ^{o}C then compound **3** (17.8 g, 0.1 mol) was added with continuous heating till the temperature reach 125 ^{o}C at this moment the whole reaction mixture is heated under reflux for 20 min. The reaction mixture was allowed to cool overnight and the formed solid product was filtered, dried and crystallized from ethanol to give compound **5**.

*General procedure for the synthesis of the pyran derivatives **7a-c***

To a solution of compound **5** (2.62 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethyl amine (0.50 mL) any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.41 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then left to cool. The formed solid product was collected by filtration.

*2-(5-Acetyl-4-phenyl-4H-pyran-2-ylamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitril (**7a**)*. Yield: 83%; m.p.: 125-128 ^{o}C; IR (KBr, cm^{-1}) ν: 3477-3320 (NH_2, NH), 3055 (CH aromatic), 2936-2850 (CH aliphatic), 2223, 2220 (2CN), 1689 (CO); ^{1}H NMR (DMSO-d_6) δ: 1.73-2.46 (m, 8H, $4CH_2$), 3.19 (s, 3H, CH_3), 4.49 (s, 2H, D_2O exchangeable, NH_2), 6.01 (s, 1H, pyran H-4), 7.28-7.39 (m, 5H, C_6H_5), 8.63 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.8 (CH_3), 29.4, 30.5, 33.2, 38.6 ($4CH_2$), 88.3 (pyran C-4), 116.2, 118.3 (2CN), 120.3, 121.3, 121.8, 122.4, 126.0, 128.4, 124.2, 130.8, 133.0, 134.2, 137.3, 139.2 (benzene, pyran, thiophene C), 165.8 (CO); MS: *m/z*, (%): 416 (22). Anal. calcd. for $C_{23}H_{20}N_4O_2S$ (416.5): C, 66.33; H,4.84; N,13.45; S,7.69. Found: C, 66.53; H, 4.92; N, 13.62; S, 7.92.

*2-(5-Acetyl-4-chlorophenyl-4H-pyran-2-ylamino)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitril (**7b**)*. Yield: 78%; m.p. 85 ^{o}C; IR (KBr, cm^{-1}) ν: 3473-3343 (NH_2, NH), 3058 (CH aromatic), 2938-2873 (CH aliphatic), 2226, 2221 (2CN), 1687 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.70-2.48 (m, 8H, $4CH_2$), 3.17 (s, 3H, CH_3), 4.48 (s, 2H, D_2O exchangeable, NH_2), 6.04 (s, 1H, pyran H-4), 7.23-7.42 (m, 4H, C_6H_4), 8.68 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.8 (CH_3), 29.4, 3 0.5, 33.2, 38.6 ($4CH_2$), 88.3 (pyran C-4), 116.1, 116.4 (2CN), 120.3, 121.3, 121.8, 122.4, 126.0, 128.4, 124.2, 130.8, 133.0, 134.2, 137.3, 139.2 (benzene, pyran, thiophene C), 165.8 (CO); MS (*m/z*, %): 450 (36). Anal. calcd. for $C_{23}H_{19}ClN_4O_2S$ (450.94): C, 61.26; H, 4.25; N, 12.42; S, 7.11. Found: C, 61.09; H, 4.31; N, 12.69; S, 7.08.

*2-(5-Acetyl-4-methoxyphenyl-4H-pyran-2-ylamino)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitril (**7c**)*. Yield: 68%; m.p. 145-148 ^{o}C; IR (KBr, cm^{-1}) ν: 3495-3318 (NH_2, NH), 3056 (CH aromatic), 2972-2893 (CH aliphatic), 2224, 2220 (2CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.72-2.40 (m, 8H, $4CH_2$), 3.11, 3.24 (2s, 6H, $2CH_3$), 4.44 (s, 2H, D_2O exchangeable, NH_2), 6.02 (s, 1H, pyran H-4), 7.28-7.36 (m, 4H, C_6H_4), 8.60 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.6 (CH_3), 29.2, 30.5, 33.7, 38.4 ($4CH_2$), 32.12 (CH_3), 88.6 (pyran C-4), 115.8, 116.2 (2CN), 120.6, 122.0, 122+.2, 122.4, 125.3, 127.0, 128.3, 130.3, 133.3, 135.0, 136.2, 139.0 (benzene, pyran, thiophene C), 165.9 (CO); MS (*m/z*, %): 446 (22). Anal. calcd. for $C_{24}H_{22}N_4O_3S$ (446.52): C, 64.56; H, 4.97; N, 12.55; S, 7.18. Found: C, 64.25; H, 4.77; N, 12.73; S, 7.16.

*General procedure for the synthesis of the pyridine derivatives **8a-c***

To a solution of compound **5** (2.62 g, 0.01 mol) in 1,4-dioxane (40 mL) containing ammonium acetate (0.50 mL) any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.41 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h then left to cool. The formed solid product was collected by filtration.

*2-(5-Acetyl-1,4-dihydro-4-phenylpyridin-2-ylamino)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonitril (**8a**)*. Yield: 77%; m.p. 102-104 ^{o}C; IR (KBr, cm^{-1}) ν: 3483-3316 (NH_2, 2NH), 3053 (CH aromatic), 2948-2862 (CH aliphatic), 2224, 2220 (2CN), 1687 (CO), 1633 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.70-2.43 (m, 8H, $4CH_2$), 3.16 (s, 3H, CH_3), 4.44 (s, 2H, D_2O exchangeable, NH_2), 6.01 (s, 1H, pyran H-4), 7.25-7.39 (m, 5H, C_6H_5), 8.60, 8.72 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.7 (CH_3), 29.0, 30.8, 32.9, 38.9 ($4CH_2$), 88.2 (pyran C-4), 116.2, 116.9 (2CN), 120.6, 121.0, 121.6, 122.0, 124.7, 127.9, 128.3, 130.5, 133.2, 133.9, 137.7, 139.3 (benzene, pyridine, thiophene C), 166.0 (CO); MS (*m/z*, %): 415 (18). Anal. calcd. for $C_{23}H_{21}N_5OS$ (415.51): C, 66.48; H, 5.09; N, 16.85; S, 7.72. Found: C, 66.50; H, 4.92; N, 16.53; S, 7.92.

2-(5-Acetyl-1,4-dihydro-4-chloro-phenylpyridin-2-ylamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitril ***(8b)***. Yield 79%; m.p.: 116-119 °C; IR (KBr, cm^{-1}) ν: 3488-3323 (NH_2, 2NH), 3058 (CH aromatic), 2938-2873 (CH aliphatic), 2226, 2221 (2CN), 1687 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.73-2.45 (m, 8H, $4CH_2$), 3.14 (s, 3H, CH_3), 4.45 (s, 2H, D_2O exchangeable, NH_2), 6.02 (s, 1H, pyran H-4), 7.26-7.45 (m, 4H, C_6H_4), 8.33, 8.65 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.4 (CH_3), 28.3, 31.8, 32.8, 37.1 ($4CH_2$), 88.6 (pyran C-4), 116.0, 116.3 (2CN), 120.3, 121.6, 121.8, 122.2, 124.8, 127.0, 128.3, 131.9, 132.8, 134.9, 138.5, 139.2 (benzene, pyran, thiophene C), 164.8 (CO); MS (*m/z*, %): 449 (38). Anal. calcd. for $C_{23}H_{20}ClN_5OS$ (449.11): C, 61.39; H, 4.48; N, 15.56; S, 7.13. Found: C, 61.42; H, 4.63; N, 15.36; S, 7.28.

2-(5-Acetyl-1,4-dihydro-4-methoxy-phenylpyridin-2-ylamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitril ***(8c)***. Yield: 83%; m.p.: 140-142 °C; IR (KBr, cm^{-1}) ν: 3495-3318 (NH_2, 2NH), 3056 (CH aromatic), 2972-2893 (CH aliphatic), 2224, 2220 (2CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.72-2.40 (m, 8H, $4CH_2$), 3.11, 3.24 (2s, 6H, $2CH_3$), 4.44 (s, 2H, D_2O exchangeable, NH_2), 6.02 (s, 1H, pyran H-4), 7.28-7.36 (m, 4H, C_6H_4), 8.32, 8.60 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 22.6 (CH_3), 29.2, 30.5, 33.7, 38.4 ($4CH_2$), 32.12 (CH_3), 88.6 (pyran C-4), 115.8, 116.4 (2CN), 120.2, 122.3, 122.8, 122.4, 125.3, 127.8, 129.1, 132.7, 133.6, 135.0, 136.2 (benzene, pyran, thiophene C), 165.6 (CO); MS (*m/z*, %): 445 (16). Anal. calcd. for $C_{24}H_{23}N_5O_2S$ (445.54): C, 64.70; H, 5.20; N, 15.72; S, 7.20. Found: C, 64.49; H, 5.26; N, 15.91; S, 7.42.

General procedure for the synthesis of the 5-amino-4-cyanothiophen-3-yl)-4-phenyl-4H-pyran derivatives ***9a-c***

To a solution of any of compound **7a** (4.15 g, 0.01 mol), **7b** (4.50 g, 0.01 mol) or **7c** (4.46 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethyl amine (1.50 mL) elemental sulfur (0.32 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-6-((3-cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)amino)-4-phenyl-4H-pyran-3-carbonitrile ***(9a)***. Yield: 70%; m.p.: 230-235 °C; IR (KBr, cm^{-1}) ν: 3453-3341 ($2NH_2$, NH), 3053 (CH aromatic), 2983, 2851 (CH aliphatic), 2228, 2223-2220 (3CN), 1636 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.43-2.51 (m, 8H, $4CH_2$), 4.38, 4.70 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.96 (s, 1H, thiophene H-5), 6.04 (s, 1H, pyran H-4), 7.28-7.46 (m, 5H, C_6H_5), 8.39 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 29.3, 30.5, 32.9, 38.3 ($4CH_2$), 88.6 (pyran C-4), 115.7, 116.0, 116.3 (3CN), 119.5, 120.8, 121.3, 122.9, 123.3, 126.9, 127.5, 130.8, 132.6, 133.5, 137.7, 138.5, 139.7, 140.6, 142.9, 145.2 (benzene, pyran, two thiophene C); MS (*m/z*, %): 496 (42). Anal. calcd. for $C_{26}H_{20}N_6OS_2$ (496.61): C, 62.88; H, 4.06; N, 16.92; S, 12.91. Found: C, 62.94; H, 4.33; N, 17.18; S, 13.07.

2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-4-(4-chlorophenyl)-6-((3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)amino)-4H-pyran-3-carbonitrile ***(9b)***. Yield 84%; m.p.: 216-218 °C; IR (KBr, cm^{-1}) ν: 3481-3342 ($2NH_2$, NH), 3052 (CH aromatic), 2948-2881 (CH aliphatic), 2226, 2223-2220 (3CN), 1689 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.76-2.48 (m, 8H, $4CH_2$), 4.36, 4.90 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.95 (s, 1H, thiophene H-5), 6.04 (s, 1H, pyran H-4), 7.30-7.48 (m, 4H, C_6H_4), 8.29 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 28.1, 31.4, 31.9, 35.8 ($4CH_2$), 88.9 (pyran C-4), 116.1, 116.7, 117.3 (3CN), 120.8, 121.9, 122.4, 123.8, 125.3, 126.9, 128.8, 130.4, 131.3, 132.7, 136.5, 137.9, 138.0, 140.8, 142.8, 143.6

(benzene, pyran, two thiophene C); MS (*m/z*, %): 531 (32). Anal. calcd. for $C_{26}H_{19}ClN_6OS_2$ (531.05): C, 58.80; H, 3.61; N, 15.83; S, 12.08. Found: C, 59.28; H, 3.94; N, 16.33; S, 11.94.

*2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-4-(4-chlorophenyl)-6-((3-cyano-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl)amino)-4H-pyran-3-carbonitrile (**9c**)*. Yield 73%; m.p.: >300 ^{o}C; IR (KBr, cm^{-1}) ν: 3472-3321 ($2NH_2$, NH), 3058 (CH aromatic), 2973, 2890 (CH aliphatic), 2227, 2222-2220 (3CN), 1688 (CO), 1633 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.68-2.42 (m, 8H, $4CH_2$), 3.24 (s, 3H, CH_3), 4.42, 4.57 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.08 (s, 1H, pyran H-4), 6.22 (s, 1H, thiophene H-5), 7.28-7.36 (m, 4H, C_6H_4), 8.34 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 28.6, 31.6, 32.9, 35.8 ($4CH_2$), 32.3 (CH_3), 88.7 (pyran C-4), 115.6, 116.5, 116.8 (3CN), 119.8, 121.6, 122.4, 123.9, 124.9, 127.5, 128.5, 130.4, 132.3, 132.9, 134.3, 135.7, 136.2, 140.9, 141.8, 143.9 (benzene, pyran, twothiophene C); MS (*m/z*, %): 526 (26). Anal. calcd. for $C_{27}H_{22}N_6O_2S_2$ (526.63): C, 61.58; H, 4.21; N, 15.98; S, 12.18. Found: C, 61.83; H, 4.47; N, 16.31; S, 12.22.

*General procedure for the synthesis of the 5-amino-4-cyanothiophen-3-yl)-4-phenyl-4H-pyridine derivatives **10a-c***

To a solution of any of compound **8a** (4.15 g, 0.01 mol), **8b** (4.49 g, 0.01 mol) or **8c** (4.45 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethyl amine (1.50 mL) elemental sulfur (0.32 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

*2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-6-((3-cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)amino)-4-phenyl-1,4-dihydropyridine-3-carbonitrile (**10a**)*. Yield: 83%; m.p. 145 ^{o}C; IR (KBr, cm^{-1}) ν: 3463-3329 ($2NH_2$, 2NH), 3056 (CH aromatic), 2967-2851 (CH aliphatic), 2227, 2222-2220 (3CN), 1631 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.48-2.39 (m, 8H, $4CH_2$), 4.43, 4.72 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.99 (s, 1H, thiophene H-5), 6.03 (s, 1H, pyridine H-4), 7.22-7.42 (m, 5H, C_6H_5), 8.54, 8.65 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 29.0, 30.8, 32.9, 38.9 ($4CH_2$), 88.2 (pyridine C-4), 116.1, 116.3, 116.8 (3CN), 120.6, 121.0, 121.6, 122.0, 124.7, 127.9, 128.3, 130.5, 133.2, 133.9, 137.7, 138.0, 139.3, 141.3, 144.2, 146.8 (benzene, pyridine, two thiophene C); MS (*m/z*, 495 (33). Anal. calcd. for $C_{26}H_{21}N_7S_2$ (495.62): C, 63.01; H, 4.27; N, 19.78; S, 12.94. Found: C, 62.88; H, 4.51; N, 19.49; S, 13.19.

*2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-6-((3-cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)amino)-4-chlorophenyl-1,4-dihydropyridine-3-carbonitrile (**10b**)*. Yield 88%; m.p. 180-182 ^{o}C; IR (KBr, cm^{-1}) ν: 3463-3313 ($2NH_2$, 2NH), 3054 (CH aromatic), 2942-2870 (CH aliphatic), 2224, 2222-2220 (3CN), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.73-2.45 (m, 8H, $4CH_2$), 4.29, 4.45 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.99 (s, 1H, thiophene H-5), 6.02 (s, 1H, pyridine H-4), 7.26-7.43 (m, 4H, C_6H_4), 8.33, 8.65 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 28.3, 31.8, 32.8, 37.1 ($4CH_2$), 88.6 (pyridine C-4), 116.0, 116.4, 116.9 (3CN), 120.3, 121.6, 121.8, 122.2, 124.8, 127.0, 128.3, 131.9, 132.8, 134.9, 138.3, 138.5, 139.2 141.2, 142.8, 143.9 (benzene, pyridine, two thiophene C); MS (*m/z*, 530 (42). Anal. calcd. for $C_{26}H_{20}ClN_7S_2$ (530.07): C, 58.91; H, 3.80; N, 18.50; S, 12.10. Found: C, 59.09; H, 3.66; N, 18.83; S, 11.89.

*2-Amino-5-(5-amino-4-cyanothiophen-3-yl)-6-((3-cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)amino)-4-methoxyphenyl-1,4-dihydropyridine-3-carbonitrile (**10c**)*. Yield 68%; m.p. 110 ^{o}C; IR (KBr, cm^{-1}) ν: 3488-3342 ($2NH_2$, 2NH), 3054 (CH aromatic), 2960, 2892 (CH aliphatic), 2225, 2222-2220 (3CN), 1633 (C=C); ^{1}H NMR (DMSO-d_6) δ: 1.68-2.42 (m, 8H, $4CH_2$), 3.26 (s,

3H, CH_3), 4.46, 4.69 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.04 (s, 1H, pyridine H-4), 6.11 (s, 1H, thiophene H-5), 7.25-7.39 (m, 4H, C_6H_5), 8.31, 8.63 (2s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 29.0, 30.8, 33.4, 36.7 ($4CH_2$), 31.8 (CH_3), 88.9 (pyridine C-4), 115.7, 116.1, 116.3 (3CN), 120.2, 122.3, 122.8, 122.4, 125.3, 127.8, 129.1, 130.9, 132.7, 133.6, 135.0, 136.2, 138.1, 140.4, 142.5, 143.2 (benzene, pyridine, two thiophene C); MS (*m/z*, %): 525 (26). Anal. calcd. for $C_{27}H_{23}N_7OS_2$ (525.65): C, 61.69; H, 4.41; N, 18.65; S, 12.20. Found: C, 62.33; H, 4.68; N, 18.89; S, 12.41.

General procedure for the synthesis of the thiophene derivatives ***12a,b***

To a solution of compound **5** (2.62 g, 0.01 mol), in 1,4-dioxane (40 mL) containing triethyl amine (1.50 mL) elemental sulfur (0.32 g, 0.01 mol) and either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-(5-Amino-4-cyanothiophen-3-yl)-N-(3-cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)acetamide ***(12a)***. Yield 80%; m.p. 120 ^oC; IR (KBr, cm^{-1}) ν: 3459-3328 (NH_2, NH), 3053 (CH aromatic), 2972, 2869 (CH aliphatic), 2224, 2220 (2CN), 1703 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6) δ: 1.65-2.41 (m, 8H, $4CH_2$), 3.88 (s, 2H, CH_2), 4.42 (s, 2H, D_2O exchangeable, NH_2), 6.18 (s, 1H, thiophene H-5), 8.33 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 29.0, 30.5, 33.1, 36.9 ($4CH_2$), 43.8 (CH_2), 116.3, 116.6 (2CN), 130.8, 133.1, 133.6, 137.1, 138.1, 142.9, 143.6 (two thiophene C), 166.2 (CO); MS (*m/z*, %): 342 (48). Anal. calcd. for $C_{16}H_{14}N_4OS_2$ (342.44): C, 56.12; H, 4.12; N, 16.36; S, 18.73. Found: C, 56.08; H, 4.33; N, 16.59; S, 18.92.

Ethyl-2-amino-4-(2-((3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)amino)-2-oxoethyl)thiophene-3-carboxylate ***(12b)***. Yield 67%; m.p. 110 ^oC; IR (KBr, cm^{-1}) ν: 3483-3330 (NH_2, NH), 3054 (CH aromatic), 2983, 2863 (CH aliphatic), 2220 (CN), 1705, 1688 (2 CO), 1632 (C=C); ^1H NMR (DMSO-d_6) δ: 1.16 (t, 3H, J = 6.95 Hz, CH_3), 1.62-2.48 (m, 8H, $4CH_2$), 3.92 (s, 2H, CH_2), 4.21 (q, 2H, J = 6.95 Hz, CH_2), 4.49 (s, 2H, D_2O exchangeable, NH_2), 6.18 (s, 1H, thiophene H-5), 8.33 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 14.2 (ester CH_3), 29.2, 30.9, 33.3, 36.9 ($4CH_2$), 43.6 (CH_2), 53.8 (ester CH_2), 116.4 (CN), 130.2, 133.3, 133.9, 135.8, 137.4, 142.6, 144.2 (two thiophene C), 166.8, 168.3 (2CO); MS (*m/z*, %): 389 (31). Anal. calcd. for $C_{18}H_{19}N_3O_3S_2$ (389.49): C, 55.51; H, 4.92; N, 10.79; S, 16.47. Found: C, 55.39; H, 5.18; N, 11.09; S, 16.62.

3-Oxo-N-(3-(4-oxo-4,5-dihydrothiazol-2-yl)-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)butanemide ***(14)***. To a solution of compound **5** (2.62 g, 0.01 mol) in acetic acid (50 mL), thioglycollic acid (0.92 g, 0.01 mol) was added. the reaction mixture was heated under reflux for 2 h then was left to cool. The solid product produced upon pouring onto ice/water mixture was collected by filtration. Yield 58%; m.p. 140 ^oC; IR (KBr, cm^{-1}) ν: 3495-3321 (NH), 2972, 2829 (CH aliphatic), 1708, 1689-1660 (3CO), 1636 (C=C); ^1H NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 1.59-2.45 (m, 8H, $4CH_2$), 3.96 (s, 2H, CH_2), 5.30 (s, 2H, thiazole CH_2), 8.33 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz): 29.6, 29.6, 32.8, 34.9 ($4CH_2$), 45.2 (CH_2), 89.2 (thiazole CH_2), 132.6, 136.2, 142.9, 144.9 (thiophene C), 164.8, 163.6, 169.0 (3CO), 170.3 (C=N); MS (*m/z*, %): 336 (24). Anal. calcd. for $C_{15}H_{16}N_2O_3S_2$ (336.43): C, 53.55; H, 4.79; N, 8.33; S, 19.06. Found: C, 53.80; H, 4.88; N, 8.58; S, 18.79.

CONCLUSION

The newly synthesized thiophene derivatives were obtained in optimal yields and their structures were confirmed using the analytical and spectral tools. The antitumor evaluations of the synthesized compounds against the three cancer cell lines MCF-7, NCI-H460 and SF-268 showed that compounds **9a**, **9c**, **12a** and **14** have the highest potencies against the three cancer cell lines among the tested compounds.

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