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Design and synthesis of new thiophene derivatives together with their antitumor evaluations

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ABSTRACT

A series of new thiophene derivatives (3a,b) have been prepared by the reaction of acetylacetone with either cyanoacetanilide or 2-cyano-*N*-(*p*-tolyl)acetamide and elemental sulfur in the presence of triethylamine as basic catalyst. The two synthesized compounds were used to further synthesize new thiophene derivatives. The structures of all the newly synthesized products have been established on the basis of analytical and spectral data. The antitumor activity of the newly thiophene derivatives was evaluated against six human cancer cell lines, namely gastric cancer (NUGC), colon cancer (DLD1), liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), breast cancer (MCF) and normal fibroblast cells (WI38).

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1. Introduction

Thiophenes are versatile reagents for many heterocyclic reactions leading to the formation of important biologically active compounds [1-6]. In addition, thiophenes derivatives belong to aromatic heterocyclic group consequently; they are important structural fragment in many pharmaceutical and chemical compounds [7,8]. Thiophenes compounds have been found to show nematocidal [9], insecticidal [10], antibacterial [11], antifungal [12], antiviral [13] and antioxidant activity [14]. In fact, many therapeutic drugs contain the thiophene nucleus as the main active moiety through the drug e.g. NSC 652287 (Figure 1) [15]. It was hoped that thiophenes compounds would be as active as the parent compound or that these analogous would, by virtue of its similar chemical structure, combine with the receptor and if not elicit a response of its own, serve effectively as a competitive inhibitor. Thus, when the thiophenes and furans derivatives are prepared, it is conceivable that such an agentmay intensify, mimic or antagonize the physiological activity of the parent substance [16].

2. Experimental

2.1. Instrumentation

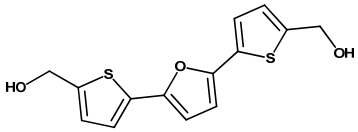

Figure 1. Chemical structure of the thiophene NSC 6522872, 5-*bis*(5-hydroxymethyl-2-thienyl)furan.

All melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FT-IR plus 460 or Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were recorded with Varian Gemini-200 (200 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. MS (EI) 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 analyser.

2.2. Synthesis

2.2.1. General procedure for the synthesis of the thiophene derivatives 3a,b

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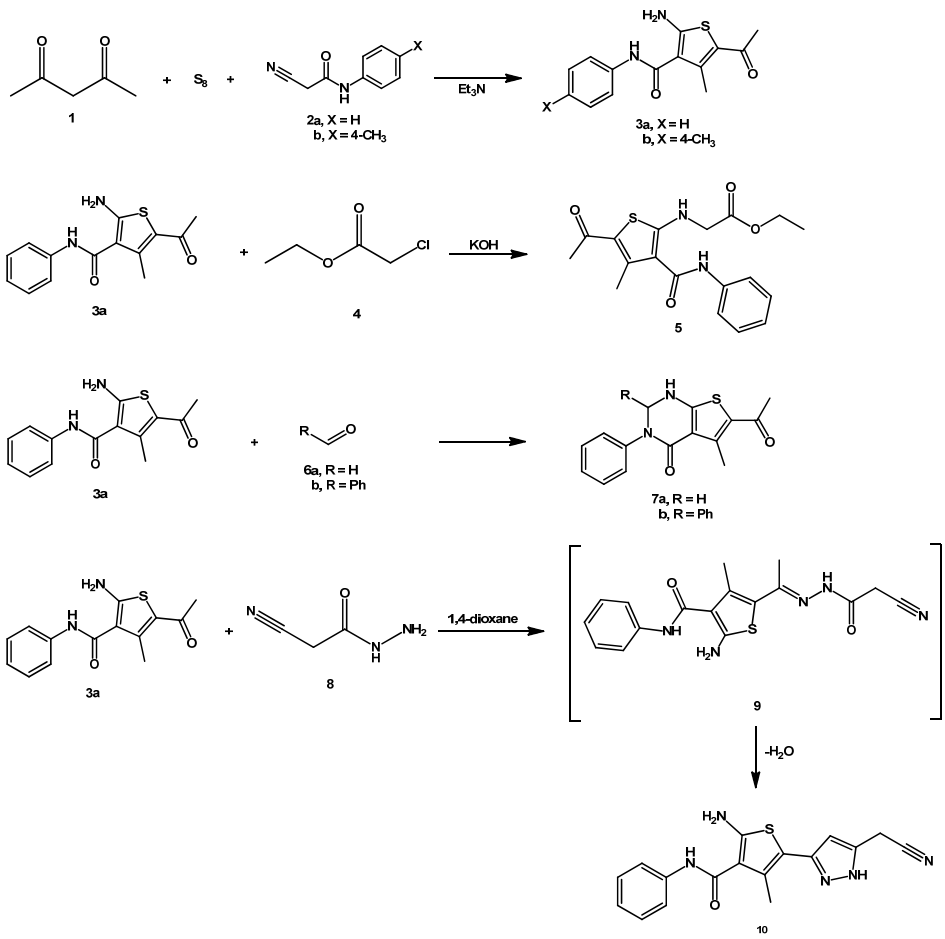


Scheme 1

To a solution of acetylacetone (1.0 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), either cyanoacetanilide (1.60 g, 0.01 mol) (**2a**) or 2-cyano-*N*-(*p*-tolyl)acetamide (**2b**) (1.74 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture, in each case, was heated under reflux for 2 h then left to cool. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration and crystallized from the suitable solvent (Scheme 1).

5-Acetyl-2-amino-4-methyl-N-phenylthiophene-3-carboxamide (**3a**): Color: Pale yellow crystals from acetic acid. Yield: 74 % (2.03 g). M.p.: 180-182 °C. IR (KBr, ν, cm^{-1}): 3473-3340 (NH_2, NH), 3054 (CH, aromatic), 2982 (CH_3), 1708, 1687 (2 CO), 1628 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.68 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 4.85 (s, 2H, NH_2), 7.31-7.37 (m, 5H, C_6H_5), 8.38 (s, 1H, NH). Anal. calcd. for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21. Found: C, 61.03; H, 5.30; N; 10.49%.

5-Acetyl-2-amino-4-methyl-N-(p-tolyl) thiophene-3-carboxamide (**3b**): Color: Yellow crystals from acetic acid. Yield: 80 % (2.31 g). M.p.: 220-222 °C. IR (KBr, ν, cm^{-1}): 3455-3332 (NH_2, NH), 3058 (CH, aromatic), 2984 (CH_3), 1705, 1688 (2 CO), 1625 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.66 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 4.87 (s, 2H, NH_2), 7.30-7.41 (m, 4H, C_6H_4), 8.36 (s, 1H, NH). Anal. calcd. for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71. Found: C, 62.31; H, 5.42; N; 9.86%.

2.2.2. Ethyl (5-acetyl-4-methyl-3-(phenylcarbamoyl) thiophen-2-yl)glycinate (5)

To a solution of compound **3a** (2.74 g, 0.01 mol) in 1,4-dioxane (40 mL) containing potassium hydroxide (0.50 g) ethyl 2-chloro acetate (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid (till pH = 6) and the formed solid product was collected by filtration. Color: White crystals from ethanol (Scheme 1). Yield: 78 % (2.81 g). M.p.: 195-197 °C. IR (KBr, ν, cm^{-1}): 3466-3323 (2 NH), 3054 (CH, aromatic), 2980 (CH_3), 1712-1686 (3 CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.13 (t, 3H, J = 7.02 Hz, CH_3), 2.66 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.02 Hz, CH_2), 5.37 (s, 2H, CH_2), 7.28-7.39 (m, 5H, C_6H_5), 8.31 (s, 1H, NH), 8.40 (s, 1H, NH). Anal. calcd. for $C_{18}H_{20}N_2O_4S$: C, 59.98; H, 5.59; N, 7.77. Found: C, 60.17; H, 5.66; N; 7.93%.

2.2.3. General procedure for the synthesis of the 2,3-dihydro thieno[2,3-d]pyrimidine derivatives 7a,b

To a solution of compound **3a** (2.74 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) either formaldehyde (0.32 g, 0.01 mol) or benzaldehyde (1.06 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h then evaporated under vacuum.

The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration (Scheme 1).

6-Acetyl-5-methyl-3-phenyl-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one (**7a**): Color: Pale yellow crystals from ethanol. Yield: 66 % (1.89 g). M.p.: 210-212 °C. IR (KBr, ν, cm^{-1}): 3458-3312 (2 NH) , 3053 (CH, aromatic), 2982 (CH_3) ,1705-1688 (2 CO), 1628 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.68 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 6.41 (s, 2H, CH_2), 7.31-7.38 (m, 5H, C_6H_5), 8.34 (s, 1H, NH). Anal. calcd. for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78. Found: C, 62.68; H, 5.04; N, 9.61%.

6-Acetyl-5-methyl-2,3-diphenyl-2,3-dihydrothieno[2,3-d]pyridin-4(1H)-one (**7b**): Color: Orange crystals from ethanol. Yield: 78 % (2.83 g). M.p.: 188-190 °C. IR (KBr, ν, cm^{-1}): 3461 (NH), 3056 (CH, aromatic), 2980 (CH_3) , 1720-1687 (2 CO), 1623 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.67 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 7.02 (s, 1H, CH), 7.27-7.35 (m, 10H, $2C_6H_5$), 8.29 (s, 1H, NH). Anal. calcd. for $C_{21}H_{18}N_2O_2S$: C, 69.59; H, 5.01; N, 7.73. Found: C, 69.39; H, 4.88; N; 7.47%.

2.2.4. 2-Amino-5-(5-(cyanomethyl)-1H-pyrazol-3-yl)-4-methyl-N-phenylthiophene-3-carboxamide (10)

To a solution of compound **3a** (2.74 g, 0.01 mol) in 1,4-dioxane (40 mL) cyanoacetyl hydrazine (1.00 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water and the formed solid product was collected by filtration (Scheme 1). Color: White crystals from acetic acid. Yield: 69 % (2.32 g). M.p.: > 300 °C. IR (KBr, ν, cm^{-1}): 3479-3322 (NH_2, 2NH), 3052 (CH, aromatic), 2984 (CH_3), 2220 (CN), 1688 (CO), 1631 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.68 (s, 3H, CH_3), 4.97 (s, 2H, NH_2), 5.30 (s, 2H, CH_2), 6.89 (s, 1H, pyrazole CH), 7.29-7.38 (m, 5H, C_6H_5), 8.27 (s, 1H, NH), 8.35 (s, 1H, NH). Anal. calcd. for $C_{17}H_{15}N_5OS$: C, 60.52; H, 4.48; N, 20.76. Found: C, 60.39; H, 4.77; N; 20.91%.

2.2.5. General procedure for the synthesis benzo[b]thiophene derivatives 13a,b

To the dry solid of compound **3a** (2.74 g, 0.01 mol) containing ammonium acetate (0.50 g) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture was heated in an oil bath at 120 °C for 45 min. The formed solid product, upon cooling, was triturated with ethanol and the formed solid was collected by filtration (Scheme 2).

2,5-Diamino-6-cyano-7-methyl-N-phenylbenzo[b]thiophene-3-carboxamide (**13a**): Color: Yellow crystals from acetic acid. Yield: 81 % (2.64 g). M.p.: 112-115 °C. IR (KBr, ν, cm^{-1}): 3488-3320 ($2NH_2$, NH), 3056 (CH, aromatic), 2986 (CH_3), 2222 (CN), 1689 (CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.20 (s, 3H, CH_3), 4.77 (s, 2H, NH_2), 5.31 (s, 2H, NH_2), 7.26-7.39 (m, 6H, C_6H, C_6H_5), 8.29 (s, 1H, NH). Anal. calcd. for $C_{17}H_{14}N_4OS$: C, 63.34; H, 4.38; N, 17.38. Found: C, 63.39; H, 4.57; N; 17.44%.

2,5-Diamino-7-methyl-3-(phenylcarbamoyl)benzo[b]thiophen-6-yl propionate (**13b**): Color: Orange crystals from acetic acid. Yield: 80 % (2.96 g). M.p.: 223-225 °C. IR (KBr, ν, cm^{-1}): 3493-3320 ($2NH_2$, NH), 3057 (CH, aromatic), 2983 (CH_3), 2224 (CN), 1689 (CO), 1626 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.16 (t, 3H, J = 7.24 Hz, CH_3), 3.19 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.24 Hz, CH_2), 4.66 (s, 2H, NH_2), 5.31 (s, 2H, NH_2), 7.31-7.42 (m, 6H, C_6H, C_6H_5), 8.29 (s, 1H, NH). Anal. calcd. for $C_{19}H_{19}N_3O_3S$: C, 61.77; H, 5.18; N, 11.37. Found: C, 61.05; H, 5.28; N; 11.60%.

2.2.6. (E)-2,5-Diamino-6-cyano-N-phenyl-7-styrylbenzo[b]thiophene-3-carboxamide (15)

To a solution of compound **13a** (3.22 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine, benzaldehyde (1.06 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration (Scheme 2). Color: Orange crystals from ethanol. Yield: 72 % (2.96 g). M.p.: 180-182 °C. IR (KBr, ν, cm^{-1}): 3488-3328 ($2NH_2$, NH), 3054 (CH aromatic), 2220 (CN), 1686 (CO), 1628 (C=C). ^{1}H NMR (400MHz, DMSO-d_6, δ, ppm): 4.48 (s, 2H, NH_2), 5.33 (s, 2H, NH_2), 6.62 (d, 1H, CH=CH), 6.70 (d, 1H, CH=CH), 7.31-7.48 (m, 11H, C_6H, $2C_6H_5$), 8.32 (s, 1H, NH). Anal. calcd. for $C_{24}H_{18}N_4OS$: C, 70.22; H, 4.42; N, 13.65. Found: C, 70.09; H, 4.69; N; 13.81%.

2.2.7. 2,5-Diamino-7-(bromomethyl)-6-cyano-N-phenyl benzo[b]thiophene-3-carboxamide (16)

A solution of compound **13a** (3.22 g, 0.01 mol) in acetic acid (40 mL) was warmed till 60 °C then bromine solution (0.50 mL, 0.01 mol) in acetic acid (8 mL) was added drop wise with continuous stirring within a period 30 min. The reaction mixture was kept to stir at room temperature for an additional 3 h and the solid product formed upon pouring onto ice/water was collected by filtration (Scheme 2). Color: Pale yellow crystals from acetic acid. Yield: 60 % (2.41 g). M.p.: 140-142 °C. IR (KBr, ν, cm^{-1}): 3464-3348 ($2NH_2$, NH), 3056 (CH aromatic), 2221 (CN), 1689 (CO), 1623 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.45 (s, 2H, NH_2), 5.36 (s, 2H, NH_2), 5.39 (s, 2H, CH_2), 7.26-7.39 (m, 6H, C_6H, C_6H_5), 8.30 (s, 1H, NH). Anal. calcd. for $C_{17}H_{13}BrN_4OS$: C, 50.88; H, 3.27; N, 13.96. Found: C, 50.72; H, 3.61; N; 13.63%.

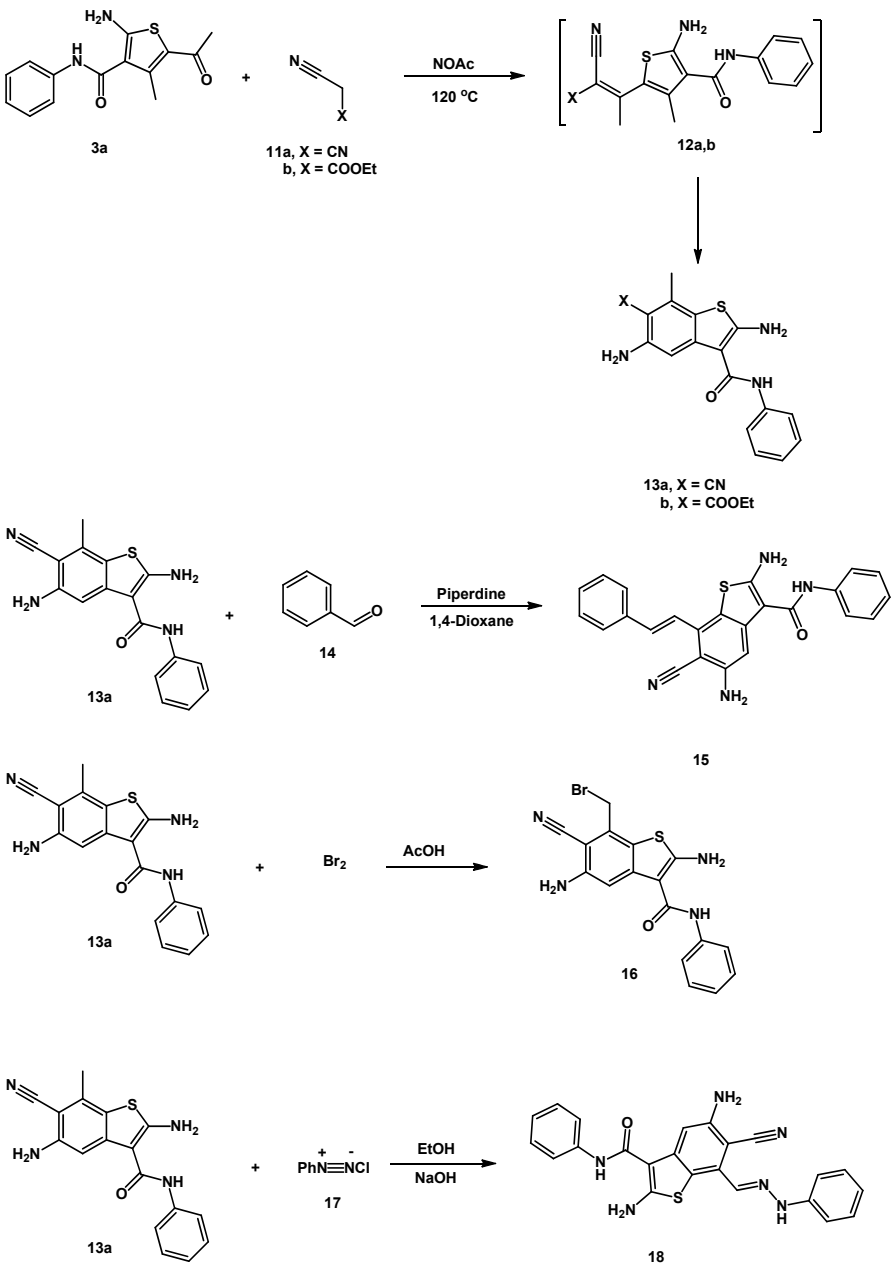
2.2.8. 2,5-Diamino-6-cyano-N-phenyl-7-((2-phenyl hydrazono)methyl)benzo[b]thiophene-3-carboxamide (18)

To a cold solution (0-5 °C) of compound **13a** (3.22 g, 0.01 mol) in ethanol (50 mL) containing sodium hydroxide benzenediazonium chloride (obtained via the addition of a cold solution of sodium nitrite (0.70 g, 0.01 mol, in water) to a cold solution of aniline (0.94 g, 0.01 mol) in concentrated hydrochloric acid (8 mL, 18 %) with continuous stirring) was added with continuous stirring. The whole reaction mixture was stirred at room temperature for 3 h and the formed solid product was collected by filtration (Scheme 2). Color: Reddish brown crystals from ethanol. Yield: 77 % (3.28 g). M.p.: 150-152 °C. IR (KBr, ν, cm^{-1}): 3481-3338 ($2NH_2$, 2NH), 3054 (CH aromatic), 2220 (CN), 1686 (CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.46 (s, 2H, NH_2), 5.33 (s, 2H, NH_2), 6.09 (s, 1H, CH), 7.28-7.43 (m, 11H, C_6H, $2C_6H_5$), 8.32 (s, 1H, NH), 8.43 (s, 1H, NH). Anal. calcd. for $C_{23}H_{18}N_6OS$: C, 64.77; H, 4.25; N, 19.71. Found: C, 64.68; H, 4.49; N; 19.57%.

2.2.9. General procedure for the synthesis of the bithiophene derivatives 19a,b

To a solution of compound **13a** (2.22 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) and elemental sulphur either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture, in each case, was heated under reflux for 1 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration (Scheme 3).

5,5′-Diamino-4′-cyano-3-methyl-N-phenyl-[2,3′-bithiophene]-4-carboxamide (**19a**): Color: Yellow crystals from acetic acid. Yield: 83 % (2.94 g). M.p.: 208-210 °C. IR (KBr, ν, cm^{-1}): 3473-3321 ($2NH_2$, NH), 3057 (CH aromatic), 2983 (CH_3), 2222 (CN), 1689 (CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.84 (s, 3H, CH_3), 4.48 (s, 2H, NH_2), 5.37 (s, 2H, NH_2), 6.89 (s, 1H, thiophene), 7.31-7.40 (m, 5H, C_6H_5), 8.30 (s, 1H, NH). Anal. calcd. for $C_{17}H_{14}N_4OS_2$: C, 57.61; H, 3.98; N, 15.81. Found: C, 57.83; H, 4.32; N; 15.77%.



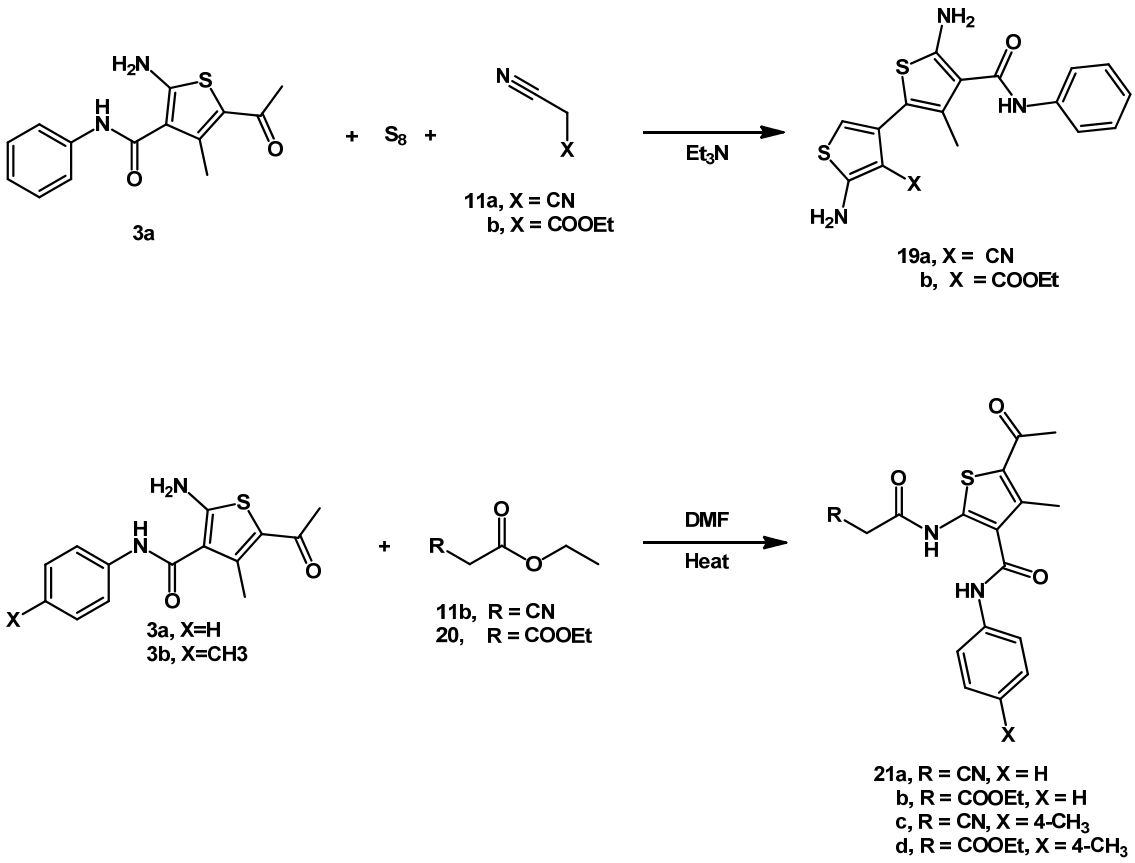
Scheme 2

Ethyl 5,5'-diamino-3-methyl-4-(phenylcarbamoyl)-[2,3'-bi thiophene]-4'-carboxylate (**19b**): Color: Yellow crystals from acetic acid. Yield: 74 % (2.96 g). M.p.: 177-179 °C. IR (KBr, ν, cm^{-1}): 3460-3342 ($2NH_2$, NH), 3054 (CH aromatic), 2980, 2877 (CH_3,CH_2), 1720, 1687 (2CO), 1632 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.14 (t, 3H, *J* = 7.22 Hz, CH_3), 2.89 (s, 3H, CH_3), 4.20 (q, 2H, *J* = 7.22 Hz, CH_2), 4.45 (s, 2H, NH_2), 5.35 (s, 2H, NH_2), 6.92 (s, 1H, thiophene), 7.28-7.43 (m, 5H, C_6H_5), 8.32 (s, 1H, NH). Anal. calcd. for $C_{19}H_{19}N_3O_3S_2$: C, 56.84; H, 4.77; N, 10.47. Found: C, 56.51; H, 4.49; N; 10.37%.

2.2.10. General procedure for the synthesis of the thiophene derivatives (21a-d)

To a solution of either compound **3a** (2.74 g, 0.01 mol) or **3b** (2.88 g, 0.01 mol) in dimethyl formamide (40 mL) either ethyl cyanoacetate (1.13 g, 0.01 mol) or diethyl malonate (1.60 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then poured onto ice/water. The solid product, so formed in each case, was collected by filtration (Scheme 3).

5-Acetyl-2-(2-cyanoacetamido)- 4-methyl-N-phenylthio phene-3-carboxamide (**21a**): Color: Yellow crystals from 1,4-dioxane. Yield: 79 % (2.70 g). M.p.: 214-216 °C. IR (KBr, ν, cm^{-1}): 3469-3360 (2NH), 3056 (CH aromatic), 2982, 2879 (CH_3,CH_2), 2220 (CN), 1721-1689 (3CO), 1634 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.86 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 5.82 (s, 2H, CH_2), 7.25-7.40 (m, 5H, C_6H_5), 8.26 (s, 1H, NH), 8.29 (s, 1H, NH). Anal. calcd. for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31. Found: C, 59.77; H, 4.67; N; 12.09%.



Scheme 3

Ethyl 3-((5-acetyl-4-methyl-3-(phenylcarbamoyl)thiophen-2-yl)amino)-3-oxopropanoate (**21b**): Color: Yellow crystals from 1,4-dioxane. Yield: 60 % (2.33 g). M.p.: 166-168 °C. IR (KBr, ν, cm^{-1}): 3482-3344 (2NH), 3053 (CH aromatic), 2980, 2883 (CH_3,CH_2), 1723-1685 (4CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.15 (t, 3H, *J* = 7.48 Hz, CH_3), 2.83 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 4.25 (q, 2H, *J* = 7.48 Hz, CH_2), 5.80 (s, 2H, CH_2), 7.29-7.37 (m, 5H, C_6H_5), 8.28 (s, 1H, NH), 8.30 (s, 1H, NH). Anal. calcd. for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21. Found: C, 58.88; H, 4.83; N; 6.93%.

5-Acetyl-2-(2-cyanoacetamido)-4-methyl-N-(p-tolyl) thio phene-3-carboxamide (**21c**): Color: Yellow crystals from 1,4-dioxane. Yield: 80 % (2.84 g). M.p.: 170-172 °C. IR (KBr, ν, cm^{-1}): 3455-3348 (2NH), 3054 (CH aromatic), 2980, 2883 (CH_3,CH_2), 2222 (CN), 1723-1686 (3CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.88 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 5.80 (s, 2H, CH_2), 7.29-7.38 (m, 4H, C_6H_4), 8.23 (s, 1H, NH), 8.32 (s, 1H, NH). Anal. calcd. for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.91; H, 4.90; N; 11.69%.

Ethyl 3-((5-acetyl-4-methyl-3-(p-tolylcarbamoyl)thiophen-2-yl)amino)-3-oxopropanoate (**21d**): Color: Yellow crystals from 1,4-dioxane. Yield: 66 % (2.66 g). M.p.: 180-182 °C. IR (KBr, ν, cm^{-1}): 3489-3364 (2NH), 3056 (CH aromatic), 2983, 2880 (CH_3,CH_2), 1720-1688 (4CO), 1630 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.13 (t, 3H, *J* = 6.99 Hz, CH_3), 2.80 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 4.24 (q, 2H, *J* = 6.99 Hz, CH_2), 5.82 (s, 2H, CH_2), 7.27-7.40 (m, 4H, C_6H_4), 8.26 (s, 1H, NH), 8.33 (s, 1H, NH). Anal. calcd. for $C_{20}H_{22}N_2O_5S$: C, 59.69; H, 5.51; N, 6.96. Found: C, 59.76; H, 5.47; N; 6.73%.

3. Results and discussions

3.1. Chemistry

In the present work, we are demonstrating the synthesis of thiophene derivatives together with their uses in heterocyclic synthesis. The cytotoxicity of the newly synthesized products were evaluated against cancer and normal cell lines. Thus, the reaction of acetylacetone with either cyanoacetanilide (**2a**) or 2-cyano-*N*-(*p*-tolyl)acetamide (**2b**) and elemental sulfur in the presence of triethylamine gave the thiophene derivatives **3a** and **3b**, respectively. The structures of the latter products were confirmed on the basis of analytical and spectral data. Thus the ^{1}H NMR spectrum of compound **3a** showed a two singlets at δ 2.68, 2.97 ppm indicating the presence of two CH_3 group, a singlet at δ 4.85 ppm indicating the presence of one NH_2 group, a multiplet at δ 7.31-7.37 ppm corresponding to phenyl group, and one singlet at δ 8.38 for NH group.

The reaction of compound **3a** with ethyl α-chloroacetate (**4**) gave the *N*-alkyl product **5**. On the other hand, the reaction of compound **3a** with either formaldehyde (**6a**) or benzaldehyde (**6b**) gave the 2,3-dihydrothieno [2,3-d]pyrimidin-4(1*H*)-one derivatives **7a** and **7b**, respectively. The analytical and spectral data of compound **7a** and **7b** were consistence with their respective structures. The ^{1}H NMR spectrum of compound **7a** showed a two singlets at δ 2.68, 2.80 ppm indicating the presence of two CH_3 group, a singlet at δ 6.41 ppm indicating the presence of one CH_2 group, a multiplet at δ 7.31-7.38 ppm corresponding to phenyl group, and one singlet at δ 8.34 for NH group.

Table 1. Cytotoxicity of the synthesized compounds against a variety of cancer cell lines [a] [IC_{50}[b] (nM)].

Compound	Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
3a	302	412	129	170	232	222	Na
3b	1255	2238	2065	429	467	180	Na
5	160	206	133	120	189	250	Na
7a	1443	527	2263	2310	230	2188	Na
7b	1320	1142	1754	3175	2048	1370	Na
10	1114	1210	1577	80	1293	126	Na
13a	120	141	159	1092	1042	1190	Na
13b	1113	2020	156	140	110	129	Na
15	30	28	208	233	1008	2170	Na
16	112	131	129	1159	189	1154	Na
18	1128	1078	1105	2120	1068	880	Na
19a	77	42	98	39	1943	590	Na
19b	20	42	120	38	129	78	Na
21a	1148	1260	2230	1287	2280	2266	Na
21b	2055	2072	1079	2693	2228	3332	Na
21c	340	633	48	290	42	1395	Na
21d	269	189	49	260	339	49	Na
CHS 828	25	2315	2067	1245	15	18	Na

[a] NUGC, gastric cancer; DLDI, colon cancer; HA22T and HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; MCF, breast cancer; WI38, normal fibroblast cells.

[b] The sample concentration that produces a 50% reduction in cell growth.

The acetyl group present in compound **3a** showed interesting activity towards condensation with hydrazide moiety. Thus, the reaction of compound **3a** with α-cyanoacetyl hydrazine (**8**) in 1,4-dioxane gave the 3-thienylpyrazole derivative (**10**) through the intermediate hydrazide-hydrazone derivative (**9**). Moreover, compound **3a** was found to be a good candidate towards Knoevenagel condensation reaction through its reaction with active methylene reagents. Interestingly, compound **3a** reacted with either malononitrile (**11a**) or ethyl cyanoacetate (**11b**) at 120 °C and the presence of ammonium acetate gave the benzo[*b*]thiophene derivatives (**13a** and **13b**), respectively. Formation of compound **13a**,**b** took place through the intermediate formation of the condensation products **12a**,**b**.

The high yield of compound **13a** encouraged us to study some of its chemical reactivity towards some chemical reagents. Interestingly the *o*-methyl group present in compound **13a** to the cyano group showed acidic nature, this appeared through the reaction of compound **13a** with benzaldehyde (**14**) in the presence of piperidine gave the benzylidene derivative **15**. On the other hand, compound **13a** reacted with bromine in acetic acid solution gave the bromomethyl derivative **16**. Moreover, the reaction of compound **13a** with benzenediazonium chloride at 0-5 °C in the presence of sodium hydroxide gave the phenylhydrazone derivative **18**. Structures of compounds **15**, **16** and **18** were confirmed on the basis of analytical and spectral data (see experimental section).

Next, we moved towards the reactivity of compound **3a** towards further thiophene formation through the use of the 2-acetyl group. Thus, the reaction of compound **3a** with either malononitrile (**11a**) or ethyl cyanoacetate (**11b**) and elemental sulphur gave the 3-thienyl thiophene derivatives **19a** and **19b**, respectively. On the other hand, compounds **3a** and **3b** reacted with either ethyl cyanoacetate (**11b**) or diethyl malonate (**20**) to give the amide derivatives **21a**-**d**, respectively. The structures of the latter products were established on the basis of analytical and spectral data (see experimental section).

3.2. Antitumor evaluations

3.2.1. Effect on the growth of human cancer cell lines

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). For comparison reasons, CHS 828 was used as standard anticancer drug. All of IC_{50} values in (nM) are listed in Table 1.

3.2.2. Structure activity relationship

From Table 1, the newly synthesized compounds were tested against the six cancer cell lines, the human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). The compounds **3a**, **5**, **13a**, **15**, **16**, **19a**, **19b**, **21c** and **21d** exhibited optimal cytotoxic effect against cancer cell lines with IC_{50}'s in the nM range. The reaction of 5-acetyl-2-amino-4-methyl-*N*-phenylthiophene-3-carboxamide (**3a**) with ethyl α-chloroacetate gave the ethyl 2-((5-acetyl-4-methyl-3-(phenylcarbamoyl) thiophen-2-yl)amino)acetate (**5**) through which the cytotoxicity decreases against the tested cancer cell lines. Such decrease in cytotoxicity is attributed to CH_2COOEt moiety. On the other hand, the reaction of compound **3a** with either formaldehyde or benzaldehyde gave the 2,3-dihydro-thieno[2,3-*d*]pyrimidin-4(1*H*)-one derivatives **7a** and **7b**, respectively. The pyrimidine derivatives **7a** and **7b** showed remarkable cytotoxicity which is more than that of compound **3a** against the tested cancer cell lines. Comparing the cytotoxicity of the compound **3a** and the 3-thienylpyrazole derivative **10** exhibited clearly that cyclization of compound **3a** to the pyrazole product **10** increases the cytotoxicity of the latter compound. The benzo[*b*]thiophene derivatives **13a** and **13b** showed potency against NUGC, HONE1 and MCF cell lines. The benzylidene derivative **15** with CH=CHPh moiety is less potent against the cell lines than compound **13a**. On the other hand bromomethyl-*N*-phenylbenzo[*b*]thiophene **16** with electronegative group the CN and Br showed high potent against NUGC, HONE1 and MCF cell lines. It is obvious that the phenyl hydrazine derivative **18** with the hydrazine moiety showed high cytotoxicity against NUGC, HEPG2, HONE1 and MCF cell lines. It is clear that for the 3-thienyl thiophene derivatives **19a** and **19b**, compound **19b** with COOEt moiety are less potent than compound **19a** with CN moiety. Finally the amide derivatives **21a-d**, compounds **21a** with CN moiety and compound **21b** with the COOEt moiety are more potent than compound **21c** and **21d**. Moreover compound **21a** showed high cytotoxicity against NUGC, HA22T, HEPG2, HONE1 and MCF cell lines as well as compound **21b** showed

high cytotoxicity against NUGC, HEPG2, HONE1 and MCF cell lines.

4. Conclusion

Many of the synthesized heterocyclic compounds were observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50} < 1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC_{50} > 10,000 nM). Among the tested compounds **3b**, **7a**, **7b**, **18**, **21a**, and **21b** was found to show the highest cytotoxic effect against the 6 cancer cell lines in the range of IC_{50} 33-442 nM. Broad spectrum antitumor activity was exhibited by compounds **3a**, **5**, **10**, **13a**, **13b** and **21c**.

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