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Original research paper

# Synthesis and structure elucidation of some novel thiophene and benzothiophene derivatives as cytotoxic agents

RAFAT M. MOHAREB[1]*
AMIRA E. M. ABDALLAH[2]
MAHER H. E. HELAL[2]
SOMIYA M. H. SHALOOF[3]

[1] *Department of Chemistry Faculty of Science, Cairo University Giza, A. R. Egypt*

[2] *Department of Chemistry Faculty of Science, Helwan University Ain Helwan, Cairo, A. R. Egypt*

[3] *Department of Chemistry Faculty of Science, Western Mountain University, Alzintan, Libya*

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Attempting to produce cyclized systems with potential anti-proliferative activity, a series of novel thiophene and benzothiophene derivatives were designed and synthesized. The reactivity of the latter derivatives towards different chemical reagents was studied. Twenty-one compounds were synthesized and evaluated as anti-cancer agents. The results showed that ethyl 5-amino-3-(4-chlorostyryl)-4-cyanothiophene-2-carboxylate (**5b**), ethyl 5-amino-4-((4-methoxyphenyl)carbonyl)-3-methylhiophene-2-carboxylate (**8c**) and 5-3-(ethoxy-3-oxopropanamido)-3-methyl-4-(phenylcarbamoyl)thiophene-2-carboxylate (**9**) were the most active compounds towards three tumor cell lines – MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) and a normal fibroblast human cell line (WI-38) compared to the anti-proliferative effects of the reference control doxorubicin.

*Keywords:* thiophene, benzothiophene, cytotoxic agents

Thiophene derivatives are a very important class of compounds with different uses, including industrial and medicinal chemistry (1–4). Further, substituted and fused thiophenes showed interesting applications in the field of medicinal chemistry (5–11).

On the other hand, benzo[*b*]thiophenes (12) are naturally occurring heterocyclic compounds (13) with diverse applications in medicinal chemistry and material science, attracting great interest in industry as well as academia. They display a wide range of biological and physiological functions such as anti-inflammatory (14), anti-fungal (15), anti-depressant (16), estrogen receptor modulating (17), anti-mitotic (18), kinases inhibiting (19, 20) and anti-cancer (21, 22). Several commercially available drugs, such as sertaconazole nitrate and benocyclidine, contain the benzo[*b*]thiophene core structure as well.

* Correspondence; e-mail: raafat_mohareb@yahoo.com

## EXPERIMENTAL

### *General*

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 Pye Unicam SP-1000 spectrophotometer (Pye Unicam, UK). $^1$H NMR and $^{13}$C NMR spectra were recorded with Varian Gemini-200 (200 MHz, Varian UK) and Jeol AS 500 MHz (Jeol, Japan) instruments in DMSO-$d_6$ as a solvent, using TMS as internal standard. Chemical shifts are expressed as $\delta$ ppm. The mass spectra were recorded with a Hewlett Packard 5988 A GC/MS system (Hewlett Packard, Agilent, USA) and GCMS-QP 1000Ex Shimadzu (EI, 70 eV) (Japan) instruments. Analytical data were obtained from a Vario EL III Elemental CHNS analyzer (Germany).

### *Syntheses*

*Ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate (**1a**) and diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**1b**). General procedure.* – Equimolar amounts of ethyl acetoacetate (1.30 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) containing a catalytic amount of triethylamine in ethanol (25 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol), were added. The reaction mixture in each case was heated under reflux for 3 hours, then cooled and neutralized by pouring into an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed in each case was collected by filtration and crystallized from ethanol to give **1a** and **1b**, respectively (Table I).

*Ethyl 5-(acetylamino)-4-cyano-3-methylthiophene-2-carboxylate (**2**).* – A solution of **1a** (2.10 g, 0.01 mol) in acetic acid/acetic anhydride (10:3 mL) was heated under reflux for 3 hours. The solid product formed upon pouring into an ice/water mixture was collected by filtration, washed with water and crystallized from ethanol to give **2** (Table I).

*Ethyl 5-amino-4-cyano-3-((2-phenylhydrazinylidene)methyl) thiophene-2-carboxylate (**3a**), ethyl 5-amino-3-(2-(4-chlorophenyl)hydrazinylidene)-methyl-4-cyanothiophene-2-carboxylate (**3b**), ethyl 5-amino-4-cyano-3-[2-(4-methoxyphenyl)hydrazinylidene]-methyl thiophene-2-carboxylate (**3c**) and ethyl 5-amino-4-cyano-3-(2-(4-methylphenyl)hydrazinylidene)methylthiophene-2-carboxylate (**3d**). General procedure.* – To a cold solution (0–5 °C) of **1a** (2.10 g, 0.01 mol) in ethanol (98 %, 20 mL) containing sodium hydroxide (10 %, 5 mL), an equimolar amount of either diazotized aniline (0.93 g, 0.01 mol), diazotiazed 4-chloroaniline (1.27 g, 0.01 mol), diazotiazed 4-methoxyaniline (1.23 g, 0.01 mol) or diazotiazed *p*-toluidene (1.07 g, 0.01 mol) [the corresponding diazonium salt, in each case, was prepared by adding a $NaNO_2$ (0.70 g, 0.01 mol) solution to a cold solution (0–5 °C) of either aniline, 4-chloroaniline, 4-methoxyaniline or *p*-toluidene in concentrated hydrochloric acid (18 μmol $L^{-1}$, 5 mL) under continuous stirring] was gradually added under stirring. The solid products formed upon cooling in an ice bath were collected by filtration, washed with water and crystallized from ethanol to give **3a-d**, respectively (Table I).

*Ethyl 4-cyano-5-((2,2-dicyanoethylidene)amino)-3-methylthiophene-2-carboxylate (**4**).* – To a mixture of **1a** (2.10 g, 0.01 mol) and ethyl orthoformate (1.48 g, 0.01 mol), a catalytic amount of piperidine was added and the reaction mixture was heated in an oil bath at 120 °C for 2

*Table I. Physicochemical and analytical data of the newly synthesized compounds*

| Compd. | Molecular formula ($M_r$) | M. p. (°C) | Yield (%) | Colour | Analysis (calcd./found) (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | S |
| **1a** | $C_9H_{10}N_2O_2S$ (210.25) | 258–260 | 75 | off white crystals | 51.41/51.48 | 4.79/ 5.11 | 13.32/13.00 | 15.25/15.43 |
| **1b** | $C_{11}H_{15}NO_4S$ (257.31) | 83-85 | 75 | orange crystals | 51.35/50.90 | 5.88/5.80 | 5.44/5.24 | 12.46/12.11 |
| **2** | $C_{11}H_{12}N_2O_3S$ (252.92) | >300 | 92 | off white crystals | 52.37/52.07 | 4.79/4.59 | 11.10/11.20 | 12.71/13.03 |
| **3a** | $C_{15}H_{14}N_4O_2S$ (314.36) | 168-170 | 70 | orange crystals | 57.31/57.00 | 4.49/4.22 | 17.82/17.47 | 10.20/10.53 |
| **3b** | $C_{15}H_{13}N_4O_2SCl$ (348.81) | 118–120 | 60 | brown crystals | 51.65/51.35 | 3.76/3.43 | 16.06/15.85 | 9.19/8.78 |
| **3c** | $C_{16}H_{16}N_4O_3S$ (344.39) | 108–110 | 80 | brown crystals | 55.80/55.40 | 4.68/4.20 | 16.27/15.90 | 9.31/8.91 |
| **3d** | $C_{16}H_{16}N_4O_2S$ (328.39) | 133–135 | 64 | orange crystals | 58.52/58.64 | 4.91/4.56 | 17.06/17.11 | 9.76/9.40 |
| **4** | $C_{13}H_{10}N_4O_2S$ (286.31) | >300 | 70 | brown crystals | 54.54/54.20 | 3.52/3.79 | 19.57/19.80 | 11.20/10.88 |
| **5a** | $C_{16}H_{14}N_2O_2S$ (298.36) | 91–93 | 71 | brown crystals | 64.41/64.19 | 4.73/4.96 | 9.39/9.17 | 10.75/11.04 |
| **5b** | $C_{16}H_{13}N_2O_2SCl$ (332.80) | 93–95 | 90 | brown crystals | 57.74/58.03 | 3.94/4.10 | 8.42/8.17 | 9.63/9.83 |
| **5c** | $C_{17}H_{16}N_2O_3S$ (328.39) | 93–95 | 90 | brown crystals | 62.18/62.11 | 4.91/4.93 | 8.53/8.58 | 9.76/9.35 |
| **6** | $C_{16}H_{14}N_2O_3S$ (314.40) | 173–175 | 94 | faint yellow crystals | 61.13/61.07 | 4.49/4.63 | 8.91/9.07 | 10.20/10.45 |
| **7** | $C_{12}H_{11}N_3O_3S$ (277.30) | 128–130 | 93 | brown crystals | 51.98/51.68 | 4.00/3.89 | 15.15/14.80 | 11.56/11.20 |
| **8a** | $C_{15}H_{16}N_2O_3S$ (304.40) | 183–185 | 70 | orange crystals | 59.19/59.54 | 59.19/59.54 | 9.20/9.50 | 10.54/10.20 |
| **8b** | $C_{16}H_{18}N_2O_3S$ (318.40) | 182–184 | 65 | orange crystals | 60.36/60.64 | 5.70/5.31 | 8.80/9.20 | 10.07/9.70 |
| **8c** | $C_{16}H_{18}N_2O_4S$ (334.40) | 118–120 | 60 | orange crystals | 57.47/57.87 | 5.43/5.73 | 8.38/8.68 | 9.59/9.89 |
| **9** | $C_{20}H_{22}N_2O_6S$ (418.50) | 118–120 | 65 | brown crystals | 57.40/57.70 | 5.30/5.60 | 6.69/6.99 | 7.66/7.94 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **10a** | $C_{18}H_{16}N_4O_2S$ (352.40) | 211–215 | 86 | black crystals | 61.35/60.95 | 4.58/4.20 | 15.90/15.50 | 9.10/9.50 |
| **10b** | $C_{20}H_{21}N_3O_4S$ (399.50) | 142–144 | 70 | white crystals | 60.13/59.83 | 5.30/4.95 | 10.52/10.36 | 8.03/8.40 |
| **11** | $C_{17}H_{18}N_2O_4S$ (346.40) | 98–100 | 76 | yellowish white crystals | 58.94/58.56 | 5.24/4.90 | 8.09/7.70 | 9.26/8.96 |
| **12** | $C_{22}H_{20}N_2O_3S$ (392.50) | 213–215 | 78 | brown crystals | 67.33/67.18 | 5.14/4.80 | 7.14/7.44 | 8.17/7.80 |

hours. The mixture was then boiled in ethanol for a few minutes, poured onto an acidified ice/water mixture and the product was crystallized from ethanol to give **4** (Table I).

*Ethyl 5-amino-4-cyano-3-styrylthiophene-2-carboxylate (**5a**), ethyl 5-amino-3-(4-chlorostyryl)-4-cyanothiophene-2-carboxylate (**5b**), ethyl 5-amino-4-cyano-3-[2-(2-methoxyphenyl)-ethenyl]thiophene-2-carboxylate (**5c**) and ethyl 5-amino-4-cyano-3-[2-(2-hydroxypentyl)ethenyl]thiophene-carboxylate (**6**). General procedure.* – To a solution of **1a** (2.10 g, 0.01 mol) containing a catalytic amount of piperidine (0.5 mL), either benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol), 4-methoxybenzaldehyde (1.36 g, 0.01 mol) or salicyaldehyde (1.22 g, 0.01 mol) was added and heated in an oil bath at 120 °C for about 2 hours, then boiled in ethanol (20 mL) for a few minutes. The solid products obtained upon pouring into an acidified ice/water mixture were crystallized from ethanol to give **5a-c** and **6**, respectively (Table I).

*Ethyl 3,6-diamino-5-cyano-4-hydroxybenzo[c]thiophene-1-carboxylate (**7**).* – To a solution of **1b** (2.57 g, 0.01 mol) in 1,4-dioxane (25 mL) containing a catalytic amount of triethylamine, malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 hours, then cooled and neutralized by pouring into an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and crystallized from 1,4-dioxane to give **7** (Table I).

*2-Ethyl 5-amino-3-methyl-4-(phenylcarbamoyl)thiophene-2-carboxylate (**8a**), ethyl 5-amino-3-methyl-4-(*p*-tolylcarbamoyl)thiophene-2-carboxylate (**8b**) and ethyl 5-amino-4-((4-methoxyphenyl)carbamoyl)-3-methylthiophene-2-carboxylate (**8c**). General procedure.* – To a solution of either 2-cyano-*N*-phenylacetamide (1.60 g, 0.01 mol), 2-cyano-*N*-(*p*-tolyl)acetamide (1.74 g, 0.01 mol) or *N*-(4-methoxyphenyl)-2-cyanoacetamide (1.90 g, 0.01 mol) [prepared by adding ethyl cyanoacetate (1.13 g, 0.01 mol) to either aniline (0.93 g, 0.01 mol), *p*-toludiene (1.07 g , 0.01 mol) or 4-methoxyaniline (1.23 g, 0.01 mol) under reflux for 2 hours, then poured into an ice/water mixture and collected by filtration] in ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL), ethyl acetoacetate (1.30 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 moL) were added. The reaction mixture was heated under reflux for 5 hours, then cooled, and neutralized by pouring into an acidified ice/water mixture. The solid product formed in each case was filtered off and crystallized from ethanol to give **8a-c**, respectively (Table I).

*Ethyl 5-(3-ethoxy-3-oxopropanamido)-3-methyl-4-(phenylcarbamoyl)thiophene-2-carboxylate (**9**).* – Equimolar amounts of **8a** (3.04 g, 0.01 mol) and malonic acid diethyl ester (1.60 g,

0.01 mol) in dimethylformamide (20 mL) were heated under reflux for 5 hours. The solid product formed upon pouring into an ice/water mixture was collected by filtration and crystallized from dimethylformamide to give **9** (Table I).

*Ethyl 3,6-diamino-5-cyano-4-(phenylamino)benzo[c]thiophene-1-carboxylate (**10a**) and diethyl 3,6-diamino-4-(phenylamino)benzo[c]thiophene-1,5-dicarboxylate (**10b**). General procedure.* – To a solution of **8a** (3.04 g, 0.01 moL) in 1,4 dioxane (25 mL) and dimethylformamide (15 mL) containing a catalytic amount of triethylamine, 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 5 hours. After cooling, the reaction mixture, in each case, was acidified by a few drops of hydrochloric acid (18 mol $L^{-1}$, 0.50 mL) and the crude product was precipitated, collected by filtration and crystallized from 1,4-dioxane to give **10a** and **10b**, respectively (Table I).

*Ethyl 5-acetamido-3-methyl-4-(phenylcarbamoyl)thiophene-2-carboxylate (**11**).* – A solution of **8a** (3.04 g, 0.01 mol) in acetic acid/acetic anhydride (10:3 mL) was heated under reflux for 3 hours. The solid product formed upon pouring into an ice/water mixture was collected by filtration, washed with water and crystallized from ethanol to give **11** (Table I).

*Ethyl 5-amino-4-(phenylcarbamoyl)-3-styrylthiophene-2-carboxylate (**12**).* – A solution of **8a** (3.04 g, 0.01 mol) containing a catalytic amount of piperidine (0.50 mL) and benzaldehyde (1.06 g, 0.01 mol) was heated in an oil bath at 120 °C for about 2 hours and then boiled in ethanol (20 mL) for a few minutes. The solid product obtained upon pouring into an acidified ice/water mixture was crystallized from ethanol to give **12** (Table I).

## In vitro *cytotoxic activity of the newly synthesized compounds*

Fetal bovine serum (FBS) and L-glutamine were obtained from Gibco Invitrogen Company (UK). RPMI-1640 medium was provided by Cambrex (USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were obtained from Sigma Chemical Company (USA).

Stock solutions of compounds **1** to **12** were prepared in DMSO and kept at –20 °C. Appropriate dilutions of the compounds were freshly prepared just prior to assays. Final concentrations of DMSO did not interfere with cell growth.

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) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grew as monolayers and were routinely maintained in RPMI-1640 medium supplemented with 5 % heat-inactivated FBS, 2 mmol $L^{-1}$ glutamine and antibiotics (penicillin 100 μg $mL^{-1}$, streptomycin 100 μg $mL^{-1}$), at 37 °C in a humidified atmosphere containing 5 % $CO_2$. Exponentially growing cells were obtained by plating $1.5 \times 10^5$ cell $mL^{-1}$ for MCF-7 and SF-268 and $0.75 \times 10^4$ cell $mL^{-1}$ 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 and the results are given in Table III.

*Table II. Spectral data of the newly synthesized compounds*

| Compd. | IR ($\nu_{max}$, $cm^{-1}$) | $^1H$ NMR (DMSO-$d_6$) ($\delta$, ppm) | $^{13}C$ NMR (DMSO-$d_6$) ($\delta$, ppm) | MS: *m/z* (%) |
|---|---|---|---|---|
| **1a** | 3401–3203 ($NH_2$), 2983–2931 ($CH_2$, $2CH_3$), 2204 (CN), 1675 (C=O), 1545, 1494 (C=C) | 1.18 (s, 3H, $CH_3$), 1.23 (t, 3H, $CH_3$), 4.15–4.18 (q, 2H, $CH_2$), 7.93 (s, 2H, $NH_2$) | 14.20 ($CH_3$), 14.66 ($CH_3$), 60.08 ($CH_2$), 114.97 (CN), 88.73, 106.95, 146.31, 152.05 (thiophene 4C), 166.64 (C=O) | 211[$M^+$+1] (19.50), 210 [$M^+$] (75.60), 208 [$M^+$-2] (7.30) |
| **1b** | 3421–3310 ($NH_2$), 2983–2949 ($2CH_2$, $3CH_3$), 1675, 1593 (2C=O), 1528, 1440 (C=C) | 1.16 (s, 3H, $CH_3$), 1.17–1.21 (t, 3H, $CH_3$), 1.22–1.28 (t, 3H, $CH_3$), 4.12–4.15 (q, 2H, $CH_2$), 4.16–4.20 (q, 2H, $CH_2$), 7.90 (s, 2H, $NH_2$) | 8.52 ($CH_3$), 14.23 ($CH_3$), 15.56 ($CH_3$), 45.64 ($CH_3$), 59.36 ($CH_2$), 59.77 ($CH_2$), 106.15, 147.47, 161.95, 164.96 (thiophene 4C), 166.36, 166.63 (2C=O) | 259 [$M^+$+2] (0.60), 258 [$M^+$+1] (2.00), 257 [$M^+$] (7.40), 256 [$M^+$-1] (3.40) |
| **2** | 3400–3260 (NH), 2970 ($CH_2$, $3CH_3$), 2223 (CN), 1730, 1709 (2C=O), 1563, 1450 (C=C) | 1.26 (s, 3H, $CH_3$) 1.28–1.31 (t, 3H, $CH_3$), 1.91 (s, 3H, $CH_3$), 4.24–4.26 (q, 2H, $CH_2$), 12.06 (s, 1H, NH) | 14.17 ($CH_3$ ester), 22.66 ($CH_3$), 60.43 ($CH_2$), 116.29 (CN), 95.80, 113.61, 143.36, 152.23, 152.22 (thiophene 4C), 161.53, 169.57 (2C=O) | 253 [$M^+$] (29.20), 252 [$M^+$–1] (20.80), 57.20 (100.00) |
| **3a** | 3397–3197 (NH, $NH_2$), 3026 (CH aromatic), 2982 ($CH_2$, $CH_3$), 2211 (CN), 1673 (C=O), 1602, 1495 (C=C), 1535 (=N-NH) | 1.21–1.26 (t, 3H, $CH_3$), 4.14–4.21 (q, 2H, $CH_2$), 6.73 (s, 1H, CH), 6.76–7.69 (m, 5H, $C_6H_5$), 7.93 (s, 2H, $NH_2$), 9.28 (s, 1H, NH) | 14.22 ($CH_3$), 60.09 ($CH_2$), 114.98 (CN), 106.93, 116.10, 120.00, 123.50, 125.00, 129.17, 129.74, 130.00, 146.33 (thiophene 4C, $C_6H_5$), 161.31 (C=N), 166.65 (C=O) | 314 [$M^+$] (2.40), 313 [$M^+$-1] (1.00), 312 [$M^+$-2] (1.00), 165 (100.00), 77 $[C_6H_5]^+$ (83.20) |
| **3b** | 3425–3201 (NH, $NH_2$), 3100 (CH aromatic), 2900 ($CH_2$, $CH_3$), 2216 (CN), 1672 (C=O), 1600, 1486 (C=C), 1520 (=N–NH) | 1.23–1.27 (t, 3H, $CH_3$), 4.24–4.31 (q, 2H, $CH_2$), 7.07 (s, 1H, CH), 7.35–7.96 (m, 4H, $C_6H_4$), 7.98 (s, 2H, $NH_2$), 8.70 (s, 1H, NH) | 14.40 ($CH_3$), 61.09 ($CH_2$), 117.22 (CN), 113.56, 114.98, 119.02, 123.00, 125.50, 128.99, 129.51, 138.62, 145.22, 160.90, (thiophene 4C, $C_6H_5$), 161.30 (C=N), 166.70 (C=O) | 351 [$M^+$+2] (25.00), 60 (100.00) |
| **3c** | 3408–3191 (NH, $NH_2$), 3060 (CH aromatic), 2985 ($CH_2$, $2CH_3$), 2214 (CN), 1670 (C=O), 1600, 1458 (C=C), 1519 (=N–NH) | 1.20–1.22 (t, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 4.22–4.29 (q, 2H, $CH_2$), 6.99 (s, 1H, CH), 7.02–7.37 (m, 4H, $C_6H_4$), 8.00 (s, 2H, $NH_2$), 9.80 (s, 1H, NH) | 14.16 ($CH_3$), 55.40 ($OCH_3$), 60.03 ($CH_2$), 115.01 (CN), 106.93, 113.74, 114.65, 120.33, 122.00, 125.45, 127.75, 129.35, 132.51, 146.25 (thiophene 4C, $C_6H_5$), 161.33 (C=N), 166.69 (C=O) | 346 [$M^+$+2] (14.30), 57 (100.00) |
| **3d** | 3433–3191 (NH, $NH_2$), 3090 (CH aromatic), 2986–2925 ($CH_2$, $2CH_3$), 2220 (CN), 1673 (C=O), 1603, 1454 (C=C), 1526 (=N–NH) | 1.21–1.25 (t, 3H, $CH_3$), 2.24 (s, 3H, $CH_3$), 4.24–4.31 (q, 2H, $CH_2$), 7.10 (s, 1H, CH), 7.22–7.31 (m, 4H, $C_6H_4$), 7.92 (s, 2H, $NH_2$), 9.05 (s, 1H, NH) | 14.46 ($CH_3$), 20.52 ($CH_3$), 61.03 ($CH_2$), 116.01 (CN), 113.90, 114.98, 118.00, 123.54, 130.11, 134.80, 138.00, 144.00, 145.23, 146.31 (thiophene 4C, $C_6H_5$), 161.08 (C=O), 166.65 (C=N) | 328 [$M^+$] (4.00), 164 (100.00), 76 $[C_6H_4]^+$ (11.10) |

| | | | | |
|---|---|---|---|---|
| **4** | 2931 (2CH, $CH_2$, $2CH_3$), 2199 (CN), 1611 (C=O), 1501, 1439 (C=C), 1543 (C=N) | 1.30 (t, 3H, $CH_3$), 1.63 (s, 3H, $CH_3$), 4.00 (s, 1H, CH), 4.17 (q, 2H, $CH_2$), 6.80 (s, 1H, CH) | | |
| **5a** | 3398–3204 ($NH_2$), 2977–2931 ($CH_2$, $CH_3$), 2205 (CN), 1678 (C=O), 1630, 1496 (C=C) | 1.21 (t, 3H, $CH_3$), 4.14–4.18 (q, 2H, $CH_2$), 4.28 (s, 1H, CH), 4.43 (s, 1H, CH), 7.24–7.56 (m, 5H, $C_6H_5$), 7.93 (s, 2H, $NH_2$) | 14.66 ($CH_3$), 60.09 ($CH_2$), 88.73 (HC=CH), 106.95 (HC=CH), 114.98 (CN), 125.00, 127.34, 128.52, 136.00, 150.00, 146.32, 161.31 (thiophene 4C, $C_6H_5$), 166.65 (C=O) | 300 [$M^+$+2] (5.70), 165 (100.0) 77 $[C_6H_5]^+$ (25.80) |
| **5b** | 3397–3204 ($NH_2$), 2979–2859 ($CH_2$, $CH_3$), 2205 (CN), 1677 (C=O), 1634, 1495 (C=C) | 1.10 (t, 3H, $CH_3$), 4.13–4.17 (q, 2H, $CH_2$), 7.00 (s, 1H, CH), 7.25 (s, 1H, CH), 7.32–7.44 (m, 4H, $C_6H_4$), 7.93 (s, 2H, $NH_2$) | 14.66 ($CH_3$), 60.07 ($CH_2$), 88.72 (HC=CH), 106.95 (HC=CH), 114.98 (CN), 128.49, 129.21, 130.43, 131.07, 132.00, 136.00, 146.30, 147.00, 148.00, 161.30 (thiophene 4C, $C_6H_5$), 166.65 (C=O) | 335 [$M^+$+2] (8.20), 334 [$M^+$+1] (13.40), 333 [$M^+$] (11.3), 332 [$M^+$-1] (10.30), 331 [$M^+$-2] (7.20), 125 (100.00), 76 $[C_6H_4]^+$ (23.70) |
| **5c** | 3399–3204 ($NH_2$), 2931–2840 ($CH_2$, $2CH_3$), 2204 (CN), 1677 (C=O), 1632, 1502 (C=C) | 1.21–1.26 (t, 3H, $CH_3$), 2.10 (s, 3H, $CH_3$), 4.13–4.20 (q, 2H, $CH_2$), 6.80 (s, 1H, CH), 6.93 (s, 1H, CH), 6.98–7.91 (m, 4H, $C_6H_4$), 7.93 (s, 2H, $NH_2$) | 114.20 ($CH_3$ ester), 55.02 ($OCH_3$), 60.07 ($CH_2$), 88.72 (HC=CH), 106.95 (HC=CH), 113.88 (CN), 114.98, 122.00, 128.79, 129.55, 132.00, 133.00, 146.30, 147.00, 158.00, 161.30 (thiophene 4C, $C_6H_5$), 166.65 (C=O) | 329 [$M^+$+1] (5.40), 328 [$M^+$] (32.40), 165 (100.00) |
| **6** | 3317–3204 (OH, $NH_2$), 2935 ($CH_2$, $CH_3$), 2204 (CN), 1676 (C=O), 1624, 1497 (C=C) | 1.17–1.19 (t, 3H, $CH_3$), 4.13–4.20 (q, 2H, $CH_2$), 6.10 (s, 1H, CH), 6.71 (s, 1H, CH), 6.73–7.01 (m, 4H, $C_6H_4$), 7.94 (s, 2H, $NH_2$), 9.49 (s, 1H, OH) | | 314 [$M^+$] (1.04), 165 (100.00), 76 $[C_6H_4]^+$ (5.92) |
| **7** | 3412–3301 (OH, $2NH_2$), 2979–2937 ($CH_2$, $CH_3$), 2205 (CN), 1664 (C=O), 1583, 1449 (C=C) | 1.16–1.18 (t, 3H, $CH_3$), 4.18–4.23 (q, 2H, $CH_2$), 7.10 (s, 2H, $NH_2$), 7.89 (s, 1H, CH benzene ring), 9.10 (s, 2H, $NH_2$), 14.23 (s, 1H, OH) | 14.22 ($CH_3$), 59.79 ($CH_2$), 115.00 (CN), 122.00, 125.00, 134.00, 136.00, 144.00, 147.39, 161.96, 164.68 (thiophene 4C, $C_6H_5$), 166.66 (C=O) | 278 [$M^+$+1] (58.30], 277 [$M^+$] (41.70), 59 (100.00) |
| **8a** | 3416–3208 (NH, $NH_2$), 3143–3057 (CH aromatic), 2961–2812 ($CH_2$, $2CH_3$), 1669, 1613 (2C=O), 1558, 1493 (C=C) | 1.10 (s, 3H, $CH_3$), 1.20 (t, 3H, $CH_3$), 4.10 (q, 2H, $CH_2$), 7.07–7.52 (m, 5H, $C_6H_5$), 7.55 (s, 2H, $NH_2$), 10.26 (s, 1H, NH) | 8.55 ($CH_3$), 26.65 ($CH_3$), 45.62 ($CH_2$), 115.87, 119.20, 119.30, 123.84, 128.84, 128.94, 138.33, 144.00, 151.00, 155.00 (thiophene 4C, $C_6H_4$), 160.94, 161.00 (2C=O) | 304 [$M^+$] (0.17], 93 (100.00), 76 $[C_6H_4]^+$ (6.39) |
| **8b** | 3405–3206 (NH, $NH_2$), 3134–3037 (CH aromatic), 2960–2922 ($CH_2$, $3CH_3$), 1662, 1615 (2C=O), 1549, 1452 (C=C) | 1.06 (s, 3H, $CH_3$), 1.15–1.20 (t, 3H, $CH_3$), 1.91 (s, 3H, $CH_3$), 4.15–4.18 (q, 2H, $CH_2$), 7.12–7.48 (m, 4H, $C_6H_4$), 7.90 (s, 2H, $NH_2$), 10.18 (s, 1H, NH) | 8.61 ($CH_3$), 20.39 ($CH_3$), 26.59 ($CH_3$), 45.73 ($CH_2$), 115.91, 119.22, 129.22, 132.85, 135.82 (thiophene 4C, $C_6H_4$), 160.40, 160.66 (2C=O) | 318 [$M^+$] (11.60), 317 [$M^+$-1] (9.80), 86 (100.00) |

| | | | |
|---|---|---|---|
| **8c** | 3426 (NH, $NH_2$), 3050 (CH aromatic), 2924 ($CH_2$, $3CH_3$), 1630, 1611 (2C=O), 1509, 1480 (C=C) | 1.16 (s, 3H, $CH_3$), 1.18–1.24 (t, 3H, $CH_3$), 2.17 (s, 3H, $CH_3$), 4.10 (q, 2H, $CH_2$), 6.89–7.46 (m, 4H, $C_6H_4$), 7.53 (s, 2H, $NH_2$), 10.12 (s, 1H, NH) | 333 [$M^+$-1] (11.40), 332 [$M^+$-2] (9.10), 108 (100.00) |
| **9** | 3427 (2NH), 2977 ($3CH_2$, $3CH_3$), 3060 (CH aromatic), 1730, 1740, 1639, 1610 (4C=O), 1544, 1495 (C=C) | 1.18–1.21 (t, 3H, $CH_3$), 1.23–1.26 (t, 3H, $CH_3$), 1.29 (s, 3H, $CH_3$), 4.15– 4.17 (q, 2H, $CH_2$), 4.20 (s, 2H, $CH_2$), 4.26 (s, 2H, $CH_2$), 7.03–7.80 (m, 5H, $C_6H_5$), 10.13 (s, 1H, NH), 12.10 (s, 1H, NH) | 420 [$M^+$+2] (0.80), 419 [$M^+$ +1] (0.80), 93 (100.00), 77 $[C_6H_5]^+$ (49.20) |
| **10a** | 3419–3207 (NH, $NH_2$), 3144–3053 (CH aromatic), 2957–2915 ($2CH_2$, $CH_3$), 2210 (CN), 1667 (C=O), 1616, 1488 (C=C) | 1.23–1.26 (t, 3H, $CH_3$), 4.15–4.17 (q, 2H, $CH_2$), 4.40 (s, 2H, $CH_2$), 7.07–7.55 (m, 6H, $C_6H_5$, CH benzene ring), 7.90 (s, 2H, $NH_2$), 10.26 (s, 1H, NH) | 353 [$M^+$+1] (15.70), 93 (100.00), 77 $[C_6H_5]^+$ (52.90) |
| **10b** | 3427–3210 (NH, $NH_2$), 3144–3103 (CH aromatic), 2964 ($3CH_2$, $2CH_3$), 1667, 1609 (2C=O), 1556, 1493 (C=C) | 1.15–1.20 (t, 3H, $CH_3$), 1.22–1.24 (t, 3H, $CH_3$), 4.13–4.15 (q, 2H, $CH_2$), 4.16–4.17 (q, 2H, $CH_2$), 4.20 (s, 2H, $CH_2$), 7.02–7.67 (m, 6H, $C_6H_5$, CH benzene ring), 7.91 (s, 2H, $NH_2$), 10.27 (s, 1H, NH) | 400 [$M^+$+1] (24.00), 184 (100.00) |
| **11** | 3264 (2NH), 3050 (CH aromatic), 2976 ($CH_2$, $3CH_3$), 1730, 1673, 1597 (3C=O), 1545, 1493 (C=C) | 1.08-1.13 (t, 3H, $CH_3$), 1.34 (s, 3H, $CH_3$), 1.90 (s, 3H, $CH_3$), 4.15–4.35 (q, 2H, $CH_2$), 7.07–7.73 (m, 5H, $C_6H_5$), 10.26 (s, 1H, NH), 11.10 (s, 1H, NH) | 346 [$M^+$] (5.60), 93 (100.00), 77 $[C_6H_5]^+$ (55.60) |
| **12** | 3441-3321 (NH, $NH_2$), 3050 (CH aromatic), 2931 ($CH_2$, $CH_3$), 1677, 1600 (2C=O), 1538, 1493 (C=C) | 1.18-1.21 (t, 3H, $CH_3$), 4.15 (s, 1H, CH), 4.17 (s, 1H, CH), 4.64–4.76 (q, 2H, $CH_2$), 7.12–8.10 (m, 10H, $2C_6H_5$), 8.29 (s, 2H, $NH_2$), 10.41 (s, 1H, NH) | 391 [$M^+$-1] (0.60), 390 [$M^+$-2] (0.60), 156 (100.00), 77 $[C_6H_5]^+$ (69.50) |

*Table III.* $IC_{50}$ *of the newly synthesized compounds against three human tumor and one normal human cell line*

| Compd. | $IC_{50}$ (μmol $L^{-1}$)[a] | | | |
|---|---|---|---|---|
| | MCF-7 | NCI-H460 | SF-268 | WI-38 |
| **1a** | 40.0 ± 1.8 | 44.3 ± 10.8 | 20.5 ± 1.1 | 10.3 ± 2.8 |
| **1b** | 2.0 ± 1.2 | 2.6 ± 1.4 | 4.4 ± 0.8 | 80.3 ± 18.4 |
| **2** | 4.6 ± 2.4 | 2.9 ± 0.8 | 1.8 ± 0.6 | 40.2 ± 10.2 |
| **3a** | 13.8 ± 0.6 | 16.5 ± 0.8 | 16.7 ± 1.6 | > 100 |
| **3b** | 22.0 ± 0.2 | 30.6 ± 1.7 | 38.4 ± 0.6 | 30.1 ± 4.6 |
| **3c** | 36.7 ± 17.5 | 42.2 ± 12.8 | 54.0 ± 9.0 | 43.5 ± 8.2 |
| **3d** | 20.0 ± 0.6 | 22.0 ± 0.4 | 31.5 ± 8.0 | 58.2 ± 12.7 |
| **4** | 44.6 ± 12.2 | 32.6 ± 8.6 | 60.4 ± 14.8 | 12.3 ± 6.1 |
| **5a** | 0.6 ± 0.2 | 0.1 ± 0.02 | 0.3 ± 0.05 | 22.8 ± 8.0 |
| **5b** | 0.03 ± 0.007 | 0.02 ± 0.008 | 0.01 ± 0.004 | > 100 |
| **5c** | 38.0 ± 1.8 | 44.0 ± 4.5 | 20.5 ± 1.1 | 68.2 ± 12.9 |
| **6** | 23.6 ± 0.4 | 24.3 ± 0.8 | 32.0 ± 0.8 | 4.2 ± 1.8 |
| **7** | 28.0 ± 4.6 | 20.0 ± 2.4 | 33.5 ± 6.0 | 36.2 ± 6.9 |
| **8a** | 35.4 ± 10.2 | 24.1 ± 0.8 | 18.9 ± 6.8 | 44.1 ± 6.3 |
| **8b** | 38.0 ± 1.8 | 12.0 ± 0.8 | 16.5 ± 4.1 | 36.6 ± 4.7 |
| **8c** | 0.01 ± 0.006 | 0.03 ± 0.002 | 0.06 ± 0.005 | > 100 |
| **9** | 0.01 ± 0.003 | 0.02 ± 0.001 | 0.01 ± 0.001 | 66.5 ± 12.7 |
| **10a** | 30.1 ± 0.6 | 17.3 ± 1.4 | 22.3 ± 1.5 | 60.5 ± 22.6 |
| **10b** | 28.0 ± 0.2 | 30.6 ± 1.4 | 38.4 ± 0.6 | 44.3 ± 10.6 |
| **11** | 28.7 ± 11.5 | 22.2 ± 10.8 | 26.0 ± 8.0 | 40.7 ± 8.3 |
| **12** | 7.0 ± 17.5 | 20.2 ± 12.8 | 33.0 ± 9.0 | 70.1 ± 22.3 |
| DMSO | 94.3 ± 6.4 | 96.4 ± 10.2 | 98.6 ± 12.2 | > 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 mean ± SEM of three independent experiments performed in duplicates.

## RESULTS AND DISCUSSION

### *Chemistry*

In the present work, we are demonstrating the synthesis of thiophene derivatives together with their cytotoxic evaluation, in continuation of our interest in the design of bioactive heterocycles (23–27). Thus, the reaction of ethyl acetoacetate with elemental sulphur and either malononitrile or ethyl cyanoacetate gave the thiophene derivatives **1a,b**, respectively. The structures of the products were based on analytical and spectral data. Thus, the $^1$H NMR spectrum of **1a** showed a singlet at $\delta$ 1.18 ppm for the $CH_3$ group, a triplet at $\delta$ 1.23 ppm and a quartet at $\delta$ 4.15–4.18 ppm indicating ethoxy group and a singlet at $\delta$ 7.93 ppm ($D_2O$ exchangeable) equivalent to the $NH_2$ group. The 2-amino group present in compound **1a** was capable of acetylation. Thus, compound **1a** reacted with acetic anhydride to give the *N*-acetyl derivative **2**.

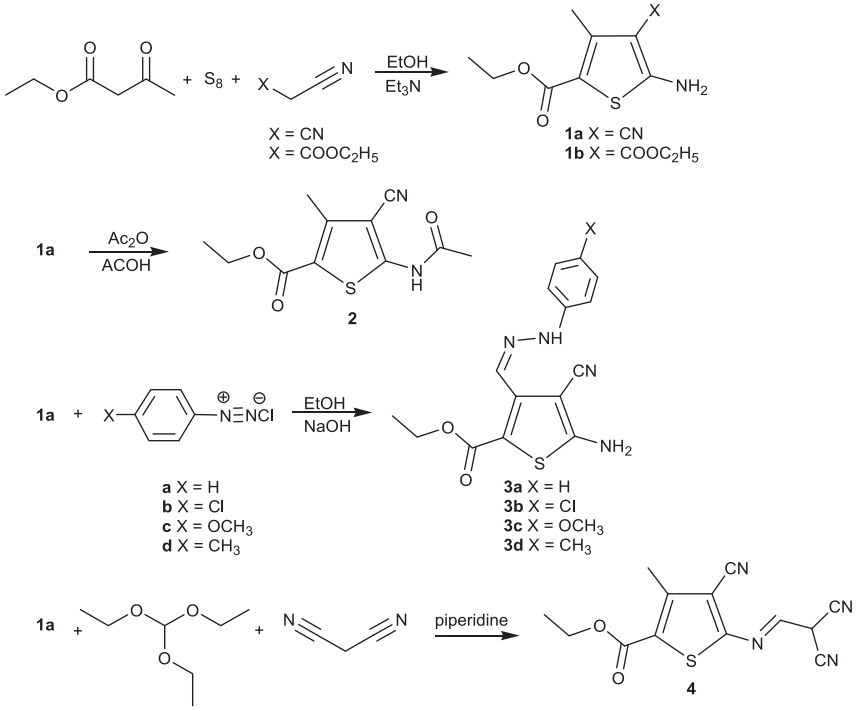
On the other hand, the methyl group, which is in the *ortho*-position to the cyano group present in compound **1a**, showed interesting reactivity towards some reagents. Thus, compound **1a** reacted with either benzene-diazonium chloride, 4-chlorobenzene, 4-methoxybenzene or 4-methylbenzene diazonium chloride to give the arylhydrazone derivatives **3a-d**, respectively.

Next, we studied the reaction of the thiophene derivative **1a** with each ethyl orthoformate and malononitrile. The reaction was carried out in a catalytic amount of piperidine to afford the *N*-methinomalononitrile derivative **4** (Scheme 1). The structure of compound **4** was based on analytical and spectral data. Thus, the $^1$H NMR spectrum showed a singlet at $\delta$ 1.63 ppm corresponding to the $CH_3$ group, a triplet at $\delta$ 1.30 ppm equivalent to the ester $CH_3$ group, a quartet at $\delta$ 4.17 ppm for the ester $CH_2$ group and two singlets at $\delta$ 4.00 and $\delta$ 6.80 ppm for the two CH groups.

Compound **1a** reacted with either benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde to give the benzal derivatives **5a-c**, respectively. Similarly, the reaction of **1a** with salicyladehyde gave the *o*-hydroxybenzal derivative **6**. The analytical and spectral data of compounds **5a-c** and **6** were consistent with their respective structures. On the other hand, the reaction of **1b** with malononitrile gave ethyl 3,6-diamino-5-cyano-4-hydroxybenzo[*c*]thiophen-1-carboxylate **7** (Scheme 2). The analytical and spectral data of the latter product were the tools of its structural elucidation. Thus, the $^1$H NMR spectrum showed a triplet at $\delta$ 1.16–1.18 ppm corresponding to the ester $CH_3$ group, a quartet at $\delta$ 4.18–4.23 ppm for the ester $CH_2$ group, a singlet indicating CH benzene ring at $\delta$ 7.89 ppm, two singlets at $\delta$ 7.10 and 9.10 ppm ($D_2O$ exchangeable) indicating the two $NH_2$ groups and a singlet for the OH group at $\delta$ 14.23 ppm.

At the other extreme, ethyl acetoacetate reacted with elemental sulfur and either 2-cyano-*N*-phenylacetamide, 2-cyano-*N*-(*p*-tolyl)acetamide, or *N*-(4-methoxyphenyl)-2-cyanoacetamide in the presence of ethanol containing a catalytic amount of triethylamine to give the thiophene derivatives **8a-c**. Mass spectra of **8a-c** displayed [$M^+$] ion peaks and [$M^+$-1] at *m*/*z* 304, 318 and 333, respectively, corresponding to their respective molecular formulae $C_{15}H_{16}N_2O_3S$, $C_{16}H_{18}N_2O_3S$ and $C_{16}H_{18}N_2O_4S$.

The 2-amino group present in **8a** was capable of amide formation; thus, the reaction of **8a** with diethylmalonate gave the (3-ethoxy-3-oxopropanamido)thiophene derivative **9**. On the other hand, the reaction of **8a** with either malononitrile or ethyl cyanoacetate in



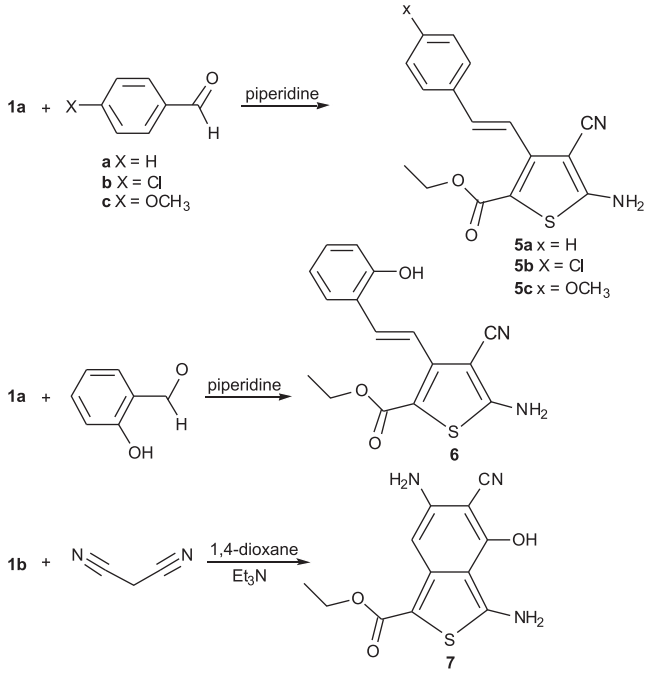

Scheme 1

refluxing 1,4-dioxane containing a catalytic amount of triethylamine gave the benzo[*c*] thiophene derivatives **10a** and **10b**, respectively. The $^1$H NMR and $^{13}$C NMR spectra were the basis of their structure elucidation. The reaction of compound **8a** with acetic anhydride gave the *N*-acetyl derivative **11**.

Moreover, the reaction of compound **8a** with benzaldehyde gave the benzalidene derivative **12** (Scheme 3). The structure of compound **12** was based on analytical and spectral data. Thus, the $^1$H NMR spectrum showed a triplet for the ester $CH_3$ group $\delta$ 1.18–1.21 ppm, two singlets at $\delta$ 4.15 and 4.17 ppm equivalent to the benzal CH, a quartet $\delta$ 4.64–4.76 ppm for the ester $CH_2$ group, a multiplet at $\delta$ 7.12–8.10 ppm for the benzene ring and two singlets at $\delta$ 8.29 and 10.41 ppm for $NH_2$ and NH groups, respectively. Mass spectrum of compound **12** showed *m*/*z* 391 [$M^+$-1] and *m*/*z* 77 $[C_6H_5]^+$ for phenyl moiety.

## In vitro *cytotoxic activity of the newly synthesized compounds*

The tumor cell growth inhibition activities of the newly synthesized thiophene systems (21 compounds in total) were assessed *in vitro* (28, 29) on three human tumor cell lines, namely, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (CNS cancer), and normal fibroblast cells (WI-38), after continuous exposure for 48 h. The results were compared to the anti-proliferative effects of the reference control doxorubicin




Scheme 2

(30). All compounds were dissolved in DMSO with the maximum concentration 0.5 % in each assay.

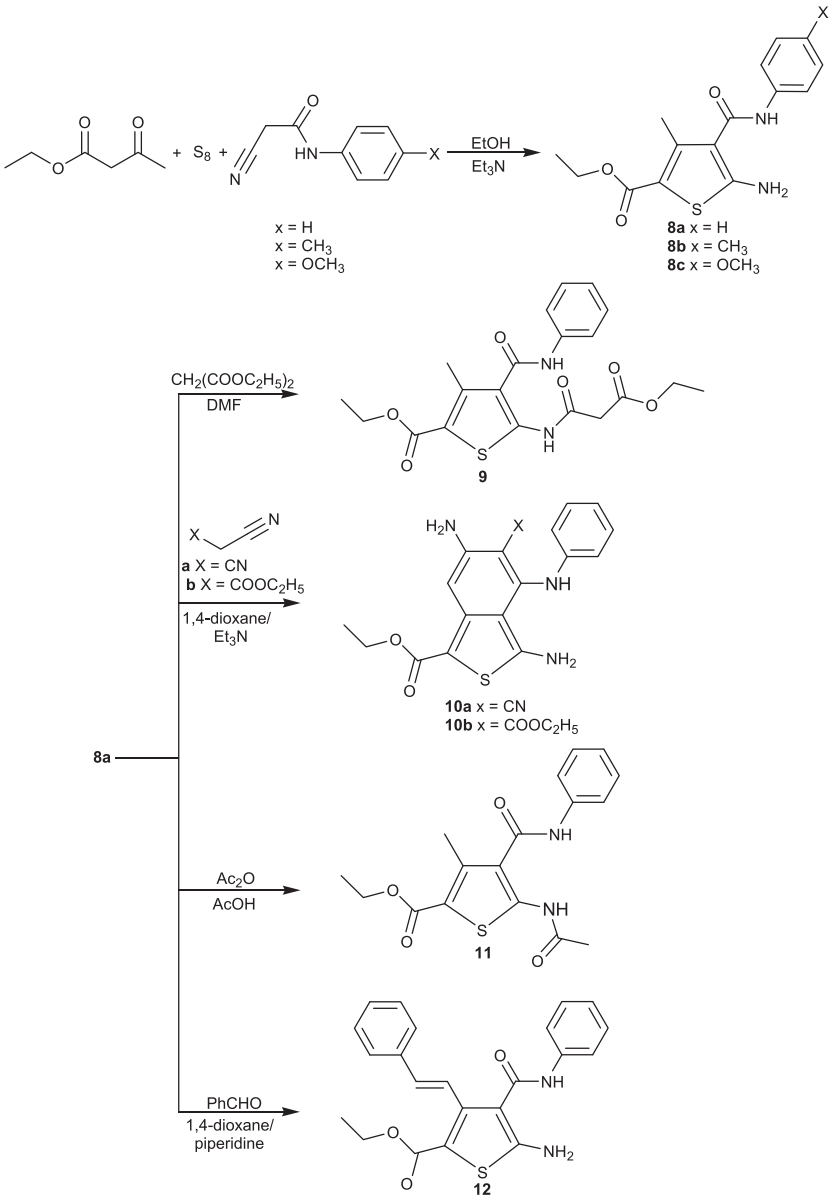
The ± SEM means of three independent experiments performed in duplicate.

The results from Table III indicate that most of the compounds demonstrated substantial growth inhibitory effects against the human tumor cells at the concentrations tested. The anti-proliferative activity of the test compounds against each of the title tumor cell lines may be arranged in a descending according to the measured concentration required to inhibit tumor cell proliferation by 50 %.

It is clear from Table III that compounds **5b**, **8c** and **9** showed significant activity against the three tumor cell lines tested. The inhibitory effects of other compounds varied, depending on the tested tumor cell, from high to medium or marginal effects. Some compounds had no impact on a specific tumor cell proliferation, but exhibited some specificity to another.

## *Structure activity relationship*

It is obvious that compounds **5b**, **8c** and **9** exhibited maximal cytotoxic effect against cancer cell lines, with $IC_{50}$'s in the μmol $L^{-1}$ range. Comparing the cytotoxicity of thiophene derivatives **1a** and **1b**, it is clear that the cytotoxicity of **1b** is higher than that of **1b**. The presence of the ethoxy group is responsible for the higher potency of **1b**. Acetylation of




Scheme 3

compound **1a** gave the *N*-acetyl derivative **2,** for which the cytotoxicity apparently increased. As regards the arylhydrazone derivatives **3a-d**, it is clear that the unsubstituted aryl derivatives showed the highest cytotoxicity among the four compounds. On the other

hand, the reaction of compound **1a** with malononitrile and ethyl orthoformate to give compound **4** resulted in a remarkable decrease of cyctotoxicity. Moreover, it is obvious for the benzalthiophene derivatives **5a-c** that compound **5b** showed high cytotoxicity due to the presence of the chloro group. The reaction of **1a** with either salicylaldehyde or malononitrile gave compound **6** or **7**, respectively, which showed a moderate increase in their cytotoxicity. On the other hand, it is clear for the other series of the thiophene amide derivatives **8a**, **8b** and **8c** that the presence of the $OCH_3$ group in compound **8c** is responsible for higher cytotoxicity than that of compound **8b**, which bears the $CH_3$ group. Moreover, compound **9** revealed higher cytotoxicity than doxorubicin; such high cytotoxicity was attributed to the presence of two ethoxy groups. It is clear from Table III that the thiophene derivatives **10a**, **10b** and **11** showed moderate cytotoxicity. It is noteworthy that the reaction of compound **8a** with benzaldehyde to give benzylidine derivative **12** resulted in remarkable increase in cytotoxicity. Our results showed that, in most cases, the electronegative Cl, $OCH_3$ and $OC_2H_5$ hydrophobic groups in the thiophene derivatives might play a very important role in enhancing the cytotoxic effect. It is clear from Table III that compounds **1a**, **4** and **6** showed cytotoxicity against the normal cell line WI-38 and low potency against the cancer cell lines.

## CONCLUSIONS

In summary, we have developed a convenient synthetic approach for novel thiophene and benzothiophene derivatives. The region selective attack by different reagents on the active center moiety in the thiophene system led to the diversity of the produced systems. Most of the newly synthesized compounds were found to be promising anti-proliferative agents. Results showed that ethyl 5-amino-3-(4-chlorostyryl)-4-cyanothiophene-2-carboxylate (**5b**), ethyl 5-amino-4-[(4-methoxyphenyl)carbamoyl]-3-methylthiophene-2-carboxylate (**8c**) and ethyl 5-(3-ethoxy-3-oxopropanamido)-3-methyl-4-(phenylcarbamoyl)thiophene-2-carboxylate (**9**) are the most active compounds against the three tumor cell lines such as MCF-7, NCI-H460 and SF-268. At the same time, they showed low potency against the normal fibroblasts human cell line (WI-38). On the other hand, compounds **1a**, **4** and **6**, although showing low potency against the cancer cell lines, showed high potency against the normal fibroblasts (WI-38) cell lines.

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