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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of pyridine, pyran and thiazole containing thiophene derivatives and their anti-tumor evaluations

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Abstract The multi-component reaction of 2-acetylthiophene with aromatic aldehydes and either malononitrile or ethyl cyanoacetate gave the pyran derivatives **4a**–**4f** and pyridine derivatives **5a**–**5f**. On the other hand, the reaction of the 2-acetylthiophene with elemental sulfur and either malononitrile or ethyl cyanoacetate gave the thiophene derivatives **6a** and **6b**; respectively. Compounds **6a** and **6b** underwent a series of heterocyclic reactions to give thiazole and thiophene derivatives. All the products were assessed for antitumor activity towards human cancer human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) cell lines. Compounds **4e**, **4f**, **5e**, **5f**, **7b**, **8b**, **10e**, **10f**, **11e**, **11f**, **14d-f**, **15d-f**, **16a,b** and **18b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Moreover, **7b**, **10e**, **14d, 15e** and **16b** showed no toxicity against shrimp larvae. Anti-proliferative cell activity against cancer cell lines of the most potent compounds showed that compounds **5f** and **10e** achieved the highest activities among the tested compounds.

Keywords Thiophene · Pyran · Pyridine · Cytotoxicity · Toxicity · Anti-proliferative

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Introduction

Multi-component reactions (MCRs) have emerged as a valuable tool in the preparation of structurally diverse chemical libraries of heterocyclic compounds (Zhang et al., 2014). They are inherently atom economical processes in which relatively complex products can be obtained in a one-pot reaction from simple starting materials, and thus they exemplify many of the desired features of an ideal synthesis. MCRs are generally much more environmentally friendly and offer access to large compound libraries with diverse functionalities with the avoidance of protection and de-protection steps for possible combinatorial surveying of structural variations. In view of the increasing interest in the preparation of a large variety of heterocyclic compound libraries, the development of new synthetically valuable MCRs with several diversity points remains a challenge for both academic and industrial institutions (Terzidis et al., 2014). Thiophene and its derivatives belong to an important class of heterocyclic compounds, which possess broad biological activities; such as anti-inflammatory (Giri et al., 2010), analgesic (Fakhr et al., 2009), antioxidant (Harinath et al., 2013), antitubercular (Karthikeyan et al., 2009), antidepressant (Mathew et al., 2014), sedative (Sable et al., 2014), antiamoebic (Sharma et al., 2005), oral analgesic (Rodenhuis et al., 2000), anti-metabolite (Martorana et al., 2015), and antineoplastic properties (Romagnoli et al., 2010). From the aforementioned reports, it seemed that the development of an efficient, rapid, and clean synthetic route towards focused libraries of such compounds is of great importance to both medicinal and synthetic chemists (Chatterjee and Roy, 2011). Hence in this paper we report uses of 2-acetylthiophene for the synthesis of novel heterocyclic compounds together with their antitumor evaluations.

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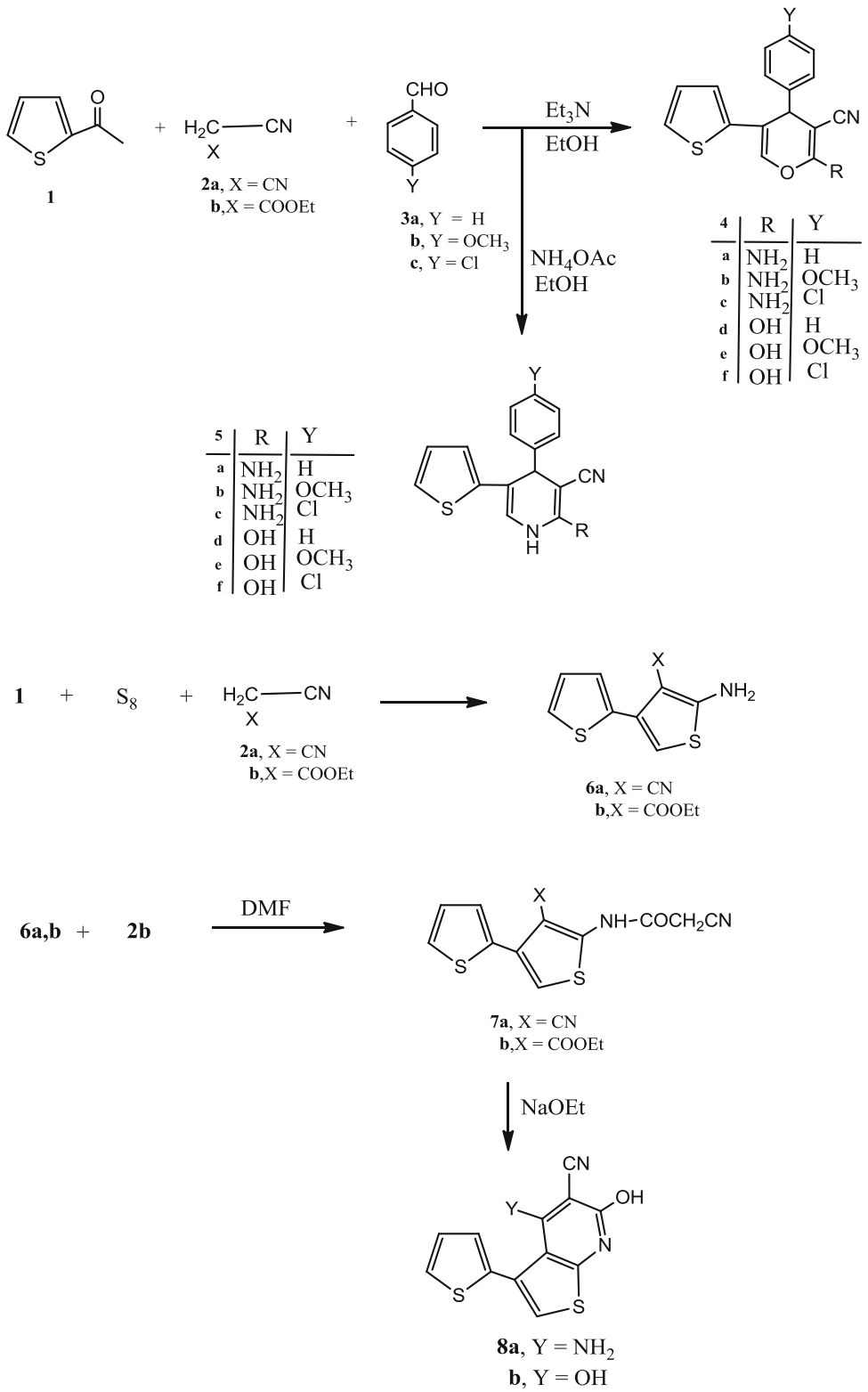
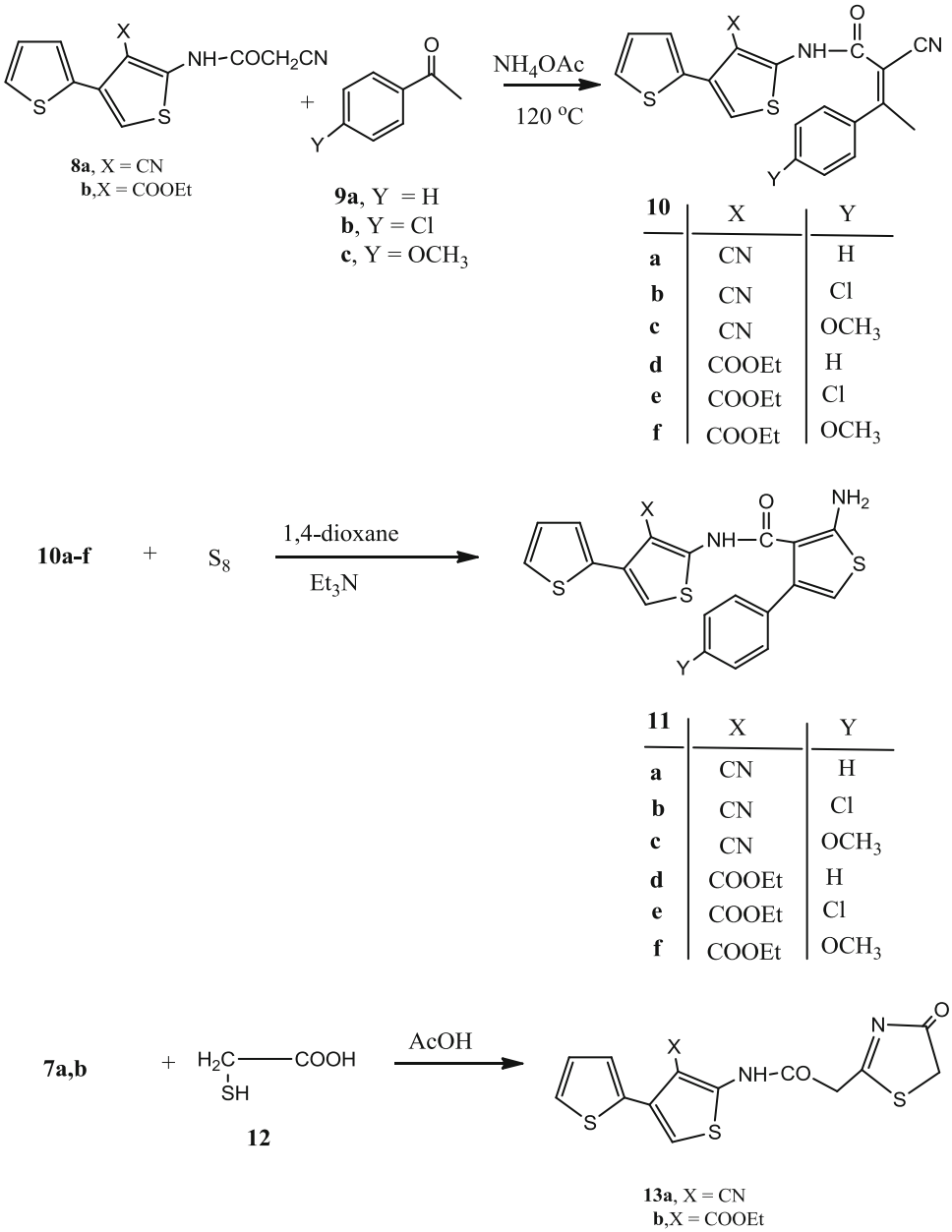
Scheme 1 Synthesis of compounds **4a–4f**; **5a–5f**; **6a**,**b** and **8a**,**b**



Results and discussion

The multi-component reaction of 2-acetylthiophene (**1**) with either of malononitrile (**2a**) or ethyl cyanoacetate (**2b**) and either of benzaldehyde (**3a**), 4-methoxybenzaldehyde (**3b**) or4-clorobenzaldehyde (**3c**) in ethanol containing triethylamine gave the pyran derivatives **4a–4f**, respectively. The structures of compounds **4a–4f** were based on their

Scheme 2 Synthesis of compounds **10a–10f**; **11a–11f** and **13a**,**b**

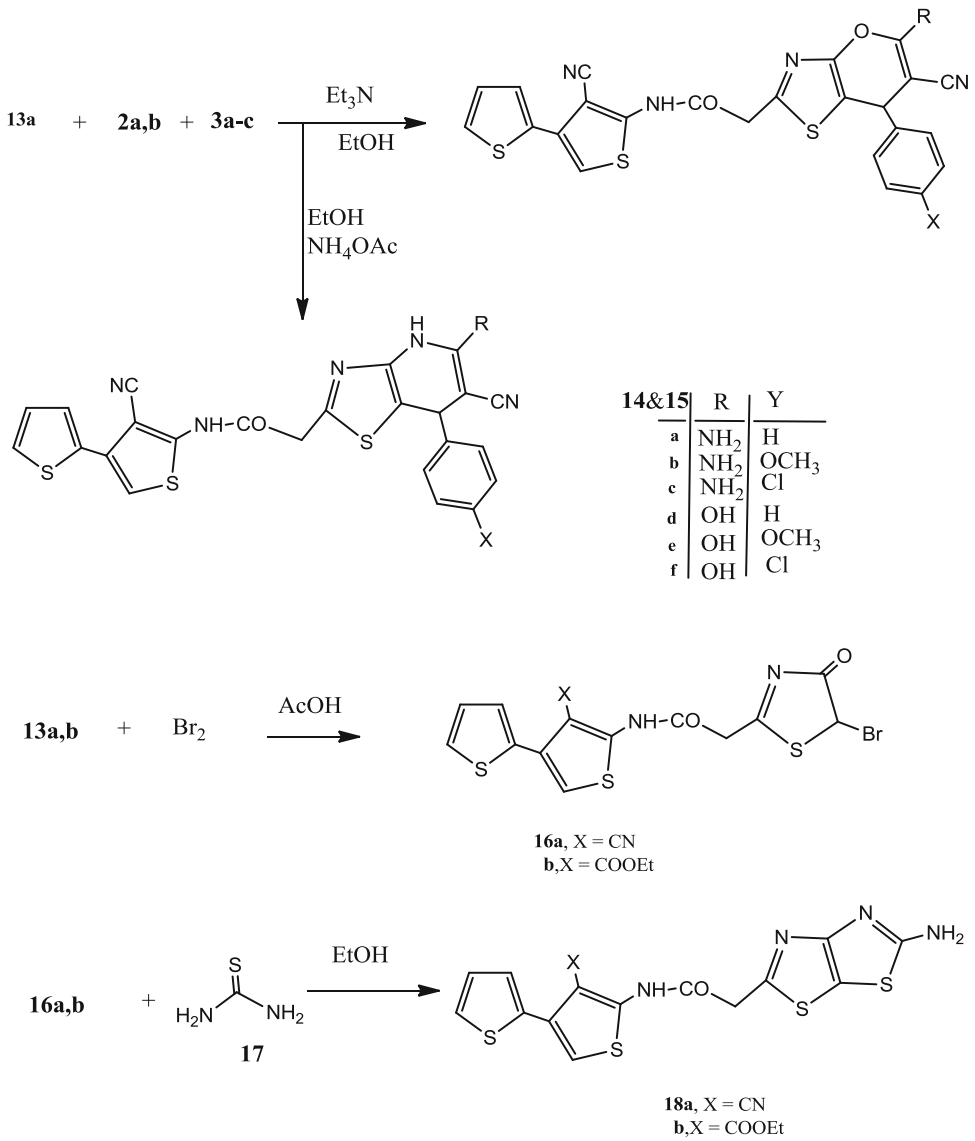


analytical and spectral data. Thus, the ^{1}HNMR spectrum of **4a** (as an example) showed the presence of a singlet at δ 4.22 ppm (D_2O exchangeable) indicating the presence of the NH_2 group, a singlet at δ 5.11 ppm corresponding to the pyran H-4, a singlet at δ 6.23 ppm indicating the presence of the pyran H-6 and a multiplet at δ 7.28–7.39 ppm for the phenyl and thiophene protons. The ^{13}C NMR spectra revealed, beside the expected peaks, the presence of δ 63.4 indicating the presence of the pyran C-4, one cyano group at δ 116.6.

On the other hand, carrying the same reaction but using a catalytic amount of ammonium acetate gave the pyridine derivatives **5a–5f**, respectively. Compound **1** with its 2-acetyl moiety underwent the Gewald's thiophene through its reaction with elemental sulfur and either maloninitrile (**2a**) or ethyl cyanoacetate (**2b**) gave the 2-thienylthiophene derivatives **6a** and **6b**, respectively (Huang et al., 1998). The reaction of compounds **6a** and **6b** with ethyl cyanoacetate provided **7a** and **7b**. Cyclization of compounds **7a** and **7b** in sodium ethoxide provided the 3-(thiophen-2-yl) thieno[2,3-*b*]pyridine **8a** and **8b** depicted in Scheme 1.

The reaction of any of compound **8a** and **8b** with either acetophenone (**9a**), 4-chloroacetophenone (**9b**) or 4-methoxyacetophenone (**9c**) in ammonium acetate in an oil bath at 120 °C gave the Knoevenagel condensation products **10a–10f**, respectively. The latter products afforded the

Scheme 3 Synthesis of compounds **14a–14f**; **15a–15f**; **16a**,**b** and **18a**,**b**



thiophene derivatives **11a–11f** upon their respective reactions with elemental sulfur in the presence of a catalytic amount of triethylamine.

The reaction of either compound **8a** or **8b** with thioglycollic acid (**12**) in acetic acid gave the thiazole derivatives **13a** and **13b**, respectively (Elghandour et al., 1992; Revelant et al., 2011) depicted in Scheme 2. The structures of compounds **13a** and **13b** were well characterized by their respective spectral data. Thus, the ^{1}H NMR spectrum of **13a** showed a singlet at δ 4.89 ppm for the CH_2 group, a singlet at δ 5.98 ppm for the thiazole CH_2 group, a singlet at δ 6.14 corresponding to the thiophene H-4, a multiplet at δ 7.23–7.40 indicating the thiophene 3CH groups and a singlet at δ 8.28 ppm for the NH group. In addition the ^{13}C NMR spectrum revealed the presence of δ 88.9 indicating the thiazole CH_2, and δ at 116.7 corresponding to the cyano group, two signals at δ 164.4, 168.9 equivalent to the two carbonyl groups and a signal at δ 173.8 for the C=N group.

Compound **13a** underwent multi-component reaction upon its reaction with either malononitrile (**2a**) or ethyl cyanoacetate (**2b**) and any of benzaldehyde (**3a**), 4-methoxybenzaldehyde (**3b**) or 4-chlorobenzaldehyde (**3c**) in ethanol containing triethylamine to give the pyrano[2,3-*d*]thiazole derivatives 1**4a**–**14f**, respectively. On the other hand carrying the same reactions but using ammonium acetate instead of triethylamine gave the pyrido[2,3-*d*]thiazole derivatives **15a**–**15f,** respectively. The reaction of either **13a** or **13b** with bromine in acetic acid gave the 5-bromothiazol-4-one derivatives **16a** and **16b**, respectively. The latter compounds as α-bromocarbonyl compound reacted with thiourea to give thiazolo[4,5-*d*]thiazole derivatives **18a** and **18b**, respectively (Scheme 3).

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

Compd	Cytotoxocity (IC_{50} in nM)[a]					
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF
4a	1041	2123	2418	1539	1433	2248
4b	2127	2280	2165	1215	2208	2132
4c	1886	1349	1560	1461	1339	1429
4d	860	448	178	189	1098	1173
4e	380	23	218	130	59	2177
4f	286	387	298	149	2190	168
5a	2288	2190	1190	1287	1773	1980
5b	1627	1892	1528	1420	1290	2290
5c	2176	2019	2660	2127	2133	1293
5d	1029	892	1020	795	2220	1166
5e	254	218	84	1241	886	531
5f	887	1187	179	1876	28	363
6a	1428	1199	1192	1153	1184	1820
6b	1154	1543	1375	1556	1287	1931
7a	1149	1365	2130	2251	2433	1389
7b	218	432	488	149	215	123
8a	889	543	299	259	2323	2098
8b	430	376	166	191	1080	83
10a	1140	2158	1060	1236	1319	1248
10b	1648	168	2140	1234	1620	1205
10c	1248	1456	1768	1886	1873	1766
10d	1208	1422	1263	2254	2418	1870
10e	45	280	1265	432	169	109
10f	432	168	98	29	108	121
11a	2188	1143	1231	1580	1433	1095
11b	1213	1243	2163	2442	1674	2043
11c	1763	1428	1533	1286	1180	1176
11d	1990	1182	1652	1092	1327	899
11e	389	48	909	387	2190	630
11f	23	29	480	287	291	320
13a	2920	1280	1773	2187	1123	3289
13b	1099	897	1029	1189	784	669
14a	2192	1265	2276	3198	1178	908
14b	2129	1129	1126	2176	1129	908
14c	1850	1298	2180	3219	2764	1170
14d	897	326	2170	1876	1265	1098
14e	786	218	831	199	139	1176
14f	69	580	1286	2180	674	1876
15a	2654	1754	1098	1344	1765	1944
15b	1876	1392	1038	1650	1829	1772
15c	2177	542	1164	930	2176	1092
15d	883	2170	1277	2109	2210	1827
15e	210	329	62	18	1022	1328
15f	64	38	1118	980	287	320
16a	198	128	190	927	190	117
16b	241	432	761	320	451	903
18a	1190	2038	1263	1269	1198	2193
18b	221	328	440	518	662	291
CHS 828	25	2315	2067	1245	15	18

The sample concentration produces a 50 % reduction in cell growth CHS-828 is a pyridylcyanoguanidine anti-tumor agent

NUGC gastric cancer, *DLDI* colon cancer, *HA22T* liver cancer, *HEPG2* liver cancer; *HONEI* nasopharyngeal carcinoma; *HR* gastric cancer; *MCF* breast cancer; *WI38* normal fibroblast cells

[a] IC_{50} against the normal fibroblast cells (WI38) are indicated as multiples of 10^4nM

In vitro cytotoxic assay

Chemicals

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

Cell cultures

The cell cultures were obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), and nasopharyngeal carcinoma (HONE1) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 mg/mL), at 37 °C in a humidified atmosphere containing 5 % CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for the seven human cancer cell lines including cells derived from 0.75×10^4 cells/mL 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.

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their in vitro cytotoxicity (Mohareb and Al-Omran, 2012; Shi and Lowary, 2011; Bryan et al., 2009) against six human cancer cell lines including cells derived from human gastric

cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF) and nasopharyngeal carcinoma (HONE1). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested ($IC_{50} = 10–1000$ nM). The reference compound used is the CHS-828 which is a pyridylcyanoguanidine antitumor agent.

Structure activity relationship

From Table 1 it is clear that the thiophene moiety was found to be crucial for the cytotoxic effect of the cyclic compounds from **4a–e** to **18a** and **18b**. Compounds **4e**, **4f**, **5e**, **5f**,**7b**, **8b**, **10e**, **10f**, **11e**, **11f**, **14d–14f**, **15d–15f**, **16a**, **16b** and **18b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of the pyran derivatives **4a–4f**, it is obvious that the cytotoxicity of compounds **4e** and **4f** were higher than that of **4a–4d**. The presence of the 4-OCH_3 moiety in compound **4e** and the 4-Cl group in **4f** were responsible for their high potency. Similarly considering the pyridine derivatives **5a–5f**, where compounds **5e** and **5f** were the most cytotoxic compounds among this series of compounds. On the other hand, the thiophene derivatives **6a** and **6b** showed low cytotoxicity. However, for the thiophene derivatives **7a** and **7b** the latter showed higher cytotoxicity than **7a** which was attributed to the presence of the ethoxycarbonyl group. The thieno[2,3-*b*]pyridine derivatives **8a** and **8b**, where compound **8a** with the 4-amino moiety showed activity towards the four cancer cell lines NUGC, DLDI, HA22T and HEPG2 with IC_{50}'s 889, 543, 299 and 259 nM, respectively. In addition, compound **8b** with the electronegative 4-OH moiety showed high potency against the five cancer cell lines NUGC, DLDI, HA22T, HEPG2 and MC7. Considering the Knoevenagel condensate products **10a–10f**, compounds **10e** with the COOEt and the Cl groups and compound **10f** with the COOEt and the OCH_3 groups showed the highest cytotoxicity towards the six cancer cell lines among such series of compounds. On the other hand, in case of compounds **11a–11f**, only compound **11f** showed the highest cytotoxic compound. The reaction of either of compound **8a** or **8b** with thioglycollic acid gave the thiazole derivatives **13a** and **13b** where the latter with the COOEt moiety showed high cytotoxicity against the three cancer cell lines DLDI, HONE1 and MCF cell lines with IC_{50}'s 897, 784 and 669 nM, respectively. Considering the pyrano[2,3-*d*]thiazole derivatives **14a–14f** and the pyrido[2,3-*d*]thiazole derivatives **15a–15f** where compounds **14d–14f** and **15d–15f** showed higher cytotoxicity than the corresponding **14a–14c** and **15a–15c**. From Table 1 it is obvious that the bromo- derivatives **16a** and **16b** showed high cytotoxicity towards the cancer cell lines. Finally, considering the thiazolo[4,5-*d*]thiazole derivatives **18a** and **18b**, it is obvious that Compound **18b** with the COOEt moiety showed higher cytotoxicity than **18a**.

Toxicity

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals' in vivo lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay (Choudhary and Thomsen, 2001) was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95 % confidence intervals (Brayn and Timothy). Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines which are the twelve compounds **4e**, **4f**, **5e**, **5f**, **7b**, **8b**, **10e**, **10f**, **11e**, **11f**, **14d–14f**, **15d–15f**, **16a**, **16b** and **18b**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials natural and synthetic organic compounds (Carballo et al., 2002). It has also been shown that, *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds (Calleja and Persoone, 1992).

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 2 that compounds **7b**, **10e**, **14d, 15e** and **16b** showed non-toxicity against the tested organisms. On the other hand, compounds **10f**, **11e, 14e, 14f, 15f, 16a** and **18b** are very toxic compounds.

Toxicity method

All toxicity tests were 96 h static renewal tests and water quality measurements (dissolved oxygen, pH, temperature, salinity) were taken in the control containers each day. Tests were run in a Revcos Environmental Chamber at 25 °C, 20 % salinity, and a 16 h light:8 h dark cycle. A media change was made every 24 h. Larvae used for all tests were one to two days old and exposed in 600 mL glass beakers containing 400 mL of media with 10 larvae/beaker and three replicates/concentration. Larvae were fed newly hatched *Artemia* after daily media change. The concentration of each compound was taken in terms 10, 100 and 100 mg/mL. Adult shrimp toxicity tests were also run to complete the grass shrimp toxicity profile. Adult shrimp (acclimated for 2 weeks before testing) were

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

Compound No.	Cons. (μg/mL)	Mortality[a]	Toxicity	LC_{50}	Upper 95 % lim.	Lower 95 % lim
4e	10	0	Harmful	216.47	255.25	121.40
	100	2	–	–	–	–
	1000	10	–	–	–	–
4f	10	0	Harmful	16.43	826.38	144.52
	100	5	–	–	–	–
	1000	10	–	–	–	–
5e	10	0	Harmful	250.49	73.26	4.58
	100	3	–	–	–	–
	1000	10	–	–	–	–
5f	10	0	Harmful	138.85	254.29	80.42
	100	4	–	–	–	–
	1000	10	–	–	–	–
7b	10	0	Non toxic	143.54	124.86	107.83
	100	0	–	–	–	–
	1000	5	–	–	–	–
8b	10	0	Harmful	89.70	228.65	–
	100	4	–	–	–	–
	1000	10	–	–	–	–
10e	10	0	Non-toxic	977.24	–	–
	100	0	–	–	–	–
	1000	3	–	–	–	–
10f	10	5	Very toxic	14.39	–	–
	100	10	–	–	–	–
	1000	10	–	–	–	–
11e	10	4	Very toxic	18.23	–	–
	100	10	–	–	–	–
	1000	10	–	–	–	–
11f	10	1	Harmful	69.29	321.22	–
	100	2	–	–	–	–
	1000	10	–	–	–	–
14d	10	0	Non-toxic	899.23	–	–
	100	0	–	–	–	–
	1000	4				
14e	10	5	Very toxic	64.09	–	–
	100	8	–	–	–	–
	1000	10	–	–	–	–
14f	10	4	Very toxic	14.87	–	–
	100	8	–	–	–	–
	1000	10	–	–	–	–
15d	10	0	Harmful	67.40	189.73	–
	100	3	–	–	–	–
	1000	10	–	–	–	–
15e	10	0	Non-toxic	890.52	-	-
	100	0	–	–	–	–
	1000	3	–	–	–	–
15f	10	7	Very toxic	10.41	–	–
	100	10	–	–	–	–

Table 2 continued

Compound No.	Cons. (μg/mL)	Mortality[a]	Toxicity	LC_{50}	Upper 95 % lim.	Lower 95 % lim
	1000	10	–	–	–	–
16a	10	5	Very toxic	14.29	–	–
	100	10	–	–	–	–
	1000	10	–	–	–	–
16b	10	0	Non-toxic	965.40	–	–
	100	0	–	–	–	–
	1000	4	–	–	–	–
18b	10	7	Very toxic	12.38	–	–
	100	9	–	–	–	–
	1000	10	–	–	–	–

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

exposed in 4-L wide mouth glass jars containing 2-L of media and 10 shrimp/jar with two replicates/concentration, modified from Delorenzo et al. (2007) and were run under conditions as described above for larvae.

Anti-proliferative cell activity against cancer cell lines

We used a panel of tumor cell lines to test the cytotoxicity of the new compounds, especially those showed high potency against the six cancer cell lines through Table 1. Importantly, this panel included the cell lines and their isogenic sub-lines with the determinants of drug resistance: murine leukemia L1210, T-lymphocyte cell lines Molt4/C8 and CEM, human leukemia R562 and its MDR subline K562/4 that over expressed P- glycoprotein, and the colon carcinoma HCT116. The above determinants alter the response of cells to many anticancer drugs including doxorubicin. Data on cytotoxic (anti-proliferative) activity are presented in Table 3 in which IC_{50} values represent the concentrations that inhibit cell proliferation by 50 %. It is clear from Table 3 that tested compounds **4e**, **4f**, **5e**, **5f**, **7b**,**8b**, **10e**, **10f**, **11e**, **11f**, **14d–14f**, **15d–15f**, **16a**, **16b** and **18b** showed high potency against the cell lines. The pyridine derivative **5f** and with the OH and OCH_3moieties and the thiophenyl-thiophene derivative **10e** with the Cl and COOEt moieties showed high potency against the tested cancer cell lines and their IC_{50}'s are higher than the reference doxorubicin. It is clear from Table 3 that the tested compounds showed high IC_{50} against K562/4 cell line than doxorubicin.

Experimental

Synthetic methods, analytical and spectral data

The starting steroid, progesterone, was purchased from Sigma Company, USA. All solvents were dried by distillation prior to using. Melting points were recorded on Buchi melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. ^{13}C NMR and ^{1}H NMR spectra were recorded on Bruker DPX200 instrument in DMSO with tetramethylsilane (TMS) as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried by the Microanalytical Data Unit at Cairo University. The progress of all reactions was monitored by TLC on 2×5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

General procedure for the synthesis of the pyran derivatives ***4a–4f***

To compound **1** (1.26 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (0.50 mL) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (0.133 g,1 mmol) and either of the aromatic aldehydes namely benzaldehyde (0.106 g, 1 mmol), 4-methoxybenzaldehyde (0.137 g, 1 mmol) 4-chlorobenzaldehyde (1.40 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 h and the formed solid product produced from the hot solution was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

2-Amino-4-phenyl-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4a**) Pale yellow crystals (ethanol), yield 75 % (1.57 g), m.p. 168–170 °C; IR (KBr) ν_{max} 3484, 3433, 3055, 2224, 1623 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 7.39$ -7.28 (8H, m, Bz, H-3′, H-4′, H-5′), 6.23 (1H, s, H-6), 5.11 (1H, s, H-4), 4.22 (2H, s, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 153.6, 150.9, 149.2, 148.8, 145.6, 129.3, 128.6, 126.2, 125.9, 124.0, 121.6,

Table 3 Anti-proliferative activity (IC_{50}) of selected compounds against variety of cell lines

Compound No.	Cytotoxocity (IC_{50} in nM)					
	L1210	Molt4/C8	CEM	K562	K562/4	HCT116
4e	1.2 ± 0.5	1.1 ± 0.03	0.5 ± 0.03	0.8 ± 0.04	0.9 ± 0.06	0.8 ± 0.03
4f	0.6 ± 0.2	0.8 ± 0.04	1.5 ± 0.4	1.0 ± 0.02	0.1 ± 0.06	1.4 ± 0.05
5e	0.2 ± 0.08	0.3 ± 0.04	0.4 ± 0.05	1.0 ± 0.05	0.5 ± 0.07	1.3 ± 0.05
5f	0.02 ± 0.002	0.02 ± 0.001	0.05 ± 0.03	0.4 ± 0.06	0.8 ± 0.07	0.2 ± 0.05
7b	1.2 ± 0.04	0.8 ± 0.03	0.9 ± 0.04	0.1 ± 0.05	0.8 ± 0.19	1.0 ± 0.5
8b	0.8 ± 0.05	0.7 ± 0.02	0.3 ± 0.08	0.6 ± 0.05	0.6 ± 0.07	0.9 ± 0.08
10e	0.05 ± 0.001	0.02 ± 0.006	0.2 ± 0.05	0.2 ± 0.06	0.8 ± 0.05	0.2 ± 0.06
10f	1.0 ± 0.07	0.3 ± 0.05	0.8 ± 0.02	0.5 ± 0.09	0.3 ± 0.05	0.6 ± 0.08
11e	0.9 ± 0.02	0.8 ± 0.03	0.8 ± 0.05	0.6 ± 0.04	0.8 ± 0.02	0.3 ± 0.04
11f	0.3 ± 0.07	0.7 ± 0.02	0.6 ± 0.01	1.1 ± 0.08	0.1 ± 0.03	0.1 ± 0.02
14d	1.3 ± 0.08	0.9 ± 0.01	1.1 ± 0.08	1.2 ± 0.05	0.5 ± 0.03	0.8 ± 0.05
14e	0.4 ± 0.01	0.2 ± 0.02	0.6 ± 0.05	0.5 ± 0.06	1.1 ± 0.08	0.5 ± 0.02
14f	0.2 ± 0.04	0.5 ± 0.06	0.2 ± 0.03	0.1 ± 0.06	0.1 ± 0.06	0.2 ± 0.03
15d	1.2 ± 0.08	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.8 ± 0.05
15e	0.9 ± 0.03	0.1 ± 0.06	1.1 ± 0.02	0.8 ± 0.02	0.8 ± 0.03	0.6 ± 0.04
15f	1.4 ± 0.02	0.6 ± 0.02	0.5 ± 0.05	0.04 ± 0.01	0.2 ± 0.02	1.3 ± 0.03
16a	0.8 ± 0.04	0.4 ± 0.01	0.8 ± 0.03	0.01 ± 0.002	0.5 ± 0.02	0.6 ± 0.07
16b	0.3 ± 0.02	0.6 ± 0.07	0.2 ± 0.02	0.6 ± 0.02	0.2 ± 0.05	0.9 ± 0.02
18b	0.7 ± 0.01	0.5 ± 0.02	0.4 ± 0.06	0.5 ± 0.06	0.7 ± 0.02	0.3 ± 0.04
Dox	0.37 ± 0.07	0.20 ± 0.02	0.06 ± 0.02	0.14 ± 0.03	7.2 ± 0.9	1.4 ± 0.1

Doxorubicin (Dox) was used as the reference drug

119.8 (C-Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.6 (CN), 63.4 (C-4); EIMMS: *m/z* 280 $[M]^+$ (28); analysis calcd for $C_{16}H_{12}N_2OS$ (280.34): C, 68.55; H, 4.31; N, 9.99, S, 11.44. Found: C, 68.41; H, 4.29; N, 10.18; S, 11.59.

2-Amino-4-phenyl-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4b**) Pale yellow crystals (ethanol); yield 80 % (2.48 g), m.p. 210–212 °C; IR (KBr) ν_{max} 3484, 3433, 3055, 2224, 1623 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): $\delta =$ 7.29–7.39 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.20 (s, 1H, H-6), 5.18 (s, 1H, H-4), 4.28 (s, 2H, D_2O exchangeable, NH_2), 3.20 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 153.8, 151.4, 150.5, 149.6, 148.5, 145.5, 129.8, 128.8, 127.3, 119.6, 125.0, 121.9, (C-Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.8 (CN), 62.9 (C-4), 26.3 (C-4′); EIMMS: *m/z* 310 $[M]^+$ (21); analysis calcd for $C_{17}H_{14}N_2O_2S$ (310.37): C, 65.79; H, 4.55; N, 9.03, S, 10.33. Found: C, 65.63; H, 4.39; N, 8.89; S, 10.49.

2-Amino-4-(4-chlorophenyl)-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4c**) Yellow crystals (ethanol); yield 77 % (2.42 g), m.p. 189–191 °C; IR (KBr) ν_{max} 3473, 3430, 3058, 2222, 1620; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 7.26$–7.41 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.22 (s, 1H, H-6), 5.18 (s, 1H, H-4), 4.28 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 154.2$, 153.7, 150.2, 149.9, 148.6, 145.8, 129.6, 128.2, 126.8, 125.2, 122.3, 119.0 (C-Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.4 (CN), 62.5 (C-4); EIMMS: *m/z* 314 $[M]^+$ (28); analysis calcd for $C_{16}H_{11}ClN_2OS$ (314.79): C, 61.05; H, 3.52; N, 8.90, S, 10.19. Found: C, 61.28; H, 3.63; N, 8.86; S, 10.22.

2-Hydroxy-4-phenyl-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4d**) Orange crystals (ethanol); yield 66 % (1.85 g), m.p. 133–136 °C; IR (KBr) ν_{max} 3520, 3422, 3058, 2220, 1628; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.30$ (s, 1H, D_2O exchangeable, OH), 7.28–7.39 (m, 8H, Bz, H-3′, H-4′, H-5′), 6.25 (s, 1H, H-6), 5.20 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 153.9$, 151.8, 150.6, 149.9, 148.8, 146.7, 130.2, 128.4, 126.8, 125.6, 123.8, 120.6 (C–Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.6 (CN), 62.6 (C-4); EIMMS: *m/z* 281 $[M]^+$ (28); analysis calcd for $C_{16}H_{11}NO_2S$ (281.33): C, 68.31; H, 3.94; N, 4.98, S, 11.40. Found: C, 63.26; H, 4.18; N, 5.31; S, 11.59.

2-Hydroxy-4-(4-methoxyphenyl)-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4e**) Yellow crystals (ethanol); yield 64 % (1.99 g), m.p. 211–213 °C; IR (KBr) ν_{max} 3528–3431, 3053, 2220, 1623; ^1H NMR (DMSO-d_6, 200 MHz): $\delta =$ 10.30 (s, 1H, D_2O exchangeable, OH), 7.28–7.40 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.24 (s, 1H, H-6), 5.21 (s, 1H, H-4),

3.15 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 153.9, 151.4, 150.3, 149.3, 148.0, 145.8, 129.6, 128.9, 127.3, 125.4, 122.6, 120.2, (C–Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.7 (CN), 62.5 (C-4), 24.7 (OCH_3), EIMMS: *m/z* 311 $[M]^+$ (32); analysis calcd for $C_{17}H_{13}NO_3S$ (311.36): C, 65.58; H, 4.21 N, 4.50, S, 10.30. Found: C, 65.80; H, 4.41; N, 4.83; S, 10.52.

4-(4-Chlorophenyl)-2-hydroxy-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**4f**) Yellow crystals (ethanol), yield 74 % (2.33 g), m.p. 116–119 °C; IR (KBr) ν_{max} 3521–3449, 3055, 2220, 1626; ^1H NMR (DMSO-d_6, 200 MHz): δ = 10.27 (s, 1H, D_2O exchangeable, OH), 7.26–7.48 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.20 (s, 1H, H-6), 5.23 (s, 1H, H-4), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 153.4, 151.5, 150.4, 149.8, 148.9, 145.3, 129.3, 128.9, 126.9, 125.8, 122.5, 120.2 (C-Bz, C-1, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.9 (CN), 62.8 (C-4); EIMMS: *m/z* 315 $[M]^+$ (26); analysis calcd for $C_{16}H_{10}ClNO_2S$ (315.77): C, 60.86; H, 3.19; N, 4.44, S, 10.15. Found: C, 60.59; H, 4.28; N, 4.63; S, 10.28.

*General procedure for the synthesis of the pyridine derivatives **5a–5f***

To compound **1** (1.26 g, 0.01 mol) in absolute ethanol (40 mL) containing ammonium acetate (1.0 g) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (0.133 g,1 mmol) and any of the aromatic aldehydes namely benzaldehyde (0.106 g, 1 mmol), 4-methoxybenzaldehyde (0.137 g, 1 mmol) 4-chlorobenzaldehyde (1.40 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 h and the formed solid product produced from the hot solution was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

2-Amino-4-phenyl-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**5a**) Pale yellow crystals (ethanol); yield 69 % (1.92 g), m.p. 155–157 °C; IR (KBr) ν_{max} 3457–3429, 3053, 2221, 1626; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.34 (s, 1H, D_2O exchangeable, NH), 7.25–7.41 (m, 8H, Bz, H-3′, H-4′, H-5′), 6.28 (s, 1H, H-6), 5.19 (s, 1H, H-4), 4.28 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 156.8, 149.2, 148.0, 146.3, 143.7, 129.6, 128.9, 125.5, 124.3, 123.5, 121.8 (Bz, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.9 (CN), 63.6 (C-4); EIMMS: *m/z* 279 $[M]^+$ (20); analysis calcd for $C_{16}H_{13}N_3S$ (279.36): C, 68.79; H, 4.69; N, 15.04, S, 11.48. Found: C, 68.59; H, 4.48; N, 15.22; S, 11.63.

2-Amino-4-phenyl-5-(thiophen-2-yl)-4H-pyran-3-carbonitrile (**5b**) Yellow crystals (ethanol); yield 80 % (2.47 g), m.p. 258–260 °C; IR (KBr) ν_{max} 3477–3420, 3054, 2220, 1580; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.32 (s, 1H, D_2O exchangeable, NH), 7.27–7.36 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.25 (s, 1H, H-6), 5.22 (s, 1H, H-4), 4.25 (s, 2H, D_2O exchangeable, NH_2), 3.15 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 154.5, 152.8, 150.7, 149.9, 144.8, 142.9, 128.6, 127.4, 126.8, 124.3, 122.7, 119.8, (Bz, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 117.0 (CN), 62.5 (C-4), 26.6 (CH_3); EIMMS: *m/z* 309 $[M]^+$ (26); analysis calcd for $C_{17}H_{15}N_3OS$ (309.09): C, 66.00; H, 4.89; N, 13.58, S, 10.36. Found: C, 65.87; H, 4.68; N, 13.40; S, 10.44.

2-Amino-4-(4-chlorophenyl)-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**5c**) Orange crystals (ethanol); yield 65 % (2.03 g); m.p. 222–225 °C; IR (KBr) ν_{max} 3487, 3420, 3055, 2220, 1620; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.30 (s, 1H, D_2O exchangeable, NH), 7.26–7.38 (m, 7H, Bz, H-3′. H-4′, H-5′), 6.28 (s, 1H, H-6), 5.15 (s, 1H, H-4), 4.25 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 153.6, 152.9, 150.2, 148.7, 147.9, 146.5, 128.8, 128.2, 126.4, 125.8, 122.6, 119.9 (Bz, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.7 (CN), 62.6 (C-4); EIMMS: *m/z* $[M]^+$ 313 (16); analysis calcd for $C_{16}H_{12}ClN_3S$ (313.80): C, 61.24; H, 3.85; N, 13.39, S, 10.22. Found: C, 61.35; H, 3.79; N, 13.51; S, 10.40.

2-Hydroxy-4-phenyl-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**5d**) Orange crystals (ethanol), yield 73 % (2.04 g), m.p. 189–191 °C; IR (KBr) ν_{max} 3566, 3420, 3053, 2223, 1624; ^1H NMR (DMSO-d_6, 200 MHz): δ = 10.33 (s, 1H, D_2O exchangeable, OH), 8.32 (s, 1H, D_2O exchangeable, NH), 7.25–7.44 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.28 (s, 1H, H-6), 5.23 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 153.4, 152.7, 150.8, 149.8, 143.5, 146.2, 132.9, 128.9, 126.8, 124.3, 122.6, 120.5 (Bz, C-2. C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.9 (CN), 62.8 (pyridine C-4), EIMMS: *m/z* $[M]^+$ 280 (28); analysis calcd for $C_{16}H_{12}N_2OS$ (280.34): C, 68.55; H, 4.31; N, 9.99, S, 11.44. Found: C, 68.72; H, 4.28; N, 10.04; S, 11.53.

2-Hydroxy-4-(4-methoxyphenyl)-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**5e**) Yellow crystals (ethanol), yield 60 % (1.86 g), m.p. 277–279 °C; IR (KBr) ν_{max} 3541–3418, 3055, 2222, 1626; ^1H NMR (DMSO-d_6, 200 MHz): δ = 10.31 (s, 1H, D_2O exchangeable, OH), 8.29 (s, 1H, D_2O exchangeable, NH), 7.26–7.38 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.19 (s, 1H, H-6), 5.24 (s, 1H, H-4), 3.13 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 152.8, 150.8, 149.3, 144.5, 142.7, 129.3, 128.7, 127.8, 125.6, 123.3, (Bz, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.7 (CN), 62.8 (C-4), 24.9 (OCH_3), EIMMS: *m/z* $[M]^+$ 310 (48); analysis calcd for $C_{17}H_{14}N_2O_2S$ (310.37): C, 65.79; H, 4.55; N, 9.03, S, 10.33. Found: C, 65.89; H, 4.72; N, 8.83; S, 10.44.

4-(4-Chlorophenyl)-2-hydroxy-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**5f**) Yellow crystals (ethanol), yield 82 % (2.72 g), m.p. 240–243 °C; IR (KBr) ν_{max} 3549–3438, 3058, 2223, 1628; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.28$ (s, 1H, D_2O exchangeable, OH), 8.26 (s, 1H, D_2O exchangeable, NH), 7.28–7.44 (m, 7H, Bz, H-3′, H-4′, H-5′), 6.21 (s, 1H, H-6), 5.24 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 154.8, 153.4, 152.6, 149.6, 148.8, 145.6, 131.6, 128.6, 127.3, 123.4, 120.8, 122.6 (Bz, C-2, C-3, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.79 (CN), 62.6 (C-4); EIMMS: *m/z* $[M]^+$ 314 (28); analysis calcd for $C_{16}H_{11}ClN_2OS$ (314.79): C, 61.05; H, 3.52; N, 8.90, S, 10.19. Found: C, 61.22; H, 3.80; N, 9.21; S, 10.24.

*Synthesis of the thiophene derivatives **6a** and **6b***

General procedure To a solution of either of compound **1** (1.26 g, 0.01 mol) in absolute ethanol (20 mL) containing triethylamine (0.50 mL) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.03 g, 0.01 mol) elemental sulfur (0.032 g, 1 mmol) was added. The reaction mixture was heated under reflux for 1 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product, in each case, was collected by filtration.

5′-Amino-[2,3′-bithiophene]-4′-carbonitrile (**6a**) Brown crystals (ethanol), yield 70 % (1.44 g), m.p. 266–268 °C; IR (KBr) ν_{max} 3449, 3422, 3056 2220, 1628; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 7.20$–7.43 (m, 3H, H-3′, H-4′, H-5′), 6.36 (s, 1H, H-5), 4.35 (s, 2H, D_2O exchangeable, NH_2), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 147.3, 144.6, 142.5, 136.6, 133.2, 129.6, 129.0, 127.5 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.5 (CN); EIMMS: *m/z* $[M]^+$ 206 (62); analysis calcd for $C_9H_6N_2S_2$ (206.29): C, 52.40; H, 2.93; N, 13.58, S, 31.09. Found: C, 52.31; H, 3.12; N, 13.49; S, 31.19.

Ethyl 5′-amino-[2,3′-bithiophene]-4′-carboxylate (**6b**) Orange crystals (ethanol), yield 83 % (2.09 g); m.p. 166–168 °C; IR (KBr) ν_{max} 3473, 3433, 3054. 1630; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 7.25$–7.39 (m, 3H, H-3′, H-4′, H-5′), 6.33 (s, 1H, H-5), 4.26 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, J = 7.26 Hz, OCH_2CH_3), 1.38 (t, 3H, J = 7.26 Hz, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 145.6, 143.2, 140.8, 136.9, 134.5, 131.8, 129.3, 127.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 56.3 (CH_2, OCH_2CH_3), 16.8 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 253 (18); analysis calcd for $C_{11}H_{11}NO_2S_2$ (253.34): C, 52.15; H, 4.38; N, 5.53, S, 25.31. Found: C, 52.34; H, 4.65; N, 5.69; S, 25.49.

*General procedure for the synthesis of the 2-α-cyanoacetamidothiophene derivatives **7a** and **7b***

To a solution of either of compound **6a** (2.06 g, 0.01 mol) or **6b** (2.53 g, 0.01 mol) in dimethylformamide (40 mL) ethyl cyanoacetate (1.03 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.

2-Cyano-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**7a**) Yellow crystals (1,4-dioxane), yield 66 % (1.80 g) m.p. 288–291 °C; IR (KBr) ν_{max} 3489–3380, 3054, 2980, 2223, 2221, 1680, 1633; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.24$ (s, 1H, D_2O exchangeable, NH), 7.29–7.38 (m, 3H, H-3′, H-4′, H-5′), 6.39 (s, 1H, H-5), 5.63 (s, 2H, $COCH_2CN$; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 148.8, 143.6, 140.7, 133.4, 130.8, 129.2, 128.3, 127.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.8 (CN), 58.4 (CH_2, $COCH_2CN$); EIMMS: *m/z* $[M]^+$ 273 (38); analysis calcd for $C_{12}H_7N_3OS_2$ (273.33): C, 52.73; H, 2.58; N, 15.37, S, 23.46. Found: C, 52.94; H, 2.39; N, 15.44; S, 23.20.

Ethyl 5′-(2-cyanoacetamido)-[2,3′-bithiophene]-4′-carboxylate (**7b**) Pale yellow crystals (ethanol), yield 70 % (2.24 g), m.p. 120–122 °C; IR (KBr) ν_{max} 3493–3421, 3056, 2987, 2869, 2220, 1705, 1680, 1633; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.35$ (s, 1H, D_2O excahangeable, NH), 7.28–7.44 (m, 3H, H-3′. H-4′, H-5′), 6.35 (s, 1H, H-5), 4.25 (q, 2H, J = 7.48 Hz, OCH_2CH_3), 1.29 (t, 3H, J = 7.48 Hz, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 146.2, 143.9, 141.2, 138.3, 136.4, 133.2, 129.9, 125.3 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 59.8 (CH_2, OCH_2CH_3), 56.2 (CH_2, $COCH_2CN$), 16.9 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 320 (21); analysis calcd for $C_{14}H_{12}N_2O_3S_2$ (320.39): C, 52.48; H, 3.78; N, 8.74, S, 20.02. Found: C, 52.66; H, 3.89; N, 8.59; S, 19.80.

*Synthesis of the thieno[2,3-b]pyridine derivatives **8a** and **8b***

A suspension of either compound **7a** (2.73 g, 0.01 mol) or **7b** (3.20 g, 0.0l mol) in sodium ethoxide solution [prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (40 mL)] was heated in a boiling water bath for 3 h then left to cool. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

4-Amino-6-hydroxy-3-(thiophen-2-yl)thieno[2,3-*b*]pyridine-5-carbonitrile (**8a**) Orange crystals (1,4-dioxane), yield 63 % (1.72), m.p. 212–215 °C; IR (KBr) ν_{max} 3520–3328, 3056, 2222, 1635; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.21$ (s, 1H, D_2O exchangeable, NH), 7.23–7.37 (m, 3H, H-3′, H-4′, H-5′), 6.12 (s, 1H, H-5′′),

4.22 (s, 2H, D_2O exchangeable, NH_2), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.7 (C-1), 154.8, 148.8, 143.6, 140.7, 133.4, 130.8, 129.2, 128.3, 127.6, 118.6 (C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.8 (CN); EIMMS: *m/z* $[M]^+$ 273 (38); analysis calcd for $C_{12}H_7N_3OS_2$ (273.33): C, 52.73; H, 2.58; N, 15.37, S, 23.46. Found: C, 52.94; H, 2.39; N, 15.44; S, 23.20.

4,6-Dihydroxy-3-(thiophen-2-yl)thieno[2,3-b]pyridine-5-carbonitrile (**8b**) Reddish brown crystals (1,4-dioxane), yield 76 % (2.08 g), m.p.177–179 °C; IR (KBr) ν_{max} 3522–3376, 3053, 2222, 1655, 1636; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.29, 10.12 (2s, 2H, D_2O excahangeable, 2OH), 7.26–7.52 (m, 3H, H-3′, H-4′, H-5′), 6.29 (s, 1H, H-5′′); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.4 (C-1), 148.6, 144.2, 140.8, 135.7, 134.8, 130.4, 127.4, 126.8, 124.8, 122.3 (C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′), 116.9 (CN); EIMMS: *m/z* $[M]^+$ 274 (46); analysis calcd for $C_{12}H_6N_2O_2S_2$ (274.32): C, 52.54; H, 2.20; N, 10.21, S, 23.38. Found: C, 52.68; H, 2.39; N, 10.44; S, 23.41.

*General procedure for the synthesis of the phenylbut-2-enamide derivatives **10a–10f***

General procedure To the dry solid of either of compound **8a** (2.73 g, 0.01 mol) or **8b** (2.74 g, 0.01 mol) any of acetophenone (1.20 g, 0.01 mol), 4-chloroacetophenone (1.54 g, 0.01 mol) or 4-methoxyacetophenone (1.50 g, 0.01 mol) and ammonium acetate (2.0 g) was added. The whole reaction mixture, in each case was heated in an oil bath at 120 °C for 30 min. and the remaining product formed, in each case, was triturated with ethanol and the formed solid product was collected by filtration.

2-Cyano-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)-3-phenylbut-2-enamide(**10a**) Orange crystals (acetic acid), yield 80 % (3.00 g), m.p. 204–206 °C; IR (KBr) ν_{max} 3458–3420, 3055, 2225–2220, 1689, 1634; ^1H NMR (DMSO-d_6, 200 MHz,): δ = 8.24 (s, 1H, D_2O exchangeable, NH), 7.23–7.46 (m, 8H, Bz,H-3′, H-4′, H-5′), 6.26 (s, 1H, H-5), 2.79 (CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.9 (C-1), 164.4 (C, CONH), 154.2, 149.7, 148.3, 145.8, 142.9, 140.3, 128.6, 128.0, 127.1, 125.9, 124.2, 120.8 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.9, 117.3 (2CN), 89.3, 96.3 (C=C), 26.9 (CH_3); EIMMS: *m/z* $[M]^+$ 375 (28); analysis calcd for $C_{20}H_{13}N_3OS_2$ (375.47): C, 63.98; H, 3.49; N, 11.19, S, 17.08. Found: C, 63.79; H, 3.62; N, 11.08; S, 16.88.

3-(4-Chlorophenyl)-2-cyano-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)but-2-enamide (**10b**) Yellow crystals (ethanol), yield 72 % (2.94 g) m.p. 188–190 °C; IR (KBr) ν_{max} 3489–3431, 3057, 2222, 2220, 1689, 1641; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.29 (s, 1H, D_2O exchangeable, NH), 7.23–7.39 (m, 8H, Bz, H-3′, H-4′, H-5′), 6.19 (s, 1H, H-5), 2.83 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.5 (C-1), 164.3 (C, CONH), 153.8, 152.8, 149.3, 146.5, 140.2, 138.4, 130.3, 127.9, 126.3, 125.2, 123.6, 121.2 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.7, 117.3 (2CN), 89.4, 96.6 (C=C), 27.3 (CH_3); EIMMS: *m/z* $[M]^+$ 409 (33); analysis calcd for $C_{20}H_{12}ClN_3OS_2$ (409.91): C, 58.60; H, 2.95; N, 10.25, S, 15.64. Found: C, 58.81; H, 3.17; N, 10.40; S, 15.83.

2-Amino-4-(4-chlorophenyl)-5-(thiophen-2-yl)-1,4-dihydropyridine-3-carbonitrile (**10c**) Orange crystals (ethanol), yield 60 % (2.34 g) m.p. 222–225 °C; IR (KBr) ν_{max} 3421, 3380, 3053, 2223, 2220, 1633; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.23–7.39 (m, 8H, Bz, H-3′, H-4′, H-5′), 6.18 (s, 1H, H-5), 3.21 (s, 3H, OCH_3), 2.83 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.9 (C-1), 164.5 (C, CONH), 153.6, 150.6, 149.7, 146.3, 142.8, 138.1, 136.8, 128.8, 127.6, 125.4, 124.6, 121.4 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.5, 117.8 (2CN), 89.4, 96.8 (C=C), 28.9 (C, OCH_3), 26.4 (C, CH_3); EIMMS: *m/z* $[M]^+$ 405 (22); analysis calcd for $C_{21}H_{15}N_3O_2S_2$ (405.49): C, 62.20; H, 3.73; N, 10.36, S, 15.82. Found: C, 62.43; H, 3.80; N, 10.51; S, 16.08.

Ethyl 5′-(2-cyano-3-phenylbut-2-enamido)-[2,3′-bithiophene]-4′-carboxylate (**10d**) Orange crystals (ethanol), yield 77 % (3.25), m.p. 166–168 °C; IR (KBr) ν_{max} 3466–3330, 3054, 2220, 1708, 1689, 1628; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.33 (s, 1H, D_2O exchangeable, NH), 7.23–7.44 (m, 8H, Bz, H-3′. H-4′, H-5′), 6.21 (s, 1H, H-5), 4.23 (q, 2H, J = 7.51 Hz, CH_2, OCH_2CH_3), 2.84 (s, 3H, CH_3), 1.12 (t, 3H, J = 7.51 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.7 (C-1), 164.8, 166.2 (C, CONH, $COOCH_2CH_3$), 154.8, 152.5, 146.3, 144.9, 142.8, 134.2, 130.5, 129.4, 125.2, 123.8, 122.5, 120.9 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.3 (CN), 89.5, 96.9 (C=C), 56.8 (CH_2, OCH_2CH_3), 19.2 (CH_3),16.4 (CH_3, OCH_2CH_3), EIMMS: *m/z* $[M]^+$ 422 (28); analysis calcd for $C_{22}H_{18}N_2O_3S_2$ (422.52): C, 62.54; H, 4.29; N, 6.63, S, 15.18. Found: C, 62.33; H, 4.29; N, 6.65; S, 15.28.

Ethyl 5′-(3-(4-chlorophenyl)-2-cyanobut-2-enamido)-[2,3′-bithiophene]-4′-carboxylate (**10e**) Orange crystals (ethanol), yield 64 % (2.92 g) m.p. 109–111 °C; IR (KBr) ν_{max} 3496–3438, 3056, 2222, 1631; ^1H NMR (DMSO-d_6, 200 MHz): δ = 8.25 (s, 1H, D_2O exchangeable, NH), 7.22–7.40 (m, 8H, Bz, H-3′. H-4′, H-5′), 6.18 (s, 1H, H -5), 4.24 (q, 2H, J = 7.23 Hz, CH_2, OCH_2CH_3), 2.65 (s, 3H, CH_3), 1.13 (t, 3H, J = 7.23 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.4 (C-1), 168.9, 165.3 (C, CONH, $COOCH_2CH_3$), 154.5, 152.2, 146.7, 144.5, 140.3, 134.8, 130.6, 129.1, 126.6, 123.3, 122.8, 120.4 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.8 (CN), 89.3, 96.2 (C=C),

56.3 (CH_2, OCH_2CH_3), 19.8 (CH_3), 16.6 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 456 (36); analysis calcd for $C_{22}H_{17}ClN_2O_3S_2$ (456.96): C, 57.82; H, 3.75; N, 6.13, S, 14.03. Found: C, 57.63; H, 4.01; N, 6.29; S, 14.41.

Ehyl 5′-(2-cyano-3-(4-methoxyphenyl)but-2-enamido)-[2,3′-bithiophene]-4′-carboxylate (**10f**) Yellow crystals (1,4-dioxane), yield 76 % (3.43 g), m.p. 177–179 °C; IR (KBr) ν_{max} 3563–3359, 3058, 2221, 1688, 1635; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.82$ (s, 1H, D_2O exchangeable, NH), 7.26–7.43 (m, 8H, Bz, H-3′, H-4′, H-5′), 6.19 (s, 1H, H-5), 4.22 (q, 2H, J = 7.52 Hz, CH_2, OCH_2CH_3), 3.11 (s, 3H, Bz–OCH_3), 2.64 (s, 3H, CH_3), 1.13 (t, 3H, J = 7.52 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.6 (C-1), 168.4, 164.8 (C, CONH, $COOCH_2CH_3$), 153.7, 152.8, 145.3, 144.9, 141.6, 133.9, 130.5, 129.8, 126.8, 123.6, 122.3, 120.3 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.8 (CN), 89.4, 96.1 (C=C), 56.4 (CH_2, OCH_2CH_3), 24.3 (OCH_3), 19.8 (CH_3), 16.8 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 452 (22); analysis calcd for $C_{23}H_{20}N_2O_4S_2$ (452.55): C, 61.04; H, 4.45; N, 6.19, S, 14.17. Found: C, 61.29; H, 4.39; N, 6.24; S, 14.30.

*General procedure for the synthesis of the thiophene derivatives **11a–11f***

To a solution of any of compound **10a** (3.75 g, 0.01 mol), **10b** (4.09 g, 0.01 mol), **10c** (4.05 g, 0.01 mol), **10d** (4.22 g, 0.01 mol), **10e** (4.56 g, 0.01 mol) or **10f** (4.52 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), elemental sulfur (0.32 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 a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-Amino-N-(4′-cyano-[2,3′-b]thiophen-5′-yl)-4-phenylthiophene-3-carboxamide (**11a**) Brown crystals (1,4-dioxane), yield 68 % (2.55 g) m.p. 144–146 °C; IR (KBr) ν_{max} 3466–3431, 3055 2228, 1686, 1631; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.31$ (s, 1H, D_2O exchangeable, NH), 7.25–7.40 (m, 8H, Bz, H-3, H-4, H-5), 6.16, 6.24 (2s, 2H, H-5′, H-5′′), 4.72 (s, 2H, D_2O exchangeable, NH_2), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 164.5 (C, CONH), 152.1, 149.2, 146.9, 145.6, 142.2, 140.4, 128.9, 128.2, 126.4, 125.3, 124.6, 122.6, (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.4 (CN), EIMMS: *m/z* $[M]^+$ 407 (28); analysis calcd for $C_{20}H_{13}N_3OS_3$ (407.53): C, 58.94; H, 3.22; N, 10.31, S, 23.60. Found: C, 58.88; H, 3.41; N, 10.08; S, 23.51.

2-Amino-4-(4-chlorophenyl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)thiophene-3-carboxamide (**11b**) Yellow crystals (1,4-dioxane), 70 % (3.08 g) m.p. 133–135 °C; IR (KBr) ν_{max} 3478–3421, 3053, 2220, 1686, 1638; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.26$ (s, 1H, D_2O exchangeable, NH), 7.24–7.41 (m, 8H, Bz, H-3, H-4, H-5), 6.12, 6.23 (2s, 2H, H-5′, H-5′′), 4.31 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 165.3 (C, CONH), 152.6, 150.5, 148.4, 145.2, 139.4, 133.9, 132.8, 128.4, 125.9, 124.1, 123.8, 121.8 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.9 (CN); EIMMS: *m/z* $[M]^+$ 441 (28); analysis calcd for $C_{20}H_{12}ClN_3OS_3$ (441.98): C, 54.35; H, 2.74; N, 9.51, S, 21.76. Found: C, 54.51; H, 2.93; N, 9.47; S, 21.53.

2-Amino-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)-4-(4-methoxyphenyl)thiophene-3-carboxamide (**11c**) Pale yellow crystals (1,4-dioxane), yield 82 % (3.58 g), m.p. 211–213 °C; IR (KBr) ν_{max} 3479, 3325, 3054, 2221, 1636; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.41$ (s, 1H, D_2O exchangeable, NH), 7.26–7.43 (m, 8H, Bz, H-3, H-4, H-5, H-5′, H-5′′), 6.11, 6.20 (2s, 2H, H-5′, H-5′′), 4.38 (s, 2H, D_2O exchangeable, NH_2), 3.19 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 164.6 (C, CONH), 153.8, 151.8,, 149.5, 164.4 146.5, 143.1, 136.9, 130.6, 129.3, 126.1, 124.5, 125.9, 121.9 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.4 (CN), 29.2 (C, OCH_3); EIMMS: *m/z* $[M]^+$ 437 (16); analysis calcd for $C_{21}H_{15}N_3O_2S_3$ (437.56): C, 57.64; H, 3.46; N, 9.60, S, 21.98. Found: C, 57.82; H, 3.60; N, 9.48; S, 22.03.

Ethyl 5′-(2-amino-4-phenylthiophene-3-carboxamido)-[2,3′-bithiophene]-4′-carboxylate (**11d**) Orange crystals (ethanol), yield 67 % (3.04 g), m.p. 211–213 °C; IR (KBr) ν_{max} 3483–3341, 3056, 2222, 1705, 1686, 1626; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.41$ (s, 1H, D_2O exchangeable, NH), 7.26–7.39 (m, 8H, Bz, H-3, H-4, H-5, H-5′, H-5′′), 6.13, 6.29 (2s, 2H, H-5′, H-5′′), 4.44 (s, 2H, D_2O exchangeable, NH_2), 4.24 (q, 2H, J = 7.41 Hz, CH_2, OCH_2CH_3), 1.14 (t, 3H, J = 7.41 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 166.3, 164.3 (C, CONH, $COOC_2H_5$), 154.2, 153.8, 146.8, 144.9, 143.8, 134.8, 130.6, 126.6, 125.8, 123.6 , 122.4, 120.9 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.6 (CN), 56.4 (CH_2, OCH_2CH_3), 16.3 (CH_3, OCH_2CH_3). EIMMS: *m/z* $[M]^+$ 454 (20); analysis calcd for $C_{22}H_{18}N_2O_3S_3$ (454.58): C, 58.13; H, 3.99; N, 6.16, S, 21.16. Found: C, 58.09; H, 4.17; N, 6.28; S, 21.35.

Ethyl 5′-(2-amino-4-(4-chlorophenyl)thiophene-3-carboxamido)-[2,3′-bithiophene]-4′-carboxylate (**11e**) Orange crystals (1,4-dioxane), yield 58 % (2.64 g), m.p. 178–179 °C; IR (KBr) ν_{max} 3484–3434, 3053, 2222, 1632; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.26$ (s, 1H, D_2O exchangeable, NH); 7.25–7.42 (m, 8H, Bz, H-3, H-4, H-5, H-5′, H-5′′), 6.14, 6.24 (2s, 2H, H-5′, H-5′′), 4.47 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, J = 6.11 Hz, CH_2, OCH_2CH_3), 1.15 (t, 3H, J = 6.11 Hz, CH_3, CH_2CH_3); ^{13}C

NMR (DMSO-d_6, 75 MHz): δ = 164.2, 166.2 (C, CONH, $COOC_2H_5$), 153.9, 152.7, 145.3, 144.8, 141.9, 134.4, 130.2, 129.5, 126.8, 124.8, 122.9, 120.9 (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 116.5 (CN), 56.2 (CH_2, OCH_2CH_3), 16.4 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 456 (29); analysis calcd for $C_{22}H_{17}ClN_2O_3S_2$: C, 57.03; H, 3.75; N, 6.13, S, 14.03. Found: C, 57.27; H, 3.59; N, 7.83; S, 14.24.

Ehyl 5′-(2-cyano-3-(4-methoxyphenyl)but-2-enamido)-[2,3′-bithiophene]-4′-carboxylate (**11f**) Yellow crystals (ethanol), yield 60 % (2.90 g), m.p. 177–179 °C; IR (KBr) ν_{max} 3563–3359 (NH_2, NH), 3058 (CH aromatic), 2221 (CN), 1688 (CO), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.83 (s, 1H, D_2O exchangeable, NH), 7.26–7.43 (m, 8H, Bz, H-3, H-4, H-5, H-5′, H-5′′), 6.19, 6.28 6.14, 6.24 (2s, 2H, H-5′, H-5′′), 4.22 (q, 2H, J = 7.52 Hz, CH_2, OCH_2CH_3), 3.11 (s, 3H, OCH_3), 2.64 (s, 3H, CH_3), 1.13 (t, 3H, J = 7.52 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 164.8, 166.3 (C, CONH, $COOC_2H_5$), 153.7, 152.9, 145.2, 144.9, 141.6, 133.6, 130.7, 128.2, 126.3, 123.8, 122.4, 121.3, (Bz, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 117.3 (CN), 56.8 (CH_2, OCH_2CH_3), 24.5 (OCH_3), 16.6 (CH_3, OCH_2CH_3), EIMMS: *m/z* $[M]^+$ 484 (28); analysis calcd for $C_{23}H_{20}N_2O_4S_3$ (484.61) : C, 57.00; H, 4.16; N, 5.78, S, 19.85. Found: C, 56.93; H, 4.29; N, 5.63; S, 20.17.

*General procedure for the synthesis of the thiazole derivatives **13a** and **13b***

To a solution of either compound **7a** (2.73 g, 0.01 mol) or **7b** (3.20 g, 0.01 mol) in acetic acid, thioglycollic acid (0.92 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water then was left over night and the formed solid product was collected by filtration.

N-(4′-Cyano-[2,3′-bithiophen]-5′-yl)-2-(4-oxo-4,5-dihydrothiazol-2-yl)acetamide (**13a**) Orange crystals (ethanol), yield 76 % (2.63 g), m.p. 155–158 °C; IR (KBr) ν_{max} 3455–3423, 3056, 2220, 1710, 1688, 1636; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.23–7.40 (m, 3H, H-1′, H-2′, H-3′), 6.14 (s, 1H, H-4), 5.98 (s, 2H, C-5), 4.89 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.8 (C, C-2), 164.4, 168.9 (C, CONH, C-3), 153.9, 150.2, 148.8, 144.6, 143.8, 141.8, 131.2, 129.3, 126.3 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 116.7 (CN), 88.9 (CH_2, C-2); EIMMS: *m/z* $[M]^+$ 347 (20); analysis calcd for $C_{14}H_9N_3O_2S_3$ (347.44): C, 48.40; H, 2.61; N, 12.09, S, 27.69. Found: C, 48.38; H, 2.79; N, 11.86; S, 27.49.

Ethyl 5′-(2-(4-oxo-4,5-dihydrothiazol-2-yl)acetamido)-[2,3′-bithiophene]-4′-carboxylate (**13b**) Pale yellow crystals (ethanol), 72 % (2.83 g), m.p. 201–203 °C; IR (KBr) ν_{max} 3476–3422, 3054, 2223, 2220, 1708, 1686, 1635; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.31 (s, 1H, D_2O exchangeable, NH), 7.29–7.46 (m, 3H, H-1′, H-2′, H-3′), 6.19 (s, 1H, thiophene H-5), 5.84 (s, 2H, C-5), 4.88 (s, 2H, CH_2), 4.21 (q, 2H, J = 7.17 Hz, CH_2, OCH_2CH_3), 1.31 (t, 3H, J = 7.17 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 173.3 (C-2), 154.0, 150.6, 144.3, 140.8, 134.7, 130.6, 128.3, 125.8 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 116.9 (2 CN), 88.6 (CH_2), 54.6 (CH_2, OCH_2CH_3), 16.8 (CH_3, OCH_2CH_3), EIMMS: *m/z* $[M]^+$ 394 (31); MS: m/e = 394 (M^+, 31 %); analysis calcd for $C_{16}H_{14}N_2O_4S_3$ (394.49): C, 48.71; H, 3.58; N, 7.10, S, 24.38. Found: C, 48.62; H, 3.49; N, 6.87; S, 24.06.

*General procedure of the thieno[4,5-b]pyran derivatives **14a–14f***

To a solution of compound **13a** (3.47 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL) either of compound **2a** (0.66 g, 0.01 mol) or **2b** (1.13 g, 0.01 mol) and any of benzaldehyde (0.106 g, 1 mmol), 4-methoxybenzaldehyde (0.137 g, 1 mmol) 4-chlorobenzaldehyde (1.40 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 4 h and the formed solid product formed upon cooling was collected by filtration.

2-(5-Amino-6-cyano-7-phenyl-7H-pyrano[2,3-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**14a**) Yellow crystals (1,4-dioxane), yield 67 % (3.35 g), m.p..144–146 °C; IR (KBr) ν_{max} 3489–3418, 3058, 2227, 2220, 1687, 1630; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.22 (s, 1H, D_2O exchangeable, NH), 7.24–7.48 (m, 8H, Bz, H-3′′, H-4′′, H-5′′), 6.13 (s, 1H, H-5′), 5.24 (s, 1H, H-5), 4.82 (s, 2H, CH_2), 4.38 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 172.0 (C, C-2), 164.3 (C, CONH), 153.6, 152.3, 150.2, 149.2, 148.6, 144.2, 143.2, 140.5, 128.0, 127.8, 128.6, 125.5, 124.6, 122.3, 120.8, 119.8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.1, 116.5 (2CN), 62.8 (CH_2); EIMMS: *m/z* $[M]^+$ 501 (39); analysis calcd for $C_{24}H_{15}N_5O_2S_3$ (501.60): C, 57.47; H, 3.01; N, 13.96, S, 19.18. Found: C, 57.37; H, 2.89; N, 13.83; S, 19.04.

2-(5-Amino-6-cyano-7-(4-methoxyphenyl)-7H-pyrano[2,3-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**14b**) Orange crystals (ethanol), yield 82 % (53 g), m.p. 160–163 °C; IR (KBr) ν_{max} 3498–3425, 3057, 2224, 2220, 1688, 1636; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.36 (s, 1H, D_2O exchangeable, NH), 7.26–7.39 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.14 (s, 1H, H-5′), 5.24 (s, 1H, H-5),

4.88 (s, 2H, CH_2), 4.79 (s, 2H, D_2O exchangeable NH_2), 3.15 (s, 3H, OCH_3), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.6 (C-2), 164.3 (C, CONH), 154.6, , 153.6, 152.8, 150.1, 148.8, 146.8, 140.2, 138.4, 130.3, 129.3, 126.7, 125.6, 124.8, 120.6, 119.6, 121.7 8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 116.4, 117.0 (2CN), 62.6 (CH_2), 27.8 (OCH_3); EIMMS: *m/z* $[M]^+$ 531 (30); analysis calcd for $C_{25}H_{17}N_5O_3S_3$ (531.63): C, 56.48; H, 3.22; N, 13.17, S, 18.09. Found: C, 56.61; H, 3.46; N, 13.38; S, 17.80.

2-(5-amino-7-(4-chlorophenyl)-6-cyano-7H-pyrano[2,3-*d*] thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**14c**) Orange crystals (ethanol), yield 70 % (3.75 g), m.p. 166–168 °C; IR (KBr) ν_{max} 3441, 3386, 3058, 2223, 2220, 1680, 1638; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.29$ (s, 1H, D_2O exchangeable, NH), 7.25–7.43 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.15 (s, 1H, H-5′), 5.24 (s, 1H, H-5), 4.88 (s, 2H, CH_2), 4.33 (s, 2H, D_2Oexchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.3 (C-2), 164.8 (C, CONH), 154.8, 151.6, 149.5, 146.2, 144.9, 142.7, 136.8, 134.2, 133.5, 131.4, 130.5, 127.6, 126.3, 123.6, 120.5, 119.8, 8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.2, 116.7 (2CN), 62.8 (CH_2), EIMMS: *m/z* $[M]^+$ 536 (17); analysis calcd for $C_{24}H_{14}ClN_5O_2S_3$ (536.05): C, 53.77; H, 2.63; N, 13.06, S, 17.95. Found: C, 53.92; H, 2.97; N, 12.84; S, 18.06.

2-(6-Cyano-5-hydroxy-7-phenyl-7H-pyrano[2,3-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)-acetamide (**14d**) Orange crystals (ethanol), yield 55 % (2.76 g), m.p. 205–208 °C; IR (KBr) ν_{max} 3573–3331, 3056, 2224, 2221, 1690, 1632; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.22$ (s, 1H, D_2O exchangeable, OH), 8.23 (s, 1H, D_2O exchangeable, NH), 7.28–7.41 (m, 8H, Bz, H-3′′, H-4′′, H-5′′), 6.16 (s, 1H, H-5′), 5.29 (s, 1H, H-5), 4.86 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.9 (C-2), 164.8 (C, CONH), 153.9, 152.4, 150.8, 149.8, 148.1, 146.4, 143.3, 140.8, 133.1, 128.6, 127.1, 126.8, 124.4, 121.3, 120.9, 119.8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.3, 116.8 (2CN), 62.3 (CH_2); EIMMS: *m/z* $[M]^+$ 502 (18); analysis calcd for $C_{24}H_{14}N_4O_3S_3$ (502.59): C, 57.35; H, 2.81; N, 11.15, S, 19.14. Found: C, 57.40; H, 2.68; N, 10.93; S, 19.27.

2-(6-Cyano-5-hydroxy-7-(4-methoxyphenyl)-7H-pyrano [2,3-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl) acetamide (**14e**) Orange crystals (1,4-dioxane), m.p. 162–164 °C; IR (KBr) ν_{max} 3487–3430, 3053, 2223, 2220, 1631; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.31$(s, 1H, D_2O exchangeable, OH), 8.28 (s, 1H, D_2O exchangeable, NH), 7.25–7.39 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.14 (s, 1H, H-5′), 5.67 (s, 2H, CH_2), 5.22 (s, 1H, H-5), 3.12 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.9 (C-2), 165.1 (C, CONH), 154.8, 152.2, 150.2, 148.6, 144.1, 142.2, 140.9, 134.8, 130.6, 129.6, 126.3, 123.8, 122.5, 122.4, 120.5, 119.8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 116.4, 117.0 (2CN), 64.2 (CH_2), 16.6 (OCH_3); EIMMS: *m/z* $[M]^+$ 532 (33); analysis calcd for $C_{25}H_{16}N_4O_4S_3$ (532.61) : C, 56.38; H, 3.03; N, 10.52, S, 18.06. Found: C, 56.41; H, 3.29; N, 10.28; S, 17.79.

2-(7-(4-Chlorophenyl)-6-cyano-5-hydroxy-7H-pyrano[2,3-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**14f**) Reddish brown crystals (ethanol), yield 80 % (4.29 g), m.p. 123–125 °C; IR (KBr) ν_{max} 3497–3328, 3054, 2226, 2220, 1689, 1631; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.29$ (s, 1H, D_2O exchangeable, OH), 8.26 (s, 1H, D_2O exchangeable, NH), 7.28–7.42 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.16 (s, 1H, H-5′), 5.71 (s, 2H, CH_2), 5.26 (s, 1H, H-5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.4 (C, C-2), 164.9 (C, CONH), 154.2, 153.7, 150.4, 148.0, 144.8, 143.2, 141.5, 136.3, 131.4, 129.9, 127.5, 123.9, 123.0, 122.2, 120.4, 119.6 8 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.3, 116.0, (2CN), 64.8 (CH_2); EIMMS: *m/z* $[M]^+$ 537 (32); analysis calcd for $C_{24}H_{13}ClN_4O_3S_3$ (537.03): C, 53.68; H, 2.44; N, 10.43, S, 17.91. Found: C, 53.72; H, 2.39; N, 10.28; S, 18.18.

*General procedure of the thieno[4,5-*b*]pyridine derivatives* ***15a–15f***

To a solution of compound **13a** (3.47 g, 0.01 mol) in ethanol (40 mL) containing ammonium acetate (0.80 g) either of compound **2a** (0.66 g, 0.01 mol) or **2b** (1.13 g, 0.01 mol) and any of benzaldehyde (0.106 g, 1 mmol), 4-methoxybenzaldehyde (0.137 g, 1 mmol) or 4-chlorobenzaldehyde (1.40 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 6 h and the formed solid product formed upon cooling was collected by filtration.

2-(5-Amino-6-cyano-7-phenyl-4,7-dihydrothiazolo[4,5-*b*] pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15a**) Brown crystals (acetic acid), yield 72 % (3.60 g), m.p. 188–190 °C; IR (KBr) ν_{max} 3469–3430, 3058, 2227, 2220 (2CN), 1687 (CO), 1630; ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.28$, 8.32(2s, 2H, D_2O exchangeable, 2NH), 7.28–7.48 (m, 8H, Bz, H-3′′, H-4′′, H-5′′), 6.12 (s, 1H, H-5′), 5.26 (s, 1H, H-5), 4.88 (s, 2H, CH_2), 4.36 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.9 (C-2), 164.5 (C, CONH), 152.9, 152.4, 150.2, 149.9, 148.1, 145.8, 143.6, 141.7, 129.9, 128.3, 127.5, 125.6, 124.8, 122.5, 121.8, 120.3 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.3, 116.8 (2CN), 62.5 (CH_2); EIMMS: *m/z*

$[M]^+$ 500 (27); analysis calcd for $C_{24}H_{16}N_6OS_3$ (500.62): C, 57.58; H, 3.22; N, 16.79, S, 19.22. Found: C, 57.48; H, 3.39; N, 17.03; S, 19.08.

2-(5-Amino-6-cyano-7-(4-methoxyphenyl)-4,7-dihydrothiazolo[4,5-*b*]pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15b**) Orange crystals (ethanol), yield 60 % (3.18 g), m.p. 211–213 °C; ; IR (KBr) ν_{max} 3479–3433, 3053, 2225, 2221, 1688; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.29$, 8.36 (2s, 2H, D_2O exchangeable, 2NH), 7.23–7.38 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.13 (s, 1H, H-5′), 5.26 (s, 1H, H-5), 4.86 (s, 2H, CH_2), 4.49 (s, 2H, D_2O exchangeable NH_2), 3.14 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.9 (C-2), 164.5 (C, CONH), 154.2, 152.7, 143.6, 141.6, 136.6, 132.6, 129.0, 126.7, 124.9, 123.4 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.3, 116.6 (2CN), 63.2 (CH_2), 54.9 (OCH_3); EIMMS: *m/z* $[M]^+$ 530 (26); analysis calcd for $C_{25}H_{18}N_6O_2S_3$ (530.64): C, 56.59; H, 3.42; N, 15.84, S, 18.13. Found: C, 56.72; H, 3.55; N, 16.01; S, 18.09.

2-(5-Amino-7-(4-chlorophenyl)-6-cyano-4,7-dihydrothiazolo[4,5-*b*]pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15c**) Orange crystals (1,4-dioxane), yield 66 % m.p. 277–279 °C; IR (KBr) ν_{max} 3459, 3328, 3058, 2224, 2220, 1689, 1630; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.24$, 8.40 (2s, 2H, D_2O exchangeable, 2NH), 7.26–7.39 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.15 (s, 1H, H-5′), 6.03 (s, 1H, H-5), 4.86 (s, 2H, CH_2), 4.75 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.6 (C-2), 164.3 (C, CONH), 154.8, 152.6, 151.6, 150.1, 149.2, 143.2, 141.8, 133.9, 132.8, 129.3, 128.3, 124.9, 123.6, 123.2, 122.6, 120.4 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.1, 116.8 (2CN), 63.4 (CH_2); EIMMS: *m/z* $[M]^+$ 535 (20); analysis calcd for $C_{24}H_{15}ClN_6OS_3$ (535.06): C, 53.87; H, 2.83; N, 15.71, S, 17.98. Found: C, 53.41; H, 2.69; N, 15.88; S, 18.21.

2-(6-Cyano-5-hydroxy-7-phenyl-4,7-dihydrothiazolo[4,5-*b*]pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15d**) Orange crystals from (1,4-dioxane), yield 68 % (3.40 g), m.p. 277–279 °C; IR (KBr) ν_{max} 3591–3320, 3053, 2228, 2220, 1689, 1634; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.26$ (s, 1H, D_2O exchangeable, OH), 8.23, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 7.23–7.45 (m, 8H, Bz, H-3′′, H-4′′, H-5′′), 6.15 (s, 1H, H-5′), 6.04 (s, 1H, H-5), 4.89 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 169.8 (C-2), 164.8 (C, CONH), 154.0, 153.9, 152.5, 151.3, 150.3, 148.7, 144.9, 143.1, 139.2, 135.6, 128.8, 127.7, 125.3, 122.9, 121.8, 120.3 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.1, 116.5 (2CN), 62.1 (CH_2); EIMMS: *m/z* $[M]^+$ 501 (33); analysis calcd for $C_{24}H_{15}N_5O_2S_3$ (501.60): C, 57.47; H, 3.01; N, 13.96, S, 19.18. Found: C, 57.39; H, 2.84; N, 14.27; S, 19.32.

2-(6-Cyano-5-hydroxy-7-(4-methoxyphenyl)-4,7-dihydrothiazolo[4,5-*b*]pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15e**) Orange crystals (ethanol), yield 58 % (3.08 g), m.p. 104–106 °C; IR (KBr) ν_{max} 3493–3428, 3056, 2224, 2220, 1630; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.22$ (s, 1H, D_2O exchangeable, OH), 8.24, 8.32 (2s, 2H, D_2O exchangeable, 2NH), 7.24–7.49 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.16 (s, 1H, H-5′), 6.08 (s, 1H, H-5), 4.85 (s, 2H, CH_2), 3.12 (s, 3H, OCH_3);); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.8 (C-2), 164.6 (C, CONH), 154.6, 153.5, 151.4, 150.3, 149.1, 147.3, 143.6, 138.6, 135.8, 130.6, 127.9, 124.6, 123.4, 122.5, 121.8, 120.4 (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 116.2, 117.4 (2CN), 62.8 (CH_2); EIMMS: *m/z* $[M]^+$ 531 (34); analysis calcd for $C_{25}H_{17}N_5O_3S_3$ (531.60) : C, 56.48; H, 3.22; N, 13.17, S, 18.09. Found: C, 56.58; H, 3.31; N, 13.40; S, 17.89.

2-(7-(4-Chlorophenyl)-6-cyano-5-hydroxy-4,7-dihydrothiazolo[4,5-b]pyridin-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**15f**) Brown crystals (ethanol), yield 55 % (2.94 g), m.p 168–171 °C; IR (KBr) ν_{max} 3483–3312, 3056, 2224, 2220, 1688, 1635; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.27$ (s, 1H, D_2O exchangeable, OH), 8.25, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 7.23–7.44 (m, 7H, Bz, H-3′′, H-4′′, H-5′′), 6.13 6.16 (s, 1H, H-5′), 6.05 (s, 1H, H-5), 4.82 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 168.4 (C-2), 164.8 (C, CONH), 153.1, 151.8, 150.6, 149.8, 146.4, 143.6, 139.5, 137.2, 136.4, 133.9, 128.3, 124.6, 123.9, 121.6, 120.4, 119.8, (Bz, C-4, C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 117.1, 116.4, (2CN), 62.4 (CH_2); EIMMS: *m/z* $[M]^+$ 536 (24); analysis calcd for $C_{24}H_{14}ClN_5O_2S_3$ (536.00): C, 53.77; H, 2.63; N, 13.06, S, 17.95. Found: C, 53.83; H, 2.48; N, 12.99; S, 18.21.

*Synthesis of the 2-bromothiazole derivatives **16a** and **16b***

To a solution of either of compound **13a** (3.47 g, 0.01 mol) or **13b** (3.94 g, 0.01 mol) in acetic acid (60 mL) at 60 °C, bromine (1.16 g, 0.01 mol) in acetic acid (10 mL) was added drop-wise. The whole reaction mixture was stirred at room temperature for 0.50 h then poured onto ice/water.

2-(5-Bromo-4-oxo-4,5-dihydrothiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**16a**) Yellow crystals (ethanol), yield 70 % (2.96 g), m.p. 89–92 °C; IR (KBr) ν_{max} 3455–3423, 3056, 2220, 1710, 1688, 1636; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.30$ (s, 1H, D_2O exchangeable, NH), 7.26–7.39 (m, 3H, H-3′′, H-4′′, H-5′′), 6.89 (s, 1H, H-5),

6.12 (s, 1H, H-4′), 4.93 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 174.5 (C-2), 166.2, 164.7 (2C, C-4, CONH), 153.1, 150.8, 148.8, 144.6, 141.8, 130.2, 126.1, 124.7 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 69.3 (CH, C-5), 63.8 (CH_2), EIMMS: *m/z* $[M]^+$ 424 (14); analysis calcd for $C_{14}H_8BrN_3O_2S_3$ (424.30): C, 39.44; H, 1.89; N, 9.86, S, 22.56. Found: C, 39.62; H, 2.02; N, 10.17; S, 22.81.

Ethyl 5′-(2-(4-oxo-4,5-dihydrothiazol-2-yl)acetamido)-[2,3′-bithiophene]-4′-carboxylate (**16b**) Pale yellow crystals (ethanol) yield, 66 % (3.11 g) m.p. 201–203 °C; IR (KBr) ν_{max} 3476–3422, 3054, 1708–1686, 1635; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.28–7.48 (m, 3H, H-3′′, H-4′′, H-5′′), 6.63 (s, 1H, H-5), 6.15 (s, 1H, H-4′), 4.79 (s, 2H, CH_2), 4.24 (q, 2H, J = 7.63 Hz, CH_2, OCH_2CH_3), 1.18 (t, 3H, J = 7.63 Hz, CH_3, OCH_2CH_3), ; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 174.3 (C-2), 164.5, 166.1 (2CO, C-4, CONH), 153.6, 152.8, 148.6, 142.8, 138.4, 131.7, 128.9, 124.3, 123.4 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 69.6 (CH, C-5), 63.6 (CH_2), 44.5 (CH_2, OCH_2CH_3), 16.4 (CH_3, OCH_2CH_3); EIMMS: *m/z* $[M]^+$ 473 (23); analysis calcd for $C_{16}H_{13}BrN_2O_4S_3$ (473.38): C, 40.60; H, 2.77; N, 5.92, S, 20.32. Found: C, 40.44; H, 3.01; N, 6.15; S, 20.29.

*General procedure for the synthesis of the thiazolo[4,5-d] thiazole derivatives **18a** and **18b***

To a solution of either of compound **16a** (4.24 g, 0.01 mol) or **16b** (4.71 g, 0.01 mol) in ethanol (50 mL) thiourea (0.76 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux and the formed solid product, upon cooling, was collected by filtration.

2-(5-Aminothiazolo[4,5-*d*]thiazol-2-yl)-N-(4′-cyano-[2,3′-bithiophen]-5′-yl)acetamide (**18a**) Yellow crystals (ethanol), yield 60 % (2.72 g) m.p. 144–147 °C; IR (KBr) ν_{max} 3488–3418, 3053, 2224, 1687, 1633; ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.29 (s, 1H, D_2O exchangeable, NH), 7.31–7.42 (m, 4H, H-3′′, H-4′′, H-5′′, H-5′), 5.21 (s, 2H, CH_2), ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 171.2, 170.3 (C-2, C-7), 164.8 (C, CONH), 122.9, 124.6, 132.7, 138.5, 142.9, 143.6, 145.1, 148.2, 149.5, 152.9 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′, C4, C-5), 116.8 (CN), 62.5 (CH_2), EIMMS: *m/z* $[M]^+$ 454 (28); analysis calcd for $C_{14}H_8BrN_5O_2S_3$ (452.34): C, 37.01; H, 1.77; N, 15.41, S, 21.17. Found: C, 36.92; H, 2.01; N, 15.03; S, 21.42.

Ethyl 5′-(2-(5-aminothiazolo[4,5-d]thiazol-2-yl)acetamido)-[2,3′-bithiophene]-4′-carboxylate (**18b**) Orange crystals (1,4-dioxane), yield 76 % (3.59 g), m.p. 188–191 °C; IR (KBr) ν_{max} 3433–3412, 3056, 1689, 1686, 1633; ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = \delta$ 8.24 (s, 1H, D_2O exchangeable, NH), 7.23–7.47 (m, 4H, H-3′′, H-4′′, H-5′′, H-5′), 5.22 (s, 2H, CH_2), 4.21 (q, 2H, J = 7.08 Hz, CH_2, OCH_2CH_3), 1.15 (t, 3H, J = 7.08 Hz, CH_3, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 171.3, 170.5 (C-2, C-7), 164.4 (C, CONH), 152.8, 148.4, 144.3, 140.1, 135.8, 131.3, 128.7, 123.6, 122.8, 120.5 (C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-"5, C4, C-5), 44.3 (CH_2, OCH_2CH_3), 16.8 (CH_3, OCH_2CH_3), EIMMS: *m/z* $[M]^+$ 473 (22); analysis calcd for $C_{16}H_{13}BrN_2O_4S_3$ (473.38): C, 40.60; H, 2.77; N, 5.92, S, 20.32. Found: C, 40.88; H, 2.94; N, 6.03; S, 20.48.

Conclusions

Through this work we succeeded to synthesis a series of pyran, thiazole derivatives incorporating thiophene ring followed by their antitumor evaluations. The results showed that compounds **4e**, **4f**, **5e**, **5f**, **7b**, **8b**, **10e**, **10f**, **11e**, **11f**, **14d–f**, **15d–f**, **16a, 16b** and **18b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. The toxicity of such compounds against shrimp larvae was measured where compounds **7b**, **10e**, **14d, 15e** and **16b** showed non toxicity against the tested organisms. The anti-proliferative activity of the most cytotoxic compounds showed that these compounds indicated high potency against the cell lines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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