Scientific paper

Synthesis, cytotoxic and anti-proliferative activity of novel thiophene, thieno[2,3-*b*]pyridine and pyran derivatives derived from 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivative

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Abstract

Novel tetrahydrobenzo[*b*]thienopyrole derivatives are synthesized from 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) through its reaction with α-chloroacetone to give the corresponding *N*-alkyl derivative **3**. Compound **3** undergoes ready cyclization in sodium ethoxide solution to give the tetrahydrobenzo[*b*]thienopyrrole **4**. The latter compound 4 is used as the key starting material for the synthesis of thiophene, thieno[2,3-*b*]pyridine and pyran derivatives. The cytotoxicity of the synthesized products towards the human cancer cell lines namely gastric cancer (NUGC), colon cancer (DLD-1), liver cancer (HA22T and HEPG-2), breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and normal fibroblast (WI-38) cell lines are measured. Compounds **4**, **7a**, **7b**, **8a**, **8b**, **10c, 10d**, **10f**, **12a**, **12b, 14b** and **15b** exhibit the optimal cytotoxic effect against cancer cell lines. Compounds **7b** and **14b** show the maximum inhibitory effect and these are much higher than the reference CHS-828 (pyridyl cyanoguanidine). On the other hand, the anti-proliferative evaluations of these compounds with high potency against the cancer cell lines L1210, Molt4/C8, CEM, K562, K562/4 and HCT116 show that compounds **7b** and **8b** give IC_{50}'s against Molt4/C8 and CEM cell lines higher than that of the reference, doxorubicin.

Keywords: tetrahydrobenzo[*b*]thiophene, pyran, thiophene, cytotoxicity, anti-proliferative activity

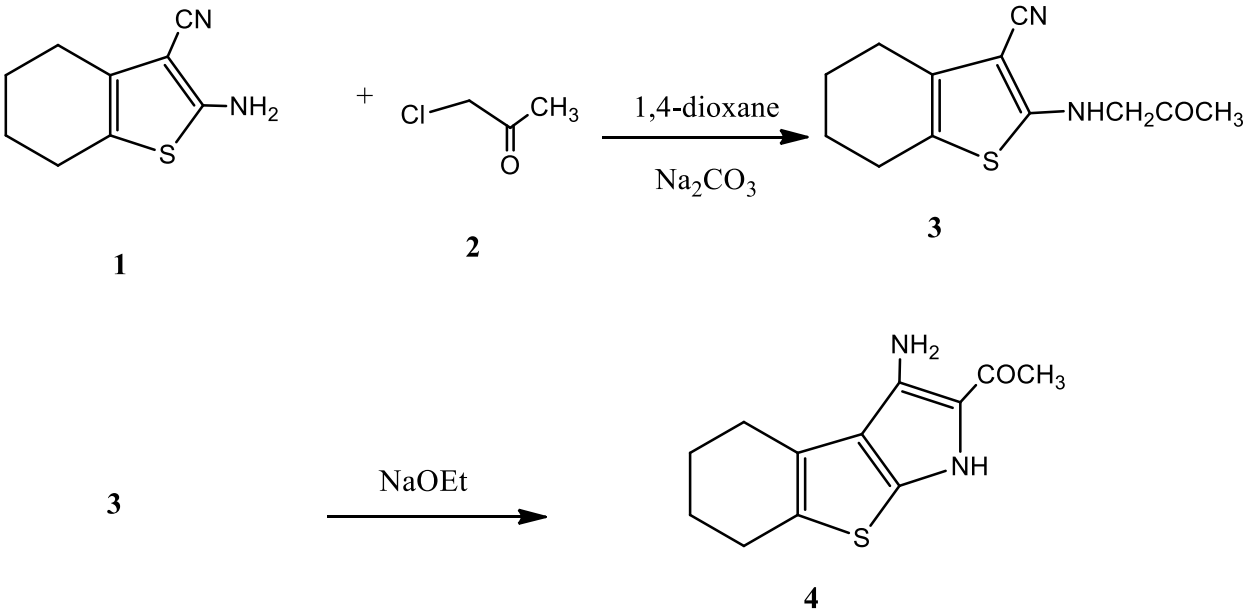
1. Introduction

Sulfur containing heterocycles paved way for the active research in the pharmaceutical Chemistry. Nowadays benzothiophene derivatives in combination with other ring systems have been used extensively in pharmaceutical applications.[1-3] A large number of compounds containing thiophene system have been investigated because of their broad spectrum of biological activities which include analgesic,[4] antibacterial,[5] antifungal,[6] antiparasitic,[7] antiviral,[8] anti-inflammatory,[9] anticonvulsant,[10] anti-nociceptive,[11] DNA cleavage,[12] herbicidal,[13] antitubercular,[14] protein kinase

inhibition,[15] respiratory syndrome protease inactivation,[16] an active ester in the peptide synthesis and agonists of peroxisome proliferator activated receptors.[17] In addition to these considerable biological applications, tetrahydrobenzo[*b*]thiophenes are important intermediates, protecting groups and final products in organic synthesis. Recently, our research group was involved through comprehensive program aiming for the synthesis of 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives followed by their antitumor evaluations.[18,19] Moreover, we reported the multi-component reactions with 3-(α-bromoacetyl)coumarin to give pyan and pyrididine derivatives.[20] In continuation of this program we are demonstrating the use of 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene for the synthesis of tetrahydrobenzo[*b*]thienopyrrole derivatives followed by their cytotoxic and the anti-proliferative evaluations.[21,22]

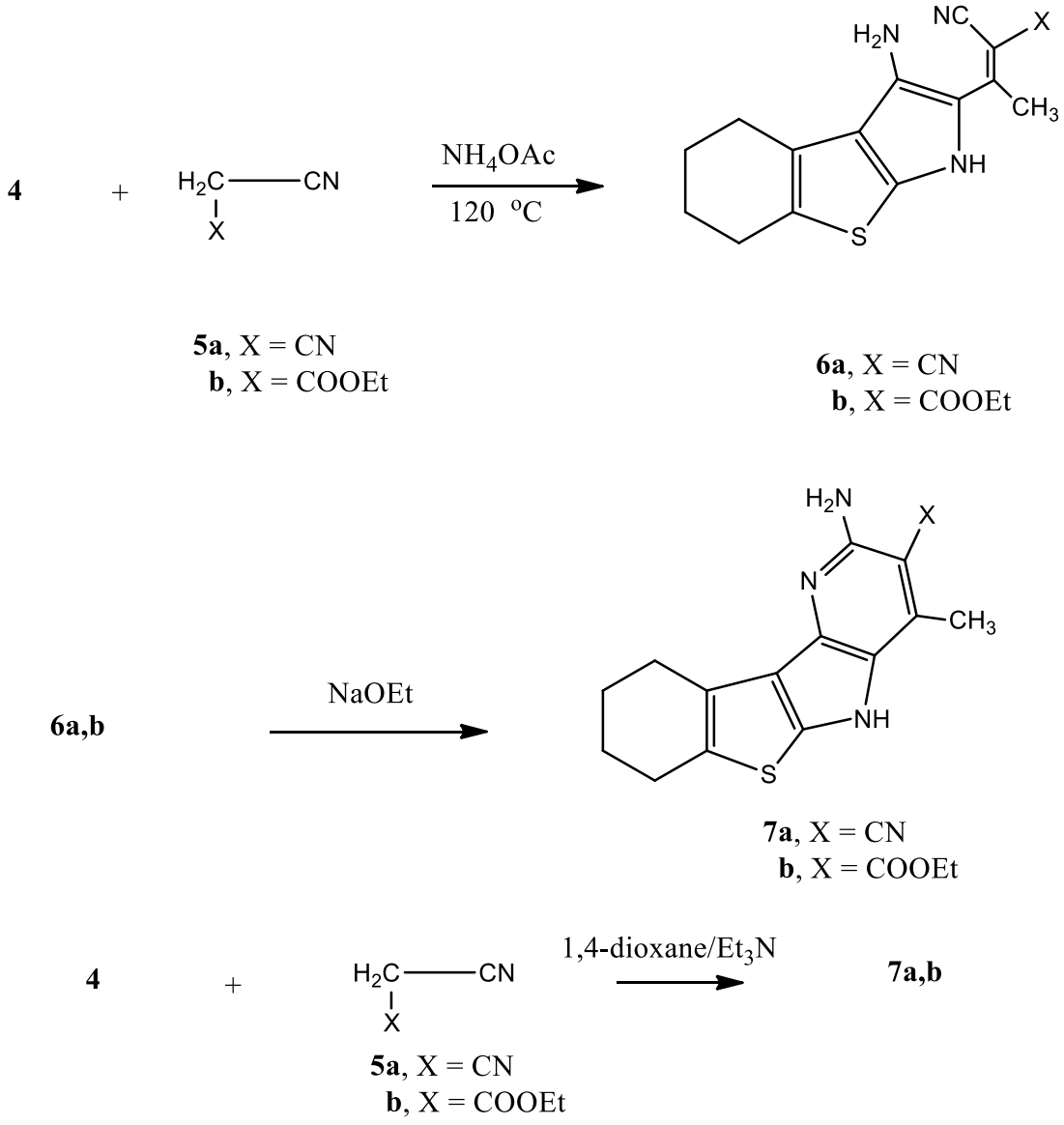
2. Results and discussion

The reaction of the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) with α-chloroacetone in the presence potassium carbonate afforded the 2-((2-oxopropyl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**3**). Compound **3** was characterized by ^{1}H-NMR and ^{13}C-NMR. Thus, the ^{1}H-NMR spectrum display the presence of beside the expected tetrahydrobenzene moiety, a singlet at δ 5.20 ppm indicating the presence of the *N*-CH_2 group, a singlet at δ 2.88 ppm assigned to the CH_3 group and a broad singlet at δ 8.30 ppm due to the NH group. Moreover, the ^{13}C-NMR spectrum showed δ: 19.6 (CH_3), 20.3, 22.0, 25.7 and 34.6 (4 CH_2), 55.6 (CH_2), 116.8 (CN), 124.1, 124.9, 128.7 and 139.5 (thiophene C), 164.8 (C=O). Compound **3** underwent ready cyclization when heated in sodium ethoxide solution in a boiling water bath to yield the 1-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)ethanone (**4**) (Scheme 1).



Scheme 1. Synthesis of compounds **3** and **4**.

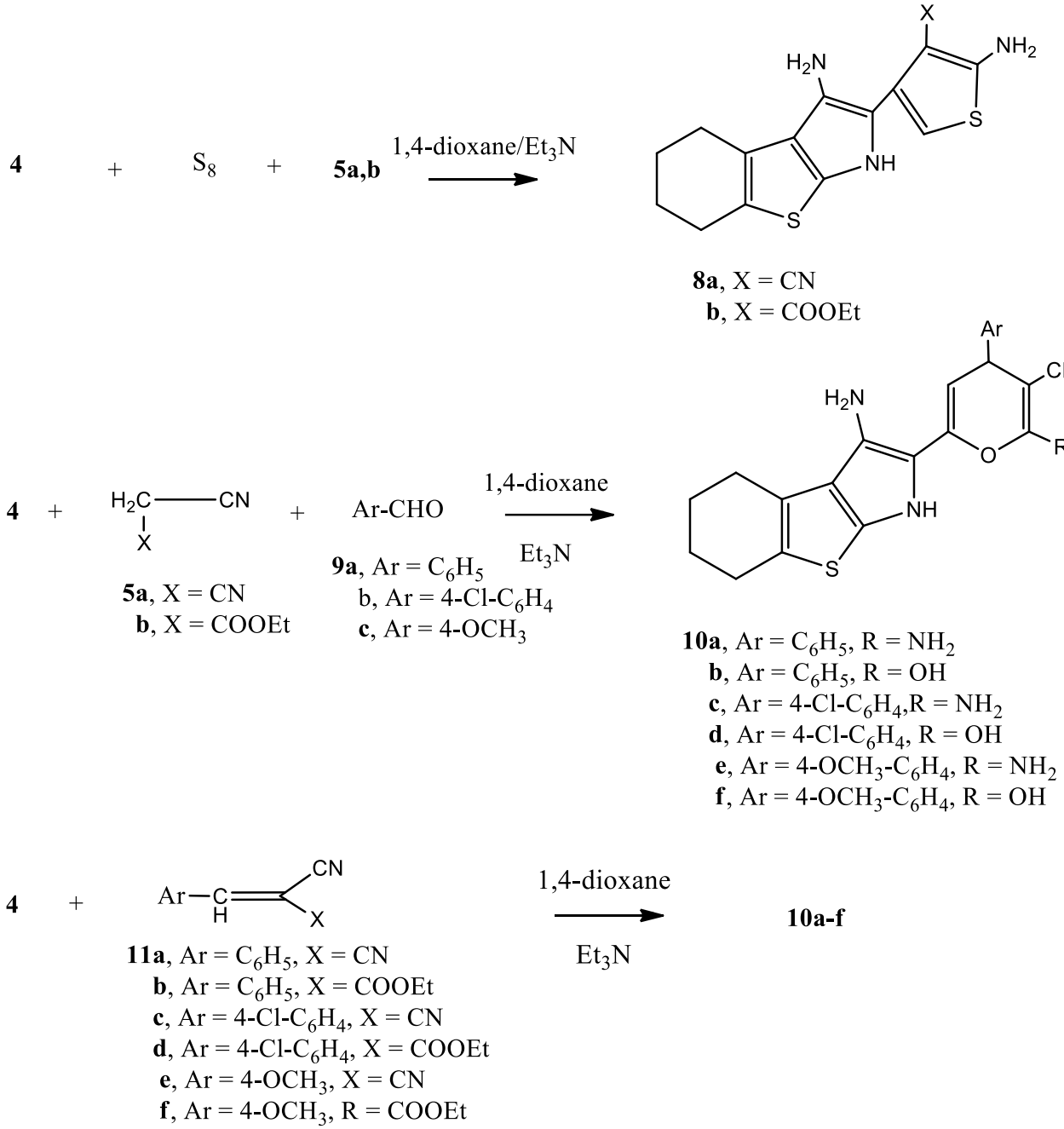
Compound **4** showed interesting reactivity towards different reagents, thus, it reacted with either malononitrile (**5a**) or ethyl cyanoacetate (**5b**) in the presence of ammonium acetate in an oil bath at 120 ^{o}C afforded the Knoevenagel condensated products **6a** and **6b**, respectively. The latter products underwent ready cyclization in sodium ethoxide solution to give the annulated products **7a** and **7b**, respectively (Scheme 2). The structures of the latter products were established on the basis of the analytical and spectral data. Thus, the ^{1}H-NMR spectrum of **7a** showed the presence of δ 2.89 ppm assigned to the CH_3 group, a singlet at δ 4.89 ppm indicating the NH_2 group and a singlet at δ 8.33 ppm confirming the presence of the NH group. Moreover, the ^{13}C-NMR spectrum showed δ 19.8 (CH_3), 20.1, 22.7, 25.2 and 34.6 (4 CH_2), 116.8 (CN), 120.1, 122.6, 123.8, 124.2, 125.3, 127.2, 135.6, 142.3 (thiophene, pyrrole, pyridine C) and 168.2 (C=N).



Scheme 2. Synthesis of compounds **6a,b and 7a,b**.,

Compound **4** was studied to produce thiophene derivatives through the Gewald's reaction[23-26] as many thiophenes were used as anticancer drugs. Thus, the reaction of compound **4** with either of malononitrile or ethyl cyanoacetate and elemental sulphur gave the thiophene derivatives **8a** and **8b**, respectively. On the other hand, the one pot reaction of compound **4** with either malononitrile or ethyl cyanoacetate and any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde gave the pyran derivatives **10a-f**, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra **10a-f** were consistent with their respective structures. Further confirmations for the structure of compounds **10a-f** were obtained through their synthesis via another synthetic root. Thus, the reaction of compound **4** with the cinnamonitrile derivatives **11a-f** in the presence of a catalytic amount of

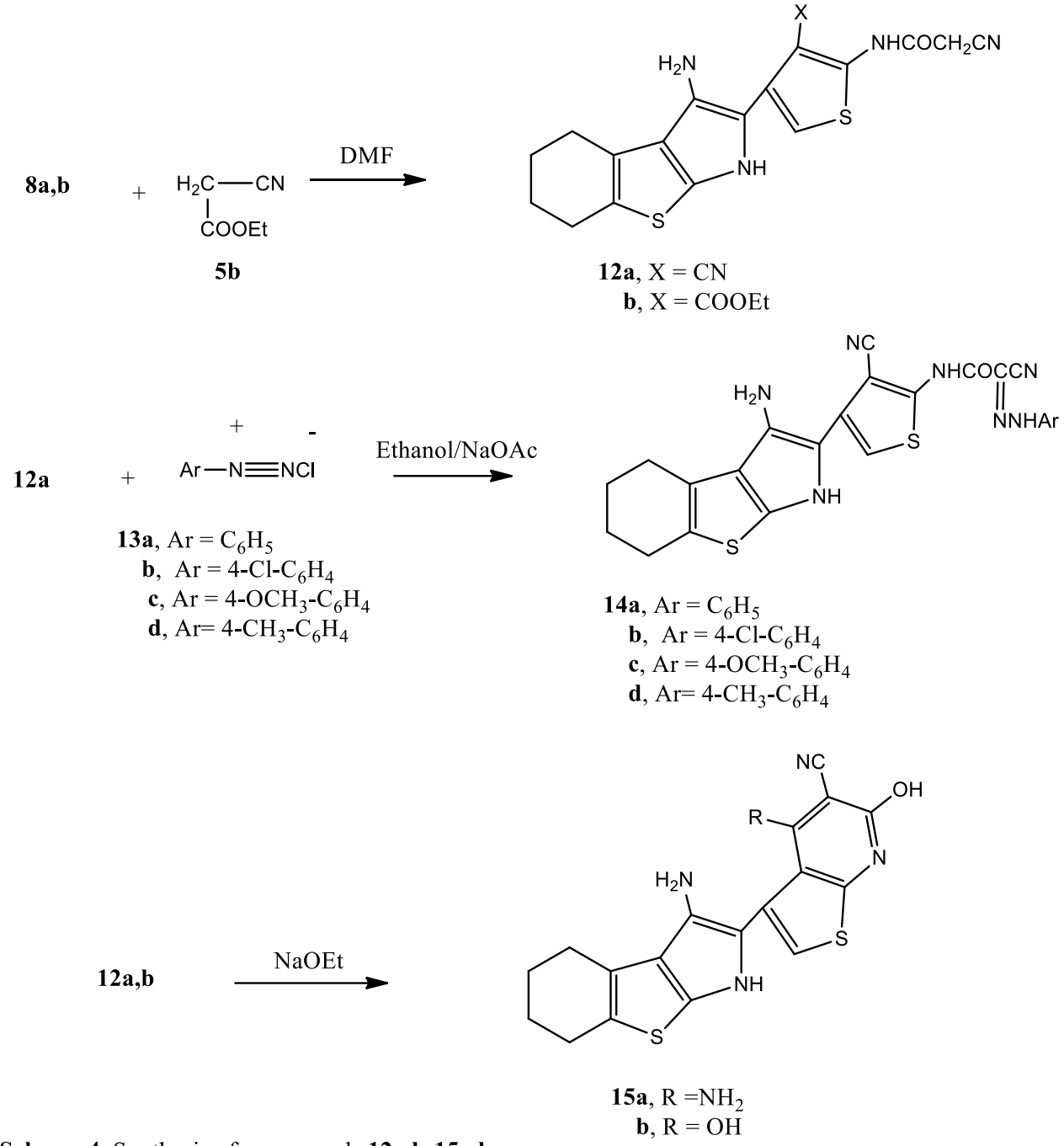
triethylamine gave the same products **10a-f**, respectively (m.p., mixed m.p. and fingerprint IR) (Scheme 3).



Scheme 3. Synthesis of compounds **8a,b and 10a-f**.

Moreover, the reaction of either of compound **8a** or **8b** with ethyl cyanoacetate in refluxing dimethylformamide afforded the 2-amido derivatives **12a** and **12b**, respectively. Formation of the latter products was explained on the condensation of ethyl cyanoacetate with the 2-aminothiophene

moiety not to the 3-aminopyrrol moiety on the basis of the ^{1}H-NMR spectra of such products. Thus, the ^{1}H-NMR spectrum of either **12a** or **12b** displayed the missing of the NH_2 group that attached to thiophene ring which is expected to appear within the range δ 5.10-5.24 ppm while that of the 3-aminopyrrole moiety existing at δ 4.81-4.83 ppm. Similar acylation of the 2-aminothiophene was reported before in literature.[27] The high yield of compound **12a**, encouraged us to make further work. Thus, the reaction of **12a** with either of the aryl diazonium salts **13a-d** gave the aryl hydrazo derivatives **14a-d**, respectively. Moreover, compounds **12a,b** underwent ready cyclization in sodium ethoxide to produce the thieno[2,3-*b*]pyridine derivatives **15a** and **15b**, respectively (Scheme 4).



Scheme 4. Synthesis of compounds **12a,b-15a,b**.

2.2.Anti-tumor cell activity

2.2.1. Chemicals and Cell cultures

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, CHS-828, penicillin, streptomycin and sulforhodamine B (SRB)

were purchased from Sigma Chemical Co. (Saint Louis, USA). The cell cultures was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human colon cancer (DLD-1), human liver cancer (HA22T and HEPG-2), human breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and normal fibroblast cells (WI-38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 10^5 cells/mL for the six human cancer cell lines including cells derived from 0.75 x 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.

2.2.2. In vitro cytotoxicity assay

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols[28,29] for their *in vitro* cytotoxicity against the six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD-1), human liver cancer (HA22T and HEPG-2), human breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and a normal fibroblast cells (WI-38). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds were observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50}=10–1000 nM). Normal fibroblasts cells (WI-38) were affected to a much lesser extent (IC_{50}>10,000 nM). The reference compound used was the CHS-828 which is the pyridyl cyanoguanidine anti-tumor agent.[30] It is a new chemotherapeutic drug in addition it has low toxicity and lacks known patterns of multidrug resistance.[31]

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

Compound No.	Cytotoxicity (IC_{50} in nM)						
	NUGC[b]	DLD-1[b]	HA22T[b]	HEPG-2[b]	HONE-1[b]	MCF-7[b]	WI-38[b]
3	2142	1222	1340	1028	1828	2246	NA
4	86	45	313	128	212	310	NA
6a	2101	2380	3258	2266	2380	3330	NA
6b	1335	1140	1072	1154	1064	1258	NA
7a	218	146	220	337	241	380	NA
7b	48	92	260	46	74	32	NA
8a	320	240	230	165	128	1265	NA
8b	48	35	53	170	49	78	NA
10a	1220	1033	2250	1275	2126	2372	NA
10b	1165	1322	2350	2221	2152	1322	NA
10c	330	532	822	442	1529	1224	NA
10d	30	62	74	39	1330	88	NA
10e	1135	2160	2160	814	780	296	NA
10f	149	2220	3210	550	2451	1286	120
12a	69	74	190	448	2871	2690	NA
12b	26	65	38	220	440	57	NA
14a	1350	1160	2290	2120	1126	2230	NA
14b	83	59	80	64	87	48	1330
14c	1480	1156	1346	1226	1275	1240	NA
14d	1245	2160	2180	2220	1869	1765	NA
15a	1845	1210	1218	1076	1270	436	Na
15b	1220	2063	377	740	253	2210	NA
CHS-828	25	2315	2067	1245	15	18	NA

[a]Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h.
[b]NUGC, gastric cancer; DLD-1, colon cancer; HA22T, liver cancer; HEPG-2, liver cancer; HONE-1, nasopharyngeal carcinoma; MCF-7, breast cancer; WI-38, normal fibroblast cells. NA: Not Active.

2.2.3. 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 **3 -15a,b**. Compounds **4**, **7a**, **7b**, **8a**, **8b**, **10c, 10d**, **10f**, **12a**, **12b, 14b** and **15b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of the tetrahydrobenzothiophene **3** and the cyclized product **4**, it is obvious that the cytotoxicity of compound **4** is higher than that of compound **3**. The presence of the pyrrol ring through the tetrahydrobenzo[*b*]thiophene in compound **4** is responsible for its high potency. The condensation reaction of compound **4** with either malononitrile or ethyl cyanoacetate to produce compounds **5a** and **5b**, respectively showed a decrease of cytotoxicity. On the other hand, the cyclization of compounds **6a** and **6b** to the benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-*b*]pyridine derivatives **7a** and **7b** showed remarkable increase of the cytotoxicity. Moreover, it is clear that compound **7b** showed more cytotoxicity than **7a**, this is attributed to the presence of the oxygen rich COOEt group. The introduction of the second thiophene moiety to compound **4** that gives both of compounds **8a** and **8b** showed high potency–especially–in case of compounds **8b** which was attributed due to the presence of the COOEt. Considering the pyran derivatives **10a-f**, the cytotoxicity of compounds **10c** and **10d** showed the highest values among the six compounds. However, compound **10c** showed high cytotoxicity against the four cancer cell lines HUGC, DLD-1, HA22T and HEPG-2, but it is of great value to notice that compound **10d** showed high cytotoxicity against five cancer cell lines and such cytotoxicity is higher than that of compound **10c**. The high cytotoxicity of compound **10d** is attributed to the presence of the OH and the Cl group as well.

The thiophene derivatives **12a** and **12b** showed high cytotoxicity similarl to that of compounds **8a,b**. Moreover, compound **12b** with the COOEt showed high potency than that of compound **12a**. The coupling of the diazonium salts **13a-d** with compound **12a** afforded the arylhydrazone derivatives **14a-d**. Compound **14b** with the Cl group showed the maximum cytotoxicity among the arylhydrazone derivatives **14a-d**. Finally, considering the thieno[2,3-*b*]pyridine derivatives **15a,b** where the presence of the OH in compound **15b** conserved an interesting cytotoxicity against the cancer cell lines HA22T, HEPG-2 and HONE-1 with the IC_{50}'s 377, 740, 253 nM, respectively. It is of great value to notice that compounds **7b**, **8b** and **12b** showed the maximum cytotoxicity among the tested compounds.

2.2.4. 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 2. 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 2 in which IC_{50} values represent the concentrations that inhibit cell proliferation by 50%. It is clear from Table 2 that tested compounds **4**, **7a**, **7b**, **8a**, **8b**, **10c**, **10d**, **10f**, **12a**, **12b, 14b** and **15b** showed high potency against the cell lines. The benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-*b*]pyridine derivative **7b** and the benzo[4,5]thieno-[2,3-*b*]pyrrol-2-yl)-thiophene derivative **8b** showed high potency against Molt4/C8 and CEM cell lines and their IC_{50}'s are higher than that of the reference doxorubicin. It is clear from Table 2 that the twelve tested compounds showed high IC_{50} against K562/4 cell line than doxorubicin.

Table 2: Anti-proliferative activity (IC_{50}) of selected compounds against variety of cell lines

Compound No.	Cytotoxicity (IC_{50} in nM)					
	L1210	Molt4/C8	CEM	K562	K562/4	HCT116
4	1.5±0.5	1.1±0.03	0.3±0.01	0.4±0.08	0.9±0.02	0.8±0.05
7a	0.4±0.1	0.8±0.04	2.0±0.4	1.8±0.03	0.9±0.06	1.3±0.02
7b	0.3±0.08	0.4±0.04	0.9±0.05	1.30±0.08	1.1±0.07	2.4±0.09
8a	1.2±0.09	0.8±0.02	0.6±0.01	0.2±0.01	0.9±0.08	1.4±0.2
8b	1.1±0.06	0.02±0.002	0.7±0.03	0.9±0.06	1.6±0.07	0.8±0.02
10c	0.8±0.05	0.4±0.02	1.3±0.05	0.6±0.02	0.02±0.01	1.2±0.08
10d	0.6±0.02	1.5±0.07	2.5±0.05	1.7±0.02	2.5±0.02	2.8±0.07
10f	1.4±0.05	0.8±0.03	2.6±0.09	0.02±0.01	2.8±0.06	0.4±0.08
12a	2.1±0.05	0.6±0.02	0.5±0.01	0.3±0.01	0.4±0.06	2.4±0.07
12b	1.8±0.09	0.9±0.04	1.8±0.6	0.7±0.06	0.8±0.06	0.9±0.08
14b	0.5±0.03	0.3±0.05	2.6±0.06	0.5±0.07	0.6±0.02	0.1±0.01
15b	0.9±0.02	0.3±0.01	0.6±0.05	2.1±0.07	2.7±1.03	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

3. Experimental

3.1. General

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. ^{13}C-NMR and ^{1}H-NMR spectra were recorded on Bruker DPX200 instrument in DMSO with 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 out by the Microanalytical Data Unit Ludwig-Maximilians-Universitat-Munchen, Germany. The progress of all reactions was monitored by TLC on 2 x 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

3.1.1. Synthesis of 2-((2-Oxopropyl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (3)

To a solution of compound **1** (1.78 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium carbonate (1.00 g) α-chloroacetone (0.94 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration and crystallized from ethanol**.**

White crystals; yield: 2.01 g (86%); mp: 182-183ºC; IR (KBr, cm^{-1}): 3465-3328 (NH), 2220 (CN), 1705 (C=O), 1615 (C=C); ^{1}H-NMR (dimethyl sulfoxide (DMSO)-d_6) δ:1.80-1.85 (m, 4H, 2CH_2), 2.22-2.26 (m, 4H, 2CH_2), 2.88 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 8.30 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 19.6, 20.3, 22.0, 25.7, 34.6, 55.6, 116.8, 124.1, 124.9, 128.7, 139.5, 164.8; MS electron impact (EI): *m/z* (%) 234 (M^+). *Anal*. Calcd for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.68. Found: C, 61.82; H, 6.22; N, 11.77; S, 13.73.

Synthesis of 1-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)ethanone (4)

A suspension of compound **3** (2.34 g, 0.01 mol) in sodium ethoxide (0.02 mol) [prepared by dissolving metallic sodium (0.46 g, 0.02 g) in absolute ethanol (20 mL] was heated in a boiling water bath for 6 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

White crystals; yield: 1.80 g (77%); mp: >300ºC; IR (KBr, cm^{-1}): 3479-3348 (NH, NH_2), 1715 (C=O), 1618 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.78-1.83 (m, 4H, 2CH_2), 2.20-2.27 (m, 4H, 2CH_2),

2.91 (s, 3H, CH_3), 4.83 (s, 2H, NH_2, D_2O exchangeable), 8.27 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 19.8, 20.2, 22.0, 25.6, 34.8, 124.0, 124.9, 128.5, 139.6, 165.6; MS (EI): *m/z* (%) 234 (M^+). *Anal*. Calcd for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.68. Found: C, 61.68; H, 5.89; N, 12.20; S, 13.83.

3.1.2. General procedure for the synthesis of thieno[2,3-*b*]pyrrol derivatives 6a and 6b

To the dry solid of compound **4** (2.34 g, 0.01 mol) either malononitrile (0.66 g, 0.01mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added followed by ammonium acetate (0.50 g, 0.01 mol).The whole reaction mixture was heated in an oil bath at 120 ºC for 1h then left to cool. The solidified product was boiled with ethanol then left to cool. The formed solid product was collected by filtration and crystallized from acetic acid.

2-(1-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)ethylidene)-malononitrile (6a)

Yellow crystals; yield: 1.92 g (68%); mp: 167-168ºC; IR (KBr, cm^{-1}): 3488-3334 (NH, NH_2), 3054 (CH aromatic), 2227, 2222 (2CN), 1620 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.79-1.86 (m, 4H, $2CH_2$), (m, 4H, $2CH_2$), 2.69 (s, 3H, CH_3), 4.86 (s, 2H, NH_2, D_2O exchangeable), 8.29 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 19.4, 20.3, 22.2, 25.6, 34.5, 116.3, 116.9, 122.3, 123.8, 124.0, 124.9, 127.2, 135.2; MS (EI): *m/z* (%) 282 (M^+). *Anal*. Calcd for $C_{15}H_{14}N_4S$: C, 63.80; H, 5.00; N, 19.84; S, 11.36. Found: C, 63.72; H, 4.93; N, 20.05; S, 11.59.

Ethyl 3-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-2-cyanobut-2-enoate (6b)

Yellow crystals; yield: 2.46 g (75%); mp: 121-122ºC; IR (KBr, cm^{-1}): 3473-3330 (NH, NH_2), 3054 (CH aromatic), 2222 (CN), 1640 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.13 (t, 3H, J = 7.26 Hz, CH_3), 1.80-1.86 (m, 4H, $2CH_2$), 2.22-2.27 (m, 4H, $2CH_2$), 2.66 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.26 Hz, CH_2), 4.88 (s, 2H, NH_2, D_2O exchangeable), 8.27 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 16.3, 19.6, 20.2, 22.5, 25.6, 34.8, 116.6, 122.0, 123.5, 124.6, 124.7, 127.2, 134.8, 166.1; MS (EI): *m/z* (%) 329 (M^+). *Anal.* Calcd for $C_{17}H_{19}N_3O_2S$: C, 61.98; H, 5.81; N, 12.76; S, 9.73. Found: C, 62.08; H, 6.07; N, 12.59; S, 9.88.

3.1.3. General procedure for the synthesis of the benzo[4',5']thieno[3',2':4,5]-pyrrolo[3,2-*b*]pyridine derivatives 7a and 7b

Method (A): A suspension of either compound **6a** (2.28 g, 0.01 mol) or **6b** (3.29 g, 0.01 mol) in sodium ethoxide (0.02 mol) [prepared by dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (20 mL) was heated in a boiling water bath for 8 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from acetic acid.

Method (B): To a solution of compound **4** (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture, in each case, was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from acetic acid.

2-Amino-4-methyl-7,8,9,10-tetrahydro-5*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-*b*]pyridine-3-carbonitrile (7a)

Yellow crystals; yield: 2.27 g (80%); mp: 232-233ºC; IR (KBr, cm^{-1}): 3474-3314 (NH, NH_2), 3056 (CH aromatic), 2220 (CN), 1626 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.76-1.84 (m, 4H, $2CH_2$), 2.21-2.26 (m, 4H, $2CH_2$), 2.89 (s, 3H, CH_3), 4.89 (s, 2H, NH_2, D_2O exchangeable), 8.33 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 19.8, 20.1, 22.7, 25.2, 34.6, 116.8, 120.1, 122.6, 123.8, 124.2, 125.3, 127.2, 135.6, 142.3, 168.2; MS (EI): *m/z* (%) 282 (M^+). *Anal*. Calcd for $C_{15}H_{14}N_4S$: C, 63.80; H, 5.00; N, 19.84; S, 11.36. Found: C, 63.66; H, 4.83; N, 20.25; S, 11.37.

Ethyl 2-amino-4-methyl-7,8,9,10-tetrahydro-5*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-*b*]pyridine-3-carboxylate (7b)

Yellow crystals; yield: 2.24 g (68%), mp: 195-196ºC; IR (KBr, cm^{-1}): 3466-3327 (NH, NH_2), 3056 (CH aromatic), 1640 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.14 (t, 3H, J = 7.07 Hz, CH_3), 1.82-1.86 (m, 4H, $2CH_2$), 2.20-2.27 (m, 4H, $2CH_2$), 2.88 (s, 3H, CH_3), 4.24 (q, 2H, J = 7.07 Hz, CH_2), 4.84 (s, 2H, NH_2, D_2O exchangeable), 8.32 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 16.2, 19.8, 20.3, 22.5, 25.6, 34.5, 55.6, 120.3, 122.4, 123.8, 124.6, 124.7, 127.6, 133.9, 143.2, 164.4, 168.9; MS (EI): *m/z* (%) 329 (M^+). *Anal*. Calcd for $C_{17}H_{19}N_3O_2S$: C, 61.98; H, 5.81; N, 12.76; S, 9.73. Found: C, 61.68; H, 5.94; N, 12.63; S, 9.90.

3.1.4. General procedure for the synthesis of [4,5]thieno[2,3-*b*]pyrrol-2-yl)thiophene derivatives 8a and 8b

To a solution of compound **4** (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g,0.01 mol) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case was heated under reflux for 2 h then was left to cool and the formed solid product, in each case, was collected by filtration and crystallized from ethanol.

2-Amino-4-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)thiophene-3-carbonitrile (8a)

Orange crystals; yield: 2.42 g (77%), mp: 141-142ºC; IR (KBr, cm^{-1}): 3462-3354 (NH, NH_2), 3053 (CH aromatic), 2221 (CN), 1628 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.78-1.84 (m, 4H, $2CH_2$), 2.23-2.28 (m, 4H, $2CH_2$), 4.80, 5.25 (2s, 4H, $2NH_2$, D_2O exchangeable), 6.11 (s, 1H, thiophene H-5), 8.26 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO--d_6) δ: 20.4, 22.9, 25.0, 34.6, 116.6, 120.3, 123.1, 123.8, 124.2, 125.3, 127.2, 139.3, 140.6, 142.3; MS (EI): *m/z* (%) 314 (M^+). *Anal*. Calcd for $C_{15}H_{14}N_4S_2$: C, 57.30; H, 4.49; N, 17.82; S, 20.40. Found: C, 57.44; H, 4.39; N, 18.04; S, 20.28.

Ethyl 2-amino-4-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-thiophene-3-carboxylate (8b)

Orange crystals; yield: 2.60 g (74%), mp: 131-132ºC. IR (KBr, cm^{-1}): 3479-3331 (NH_2), 3053 (CH aromatic), 1690 (C=O), 1632 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.13 (t, 3H, J = 6.83 Hz, CH_3), 1.81-1.87 (m, 4H, $2CH_2$), 2.22-2.25 (m, 4H, $2CH_2$), 4.23 (q, 2H, J = 6.83 Hz, CH_2), 4.81, 5.03 (2s, 4H, $2NH_2$, D_2O exchangeable), 6.13 (s, 1H, thiophene H-5), 8.30 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 16.0, 20.0, 22.7, 25.6, 34.5, 55.6, 120.8, 122.7, 123.8, 124.6, 124.9, 127.6, 133.9, 143.5, 164.2; MS (EI): *m/z* (%) 361 (M^+). *Anal*. Calcd for $C_{17}H_{19}N_3O_2S_2$: C, 56.48; H, 5.30; N, 11.62; S, 17.74. Found: C, 56.71; H, 5.55; N, 11.42; S, 17.49.

3.1.5. General procedure for the synthesis of pyran derivatives 10a-f

Method (A): General procedure: To a solution of compound **4** (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL), either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and either of benzaldehyde (1.06 g, 0.1 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 1 h and the formed solid product produced from the hot solution was

collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Method (B): To a solution of compound **4** (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL), either of the cinnamonitrile derivatives **11a-f** (0.01 mol) were added. The reaction mixture was heated under reflux for 2 h and the formed solid product produced from the hot solution was collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

2-Amino-6-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-4-phenyl-4*H*-pyran-3-carbonitrile (10a)

Pale yellow crystals; yield: 3.10 g (80%); mp: 167-168ºC; IR (KBr, cm^{-1}): 3489-3321 (NH, NH_2), 3056 (CH aromatic), 2220 (CN), 1630 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.76-1.85 (m, 4H, $2CH_2$), 2.21-2.27 (m, 4H, $2CH_2$), 4.83, 5.41 (2s, 4H, $2NH_2$, D_2O exchangeable), 5.66-5.90 (2d, 2H, pyran H-4, H-5), 7.28-7.38 (m, 5H, C_6H_5), 8.24 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.6, 22.9, 25.3, 34.8, 39.3, 116.9, 120.6, 122.8, 123.8, 123.9, 125.3, 126.9, 127.2, 129.4, 130.8, 139.3, 140.6, 141.8, 142.3; MS (EI): *m/z* (%) 388 (M^+). *Anal*. Calcd for $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42; S, 8.25. Found: C, 67.93; H, 5.32; N, 14.60; S, 8.44.

6-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-2-hydroxy-4-phenyl-4*H*-pyran-3-carbonitrile (10b)

Pale yellow crystals; yield: 2.57 g (66%), mp: 264-265ºC; IR (KBr, cm^{-1}): 3520-3341 (NH, NH_2, OH), 3055 (CH aromatic), 2222 (CN), 1632 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.77-1.86 (m, 4H, $2CH_2$), 2.20-2.27 (m, 4H, $2CH_2$), 4.86 (s, 2H, NH_2, D_2O exchangeable), 5.68-5.87 (2d, 2H, pyran H-4, H-5), 7.30-7.41 (m, 5H, C_6H_5), 8.22 (s, 1H, NH, D_2O exchangeable), 10.30 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.4, 22.7, 25.4, 34.8, 39.9, 116.7, 120.8, 122.8, 123.3, 123.9, 125.7, 126.9, 127.0, 130.4, 133.6, 139.3, 140.8, 142.0, 142.7; MS (EI): *m/z* (%) 389 (M^+). *Anal*. Calcd for $C_{22}H_{19}N_3O_2S$: C, 67.84; H, 4.92; N, 10.79; S, 8.23. Found: C, 67.60; H, 4.69; N, 10.99; S, 8.40.

2-Amino-6-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-4-(4-chlorophenyl-4*H*-pyran-3-carbonitrile (10c)

Pale yellow crystals; yield: 2.87 g (68%); mp: 274-275°C; IR (KBr, cm^{-1}): 3474-3330 (NH, NH_2), 3055 (CH aromatic), 2220 (CN), 1633 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.78-1.85 (m, 4H, $2CH_2$), 2.18-2.25 (m, 4H, $2CH_2$), 4.86, 5.40 (2s, 4H, $2NH_2$, D_2O exchangeable), 5.68-5.73 (2d, 2H, pyran H-4, H-5), 7.30-7.38 (m, 4H, C_6H_4), 8.26 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.3, 22.8, 25.5, 34.8, 39.7, 116.7, 120.4, 122.6, 123.9, 124.3, 125.3, 126.9, 128.8, 130.6, 139.0, 140.9, 142.8, 144.3; MS (EI): *m/z* (%) 423 (M^+). *Anal*. Calcd for $C_{22}H_{19}ClN_4OS$: C, 62.48; H, 4.53; N, 13.25; S, 7.58. Found: C, 62.22; H, 4.72; N, 13.51; S, 7.28.

6-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-4-(4-chlorophenyl)-2-hydroxy-4*H*-pyran-3-carbonitrile (10d)

Yellow crystals; yield: 3.21 g (76%), mp: 222-223°C; IR (KBr, cm^{-1}): 3541-3333 (NH, NH_2), 3055 (CH aromatic), 2220 (CN), 1626 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.78-1.87 (m, 4H, $2CH_2$), 2.21-2.28 (m, 4H, $2CH_2$), 4.83 (s, 2H, NH_2, D_2O exchangeable), 5.65-5.72 (2d, 2H, pyran H-4, H-5), 7.30-7.41 (m, 4H, C_6H_4), 8.24 (s, 1H, NH, D_2O exchangeable), 10.28 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.2, 22.6, 25.8, 34.3, 39.8, 116.5, 120.2, 122.6, 123.7, 123.9, 125.7, 126.9, 127.4, 130.2, 139.3, 141.3, 142.0, 142.8; MS (EI): *m/z* (%) 424 (M^+). *Anal*. Calcd for $C_{22}H_{18}ClN_3O_2S$: C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 62.09; H, 4.46; N, 9.75; S, 7.39.

2-Amino-6-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-4-(4-methoxyphenyl-4*H*-pyran-3-carbonitrile (10e)

Orange crystals; yield: 3.01 g (72%), mp: 167-168°C; IR (KBr, cm^{-1}): 3531-3312 (NH, NH_2), 3058 (CH aromatic), 2223 (CN), 1628 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.74-1.86 (m, 4H, $2CH_2$), 2.20-2.28 (m, 4H, $2CH_2$), 3.01 (s, 3H, OCH_3), 4.86, 5.22 (2s, 4H, $2NH_2$, D_2O exchangeable), 5.67-5,74 (2d, 2H, pyran H-4, H-5), 7.32-7.38 (m, 4H, C_6H_4), 8.25 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.0, 22.8, 25.8, 34.8, 30.8, 39.6, 116.9, 120.6, 122.6, 123.4, 123.9, 125.7, 126.9, 127.6, 130.4, 139.4, 141.7, 142.3, 143.6; MS (EI): *m/z* (%) 418 (M^+). *Anal*. Calcd for $C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39; S, 7.66. Found: C, 66.24; H, 5.48; N, 13.19; S, 7.80.

6-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-2-hydroxy-4-(4-methoxyphenyl)-4*H*-pyran-3-carbonitrile (10f)

Orange crystals; yield: 3.01 g (70%), mp: 229-230°C; IR (KBr, cm^{-1}): 3566-3332 (NH, NH_2, OH), 3056 (CH aromatic), 2220 (CN), 1626 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.74-1.86 (m, 4H, $2CH_2$),

2.22-2.29 (m, 4H, $2CH_2$), 3.08 (s, 3H, OCH_3), 4.83 (s, 2H, NH_2, D_2O exchangeable), 5.64, 5.71 (2d, 2H, pyran H-4, H-5), 7.30-7.44 (m, 4H, C_6H_4), 8.23 (s, 1H, NH, D_2O exchangeable), 10.32 (s, 1H, D_2O exchangeable, OH); ^{13}C-NMR (DMSO-d_6) δ: 20.5, 22.8, 25.3, 34.5, 30.8, 39.1, 116.9, 120.6, 122.6, 123.4, 123.9, 125.7, 126.9, 127.6, 130.6, 139.4, 141.7, 142.3, 143.9; MS (EI): *m/z* (%) 419 (M^+). *Anal*. Calcd for $C_{23}H_{21}N_3O_3S$: C, 65.85; H, 5.05; N, 10.02; S, 7.64. Found: C, 66.19; H, 5.17; N, 10.22; S, 7.59.

3.1.7. General procedure for the synthesis of benzo[4,5]thieno-[2,3-*b*]pyrrol-2-yl)-2-(2-cyanoacetamido)thiophene derivatives 12a and 12b

To a solution of either compound **8a** (3.14 g, 0.01 mol) or **8b** (3.61 g, 0.01 mol) in dimethylformamide (40 mL) ethyl cyanoacetate was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water. The formed solid product was collected by filtration and crystallized from ethanol.

N-(4-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-3-cyano-thiophen-2-yl)-1-cyanoacetamide (12a)

Yellow crystals; yield: 3.43 g (90%), mp: 184-185ºC; IR (KBr, cm^{-1}): 3482-3323 (NH, NH_2), 3055 (CH aromatic), 2225, 2220 (2CN), 1705 (C=O), 1630 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.79-1.83 (m, 4H, $2CH_2$), 2.25-2.26 (m, 4H, $2CH_2$), 4.83 (s, 2H, NH_2, D_2O exchangeable), 5.20 (s, 2H, CH_2), 6.20 (s, 1H, thiophene H-5), 8.28, 8.32 (2s, 2H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.3, 22.9, 25.4, 34.7, 52.7, 116.9, 117.2, 120.3, 123.1, 124.1, 124.6, 125.3, 127.2, 138.8, 141.2, 142.6, 168.2; MS (EI): *m/z* (%) 381 (M^+). *Anal*. Calcd for $C_{18}H_{15}N_5OS_2$: C, 56.67; H, 3.96; N, 18.36; S, 16.81. Found: C, 56.88; H, 3.58; N, 18.56; S, 16.93.

Ethyl 4-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno-[2,3-*b*]pyrrol-2-yl)-2-(2-cyano-acetamido)thiophene-3-carboxylate (12b)

Orange crystals; yield: 2.99 g (70%); mp: 194-195ºC; IR (KBr, cm^{-1}): 3453-3320 (NH, NH_2), 3056 (CH aromatic), 2223, 1702, 1688 (2C=O), 1632 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.13 (t, 3H, J = 6.83 Hz, CH_3), 1.81-1.87 (m, 4H, $2CH_2$), 2.22-2.25 (m, 4H, $2CH_2$), 4.23 (q, 2H, J = 6.83 Hz, CH_2), 4.81 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 2H, CH_2), 6.23 (s, 1H, thiophene H-5), 8.30, 8.34 (s, 2H, 2NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 16.0, 20.3, 22.2, 25.6, 34.8, 47.1, 51.4, 116.5, 120.4, 122.7, 123.8, 124.3, 124.9, 127.6, 133.9, 143.8, 164.3, 170.2; MS (EI): *m/z* (%) 428

(M^+). *Anal*. Calcd for $C_{20}H_{20}N_4O_3S_2$: C, 56.06; H, 4.70; N, 13.07; S, 14.97. Found: C, 56.22; H, 4.53; N, 13.31; S, 15.07.

3.1.8. General procedure for the synthesis of hydrazoacetamide derivatives 14a-d

To a cold solution (0-5 ^oC) of compound **12a** (3.81 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.50 g, 0.50 mol) either benzenediazonium chloride (0.01 mol), 4-chlorobenzene-diazonium chloride (0.01 mol), 4-methoxybenzenediazonium chloride (0.01 mol) or 4-methylaniline (0.01 mol) [prepared by adding a cold solution of sodium nitrite (0.70 g, in water (10 mL)) to a cold solution (0-5 ^oC) of either aniline oil (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) 4-methoxybenzenediazonium chloride (1.24 g, 0.01 mol) or 4-methylaniline (1.07 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added with continuous stirring. The whole reaction mixture was left at room temperature for 1 h then the formed solid product was collected by filtration and crystallized from acetic acid.

2-((4-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-3-cyanothiophen-2-yl)amino)-2-oxo-*N'*-phenylacetohydrazonoyl cyanide (14a)

Red crystals; yield: 3.78 g (78%), mp: 223-224^oC; IR (KBr, cm^{-1}): 3475-3320 (NH), 3053 (CH aromatic), 2223, 2220 (2CN), 1708 (C=O), 1630 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.77-1.85 (m, 4H, $2CH_2$), 2.25-2.28 (m, 4H, $2CH_2$), 4.80 (s, 2H, NH_2, D_2O exchangeable), 6.15 (s, 1H, thiophene H-5), 7.25-7.41 (m, 5H, C_6H_5), 8.25, 8.30, 8.56 (3s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.5, 22.9, 25.8, 34.7, 116.7, 117.0, 120.2, 121.7, 123.1, 124.0, 124.1, 124.6, 125.3, 126.9, 127.2, 129.3, 133.1, 138.8, 141.2, 142.8, 164.2, 168.7; MS (EI): *m/z* (%) 485 (M^+). *Anal*. Calcd for $C_{24}H_{19}N_7OS_2$: C, 59.36; H, 3.94; N, 20.19; S, 13.21. Found: C, 59.42; H, 3.72; N, 20.53; S, 13.08.

2-((4-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-3-cyanothiophen-2-yl)amino)-*N*'-(4-chlorophenyl)-2-oxoacetohydrazonoyl cyanide (14b)

Red crystals; yield: 4.41 g (85%), mp: 194-195^oC; IR (KBr, cm^{-1}): 3488-3315 (NH, NH_2), 3056 (CH aromatic), 2225, 2220 (2CN), 1710 (C=O), 1628 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.79-1.85 (m, 4H, $2CH_2$), 2.23-2.27 (m, 4H, $2CH_2$), 4.83 (s, 2H, NH_2, D_2O exchangeable), 6.12 (s, 1H, thiophene H-5), 7.28-7.39 (m, 4H, C_6H_4), 8.23, 8.32, 8.42 (3s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.6, 22.4, 25.8, 34.9, 116.8, 117.3, 120.0, 121.4, 123.1, 124.0, 124.1, 124.8, 125.3, 127.2,

138.8, 140.4, 141.2, 143.4, 164.8, 168.6; MS (EI): *m/z* (%) 520 (M^+). *Anal*. Calcd for $C_{24}H_{18}ClN_7OS_2$: C, 55.43; H, 3.49; N, 18.85; S, 12.33. Found: C, 55.70; H, 3.62; N, 18.59; S, 12.48.

2-((4-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-3-cyanothiophen-2-yl)amino)-*N*'-(4-methoxyphenyl)-2-oxoacetohydrazonoyl cyanide (14c)

Reddish brown crystals; yield: 4.63 g (90%); mp: 168-169ºC; IR (KBr, cm^{-1}): 3462-3335 (NH, NH_2), 3053 (CH aromatic), 2227, 2221 (2CN), 1720 (C=O), 1638 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.74-1.82 (m, 4H, $2CH_2$), 2.21-2.28 (m, 4H, $2CH_2$), 3.38 (s, 3H, OCH_3), 4.88 (s, 2H, NH_2, D_2O exchangeable), 6.13 (s, 1H, thiophene H-5), 7.31-7.42 (m, 4H, C_6H_4), 8.21, 8.32, 8.45 (3s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.8, 22.7, 25.8, 34.3, 55.3, 116.3, 117.0, 120.3, 121.4, 123.8, 124.0, 124.0, 124.8, 125.9, 127.0, 133.2, 138.2, 140.8, 141.9, 164.9, 168.6; MS (EI): *m/z* (%) 516 (M^+). *Anal.* Calcd for $C_{25}H_{21}N_7O_2S_2$: C, 58.24; H, 4.11; N, 19.02; S, 12.44. Found: C, 58.40; H, 4.26; N, 19.11; S, 12.29.

2-((4-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-3-cyanothiophen-2-yl)amino)-2-oxo-*N*'-(p-tolyl)acetohydrazonoyl cyanide (14d)

Reddish brown crystals; yield: 3.44 g (69%); mp: 129-130ºC; IR (KBr, cm^{-1}): 3482-3318 (NH, NH_2), 3057 (CH aromatic), 2227, 2220 (2CN), 1712 (C=O), 1630 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.76-1.83 (m, 4H, $2CH_2$), 2.23-2.28 (m, 4H, $2CH_2$), 2.65 (s, 3H, CH_3), 4.86 (s, 2H, NH_2, D_2O exchangeable), 6.11 (s, 1H, thiophene H-5), 7.30-7.39 (m, 4H, C_6H_4), 8.23, 8.30, 8.48 (3s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.4, 22.9, 23.3, 25.8, 34.6, 116.4, 117.3, 120.6, 122.8, 123.8, 124.0, 124.3, 124.8, 125.2, 126.4, 138.8, 140.6, 141.7, 143.9, 164.6, 168.7; MS (EI): *m/z* (%) 500 (M^+). *Anal*. Calcd for $C_{25}H_{21}N_7OS_2$: C, 60.10; H, 4.24; N, 19.62; S, 12.84. Found: C, 60.32; H, 4.52; N, 19.48; S, 12.64.

3.1.9. General procedure for the synthesis of thieno[2,3-*b*]pyridine derivatives 15a and 15b

A suspension of either compound **12a** (3.81 g, 0.01 mol) or **12b** (4.28 g,0.01 mol) in sodium ethoxide (0.02 mol) [prepared by dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (20 mL] was heated in a boiling water bath for 12 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

4-Amino-3-(3-amino-4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-2-yl)-6-hydroxy-thieno[2,3-*b*]pyridine-5-carbonitrile (15a)

Yellow crystals; yield: 2.29 g (60%); mp: > 300 °C; IR (KBr, cm^{-1}): 3593-3355 (NH, NH_2, OH), 3056 (CH aromatic), 2224 (CN), 1635 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.75-1.85 (m, 4H, $2CH_2$), 2.23-2.27 (m, 4H, $2CH_2$), 4.68, 5.09 (2s, 4H, $2NH_2$, D_2O exchangeable), 6.16 (s, 1H, thiophene H-5), 8.28 (s, 1H, NH, D_2O exchangeable), 9.90 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 20.8, 22.9, 25.8, 34.7, 116.7, 120.2, 121.7, 123.1, 124.1, 124.6, 125.3, 126.5, 127.0, 129.6, 138.8, 142.8, 144.5, 162.8; MS (EI): *m/z* (%) 381 (M^+). *Anal.* Calcd for $C_{18}H_{15}N_5OS_2$: C, 56.67; H, 3.96; N, 18.36; S, 16.81. Found: C, 56.93; H, 3.65; N, 18.48; S, 17.09.

3-(3-Amino-4,5,6,7-tetrahydro-1*H*-benzo-4,5]thieno[2,3-*b*]pyrrol-2-yl)-4,6-dihydroxy-thieno[2,3-*b*]pyridine-5-carbonitrile (15b)

Yellow crystals; yield: 2.79 g (73%) g); mp: 289-290°C; IR (KBr, cm^{-1}): 3578-3345 (NH, NH_2, OH), 3056 (CH aromatic), 2222 (CN), 1628 (C=C); ^{1}H-NMR (DMSO-d_6) δ: 1.79-1.85 (m, 4H, $2CH_2$), 2.23-2.27 (m, 4H, $2CH_2$), 4.86 (s, 2H, NH_2, D_2O exchangeable), 6.17 (s, 1H, thiophene H-5), 8.26 (s, 1H, NH, D_2O exchangeable), 10.29, 10.34 (2s, 2H, D_2O exchangeable, 2OH); ^{13}C-NMR (DMSO-d_6) δ: 20.3, 22.8, 25.8, 34.7, 116.6, 120.2, 121.6, 123.1, 124.7, 124.1, 124.8, 125.3, 126.8, 127.5, 133.2, 140.8, 143.8, 144.2, 162.9; MS (EI): *m/z* (%) 382 (M^+). *Anal*. Calcd for $C_{18}H_{14}N_4O_2S_2$: C, 56.53; H, 3.69; N, 14.65; S, 16.77. Found: C, 56.72; H, 3.46; N, 14.80; S, 16.37.

4. Conclusions

Novel 4,5,6,7-tetrahydro-1*H*-benzo[4,5]thieno[2,3-*b*]pyrrol-derivatives were synthesized in good yields. Some compounds were used to produce annulated products. The cytotoxicity of the newly synthesized compounds indicate that compounds **4**, **7a**, **7b**, **8a**, **8b**, **10c, 10d**, **10f**, **12a**, **12b, 14b** and **15b** showed the highest potency among the tested compounds. In addition, the anti-proliferative evaluations of these twelve compounds indicated that the benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-*b*]pyridine derivative **7b** and the benzo[4,5]thieno-[2,3-*b*]pyrrol-2-yl)-thiophene derivative **8b** showed high potency against Molt4/C8 and CEM cell lines and their IC_{50}'s are higher than the reference drug "doxorubicin".

5. Acknowledgments

R. M. Mohareb would like to thank the Alexander von Humboldt for affording him regular fellowships in Germany for doing research and completing this work.

6. References

1. S. Xue, H. Guo, M. Liu, J. Jin, D. Ju, Z. Liu, Z. Li, *Eur. J. Med. Chem.* **2015**, *26*, 151-161. http://dx.doi.org/10.1016/j.ejmech.2015.04.016

2. L. Ye, J. He, Z. Hu, Q. Dong, H. Wang, F. Fu, J. Tian, *Food Chem. Toxicol.* **2013**, *52*, 200-206. http://dx.doi.org/10.1016/j.fct.2012.11.004

3. S. Hama, S. Utsumi, Y. Fukuda, K. Nakayama, Y. Okamura, H. Tsuchiya, K. Fukuzawa, H. Harashima, K. Kogure, *J. Controlled Release*. **2012**, *161*, 843-851. http:// 10.1016/j.jconrel.2012.05.031

4. I. M. Fakhr, M. A. Radwan, S. El-Batran, O. M. Abd El-Salam, S. M. El-Shenawy, *Eur. J. Med. Chem.* **2009**, *44*, 1718-1725. http:// 10.1016/j.ejmech.2008.02.034

5. S. Bondock, W. Fadaly, M. A. Metwally, *Eur. J. Med. Chem.* **2010**, *45*, 3692-3701. http://dx.doi.org/10.1016/j.ejmech.2010.05.018

6. C. K. Ryu, S. K. Lee, J. Y. Han, O. J. Jung, J. Y. Lee, S. H. Jeong, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2617-2620. http://doi.org/10.1016/j.bmcl.2005.03.042

7. L. J. Gonzalez, C. E. Stephens, T. Wenzler, R. Brun, F. A. Tanious, W. D. Wilson, T. Barszcz, K. A. Werbovetz, D. W. Boykin, *Eur. J. Med. Chem.* **2007**, *42*, 552-557. http://dx.doi.org/10.1016/j.ejmech.2006.11.006

8. J. B. Hudson, E. A. Graham, N. Miki, G. H. N. Towers, L. L. Hudson, R. Rossi, A. Carpita, D. Neri, *Chemosphere*. **1989**, *19*, 1329-1343. http://dx.doi.10.1016/0045-6535(89)90080-5

9. K. I. Molvi, K. K. Vasu, S. G. Yerande, V. Sudarsanam, N. Haque, *Eur. J. Med. Chem*. **2007**, *42*, 1049-1058. http://dx.doi.10.1016/j.ejmech.2007.01.007

10. R. Kulandasamy, A. V. Adhikari, J. P. Stables, *Eur. J. Med. Chem.* **2009**, *44*, 4376-4384. http://dx.doi.10.1016/j.ejmech. 2009.05.026

11. H. J. Jung, Y. S. Song, C. J. Lim, E. H. Park, *J. Ethanopharmacol.* **2009**, *126***,** 355-360. http://dx.doi.10.1016/j.jep.2009.08.031

12. W. O. Chi, L. H. Chang, *Inorg. Chim. Acta*. **1986**, *125*, 203-206. http://dx.doi. 10.1016/S0020-1693(00)81212-8.

13. D. C. S. Friedman, P. Friedman, *J. Mol. Struct. Theochem*, **1995**, *333***,** 71-78. http://dx.doi. 10.1016/0166-1280(94)03930-J

14. M. K. Parai, G. Panda, V. Chaturvedi, Y. K. Manju, S. Sinha, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 289-292. http://dx.doi. 10.1016/j.bmcl.2007.10.083

15. D. Caridha, A. K. Kathcart, D. Jirage, N. C. Waters, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3863-3867. http://dx.doi.org/10.1016/j.bmcl.2010.05.039

16. G. Fear, S. Komarnytsky, I. Raskin, *Pharm. Therap.* **2007**, *113*, 354-368. http://dx.doi. 10.1016/j.pharmthera.2006.09.001

17. B. O. Al-Najjar, H. A. Wahab, T. S. T. Muhammad, A. C. Shu-Chien, N. A. A. Noruddin, M. O. Taha, *Eur. J. Med. Chem.* **2011**, *46*, 2513-2529. http://dx.doi.org/10.1016.j.ejmech.2011.03.040

18. R. M Mohareb, N. S. Abbas, A. R. Ibrahim, *Acta Chim. Solv.* **2013**, *60*, 583-594. http://dx.doi.org/ 0.17344/acsi.2013.1418

19. R. M. Mohareb, E. Z. El-Arab, K. A. El-Sharkawy, *Sci. Pharm*. **2009**, ***77***, 355–366. http://dx.doi.org/10.3797/scipharm.0901-20

20. R. M. Mohareb, N. Y. Megally Abdo, *Molecules.* **2015**, *20*, 11535-11553. doi:10.3390/molecules200611535

21. R. M. Mohareb, N. Y. Megally Abdo, *Chem. Pharm. Bull.* **2015**, *63*, 678–687. http://dx.doi.org/10.1248/cpb.c15-00115

22. R. M. Mohareb, M. Y. Zaki, N. S. Abbas, *Steroids.* **2015**, *98*, 80–91. 10.1016/j.steroids.2015.03.001

23. R. M. Mohareb, F. Al-Omran, *Steroids.* **2012**, *77*, 1551-1559. http://dx.doi.org/10.1016/j.steroids.2012.09.007

24. B. P. McKibben, C. H. Cartwright, A. L. Castelhano, *Tetrahedron Lett*. **1999**, *40*, 5471-5474. http://dx.doi.org/10.1016/S0040-4039(99)01108-9

25. K. Balamurugan, S. Perumal, S. K. Reddy, P. Yogeeswari, D. Sriram, *Tetrahedron Lett.* **2009**, *50*, 6191-6195. http://dx.doi.org/10.1016/j.tetlet.2009.08.085

26. R. M. Mohareb, A. E. Mahmoud, M. A. Abdelaziz, *Med. Chem. Res.* **2014**, *23*, 564-579. http://dx.doi.org/10.1007/s00044-013-0664

27. D. Briel, A. Rybak, C. Kronbach, K. Unverferth, *Eur. J. Med. Chem.* **2010**, *45*, 69-77. http://dx.doi.org/10.1016/j.ejmech.2009.09.025

28. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Waigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst*. **1991**, *83*, 757-766. http://dx.doi.org/10.1093/jnci/83.11.757

29. K. D. Paull, H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, M. R. Boyd, *J. Natl. Cancer Inst.* **1989**, *81*, 1088-1092. http://dx.doi.org/10.1093/jnci/81.14.1088

30. L. S. Olsen, P. J. Hjarnaa, S. Latini, P. K. Holm, R. Larsson, E. Bramm, L.Binderup, M. W. Madsen, *Int. J. Cancer*. **2004**, *111*, 198-205. http://dx.doi.org/10.1002/ijc.20255

31. A. Svensson, U. Bäckman, E. Jonsson, R. Larsson, R. Christofferson, *Pediatr Res.* **2002**, *51*, 607–611. http://dx.doi.org/10.1203/00006450-200205000