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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and cytotoxicity of fused thiophene and pyrazole derivatives derived from 2-*N*-acetyl-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene

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Abstract The reaction of 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene with chloroacetyl chloride gave the 2-chloroacetamido derivative **3**. The latter reacted with hydrazine hydrate to give the hydrazine derivative **5** which was used to form the hydrazone derivatives **7a, b** and **9a, b** via its reaction with some carbonyl compounds. Moreover, it produced the pyrazole derivatives **11a, b** through its reaction with either acetylacetone or ethyl acetoacetate. On the other hand, compounds **5** and **3** were used to form some thiazole, pyridine, and fused derivatives. The cytotoxicity of the newly obtained products was evaluated against some of the human cancer and normal cell lines where the results showed that compounds **3**, **11b**, **13**, **18c**, **18d**, **21**, **23,** and **24** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range.

Keywords Benzo[*b*]thiophene · Pyridine · Pyrazole · Thiazole · Cytotoxicity

Introduction

Cancer is a major health problem worldwide. The relative mortality rate caused by cancer is still very high in the developed countries, accounting for more than 20 % of all deaths. Among the various types of malignant tumors, breast cancer is the second leading cause of death in women (Walker *et al.*, 2014; Ament *et al.*, 2014). In the US and other western countries, alcohol-related cirrhosis, and possibly nonalcoholic fatty liver disease associated with obesity, is account for the majority of liver cancer cases. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are associated with less than half of liver cancer cases in the US, although they are the major risk factors for the disease worldwide (Upreti *et al.*, 2014). Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited mainly due to undesirable side effects and a limited choice of available anticancer drugs (Goutham Vemana et al. 2014). 2-Aminothiophenes exhibit several pharmacological activities and have therefore attracted considerable attention from medicinal and synthetic organic chemists (Behbehani *et al.*, 2012; Du *et al.*, 2013; Padmavathi *et al.*, 2011; Wang *et al.*, 2013; Titchenell *et al.*, 2013; Aurelio *et al.*, 2011; Perspicace *et al.*, 2013; Duval *et al.*, 2005). They have been described to present anti-inflammatory (Madhusudana *et al.*, 2012), insecticidal (Campaigne, 1984), antibacterial (Dewal *et al.*, 2012), antifungal (Stephens *et al.*, 2001), antiviral (Goncales *et al.*, 2005), and antinociceptive activities (Crouse *et al.*, 2004). Recently some other compounds derived from benzo[*b*]thiophene series showed as potent tubulin polymerization inhibitors (Romagnoli *et al.,* 2010). Some similar compounds from benzothiophene series namely 3-(aryl)benzothieno[2,3-*c*]pyran-1-ones (tricyclic lactones) were prepared by a tandem one-pot Sonogashira coupling and intramolecular cyclization. Furthermore, the tetrahydrobenzo[*b*]thiophene nucleus was adopted as a scaffold for many antimicrobial (Refat and Fadda, 2013), antiviral (Dewal *et al.*, 2012), antiarrhythmic, (Amr *et al.*, 2010), and antitumor (Abbas *et al.*, 2013) activities. A structure activity relationship was established for the heterocyclic compounds derived from

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benzo[*b*]thiophene series with antitumor properties were prepared (Ferreirra *et al*., 2009), as well as a series of *N*-(2-(5-fluoro-2-(4-fluorophenylthio)benzo[*b*]thiophen-3-yl)ethyl) acylamides, which were evaluated for their binding affinity and intrinsic activity at melatonin receptors (Mésangeau *et al*., 2011). Also, benzothiophenes offer interest to pharma industry as scaffolds for synthesis of raloxifene and relevant structural analogs of selective estrogen receptor modulators (SERM) (Dadiboyena, 2012). As a part of our continuing search for potential anticancer agents related to heterocyclic thiophenes, we have previously reported synthesis and strong inhibitory activities on several human tumor cell lines of thiophene derivatives (Mohareb and Al-Omran, 2012; Mohareb *et al*., 2013).

Results and discussion

The present investigation emphasized mainly on two important things: of these one is to the synthesis of heterocyclic compounds bearing the tetrahydrobenzo[*b*]thiophene moiety and the other is to determine their cytotoxicity against cancer and normal cell lines. Throughout our program, some of our synthesized products were good candidates as target anticancer agents. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Schemes 1, 2 and 3. In the present work, we studied the uses of the tetrahydrobenzo[*b*]thiophene derivative **1** in heterocyclic chemistry through the one-pot reaction to form novel heterocyclic rings derived from the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) as potentially anticancer agents. The obtained products were important in a different strategy, being used to obtain new heterocyclic derivatives of the tetrahydrobenzo[*b*]thiophene together with the comparison of their cytotoxic activities toward human cancer normal cell lines. Thus, the reaction of the 4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) with chloroacetyl chloride (**2**) gave the 2-chloro-*N*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)acetamide (**3**). The structure of compound **3** was based on the analytical and spectral data (see experimental section). Compound **3** was a good candidate for many heterocyclic transformations. Thus, the reaction of **3** with hydrazine hydrate gave the hydrazino derivative **5**. The high yield of compound **5** encouraged us to study its reactivity toward some chemical reagents. Thus, compound **5** reacted with either cyclopentanone (**6a**) or cyclohexanone (**6b**) to give the hydrazone derivatives **7a** and **7b**, respectively. The analytical and spectral data were the tools of their structural elucidations. Similarly, the reaction of compound **5** with either benzaldehyde (**8a**) or acetophenone (**8b**) gave the hydrazone derivatives **9a** and **9b**, respectively. On the other hand, the reaction of compound **5** with either acetylacetone (**10a**) or ethyl acetoacetate (**10b**) afforded the pyrazole derivatives **11a** and **11b**, respectively. Compound **5** reacted with ethyl cyanoacetate (**12**) in 1,4-dioxane to give the tetrahydrobenzo[4,5]thieno[2,3-*b*]thieno[2,3-*b*]pyridine derivative **13**. Compound **5** reacted with malononitrile (**14**) to afford the 2,5-diaminopyrazole derivative **15**.

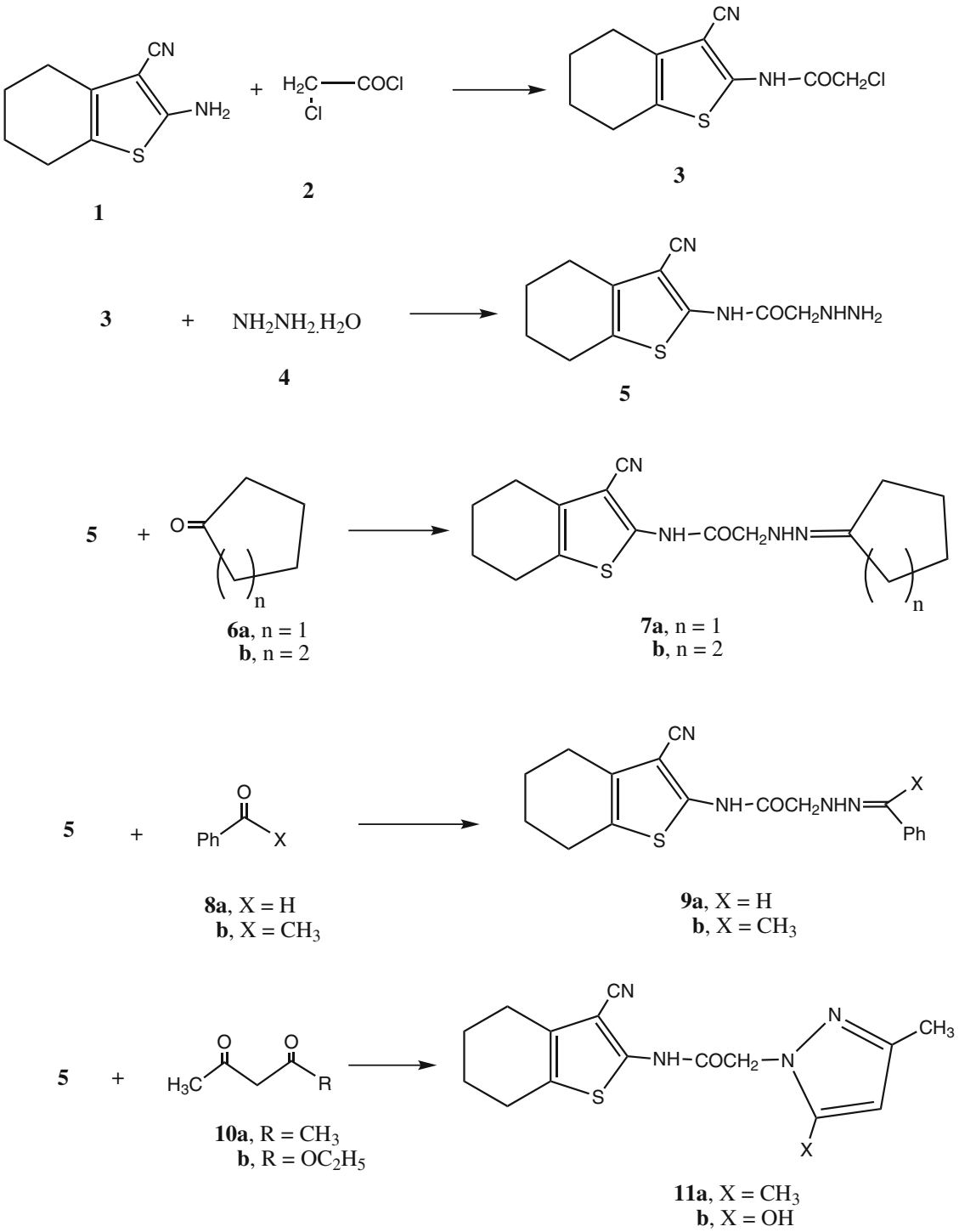
In our strategy, we moved through studying the reactivity of compound **3** as an α-halocarbonyl compound to react with potassium sulfide salts of some active methylene compounds via the well-known Hantzsch reaction (Fadda *et al.*, 2009). Thus, the reaction of the active methylene reagents namely malononitrile (**14**), ethyl cyanoacetate (**12**), acetylacetone (**10a**), or ethyl acetoacetate (**10b**) with phenylisothiocyanate (**16**) in dimethylformamide containing potassium hydroxide gave the intermediate potassium sulfide salts **17a-d**. The reaction of the latter intermediates with compound **3** at room temperature gave the thiazole derivatives **18a-d**, respectively. Analytical and spectral data of the latter products were consistent with their respective structures (see experimental section). Compound **3** reacted with aniline to give the α-anilino derivative **20** which underwent ready cyclization in sodium hydroxide to give the 4-amino-3-chloro-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*b*]pyridine-2-ol (**21**). The latter compound reacted with aniline to give the 3-anilino derivative **22** its structure was based on the analytical and spectral data (see experimental section). Further confirmation for the structure of compound **22** was obtained through its synthesis via another reaction root. Thus, the cyclization of compound **21** in sodium ethoxide solution gave the same product **20** (m.p., mixed m.p., and finger print IR). Compound **22** reacted with phenylisothiocyanate (**16**) in 1,4-dioxane solution to give the thiourea derivative **23**, and the latter underwent ready cyclization when being heated in sodium ethoxide solution to give the annulated derivative **24**. The analytical and spectral data of **24** are in agreement with its structure.

Biological evaluation

In vitro cytotoxic assay

Chemicals

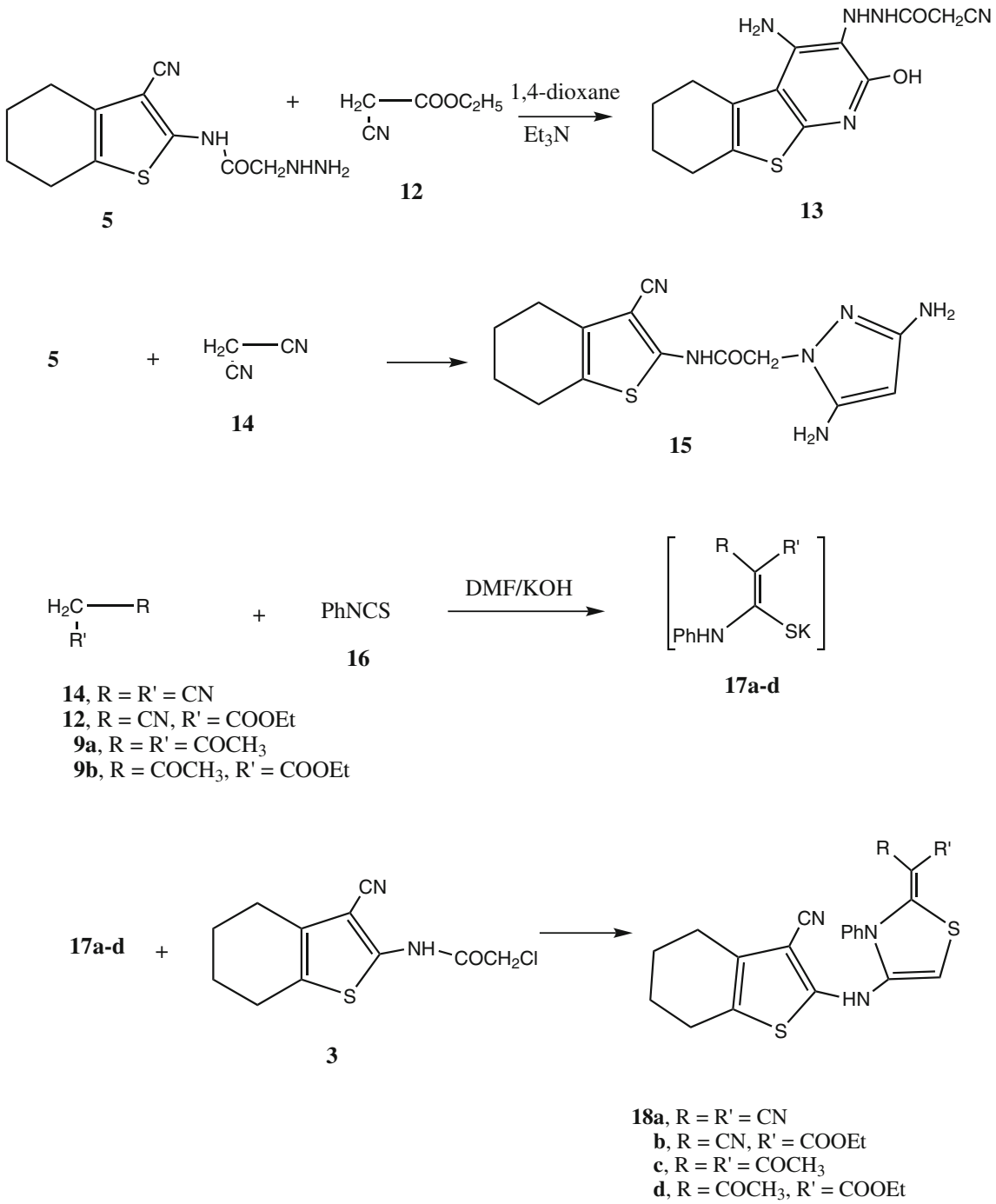
Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis,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



Scheme 1 Synthesis of compounds **3–11a,b**

Cell cultures

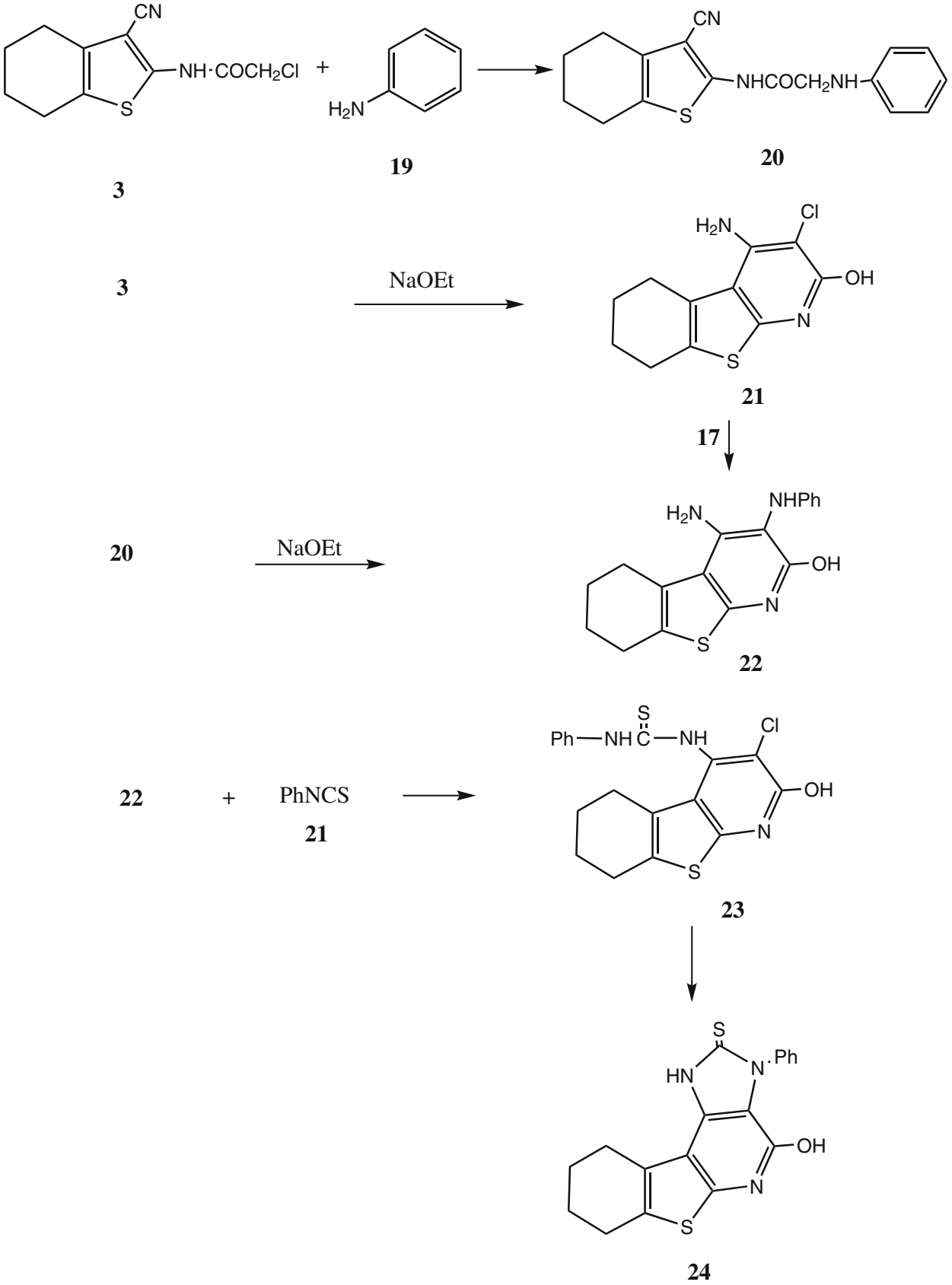
The cell cultures was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1), and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS, 2 μM glutamine and antibiotics (penicillin 100 U/mL, streptomycin



Scheme 2 Synthesis of compounds **13–15** and **18a–d**

100 lg/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 six 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 (Leopoldo *et al*., 2013; Pranati Samadder *et al*., 2014; Vizzotto *et al*., 2014) against six human cancer cell lines including cells derived from



Scheme 3 Synthesis of compounds **20–24**

human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1), and a normal fibroblast cells (WI38).

Each cell line was incubated with four concentrations (6–62 μg/ml or the equivalent nM/mL) for each compound and was used to create compound concentration versus survival fraction curves. The response parameter (IC_{50}) was calculated for each cell line (Table 1). The IC_{50} value corresponds to the compound's concentration causing a net 50 % loss of initial cells at the end of the incubation period (48 h). The antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor compounds and tumor selective agents. In the present study, the active compound showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity.

Some heterocyclic compounds were observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50} = 10–1,000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC_{50} > 10,000 nM). The reference compound used is the CHS-828 which is the pyridyl cyanoguanidine antitumor agent.

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

Compd. no.	Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
3	112	24	241	128	220	36	na
5	2253	2690	2166	3309	2213	2318	na
7a	2208	2128	2153	1236	1138	2240	na
7b	2114	1640	2173	2859	3266	2285	na
9a	2166	4840	1320	3266	4385	1428	na
9b	1629	1294	2469	1244	3027	2236	na
11a	2213	2146	2120	2110	3290	1368	na
11b	120	140	203	37	44	219	na
13	38	35	98	308	210	103	na
15	1220	240	820	630	408	254	na
18a	1535	2480	1216	1683	3076	1328	na
18b	2270	3129	1155	2424	1242	1252	na
18c	323	608	282	523	201	348	na
18d	328	126	1483	263	531	280	na
20	1254	3760	2175	2208	4236	2270	na
21	37	88	320	122	280	37	na
22	2379	2125	3280	2312	1288	3260	na
23	28	42	69	235	273	528	na
24	80	166	228	262	120	2170	na
CHS 828	25	2315	2067	1245	15	18	na

The sample concentration produces a 50 % reduction in cell growth
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

Structure activity relationship

From Table 1, it is clear that the 4,5,6,7-tetrahydro[*b*]thiophene moiety was found to be crucial for the cytotoxic effect of cyclic compounds **3**–**24**. Compounds **3**, **11b**, **13**, **18c**, **18d**, **21**, **23,** and **24** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of compounds **3** and **4**, it is obvious that the cytotoxicity of **3** is the higher than that of **4**. Thus, substituting the chloroacetyl group by the hydrazinoacetyl group decreased the cytotoxicity. However, the hydrazone derivatives **7a** and **7b** are more potent than **5,** and it is remarkable that compound **7a** showed high cytotoxicity against HA22T, HEPG2, HONE1, and MCF cell lines with IC_{50} values 153, 263, 138, and 240 nM, respectively. Considering the pyrazole derivatives **11a** and **11b**, it is clear that compound **11b** bearing the OH group showed much cytotoxicity over **11a**. It is of great value to notice that the *N*′-(4-Amino-2-hydroxy-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-*b*]pyridine-3-yl)-2-cyanoacetohydrazide (**13**) showed high cytotoxicity that is attributed to the presence of the thieno[2,3-*b*]pyridine moiety. Considering the thiazole derivatives **18a–d**, it is clear that compounds **18c** and **18d** are the most active compounds among the four thiazoles **18a–d**, and it is clear that the presence of the C=O moiety is responsible of the increase of the cytotoxicity of **18c** and **18d**. On the other hand, compound **18d** showed less potency against the HA22T cell line. The amide derivative **20** showed low cytotoxicity against the six cancer cell lines. The cyclized form of compound **3** produced the 4-amino-3-chloro-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-*b*]pyridine-2-ol (**21**) showed high cytotoxicity against the six cancer cell lines. The high cytotoxicity of compound **21** is attributed to the presence of the theino[2,3-*b*]pyridine moiety together with the electronegative chloro group. However, cyclization of compound **20** produced compound **22** with very low cytotoxicity. The phenylthiourea derivative **23** showed high cytotoxicity against the six cancer cell lines but its cyclized form the benzo[4,5]thieno[2,3-*b*]imidazo[4,5-*d*]pyridine derivative **24** showed high cytotoxicity against the five cell lines NUGC, DLDI, HA22T, HEPG2, and HONE1, but it showed less potency against MCF cell line.

It is very clear from our present finding that the newly synthesized products with halogen substituted pattern OCH_3, Cl, or COOEt show greater cytotoxic property thus, compounds **3, 11b, 13, 18c, 18d, 21, 23, and 24** showed the optimal cytotoxicity.

Table 2 Toxicity of the most potency compounds against shrimp larvae

Compound no.	Cons. (μg/ml)	Mortality[a]	Toxicity	LC_{50}	Upper 95 % lim.	Lower 95 % lim.
3	10	4	Harmful	320.13	250.26	120.28
	100	5				
	1,000	10				
11b	10	3	Harmful	18.26	660.15	168.33
	100	6				
	1,000	8				
13	10	1	Harmful	266.29	77.22	120.14
	100	6				
	1,000	10				
18c	10	2	Harmful	118.36	253.17	84.40
	100	9				
	1,000	10				
18d	10	0	Harmful	120.00	124.3	158.32
	100	8				
	1,000	10				
21	10	0	Non-toxic	886.27	–	–
	100	1				
	1,000	10				
23	10	0	Non-toxic	950.11	–	–
	100	0				
	1,000	5				
24	10	6	Very toxic	12.00	–	–
	100	9				
	1,000	10				

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

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 Thomsenm, 2001) was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95 % confidence intervals (Brayn *et al*., 2009). Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines which are the eight compounds **3**, **11b**, **13**, **18c**, **18d**, **21**, **23,** and **24**. 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 (Carballo *et al*., 2002), natural and synthetic organic compounds. 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 the 4-amino-3-chloro-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*b*]pyridine-2-ol (**21**) and the 1-(3-chloro-2-hydroxy-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*b*]pyridine-4-yl)-3-phenylthiourea (**23**) showed non-toxicity against the tested organisms.

Experimental

Chemistry

All melting points are uncorrected. IR spectra were recorded for KBr disks on a Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Varian EM-390–200 MHz in DMSO as solvent using TMS as internal standard and chemical shifts

are expressed as δ. Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt.

*2-Chloro-N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)acetamide (**3**)*

To a solution of compound **1** (1.80 g, 0.01 mol) in 1,4-dioxane, chloroacetyl chloride (1.13 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. Crystallized from ethanol to give white crystals, yield 2.23 g (88 %), m.p. 188–191 °C. *Anal*. Calculated for $C_{11}H_{11}ClN_2OS$ (254.74): C, 51.86; H, 4.35; Cl, 13.92; N, 11.00; S, 12.59 %. Found: C, 51.69; H, 4.57; Cl, 14.26; N, 10.85; S, 12.32 %. IR, υ: 3477–3321 (NH), 2220 (CN), 1702 (CO), 1623 (C=C). ^{1}H-NMR, δ: 1.78–1.84 (m, 4H, $2CH_2$), 2.56–2.74 (m, 4H, $2CH_2$), 5.58 (s, 2H, CH_2), 8.43 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.8, 20.5, 21.6, 24.6 ($4CH_2$), 45.8 (CH_2), 116.8 (CN), 119.0, 129.7, 134.2, 136.0 (thiophene C), 166.9 (C=O).

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

To a solution of compound **3** (2.54 g, 0.01 mol) in 1,4-dioxane (40 mL), hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then left to cool. The solid product formed upon leaving the reaction mixture overnight was collected by filtration. Crystallized from ethanol to give white crystals, yield 2.23 g (88 %), m.p. 166–168 °C. *Anal*. Calculated for $C_{11}H_{14}N_4OS$ (250.32): C, 52.78; H, 5.64; N, 22.38; S, 12.81 %. Found: C, 52.93; H, 5.84; N, 22.48; S, 13.02 %. IR, υ: 3489–3339 (NH_2, 2NH), 2223 (CN), 1719 (CO), 1623 (C=C). ^{1}H-NMR, δ: 1.72–1.81 (m, 4H, $2CH_2$), 2.53–2.76 (m, 4H, $2CH_2$), 4.98 (s, 2H, CH_2), 6.77 (s, 2H, D_2O exchangeable, NH_2), 8.21, 8.28 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.5, 20.7, 21.5, 24.7 ($4CH_2$), 38.6 (CH_2), 116.4 (CN), 119.3, 129.6, 133.6, 136.6 (thiophene C), 164.4 (C=O).

*N-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-2-(2-cyclopentylidene-hydrazinyl)-acetamide (**7a**) and N-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]-thiophen-2-yl)-2-(2-cyclohexaylidene-hydrazinyl)acetamide (**7b**)*

General procedure: To a solution of compound **5** (2.50 g, 0.01 mol) in 1,4-dioxane (40 mL) either cyclopentanone (0.84 g, 0.01 mol) or cyclohexanone (0.98 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then evaporated under vacuum, and the remaining product was triturated with ethanol and the formed solid product, in each case, was collected by filtration.

*Compound **7a*** Crystallized from ethanol to give yellow crystals, yield 2.53 g (80 %), m.p. 261–263 °C. *Anal*. Calculated for $C_{16}H_{20}N_4OS$ (316.42): C, 60.73; H, 6.37; N, 17.71; S, 10.13 %. Found: C, 60.84; H, 6.67; N, 17.84; S, 9.94 %. IR, υ: 3469–3342 (2 NH), 2220 (CN), 1721 (CO), 1660 (C=N), 1620 (C=C). 1H-NMR, δ: 1.70–1.82 (m, 8H, $4CH_2$), 2.52–2.78 (m, 8H, $4CH_2$), 4.98 (s, 2H, CH_2), 8.21, 8.32 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.5, 20.7, 21.5, 24.7 24.8, 24.9, 32.0, 34.8 ($8CH_2$), 50.3 (CH_2), 116.8 (CN), 120.5, 128.4, 134.2, 137.2 (thiopohene C), 166.2 (C=O).

*Compound **7b*** Crystallized from 1,4-dioxane to give pale yellow crystals, yield 2.54 g (77 %), m.p. 248–250 °C. *Anal*. Calculated for $C_{17}H_{22}N_4OS$ (330.45): C, 61.79; H, 6.71; N, 16.95; S, 9.70 %. Found: C, 61.92; H, 6.85; N, 16.74; S, 9.88 %. IR, υ: 3473–3342 (2 NH), 2223 (CN), 1720 (CO), 1662 (C=N), 1622 (C=C). 1H-NMR, δ: 1.72–1.82 (m, 8H, $4CH_2$), 2.56–2.93 (m, 10H, $5CH_2$), 4.74 (s, 2H, CH_2), 8.22, 8.31 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.5, 20.7, 21.6, 24.7, 24.8, 32.0, 34.8, 48.02 (9 CH_2), 50.6 (CH_2), 116.7 (CN), 120.2, 128.4, 134.5, 137.8 (thiophene C), 166.6 (C=O).

*2-(2-Benzylidenehydrazinyl)-N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)acetamide (**9a**) and N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-2-(2-(1-phenylethylidene)hydrazinyl)acetamide (**9b**)*

General procedure To a solution of compound **5** (2.50 g, 0.01 mol) in dimethylformamide (20 mL) either benzaldehyde (1.08 g, 0.01 mol) or acetophenone (1.20 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.

*Compound **9a*** Crystallized from ethanol to give yellow crystals, yield 2.43 g (72 %), m.p. 177–179 °C. *Anal*. Calculated for $C_{18}H_{18}N_4OS$ (338.43): C, 63.88; H, 5.36; N, 16.56; S, 9.47 %. Found: C, 63.64; H, 5.49; N, 16.28; S, 9.77 %. IR, υ: 3439–3312 (2 NH), 2226 (CN), 1683 (CO), 1662 (C=N), 1624 (C=C). ^{1}H-NMR, δ: 1.71–1.88 (m, 4H, $2CH_2$), 2.46–2.93 (m, 4H, $2CH_2$), 4.78 (s, 2H, CH_2), 6.05 (s, 1H, N = CH), 7.29–7.38 (m, 5H, C_6H_5), 8.23, 8.36 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.5, 20.3, 21.5, 24.8 ($4CH_2$), 50.8 (CH_2), 116.9 (CN), 120.6, 122.3, 124.

8, 127.2, 128.9, 133.6, 137.9 (thiophene, C_6H_5 C), 164.8 (C=N), 172.3 (C=O).

*Compound **9b*** Crystallized from acetic acid to give yellow crystals, yield 2.39 g (68 %), m.p. 210–213 °C. *Anal*. Calculated for $C_{19}H_{20}N_4OS$ (352.45): C, 64.75; H, 5.72; N, 15.90; S, 9.10 %. Found: C, 64.88; H, 5.60; N, 16.19; S, 9. 26 %. IR, υ: 3430–3342 (2 NH), 2222 (CN), 1684 (CO), 1664 (C=N), 1622 (C=C). ^{1}H-NMR, δ: 1.74–1.87 (m, 4H, $2CH_2$), 2.43–2.90 (m, 4H, $2CH_2$), 3.05 (s, 3H, CH_3), 4.74 (s, 2H, CH_2), 7.31–7.39 (m, 5H, C_6H_5), 8.24, 8.39 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.8, 20. 5, 21.5, 24.6 ($4CH_2$), 50.5 (CH_2), 116.8 (CN), 120.8, 122.0, 124.9, 126.6, 128.5, 133.9, 138.3 (thiophene, C_6H_5 C), 164.3(C=N), 172.6 (C=O).

*N-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetamide (**11a**) and N-(3-Cyano-4,5,6,7-tetrahydrobenzo-[b]thiophen-2-yl)-2-(5-hydroxy-3-methyl-1H-pyrazol-1-yl)acetamide (**11b**)*

General procedure To a solution of compound **5** (2.50 g, 0.01 mol) in 1,4-dioxane (20 mL) containing triethylamine (0.50 mL) either acetylacetone (1.0 g, 0.01 mol) or ethyl acetoacetate (1.30 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.

*Compound **11a*** Crystallized from ethanol to give yellow crystals, yield 1.88 g (60 %), m.p. 260–263 °C. *Anal*. Calculated for $C_{16}H_{18}N_4OS$ (314.41): C, 61.12; H, 5.77; N, 17.82; S, 10.20 %. Found: C, 61.48; H, 5.73; N, 18.05; S, 10.41 %. IR, υ: 3458–3331 (NH), 2220 (CN), 1680 (CO), 1632 (C=C). ^{1}H-NMR, δ: 1.72–1.87 (m, 4H, $2CH_2$), 2.28, 2.49 (2s, 6H, $2CH_3$), 2.47–2.92 (m, 4H, $2CH_2$), 4.98 (s, 2H, CH_2), 8.21 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.3, 20.2, 21.5, 25.2 ($4CH_2$), 54.6 (CH_2), 116.7 (CN), 126. 3, 127.3, 128.9, 133.6, 137.9 (thiophene, pyrazole C), 164. 4 (C=N), 172.8 (C=O).

*Compound **11b*** Crystallized from ethanol to give orange crystals, yield 1.83 g (58 %), m.p. 230–232 °C. *Anal*. Calculated for $C_{15}H_{16}N_4O_2S$ (316.38): C, 56.94; H, 5.10; N, 17.71; S, 10.14 %. Found: C, 56.72; H, 4.82; N, 17.91; S, 10.29 %. IR, υ: 3520–3321 (OH, NH), 2222 (CN), 1687 (CO), 1630 (C=C). ^{1}H-NMR, δ: 1.71–1.89 (m, 48H, $2CH_2$), 2.22 (s, 3H, CH_3), 2.43–2.90 (m, 4H, $2CH_2$), 4.96 (s, 2H, CH_2), 8.20 (s, 1H, D_2O exchangeable, NH), 10.37 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.4, 20.6, 21.5, 25.5 ($4CH_2$), 54.8 (CH_2), 116.4 (CN), 126.2, 127.3, 128.5, 133.86, 137.6 (thiophene, pyrazole C), 164.6 (C=N), 172.5 (C=O).

*N′-(4-Amino-2-hydroxy-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-b]pyridine-3-yl)-2-cyanoacetohydrazide (**13**)*

To a solution of compound **5** (2.50 g, 0.01 mol) in 1,4-dioxane (20 mL) containing triethylamine (0.50 mL) ethyl cyanoacetate (1.13 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.

*Compound **13*** Crystallized from acetic acid to give buff crystals, yield 1.74 g (55 %), m.p. >300 °C. *Anal*. Calculated for $C_{14}H_{15}N_5O_2S$ (317.37): C, 52.98; H, 4.76; N, 22. 07; S, 10.10 %. Found: C, 53.22; H, 4.63; N, 21.83; S, 10. 30 %. IR, υ: 3560–3312 (OH, NH_2, 2NH), 1683 (CO), 1626 (C=C). ^{1}H-NMR, δ: 1.66–1.86 (m, 4H, $2CH_2$), 2. 40–2.88 (m, 4H, $2CH_2$), 4.64 (s, 2H, CH_2), 5.21 (s, 2H, D_2O exchangeable, NH_2), 8.03, 8.33 (2s, 2H, D_2O exchangeable, 2NH), 10.41 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.6, 20.8, 21.6, 25.8 ($4CH_2$), 56.0 (CH_2), 126.2, 127.3, 128.5, 133.2, 137.6, 143.2, 148.7 (thiophene, pyridine C), 166.8 (C=N), 177.1 (C=O).

*Compound **15*** Crystallized from 1,4-dioxane to give yellow crystals, yield 2.62 g (83 %), m.p. 288–290 °C. *Anal*. Calculated for $C_{14}H_{16}N_6OS$ (316.38): C, 53.15; H, 5. 10; N, 26.56; S, 10.13 %. Found: C, 53.40; H, 4.88; N, 26. 73; S, 10.29 %. IR, υ: 3488–3332 (2 NH_2, NH), 2222 (CN), 1688 (CO), 1628 (C=C). ^{1}H-NMR, δ: 1.65–1.89 (m, 4H, $2CH_2$), 2.42–2.86 (m, 4H, $2CH_2$), 4.76, 4.93 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.23 (s, 2H, CH_2), 6.88 (s, 1H, pyrazole H-4), 8.26 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.9, 20.5, 21.9, 25.4 ($4CH_2$), 57.3 (CH_2), 124.8, 126.2, 129.9, 134.8, 139.2, 140.7, 147.3 (thiophene, pyrazole C), 164.3 (C=N), 174.0 (C=O).

*2-(4-((3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)amino)-3-phenyl-thiazol-2(3H)-ylidene)malononitrile (**18a**), ethyl 2-cyano-2-(4-((3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)amino-3-phenylthiazol-2(3H)-ylidene)acetate (**18b**), 2-((2-(2,4-dioxopentan-3-ylidene)-3-phenyl-2,3-dihydro-thiazol-4-yl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**18c**) and ethyl 2-(4-((3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)amino-3-phenylthiazol-2(3H)-ylidene)-3-oxobutanoate (**18d**)*

General procedure A solution of either malononitrile (0. 66 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol), acetylacetone (1.0 g, 0.01 mol) or ethyl acetoacetate (1. 30 g, 0.01 mol) in dimethylformamide (30 mL) containing potassium hydroxide (0.56 g) was stirred at room

overnight. On the second day, compound 3 (2.54 g, 0. 01 mol) was added, and the whole reaction mixture was stirred at room temperature for an additional night. The solid product produced upon pouring onto ice/water containing hydrochloric acid (till pH 6) was collected by filtration.

*Compound **18a*** Crystallized from ethanol to give pall yellow crystals, yield 2.08 g (66 %), m.p. 144–147 °C. *Anal*. Calculated for $C_{21}H_{15}N_5S_2$ (401.51): C, 62.82; H, 3. 77; N, 17.44; S, 15.97 %. Found: C, 62.62; H, 4.18; N, 17. 29; S, 16.13 %. IR, υ: 3452–3312 (NH), 3055 (CH aromatic), 2227, 2220 (2CN), 1628 (C=C). ^{1}H-NMR, δ: 1. 65–1.87 (m, 8H, $4CH_2$), 2.40–2.86 (m, 8H, $4CH_2$), 6.23 (s, 1H, thiazole H-5), 7.31-7.42 (m, 5H, C_6H_5), 8.32 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.4, 20.8, 21.6, 25. 9 ($4CH_2$), 109.8, 114.2 (C=C), 116.6, 117.4 (2CN), 121.6, 124.8, 126.2, 127.8, 129.9, 134.8, 139.2, 142.4, 143.2, 147. 3 (thiophene, pyrazole, C_6H_5 C).

*Compound **18b*** Crystallized from ethanol to give orange crystals, yield 2.95 g (66 %), m.p. 120–122 °C. *Anal*. Calculated for $C_{23}H_{20}N_4O_2S_2$ (448.56): C, 61.59; H, 4.49; N, 12.49; S, 14.30 %. Found: C, 61.69; H, 4.36; N, 12.73; S, 14.52 %. IR, υ: 3466–3329 (NH), 3053 (CH aromatic), 2223 (CN), 1720 (CO), 1639 (C=C). ^{1}H-NMR, δ: 1.13 (t, 3H, $J = 6.09$ Hz, CH_3), 1.66–1.84 (m, 8H, $4CH_2$), 2.42–2. 87 (m, 8H, $4CH_2$), 4.23 (q, 2H, $J = 6.09$ Hz, CH_2), 6.25 (s, 1H, thiazole H-5), 7.31–7.40 (m, 5H, C_6H_5), 8.38 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.4, 20.8, 21.6, 25. 9 ($4CH_2$), 109.5, 114.7 (C=C), 116.8 (CN), 122.06, 124.8, 126.6, 127.8, 131.2, 134.2, 139.6, 142.2, 143.8, 147.9 (thiophene, pyrazole, C_6H_5 C).

*Compound **18c*** Crystallized from ethanol to give orange crystals, yield 3.04 g (70 %), m.p. 210–212 °C. *Anal*. Calculated for $C_{23}H_{21}N_3O_2S_2$ (435.56): C, 63.42; H, 4.86; N, 9.65; S, 14.72 %. Found: C, 63.71; H, 4.59; N, 9.42; S, 14.39 %. IR, υ: 3470–3341 (NH), 3051 (CH aromatic), 2220 (CN), 1680–1688 (2CO), 1636 (C=C). ^{1}H-NMR, δ: 1. 68–1.85 (m, 8H, $4CH_2$), 2.41–2.83 (m, 8H, $4CH_2$), 2.83, 2. 90 (2s, 6H, $2CH_3$), 6.29 (s, 1H, thiazole H-5), 7.28–7.38 (m, 5H, C_6H_5), 8.36 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.6, 20.98, 21.8, 25.8 ($4CH_2$), 109.8, 114.3 (C= C), 116.5 (CN), 122.5, 124.9, 126.8, 127.9, 131.4, 134.5, 139.8, 142.0, 143.6, 147.5 (thiopohene, pyrazole, C_6H_5 C).

*Compound **18d*** Crystallized from ethanol to give orange crystals, yield 3.63 g (77 %), m.p. 140–142 °C. *Anal*. Calculated for $C_{24}H_{23}N_3O_3S_2$ (465.59): C, 61.91; H, 4.98; N, 9.03; S, 13.77 %. Found: C, 62.19; H, 4.69; N, 9.19; S, 14.02 %. IR, υ: 3459–3316 (NH), 3051 (CH aromatic), 2220 (CN), 1683, 1689 (2CO), 1633 (C=C). ^{1}H-NMR, δ: 1. 14 (t, 3H, $J = 7.09$ Hz, CH_3), 1.62–1.84 (m, 8H, $4CH_2$), 2. 43–2.88 (m, 8H, $4CH_2$), 2.84 (s, 3H, CH_3), 4.22 (q, 2H, $J = 7.09$ Hz, CH_2), 6.32 (s, 1H, thiazole H-5), 7.29–7.44 (m, 5H, C_6H_5), 8.33 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR, δ: 19.8, 20.6, 21.5, 25.7 ($4CH_2$), 111.3, 115.8 (C= C), 117.1 (CN), 123.5, 124.6, 125.28, 128.3, 133. 2, 134.5, 139.8, 142.8, 143.4, 147.6 (thiopohene, pyrazole, C_6H_5 C), 166.4, 172.0 (2 CO).

*N-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-2-(phenylamino)-acetamide (**20**)*

Equimolar amounts of compound 3 (2.54 g, 0.01 mol) and aniline (0.93 g, 0.01 mol) in 1,4-dioxane (40 mL) was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

*Compound **20*** Crystallized from ethanol to give colorless crystals, yield 2.43 g (82 %), m.p. 196–198 °C. *Anal*. Calculated for $C_{17}H_{17}N_3S_2$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30 %. Found: C, 65.81; H, 5.80; N, 13.63; S, 16.22 %. IR, υ: 3489–3338 (NH), 3052 (CH aromatic), 2225 (CN), 1695 (CO), 1638 (C=C). ^{1}H-NMR, δ: 1.63–1. 87 (m, 8H, $4CH_2$), 2.43–2.88 (m, 8H, $4CH_2$), 5.77 (s, 2H, CH_2), 7.26–7.39 (m, 5H, C_6H_5), 8.24, 8.36 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C-NMR, δ: 19.6, 20.6, 21.8, 25.6 ($4CH_2$), 56.4 (CH_2), 116.8 (CN), 120.8, 124.2, 126.8, 127. 8, 128.3, 142.4, 143.6, 144.2 (thiophene, C_6H_5 C).

*4-Amino-3-chloro-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-b]pyridine-2-ol (**21**) and 4-amino-3-(phenylamino)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-b]pyridine-2-ol (**22**)*

General procedure A suspension of either compound **3** (2.54 g, 0.01 mol) or **20** (3.11 g, 0.01 mol) in sodium ethoxide solution [prepared by dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (30 mL)] was heated in a boiling water bath for 6 h. The formed solid product upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

*Converting compound **21** into **22***

To a solution of compound **21**(2.54 g, 0.01 mol) in 1,4-dioxane (30 mL) aniline (0.93 g, 0.01 mol) was added and the reaction mixture was heated under reflux for 6 h. After reaching room temperature, the reaction contents were poured onto ice/water and the formed solid product was collected by filtration.

*Compound **21*** Crystallized from ethanol to give yellow crystals, yield 1.93 g (76 %), m.p. 120–122 °C. *Anal*.

Calculated for $C_{11}H_{11}ClN_2OS$ (254.74): C, 51.86; H, 4.35; N, 11.00; S, 12.59 %. Found: C, 51.74; H, 4.42; N, 11.32; S, 12.73 %. IR, υ: 3520–3318 (OH, NH), 3052 (CH aromatic), 1622 (C=C). ^{1}H-NMR, δ: 1.61–1.88 (m, 8H, $4CH_2$), 2.42–2.89 (m, 8H, $4CH_2$), 4.69 (s, 2H, D_2O exchangeable, NH_2), 10.02 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.8, 20.8, 21.8, 25.9 ($4CH_2$), 122.3, 124.2, 126.6, 133.2, 138.0, 142.4, 143.2, 144.6 (thiophene, pyridine C), 172.2 (C=N).

Compound ***22*** Crystallized from ethanol to give yellow crystals, yield 2.05 g (66 %), m.p. 198–200 °C. *Anal*. Calculated for $C_{17}H_{17}N_3OS$ (311.11): C, 65.57; H, 5.50; N, 13.49; S, 10.30 %. Found: C, 65.77; H, 5.69; N, 13.73; S, 10.13 %. IR, υ: 3541–3330 (OH, NH_2, NH), 3056 (CH aromatic), 1618 (C=C). ^{1}H-NMR, δ: 1.63–1.87 (m, 8H, $4CH_2$), 2.43–2.90 (m, 8H, $4CH_2$), 4.67 (s, 2H, D_2O exchangeable, NH_2), 7.28–7.38 (m, 5H, C_6H_5), 8.33 (s, 1H, D_2O exchangeable, NH), 10.02 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.4, 20.7, 21.8, 25.4 ($4CH_2$), 120.2, 122.1, 123.5, 125.6, 128.9, 133.2, 138.0, 142.4, 143.0, 144.8 (thiophene, pyridine, C_6H_5, C), 171.2 (C=N).

*1-(3-Chloro-2-hydroxy-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-b]pyridine-4-yl)-3-phenylthiourea (**23**)*

To a solution of compound 22 (3.11 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (0.50 mL) phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then evaporated under vacuum, and the remaining product was triturated by diethyl ether, and the solidified product was collected by filtration.

Compound ***23*** Crystallized from ethanol to give orange crystals, yield 3.34 g (86 %), m.p. 205–208 °C. *Anal*. Calculated for $C_{18}H_{16}ClN_3OS_2$ (389.92): C, 55.45; H, 4.14; N, 10.78; S, 16.45 %. Found: C, 55.39; H, 4.42; N, 10.58; S, 16.29 %. IR, υ: 3555–3323 (OH, 2 NH), 3058 (CH aromatic), 1625 (C=C). ^{1}H-NMR, δ: 1.60–1.87 (m, 8H, $4CH_2$), 2.44–2.95 (m, 8H, $4CH_2$), 7.32–7.39 (m, 5H, C_6H_5), 8.28, 8.36 (s, 1H, D_2O exchangeable, 2NH), 10.29 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.2, 20.9, 21.8, 25.6 ($4CH_2$), 120.8, 121.8, 123.5, 125.26, 129.3, 133.2, 138.60, 142.8, 143.6, 145.2 (thiophene, pyridine, C_6H_5, C), 171.2 (C=N).

*4-Hydroxy-3-phenyl-7,8,9,10-tetrahydro-1H-benzo [4,5]thieno[2,3-b]imidazo[4,5-d]pyridine-2(3H)-thione (**24**)*

A suspension of compound **23** (3.53 g, 0.01 mol) in sodium ethoxide solution [prepared by dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (30 mL)] was heated in a boiling water bath for 12 h. The formed solid product upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

Compound ***24*** Crystallized from ethanol to give Pall yellow crystals, yield 2.47 g (70 %), m.p. >300 °C. *Anal*. Calculated for $C_{18}H_{15}N_3OS_2$ (353.46): C, 61.16; H, 4.28; N, 11.89; S, 18.14 %. Found: C, 61.38; H, 4.11; N, 12.08; S, 17.94 %. IR, υ: 3538–3303 (OH, NH), 3056 (CH aromatic), 1633 (C=C), 1200-1190 (C=S). ^{1}H-NMR, δ: 1.62–1.87 (m, 8H, $4CH_2$), 2.41–2.83 (m, 8H, $4CH_2$), 7.34–7.37 (m, 5H, C_6H_5), 8.48 (s, 1H, D_2O exchangeable, NH), 10.32 (s, 1H, D_2O exchangeable, OH). ^{13}C-NMR, δ: 19.6, 20.59, 21.8, 25.9 ($4CH_2$), 120.5, 122.6, 123.5, 126.3, 127.4, 128.7, 129.3, 133.2, 138.6, 143.8, 144.8, 146.2, 149.2 (thiophene, imidazole, pyridine, C_6H_5, C), 172.0 (C=N).

Conclusions

Novel 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives were synthesized in good yields. Some compounds were used to produce annulated products. The antitumor evaluation of the newly synthesized products showed in every case it was observed that molecules with electronegative substitutions as compounds **3, 11b, 13, 18c, 18d, 21, 23, and 24** showed higher cytotoxicity because them were either oxygen or chlorine substituted as well as comprised with similar structural features. The toxicity of the compounds of optimal cytotoxicity showed that compounds **21** and **32** are non toxic against shrimp larvae.

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References

Abbas SE, Abdel Gawad NM, George RF, Akar YA (2013) Synthesis, antitumor and antibacterial activities of some novel tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine derivatives. Eur J Med Chem 65:195–204

Ament SMC, Gillissen F, Maessen JMC, Dirksen CD, Bell AVR, Vissers YL, Weijden TV, Meyenfeldt MF (2014) Sustainability of short stay after breast cancer surgery in early adopter hospitals. Breast 23:429–434

Amr AE, Sherif MH, Assy MG, Al-Omar MA, Ragab I (2010) o Antiarrhythmic, serotonin antagonist and antianxiety activities of novel substituted thiophene derivatives synthesized from 2-amino-4,5,6,7-tetrahydro-*N*-phenylbenzo[*b*]thiophene-3-carboxamide. Eur J Med Chem 45:5935–5942

Aurelio L, Christopoulos A, Flynn BL, Scammells PJ, Sexton PM, Valant C (2011) The synthesis and biological evaluation of 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophenes as allosteric modulators of the A1 adenosine receptor. Bioorg Med Chem Lett 21:3704–3807

Behbehani H, Ibrahim HM, Makhseed S, Elnagdi MH, Mahmoud H (2012) 2-Aminothiophenes as building blocks in heterocyclic synthesis: Synthesis and antimicrobial evaluation of a new class of pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine, quinoline and pyridin-2-one derivatives. Eur J Med Chem 52:51–65

Brayn B, Timothy M, Tore S (2009) General and applied toxicology, vol 6, 3rd edn. Wiley, New york, p 52

Calleja MC, Persoone G (1992) The potential ofecotoxicological tests for the prediction of acute toxicityin man as evaluated on the 1st 10 chemicals on the meic program. ATLA 20:396–405

Campaigne E (1984) Thiophenes and their benzo derivatives: (iii) synthesis and applications. Compreh Het Chem 4:863–934

Carballo JL, Inda ZLH, Pérez P, Grávalos MDG (2002) A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol 2:17

Choudhary MI, Thomsen WJ (2001) Bioassay techniques for drug development. Harwood Academic Publishers, Amsterdam, pp 9–10

Crouse KA, Chew KB, Tarafder MTH, Kasbollah A, Ali AM, Yamin BM, Fun HK (2004) Synthesis, characterization and bio-activity of S-2-picolyldithiocarbazate (S2PDTC), some of its Schiff bases and their Ni(II) complexes and X-ray structure of S-2-picolyl-β-*N*-(2-acetylpyrrole)dithiocarbazate. Polyhed 23:161–168

Dadiboyena S (2012) Recent advances in the synthesis of raloxifene: a selective estrogen receptor modulator. Eur J Med Chem 51:17–34

Dewal MB, Wani AS, Vidaillac C, Oupický D, Rybak MJ, Firestine SM (2012) Thieno[2,3-d]pyrimidinedione derivatives as antibacterial agents. Eur J Med Chem 51:145–153

Du QS, Shi YX, Lia PF, Zhao ZJ, Zhua WP, Qian XH, Li BJ (2013) Novel plant activators with thieno[2,3-*d*]-1,2,3-thiadiazole-6-carboxylate scaffold: Synthesis and bioactivity. Chin Chem Lett 24:967–969

Duval E, Case A, Stein RL, Cuny GD (2005) Structure–activity relationship study of novel tissue transglutaminase inhibitors. Bioorg Med Chem. Lett 15:1885–1889

Fadda AA, Abdel-Latif EA, El-Mekawy RE (2009) Synthesis and molluscicidal activity of some new thiophene, thiadiazole and pyrazole derivatives. Eur J Med Chem 44:1250–1256

Ferreirra AP, da Silva JLF, Duarte MP, da Piedade MFM, Robalo MP, Harjivan SG, Marzano C, Gandin V, Marques MM (2009) Synthesis and characterization of new organometallic Benzo[*b*]thiophene derivatives with potential antitumor properties. Organometalics 28:5412–5423

Goncales CEP, Araldi D, Panatieri RB, Rocha JBT, Zeni G, Nogueira CW (2005) Antinociceptive properties of acetylenic thiophene and furan derivatives: evidence for the mechanism of action. Life Sci 76:2221–2234

Goutham Vemana G, Kenneth G, Nepple KG, Joel Vetter J, Gurdarshan Sandhu G, Seth A, Strope SA (2014) Defining the potential of neoadjuvant chemotherapy use as a quality indicator for bladder cancer care. J Urol 192:43–49

Leopoldo C, Baratto LC, Mariana V, Porsani MV, Ida C, Pimentel IC, Adaucto B, Pereira Netto AB, Reinhard Paschke R, Brás H, Oliveira BH (2013) Preparation of betulinic acid derivatives by chemical and biotransformation methods and determination of cytotoxicity against selected cancer cell lines. Eur J Med Chem 68:121–131

Madhusudana K, Shireesha B, Naidu VGM, Ramakrishna S, Narsaiah B, Rao AR, Diwan PV (2012) Anti-inflammatory potential of thienopyridines as possible alternative to NSAIDs. Eur J Pharmacol 678:48–54

Mésangeau C, Fraise M, Delagrange P, Caignard DH, Boutin JA, Berthelot P, Yous S (2011) Preparation and pharmacological evaluation of a novel series of 2-(phenylthio)benzo[*b*]thiophenes as selective MT2 receptor ligands. Eur J Med Chem 46:1835–1840

Mohareb RM, Al-Omran F (2012) Reaction of pregnenolone with cyanoacetylhydrazin Novel synthesis of hydrazide–hydrazone, pyrazole, pyridine, thiazole, thiophene derivatives and their cytotoxicity evaluations. Steroids 77:1551–1559

Mohareb RM, El-Sayed NEE, Abdelaziz MA (2013) The Knoevenagel reactions of pregnenolone with cyanomethylene reagents: Synthesis of thiophene, thieno[2,3-*b*]pyridine, thieno[3,2-*d*]isoxazole derivatives of pregnenolone and their in vitro cytotoxicity towards tumor and normal cell lines. Steroids 78:1209–1219

Padmavathi V, Reddy GD, Reddy SN, Mahesh K (2011) Synthesis and biological activity of 2-(bis((1,3,4-oxadiazolyl/1,3,4-thiadiazol-yl)-methylthio)methylene)malononitriles. Eur J Med Chem 46:1367–1373

Perspicace E, Hureaux VJ, Ragno R, Ballante F, Sartini S, Motta CL, Settimo FD, Chen B, Kirsch G, der Schnei S, Faivre B, Hesse S (2013) Design, synthesis and biological evaluation of new classes of thieno[3,2-*d*]pyrimidinone and thieno[1,2,3]triazine as inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2). Eur J Med Chem 63:765–781

Pranati Samadder P, Yaozu XuY, Frank Schweizer F, Gilbert Arthur G (2014) Cytotoxic properties of D-gluco-, D-galacto- and D-manno-configured 2-amino-2-deoxy-glycerolipids against epithelial cancer cell lines and BT-474 breast cancer stem cells. Eur J Med Chem 78:225–235

Refat HM, Fadda AA (2013) Synthesis and antimicrobial activity of some novel hydrazide, benzochromenone, dihydropyridine, pyrrole, thiazole and thiophene derivatives. Eur J Med Chem 70:419–426

Romagnoli R, Baraldi PG, Carrion MD, Cruz-Lopez O, Tolomeo M, Grimaudo S, Cristina AD, Pipitone MR, Balzarini J, Brancale A, Hamel E (2010) Substituted 2-(30,40,50- trimethoxybenzoyl)-benzo[*b*]thiophene derivatives as potent tubulin polymerization inhibitors. Bioorg Med Chem 18:5114–5122

Stephens CE, Felder TM, Sowell JW, Andrei G, Balzarini J, Snoeck R, Clercq ED (2001) Synthesis and antiviral/antitumor evaluation of 2-amino- and 2-carboxamido-3-arylsulfonylthiophenes and related compounds as a new class of diarylsulfones. Bioorg Med Chem 9:1123–1132

Titchenell PM, Showalter HD, Pons JF, Barber AJ, Jin Y, Antonetti DA (2013) Synthesis and structure–activity relationships of 2-amino-3-carboxy-4-phenylthiophenes as novel atypical protein kinase C inhibitors. Bioorg Med Chem Lett 13:3034–3038

Upreti SR, Gurung S, Patel M, Dixit SM, Krause LK, Shakya G, Wannemuehler K, Rajbhandari R, Bohara R, Schluter WW (2014) Prevalence of chronic hepatitis B virus infection before and after implementation of a hepatitis B vaccination program among children in Nepal. Vaccine 32:4304–4309

Vizzotto M, Porter W, Byrne D, Zevallos LC (2014) Polyphenols of selected peach and plum genotypes reduce cell viability and inhibit proliferation of breast cancer cells while not affecting normal cells. Food Chem 164:363–370

Walker MJ, Mirea L, Glendon G, Ritvo P, Andrulis IL, Knight JA, Chiarelli AM (2014) Relevance date relevance—selected all access types all access types. Breast 23:482–488

Wang W, Shangguan S, Qiu N, Hu C, Zhang L, Hu Y (2013) Design, synthesis and biological evaluation of novel 3,4,5-trisubstituted aminothiophenes as inhibitors of p53–MDM2 interaction. Part 1. Bioorg Med Chem 21:2879–2885