Chemistry of Heterocyclic Compounds, Vol. 49, No. 3, June, 2013 (Russian Original Vol. 49, No. 3, March, 2013)

DESIGN, SYNTHESIS, AND CYTOTOXICITY OF PYRIDINE, PYRAZOLE, AND THIAZOLE DERIVATIVES DERIVED FROM *N*-ALKYL-4,5,6,7-TETRAHYDRO-1-BENZOTHIOPHENE

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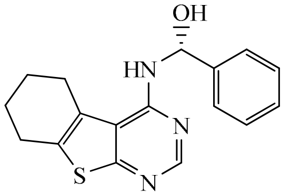
The reaction of 4,5,6,7-tetrahydro-1-benzothiophene derivatives with ethyl acetoacetate gave oxobutanamide derivatives. The reactivity of these products towards some chemical reagents was studied to afford new heterocyclic derivatives. The cytotoxicity of the newly synthesized compounds was evaluated against three human tumor cells lines and three normal cell lines. The results showed that some of these compounds exhibit high inhibitory effects towards the three tumor cell lines and the normal cell lines.

Keywords: pyridine, tetrahydro-1-benzothiophene, thiazole, thiophene, cytotoxocity.

Aromatic thiophenes are the most promising small-molecule selective protein kinase inhibitors [1-4]. Recently [5], a tricyclic compound, tetrahydro-1-benzothieno[2,3-*d*]pyrimidine, was identified as an initial hit with an enzyme inhibition IC_{50} 2.6 μM.



In recent years, thiophenes and their fused derivatives showed promising results as anticancer agents [6-9]. Moreover, Mohareb et al. [10] reported that 2-acylamino-4,5,6,7-tetrahydro-1-benzothiophene derivatives were tested against three human tumor cells lines, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and human glioblastoma (SF-268). The results showed that most of the tested compounds exhibit high inhibitory effects towards all three tumor cell lines. To our knowledge, some research has been done on the variation of substitution at positions 2 and 3 of the tetrahydro-1-benzothiophene nucleus

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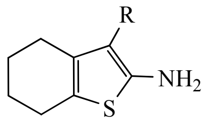
Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 3, pp. 423-435, March, 2013. Original article submitted July 23, 2012.

[8, 11-13]. In light of these observations, our efforts towards drug discovery prompted us to design, synthesize, and evaluate the cytotoxocity of some new tetrahydro-1-benzothiophene derivatives. In the present work, a new series of thiophene derivatives has been synthesized by incorporation of a variety of ring systems such as pyridine, thiazole, and thiophene at position 2 of the benzothiophene ring and either cyano or ethyl carboxylate group at position 3. The newly synthesized products have been evaluated as to their antitumor activity against the three cancer cell lines mentioned above. Some of them were tested for activity towards human normal cell lines. The comparison between the activity of the newly synthesized thiophene derivatives towards tumor and normal cell lines will direct future research towards the synthesis of good anticancer agents.

In an attempt to obtain an antitumor agent with high activity, the substitution pattern at positions 2 and 3 of the thiophene pharmacophore was selected in order to alter the electronic environment and thus affect the lipophilicity of the target molecules. Our aim was the acylation of the 2-amino groups in the known tetrahydro-1-benzothiophene nucleus **1a**,**b** and the introduction of 1,3-dicarbonyl [14], α,β-unsaturated carbonyl groups [15], and a nitrogen- or sulfur-containing heterocyclic ring [16] that are known to contribute to the enhancement of antitumor activity. The incorporation of a heterocyclic ring at position 2 of the thiophene pharmacofore was of great importance in order to increase the lipophilicity and thus radically modify the bioavailability and efficacy of the synthesized compounds. Therefore, in the present work we want to provide a comparison between some previously reported [17-20] tetrahydro-1-benzothiophenes **1a**-**e** and our newly synthesized compounds derived from the synthons **1a**,**b**.

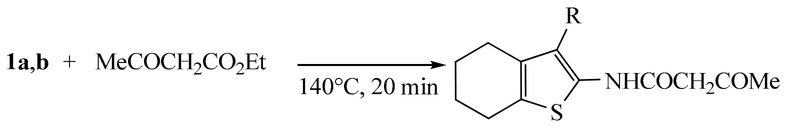
In the present work, we are considering the reported thiophene derivatives **1a**-**e** where antitumor reactivity will be compared to the newly synthesized compounds derived from the acylated derivatives of synthons **1a**,**b**.



1a–e

a R = CN, **b** R = CO_2Et, **c** R = $CONH_2$,
d R = CONHPh, **e** R = CONH(4-ClC_6H_4)

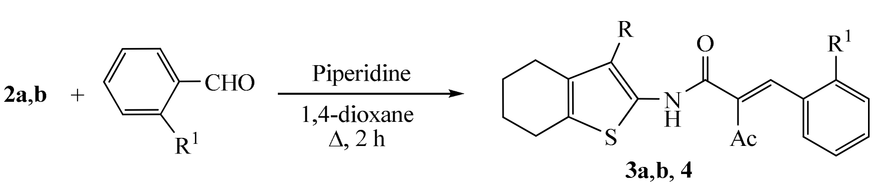
Thus, the reaction of compounds **1a**,**b** with ethyl acetoacetate at 140°C afforded the *N*-acyl derivatives **2a**,**b**, respectively. The structures of these and other synthesized compounds were assigned on the basis of their analytical and spectral data (Tables 1 and 2). Thus, the ^1H NMR spectrum of compound **2a** showed signals for all of the aliphatic groups and a singlet (D_2O exchangeable) at 11.57 ppm corresponding to the NH group.



2a,b

2 a R = CN, **b** R = CO_2Et

The reactivity of compounds **2a**,**b** towards different electrophilic reagents to form benzylidene, hydrazo, pyridine, and thiophene derivatives was studied with a view of investigating their biological activity. Thus, the reaction with benzaldehyde or salicylaldehyde gave the corresponding arylidene derivatives **3a**,**b** and **4**, respectively.

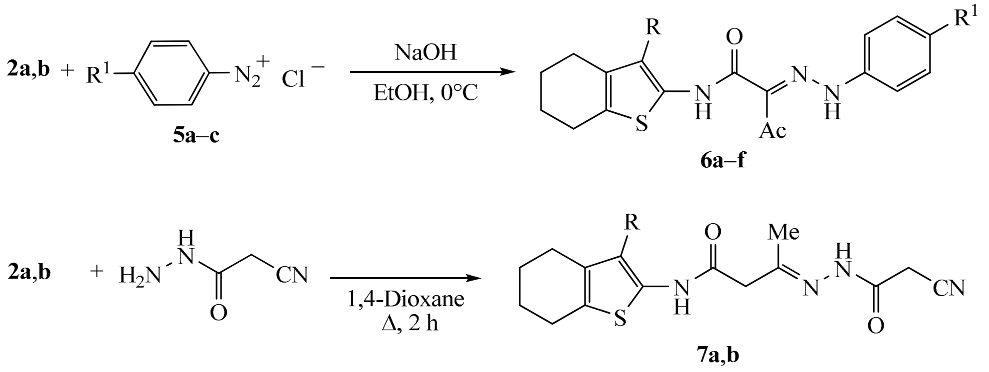


3a,b, 4

3a, **4** R = CN, **3b** R = CO_2Et; **3a**,**b** R^1 = H; **4** R^1 = OH

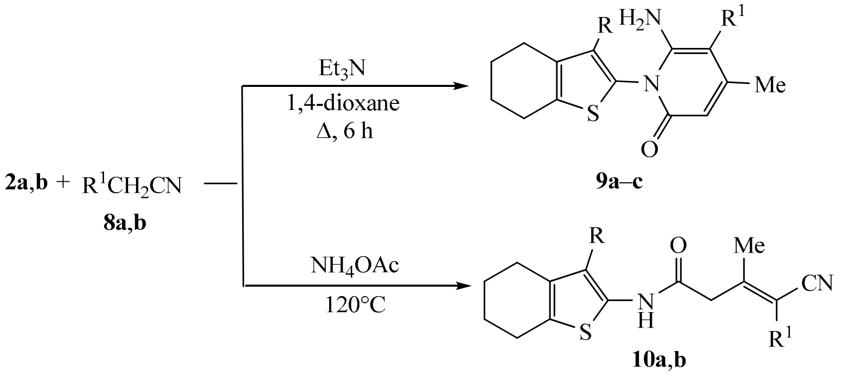
The reaction of compounds **2a** or **2b** with aromatic diazonium salts **5a-c** gave arylhydrazo derivatives **6a-f**. The analytical and spectral data of the latter products were consistent with the proposed structures.

The reactivity of compounds **2a**,**b** towards formation of hydrazide-hydrazone derivatives was studied on the example of the reaction with cyanoacetylhydrazine which gave hydrazones **7a**,**b**. The ^{1}H NMR spectra were the basis of the structure elucidation of hydrazones **7a**,**b**. Thus, the ^{1}H NMR spectrum of compound **7a** showed signals of all expected aliphatic groups and two singlets (D_2O exchangeable) at 10.18 and 11.01 ppm for the two NH groups.



6a–c, **7a** R = CN, **6d–f**, **7b** R = CO_2Et; **5a**, **6a**,**d** R^1 = H; **5b**, **6b**,**e** R^1 = Me; **5c**, **6c**,**f** R^1 = Cl

Next, we moved towards studying the reactivity of compounds **2a**,**b** towards active methylene reagents under different conditions. Thus, the reaction of compound **2a** with either malononitrile (**8a**) or ethyl cyanoacetate (**8b**) in 1,4-dioxane in the presence of triethylamine afforded pyridine derivatives **9a**,**b**, respectively [21]. The ^{1}H NMR spectrum of compound **9a** showed, besides the expected signals of the cyclohexene moiety, a singlet at 2.77 ppm corresponding to the CH_3 group, a singlet at 3.34 ppm (D_2O exchangeable) for one NH_2 group, and a singlet at 6.01 ppm corresponding to the pyridine H-3. Similarly, the reaction between compounds **2b** and **8a** in 1,4-dioxane/Et_3N solution gave the pyridine derivative **9c**. On the other hand, carrying out the same reactions of compound **2a** with compounds **8a**,**b** but in the presence of ammonium acetate in an oil bath at 120°C gave the Knoevenagel condensation products **10a**,**b**. The analytical and spectral data of the latter products were in agreement with their respective structures (Tables 1, 2).



9a,**b**, **10a**,**b** R = CN; **9c** R = CO_2Et; **8**, **9**, **10 a** R^1 = CN; **8**, **9**, **10 b** R^1 = CO_2Et; **9c** R^1 = CN

The reaction of either compound **2a** or **2b** with acetylacetone gave the pyridine derivatives **11a** and **11b**, respectively. The ^{1}H NMR spectrum of compound **11a** showed three singlets at 2.41, 2.63, and 3.59 ppm corresponding to the three CH_3 groups and a singlet at 6.91 ppm indicating the presence of the pyridine H-5 atom.

TABLE 1. Physicochemical Characteristics of the Synthesized Compounds

Com-pound	Empirical formula	Found, % Calculated, % C	 H	 N	 S	Mp*, °C	Yield, %
2a	$C_{13}H_{14}N_2O_2S$	59.60 59.52	5.60 5.38	10.59 10.68	11.89 12.22	217-219	78
2b	$C_{15}H_{19}NO_4S$	58.02 58.23	5.35 6.19	4.73 4.53	10.14 10.36	210-212	66
3a	$C_{20}H_{18}N_2O_2S$	68.38 68.55	5.44 5.18	8.28 7.99	8.84 9.15	144-145	70
3b	$C_{22}H_{23}NO_4S$	66.44 66.48	6.07 5.83	3.82 3.52	7.71 8.07	138-139	83
4	$C_{20}H_{18}N_2O_3S$	65.37 65.55	5.17 4.95	7.93 7.64	9.16 8.75	189-190	62
6a	$C_{19}H_{18}N_4O_2S$	61.99 62.28	5.12 4.95	14.84 15.29	9.05 8.75	170-171	77
6b	$C_{20}H_{20}N_4O_2S$	63.09 63.14	5.18 5.30	15.07 14.73	8.18 8.43	251-252	80
6c	$C_{19}H_{17}ClN_4O_2S$	57.23 56.93	4.52 4.27	14.11 13.98	7.83 8.00	240-241	71
6d	$C_{21}H_{23}N_3O_4S$	60.95 61.00	5.72 5.61	9.84 10.16	7.69 7.75	183-184	64
6e	$C_{22}H_{25}N_3O_4S$	61.74 61.81	6.01 5.89	10.02 9.83	7.09 7.50	100-102	60
6f	$C_{21}H_{22}ClN_3O_4S$	56.49 56.31	4.60 4.95	9.66 9.38	6.80 7.16	139-140	88
7a	$C_{16}H_{17}N_5O_2S$	56.21 55.96	5.12 4.99	19.92 20.39	9.26 9.34	222-223	87
7b	$C_{18}H_{22}N_4O_4S$	55.64 55.37	5.89 5.68	14.15 14.35	8.52 8.21	120-121	79
9a	$C_{16}H_{14}N_4OS$	61.69 61.92	4.67 4.55	17.83 18.05	10.11 10.33	268-269	58
9b	$C_{18}H_{19}N_3O_3S$	60.34 60.49	5.38 5.36	12.04 11.76	8.68 8.97	110-111	70
9c	$C_{18}H_{19}N_3O_3S$	60.72 60.49	5.66 5.36	12.06 11.76	8.62 8.97	133-134	76
10a	$C_{16}H_{14}N_4OS$	61.44 61.92	4.83 4.55	17.88 18.05	10.01 10.33	280-283	75
10b	$C_{18}H_{19}N_3O_3S$	60.34 60.49	5.38 5.36	12.04 11.76	9.36 8.97	184-185	59
11a	$C_{18}H_{18}N_2O_2S$	66.02 66.23	5.84 5.55	8.69 8.58	10.27 9.82	216-217	62
11b	$C_{20}H_{23}NO_4S$	64.01 64.32	6.49 6.21	3.49 3.75	8.82 8.59	180-181	70
15	$C_{22}H_{19}N_3O_3S_2$	59.28 60.39	5.19 4.38	9.52 9.60	14.52 14.66	200-201	77
16a	$C_{23}H_{21}N_3O_2S_2$	62.02 63.42	5.08 4.86	9.92 9.65	14.41 14.72	277-278	80
16b	$C_{26}H_{28}N_2O_5S_2$	60.73 60.92	5.29 5.51	5.05 5.46	12.92 12.51	170-171	80
16c	$C_{30}H_{28}N_2O_4S_2$	65.88 66.15	5.15 5.18	4.99 5.14	12.04 11.77	220-221	60
16d	$C_{25}H_{26}N_2O_4S_2$	62.16 62.22	5.67 5.43	6.29 5.80	12.93 13.29	160-161	74

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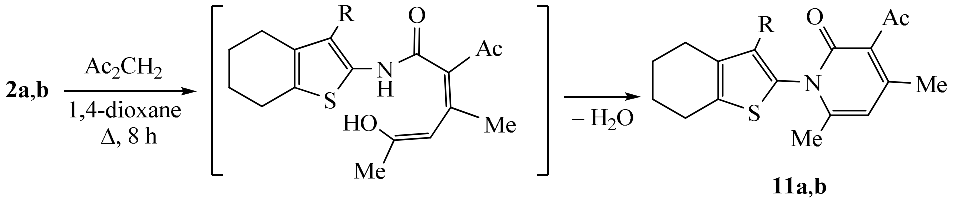
* Recrystallization solvents: ethanol (compounds **2a**,**b**, **3a**,**b**, **4**, **6a-f**, **7a**,**b**, **11a**,**b**, **15**, and **16a-d**)



11a R = R^1 = CN; **11b** R = R^1 = CO_2Et

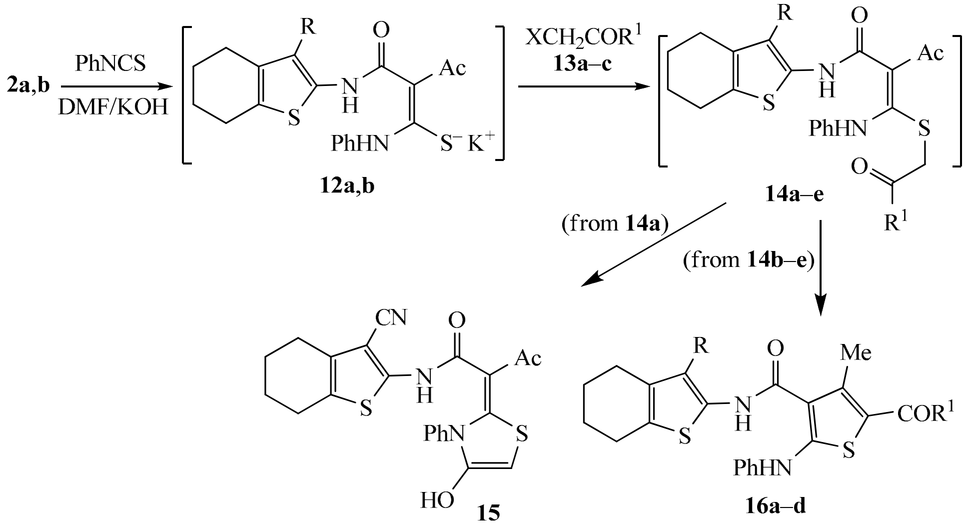
TABLE 2. Spectral Characteristics of the Obtained Compounds

Compound	IR spectrum, ν, cm^{-1}	^{1}H NMR spectrum (DMSO-d$_6$), δ, ppm (*J*, Hz)	MS, *m*/*z* (I_{rel}, %)
1	2	2	3
2a	3445-3224 (NH); 2936, 2850 (CH_3, CH_2); 2217 (CN); 1699-1690 (CO)	1.74-1.79 (4H, m, 2CH_2); 2.44-2.47 (4H, m, 2CH_2); 3.31 (3H, s, CH_3); 3.76 (2H, s, CH_2); 11.57 (1H, s, NH)	262 $[M]^+$ (15), 163 (100)
2b	3433-3270 (NH); 2928, 2850 (CH_3, CH_2); 1688-1644 (CO)	1.26 (3H, t, *J* = 7.0, CH_3); 1.68-1.71 (4H, m, 2CH_2); 2.24-2.28 (4H, m, 2CH_2); 3.32 (3H, s, CH_3); 3.85 (2H, s, CH_2); 4.24 (2H, q, *J* = 7.0, CH_2); 10.30 (1H, s, NH)	309 $[M]^+$ (15), 210 (100)
3a	3435-3370 (NH); 2932, 2850 (CH_3, CH_2); 1688-1646 (CO)	1.70-1.73 (4H, m, 2CH_2); 2.42-2.48 (4H, m, 2CH_2); 3.02 (3H, s, CH_3); 6.91 (1H, s, CH); 7.21-7.57 (5H, m, H Ph); 11.80 (1H, s, NH)	350 $[M]^+$ (7), 146 (100)
3b	3428-3278 (NH); 2928 (CH_3); 1690-1641 (C=O)	1.23 (3H, t, *J* = 6.7, CH_3); 1.77-1.80 (4H, m, 2CH_2); 2.58-2.61 (4H, m, 2CH_2); 2.69 (3H, s, CH_3); 4.25 (2H, q, *J* = 6.7, CH_2); 5.24 (1H, s, CH); 7.02-7.30 (5H, m, H Ph); 10.81 (1H, s, NH)	397 $[M]^+$ (18), 146 (100)
4	3580-3320 (OH, NH); 3054 (CH Ar); 2932, 2864 (CH_3, CH_2); 2214 (CN); 1680-1666 (CO)	1.79-1.83 (4H, m, 2CH_2); 2.52-2.56 (4H, m, 2CH_2); 2.71 (3H, s, CH_3); 6.91 (1H, s, CH); 7.28-7.81 (4H, m, H Ar); 8.80 (1H, s, NH); 11.47 (1H, s, OH)	366 $[M]^+$ (7), 162 (100)
6a	3738-3437 (NH); 3005 (CH Ar); 2936, 2856 (CH_3, CH_2); 2207 (CN); 1688-1646 (CO); 1509 (C=C)	1.76-1.80 (4H, m, 2CH_2); 2.50-2.54 (4H, m, 2CH_2); 3.30 (3H, s, CH_3); 7.23-7.62 (5H, m, H Ph); 12.13 (1H, s, NH); 12.79 (1H, s, NH)	366 $[M]^+$ (35), 189 (100)
6b	3520-3422 (NH); 3021 (CH Ar); 2929 (CH_3); 2210 (CN); 1688-1683 (CO)	1.77-1.80 (4H, m, 2CH_2); 1.93 (3H, s, CH_3); 2.49-2.53 (4H, m, 2CH_2); 3.17 (3H, s, CH_3); 7.22-7.48 (4H, m, H Ar); 10.13 (1H, s, NH); 10.22 (1H, s, NH)	380 $[M]^+$ (63), 91 (100)
6c	3560-3433 (NH); 3087 (CH Ar); 2938 (CH_3); 2215 (CN); 1720-1694 (CO)	1.77-1.81 (4H, m, 2CH_2); 2.49-2.54 (4H, m, 2CH_2); 3.74 (3H, s, CH_3); 7.48-7.65 (4H, m, H Ar); 10.47 (1H, s, NH); 10.63 (1H, s, NH)	400 $[M]^+$ (48), 223(100)
6d	3520-3420 (NH); 3092 (CH Ar); 1688-1677 (C=O)	1.25 (3H, t, *J* = 6.9, CH_3); 1.72-1.76 (4H, m, 2CH_2); 2.45-2.49 (4H, m, 2CH_2); 2.72 (3H, s, CH_3); 4.27 (2H, q, *J* = 6.9, CH_2); 7.45-7.61 (5H, m, H Ph); 10.26 (1H, s, NH); 10.29 (1H, s, NH)	413 $[M]^+$ (55), 189 (100)
6e	3520-3433 (NH); 3089 (CH Ar); 2930 (CH_3); 1690-1665 (C=O)	1.28 (3H, t, *J* = 7.1, CH_3); 1.72-1.78 (4H, m, 2CH_2); 2.38-2.42 (4H, m, 2CH_2); 2.50 (3H, s, CH_3); 2.61 (3H, s, CH_3); 4.35 (2H, q, *J* = 7.1, CH_2); 7.23-7.47 (4H, m, H Ar); 11.20 (1H, s, NH); 12.01 (1H, s, NH)	427 $[M]^+$ (46), 91 (100)
6f	3520-3424 (NH); 3092 (CH Ar); 2933 (CH_3); 1689-1677 (C=O)	1.25 (3H, t, *J* = 6.2, CH_3); 1.72-1.76 (4H, m, 2CH_2); 2.45-2.49 (4H, m, 2CH_2); 2.55 (3H, s, CH_3); 4.26 (2H, q, *J* = 6.2, CH_2); 7.45-7.61 (4H, m, H Ar); 10.23 (1H, s, NH); 10.82 (1H, s, NH)	447 $[M]^+$ (22), 223 (100)
7a	3434-3263 (NH); 3088 (CH Ar); 2930 (CH_3); 2216 (CN); 1686-1680 (CO)	1.81-1.85 (4H, m, 2CH_2); 2.54-2.57 (4H, m, 2CH_2); 3.34 (3H, s, CH_3); 3.41 (2H, s, 2CH_2); 3.74 (2H, s, CH_2); 10.18 (1H, s, NH); 11.01 (1H, s, NH)	343 $[M]^+$ (11), 177 (100)
7b	3404, 3297 (NH); 2933, 2852 (CH_3, CH_2); 1690-1656, 1705 (C=O)	1.23 (3H, t, *J* = 7.2, CH_3); 1.71-1.75 (4H, m, 2CH_2); 2.50-2.55 (4H, m, 2CH_2); 2.69 (3H, s, CH_3); 3.11 (2H, s, 2CH_2); 3.14 (2H, s, 2CH_2); 4.24 (2H, q, *J* = 7.2, CH_2); 10.92 (1H, s, NH); 11.22 (1H, s, NH)	390 $[M]^+$ (30), 224 (100)

TABLE 2 (continued)

1	2	2	3
9a	3458-3334 (NH_2); 2932, 2852 (CH_3, CH_2); 2220, 2204 (CN); 1688 (CO); 1648 (C=C)	1.72-1.77 (4H, m, $2CH_2$); 2.56-2.59 (4H, m, $2CH_2$); 2.77 (3H, s, CH_3); 3.34 (2H, s, NH_2); 6.01 (1H, s, H-5 Py)	310 $[M]^+$ (25), 148 (100)
9b	3741-3221 (NH_2); 3088 (CH Py); 2933, 2854 (CH_3, CH_2); 2213 (CN); 1687, 1800 (CO)	1.33 (3H, t, *J* = 6.0, CH_3); 1.74-1.79 (4H, m, $2CH_2$); 2.50-2.53 (4H, m, $2CH_2$); 2.95 (3H, s, CH_3); 3.93 (2H, s, NH_2); 4.27 (2H, q, *J* = 6.0, CH_2); 6.98 (1H, s, H-5 Py)	357 $[M]^+$ (5), 148 (100)
9c	3734, 3343 (NH_2); 2933 (CH_3); 2216 (CN); 1705, 1685 (CO); 1650 (C=C)	1.22 (3H, t, *J* = 6.1, CH_3); 1.70-1.75 (4H, m, $2CH_2$); 2.55-2.59 (4H, m, $2CH_2$); 2.59 (3H, s, CH_3); 3.40 (2H, s, NH_2); 4.32 (2H, q, *J* = 6.1, CH_2); 6.26 (1H, s, H-5 Py)	357 $[M]^+$ (16), 148 (100)
10a	3418 (NH); 3051 (CH Ar); 2923, 2851 (CH_3, CH_2); 2225-2202 (CN); 1680 (CO)	1.76-1.81 (4H, m, $2CH_2$); 1.90 (3H, s, CH_3); 2.50-2.56 (4H, m, $2CH_2$); 5.98 (2H, s, CH_2); 8.58 (1H, s, NH)	310 $[M]^+$ (9), 177 (100)
10b	3488-3428 (NH); 2993, 2932 (CH_3, CH_2); 2217, 2222 (CN); 1725, 1681 (CO); 1655 (C=C)	1.39 (3H, t, *J* = 6.8, CH_3); 1.74-1.78 (4H, m, $2CH_2$); 2.50 (3H, s, CH_3); 2.52-2.56 (4H, m, $2CH_2$); 3.98 (2H, s, CH_2); 4.20 (2H, q, *J* = 6.8, CH_2); 11.82 (1H, s, NH)	357 $[M]^+$ (16), 224 (100)
11a	3043 (CH Ar); 2933, 2848 (CH_3, CH_2); 2208 (CN); 1678, 1655 (CO)	1.57-1.59 (4H, m, $2CH_2$); 2.41 (3H, s, CH_3); 2.58-2.61 (4H, m, $2CH_2$); 2.63 (3H, s, CH_3); 3.59 (3H, s, CH_3); 6.91 (1H, s, H-5 Py)	326 $[M]^+$ (40), 164 (100)
11b	3043 (CH Ar); 2930, 2854 (CH_3, CH_2); 1687-1705 (CO)	1.28 (3H, t, *J* = 7.1, CH_3); 1.70-1.78 (4H, m, $2CH_2$); 2.46-2.48 (4H, m, $2CH_2$); 2.50 (3H, s, CH_3); 2.65 (3H, s, CH_3); 3.34 (3H, s, CH_3); 4.27 (2H, q, *J* = 7.1, CH_2); 7.26 (1H, s, H-5 Py)	373 $[M]^+$ (60), 164 (100)
15	3555-3438 (OH, NH); 3068 (CH Ar); 2931, 2850 (CH_3, CH_2); 2213 (CN); 1696, 1659 (CO)	1.58-1.62 (4H, m, $2CH_2$); 2.58-2.63 (4H, m, $2CH_2$); 2.72 (3H, s, CH_3); 5.61 (1H, s, H-5 thiazole); 7.05-7.57 (5H, m, H Ph); 10.22 (1H, s, NH); 11.30 (1H, s, OH)	437 $[M]^+$ (3), 260 (100)
16a	3457-3330 (NH); 2993, 2930 (CH_3, CH_2); 2201 (CN); 1646-1705 (CO)	1.77-1.80 (4H, m, $2CH_2$); 2.22 (3H, s, CH_3); 2.58-2.62 (4H, m, $2CH_2$); 3.14 (3H, s, CH_3); 7.01-7.40 (5H, m, H Ph); 10.05 (1H, s, NH); 12.02 (1H, s, NH)	435 $[M]^+$ (2), 258 (100)
16b	3429-3229 (NH); 2929 (CH_3); 1690-1662 (C=O)	1.22 (3H, t, *J* = 7.3, CH_3); 1.29 (3H, t, *J* = 6.6, CH_3); 1.71-1.73 (4H, m, $2CH_2$); 2.47-2.49 (4H, m, $2CH_2$); 2.50 (3H, s, CH_3), 4.19 (2H, q, *J* = 7.3, CH_2); 4.32 (2H, q, *J* = 6.6, CH_2); 7.31-7.61 (5H, m, H Ph), 10.20 (1H, s, NH); 10.34 (1H, s, NH)	512 $[M]^+$ (33), 288 (100)
16c	3430-3255 (NH); 3050 (CH Ar); 2930 (CH_3); 1687-1665 (C=O)	1.36 (3H, t, *J* = 6.1, CH_3); 1.70-1.75 (4H, m, $2CH_2$); 2.48-2.53 (4H, m, $2CH_2$); 2.65 (3H, s, CH_3); 4.17 (2H, q, *J* = 6.1, CH_2); 7.09-7.68 (10H, m, H Ph); 10.25 (1H, s, NH); 11.58 (1H, s, NH)	544 $[M]^+$ (20), 105 (100)
16d	3550-3432 (NH); 3071 (CH Ar); 2927 (CH_3); 1680-1665 (C=O)	1.30 (3H, t, *J* = 5.9, CH_3); 1.72-1.75 (4H, m, $2CH_2$); 2.42-2.48 (4H, m, $2CH_2$); 2.88 (3H, s, CH_3); 3.30 (3H, s, CH_3); 4.20 (2H, q, *J* = 5.9, CH_2); 7.09-7.42 (5H, m, H Ph); 10.06 (1H, s, NH); 11.55 (1H, s, NH)	482 $[M]^+$ (22), 258 (100)

It has been reported [22-24] that the reaction of active methylene reagents with phenylisothiocyanate in basic dimethyl formamide gives intermediate potassium sulfide salts that undergo heterocyclization with α-halocarbonyl compounds to give either thiophene or thiazole derivatives, depending on the reaction conditions or the nature of the α-halocarbonyl compound. In a similar way, we expected the reaction of either compound **2a** or **2b** with phenylisothiocyanate in DMF/KOH solution to give the salts **12a**,**b** that would further react with the α-halocarbonyl derivatives **13a-c** to yield intermediate thioether derivatives of the type **14a-e** [23]. The reaction of compound **2a** with phenylisothiocyanate, and further with ester **13a**, produced the thiazole derivative **15**. The ^{1}H NMR spectrum of compound **15** showed, besides the expected CH_2 and Ph group signals, a singlet at 2.72 ppm for the CH_3 group, a singlet at 5.61 ppm indicating the presence of the thiazole H-5 proton, and two singlets at 10.22 and 11.30 ppm corresponding to the NH and OH groups, respectively. Formation of the thiazole ring of compound **15** most likely took place *via* ethanol elimination from the intermediate **14a** due to the presence of the good ethoxy leaving group. On the other hand, the reaction of either nitrile **2a** with chloroacetone (**13c**) or ester **2b** with α-halocarbonyl compounds **13a-c** afforded the thiophene derivatives **16a-d**, which were formed *via* water elimination from the intermediates **14b-e**.



12a, **14a**,**b**, **16a** R = CN; **12b**, **14c–e**, **16b–d** R = CO_2Et; **13a**, **14a**,**c**, **16b** R^1 = OEt; **13b**, **14d**, **16c** R^1 = Ph; **13c**, **14b**,**e**, **16a**,**d** R^1 = Me; **13 a**,**c** X = Cl; **b** X = Br

A concurrent formation of thiazoles of the type **15** and thiophenes of the type **16a-d** has been also reported in the literature [25]. It is important to note that in such reaction, although the same reagent ethyl chloroacetate (**12a**) was used, thiazole derivative **15** and thiophene derivative **16a** were alternatively formed. Such findings are complementary to those reported earlier [26].

All synthesized compounds **2-16** were evaluated for their capacity to inhibit the *in vitro* growth of breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and human glioblastoma (SF-268) cell lines, after 48 h continuous exposure (Table 3). The *In vitro* Anticancer Drug Discovery Screen, a procedure adopted by the National Cancer Institute (NCI, USA), was employed, which uses the protein-binding dye sulforhodamine B to assess cell growth [27]. The dose–response curves were obtained and, in each case, the concentration of the compound that inhibits 50% of the net cell growth (IC_{50}) was calculated [27]. The above-mentioned three cancer cell lines were selected as our compounds are electron-rich systems substituted with electronegative groups and many reports from previous work [28-31] used such cell lines together with the use of doxorubicin, which was shown to be the best positive control against these three cell lines.

TABLE 3. Effect of the Synthesized Compounds on the Growth of Human Tumor Cell Lines

Compound	Inhibitory concentration IC_{50}, μM		
	MCF-7	NCI-H460	SF-268
1b	20.0 ± 2.2	26.3 ± 2.4	24 ± 4.6
1c	26.2 ± 2.9	24.8 ± 4.6	28.2 ± 12.6
1d	34.2 ± 12.6	33.7 ± 6.6	44.2 ± 8.2
1e	22.2 ± 4.5	22.8 ± 4.2	18.0 ± 4.4
2a	10.0 ± 0.6	8.4 ± 2.4	8.8 ± 4.8
2b	8.0 ± 4.2	6.3 ± 2.6	8.0 ± 1.8
3a	0.2 ± 0.09	0.8 ± 0.08	0.2 ± 0.06
3b	30.2 ± 10.9	22.7 ± 2.8	40.2 ± 6.0
4	0.02 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
6a	2.2 ± 0.8	4.6 ± 0.4	1.2 ± 0.8
6b	30.0 ± 2.5	22.0 ± 4.6	18.5 ± 2.8
6c	10.0 ± 0.8	8.3 ± 2.8	16.5 ± 4.0
6d	30.4 ± 2.8	20.1 ± 4.6	36.3 ± 4.5
6e	0.01 ± 0.008	0.01 ± 0.006	0.08 ± 0.08
6f	77.8 ± 10.0	64.2 ± 8.4	70.2 ± 12.6
7a	0.4 ± 0.1	0.2 ± 0.01	0.1 ± 0.02
7b	14.2 ± 8.2	10.0 ± 2.6	10.2 ± 4.8
9a	4.2 ± 0.8	2.6 ± 0.4	4.2 ± 1.8
9b	32.0 ± 2.5	24.0 ± 4.6	26.5 ± 2.8
9c	0.01 ± 0.008	0.01 ± 0.006	0.08 ± 0.08
10a	10.0 ± 0.8	8.3 ± 2.8	16.5 ± 4.0
10b	44.4 ± 6.8	26.1 ± 2.6	34.3 ± 2.5
11a	70.8 ± 10.0	66.2 ± 8.4	74.2 ± 12.6
11b	10.2 ± 8.2	8.0 ± 2.6	6.2 ± 2.8
15	0.4 ± 0.1	0.2 ± 0.01	0.1 ± 0.02
16a	20.4 ± 2.8	20.1 ± 0.6	36.3 ± 0.5
16b	0.04 ± 0.008	0.02 ± 0.006	0.06 ± 0.08
16c	30.8 ± 10.0	22.2 ± 8.4	12.2 ± 4.6
16d	8.2 ± 2.2	6.0 ± 1.6	8.2 ± 2.8
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Some selected compounds were also evaluated against three normal cell lines: human fibroblast (WI-38), normal prostate epithelial cells (PrEC), and normal colon mucosal (NCM 460) cells. To further characterize the possible differential effects of the obtained compounds on tumor and normal cells, we compared cell viability (scored as membrane integrity by the trypan blue exclusion assay) [32] after treatment with the synthesized compounds.

The normal PrEC cells showed minimal loss of viability up to at least 25 μM of the tested compound (i.e., about 75×IC_{50}) even after a 24 h continuous treatment (Table 4). Other normal cell lines showed similar marginal decreases in cell viability.

The results show that all compounds were able to inhibit the growth of both human tumor and normal cell lines in a dose-dependent manner (Table 3, 4). Although in most cases the growth inhibiting effect was moderate, a more pronounced effect was found with compounds **3a**, **4**, **6e**, **7a**, **9c**, **15**, and **16b** where such

TABLE 4. Effect of the Obtained Compounds on the Growth of Normal Cell Lines

Compound	Inhibitory concentration IC_{50}, μM		
	WI-38	PrEC	NCM 460
2a	3.11 ± 0.08	2.81 ± 0.70	4.21 ± 1.12
2b	0.18 ± 0.08	0.67 ± 0.02	0.24 ± 0.02
3a	0.14 ± 0.006	0.03 ± 0.002	0.04 ± 0.006
3b	6.33 ± 1.13	4.42 ± 0.9	2.28 ± 0.4
4	0.06 ± 0.003	0.08 ± 0.005	0.01 ± 0.004
6a	0.36 ± 0.02	0.22 ± 0.02	0.42 ± 0.01
6e	0.18 ± 0.03	0.29 ± 0.01	0.49 ± 0.004
7a	0.88 ± 0.01	1.33 ± 0.09	1.68 ± 0.03
9c	0.18 ± 0.04	0.13 ± 0.02	0.28 ± 0.01
15	0.21 ± 0.01	0.38 ± 0.05	0.49 ± 0.08
16a	3.47 ± 0.28	5.45 ± 2.03	5.09 ± 1.74
16b	0.18 ± 0.03	0.17 ± 0.04	0.28 ± 0.06

compounds showed the best results, exhibiting submicromolar inhibitory effect against the three tumors, but also against normal cell lines. Compounds **4**, **6e** and **9c** showed higher inhibitory effect towards the tumor cell lines than the reference control doxorubicin. However, only compound **7a** showed selective inhibition of the tumor cell lines.

In summary, a series of new 2-substituted 4,5,6,7-tetrahydro-1-benzothiophene derivatives was synthesized, an *in vitro* cell viability assay was employed to investigate the inhibition effect of 31 compounds against three tumor cell lines, and eleven selected compounds were also evaluated against normal cell lines. It was found that some of the compounds achieved promising cytotoxicity with IC_{50} values lower than 5 μM against some cancer cell lines.

EXPERIMENTAL

IR spectra were recorded in KBr pellets on a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer at 200 MHz in DMSO-d_6 using TMS as internal standard. Mass spectra were recorded with a Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. Elemental analysis data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt. Antitumor activity evaluation of the newly synthesized products was performed by a research group at the National Research Center (Medicinal Section) and the National Cancer Institute at Cairo University. Fetal bovine serum and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, and sulforhodamine B were from Sigma Chemical Co. (Saint Louis, MO, USA). Stock solutions of all compounds were prepared in DMSO and kept at -20°C. Appropriate dilutions of the compounds were freshly prepared just prior to assays. The final concentrations of DMSO did not interfere with the cell growth. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). WI-38 and PrEC cells were purchased from the American Type Culture, and NCM 460 cells were obtained from In Cell Corporation LLC. Gen-Probe kits were obtained from Clonetics.

***N*-(3-Cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-3-oxobutanamide (2a) and Ethyl 2-[(3-oxobutanoyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (2b) (General Method)**. Ethyl acetoacetate (13.0 g, 0.1 mol) was heated to 140°C, then compound **1a** (17.8 g, 0.1 mol) or **1b** (22.5 g, 0.1 mol) was added with continuous heating until the temperature reached 125°C, and the whole reaction mixture was heated under reflux for 20 min, then left to cool. The formed solid product was triturated with ethanol and collected by filtration.

2-Benzylidene-*N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-3-oxobutanamide (3a) and Ethyl 2-(2-Benzylidene-3-oxobutanamido)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (3b) (General Method). Benzaldehyde (1.06 g, 0.01 mol) was added to a solution of compound **2a** (2.62 g, 0.01 mol) or **2b** (3.09 g, 0.01 mol) in 1,4-dioxane (40 ml) containing piperidine (0.5 ml). The reaction mixture was heated under reflux for 2 h, then poured onto ice/water containing a few drops of hydrochloric acid to reach pH 6. The solid product was collected by filtration.

***N*-(3-Cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-2-(2-hydroxybenzylidene)-3-oxobutanamide (4)**. Salicylaldehyde (1.22 g, 0.01 mol) was added to a solution of compound **2a** (2.62 g, 0.01 mol) in 1,4-dioxane (40 ml) containing piperidine (0.5 ml). The reaction mixture was heated under reflux for 3 h, then worked up as above.

2-(2-Arylhydrazinylidene)-*N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-3-oxobutanamides 6a-c and Ethyl 2-[2-(2-Arylhydrazinylidene)-3-oxobutanamido]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylates 6d-f (General Method). A solution of the corresponding diazonium salt **5a-c** (0.01 mol), prepared by adding sodium nitrite solution (0.70 g, 0.01 mol) to a stirred cold solution of the corresponding aniline (0.01 mol) in concentrated hydrochloric acid (20 ml), was added with stirring to a cold (0-5°C) solution of compound **2a** (2.62 g, 0.01 mol) or **2b** (3.09 g, 0.01 mol) in ethanol (40 ml) containing 5% sodium hydroxide (5 ml). The reaction mixture was maintained at room temperature for 1 h, and the formed solid product was collected by filtration.

3-[2-(Cyanoacetyl)hydrazinylidene)]-*N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)butanamide (7a) and Ethyl 2-{3-[2-(Cyanoacetyl)hydrazinylidene]butanamido}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (7b) (General Method). Cyanoacetylhydrazine (1.0 g, 0.01 mol) was added to a solution of either compound **2a** (2.62 g, 0.01 mol) or **2b** (3.09 g, 0.01 mol) in 1,4-dioxane (40 ml). The reaction mixture was heated under reflux for 2 h, then concentrated under vacuum. The residue was triturated with ethanol, and the formed solid product was collected by filtration.

2-Amino-1-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-4-methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (9a) and Ethyl 2-Amino-1-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-4-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (9b) (General Method). Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added to a solution of compound **2a** (2.62 g, 0.01 mol) in 1,4-dioxane (40 ml) containing triethylamine (0.50 ml). The reaction mixture was heated under reflux for 4 h, then poured onto ice/water containing 18 M hydrochloric acid (0.50 ml) to reach pH 6, and the formed solid product was collected by filtration.

Ethyl 2-(6-Amino-5-cyano-4-methyl-2-oxopyridin-1(2*H*)-yl)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (9c). Malononitrile (0.66 g, 0.01 mol) was added to a solution of compound **2b** (3.09 g, 0.01 mol) in 1,4-dioxane (40 ml) containing triethylamine (0.50 ml). The reaction mixture was heated under reflux for 6 h, then poured onto ice/water containing hydrochloric acid (0.5 ml), and the formed solid product was collected by filtration.

4,4-Dicyano-*N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-3-methylbut-3-enamide (10a) and Ethyl 2-Cyano-5-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-ylamino)-3-methyl-5-oxopent-2-enoate (10b) (General Method). Either malononitrile (0.66 g, 0.01 mol) or ethyl cycanoacetate (1.13 g, 0.01 mol) was added to a dry solid compound **2a** (2.62 g, 0.01 mol). Ammonium acetate (0.50 g) was added to the reaction mixture. The reaction mixture was heated in an oil bath at 120°C for 15 min, then left to cool. The residue was triturated with ethanol, and the formed solid product was collected by filtration.

2-(3-Acetyl-4,6-dimethyl-2-oxopyridin-1(2*H*)-yl)-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile (11a) and Ethyl 2-(3-Acetyl-4,6-dimethyl-2-oxopyridin-1(2*H*)-yl)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (11b) (General Method). Acetylacetone (1.0 g, 0.01 mol) was added to a solution of compound

2a (2.62 g, 0.01 mol) or **2b** (3.09 g,0.01 mol) in 1,4-dioxane (40 ml). The whole reaction mixture was heated under reflux for 8 h, then concentrated under vacuum. The residue was triturated with ethanol, and the formed solid product was collected by filtration.

***N*-(3-Cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-2-(4-hydroxy-3-phenylthiazol-2(3*H*)-ylidene)-3-oxobutanamide (15), 5-Acetyl-*N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-4-methyl-2-(phenylamino)thiophene-3-carboxamide (16a), and Ethyl 2-[5-Acyl-4-methyl-2-(phenylamino)thiophene-3-carboxamido]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylates 16b-d (General Method)**. Potassium hydroxide (0.56 g, 0.01 mol) and then phenylisothiocyanate (1.30 g, 0.01 mol) were added to a solution of compound **2a** (2.62 g, 0.01 mol) or **2b** (3.09 g, 0.01 mol) in dimethyl formamide (30 ml). The reaction mixture was stirred at room temperature overnight. On the next day, the corresponding α-halocarbonyl compound **13a**, **13b** (with **2b** only), or **13c** (0.01 mol) was added with continuous stirring overnight at room temperature. The reaction mixture was poured onto ice/water containing a few drops of 18 M hydrochloric acid (0.50 ml) to reach pH 6. The solid product formed in each case was collected by filtration.

Tumor Cell Cultures. The human tumor cells grow as a monolayer and were routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 μM glutamine, and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/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 MCF-7 and SF-268 and 0.75×10^4 cells/ml for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Normal Cell Cultures. All cell lines were tested regularly for *Mycoplasma* contamination by the DNA hybridization method using a Gen-Probe kit [33].

Cell Growth Assay. Exponentially growing cells in 96-well plates were exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period, adherent cells were fixed, washed, and stained with sulforhodamine B [33]. The bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Winooski, Vermont, USA).

REFERENCES

1. J. W. Janetka, L. Almeida, S. Ashwell, P. J. Brassil, K. Daly, C. Deng, T. Gero, R. E. Glynn, C. L. Horn, S. Ioannidis, P. Lyne, N. J. Newcombe, V. B. Oza, M. Pass, S. K. Springer, M. Su, D. Toader, M. M. Vasbinder, D. Yu, Y. Yu, and S. D. Zabludoff, *Bioorg. Med. Chem. Lett.*, **18**, 4242 (2008).
2. D.Ye, Y. Zhang, F. Wang, M. Zheng, X. Zhang, X. Luo, X. Shen, H. Jiang, and H. Liu, *Bioorg. Med. Chem.*, **18**, 1773 (2010).
3. J. Malmström, J. Viklund, C. Slivo, A. Costa, M. Maudet, C. Sandelin, G. Hiller, L. L. Olsson, A. Aagaard, S. Geschwindner, Y. Xue, and M. Vasänge, *Bioorg. Med. Chem. Lett.*, **22**, 5919 (2012).
4. K. A. Emmitte, G. M. Adjebang, C. W. Andrews, J. G. B. Alberti, R. Bambal, S. D. Chamberlain, R. G. Davis-Ward, H. D. Dickson, D. F. Hassler, K. R. Hornberger, J. R. Jackson, K. W. Kuntz, T. J. Lansing, R. A. Mook, Jr., K. E. Nailor, M. A. Pobanz, S. C. Smith, C.-M. Sung, and M. Cheung, *Bioorg. Med. Chem. Lett.*, **19**, 1694 (2009).
5. C.-H. Wu, M. S. Coumar, C.-Y. Chu, W.-H. Lin, Y.-R. Chen, C.-T. Chen, H.-Y. Shiao, S. Rafi, S. Y. Wang, H. Hsu, C.-H. Chen, C.-Y. Chang, T.-Y. Chang, T.-W. Lien, M.-Y. Fang, K.-C. Yeh, C.-P. Chen, T.-K. Yeh, S.-H. Hsieh, J. T.-A. Hsu, C.-C. Liao, Y.-S. Chao, and H.-P. Hsieh, *J. Med. Chem.*, **53**, 7316 (2010).

6. L. D. Jennings, S. L. Kincaid, Y. D. Wang, G. Krishnamurthy, C. F. Beyer, J. P. McGinnis, M. Miranda, C. M. Discafani, and S. K. Rabindran, *Bioorg. Med. Chem. Lett.*, **15**, 4731 (2005).
7. R. Romagnoli, P. G. Baraldi, M. K. Jung, M. A. Iaconinoto, M. D. Carrion, V. Remusat, D. Preti, M. A. Tabrizi, F. Francesca, E. D. Clercq, J. Balzarini, and E. Hamel, *Bioorg. Med. Chem. Lett.*, **15**, 4048 (2005).
8. A. Nakhi, R. Adepu, D. Rambabu, R. Kishore, G. R. Vanaja, A. M. Kalle, and M. Pal, *Bioorg. Med. Chem. Lett.*, **22**, 4418 (2012).
9. M. S. Al-Said, M. S. Bashandy, S. I. Al-qasoumi, and M. M. Ghorab, *Eur. J. Med. Chem.*, **46**, 137 (2011).
10. H. Z. Shams, R. M. Mohareb, M. H. Helal, and A. E. Mahmoud, *Molecules*, **16**, 52 (2011).
11. S. V. Gupta, K. Baheti, R. Bora, D. Dekhane, M. Chhabría, M. Shingare, S. Pawar, C. J. Shishoo, and S. N. Thore, *Eur. J. Med. Chem.*, **44**, 4721 (2009).
12. B. V. Ashalatha, B. Narayana, K. V. Raj, and N. S. Kumari, *Eur. J. Med. Chem.*, **42**, 719 (2007).
13. E. Campaigne, in: *Comprehensive Heterocyclic Chemistry*, Vol. 4, (1984), p. 863.
14. G. Bose, K. Bracht, P. J. Bednarski, M. Lalk, P. Langer, *Bioorg Med. Chem.*, **14**, 4694 (2006).
15. H. Behbehani, H. M. Ibrahim, S. Makhseed, M. H. Elnagdi, and H. Mahmoud, *Eur. J. Med. Chem.*, **52**, 51 (2012).
16. L. D. Jennings, S. L. Kincaid, Y. D. Wang, G. Krishnamurthy, C. F. Beyer, J. P. McGinnis, M. Miranda, C. M. Discafani, and S. K. Rabindran, *Bioorg. Med. Chem. Lett.*, **15**, 4731 (2005).
17. K. Gewald, E. Schinke, and H. Böttcher, *Chem. Ber.*, **99**, 94 (1966).
18. K. Gewald, *Z. Chem.*, **2**, 305 (1962).
19. G. Nikolakopoulos, H. Figler, J. Linden, and P. J. Scammells, *Bioorg. Med. Chem.*, **14**, 2358 (2006).
20. A. E. E. Amr, M. H. Sherif, M. G. Assy, M. A. Al-Omar, and I. Ragab, *Eur. J. Med. Chem.*, **45**, 5935 (2010).
21. A. Habashi, N. S. Ibrahim, R. M. Mohareb, and S. M. Fahmy, *Liebigs Ann. Chem.*, 1632 (1986).
22. M. A. Gouda, M. A. Berghot, G. E. Abd El-Ghani, and A. M. Khalil, *Eur. J. Med. Chem.*, **45**, 1338 (2010).
23. A. A. Fadda, E. Abdel-Latif, and R. E. El-Mekawy, *Eur. J. Med. Chem.*, **44**, 1250 (2009).
24. R. M. Mohareb, S. El-Kousy, and A. M. El-Torgoman, *Collect. Czech. Chem. Commun.*, **57**, 1747 (1992).
25. R. M. Mohareb and F. El-Omran, *Steroids*, **77**, 1551 (2012).
26. A. M. Khalil, M. A. Berghot, and M. A. Gouda, *Eur. J. Med. Chem.*, **44**, 4434 (2009).
27. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, and J. M. Boyd, *J. Natl. Cancer Inst.*, **83**, 757 (1991).
28. R. M. Mohareb and H. E. Moustafa, *Acta Pharm.*, **61**, 51 (2011).
29. R. M. Mohareb and J. Schatz, *Bioorg. Med. Chem.*, **19**, 2707 (2011).
30. J. Benites, J. A. Valderrama, F. Rivera, L. Rojo, N. Campos, M. Pedro, and M. S. J. Nascimento, *Bioorg. Med. Chem.*, **16**, 862 (2008).
31. N. A. Monteiro-Riviere, A. O. Inman, and L. W. Zhang, *Toxicol. Appl. Pharm.*, **234**, 222 (2009).
32. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenny, and M. R. Boyd, *J. Natl. Cancer Inst.*, **82**, 1107 (1990).
33. J. M. Woynarowski, C. Napier, S. K. Koester, S. F. Chen, D. A.Troyer, W. Chapman, and J. R. MacDonald, *Biochem. Pharmacol.*, **54**, 1181 (1997).