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Antitumor Activity of Novel Pyridine, Thiophene and Thiazole Derivatives

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2-Cyano-*N*′-[1-(2,5-dimethoxyphenyl)]ethylideneacetohydrazide **1** was obtained via reaction of cyanoacetic acid hydrazide with 2,5-dimethoxyacetophenone. A number of novel pyridines **2a-j**, **3**, **4**, thiophenes **5-9** and thiazoles **10-12** were prepared by using the hydrazide-hydrazone derivative **1** as a starting material. The structure of the newly synthesized compounds was characterized by elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. All the target compounds were subjected to *in vitro* antitumor activity against Ehrlich Ascites Carcinoma (EAC) cells. Compounds **2j** and **6** showed a higher activity with IC_{50} values (54.54, 61.57 μM), 8 when compared with a reference drug IC_{50} value (68.99 μM), while compound **5** is nearly as active as Doxorubicin (CAS 23214-92-8).

Key words: Dihydropyridine, Thiophene, Thiazole, Antitumor activity

INTRODUCTION

A large number of hydrazone and hydrazide-hydrazone derivatives have been found to exhibit a wide variety of pharmacological especially anticancer activity (Krasnaya, 2004; Xia et al., 2008; Liu et al., 2009; Zheng et al., 2009; Pavan et al., 2010). Furthermore, it is well known that the Pirfenidone (PFD) (**I**), 5-methyl-1-phenyl-2(1H) pyridine is an agent with demonstrated antifibrotic activity in several organs in experimental animals, including the lung, kidney and uterus. A phase II clinical study showed PFD to be a promising agent for treatment of idiopathic pulmonary fibrosis initiated in mice treated with cyclophosphamide (Kehrer and Margolin, 1997; Card et al., 2003). Also Milrinone WIN 47203 (**II**) and their analogues are cardiotonic agents for the treatment of heart failure (Pastelin et al., 1983), some 2-pyridones are also reported to possess antitumor activity (French et al., 1974; Srivastava and Robins, 1983; Liu et al., 1992). Additionally, several types of benzothiophene (**III**) and their derivatives were reported to possess cytotoxicity (Pinney et al., 1999; Romagnoli et al., 2006; Romagnoli et al., 2010). On the other hand, Tiazofurin (**IV**) is a high-priority candidate for clinical trials with potential importance for treatment of the lung and metastases (Robins et al., 1982; Tricot et al., 1989; Weber et al., 2003). Based on the above and as a continuation of our work on the syntheses of novel anticancer agents (Ghorab et al., 2009, 2010, 2011; Al-Said et al., 2010; Shaaban et al., 2010), we reported the syntheses of a new series of 2-pyridones, thiophenes, and thiazoles as analogs to the biologically active compounds (**I-IV**).

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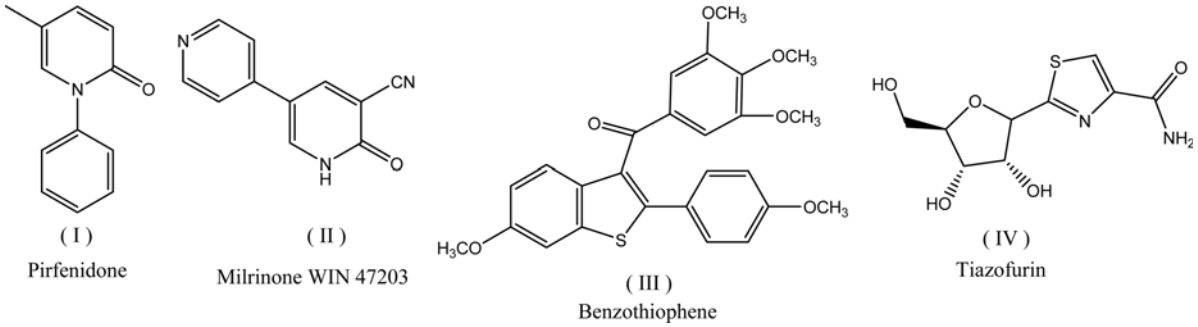
MATERIALS AND METHODS

Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck) were used for thin layer chromatography, and dichloromethane/methanol (9.5:0.5 mL) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded in KBr using IR-470 Shimadzu spectrometer (Shimadzu). ^{1}H-NMR spectra (in DMSO-d_6) were re-



corded on Bruker Ac-300 ultra shield NMR spectrometer (Bruker, δ ppm at 300 MHz, using TMS as internal standard). Electron impact Mass Spectra were recorded on a Shimadzu Gc-Ms-Qp 5000 instrument (Shimadzu). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus). All compounds were within ± 0.4% of the theoretical values.

Cyano-*N'*-[1-(2,5-dimethoxyphenyl)ethylidene]acetohydrazide (1)

A mixture of 2-cyanoacetohydrazide (1.0 g, 0.01 mol) and 2,5-dimethoxyacetophenone (1.8 g, 0.01 mol) in 1,4-dioxane (20 mL) was refluxed for 2 h. The reaction mixture was cooled and the solid product formed upon pouring it onto the ice which was crystallized from the ethanol. Yield, 90%; m.p. 71-73°C; IR (KBr, cm^{-1}): 3209 (NH), 3038 (CH arom.), 2961, 2836 (CH aliph.), 2258 (C≡N), 1683 (C=O), 1634 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.2 [s, 3H, CH_3], 3.79, 3.80 [2s, 6H, $2OCH_3$], 4.1 [s, 2H, CH_2], 6.9-7.9 [m, 3H, Ar-H], 10.9 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 17.7, 24.8, 55.4 (2), 113.2, 114.9, 115.6, 116.0, 119.5, 152.8, 154.9, 165.6, 198.4. MS m/z (%): 261 [M^+] (21.3), 163 (100). Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.50; H, 5.40; N, 15.70.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl)ethylideneamino]-6-oxo-4-(substituted aryl)-1,6-dihydropyridine-3-carboxylate (2a-j)

To a solution of compound **1** (2.61 g, 0.01 mol), in 1,4-dioxane (30 mL) containing triethylamine (1 mL) and ethylbenzalacetate derivatives (0.01 mol) was added. The reaction mixture was refluxed for 4 h, which was then poured onto ice water and the obtained solid was recrystallized from dioxane to give (**2a-j**).

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl)ethylideneamino]-6-oxo-4-*P*-tolyl-1,6-dihydropyridine-3-carboxylate (2a)

Yield, 69%; m.p. 260-262°C; IR (KBr, cm^{-1}): 3447, 3320 (NH_2), 3092 (CH arom.), 2939, 2835 (CH aliph.), 2217 (C≡N), 1710, 1685 (2C=O), 1603 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3], 2.2 [s, 3H, CH_3], 2.4 [s, 3H, CH_3 tolyl], 3.78, 3.80 [2s, 6H, $2OCH_3$], 4.4 [q, 2H, CH_2 ester], 6.7 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.4 [m, 7H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 13.7 (2), 25.3, 54.6 (2), 62.7, 92.4, 113.6, 114.1, 115.4, 116.3, 117.9, 119.3, 127.1 (2), 128.0 (2), 129.9, 139.0, 154.6, 155.2, 156.0, 168.5, 168.9, 169.9, 198.6. Anal. Calcd for $C_{26}H_{26}N_4O_5$: C, 65.81; H, 5.52; N, 11.81. Found: C, 65.50; H, 5.20; N, 11.60.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl)ethylideneamino]-6-oxo-4-(2-hydroxyphenyl)-1,6-dihydropyridine-3-carboxylate (2b)

Yield, 72%; m.p. 150-152°C; IR (KBr, cm^{-1}): 3446 (br, OH), 3380, 3250 (NH_2), 3074 (CH arom.), 2940, 2834 (CH aliph.), 2220 (C≡N), 1743, 1680 (2C=O), 1610 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.2 [t, 3H, CH_3 ester], 2.1 [s, 3H, CH_3], 3.72, 3.79 [2s, 6H, $2OCH_3$], 4.3 [q, 2H, CH_2 ester], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.0 [m, 7H, Ar-H], 13.9 [s, 1H, OH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 17.7, 55.4, 66.3, 73.1, 113.9, 114.8, 115.6, 116.0, 117.9, 119.4, 121.7, 122.9, 128.5, 129.2, 151.2, 151.4, 152.7, 155.5, 162.0, 163.7, 164.6, 198.4. MS m/z (%): 467 [M^+] (5.3), 40 (100). Anal. Calcd for $C_{25}H_{24}N_4O_6$: C, 63.02; H, 5.08; N, 11.76. Found: C, 62.70; H, 5.30; N, 11.50.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl)ethylideneamino]-6-oxo-4-(4-hydroxyphenyl)-1,6-dihydropyridine-3-carboxylate (2c)

Yield, 68%; m.p. 157-159°C; IR (KBr, cm^{-1}): 3322 (br, OH), 3210, 3160 (NH_2), 3025 (CH arom.), 2940, 2839 (CH aliph.), 2228 (C≡N), 1714, 1654 (2C=O), 1588 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ester], 2.2 [s, 3H, CH_3], 3.71, 3.75 [2s, 6H, $2OCH_3$], 4.2 [q, 2H, CH_2 ester], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.9-8.1 [m, 7H, Ar-H], 10.8 [s, 1H, OH]. ^{13}C-NMR (DMSO-d_6): 13.9, 55.9, 61.8, 97.0, 114.5, 115.3, 115.6, 116.3, 116.9, 117.2, 123.1, 129.2, 152.1, 152.8, 153.5, 154.5, 161.8, 162.2, 162.5, 162.8. MS m/z (%): 476 [M^+] (10.9), 172 (100). Anal. Calcd for $C_{25}H_{24}N_4O_6$: C, 63.02; H, 5.08; N, 11.76. Found: C, 63.30; H, 4.80; N, 12.10.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-(2-methoxyphenyl)-1,6-dihydropyridine-3-carboxylate (2d)
Yield, 66%; m.p. 126-128°C; IR (KBr, cm^{-1}): 3408, 3320 (NH_2), 3046 (CH arom.), 2935, 2836 (CH aliph.), 2218 (C≡N), 1718, 1670 (2C=O), 1603 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ester], 2.3 [s, 3H, CH_3], 3.72, 3.77, 3.8 [3s, 9H, 3OCH_3], 4.3 [q, 2H, CH_2 ester], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.0 [m, 7H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 13.9, 55.7, 56.1, 62.0, 81.9, 114.2, 114.4, 114.7, 114.9, 116.2, 116.7, 117.0, 119.5, 123.9, 128.3, 129.5, 151.2, 152.8, 153.5, 154.4, 162.3, 162.6, 162.9, 163.5. MS m/z (%): 490 [M^+] (10.8), 48 (100). Anal. Calcd for $C_{26}H_{26}N_4O_6$: C, 63.66; H, 5.34; N, 11.42. Found: C, 63.30; H, 5.60; N, 11.10.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-(4-methoxyphenyl)-1,6-dihydropyridine-3-carboxylate (2e)
Yield, 76%; m.p. 110-112°C; IR (KBr, cm^{-1}): 3420, 3370 (NH_2), 3038 (CH arom.), 2935, 2836 (CH aliph.), 2218 (C≡N), 1705, 1684 (2C=O), 1603 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ester], 2.3 [s, 3H, CH_3], 3.72, 3.75, 3.8 [3s, 9H, 3OCH_3], 4.3 [q, 2H, CH_2 ester], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.0 [m, 7H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 13.7, 55.6, 62.9, 81.8, 113.9, 114.7, 115.2, 116.1, 116.7, 117.0, 119.4, 124.6, 128.0, 152.8, 153.5, 154.3, 159.4, 162.3, 162.9, 163.5, 164.1. MS m/z (%): 490 [M^+] (4.9), 148 (100). Anal. Calcd for $C_{26}H_{26}N_4O_6$: C, 63.66; H, 5.34; N, 11.42. Found: C, 63.40; H, 5.10; N, 11.70.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-styryl-1,6-dihydropyridine-3-carboxylate (2f)
Yield, 63%; m.p. 136-138°C; IR (KBr, cm^{-1}): 3446, 3310 (NH_2), 3049 (CH arom.), 2939, 2835 (CH aliph.), 2217 (C≡N), 1740, 1699 (2C=O), 1609 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ester], 2.3 [s, 3H, CH_3], 3.73, 3.78 [2s, 6H, 2OCH_3], 4.3 [q, 2H, CH_2 ester], 6.7 [s, 2H, NH_2 exchangeable with D_2O], 6.8, 6.9 [2d, 2H, CH = CH, J = 7.1, 7.6 Hz], 7.0-7.9 [m, 8H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 14.4, 55.8, 62.9, 93.4, 103.2, 114.5, 114.8, 115.1, 116.2, 119.5, 127.1, 127.6, 128.6, 129.1, 134.6, 138.9, 151.1, 152.8, 155.5, 155.8, 158.9, 161.7, 165.6, 198.4. MS m/z (%): 486 [M^+] (5.3), 77 (100). Anal. Calcd for $C_{27}H_{26}N_4O_5$: C, 66.65; H, 5.39; N, 11.52. Found: C, 66.40; H, 5.70; N, 11.20.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-(benzo[d][1,3]dioxol-5-yl)-1,6-dihydropyridine-3-carboxylate (2g)
Yield, 59%; m.p. 170-172°C; IR (KBr, cm^{-1}): 3413, 3310 (NH_2), 3093 (CH arom.), 2915, 2839 (CH aliph.), 2216 (C≡N), 1724, 1686 (2C=O), 1616 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ethyl], 2.2 [s, 3H, CH_3], 3.75, 3.81 [2s, 6H, 2OCH_3], 4.3 [q, 2H, CH_2 ester], 6.1 [s, 2H, O-CH_2-O], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.0 [m, 6H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 13.9, 55.5, 62.1, 98.9, 102.3, 109.0, 113.4, 113.9, 114.3, 115.4, 116.0, 116.6, 116.9, 119.5, 126.2, 148.5, 151.0, 152.8, 153.5, 154.3, 154.7, 162.1, 190.8, 198.4. MS m/z (%): 504 [M^+] (9.6), 47 (100). Anal. Calcd for $C_{26}H_{24}N_4O_7$: C, 61.90; H, 4.80; N, 11.11. Found: C, 61.60; H, 4.40; N, 11.40.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl)ethylideneamino]-6-oxo-4-(3-nitrophenyl)-1,6-dihydropyridine-3-carboxylate (2h)
Yield, 65%; m.p. 178-180°C; IR (KBr, cm^{-1}): 3447, 3290 (NH_2), 3086 (CH arom.), 2940, 2836 (CH aliph.), 2207 (C≡N), 1742, 1699 (2C=O), 1636 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.2 [t, 3H, CH_3 ester], 2.2 [s, 3H, CH_3], 3.71, 3.78 [2s, 6H, 2OCH_3], 4.2 [q, 2H, CH_2 ester], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.1 [m, 7H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 14.9 (2), 56.4 (2), 63.7, 91.3, 113.6, 115.7, 116.5, 116.9, 118.0, 119.4, 121.6, 122.8, 128.1, 134.1, 135.7, 145.9, 154.6, 154.9, 156.4, 164.5, 167.0, 168.9, 198.2. MS m/z (%): 505 [M^+] (4.4), 40 (100). Anal. Calcd for $C_{25}H_{23}N_5O_7$: C, 59.40; H, 4.59; N, 13.85. Found: C, 59.70; H, 4.20; N, 13.50.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-(2,4-dichlorophenyl)-1,6-dihydropyridine-3-carboxylate (2i)
Yield, 76%; m.p. 95-97°C; IR (KBr, cm^{-1}): 3447, 3320 (NH_2), 3091 (CH arom.), 2940, 2835 (CH aliph.), 2218 (C≡N), 1745, 1696 (2C=O), 1585 (C=N), 751 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 1.2 [t, 3H, CH_3 ester], 2.2 [s, 3H, CH_3], 3.70, 3.79 [2s, 6H, 2OCH_3], 4.2 [q, 2H, CH_2 ester], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.7 [m, 6H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 14.3, 55.4, 66.3, 93.8, 114.0, 114.6, 115.2, 115.9, 116.1, 119.5, 127.5, 130.4, 132.7, 133.4, 134.3, 135.2, 151.9, 152.8, 153.2, 164.2, 165.7, 166.8, 198.4. MS m/z (%): 529 [M^+] (3.4), 47 (100). Anal. Calcd for $C_{25}H_{22}Cl_2N_4O_5$: C, 56.72; H, 4.19; N, 10.58. Found: C, 56.40; H, 4.50; N, 10.20.

Ethyl-2-amino-5-cyano-1-[1-(2,5-dimethoxyphenyl) ethylideneamino]-6-oxo-4-(2,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylate (2j)
Yield, 81%; m.p. > 300°C; IR (KBr, cm^{-1}): 3481, 3290 (NH_2), 3100 (CH arom.), 2941, 2835 (CH aliph.), 2208 (C≡N), 1701, 1683 (2C=O), 1593 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.3 [t, 3H, CH_3 ester], 2.2 [s, 3H, CH_3], 3.72, 3.75, 3.81, 3.86, 3.91 [5s, 15H, 5OCH_3], 4.2 [q, 2H, CH_2 ester], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 6.9-7.8

[m, 5H, Ar-H]. ^{13}C-NMR (DMSO-d_6): 14.0, 55.7, 56.1, 56.6, 61.8, 96.1, 107.7, 111.4, 113.0, 113.2, 114.4, 115.5, 116.9, 117.4, 117.7, 143.1, 151.3, 153.5, 154.8, 155.6, 156.0, 156.5, 158.3, 162.8, 186.7. MS m/z (%): 550 [M^+] (3.5), 48 (100). Anal. Calcd for $C_{28}H_{30}N_4O_8$: C, 61.08; H, 5.49; N, 10.18. Found: C, 61.40; H, 5.20; N, 9.80.

4-Amino-6-substituted-1-(1-(2,5-dimethoxyphenyl) ethylideneamino)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3,4)

A mixture of **1** (2.61 g, 0.01 mol) with either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) in dioxane (20 mL) containing triethylamine (1 mL) was refluxed for 6 h. The obtained solid was then recrystallized from ethanol to give **3** and **4**, respectively.

4,6-Diamino-1-(1-(2,5-dimethoxyphenyl)ethylideneamino)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3)

Yield, 82%; m.p. > 300°C; IR (KBr, cm^{-1}): 3410, 3331 (NH_2), 3100 (CH arom.), 2919, 2862 (CH aliph.), 2215 (C≡N), 1647 (C=O), 1575 (C=N). ^{1}H NMR (DMSO-d_6): δ 2.5 [s, 3H, CH_3], 3.5 [s, 6H, 2OCH_3], 4.1 [s, 3H, CH + NH_2 exchangeable with D_2O], 7.3-7.5 [m, 5H, Ar-H + NH_2]. ^{13}C-NMR (DMSO-d_6): 13.6, 56.8 (2), 72.0, 86.3, 113.6, 114.4, 115.9, 116.2, 119.0, 145.6, 154.8, 155.0, 166.4, 180.6, 198.0. MS m/z (%): 327 [M^+] (7.2), 44 (100). Anal. Calcd for $C_{16}H_{17}N_5O_3$: C, 58.17; H, 5.23; N, 21.39. Found: C, 57.80; H, 5.50; N, 21.70.

4-Amino-6-hydroxy-1-(1-(2,5-dimethoxyphenyl)ethylideneamino)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4)

Yield, 77%; m.p. 119-121°C; IR (KBr, cm^{-1}): 3460 (br, OH), 3216, 3190 (NH_2), 3032 (CH arom.), 2965, 2836 (CH aliph.), 2259 (C≡N), 1682 (C=O), 1631 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.2 [s, 3H, CH_3], 3.73, 3.76 [2s, 6H, 2OCH_3], 4.1 [s, 2H, NH_2 exchangeable with D_2O], 6.7 [s, 1H, CH], 6.9-7.2 [m, 3H, Ar-H], 10.9 [s, 1H, OH]. ^{13}C-NMR (DMSO-d_6): 13.7, 55.9, 61.8, 66.3, 114.9, 115.5, 115.9, 116.1, 119.4, 153.3, 154.9, 164.1, 164.2, 165.6, 198.3. MS m/z (%): 328 [M^+] (2.6), 47 (100). Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.20; H, 4.60; N, 16.70.

3,5-Diamino-4-cyano-*N′*-[1-(2,5-dimethoxyphenyl) ethylidene)thiophene-2-carbohydrazide (5) and Ethyl 2,4-diamino-5-[2-(1-(2,5-dimethoxyphenyl)-ethylidene)hydrazinecarbony)thiophene-3-carboxylate (6)

To a solution of **1** (2.61 g, 0.01 mol) and ethanol (30 mL) containing triethylamine (1 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) with elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was refluxed for 2 h, which was then poured onto ice water and the obtained solid was recrystallized from dioxane to give **5** and **6**, respectively.

5: Yield, 59%; m.p. 144-146°C; IR (KBr, cm^{-1}): 3390, 3328, 3220 (2NH_2), 3074 (CH arom.), 2932, 2838 (CH aliph.), 2211 (C≡N), 1636 (C=O), 1593 (C=N). ^{1}H-NMR (DMSO-d_6): δ 3.6 [s, 3H, CH_3], 3.72, 3.76 [2s, 6H, 2OCH_3], 6.4 [s, 2H, NH_2 exchangeable with D_2O], 6.8-7.1 [m, 5H, Ar-H + NH_2], 12.0 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 17.4, 55.7, 85.8, 113.9, 114.5, 115.8, 119.6, 122.2, 128.0, 150.5, 151.4, 152.6, 152.7, 164.5, 198.4. MS m/z (%): 359 [M^+] (7.7), 47 (100). Anal. Calcd for $C_{16}H_{17}N_5O_3S$: C, 53.47; H, 4.77; N, 19.49. Found: C, 53.10; H, 4.40; N, 19.20.

6: Yield, 63%; m.p. 148-150°C; IR (KBr, cm^{-1}): 3411, 3333, 3245 (2NH_2), 3100 (CH arom.), 2925, 2836 (CH aliph.), 1734, 1653 (2C=O), 1593 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.2 [t, 3H, CH_3 ester], 3.6 [s, 3H, CH_3], 3.70, 3.77 [2s, 6H, 2OCH_3], 4.2 [q, 2H, CH_2 ester], 5.9 [s, 2H, NH_2 exchangeable with D_2O], 6.9-7.4 [m, 5H, Ar-H + NH_2], 12.4 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): Anal. Calcd for $C_{18}H_{22}N_4O_5S$: C, 53.19; H, 5.46; N, 13.78. Found: C, 52.80; H, 5.10; N, 13.40.

3-Amino-*N′*-[1-(2,5-dimethoxyphenyl)ethylidene)-5,6-dihydro-4H-cyclopenta-[b]thiophene-2-carbohydrazide (7), 3-amino-*N′*[1-(2,5-dimethoxy-phenyl) ethylidene]-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carbohydrazide (8) and 3-amino-*N′*[1-(2,5-dimethoxyphenyl)ethylidene)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-2-carbohydrazide (9)

To a solution of **1** (2.61 g, 0.01 mol) in ethanol (30 mL) containing triethylamine (1 mL) with cyclopentanone or cyclohexanone, and/or cycloheptanone (0.01 mol) with elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was refluxed for 2 h, which was then poured onto ice water and the isolated products were recrystallized from dioxane to give **7-9**, respectively.

7: Yield, 59%; m.p. 126-128°C; IR (KBr, cm^{-1}): 3420, 3308, 3210 (NH, NH_2), 2938, 2833 (CH aliph.), 1669 (C=O), 1576 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.0-2.5 [m, 6H, 3CH_2 cyclo], 3.0 [s, 3H, CH_3], 3.71, 3.76 [2s, 6H, 2OCH_3], 5.2 [hump, 2H, NH_2 exchangeable with D_2O], 6.9-7.2 [m, 3H, Ar-H], 10.3 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 14.6, 25.4, 28.3, 34.6, 56.2 (2), 113.9, 114.6, 116.7, 118.4, 125.8, 140.3, 143.7, 145.2, 151.5, 152.9, 164.7, 198.0. MS m/z (%): 359 [M^+] (46.8), 43 (100). Anal. Calcd for $C_{18}H_{21}N_3O_3S$: C, 60.15; H, 5.89; N, 11.69. Found: C, 60.50; H, 5.50; N, 11.30.

8: Yield, 63%; m.p. 72-74°C; IR (KBr, cm^{-1}): 3370,

3332, 3260 (NH, NH_2), 3047 (CH arom.), 2930, 2833 (CH aliph.), 1653 (C=O), 1576 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.6-2.5 [m, 8H, $4CH_2$ cyclo], 3.6 [s, 3H, CH_3], 3.72, 3.76 [2s, 6H, $2OCH_3$], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.6 [m, 3H, Ar-H], 10.9 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 14.0, 23.9, 25.5, 26.8, 55.4, 113.9, 114.9, 119.4, 119.6, 127.1, 143.3, 151.0, 152.7, 154.3, 157.2, 170.3, 198.4. MS m/z (%): 373 [M^+] (7.6), 48 (100). Anal. Calcd for $C_{19}H_{23}N_3O_3S$: C, 61.10; H, 6.21; N, 11.25. Found: C, 61.50; H, 6.50; N, 11.60.

9: Yield, 65%; m.p. 101-103°C; IR (KBr, cm^{-1}): 3420, 3330, 3210 (NH, NH_2), 2929, 2833 (CH aliph.), 1684 (C=O), 1636 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.2-2.5 [m, 10H, $5CH_2$ cyclo], 3.6 [s, 3H, CH_3], 3.8 [s, 6H, $2OCH_3$], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.1-7.3 [m, 3H, Ar-H], 10.9 [s, 1H, NH]. ^{13}C-NMR (DMSO-d_6): 17.7, 23.6, 24.8, 29.8, 31.5, 55.4, 114.0, 114.9, 116.2, 119.5, 128.1, 132.1, 132.9, 149.6, 151.2, 152.8, 164.8, 198.4. MS m/z (%): 387 [M^+] (9.2), 63 (100). Anal. Calcd for $C_{20}H_{25}N_3O_3S$: C, 61.99; H, 6.50; N, 10.84. Found: C, 61.60; H, 6.20; N, 10.50.

4-Amino-*N′*-[1-(2,5-dimethoxyphenyl)ethylidene)]-3-substitutedphenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (10-12)

To a solution of compound **1** (2.61 g, 0.01 mol) in ethanol (20 mL) containing triethylamine (1 mL) and elemental sulfur (0.32 g, 0.01 mol), aryl isothiocyanate (0.01 mol) was added. The reaction mixture was refluxed for 3 h, and the solid that was obtained upon pouring onto ice water was recrystallized from dioxane to give **10-12**, respectively.

4-Amino-*N′*-[1-(2,5-dimethoxyphenyl)ethylidene)-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (10)

Yield, 87%; m.p. 240-242°C; IR (KBr, cm^{-1}): 3425, 3301, 3160 (NH, NH_2), 3091 (CH arom.), 2940, 2829 (CH aliph.), 1630 (C=O), 1592 (C=N), 1292 (C=S). ^{1}H-NMR (DMSO-d_6): δ 2.2 [s, 3H, CH_3], 3.78, 3.79 [2s, 6H, $2OCH_3$], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.6 [m, 8H, Ar-H], 10.6 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 17.6, 55.4, 80.2, 113.2, 113.3, 114.2, 115.6, 128.7, 128.9, 130.0, 134.8, 148.8, 151.3, 152.8, 153.9, 163.4, 190.3. MS m/z (%): 428 [M^+] (5.2), 47 (100). Anal. Calcd for $C_{20}H_{20}N_4O_3S_2$: C, 56.06; H, 4.70; N, 13.07. Found: C, 56.30; H, 4.40; N, 13.40.

4-Amino-3-(4-chlorophenyl)-*N′*-[1-(2,5-dimethoxyphenyl)ethylidene)-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (11)

Yield, 91%; m.p. 186-188°C; IR (KBr, cm^{-1}): 3440, 3285, 3164 (NH, NH_2), 3056 (CH arom.), 2940, 2836 (CH aliph.), 1644 (C=O), 1598 (C=N), 1234 (C=S), 724 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 2.2 [s, 3H, CH_3], 3.78, 3.79 [2s, 6H, $2OCH_3$], 6.96 [s, 2H, NH_2 exchangeable with D_2O], 6.97-7.6 [m, 7H, Ar-H], 10.6 [s, 1H, NH]. ^{13}C-NMR (DMSO-d_6): 17.6, 55.4, 80.2, 113.2, 114.2, 115.0, 115.6, 128.6, 131.0, 133.8, 134.7, 148.8, 151.3, 152.8, 153.9, 163.4, 190.2. MS m/z (%): 462 [M^+] (13.4), 66 (100). Anal. Calcd for $C_{20}H_{19}ClN_4O_3S_2$: C, 51.89; H, 4.14; N, 12.10. Found: C, 51.50; H, 4.40; N, 12.40.

4-Amino-3-(4-bromophenyl)-*N′*-[1-(2,5-dimethoxyphenyl)ethylidene)-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (12)

Yield, 85%; m.p. 190-192°C; IR (KBr, cm^{-1}): 3441, 3285, 3162 (NH, NH_2), 3094 (CH arom.), 2940, 2839 (CH aliph.), 1644 (C=O), 1250 (C=S). ^{1}H-NMR (DMSO-d_6): δ 2.2 [s, 3H, CH_3], 3.78, 3.79 [2s, 6H, $2OCH_3$], 6.96 [s, 2H, NH_2 exchangeable with D_2O], 6.98-7.8 [m, 7H, Ar-H], 10.6 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 17.6, 55.4, 80.2, 113.2, 114.2, 115.6, 123.4, 128.6, 131.3, 133.1, 134.2, 148.8, 151.3, 152.8, 153.8, 163.4, 190.2. MS m/z (%): 507 [M^+] (13.8), 64 (100). Anal. Calcd for $C_{20}H_{19}BrN_4O_3S_2$: C, 47.34; H, 3.77; N, 11.04. Found: C, 47.00; H, 3.40; N, 10.70.

Antitumor activity *(in vitro* study)

Reagents

Doxorubicin, the reference drug that was used in this study, is one of the most effective antitumor agents used to produce regressions in acute leukemia's, Hodgkin's disease and other lymphomas. The relationship between survival ratio and drug concentration was plotted to obtain the survival curve of the Ehrlich Ascites Carcenoma (EAC) cells. The response which was IC_{50} value (Table I) that corresponds to the compound concentration causing (50%) mortality in net cells.

Procedure

The Ehrlich Ascites Carcinoma (EAC) cells were obtained by needle aspiration of the ascetic from preinoculated mice under aseptic conditions (Uma Devi et al., 1999). Tumor cells suspension (2.5×10^6 per mL) was prepared in RPMI-1640 media. Tested compounds were prepared with various dilutions by dissolving: 100, 50, 25, 10 μg in DMSO (1 mL). In a set of sterile test tubes 0.8 mL RPMI-1640 media containing (glutamine, fetal calf serum as nutrient, streptomycin and penicillin) 0.1 mL of each of the tested compounds (corresponding to 100, 50, 25, 10 μg) were then 0.1 mL of tumor cell suspension (2.5×10^5) was added. The test tubes then incubated at 37°C for 2 h. Trypan blue exclusion test (Takemoto et al., 1982; Raffa et al., 2004) was carried out to calculate the percentage of

non-viable cells after 2 h of incubation (Brusick, 1984).

$$\% \text{ of non-viable cells} = \frac{\text{No. of non-viable cells}}{\text{Total No. of cells}} \times 100$$

The results of *in vitro* cytotoxic activity experiments are presented in Table I.

Biological testing (Animals, chemicals and facilities)

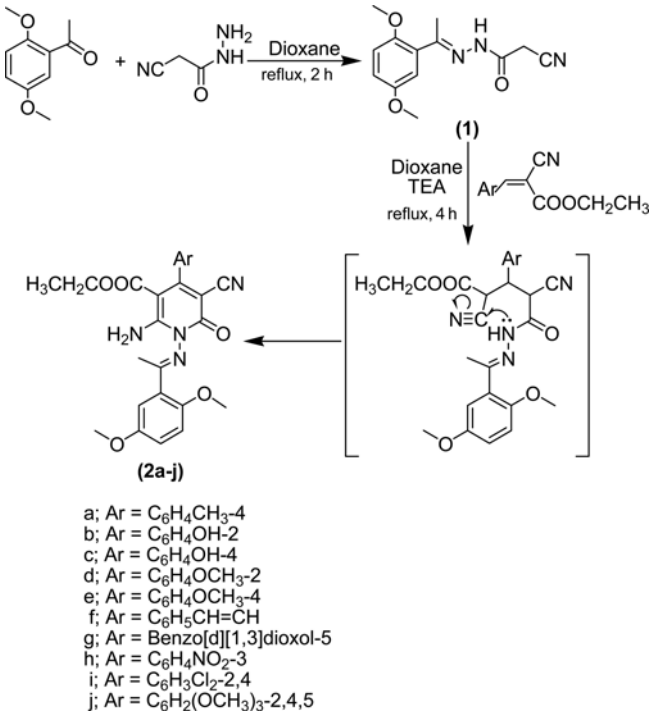
EAC cell line was maintained in female Swiss albino mice weighing 25-30 g (the holding company for biological products and vaccines. VACSERA) were housed at a constant temperature (24 ± 2°C) with alternating 12 h-light and dark cycles and was fed standard laboratory food (Milad Co) along with water *ad libitum*. All chemicals and reagents were of the highest grade commercially available. Animal house and biochemical have been made available by the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. Animal care and handling was according to the guidelines set by the world heath organization, Genneva, Switherland and was approved from the committee for animal care at NCRRT, AEA.

RESULTS AND DISCUSSION

Chemistry

The sequence of reactions followed in the synthesis of the target compounds is illustrated in (Schemes 1-3). The starting material 2-cyano-*N'*-[1-(2,5-dimethoxyphenyl)ethylideneacetohydrazide **1** was obtained via the reaction of cyanoacetic acid with 2,5-dimethoxyacetophenone (Scheme 1). The structure of hydrazide-hydrazone derivative **1** was confirmed by the elemental analysis, IR, ^{1}H-NMR and mass spectral data. IR spectrum showed the presence of bands at 3209 cm^{-1} (NH), 3038, 2258 cm^{-1} (C≡N), 1683 cm^{-1} (C=O). ^{1}H-NMR spectrum of **1** revealed signals at d 2.2 ppm corresponding to CH_3 group, a singlet at 4.1 ppm for CH_2 group, 3.79, 3.80 ppm for 2 OCH_3 groups. Mass spectrum of **1** showed a molecular ion peak m/z 261 (21.3), with a base peak that appeared at m/z 163 (100).

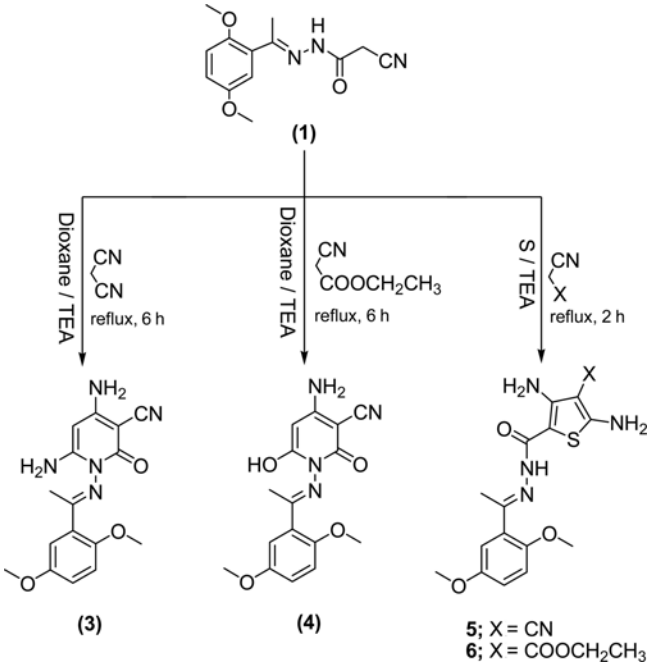
Evidence for the structure of compound **1** was obtained through studying its chemical reactivity via some chemical reactions. Thus, interaction of compound **1** with ethyl a-cyanocinnamate derivatives gave the 1,6-dihydropyridine derivatives **2a-j**. Structures of **2a-j** were based on analytical and spectral data. Thus, IR spectrum of **2a** showed bands at 3447, 3320 cm^{-1} (NH_2), 2217 cm^{-1} (C≡N), 1720, 1685 cm^{-1} (2C=O). ^{1}H-NMR spectrum of **2a** revealed a triplet at 1.3 ppm for CH_3 ester and a quartet at 4.4 ppm for CH_2 ester. IR



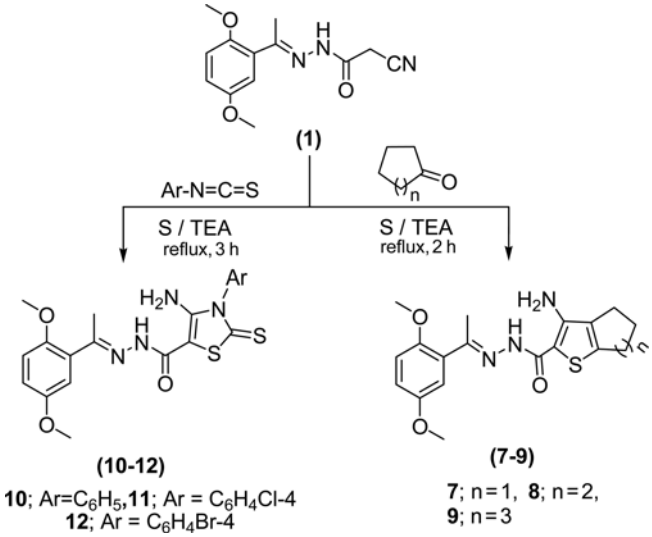
Scheme 1

spectrum of **2b** revealed bands at 3446 cm^{-1} (OH), 3380, 3250 cm^{-1} (NH_2), 2220 cm^{-1} (C≡N), 1743, 1680 cm^{-1} (2C=O). IR spectrum of **2c** showed bands at 3322 cm^{-1} (OH), 3210, 3160 cm^{-1} (NH_2), 2228 cm^{-1} (C≡N). Interaction of compound **1** with malononitrile and/or ethyl cyanoacetate furnished the corresponding 1,2-dihydropyridine-2-one derivatives **3** and **4** (Scheme 2). Analytical and spectral data are consistent with the proposed structures. IR spectrum of **3** showed bands at 3410, 3331, 3230 cm^{-1} (NH_2), 2215 cm^{-1} (C≡N), 1647 cm^{-1} (C=O). ^{1}H-NMR spectrum of **3** exhibited signals at 4.1, 7.5 ppm for ($2NH_2$) groups. Mass spectrum of **3** showed a molecular ion peak m/z at 327 [M$^+$] (6.9), with a base peak at m/z 44 (100). IR spectrum of **4** revealed bands at 3410 cm^{-1} (OH), 3240, 3216 cm^{-1} (NH_2), 2258 cm^{-1} (C≡N), 1682 cm^{-1} (C=O), ^{1}H-NMR spectrum of **4** revealed signals at 4.1 ppm for (NH_2) group and 10.9 ppm for (OH) group. Mass spectrum of **5** showed a molecular ion peak m/z at 328 [M$^+$] (2.5), with a base peak m/z at 47 (100).

On the other hand, the reaction of compound **1** with either malononitrile or ethyl cyanoacetate and elemental sulfur in ethanol containing triethylamine gave the corresponding thiophene derivatives **5** and **6**, respectively (Scheme 2). The reaction goes in parallel to the reported Gewald's thiophene synthesis (Mckibben et al., 1999). IR spectrum of **5** exhibited bands at 3380, 3328, 3210 cm^{-1} ($2NH_2$), 2211 cm^{-1} (C≡N), 1636 cm^{-1} (C=O). ^{1}H-NMR spectrum of **5** revealed signals at 6.4,



Scheme 2



Scheme 3

7.1 ppm for ($2NH_2$) groups. Mass spectrum of **5** revealed a molecular ion peak m/z at 359 [M^+] (7.6), with a base peak at 47 (100). IR spectrum of **6** showed bands at 3411, 3333, 3245 cm^{-1} ($2NH_2$), 1734, 1653 cm^{-1} (2C=O). ^{1}H-NMR spectrum of **6** showed signals at 1.2 ppm triplet for CH_3 ester and 4.2 ppm quartet for CH_2 ester. Mass spectrum of **6** revealed a molecular ion peak m/z at 374 [M^+] (3.5), with a base peak m/z at 62 (100). Also, the reaction of **1** with cyclopentanone or cyclohexanone, and/or cycloheptanone and elemental sulfur gave the corresponding thiophene derivatives **7-9**, respectively (Scheme 3). The formation of **7-9** took place according to the similar reported reactions of cyclohexanone with methylene reagents and elemental sulfur (Scrowston, 1981). IR spectrum of **7** showed bands at 3420, 3308, 3210 cm^{-1} (NH, NH_2), 1669 cm^{-1} (C=O). IR spectrum of **8** revealed bands at 3370, 3332, 3260 cm^{-1} (NH, NH_2), 1653 cm^{-1} (C=O). ^{1}H-NMR spectrum of **7** revealed singlet at 6.9 due to NH_2 group. Mass spectrum of **8** showed a molecular ion peak m/z at 373 [M^+] (7.7), with a base peak m/z at 48 (100).

Finally, the reaction of compound **1** with elemental sulfur and aryl isothiocyanate in ethanol containing triethylamine yielded the corresponding thiazole derivatives **10-12**, respectively (Scheme 3). The formation of the later products took place in parallel to the reported method (Tormos et al., 1992). Structure of compounds **10-12** was confirmed based on analytical and spectral data. IR spectrum of **10** exhibited bands at 3445, 3380, 3310 cm^{-1} (NH, NH_2), 1684 cm^{-1} (C=O), 1292 cm^{-1} (C=S). ^{1}H-NMR spectrum of **10** revealed signals at 6.9 ppm corresponding to NH_2 group. Mass spectrum of **10** revealed a molecular ion peak m/z at 428 [M^+] (5.2), with a base peak at 47 (100).

Antitumor activity

The relationship between the surviving fraction and compound concentration was plotted to obtain the survival curve of EAC cells. The response parameter calculated was the IC_{50} which corresponds to the compound concentration causing 50% mortality in net cells. The results of antitumor activity indicated that 1,6-dihydropyridine **2j** having 2,4,5-trimethoxyphenyl with cyano group at 4-position and thiophene **6** carrying o-amino ester with hydrazine carbonyl moiety at 5-position were found to exert the most powerful effect on EAC cells *in vitro* IC_{50} values (54.54 and 61.57 μM) compared with that of the positive control doxorubicin (CAS-23214-92-8) with IC_{50} value (68.99 μM). On the other hand, thiophene derivative **5** containing o-amino carbonitrile with hydrazine-carbonyl moiety at 5-position (IC_{50} value = 69.63 μm) is nearly as active as doxorubicin as positive control. Compounds **8**, **2h**, **2e**, **7**, **10**, **4** and **2g** showed to have moderate activity.

In conclusion, the present data showed that 1,6-dihydropyridine and thiophene nucleus exhibited promising *in vitro* cytotoxic activity against EAC cells, especially compounds **2j** containing 2,4,5-trimethoxyphenyl at 4-position and **6** carrying o-amino with hydrazine carbonyl at 5-position. Compounds **2j** and **6** exhibited the highest *in vitro* cytotoxic when compared with the other tested compounds and doxorubicin as a reference drug. Also, compound **5** is nearly as active as positive control.

Table I. *In vitro* cytotoxic activity of the newly synthesized compounds **1-12**

Compd. No.	Non-viable cells (%)[a]					
	Concentration (μg/mL)					
	100	50	25	10	IC_{50}[b] (μg/mL)	IC_{50} (μM)
Doxorubicin	100	60	39	20	37.5	68.99
1	90	50	50	40	50	191.57
2a	40	35	15	5	>100*	-
2b	60	35	10	5	80	168.06
2c	55	40	30	20	82	172.26
2d	30	20	5	5	>100*	-
2e	60	50	40	20	50	102.04
2f	25	10	15	5	>100*	-
2g	60	55	40	25	56	111.11
2h	70	50	20	5	50	99.00
2i	35	30	20	10	>100*	-
2j	100	70	45	30	30	54.54
3	60	40	25	20	75	229.35
4	60	40	35	10	75	106.70
5	100	95	50	40	25	69.63
6	70	65	50	30	25	61.57
7	60	55	45	30	37.5	104.45
8	80	65	45	40	31	83.11
9	85	75	40	40	47	121.44
10	80	70	35	30	45.5	106.30
11	40	20	20	5	>100*	-
12	50	30	25	10	100	197.23

[a]Mean of non-viable percentage of three repeated experiments; [b]IC_{50} value: corresponds to the compound concentration causing 50% mortality in net cells. *Compounds with IC_{50} > 100 μg/mL are considered to be inactive.

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