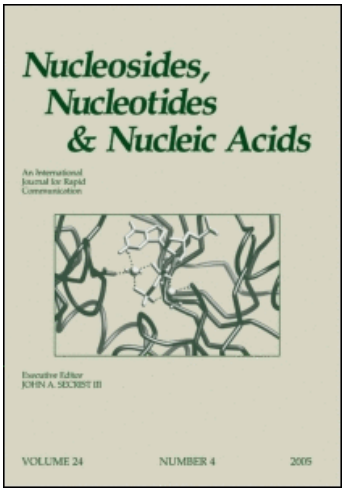

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Synthesis and In Vitro Antitumor Activity of New Substituted Thiopyrimidine Acyclic Nucleosides and Their Thioglycoside Analogs

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SYNTHESIS AND IN VITRO ANTITUMOR ACTIVITY OF NEW SUBSTITUTED THIOPYRIMIDINE ACYCLIC NUCLEOSIDES AND THEIR THIOGLYCOSIDE ANALOGS

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□ *Some new thiopyrimidine acyclic nucleosides and thioglycoside derivatives* ***3a-c***, ***4a-c***, ***6a,b***, *and* ***7a,b*** *were synthesized. The cytotoxicity and antitumor evaluation of all prepared compounds have been tested in vitro against Ehrlich's ascites carcinoma cell line and their activity against glutathione peroxidase and catalase were reported. The role of the prepared compounds as free radical regulators and the therapeutic antitumor effect of a balanced generation of free radicals are discussed. Compounds* ***2***, ***3b***, ***3c***, ***4a***, *and* ***4c*** *inhibited significantly in a dose dependent manner the growth of Ehrlich ascites carcinoma cells while the other compounds did not show any antitumor activity even at higher concentrations.*

Keywords Thiopyrmidines; acyclic nucleosides; thioglycosides; free radicals; antitumor activity

INTRODUCTION

Pyrimidines have been recognized as important heterocyclic compounds due to their diverse biological activities such as Tie-2 kinase inhibitors,[1] HIV-1 inhibitor,[2] antimalarial,[3] secretive adenosine A1 receptor antagonist,[4] antibacterial,[5] anticancer,[6] analgesic,[7] cardiovascular,[8] and antiallergic[9] activities. The thio analogues of pyrimidine bases, including 2-thiouracil, are minor components of *t*-RNA. Their *S*-, *N*- or *S,N*-disubstituted analogs have shown therapeutic properties,

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especially antiviral, antithyroid and antitumor activities[10–12] due to their incorporation into polynucleic acids[13] and therefore act as potential inhibitors of protein and polynucleic acid syntheses.[14] On the other hand, nucleoside analogs are structurally, metabolically, and pharmacodynamically related agents that have diverse biological actions and therapeutic effects including antiviral[15–17] and antitumor[18–20] activities. Furthermore, the glycosylthio heterocycles[21–23] and acyclic nucleoside[23–25] analogues including modifications of both the acyclic glycon and aglycon parts have stimulated extensive research as biological inhibitors.[26–29] The importance of substituted hydroxylalkyl chain conformation in the interaction of acyclic nucleosides with enzymes has been demonstrated.[30] Moreover, the 5-substituted derivatives of pyrimidine acyclonucleoside analogs have exhibited pronounced inhibitory properties with respect to uridine phosphorylase and have enhanced antitumor action.[19,20]

Owing to the above facts, the aim of the present work is to synthesize new substituted thiopyrimidine nucleobases and their acyclic nucleoside analogs as well as evaluation of the cytotoxicity of these compounds in vitro against Ehrlich's ascites carcinoma cell line. In addition, their activity against glutathione peroxidase and catalase was studied. The role of the prepared compounds as free radical regulators and the therapeutic antitumor effect of a balanced generation of free radicals were discussed.

RESULTS AND DISCUSSION

Reaction of cinnamaldehyde, ethyl cyanoacetate and thiourea in ethanol afforded 1,2,3,4-tetrahydro-4-oxo-6-styryl-2-thioxopyrimidine-5-carbonitrile (**2**). Reaction of compound **2** with methyl iodide, ethyl iodide, chloroethyl methyl ether afforded the alkylthio derivatives **3a-c**, respectively. The ^{1}H NMR spectrum of compound **3a**, as a representative example, showed the presence of the methyl group at δ 2.77 ppm, the aromatic proton signals at δ 7.47–7.75 ppm in addition to the peak corresponding to the NH group at δ 8.33 ppm. Its ^{13}C NMR spectrum showed the presence of the methyl group, the aromatic carbon atoms in addition to the C=O and C=N groups. The absence of the peak corresponding to C=S group indicates that the alkylation had taken place at the sulfur atom to give the alkythio derivatives.

In order to synthesize a number of *N*-substituted acyclic nucleoside analogs, we used 1,6-dihydro-2-(methylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (**3a**) as a nucleobase. Thus, reaction of **3a** with chloroacetaldehyde dimethylacetal, chloroethyl methyl ether or 3-chloropropane-1,2-diol gave the corresponding *N*-acyclic nucleoside analogs **4a-c** (see Scheme 1). The IR spectrum of compound **4a**, as a representative example, showed the characteristic peaks for the C=O and CN groups and its ^{1}H NMR showed

two OCH_3 groups in addition to the CH_2 signal as doublet which agreed with the assigned structure.

On the other hand, treatment of compound **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galacto- or glucopyranosyl bormide (**5a,b**) afforded the corresponding thioglycosides (**6a,b**) (see Scheme 1). Their IR spectra showed characteristic absorption bands in the carbonyl frequency region corresponding to the *O*-acetyl groups and the ^{1}H NMR spectra showed the anomeric proton of the sugar moiety in the range δ 5.75–5.80 ppm as doublet, with a coupling constants equal to 9.4 and 9.5 Hz for **6a** and **6b**, respectively indicating the β-orientation of the thioglycosidic bond. The attachment of the glycosyl residues to the sulfur rather than to the nitrogen has been supported by the value of the chemical shift of the anomeric protons which otherwise should appear at a lower field. The anomeric proton of β-*N*-glucosides having an adjacent C=S, was reported to appear at higher chemical shift (δ 6.9–7.2 ppm) due to the anisotropic deshielding effect of the C=S.[31–34] Furthermore, the ^{13}C NMR spectrum of **6a** showed a signal at δ 78.14 corresponding to the anomeric C-1 which also confirmed the β-configuration. The absence of a peak corresponding to the C=S group indicates that the attachment of the sugar at the sulfur atom.

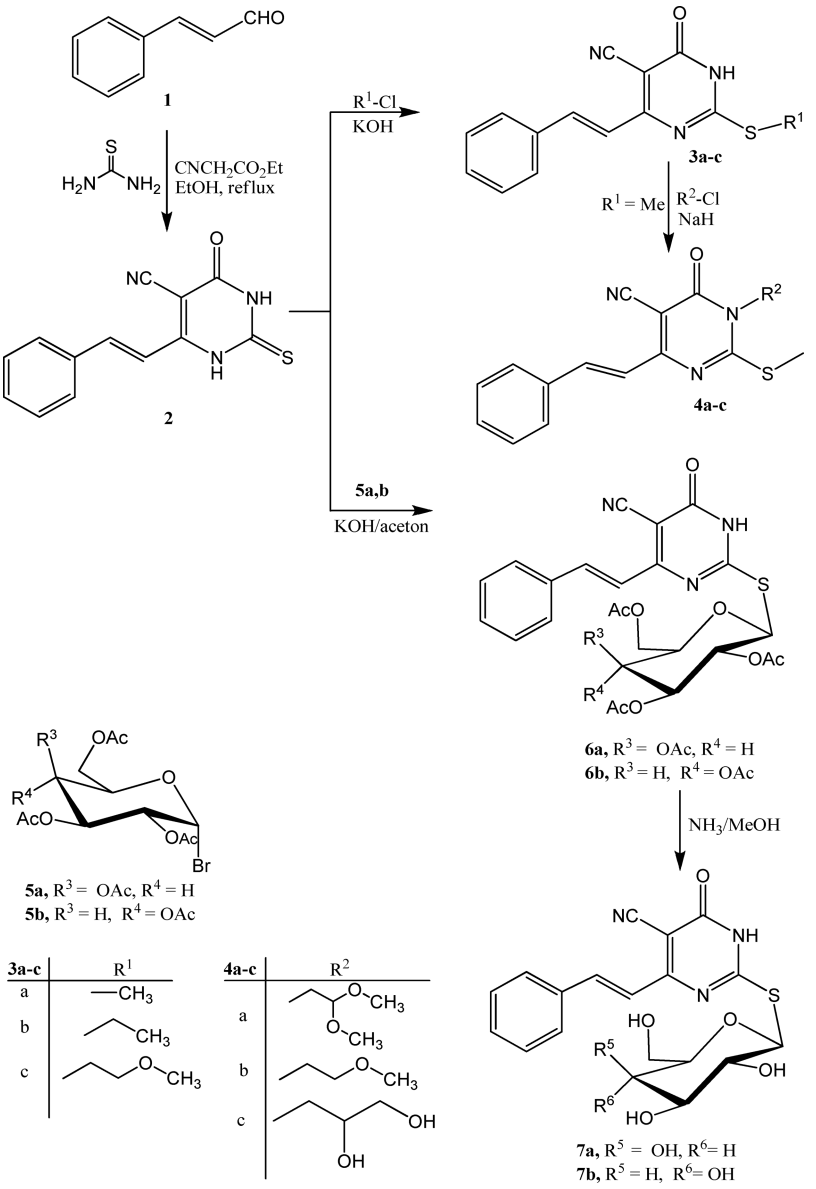
When compounds **6a,b** were treated with methanolic ammonia at 0°C, the deacetylated thioglycoside derivatives **7a,b** were obtained in moderate yields. The IR spectra of the deacetylated thioglycoside **7a,b** showed characteristic absorption bands corresponding to the OH groups for **7a** and **7b**, respectively and their ^{1}H NMR agreed with the assigned structures. (See Scheme 1.)

Antitumor Activity

The tested compounds were screened in vitro using a single tumor (Ehrlich ascites carcinoma cells). The results of the present study indicated that compounds **2, 3b, 3c, 4a,** and **4c** could inhibit the growth of Ehrlich ascites carcinoma cells significantly in culture, while the other compounds did not show any antitumor activity even at higher concentrations.

As shown in Table 1, compounds **2, 3b, 3c, 4a,** and **4c** inhibited in dose dependent manner the growth of Ehrlich ascites carcinoma cells. It is observed that there was gradual decrease in the viability with increasing the concentration in a dose dependent type. These compounds were found to inhibit 50% proliferation of EAC cells in short term culture at concentrations of 13.7, 12.3, 6.2, 13, and 23 μg/ml, respectively. Compounds increased the NO and TBARS concentrations in the culture medium in dose dependent manner.

GSH-Px and CAT data for compounds **2, 3b, 3c, 4a,** and **4c** are given in Table 1, which showed that these compounds were GSH-Px inhibitors, and also prevent H_2O_2 degradation by inhibiting catalase activity. On the other



SCHEME 1 Synthesis route of substituted thiopyrimidine derivatives **2–7**.

hand, reactive oxygen species (ROS), including hydroxyl radicals ($^{\cdot}OH$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of some enzymes, and a massive protein oxidation and degradation.[33,36]

Our results revealed that, compounds **4a** and **4c** were more active anticancer compounds than compounds **2, 3b,** and **3c**. The activity of

TABLE 1 Effect of the synthesized compounds on the growth of Ehrlich ascites carcinoma cells in tissue culture and on the concentration of nitric oxide (NO), and thiobarbituric acid reactive substances (TBARS), as well as the activity of catalase (CAT) and glutathione peroxidase (GSH-Px)

Compound	Concentration (μg/ml)	% Inhibition of cell growth after 24 hours	IC_{50} (μg/ml)	NO (%)	TBARS (%)	CAT Inhibition, %	GSH-Px Inhibition, %
2	5	10.00		15.50 ± 1.20	20.00 ± 2.08	13.00 ± 1.68	12.00 ± 1.08
	10	27.00	13.70	24.75 ± 2.06	30.00 ± 4.65	21.00 ± 3.03	26.00 ± 3.03
	15	58.00		60.50 ± 5.83	66.00 ± 8.17	26.75 ± 3.12	33.00 ± 3.24
3b	5	20.00		20.50 ± 4.84	23.00 ± 2.65	34.75 ± 3.17	16.00 ± 2.48
	10	35.00	12.30	60.00 ± 6.18	26.00 ± 3.34	42.50 ± 4.63	29.00 ± 3.40
	15	70.00		80.50 ± 6.18	73.00 ± 5.15	46.00 ± 5.11	39.00 ± 5.48
3c	5	45.00		35.00 ± 6.18	30.00 ± 3.67	41.00 ± 4.02	28.00 ± 2.49
	10	75.00		90.00 ± 9.71	42.00 ± 3.34	52.00 ± 5.07	30.00 ± 4.32
	20	90.00	6.20	150.00 ± 13.85	80.00 ± 7.36	57.00 ± 6.44	36.00 ± 3.14
4a	10	42.00		160.00 ± 12.42	12.00 ± 1.22	32.00 ± 3.03	18.00 ± 2.20
	20	68.00	13.00	280.00 ± 35.60	23.00 ± 1.58	40.00 ± 5.40	22.00 ± 3.48
	30	80.00		350.00 ± 33.41	45.00 ± 6.45	62.00 ± 4.81	47.00 ± 5.40
4c	25	55.00		150.00 ± 13.70	15.00 ± 1.83	30.00 ± 2.65	21.00 ± 1.96
	50	70.00	23.00	400.00 ± 55.82	36.00 ± 3.89	42.00 ± 6.18	34.00 ± 2.80
	100	91.00		800.00 ± 57.60	46.00 ± 6.47	70.00 ± 9.79	52.00 ± 4.80

IC_{50} concentration (μg/ml) providing 50% cell killing effect.

compounds **4a** and **4c** is due to an increase in NO level, which leads to apoptosis, whereas in compounds **2, 3b, and 3c**, an increase in oxygen radical level leads to necrosis.[37]

The regulation of the glutathione peroxidase and catalase enzymes may affect free radical balance in tumor. Glutathione peroxidase may scavenge various peroxides, while catalase causes hydrogen peroxide degradation.[37] Compounds **2, 3b, 3c, 4a,** and **4c** revealed inhibition of glutathione peroxidase and catalase activities. Compounds **4a** and **4c** demonstrated the greatest inhibition of glutathione peroxidase and catalase enzymes.

The mechanism of action of these compounds can be explained by the fact that these compounds may catalyze the conversion of superoxide and hydrogen peroxide to free radical species that attack cellular membranes, proteins and DNA causing cell damage.[38]

Moreover, Huang et al.[39] observed that selective inhibition of superoxide dismutase kills human cancer cells but not normal cells, suggesting that regulation of free radical-producing agents may also have important clinical applications. This mechanism for the effects of ROS generating anticancer agents is only beginning to be understood, as previously the mechanism of most anticancer agents was believed to be due mainly to direct interaction with DNA and interference with DNA regulatory machinery (e.g., topoisomerases, helicases) and to the initiation of DNA damage via production of ROS.[40]

CONCLUSION

According to the obtained results, there is a correlation between the efficiency in generating free radicals and the ability of the tested compounds to suppress the growth of cancer cells, and we propose that the antitumor effect of the tested compounds stems from the fact that they are able to regulate the free radical balance inside the cells. Furthermore, structure-activity relationship showed that the increase in NO level in compounds **4a** and **4c** leading to apoptosis give rise to their high antitumor activity with respect to other tested compounds.

EXPEREMINTAL

Chemistry

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt. ^{1}H NMR and ^{13}C NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal

reference (Faculty of Science, Cairo University, Cairo, Egypt). Microanalyses were operated using Mario Elmentar apparatus, Organic Microanalysis Unit, National Research Centre, Cairo, Egypt and the results were within the accepted range of the calculated values. Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Antitumer activity evaluation was carried out at Biochemistry Department, Division of Genetic Engineering and Biotechnology, National Research Center, Cairo, Egypt.

1,2,3,4-Tetrahydro-4-oxo-6-styryl-2-thioxopyrimidine-5- carbonitrile (2). To a solution of cinnamaldehyde (6.60 g, 50 mmol) and thiourea (3.81 g, 50 mmol) in ethanol (75 mL) was added ethylcyanoacetate (5.66 g, 50 mmol) and the mixture was refluxed for 6 hours. The solvent was concentrated under reduced pressure and water (100 mL) was added. The resulting solid was filtered, washed with ice cold water and then recrystallized from ethanol to afford 10.3 g compound of **2** (81%); m.p. 262–263°C; IR (KBr): 3395 (NH), 2212 (CN), 1668 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 10.12 (s, 1 H, NH), 8.33 (s, 1 H, NH), 8.07 (d, 1 H, *J* 6.5 Hz, CH), 7.72 (m, 2 H, Ar-2H), 7.45 (m, 3 H, Ar-3H), 7.29 (d, 1 H, *J* 6.5 Hz, CH). Analysis calcd. for $C_{13}H_9N_3OS$: C, 61.16; H, 3.55; N, 16.46. Found: C, 60.95; H, 3.49; N, 16.35%.

1,6-Dihydro-2-(alkylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (3a,b,c). To a well stirred solution of compound **2** (2.55 g, 10 mmol) and potassium hydroxide (0.56 g, 10 mmol) in alcoholic mixture of ethanol (10 mL) and water (20 mL) was added methyl iodide or ethyl iodide (10 mmol mole). The reaction mixture was stirred at 60°C for 4 hours then cooled and the precipitated solid was filtered, washed with water and crystallized from ethanol to give compounds **3a** or **3b** in 77–79% yields.

1,6-Dihydro-2-(methylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (3a). Pale yellow powder (77%); m.p. 294–296°C; IR (KBr): 3395 (NH), 2213 (CN), 1667 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.33 (s, 1 H, NH) 8.05 (d, 1 H, *J* 6.5 Hz, CH), 7.75 (m, 2 H, Ar-2H), 7.47 (m, 3 H, Ar-3H), 7.21 (d, 1 H, *J* 6.5 Hz, CH), 2.77 (s, 3 H, SCH_3). 168.79 (pyrimidyl-C), 163.24 (C=N), 160.50 (C=O), 142.04 (CH), 134.50 (Ar-C), 129.14 (Ar-2C), 128.68 (Ar-2C), 128.47 (Ar-C), 121.56 (CH), 115.10 (CN), 93.09 (pyrimidyl-C), 13.32 (CH_3). Analysis calcd. for $C_{14}H_{11}N_3OS$: C, 62.43; H, 4.12; N, 15.60. Found: C, 62.22; H, 3.91; N, 15.88%.

1,6-Dihydro-2-(ethylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (3b). Pale yellow powder (79%); m.p. 288–289°C; IR (KBr): 3396 (NH), 2212 (CN), 1670 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.07 (s, 1 H, NH), 8.01 (d, 1 H, *J* 6.5 Hz, CH), 7.76 (m, 2 H, Ar-2H), 7.47 (m, 3 H, Ar-3H), 7.22 (d, 1 H, *J* 6.5 Hz, CH), 4.01 (q, 2 H, *J* 3.5 Hz, CH_2), 1.43 (t, 3 H, *J* 3.5 Hz, CH_3). Analysis calcd. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.75; H, 63.75; N, 14.46%.

2-(2-Methoxyethylthio)-1,6-dihydro-6-oxo-4-styrylpyrimidine-5-carbonitrile (3c): 2.25 g (72% yield); m.p. 289–290°C; IR (KBr): 3410 (NH), 2206 (CN), 1657 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.21 (s, 1 H, NH), 7.74 (d, 1 H, *J* 6.5 Hz, CH), 7.42 (m, 2 H, Ar-2H), 7.39 (m, 3 H, Ar-3H), 7.28 (d, 1 H, *J* 6.5 Hz, CH), 3.40 (t, 2 H, *J* 5.4 Hz, CH_2), 3.27 (s, 3 H, OCH_3), 3.25 (t, 2 H, *J* 5.4 Hz, CH_2). Analysis calcd. for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41. Found: C, 60.96; H, 4.61; N, 13.09%.

General Procedure for the Synthesis of Compounds 4a and 4b

To a well stirred solution of compound **3a** (2.69 g, 10 mmol) and sodium hydride (0.25 g, 10 mmol) in *N,N*-dimethylformamide (15 mL) was added chloroacetaldehyde dimethylacetal or chloroethyl methyl ether (0.01 mole). The reaction mixture was stirred at room temperature for 10 hours and then poured on ice-cold water. The precipitated solid was filtered, washed with water and recrystallized from ethanol to give compounds **4a,b** in 71–76% yields.

1,6-Dihydro-1-(2,2-dimethoxyethyl)-2-(methylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (4a). Pale yellow powder (76%); m.p. 288–290°C; IR (KBr): 2215 (CN), 1667 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.07 (d, 1 H, *J* 6.5 Hz, CH), 7.60 (m, 2 H, Ar-2H), 7.37 (m, 3 H, Ar-3H), 7.24 (d, 1 H, *J* 6.5 Hz, CH), 4.58 (t, 1 H, *J* 5.8 Hz, CH), 4.52 (d, 2 H, *J* 5.8 Hz, CH_2), 3.41 (s, 3 H, OCH_3), 3.39 (s, 3 H, OCH_3), 2.57 (s, 3 H, CH_3). 168.33 (pyrimidyl-C), 164.11 (C=N), 162.55 (C=O), 143.24 (CH), 135.25 (Ar-C), 129.18 (Ar-2C), 128.33 (Ar-2C), 128.14 (Ar-C), 123.08 (CH), 116.12 (CN), 96.24 (CH), 92.15 (pyrimidyl-C), 49.88 (2OCH_3), 46.95 (CH_2), 14.32 (CH_3). Analysis calcd. for $C_{18}H_{19}N_3O_3S$: C, 60.49; H, 5.36; N, 11.76. Found: C, 60.71; H, 5.48; N, 11.57%.

4,5-Dihydro-5-(2-methoxyethyl)-6-(methylthio)-4-oxo-2-styrylpyridine-3-carbonitrile (4b). Pale yellow powder (71%); m.p. 258–259°C; IR (KBr): 2215 (CN), 1654 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.07 (d, 1 H, *J* 6.5 Hz, CH), 7.60 (m, 2 H, Ar-2H), 7.37 (m, 3 H, Ar-3H), 7.24 (d, 1 H, *J* 6.5 Hz, CH), 3.82 (t, 2 H, *J* 5.6 Hz, CH_2), 3.73 (t, 2 H, *J* 5.6 Hz, CH_2), 3.37 (s, 3 H, OCH_3), 2.62 (s, 3 H, CH_3). Analysis calcd. for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83. Found: C, 62.05; H, 5.15; N, 13.11%.

1,6-Dihydro-1-(2,3-dihydroxypropyl)-2-(methylthio)-6-oxo-4-styrylpyrimidine-5-carbonitrile (4c): To a stirred solution of compound **3a** (2.69 g, 10 mmol) and potassium hydroxide (0.56 g, 10 mmol) in ethanol (25 mL) was added 3-chloropropane-1,2-diol (1.11 g, 10 mmol) and the reaction mixture was heating under reflux for 3 hours. The solvent was concentrated under reduced pressure then cooled and the precipitated solid was filtered, and crystallized from ethanol to give 2.34 g of compound **4c** as yellow powder (71% yield); m.p. 287–289°C; IR (KBr): 3380 (OH), 2216 (CN), 1662 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 8.11 (d, 1 H, *J* 6.5 Hz, CH), 7.57

(m, 2 H, Ar-2H), 7.31 (m, 3H, Ar-3H), 7.22 (d, 1H, *J* 6.5 Hz, CH), 4.57 (d, 2H, *J* 5.8 Hz, CH_2), 4.53 (m, 1 H, CH), 4.32 (d, 1 H, *J* 5.8 Hz, OH), 4.22 (m, 2 H, CH_2), 4.11 (t, 1 H, *J* 5.2 Hz, OH), 2.58 (s, 3 H, CH_3). 168.22 (pyrimidyl-C), 163.14 (C=N), 163.52 (C=O), 142.70 (CH), 134.21 (Ar-C), 128.19 (Ar-2C), 128.24 (Ar-2C), 127.97 (Ar-C), 127.97 (Ar-C), 118.72 (CN), 91.18 (pyrimidyl-C), 72.78 (CH), 62.43 (CH_2), 45.87 (CH_2), 14.32 (CH_3). Analysis calcd. for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24. Found: C, 59.15; H, 5.11; N, 12.18%.

1,6-Dihydro-2-[(2,3,4,6-tetra-O-acetyl-β-D-glycopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (6a,b). General procedure: To a solution of 1,2,3,4-tetrahydro-4-oxo-6-styryl-2-thioxopyrimidine-5-carbonitrile (**2**) (2.55 g, 10 mmol) in aqueous potassium hydroxide [10 mmol in distilled water (16 mL)] was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galacto (**5a**) or gluco- (**5b**) pyranosyl bromide (10 mmol) in acetone (30 mL). The reaction mixture was stirred at room temperature until reaction was judged complete by TLC using chloroform/methanol 99.5:0.5. The solvent was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove potassium bromide formed. The product was dried, and crystallized from ethanol.

1,6-Dihydro-2-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (6a). Yellow powder (75%); m.p. 166–168°C; IR (KBr): 3398 (NH), 2210 (CN), 1740 (C=O), 1658 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 7.89 (s, 1 H, NH), 7.46 (m, 2 H, Ar-2H), 7.31 (m, 3 H, Ar-3H), 7.28 (d, 1 H, *J* 6.5 Hz, CH), 5.80 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.24 (t, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 5.15 (dd, 1 H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.3 Hz, H-3), 4.93 (t, 1 H, $J_{3,4}$ 9.3 Hz, H-4), 4.10 (dd, 1 H, $J_{6,6'}$ 11.2 Hz, $J_{5,6}$ 3.5 Hz, H-6), 4.05 (dd, 1 H, $J_{6,6'}$ 11.2 Hz, $J_{5,6'}$ 2.8 Hz, H-6′), 3.98 (m, 1 H, H-5), 1.96, 2.03, 2.11, 2.13 (4s, 12 H, $4CH_3$). 168.79 (pyrimidyl-C), 164.14, 164.32, 164.67, 165.35, 169.45 (5CO), 163.33 (C=N), (C=O), 142.11 (CH), 134.65 (Ar-C), 134.65 (Ar-C), 134.65 (Ar-C), 128.41 (Ar-C), 122.51 (CH), 116.15 (CN), 92.29 (pyrimidyl-C), 78.14 (C-1), 72.48 (C-2), 71.65 (C-3), 68.66 (C-4), 64.21 (C-5), 62.98 (C-6), 15.27, 15.23, 15.44, 15.65 ($4CH_3$). Analysis calcd. for $C_{27}H_{27}N_3O_{10}S$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.04; H, 4.42; N, 7.35%.

1,6-Dihydro-2-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (6b). Yellow powder (77%); m.p. 159–161°C; IR (KBr): 3305 (NH), 2208 (CN), 1735 (C=O), 1658 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 7.92 (s, 1 H, NH), 7.72 (d, 1 H, *J* 6.5 Hz, CH), 7.51 (m, 2 H, Ar-2H), 7.34 (m, 3 H, Ar-3H), 7.25 (d, 1 H, *J* 6.5 Hz, CH), 5.75 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 5.27 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 5.18 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 9.2 Hz, H-3), 4.62 (t, 1 H, $J_{3,4}$ 9.2 Hz, H-4), 4.33 (m, 1 H, H-5), 4.18 (dd, 1 H, $J_{6,6'}$ 11.4 Hz, $J_{5,6}$ 3.5 Hz, H-6), 4.10 (dd, 1 H, $J_{6,6'}$ 11.4 Hz, $J_{5,6'}$ 2.8 Hz, H-6′), 1.93, 2.04, 2.11, 2.15 (4s, 12 H, $4CH_3$). Analysis calcd. for $C_{27}H_{27}N_3O_{10}S$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.15; H, 4.36; N, 7.24%.

1,6-Dihydro-2-[(β-D-glycopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (7a,b). General procedure: Dry gaseous ammonia was passed through a solution of a protected nucleoside **6a,b** (10 mmol) in dry methanol (20 mL) at 0°C for 0.5 hours, and then the mixture was stirred at 0°C for about 5 hours. The solvent was evaporated under reduced pressure at 40°C to give a solid residue, which was crystallized from ethanol to afford the desired products.

1,6-Dihydro-2-[(β-D-galactopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (7a). Pale yellow powder (67%); m.p. 232–233°C; IR (KBr): 3366 (NH), 3282 (NH), 2215 (CN), 1663 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 7.88 (s, 1 H, NH), 7.73 (d, 1 H, J 6.5 Hz, CH), 7.51 (m, 2 H, Ar-2H), 7.34 (m, 3 H, Ar-3H), 7.32 (d, 1 H, J 6.5 Hz, CH), 5.42 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 5.14 (t, 1 H, J 5.8 Hz, OH), 5.05 (m, 1 H, OH), 4.95 (d, 1 H, J 6.2 Hz, OH), 4.82 (m, 1 H, OH), 4.44 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 4.25 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-3), 3.92 (m, 1 H, H-4), 3.52 (m, 1 H, H-6′), 3.48 (dd, 1 H, $J_{6,6'}$ 11.4 Hz, $J_{5,6}$ 3.4 Hz, H-6), 3.41 (m, 1 H, H-5). Analysis calcd. for $C_{19}H_{19}N_3O_6S$: C, 54.67; H, 4.59; N, 10.07. Found: C, 54.37; H, 4.42; N, 10.22%.

1,6-Dihydro-2-[(β-D-glucopyranosyl)thio]-6-oxo-4-styrylpyrimidine-5-carbonitrile (7b). Pale yellow powder (69%); m.p. 237–238°C; IR (KBr): 3345 (NH), 3278 (NH), 2212 (CN), 1660 (C=O); ^{1}H NMR (DMSO-d_6, 300 Mz), δ_H: 7.92 (s, 1 H, NH), 7.72 (d, 1H, J 6.5 Hz, CH), 7.53 (m, 2 H, Ar-2H), 7.38 (m, 3 H, Ar-3H), 7.35 (d, 1 H, J 6.5 Hz, CH), 5.39 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 5.17 (t, 1 H, J 5.8 Hz, OH), 5.11 (t, 1 H, J 6.4 Hz, OH), 4.92 (d, 1 H, J 6.2 Hz, OH), 4.79 (m, 1 H, OH), 4.47 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 4.22 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.94 (m, 1 H, H-4), 3.42 (m, 1 H, H-5′), 3.47 (m, 2 H, H-6,6′). Analysis calcd. for $C_{19}H_{19}N_3O_6S$: C, 54.67; H, 4.59; N, 10.07. Found: C, 54.33; H, 4.29; N, 10.19%.

ANTITUMOR ACTIVITY

Chemicals

Dimethylsulphoxide (DMSO) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used were of analytical grade.

Cell Line

Ehrlich's ascites carcinoma cells (EAC) were obtained from the National Cancer Institute, Cairo University, Egypt. The cells were maintained by intraperitoneal inoculation of 1×10^6 viable cells in mice.

The estimation of in vitro tumor cell growth inhibition was assessed by incubating 1×10^6 EAC cells in 1 ml phosphate buffer saline with varying concentrations of the prepared compounds at 37°C for 3 hours

in CO_2 atmosphere (Table 1). In all the cellular experiments, results were compared with untreated cells.

In Vitro Cytotoxicity Assay

The effect of the prepared compounds on the growth of EAC was estimated by MTT assay according to Mosmann.[41] The cytotoxic activity of the compounds was determined by the MTT colorimetric assay. The MTT colorimetric assay is based on the mitochondrial reduction of tetrazolium salt by living cells. The viable cell number is proportional to the production of formazan salts. The crystals of formazan were dissolved in DMSO and the optical density was measured spectrophotometrically (Microplates reader, Asys Hitech, Austria).

Cells (1×10^5 cells/well) were suspended in serum-free media. The cells were plated separately in a sterile flat bottom 96-well microplate, and treated with 20 μl of the drug with a final concentration as mentioned in Table 1, for 24 hours at 37°C, in a humidified 5% CO_2 atmosphere. The control cells without test compounds were cultured on separate plate. After incubation, media were removed and 40 μl MTT solution/well were added and incubated for an additional 4 hours. MTT crystals were solubilized by adding 200 μl of DMSO/well and plate was shacked at room temperature. The results were determined photometrically using microplate ELISA reader and the absorbance was 570 nm. The concentration of nitric oxide (NO), and thiobarbituric acid reactive substances (TBARS) as indicator of lipid peroxidation were determined according to Granger et al.[42]; Conrad et al.[43] respectively. The activity of catalase (CAT) and glutathione peroxidase (GSH-Px) were determined according to Aebi[44]; Paglia and Valentine,[45] respectively.

Statistical Analysis

All experiments were repeated four times for each concentration and the data was represented as Mean ± Standard error (S.E.) and transformed to percentage of untreated cells.

Percentage of relative viability was calculated using the following equation:

$$(\text{absorbance of treated cells})/\text{Absorbance of control cells} \times 100.$$

Cytotoxic concentration was expressed by half maximal inhibitory concentration (IC_{50}). IC_{50} calculations were performed using Microsoft Excel and Microcal Origin software for PC.

REFERENCES

1. Matloobi, M.; Kappe, C.O. Microwave-assisted solution- and solid-phase synthesis of 2-amino-4-arylpyrimidine derivatives. *J. Comb. Chem.* **2007**, 9, 275–284.
2. Gadhachanda, V.R.; Wu, B.; Wang, Z.; Kuhen, K.L.; Caldwell, J.; Zondler, H.; Walter, H.; Havenhand, M.; He, Y. 4-Aminopyrimidines as novel HIV-1 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, 17, 260–265.
3. Agarwal, A.; Srivastava, K.; Purib, S.K.; Chauhana, P.M.S. Synthesis of 2,4,6-trisubstituted pyrimidines as antimalarial agents. *Bioorg. Med. Chem.* **2005**, 13, 4645–4650.
4. Chang, L.C.W.; Spanjersberg, R.F.; Jvon Frijtag Drabbe Künzel, K.; Mulder- Krieger, T.; Van DenHout, G.; Beukers, M.W.; Brussee, J.; IJzerman, A.P. 2,4,6-Trisubstituted pyrimidines as a new class of selective adenosine a1 receptor antagonists. *J. Med. Chem.* **2004**, 47, 6529–6540.
5. Hegab, M.I.; Hassan, N.A.; Rashad, A.E.; Fahmy, A.A. Abdel-Megeid, F.M.E. Synthesis, reactions, and antimicrobial activity of some fused thieno[2,3-d]pyrimidine derivatives. *Phosphorus, Sulfur, Silicon and Related Elements* **2007**, 182, 1535–1556.
6. Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A.; Glivec, S.T. rationally developed targeted anti cancer drug. *Nat. Rev. Drug Disc.* **2002**, 1, 493–502.
7. Zaki, M.E.A.; Soliman, H.A.; Hiekal, O.A.; Rashad, A.E. Pyrazolopyranopyrimidines as a class of anti-inflammatory agents. *Z. Naturforsch.* **2006**, C 61c, 1–5.
8. Atwal, K. Preparation of 2-amino dihydropyrimidin-5-carboxylates as cardiovascular agents. US Patent US, 1988, 4,769,371 (Cl 514–275, C 07 D 239/42) 6 September, Appl. 45956 01 March 1987, 14 pp.
9. Ozeki, K.; Ichikawa, T.; Hiroyuki, T.; Tanimury, K.; Sato, M.; Yaginuna, H. Studies on antiallergy agents. III. Synthesis of 2-anilino-1, 6-dihydro-6-oxo-5-pyrimidinecarboxylic acids and related compounds. *Chem. Pharm. Bull.* **1989**, 37, 1780–1787.
10. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Inouye, N.; Baba, M.; Shigeta, S.; Walker, R.T.; De Clercq, E. Synthesis and antiviral activity of 6-benzyl analogs of 1-[(2-hydroxyethoxy)methyl]-5-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J. Med. Chem.* **1995**, 38, 2860–2865.
11. Hawthorne, M.F. Die rolle der chemie in der entwicklung einer krebstherapie durch die borneutroneneinfangreaktion. *Angew. Chem.* **1993**, 105, 997–1033.
12. Nugent, R.A.; Schlachter, S.T.; Murphy, M.I.; Cleek, G.J.; Poel, T.J.; Wishka, D.G.; Graber, D.R.; Yagi, Y.; Kaiser, B.J.; Olmsted, R.A.; Kopta, L.A.; Swaney, S.M.; Poppe, S.M.; Morris, J.; Tarpley, W.G.; Thomas, R.C. Pyrimidine thioethers: A novel class of HIV-1 reverse transcriptase inhibitors with activity against BHAP-resistant HIV. *J. Med. Chem.* **1998**, 41, 3793–3803.
13. Holland, J.F.; Guthrie, R.; Shecke, P.; Tierkelman, H. Inhibition of tumor growth in mice by a series of 2-substituted thiopyrimidines. *Cancer Res.* **1958**, 18, 776–780.
14. Albot, J.; Goodgame, D.M.L.; Jeeves, I. Complexes of pyrimidine-2-thione with some bivalent metal halides of the first transition series. *J. Chem. Soc. Dalton Trans.* **1978**, 880–884.
15. Remy, R.J.; Secrist, J. A. Acyclic nucleosides other than acyclovir as potential antiviral agents. *Nucleosides, Nucleotides* **1985**, 44, 411–427
16. Abdel-Aal, M.T.; El-Sayed, W.A.; El-Ashry, E.S.H. Synthesis and antiviral evaluation of some sugar arylglycinoylhydrazones and their oxadiazoline derivatives. *Arch. Pharm. Chem. Life Sci.* **2006**, 339, 356–663.
17. Rashad, A.E.; Hegab, M.I.; Abdel-Megeid, R.E.; Micky, J.A.; Abdel-Megeid, F.M.E. Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives. *Bioorg. Med. Chem.* **2008**, 16, 7102–7106.
18. Chu, M.Y.W.; Naguib, F.N.M.; Iltzsch, M.H.; El Kouni, M.H.; Chu, S.H.; Cha, S.; Calabresi, P. Potentiation of 5-fluoro-2′-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine. *Cancer Res.* **1984**, 44, 1852–1856.
19. Park, K.S.; El Kouni, M.H.; Krenitsky, T.A.; Chu, S.H.; Cha, S. Inhibition of uridine phosphorylase from *Escherichia coli* by benzylacyclouridines. *Biochem. Pharmacol.* **1986**, 35, 3853–3855.
20. Lin, T.-S.; Liu, M.-C. Synthesis of 1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil and its amino analogue, new potent uridine phosphorylase inhibitors with high water solubility. *J. Med. Chem.* **1985**, 28, 971–973.
21. Brajeswar, P.; Walter, K. S-, N-, and O-glycosyl derivatives of 2-acetamido-2-deoxyglucose with hydrophobic aglycons as potential chemotherapeutic agents and N-acetyl-β–glucosaminidase inhibitors. *Carbohydr. Res.* **1984**, 126, 27–43.

22. Kuhn, C.S.; Lehmann, J.; Steck, J. Syntheses and properties of some photolabile β-thioglycosides. Potential photoaffinity reagents for β-glycoside hydrolases. *Tetrahedron* **1990**, 46, 3129–3134.
23. a) El Ashry, E.S.H.; Awad, L.F.; Atta, I.A. Synthesis and role of glycosylthio heterocycles in carbohydrate chemistry. *Tetrahedron* **2006**, 62, 2943–2998. b) Mereyala, H.B.; Gurijala, V.R. Use of 2-pyridyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-gluco-pyranoside as a glycosyl donor and methyl iodide as an activator for the synthesis of 1,2-trans-linked saccharides. *Carbohydr. Res.* **1993**, 242, 277–280. c) El Ashry, E.S.H.; Awad, L.F.; Abdel-Hamid, H.; Atta, I.A. Synthesis of interglycosidically S-linked 1-thio-oligosaccharides under microwave irradiation. *J. Carbohydr. Chem.* **2005**, 24, 745–753.
24. El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 1. *seco*-Nucleosides. *Advances in Heterocyclic Chemistry* **1996**, 67, 391–438.
25. a) El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 2. *diseco*-Nucleosides. *Advances in Heterocyclic Chemistry* **1997**, 68, 1–88. b) El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: part 3. *tri-*,*tetra-*, and *pentaseco*-Nucleosides. *Advances in Heterocyclic Chemistry* **1997**, 69, 129–215.
26. El Ashry, E.S.H.; Rashed, N.; Shobier, A.H. Glycosidase inhibitors and their chemotherapeutic value. Part1. *Pharmazie.* **2000**, 55, 251–262.
27. El Ashry, E.S.H.; Rashed, N.; Shobier, A.H. Glycosidase inhibitors and their chemotherapeutic value. Part 2. *Pharmazie.* **2000**, 55, 331–348.
28. El Ashry, E.S.H.; Rashed, N.; Shobier, A.H. Glycosidase inhibitors and their chemotherapeutic value. Part 3. *Pharmazie.* **2000**, 55, 403–415.
29. El Ashry, E.S.H.; Rashed, N.; Awad, L.F.; Ramadan, E.; Abdel-Mageed, S.M.; Rezki, N. Novel regioselective hydroxyl-alkylation of 4,5-diphenylimidazole-2-thione and a competitive intramolecular ring closure of the S-hydroxyalkyl-imidazoles to imidazo[2,1-b]thiazines and thiazoles. Role of catalyst, microwave irradiation, and solid support. nucleosides, *Nucleotides & Nucleic Acids* **2007**, 26, 423–435.
30. Alvarez, R.; Velazquez, S.; San-Felx, A.; Aquaro, S.; De Clercq, E.; Perno, C.-F.; Karlsson, A.; Balzarini, J.; Camarasa, J. 1,2,3-Triazole-[2,5-Bis-O-(tert-butyldimethylsilyl)-.beta.-D-ribofuranosyl]-3′-spiro-5′′-(4′′-amino-1′′,2′′-oxathiole 2′′,2′′-dioxide) (TSAO) Analogs: Synthesis and Anti-HIV-1 Activity. *J. Med. Chem.* **1994**, 37, 4185–4194.
31. Mansour, A.K.; Ibrahim, Y.A.; Khalil, N.S.A.M. Selective synthesis and structure of 6-arylvinyl-2- and 4-glucosyl-1,2,4-triazines of expected interesting biological activity. *Nucleosides Nucleotides Nucleic Acids* **1999**, 18, 2256–2283.
32. El-Sayed, W.A.; Fathi, N.M.; Gad, W.A.; El-Ashry, E.S.H. Synthesis and antiviral evaluation of some 5-N-arylaminomethyl-2-glycosylsulphanyl-1,3,4-oxadiazoles and their analogs against hepatitis A and herpes simplex viruses. *J. Carbohydr. Chem.* **2008**, 27, 357–372.
33. Abdel-Aal, M.T.; El-Sayed, W.A.; El-Kosy, S.M.; El-Ashry, E.S.H. Synthesis and antiviral evaluation of novel 5-(N-Arylaminomethyl-1,3,4-oxadiazol-2-yl)hydrazines and their sugars, 1,2,4-triazoles, tetrazoles and pyrazolyl derivatives. *Arch. Pharm. Chem. Life Sci.* **2008**, 341, 307–313.
34. Ibrahim, Y.A. Facile approach for the selective glycosidation of cyclic asymmetric amides and thioamides. *Carbohydr. Lett.* **1996**, 1, 425–432.
35. Stamler, J.S.; Hausladen, A. Oxidative modifications in nitrosative stress. *Nat. Struct. Biol.* **1998**, 5, 247–249.
36. Jourd'heuil, D.; Mills, L.; Miles, A.M.; Grisham, M.B. Effect of nitric oxide on hemoproteincatalyzed oxidative reactions. *Nitric Oxide* **1998**, 2, 37–44.
37. Arsenyan, P.; Shestakova, I.; Rubina, K.; Domracheva, I.; Nesterova, A.; Vosele, K.; Pudova, O.; Lukevics, E. Organoammonium hydroselenite: antitumor action through radical balance regulation. *Europ. J. Pharmacol.* **2003**, 465, 229–235.
38. Gutteridge, J.M.C.; Rowley, D.A.; Halliwell, B. Superoxide dependent formation of hydroxyl radical and lipid peroxidation in the presence of iron salt: detection of catalytic iron and antioxidant activity in extracellular fluids. *Biochem. J.* **1982**, 206, 605–609.
39. Huang, P.; Feng, L.; Oldham, E.A.; Keating, M.J.; Plunkett, W. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* **2000**, 407, 390–395.
40. Gewirtz, D.A. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin, and daunorubicin. *Biochem. Pharmacol.* **1999**, 57, 727–741.
41. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, 65, 55–63.

42. Granger, D.L.; Taintor, R.R.; Boockvar, K.S. Determination of nitrate and nitrite in biological samples using bacterial nitrate reductase coupled with the Griess reaction. *Methods Companion Meth. Enzymol.* **1995**, 7, 78–83.
43. Conrad, C.C.; Grabowski, D.T.; Walter, C.A.; Sabla, S.; Richardson, A. Using MT $^{-/-}$ mice to study metallothionein and oxidative stress. *Free Rad. Biol. Med.* **2000**, 28, 447–462.
44. Aebi, H. Catalase. In: *Method of Enzymatic Analysis.* Academic: New York, 1984, Vol. 2, pp. 673–684.
45. Paglia, E.D.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, 70, 158–169.