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Synthesis and cytotoxicity of silylalkylthio-substituted *N*-heterocycles and their hydroselenites

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New hydroselenites of the different silylalkylthio-substituted *N*-heterocycles have been prepared by the reaction of selenium dioxide with *N*-heterocycles in an aqueous medium. Their structure was confirmed by ^{1}H, ^{13}C, and ^{77}Se NMR data. Most of these silylalkylthio-substituted *N*-heterocycles and their hydroselenites have an expressed cytotoxic activity on the MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), and Neuro 2A (mouse neuroblastoma) cell lines. Some of the hydroselenites exhibit free-radical protection simultaneously with a high cytotoxic effect. The substances studied were also active *in vivo* against sarcoma S-180. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: antitumor activity; cytotoxicity; morphology; selenium; silicon

INTRODUCTION

Selenium is an essential trace element that has been shown to have anticarcinogenic activity. One mechanism that has been proposed for this activity is a cytotoxic effect of selenium on tumor cells.[1–3] A number of selenium compounds have been found to inhibit tumorigenesis in a variety of animal and cell models. Reactive oxygen species (ROS) mediate apoptosis induced by Na_2SeO_3, and mitochondria may be a major source of Na_2SeO_3-induced ROS.[4] The combination of ascorbic acid and sodium selenite may be a potent anticancer treatment option for human hepatoma cells. It may induce the redifferentiation of hepatoma cells and inhibit cell growth by virtue of enhancing the activities of antioxidative enzymes and by reducing the formation of hydrogen peroxide (H_2O_2), and altering the cell redox status.[5] Ammonium selenite–cisplatin conjugate $(NH_3)_4PtCl_2SeO_3$ may specifically inhibit telomerase activity in endometrial cancer cells.[6] The majority of organic base hydroselenites tested exhibited high activity *in vitro* on the tumor cell lines investigated. *o*-Phenanthrolinium selenite and imidazolinium selenite exhibit the highest cytotoxic effect on HT-1080, MG-22A, B16 and Neuro 2A cell lines. Besides that, most of the selenites synthesized are very active (0.5–0.6 μg ml^{-1}) against mouse melanoma B16. In a series of ethanolamine derivatives, *N*-methylethanolammonium selenite is more active in experiments with Neuro 2A ($TD_{50} = 1$ μg ml^{-1}), while triethanolammonium selenite effectively inhibits HT-1080 ($TD_{50} = 2.3$ μg ml^{-1}), being less toxic against normal cells 3T3 ($TD_{50} = 47.7$ μg ml^{-1}).[7] Hydroselenites are able to activate the nitric oxide generation level in various tumor cell cultures. The activation of nitric oxide production is especially increased on HT-1080 and MG-22A cell lines. The amount of nitric oxide depends on the type of tumor and the cation structure. According to the literature data and our results, we propose that the antitumor effect of hydroselenites stems from the fact that they are able to regulate the free radical balance *in vitro* and *in vivo*.[8] All the compounds studied are glutathione peroxidase inhibitors. Some derivatives may also prevent H_2O_2 degradation by inhibiting catalase. In general, tumor cells die by the apoptotic route under the action of selenium derivatives. Nitric oxide radical regulation plays the main role. A correlation between the cell morphology results and antitumor activity was found. Human fibrosarcoma HT-1080 cell apoptosis caused by hydroselenite testifies that the compound is able to inhibit tumor growth.[9]

Taking into account the importance of selenium as a trace element in the organism, our present investigation is connected with synthesis and antitumor activity studies in a series of hydroselenites of different silylalkylthio-substituted *N*-heterocycles.

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MATERIALS AND METHODS

Instrumental

^{1}H, ^{13}C, and ^{77}Se NMR spectra were recorded on a Varian 200 Mercury spectrometer at 200 MH_3, 50.3 MH_3 and 39.74 MHz respectively at 303 K in $CDCl_3$ or DMSO-d_6 solution. The ^{1}H and ^{13}C chemical shifts are given relative to tetramethylsilane; ^{77}Se chemical shifts relative to dimethyl selenide. Mass spectra were recorded on a Hewlett Packard apparatus (HP-6890, GC with HP5MS, 70 eV).

Synthesis

*Preparation of silylalkylthio-substituted N-heterocycles (**1–10**)*[10,11]

Finely powdered dry K_2CO_3 (25 mmol, 3.5 g) was added to a solution of 10 mmol of the thiol (**1** or **6**), 10 mmol of corresponding silyl-substituted alkyl iodide and 18-crown-6 (1 mmol, 264 mg) in 25 ml of toluene. The mixture was refluxed with stirring to achieve the disappearance of the substrates (gas chromatography control), filtered over the thin silica gel layer and concentrated under reduced pressure. The residue was purified by column chromatography using a toluene–ethyl acetate mixture as eluent.

2-[(Trimethylsilylmethyl)thio]quinoline (**1**). MS, *m/z* (I, %): 247 (M^+, 30).^{1}H NMR δ ppm: 0.16 (s, 9H, $SiMe_3$); 2.54 (s, 2H, CH_2); 7.12–7.28, 7.33–7.44, 7.56–7.72, 7.78–7.98 (m, 6H, ring protons).^{13}C NMR δ ppm: −1.56, 14.86, 120.50 (C-3), 124.97 (C-6), 125.26 (C-4a), 125.89 (C-5), 127.56 (C-7), 127.84 (C-8), 128.19 (C-4), 129.00 (C-8a), 129.49 (C-2). Anal. Found: C, 63.16; H, 6.88; N, 5.51; S, 13.10. Calc. for $C_{13}H_{17}NSSi$: C, 63.10; H, 6.93; N, 5.66; S, 12.96%.

2-[(3-Trimethylsilylpropyl)thio]quinoline (**6**). MS, *m/z* (I, %): 275 (M^+, 3).^{1}H NMR δ ppm: 0.01 (s, 9H, $SiMe_3$); 0.76 (m, 2H, $SiCH_2$); 1.80 (m, 2H, $CH_2CH_2CH_2$); 3.35 (m, 2H, SCH_2); 7.20–8.00 (m, 6H, ring protons). ^{13}C NMR δ ppm: −1.68, 16.59, 24.30, 33.20, 121.04 (C-3), 125.03 (C-6), 125.87 (C-4a), 127.55 (C-5), 128.01 (C-7), 129.48 (C-8), 135.11 (C-4), 148.36 (C-8a), 159.60 (C-2). Anal. Found: C, 65.32; H, 7.61; N, 5.10; S, 11.72. Calc. for $C_{15}H_{21}NSSi$: C, 65.40; H, 7.68; N, 5.08; S, 11.64%.

Preparation of hydroselenites of silylalkylthio-substituted N-heterocycles

To a suspension of selenium dioxide (1 mmol, 110 mg) in 1 ml of water and 1ml of ethanol, an equimolar amount of the corresponding silicon-containing heterocyclic sulfide **11–19** was added. The reaction mixture was stirred at room temperature until homogenization and then evaporated. The residue was washed with diethyl ether and recrystallized from ethanol or purified on silica gel.

2-[(Trimethylsilylmethyl)thio]quinolinium hydroselenite (**11**).^{1}H NMR δ ppm: 0.14 (s, 9H, $SiMe_3$); under DMSO (m, 2H, CH_2); 6.4 (bs, 1H, NH); 7.48 (m, 2H, H-5, H-6); 7.70 (m, 1H, H-7); 7.87 (m, 2H, H-3, H-4); 8.13 (m, 1H, H-8)^{13}C NMR δ ppm: −1.6, 14.1, 120.50 (C-3), 125.3 (C-6), 125.7 (C-4a), 127.3 (C-5), 128.0 (C-7), 129.9 (C-8), 135.6 (C-4), 147.6 (C-8a), 160.4 (C-2). ^{77}Se NMR δ ppm: 1321.88. Anal. Found: C, 41.41; H, 5.12; N, 3.80; S, 8.41. Calc. for $C_{13}H_{19}NO_3SSeSi$: C, 41.48; H, 5.09; N, 3.72; S, 8.52%.

N−Methyl-2-[(trimethylsilylmethyl)thio]imidazolinium hydroselenite (**12**).^{1}H NMR δ ppm: 0.06 (s, 9H, $SiMe_3$); 2.30 (s, 2H, CH_2); 3.56 (s, 3H, NCH_3); 4.70 (bs, 1H, NH); 6.91 and 7.21 (each d, 1H, $J = 1.24$ Hz, imidazole protons).^{13}C NMR δ ppm: −1.58, 18.41, 123.16 (C-5), 127.44 (C-4), 142.30 (C-2), 32.89 (NCH_3).^{77}Se NMR δ ppm: 1333.31. Anal. Found: C, 29.12; H, 5.47; N, 8.41; S, 9.65. Calc. for $C_8H_{18}N_2O_3SSeSi$: C, 29.17; H, 5.51; N, 8.51; S, 9.74%.

2-[(Trimethylsilylmethyl)thio]benzothiazolium hydroselenite (**13**).^{1}H NMR δ ppm: 0.16 (s, 9H, $SiMe_3$); 2.63 (s, 2H, SCH_2); 5.07 (bs, 1H, NH); 7.33 (t, 1H, $J = 7.2$ Hz, H-6); 7.46 (t, 1H, $J = 6.8$ Hz, H-5); 7.84 (d, 1H, $J = 8.0$ Hz, H-7); 7.99 (d, 1H, $J = 7.6$ Hz, H-3).^{13}C NMR δ ppm: −1.84, 18.26, 120.97 (C-7), 121.69 (C-4), 124.15 (C-6), 126.31 (C-5), 134.70 (C-7a), 153.10 (C-3a), 169.92 (C-2).^{77}Se NMR δ ppm: 1325.19. Anal. Found: C, 36.41; H, 4.87; N, 3.55; S, 16.18. Calc. for $C_{12}H_{19}NO_3S_2SeSi$: C, 36.35; H, 4.83; N, 3.53; S, 16.18%.

6-[(Trimethylsilylmethyl)thio]-9-(trimethylsilylmethyl) purinium hydroselenite (**14**).^{1}H NMR δ ppm: 0.04 and 0.14 (each s, 9H, $SiMe_3$); 2.56(s, 2H, SCH_2); 3.88 (s, 2H, NCH_2); 4.62 (bs, 1H, NH); 8.34 (s, 1H, H-8); 8.70 (s, 1H, H-2).^{13}C NMR δ ppm: −2.57, −1.8, 12.4, 34.4, 130.4 (C-5), 144.4 (C-8), 148.1 (C-4), 151.1 (C-2), 160.4 (C-6). ^{77}Se NMR δ ppm: 1321.58. Anal. Found: C, 34.43; H, 5.80; N, 12.35; S, 7.08. Calc. for $C_{13}H_{26}N_4O_3SSeSi_2$: C, 34.42; H, 5.78; N, 12.35; S, 7.07%.

2-[(3-Trimethylsilylpropyl)thio]quinolinium hydroselenite (**15**).^{1}H NMR δ ppm: 0.04 (s, 9H, $SiMe_3$); 0.73 (m, 2H, $SiCH_2$); 1.78 (m, 2H, $CH_2CH_2CH_2$); 3.34 (t, 2H, $J = 7.2$ Hz, SCH_2); 7.38–7.97 (m, 5H, ring protons); 8.19 (m, 1H, H-8).^{13}C NMR δ ppm: −1.58, 15.9, 24.0, 32.3, 121.0 (C-3), 125.3 (C-6), 125.7 (C-4a), 127.3 (C-5), 128.0 (C-7), 130.0 (C-8), 135.9 (C-4), 147.7 (C-8a), 159.0 (C-2).^{77}Se NMR δ ppm: 1322.12. Anal. Found: C, 44.61; H, 5.77; N, 3.50; S, 7.95. Calc. for $C_{15}H_{23}NO_3SSeSi$: C, 44.54; H, 5.73; N, 3.46; S, 7.93%.

N-Methyl-2-[(3-trimethylsilylpropyl)thio]imidazolinium hydroselenite (**16**).^{1}H NMR δ ppm: 0.03 (s, 9H, $SiMe_3$); 0.09 (s, 9H, $SiMe_3$); 0.71 (m, 2H, CH_2Si); 1.68 (m, 2H, $CH_2CH_2CH_2Si$); 3.11 (t, 2H, $J = 7.2$ Hz, SCH_2); 3.72 (s, 3H, NCH_3); 6.18 (bs, 1H, NH); 7.11 and 7.38 (each d, 1H, $J = 1$ Hz, in the imidazole ring).^{13}C NMR δ ppm: −1.16, 15.39, 24.20, 37.07, 123.11 (C-5), 127.94 (C-4), 140.27 (C-2), 32.98 (NCH_3).^{77}Se NMR δ ppm: 1330.24. Anal. Found: C, 33.65; H, 6.22; N, 7.87; S, 9.04. Calc. for $C_{10}H_{22}N_2O_3SSeSi$: C, 33.61; H, 6.20; N, 7.84; S, 8.97%.

2-[(3-Trimethylsilylpropyl)thio]pyrimidinium hydroselenite (**17**).^{1}H NMR δ ppm: 0.05 (s, 9H, $SiMe_3$); 0.70 (m, 2H, CH_2Si); 1.74 (m, 2H, CH_2 $\underline{CH_2}$ CH_2); 3.19 (t, 2H, $J = 7.2$ Hz, SCH_2); 3.98 (bs, 1H, NH); 7.27 (t, 1H, $J = 4.8$ Hz, H-5); 8.70 (d, 2H, $J = 4.8$ Hz, H-4, H-6).^{13}C NMR δ ppm: −1.70, 15.8, 23.7, 33.4, 117.0 (C-5), 157.6 (C-4, C-6), 171.2 (C-2).^{77}Se NMR δ ppm: 1322.30. Anal. Found: C, 34.04; H, 5.15; N, 7.94; S, 9.07. Calc. for $C_{10}H_{18}N_2O_3SSeSi$: C, 33.99; H, 5.13; N, 7.93; S, 9.07%.

2-[(3-Trimethylsilylpropyl)thio]benzothiazolium hydroselenite (**18**).^{1}H NMR δ ppm: −0.01 (s, 9H, $SiMe_3$); 0.66 (m,

2H, CH_2Si); 1.78 (m, 2H, $CH_2CH_2CH_2$); 3.35 (t, 2H, $J = 7.2$ Hz, SCH_2); 6.13 (bs, 1H, NH); 7.30–7.40 (m, 2H, H-5, H-6); 7.84 (m, 1H, H-7); 7.98 (m, 1H, H-4).^{13}C NMR δ ppm: −1.7, 15.6, 23.9, 36.2, 121.1 (C-7), 121.7 (C-4), 124.4 (C-6), 126.4 (C-5), 134.5 (C-7a), 152.8 (C-3a), 166.9 (C-2).^{77}Se NMR δ ppm: 1322.18. Anal. Found: C, 38.00; H, 5.11; N, 3.34; S, 15.52. Calc. for $C_{13}H_{21}NO_3S_2SeSi$: C, 38.04; H, 5.16; N, 3.41; S, 15.62%.

6-[(3-Trimethylsilylpropyl)thio]-9-(3-trimethylsilylpropyl) purinium hydroselenite (**19**).^{1}H NMR δ ppm: −0.05 and −0.04 (both s, 9H, $SiMe_3$); 0.42 (m, 2H, $S(CH_2)_2CH_2Si$); 0.66 (m, 2H, $N(CH_2)_2CH_2Si$); 1.75 (m, 4H, $CH_2CH_2CH_2$); 3.35 (t, 2H, $J = 7.2$ Hz, SCH_2); 4.21 (t, 2H, $J = 7.2$ Hz, NCH_2); 7.48 (s, 1H, H-8); 8.70 (s, 1H, H-2).^{13}C NMR δ ppm: −1.79, −1.65, 12.9, 15.8, 24.0, 24.2, 31.1, 46.2, 130.7 (C-5), 144.6 (C-8), 148.4 (C-4), 151.3 (C-2), 159.5 (C-6).^{77}Se NMR δ ppm: 1322.42. Anal. Found: C, 40.01; H, 6.70; N, 10.82; S, 6.21. Calc. for $C_{17}H_{34}N_4O_3SSeSi_2$: C, 40.06; H, 6.72; N, 10.99; S, 6.29%.

In vitro cytotoxicity assay

Monolayer tumor cell lines—MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma), normal mouse fibroblasts (NIH 3T3), and hamster endothelial cells (BHK 21)—were cultured in standard medium (Dulbecco's modified Eagle's medium; DMEM) without an indicator ('Sigma') and supplemented with 10% heat-inactivated fetal bovine serum ('Sigma'). Tumor cell lines were taken from the European Collection of Cell Culture (ECACC). After the ampoule had thawed, cells from one to four passages were used in three concentrations test compound: 1, 10 and 100 μg ml^{-1}. About $(2–5)\times10^4$ cells ml^{-1} (depending on the nature of the line) were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl.The control cells without test compounds were cultured on separate plate. The plates were incubated for 72 h, 37 °C, 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT). The quantity on the control plate was taken in calculations for 100%.[12,13] The concentration of NO was determined according to the Gryess method (by NO_2 level in the culture medium). Sodium nitrite standard solution was used for the calibration curve.[12] The TD_{50} was calculated using the program Graph Pad Prisim® 3.0, $r < 0.05$.

In vivo activity assay

The compounds were tested *in vivo* against sarcoma S-180 cells. Sarcoma S-180 (5×10^6) cells were inoculated *s.c.* into male ICR mice (6 weeks old, 18–20 g) on day 0. Drugs were administered *i.p.*; the treatment was started 24 h after tumor transplantation. The number of mice used in each group was between six and ten. The daily dose was 10 mg kg^{-1}; duration of treatment was 9 days. The efficacy of the treatment was estimated by the ellipsoid formula; the tumor volume V of the control group was taken as 100% in calculations. V was calculated from the equation: $V = 4\pi ab^2/3$, were a and b are the ellipsoid maximum and minimum diameters (the calculated volume was halved in the case of flat tumor shapes).[14]

RESULTS AND DISCUSSION

Chemistry

It has been shown that silylalkylthio-substituted *N*-heterocycles**1–10** readily react with selenic acid, H_2SeO_3 (formed during solution of selenium(IV) oxide in water), to give the corresponding ammonium hydroselenites **11–19** (Scheme 1). The reaction proceeds easily at room temperature with good to excellent yields (67–94%). In the case of quaternization by 2-[(trimethylsilylmethyl)thio]pyrimidine (**3**) the reaction with selenic acid proceeds with partial desilylation of the trimethylsilyl group. The ^{1}H, ^{13}C, and ^{77}Se NMR data confirm the formation of hydroselenites. The selenium signal in the ^{77}Se NMR spectra for all compounds appears in the 1321.9–1333.3 ppm region. The chemical shift of selenic acid, H_2SeO_3 (1292 ppm)[15], is similar to that for the selenites **11–19** studied.

Antitumor activity

The results of these experiments are summarized in Table 1. The majority of silylalkylthio-substituted *N*-heterocycles tested exhibited high activity *in vitro* on human fibrosarcoma HT-1080, mouse hepatoma MG-22A, mouse melanoma B16, and mouse neuroblastoma Neuro 2A tumor cell lines. There is no correlation between silylalkylthio chain length and cytotoxic activity. 2-[(Trimethylsilylmethyl)thio]quinoline (**1**) exhibits high cytotoxic effect on all cell lines (1.5–8.5 μg ml^{-1}), the prolongation of the methylene chain between silicon and sulfur from one to three (**6**) decreases activity on HT-1080 and Neuro 2A cell lines. The *N*-methylimidazole derivative **2** is less active than the trimethylsilylpropyl analogue **7** (1.0–2.0 μg ml^{-1}). The introduction of a pyrimidine group in the place of *N*-methylimidazole strongly decreases cytotoxicity on the tumor cell lines studied. Besides that, the 2-[(trimethylsilylmethyl)thio]benzothiazole (**4**, 2.0–9.0 μg ml^{-1}) is much more active than the trimethylsilylpropyl derivative **9**. The silylalkylated purines **5** (1.5–11.0 μg ml^{-1}) and **10** (2.0–13.0 μg ml^{-1}) have comparable cytotoxic action. It should be noted that the same

$$\underset{\textbf{1–10}}{(N\text{-Het})—S(CH_2)_nSiMe_3} \xrightarrow{H_2SeO_3} \underset{\textbf{11–19}}{H(N\text{-Het})^+—S(CH_2)_nSiMe_3\ HSeO_3^-}$$

Scheme 1.

concentrations of silylalkylthio-substituted *N*-heterocycles **1**–**10** are toxic both against tumor and normal mouse fibroblast and hamster endothelial cells. A preliminary analysis of the structure–activity relationship for the cytotoxic action clearly indicates the strong influence of the introduction of selenium on toxic effects *in vitro* (Table 1). 2-[(Trimethylsilylmethyl)thio]quinolinium hydroselenite (**11**) shows specific cytotoxic activity on HT-1080 (TD_{50} = 0.42 μg ml^{-1}); however, the trimethylsilylpropyl analogue **15** has no selectivity on the cell lines studied (TD_{50} = 1.0–3.5 μg ml^{-1}). Inspection of trimethylsilylthio-substituted *N*-methylimidazolinium hydroselenite **12** and benzothiazolium hydroselenite **13** cytotoxic data shows extended activity against human fibrosarcoma, mouse melanoma and neuroblastoma (0.4–3.5 μg ml^{-1}), except mouse hepatoma MG-22A. The introduction of the trimethylsilylpropyl chain (**16**, **18**) leads to high cytotoxicity on MG-22A (1.0–5.5 μg ml^{-1}). Silylalkylated purinium hydroselenites **14** (1.3–7.5 μg ml^{-1}) and **19** (1.0–5.0 μg ml^{-1}) exhibit similar cytotoxic activity.

Table 1. *In vitro* cell cytotoxicity and the ability of intracellular NO generation caused by substituted *N*-heterocycles **1**–**10** and their hydroselenites **11**–**19**[a]

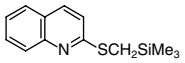
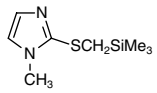
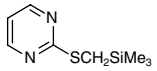
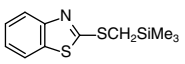
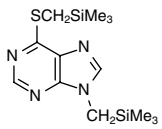
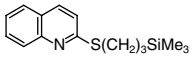
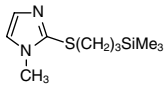
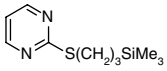
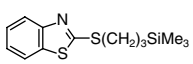
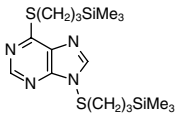
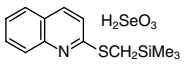
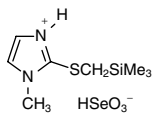
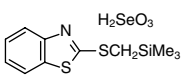
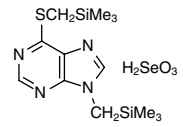
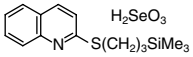
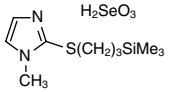
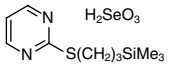
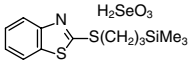
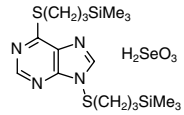
		HT-1080		MG-22A		B16		Neuro 2A		NIH 3T3	BHK 21
		TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	TD_{50}
1		2	250	3.5	125	1.5	150	8.5	500	20	6
2		20.5	300	15.5	400	5.5	650	13	800	**	4
3		*	13	120	14	9.5	300	20	60	**	21
4		2	450	6	200	3	150	9	500	2.5	37
5		1.5	650	4	250	11	350	2	550	4.5	22
6		72	250	3.8	200	2.5	1100	28	1100	**	27
7		1	500	1	300	1.5	250	2	600	1	12
8		18	450	2	300	1.7	300	*	11	*	12
9		32	117	22	350	*	9	*	18	*	67
10		13	450	2	500	4	300	12	500	23	3
11		0.42	250	18.5	450	4	600	4	750	122	2.4
12		0.4	300	47	43	3.5	650	2	800	7	2
13		3.5	30	22	40	3	300	1.2	400	4	1.9

Table 1. (*Continued*)

		HT-1080		MG-22A		B16		Neuro 2A		NIH 3T3	BHK 21
		TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	NO 100%	TD_{50}	TD_{50}
14		1.3	250	7.5	300	3.5	250	17	217	2	7
15		2	250	3.5	450	1	250	17	500	4	26
16		1	500	1	250	1.25	300	1.67	40	0.15	0.22
17		1	250	7.5	600	1	200	*	11	*	1.4
18		2	200	5.5	400	3	150	11	500	2.5	35
19		1	40	18.5	250	4	300	5	500	13	15

[a] TD_{50}: concentration (μg ml^{-1}) providing 50% cell killing effect [(CV+MTT)/2]. NO concentration (%) (CV: coloration).
* no cytotoxic effect.
** not tested.

The role of NO in biosystems has attracted considerable attention in the last decade. NO is formed by enzymatic and non-enzymatic mechanisms. Because of its low molecular weight and high lipophilicity, NO has good diffusion properties. It may act not only in the cell where it is produced, but also in nearby tissues. Biologically produced NO originates from oxygen and L-arginine in a reaction catalyzed by NO synthase. NO, a long-lived radical with a wide range of actions, is known as a regulator of a variety of biological processes.[16] The NO level was determined according to Ref 12, and NO release was defined using the Greyss reagent (by NO_2 concentration in the culture medium). The yield of nitrite was expressed as nanomoles of NO_2/200 μl of cultural medium in testing plates for 100% alive cells after CV coloration assay (selenites concentration 100 μg ml^{-1}). It was shown (Table 1) that compounds **1**–**10** readily increase NO concentration in the culture medium on all cell lines. This effect is especially expressed in the case of 2-[(3-trimethylsilylpropyl)thio]quinoline (**6**, $TG_{100} = 1100\%$) on B16 and Neuro 2A tumor cell lines. In the series of hydroselenites **11**–**19,** free-radical protection was also present. 2-[(Trimethylsilylmethyl)thio]benzothiazolium hydroselenite (**13**) inhibits nitric oxide generation by 70% ($TG_{100} = 30\%$ on HT-1080). *N*-Methylimidazolinium hydroselenite **12** ($TG_{100} = 43\%$ on MG-22A), trimethylsilylpropyl analogue **26** ($TG_{100} = 40\%$ on Neuro 2A), and purinium hydroselenite **19** ($TG_{100} = 40\%$ on HT-1080) also exhibit free-radical protection activity simultaneously with high cytotoxic effect (Table 1). In the case of the hydroselenites **11**–**19**, the role of thiol is played by glutathione. Moreover, hydroselenites may regulate the level of glutathione in the cell, because selenium-containing derivatives are glutathione peroxidase inhibitors. According to our results, there is no correlation between the ability to inhibit glutathione peroxidase and the efficacy to generate NO. The increase in NO depends on the type of tissue, the prodrug dose, etc. According to the literature, the conversion of L-arginine to L-citrulline with the production of NO is catalyzed by heme iron.[17] Our results indicate that the organoammonium hydroselenites investigated may be promising antitumor agents with an action mediated by the regulation of radical formation. The hydroselenite compounds **11**–**19** disrupt the different radical-dependent systems and slowly normalize the biochemical processes in the cell.

Antitumor activity of silylalkylthio-substituted *N*-heterocycles **1**–**10** against sarcoma S-180 was determined for male ICR mice (18–20 g). 2-[(Trimethylsilylmethyl)thio]quinoline (**1**), increases sarcoma growth by 45% at a dose of 2 mg kg^{-1} in 9 days. Besides, prolongation of the silylalkyl chain leads to the opposite effect (**6**, inhibition by 12%). Trimethylsilylmethyl pyrimidine **3** has no effect on the speed of S-180 growth; however, the silylpropyl analogue **8** undesirably increases tumor growth by 87%. The best result in the series of hydroselenites of silylalkylthio-substituted *N*-heterocycles **1**–**10** is shown by 6-[(3-trimethylsilylpropyl)thio]-9-(3-trimethylsilylpropyl)purine (**10**, inhibition by 51%). Generally, the conversion of bases

1–10 into their hydroselenites (**11**–**19**) leads to more effective tumor growth inhibition. Inspection of the *in vivo* activity of hydroselenites **13**–**17** shows sarcoma inhibition by 10–39% in 9 days. In the series of hydroselenites **11**–**19,** 6-[(trimethylsilylmethyl)thio]-9-(trimethylsilylmethyl)purinium hydroselenite (**14**, inhibition by 39%) shows the greatest level of tumor growth inhibition, but introduction of a silylpropyl chain into the molecule (**19**) completely removed sarcoma S-180 inhibiting properties.

CONCLUSIONS

It has been found that silylalkylthio-substituted *N*-heterocycles and their hydroselenites show significant potency *in vitro* against different tumor cell lines. Inspection of the cytotoxicity shows that hydroselenites are more active than the initial *N*-heterocycles. Some of the hydroselenites exhibit free-radical protection simultaneously with a high cytotoxic effect. *In vivo* evaluation of silylalkylated *N*-heterocycles and their hydroselenites confirmed antitumor activity against sarcoma S-180.

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