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Synthesis, structure and cytotoxicity of organoammonium selenites†

Edmunds Lukevics*, Pavel Arsenyan, Irina Shestakova, Ilona Domracheva, Iveta Kanepe, Sergey Belyakov, Juris Popelis and Olga Pudova

Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia

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New selenites of different organic bases have been prepared by the reaction of selenium dioxide with aliphatic and heterocyclic amines in an aqueous medium. Their structure was confirmed by ^{1}H, ^{13}C and ^{77}Se NMR data and, in the case of triethanolammonium hydroselenite [$HN^{+}(CH_2CH_2OH)_3$ $HSeO_3$], by X-ray analysis. Most of these selenites 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. The substances studied were also active *in vivo* against sarcoma S-180. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: antitumor activity; cytotoxicity; morphology; selenium; X-ray

INTRODUCTION

Selenium ranks 70th in abundance among the elements and constitutes approximately 10^{-5}% of the Earth's crust.[1] In biology it was long considered as a dangerous poison, until it was identified as an essential trace element for animals. The fundamental importance of selenium for biology has become increasingly clear as new research has shown an unsuspected role of this element for human health.[2,3] As selenocysteine, selenium is a component of selenoproteins, some of which have important enzymic functions, in this context being best known as an antioxidant and catalyst in the reduction of hydrogen peroxide; and the family of selenium-dependent glutathione peroxidases is involved on the processing of lipid or phospholipid hydroperoxides to harmless products.[4] Selenium has additional important effects, particularly in relation to the immune function.[5] Selenium deficiency is also linked to the progress of some viral infections.[6] Administration of trace quantities of Na_2SeO_3 or Na_2SeO_4 can successfully treat Keshan disease.[7]

Organic and inorganic selenium derivatives are effective in inhibition of a wide range of tumors. There is evidence that selenium protects against certain human cancers.[3,8–11] In order to investigate the relationship between serum selenium content and the risk of cancer, some thousands of serum samples were obtained from cancerous and non-cancerous patients. In this cohort, the incidence of cancer in the non-cancerous patients was followed for the subsequent 3 years. The serum selenium level of non-cancerous patients who later developed cancer during the 3 years was determined and compared with that of the non-cancerous patients. A higher incidence of cancer was observed in the lower serum selenium patients of the non-cancerous group. The serum selenium level in cancerous patients was significantly lower than in non-cancerous patients. These results suggest that the low serum selenium level in cancerous patients may not be induced by the tumor, but it was more likely already present before the tumor. The ratio between the non-cancerous and cancerous groups was estimated to be 1.95:1, suggesting a higher risk of cancer in the low serum selenium group.[10]

Taking into account the importance of the selenium element in the organism, pyridinium, quinolinium, bipyridinium, phenanthrolinium, imidazolinium, benzimidazolinium and ethanolammonium selenites were synthesized to study the influence of cation structure on cytotoxic effect. Their antitumor activity on HT-1080, MG-22A, B-16 and Neuro 2A cell lines and *in vivo* (S-180) was investigated.

*Correspondence to: E. Lukevics, Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia.
E-mail: pavel.arsenyan@lycos.com

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MATERIALS AND METHODS

Crystal data determination

The crystals of compound **13** are monoclinic, space group

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$P2_1$; the lattice constants are as follows: $a = 8.043(1)$, $b = 5.3468(8)$, $c = 12.543(1)$ Å, $\beta = 93.66(1)°$, $Z = 2$, $V = 538.3(1)$ Å^3, $F(000) = 284$, $D_c = 1.716(1)$ g cm^{-3}; Mo Kα radiation, $\lambda = 0.710\ 69$ Å, $\mu = 3.494$ mm^{-1}. A single crystal of dimensions $0.25 \times 0.40 \times 0.40$ mm^3 was used for X-ray measurements at room temperature on a Syntex P 2_1 diffractometer. The intensity data were collected to a maximum 2θ of 50° by θ–2θ scans. The total number of independent reflections measured was 1077, of which 979 reflections were considered as observed by criterion $|F| > 4.0\sigma(F)$. No absorption correction was applied. The structure was solved by the heavy-atom method and refined by full-matrix least squares with anisotropic temperature factors to $R = 0.0434$ for observed data ($R = 0.0479$ for all data; goodness of fit is 1.061). Flack's x parameter is 0.02(3). All calculations were carried out using the SHELXL-93 program.[12] Atomic coordinates and components of temperature factor tensors are deposited at the Cambridge Crystallographic Data Centre (the CCDC deposition number is 141712).

Instrumental

^{1}H, ^{13}C, and ^{77}Se NMR spectra were recorded on a Varian 200 Mercury spectrometer at 200 MHz, 50.3 MHz and 39.74 MHz respectively at 303 K in D_2O–Me_3COH solution. The ^{1}H and ^{13}C chemical shifts are given relative to tetramethylsilane (TMS); ^{77}Se is relative to dimethyl selenide.

Synthesis of hydroselenites

To a solution of the base (0.02 mol) in 50 ml of water an equimolar amount of selenium dioxide was added. The reaction mixture was stirred for 1 h at room temperature. The residue was recrystallized from ethanol or purified on silica gel.

Pyridinium hydroselenite (1)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.25–7.32 (2H, m, arom); 7.62–7.70 (1H, m, arom); 8.6–8.63 (2H, m, arom). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 125.45, 139.1, 145.2. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1283.7. Found: C, 29.01; H, 3.21; N, 6.65. Calc. for $C_5H_7NO_3Se$: C, 28.86; H, 3.39; N, 6.73%.

4-Hydroximinomethylpyridinium hydroselenite (2)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.71 (2H, dd, $J = 1.6$ Hz, $J = 4.4$ Hz); 8.74 (2H, dd, $J = 1.6$ Hz, $J = 4.4$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 116.0, 119.8, 125.5, 148.9. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1282.4. Found: C, 28.71; H, 2.85; N, 11.28. Calc. for $C_6H_7N_2O_4Se$: C, 28.82; H, 2.82; N, 11.2%.

4-Amidohydroximinomethylpyridinium hydroselenite (3)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 8.2 (2H, dd, $J = 1.8$ Hz, $J = 6$ Hz); 8.5 (2H, dd, $J = 1.8$ Hz, $J = 6$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 122.8, 142.0, 146.3, 150.9. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1281.6. Found: C, 27.11; H, 3.14; N, 15.8. Calc. for $C_6H_8N_3O_4Se$: C, 27.18; H, 3.04; N, 15.85%.

Quinolinium hydroselenite (4)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.85–7.92 (1H, m); 8.0–8.16 (4H, m); 9.02 (1H, d, $J = 8.2$ Hz); 9.14 (1H, d, $J = 5.2$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 118.0; 119.6, 126.5, 127.0, 128.0, 141.9, 145.2. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1287.4. Found: C, 41.75; H, 3.43; N, 5.30. Calc. for $C_9H_9NO_3Se$: C, 41.88; H, 3.51; N, 5.43%.

6-Bromoquinolinium hydroselenite (5)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.81–7.9 (1H, m); 8.1–8.26 (3H, m); 9.22 (1H, d, $J = 8.2$ Hz); 9.28 (1H, d, $J = 5.2$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 118.4; 120.6, 127.5, 127.8, 131.0, 143.9, 148.1. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1288.5. Found: 32.11; H, 2.25; N, 4.03. Calc. for $C_9H_8BrNO_3Se$: C, 32.07; H, 2.39; N, 4.16%.

o-Phenanthrolinium hydroselenite (6)

M.p. 123–124 °C (from ethanol–water). ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.5 (2H, s); 7.78 (2H, dd, $J = 4.8$ Hz, $J = 8.2$ Hz); 8.34 (2H, dd, $J = 1.4$ Hz, $J = 8.2$ Hz); 8.78 (2H, dd, $J = 1.2$ Hz, $J = 4.8$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 124.1, 125.9, 127.9, 136.0, 140.0, 146.0. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1291.8. Found: C, 46.42; H, 3.12; N, 9.16. Calc. for $C_{12}H_{10}N_2O_3Se$: C, 46.62; H, 3.23; N, 9.06%.

2,2′-Bipyridinium hydroselenite (7)

M.p. 53–54 °C (from ethanol). ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.86 (2H, dt, $J = 2.8$ Hz; $J = 5.6$ Hz, $J = 11.2$ Hz); 8.31–8.37 (4H, m); 8.8 (2H, d, $J = 5.2$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 121.6, 124.9, 140.8, 144.4, 146.0. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1290.1. Found: C, 42.12; H, 3.55; N, 9.88. Calc. for $C_{10}H_{10}N_2O_3Se$: C, 42.12; H, 3.53; N, 9.82%.

4,4′-Bipyridinium hydroselenite (8)

M.p. 184–185 °C (from ethanol). ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 8.14 (4H, dd, $J = 1.2$ Hz, $J = 5$ Hz); 8.85 (4H, dd, $J = 1.2$ Hz, $J = 5$ Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 126.8, 148.8, 151.7. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1290.4 Found: C, 42.1; H, 3.47; N, 9.78. Calc. for $C_{10}H_{10}N_2O_3Se$: C, 42.12; H, 3.53; N, 9.82%.

Imidazolinium hydroselenite (9)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.49 (2H, s); 8.67 (1H, s). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 117.6, 118.2, 132.6. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1288.4. Found: C, 18.15; H, 3.07; N, 14.18. Calc. for $C_3H_6N_2O_3Se$: C, 18.28; H, 3.07; N, 14.21%.

Benzimidazolinium hydroselenite (10)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 7.58 (4H, dqui, J = 3.2 Hz, J = 25.2 Hz); 9.00 (1H, s). ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1323.9. Found: C, 33.04; H, 3.40; N, 11.12. Calc. for $C_7H_8N_2O_3Se$: C, 34.03; H, 3.26; N, 11.34%.

N-Methylethanolammonium hydroselenite (11)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 2.74 (3H, s); 3.17 (2H, t, J = 5.4 Hz); 3.84 (2H, t, J = 5.4 Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 30.8, 48.6, 54.6. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1289.4. Found: C, 17.55; H, 5.33; N, 6.71. Calc. for $C_3H_{11}NO_4Se$: C, 17.65; H, 5.43; N, 6.86%.

N-Methyldiethanolammonium hydroselenite (12)

The pure product was isolated by column chromatography on silica gel with ethanol as eluent. Oil. ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 2.96 (3H, s); 3.37 (4H, t, J = 5 Hz); 3.94 (4H, t, J = 5 Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 38.7, 53.3, 55.5. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1293.3. Found: C, 24.03; H, 5.93; N, 5.43. Calc. for $C_5H_{15}NO_5Se$: C, 24.20; H, 6.09; N, 5.65%.

Triethanolammonium hydroselenite (13)

M.p. 94 °C (from ethanol–water). ^{1}H NMR (Me_3COH–D_2O/TMS) δ (ppm): 3.51 (6H, t, J = 4.2 Hz); 3.99 (6H, t, J = 4.2 Hz). ^{13}C NMR (Me_3COH–D_2O/TMS) δ (ppm): 53.3, 53.6. ^{77}Se NMR (D_2O/SeO_2) δ (ppm): 1293.0. Found: C, 25.88; H, 6.15; N, 5.03. Calc. for $C_6H_{17}NO_6Se$: C, 25.90; H, 6.21; N, 4.94%.

In vitro cytotoxicity assay

Monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and normal mouse fibroblast cells were cultivated with standard medium (Dulbecco's modified Eagle's medium) without an indicator ('Sigma') supplemented with 10% heat-inactivated fetal bovine serum ('Sigma'). After the ampoule was defrosted the cells were used for only one to four passages. Cells in the range of (2–5) $\times 10^4$ (cells/ml) (depending on line nature) were placed on 96-well plates immediately after compounds were inoculated to wells. The control cells, without test compounds, were cultivated on separate plates. The plates were cultivated for 72 h, at 17 °C, in 5% CO_2. The quantity of surviving cells was determined using crystal violet (CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT). The quantity of live cells on the control plate was taken in calculations as 100%.[13,14] The concentration of NO was determined according to the Gryess method (by NO_2 level in cultural medium). Sodium nitrite standard solution was used for the calibration curve.[13]

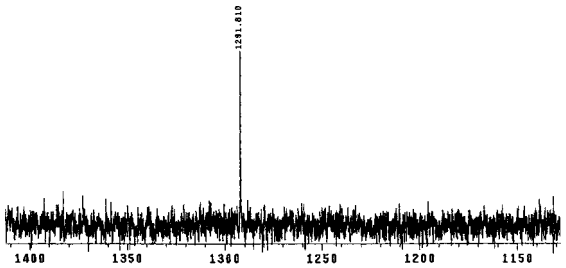


Figure 1. ^{77}Se NMR spectrum of *o*-phenanthrolinium hydroselenite (**6**).

In vivo activity assay

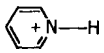
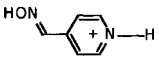
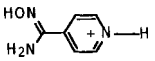
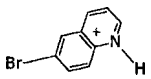
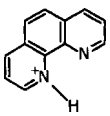
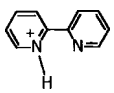
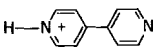
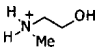
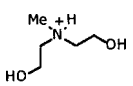
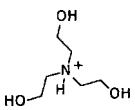
The compounds were tested *in vivo* against sarcoma S-180 cells. Sarcoma S-180, 5×10^6 cells were inoculated *s.c.* to male ICR mice (6 weeks old, 18–20 g) on day 0. Drugs were administered *i.p.*; the treatment was started 4 h after tumor transplantation. The number of mice used in each group was between six and ten. The efficacy of the treatment was estimated by the ellipsoid formula; the volume V (mm^3) of tumor in the control group was taken in calculations as 100%.

RESULTS AND DISCUSSION

Chemistry

An aqueous solution of ammonia reacts with selenium dioxide to form $NH_4H_3(SeO_3)_2$.[15] $(NH_4)_2Se_2O_5$ crystallizes from a selenoic acid solution, half-neutralized with NH_4OH, above 32 °C.[16] We have shown that aromatic and aliphatic amines readily react with selenic acid (H_2SeO_3), forming during solution of selenium(IV) oxide in water, to give the corresponding ammonium hydroselenites **1–13**. Reaction proceeds easily at room temperature with good to excellent yields (67–94%) (Scheme 1, Table 1). The ^{1}H, ^{13}C, and ^{77}Se NMR data confirm the formation of ammonium selenites. The selenium signal in the ^{77}Se NMR spectra for all compounds appears in the 1281.6–1293.3 ppm region. A typical ^{77}Se NMR spectrum for *o*-phenanthrolinium hydroselenite (**6**) is presented in Fig. 1. The chemical shift of H_2SeO_3 (1292 ppm)[17] is similar to that for the selenites **1–13** The molecular structure of triethanolammonium hydroselenite (**13**) (the compound that provided the best crystals) was determined by X-ray diffraction (Fig. 2). Three intramolecular hydrogen N—H···O bonds determine the conformation of the cation **13** framework. A similar situation occurs in other

Table 1. Triorganylammonium selenites **1–13**

No.	$BaseH^+$	Compound	Yield (%)
1		*N*-Pyridinium hydroselenite	68
2		4-Hydroximinomethylpyridinium hydroselenite	80
3		4-Amidohydroximinomethylpyridinium hydroselenite	94
4		Quinolinium hydroselenite	87
5		6-Bromoquinolinium hydroselenite	67
6		*o*-Phenanthrolinium hydroselenite	78
7		2,2′-Bipyridinium hydroselenite	81
8		4,4′-Bipyridinium hydroselenite	80
9		Imidazolinium hydroselenite	94
10		Benzimidazolinium hydroselenite	80
11		*N*-Methylethanolammonium hydroselenite	79
12		*N*-Methyldiethanolammonium hydroselenite	76
13		Triethanolammonium hydroselenite	75

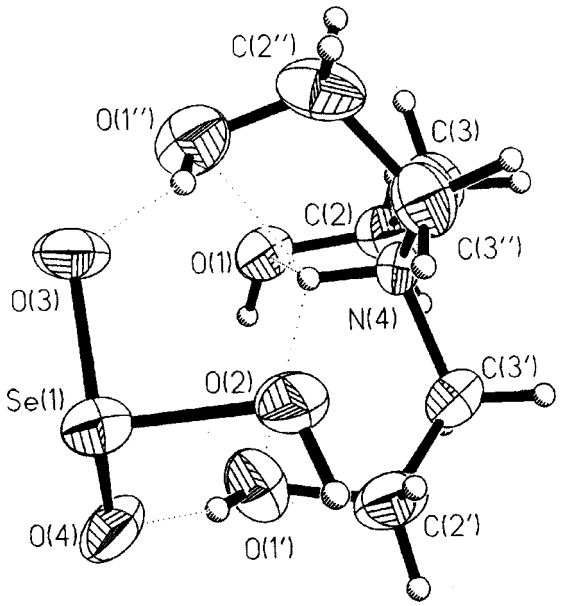


Figure 2. Molecular structure of the triethanolammonium hydroselenite (**13**).

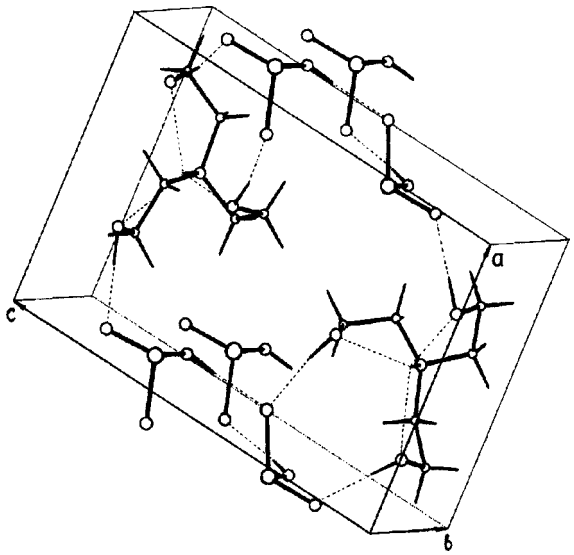


Figure 3. Unit cell of the triethanolammonium hydroselenite (**13**).

triethanolammonium salts. By means of these intramolecular bonds the triethanolammonium cations of chloride,[18] bromide,[19] iodide,[20] nitrate,[21] perchlorate,[22] *p*-chlorophenylthioacetate,[23] tetrachloroantimonate,[24] as well as selenite **13**, form a tricyclic system analogous to atranes.[25–27] The deviation of the nitrogen atom from the C3, C3′, C3″ plane in compound **13** is 0.425(6) Å. It should be noted that the tricyclic system is not formed in the triethanolamine structure,[28,29] where intramolecular hydrogen bonds are absent. The crystal structure of **13** is stabilized by intermolecular hydrogen bonds of the O—H···O type. The packing diagram for the structure **13** is illustrated in Fig. 3. Table 2 gives the parameters of the hydrogen bonds in selenite **13**.

$$\text{Base} + SeO_2 + H_2O \longrightarrow (\text{BaseH})^+HSeO_3^-$$

Scheme 1.

Antitumor activity

Cytotoxic activity of the synthesized selenites **1–13** was tested *in vitro* on four monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and normal mouse fibroblast cells. Concentrations providing 50% of tumor death effect (TD_{50}) were determined according to known procedures[30] using 96-well plates and two independent coloration methods: (a) coloration with CV, specifying the integrity of cell membranes; (b) coloration with MTT, characterizing the redox activity in cells.

The results of these experiments are summarized in Table 3. The majority of compounds tested exhibited high activity *in vitro* on the tumor cell lines investigated. *o*-Phenanthrolinium selenite (**6**) and imidazolinium selenite (**9**) have the highest cytotoxic effect, on HT-1080, MG-22A, B16 and Neuro 2A cell lines. Besides that, most of the selenites synthesized (**3, 5, 7, 8** and **9**) are very active (0.5–0.6 μg ml^{-1}) against mouse melanoma B16. It should be noted that, in the same concentrations, selenite **6** is toxic against both tumor and normal mouse fibroblast cells. Compound **9** more selectively inhibits tumor cells. 2,2′-Bipyridinium selenite (**7**) and its 4,4′-isomer **8** have a comparable cytotoxic activity; however, 4,4′-bipyridinium selenite (**8**) is considerably less

Table 2. Hydrogen bond parameters in triethanolammonium hydroselenite (**13**)

Hydrogen bridge D—H···A	D···A (Å)	D—H (Å)	H···A (Å)	D—H···A (deg)	Atom A symmetry
N4—H4···O1	2.778(7)	0.957	2.297	110.3	*x, y, z*
N4—H4···O1′	2.735(7)	0.957	2.296	107.1	*x, y, z*
N4—H4···O1″	2.806(7)	0.957	2.335	109.6	*x, y, z*
O1′—H1′···O4	2.680(6)	0.934	1.758	168.3	$-x, y-\frac{1}{2}, 1-z$
O1″—H1″···O3	2.722(6)	1.023	1.784	150.5	$-x, y-\frac{1}{2}, 1-z$
O1—H1···O1	2.684(6)	0.995	1.689	179.7	$1-x, y-\frac{1}{2}, 1-z$
O2—H2···O4	2.687(8)	0.887	1.802	174.6	$-x, y+\frac{1}{2}, 1-z$

Table 3. *In vitro* cell cytotoxicity and the ability of intracellular NO generation caused by selenites **1–13**

No.	HT-1080			MG-22A			Neuro 2A			B16			3T3	
	TD_{50} (μg ml^{-1})		TG_{100}	TD_{50} (μg ml^{-1})		TG_{100}	TD_{50} (μg ml^{-1})		TG_{100}	TD_{50} (μg ml^{-1})		TG_{100}	TD_{50} (μg ml^{-1})	
	CV[a]	MTT[b]	NO (%)[c]	CV[a]	MTT[b]	NO (%)[c]	CV[a]	MTT[b]	NO (%)[c]	CV[a]	MTT[b]	NO (%)[c]	CV[a]	MTT[b]
1	4.2	4.7	100	6.7	8	67	4	3	300	7	7	100	–[d]	–[d]
2	–[e]	–[e]	5	–[e]	–[e]	7	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
3	4.4	2.7	24	5.5	3.6	13	3.3	2.3	38	0.6	0.7	50	6.5	6.6
4	5.6	6.2	150	5	10	200	9	10	300	52.5	44	19	–[e]	46.2
5	5.3	5	38	6	5.4	21	2	1.6	33	0.5	0.8	80	–[e]	–[e]
6	0.6	0.5	50	0.1	0.7	750	0.5	0.6	200	1.5	6	42	0.8	0.9
7	5	5	50	3	3.3	43	3	1	45	0.6	0.7	150	8.2	12
8	5.6	3.3	38	4.8	3.6	21	0.8	0.9	38	0.5	0.6	200	–[e]	–[e]
9	0.85	0.8	70	0.9	0.8	25	0.8	0.8	31	0.5	0.6	133	9	8.2
10	5.3	4	200	7.2	7.2	200	7.9	5.9	21	4.7	5.6	250	3.7	4.2
11	6.5	7	350	6	10	40	1.7	1	400	6.6	5.7	125	13	9.2
12	4.25	4.5	400	7	10	88	6	5	267	58.4	7.6	30	10.7	13.4
13	3.15	4	150	7.6	6.7	100	7	10	32	80	140	32	47.7	50

[a] CV: coloration.
[b] MTT: coloration.
[c] NO concentration (CV: coloration).
[d] Not tested.
[e] No cytotoxic effect.

toxic on fibroblasts than its 2,2′-analogue **7**. In the series of the ethanolamine derivatives **11–13**, *N*-methylethanolammonium selenite (**11**) is more active in experiments with Neuro 2A (TD_{50} = 1 μg ml^{-1} in the MTT test), whereas triethanolammonium selenite (**13**) effectively inhibits HT-1080 (TD_{50} = 2.3 μg ml^{-1} in the CV test) and is less toxic against normal 3T3 cells (TD_{50} = 47.7 μg ml^{-1} in the CV test).

The NO level (as a possible indicator of cytoxic activity) was determined according to Ref. 13. NO release was defined using the Gryess reagent (by NO_2 concentration in the cultural medium). The yield of nitrite was expressed as NO_2 nmol/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 3) that compounds **1, 4**, and **10–13** readily increase NO concentration in the cultural medium on the HT-1080 line. This effect is especially expressed in the case of ethanolammonium selenites **11** and **12** (TG_{100} = 350–400%). Hydroselenites **6** and **11** show the highest NO generation in MG-22A (TG_{100} = 750%) and Neuro 2A (TG_{100} = 400%) cell lines respectively. There is no correlation between NO generation ability and the cytotoxic effects of the salts studied, which is obviously connected with specific tissue features of the cell lines.

However, according to our investigations, the ammonium selenites studied change the cell phenotype. The changes in normal cell phenotype and the combination of high cytotoxicity can lead to an unfavorable influence on experiments *in vivo*. Some of the substances studied changed fibroblast morphology: increasing cell sizes and forming stretched cell units. That is why we have studied *in vivo* two compounds: *o*-phenathrolinium hydroselenite (**6**; salt with high *in vitro* cytotoxicity) and triethanolammonium hydro-

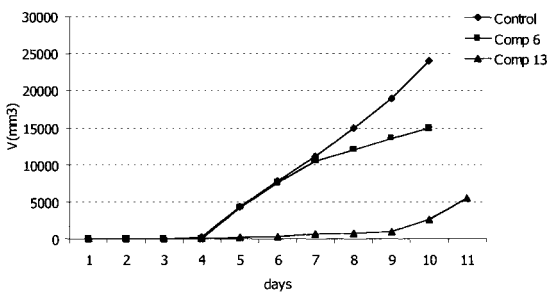


Figure 4. Inhibition of sarcoma S-180 volume growth *V* by hydroselenites **6** and **13**.

Table 4. Antitumor activity of hydroselenites (ICR mice; S-180)

Compound	Dose (mg kg^{-1})	Scheme of administration, *i.p.* (days)	Inhibition of S-180 growth (control 9 days) (%)
6	10	1, 2, 4, 7, 8	39 ± 6.2
13	10	1, 2, 4, 7, 8, 9	81 ± 3.8
13	8	1, 2, 3, 4, 7, 8, 9, 10	73 ± 2.5

selenite (**13**; derivative with slight influence on normal cell morphology and medium cytotoxicity) (Table 3).

o-Phenanthrolinium hydroselenite (**6**), the most active *in vitro*, and triethanolammonium selenite (**13**), the most selective, were selected for extended investigations of antitumor activity against sarcoma S-180 determined for male ICR mice (18–20 g). These compounds are very toxic (LD_{50} = 12.9 mg kg^{-1} for *o*-phenanthrolinium hydroselenite (**6**) and 25.8 mg kg^{-1} for triethanolammonium hydroselenite (**13**), *i.p.*). A dose of 10 mg kg^{-1} of selenite **6** has undesirable effects and leads to tonus decrease after injection. However, a 39% inhibition of tumor growth was observed. The same concentration of triethanolammonium selenite (**13**) inhibited tumor growth by 81%. In a dose of 8 mg kg^{-1} the tumor inhibition by compound **13** was slightly decreased (73%), however, in this case the undesirable influence was not detected (Fig. 4, Table 4).

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