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Amino-acid hydroselenites: synthesis and cytotoxicity†

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Amino acids, di- and tri-peptides readily react with selenic acid (H_2SeO_3, formed during solution of selenium(IV) oxide in water) to give the corresponding ammonium hydroselenites. Most selenites synthesized are very active (0.4–11 μg ml^{-1}) against mouse hepatoma MG-22A and readily increase NO concentration in the cultural medium on the HT-1080 line (up to TG_{100} = 1500%). The amino-acid hydroselenites studied influenced the cell phenotype. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: cell phenotype; cytotoxicity; hydroselenite; selenium

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.[1,2] Brief exposure of HeLa cells to micromolar concentrations of selenite resulted in significant inhibition of tumor cell colony formation, indicating that this is an assay for selenite cytotoxicity.[3] There was no significant difference in the potency of inhibition of development of mammary tumors, drug resistant and drug non-resistant human ovarian tumor cells by selenate, selenite, selenium dioxide, selenomethionine and selenocysteine.[4–7] Sodium selenite and ebselen (a stimulator and a mimic respectively of glutathione (GSH) peroxidase activity) displayed remarkable protective effects against 15(*S*)-hydroperoxyeicosatetraenoic acid (15-HPETE)-induced cytotoxicity. These results suggest that intracellular GSH plays a pivotal role in the protection against 15-HPETE-induced endothelial cell injury, and that decreased activity of GSH peroxidase activity is involved in 15-HPETE-induced cytotoxicity.[8] Selenocysteine and sodium selenite demonstrated significant redox chemistry, including depletion of cellular GSH, stimulation of GSH reductase, and stimulation of oxygen consumption. The interaction of these two compounds with GSH suggests an intriguing potential role for them in cancer therapy.[9] 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 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 (IC_{50} = 1 μg ml^{-1}), whereas triethanolammonium selenite effectively inhibits HT-1080 (IC_{50} = 2.3 μg ml^{-1}) and is less toxic against normal 3T3 cells (IC_{50} = 47.7 μg ml^{-1}). The substances studied were also active *in vivo* against sarcoma S-180.[10]

In the current part of the work we present the synthesis of various hydroselenites of amino acids, di- and tri-peptides. Their cytotoxic activity *in vitro* on HT-1080, MG-22A, B-16, Neuro 2A, BHK 21 and NIH 3T3 cell lines was determined.

MATERIALS AND METHODS

Instrumental

^{1}H and ^{77}Se NMR spectra were recorded on a Varian 200 Mercury spectrometer at 200 MHz and 39.74 MHz respectively at 303 K in D_2O–Me_3COH solution. The ^{1}H chemical shifts are given relative to tetramethylsilane; ^{77}Se is relative to dimethyl selenide. Elemental analyses (C, N, H) of hydroselenites are in agreement with calculated values.

Synthesis of hydroselenites

To a solution of the amino acid (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.

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Table 1. Triorganylammonium selenites **1–12**

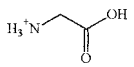
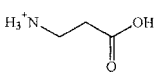
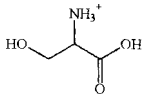
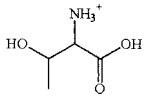
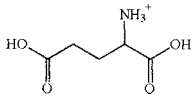
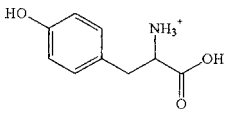
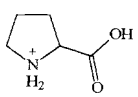
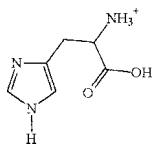
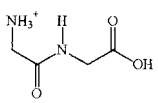
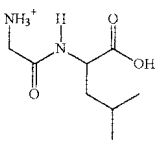
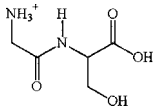
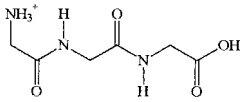
No.	Amino-acid$H^+HSeO_3^-$	Yield (%)	M.p. (°C)	^{1}H NMR	^{77}Se NMR
1		91	116–118	4.59 (2H, m).	1318.6
2		88	–	2.74 (2H, t, J = 6.6 Hz); 3.25 (2H, t, J = 6.6 Hz).	1320.7
3		76	181–183	3.99 (2H, m); 4.11 (1H, m).	1314.6
4		84	>230	2.36 (3H, d, J = 7.2 Hz); 3.81 (1H, m); 4.32 (1H, m).	1312.5
5		75	126–128	2.74 (2H, m); 3.25 (2H, t, J = 6.6 Hz); 4.25 (1H, m).	1318.2
6		81	182–185 (decomp.)	4.70–4.4.82 (3H, m); 6.88 (2H, d, J = 8.2 Hz); 7.17 (2H, d, J = 8.2 Hz).	1314.3
7		86	217 (decomp.)	1.76–1.87 (3H, m); 2.10–2.21 (1H, m); 3.07–3.23 (2H, m); 3.79–3.86 (1H, m).	1336.4
8		74	142 (decomp.)	3.34 (2H, d, J = 6.8 Hz); 4.05 (1H, t, J = 6.8 Hz); 7.39 (1H, s); 8.65 (1H, s).	1322.8
9		90	–	3.89 (2H, s); 4.00 (2H, s).	1319.2
10		94	121–123	1.65 (6H, d, J = 6.8 Hz), 2.23–2.45 (3H, m); 3.94 (2H, s); 4.12 (1H, d, J = 7.1 Hz).	1318.8

Table 1. continued.

No.	Amino-acid$H^+HSeO_3^-$	Yield (%)	M.p. (°C)	^{1}H NMR	^{77}Se NMR
11		95	–	3.90 (2H, s); 3.92–3.94 (1H, m); 4.79 (2H, m).	1316.23
12		85	–	3.91 (2H, s); 3.96 (2H, s); 4.05 (2H, s).	1319.5

In vitro cytotoxicity assay

Monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma), NIH 3T3 (normal mouse embrion fibroblasts) and BHK 21 (hamster neuroblasts) 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 only for one to four passage. 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 the 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, coloration of live cell membranes) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT, coloration shows the activity of mitochondrial enzymes in live cells). The quantity of live cells on the control plate was taken in calculations as 100%.[11,12] The concentration of NO was determined according to the Gryess method (by NO_2 level in the cultural medium). Sodium nitrite standard solution was used for the calibration curve.[11]

RESULTS AND DISCUSSION

Chemistry

We have shown that amino acids, di- and tri-peptides readily react with selenic acid (H_2SeO_3), forming during solution of selenium(IV) oxide in water, to give the corresponding ammonium hydroselenites **1–12**. Reaction proceeds easily at room temperature with good to excellent yields (74–95%) (Table 1). The ^{1}H and ^{77}Se NMR data confirm the formation of amino-acid hydroselenites. The selenium signal in the ^{77}Se NMR spectra for all compounds appears in the 1312.5–1336.4 ppm region.

Table 2. *In vitro* cell cytotoxicity and the ability of intracellular NO generation caused by hydroselenites **1–12**[a]

No.	Amino acid$^+$ $HSeO_3^-$	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	Gly H^+	2	950	3	650	20.5	192	4	186	5	4.5
2	β-Ala H^+	3.6	800	0.4	650	2.5	63	–[b]	–[b]	1.1	3.1
3	Ser H^+	10	1150	3	117	31.5	74	3	144	2.5	31.5
4	Tre H^+	13	200	36.5	550	65	71	34	1300	30	36.5
5	Glu H^+	23	650	25	133	100	24	89	31	50	25
6	Tyr H^+	35	183	40	76	–[c]	20	72	50	32.5	20
7	Pro H^+	1.7	1500	1.25	1300	3	1150	3	700	1.5	2.5
8	His H^+	5	340	4	325	54	32	–[c]	50	5.5	3
9	Gly–Gly H^+	20	800	11	100	–[c]	18	–[c]	14	47	40
10	Gly–Leu H^+	15	440	16.5	208	100	44	100	21	52	48
11	Gly–Ser H^+	15	600	–[c]	41	–[c]	12	–[c]	16	20	10.5
12	Gly–Gly–Gly H^+	10	600	3.5	73	100	23	53	45	21	5

[a] TD_{50}: concentration (μg ml^{-1}) providing 50% cell killing effect [(CV + MTT)/2]; NO concentration (%) (CV: coloration).
[b] Not tested.
[c] No cytotoxic effect.

Cytotoxic activity

Cytotoxic activity of the synthesized selenites **1–12** 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 fibroblast cells (NIH 3T3 and BHK 21). Concentrations providing 50% of tumor death effect (TD_{50}) were determined according to known procedures[11] 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 2. A preliminary analysis of the structure–activity relationship for the cytotoxic action clearly indicates the strong influence of the amino acid nature on toxic effects *in vitro*. Glycine (**1**), *β*-alanine (**2**) and proline (**7**) hydroselenites have the highest cytotoxic effect, on HT-1080, MG-22A, B16 and Neuro 2A cell lines. Besides that, most selenites synthesized (**2, 3, 7, 8** and **12**) are very active (0.4–11 $\mu g\ ml^{-1}$) against mouse hepatoma MG-22A. Unfortunately, all amino-acid hydroselenites **1–12** are toxic against both tumor and normal mouse fibroblast cells. However, the tripeptide hydroselenite **12** inhibits MG-22A ($TD_{50} = 3.5\ \mu g\ ml^{-1}$) tumor cells more selectively than NIH 3T3 ($TD_{50} = 21.0\ \mu g\ ml^{-1}$). Tyrosine **6**, glycylglycine **9** and glycylserine **11** did not express cytotoxic effects on all the cell lines studied, but they possessed medium toxicity on the NIH 3T3 and BHK 21 normal cells. In the series of glycine derivatives **1**, **9** and **12**, the initial glycine **1** ($TD_{50} = 3.0\ \mu g\ ml^{-1}$) is more active than its dipeptide **9** ($TD_{50} = 11.0\ \mu g\ ml^{-1}$) and tripeptide **12** ($TD_{50} = 3.5\ \mu g\ ml^{-1}$) on MG-22A.

The NO level was determined according to Ref. 11. 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 (hydroselenites concentration 100 $\mu g\ ml^{-1}$). The NO radical, which is relatively stable and can pass cellular membranes, is involved in a multitude of biological effects, such as regulation of the vascular tone, antiplatelet and antileukocyte activity, and modulation of cell growth.[13] It has also been implicated in the induction and the inhibition of apoptosis. Modulation of apoptosis by NO can lead to physiological or pathophysiological consequences. NO is involved in natural tumor defense mechanisms.[14–16] It was shown (Table 2) that compounds **1–3**, **7**, **9**, **11** and **12** readily increase NO concentration in the cultural medium on the HT-1080 line (up to $TG_{100} = 1500\%$). This effect is especially expressed in the case of proline hydroselenite **7** on all the tumor cell lines studied ($TG_{100} = 700–1500\%$). NO correlation between NO generation ability and cytotoxic effect of the salts studied was found.

According to our investigations, some of the amino-acid hydroselenites studied influenced the cell phenotype. Serine (**3**), treonine (**4**), tyrosine (**6**), histidine (**8**), glycylglycine (**9**), glycylserine (**11**) and diglycylglycine (**12**) hydroselenites changed NIH 3T3 fibroblast morphology, increasing cell size, starting at a concentration of 1 $\mu g\ ml^{-1}$. Treonine (**4**), glutamic acid (**5**) and proline (**7**) hydroselenites transformed BHK 21 cells (substance concentration 100 $\mu g\ ml^{-1}$). Also, glycine (**1**), serine (**3**) and glycylserine (**11**) hydroselenites were able to change the mouse melanoma B16 cell phenotype. This means that only two compounds, *β*-alanine (**2**) and glycyleucine (**10**), had no influence on cell morphology.

REFERENCES

1. Flohé L, Andreesen JR, Brigelius-Flohé R, Maiorino M and Ursini F. *IUBMB Life* 2000; **49**: 411.
2. Rayman MP. *Lancet* 2000; **356**: 233.
3. Caffrey PB and Frenkel GD. *Mol. Pharmacol.* 1991; **39**: 281.
4. Ip C and White G. *Carcinogenesis* 1987; **8**: 1763.
5. Caffrey PB and Frenkel GD. *Cancer Lett.* 1997; **121**: 177.
6. Caffrey PB and Frenkel GD. *Cancer Lett.* 1992; **52**: 4812.
7. Caffrey PB and Frenkel GD. *Cancer Lett.* 1994; **81**: 59.
8. Ochi H, Morita I and Murota S. *Arch. Biochem. Biophys.* 1992; **294**: 407.
9. Batist G, Katki AG, Klecker RW Jr and Myers CE. *Cancer Res.* 1986; **46**: 5482.
10. Lukevics E, Arsenyan P, Shestakova I, Domracheva I, Kanepe I, Belyakov S, Popelis J and Pudova O. *Appl. Organomet. Chem.* this issue.
11. Riddell RJ, Clothier RH and Balls MF. *Chem. Toxicol.* 1986; **24**: 469.
12. Fast DJ, Lynch RC and Leu RW. *J. Leucocyt. Biol.* 1992; **52**: 255.
13. Lopez Farre A, Rodriguesfeo JA, Demiguel LS, Rico L and Casado S. *Int. J. Biochem. Cell Biol.* 1998; **30**: 1095.
14. Lopez Farre A, De Miguel LS, Caramelo C, Gomez Macias J, Garcia R, Mosquera JR, De Frutos T, Millas I, Rivas F, Echezarreta G and Casado S. *Am. J. Physiol.* 1997; **272**: H760.
15. Brockhaus F and Bruene B. *Exp. Cell Res.* 1998; **238**: 33.
16. Edmiston KH, Shoji Y, Mizoi T, Ford R, Nachman A and Jessup JM. *Cancer Res.* 1998; **58**: 1524.

