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In vitro antitumour activity of some organogermanium radioprotectors

Benoît Célariès[1], Marcel Gielen[2], Dick de Vos[3] and Ghassoub Rima[1]*

[1]Laboratoire d'Hétérochimie Fondamentale et Appliquée, UMR 5069–CNRS, Université Paul Sabatier, 118, route de Narbonne, F-31062 Toulouse cedex 4, France
[2]POSC Department, Faculty of Applied Sciences, Free University of Brussels VUB; Pleinlaan 2, B-1050 Brussels, Belgium
[3]PCH Nederland, Pharmachemie BV, Medical Department, PO Box 552, NL-2003 RN Haarlem, The Netherlands

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Four germanium derivatives of 2,2′-oxydiethanethiol and 2,2′-thiodiethanethiol have been synthesized and characterized by ^{1}H and ^{13}C NMR, mass spectroscopy and elemental analysis. The antitumour activity of one of them is comparable to those of *cis*-platin and etoposide. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: antitumour activity; selenagermaadamantane; organogermanium radioprotector

INTRODUCTION

Many organometallic compounds of group 14, containing tin, germanium or silicon, are biologically active[1–9] and some of them display *in vitro* antitumour activity against tumour cell lines of human origin.[10–13]

Several organogermanium compounds containing chalcogen atoms (sulfur or selenium) have been studied *in vivo* for their radioprotective activity.[14]

Some thia- and selena-silaadamantanes have been found to possess good antitumour properties. These organometallic derivatives have been screened *in vivo* in mice at a lower dose than that of Ge-132.[15,16]

In this paper we report the synthesis, characterization and antitumour properties of four organogermanium compounds also containing sulfur or selenium. Their *in vitro* antitumour activity has been screened against seven tumour cell lines of human origin.

EXPERIMENTAL

General procedures

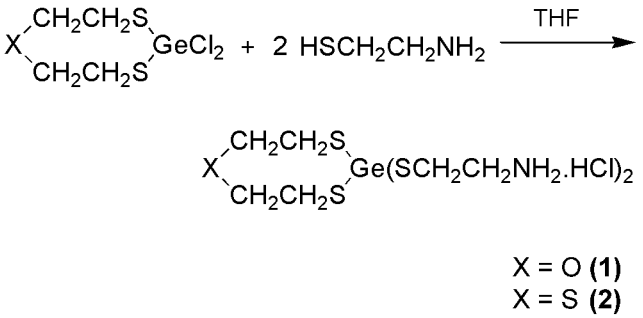
All manipulations were performed under an inert atmosphere of argon using standard Schlenck, glove box and high-vacuum-line techniques. All solvents used were freshly dried using standard techniques and all glassware was oven-dried. ^{1}H NMR spectra were recorded on a Bruker AC 80 spectrometer operating at 80.13 MHz (chemical shifts ppm relative to internal Me_4Si) and ^{13}C NMR spectra, on an AC 200 spectrometer (50.32 MHz). The multiplicity of the ^{13}C NMR signals was determined by the APT technique. Mass spectra under electron impact (EI) or chemical ionization (CI/CH_4) conditions at 70 and 30 eV were obtained on Hewlett-Packard 5989 and Nermag R10-10H spectrometers. IR and UV spectra were recorded on Perkin–Elmer 1600 FT-IR and Lambda-17 spectrophotometers. Melting points were taken uncorrected on a Leitz Biomed hot-plate microscope apparatus or, in capillary tubes, on a digital Electrothermal apparatus. Elemental analyses (C, H, N) were performed at the Laboratoire de Microanalyse de l'Ecole Nationale Supérieure de Chimie, Toulouse.

Synthesis of compound 1

To a stirred solution of cysteamine (1.97 g, 25.56 mmol) in 35 ml of anhydrous tetrahydrofuran (THF) was added dropwise a solution of 2,2′-dichloro-6-chalcogena-1,3,2-dithiagermocane (3.78 g, 12.78 mmol) in 40 ml of THF. The reaction mixture was refluxed under an argon atmosphere for 2 h. The white precipitate was filtered and dried under vacuum to give **1** (5.30 g, 92% yield): m.p. 250–260 °C (dec.). ^{1}H NMR (DMSO-d_6; δ, ppm): 2.80–3.30 (m, 16H, CH_2); 8.37 (s, 6H, NH_3^+).Anal. Found: C, 22.10; H, 5.13. Calc. for $C_8H_{22}Cl_2GeN_2OS_4$: C, 22.14; H, 5.11%.

Compound **2** was prepared analogously (96% yield): m.p. 80–90 °C (dec.). ^{1}H NMR (DMSO-d_6; δ, ppm): 2.80–3.10 (m, 12H, CH_2N and CH_2S); 3.52–3.69 (m, 4H, CH_2O); 8.00 (s, 6H,

*Correspondence to: G. Rima, Laboratoire d'Hétérochimie Fondamentale et Appliquée, UMR 5069–CNRS, Université Paul Sabatier, 118, route de Narbonne, F-31062 Toulouse cedex 4, France.
E-mail: rima@chimie.ups-tlse.fr



Scheme 1.

NH_3^+); Anal. Found: C, 21.29; H, 5.00. Calc. for $C_8H_{22}Cl_2GeN_2S_5$: C, 21.35; H, 4.93%.

Synthesis of compound 3

A solution of 2,2′-dichloro-1,3,6,2-trithiagermocane (5.59 g, 18.90 mmol) in 100 ml of pyridine was added dropwise, with stirring, to a solution of NaSH (2.12 g, 37.79 mmol) in 150 ml of anhydrous pyridine at 80 °C. The reaction mixture was stirred under an argon atmosphere for 16 h at room temperature. Evaporation under reduced pressure leads to a yellow residue which was washed with 5×25 ml of anhydrous THF. After filtration, the solid residue was purified by stirring it overnight in 50 ml of dry methanol. The white precipitate was filtered and dried under vacuum to give **3** (4.80 g, 99% yield): m.p. 180–190 °C (dec.). ^{1}H NMR (DMSO-d_6; δ, ppm): 2.85–2.98 (m, 4H, CH_2S); 3.07–3.21 (m, 4H, CH_2S). Anal. Found: C, 18.79; H, 2.98. Calc. for $C_{12}H_{24}Ge_3S_{12}$: C, 18.70; H, 3.06%.

Synthesis of hexaselenatetrakis(isoamylgerma)adamantane (4)

A solution of $LiEt_3BH$ (57.20 mmol in 57.2 ml of THF) was added dropwise to elemental selenium (2.26 g, 28.60 mmol) via a syringe. The mixture was stirred for 1 h at room temperature. A solution of trichloroisoamylgermane (4.77 g, 19.07 mmol) in 25 ml of anhydrous THF was added at 0 °C for 1 h. The reaction mixture was then allowed to warm to room temperature and was stirred until the red colour of the selenium salt had disappeared (5 days). The solvent was removed *in vacuo* and the residue was extracted with toluene. After filtration and concentration of the solution, the solid residue was crystallized from pentane to afford **4** (4.5 g, 90%). ^{1}H NMR ($CDCl_3$): 0.90 (d, 24H, $J = 5.4$ Hz, $(CH_3)_2CH$); 1.20–2.11 (m, 20H, CH_2CH_2CH); ^{13}C NMR ($CDCl_3$): 21.96 (CH_3); 29.70 (CH); 31.37 (CH_2); 32.69 (CH_2). Mass spectrum (CI/CH_4): *m/z* 1079 $[M + 29]^+$. Anal. Found: C, 22.93; H, 4.23. Calc. for $C_{20}H_{44}Ge_4Se_6$: C, 22.90; H, 4.20%.

This compound has been shown by X-ray diffraction to have an adamantane-type structure, but the quality of the crystal does not allow structure refinement.

General synthesis of $X(CH_2CH_2S)_2GeCl_2$, X = O, S

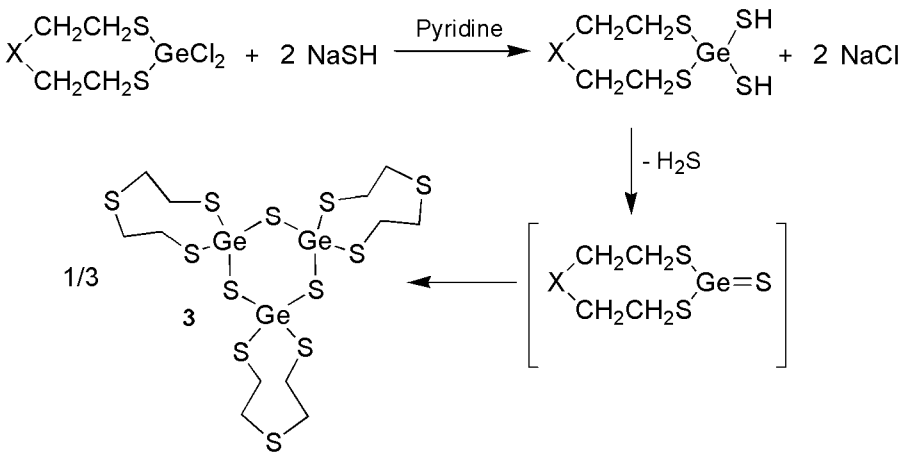
To a stirred mixture of 2,2′-thiodiethanethiol (4.17 g, 27.04 mmol) or 2,2′-oxydiethanethiol (3.74 g, 27.04 mmol) and triethylamine (6.02 g, 59.50 mmol) in 125 ml of anhydrous THF was added dropwise a solution of $GeCl_4$ (5.80 g, 27.04 mmol) in 75 ml of THF. The reaction mixture was refluxed under argon for 2 h. The white precipitate was filtered and dried under vacuum to give the expected reaction products (70–75% yield).

X = O

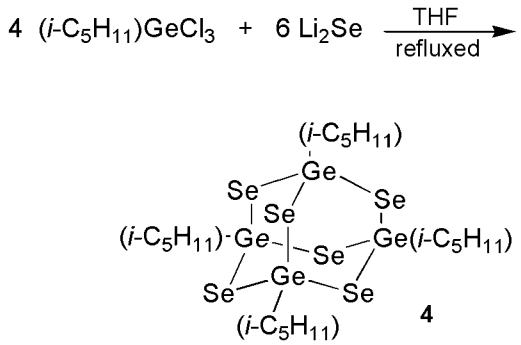
M.p. 117–120 °C. ^{1}H NMR ($CDCl_3$; δ, ppm): 3.09–3.26 (m, 4H, CH_2S); 3.69–3.87 (m, 4H, CH_2O). Mass spectrum (EI: 10 eV, 120 °C): *m/z* 280 $[M]^{+\cdot}$. Anal. Found: C, 17.21; H, 2.83. Calc. for $C_4H_8Cl_2GeOS_2$: C, 17.17; H, 2.88%.

X = S

M.p. 105–107 °C. ^{1}H NMR ($CDCl_3$; δ, ppm): 2.84–3.08 (m, 4H,



Scheme 2.



Scheme 3.

CH_2S); 3.09–3.20 (m, 4H, CH_2S). Mass spectrum (EI: 10 eV, 105 °C): *m/z* 296 $[M]^{+\cdot}$. Anal. Found: C, 16.19; H, 2.69. Calc. for $C_4H_8Cl_2GeS_3$: C, 16.24; H, 2.73%.

RESULTS AND DISCUSSION

Synthesis of compounds 1 and 2

The action of 2,2′-dichloro-6-chalcogena-1,3,2-dithiagermocane[17] on two equivalents of cysteamine in refluxing anhydrous THF gave the corresponding monocyclic derivatives (Scheme 1) in yields of 92–95%.

Synthesis of compound 3

The action of two equivalents of NaSH on the 2,2′-dichloro-1,3,6,2-trithiagermocane[17] in anhydrous pyridine gave the corresponding trimer of the germathione with the elimination of hydrogen sulfide (Scheme 2) in 99% yield.

Synthesis of selenagermaadamantane (4)

Treatment of isoamyltrichlorogermane with lithium selenide gave selenagermaadamantane (**4**)[14,18] (Scheme 3).

Antitumour activity

Compounds **1**–**4** were screened *in vitro* against a panel of seven human cancer cell lines: A498 (a renal cancer); EVSA-T (a mammary cancer); H226 (a non-small cell lung cancer); IGROV (an ovarian cancer); M19 MEL (a melanoma); MCF-7 (a mammary cancer); and WiDr (a colon cancer). The inhibition doses (ID_{50}) given in Table 1 are compared with those of some reference compounds used clinically: etoposide (ETO), 5-fluorouracil (5-FU), doxorubicin (DOX), methotrexate (MTX) and *cis*-platin (CPT).

Only one derivative, compound **4**, has a moderate *in vitro* antitumour activity. It is indeed characterized by ID_{50} values similar to those obtained for ETO and CPT. It is, however, less active than DOX, MTX or taxol for the cancer cell lines studied.

Table 1. ID_{50} values (ng ml^{-1}) of compounds **1**–**4**, together with those of some reference compounds used clinically, against several tumour cell lines

	A498	EVSA-T	H226	IGROV	M19 MEL	MCF-7	WiDr
1	>62500	>62500	23400	>62500	31300	54100	>62500
2	>62500	27100	26900	33400	22900	36500	>62500
3	35000	43400	14600	27500	20500	22000	153000
4	558	1155	212	991	699	814	1020
DOX	90	8	199	60	16	10	11
CPT	2253	422	3269	169	558	699	967
5-FU	143	475	340	297	442	750	225
MTX	37	5	2287	7	23	18	<3
ETO	1314	317	3934	580	505	2594	159

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