Synthesis of new organometallic compounds and their radioprotective activity evaluation

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Abstract Further to our work concerning organometallic compounds active in chemical radioprotection, we report the synthesis and pharmacological study (radioprotective activity, toxicity) of new germathiazolidines and germadithioiacetals derived from cysteamine, methylcysteamine and N-substituted cysteamine. A germylated oxide and sulfide with methylcysteamine hydrochloride as ligand were also investigated.
A notable decrease in the toxicity and a fairly large increase in the radioprotective activity of these new organogermylated compounds were observed compared with cysteamine, methylcysteamine and N-substituted cysteamine.

Keywords: germathiazolidines, germadithioacetals, germylared sulfide, toxicity, radioprotective activity.

1. Introduction

During a research program in the field of the pharmacological activity of organogermanium compounds, several derivatives were chemically synthesized and tested for their radioprotection properties. The great majority of these compounds were metalla-thiazolidines and -dithioacetals of *N*-substituted cysteamine, methylcysteamine, *N*-(2-thioethyl)-1,3-diaminopropane and naphthylmethyl-imidazoline. Seventy compounds of these derivatives have a dose reduction factor (DRF) between 1.4 and 1.75 [1-7].

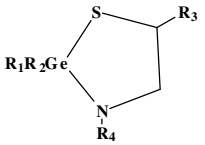
In Fig. 1 some compounds synthesized in our laboratory by way of example are cited.

We have broadened our research program and completed our study on the germathiazolidines and germadithioacetals series.

Various organogermanium compounds of these types with different substituents on germanium and nitrogen have been prepared and tested.

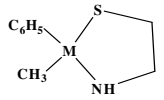
In this work the study of the synthesis, toxicity and radioprotective activity of some new germathiazolidines and germadithioacetals, and a germylated oxide and sulfide, as listed below is presented.

(a) Germathiazolidines

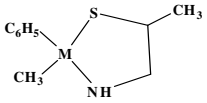


$R_1 = R_2 = i\text{-}C_5H_{11}$, $R_3 = H$
$R_4 = -(CH_2)_3NHCH_2CH=CH_2$ **1**

$R_1 = p\text{-}CH_3\text{-}C_6H_4$, $R_2 = CH_3$, $R_3 = H$
$R_4 = -(CH_2)_3NHCH_2CH=CH_2$ **2**



M = Si, DRF = 1.6
M = Ge, DRF = 1.6



M = Si, DRF = 1.5
M = Ge, DRF = 1.75

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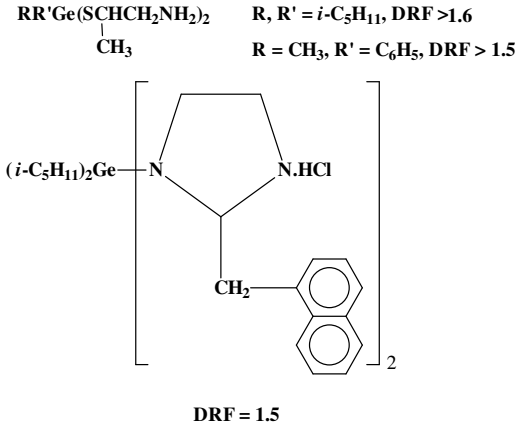
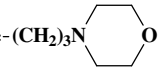
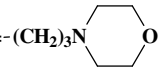


Fig.1: Some germanium- and silicon containing heterocyclic and their dose reduction factors (DRF)

$R_1 = R_2 = i\text{-}C_5H_{11}$, $R_3 = H$

$R_4 =$  **3**

$R_1 = R_2 = n\text{-}C_6H_{13}$, $R_3 = H$

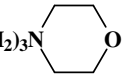
$R_4 =$  **4**

$R_1 = R_2 = n\text{-}C_6H_{13}$, $R_3 = CH_3$, $R_4 = H$ 5

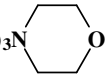
$R_1 = R_2 =$ **, $R_3 = R_4 = H$ 6**

$R_1 = R_2 =$ **, $R_3 = CH_3$, $R_4 = H$ 7**

$R_1 = R_2 = -SCH(CH_3)CH_2NH_2.HCl$

$R_3 = H$,  **8**

$R_1 = R_2 = -SCH_2CH_2NH_2.HCl$

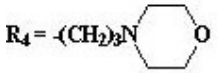
$R_3 = H, R_4 =$  **9**

(b) Germadithioacetals

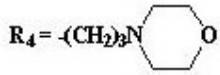
$R_1R_2Ge\ [SCH(R_3)CH_2NHR_4]_2$

$R_1 = p\text{-}CH_3C_6H_4$, $R_2 = CH_3$, $R_3 = H$
$R_4 = -(CH_2)_3NHCH_2CH{=}CH_2$ 10

$R_1 = R_2 = i\text{-}C_5H_{11}$, $R_3 = H$

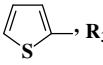
$R_4 =$  **11**

$R_1 = R_2 = n\text{-}C_6H_{13}$, $R_3 = H$

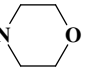
$R_4 =$  **12**

$R_1 = R_2 = n\text{-}C_6H_{13}$, $R_3 = CH_3$,
$R_4 = H$ 13

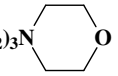
$R_1 = R_2 =$ **, $R_3 = R_4 = H$ 14**

$R_1 = R_2 =$  **, $R_3 = CH_3$, $R_4 = H$ 15**

$R_1 = R_2 = -SCH(CH_3)CH_2NH_2.HCl$, $R_3 = H$

$R_4 =$  **16**

$R_1 = R_2 = -SCH_2CH_2NH_2.HCl$, $R_3 = H$

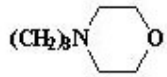
$R_4 =$  **17**

(c) Germylated oxide and sulfide (i.e. a germoxane and a germathiane)

$([HCl.H_2NCH_2CH(CH_3)S]_2GeX)_3$

X = O 18
X = S 19

(d) N-Substituted R-$NHCH_2CH_2SH$

R =  **20**

$R = CH_2{=}CHCH_2NH(CH_2)_3$- 21

2. Experimental

All the syntheses were performed under nitrogen or argon. Solvents were freshly under nitrogen or argon. Solvents were freshly distilled from sodium/benzophenone before use. IR spectra

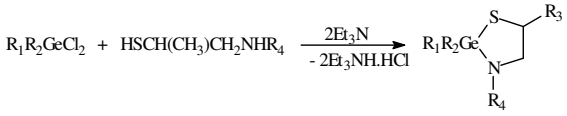
were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. ^{1}H-NMR spectra were recorded on a Brüker AC-80 spectrometer. Mass spectra under electron impact (EI) conditions at 70 eV, were recorded on a Hewlett-Packard 5989 spectrometer. Elemental analyses (C, H, N) were performed at the Laboratoire de Microanalyses de l'Ecole Nationale supérieure de Chimie de Toulouse.

2.1. Syntheses of germathiazolidines

These compounds were prepared by two methods: A and B.

Method A (compound 1) - Scheme 1

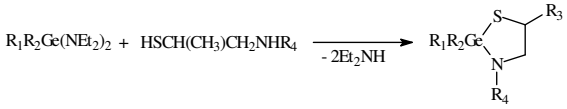
To a solution of di(isoamyl)dichlorogermane (4 g, 14 mmol) were added *N*-substituted cysteamine (0.44 g, 14 mmol) and triethylamine (3.03 g, 30 mmol) in 50 mL of tetrahydrofuran (THF) freshly distilled. After the mixture was refluxed for 4 h with stirring and cooled to room temperature it was filtered under argon and the filtrate concentrated *in vacuum*.



Scheme 1

Method B (compound 2) - Scheme 2

Bis(diethylamino)di-isoamylgermane (2 g, 5.6 mmol) was dissolved in 50 ml of THF and *N*-substituted cysteamine (0.975 g, 5.6 mmol) was added from a syringe. The solution was refluxed under argon atmosphere for 3 h with stirring, and concentrated *in vacuum*.



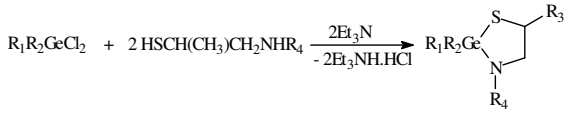
Scheme 2

2.2 Syntheses of germadithioacetals

Two methods: C and D have been applied to synthesize these compounds.

Method C (compound 12) - Scheme 3

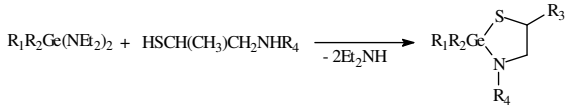
To a stirred mixture of *N*-substituted cysteamine (2.88 g, 14.1 mmol), triethylamine (1.6 g, 15.84 mmol) and 70 mL of THF was added slowly, a solution of dichlorodihexylgermane (2.21g, 7.05 mmol) in 40 mL of THF. The reaction mixture was refluxed for 8 h, filtered at ambient temperature under argon, and concentrated *in vacuo*.



Scheme 3

Method D (compound 13) - Scheme 4

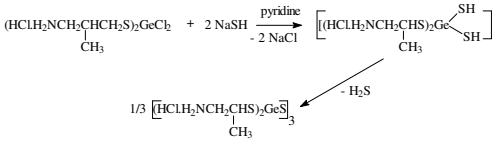
To a solution of methylcysteamine (0.945 g, 10.38 mmol) in 50 mL of THF was added dropwise a solution of the bis(diethylamino)dihexylgermane (2 g, 5.19 mmol) in 50 mL with of anhydrous THF a stirring. The mixture was refluxed for 4 h. Removal of the solvent and work-up was as before.



Scheme 4

2.3. Synthesis of germylated sulfide

The action of NaSH on $[HCl.H_2NCH_2CH(CH_3)S]_2GeCl_2$ in anhydrous pyridine leads to the corresponding product



Scheme 5

The germylated derivatives described have generally a radioprotective activity greater than that of the basic organic derivatives, and a lower toxicity.

For example, compounds **3** and **4** have DRF values of 1.3 and 1.5, compared with **20** (DRF = 1.2) and **5** (DRF = 1.5), compared with methylcysteamine DRF.

Furthermore we note the low toxicity of compounds **3**, **4** and **5** (LD_{50}, 800, 1500 and 1500 $mg.kg^{-1}$) compared with **20** (LD_{50}, 450 $mg.kg^{-1}$) and methylcysteamine (LD_{50}, 500 $mg.k^{-1}$).

Noteworthy also are the compounds **11**, **12**, **13** and **14**, which have an interesting radioprotective activity (DRF 1.4 – 1.6), compared with derivative **20** (DRF = 1.2). Derivatives **13** and **14**, compared

with cysteamine and methylcysteamine, have a greater radioprotective activity and a lower toxicity, in spite of lower injected dosages in the case of the germylated derivatives (expressed in mmol fractions): derivative **11** LD_{50} 900 $mg.kg^{-1}$ (1.45 mmol); derivative **12** LD_{50} 800 $mg.k^{-1}$(1.23 mmol) compared with derivative **20** LD_{50} 450 $mg.k^{-1}$ (2.2 mmol); derivative **13** LD_{50} 800 $mg.k^{-1}$(1.89 mmol), compared with methylcysteamine LD_{50} 500 $mg.k^{-1}$ (3.92 mmol); derivative **14** LD_{50} 500 $mg.kg^{-1}$ (1.28 mmol), compared with cysteamine LD_{50} 450 $mg.k^{-1}$ (3.96 mmol).

Another very interesting result was obtained with the germylated **18** [DRF 1.6, LD_{50} 1000 $mg.kg^{-1}$ (0.93 mmol)], compared with methylcysteamine.

2.4. Pharmacology: evaluation of radioprotection

Male CD1 mice (Charles River France), 25 g body weight, were used. Compounds were injected intraperitoneally 15 or 90 min before irradiation. The irradiation dose was $LD_{100}/30$, days for non treated control mice (8.5, 9 or 9.5, according to the irradiation date) or a 2 Gy greater dose. The injected dose of compound was equal to either one-half or one-eighth of the LD_{50} value which has been determined previously. The radioprotective effect was evaluated by the dose reduction factor (DRF), which is the ratio between the $LD_{50}/30$ days of treated mice and that of control mice (between 7.5 and 8.5 Gy, according to the date).

Irradiation was applied using a cobalt-60 source at a dose rate of 7-8 Gy min^{-1} according the date. During irradiation, animals were placed in a Plexiglass box with 30 cells in a homogeneous field 28.5 cm x 28.5 cm in area. Dosimetry was checked with an ionization chamber dosimeter. The different LD_{50} values were determined by probit analysis.

3. Results and discussions

Germathiazolidines of *N*-substituted cysteamine and methylcysteamine were prepared according to two methods of heterocyclization already described in the literature [6].

The action of the diorganogermanium dichloride (in stoichiometric amounts) on *N*-substituted cysteamine or methylcysteamine in refluxing anhydrous THF in the presence of freshly distilled triethylamine gave by a cyclization reaction, with elimination of hydrochloric acid from M-Cl, SH and NH groups, the corresponding products in yields of 51-89% (Scheme 1).

The reaction of *N*-substituted cysteamine and methylcysteamine, in stoichiometric amounts, with the bis(diethylamino)dialkylgermane in anhydrous THF resulted in the cleavage of Ge-N bonds by the NH and SH groups, forming the corresponding germathiazolidines in good yields (83-89%) (Scheme 2).

The action of the diorganogermanium dichloride on 2 mol of *N*-substituted cysteamine or methylcysteamine in refluxing anhydrous THF in the presence of triethylamine gave the acyclic derivatives (Scheme 3) in yields of 42-87%.

The reaction of 2 mol of *N*-substituted cysteamine or methylcysteamine with the bis(diethylamino) dialkylgermane in anhydrous THF (a cleavage reaction of the Ge-N bonds by the SH groups) gave the corresponding germylated derivatives (Scheme 4) in yields of 52-80%.

4. Conclusions

In this work, several silicon or germanium organometallic compounds have been synthesized: a great majority presents an interesting radioprotective activity.

In many cases, when the structure was cyclic, we observed a delayed effect. The more important radioprotective activity and the generally lower toxicity of the silicon or germanium derivatives compared to basic organic materials. The results show clearly the important contribution of silicon and germanium in the origin of the radioprotective properties of these structures.

In short, the radioprotective activity of germa-thiazolidines, germadithioacetals, and the germylated sulfide and oxide derived from cysteamine, methylcysteamine and *N*-substituted cysteamine can be increased compared with unsubstituted organic derivatives by the presence of organometallic groups which increase the hydrosolubility, the lipophilicity and the activity of these molecules, thereby favoring their passage through the cellular membranes.

These derivatives are generally less toxic and more active than the basic organic derivatives.

The results presented in this paper confirm the positive contribution of germanium in this field in agreement with previous work and the interesting biological activity of organogermanium compounds. We also observed that organogermylated groups decrease the toxicity of the basic molecules to which they are attached.

5. Acknowledgements

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6. References

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