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NOTE

Neurotropic and Antitumor Activities of Silyl- and Germyl-isoxazolin-2-yl Derivatives

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Neurotropic and antitumor activities of the pyridyl-substituted 5-Si(Ge)-isoxazolines and (3-alkyl-4-triphenylgermylisoxazolin-2-yl)-5-carboxylic acid ethyl esters have been investigated. It has been shown that silylisoxazolin-2-yl derivatives are more potent in protection against hypoxia and Corazole convulsions than their analogues. Germylisoxazolin-2-yl compounds are stronger tumor growth inhibitors and NO-inducers than their silyl analogues. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: silylisoxazolines; germylisoxazolines; neurotropic; antitumour

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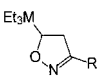
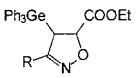
INTRODUCTION

Silyl-substituted isoxazolines show a wide spectrum of biological activity.[1] Germanium-containing heterocycles are usually less toxic than their carbon and silicon analogues.[2–4] We have performed a comparative study of the vasodilating, anticoagulant and cardioprotective activity of 5-Si(Ge)-substituted isoxazolin-2-yl derivatives. The substitution of the silicon atom for the germanium does indeed lead to a significant increase in vasodilating, antithrombotic and cardioprotective activity. The insertion of a methylene group between Ge and the isoxazoline ring reduces the vasodilating activity. The most active isoxazoline, 3-(5′-triethylgermyl-3′-isoxazolinyl)pyridine hydrochloride, protects the heart from rhythm disturbances and lethality during ischaemia reperfusion.[5] As has been found previously, the triphenylgermyl derivatives of another nitrogen-containing five-membered heterocycle (substituted 1,2,4-triazolinones) are effective against experimental gastric carcinoma MGC-803.[6] Therefore, we decided to investigate the antitumor (and also neurotropic) properties of the following Si(Ge)-substituted isoxazolin-2-yl compounds.





1—8: M = Si, Ge; R = 2-Py, 3-Py, 4-Py 9—10: R = Me, Et

EXPERIMENTAL

The Si(Ge)-substituted isoxazolines **1**–**10** (Table 1) were prepared by cycloaddition of nitrile oxides to vinyl- and ally-germanes (silanes). Their synthesis and characterization are described in Refs 5 and 7.

Neurotropic activity was studied on Icr:Ice and BALB/c mice. Ambient temperature (22 ± 1 °C) was maintained in the laboratory and in the animal colony. The tested substances were administered intraperitoneally as aqueous suspensions prepared with the aid of Tween 80. Control animals received injections of equal amounts of distilled water with Tween 80. Tests were carried out and determined according to Refs 8 and 9.

Acute toxicity was evaluated in male ICR-JCL mice (19–23 g). The compounds were dissolved–suspended in a 0.6% solution of Tween 80 and injected intraperitoneally.

A conventional reflex of passive avoidance test was applied to evaluate the influence of the studied substances on memory and antiamnestic activity. Retrogradal amnesia was caused transcorneally by

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Table 1 The silyl(germyl)isoxazolines-2 investigated

No.	Compound	Yield (%)	Ref.
1	2-(5′-Triethylsilyl-3′-isoxazolinyl)pyridine hydrobromide	52	5
2	3-(5′-Triethylsilyl-3′-isoxazolinyl)pyridine hydrobromide	44	5
3	4-(5′-Triethylsilyl-3′-isoxazolinyl)pyridine hydrobromide	44	5
4	2-(5′-Triethylgermyl-3′-isoxazolinyl)pyridine hydrochloride	70	5
5	3-(5′-Triethylgermyl-3′-isoxazolinyl)pyridine hydrochloride	47	5
6	4-(5′-Triethylgermyl-3′-isoxazolinyl)pyridine hydrochloride	45	5
7	2-(5′-Triethylgermylmethyl-3′-isoxazolinyl)pyridine hydrochloride	43	5
8	3-(5′-Triethylgermylmethyl-3′-isoxazolinyl)pyridine hydrobromide	45	5
9	(3-Methyl-4-triphenylgermylisoxazolinyl-2)-5-carboxylic acid ethyl ester	94	7
10	(3-Ethyl-4-triphenylgermylisoxazolinyl-2)-5-carboxylic acid ethyl ester	90	7

maximal electric shock administered just after learning.

According to recent investigations there is a strong assumption that excessive induction of NO biosynthesis in cells could lead to their irreversible damage.[10] Potential cytotoxic activity of the synthesized Si(Ge)-substituted isoxazolines-2 based on such a biological effect was tested *in vitro* on three standard monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), and normal mouse fibroblast cells. This was done according to a known procedure[11] using 96-well plates. Two independent colorimetric methods were employed: (a) coloration with Crystal Violet (CV), specifying the integrity of cell membranes; and (b) coloration with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), characterizing the redox activity of mitochondrial enzymes in cells.

Table 2 Acute toxicity, rotating-rod, tube and traction tests of silyl(germyl)isoxazolin-2-yl compounds in white mice (i.p)

		(EC_{50}, mg kg^{-1})		
No.	LD_{50} (mg kg^{-1})	Rotating-rod test	Tube test	Traction test
1	447 (313–596)	178 (112–253)	178 (112–253)	282 (183–372)
2	515 (362–692)	141 (92–209)	69 (24.2–130.3)	274 (99–324)
3	325 (219–455)	54.7 (20–102.7)	70.8 (50.1–92.5)	60.0 (31.7–93)
4	410 (268–552)	89 (56–129.1)	51.5 (36.2–69.2)	90.8 (50.1–92.5)
5	355 (249–461)	178 (136–230)	163 (109–227)	205 (146–288)
6	355 (202–508)	44.7 (31.3–59.6)	70.8 (50.1–92.5)	>100
7	708 (501–925)	355 (202–508)	325 (219–455)	410 (211–622)
8	515 (362–692)	163 (109–227)	141 (68–209)	141 (92–209)
9	>500	258 (168–357)	89 (63.1–119.7)	>250
10	>500	>250	178 (136–230)	>250

Table 3 Neurotropic activity of silyl(germyl)isoxazolin-2-yl compounds

	Activity (% of control)				
No.	Hypoxia	Corazole spasms	Phenamine stereotype	Ethanol anaesthesia	Thiopental anaesthesia
1	118	145.5	100.4	79.3	133.3
2	143	205	88.4	119	144.4
3	131	265	155.3	73.6	87.3
4	125.5	156	113	99	209
5	136	174	28.5	336.7	74.1
6	102	114	117.8	216.7	138.8
7	134	140	91	72	133
8	113.7	120.7	94.1	30.2	119
9	—	139	204	84.6	104.5
10	—	125	112.3	176.1	103

RESULTS AND DISCUSSION

The experimental evaluations of acute toxicity and neurotropic properties are presented in Tables 2 and 3.

The compounds investigated have a medium toxicity. The most toxic compound is **3**, 4-(5′-triethylgermyl-3′-isoxazolinyl)pyridine hydrobromide (325 mg kg^{-1}). The introduction of a methylene group between the triethylgermyl group and the isoxazoline ring decreases the toxicity 2.2-fold for **7**. Triethylsilylisoxazolines are a little more toxic than the germyl analogues. All compounds exhibit low activity in rotating-rod, tube and traction tests.

The 2-pyridyl-substituted germylisoxazoline **4** was the most active in increasing the duration of thiopental anaesthesia by 109%, while the 3-pyridyl-substituted analogue **5** decreased it by 25.9% (Table 3). Silylisoxazolines decreased ethanol anaesthesia, but their germyl analogues showed a prolongation effect. The introduction of the methylene group between the triethylgermyl group and the isoxazoline ring caused an opposite effect — shortening the anaesthesia time by 28% for **7** and by 70% for **8**.

Silyl- and germyl-isoxazolin-2-yl compounds (**1** – **10**) possess low antihypoxic activity (Table 3). 4-(5′-Triethylsilyl-3′-isoxazolinyl)pyridine hydrobromide **3** is 1.3 times more active than the corresponding germyl analogue **6**.

The stimulating effects of phenamine are strengthened under the influence of silylisoxazoline **3** and germylisoxazoline **6** by 55.3 and 17.8%, respectively, but triphenylgermylisoxazoline **9** exhibits the highest effect (by 104%).

All isoxazoline derivatives tested possess anti-Corazole potency. The most active is silylisoxazoline **3** (165%). Silyl derivative **3**, germyl derivative **6** and germylmethylisoxazolines **7** and **8** in comparatively small doses (50 mg kg^{-1}) completely prevent the retrogradal amnesia caused by electric shock.

The isoxazolines tested have medium activity on

Table 4 Antitumor activity of isoxazolines

	HT-1080			MG-22A			B16		
	IC_{50} (μg ml^{-1})		NO (%)	IC_{50} (μg ml^{-1})		NO (%)	IC_{50} (μg ml^{-1})		NO (%)
No.	CV	MTT	CV	CV	MTT	CV	CV	MTT	CV
1	nce[a]	nce	26	100	100	46	nce	nce	nce
2	80	nce	38	70	55	55	nce	nce	nce
4	45	50	850	13	20	850	100	100	42
7	40	45	850	23	23	450	nce	nce	22
9	50	77	126	60	80	177	12	75	157
10	37	1	77	10	3	129	65	nce	67

[a] nce, no cytotoxic effect was detected.

HT-1080 and MG-22A cell tumor cultures, and low activity on B16 (Table 4). The most active antitumor substance is triphenylgermylisoxazoline **10** (IC_{50} MTT). Germylisoxazolines **4** and **7** are strong NO-inducers (850%) on HT-1080 and MG-22A.

Germylisoxazolin-2-yl derivatives are stronger tumor growth inhibitors and NO-inducers than their silyl analogues.

REFERENCES

1. E. Lukevics, V. Veveris and V. Dirnens, *Appl. Organometal. Chem.* **11**, 805 (1997).
2. E. Lukevics, L. Ignatovich and S. Germane, *Khim. Geterotsikl. Soed.* **10**, 1412 (1995).
3. E. Lukevics and L. Ignatovich, *Main Group Met. Chem.* **1**, 133 (1994).
4. E. Lukevics, T. Gar, L. Ignatovich and F. Mironov, *Biological Activity of the Germanium Containing Compounds*, Zinatne, Riga, 1990 (in Russian).
5. E. Lukevics, P. Azrenyan and M. Vevezis, *Metal-Based Drugs* **5**, 251 (1998).
6. F. Li, Z. Zhang and H. Gao, *Metal-Based Drugs* **3**, 251 (1998).
7. E. Lukevics, P. Arsenyan, S. Belyakov and J. Popelis, *J. Organometal. Chem.* **258**, 155 (1998).
8. E. Lukevics, L. Ignatovich, N. Porsiurova and S. Germane, *Appl. Organometal. Chem.* **2**, 115 (1988).
9. E. Lukevics, L. Ignatovich, N. Shilina and S. Germane, *Appl. Organometal. Chem.* **6**, 261 (1992).
10. J. F. Kerwin Jr, F. R. Lancaster Jr and P. L. Feldman, *J. Med. Chem.* **38**, 4343 (1995).
11. R. J. Riddell, R. H. Clothier and M. F. Balls, *Chem. Toxicol.* **24**, 469 (1986).

