CYTOTOXIC ACTIVITY OF SILYL- AND GERMYL-SUBSTITUTED 4,4-DIOXO-3a,6a-DIHYDROTHIENO[2,3-*d*]ISOXAZOLINES-2

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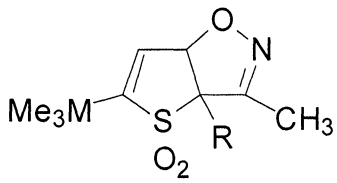
ABSTRACT

The [2+3] dipolar cycloaddition of nitrile oxides to the double C=C bonds of thiophene-1,1-dioxides leads to formation of the fused isoxazolines-2 (**1**, **2**). Tumor growth inhibition of these compounds strongly depends on the nature of group IV A element increasing from slightly active *tert*-butyl derivatives to silicon and germanium containing analogues. The products of benzonitrile oxide cycloaddition have greater cytotoxic effect than the compounds obtained from the cycloaddition reaction of 2,5-disubstituted thiophene-1,1-dioxides with acetonitrile oxide. Fused silyl substituted isoxazolines-2 are stronger NO-inducers than their germyl and *tert*-butyl analogues.

INTRODUCTION

The interest in silyl substituted thiophene-1,1-dioxides stems from the fact that they are useful synthetic intermediates for the preparation of various types of organic compounds by Diels-Alder cycloaddition [1], amine induced ring-opening reaction [2], or coupling of bromothiophene-1,1-dioxides with thienyl stannanes in the presence of a palladium (0) catalyst [3]. It has been shown that unsubstituted thiophene-1,1-dioxide prepared *in situ* is a quite reactive dipolarophile in the [2+3] cycloaddition reactions with N,α-diphenylnitrone [4], benzonitrile [4, 5] and mesitonitrile [4, 5] oxides yielding mono- and diisoxazolines-2 and N-substituted isoxazolidines. Moreover, our recent studies indicate that silyl- and germyl-containing isoxazolines have gained a great deal of attention as compounds possessing a wide spectrum of the biological properties. The vasodilating, anticoagulant and cardioprotective activity of 5-Si-(Ge)substituted isoxazolines-2 has been studied in *vitro* and in *vivo* [6, 7]. The most active isoxazoline - 3-(5`-triethylgermyl-3`-isoxazolino)pyridine hydrochloride protected the heart from rhythm disturbances and lethality during ischemia-reperfusion [7]. It has been shown that silylisoxazolines-2 are more potent in protection against hypoxia and corazole convulsions than germanium analogues. However, germylisoxazolines-2 are stronger tumor growth inhibitors and NO-inducers than their silicon analogue [8].

This work presents the results of cytotoxic activity for fused isoxazolines-2 bearing a group 14 element as substituent (**1**, **2**) in function of the nature of the group 14 element.



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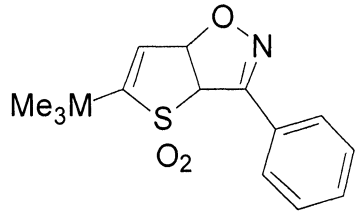

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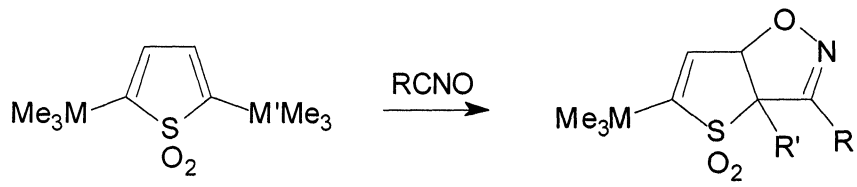
MATERIALS AND METHODS

CHEMISTRY

Seven *tert*-butyl-, trimethylsilyl-, and trimethylgermyl-substituted 4,4-dioxo-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2 **1** and **2** (Table 1) were prepared by the [2+3] dipolar

cycloaddition of aceto- and benzonitrile oxides to 2,5-disubstituted thiophene-1,1-dioxides. Their synthesis and characterization are given in ref. [9].



M, M'=C, Si, Ge; R=Me, Ph; R'=H, Me_3Ge

Table 1. Investigated Me_3C, Me_3Si, Me_3Ge substituted 4,4-dioxo-3a,6a-dihydrothieno[2,3-*d*]isoxazolines-2

Compound	Type	M	R	Yield (%)
4,4-dioxo-3-methyl-5-*tert*-butyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**1a**	C	H	80
4,4-dioxo-3-methyl-3a-trimethylgermyl-5-*tert*-butyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**1b**	C	Me_3Ge	58
4,4-dioxo-3-methyl-3a-trimethylgermyl-5-trimethylsilyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**1c**	Si	Me_3Ge	45
4,4-dioxo-3-methyl-3a,5-bis(trimethylgermyl)-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**1d**	Ge	Me_3Ge	67
4,4-dioxo-3-phenyl-5-*tert*-butyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**2a**	C	-	84
4,4-dioxo-3-phenyl-5-trimethylsilyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**2b**	Si	-	77
4,4-dioxo-3-phenyl-5-trimethylgermyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2	**2c**	Ge	-	85

IN VITRO CYTOTOXITY ASSAY

Monolayer cells lines were cultivated for 72 h in DMEM standard medium without an indicator and antibiotics. After the ampoule was defreezed not more than four passages were performed. The control cells and cells with tested substances in the range of 2-5 10^4 cell/mL concentration (depending on line nature) were placed on a separate 96 wells plates. Solutions containing test compounds were diluted and added in wells to give the final concentrations of 50, 25, 12.5, and 6.25 μg/mL Control cells were treated in the same manner only in the absence of test compounds. Plates were cultivated for 72 h. A quantity of survived cells was determined using crystal violet (CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT) coloration which was assayed by multiscan spectrofotometer. The quantity of alive cells on control plate was taken in calculations for 100% [10, 11]. Concentration of NO was determined according to [10].

RESULTS AND DISCUSSION

Potential cytotoxic activity of synthesized fused isoxazolines **1** and **2** was tested *in vitro* on four monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibroblastoma), B16 (mouse melanoma), Neuro 2A (mouse neiroblastoma). Concentrations providing 50% of tumor death effect were determined according to the known procedure [12] using 96 well plates.

The experimental evaluation of cytotoxicity properties is presented in Table 2. A preliminary analysis of the structure-activity relationship for the cytotoxic action clearly indicates the strong influence of the Me_3M (M=C, Si, Ge) group in position 5 of fused isoxazolines **1** and **2**. Derivatives bearing *tert*-butyl substituent (**1a,b** and **2a**) have a slight cytotoxic effect (> 10 μg/mL). The substitution of the *tert*-butyl group by trimethylsilyl or

Table 2. *In vitro* cell cytotoxicity and the ability of intracellular NO generation caused by the fused isoxazolines **1**and **2**

N°	Cell lines											
	HT- 1080			MG- 22A			Neuro 2A			B16		
	IC_{50} CV [a]	IC_{50} MTT [b]	NO %, CV [c]	IC_{50} CV	IC_{50} MTT	NO %, CV	IC_{50} CV	IC_{50} MTT	NO %, CV	IC_{50} CV	IC_{50} MTT	NO %, CV
1a	>>10	>>10	3	>10	>10	9	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
1b	>10	>10	6	>10	>10	6	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
1c	5.5	5	63	5.2	4	38	8.4	6	14	8.4	7.3	8
1d	7	6.3	24	6.6	6	16	7.6	2	10	4	4	60
2a	>10	>10	6	6.3	6	9	>10	>10	3	>10	>10	5
2b	0.3	1.3	200	0.2	0.6	250	4.6	0.9	38	4.8	3.7	75
2c	1	3	50	0.5	0.5	100	4.3	0.9	21	4	3.2	67

[a] Concentration (μg/mL) providing 50% cell killing effect (CV: coloration).
[b] Concentration (μg/mL) providing 50% cell killing effect (MTT: coloration).
[c] NO Concentration (%) (CV: coloration).
[d] Not tested

trimethylgermyl ones leads to considerable increase of cytotoxicity. It must be noted that the activity of silicon- and germanium-containing compounds (**1c** and **1d**) depends on the tumor type. 5-Trimethylsilyl-substituted fused isoxazoline **1c** is more active than the germanium analogue **1d** in tests on HP-1080 and MG-22A cell lines. However, the germanium compound **1d** has greater cytotoxic effect on Neuro 2A and B16 cell lines than the silicon derivative **1c.** Comparison of the tumor growth inhibition for derivatives **1** and **2** shows a higher activity of the condensed isoxazolines **2** containing a phenyl group in position 3 with respect to 4,4-dioxo-3-methyl-3a-trimethylgermyl-5-Me_3M-3a,6a-dihydrothieno[2,3-*d*]isoxazolines **1b-d**. Silyl- and germyl-substituted fused isoxazolines have a medium NO-induction ability, 4,4-dioxo-3-phenyl-5-trimethylsilyl-3a,6a-dihydrothieno[2,3-*d*]isoxazoline-2 (**2b**) being the most active (250% in the MG-22A test).

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