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Synthesis, Vasodilating and Antithrombotic Activity of Pyridyl-substituted Silylisoxazolines

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3 - (2-Pyridyl) - 5 - phenyldimethylsilylisoxazoline, 3-(3-pyridyl) - 5 - phenyldimethylsilylisoxazoline and 3-(4-pyridyl) - 5 - phenyldimethylsilylisoxazoline were obtained by the [2 + 3] cycloaddition reaction of pyridyl nitrile oxides to phenyldimethylvinylsilane. The condensation of 3-pyridyl-substituted 5-triethoxysilylisoxazolines with triethanolamine afforded 3 - (2-pyridyl) - 5 - silatranylisoxazoline, 3 - (3 - pyridyl)-5-silatranylisoxazoline and 3-(4-pyridyl)-5-silatranylisoxazoline (12). In experiments *in vivo* and *in vitro* the vasodilating, antiarrhythmic and antithrombotic properties of pyridyl-substituted silylisoxazolines, their influence on the haemodynamic parameters in anaesthetized animals and their acute toxicity have been studied. It has been found that pyridyl-substituted silylisoxazolines possess vasodilating and antithrombotic properties. In experiments on the noradrenaline-preconstricted isolated rabbit ear artery, 3-(2-pyridyl)- and 3 - (4 - pyridyl) - 5 - phenyldimethylsilylisoxazoline exhibited pronounced vasodilating activity. 3 - (2 - Pyridyl) - and 3 - (3 - pyridyl) - 5 - phenyldimethylsilylisoxazoline and 3-(2-pyridyl)-5-silatranylisoxazoline prolonged blood coagulation time. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Contraction of vascular smooth muscle is one of the factors controlling the resistance of blood flow in the arterial system. The process of relaxation can be triggered by a number of substances occurring in the organism, such as acetylcholine, histamine and bradykinin. It has been shown that acetylcholine interacts with muscarinic cholinoreceptors in the endothelium, which, in turn, produces endothelium-derived relaxing factors: endothelium-derived nitric oxide and endothelium-dependent hyperpolarization.[1]

Nowadays intensive studies concerning the role of the endothelium in the regulation of tone and in the relaxation of blood vessels, and also a search for new substances affecting these processes, are being carried out. The clinical observations show that the functional state of the endothelium cells influences considerably the emergence and course of cardiovascular diseases.[2]

On the other hand, the silicon-containing compound *p*-fluorohexahydrosiladifenidol exhibits highly specific binding with muscarinic cholinoreceptors, bringing about vasodilation (M_3 subtype of muscarinic cholinoreceptor[1,3,4]).

Therefore, in our study aiming at the discovery of new compounds which can affect endothelium-regulated processes, we have synthesized silicon-containing isoxazolines substituted in position 3 with the pyridyl group, and we have investigated them for cardiovascular activity.

EXPERIMENTAL

Chemistry

Synthesis of pyridyl-substituted 5-phenyldimethylsilylisoxazolines 4–6

An equimolar amount of triethylamine in benzene (20 ml) was gradually added to a solution of

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phenyldimethyl(vinyl)silane (0.02 mol) and pyridylhydroxamic chloride (0.02 mol) in benzene (30 ml) at 70 °C. After addition of the amine the mixture was stirred for 4 h at the same temperature. When the temperature of the reaction mixture had fallen to ambient, triethylamine hydrochloride was filtered off. The solvent was evaporated *in vacuo* and the residue was extracted with hexane. During the cooling of the hexane solution pure pyridyl-substituted 5-phenyldimethylsilylisoxazoline precipitated, which was then filtered off and dried over air. The compound characteristics and analytical data are summarized in Tables 1 and 2.

Synthesis of pyridyl-substituted 5-silatranylisoxazolines

An equimolar amount of triethylamine in benzene (50 ml) was gradually added to a solution of triethoxy(vinyl)silane (0.04 mol) and pyridylhydroxamic chloride (0.04 mol) in benzene (50 ml) at 70 °C. After the addition of amine the mixture was stirred for 4 h at the same temperature. When the temperature had fallen to ambient, triethylamine hydrochloride was filtered off. The solvent and other unreacted materials were removed firstly using a rotary evaporator and then at 100 °C under 1 mmHg. The residue, without additional purification, was dissolved in toluene and an equimolar amount of triethanolamine was added. Then the mixture was heated at 100 °C and the calculated amount of ethanol distilled off. After cooling the silatrane formed was filtered off, recrystallized and dried over air.

Pharmacology

Vasodilating activity

For the experiments on the isolated perfused rabbit-ear blood vessels, the modified classical method was used.[7,8] Rabbits of both sexes (2.6–3.3 kg) were sacrificed by i.v. injection of pentobarbital sodium (80 mg kg^{-1}). The central ear artery was dissected free at the base of the ear, cannulated with polyethylene tubing and perfused at a constant flow rate (2 ml min^{-1}) from a four-channel peristaltic pump (Geming, Italy).

The perfusion fluid contained (mmol): NaCl 136.9; KCl 2.68; $CaCl_2$ 1.8; $MgCl_2$ 1.05; $NaHCO_3$11.9; NaH_2PO_4 0.42; glucose 5.6 (pH 7.35 at 22 °C). Intraluminal inflow perfusion

Table 1 Pyridylsilyl- and pyridylsilatrany-isoxazoline derivatives **4**–**6** and **10**–**12**

				Elemental analysis, Calculated/Found (%)		
Compound	Yield (%)	M.p. (°C)	Formula	C	H	N
4[a]	50	96[b]	$SiC_{16}H_{18}N_2O \cdot HCl$	60·26 / 59·91	6·01 / 5·97	8·78 / 8·80
5[a]	53	112[b]	$SiC_{16}H_{18}N_2O \cdot HCl$	60·26 / 59·79	6·01 / 5·97	8·78 / 8·74
6	60	72[c]	$SiC_{16}H_{18}N_2O$	71·97 / 72·11	4·03 / 3·98	9·33 / 9·30
10	80	215[d]	$SiC_{14}H_{19}N_3O_4$	52·32 / 52·07	5·97 / 5·93	13·06 / 12·87
11	60	207[b]	$SiC_{14}H_{19}N_3O_4$	52·32 / 52·25	5·97 / 5·94	13·06 / 12·90
12	70	216[b]	$SiC_{14}H_{19}N_3O_4$	52·32 / 52·13	5·97 / 5·91	13·06 / 12·86

[a] HCl salt. [b] From MeOH. [c] From hexane. [d] From THF.

pressure was measured with a Statham P23J transducer and recorded on a DMP-4B physiograph (Narco Bio-Systems, USA). As the flow rate remained constant, the changes in perfusion pressure reflected changes in blood vessel resistance, i.e. the degree of vasoconstriction or relaxation. Vasoconstriction was caused by the intraluminal infusion of noradrenaline (10 μmol). The compounds under investigation were dissolved in dimethylacetamide and then diluted in the perfusion fluid. Responses to these compounds at various concentrations (10 and 50 μmol l^{-1}) were tested: they are expressed as % relaxation (% changes in the perfusion pressure) without and with the investigated compounds or solvent.

Thrombos formation time

Blood coagulation time was detected using the Moravic method.[8] White male mice of the ICR-JCL strain (20–22 g) were used in the experiments (five mice in every group). The compounds studied were administered orally (p.o.) 60 min before the experiments. Blood was sampled from the jugular vein from the previously anaesthetized mice [urethane, 900 mg kg^{-1}, intraperitoneally (i.p.)]. The degree of inhibition and the blood coagulation time (for every compound in two different doses) were expressed in a percentage ratio in comparison with the control (the blood coagulation time caused by the solvent, a 0.6% solution of Tween 80).

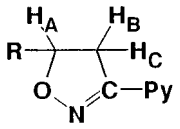
Bleeding time

The bleeding time was measured following Kopley and Roskam.[8] White male mice of the ICR-JCL strain (22–22 g) were used in the experiments (five mice in every group). The compound studied or solvent (0.6% solution of Tween 80) was administered p.o. 60 min before the test. The bleeding was obtained from the tails of the anaesthetized mice (urethane 900 mg kg^{-1}, i.p.).

Haemodynamic parameters

In acute trials on anaesthetized (α-glucochloralose, 80 mg kg^{-1}, and urethane, 200 mg kg^{-1},

Table 2 ^{1}H NMR spectroscopy data for pyridylsilyl- and pyridylsilatrany-isoxazoline derivatives **4**–**6** and **10**–**12** ($CDCl_3$/TMS)



			Chemical shift δ (ppm)					Coupling constant (J_{HH} (Hz)		
Compd	R	Py isomer	H_A	H_B	H_C	H_R	H_{Py}	AC	AB	BC
4[a]	$PhMe_2Si$	2	4.39	3.68	3.17	0.38(CH_3) 0.39(CH_3) 7.32–7.5(C_6H_5)	7.32–7.80	11.2	16.0	−16.0
5[a]	$PhMe_2Si$	3	4.44	3.72	3.27	0.38(CH_3) 0.39(CH_3) 7.26–7.47(C_6H_5)	7.26–9.16	10.8	15.0	−15.0
6	$PhMe_2Si$	4	4.38	3.40	3.02	0.38(CH_3) 0.39(CH_3) 7.31–7.64(C_6H_5)	7.49($H^{3,5}$pyr)[b] 8.64($H^{2,6}$pyr)	10.7	15.8	−15.8
10	$N(CH_2CH_2O)_3Si$	2	3.98	3.49	3.45	2.89(CH_2N) 3.84(CH_2O)	7.16–8.64	10.3	16.0	−16.0
11	$N(CH_2CH_2O)_3Si$	3	3.93	3.35	3.31	2.91(CH_2N) 3.87(CH_2O)	7.20–8.82	11.0	14.5	−14.5
12	$N(CH_2CH_2O)_3Si$	4	3.98	3.31	3.27	2.91(CH_2N) 0.87(CH_2O)	7.53($H^{3,5}$pyr) 8.60($H^{2,6}$pyr)	11.0	15.0	−15.0

[a] HCl salt. [b] Abbreviation: pyr, pyridine.

i.p.) male and female cats weighing 2.8–3.8 kg, the arterial blood pressure (RP-1500 transducer), the blood flow rate in the left common carotid artery (MFV-1200 electromagnetic flowmeter, Nihon Kohden, Japan) and the heart rate, triggered from the blood presure, were measured. All data were registered on a DMP-4B physiograph. The compounds being investigated were dissolved in dimethylacetamide and then diluted with a 0.9% solution of NaCl. The solutions of compounds or solvent in a volume not exceeding 1 ml were injected intravenously (i.v.) via a cannula.

Antiarrhythmic activity

Antiarrhythmic activity was tested on experimental calcium chloride-induced arrhythmia in male ICR-JCL mice (19–23 g). The compounds being tested, or solvents, were administered i.p. 15 min before i.v. infusion of a 2% solution of $CaCl_2$ at a constant rate (0.02 ml s^{-1}) at a dose of 180 mg kg^{-1}. The number of animals protected from $CaCl_2$-induced arrhythmia by the investigated compounds was determined.

Acute toxicity

The 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 i.p. To reduce the number of animals being used the maximal dose (400 mg kg^{-1}, i.p.) was used. If possible the LD_{50} was calculated at which 50% of the animals died.

Statistical analysis

The results were presented as mean or mean +SEM for each group. The statistical analysis was performed using Student's *t* test for unpaired data and the chi-squared (χ^2) test. The results were considered as significant at $P < 0.05$.

RESULTS

Chemistry

Synthesis of 3-pyridyl-5-silyl-substituted isoxazolines

Isoxazoline derivatives **4**–**6**, summarized in Tables 1 and 2, were prepared by the reaction of a pyridylhydroxamic chloride **1**–**3** with phenyldimethylvinylsilane in the presence of triethylamine (Eqn [1]).

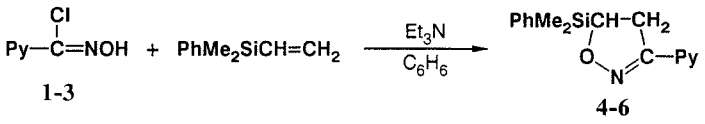
Hydroxamic chlorides were obtained by the method described in Refs 5 and 6, and were transformed to cyanopyridine oxide [Py–C≡N^+–O^-] under reaction conditions in the presence of triethylamine. The product formed interacted with vinylsilanes in the [2+3] cycloaddition reaction, affording the derivatives of 3-pyridyl-5-silyl-substituted isoxazolines.

3-Pyridyl-5-silatranylisoxazolines **10**–**12** (described in Tables 1 and 2) were synthesized by the condensation of triethoxysilylisoxazolinylpyridines **7**–**9** with triethanolamine (Eqn [2]).

Pharmacology

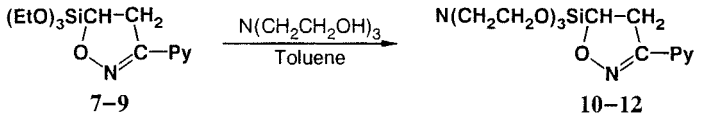
All the compounds under study (especially compounds **4** and **6**) caused the relaxation of the noradrenaline-preconstricted blood vessel of a rabbit's ear (Table 3).

Compounds **4**, **5** and **6** exhibited compara-



1-3 4-6

Py: 2-pyridyl (**1**,**4**); 3-pyridyl (**2**, **5**); 4-pyridyl (**3**, **6**) [1]



7–9 10–12

Py: 2-pyridyl (**7**,**10**); 3-pyridyl (**8**, **11**); 4-pyridyl (**9**, **12**) [2]

tively more expressed vasodilation than the corresponding silatranyl derivatives **10**–**12**. Table 4 summarizes the data on the anticoagulant activity of the 3-pyridyl-5-silyl-substituted isoxazolines. It has been shown that these compounds have no significant influence on the bleeding time. Compounds **4** and **5** prolong the coagulation time more considerably (2.6 to 2.76 times against the control). Compound **10** also revealed statistically significant anticoagulant properties in this experiment (see Table 4).

In trials on anaesthetized cats, we investigated the acute cardiovascular effects of pyridyl-substituted silylisoxazolines (Table 5). It has been shown that the compounds investigated (0.3 to 3 mg kg^{-1}, i.v.) did not cause significant changes in the haemodynamic parameters (mean blood pressure, heart rate and blood flow in the carotid artery). However, compounds **4**, **6** and **10**

Table 3 Vasodilating activity of the investigated compounds in rabbit's ear artery

Compound	Concentration (μmol)	Relaxation (%)
4	10	20
	50	42*
5	10	5
	50	25
6	10	22
	50	40*
10	10	15
	50	25
11	10	5
	50	12
12	10	12
	50	20
Solvent	—	8

* Significantly differ from the control (solvent) ($P < 0.05$).

Table 4 Anticoagulant activity of the investigated compounds

Compound	Dose (mg kg^{-1}, p.o.)	Bleeding time (min)	Increase in coagulation time (%)
4	30	7.7 ± 1.8	50
	90	9.5 ± 2.23	160*
5	30	6.5 ± 0.9	90
	90	8.7 ± 1.73	176*
6	30	5.85 ± 1.2	20
	90	6.7 ± 1.15	45
10	30	8.3 ± 0.77	55
	90	5.5 ± 0.96	115*
11	30	8.07 ± 0.83	60
	90	5.95 ± 0.68	85
12	30	5.25 ± 0.75	15
	90	8.3 ± 1.77	35
Control	—	6.1 ± 1.58	0

* Significantly differ from the control ($P < 0.05$).

Table 5 Cardiovascular effects of the investigated compounds on anaesthetized cats

Compound	Dose (mg kg^{-1}, i.v.)	Increase in blood pressure (%)[a]	Increase in heart rate (%)[a]	Increase in blood flow (%)[a]
4	0.3	±0	±0	+3
	1.0	−5	±0	+7
	3.0	+10 to −8	−3	+25
5	0.3	±0	−3	±0
	1.0	±3	±0	+5
	3.0	−10	±3	−5
6	0.3	−4	±0	+10
	1.0	−10	+5	+5
10	0.3	±0	±0	±0
	1.0	±3	±0	+5
	3.0	−5	±3	+5
11	0.3	±0	±0	±3
	1.0	±5	±0	±5
Solvent	0.3 ml	±0	±0	±0
	1.0 ml	±3	±3	−4

[a] Haemodynamic parameters (mean blood pressure, heart rate and blood flow in the carotid artery) are expressed as % change—predrug/postdrug: positive values indicate an increase, negative values a decrease.

diminished blood pressure and at the same time increased blood flow (Table 5). These findings were evidence for the vasodilating and more or less expressed cardiotonic properties of the investigated compounds. Table 6 summarizes the date on the antiarrhythmic activity and acute toxicity of pyridyl-substituted silylisoxazolines. The compounds under study did not protect from $CaCl_2$-induce arrhythmia. The acute toxicity of 3-pyridyl-5-silyl-substituted isoxazolines tested i.p. in mice was low ($LD_{50} > 400$ mg kg^{-1} (Table 6).

DISCUSSION

Contraction of the vascular smooth muscle is one of the factors controlling the resistance of blood flow in the arterial system. Smooth-muscle relaxation (which is a positive process rather than just the absence of contraction) requires activation of the enzyme guanylate cyclase and is accompanied by the conversion of guanosine triphosphate into cyclic guanosine monophosphate (cGMP).[10, 11] The process of relaxation can be triggered by a number of substances occurring in the organism, such as acetylcholine (Ach) and bradykinin. It was shown that Ach acted upon the endothelium[12] which, in turn, produced a 'second messenger'—an endothelium-derived relaxing factor (EDRF).[13] The EDRF diffused from the endothelium into the underlying muscle cells and activated guanylate cyclase. During recent years there have been numerous investigations dedicated to the chemical identity of the EDRF and nitric oxide.[14–17] Nitric oxide (NO) causes the relaxation of vascular smooth muscles and the inhibition of platelet aggregation involving cGMP.[17,18]

Experimental investigations showed that NO-related relaxation in the vascular bed in response to Ach is mediated by the activation of muscarinic cholinoreceptor (subtype M_3).[1]

On the other hand, endothelium cell-lining blood vessels separate platelets in circulating blood from the thrombogenic structures in the vascular wall. In the case of rupture by atherosclerotic plaque, or other lesions of the vascular wall, the numerous external stimuli interact with the specific receptors on the platelet membrane and trigger platelet aggregation. A series of pyridine derivatives may be designed and synthesized as antithrombotic agents—inhibitors of thromboxane A_2 synthase and thromboxane A_2 receptor antagonists. The substances containing a 3-pyridyl group have been found to be optimal regarding the dual mode of action.[19] It is postulated that the pyridine or imidazole moiety forms a complex via the nitrogen atom with the haem group of the catalytic site of thromboxane A_2 synthase.[20]

Silyl-substituted isoxazolines have been shown to possess vasodilating activity towards the endothelium in the experiments *in vitro* on an isolated rabbit's ear blood vessel. Obviously, this is explained by the endothelium-dependent vasorelaxation response attributed to agonist-stimulated NO production. We have not studied the binding of these compounds with the specific cholinoreceptors. However, it is clear that the vasodilation is achieved via M cholinoreceptors and specifically via M_3. It is indirectly confirmed by toxicological study of 3-pyridyl-5-silyl-substituted isoxazolines (lack of muscarinic-like reactions: salivation, etc.).

Table 6 Antiarrhythmic activity and acute toxicity of the investigated compounds in experiments on mice

Compound	Dose (mg kg^{-1}, i.p.)	Anti-arrhythmic activity, Y/N[a]	Acute toxicity, LD_{50} (mg kg^{-1}, i.p.)
4	30	1/5	>400
	90	0/5	
5	30	0/5	>400
	90	1/5	
6	30	1/5	>400
	90	2/5	
10	10	1/5	
	30	0.5	>400
	60	1/5	
11	10	0/5	
	30	1/5	>400
	60	0/5	
12	30	0/5	>400
	90	1/5	
Procainamide	30	1/5	360 (257–504)
	90	3/5*	
Solvent/ Control	—	0/10	

[a] Ratio Y/N, where Y = number of mice protected against $CaCl_2$-induced arrhythmia; N = number of animals in experiment.
* Significantly differ from the control ($P < 0.05$).

Perhaps the presence of the NO group in the structure of the isoxazoline derivatives is responsible for the occurrence of vasodilation. The assumption that silyl-substituted isoxazolines may act as NO donors is supported by the high anticoagulant activity of these compounds (Table 4).

The results of a study of antithrombotic agents containing the 3-pyridyl group must be taken into consideration when discussing the possible mechanism of action of 3-pyridyl-5-silyl-substituted isoxazolines.[19,20] It may be suggested that the presence of the silyl group does not decrease the interaction of silyl-substituted isoxazolines with thromboxane A_2 receptor or/and influences the thromboxane A_2 synthase.

It may be concluded that pyridyl-substituted silylisoxazolines possess valsodilating and antithrombotic activity. Some leading compounds of the 3-pyridyl-5-silyl-substituted isoxazoline series have been found, for which it would be reasonable to carry out a more detailed pharmacological study.

REFERENCES

1. A. S. O. Adeagbo and C. R. Triggle, *J. Cardiovasc. Pharmacol.* **21**, 423 (1993).
2. H. Drexler and B. Horning, *J. Cardiovasc. Pharmacol.* **27**, 9 (1996).
3. E. Bungardt, E. Vockert, R. Feifel, U. Moser, R. Tacke, E. Mutschler, G. Lambrecht and A. Surprenant, *Eur. J. Pharmacol.* **213**, 53 (1992).
4. E. Barocelli, M. Chiavarini, V. Ballabeni, F. Bordi and M. Impicciatore, *Br. J. Pharmacol.* **108**, 393 (1993).
5. R. Paul and S. Tchelitcheff, *Bull. Soc. Chim. Fr.* 2215 (1962).
6. M. Kocevar, S. Polanc, M. Sollner, M. Tisler and B. Vercek, *Synth. Commun.* **18**, 1427 (1988).
7. R. Blattner, H. G. Classen, H. Dehnert and H. G. Doring, *Experiments on Isolated Smooth Muscle Preparations*, Hugo Sachs Elektronik KG, (BRD), 1980.
8. S. K. Wilson, O. S. Steinsland and S. H. Nelson, *J. Cardiovasc. Pharmacol.* **23**, 127 (1994).
9. Y. Thodorov, Klinischeskie Laboratornye Issledovania v Pediatrii. Medic. Phys., Sophia, 1966.
10. J. A. Michell, U. Forstermann, T. D. Warnen, J. S. Pollock, H. H. Schmidt, M. Hellen and F. Murad, *Biochem. Biophys. Res. Commun.* **176**, 14 (1991).
11. L. J. Ignarro, *Hypertension* **16**, 477 (1990).
12. R. F. Furchgott and J. V. Zawadzki, *Nature (London)* **288**, 373 (1980).
13. R. F. Furchgott, *Circ. Res.* **53**, 557 (1983).
14. R. M. J. Palmer, A. G. Ferige and S. Moncada, *Nature (London)* **327**, 524 (1987).
15. R. M. J. Palmer, D. D. Rees, D. S. Ashton and S. Moncada, *Biochem. Biophys. Res. Commun.* **153**, 1251 (1988).
16. I. Sakuma, D. Stuehr, S. S. Gross, C. Nathan and R. Levi, *Proc. Natl. Acad. Sci. USA* **85**, 8664 (1988).
17. A. R. Butter and D. Z. H. Williams, Chem. Soc. Rev., 233 (1993).
18. M. G. Doni, A. Alexandre, E. Tadoin, S. Bertoncello and R. Deana, *Cardioscience* **2**(3), 161 (1991).
19. R. Soyka, A. Heckel, J. Nickl, W. Eisert, T. H. Muller and H. Weisenberger, *J. Med. Chem.* **37** , 26 (1994).
20. M. Hecker, M. Haurand, V. Ulrich and S. Terao, *Eur. J. Biochem.* **157**, 217 (1986).

