Synthesis and Cytotoxicity of Silicon and Germanium Containing Pyridine Oxime O-Ethers

Edgars Abele*, Ramona Abele, Pavel Arsenyan, Irina Shestakova, Iveta Kanepe, Inga Antonenko, Juris Popelis, and Edmunds Lukevics*

*Latvian Institute of Organic Synthesis,
21 Aizkraukles Street, Riga, LV-1006, Latvia; Fax: (371) 7550338; E-mail: abele@osi.lv*

ABSTRACT

Silicon and germanium containing pyridine aldoxime, ketoxime and amidoxime O-ethers have been prepared using phase transfer catalytic systems oxime / alkyl halide / solid KOH / 18-crown-6 / benzene and oxime / alkyl halide / solid K₂CO₃ or Cs₂CO₃ / 18-crown-6 / toluene. Cytotoxic activity of silicon and germanium containing pyridine oxime O-ethers was tested *in vitro* on two monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma). O-[3-Triethylsilylpropyl]- and O-[3-(1-methyl-1-silacyclopentyl)propyl] oximes of pyridine aldehydes and ketones exhibit high cytotoxicity. Presence of methyl group in the pyridine ring considerably decreased activity of amidoxime O-ethers. Oxime ethers containing two elements are essentially inactive. For 2-acetylpyridine oxime ethers the activity increases in order of alkyl substituents: Et₃GeCH₂CH₂SiMe₂CH₂ < Et₃SiCH₂CH₂CH₂ < (CH₂)₄SiCH₂CH₂CH₂. Cytotoxicity of ketoxime O-ethers is considerably lower in comparison with aldoxime O-ethers.

INTRODUCTION

Pyridine oxime O-ethers containing different heteroatomic groups are of interest as biologically active compounds. For example, pyridine oxime amino derivatives exhibit antiandrogenic /1,2/, antibacterial /3/, antidotal against organophosphate poisoning /4,5/, fungicidal and pesticidal /6/ activities. Sulfur derivatives of pyridine oxime O-ethers display vasodilating, antiulcer /7/, trombocytes aggregation inhibiting /8/, antibacterial /9/, cytotoxic /9/, antidotal of organophosphate poisoning /10/, fungicidal /11/ and herbicidal /12, 13/ activities. Phosphorus containing oxime ethers are reactivators of acetylcholinesterase /14/ and exhibit insecticidal /15/ activity. Fungicidal and insecticidal properties of pyridine aldehyde O-

* Dr. Hab. Chem. Edgars Abele and Prof. Edmunds Lukevics; Latvian Institute of Organic Synthesis, 21 Aizkraukles Street, Riga, LV-1006, Latvia; Tel: (371) 7551822, Fax: (371) 7550338; E-mail: abele@osi.lv

(triethylstannyl)oximes are also described /16/. Cytotoxicity of silicon and germanium derivatives of pyridine oxime O-ethers has not been investigated till now and is the aim of the present work.

MATERIALS AND METHODS

Chemistry

^1H NMR spectra were recorded on a Varian 200 Mercury instrument using CDCl_3 as solvent and hexamethyldisiloxane (HMDSO) as internal standard. Mass spectra were registered on a GC-MS HP 6890 (70 eV). GC analysis was performed on a Chrom-5 instrument equipped with flame-ionization detector using glass column packed with 5 % OV-101 / Chromosorb W-HP (80-100 mesh) (1.2 m x 3 mm). Pyridine oximes **1-3** and **13-16** were obtained according to the procedures described in /17, 18/. 1-(3-Iodopropyl)-1-methylsilacyclopentane was obtained by Grignard reaction /19, 20/. $\text{MeEt}_2\text{SiCH}_2\text{CH}_2\text{Si}(\text{Me}_2)\text{CH}_2\text{I}$ and $\text{Et}_3\text{GeCH}_2\text{CH}_2\text{Si}(\text{Me}_2)\text{CH}_2\text{I}$ were prepared as described in reference /21/. The purity of all synthesized compounds was determined by HPLC and content of impurities was not higher than 2.5%.

General procedure for alkylation of pyridine aldoximes and ketoximes 1-3.

Haloalkylsilane (1 mmol) was added to a suspension of pyridine oxime **1-3** (1 mmol), 18-crown-6 (0.026 g, 0.1 mmol) and powdered KOH (0.168 g, 3 mmol) in benzene (1 ml). The reaction mixture was stirred for 3-6 h at reflux (GLC control). The resulting mixture was filtered, benzene was removed under reduced pressure and the residue was purified by column chromatography (benzene-ethyl acetate in different ratio was used as eluent) to give products **4-12**. The results can be seen in Tables 1, 3 and 4.

General procedure for alkylation of pyridine amidoximes 13-16

Haloalkylsilane (1 mmol) was added to a suspension of pyridine amidoxime **13-16** (1 mmol), 18-crown-6 (0.026 g, 0.1 mmol) and powdered K_2CO_3 (0.41g, 3 mmol) (in the synthesis of compounds **19,20** Cs_2CO_3 was used) in toluene (2 ml). The reaction mixture was stirred for 6-12 h at reflux (GLC control). The resulting mixture was filtered, benzene was removed under reduced pressure and the residue was purified by column chromatography (benzene-ethyl acetate in different ratio was used as eluent) to give products **17-25**. The results can be seen in Tables 2-4.

In vitro cytotoxicity assay

Monolayer cell lines were cultivated for 72 h in DMEM standard medium without an indicator and antibiotics. After the ampoule was unfrozen not more than four passages were performed. The control cells and cells with tested substances in the range of $(2-5) \times 10^4$ cell/mL concentration (depending on line nature) were placed on 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 $\mu\text{g/mL}$. Control cells were treated in the same manner only in the absence of test compounds. Plates were cultivated for 72 h. The amount of surviving cells was determined using crystal violet (CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) coloration, which was assayed by multiscan spectrophotometer. The quantity of live cells on control plate was taken in calculations for 100% [22, 23]. Concentration of NO was determined according to [22].

RESULTS AND DISCUSSION

Chemistry

Alkylation of pyridine oximes with silicon and germanium containing alkyl halides was studied. The known methods for the preparation of oxime O-ethers are based on oxime alkylation in the presence of NaH / DMF [24] or alkali metal alkoxide [25, 26]. The oxime alkylation reactions in the phase transfer catalytic (PTC) systems oxime / RX (X = Br, I) / 10 % aqueous NaOH / R_4NX (R = n-Bu, n-Oct; X = Br, HSO_4) / PhH [27, 28], oxime / RX (X = Br, I) / solid K_2CO_3 / 18-crown-6 / PhH [29], oxime / RCl / solid KOH or K_2CO_3 / solid KI / 18-crown-6 / PhMe or PhH [30] and oxime O-acetate or benzoate / RX (X = Br, I) / solid KOH / 18-crown-6 / PhH [31] considerably simplifies the preparation of their O-ethers.

The PTC system iodo- or bromomethylsilane / solid KOH / 18-crown-6 / benzene was found to be the most active in the synthesis of oxime ethers **4 - 12** from pyridine aldioximes and ketoximes **1 - 3** (Table 1, 3, 4). Synthesis of all pyridine oxime O-ethers proceeded stereoselectively with formation of only *E*-isomers (yields up to 65%).

However, the attempts to alkylate the pyridine amidoximes **13-16** with silicon containing alkyl halides in PTC system (solid KOH / 18-crown-6 / benzene) were ineffective. Replacement of the solid KOH / 18-crown-6 / benzene to solid K_2CO_3 (or Cs_2CO_3) / 18-crown-6 / toluene increased the yield of pyridine amidoxime O-ethers **17-25** (Tables 2-4). The *E*-isomers of products **17-25** were isolated by column chromatography in 40-79 % yields.

Table 1
Synthesis of pyridine aldoxime and ketoxime O-ethers **4-12**

Oxime	Pyridyl	R	SiR'R''R'''	n	X	Reaction time, h	Product	Yield ^a , %
1	2-	H	Si(Me ₂)CH ₂ CH ₂ GeEt ₃	1	I	3	4	42
1	2-	H	SiEt ₃	3	Br	6	5	48
1	2-	H	1-methyl-1-silacyclopentyl	3	I	6	6	31
2	2-	Me	Si(Me ₂)CH ₂ CH ₂ SiEt ₂ Me	1	I	3	7	51
2	2-	Me	Si(Me ₂)CH ₂ CH ₂ GeEt ₃	1	I	3	8	56
2	2-	Me	SiEt ₃	3	Br	6	9	41
2	2-	Me	1-methyl-1-silacyclopentyl	3	I	6	10	65
3	4-	H	SiEt ₃	3	Br	6	11	62
3	4-	H	1-methyl-1-silacyclopentyl	3	I	6	12	64

^a Yields of isolated products

Table 2
Synthesis of pyridine amidoxime O-ethers **17-25**

Oxime	Pyridyl	R	SiR'R''R'''	n	X	Time, h	Product	Yield ^a , %
13	3-	H	SiEt ₃	3	Br	6	17	40
13	3-	H	1-methyl-1-silacyclopentyl	3	I	7	18	65
14	4-	H	SiEt ₃	3	Br	12	19	40
14	4-	H	1-methyl-1-silacyclopentyl	3	I	12	20	33
15	2	3-Me	SiEt ₃	3	Br	8	21	49
15	2	3-Me	1-methyl-1-silacyclopentyl	3	I	6	22	59
15	2	3-Me	Si(Me ₂)CH ₂ CH ₂ SiEt ₂ Me	1	I	8	23	46
16	2	6-Me	SiEt ₃	3	Br	7	24	60
16	2	6-Me	Si(Me ₂)CH ₂ CH ₂ GeEt ₃	1	I	9	25	64

^a Yields of isolated products

***In Vitro* cytotoxicity**

Cytotoxic activity of silicon and germanium containing pyridine oxime O-ethers was tested *in vitro* on two monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma). Concentrations providing 50% of tumor death effect were determined according to the known procedure [32] using 96 well plates.

Table 3
Mass spectroscopic data of pyridine oxime O-ethers

Compound	M/z (intensity, %)
4	337 (< 1, $M^+ - C_3H_7$), 219 (4), 193 (63), 163 (100), 149 (5), 133 (16), 119 (9), 103 (14), 92 (12), 78 (10), 59 (21).
5	279 (< 1, M^+), 249 (23), 233 (8), 145 (7), 115 (21), 103 (100), 87 (41), 78 (74), 59 (30), 51 (14)
6	262 (2, M^+), 217 (9), 203 (89), 192 (8), 184 (18), 175 (17), 165 (10), 148 (40), 133 (13), 119 (18), 101 (84), 87 (19), 78 (100), 61 (23), 45 (37)
7	321 (< 1, $M^+ - Me$), 291 (3), 207 (51), 177 (100), 136 (16), 119 (13), 101 (14), 87 (15), 78 (45), 59 (26), 45 (26)
8	367 (< 1, $M^+ - Et$), 207 (73), 177 (100), 161 (5), 147 (5), 136 (17), 103 (130), 78 (47), 59 (16), 43 (7)
9	291 (< 1, M^+), 247 (7), 133 (25), 119 (72), 87 (22), 78 (100), 59 (17), 51 (10)
10	276 (< 1, M^+), 231 (5), 133 (18), 119 (50), 99 (18), 78 (100), 45 (10)
11	249 (35, $M^+ - Et$), 115 (53), 103 (100), 87 (62), 75 (66), 59 (34)
12	262 (5, M^+), 233 (5), 184 (41), 133 (5), 101 (100), 87 (13), 78 (23), 61 (13), 51 (18), 43 (11)
17	293 (2, M^+), 264 (12), 222 (4), 137 (23), 120 (100), 103 (68), 87 (34), 78 (30), 59 (28), 51 (16)
18	276 (4, M^+), 248 (12), 234 (14), 220 (13), 193 (8), 163 (10), 137 (20), 129 (9), 120 (100), 102 (66), 87 (27), 78 (66), 59 (24), 45 (46)
19	293 (2, M^+), 264 (51), 222 (5), 137 (23), 120 (100), 103 (81), 87 (43), 78 (36), 59 (33), 43 (10)
20	277 (6, M^+), 248 (47), 234 (34), 220 (10), 193 (8), 178 (12), 148 (45), 137 (33), 129 (10), 120 (93), 102 (100), 87 (29), 78 (77), 59 (26), 51 (50), 43 (45)
21	307 (2, M^+), 262 (6), 162 (13), 151 (10), 134 (100), 118 (16), 103 (18), 92 (36), 75 (13), 59 (18)
22	290 (2, M^+), 276 (6), 177 (16), 162 (22), 145 (10), 134 (100), 119 (26), 101 (20), 92 (66), 65 (29), 45 (22)
23	306 (2, $M^+ - Et - Me$), 222 (75), 192 (89), 134 (13), 119 (16), 101 (16), 92 (20), 74 (100), 59 (26), 45 (25)
24	307 (3, M^+), 278 (7), 262 (10), 178 (10), 162 (41), 151 (24), 134 (100), 119 (32), 103 (34), 92 (66), 75 (22), 59 (35), 43 (12)
25	396 (< 1, $M^+ - Me$), 222 (100), 192 (46), 133 (14), 119 (28), 103 (14), 92 (14), 74 (97), 59 (17)

Table 4
¹H NMR data of pyridine oxime O-ethers

Ether	δ ppm, CDCl ₃ / HMDSO)
4	0.06 (s, 6H, Si(CH ₃) ₂), 0.67 (m, 10H, SiCH ₂ and GeCH ₂), 0.99 (t, 9H, J = 7,6 Hz, GeCH ₂ CH ₃), 4.06 (s, 2H, OCH ₂), 7.23 (m, 1H, 5-H), 7.62 – 7.79 (m, 2H, 3-H and 4-H), 8.13 (s, 1H, CH), 8.59 (m, 1H, 6-H)
5	0.52 (m, 8H, SiCH ₂), 0.92 (t, 9H, J = 7.4 Hz, CH ₃), 1.60 (m, 2H, CH ₂ CH ₂ CH ₂), 4.16 (t, 2H, J = 7.0 Hz, OCH ₂), 7.26 (m, 1H, 5-H), 7.62 - 7.80 (m, 2H, 3-H and 4-H), 8.16 (s, 1H, CH), 8.61 (m, 1H, 6-H)
6	0.08 (s, 3H, CH ₃), 0.59 (m, 6H, SiCH ₂), 1.55 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 4.18 (t, 2H, J = 7.0 Hz, OCH ₂), 7.25 (m, 1H, 5-H), 7.67 - 7.80 (m, 2H, 3-H and 4-H), 8.16 (s, 1H, CH), 8.60 (m, 1H, 6-H)
7	-0.09 (s, 3H, SiCH ₃), 0.10 (s, 6H, Si(CH ₃) ₂), 0.51 (m, 8H, SiCH ₂), 0.91 (t, 6H, J = 7,6 Hz, SiCH ₂ CH ₃), 2.31 (s, 3H, CCH ₃), 4.06 (s, 2H, OCH ₂), 7.36 (m, 1H, 5-H), 7.45 (m, 1H, 3-H), 7.90 (m, 1H, 4-H), 8.59 (m, 1H, 6-H)
8	0.10 (s, 6H, Si(CH ₃) ₂), 0.72 (m, 10H, SiCH ₂ and GeCH ₂), 0.99 (t, 9H, J = 7.2 Hz, GeCH ₂ CH ₃), 2.29 (s, 3H, CCH ₃), 4.05 (s, 2H, OCH ₂), 7.21 (m, 1H, 5-H), 7.64 (m, 1H, 3-H), 7.89 (m, 1H, 4-H), 8.58 (m, 1H, 6-H)
9	0.54 (m, 8H, SiCH ₂), 0.93 (t, 9H, J = 7.6 Hz, CH ₃), 1.60 (m, 2H, CH ₂ CH ₂ CH ₂), 2.35 (s, 3H, CH ₃), 4.17 (t, 2H, J = 7.0 Hz, OCH ₂), 7.25 (m, 1H, 5-H), 7.61 (m, 1H, 3-H), 7.87 (m, 1H, 4-H), 8.58 (m, 1H, 6-H)
10	0.09 (s, 3H, CH ₃), 0.60 (m, 6H, SiCH ₂), 1.55 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 2.32 (s, 3H, CH ₃), 4.18 (t, 2H, J = 7.0 Hz, OCH ₂), 7.25 (m, 1H, 5-H), 7.65 (m, 1H, 3-H), 7.89 (m, 1H, 4-H), 8.59 (m, 1H, 6-H)
11	0.54 (m, 8H, SiCH ₂), 0.93 (t, 9H, J = 7.6 Hz, CH ₃), 1.65 (m, 2H, CH ₂ CH ₂ CH ₂), 4.15 (t, 2H, J = 7.0 Hz, OCH ₂), 7.42 (m, 2H, 3-H and 5-H), 8.01 (s, 1H, CH), 8.62 (m, 2H, 2-H and 6-H)
12	0.09 (s, 3H, CH ₃), 0.59 (m, 6H, SiCH ₂), 1.55 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 4.16 (t, 2H, J = 7.0 Hz, OCH ₂), 7.43 (m, 2H, 3-H and 5-H), 8.01 (s, 1H, CH), 8.62 (m, 2H, 2-H and 6-H)
17	0.52 (m, 8H, SiCH ₂), 0.92 (t, 9H, J = 7.0 Hz, CH ₃), 1.66 (m, 2H, CH ₂ CH ₂ CH ₂), 4.07 (t, 2H, J = 7.0 Hz, OCH ₂), 4.77 (bs, 2H, NH ₂), 7.22 (m, 1H, 5-H), 7.91 (m, 1H, 4-H), 8.53 (m, 1H, 6-H), 8.85 (m, 1H, 2-H)
18	0.26 (s, 3H, CH ₃), 0.76 (m, 6H, SiCH ₂), 1.78 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 4.21 (t, 2H, J = 7.2 Hz, OCH ₂), 5.00 (bs, 2H, NH ₂), 7.48 (m, 1H, 5-H), 8.08 (m, 1H, 4-H), 8.77 (m, 1H, 6-H), 8.98 (m, 1H, 2-H)

Table 4 (continued)

19	0.52 (m, 8H, SiCH ₂), 0.93 (t, 9H, J = 7.4 Hz, CH ₃), 1.65 (m, 2H, CH ₂ CH ₂ CH ₂), 4.06 (t, 2H, J = 7.0 Hz, OCH ₂), 4.79 (bs, 2H, NH ₂), 7.51 (m, 2H, 3-H and 5-H), 8.62 (m, 2H, 2-H and 6-H)
20	0.09 (s, 3H, CH ₃), 0.59 (m, 6H, SiCH ₂), 1.55 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 4.07 (t, 2H, J = 6.8 Hz, OCH ₂), 4.79 (bs, 2H, NH ₂), 7.51 (m, 2H, 3-H and 5-H), 8.63 (m, 2H, 2-H and 6-H)
21	0.52 (m, 8H, SiCH ₂), 0.93 (t, 9H, J = 6.8 Hz, CH ₃), 1.72 (m, 2H, CH ₂ CH ₂ CH ₂), 2.56 (s, 3H, CH ₃ in ring), 4.03 (t, 2H, J = 7.0 Hz, OCH ₂), 5.47 (bs, 2H, NH ₂), 7.09 (m, 1H, 5-H), 7.45 (m, 1H, 4-H), 8.36 (m, 1H, 6-H)
22	0.08 (s, 3H, CH ₃), 0.60 (m, 6H, SiCH ₂), 1.55 (m, 6H, CH ₂ (CH ₂) ₂ CH ₂ in silacycle and CH ₂ CH ₂ CH ₂), 2.59 (s, 3H, CH ₃ in ring), 4.07 (t, 2H, J = 6.8 Hz, OCH ₂), 5.50 (bs, 2H, NH ₂), 7.17 (m, 1H, 5-H), 7.53 (m, 1H, 4-H), 8.40 (m, 1H, 6-H)
23	-0.15 (s, 3H, Et ₂ SiCH ₃), 0.04 (s, 6H, Si(CH ₃) ₂), 0.43 (m, 8H, SiCH ₂), 0.82 (t, 6H, J = 7.6 Hz, SiCH ₂ CH ₃), 2.54 (s, 3H, CH ₃ in ring), 3.90 (s, 2H, OCH ₂), 5.42 (bs, 2H, NH ₂), 7.10 (m, 1H, 5-H), 7.45 (m, 1H, 4-H), 8.34 (m, 1H, 6-H)
24	0.53 (m, 8H, SiCH ₂), 0.90 (t, 9H, J = 6.8 Hz, CH ₃), 1.71 (m, 2H, CH ₂ CH ₂ CH ₂), 2.51 (s, 3H, CH ₃ in ring), 4.05 (t, 2H, J = 7.0 Hz, OCH ₂), 5.53 (bs, 2H, NH ₂), 7.04 (m, 1H, 5-H), 7.56 (m, 2H, 4-H and 3-H)
25	0.17 (s, 6H, Si(CH ₃) ₂), 0.66 (m, 10H, SiCH ₂ and GeCH ₂), 1.02 (t, 9H, J = 7.0 Hz, GeCH ₂ CH ₃), 2.63 (s, 3H, CH ₃ in ring), 4.05 (s, 2H, OCH ₂), 5.60 (bs, 2H, NH ₂), 7.19 (m, 1H, 5-H), 7.68 (m, 2H, 4-H and 3-H)

The experimental evaluation of cytotoxicity is presented in Table 5. A preliminary analysis of the structure-activity relationship for the cytotoxic action clearly indicates the strong influence of the silicon and germanium containing alkyl substituent on toxic effects *in vitro*. In the series of O-silyl(germyl)alkyl pyridine aldoximes and ketoximes (4-12) the TD₅₀ values change from 1.85 µg/mL (silacyclopentyl derivative 6 on human fibrosarcoma HT – 1080 cell line) to non cytotoxic compounds (>100 µg/mL for 4, 7, and 8). Oxime ethers containing two elements are essentially inactive. Silacyclopentyl derivative 6 exhibits the higher cytotoxicity on HT – 1080 as compared to triethylsilylpropyl substituted pyridine aldoxime 5. For pyridine ketoxime ethers (8-10) the activity increases in order of alkyl substituents: Et₃GeCH₂CH₂SiMe₂CH₂ < Et₃SiCH₂CH₂CH₂ < (CH₂)₄SiCH₂CH₂CH₂. Cytotoxicity of ketoxime O-ethers is considerably lower in comparison with aldoxime O-ethers.

In the cases of O-ethers of pyridine amidoximes the highest cytotoxicity was exhibited by unsubstituted pyridine derivatives (17-20). The presence of methyl group in the pyridine ring considerably decreased the activity of amidoxime O-ethers.

Table 5*In vitro* cell cytotoxicity and the ability of intracellular NO-generation by pyridine oxime O-ethers.

N°	HT - 1080		MG - 22A	
	TD ₅₀ ^a	NO, % CV ^b	TD ₅₀ ^a	NO, % CV ^b
4	>100	3	>100	6
5	3.5	350	5	350
6	1.85	400	6	500
7	>100	3	>100	4
8	>100	8	>100	4
9	62	350	29	500
10	28	175	27.5	300
11	3.65	350	3.5	350
12	3.15	300	5.5	200
17	5.4	300	4.3	300
18	4.85	250	24.5	200
19	2.5	550	4.5	330
20	6	300	4.5	300
21	21.5	300	37	350
22	>100	9	>100	9
23	6.35	250	22	450
24	>100	4	>100	6
25	>100	4	>100	5

^a Concentration (μg/mL) providing 50% cell killing effect [(CV+MTT)/2]^b NO Concentration (%) (CV: coloration)

The NO level was determined accordingly [22], NO release was defined using the Greys reagent (by NO₂ concentration in the cultural medium). The yield of nitrite was expressed as NO₂ nmol/200 μL of cultural medium in testing plates for 100% alive cells after CV coloration assay (pyridine derivatives concentration 100 μg/mL). It was shown (Table 5) that compounds 6, 9, and 19 readily increase NO concentration in the cultural medium (TG₁₀₀=500%) in HT-1080 and MG - 22A cell lines.

REFERENCES

1. Scherico Ltd. *Brit. Pat.* 1070964 (1967); *Chem. Abstr.*, **68**,104994x (1968).
2. F.J. Villant, *US Pat.* 3290320 (1966); *Chem. Abstr.*, **66**, 46340b (1967).
3. C.Y. Hong, Y.K. Kim, S.H. Kim, J.H. Chang, H. Chou, D.H. Nam, A.R. Kim, J.H. Lee and K.S. Park, *US Pat.* 5776944 (1998); *Chem. Abstr.*,**129**, 122580s (1998).
4. T. Nishimura, C. Yamazaki and T. Ishiura, *Bull. Chem. Soc. Jap.*, **40**, 2434 (1967).
5. I. Bulete, *Rom. Pat.* 51935 (1970); *Chem. Abstr.*, **73**, 120678f (1970).
6. F. Rohl, V. Harries, E. Ammermann, G. Lorenz, S. Strathmann, A. Ptock, H. Sauter, W. Grammenos, T. Grote, H. Bayer, R. Kirstgen, K. Oberdorf, B. Muller and R.Muller, *PCT Int. WO Pat.* 9812179 (1998); *Chem. Abstr.*, **128**, 257334q (1998).
7. C. Malen, B. Danree and X.Pascaud, *Ger. Pat.* 2217180 (1972); *Chem. Abstr.*, **78**, 16052q (1973).
8. U. Niewoehner, U.E. Mueller, E. Perzborn, E. Bischoff and H.G. Dellweg, *Eur. Pat.* 471259 (1992); *Chem. Abstr.*, **116**, 214360e (1992).
9. F. Trecourt, B. Gervais, O. Mongin, C. Le Gal, F. Mongin and G. Queguiner, *J. Org. Chem.*, **63**, 2892 (1998).
10. F. Hauschild, R. Schmiedel and W.D. Wiezorek, *East. Ger. Pat.* 38036 (1965); *Chem. Abstr.*, **63**, 13225c (1965).
11. C. Fest, W. Brandes, G. Haenssler and P. Reinecke, *Ger. Pat.* 3608383 (1987); *Chem. Abstr.*, **107**, 217494b (1987).
12. W. Fuehrer, J. Stetter, H. Foerster, L. Eue and R.R. Schmidt, *Ger. Pat.* 3221215 (1983); *Chem. Abstr.*, **100**, 138961d (1984).
13. T. Goshima, Y. Kitagawa, S. Kaji, H. Hayakawa and A. Watanabe, *Jpn. Pat.* 0499767 (1992); *Chem Abstr.*, **117**, 131079q (1992).
14. R.A. Andersen, J.A.B. Barstad and K. Laake, *Progr. Brain Res.*, **36**, 189 (1972).
15. Ishikara Sangyo Kaisha, Ltd. *Jpn. Pat.* 8183495 (1981); *Chem. Abstr.*, **95**, 132689c (1981).
16. Farbenfabriken Bayer A.-G. *Brit. Pat.* 945068 (1963); *Chem. Abstr.*, **60**, 12051c (1964).
17. S. Ginsburg and I.B. Wilson, *J. Am. Chem. Soc.*, **79**, 481 (1957).
18. E. Bernasek, *J. Org. Chem.*, **22**, 1263 (1957).
19. N.S. Nametkin, K.S. Vdovin, K.S. Pushchevaya, V.I. Zavyalov, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1453 (1965).
20. J.W. Wilt and C.F. Dockus, *J. Amer. Chem. Soc.*, **92**, 5813 (1970).
21. K. Rubina, E. Abele, P. Arsenyan, R. Abele, M. Veveris and E. Lukevics, *Metal-Based Drugs*, **8**, 85 (2001).
22. D.J. Fast, R.C. Lynch and R.W. Leu, *J. Leukocyt. Biol.*, **52**, 255 (1992).
23. P.J. Freshney, *Culture of Animal Cells (A Manual of Basic Technique)*, Wiley-Liss, New York, 1994, pp. 296-297.
24. Z. Wimmer, D. Saman, J. Smolinkova and M. Romanuk, *Lieb. Ann. Chem.*, 1091 (1988).
25. I. Hadegorn, W.-H. Gundel, J. Hosse and Chr. Fenter, *Arzneim.-Forsch. (Drug Res.)*, **26**, 1273 (1976).

26. A. Steinhards and W. Mathes, *US. Pat.* 2924604 (1958); *Chem. Abstr.*, **56**, 736d (1961).
27. K. Rubina, Yu. Goldberg, A. Gaukhman and M. Shymanska, *Synth. Commun.*, **19**, 3129 (1989).
28. K. Rubina, Yu. Popelis, Yu. Goldberg and M. Shymanska, *Chem. Heterocycl. Comp.*, **28**, 1406 (1992).
29. E. Abele, Yu. Popelis, E. Lukevics, M. Shymanska and Yu. Goldberg, *Chem. Heterocycl. Comp.*, **30**, 14 (1994).
30. E. Abele, R. Abele, K. Rubina, J. Popelis, I. Sleikša and E. Lukevics, *Synth. Commun.*, **28**, 2621 (1998).
31. E. Ābele, R. Ābele, J. Popelis and E. Lukevics, *Latv. J. Chem.*, N 2, 61 (1998).
32. R.J. Riddell, R.H. Clothier, M. Fd.Balls, *Chem. Toxicol.*, **24**, 469 (1986).