

## SYNTHESIS AND BIOLOGICAL ACTIVITY OF SILYL- AND GERMYSUBSTITUTED TRIFLUOROACETYLFURANS

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### ABSTRACT

A series of silyl, germyl and alkyl substituted trifluoroacetyl furans has been synthesized under Friedel-Crafts electrophilic acylation conditions. Biological investigations have demonstrated that germyl derivatives of trifluoroacetyl furan are more toxic than the silicon analogues. 5-Triethylgermyl-2-trifluoroacetyl furan was the most toxic compound ( $LD_{50}$  11.2 mg kg<sup>-1</sup>, *i.p.* for white mice), 200 times more toxic than the silicon analogue. 5-*t*-Butyl- and 5-trimethylsilyl-2-trifluoroacetyl furan prolong the duration of ethanol anaesthesia by 220 and 140%. 5-Triethylgermyl-2-trifluoroacetyl furan exhibited high anesthetic activity in hexobarbital test (prolonged the duration by 137%). Some of compounds influenced muscle tone and locomotor coordination parameters. 5-Triethylgermyl-2-trifluoroacetyl furan exhibited analgesic activity ( $ED_{50}$  0.9 mg kg<sup>-1</sup>).

### INTRODUCTION

The introduction of the trifluoroacetyl moiety into aromatics and heteroaromatics has been generally realized by Friedel-Crafts acylation and by reaction of organomagnesium or organolithium reagents with ethyl trifluoroacetate, trifluoroacetic acid or its salts [1-7 and ref. therein].

Friedel-Crafts electrophilic acylation of aryl(hetaryl)silanes, -germanes and -stannanes was accompanied by *ipso*-substitution of trialkylsilyl, -germyl or -stannyl group. Reactions of this kind have gained currency in organic synthesis primarily due to the fact that they provide a unique possibility for regiospecific insertion of various groups (halogen, nitro, alkyl, acyl, etc.) into the aromatic or heterocyclic ring [8,9].

We have realized the electrophilic acylation of organometallic furans leading to corresponding 5-trialkylgermyl(silyl)-2-trifluoroacetyl derivatives.

On the other hand, trifluoromethyl ketones have generated much interest as components of potent enzyme inhibitors [10]. A series of trimethylsilylated aliphatic and aromatic trifluoromethylketones was synthesized and evaluated for anti-acetylcholinesterase activity. One compound in this series – 3-(trimethylsilyl)trifluoroacetylbenzene (MDL-73745, Zifrosilone) was selected for further investigation for the treatment of Alzheimer's disease [10-12].

The present investigation elucidates the role of the substituent, its influence on psychotropic and antitumor activity of 5-R<sub>3</sub>M(M=C, Si, Ge)-substituted 2-trifluoroacetyl furans.

### MATERIALS AND METHODS

#### Chemistry

<sup>1</sup>H NMR spectra were recorded on a Varian 200 Mercury instrument (200 MHz) using CDCl<sub>3</sub> as a solvent and hexamethyldisiloxane (HMDSO) as internal standard. Mass spectra were registered on GC-MS HP 6890 (70 eV). GC analysis was performed on a Varian instrument equipped with flame-ionization detector using column packed with 5% OV-17 Chromosorb W-HP (80-100 mesh). Trifluoroacetic anhydride was distilled prior use.

#### ACYLATION OF 2-TRIMETHYLSILYL FURAN BY TRIFLUOROACETIC ANHYDRIDE (method a).

A mixture of 0.015 mol of 2-trimethylsilyl furan, 0.020 mol trifluoroacetic anhydride and 0.03 mmol I<sub>2</sub> was stirred for 32 h in a "Pierce" vial at 70°C. Process was controlled by GC until starting material disappeared. The mixture was cooled, supplemented with 10 ml of distilled water, stirred for 10 min and titrated with 10% KOH to pH 7. After washing with water solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and water, it was dried with anhydrous magnesium sulphate. The solvent was removed by distillation and the residue was fractionated in vacuum. A fraction boiling at 114-116°C/11mm Hg was collected giving 5-trimethylsilyl-2-trifluoroacetyl furan (**2**) (48%) as light yellow oil. <sup>1</sup>H NMR and mass spectral data of compound **2** are presented in Table 1.

#### ACYLATION OF 2-TRIMETHYLGERMYL FURAN BY TRIFLUOROACETIC ANHYDRIDE (method b).

A mixture of 0.005 mol 2-trimethylgermyl furan and 0.0065 mol trifluoroacetic anhydride was stirred for 2 h in "Pierce" vial at 70°C. The excess of trifluoroacetic anhydride was removed under vacuum at room temperature. Aqueous saturated NaHCO<sub>3</sub> (4 ml) was added and the crude product was extracted with Et<sub>2</sub>O (3x15ml) and dried by Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed and residue was purified by column chromatography using the mixture benzene-hexane (1:1) as eluent. Evaporation of eluent afforded the 5-

trimethylgermyl-2-trifluoroacetyl furan (**4**) (26%) as yellow oil.  $^1\text{H}$  NMR and mass spectral data of compound **4** are presented in Table 1.

Our attempts to synthesize the compound **4** by method **a** were unsuccessful. 5-*t*-Butyl-2-trifluoroacetyl furan (**1**) was prepared and isolated by method **b** (eluent hexane), the compounds **3** and **5** – by method **a**.

#### Pharmacology

The neurotropic activity was studied on ICR-JCL mice (19-23 g). Ambient temperature (22 °C) was maintained in the laboratory and in the animal colony. The tested substances were administered intraperitoneally (*i.p.*) as aqueous suspensions prepared with the aid of Tween 80, 30 or 60 min prior to the assay. Control animals received injections of equal amounts of distilled water with Tween 80. Tests were performed according to Ref.13.

Conventional reflex of passive avoidance was applied to evaluate the influence of the substances in question on memory and anti-amnesic activity. Retrogradal amnesia was caused transcorneally by maximal electric shock administered just after learning.

**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 defrosted not more than four passages were performed. The control cells and cells with tested substances in the range of  $2\text{-}5 \cdot 10^4$  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. A quantity of survived 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 alive cells on control plate was taken in calculations for 100% [14, 15]. Concentration of NO was determined according to [14].

#### RESULTS AND DISCUSSION

Trifluoroacetylations of trialkyl(2-furyl)silanes, –germanes and their carbon analogue were realized under Friedel-Crafts conditions using trifluoroacetic anhydride. Yields and reaction conditions are summarized in Table 1.

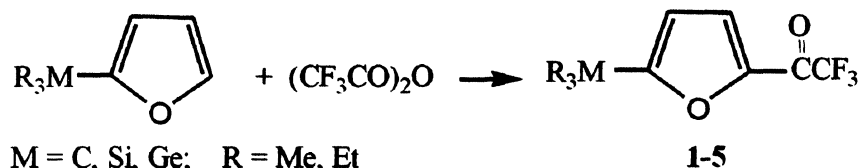


Table 1. Synthesis of Trifluoroacetyl furans **1-5**

No.	R <sub>3</sub> M	Reaction time, h	T, °C	Yield, %	$^1\text{H}$ NMR	MS-GC (m/z)
<b>1.</b>	Me <sub>3</sub> C	24	20	72	1.33(s,9H,CMe <sub>3</sub> ), 6.29 (d,1H,H <sup>4</sup> ), 7.41(m,1H,H <sup>3</sup> )	220 (M <sup>+</sup> ,10), 205(M <sup>+</sup> -Me,100)
<b>2.</b>	Me <sub>3</sub> Si	32	70	48	0.35(s,9H,SiMe <sub>3</sub> ), 6.82 (d,1H,H <sup>4</sup> ), 7.50(m,1H,H <sup>3</sup> )	236 (M <sup>+</sup> ,30), 221(M <sup>+</sup> -Me,90), 167(30),143(100)
<b>3.</b>	Et <sub>3</sub> Si	26	70	51	0.44-1.27(m,15H,SiEt <sub>3</sub> ), 6.78(d,1H,H <sup>4</sup> ),7.56(m,1H,H <sup>3</sup> )	278(M <sup>+</sup> ,17), 249(M <sup>+</sup> -Et,100), 221(90), 171(50)
<b>4.</b>	Me <sub>3</sub> Ge	2	70	26	0.49(s,9H,GeMe <sub>3</sub> ), 6.74 (d,1H,H <sup>4</sup> ), 7.48(m,1H,H <sup>3</sup> )	282 M <sup>+</sup> , 10), 267(M <sup>+</sup> -Me,100) 123(50), 97(40)
<b>5.</b>	Et <sub>3</sub> Ge	1	55	64	0.89-1.05(m,15H,GeEt <sub>3</sub> ), 6.76(d,1H,H <sup>4</sup> ),7.52(d,1H,H <sup>3</sup> )	324(M <sup>+</sup> ,5), 295(M <sup>+</sup> -Et,100),267(50), 239(66),97(20)

Biological investigations have demonstrated that the toxicity of trifluoroacetyl furan derivatives **1-5** strongly depends on the substituent at the position 5. Germanium derivatives **4,5** are more toxic compounds than corresponding silicon derivatives **2,3**. 5-Triethylgermyl-2-trifluoroacetyl furan (**5**) was the most toxic compound ( $LD_{50}$  11.2 mg kg<sup>-1</sup>), 200 times more toxic than the silicon analogue (Table 2). It is interesting to note that trimethyl derivatives of silicon and their germanium analogues have comparable toxicity, but substitution of the methyl group by ethyl dramatically changes the toxicity (see Table 2). The neurotropic activity of 5-trialkylgermyl-2-trifluoroacetyl furans depends on the alkyl substituent at the germanium atom: the 5-triethylgermyl derivative **5** exhibits the highest activity in the hexobarbital anaesthesia test and prolongs its duration by 137% (Table 2). The 2-trimethylgermyl derivative **4** exhibits a stimulating activity in the ethanol anaesthesia and completely prevents animals from retrogradal amnesia (RA) (Table 2).

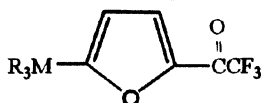


Table 2. Acute Toxicity and Neurotropic Activity of

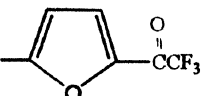
R <sub>3</sub> M	LD <sub>50</sub> , mg kg <sup>-1</sup>	Hypoxia %	Corazol induced spasms, %	Phenamine, stereotype, %	Anaesthesia, % of control		RA, %
					Hexo- barbital	Ethanol	
Me <sub>3</sub> C ( <b>1</b> )	22	120	175	61	133	320	60
Me <sub>3</sub> Si ( <b>2</b> )	112	100	224.7	44	86	240	16.7
Et <sub>3</sub> Si ( <b>3</b> )	2240	128	94	90	89	74	50
Me <sub>3</sub> Ge ( <b>4</b> )	71	113	149	58	96	51	100
Et <sub>3</sub> Ge ( <b>5</b> )	11.2	109	129	84	237	71	80

Table 3. Effects of Trifluoroacetyl furans **1-5** on Locomotor Coordination and Muscle Tone

R <sub>3</sub> M	ED <sub>50</sub> (mg kg <sup>-1</sup> )				
	Test:				
	Rotating rod	Tube	Traction	Hypothermia	Analgesia
Me <sub>3</sub> C ( <b>1</b> )	4 (3-6)	9 (6-12)	9 (6-12)	10 (7-14)	10 (7-14)
Me <sub>3</sub> Si ( <b>2</b> )	4.1 (2.7-5.5)	2.8 (1.8-3.7)	35.5 (20.2-50.8)	4.5 (3.1-6.0)	28.2 (15.9-41.9)
Et <sub>3</sub> Si ( <b>3</b> )	447 (31.3-59.6)	89 (63.1-119.6)	>250	112 (79-147)	>250
Me <sub>3</sub> Ge ( <b>4</b> )	7 (50-93)	14 (7-21)	14 (7-21)	7 (5-9)	14 (5-26)
Et <sub>3</sub> Ge ( <b>5</b> )	>5	>5	>5	11.5 (8-15)	0.9 (0.6-1.2)

The silicon and carbon analogues **1,2** prolonged the ethanol anaesthesia by 140 and 220%, correspondingly. They had higher anti-Corazol potency but they did not prevent animals from retrogradal amnesia. The pharmacological effects of phenamine are diminished by all the trifluoroacetyl derivatives (Table 2). The effect of trifluoroacetyl furan derivatives **1-5** on locomotor coordination and muscle tonus depends on the substituent and differs to some extent (Table 3). Thus, compounds **1, 2** and **4** in rotating-rod, tube and

traction tests have  $ED_{50}$  in the 4 - 35.5  $mg\ kg^{-1}$  range, but compound **3** in the range 89 - 447  $mg\ kg^{-1}$ . The 5-trimethylsilyl-2-fluoroacetyl-furan (**2**) has the highest hypothermic action, but the 5-triethylgermyl derivative **5** has the highest analgesic activity ( $EC_{50}$  0.9  $mg\ kg^{-1}$ ) (Table 3).

Table 4. Cytotoxicity of 

Cell line	Method	$R_3M$				
		$Me_3C$ (1)	$Me_3Si$ (2)	$Et_3Si$ (3)	$Me_3Ge$ (4)	$Et_3Ge$ (5)
HT-1080	CV	nce	nce	nce	nce	72*
	MTT	nce	nce	nce	nce	72
	**NO	4	6	5	18	250
MG-22A	CV	nce	nce	nce	nce	6
	MTT	nce	nce	nce	nce	10
	NO	5	7	15	14	250
Neuro 2A	CV	nce	-	-	nce	3.3
	MTT	nce	-	-	nce	4
	NO	5	-	-	18	300

\*  $IC_{50}$  ( $\mu g\ mL^{-1}$ ) providing 50% cell killing effect (CV: coloration; MTT: coloration);

\*\*NO – concentration (%) (CV: coloration);

nce - no cytotoxic effect; - Not tested.

Potential cytotoxic activity of synthesized compounds **1-5** was tested *in vitro* on three monolayer tumor cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), Neuro 2A (mouse neuroblastoma). Concentrations providing 50% of tumor death effect ( $IC_{50}$ ) were determined according to the known procedure [16] using 96 well plates.

The compounds **1-5** have low activity on HT-1080, MG-22A and Neuro 2A tumor cell cultures (Table 4). The most active antitumor substance is 5-triethylgermyl-2-trifluoroacetyl-furan ( $IC_{50}$  on Neuro 2A 3.3  $\mu g\ mL^{-1}$ ). Germyl derivative **5** is stronger tumor growth inhibitor and NO-inducer than corresponding silyl analogue **2**.

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