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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME TRIFLUOROMETHYL DERIVATIVES OF 5-*tert*-BUTYL-2-FURYLMETHYLIDENE-ANILINES AND THEIR SILYL ANALOGS

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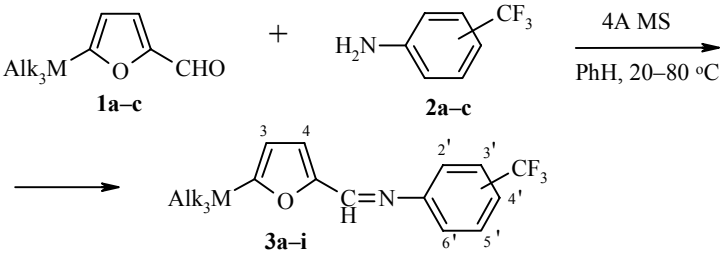
We have synthesized the corresponding aldimines by condensation of 5-tert-butylfurfural and its trimethyl- and triethylsilyl analogs with 2-, 3-, and 4-trifluoromethylanilines in the presence of 4A molecular sieves. We have studied their neurotropic and antitumor activity. The phenamine motor activity test showed that tert-butyl derivatives exhibit high efficacy and also shorten the ethanol narcosis time. Some silyl derivatives exhibit significant anti-corazole activity. The tert-butyl derivatives have high cytotoxicity toward human lung fibrosarcoma cells (the 3-trifluoromethyl-substituted derivative) and mouse hepatoma cells (the 2-trifluoromethyl-substituted derivative).

Keywords: furan Schiff's bases, silyl derivatives, trifluoromethylanilines, neurotropic activity, cytotoxicity.

Derivatives of heterocyclic compounds, including furan compounds that have trifluoromethyl and silyl groups, have attracted interest as valuable synthons and potentially biologically active compounds. The aim of this work was to obtain a series of Schiff's bases containing the indicated groups and to study their biological activity. The target azomethines were synthesized using an efficient method we developed [1-4]: by condensations of heterocyclic aldehydes with amines in the presence of molecular sieves.

Trimethyl- and triethylsilyl derivatives of furfural **1b,c** react with 3- and 4-trifluoromethylanilines **2b,c** even at room temperature. The same aldehydes react with amine **2a** only when heated (80°C), as does aldehyde **1a** with all amines.



1a, **3a–c** M = C, Alk = Me; **1b**, **3d–f** M = Si, Alk = Me, **1c**, **3g–i** M = Si, Alk = Et;
CF_3 position: **2a**, **3a**, **3d**, **3g** 2'-; **2b**, **3b**, **3e**, **3h** 3'-; **2c**, **3c**, **3f**, **3i** 4'-

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The data obtained (Table 1) show that the minimum activity is exhibited by *tert*-butyl-substituted aldehyde **1a** and aniline **2a**, where the 2-trifluoromethyl group probably causes steric hindrance during condensation.

The biological properties of the synthesized compounds were determined by various methods: the neurotropic activity was determined in white mice of the ICR line (Table 2), while the cytotoxicity was determined on tumor cells *in vitro* (Table 3).

The studied aldimines, except for compound **3f** (LD_{50} 365 mg/kg) are low-toxicity compounds with LD_{50} higher than 1000 mg/kg. Therefore the therapeutic index of neurotropically active compounds in the studied series may be rather high.

Study of the motor activity of animals in "rotating rod" and "tube" tests showed that only imine **3b** has pronounced depriming properties (ED_{50} 14.5 mg/kg in the "tube" test). In the other compounds, this activity is significantly lower or is virtually absent. Most compounds exhibit analgesic properties (except for **3d,g**); the maximum effect in this test occurred for **3e,i,b**. None of the synthesized compounds (except for the highly toxic **3f**) affected traction (crossbar pullup test).

Almost all the studied compounds **3a-h** reduce the lifespan of the mice somewhat under hypoxic hypoxia conditions: 75%-99% (compared with control, 100%). The studied compounds have little effect on phenamine-induced hyperthermia: the data are close to the control values (100%)).

Tert-butyl derivatives **3a-c**, in particular **3b** and also triethylsilyl **3g** appreciably shorten the ethanol narcosis time. The duration of hexenal narcosis increases in the presence of most of the compounds.

Anticonvulsive properties with respect to corazole-induced convulsions are exhibited by almost all the synthesized imines, especially triethylsilyl derivatives **3g,h**. The studied compounds, depending on the structure, specifically affect the pharmacological effects of phenamine. Thus *tert*-butyl derivatives **3a-c** significantly enhance the stimulating activity of phenamine, by a factor of 5.39, 3.17, and 8.59 respectively. The silyl derivatives either do not affect the action of phenamine (**3f,g**) or else antagonize it (**3d,e** and especially **3h**).

The aim of the cytotoxic studies was to determine the ability of the synthesized compounds to inhibit growth of tumor cells *in vitro*, and also to determine the activity of these compounds during intracellular synthesis of nitric oxide radicals, an elevated concentration of which is the reason for cell death [5]. The concentrations of the compounds resulting in 50% cell death (IC_{50}) were determined by the standard procedure [6] on two tumor cell lines: HT-1080 (human lung fibrosarcoma) and MG-22A (mouse hepatoma).

Among the studied compounds, only the imine **3e** did not exhibit cytotoxicity. The rest of the silyl derivatives have a moderate cytotoxic effect. However, many of them showed a high level of NO generation, in particular the triethylsilyl derivatives **3g,i** on the HT-1080 cell line and the imine **3d** on MG-22A cells. The highest cytotoxic activity is observed in the carbon analogs, the *tert*-butyl derivatives; in this case, their action is

TABLE 1. Characteristics of Condensation Reactions

Compound	Temperature, °C	Time, h	Purity, % (GLC)	Yield, %
3a	80	22	98.5	43
3b	80	8	98	63
3c	80	8	99	71
3d	20, 80	48, 3	100	72
3e	20	20	100	100
3f	20	20	100	94
3g	20, 80	48, 6	100	38
3h	20	20	100	89
3i	20	20	100	100

TABLE 2. Toxicity and Neurotropic Activity of Aldimines **3a-i**

Imine	LD_{50}*, mg/kg	Test									
		ED_{50}*[2], mg/kg				M, % of control (100%); dose - 5 mg/kg					
		rotating rod	tubes	analgesia	traction	hypoxic hypoxia	phenamine-induced hyperthermia	phenamine-induced hyperactivity	hexenal narcosis	ethanol narcosis	corazole-induced convulsions
3a	—	—	—	—	—	76	94	539	144	59	124
3b	—	>1000	14.5 (±23.9)	83.9 (±53.7)	>1000	99	98	317	88	25	104
3c	—	>1000	551.0 (±144.0)	178.1 (±406.0)	>1000	75	101	859	159	55	91
3d	>1000	>1000	>1000	>1000	>1000	92	101	52	90	103	154
3e	—	528.8 (±176.0)	>1000	77.0 (±17.3)	>1000	87	103	60	103	79	122
3f	365 (±168)	158.0 (±51.7)	194.0 (±25.0)	137.0 (±40.1)	208.0 (±40.3)	89	101	101	147	117	123
3g	>1000	628.8 (±281.0)	>1000	>1000	>1000	88	102	109	123	48	187
3h	>1000	>1000	>1000	177.0 (±299.0)	>1000	93	102	17	93	62	210
3i	>1000	>1000	>1000	81.6 (±47.7)	>1000	104	103	147	147	131	128

* Median lethal dose.

*[2] Median effective dose (the mean statistical error is indicated within the parentheses).

TABLE 3. *In vitro* Cytotoxicity of Aldimines **3a-i***

Imine	Cell line					
	HT-1080			MG-22A		
	IC_{50}		TG_{100}	IC_{50}		TG_{100}
	CV	MTT		CV	MTT	
3a	42	124	23	8	0.43	100
3b	17	0.15	400	5	12	150
3c	28	>100	300	5	4	7
3d	11	28	400	7	9	500
3e	>100	>100	7	>100	>100	12
3f	34	>100	67	60	37	26
3g	11	19	450	12	20	200
3h	20	30	200	22	19	200
3i	14	18	450	8	15	250

* IC_{50} is the concentration resulting in 50% cell death, μg/mL; CV means staining with crystal violet; MTT means staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TG_{100} is the specific NO-generating capacity.

specific on different cells and in different tests. The maximum cytotoxicity is typical of the 3-trifluoromethyl derivative **3b**: IC_{50} = 0.15 μg/ml on the HT-1080 cell line, and also for the 2-trifluoromethyl derivative **3a**: IC_{50} = 0.43 μg/ml on the MG-22A cell line. In both cases, this cytotoxicity was exhibited when the mitochondrial enzymes were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which suggests enhanced oxidation–reduction properties in the presence of the test compounds. Compound **3c** showed appreciable cytotoxic activity on the mouse hepatoma cell line for staining both with MTT (IC_{50} = 4 μg/ml) and in the test staining the cell membranes with crystal violet (IC_{50} = 5 μg/ml). In this case, in all the cytotoxicity tests no morphological changes occurred in the cells.

EXPERIMENTAL

The ^{1}H NMR spectra were studied on a Varian Mercury (200 MHz) spectrometer for solutions in $CDCl_3$, internal standard TMS. The mass spectra were obtained on an HP 6890 GC-MS instrument equipped with an HP-5 MS capillary column (30.0 m × 250 μm × 0.25 μm), with temperature programming from 70 to 260°C (10°C/min).

The benzene was distilled over CaH_2 before use. The anilines were obtained from Acros and used without additional purification. In this work, we used 4A molecular sieves (VEB Laborchemie Apolda).

General Procedure for Synthesis of Aldimines 3a-i. Dry benzene (10 ml) and each of the starting aldehyde and amine (5 mmol) were placed into a round-bottomed flask with a reflux condenser. Then freshly calcined molecular sieves (5 g) were added and the reaction was carried out at room temperature or with heating on a water bath at 80°C under an argon atmosphere. Samples were periodically withdrawn and analyzed using TLC on Kieselgel 60 F254 plates in a 3:1 hexane–ethyl acetate system and also by GLC-MS. Practically complete conversion to the corresponding products occurred within a certain time period that depended on the substrates (Table 1). At the end of the reaction, the sieves were filtered out and washed with benzene; the filtrate was evaporated off under reduced pressure (40°C/15 mm) and slight residues of the starting materials were removed under vacuum (45-50°C/0.1 mm). The products were yellow oily materials.

The Neurotropic Activity was studied in mice of the ICR line. The experimental procedure is described in detail in [7, 8].

The Cytotoxic Properties of the compounds were studied on cultures of monolayer tumor cells according to the procedure in [6]. The number of live cells were determined by two independent colorimetric methods from the intensity of staining of the cell membranes with crystal violet and staining of mitochondrial enzymes with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, characterizing the level of their oxidation–reduction properties.

The specific NO-generating capacity of the test substances, extrapolated to 100% live cells, was calculated from the equation:

$$TG_{100} = G_{EX} \cdot 100/C \text{ (nmol} \cdot 10^2/200 \text{ µl)},$$

where GEX is the NO concentration (nanomoles) in 200 microliters (the volume of a well in the plate) of culture medium, generated by live cells after incubation with 50 µg/ml of the test substance, according to the method in [9]; *C* is the percentage of live cells after incubation with 50 µg/ml of the test substance, determined from the intensity of staining of the cell membranes with crystal violet [6].

N-(5-*tert*-Butyl-2-furylmethylidene)-2-trifluoromethylaniline (3a). Mass spectrum, *m*/*z* (I_{rel}, %): 296 (7, $[M + H]^+$, 295 (37, M^+), 281 (15), 280 (100, $[M - Me]^+$), 172 (36, $[HCNC_6H_4CF_3]^+$), 145 (42, $[C_6H_4CF_3]^+$), 126 (8), 125 (7), 109 (15), 95 (15), 81 (9), 79 (16), 65 (8), 53 (11). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.33 (9H, s, $3CH_3$); 6.18 (1H, d, *J* = 4.0, H-4); 6.95 (1H, d, *J* = 4.0, H-3); 6.70 (1H, d, *J* = 8.2, H-3'); 7.20 (1H, t, *J* = 8.2, H-5'); 7.51 (1H, t, *J*= 8.2, H-4'); 7.64 (1H, d, *J* = 8.2, H-6'); 8.09 (1H, s, CH=N).

N-(5-*tert*-Butyl-2-furylmethylidene)-3-trifluoromethylaniline (3b). Mass spectrum, *m*/*z* (I_{rel}, %): 296 (5, $[M + H]^+$), 295 (37, M^+), 281 (17), 280 (100, $[M - Me]^+$), 172 (45, $[HCNC_6H_4CF_3]^+$), 145 (47, $[C_6H_4CF_3]^+$), 140 (5), 126 (10), 109 (15), 95 (12), 81 (10), 79 (17), 65 (6), 53 (11). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.38 (9H, s, $3CH_3$); 6.20 (1H, d, *J* = 4.0, H-4); 6.98 (1H, d, *J* = 4.0, H-3); 7.29-7.53 (4H, m, H-2', H-4', H-5', H-6'); 8.18 (1H, s, CH=N).

N-(5-*tert*-Butyl-2-furylmethylidene)-4-trifluoromethylaniline (3c). Mass spectrum, *m*/*z* (I_{rel}, %): 296 (4, $[M + H]^+$), 295 (37, M^+), 281 (15), 280 (100, $[M - Me]^+$), 172 (37, $[HCNC_6H_4CF_3]^+$), 145 (40, $[C_6H_4CF_3]^+$), 126 (9), 109 (12), 95 (12), 81 (7), 79 (9), 65 (5), 53 (8). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.33 (9H, s, $3CH_3$); 6.18 (1H, d, *J* = 4.0, H-4); 6.93 (1H, d, *J* = 4.0, H-3); 7.22 (2H, d, *J* = 8.8, H-3', H-5'); 7.60 (2H, d, *J* = 8.8, H-4', H-6'); 8.09 (1H, s, CH=N).

N-(5-Trimethylsilyl-2-furylmethylidene)-2-trifluoromethylaniline (3d). Mass spectrum, *m*/*z* (I_{rel}, %): 312 (23, $[M + H]^+$), 311 (100, M^+), 296 (6, $[M - Me]^+$), 200 (86), 172 (16, $[HCNC_6H_4CF_3]^+$), 152 (21), 145 (23, $[C_6H_4CF_3]^+$), 126 (13), 125 (10), 107 (5), 95 (5), 81 (6), 77 (30). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.33 (9H, s, $3CH_3$); 6.78 (1H, d, *J* = 4.0, H-4); 7.02 (1H, d, *J* = 8.0, H-3'); 7.09 (1H, d, *J* = 4.0, H-3); 7.24 (1H, t, *J* = 8.0, H-5'); 7.47 (1H, t, *J* = 8.0, H-4'); 7.66 (1H, d, *J* = 8.0, H-6'); 8.18 (1H, s, CH=N).

N-(5-Trimethylsilyl-2-furylmethylidene)-3-trifluoromethylaniline (3e). Mass spectrum, *m*/*z* (I_{rel}, %): 312 (22, $[M + H]^+$), 311 (100, M^+), 296 (32, $[M - Me]^+$), 268 (5), 252 (5), 230 (5), 218 (14), 202 (52), 230 (5), 218 (14), 202 (52), 172 (12, $[HCNC_6H_4CF_3]^+$), 152 (15), 145 (32, $[C_6H_4CF_3]^+$), 141 (16), 126 (10), 125 (10), 95 (10), 77 (51), 73 (10), 59 (9). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.33 (9H, s, $3CH_3$); 6.73 (1H, d, *J* = 4.0, H-4); 7.02 (1H, d, *J* = 4.0, H-3); 7.27-7.55 (4H, m, H-3', H-4', H-5', H-6'); 8.27 (1H, s, CH=N).

N-(5-Trimethylsilyl-2-furylmethylidene)-4-trifluoromethylaniline (3f). Mass spectrum, *m*/*z* (I_{rel}, %): 312 (11, $[M + H]^+$), 311 (53, M^+), 296 (100, $[M - Me]^+$), 200 (86), 178 (4), 145 (19, $[C_6H_4CF_3]^+$), 141 (12), 126 (7), 95 (5), 77 (7), 73 (6), 59 (9). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.33 (9H, s, $3CH_3$); 6.75 (1H, d, *J* = 4.0, H-4); 7.04 (1H, d, *J* = 4.0, H-3); 7.26 (2H, d, *J* = 8.6, H-3', H-5'); 7.64 (1H, d, *J* = 8.6, H-4', H-6'); 8.29 (1H, s, CH=N).

N-(5-Triethylsilyl-2-furylmethylidene)-2-trifluoromethylaniline (3g). Mass spectrum, *m*/*z* (I_{rel}, %): 354 (5, $[M + H]^+$), 353 (30, M^+), 324 (11, $[M - Et]^+$), 201 (13), 200 (100), 172 (10, $[HCNC_6H_4CF_3]^+$), 152 (10), 145 (10, $[C_6H_4CF_3]^+$), 126 (9), 125 (9), 105 (12), 95 (6), 77 (28), 59 (5). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.84 (6H, q, *J* = 7.2, $3CH_2$); 0.98 (9H, t, *J* = 7.2, $3CH_3$); 6.76 (1H, d, *J* = 3.4, H-4); 7.03 (1H, d, *J* = 7.8, H-3'); 7.09 (1H, d, *J* = 3.4, H-3); 7.24 (1H, t, *J* = 7.8, H-5'); 7.52 (1H, t, *J* = 7.8, H-4'); 7.65 (1H, d, *J* = 7.8, H-6'); 8.23 (1H, s, CH=N).

N-(5-Triethylsilyl-2-furylmethylidene)-3-trifluoromethylaniline (3h). Mass spectrum, *m*/*z* (I_{rel}, %): 354 (22, $[M + H]^+$), 353 (82, M^+), 325 (17), 324 (68, $[M - Et]^+$), 296 (12), 248 (7), 238 (7), 220 (19), 218 (37), 203 (25), 202 (100), 184 (15), 172 (28, $[HCNC_6H_4CF_3]^+$), 152 (27), 145 (59, $[C_6H_4CF_3]^+$), 133 (33), 126 (25), 125 (23), 105 (53), 95 (30), 77 (75), 59 (22). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.85 (6H, q, *J* = 7.2, $3CH_2$); 1.00 (9H, t, *J* = 7.2, $3CH_3$); 6.80 (1H, d, *J*= 4.0, H-4); 7.09 (1H, d, *J* = 4.0, H-3); 7.31-7.58 (4H, m, H-3', H-4', H-5', H-6'); 8.28 (1H, s, CH=N).

N-(5-Triethylsilyl-2-furylmethylidene)-4-trifluoromethylaniline (3i). Mass spectrum, *m*/*z* (I_{rel}, %): 353 (23, M^+), 334 (5, $[M - F]^+$), 325 (25), 324 (100, $[M - Et]^+$), 296 (5), 266 (7), 248 (2), 238 (2), 220 (7), 145 (18, $C_6H_4CF_3]^+$), 133 (3), 126 (7), 95 (5), 77 (4), 59 (6). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.85 (6H, q, *J* = 7.2, $3CH_2$); 0.99 (9H, t, *J* = 7.2, $3CH_3$); 6.78 (1H, d, *J*= 4.0, H-4); 7.04 (1H, d, *J* = 4.0, H-3); 7.24 (2H, d, *J* = 8.4, H-3', H-5'); 7.62 (1H, d, *J* = 8.4, H-4', H-6'); 8.22 (1H, s, CH=N)

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