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Short communication

Synthesis, toxicological and pharmacological assessment of esters of carbonic and carbamic acids with 2-aryl-4-hydroxyethylmorpholines

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Summary — The synthesis of 15 original morpholine derivatives from 4-hydroxyethyl-2-arylmorpholines is described. The structures of the synthesized esters were proved by IR, ^{1}H-NMR and ^{13}C-NMR data. Acute toxicity studies of the compounds were performed on mice. A comparative pharmacological study of the in vivo effect on the central nervous system was realized by certain screening tests: hexobarbital-induced sleeping time, locomotor activity and behavior despair test for antidepressants. The results indicate that some of the compounds are less toxic than the reference drug Moclobemide (Aurorix). Compound **2d**, 2-(4-bromphenyl)-4-(2-phenyloxycarbonyloxyethyl)morpholine, has a low toxicity and weak dose-dependent antidepressive activity. This compound does not influence hexobarbital-induced sleeping time or locomotor activity of mice.

4-hydroxyethyl-2-arylmorpholine ester / acute toxicity / antidepressant activity / Moclobemide

Introduction

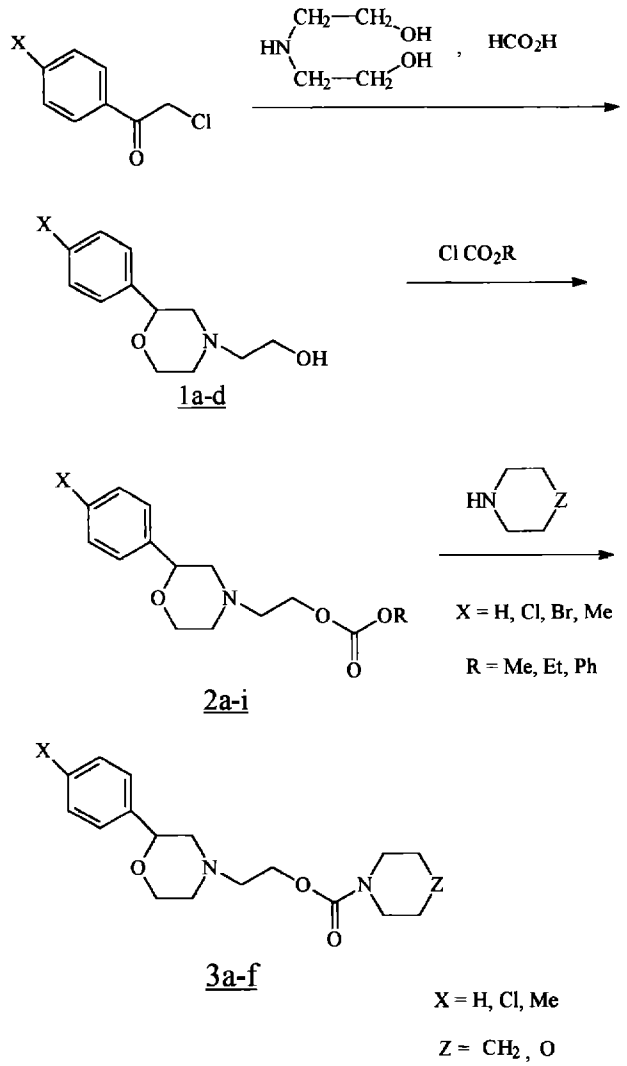
The biologically activite compounds [1–3] and drugs Oxaflozan and Moclobemide (antidepressants) include a morpholine ring in their structure. Selective and reversible inhibitors of monoaminooxidase A (MAO-A) were among the first drugs used in the treatment of depression [4, 5]. A prominent example is Moclobemide (4-[2-(4-chlorophenylcarbamoyl)ethyl]morpholine) [6]. By chemical modification of the structure of the drug Moclobemide, it is possible to obtain new compounds with comparable activity and lower toxicity than Moclobemide. We report here the preparation of 15 modified Moclobemide derivatives and describe their pharmacological and toxicological effects.

Chemistry

The basic starting compounds, 2-arylmorpholinethanols **1a–d**, were used for the preparation of carbonic **2a–i** and carbamic **3a–f** esters (scheme 1). We synthesized 2-aryl-4-hydroxyethylmorpholines by the unusual Leuckart–Wallach reaction, in which 2-halogenoketones reacted with *N,N*-diethanolamine and formic acid [7]. Further, the 2-arylmorpholinethanols were acylated with phenyl-, methyl- or ethylchloroformate in anhydrous benzene at a molar ratio of 1:1.1 to yield the esters of carbonic acid **2a–i** in 40–50%. 4-(2-Phenyloxycarbonyloxyethyl)-2-arylmorpholines **2a–d** were then reacted with piperidine or morpholine in anhydrous ether at a 1:1 molar ratio to produce esters of carbamic acid **3a–f** in 65–90% yield.

The structure of 4-(2-phenyloxy(alkyloxy)carbonyloxyethyl)-2-arylmorpholines **2a–i** and 4-(2-piperidinyl(morpholinyl)carbonyloxyethyl)-2-arylmorpholines was confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. IR spectra of the compounds (as HCl salts) were recorded on solid state in Nujol. The carbonyl groups absorb in the 1700–1780 cm^{-1} region. All carbonic esters have an intensive absorbtion 1200–1285 cm^{-1} region. The observed absorbtion in the 2200–2800 cm^{-1} region is due to the protonated nitrogen in the salts (NH^+ function).

^{1}H-NMR spectra of the compounds **2a–i** and **3a–f**, recorded at 100 Mhz, confirm the structures. In the 4.65–4.50 ppm region, we observed the signals of the CH group of the morpholine ring as a doublet of doublets. The signals of the OCH_2 group of the morpholine structure are at 3.8–4.0 ppm. The signals of



Scheme 1.

methylene groups attached to the carbonate or carbamate groups are in the region of the 4.40–4.25 ppm. All the NCH_2 groups are in the range 2.1–2.8 ppm. The signals of the other protons of methoxy, ethoxy and methyl groups are in their usual regions. The aromatic protons appear as two doublets (A_2B_2 system) in the case of *para*-bromophenyl and *para*-methylphenyl derivatives and as a singlet in the *para*-chlorophenyl derivatives. A good correlation between the chemical shifts of the carbon atoms and their position in the molecule was observed in the ^{13}C spectra of the compounds. Special techniques, such as *J*-coupled spin-echoes and DEPT were employed to confirm the assignment of certain carbon atoms. The signals of the aromatic carbon atoms are in their usual region, 121–140 ppm; the signal of the tertiary carbon atom in the morpholine ring is at 77–78 ppm. The other sp^3 carbon atoms of the morpholine group are in the region 52–67 ppm. The typical chemical shifts of the ^1H and ^{13}C spectra in $CDCl_3$ solvent are shown in tables I and II.

Pharmacology

The new compounds were assayed for acute toxicity, influence on locomotor activity, hexobarbital sleeping time. Compounds were tested for in vivo antidepressive activity using the behavior despair test. Moclobemide was used as a reference compound.

Results and discussion

The experimental data on the acute toxicity (LD_{50}) of the compounds was compared with that of Moclobemide, which showed that some of the compounds had significantly lower acute toxicity (table III). Compounds **2b–d** were less toxic than the standard.

The effects of the compounds in doses one-tenth of the LD_{50} on hexobarbital-induced sleeping time are shown in table IV. All the compounds tested (except **2d**, **3c** and **3d**) significantly increased hexobarbital sleeping time.

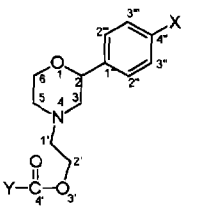
As shown by in vivo experiments on the influence of the compounds on locomotor activity of mice, all the tested compounds (except **3d**) and Moclobemide decrease the locomotor activity (table V).

In vivo antidepressive activity in the behavior despair test was shown by compounds **2c** and **2d**. The compound **2c** shows antidepressive activity at a dose of only one-tenth of the LD_{50}. Compound **2d** dose-dependently decreased the time of immobilization in doses of one-twentieth, one-tenth and one-fifth of the LD_{50}. None of the other compounds showed antidepressive activity at doses of one-tenth of the LD_{50}. The reference compound Moclobemide expressed antidepressive activity at doses 28, 59 and 118 mg/kg body weight ip (table IV).

Conclusions

The results of the in vivo pharmacological screening show that compound **2d** has an appreciable antidepressive effect, which is comparable to Moclobemide. The acute toxicity of compound **2d** is signifi-

Table I. ^{1}H chemicals shifts[a] (δ-values) of the compounds **2a–i** and **3a–f**.



Compound	*H-2*	*4H-3,5*	*2H-6*	*2H-1'*	*2H-2'*	*H2'', 2''', 3'', 3'''*	*H-Y, H-X*
2a	4.60 dd	2.20–2.60 m	3.85–4.05 m	2.80 t	4.40 t	7.33 s	7.22–7.00 m
2b	4.55 dd	2.20–2.65 m	3.84–4.03 m	2.79 t	4.41 t	7.16, 7.12 dd A_2B_2	7.21–6.98 m
2c	4.65 dd	2.20–2.65 m	3.83–4.04 m	2.81 t	4.40 t	7.30 s	7.22–7.00 m
2d	4.65 dd	2.21–2.65 m	3.84–4.05 m	2.80 t	4.40 t	7.44 d, 7.12 d	7.22–6.99 m
2e	4.65 dd	2.18–2.63 m	3.83–4.04 m	2.78 t	4.37 t	7.45 d, 7.13 d	3.81 s
2f	4.63 dd	2.19–2.64 m	3.84–4.05 m	2.78 t	4.36 t	7.46 d, 7.13 d	1.21 t, 4.21 q
2g	4.64 dd	2.21–2.65 m	3.82–4.03 m	2.77 t	4.38 t	7.30 s	3.80 s
2h	4.64 dd	2.19–2.63 m	3.83–4.05 m	2.77 t	4.38 t	7.30 s	1.22 t, 4.20 q
2i	4.56 dd	2.20–2.64 m	3.82–4.04 m	2.78 t	4.37 t	7.26, 7.12 dd A_2B_2	3.80 s, 2.30 s
3a	4.59 dd	2.17–2.60 m	3.83–4.04 m	2.75 t	4.26 t	7.31 s	3.70–3.60 m, 3.45–3.35 m
3b	4.58 d	2.16–2.59 m	3.83–4.04 m	2.75 t	4.27 t	7.31 s	3.39–3.33 m, 1.55–1.20 m
3c	4.56 dd	2.16–2.59 m	3.82–4.03 m	2.76 t	4.25 t	7.25, 7.12 dd A_2B_2	3.70–3.60 m, 3.46–3.36 m
3d	4.56 dd	2.15–2.60 m	3.82–4.03 m	2.76 t	4.26 t	7.25, 7.12 dd A_2B_2	3.40–3.33 m, 1.55–1.19 m
3e	4.63 dd	2.15–2.60 m	3.84–4.05 m	2.75 t	4.26 t	7.30 s	3.72–3.61 m, 3.45–3.35 m
3f	4.64 dd	2.15–2.59 m	3.84–4.05 m	2.75 t	4.25 t	7.29 s	3.40–3.32 m, 1.54–1.20 m

[a]Examples of coupling constants J (Hz): **2a**: H-2, dd, J_1 = 10.5, J_2 = 2.0; H-1', t, J = 7.0; H-2', t, J = 7.0; **2e**: H-2'', 2''', d, J = 6.9; H-3'', 3''', d, J = 6.9.

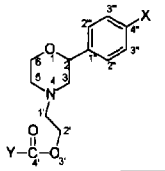
cantly lower than the standard drug Moclobemide. On the other hand, this compound fails to significantly influence barbiturate-induced hypnosis. This could be an advantage in comparison to Moclobemide, because this compound does not possess sedative effects. For this reason it appears to be very promising for further detailed pharmacological and toxicological experiments. More 2-arylmorpholine derivatives will be synthesized in order to elucidate a structure–activity relationship.

Experimental protocols

Chemistry

Melting points were measured on Boetius hot plate microscope (Germany) and were corrected. IR spectra (Nujol) were recorded on a UR 20 (Karl Zeiss, Jena) apparatus. ^{1}H-NMR spectra were recorded at room temperature on Brucker WP 100 (100 MHz) spectrometer in $CDCl_3$. Chemical shifts are given in ppm; TMS was used as internal standard. ^{13}C-NMR spectra were recorded at room temperature on Brucker WP 00 (25.18 MHz). TLC was performed on 0.25 mm precoated plates Kieselgel 60 Merck

Table II. ^{13}C chemical shifts (δ-values) of the compounds **2a–i** and **3a–f**.



Compound	*C-2*	*C-3,5*	*C-6*	*C-1'*	*C-2'*	*C-4'*	*C-1''*	*C-2'', 2'''*	*C-3'', 3'''*	*C-4''*	*C-Y (C-X)*
2a	78.0	52.3, 54.0	66.6	59.8	68.8	153.5	140.1	126.4	128.2	125.8	121.3, 125.3, 124.5, 151.2
2b	77.9	52.1, 54.5	66.2	59.7	69.0	154.3	140.0	128.7	126.8	137.1	121.3, 125.2, 124.6, 151.4 (21.5)
2c	78.0	53.0, 54.9	66.8	59.9	68.7	154.1	139.7	128.8	127.5	134.4	121.4, 125.4, 124.7, 150.9
2d	78.1	52.8, 54.5	66.3	60.0	68.9	154.5	139.8	130.1	128.3	122.1	121.4, 125.2, 124.6, 151.1
2e	77.9	53.0, 54.9	66.5	59.6	67.3	153.7	139.8	131.1	127.9	121.8	55.3
2f	78.0	52.9, 54.8	66.6	60.1	67.8	153.6	140.0	131.0	128.1	121.9	64.2, 15.1
2g	78.1	53.1, 54.9	66.0	59.4	67.3	154.0	139.6	128.7	128.0	134.0	55.1
2h	78.1	53.1, 54.8	66.1	59.6	67.5	154.1	139.5	129.0	127.1	137.0	64.3, 15.2
2i	78.1	52.8, 54.7	66.2	60.0	67.4	154.2	140.3	129.0	126.1	139.6	55.0 (21.2)
3a	77.9	52.7, 57.5	66.5	60.7	67.3	155.2	139.9	128.3	126.1	127.4	44.4, 64.0
3b	78.0	53.0, 57.0	67.0	60.9	62.6	155.3	140.0	128.2	126.2	138.5	44.8, 25.5, 24.1
3c	78.0	53.0, 57.1	66.6	60.7	68.0	155.3	138.1	128.3	127.8	136.8	44.5, 64.0 (21.1)
3d	78.0	53.0, 57.4	67.0	61.0	63.1	155.5	138.6	128.8	127.9	137.5	44.8, 25.6, 24.2 (21.2)
3e	78.0	53.0, 57.3	66.9	61.1	67.0	155.1	139.1	128.8	127.6	134.1	44.3, 64.1
3f	78.1	52.9, 57.6	67.0	60.9	63.0	155.4	139.3	128.7	127.9	134.3	44.7, 25.6, 24.1

Table III. Acute toxicity (LD_{50}) of the compounds and Moclobemide.

Compound	*LD_{50} (mg/kg ip) and 95% confidence interval*
2a	913.2[a] (789.1–1071.2)
2b	1154.5[a] (1012.4–1316.5)
2c	1193.0[a] (791.3–1542.7)
2d	1032.5[a] (907.1–1175.3)
2e	731.2[a] (623.5–864.8)
2f	449.6[a] (391.7–504.5)
2g	631.5 (542.1–714.4)
2i	621.7 (508.4–742.1)
3a	688.2[a] (597.3–789.7)
3b	346.4[a] (282.0–424.1)
3c	858.3[a] (750.2–981.1)
3d	652.6[a] (603.9–704.6)
3e	410.7[a] (344.1–492.5)
3f	907.9[a] (759.5–1085.4)
Moclobemide	591.2 (503.7–671.6)

[a] $P \leq 0.05$, statistically significant difference compared to Moclobemide.

Table IV. Effect of the compounds on hexobarbital sleeping time.

Compound	*Dose one-tenth of LD_{50} (mg/kg ip)*	*Sleeping time (min) ($x \pm SD$)*
Control	–	25.8 ± 5.3
2a	91	51.1 ± 6.8[a]
2b	115	37.6 ± 5.4[a]
2c	119	41.9 ± 9.7[a]
2d	103	36.3 ± 8.4
2e	73	39.5 ± 7.7[a]
2f	45	43.1 ± 9.3[a]
2g	63	47.2 ± 10.4[a]
2i	62	37.8 ± 7.3[a]
3a	69	61.3 ± 9.7[a]
3b	35	53.7 ± 6.4[a]
3c	86	31.4 ± 4.2
3d	65	27.4 ± 6.2
3e	41	53.5 ± 8.9[a]
3f	31	69.8 ± 9.7[a]
Moclobemide	59	42.3 ± 7.9[a]

[a] $P \leq 0.05$, statistically significant compared to the control group.

(Germany) with chloroform/ether petroleum/acetone/methanol (4:4:1.5:0.5) and detected with reactive of Dragendourf. The novel structures were supported by microanalyses (Microanalytical Unit, Faculty of Pharmacy, Sofia) and the characteristic IR and NMR data quoted.

General method for 2-aryl-4-(2-hydroxyethyl)morpholines ***1a–d***

The appropriate 2-chloroketone (50 mmol) was added to a mixture of 100 mmol *N,N*-diethanolamine and 100 mmol formic acid. The reaction mixture was heated under reflux for 15 h at 180 °C. The mixture was acidified with diluted HCl (1:1) to pH 5–5.5. Non-basic products were extracted with benzene (3 × 50 mL). The water layer was treated with charcoal and the clear solution obtained upon filtration was treated with 30% sodium hydroxide to alkaline (pH 8–9). The obtained 2-aryl-4-(2-hydroxyethyl)morpholine was extracted with ether (3 × 50 mL). The ethereal solution was dried over Na_2SO_4, filtered and evaporated on rotary evaporator. The crude product was distilled under vacuum (oil pump), or recrystallized in the case of solid products [3].

General method for 4-(2-phenyloxy(alkyloxy)carbonyloxyethyl)-2-arylmorpholines ***2a–i***

4-(2-Hydroxyethyl)-2-arylmorpholine (5 mmol) was dissolved in 30–40 mL anhydrous benzene and a solution of phenyl-, methyl- or ethylchloroformate (6 mmol), dissolved in 5 mL anhydrous benzene, was added dropwise. The reaction mixture was stirred for 2 h and left overnight at room temperature. After filtration the separated product was recrystallized from absolute ethanol / acetone (2:1). Yield 40–45% (table VII).

Table V. Effect of the compounds on the total locomotor activity of mice over 90 min observation.

Compound	*Dose one-tenth of LD_{50} (mg/kg ip)*	*Total locomotor activity in arbitrary units ± SD*
Control	–	2120 ± 232
2a	91	631 ± 78[a]
2b	115	965 ± 118[a]
2c	119	381 ± 55[a]
2d	103	1524 ± 265[a]
2e	73	429 ± 88[a]
2f	45	997 ± 156[a]
2g	63	1035 ± 234[a]
2i	62	1125 ± 286[a]
3a	69	282 ± 41[a]
3b	35	427 ± 73[a]
3c	86	1590 ± 461[a]
3d	65	1852 ± 340
3e	61	1420 ± 256[a]
3f	41	1561 ± 321[a]
Moclobemide	59	1368 ± 345[a]

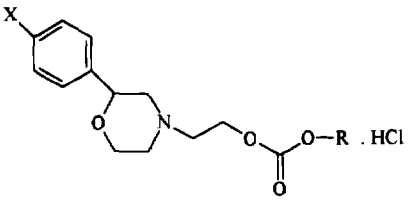
[a]$P \leq 0.05$, statistically significant compared to the control group.

Table VI. Effect of the compounds on the time of immobilization by behavior despair test.

Compound	*Dose (mg/kg ip)*	*Time of immobilization (s) ± SD*
Control	–	242 ± 26
2c	119	158 ± 27[a]
2d	51.5	145 ± 34
2d	103	115 ± 21[a]
2d	206	84 ± 32[a,b]
Moclobemide	28.5	163 ± 39[a]
Moclobemide	59	127 ± 26[a]
Moclobemide	118	101 ± 17[a,b]

[a]$P \leq 0.05$, statistically significant compared to the control group. [b]$P \leq 0.05$, statistically significant compared to the group treated with dose of one-twentieth LD_{50}.

Table VII. Carbonic acid esters.



Compound	*X*	*R*	*Mp (°C)*	*Yield (%)*	*Formula (molecular mass)*
2a	H	Ph	192–195	56	$C_{19}H_{22}ClNO_4$ (363.84)
2b	Me	Ph	205–208	40	$C_{20}H_{24}ClNO_4$ (377.87)
2c	Cl	Ph	176–178	43	$C_{19}H_{21}Cl_2NO_4$ (398.29)
2d	Br	Ph	200–201	52	$C_{19}H_{21}BrClNO_4$ (442.74)
2e	Br	Me	141–143	56	$C_{14}H_{19}BrClNO_4$ (380.67)
2f	Br	Et	154–157	52	$C_{15}H_{21}BrClNO_4$ (394.7)
2g	Cl	Me	171–173	45	$C_{14}H_{19}Cl_2NO_4$ (336.22)
2h	Cl	Et	118–120	55	$C_{15}H_{21}Cl_2NO_4$ (350.24)
2i	Me	Me	161–163	51	$C_{15}H_{22}ClNO_4$ (315.8)

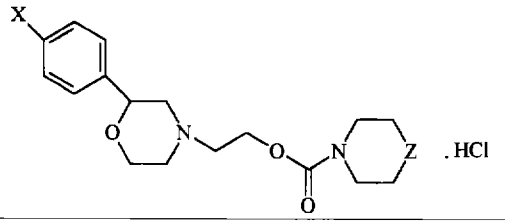
General method for 4-(2-piperidinyl(morpholinyl)carbonyl-oxyethyl-2-arylmorpholines ***3a–f***

Piperidine or morpholine (5 mmol) was slowly added with stirring to 5 mmol of 4-(2-phenyloxycarbonyloxyethyl)-2-aryl-morpholine dissolved in 20 mL anhydrous benzene. The mixture was heated for 12 h at 80 °C. The solution was treated with 5% sodium hydroxide, rinsed in H_2O and the benzene was distilled under vacuum. The crude product was dissolved in absolute ether and saturated HCl/ether was added dropwise (pH 5.5). After distillation of the solvent the residual solid was recrystallized from absolute ethanol (table VIII).

Pharmacology

The experiments were conducted on 680 male white mice with body weight 12–22 g. Acute toxicity (LD_{50}) of the studied compounds was assessed by dissolving them in saline (0.9% NaCl), and administering them to mice via intraperitoneal (ip) route. LD_{50} was evaluated for four or five different doses, each on the six animals and calculated by the method of Litchfield–Wilcoxon [8].

Table VIII. Carbamic acid esters.



Compound	*X*	*Z*	*Mp (°C)*	*Yield (%)*	*Formula (molecular mass)*
3a	H	O	167–170	65	$C_{17}H_{25}ClN_2O_4$ (356.85)
3b	H	CH_2	161–164	75	$C_{18}H_{27}ClN_2O_3$ (354.88)
3c	Me	O	165–167	68	$C_{18}H_{27}ClN_2O_4$ (370.88)
3d	Me	CH_2	179–182	75	$C_{19}H_{29}ClN_2O_3$ (368.90)
3e	Cl	O	157–160	71	$C_{17}H_{24}Cl_2N_2O_4$ (391.29)
3f	Cl	CH_2	159–161	90	$C_{18}H_{26}Cl_2N_2O_3$ (389.32)

Influence on hexobarbital-induced sleeping time (HBST)

The studied compounds were administered to male mice ip at doses of one-tenth of the LD_{50} (the same volume, 0.1 mL/10 g body weight of the solvent 0.9% NaCl, was administered to the controls). The solution of hexobarbital sodium at a dose of 80 mg/kg body weight was administered ip to the animals 30 min after the administration of the compound solutions. Sleeping time was measured in minutes by observing the righting reflex recovery.

Influence on locomotor activity

A group of six animals was put in the actometer (Activity Cage, Ugo Basile, Italy) and the locomotor activity in arbitrary units was determined at 10 min intervals for 90 min. The tested compounds at a dose of one-tenth of the LD_{50} were administered to the animals and they were tested in the apparatus for 90 min under the same conditions. The total locomotor activity was compared to that of the control (vehicle-treated) group.

The antidepressant activity was examined using the screening test behavior despair [9], calculating the time of immobilization of the mice in seconds over a 5 min observation period. The results of pharmacological experiments underwent statistical processing by the Student's-Fisher t-test at $P \leq 0.05$.

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