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SYNTHESIS AND ANTI-ARRHYTHMIC ACTIVITY OF THE CHLORIDE AND SALICYLATE SALTS OF MORPHOLINOACETIC ACID ORTHO-TOLUIDIDE

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Acylation of *ortho*-toluidine hydrochloride by chloroacetylchloride followed by amination by morpholine in the presence of an excess of Et_3N produced morpholinoacetic acid *ortho*-toluidide and its salicylate salt. The acute toxicity and anti-arrhythmic and antifibrillatory activities of these compounds were studied. The biological activities indicated that these compounds were promising for further studies and evaluation of their potential for implementation into medical practice.

Keywords: arylamide, acylation, morpholine, PMR spectroscopy, effective anti-arrhythmic dose, lidocaine.

Arylamides of aminocarboxylic acids are used in medical practice as anesthetics and anti-arrhythmics, e.g., lidocaine and trimecaine [1 – 3]. However, their therapeutic effects are short-lived because of rapid hepatic destruction [4]. Therefore, compounds that retain their value and are stable with longer anti-arrhythmic activity are highly sought among arylamides of aminocarboxylic acids.

The chloride (**I**) and salicylate (**II**) salts of morpholinoacetic acid *ortho*-toluidide (**III**) were synthesized in order to study the effect of the acid anion on the manifestation of biological activity (Scheme 1).

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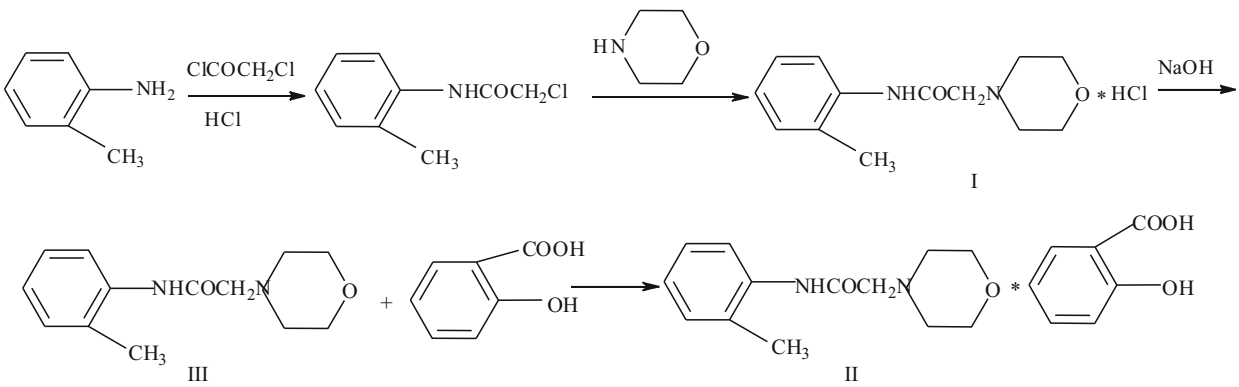
EXPERIMENTAL CHEMICAL PART

4-{[(2-Methylphenyl)carbamoyl]methyl}morpholin-4-ium chloride (I). *ortho*-Toluidine (21.4 g, 0.2 mol) was acylated by chloroacetylchloride (12.43 g, 0.11 mol). The resulting precipitate of chloroacetyl *o*-toluidide was recrystallized from hexane (yield 27.5 g, 81.1%; mp 102 – 104°C) and heated with morpholine (8.71 g, 0.1 mol) in the presence of an excess of Et_3N (10.1 g, 0.1 mol). The precipitate of

Схема



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Et_3NHCl was filtered off. The excess of amine was distilled off. The resulting *o*-toluidide **III** was dissolved in Et_2O. The solution was saturated with gaseous HCl [5]. Salt **I** crystallized from DMF–Me_2CO (1:1). Yield of **I**, 20.1 g (85.89%), mp 216 – 218°C. Next, salt **I** was used to isolate the free base by treatment with base and extraction by Et_2O. Then, the Et_2O was distilled off. The resulting free base **III** was dried over $CaCl_2$.

4-{[(2-Methylphenyl)carbamoyl]methyl}morpholin-4-ium 2-methylbenzoate (II). A solution of **III** (2.7 g, 0.01 mol) in anhydrous Et_2O (15 mL) at 10 – 20°C was stirred for 1 h, treated with salicylic acid (1.22 g, 0.01 mol) in anhydrous Et_2O (10 mL), and stirred for 30 min. The resulting precipitate of salicylate **II** was filtered off, dried in air, and recrystallized from hexane, mp 86 – 88°C, yield 3.7 g (90.68%).

Compound **II** was a white crystalline compound that was soluble in H_2O, EtOH, MeCN, and DMF and insoluble in Me_2CO, hexane, and C_6H_6.

The structures of **I** and **II** were confirmed using PMR spectroscopy in $CDCl_3$ with HMDS internal standard (BS-567A, 100 MHz).

Compound **I**: 2.244 (s, 3H, CH_3), 2.560 – 2.851 (m, 4H, $N(\underline{CH_2}CH_2)_2O$), 3.116 (s, 2H, CH_2), 3.664 – 3.912 (m, 4H, $N(CH_2\underline{CH_2})_2O$), 6.900 – 7.976 (m, 4H, C_6H_4), 9.089 (s, 1H, -NH-), 10.601 (s, 1H, HCl).

Compound **II**: 2.263 (s, 3H, CH_3), 2.671 – 2.755 (m, 4H, $N(\underline{CH_2}CH_2)_2O$), 3.265 (s, 2H, CH_2), 3.728 – 3.812 (m, 4H, $N(CH_2\underline{CH_2})_2O$), 6.808 – 7.986 (m, 4H, C_6H_4), 9.210 (s, 1H, -NH-), 10.045 (δ, 2H, COOH, OH).

EXPERIMENTAL PHARMACOLOGICAL PART

Acute toxicity was determined using i.v. injection. The median lethal dose was determined using the Prozorovskii method [6]. Tests were conducted on white male mice (18 – 24 g). The experiments used four animal groups with two observations in each. Each pair was injected with one dose of compound in increasing order (compound **I** was injected at doses of 158, 200, 250, and 316 mg/kg; compound **II**, 100, 126, 158, and 200 mg/kg). The number of dead mice in each group was noted.

Anti-arrhythmic activity was studied in mice using an arrhythmia model induced by i.v. injection of $CaCl_2$ solution (3%, GUP Immunopreparat, Ufa) at a dose of 280 mg/kg [7, 8]. The effective anti-arrhythmic doses were determined by the Prozorovskii method [6]. Test compounds were injected in increasing doses, each to two animals (compound **I** was injected at doses of 5, 6.3, 7, and 10 mg/kg; compound **II**, 7.9, 10, 12.6, and 15.8 mg/kg) 2 min before inducing arrhythmia. Their activities were assessed from the ability to prevent lethal disruption of cardiac rhythm.

Antifibrillatory activity was studied using an acute coronary occlusion model in rats [7, 9]. This model was adequate because it reproduced conditions similar to sudden cardiac death. In the first step, an occluding device consisting of a polyethylene tube with a ligature drawn through it was implanted into the chest cavity of rats under ether anesthesia. The ligature was fed through the myocardium under the descending coronary artery, which enabled the coronary vessels to be occluded by tightening one of the ligature ends. Occlusion was induced in awake animals 5 – 7 d after the operation. The EKG in the second standard lead was recorded during the whole test. The frequency of fibrillation and death was calculated. Tests were conducted on white rats of both sexes (180 – 220 g). Each group contained 10 animals. The reference drug was lidocaine in solution for injection (OAO Biokhimik, Saransk). Drugs were injected i.v. 2 min before occlusion at the effective anti-arrhythmic doses.

TABLE 1. Influence of Compounds on Course of Acute Coronary Occlusion in Awake Rats

Compound	Number of animals in group	Anti-arrhythmic activity			Antifibrillatory activity	
		ED_{50}, mg/kg, i.v.		arrhythmia frequency at ED_{50}, %	frequency of ventricular fibrillations at ED_{50}, %	death, %
		average parameters	confidence intervals			
Control	10	–	–	87.0	66.0	53.0
II	10	11.2	(9.2 ± 13.4)	20.0 $p < 0.01$ $p' > 0.05$	0 $p < 0.01$ $p' < 0.05$	0 $p < 0.01$
I	10	6.4	(4.9 ± 8.6)	60.0 $p > 0.05$ $p' > 0.5$	0 $p < 0.01$ $p' < 0.05$	0 $p < 0.01$
Lidocaine	10	7.7	(5.9 ± 9.4)	50.0 $p > 0.05$	30.0 $p = 0.05$	0 $p < 0.01$

$p < 0.05$ is significance of differences vs. the control; $p' < 0.05$ is significance of differences vs. lidocaine.

Anti-arrhythmic activity was also studied in mice using an arrhythmia model induced by strophanthin K (AO Galichfarm, Lviv, solution for injection) that was injected i.v. at a dose of 1 mg/kg 2 min after injecting the test compound at a dose equal to ED_{50}. The frequency of animal death was calculated [7, 10].

Results were processed by variational statistics using the Fisher–Student method [11].

RESULTS AND DISCUSSION

The studies showed that the synthesized compounds had low toxicity and pronounced anti-arrhythmic activity.

The LD_{50} of **II** was 146 mg/kg. The effective anti-arrhythmic dose calculated during the experiment for the $CaCl_2$-induced cardiac rhythm disruption model in mice (ED_{50}) was 11.2 mg/kg, i.e., the anti-arrhythmic index (LD_{50}/ED_{50}) was 13.0. For **I**, LD_{50} was 231 mg/kg; ED_{50}, 6.4 mg/kg; which meant that the anti-arrhythmic index was 36.1. The same index for reference drug lidocaine was 5.1.

Table 1 lists data obtained from studies of the course of acute coronary occlusion in awake rats.

Arrhythmia was observed in control tests with i.v. injection of normal saline in 87% of tests; fibrillation, in 66% of the animals. The outcome was lethal for most rats (53%). The ST segment in the EKG shifted sharply, more often above the isoelectric line even in the first seconds of the occlusion. Then, the shift lessened somewhat and rose again, after which arrhythmia developed up to fibrillation.

Arrhythmia was observed in 50% of cases with i.v. injection of lidocaine at a dose of 7.7 mg/kg. Spikes of ventricular fibrillation were observed in only 30% of the animals. This was statistically significantly less than in the control tests.

More pronounced effects were found if compounds **I** and **II** were injected at doses equal to ED_{50}. They completely protected the animals from manifestation of fibrillation. This distinguished them statistically significantly from not only the controls but also from the experimental effect of lidocaine. Furthermore, arrhythmia developed in only 20% of tests if compound **II** was used at a dose of 11.2 mg/kg. This differed statistically significantly from the controls. However, the frequency of their occurrence was 2.5 times less than in the tests with lidocaine whereas compound **I** did not prevent manifestation of arrhythmia. Lethal outcomes after coronary artery occlusion in awake rats were not recorded in tests with compounds **II** and **I** and lidocaine injected into the animals.

It was found that cardiac rhythm was disrupted in all animals after i.v. injection to white mice of strophanthin K. Lethal outcomes occurred in 62% of cases in the controls with developed arrhythmia. Only 30% of animals died if compound **II** was used. However, the decrease of this parameter was not significant compared with the control ($p > 0.05$) and lidocaine, which statistically significantly prevented animal deaths (20%, $p = 0.05$). Injection of **I** completely prevented development of strophanthin arrhythmia, which was 30 times more effective than **II** and 20 times more effective than lidocaine ($p < 0.05$).

Thus, the synthesized compounds exhibited pronounced anti-arrhythmic effects that were comparable with those of lidocaine in various induced experimental models. The results indicated that further expanded pharmacological studies of these compounds are warranted.

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