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INFLUENCE OF A SERIES OF 1,4-DICARBONIC ACID DERIVATIVES ON THE BLOOD CLOTTING TIME

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The effect of six monosubstituted amides and eight acylhydrazides of succinic, maleic, citraconic and phthalic acids on hemostasis on the APG4-02-P coagulometer was studied. The experiments were performed on citrated (3.8%) blood (9:1) of rabbits. To determine the activity, 50 µl of blood was placed in the cuvette and 50 µl of 0.2% solution of the test compound was added. In the control, instead of the substance, 50 µl of isotonic sodium chloride solution was added. As the reference preparation, 50 µl of heparin was used at a concentration of 1 U/ml of blood or 50 µl of the solution of the Ethamsylate preparation at concentration of 0.2%. The degree of influence of the compounds on hemostasis was determined by the change in the clotting time of citrated blood in the control and experiment. Among the fourteen studied derivatives of 1,4-dicarboxylic acids, ten compounds had an effect on hemostasis. Five compounds exhibited anticoagulant effects. Five compounds exhibited a hemostatic effect. The effect of the two compounds is similar to that of Ethamsylate.

Keywords: amides and hydrazides of 1,4-dicarboxylic acids, direct anticoagulant and emostatic activity

Medicines that affect hemostasis are used in various fields of medicine. Anticoagulants are widely used in surgical and therapeutic practice for prevention and treatment of diseases that cause thrombosis. However, the currently available direct anticoagulants have a number of disadvantages: reactions at the injection sites, the development of thrombocytopenia, the risk of hemorrhagic complications, increased thrombus formation after withdrawal of medication, and the high cost of medications [1,2].

Prevention and control of bleeding are critical for hematology, surgery, traumatology, oncology and obstetrics, since the use of hemostatic agents is necessary to stop serious bleeding. For this purpose, the medicines that have different mechanisms of influence on blood clotting are used. The hemostatics currently used have a number of disadvantages that limit their use [3–5].

All of this determines the relevance of the search and study of new compounds that affect hemostasis. There is evidence in the literature that amides and hydrazides of 1,4-dicarboxylic acids have hemostatic and anticoagulant activities [6–13].

The purpose of our study is to continue studying the effect on hemostasis of monosubstituted amides and hydrazides of succinic, maleic, citraconic and phthalic acids obtained by known methods [12–14], as well as to identify the biological action – structure of compounds relationship.

MATERIALS AND METHODS

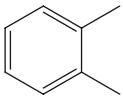
The objects of study of biological activity were monosubstituted amides and hydrazides

of succinic, maleic and citraconic acids. General formula of the studied compounds:

RCOX-YCOOH,

where

X-Y: -CH_2-CH_2-, -CH=CH-, -CH=C(CH_3)-,



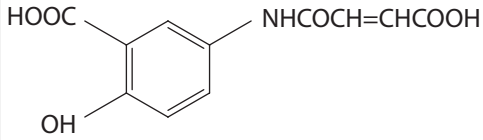
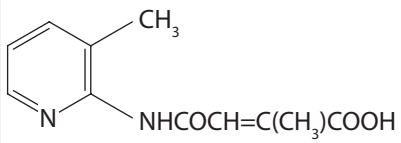
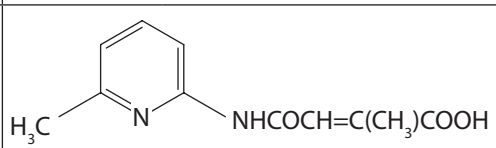
R: acyl-NH-, acyl-CONHNH- (**acyl:** aliphatic, aromatic, and heterocyclic substituents, see Table).

The effect of the compounds on hemostasis was studied using an APG4-02-P coagulometer. For the study, citrated (3.8%) blood (9:1) of rabbits was used. To determine the activity, 50 µl of blood was placed in the cuvette and 50 µl of a 0.2% solution of the test compound was added; for control, 50 µl of isotonic sodium chloride solution was added instead of the substance. As reference drugs, 50 µl of heparin was added at concentration of 1 U/ml of blood or 50 µl of solution of the Ethamsylate medicine at concentration of 0.2%. The samples were then incubated for 60 seconds, 50 µl of 1% calcium chloride solution was added, and blood clotting time was measured. Each compound was studied using the blood of 10 animals.

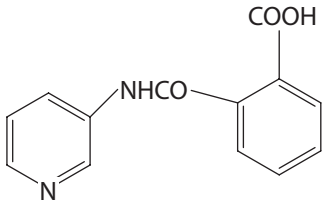
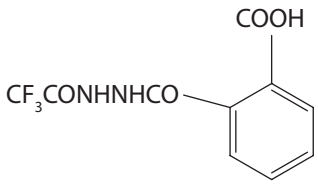
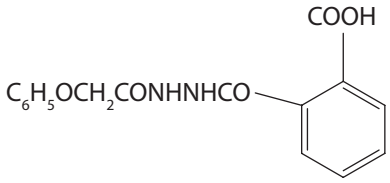
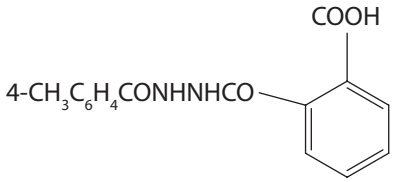
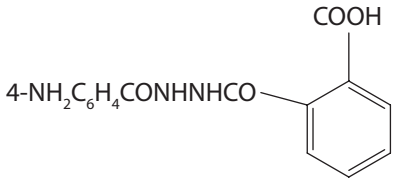
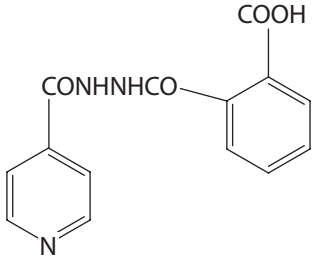
The results of the study of the effect of compounds on blood clotting were processed by the method of variation statistics according to the Fisher-Student method using the statistical

Table

EFFECT OF COMPOUNDS ON THE BLOOD CLOTTING TIME

No.	Compound formulas	Number of rabbits, control	Clotting time, sec control	Number of rabbits, experiment	Clotting time, sec experiment	% coagulability change	P
1.	4-$CH_3CONHC_6H_4NHCO\ CH_2$-H_2COOH	10	163.1±4.88	10	185.1±7.59	-13.3	<0.05
2.	$(C_6H_5)_2C$=NNHCOCH_2-CH_2COOH	10	127.9±4.93	10	103.2±5.84	**+19.3**	<0.02
3.	4-$CH_3C_6H_4CONHNHCO\ CH_2$-H_2COOH	10	116.3±4.80	10	118.2±4.07	-1.6	>0.05
4.	2-$CF_3C_6H_5NHCOCOOH$	10	95.3±7.14	10	101.1±4.64	-6.1	>0.05
5.		10	125.1±2.30	10	135.9±3.98	-8.6	<0.05
6.		10	121.5±4.07	10	91.9±2.98	**+24.4**	<0.02
7.		10	110.8±3.80	10	125.4±5.13	-13.2	<0.05

End of the table

No.	Compound formulas	Number of rabbits, control	Clotting time, sec control	Number of rabbits, experiment	Clotting time, sec experiment	% coagulability change	P
8.	2-OH-$C_6H_5CONHNHCOCH{=}C(CH_3)COOH$	10	105.1±4.70	10	138.7±3.97	-31.9	<0.001
9.			145.1±3.08	10	119.1±3.29	**+17.9**	<0.001
10.		10	98.1±7.39	10	100.5±6.77	-2.4	>0.05
11.		10	115.4±5.14	10	99.6±5.48	+13.7	>0.05
12.		10	141.6±5.09	10	120.2±4.23	**+15.1**	<0.01
13.		10	92.7±3.05	10	72.6±3.80	**+21.7**	<0.01
14.		10	122.1±4.46	10	138.1±3.94	-13.6	<0.05
References	Ethamsylates	10	144.1±7.83	10	121.0±7.20	+16.0	<0.05
	Heparin	10	145.7±9.64	10	618.3±55.88	-324.4	<0.001

processing program StatBase [15] and are shown in the table.

The assessment of biological activity in animal experiments was carried out in accordance with the requirements of the Pharmacological Committee set out in the Guidelines for Preclinical Studies of Medicines [16]. Animal management was in compliance with the rules of good laboratory practice in preclinical studies in the Russian Federation (GOST R 51000.3–96, General requirements for testing laboratories) and the Order of the Ministry of the Russian Federation No. 267 dated 19.06.2003 "On approval of the rules of good laboratory practice «(GLP), in compliance with international recommendations of the European Convention for protection of vertebrate animals used in experimental studies (1997).

RESULTS AND DISCUSSION

Among 14 compounds studied, five derivatives of 1,4-dicarboxylic acids have a hemostatic effect, two of them (compounds 6, 13) exceed the activity of the Ethamsylate medicine, two compounds (2 and 9) effect similarly to the reference drug. The activity of 3-Methyl-2-pyridylamide citraconic acid (compound 6) is 1.5 times higher than the activity of the Ethamsylate medicine, while the same derivatives of maleic and phthalic acids have an anticoagulant effect, and the derivative of succinic acid does not affect hemostasis [8,10]. Transfer of the methyl group from position 3 (compound 6) to position 6 (compound 7) leads to change from the hemostatic effect on blood clotting to anticoagulant one. 6-Methyl-2-pyridylamide of phthalic acid has an anticoagulant effect, and the same amide of succinic acid is inactive [8]. 4-Aminobenzoylhydrazide of phthalic acid (compound 13) is 1.35 times higher than the activity of Ethamsylate, the same derivative of maleic acid is less active [8], and potassium, sodium and ammonium salts of phthalic and succinic acids do not affect hemostasis [8,10]. Five compounds (1, 5, 7, 8, 14) showed an anticoagulant effect, significantly inferior to the action of heparin. 2-hydroxybenzoylhydrazide of citraconic acid (compound 8) has the greatest anticoagulant activity although previously only a hemostatic effect was found for citraconic acid derivatives [11]. Isonicotinoylhydrazide of phthalic acid (compound 14) showed a slight anticoagulant effect, the same maleic acid derivative is inactive, and isonicotinoylhydrazide of citraconic acid showed a hemostatic effect exceeding the activity of Ethamsylate [8,10]. Phenoxyacetylhydrazide of phthalic acid (compound 11) does not affect hemostasis, whereas methoxyacetylhydrazide of this acid has a hemostatic effect [10], that is, the replacement of the methoxyl group with the phenoxy group leads to a loss of activity.

CONCLUSIONS

1. Monosubstituted amides and hydrazides of 1,4-dicarboxylic acids are promising compounds that affect hemostasis, and are characterized by anticoagulant and hemostatic activity.

2. Among the substances studied, five compounds with hemostatic effect (two of them exceed the effect of Ethamsylate) and five compounds with direct anticoagulant activity were found.

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