Biological Activity of Some Derivatives of β-Cyclodextrin

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New compounds of β-cyclodextrin containing covalently bound (conjugated) residues of acetylsalicylic and 1-(4-isobutylphenyl)-propionic acids were synthesized in the reaction of chlorides of the corresponding acids with β-cyclodextrin. We studied antiplatelet and antiphlogistic properties of these substances. It was shown that new compounds are comparable and in some cases are superior to the reference drugs acetylsalicylic acid and ibuprofen by anti-inflammatory and antiaggregant activities.

Key Words: *β-cyclodextrin conjugates; acetylsalicylic acid; ibuprofen; antiphlogistic and antiplatelet properties*

The inclusion compounds, cyclodextrin clathrates (CC) with known pharmacological agents, exhibit a wide range of biological activities and are of practical interest [5,8].

CC are regular cyclic oligosaccharides containing D-glucopyranose fragments bound via α-1-4-glycosidic bonds. CC are relatively inexpensive, biodegradable, and nontoxic and due to these properties they are widely used in various fields of chemistry, especially supramolecular chemistry, fine organic synthesis, and in some interdisciplinary areas. CC are of particular interest due to their cyclic structure and the presence of internal hydrophobic cavity, capable to form the host–guest clathrate complexes with various organic substrates [6].

Because of the capacity for inclusion of hydrophobic compounds, CC are widely used in pharmacology as a system for drug delivery via molecular encapsulation. Cyclodextrin skeleton protects the included drug from biodegradation, improves its solubility and targeted delivery to the desired location for the required time period [7,8,10,11]. Covalent "binding" (conjugation) of drugs to CC and creation of drugs of this basis are recent and promising opportunities in the pharmaceutical use of CC. This technique provides the basis for obtaining more efficient drugs with less pronounced side effects [2].

The aim of the work was the synthesis of new β-CC derivatives: β-CC–acetylsalicylic acid (AA) conjugate (compound I), and β-CC–1-(4-isobutylphenyl)-propionic acid conjugate (compound II; Fig. 1), and pharmacological testing of these compounds as anti-inflammatory and antiaggregant agents.

MATERIALS AND METHODS

The synthesized compounds were tested for their antiaggregant activity on platelets of platelet-rich plasma (PRP); AA and ibuprofen served as the reference drugs.

PRP and washed platelet suspension was used for photometric studies of platelet function. The blood was drawn at fast from the jugular vein with plastic syringes containing 3.8% (0.11 M) sodium citrate (9:1 blood:anticoagulant ratio). All manipulations with blood, PRP, and washed platelet suspension were carried out in polyethylene tubes. For preparing PRP, the blood was centrifuged in plastic tubes at room temperature for 10 min to precipitate red and white

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TABLE 1. Antiplatelet Activity of Compounds I and II

Sample	Concentration of anti-inflammatory drug in the weighed portion, mM	Amplitude, %	Rate, %/min
Control	-	38.0±8.7	48.0±6.5
AA	0.05	24.7±4.9	27.9±6.5
Compound I	0.02	13.0±3.5*	13.0±6.1*
Ibuprofen	0.04	25.4±3.9	23.4±4.2*
Compound II	0.026	35.8±4.8	18.7±7.7*

Note. Here and in Table 2: *$p<0.05$ in comparison with the control.

blood cells. The platelet count in PRP was determined in a Goryaev chamber under a MBI-15 microscope with phase-contrast attachment and adjusted to 2×10^{11}-2.5×10^{11} platelets/liter with platelet-poor plasma. PRP was obtained by repeated centrifugation of blood for 10 min and stored at 4°C.

Platelet aggregation was evaluated on a PICA 330 aggregometer and dual channel LaborAPACT aggregometer with graphic recording and automatic calculation of the amplitude and maximum rate of aggregation. Agregatogramms were analyzed by amplitude (maximum decrease in optical density, in %) and maximum rate of aggregation (in %/min). The experimental sample was compared with the control in 6 parallel tests. Thrombin in a final concentration of 0.5 U/ml was used as inductor of platelet aggregation. The blood was incubated with the samples for 5 min at 37°C.

The recommended dose of AA as an anticoagulant for humans is 0.0625 g/day; the concentration of drug in the blood is 0.05 mmol/liter [4]. Anticoagulant activity of ibuprofen in clinical practice is not taken into account. In this regard, the chosen concentration of ibuprofen was similar to the concentration of AA: 0.04 mmol/liter. Our preliminary studies showed that pharmacological activity of compounds I and II (Fig. 1) is higher than that of the reference samples. For this reason, the concentrations of the investigated conjugates were deliberately reduced (Table 1).

Anti-inflammatory properties of compounds I and II were studied on a model of acute inflammatory edema in mice weighing 18-20 g (7 animals in each group) after subcutaneous injection of 50 µl 1% formalin in the paw. The volume of the paw was measured with a water plethysmometer before injection of the phlogogenic agent and then 1, 2, 4, and 24 h after administration.

Anti-inflammatory effect was evaluated by the degree of edema inhibition in comparison with that

TABLE 2. Antiphlogistic Activity of Compounds I and II and Average Therapeutic Dose of AA, Ibuprofen, and Ortofen ($M\pm m$)

Compound	Dose, mg/kg	Increase of formalin-induced edema, %				Peritoneal exudate, ml
		1 h	2 h	4 h	24 h	
Control	-	24.73±11.31	31.55±5.73	7.21±12.34	35.48±9.38	3.17±0.51
Compound I	50	22.39±5.43	28.71±12.40	31.22±9.38*	17.43±7.31*	2.19±0.89
	100	17.81±7.30	22.41±2.54*	27.11±11.37*	15.80±8.31*	1.92±0.63*
	150	16.11±2.75	19.78±9.31	25.41±8.45*	16.91±9.83	1.74±1.03
AA	28	21.14±9.35	25.16±7.16	32.71±10.81*	26.18±9.39	2.21±0.51
Compound II	7	17.10±4.21	27.66±1.18	31.21±6.21*	27.31±8.96	2.03±0.37*
	15	13.30±2.93	20.11±3.12*	23.21±10.41*	23.53±5.69	1.81±0.52*
	25	13.10±1.77	13.10±7.81*	23.68±14.35*	19.88±4.31*	1.66±0.61*
Ibuprofen	5	17.70±6.33	22.01±4.18	34.16±5.11*	29.21±1.61	2.11±0.69
Ortofen	15	16.35±8.15	21.71±7.73	17.39±6.88*	14.15±10.25*	1.90±0.41*

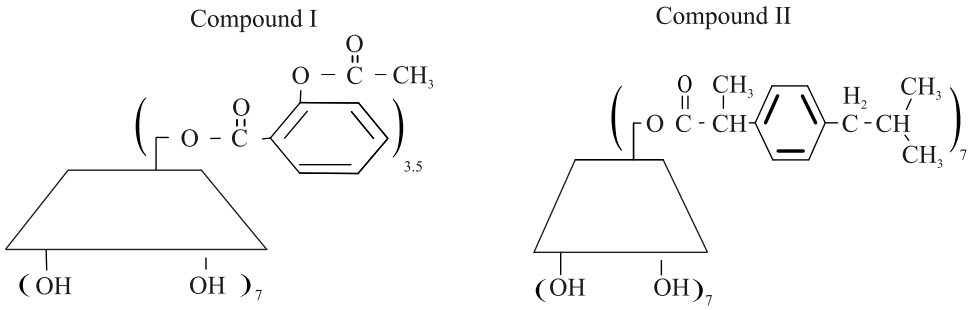

Fig. 1. Chemical formula and characteristics of compounds I and II. Compound I: NMR spectrum ^{1}H (DMSO-d_6), δ, ppm: 2.05-2.35 m (10.5H, C(O)CH_3), 3.13-4.08 m (42H; C^2H-C^5H, C^6H_2), 4.23-4.59 m (3.5H, C^6OH), 4.81 br.s. (7H, C^1H), 5.60-6.04 m (14H; C^2OH, C^3OH), 7.05-8.11 m (14H, CH_{arom}). NMR^{13}C (DMSO-d_6), δ, ppm: 20.8 (C(O)$\underline{C}H_3$), 59.4 (C^6OH), 63.9 [$\underline{C}^6$OC(O)], 68.9 [$\underline{C}^5C^6$OC(O)], 71.5-74.0 (C^2, C^3, C^5), 80.1-83.0 (C^4), 101.0-103.1 (C^1), 122.1-135.0 ($C_{arom.}$), 150.1 [$\underline{C}$OC(O)], 169.2 [C(O)]. Experimental, %: C 51.11; H 5.45. $C_{73.5}H_{91}O_{45.5}$. Calculated, %: C 51.85; H 5.39.

Compound II: NMR spectrum ^{1}H (DMSO-d_6), δ, ppm: 0.78-0.85 m [42H, $(CH_3)_2$], 1.24-1.30 m (21H, CH_3), 1.72-1.81 m [7H, C$\underline{H}(CH_3)_2$], 2.37 br.s. (14H, CH_2), 3.13-3.77 m (42H; C^1H-C^5H, C^6H_2), 3.47-3.51 m (5H, CH), 4.82 d (7H, C^1H), 5.66-5.82 m (14H; C^2OH, C^3OH), 6.96-7.22 m (28H, $CH_{arom.}$). NMR^{13}C (DMSO-d_6), δ, ppm: 18.2 (CH_3), 22.2 [$(CH_3)_2$], 29.7 [$\underline{C}H(CH_3)_2$], 40.3 ($\underline{C}HCH_3$), 44.3 (CH_2), 63.2 [$\underline{C}^6$OC(O)], 69.3 [$\underline{C}^5C^6$OC(O)], 72.0-73.0 (C^2, C^3, C^5), 81.8 (C^4), 102.1 (C^1), 127.1-139.6 ($CH_{arom.}$), 174.2 [C(O)]. Experimental, %: C 64.64; H 7.55. $C_{133}H_{182}O_{42}$. Calculated, %: C 65.13; H 7.48.

in controls after intragastric administration of the test substances (experimental group) and saline (control group). In parallel, antiphlogistic activity of the test compounds was compared to that of ibuprofen, AA, and ortofen on albino outbred rats weighing 180-200 g (5 animals per group) with experimental peritonitis induced by intraperitoneal injection of 0.2% silver nitrate (1 ml/100 g) [3].

Compound I was obtained by adding dropwise 0.44 g (2.20 mmol) AA chloride in 2 ml to a solution of 0.50 g (0.441 mmol) β-CC in 8 ml pyridine with stirring; the mixture was left for 24 h at 20°C. Pyridine hydrochloride was filtered, the filtrate was evaporated, the residue was ground in ethanol (10 ml), precipitate was filtered, washed with ethanol (2×5 ml), and dried under vacuum. The yield of compound I was 0.47 g (63%), melting point 203-205°C, R_f 0.78 (on aluminum plates with fixed layer of silica gel in acetonitrile–water 5:2 system).

For preparing compound II, 0.69 g (3.09 mmol) 1-(4-isobutylphenyl)-propionyl chloride in 2 ml of benzene was added dropwise with stirring to a solution of 0.50 g (0.441 mmol) β -CC in 8 ml pyridine and kept for 24 h at 20°C. Pyridine hydrochloride precipitate was filtered, the filtrate evaporated, the residue was ground in ether (5 ml), and the precipitate was filtered and washed with water (2×5 ml). Residual water was removed by azeotropic distillation with benzene and dried under vacuum (1 mm Hg) for 3 hours at 60°C. The yield of compound II was 0.70 g (65%), melting point 157-160°C, R_f 0.59 (on aluminum plates with fixed layer of silica gel in an ethyl acetate–acetic acid–water 3:1:3 system).

The data were processed using Student's *t*-test [1]. The normality of the data was previously checked.

RESULTS

Despite the lower concentrations of the studied conjugates, the maximum decrease in platelet optical density and maximum rate of platelet aggregation observed in experiments with new compounds were not inferior to the corresponding values in the control and reference samples and even surpassed them (Table 1). Thus, antiplatelet activity of the test substances essentially depends on the location and number of introduced drug residues, which necessitates further pharmacological tests of this compounds.

Compounds I and II protected from biodegradation can circulate in the blood for a longer time compared to the "unprotected" drugs. In our model, the rate of hydrolysis of compounds I and II probably increases during the formation of edema in the inflammatory focus, which leads to the more targeted delivery of the anti-inflammatory agent to the pathological zone (Table 2).

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