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Synthesis and inhibitory effect of threo-DL-phenylserine derivatives in rat experimental adjuvant arthritis model

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New threo-DL-phenylserine derivatives **5–12** were synthesised from the corresponding amino acid esters **1** and succinic (butanedioic) acid derivatives **2**, **3** and *N*-2-fluorenylsulfonyl-β-alanine (**4**). Acylation of phenylserine esters with *N*-(R^1-phenyl)succinic acid amide (**2**) was performed in presence of dicyclohexylcarbodiimide (DCC) at 0 °C (Scheme). The reaction proceeded smoothly and yields of compounds **5–9** were good (65–75%), except for compound **6** (40%). The fluorenyl derivative **12** was obtained in an analogous way using the fluorenyl derivative **4**. Compound **10** was prepared by direct acylation of **1** with succinic anhydride **3**, and the salt **11** was obtained by treatment of **10** with 4,5-dihydro-1,3-thiazol-2-amine. Recrystallization from ethanol afforded pure compounds. IR and ^{1}H NMR spectral and elemental analysis data confirmed structures of the synthesized compounds.

The results of anti-inflammatory investigation are summarized in the Table. It should be noted that compounds showed inhibitory effects on joint swelling during the experiment. The best anti-inflammatory effect was achieved in rats receiving preparation **9**. The intensity of joint swelling at the end of experiment was suppressed by 50.7% ($P < 0.002$), ESR – by 50% ($P < 0.002$) and count of leukocytes – by 25% ($P < 0.001$). Polyarthritis characterizing the development of the autoimmune process was absent in this treated group and it developed in 50% of rats in the control group.

Compound **12** also reduced the intensity of joint swelling by 31.3% ($P < 0.05$) at the end of the experiment but did not show any significant effect on blood indices. Polyarthritis developed only in 20% rats given this preparation. The preparation **5** considerably suppressed joint swelling until day 10. Polyarthritis developed in 40% of rats treated with preparation **5**, and it significantly decreased the count of leukocytes in comparison to the control group.

No essential differences in body and internal organs (liver, kidney, spleen, and thymus) weight were observed among the treated and control groups at the end of experiment. Thus, among of all compounds tested preparation **9** caused the most potent anti-inflammatory activity in the experiment performed. It suppressed joint swelling, development of polyarthritis and showed beneficial effect on blood indices. The results obtained may be useful in search of new non-steroidal anti-inflammatory drugs that are mainstay in treatment of inflammation and pain [1].

Experimental

1. Chemistry

M.p.'s were determined in open capillaries and are uncorrected. IR spectra were recorded on a Specord 75 instrument (Germany) in KBr pellets, ^{1}H NMR spectra – on a Hitachi R-22 spectrometer (90 MHz, Japan) using HMDS as an internal reference ($\delta = 0.05$ ppm to TMS) in DMSO-d_6 solu-

Scheme

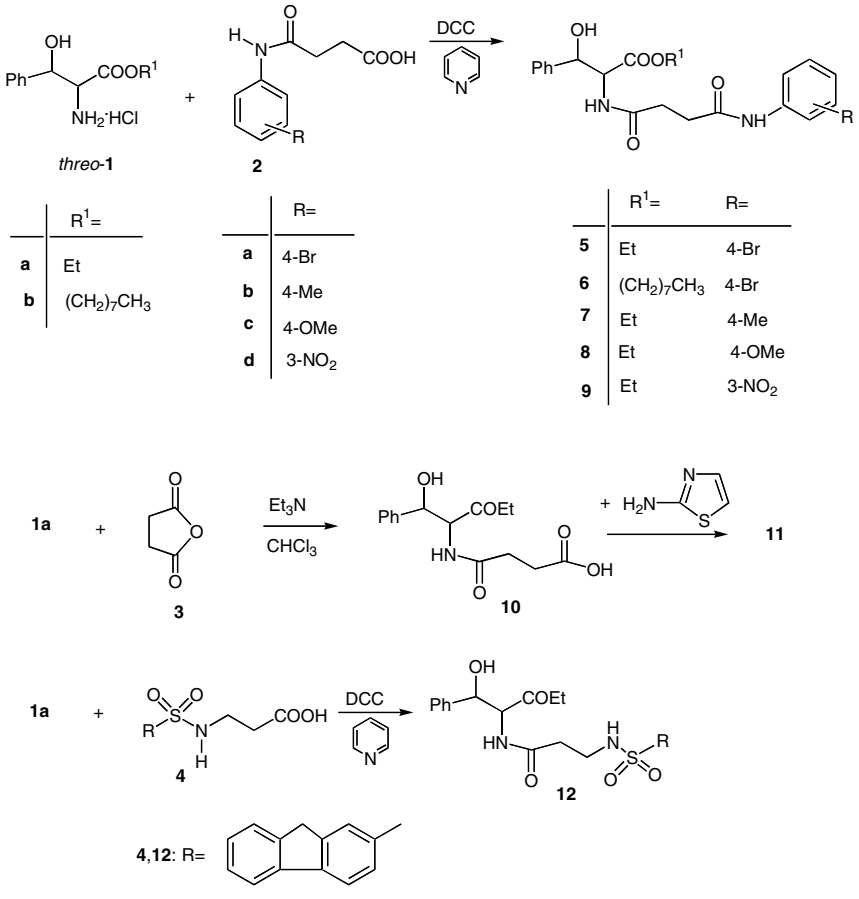

Table 1: Biological activity of compounds 5 and 7–12 in rats with adjuvant arthritis

Compd.	Dose (mg/kg)	Deviation from control (%)								
		Joint swelling (on day of experiment)						Blood indices		
		3	5	8–10	12	15	17	ESR	Leukocytes	Erythrocytes
5	30	−33.0**[a]	−27.0*	−26.5*	−25.1	−19.8	−11.5	−11.9	−25.3***	−4.4
7	25	−24.9*	−21.1	+1.0	−11.6	−3.0	−3.0	+4.1	−23.3	+15.1
8	25	−9.5	−16.2	+4.8	−10.3	+7.4	+10.0	+7.1	−23.6	+14.9
9	30	−36.4**	−32.2**	−25.7	−26.0	-43.7^{+}	-50.7^{+}	-50.0^{++}	−24.9***	+6.9
10	25	−22.0*	−12.0	+2.4	−10.5	+1.3	+2.6	-58.9^{+}	−23.9	+17.6
11	25	−3.7	−12.7	+4.04	−20.6	−3.0	−17.8	−42.8*	−25.7	+9.2
12	30	−27.3**	+1.0	−7.9	−18.2	−29.8	−31.3*	−18.8	−9.6	+1.1

[a] Differences are significant in comparison with control group. *P < 0.05, **P < 0.01, +P< 0.02, ++P < 0.002, ***P < 0.001.

tion. Chemical shifts δ are reported in ppm, coupling constants (J) are given in Hz. Multiplicity of signals is expressed as s (singlet) or bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet).

Starting threo-DL-phenylserine esters were synthesised from a commercially available corresponding acid (Chemapol, Prague, Czech Republic) and ethanol under reflux in the presence of gaseous HCl, or octanol in presence of p-toluenesulfonic acid in benzene, respectively.

1.1. N-(N′-Arylsuccinyl)-threo-D,L-phenylserine esters (**5–9**, **12**)

To a cooled mixture of 10 mmol of threo-DL-phenylserine ethyl ester hydrochloride (**1**, $R^1 = C_2H_5$) or octyl ester *p*-toluenesulfonate (**1**, $R^1 = C_8H_{17}$) and 10 mmol *N*-(*R*-phenyl)butanedioic acid amide (**2**) or *N*-2-fluorenylsulfonyl-β-alanine (**4**) in 10 ml of anhydrous pyridine was added 11 mmol of DCC at 0 °C. The reaction mixture was kept overnight in a refrigerator. The precipitate formed was filtered off and the solution poured into ice water. The product (**5–9**, and **12**) was filtered off, washed with water and recrystallized from ethanol.

5 ($C_{21}H_{23}O_5N_2Br$): yield 72%, m.p. 171–173 °C; **6** ($C_{27}H_{35}O_5N_2Br$), yield 40%, m.p. 45–46 °C; **7** ($C_{22}H_{26}O_5N_2$), yield 75%, m.p. 177–179 °C. **8** ($C_{22}H_{26}O_6N_2$) yield 63%, m.p. 169–171 °C.

9 ($C_{21}H_{23}O_7N_3$): yield of 67%, m.p. 174–176 °C; IR ν, cm^{-1}:1640 (CON), 1733 (COO), 3260–3270 (NH, OH); ^{1}H NMR δ: 1.0 (3 H, t; J = 7.0, CH_3); 2.40 (4 H, m, $(COCH_2)_2$); 3.95 (2 H, q, J = 7.0, CH_2,); 4.50 (1 H, dd, J = 4.6, 8.5, α-CH); 5.0 (1 H, dd, J = 4.5, 9.0, β-CH); 5.50 (1 H, d, J = 5.0, OH); 8.10 (1 H, d, J = 8.5, NH); 7.10–7.90 (8 H, m, aromatic signals); 10.3 (1 H, s, CONH).

12 ($C_{27}H_{28}O_6N_2S$): yield 77%, m.p. 166–168 °C; IR ν, cm^{-1}:1150, 1330 (SO_2), 1645 (CON), 1725 (COO), 3200–3340 (NH, OH); ^{1}H NMR δ: 1.10 (3 H, t; J =7.5, CH_3); 2.3–2.7 (4 H, m, $(COCH_2)_2$); 4.05 (2 H, q, J = 7.5, CH_2); 4.50 (1 H, dd, J = 4.5, 8.5, α-CH); 5.0 (1 H, d, J = 4.5, β-CH); 5.80 (1 H, d, J = 4.5, OH); 7.15–8.25 (16 H, m, aromatic signals and NH).

1.2. N-Succinyl-threo-D,L-phenylserine ethyl ester (**10**)

A mixture of 20 mmol of threo-DL-phenylserine ethyl ester hydrochloride (**1**, $R^1 = C_2H_5$) and 20 mmol of died triethylamine was dissolved in 25 ml of $CHCl_3$. Water was added to a mixture, organic layer was separated and dried over anh. $MgSO_4$. After addition to a solution of 22 mmol of succinic anhydride (**3**) it was heated until dissolution of reagents and left overnight in refrigerator. The crystals were filtered off, washed with diethyl ether and recrystallized from ethanol. **10** ($C_{15}H_{19}O_6N$): yield 68%, m.p. 153–155 °C; IR ν, cm^{-1}: 1645 (CON), 1700 (COOH), 1733 (COO), 3340 (NH, OH); ^{1}H NMR δ: 1.0 (3 H, t; CH_3, J = 7.0); 2.2 (4 H, m, $(COCH_2)_2$); 3.95 (2 H, q, J = 7.0); 4.2 (1 H, dd, J = 4.5, 8.5, α-CH); 4.7 (1 H, bs, β-CH); 5.45 (1 H, bs, OH); 8.0 (1 H, d, J = 8.5, NH); 7.2 (5 H, m, aromatic signals).

1.3. Thiazolinium salt of N-succinyl-threo-D,L-phenylserine ethyl ester (**11**)

A mixture of 5 mmol of threo-DL-phenylserine ethyl ester (**10**) was dissolved in 6 ml of hot ethanol and 5.5 mmol of 4,5-dihydro-1,3-thiazol-2-amine in 3 ml of ethanol was added. The reaction mixture was filtered and kept overnight in a refrigerator. The crystals (**11**) were filtered off and washed with diethyl ether. **11** ($C_{18}H_{25}O_6N_3S$): yield 91%, m.p. 136–138 °C.

2. Pharmacology

2.1. Animals

Experiments were performed on 80 male Wistar rats weighting 160–200 g at the baseline. The animals were purchased from Bioreglament (Vilnius, Lithuania) and kept under standardized conditions and allowed free access to standard rat chow and water during experiment. After a resting period of one week, animals were subjected to the experimental protocol. All experimental groups consisted of 10 animals.

2.2. Induction and evaluation of AA and other parameters

Adjuvant arthritis (AA) was produced by an injection of 0.1 ml of complete Freund's adjuvant (Sigma, St. Louis, MO USA) into the left hind paw on day 0. To evaluate the progression of the disease, two parameters were defined: the swelling of the hind paws determined plethysmographically and the development of polyarthritis. Paw volume and body weights were measured 3 times a week and the percentage of deviation was determined. At the end of experiment the animals were killed by decapitation preceded by narcosis. The internal organs' weight and ESR were determined. Leukocytes and erythrocytes were counted with a Picoscale hematological analyzer.

2.3. Preparations and treatment schedule

Compounds **5**, **7–12** were used throughout the study. All preparations were prepared *ex tempore* as a fine homogenized suspension in sterile 0.85 NaCl solution with 5 drops of Tween®-20 and were injected intraplantarly (volume 0.15 ml) into the right hind paw in doses 30 mg/kg **5**, **9**, and **12** and 25 mg/kg **7**, **8**, **10**, and **11**. Control group received sodium saline with same amount of Tween®-20. Treatment was started since the AA inducing day and continued to day 16. The experiment lasted 17 days.

2.4. Statistical analysis

The results were expressed as mean value ± S.E.M. Differences between control and treated groups were statistically analyzed by Student's test with $P < 0.05$ considered as significant. The percentage of deviation from the control group was derived by the following formula: (T-C)/C × 100, where T is the data on the tested group and C is the data on the control.

References

1 Kavanaugh, A.: Biodrugs **7**, 119 (1997)

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