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Synthesis of *N*-arylsulfonyl DL-phenylserine derivatives exhibiting anti-inflammatory activity in experimental studies

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Abstract

Synthesis and evaluation of anti-inflammatory activity in rats with adjuvant arthritis of aryl sulfonyl derivatives of nonproteinogenic aromatic amino acids is reported. The studied compounds were synthesized by introducing residues of benzene-, *p*-toluene-, and *p*-bromobenzene sulfonic acids into *threo*-DL-phenylserine and *erythro*-DL-*p*-nitrophenylserine structures. From the set of 12 compounds tested in animal screening, *N*-(*p*-bromobenzenesulfonyl)-*erythro*-DL-*p*-nitrophenylserine ethyl ester **12** demonstrated the most pronounced anti-inflammatory activity. This compound inhibited inflammation process in polyarthritis phase by 53% ($P < 0.001$) though it was slightly toxic ($LD_{50} > 6000$ mg kg^{-1} for mice).
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1. Introduction

Continuing interest in nonsteroidal anti-inflammatory drugs (NSAIDs) has been evidenced in several substantial articles published during the last few years [1–5]. Though the mechanism of suppression of inflammation is not completely ascertained, the NSAIDs provide well-established anti-inflammatory therapy acting via inhibition of cyclooxygenases (COX-1 and COX-2) [6,7]. The evaluation of the role of COX-1 and COX-2 in inflammation has stimulated renewed interest in the field of NSAIDs. COX-2 inhibitors can be placed in three structural classes, among which compounds containing sulfonamide group are of current interest [1,4,8]. In addition, recently a series of amino acid derivatives have been synthesized and showed anti-inflammatory properties [5]. Earlier we have synthesized several *N*-substituted amino acids derivatives and it was found that the anti-inflammatory effect depended mainly on the nature of amino acids [9,10]. Considering the foregoing we performed a synthesis of modified nonproteinogenic amino acid phenylserine derivatives containing sulfonamide group. In this study the hydrophobic and stereoelectronic properties of isomeric *threo*- and *erythro*-phenylserines were altered by introducing in their structures residues of arylsulfonic acids and also modifying carboxyl group by synthesis of the corresponding alkyl esters, hydrazide and organic salts.

2. Experimental

2.1. Chemistry

Melting points were determined in open glass capillaries and are uncorrected. IR spectra were obtained on a Specord 71 IR spectrophotometer (Germany) in KBr pellets. ^{1}H NMR spectra were recorded on a Hitachi R-22 90 MHz spectrometer (Japan). Chemical shifts (δ) are given in ppm relative to hexamethyldisiloxane and signal multiplicities are as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. Microanalyses of the synthesized compounds for C, H, N, Br and S were within ±0.4% of the calculated values.

threo-DL-Phenylserine was purchased from Chemapol (Czech Republic), 2-amino-2-thiazoline from Aldrich.

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Compounds **1** and **6** were prepared according to the literature methods, Refs. [11] and [12], respectively.

2.1.1. *N-Benzenesulfonyl-threo-DL-phenylserine (2)*

To a vigorously stirred solution of 1.81 g (10 mmol) of *threo*-DL-phenylserine in 20 ml (20 mmol) 1N KOH in water simultaneously dropwise was added solution of 1.4 ml (11 mmol) benzenesulfonyl chloride in 10 ml of Et_2O and 12 ml (12 mmol) of 1N $NaHCO_3$ solution in water over a period of 15 min at 0 °C. The reaction mixture was stirred for 3 h at room temperature maintaining it alkaline during the course of the reaction. Then etheral layer was separated, the aqueous layer was poured into crushed ice and acidified with 1N HCl to pH 3. The precipitated product was filtered off, washed with water and recrystallized from ethanol to yield 1.96 g (61%) of white crystals, m.p. 158–159 °C. IR ν, cm^{-1}: 1158 and 1328 (S=O), 1725 (C=O); ^{1}H NMR (CD_3OD): δ 4.1 (d, 1H, $J = 4$ Hz, α-CH), 5.1 (d, 1H, $J = 3$ Hz, β-CH), 7.0–7.7 (m, 10 H, aromatic protons).

2.1.2. *Diethanolamine and 2-amino-2-thiazoline salts of N-benzenesulfonyl-threo-DL-phenylserine (3 and 8)*

A mixture of 3.21 g (10 mmol) or 6.42 g (20 mmol) of *N*-benzenesulfonyl *threo*-DL-phenylserine **2** and 2.31 g (22 mmol) of diethanolamine or 2.15 g (20 mmol) of 2-amino-2-thiazoline was dissolved in 10 or 30 ml of ethanol, respectively, at room temperature. The solution was filtered and kept at room temperature for 1 h and then for 5 h in a refrigerator. The formed precipitate was filtered off and washed with ether.

3: Yield 3.64 g (68%), m.p. 154–155 °C. IR ν, cm^{-1}: 1085 (S=O), 1580 and 1622 (C=O); ^{1}H NMR (CD_3OD): δ 3.1 (t, 2H, $J = 5$ Hz, CH_2), 3.8 (t, 2H, $J = 5$ Hz, CH_2), 3.9 (m, 1H, α-CH), 5.1 (m, 1H, β-CH), 7.0–7.7 (m, 10 H, aromatic protons).

8: Yield 6.60 g (78%), m.p. 192–194 °C; IR ν, cm^{-1}: 1165 (S=O), 1580 and 1635 (C=O); ^{1}H NMR ($(CD_3)_2SO$): δ 3.4–3.7 (m, 4H, $(CH_2)_2$), 3.7 (d, 1H, α-CH), 4.9 (d, 1H, β-CH), 6.4 (br s, 4H, NH, COOH, NH_2), 7.1–7.7 (m, 10 H, aromatic protons).

2.1.3. *General procedure for synthesis of N-arylsulfonyl derivatives of phenylserine esters (4, 5, 9–12)*

To a stirred mixture of 10 mmol *threo*-DL-phenylserine ethyl ester hydrochloride [13], toluenesulfonates of *threo*-DL-phenylserine 1-octyl [10] and 1-tetradecyl [11] esters, and *erythro*-DL-*p*-nitrophenylserine ethyl ester hydrochloride [14], respectively, and 3.1 ml (22 mmol) of triethylamine in 30 ml of chloroform a solution of 10 mmol of the corresponding arylsulfonyl chloride in 10 ml of $CHCl_3$ was added dropwise over 10 min at 0–5 °C temperature. The reaction mixture was refluxed for 2 h, then cooled, and chloroform was added. The separated organic layer was washed with water, 1N HCl, and water, and dried over anh. $MgSO_4$. Solvent was evaporated in vacuo and solid residue recrystallized from ethanol–hexane (**4**), ethyl ether–hexane (**5**, **9**, **10**), ethanol–water (**11**, **12**) to yield pure arylsulfonyl derivatives.

2.1.3.1. *N-Benzenesulfonyl-threo-DL-phenylserine ethyl ester (4).*
Yield 73%, m.p. 121–123 °C; IR ν, cm^{-1}: 1165 and 1338 (S=O), 1708 (C=O); ^{1}H NMR ($(CD_3)_2CO$): δ 0.9 (t, 3H, $J = 7$ Hz, CH_3), 3.7 (q, 2H, $J = 7$ Hz, CH_2), 4.0 (d, 1H, $J = 7$ Hz, α-CH), 4.5 (s, 1H, OH), 5.0 (m, 1H, β-CH), 6.5 (s, 1H, NH), 7.0–7.6 (m, 10H, aromatic protons).

2.1.3.2. *N-Benzenesulfonyl-threo-DL-phenylserine octyl ester (5).*
Yield 70%, m.p. 46–48 °C, IR ν, cm^{-1}: 1167 and 1343 (S=O), 1693 and 1743 (C=O); ^{1}H NMR ($(CD_3)_2CO$): δ 0.8 (m, 3H, CH_3), 1.25 (m, 12H, $(CH_2)_6$), 3.7 (t, 2H, CH_2O), 4.1 (m, 1H, α-CH), 5.1 (d, 1H, β-CH), 7.1–7.7 (m, 10H, aromatic protons).

2.1.3.3. *N-p-Toluenesulfonyl-threo-DL-phenylserine 1-octyl ester (9).*
Yield 74%, m.p. 82–84 °C, IR ν, cm^{-1}: 1162 (S=O), 1695 and 1720 (C=O); ^{1}H NMR ($(CD_3)_2CO$): δ 0.9 (m, 3H, CH_3), 1.3 (m, 12H, $(CH_2)_6$), 2.4 (s, 3H, CH_3), 3.8 (t, 2H, CH_2O), 4.1 (m, 1H, α-CH), 5.0 (m, 1H, β-CH), 6.4 (s, 1H, NH), 7.1–7.5 (m, 10H, aromatic protons).

2.1.3.4. *N-p-Toluenesulfonyl-threo-DL-phenylserine 1-tetradecyl ester (10).*
Yield 79%, m.p. 63–65 °C, IR ν, cm^{-1}: 1158 (S=O), 1706 (C=O); ^{1}H NMR ($(CD_3)_2CO$): δ 0.9 (m, 3H, CH_3), 1.85 (m, 24H, $(CH_2)_{12}$), 2.4 (s, 3H, CH_3), 3.8 (t, 2H, CH_2O), 4.1 (d, 1H, α-CH), 5.0 (d, 1H, β-CH), 6.4 (s, 1H, NH), 7.1–7.5 (m, 10H, aromatic protons).

2.1.3.5. *N-p-Toluenesulfonyl-erythro-DL-p-nitrophenylserine ethyl ester (11).*
Yield 72%, m.p. 159–160 °C, IR ν, cm^{-1}: 1163 and 1315 (S=O), 1350 and 1515 (NO_2), 1742 (C=O); ^{1}H NMR ($(CD_3)_2SO$): δ 1.1 (t, 3H, $J = 7$ Hz CH_3), 2.3 (s, 3H, CH_3), 3.8 (m, 2H, CH_2), 3.95 (m, 1H, α-CH), 4.8 (d, 1H, β-CH), 7.4–8.0 (m, 10H, aromatic protons).

2.1.3.6. *N-p-Bromobenzenesulfonyl-erythro-DL-p-nitrophenylserine ethyl ester (12).*
Yield 64%, m.p. 165–166 °C, IR ν, cm^{-1}: 1170 (S=O), 1350 and 1520 (NO_2), 1742 (C=O); ^{1}H NMR ($(CD_3)_2SO$): δ 1.1 (t, 3 H, $J = 7$ Hz, CH_3), 3.8 (q, 2H, $J = 7$ Hz, CH_3), 3.9 (m, 1H, α-CH), 4.8 (d, 1H, β-CH), 7.4–8.1 (m, 10H, aromatic protons).

2.1.3.7. *N-Benzenesulfonyl-threo-DL-phenylserine hydrazide (7).*
A mixture of 5.20 g (15 mmol) of *N*-benzenesulfonyl-*threo*-DL-phenylserine ethyl ester (**4**) and 1.60 g (32 mmol) of hydrazine monohydrate was

refluxed in 15 ml of absolute ethanol for 2 h. The reaction mixture was cooled, formed crystals were filtered off, washed with water and recrystallized from ethanol to yield 3.57 g (71%) of white crystals of hydrazide **7**, m.p. 178–180 °C; IR ν, cm^{-1}: 1168 and 1337 (S=O), 1657 and 1680 (C=O); ^1H NMR ($(CD_3)_2SO$): δ 3.7 (br s, 1H, α-CH), 4.7 (m, 1H, β-CH), 5.4 (d, 1H, $J = 5$ Hz, OH), 7.0–7.5 (m, 10H, aromatic protons), 8.8 (m, 3H, $NHNH_2$).

2.2. *Biological evaluation*

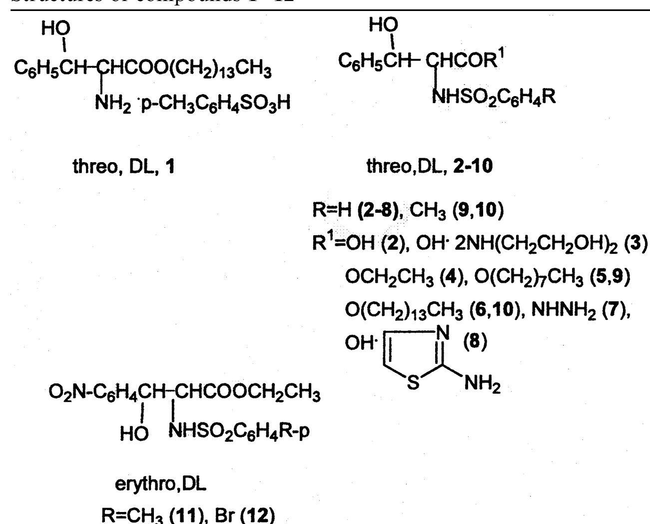
2.2.1. *Animals*

All young animals were clinically healthy. The mice 420 Balb/c for toxicity testing were obtained from the Grindex Latvian State Pharmaceutical Company, Riga. The anti-inflammatory testing was performed on male Wistar rats weighting 160–200 g. The rats 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 1 week animals were subjected to the experimental protocol. All experimental groups consisted of 10 animals.

2.2.2. *Toxicity testing*

The survival of mice (4–6 weeks old) administered intraperitonically the graduated dose levels of each compound was observed for 14 days [15]. The LD_{50} value was determined by an accepted method of Litchfield and Wilcoxon [16].

Table 1
Structures of compounds **1**–**12**



2.2.3. *Induction and evaluation of adjuvant arthritis (AA) and other parameters*

AA was induced 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, namely, the swelling of the hind paws determined plethysmographically and the development of polyarthritis. The paw volume and body weights were measured three times per week and the percentage of deviation was determined. At the end of the experiment the animals were killed by decapitation preceded by narcosis. The weight of internal organs was determined. The blood indices were determined on a Picoscale hematological analyzer.

2.3. *Preparations and treatment schedule*

Compounds **1**–**12** for testing were prepared ex tempore as a fine homogenized suspension in 1% starch paste and were used peroral by aid of the probe in the stomach in 1 ml volume. The therapeutical doses of compounds are indicated in Table 2. The control group received only 1% starch paste in the same amount. Treatment was started since the AA inducing day and continued to day 16. The experiment lasted for 17 days. The percentage of deviation from the control group was derived by the following formula: $(T-C)/C \times 100$, where T is the data on the tested group, and C is the data on the control.

3. Results and discussions

3.1. *Synthesis*

The synthesis of *threo*-DL-phenylserine 1-tetradecyl ester (**1**) and *N*-benzenesulfonyl *threo*-DL-phenylserine 1-tetradecyl ester (**6**) has been described earlier in Refs. [11] and [12], respectively. *N*-Benzenesulfonyl-*threo*-DL-phenylserine (**2**) was obtained by a reaction of *threo*-DL-phenylserine with benzenesulfonyl chloride in aqueous alkaline solution. Diethanolamine and 2-amino-2-thiazoline salts of *N*-benzenesulfonyl-*threo*-DL-phenylserine **3** and **8** were obtained by reaction of the former with **2** in ethanol. Arylsulfonyl derivatives of phenylserine esters **4**, **5**, and **9**–**12** were obtained by condensation of benzene-, *p*-toluene- and *p*-bromobenzene- sulfonylchlorides with *threo*-DL-phenylserine ethyl ester hydrochloride [14], toluenesulfonates of *threo*-DL-phenylserine 1-octyl [10] and 1-tetradecyl [11] esters and *erythro* DL-*p*-nitrophenylserine ethyl ester hydrochloride [14], respectively, in presence of triethylamine in ethanol. *N*-Benzenesulfonyl-*threo*-DL-phenylserine hydrazide (**7**) was obtained in a reaction of hydrazine monohydrate with **4** in ethanol. IR, ^1H NMR spectroscopy and elemental analysis proved the structures of

Table 2
Biological properties of compounds **1**–**12** in rats with AA

Comp.	LD_{50} for mice (mg/kg, i.p.)	TD (mg/kg p.o.)	Divergence from control (%), (P)															
			Joint swelling (on day of experiment)							Weight of					Blood			
			3	5	8	10	12	15	17	Body	Liver	Kidney	Spleen	Thymus	ESR	Erythrocytes	Leukocytes	Haemoglobin
1	1540	100	−5	−14	−15	−36 [a]	−38 [a]	−12	−11	6	39 [a]	6	29	12	36 [b]	−13	87 [a]	−90 [c]
2	1200	120	−29 [b]	−38 [a]	−35 [b]	−30 [d]	−35 [d]	−22	−11	−8	−2	−3	−10	−11	−25	−16	−36 [a]	10
3	> 2000	200	1	3	9	12	3	10	−2	−1	−1	17	1	−4	26	−18	−43	5
4	1500	150	−24 [b]	−23 [c]	−16	7	−2	−39	−25	−13	−12	−11	−35 [c]	−32 [c]	−32 [c]	4	−17	20
5	1400	140	−3	−22	−36 [a]	−40 [b]	−20	−8	−19	9	−9	−2	−16	14	−43 [c]			
6	1000	100	1	−13	−14	−16	−26	−28 [c]	−32 [c]	−6	3	2	8	−6	−19	−15	83 [a]	13
7	1500	150	−31	−38 [a]	−18	−2	4	−3	−2	−11	−6	−12	−15	−26 [d]	−43 [b]	−6	−18	11
8	1000	100	0	−11	−34	−22	−5	9	−10	−3	−28	−4	−18	−2	28			
9	1400	140	−10	−26 [b]	−40 [a]	−36 [b]	−15	−2	−21	3	−12	−3	−16	26	−1			
10	> 1000	100	−11	−9	−13	1	11	7	−5	11	8	5	8	10	−27	−4	0	8
11	> 6000	600	−7	0	−2	−4	1	−14	−25	−3	−19	−11	−28	−46				
12	> 6000	600	−6	–	−2	−22		−45 [b]	−53 [a]	−24	−30 [d]	−22 [b]	−49 [d]	−20				
ASA	1400	120	−31 [a]	−46 [a]	−22 [d]	−24	−31 [d]	−14	−23	−11	2	−10	−5	−26 [c]	−8	4	−31 [a]	13

Abbreviations: LD_{50}, half lethal dose; TD, therapeutical dose; i.p., intraperitoneal; p.o., per oral; "–" untested; ESR, erythrocyte sedimentation rate; ASA, acetylsalicyclic acid; P, reliability coefficient calculated by the variation statistical method [16], in case P is not indicated, consider $P > 0.05$, i.e. data are unreliable.

[a] $P < 0.001$.

[b] $P < 0.01$.

[c] $P < 0.02$.

[d] $P < 0.05$.

the synthesized compounds presented in Table 1. It is interesting to note that chemical shifts in ^{1}H NMR spectra are dependent on the solvent used, and splitting of exchangeable protons is observed in aprotic solvents.

3.2. Biological testing

The results obtained in testing compounds **1**–**12** in rats with AA are presented in Table 2. The acute toxicity of examined compounds for mice were moderate ($LD_{50} = 1000 \div 2000$ mg kg^{-1}) for compounds **1**–**10** or slightly toxic ($LD_{50} > 6000$ mg kg^{-1}) for compounds **11** and **12**. The nature of moieties of aromatic sulfonic acids introduced into structure of the investigated amino acids and modification of the carboxyl group had strong influence on the remission of inflammatory process. Thus, *p*-toluenesulfonate of tetradecyl ester of *threo*-DL-phenylserine **1** inhibited the development of inflammatory process after exudation state of AA, i.e. in the middle of experiment. Introducing of benzenesulfonyl group into the structure **1** afforded compound **6**, which increased remission of inflammation process in polyarthritis phase, while the *p*-toluenesulfonyl analogue **10** did not exhibit anti-inflammatory properties. The latter contains a long ester function chain that presumably prevents binding to COX sites. *N*-Benzenesulfonyl-*threo*-DL-phenylserine **2** possessed significant anti-inflammatory activity in exudation phase as well as after this stage, though the diethanolamine salt of this sulfonyl derivative **3** was inactive. *N*-Benzenesulfonyl-*threo*-DL-phenylserine ethyl ester **4** and its hydrazide **7** inhibited inflammatory process in initial phase of experiment and this inhibition effect was more expressed for hydrazide derivative **7**. *N*-Benzenesulfonyl- and *N*-toluenesulfonyl-*threo*-DL-phenylserine 1-octyl esters **5** and **9** similarly increased remission of inflammation process after exudation phase. This inflammation phase is less expressed effected by the 2-amino-2-thiazoline salt of *N*-benzenesulfonyl derivative **8**. By examining two sulfonyl derivatives of *erythro*-DL-p-nitrophenylserine **11** and **12**, pronounced anti-inflammatory effect possess only *N*-(*p*-bromobenzenesulfonyl)-*erythro* ethyl ester **12**, while its *p*-toluenesulfonyl analogue **11** was inactive. The *p*-bromobenzenesulfonyl derivative **12** inhibited inflammation process in polyarthritis phase by 53% ($P < 0.001$). This compound in experimental studies decreased the weight of liver, kidney and spleen. This can be related with immunosuppression activity of compound **12**.

Summing up, from a series of the twelve *threo*- and *erythro*-phenylserine derivatives evaluated in animal screening, nine compounds demonstrated anti-inflammatory effect. Although results obtained do not permit a definite conclusion to be drawn regarding quantitative structure–activity relationship for the studied compounds, it follows that the sulfonamides with a free carboxyl group or short ester function possessed the most pronounced activity. This could be accounted for by the deeper insertion of the SO_2 group into the COX-2 primary binding site. Further structural modifications will provide more detailed SAR of this class of compounds.

The best result in rats experimental AA model was achieved for compounds **2** and **12**. *N*-Benzenesulfonyl-*threo*-DL-phenylserine **2** by all parameters of biological activity is similar to acetylsalicylic acid. *N*-(*p*-Bromobenzenesulfonyl) derivative **12** is the most potent anti-inflammatory agent from the set of tested compounds. This compound is slightly toxic ($LD_{50} > 6000$ mg kg^{-1}) and substantially inhibited development of polyarthritis, while ASA did not affect the inflammation process in polyarthritis phase. These findings may serve as a basis for further investigation of this class compounds in the treatment of inflammation.

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