SYNTHESIS AND ANTIINFLAMMATORY AND ANALGESIC ACTIVITY OF NAPROXEN AMIDES WITH AMINO ACID DERIVATIVES

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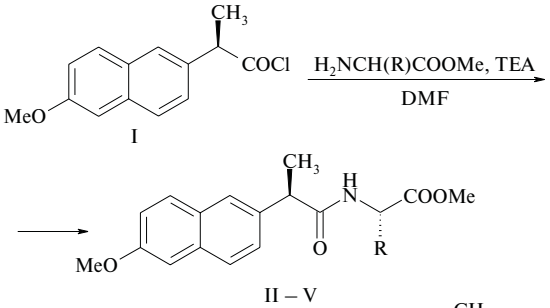
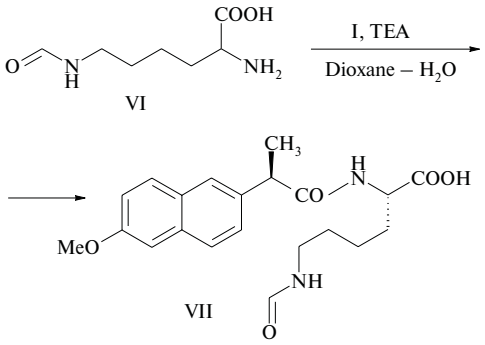
In recent years, there have been attempts to modify the well-known nonsteroidal antiinflammatory drugs by reactions with natural amino acids, aimed at eliminating undesired side effects of the drug action [1]. In particular, the synthesis of naproxen amide with glycine was reported in [2, 3].

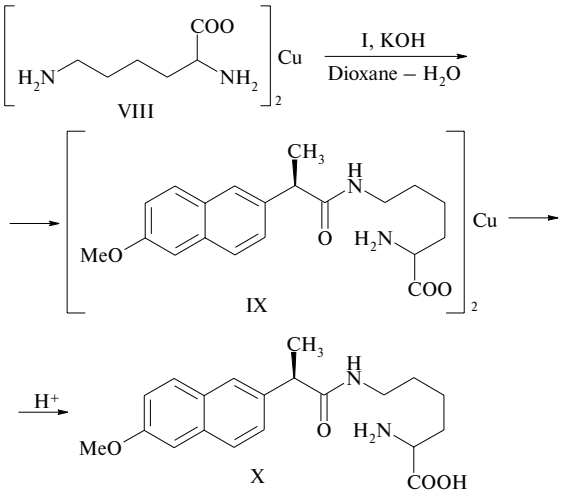
The aim of this study was to obtain naproxen amides with some amino acid derivatives and characterize the products with respect to the antiinflammatory and analgesic activity and acute toxicity. The synthesis of naproxen amides (II – V) with methyl esters of (*S*)-methionine, (*S*)-phenylalanine, (*S*)-histidine, and (*S*)-leucine was based on the condensation of (*S*)-naproxen chloroanhydride (I) with the corresponding amino acid esters in DMF in the presence of triethanolamine (TEA) [4]:



The condensation of naproxen chloroanhydride with N^{ε}-formyl-*L*-lysine (VI) was performed in an aqueous dioxane solution in the presence of TEA:



The condensation of naproxen chloroanhydride I with a copper complex of *L*-lysine (VIII) in an aqueous dioxane solution in the presence of KOH, followed by decomposition of the intermediate copper complex IX in a dilute hydrochloric acid solution, led to (*S*)-naproxen amide with *L*-lysine (X):



The possibility of selective hydrolysis of an ester group with the formation of amide (XI) with a free carboxy group was demonstrated by the alkaline hydrolysis of amide V in an aqueous acetone solution at 0°C:

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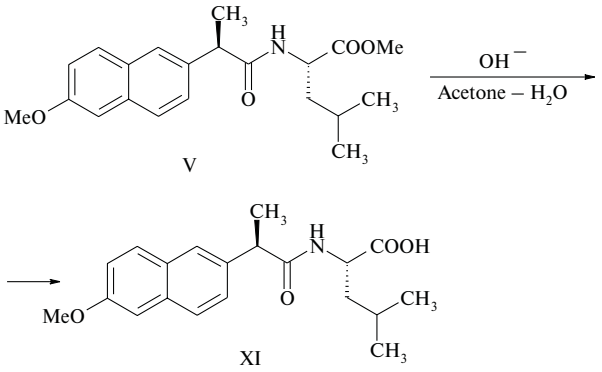

The proposed structures of amides II – V, VII, X, and XI were confirmed by the results of elemental analyses and by ^{1}H NMR data. It should be noted that, according to the ^{1}H NMR data, the synthesized compounds represent individual (*S*,*S*)-diastereomers. Amides II – V, VII, X, and XI appear as colorless crystalline substances. Compounds II – V, VII, and XI are insoluble in water and well soluble in organic solvents. Compound X is soluble only in diluted acids (hydrochloric, trifluoroacetic).

The UV spectra of compounds II – V, VII, X, and XI display five absorption maxima related to the naphthalene nucleus, including one intense peak at λ = 233 – 235 nm and somewhat less intense, at λ = 264, 274, 320, and 333 – 335 nm.

Some physicochemical characteristics of the synthesized amides are presented in Table 1. The data of elemental analyses agree with the results of analytical calculations using empirical formulas.

EXPERIMENTAL CHEMICAL PART

The purity of the products was checked by TLC on Sorbfil eluted in chloroform – methanol (9 : 1) and developed by exposure to UV light. The melting points were determined on a Boetius heating table. The UV absorption spectra were recorded with a Specord UV- VIS spectrophotometer (Germany) using ethanol solutions. The ^{1}H NMR spectra were measured on a Bruker DRX400 (Germany) with a working frequency of 400 MHz using TMS as the internal standard. The values of specific rotation of the polarization plane were determined on an A1-EPO polarimeter (Russia). The initial naproxen chloroanhydride (I), or (*S*)-2-(6-methoxy-2-naphthyl)propionyl chloride, was synthesized as described in [4].

General method for the synthesis of amides II – V. To a solution of 5.4 mmole of an amino acid methyl ester hydrochloride in 10 ml DMF was added 0.75 ml (5.4 mmole) TEA. To this suspension, cooled to 0°C on an ice-cold bath, was added dropwise (with stirring and cooling) a solution of 1.47 g (5.4 mmole) of naproxen chloroanhydride I in 5 ml DMF and (simultaneously, in two portions) 0.75 ml (5.4 mmole) of TEA. Still on the ice-cold bath, the reaction mass was stirred for 2 h and then allowed to stand at room temperature overnight. Then the mass was poured into 150 ml of ice-cold water. The precipitated oil was extracted with ethyl acetate. The extract was sequentially washed with a 5% $NaHCO_3$ solution, water, 1 N HCl, and water, dried over Na_2SO_4, and evaporated to dryness. Finally, the residue was recrystallized from a hexane – ethyl acetate mixture.

N-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-methionine methyl ester (II). Yield, 1.52 g (75%); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.78 – 7.10 (m, 6H, H_{arom}), 6.11 (d, 1H, NH), 4.67 (m, 1H, C_αH methionine), 3.91 (s, 3H, OCH_3), 3.75 (q, 1H, CH naproxen), 3.65 (s, 3H, $COOCH_3$), 2.38 (m, 2H, $C\underline{H}_2CH$), 1.99 (m, 2H, S-CH_2), 1.97 (s, 3H, S-CH_3), 1.61 (d, 3H, CH–$C\underline{H}_3$).

N-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-phenylalanine methyl ester (III). Yield, 1.26 g (60%); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.70 – 6.83 (m, 11H, H_{arom}), 5.78 (d, 1H, NH), 4.80 (2t, 1H, C_αH phenylalanine), 3.93 (s, 3H, OCH_3), 3.69 (q, 1H, CH naproxen), 3.65 (s, 3H, $COOCH_3$), 3.05, 2.97 (2dd, 2H, $C\underline{H}_2$–C_6H_4 phenylalanine), 1.58 (d, 3H, CH–$C\underline{H}_3$).

N-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-histidine methyl ester (IV). Yield, 1.23 g (60%); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 8.12 (d, 1H, NH), 7.71 – 7.05 (m, 6H, H_{arom}), 7.40 (d, 1H, C^2H imidazole), 6.69 (s, 1H, C^4H imidazole), 4.51 (m, 1H, C_αH histidine), 3.87 (s, 3H, OCH_3), 3.76 (q, 1H, CH naproxen), 3.52 (s, 3H, $COOCH_3$), 2.90 (m, 2H, $C\underline{H}_2$ histidine), 1.39 (d, 3H, CH–$C\underline{H}_3$).

N-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-leucine methyl ester (V). Yield, 1.54 g (80%); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.74 – 7.12 (m, 6H, H_{arom}), 5.73 (d, 1H, NH), 4.60 (2t, 1H, C_αH leucine), 3.92 (s, 3H, OCH_3), 3.73 (q, 1H, CH naproxen), 3.63 (3H, $COOCH_3$), 1.65 (m, 2H, $C\underline{H}_2CH$), 1.60 (d, 3H, CH–CH_3 naproxen), 1.40 (m, 1H, $CH_2C\underline{H}$), 0.89, 0.87 (2d, 6H, $(CH_3)_2$).

N^α-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-N^ε-formyl-(2*S*)-lysine (VII). To a solution of 1.0 g (5.74 mmole) of H^ε-formyl-*L*-lysine in 32 ml of a dioxane – water (5 : 3) mixture was added 1.6 ml (11.48 mmole) TEA. To this suspension, cooled to 0°C on an ice-cold bath, was added dropwise (with stirring and cooling) a solution of 1.42 g (5.34 mmole) of naproxen chloroanhydride I in 20 ml

TABLE 1. Melting Points and Specific Rotation of Compounds II – V, VII, X, and XI

Compound	M.p., °C	$[\alpha]_D^{20}$, deg	Empirical formula
II	106 – 107	+ 15.7 (*c*, 1.84; chloroform)	$C_{20}H_{25}NO_4S$
III	106 – 107	+ 8.6 (*c*, 2.0; acetone)	$C_{24}H_{25}NO_4$
IV	211 – 213	– 8.4 (*c*, 1.0; methanol)	$C_{21}H_{23}N_3O_4$
V	99 – 101	+ 27.5 (*c*, 2.0; chloroform)	$C_{21}H_{27}NO_4$
VII	129 – 131	–9.5 (*c*, 1.2; acetone)	$C_{21}H_{26}N_2O_5$
X	225 – 227	+ 34.2 (*c*, 2.0; 1 N CF_3COOH)	$C_{20}H_{26}N_2O_4$
XI	135 – 137	–15.0 (*c*, 1.0; methanol)	$C_{20}H_{25}NO_4$

dioxane. Still on the ice-cold bath, the reaction mass was stirred for 2 h and then allowed to stand at room temperature overnight. Then the mass was poured into 100 ml of ice-cold water and acidified with 1 N HCl to pH 1. The precipitated oil was extracted with ethyl acetate. The extract was washed with a saturated NaCl solution, dried over Na_2SO_4, and evaporated to dryness. Finally, the residue was recrystallized from a hexane – acetone mixture.

Yield of compound VII, 1.88 g (85%); ^{1}H NMR spectrum in acetone-d_6 (δ, ppm): 8.11 (s, 1H, HCO), 7.78 – 7.04 (m, 6H, H_{arom}), 7.2 (d, 1H, NH), 4.41 (m, 1H, C_αH lysine), 3.90 (s, 3H, OCH_3), 3.9 (q, 1H, CH naproxen), 3.21 (q, 2H, $NHC\underline{H}_2CH_2$), 1.90 – 1.55 (m, 6H, $CH_2C\underline{H}_2C\underline{H}_2CH$), 1.50 (d, 3H, $CH–C\underline{H}_3$ naproxen).

N^ε[-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-lysine (X). To a solution of 1.18 g (6.46 mmole) of *L*-lysine hydrochloride and 0.96 g (14.53 mmole) KOH in 20 ml of water was added a solution of 0.81 g (3.23 mmole) $CuSO_4 \cdot 5H_2O$ in 10 ml water. To the resulting solution of an *L*-lysine copper complex (VIII), cooled to 0°C on an ice-cold bath, was added dropwise (with stirring and cooling) a solution of 2.0 g (8.08 mmole) of naproxen chloroanhydride I in 30 ml dioxane. Still on the ice-cold bath, the reaction mass was stirred for 2 h and then allowed to stand at room temperature overnight. The precipitated copper complex IX was separated by filtration, washed on the filter sequentially with water, 5% $NaHCO_3$ solution, and water (to pH 7.0), and ethanol, and dried in air to obtain 1.98 g (79%) of copper complex IX. The blue-violet residue of IX was stirred in 20 ml of 1 N HCl for 2 h at room temperature. The resulting white precipitate was separated by filtration, washed on the filter with (to pH 7.0) and ethanol, and dried over P_2O_5. The resulting amide X was reprecipitated from 2 N trifluoroacetic acid.

Yield of compound X, 1.39 g (60%); ^{1}H NMR spectrum in DMSO-d_6/CF_3COOD (δ, ppm): 7.54 (s, 3H, NH_3^+), 7.51 – 6.89 (m, 6H, H_{arom}), 7.32 (bs, 1H, NH), 3.74 (s, 1H, C_αH lysine), 3.68 (q, 1H, CH naproxen), 3.63 (s, 3H, OCH_3), 3.05 (bs, 2H, $NHC\underline{H}_2CH_2$), 1.72 (m, 2H, $C\underline{H}_2CH$), 1.35 – 1.15 (m, 4H, CH_2CH_2), 1.35 (d, 3H, $CH–C\underline{H}_3$ naproxen).

TABLE 2. Acute Toxicity, Antiinflammatory, and Analgesic Activity of Naproxen Amides with *L*-Amino Acid Derivatives

Compound	LD_{50}, mg/kg	Antiinflammatory activity (carrageenan edema)*			Analgesic activity (vinegar convulsions)*		
		Dose, mg/kg	Percentage edema growth relative to initial background, %	Edema growth inhibited relative to control,%	Dose, mg/kg	Number of convulsions	Convulsions inhibited relative to control, %
II	> 3000	25	59.76 ± 4.93 *n* = 10 *P* > 0.05	19.6	103	13.50 ± 2.00 *n* = 6 *P* > 0.05	39.1
III	...	25	54.65 ± 2.96 *n* = 10 *P* < 0.05	26.4	106	21.50 ± 3.15 *n* = 6 *P* > 0.5	3.0
IV	> 3000	25	52.12 ± 4.45 *n* = 10 *P* < 0.01	29.8	105	9.83 ± 4.34 *n* = 6 *P* < 0.05	55.7
V	> 3000	23	51.76 ± 13.13 *n* = 6 *P* > 0.05	30.3	98	8.00 ± 3.47 *n* = 6 *P* < 0.02	63.9
VII	...	25	54.68 ± 8.77 *n* = 10 *P* > 0.05	26.4	105	13.83 ± 2.02 *n* = 6 *P* > 0.5	37.6
X	> 3000	23	55.52 ± 3.31 *n* = 10 *P* < 0.02	25.3	98	5.17 ± 2.32 *n* = 6 *P* < 0.002	76.7
XI	...	22	60.17 ± 9.28 *n* = 6 *P* > 0.1	19.0	94	3.67 ± 1.52 *n* = 6 *P* < 0.001	83.5
Naproxen	547 (400 – 750)	15	38.95 ± 1.52 *n* = 10 *P* < 0.001	47.6	63	5.50 ± 2.66 *n* = 6 *P* < 0.01	75.2
Control	–	–	74.28 ± 6.00 *n* = 10	–	–	22.17 ± 3.36 *n* = 6	–

* *n*, number of test animals; *P* is the confidence level of differences relative to control.

TABLE 3. Dynamics of the Antiinflammatory Activity of Compound IV upon Peroral Administration

Compound	Dose, mg/kg	Antiinflammatory activity upon drug introduction,* tested after											
		1 h		2 h		3 h		4 h		8 h		24 h	
		A	B	A	B	A	B	A	B	A	B	A	B
IV	25	27.07 ± 2.67 *n* = 6 *P* > 0.1	23.1	34.05 ± 4.20 *n* = 6 *P* < 0.02	43.7	50.11 ± 5.01 *n* = 6 *P* < 0.01	30.6	70.23 ± 9.28 *n* = 6 *P* > 0.5	7.1	57.09 ± 6.47 *n* = 6 *P* > 0.5	–2.9	24.94 ± 3.51 *n* = 6 *P* < 0.001	–129.2
Naproxen	15	31.65 ± 6.69 *n* = 6 *P* > 0.5	10.1	42.03 ± 6.53 *n* = 6 *P* = 0.1	30.5	58.10 ± 4.90 *n* = 6 *P* > 0.05	19.6	54.20 ± 3.01 *n* = 6 *P* < 0.002	28.3	36.40 ± 2.55 *n* = 6 *P* < 0.01	34.4	8.05 ± 1.27 *n* = 6 *P* > 0.1	26.0
Control	–	35.22 ± 5.24 *n* = 10	–	60.44 ± 8.17 *n* = 10	–	12.24 ± 4.65 *n* = 10	–	75.57 ± 4.44 *n* = 10	–	55.48 ± 4.68 *n* = 10	–	10.88 ± 1.50 *n* = 10	–

* *n* is the number of test animals; *P* is the confidence level of differences relative to control; A, percentage edema growth relative to initial background; B, percentage edema-growth inhibition relative to control.

N-[(2*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(2*S*)-leucine (XI). To a solution of 0.36 g (1 mmole) of ester V in 3 ml acetone, cooled to 0°C on an ice-cold bath, was added dropwise (with stirring and cooling) 2.4 ml (2.4 mmole) of 1 N NaOH solution, and the reaction mixture was kept on the cool bath for 18 h. To this mixture was added 3 ml of diethyl ether, after which the aqueous layer was separated, washed again with diethyl ether, and neutralized with 1 N HCl to pH 6. The precipitate was separated by filtration, washed on the filter with water, and recrystallized from a hexane – acetone (4 : 3) mixture.

Yield of compound X, 0.24 g (70%); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.73 – 7.12 (m, 6H, H_{arom}), 5.76 (d, 1H, NH), 4.54 (m, 1H, C_αH leucine), 3.91 (s, 3H, OCH_3), 3.76 (q, 1H, CH naproxen), 1.60 (d, 3H, CH–C$\underline{H}_3$ naproxen), 1.57 (m, 2H, C$\underline{H}_2$CH), 1.42 (m, 1H, $CH_2C\underline{H}$), 0.87, 0.86 (2d, 6H, $(CH_3)_2$).

EXPERIMENTAL PHARMACOLOGICAL PART

The antiinflammatory activity was studied on a group of both male and female rats weighing 180 – 220 g. The model edema was induced by subplantar 0.1-ml injections of a 1% aqueous carrageenan solution [5]. The synthesized compounds suspended in a 2% starch jelly were intraperitoneally injected 1 h before model edema induction. The drug dose corresponded to ED_{50} for naproxen (15 mg/kg [6]), with a coefficient taking into account the molecular mass of each amide. Animals in the control group received pure 2% starch jelly. The results, evaluated as an increase in the foot volume relative to the initial value (measured before test), were determined oncometrically 4 h after carrageenan injection. From these data, the percentage edema growth relative to the initial volume and a percentage inhibition of the model edema growth relative to that in the control group were calculated.

For the most active compound, the antiinflammatory action with respect to the carrageenan edema development was also studied upon peroral administration, with the antiinflammatory effect determined 1, 2, 3, 4, 8, and 24 h after model edema induction [7].

The analgesic activity was studied on a group of male and female white mongrel mice weighing 16 – 18 g on the model of vinegar convulsions [8]. The synthesized compounds were introduced perorally with a 2% starch jelly; Animals in the control group received pure starch jelly. The model convulsions were induced, 1 h after treatment with the synthesized compounds, by intraperitoneal injections of 0.75% aqueous acetic acid solution in a dose of 0.25 ml per 10 g animal body weight. The test results were evaluated by counting the number of convulsions over a time period of 10 min and by calculating the percentage inhibition of convulsions relative to control.

The acute toxicity for intraperitoneal injections was determined in a group of male and female white mice weighing 16 – 20 g and evaluated as LD_{50} [9].

The experimental data were statistically processed in terms of the Student criterion [10]. The effects were considered as reliable for $p < 0.05$. It was established that, of the total of seven derivatives studied, only two naproxen amides – with *L*-histidine methylate (IV) and phenylalanine methylate (III) – exhibit reliable antiinflammatory activity (Table 2). Investigation of the dynamics of the antiinflammatory action for the most active compound IV upon peroral administration showed a reliable effect 2 and 3 h after introduction (Table 3), while the maximum effect of naproxen was observed 4 and 8 h after treatment.

Four compounds (IV, V, X, and XI) of the total of seven studied exhibited a reliable analgesic effect (Table 2). Most of the synthesized compounds are inferior to naproxen with respect to the acute toxicity (Table 2).

Thus, the most promising results were observed for naproxen amide with methyl ester, which, while possessing less pronounced antiinflammatory and analgesic properties, exhibits a somewhat lower acute toxicity.

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REFERENCES

1. M. F. Otis, L. Levesque, F. Marceau, et al., *Inflammopharmacology*, **1**(3), 201 – 212 (1992).
2. P. Singh, L. L. Hingorani, and G. K. Trivedi, *Indian J. Chem.*, *Sect. B*, **29B**(6), 551 – 555 (1990).
3. V. R. Shambhag, A. M. Crider, R. Gokhale, et al., *J. Pharm. Sci.*, **81**(2), 378 – 382 (1998).
4. G. L. Levit, M. A. Koroleva, and V. P. Krasnov, *Zh. Org. Khim*., **34**(2), 149 – 154 (1992).
5. C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. (N. J.)*, **111**(3), 544 – 547 (1962).
6. R. D. Syubaev, M. D. Mashkovskii, G. Ya. Shvarts, and V. I. Pokryshkin, *Khim.-Farm. Zh*., **19**(1), 33 – 39 (1985).
7. F. P. Trinus, B. M. Klebanov, V. I. Kondratenko, et al. (eds.), *Methodological Recommendations on the Experimental (Preclinical) Investigation of Nonsteroidal Antiinflammatory Pharmaceuticals* [in Russian], Pharmaceutical Committee of the USSR Ministry of Public Health, Moscow (1982).
8. R. Koster, M. Anderson, and E. J. de Beer, *Fed. Proc.*, **18**(7), 412 (1959).
9. V. B. Prozorovskii, *Farmakol. Toksikol*., No. 4, 497 – 502 (1978).
10. M. L. Belen'kii, *Elements of the Quantitative Evaluation of the Pharmacological Effect* [in Russian], Medgiz, Leningrad (1963).