UDC 547.461+615.778

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3,4-DIMETHYLPHENYLOXAMOYL AMINOACIDS AND THEIR SALTS WITH D(+)-GLUCOSAMINE

K.M.Alrahawi, G.P.Petyunin*, L.N.Maloshtan**

Sana'a University (Yemen)
* Kharkov Medical Academy of Post-Graduate Education (Ukraine)
58 Korchagintsev str., Kharkiv, 61176, Ukraine. E-mail: office@med.edu.ua
** National University of Pharmacy (Kharkiv, Ukraine)

Key words: synthesis; oxamoylaminoacid; optical activity; D-glucosamine's salt

New 3,4-dimethylphenyloxamoylaminoacids and their salts with D-(+)- glucosamine were synthesized. Convenient method of synthesis of optically active oxamoylaminoacids were discovered. The biological screening showed that some compounds have significant hepatoprotective, antiinflammatory and immunodepressant activities with low toxicity.

СИНТЕЗ ТА БІОЛОГІЧНА АКТИВНІСТЬ 3,4-ДИМЕТИЛ-ФЕНІЛОКСАМОЇЛАМІНОКИСЛОТ ТА ЇХ СОЛЕЙ З D(+)-ГЛЮКОЗАМІНОМ
Х.М.Альрахаві, Г.П.Петюнін, Л.М.Малоштан
Синтезовані 3,4-диметилоксамоїламінокислоти та їх солі з D-(+)глюкозаміном. Знайдений зручний спосіб синтезу оптично активних оксамоїламінокислот. Біологічний скринінг показав наявність у них гепатопротекторної, протизапальної та імунодепресивної активності при низькій токсичності.

СИНТЕЗ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ 3,4-ДИМЕТИЛ-ФЕНИЛОКСАМОИЛАМИНОКИСЛОТ И ИХ СОЛЕЙ С D-(+)-ГЛЮКОЗАМИНОМ
Х.М.Альрахави, Г.П.Петюнин, Л.Н.Малоштан
Синтезированы 3,4-диметилоксамоиламинокислоты и их соли с D(+)глюкозамином. Найден удобный метод синтеза оптически активних оксамоиламинокислот. Биологический скрининг показал наличие у них гепатопротекторной, противовоспалительной и иммунодепрессантной активности при низкой токсичности.

It is known that acyl derivatives of amino acids exhibit various pharmacological activities [1-6].

Taking into account these properties, it was interesting from the biological stand point to use oxamic acid derivatives and amino acids- which are considered biologically active compounds- as initial substances for obtaining new biologically active substances containing them in one molecule.

In continuation of the previous work [7-9] on the search for biologically active substances among the derivatives of oxamoylaminoacids, we synthesized 3,4-dimethylphenyl-oxamoylaminoacids and their salts with D-(+)-glucosamine base and studied their physico-chemical and biological properties. 3,4-dimethylphenyloxamoylaminoacids (1-11, table 1) was obtained by three methods using ethyl ester of 3,4-dimethylphenyloxamic acid and corresponding amino acids (scheme 1).

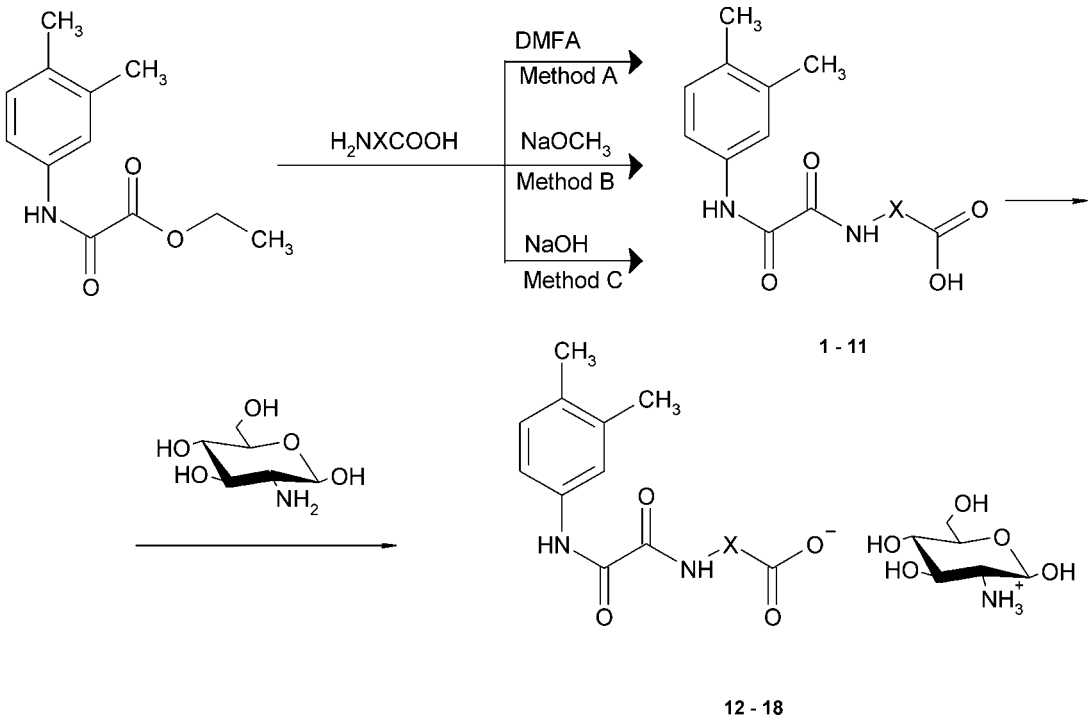
It was established that method A was not suitable for the synthesis of 3,4-dimethylphenyl-oxamoylserine and 3,4-dimethylphenyloxamoyltyrosine because of high boiling temperature of dimethylformamide and thermolability of these amino acids. Also this method was not suitable for synthesis of oxamoyl-β-alanine due to low yield and for oxamoylvaline and oxamoyl-γ-butyric acid as well.

Method B was conducted on cooling and can be used for synthesis of oxamoyl derivatives of thermolabile amino acids (serine) and was suitable for synthesis of oxamoyl-β-alanine, oxamoyl-γ-butyric acid and oxamoylvaline. However, this method has a limitation, which is unsuitability for synthesis of oxamoylleucine and oxamoylisoleucine because of insolubility of these amino acids in methanolic solution of sodium methoxide.

Method C also was conducted on cooling in the presence of aqueous sodium hydroxide. Using this method the oxamoyl of glycine, α-alanine, serine and valine were obtained with low yield.

As is known, the stereochemistry — optical activity-exerts pronounced effects on biological activities. As far as most of the amino acids contain in their structure chiral center, it was interesting to obtain their oxamoyl derivatives, but it should be noted that optically active α-amino acids in chemical reactions may be accompanied by racemization [10].

Optically active 3,4-dimethylphenyloxamoylaminoacids were obtained using dextrorotatory optically

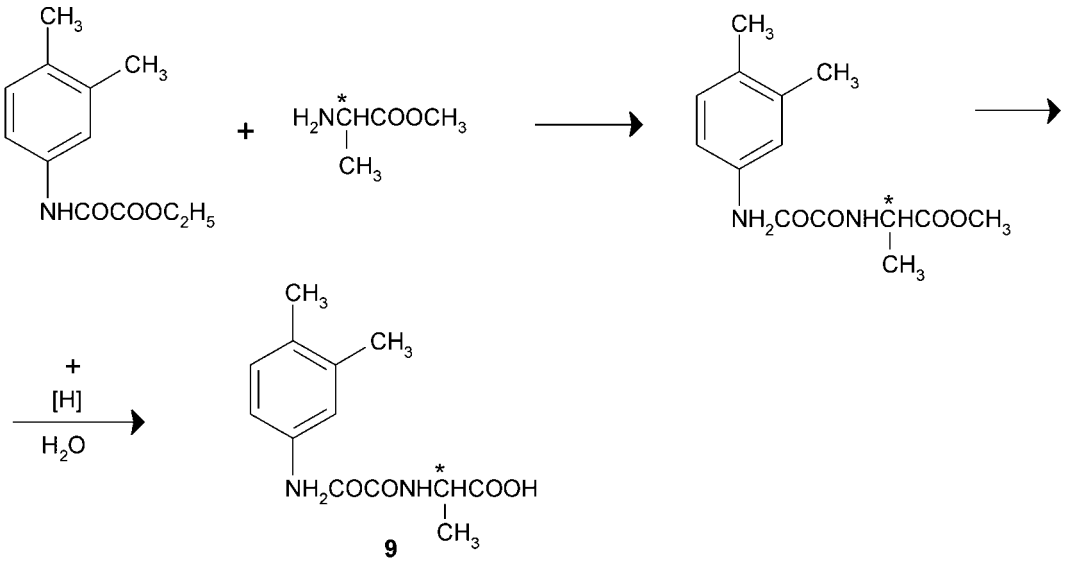


Scheme 1

active amino acids: L(+)-alanine, L(+)-serine, L(+)-valine. Synthesis of 3,4-dimethylphenyloxamoylalanine was carried out according to methods previously described A,B and C. It was shown that method A and C resulted in racemate products. This is caused by the high temperature in method A, and by the alkaline medium in method C [11]. When the reaction was conducted in the presence of absolute methanol (method B) the optically active compound 3,4-dimethylphenyloxamoylalanine 9 was obtained. In addition this compound was obtained by counter synthesis, interaction of ethyl ester of 3,4-dimethylphenyloxanylic acid with methyl ester of L(+)-alanine, then the obtained methyl ester of 3,4-dimethylphenyloxamoylalanine was hydrolyzed to 3,4-dimethylphenyloxamoylalanine. This chemical conversion is described in the following scheme 2.

In accordance with this result, optically active dimethylphenyloxamoylserine 10 and 3,4-dimethylphenyloxamoylvaline 11 were obtained by method B. The physico-chemical properties of the optically active oxamoylaminoacids 9-11 are similar to optically inactive analogues 2, 5, 6, but they are optically active and their specific rotation values are presented in table 1.

The obtained 3,4-dimethylphenyloxamoylaminoacids (1-11, table 1) have the form of white crystalline substances well soluble in polar organic solvent and alkaline solution, while insoluble in water (except compound 5). The purity and individuality of the synthesized compounds were checked by the method



Scheme 2

Table 1

Physicochemical Characteristic of Synthesized Compounds 1-11

Compd.	X	Method, Yield, %			M.p., °C	Found, % N	Empirical formula	Calculated, % N	Rf		pKa	$[\alpha]_D^{20}$ (2% DMFA)
		A	B	C					I	II		
1	CH_2	86	-	50	204-206	11,10	$C_{12}H_{14}N_2O_4$	11,20	0,66	0,76	5,65	-
2	$CHCH_3$	99	-	60	200-202	10,58	$C_{13}H_{15}N_2O_4$	10,60	0,71	0,82	5,14	-
3	CH_2CH_2	-	99	-	207-209	10,70	$C_{13}H_{15}N_2O_4$	10,60	0,72	0,81	4,75	-
4	$CH_2CH_2CH_2$	-	78	-	158-160	10,00	$C_{14}H_{18}N_2O_4$	10,07	0,66	0,78	5,73	-
5	CH_2CH_2OH	-	-	50	200-202	9,98	$C_{13}H_{16}N_2O_5$	10,00	0,67	0,76	4,55	-
6	$CHCH(CH_3)_2$	-	-	40	185-186	9,44	$C_{15}H_{20}N_2O_4$	9,58	0,71	0,81	4,77	-
7	$CHCH_2CH(CH_3)_2$	82	-	-	181-183	9,20	$C_{16}H_{22}N_2O_4$	9,15	0,77	0,93	5,16	-
8	$CHCH(CH_3)CH_2CH_3$	96	-	-	172-174	9,25	$C_{16}H_{22}N_2O_4$	9,15	0,80	0,93	5,12	-
9	$CHCH_3$	-	98	-	200-202	10,79	$C_{13}H_{15}N_2O_4$	10,60	0,71	0,82	5,14	-8,5
10	CH_2CH_2OH	-	94	-	200-202	9,98	$C_{13}H_{16}N_2O_5$	10,00	0,67	0,76	4,55	-10,0
11	$CHCH(CH_3)_2$	-	50	-	185-186	9,44	$C_{15}H_{20}N_2O_4$	9,58	0,71	0,81	4,77	-17,5

of thin layer chromatography eluted with two systems of solvents (table 1).

Since the obtained substances are acids the ionization constants (pKa) were determined (table 1) and the obtained data confirms the presence of one carboxylic group in the structures of the compounds 1-11.

The proposed structures of the synthesized compounds were confirmed by the results of UV, IR and ^{1}H NMR spectroscopies (table 2).

The obtained acids 1-8 were used for the synthesis of biological active salts with D(+)-glucosamine 12-18. The obtained salts (12-18, table 3) appeared as white crystalline substances, readily soluble in water with the formation of neutral solution, which was sparingly soluble in polar organic solvents. Adding mineral acid into the solutions of the obtained salts leads to formation of the precipitate of initial compounds 3,4-dimethylphenyloxamoylaminoacids.

Table 2

Parameters of UV, IR and ^{1}H NMR Spectra of Synthesized Compounds 1-8

Compound	UV-Spectra λ_{nm} (lgε)	IR-Spectrum (KBr), ν, см$^{-1}$	^{1}H NMR Spectrum, δ, ppm
1	205 (4,39); 276 (4,39)	3338,3277 γNH; 3044-2476γOH (COOH); 2920,2853γC-H 1725 γCO(COOH); 1661 γCO; 1520CONH; 1595 γC=C	2,00-2,10s(6H, CH_{3arom}); 3,65-3,88d (2H, CH_2); 6,92-7,14d (1H$_{arom}$); 7,36-7,59t (2H$_{arom}$); 8,89-9,10t (1H, NHCH$_2$); 10,25-10,49s (1H, NHCO)
2, 9	202 (3,24); 279(2,99)	3318,3284 γNH; 3028-2540γOH (COOH); 2927,2851γC-H; 1710 γCO (COOH); 1659 γCO; 1524 CONH; 1596 γC=C	1,25-1,45d (3H, CH_3); 2,04-2,25s (6H, CH_{3arom}); 4,20-4,40m (1H, CH); 6,95-7,15d (1H$_{arom}$); 7,42-7,63t (2H$_{arom}$); 8,90-9,10d (1H, NHCH); 10,33-10,54s (1H, NHCO)
3	203 (3,29); 278 (3,09)	3310,3280 γNH; 3050-2530γOH (COOH); 2925,2852γC-H; 1715 γCO (COOH); 1660 γCO; 1522CONH; 1596 γC=C	1,24-1,49t (2H, CH$_2$COOH); 2,1-2,2s (6H, CH_{3arom}); 4.15-4.40 m (2H, NHCH$_2$CH$_2$); 6,95-7,15d (1H$_{arom}$); 7,40-7,60t (2H$_{arom}$); 8,90-9,10t (1H, NHCH); 10,32-10,55s (1H, NHCO)
4	203 (4,217) 278 (2,98)	3340,3285 γNH; 3030-2520γOH (COOH); 2928,2853γC-H; 1700 γCO (COOH); 1662 γCO; 1525CONH; 1596 γC=C	1,60-1,80m (2H, βCH$_2$); 2,09-2,29m (6H, CH_{3arom}, 2HαCH$_2$); 3,05-3,25q (2H, γCH$_2$); 6,94-7,15d (1H$_{arom}$); 7,42-7,63t (2H$_{arom}$); 8,83-9,03t (1H, NHCH$_2$); 10,24-10,34s (1H, NHCO)
5, 10	201 (3,22); 278 (2,95)	3450γOHacc. 3335,3280γNH; 3100-2625γOH (COOH); 2930,2854γC-H; 1725γCO (COOH); 1645γCO; 1532CONH; 1596 γC=C	2,05-2,20s (6H, 3CH_{3arom}); 3,68-3,87m (2H, CH$_2$OH); 4,20-4,39m (1H, CH); 6,98-7,14d (1H$_{arom}$); 7,43-7,61t (2H$_{arom}$); 8,49-8,65d (1H, NHCH); 10,43-10,60s (1H, NHCO)
6, 11	201 (3,19); 278 (2,93)	3335,3285 γNH; 3125-2600γOH; (COOH); 2923,2850 γC-H; 1723γCO (COOH); 1655 γCO; 1523CONH; 1595 γC=C	0,82-0,92d (6H, CH_3); 2,04-2,25s (6H, CH_{3arom}); 2,16-2,35m [CH(CH_3)$_2$]; 4,19-4,40m (1H, NHCH); 6,95-7,15d (1H$_{arom}$); 7,42-7,60t (2H$_{arom}$); 8,90-9,10d (1H, NHCH); 10,33-10,55s (1H, NHCO)
7	203 (4,24) 278 (3,00)	3325,3280 γNH; 3130-2660γOH; (COOH); 2940,2850 γC-H; 1728γCO (COOH); 1670 γCO; 1510CONH; 1595 γC=C	0,62-0,84t (6H, CH_3 aliph); 1.40-1.62t (2H, CH_2); 1,70-1,93m (1H, CH(CH_3)$_2$; 2,00-2,23s (6H, CH_{3arom}); 4,20-4,42q (1H, NHCH) 6,95-7,08d (1H$_{arom}$); 7,39-7,60t (2H$_{arom}$) 8,82-9,05d (1H, NHCH); 10,35-10,59s (1H, NHCO)
8	202 (4,21) 278 (2.96)	3325,3280 γNH; 3130-2660γOH; (COOH); 2940,2850 γC-H; 1728γCO (COOH); 1670 γCO; 1510CONH; 1595 γC=C	0,70-0,94m (6H, CH_3aliph.); 1,14-1,38m (2H, CH_2); 1,80-2,00m (1H, CHCH$_3$); 2,05-2,28s (6H, CH_{3arom}); 4,14-4,35t (1H, NHCH); 6,93-7,15d (1H$_{arom}$); 7,40-7,15t (2H$_{arom}$); 8,24-8,47dd (1H, NHCH); 10,40-10,63d (1H, NHCO)

Table 3

Physical Constants of Synthesized Compound 12-18

Compound	X	Yeild, %	M.p (decomp.), °C	Found N, %	Empirical formula	Calculd. N, %
12	CH_2	82	160	9.70	$C_{18}H_{27}N_3O_9$	9,79
13	$CHCH_3$	79	125	9.33	$C_{19}H_{29}N_3O_9$	9,48
14	CH_2CH_2	84	160	9.50	$C_{19}H_{29}N_3O_9$	9,48
15	$CH_2CH_2CH_2$	88	158	9.22	$C_{20}H_{31}N_3O_9$	9,19
16	CH_2CH_2OH	90	185	8,99	$C_{19}H_{29}N_3O_{10}$	9,15
17	$CHCH(CH_3)_2$	85	146	8,85	$C_{21}H_{33}N_3O_9$	8,91
18	$CHCH(CH_3)CH_2CH_3$	83	156	8,58	$C_{22}H_{35}N_3O_9$	8,65

The UV spectra of the salts 12-18 were determined by anions and were found to be identical to the spectra of acids. Their IR spectra contained broad absorption band due to stretching vibration of associated NH and OH groups in the region of 3430-3251 cm^{-1}, stretching absorption bands of CO of COOH group in the region of 1710-1680 cm^{-1}, CO amid I and amide II in the region of 1640-1620, 1530-1510 cm^{-1} respectively and groups of stretching absorption bands in the region of 3000 cm^{-1} belonging to ammonium groups. The hydrocarbon ring of D-(+)-glucosamine exists in pyranose form and shows two bands in IR spectrum asymmetric at 920 cm^{-1} and symmetric at 770 cm^{-1} due to pyranose vibration.

Experimental part

The UV-spectra were measured on a UV-160 IPC (Shimadzu) spectrophotometer using samples (10^{-4} mol) dissolved in methanol or ethanol. The IR spectra were obtained on a IR-FTIR-8300 (Shimadzu) spectrophotometer in tablets of potassium bromide, at the range of 4000-400 cm^{-1}. The ^{1}HNMR-spectra were recorded on spectrophotometer "Varian Mercury-VX-200" (200 MHz), using DMSO-d_6 as solvent and TMS as the internal standard.

The ionization constants at various concentrations were determined by potentiometric titration with a potassium hydroxide solution. The measurements were performed on FTI-6 Universal Digital pH-meter (England) at a 0,005 mol concentration of samples in 50% dioxane. The values were determined by the half-neutralization potential.

The melting temperatures were determined on melting point apparatus SMP3 (England).

TLC patterns were obtained on aluminium plates with 0,25 mm silica gel 60 F_{254} layer (Merk, Germany) visualized under UV illumination and developed with selective and non-selective reagents.

3,4-Dimethylphenyloxamoyl-α-Alanine (2)

Method A. Mixture of 0,01 mole of α-alanine and 0,01 mole of ethyl ester of 3,4-dimethyloxanylic acid in 5 ml dimethylformamide was heated with reflex air condenser until dissolving the amino acid. The reaction mixture was added into water, the formed precipitate filtered and crystallized from ethanol. Analogous procedure compounds 1, 7 and 8 were synthesized.

Method B. To a solution of 0,01 mole of ethyl ester of 3,4-dimethyloxanylic acid in 10 absolute methanol was added a solution of 0,01 mole of α-alanine in sodium methoxide (obtained from 0.01 g metal sodium and 10 ml absolute methanol) and allowed to stand to neutral pH. Then the mixture was acidified to pH=2 by hydrochloric acid (1:1), the formed precipitate was filtered and crystallised from ethanol, analogous procedure compounds 3, 4 9-11 were synthesized.

Method C. 0,01 mole of methanolic solution of ethyl ester of 3,4-dimethyloxanylic acid was added into aqueous solution of 0,01 mole of α-alanine and 0,01 potassium hydroxide, the mixture allowed to stand until a neutral pH, the formed precipitate was dissolved in water and acidified with HCl (1:1) to pH=2, the precipitate was filtered and crystallized from ethanol compound 1, 5, 6 were obtained using this method.

2-D-(+)-Glucosamonium 3,4-Dimethylphenyloxamoylglycinate (12)

0.05 mole of D(+)-glucosaminium chloride was dissolved by heating in potassium hydroxide solution(obtained from 0.05 mole of potassium hydroxide and 10 ml 50% aqueous ethanol). The precipitate of potassium chloride was filtered, then the filtrate was added into a solution of 0.05 mole of 3,4-dimethyloxanyloylglycine in 15 ml ethanol. The reaction mixture was allowed to stand for one night. The formed precipitate (salt) was filtered, washed by diethyl ether and dried. Other salts were similarly obtained.

Biological Activity of 3,4-dimethylphenyloxamoyl-aminoacids

Hepatoprotector Activity

Hepatoprotector activity was studied on the acute hepatitis model in comparison with Silymarin [12-14]. The experiment showed that 100% survival rate was found in animals which received substances 12 and 13, while animals receiving silymarin had survival rate of 75% (table 4).

As a result of the experiment, it was established that substances 12 and 13 exhibited hepatoprotective activity (table 4) and can be recommended for further investigation.

The Immunodepression Activity

The immune activity was studied on parameters of cellular immunity by the conventional method [15].

Table 4

Hepatoprotector Activity of Compounds 12-18

Compound (10 mg/kg)	Liver's coefficient	% survival rate	AST mmole/L	ALT mmole/L	TBARS mic mole/L
12	4,59±0,32	100	1,16±0,03	1,53±0,03	44,33±2,06
13	4,43±0,33	100	1,10±0,05	1,41±0,06	46,31±1,55
14	5,07±0,52	75	1,28±0,03	1,64±0,01	55,0±1,88
15	5,13±0,18	75	1,15±0,05	1,50±0,10	58,4±1,89
16	4,60±0,10	50	1,13±0,06	1,65±0,020	52,31±2,13
17	4,60±0,20	50	1,42±0,02	1,66±0,031	49,92±3,35
18	4,40±0,32	75	1,31±0,015	1,63±0,023	59,32±2,55
Silymarine 25 mg/kg	4,60±0,06	75	1,15±0,03	1,63±0,07	49,71±1,87

Table 5

Influence of Investigated Substances on Parameters of Specific Immunity

Compound	Spleen weight coefficient	Amount of karyocytes, million/1mg of tissue • 10^6	Amount of bone morrow cells, million/1mg of tissue • 10^6
1	1,41±0,27	56,86±0,85	50,66±0,88
3	1,33±0,25	56,50±2,45	48,91±0,93
5	0,99±0,14	17,32±0,88	45,16±2,45
6	1,65±0,55	53,30±1,45	52,66±0,72
8	1,15±0,19	49,71±0,97	50,91±1,34
Control	1,05±0,13	51,32±0,88	47,00±1,52

The results of the investigation are demonstrated in table 5.

Analysis of obtained results showed that substance 5 exhibited the most expressed inhibiting effect on lymphoid organs (spleen), and significantly reduced the amount of karyocytes in comparison with the control group.

Anti-inflammatory Activity

Anti-inflammatory activity was measured using carrageenin-induced rat paw oedema assay [16, 17]. It was established that compounds 5, 6, 10, 12 produced the highest anti-inflammatory activity. The most expressed anti-inflammatory activity was observed for compound 6 which was more than Diclofenac sodium during all the period of the experiment. Substance 10 showed equal anti-inflammatory effect with Diclofenac sodium.

Toxicity of the Most Biologically Active Compounds

According to the classic method [18] acute toxicity of the most pharmacologically active substances 5, 6, 12 and 13 was investigated, and the toxicity was evaluated by the conventional method [16]. It was found that all of test compounds have low toxicity (LD 50≥1000 mg/kg).

Conclusion

1. The synthesis of the new oxamoylaminoacids and their salts with D-(+)-glucosamine has been achieved.

2. The convenient method to synthesize the optically-active oxamoylaminoacids has been found.

3. It has been established that the synthesized compounds showed a hepatoprotective, anti-inflammatory, immunodepressant activity and low toxicity.

References

1. *Li W., Jing F., Wei L. // Carbohydrate Res. — 2008. — Vol. 343. — P. 685-694.*
2. *Bruneau C., Renaud J., Jerphagnon T. // Coord. Chem. Rev. —2008. — Vol. 252, №5. — P. 532-544.*
3. *Пат. Евр. EP1130108 European Patent Application, Sept. 2001.*
4. *Пат. Японии JP2005239601 Patent Abstracts of Japan, Sept. 2005.*
5. *Galeazzi R., Martelli G., Mobbili G. et al. // Tetrahedron. — 2006. — Vol. 62, №44. — P. 10450-10455.*
6. *Iizuka T., Funayama H., Kusano G. et al. // J. Pharm. Soc. of Japan. — 2003. — Vol. 123, №11. — P. 963-971.*
7. *Каращук Ю.В. Синтез и биологическая активность оксамоиламинопропионовых кислот: Автореф. дис. ... канд. фармац. наук. — Х., 1990. — 22 с.*
8. *А.с. 1782000 (1992). СССР // Б.И. — 1992. — №24.*
9. *Абдуллаева Б.М. Синтез и биологическая активность производных оксалил- и аренсульфониламинокислот: Автореф. дис. ... канд. фармац. наук. — Х., 1991. — 23 с.*
10. *Потапов В.М. Стереохимия. — М.: Химия, 1978. — 696 с.*

11. Jakubke H.-D., Jtschkeit H. Aminosaure, Peptide, Proteine. —B.: Akademie-Verlag, 1982. — 456 S.
12. Zimmerman H.J., Seeff L.B. Enzymes in Hepatic Disease. — Philadelphia: Lea and Febiger, 1970. — 138 p.
13. Vogel G. In Natural Substances with Effects on the Liver. — Springer Verlag: New York, 1977. — 249 p.
14. Miguez M., Anundi I., Sainz-Pardo L. et al. // Chem. Biol. Interact. — 1994. —Vol. 91. — P. 183-186.
15. Veereshwarayya V., Thiruvengadam D. // J. Ethno Pharmacol. — 2004. — Vol. 90. — P.151-154.
16. Winter C.A., Risley E.A., Proc G.W. // Soc. Exp. Biol. Med. — 1966. — Vol. 111. — P. 544-548.
17. Duffy J.C., Dearden J.C., Rostorn C. // J. Pharm. Pharmacol. — 2001. —Vol. 53. — P.1505-1514.
18. Саноцкий И.В. Методы определения токсичности и опасности химических веществ. — М.: Медицина, 1970. —142 с.

Надійшла до редакції 05.05.2008 р.