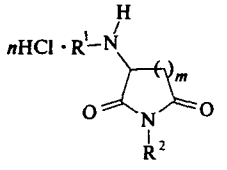
SYNTHESIS AND ANTIAGGREGATIVE ACTIVITY OF DERIVATIVES OF 3-AMINOPIPERIDINE-2,6-DIONES AND 3-AMINOPYRROLIDINE-2,5-DIONES

A. A. Krys'ko,[1] V. M. Kabanov,[1] T. A. Kabanova,[1] M. V. Belikova,[1] and A. V. Mazepa[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 32, No. 6, pp. 18 – 20, June, 1998.

Original article submitted May 20, 1997.

The search for new antithrombic and antiaggregative agents possessing high activity and low toxicity is a currently important task of pharmaceutical chemistry [1]. A large number of potential antiaggregants have been described in the literature by now, which can be divided into several groups according to the mechanism of their action. In one group, the mechanism is related to the arachidonic acid metabolism. Other groups are characterized by influence upon the ADP-dependent aggregation pathway or inhibition of the thrombocyte aggregation factor [2]. However, there are many compounds of different structures, also possessing the antiaggregative properties, whose mechanism of action is still unclear. A group of potential antiaggregants includes pyridyloxazoles and their 4,5-dihydro analogs [3], imidazoquinolin-2-one [4], and piperidine-3-carboxamides [5]. Previously we have reported that antiaggregative activity is also observed in a group of derivatives of 3-aminopiperidine-2,6-diones [6]. In order to find new effective antiaggregants among these compounds and their analogs – 3-aminopyrrolidine-2,5-diones, we have synthesized a series of compounds (I – XIII) and studied their properties;



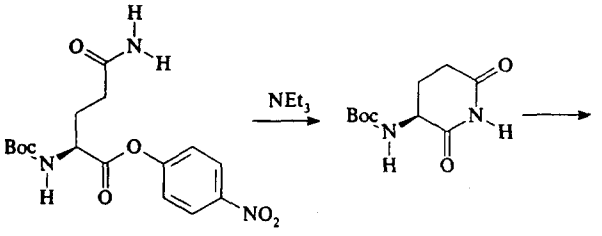
I – XIII

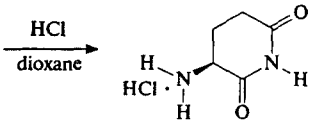
Compound	R^1	R^2	*n*	*m*
I	H	H	1	2
II	Boc	2-pyridyl	0	2
III	Boc	3-pyridyl	0	2
IV	Boc	4-pyridyl	0	2
V	Boc	2-pyridyl	0	1
VI	Boc	3-pyridyl	0	1

[1] Bogatskii Institute of Physical Chemistry, National Academy of Sciences of Ukraine, Odessa, Ukraine.

Compound	R^1	R2	*n*	m
VII	H	2-pyridyl	2	2
VIII	H	3-pyridyl	2	2
IX	H	4-pyridyl	2	2
X	H	2-pyridyl	2	1
XI	H	3-pyridyl	2	1
XII	Pro	2-pyridyl	2	2
XIII	Thz	2-pyridyl	2	2

3-Aminopiperidine-2,6-dione (I) was obtained by cyclization of Boc – Glu *p*-nitrophenyl ester (Boc–Glu–ONp) in the presence of triethylamine [7]:



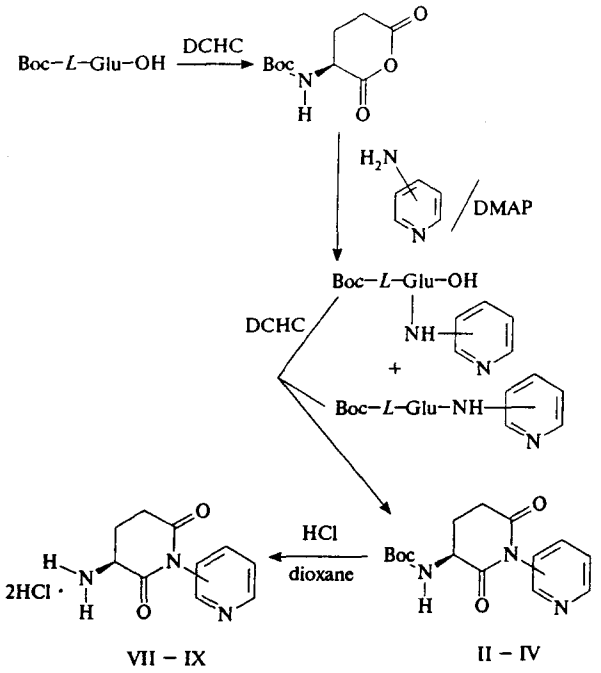


The synthesis of 1-pyridyl-substituted 3-aminopiperidine-2,6-diones and 3-aminopyrrolidine-2,5-diones was based on the formation of intramolecular anhydrides of N-protected dicarboxylic amino acids, followed by their reactions with 2-, 3-, or 4-aminopyridines.

Compounds V, VI, X, and XI were synthesized by the same scheme using Boc–Asp–OH as the initial compound.

The synthesis of compounds XII and XIII was conducted via the activated ester pathway based on the condensation of 3-aminopiperidine-2,6-dione VII with five-membered cyclic amino acids in the presence of triethylamine.

0091-150X/98/3206-0304$20.00



EXPERIMENTAL CHEMICAL PART

The target compounds were synthesized using amino acids purchased from Merck, Sigma, and Reanal. The mass spectra were recorded with an MX-1321 spectrometer (ionizing electron impact energy, 70 eV; ionization chamber temperature, 200°C). The optical rotation measurements were performed on a Perkin-Elmer Model 241-MC spectropolarimeter. The purity of compounds was checked by column chromatography, using a chromatograph (DuPont Instruments) equipped with Zorbax C8 and C18 columns, and by TLC on Silufol (Kavalier) plates eluted in the systems benzene – acetone – acetic acid, 100 : 50 : 1 (A) or butanol – acetic acid – water, 4 : 1:1 (B). The brief notation of amino acids is given according to the IUPAC – IUB Nomenclature Recommendations [8], the other compounds being N,N′-dicyclohexylcarbodiimide (DCHC), 1-hydroxybenzotriazole (HBT), and 4-dimethylaminopyridine (DMAP). The yields of the reaction products and characteristics of the synthesized compounds are given in Table 1. The data of elemental analyses agree with the results of calculations according to the empirical formulas.

3-Aminopiperidine-2,6-dione hydrochloride (I). To a solution of 1.1 g (3 mmole) Boc–Glu–ONp in 5 ml anhydrous DMF was added 0.42 ml (3 mmole) triethylamine and the mixture was allowed to stand for 24 h. The solvent was distilled off in vacuum (≤ 45°C). The residue was mixed with 30ml ethyl acetate and washed sequentially with water (2 × 20 ml) and 5% aqueous $NaHCO_3$ (3 × 30 ml). The ethyl acetate solution was dehydrated and evaporated. The residue was triturated with anhydrous ether, filtered, mixed with 10 ml of a 4 N HCl solution in dioxane, and allowed to stand for 1 h. Then the solvent was distilled off at a reduced pressure and the residue dried over KOH in vacuum.

1-Pyridyl-3-(*tert*-butylhydroxycarbonylamino)piperidine-2,6-diones (II – IV). To a solution of 2 g (8 mmole) Boc – Glu – OH in anhydrous ethyl acetate cooled to 0°C was added 1.724 g (8.4 mmole) DCHC. The mixture was stirred for 30 min and filtered. To the filtrate was added 0.902 g (8 mmole) of aminopyridine and 1 g (8 mmole) DMAP, and the reaction mass was stirred for 1 h. Then was added another portion (1.724 g, 8.4 mmole) of DCHC and the mixture again stirred for 30-min and filtered to separate the residue. Then was added 40 ml ethyl acetate and the mixture was washed sequentially with 5% AcOH (2 × 10 ml), water, 5% aqueous $NaHCO_3$ (2 × 15 ml), and saturated NaCl solution. The ethyl acetate solution was dried over $MgSO_4$ and the solvent was distilled in vacuum. The residue was chromatographed on a silica gel column (25 × 3 cm) eluted with chloroform and chloroform – methanol, 9 : 1.

1-Pyridyl-3-(*tert*-butylhydroxycarbonylamino)pyrrolidine-2,5-diones (V, VI). Compounds V and VI were obtained by an analogous method proceeding from Boc – Asp – OH.

Dihydrochlorides of 1-pyridyl-3-aminopiperidine-2,6-diones (VII – IX) and 1-pyridyl-3-aminopyrrolidine-2,5-diones (X, XI). To 3 mmole Boc-derivatives II – VI was added 5 ml of 4 N HCl solution in dioxane and the mixture was allowed to stand for 1 h. Then dioxane was distilled off at a reduced pressure and the residue dried in vacuum over KOH.

Dihydrochloride of 1-(2-pyridyl)-3-(*L*-prolylamino)-piperidine-2,6-dione (XII). Compound VII (0.305 g, 1.1 mmole) was dissolved in a mixture of 5 ml anhydrous DMF and 0.334 ml (2.4 mmole) freshly distilled triethylamine. To this solution was added a mixture of 0.237 g (1.1 mmole) Boc–Pro, 0.149 g (1.1 mmole) HBT, and 0.227 g (1.1 mmole) DCHC in 5 ml THF. The mixed solution was allowed to stand for 3 h and then filtered. The filtrate was

TABLE 1. Yields and Physicochemical Characteristics of Synthesized Compounds

Compound	Yield, %	Empirical formula	M.p., °C	m/z, (M^+)	$[\alpha]_D^{20}$	R_f
I	77	$C_5H_9N_2O_2$	hygr.	128	+ 12.21*	0.33 (B)
II	85	$C_{15}H_{19}N_3O_4$	206 – 208	305	– 12.2**	0.55 (A)
III	78	$C_{15}H_{19}N_3H_4$	215 – 216	305	+ 9.5**	0.52 (A)
IV	79	$C_{15}H_{19}N_3O_4$	201 – 204	305	– 27.4**	0.53 (A)
V	83	$C_{14}H_{17}N_3O_4$	221 – 223	291	– 35.1**	0.43 (A)
VI	67	$C_{14}H_{17}N_3O_4$	212 – 215	291	– 28.2**	0.37 (A)
VII	82	$C_{10}H_{13}Cl_2N_3O_2$	hygr.	205	– 7.4*	0.23 (B)
VIII	83	$C_{10}H_{13}Cl_2N_3O_2$	hygr.	205	– 24.4*	0.30 (B)
IX	82	$C_{10}H_{13}Cl_2N_3O_2$	hygr.	205	– 14.7*	0.25 (B)
X	81	$C_9H_{11}Cl_2N_3O_2$	hygr.	191	– 19.5*	0.25 (B)
XI	61	$C9H_{11}Cl_2N_3O_2$	hygr.	191	– 30.6*	0.27 (B)
XII	61	$C_{15}H_{20}Cl_2N_4O_3$	hygr.	302	+ 48.0*	0.12 (B)
XIII	67	$C_{14}H_{18}Cl_2N_4O_5S$	hygr.	320	– 87.2*	0.46 (B)

* c = 1, H_2O
** c = 1, MeOH.

TABLE 2. Antiaggregative Activity of Derivatives of 3-Aminopiperidine-2,6-diones and 3-Aminopyrrolidine-2,5-diones

Compound	IC_{50} (10^{-4} M)
I	26.5 ± 1.8
II	8.0 ± 0.7
III	6.6 ± 0.5
IV	8.2 ± 0.5
VII	8.9 ± 0.7
VIII	3.6 ± 0.2
IX	7.0 ± 0.5
X	9.5 ± 0.6
XI	8.0 ± 0.7
XII	8.0 ± 0.7
XIII	10.0 ± 0.9
Arg–Gly–Asp (RGD)	36.1 ± 2.9
Acetylsalicylic acid	25.6 ± 1.7

evaporated on a rotor evaporator. To the oily residue was added 20 ml of chloroform and the mixture was washed sequentially with 5% AcOH (2 × 10 ml), water, 5% aqueous $NaHCO_3$ (2 × 15 ml), and saturated NaCl solution. The chloroform solution was dried over $MgSO_4$ and the solvent was distilled in vacuum. To the residue was added 5 ml of absolute ether and the mixture was cooled. The precipitate was dissolved in 5 ml of a 4 N HCl solution in dioxane, and allowed to stand for 30 min. Then the solvent was distilled off and the residue triturated with absolute ether.

A similar procedure was used to obtain **dihydrochloride of 1-(2-pyridyl)-3-(*L*-4-thiazolidinecarbonylamino)piperidine-2,6-dione (XIII).**

EXPERIMENTAL PHARMACOLOGICAL PART

The antiaggregative properties were studied for a group of 11 compounds including piperidine derivatives (I – IV, VII – IX, XII, XIII) and pyrrolidine derivatives (X, XI). Note that some compounds contain protective Boc groups (II – IV) and cyclic amino acids (XII, XIII).

The samples of thrombocyte-rich plasma (250 μl), obtained from a citrate blood of male rats by centrifuging for 15 min at 200 g, were incubated with various concentrations of the synthesized compounds at 37°C for 5 min before introducing ADP (10 μM). The aggregation of thrombocytes was measured during 2 min (to maximum aggregation) by monitoring the light scattering with a Thromlite 1006A aggregometer (Biokhimmak company, Moscow) connected to a recorder. Each compound was tested 5 – 6 times. The antiaggregative activity was expressed as the inhibiting concentration (IC_{50}) at which the degree of aggregation decreased to half of the initial level.

RESULTS AND DISCUSSION

As is seen from the data presented in Table 2, all the compounds studied are capable of inhibiting to greater or lesser extent the ADP-induced thrombocyte aggregation. The antiaggregative effect observed for most of the compounds (except I) exceeded the action of RGD-peptide and acetylsalicylic acid.

The antiaggregative activity of 3-aminopiperidine-2,6-dione (I) is comparable to that of RGD-peptide and acetylsalicylic acid. Thus, the introduction of substituents in positions 1 and 3 in both piperidine derivatives (I – IV, VII – IX, XII, XIII) and pyrrolidine derivatives (X, XI) increases the activity 3 – 10 times as compared to that of RGD-peptide. The maximum contribution to this increase is apparently related to the pyridine residue in position 1, in agreement with the data reported on the positive effect of pyridine residues on the antiaggregative activity [3].

ACKNOWLEDGMENTS

The work was supported by the PECO Program (Biomed, project No. ERBCIPDCT 940 247).

REFERENCES

1. J. G. Lombardi (ed.), *Nonsteroidal Antiinflammatory Drugs*, Vol. 5, Wiley Inc., New York (1985), pp. 78 – 103.
2. M. Ferstrate, G. Fermilen, and I. N. Bokarev (eds.), *Thromboses* [Russian translation], Meditsina, Moscow (1986), p. 61.
3. O. V. Demina, P. V. Vrzhats, A. A. Khodonov, et al., *Bioorg. Khim.*, **21**(12), 933 – 940 (1995).
4. N. A. Meanwell, P. Hewawasam, J. A. Thompson, et al., *J. Med. Chem.*, **36**, 3251 – 3264 (1993).
5. X. Zheng, S. R. Salgia, W. B. Thompson, et al., *J. Med. Chem.*, **38**, 180 – 188 (1995).
6. S. A. Andronati, V. M. Kabanov, T. L. Karaseva, et al., *Peptides-94. Proc. 23rd Eur. Peptide Symp.*, ESCOM (1995) p. 383.
7. A. A. Mazurov, S. A. Andronati, T. I. Korotenko, et al., *Int. J. Peptide Protein Res.*, **42**, 14 – 19 (1993).
8. Bul. IUPAC-IUB Joint Comission on Biochemical Nomenclature, *Pure Appl. Chem.*, **56**, 595 – 624 (1984).