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4-(Isoindolin-5-yl)amino-4-oxobutyryl-β-alkoxyphenyl-β-alanines, New RGDF Mimetics. Synthesis, Affinity to Fibrinogen Receptors, Antiaggregatory Properties, and Their Correlation with the Hydrophobicity

S.A. Andronati, A.A. Krys'ko, B.M. Chugunov, T.A. Kabanova, and A.G. Artemenko

Bogatskii Physicochemical Institute, National Academy of Sciences of Ukraine, Odessa, 6508 Ukraine
e-mail: peptides@paco.net

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Abstract—A new series of RGDF mimetics, derivatives of 4-(isoindolin-5-yl)amino-4-oxobutanoic acid, was synthesized. The compounds obtained inhibit efficiently the thrombocytes aggregation in experiments in vitro; their biological targets are fibrinogen receptors. The antiaggregatory activity and the affinity correlate with the hydrophobicity of the compounds.

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The search for efficient inhibitors of thrombocytes aggregation is the line of research that gains the highest momentum within the last decade aiming at solving the problem of the thromboprophylaxis and thrombosis treatment. The greatest attention is drawn to antiaggregants, the antagonists of the fibrinogen receptors (integrin $\alpha_{IIb}\beta_3$). The integrin $\alpha_{IIb}\beta_3$ is a glycoprotein complex located on the surface of the activated thrombocytes. The binding site in the fibrinogen for integrin $\alpha_{IIb}\beta_3$ is the tripeptide sequence Arg–Gly–Asp (RGD in the single-letter code). Quite a number of RGDF peptides, RGDF pseudopeptides, and RGDF mimetics are known now recognizing integrin $\alpha_{IIb}\beta_3$ with a high affinity and blocking these receptors. However the problem is far from being solved [1]. Many aspects of the ligand-receptor interaction and of the subsequent pharmacological (physiologic) consequences are unknown. It is assumed [1] that to enter in an efficient interaction with the $\alpha_{IIb}\beta_3$ the RGDF mimetic should possess a basic and an acidic site for binding with the receptor. The distance between the sites in the ligand molecule should fall into the range from 10 to 15 A [2]. The selective binding of the antagonists to the $\alpha_{IIb}\beta_3$ is likely to be favored by the predominantly stretched conformation of the ligand. The presence of a hydrophobic fragment in the linker between the basic and acidic sites of the RGDF mimetic is regarded as increasing its affinity to the receptor and consequently as enhancing its antiaggregatory activity [3].

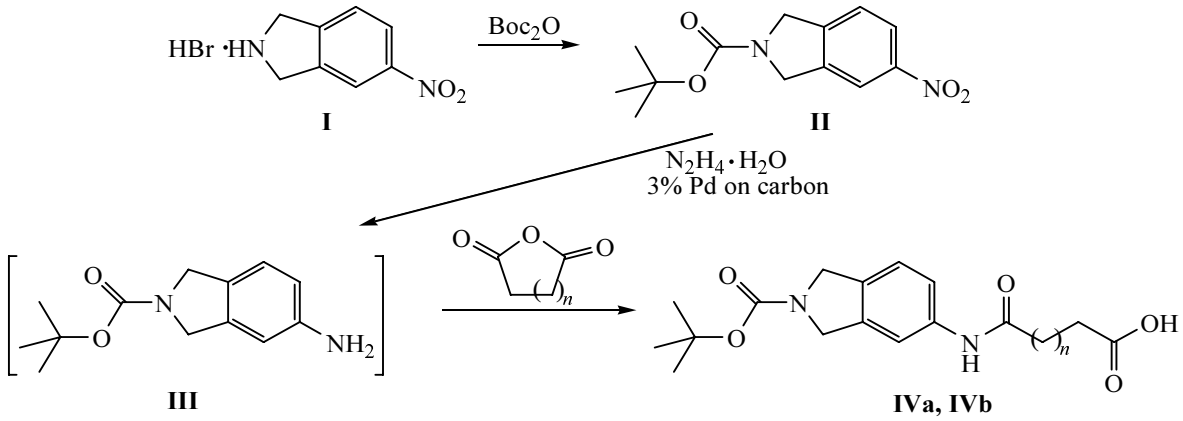
The imitation of the δ-guanidine group from the arginine residue in designing the active and selective RGDF mimetics, antagonists of the fibrinogen receptor $\alpha_{IIb}\beta_3$, is performed with the use of moieties containing residues of *p*-benzamidine, piperidine, *p*-benzguanidine, etc. [1, 2]. The cited fragments may be regarded as bioisosteric to the side function of the arginine residue. Two open-chain RGDF mimetics were formerly synthesized based on 4-(isoindolin-5-yl)amino-4-oxobutanoic acid simulationg the Arg–Gly site. They exhibited *in vitro* a high antiaggregatory activity and the affinity to $\alpha_{IIb}\beta_3$ in a suspension of human thrombocytes subjected to ablution [4].

We report here on the synthesis of an RGDF mimetics series containing as Arg–Gly of the mimetic a residue of the 4-(isoindolin-5-yl)amino-4-oxobutanoic acid and as a substitute for the fragment Asp–Phe the residues of β-alkoxyphenyl(or dialkoxyphenyl)-β-alanines.

The target of the research was the preparation of potentially highly active antagonists of integrin $\alpha_{IIb}\beta_3$ possessing an antiaggregatory activity, and the study of relations between the structure and the properties of compounds obtained. In particular, the effect of the lipophilicity of the mimetics on their biological characteristics (the affinity to $\alpha_{IIb}\beta_3$ and the antiaggregatory activity) would be estimated.

β-Alanines with various substituents attached to the β-position are successfully employed in the synthesis of

Scheme 1.



$n = 1$ (**a**), 2 (**b**).

the RGDF mimetics, blockaders of the fibrinogen receptors. The residue of the β-substituted β-alanine is believed to imitate the site Asp–Phe [5]. An aromatic substituent makes it possible to bind additionally the ligand with $\alpha_{IIb}\beta_3$, and the carboxy group of the β-alanine simulates the side chain of the aspartic acid residue. To reveal the effect of the hydrophobicity of bioisosteric sites simulating the fragment Asp–Phe on the antiaggregatory characteristics of the RGDF mimetics we synthesized compounds **VII** of essentially different hydrophobicity.

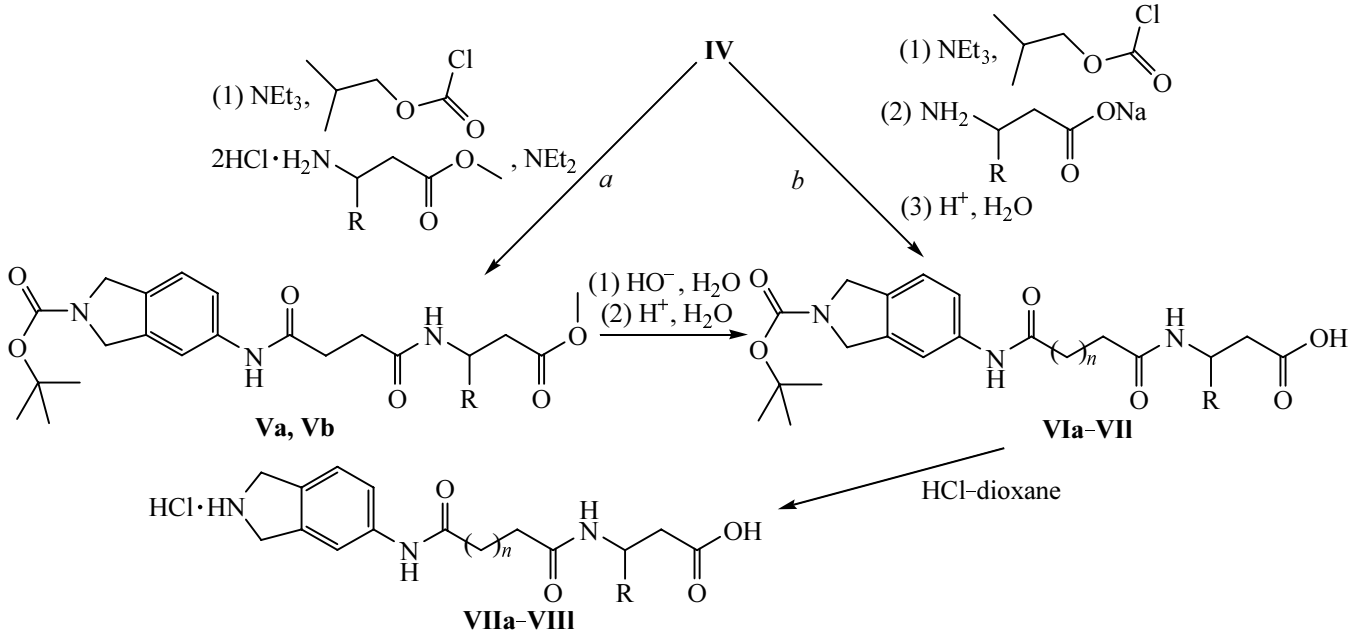
We used the 5-nitroisoindoline (**I**) as an initial compound for designing the RGDF mimetics based on the derivatives of 4-(isoindolin-5-yl)amino-4-oxobutanoic acid. The blocking of the amino group in the 5-nitroisoindoline (**I**), the reduction of the nitro group in the N-BOC-5-nitroisoindoline (**II**) obtained followed by acylation of amine **III** with succinic anhydride led to the formation of 4-(*N*-Boc-isoindolin-5-yl)-amino-4-oxobutanoic acid (**IVa**) (Scheme 1) [4].

We formerly synthesized RGDF mimetics **VIIa** and **VIIb** by condensation of acid **IVa** with the β-alanines esters followed by the hydrolysis of ester groups in compounds **V** and removing BOC-protection in the last stage (method *a*) [4]. The application of a salt protection on the N-components in the building-up of the RGDF mimetics skeleton (method *b*) eliminated one reaction stage and also gave compounds **VI** in high yields and of high purity without chromatographic separation. We chose for condensation of acid **IVa** with the β-alanines sodium salts the procedure using mixed anhydrides (Scheme 2). The condensation of the β-alanines with compound **IVa** yielded protected mimetics **VI**, and the removal of the BOC protection resulted in the target mimetics **VII**. The RGDF mimetics obtained are chemically stable compounds both as dry substances and in water solutions at the normal conditions. Compounds **VII** are well soluble in water; that is important for in vitro experiments [4].

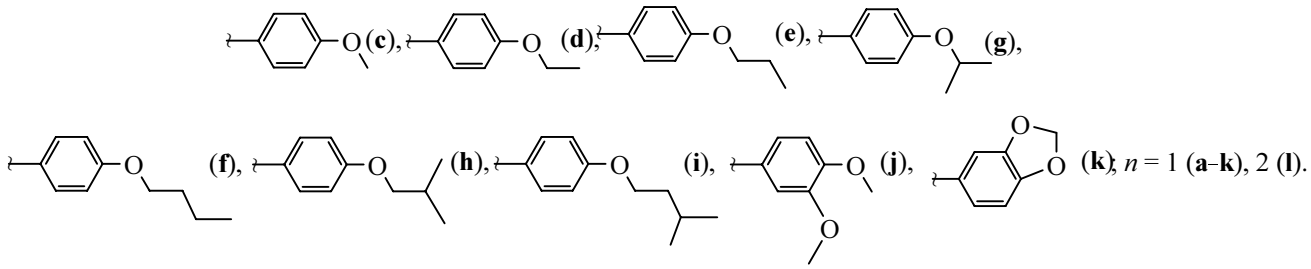
The structure of compounds obtained was confirmed by their mass and ^{1}H NMR spectra. In the ^{1}H NMR spectra of the compounds all the characteristic proton signals were present with the appropriate integral intensities. The electron-impact (ionizing voltage 70 eV) mass spectra of compounds **I–IV** contained molecular ion peaks of low intensity, and also fragment ions corresponding to the primary degradation processes. We failed to measure the electron-impact mass spectra of **V–VII** due to the extensive thermal degradation. Compounds **V–VII** are unstable with respect to the electron impact ($I_{M^+\cdot} < 0.1\%$), and therefore we measured their FAD mass spectra. In the FAD mass spectra we observed ion peaks corresponding to $[M + H]^+$ and $[M + Na]^+$.

The RGDF mimetics synthesized, derivatives of the 4-(isoindolin-5-yl)amino-4-oxobutanoic acid, exhibited a high antiaggregatory activity in vitro both on the human blood serum rich in thrombocytes or on the suspension of human thrombocytes subjected to ablution. The experiments were carried out by Born method using blood samples from three donors [6]. To reveal the molecular mechanism of the antiaggregatory action of RGDF mimetics **VII** we investigated their effect on the specific binding of fluorescein-labeled fibrinogen (FITC-Fg) using the suspension of human thrombocytes subjected to ablution by procedure [7]. FITC-Fg prepared as described in [8] is specifically bound to the receptors on the thrombocytes with a dissociation constant 1.02 μmol. Compounds **VII** inhibited the binding of FITC-Fg with $\alpha_{IIb}\beta_3$ on the suspension of human thrombocytes subjected to ablution (see the table).

Scheme 2.



V, R = H (**a**), C_6H_5 (**b**); **VI, VII**, R = H (**a, l**), C_6H_5 (**b**),



n = 1 (**a**–**k**), 2 (**l**).

Characteristics of RGDF mimetics based on 4-(isoindolin-5-yl)amino-4-oxobutanoic acid

Compound no.	Antiaggregatory activity in experiments in vitro using human blood serum rich in thrombocytes, IC_{50}, μmol/l	Antiaggregatory activity in experiments in vitro using the suspension of human thrombocytes subjected to ablution, IC_{50}, μmol/l	Inhibition of binding of FITC-Fg with $\alpha_{IIb}\beta_3$ on the surface of activated human thrombocytes, IC_{50}, μmol/l	log*P*
VIIa	2.760	2.0900	0.01400	0.08
VIIb	0.860	0.2500	0.00830	1.90
VIIc	0.160	0.0640	0.00260	1.86
VIId	0.150	0.1080	0.00280	2.28
VIIe	0.150	untested	untested	2.73
VIIf	0.105	0.0660	0.00600	2.78
VIIg	0.210	0.0876	0.00170	3.18
VIIh	0.029	0.0265	0.00080	3.00
VIIi	0.050	0.0370	0.00135	3.45
VIIj	0.890	0.2100	0.00900	1.51
VIIk	0.031	0.0270	0.00022	1.57
VIIl	0.040	untested	untested	0.53

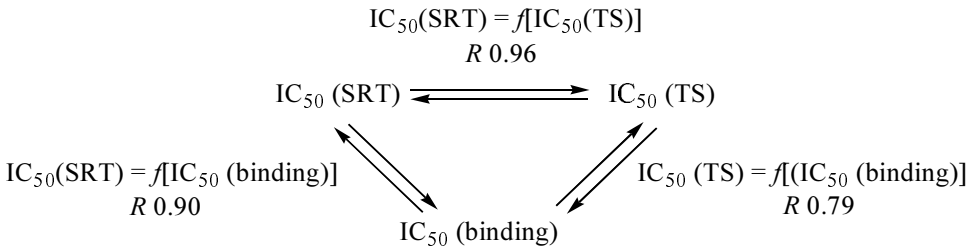


Fig. 1. Mutual correlation of the IC_{50} values.

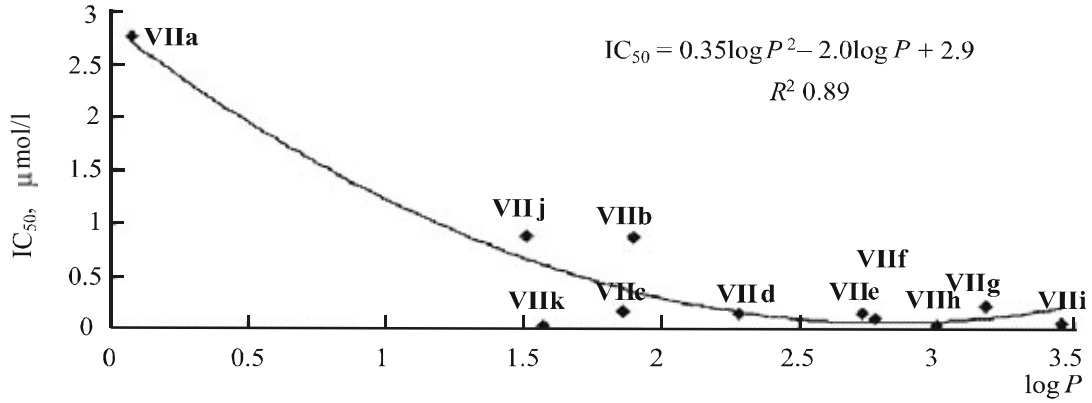


Fig. 2. Values of IC_{50} (SRT) as a function of lg*P* for mimetics **VIIa–VIIk**.

Note also that the values of IC_{50} for all three experiments are in a sufficiently good correlation (Fig. 1).

We formerly showed that introducing a phenyl into the β-position of the β-alanine residue enhanced the antiaggregatory activity of compound **VIIb** 3-fold, and its affinity 2-fold. The presence of nonbranched alkoxy substituents in the *para*-position of the phenyl group in the β-alanine moiety of the RGDF mimetics (compounds **VIIc–VIIf**) results in insignificant increase in the antiaggregatory activity and affinity. The isoalkoxy analogs, compounds **VIIg–VIIi**, proved to be more active RGDF mimetics. The 3,4-dimethoxyphenyl substituent in the β-position of the β-alanine residue (compound **VIIj**) possessed virtually the same antiaggregatory activity and affinity to the fibrinogen receptors as the phenyl-substituted derivative **VIIb**. The derivative of 3-[5-(3,4-methylenedioxyphenyl)]-β-alanine **VIIk** is distinguished by a high antiaggregatory activity and affinity in the series of RGDF mimetics **VII**.

Formerly QSAR investigation was successfully carried out on antagonists of $\alpha_{IIb}\beta_3$, derivatives of 4-(4-amidinophenoxy)butanoyl-aspartyl-valine, applying a hydrophobic descriptor, the logarithm of the micelle-water distribution factor [9]. To estimate the hydrophobicity effect of RGDF mimetics **VIIa–VIIk** on their activity we calculated their distribution factors in a system water–octanol (log*P*) by procedure [10] (see the table). The biological activity was evaluated by the constants of the half-inhibition of the thrombocytes aggregation in the *in vitro* experiments with the use of the human blood serum rich in thrombocytes, IC_{50} (SRT), on the suspension of human thrombocytes subjected to ablution, IC_{50} (TS), and the constant of the half-inhibition of FITC-Fg binding with the fibrinogen receptor, IC_{50} (binding). The relation of IC_{50} (SRT) values for compounds **VIIa–VIIk** to their hydrophobicity (Fig. 2) estimated by means of the regression analysis is expressed by equation (1). Likewise for experiments with therombocytes suspension and for inhibition of FITC-Fg binding with the fibrinogen receptors equations (2) and (3) were respectively derived.

$$IC_{50}\,(SRT) = 0.35\log P^2 - 2.0\log P + 2.9\ (R^2 = 0.89) \quad (1)$$

$$IC_{50}\,(TS) = 0.36\log P^2 - 1.7\log P + 2.2\ (R^2 = 0.95) \quad (2)$$

$$IC_{50}\,(binding) = 0.00097\log P^2 - 0.0069\log P + 0.014\ (R^2 = 0.60) \quad (3)$$

The antiaggregatory activity and affinity of the RGDF mimetics we synthesized are comparable or higher than

the characteristics of the known RGDF mimetics containing a *p*-benzamidine group as a bioisoester structure of the arginine side function. It should be emphasized that among the β-(*p*-alkoxyphenyl)-β-alanines the best isoester structure for the fragment Asp–Phe of the RGDF sequence proved to be the β-(*p*-isopropoxyphenyl)-β-alanine residue. The derivative of the β-(*p*- isopropoxyphenyl)-β-alanine and 4-(isoindolin-5-yl)amino-4-oxobutanoyl **VIIh** exhibited the maximum antiaggregatory activity and affinity to the $\alpha_{IIb}\beta_3$ receptors on the surface of the human thrombocytes subjected to ablution. The promising prospects of looking for RGDF mimetics among new derivatives of 5-(isoindolin-5-yl)amino-5-oxopentanoic acid as potential highly active antagonists for $\alpha_{IIb}\beta_3$ receptors also should be noted.

The experimental data on the antiaggregatory activity and inhibition of FITC-Fg binding with the fibrinogen receptors indicate that the new RGDF mimetics based on 4-(isoindolin-5-yl)amino-4-oxobutanoic acid and β-alkoxyphenyl-substituted β-alanines may be regarded as highly active inhibitors of the thrombocytes aggregation and as antagonists of the $\alpha_{IIb}\beta_3$ receptors.

EXPERIMENTAL

^{1}H NMR spectra were registered on a spectrometer Varian WXP-300 at operating frequency 299.95 MHz using tetramethylsilane as internal reference. Mass spectra FAB were measured on VG 7070 instrument applying glycerol matrix, ionization with a Xe atoms beam of 8 kW energy. TLC was carried out on Silufol (Kavalier, Czechia) and Kieselgel 60 plates (Merck, Germany) in solvent systems: 10% ethyl acetate solution in hexane (A), benzene–acetone–acetic acid, 100:50:1 (B), chloroform–ethyl acetate–methanol, 9:3:2 (C), ammonia–dioxane, 1:5 (D). Spots were visualized with ninhydrin and chlorotoluidine reagents.

***N*-*tert*-Butyloxycarbonyl-5-nitroisoindoline (II)**. A suspension was prepared of 1 g (4.08 mmol) of 5-nitroisoindoline hydrobromide in 10 ml of 5% Na_2CO_3 solution. To the suspension a solution of 0.873 g (4 mmol) of di-*tert*-butyl pyrocarbonate in 30 ml of chloroform was added. The reaction mixture was stirred for 5 h at room temperature, then it was diluted with 50 ml of chloroform. The organic layer was separated, the water layer was extracted with chloroform (2×50 ml). The combined organic solutions were washed in succession with 50 ml of 1 M HCl solution and with water, then dried with anhydrous Na_2SO_4. The drying agent was filtered off, the solvent was evaporated on the rotary evaporator. The oily residue obtained was recrystallized from hexane. Yield 94%, R_f 0.61 (A), mp 159–162°C. ^{1}H (DMSO-d_6), δ, ppm: 1.47 s (9H), 4.67 s (4H), 7.59 d.d (1H, *J* 2.5, 8.4 Hz), 8.18 d.d (1H, *J* 1.9, 8.4 Hz), 8.23 s (1H). Mass spectrum, *m/z*: 265 [*M* + H].

4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutanoic acid (IVa). In dioxane was dissolved 5 g (18.9 mmol) of compound **II**. To the solution obtained 0.3 g Pd (3%) on carbon was added. Then to this suspension was added dropwise at 60°C 9.4 ml (18.9 mmol) of 50% hydrazine hydrate solution, the reaction mixture was stirred at the same temperature till the gas liberation ceased. Then the solvent was evaporated in a vacuum. The oily residue was dried for 3 h at 60°C (3 mm Hg). The residue was dissolved in 50 ml of chloroform. To the solution of compound **III** obtained was added a solution of 1.96 g (18.9 mmol) of succinic anhydride in 30 ml of chloroform, the mixture was boiled for 30 min and left standing for 6 h at room temperature. The separated precipitate was filtered off, washed with 30 ml of chloroform, and dried at 60°C. Yield 72%, R_f 0.6 (C), mp 205–207°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.49 s (9H), 2.62–2.70 m (4H), 4.58 d.d (4H, *J* 5.9, 10.0 Hz), 7.26 d.d (1H, *J* 4.8, 9.1 Hz), 7.53 d (1H, *J* 9.1 Hz), 7.75 d (1H, *J* 9.1 Hz), 10.12 s (1H). Mass spectrum, *m/z*: [*M* + H] 335.

5-(2-BOC-isoindolin-5-yl)amino-5-oxopentanoic acid (IVb) was prepared similarly. Yield 89%, R_f 0.73 (C), mp 122–125°C. ^{1}H NMR spectrum (DMTA-d_7), δ, ppm: 1.45 s (9H), 1.80 m (2H), 2.27 t (2H, *J* 7.4 Hz), 2.35 t (2H, *J* 7.4 Hz), 4.53 t (4H, *J* 8.1 Hz), 7.22 d.d (1H, *J* 4.0, 8.2 Hz), 7.38–7.45 m (1H), 7.63 d (1H, *J* 19.0 Hz), 9.94 s (1H), 12.09 s (1H). Mass spectrum, *m/z*: 349 [*M* + H].

***N*-[4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-β-alanine methyl ester (Va)**. To a suspension of 1 g (2.99 mmol) of compound **IVa** in chloroform was added 0.9 ml (6.58 mmol) of triethylamine. The mixture was cooled to –12°C, and isobutyl chloroformate was added thereto. The stirring continued for 30 min at –10°C. Then 0.459 g (3.29 mmol) of β-alanine methyl ester hydrochloride was added, the reaction mixture was stirred for 15 min at –10°C and 1 h at room temperature. The reaction mixture was diluted with 100 ml of chloroform, washed in succession with 50 ml of 1 M HCl solution, 50 ml of 5% $NaHCO_3$ solution, and 50 ml of water. The organic layer was dried with anhydrous Na_2SO_4. The drying agent was filtered off, the solvent was evaporated on the rotary evaporator. Yield 78%, R_f 0.45 (B), mp 145–148°C. ^{1}H NMR spectrum (DMF-d_7), δ, ppm:

1.49 s (9H), 2.50–2.56 m (4H), 2.66 t (2H, *J* 6.8 Hz), 3.41 q (2H, *J* 6.8 Hz), 3.63 s (3H), 4.58 d.d (4H, *J* 6.3, 9.8 Hz), 7.26 d.d (1H, *J* 4.8, 8.7 Hz), 7.53 d (1H, *J* 8.7 Hz), 7.75 d (1H, *J* 8.7 Hz), 8.00 t (1H, *J* 5.6 Hz), 10.08 s (1H). Mass spectrum, *m/z*: 420 [*M* + H].

***N*-[4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-β-phenyl-β-alanine methyl ester (Vb)** was prepared analogously. Yield 82%, R_f 0.52 (B), oily substance. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.45 s (9H), 2.41–2.57 m (4H), 2.73–2.77 m (2H), 3.55 s (3H), 4.53 t (4H, *J* 7.3 Hz), 5.22 q (1H, *J* 7.8 Hz), 7.19–7.33 m (6H), 7.40 d.d (1H, *J* 1.4, 8.4 Hz), 7.61 d (1H, *J* 19.6 Hz), 8.47 d (1H, *J* 8.4 Hz), 9.97 s (1H). Mass spectrum, *m/z*: 496 [*M* + H].

***N*-[4-(N-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-β-alanine (VIa)**. (a) In 30 ml of ethanol was dissolved 0.5 g (1.19 mmol) of compound **Va**, and to the solution obtained 1 ml of 1 M NaOH solution was added. The mixture was stirred for 1 h at room temperature, then it was poured into water, acidified with 1 M HCl solution till acidic reaction, and the reaction product was extracted into chloroform (3×50 ml). The combined organic solutions were washed with water and dried with anhydrous Na_2SO_4. The drying agent was filtered off, the solvent was evaporated on the rotary evaporator. The residue obtained was ground with anhydrous ethyl ether. Yield 86%, R_f 0.70 (C), mp 142–148°C. ^{1}H NMR spectrum (DMF-d_7), δ, ppm: 1.49 s (9H), 2.47–2.56 m (4H), 2.67 t (2H, *J* 6.8 Hz), 3.40 q (2H, *J* 6.8 Hz), 4.58 d.d (4H, *J* 6.7, 9.4 Hz), 7.26 m (1H), 7.53 d (1H, *J* 8.1 Hz), 7.75 d (1H, *J* 8.1 Hz), 7.97 t (1H, *J* 5.6 Hz), 10.10 s (1H). Mass spectrum, *m/z*: 406 [*M* + H], 428 [*M* + Na].

***N*-[4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-β-phenyl-β-alanine (VIb)** was obtained in the same way. Yield 84%, R_f 0.81 (C), mp 138–141°C. ^{1}H NMR spectrum (DMF-d_7), δ, ppm: 1.49 s (9H), 2.57–2.69 m (4H), 2.83 m (2H), 4.58 t (4H, *J* 6.1 Hz), 5.39 q (1H, *J* 7.5 Hz), 7.21–7.45 m (6H), 7.53 d (1H, *J* 8.1 Hz), 7.72 d (1H, *J* 8.1 Hz), 8.46 d (1H, *J* 8.1 Hz), 10.07 s (1H). Mass spectrum, *m/z*: 482 [*M* + H], 504 [*M* + Na].

***N*-[4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-β-alanine (VIa)**. *b.* To a solution of 0.50 g (1.5 mmol) of compound **IVa** in 5 ml of DMF cooled to –10°C was added 0.21 ml (1.5 mmol) of triethylamine, then 0.2 ml (1.5 mmol) isobutyl chloroformate. The reaction mixture was stirred for 30 min at –10°C, then 0.19 g (1.7 mmol) of β-alanine sodium salt was added. The mixture was stirred for 30 min at –10°C, then 2 h more at room temperature. On completion of the reaction the mixture was diluted with water, acidified with with 1 M HCl solution till acidic reaction, and the reaction product was extracted into chloroform (3×50 ml). The combined organic solutions were washed with water (30 ml) and dried with anhydrous Na_2SO_4. The drying agent was filtered off, the solvent was evaporated on the rotary evaporator. The oily residue was ground with ether. Yield 82%.

Likewise were prepared compounds **VIb–VII**.

***N*-[4-(*N*-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-phenyl-β-alanine (VIb)**. Yield 77%.

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-methoxyphenyl)-β-alanine (VIp)**. Yield 75%, R_f 0.70 (C), mp 155–160°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.49 s (9H), 2.54–2.68 m (4H), 2.78–2.86 m (2H), 3.76 s (3H), 4.58 t (4H, *J* 7.4 Hz), 5.32 q (1H, *J* 7.6 Hz), 6.87 d (1H, *J* 8.7 Hz), 7.25 d.d (1H, *J* 5.0, 8.1 Hz), 7.33 d (1H, *J* 8.7 Hz), 7.51 d (1H, *J* 8.1 Hz), 7.71 d (1H, *J* 8.1 Hz), 8.37 d (1H, *J* 7.6 Hz), 10.06 s (1H). Mass spectrum, *m/z*: 512 [*M* + H].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-ethoxyphenyl)-β-alanine (VId)**. Yield 74%, R_f 0.69 (C), mp 205–210°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.32 t (3H, *J* 7.0 Hz), 1.49 s (9H), 2.53–2.67 m (4H), 2.77–2.86 m (2H), 3.99 q (2H, *J* 7.0 Hz), 4.57 t (4H, *J* 6.5 Hz), 5.31 q (1H, *J* 7.8 Hz), 6.84 d (2H, *J* 8.7 Hz), 7.24–7.28 m (1H), 7.32 d (1H, *J* 8.6 Hz), 7.52 d (1H, *J* 8.6 Hz), 7.72 (1H, *J* 8.1 Hz), 8.42 d (1H, *J* 8.4 Hz), 10.10 s (1H). Mass spectrum, *m/z*: 526 [*M* + H], 548 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-propoxyphenyl)-β-alanine (VIe)**. Yield 81%, R_f 0.77 (B), oily substance. Mass spectrum, *m/z*: 540 [*M* + H], 562 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isopropoxyphenyl)-β-alanine (VIf)**. Yield 78%, R_f 0.73 (C), mp 146–152°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.25 d (6H, *J* 5.9 Hz), 1.49 s (9H), 2.54–2.68 m (4H), 2.78–2.87 m (2H), 4.54–4.60 m (5H), 5.31 q (1H, *J* 7.5 Hz), 6.83 d (2H, *J* 8.6 Hz,), 7.24–7.28 m (1H), 7.32 d (1H, *J* 8.6 Hz), 7.52 d (1H, *J* 8.4 Hz), 7.73 (1H, *J* 8.4 Hz), 8.41 d (1H, *J* 8.1 Hz), 10.10 s (1H). Mass spectrum, *m/z*: 540 [*M* + H], 562 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-butoxyphenyl)-β-alanine (VIg)**. Yield 80%, R_f 0.75 (C), oily substance. ^{1}H NMR spectrum

(DMSO-d_6), δ, ppm: 0.94 t (3H, *J* 7.5 Hz), 1.41–1.46 m (2H), 1.49 s (9H), 1.65–1.74 m (2H), 2.54–2.67 m (4H), 2.77–2.85 m (2H), 3.94 t (2H, *J* 6.4 Hz), 4.57 t (4H, *J* 6.5 Hz), 5.32 q (1H, *J* 7.4 Hz), 6.85 d (2H, *J* 8.7 Hz), 7.23–7.28 m (1H), 7.32 d (1H, *J* 8.6 Hz), 7.51 d (1H, *J* 7.6 Hz), 7.72 (1H, *J* 7.61 Hz), 8.37 d (1H, *J* 8.1 Hz), 10.16 s (1H). Mass spectrum, *m/z*: 554 [*M* + H], 576 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isobutyloxyphenyl)-β-alanine (VIh)**. Yield 74%, R_f 0.69 (B), mp 183–190°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.98 d (6H, *J* 6.5 Hz), 1.49 s (9H), 1.94–2.06 m (1H), 2.54–2.70 m (4H), 2.78–2.86 m (2H), 3.71 d (2H, *J* 6.2 Hz), 4.57 t (4H, *J* 6.5 Hz), 5.31 q (1H, *J* 7.5 Hz), 6.85 d (2H, *J* 8.5 Hz), 7.24–7.28 m (1H), 7.32 d (1H, *J* 8.5 Hz), 7.52 d (1H, *J* 8.1 Hz), 7.72 (1H, *J* 8.1 Hz), 8.42 d (1H, *J* 8.7 Hz), 10.10 s (1H). Mass spectrum, *m/z*: 554 [*M* + H], 576 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isopentyloxyphenyl)-β-alanine (VIi)**. Yield 73%, R_f 0.72 (C), oily substance. Mass spectrum, *m/z*: 568 [*M* + H], 590 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(3,4-dimethoxyphenyl)-β-alanine (VIj)**. Yield 76%, R_f 0.62 (C), mp 138–146°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.49 s (9H), 2.52–2.69 m (4H), 2.78–2.88 m (2H), 3.77 s (3H), 3.80 s (3H), 4.57 t (4H, *J* 6.5 Hz), 5.33 q (1H, *J* 7.7 Hz), 6.86–6.94 m (2H), 7.06–7.09 m (1H), 7.23–7.27 m (1H), 7.51 d (1H, *J* 8.5 Hz), 7.70 d (1H, *J* 8.5 Hz), 8.38 d (1H, *J* 8.4 Hz), 10.06 s (1H). Mass spectrum, *m/z*: 542 [*M* + H], 564 [*M* + Na].

***N*-[4-(2-Boc-isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(3,4-methylenedioxyphenyl)-β-alanine (VIk)**. Yield 76%, R_f 0.63 (B), mp 129–132°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.49 s (9H), 2.54–2.70 m (4H), 2.78–2.87 m (2H), 4.57 t (4H, *J* 6.5 Hz), 5.14 q (1H, *J* 7.6 Hz), 6.02 s (2H), 6.79–6.90 m (2H), 6.99 s (1H), 7.23–7.27 m (1H), 7.51 d (1H, *J* 7.8 Hz), 7.72 d (1H, *J* 7.8 Hz), 8.38 d (1H, *J* 8.4 Hz), 10.05 s (1H). Mass spectrum, *m/z*: 526 [*M* + H], 548 [*M* + Na].

***N*-[5-(2-Boc-isoindolin-5-yl)amino-5-oxopentanoyl]-β-alanine (VII)**. Yield 68%, R_f 0.61 (C), mp 95–97°C. ^{1}H NMR spectrum (DMF-d_7), δ, ppm: 1.49 s (9H), 1.92 m (2H), 2.24 t (2H, *J* 7.3 Hz), 2.40 t (2H, *J* 7.3 Hz), 2.48 t (2H, *J* 6.7 Hz), 3.40 q (2H, *J* 6.7 Hz), 4.55–4.60 m (4H), 7.24–7.28 m (1H), 7.74 d (1H, *J* 6.5 Hz), 7.02 t (1H, *J* 5.0 Hz), 10.02 s (1H). Mass spectrum, *m/z*: 420 [*M* + H], 442 [*M* + Na].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-β-alanine hydrochloride (VIIa)**. A suspension of 0.20 g (0.49 mmol) of compound **VIa** in 10 ml of 4 M HCl solution in dioxane was stirred for 1 h at room temperature and then evaporated. The solid residue was dried for 5 h at 40°C (3 mm Hg). Yield 98%, R_f 0.60 (D), mp 197–200°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.35–2.41 m (4H), 2.53–2.61 m (2H), 3.23 q (2H, *J* 6.2 Hz), 4.40–4.48 m (4H), 7.29 d (1H, *J* 8.2 Hz), 7.48 d (1H, *J* 8.2 Hz), 7.75 s (1H), 8.02 t (1H, *J* 4.7 Hz), 10.06 s (2H), 10.22 s (1H). Mass spectrum, *m/z*: 306 [*M* + H], 328 [*M* + Na].

Likewise were synthesized compounds **VIIb–VIIl**.

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-phenyl-β-alanine hydrochloride (VIIb)**. Yield 99%, R_f 0.63 (D), mp 150–153°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.42–2.56 m (4H), 2.6–2.69 m (2H), 4.40–4.47 m (4H), 5.39 q (1H, *J* 7.3 Hz), 7.19–7.34 m (6H), 7.47 d (1H, *J* 8.1 Hz), 7.74 s (1H), 8.51 d (1H, *J* 8.1 Hz), 10.05 t (2H, *J* 5.4 Hz), 10.20 s (1H). Mass spectrum, *m/z*: 382 [*M* + H], 404 [*M* + Na].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-methoxyphenyl)-β-alanine hydrochloride (VIIc)**. Yield 95%, R_f 0.61 (D), hygroscopic substance, mp 128–131°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.39–2.45 m (2H), 2.51–2.56 m (2H), 2.63 d.d (2H, *J* 4.7, 7.6 Hz), 3.72 s (3H), 4.41–4.47 m (4H), 5.12 q (1H, *J* 7.6 Hz), 6.84 d (1H, *J* 8.5 Hz), 7.23 d (1H, *J* 8.5 Hz), 7.29 d (1H, *J* 8.3 Hz), 7.45 d (1H, *J* 8.3 Hz), 7.74 s (1H), 8.38 d (1H, *J* 8.1 Hz), 9.78 s (2H), 10.14 s (1H), 12.19 br.s (1H). Mass spectrum, *m/z*: 412 [*M* + H], 434 [*M* + Na], 456 [*M* + 2Na – H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-ethoxyphenyl)-β-alanine hydrochloride (VIId)**. Yield 96%, R_f 0.63 (D), mp 121–127°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.30 t (3H, *J* 6.7 Hz), 2.36–2.44 m (2H), 2.54–2.66 m (4H), 3.97 q (2H, *J* 6.7 Hz), 4.40–4.46 m (4H), 5.12 q (1H, *J* 7.5 Hz), 6.82 d (2H, *J* 8.4 Hz), 7.20 d (2H, *J* 8.4 Hz), 7.28 d (1H, *J* 8.2 Hz), 7.46 d (1H, *J* 8.2 Hz), 7.73 s (1H), 8.402 d (1H, *J* 8.4 Hz), 9.99 s (2H), 10.18 s (1H). Mass spectrum, *m/z*: 426 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-propoxyphenyl)-β-alanine hydrochloride**

(VIIe). Yield 94%, R_f 0.62 (D), hygroscopic substance. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.96 t (3H, *J* 7.3 Hz), 1.64–1.76 m (2H), 2.39–2.45 m (4H), 2.64 t (2H, *J* 6.0 Hz), 3.88 t (2H, *J* 6.4 Hz), 4.40–4.47 m (4H), 5.12 q (1H, *J* 7.6 Hz), 6.84 d (2H, *J* 8.4 Hz), 7.22 d (2H, *J* 8.4 Hz), 7.30 d (1H, *J* 8.4 Hz), 7.44 C (1H), 8.39 d (1H, *J* 8.4 Hz), 9.94 C (2H), 10.17 C (1H). Mass spectrum, *m/z*: 440 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isopropoxyphenyl)-β-alanine hydrochloride (VIIf)**. Yield 94%, R_f 0.62 (D), mp 150–152°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.25 d (6H, *J* 6.0 Hz), 2.40–2.45 m (2H), 2.52–2.69 m (4H), 4.40–4.47 m (4H), 4.55 m (1H), 5.12 q (1H, *J* 7.3 Hz), 6.82 d (2H, *J* 8.5 Hz), 7.20 d (2H, *J* 8.5 Hz), 7.30 d (1H, *J* 8.4 Hz), 7.46 d (1H, *J* 8.4 Hz), 7.74 s (1H), 8.39 (1H, *J* 8.1 Hz), 9.99 br.s (2H), 10.17 s (1H). Mass spectrum, *m/z*: 440 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-butoxyphenyl)-β-alanine hydrochloride (VIIg)**. Yield 92%, R_f 0.61 (D), hygroscopic substance. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.92 t (3H, *J* 7.3 Hz), 1.35–1.47 m (2H), 1.62–1.71 m (2H), 2.39–2.43 m (2H), 2.52–2.55 m (2H), 2.60–2.68 m (2H), 3.91 t (2H, *J* 6.4 Hz), 4.41–4.46 m (4H), 5.12 q (1H, *J* 7.8 Hz), 6.84 d (2H, *J* 8.7 Hz), 7.21 d (2H, *J* 8.7 Hz), 7.29 d (1H, *J* 8.1 Hz), 7.45 d (1H, *J* 8.1 Hz), 7.73 s (1H), 8.41 d (1H, *J* 8.1 Hz), 10.03 s (2H), 10.19 s (1H). Mass spectrum, *m/z*: 454 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isobutyloxyphenyl)-β-alanine hydrochloride (VIIh)**. Yield 95%, R_f 0.76 (D), mp 133–136°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.92 d (6H, *J* 6.5 Hz), 1.91–2.05 m (1H), 2.39–2.45 m (2H), 2.52–2.72 m (4H), 3.70 d (2H, *J* 6.5 Hz), 4.41–4.48 m (4H), 5.12 q (1H, *J* 7.5 Hz), 6.85 d (2H, *J* 8.4 Hz), 7.22 d (2H, *J* 8.4 Hz), 7.29 d (1H, *J* 8.3 Hz), 7.46 d (1H, *J* 8.3 Hz), 7.74 s (1H), 8.39 d (1H, *J* 8.1 Hz), 10.01 s (2H), 10.17 s (1H). Mass spectrum, *m/z*: 454 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(*p*-isopentyloxyphenyl)-β-alanine hydrochloride (VIIi)**. Yield 97%, R_f 0.67 (D), hygroscopic substance. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.92 d (6H, *J* 6.5 Hz), 1.59 q (2H, *J* 6.5 Hz), 1.70–1.81 m (1H), 2.38–2.44 m (2H), 2.52–2.71 m (4H), 3.05 t (2H, *J* 6.5 Hz), 4.40–4.47 m (4H), 5.12 q (1H, *J* 7.2 Hz), 6.84 d (2H, *J* 8.2 Hz), 7.22 d (2H, *J* 8.2 Hz), 7.29 d (1H, *J* 8.0 Hz), 7.46 d (1H, *J* 8.0 Hz), 7.74 s (1H), 8.37 d (1H, *J* 8.1 Hz), 9.92 s (2H), 10.15 s (1H). Mass spectrum, *m/z*: 468 [*M* + H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(3,4-dimethoxyphenyl)-β-alanine hydrochloride (VIIj)**. Yield 95%, R_f 0.620 (D), mp 131–137°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.40–2.46 m (2H), 2.52–2.57 m (2H), 2.64 d (2H, *J* 7.6 Hz), 3.70 s (3H), 3.73 C (3H), 4.40–4.47 m (4H), 5.12 q (1H, *J* 7.6 Hz), 6.78–7.87 m (2H), 6.94 s (1H), 7.20 d (1H, *J* 8.1 Hz), 7.44 d (1H, *J* 8.1 Hz), 7.73 s (1H), 8.39 d (1H, *J* 8.4 Hz), 9.90 t (1H, *J* 4.5 Hz), 10.17 s (1H). Mass spectrum, *m/z*: 442 [*M* + H], 464 [*M* + Na], 486 [*M* + 2Na – H].

***N*-[4-(isoindolin-5-yl)amino-4-oxobutyryl]-D,L-β-(3,4-methylenedioxyphenyl)-β-alanine hydrochloride (VIIk)**. Yield 95%, R_f 0.70 (D), mp 135–138°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.39–2.44 m (2H), 2.53–2.56 m (2H), 2.61–2.65 (2H), 4.40–4.47 m (4H), 5.10 q (1H, *J* 7.7 Hz), 5.97 s (2H), 6.75–6.90 m (3H), 7.29 d (1H, *J* 8.2 Hz), 7.45 d (1H, *J* 8.2 Hz), 7.74 s (1H), 8.39 d (1H, *J* 9.0 Hz), 9.90 s (2H), 10.15 s (1H). Mass spectrum, *m/z*: 426 [*M* + H].

***N*-[5-(isoindolin-5-yl)amino-5-oxopentanoyl]-β-alanine hydrochloride (VIIl)**. Yield 93%, R_f 0.61 (D), hygroscopic substance. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.79 m (2H), 2.11 t (2H, *J* 7.3 Hz), 2.30–2.40 m (4H), 3.23 q (2H, *J* 6.3 Hz), 4.40–4.48 m (4H), 7.29 d (1H, *J* 8.4 Hz), 7.48 d (1H, *J* 8.4 Hz), 7.77 s (1H), 7.95 t (1H, *J* 5.3 Hz), 10.05 s (2H), 10.16 s (1H). Mass spectrum, *m/z*: 320 [*M* + H].

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