Novel Fibrinogen Receptor Antagonists – RGDF Mimetics, 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric Acid Derivatives

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Abstract: Two novel RGDF mimetics were synthesized with the use of 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid as a new surrogate of Arg-Gly motif. The synthesized compounds have demonstrated a high potency to inhibit platelet aggregation *in vitro* and to block FITC-Fg binding to $\alpha_{IIb}\beta_3$ on washed human platelets.

Key Words: Fibrinogen receptor antagonists, $\alpha_{IIb}\beta_3$, GP IIb/IIIa, RGD mimetics, 1,2,3,4-tetrahydro-isoquinoline, platelet aggregation.

INTRODUCTION

Occlusive thrombi formation plays a key role in progression of the cardio-vascular diseases (myocardial infarction, ischemic stroke, thromboembolisms). For prophylaxis and treatment of the thromboses, anticoagulants, fibrinolytics and antiaggregants are applied. The latter inhibit aggregation and adhesion of platelets. The antagonists of fibrinogen receptors ($\alpha_{IIb}\beta_3$) arranged on the platelet surface have gained a greatest interest among antiaggregants. These receptors are glycoprotein complexes (GP IIb/IIIa), which belong to the integrin superfamily. Formation of supramolecular complexes fibrinogen-$\alpha_{IIb}\beta_3$ leads to platelet aggregation and platelet thrombi formation. The sequence Arg-Gly-Asp (RGD) is responsible for the binding of fibrinogen to its receptor. RGD mimetics are of great interest for researchers as perspective and available synthetic $\alpha_{IIb}\beta_3$ antagonists. The design of RGD mimetics is based on the bioisosteric replacement of RGD sequence fragments.

Bioisosteres of arginine side function, fragments containing the residues of *p*-benzamidine, piperidine, *p*-benzguanidine, *p*-benzmethyleneamine etc. [1], are used for obtaining of arginine surrogates for the design of potent and selective RGDF mimetics, $\alpha_{IIb}\beta_3$ receptor antagonists.

In particular, *p*-benzoylamidine moiety was proposed as an arginine mimetic by the authors [2, 3] who had synthesized the derivatives based on 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold, which were found to be potent $\alpha_{IIb}\beta_3$ antagonists.

It was reported by the authors [4] about obtaining of the series of RGDF mimetics – ABAS, based on (aminobenzamidino)succinyl (**1**) as Arg-Gly surrogate. Subsequently, the novel Arg-Gly isostere, the fragment of 4-(isoindoline-5-yl)amino-4-oxobutyric acid (**2**) (Fig. **1**), was proposed by us, and potent $\alpha_{IIb}\beta_3$ antagonists - RGDF mimetics, containing this acid residue, were synthesized in our laboratory [5]. Tetrahydroquinolines cores are used for replacement of Arg δ-guanidine group upon the design of $\alpha_V\beta_3$ antagonists [6]. However, there is no information concerning application of 1,2,3,4-tetrahydroquinoline cycles for Arg δ-guanidine modeling upon the creation of $\alpha_{IIb}\beta_3$ antagonists.

Now, the fragment of 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid (**3**) (Fig. **1**) is proposed by us as Arg-Gly mimetic in the design of potentially active $\alpha_{IIb}\beta_3$ antagonists. The synthesis of this Arg-Gly surrogate is sufficiently simple and short-staged.

It is considered that the residue of β-substituted β-amino acid simulates optimally Asp-Phe moiety [1, 7]. The carboxylic group of β-amino acid imitates the aspartic acid side chain. The aim of present research is to estimate the possibility of use of 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid (**3**) residue for obtaining of novel RGDF mimetics - $\alpha_{IIb}\beta_3$ receptor antagonists, and also, to propose the utilization of noted above Arg-Gly surrogate for the further design of potent $\alpha_{IIb}\beta_3$ receptor antagonists.

METHODS AND MATERIALS

The ^{1}H-NMR spectra were obtained in DMSO-d_6 (99.9%) at 25 °C on a Varian WSP-300 spectrometer operating at 299.95 MHz, using TMS as an internal standard. Mass-spectra FAB were recorded on a VG 7070 apparatus using glycerol matrix, ionization was achieved by Xe atoms beam with the energy of 8 kV.

2-Boc-7-nitro-1,2,3,4-tetrahydro-isoquinoline (5)

To a solution of **4** [8] (4 g, 0.0186 mol) in water (20 mL) was added NaOH (1 g, 0.025 mol), and then a solution of Boc_2O (4.06 g, 0.0186 mol) in chloroform (50 mL) was added. The mixture was stirred at room temperature for 2 h. The organic layer was separated and successively washed with water (20 mL), 1 M solution of HCl (20 mL), and 5% solution of $NaHCO_3$ (30 mL), respectively. Then chloroform

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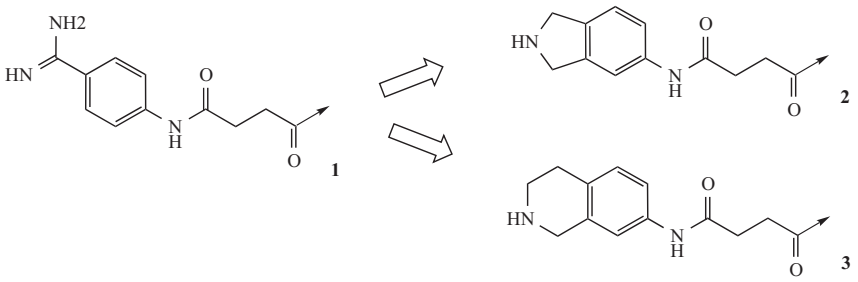


Fig. (1). Structures of Arg-Gly mimetics: (aminobenzamidino)succinyl (**1**), fragments of 4-(isoindoline-5-yl)amino-4-oxobutyric (**2**) and 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acids (**3**).

phase was dried over Na_2SO_3, filtered and evaporated to dryness. Yield 98%. Mp 93-95 °C. R_f = 0.46 (20% ethyl acetate/hexane). ^{1}H NMR (300 MHz, DMSO-d_6) 1.44 (s, 9H), 2.90 (t, *J* = 5.8 Hz, 2H), 3.58 (t, *J* = 5.8 Hz, 2H), 4.63 (s, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 8.02 (dd, *J* = 2.5 Hz, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 2.5 Hz, 1H). FAB-MS 301 (M+Na, 100%).

2-Boc-7-amino-1,2,3,4-tetrahydro-isoquinoline (6)

To a solution of **5** (2 g, 0.0072 mol) in ethyl alcohol (30 mL) was added 3% Pd – C (0.1 g). This mixture was brought to boiling under stirring, and 95% hydrazine hydrate (50 ml) was added dropwise. After addition was completed, the reaction mixture was refluxed for additional 30 min, the solvent was evaporated to dryness, and the oily residue was dried at 50 °C and the pressure of 2 mm of Hg for 1 h. Yield 95%. Mp 59-61 °C. R_f = 0.43 (30% ethyl acetate/hexane). ^{1}H NMR (300 MHz, DMSO-d_6) 1.42 (s, 9H), 2.57 (t, *J* = 5.9 Hz, 2 H), 3.48 (t, *J* = 5.9 Hz, 2H), 4.32 (s, 2H), 4.88 (s, 2H), 6.31 (s, 1H), 6.40 (dd, *J* = 2.2 Hz, *J* = 8.1 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H). FAB-MS 247 (M-H, 51%).

4-(2-Boc-1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid (7)

A solution of **6** (1 g, 0.004 mol) and succinic anhydride (0.4 g, 0.004 mol) in abs. chloroform (20 mL) was refluxed for 1 h. Then the solution was successively washed with water (20 mL), 1 M HCl (20 mL), and water (20 mL), respectively. The organic phase was separated, dried over Na_2SO_4, filtered and evaporated to dryness. Yield 90%. The oil. R_f = 0.38 (100:50:1, benzene/acetone/acetic acid). ^{1}H NMR (300 MHz, DMSO-d_6) 1.42 (s, 9H), 2.49 – 2.54 (m, 4H), 2.70 (t, *J* = 5.8 Hz, 2H), 3.52 (t, *J* = 5.8 Hz, 2H), 4.44 (s, 2H), 7.06 (d, *J* = 8.1 Hz, 1H), 7.28 – 7.31 (m, 1H), 7.45 (s, 1H), 9.89 (s, 1H), 12.12 (s, 1H). FAB-MS 349 (M+H, 42%), 372 (M+Na, 15%).

4-(2-Boc-1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyryl-β-alanine (8a)

A solution of **7** (1 g, 0.0029 mol), SuOH (0.33 g, 0.0029 mol) and DCC (0.59 g, 0.0029 mol) in abs. acetonitrile (10 mL) was stirred for 30 min. The resulting precipitate was removed by filtration, and the filtrate was evaporated under vacuum in such a way that the temperature did not exceed 20 °C. The oily residue was redissolved in chloroform (50 mL) and successively washed with water (20 mL), 1 M HCl (20 mL), and 5% solution of $NaHCO_3$ (30 mL), respectively. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to dryness to give oily product 4-(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-yl-amino)-4-oxo-butyric acid succinimide ether. (84 % yield).

To a solution of $NaHCO_3$ (0.132 g, 0.0016 mol) and β-alanine (0.137 g, 0.0016 mol) in water (10 mL) was added in a stream a solution of 4-(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-yl-amino)-4-oxo-butyric acid succinimide ether (0.5 g, 0.0011 mol) in *iso*-propyl alcohol (10 mL). The mixture was stirred at room temperature for 6 h and concentrated under vacuum. The oily residue was dissolved in chloroform (50 mL) and washed sequentially by water (20 mL), 1 M HCl solution (20 mL) and 5 % $NaHCO_3$ solution (30 mL). The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by flash-chromatography on Kieselgel 60 (Merk) using the eluents: chloroform and 5 % solution of ethyl alcohol in chloroform (by volume). The fractions, containing the pure product, were combined and evaporated. The residue was dried under vacuum over $CaCl_2$. Yield 75 %. The oil. R_f = 0.62 (9:3:2, chloroform/ethyl acetate/methyl alcohol). ^{1}H NMR (300 MHz, DMSO-d_6) 1.42 (s, 9H), 2.37 (t, *J* = 6.9 Hz, 4H), 2.49 – 2.55 (m, 2H), 2.70 (t, *J* = 5.6 Hz, 2H), 3.24 (q, *J* = 6.4 Hz, 2H), 3.53 (t, *J* = 5.6 Hz, 2H), 4.44 (s, 2H), 7.06 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.43 (s, 1H), 7.93 (s, 1H), 9.85 (s, 1H), 12.18 (s, 1H). FAB-MS 420 (M+H, 70%), 442 (M+Na, 11%).

4-(2-Boc-1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyryl-D,L-β-phenyl-β-alanine (8b)

Yield 81%. The oil. R_f = 0.58 (9:3:2, chloroform/ethyl acetate/methyl alcohol). ^{1}H NMR (300 MHz, DMSO-d_6) 1.42 (s, 9H), 2.43 – 2.54 (m, 4H), 2.66 – 2.71 (m, 4H), 3.52 (t, *J* = 5.4 Hz, 2H), 4.44 (s, 2H), 5.19 (q, *J* = 7.4 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 7.21 – 7.44 (m, 7H), 8.43 (d, *J* = 8.4 Hz, 1H), 9.87 (s, 1H), 12.24 (s, 1H). FAB-MS 296 (M+H, 73%).

4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyryl-β-alanine hydrochloride (9a)

Boc-derivative of **8a** (0.3 g, 0.0007 mol) was suspended in 4 M solution of HCl in dioxane (10 mL) and stirred at room temperature for 1 h. Then the solvent was evaporated,

and the residue was dried at 40 °C and a pressure of 2 mm of Hg for 5 h. Yield 96%. The substance is hygroscopic. R_f = 0.47 (20% ammonia/methyl alcohol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 – 2.37 (m, 4H), 2.49 – 2.52 (m, 2H), 2.90 – 2.93 (m, 2H), 3.20 -3.29 (m, 4H), 4.17 (s, 2H), 7.09 (d, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.49 (s, 1H), 7.97 (s, 1H), 9.63 (s, 2H), 10.08 (s, 1H). FAB-MS 320 (M+H, 96%), 342 (M+Na, 65%), 364 (M-H+2Na, 10%).

4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyryl-D,L-β-phenyl-β-alanine hydrochloride (9b)

Yield 98%. The substance is hygroscopic. R_f = 0.45 (20% ammonia/methyl alcohol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.41 – 2.54 (m, 4H), 2.67 (d, *J* = 6.9 Hz, 2H), 2.93 (t, *J* = 5.8 Hz, 2H), 3.30 – 3.33 (m, 2H), 4.20 (s, 2H), 5.18 (q, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.20 – 7.31 (m, 6H), 7.50 (s, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 9.52 (s, 2H), 10.06 (s, 1H). FAB-MS 396 (M+H, 100%).

***In vitro* Inhibition of Platelet Aggregation in Human Platelet-Rich Plasma**

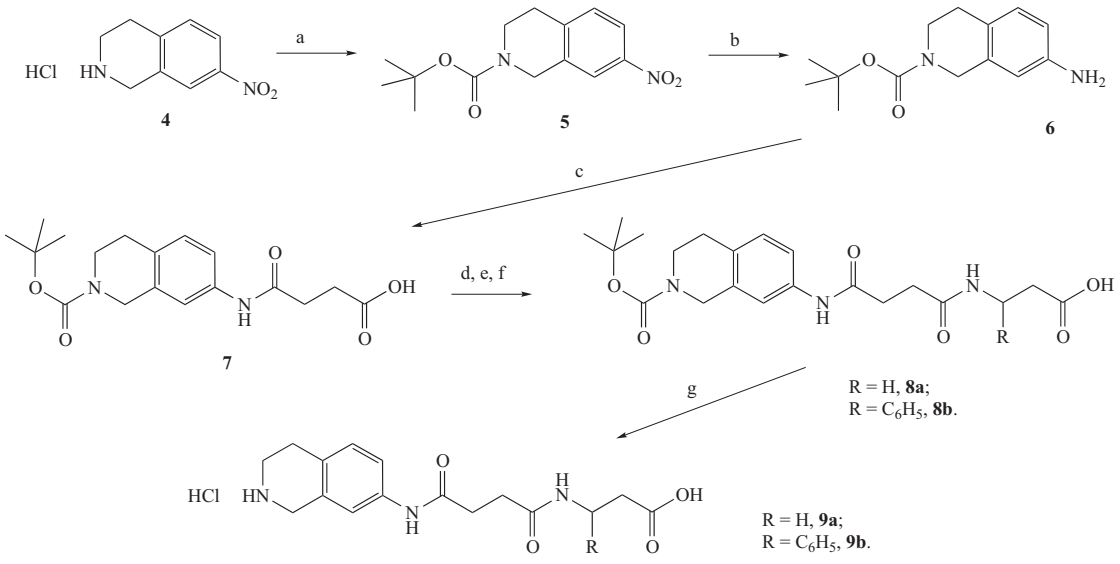
Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 150 g for 10 min, and the platelet count was adjusted to $1 \cdot 10^8$ platelets/mL with time matched platelet-poor plasma (PPP). PRP (250 µl) was preincubated with 50 µl of various concentrations of compounds to be tested, or saline, for 2 min at 37 °C prior to the addition of ADP (10 µl). Platelet aggregation was measured by the change of light transmittance (PPP represents 100%) under stirring conditions (1000 rpm) on a "THROMLITE-1006 A" aggregometer. The ability of the compounds to inhibit platelet aggregation was measured and the IC_{50} was determined as the concentration of the compound required to produce 50% inhibition of the response to ADP.

***In vitro* Inhibition of Fluorescein Isothiocyanate-Labeled Fibrinogen (FITC-Fg) Binding to Activated Human Platelets**

Fibrinogen (Sigma) was labeled with fluorescein isothiocyanate (ACROS) according to Hantgan R. [9]. Fresh platelet concentrate was centrifuged (900g, 15 min), and the platelet pellet was washed at pH 5.6 in the presence of PGE_1 and finally resuspended carefully in Tyrode's buffer, containing BSA, pH 7.4. Platelet suspension was incubated for 30 min with peptidomimetics at concentrations in the range from 0.001 nM to 1 µM at 37 °C. FITC-Fg was added after addition of agonist (ADP, 2 µM). Following 60 min incubation at room temperature (under protection with the light), the reaction mixtures were layered onto a 20% sucrose cushion and centrifuged at 6000 rpm for 15 min. The tips of each tube were clipped off, and the platelet pellet, containing the bound fibrinogen, solubilized with a 3% SDS solution. The bound FITC-Fg was quantitated spectrofluorimetrically.

RESULTS AND DISCUSSION

7-Nitro-1,2,3,4-tetrahydro-isoquinoline (**4**) [8] was used as a starting compound for the synthesis of RGDF mimetics based on 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid. Amino group of the compound **4** was protected with Boc_2O. The reduction of nitro group of the compound **5** and acylation of amino derivative **6** with succinic anhydride led to the 4-(2-Boc-1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid (**7**). The activation of carboxylic group of the compound **7** using DCC and SuOH, followed by interaction with the corresponding β-amino acids in the presence of $NaHCO_3$, gave the Boc derivatives **8a** and **8b**. Final deprotection afforded the target compounds **9a** and **9b** (Scheme **1**).



Scheme 1. Synthesis of the target compounds **9a** and **9b**. a) Boc_2O; b) $N_2H_4 \cdot H_2O$, 3% Pd on carbon; c) succinic anhydride; d) DCC, SuOH; e) β-amino acids, $NaHCO_3$, H_2O; f) 1 N HCl in H_2O; g) 4 M solution of HCl in dioxane.

The obtained RGDF mimetics **9a** and **9b** are chemically stable under normal conditions and have a high solubility in water, that makes them more convenient for *in vitro* bioassays [10]. Synthesized RGDF mimetics displayed a high *in vitro* antiaggregative activity on human blood platelet rich plasma. The IC_{50} values are of 0.030 ± 0.0016 μM (for **9a**) and 0.013 ± 0.0010 μM (for **9b**). The experiments were carried out by Born's method on blood samples, obtained from at least three different donors [11]. The ADP (10 μM final concentration) was used as platelet aggregation inductor.

In order to reveal the molecular mechanism of antiaggregative action possessed by RGDF mimetics of general formula **9**, it was examined their influence on fluorescein isothiocyanate labeled fibrinogen (FITC-Fg) binding to its receptor in the suspension of washed human platelets by Xia Z *et al*. procedure [12]. FITC-Fg was obtained by Hantgan R [9]. It specifically bound to its platelet receptor with the dissociation constant (K_d) of 1.02 μM. The compounds **9a** and **9b** were established to block FITC-Fg binding to $\alpha_{IIb}\beta_3$ on human platelets with IC_{50} values of 0.0012 ± 0.00014 μM (for **9a**) and 0.0010 ± 0.00012 μM (for **9b**). The binding curves for **9a** and **9b** are shown in Fig. **2**.

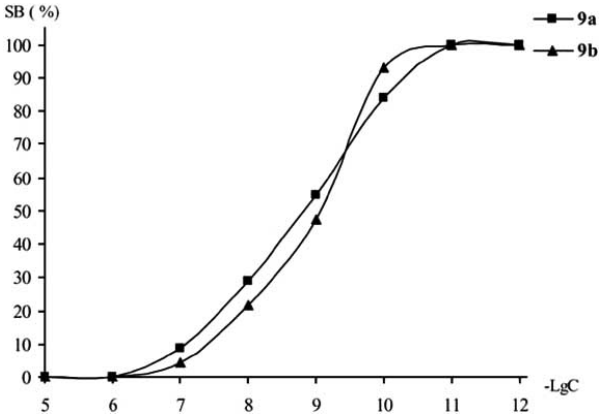
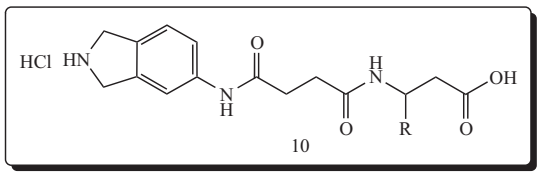


Fig. (2). Inhibition of FITC-Fg binding to ADP-stimulated $\alpha_{IIb}\beta_3$ on human platelets by compounds **9a** (■) and **9b** (▲).

It should be emphasized that 2-fold increase in the antiaggregative activity was achieved with phenyl introduction in β-position of β-amino acid residue. The case of derivatives (**10**) of 4-(isoindoline-5-yl)amino-4-oxobu-tyric acid obtained by us earlier gives the opportunity to trace the similar effect: the compound without a substituent in β-position of β-amino acid inhibited aggregation in human platelet rich plasma with IC_{50} of 2.75 μM, and the introduction of phenyl in those position led to improved activity with an IC_{50} value of 0.86 μM [5].



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Data obtained for the synthesized RGDF mimetics on their antiaggregative effects and affinities for fibrinogen receptor are considered to be well within the range of that reported for known RGDF mimetics, containing *p*-benzamidine group as a bioisostere of arginine side function, and even greater than corresponding values for compounds of ABAS series [4].

In brief, the novel RGDF mimetics based on 4-(1,2,3,4-tetrahydro-isoquinoline-7-yl)amino-4-oxo-butyric acid (**3**) were found to inhibit effectively platelet aggregation in a human platelet rich plasma and to be potent blockers of platelet $\alpha_{IIb}\beta_3$. This raises the possibility that the search for effective $\alpha_{IIb}\beta_3$ antagonists among these derivatives would be promising. 1,2,3,4-Tetrahydroisoquinoline is considered to be more suitable for mimicking of arginine δ-guanidine group, compared to isoindoline.

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