Derivatives of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic Acid as Novel Fibrinogen Receptor Antagonists

Olga L. Malovichko[a], Andrei A. Krysko[a,*], Tatyana A. Kabanova[a], Sergei A. Andronati[a], Vladimir I. Grishkovets[b], Vadim V. Kachala[c] and Denis A. Panov[b]

[a]*Department of Medicinal Chemistry, A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine. 86 Lustdorfskaya doroga, 65080, Odessa, Ukraine;* [b]*V.I. Vernadskiy Tavricheskiy National University, Simferopol, Ukraine;* [c]*N.D. Zelinsky Institute of Organic Chemistry of Russian Academy of Sciences, Moscow, Russia*

Abstract: It has been proposed a novel method for obtaining of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid as Arg-mimetic within the framework of search for novel fibrinogen receptor antagonists. New $\alpha_{IIb}\beta_3$ antagonists were prepared on a base of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid. Their high antiaggregatory activities in a human platelet rich plasma and ability to block FITC-Fg binding to $\alpha_{IIb}\beta_3$ on washed human platelets were estimated.

Key Words: fibrinogen receptor antagonists, $\alpha_{IIb}\beta_3$, RGD mimetics, 1,2,3,4-tetrahydroisoquinoline, platelet aggregation.

INTRODUCTION

Severe cardio-vascular disorders like a myocardial infarction or a stroke often result from alterations in platelet aggregatory behavior [1]. Activation of platelets by various agonists (ADP, thrombin, collagen etc.) leads to their aggregation through binding of the fibrinogen to its platelet receptor (integrin $\alpha_{IIb}\beta_3$) [2], mediated by recognition of RGD (Arg-Gly-Asp) fragment in the fibrinogen molecule [3]. Several classes of highly potent $\alpha_{IIb}\beta_3$ antagonists (RGD containing peptides, RGD mimetics and monoclonal antibodies) have been disclosed in the scientific literature, but only limited number of their representatives (eptifibatide [4], tirofiban [5], lamifiban [6] and abciximab [7]) have successfully passed clinical trials and showed significant potential as a novel therapy for thrombotic disorders.

Replacement of key fragments of RGD sequence on the base of bioisosteric similarity is a principal approach for the design of RGD mimetics. Various moieties incorporating *p*-benzamidine, piperidine, *p*-benzguanidine etc., groups were utilized as an arginine side chain bioisosteres at the preparation of potent non-peptide $\alpha_{IIb}\beta_3$ antagonists [8].

Our efforts were concentrated on obtaining high active fibrinogen receptor antagonists based on isoindoline [9, 10] or 1,2,3,4-tetrahydroisoquinoline [11, 12] groups as a surrogate of arginine (Fig. **1**).

Also, as we have learned from the literature, others [13] have prepared a series of RGDF mimetics using *p*-amidinobenzyl as a replacement of arginine. In this paper, the fragment of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid (Fig. **2**) is presented as an arginine mimick towards the development of non-peptide $\alpha_{IIb}\beta_3$ antagonists. The main difference of mentioned fragment from previously reported Arg-mimetic [11, 12] is that amine function in position 7 of 1,2,3,4-tetrahydroisoquinoline cycle, has been changed by carboxylic one.

β-Amino acids and their derivatives not only exhibit broad spectrum of biological activity but are also the building blocks for the synthesis of RGDF mimetics. In particular, the residues of β-substituted β-amino acids are successfully used for bioisosteric replacement of AspPhe fragment [8, 14].

Our goal in this study was to synthesize potent integrin $\alpha_{IIb}\beta_3$ antagonists based on 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid, which would exhibit antiaggregative properties.

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 300 MHz, using TMS as an internal standard. NMR spectra for compound **8** have been obtained on a Bruker DRX-500, with 500 MHz operating frequency of the instrument, in DMSO-d_6 solution. For two-dimensional HSQC and HMBC experiments, Bruker standard program packages have been applied. Mass-spectra FAB were recorded on a VG 7070 apparatus using *p*-nitrobenzyl alcohol matrix, ionization was achieved by Xe atoms beam with the energy of 8 kV. Mass-spectra of compounds have been recorded on MX-1321 mass-spectrometer (70 eV ionizating voltage, ionization chamber temperature 200 ºC).

2,7-Diacetyl-1,2,3,4-tetrahydroisoquinoline (5)

Compound **4** (20.6 g; 0.117 mol) was dissolved in anhydrous CCl_4 (300 mL). Then anhydrous $AlCl_3$ (86.43 g; 0.643 mol) was added to the solution at stirring, and the mixture was cooled to -5 ºC. At a temperature below 0 ºC, AcCl (28.4 mL; 0.398 mol) was added dropwise. After completion of adding, the reaction mixture was heated to 40 ºC, kept at

*Address correspondence to this author at the Department of Medicinal Chemistry, A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine. 86 Lustdorfskaya doroga, 65080, Odessa, Ukraine Tel.: +38-048-765-92-30; fax: +38-048-765-96-02;
E-mail: peptides@paco.net

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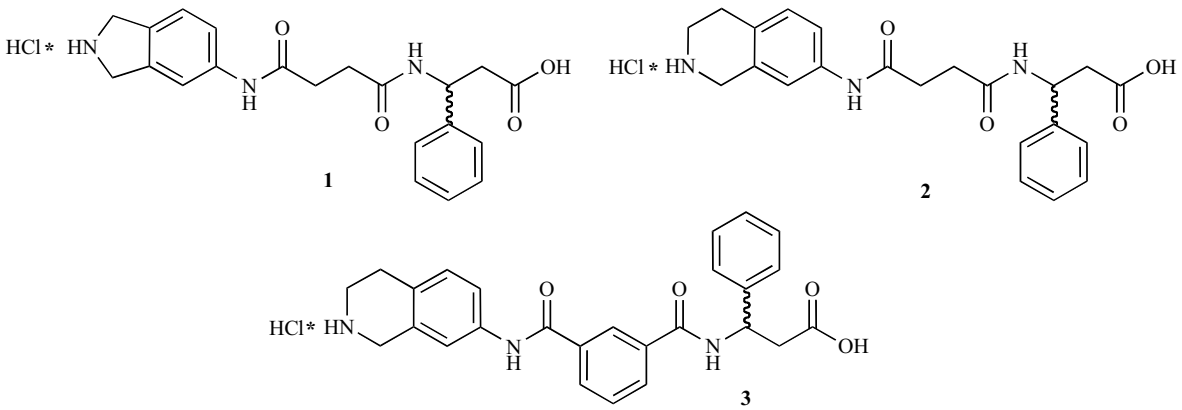


Fig. (1). Structures of mimetics **1**, **2** and **3**.

this temperature for 5 h, and left overnight. Then the mixture was poured onto a mixture of crushed ice (100 g) and concentrated hydrochloric acid (50 mL). The aqueous phase was extracted with chloroform (3 × 100 mL). The chloroform phase was dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated to dryness. Yield 24.8 g (97 %). Oil. R_f = 0.41 (A). Mass-spectrum, *m/e* (I %): 217 (100), 202 (12), 174 (49), 159 (22), 158 (23), 146 (20), 143 (17), 103 (14), 77 (16), 43 (98). ^{1}H NMR (300 MHz, DMSO-d_6) 2.09 (s, 3H), 2.55 (s, 3H), 2.82 (t, *J* = 5.9 Hz, 2H), 2.93 (t, *J* = 5.9 Hz, 2H), 3.64 – 3.69 (m, 2H), 7.31 – 7.35 (m, 1H), 7.73 – 7.81 (m, 2H). Anal. calcd for $C_{13}H_{15}NO_2$: C, 71.87 %; H, 6.96 %; N, 6.45 %. Found: C, 71.65 %; H, 7.03 %; N, 6.64 %.

2-Acetyl-1,2,3,4-tetrahydroisoquinoline-7-carboxylic Acid (6)

Compound **5** (6.0 g; 0.026 mol) was suspended in a cold water (100 mL), then NaOH (16.58 g; 0.414 mol) was added, and the suspension was cooled to -5 ºC. At the temperature kept below 0 ºC, Br_2 (4.43 mL; 0.086 mol) was added dropwise. After completion of the addition of Br_2, the reaction mixture was stirred for 1 h at 0 ºC and washed with chloroform (50 mL). The aqueous phase was acidified with cooled concentrated HCl to pH = 4 – 5. The reaction mixture was maintained at 0 ºC for 3 h, the precipitate formed was collected by filtration, recrystallized from water (50 mL) and dried in exsiccator over $CaCl_2$. Yield 3.3 g (97 %). M.p. 182-183 ºC. R_f = 0.44 (B). Mass-spectrum, *m/e* (I %): 219 (100), 176 (52), 160 (22), 161 (25), 148 (44), 130 (18), 131 (23), 117 (19), 103 (15), 77 (16), 43 (48). ^{1}H NMR (300 MHz, DMSO-d_6) 2.09 (s, 3H), 2.93 (t, *J* = 5.9 Hz, 2H), 3.67 (t, *J* = 5.9 Hz, 2H), 4.65 (s, 1H), 4.71 (s, 1H), 7.28 – 7.32 (m, 1H), 7.73 – 7.80 (m, 2H), 12.89 (s, 1H). Anal. calcd for $C_{12}H_{13}NO_3$: C, 65.74 %; H, 5.98 %; N, 6.39 %. Found: C, 65.53 %; H, 6.12 %; N, 6.61 %.

1,2,3,4-tetrahydroisoquinoline-7-carboxylic Acid Hydrochloride (7)

Compound **6** (17.2 g; 0.079 mol) was refluxed with HCl (350 mL) for 10 h. Then the solution was cooled to room temperature and left overnight. The precipitate formed was collected by filtration and recrystallized from water (40 mL). On filter, the product was washed with boiling acetone. Yield 10.79 g (64 %). M.p. 339 - 341 ºC. R_f = 0.67 (D). Mass-spectrum, *m/e* (I %): 177 (71), 176 (100), 148 (89), 130 (26), 131 (37), 103 (18), 77 (19), 51 (10), 36 (42). ^{1}H NMR (300 MHz, DMSO-d_6) 3.09 (t, *J* = 6.0 Hz, 2H), 3.35 (t, *J* = 6.0 Hz, 2H), 4.31 (s, 2H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.77 – 7.83 (m, 2H), 9.88 (s, 2H), 12.97 (s, 1H). Anal. calcd for $C_{10}H_{11}NO_2 \cdot HCl$: C, 56.21 %; H, 5.66 %; N, 6.56 %. Found: C, 56.33 %; H, 5.71 %; N, 6.73 %.

2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carboxylic Acid (8)

To the solution of **7** (4.0 g; 0.018 mol) in water (50 mL) was added NaOH (0.75 g; 0.018 mol) and $NaHCO_3$ (3.14 g; 0.037 mol). The mixture was stirred until the whole precipitate dissolved, then the solution of Boc_2O (4.08 g; 0.018 mol) in *iso*-propyl alcohol (10 mL) was added dropwise under intensive stirring. After completion of adding, the reaction mixture was stirred for 2 h. Then the solution volume was evaporated to half *in vacuo*, brought to 100 mL with a water, and washed with hexane (2 × 50 mL). The chloroform phase was washed by 1N HCl (2 × 50 mL) and water (50 mL), then dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. Yield 4.33 g (87 %). M.p. 151 - 153 ºC. R_f = 0.55 (B). Mass-spectrum, *m/e* (I %): 221 (20), 220 (38), 204 (10), 176 (24), 148 (12), 57 (100), 41 (14). FAB-MS 278 (M+H,

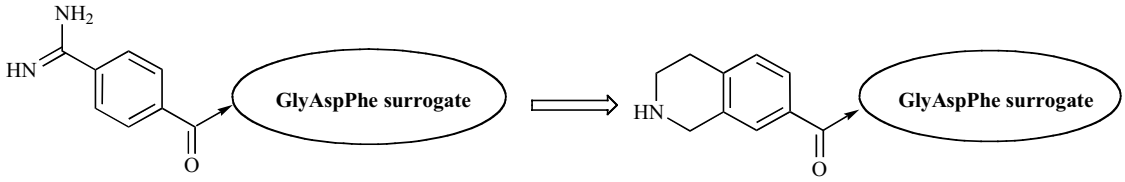


Fig. (2). Schematic structures of RGDF mimetics containing *p*-amidinobenzyl or fragment of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid.

100 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.44 (s, 9H), 2.84 (t, *J* = 5.9 Hz, 2H), 3.57 (t, *J* = 5.9 Hz, 2H), 4.56 (s, 2H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.75 (m, 2H), 12.82 (s, 1H). Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97 %; H, 6.91 %; N, 5.05 %. Found: C, 64.75 %; H, 6.81 %; N, 5.28 %.

3-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]propionic Acid (9a)

To the solution of **8** (0.8 g; 0.0029 mol) and SuOH (0.4 g; 0.0034 mol) in THF (10 mL) was added the solution of DCC (0.71 g; 0.0034 mol) in THF (10 mL). The mixture was stirred for 2 h at 5 ºC, then filtered. Solvent was evaporated to dryness. Oily residue of Su-ester of acid **9a** was dried *in vacuo* to constant weight. The solution of Su-ester of acid **9a** in ethyl alcohol (10 mL) was mixed with the solution of β-alanine (0.34 g; 0.0038 mol) and $NaHCO_3$ (0.323 g; 0.038 mol) in water (10 mL). The reaction mixture was stirred for 1 h, the solution was evaporated to half *in vacuo*, brought to 20 mL with a water and washed with chloroform (50 mL). The aqueous phase was acidified to pH 4-5 with concentrated HCl. The product was extracted from water by chloroform (2 × 50 mL). The organic layer was washed by 1 N HCl (2 × 20 mL), water (20 mL), then dried with Na_2SO_4, filtered, and the solvent was evaporated *in vacuo*. The resulting oily residue was triturated with hexane (10 mL) and the precipitate was collected by filtration and dried. Yield 0.77 g (77 %). M.p. 120 - 121 ºC. R_f = 0.51 (B). FAB-MS 349 (M+H, 80 %), 371 (M+Na, 12 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 2.51 – 2.55 (m, 2H), 2.81 (t, *J* = 5.5 Hz, 2H), 3.45 (q, *J* = 5.9 Hz, 2H), 3.56 (t, *J* = 5.5 Hz, 2H), 4.53 (s, 2H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.62 – 7.65 (m, 2H), 8.44 (t, *J* = 4.5 Hz, 1H), 12.21 (s, 1H). Anal. calcd for $C_{18}H_{24}N_2O_5$: C, 62.05 %; H, 6.94 %; N, 8.04 %. Found: C, 61.94 %; H, 6.73 %; N, 8.21 %.

4-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]butyric Acid (9b)

The compound **9b** was prepared from **8** and γ-aminobutyric acid in a similar way as for **9a** to yield the product (90 %) as an oil. R_f = 0.46 (B). FAB-MS 363 (M+H, 100 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 1.75 (m, *J* = 6.6 Hz, 2H), 2.27 (t, *J* = 6.6 Hz, 2H), 2.81 (t, *J* = 5.1 Hz, 2H), 3.27 (q, *J* = 6.6 Hz, 2H), 3.56 (t, *J* = 5.1 Hz, 2H), 4.54 (s, 2H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.62 – 7.66 (m, 2H), 8.40 (t, *J* = 4.1 Hz, 1H), 12.07 (s, 1H). Anal. calcd for $C_{19}H_{26}N_2O_5$: C, 62.97 %; H, 7.23 %; N, 7.73 %. Found: C, 62.81 %; H, 7.35 %; N, 7.86 %.

3-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]propionyl-β-alanine (10a)

The compound **10a** was prepared from **9a** and β-alanine in a similar way as for **9a** to yield the product (75 %) as an oil. R_f = 0.42 (B). FAB-MS 420 (M+H, 94 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 2.32 – 2.40 (m, 4H), 2.81 (t, *J* = 5.6 Hz, 2H), 3.34 (q, *J* = 6.3 Hz, 2H), 3.56 (t, *J* = 5.6 Hz, 2H), 4.53 (s, 2H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.60 – 7.63 (m, 2H), 7.97 (t, *J* = 4.9 Hz, 1H), 8.40 (t, *J* = 5.5 Hz, 1H), 12.19 (s, 1H). Anal. calcd for $C_{21}H_{29}N_3O_6$: C, 60.13 %; H, 6.97 %; N, 10.02 %. Found: C, 60.31 %; H, 6.74 %; N, 10.11 %.

3-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]propionyl-D,L-β-phenyl-β-alanine (10b)

The compound **10b** was prepared from **9a** and β-phenyl-β-alanine in a similar way as for **9a** to yield the product (79 %) as an oil. R_f = 0.48 (B). FAB-MS 496 (M+H, 85 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 2.39 (t, *J* = 7.1 Hz, 2H), 2.64 – 2.68 (m, 2H), 2.81 (t, *J* = 5.5 Hz, 2H), 3.39 – 3.45 (m, 2H), 3.56 (t, *J* = 5.5 Hz, 2H), 4.53 (c, 2H), 5.21 (q, *J* = 7.7 Hz, 1H), 7.20 – 7.30 (m, 6H), 7.59 – 7.63 (m, 2H), 8.39 – 8.47 (m, 2H), 12.25 (s, 1H). Anal. calcd for $C_{27}H_{33}N_3O_6$: C, 65.44 %; H, 6.71 %; N, 8.48 %. Found: C, 65.33 %; H, 6.59 %; N, 8.65 %.

4-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]butyryl-β-alanine (10c)

The compound **10c** was prepared from **9b** and β-alanine in a similar way as for **9a** to yield the product (70 %) as an oil. R_f = 0.4 (B). FAB-MS 434 (M+H, 87 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 1.70 - 1.77 (m, 2H), 2.11 (t, *J* = 6.8 Hz, 2H), 2.36 (t, *J* = 7.8 Hz, 2H), 2.81 (t, *J* = 5.7 Hz, 2H), 3.23 (m, 4H), 3.56 (t, *J* = 5.7 Hz, 2H), 4.53 (s, 2H), 7.23 (d, *J* = 8.7 Hz, 1H), 7.61 – 7.63 (m, 2H), 7.90 (t, *J* = 4.7 Hz, 1H), 8.38 (t, *J* = 5.0 Hz, 1H), 12.22 (s, 1H). Anal. calcd for $C_{22}H_{31}N_3O_6$: C, 60.95 %; H, 7.21 %; N, 9.69 %. Found: C, 61.11 %; H, 7.29 %; N, 9.45 %.

4-[(2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]butyryl-D,L-β-phenyl-β-alanine (10d)

The compound **10d** was prepared from **9b** and β-phenyl-β-alanine in a similar way as for **9a** to yield the product (81 %) as an oil. R_f = 0.50 (B). FAB-MS 510 (M+H, 98 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.43 (s, 9H), 1.67 - 1.77 (m, 2H), 2.15 (t, *J* = 6.7 Hz, 2H), 2.66 (d, *J* = 7.8 Hz, 2H), 2.81 (t, *J* = 5.8 Hz, 2H), 3.23 (q, *J* = 6.7 Hz, 2H), 3.56 (t, *J* = 5.8 Hz, 2H), 4.53 (s, 2H), 5.20 (q, *J* = 7.8 Hz, 1H), 7.19 – 7.32 (m, 6H), 7.63 – 7.65 (m, 2H), 8.35 – 8.39 (m, 2H), 12.21 (s, 1H). Anal. calcd for $C_{28}H_{35}N_3O_6$: C, 65.99 %; H, 6.92 %; N, 8.25 %. Found: C, 66.10 %; H, 6.77 %; N, 8.31 %.

3-[(1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]propionyl-β-alanine hydrochloride (11a)

Boc-derivative **10a** (0.3 g; 0.0007 mol) was dissolved in anhydrous chloroform (15 mL), and the stream of dry HCl was passed through the solution for 1 h. The solvent was evaporated, and the solid residue was dried *in vacuo* for 2 h at 40 ºC (2 mm Hg). Yield 0.22 g (90 %). The substance is hygroscopic. R_f = 0.3 (D). FAB-MS 420 (M+H, 100 %). ^{1}H NMR (300 MHz, DMSO-d_6) 2.31 – 2.40 (m, 4H), 2.84 (t, *J* = 5.4 Hz, 2H), 3.30 – 3.33 (m, 2H), 3.41 -3.44 (m, 2H), 3.55 (t, *J* = 5.5 Hz, 2H), 4.31 (s, 2H), 7.25 (d, *J* = 8.7 Hz, 1H), 7.61 – 7.65 (m, 2H), 7.81 (t, *J* = 5.1 Hz, 1H), 8.43 (t, *J* = 5.7 Hz, 1H), 9.62 (s, 2H). Anal. calcd for $C_{16}H_{21}N_3O_4$·HCl: C, 54.01 %; H, 6.23 %; N, 11.81 %. Found: C, 54.14 %; H, 6.29 %; N, 11.60 %.

3-[(1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]propionyl-D,L-β-phenyl-β-alanine hydrochloride (11b)

The compound **11b** was prepared from **10b** in a similar way as for **11a** to yield the product (98 %) as a hygroscopic solid. R_f = 0.40 (D). FAB-MS 396 (M+H, 100 %). ^{1}H NMR (300 MHz, DMSO-d_6) 2.40 (t, *J* = 6.8 Hz, 2H), 2.62 – 2.71

(m, 2H), 3.05 (t, *J* = 5.5 Hz, 2H), 3.36 – 3.45 (m, 4H), 4.28 (s, 2H), 5.21 (q, *J* = 7.87 Hz, 1H), 7.21 – 7.31 (m, 6H), 7.69 – 7.71 (m, 2H), 8.50 – 8.54 (m, 2H), 9.59 (s, 2H). Anal. calcd for $C_{22}H_{25}N_3O_4 \cdot HCl$: C, 61.18 %; H, 6.07 %; N, 9.73 %. Found: C, 61.11 %; H, 6.03 %; N, 9.79 %.

4-[(1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]butyryl-β-alanine hydrochloride (11c)

The compound **11c** was prepared from **10c** in a similar way as for **11a** to yield the product (95 %) as a hygroscopic solid. R_f = 0.35 (D). FAB-MS 334 (M+H, 97 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.70 (m, *J* = 7.0 Hz, 2H), 2.12 (t, *J* = 6.7 Hz, 2H), 2.36 (t, *J* = 7.8 Hz, 2H), 2.90 (t, *J* = 5.7 Hz, 2H), 3.21 – 3.25 (m, 4H), 3.58 (t, *J* = 5.7 Hz, 2H), 4.30 (s, 2H), 7.24 (d, *J* = 8.6 Hz, 1H), 7.61 – 7.63 (m, 2H), 7.95 (t, *J* = 4.7 Hz, 1H), 8.37 (t, *J* = 5.3 Hz, 1H), 9.61 (s, 2H). Anal. calcd for $C_{17}H_{23}N_3O_4 \cdot HCl$: C, 55.21 %; H, 6.54 %; N, 11.36 %. Found: C, 55.29 %; H, 6.49 %; N, 11.49 %.

4-[(1,2,3,4-tetrahydroisoquinoline-7-carbonyl)amino]butyryl-D,L-β-phenyl-β-alanine hydrochloride (11d)

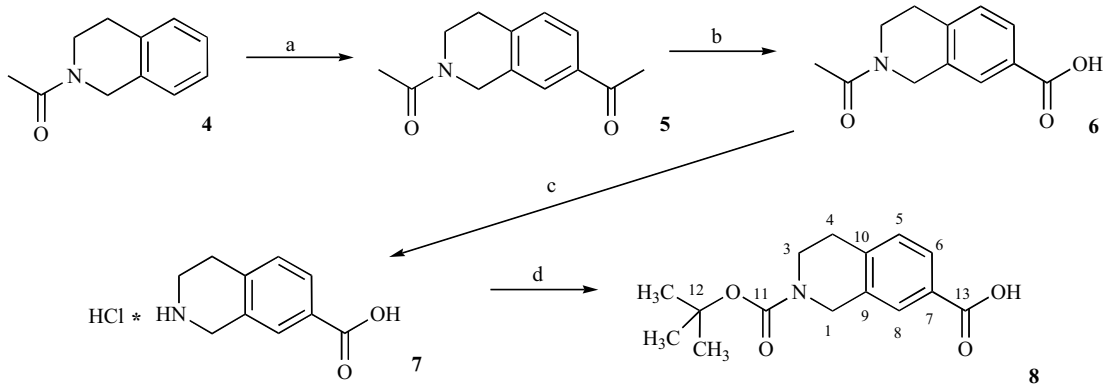
The compound **11d** was prepared from **10d** in a similar way as for **11a** to yield the product (98 %) as a hygroscopic solid. R_f = 0.38 (D). FAB-MS 410 (M+H, 100 %). ^{1}H NMR (300 MHz, DMSO-d_6) 1.71 (m, *J* = 7.2 Hz, 2H), 2.15 (t, *J* = 6.6 Hz, 2H), 2.65 – 2.68 (m, 2H), 3.04 (t, *J* = 5.9 Hz, 2H), 3.22 (m, *J* = 6.6 Hz, 2H), 3.35 – 3.37 (m, 2H), 4.28 (s, 2H), 5.19 (q, *J* = 7.8 Hz, 1H), 7.19 – 7.32 (m, 6H), 7.72 – 7.74 (m, 2H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.54 (t, *J* = 5.3 Hz, 1H), 9.61 (s, 2H). Anal. calcd for $C_{23}H_{27}N_3O_4 \cdot HCl$: C, 61.95%; H, 6.33 %; N, 9.42 %. Found: C, 62.07 %; H, 6.19 %; N, 9.31 %.

RESULTS AND DISCUSSION

Synthesis of the 2-Boc-1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid (**8**) is shown in Scheme **1**. Acylation of 2-acetyl-1,2,3,4-tetrahydroisoquinoline (**4**) with acetyl chloride at the presence of aluminium chloride gave 2,7-diacetyl-1,2,3,4-tetrahydroisoquinoline (**5**), then its oxidation and removal of the acetyl group afforded 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid (**7**). Interaction of the latter with Boc_2O resulted in the acid **8**.

The structure of acid **8** has been confirmed by data of NMR (^{1}H, ^{13}C) spectroscopy and FAB mass-spectrometry. Signal assignments in NMR (^{1}H, ^{13}C) spectra have been carried out by the use of two-dimensional correlative HSQC and HMBC procedures.

Signals of three aromatic protons have been identified in NMR spectra of the acid **8**. Two of these signals, at 7.24 ppm and 7.72 ppm, are doublets with $J \approx 8$ Hz corresponding to H-5 and H-6 protons, and one signal, at 7.73 ppm, is a pseudosinglet located at the *para*- and *meta*-positions regarding to H-8 atom. However, the character of signals splitting for aromatic protons would be the same for COOH group location, both in position 6 and in position 7. Therefore, this question could not be solved on the base of analysis of signal multiplicity for aromatic protons. For its solution, aromatic protons signals have to be correlated with aliphatic proton signals of the neighbouring heterocyclic ring. In this ring, three groups of six protons (two protons in each group) located in high field region appeared as a pseudosinglet at 4.54 ppm and two pseudotriplets at 3.55 ppm and 2.81 ppm. In the HMBC spectrum, cross-peaks at 154 ppm from the Boc-group carbonyl C-atom have been found to be with the protons at 4.54 ppm and 3.55 ppm, consequently, the latters have been attributed to H-1,1' and H-3,3' protons. The remained signal at 2.81 ppm, undoubtedly, belongs to H-4,4' protons. The signal at 4.54 ppm, by signal splitting character, has to be related to H-1,1' (pseudosinglet), and the signal at 3.55 ppm – to H-3,3' ($J_{3,4} \approx J_{3,4'} \approx 5.5$ Hz). The similar spin coupling constants have been observed for the H-4,4' proton splitting. The signals of directly bound with them C-1, C-3 and C-4 carbon atoms have been correlated over HSQC spectrum (Table **1**). In the HMBC spectrum, the cross-peak from the C-1 signal (45.2 ppm) has been found to be with aromatic proton signal at 7.73 ppm, thus, this signal indisputably belongs to the H-8 proton. Analogously, relation from C-4 signal has been accomplished for H-5 proton signal located at 7.24 ppm. Signals of corresponding C-atoms have been found over HSQC spectrum. From above stated, the remained signal of proton at 7.72 ppm might be either H-6 proton signal (for 7-carboxylic acid), or H-7 proton signal (for 6-carboxylic acid). However, multiplicity of H-8 (pseudosinglet) and H-5 (doublet with $J \approx 8$ Hz) signals indicate that H-8 have no vicinal protons, however H-5 has one vicinal proton (H-6). Consequently, COOH group is located at the C-7 carbon atom. Furthermore, the COOH group location has been proved by HMBC spectrum analysis according to



Scheme 1. Synthesis of the acid **8**. a) AcCl, $AlCl_3$; b) Br_2, NaOH; c) HCl, H_2O; (d) Boc_2O.

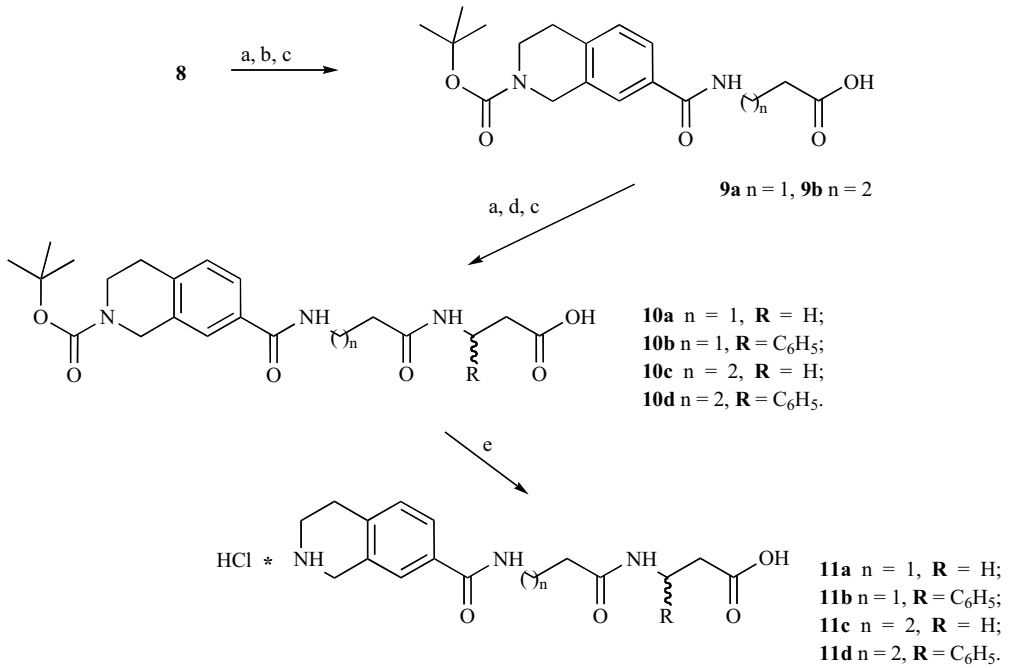
Table 1. Chemical Shifts for ^{1}H and ^{13}C in the Compound 8 (DMSO-d_6)

C-atom	δ (ppm)	H-atom	δ (ppm)
1	45.2	1, 1'	4.54
3	41.2	3, 3'	3.55
4	28.4	4, 4'	2.81
5	128.9	5	7.24
6	127.1	6	7.72
7	127.3	-	-
8	128.8	8	7.73
9	139.8	-	-
10	139.8	-	-
11	154.0	-	-
12	79.1	-	-
13	167.2	-	-
$-CH_3$	28.1	$-CH_3$	-

the presence of cross-peaks between carboxylic the C-13 atom and H-8, and H-6 protons, as well as to the absence of cross-peaks with H-5 proton.

The synthesis of target RGDF mimetics is presented in Scheme **2**. The acid **8** has been used as a starting compound. Condensation of acid **8** with β-alanine or γ-aminobutyric acid has been conducted by the DCCI method with addition of SuOH what resulted in obtaining the pseudopeptides **9a** and **9b**. Boc-derivatives **10** have been obtained analogously. Acidolytic removing of Boc-groups from compounds **10** afforded target RGDF mimetics **11**. Structures of all compounds obtained for the first time are confirmed by ^{1}H NMR spectroscopy and FAB mass-spectrometry.

A high antiaggregative activity of the mimetics **11** (Table **2**) have been revealed *in vitro* on a platelet-rich plasma (PRP) of human blood. Bioassays have been conducted by



Scheme 2. Synthesis of the target compounds **11**. a) DCC, SuOH; b) β-alanine or γ-aminobutyric acid, $NaHCO_3$, H_2O; c) 1 N HCl in H_2O; d) β-alanine or D,L-β-phenyl-β-alanine; e) 4 M solution of HCl in dioxane.

Table 2. Biological Properties of RGDF Mimetics and RGDS Peptide

Compds.	Antiaggregative activity human PRP, IC_{50}, nM[a]	Inhibition of FITC-Fg binding to $\alpha_{IIb}\beta_3$ on the surface of human activated platelets, IC_{50}, nM[a]
1	860.0 (± 120.0) [9,10]	8.3 (± 1.40) [9,10]
2	13.0 (± 1.0) [11]	1.0 (± 0.12) [11]
3	25.0 (± 1.7) [12]	1.0 (± 0.41) [12]
11a	1100.0 (± 100.0)	22.0 (± 2.00)
11b	14.0 (± 2.0)	10.5 (± 1.40)
11c	15.0 (± 1.8)	9.5 (±1.50)
11d	6.4 (± 1.1)	0.8 (± 0.12)
RGDS	31000.0 (± 2000.0)	13000 (±1600.00)

[a]Values are means of three experiments, standard deviation is given in parentheses.

Born's method using ADP as agonist of aggregation [15]. Antiaggregative properties of the novel $\alpha_{IIb}\beta_3$ antagonists containing 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid residue as an Arg mimetic are comparable to those of their analogs, 7-amino-1,2,3,4-tetrahydroisoquinoline derivatives. It should be noted that the replacement of the β-alanine residue by the γ-aminobutyric acid residue in the linker moiety leads to more active RGD mimetics - $\alpha_{IIb}\beta_3$ antagonists. The assessment of inhibitory potency of RGDF mimetics **11** towards specific binding of fluoresceinisothiocyanate-labeled fibrinogen (FITC-Fg) to fibrinogen receptor has been fulfilled using suspension of human washed platelets by the method [16]. Dissociation constant (K_d) of FITC-Fg (obtained by the procedure [17]) has been found to be of 1.02 μM. Table **2** contains the data indicating the high affinities of compounds **11** for $\alpha_{IIb}\beta_3$ receptor that obviously prove the molecular mechanism for antiaggregatory action of the obtained mimetics.

The residue of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid has been successfully employed by us as an Arg mimetic for the preparation of potent $\alpha_{IIb}\beta_3$ antagonists preparation. Promising results obtained on antiaggregatory activities and affinities of the novel RGDF mimetics on the base of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid suggest that these compounds may have a potential for treatment of thrombotic disorders in humans.

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