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Novel fibrinogen receptor antagonists. RGDF mimetics, derivatives of 4-(isoindoline-5-yl)amino-4-oxobutyric acid

Andrei A. Krysko,* Boris M. Chugunov, Olga L. Malovichko, Sergei A. Andronati, Tatyana A. Kabanova, Tamara L. Karaseva and Anna V. Kiriyak

Department of Medicinal Chemistry, A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorfskaya doroga, 65080 Odessa, Ukraine

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Abstract—The novel RGDF mimetics **9a** and **9b** were synthesized with the use of 4-(isoindoline-5-yl)amino-4-oxobutyric acid as a 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.

Last decade, interest of medicinal chemists essentially focuses on the design, synthesis and development of compounds, which prevent a thrombosis through the inhibition of platelet aggregation. A fundamental physiological role of a platelet, as a blood element, is the interaction with fibrinogen, which leads to the platelet aggregation and thereby initiates the formation of hemostatic clot, preventing bleeding at the site of vascular injury. It is established that a platelet membrane-bound glycoprotein GP IIb/IIIa (or $\alpha_{IIb}\beta_3$ integrin) is highly responsible for this aggregation process. This glycoprotein, which serves as a receptor for plasma protein fibrinogen is a member of superfamily of cell adhesion proteins, called integrins, which bind Arg-Gly-Asp (RGD) sequences occurring in fibrinogen. Various RGD containing peptides are able to block the native ligand from binding to its receptor. Therefore, the RGD sequence is a lead structure for the design of novel platelet aggregation inhibitors.[1]

The fragment containing the residues of *p*-benzamidine, piperidine, *p*-benzguanidine, *p*-benzmethyleneamine, etc. are known to be useful as surrogates of arginine δ-guanidine group in the design of active and selective RGDF mimetics.[2] Named fragments may be referred as bioisosters of the side function of arginine residue.

The synthesis of the series of RGDF mimetics—(aminobenzamidino)succinyl derivatives, has been reported.[3] The compounds of this series have demonstrated a high affinity for platelet fibrinogen receptor and effectively inhibited platelet aggregation. The (aminobenzamidino)succinyl (**1**) residue has been recognized as a mimetic of Arg-Gly moiety of RGDF sequence (Fig. 1).

In the present study, we have proposed the residue of 4-(isoindoline-5-yl)amino-4-oxobutyric acid (**2**) as Arg-Gly surrogate.

β-Alanines with different substituents in β position are successfully applied in the synthesis of RGDF mimetics—fibrinogen receptor blockers.[2,4] The residue of β-substituted β-alanine is considered as a surrogate of Asp-Phe motif. Carboxylic group of β-alanine mimics the side chain of aspartic acid residue. β-Substituted β-alanines have been used by us as starting blocks for preparation of novel RGDF mimetics.

The aim of the present study was to ascertain the possibility of use of 4-(isoindoline-5-yl)amino-4-oxobutyric acid (**2**) for obtaining of novel RGDF mimetics, $\alpha_{IIb}\beta_3$ antagonists, as well as, to offer the application of Arg-Gly surrogate for the further design of potent $\alpha_{IIb}\beta_3$ antagonists. The synthesis of this Arg-Gly surrogate is rather simple and short staged.

Keywords: Fibrinogen receptor antagonists; GP IIb/IIIa; RGD mimetics; Isoindoline; Platelet aggregation.
* Corresponding author. Tel.: +38 0482 663041; fax: +38 0482 652012; e-mail: peptides@paco.net

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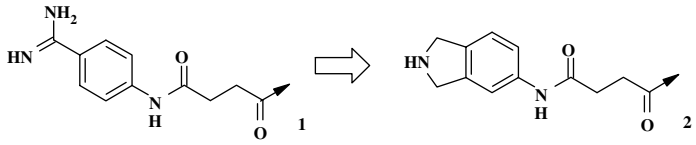


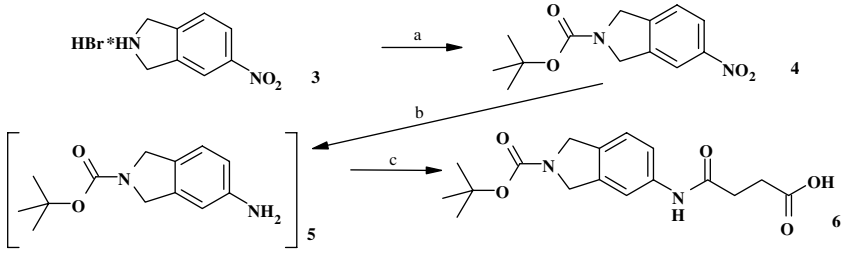
Figure 1. Structures of Arg-Gly mimetics: (aminobenzamidino)succinyl (**1**) and fragment of 4-(isoindoline-5-yl)amino-4-oxobutyric acid (**2**).

To obtain RGDF mimetics on the base of 4-(isoindoline-5-yl)amino-4-oxobutyric acid derivatives, we have used 5-nitroisoindoline (**3**). Blocking of 5-nitroisoindoline amino group, then reduction of nitro group in the obtained N-Boc-5-nitroisoindoline (**4**) followed by acylation of the amine **5** with succinic anhydride, afforded 4-(N-Boc-isoindoline-5-yl)amino-4-oxobutyric acid (**6**) (Scheme 1).[5]

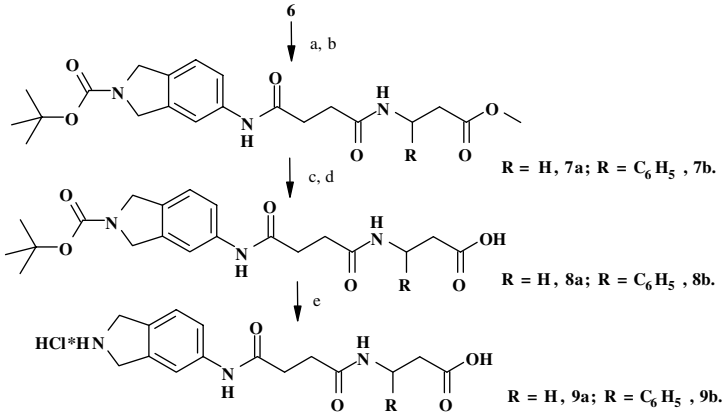
The method of mixed anhydrides selected for condensation of acid **6** with β-alanines esters has brought us to acceptable results (Scheme 2).[6] The condensation of β-alanines esters with the compound **6** gave the protected mimetics **7a** and **7b**. Subsequent saponification of their ester groups and elimination of Boc-protective groups yielded the target mimetics **9a** and **9b**. The use of given schemes of synthesis makes it possible to obtain the products of all stages with high yields and of a high purity, without HPLC processing. RGDF mimetics obtained are chemically stable substances, both in dried form and in water solution at normal conditions. The compounds **9a** and **9b** are highly water soluble. This fact is substantial for in vitro bioassays.[7]

The synthesized RGDF mimetics, derivatives of 4-(isoindoline-5-yl)amino-4-oxobutyric acid, have showed a high antiaggregative in vitro activity in human platelet rich plasma. The IC_{50} values are of 2.75 μM (for **9a**) and 0.86 μM (for **9b**). It should be noted that an IC_{50} value for RGDF peptide is of 21 μM. In bioassays on the suspension of human washed platelets IC_{50} values are of 2.09 μM (for **9a**) and 0.27 μM (for **9b**). The experiments have been carried out by Born's method in blood samples of at least three different donors.[8] At both versions of the experiment, ADP in a final concentration of 10 μM was used as an inductor of the platelet aggregation.

To define the mechanism of antiaggregatory action of RGDF mimetics **9a** and **9b**, their influence on fluoresceinisothiocyanate labeled fibrinogen (FITC-Fg) binding to its receptor on the suspension of washed human platelets was assessed by the procedure of Xia et al.[9] FITC-Fg obtained by method described by Hantgan, R.[10] specifically binded to its platelet receptor with a value K_d (dissociation constant) of 1.02 μM. It was established that compounds **9a** and **9b** had inhibited



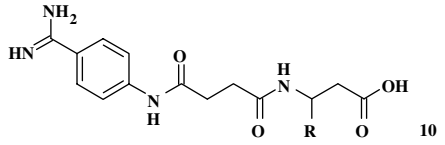
Scheme 1. Reagents and conditions: (a) Boc_2O; (b) $N_2H_4{\cdot}H_2O$, 3% Pd in carbon; (c) succinic anhydride.



Scheme 2. Reagents and conditions: (a) Et_3N, isobutyl chloroformate; (b) β-amino acid methyl ester, Et_3N; (c) 1 M NaOH, H_2O; (d) 1 M HCl, H_2O; (e) 4 M HCl in dioxane.

FITC-Fg binding to its receptor in suspension of washed human platelets with IC_{50} values of 0.014 μM (for **9a**) and 0.0083 μM (for **9b**). At that, K_i values were of 0.0071 μM and 0.0042 μM for compounds **9a** and **9b**, respectively.

The introduction of phenyl in β position of β-alanine residue has led to 10-fold enhancement in the antiaggregatory activity and affinity for $\alpha_{IIb}\beta_3$ of the compound **9b**. Similar tendency can be observed, also, for ABAS series (**10**): at R=H, an IC_{50} value is of 1.09 μM (Collagen-induced platelet aggregation, dog platelet-rich plasma), and at R=Ph, $IC_{50} = 0.294$ μM (the similar experimental conditions).[3]



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The values for antiaggregative activity and affinity for $\alpha_{IIb}\beta_3$ of RGDF mimetics synthesized by us are comparable with the values obtained for well-known RGDF mimetics containing *p*-benzamidine group as argrinine side function bioisoster. Furthermore, it should be mentioned that further synthesis of the analogs in the series of 4-(isoindoline-5-yl)amino-4-oxobutyric acid (**2**) derivatives is promising for the purposes of SAR studies.

The experimental data obtained for antiaggregatory activity and inhibition of FITC-Fg binding to fibrinogen receptor by the novel RGDF mimetics on the base of 4-(isoindoline-5-yl)amino-4-oxobutyric acid give the possibility to consider them as potent platelet aggregation inhibitors and $\alpha_{IIb}\beta_3$ antagonists.

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5. For **4**: mp 159–162 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.47 (s, 9H), 4.67 (s, 4H), 7.59 (dd, J = 2.5 Hz, J = 8.4 Hz, 1H), 8.18 (dd, J = 1.9 Hz, J = 8.4 Hz, 1H), 8.23 (s, 1H). MS (FAB): 265 (M+H). For **6**: mp 205–207 °C. ^{1}H NMR (300 MHz, DMFA-d_7): δ 1.49 (s, 9H), 2.62–2.70 (m, 4H), 4.58 (dd, J = 5.9 Hz, J = 10.0 Hz, 4H), 7.26 (dd J = 4.8 Hz, J = 9.1 Hz, 1H), 7.53 (d, J = 9.1 Hz, 1H), 7.75 (d, J = 9.1 Hz, 1H), 10.12 (s, 1H). MS (FAB): 335 (M+H).
6. For **7a**: mp 145–148 °C. ^{1}H NMR (300 MHz, DMFA-d_7) δ 1.49 (s, 9H), 2.50–2.56 (m, 4H), 2.66 (t, J = 6.8 Hz, 2H), 3.41 (q, J = 6.8 Hz, 2H), 3.63 (s, 3H), 4.58 (dd, J = 6.3 Hz, J = 9.8 Hz, 4H), 7.26 (dd J = 4.8 Hz, J = 8.7 Hz, 1H), 7.53 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 8.00 (t, J = 5.6 Hz, 1H), 10.08 (s, 1H). MS (FAB): 420 (M+H). For **7b**: The oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45 (s, 9H), 2.41–2.57 (m, 4H), 2.73–2.77 (m, 2H), 3.55 (s, 3H), 4.53 (t, J = 7.3 Hz, 4H), 5.22 (q, J = 7.8 Hz, 1H), 7.19–7.33 (m, 6H), 7.40 (dd, J = 1.4 Hz, J = 8.4 Hz, 1H), 7.61 (d, J = 19.6 Hz, 1H), 8.47 (d, J = 8.4 Hz, 1H), 9.97 (s, 1H). MS (FAB): 496 (M+H). For **8a**: mp 142–148 °C. ^{1}H NMR (300 MHz, DMFA-d_7) δ 1.49 (s, 9H), 2.47–2.56 (m, 4H), 2.67 (t, J = 6.8 Hz, 2H), 3.40 (q, J = 6.8 Hz, 2H), 4.58 (dd, J = 6.7 Hz, J = 9.4 Hz, 4H), 7.26 (m, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.97 (t, J = 5.6 Hz, 1H), 10.10 (s, 1H). MS (FAB): 406 (M+H), 428 (M+Na). For **8b**: mp 138–141 °C. ^{1}H NMR (300 MHz, DMFA-d_7) δ 1.49 (s, 9H), 2.57–2.69 (m, 4H), 2.83 (m, 2H), 4.58 (t, J = 6.1 Hz, 4H), 5.39 (q, J = 7.5 Hz, 1H), 7.21–7.45 (m, 6H), 7.53 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 8.46 (d, J = 8.1 Hz, 1H), 10.07 (s, 1H). MS (FAB): 482 (M+H), 504 (M+Na). For **9a**: mp 197–200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.35–2.41 (m, 4H), 2.53–2.61 (m, 2H), 3.23 (q, J = 6.2 Hz, 2H), 4.40–4.48 (m, 4H), 7.29 (d, J = 8.2 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.75 (s, 1H), 8.02 (t, J = 4.7 Hz, 1H), 10.06 (s, 2H), 10.22 (s, 1H). MS (FAB): 306 (M+H), 328 (M+Na). For **9b**: mp 150–153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42–2.56 (m, 4H), 2.6–2.69 (m, 2H), 4.40–4.47 (m, 4H), 5.39 (q, J = 7.3 Hz, 1H), 7.19–7.34 (m, 6H), 7.47 (d, J = 8.1 Hz, 1H), 7.74 (s, 1H), 8.51 (d, J = 8.1 Hz, 1H), 10.05 (t, J = 5.4 Hz, 2H), 10.20 (s, 1H). MS (FAB): 382 (M+H), 404 (M+Na).
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