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RGD mimetics containing phthalimidine fragment as novel ligands of fibrinogen receptor

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ABSTRACT

The novel RGD mimetics with phthalimidine central fragment were synthesized with the use of 4-piperidine-4-yl-butyric, 4-piperidine-4-yl-benzoic, 4-piperazine-4-yl-benzoic and 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acids as surrogates of Arg motif. The synthesized compounds potently inhibited platelet aggregation in vitro and blocked FITC-Fg binding to $\alpha_{IIb}\beta_3$ integrin in a suspension of washed human platelets. The key $\alpha_{IIb}\beta_3$ protein–ligand interactions were determined in docking experiments.

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Over the last twenty years, the fibrinogen receptor GP IIb/IIIa (or integrin $\alpha_{IIb}\beta_3$), has attracted a considerable attention as a promising therapeutic target and its antagonists were applied for treatment of thrombotic disorders such as unstable angina, myocardial infarction, ischemic disease, atherosclerosis, and stroke.[1] In the majority of cases, the design of $\alpha_{IIb}\beta_3$ antagonists has been based on the modeling of Arg-Gly-Asp (RGD) sequence.[1b,2] The main binding sites of RGD sequence are δ-guanidine of arginine and β-carboxylic group of aspartyl. Previously, to create non-peptide fibrinogen receptor antagonists mimicking RGD sequence, *p*-benzamidine, piperidine, isoindoline, tetrahydroisoquinoline[1b,2,3] and β-alanine[4] containing fragments were successfully utilised as bioisosteres of arginine and Asp moieties correspondingly. This approach led the development of Tirofiban (Aggrastat®) approved for treatment of patients with unstable angina.[5] Numerous bicyclic scaffolds have been also used as RGD peptidomimetic blocks including indole,[6] 3,4-dihydro-2*H*-benzopyran,[7] tetrahydronaphthalene,[7b] 1,2,3,4-tetrahydroisoquinoline,[7c] 3-oxo-1,4-benzodiazepine,[8] 3,4-dihydro-2*H*-1,4-benzoxazine,[9] benzimidazole, benzoxazole,[10] 1,2,4-triazolo[3,4-*a*]pyridine,[11] etc.[1b]

Phthalimidines (2,3-dihydroisoindol-1-one) exhibit a wide spectrum of biological activities, for example, antagonism of serotonin 5-HT[12a–c] and dopamine D[12c] receptors, inhibition of TNF-α[12d] and thromboxane A2[12e] as well as documented antifungal and antibacterial activity.[12f] Indobufen, a phthalimidine derivative, is a platelet aggregation inhibitor.[12g] The antagonist of $\alpha_{IIb}\beta_3$ **L-709,780** containing phthalimidine fragment inhibits ADP-induced platelet aggregation of human gel-filtered platelets with an IC_{50} of 0.025 μM.[12h]

This Letter describes the synthesis of new RGD mimetics containing a phthalimidine fragment and the study of their anti-aggregative properties. Furthermore, we demonstrate the utility of 4-piperidine-4-yl-butyric, 4-piperidine-4-yl-benzoic, 4-piperazine-4-yl-benzoic and 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid residues as Arg surrogates for RGD mimetic design.

Initial Boc derivatives of 4-piperidine-4-yl-butyric (**6a**),[13] 4-piperidine-4-yl-benzoic (**6b**),[14] 4-piperazine-4-yl-benzoic (**6c**)[15] and 1,2,3,4-tetrahydroisoquinoline-7-carboxylic (**6d**)[16] acids have been synthesized using previously published methods.

The synthesis of aminophthalimidine building blocks **5a–d** is shown in Scheme 1 and the synthesis of target RGD mimetics **8a–i** is presented in Scheme 2. The central step of formation of phthalimidines **5a–d** is the reduction of phthalimides **1a–d** using zinc amalgam. The nitro derivatives **3a–d** obtained by nitration of the compounds **2a–d** were further reduced by H_2/Pd(C).

Condensation of acids **6a–c** with amines **5a–d** was carried out using HATU. Subsequent saponification of ester groups of compounds **7a–n** and elimination of Boc-protective groups yielded

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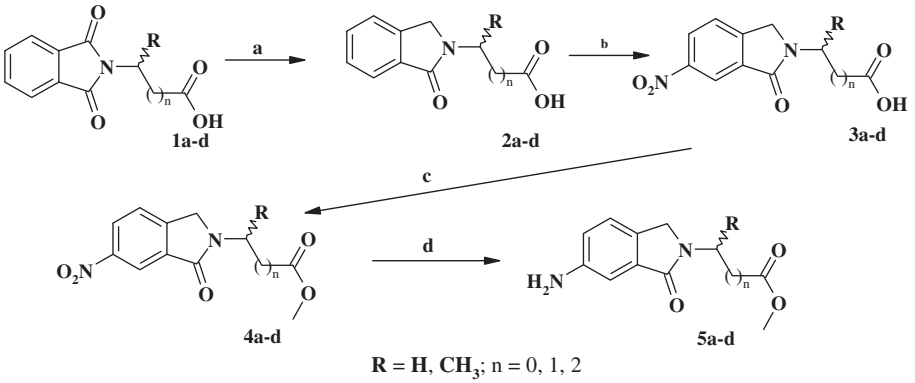
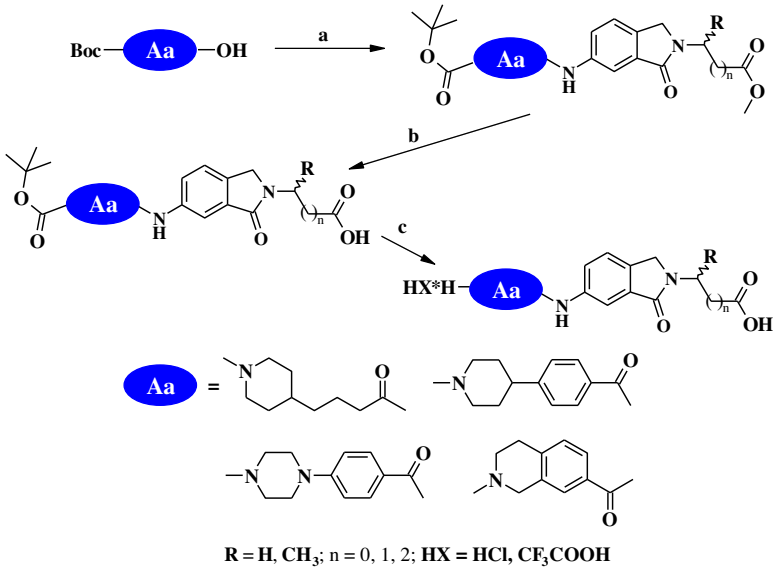


Scheme 1. Synthesis of aminophthalimidines. Reagents and conditions: (a) Zn (Hg), HCl, reflux, 4 h, 65–70%; (b) HNO_3, H_2SO_4, −5 °C, 6 h, 54–65%; (c) MeOH (H_2SO_4), reflux, 3 h, 74–87%; (d) H_2/Pd (C), MeOH, room temperature, 7 h, 82–92%.



Scheme 2. Synthesis of RGD mimetics. Reagents and conditions: (a) NEt_3, HATU, **5a–d**, room temperature, overnight, 46–58%; (b) (i) 1 M NaOH, H_2O, MeOH, room temperature, overnight; (ii)1 M HCl, 73–81%; (c) CH_2Cl_2, HCl gas, 0 °C, 1 h or CH_2Cl_2, TFA, 0 °C, 2 h, 92–96%.

the target mimetics **9a–n**. Mimetics **9h–k** were obtained only as racemic mixtures in order to reveal potent compounds and to determinate general characteristics of structure–activity relationships.

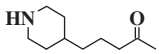
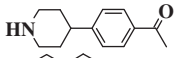
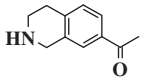
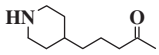
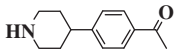
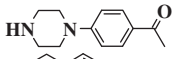
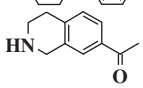
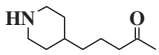
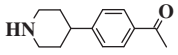
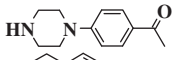
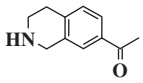
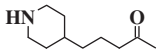
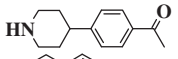
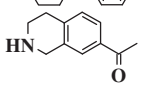
Biological activity was assessed in vitro by measuring the ability of synthesized compounds to inhibit the binding of fluoresceinisothiocyanate-labeled fibrinogen (FITC-Fg)[17] to $\alpha_{IIb}\beta_3$ (in a suspension of human washed platelets).[18] Functional activity was determined by measuring the inhibition of ADP induced platelet aggregation in human platelet-rich plasma (PRP) by Born's method.[19]

Experimental data (Table 1) demonstrate that mimetics **9** are potent inhibitors of FITC-Fg binding to $\alpha_{IIb}\beta_3$ in activated platelets. We found that peptidomimetics **9d–k** with the β-alanine fragment exhibited higher antiaggregative activities compared to compounds **9a–c** and **9l–n**. This suggests that β-amino acids might be the optimal blocks for the $\alpha_{IIb}\beta_3$ antagonists design. The compounds **9f** and **9g** displayed the highest antiaggregative activity among the synthesized mimetics with unsubstituted β-alanine as the Asp bioisostere (Table 1), whereas the antiaggregative activity of **9g** exceeds that of **9d** nearly fivefold, and that of **9e** nearly ninefold.

Comparison of antiaggregative properties of the mimetic **9f** with the previously described compound **10** (Fig. 1) containing fragments of 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid and β-alanine, demonstrated that these two antagonists inhibit platelet aggregation with similar potencies. The affinity of the mimetic **9g** for $\alpha_{IIb}\beta_3$ on activated platelets was slightly higher than the affinity of the mimetic **10**. The antiaggregative activity of **9f** was found to be twice higher than that of **9g**. Replacing β-alanine with β-methyl-β-alanine leads to compounds **9h–k**. Their antiaggregative properties were generally similar to those of unsubstituted ones, with the only exception of the mimetic **9k**. This compound possessed the highest antiaggregative activity among all compounds synthesized in this study. Furthermore, antiaggregative activity of **9k** is comparable to those of compounds **11** and **12** containing bicyclic cores reported in the literature, but in the case of **L-709,780** the comparison is not valid because of the difference in biomaterials used in the assays.

Ligand to protein docking study for all synthesized compounds has been performed with the MOE[20] program. The structure of $\alpha_{IIb}\beta_3$ receptor-Tirofiban complex (**2VDM**) was extracted from the Protein Data Bank.[21] For docking, all water molecules were removed from the binding pocket of **2VDM** with the exception of two molecules coordinated with the metal ion-dependent adhesive site (MIDAS) of $\alpha_{IIb}\beta_3$ receptor — Mg^{2+}, which is involved in the interaction with carboxyl group of Tirofiban. Then, structure of the complex was optimized using the MMFF94x force field. For initial ligand Tirofiban,

Table 1
Biological properties of RGD mimetics **9** and RGDS peptide

Compound	HX	Aa	*n*	R	IC_{50}, μM (PRP)[a]	IC_{50}, μM (FITC-Fg/$\alpha_{IIb}\beta_3$)[b]
9a	TFA		0	H	66.0 ± 9.0	—
9b	TFA		0	H	24.0 ± 3.0	0.27 ± 0.06
9c	HCl		0	H	120.0 ± 20.0	1.2 ± 0.1
9d	TFA		1	H	5.9 ± 0.6	0.0055 ± 0.009
9e	TFA		1	H	9.6 ± 1.9	0.0068 ± 0.0012
9f	TFA		1	H	0.54 ± 0.06	—
9g	HCl		1	H	1.1 ± 0.1	0.0065 ± 0.0005
9h	HCl		1	CH_3	5.4 ± 1.0	0.35 ± 0.03
9i	HCl		1	CH_3	6.2 ± 1.2	—
9j	HCl		1	CH_3	3.74 ± 0.51	0.037 ± 0.08
9k	HCl		1	CH_3	0.086 ± 0.007	0.0065 ± 0.0012
9l	TFA		2	H	51.0 ± 30.0	—
9m	TFA		2	H	410.0 ± 60.0	—
9n	HCl		2	H	330.0 ± 50.0	—
	RGDS				31.0 ± 2.0	13.0 ± 1.6

[a] Concentration required to reduce ADP-induced human platelet aggregation response by 50%.
[b] Concentration required to reduce binding of FITC-Fg to $\alpha_{IIb}\beta_3$ in suspensions of washed human platelets by 50%. The IC_{50} values are expressed as averages of at least two determinations.

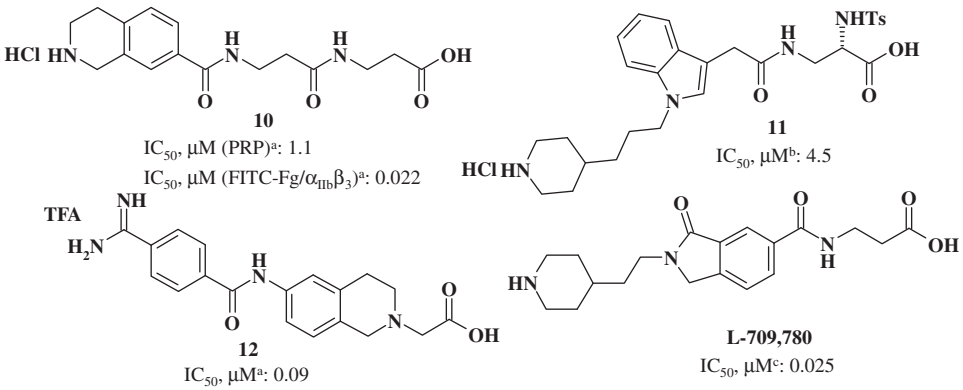


Figure 1. Structure and in vitro activities of $\alpha_{IIb}\beta_3$ antagonists **10**,[16] **11**,[6] **12**[7c] and **L-709,780**.[12h] [a]See Table 1; [b]inhibition of guinea-pig PRP aggregation induced by collagen; [c]inhibition of ADP-induced platelet aggregation of human gel-filtered platelets.

which was a part of **2VDM**, re-docking had been carried out with good result (RMSD = 0.6 Å). Thus $\alpha_{IIb}\beta_3$ cavity was prepared, and all further studies of compounds **9** docking were carried out for this binding site of the receptor.

The docking studies have revealed some general patterns for binding of mimetics **9** to their receptor $\alpha_{IIb}\beta_3$. It has been shown that the nitrogen atom of Arg-isosteres (the fragments of 4-piperidine-4-yl-butyric, 4-piperidine-4-yl-benzoic, 4-piperazine-4-yl-benzoic and 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acids) interacts with two amino acid residues of α chain of fibrinogen receptor, namely with carboxyl group of D224 side chain and S225 amide bond. Carboxyl group of mimetics **9** is involved in coordination sphere of Mg^{2+}, and also interacts with Y122 amide bond and amide group of N215 side chain incorporated in the $\alpha_{IIb}\beta_3$ β-chain. These interactions are illustrated in Figure 2 using the complex of **9g** as an example.

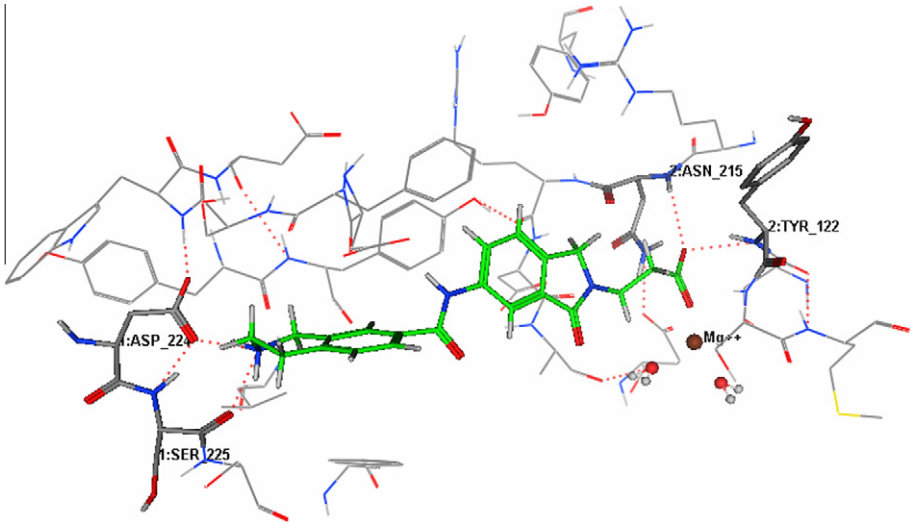


Figure 2. Binding of mimetic **9g** to the $\alpha_{IIb}\beta_3$ receptor observed in docking experiments.

Docking studies revealed that *S*-enantiomers of compounds **9i** and **9j** bind to $\alpha_{IIb}\beta_3$ stronger than corresponding *R*-enantiomers. In the case of compounds **9h** and **9k,** no significant differences in values of scoring function for *S* and *R*-enantiomers was observed. Detailed investigation of the relationship between the stereochemistry of the mimetics **9** and their affinity for $\alpha_{IIb}\beta_3$ will be the purpose of future studies.

To summarize, for a series of 4-piperidine-4-yl-butyric, 4-piperidine-4-yl-benzoic and 1,2,3,4-tetrahydroisoquinoline-7-carboxylic acids derivatives, no significant influence of Arg isostere structure on affinity for $\alpha_{IIb}\beta_3$ was observed. At the same time, the compounds **9d**–**k** have demonstrated a high affinity for $\alpha_{IIb}\beta_3$ and acceptable antiaggregative activity. A combination of tetrahydroisoquinoline and β-methyl-β-alanine as Arg and Asp bioisosteres, respectively, within the given phthalimidine series, leads to the best prospective inhibitor of the platelet aggregation. It has been shown by docking studies that α:D224, α:S225 and β:Y22, β:N215 residues of $\alpha_{IIb}\beta_3$ integrin play the key role in binding of the mimetics **9** to the receptor.

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