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Beta-Amino Acid Derivatives As Orally Active Non-Peptide Fibrinogen Receptor Antagonists

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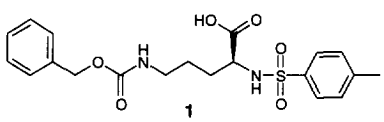
Abstract: The ornithine sulfonamide **1** was identified by random screening as weak fibrinogen receptor antagonist. Homologation of the carboxylic acid function and further structure activity studies led to the development of novel beta-amino acid derivatives, which are potent and orally active antagonists of the platelet fibrinogen receptor GPIIb/IIIa. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

The final common process induced by all platelet agonists is the exposure of the activated receptor for fibrinogen, the glycoprotein IIb/IIIa (GPIIb/IIIa). As a consequence fibrinogen is able to bind to its receptor and in this way platelets are cross-linked and platelet aggregation is initiated.[1] In pathologic conditions, the same processes can lead to deposition of platelets on thrombogenic surfaces followed by the formation of platelet aggregates and growth of a thrombus. These events are key steps in the development of disorders such as unstable angina, myocardial infarction, transient ischemic attacks and stroke.[2] The inhibition of the fibrinogen-GPIIb/IIIa interaction represents a well established intervention point in the treatment of thrombosis[3] and the efficacy of GPIIb/IIIa antagonists in preventing arterial thrombus formation has been recently demonstrated with the chimeric Fab fragment of the anti-GPIIb/IIIa monoclonal antibody 7E3 in high-risk angioplasty patients in a phase III clinical trail.[4]

Over the last years, many potent and selective GPIIb/IIIa antagonists, including snake venom polypeptides, cyclic and linear peptides, as well as non-peptide inhibitors have been described.[5] These inhibitors are mainly derivatives or mimics of the Arg-Gly-Asp (RGD) recognition motif present in various GPIIb/IIIa ligands.[6] Due to lack of oral availability and short duration of action the first generation of non-peptide antagonist is not suitable for the chronic treatment of patients with recurrent vascular events.[5] More recently several reports of a second generation of orally active inhibitors of GPIIb/IIIa have been published.[5,7]

Beside our efforts to design non-peptide GPIIb/IIIa antagonists using the RGD sequence as a lead structure, we have set up a high capacity GPIIb/IIIa-fibrinogen binding assay. Screening of our in house compound collection yielded the ornithine sulfonamide **1** as a weak inhibitor of fibrinogen binding to GPIIb/IIIa (IC_{50} = 20 μM).



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Homologation of the carboxylic acid function and replacement of the Z-protection group with a benzamidine moiety resulted in a novel class of beta-amino acid-type antagonists of the fibrinogen receptor. Further structural modifications gave highly potent and orally active inhibitors of GPIIb/IIIa.

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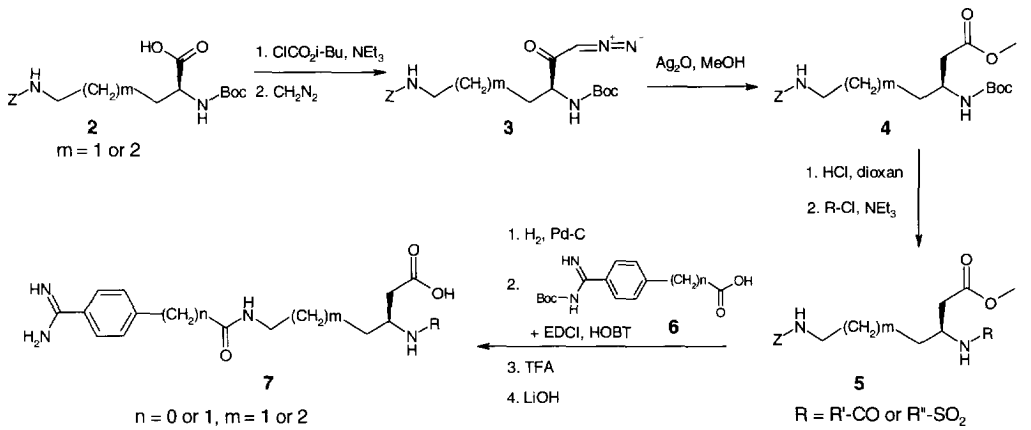
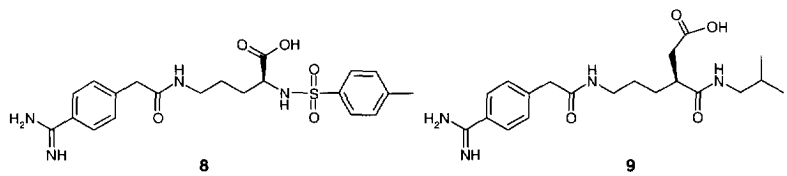
Chemistry

The beta-amino acid derivatives were prepared as outlined in Scheme 1. The reaction of BOC-, Z-diprotected ornithine (**2**, m=1) or lysine (**2**, m=2) with diazomethane via a mixed anhydride gave the diazo ketones **3,** which were converted through a silver catalysed Wolff rearrangement[8] to the BOC-, Z-diprotected beta-amino esters **4** (70 - 80 % yield from **2**). Deprotection and acylation of the beta-amino group yielded the amides or sulfonamides **5** quantitatively. The Z-protecting group on the nitrogen was removed through hydrogenation and the resulting amines were coupled with benzamidine carboxylic acids **6**[9] under standard coupling conditions (yields 50-70 %). Removal of the amidine Boc-protecting group and saponification of the methyl ester with lithium hydroxide gave the benzamidine beta-amino acid derivatives **7** (detailed structures listed in Table 1) as enantiomerically pure compounds in yields of 50 - 70 %.[10]

Scheme 1. Preparation of beta-amino acid GPIIb/IIIa antagonists



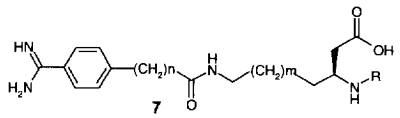
For SAR purposes the ornithine derivative **8** and Gly-Asp ethylene isostere **9** were synthesised. Starting from γ-Z-protected ornithine methyl ester, the sulphonamide **8** was obtained using a similar chemistry as described in Scheme 1. Enantioselective α-alkylation[11] of 4-azido-pentanoic acid with tert-butyl bromo-acetate gave a Gly-Asp ethylene isostere which was converted to **9** using standard reaction conditions.



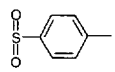
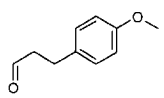
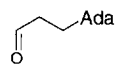
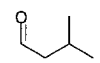
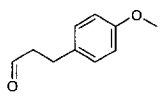
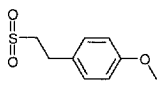
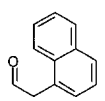
Results and Discussion

The *in vitro* activities of the compounds listed in Table 1 were assessed in two different assay systems : (a) inhibition of ADP-induced aggregation of human blood platelets,[12] (b) inhibition of fibrinogen binding to immobilised human GPIIb/IIIa.[13] To compare the *in vivo* efficacy of the different inhibitors, *ex vivo* platelet aggregation was measured in guinea pigs up to 5 h after intraduodenal (i.d.) application of the compounds. The number of platelets was determined before and 1 min after ADP addition to the blood sample, giving a percentage of inhibition of platelet aggregation.[14] The *in vivo* results are summarised in Table 1.

Table 1. *In vitro* and *in vivo* activities of beta-amino acid-type GPIIb/IIIa antagonists



7

no.	m, n	R	platelet aggregation IC_{50} (nM)	GPIIb/IIIa-FG IC_{50} (nM)	guinea pigs: ex vivo platelet aggregation, i.d. dose : % inhibition after 5 h
8	-, -	see compound 8	31000	48000	not tested (n.t.)
9	-, -	see compound 9	950	7.9	n.t.
10	1, 1		640	7.6	10 mg/kg : 10 %
11	1, 1		100	1.7	1 mg/kg : 25 % 3 mg/kg : 75 %
12	1, 1		31	1.1	1 mg/kg : 10 % 3 mg/kg : 10 %
13#	1, 1		67	2.2	n.t.
14#	1, 1		61	0.37	3 mg/kg : 10 %
15	2, 0		15	0.54	1 mg/kg : 65 % 3 mg/kg : 75 %
16	2, 0		18	0.36	0.3 mg/kg : 45 % 1 mg/kg : 90 %
17	2, 0		79	1.0	n.t.
18	2, 0		18	0.36	1 mg/kg : 40 %

#: Ada = Adamant-1-yl

An explanation for the weak binding activity of the screening hit **1** is the lack of a basic functional group which is the second major requirement for binding activity to GPIIb/IIIa beside the carboxylic acid.[5] Therefore the introduction of a benzamidine moiety was a logical step towards improvement of the activity of **1**. However, the resulting sulfonamide **8** showed no improvement in the *in vitro* assays. A drastic increase in activity in the GPIIb/IIIa-FG binding assay (6000-fold) and platelet aggregation assay (50-fold) was obtained through prolongation of the carboxylic side chain in **8** by one methylene group to give the beta-amino acid derivative **10.**

Variations of the substituents on the beta-nitrogen in **10** further improved the *in vitro* activity. Replacement of the tosyl group in **10** by isobutylcarbonyl (**11**) or p-methoxyphenethylcarbonyl (**12**) resulted in a 7 - 20-fold activity increase in the platelet aggregation and fibrinogen binding assay. Bulky adamantyl groups, which significantly increased the *in vitro* activity of our previously reported gamma-lactam containing fibrinogen receptor antagonists [9] also gave an increase in activity in this series of inhibitors. While derivative **13** with a methylenadamantylcarbonyl moiety showed similar *in vitro* potency as **11**, the ethylenadamantylcarbonyl containing inhibitor **14** had a 5-fold improved activity in the fibrinogen binding assay compared to **11**. This increase in potency was not seen in the platelet aggregation assay.

A comparison of the *in vitro* results of the beta-amino acid derivative **11** with the corresponding compound **9**, which constitutes a RGD mimetic containing an ethylene Gly-Asp isostere, shows that the inverted amide bond in **11** increases activity in the platelet aggregation and fibrinogen binding assay 7 - 9-fold. The improvement in activity might be due to either an optimised hydrogen bonding of the inverted amide with the GPIIb/IIIa receptor backbone or to a conformational change in the inhibitor which increases the binding affinity.

Variation of the position of the amide adjacent to the benzamidine group influences the *in vitro* activity. The inhibitor **15** with the amide next to the benzamidine unit, has a 3 - 6-fold increased activity in both *in vitro* assays compared to the analogue compound **11** in which the amide is shifted by one methylene group. The overall distance between the benzamidine and the carboxylic acid in both molecules is the same, therefore again an improved hydrogen bonding or an optimised conformation may account for the activity increase.

Replacement of the isobutyl group in **15** by aryl-alkyl substituents (**16, 18**) did not further increase the *in vitro* activity and the introduction of a p-methoxyphenethylsulfonyl group (**17**) reduced platelet aggregation and binding activity compared to **15**.

The importance of the *S*-configuration at the stereogenic centre in the inhibitors on binding and platelet aggregation activity was demonstrated with the *R*-enantiomer of **15.** The change in stereochemistry from *S* to *R* resulted in a 50-fold loss of binding activity to GPIIb/IIIa and a 80-fold reduction in the inhibition of platelet aggregation (data not shown).

Several of the potent GPIIb/IIIa inhibitors have been evaluated in the guinea pig *ex vivo* platelet aggregation model. The compounds were dosed between 0.3 and 10 mg/kg i.d. and the inhibition of the *ex vivo* platelet aggregation was measured 5 h later. In parallel, bleeding time measurements were performed on small mesenteric arteries on the surface of the jejunum (data not shown). As seen in Table 1, there are substantial differences in the *in vivo* activities of the various inhibitors. Best results were obtained with the isobutyl derivatives **11** and **15** and the p-methoxyphenethyl containing compound **16**. Interestingly, compound **12**, which differs from **16** only in the position of the amide adjacent to the benzamidine moiety, gave only 10 % inhibition at 3 mg/kg. A similar trend is seen with **11** and **15**, compound **15** with the amide directly bound to the benzamidine showed a 3 times better inhibition at 1 mg/kg. However at 3 mg/kg both compounds gave 75 %

inhibition. Replacement of isobutyl in **11** or **15** by naphthyl (**18**) or adamantyl (**14**) groups drastically reduced the *in vivo* activity.

GPIIb/IIIa antagonist of different structural classes can discriminate to differing degrees between inhibition of aggregation and bleeding.[5b,14] Differences in the prolongation of the bleeding time was also found with our inhibitors. A profound prolongation (>10 min) of the mesenteric bleeding time was observed with **16** at 1 mg/kg, while the isobutyl derivative **15** gave only a marginal prolongation (2-3 x control) at 3 mg/kg. In an *ex vivo* platelet aggregation model in rhesus monkeys, where **15** and **16** were found to be orally active at 1 - 3 mg/kg [15,16] again, **15** showed less effect on bleeding time than **16**. Based on these results it was decided to further develop **15** (GPI 562) as an orally active inhibitor of GPIIb/IIIa. GPI 562 is currently under clinical evaluation.

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13. GPIIb/IIIa was isolated from membranes of human platelets by triton X-100 extraction and purified and immobilized by adsorption to microtiter plates. Biotin-labelled fibrinogen was allowed to bind to the GP IIb/IIIa coated plates in the presence or absence of inhibitor. Bound fibrinogen was detected with Streptavidin-biotinylated horseradish peroxidase.
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