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# FIBRINOGEN RECEPTOR ANTAGONISTS CONTAINING A GAMMA-LACTAM GLY-ASP ISOSTERE

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***Abstract:*** A γ-lactam bridged Gly-Asp dipeptide isostere was synthesised and incorporated into RGDX tetrapeptides as a replacement for GD to obtain conformationally constrained fibrinogen receptor antagonists.

## *Introduction*

A decisive step in thrombus formation is the crosslinking of activated blood platelets by fibrinogen molecules.[1] The activation of the platelets by agonists such as thrombin or adenosine diphosphate (ADP) leads to exposure of the fibrinogen receptor GPIIb/IIIa in an active form.[2] GPIIb/IIIa belongs to a family of adhesion receptors known as integrins. Additional ligands, apart from fibrinogen, are fibronectin, vitronectin and the von Willebrand factor. These ligands play an important role in haemostatic processes by mediating the adhesion and aggregation of platelets.[3] In pathological situations, the same processes can lead to thrombotic disorders. Therefore the blockade of GPIIb/IIIa may be useful in prevention of myocardial infarction, unstable angina or stroke.[4]

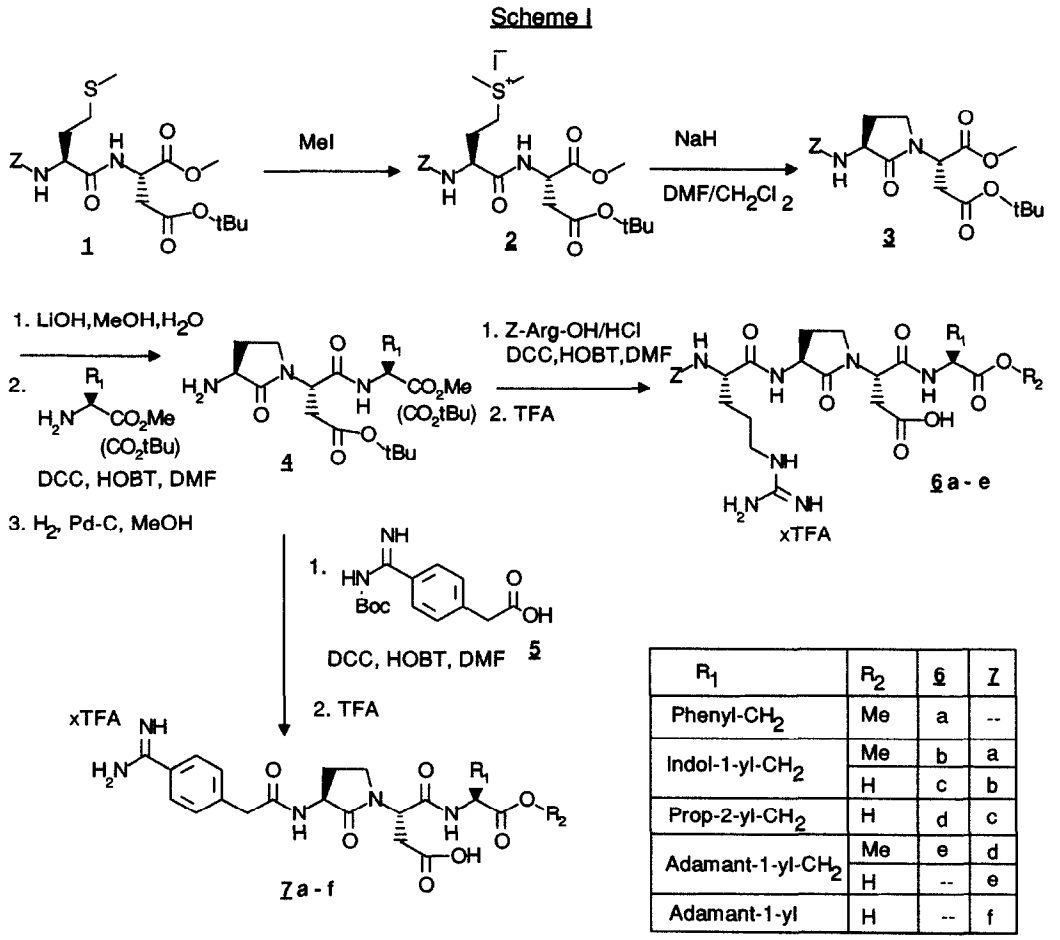
The binding of fibrinogen, and of the other ligands, is mediated by the tripeptide sequence Arg-Gly-Asp (RGD).[5] Fibrinogen possesses an additional sequence at the C-terminus of the γ-chain with affinity for the fibrinogen receptor.[6] Besides monoclonal antibodies,[7] RGD containing polypeptides isolated from snake venoms[8] and small linear and cyclic RGD containing peptides[9] are able to block the platelet fibrinogen receptor. Recently non-peptide fibrinogen receptor antagonists have been reported.[10]

During the course of our work on inhibition of fibrinogen binding to GPIIb/IIIa, we were looking for dipeptide isosteres with restricted flexibility as a replacement for GD in RGDX tetrapeptides. γ-Lactam dipeptide isosteres were successfully used as a conformational constraint in peptides.[11] Information may be obtained about the bioactive conformation of a peptide, and biological potency may be increased. We report here on the synthesis of a γ-lactam bridged Gly-Asp isostere **3** which was incorporated instead of GD in a variety of RGDX peptides and related compounds having a p-amidino phenylacetic acid instead of the N-terminal arginine.

### *Synthesis*

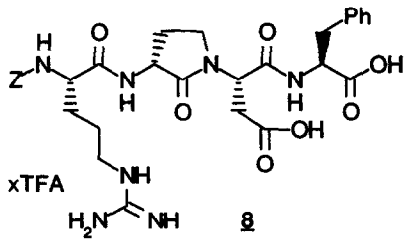
The target compounds **6a** -**e** and **7a** - **f** were prepared according to Scheme I .
The protected γ-lactame Gly-Asp unit **3**[12] was obtained in 60 % yield starting from the protected dipeptide **1**, following a procedure published by R.M.Freidinger.[13] With two equivalents of NaH, as described by Freidinger, the methyl ester was partially hydrolysed during the cyclisation and the NMR analysis of **3** revealed a complete racemisation in the aspartic acid-derived moiety. However, if only 1 equivalent NaH was used and the reaction was quenched after 3 h at 0 °C with aqueous 2N HCl, only the methyl ester **3** was isolated. The NMR analysis showed 10 - 15 % racemisation at the Asp portion under these conditions. The second diastereomere was removed by chromatography or crystallisation later on in the synthesis. After hydrolysis of the methyl ester in **3** with lithium hydroxide, the resulting acid was coupled with a variety of methyl or *tert.*-butyl esters of natural and

Scheme I




| $R_1$ | $R_2$ | 6 | 7 |
|---|---|---|---|
| Phenyl-$CH_2$ | Me | a | -- |
| Indol-1-yl-$CH_2$ | Me | b | a |
| | H | c | b |
| Prop-2-yl-$CH_2$ | H | d | c |
| Adamant-1-yl-$CH_2$ | Me | e | d |
| | H | -- | e |
| Adamant-1-yl | H | -- | f |

unnatural amino acids[14] using standard coupling conditions. The protecting group at the primary amine was removed by hydrogenation in the presence of Pd-C. The free amines 4 were reacted with Z-arginine to give after a final deprotection with trifluoroacetic acid (TFA) the γ-lactam containing tetrapeptides 6a - e. Coupling of 4 with BOC-protected p-amidino phenylacetic acid[15] 5 yielded after removal of the protection groups with TFA the benzamidino compounds 7a - f. The analog compounds 8 with (*R*)-configuration in the lactam ring or 9 with the (*R*)-configuration in the Asp moiety were prepared in a similar manner using as a starting material Z-(*D*)-Met-Asp(*tert*-Bu)OMe or Z-Met-(*D*)-Asp(*tert*-Bu)OMe respectively.



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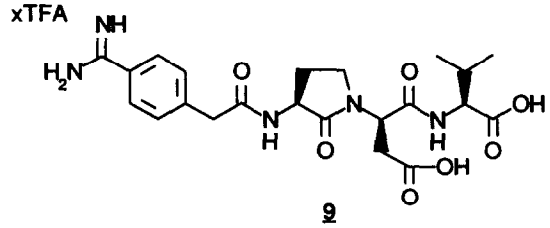

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***Biological Evaluation***

The biological activity of our fibrinogen receptor antagonists was assessed in two different in vitro test systems:

a) Inhibition of ADP-induced aggregation of human blood platelets in the presence of fibrinogen: Blood platelets were isolated from fresh whole blood by centrifugation. The washed platelets were resuspended to a final concentration of $3x10^8$ platelets/ml, incubated with inhibitor or solvent (control) and fibrinogen (0.1mg/ml) and aggregation was initiated by adding 10 μM ADP. The ability of the platelets to aggregate in the presence or absence of inhibitors was quantified using an aggregometer.

b) Inhibition of fibrinogen binding to purified immobilised GPIIb/IIIa: GPIIb/IIIa was isolated from membranes of human blood platelets by triton X-100 extraction and purified by chromatography on ion exchangers and by gel filtration. The receptor protein thus obtained was used to coat microtiter plates. Fibrinogen was biotinylated and the binding to the receptor in the presence or absence of inhibitor was quantified.

The $IC_{50}$ values of our inhibitors determined in these assays are shown in Table I. A good correlation between the order of potency in the fibrinogen binding assay and the platelet aggregation was observed. The 50-100 fold lower $IC_{50}$ values in binding assay compared to the platelet aggregation test is due to different fibrinogen concentrations present (aggregation : 300 nM, binding : 3 nM).

***Discussion***

The results given in Table I demonstrate that a γ-lactam GD isostere can replace GD in RGDX peptides without significant loss of biological activity. The γ-lactam compound **6a**, compared to the analog tetrapeptide Z-Arg-Gly-Asp-Phe-OMe, is only 6-times less active in the aggregation and binding assay, while the related peptides Z-Arg-Ala-Asp-Phe-OMe and Z-Arg-(*D*)-Ala-Asp-Phe-OMe, substituted at the α-center at glycine, are not tolerated by the receptor as reflected in the low activities. In the γ-lactame isosteres the substitution at the α-center of glycine is tolerated. However, the (*S*)-configuration in the lactame ring as well as in the Asp moiety of compounds **6** and **7** is necessary to get a bioactive conformation. Compound **8** with (*R*)-configuration in the lactam ring and **9** with (*R*)-configuration in the Asp-moiety have biological activity in our assays.

Table I. Affinity and Antiaggregatory Activity

| Compound | $IC_{50}$(μM) platelet aggreg. | $IC_{50}$ (μM) GPIIb/IIIa-FG |
|---|---|---|
| | | |
| Z-Arg-Gly-Asp-Phe-OMe | 13.0 | 0.27 |
| Z-Arg-Gly-Asp-Val-OH | 3.10 | 0.11 |
| Z-Arg-Ala-Asp-Phe-OMe | >100 | 78.0 |
| Z-Arg-(D)Ala-Asp-Phe-OMe | >100 | >100 |
| **6a** | 85.1 | 0.77 |
| **6b** | 25.1 | 1.22 |
| **6c** | 10.0 | 1.07 |
| **6d** | 12.8 | 0.56 |
| **6e** | 4.00 | 0.51 |
| **7a** | 4.40 | 0.12 |
| **7b** | 0.74 | 0.02 |
| **7c** | 0.36 | 0.04 |
| **7d** | 0.36 | 0.02 |
| **7e** | 0.10 | 0.005 |
| **7f** | 0.04 | 0.002 |
| **8** | >100 | >100 |
| **9** | >100 | >100 |
| Echistatin[8] | 0.02 | 0.002 |

The p-amidinophenyl moiety, a known mimic of the arginine side chain[16] and successfully used in non-peptide fibrinogen antagonists,[10] is a good replacement for arginine in the γ-lactam GPIIb/IIIa antagonists. Antiaggregatory activities as well as affinities to GPIIb/IIIa are enhanced by a

factor 10 - 30. Like with RGDX tetrapetides, an additional increase in activity by a factor of 3 - 5 is gained with a free acid instead of the methyl ester at the C-terminus as demonstrated in **6b** / **6c** , **7a**/**7b** and **7d**/**7e**. For both, the arginine and the benzamidino γ-lactams, an increase in the steric demand of the side chain in the terminal amino acids leads to an improvement in potency. The adamantylalanine containing compounds **6e** and **7d** are 7 - 10 times more potent in the aggregation assay than **6b** and **7a** with a tryptophan. The most active compound **7f** with adamantylglycine at the C-terminus shows $IC_{50}$ values in the aggregation and binding assay in the same range as the snake venom echistatin,[8] one of the most potent natural occurring GPIIb/IIIa antagonists. It is interesting to note, that replacement of the γ-lactam Gly-Asp moiety in **7f** with a Gly-Asp dipeptide reduces the activity in the aggregation assay by a factor of 3. This indicates that the γ-lactam is not only a mimic of Gly-Asp, it also stabilises the bioactive conformation in these molecules.

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