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# PLATELET GLYCOPROTEIN IIb/IIIa RECEPTOR ANTAGONISTS DERIVED FROM ISOXAZOLIDINES

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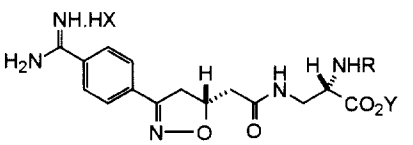
**Abstract:** A series of isoxazolidines has been synthesized as mimetics of the RGD sequence and was evaluated as antagonists of the platelet glycoprotein IIb/IIIa receptor. These compounds were shown to be highly potent GPIIb/IIIa antagonists, exhibiting submicromolar potencies. 

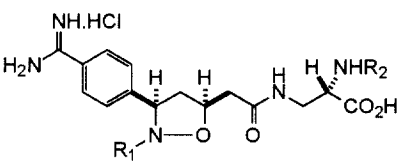
Platelet rich thrombus formation is involved in various vasoocclusive disorders such as unstable angina, acute myocardial infarction, and reocclusion following angioplasty.[1,2,3] The final, common event leading to thrombus formation, independent of the mechanism of platelet activation, is the binding of fibrinogen to its platelet receptor glycoprotein integrin (GPIIb/IIIa).[4,5,6] A major determinant of the GPIIb/IIIa-fibrinogen interaction is receptor recognition of the tripeptide sequence Arg-Gly-Asp (RGD).[7,8] Thus, the RGD motif has been actively pursued as a template for structure-based drug design, and considerable efforts have been expended to design peptides and peptidomimetics that contain or mimic the RGD sequence as antagonists of fibrinogen binding to activated platelets.[9,10]

Many examples of potent GPIIb/IIIa antagonists have been described, including those incorporating centrally constrained RGD mimics, for example, drug candidates **1a**[11,12] and **1b.**[13]




**1a (DMP754)**
X = OAc, Y = Me, R = $CO_2n$-Bu

**1b (DMP802)**
X = $SO_3CH_3$, Y = H
R = 3,5-dimethylisoxazol-4-ylsulfonyl




**2**

$R_1$ = Me, *i*-Pr, Bn, Ph
$R_2$ = $SO_2$Ar, $CO_2n$-Bu

As a part of our research program directed toward the design and synthesis of clinically useful antithrombotic agents, we discovered a novel series of isoxazolidine-based heterocycles **2**, shown to be inhibitors of fibrinogen-mediated platelet aggregation. We herein report the synthesis and biological activity of these novel GPIIb/IIIa antagonists.

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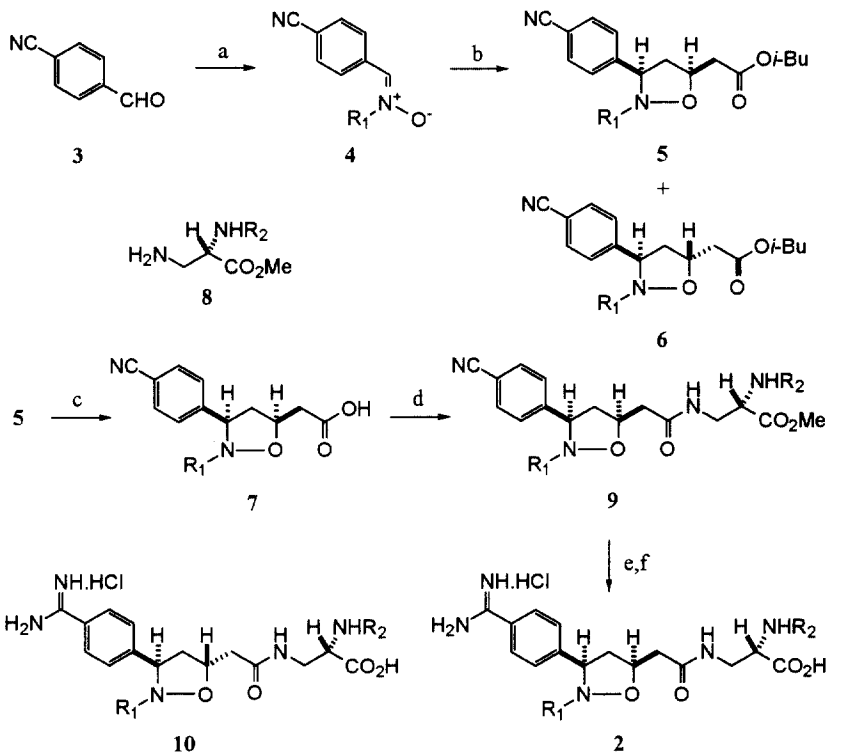
## Synthesis

The isoxazolidine-based GPIIb/IIIa antagonists of general structure **2** were synthesized as shown in Scheme 1. Treatment of 4-cyanobenzaldehyde with an N-substituted hydroxylamine yields the nitrone **4**, which is treated with the dipolarophile *i*-butyl vinylacetate to afford the isoxazolidinyl acetates **5**, **6** as a *cis* and *trans* mixture with the *cis* isomer predominant, as shown by NOE measurements. The *cis/trans* ratio varies with the $R_1$ substituent. For example, in the case of $R_1$ = methyl, the *cis/trans* ratio is around 3; while for $R_1$ = benzyl, the ratio is increased to 10. The two isomers are separated by chromatography and transformed to the target molecules separately. Basic hydrolysis of **5** with LiOH in aqueous THF yields the key intermediate acid **7**, which is coupled to the diaminoester **8**[14] using PyBOP to yield **9**. A Pinner reaction, ammonia treatment and ester hydrolysis sequence carried out on **9** yields the target RGD mimics **2**. A related series of reactions are employed for conversion of the *trans* series **6** to the corresponding *trans* RGD mimics **10**.

**Scheme 1**




(a) $R_1NHOH.HCl$, $Na_2CO_3$, $CH_2Cl_2$, rt, 80-95%; (b) *i*-Butyl vinylacetate, 100 °C, 12-18 h, 70-85%; (c) LiOH, THF, $H_2O$, 75-90%; (d) **8**, $NEt_3$, PyBOP, DMF, 0 °C, 80-95%; (e) MeOH, HCl(g), $CHCl_3$, 0 °C-rt, 24 h; then , $(NH_4)_2CO_3$, MeOH, rt, 4-8 h, 40-80%; (f) 4 N HCl in $H_2O$, 48 h, 80-90%

## Biological Activity

The compounds were evaluated as platelet aggregation inhibitors by measuring their effect on the ADP stimulated aggregation of human platelets *in vitro*.[15] These results are presented in Table 1.

**Table 1.** *In vitro* activity of isoxazolidine analogs in the inhibition of platelet aggregation using human platelet-rich plasma

| Compound | $R_1$ | $R_2$ | $IC_{50}(\mu M)$ |
|---|---|---|---|
| **2a** | Me |  | 0.028 |
| **10** | Me |  | 1.5 |
| **2b** | $C_6H_5CH_2$ |  | 0.19 |
| **2c** | *i*-Pr |  | 0.14 |
| **2d** | Ph |  | 0.53 |
| **2e** | Me |  | 0.058 |
| **2f** | Me |  | 0.084 |
| **2g** | Me | $CO_2n$-Bu | 0.164 |

A key element of our design for potent GPIIb/IIIa receptor inhibitors is the incorporation of a structural constraint within the molecule to direct the key vectors of the N and C terminal side chains.[10,16,17] Control of the stereochemistry of this key central constraint may lead to enhanced potencies. Indeed, the *cis* and *trans* isomers of this class of isoxazolidines exhibit quite different activity profiles. As shown in Table 1, the *cis* isomer **2a** is over 50-fold more potent than its corresponding *trans* isomer **10**, suggesting the *cis* isomer, due to its favorable geometry, has a more efficient interaction of its N and C terminal chains with the fibrinogen receptor than the *trans* cogener.

To investigate the effect of substitution on the isoxazolidine nitrogen on potency, various $R_1$-substituted analogs were prepared. When the methyl group in **2a** is replaced with benzyl (**2b**) or isopropyl (**2c**), a decrease of around 5-fold in potency is observed, while with phenyl (**2d**), a very significant decrease is found, indicating simple alkyl substituents on the isoxazolidine nitrigen are preferred for activity. Different sulfonamides are comparable in activity; for example, **2e** and **2f** are only slightly less potent than **2a**. We made one carbamate analog, the *n*-butylcarbamate **2g**. This compound is significantly less active than the corresponding sulfonamides, in contrast to other related series.[11]

*In vivo* studies of these isoxazolidine-based GPIIb/IIIa inhibitors with regard to duration and potency revealed attractive phamacological profiles. For example, when administered intravenously at a dose of 0.025mg/kg in a canine model,[18] **2a** maintained over 50% *ex vivo* platelet inhibition after 12 h, implicating a potential value of this series of compounds as clinically useful antithrombotic agents.

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