PLATELET GLYCOPROTEIN IIb/IIIa RECEPTOR ANTAGONISTS DERIVED FROM ISOXAZOLIDINES

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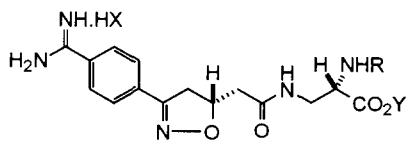
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Abstract: A series of isoxazolidines has been synthesized as mimetics of the RGD sequence and was evaluated as antagonists of the platelet glycoprotein IIb/IIIa receptor. These compounds were shown to be highly potent GPIIb/IIIa antagonists, exhibiting submicromolar potencies. © 1998 The DuPont Pharmaceuticals Company. Published by Elsevier Science Ltd. All rights reserved.
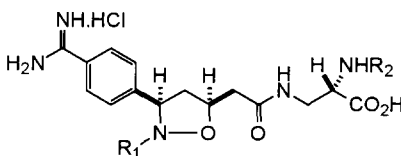
Platelet rich thrombus formation is involved in various vasoocclusive disorders such as unstable angina, acute myocardial infarction, and reocclusion following angioplasty.[1,2,3] The final, common event leading to thrombus formation, independent of the mechanism of platelet activation, is the binding 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$_2$n-Bu

1b (DMP802)
X = SO$_3$CH$_3$, Y = H
R = 3,5-dimethylisoxazol-4-ylsulfonyl

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R$_1$ = Me, i-Pr, Bn, Ph
R$_2$ = SO$_2$Ar, CO$_2$n-Bu
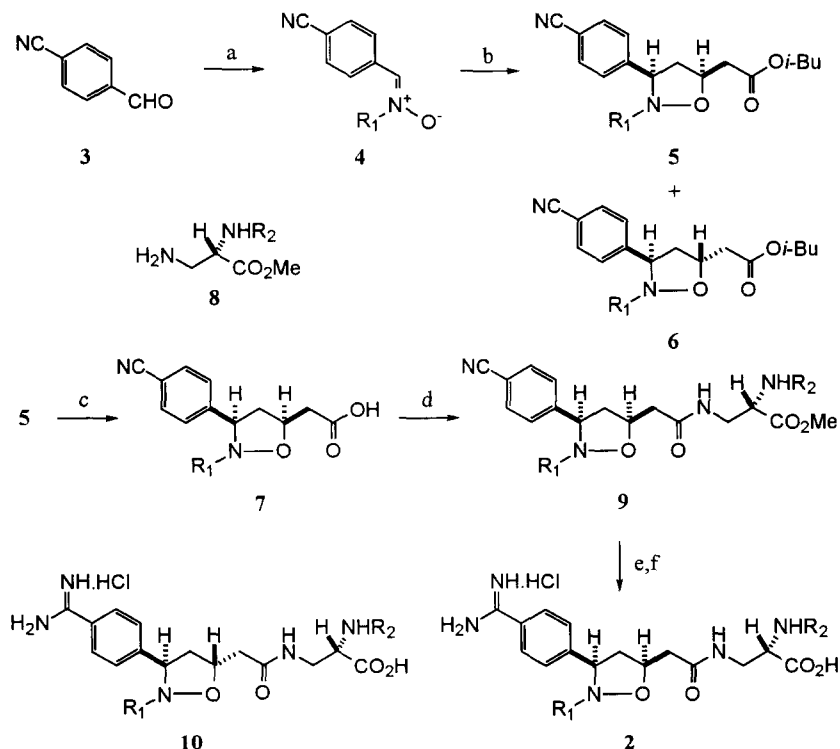
As a part of our research program directed toward the design and synthesis of clinically useful antithrombotic agents, we discovered a novel series of isoxazolidine-based heterocycles **2**, shown to be inhibitors of fibrinogen-mediated platelet aggregation. We herein report the synthesis and biological activity of these novel GPIIb/IIIa antagonists.

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	SO_2 (3-substituted phenyl)	0.028
10	Me	SO_2 (3-substituted phenyl)	1.5
2b	$C_6H_5CH_2$	SO_2 (3-substituted phenyl)	0.19
2c	*i*-Pr	SO_2 (3-substituted phenyl)	0.14
2d	Ph	SO_2 (3-substituted phenyl)	0.53
2e	Me	SO_2 (2-substituted phenyl)	0.058
2f	Me	SO_2 (N–O isoxazole)	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.

References and Notes

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