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Ring Constrained Analogues of β-Alanine-Containing GPIIb/IIIa Receptor Antagonists

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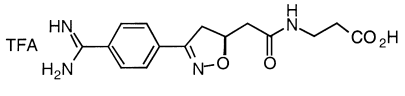
Abstract—A series of ring constrained analogues of the GPIIb/IIIa receptor antagonist XR299 (**1**) was investigated as potential inhibitors of glycoprotein IIb/IIIa, a platelet receptor that plays a key role in platelet aggregation and platelet adhesion. Ring size was found to have a large effect on in vitro potency. Selected compounds showed good in vitro activity, a preference for binding to activated platelets, and modest duration of action when dosed iv as a racemate in a canine model.

The utility of glycoprotein IIb/IIIa antagonists (GPIIb/IIIa, α_{IIb}/β_3) for the treatment of thrombotic disorders has been extensively demonstrated in the clinical trials of the monoclonal antibody c7E3 (ReoPro®),[1] the peptide Integrilin®,[2] and the nonpeptides Aggrastat®,[3–5] and lamifiban.[6] While these drugs are finding use as treatments in acute clinical settings, nonpeptide GPIIb/IIIa antagonists are showing promise for the chronic treatment of thrombotic disorders. Xemilofiban[7–9] and sibrafiban[10,11] have undergone clinical trials in an unstable angina setting.Both of these compounds are prodrugs of RGD mimetics.

Previous reports from these laboratories described the discovery of the novel 3,5-disubstituted isoxazoline XR299 (**1**)[12] as a nonpeptide glycoprotein IIb/IIIa receptor antagonist. Within this series it was shown that the benzamidine was the preferred basic moiety, and a 16 atom separation of this group from the acidic residue was optimal. The effect of placing lipophilic substituents alpha and beta to the carboxylate moiety has also been reported.[13,14]



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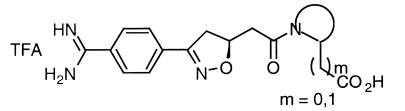

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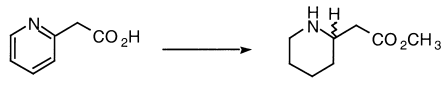
As an extension of this work, ring constrained β-alanine moieties (**2**) were explored in an effort to examine the tolerability of the receptor to a rigid alanine replacement. In view of the increased in vitro potency observed with beta substitution, the β-alanine was cyclized from the beta position onto the central amide nitrogen.

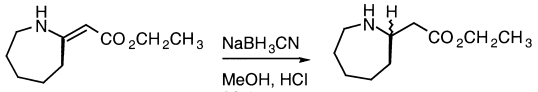
Chemistry

Synthesis of the target compounds was accomplished starting from the cyclic amines. Ethyl piperidineacetate was prepared from 2-pyridineacetic acid **3** by hydrogenation over PtO_2/AcOH/MeOH/HCl to give the necessary ester **4** (76%).



3 4

Ethyl azepineacetate **6** was obtained by sodium cyanoborohydride reduction of (alphahexahydroazepinylidene)-2-acetate **5**, while the five-membered ring analogue was synthesized from pyrrole, following the procedure of Fukawa et al.[15] The other cyclic amines employed were commercially available.



5 6

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The previously reported Boc-protected isoxazoline **7**[12] was coupled to the appropriate cyclic amine using TBTU as the coupling reagent (Scheme 1). This reaction was relatively slow, requiring a minimum of twelve hours to reach completion. Purification of the intermediate **8**, deprotection of the amidine under acidic conditions (TFA/CH_2Cl_2), and ester cleavage using either saponification ($LiOH/H_2O$) or hydrolysis (6 N HCl) conditions gave the desired products **9** in 45–64% overall yield. The final products were then isolated as mixtures of isomers using preparative HPLC.[16]

Biological Activity/Discussion

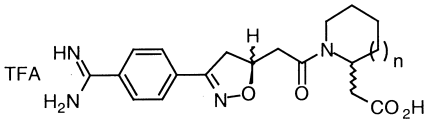
The compounds were evaluated for their in vitro antiplatelet activity.[17] As shown in Table 1, ring size was found to be key to activity. The piperidine **11** was the most potent of the 5-, 6- and 7-membered ring analogues, with an hPRP IC_{50} of 0.28 μM. Both the five- and seven-membered ring systems displayed a significant loss of in vitro potency, the seven membered ring being the poorest in this assay. Synthesized as a mixture of four isomers, piperidine **11** (hPRP $IC_{50} = 0.28$ μM) had a similar PRP IC_{50} to XR299 (**1**).

The piperidine system was further examined for optimal placement of the carboxyl moiety and overall separation of the acidic and basic groups (Table 2). The 2-carboxy piperidine **13** is 100-fold less potent compared to the longer piperidine **11**, while the 3-carboxypiperidine **14** shows only a 10-fold loss in activity (hPRP $IC_{50} =$ 5.2 μM) versus **11**. Placement of the carboxyl at the 4-position of the piperidine ring (**15**) resulted in a 10-fold loss of potency. Further restricting the ring conformation via an sp^2 center incorporated in the structure as in **16** had no apparent effect on in vitro potency as compared to analogue **14**.

As the racemic piperidyl system **11** exhibited submicromolar in vitro activity, the esters of the four isomers were separated to >98% de by preparative HPLC[18] (Table 3). Following hydrolysis of the esters, the four isomers were evaluated in vitro. The preferred isomer SV873 showed an hPRP $IC_{50} = 0.11$ μM, a 3-fold improvement over the isomeric mixture. However, this isomer is less potent than optically active parent (*R*)-XR299 (hPRP $IC_{50} = 0.06$ μM).

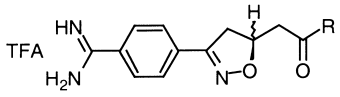
As high affinity binding of GPIIb/IIIa antagonists to unactivated platelets can contribute to in vivo duration,[19–22] the four isomers were tested in human platelet binding selectivity assays.[23] SV873 shows similar dissociation kinetics to (*R*)-XR299, that is, it shows a

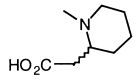
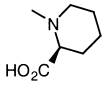
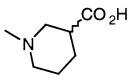
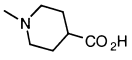
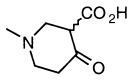
Table 1. Ring size comparisons

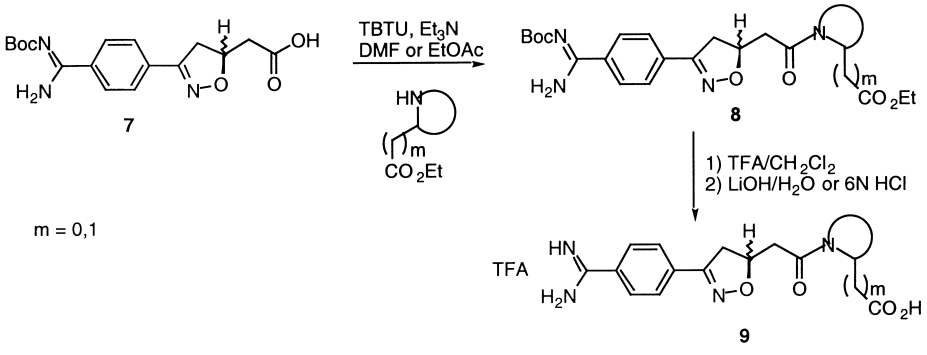


Compound	**10**	**11**	**12**	**1**
n	0	1	2	—
IC_{50} (μM, hPRP)	1.1	0.28	3.7	0.24

Table 2. Ring substitution and overall length effects

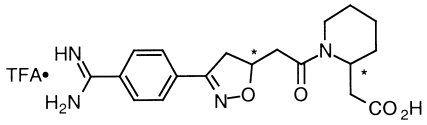


Compound	R	hPRP IC_{50} (μM)
11		0.28
13		39
14		5.2
15		2.5
16		6.4



Scheme 1.

Table 3. Isomers of **17**



Compound	hPRP IC_{50} (IC_{50}, μM)	K_d, activated platelets[15] (IC_{50}, μM)	K_d, unactivated platelets[15] (IC_{50}, μM)	Unactivated/activated
Isomer A	>100	9.800	—	—
Isomer B	3.8	0.302	5.800	19
Isomer C (SV873)	0.11	0.002	0.064	32
Isomer D	>100	>5.000	>5.000	—
(*R*)-XR299	0.06 ± 0.0092	0.004 ± 0.0018	0.112 ± 0.0502	28

preference for binding to activated platelets with a ratio for unactivated to activated platelets of approximately 30.

The ethyl ester of piperidyl-acetic acid **11** taken in vivo as the mixture of four isomer shows 100% ex vivo inhibition of aggregation 3 h after dosing iv at 1 mg/kg in a canine model. By 5 h the ex vivo inhibition of aggregation was still 65%.

In conclusion, we have demonstrated that cylization of the β-alanine side chain of XR299 can result in potent compounds. Upon examination of the 5-, 6-, and 7-membered rings, it was determined that the optimal ring size in this series was the 6-membered ring (**11**). The addition of further ring constraints to the piperidine ring did not improve in vitro potency. This effect may be the result of several factors including lipophilic and/or steric considerations, conformational constraints, or *N*-alkylation. When examined as a discrete isomer, the piperidine SV873 had good in vitro potency and a preference for binding to activated platelets. When tested in vivo in dog, the ethyl ester of **11** demonstrated a potency and a duration of action similar in profile to the acyclic parent, XR299.

Acknowledgements

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17. For platelet aggregation assay protocol, see: Mousaka, S. A.; Bozarth, J. M.; Forsythe, M. S.; Lorelli, W.; Ramachandran, N.; Jackson, S.; De Grado, W. F.; Reilly, T. M. *Cardiology* **1993**, *83*, 374.
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