Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2201–2204

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Pharmacology of Modified Amidine Isoxazoline Glycoprotein IIb/IIIa Receptor Antagonists

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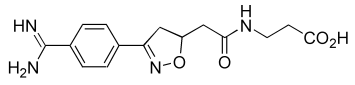
Received 9 March 2001; accepted 7 June 2001

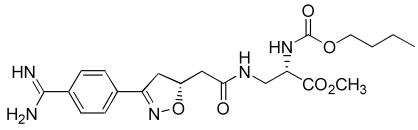
Abstract—Selective antagonism of the platelet GPIIb/IIIa receptor represents an attractive mechanism for the prevention and treatment of a number of thrombotic disease states. The antiplatelet activity of the oral GPIIb/IIIa receptor antagonists DMP 754 and DMP 802 have been disclosed. In this paper, the synthesis and biological evaluation of a series of potent *N*-substituted benzamidine isoxazolines are explored. The effect of benzamidine substitution on the duration of antiplatelet efficacy in dog is presented.

Blood vessel injury, either by mechanical means or through the pathophysiology of atherosclerosis, results in the activation and adhesion of platelets to the disrupted surface and to one another, increasing the risk of thrombosis related morbidity and mortality. Platelet glycoprotein IIb/IIIa (GPIIb/IIIa, α_{IIb}/β_3) is a membrane-bound protein that upon activation binds fibrinogen. Fibrinogen binding to GPIIb/IIIa is the final common event leading to platelet aggregation. As was shown clinically with the 7E3 monoclonal antibody,[1] the peptide Integrelin®,[2] and the nonpeptides Aggrastat®,[3] and lamifiban,[4] the prevention of GPIIb/IIIa mediated platelet aggregation represents an attractive antithrombotic strategy. While these drugs are finding use as treatments in acute clinical settings, nonpeptide GPIIb/IIIa antagonists are being examined for the chronic treatment of thrombotic disorders.[5]

Previous reports described the discovery of the novel 3,5-disubstituted isoxazoline XR299 (**1**) as a nonpeptide GPIIb/IIIa receptor antagonist.[6] Substitution of the β-alanine side chain at both the α- and β-positions was explored and it was shown that α substitution especially, with both carbamates and sulfonamides, had a pronounced effect on in vitro and in vivo potency and duration of action. Substitution at this position led to the clinical candidates DMP 754,[7] an α-substituted carbamate, and DMP 802,[8] an α-substituted sulfonamide. Similar α-substituents have proven effective in other series.[9]



1 (XR299)



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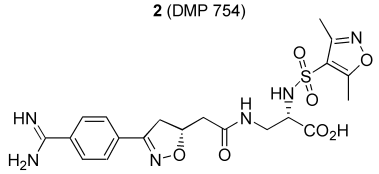

3 (DMP 802)

As the α-sulfonamide DMP 802 exhibited a significantly longer in vivo duration of action (dog model) as compared to the α-carbamate DMP 754, we wanted to define a series of compounds characterized by an intermediate duration of antiplatelet efficacy. In an effort to explore the effects on potency and duration of action of modified benzamidines in the isoxazoline series, a number of *N*-substituted amidines were synthesized. The effects of this type of substitution were explored in the

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00406-1

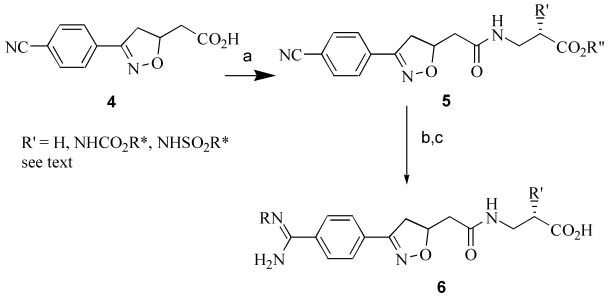
unsubstituted β-alanine series as well as in the α substituted β-alanine series.

The previously reported isoxazoline nitrile **4**[6] was coupled to the appropriately substituted β-alanine to afford the amide nitrile intermediate **5**, which was converted to imidate by exposure to methanol or ethanol saturated with HCl gas. This intermediate was not isolated but immediately converted to the substituted amidine by treatment with the appropriate alkyl or benzyl amine in ethanol. Hydrolysis or saponification of the ester afforded the desired amidine substituted analogue **6**. This methodology was applicable to both the racemic and optically active compounds (Scheme 1).

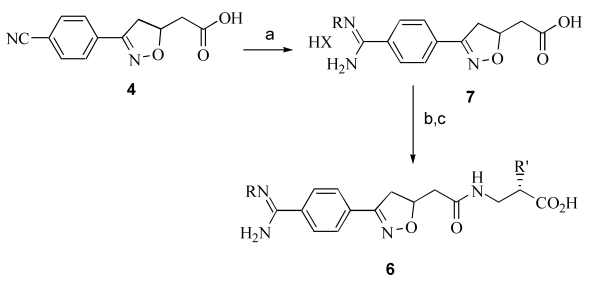
Alternately, the nitrile **4** was subjected to Pinner conditions to afford the *N*-substituted amidine **7**. Following peptide coupling and ester cleavage as described above, the desired analogues were obtained in good yields (Scheme 2).

Substitution on the amidine nitrogen in the isoxazoline series bearing an unsubstituted β-alanine side chain results in compounds that can maintain good in vitro potency[10] (Table 1). Potency was observed with small alkyl groups such as ethyl (**7**) (hPRP $IC_{50} = 0.089\,\mu M$) while a chain length of four carbons (**9**) correlates to an enhancement of in vitro activity compared to that observed for **XR299**. Compounds with longer chain lengths (**10**) begin to show a loss of potency, as do branched alkanes (**11**).

Benzyl substitution on the amidine (**12**) also maintained reasonable in vitro potency. Although the *R* isoxazoline isomer (**13**) exhibited similar in vitro potency as compared to the racemate, subsequent *N*-benzyl analogues were synthesized as the *R* isomers as this isomer was shown to be the more active isomer in previous isoxazoline work.[8–10] The single isomer *N*-benzyl analogues with both *ortho*-electron donating (**14**) and *meta*-electron withdrawing (**15**) moieties on the benzyl groups showed in vitro potency in the low nanomolar range. These compounds also compare favorably with the *R* isomer of **XR299**.[6]



Scheme 1. Synthesis of *N*-substituted amidines. Reagents: (a) TBTU/Et_3N/DMF/α-substituted β-alanine; (b) (i) EtOH/HCl; (ii) RNH_2/EtOH; (c) LiOH/H_2O/MeOH or 6 N HCl.



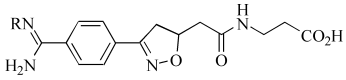
Scheme 2. Alternate synthesis of *N*-substituted amidines. Reagents: (a) (i) EtOH/HCl; (ii) RNH_2/EtOH; (b) TBTU/Et_3N/DMF/substituted β-alanine; (c) LiOH/H_2O/MeOH or 6 N HCl.

Analogues incorporating substitution on an amidine nitrogen in conjunction with substitution at the α-position of the β-alanine maintained good in vitro potency compared to the unsubstituted amidine analogues (Table 2). Both straight-chain alkyl (**16**, **17**, **19**, and **22**) and benzyl substitution (**23**, **24**, and **25**) are tolerated in this series giving compounds that are in the 0.016–0.082 μM range in the hPRP assay.

When the methyl ester of **7** was dosed at 0.025 mg/kg iv in a canine model, the substituted amidine bearing an unsubstituted β-alanine side chain showed maximal ex vivo inhibition of aggregation of 70% at 30 min and was down to less than 20% inhibition of aggregation at 5 h.

However, the α-substituted β-alanine series with substitution on the amidine nitrogen exhibited good ex vivo potency when dosed at 0.025 mg/kg iv in a canine model (Fig. 1). In examining a series of *N*-substituted analogues of **20** (SD270), it was seen that duration of action varied with the amidine substituent. The ethyl *N*-substituted

Table 1. Substituted amidines analogues of XR299



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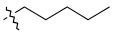
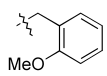
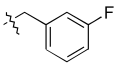
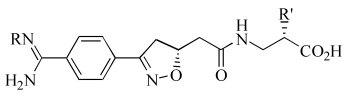
Compd	R	Stereochemistry	HPRP IC_{50} (μM)
XR299	H	*R,S*	0.24 ± 0.063
XR299(R)	H	*R*	0.06
7		*R,S*	0.089
8		*R*	0.12
9		*R,S*	0.02
10		*R,S*	0.3
11		*R,S*	1.3
12		*R,S*	0.20
13		*R*	0.087
14		*R*	0.032
15		*R*	0.012

Table 2. Substituted amidines with side chain substitution



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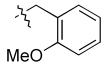
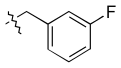
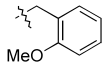
Compd	R	R′	hPRP IC_{50} (μM)
DMP 754	H	$NHCO_2nBu$	0.050±0.003
16		$NHCO_2nBu$	0.082
17		$NHCO_2nBu$	0.062
18	H	$NHSO_2m$-tolyl	0.050
19		$NHSO_2m$-tolyl	0.051
20 (SD270)	H	$NHSO_2o$-tolyl	0.054
21		$NHSO_2o$-tolyl	0.043
22		$NHSO_2o$-tolyl	0.016
23		$NHSO_2o$-tolyl	0.040
24		$NHSO_2$(3,5-diMeIsoxazole	0.055
25		$NHSO_2$(3,5-diMeIsoxazole	0.055

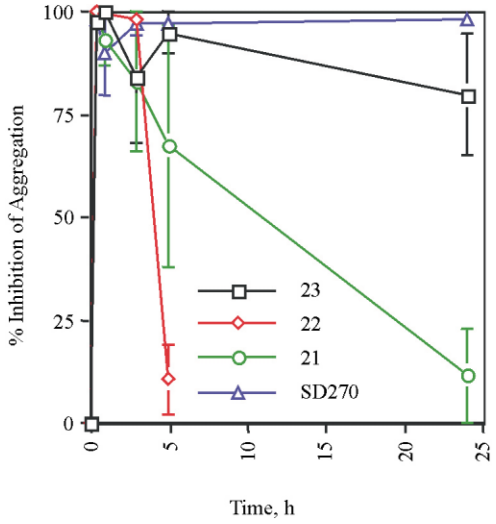


Figure 1. Compounds **21**, **22**, **23**, and SD270 dosed at 0.025 mg/kg iv in a canine model.

analogue **21** gave an intermediate duration compound, similar to the α-substituted carbamate DMP 754 (0.025 mg/kg results in 40% ex vivo platelet aggregation at 5 h),[7] with maximum inhibition of aggregation achieved at 1 h with minor inhibition of aggregation at 24 h. The *n*-butyl *N*-substituted analogue **22** showed a duration of only 5 h while the *o*-methoxybenzyl *N*-substituted analogue **23** maintained approximately 80% inhibition of aggregation at 24 h, longer in duration of action than the clinical candidate DMP 754. However, all of the *N*-substituted analogues had an observed shorter duration of action ex vivo compared to the unsubstituted amidine analogue SD270. Interestingly, substitution on the amidine results in a desirable 3- to 25-fold increase in water solubility.

We have shown that amidine substitution in the isoxazoline series results in compounds that are potent in vitro GPIIb/IIIa inhibitors. When evaluated ex vivo in a canine model, amidine substitution allowed for the modulation of duration of action within the sulfonamide series albeit all of the substituted amidines evaluated had shorter duration of action compared to the unsubstituted amidine **20** (SD270). A select analogue showed intermediate duration of action compared to the clinical candidate DMP754 and analogue **20**. The effect on duration of action varies with the chain length of the *N*-substituent—the butyl-substituted amidine being shorter in duration of action, while the benzyl-substituted amidine conferred the longest duration of action of the substituted amidine analogues evaluated. Substitution of the amidine presents an interesting manifold through which one can modify duration of action of the isoxazoline series of GP IIb/IIIa inhibitors while modifying the molecule's physical characteristics.

Acknowledgements

The authors would like to thank Mark Forsythe and Jeff Bozarth for performing the in vitro assays. We also thank Lori Foster, Sandra Flint, Tracy Bozarth, and Karen Schlingmann for conducting in vivo studies.

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