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# ISOXAZOLINE GPIIb/IIIa ANTAGONISTS BEARING A PHOSPHORAMIDATE

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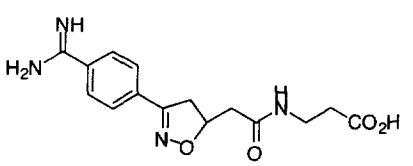
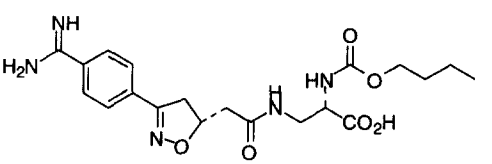
**Abstract:** Isoxazolinylacetamides bearing a phosphoramidate group α- to the carboxylate moiety (**3**) were prepared and evaluated for in vitro antiplatelet efficacy. They were found to bind GPIIb/IIIa with high affinity and were potent antagonists of ADP mediated platelet aggregation. 

The activation and aggregation of platelets plays a central role in the pathogenesis in acute ischemic syndromes characteristic of coronary artery, cerebrovascular, and peripheral arterial vascular diseases.[1] Platelet aggregation is affected by the binding of fibrinogen or von Willebrand factor (vWF) to the platelet membrane bound receptor, glycoprotein IIb/IIIa (GPIIb/IIIa, $\alpha_{IIb}\beta_3$).[2] The clinical benefit of antagonism of GPIIb/IIIa in lowering the risk of ischemic events in patients with unstable angina or undergoing revascularization procedures has been demonstrated using parenteral agents in an acute setting.[3] The use of orally active GPIIb/IIIa antagonists for chronic use is currently under evaluation.

In previous reports, we described the discovery of a series of isoxazolinylacetamide GPIIb/IIIa antagonists represented by XR299 (**1**) and XV459 (**2**).[4] Ester derivatives of these compounds are orally active in canine, and in the case of **2**, have an impressive duration of action. As a result of constructing the SAR within this series with respect to the lipophilic substituent α- to the carboxylate, we wish to describe the synthesis and in vitro antiplatelet activity of a novel series of phosphoramidates (**3**).



XR299, **1**



XV459, **2**

## Chemistry

Full details of the synthesis of α-amino ester **4**, the key intermediate used to prepare the phosphoramidates, have been described.[4b] Phosphorylation of amine **4** using a variety of agents, including phosphites and phosphoryl chlorides, afforded the phosphoramidates **5** in 42–90% yield. In particular, use of the trialkylphosphite-$I_2$ system[5] offered operational simplicity and good to excellent yields. This underutilized method holds the advantages of commercial availability of a wide variety of trialkyl- and triarylphosphites. Additionally, phosphites are generally more agreeable reagents to handle and store relative to highly toxic, corrosive

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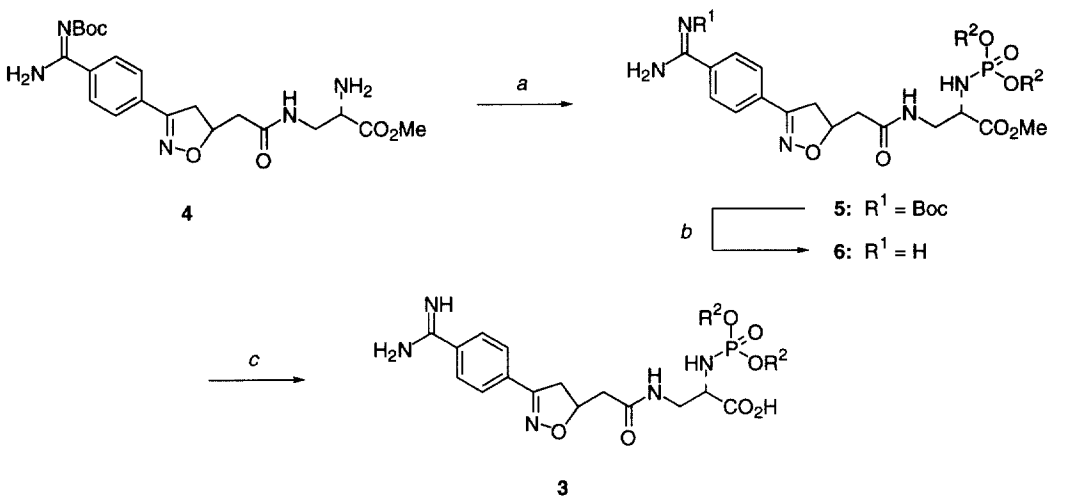
phosphoryl halides. Cleavage of the Boc-group using TFA in dichloromethane afforded the amidines **6**. Hydrolysis of the ester functionality represented a challenge, as phosphoramidates are not robust functionality when subjected to extremes of pH. Under alkaline conditions the P-O bond is labile, while under acidic conditions the P-N bond is unstable. Hydrolysis of these esters was successfully accomplished using rabbit liver esterase (RLE) at pH 7 (Scheme 1). Using this method, examples **3a–e** were prepared (Table 1). Attempts at preparation of the diphenyl derivative were unsuccessful, as the ester intermediate **6** was refractory to the effects of esterase, likely due to steric hindrance. Other methods investigated for this hydrolysis resulted in no reaction, decomposition, or the loss of phenyl signals in the $^1$H NMR spectrum. The phosphoramidic acid **3f** was prepared from **5d** via reaction under aqueous conditions at pH 4. Under these conditions cleavage of the Boc group was anticipated. Surprisingly, concomitant hydrolysis of the *n*-butyl groups and methyl ester also occurred.

**Scheme 1.** Synthesis of Phosphoramidates[a]




[a]Reagents: (a) $P(OR^2)_3$, $I_2$, $CH_2Cl_2$, then **4**; (b) TFA, $CH_2Cl_2$; (c) RLE, HEPES, pH 7.0.

## In Vitro Antiplatelet Efficacy

The compounds were assayed for in vitro antiplatelet efficacy against ADP (10 μM) mediated aggregation using light transmittance aggregometry in human platelet rich plasma (PRP) with citrate as the anticoagulant.[6] As shown in Table 1, phosphoramidic acid **3f** was the weakest compound in this assay. Phosphoramidates **3a–e** had approximately 2-fold greater potency than **3f**, however, little additional SAR information was gained from the series. As shown using a GPIIb/IIIa ELISA,[6] the phosphoramidates bound with high affinity to purified GPIIb/IIIa receptor, however, relative to **1**, the presence of the phosphoramidate did not appear to significantly increase receptor affinity.

The effect of the $Ca^{2+}$ concentration on the apparent potency of GPIIb/IIIa antagonists was recently highlighted with respect to clinical studies of Integrilin®,[7] a cyclic peptide intended for use in an acute setting. Citrate anticoagulation is a standard clinical protocol and is the method typically employed in assays of in vitro antiplatelet efficacy. Citrate is a weak chelator of $Ca^{2+}$, leaving enough bound to the high affinity sites on

GPIIb/IIIa to support platelet aggregation. In citrated human PRP ($[Ca^{2+}]$ = 40–50 μmol/L), Integrilin® had an $IC_{50}$ of 140 nM. When PPACK, heparin, or hirudin was substituted for citrate (conditions under which the $[Ca^{2+}]$ = physiological concentration of 1 mmol/L), the $IC_{50}$ of Integrilin® increased to approximately 600 nM. This finding is especially critical to the establishment of clinical dosing regimens. In heparinized human PRP, the phosphorylated derivatives **3** had twofold lower antiplatelet potency as compared to that observed in citrated PRP. For comparison, the antiplatelet potency of butyl carbamate **2** is independent of the method of anticoagulation employed.

**Table 1.** In Vitro Antiplatelet Efficacy of Phosphoramidates and Related Agents

| | | hPRP $IC_{50}$ ± SEM (nM)[a] | | GPIIb/IIIa ELISA | GPP + Fg |
|---|---|---|---|---|---|
| cmpd | R | citrate | heparin | $IC_{50}$ ± SEM (nM)[a] | $IC_{50}$ (nM) |
| **3a** | Et | 117 ± 21 | 240 ± 24 | 1.7 ± 0.70 | 102 |
| **3b** | Me | 105 ± 24 | 197 ± 27 | 0.55 ± 0.17 | 54 |
| **3c** | $CH_2$=$CHCH_2$ | 122 ± 20 | 224 ± 32 | 1.5 ± 1.3 | 46 |
| **3d** | *n*-Bu | 114 ± 28 | 221 ± 35 | 1.2 ± 0.56 | 55 |
| **3e** | *i*-Pr | 103 ± 31 | 210 ± 31 | 0.86 ± 1.0 | 73 |
| **3f** | H | 260 ± 2.3 | 376 ± 54 | 2.6 ± 0.81 | 229 |
| **1** | --- | 240 ± 6.3 | 560 ± 38 | 1.1 ± 0.80 | 140 |
| **2** | --- | 50 ± 0.3 | 66 ± 5.1 | 0.25 ± 0.05 | 21 |

[a]Results are an average of 3 measurements

Determinations of in vitro antiplatelet efficacy performed in PRP may be misleading with regard to absolute ligand-receptor affinity due to possible competition for ligand binding by plasma protein. This is at once a benefit and a limitation of screening in PRP. Performing studies of in vitro antiplatelet efficacy in gel filtered platelets (GPP) removes this limitation, however, this assay tends to overestimate antiplatelet potency due to the reduction in the concentration of competing ligands present. Since fibrinogen (Fg) is the most significant of the naturally occurring GPIIb/IIIa ligands with respect to the support of platelet aggregation,[8] its concentration in our GPP preparations was adjusted to 1 mg/mL (physiological concentration = 1–2 mg/mL). The use of higher concentrations of Fg was not practical due to the limited solubility of Fg in GPP. In the GPP + Fg assay, **1** and **2** had values of 140 and 21 nM, respectively. For comparison, the GPP $IC_{50}$'s for **1** and **2** were 10[4a] and 11 nM,[4b] respectively. In the GPP + Fg assay, compounds **3a–f** had potencies comparable to those observed in the PRP assay (Table 1), suggesting that protein binding levels for this series were not high enough to significantly effect the antiplatelet potency observed in PRP. Currently, we are investigating the nature of the relationship between the difference in PRP and GPP + Fg values and measured human plasma protein binding.

In conclusion, phosphoramidates **3** prepared from α-amino ester **4** are high affinity ligands to the GPIIb/IIIa receptor, and potent inhibitors of ADP stimulated platelet aggregation, as measured in PRP using light transmittance aggregometry. As shown in heparinized PRP, the potency of these derivatives was somewhat modulated by the $Ca^{2+}$ concentration, in contrast to **2**. The comparable results observed from the PRP-citrate and GPP + Fg assays is suggestive of low protein binding levels for this series.

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