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SYNTHESIS AND ANTIPLATELET EFFECTS OF AN ISOXAZOLE SERIES OF GLYCOPROTEIN IIb/IIIa ANTAGONISTS

Chu-Biao Xue,* John Roderick, Shaker Mousa, Richard E. Olson, and William F. DeGrado#

Chemical and Physical Sciences and Cardiovascular Diseases, The DuPont Pharmaceuticals Company, Experimental Station, P. O. Box 80500, Wilmington, DE 19880, U.S.A.

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Abstract: Despite the excellent in vitro potency of a series of benzamide glycoprotein IIb/IIIa antagonists, which have been reported previously, poor in vivo potency in the inhibition of platelet aggregation was observed when the most potent inhibitor **XU057** was dosed intravenously to dogs. In this communication, we report that replacement of the benzamide in **XU057** with an isoxazolecarboxamide resulted in significant improvement in in vivo potency. More importantly, the analogue **XU065** showed an excellent oral antiplatelet effect in dogs.

Glycoprotein IIb/IIIa (GPIIb/IIIa) is a heterodimeric membrane protein present on the surface of platelets that mediates platelet adherence and aggregation.[1] In response to platelet activation by a variety of agonists, such as ADP, thrombin, and collagen, this protein undergoes a substantial conformational change that results in an increased affinity for fibrinogen, a multivalent plasma protein.[2,3] The binding of multiple GPIIb/IIIa molecules to a single molecule of fibrinogen leads to crosslinking of the platelets, which causes platelets to aggregate. The pathophysiological consequences of this process include myocardial infarction, unstable angina, transient ischemic attack, and stroke.[4,5] Thus, inhibition of platelet aggregation by selectively blocking the association of fibrinogen with GPIIb/IIIa represents an attractive antithrombotic strategy.

The Arg-Gly-Asp (RGD) sequence present in fibrinogen[6,7] has been proposed as a recognition site for the binding of fibrinogen to GPIIb/IIIa , and platelet aggregation is inhibited in the presence of small RGD-containing peptides.[8–12] Thus, the RGD sequence has provided a starting point for the successful development of highly potent antagonists of GPIIb/IIIa, and a number of RGD-containing cyclic peptide and nonpeptide RGD mimetics have been reported as potential antithrombotics.[13,14]

In our efforts to identify nonpeptide RGD mimetics, several novel series of GPIIb/IIIa inhibitors with 2,3-diaminopropionic acid derivatives as surrogates of aspartic acid have been discovered.[15–17] Despite the excellent in vitro activity of the benzamide series of compounds,[16] poor activity was observed in the ex vivo inhibition of ADP induced platelet aggregation in dogs. In this communication, we report that replacement of the benzamide core of the most active compound **XU057** in this series with an isoxazolecarboxamide resulted in a significant improvement in in vivo activity. More importantly, the isoxazolecarboxamide analogue **XU065** showed a dose dependent antiplatelet effect following oral administration to dogs.

#Present address: Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, U.S.A.

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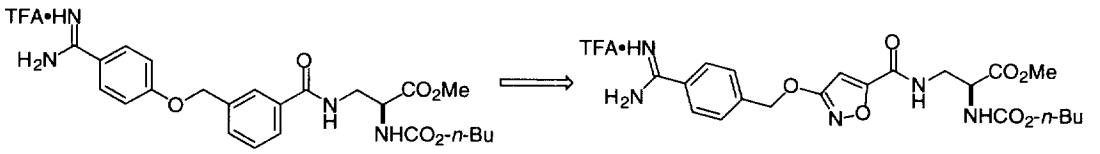
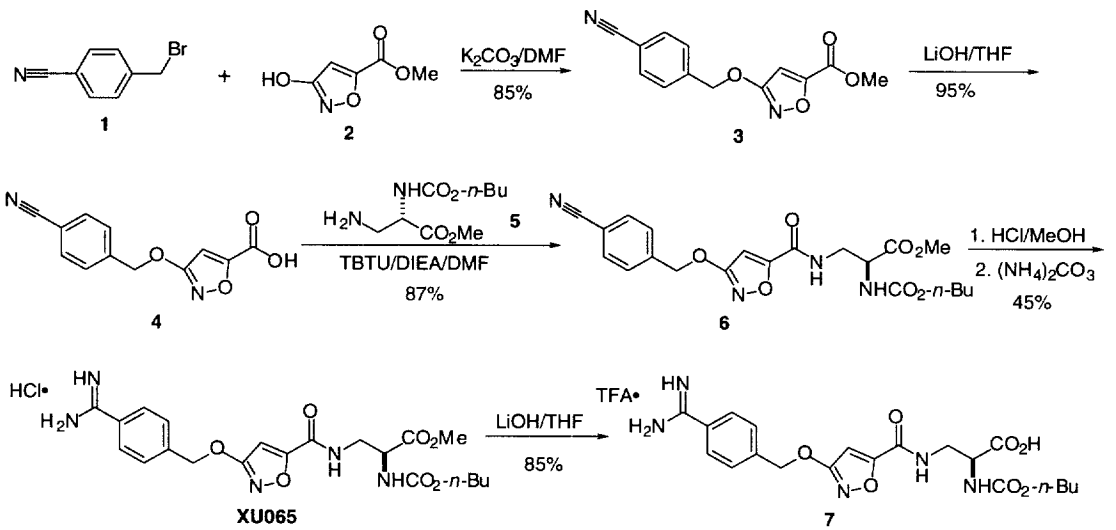


Chemistry

The synthesis of **XU065** is outlined in Scheme 1. Alkylation of methyl-3-hydroxy-5-isoxazolecarboxylate **2** (Aldrich) with 4-cyanobenzyl bromide **1** in DMF at 60 °C using potassium carbonate as base produced the ether derivative **3**, which was saponified to give the carboxylic acid **4**. Coupling of **4** with methyl-N^2-(*n*-butoxycarbonyl)-L-2,3-diaminopropionate **5**, which was prepared as described previously,[16] yielded the amide intermediate **6**. A Pinner reaction of **6** using anhydrous hydrogen chloride in methanol followed by treatment with ammonium carbonate in methanol afforded **XU065**. Saponification of **XU065** produced the corresponding acid **7**.

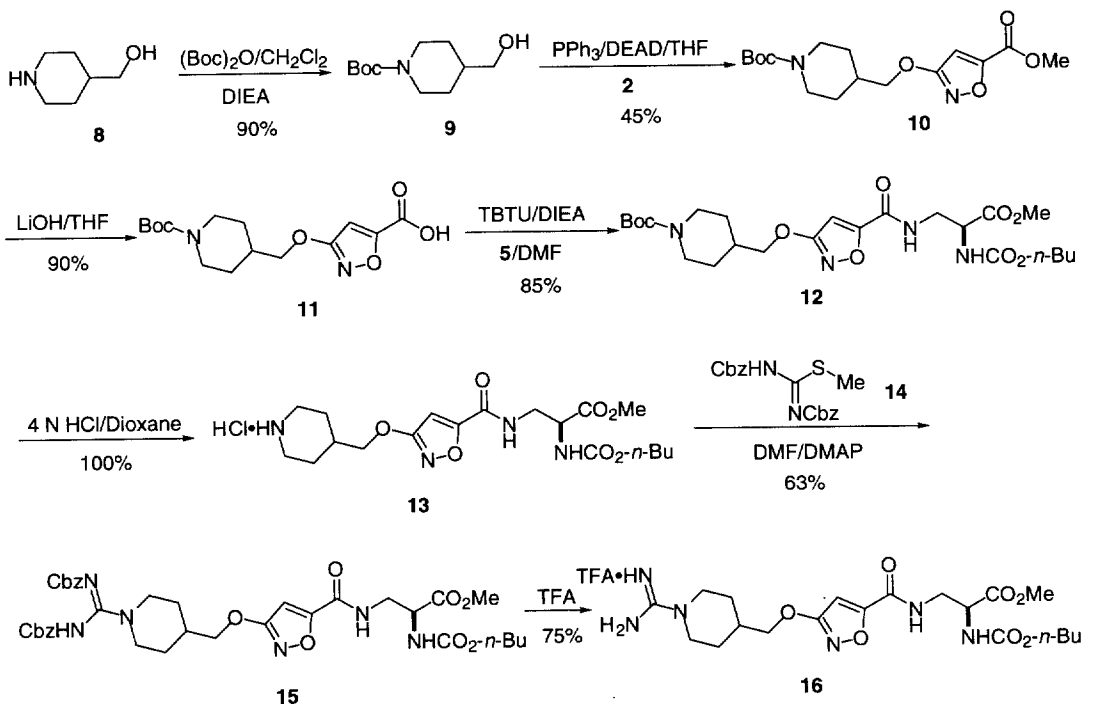
Scheme 1



The formamidinopiperidine analogue **16** of this series was prepared using the method shown in Scheme 2. 4-Hydroxymethylpiperidine **8** was reacted with di-*t*-butyl-dicarbonate to give the *N*-Boc protected derivative **9**. A Mitsunobu reaction of **9** with methyl 3-hydroxy-5-isoxazolecarboxylate **2** produced the ether compound **10**, which was saponified to give the acid **11**. Coupling of **11** with 2,3-diaminopropionic acid derivative **5** yielded the amide **12**. The Boc group of **12** was removed using 4 N HCl/dioxane and the resulting amine was reacted with S-methyl-*N*,*N*'-bis(benzyloxycarbonyl)isothiourea **14** to give the *N*-bis(benzyloxycarbonyl)amidinopiperidine

derivative **15**. The two Cbz groups were removed using TFA at an elevated temperature (60 °C) to give the *N*-amidinopiperidine compound **16**.

Scheme 2



Results and Discussion

We have reported the discovery of a novel series of fibrinogen receptor antagonists with benzamide as a core and 2,3-diaminopropionic acid derivatives as surrogates of aspartic acid.[16] The most potent inhibitor among the analogues prepared is the *n*-butylcarbamate **XU057**. Because of its excellent in vitro potency, **XU057** was studied in dogs.[18] As shown in Figure 1, the administration of an iv bolus of 0.5 mg/kg of **XU057** to dogs resulted in an 80% inhibition of ex vivo platelet aggregation, which quickly declined to approximately 25% over 1 h. Our efforts to optimize this series focused on replacement of the benzamide core with heterocycles and on modification of the benzamidine. Thus, the phenyl ring in **XU057** was replaced with an isoxazole ring to afford **XU065**. Using the human platelet rich plasma assay (human PRP assay) **XU065** showed an in vitro activity comparable to that of **XU057** with an IC_{50} of 50 nM (Table 1) after esterase conversion to the corresponding free acids. When the 4-amidinophenyl residue of **XU065** was replaced with a *N*-amidinopiperidin-4-yl group to afford compound **16**, a fourfold loss in the inhibitory activity resulted.

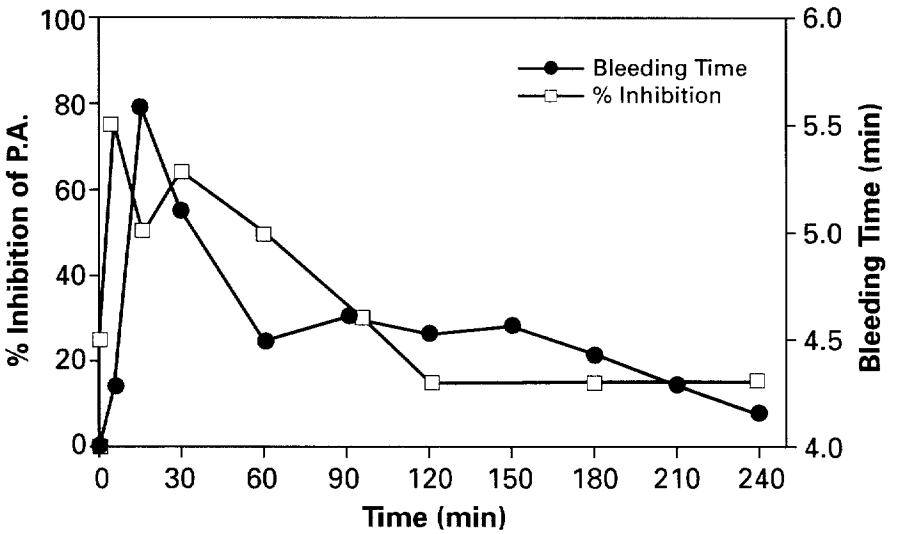
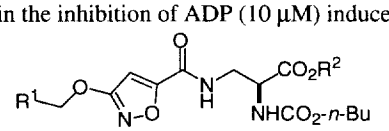


Figure 1. Inhibition of ADP (100 μM) induced ex vivo platelet aggregation in dogs dosed intravenously with **XU057** at 0.5 mg/kg.

Table 1. In vitro potency in the inhibition of ADP (10 μM) induced platelet aggregation



Compd[a]	R^1	R^2	Human PRP IC_{50} ± SEM (nM)[b]
XU057			32 ± 5
XU065	4-Amidinophenyl	Me	50 ± 7
7	4-Amidinophenyl	H	60 ± 10
16	*N*-Amidinopiperidin-4-yl	Me	200 ± 20

[a]Methyl ester was converted to the free acid form prior to the PRP assay using porcine liver esterase.[16]

[b]Inhibition of platelet aggregation was determined in three donors. See ref 16 for assay protocol.

The in vivo antiplatelet effects of **XU065** were evaluated in dogs.[18] Compared with **XU057**, significant improvement in in vivo activity was observed with **XU065**. Following an iv bolus administration of **XU065** to dogs at a dose of 0.5 mg/kg, **XU065** exhibited a maximum inhibition of ex vivo ADP (100 μM) induced platelet aggregation, which was maintained for over 3 h (Figure 2). More importantly, **XU065** demonstrated a dose dependent inhibition of ex vivo platelet aggregation after oral administration to dogs. At an oral dose of 0.5

mg/kg, **XU065** showed a measurable inhibition of ex vivo platelet aggregation. Over 60% of inhibition was achieved when the dose was increased to 0.8 mg/kg. Maximal inhibition of platelet aggregation was achieved and maintained for up to 5 h after an oral dose of 1.6 mg/kg.

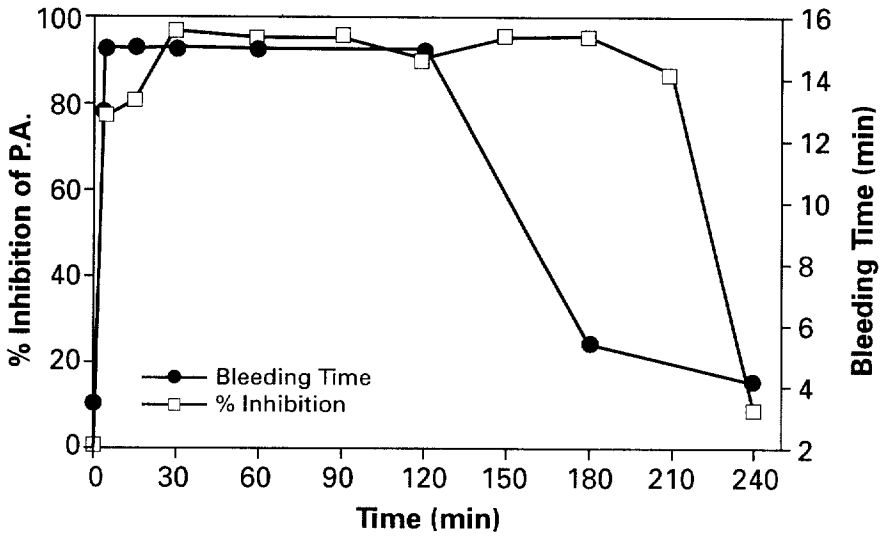


Figure 2. Inhibition of ADP (100 μM) induced ex vivo platelet aggregation in dogs dosed intravenously with **XU065** at 0.5 mg/kg.

Conclusion

Replacement of the benzamide core in **XU057** with an isoxazolecarboxamide afforded **XU065**, which showed a significant improvement in in vivo antiplatelet activity over **XU057** following an iv administration to dogs. More importantly, **XU065** is orally active in dogs and demonstrated a dose dependent inhibition of ex vivo ADP (100 μM) induced platelet aggregation.

References and Notes

1. Kieffer, N.; Phillips, D. R. *Annu. Rev. Cell Biol.* **1990**, *6*, 329.
2. Plow, E. F.; Ginsberg, M. H. *Prog. Hemost. Thromb.* **1989**, *9*, 117.
3. Phillips, D. R.; Charo, I. F.; Scarborough, R. M. *Cell* **1991**, *65*, 359.
4. Falk, E. *Circulation* **1985**, *71*, 699.
5. Coller, B.S. *New Eng. J. Med.* **1990**, *322*, 33.
6. Gartner, T. K.; Bennett, J. S. *J. Biol. Chem.* **1985**, *260*, 11891.
7. Pytela, R.; Pierschbacher, M. D.; Ginsberg, M. H.; Plow, E. F.; Ruoslahti, E. *Science* **1986**, *231*, 1559.
8. Gan, Z.-R.; Gould, R. J.; Jacobs, J. W.; Friedman, P. A.; Polokoff, M. A. *J. Biol. Chem.* **1988**, *263*, 19827.

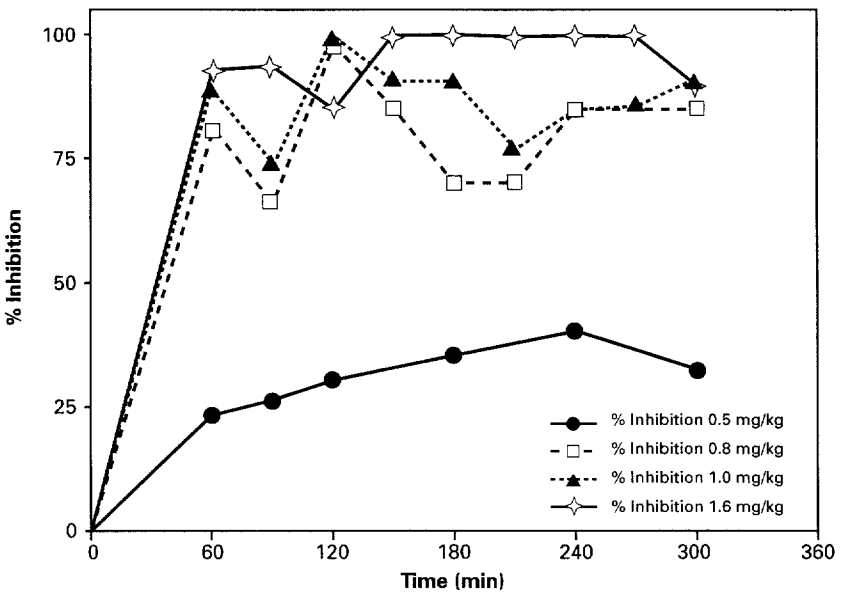


Figure 3. Inhibition of ADP (100 μM) induced ex vivo platelet aggregation in dogs dosed orally with **XU065**.

9. Shebuski, R. J.; Ramjit, R. J.; Bencin, G. H.; Polokoff, M. A. *J. Biol. Chem.* **1989**, *264*, 21550.
10. Gould, R. J.; Polokoff, M. A.; Friedman, P. A.; Huang, T.-F.; Holt, J. C.; Cook, J. J.; Niewiarowski, S. *Proc. Soc. Exp. Biol. Med.* **1990**, *195*, 168-171.
11. Haverstick, D. M.; Cowan, J. F.; Yamada, K. M.; Santoro, S. A. *Blood* **1985**, *66*, 946.
12. Beer, J. H.; Springer, K. T.; Coller, B. S. *Blood* **1992**, *79*, 117.
13. Feuerstein, G.; Ruffolo, R. R. Jr.; Samanen, J. *Pharmacology Reviews and Communications* **1996**, *8*, 257.
14. Samanen, J. *Ann. Rep. Med. Chem.* **1996**, *31*, 91.
15. Xue, C.-B.; Rafalski, M.; Roderick, J.; Eyermann, C. J.; Mousa, S.; Olson, R. E.; DeGrado, W. F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 339.
16. Xue, C.-B.; Roderick, J.; Jackson, S.; Rafalski, M.; Rockwell, A.; Mousa, S.; Olson, R.; DeGrado, W. F. *Bioorg. Med. Chem.* **1997**, *5*, 693.
17. Xue, C.-B.; Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Batt, D. G.; Cain, G. A.; Sworin, M.; Rockwell, A. L.; Roderick, J. J.; Wang, S.; Orwat, M. J.; Frietze, W. F.; Bostrom, L. L.; Liu, J.; Higley, C. A.; Rankin, F. W.; Tobin, A. E.; Emmett, G.; Lalka, G. K.; Sze, J. Y.; DiMeo, S. V.; Mousa, S. A.; Thoolen, M. J.; Racanelli, A. L.; Hausner, E. A.; Reilly, T. M.; DeGrado, W. F.; Wexler, R. R.; Olson, R. E. *J. Med. Chem.* **1997**, *40*, 2064.
18. **XU057** or **XU065** itself is inactive in the inhibition of platelet aggregation. After intravenous or oral administration to dogs, they were rapidly hydrolyzed to their corresponding carboxylic acids to exhibit the observed inhibition of ADP-induced ex vivo platelet aggregation as shown in Figures 1-3.