Orally Active Fibrinogen Receptor Antagonists. 2. Amidoximes as Prodrugs of Amidines†

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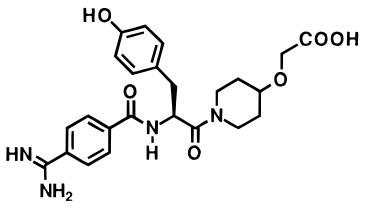
The potent and selective GP IIb-IIIa antagonist lamifiban (**1**, Ro 44-9883) is currently in clinical development as an injectable antithrombotic agent for treating and preventing acute coronary syndromes. However, for secondary prevention of thrombotic occlusions, orally active inhibitors are needed. By means of a prodrug strategy, the modest oral absorption of **1** in mice was improved by a factor of 9. In addition, these studies demonstrated that an amidoxime group can serve as a prodrug functionality for an amidino group. Application of this principle to the structurally related amidino carboxylate **13** led to the amidoxime ester **18** which was absorbed approximately 20 times better, after oral administration to mice, than **13**. Due to the modification of the amidino group as well as of the carboxylate group, **18** completely lost its ability to interact with purified platelet GP IIb-IIIa. After oral administration of **18** to rats, dogs, and rhesus monkeys, the bioavailability of the active derivative **13** was 26 ± 5, 25 ± 6, and 33 ± 6%, respectively, and the elimination half-life was 4.1 ± 1.7, 11.4 ± 1.1, and 5.1± 1.4 h, respectively. On the basis of these properties, the orally active **18** (Ro 48-3657), a double prodrug of the potent and selective non-peptide GP IIb-IIIa antagonist **13** (Ro 44-3888), was selected as clinical candidate for evaluation as a prophylactic agent in patients at high risk for arterial thrombosis.

Introduction

Human blood platelets play an important role not only in normal hemostasis but also in arterial thrombosis, particularly under conditions of high shear stress typical of narrowed atherosclerotic arteries.[1−3] The deposition of platelets on thrombogenic surfaces such as ruptured atherosclerotic plaques followed by the formation of occlusive platelet rich aggregates are crucial events leading to disorders such as unstable angina, myocardial infarction, transient ischemic attacks, and stroke.[4,5] The interaction of tissue-bound von Willebrand factor (vWF) with glycoprotein (GP) Ib-IX on the platelet surface not only is responsible for the adhesion of platelets to exposed subendothelium but also triggers the generation of activation signals.[6−8] As a consequence, the glycoprotein IIb-IIIa complex (GP IIb-IIIa) becomes competent to bind adhesive proteins such as fibrinogen, vWF, or fibronectin present in plasma. In this way, the platelets are cross-linked and thrombus growth is initiated.[9] Due to the release and formation of a variety of platelet activators (e.g., ADP, thrombin, collagen, serotonin, thromboxane A_2, epinephrine), additional platelets are activated and recruited to the growing thrombus.[10−13] The efficacy of existing antiplatelet agents, such as aspirin which inhibits only one agonist pathway, is limited.[14−16] It is expected that the higher intrinsic potency of GP IIb-IIIa antagonists compared to aspirin will translate into better clinical efficacy in preventing arterial thrombus formation.[14,17] Indeed, this has been demonstrated with the chimeric Fab fragment of the anti-GP IIb-IIIa monoclonal antibody 7E3 (c7E3) in high-risk angioplasty patients in a phase III clinical trial.[18,19]

In the meantime, a large number of potent and selective GP IIb-IIIa antagonists, including snake venom polypeptides and linear and cyclic peptides, as well as non-peptide inhibitors have been identified.[20−32] Most of these agents seem to mimic the Arg-Gly-Asp (RGD) recognition sequence present in various ligands of receptors of the integrin family.[33] The usefulness of several of these low molecular weight GP IIb-IIIa antagonists, e.g., integrelin,[34,35] MK-383,[36] or lamifiban (Ro 44-9883, **1**),[37] as intravenous treatments of acute thrombosis is currently being assessed in clinical trials. However, for the chronic treatment of patients with recurrent vascular events, compounds with a sufficiently high bioavailability and a reasonably long half-life in plasma after oral administration are needed. The amidinodicarboxylate **2**[38] is an early example of a potent, selective non-peptide GP IIb-IIIa antagonist that exhibits oral activity in dogs.[39] However, the absolute



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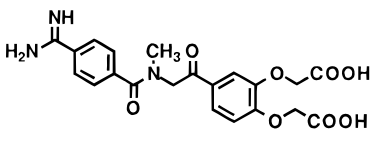

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† Abbreviations: ADP, adenosine 5′-diphosphate; AUC, area under the concentration−time curve; EtOEt, diethyl ether; FG, fibrinogen; GP, glycoprotein; h-PRP, human platelet rich plasma; iv, intravenous; po, per os; THF, tetrahydrofuran.

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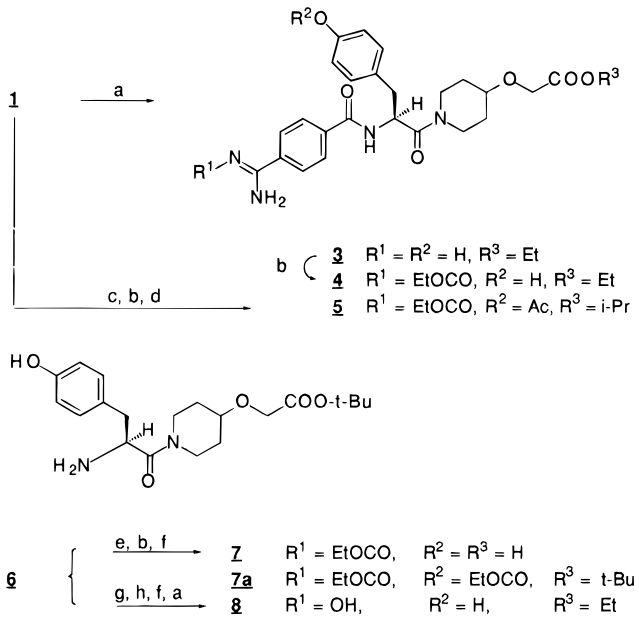
Scheme 1. Preparation of Prodrug Derivatives of **1**[a]



[a] (a) EtOH, H_2SO_4; (b) EtOCOCl, NaOH, CH_2Cl_2; (c) 2-propanol, H_2SO_4; (d) Ac_2O, K_2CO_3; (e) 4-$NH_2C(NH)C_6H_4COCl$, pyridine; (f) HCOOH; (g) 4-NCC_6H_4COCl, $NaHCO_3$, CH_2Cl_2; (h) $NH_2OH \cdot HCl$, Na, MeOH.

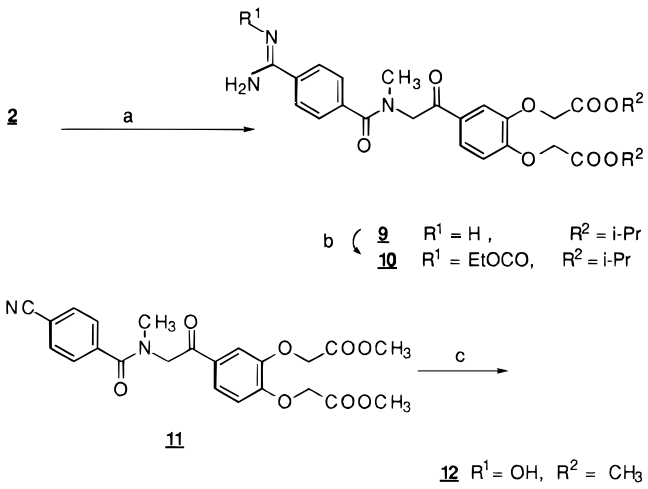
bioavailability of **2** is not high enough for oral use in humans. Recent reports describing orally active GP IIb-IIIa antagonists[40−51] have prompted us to disclose our prodrug approach to increase the oral bioavailability of **1**, **2**, and related compounds. One of these is the orally active alanine derivative **18** (Ro 48-3657) which has been selected as a clinical candidate. The results demonstrate that an amidoxime group can serve as a prodrug functionality for an amidino group.

Chemistry

The target compounds indicated in Tables 1−3 were synthesized as outlined in Schemes 1−3. Starting with the amidino carboxylate **1**[38] (Scheme 1), straightforward esterification and ethoxycarbonylation[52,53] protocols were used for the preparation of **3**−**5**. In analogy to the X-ray structure of 2-amino-*N*-benzoylbenzamidine,[54] all acylated benzamidines described in this report are shown in the *Z*-(acylimino)amine form rather than in the tautomeric (acylamino)imine form. The tyrosine derivative **6**, obtained by coupling of *N*-Z-Tyr-OH with *tert*-butyl (4-piperidinyloxy)acetate followed by catalytic hydrogenation as described earlier,[38] was acylated with 4-amidinobenzoyl chloride and subsequently with ethoxycarbonyl chloride. Before deprotection to **7** with formic acid, the side product **7a** was removed by chromatography. Acylation of the same tyrosine derivative **6** with 4-cyanobenzoyl chloride followed by treatment with hydroxylamine hydrochloride in the presence of sodium methoxide[55,56] and transesterification yielded the amidoxime derivative **8**. On the basis of the X-ray structure determined for **18** (see below) as well as for pyridine 2-amidoxime,[57] all amidoximes discussed in this report are shown in the *Z*-form.

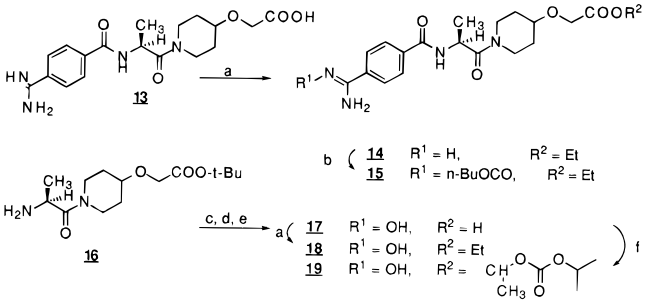
Using the amidinodicarboxylate **2**[38] and the nitrile derivative **11**[38] (Scheme 2), similar standard transformations as described above furnished the target compounds **9**, **10**, and **12**.

Scheme 2. Preparation of Prodrug Derivatives of **2**[a]



[a] (a) 2-Propanol, H_2SO_4; (b) EtOCOCl, NaOH, CH_2Cl_2; (c) $NH_2OH \cdot HCl$, Na, MeOH.

Scheme 3. Preparation of Prodrug Derivatives of **13**[a]



[a] (a) EtOH, H_2SO_4; (b) *n*-BuOCOCl, NaOH, CH_2Cl_2; (c) 4-NCC_6H_4COCl, $NaHCO_3$, CH_2Cl_2; (d) $NH_2OH \cdot HCl$, Na, CH_3OH; (e) HCOOH; (f) 1-iodoethyl isopropyl carbonate, dicyclohexylamine, DMF.

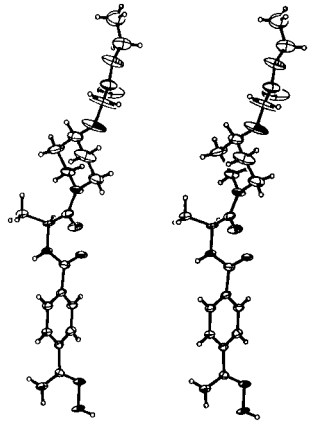


Figure 1. Stereorepresentation of the molecular structure of **18** (Ro 48-3657) as derived from the X-ray analysis.

The amidino carboxylate **13**[38] and the alanine derivative **16**[38] (Scheme 3) were converted to the corresponding prodrugs **14** and **15** as well as **17** and **18**, respectively, using the same method as outlined above. As already mentioned, the X-ray structure of **18** (Figure 1) shows that the amidoxime function is *Z*-configurated around the C−N double bond. In addition, in the crystalline state the oxyacetic acid side chain prefers an axial position at the piperidine ring. Finally, alkylation of the amidoxime acid **17** with 1-iodoethyl isopropyl carbonate[58] yielded the ester **19** as a mixture of diastereoisomers.

Results and Discussion

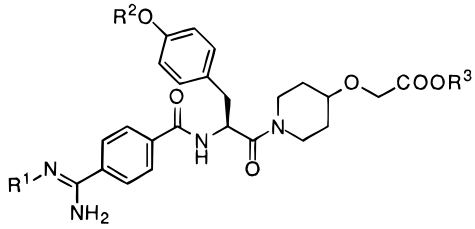
Assay Systems. For assessing the *in vitro* activity of the compounds, inhibition of platelet aggregation in human platelet rich plasma (h-PRP) induced by ADP as well as inhibition of fibrinogen binding to immobilized GP IIb-IIIa (integrin $\alpha_{IIb}\beta_3$) was used as described earlier.[38] The oral activity of the compounds was initially determined in mice.

Per experiment, a given compound was orally administered to two mice. The first animal was sacrificed after 1 h and the second after 3 h, and plasma was prepared. Since GP IIb-IIIa antagonists are generally very species selective, the inhibitory potency of the mice plasma was assessed in a hybrid bioassay. The mouse plasma was mixed with h-PRP, and the extent of ADP-induced platelet aggregation was determined. From the IC_{50} value (μL of plasma/400 μL), the oral dose (ID_{50}) was calculated that would result in 50% inhibition of human platelet aggregation. The ID_{50} values presented in Tables 1−3 were calculated from experiments, where about 100 mg/kg of a given compound was orally administered. The ID_{50} values determined from the '1 h mice' and the '3h mice' were pooled since no significant differences between the two groups were detected among the compounds presented in Tables 1−3. For selected compounds with low ID_{50} values, i.e., with good oral activity in mice, we investigated whether the ID_{50} was dependent on the dose administered. Compounds **15**, **18**, and **19** were, in addition to 100 mg/kg, also dosed at 10 and 1 mg/kg. For all three compounds the ID_{50} values derived from the 1 mg/kg experiments were only about 1.5-fold lower than those determined from the 100 or 10 mg/kg experiments. For example, compound **18** showed an ID_{50} of 0.25 ± 0.04 and 0.17 ± 0.02 mg/kg for the experiments dosed at 100 and 1 mg/kg, respectively. Thus, the ID_{50} was largely independent of the dose administered.

Prodrug Derivatives of 1 and 2. As shown by the ID_{50} values given in Tables 1 and 2, the potent fibrinogen receptor antagonists **1** and **2** exhibited similar oral activities in mice. In a rat experiment, the absolute bioavailability F (F (in %) = $[AUC_{po}]/[AUC_{iv}] \times [dose_{iv}]/[dose_{po}] \times 100$) of **1** was estimated to be about 1% (results not shown). To improve the oral bioavailability of compounds such as **1** or **2**, several strategies might be pursued as follows: (i) synthesis of structurally related novel scaffolds, (ii) replacement of polar and/or charged functional groups (e.g., amidino and/or carboxylate function), or (iii) modification of polar and/or charged functional groups by means of a prodrug strategy. This approach would also protect the gastrointestinal tract from exposure to high concentrations of a potent platelet inhibitor, provided the prodrug is not a competent GP IIb-IIIa antagonist. Application of a prodrug strategy to **1** seemed especially attractive since this compound is already in clinial trials for intravenous administration.[37] Esterification of the carboxylate group in **1** did not yield a substantial increase in oral activity in mice (**3**, Table 1). Only the simultaneous masking of the amidino function by an ethoxycarbonyl group to give the uncharged derivative **4** resulted in the desired increase of oral activity. A similar observation was reported for the GP IIb-IIIa antagonist BIBU 52 and its double prodrug BIBU 104.[44] Further improvement was possible by acetylation of the phenolic hydroxyl group of **4** to give the triple prodrug **5** which is devoid of any *in vitro* activity. Interestingly, modification of the amidino group alone, as demonstrated in **7**, did not result in a significant increase in oral activity in mice.

Although completely inactive in inhibiting FG binding to GP IIb-IIIa as well as in inhibiting platelet aggregation, the amidoxime ethyl ester **8**, which might be considered as intermediate in the synthesis of compounds like **3**,[40] exhibited oral activity in the mouse assay. Obviously, the double prodrug **8** was absorbed and converted to an active compound *in vivo*, most probably to the amidino acid **1**. The metabolic reduction of benzamidoxime derivatives to benzamidines, *in vivo* as well as *in vitro*, is well documented.[59−62] The pK_a values of benzamidoxime,[63] i.e., pK_{a1} approximately 13 (deprotonation) and pK_{a2} approximately 5 (protonation),

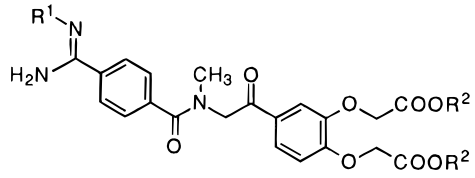
Table 1. Prodrug Derivatives of **1**



	R^1	R^2	R^3	inhibtn of FG binding to GP IIb-IIIa IC_{50} (nM)[a]	inhibtn of platelet aggregation (h-PRP/ADP) IC_{50} (nM)[b]	oral activity in mice ID_{50} (mg/kg)[c] mean ± SEM (n)
1	H	H	H	1.4	33	8.5 ± 0.8 (12)
3	H	H	Et	51	63	6.9 ± 1.1 (4)
4	EtOCO	H	Et	>8000	39 800	1.4 ± 0.4 (4)
5	EtOCO	CH_3CO	*i*-Pr	>10 000	>100 000	0.9 ± 0.3 (4)
7	EtOCO	H	H	90	1950	10.7 ± 2.8 (2)
8	OH	H	Et	>10 000	>100 000	2.6 ± 0.7 (5)

[a] IC_{50} values were calculated with a log linear regression analysis. The mean value of at least two independent experiments is shown. In examples with more than five independent experiments, the SD was always lower than ±25% of the mean. [b] Inhibition of ADP-induced platelet aggregation was determined in h-PRP of at least two donors. In examples with more than five determinations, the SD was always lower than ±20% of the mean. [c] The indicated ID_{50} values represent means ± SEM. Each ID_{50} value was calculated as described in the Experimental Section. The ID_{50} values indicate the oral dose (mg/kg) administered to mice that, when their plasma is tested in the hybrid bioassay, results in 50% inhibition of human platelet aggregation.

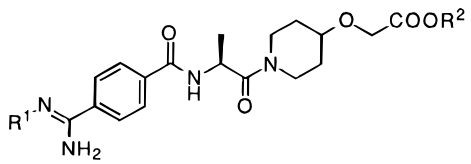
Table 2. Prodrug Derivatives of **2**

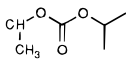


	R^1	R^2	inhibtn of FG binding to GP IIb-IIIa IC_{50} (nM)[a]	inhibtn of platelet aggregation (h-PRP/ADP) IC_{50} (nM)[b]	oral activity in mice ID_{50} (mg/kg)[c] mean ± SEM (n)
2	H	H	0.4	51	11.2 ± 1.8 (4)
9	H	*i*-Pr	900	345	11.0 ± 2.1 (4)
10	EtOCO	*i*-Pr	>10 000	>100 000	1.4 ± 0.4 (5)
12	OH	CH_3	>10 000	>100 000	0.7 ± 0.2 (6)

[a−c] See corresponding footnotes in Table 1.

Table 3. Prodrug Derivatives of **13**



	R^1	R^2	inhibtn of FG binding to GP IIb-IIIa IC_{50} (nM)[a]	inhibtn of platelet aggregation (h-PRP/ADP) IC_{50} (nM)[b]	oral activity in mice ID_{50} (mg/kg)[c] mean ± SEM (*n*)
13	H	H	1.9	38	5.2 ± 1.5 (10)
14	H	Et	163	500	1.8 ± 0.4 (6)
15	*n*-BuOCO	Et	>10 000	>100 000	0.30 ± 0.05 (6)
17	OH	H	594	13 800	5.1 ± 1.6 (6)
18	OH	Et	>10 000	>100 000	0.25 ± 0.04 (6)
19	OH		>10 000	>60 000	0.34 ± 0.10 (5)

[a–c] See corresponding footnotes in Table 1.

indicate that **8** remains uncharged over a wide pH range. This property which also characterizes compounds **4** and **5** might enhance oral absorption.

On the basis of the results shown in Table 1, the prodrug concept was then applied to the amidino dicarboxylate **2** (Table 2). Again, only the modification of the amidino group and the carboxylate groups to give **10** or **12** was successful, while after esterification of **2** to **9** no improvement of the oral activity in mice was observed. Contrary to the derivatives of **1** (Table 1), the amidoxime dimethyl ester **12** is superior to the other derivatives shown in Table 2 with respect to the oral activity in mice.

Prodrug Derivatives of 13. We then focused our attention on prodrugs of the alanine derivative **13** (Table 3), a highly potent and selective FG receptor antagonist structurally related to **1** but with a lower molecular weight.[38] According to the ID_{50} (Table 3) **13** showed better oral activity as compared to **1**. Following the trend already discussed above, the ethyl ester **14** exhibited a 3 times higher oral activity than **13** in the mouse model. A further 6−7-fold gain in oral activity was again observed with the (*n*-butoxycarbonyl)amidino derivative **15** as well as with the amidoxime ethyl ester **18**, while the amidoxime acid **17** showed about the same activity after oral administration as the amidino acid **13**. Replacement of the ethyl ester in **18** by an [(isopropoxycarbonyl)oxy]ethyl ester to give **19** (as a mixture of diastereoisomers) did not lead to a further improvement of oral activity in mice. Therefore, this compound was not further investigated. Since the bioavailability of **13** after oral administration of **15** to rats ($F = 9.8\%$, $n = 2$), dogs ($F = 10.1\%$, $n = 3$), and rhesus monkeys ($F = 4.0\%$, $n = 2$) was clearly lower as compared to **18**, the pharmacokinetics of the latter compound were characterized in more detail. The results are summarized in Table 4.

Oral bioavailability F was calculated by comparing the individual AUCs of **13** after oral administration of the double prodrug **18** with the mean value after intravenous administration of the active drug **13**, i.e., F (in %) = [AUC(**18**)$_{po}$]/[AUC (**13**)$_{iv}$] × [dose(**13**)$_{iv}$]/[dose(**18**)$_{po}$] × 100, where the oral dose was corrected by the corresponding molecular weights of **13** and **18**. The plasma concentrations were determined either by HPLC (rat, dog, and selected monkey samples; detection limit 2.5 ng/mL) or by a solid phase FG binding assay (monkey samples; detection limit 0.5 ng/mL).

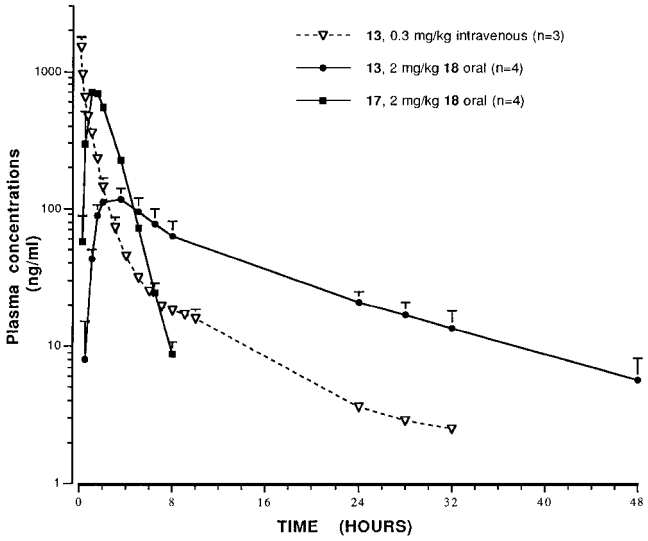


Figure 2. Plasma levels of **13** upon iv and po administration of **13** and **18**, respectively, and plasma levels of **17** upon po administration of **18** versus time as determined by HPLC in dogs. Data are mean ± SD.

Table 4. Pharmacokinetic Parameters of **13** after Oral Administration of **18**

species (*n*)	dose (mg/kg)	$t_{1/2\beta}$ (h)[a,b]	F (%)[a,c]
rat (3)	4	4.1 ± 1.7	26 ± 5
dog (4)	2	11.4 ± 1.1	25 ± 6
rhesus monkey (5)	1	5.1 ± 1.4	33 ± 6

[a] Data are mean ± SD. [b] The apparent elimination half-life ($t_{1/2\beta}$) was determined from the linear portion of the log plasma concentration−time profile. [c] The bioavailability (F) of **13** following po administration of **18** was calculated accounting for the differences in molecular weights and doses.

After oral administration of **18** in solution at a dose of 4 mg/kg to male rats ($n = 3$), the active drug **13** as well as the amidoxime acid **17** were detected in plasma. While **13** showed a relatively long elimination half-life (Table 4), **17** disappeared more rapidly ($t_{1/2\beta} = 0.35 \pm 0.025$ h, mean ± SD). It was gratifying to see that **18** was absorbed and metabolized to the active drug **13**, suggesting that the antiplatelet activity seen in the mouse assay of **18** was due to *in vivo* metabolism to **13**. The metabolic consequence of *in vivo* production of the amidoxime acid **17** remains to be elucidated. Despite the two metabolic steps involved, the interindividual variability of the plasma concentrations of **13** was low (in eight rats dosed at 5 mg/kg the coefficient of variation, CV, of AUC(**13**) was ≤10%). Compared to the tyrosine derivative **1**, a more than 25-fold increase of the bioavailability in rats was achieved.

When compound **18** was given orally to fasted male beagle dogs as 25 mg tablets, a pharmacokinetic pattern similar to that described above for rats was observed. As depicted in Figure 2, peak plasma levels of **17** were significantly higher and reached earlier than peak plasma levels of **13**. Again, the half-life of **13** was significantly longer (Table 4) than that of **17** ($t_{1/2\beta} = 0.96 \pm 0.07$ h, mean ± SD).

Comparison of the AUCs in rhesus monkeys after intravenous or oral administration of **13** or **18**, respectively, is shown in Figure 3. After administration of **18**

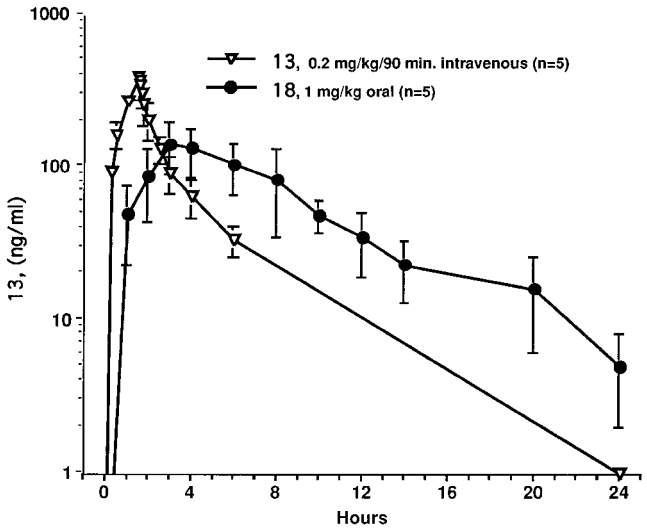


Figure 3. Plasma levels of **13** upon iv and po administration of **13** and **18**, respectively, versus time as determined by bioassay in rhesus monkeys. Data are mean ± SD.

as either a pressed tablet ($n = 2$) or an enteric coated capsule ($n = 3$), no significant differences in the plasma concentrations were seen. Therefore, the data from the two groups were pooled (Table 4). Compared to the experiments in dogs, the half-life of **13** was shorter while its bioavailability was higher in this primate model of oral activity. In addition to solid phase FG binding assay, plasma samples from those animals dosed with **18** formulated as a pressed tablet were also analyzed by reverse phase HPLC (Supporting Information). The concentrations of **13** determined by these two independent methods are highly correlatable (slope = 0.989, $R = 0.94$). Since the enantiomer of **13** is 10-fold less potent than **13**,[38] these data suggest that **18**, upon absorption and metabolism to **13**, is stereochemically stable *in vivo*. HPLC analysis also indicated the presence of **17** having a similar plasma disposition seen in the rats and dogs described above.

Oral administration of **18** to dogs and rhesus monkeys had no effect on complete blood cell counts. Furthermore, iv administration of **13** or po administration of **18** to rhesus monkeys had no effect on heart rate or mean arterial pressure.

Conclusions

In summary, by pursuing a prodrug approach we were able to improve the oral bioavailability of potent and selective GP IIb-IIIa antagonists such as **13** by a factor of approximately 20. With double prodrugs like the amidoxime ethyl ester **18**, we have demonstrated that an amidoxime group can serve as a prodrug functionality for an amidino group. Although the mechanistic details for this reduction remain to be elucidated, these findings might be of interest in those cases where highly basic, charged amidino or guanidino groups have to be replaced by neutral surrogates. The similar bioavailability of **13** after oral administration of **18** in rats, dogs, and rhesus monkeys suggests the absence of a species dependent conversion of the double prodrug to the active moiety. In addition, a favorable elimination half-life of **13** after oral administration of **18** was observed in the same three animal species. On the basis of these properties, the orally active **18** (Ro 48-3657), a double prodrug of the potent and selective non-peptide GP IIb-IIIa antagonist **13** (Ro 44-3888), was selected as a clinical candidate for evaluation as a prophylactic agent in patients at high risk for arterial thrombosis.

Experimental Section

Chemistry. Reagent grade solvents were used without further purification. Evaporation implies the removal of solvent by use of a Büchi rotary evaporator at 40−50 °C *in vacuo*. All organic extracts were dried over Na_2SO_4 or $MgSO_4$. Normal phase silica gel used for flash chromatography (FC) was Kieselgel-60 (230−400 mesh); reverse phase silica gel used was Lichroprep RP 18 (40−63 μm); both were supplied by E. Merck, A.G., Darmstadt, Germany. TLC plates coated with silica gel 60 F_{254} (Merck) were used; detection by UV light (254 nm) or I_2 vapor was applied. Melting points were determined with a Büchi 510 or Electrothermal 9100 apparatus and are uncorrected. Proton NMR spectra (NMR) were recorded on a Bruker AC 250 spectrometer; δ values in ppm relative to tetramethylsilane are given. IR spectra (IR) were recorded with the compound (neat) on a sodium chloride disk or as KBr pellets using a Nicolet 7199-FT-IR spectrometer. Optical rotations, $[\alpha]^{20}_D$, were determined with a Perkin Elmer 241 polarimeter, c in g/100 mL. Mass spectra (MS) were recorded with an instrument of type API III from Perkin Elmer Sciex using the pneumatically assisted electrospray technique. Results of elemental analysis were within 0.4% of the theoretical values unless otherwise noted.

(*S*)-[[1-[2-[[4-(Aminoiminomethyl)benzoyl]amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic Acid Ethyl Ester (14) (Standard procedure A). To a solution of the amidino acid **13**[38] (4.85 g, 12.9 mmol) in EtOH (50 mL) was added concentrated sulfuric acid (3 mL). After 14 h at 23 °C, the solvents were removed *in vacuo* to a volume of about 10 mL, water was added (25 mL), and the resulting solution was neutralized by careful addition of solid $NaHCO_3$ in portions. After evaporation of the solvents, the residue was passed through silica gel RP-18 (180 g, H_2O/EtOH, 0−5%). The pure fractions were combined and the solvents evaporated, and the residue was triturated with EtOH to give the hemisulfate salt of **14** (4.39 g, 75%): mp 196−198 °C; $[\alpha]^{20}_D = +36.5°$ ($c = 0.6$, EtOH); NMR (DMSO-d_6) δ 1.19 and 1.20 (2 t, $J = 7$ Hz, 3 H, rotamers), 1.30 (d, $J = 5$ Hz, 3 H), 1.40 (m, 2 H), 1.85 (m, 2 H), 2.92−3.45 (m, 2 H), 3.52−3.88 (m, 3 H), 4.10 (m, 2 H), 4.14 (2 s, 2 H, rotamers), 4.98 (m, 1 H), 7.88 (part of *AA'BB'*, 2 H), 8.10 (part of *AA'BB'*, 2 H), 8.92 (2 d, $J = 7$ Hz, NH, rotamers), 9.94 (bs, 2 × NH_2); MS *m/z* (FAB) 405 $(M + H)^+$. Anal. ($C_{20}H_{28}N_4O_5 \cdot 0.5\ H_2SO_4$) C, H, N.

Compounds **3** and **9** were prepared from **1**[38] and **2**,[38] respectively, according to standard procedure A using the corresponding alcohols.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino[(*n*-butoxycarbonyl)imino]-methyl]benzoyl]amino]-1-oxopropyl]-4-piperidinyl]oxy]-acetic Acid Ethyl Ester (15) (Standard procedure B). A solution of the hemisulfate salt of **14** (3.71 g, 8.2 mmol) in water (100 mL) was neutralized by addition of solid $NaHCO_3$ in portions at 5 °C. After addition of CH_2Cl_2 (100 mL), the reaction mixture was treated with *n*-butyl chloroformate (2.05 g, 15 mmol). With vigorous stirring aqueous NaOH (0.5 N, 40 mL) was added dropwise within 1 h. After transferring the reaction mixture to a separatory funnel, the layers were separated. The organic extracts were washed with water and dried and the solvents evaporated. After chromatography (silica gel, EtOAc) **15** (2.3 g, 56%) was obtained as a colorless foam: $[\alpha]^{20}_D = +36.2°$ ($c = 1$, EtOH); NMR ($CDCl_3$) δ 0.95 (t, $J = 7$ Hz, 3 H), 1.30 (t, $J = 7$ Hz, 3 H), 1.35−1.50 (m, 2 H), 1.44 (d, $J = 7$ Hz, 3 H), 1.60−2.00 (m, 6 H), 3.29−4.00 (m, 5 H), 4.14 (bs, 2 H), 4.23 (2 q, $J = 7$ Hz, 4 H), 5.06 (m, 1 H), 7.56 (m, NH), 7.89 (*AA'BB'* system, 4 H), 8.17 and 9.90 (2 bs, NH_2); MS *m/z* (EI) 505 $(M + H)^+$. Anal. ($C_{25}H_{36}N_4O_7 \cdot 1.25\ C_4H_8O_2$) C, H, N.

Compounds **4** and **10** were prepared from **3** and **9**, respectively, according to standard procedure B using ethyl chloroformate.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino[(ethoxycarbonyl)imino]methyl]benzoyl]amino]-3-(4-acetoxyphenyl)propionyl]piperi-

din-4-yl]oxy]acetic Acid Isopropyl Ester (5). Using standard procedure A (with 2-propanol) and standard procedure B (with ethyl chloroformate) **1** (4.68 g, 10 mmol) was converted to (*Z*)-(*S*)-[[1-[2-[[4-[amino[(ethoxycarbonyl)imino]methyl]benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]-acetic acid isopropyl ester (1.20 g, 21% overall) as a colorless foam: MS *m*/*z* (FAB) 583 $(M + H)^+$.

To a solution of this material (291 mg, 0.5 mmol) in acetone (10 mL) was added acetic anhydride (102 mg, 1 mmol). After 40 min at 23 °C, solid K_2CO_3 (173 mg, 1.25 mmol) was added. After 1 h the volume was reduced to 2 mL and the residue distributed between water and EtOAc. The organic extracts were washed with water and dried and the solvents evaporated. The residue was purified by chromatography (silica gel, EtOAc) to give **5** (155 mg, 50%) as a colorless foam: NMR ($CDCl_3$) δ 1.24 and 1.25 (2 t, $J = 7$ Hz, 6 H), 1.37 (t, $J = 7$ Hz, 3 H), 1.45−1.90 (m, 4 H), 2.27 and 2.28 (2 s, 3 H, rotamers), 2.90−3.34 (m, 3 H), 3.40−3.80 (m, 4 H), 4.02 and 4.05 (2 bs, 2 H, rotamers), 4.24 (q, $J = 7$ Hz, 2 H), 5.07 (m, 1 H), 5.34 (m, 1 H), 7.01 (m, 2 H), 7.17−7.32 (m, 3 H), 7.80 (m, 2 H), 7.91 (m, 2 H), 9.70 (bs, NH_2); MS *m*/*z* (FAB) 625 $(M + H)^+$. Anal. ($C_{32}H_{40}N_4O_9 \cdot 1.2\ C_4H_8O_2$) H, C: calcd, 60.52; found, 59.55. N: calcd, 7.68; found, 8.22.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino[(ethoxycarbonyl)imino]methyl]benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic Acid (7). The amine **6** was acylated with 4-amidinobenzoyl chloride as described earlier[38] to give (*S*)-[[1-[2-[[4-(aminoiminomethyl)benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic acid *tert*-butyl ester: MS *m*/*z* (FAB) 525 $(M + H)^+$.

This material (787 mg, 1.5 mmol) was treated with ethyl chloroformate (216 mg, 2 mmol) according to standard procedure B to give, after chromatography (silica gel, CH_2Cl_2/MeOH, 49:1), **7a** (170 mg, 17%; mp 72−73 °C; MS *m*/*z* (FAB) 669 $(M + H)^+$) together with the desired monoacylated product (180 mg, 20%) which was dissolved in formic acid (5 mL) and heated to 50 °C. After 1 h the solvents were removed by evaporation, and the residue was crystallized upon addition of CH_3CN to give **7** (115 mg, 71%): mp 134−135 °C; NMR (DMSO-d_6) δ 1.22 (t, $J = 7$ Hz, 3 H), 1.20−1.80 (m, 4 H), 2.75−3.98 (m, 7 H), 4.07 (q, $J = 7$ Hz, 2 H), 4.11 (s, 2 H), 5.08 (m, 1 H), 6.64 (part of *AA'BB'*, 2 H), 7.07 (part of *AA'BB'*, 2 H), 7.90 (part of *AA'BB'*, 2 H), 8.02 (part of *AA'BB'*, 2 H), 8.85 (m, 1 H), 9.20 (bs, NH_2, 2 OH); MS *m*/*z* (FAB) 539 $(M - H)^-$. Anal. ($C_{27}H_{32}N_4O_8$) H, N, C: calcd, 59.99; found, 59.27.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino(hydroxyimino)methyl]benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]-acetic Acid Ethyl Ester (8). The amine **6** (3.65 g, 9.64 mmol) in CH_2Cl_2/saturated $NaHCO_3$ (1:1, 140 mL) was acylated with 4-cyanobenzoyl chloride (1.75 g, 10.6 mmol) as described earlier[38] to give (*S*)-[[1-[2-[(4-cyanobenzoyl)amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic acid *tert*-butyl ester as a colorless foam (3.10 g, 63%): IR (KBr) 2231, 1746, 1628 cm^{-1}; MS *m*/*z* (FAB) 508 $(M + H)^+$.

Standard procedure C: To a solution of Na (124 mg, 5.4 mmol) in MeOH (100mL) were successively added $NH_2OH \cdot$ HCl (392 mg, 5.63 mmol) and (*S*)-[[1-[2-[(4-cyanobenzoyl)-amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic acid *tert*-butyl ester (1.90 g, 3.75 mmol). After 14 h at 23 °C, the solvents were evaporated, and the residue was purified by chromatography (silica gel, CH_2Cl_2/CH_3OH, 15:1) to give (*Z*)-(*S*)-[[1-[2-[[4-[amino(hydroxyimino)methyl]benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic acid *tert*-butyl ester as a colorless foam (930 mg, 46%): MS *m*/*z* (FAB) 541 $(M + H)^+$.

A solution of this material (930 mg, 1.72 mmol) in formic acid (20 mL) was stirred at 23 °C. After 5 h the solvents were evaporated; the residue was washed with water, evaporated, and triturated with EtOEt to give (*Z*)-(*S*)-[[1-[2-[[4-[amino-(hydroxyimino)methyl]benzoyl]amino]-3-(4-hydroxyphenyl)propionyl]piperidin-4-yl]oxy]acetic acid as an amorphous solid (800 mg, 96%): MS *m*/*z* (FAB) 485 $(M + H)^+$.

This material (350 mg, 0.72 mmol) was esterified in EtOH (50 mL) in the presence of concentrated sulfuric acid according to standard procedure A to give, after trituration with EtOEt, **8** (120 mg, 33%): mp 107−110 °C; IR (KBr) 3369, 1746, 1627 cm^{-1}; NMR (DMSO-d_6) δ 1.18 and 1.19 (2 t, $J = 7$ Hz, 3 H, rotamers), 1.20−1.80 (m, 4 H), 2.75−3.98 (m, 7 H), 4.09 (m, 2 H), 4.11 (bs, 2 H), 5.04 (m, 1 H), 5.89 (bs, NH_2), 6.63 (part of *AA'BB'*, 2 H), 7.07 (part of *AA'BB'*, 2 H), 7.73 (part of *AA'BB'*, 2 H), 7.83 (part of *AA'BB'*, 2 H), 8.74 (m, 1 H), 9.17 (bs, OH), 9.80 (bs, OH); MS *m*/*z* (FAB) 513 $(M + H)^+$. Anal. ($C_{26}H_{32}N_4O_7$) H, C: calcd, 60.93; found, 60.52. N: calcd, 10.93; found, 10.37.

Compound **11**[38] was converted to the corresponding amidoxime **12** according to standard procedure C.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino(hydroxyimino)methyl]benzoyl]-amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic Acid (17). The amine **13**[38] (43 g, 0.15 mol) in CH_2Cl_2/saturated $NaHCO_3$ (1:1, 600 mL) was acylated with 4-cyanobenzoyl chloride (26 g, 0.16 mol) as described earlier[38] to give (*S*)-[[1-[2-[(4-cyanobenzoyl)amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic acid *tert*-butyl ester (50 g, 80%): mp 134−137 °C (from EtOEt); $[\alpha]^{20}_D = +39.6°$ (*c* = 1.0, EtOH); MS *m*/*z* (EI) 359 $(M - C_4H_8)^+$.

This material (50 g, 0.12 mol) was converted to (*Z*)-(*S*)-[[1-[2-[[4-[amino(hydroxyimino)methyl]benzoyl]amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic acid *tert*-butyl ester according to standard procedure C (46 g, 85%): mp 193−194 °C (from MeOH); MS *m*/*z* (FAB) 449 $(M + H)^+$.

This material (46 g, 0.10 mol) in formic acid (150 mL) was heated to 80 °C. After 3 h the solvents were evaporated, and the residue was crystallized upon addition of EtOAc (150 mL). The resulting suspension was filtered; the filter cake was washed with EtOAc (50 mL) and dried *in vacuo* to give **17** (34.5 g, 86%): mp 176−178 °C; $[\alpha]^{20}_D = +44.2°$ (*c* = 1.0, H_2O); NMR (DMSO-d_6) δ 1.27 (bd, $J = 5$ Hz, 3 H), 1.43 (m, 2 H), 1.85 (m, 2 H), 2.95−3.45 (m, 3 H), 3.55−3.80 (m, 2 H), 4.04 (2 s, 2 H, rotamers), 4.96 (m, 1 H), 5.90 (bs, NH_2), 7.75 (part of *AA'BB'*, 2 H), 7.88 (part of *AA'BB'*, 2 H), 8.62 (m, NH, rotamers), 9.79 (bs, N-OH), 12.59 (bs, COOH); MS *m*/*z* (FAB) 391 $(M - H)^-$. Anal. ($C_{18}H_{24}N_4O_6$) C, H, N.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino(hydroxyimino)methyl)benzoyl]-amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic Acid Ethyl Ester (18). Concentrated sulfuric acid (15 mL) was added with ice cooling to a suspension of **17** (19.6 g, 50 mmol) in EtOH (300 mL). After removal of the ice bath, the solution was stirred for 14 h at 23 °C. Subsequently, the solvents were evaporated, and the residue was dissolved in water (300 mL). At ice bath temperature the solution was neutralized upon addition of an aqueous solution (28%) of sodium hydroxide. The precipitates were collected by filtration, washed with water, and recrystallized from EtOH to give **18** (11.7 g, 56%): mp 201−203 °C; $[\alpha]^{20}_D = +75.3°$ (*c* = 0.9, AcOH); NMR (DMSO-d_6) δ 1.19 (2 t, $J = 7$ Hz, 3 H, rotamers), 1.29 (bd, $J = 6$ Hz, 3 H, rotamers), 1.42 (m, 2 H), 1.82 (m, 2 H), 2.90−3.33 (m, 2 H), 3.52−4.04 (m, 3 H), 4.09 (m, 4 H), 4.94 (m, 1 H), 5.90 (bs, NH_2), 7.75 (part of *AA'BB'*, 2 H), 7.88 (part of *AA'BB'*, 2 H), 8.68 (m, NH, rotamers), 9.80 (bs, N-OH); MS *m*/*z* (FAB) 421 $(M + H)^+$. Anal. ($C_{20}H_{28}N_4O_6$) C, H, N.

X-ray Crystallographic Structure Analysis of 18. From EtOH, crystals of **18** ($C_{20}H_{28}N_4O_6$, formula weight 420.46) formed in the monoclinic space group $P2_1$. A representative crystal was surveyed, and a total of 1634 reflections with 2θ less than 113.5° were measured at 173 K on a Siemens P4/RA, M18XHF diffractometer using monochromatic copper radiation. The structure was solved by direct methods using the SHELXS-86 package; refinement was facilitated by the SHELXL-93[64] system. Molecules/unit cell $Z = 2$; cell dimensions $a = 11.390(4)$ Å, $b = 5.386(2)$ Å, $c = 16.793(7)$ Å, $\beta = 90.04(3)°$; calculated density 1.355 g cm^{-3}. Full-matrix least-squares refinement was conducted with anisotropic temperature factors for all atoms except hydrogen which were included at calculated positions with isotropic temperature factors. The final *R*-factor of 0.066 was obtained for 1542 observed reflections ($I > 2\sigma(I)$); largest difference peak and hole were 0.61 and −0.35 e$Å^{-3}$, respectively. Coordinates, anisotropic temperature factors, bond lengths, and angles are available as Supporting Information and will be deposited at the Cambridge Crystallographic Data Centre.

(*Z*)-(*S*)-[[1-[2-[[4-[Amino(hydroxyimino)methyl]benzoyl]-amino]-1-oxopropyl]-4-piperidinyl]oxy]acetic Acid (*R*/*S*)-1-[(Isopropoxycarbonyl)oxy]ethyl Ester (19). To a solu-

tion of **17** (780 mg, 2 mmol) in *N,N*-dimethylformamide (20 mL) were added at 0 °C dicyclohexylamine (400 mg, 2.2 mmol) and 1-iodoethyl isopropyl carbonate[58] (620 mg, 2.4 mmol). After 14 h at 23 °C, the solvents were evaporated *in vacuo* at 60 °C. The residue was dissolved in EtOAc, and the resulting solution was washed twice with water, dried, and evaporated. After chromatography (silica gel, EtOAc) and crystallization (EtOEt), **19** was obtained as colorless powder (220 mg, 21%): mp 113 °C dec; NMR ($CDCl_3$) δ 1.32 (d, $J = 7$ Hz, 6 H), 1.47 (d, $J = 6$ Hz, 3 H), 1.54 (2 d, $J = 6$ Hz, 3 H, diastereomers), 1.58−1.98 (m, 4 H), 3.35−4.05 (m, 5 H), 4.15 and 4.16 (2 s, 2 H), 4.86 (m, 3 H), 5.26 (m, 1 H), 6.82 (2 q, $J = 6$ Hz, 1 H, diastereomers), 7.38 (part of *AA'BB'*, 2 H), 7.61 (part of *AA'BB'*, 2 H), 8.42 (m, 1 H), 8.98 (bs, 1 H); MS *m/z* (FAB) 523 $(M + H)^+$. Anal. ($C_{24}H_{34}N_4O_9$) C, H, N.

Pharmacology. *In Vitro* Assay Systems. The inhibition of platelet aggregation in h-PRP induced by ADP as well as the inhibition of fibrinogen binding to immobilized GP IIb-IIIa (integrin $\alpha_{IIb}\beta_3$) was used as described earlier.[38]

Assessment of Oral Activity in Mice. Mice were used for the first assessment of the oral activity of each compound. Per experiment, about 100 mg/kg of a given compound, suspended in 10% gelatin (particle size < 1 μm), was orally administered to two mice. The first animal was sacrificed after 1 h and the second after 3 h to prepare plasma. Since GP IIb-IIIa antagonists are generally very species selective, *ex vivo* aggregation in mouse PRP could not be used to determine the amount of the active constituent(s) in the plasma. The inhibitory potency of these mice plasma samples was therefore assessed in a hybrid bioassay. Serial dilutions of each mouse plasma (100 μL) were mixed with human PRP (300 μL), and platelet aggregation was induced by ADP (10 μM final concentration). The volume of plasma required to inhibit 50% of aggregation was determined (IC_{50}). From the IC_{50} value (μL of plasma/400 μL), the dilution factor for each mouse plasma was determined. The orally administered dose (mg/kg) was then divided by this dilution factor to calculate the oral dose (ID_{50}) that results in 50% inhibition of ADP-induced aggregation in human PRP.

Pharmacokinetics in Rats. An intravenous infusion of **13** (Ro 44-3888; 4 mg/kg) was given to three male rats, and **18** (Ro 48-3657; 4 mg/kg) as solution (stock solution: 20 mg was dissolved in 2.5 mL of 0.1 N HCl, neutralized with 2.5 mL of NaOH, and adjusted to 10 mL with water) was administered orally to another three male rats. Blood samples were drawn into vials containing EDTA and NaF at specified times until 48 h postdosing. After collection all blood samples were immediately centrifuged at 4000*g* for 5 min at 4 °C. The plasma was removed and stored frozen at −20 °C until analysis. The plasma concentrations of **13** and **17** were measured using a sensitive and highly automated HPLC method involving column switching, gradient elution, and UV detection. The plasma samples were deproteinated by adding perchloric acid. The quantification limit was 2.5 ng/mL for both compounds. The pharmacokinetic parameters were evaluated by noncompartmental analysis using TopFit,[65] a pharmacokinetic analysis package.

Pharmacokinetics in Dogs. An intravenous infusion of **13** (Ro 44-3888; 0.3 mg/kg) was given to three fasted male beagle dogs, and **18** (Ro 48-3657; 2 mg/kg) was orally administered in tablet form to four fasted male beagle dogs. Blood samples were drawn into vials containing EDTA and NaF. Plasma was prepared, and the concentrations of **13** and **17** were determined by HPLC as described above.

Pharmacokinetics in Rhesus Monkeys. Five conscious rhesus monkeys weighing 3.8−4.5 kg were given an intravenous infusion of **13** (Ro 44-3888; 0.2 mg/kg over 90 min) via a cephalic vein catheter. Following a 48 h washout period, the monkeys were given an oral dose of **18** (Ro 48-3657; 1 mg/kg) in either a pressed tablet ($n = 2$) or an enteric coated capsule ($n = 3$) form. At specified times, up to 24 h after iv and po dosing, blood samples were collected from a saphenous vein catheter (<6 h) or femoral venipuncture (>6 h) and distributed into tubes containing K_2EDTA (4.2 mM final concentration). Shortly after collection, whole blood samples which were kept on wet ice were centrifuged at 10000*g* for 4 min. The resulting plasma was transferred to fresh tubes and stored at −70 °C. Prior to assay, plasma samples were thawed and filtered through Microcon-10 microconcentrators (Amicon Inc., Beverly, MA). The protein poor filtrate was analyzed for anti-GP IIb-IIIa activity using the solid phase fibrinogen binding assay as described earlier.[38] The compound **13** (Ro 44-3888) was used to generate the assay standard curve, and test results are expressed in ng/mL of **13** (Ro 44-3888). The lower limit of detection in this assay system was approximately 0.5 ng/mL. The extraction procedure was validated by recovery studies from spiked whole blood. The half-life of **13** was determined using TopFit.[65] The half-life was estimated using the last four data points, and the bioavailability of **13** following po administration of **18** was calculated accounting for the differences in the molecular weights and doses.

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Supporting Information Available: Experimental details and characterization data (NMR spectra, mass spectra, IR spectra, melting points, and microanalytical) for all compounds contained in Schemes 1−3 as well as in Tables 1−3 which do not appear in the Experimental Section and crystallographic data (coordinates, anisotropic temperature factors, bond lengths, and angles) of **18** (6 pages). Ordering information is given on any current masthead page.

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