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Design of a Potent and Orally Active Nonpeptide Platelet Fibrinogen Receptor (GPIIb/IIIa) Antagonist

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Abstract—The direct design of the potent nonpeptide platelet fibrinogen receptor (GPIIb/IIIa) antagonist, 8-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1*H*-1,4-benzodiazepine-2-acetic acid, (**3**) (SB 207448), based on the structure and conformation of the potent and highly constrained cyclic peptide antagonist SK&F 107260 (**2**), has been reported [Ku *et al., J. Am. Chem. Soc.* **1993**, *115*, 8861]. While **3** displayed *in vivo* activity in the conscious dog following intravenous administration, it was not active following intraduodenal administration; activity was measured with an *ex vivo* platelet aggregation assay. The secondary amide in **3** was N-methylated in the expectation of increased absorption and bioavailability. The resulting tertiary amide, **4** (SB 208651), also showed high binding affinity for human GPIIb/IIIa and potent antiaggregatory activity in human platelet-rich plasma. Most importantly, **4** was active *in vivo* following intravenous and intraduodenal administration. Comparison of the iv and id inhibition curves suggests an apparent bioavailability of approximately 10 %. Thus, **4** represents the first orally active compound in this series of potent, nonpeptide fibrinogen receptor antagonists.

Introduction

Platelet aggregation and thrombus formation contribute significantly to cardiovascular, cerebrovascular and peripheral vascular diseases. Aggregation is initiated by activation of the platelet and the platelet fibrinogen receptor (GPIIb/IIIa) which subsequently binds to fibrinogen, a dimeric plasma protein, to initiate platelet aggregation. The key role of GPIIb/IIIa in platelet aggregation is evidenced by studies showing that antibodies to GPIIb/IIIa prevent platelet aggregation in animals and in man.[1] Alternatively, the binding of activated GPIIb/IIIa to fibrinogen can be blocked with small molecules, *vide infra*, which are expected to have advantages over the use of antibodies.[2]

One approach to small molecule antagonists is suggested by the fact that the binding of activated GPIIb/IIIa to fibrinogen is primarily mediated by several short sequences located in the α and γ chains of the latter. These sequences include a dodecapeptide (400–411) on the γ chains[3,4] and two Arg-Gly-Asp (RGD)-containing tetrapeptides, Arg-Gly-Asp-Phe (RGDF; 95–98) and Arg-Gly-Asp-Ser (RGDS; 572–575), on the α-chains.[5–8] Of particular interest for a small molecule strategy, peptides corresponding to these sequences can block the binding of GPIIb/IIIa to fibrinogen and prevent platelet aggregation.[3,5,9–11]

Extending these observations, we showed that Ac-Arg-Gly-Asp-Ser-NH_2 (**1**), (Figure 1), a moderately potent GPIIb/IIIa antagonist, blocked thrombus formation in the canine coronary artery (Folts model) after intracoronary infusion, albeit at high concentrations. Taken together, these results suggested that small RGD peptides with GPIIb/IIIa antagonist activity could be useful antiaggregatory agents, if their potency could be improved.[11]

Subsequently, structural modification of **1** in our laboratories led to the discovery of a family of small, cyclic RGD-containing peptides that displayed affinities for GPIIb/IIIa comparable to that of fibrinogen itself, and had potent *in vitro* antiaggregatory activity. These peptides include cyclo(*S,S*)-[Ac-Cys-(N^{α}-Me)Arg-Gly-Asp-Pen]-NH_2 (SK&F 106760), and cyclo(*S,S*)-[Mba-(N^{α}-Me)Arg-Gly-Asp-Man], **2** (SK&F 107260), Figure 1.[12–15] Furthermore, SK&F 106760, a member of this series which displayed potent *in vivo* antiaggregatory activity, completely inhibited platelet-dependent coronary artery thrombosis in the dog following intravenous infusion.[2,16,17]

While potentially useful for acute treatment, peptidic antagonists of GPIIb/IIIa are generally thought to lack the oral bioavailability and duration of action required for

chronic administration. Consequently, there is keen interest in the discovery of orally active nonpeptide and semipeptide GPIIb/IIIa antagonists.[18,19]

As part of our overall approach to discovering nonpeptide GPIIb/IIIa antagonists, we studied the solution conformation of the (N^{α}-Me)Arg-Gly-Asp peptide backbone in the potent and conformationally constrained cyclic peptide antagonists, SK&F 106760 and SK&F 107260 (**2**) using ^{1}H NMR data in a constrained distance geometry search procedure. An X-ray crystal structure for SK&F 107260 was also obtained. The results suggested that the dominant conformer of the peptide backbone in solution contains a turn-extended-turn conformation with a C_7-like turn at Asp.[20]

The structural and conformational data derived from these studies were then used in the design of the potent nonpeptide antagonist **3** (SB 207448), Figure 1. In brief, **3** was designed to mimic the structure and extended C_7 turn conformation of the Gly-Asp moiety in **2** by using the constraint inherent in a 1,4-benzodiazepine-2-acetic acid moiety to replace the constraint provided by the macrocyclic ring in **2**. The design of **3** was completed with the selection of a 4-(amidino)phenyl group,[21,22] attached at the 8-position, to mimic the Arg side chain in **2**. The resulting putative Arg-Gly-Asp mimetic **3** was synthesized and shown to be a very potent human platelet fibrinogen receptor antagonist displaying GPIIb/IIIa affinity and *in vitro* antiaggregatory activity comparable to that seen with **2**.[23]

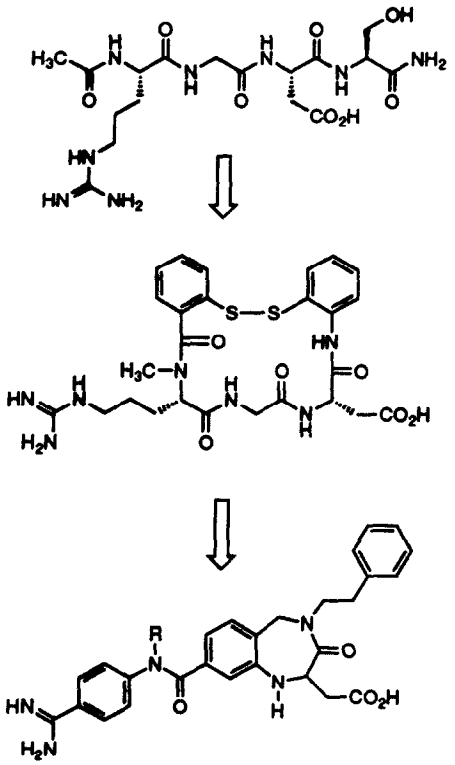
In this paper, we report that N-methylation of the secondary amide in **3** resulted in a compound, **4** (SB 208651), Figure 1, which shows similar *in vitro* potency, and which, unlike **3**, is active *in vivo* following intravenous and intraduodenal administration.

Strategy[24]

The *N*-methyl amide **4** was selected as one of the first analogs of **3** to be synthesized in the expectation that it might be better absorbed and more bioavailable. The rationale was based in part on reports that the movement of peptides across confluent layers of Caco-2 cells is inversely correlated with their hydrogen bonding ability. It was shown that N-methylation of secondary amide (peptide) bonds, which reduces hydrogen bonding ability, resulted in improved flux. For example, sequential N-methylation of the secondary amides in Ac-(D-Phe)$_3$-NH_2 was accompanied by substantial increases in permeability.[25-27,28] The ability of nonpeptides to cross the blood–brain barrier is also improved by removing polar groups not essential for activity or by reducing the polarity of groups considered necessary for activity.[29]

Chemistry

The substituted 1,4-benzodiazepine **12**, the precursor of **3** and **4**, was synthesized as shown in Scheme I.[23,30] The *tert*-butyl benzoate **5**, which contains appropriate functionality for elaboration of the diazepine ring and a carboxyl for subsequent attachment of the (amidino)phenyl-amido side chain, was synthesized as described.[31,32] The phenylethylamino group was introduced by treating **5** with phenethylamine to give **6**. Protection of the benzylic



Ac-Arg-Gly-Asp-Ser-NH_2 (1)

SK&F 107260 (2)

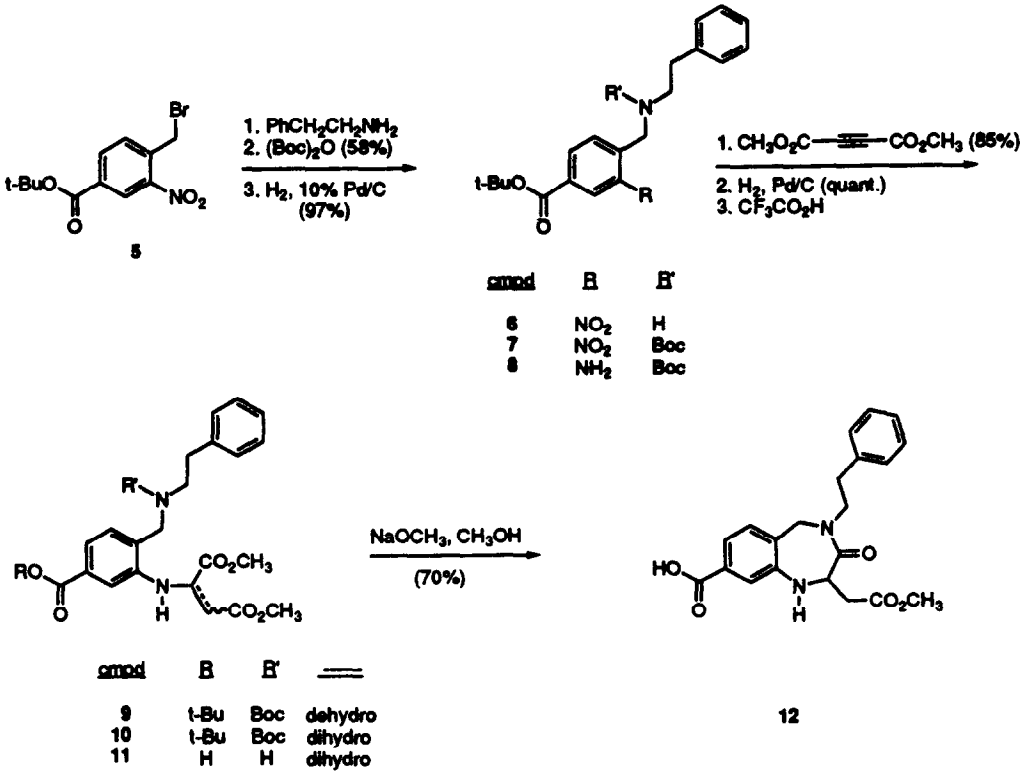
SB 207448 R = H (3)
SB 208651 R = CH_3 (4)

Figure 1. Key GPIIb/IIIa antagonists leading to **3** and **4**.

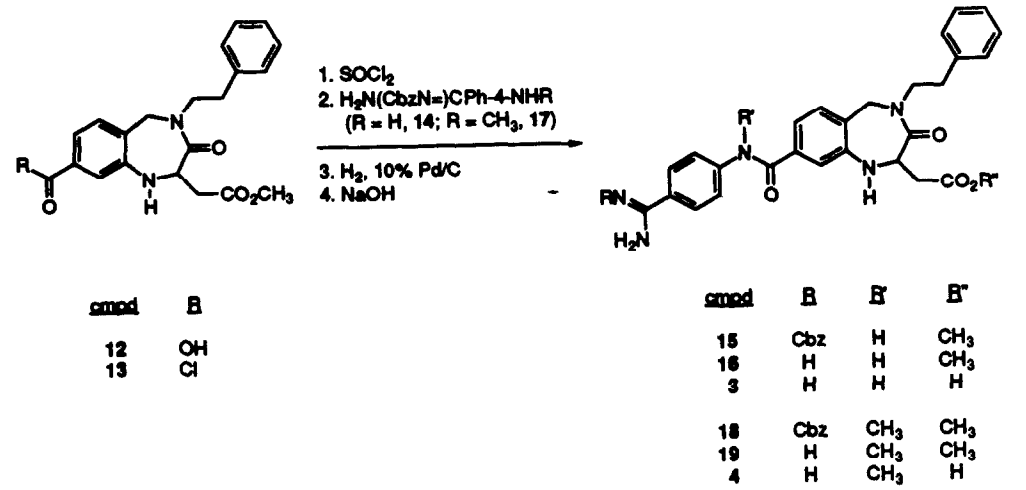
amine as the Boc derivative gave **7**, and catalytic hydrogenation of the nitro group afforded the aniline **8**. The four carbons needed to complete construction of the diazepine ring were introduced by Michael-type addition of **8** to dimethyl acetylenedicarboxylate[33,34] to afford the dehydro-Asp analog **9**. The double bond in **9** was reduced catalytically to give **10**, and the *N*-Boc and *O*-*tert*-butyl groups were removed with trifluoroacetic acid to give **11**. Finally, the diazepine ring was closed with sodium methoxide in methanol to afford the desired **12**. This synthesis has been used to provide **12** in an overall yield of 37 % from commercially available 4-(bromomethyl)-benzoic acid, the precursor of **5**.

Compounds **3** and **4** were synthesized from **12** by condensation of the acid chloride **13** with 4-(Cbz-amidino)aniline (**14**) and 4-(Cbz-amidino)-*N*-methylaniline (**17**), respectively (Scheme II). The resulting Cbz-methyl esters, **15** and **18**, were deprotected to afford the methyl esters, **16** and **19**, which were saponified to give **3** and **4**. The 4-(Cbz-amidino)anilines, **14** and **17**, were prepared by reaction of 4-(amidino)aniline and 4-(amidino)-*N*-methylaniline, respectively, with benzyl chloroformate. 4-Amidino-*N*-methylaniline was prepared from 4-cyano-*N*-methylaniline using the general method of Garigipati.[35]

The (*R*)- and (*S*)-enantiomers of **3** and **4** were synthesized from (*R*)- and (*S*)-**12** by the method of Scheme III. (*R*)- and (*S*)-**12** were obtained by chiral HPLC resolution of the racemic *tert*-butyl ester, **20**, prepared from **12** with dimethylformamide di-*tert*-butyl acetal. Removal of the



Scheme I.



Scheme II.

tert-butyl groups from the resulting (*R*)- and (*S*)-**20** with hydrogen chloride gave the desired starting materials. The optical purity of the (*R*)- and (*S*)-**20** was determined to be > 99 % by chiral HPLC. The absolute stereochemistry of (*R*)-**12** was established by first converting it to the bromoanilide **21**. X-Ray crystal structure analysis confirmed the structure of **21** and allowed assignment of the absolute stereochemistry at C(2) as *R* (Figure 2). Following the conversion of (*R*)- and (*S*)-**20** to (*R*)- and (*S*)-**3**, the optical purity of the products was also shown by chiral HPLC to be > 99 %, i.e. the transformation of (*R*)- and (*S*)-**20** to (*R*)- and (*S*)-**3** proceeded without racemization. By analogy, it is assumed that the conversion of (*R*)- and (*S*)-**20** to (*R*)- and (*S*)-**4** also proceeded without racemization.

Biological assays

The *N*-methyl amide **4** was evaluated for binding affinity to human GPIIb/IIIa versus biotinylated-Fg[36] and ^{3}H-SK&F 107260,[15,37,38] and for inhibition of human platelet aggregation induced by ADP in platelet rich plasma; the results were compared with previously reported values for **3**. The binding affinity of the (*R*)- and (*S*)-enantiomers of **3** and **4** for GPIIb/IIIa, and for human $\alpha_v\beta_3$ (vitronectin receptor)[39] was also evaluated. The *in vivo* activity of **3** and **4** was assessed by intravenous and intraduodenal administration in the conscious dog; antiaggregatory activity was measured using an *ex vivo* assay for inhibition of platelet aggregation induced by collagen in whole blood. The ability of **3** and **4** to transit rabbit ileum was measured *in vitro* in side-by-side diffusion chambers and flux was monitored by HPLC.[40]

Results and Discussion

In vitro *activity*

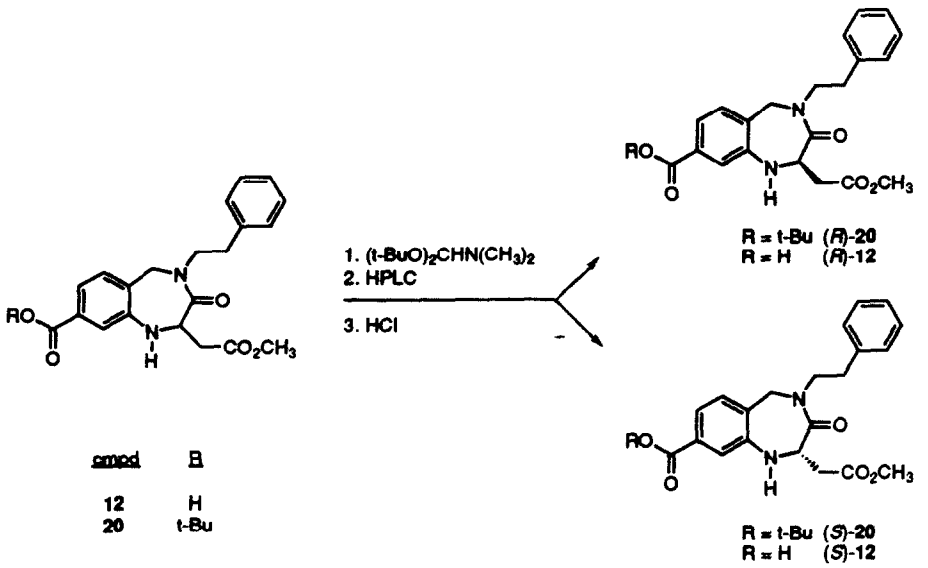
Evaluation of **4** for binding to GPIIb/IIIa versus biotinylated-Fg and ^{3}H-SK & F 107260 shows that it exhibits very high affinity with K_i values comparable to those obtained for **2** and **3** (Table 1). Binding competition studies with the (*R*)- and (*S*)-enantiomers of **3** and **4** show that affinity for GPIIb/IIIa resides in the (*S*)-enantiomers. Thus, the stereochemical requirement for activity in the Asp-mimetic region of these nonpeptide antagonists is the same as that observed in the Asp residue in the cyclic peptide series.[12] The *N*-methyl amide **4** also showed high antiaggregatory potency in human platelet rich plasma where it was comparable to **2** and slightly more potent than **3**. Therefore, the secondary amide **3** and the tertiary *N*-methyl amide **4** show similar *in vitro* activities that are comparable to the peptide antagonist **2**.

The peptide antagonist, **2**, showed high affinity for the human vitronectin receptor ($\alpha_v\beta_3$), an integrin that shares the same β_3 subunit present in GPIIb/IIIa.[41] In contrast to **2**, **3** and **4** displayed 10^5-fold lower affinity for $\alpha_v\beta_3$ than for GPIIb/IIIa. Similar to the observation obtained for GPIIb/IIIa, the (*S*)-enantiomers of **3** and **4** were the active forms in binding to $\alpha_v\beta_3$.

The results show that the *N*-methyl group in **4** has little effect on *in vitro* activity compared to **3**. The simplest explanation for this is that **3** and **4** bind to GPIIb/IIIa in a similar orientation. Although the *N*-methyl amide in **4** can exist in the *cis* or *trans* geometry,[42,43] secondary amides, as in **3**, highly favor the *trans* geometry. Thus we favor the *trans*-amide conformers of **3** and **4** as the primary contributors to the interaction with GPIIb/IIIa.

In vivo *activity*

The *in vivo* activity of **3** was first assessed by intravenous infusion (0.3 mg/kg) for 30 min in the conscious dog. The infusion resulted in a complete block of *ex vivo* platelet aggregation followed by a return to control reaching 50 % inhibition at 30 min after completion of the infusion, and returning to baseline at 90 min after completion of the infusion. The oral activity of **3** was assessed using a 10-fold higher dose (3 mg/kg) administered intraduodenaly as a bolus. In contrast to the anti-



Scheme III.

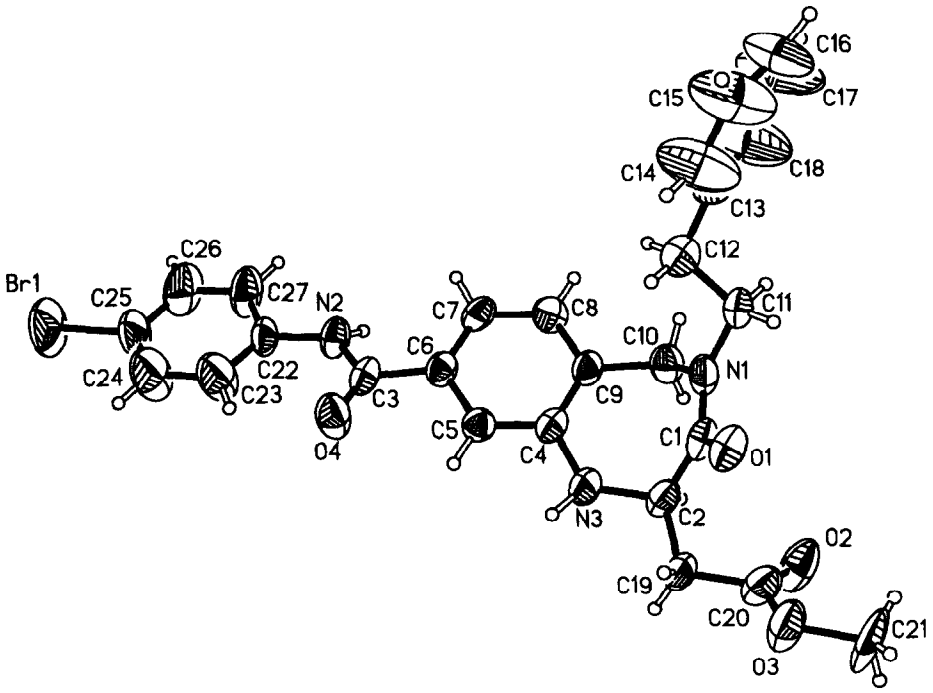


Figure 2. A view of the structure of **21** as determined by single crystal X-ray diffraction. Nonhydrogen atoms are drawn as principal ellipses at the 50 % probability level; hydrogen atoms as spheres of arbitrary size.

Table 1. Antiaggregatory activity and GPIIb/IIIa and $\alpha_v\beta_3$ binding inhibition

		binding inhibition		
		human GPIIb/IIIa		human $\alpha_v\beta_3$
cmpd	antiaggregatory potency[a] human PRP/ADP IC_{50} (nM)	Biotinyl-Fg[b] K_i (nM)	^{3}H-SK&F 107260[c] K_i (nM)	^{3}H-SK&F 107260[d] % inhib at 100 μM or K_i (nM)
3	150 ± 40	1.5 ± 0.2	2.8 ± 0.12	17 ± 2
(*R*)-**3**			500 ± 40	nse[e]
(*S*)-**3**			1.8 ± 0.1	23 ± 1
4	65 ± 3	1.4 ± 0.3	1.6 ± 0.20	28 ± 1
(*R*)-**4**			120 ± 10.5	nse[e]
(*S*)-**4**			1.2 ± 0.08	44 ± 3
2	57 ± 11	0.62 ± 0.4	2.08 ± 0.10	2.86 ± 1.1[f]

[a]Inhibition of platelet aggregation in human platelet rich plasma induced by ADP.
[b]Inhibition of biotinylated-Fg binding to GPIIb/IIIa purified from human platelets, immobilized on microtiter plates (ELISA).
[c]Inhibition of ^{3}H-SK&F 107260 binding to GPIIb/IIIa purified from human platelets, reconstituted in liposomes.
[d]Inhibition of ^{3}H-SK&F 107260 binding to $\alpha_v\beta_3$ purified from human platelets, immobilized on microtiter plates.
[e]No significant effect at 100 μM.
[f]K_i.

aggregatory activity seen following intravenous infusion, intraduodenal administration did not result in detectable inhibition of *ex vivo* platelet aggregation over a period of 5 h, Figure 3.

Intravenous infusion of **4**, using the same dose and protocol employed for **3**, resulted in an inhibition of *ex vivo* platelet aggregation that was similar in degree and duration. In contrast, intraduodenal administration of **4** (3 mg/kg) as a bolus resulted in the rapid onset of inhibition of *ex vivo* platelet aggregation that reached ~80 % after 60 min. Subsequently, inhibition returned to 50 % of control after 90 min and to baseline approximately 4 h after administration of the bolus dose, Figure 4. Comparison of the intravenous and intraduodenal activity curves shows that **4** had an apparent oral bioavailability of approximately 10 % in the conscious dog as determined by *ex vivo* pharmacological activity.

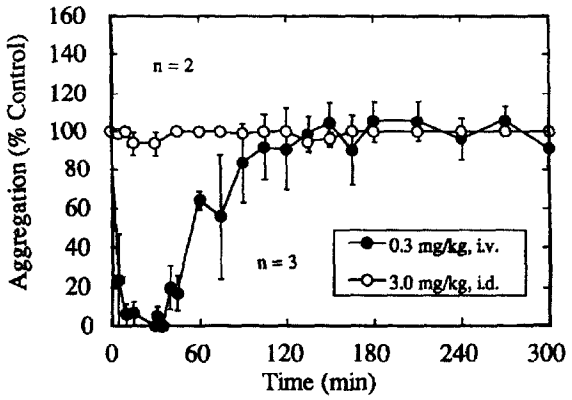


Figure 3. *In vivo* activity of SB 207448.

Two observations suggest that the increase in bioavailability of **4** versus **3** is primarily due to an increase in intestinal permeability rather than to differences in clearance. First, the ability of **3** and **4** to transit rabbit ileum was measured *in vitro* (Table 2). The results show that the ileal permeability of **4** was higher than that of **3**, which was not measurable under the conditions used. The intestinal permeabilities for mannitol and diazepam are shown for comparison purposes; these compounds have moderate and high bioavailabilities in man, respectively.[44,45] Secondly, the *ex vivo* inhibition curves for **3** and **4** following intravenous infusion are similar, which suggests that their rates of clearance are similar.

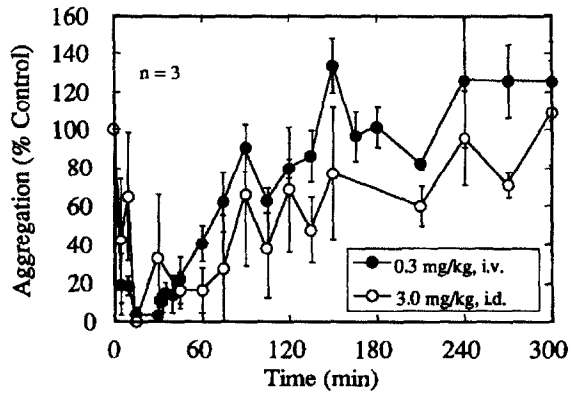


Figure 4. *In vivo* activity of SB 208651.

Table 2. *In vitro* transport across rabbit ileum

compound	ileal permeability (cm/h)
3	<0.0001
4	0.0120 ± 0.0018
mannitol	0.0192 ± 0.0013
diazepam	0.133 ± 0.012

In conclusion, the observation of oral activity for **4**, a close analog of **3**, extends our previously reported work towards the discovery of a potent and orally active nonpeptide GPIIb/IIIa antagonist.[46] The effect of the structural modification of **4** on *in vivo* potency, duration and bioavailability is being examined.

Experimental Section

General

Melting points were measured with a Thomas-Hoover melting point apparatus. ^{1}H NMR Spectra were recorded with a Bruker AM-250, Bruker AC-400, Bruker AMX400 or Bruker AMX500 spectrometer and shifts are reported as ppm downfield from Me_4Si with multiplicity, number of protons and coupling constant(s) in Hertz indicated parenthetically. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Mass spectra were taken on either VG 70 FE, PE Syx API III or VG ZAB HF instruments. TLC were taken on Analtech silica gel GF plates or E. Merck silica gel 60-F-254 plates. Chromatography refers to flash chromatography using E. Merck Kieselgel 60, 230–400 mesh silica gel. Analytical HPLC was carried out on Rainin or Beckman Chromatographs. Semi-preparative HPLC was carried out on a Rainin Chromatograph using a 10 μ (50 × 250 mm) YMC-ODS AQ column.

Methyl (R,S)-8-carboxy-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (12)

tert-*Butyl 4-[N-(tert-butoxycarbonyl)-N-(2-phenylethyl)-aminomethyl]-3-nitrobenzoate (7).* A solution of *tert*-butyl 4-bromomethyl-3-nitrobenzoate (**5**)[31,32] (1.6 g, 5 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 15 min to a solution of phenethylamine (1.89 g, 15 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at rt under argon for 24 h and concentrated *in vacuo* to give **6**, which was dissolved in THF (50 mL) and treated with a solution of Et_3N (2.5 g, 25 mmol) and di-*tert*-butyl dicarbonate (4.4 g, 20 mmol) in THF (50 mL). The resulting mixture was stirred overnight at rt under argon and concentrated *in vacuo*. The residue was dissolved in EtOAc, washed with H_2O and dried (Na_2SO_4). The organic phase was concentrated *in vacuo* and the residue was triturated with EtOAc:hexane (15:85) to give **7** (0.87 g, 37 %): mp 110–113 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 8.56 (s, 1H), 8.25 (d, 1H, J = 7.2), 7.45 (d, 1H, J = 7.2), 7.1–7.45 (m, 5H), 4.7 (s, 1H), 4.68 (s, 1H), 3.35–3.58 (m, 2H), 2.78–2.96 (m, 2H), 1.60 (s, 9H), 1.49 (s, 6H), 1.35 (s, 3H). The filtrate was concentrated *in vacuo* and the residue was chromatographed (silica gel, 15:85 EtOAc:hexane) to yield additional **7** (0.5 g, 21 %): mp 113–115 °C. A sample was recrystallized from hexane: mp 117.5–118.5 °C. Anal. calcd for $C_{25}H_{32}N_2O_6$: C, 65.77; H, 7.07; N, 6.14; found C, 65.87; H, 6.97; N, 6.11.

tert-*Butyl 3-amino-4-[N-(tert-butoxycarbonyl)-N-(2-phenylethyl)aminomethyl]benzoate (8).* A mixture of **7** (1.3 g, 2.8 mmol) and 10 % Pd/C (0.32 g) in EtOH (125 mL) was shaken under a hydrogen atmosphere (40 psi) for 50 min. The mixture was filtered and the filtrate was concentrated *in vacuo* to give **8** (1.15 g, 97 %): mp 105–106 °C; ^{1}H NMR (250 MHz, $CDCl_3$): 7.1–7.35 (m, 7H), 7.02 (d, 1H, J = 7.2), 4.3 (s, 2H), 3.29 (t, 2H, J = 7.9), 2.65 (t, 2H, J = 7.9), 1.59 (s, 9H), 1.45 (s, 9H). A sample was crystallized from hexane: mp 106.5–109 °C. Anal. calcd

for $C_{25}H_{34}N_2O_4$: C, 70.40; H, 8.03; N, 6.57; found C, 70.57; H, 7.92; N, 6.53.

tert-Butyl (E/Z)-4-[N-(tert-butoxycarbonyl)-N-(2-phenylethyl)aminomethyl]-3-[(1,4-dimethoxy-1,4-dioxo-2-buten-2-yl)amino]benzoate (9). A solution of **8** (1.15 g, 2.7 mmol) in CH_3OH (50 mL) was treated with dimethyl acetylenedicarboxylate (0.45 g, 3.2 mmol), and the resulting solution was heated to reflux under argon for 1 h. The mixture was concentrated *in vacuo* and the residue was chromatographed (silica gel, EtOAc:hexane 20:80) to give **9** (1.3 g, 85 %): ^1H NMR (250 MHz, $CDCl_3$): 9.45 (s, 1H), 7.7 (d, 1H, J = 7.3), 7.35 (s, 1H), 7.08–7.35 (m, 6H), 5.55 (s, 1H), 4.55 (s, 2H), 3.75 (s, 3H), 3.65 (s, 3H), 3.35 (m, 2H), 2.71–2.88 (m s, 2H), 1.56 (s, 9H), 1.48 (s, 9H).

tert-Butyl (R,S)-4-[N-(tert-butoxycarbonyl)-N-(2-phenylethyl)aminomethyl]-3-[(1,4-dimethoxy-1,4-dioxobut-2-yl)amino]benzoate (10). A solution of **9** (1.3 g, 2.3 mmol) in CH_3OH (100 mL) containing 10 % Pd/C (0.38 g) was shaken in a hydrogen atmosphere (40 psi) for 4.5 h. The mixture was filtered and the filtrate was concentrated *in vacuo* to yield **10** (1.3 g, ~100 %): ^1H NMR (250 MHz, $CDCl_3$): 7.16–7.35 (m, 5H), 7.09 (d, 2H, J = 6.5), 7.05 (d, 1H, J = 6.5), 5.86 (br s, 1H), 4.65 (q, 1H, J = 5.3), 4.41 (d, 1H, J = 13.7), 4.28 (d, 1H, J = 13.7), 3.69 (s, 6H), 3.35 (t, 2H, J = 9.8), 2.85 (m, 2H), 2.65 (m, 2H), 1.56 (s, 9H), 1.45 (s, 9H).

Methyl (R,S)-8-carboxy-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (12). A solution of **10** (1.3 g, 3 mmol) in CH_2Cl_2 (50 mL) and TFA (50 mL) was kept at rt overnight under argon. The mixture was concentrated *in vacuo* to give a residue containing crude (*R,S*)-3-[(1,4-dimethoxy-1,4-dioxobut-2-yl)amino]-4-[*N*-(2-phenylethyl)aminomethyl]benzoic acid trifluoroacetate, **11**. The residue was dissolved in anhydrous CH_3OH (70 mL), treated with methanolic $NaOCH_3$ (1.6 mL, 7 mmol), and heated to reflux for 8 h. The mixture was then stirred at rt for 14 h, treated with 1 N HCl in Et_2O (7.5 mL), concentrated *in vacuo*, treated with CH_2Cl_2 (3 × 20 mL), and concentrated *in vacuo*. The residue was dissolved in CH_3OH:CH_2Cl_2:AcOH (10:90:0.4, 15 mL), filtered and chromatographed (silica gel, CH_3OH:CH_2Cl_2:AcOH 10:90:0.4) to give **12** (0.66 g, 70 %): mp 90–91 °C. ^1H NMR (400 MHz, DMSO-d_6): 7.1–7.35 (m, 6H), 7.05 (s, 2H), 6.14 (d, 1H, J = 5.3), 5.44 (d, 1H, J = 15.7), 5.05 (m, 2H), 4.04 (d, 1H, J = 15.7), 3.65 (s, 3H), 3.62 (m, 2H), 2.84 (dd, 1H, J = 15.8, 9.4), 2.6–2.75 (m, 3H). Anal. calcd for $C_{21}H_{22}N_2O_5 \cdot 0.25H_2O$: C, 65.19; H, 5.86; N, 7.24; found: C, 65.22; H, 5.90; N, 6.97.

4-[N-(Benzyloxycarbonyl)aminoiminomethyl]aniline (14). Aqueous 5 N NaOH (28.8 mL, 144 mmol) was added to a suspension of 4-aminobenzamidine dihydrochloride (Aldrich) (10.0 g, 48 mmol) in a mixture of THF (250 mL) and H_2O (50 mL) at 0 °C. A solution of benzyl chloroformate (8.2 g, 48 mmol) in THF (15 mL) was added rapidly dropwise, and the resulting mixture was stirred vigorously for 1 h at 0 °C. The aqueous layer was separated and the organic layer was concentrated *in vacuo* to ~20 mL. The resulting suspension was partitioned between EtOAc (500 mL) and H_2O (125 mL). The organic layer was dried (Na_2SO_4), concentrated *in vacuo*, and the residue was recrystallized from $CHCl_3$ to give **14** (6.0 g, 48 %) as a yellow solid: mp 147–148 °C. ^1H NMR (400 MHz, DMSO-d_6): 9.19 (br s, 1H), 8.65 (br s, 1H), 7.78 (d, 2H, J = 10.5), 7.35 (m, 5H), 6.55 (d, 2H, J = 10.5), 5.82 (s, 2H), 5.05 (s, 2H); MS(ES) m/z 270.2 $[M + H]^+$. Anal. calcd for $C_{15}H_{15}N_3O_2 \cdot 0.25H_2O$: C, 65.80; H, 5.71; N, 15.35; found: C, 65.65; H, 5.58; N, 15.59.

(R,S)-8-[[[4-(Aminoiminomethyl)phenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetic acid dihydrochloride (3)

Methyl (R,S)-8-chlorocarbonyl-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate hydrochloride (13). A mixture of **12** (0.2 g, 0.5 mmol) and thionyl chloride (6 mL) was heated to reflux under argon for 15 min. The mixture was concentrated *in vacuo* and the residue was reconcentrated from CH_2Cl_2 (3 × 20 mL), to give **13** as a yellow solid.

Methyl (R,S)-8-[[4-[N-(benzyloxycarbonyl)-(aminoiminomethyl)phenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (15). To a solution of **14** (0.13 g, 0.5 mmol) and diisopropylethylamine (62 mg, 0.5 mmol) in CH_2Cl_2 (25 mL) was added a solution of **13** (0.22 g, 0.5 mmol) in CH_2Cl_2 (5 mL). The mixture was kept at rt for 20 h, treated with diisopropylethylamine (0.15 g), and washed with H_2O. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by preparative TLC (silica gel, CH_3OH:CH_2Cl_2 5:95) to give **15** (0.14 g, 46 %). ^1H NMR (400 MHz, CD_3OD): 7.89 (d, 2H, J = 10.5), 7.82 (d, 2H, 10.5), 7.43 (d, 2H, 9.4), 7.05–7.39 (m, 10H), 7.01 (d, 1H, J = 10.3), 5.48 (d, 1H, J = 15.7), 5.12 (dd, 1H, J = 10.5, 5.3), 3.91 (d, 1H, J = 15.7), 3.76 (m, 1H), 3.74 (s, 3H), 3.66 (m, 1H), 2.95 (dd, 1H, J = 15.7, 10.5), 2.77 (m, 2H), 2.69 (dd, 1H, J = 15.7, 5.3); MS(ES) m/z 634 $[M + H]^+$. Anal. calcd for $C_{36}H_{35}N_5O_6 \cdot HCl \cdot 2.5H_2O$: C, 60.45; H, 5.78; N, 9.79; found C, 60.51; H, 5.58; N, 9.84.

Methyl (R,S)-8-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate hydrochloride (16). A solution of **15** (0.13 g, 0.2 mmol) and 10 % Pd/C (0.1 g) in CH_3OH (50 mL) and 1 N HCl in Et_2O (1.0 mL) was shaken under a hydrogen atmosphere (30 psi) at rt for 30 min. The mixture was filtered and the filtrate was concentrated *in vacuo* to give **16**. ^1H NMR (CD_3OD, 400 MHz): 8.01 (d, 2H, J = 9.5), 7.82 (d, 2H, J = 9.5), 7.04–7.25 (m, 8H), 5.46 (d, 1H, J = 15.7), 5.15 (m, 1H), 3.94 (d, 1H, J = 15.7), 3.75 (m, 1H), 3.71 (s, 3H), 3.65 (m, 1H), 2.95 (dd, 1H, J = 15.7, 10.5), 2.75 (t, 2H, J = 6.3), 2.65 (dd, 1H, J = 15.7, 5.3); MS(ES) m/z 500.2 $[M + H]^+$.

(R,S)-8-[[[4-(Aminoiminomethyl)phenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate dihydrochloride (3). A solution of **16** (0.1 g, 0.175 mmol) in a mixture of CH_3OH (20 mL), H_2O (2 mL) and 1 N NaOH (1 mL) was stirred at rt for 19 h. The mixture was acidified to pH 1 with 3 N aqueous HCl, concentrated *in vacuo*, and purified by HPLC: R_t 21.19 min (YMC ODS-AQ®, 50 × 250 mm, 1.5 mL/min, 33 % CH_3CN:H_2O:0.1 % TFA, UV detection at 220 nm). Fractions containing product were pooled and lyophilized, redissolved in H_2O (70 mL), 6 N aqueous HCl (2 mL) and CH_3CN (5 mL) and lyophilized to give **3** (0.37 g, 40 %). ^1H NMR (400 MHz, CD_3OD): 9.20 (s, 2H), 8.75 (s, 2H), 8.01 (d, 2H, J = 10.5), 7.81 (d, 2H, J = 10.5), 7.06–7.35 (m, 7H), 7.05 (d, 1H, J = 6.3), 5.51 (d, 1H, J = 15.7), 5.12 (dd, 1H, J = 10.5, 5.3), 3.95 (d, 1H, J = 15.7), 3.78 (m, 1H), 3.65 (m, 1H), 2.95 (dd, 1H, J = 15.7, 10.5), 2.75 (m, 2H), 2.65 (dd, 1H, J = 15.7, 5.3); MS(ES) m/z 486 $[M + H]^+$. Anal. calcd for $C_{27}H_{27}N_5O_4 \cdot 2.0HCl \cdot 0.4H_2O$: C, 57.33; H, 5.31; N, 12.30; found C, 57.71; H, 5.31; N, 11.90.

4-[N-(Benzyloxycarbonyl)aminoiminomethyl]-N-methylaniline (17)

4-(Aminoiminomethyl)-N-methylaniline. A solution of $Al(CH_3)_3$ in toluene (2.0 M, 51 mL, 0.1 mol) was added over 4 min to a suspension of powdered NH_4Cl (5.46 g, 0.102 mol) in dry toluene (51 mL) in a flame-dried flask at 0 °C under argon. The ice bath was removed and the reaction was allowed to stir at rt until gas evolution ceased (1 h). 4-Cyano-*N*-methylaniline (4.49 g, 34 mmol) was added and the reaction was warmed to 80 °C. Gas evolution occurred on warming. After 23 h at 80 °C, the reaction was cooled to rt and poured into a stirred slurry of silica gel (170 g) in $CHCl_3$ (500 mL), causing a significantly exothermic reaction. The resulting mixture was stirred for 0.5 h, filtered, and the filter pad was washed with CH_3OH (1 L). The filtrate was concentrated to a yellow solid which was dried under high vacuum at 50–60 °C for 0.5 h to give the title compound which was used without further purification.

4- [N- (Benzyloxycarbonyl) aminoiminomethyl] -N-methylaniline (17). 4-(Aminoiminomethyl)-*N*-methylaniline and benzyl chloroformate were reacted using the procedure described for **14** and the product was recrystallized from EtOAc:hexane to give **17** (6.5 g, 67 %): mp 141–143 °C. TLC R_f 0.49 (silica gel, 3:2 EtOAc:hexane); IR ($CHCl_3$) 3500, 3450, 3310, 1648, 1608, 1575, 1493, 1263, 1141 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.77 (d, 2H, J = 11.5), 7.45 (m, 2H), 7.27–7.38 (m, 3H), 6.56 (d, 2H, J = 11.5), 5.20 (s, 2H), 4.28 (br s, 1H), 2.88 (s, 3H); MS(ES) m/z 284.2 $[M + H]^+$.

(R,S)-8-[[[4-(Aminoiminomethyl) phenyl] methylamino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetic acid (4)

Compounds **12** and **17** were reacted using the general procedure described for **3** to give the following compounds:

Methyl (R,S)-8-[[[4-[N-(benzyloxycarbonyl)-aminoiminomethyl]phenyl]methylamino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (18). (441.6 mg, 68 %). TLC R_f 0.38 (silica gel, 9:1 EtOAc:toluene); IR ($CHCl_3$) 3160–3540, 3490, 3300, 1733, 1655, 1616, 1577, 1500, 1443, 1380, 1271, 1149, 1110 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.71 (d, 2H, J = 8.5), 7.02–7.46 (m, 12H), 6.62 (d, 1H, J = 1.4), 6.56 (d, 1H, J = 7.8), 6.38 (dd, 1H, J = 7.8, 1.4), 5.19 (s, 2H), 5.18 (d, 1H, J = 16.8), 4.85–4.93 (m, 1H), 4.17 (d, 1H, J = 5.3), 3.71 (s, 3H), 3.57-3.70 (m, 2H), 3.53 (d, 1H, J = 16.8), 3.44 (s, 3H), 2.90 (dd, 1H, J = 16.1, 6.9), 2.63–2.79 (m, 2H), 2.58 (dd, 1H, J = 16.1, 6.3); MS(ES) m/z 648.4 $[M + H]^+$.

Methyl (R,S)-8-[[[4-(aminoiminomethyl)phenyl]methylamino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate hydrochloride (19). ^1H NMR (400 MHz, CD_3OD): 9.23 (s, 2H), 8.73 (s, 2H), 7.69 (d, 2H, J = 8.7), 7.38 (d, 2H, J = 8.7), 7.08–7.25 (m, 5H), 6.76 (d, 1H, J = 7.8), 6.74 (s, 1H), 6.48 (d, 1H, J = 7.8), 5.31 (d, 1H, J = 16.5), 4.95 (m, 1H), 3.87 (d, 1H, J = 16.5), 3.69 (s, 3H), 3.62 (m, 2H), 3.48 (s, 3H), 2.91 (dd, 1H, J = 16.7, 8.7), 2.62–2.75 (m, 3H).

(R,S)-8-[[[4-(Aminoiminomethyl)phenyl]methylamino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetic acid trifluoroacetate (4). (403.4 mg, 68 %). HPLC k' 9.1 (PRP-1® column; 25 % CH_3CN:H_2O: 0.1 % TFA); ^1H NMR (400 MHz, CD_3OD): 7.67 (m, 2H), 7.37 (m, 2H), 7.06–7.25 (m, 5H), 6.74 (d, 1H, J = 7.8), 6.64 (d, 1H, J = 1.6), 6.40 (dd, 1H, J = 7.8, 1.6), 5.33 (d, 1H, J = 16.9), 5.01 (dd, 1H, J = 9.0, 5.1), 3.77 (d, 1H, J = 16.9), 3.53–3.71 (m, 2H), 3.46 (s, 3H), 2.88 (dd, 1H, J = 16.7, 9.0), 2.60–2.75 (m, 2H), 2.58 (dd, 1H, J = 16.7, 5.1); MS(ES) m/z 500.2 $[M + H]^+$. Anal. calcd for $C_{28}H_{29}N_5O_4 \cdot 1.5TFA \cdot H_2O$: C, 54.07; H, 4.76; N, 10.17; found: C, 53.73; H, 4.94; N, 9.84.

Methyl (R)- and (S)-8-carboxy-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate: (R)- and (S)-12

Methyl (R,S)-8-tert-butoxycarbonyl-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (20). Dimethylformamide di-*tert*-butyl acetal (24.4 g, 0.12 mol) was added dropwise over 20 min to a vigorously stirred suspension of **12** (11.4 g, 0.03 mol) in dry toluene (45 mL) at 80 °C under argon. The resulting solution was stirred at 80 °C for 30 min, cooled to rt, and washed with H_2O (45 mL). The organic layer was diluted with toluene (150 mL), washed with saturated $NaHCO_3$ (45 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give **20** (6.2 g, 47 %) as a pale yellow solid: mp 166–167 °C. ^1H NMR (400 MHz, $CDCl_3$): 7.2 (m, 7H), 6.85 (d, 1H, J = 9.4), 5.30 (d, 1H, J = 16.7), 4.98 (q, 1H, J = 5.3), 3.75 (s, 3H), 3.72 (m, 3H), 3.00 (dd, 1H, J = 10.5, 5.3), 2.81 (m, 1H), 2.65 (dd, 1H, J = 10.5, 5.3), 1.57 (s, 9H); MS(ES) m/z 439.2 $[M + H]^+$. Anal. calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39; found C, 68.73; H, 6.93; N, 6.34.

Methyl (R)- and (S)-8-tert-butoxycarbonyl-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (R)- and (S)-20. Compound **20** was resolved by preparative HPLC (Chiralpak AS®, 21.2 × 250 mm, 75:25 hexane:ethanol, 10 mL/min, UV detection at 280 nm) to give:

(*S*)-**20**: HPLC R_t 7.8 min, *e.e.* > 99 % (Chiralpak AS®, 4.6 × 250 mm, 70:30 hexane:ethanol, 1.0 mL/min, UV detection at 210 nm); $[\alpha]_D$ −78.5° (*c* 1.0, EtOAc).

(*R*)-**20**: HPLC R_t 10.2 min, *e.e.* > 99 % (Chiralpak AS®, 4.6 × 250 mm, 70:30 hexane:ethanol, 1.0 mL/min, UV detection at 210 nm); $[\alpha]_D$ +78.1° (*c* 1.0, EtOAc).

Methyl (R)- and (S)-8-carboxy-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (R)- and (S)-12. (*R*)- or (*S*)-**20** (250 mg, 0.57 mmol) was dissolved in 4 M HCl in dioxane (10.0 mL) at rt. After 16 h, the mixture was concentrated *in vacuo* and the residue was triturated in Et_2O (25 mL) to give the title compounds. ^1H NMR spectra were identical to **12**; HPLC R_t 15.6 min (YMC ODS AQ®, 6 × 250 mm, 50 % CH_3CN:H_2O:0.1 % TFA, 1.5 mL/min, UV detection at 220 nm).

Compounds (R)- and (S)-3 and 4

The title compounds were synthesized from (*R*)- and (*S*)-**12** using the general procedure described for **3**. NMR and mass spectra were identical:

(*R*)-**3**: $[\alpha]_D$ +40.7° (*c* 1.0, CH_3OH); HPLC R_t 14.95 min, *e.e.* > 99.9 % (CHIRALCEL OD-R®, 4.6 × 250 mm, 37 % CH_3CN:H_2O:0.1 % TFA, 0.7 mL/min, UV detection at 220 nm). Anal. calcd for $C_{27}H_{27}N_5O_4 \cdot 1.2TFA \cdot 1.0H_2O$: C, 55.14; H, 4.75; N, 10.94; found C, 55.01; H, 4.67; N, 10.82.

(*S*)-**3**: $[\alpha]_D$ −40.7° (*c* 1.0, CH_3OH); HPLC R_t 10.87 min, *e.e.* > 99.9 % (CHIRALCEL OD-R®, 4.6 × 250 mm, 37 % CH_3CN:H_2O:0.1 % TFA, 0.7 mL/min, UV detection at 220 nm). Anal. calcd for $C_{27}H_{27}N_5O_4 \cdot 1.4TFA \cdot 1.0H_2O$: C, 53.97; H, 4.62; N, 10.56; found C, 53.77; H, 4.59; N, 10.33.

(*R*)-**4**: $[\alpha]_D$ +59.6° (*c* 1.0, CH_3OH). Anal. calcd for $C_{28}H_{29}N_5O_4 \cdot 1.1TFA \cdot 1.0H_2O$: C, 56.41; H, 5.03; N, 10.89; found C, 56.61; H, 4.9; N, 10.88.

(*S*)-**4**: $[\alpha]_D$ −59.4° (*c* 1.0, CH_3OH). Anal. calcd for $C_{28}H_{29}N_5O_4 \cdot 1.2TFA \cdot 1.0H_2O$: C, 55.80; H, 4.96; N, 10.70; found C, 55.79; H, 4.74; N, 10.59.

Methyl (R)-8-[[[4-bromophenyl]amino]carbonyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1H-1,4-benzodiazepine-2-acetate (21)

Compound (*R*)-**12** was reacted with thionyl chloride, and the acid chloride was treated with 4-bromoaniline as described for the preparation of **3**. The product was purified by HPLC R_t 25.21 min, (YMC ODS-AQ®, 50 × 250 mm, 10 μm, 60 % CH_3CN:H_2O:0.1 % TFA, 90 mL/min, UV detection at 220 nm) to give **21** (0.20 g, 67 %). $[\alpha]_D$ +50.8° (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): 9.02 (s, 1H), 7.62 (d, 2H, *J* = 10.5), 7.45 (d, 2H, *J* = 10.5), 7.15 (m, 3H), 7.04 (s, 2H), 6.95 (d, 2H, *J* = 6.3), 6.75 (d, 1H, *J* = 6.3), 5.75 (d, 1H, *J* = 15.7), 4.95 (t, 1H, *J* = 5.3), 3.71 (s, 3H), 3.54 (m, 3H), 2.95 (dd, 1H, *J* = 16.7, 8.4), 2.65 (dd, 1H, *J* = 16.7, 5.3), 2.55 (m, 2H); MS(ES) m/z 536.2 $[M + H]^+$.

X-Ray procedures and data

Crystal data for **21**: ambient temperature data (295 K) from a crystal (0.02 × 0.13 × 0.14 mm) grown from an ethanol/isopropanol mixture were collected on an Enraf Nonius CAD4 diffractometer equipped with graphite monochromated copper radiation (lka = 1.54178 Å). The space group is $P2_1$ with *a* = 10.596(2) Å, *b* = 9.586(2) Å, *c* = 14.793(3) Å, *b* = 92.57(3)°, *V* = 1501.1(5) Å^3, *Z* = 2, dcalc = 1.29 g/cm^3 based on M_r = 582.49 for $[C_{29}H_{32}BrN_3O_5]$ which includes one molecule of ethanol, *m* = 2.19 cm^{-1}, *F*(000) = 604. Nearly a full sphere of data (3536) were measured on the diffractometer using variable speed *w*-2q scans, 2q max = 120°. Intensities were corrected for Lorentz and polarization effects and for an 8 % decrease in the intensity of standards measured at regular intervals. A unique set of data (3237) were obtained by averaging non-Friedel related symmetry equivalents (R_{int} = 0.053). The structure was solved with SHELXTL-PLUS and refined with SHELXL-92.[47,48] Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included as fixed contributions in the final model. The full matrix refinement (on F^2) with the weights, *w*, defined as $1/[s^2(Fo^2) + (0.1108P)^2 + 1.37P]$ where $P = [MAX(Fo^2,0) + 2Fc^2]/3$. Converged (max *D/s* = 0.05) to final crystallographic residuals of *R* = 0.069, *wR2* = 0.172, *G.O.F.* = 1.029 with 343 variables and 1957 observations [$I \geq 2s(I)$] or *R* = 0.140, *wR* = 0.217 for all data. Maximum excursions in a final difference Fourier map were within ± 0.374 e/Å^3. Neutral atom scattering factors, including values of Df' and Df" from the International Tables for X-ray Crystallography,[49] were used. The configuration assignment was based on refinement of an absolute structure parameter[50] [*x* = 0.03(5)] and on Hamilton's *R*-factor ratio test[51] (99.99 % confidence level).

Pharmacology methods

In vitro inhibition of biotinylated-fibrinogen binding to purified human platelet fibrinogen receptor was carried out as described.[36]

In vitro inhibition of ^3H-SK&F 107260 binding to purified human platelet fibrinogen receptor was carried out as described.[15,37,38]

In vitro inhibition of ^3H-SK&F 107260 binding to purified, human vitronectin ($\alpha_v\beta_3$) receptor was carried out as described.[39]

In vitro *human platelet aggregation.* Blood was drawn from the antecubital vein of normal human volunteers, who had not taken a cyclooxygenase inhibitor within the previous 14 days, into a plastic syringe containing one part 3.8 % trisodium citrate to nine parts blood. Platelet rich plasma was prepared by centrifuging the blood at 200 *g* for 10 min at rt. The platelet rich plasma was drawn off and the remaining blood was centrifuged at 2400 *g* for 5 min at rt to make platelet poor plasma. Platelet count was measured with a model ZB1 Coulter Counter (Coulter Electronics Inc., Hialeah, FL) and was adjusted to 300,000/μL using platelet poor plasma. Platelet aggregation was studied in a Chrono-Log model 400VS Lumi Aggregometer (Chrono-Log, Havertown, PA) using platelet rich plasma stirred at 1200 rpm and maintained at 37 °C, with platelet poor plasma as the 100 % transmission standard. Concentration–response curves for the ability of compounds to inhibit platelet aggregation, measured as the maximum change in light transmission, induced by a maximal concentration of adenosine diphosphate (10 μM), were constructed and the IC_{50} was determined as the concentration of antagonist required to produce 50 % inhibition of the response to the agonist.

In vivo *antiaggregatory activity.* Male mongrel dogs (8–12 kg) were surgically prepared with chronically implanted access ports in the duodenum and jejunum to allow direct delivery of drug to the small intestine without passing through the stomach. On the day of the experiment, the dogs were supported in a sling and a catheter was inserted in a cephalic vein for the withdrawal of blood samples. Venous blood samples were drawn into a plastic syringe containing one part 3.8 % trisodium citrate to nine parts blood. Samples were drawn immediately before and at various time points during and after the intravenous infusion of compounds at a dose of 0.3 mg/kg given over 30 min and before and after bolus intraduodenal administration of compounds at 3.0 mg/kg.

Ex vivo whole blood platelet aggregation was studied by impedance aggregometry in a Chrono-Log model 570VS Whole-Blood Aggregometer (Chrono-Log, Havertown, PA). Platelet aggregation was induced by collagen (5 μg/mL) in 1 mL citrated whole blood stirred at 1200 rpm and maintained at 37 °C and was measured as the initial slope of the increase in impedance produced by the platelet aggregate forming between the two impedance electrodes in Ohm/min.

Intestinal permeability studies.[40] ^{3}H-Diazepam (84.5 Ci/mmol) and ^{14}C-mannitol (49.3 mCi/mmol) were purchased from New England Nuclear Products (Boston, MA). Solutions for both mucosal and serosal solutions were prepared in pH 7.4 oxygenated (O_2:CO_2, 95:5) bicarbonate buffer as described previously.[52] The serosal solution included 8 mM D-glucose, to maintain tissue viability, and 2 mM mannitol, and the mucosal solution contained 10 mM mannitol. The concentration of mucosal solutions containing **3**, **4** and diazepam were 20, 200 and 100 μM, respectively.

Segments of ileum from male New Zealand White rabbits (3–5 kg) were stripped of the underlying musculature[52] and were mounted in diffusion cells (Precision Instrument Design, Tahoe City, CA) with 1.78 cm^2 exposed surface area. Tissue integrity was monitored by monitoring the flux of the paracellular flux marker, mannitol. The temperature of the diffusion cells was maintained at 37 °C in an aluminum block heater and the reservoirs were filled with warmed (37 °C) mucosal and serosal solutions, and circulation was maintained by a constant O_2/CO_2 gas lift.

Samples were taken from both mucosal and serosal solutions at appropriate time points. Samples from the receiver chamber were replaced with an equal volume of fresh receiver solution to maintain constant volume. Compounds **3** and **4** were detected and quantified by HPLC: **3** R_t 3.3 min (Beckman ODS®, 4.6 × 150 mm, 5 μm, 30 % CH_3CN:H_2O:0.1 % TFA, UV detection at 203 nm]; **4** R_t 5.4 min (Beckman ODS, 4.6 × 150 mm, 5 μm, 23 % CH_3CN:H_2O:0.1% TFA, UV detection at 203 nm). Radiolabeled compounds were determined in a β scintillation counter (Beckman Instruments, Fullerton, CA).

Supplementary material available—For **21**, tables of fractional atomic coordinates, anisotropic thermal parameters for non-hydrogen atoms and tables containing additional metrical details (8 pages) as well as listings of structure factors (8 pages) have been included with the deposited supplementary material. Ordering information is given on any current masthead page.

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