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Communications to the Editor

Potent, Selective, Orally Active 3-Oxo-1,4-benzodiazepine GPIIb/IIIa Integrin Antagonists

James M. Samanen,*,† Fadia E. Ali,† Linda S. Barton,† William E. Bondinell,† Joelle L. Burgess,† James F. Callahan,† Raul R. Calvo,† Wenting Chen,‡ Lichong Chen,† Karl Erhard,† Giora Feuerstein,∥ Richard Heys,‡ Shing-Mei Hwang,§ Dalia R. Jakas,† Richard M. Keenan,† Thomas W. Ku,† Chet Kwon,† Chao-Pin Lee,▽ William H. Miller,† Kenneth A. Newlander,† Andrew Nichols,∥ Michael Parker,† Catherine E. Peishoff,⊥ Gerald Rhodes,# Steven Ross,† Arthur Shu,‡ Richard Simpson,# Dennis Takata,† Tobias O. Yellin,† Irene Uzsinskas,† Joseph W. Venslavsky,† Chuan-Kui Yuan,† and William F. Huffman†

Department of Medicinal Chemistry, Radiochemistry Department, Department of Cellular Biochemistry, Pharmaceutical Sciences, Pharmacology Department, Department of Physical/Structural Chemistry, and Department of Pharmacokinetics, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406

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The role of GPIIb/IIIa antagonists in the prevention of thrombosis has gained increasing attention in recent years.[1,2] Since chronic oral administration of GPIIb/IIIa antagonists could, in principle, address myocardial infarction and stroke, the number one and number three leading causes of death in the United States,[3] intense interest has developed over the discovery of orally active GPIIb/IIIa antagonists. The peptide SK&F 107260[4] (**1**, depicted in Table 1), shown to contain a turn−extended-turn conformation about the critical residues (NMe)Arg-Gly-Asp,[5] was one of a series of cyclic peptide antagonists that defined a peptide pharmacophore model[6] which was employed in the design[7] of a series of 8-substituted 3-oxo-1,4-benzodiazepine GPIIb/IIIa antagonists, typified by the 8-(*p*-amidinophenyl)amido compounds **2** and **3**.[7,8] Extensive investigations of the 8-substituted analogs have led to the discovery of several analogs which displayed oral activity but with limited oral duration of action.

We have concurrently explored a series of 7-substi-

Table 1. *In Vitro* Activities[a] of Peptide and Nonpeptide GPIIb/IIIa Antagonists

Compound No.	Configuration of Benzodiazepine at Position 2		Platelet Aggregation hPRP / ADP[b] IC_{50} (nM)	Binding Inhibition ^{3}H-**1** / hGPIIb/IIIa[c] Ki (nM)
1			57 ± 11	2.8 ± 0.1
	R[1]			
2	H	R,S	150 ± 40	2.8 ± 0.12
3	CH_3	R,S	65 ± 3	1.6 ± 0.20
	R[1]			
4	H	R,S	380 ± 20	26 ± 2
5	CH_3	R,S	160 ± 49	90 ± 10
6		R,S	60 ± 16	8.0 ± 0.3
	R[2]			
7	CH_2CH_2Ph	R,S	20 ± 4	1.5 ± 0.1
8	CH_3	R,S	106 ± 10	4.0 ± 0.3
9	CH_3	S	28 ± 12	2.5 ± 0.1
10	CH_3	R	8167 ± 237	1530 ± 23

[a] Methods are described in Bondinell et al.[8] [b] Patelet aggregation in human platelet-rich plasma induced by ADP, as in Bondinell et al.[8] [c] Binding of [^{3}H]-**1** to GPIIb/IIIa purified from human platelets, reconstituted in liposomes, as in Bondinell et al.[8]

† Department of Medicinal Chemistry.
‡ Radiochemistry Department.
§ Department of Cellular Biochemistry.
▽ Pharmaceutical Sciences.
∥ Pharmacology Department.
⊥ Department of Physical/Structural Chemistry.
Department of Pharmacokinetics.

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Table 2. Effects of Intravenous and Intraduodenal Doses of GPIIb/IIIa Antagonists on *ex Vivo* Platelet Aggregation in the Conscious Dog[a]

compd no.	dose (mg/kg)	route of admin[b]	maximal inhibition[c] (%)	time (min) to maximal inhibition	duration (min) of maximal inhibition	inhibition (%) at 390 min
7	0.3	iv	90−100	10	~140	control[d]
	3.0	id	70−90	30	~140	control
8	0.3	iv	90−100	5	~240	50
	3.0	id	90−100	10	~240	60
9	0.1	iv	90−100	5	~240	50
	1.0	id	90−100	30	~240	90

[a] Methods are described in Bondinell et al.[8] [b] Route of administration: iv = intravenous, bolus dose; id = intraduodenal catheter, short infusion. [c] Maximal inhibition of platelet aggregation *ex vivo*, canine whole blood/collagen. [d] Returned to control level of platelet aggregation prior to 390 min.

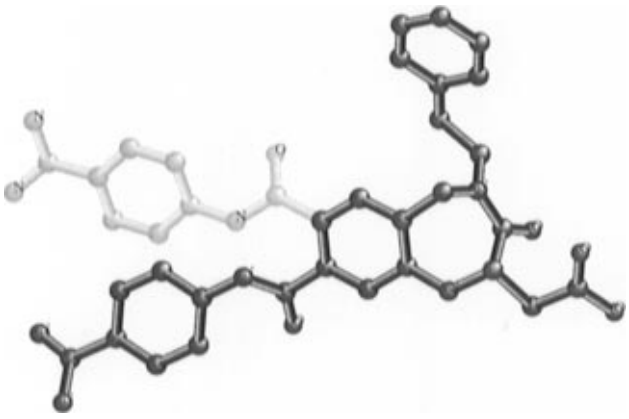


Figure 1. Overlay of 7-substituted 1,4-benzodiazepine **4** and 8-substituted 1,4-benzodiazepine **2**, showing that the amidine in **4** cannot readily access the same space as the amidine in **2** when both benzodiazepine templates are superimposed, as described in Ku et al.[9] Models of **2** and **4** were prepared in SYBYL (Tripos Associates) from the X-ray crystal structure of the benzodiazepine nucleus (Ku et al.).[5] The conformations shown for **2** and **4** were minimized via SYBYL.

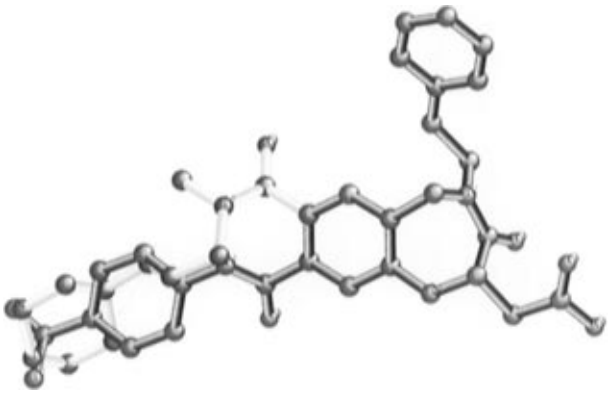


Figure 2. Overlay of 7-substituted 1,4-benzodiazepine **6** and 8-substituted 1,4-benzodiazepine **2**, showing that the piperidine in **6** is capable of accessing the same space as the amidine in **2**. The conformation of **2** had been previously shown to allow for an overlay of the amidine and carboxylate groups in **2** with the guanidine and carboxylate groups in cyclic peptide **1**, described in Ku et al.[5] Models of **2** and **6** were prepared as described in Figure 1.

tuted 3-oxo-1,4-benzodiazepines, keeping in mind our initial overlay hypothesis as a basic design guide. In an earlier publication, we showed that an amidinophenyl group in position 7 of the potent benzodiazepine GPIIb/IIIa antagonist **4** occupies a different region of space than the amidinophenyl group in position 8 of **2**, Figure 1, suggesting two hypotheses:[9] (a) The receptor group(s) that bind the cationic groups in RGD-related peptides and nonpeptide mimetics can occupy two different regions of space due to receptor flexibility (i.e., an expanded cationic site), or (b) there are two distinct sets of receptor binding groups that bind the cationic groups. While N-methylation of the 8-(*p*-amidinophenyl)amido compound **2** gave rise to the orally active *N*-methylamide **3**, the equivalent *N*-methylation of the 7-(*p*-amidinophenyl)amido compound **4** afforded the potent antagonist **5**, Table 1, which displayed *in vivo* activity after intravenous administration at 0.3 mg/kg but displayed no *in vivo* activity after intraduodenal administration at 3 mg/kg (data not shown).

We sought a new series of compounds in which a 7-substituent could position a cationic group in the same region of space accessed by the cationic 8-substituent of **4**. In this way it might be possible to merge aspects of the structure−activity relationships (SAR) of the 7- and 8-substituted series, allowing for the discovery of

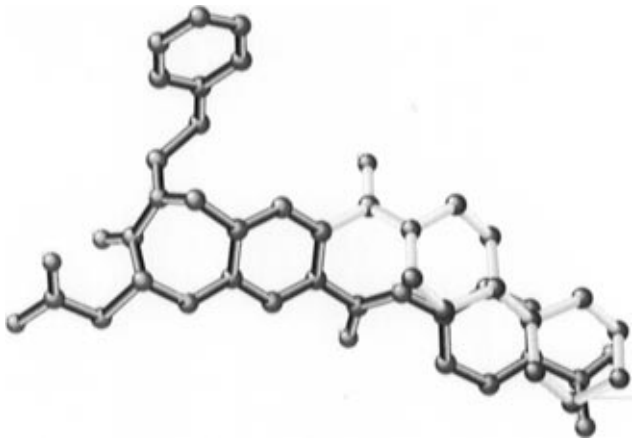


Figure 3. Overlay of 7-substituted 1,4-benzodiazepine **7** and 8-substituted 1,4-benzodiazepine **2**, showing that the bipiperidine in **7** is capable of accessing the same space as the amidine in **2**, as described in Figure 2.

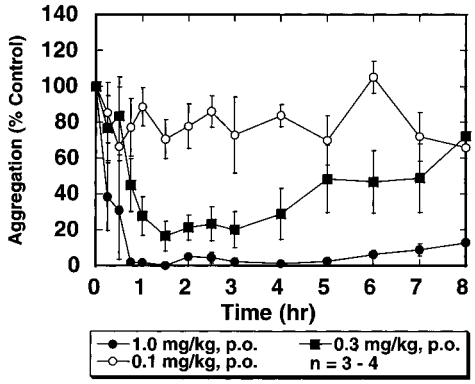
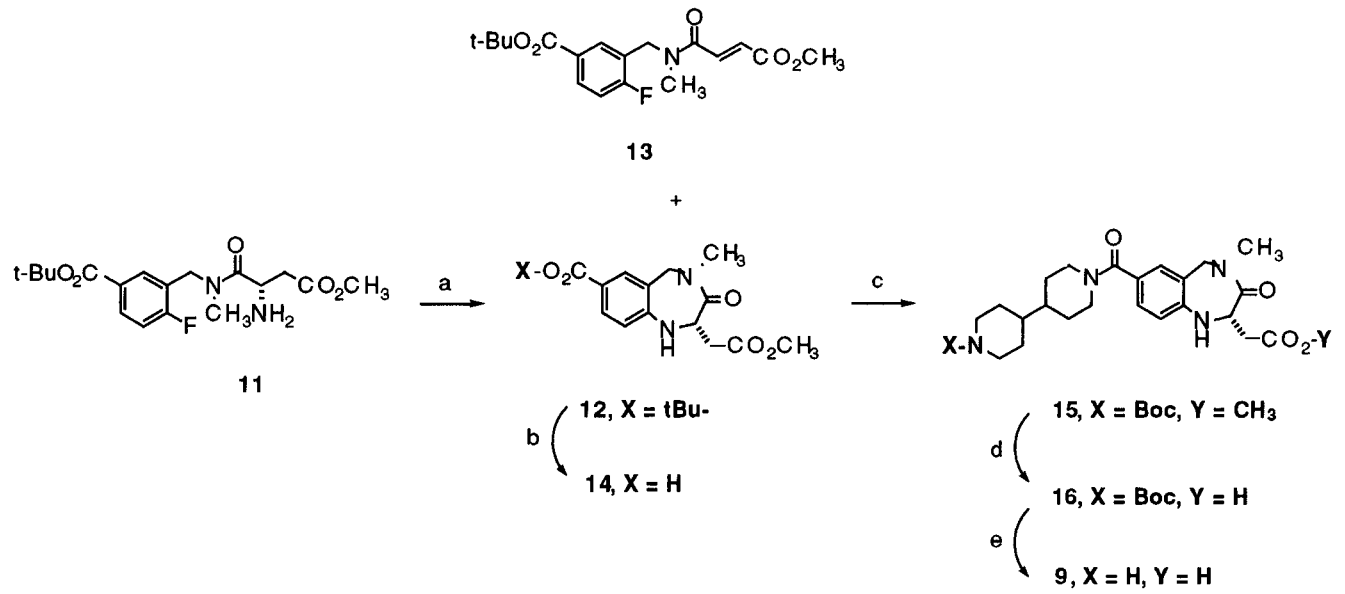


Figure 4. Percent platelet aggregation (canine whole blood stimulated with collagen) *ex vivo* after (○) 0.1 mg/kg, (■) 0.3 mg/kg, or (●) 1.0 mg/kg oral (po) administration of **9** to the conscious dog.

Scheme 1. Homochiral Synthesis[12] of 3-Oxo-1,4-benzodiazepine **9**[a]



[a] (a) 0.1 M **11** in anhydrous DMSO, 125 °C (47% of **12**, 28% of **13**); (b) 1:1 TFA/CH_2Cl_2, anisole (95%); (c) 1-BOC-4,4′-bipiperidine, EDC, (*i*-Pr)$_2$NEt, DMF (94%); (d) 2.0 N NaOH (2 equiv), 1:1 MeOH/THF, then AcOH (81%); (e) 4 M HCl in dioxane, $CHCl_3$, then neutralization of excess reagent with ca. 1.0 N KOH in EtOH to give **9**·HCl (75%) and precipitation from aqueous solution at pH 6.8 (83%).

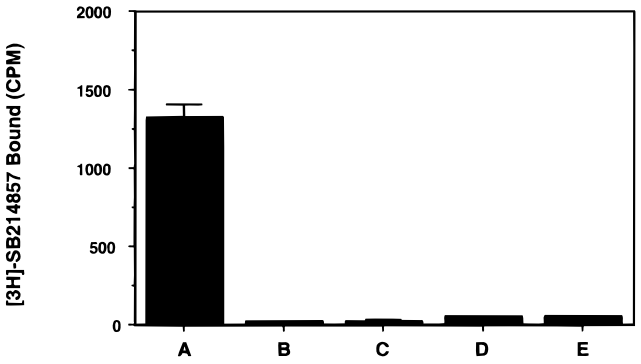


Figure 5. Reversible binding of [^{3}H]-**9** to purified GPIIbIIIa.[15] GPIIb/IIIa was incubated with radioligand [^{3}H]-**1** or [^{3}H]-**9** in the presence or absence of unlabeled **1** or **9** for 2 h. Alternatively, GPIIb/IIIa was pre-equilibrated with radioligand for 1 h, 2 μM unlabeled **1** or **9** was then added, and the samples were incubated for an additional 1 h; 0.5 and 1 μg of the lentil lectin-purified GPIIb/IIIa was used in these radioligand binding assays for **1** and **9**, respectively. In experiments A−E, the conditions were varied as follows: (A) 2 nM [^{3}H]-**9** incubated with GPIIb/IIIa for 2 h (showing total binding of [^{3}H]-**9**), (B) 2 nM [^{3}H]-**9** and 1 μM of cold **9** incubated together with GPIIb/IIIa for 2 h (showing reversibility of binding by **9**), (C) 2 nM [^{3}H]-**9** and 1 μM **1** incubated together with GPIIb/IIIa for 2 h (showing reversibility of binding by **1**), (D) 2 nM [^{3}H]-**9** incubated with GPIIb/IIIa for 1 h and then 1 μM **9** for 1 h (again, showing reversibility of binding by **9**), and (E) 2 nM [^{3}H]-**9** incubated with GPIIb/IIIa for 1 h and then 1 μM **1** for 1 h (again, showing reversibility of binding by **1**).

new compounds that could display high affinity for GPIIb/IIIa but contain structures that would possess enhanced pharmacokinetic properties. One solution from that search was the (piperidinylpropyl)amide **6**. A piperidinyl GPIIb/IIIa antagonist has been described by Hartman et al.[10] and in our own work where 8-(piperidinylethyl)amide 1,4-benzodiazepine analogs were found to display potent GPIIb/IIIa antagonist activity.[11] Compound **6** displayed high affinity for GPIIb/IIIa and high potency in the platelet aggregation assay, Table 1. The cationic amine of the piperidinylpropyl group could access the same region of space as the 8-amidinophenyl group in **2**, albeit in a somewhat contorted conformation, Figure 2. Other orientations are also possible via isomerism of the amide linkage. The more rigid bipiperidinylcarbonyl group in the 7-substituted compound **7** could hold the terminal piperidine into a more fixed orientation and avoid the ambiguity of amide bond isomerism. As can be seen in the overlay shown in Figure 3, the 7-bipiperidinyloxo group in **7** is capable of positioning its cationic amine in the same region of space as the cationic amine in the 8-(amidinophenyl)-amido group of **2**. Compound **7** proved to be a potent GPIIb/IIIa antagonist *in vitro*, Table 1, as well as in the conscious dog, Table 2, comparable now to the 8-substituted benzodiazepine **2**.[8]

Continued exploration of the 7-bipiperidinyloxo series led to an investigation of position 4. Although replacement of the 4-phenethyl group with 4-Me, as in **8**, resulted in a decrease in potency *in vitro*, Table 1, the 4-Me modification resulted in an unexpected gain in duration of antiaggregatory activity *in vivo*, Table 2.

Both enantiomers of **8** were prepared by homochiral synthesis from (*R*)- and (*S*)-aspartic acid.[12] The key step in the synthesis of both enantiomers involved the intramolecular displacement of the aryl fluoride **11** to construct the seven-membered ring of the 1,4-benzodiazepine system, as exemplified in the synthesis of the *S*-enantiomer, Scheme 1.[13] By this reaction, the desired cyclization product **12** was obtained without racemization, together with the elimination product **13**. To complete the synthesis, the carboxylic acid **14** was coupled with 1-BOC-4,4′-bipiperidine[14] to afford, after protecting group removal, zwitterionic SB 214857 **9**, in good overall yield and high enantiomeric purity. As seen in Table 1, the *S*-enantiomer **9** is considerably more potent *in vitro* than the *R*-enantiomer **10**.[15]

SB 214857, **9**, is a potent antiaggregatory agent after intravenous and intraduodenal administration to conscious dogs, more potent than either **7** or **8**, Table 2, and displays an *extended* duration of action. The compound is also orally active, Figure 4. After po

administration of compound **9** at 0.1 mg/kg, platelet aggregation *ex vivo* was inhibited by 80% between 90 min and 3 h. After po administration of compound **9** at 1.0 mg/kg, however, platelet aggregation was inhibited by >80% for the duration of the experiment, 8 h.

This increased activity *in vivo* for the 4-Me compounds **8** and **9** over the 4-phenethyl compound **7** is counterintuitive, since the decreased affinity and lipophilicity of the smaller methyl group would suggest diminished *in vivo* activity. In hindsight, we believe that the smaller size of the 4-Me group and diminished clearance are the factors that favor the 4-Me over the 4-phenethyl group.

The several modifications to the benzodiazepine **2** that led to **9** raise the question of whether **9** is still a nonpeptide mimetic of peptide **1**. That **9** may be regarded as a nonpeptide mimetic of the peptide **1** comes from the fact that both compounds (a) position cationic amine and anionic carboxylate groups in the same regions of space and (b) require an (*S*)-acetic acid side chain. We have also found that a derivative of **9** bearing tritium in the benzo group displays specific, saturable binding to human GPIIb/IIIa with a K_d of 2 nM, identical with the K_i of inhibition of [^{3}H]-**1** binding to GPIIb/IIIa by cold **9**.[16] Tritiated **9** binding, furthermore, is completely reversed by either cold **9** or cold **1**, even after preincubation for 1 h, Figure 5. We have also found that the K_i values for a series of GPIIb/IIIa antagonists, determined in either a [^{3}H]-**9** or [^{3}H]-**1** competition binding assay, are similar.[16] Thus, we observe no appreciable difference between the binding of SB 214857 and SK&F 107260 to purified human GPIIb/IIIa. Site-directed mutagenesis experiments in the G-protein-coupled receptor area suggest that, in some cases,[17,18] nonpeptide ligands can bind to sites that are similar to the peptide binding site or, in other cases,[19,20] distinct from the peptide binding site. SB 214857, however, appears to be an example where the nonpeptide ligand binds to the same receptor binding site as the peptide and, as such, is a true peptidomimetic.

In conclusion, from our series of 3-oxo-1,4-benzodiazepines, we have discovered a potent, selective, orally active GPIIb/IIIa antagonist that displays an extended duration of action.[20] Furthermore, with (a) the discovery of high affinity in 7-(bipiperidinylamido)-1,4-benzodiazepines and (b) their apparent mimicry of the original peptide ligand, we have shown that the original design hypothesis can continue to serve as a guide in analog design during subsequent studies, such as the search for analogs with enhanced oral duration. SB 214857 is currently undergoing clinical investigation.

Supporting Information Available: Elemental analytical data and additional figures showing the effects of compounds **3**, **5**, and **7**−**9** on *ex vivo* platelet aggregation in the conscious dog (1 page). Ordering information is given on any current masthead page.

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