Fused Bicyclic Gly-Asp β-Turn Mimics with Specific Affinity for GPIIb-IIIa

Matthew J. Fisher,* Ann E. Arfstan,† Ulrich Giese,‡ Bruce P. Gunn, Cathy S. Harms, Vien Khau, Michael D. Kinnick, Terry D. Lindstrom, Michael J. Martinelli, Hans-Jürgen Mest,‡ Michael Mohr,‡ John M. Morin, Jr., Jeffrey T. Mullaney, Anne Nunes, Michael Paal,‡ Achim Rapp,‡ Gerd Rühter,‡ Ken J. Ruterbories, Daniel J. Sall, Robert M. Scarborough,† Theo Schotten,‡ Birgit Sommer,‡ Wolfgang Stenzel,‡ Richard D. Towner, Suzane L. Um, Barbara G. Utterback, Robert T. Vasileff, Silke Vöelkers,‡ Virginia L. Wyss, and Joseph A. Jakubowski

Eli Lilly and Company, Indianapolis, Indiana 46028, Lilly Research Laboratories, Hamburg, Germany, and COR Therapeutics, Inc., South San Francisco, California 94080

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Disubstituted isoquinolones **2** and **3** have affinity for GPIIb-IIIa and represent leads for further structural evaluation. Structure−activity studies centered on the bicyclic β-turn mimic contained in these molecules indicated that this moiety could accommodate a variety of modifications. Specifically, monocyclic, 6,5-bicyclic, and 6,7-bicyclic structures provide compounds with affinity for GPIIb-IIIa. Within the 6,6-series, isoquinoline, tetralin, tetralone, and benzopyran nuclei yield potent antagonists that are specific for GPIIb-IIIa. Attachment of the arginine isostere (benzamidine) to the supporting nucleus can be accomplished with an ether or amide linkage, although the latter enhances activity. Several compounds in this series provided measurable blood levels after oral dosing. Conversion of the acid moiety in these molecules to an ester generally provided compounds which gave greater systemic exposure after oral administration. Absolute bioavailabilities in the rat for the ethyl ester prodrug derivatives of the tetralin, tetralone, and benzopyran analogues of **3** were 28%, 23%, and 24%, respectively.

Introduction

The design and development of agents which control thrombosis by modulating platelet aggregation has received significant attention over the past decade.[1] The primary physiological role of the platelet is to copolymerize with fibrinogen and thus aggregate at sites of injury forming a hemostatic plug that seals off leaks in the vasculature.[2] It has been shown that the membrane-associated glycoprotein (GP) IIb-IIIa is primarily responsible for this aggregation phenomenon.[3−5] This receptor, which is a member of a family of adhesive proteins collectively known as integrins, binds to Arg-Gly-Asp (RGD) sequences contained in the soluble plasma protein fibrinogen.[6−9] This critical interaction between GPIIb-IIIa and fibrinogen can be inhibited by either acyclic or cyclic RGD-containing peptides.[10−19] These observations have formed the basis for the use of the RGD motif as a lead structure in the design of novel inhibitors of platelet aggregation.[20−36]

We have recently described our initial studies toward the design and refinement of non-peptide antagonists of GPIIb-IIIa.[37,38] Our approach was initiated with the preparation of small cyclic peptides which incorporated an RGD sequence, yielding a family of compounds with high affinity for GPIIb-IIIa.[39] We subsequently evaluated one of the most potent peptides (**1**) by ^{1}H NMR and determined that in aqueous solution, the critical RGD sequence in this molecule primarily exists in a well-defined type II′ Gly-Asp β-turn.[38] This conformation, which defined the relationship of the critical arginine and aspartate side chains, formed the foundation for our design of non-peptide GPIIb-IIIa antagonists. Initial compounds employed an isoquinolone moiety as a β-turn mimic with the aspartate and arginine side chains attached at the 2- and 6-positions, respectively. Structure−activity studies (SAR) in this series demonstrated that potent GPIIb-IIIa receptor antagonism could be obtained with compounds that contained an acetic acid residue at C2 and a benzamidine tethered from C6 of the isoquinolone nucleus. Data from this investigation suggested that 2,6-disubstituted isoquinolones **2** and **3** were leads suitable for further investigation (Figure 1).[40]

Originally, our choice of an isoquinolone nucleus was based on the fact that its overall shape closely resembled the Gly-Asp β-turn determined for peptide **1** and that the resident functionality of this ring system allowed ready attachment of the arginine and aspartate side chains. As our initial investigations focused solely on side chain optimization, we remained interested in the SARs afforded by the supporting nucleus. We were particularly interested to see if a monocyclic analogue of our lead isoquinolone would have activity, and within the constraints of a bicyclic β-turn mimic, we were interested in what effect would result from changing the size of the ring to which the aspartate isostere was appended. Finally, the functionality within the B-ring (see Figure 1) of our isoquinolone leads also offered several readily accessible areas for modification. We sought to understand the role of the cyclic amide and probe ring conformation by evaluating compounds with differing hybridization at C2 and C3 of the bicyclic nucleus. Key goals for this investigation were to gain insight into the relationship between the β-turn mimic and in vitro activity and to determine if active compounds from this series would afford measurable plasma

‡ Lilly Research Laboratories.
† COR Therapeutics, Inc.

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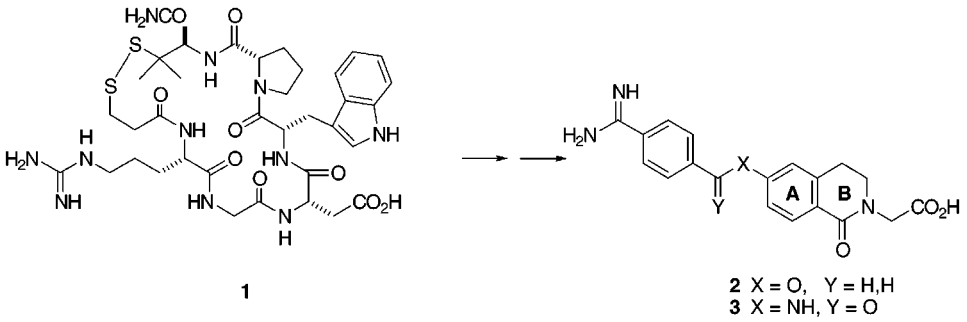
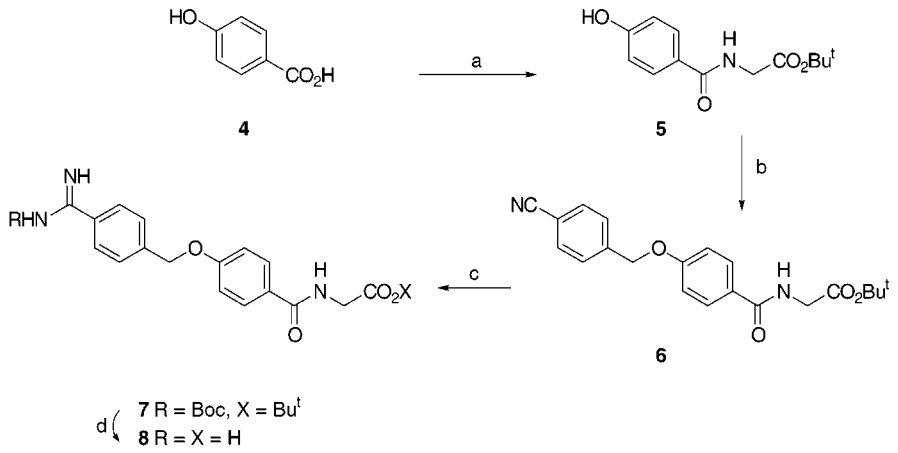


Figure 1. Lead peptide **1** and derived non-peptide β-turn mimics **2** and **3**. Activity data for compounds **2** and **3** are located in Tables 2 and 3, respectively.

Scheme 1[a]



[a] (a) EEDQ, *tert*-butylglycine; (b) Triton B, α-bromo-*p*-tolunitrile; (c) H_2S, MeI, NH_4OAc, Boc_2O; (d) TFA.

levels in animals following oral administration. For this study, we chose to initially examine relevant structural modifications in the oxo-linked series since the required synthetic intermediates for the desired target molecules were readily accessible. Structural motifs that provided greater intrinsic activity than the lead oxo-linked isoquinolone **2** were then synthesized and evaluated in the more active amide-linked series. In this report, we disclose our observations regarding the in vitro SARs that we have defined for the β-turn mimic contained in our lead isoquinolones **2** and **3** and report on the oral activity of the most potent compounds prepared in this series.

Chemistry

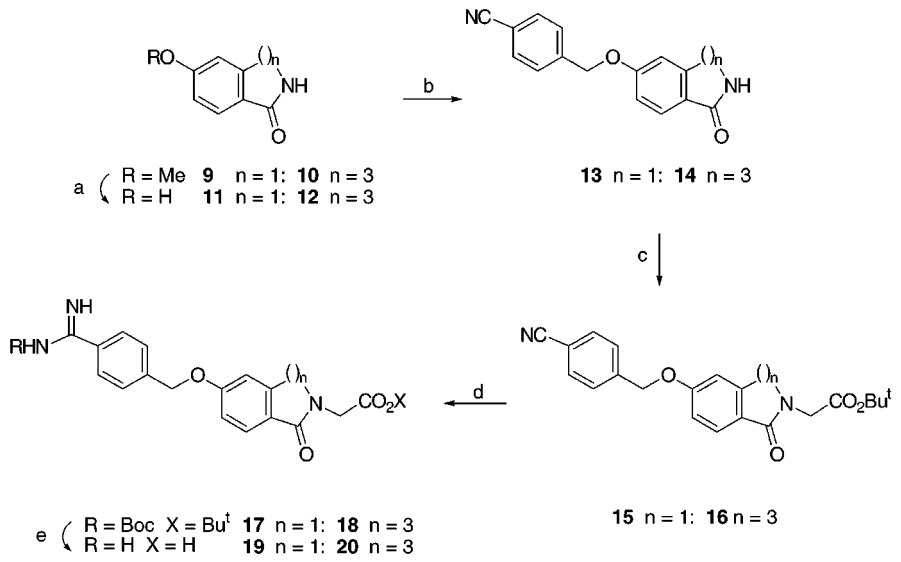
The synthesis of the monocyclic derivative of isoquinolone **2** is illustrated in Scheme 1. 4-Hydroxybenzoic acid (**4**) and *tert*-butylglycine were coupled using EEDQ, producing amide **5**. The phenol moiety in this molecule was then alkylated with α-bromo-*p*-tolunitrile which provided adduct **6**. The nitrile moiety was subsequently converted into a Boc-protected amidine (**7**) using a modified thio-Pinner sequence previously described.[38] Simultaneous deprotection of the *tert*-butyl ester and the Boc-amidine moieties with neat TFA provided the desired amidino acid **8** as the TFA salt.

Compounds in which the B-ring of isoquinolone **2** (see Figure 1) was expanded or contracted by one carbon were prepared as described in Scheme 2. Demethylation of methoxyisoindol-1-one **9**[41] and methoxybenzazepin-1-one **10**[42] was accomplished with BBr_3 at room temperature which provided phenols **11** and **12**. Alkylation of these compounds selectively on oxygen was accomplished with α-bromo-*p*-tolunitrile using Triton B which provided the monosubstituted intermediates **13** and **14**. Alkylation of the lactam nitrogen contained in these molecules was effected by first deprotonating with NaH and then quenching the resultant anion with *tert*-butyl bromoacetate which yielded disubstituted compounds **15** and **16**. These materials were then transformed into the desired amidino acids **19** and **20** using protocols similar to that outlined for the preparation of **8**.

One example of an unsaturated isoquinolone was prepared as outlined in Scheme 3. Treatment of 5-methoxyindanone with sodium methoxide and butyl nitrite afforded a mixture of the desired isoquinolone **23** and oxime **22**.[43] Treatment of **23** with aqueous HI at reflux effected reduction of the hydroxamic acid and demethylation of the C6-methoxy group providing intermediate **24** in good yield. This material was transformed into the desired 2,6-difunctionalized compound **28** by utilizing a sequence similar to that described for the preparation of **8**.

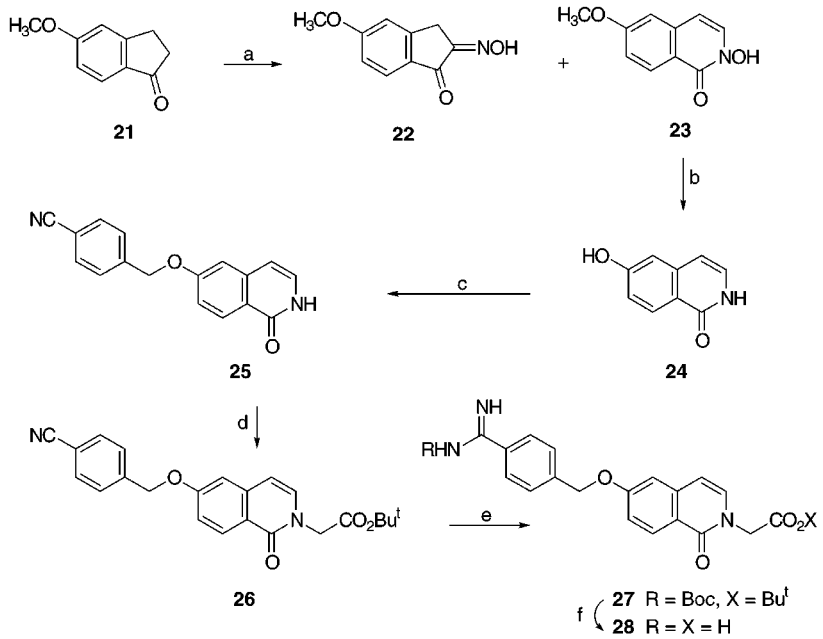
Compound **37**, which contains an isomeric 3-oxoisoquinolone nucleus, was synthesized as shown in Scheme 4. Treatment of lactone **29**[44] with saturated methanolic HBr provided bromo ester **30**. This material was reacted directly with lithium azide to afford intermediate **31**. The crude product from this reaction was immediately treated with triphenylphosphine which formed lactam **32** as a crystalline solid. This intermediate was transformed into the desired target compound **37** by functionalization at the 2- and 6-positions in a manner

Scheme 2[a]



[a] (a) BBr_3; (b) Triton B, α-bromo-*p*-tolunitrile; (c) NaH, *tert*-butyl bromoacetate; (d) H_2S, MeI, NH_4OAc, Boc_2O; (e) TFA.

Scheme 3[a]



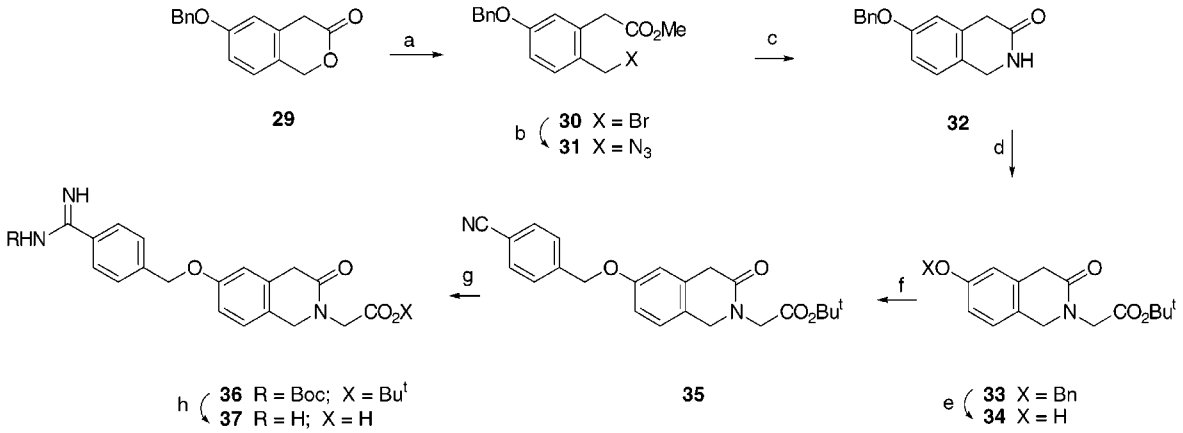
[a] (a) NaOMe, butyl nitrite; (b) HI reflux; (c) α-bromo-*p*-tolunitrile, K_2CO_3; (d) NaH, *tert*-butyl bromoacetate; (e) H_2S, MeI, NH_4OAc, Boc_2O; (f) TFA.

analogous to that previously described for the preparation of compound **8**.

Compounds that contain either a 6-oxo- or 6-aminoisoquinoline were prepared from the known isoquinolone intermediates **38** and **39** as outlined in Scheme 5. Reduction of these compounds with LAH yielded the requisite isoquinoline derivatives **40** and **41**. Functionalization of the secondary nitrogen in these molecules was accomplished by alkylation with *tert*-butyl bromoacetate in the presence of K_2CO_3 affording **42** and **43**. The *p*-cyanophenyl moiety was appended to the 6-position of **43** and **44** via alkylation or acylation to afford **45** and **48**, respectively. Amidine formation in this series was complicated by the fact that the tertiary amine present in these molecules was susceptible to quaternization during the course of our standard thio-Pinner sequence. Thus, we protected the amine as its TFA salt prior to alkylation of the intermediate thioamide with excess methyl iodide. This modification of our standard sequence allowed for the preparation of the desired Boc-protected amidines **46** and **49** in reasonable yield. Deprotection of these compounds was accomplished with neat TFA, which provided the target molecules **47** and **50** as the corresponding salts.

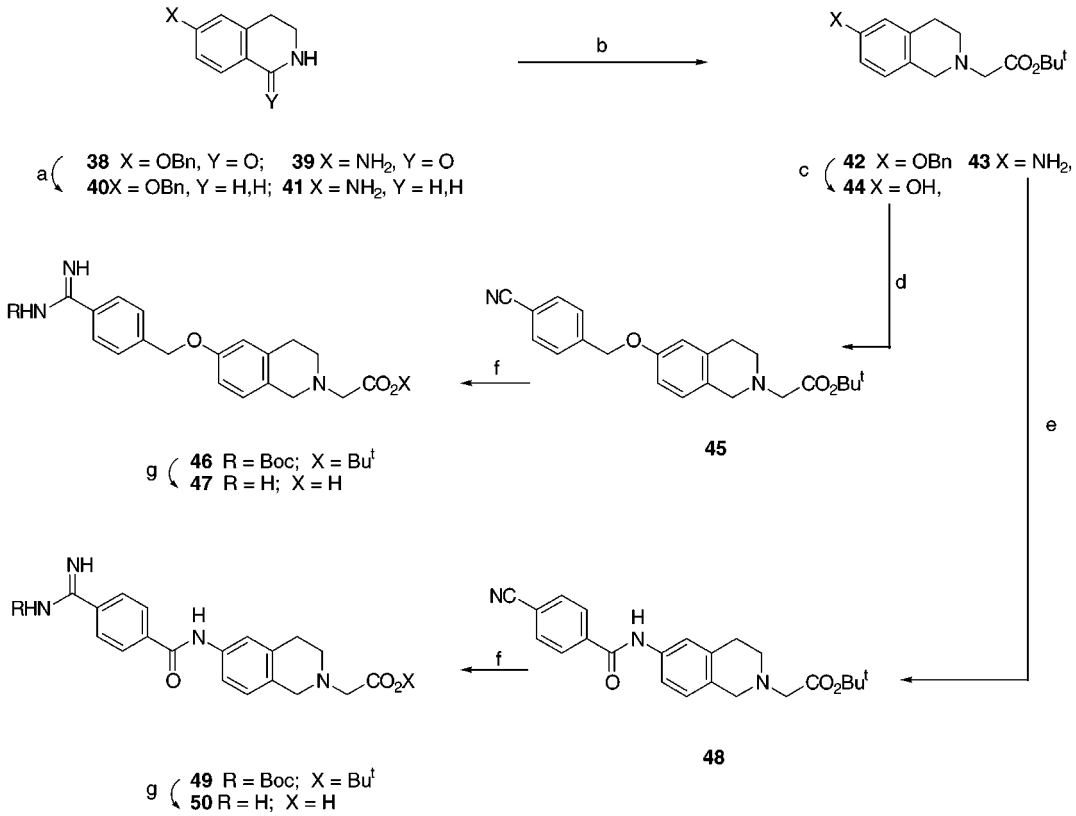
The methods used for the construction of compounds containing tetralone nuclei are outlined in Schemes 6 and 7. Preparation of compound **56**, which contains a 6-oxotetralone nucleus begins with the condensation of 6-hydroxytetralone (**51**) and glyoxylic acid in the presence of NaOH which afforded adduct **52**.[45] The α,β-unsaturated ester was reduced with zinc and acetic acid, and the formed saturated carboxylic acid was subsequently converted to ester **53** by treatment with diphenyl diazomethane. Phenol **53** was then alkylated with

Scheme 4[a]



[a] (a) HBr/MeOH; (b) lithium azide; (c) Ph_3P; (d) NaH, *tert*-butyl bromoacetate; (e) Pd/C; (f) α-bromo-*p*-tolunitrile, K_2CO_3; (g) H_2S, MeI, NH_4OAc, Boc_2O; (h) TFA.

Scheme 5[a]



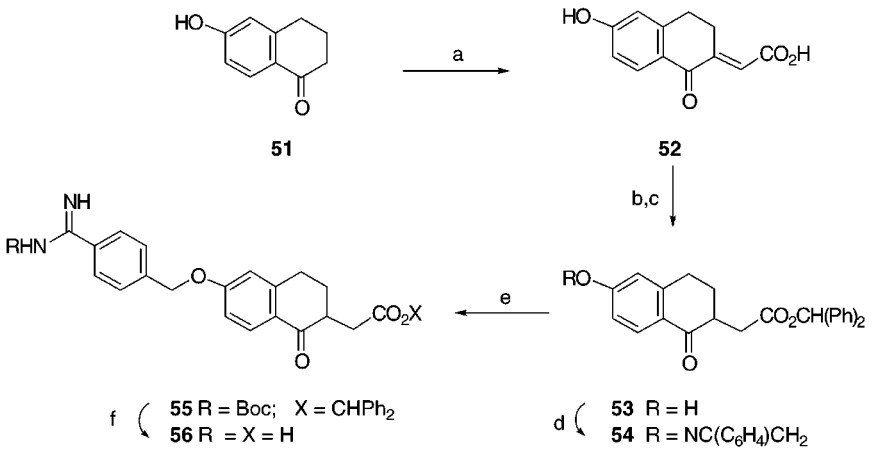
[a] (a) LAH; (b) *tert*-butyl bromoacetate, K_2CO_3; (c) H_2, Pd/C; (d) α-bromo-*p*-tolunitrile; (e) *p*-cyanobenzoic acid, EDCI; (f) H_2S, TFA−MeI, NH_4OAc, Boc_2O, (g) TFA.

α-bromo-*p*-tolunitrile which provided the 2,6-disubstituted compound **54**. Conversion of the nitrile moiety in this molecule to a Boc-protected amidine (**55**) was accomplished using protocols similar to that described for the preparation of **8**. Simultaneous deprotection of the ester and amidine functional groups was accomplished with neat TFA to afford the desired amidino acid **56** as the TFA salt.

Synthesis of the 6-amino-1-tetralone (Scheme 7) began with acidic hydrolysis of the acetamide contained in the tetralone **57**.[45] This material was then esterified with ethanolic HCl which provided aniline **58**. Acylation of aniline **58** with 4-cyanobenzoic acid yielded **59**, which was then converted to the Boc-protected amidine **60** using the same conditions outlined for the preparation of **8**. Treatment of this material with aqueous LiOH in THF afforded the protected amidino acid **62** after careful neutralization. This material was not characterized; rather it was subjected to deprotection with neat TFA yielding amidino acid **63** as a white solid.

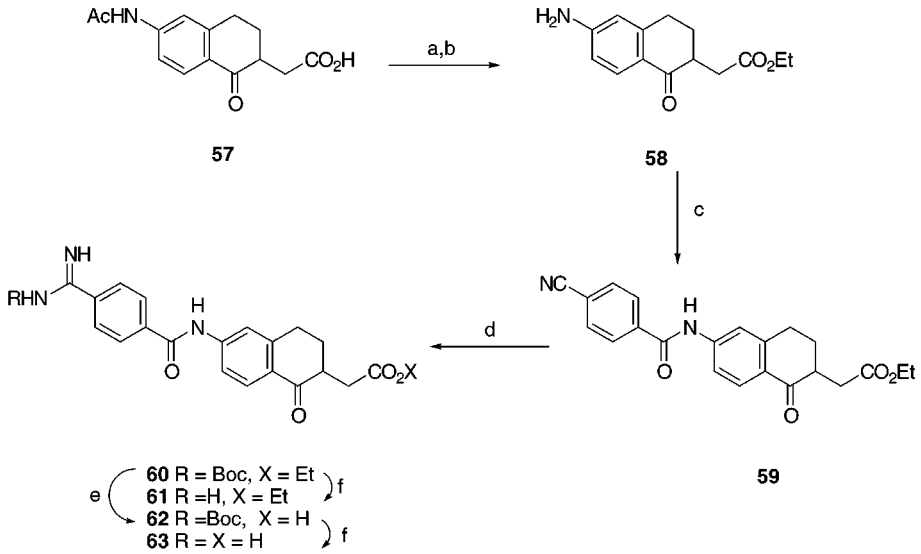
The construction of compounds that contain tetralin nuclei is outlined in Schemes 8 and 9. Synthesis of a 2-substituted-6-oxotetralin began with the reaction of commercially available 6-methoxytetralone (**64**) with the sodium salt of triethyl phosphonoacetate which provided ester **65** as a mixture of olefin isomers. Saturation of the double bond was accomplished with hydrogen in the presence of palladium on carbon, and the 6-methoxy

Scheme 6[a]



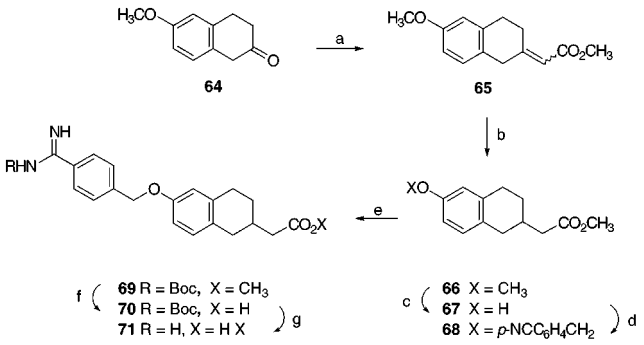
[a] (a) NaOH, glyoxylic acid; (b) Zn, HOAc; (c) diphenyl diazomethane; (d) α-bromo-*p*-tolunitrile; (e) H_2S, MeI, NH_4OAc, Boc_2O; (f) TFA.

Scheme 7[a]



[a] (a) Aqueous HCl; (b) HCl/EtOH; (c) *p*-cyanobenzoic acid/EDCI; (d) H_2S, MeI, NH_4OAc, Boc_2O; (e) LiOH, (f) TFA.

Scheme 8[a]



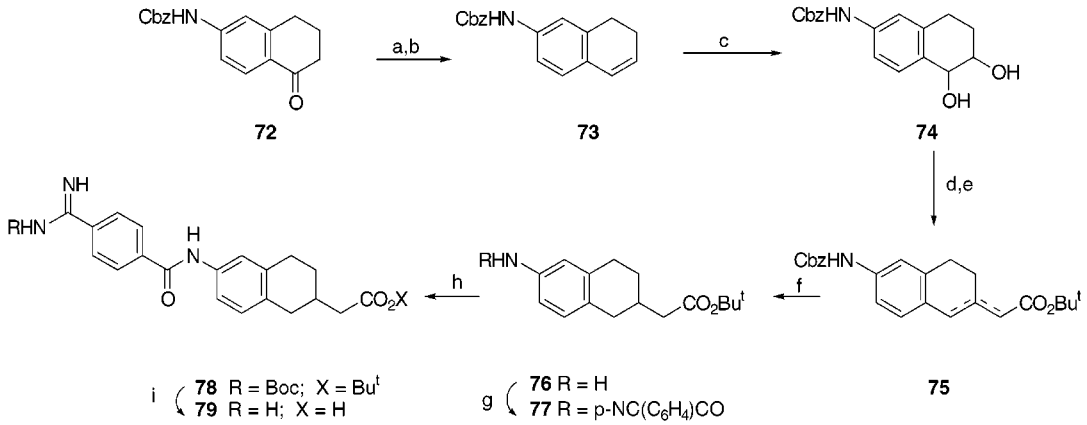
[a] (a) Methyl diethyl phosphonoacetate; (b) H_2, Pd/C; (c) BBr_3; (d) α-bromo-*p*-tolunitrile; (e) H_2S, MeI, NH_4OAc, Boc_2O; (f) NaOH; (g) TFA.

group was demethylated with BBr_3. Phenol **67** was alkylated with α-bromo-*p*-tolunitrile which provided the 2,6-disubstituted tetralin **68**. Conversion of the nitrile moiety of **68** to a Boc-protected benzamidine was accomplished with standard thio-Pinner conditions. Conversion of intermediate **69** into the desired amidino acid **71** was accomplished using procedures similar to that employed for the preparation of compound **63**.

Synthesis of the 6-aminotetralin **79** began by reduction of **72** with $NaBH_4$ forming the corresponding benzylic alcohol, which was subsequently dehydrated with TsOH in refluxing toluene to yield olefin **73**. This material was then allowed to react with OsO_4 to produce vicinal diol **74**. Treatment of a toluene solution of **74** with TsOH at reflux produced an intermediate 2-tetralone which was not purified; rather it was reacted crude with the sodium salt of *tert*-butyl diethyl phosphonoacetate which provided a mixture of olefin isomers **75**. Catalytic hydrogenation of this material afforded the desired 6-aminotetralin **76** in good yield. Conversion of this intermediate into the desired product **79** was accomplished in a manner similar to that outlined for the preparation of **63**.

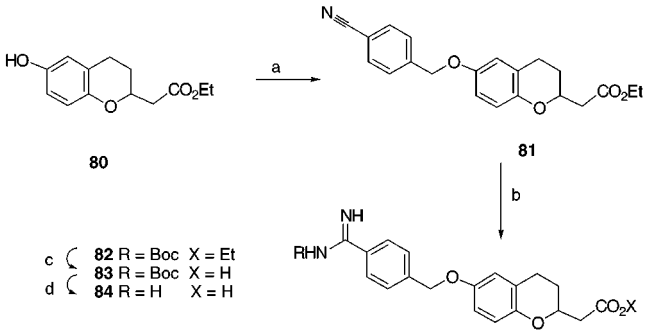
Compounds that contain a benzopyran nucleus were prepared as outlined in Schemes 10 and 11. Preparation of ether-linked derivative **84** began with alkylation of the known 6-hydroxybenzopyran **80**[46] with α-bromo-*p*-tolunitrile which afforded intermediate **81**. This compound was converted first to the Boc-protected amidine **82** and then to the fully deprotected compound **84** using the protocol described for the preparation of **63**. Synthesis of the amide-linked congener of **84** began with

Scheme 9[a]



[a] (a) $NaBH_4$; (b) TsOH; (c) OsO_4; (d) TsOH; (e) *tert*-butyl diethyl phosphonoacetate; (f) Pd/C, H_2; (g) *p*-cyanobenzoic acid, EDCI; (h) H_2S, MeI, NH_4OAc, Boc_2O; (i) TFA.

Scheme 10[a]



[a] (a) K_2CO_3, α-bromo-*p*-tolunitrile; (b) H_2S, MeI, NH_4OAc, Boc_2O; (c) NaOH; (d) TFA.

the palladium-catalyzed hydrogenation of commercially available 6-nitrocoumarin (**85**) in the presence of di-*tert*-butyl dicarbonate which provided compound **86**. Partial reduction of the lactone **86** with DIBAH gave rise to an intermediate lactol that was immediately reacted with (ethoxycarbonyl)triphenylphosphorane which yielded benzopyran **87**. The Boc group was then removed with TFA giving rise to aniline **88**. Acylation of this material with 4-cyanobenzoic acid provided the 2,6-disubstituted benzopyran **89**. Benzamidine formation in this series was accomplished using the same protocol described for the tetralone **63**. Formation of the parent compound **93** was accomplished by first hydrolyzing the ester with aqueous NaOH and then removing the amidine protecting group with TFA.

Results

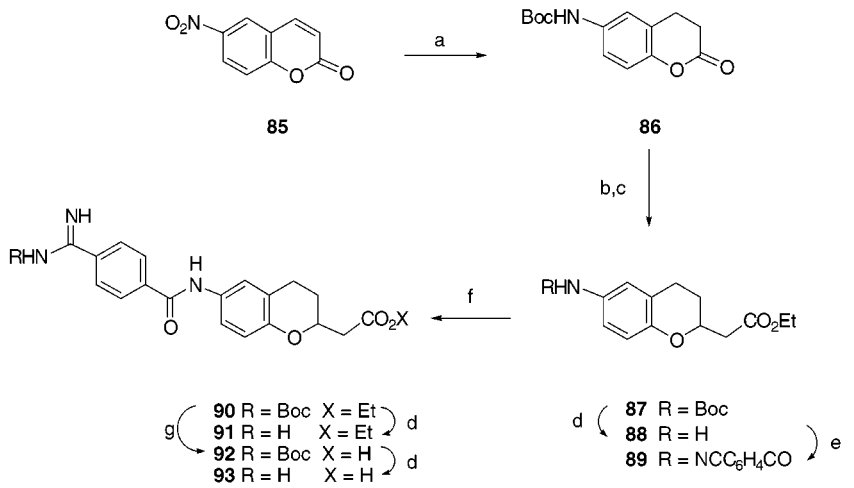
Compounds were evaluated for receptor affinity in an ELISA type assay that measured the inhibition of biotinylated fibrinogen binding to immobilized human GPIIb-IIIa. Compounds with sufficient activity in this assay (IC_{50} < 0.5 μM) were then examined in a functional assay that measured the agents' ability to inhibit aggregation of human platelets activated by ADP (5 μM) in human platelet-rich plasma (PRP). Compounds **2** and **3**, which were developed during our previous investigations, served as activity references for this study.[38] Compounds with IC_{50} values less than 0.2 μM in PRP were further evaluated in vivo in rats and/or guinea pigs in an effort to determine if measurable blood levels could be achieved after oral dosing. Selected compounds were also evaluated for cross-reactivity with $\alpha_V\beta_3$ in an ELISA type assay that measured the inhibition of vitronectin binding to purified immobilized human vitronectin receptor ($\alpha_V\beta_3$).

Evaluation of the β-turn mimic contained in our initial lead compounds began with an effort to define the relationship between the size of the aliphatic ring (B-ring) and activity (Table 1). Monocyclic compound **8** was found to be 8-fold less potent in the ELISA assay (IC_{50} = 5.0 μM) than the biclycic congener **2** from which it was modeled. Compound **19**, which contains a 6,5-ring system, provided 4-fold greater activity than monocyclic compound **8** but 2-fold less activity than its one-carbon homologue **2**. Disubstituted benzazepine **20** was 8-fold more potent than either the monocyclic compound **8** or the compound containing a 6,5-nucleus (**19**).

We next investigated the contributions of the functionality contained in the lactam ring of **2** (Table 2). Introduction of unsaturation at positions 3 and 4 yielded derivative **28** which was found to be 7-fold less potent in the ELISA (IC_{50} = 4.2 μM) than its saturated congener. Shifting the amide carbonyl of isoquinolone **2** from the 1-position to the 3-position provided isomeric isoquinolone **37**, which was determined to be 10-fold less active in the ELISA (IC_{50} = 5.8 μM) than the 1-oxo derivative **2**. Reduction of the lactam of **2** provided isoquinoline **47** which is 20-fold more potent in the ELISA (IC_{50} = 0.033 μM) than the corresponding 1-oxo compound **2**. Replacement of the isoquinolone nitrogen of **2** by carbon affords tetralone **56** which was roughly equipotent with **47** in the ELISA assay and has similar activity in PRP. Removal of all functionality in the B-ring of **2** provides tetralin derivative **71** which was 2-fold more potent in the ELISA (IC_{50} = 0.26 μM) than **2** but 10-fold less active than either **47** or **56**. Benzopyran **84** provided potency similar to isoquinoline **47** and tetralone **56** and greater activity than either **2** or the tetralin derivative **71**.

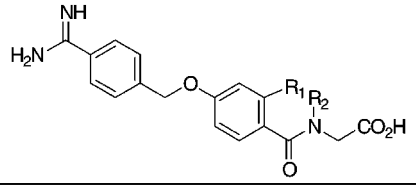
These data demonstrate that the oxo-linked tetralin (**71**), tetralone (**56**), isoquinoline (**47**), and benzopyran (**84**) nuclei afford compounds with greater affinity for GPIIb-IIIa than the lead isoquinolone **2**. We therefore chose to evaluate these nuclei in the amide-linked series (Table 3). As expected from previous studies, amide-linked isoquinoline **50** afforded a 6-fold increase in

Scheme 11[a]



[a] (a) Pd/C, Boc_2O; (b) DIBAH; (c) (ethoxycarbonyl)triphenylphosphorane; (d) TFA; (e) 4-cyanobenzoic acid, EDCI; (f) H_2S, MeI, NH_4OAc, Boc_2O; (g) NaOH.

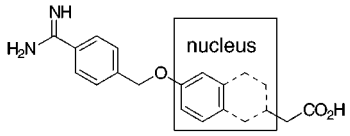
Table 1. Activity Data for Compounds with Varying Ring Size

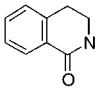
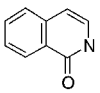
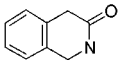
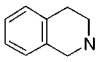


compd	R_1	R_2	ELISA IC_{50} (μM)[a]
8	H	H	5.0
19		CH_2	1.2
2		$(CH_2)_2$	0.6
20		$(CH_2)_3$	0.6

[a] Concentration required to reduce binding of fibrinogen to purified human GPIIb-IIIa by 50%. The IC_{50} values are expressed as the average of at least two determinations. The average error for the IC_{50} determinations was ±15%.

Table 2. Activity Data for 6-Oxo-Linked Compounds with Varying Nuclei



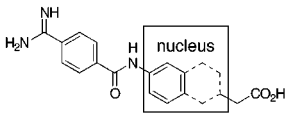
compd	nucleus	ELISA IC_{50} (μM)[a]	PRP IC_{50} (μM)[b]
2		0.6	nd
28		4.2	nd
37		5.8	nd
47		0.03	0.33
56		0.027	0.45
71		0.26	33
84		0.039	2.5

[a] As in Table 1. [b] Concentration required to reduce ADP-induced human platelet aggregation response by 50%. The IC_{50} values are expressed as the average of at least two determinations. The average error for the IC_{50} determinations was ±16%. nd, not determined.

GPIIb-IIIa binding affinity (ELISA IC_{50} = 0.005 μM) and a 2-fold activity increase in PRP (IC_{50} = 0.17 μM) relative to **47**. Activity gains were also observed for the amide-linked tetralin derivative **79** which was roughly 50-fold more active in the ELISA (IC_{50} = 0.005 μM) and 170-fold more potent in PRP (IC_{50} = 0.19 μM) than its oxo-linked counterpart **71**. Replacement of the ether linkage with an amide linkage was also beneficial for the tetralone derivative **63** as well. Activity increased in the ELISA by 16-fold (IC_{50} = 0.002 μM) and by 7.5-fold in PRP (IC_{50} = 0.06 μM) relative to **56**. Intrinsic activity for benzopyran **93** was found to be similar to that for other amide-linked compounds (ELISA IC_{50} = 0.002 μM) with functional activity comparable to that of analogues **50** and **79** but slightly less than that of isoquinolone **3** or tetralone **63**.

The compounds described in Table 3 all afforded in vitro activity in human PRP sufficient for further evaluation in vivo. Since one of our goals was the development of agents that would provide pharmacological concentrations of antagonist in plasma after oral administration, we further evaluated compounds of interest by determining the plasma concentrations and degree of exposure (as defined by the area under the curve (AUC) for the time course studied) which resulted from a single oral dose. We were unable to use pharmacodynamic (platelet aggregation) measurements for these studies since pilot experiments with rodent platelets indicate that several of the more potent compounds showed poor inhibition of ADP-induced platelet aggregation in rodent PRP (data not shown). This is

Table 3. Activity Data for 6-Amide-Linked Compounds with Varying Nuclei



compd	nucleus	ELISA IC_{50} (μM)[a]	PRP, IC_{50} (μM)[b]	PRP, IC_{50} (ng/mL)[b]
3		0.003 ± 0.0003	0.09 ± 0.04	32± 15
50		0.005 ± 0.001	0.17 ± 0.07	60± 24
63		0.002 ± 0.0002	0.06 ± 0.007	20 ± 2.4
79		0.005 ± 0.001	0.19 ± 0.04	67 ± 17
93		0.002 ± 0.0008	0.19 ± 0.06	27 ± 21

[a] Concentration required to reduce binding of fibrinogen to purified human GPIIb-IIIa by 50%. [b] Concentration required to reduce ADP-induced human platelet aggregation response by 50%.

Table 4. Blood Level Data Obtained After a Single 10 mg/kg Oral Dose in the Guinea Pig

compd	nucleus	ester	AUC (ng·h/mL)	C_{max} (ng/mL)
3	isoquinolone	none	400	126
79	tetralin	none	903	252
94	isoquinolone[a]	ethyl	543	177
96	tetralin[a]	ethyl	3697	1167
99	isoquinoline[a]	ethyl	1583	469
61	tetralone[a]	ethyl	3988	1147

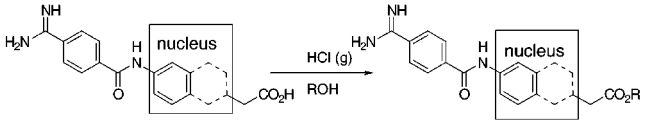
[a] Tabulated values derived from detection and analysis of parent free acid in plasma.

consistent with previous reports of species specificity with GPIIb-IIIa antagonists.[47] Accordingly, we devised a pharmacokinetic approach in which compound blood levels were determined by HPLC analysis at 1-, 2-, and 5-h time points. We chose to test compounds of interest in the guinea pig and/or rat after a single oral dose of 10 mg/kg.

Analysis of isoquinolone **3** in the guinea pig according to the above protocol indicated that measurable blood levels (Table 4) were achieved after oral administration of 10 mg/kg. The observed C_{max} was 126 ng/mL, and AUC for the 5-h time period was 400 ng·h/mL. Results for the tetralin **79** were approximately 2-fold greater with a C_{max} of 252 ng/mL and an AUC for the 5 h of 903 ng·h/mL. While these results were encouraging, the moderate plasma levels obtained with amidino acids **3** and **79** suggested that the extent of oral absorption was poor. We hypothesized that the zwitterionic character of these molecules might be in part responsible for the limited exposure obtained after oral dosing. We therefore elected to test this possibility by masking the carboxylate moiety found in **3** and **79** as an ester prodrug.

Esterification was readily accomplished by dissolving the parent amidino acid in the desired alcohol and then treating the resulting solution with excess anhydrous HCl (see Table 5). Isolation was accomplished by concentration which provided the desired prodrug compounds as the hydrochloride salt. Oral administration of the ethyl ester analogue of **3** (**94**) provided a 1.4-fold improvement of the C_{max} (177 ng/mL) of the parent isoquinolone **3** and a 1.3-fold increase in the AUC for the 0−5-h time course studied. Conversion of tetralin derivative **79** to the ethyl ester prodrug **96** provided more substantial gains in that the C_{max} increased by 4.6-fold (3697 ng/mL) and the AUC increased by 4-fold.[48]

Table 5. Conversion of Parent Free Acids to Ester Prodrugs



compd	nucleus	R
94	isoquinolone	Ethyl
95	tetralin	Methyl
96	tetralin	Ethyl
97	tetralin	Propyl
98	tetralin	Butyl
99	isoquinoline	Ethyl

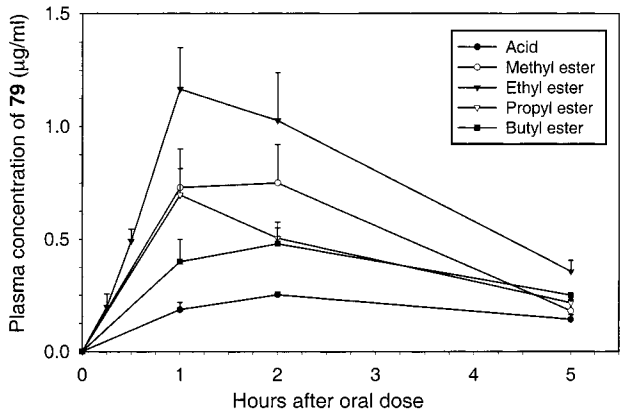


Figure 2. Plasma concentration of tetralin **79** in guinea pig following a single oral dose (10 mg/kg) of various ester prodrugs.

Encouraged by these results, we then evaluated the impact that the alkyl portion of the ester had on exposure in the tetralin series. Simple alkyl ester homologues of **96** were evaluated orally at 10 mg/kg in the guinea pig, and the results are shown in Figure 2. These data indicate that the alkyl portion of the ester moiety can affect exposure. Each ester derivative provided an increase in AUC relative to the parent acid with the ethyl analogue proving to be optimum of those tested. As a result, we continued our oral testing efforts with compounds in which the carboxylate was esterified. We focused our efforts on ethyl ester analogues as this modification provided the greatest increase in AUC relative to the parent acid for the tetralin and because the alcohol product of ester hydrolysis is presumably less toxic.

The ethyl ester of isoquinoline **50** (**99**) afforded a 2.9-fold increase in AUC relative to the related isoquinolone ester **94** but provided roughly one-half the exposure of the tetralin ester **96**. Tetralone ester **61** provided an exposure profile that was equivalent to that of tetralin **96** and better than that of either isoquinolone **94** or isoquinoline **99**. The tetralin ester **96** and tetralone ester **61** were further studied orally at 10 mg/kg in the rat

Table 6. Blood Levels Obtained After a Single 10 mg/kg Oral Dose of Compounds **96**, **61**, and **91** in the Rat

compd	nucleus	ester	AUC (ng·h/mL)	C_{max} (ng/mL)
96	tetralin	ethyl[a]	15910	5055
61	tetralone	ethyl[a]	11243	3630
91	benzopyran	ethyl[a]	14835	4850

[a] See note on Table 4.

(Table 6). Comparison of the data in Tables 4 and 6 demonstrates that the tetralone **61** and tetralin **96** behaved similarly in both species. However, the data shows that the rat provides somewhat higher levels of exposure after oral administration of these compounds. The ethyl ester of benzopyran **93** (**91**) produced an AUC of 14835 ng·h/mL and a C_{max} of 4850 ng/mL in the rat which was comparable to that achieved with **61** and **96**.

Encouraged by these results, we chose to determine the absolute bioavailability of the tetralin **96**, tetralone **61**, and benzopyran **91** in the rat. This was accomplished by determining a companion pharmacokinnetic profile which resulted from an intravenous administration of the parent free acid of each ester analogue. Comparison of the oral and intravenous exposure profiles for these compounds affords an absolute bioavailability in the rat of 28%, 23%, and 24% for the tetralin **96**, tetralone **61**, and benzopyran **91**, respectively. The bioavailability afforded by these compounds makes them attractive candidates for further investigations. As a prelude to these studies, the parent acids of the tetralin **96** (**79**), tetralone **61** (**63**), and benzopyran **91** (**93**) were evaluated for cross-reactivity with the related integrin $\alpha_v\beta_3$. No appreciable binding was observed at micromolar concentrations indicating that these compounds have high selectivity for human GPIIb-IIIa.

Discussion

Replacement of the bicyclic β-turn mimic found in lead isoquinolone **2** with a monocyclic derivative affords compound **8**, which has measurable affinity for GPIIb-IIIa (IC_{50} = 6 μM) but is approximately 10-fold less active than its bicyclic congener. Comparison of compounds **2** and **8** reveals that the overall distance between the acidic and basic moieties, and their spatial relationship, is approximately the same for these two compounds, thus accounting for the activity observed for monocyclic compound **8**. The data does however suggest that the conformational restriction afforded by the bicyclic motif contained in **2** is advantageous for activity in this family of molecules. Within the bicyclic series, the size of the B-ring (see Figure 1) also appears to be a factor that influences activity. The data demonstrates that a ring size of five (**19**) provides lower affinity for GPIIb-IIIa than ring sizes of six (**2**) or seven (**20**).

Incorporation of unsaturation into the lactam ring provides compound **28** which was 7-fold less potent than the lead isoquinolone **2**. While the structural difference between these two compounds initially appears minimal, comparison of models of isoquinolone **2** with the unsaturated compound **28** demonstrates that the lactam ring of this latter compound is less flexible, effectively forcing the aspartate isostere to reside coplanar with the aromatic A-ring. A similar coplanar relationship between the aromatic ring and the aspartate isostere is also seen for compound **19** which contains a 6,5-ring system. The role of the amide functionality in the B-ring of **2** was probed by moving the amide carbonyl from position 1 to position 3. Examination of models indicates that this transposition of the amide carbonyl changes the conformation of the B-ring from essentially flat to that of a boat, thus imparting a cup shape to the molecule. The lower activity afforded by **37** suggests that the conformation provided by this lactam isomer is less desirable than that of the related isoquinolone **2**.

The SARs afforded by the B-ring were further examined in the oxo-linked series with the incorporation of sp^3 hybridization at C1 and C2. These modifications afforded an increase in ELISA activity relative to isoquinolone **2**. Removal of the sp^2 character provided by the amide moiety allows the B-ring in these 6,6-systems to adopt a twist-chair conformation. Comparison of models of compounds **47**, **56**, and **71** (assuming that the aspartate isostere will occupy the pseudoequatorial position) reveals that the aspartate isostere adopts an overall topography similar to that of isoquinolone **2**. In contrast to the lactam leads, the B-ring of these latter compounds provides additional conformational flexibility, which may be in part responsible for the increase in potency observed for these molecules.

In the oxo-linked series, the functionality contained in the B-ring had some influence on activity. Isoquinoline **47** demonstrates that an additional basic residue proximal to the aspartate isostere is well-tolerated. Comparison of the activity data derived from the isoquinoline **47** and tetralone **56** with the tetralin derivative **71** indicates that the polar functionality at C1 or C2 in the former compounds improved activity. This observation is consistent with the fact that benzopyran **84** affords greater activity than tetralin **71**.

In accord with our earlier findings, linkage of the benzamidine to the supporting nucleus through an amide bond provides an increase in activity over an ether linkage. It is however interesting to note that in the amide-linked series, all nuclei provided similar intrinsic activity. This is in contrast to what was observed in the oxo-linked series where lack of polar functionality in the B-ring (compare **71** with **56** and **84**) and sp^2 rather than sp^3 hybridization at C2 (compare **2** with **47** and **56**) negatively impact activity. The amide linkage, in effect, provides the opportunity to incorporate a wider variety of functional groups into the β-turn mimic without sacrificing activity.

The data presented in this report are consistent with the literature in that they demonstrate a variety of central constraints can be employed in the design and preparation of compounds with affinity for GPIIb-IIIa.[1] Recently, a number of similarly substituted bicyclic inhibitors have been described.[29,33,40] The design of these compounds generally follows from structural information obtained during the spectroscopic analysis of potent RGD-containing cyclic peptides. These reports have suggested that a turn-extended-turn conformation of the RGD sequence predominates in solution for the peptides examined. [29,33] Several studies have demonstrated that mimicking this conformation with a bicyclic nucleus that is angularly substituted (see Figure 3) with an arginine and aspartate isostere affords compounds with exquisite

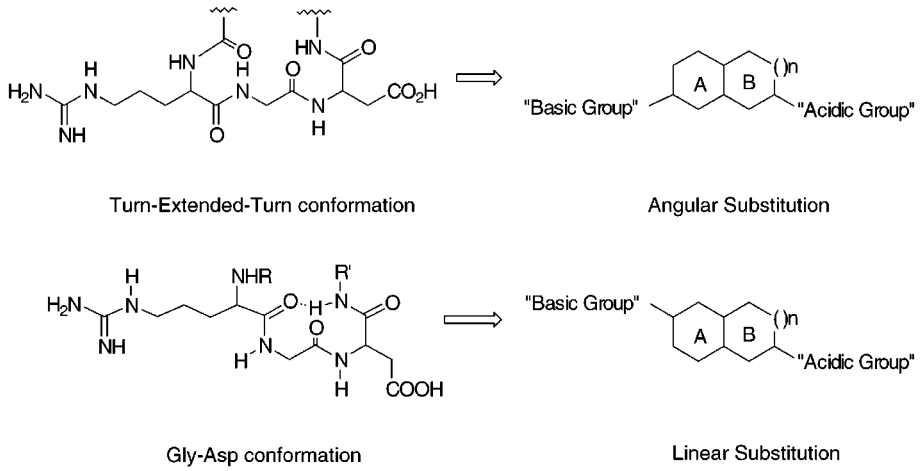


Figure 3. Comparison of turn-extended-turn and Gly-Asp conformational models.

potency and selectivity.[29,33] As we have discussed, our design strategy utilized a Gly-Asp conformation, which requires a linear substitution of the bicyclic β-turn mimic. The activity data for the linearly substituted compounds contained in this report demonstrate that a Gly-Asp conformation can also be utilized as a tool in the design of potent inhibitors of GPIIb-IIIa.

Okumura et al. have demonstrated that 2,7-substituted 6,6-bicyclic nuclei also can be effectively utilized in the design of potent antagonists of GPIIb-IIIa.[40] We have previously shown that the combination of an ether-linked benzamidine and an isoquinolone nucleus was sensitive to substitution patterns, with 2,6-substitution (linear) providing higher affinity for GPIIb-IIIa than 2,7-substitution (angular). Comparison of the data in this report with that disclosed[40] suggests that this observation may not be generalized to include all 6,6-nuclei. The fact that both 2,6- and 2,7-substituted nuclei can be optimized to yield potent inhibitors of GPIIb-IIIa provides additional evidence to support the notion that GPIIb-IIIa contains an extended cation binding site.[49]

While potency has been an important end point for all of these studies, selectivity for GPIIb-IIIa has also been a consideration. It is known that the RGD motif is recognized by other integrins, such as the closely related integrin $\alpha_V\beta_3$, and it has been suggested that ligand specificity among these receptors may be a function of the conformation afforded by the RGD sequence (or RGD mimic) in question.[50,51] It has been proposed that a Gly-Asp motif is an important design element to consider for the preparation of compounds with selectivity for $\alpha_V\beta_3$ relative to GPIIb-IIIa.[52] In contrast to this proposal, we have determined that several of our Gly-Asp mimics provide potent affinity for GPIIb-IIIa with little or no cross-reactivity for $\alpha_V\beta_3$. These data suggest that the presence of a Gly-Asp conformation in an RGD mimic is insufficient to confer selectivity for $\alpha_V\beta_3$ relative to GPIIb-IIIa.

Examination of the data presented in Tables 4 and 6 indicates that all of the compounds evaluated in this study provided measurable plasma levels after oral administration of 10 mg/kg. Isoquinolone **3** and tetralin **79** provided maximum blood levels of 126 and 252 ng/mL, respectively. Consistent with other observations in the literature, our results indicate that greater exposure was obtained with compounds in which the acid moiety had been masked by esterification.[48] Examination of the data contained in Figure 2 suggests that the nature of the ester prodrug can have an effect on oral availability. The observed trend for the tetralin series indicates that oral exposure decreased as the size of the alkyl portion of the ester increased. While we were able to obtain μg/mL blood levels with several molecules in this series, we were unable to look for efficacy surrogates in either the rat or guinea pig as these compounds have greatly diminished activity toward platelets from these species relative to that from humans. However, the fact that the human PRP IC_{50} values for the compounds listed in Table 3 range from 20 to 70 ng/mL indicates that if exposure in humans was similar, a single oral dose of 10 mg/kg would provide supra pharmacological plasma concentrations of active agent.

Conclusion

We have demonstrated that a variety of potent inhibitors of GPIIb-IIIa can be obtained through systematic modification of the lactam ring contained in lead isoquinolones **2** and **3**. We have demonstrated that this series of compounds benefits from a bicyclic central nucleus and that isoquinoline, tetralin, tetralone, and benzopyran ring systems provide active molecules. SARs suggest that completely planar ring systems in this series are less effective. Greater activity is observed with a variety of bicyclic ring systems that provide a B-ring which affords some conformational flexibility. Activity also appears to benefit from the incorporation of a polar group at positions C1 or C2 of the bicyclic core. Linkage of the benzamidine moiety to the supporting nucleus with an amide provides greater potency than the corresponding ether linkage, and activity for the amide-linked series is less sensitive to structural manipulation of the β-turn mimic.

Measurable blood levels were obtained after oral dosing of two of the most potent amidino acids. Exposure levels were however modest, and in an effort to improve this, the compounds were given as the corresponding ester prodrugs. These compounds, which lack the zwitterionic character possessed by the parent amidino acid, provided increased exposure. Evaluation of a series of amide-linked ester prodrugs in both the rat and guinea pig identified several compounds that provided substantial blood levels after oral administration of 10 mg/

kg. Efforts are underway to evaluate selected compounds for efficacy in a relevant animal model, and these studies will be reported shortly.

Experimental Section

All starting materials were commercially available or previously reported in the literature unless noted. All reactions were run in an atmosphere of dry nitrogen, and solvents and reagents were used without purification with the exception of THF which was distilled from sodium/benzophenone. Nuclear magnetic resonance spectra for characterization of synthesis products were recorded at 300 MHz on GE QE-300 and Bruker AC-300 spectrometers or at 400 MHz on a Varian Mercury spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane. High-resolution mass spectra were recorded on a VG Analytical ZAB2-SE instrument, and FD mass spectra were recorded on a MAT-731 instrument. Infrared spectra were recorded on a Nicolet DX10 FT-IR spectrometer with the medium noted in the individual experiment. Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed at Lilly Research Laboratories by the Physical Chemistry Department and are within ±0.4% of theory unless otherwise noted.

4-Hydroxybenzoylglycine 1,1-Dimethylethyl Ester (5). 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (19.7 g, 79.6 mmol) was added to a solution of 4-hydroxybenzoic acid (**4**) (10.0 g, 72.4 mmol) in 400 mL of CH_3CN and 100 mL of THF at room temperature. The mixture was stirred at room temperature for 0.25 h; then glycine *tert*-butyl ester (9.5 g, 72.4 mmol) was added, and the mixture stirred at room temperature for 72 h. The solvent was removed in vacuo. The residue was diluted with EtOAc, washed with 1 N HCl, H_2O, and saturated aqueous $NaHCO_3$, and then extracted five times with 1 N NaOH. The combined aqueous extracts were washed with EtOAc, acidified to pH 2 with concentrated HCl, and extracted five times with methylene chloride. The combined organic extracts were washed with saturated brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to afford 4.5 g (25%) of **5** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.0 (s, 1H), 8.55 (br t, 1H, J = 6 Hz), 7.75 (d, 2H, J = 8 Hz), 6.82 (d, 2H, J = 8 Hz), 3.85 (d, 2H, J = 6 Hz), 1.4 (s, 9H); IR (KBr) 3396, 3123, 2982, 1725, 1636, 1602, 1581, 1544, 1508, 1435, 1382, 1367, 1290, 1232, 1182, 11698, 855, 775 cm^{-1}; MS (FD) *m/e* 251. Anal. ($C_{13}H_{17}NO_4$) C,H,N.

4-[(4-Cyanophenyl)methoxy]benzoylglycine 1,1-Dimethylethyl Ester (6). Triton B (40% in methanol) (1.88 mL, 4.18 mmol) was slowly added to a solution of **5** (1.00 g, 3.98 mmol) in 50 mL of DMF at −5 °C. The mixture was stirred at −5 °C for 0.5 h; then α-bromo-*p*-tolunitrile (0.82 g, 4.18 mmol) was added, and the resultant mixture was stirred at −5 °C for an additional 4 h. The reaction mixture was diluted with EtOAc, washed three times each with 1 N HCl, H_2O, 1 N NaOH, H_2O, and saturated brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The oily white residue was recrystallized twice from EtOAc/Et_2O to afford 0.83 g (57%) of **6** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 (br t, 1H, J = 6 Hz), 7.8 (m, 4H), 7.62 (d, 2H, J = 8 Hz), 7.05 (d, 2H, J = 8 Hz), 5.25 (s, 2H), 3.82 (d, 2H, J = 6 Hz), 1.36 (s, 9H); IR (KBr) 3388, 2229, 1735, 1643, 1607, 1533, 1504, 1369, 1252, 1232, 1178, 1153, 1019, 880, 837, 827, 765, 558 cm^{-1}; MS (FD) *m/e* 366. Anal. ($C_{21}H_{22}N_2O_4$) C,H,N.

4-[[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]methoxy]benzolyglycine 1,1-Dimethylethyl Ester (7). This compound was prepared starting from nitrile **6** by employing the thio-Pinner sequence previously described:[38] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.8 (d, 2H, J = 8 Hz), 7.66 (d, 2H, J = 8 Hz), 7.3 (d, 2H, J = 8 Hz), 7.2(m, 2H), 6.85 (br t, 1H, J = 6 Hz), 6.82 (d, 2H, J = 8 Hz), 5.0 (s, 2H), 4.02 (d, 2H, J = 6 Hz), 1.44 (s, 9H), 1.43 (s, 9H); IR (KBr) 3374, 2979, 1745, 1617, 1576, 1532, 1503, 1456, 1392, 1367, 1280, 1252, 1226, 1164, 1017, 999, 843, 768 cm^{-1}; MS (EI) *m/e* 484. Anal. ($C_{26}H_{33}N_3O_6$) C,H,N.

4-[[4-(Aminoiminomethyl)phenyl]methoxy]benzoylglycine Trifluoroacetate (8). A solution of **7** (0.20 g, 0.42 mmol) in 4 mL of TFA was stirred at room temperature for 3 h. The solvent was removed in vacuo and the residue triturated with Et_2O and dried in vacuo to afford 0.16 g (90%) of **8** as a white powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.55 (br s, 1H), 9.3 (s, 2H), 9.0 (s, 2H), 8.7 (t, 1H, J = 6 Hz), 7.85 (t, 4H, J = 8 Hz), 7.7 (d, 2H, J = 8 Hz), 7.1 (d, 2H, J = 8 Hz), 5.35 (s, 2H), 3.9 (d, 2H, J = 6 Hz); IR (KBr) 3332, 3106, 1737, 1670, 1637, 1607, 1575, 1548, 1505, 1450, 1420, 1390, 1305, 1262, 1191, 1133, 1044, 844; MS (FAB) *m/e* 328. Anal. ($C_{19}H_{18}N_3O_6F_3$) C,H,N.

6-Amino-1,2,3,4-tetrahydroisoquinolineacetic Acid 1,1-Dimethyl Ester (44). A mixture of 6-aminoisoquinolone (**40**)[52] (1.0 g, 6.17 mmol) and lithium aluminum hydride (0.18 g, 18.5 mmol) in THF (50 mL) was maintained at reflux for 72 h. The mixture was then treated sequentially with H_2O (0.185 mL), 15% NaOH (0.185 mL), and H_2O (0.55 mL).[52] The resulting mixture was filtered and the filtrate concentrated to dryness. The residue was chromatographed on silica gel (25% MeOH/$CHCl_3$ to 25% MeOH/10% Et_3N/$CHCl_3$) which afforded 0.6 g of the desired amine **41** as a yellow semisolid. This material darkened appreciably upon standing and, as a result, was used immediately in the next step.

A portion of the material obtained in the previous step (0.20 g, 1.35 mmol) was treated with a mixture of *tert*-butyl bromoacetate (0.2 mL, 1.35 mmol), K_2CO_3 (0.19 g, 1.35 mmol), and CH_3CN (3 mL). After stirring for 24 h at room temperature, the above mixture was diluted with EtOAc (50 mL) and washed with H_2O (3 × 10 mL). The organic material was dried (K_2CO_3) and concentrated. Chromatography on silica gel (EtOAc:TEA:MeOH, 90/5/5) provided 0.24 g (70%) of **44** as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.79 (s, 4H), 3.25 (s, 2H), 3.64 (s, 2H), 6.38 (d, J = 2.4 Hz, 1H), 6.43 (dd, J = 2.4, 8.3 Hz, 1H), 6.76 (d, J = 8.2 Hz, 1H); IR ($CHCl_3$) 3007, 1736, 1624, 1369, 1150 cm^{-1}; MS (ES) *m/e* 263 (MH^+). Anal. ($C_{15}H_{22}N_2O_2$) C,H,N.

6-[(4-Cyanobenzoyl)amino]-1,2,3,4-tetrahydroisoquinolineacetic Acid 1,1-Dimethylethyl Ester (48). A solution of **44** (0.038 g, 0.144 mmol), 4-cyanobenzoic acid (0.021 g, 0.144 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.041 g, 0.217 mmol), DMAP (cat.), and CH_2Cl_2 (2 mL) was maintained at room temperature for 12 h. The solution was diluted with EtOAc (50 mL), washed with H_2O, and then concentrated. Chromatography (silica gel, EtOAc) provided 0.034 g (60%) of **48** as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 9H), 2.95 (m, 4H), 3.33 (s, 2H), 3.79 (s, 2H), 7.03 (d, J = 8.2 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.45 (s, 1H), 7.75 (d, J = 8.1 Hz, 2H), 7.95 (d, J = 8.1 Hz, 2H); IR (KBr) 3300, 2233, 1730, 1647, 1153 cm^{-1}; MD (FD) *m/e* 391. Anal. ($C_{23}H_{25}N_3O_3$) C,H,N.

6-[[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]iminomethyl]benzoyl]amino]-1,2,3,4-tetrahydroisoquinolineacetic Acid 1,1-Dimethylethyl Ester (49). A solution of **48** (0.10 g, 0.265 mmol) in triethylamine (1 mL) and pyridine (10 mL) was saturated with H_2S(g) and allowed to stand at room temperature overnight. The solution was then diluted with H_2O and the resulting mixture was extracted with EtOAc (3 × 25 mL). The extracts were combined and concentrated. The crude residue was taken up in acetone and treated with trifluoroacetic acid (0.05 mL, 0.53 mmol) and methyl iodide (0.08 mL, 1.33 mmol). The resulting solution was maintained at 60 °C for 2 h and then concentrated to dryness. The resulting solid was taken up in MeOH (1 mL) and treated with anhydrous NH_4OAc (0.051 g, 0.66 mmol). This solution was maintained at 60 °C for 2 h and then concentrated. This material was dissolved in THF/H_2O (1:1 10 mL) and treated with K_2CO_3 (0.22 g, 1.59 mmol) and di-*tert*-butyl dicarbonate (0.29 g, 1.32 mmol). After 1 h at room temperature, this mixture was diluted with EtOAc (50 mL), washed with H_2O, and then concentrated. Chromatography (silica gel, hexanes−EtOAc, 1:4) provided 0.34 g of **49** as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.52 (s, 9H), 2.87 (m, 4H), 3.29 (s, 2H), 3.74 (s, 2H), 6.96 (d, J = 8.2 Hz, 1H), 7.39 (br d,

J = 8.2 Hz, 1H), 7.43 (s, 1H), 7.82 (s, 4H), 8.18 (br s, 1H); IR (KBr) 1730, 1550, 1421, 1153 cm^{-1}; MS (FAB) *m/e* 509 (MH^+). Anal. ($C_{23}H_{36}N_4O_5$) C,H,N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-1,2,3,4-tetrahydroisoquinolineacetic Acid Trifluoroacetate (50). This compound was prepared from **49** using the same procedure employed for the preparation of **8**: ^{1}H NMR (300 MHz, CD_3OD) δ 3.26 (m, 2H), 3.65 (m, 2H), 4.11 (s, 2H), 4.50 (s, 2H), 7.20 (d, J = 8.47 Hz, 1H), 7.61 (m, 2H), 7.72 (s, 1H), 7.92 (d, J = 8.3 Hz, 2H), 8.12 (d, J = 8.2 Hz, 2H); IR (KBr) 3301, 1672, 1202, 1135 cm^{-1}; MS (FAB) *m/e* 353. Anal. ($C_{23}H_{22}N_4O_7F_6$) C,H,N.

6-Amino-1,2,3,4-tetrahydro-1-oxonaphthalene-2-acetic Acid Ethyl Ester (58). A mixture of **57**[45] (20 g, 77.2 mmol) and concentrated HCl (100 mL) was maintained at reflux for 0.5 h and then allowed to cool to room temperature. This mixture was diluted with H_2O (300 mL) and cooled to 0 °C. The mixture was neutralized to pH 4 by the careful addition of solid Na_2CO_3. The formed precipitate was collected by filtration and dried in vacuo. This material was dissolved in EtOH (250 mL) and the formed solution was saturated with HCl (g). After 2 h, the solution was concentrated to dryness in vacuo. The formed solid was dissolved in H_2O and the mixture saturated with $NaHCO_3$. This mixture was then extracted with EtOAc and the extracts were combined and concentrated. The crude solid was recrystallized from EtOAc/hexanes giving 10.0 g of **58** as a tan solid (mp 122−124 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (t, J = 7.1 Hz, 3H), 1.92 m, 1H), 2.14 (m, 1H), 2.34 (m, 2H), 2.80 (m, 1H), 2.95 (m, 3H), 4.13 (q, J = 7.1 Hz, 2H), 6.38 (s, 1H), 6.52 (dd, J = 1.8, 8.3 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H); IR (KBr) 1743, 1654, 1579, 1357 cm^{-1}; MS (FAB) *m/e* 248.1293 (248.1286 calcd for $C_{14}H_{18}NO_3$).

6-[[(4-Cyanophenyl)carbonyl]amino]-1,2,3,4-tetrahydro-1-oxonaphthylene-2-acetic Acid Ethyl Ester (59). This compound was prepared using the protocol outlined for the preparation of compound **48**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7.1 Hz, 3H), 2.0 (m, 1H), 2.25 (m, 1H), 2.45 (m, 1H), 3.15 (m, 4H), 4.20 (q, J = 7.1 Hz, 2H), 7.25 (s, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 8.1 Hz, 2H), 8.01 (d, J = 8.1 Hz, 2H), 8.05 (d, J = 8.6 Hz, 1H); IR (KBr) 3309, 2233, 1716, 1685, 1584, 1540 cm^{-1}; MS (FAB) *m/e* 377. Anal. ($C_{22}H_{20}N_2O_4$) C,H,N.

6-[[[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]carbonyl]amino]-1,2,3,4-tetrahydro-1-oxonaphthylene-2-acetic Acid Ethyl Ester (60). This compound was prepared from **59** using the thio-Pinner sequence previously reported:[38] ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.1 Hz, 3H), 1.59 (s, 9H), 2.0 (m, 1H), 2.22 (m, 1H), 2.45 (m, 1H), 3.0 (m, 4H), 4.20 (q, J = 7.1 Hz, 2H), 7.40 (dd, J = 2.1, 8.6 Hz, 1H), 7.80−7.95 (m, 5H), 8.05 (d, J = 8.6 Hz, 1H), 8.32 (br s, 1H); IR (KBr) 3434, 1736, 1669, 1147 cm^{-1}; MS (FAB) *m/e* 522. Anal. ($C_{27}H_{31}N_3O_6$) C,H,N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-1,2,3,4-tetrahydro-1-oxonaphthylene-2-acetic Acid Ethyl Ester Hydrochloride (61). This compound was prepared from **60** by treatment with neat TFA at room temperature for 1 h followed by concentration. The crude material was then dissolved in 1 N aqueous HCl and lyophilized giving the desired ester **61** as the hydrochloride salt: ^{1}H NMR (300 MHz, CD_3OD) δ 1.24 (t, J = 7.3 Hz, 3H), 1.97 (m, 1 h), 2.20 (m, 1H), 2.24 (dd, J = 6.2, 16.4 Hz, 1H), 2.79 (dd, J = 6.3 Hz, 16.4 Hz, 1H), 3.0 (m, 3H), 4.12 (q, J = 7.3 Hz, 2H), 7.63 (dd, J = 1.8, 8.5 Hz, 1H), 7.77 (s, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 8.15 (d, J = 8.2 Hz, 2H); IR (KBr) 3094, 1730, 1697, 1536, 1277 cm^{-1}; MS (FAB) *m/e* 394. Anal. ($C_{22}H_{24}N_3O_4Cl$) C,H,N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-1,2,3,4-tetrahydro-1-oxonaphthylene-2-acetic Acid Trifluoroacetate (63). A mixture of **60** (0.10 g, 0.19 mmol), NaOH (0.015 g, 0.383 mmol), and EtOH (5 mL) was maintained at room temperature for 4 h and then concentrated. The residue was taken up in H_2O (10 mL) and carefully neutralized (pH 5) with 10% aqueous $KHSO_4$. The formed precipitate was collected by filtration and dried under vacuum. This material was then dissolved in TFA (10 mL) and allowed to stand for 1 h at room temperature. The material was then concentrated and the residue was triturated with Et_2O forming a solid which was collected by filtration: ^{1}H NMR (300 MHz, CD_3OD) δ 2.0 (m, 1H), 2.25 (m, 1H), 2.50 (dd, J = 6.4,16.5 Hz, 1H), 2.86 (dd, J = 5.8, 16.5 Hz, 1H), 2.90−3.20 (m, 3H), 7.62 (dd, J = 1.9, 8.6 Hz, 1H), 7.78 (s, 1H), 7.94 (m, 3H), 8.14 (d, J = 8.3 Hz, 2H); IR (KBr) 3330, 3108, 1669, 1538 cm^{-1}; MS (FAB) *m/e* 366. Anal. ($C_{22}H_{20}N_3O_6F_3$) C,H,N.

7-[[(Phenylmethoxy)carbonyl]amino]-1,2-dihydronaphthylene (73). A solution of **72**[45] (5.0 g, 16.9 mmol) and EtOH (30 mL) was treated with $NaBH_4$ (0.64 g, 16.9 mmol) at room temperature. After 5 h, the solution was diluted with H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The extracts were combined and concentrated. The crude residue was taken up in benzene (50 mL) and treated with TsOH (0.1 g). The resulting mixture was maintained at reflux for 1 h with removal of H_2O and then allowed to cool to room temperature. The mixture was diluted with EtOAc (300 mL) and washed with saturated aqueous $NaHCO_3$. The organic material was dried ($MgSO_4$), filtered, and concentrated. Chromatography (silica gel, hexanes−EtOAc, 3:1) provided 3.9 g of **73** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (m, 2H), 2.78 (t, J = 8.13 Hz, 2H), 5.21 (s, 2H), 5.97 (m, 1H), 6.42 (d, J = 9.6 Hz, 1H), 6.65 (br s, 1H), 6.96 (d, J = 8.1 Hz, 1H), 7.12 (d, J = 8.1 Hz, 1H), 7.21 (s, 1H), 7.38 (m, 5H); IR (KBr) 3307, 1718, 1694, 1535, 1228 cm^{-1}; MS (FD) *m/e* 279. Anal. ($C_{18}H_{17}NO_2$) C,H,N.

1,2-Dihydroxy-6-[[(phenylmethoxy)carbonyl]amino]-1,2,3,4-tetrahydronaphthylene (74). A mixture of **73** (3.9 g, 13.97 mmol) *N*-methylmorpholine *N*-oxide (2.07 g, 15.37 mmol), *tert*-butyl alcohol (7.5 mL), acetone (7.5 mL), H_2O (10 mL), and OsO_4 (cat.) was stirred at room temperature for 6 h. The mixture was then diluted with EtOAc (100 mL) and washed sequentially with 1 N aqueous sodium bisulfite and H_2O. The organic material was dried ($MgSO_4$), filtered, and concentrated affording **74** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (m, 1H), 1.95 (m, 1H), 2.74 (m, 1H), 2.90 (m, 1H), 3.97 (m, 1H), 4.62 (d, J = 3.9 Hz, 1H), 5.16 (s, 2H), 6.69 (br s, 1H), 7.12 (d, J = 8.3 Hz, 1H), 7.21 (s, 1H), 7.35 (m, 6H); IR (KBr) 3419, 33.21, 1741, 1713, 1545, 1224 cm^{-1}; MS (FD) *m/e* 313. Anal. ($C_{18}H_{19}N0_4$) C,H,N.

Product Mixture 75. A mixture of diol **74** (1.33 g, 4.24 mmol), TsOH (cat.), and toluene (20 mL) was heated at reflux with removal of H_2O for 1 h and then allowed to cool to room temperature. The solution was then washed with 0.1 N NaOH, dried (K_2CO_3), and concentrated. In a separate reaction vessel, a mixture of diethyl *tert*-butyl phosphonoacetate (1.6 g, 6.37 mmol), NaH (0.25 g of a 60% dispersion in oil, 6.37 mmol), and THF (10 mL) was allowed to react and then cooled to −78 °C. A THF (10 mL) solution of the crude dehydration product was then added and the resulting mixture was allowed to warm to room temperature. The reaction mixture was diluted with EtOAc (50 mL) and washed with H_2O (2 × 20 mL). The organic material was dried ($MgSO_4$), filtered, and concentrated. Chromatography (silica gel, hexanes−EtOAc, 5:1) provided 0.7 g of **75** as a clear oil. Characteristic data for the product mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.29 (br t, J = 8.3 Hz, 2H), 2.79 (br t, J = 8.3 Hz, 2H), 3.07 (s, 2H), 5.17 (s, 2H), 6.25 (s, 1H), 6.61 (br s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 7.24 (br s, 1H), 7.35 (m, 5H); IR ($CHCl_3$) 1725, 1521, 1369, 1145, 1055 cm^{-1}; MS (ES) *m/e* 394 (MH+). Anal. ($C_{24}H_{27}NO_4$) C,H,N.

6-Amino-2-tetralinacetic Acid 1,1-Dimethylethyl Ester (76). A mixture of **75** (1.5 g, 3.81 mmol), Pd/C (10%, 1.0 g), and EtOH (30 mL) was stirred under an atmosphere of H_2 (balloon) for 2 h, then filtered, and concentrated. The crude residue was purified by chromatography (silica gel, hexanes−EtOAc, 2:1) giving 0.77 g (77%) of **76** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.4 (m, 1H), 1.47 (s, 9H), 1.95 (m, 1H), 2.1−2.3 (m, 3H), 2.40 (m, 1H), 2.75 (m, 3H), 6.45 (s, 1H), 6.49 (d, J = 8.1 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H); IR ($CHCl_3$) 1719, 1624, 1506, 1149 cm^{-1}; MS (FAB) *m/e* 262. Anal. ($C_{16}H_{23}NO_2 \cdot 0.15H_2O$) C,H,N.

6-[(4-Cyanobenzoyl)amino]-2-tetralinacetic Acid 1,1-Dimethylethyl Ester (77). A mixture of **76** (1.18 g, 4.52 mmol), 4-cyanobenzoic acid (0.73 g, 4.97 mmol), EDCI (1.29 g, 6.78 mmol), and CH_2Cl_2 (5 mL) was stirred at room temperature for 2 h. The mixture was then diluted with EtOAc (150 mL), washed with H_2O (2 × 25 mL), and concentrated. Chromatography (silica gel, 3:1 hexanes/EtOAc) provided 1.38 g (78%) of **77** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 1.46 (m, 1H), 1.95 (m, 1H), 2.25 (m, 3H), 2.45 (m, 1H), 2.95 (m, 3H), 7.05 (d, J = 8.2 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.41 (s, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.88 (br s, 1H), 7.98 (d, J = 8.2 Hz, 1H); IR (KBr) 2980, 2234, 1719, 1647, 1149 cm^{-1}; MS (FD) *m/e* 390. Anal. ($C_{24}H_{26}N_2O_3$) C,H,N.

6-[[[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]carbonyl]amino]-2-tetralinacetic Acid 1,1-Dimethylethyl Ester (78). This compound was prepared from **77** using the thio-Pinner sequence previously described:[38] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9H), 1.54 (s, 9H), 1.60 (m, 1H), 1.95 (m, 1H), 2.22 (m, 3H), 2.41 (m, 1H), 2.80 (m, 3H), 7.04 (d, J = 8.2 Hz, 1H), 7.3 (d, J = 8.1 Hz, 1H), 7.4 (s, 1H), 7.90 (m, 4H); IR (KBr) 1718, 1667, 1613, 1527, 1286 cm^{-1}; MS (FAB) *m/e* 508. Anal. ($C_{29}H_{37}N_3O_5$) C,H,N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-2-tetralinacetic Acid Trifluoroacetate (79). This compound was prepared from **78** using the same procedure employed for the preparation of **8**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.5 (m, 1H), 2.0 M, 4H), 2.21 (m, 1H), 2.39 (m, 2H) 2.45 (m, 1H), 2.90 (m, 3H), 7.04 (d, J = 7.7 Hz, 1H), 7.40 (m, 2H), 7.91 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.3 Hz, 1H); IR (KBr) 3322, 3104, 1712, 1667, 1141 cm^{-1}; MS (FAB) *m/e* 352. Anal. ($C_{22}H_{22}N_3O_5F_3 \cdot 0.2H_2O$) C,H,N.

6-[[(1,1-Dimethylethoxy)carbonyl]amino]-3,4-dihydro-2-oxo-2*H*-benzopyran (86). In a 1.5-L Parr bottle, 10 g of 20% Pd/C was washed with 100 mL of anhydrous THF overnight. After decantation of the palladium catalyst, the THF was removed and replaced by 900 mL of anhydrous THF. Then, 90.5 g (0.47 mol) of 6-nitrocoumarin, 155 g (0.71 mol) of di-*tert*-butyl dicarbonate, and 43 g of 0.3-nm molecular sieves were added and the reaction mixture was shaken with a Parr apparatus at a pressure of 25 psi for 1.5 h while the temperature was maintained below 60 °C. After shaking at room temperature at a pressure of 40 psi for 24 h, 10 g of 20% Pd/C, previously washed with THF, and 20 g of 0.3-nm molecular sieves were added and the hydrogen pressure was maintained for 46 h. The reaction mixture was filtered through Celite which was then washed with 200 mL of EtOAc. The organic layer was concentrated to dryness and the resulting solid crystallized from 400 mL of EtOAc to afford 86 g (70%) of **86**. The filtrate was concentrated to a volume of 100 mL to afford 26 g (21%) of **86** as a second crop: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.84 (dd, J = 10.3 Hz, 2.5 Hz, 1H), 6.98 (s, 1H), 6.87 (d, J = 10 Hz, 1H), 2.90 (t, J = 7.5 Hz, 2H), 2.70 (td, J = 7.5 Hz, 2.5 Hz, 2H), 1.46 (s, 9H); IR ($CDCl_3$) 1769, 1757, 1723, 1523, 1148 cm^{-1}; MS (FD) *m/e* 263. Anal. ($C_{14}H_{17}NO_4$) C,H,N.

6-[[(1,1-Dimethylethoxy)carbonyl]amino]-2[2*H*]-chromaneacetic Acid Ethyl Ester (87). To a solution of 52.66 g (0.2 mol) of **86** in 500 mL of CH_2Cl_2 under nitrogen at −78 °C was added dropwise 240 mL (0.24 mol) of a toluene solution of 1 M DIBAH within 1 h. After the reaction mixture was maintained at −75 °C for 1 h and then at −65 °C for 30 min, 60 mL of methanol and then 90 mL of water were added dropwise. The slurry was stirred at room temperature for 2 h and filtered through 100 g of Celite. The cake was washed four times with 250 mL of dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated to dryness to afford 53 g (100%) of crude reduction product. This material was of sufficent purity for use in the next step. To a solution of 53 g of this material in 500 mL of toluene was added 70 g (0.2 mol) of (carbethoxymethylene)triphenylphosphorane and the resulting solution maintained at reflux under nitrogen for 22 h. After cooling to room temperature and evaporation of toluene under reduced pressure, the resulting oil (125 g) was purified by filtration on 1 kg of silica gel eluting first with pure cyclohexane and then with cyclohexane/EtOAc (from 95:5 to 80:20, v/v) to yield 51 g of **87** (76%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (br s, 1H), 6.70 (dd, J = 8.9 Hz, 2.5 Hz, 1H), 6.48 (d, J = 8.9 Hz, 1H), 6.16 (s, 1H), 4.21 (m, 1H), 3.98 (q, J = 7.2 Hz, 2H), 2.64 (ddd, J = 16.5 Hz, 10.4 Hz, 5.2 Hz, 1H), 2.55 (dd, J = 15.4 Hz, 7.4 Hz, 1H), 2.52 (ddd, J = 16.5 Hz, 5.2 Hz, 4.1 Hz, 1H), 2.36 (dd, J = 15.4 Hz, 6.1 Hz, 1H), 1.84 (dm, J = 13.5 Hz, 1H), 1.54 (m, 1H), 1.29 (s, 9H), 1.07 (t, J = 7.2 Hz, 3H); IR (KBr) 3358, 1736, 1698, 1526, 1238 cm^{-1}; MS (ES) *m/e* 335. Anal. ($C_{18}H_{25}NO_5$) C,H,N.

6-Amino-2[2*H*]-chromaneacetic Acid Ethyl Ester (88). A mixture of **87** (1.0 g, 2.98 mmol) was dissolved in anhydrous TFA (10 mL) and allowed to stand at room temperature for 1 h. This solution was then concentrated to dryness and the residue dissolved in H_2O (10 mL). This solution was made basic with 1 N NaOH and then extracted with EtOAc (3 × 20 mL). The extracts were dried (Na_2SO_4), filtered, and concentrated to afford essentially pure **88**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.3 Hz, 3H), 1.74 (m, 1H), 2.02 (m, 1H), 2.55 (dd, J = 5.8, 15.1 Hz, 1H), 2.85−2.60 (m, 3H), 4.18 (q, J = 7.3 Hz, 2H), 4.38 (m, 1H), 6.40 (d, J = 1.8 Hz, 1H), 6.46 (dd, J = 1.8, 8.3 Hz, 1H), 6.06 (d, J = 8.3 Hz, 1H); IR ($CHCl_3$) 2985, 1729, 1624, 1499, 1086 cm^{-1}; MS (ES) *m/e* 253. Anal. ($C_{13}H_{17}NO_3$) C,H,N.

6-[(4-Cyanobenzoyl)amino]-2[2*H*]-chromaneacetic Acid Ethyl Ester (89). This compound was prepared from **88** and 4-cyanobenzoic acid according to the procedure outlined for the preparation of **77**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, J = 7.23 Hz, 3H), 1.80 (m, 1H), 2.10 (m, 1H), 2.61 (dd, J = 5.8, 15.6 Hz, 1H), 2.80 (m, 2H), 2.95 (m, 1H), 4.20 (q, J = 7.3 Hz, 2H), 4.29 (m, 1 h), 6.79 (d, J = 8.7 Hz, 1H), 7.20 (br d, J = 8.7 Hz, 1H), 7.45 (s, 1H), 7.63 (s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.94 (d, J = 8.3 Hz, 2H); IR (KBr) 3270, 2228, 1726, 1645, 1533, 1188 cm^{-1}; MS (ES) *m/e* 364. Anal. ($C_{21}H_{20}N_2O_4 \cdot 0.24H_2O$) C,H,N.

6-[[[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]carbonyl]amino]-2[2*H*]-chromaneacetic Acid Ethyl Ester (90). This compound was prepared from **89** utilizing the thio-Pinner sequence previously disclosed:[38] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.53 (s, 9H), 1.75 (m, 1H), 2.05 (m, 1H), 2.76 (dd, J = 5.8, 15.6 Hz, 1H), 2.80 (m, 3H), 4.19 (q, J = 7.3 Hz, 2H), 4.25 (m, 2H), 6.75 (d, J = 8.9 Hz, 1H), 7.23 (m, 1H), 7.47 (s, 1H), 7.73 (s, 4H), 8.35 (br s, 1H); IR ($CHCl_3$) 1729, 1660, 1613, 1528, 1497, 1286 cm^{-1}; MS (ES) *m/e* 481. Anal. ($C_{26}H_{31}N_3O_6 \cdot 1.3H_2O$) C, H, N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-2[2*H*]-chromaneacetic Acid Ethyl Ester Trifluoroacetate (91). The title compound was prepared from **90** by reaction with neat TFA at room temperature for 1 h followed by concentration. The crude residue was triturated with Et_2O yielding a white solid which was collected by filtration: ^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (t, J = 7.3 Hz, 3H), 1.77 (m, 1H), 2.10 (m, 1H), 2.60−3.0 (m, 4H), 4.18 (q, J = 7.3 Hz, 2H), 4.40 (m, 1H), 6.72 (d, J = 8.7 Hz, 1H), 7.31 (m, 1H), 7.40 (m, 1H), 7.89 (d, J = 8.3 Hz, 2H), 8.05 (d, J = 8.3 Hz, 2H); IR (KBr) 3314, 3089, 1735, 1669, 1205 cm^{-1}; MS (ES) *m/e* 381. Anal. ($C_{23}H_{24}N_3O_6F_3 \cdot 0.38H_2O$) C, H, N.

6-[[[4-(Aminoiminomethyl)phenyl]carbonyl]amino]-2[2*H*]-chromaneacetic Acid Trifluoroacetate (93). This compound was prepared from **90** using the procedure outlined for the preparation of **63**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.75 (m, 1H), 2.10 (m, 1H), 2.60−2.84 (m, 3H), 2.90 (m, 1H), 4.42 (m, 1H), 6.74 (d, J = 8.8 Hz, 1H), 7.33 (dd, J = 2.9, 8.7 Hz, 1H), 7.41 (d, J = 2.9 Hz, 1H), 7.92 (d, J = 8.3 Hz, 2H), 8.13 (d, J = 8.3 Hz, 2H); IR (KBr) 3321, 3101, 1715, 1667, 1496, 1205 cm^{-1}; MS (ES) *m/e* 354 (MH^+). Anal. ($C_{21}H_{20}N_3O_6F_3 \cdot 0.4H_2O$) C,H,N.

Biological Assays. 1. Solid-Phase Ligand Binding Assays. The ability of the compounds to antagonize the binding of fibrinogen to GPIIb-IIIa and vitronectin to $\alpha_v\beta_3$ was determined by ELISA (enzyme-linked immunoadsorbent assay) as previously described.[54,55]

2. Platelet Aggregation Assay. Assessment of functional blockade of GPIIb-IIIa was made using ADP-induced platelet aggregation in human platelet-rich plasma (PRP). Varying

concentrations of test compound or vehicle were incubated in human PRP for 1 min prior to the addition of ADP (5 mM), and the aggregation response was followed turbidometrically.[56]

3. Oral Dosing of Compounds to Rats and Guinea Pigs. Animals were fasted overnight and dosed by oral gavage. The compounds were formulated in 50% poly(ethylene glycol) 300 at 3 mg/mL and administered at 10 mg/kg. Blood was collected under Isoflurane anesthesia into 3.8% trisodium citrate (9:1), and plasma was prepared for subsequent analysis by HPLC.

4. Plasma Analysis Procedure. Plasma (0.2 mL) was mixed with 0.8 mL of pH 1 phosphate buffer containing internal standard and then extracted using IST Isolute C2 (EC) extraction cartridges. The extracts were analyzed by HPLC using an acetonitrile/pH 3 sodium dodecyl sulfate mobile phase and an Inertsil 150- × 3.2-mm column maintained at 40 °C with UV detection at 235 nm. The standard curves for tested analogues were linear from 10 to 5120 ng/mL.

Supporting Information Available: Complete experimental procedures for the preparation of compounds **19**, **20**, **28**, **37**, **47**, **56**, **71**, **84**, and **94**−**99**. This information is available free of charge via the Internet at http://pubs.acs.org.

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