HETEROCYCLES, Vol. 62, 2004, pp. 543 - 557
Received, 6th August, 2003, Accepted, 7th October, 2003, Published online, 7th November, 2003

A CONCISE SYNTHESIS OF AN INDENOPYRROLIDINE-BASED DUAL $\alpha_V\beta_3/\alpha_V\beta_5$ INTEGRIN ANTAGONIST

Diane K. Luci, Rosemary J. Santulli, Diane A. Gauthier, Brett A. Tounge, Shyamali Ghosh, Jef C. Proost, William A. Kinney,* Bart De Corte, Robert A. Galemmo, Jr., Joan M. Lewis, Warren E. Dorsch,[†] Michael W. Wagaman,[†] Bruce P. Damiano, and Bruce E. Maryanoff

Wkinney1@prdus.jnj.com, Johnson & Johnson Pharmaceutical Research & Development, Spring House, PA 19477-0776, and [†]C. B. Research and Development, Inc., New Castle, DE, U.S.A.

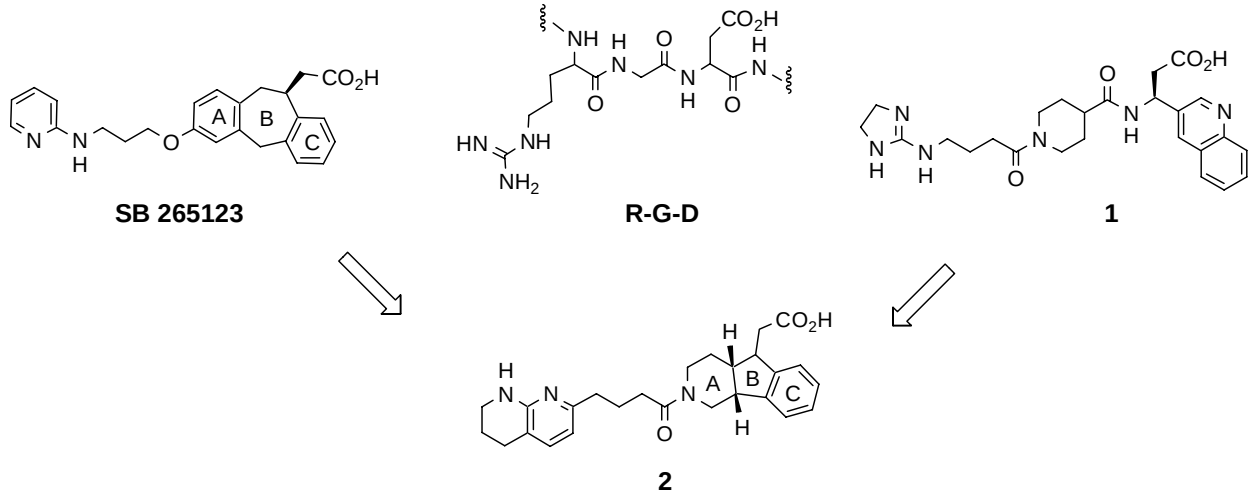
Abstract – A new class of dual $\alpha_V\beta_3/\alpha_V\beta_5$ integrin antagonists containing a central *cis*-fused cyclopentane ring was identified. Because of its increased structural rigidity, the indenopyrrolidine ring system provides insight into the active conformation of other $\alpha_V\beta_3$ ligands. A concise synthesis of the indenopyrrolidine ring system was accomplished by 1,3-dipolar cycloaddition. Individual isomers of the most active compound were separated by chiral HPLC and their biological activities were compared.

INTRODUCTION

The $\alpha_V\beta_3/\alpha_V\beta_5$ integrins are cell adhesion receptors that recognize matrix proteins specifically containing the cell-adhesion tripeptide motif arginine-glycine-aspartic acid (RGD). Both integrins are expressed on endothelial cells, smooth muscle cells, osteoclasts and tumor cells and behave as mediators of cell adhesion, migration, and survival. Expression of these integrins is typically minimal on normal blood vessels, but is significantly up-regulated in response to a variety of stimuli and play a key role in angiogenesis. From results with preclinical pharmacological models, antagonists to $\alpha_V\beta_3/\alpha_V\beta_5$ integrins may be useful for treating osteoporosis, growth and metastasis of malignant tumors, diabetic retinopathy, arthritis, and coronary restenosis.[1] The related integrin $\alpha_{IIb}\beta_3$, which is vital to clotting and thrombosis, also recognizes the RGD sequence. Thus, in order to limit the bleeding liability of a dual $\alpha_V\beta_3/\alpha_V\beta5$ antagonist, high selectivity versus $\alpha_{IIb}\beta_3$ is desired.

Since the early integrin antagonists were based on the peptide RGD motif, it initially proved difficult to obtain acceptable oral bioavailability. One notable exception was the potent tricyclic dual antagonist SB-265123 ($\alpha_V\beta_3$ K_i = 4 nM, $\alpha_V\beta_5$ K_i = 1.3 nM, $\alpha_{IIb}\beta_3$ K_i = 9 μM), which is reported to have 100% oral bioavailability in rats (Figure 1).[2] It contains a guanidine mimetic of R and the carboxylic acidic group of D, but has a highly lipophilic, conformationally constrained core structure. The central seven-membered ring would be expected to exist in two rapidly interconverting puckered conformations in which the A and C rings are almost perpendicular to each other. It has not been reported which conformation is responsible for biological activity. We sought to find another ring system that would position the C-ring, carboxylic acid, and basic tail in similar orientations, to obtain a potent, orally bioavailable dual antagonist. It had been shown[3] that **1** ($\alpha_V\beta_3$ IC_{50} = 3.2 ± 0.9 nM, $\alpha_V\beta_5$ IC_{50} = 490 ± 160 nM, $\alpha_{IIb}\beta_3$ IC_{50} = 120 ± 22 nM) is a potent antagonist of $\alpha_V\beta_3$, suggesting that the piperidineamide can be a substitute for the oxyphenyl group of SB-265123 (Figure 1). Target (**2**) would be a prototype combining the piperidine A-ring and phenyl C-ring, but with a five-membered B-ring. This arrangement would display a similar perpendicular architecture for the A- and C-rings. Because five-membered rings are more stable in the *cis*-orientation, the synthesis of the desired diastereomer was expected to be straightforward. However, we did not know a priori which relative stereochemistry would be required at the position beta to the carboxylic acid in **2**. The tetrahydronaphthyridine basic group was chosen because others had shown it to be beneficial for oral bioavailability.[4] In addition to being more lipophilic than **1**, it was hoped that targets such as **2** would show improved activity for $\alpha_V\beta_5$ and better selectivity over $\alpha_{IIb}\beta_3$.

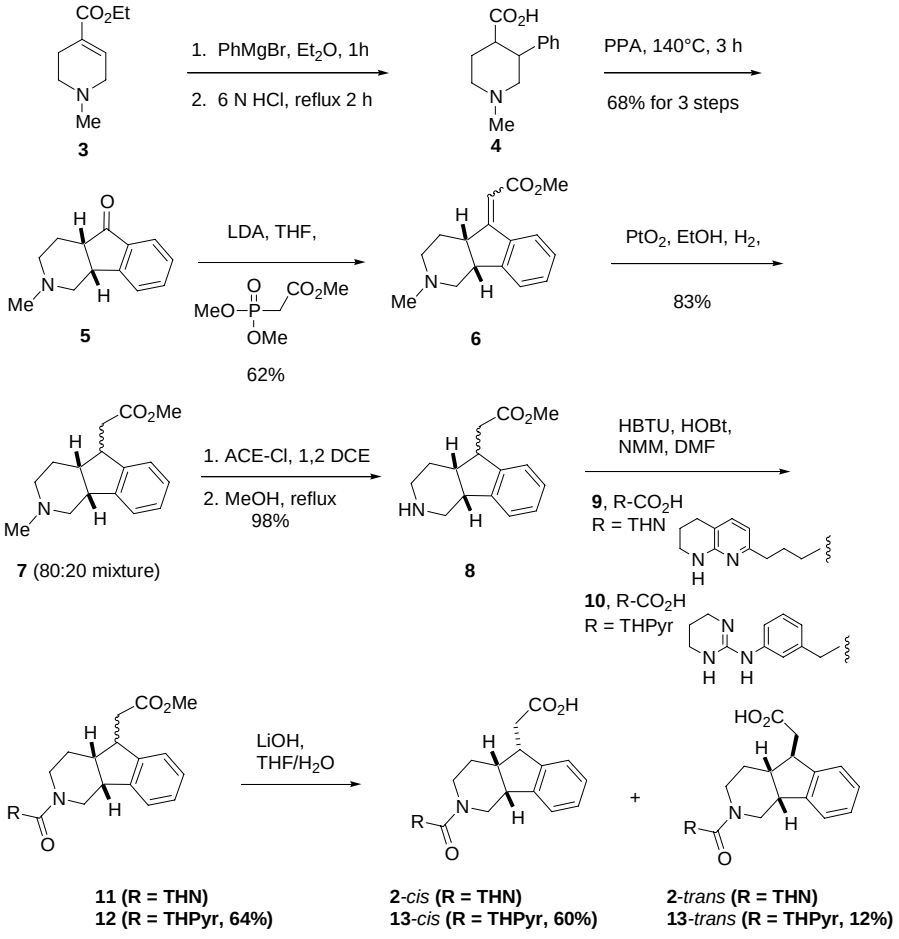
Figure 1



RESULTS AND DISCUSSION

The synthesis of indenopiperidines (**2**) and (**13**) are depicted below (Scheme 1). The tricyclic ring system in target (**5**) was constructed in three steps according to a literature method.[5] Ketone (**5**) was treated with the Horner-Emmon's reagent trimethylphosphonoacetate to afford **6** as a mixture of *E*- and *Z*-isomers in good yield. Hydrogenation afforded a mixture of diastereomers with the *cis*-isomer predominant (80:20 ratio by NMR and HPLC), which would be formed by delivery of hydrogen from the most accessible β-face. The *N*-methyl group in **7** was conveniently removed by heating with ACE-Cl to yield **8**. Finally, we added the tetrahydronaphthyridine (THN) carboxylic acid (**9**)[6] or tetrahydropyrimidine (THPyr) acid (**10**)[6] to afford the esters (**11**) and (**12**), which were saponified to **2** and **13**, respectively. The isomers of **13** were separable in the final step with **13**-*cis* in the majority, whereas **2** was tested as a (60:40) mixture.

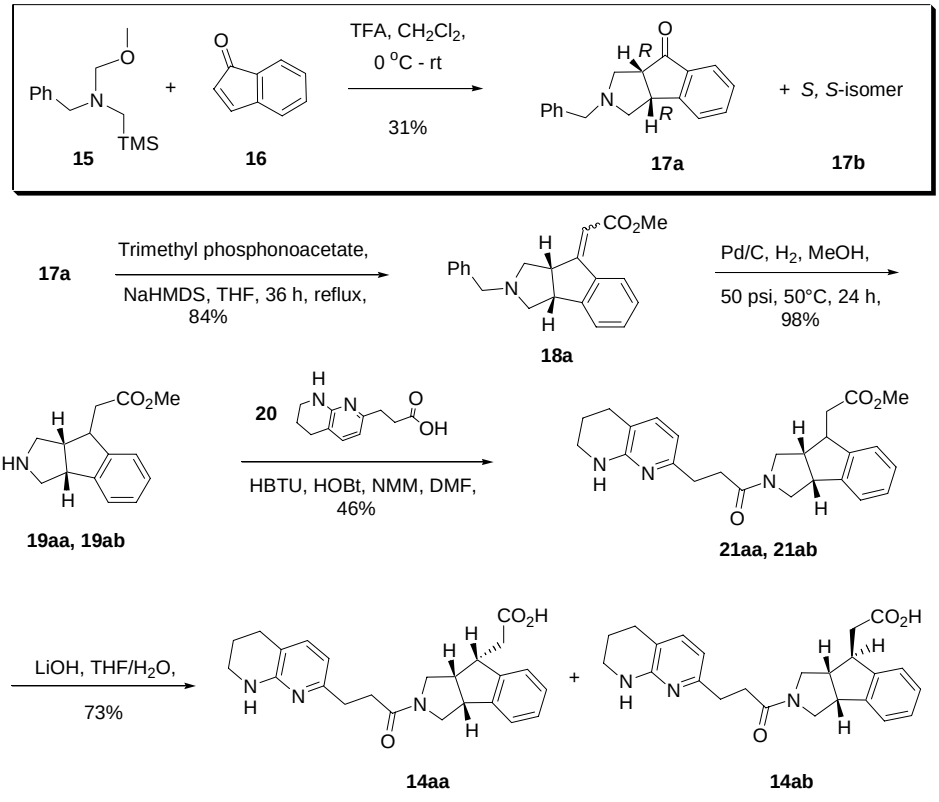
Scheme 1



It was also of interest to prepare the indenopyrrolidine structures such as **14** (Scheme 2). The required indenopyrrolidine (**17**) was initially prepared by the seven-step literature procedure,[7] but it proved more

convenient to make it in a novel way by dipolar cycloaddition. The 1,3-dipolar cycloaddition of nitrones to indenones has precedence in the synthesis of the related indenoisoxazolidines.[8] The required ylide, prepared in situ by reacting **15** with trifluoroacetic acid, reacted with indenone (**16**)[9] to afford **17** in modest yield directly. Compound **17** synthesized by 1,3-dipolar cycloaddition was indistinguishable (^{1}H NMR, HPLC, MS) from material obtained by the alternative method.[7] Ketone (**17**) was initially taken forward to **14** as a mixture of enantiomers, but **17** was also separated into its two enantiomers (**17a**) and (**17b**) by chiral HPLC. For example ketone (**17a**) (assumed *R*, *R* as shown) was elaborated as before to afford a mixture of *E*- and *Z*-isomers (**18a**). The double bond was reduced and the benzyl group removed by treating this intermediate with hydrogen at elevated temperature in the presence of palladium to give amines (**19aa**) and (**19ab**) in roughly equal amounts (the second letter denotes the stereoisomer at the third chiral center). The mixture **19aa** and **19ab** was then combined with THN acid (**20**)[6] and hydrolyzed to afford the targets (**14aa**) and (**14ab**), which were tested as a mixture and found to be active; compounds (**14ba**) and (**14bb**), prepared from **17b**, were inactive. Separation of enantiomers (**14aa**) from **14ab** was accomplished by chiral chromatography.

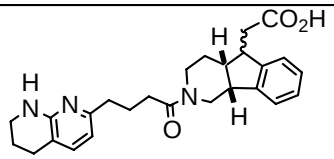
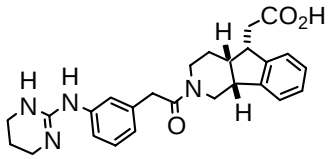
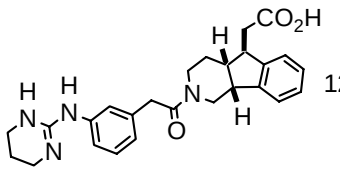
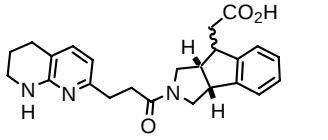
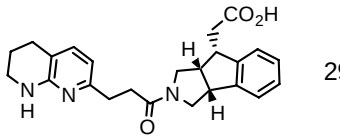
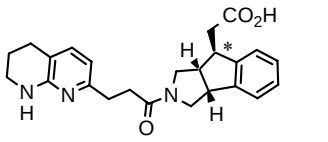
Scheme 2



Compounds (**2**) and (**13**) were evaluated in the $\alpha_V\beta_3$, $\alpha_V\beta_5$, and $\alpha_{IIb}\beta_3$ integrin binding assays.[10] Compound (**2**) had promising activity at antagonizing $\alpha_V\beta_3$ and, in addition, it had dual activity and was

quite selective (>500-fold) relative to $\alpha_{IIb}\beta_3$ (Table). Although the potency was not what we required, it was a good starting point. The major diastereomer of **13**-*cis* was slightly more potent than **13**-*trans* at $\alpha_V\beta_3$, but less so at $\alpha_V\beta_5$. Therefore, the acetic acid group in the β-orientation leads to better dual activity. It was thought that the moderate potency of **2** and **13** were due to improper alignment of the basic tail relative to the carboxylic acid functionality, as compared to SB-265123. By replacing the piperidine A-ring with a pyrrolidine, the basic appendage would be brought in closer proximity to the carboxylic acid in target (**14**).

Table

		IC$_{50}$ (nM)		
		$\alpha_v\beta_3$[a]	$\alpha_v\beta_5$[a]	$\alpha_{IIb}\beta_3$[b]
2		85 ± 8	230 ± 40	> 50,000
13-*cis*		66 ± 7	1,020 ± 180	17,700 ± 300
13-*trans*		126 ± 40	217 ± 5	18,000 ± 800
14		26 ± 8	15 ± 2	>50,000
14aa		290 ± 90	260 ± 60	>50,000
14ab		17 ± 3	5.8 ± 1.2	>50,000

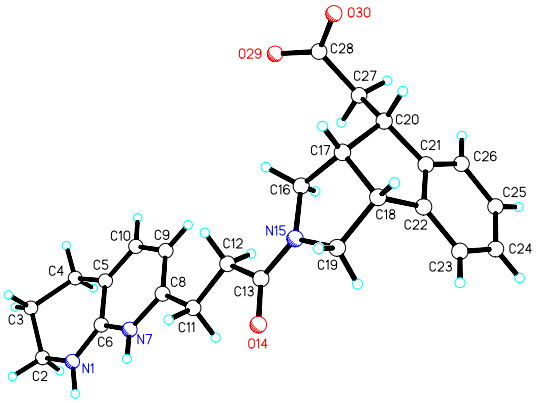
[a] Inhibition of human vitronectin binding to immobilized $\alpha_V\beta_3$ and $\alpha_V\beta_5$ (N ≥ 3).[10]

[b] Inhibition of biotinylated fibrinogen binding to immobilized $\alpha_{IIb}\beta_3$ (N ≥ 3).[10]

Compound (**14**) was initially tested a mixture of four isomers (ratio: 1:1:1:1) and found to have good potency and selectivity. The homolog of **14** prepared from THN acid (**9**) was less potent ($\alpha_V\beta_3$ IC$_{50}$ =

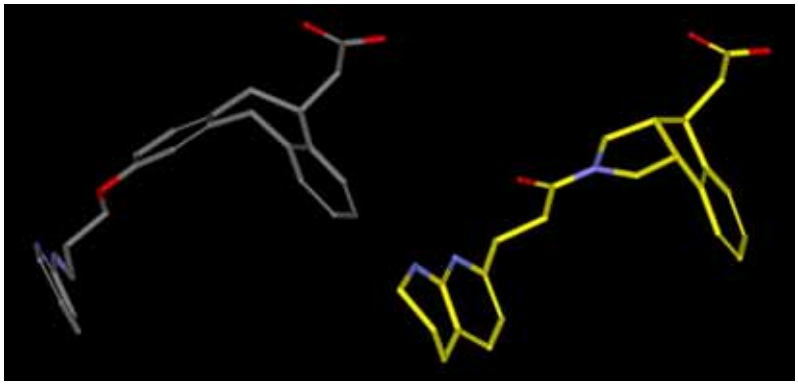
0.41 μM), whereas THN acid (**9**) was a useful length in the piperidine series (**2**). Because of the promising potency of **14**, an effort was made to isolate the active isomer or isomers. The mixture of isomers with the acetic acid group in the α- and β-orientations was prepared from the individual chiral *cis*-pyrrolidines (**17a**) and (**17b**). The mixture of isomers (**14ba**) and (**14bb**) was inactive at 1 μM. Biological activity resided in isomers (**14aa**) and (**14ab**), which were separated by chiral chromatography and evaluated individually; the most potent isomer was **14ab**. Compound (**14aa**) was crystalline and an

Figure 2. X-Ray structure of **14aa.**



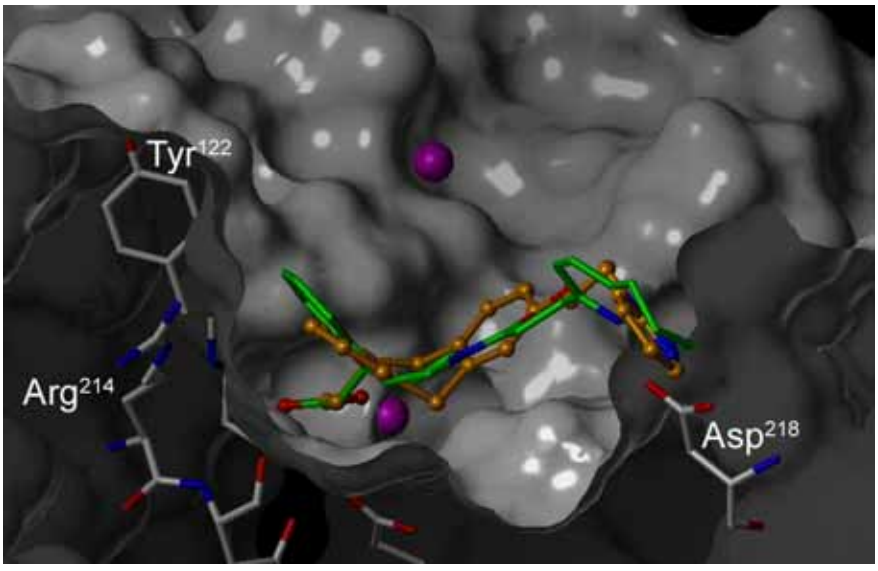
X-Ray structure was obtained,[11] which proved the relative stereochemistry of **14aa** and **14ab** (Figure 2). The relative stereochemistry of **14ab** is the same as **13**-*trans* in the piperidine series, which had the best dual activity. The absolute stereochemistry drawn for **14ab** (Table) is consistent with the known stereochemistry of the active isomer SB-265123 at the center beta to the carboxylic acid. If this is correct, then **14ab** is locked in a right turn conformation (Figure 3).[12] This would imply that the active conformer of SB-265123 is also the right turn form.

Figure 3. Comparison of the Right Turn Conformations of SB-265123 (gray) and **14ab** (yellow).



A comparison of the docking of **14ab** and SB-265123 to an $\alpha_V\beta_3$ receptor model was done to evaluate their three dimensional geometries and to test whether the right turn conformation was an acceptable binding mode (Figure 4). The model was constructed[13] based on the published X-Ray structure for the $\alpha_V\beta_3$ receptor/RGD ligand (cyclic-**Arg**-**Gly**-**Asp**-*D*-Phe-N-MeVal) complex.[14] A similar modeling study was recently reported for a biphenyl-arylsulfonamide based integrin antagonist.[15] In the present study, the key interactions seen in the X-Ray structure between the RGD ligand and the protein are preserved with the docked poses of **14ab** and SB-265123. For both compounds, the carboxylic acid moiety is positioned for interaction with the so called metal ion-dependent adhesion site (MIDAS)[14] and the basic moiety is positioned to form H-bond contacts to Asp^{218}. As is evident in Figure 4, the right turn structural motifs for **14ab** and SB-265123 fit with good overlap in the binding site. The phenyl rings of the turn motif are oriented in a similar position to the phenyl ring of the RGD ligand (*D*-Phe). However, for **14ab** and SB-265123, the ring does not extend far enough out to interact with Tyr^{122}, as positioned in the X-Ray structure.

Figure 4. Compounds (**14ab**) (green) and SB-265123 (bronze) docked into the binding pocket of $\alpha_v\beta_3$. The Mn^{2+} atom of MIDAS is shown in purple.



Compound (**14ab**) is a potent and selective $\alpha_V\beta_3/\alpha_V\beta_5$ antagonist. The mixture of four isomers (**14**) was not well absorbed orally in the rat (C_{max} = 0.06 μM at 15 mg/kg). However, **14** had high aqueous solubility (> 1 mg/mL at pH 2.0 and 7.4), medium absorption potential in CACO-2 (0.70 x 10^{-6} cm/s), and good metabolic stability in human liver microsomes ($t_{1/2}$ > 100 min), indicating good pharmacokinetic (PK) potential in the series.[16] Furthermore, the structure has no Lipinski's rule-of-5 violations.[17]

In conclusion, a potent dual $\alpha_V\beta_3/\alpha_V\beta_5$ antagonist (**14ab**) with a novel tricyclic indenopyrrolidine core, was identified. A concise synthesis was developed to prepare the required 5-5-6 ring system in one step. The structural rigidity of the indenopyrrolidine core provides information about the active conformation of other $\alpha_V\beta_3$ ligands. Although **14ab** lacks oral bioavailablity, it has favorable *in vitro* PK and physicochemical attributes, which could lead to a viable drug candidate in due course.

EXPERIMENTAL

All analytical HPLC was done on the Hewlett Packard 1100 series HPLC at wavelengths of 220 and 254 nm unless otherwise stated. The method used was a gradient of 10 to 90% over 4 min with 2 min equilibration. The mobile phase was acetonitrile with 0.16% TFA/water with 0.16 % TFA and the column was a Kromasil 5 cm x 2.1 mm; 3 μm. Purifications were carried out on the Gilson 333 Prep with the 215 liquid handler. The mobile phase was 10 to 90% gradient over 1 hour acetonitrile with 0.16 % TFA and H_2O with 0.16% TFA, and a Kromasil (10 μm, 100 Å, C18, 250 x 50.00 mm) filled Phenomenex column was employed. The UV triggered chiral preparative HPLC work was accomplished using a Dynamic Axial Compression type Prochrom LC50 column, which was filled with 500 grams of stationary phase. A Prep LC 4000 (Waters) quaternary gradient low pressure mixing pump, a K-2500 UV detector (KNAUER), a 233 XL auto injector (Gilson), a 402 Syringe pump (Gilson), a 202 fraction collector (Gilson), an rh.7030L fraction collector valve (Gilson), and Unipoint control software (Gilson) were utilized.

(3-Methyl-1,2,3,4,4a,9a-hexahydro-3-azafluoren-9-ylidene)acetic acid methyl ester (6)

A solution of trimethyl phosphonoacetate (3.9 mL, 24 mmol) in THF (40 mL) was chilled to –78 °C before 2 M LDA in THF (9.7 mL, 19 mmol) was added dropwise. This solution was stirred at –78 °C for 30 min, warmed to rt, and heated to 40 °C for 1 h. The solution was allowed to come to rt before a solution of **5**[5] (2.44 g, 12.1 mmol) in 40 mL of THF was added over a 30 min period. The reaction mixture was heated to reflux for 24 h, cooled to rt, and concentrated under high vacuum. The brown oil was taken up in 100 mL of ethyl acetate and washed with 50 mL of 1N sodium hydroxide solution. The basic layer was washed with additional ethyl acetate (2 x 75 mL), and the organic layers were combined, washed 1 time with brine, dried over sodium sulfate, and concentrated. The brown oil was purified on silica gel with 5% methanol in dichloromethane to give **6** (2.02 g, 7.86 mmol, 62%) as a mixture of isomers (60/40); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.65 (d, 0.5 H, J = 8 Hz), 7.75 (d, 0.5 H, J = 8 Hz), 7.42-7.27 (m, 3 H), 6.23 (s, 0.4 H), 5.88 (s, 0.6 H), 3.96-3.92 (m, 0.5 H), 3.83-3.78 (m, 0.5 H), 3.77 (s, 3 H), 3.31-3.25 (m, 1 H), 2.97 (q, 0.5 H, J = 5 Hz), 2.66 (q, 0.5 H, J = 5 Hz), 2.46-2.31 (m, 2 H), 2.29 (s,

1.2 H), 2.24 (s, 1.8 H), 2.17-2.14 (m, 0.5 H), 2.05-1.90 (m, 1 H), 1.82-1.76 (m, 0.5 H), 1.46-1.22 (m, 1 H); ^{13}C NMR ($CDCl_3$, 300 MHz) ppm 167.6, 166.9, 165.6, 161.3, 150.6, 149.0, 139.4, 137.3, 131.6, 131.1, 129.4, 127.4, 127.3, 123.7, 123.6, 122.9, 111.1, 107.9, 58.3, 55.2, 53.8, 53.1, 51.5, 51.4, 47.2, 46.8, 46.0, 43.1, 42.6, 41.8, 28.3, 27.7; MS (ES^+) m/z (relative intensity) 258.1 ($(M + H)^+$, 100), 299.1 ($(M + MeCN)^+$, 20).

(3-Methyl-2,3,4,4a,9,9a-hexahydro-1*H*-3-azafluoren-9-yl)acetic acid methyl ester (7)

The reduction of **6** (990 mg, 3.85 mmol) was carried out on a Parr apparatus under 44 psi of hydrogen with 20-mol% of platinum (IV) oxide (172 mg, 0.758 mmol) in 30 mL of ethanol over a 24 h period. The reaction was filtered through a pad of Celite®, concentrated, and purified on silica gel with 7% methanol in dichloromethane to give **7** (830 mg, 83%) as a clear yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24-7.08 (m, 4 H), 3.77 (s, 2.4 H), 3.74 (s, 0.6 H), 3.71-3.52 (m, 2 H), 3.42 (d, 1 H, J = 10 Hz), 3.16 (t, 1 H, J = 10 Hz), 2.91 (d, 0.5 H, J = 6 Hz), 2.86 (d, 0.5 H, J = 6 Hz), 2.61-2.49 (m, 2 H), 2.37- 2.29 (m, 1 H), 2.25 (s, 3 H), 1.85 (t, 1 H, J = 2 Hz), 1.47-1.43 (m, 1 H), 1.27-1.10 (m, 1 H); MS (ES^+) m/z (relative intensity) 260.5 ($(M + H)^+$, 100), 301.3 ($(M + MeCN)^+$, 20).

(2,3,4,4a,9,9a-Hexahydro-1*H*-3-azafluoren-9-yl)acetic acid methyl ester (8)

Compound **7** (830 mg, 3.20 mmol) was dissolved in 1,2-dichloroethane (32 mL) and chilled to 4 °C before 1-chloroethyl chloroformate (ACE-Cl, 675 μL, 6.40 mmol) was added. The reaction mixture was stirred at 4°C for 15 min and allowed to warm to rt before heating to reflux for 24 h. The solution was concentrated and the residue was dissolved in dry methanol (32 mL), and heated to 50 °C for 2 h. The reaction mixture was concentrated to give **8** (765 mg, 3.12 mmol, 98%) as a light tan solid that was used was used without purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.23 (m, 3 H), 7.12 (d, 1 H, J = 6 Hz), 3.76 (s, 2.4 H) ,3.74 (s, 0.6 H) 3.75-3.67 (m, 2 H), 3.44-3.40 (m, 2 H), 2.93-2.82 (m, 3 H), 2.52 (m, 1 H), 1.87-1.26 (m, 4 H); MS (ES^+) m/z (relative intensity) 246.1 ($(M + H)^+$, 100), 287.1 $(M + MeCN)^+$, 20); Anal. Calcd for $C_{15}H_{19}NO_2$: C, 59.20; H, 7.47; N, 4.37. Found: C, 59.19; H, 7.47; N, 4.03.

(3-{2-[3-(1,4,5,6-Tetrahydropyrimidin-2-ylamino)phenyl]acetyl}-2,3,4,4a,9,9a-hexahydro-1*H*-3-azafluoren-9-yl)acetic acid methyl ester (12)

[3-(1,4,5,6-Tetrahydropyrimidin-2-yl-amino)phenyl]acetic acid (**10**)[6] (217 mg, 0.813 mmol) was dissolved in 5 mL of DMF and treated with N-methylmorpholine (178 μL, 1.63 mmol) before the mixture was chilled to 0 °C. The reaction mixture was treated with 20-mol % of 1-hydroxybenzotriazole (22 mg), and stirred in an ice bath for an additional 10 min, before a slurry of **8** (200 mg, 0.813 mmol) in DMF (5 mL) and N-methyl morpholine (178 μL, 1.63 mmol) were added. The reaction mixture was stirred for 30 min at

0°C and treated with O-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (463 mg, 1.22 mmol). The reaction mixture was stirred for 36 h at rt, and the suspension was concentrated under high vacuum. Purification on silica gel with 10% methanol in dichloromethane gave **12** (240 mg, 0.522 mmol, 64%, mp 96-97 ^{o}C) as a white solid. MS (ES^+) m/z (relative intensity) 461.5 ($(M + H)^+$, 100).

3-{2-[3-(1,4,5,6-Tetrahydropyrimidin-2-ylamino)phenyl]acetyl}-2,3,4,4a,9,9a-hexahydro-1*H*-3-azafluoren-9-yl)acetic acid (13-*cis* & 13-*trans*)

A mixture of lithium hydroxide monohydrate (44.2 mg, 1.04 mmol) in water (3 mL) was added to a solution of **12** (240 mg, 0.522 mmol) in THF (3 mL). This mixture was allowed to stir for 3 h at rt before the mixture was adjusted to pH 1 with TFA. The reaction mixture was concentrated, purified by Gilson Prep, and dried to give **13**-*cis* (142 mg, 0.318 mmol, 60%) and **13**-*trans* (28 mg, 0.063 mmol, 12%); **(13-*cis*)** ^{1}H NMR (CD_3OD, 300 MHz) δ 7.43-7.34 (m, 1 H), 7.28-7.16 (m, 4 H), 7.03 (t, 1 H, J = 8 Hz), 6.86-6.84 (m, 1 H), 6.80 (s, 1 H), 5.18 (d, 1 H, J = 14 Hz), 4.55 (d, 0.5 H, J = 14 Hz), 4.33 (d, 0.5 H, J = 13 Hz), 3.91-3.56 (m, 5 H), 3.38 (bs, 5 H), 3.17-3.03 (m, 2 H), 2.89-2.78 (m, 2 H), 2.41-2.32 (m, 1 H), 2.00 (s, 2 H), 1.42 (d, 1 H, J = 13 Hz), 0.78-0.67 (m, 1 H); MS (ES^+) m/z (relative intensity) 447 ($(M + H)^+$, 100); Anal. Calcd for $C_{26}H_{30}N_4O_3 \cdot 0.84\ H_2O$ 1.8 TFA: C, 53.25; H, 5.05; N, 8.39; F, 15.44; H_2O, 2.27. Found: C, 52.91; H, 4.75; N, 8.49; F, 15.09; H_2O, 1.98; HRMS (FAB^+) m/z Calcd for $C_{26}H_{31}N_4O_3$: 447.2396; Found 447.2403. **(13-*trans*)** ^{1}H NMR (CD_3OD, 300 MHz) δ 7.43-6.88 (m, 9 H), 4.39 (d, 1H, J = 14 Hz), 3.9-3.34 (m, 10 H), 330-3.21 (m, 2 H), 2.61-2.40 (m, 2 H), 2.00 (s, 2 H), 1.85-1.78 (m, 1 H), 1.76-1.61 (m, 1 H), 1.32-1.25 (m, 1 H); MS (ES^+) m/z (relative intensity) 447.2 ($(M + H)^+$, 100); HRMS (FAB^+) m/z Calcd for $C_{26}H_{31}N_4O_3$: 447.2396; Found 447.2409.

[3-(4,5,6,7,8-Tetrahydro[1,8]naphthyrin-2-ylbutyryl)-2,3,4,4a,9,9a-hexahydro-1*H*-3-azafluoren-9-yl)acetic acid (2)

Using the methods described for **13** above, **2** was prepared from **8** and **9**[6] as a (60:40) mixture of diastereomers (101 mg, 31% for 2 steps, mp 122-124 ^{o}C); ^{1}H NMR (DMSO-D_6, 500 MHz) δ 13.6 (br s, 1 H), 8.24 (br s, 0.4 H), 8.20 (br s, 0.6 H), 7.61 (d, 0.4 H, J = 7 Hz), 7.47 (d, 0.6 H, J = 7 Hz), 7.29 (d, 0.4 H, J = 7 Hz),7.24-7.20 (m, 0.6 H), 7.18-7.12 (m, 3 H), 6.61 (d, 0.4 H, J = 7 Hz), 6.20 (d, 0.6 H, J = 7 Hz), 4.94 (d, 1 H, J = 14 Hz), 4.34 (d, 0.5 H, J = 14 Hz), 4.21 (d, 0.5 H, J = 13 Hz), 3.65 (d, 0.6 H, J = 14 Hz), 3.52-3.398 (m, 3.4 H), 3.22-3.19 (m, 1 H), 3.03-2.91(m, 1 H), 2.85-2.80 (m, 1 H), 2.76-2.63 (m, 1.6 H), 2.59-2.41 (m, 4 H), 2.39-2.31 (m, 1.4 H), 2.24-2.18 (m, 0.6 H), 2.12-2.06 (m, 0.4 H), 1.87-1.639 (m, 4 H), 1.44-1.41 (m, 1 H), 0.88-0.70 (m, 1 H); ^{13}C NMR (DMSO-D_6, 500 MHz) ppm 173.8, 169.6, 169.5, 158.6 (TFA), 158.3 (TFA), 151.0, 147.8, 147.5, 145.0,144.8, 142.6, 142.3, 141.1, 140.9, 126.6, 126.5, 126.2, 123.5, 123.2, 123.0, 122.9, 118.8 110.2, 110.1, 43.7, 43.1, 42.9, 41.9, 40.6, 40.2, 32.4, 31.1, 30.6, 24.8, 23.8,

18.9; MS (ES^+) m/z (relative intensity) 434.3 ($(M + H)^+$, 100); HRMS (FAB^+) m/z Calcd for $C_{26}H_{32}N_3O_3$: 434.2444; Found 434.2447.

2-Benzyl-2,3,3a,8a,-tetrahydro-1*H*-2-azacyclopenta[a]inden-8-one (17)

To a solution of **15** (1.6 g, 6.9 mmol, available from Aldrich) in dichloromethane (15 mL) at 0°C was added indenone (**16**,[9] 750 mg, 5.8 mmol) in dichloromethane (5 mL), followed by a 1 M solution of trifluoroacetic acid in dichloromethane (0.5 mL). The reaction was allowed to warm to rt and stirred overnight. The solution was quenched with saturated sodium bicarbonate and then washed with brine. The organic layer was dried over sodium sulfate and the solvent removed to give a yellow oil. Purification using a Biotage 40S unit (elution with 8:1 heptanes/ethyl acetate) provided the product (**17**) (470 mg, 31%) as a light yellow oil; ^1H NMR (300 MHz $CDCl_3$) δ 7.63 (d, 1 H, J = 8 Hz), 7.50 (dd, 1 H, J = 7.7 and 7.0 Hz), 7.39-6.85 (m, 7 H), 3.70 (t, 1H, J = 7.0 Hz), 3.44 (s, 2 H), 3.12 (d, 1 H, J = 9.0 Hz), 3.00 (dd, 1 H, J = 8.0 Hz and J = 7.0 Hz), 2.83 (d, 1H, J = 9 Hz), 2.54 (t, 1 H, J = 17 Hz), 2.42 (t, 1 H, J = 17 Hz); HPLC R_f = 2.444 min, 220 nm & 254 nm; MS (ES^+) m/z (relative intensity) 264.1 ($(M + H)^+$, 100); Anal. Calcd for $C_{18}H_{17}NO$-0.5H_2O: C, 79.38; H, 6.66; N, 5.14. Found: C, 79.67; H, 6.59; N, 5.31.

The enantiomers of **17** were separated on a chiral column: Chiralpak® AD, Amylose tris-(3,5-dimethylphenylcarbamate) coated on a 20 μm silica-gel, 500 g; 5 cm ID; 41 cm length; using an isocratic solvent mixture of hexane /ethanol: 80/20 vol/vol% at 80 mL/min.; and detection at a wavelength of 220 nM. Enantiomer (**17a**) (first eluting compound) had an $\alpha_{[D]}^{23}$ = +14° (*c* 0.50, CH_3OH) and enantiomer (**17b**) had an $\alpha_{[D]}^{23}$ = –14° (*c* 0.50, CH_3OH).

(2-Benzyl-2,3,3a,8a-tetrahydro-1*H*-2-azacyclopenta[*a*]inden-8-ylidene)acetic acid methyl ester (18a)

A solution of trimethyl phosphonoacetate (2 mL, 12 mmol) in THF (50 mL) was chilled to 0 °C before 1M sodium hexamethyldisilazide in THF (12 mL, 12 mmol) was added dropwise. This solution was stirred at 0°C for 30 min, then allowed to warm to rt before adding **17** (1.04 g, 3.80 mmol) in 40 mL of THF dropwise over a 30 min period. The reaction mixture was heated to reflux for 36 h, cooled to rt, and concentrated under high vacuum. The brown oil was taken up in ethyl acetate (100 mL), washed with 1N sodium hydroxide solution (50 mL) and separated. The basic layer was extracted with ethyl acetate (2 x 75 mL); the organic layers were combined, washed with brine, dried over sodium sulfate, and concentrated. The brown oil was purified on silica gel with 30% ethyl acetate in hexane to give **18a** (1.01 g, 84%, 1:1 ratio) as a mixture of *E, Z*-isomers; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.68 (d, 1 H, J = 7 Hz), 7.50 (m, 1 H), 7.40-7.31 (m, 2 H), 7.22-7.06 (m, 5 H), 6.27 (s, 0.5 H), 5.83 (s, 0.5 H), 3.70 (s, 1.5 H), 3.69 (s, 1.5 H), 3.78-3.69

(overlapping m, 1 H), 3.47 (s, 2 H), 3.16 (d, 1 H, J = 9.0 Hz), 3.01 (m, 1 H), 2.88 (d, 1H, J = 9 Hz), 2.62-2.48 (m, 2 H); HPLC R_f = 3.202 min, 220 nm; MS (ES^+) m/z (relative intensity) 320.2 ($(M + H)^+$, 100).

(1,2,3,3a,8,8a-Hexahydro-2-azacyclopenta[*a*]inden-8-yl)acetic acid methyl ester (19aa and **19ab)**

Compound (**18a**) (1.01 g, 3.20 mmol) was taken up in methanol (40 mL), and 5% palladium on carbon (140 mg) was added. The reaction mixture was placed under 40 psi of hydrogen on a Parr shaker and heated to 50 °C for 24 h. The reaction mixture was cooled to rt and filtered through a pad of Celite®. The filtrate was concentrated and placed on high vacuum overnight. The mixture of reduced compounds (**19aa**) and **(19ab**) was obtained (720 mg, 98%) and was used without purification. MS (ES^+) m/z (relative intensity) 232.0 ($(M + H)^+$, 100).

[2-(3-5,6,7,8-Tetrahydro[1,8]naphthyrin-2-yl-propionyl)-1,2,3,3a,8,8a-hexahydro-2-azacyclopenta-[*a*]inden-8-yl]acetic acid methyl ester (21aa and **21ab)**

4-(5,6,7,8-Tetrahydro[1,8]naphthyridin-2-yl)propionyl acid (**20**,[6] 760 mg, 3.13 mmol) was dissolved in DMF (20 mL) and 3 equivalents of *N*-methylmorpholine (1.03 mL, 9.39 mmol) were added. The reaction mixture was chilled to 4 °C and 1-hydroxybenzotriazole (86 mg) was added. The reaction mixture was stirred for an additional 30 min before a solution of **19aa** and **19ab** (720 mg, 3.13 mmol) and *N*-methylmorpholine (1.03 mL, 9.39 mmol) in DMF (20 mL) was introduced. This reaction mixture was allowed to stir for 30 min in an ice bath before treating with O-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (1.78 g, 4.70 mmol). The solution was stirred in an ice bath for 3 h before pouring the mixture into 1 N sodium hydroxide solution (50 mL) and ethyl acetate (50 mL). The organic layer was separated, washed with 1 N sodium hydroxide solution (3 x 50 mL), dried with sodium sulfate and concentrated. The compound was purified on the Gilson Prep with a gradient of 15-90% acetonitrile in water (0.16% TFA). The esters (**21aa**) and (**21ab**) (600 mg, 1.43 mmol) were recovered as a mixture of diastereomers (1:1) in a 46 % yield as a clear oil; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.13 (m, 5 H), 6.49-6.43 (m, 1 H), 4.11-3.97 (m, 2 H), 3.77 (s, 3 H), 3.49 (bs, 2 H), 3.29 (t, 0.5 H, J =17 Hz), 3.13 (t, 0.5 H, J = 17 Hz), 2.99-2.83 (m, 3 H), 2.79-2.62 (m, 3 H) 2.60-2.43 (m, 2 H), 1.93 (bs, 2 H); MS (ES^+) m/z (relative intensity) 420.1 ($(M + H)^+$, 100).

[2-(3-5,6,7,8-Tetrahydro[1,8]naphthyrin-2-yl-propionyl)-1,2,3,3a,8,8a-hexahydro-2-azacyclopenta-[*a*]inden-8-yl]acetic acid (14aa and **14ab)**

The mixture of esters (**21aa**) and (**21ab**) (600 mg, 1.43 mmol) was dissolved in THF/water (1:1, 30 mL) and treated with lithium hydroxide monohydrate (300 mg, 7.15 mmol). The reaction mixture was stirred for 1 h and quenched with TFA to pH 1. The acid was purified on the Gilson 333 Prep unit to give **14aa**

and **14ab** (545 mg, 73%, ratio 1:1) as the TFA salt; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.51- 7.44 (m, 1 H), 7.29-7.21 (m, 4 H), 6.56-6.49 (m, 1 H), 3.97-3.63 (m, 5 H), 3.45 (br s, 3 H), 3.27 (t, 0.5 H, J = 13 Hz), 3.14 (t, 0.5 H, J = 11 Hz), 3.03-2.85 (m, 4 H), 2.79-2.44 (m, 5 H), 1.94-1.89 (m, 2H); ^{13}C NMR (CD_3OD, 500 MHz) δ 176.1, 172.1, 152.4, 148.8, 145.8, 142.6, 130.2, 129.1, 128.8, 126.1, 125.6, 124.9, 120.8, 111.7, 53.0, 47.6, 46.5, 42.2, 33.8, 33.6, 28.6, 26.4; HPLC R_f = 2.794 min, 220 nm; MS (ES^+) m/z (relative intensity) 406.4 ($(M + H)^+$, 100); HRMS (FAB^+) m/z Calcd for $C_{24}H_{28}N_3O_3$: 406.2126; Found 406.2131.

The diastereomers (**14aa**) and (**14ab**) were separated using a Chiralpak® OD column, Cellulose tris-(3,5-dimethylphenylcarbamate) coated on a 20 μm silica-gel, 500 g; 5 cm ID; 41 cm length; using an isocratic solvent mixture of hexane (+5% ethanol +0.1% TFA + 0.02% TEA) /methanol /ethanol: 85/9.75/5.25 vol/vol/vol% at 80 mL/min; and detection at a wavelength of 215 nM. Optical rotation: **14aa** (first eluting) $[\alpha]_D^{23}$ = -18.2° (*c* 0.10, CH_3OH) and **14ab** $[\alpha]_D^{23}$ = +18.7° (*c* 0.10, CH_3OH). The NMR sample of **14aa** in CD_3OD crystallized over time so the sample was analyzed by X-Ray.[11]

ACKNOWLEDGMENTS

The authors would like to acknowledge the important contributions of Charles Reynolds (molecular modeling), William Hageman (*in vitro* pharmacokinetics), John Masucci (rat pharmacokinetics), and Victor Day (X-Ray crystallography) to our work.

DEDICATION

The paper is dedicated to Leo A. Paquette on the occasion of his 70th birthday.

REFERENCES AND NOTES

1. W. H. Miller, R. M. Keenan, R. N. Willette, and M. W. Lark, *DDT*, 2000, **5**, 397.
2. W. H. Miller, W. E. Bondinell, R. D. Cousins, K. F. Erhard, D. R. Jakas, R. M. Keenan, T. W. Ku, K. A. Newlander, S. T. Ross, R. C. Haltiwanger, J. Badbeer, F. H. Drake, M. Gowen, S. J. Hoffman, S-M. Hwang, I. E. James, M. W. Lark, B. Lechowska, D. J. Rieman, G. B. Stroup, J. A. Vasko-Moser, D. L. Zembryki, L. M. Azzarano, P. C. Adams, K. L. Salyers, B. R. Smith, K. W. Ward, K. O. Johanson, and W. F. Huffman, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1807.
3. The synthesis of **1** is contained in M. J. Costanzo, W. J. Hoekstra, and B. E. Maryanoff, WO2001/23376, 2001, Apr 5, and will also be published separately in manuscript form.
4. P. J. Coleman, B. C. Askew, J. H. Hutchinson, D. B. Whitman, J. J. Perkins, G. D. Hartman, G. A. Rodan, C-T. Leu, T. Prueksaritanont, C. Fernandez-Metzler, K. M. Merkle, J. J. Lynch, S. B. Rodan, and M. E. Duggan, *Biorg. Med. Chem. Lett.,* 2002, **12**, 2463.

5. V. Paragamian, U. S. Patent 3,462,443, 1969, Aug 19; C. E. Cook, M. C. Wani, J. M. Jump, Y-W. Lee, P. A. Fail, S. A. Anderson, Y-Q. Gu, and V. Petrow, *J. Med. Chem.,* 1995, **38**, 753.
6. B. C. Askew, P. J. Coleman, M. E. Duggan, W. Halczenko, G. D. Hartman, C. A. Hunt, J. H. Hutchinson, R. S. Meissner, M. A. Patane, G. R. Smith, and J. Wang, U. S. Patent 6,048,861, 2000, Apr. 11; N. S. Chandrakumar, B. N. Desai, B. Devadas, R. Huff, I. K. Khanna, S. N. Rao, J. G. Rico, T. E. Rogers, P. G. Ruminski, M. A. Russell, Y. Yu, A. F. Gasiecki, J. W. Malecha, and J. M. Miyashiro, WO99/52896, 1999, Oct. 21; M. E. Duggan, G. D. Hartman, R. S. Meissner, and J. J. Perkins, U. S. Patent 6,410,526, 2002, Jun. 25.
7. R. Achini, *Helv. Chim. Acta,* 1981, **64**, 2203.
8. D. A. Nugiel, *Tetrahedron Lett.,* 2001, **42**, 3545 and references sited therein.
9. Can be prepared in one step from indanone: K. C. Nicolaou, T. Montagnon, and P. S. Baran, *Angew. Chem., Int. Ed.,* 2002, **41**, 1386.
10. Human $\alpha_v\beta_3$ or $\alpha_v\beta_5$ (Chemicon International Inc.) dissolved in Tris buffer (20 mM Tris, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μM $MnCl_2$, 150 mM NaCl) was immobilized (1 μg/mL) on Immulon 96 well plates (Dynex Technologies) overnight at 4°C. Plates were blocked with 3% BSA in Tris buffer for 2 h at 37°C. Plates were then rinsed 2X in assay buffer (Tris buffer with 0.3% BSA and 0.2% Tween-20). Five minutes prior to the addition of 4 nM vitronectin (Sigma Chemical Co.), compounds were added to wells in duplicate. Following 3 h at 37°C, plates were washed 5 times in assay buffer. An anti-human vitronectin IgG rabbit polyclonal antibody (Calbiochem Inc.) was added (1:2000) and plates were incubated for 1 h at room temperature. VectaStain ABC peroxidase kit reagents (Vector Laboratories) employing a biotin labeled anti-rabbit IgG, were utilized for detection of bound antibody (490 nm); J. Hoekstra, M. P. Beavers, P. Andrade-Gordon, M. F. Evangelisto, P. M. Keane, J. B. Press, K. A. Tomko, F. Fan, M. Kloczewiak, K. H. Mayo, K. A. Durkin, and D. C. Liotta, *J. Med. Chem.,* 1995, **38**, 1582.
11. The X-Ray structure was obtained from Crystalytics Company, Lincoln, Nebraska.
12. The right turn is defined as the clockwise rotation from the A-ring to C-ring with the carboxylic acid group directed away from the viewer.
13. Each ligand was docked into the X-Ray structure by aligning the basic and acidic motifs. In order to place the turn motif, energies were calculated for several conformations in which the turn was rotated about the long axis of the molecule. (Energy minimization runs: Polak-Ribier Conjugate (PRCG) method, in water, OPLS-AA, constant dielectric constant of 1, sidechain atoms in a 8.0 Å radius around the active site and the ligand were allowed to move, and all other atoms were held frozen) The minimum energy poses from these studies are shown in Figure 4. All the simulations were performed using MacroModel (Schrödinger, Inc., Portland, OR., 2003).

14. J-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, and M. A. Arnaout, *Science*, 2002, **296**, 151.
15. K. Urbahns, M. Härter, A. Vaupel, M. Albers, D. Schmidt, U. Brüggemeier, B. Stelte-Ludwig, C. Gerdes, and H. Tsujishita, *Biorg. Med. Chem. Lett.,* 2003, **13**, 1071.
16. These in vitro pharmacokinetic assays were preformed at Absorption Systems, Exton, PA.
17. C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, *Adv. Drug Delivery Rev.,* 1997, **23**, 3.