Design, Synthesis, and Biological Evaluation of Novel Potent and Selective $\alpha_v\beta_3/\alpha_v\beta_5$ Integrin Dual Inhibitors with Improved Bioavailability. Selection of the Molecular Core

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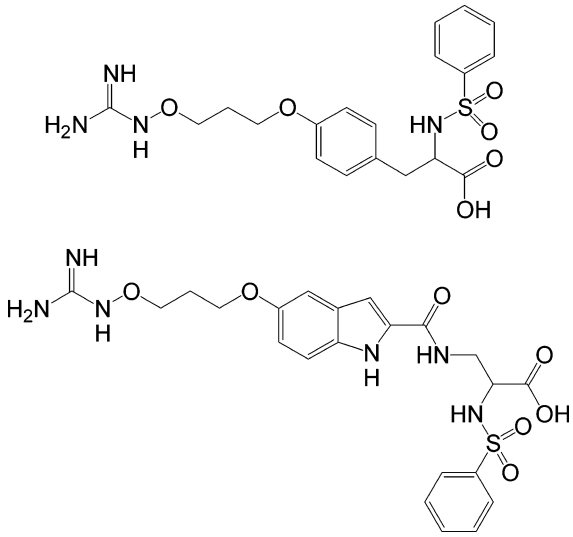
A novel series of potent and selective $\alpha_v\beta_3/\alpha_v\beta_5$ dual inhibitors was designed, synthesized, and evaluated against several integrins. These compounds were synthesized through a Mitsunobu reaction between the guanidinium mimetics and the corresponding central templates. Guanidinium mimetics with enhaced rigidity (i.e., (2-pyridylamino)propoxy versus the 2-(6-methylamino-2-pyridyl)ethoxy) led to improved activity toward $\alpha_v\beta_3$. Exemplary oral bioavailability in mice was achieved using the indole central scaffold. Although, oral bioavailability was maintained when the indole molecular core was replace with the bioisosteric benzofuran or benzothiophene ring systems, it was found to not significantly impact the integrin activity or selectivity. However, the indole series displayed the best in vivo pharmacokinetic properties. Thus, the indole series was selected for further structure−activity relationships to obtain more potent $\alpha_v\beta_3/\alpha_v\beta_5$ dual antagonist with improved oral bioavailability.

Introduction

Integrins are a family of heterodimeric cell surface receptors responsible for the regulation of cell attachment to the extracellular matrix.[1] The noncovalent union of two different subunits, called unit α and unit β, forms these heterodimeric receptors. Both subunits are type I membrane proteins with large extracelullar segments.[1] In mammals, there are 19 α and 8 β subunits, which assemble into 26 different receptors.[2] Several integrins have been the focus of attention in the past decade due to their capacity to regulate cell mobility and cell−cell interaction. In this sense, II_bIII_a, the platelet fibrinogen receptor, has been involved in thrombosis,[3] or $\alpha_4\beta_1$, the very late antigen-4 (VLA-4), has been involved in inflammatory response through the adhesion, migration, and activation of leukocytes.[4] Two additional integrins that have received considerable attention have been $\alpha_v\beta_3$, the vitronectin receptor[5] that is up-regulated on endothelial cells during tumor angiogenesis and on smooth muscle cells mobility during proliferation, and $\alpha_v\beta_5$, involved also in angiogenesis.[6] These receptors represent an interesting therapeutic target because of their important role in pathologies as diverse as osteoporosis,[7] restenosis,[8] acute renal failure,[9] ocular diseases,[10] tumor-induced angiogenesis,[11] metastasis formation,[12] and sickle cell anemia.[13]

Many of the receptors from the integrin family recognize the same core amino acid sequence Arg-Gly-Asp (RGD)[14] contained in a number of matrix proteins (fibronectin, fibrinogen, vitronectin, osteopontin, etc.), and as consequence, some of the integrin receptors, such as $\alpha_v\beta_3$, are promiscuous to a number of these matrix proteins.[15]

Chart 1



A number of groups have reported small molecule inhibitors designed as RGD mimetics.[16] The difficulty in obtaining $\alpha_v\beta_3$ inhibitors with good oral properties has been recognized for many years as one of the major hurdles to be overcome.[17] One of the reasons is the zwiterionic character of these RGD mimetics. We have recently reported a first series of potent $\alpha_v\beta_3/\alpha_v\beta_5$ integrin inhibitors with an *O*-guanidine moiety as a basic ending (Chart 1).[18] However, the poor bioavailability of these compounds hampered their development as drug candidates and prompted us to develop other templates to identify a novel series of compounds with improved oral bioavailability. A number of teams have already reported $\alpha_v\beta_3/\alpha_v\beta_5$ inhibitors with good oral bioavailability.[17] The majority of these compounds incorporate basic ending groups with relatively low pK_a

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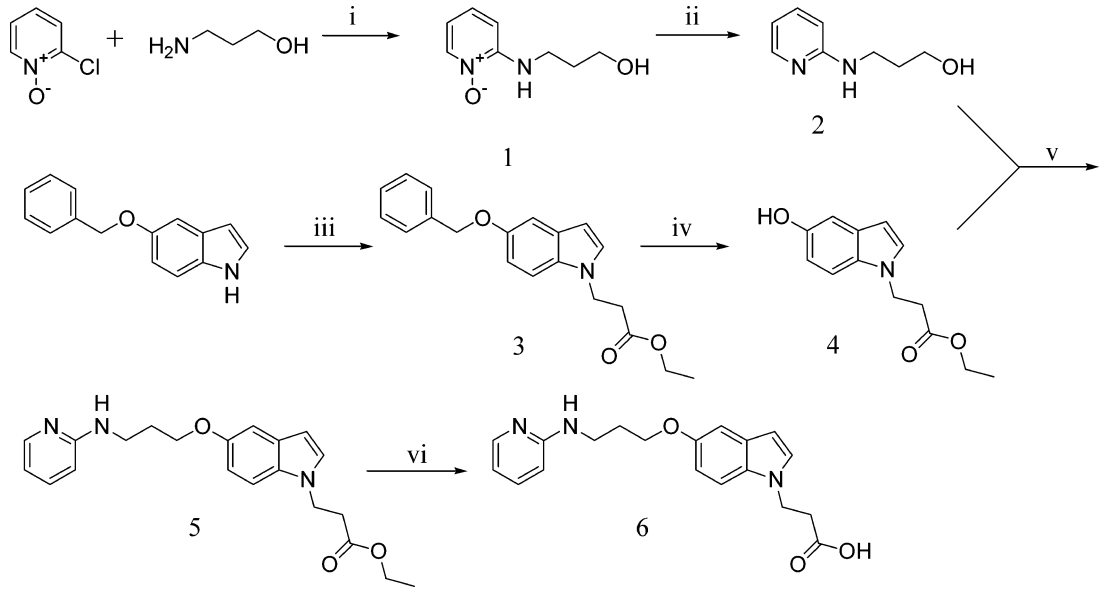
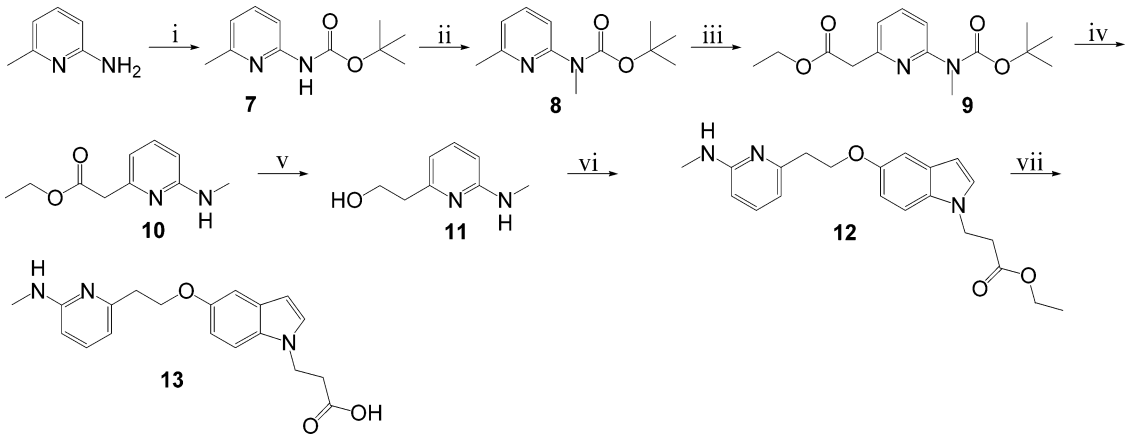
Scheme 1. Synthesis of Indole **6**[a]



[a] Reagents and conditions: (i) $NaHCO_3$, *tert*-amyl alcohol, reflux; (ii) H_2, Pd/C, cyclohexanes−ethanol, room temperature; (iii) NaH/DMF, ethyl 3-bromopropionate, room temperature; (iv) H_2, Pd/C, EtOH, room temperature; (v) *n*-Bu_3P/THF, 1,1-(azodicarbonyl)dipiperidine, room temperature; (vi) LiOH, MeOH−H_2O, room temperature.

Scheme 2. Synthesis of Indole **13**[a]



[a] Reagents and conditions: (i) di-*tert*-butyl dicarbonate, 60 °C; (ii) NaH, MeI, DMF, 0 °C to room temperature; (iii) LDA, diethyl carbonate, THF, −78 °C to 0 °C; (iv) TFA, CH_2Cl_2, 0 °C to room temperature; (v) $LiAlH_4$, THF, 0 °C to room temperature; (vi) 4, PPh_3, DIAD, THF, 0 °C to room temperature; (vii) NaOH, MeOH−H_2O, room temperature.

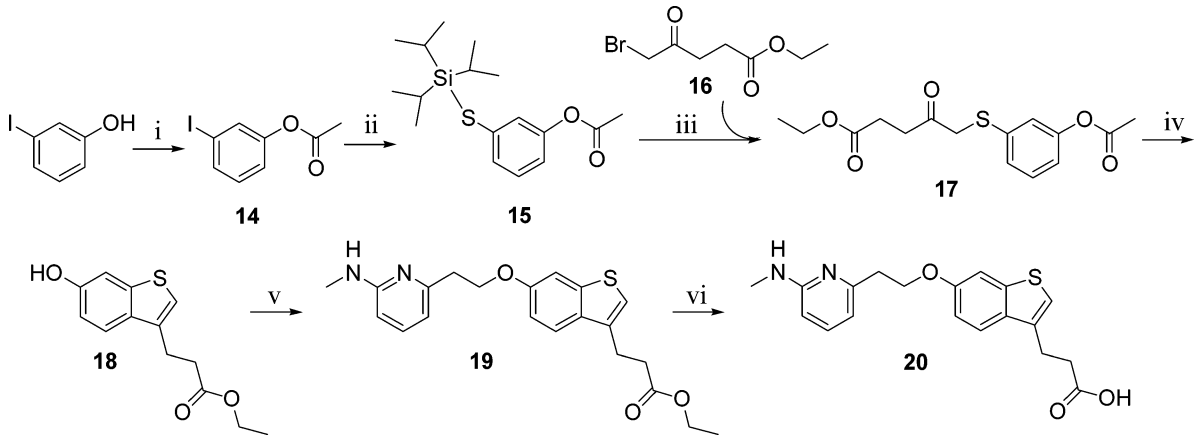
values (e.g., aminopyridine and analogues). The reduction of the total number of heteroatoms in the molecule, for example by avoiding sulfonamide substitution near of the acidic ending, is another approach that has successfully led to compounds with improved pharmacokinetic (PK) properties. In view of these promising results, we decided to incorporate those premises in our lead series, while studying the possibility of improving the druglike properties of the resulting compounds by modifying the central molecular core. In this paper, we report the synthesis and biological evaluation of a novel series of $\alpha_v\beta_3/\alpha_v\beta_5$ dual inhibitors, which incorporate different bioisosteric ring bicycles (indole, benzoxazole, and benzothiophene) and which have ultimately led to the identification of compounds with a better PK profile and an improved selectivity profile toward $\alpha_5\beta_1$ and $\alpha_{IIb}\beta_{IIIa}$ intergins.

Chemical Synthesis

Reaction of 2-chloropyridine-*N*-oxide hydrochloride and 3-aminopropanol in the presence of sodium bicarbonate produces 2-[(3-hydroxypropyl)amino]pyridine *N*-oxide (**1**) (Scheme 1), which was reduced by catalytic hydrogenation to afford 2-[(3-hydroxypropyl)amino]-pyridine **(2)** (88% yield). The indole scaffold was produced by N-alkylation of 5-benzyloxyindole with ethyl 3-bromopropionate in the presence of sodium hydride in dimethylformamide (DMF). The benzyloxy-protected group was cleaved under standard hydrogenolysis conditions leading to **4** (53% yield), which was subsequently coupled with **2** through a Mitsunobu reaction to give the propionate ester **5** in 60% yield. Hydrolysis of the ester **5** with lithium hydroxide provided the target 3-{5-[3-(2-pyridylamino)propoxy]indol-1-yl}propionic acid (**6**).

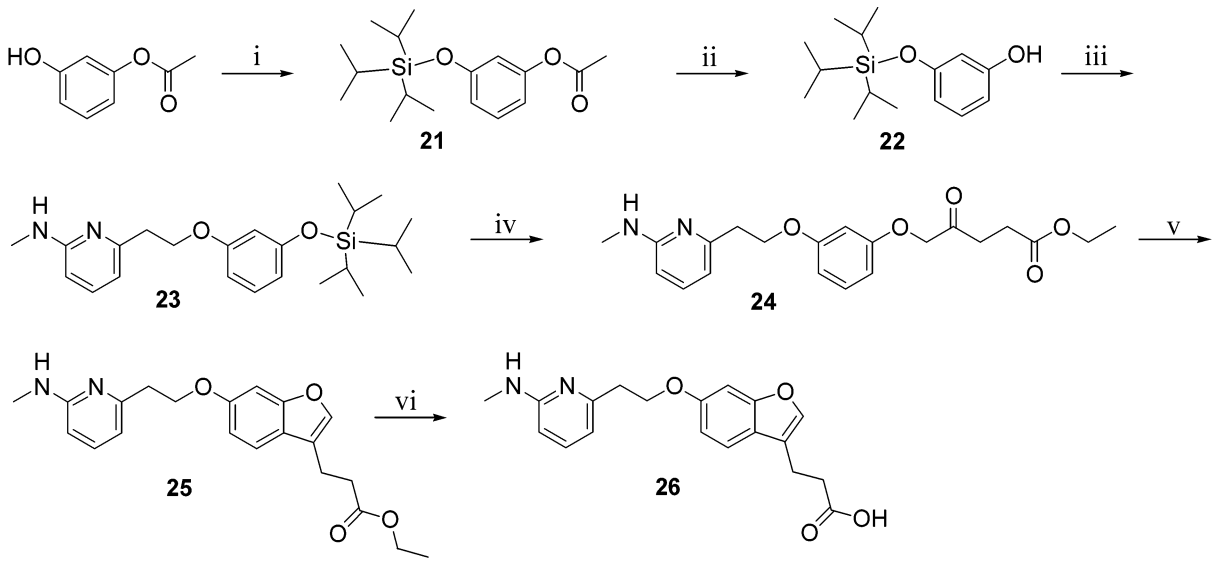
Indole **13** was synthesized as outlined in Scheme 2. 2-Amino picoline was first Boc protected and subsequently N-alkylated to give **8** in 57% yield. The carbonylation of **8** with diethyl carbonate and lithium diisopropyl amide (LDA) provided the ethyl 2-{6-[*N*-(*tert*-butoxycarbonyl)-*N*-methylamino]-2-pyridyl}-acetate **9** in 60% isolated yield. After removal of the Boc protection, compound **10** was reduced with lithium

Scheme 3. Synthesis of Benzothiophene **20**[a]



[a] Reagents and conditions: (i) acetyl chloride, K_2CO_3, DMF, room temperature; (ii) triisopropylsilanethiol, NaH, $Cl_2Pd(Ph_3)_2$,toluene/THF, reflux; (iii) TBAF, THF, room temperature; (iv) H_2SO_4 concentrated, 0 °C; (v) **11**, *N*-methylmopholine, Ph_3P, DIAD, THF, room temperature; (vi) NaOH, H_2O/THF, room temperature.

Scheme 4. Synthesis of Benzofurane **26**[a]



[a] Reagents and conditions: (i) TIPSiCl, $LiN(SiMe_3)_2$, THF, −78 °C to room temperature; (ii) NaOH, H_2O/THF, room temperature; (iii) **11**, Ph_3P, DIAD, THF, 0 °C to room temperature; (iv) **16**, TBAF, THF, room temperature; (v) H_2SO_4 concentrated, 0 °C; (vi) NaOH, H_2O, THF, room temperature.

aluminum hydride to produce the basic ending chain **11** in 70 % yield. The condensation of the alcohol **11** with the phenol **4** was realized by a Mitsunobu reaction to afford the protected ester **12**, which was eventually deprotected using sodium hydroxide at the last step of the reaction sequence, to produce the target indole **13**.

The benzothiophene derivative was prepared according to the synthesis depicted in Scheme 3. Protection of 3-iodophenol with acetyl chloride followed by a palladium-mediated cross-coupling reaction with triisopropylsilanethiol and iodo **14** afforded the corresponding silanethiol acetate **15** in 52% yield. After the in situ deprotection of the triisopropylsylil moiety with tetrabutylammonium fluoride, the thio intermediate was subsequently S-alkylated with the α-bromoketone **16** to furnish the diester **17** in 73% yield. Cyclization and deprotection of **17** were performed by treatment with concentrate sulfuric acid to give **18** (19% yield). Intermediate **18** was then coupled with alcohol **11** through a Mitsunobu reaction to produce **19**, which was ultimately deprotected to afford the final thiophene **20**.

In the case of the benzofurane **26**, the resorcinol monoacetate was reacted with triisopropylsilyl chloride in the presence of lithium bis(trimethylsilyl)amide to give **21** (Scheme 4). Hydrolysis of the acetyl protecting group followed by the reaction with alcohol **11** under Mitsunobu general conditions yielded ether **23** (28% yield). Intermediate **23** was then deprotected in situ with tetrabutylammonium fluoride and was subsequently reacted with ethyl 5-bromo-4-oxovalerate to give the ester **24**. Cyclization of **24** using concentrated sulfuric acid furnished the benzofurane **25**, which was eventually hydrolyzed by treatment with sodium hydroxide to afford the target compound **26** in 74% yield.

Biological Activity

All of the compounds synthesized have been evaluated on a panel of integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, and $\alpha_{IIb}\beta_{IIIa}$) for their ability to antagonize integrin−ligand interactions. To predict their propensity for GI absorption, a CACO-2 permeability evaluation was also determined. All of these data are depicted in Table 1.

Table 1. In Vitro Inhibition of Protein Binding and CACO-2 Cell Permeability of Compounds **6**, **13**, **20**, and **26**

compound number	ELISA IC_{50} (μM)[a]				CACO-2 permeability (AB cm/s)
	$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_5\beta_1$	II_bIII_a	
6	0.47 (±0.03)	0.0025 (±0.0007)	>20	>20	6×10^{-6} ($\pm 1 \times 10^6$)
13	0.049 (±0.012)	0.21 (±0.04)	15 (±3)	>20	6×10^{-6} ($\pm 1 \times 10^6$)
20	0.030 (±0.003)	0.14 (±0.05)	7.8 (±2.5)	>20	16×10^{-6} ($\pm 1 \times 10^6$)
26	0.033 (±0.002)	0.42 (±0.24)	>20	>20	17×10^{-6} ($\pm 1 \times 10^6$)

[a] In vitro inhibition ($n \geq 3$, mean (SD)), as measured by ELISA, of $\alpha_v\beta_3$-vitronectin, $\alpha_v\beta_5$-vitronectin, $\alpha_5\beta_1$-fibronectin, and II_bIII_a-fibrinogen protein interactions.

As seen by comparison of compounds **6** and **13**, switching the position of the pyridine ring has a beneficial impact on the activity against $\alpha_v\beta_3$ ($IC_{50-\alpha v\beta 3}$ = 0.47 and 0.049 μM, respectively) but considerably decreases the activity against $\alpha_v\beta_5$ ($IC_{50-\alpha v\beta 5}$ = 0.0025 and 0.21 μM, respectively). Interestingly, the selectivity profile toward the other integrins ($\alpha_5\beta_1$, $\alpha_{IIb}\beta_{IIIa}$) is not significantly altered by this modification. Further bioisosteric replacement of the indole template by the bicyclic benzothiophene or benzofurane ring systems does not dramatically affect the activity against $\alpha_v\beta_3$ or the selectivity profile of the resulting compounds (**20** and **26**, respectively). As seen in Table 1, all of these compounds (**6**, **13**, **20**, and **26**) exhibited a very similar CACO-2 permeability profile. However, the benzothiophene **20** and benzofurane **26** derivatives appeared to be slightly more permeable that the indoles **6** and **13**. No evidence of efflux activity has been seen with these four compounds (data not shown).

To assess whether the bioisosteric replacement of the central molecular core of these $\alpha_v\beta_3$ antagonists would affect their pharmacokinetic profile, a full mouse PK study was undertaken. These data are reported in Table 2 and illustrated in Figures 1 and 2.

Consistent with the very promising in vitro CACO-2 permeability data, all three compounds (**13**, **20**, and **26**) exhibited rapid and nearly complete absorption with T_{max} values between 15 and 30 min and good C_{max} values (38.3, 27.5, and 16.2 μg/mL, respectively) after oral administration. The indole analogue **13** showed the best overall pharmacokinetic profile after iv and oral administration with low systemic clearance in relation to liver blood flow (liver blood flow = 90 mL/(min/kg)),[19] moderate volume of distribution (total body water = 725 mL/kg),[19] and the highest AUC_{inf} value. The benzofuran was the worst due to a small volume of distribution and high clearance. Overall, the pharmacokinetic behavior highlighted the indole-containing derivatives as the most promising series for the development of novel potent, selective, and oral bioavailable $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists. Further studies identified the indole scaffold as superior in a second species, dog (data not shown).

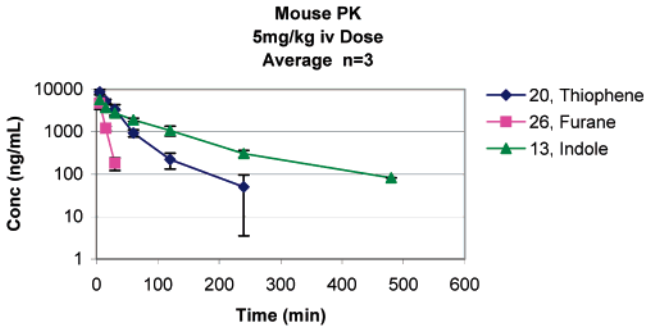


Figure 1.

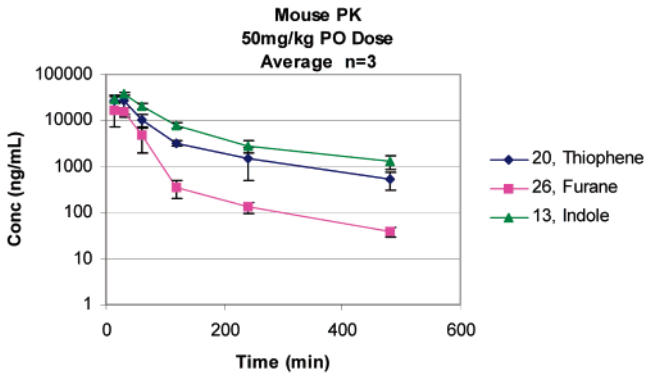


Figure 2.

In summary, a novel series of $\alpha_v\beta_3/\alpha_v\beta_5$ inhibitors was synthesized and evaluated in vivo and in vitro for their potency and selectivity profiles against $\alpha_v\beta_5$ and $\alpha_{IIb}\beta_{IIIa}$. Interestingly, it was observed that the switch of the position of the pyridine ring of the basic ending has a positive effect in terms of activity against $\alpha_v\beta_3$. Although the bioisosteric replacement of the indole ring with benzofuran or benzothiophene is well-tolerated in vitro in terms of $\alpha_v\beta_3$ potency or in the CACO-2 permeability, the indole **13** appears to have a much better pharmacokinetics profile than the benzothiophene (**20**) or the benzofuran (**26**). Thus, we have selected the indole series to perform further SAR studies (manuscript in preparation).

Table 2. PK Parameters Determination for Compounds **13**, **20**, and **26**[a]

	compound number					
	13		**20**		**26**	
	5 mpk iv	50 mpk po	5 mpk iv	50 mpk po	5 mpk iv	50 mpk po
C_{max} (μg/mL) (n = 3)		38.3 (±3.7)		27.5 (±8.4)		16.2 (±8.8)
T_{max} (h) (n = 3)		0.25−0.50		0.25−0.50		0.25−0.50
$t_{1/2}$ (h)	1.56	2.39	0.74	2.34	0.09	1.92
CL (mL/(min/kg))	11.74		16.5		65.1	
Vz (L/kg)	1.58		1.05		0.50	
AUC_{inf} (μg/(mL·min))	426.0	3837.6	302.2	2201.8	76.9	877.3
% ext AUC	3	7	1	5	2	1
MRT (h)	1.72		0.56		0.11	
F		90%		73%		114%

[a] PK parameters were calculated from the mean curve prepared with data from three animals at each time point.

Experimental Section

Chemical Synthesis. Reagents used for synthesis were purchased from Sigma-Aldrich (Milwaukee, WI) and Lancaster (Windham, NH). All of the solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed on Geduran Silical gel60 (40−63 μm, Merck). The spots were visualized either under UV light ($\lambda = 254$ nm) or by spraying with molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/ $(NH_4)Ce(SO_4)_4\cdot 2H_2O$, 90/10/25/1 v/v/w/w) and charring at 140 °C for a few minutes. All of the chemical yields are unoptimized and generally represent the result of a single experiment.

^{1}H NMR spectra were recorded on a Brucker B-ACS-120 (400 MHz) spectrophotometer at room temperature. Chemical shifts are given in ppm (δ), coupling constants (J) are in hertz (Hz), and signals are designed as follows: s, singled; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; m, multiplet; br s, broad singlet.

LC-MS/ELS was performed on a system consisting of an electrospray source on a Finnigan LCQ ion trap mass spectrometer, a SEDEX 75C evaporative light scattering detector, a Shimadzu LC-10ADvp binary gradient pumping system, a Gilson 215 configured as an autosampler, and a Princeton Chromatography HTS HPLC columm (5 μm, 50 mm × 3.0 mm).

Accurate mass analysis was performed using a Micromass Autospec OATOF high-resolution magnetic sector mass spectrometer. Compounds were ionized using fast atom bombardment (FAB) ionization with poly(ethylene glycol) (PEG) matrix. Samples were prepared by dissolving approximately 0.2 mg of compound into 200 μL of PEG deposited on the FAB target. Mass measurement was performed using voltage scanning and bracketing the molecular ion of interest with two PEG reference peaks.

The purity of the final compounds was analyzed with a 218 PrepStar Varian HPLC system connected to a ProStar Varian UV detector and following the peaks at $\lambda = 254$ nm. The flow was 1 mL/min, and the gradient was from 5% acetonitrile/water (with 0.1% of trifluoroacetic acid), until 95% over a period of 23 min. The columns for the analysis were Betabasic C18 (5 μm, 150 Å, 150 mm × 4.6 mm) and Betabasic C8 (5 μm, 150 Å, 150 mm × 4.6 mm). All of the the final compounds had 95% or greater purity (Supporting Information).

2-(3-Hydroxypropyl)aminopyridine *N*-Oxide (1). A mixture of 2-chloropyridine-*N*-oxide hydrochloride (3.32 g, 20 mmol), 3-amino-1-propanol (3.06 mL, 40 mmol), and $NaHCO_3$ (8.4 g, 100 mmol) in *tert*-amyl alcohol (20 mL) was heated to reflux. After being stirred overnight, the reaction mixture was cooled, diluted with methylene chloride (100 mL), and suction filtered to remove the insoluble materials. The filtrate was concentrated and reconcentrated from methylene chloride twice. The residue was recrystallized from ethyl acetate and hexane, collected by filtration, washed with ethyl acetate, and dried under high vacuum to give the title compound as a pale yellow solid (3.2 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, $J = 6.5$ Hz, 1H), 7.32 (br s, 1H), 7.21 (t, $J = 8.6$ Hz, 1H), 6.64 (d, $J = 8.5$ Hz, 1H), 6.53 (t, $J = 6.7$ Hz, 1H), 3.75 (t, $J = 5.8$ Hz, 2H), 3.47 (q, $J = 6.2$ Hz, 2H), 1.86 (t, $J = 6.0$ Hz, 2H).

2-(3-Hydroxypropyl)aminopyridine (2). A mixture of **1** (3.0 g, 17.9 mmol), as prepared in the preceding step, cyclohexene (10 mL, 100 mmol), and 10% palladium(0) on carbon (300 mg) in ethanol (50 mL) was heated to reflux. After 2 days, the reaction mixture was cooled. The catalyst was removed by filtration through Celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (silica gel, 5% methanol in methylene chloride) to give **2** as a colorless oil (2.4 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, $J = 5.0$ Hz, 1H), 7.37 (t, $J = 7.8$ Hz, 1H), 6.54 (d, $J = 6.0$ Hz, 1H), 6.39 (t, $J = 8.0$ Hz, 1H), 4.69 (br s, 2H), 3.65 (t, $J = 5.5$ Hz, 2H), 3.53 (q, $J = 5.9$ Hz, 2H), 1.77 (t, $J = 5.6$ Hz, 2H).

Ethyl 3-(5-Benzyloxy)indolylpropanoate (3). A solution of 5-benzyloxyindole (1.30 g, 5.82 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (25 mL) under nitrogen and treated with a 60% suspension of sodium hydride in mineral oil (0.60 g, 15 mmol). After being stirred 1 h at ambient temperature, the reaction mixture was treated with ethyl 3-bromopropionate (1.00 mL, 6.96 mmol) and stirred an additional 18 h. The reaction was then treated with additional sodium hydride (0.3 g, 7.5 mmol) and stirred two more hours, and the solvent removed in vacuo. The crude product was dissolved in methylene chloride, washed with 10% aqueous HCl, water, and brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated, and the residue was purified by flash column chromatography (1:1 methylene chloride/ethyl acetate eluant) giving compound **3** as a yellow oil (0.96 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (br d, $J = 7.2$ Hz, 2H), 7.37 (m, 2H), 7.32 (m, 1H), 7.24 (br d, $J = 8.8$ Hz, 1H), 7.15 (d, $J = 2.4$ Hz, 1H), 7.10 (m, 1H), 6.96 (dd, $J = 8.8$ Hz, 2.4 Hz, 1H), 6.38 (m, 1H), 5.09 (s, 2H), 4.44 (t, $J = 6.9$ Hz, 2H), 4.21 (q, $J = 7.1$ Hz, 2H), 2.92 (t, $J = 6.9$ Hz. 2H), 1.26 (m, 3H).

Ethyl 3-(5-Hydroxyindolyl)propanoate (4). A solution of **3** (0.94 g, 2.90 mmol) and 10% palladium(0) on carbon (97 mg) in reagent ethanol (40 mL) was stirred under hydrogen at ambient pressure and temperature for 18 h. The reaction was filtered over Celite, and the evaporated filtrate was purified by flash column chromatography (10% ethyl acetate in methylene chloride eluant) to yield **4** as colorless oil. (0.36 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.18 (d, $J = 8.7$ Hz, 1H), 7.10 (d, $J = 3.0$ Hz, 1H), 7.01 (d, $J = 1.9$ Hz, 1H), 6.78 (dd, $J = 8.7$ Hz, 2.2 Hz, 1H), 6.34 (d, $J = 3.0$ Hz, 1H), 4.86 (s, 1H), 4.43 (t, $J = 6.9$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 2.92 (t, $J = 6.9$ Hz, 2H), 1.27 (t, $J = 7.1$ Hz, 3H).

Ethyl 3-{5-[3-(2-Pyridylamino)propoxy]indolyl}propanoate (5). A solution **4** (0.35 g, 1.51 mmol) and **2** (0.24 g, 1.58 mmol) in anhydrous tetrahydrofuran (25 mL) was treated with tri-*n*-butylphosphine (0.43 mL, 1.72 mmol) and 1,1-(azodicarbonyl)dipiperidine (0.43 g, 1.70 mmol) at ambient temperature. After 18 h, the reaction was concentrated in vacuo, and the crude product purified by flash column chromatography (1:1 methylene chloride/ethyl acetate eluant)) to yield compound **5** as a yellow oil (0.33 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (dd, $J = 5$ Hz, 1 Hz, 1H), 7.40 (m, 1H), 7.24 (d, $J = 8.8$ Hz, 1H), 7.11 (d, $J = 3.1$ Hz, 1H), 7.09 (d, $J = 2.4$ Hz, 1H), 6.89 (dd, $J = 8.8$ Hz, 2.4 Hz, 1H), 6.55 (m, 1H), 6.41 (d, $J = 8.4$ Hz, 1H), 6.39 (d, $J = 3.0$ Hz, 1H), 4.76 (br m, 1H), 4.45 (t, $J = 6.9$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 4.12 (m, 2H), 3.53 (dd, $J = 12.6$ Hz, 6.5 Hz, 2H), 2.93 (t, $J = 6.9$ Hz, 2H), 2.12 (quint., $J = 6$ Hz, 2H), 1.27 (m, 3H).

3-{5-[3-(2-Pyridylamino)propoxy]indolyl}propanoic Acid Ammonium Salt (6). Compound **5** (0.33 g, 0.90 mmol) was dissolved in methanol (10 mL) and treated with 1 N aqueous LiOH (2 mL) at ambient temperature. After 18 h, the reaction was acidified with 10% aqueous HCl and concentrated in vacuo, and the crude product was purified by flash column chromatography (15% methanol in methylene chloride eluant), giving a very hygroscopic solid. This was dissolved in a mixture of methylene chloride and methanol (saturated with ammonia gas) and filtered, and the filtrate was concentrated in vacuo to yield **6** as a stable, pale yellow solid (0.14 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.92 (m, 1H), 7.59 (m, 1H), 7.37 (d, $J = 8.9$ Hz, 1H), 7.28 (d, $J = 3.1$ Hz, 1H), 7.04 (d, $J = 2.3$ Hz, 1H), 6.78 (dd, $J = 8.9$ Hz, 2.3 Hz, 1H), 6.75 (d, $J = 9.7$ Hz, 1H), 6.63 (br t, $J = 6.3$ Hz, 1H), 4.34 (t, $J = 6.8$ Hz, 2H), 4.05 (t, $J = 6.2$ Hz, 2H), 3.45 (dd, $J = 12.5$ Hz, 6.6 Hz, 2H), 2.71 (t, $J = 6.8$ Hz, 2H), 2.02 (quint., $J = 6.5$ Hz, 2H). Mass spectrum (LCMS, ESI+) Calcd for $C_{19}H_{21}N_3O_3$: 339.4 (M + H). Found: 340.1. HSMS (FAB+) Calcd for $C_{19}H_{22}N_3O_3$: 340.166117 (MH+). Found: 340.166819.

(*tert*-Butoxy)-*N*-[6-methyl-(2-pyridyl)]carboxamide (7). A mixture of 2-amino-picoline (6.0 g, 5.5 mmol) and di-*tert*-butyl dicarbonate (13.3 g, 6.0 mmol) was heated to 60 °C overnight (16 h). The reaction was cooled, poured into saturated NH_4Cl (250 mL), and extracted with ethyl acetate (2 × 250 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give a yellow oil (crude 12.3 g), which was used directly in the next reaction.

(*tert*-Butoxy)-*N*-methyl-*N*-[6-methyl-(2-pyridyl)]carboxamide (8). To a suspension of NaH (2.63 g 6.6 mmol) in 200 mL of *N,N*-dimethylformamide at 0 °C was added a solution of (*tert*-butoxy)-*N*-[6-methyl-(2-pyridyl)]carboxamide **7** (12.3 g, crude), as prepared in the preceding step, in 50 mL of *N,N*-dimethylformamide. The reaction stirred at 0 °C for 15 min then at ambient temperature for 1 h. Then, iodomethane (10.22 g, 7.2 mmol) was added, and the mixture was stirred at ambient temperature overnight (16 h). The reaction mixture was concentrated in vacuo, diluted with saturated NH_4Cl (400 mL), and extracted with ethyl acetate (2 × 250 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography on silica gel (10% ethyl acetate in hexane) to give **8** as a yellow oil (7.56 g, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.63 (t, J = 7.2 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 6.9 Hz, 1H), 3.27 (s, 2H), 2.42 (s, 3H), 1.45 (s, 9H).

Ethyl 2-{6-[(*tert*-butoxy)-*N*-methylcarbonylamino]-2-pyridyl}acetate (9). Lithium diisopropylamide (6.6 mmol) was prepared in tetrahydrofuran (60 mL) and cooled to −78 °C, and (*tert*-butoxy)-*N*-methyl-*N*-[6-methyl-(2-pyridyl)]carboxamide **(8)** (7.56 g, 3.3 mmol) was dissolved in tetrahydrofuran (100 mL) and added dropwise over 30 min. The mixture was stirred for 15 min then diethyl carbonate (6.24 g, 5.3 mmol) was added. The solution was stirred for an additional 15 min, and then allowed to warm to 0 °C over 2 h. The reaction was quenched with saturated NH_4Cl solution (200 mL). The mixture was allowed to warm to ambient temperature and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (silica gel, 10% ethyl acetate in hexane) to yield **9** as yellow oil (5.51 g, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.71 (t, J = 7.9 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.07 (d, J = 7.4 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.78 (s, 2H), 2.54 (s, 3H), 1.46 (s, 9H), 1.18 (t, J = 7.1 Hz, 3H).

Ethyl 2-[6-(methylamino)-2-pyridyl]acetate (10). A solution of **9** (5.51 g, 1.9 mmol) in methylene chloride (25 mL) was stirred in an ice bath at 0 °C. Trifluoroacetic acid (10 mL) was then added, and the solution was allowed to warm to ambient temperature and stirred overnight (16 h). The reaction mixture was concentrated, 10% aqueous K_2CO_3 (300 mL) was added, and the mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield **10** as bright yellow oil (3.4 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.32 (t, J = 7.2 Hz, 1H), 6.40 (d, J = 7.0 Hz, 1H), 6.29 (d, J = 8.3 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 3.56 (s, 2H), 2.71 (d, J = 4.9 Hz, 3H), 1.17 (t, J = 7.1 Hz, 3H).

2-[6-(Methylamino)-2-pyridyl]ethan-1-ol (11). To a suspension of lithium aluminum hydride (1.8 g, 4.9 mmol) in tetrahydrofuran (50 mL) was added dropwise a solution of **10** (3.5 g, 1.9 mmol) in tetrahydrofuran (50 mL) at 0 °C. After the addition was completed, the reaction mixture was stirred at 0 °C for 30 min and then stirred at ambient temperature for 2 h. The reaction mixture was then cooled back to 0 °C, quenched with H_2O (1.8 mL), 10% NaOH (1.8 mL), and H_2O (3.0 mL), and allowed to warm back to ambient temperature. The solids were removed by filtration through Celite and washed with tetrahydrofuran (100 mL). The filtrate was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography on silica gel (3% methanol in methylene chloride) to yield **11** as yellow oil (2.1 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (t, J = 7.8 Hz, 1H), 6.41 (d, J = 7.2 Hz, 1H), 6.26 (d, J = 8.3 Hz, 1H), 4.51 (br s, 1H), 3.96 (t, J = 5.2 Hz, 2H), 2.89 (d, J = 5.1 Hz, 3H), 2.84 (t, J = 5.4 Hz, 2H).

Methyl 3-(5-{2-[6-(Methylamino)-2-pyridyl]ethoxy}-indolyl)propanoate (12). Diisopropyl azodicarboxylate (0.19 g, 0.94 mmol) was added to a solution of 2-[6-(methylamino)-2-pyridyl]ethan-1-ol **11** (0.10 g, 0.66 mmol), methyl 3-(5-hydroxyindolyl)propanoate (**4**) (0.10 g, 0.46 mmol), and triphenylphosphine (0.24 g, 0.92 mmol) in tetrahydrofuran (5.0 mL) at 0 °C in an ice bath. After the mixture was stirred at ambient temperature overnight (16 h), the reaction was concentrated and the residue was purified by flash chromatography on silica gel (20%−30% ethyl acetate in hexane) to yield **12** as yellow oil (0.023 g, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 (t, J = 7.3 Hz, 1H), 7.20 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 3.1 Hz, 1H), 6.87 (dd, J = 2.4, 8.9 Hz, 1H), 6.56 (d, J = 7.2 Hz, 1H), 6.37 (d, J = 3.1 Hz, 1H), 6.24 (d, J = 8.2 Hz, 1H), 4.56 (br s, 1H), 3.40 (t, J = 6.9 Hz, 2H), 4.34 (t, J = 7.0 Hz, 2H), 3.65 (s, 3H), 3.10 (t, J = 7.0 Hz, 2H), 2.89 (d, J = 4.8 Hz, 2H), 2.80 (t, J = 6.9 Hz, 2H).

3-(5-{2-[6-(Methylamino)-2-pyridyl]ethoxy}indolyl)-propanoic Acid (13). To a solution of methyl 3-(5-{2-[6-(methylamino)-2-pyridyl]ethoxy}indolyl)propanoate **12** (0.023 g, 0.65 mmol) in methanol (3 mL) was added sodium hydroxide (0.15 g, 3.8 mmol) in H_2O (0.5 mL), and the reaction was stirred for 6 h at ambient temperature. After the solvent was evaporated in vacuo, the residue is taken up in H_2O (5 mL) and acidified to pH 4−5 with 10% HCl and extracted with a mixture of ethyl acetate and butanol (2 × 50 mL) and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield **13** as a solid (0.018 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ: 7.52 (t, J = 7.3 Hz, 1H), 7.25 (d, J = 8.9 Hz, 1H), 7.14 (d, J = 3.1 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 6.81 (dd, J = 8.9, 2.4 Hz, 1H), 6.60 (d, J = 7.3 Hz, 1H), 6.38 (d, J = 8.6 Hz, 1H), 6.33 (d, J = 3.2 Hz, 1H), 4.38 (t, J = 7.0 Hz, 2H), 4.24 (t, J = 6.6 Hz, 2H), 3.06 (t, J = 6.6 Hz, 2H), 2.89 (s, 3H), 2.77 (t, J = 6.9 Hz, 2H). Mass spectrum (LCMS, ESI+) Calcd for $C_{19}H_{21}N_3O_3$: 340.3 (M + H). Found: 340.9. HSMS (FAB+) Calcd for $C_{19}H_{22}N_3O_3$: 340.166117 (MH+). Found: 340.166546.

3-Iodophenyl Acetate (14). A solution of 3-iodophenol (3 g, 13.6 mmol), acetyl chloride (2.9 mL, 40.9 mmol), and potassium carbonate (9.42 g, 68.2 mmol) in *N,N*-dimethylformamide (75 mL) was stirred for 16 h at room temperature. The mixture was partitioned between water and ethyl acetate. The organic layer was washed with 1N NaOH, dried over magnesium sulfate, and evaporated under vacuum. The crude product was chromatographed over silica gel, eluting with 20% ethyl acetate/hexanes to yield 2.3 g (65%) of **14**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (m, 1H), 7.46 (m, 1H), 7.08 (m, 2H) 2.29 (s, 3H).

3-[1,1-Bis(methylethyl)-2-methyl-1-silapropylthio]phenyl Acetate (15). Triisopropylsilanethiol (2.91 mL, 13.5 mmol) was added dropwise to a suspension of sodium hydride (325 mg, 13.5 mmol) in THF (10 mL). After the evolution of hydrogen ceased, a solution of **14** (2.37 g, 9.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.04 g, 0.9 mmol) in toluene (90 mL) was added. After being refluxed for 16 h under argon, the reaction was cooled to room temperature, and the solvent was evaporated under vacuum. The resulting residue was dissolved in ethyl acetate, washed with 1 N NaOH and brine, dried with sodium sulfate, filtered, and evaporated under vacuum. The crude product was chomatrographed over silica gel to yield 1.53 g (52%) of **15**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 (m, 2H), 7.23 (t, J = 2.4 Hz, 1H), 6.94 (dd, J = 1.2, 8.4 Hz, 1H), 2.28 (s, 3H), 1.25 (m, 3H), 1.08 (d, J = 7.2 Hz, 18 H).

Ethyl 5-Bromo-4-oxopentanoate (16). (Trimethylsilyl)-diazomethane (34 mL, 67 mmol, 2.0 M solution in hexanes) was added dropwise to a solution of ethyl succinyl chloride (5 g, 30.3 mmol) in acetonitrile (60 mL) over a period of 30 min. After the mixture was stirred for 2 h, hydrogen bromide (14 mL, 30% solution in acetic acid) was slowly added over 15 min. After the reaction was stirred for an additional 1 h, the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate and washed with 1 N NaOH and brine. The organic layer was dried with sodium sulfate, filtered, and evaporated under vacuum to yield 4.3 g (64%) of **16**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.13 (c, J = 7.2 Hz, 2H), 3.96 (s, 2H), 2.95 (t, J = 6.4 Hz, 2H), 2.65 (t, J = 6.4 Hz, 2H), 1.24 (t, J = 7.2 Hz, 1H).

Ethyl 5-(3-Acetyloxyphenylthio)-4-oxopentanoate (17). Tetrabutylammonium fluoride (7 mL, 7.0 mmol, 1 M in THF) was added to a solution of **15** (1.53 g, 4.7 mmol) in THF (10 mL) under argon at room temperature. The reaction was

stirred for 15 min, followed by addition of a solution of **16** (1.15 g, 5.17 mmol) in THF (5 mL). After the reaction was stirred for 3 h, the solvent was removed under vacuum, and the crude product was chromatographed over silica gel to yield 920 mg (73%) of **17**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.29 (t, J = 8.0 Hz, 1H), 7.18 (dd, J = 0.8, 7.6 Hz, 1H), 7.07 (t, J = 1.6 Hz, 1H), 6.94 (dd, J = 1.2, 8.0 Hz, 1H), 4.12 (c, J = 7.2 Hz, 2H), 3.75 (s, 2H), 2.89 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 2.29 (s, 3H), 1.24 (t, J = 7.2 Hz, 1H).

Ethyl 3-(6-Hydroxybenzo[*b*]thiophen-3-yl)propanoate (18). Concentrated sulfuric acid (20 mL) was cooled in an ice−water bath to 0 °C and added to a flask containing **17** (920 mg, 3.4 mmol) at 0 °C. The reaction was stirred at 0 °C for 15 min and then poured over ice. The mixture was extracted with ethyl acetate, dried, filtered, and evaporated under vacuum to yield 700 mg (82%) of **18**. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.59 (s, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 2.4 Hz, 1H), 7.08 (s, 1H), 6.89 (dd, J = 2.4, 8.4 Hz, 1H), 4.06 (c, J = 7.2 Hz, 2H), 2.99 (t, J = 6.8 Hz, 2H), 2.70 (t, J = 6.8 Hz, 2H), 1.16 (t, J = 7.2 Hz, 1H).

Ethyl 3-(6-{2-[6-(Methylamino)-2-pyridyl]ethoxy}benzo-[*b*]thiophen-3-yl)propanoate (19). Ethyl 3-(6-hydroxybenzo-[*b*]thiophen-3-yl)propanoate (**18**) (100 mg, 0.4 mmol) and 4-methylmorpholine (0.05 mL, 0.44 mmol) were dissolved in THF (5 mL) and stirred for 5 min. 2-[6-(Methylamino)-2-pyridyl]ethan-1-ol (**11**) (91 mg, 0.6 mmol), triphenylphosphine (210 mg, 0.8 mmol), and diisopropyl azodicarboxylate (0.16 mL, 0.8 mmol) were added to the mixture sequentially. After being stirred overnight under argon, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was dried with sodium sulfate, filtered, and evaporated under vacuum. The crude product was chromatographed over silica gel to yield 30 mg (19%) of **19**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 7.2, 8.0 Hz, 1H), 7.35 (d, J = 2.0 Hz, 1H), 7.07 (dd, J = 2.4, 8.8 Hz, 1H), 6.93 (m, 1H), 6.56 (d, J = 7.2 Hz, 1H), 6.25 (d, J = 8.0 Hz, 1H), 4.40 (t, J = 6.8 Hz, 2H), 4.15 (c, J = 7.2 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.90 (m, 5H), 2.74 (t, J = 6.4 Hz, 2H), 1.25 (t, J = 7.2 Hz, 1H).

3-(6-{2-[6-(Methylamino)-2 -pyridyl]ethoxy}benzo[*b*]-thiophen-3-yl)propanoic Acid (20). NaOH (10 mL, 1 N) was added to a solution of **19** and THF (10 mL). The reaction was stirred at room temperature for 16 h. The mixture was diluted with water and ethyl acetate. The separated aqueous layer was neutralized with 1 N HCl to pH = 6.5. The resulting precipitate was filtered, washed with distilled water, and dried to yield 74 mg (55%) of **20** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.67 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 7.31 (dd, J = 7.2, 8.0 Hz, 1H), 7.18 (s, 1H), 7.00 (dd, J = 2.4, 8.0 Hz, 1H), 6.45 (d, J = 7.2 Hz, 1H), 6.37 (m, 1H), 6.27 (d, J = 8.0 Hz, 1H), 4.36 (t, J = 6.4 Hz, 2H), 2.99 (t, J = 6.4 Hz, 2H), 2.90 (d, J = 8.0 Hz, 3H), 2.64 (t, J = 6.4 Hz, 2H). Mass spectrum (LCMS, ESI) Calcd for $C_{19}H_{21}N_2O_3S$: 357.1 (M + H). Found: 357.3. HSMS (FAB+) Calcd for $C_{19}H_{21}N_2O_3S$: 357.127290 (MH+). Found: 357.128119.

3-[1,1-Bis(methylethyl)-2-methyl-1-silapropoxy]phenyl Acetate (21). Lithium bis(trimethylsilyl)amide (73 mL, 1 M solution in THF) was added dropwise to a solution of resorcinol monoacetate (10 g, 65.7 mmol) in THF (100 mL) at 78 °C under argon. The solution was stirred for 10 min, and then triisopropylsilyl chloride (15.5 mL, 73 mmol) was added via syringe. After being stirred at room temperature overnight, the mixture was partitioned between water and ethyl acetate. The organic layer was dried, filtered, and evaporated under vacuum to yield 13 g of crude 3-[1,1-bis(methylethyl)-2-methyl-1-silapropoxy]phenyl acetate (**21**), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.19 (t, J = 8.0 Hz, 1H), 6.75 (m, 1H), 6.68 (m, 1H), 6.63 (t, J = 4.0 Hz, 1H), 2.29 (s, 3H), 1.25 (m, 3H), 1.11 (d, J = 7.0 Hz, 18H).

3-[1,1-Bis(methylethyl)-2-methyl-1-silapropoxy]phenol (22). An aqueous (50 mL) solution of NaOH (3.25 g, 81 mmol) was added to a solution of **21** (5 g, 16.2 mmol) in THF (50 mL). After being stirred overnight at room temperature, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried, filtered, and evaporated under vacuum. The crude product was chromatographed over silica gel to yield 3.89 g (90%) of **22**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.06 (t, J = 8.0 Hz, 1H), 6.42 (m, 3H), 1.28 (m, 3H), 1.10 (d, J = 7.0 Hz, 18H).

[6-(2-{3-[1,1-Bis(methylethyl)-2-methyl-1-silapropoxy]-phenoxy}ethyl)(2-pyridyl)]methylamine (23). To a stirred solution of **22** (200 mg, 0.75 mmol), **11** (104 mg, 0.68 mmol), triphenylphosphine (199 mg, 0.75 mmol), and THF (25 mL) was added diethyl azodicarboxylate (0.12 mL, 0.75 mmol) at 0 °C. The reaction was stirred overnight under argon. The solvent was removed under vacuum, and the crude product was chromatographed over silica gel to yield 76 mg (28%) of **23**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 (m, 1H), 7.07 (t, J = 8.0 Hz, 1H), 6.5 (m, 3H), 6.25 (d, J = 8 Hz, 1H), 4.27 (t, J = 6.8 Hz, 2H), 3.06(t, J = 6.8 Hz, 2H), 2.90 (d, J = 8.0 Hz, 3H), 1.28 (m, 3H), 1.10 (d, J = 7.0 Hz, 18H).

Ethyl 5-(3-{2-[6-(Methylamino)(2-pyridyl)]ethoxy}-phenoxy)-4-oxopentanoate (24). To a solution of **23** (1.60 g, 4.0 mmol) in THF (30 mL) under argon at room temperature was added tetrabutylammonium fluoride (4.4 mL, 4.4 mmol, 1 M in THF). After the mixture was stirred for 15 min, a solution of **16** (0.98 g, 4.4 mmol) in THF (5 mL) was added. The mixture was stirred for an additional 3 h. The solvent was removed under vacuum, and the remaining residue was chromatographed over silica gel to yield 860 mg (56%) of **24**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (m, 1H), 7.16 (t, J = 8.2 Hz, 1H), 7.08 (t, J = 7.9 Hz, 1H), 6.64 (m, 1H), 6.45 (m, 3H), 6.26 (dd, J = 8.2, 2.3 Hz, 1H), 4.57 (s, 2H), 4.30 (t, J = 6.8 Hz, 2H), 4.13 (c, J = 7.2 Hz, 2H), 3.07 (m, 2H), 2.91 (m, 5H), 2.63 (t, J = 6.6 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H).

Ethyl 3-(6-{2-[6-(Methylamino)-2-pyridyl]ethoxy}benzo-[*b*]furan-3-yl)propanoate (25). Concentrated sulfuric acid (3 mL) was cooled in an ice−water bath to 0 °C and added to a flask containing **24** (190 mg, 0.5 mmol) at 0 °C. The reaction was stirred 15 min and then poured over ice. The solution was neutralized with solid sodium hydrogencarbonate (pH = 7), and the product was extracted with ethyl acetate. The organic layer was dried, filtered, and evaporated under vacuum to yield 104 mg (57%) of **25**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (m, 3H), 7.01 (d, J = 2.1 Hz, 1H), 6.87 (dd, J = 8.5, 2.1 Hz, 1H), 6.54 (d, J = 7.2 Hz, 1H), 6.23 (d, J = 8.2 Hz, 1H), 4.68 (br s, 1H), 4.15 (c, J = 7.4 Hz, 2H), 3.09 (t, J = 6.9 Hz, 2H), 2.97 (t, J = 6.9 Hz, 2H), 2.87 (d, J = 5.1 Hz, 3H), 2.68 (t, J = 6.9 Hz, 2H), 1.26 (t, J = 7.4 Hz, 1H).

3-(6-{2-[6-(Methylamino)-2-pyridyl]ethoxy}benzo[*b*]-furan-3-yl)propanoic Acid (26). NaOH (4 mL, 1 N) was added to a solution of **25** in THF (4 mL) and stirred for 16 h. The reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was neutralized with 1 N HCl (pH = 6.5). The resulting precipitate was filtered, rinsed with distilled water, and dried to yield 70 mg (74%) of **26** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.54 (dd, J = 7.3, 8.6 Hz, 1H), 7.34 (m, 2H), 6.99 (d, J = 2.0 Hz, 1H), 6.77 (dd, J = 2.0, 8.6 Hz, 1H), 6.53 (d, J = 7.1 Hz, 1H), 6.37 (d, J = 7.1 Hz, 1H), 6.27 (d, J = 8.5 Hz, 1H), 4.19 (t, J = 6.5 Hz, 2H), 3.09 (t, J = 6.5 Hz, 2H), 2.94 (m, 2H), 2.87 (s, 3H), 2.69 (t, J = 6.5 Hz, 2H). Mass spectrum (LCMS, ESI) Calcd for $C_{19}H_{21}N_2O_4$: 341.1 (M + H). Found: 341.4. HSMS (FAB+) Calcd for $C_{19}H_{21}N_2O_4$: 341.150132 (MH+). Found: 341.150855.

In Vitro Inhibition of Protein−Protein Binding. $\alpha_{IIb}\beta_{IIIa}$-Fibrinogen Assay. The assay is based on the method of Dennis.[20] Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at 4 °C with 100 μL/well of 10 μg/mL human fibrinogen (Calbiochem) in 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM $CaC1_2$, and 0.02% NaN_3 (TAC buffer). Plates were subsequently emptied and blocked for 1 h at 37 °C with 150 μL/well of TAC buffer containing 0.05% Tween 20 and 1% bovine serum albumin (TACTB buffer). After being washed three times with 300 μL/well of 10 mM Na_2HPO_4 pH 7.5, 150 mM NaCl, and 0.01% Tween 20 (PBST buffer), controls or test compounds (0.027−20.0 μM) were mixed with 40 μg/mL human GPIIbIIIa (Enzyme Research Laboratories) in TACTB buffer,

and 100 μL/well of these solutions was incubated for 1 h at 37 °C. The plate was then washed five times with PBST buffer, and 100 μL/well of a monoclonal anti-GPIIbIIIa antibody in TACTB buffer (1 μg/mL, Enzyme Research Laboratories) was incubated at 37 °C for 1 h. After the plate was washed 5 times with PBST buffer, 100 μL/well of goat anti-mouse IgG conjugated to horseradish peroxidase (Kirkegaard & Perry) was incubated at 37 °C for 1 h (25 ng/mL in PBST buffer), followed by a 6-fold PBST buffer wash. The plate was developed by adding 100 μL/well of 0.67 mg of *o*-phenylenediamine dihydrochloride per milliliter of 0.012% H_2O_2, 22 mM sodium citrate, and 50 mM sodium phosphate, pH 5.0 at room temperature. The reaction was stopped with 50 μL/well of 2 M H_2SO_4, and the absorbance at 492 nm was recorded. Percent (%) inhibition was calculated from an average of three separate determinations relative to buffer controls (no test compound added), and a four parameter fit was used to estimate the half-maximal inhibition concentration (IC_{50}).

$\alpha_v\beta_3$-Vitronectin Assay. The assay was based on the method of Niiya,[21] and all steps were performed at room temperature. Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at room temperature with 100 μL/well of 0.4 μg/mL human $\alpha_v\beta_3$ (Chemicon) in TS buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$). Plates were subsequently emptied and blocked for 2 h with 150 μL/well of TS buffer containing 1% BSA (TSB buffer) and washed three times with 300 μL/well of PBST buffer. Controls or test compounds (0.0001−20.0 μM) were mixed with 1 μg/mL of human vitronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μL/well of these solutions (in TSB buffer) were incubated for 2 h. The plate was then washed five times with PBST buffer, and 100 μL/well of 0.25 μg/mL NeutrAvidin−horseradish peroxidase conjugate (Pierce) in TSB buffer was added to the plate and incubated for 1 h. After a 5-fold PBST buffer wash, the plate was developed, and results were calculated as described for the IIbIIIa-fibrinogen assay.

$\alpha_v\beta_5$-Vitronectin Assay. The assay is similar to the $\alpha_v\beta_3$-vitronectin assay, and all steps were performed at room temperature. Costar 9018 flat-botom 96-well ELISA plates were coated overnight at room temperature with 100 μL/well of 1 μg/mL human $\alpha_v\beta_5$ (Chemicon) in TS buffer. Plates were blocked for 2 h with 150 μL/well of TSB buffer and washed three times with 300 μL/well of PBST buffer. Controls or test compound (0.0001−20 μM) were mixed with 1 μg/mL of human vitronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μL/well of these solutions (in TSB buffer) was incubated for 2 h. The plate was then washed five times with PBST buffer, and 100 μL/well of 0.25 μg/mL NeutrAvidin−horseradish peroxidase conjugate (Pierce) in TSB buffer was added to the plate and incubated at for 1 h. After a 5-fold PBST buffer wash, the plate was developed, and results were calculated as described for the IIbIIIa-fibrinogen assay.

$\alpha_5\beta_1$-Fibronectin Assay. Costar 9018 flat-botom 96-well ELISA plates were coated overnight at room temperature with 100 μL/well of 3 μg/mL human $\alpha_5\beta_1$ (Chemicon) in TS buffer. Plates were subsequently emptied, blocked for 2 h at 30 °C with 150 μL/well of TSB buffer, and washed 3 times with 300 μL/well of PBST buffer. Controls or test compounds (0.0001−20 μM) were mixed with 1 μg/mL of human fibronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μL/well of these solutions (in TSB buffer) was incubated for 2 h at 30 °C. The plate was then washed three times with PBST buffer, and 100 μL/well of 0.25 μg/mL NeutrAvidin−horseradish peroxidase conjugate (Pierce) in TSB buffer was added to the plate and incubated at for 1 h at 30 °C. After a 6-fold PBST buffer wash, the plate was developed, and results were calculated as described for the IIbIIIa-fibrinogen assay.

Supporting Information Available: HPLC data for the compounds under investigation. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hynes, R. O. Integrins: Versatility, Modulation and Signaling in Cell Adhesion. *Cell* **1992**, *69*, 11−25.

(2) M. J. Humphries, Integrin Structure, *Biochem. Soc. Trans.* **2000**, *28*, 311−339.

(3) (a) Iwao Ojima, Subrata Chakravarty and Qing Dong, Antithrombotic agents: From RGD to peptide mimetics, *Bioorg. Med. Chem.* **1995**, *3*, 337−360. (b) Samanen, J, GPIIb/IIIa Antagonists. *Annu. Rep. Med. Chem.* **1996**, *31*, 91−100.

(4) Elices, M. J. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*; Mousa, S. A., Ed.; Springer and R. G. Landes Co.: Berlin, Germany, 1998; p 133.

(5) (a) Xiong, J.-P.; Stehe, T.; Diefenbach, B.; Zhang, R.; Dunker, R.; Scott, D. L.; Joachimiak, A.; Goodman, S. L.; Arnaout, M. A.; Crystal Structure of the Extracellular Segment of Integrin $\alpha_v\beta_3$. *Science* **2001**, *294*, 339−345. (b) Xiong, J.-P.; Stehle, T.; Zhang, R.; Joachimiak. A.; Frech, M.; Goodman, S. L.; Arnaout, M. A. Crystal Structure of the Extracellular Segment of Integrin $\alpha_v\beta_3$ in Complex with an Arg-Gly-Asp Ligand. *Science* **2002**, *296*, 151−155. (c) Gottschalk, K. E.; Gunther, R.; Kessler, H. A Three-State Mechanism of Integrin Activation and Signal Transduction for Integrin $\alpha_v\beta_3$. *ChemBioChem* **2002**, *5*, 470−473. (d) Feuston, B. P.; Culberson, J. C.; Duggan, M. E.; Hartman, G. D.; Leu, C.-T.; Rodan, S. B. Binding Model for Nonpeptide Antagonists of $\alpha_v\beta_3$ Integrin. *J. Med. Chem.* **2002**, *45*, 5640−5648.

(6) (a) Cardo-Vila, Marina; Arap, Wadih; Pasqualini, Renata. $\alpha_v\beta_5$ integrin-dependent programmed cell death triggered by a peptide mimic of annexin V. *Mol. Cell* **2003**, *11*, 1151−1162. (b) Eskens, F. A. L. M.; Dumez, H.; Hoekstra, R.; Perschl, A.; Brindley, C.; Bottcher, S.; Wynendaele, W.; Drevs, J.; Verweij, J.; van Oosterom, A. T. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in patients with advanced solid tumours. *Eur. J. Cancer* **2003**, *39*, 917−926. (c) Eskens, F. A. L. M.; Dumez, H.; Hoekstra, R.; Perschl, A.; Brindley, C.; Bottcher, S.; Wynendaele, W.; Drevs, J.; Verweij, J.; van Oosterom, A. T. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in patients with advanced solid tumours. *Eur. J. Cancer* **2003**, *39*, 917−926.

(7) (a) Duong, L. T.; Rodan, G. A. Regulation of osteoclast formation and function. *Rev. Endocrinol. Metab. Dis.* **2001**, *2*, 95−104. (b) For a review on the role of $\alpha_v\beta_3$ in osteoporosis see: Robey, P. G. *Ann. Rep. Med. Chem.* **1993**, *28*, 227. (c) Engleman, V. W.; Nickols, G. A.; Ross, F. P.; Horton, M. A.; Griggs, D. W.; Settle, S. L.; Ruminski, P. G.; Teitelbaum, S. L. A Peptidomimetic Antagonist of the $\alpha_v\beta_3$ Integrin Inhibits Bone Resorption in Vitro and Prevents Osteoporosis in Vivo. *J. Clin. Invest.* **1997**, *99*, 2284−2292.

(8) (a) Matsuno, H.; Stassen, J. M.; Vermylen, J.; Deckmyn, H. Inhibition of Integrin Function by a Cyclic RGD-Containing Peptide Prevents Neointima Formation. *Circulation* **1994**, *90*, 2203−2206. (b) Choi, E. T.; Engel, L.; Callow, A. D.; Sun, S.; Trachtenberg, J.; Santoro, S.; Ryan, U. S. Inhibition of Neointimal Hyperplasia by Blocking $\alpha_v\beta_3$ Integrin with a Small Peptide Antagonist GpenGRGDSPCA. *J. Vasc. Surg.* **1994**, *19*, 125−134.

(9) Noiri, E.; Gailit, J.; Sheth, D.; Magazine, H.; Gurrath, M.; Müller, G.; Kessler, H.; Goligorsky, M. S. Cyclic RGD Peptides Ameliorate Ischemic Acute Renal Failure in Rats. *Kidney Int.* **1994**, *46*, 1050−1058.

(10) (a) Hammes, H.-P.; Brownlee, M.; Jonczyk, A.; Sutter, A.; Preissner, K. T. Subcutaneous Injection of a Cyclic Peptide Antagonist of Vitronectin Receptor-Type Integrins Inhibits Retinal Neovascularization. *Nat. Med.* **1996**, *2*, 529. (b) Friedlander, M.; Theesfeld, C. L.; Sugita, M.; Fruttiger, M.; Thomas, M. A.; Chang, S.; Cheresh, D. A., Involvement of Integrines $\alpha_v\beta_3$ in Ocular Neovascular Diseases, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9764.

(11) (a) Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. Integrin $\alpha_v\beta_3$ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels, *Cell* **1994**, *79*, 1157. (b) Strömblad, S.; Cheresh, D. A. Integrins, angiogenesis and vascular cell survival. *Chem. Biol.* **1996**, *3*, 881−885.

(12) Yun, Z.; Menter, D. G.; Nicolson, G. L. Involvement of Integrin $\alpha_v\beta_3$ in Cell Adhesion, Motility, and Liver Metastasis of Murine RAW117 Large Cell Lymphoma. *Cancer Res.* **1996**, *56*, 3103−3111.

(13) Kaul, D. K.; Tsai, H. M.; Liu, X. D.; Nakada, M. T.; Nagel, R. L.; Coller, B. S. Monoclonal Antibodies to $\alpha_v\beta_3$ (7E3 and LM609) Inhibit Sickle Red Blood Cell−Endothelium Interactions Induced by Platelet-Activating Factor. *Blood* **2000**, 95, 368−374.

(14) Goodman, S. L.; Holzemann, G.; Sulyok, G. A. G.; Kessler, H. Nanomolar Small Molecule Inhibitors for $\alpha_v\beta_6$, $\alpha_v\beta_5$, and $\alpha_v\beta_3$ Integrins. *J. Med. Chem.* **2002**, *45*, 1045−1051.

(15) Varner, J. A.; Cheresh, D. A. Tumor Angiogenesis and the Role of Vascular Cell Integrin $\alpha_v\beta_3$. *Important Adv. Oncol*. **1996**, 69−87.

(16) (a) Ahrens, Ingo; Peter, Karlheinz; Bode, Christoph. Use of GPIIb/IIIa Inhibitors in Cardiovascular Medicine. *Expert Rev. Cardiovasc. Ther.* **2003**, *1*, 233−242. (b) Giavazzi, Raffaella; Nicoletti, Maria Ines. Small Molecules in Anti-Angiogenic Therapy. *Curr. Opin. Invest. Drug* **2002**, *3* , 482−491, (c) Adams, S. P.; Lobb, R. R. Small-Molecule VLA-4 Antagonists, *Prog. Respir. Res.* **2001**, 31, 302−305 (New Drugs for Asthma, Allergy and COPD). (d) Kerr, J. S.; Slee, A. M.; Mousa, S. A. Small Molecule α_v Integrin Antagonists: Novel Anticancer Agents. *Expert Opin. Invest. Drugs* **2000**, *9*, 1271−1279.

(17) (a) Brashear, K. M.; Coleman, P. J.; Hutchinson, J. H.; Duggan, M. E.; Hartman, G. D.; Whitman, D. B.; Smith, G. R.; Rodan, S. B.; Rodan, G. A.; Leu, C.-T.; Duong, L.; Kimmel, D.; Prueksaritanont, T.; Fernandez-Metzler, C.; Ma, B.; Lynch, J. J. Non-Peptide $\alpha_v\beta_3$ Receptor Antagonists: Identification of Potent, Orally Active RGD Mimetics for the Treatment and Prevention of Osteoporosis. Abstracts of Papers, 226th ACS National Meeting, New York, United States, September 7−11, 2000. (b) Phenylbutyrates as Potent, Orally Bioavailable Vitronectin Receptor (Integrin $\alpha_v\beta_3$) antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1483−1486. (c) Coleman, P. J.; Askew, B. C.; Hutchinson, J. H.; Whitman, D. B.; Perkins, J. J.; Hartman, G. D.; Rodan, G. A.; Leu, C.-T.; Prueksaritanont, T.; Fernandez-Metzler, C.; Merkle, K. M.; Lynch, R.; Lynch, J. J.; Rodan, S. B.; Duggan, M. E. Non-Peptide $\alpha_v\beta_3$ Antagonists. Part 4: Potent and Orally Bioavailable Chain-Shortened RGD Mimetics. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2463−2465. (d) Lark, M. W.; Stroup, G. B.; Hwang, S.-M.; James, I. E.; Rieman, D. J.; Drake, F. H.; Bradbeer, J. N.; Mathur, A.; Erhard, K. F.; Newlander, K. A.; Ross, S. T.; Salyers, K. L.; Smith, B. R.; Miller, W. H.; Huffman, W. F.; Gowen, M., Design and Characterization of Orally Active Arg-Gly-Asp Peptidomimetic Vitronectin Receptor Antagonist SB 265123 for Prevention of Bone Loss in Osteoporosis. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 612−617. (e) Keenan, R. M.; Miller, W. H.; Barton, L. S.; Bondinell, W. E.; Cousins, R. D.; Eppley, D. F.; Hwang, S.-M.; Kwon, C.; Lago, M. A.; Nguyen, T. T.; Smith, B. R.; Uzinskas, I. N.; Yuan, Catherine C. K. Orally Bioavailable Nonpeptide Vitronectin Receptor Antagonists Containing 2-Aminopyridine Arginine Mimetics. *Bioorg. Med. Chem. Lett.* **1999**, *9* , 1801−1806.

(18) (a) Manthey, C. L.; Lee, Y.; Wang, A.; Crysler, C.; Zhao, S.; Bone, R. F.; Soll, R. M.; Tomczuk, B. E. Characterization of *O*-Guanidines as Novel $\alpha_v\beta_3$ Inhibitors. *Proc. Am. Assoc. Cancer Res*. **2001**, *42*, March 24−28, 368. (b) Marugan, J. J.; Haslow, K. D.; Crysler, C. Design, Synthesis and Biological Evaluation of Novel $\alpha_v\beta_3$ Integrin Ligands. *Bioorg. Med. Chem. Lett.* **2004**, *17*, 4553−4555.

(19) Davies, B.; Morris, T. Physiological Parameters in Laboratory Animals and Humans. *Pharm. Res.* **1993**, *10*, 1093−1095.

(20) Dennis, M. S.; Carter, P.; Lazarus, R. A. Binding Interactions of Kistrin with Platelet Glycoprotein IIb-IIIa: Analysis by Site-Directed Mutagenesis. *Proteins* **1993**, *15*, 312−321.

(21) Niiya, K.; Hodson, E.; Bader, R.; Byers-Ward, V.; Koziol, J. A.; Plow E. F.; Ruggeri, Z. M. Increased Surface Expression of the Membrane Glycoprotein IIb/IIIa Complex Induced by Platelet Activation. Relationship to the Binding of Fibrinogen and Platelet Aggregation. *Blood* **1987**, *70*, 475−483.

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