Nonpeptide $\alpha_v\beta_3$ Antagonists. 8. In Vitro and in Vivo Evaluation of a Potent $\alpha_v\beta_3$ Antagonist for the Prevention and Treatment of Osteoporosis

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3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]-imidazolidin-1-yl}propionic acid **6** was identified as a potent and selective antagonist of the $\alpha_v\beta_3$ receptor. This compound has an excellent in vitro profile (IC_{50} = 0.08 nM), a significant unbound fraction in human plasma (12%), and good pharmacokinetics in rat, dog, and rhesus monkey. On the basis of the efficacy shown in three in vivo models of bone turnover, the compound was selected for clinical development. To support the ongoing metabolism and safety studies, a novel strategy was employed in which a series of oxidized derivatives of **6** were prepared by exposure of **6** (or the methyl ester) to chemical oxidizing agents. These products proved useful in the identification of active metabolites generated by either in vitro or in vivo metabolism.

Introduction

Osteoporosis is primarily a disease of the elderly, characterized by a significant reduction in bone mass leading to a decrease in mechanical bone strength and, consequently, an increase in the risk of fracture.[1] Fractures in this population are a significant contributor to mortality. Despite the fact that there are a number of proven therapies for the management of osteoporosis, the disease is significantly under-treated and there is still a need for effective new medicines.[2,3] In recent years, it has been shown that the $\alpha_v\beta_3$ integrin is involved in the bone remodeling process. Highly expressed in osteoclasts, this receptor is implicated in the adhesion, activation, and migration of osteoclasts on the bone surface as well as osteoclast polarization.[4−6] A number of arg-gly-asp (RGD)-containing proteins, including osteopontin, bone sialoprotein, vitronectin, and fibrinogen, are known to bind to $\alpha_v\beta_3$. Antibodies to $\alpha_v\beta_3$, RGD-peptides, and small-molecule $\alpha_v\beta_3$ antagonists have been shown to be efficacious in in vitro and in vivo models of bone resorption, providing strong evidence that inhibitors of this integrin would be useful agents for the treatment of osteoporosis.[7−16]

In previous publications from this laboratory, we have described the evolution of small-molecule $\alpha_v\beta_3$ antagonists that were crafted as mimetics of the RGD-triad that incorporate the key pharmacophores (see Figure 1). Using a pyrrolidinone scaffold as a conformational lock, and replacing the guanidine group of the arginine with a tetrahydronaphthyridine (THN), compounds of general structure **1** were identified as potent and orally bioavailable zwitterionic antagonists.[17] Despite extensive SAR studies, further advances in this series were stymied, as increases in potency were not accompanied by acceptable pharmacokinetics. A paradigm shift in our approach to this problem occurred when it was found that the deletion of an amide bond, to afford analogues

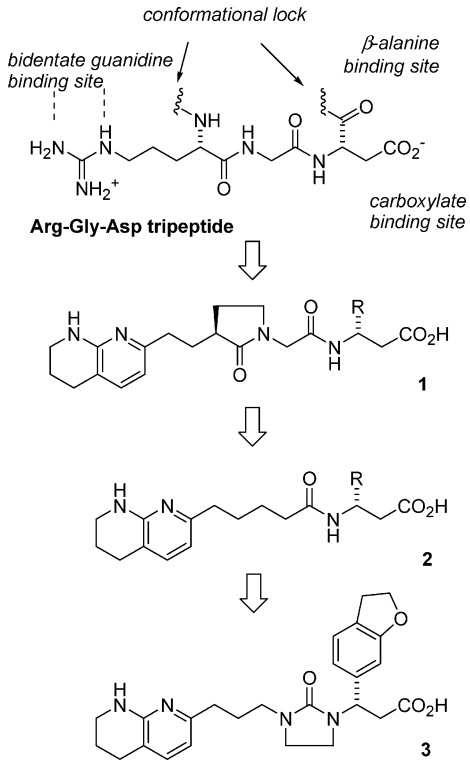

Figure 1. Evolution of imidazolidinone **3** from the RGD-tripeptide sequence.

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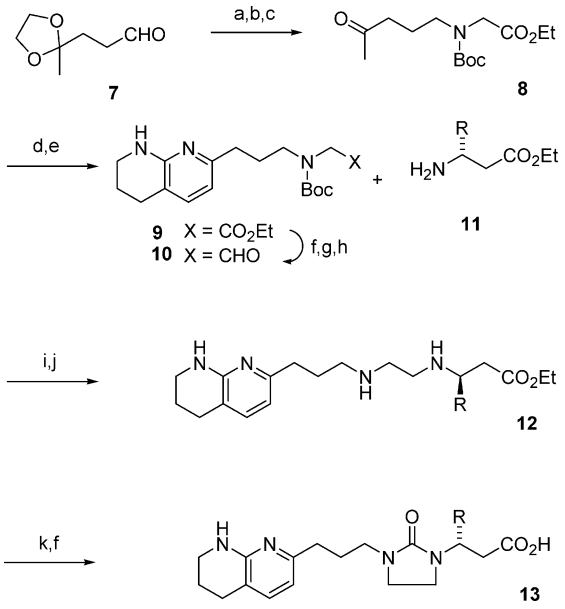
Scheme 1[a]



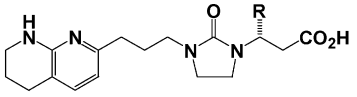
[a] Reagents: (a) $H_2NCH_2CO_2Et$, $NaB(OAc)_3H$, Et_3N, CH_2ClCH_2Cl; (b) Boc_2O, DMAP, THF; (c) acetone, TsOH; (d) 2-amino-3-formylpyridine, proline, EtOH; (e) Pt_2O, H_2, EtOH; (f) aq NaOH, EtOH; (g) EDC, HOBT, Et_3N, CH_3NHOMe, $CHCl_3$; (h) DIBAL, THF; (i) $NaB(OAc)_3H$, Et_3N, 4 Å sieves, CH_2Cl_2; (j) HCl(g), dioxane; (k) $COCl_2$, aq $NaHCO_3$, CH_2Cl_2.

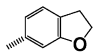
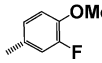
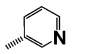
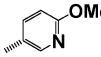
such as **2**, furnished a novel class of antagonists with improved pharmacokinetic properties.[18] Interestingly, they are typified as being "chain-shortened", i.e., they possess two fewer atoms in the chain between the carboxy and the N-terminal groups compared to the number of atoms between the guanidine and carboxylic acid of the RGD tripeptide sequence. Intensive SAR efforts then focused on the incorporation of cyclic constraints in an attempt to further improve potency. On the basis of this rationale, we recently discovered and reported on a novel series of potent $\alpha_v\beta_3$ antagonists that incorporate an imidazolidinone core.[19] These constrained, chain-shortened analogues have excellent in vitro potency and display good pharmacokinetics in rats and dogs. From this series, compound **3** was identified and shown to be efficacious in rat models of bone resorption. Despite this, compound **3** was considered to be unsuitable for clinical development in part due to extensive binding to human plasma proteins (>98%). This is significant, as our in vivo studies have shown a strong correlation between free drug concentration and in vivo efficacy. In this manuscript, we describe further SAR studies leading to the identification of a potent $\alpha_v\beta_3$ antagonist that has been selected for evaluation in the treatment of osteoporosis in human clinical trials.[20] In support of development activities, a strategy was employed to chemically oxidize the compound to generate novel derivatives to confirm the identity of metabolites and degradates.

Chemistry

Imidazolidinones of general structure **13** were prepared by the route outlined in Scheme 1. The aldehyde **7** was converted into the glycine derivative **8** via a three-step procedure (reductive amination, Boc-protection, and deketalization).[21] Methyl ketone **8** then underwent a Friedlander condensation with 2-amino-3-formylpyridine.[22] The resulting naphthyridine immediately underwent regioselective hydrogenation in the presence of Pt_2O and H_2 gas to afford **9**. Conversion of the ester **9** to the corresponding aldehyde **10** was achieved using standard chemistry in a three-step process. This aldehyde **10** was reductively aminated with the appropriate β-alanine ester **11** and the product deprotected to yield the diamine **12**. The reaction of **12** with phosgene and $NaHCO_3$ to form the imidazolidinone core was followed by saponification to afford the desired compound **13**. The β-alanine esters **11** were prepared using the method of Davies by the asymmetric addition of *R*- or *S*-α-methylbenzylbenzylamine to the styryl precursor followed by deprotection of the amine.[23]

Table 1. In Vitro and in Vivo Properties of Imidazolidinone-Based $\alpha_v\beta_3$ Antagonists



R	compound	SPAV3 IC_{50}(nM)[a]	h-protein binding	logP	Dog PK[b] F	T1/2 hr	CL ml/min/kg
	**3**	0.1	98%	0.8	75%	2.7	4.1
	**4**	0.35	95%	0.82	94%	2.6	5.1
	**5**	0.25	61%	-0.55	10%	0.9	70
	**6**	0.08	88%	0.21	64%	3.5	6.4

[a] Binding to the $\alpha_v\beta_3$ receptor using a scintillation-proximity assay (SPAV3), $n = 2$ for **4** and **5**, $n = 82$ for **3**, $n = 226$ for **6**.[b] Dog PK: compounds dose at 0.2 mpk iv and 1 mpk po in water.

Results and Discussion

Compounds were prepared focusing on modifications to the 3-substituents (Table 1) with the intent of preparing potent analogues with acceptable pharmacokinetics and plasma protein binding. The choice of 3-substituents utilized previously defined SAR relating the structural features that contribute to potency at $\alpha_v\beta_3$ to the effect on lipophilicity (as measured by the log *P* value).[24] On the basis of this, compound **4** (containing a potency-enhancing 4-methoxy substituent on the fluorophenyl ring) was synthesized and was found to have a similar in vitro and in vivo profile to **3**. Both compounds are potent, have nearly identical log *P* values, and, in the dog, both have comparable PK parameters (good bioavailability, low clearance, and a half-life of 2.5 h).[25] The free fraction of **4** was marginally improved over that for **3** (5% versus 2% respectively); however, this was not considered to be acceptable.[26]

To more dramatically decrease log *P* and evaluate the effect on protein binding, a pyridyl substituent was selected. In a related series of $\alpha_v\beta_3$ antagonists, the 3-pyridyl group is known to significantly reduce the log *P* and the degree of binding to human plasma proteins while maintaining in vitro potency.[24,27] When this modification was incorporated the resulting compound (**5**) had an IC_{50} of 0.25 nM in the $\alpha_v\beta_3$ binding assay

(SPAV3) and, as expected, was considerably more polar than **3** with a log *P* value of −0.55.[28] In conjunction with this, the human plasma protein binding value was 61%, a significant improvement over **3** and **4**. However, in the dog, the clearance was very high (70 mL/min/kg), the half-life was short (0.9 h), and the bioavailability was low (10%), thus yielding a less than optimal PK profile.

We hypothesized that the poor bioavailability of **5** was related, in part, to the basicity of the pyridine ring as nonbasic substituents in this (and related) series generally resulted in compounds with acceptable PK. Further, we speculated that compounds with polarity that are intermediate between that of **3** and **5** would prove to be optimal. So, starting with **5**, we sought to attenuate the basicity and lipophilicity of the pyridyl group by incorporation of a 2-methoxy group (the pK_a of 2-methoxypyridine is 3.2, 2 log units lower than that of pyridine which has a pK_a of 5.2).[29] This would also have potential beneficial effects on potency, as a 2-methoxy substituent was known to enhance potency in the fluorophenyl series (c.f. compound **4**). This compound was prepared (compound **6**) and was found to be the most potent compound of the series with an IC_{50} of 0.08 nM in the SPAV3 assay. Importantly, the log *P* was within the targeted range at 0.21 and the binding to human plasma proteins at 88% represents a significant improvement over that for **3** (98%). When **6** was dosed to dogs it was found to be bioavailable (64%) with low clearance (6.4 mL/min/kg) and an increased half-life (3.5 h) over that of **3**, **4**, and **5**. The PK parameters of **6** were also measured in rat and rhesus monkey. In rat, the pharmacokinetic profile of **6** was not as good as was seen for dog. The bioavailability was 26% and the clearance rate was high at 47 mL/min/kg. However, the $T_{1/2}$ was 3 h, and based on this profile, BID oral dosing of **6** would provide suitable coverage for proof-of-concept studies in rat. The PK in the rhesus monkey proved to be similar to dog, the bioavailability was 74%, the clearance was low (9 mL/min/kg), and the half-life was about 2 h.

Selectivity against two closely related integrins was also determined.[28,30] Against $\alpha_v\beta_5$, **6** had an IC_{50} of 10 nM in an $\alpha_v\beta_5$ binding assay, demonstrating **6** to display approximately 100-fold selectivity for $\alpha_v\beta_3$ over $\alpha_v\beta_5$. In a platelet aggregation assay that indirectly measures the affinity of compound **6** for the fibrinogen receptor $\alpha_{IIB}\beta_3$, the IC_{50} of **6** was found to be 34.7 μM.

In Vivo Studies. On the basis of the excellent in vitro and PK profile, compound **6** was assessed for in vivo efficacy as an inhibitor of bone resorption in three different animal models. In the ovariectomized (OVX) rat model, female rats experience accelerated bone loss that parallels estrogen-deficiency bone loss in postmenopausal women.[31] The compound was dosed orally at 10 and 30 mpk (mg/kg body weight) b.i.d. for 28 days in OVX rats and compared to vehicle control and sham-operated rats (Table 2). The bone mineral density (BMD) was measured by dual energy X-ray absorptiometry at both the distal femoral metaphysis (DFM) and the central femur. To compensate for differences in bone size among animals, the results are expressed as the ratio of the BMD of the distal femoral metaphysis compared to the BMD of the central femur (DFM:Central ratio). The DFM:cenral ratio for drug-treated animals was not significantly different from that of sham-operated rats. Vehicle treated OVX rats showed a statistically significant decrease in the DFM:central ratio from sham-operated rats (12.3%; $P < 0.01$) and drug-treated rats dosed at both doses (8.9% and 12.8% for the 10 and 30 mpk doses, respectively). Thus **6** proved to be fully efficacious in the OVX rat model at the doses tested.

Table 2. Effect of **6** on Ovariectomized Female Rats[a]

group	BMD ratio[b] (mg/cm^2 ± SEM)	% increase over vehicle
sham	0.955 ± 0.085	12.3% ($P < 0.01$)
OVX + vehicle	0.865 ± 0.056	-
OVX + **6** (10 mpk)	0.908 ± 0.054	8.9% ($P < 0.06$)
OVX + **6** (30 mpk)	0.951 ± 0.071	12.8% ($P < 0.01$)

[a] Female rats were ovariectomized and, within 24 h, were dosed orally with **6** at 10 and 30 mpk b.i.d. for 28 days and compared to sham-operated and OVX rats given vehicle ($n = 10$ for each group). [b] BMD: bone mineral density. Results are expressed as ratio of BMD of distal femoral metaphysis to BMD of cortex (trabecular/cortical BMD ratio).

Table 3. Effect of **6** on Growing Young Male Rats[a]

group[b]	DFM BMD[c] (mg/cm^2)	% increase over vehicle
vehicle	118.0 ± 6.5	-
Alendronate	149.7 ± 8.3	18.4% ($P < 0.0002$)
6	142.2 ± 6.5	20.5% ($P < 0.0002$)

[a] Young male rats were dosed with alendronate or **6** for 10 days and compared to vehicle treated rats. [b] Vehicle ($n = 13$), alendronate dosed at 0.005 mpk sc, qd ($n = 8$); **6** dosed by constant infusion using minipump to give a steady-state concentration of 800 nM ($n = 15$). [c] DFM BMD distal femoral metaphysis bone mineral density.

A second rat model was employed wherein compound **6** was administered to young, rapidly growing male rats by minipump infusion over 10 days. At the end of the experiment, the BMD at the distal femoral metaphysis was measured and compared to vehicle treated animals. Alendronate (0.005 mpk sc, qd) was used as the positive control. As can be seen in Table 3, there is a significant increase in BMD among the treated rats with both the Alendronate and the drug-treated groups being statistically different from the vehicle control group. Alendronate, given at a dose relevant to the human clinical dose, produced an 18.4% increase in BMD when compared to the vehicle-treated group. This increase was matched (20.5% increase in BMD) with **6** administered at a steady-state serum concentration of 800 nM. Thus, at this dose, compound **6** is as efficacious as Alendronate in inhibiting bone resorption in this model.

An OVX rhesus monkey model was utilized to monitor the effect of **6** on urinary markers of bone degradation.[32] The urine was collected and analyzed for n-telopeptides (NTx) and creatinine (Cre) with the results being expressed as uNTx/Cre. When **6** was administered orally once-daily for 2 weeks to OVX rhesus monkeys (Figure 2) that had been habituated to the dosing protocol, there was a 39% reduction in the level of NTx excreted in the urine when compared to placebo controls. When the drug was withdrawn, the NTx levels returned to baseline levels after 2 days.

Thus compound **6** is efficacious in three different animal models of bone turnover. On the basis of this, and the excellent in vitro profile, **6** was selected as a potential clinical candidate for the prevention and treatment of osteoporosis.

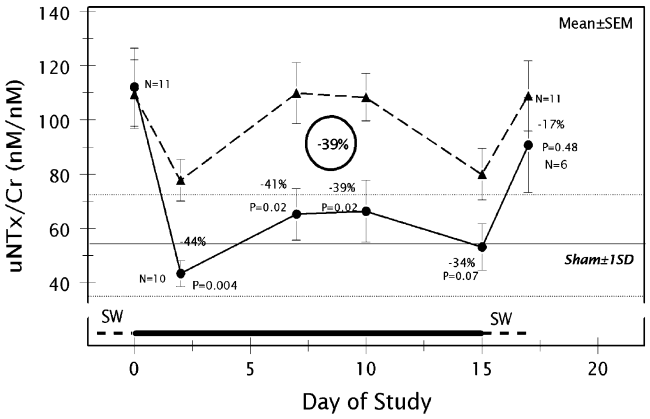


Figure 2. Urinary NTx levels (normalized for creatinine) from adult OVX Rhesus monkeys showing effect of compound **6** (●) dosed at 15 mpk/day, $N = 11$, compared to vehicle controls (▲), $N = 10$.

Further Studies on 6. In support of the planned development of this compound, it was decided to synthesize novel oxidized derivatives of **6**. We felt that such compounds would prove useful as standards for the identification of potential in vitro and in vivo metabolites. Since the in vitro metabolism studies using liver microsomes from several species showed minimal metabolism (<10%), the availability of synthetic standards could be critical to the elucidation of the identity of metabolites that are formed. Additionally, they would be useful for identifying impurities arising from the synthesis of bulk drug and degradation products arising from compound stability studies. The strategy chosen was a simple one, since compound **6** has numerous potential sites for oxidation, probe its reactivity to a variety of oxidants and isolate the products. The chemistry used to achieve these transformations is shown in Schemes 2 and 3.

Direct oxidation of the 2-methoxypyridyl derivative **6** with 1 equiv of $KMnO_4$ and 1 equiv of KOH in water yielded a mixture with two main components. Reverse phase HPLC provided each component in pure form. The first eluted peak was identified by extensive NMR analysis and mass spectroscopy to be the lactam **14**. Further elution then afforded a second compound that proved to be highly unstable. Proton and carbon NMR analysis along with mass spectroscopy data indicated that the structure is the aminal **15** as a 1:1 mixture of diastereomers. Confirmation of this was provided by further oxidation of the aminal using the same conditions, demonstrating that it was smoothly converted into **14**. The aminal **15** (IC_{50} of 0.4 nM in the SPAV3 assay) proved to be almost as potent to **6** while the lactam **14** showed a significant decrease in potency (IC_{50} of 141 nM). The aminal **15** decomposed on standing to provide a mixture of compounds of which the major component was identified as the naphthyridine analogue **16**. It was found that this compound was more conveniently prepared by oxidation of **6** using CrO_3 in pyridine at room-temperature overnight. This naphthyridine derivative was about 50-fold less potent than **6** with an IC_{50} of 3.7 nM in the SPAV3 assay. Potential in vivo oxidative demethylation of the pyridine *O*-methyl group would provide the corresponding pyridone. This compound was readily synthesized by brief treatment of **6** with pyridine hydrochloride at 125 °C followed by purification to provide the demethylated derivative **17** in good yield. The pyridone β-substituent resulted in a significant reduction in potency (SPAV3 $IC_{50} = 13$ nM).

Analogues containing oxidative modifications to the methylene chain linking the THN group to the imidazolidinone were synthesized as shown in Scheme 3. The acid **6** was derivatized as the methyl ester (**18**) by treatment with concentrated H_2SO_4 in methanol. Reaction with *m*-CPBA gave rise to the *N*-oxide **19** in which the aromatic nitrogen of the THN group was chemoselectively oxidized. This was then saponified to afford **20** (IC_{50} of 13 nM in the SPAV3 assay). The *N*-oxide of **19** was then used as a handle to direct the oxidation into the benzylic position of the propyl chain via a rearrangement reaction. Thus, upon treatment of **19** with acetic anhydride at 90 °C followed by saponification a 1:1 mixture of alcohols was formed. These were separated by HPLC using a Chiralpak AD column to give the individual diastereomers **21a** and **21b**. Interestingly, there is approximately a 20-fold difference in potency between the two diastereomers with **21a** and **21b** having IC_{50}s of 0.2 nM and 3.4 nM, respectively. The stereochemistry at the alcohol center was not determined. Also isolated, as a minor byproduct from this two-step procedure, was the unsaturated analogue **22** which, at 0.05 nM IC_{50}, is the most potent compound prepared in this series. Finally, reaction of the mixture of alcohols **21a** and **21b** with MnO_2 proceeded smoothly to give the ketone compound **23** ($IC_{50} = 3$ nM). The structures of compounds **19** through **23** were all confirmed by NMR and mass spectroscopy.

The utility of the available oxidized derivatives of **6** was demonstrated when small amounts of metabolites of **6** were isolated.[33] The available spectral data indicated that oxidation had occurred at the methylenes of the naphthyridine ring. Potentially, oxidation could have occurred at C-5 or C-7 (THN ring numbering) and, given the similarity of the chemical shifts involved coupled with the miniscule amounts isolated, definitive identification was not possible. Comparison to the authentic derivatives proved that the metabolites were identical to compounds **14** and **15**. Compounds **16**, **17**, and **21** were also identified as biliary metabolites of compound **6**. Interestingly, all of the compounds identified as products of in vitro or in vivo metabolism of **6** are potent antagonists of the $\alpha_v\beta_3$ receptor.

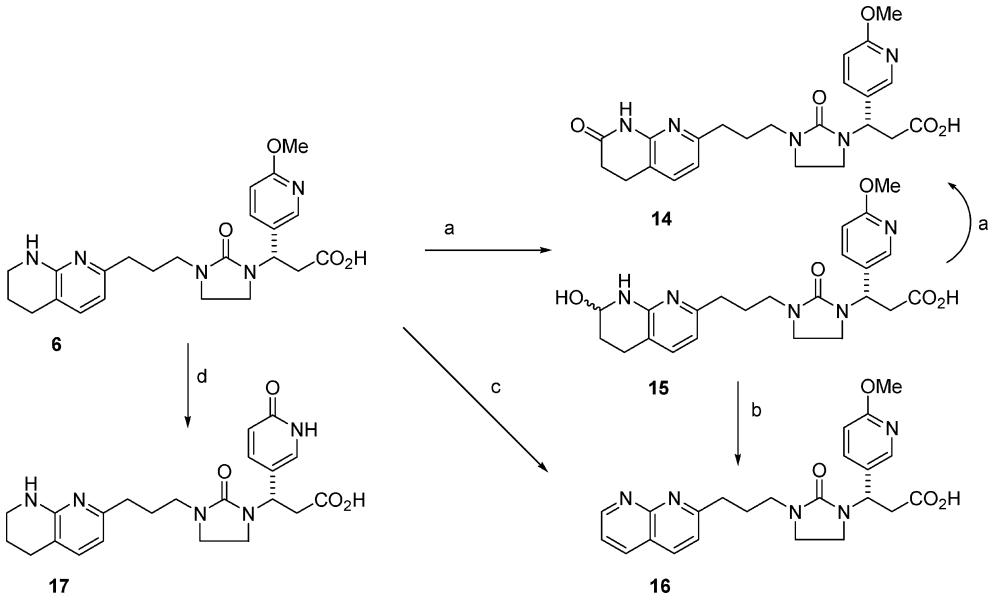
Conclusion

We have identified **6** as a potent, selective antagonist of the $\alpha_v\beta_3$ receptor. This compound has an excellent in vitro profile, a significant unbound fraction in human plasma and good pharmacokinetics in rat, dog, and rhesus monkey. On the basis of this, and the efficacy shown in in vivo models of bone turnover, the compound was selected for further development. To support the ongoing metabolism and safety studies, a novel, proactive strategy was employed in which a series of oxidized derivatives of **6** were prepared upon exposure to chemical oxidizing agents. These products proved useful in the identification of active metabolites generated by either in vitro or in vivo metabolism.

Experimental Section

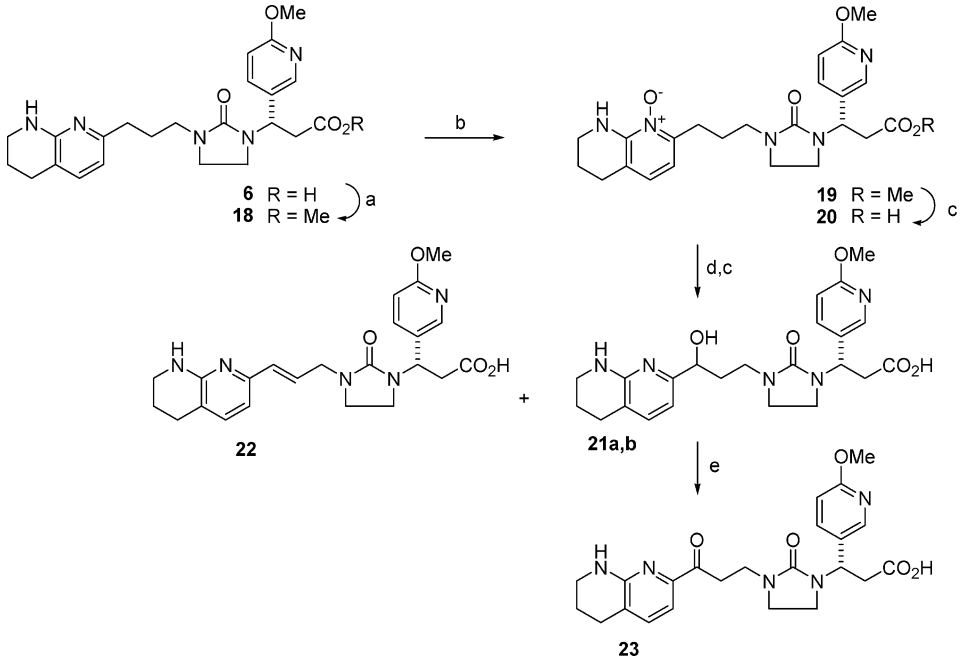
General. All commercially available chemicals and solvents were used without further purification. All new compounds gave satisfactory ^{1}H NMR and mass spectrometry analysis.

Scheme 2[a]



[a] Reagents: (a) $KMnO_4$, KOH, H_2O; (b) dehydration on standing; (c) CrO_3−pyridine; (d) pyr·HCl, Δ.

Scheme 3[a]



[a] Reagents: (a) concd H_2SO_4, MeOH; (b) m-CPBA; (c) aq LiOH; (d) TFAA, Δ; (e) MnO_2.

IH NMR spectra were recorded on a Varian Unity 300 or 500 MHz spectrometer, and chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard. High-resolution mass spectral analysis was performed on a Bruker-daltonics BioApex 3T mass spectrometer.

Typical Procedure for the Preparation of 3-Aryl-*β*-alanine Ethyl Esters 11. 3(*S*)-(6-Methoxypyridin-3-yl)-*β*-alanine Ethyl Ester. Step 1: 5-Bromo-2-methoxypyridine. To a solution of KOH (4.2 g, 0.075 mol) in water (750 mL) was added 2-methoxypyridine (16.4 g, 0.15 mol) followed by a dropwise addition of bromine (24 g, 0.15 mol) in 1 N aqueous KBr (750 mL), and the resulting solution was stirred at room temperature for 5 h. Solid $NaHCO_3$ was added until basic, and the solution was extracted with $CHCl_3$ (3 × 500 mL). The organic layer was washed with 10% $NaHSO_3$, then brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting dark oil (20.2 g) was predominantly the desired compound (containing ∼5% of the 3-bromo-2-methoxypyridine isomer) and was used as such in the next step. ^{1}H NMR ($CDCl_3$): δ 3.91 (3H, s), 6.66 (1H, dd), 7.62 (1H, dd), 8.20 (1H, d).

Step 2: Ethyl (6-Methoxypyridin-3-yl)acrylate. A solution of 5-bromo-2-methoxypyridine (74.3 g, 0.4 mol), ethyl acrylate (150 mL, 1.4 mol), triethylamine (150 mL, 1.08 mol), palladium acetate (10 g, 0.045 mol), and tri-*o*-tolylphosphine (20 g, 0.066 mol) in 100 mL acetonitrile was degassed with argon for 10 min. The mixture was heated at 90 °C for 12 h then the volatiles removed in vacuo. Toluene (300 mL) was added and the mixture concentrated again. Diethyl ether (300 mL) was added and the mixture filtered through a pad of silica gel eluting with 800 mL of diethyl ether. After removal of the solvent, the residue was chromatographed (silica gel; EtOAc/hexane 1:19 then 1:9), which gave the desired compound as a yellow solid (63.5 g, 77%). ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, t), 3.97 (3H, s), 4.26 (2H, q), 6.34 (1H, d), 6.76 (1H, d), 7.63 (1H, d), 7.77 (1H, dd), 8.27 (1H, q).

Step 3: *N*-Benzyl-(*R*)-α-methylbenzyl-3(*S*)-(6-methoxypyridin-3-yl)-*β*-alanine Ethyl Ester. To a solution of *N*-benzyl-(R)-α-methylbenzylamine (97.5 g, 462 mmol), in THF (750 mL) at 0 °C was added *n*-butyllithium (2.5M hexanes; 178.5 mL, 446 mmol). The dark violet solution was stirred at 0 °C for 20 min then cooled to −78 °C and the ethyl (6-methoxypyridin-3-yl)acrylate (63.7 g, 308 mmol) in THF (250 mL) was added over 60 min. The resulting solution was stirred at −78 °C for 1 h then cannulated into saturated NH_4Cl and extracted with EtOAc, washed with water, then brine, dried (Na_2SO_4) and concentrated to give an oil. Column chromatography (silica gel; EtOAc/ Hexane 1:9 then 1:4) gave the desired compound contaminated with *N*-benzyl-α-methylbenzylamine. This oil was taken up in 5% AcOH in water and extracted with diethyl ether (4×). The organic layer was dried ($MgSO_4$) and the solvent removed to give the desired compound (102.9 g, 80%). ^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t), 1.27 (3H, d), 2.52 (1H, dd), 2.62 (1H, dd), 3.66 (1H, d), 3.70 (1H, d), 3.93 (3H, s), 3.95 (2H, m), 4.41 (1H, dd), 6.74 (1H, d), 7.15−7.45 (10H, m), 7.64 (1H, dd), 8.15 (1H, d).

Step 4: 3(*S*)-(6-Methoxypyridin-3-yl)-*β*-alanine Ethyl Ester. To a degassed (argon) solution of the ester from step 3 (70 g), in EtOH (250 mL), HOAc (25 mL), and water (2 mL) was added 20% $Pd(OH)_2$ on carbon. The mixture was placed under hydrogen using a balloon and then stirred for 24 h. After degassing with argon and filtering through Celite, the solvent was removed in vacuo to afford a waxy solid. This was dissolved in 200 mL water and extracted with diethyl ether (2 × 200 mL). The aqueous layer was then treated with solid K_2CO_3 until fully saturated and extracted with EtOAc (4 × 200 mL). After being dried over $MgSO_4$, the solvent was removed under reduced pressure to give the title compound as an oil which solidified on standing (31.1 g, 83%). ^{1}H NMR ($CDCl_3$): δ 1.23 (3H, t), 2.61 (1H, dd), 2,68 (1H, dd), 3.92 (3H, s), 4.15 (2H, q) 4.41 (1H, dd), 6.93 (1H, d), 7.62 (1H, dd), 8.13 (1H, d).

The following *β*-alanines were also prepared using the above procedures:[24] 3(*S*)-(2,3-dihydrobenzofuran-6-yl)-*β*-alanine ethyl ester; 3(*S*)-(3-fluoro-4-methoxyphenyl)-*β*-alanine ethyl ester; 3(*S*)-(pyrid-3-yl)-*β*-alanine ethyl ester.

[*tert*-Butoxycarbonyl-(4-oxopentyl)amino]acetic Acid Ethyl Ester 8. To a solution of the aldehyde **7** (31.7 g, 0.22 mol) in 1 L of 1,2-dichloroethane at 0 °C were added glycine ethyl ester hydrochloride (61.5 g, 0.44 mol), triethylamine (107 mL, 0.77 mol), and $NaB(OAc)_3H$ (65.3 g, 0.308 mol). The mixture was allowed to warm to room temperature and stirred for 1.5 h. After concentration to one-third the initial volume, EtOAc was added and then the solution washed with 10% K_2CO_3 and brine and dried over Na_2SO_4. Following evaporation of the solvent, the residue was purified by column chromatography (silica gel; 5% MeOH in EtOAc) to give a yellow oil (24.2 g, 48%). ^{1}H NMR ($CDCl_3$): δ 1.29 (6H, m), 1.53−1.67 (4H, m), 2.62 (2H, t), 3.93 (4H, m), 4.03 (1H, d), 4.21 (3H, m) 6.01 (1H, br s).

To a solution of the amine (24 g, 0.104 mol) in THF (100 mL) were added DMAP (trace), triethylamine (20 drops), and Boc_2O (23.8 g, 0.109 mol). After 4 h, removal of the solvent in vacuo gave a colorless oil (35.1 g, 96%).

This oil (35 g, 0.1 mol) in acetone (600 mL) was treated with *p*-toluenesulfonic acid (1 g) and the mixture heated at reflux for 2 h. After cooling, the mixture was concentrated to one-fifth the initial volume and then EtOAc added. The solution was washed with sat. $NaHCO_3$ and brine and dried (Na_2SO_4) and the solvent removed to give **8** as a yellow oil (28.6 g, 92%). ^{1}H NMR (mixture rotamers; $CDCl_3$): δ 1.28 (3H, m), 1.42−1.51 (9H, s), 1.78 (2H, m), 2.14 (3H, s), 2.52 (2H, m), 3.3 (2H, m), 3.83 (1.15H, s), 3.92 (0.85H, s), 4.20 (2H, m).

{*tert*-Butoxycarbony-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)propyl]amino}acetic Acid Ethyl Ester 9. A solution of **8** (28 g, 97.4 mmol), 2-amino-3-formylpyridine (15.5 g, 127 mmol), and proline (11.2 g, 97.4 mmol) in ethanol (250 mL) was heated at reflux for 15 h. After cooling and evaporation of the volatiles, the residue was chromatographed (silica gel; 1:1 chloroform/ethyl acetate) to give the naphthyridine as a yellow oil (24.3 g, 67%).

This oil (24.3 g, 65.1 mmol), platinum oxide (4 g), and ethanol (130 mL) was stirred under a balloon of hydrogen gas for 6 h. Following filtration and evaporation, the residue was chromatographed (silica gel; ethyl acetate) to give **9** as a yellow oil (19.3 g, 78%). ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, m), 1.43 (9H, m), 2.72 (4H, m), 2.51 (2H, m), 2.63 (2H, m), 3.32 (4H, m), 3.9 (2H, s), 4.18 (2H, q), 4.74 (1H, br s), 6.37 (1H, m), 7.05 (1H, d).

{*tert*-Butoxycarbony-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)propyl]amino}acetaldehyde 10. To a solution of **9** (36 g, 95 mmol) in ethanol (300 mL) was added 1 N NaOH (109 mL, 109 mmol), and the mixture stirred for 1 h at 50 °C. HCl (12 N) (10.3 mL, 120 mmol) was added in 50 mL of EtOH and the mixture concentrated in vacuo to give an oily residue. The residue was evaporated from ethanol three times and then from acetonitrile three times to produce a yellow solid that was dried under vacuum (<2 mmHg) for 2 h. This solid was then slurried in acetonitrile (180 mL), chloroform (180 mL), and NMM (68 mL, 620 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (18.6 g, 197 mmol), HOBT (16.7 g, 123 mmol), and EDC (23.7 g, 123 mmol) were added. After being stirred for 15 h, the mixture was evaporated to dryness and the residue slurried in EtOAc, washed with sat. $NaHCO_3$ and brine, and dried over Na_2SO_4. Removal of the solvent in vacuo gave a yellow oil (43.1 g, quantitative). ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, m), 1.88 (4H, m), 2.53 (2H, m), 2.64 (2H, m), 3.18 (3H, s), 3.37 (4H, m), 3.69 (3H, m), 4.81 (1H, br s), 6.38 (1H, m), 7.05 (1H, m).

To a stirred solution of this oil (40 g, 95 mmol) in THF (300 mL) at −78 °C was added DIBAL (1.0M in hexanes, 143 mL, 143 mmol) dropwise over 20 min. After 1 h, the mixture was warmed to room temperature and quenched with 30 mL of MeOH, and 300 mL of 1.0M Rochelle's salt was added. The mixture was stirred for 1 h and then diluted with ether. After 30 min of stirring, the organic layer was separated and dried over $MgSO_4$. Removal of the solvent gave the aldehyde **10** as an amorphous solid that was used as such (29.6 g, 97%). TLC Rf = 0.34 (silica; 70:25:5 chloroform/EtOAc/MeOH).

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid 6. Step 1. 3(*S*)-(2-[*tert*-Butoxycarbonyl-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]-amino}ethylamino)-3-(2-methoxypyridin-5-yl)propionic Acid Ethyl Ester. To a solution of the 3(*S*)-(6-methoxypyridin-3-yl)-*β*-alanine ethyl ester (6.85 g, 30.5 mmol) in 2-propanol (300 mL) at room temperature were added acetic acid (1.75 mL, 30.5 mL), NaOAc (24.6 g, 0.3 mol), and 4 Å molecular seives (5 g). The aldehyde **10** (8.1 g, 24.3 mmol) in 2-propanol (150 mL) was added, the mixture stirred for 15 min and then cooled to 0 °C, and $NaCNBH_3$ (5.66 g, 90 mmol) added in one lot. The resulting mixture was allowed to warm to room temperature and stirred for 16 h before being filtered through Celite. After removal of the solvent in vacuo, the residue was treated with 10% aqueous $KHSO_4$ for 30 min, basified with solid K_2CO_3 (to pH ∼10), and extracted with 3 × 200 mL EtOAc. The EtOAc layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give an oil (14.5 g). Column chromatography (silica gel; 5% MeOH in $CHCl_3$) gave the title compound as an oil (4.06 g) contaminated with ∼7% of the *β*-alanine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (3H, t), 1.42 (9H, s), 1.7−2 (4H, br m), 2.5−2.8 (8H, m), 3.42 (2H, m), 3.92 (3H, s), 4.06 (2H, q), 5.0−5.4 (1H, bs), 6.36 (1H, br s), 6.72 (1H, d), 7.12 (1H, br s), 7.58 (1H, dd), 8.07 (1H, d).

Step 2. 3(*S*)-(6-Methoxypyridin-2-yl)-3-{2-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]amino}ethylamino)propionic Acid Ethyl Ester. A solution of the ester from step 1 (14.1 g, 26 mmol) in EtOAc (350 mL) cooled to −20 °C was treated with HCl (gas) for 10 min then stoppered and stirred at 0 °C for 1.5 h. The volatiles were removed in vacuo, and the residue was taken up in 150 mL water and treated with solid K_2CO_3 to pH ∼10. This solution was extracted with EtOAc (3 × 150 mL), washed with brine, dried

(Na_2SO_4), and concentrated to give an oil. Column chromatography (silica gel; 5%MeOH in $CHCl_3$ followed by 5% MeOH in $CHCl_3$ saturated with NH_3) gave the title compound as a viscous oil (8.03 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (3H, t), 1.8−1.95 (4H, m), 2.5−2.8 (12H, m), 3.39 (2H, m), 3.92 (3H, s), 4.09 (2H, q), 5.01 (1H, bs), 6.34 (1H, d), 6.72 (1H, d), 7.06 (1H, d), 7.59 (1H, dd), 8.07 (1H, d).

Step 3. 3(*S*)-(6-Methoxypyridin-2-yl)-3-{2-oxo-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid Ethyl Ester. To a solution of the diamine from step 2 (8.03 g, 18.2 mmol), DIPEA (9.5 mL, 54.6 mmol), and DMAP (250 mg) in 1,2-dichloroethane (150 mL) cooled to −20 °C was added *p*-nitrophenyl chloroformate (3.85 g, 19.1 mmol) in 1,2-dichloroethane (25 mL) dropwise such that the internal temperature remained below −15 °C. The resulting mixture was allowed to warm to 0 °C, stirred for 45 min, and then heated to reflux for 4 h. After cooling, the solvent was evaporated in vacuo and the residue taken up in EtOAc and washed successively with 10% K_2CO_3 (6 × 150 mL) and brine. The EtOAc layer was dried (Na_2SO_4) and concentrated in vacuo to give an oil. Column chromatography (silica gel; 5% EtOH in CH_2Cl_2) gave the title compound as an oil (5.28 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (3H, t), 1.8−1.95 (4H, m), 2.52 (2H, dd), 2.68 (1H, dd), 2.9−3.1 (3H, m), 3.15−3.3 (5H, m), 3.39 (2H, m), 3.92 (3H, s), 4.11 (2H, q), 4.8 (1H, bs), 5.42 (1H, t), 6.34 (1H, d), 6.72 (1H, d), 7.03 (1H, d), 7.60 (1H, dd), 8.08 (1H, d).

Step 4. Compound 6. To a solution of the ester from step 3 (3.48 g, 7.4 mmol) in MeOH (50 mL) and water (30 mL) at room temperature was added 1 N NaOH (22.3 mL, 22.3 mmol) and the mixture stirred for 16 h. After removal of the solvent in vacuo, the residue was treated with 1 N HCl (25 mL) and the solvent removed again. Column chromatography of the residue (silica gel; EtOAc/EtOH/aq NH_4OH/ H_2O 20:10:1:1) gave a gum which was crystallized from water and filtered to give **6** as a white solid (3.04 g, 93%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.75−2.1 (4H, m), 2.55−3.1 (8H, m), 3.28 (1H, q), 3.3 (1H, m), 3.4−3.55 (3H, m), 3.63 (1H, q), 3.85 (3H, s), 5.47 (1H, dd), 6.55 (1H, d), 6.80 (1H, d), 7.48 (1H, d), 7.68 (1H, d), 8.09 (1H, d). Exact mass (FAB, M + H) found: 440.2297; $C_{23}H_{29}N_5O_4$ + H requires 440.2293. Anal. for $C_{23}H_{29}N_5O_4$: Calcd C 62.85; H 6.65; N 15.94. Found: C 62.51; H 6.76; N 16.04.

Compounds **3**−**5** were prepared using similar procedures used for the preparation of **6**:

3(*S*)-(Dihydrobenzofuran-6-yl)-3-{2-oxo-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid 3. ^{1}H NMR (300 MHz, CD_3OD) δ 1.93 (4H, m), 2.67 (2H, t), 2.80 (2H, t), 2.97 (3H, m), 3.14−3.53 (9H, m), 4.53 (1H, t), 5.38 (1H, t), 6.62 (1H, d), 6.72 (1H s), 6.81 (1H, d), 7.18 (1H, d), 7.55 (1H, d). High res MS (FAB, M + H) found: 451.2354; $C_{25}H_{30}N_4O_4$ + H requires 451.2340.

3(*S*)-(4-Methoxy-3-fluorophenyl)-3-{2-oxo-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-propyl]imidazolidin-1-yl}propionic Acid 4. ^{1}H NMR (300 MHz, CD_3OD) δ 1.86 (2H, m), 1.95 (2H, m), 2.66 (2H, t), 2.82 (2H, t), 3.01 (2H, m), 3.05 (1H, q), 3.2−3.2 (7H, m), 3.86 (3H, s), 5.39 (1H, t), 6.63 (1H, d), 7.0−7.2 (3H, m), 7.57 (1H, d). High res MS (FAB, M + H) found: 457.2246; $C_{24}H_{29}N_4O_4F$ + H requires 457.2246. Anal. for $C_{24}H_{29}N_4O_4F$·1.55TFA·0.3H_2O: Calcd C 50.96; H 4.92; N 8.77. Found: C 50.96; H 4.92; N 8.76.

3(*S*)-(Pyridin-3-yl)-3-{2-oxo-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-propyl]imidazolidin-1-yl}propionic Acid 5. ^{1}H NMR (300 MHz, CD_3OD) δ 1.93 (3H, m), 2.05 (1H, m), 2.60 (2H, m), 2.77 (4H, m), 3.01 (2H, m), 3.18 (2H, m), 3.46 (3H, m), 3.63 (2H, m), 5.57 (1H, m), 6.55 (1H, d), 7.46 (2H, m), 7.85 (1H, d), 8.47 (1H, m), 8.54 (1H, m). High res MS (FAB, M + H) found: 410.2192; $C_{22}H_{27}N_5O_3$ + H requires 410.2211.

3(*S*)-(2(1*H*)-Pyridone-5-yl)-3-{2-oxo-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}-propionic Acid 17. The acid **6** (100 mg, 0.23 mmol) and pyridine hydrochloride (300 mg) were placed in a sealed vial and heated at 125 °C for 2.5 min. The molten mixture was cooled and purified by reverse phase HPLC (C18 column; water/acetonitrile + 0.1% TFA; gradient) to give (after lyophilization) **17** as a TFA salt. ^{1}H NMR (300 MHz, CD_3OD) δ 1.86 (2H, m), 1.95 (2H, m), 2.66 (2H, t), 2.81 (2H, t), 2.96 (1H, m), 3.15 (1H, m), 3.23 (2H, t), 3.35−3.55 (5H, m), 5.23 (1H, t), 6.55 (1H, d), 6.64 (1H, d), 7.44 (1H, d), 7.58 (1H, d), 7.65 (1H, dd). Exact mass (FAB, M + H) found: 426.2141; $C_{24}H_{29}N_4O_4F$ + H requires 426.2136. Anal. for $C_{22}H_{27}N_5O_4F$·2.2TFA·0.65H_2O: Calcd C 46.06; H 4.45; N 10.24. Found: C 46.08; H 4.47; N 10.18.

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-[3-(7-hydroxy-5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid 15. To a solution of **6** (0.88 g, 2 mmol) in water (20 mL) at room temperature were added 1 N NaOH (2.0 mL, 2 mmol) and then $KMnO_4$ (0.316, 2 mmol), and the mixture was stirred for 16 h. After filtration, the solution was purified by HPLC chromatography (DeltaPak C-18 column; 0.1% NH_4HCO_3 (aq) and acetonitrile; gradient elution). Collection of the second eluting peak followed by lyophilization afforded **15** as a white solid (1:1 mixture of 7-hydroxy epimers). ^{1}H NMR (600 MHz, CD_3OD) δ 1.70 (1H, m), 1.86 (1H, m), 2.07 (1H, m), 2.12 (1H, m), 2.61 (1H, m), 2.71 (2H, m), 2.75−3.0 (6H, m), 3.17 (1H, q), 3.48 (1H, m), 3.62 (1H, m), 3.90 (3H, s), 4.73 (1H, m), 5.46 (1H, br d), 6.69 (1H, d), 6.80 (1H, dd), 7.54 (1H, m), 7.67 (1H, dd), 8.09 (1H, d). Mass spectrum: found 456.1 $(M + H)^+$.

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-[3-(7-oxo-5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid 14. Following the procedure described above but collecting the first eluted peak following HPLC chromatography and lyophilization gave **14** as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 1.90 (2H, m), 2.57 (2H, m), 2.65 (2H, m), 2.87 (2H, m), 2.93 (3H, m), 3.13 (1H, m), 3.26 (3H, m), 3.40 (1H, q), 3.89 (3H, s), 5.37 (1H, t), 6.78 (1H, d), 6.82 (1H, d), 7.39 (1H, d), 7.67 (1H, dd), 8.11 (1H, d). Exact mass (FAB, M + H) found: 454.2066; $C_{23}H_{27}N_5O_5$ + H requires 454.2085.

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-([1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid 16. To a solution of **6** (0.44 g, 1 mmol) in pyridine (5 mL) at room temperature was added CrO_3 (0.1 g, 1 mmol), and the mixture was stirred for 16 h. The mixture was diluted with water (100 mL) and filtered through Celite and the solvent removed in vacuo. The residue was purified by HPLC chromatography (DeltaPak C-18 column; 0.1% NH_4HCO_3 (aq) and MeOH; gradient elution) to give (after lyophilization) **16** as a pale yellow solid. ^{1}H NMR (300 MHz, CD_3OD) δ 2.12 (2H, m), 2.65−2.85 (4H, m), 3.03 (2H, t), 3.1−3.4 (4H, m), 3.75 (3H, s), 5.38 (1H, t), 6.75 (1H, d), 7.53 (1H, d), 7.74 (1H,dd), 7.64 (1H, dd), 8.09 (1H, d), 8.22 (1H, d), 8.36 (1H, dd), 8.98 (1H, dd). Mass spectrum: found 436.0 $(M + H)^+$. Exact mass (FAB, M + H) found: 436.1978; $C_{23}H_{27}N_5O_5$ + H requires 436.1980.

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-[3-(1N-oxide-5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)propyl]imidazolidin-1-yl}propionic Acid Ethyl Ester 19. To a solution of **18** (1.51 g, 3.2 mmol) in CH_2Cl_2 (20 mL) was added m-CPBA (70%; 0.96 g, 3.9 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (5×) and brine and dried over Na_2SO_4. The solvent was removed and the residue purified by silica gel chromatography ($CHCl_3$/MeOH 97:3) to afford the *N*-oxide **19** as a viscous oil. Mass spectrum: found 484.1 $(M + H)^+$.

Step 2. Compound 20. To a solution of the ester from step 1 (0.26 g, 0.5 mmol) in MeOH (7.5 mL) was added 1 N NaOH (1.5 mL, 1.5 mmol), and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo, the residue dissolved in water (5 mL), and 1 N HCl added (1.5 mL). After extraction with CH_2Cl_2 (4 × 20 mL), the organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give **20** as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.7−2.1 (4H, m), 2.7−3.1 (8H, m), 3.21 (1H, m), 3.5 (4H, m), 3.81 (1H, ddd), 3.91 (3H, s), 5.54 (1H, dd), 5.4−5.7

(1H, br), 6.40 (1H, d), 6.71 (1H, d), 6.87 (1H, s), 6.97 (1H, d), 7.53 (1H, dd), 8.06 (1H, d). Mass spectrum: found 456.1 $(M + H)^+$.

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3 (R or S)-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-3-hydroxypropyl]imidazolidin-1-yl}propionic Acid 21a,b and 3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-2-propenyl]imidazolidin-1-yl}propionic Acid 22. Step 1 3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-[3-(8-acetyl-5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-3-acetoxypropyl]imidazolidin-1-yl}propionic Acid Ethyl Ester. A mixture of the *N*-oxide ester (**19**; 0.24 g, 0.5 mmol) in acetic anhydride (2 mL) was heated to 90 °C for 7.5 h then poured onto ice and neutralized with $NaHCO_3$. The mixture was extracted with EtOAc (3×), washed with brine, and dried (Na_2SO_4) and the solvent removed. Purification of the residue by column chromatography (silica gel; 3% MeOH in $CHCl_3$) afforded the title compound as a viscous oil. Mass spectrum: found 568.2 $(M + H)^+$.

Step 2. Compounds 22, 21a, and 21b. To a solution of the diester from step 1 (0.75 g, 1.32 mmol) in EtOH (10 mL) was added 1 N NaOH (6 mL, 6 mmol), and the solution was heated to reflux for 4 h. The mixture was concentrated in vacuo and the purified by reverse phase HPLC (C18 column; water/acetonitrile + 0.1% TFA; gradient) to give the alkene **22** as the first eluted compound. ^{1}H NMR (500 MHz, CD_3OD) δ 1.95 (2H, m), 2.83 (2H, t), 3.01 (2H, dd), 3.07 (1H, dd), 3.15 (1H, q), 3.36 (2H, m), 3.50 (2H, m), 3.90 (3H, s), 3.96 (1H, dd), 4.03 (1H, dd), 5.39 (1H, t), 6.43 (1H, d), 6.54 (1H, dt), 6.82 (2H, dd), 7.61 (1H, d), 7.73 (1H,dd), 8.13 (1H, d). Exact mass (FAB, M + H) found: 438.2128; $C_{23}H_{27}N_5O_4$ + H requires 438.2136.

Continued elution gave (after lyophilization) the alcohols **21a**,**b** as a mixture of diastereomers. Mass spectrum: found 456.1 $(M + H)^+$. The mixture was further separated by HPLC chromatography using Chiralpak AD column eluting with hexane + 0.2% TFA/2-propanol/ethanol 70/25/5 to give (after lyophilization) the faster eluting diastereomer **21a** as a TFA salt. ^{1}H NMR (500 MHz, CD_3OD) δ 1.88 (1H, septet), 1.95 (3H, m), 2.82 (2H, t), 3.01 (2H, m), 3.08 (1H, q), 3.3−3.4 (4H, m), 3.45 (1H, m), 3.51 (2H, t), 3.90 (3H, s), 4.41 (1H, dd), 5.37 (1H, t), 6.72 (1H, d), 6.82 (1H, d), 7.61 (1H, d), 7.72 (1H,dd), 8.12 (1H, d).

Continued elution afforded (after lyophilization) the slower eluting diastereomer **21b** as a TFA salt. ^{1}H NMR (500 MHz, CD_3OD) δ 1.89 (1H, septet), 1.95 (3H, m), 2.82 (2H, t), 3.01 (2H, m), 3.07 (1H, q), 3.24 (1H, m), 3.3−3.4 (3H, m), 3.45 (1H, m), 3.51 (2H, t), 3.90 (3H, s), 4.71 (1H, dd), 5.37 (1H, t), 6.71 (1H, d), 6.82 (1H, d), 7.59 (1H, d), 7.72 (1H,dd), 8.12 (1H, d).

3(*S*)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)-3-oxopropyl]imidazolidin-1-yl}propionic Acid 23. A mixture of the diasteromeric alcohols **21a** and **21b** (0.35 g, 0.77 mmol) in acetone (5 mL) was treated with MnO_2 (2.0 g) and allowed to stir at room temperature for 16 h. A further 1.0 g of MnO_2 was added and the mixture stirred for 2 h. After filtration through Celite, the solvent was removed and the residue purified by reverse phase HPLC (C18 column; water/acetonitrile + 0.1% TFA; gradient) to give (after lyophilization) the ketone **23** as a TFA salt. ^{1}H NMR (500 MHz, CD_3OD) δ 1.99 (2H, m), 2.9−3.0 (4H, m), 3.06 (1H, m), 3.21 (2H, t), 3.4 (3H, m), 3.55 (4H, m), 3.90 (3H, s), 5.32 (1H, t), 6.81 (1H, d), 7.55 (1H, d), 7.69 (1H, dd), 7.78 (1H, d), 8.09 (1H, d). Exact mass (FAB, M + H) found: 454.2102; $C_{23}H_{27}N_5O5_4$ + H requires 454.2085.

Ovariectomized Rat Model. Female Sprague−Dawley rats aged 7 months (body weight 325 g) were used. They were ovariectomized (OVX) by a dorsal approach, then started on treatment the next day with 0, 10, or 30 mg/kg/d po, b.i.d. Treatment continued for 28 days. At necropsy, right femurs were collected and placed in 70% ethanol. They were defleshed and analyzed (bone mineral density, BMD) by dual energy X-ray absorptiometry (DXA). BMD of the central femur (CF), the distal 25% of the femur, and the distal femoral metaphysis (DFM, 12−25% of the distance from distal to proximal end) was conducted. To compensate for differences in bone size among animals, the results are expressed as the ratio of DFMBMD to CFBMD (DFM:Central).

Significant bone loss occurred after ovariectomy ($P < 0.01$). Estrogen deficiency bone loss was fully prevented by 30 mg/kg/d ($P < 0.01$). It was also partially prevented by 10 mg/kg/d ($0.10 < P < 0.06$).

Growing Rat Model. Growing male Sprague−Dawley rats weighing 160 g were randomized to treatment groups and treated for 10 days with compound. Alendronate was administered at 0.005 mpk using subcutaneous dosing ($N = 13$). Compound **6** was dissolved in water using a minimum amount (<1 equiv) of 1 N HCl to dissolve to give a final concentration of 60 mg/mL. This solution was administered subcutaneously using implanted osmotic minipumps ($N = 15$). The pharmacokinetic profile of **6** under this dosing regimen was measured in a parallel set of three rats. At necropsy, the right femur was removed, defleshed, and stored in 70% ethanol. BMD analysis of the DFM was performed using DXA as described above for the OVX rat assay.

Significant increases in BMD compared to vehicle treated rats were observed for both drug treated groups ($P < 0.0002$). Alendronate resulted in an 18.4% increase and **6** resulted in a 20.5% increase in BMD of the DFM, respectively.

Ovariectomized Rhesus Monkey Model. Twenty-one female rhesus monkeys aged 12.5 years were ovariectomized by a dorsal approach and allowed to acclimate for eighteen months. Groups were given 0 ($N = 11$) or 15 mg/kg/d ($N = 10$) po, q.d., for fifteen days. Two days of oral vehicle dosing were conducted just before and just after compound dosing. Urine was collected before the first dose, and on the second, seventh, tenth, fifteenth, and seventeenth days of dosing. The urine was analyzed for n-telopeptides (NTx) and creatinine (Cre). The results are expressed as uNTx/Cre.

uNTx/Cre was 34−44% lower (mean 39%) than in vehicle-treated animals during the treatment period and in the range of intact rhesus monkeys of the same age. uNTx/Cre did not differ significantly between the two groups just before compound dosing or at 2 days after completion of compound dosing.

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(25) log *P*: Measured from octanol, pH 7.4 buffer.

(26) Protein binding: Human plasma binding measured in a high-throughput manner using ultrafiltration of diluted plasma in a pH 7.4 buffer. The analysis is conducted at room temperature, and solutions are analyzed by HPLC.

(27) Compounds were screened for their affinity to bind to the $\alpha_v\beta_3$ receptor using a scintillation-proximity assay (SPAV3 assay). This binding assay utilizes the displacement of 2(*S*)-(4-^{125}iodobenzenesulfonylamino)-3-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)ethyl]benzoylamino}propionic acid[34] from purified, recombinant human $\alpha_v\beta_3$. A similar SPA assay was used to counterscreen for binding to the $\alpha_v\beta_5$ receptor (SPAV5). In this case the displacement of the same radioligand from purified, recombinant $\alpha_v\beta_5$ was measured. See: Duggan, M. E.; Duong, L. T.; Fisher, J. E.; Hamill, T. G.; Hoffman, W. F.; Huff, J. R.; Ihle, N. C.; Leu, C.-T.; Nagy, R. M.; Perkins, J. J.; Rodan, S. B.; Weslowski, G.; Whitman, D. B.; Zartman, A. E.; Rodan, G. A.; Hartman, G. D. Nonpeptide $\alpha_v\beta_3$ Antagonists. 1. Transformation of a Potent, Integrin-Selective $\alpha_{IIb}\beta_3$ Antagonist into a Potent $\alpha_v\beta_3$ Antagonist. *J. Med. Chem*. **2000**, *43*, 3736−3745.

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