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Nonpeptide $\alpha_v\beta_3$ antagonists. Part 10: In vitro and in vivo evaluation of a potent 7-methyl substituted tetrahydro-[1,8]naphthyridine derivative

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Abstract—Subtle modifications were incorporated into the structure of clinical candidate **1**. These changes were designed to maintain potency and selectivity while inducing changes in physical properties leading to improved pharmacokinetics in three species. This approach led to the identification of **4** as a potent, selective $\alpha_v\beta_3$ receptor antagonist that was selected for clinical development based on an improved PK profile and efficacy demonstrated in an in vivo model of bone turnover.

Osteoporosis is a disease most commonly found in postmenopausal women, where the combination of estrogen deficiency and aging results in the loss of a significant portion of the peak bone mass attained as a young adult. The characteristic skeletal fragility and high incidence of fracture are due to a decrease in mechanical bone strength resulting from an imbalance between the activities of bone-resorbing osteoclast cells and bone-depositing osteoblast cells.[1–4] One potential approach to combatting this disease is to reduce the rate of osteoclast-mediated bone resorption by antagonism of the integrin $\alpha_v\beta_3$.[5] $\alpha_v\beta_3$ is highly expressed in osteoclasts and is thought to be involved in the adhesion, activation, and migration of osteoclasts on the bone surface as well as osteoclast polarization.[6,7] Several proteins that possess the three-amino acid sequence arginine–glycine–aspartic acid (RGD), including osteopontin, bone sialoprotein, vitronectin, and fibrinogen, bind with high affinity to $\alpha_v\beta_3$.[8] Antibodies to $\alpha_v\beta_3$, RGD-peptides, and small molecule mimetics of RGD have been shown to be efficacious in in vitro and in vivo models of bone resorption.[9] These results suggest that antagonism of the RGD binding domain of $\alpha_v\beta_3$ by high affinity nonpeptidic RGD mimetics may result in a treatment for osteoporosis.

Previous reports from this laboratory have described the chain-shortened imidazolidinone **1** as an orally bioavailable zwitterionic antagonist that was selected for evaluation in human clinical trials.[10] Compound **1** was projected to display a pharmacokinetic profile in humans to support twice daily oral dosing. Satisfied with the in vitro potency and selectivity of **1**, we sought to identify a related compound with improved pharmacokinetics that could be effective in humans following once-a-day administration. Herein, we describe modifications to the *N*-terminus of **1** that were designed to increase lipophilicity and block a potential site of human metabolism. These efforts resulted in compound **4**, which showed an enhanced pharmacokinetic profile and was chosen for clinical development.

Compound **1** was selected as a clinical candidate for the prevention and treatment of osteoporosis based on

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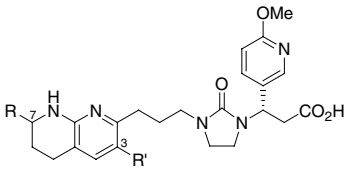
excellent in vitro potency and selectivity, a good pharmacokinetic profile and efficacy shown in three different in vivo models of bone resorption.[10] After extensive SAR studies around aryl substituents on the β-alanine *C*-terminus of the imidazolidinone core series, 2-methoxypyridine was found to possess a desirable balance between basicity, polarity, and the degree of binding to human plasma proteins.[11,12] Typically, polar compounds in the imidazolidinone series, as defined by a log *P* value approaching zero, suffered from poor pharmacokinetic profiles due to low bioavailability. Lipophilic compounds with a log *P* value approaching 1.0 usually displayed good oral bioavailability and half-life, but high protein binding reduced in vivo efficacy due to low free drug concentration.

We sought to introduce conservative modifications to the *N*-terminus of **1** that would not adversely alter the in vitro potency and selectivity, but would induce favorable changes in physical properties. The methyl group was chosen as a small substituent that could enhance lipophilicity without having a dramatic effect on free drug concentration. In a related series, 7-methyl substitution on the tetrahydro-[1,8]naphthyridine (THN) ring was found to be 2-fold more potent than 6-methyl substitution. In this series, substitution at the C-3 position of the THN ring was extensively studied showing that electron withdrawing groups decreased binding affinity while electron donating groups increased potency.[13] Substitution on the unsaturated ring of the THN may largely affect the binding affinity and physical properties of the *N*-terminus by modulation of the pK_a. When 3-methyl substitution was employed in the imidazolidinone series, the substitution had the desired effect on in vitro potency and physical properties. Compound **2** possessed comparable potency to **1** while having less protein binding in spite of being more lipophilic (Table 1). This substitution had detrimental effects on the pharmacokinetic profile of **2**; clearance increased dramatically while half-life decreased. Substitution with slightly larger aliphatic groups such as ethyl caused a 10–20-fold loss of selectivity versus the related integrin $\alpha_v\beta_5$ when compared with **1** and **2**.

Table 1. In vitro binding affinity and physical properties of THN substituted $\alpha_v\beta_3$ antagonists



Compd	R	R′	SPAV3[a] IC_{50} (nM)	PB (%)[b]	Log*P*
1	H	H	0.08	88	0.21
2	H	CH_3	0.13	78	0.62
3	(*S*)-CH_3	H	0.11	92	0.56
4	(*R*)-CH_3	H	0.11	95	0.69

[a] Binding to the $\alpha_v\beta_3$ receptor using a scintillation-proximity assay (SPAV3), $n = 235$ for **1**, $n = 2$ for **2** and **3**, $n = 7$ for **4**.
[b] Human plasma protein binding.

Although in vitro metabolism studies on **1** using liver microsomes from several species showed minimal metabolism, the C-7 methylene position of the THN ring was identified as a site of oxidative metabolism. The C-7 hydroxylated metabolite could undergo further oxidation to yield a lactam or eliminate and aromatize to give the fully unsaturated naphthyridine.[10] We focused on placing a methyl substituent at the C-7 position of the THN ring knowing that this substitution was tolerated in a related series and that blocking a site of metabolism could improve pharmacokinetics. The first synthesis of a 7-methyl substituted THN ring in the imidazolidinone series yielded a racemic compound, which was separated by chiral chromatography to give **3** and **4**.[14] Both **3** and **4** were equipotent in the $\alpha_v\beta_3$ binding assay (SPAV3) and their physical properties were similar.[15] Later asymmetric synthesis established the absolute stereochemistry at the C-7 methyl position to be *S* for compound **3** and *R* for compound **4**. We concentrated further studies on diastereomer **4**.

Like compound **1**, **4** displayed good selectivity against several related integrins. Compound **4** displayed a 50-fold selectivity for $\alpha_v\beta_3$ over $\alpha_v\beta_5$ as demonstrated by an IC_{50} of 5 nM ($n = 7$) in an $\alpha_v\beta_5$ binding assay.[14] Affinity for the fibrinogen receptor $\alpha_{IIB}\beta_3$, as measured by a platelet aggregation assay, was found to be weak with an $IC_{50} > 10\,\mu M$.[16] While the addition of the C-7 methyl group on the THN ring did not adversely alter the vitro potency and selectivity, the physical properties of **4** were changed. As compared to compound **1**, Log*P* increased favorably from 0.21 to 0.69 while protein binding increased slightly from 88% to 95%. This compound displayed improved pharmacokinetics in three species (Table 2). Clearance remained high in rats, but slightly improved over compound **1**. Half-life decreased slightly, but oral bioavailability doubled (50% vs 26%). The pharmacokinetic profile in dog and monkey was characterized by low clearance (1.7 and 3.5, respectively) and high oral bioavailability (83% and 75%) while $t_{1/2}$ was unchanged from that observed for **1**. The pharmacokinetic profile of compound **4** was highlighted by reduced clearance, comparable half-life, and improved bioavailability.

On the basis of its excellent in vitro potency, selectivity, and improved pharmacokinetic profile, **4** was chosen for study in a rodent model of bone resorption. Compound **4** was administered in two different experiments to

Table 2. Pharmacokinetics in several species[a]

Species	Compd	CL (mg/min/kg)	$T_{1/2}$ (h)	*F* (%)
Rat	**1**	47	3	26
	4	41	1–2	50
Rhesus monkey	**1**	9.0	2	74
	4	3.5	2	75
Dog	**1**	6.4	3.5	64
	2	18	2.2	55
	4	1.7	4	83

[a] Compounds dosed at 0.2 mpk iv and 1 mpk po in water.

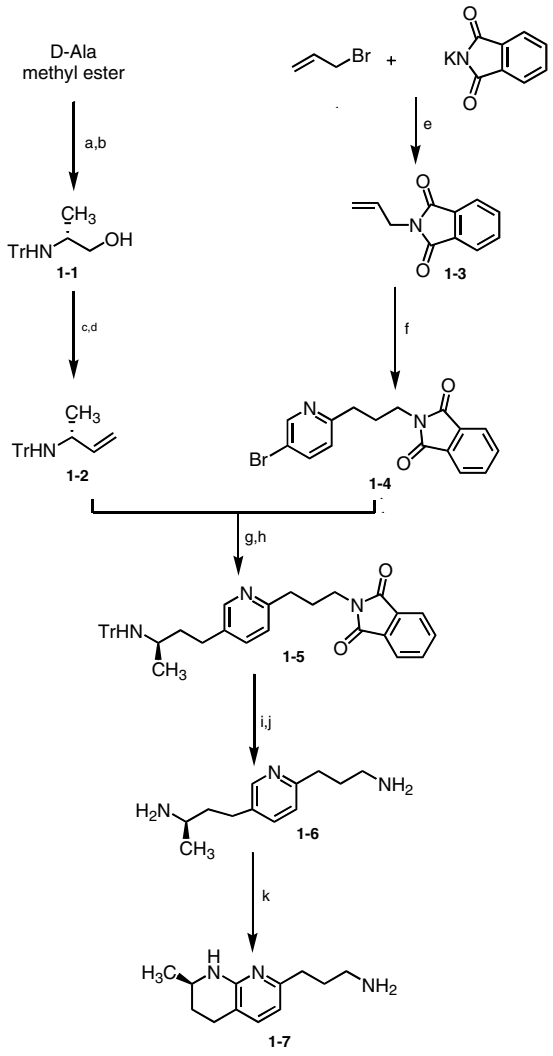
Table 3. Effect of **1** and **4** on growing young male rats

Compd	Free C_{ss}[a] (nM)	DFMBMD[b] (mg/cm^2)	% Increase over vehicle
Vehicle	—	112.5 ± 5.8	—
1	170	133.0 ± 7.5[c]	+18
4	190	137.0 ± 10.0[c]	+22
4	560	147.9 ± 7.3[c,d]	+32
4	1440	161.0 ± 13.7[c,d]	+43
Vehicle	—	118.6 ± 9.2	—
1	390	145.0 ± 10.0[c]	+22
1	1550	157.2 ± 12.3[c,e]	+33
4	170	141.1 ± 9.7[c]	+19
4	1100	156.6 ± 11.8[c,f]	+32

[a] Free C_{ss} (nM)—free steady state serum concentration.
[b] DFMBMD—distal femoral metaphysis bone mineral density.
[c] >Vehicle ($P < 0.0001$).
[d] >190 nM **4** ($P < 0.0001$).
[e] >390 nM **1** ($P < 0.002$).
[f] >170 nM **4** ($P < 0.0001$).

young, rapidly growing male rats by minipump infusion over ten days (Table 3). Compound **1** was used as a positive control and at the end of the experiment bone mineral density at the distal femoral metaphysis was measured and compared to that of vehicle-treated animals. Both compounds produced a significant increase in distal femoral metaphysis bone mineral density (DFMBMD), which was significantly different than the vehicle control group. In the first experiment, compound **4** increased DFMBMD in an exposure-dependent fashion, where free steady state serum concentration (C_{ss}) varied from 190 to 1440 nM with a maximum effect at +43% above the vehicle. The DFMBMD increase achieved with **4** at 190 nM (+22%) was approximately the same as seen with **1** at 170 nM (+18%). In the second experiment, both **1** and **4** produced statistically significant, exposure-dependent increases in DFMBMD. The C_{ss} of **1** varied from 390 to 1550 nM with a maximum effect at +33% while a lower C_{ss} of **4** (170–1100 nM) was able to produce similar effects (maximum at +32%). These data suggest that **4** is at least as efficacious as **1** for inhibiting bone resorption in rats.

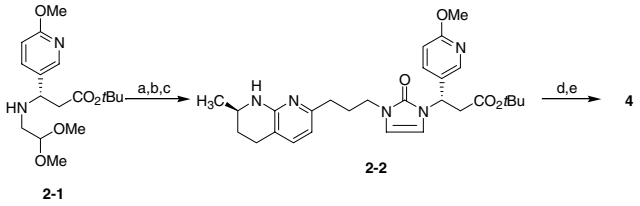
The asymmetric synthesis of **4** was accomplished using a three part convergent synthesis, which utilized the *C*-terminal intermediates developed for the process scale preparation of compound **1**.[17] The synthesis of the *N*-terminus of **4** (Scheme 1) is accomplished in 10 steps from commercially available materials. D-Alanine methyl ester was trityl protected and reduced to the alcohol **1**–**1** with LAH. Oxidation by pyridine–SO_3 yielded the aldehyde, which was immediately used in a Wittig reaction with methyl triphenylphosphonium bromide to give **1**–**2** in 93% yield for four steps.[18] Allyl bromide was coupled to potassium phthalimide by heating in DMF followed by cross-coupling to 2,5-dibromopyridine to give **1**–**4** in 50% yield. Heck coupling of **1**–**2** and **1**–**4** gave **1**–**5**, which was fully deprotected and subsequently cyclized via an intramolecular Chichibabin reaction to yield the key intermediate **1**–**7** in 60% yield for the last four steps.



Scheme 1. Reagents and conditions: (a) trityl chloride, Et_3N, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) pyridine–SO_3, Et_3N, DMSO, CH_2Cl_2; (d) methyl triphenylphosphonium bromide, *n*-BuLi, THF, 0 °C; (e) DMF, 70 °C; (f) 9-BBN, 2,5-dibromopyridine, K_2CO_3, DPPF, $Pd(OAc)_2$, DMF; (g) 9-BBN, THF; (h) **1**–**4**, K_2CO_3, DPPF, $Pd(OAc)_2$, DMF; (i) HCl, EtOAc; (j) hydrazine, EtOH, MTBE; (k) NaH, xylenes.

The *C*-terminal intermediate **2**–**1** was converted to the chlorocarbamate with triphosgene and then reacted directly with **1**–**7** to give an unsymmetric urea that was cyclized to the imidazoline-2-one **2**–**2** by the addition of 2 M sulfuric acid to the reaction mixture.[17] Catalytic hydrogenation smoothly reduced the olefin and this was followed by hydrolysis of the ester with 6 M sulfuric acid to give **4** (Scheme 2). The original chiral resolution of **3** and **4** was performed on the *tert*-butyl ester following hydrogenation. Analysis of the *tert*-butyl ester of **4** showed 98.8% de was obtained, which is comparable to the ee of the D-alanine methyl ester starting material.

In summary, we have incorporated subtle changes to the structure of clinical development candidate **1** to effect changes in physical properties and improve pharmacokinetics. A strategically placed methyl group on the *N*-terminus of the molecule was found to slightly increase lipophilicity without dramatically increasing



Scheme 2. Reagents and conditions: (a) triphosgene, Et_3N, THF; (b) **1**–**7**, Et_3N, THF, 40 °C; (c) 2 M H_2SO_4; (d) $Pd(OH)_2$, EtOH, AcOH, H_2 (70 psi); (e) 6 M H_2SO_4.

protein binding. On the basis of an improved pharmacokinetic profile in three species and efficacy demonstrated in an in vivo model of bone turnover, **4** was selected for clinical development.

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