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Non-peptide $\alpha_v\beta_3$ Antagonists: Identification of Potent, Chain-Shortened RGD Mimetics that Incorporate a Central Pyrrolidinone Constraint

James J. Perkins,[a,*] Le T. Duong,[b] Carmen Fernandez-Metzler,[d] George D. Hartman,[a] Donald B. Kimmel,[b] Chih-Tai Leu,[b] Joseph J. Lynch,[d] Thomayant Prueksaritanont,[c] Gideon A. Rodan,[b] Sevgi B. Rodan,[b] Mark E. Duggan[a] and Robert S. Meissner[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Department of Bone Biology and Osteoporosis Research, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA*
[d]*Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—Antagonists of the integrin receptor $\alpha_v\beta_3$ are expected to have utility in the treatment of osteoporosis through inhibition of bone resorption. A series of potent, chain-shortened, pyrrolidinone-containing $\alpha_v\beta_3$ receptor antagonists is described. Two sets of diasteromeric pairs of high-affinity antagonists demonstrated marked differences in log P values, which translated into differing dog pharmacokinetic properties. One member of this set was demonstrated to be effective in reducing bone resorption in rats.

Introduction

The vitronectin receptor $\alpha_v\beta_3$ is a member of the integrin family of receptors and is highly expressed in osteoclasts, the cells responsible for the resorption of bone.[1,2] Antibodies to $\alpha_v\beta_3$ and the peptide echistatin have been shown to inhibit bone resorption in vitro and in vivo.[3–8] More recently, small molecule mimetics of the RGD amino acid triad have been reported to inhibit bone resorption in vivo.[9–16] These results suggest that an orally active, small molecule $\alpha_v\beta_3$ antagonist may have utility in the treatment of osteoporosis.

Previously, several reports from these laboratories described our work toward 'chain-shortened' RGD mimetics as potential inhibitors of bone resorption.[16,17] The chain-shortened mimetics are potent antagonists that offer significant improvements in pharmacokinetic profiles as compared to their 'full-length' analogues. Herein, we describe pyrrolidinone-constrained versions of these chain-shortened compounds and their comparative in vitro potencies and pharmacokinetic profiles.

*Corresponding author. Tel.: +1-215-652-4407; fax: +1-215-652-7310; e-mail: robert_meissner@merck.com

Chemistry

The stereoselective synthesis of the chain-shortened pyrrolidinone antagonists are described in Scheme 1. Commercially available 6-oxo-heptanoic acid **1** is converted to the acyl oxazolidinone by addition of the lithium anion of (*S*)-4-benzyl-2-oxazolidinone to the mixed anhydride of **1**. The resulting ketone is then protected as the ketal **2** by treatment with ethylene glycol and catalytic TsOH in refluxing toluene. Stereoselective addition of allyl bromide to the acyl oxazolidinone followed by ozonolysis afforded the aldehyde intermediate **4**. A facile one pot reductive amination/cyclization of the aldehyde with the dihydrobenzofuran (DHBF)-substituted β-alanine ester[18] was followed by unmasking of the ketal with TsOH/acetone to afford the methyl ketone **5**. Friedländer condensation of **5** with 2-amino-3-formylpyridine produced the adduct **6**. Regioselective hydrogenation of the napthyridine followed by hydrolysis of the ester provided the target acid **7**. The alternate diastereomer (**10**) was prepared with the use of the (*R*)-oxazolidinone chiral auxiliary, and the analogues **11** and **12** were prepared with the corresponding quinoline-containing β-alanine.[18]

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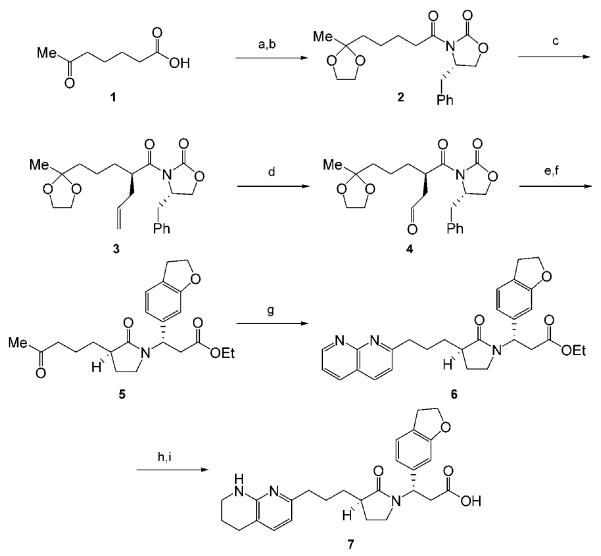


Scheme 1. (a) Isobutyl chloroformate, NEt_3, THF, then Li(*S*)-(+)-4-benzyl-2-oxazolidinone; (b) ethylene glycol, TsOH, toluene, reflux; (c) LiHMDS, THF, then allyl bromide; (d) ozone, CH_2Cl_2, then PPh_3; (e) ethyl-3(*S*)-3-amino-3-(2,3-dihydro-1-benzofuran-6-yl)propionate, $NaB(OAc)_3H$, CH_2Cl_2; (f) TsOH, acetone, reflux; (g) 2-amino-3-formylpyridine, proline, EtOH, reflux; (h) 10% Pd/C, EtOH, H_2 (1 atm); (i) 1 N NaOH, EtOH.

Results and Discussion

The affinities of the $\alpha_v\beta_3$ antagonists for human $\alpha_v\beta_3$ were determined in a competitive binding assay[19] displacing a high affinity ^{125}I-labelled nonpeptide from purified recombinant human $\alpha_v\beta_3$ bound to scintillation proximity beads (SPAV3), and are listed in Table 1 as IC_{50} values. We have recently described[16] a pair of chain-shortened $\alpha_v\beta_3$ antagonists **8** and **9** that show high affinity for the $\alpha_v\beta_3$ receptor while offering favorable pharmacokinetic profiles in dogs. These profiles are characterized by high oral bioavailability, low clearance and moderate plasma half-lives. The shortening of the chain length between the charged centers from that contained in earlier peptide-derived 'full-length' antagonists was a crucial factor in obtaining the desirable pharmacokinetic properties. In addition, the aryl substituent of the β-alanine portion of these antagonists was shown to be favored in this series for achieving desirable receptor affinity in conjunction with the good pharmacokinetic profile. Our efforts then turned to focus on the design of additional analogues that would offer further enhanced receptor affinity while maintaining favorable pharmacokinetic profiles.

It was quickly determined that incorporation of a pyrrolidine as a constraint of the amide-bond of compound **7** provided analogues with enhanced affinity for the $\alpha_v\beta_3$ receptor. The (*R*)-pyrrolidinone (**7**) had an SPAV3 IC_{50} of 0.39 nM, while the slightly more potent (*S*)-antipode **10** had an IC_{50} of 0.22 nM. While the pyrrolidinone configuration had only modest effects on in vitro potency, the pyrrolidinone stereochemistry had a profound effect on the physical properties of the diastereomers. The (*R*)-pyrrolidinone **7** had a measured log P of 0.88 while the (*S*)-isomer **10** was much less lipophilic, with a log P of −0.12. The quinoline analogues **11** and **12** were also prepared, and while both diastereomers had higher receptor affinities than their amide parent **9**, the (*R*)-pyrrolidinone **11** proved to be slightly more potent than the (*S*)-analogue **12**. The relationship between lipophilicity and pyrrolidinone stereochemistry seen with the previous pair was maintained, however, as the (*R*)-isomer **11** had the higher log P value of 1.03.

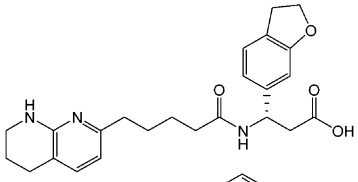
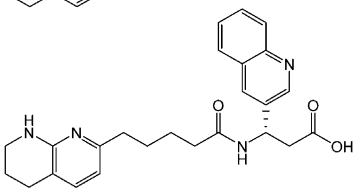
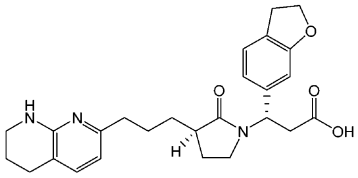
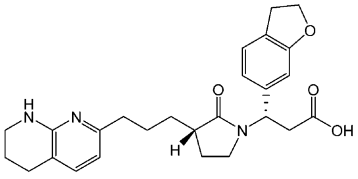
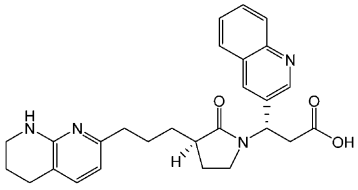
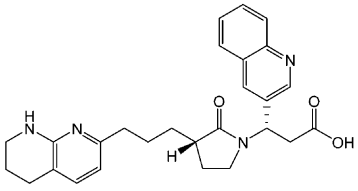
The pharmacokinetic profile for the $\alpha_v\beta_3$ antagonists following their oral administration to dogs is detailed in Table 1. DHBF-(*R*)-pyrrolidinone **7** demonstrated an oral bioavailability (F) of 52%, a clearance (Cl) of 2.1 mL/min/kg and a plasma half-life ($t_{1/2}$) of 8.0 h. Compared to the amide parent **8**, **7** offered similar F and Cl, and an improved $t_{1/2}$. The more polar diastereomer **10**, however, had a much poorer profile, with low F, higher Cl, and a short $t_{1/2}$. The same relationship between relative polarity and pharmacokinetic profile was demonstrated for the quinoline-containing diastereomeric pair of analogues **11** and **12**. In an effort to more closely examine the relationship between log P and pharmacokinetic performance, an in-depth study of the in vitro and in vivo disposition of the isomers **11** and **12** was carried out in rats.[20] Among the conclusions of these studies was the finding that the absorption, transport, and metabolism of the pair was quantitatively different and that the physicochemical properties were an important, but not exclusive cause of these differences. In light of these biological differences of the pyrrolidinone diastereomers, we sought to identify physical property differences that could explain the phenomenon. In our hands, modeling studies did not identify preferred conformations which differentiated the diastereomers **7** and **10** in any profound way, and solution NMR studies of the pair did not show any markedly different hydrogen bonding patterns.

Because compound **7** displayed excellent affinity for the $\alpha_v\beta_3$ receptor and attractive pharmacokinetic parameters in dogs, it was selected to be evaluated in an in vivo model of osteoporosis. Our model utilized young, rapidly growing male rats, and measured the effect of resorption inhibitors on the remodeling of the femur during normal growth.[21] In this assay, a twice-daily 30 mpk oral dose of **7** given for 10 consecutive days was demonstrated to increase bone density.[22]

Conclusion

We have prepared a new series of chain-shortened pyrrolidinone-containing $\alpha_v\beta_3$ antagonists that have high affinity for the $\alpha_v\beta_3$ receptor. An efficient and stereoselective route to this class of antagonists is described, utilizing an asymmetric oxazolidinone alkylation and Friedländer condensation as key steps. Two diastereomeric pairs of pyrrolidinones were prepared by varying the β-alanine aryl substituent and pyrolidinone stereochemistry, and each pair possessed enhanced receptor affinity as compared to their amide parents. Noteworthy was the finding that the diastereomeric pairs showed

Table 1. $\alpha_v\beta_3$ Antagonists

		SPAV3 (IC_{50}, nM)	Log P	Dog pharmacokinetics[a]		
				Oral F (%)	Cl (mL/min/kg)	$t_{1/2}$ (h)
8		3.0	0.53	99	1.2	2.5
9		1.6	0.77	65	5.8	2.4
7		0.39	0.88	52	2.1	8
10		0.22	−0.12	11.5	22.9	1.5
11		0.13	1.03	39	2.52	2.2
12		0.25	−0.42	9.5	15.7	2

[a]Compounds were dosed at 1 mpk po and 0.2 mpk iv to dogs ($n=2$).

similar affinities for the $\alpha_v\beta_3$ receptor, but markedly different polarities as measured by log P determinations. In each case, the (*R*)-pyrrolidinone was more lipophilic and offered improved pharmacokinetics in dogs when compared to the (*S*)-isomer. Compound **7** was further demonstrated to be effective at reducing bone resorption in a 10-day rat model of osteoporosis.

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References and Notes

1. Rodan, S. B.; Rodan, G. A. *J. Endocr*. **1997**, *154*, S47.
2. Clover, J.; Dodds, R. A.; Gowen, M. *J. Cell Sci*. **1992**, *103*, 267.
3. (a) Pytela, R.; Pierschbacher, M. D.; Ginsberg, M. H.; Plow, E. F.; Ruoslahti, E. *Science* **1986**, *231*, 1559. (b) Miyauchi, A.; Alvarez, J.; Greenfield, E. M.; Teti, A.; Grano, M.; Colucci, S.; Zambonin-Zallowe, A.; Ross, F. P.; Teitelbaum, S. L.; Cheresh, D.; Hruska, K. *J. Biol. Chem*. **1991**, *266*, 20369.
4. Ross, F. P.; Chappel, J.; Alvarez, J. I.; Sander, D.; Butler, W. T.; Farach-Carson, M. C.; Mintz, K. A.; Robey, P. G.; Teitelbaum, S. L.; Cheresh, D. A. *J. Biol. Chem*. **1993**, *268*, 9901.
5. Fisher, J. E.; Caulfield, M. P.; Sato, M.; Quartuccio, H. A.; Gould, R. J.; Garsky, V. M.; Rodan, G. A.; Rosenblatt, M. *Endocrinol*. **1993**, *132*, 1411.

6. Yamamoto, M.; Fisher, J. E.; Gentile, M.; Seedor, J. G.; Leu, C.-T.; Rodan, S. B.; Rodan, G. A. *Endocrinol*. **1998**, *139*, 1411.
7. Chambers, T. J.; Fuller, K.; Darby, J. A.; Pringle, J. A.; Horton, M. A. *In Vitro Bone Miner*. **1986**, *1*, 127.
8. Crippes, B. A.; Engleman, V. W.; Settle, S. L.; Delarco, J.; Ornberg, R. L.; Helfrich, M. H.; Horton, M. A.; Nickols, G. A. *Endocrinol*. **1996**, *137*, 918.
9. Engleman, V. W.; Nickols, G. A.; Ross, F. P.; Horton, M. A.; Griggs, D. W.; Settle, S. L.; Ruminski, P. G.; Teitelbaum, S. L. *J. Clin. Invest*. **1997**, *99*, 2284.
10. Lark, M. W.; Stroup, G. B.; Cousins, R. D.; James, I. E.; Hwang, S. M.; Bradbeer, J.; Hoffman, S.; Lechowska, B.; Vasko-Moser, J.; Zembryki, D.; Votta, B.; Lee-Rykaczewski, L.; Perng, D.; Salyers, K.; Smith, B.; Liang, X.; Dodds, R.; Newlander, K. A.; Ross, S. T.; Erhard, K.; Huffman, W. F.; Drake, F. H.; Miller, W. H.; Gowen, M. *Bone* **1998**, *22*, 28S.
11. 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. *J. Pharm. Exp*. **1999**, *91*, 612.
12. Miller, W. H.; Alberts, D. P.; Bhatnagar, P. K.; Bondinell, W. E.; Callahan, J. F.; Calvo, R. R.; Cousins, R. D.; Erhart, K. F.; Heerding, D. A.; Keenan, R. M.; Kwon, C.; Manley, P. J.; Newlander, K. A.; Ross, S. T.; Samanem, J. M.; Uzinskas, I. N.; Venslavsky, J. W.; Yuan, C. C.-T.; Haltiwanger, R. C.; Gowan, M.; Hwang, S.-M.; James, I. E.; Lark, M. W.; Rieman, D. J.; Stroup, G. B.; Azzarano, L. M.; Salyers, K. L.; Smith, B. R.; Ward, K. W.; Johanson, K. O.; Huffman, W. F. *J. Med. Chem*. **2000**, *43*, 22.
13. Lark, M. W.; Stroup, G. B.; Dodds, R. A.; Kapadia, R. J.; Hoffman, S.; Hwang, S. M.; James, I. E.; Lechowska, B.; Liang, X.; Rieman, D. J.; Salyers, K. L.; Ward, K.; Smith, B. R.; Miller, W. H.; Huffman, W. F.; Gowen, M. *J. Bone Miner. Res*. **2001**, *16*, 319.
14. 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.; Wesolowski, G.; Whitman, D. B.; Zartman, A. E.; Rodan, G. R.; Hartman, G. D. *J. Med. Chem*. **2000**, *43*, 3736.
15. For reviews, see: (a) Hartman, G. D.; Duggan, M. E. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1281. (b) Duggan, M. E.; Hutchinson, J. H. *Exp. Opin. Ther. Pat*. **2000**, *10*, 1367.
16. 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. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2463.
17. Brashear, K. M.; Hunt, C. A.; Kucer, B. T.; Duggan, M. E.; Hartman, G. D.; Rodan, G. A.; Rodan, S. B.; Leu, C.-T.; Prueksaritanont, T.; Fernandez-Metzler, C.; Barrish, A.; Homnick, C. F.; Hutchinson, J. H.; Coleman, P. J. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3483.
18. Preparation of 3(*S*)-β-alanine esters: (a) 3(*S*)-quinoline: Duggan, M. E.; Meissner, R. S.; Perkins, J. J. WO 99/30709. (b) 3(*S*)-Dihydrobenzofuranyl (DHBF): Coleman, P. J.; Hutchinson, J. H.; Hunt, C. A.; Lu, P.; Delaporte, E.; Rushmore, T. *Tetrahedron Lett*. **2000**, *41*, 5803.
19. Scintillation proximity bead-based binding assay utilizing displacement of 2(*S*)-(4-^{125}Iodo-benzenesulfonylamino)-3-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethyl]-benzoylamino}-propionic acid from purified, recombinant human $\alpha_v\beta_3$ receptor. For a detailed assay protocol, see ref 14.
20. Prueksaritanont, T.; Meng, Y.; Ma, B.; Leppert, P.; Hochman, J.; Tang, C.; Perkins, J.; Zrada, M.; Meissner, R.; Duggan, M. E.; Lin, J. H. *Xenobiotica* **2002**, *32*, 207.
21. Assay endpoint is the bone mineral density of the distal femoral metaphysis. Details of this rat bone resorption assay will be provided in forthcoming publications. All animal studies were approved by the Merck Research Laboratories IACUC.
22. Compound **7** produced a 5.6% increase in bone mineral density after 10 days as compared to vehicle dosing.