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BENZIMIDAZOLE DERIVATIVES AS ARGININE MIMETICS IN 1,4-BENZODIAZEPINE NONPEPTIDE VITRONECTIN RECEPTOR (αvβ3) ANTAGONISTS

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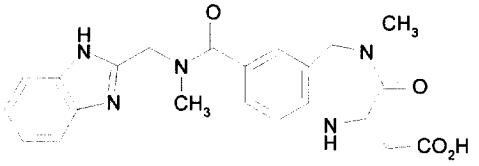
Abstract: In a 3-oxo-1,4-benzodiazepine-2-acetic acid series of vitronectin receptor (αvβ3) antagonists containing a benzimidazole as a novel arginine mimetic, we examined the effects of benzimidazole modifications and amide substitutions on both activity and pharmacokinetics.

We have been investigating the hypothesis that RGD-based peptidomimetic antagonists of the vitronectin receptor αvβ3 can lead to novel therapeutics for a variety of remodeling disorders such as osteoporosis, restenosis, and the angiogenesis component of cancer.[1] Our initial studies[2] demonstrated that highly potent and selective nonpeptide αvβ3 antagonists could be designed by consideration of the conformations of constrained RGD peptides and peptidomimetics in an approach similar to that used in the identification of highly potent and selective nonpeptide fibrinogen receptor (αIIbβ3) antagonists.[3] Remarkably, potent nonpeptide benzodiazepine antagonists could be designed with selectivity for either αIIbβ3 or αvβ3 simply by altering the length and nature of the Arg mimetic. We reported that compound **1** (SB 223245), containing the benzimidazolemethyl group as a novel arginine mimetic, was a potent and selective vitronectin receptor antagonist.[2]



SB 223245 (1)

In this and in the following communication,[4] we describe the results of further studies in the 3-oxo-1,4-benzodiazepine-2-acetic acid series of αvβ3 antagonists. These studies were directed towards the identification

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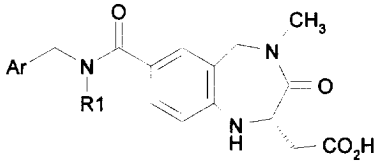
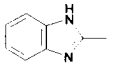
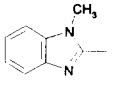
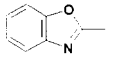
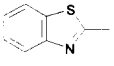
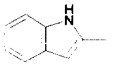
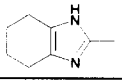
of optimal features for both potency and pharmacokinetics. In this report, we describe studies on analogs containing the novel benzimidazole arginine mimetic, focusing on modifications to the benzimidazole ring as well as substitution of the two amides. In the following report,[4] we describe our efforts to identify other arginine mimetics which would lead to potent and selective nonpeptide vitronectin receptor antagonists.

Chemistry

The benzodiazepine analogs described in this paper were prepared by coupling selected aminomethylheterocycles to the 7-substituted carboxylic acid[5] as previously described.[2] The benzimidazole intermediates for the analogs containing aryl ring substituents listed in Table 2 were prepared from Boc-sarcosine and the appropriately substituted 1,2-phenylenenediamine.[6] For the compounds containing larger subsitituents on the linking amide in Table 2, the benzimidazole intermediates were prepared by alkylation of the requisite amine with 2-N-Boc-protected bromomethylbenzimidazole. The compounds in Table 3 with different groups at position 4 were prepared in racemic fashion according to a published procedure for the synthesis of the 4-phenethyl analog.[7] Although the tables compare chiral and racemic compounds, we have shown that αvβ3 activity in this series resides almost exclusively in the (*S*)-enantiomer.[2]

Table 1. Benzimidazole Analogs



No.	Ar	R1	αvβ3 binding K_i (nM)	αIIbβ3 binding K_i (nM)
1		CH_3	2	30000
2		CH_3	70	28000
(±)3		CH_3	1100	30000
(±)4		CH_3	480	29000
(±)5		H	110	>50000
(±)6		H	2900	47000
7		CH_3	5	2750

Results and Discussion

In our initial studies, we examined several alterations to the benzimidazole arginine mimetic found in **1** for their effects on binding to both αvβ3[8] and αIIbβ3[9] (Table 1). For all of these analogs, we observed good to excellent selectivity for binding to αvβ3 over αIIbβ3. For αvβ3 activity, a free N-H group appears to be important, as methylation of the benzimidazole nitrogen to furnish **2** caused a dropoff in activity. The oxazole **3** and thiazole **4** had even lower affinity, suggesting that the amidine-like arrangement of nitrogens found in the benzimidazole is preferred. The reduction in activity for the indole analog **5**, which retains the key N-H but lacks one of the ring nitrogens, supports this conclusion. In addition, the imidazole analog **6** loses activity relative to **1**, indicating that a fused ring, either aromatic or aliphatic as in **7**, is an important receptor binding element.

The results in Table 1 established that the benzimidazole ring in our 1,4-benzodiazepine series of αvβ3 antagonists was preferred over other heterocyclic rings, conferring both high affinity for αvβ3 as well as selectivity over αIIbβ3. Our further investigations focused on structural modifications to benzimidazole-containing analogs. These potent αvβ3 antagonists were best evaluated using both a binding assay, which uses human αvβ3 isolated from platelets,8 and an αvβ3 cell adhesion assay, which measures the adhesion of HEK 293 cells transfected with human αvβ3 to vitronectin-coated plates.[10] In this way, we could examine both the intrinsic affinity of a compound for the receptor as well as its activity in a cellular context.

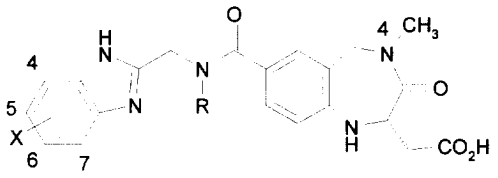
Table 2 shows the results of introducing substituents on the benzimidazole ring. In general, the analogs containing substituted benzimidazoles had activity in both the binding and cell adhesion assays that was comparable to that of the unsubstituted analog **1**. However, introduction of increased bulk (cf. **15** vs. **16**) can lead to a loss of potency. Further, compounds containing either an electron-withdrawing group (**8–13**) or an electron-donating group (**16**, **18**, **19**) had similar activity, suggesting the basicity of the benzimidazole nitrogens is not playing a large role in interactions with the receptor.

In following up on our earlier observation that methylation of the linking amide resulted in an increase in binding affinity,[2] we also prepared a series of analogs in which the linking amide was substituted by a limited range of larger functionality (Table 2). We found that relatively large groups were tolerated at this position, unless the bulk was introduced close to the amide as in **24**. Even introduction of polar groups (i.e., **23** and **25**) led to only a slight loss of activity.

We also studied a variety of substituents on the seven-membered ring amide (the 4-position of the 1,4-benzodiazepine nucleus) while maintaining the unsubstituted benzimidazole ring (Table 3). In general, substitution at the 4-position had little effect on activity in either the binding or adhesion assays, suggesting that the substituent at the 4-position has little interaction with the receptor. Interestingly, for analogs containing

different substitutents at the 4-position, we observed a decrease in activity on going from N-methyl to N-H on the linking amide, as much as tenfold in certain cases (cf. **27** vs. **28**, **32** vs. **33**, **34** vs. **35**, and **38** vs. **39**).

Table 2. Substituted Benzimidazoles

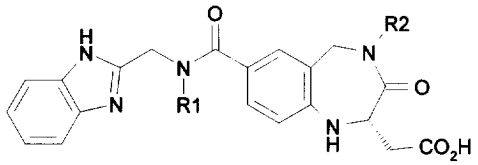


No.	X	R	αvβ3 binding K_i (nM)	αvβ3/293 adh. IC_{50} (nM)
1	H	CH_3	2	145
(±)8	5(6)-Cl	CH_3	11	900
9	5,6-F_2	CH_3	2.5	540
10	5(6)-CF_3	CH_3	8.5	1000
11	4(7)-NO_2	CH_3	13	1100
(±)12	5(6)-NO_2	CH_3	14	3000
(±)13	5(6)-CN	CH_3	15	690
14	4(7)-CH_3	CH_3	2	480
15	5-OCH_3, 6-OCH_3	CH_3	180	-
16	5,6-(OCH_2O)	CH_3	9	690
17	4-OCH_3, 7-OCH_3	CH_3	1300	-
(±)18	5(6)-NH_2	CH_3	19	900
19	4(7)-NH_2	CH_3	5.5	810
20	H	H	15	1650
21	H	*n*Bu	3.5	520
22	H	CH_2CH_2Ph	5	280
23	H	CH_2CO_2H	24	6000
24	H	cyclo(C_6H_{11})	750	-
25	H	CH_2CN	4	810
26	H	CH_2-(2-benzimidazoyl-methyl)	2.5	480

In summary, we have examined the structure–activity relationships in benzimidazole-substituted 3-oxo-1,4-benzodiazepine-2-acetic acid series of vitronectin receptor (αvβ3) antagonists. For the benzimidazole group, we found that a free N-H, an amidine-like disposition of nitrogens, and a fused aromatic or aliphatic ring are all important for optimal binding to the vitronectin receptor. Incorporation of substitution into the benzimidazole ring did not lead to significant improvements in biological activity. We also examined substitution of both the linking amide and the seven-membered ring amide, and found that although a wide variety of substituents is

tolerated at each site, none of these modifications resulted in significant improvements in activity. Compound **1** with an unsubstituted benzimidazole ring and substituted with only a methyl group at both amides remains the most active compound in this series.

Table 3. Position 4 Substitution



No.	R1	R2	αvβ3 binding K_i (nM)	αvβ3/293 adh. IC_{50} (nM)
(±)20	H	CH_3	24	3600
27	CH3	H	6.5	500
28	H	H	30	5000
29	CH3	CH_2CH_2Ph	4	90
(±)30	H	CH_2CH_2Ph	9	750
(±)31	H	$CH(CH_3)_2$	20	5100
(±)32	CH3	$CH_2CH_2OCH_3$	3	340
(±)33	H	$CH_2CH_2OCH_3$	28	3000
(±)34	CH3	$CH_2CH_2C(CH_3)_3$	1.6	180
(±)35	H	$CH_2CH_2C(CH_3)_3$	15	1400
(±)36	CH3	CH_2CH_2(3,4-OCH_2O)Ph	6	250
(±)37	H	CH_2CH_2(3,4-OCH_2O)Ph	6.5	670
(±)38	CH3	CH_2Ph	1.9	510
39	H	CH_2Ph	15	1500
40	H	CH_2CH_2(3-pyridyl)	10	350
(±)41	H	CH_2CO_2H	27	4500

Although the benzimidazole ring represents a novel, non-basic arginine mimetic, the oral and iv pharmacokinetic profile for compound **1** proved to be unsatisfactory,[11] preventing progression to in vivo disease models. The structural modifications described in this paper which altered the basicity, polarity, and lipophilicity properties of **1** did not lead to appreciable improvement in either oral bioavailability or iv half-life. Studies on an alternate heterocyclic arginine mimetic helped to address these shortcomings and are the subject of the following paper in this issue.[4]

Acknowledgments: We thank the Department of Physical and Structural Chemistry for mass spectral and elemental analysis support.

References and Notes

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8. Binding affinity for human αvβ3 was determined in a competitive binding assay employing a tritiated RGD peptide as the displaced ligand. The K_i values represent the means of values determined in two to three separate experiments. Typical assay variability was ±10%. See Wong, A.; Hwang, S. M.; McDevitt, P.; McNulty, D.; Stadel, J. M.; Johanson, K. *Mol. Pharm.* **1996**, *50*, 529.
9. Binding affinity for human αIIbβ3 was determined in a competitive binding assay employing a tritiated RGD peptide as the displaced ligand. The K_i values represent the means of values determined in two to three separate experiments. Typical assay variability was ±10%. See Ali, F. E.; Bennett, D. B.; Calvo, R. R.; Elliott, J. D.; Hwang, S.-M.; Ku, T. W.; Lago, M. A.; Nichols, A. J.; Romoff, T. T.; Shah, D. H.; Vasko, J. A.; Wong, A. S.; Yellin, T. O.; Yuan, C.-K.; Samanen, J. M. *J. Med. Chem.* **1994**, *37*, 769.
10. The IC_{50} values for the HEK293 cell adhesion assay were determined in two experiments of triplicates with compound **29** as internal standard. Typical assay variability was ±20%. For a description of the assay, see: Kumar, C. S.; James, I. E.; Wong, A.; Mwang, V.; Feild, J. A.; Nuthulaganti, P.; Connor, J. R.; Eichman, C.; Ali, F.; Hwang, S. M.; Rieman, D.; Drake, F. H.; Gowen, M. *J. Biol. Chem.* **1997**, *272*, 16390.
11. In rats, the following pharmacokinetic parameters were measured for **1**: oral bioavailability (F) = 3–7%; $t_{1/2}$ = 9–16 min.; plasma clearance = 35 mL/min/kg.