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DISCOVERY OF AN IMIDAZOPYRIDINE-CONTAINING 1,4-BENZODIAZEPINE NONPEPTIDE VITRONECTIN RECEPTOR (αvβ3) ANTAGONIST WITH EFFICACY IN A RESTENOSIS MODEL

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Abstract: In the 3-oxo-1,4-benzodiazepine-2-acetic acid series of vitronectin receptor (αvβ3) antagonists, a compound containing an imidazopyridine arginine mimetic was discovered which had sufficient potency and iv pharmacokinetics for demonstration of efficacy in a rat restenosis model.

One potential therapeutic application of RGD-based peptidomimetic antagonists of the vitronectin receptor (αvβ3) is the treatment of restenosis following percutaneous transluminal coronary angioplasty (PCTA).[1] Despite a high initial success rate in restoring blood flow in stenosed blood vessels, PCTA is plagued by long term (3–6 months) restenosis in more than one-third of the patients treated.[2] The vitronectin receptor, a member of the integrin superfamily of adhesion receptors, has been implicated in vascular smooth muscle cell (SMC) migration into the neointima, a necessary step for restenosis. Studies have demonstrated that blocking αvβ3 inhibits in vitro SMC and endothelial cell adhesion and migration,[3,4] and in vivo experiments have suggested that RGD-containing peptides with selectivity against αvβ3 or antagonizing both αvβ3 and αIIbβ3 reduce neointima formation following PCTA.[5,6] Moreover, a clinical trial using a monoclonal anti-β3 antibody significantly reduced restenosis in patients for up to six months after PCTA.[7] The results, taken together, suggest that the association of cell surface αvβ3 with extracellular matrix proteins coordinates, in part, vascular remodeling in response to PCTA and that this integrin could represent a potential therapeutic target for preventing restenosis.[8]

Our previous efforts on nonpeptide αvβ3 antagonists led to the discovery of potent 3-oxo-1,4-benzodiazepines containing a benzimidazole as a novel arginine mimetic.[9] Although potent in both binding and adhesion assays, these molecules possessed inadequate oral and iv pharmacokinetic profiles to allow progression to in vivo disease models.[10] In this communication, we report the results of our continued

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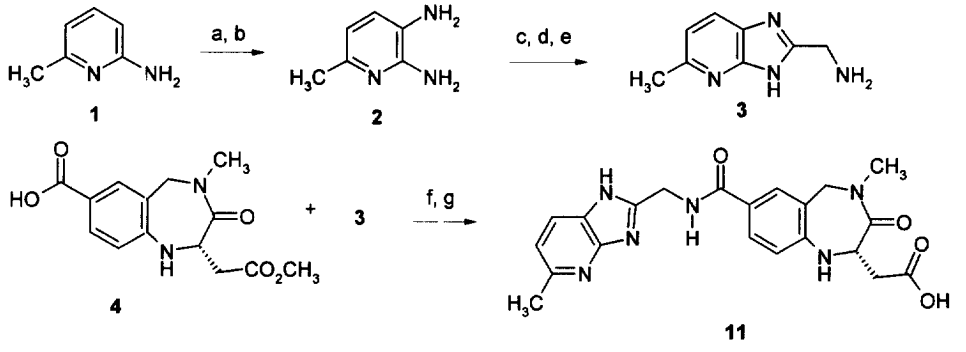
investigation of novel heterocyclic arginine mimetics which has led to the identification of the imidazopyridine analog **11**, a nonpeptide αvβ3 antagonist with efficacy in an animal model of restenosis.

In our previous studies on benzimidazole-containing nonpeptide αvβ3 antagonists,[10] we found that a free N-H, an amidine-like disposition of nitrogens, and a fused aromatic or aliphatic ring were all important for optimal binding to αvβ3. Subsequently, we were interested in examining the effect on biological activity and pharmacokinetics of introducing nitrogen atoms into the fused aromatic ring of the benzimidazole arginine mimetic. The resultant imidazopyridine ring system would possibly afford enhanced oral bioavailability or improved iv pharmacokinetics in our series of 1,4-benzodiazepine αvβ3 antagonists.

Chemistry

The benzodiazepine analogs described herein were prepared by coupling selected aminomethylheterocycles to the 7-substituted carboxylic acid as previously described.[9,10] The requisite aminomethylheterocycles were prepared from a protected sarcosine or glycine and the appropriately substituted 1,2-diaminoheterocycle. Scheme 1 depicts a representative synthesis of 2-aminomethyl-5-methyl-1(*H*)-imidazo-4-pyridine used in the synthesis of **11**.

Scheme 1



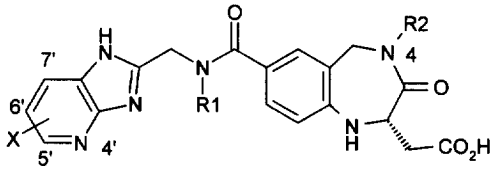
(a) H_2SO_4, HNO_3, 80 °C (26%); (b) H_2, 10% Pd/C, MeOH (97%); (c) Cbz-glycine, isobutylchloroformate, TEA, THF, rt; (d) AcOH, 110 °C; (e) H_2, 10% Pd/C, MeOH (31% for three steps); (f) EDC, HOBt · H_2O, (*i*-Pr)$_2$NEt, DMF (48%); (g) 1 N NaOH, MeOH (95%).

Results and Discussion

As with the benzimidazole analogs,[10] the compounds described in this paper were evaluated using both a binding assay, which uses human αvβ3 isolated from platelets,[11] and an αvβ3 cell adhesion assay, which measures the adhesion of HEK 293 cells transfected with human αvβ3 to vitronectin-coated plates.[12] 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. In addition, certain compounds were also tested for their inhibitory activity in a rat aortic smooth muscle cell (RASMC) migration assay. Finally, key pharmacokinetic data (oral bioavailability, iv

clearance, half-life) were obtained on selected molecules in order to help choose a compound for in vivo evaluation.

Table 1. In vitro activity of imidazopyridine vitronectin receptor antagonists



No.	X	R1	R2	αVβ3 binding K_i (nM)	HEK 293 adh. IC_{50} (nM)
5	(4′-carba)	CH_3	CH_3	2	145
6	H	CH_3	CH_3	3	270
(±)7	(5′-aza)	CH_3	CH_3	19	1500
8	(4′,6′-diaza)	CH_3	CH_3	23	5000
9	5′-CH_3, 7′-CH_3	CH_3	CH_3	710	-
10	5′-CH_3	CH_3	CH_3	3	450
11	5′-CH_3	H	CH_3	45	9000
12	5′-CH_3	CH_3	H	10	2500
(±)13	5′-CH_3	H	H	90	20000
(±)14	5′-CH_3	CH_3	CH_2Ph	13	160
(±)15	5′-CH_3	H	CH_2Ph	60	-
(±)16	H	CH_3	$CH_2CH_2OCH_3$	2.5	500
(±)17	5′-CH_3	H	$CH_2CH_2OCH_3$	50	10000
(±)18	H	CH_3	$CH(CH_3)_2$	4	2000
(±)19	H	CH_3	$CH_2CH_2C(CH_3)_3$	2.5	250

The results for a variety of aza-benzimidazole analogs are shown in Table 1. Compound **5**, a previously reported[9] benzimidazole-containing analog is included for comparison. The substitution of nitrogen for carbon to generate the imidazopyridine analog **6** resulted in retention of activity in both the binding and adhesion assays, while the isomeric imidazopyridine **7** and imidazopyrimidine **8** both had slightly weaker activity. Within the 4′-aza series, the 5′-methyl analog **10** also had good receptor binding activity, although addition of a 7′-methyl group (**9**) led to greatly reduced activity, possibly due to increased steric hindrance about the imidazole nitrogen. Consistent with our observations in the benzimidazole series,[10] removal of the linking amide methyl group from **10** to give **11** led to 20-fold reduction in activity in both the binding and adhesion assays. We observed a similar reduction in activity for the unsubstituted amide compared to its N-methyl counterpart for a number of R2 groups at position four of the benzodiazepine template (cf. **12** vs. **13**, **14** vs. **15**, **16** vs. **17**). At this position of the benzodiazepine, good activity was found for a number of hydrophobic groups, which

were examined in an effort to increase lipophilicity to aid oral absorption in this highly polar series of compounds.

In order to identify candidates for progression to the rat restenosis model, we tested a number of imidazopyridine-containing αvβ3 antagonists for efficacy in blocking vitronectin-induced migration of rat aortic smooth muscle cells (RASMC).[13] We have shown previously that vitronectin-induced RASMC migration could be inhibited by F11, a monoclonal antibody to the rat β_3 integrin subunit.3 In addition, we examined the pharmacokinetic profiles in rats for selected analogs. The data, summarized in Table 2, showed low oral bioavailability (<10%) for both the benzimidazole (**5**) and imidazopyridine analogs (**10**, **11**, **16**, **19**). However, compound **11** demonstrated very low plasma clearance, resulting in an elevated maximal concentration (C_{max}) and a prolonged half-life ($T_{1/2}$). For this reason, we selected **11**, although not among the more potent inhibitors of smooth muscle cell migration, as an appropriate test compound for in vivo evaluation in a rat restenosis model using parenteral administration.

Table 2. Rat in vitro activity and pharmacokinetic data for selected imidazopyridine αvβ3 antagonists

No.	IC_{50} RASMC migration (nM)	C_{max} (ng/mL)	$T_{1/2}$ (min)	Clearance (mL/min/kg)	oral bioavailability (%)
5	370	819 ± 445	9–16	35	3–7
10	170	1382, 1227	22–25	17, 19	2–4
11	1600	9580 ± 7200	92 ± 41	1.8 ± 1.5	8 ± 6
16	230	692 ± 71	25 ± 7	31 ± 3	4 ± 1
19	470	555 ± 85	14 ± 1	55 ± 11	<1

The effect of compound **11** on balloon injury-induced neointima formation was examined in the rat carotid artery model.[14] The day before the carotid artery balloon injury, two osmotic pumps (Alzet™, one 7-day pump releasing 10 μL/h and one 14-day pump releasing 5 μL/h; the dose of **11** was 10.8 mg/rat/day for the first 7 days and 3.6 mg/rat/day for the next seven days.) filled with a solution of **11** or vehicle were embedded in each rat abdominal cavity. Determination of the plasma level of **11** over the course of the experiment indicated that a constant plasma level of approximately 38 μM was reached during the first 7 days. Although the concentration of **11** declined when the dose was reduced over the last seven days, the final plasma level of **11** remained around 10 μM when the animals were sacrificed, approximately an order of magnitude higher than its IC_{50} for inhibition of RASMC migration.

Arteries from both treated and control groups were removed 14 days after angioplasty. Figure 1 shows representative stained cross-sections of the left common carotid artery (CA) from a sham-operated rat (I), the left common CA (angioplasty) from a vehicle-treated rat (II), and the left common CA (angioplasty) from a rat treated with **11** (III). Clearly, the extensive neointimal formation induced by the balloon injury (cf: II vs. I) was

reduced by treatment with **11** (cf: II vs. III). The total neointima lesion volume of the left common carotid artery in animals treated with **11** (n = 27) was reduced by 35.1% ($P < 0.05$) compared to the vehicle-treated group (n = 30). This treatment did not alter the cross-sectional area of the media (0.126 ± 0.01 mm^2 in vehicle and 0.134 ± 0.003 mm^2 in treated), and the mean of the ratios of neointima vs. media in the treated rats decreased by 36.2% as shown in Figure 2 (0.68 ± 0.08 in vehicle vs. 0.43 ± 0.07 in treated, $P<0.05$).

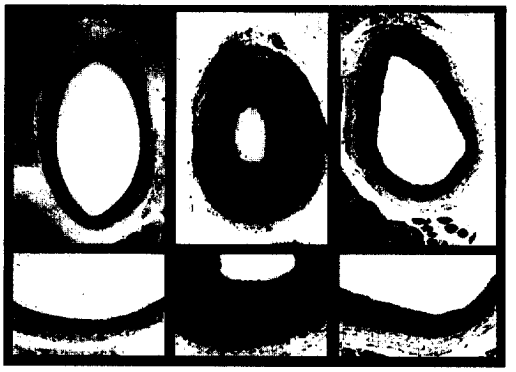


Figure 1. Representative cross sections of the left common carotid artery (CA) from a sham-operated rat (I), from a rat which underwent baloon injury and was treated with vehicle (II), and from a rat which underwent baloon injury and was treated with compound **11** (III).

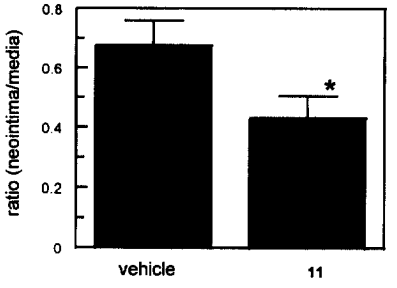


Figure 2. The ratios of cross-sectional areas of the neointima to the media from rats treated with vehicle or compound **11** ($P < 0.05$).

In conclusion, further investigation of novel arginine mimetics has led to the discovery of potent αvβ3 antagonists containing an imidazopyridine arginine mimetic. One of these analogs (**11**), had low clearance in pharmacokinetic studies in rats and was progressed to an in vivo model of restenosis. In this model, a statistically significant reduction of neointimal hyperplasia was evident, demonstrating the potential of nonpeptide αvβ3 antagonists for the treatment of restenosis following PCTA. Our further efforts in this area targetted at identification of a second generation of αvβ3 antagonists with both optimal potency as well as good oral bioavailability and iv pharmacokinetics will be the subject of future reports from these laboratories.

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