Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1801–1806

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ORALLY BIOAVAILABLE NONPEPTIDE VITRONECTIN RECEPTOR ANTAGONISTS CONTAINING 2-AMINOPYRIDINE ARGININE MIMETICS

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Received 11 March 1999; accepted 12 May 1999

Abstract: A peptide RGD analog containing a novel 2-aminopyridine arginine mimetic was discovered to have good affinity and selectivity for the vitronectin receptor. Incorporation of the 2-aminopyridine arginine mimetic into the 3-oxo-1,4-benzodiazepine-2-acetic acid integrin antagonist series led to novel and potent nonpeptide vitronectin receptor antagonists with promising levels of oral bioavailability.

In our previous studies on nonpeptide Arg-Gly-Asp (RGD) mimetic antagonists of the vitronectin receptor ($\alpha_v\beta_3$) as potential therapeutics for disorders such as osteoporosis, restenosis, and cancer,[1] we had shown that potent nonpeptide benzodiazepine antagonists could be designed with selectivity for either the vitronectin receptor ($\alpha_v\beta_3$) or the platelet fibrinogen receptor ($\alpha_{IIb}\beta_3$) simply by altering the length and nature of the Arg mimetic.[2] These studies revealed that compounds containing the nonbasic benzimidazole group[3] or related azabenzimidazole group[4] as novel arginine mimetics are potent $\alpha_v\beta_3$ antagonists and show good selectivity over the closely related integrin $\alpha_{IIb}\beta_3$. We were also interested in exploring alternative nonbasic heterocyclic arginine mimetics as a strategy to discover additional potent and selective nonpeptide $\alpha_v\beta_3$ antagonists with the opportunity for enhanced oral bioavailability. Herein, we report on our investigation of 2-aminopyridines,[5] a ring system with a neutral pKa value[6] which presents the desired amidine-like disposition of nitrogen atoms favored by $\alpha_v\beta_3$.[3,4]

Chemistry

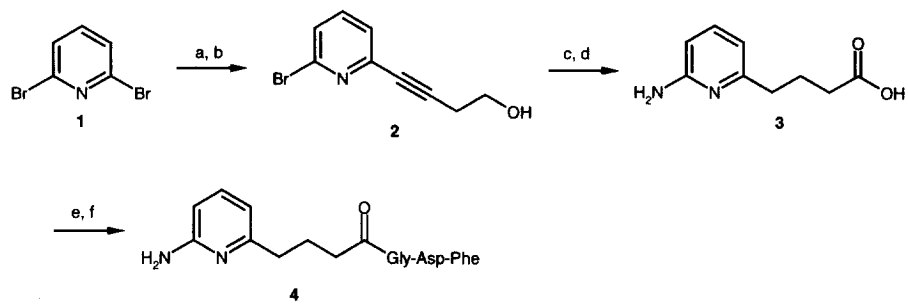
For our initial studies on RGD peptide-based $\alpha_v\beta_3$ antagonists, we chose to examine a 2-aminopyridine containing a linker at the 6-position as an atom-for-atom mimic of the arginine side chain. The synthesis of the requisite 2-aminopyridine-containing arginine mimetic is shown in Scheme 1. Palladium catalyzed coupling of 3-butyn-1-ol to 2,6-dibromopyridine **1** led to a good yield of the monodisplacement product **2**. Reduction of the alkyne was followed by oxidation to the acid and displacement of the bromide to give **3**, which was converted under standard conditions to the peptide RGD analog **4**.

The synthesis of **7** (Scheme 2) illustrates the method for the preparation of the 2-amino-6-(aminomethyl)pyridine arginine mimetics. 2-Amino-6-picoline (**5**) was converted to the corresponding

PII: S0960-894X(99)00282-6

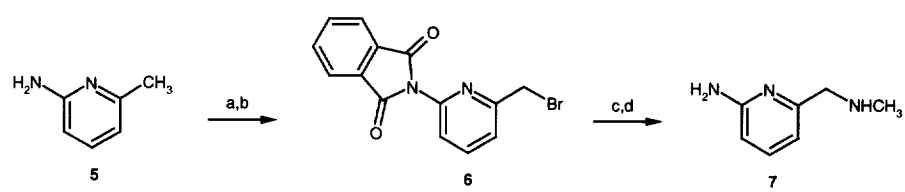
phthalimide, and the methyl group was brominated to afford **6**. Displacement of the bromide with methylamine, followed by hydrazinolysis of the phthalimide gave **7**. The 2-(aminoethyl)aminopyridine arginine mimetics were prepared by modification of the procedure reported for the preparation of related 2-aminopyridine derivatives[5a] as illustrated in Scheme 3. 2-Chloropyridine N-oxide hydrochloride (**8**) was reacted with N-Boc-ethylenediamine to afford **9**. Reduction of the N-oxide by transfer hydrogenation, followed by removal of the Boc group, gave **10**. For both types of aminopyridine arginine mimetics, coupling to the 7-substituted benzodiazepine carboxylic acid as previously described[2] afforded the desired analogs.

Scheme 1



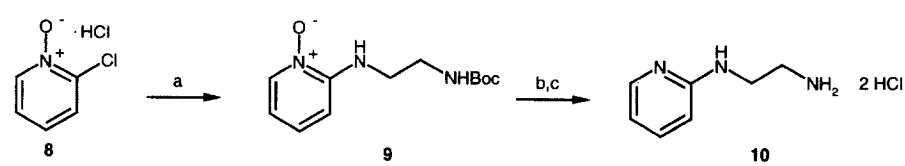
(a) 3-butyn-1-ol, $(Ph_3P)_3PdCl_2$, CuI, Et_3N (51%); (b) H_2 (1 atm), PtO_2, Et_3N, EtOH (82%); (c) Jones reagent, acetone (95%); (d) KNH_2, NH_3 (71%); (e) Gly-Asp(OBn)-Phe(OBn), HOBt, EDC, $(i\text{-}Pr)_2Net$, DMF (27%); (f) H_2 (1 atm), 10% Pd/C, MeOH (90%).

Scheme 2



(a) phthalic anhydride, $ZnCl_2$, $ClCH_2CH_2Cl$, reflux (12%); (b) NBS, CCl_4, reflux (19%); (c) CH_3NH_2 (gas), EtOH, 0 °C; (d) NH_2NH_2, EtOH (32% for two steps).

Scheme 3



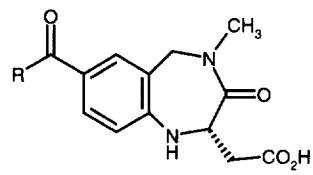
(a) N-Boc-ethylenediamine, $NaHCO_3$, *tert*-amyl alcohol, reflux (89%); (b) cyclohexene, 10% Pd/C, i-PrOH, reflux (78%); (c) 4 N HCl/dioxane, CH_2Cl_2, 0 °C to room temperature (95%).

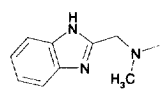
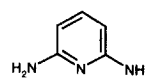
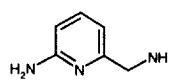
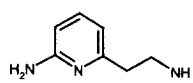
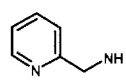
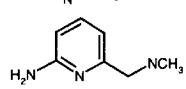
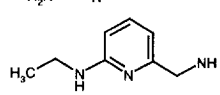
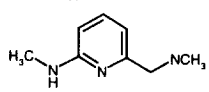
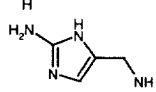
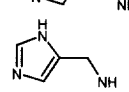
Results and Discussion

Our initial investigation of a 2-aminopyridine arginine mimetic was carried out in a peptide RGD antagonist series. Evaluation of **4** in binding assays for both $\alpha_v\beta_3$[7] and $\alpha_{IIb}\beta_3$[8] revealed that incorporation of

the 2-aminopyridine arginine mimetic results in high affinity for $\alpha_v\beta_3$ (K_i = 20 nM) with a good degree of selectivity over $\alpha_{IIb}\beta_3$ (K_i = 12,000 nM). These data encouraged us to investigate analogous 2-aminopyridine arginine mimetics in our benzodiazepine series of nonpeptide $\alpha_v\beta_3$ antagonists linked either at the 6-position of the pyridine (Table 1) or via the 2-amino substituent (Table 2).

Table 1. Binding Data for 6-linked 2-Aminopyridine Analogs

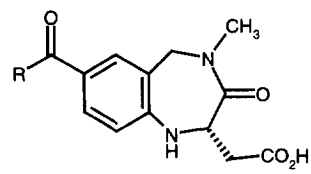


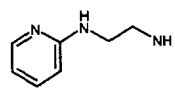
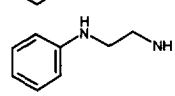
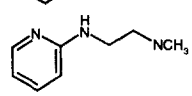
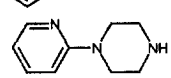
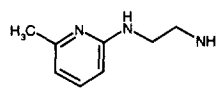
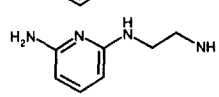
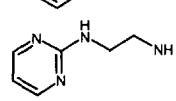
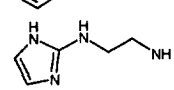
No.	R	$\alpha_v\beta_3$ K_i (nM)	$\alpha_{IIb}\beta_3$ K_i (nM)
11		2	30000
(±)12		150	160000
13		28	8000
14		85	8500
(±)15		8000	41500
16		35	32000
17		9	14000
18		11	3650
19		2	1100
(±)20		490	11000

The results shown in Table 1 reveal that incorporation of the 2-aminopyridine group into the benzodiazepine Gly-Asp mimetic affords potent and selective nonpeptide $\alpha_v\beta_3$ antagonists. Comparison of **12-14** shows that a single methylene group as a spacer between the amide and the pyridine gives the best activity. Compound **15** demonstrates the need for the 2-amino substituent on the pyridine ring, consistent with previous data that suggested an amidine-like disposition of nitrogens was favored by $\alpha_v\beta_3$.[3,4] Methylation of

the linking amide (**16**) does not increase activity, in contrast to results obtained for the corresponding analogs containing a benzimidazole arginine mimetic.[3] However, alkylation of the 2-amino group (**17 and 18**) leads to a slight increase in activity. Finally, the analogous aminoimidazole in **19** is also an excellent mimetic for arginine.[9] As in the case of the 2-aminopyridine, removal of the amino group from the imidazole (**20**) results in a significant loss of activity.

Table 2. Binding Data for N-Linked 2-Aminopyridine Analogs



No.	R	$\alpha_v\beta_3$ Ki (nM)	$\alpha_{IIb}\beta_3$ Ki (nM)
21		3.5	28000
(±)22		22000	>50000
23		33	20000
(±)24		1250	40500
25		280	2300
26		2	5000
27		3000	>50000
28		1.5	8200

The isomeric N-linked 2-aminopyridine[5a] retains the good potency and selectivity for $\alpha_v\beta_3$ (**21**, Table 2). Removal of the pyridine nitrogen (**22**) causes a loss of activity, and methylation of the linking amide nitrogen in this series results in a less potent analog (**23**). Incorporation of the 2-piperazinylpyridine (**24**) causes a more dramatic dropoff in activity, suggesting either a need for a free amino N-H, or an unfavorable conformational constraint. A methyl group is not tolerated at the 6-position of the pyridine ring (**25**), but the related amino substituted analog **26** is a highly potent $\alpha_v\beta_3$ antagonist. In a cursory examination of alternate heterocycles, replacement of the pyridine with a pyrimidine (**27**) is ineffective, but the corresponding imidazole analog **28** has high affinity for $\alpha_v\beta_3$ similar to its isomer **19** (Table 1).

Because the potential clinical utility for a nonpeptide $\alpha_v\beta_3$ antagonist would likely involve treatment of a chronic condition, the ultimate aim is to identify orally bioavailable compounds. Historically, it has proven difficult to discover nonpeptide RGD mimetics with >10% oral bioavailability, unless a prodrug strategy is employed that masks the highly polar guanidine and/or carboxylate functionalities.[10] However, a number of compounds from this study display a promising level of oral bioavailability in pharmacokinetic studies in both rats and dogs (Table 3), which may be a result of the nonbasic 2-aminopyridine arginine mimetic.[6] These results are consistent with previous results from our laboratories, in which we had shown that incorporation of a 2-aminopyridine arginine mimetic into nonpeptide $\alpha_{IIb}\beta_3$ antagonists conferred enhanced permeability in vitro as well as increased oral bioavailability.[11]

Although the level of oral bioavailability for the compounds with an aminopyridine arginine mimetic in Table 3 represents an encouraging improvement compared to the benzimidazole-containing vitronectin receptor antagonists previously reported,[4] additional improvement is clearly needed to advance this series of compounds. Our further investigations into 2-aminopyridine arginine mimetics which resulted in highly potent nonpeptide $\alpha_v\beta_3$ antagonists with excellent levels of oral bioavailability are reported in the following paper in this issue.

Table 3. Pharmacokinetic data for selected 2-aminopyridine $\alpha_v\beta_3$ antagonists.[12]

No.	species	iv $T_{1/2}$ (min)	iv Clearance (mL/min/kg)	oral bioavailability (%)
13	rat	45±15	63±7	4-13
13	dog	47-50	7.8-9.5	10
16	rat	24±2	32±4	5-16
16	dog	38-41	7-10	10
21	rat	31-39	29-36	4-8
21	dog	27-40	34-66	8-14

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

References and Notes

1. For recent reviews of $\alpha_v\beta_3$, see: (a) Samanen, J.; Jonak, Z.; Rieman, D.; Yue, T.-L. *Curr. Pharmaceut. Design* **1997**, *3*, 545. (b) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374. (c) Horton, M. A. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 721.

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5. The 2-aminopyridine ring system has previously found utility as an arginine replacement in the discovery of novel thrombin inhibitors. See: (a) Misra, R. N.; Kelly, Y. F.; Brown, B. R.; Roberts, D. G. M.; Chong, S.; Seiler, S. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2165. (b) Feng, D.-M.; Gardell, S. J.; Lewis, S. D.; Bock, M. G.; Chem, Z.; Freidinger, R. M.; Naylor-Olsen, A. M.; Ramjit, H. G.; Woltmann, R.; Baskin, E. P.; Lynch, J. J.; Lucas, R.; Shafer, J. A.; Dancheck, K. B.; Chen, I-W.; Mao, S.-S.; Krueger, J. A.; Hare, T. R.; Mulichack, A. M.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 3726.

6. See Albert, A. in *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic: NY, 1963, Vol. 1, pp 2-103.

7. Binding affinity for human $\alpha_V\beta_3$ 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*, 529-537.

8. Binding affinity for human $\alpha_{IIb}\beta_3$ 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.

9. A preliminary account on the use of aminoimidazoles as guanidine mimetics in $\alpha_V\beta_3$ antagonists has been reported. See Wityak, J.; Pitts, W. J.; Tobin, A. E.; Estrella, M. J.; Harlow, P. P.; Corjay, M. H.; Mousa, S. A.; Wexler, R. R.; Jadhay, P. K. Book of Abstracts, 215th ACS National Meeting, March 29-April 2, 1998, MEDI-088.

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12. Pharmacokinetic parameters were determined using non-compartmental analysis of plasma concentration of test compound versus time data determined in two to three separate animals. Oral bioavailability was calculated from the dose-normalized i.v. and p.o. AUC values, where AUC is the area under the plasma concentration versus time curve. See: Gibaldi, M.; Perrier, D. Absorption kinetics and bioavailability. In *Pharmacokinetics*, 2nd ed.; Swarbrick, J., Ed.; Marcel Dekker: New York, 1982: Vol. 15, pp 145-198.