Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 205–208

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Biphenyls as Potent Vitronectin Receptor Antagonists

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Received 12 July 2001; revised 17 September 2001; accepted 22 October 2001

Abstract—Vitronectin receptor ($\alpha_V\beta_3$) antagonism has been implicated as a mechanism for the treatment of restenosis following balloon angioplasty. In this work we present results from screening of a focused combinatorial library based on a biphenyl moiety. Our SAR studies led to the identification of compounds with subnanomolar activity, selectivity towards the related GPIIbIIIa receptor and functional activity on human smooth muscle cell migration.

Coronary stenting and balloon angioplasty are the most frequently used percutaneous revascularisation procedures for the treatment of coronary heart disease and myocardial infarction. Despite a high initial success rate in restoring blood flow, percutaneous interventions are plagued by arterial reocclusion within 6 months after surgery, a clinical problem also referred to as restenosis.[1]

The vitronectin receptor $\alpha_V\beta_3$ is a member of the integrin superfamily of cellular adhesion receptors.[2] Vitronectin receptor-mediated migration of coronary smooth muscle cells (SMC's) into the neointima is regarded as a key event of restenosis in both stented and non-stented arteries. Specific antagonists of the related platelet receptor GPIIbIIIa to date have failed to show anti-restenotic properties. However, the long term mortality benefits observed after administration of a mixed $\alpha_V\beta_3$/GPIIbIIIa antibody support the relevance of vitronectin receptor-mediated SMC migration inhibition for the treatment of restenosis.[3]

Like many integrins, $\alpha_V\beta_3$ is known to recognise a continous tripeptide epitope, the arginine-glycine-aspartic acid (RGD) motif. We therefore investigated small molecule peptidomimetics as $\alpha_V\beta_3$ antagonists.

A critical element in the design of RGD mimetics is the correct spatial presentation of arginine and aspartic acid residues around a central core. The discovery of c-RGDfV, a cyclic peptide with known 3-D structure bodes well for the use of beta-turn mimetic templates in the development of vitronectin receptor antagonists.[4]

Whereas biphenyls have been utilised as beta-turn mimetics at several occasions,[5] a recent molecular dynamics simulation predicted a relatively poor beta-turn stabilisation/imitation potential for biphenyl structures.[6] Furthermore, a recent report of a combinatorial biphenyl library screened against the vitronectin receptor, furnished only disappointing results with activities in the micromolar range (Fig. 1).[7]

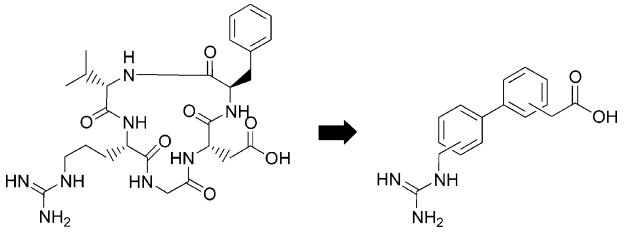

Figure 1. Design of biphenyl-based vitronectin receptor antagonists from *Kessler's* peptide c(RGDfV).

In this communication, we wish to describe a new biphenyl series of small molecule RGD mimetics with potent vitronectin receptor antagonism in the nanomolar range.

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00717-X

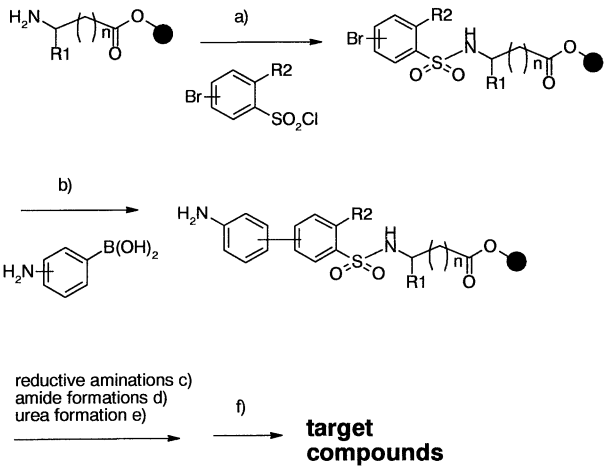
Chemistry[8]

For lead structure identification, we decided to synthesise a targeted combinatorial biphenyl library on solid support (Scheme 1). The acidic group is ideally suited for traceless resin attachment and functions well as a glutamate mimetic. Diversity has been obtained by using a set of immobilised amino acids, synthesising 3,3-, 3,4-, 4,3- and 4,4-biphenyls and exploiting the penultimate aniline for reductive aminations, amide couplings and urea formations. This strategy gave rise to 3500 single compounds, that were screened against the vitronectin receptor.

Whereas compounds **1**–**21, 24** and **27**–**32** were synthesised on solid support, the residing derivatives were obtained from liquid-phase chemistry, starting with amino acid esters. Final deprotection was achieved by LiOH, in water/dimethoxyethane. Enantiomers were synthesised separately using chiral building blocks with known configuration. Materials **20**, **21** and **24** were obtained using 3-formyl-phenyl boronic acid under the Suzuki-coupling conditions of Scheme 1. Compounds **22** and **23** were synthesised by heterocycle formation starting from the corresponding thioureas **II** and **IV**. HgO-mediated ring closure of **II** furnished benzimidazole **III**, whereas the aminohistidine **VIIa** was prepared by cyclising enamine formation of the corresponding diethyl acetal precursor **VI**. The alternative aminohistidine **VIIb** was obtained as a side product (Scheme 2). Materials **25** and **26** were synthesised using the corresponding pyrazine-5,10-diones as acylating agents (THF, DMF, 4 equiv pyridine).

Biology

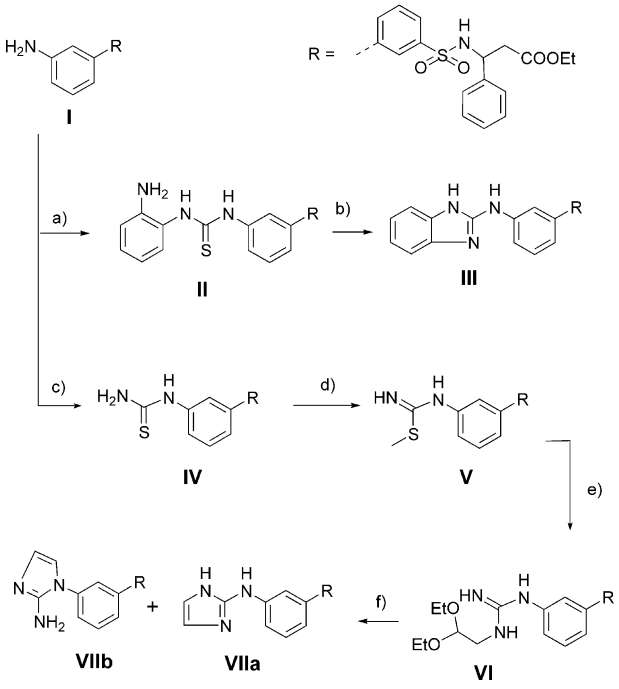
All compounds were evaluated for their in vitro activity in an $\alpha_V\beta_3$ scintillation bead assay using ^{125}I labelled echistatin as a radioligand. A GPIIbIIIa counterscreen was performed similarly.[9] Functional activity was tested in a migration assay with human coronary SMCs on vitronectin-coated plates using a horizontal measuring arrangement. Half of a confluent SMC layer was scraped off. After activation with PDGF-BB (1 nM) migration from the leading edge of the scratch was quantified, measuring migration distance and cell density of migrated cells after 24 h incubation.[10]



Scheme 1. Combinatorial synthesis of a biphenyl vitronectin receptor antagonist library: (a) pyridine/DMF; (b) $(Pd(PPh_3)_2Cl_2$, DMF/H_2O, $K_2CO_3)$, 85 °C; (c) R-CHO, $HC(OMe)_3$, Bu_4NBH_4; (d) DIC, DMF; (e) DIPEA, 4-nitrophenylchloroformate, THF/DCM (1:1), then: R-NH_2, DIPEA, DMF; (f) TFA/DCM (1:1); (● = Wang resin).

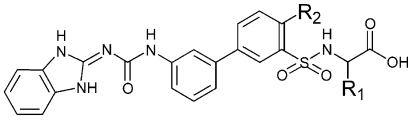
Results

The vast majority of the biphenyl library members turned out to be inactive, emphasising the highly



Scheme 2. Synthesis of heterocyclic biphenyl vitronectin receptor antagonists: (a) (1) thiophosgene, toluene, reflux, 2 h; (2) *o*-phenylendiamine, THF/toluene (1:1) 12 h rt 100%; (b) yellow HgO, $CHCl_3$, 6 h, reflux, 68%; (c) (1) thiophosgene, toluene reflux, 1.5 h; (2) NH_3, THF/toluene (1:1), rt, 30 min, 92%; (d) iodomethane, MeOH, reflux 2 h, 98%; (e) aminoacetaldehyde–diethylacetal, *n*-propanol, reflux 6 h, 88%; (f) 6 N aq HCl, 40% (VIIa), 10% (VIIb).

Table 1. Structure–activity relationship of biphenyl vitronectin receptor antagonists[a]



Compd	Configuration	R1	R2	K_i (nM)
1	*S*	Me	H	2000
2	*R*	Me	H	> 10,000
3		H	H	> 10,000
4	*S*	CH_2Ph	H	30
5	*R*	CH_2Ph	H	> 10,000
6	*S*	CH_2-*p*-$C_6H_4NO_2$	H	1500
7	*S*	CH_2-*p*-$C_6H_4NH_2$	OMe	1100
8	*S*	CH_2Ph	OMe	13

[a] K_i values are medians of three dose–response curves.

defined structural prerequisites of the $\alpha_V\beta_3$/ligand interaction. However, a small set of the urea sublibrary (Scheme 1, condition e) tended to demonstrate both, useful binding affinities and first SAR trends. This class was therefore selected for further investigations.

The first active compound identified from our library was the alanine derivative **1**, displaying an encouraging K_i of 2000 nM to the vitronectin receptor (Table 1). The activity resided exclusively on the *S*-enantiomer; the *R*-enantiomer **2** was practically inactive. The novel benzimidazolyl urea motif has to our knowledge not been described as an arginine mimetic hitherto. The lack of basicity of this novel guanidine mimetic is attractive for the development of vitronectin receptor antagonists with drug like physicochemical properties.

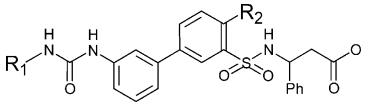
Substituents in close vicinity to the carboxylic group of the RGD motif have been reported to afford improved binding affinities to the related GPIIbIIIa receptor. Similar observations have been made for $\alpha_V\beta_3$.[11] As the corresponding glycine derivative **3** was inactive, we speculated, that further substitutions of the methyl group could result in similar activity enhancements in our series.

Indeed, the *S*-phenylalanin derivative **4** displayed a 100-fold improvement in binding affinity. Additional methoxy substitution on the biphenyl nucleus further enhanced activity, furnishing **8**. Interestingly, further enlargement of the phenyl ring by *para*-substitutions led to decreased activity (**6**,**7**).

Whereas alpha-phenylalanine derivatives showed activity exclusively in combination with benzimidazolyl ureas, the SAR of beta-phenylalanine derived compounds was more tolerant towards urea substitutions (Table 2). The unsubstituted urea (**9**) was itself inactive, but even small aliphatic substituents improved the activity to submicromolar levels. Methoxy substitution of benzyl derivative (**12**) gave rise to a 10-fold improvement of binding affinity (**15**). The closely related 3-picolyl derivative **18** turned out to be equally potent as **8**, the most effective derivative of the alpha-phenylalanine series.

We also examined whether omission of the urea fragment would still be compatible with vitronectin receptor affinity (Table 3). Indeed, the benzimidazole compounds **20**–**22** showed binding affinities in the submicromolar range. In particular, aminobenzimidazole **22** had a 4 nM affinity to $\alpha_V\beta_3$. The corresponding aminoimidazole

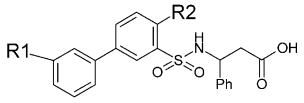
Table 2. Structure–activity relationship of biphenyl vitronectin receptor antagonists[a]

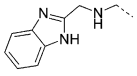
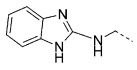
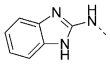
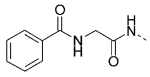


Compd	R1	R2	K_i (nM)
9	H	H	>10,000
10	Me	H	2000
11	*c*-Prop	H	700
12	CH_2Ph	H	220
13	*n*-Prop	OMe	100
14	*i*-Bu	OMe	90
15	CH_2–Ph	OMe	20
16	3-Pyridyl	OMe	60
17	2-Picolyl	OMe	200
18	3-Picolyl	OMe	14
19	4-Picolyl	OMe	25

[a]The beta-amino acids were racemic. K_i values are medians of three dose–response curves.

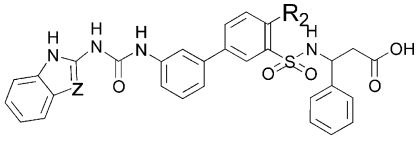
Table 3. Structure–activity relationship of biphenyl vitronectin receptor antagonists[a]



Compound	R1	R2	K_i (nM)
20		H	90
21		OMe	60
22		H	4
23		H	180
24		OMe	360
25		OMe	350
26		OMe	300
27		OMe	280

[a]The beta-amino acids were racemic. K_i values are medians of three dose–response curves.

Table 4. Structure–activity relationship of biphenyl vitronectin receptor antagonists[a]



Compd	Z	R2	Config.	K_i (nM)
28	NH	H	*R*/*S*	4
29	NH	OMe	*R*/*S*	1.4
30	NH	OMe	*R*	0.7
31	NH	OMe	*S*	>2000
32	S	OMe	*R*/*S*	>2000

[a]K_i values are medians of three dose–response curves.

showed a 45-fold drop in activity, suggesting **22**'s additional benzene ring to interact favourably with $\alpha_V\beta_3$. This observation is consistent with the SAR for benzodiazepine-based vitronectin receptor antagonists.[12] A direct comparison of **22** and **28** (Table 4) indicates that the urea moiety in **28** can be omitted without loss of activity. As observed in the alpha-phenylalanine series, methoxy substitution confers enhanced activity resulting in **29**, which was chosen for further biological characterisation.

Replacing the benzimidazole moiety of **29** by a benzothiophene resulted in total loss of activity (**32**), again confirming the importance of NH groups as arginine mimetics. The racemate **29** showed a eudismic ratio of at least 2000, the *R*-enantiomer **30** representing the active configuration. Compound **30** showed a >300 fold selectivity to the related platelet GPIIbIIIa receptor ($K_i = 300$ nM). When it was applied to SMC's freshly isolated from human aorta, **30** inhibited PDGF-induced migration with an IC_{50} of 30 nM. The drop in activity by two orders of magnitude upon moving from binding affinity determination to functional SMC migration inhibition in a cellular context has also been observed in other series.[12]

In summary, we have discovered a potent series of vitronectin receptor antagonists based on a biphenyl motif. The compounds exhibit clear SAR, which led to the identification of **30** with selectivity to GPIIbIIIa and functional activity on SMC migration.

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