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Biphenyls as Potent Vitronectin Receptor Antagonists. Part 2: Biphenylalanine Ureas

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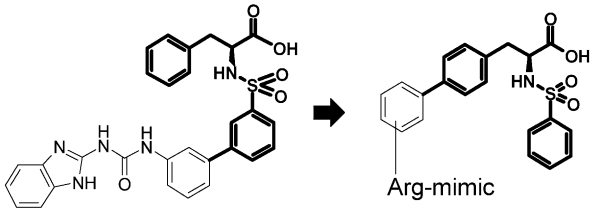
Abstract—Vitronectin receptor ($\alpha_V\beta_3$) antagonism has been implicated in a variety of disease states, like restenosis, osteoporosis and cancer. In this work, we present the development of a novel class of biphenyl vitronectin receptor antagonists. Identified from a focused combinatorial library based on *para*-bromo phenylalanine, these compounds show nanomolar affinity to the vitronectin receptor and display unprecedented SAR. Their binding mode can be rationalized by computational docking studies using the X-ray structure of $\alpha_V\beta_3$.

The vitronectin receptor $\alpha_V\beta_3$ is a member of the integrin superfamily of cellular adhesion receptors. Small molecule non-peptide antagonists of this receptor are of continued interest for the oral treatment of restenosis, cancer and osteoporosis.[1]



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In an earlier communication, we described potent vitronectin receptor antagonists based on a biphenyl moiety. Designed from Kessler's peptide *c*-RGDfV, the discovery of biphenyls such as **1** ($K_i = 30$ nM) has illustrated the versatility of the biphenyl fragment as a beta turn mimetic.[2]

Here, we wish to report a novel class of biphenylalanine ureas with high binding affinity to $\alpha_V\beta_3$. In contrast to **1**, the compounds result from an alternative distribution of functional groups around the common (*S*)-phenylalanine core and display unprecedented SAR.

Chemistry

In order to fully exploit the medicinal chemistry of biphenylalanine derivatives we synthesized a targeted combinatorial library of 500 members using *p*-bromo phenylalanine (*p*-Br-F) as a central building block (Scheme 1).[3] We immobilized *p*-Br-F on solid support via the acidic group, anticipating this moiety to be a suitable mimic of aspartic acid, a known critical element of the RGD pharmacophore. Immobilised *p*-Br-F (**I**) was generated by coupling the fMOC-protected amino acid to Wang resin followed by capping (2,6-dichlorobenzoyl chloride, pyridine, DMF) and deprotection (20% piperidine, DMF, rt). It has been demonstrated earlier, that substituents in direct neighborhood to the carboxylic group have significant impact on the potency of β_3-integrin antagonists.[4] Therefore, substituents attached to *p*-Br-F's amino group were varied broadly,

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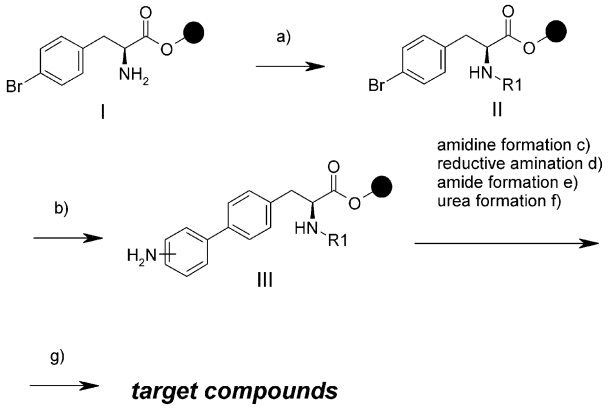


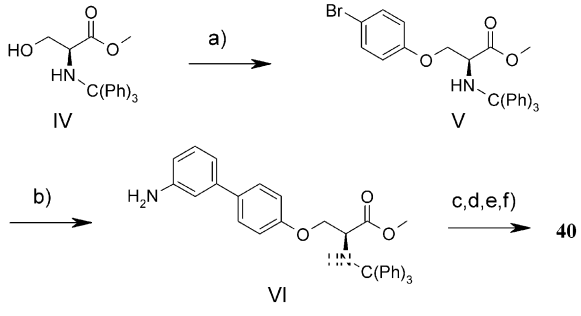
Scheme 1. Combinatorial synthesis of a biphenyl vitronectin receptor antagonist library: (a) diisopropyl ethylamine/THF 1 + 10, sulfonyl chloride or chloroformate reagent; for amide couplings: DIC, DMF, rt, 3 h; (b) (1) 3-nitro-benzene boronic acid, $Pd(PPh_3)_2Cl_2$, PPh_3, xylene/H_2O, Na_2CO_3, 85 °C; (2) $SnCl_2$ dihydrate, *N*-methyl pyrrolidone; (c) guanidinium group formation: MeS-C = N(BOC)-NH(BOC), $HgCl_2$, DMF, or: (1) diisopropyl ethylamine/THF 1 + 10, thiophosgene, then: ethylene diamine, DMF; (d) R-CHO, $HC(OMe)_3$, Bu_4NBH_4; (e) carboxylic acid, DIC, DMF, rt, 3 h; (f) diisopropyl ethylamine 4-nitrophenylchloroformate, THF/dichloromethane (1:1), then: R-NH_2, diisopropyl ethylamine, DMF; (g) TFA/dichloromethane (1:1); (● = Wang resin).

using amide, urea, urethane and sulfonamide coupling chemistry. The bromo atom of *p*-Br-F was then exploited for Suzuki coupling reactions with amino-substituted phenylboronic acids that, in turn were used for amide and urea formations.[3]

The serine ether **40** was prepared in solution from *N*-tritylated serine methyl ester following Scheme 2. The orthogonally protected intermediate VI allowed for late stage introduction of amino group modifications as well as for the synthesis of a variety of arginine mimics.[5,6]

Results

A first hit compound from the library was the ethoxy urethane **2**, with clear submicromolar binding affinity to the vitronectin receptor. Whereas the introduction of larger acyclic substituents (**3**,**4**) led to decreased activity,

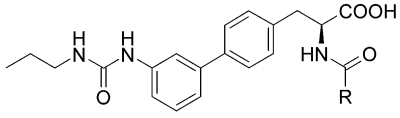


Scheme 2. Synthesis of biphenyl ether vitronectin receptor antagonist **40**: (a) 4-bromophenol, PPh_3, DEAD, toluene, rt, 15 h, 70%; (b) $AsPh_3$, $Pd(Ph_3P)_2Cl_2$, 3-amino phenyl boronic acid, Cs_2CO_3 (3 eq.), DME, rt, 3 h (40%); (c) *n*-propyl isocyanate, DABCO, toluene, 14 h rt (70%); (d) MeOH, HCl, dioxane, rt, 2 h, 30%; (e) mesitylsulfonyl chloride, dichloromethane, diisopropyl ethylamine, rt, 1 day, 40%; (f) NaOH (1 M), *i*-PrOH, 60 °C, 4 h, 50%.

cyclic substituents such as cyclopentyl (**5**), and phenyl urethanes (**6**) showed comparable potency. All amides prepared in the library (e.g., **8**, further data not shown) were inactive and ureas (**9**,**10**) were also considerably less potent, indicating the importance of the urethane oxygen for binding affinity in this series.

Whereas urethanes were the most potent derivatives in the series of Table 1, none of the derivatives showed activities below 100 nM. Only the replacement of the urethane moiety in **2** by sulfonamides led to a first example of double-digit nanomolar affinity (**11**) (Table 2). The substituted aryl sulfonamide **12** was almost equally effective and exhibited clear SAR that led to the identification of single digit nanomolar derivatives. Omitting the *para*-Cl substituent in **12** led to loss of activity (**13**), however potency could be retained by *ortho*-methyl substitution (**15**). The lower activity of its isomer (**20**) indicates the importance of a direct linkage between the aromatic ring and the sulfonamide moiety. The introduction of the 2,4,6-trimethylphenyl moiety greatly enhanced the affinity of our leads (**21**).[7]

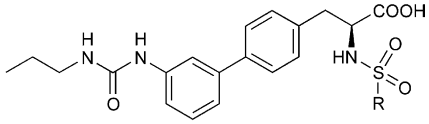
Table 1. Structure–activity relationship of urethane, amide and urea biphenyl vitronectin receptor antagonists



Compd	R	K_i (nM)
2	*O*-Et	120
3	*O*-Neopentyl	800
4	*O*-Isobutyl	900
5	*O*-Cyclopentyl	210
6	*O*-CH_2–C_6H_5	800
7	*O*-(4-MeO)–C_6H_4	300
8	4-MeO–C_6H_4	> 10,000
9	HN–CH_2–C_6H_5	850
10	NH_2	560

K_i values are means of three dose–response curves.

Table 2. Exploration of the sulfonamide moiety of biphenyl vitronectin receptor antagonists



Compd	R	K_i (nM)
11	Me	75
12	2-CF_3-4-Cl–C_6H_3	100
13	2-CF_3–C_6H_4	> 10,000
14	2-Me–SO_2–C_6H_4	> 10,000
15	2-Me–C_6H_4	70
16	2,5-Dimethoxy-C_6H_3	500
17	3,4-Dimethoxy-C_6H_3	750
18	2-Methoxy-5-Cl–C_6H_3	1700
19	2,3-Cl_2–C_6H_3	170
20	CH_2–C_6H_5	300
21	2,4,6-Trimethyl-C_6H_2	4
22	(*S*)-Camphor-10-yl	1

K_i values are means of three dose–response curves.

Although a camphor derivative (**22**)[8] showed even higher potency, **21** was selected for further SAR studies. The camphor residue was expected to give rise to structurally complicated metabolites in vivo and was not investigated further.

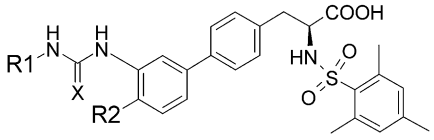
The SAR of the urea moiety was also investigated (Table 3). The simple ammonia derivative **23** showed weaker activity than the corresponding guanidine derivative **24**. We expected this result, as the latter is structurally close to the arginine side chain of the RGD motif. However, it turned out, that the introduction of small aliphatic substituents greatly improved activity of the urea class with an optimum for *n*-propyl and *cyclo*-propyl ureas (**21**, **26**). Larger aliphatic substituents (**30**, **31**) as well as fluorinated derivatives (**32**, **33**, **34**) showed decreased activity. A methyl group was introduced adjacent to the urea fragment in order to investigate possible conformational effects of this moiety. However, as the direct comparison of **26** and **27** demonstrates, potency was only marginally affected, indicating little consequences for the active conformation.

Ureas, arising from secondary aliphatic amines were generally inactive (data not shown), indicating the importance of two hydrogen bond donors in this moiety. Surprisingly, the introduction of 2-benzimidazolyl ureas, which proved beneficial in **1** and its congeners,[2] did not result in binding affinity at all (**38**).

It has been repeatedly suggested that the correct spatial arrangement, in particular the distance between the carboxylic acid and the arginine group of the RGD motif is critical for β_3 integrin receptor affinity.[9,10] In an effort to investigate the effect of shortening this distance, the *meta*/*meta* isomer of **21** has been prepared (**39**). This material was inactive, indicating the difference to **1** and its active *meta*/*meta* derivatives.

In contrast, the introduction of an additional ether atom (**40**), resulting in a prolonged RGD motif, did not affect activity in comparison to **21**, indicating the flexibility of the vitronectin receptor pharmacophore (Table 4).

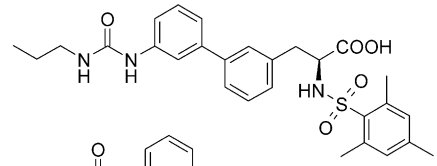
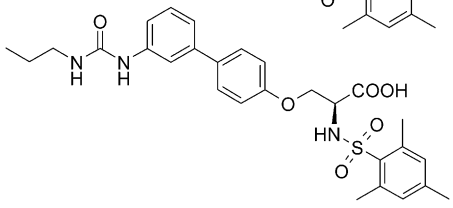
Table 3. Structure–activity relationship of substituted biphenyl ureas



Compd	X	R1	R2	K_i(nM)
23	O	H	H	80
24	NH	H	H	11
25	O	Me	H	160
26	O	*c*-Pr	H	2.5
27	O	*c*-Pr	Me	10
28	O	*i*-Pr	H	14
29	O	*i*-Bu	H	10
30	O	2-Bu	H	74
31	O	*c*-Pent	Me	100
32	O	$F_3C–CH_2$	H	20
33	O	$F_3CS–CH_2–CH_2$	H	8
34	O	$F_3C–CF_2–CH_2$	H	80
35	O	$C_6H_5–CH_2$	H	60
36	O	*o*-Pyridyl–CH_2	H	> 10,000
37	O	4- F_3C-C_6H_4–CH_2	H	1000
38	O	2-Benzimidazolyl	H	> 10,000

K_i values are means of three dose–response curves.

Table 4. The effect of chain-length variation for biphenyl vitronectin receptor antagonists.

Compd	Structure	K_i (nM)
39		> 10,000
40		4

K_i values are means of three dose–response curves

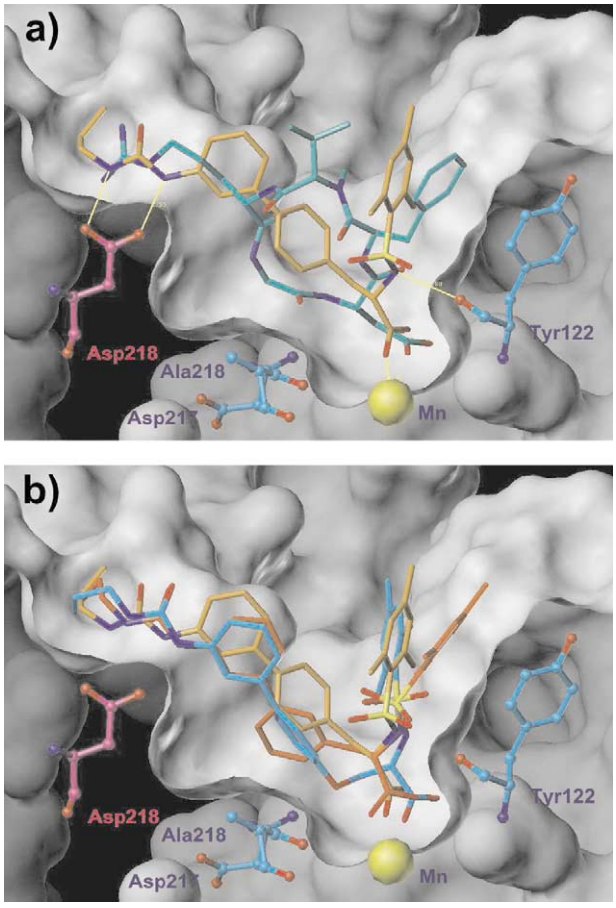


Figure 1. Surface representation of the vitronectin receptor ligand-binding site, with ligands shown as stick models. The Mn^{2+} ion of MIDAS is indicated as a yellow sphere. Only receptor amino acids involved in ligand binding interactions are shown (α_V: magenta, β_3: cyan): (a) cyclic Arg-Gly-Asp-DPhe-(*N*-methyl)-Val (green) and **21** (orange), yellow lines indicate hydrogen bonds between the receptor and the ligands; (b) **21** (orange), **39** (red), and **40** (cyan).

We used computational docking experiments to gain further insight into the SAR trends of **21**, **39** and **40.** After alignment with the recently described FAME routine,[11] the compounds were docked into the X-ray structure of the vitronectin receptor bound to the ligand *c*-RGDf(N-Me)V[12,13] (Fig. 1a). The study suggests a binding mode of **21** similar to that of the cyclic pentapeptide. The carboxylic acid moiety would interact with MIDAS in the β_3 chain whereas both urea NH groups form hydrogen bonds with Asp218 in the α_V chain. Further contacts include a hydrophobic biphenyl/Ala218 interaction as well as a hydrogen bond from the sulfonamide NH to Tyr122. Additionally, the 2,4,6-trimethylphenyl group interacts with Tyr122, presumably via π-stacking.

Figure 1b shows the superposition of **39** and **40** in the ligand-binding site of the receptor. Surprisingly, **39**'s urea and carboxylic acid moiety could well interact with the receptor. The reduced distance between both motifs was apparently not the reason for **39**'s inability to bind to $\alpha_V\beta_3$. The model suggests however that one of **39**'s *meta*-substituted phenyl rings would clash sterically with Ala218 from the beta 3 subunit.[14] In addition, the binding event would entail a reduction of the biphenyl torsion angle from 44° (**21**) and 57° (**40**) towards a more periplanar, higher energy conformation (27°). In contrast, the more flexible **40** can easily adopt the correct binding conformation, avoiding any steric contact with Ala218.

In conclusion, we have identified a novel, independent class of biphenyl vitronectin receptor antagonists with single digit nanomolar affinities from a combinatorial library. Additionally, we demonstrated the usefulness of docking simulations to the vitronectin receptor structure in rationalising SAR trends.

Acknowledgements

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13. The X-ray structure of the receptor in complex with the cyclic peptide was taken from the Protein Data Bank (PDB: 1L5G). The CONCORD 4.0.6 module in SYBYL 6.8 generated the 3-D structures of **21**, **39** and **40**. The alignment with the cyclic peptide was generated using the FAME program.[11] After removal of the peptide co-ordinates, these structures were docked to the vitronectin receptor-binding pocket. The resulting complexes were then energy minimized using the MMFF94s force field (ε = 1r) until criteria were reached (potential gradient RMS <0.05 kcal/(mol Å). The co-ordinates of the Mn^{2+} ions, the ion chelating amino acids as well as all distant residues (5 Å) were kept constant. The docking procedure was performed using SYBYL 6.8 (Tripos Inc., MO, USA).
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