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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6151–6154
Bioorganic & Medicinal Chemistry Letters

Biphenyls as potent vitronectin receptor antagonists. Part 3: Squaric acid amides

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Received 7 August 2007; revised 6 September 2007; accepted 7 September 2007
Available online 14 September 2007

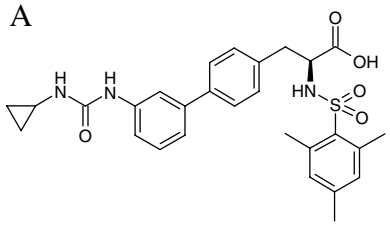
Abstract—Vitronectin receptor ($\alpha_V\beta_3$) antagonists have been implicated as a possible new treatment of restenosis following balloon angioplasty. In this work we investigate a series of novel arginine mimetic scaffolds leading to new insight of the $\alpha_V\beta_3$/ligand interaction. Squaric acid amide **10** is a subnanomolar $\alpha_V\beta_3$ antagonist with improved potency on human smooth muscle cell migration.

The integrin receptor $\alpha_V\beta_3$ has been implicated in a variety of vascular-mediated disorders such as restenosis after percutaneous transluminal coronary angioplasty, in-stent restenosis, or transplant coronary vasculopathy.[1] This receptor binds to proteins and peptides containing a characteristic continuous tripeptide epitope, also referred to as the arginine–glycine–aspartic acid (RGD) motif. The recent discovery of non-peptide RGD mimetics as potent and selective $\alpha_V\beta_3$ inhibitors represents a hallmark in the understanding of vitronectin receptor pharmacology and function.

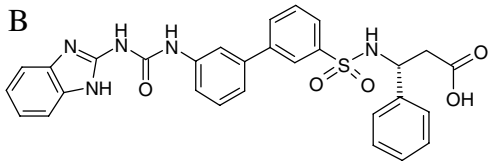
In preceding communications, we have described two novel classes of biphenyl vitronectin receptor antagonists exemplified by the α-amino acid **A** and the β-amino acid **B** with potencies of 2.5 and 4 nM, respectively.[2,3]

Despite their obvious structural differences, both materials share a sulfonamide and a biphenyl moiety as a common structural feature. Likewise, both compounds exhibit urea fragments as uncharged arginine mimetics. In an effort to broaden and compare the structure–activity relationship of the **A** series, we synthesized a variety of arginine bioisosters and investigated their binding affinity to $\alpha_V\beta_3$.

A



B



Keywords: Vitronectin; Urea; Arginine; Guanidine mimetic; Squaric acid amide; $\alpha_V\beta_3$; RGD; Restenosis; Biphenyl; Smooth muscle cell.
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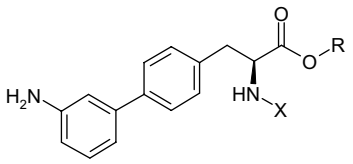
doi:10.1016/j.bmcl.2007.09.039



Ia: X= SO_2-2,4,6-trimethylphenyl, R=Me
Ib: X= SO_2-2,4,6-trimethylphenyl, R=*Wang* resin
Ic: X= SO_2-methyl, R=Me
Id: X= SO_2-(*S*)-camphoryl, R=Me

Ia —a), b)→ **1-4**
Ia —c), b)→ **5, 6**
Ib —d),e)→ **7**
Ib —f), g), e)→ **8**
Ia —i), j), b)→ **9**
Ia —k), l), b)→ **10-12**
Ic —k), l), b)→ **13**
Id —k), l), b)→ **14**

Scheme 1. Synthesis of biphenyl vitronectin receptor antagonists: (a) carboxylic acid, di-isopropyl carbodiimide, DMF, rt; (b) LiOH, dimethoxyethane, water (1 + 1), rt (40–90%); (c) 5H,10H-dipyrrolo[1,2-*a*:1,2-*d*]pyrazin-5,10-dione, pyridine (for **5**) 5H,10H-diimidazo[1,2-*a*:1,2-*d*]pyrazin-5,10-dione (for **6**), pyridine (4 equiv), THF/DMF (5:1), rt, 72 h, 70%; (d) $HgCl_2$, 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, DMF, 12 h, rt; (e) TFA/CH_2Cl_2 1 h, rt; (f) thiophosgene, THF, rt, 2 h; (g) ethylenediamine, DMF, 70 °C, 12 h; (i) 3 equiv 3,4-bis(methylthio)-1,2,5-thiadiazole-1-oxide,[5] *n*-propanol, 20 h reflux (40%), then: 10 equiv cyclopropylamine, *n*-propanol, 2 h, 50 °C (90%); (j) 10 equiv primary amine, ethanol reflux; (k) 5 equiv 3,4-diethoxy-3-cyclobutene-1,2-dione, *n*-propanol, 20 h reflux; (l) 10 equiv primary amine, *n*-propanol reflux.

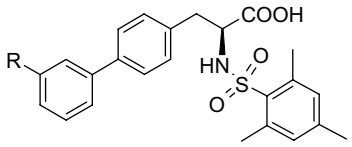
All new compounds were synthesized using the central intermediates **Ia–d** (Scheme 1).[4] The thiadiazole oxide **9** was prepared using the corresponding bis(methylthio)-substituted heterocycle as a building block.[5]

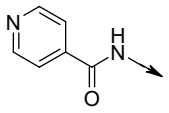
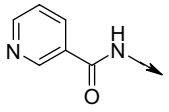
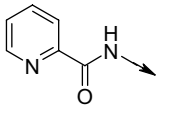
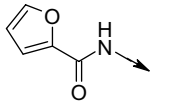
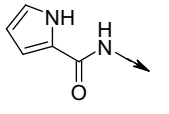
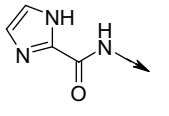
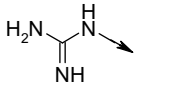
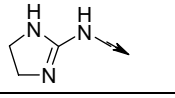
Table 1 summarizes SAR trends observed in the class of heterocyclic amides derived from biphenyl **A**. The pyridine and furan amides **1–4** were only weak inhibitors, suggesting the importance of **A**'s distal hydrogen bond donor for optimal interaction with $\alpha_V\beta_3$.

Consistently, the pyrrole and imidazole amides **5** and **6** display higher binding affinities, suggesting a direct involvement of their heterocyclic NH fragments with $\alpha_V\beta_3$. In contrast to these two examples from the **A**-series, the corresponding pyrrole and imidazole amides of the **B**-series showed much weaker potency probably indicating the need for an additional, aromatic ring to attain activity within their structural context.[2]

Compound **7** has been described in an earlier communication.[4] Its guanidinium group might be regarded as an 'ideal' atom-to-atom mimetic of the arginine side chain in the natural RGD motif. The heterocyclic amide **6** exhibited 10-fold higher affinity to $\alpha_V\beta_3$ than **7**. Apparently, $\alpha_V\beta_3$ is tolerating the enlarged distance between the two NH groups within **6**. In an effort to take advantage of this observation, we synthesized the thiadiazole **9**[5] and the squaric acid amide **10**[7] assuming that such moieties would display similar geometries.

Table 1. Structure–activity relationship of amide and guanidinium-substituted biphenyl vitronectin receptor antagonists. K_i values are medians of 3 dose–response curves



Compound	R	K_i (nM)
1		700
2		400
3		>2000
4		650
5		33
6		1
7		11
8		1.3

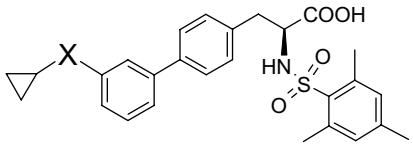
The affinity improvement for **8**, after incorporation of an ethylene bridge into **7**'s guanidine structure, has been observed in other series as well.[6] Results indicated in Table 2 show that, whereas the thiadiazole modification retained activity comparable to **7**, the squaric acid amide **10** showed clear subnanomolar potency.

A systematic investigation of a set of squaric acid amides displayed clear SAR within the series (Table 3).

The introduction of larger, benzylic substituents into the R1 position reduced affinity into the double-digit nanomolar range (**11** and **12**). Similarly, replacing the trimethylphenyl moiety with a simple methyl group reduces affinity (**13**). Consistent with observations made earlier, camphoryl residues enhance potency (**14**).[3]

The affinities of the urea counterparts of **10**, **12**, **13**, and **14** have been described earlier.[3] When we compared the

Table 2. Discovery of squaric acid amides. K_i values are medians of 3 dose–response curves



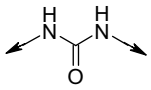
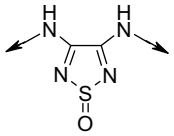
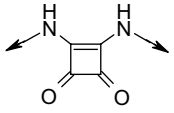
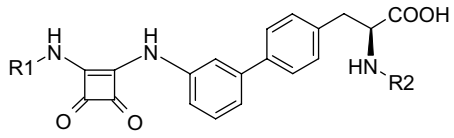
Compound	X	K_i (nM)
A		2.5
9		18
10		0.5

Table 3. Structure–activity relationship of squaric acid amides as vitronectin receptor antagonists. K_i values are medians of 3 dose–response curves



Compound	R1	R2	K_i (nM)
11	2-Pyridyl–CH_2	SO_2–2,4,6-trimethylphenyl	11
12	Ph–CH_2	SO_2–2,4,6-trimethylphenyl	25
13	*c*-Propyl	SO_2–CH_3	10
14	*c*-Propyl	SO_2–(S)-camphoryl	0.1

four corresponding urea/squaric acid amide pairs, we found that the latter are preferred in all cases by roughly half an order of magnitude (**10**: 2.5 nM vs 0.5 nM, **12**: 60 nM vs 25 nM, **13**: 75 nM vs 10 nM, and **14**: 1 nM/0.1 nM).

In our previous report we have shown that docking our compounds into the X-ray structure of $\alpha_V\beta_3$ can assist in rationalizing SAR trends (Fig. 1a).[3] Assuming that D218 and the Ca^{2+} ion are main interaction partners for our molecules, we docked derivatives **A** and **10** into $\alpha_V\beta_3$ and realized that **10**'s cyclopropyl ring would even better fit into the hydrophobic cleft defined by D150, F177, Q180, and T212 of the α_V side chain, possibly explaining its higher potency (Fig. 1b).

A direct comparison of several in vitro parameters of **10** and its urea congener **A** is shown in Table 4. The selectivity to GPIIbIIIa appears to be similar, if not better for the squaric acid derivative (**A**: 51-fold, **10**: 94-fold). The improved binding affinity to isolated $\alpha_V\beta_3$ appar-

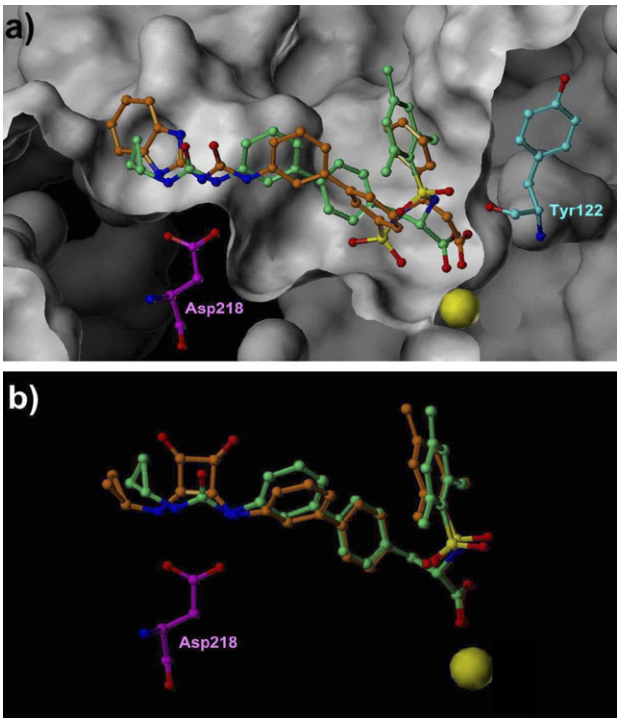
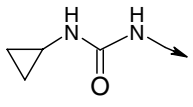
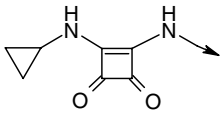


Figure 1. (a) Surface representation of the vitronectin receptor ligand-binding site, with compounds **A** (green) and **B**[2] (orange) shown as ball-and-stick models. The Ca^{2+} ion in the MIDAS is indicated as a yellow sphere. Only receptor amino acids involved in ligand binding are shown (α_V: magenta, β_3: cyan). (b) Comparison of the docked structures of urea **A** (green) and squaric acid **10** (orange).

Table 4. Comparison of in vitro parameters for urea **A** and squaric acid amide **10**



A



10

K_i ($\alpha_V\beta_3$)	2.5 nM	0.5 nM
K_i (GPIIbIIIa)[2]	128 nM	47 nM
HEK 293[8]	85 nM	13 nM
SMC[2]	390 nM	10 nM
K_d (HSA)[9]	740 nM	420 nM
Membrane affinity[10]	2410-fold	24,000-fold

ently translates into improved cellular adhesive antagonism with $\alpha_V\beta_3$-transfected HEK 293 cells.[8] Interestingly, **10** also shows improved activity in terms of smooth muscle cell (SMC) migration inhibition. The improvement in this functional response is, to our view, however too large (39-fold) to be explained solely by improved $\alpha_V\beta_3$ affinity (5-fold). We also determined the membrane affinities and realized that **10** is about 10-fold more lipophilic than **A**.[9,10] While other contributing factors such as the interaction with other α_V-integrins cannot be excluded, we hypothesize that the combination of improved receptor affinity and higher lipophilicity accounts for the highly potent SMC migration inhibition of **10**.

When tested for degradation by rat liver microsomes, **10** showed a clearance of 3.9 L/h kg (extrapolated from in vitro) characterizing this molecule as a high clearance compound.[11] Further, guanidine mimetics are therefore needed to identify biphenyl vitronectin antagonists allowing for investigations in vivo.

In summary, we have discovered several novel arginine mimetics, useful for the development of $\alpha_V\beta_3$ inhibitors. To the best of our knowledge, the structural elements of **9** and **10** have not been described in the context of $\alpha_V\beta_3$ inhibition hitherto.[12] In particular the squaric acid amide series showed picomolar binding affinity that translated well into improved functional activity on SMCs.

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