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Structure–activity relationship of a series of non peptidic RGD integrin antagonists targeting $\alpha_5\beta_1$: Part 2

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ABSTRACT

Potent antagonists of the integrin $\alpha_5\beta_1$, which are RGD mimetics built from tyrosine are described. This paper describes the optimization of in vitro potency obtained by variation of two parts of the molecule, the central aromatic core and the amide moiety.

Integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are members of a complex family of heterodimeric glycoprotein receptors that regulate a number of cellular processes such as cell adhesion, migration and survival. They interact with a family of matrix proteins including fibronectin, vitronectin and fibrinogen, through specific motifs known as RGD motifs. A key role is the regulation of angiogenesis, through complex processes that are not yet fully clarified.[1] Several integrins have been associated with cancer, amongst which $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are the most studied in clinic at present, with a diversity of inhibitors including small peptides (Cilengitide and ATN161) and antibodies (Abegrin and Volociximab).[2–5]

RGD integrins such as $\alpha_5\beta_1$ are challenging to inhibit, due to the protein–protein nature of their interaction with their ligands. Peptide–peptide interactions are notoriously challenging to mimic with non peptidic small molecules and the first attempts to inhibit $\alpha_5\beta_1$ resulted in relatively large molecules of MW >600 g/mol.[6,7] An additional complexity is that the RGD sequence to mimic contains both an acidic and a basic function, which restrained most of the research effort on zwitterionic approaches.[8] We reported in a previous communication our effort to develop a series of orally available $\alpha_5\beta_1$ inhibitors from a tyrosine based zwitterionic scaffold.[9] During optimization of the linker between the core and the basic group, as well as looking at the best basic moieties to optimize their interactions with the α_5 subunit, we also worked on optimizing the amide moiety and assessed a diversity of central cores. Our SAR findings on the core and the amide are the topic of this letter.

With the exception of compound **23**, the synthesis of compounds **1–34** was described previously and is summarized on Scheme 1.[10] To study the influence of the amide, the basic chain was introduced first and a peptidic coupling allowed the synthesis of compounds **1–17** in a parallel synthesis fashion. On the contrary, when the core was diversified, each synthesis was linear. The basic chains were introduced either by Mitsunobu for O-linked chains or by Heck coupling followed by hydrogenation for C-linked chains. Benzofuran **23** was made from an iodo tyrosine derivative used for the synthesis of compound **24**. The iodo phenol reacted with an acetylenic derivative to form the benzofuran moiety in a palladium catalyzed annulation reaction.[11]

Amide variation: Literature on $\alpha_4\beta_1$ inhibitors reported that ortho substituted phenyl amides with halogen or alkyl groups gave optimal potency. In particular 2,6 di-ortho substituted phenyls enabled an ideal orientation of the aromatic moiety inside the hydrophobic pocket of the β_1 subunit.[12] Since the amide group is modeled to be positioned in the β_1 unit, it was logical to assume that the knowledge of $\alpha_4\beta_1$ would transfer well to $\alpha_5\beta_1$. Indeed, ortho monosubstitution with a chloride in compounds **2** and **3** or small alkyls in compounds **6–8** provided an average 10-fold improvement of both binding and cell adhesion potencies

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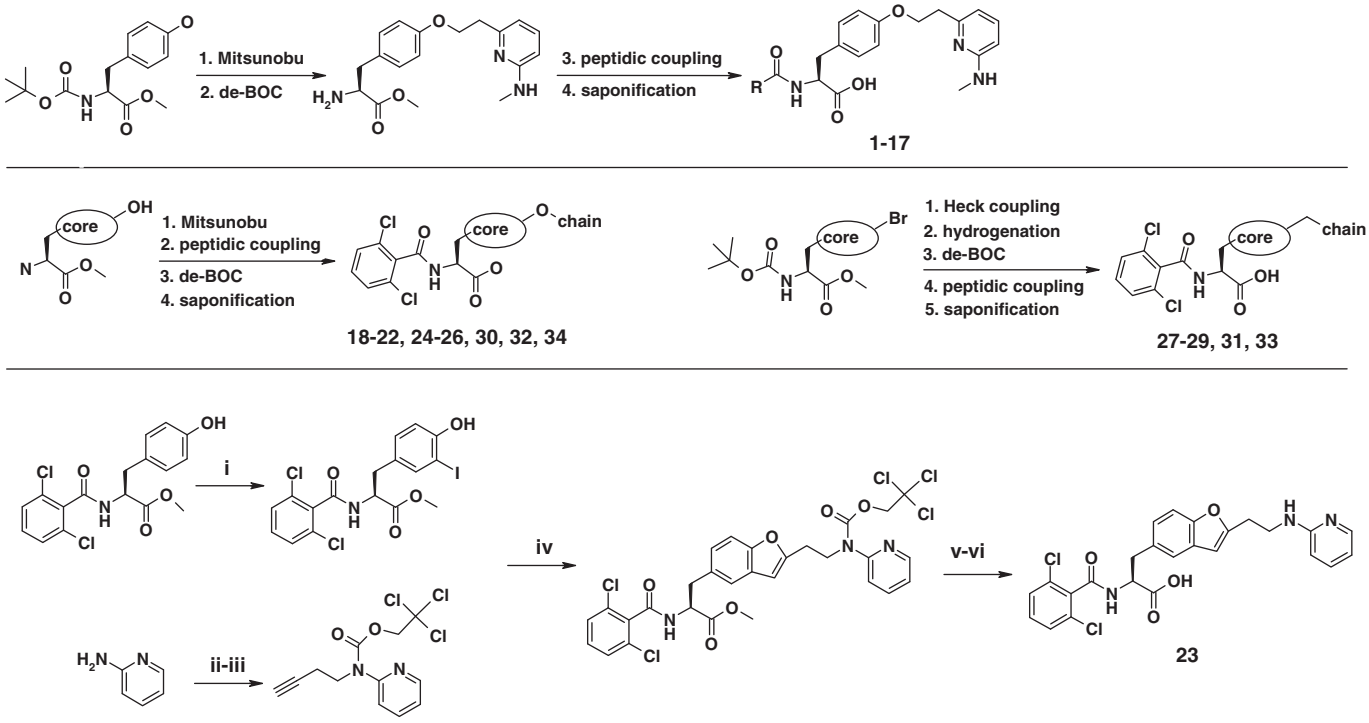
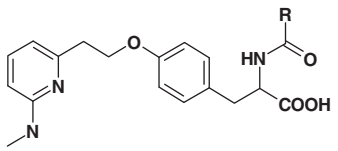


Scheme 1. General synthesis of compounds **1**–**34**. Reagents and conditions: (i) benzyltrimethylammonium dichloroiodate, DMF, 0–20 °C, 40%; (ii) 2,2,2-trichloroethylchloroformate, NEt_3, CH_2Cl_2, 0–20 °C, 29%; (iii) propargyl alcohol, di-*t*-butylazodicarboxylate, PPh_3, THF, 20 °C, 74%; (iv) NEt_3, cat. CuI, cat. $PdCl_2(PPh_3)_2$, DMF, 65 °C, 23%; (v) Zn, H_2O/THF/AcOH, 20 °C, 61%; (vi) LiOH, H_2O/DMF, 20 °C, 51%.

Table 1
Amide optimization on compounds **1**–**17**



Compound	R	Binding IC_{50}, μM[a]	K562 cell adhesion no HSA IC_{50}, μM[a]	K562 cell adhesion 630 μM HSA IC_{50}, μM[a]	% Free human	% Free rat
1	Ph	0.049	0.43	—	—	—
2	2-Cl Ph	0.003	0.042	>26	0.4	0.5
3	2-Cl, 4-F Ph	0.002	0.033	11[b]	0.2	0.3
4	2-Cl, 6-F Ph	<0.001	0.019	1.5	0.6	0.5
5	2-OMe, 4-F Ph	0.13	0.82	>100[b]	—	—
6	2-Me, 4-F Ph	0.009	0.036	24[b]	—	0.5
7	2-Et, 4-F Ph	0.004	0.024	5.0[b]	—	0.4
8	2-cPr, 4-F Ph	0.003	0.039	7.7[b]	—	0.4
9	2,6-Di Me Ph	0.001	<0.023	0.42[b]	—	2.2
10	2,4,6-Tri Me Ph	0.001	0.022	1.5[b]	—	0.5
11	2,6-Di Cl Ph	<0.001	0.007	0.17[b]	1.0	1.1
12	3,5-Di Cl 4-pyridyl	<0.001	<0.004	0.055[b]	2.4	3.3
13	3-Cl 2-thiophenyl	0.012	0.052	36[b]	0.1	0.2
14	3,5 Di-Me 4-isoxazolyl	0.001	<0.008	1.1	—	3.8
15	2-Me cyclohexyl	0.007	0.071	29[b]	—	0.9
16	1-Me cyclohexyl	0.005	0.039	7.5[b]	0.25	0.9
17	1-MeO 4-tetrahydropyranyl	0.004	0.050	0.48[b]	—	21.2

[a] In all tables, unless stated otherwise, numbers are a geometric mean of 2 or more values.
[b] $n = 1$ value.

compared to the unsubstituted phenyl amide **1** (Table 1). However, an ortho-OMe did not provide a similar advantage (**5** vs **1**). Since X-ray structures in the Cambridge Crystallography Structural Database show that both ortho-Cl and ortho-OMe phenyl amides maintain the ortho substituent on the same side than the NH of the amide and in the same plane, the potency difference between Cl and OMe is presumably due to an electronic effect rather than a conformational effect. Moreover, the difference of potency between the ethyl **7** or the cyclopropyl **8** and the methoxy **6** suggested an electronic effect rather than a steric effect. Further potency was gained with di-ortho substitution, which is known to force the aryl ring to twist by approximately 80°. Such

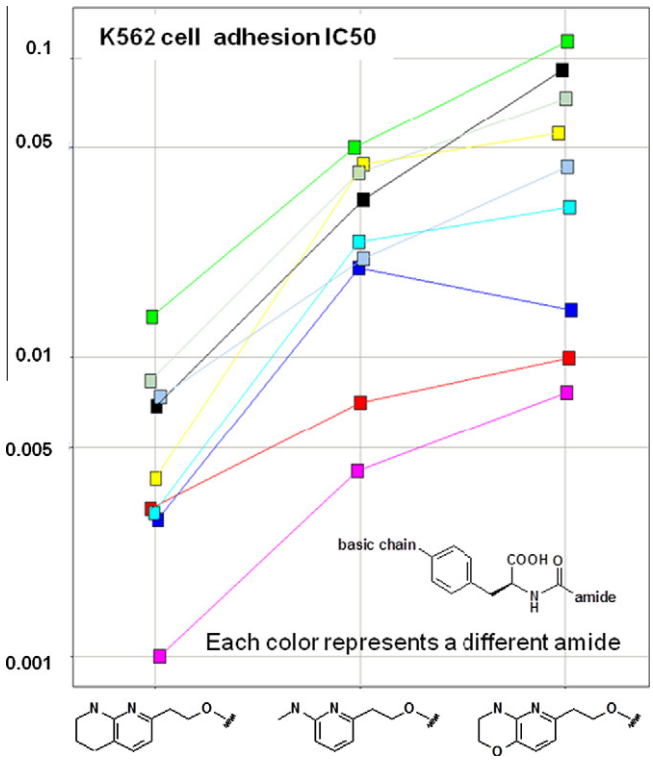


Figure 1. Transposition of amide SAR for different basic chains.

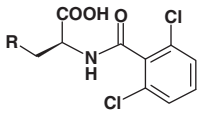
compounds (**4**, **9**, **10**, **11**) consistently reached a threefold improvement in cell potency in the adhesion assay without human serum albumin (HSA) compared to monosubstituted analogs (for instance **11** vs **2**). This gain of potency was even more noticeable in the cell adhesion assay with HSA, because di ortho substituted compounds were both more potent and less bound to albumin than their mono ortho substituted analogs (see **11** vs **2**).[13] *Para* substitution had little influence on potency (see for instance **3** vs **2**) but could offer interesting PK modulations as will be described in a forthcoming paper. Heteroaryl amides were also explored. Again, ortho substitution with chloride or small alkyl groups was found beneficial on both 5- and 6-membered heteroaryls (compounds **12–14**), with some of these examples being among our most potent compounds in cell. Cycloalkyls were also well tolerated, in particular those with steric hindrance close to the amide carbonyl (2-substitution in **15**; 1-substitution in **16** and **17**).

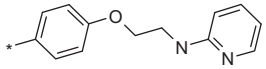
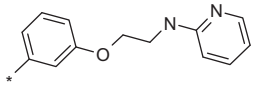
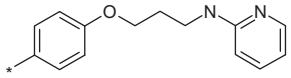
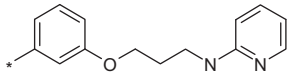
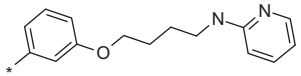
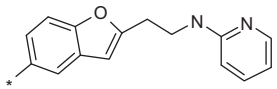
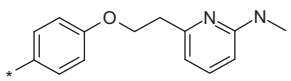
The amide SAR transferred when the basic chain on the left hand side of the molecules was varied. This is represented by a plot of matched pairs with 3 different basic chains and 9 different amides (Fig. 1).

Core variation: Due to the flexible linker and based on the homology model allowing some flexibility, one could have expected to maintain reasonable activity when moving from the para-tyrosine to the meta-tyrosine core. However, comparison of compounds **19** with **18** and **21** with **20** clearly illustrated a significant drop off in potency with a *meta* linker (Table 2). Compound **22** with a longer linker was also poorly active. The benzofurane **23**, a cyclized version of compound **20**, was more potent than the *meta*-tyrosine examples, but still 35-fold less potent than compound **20** in cells.

Substitution on the phenyl ring was tolerated and guided by the homology model which suggested that there was limited room to add substituents. Mono Me or F examples **24** and **25** remained potent, although showing a 5- to 10-fold drop off in cell potency compared to compound **11**. Di-Me substitution was less tolerated (**26** vs **11**).

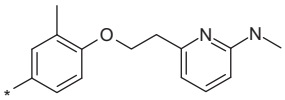
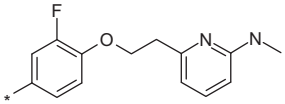
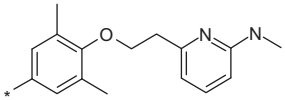
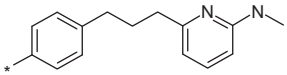
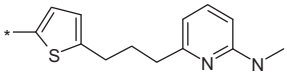
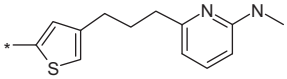
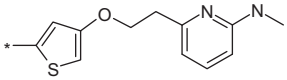
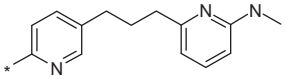
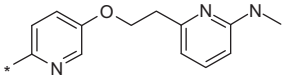
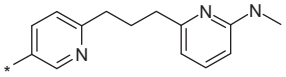
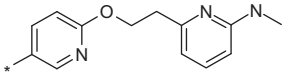
Table 2
Core optimization on compounds **18–33**



Compound	R	Binding IC_{50}, μM[a]	K562 cell adhesion IC_{50}, μM[a]
18		0.082	2.5
19		5.9	>290[b]
20		0.011	0.12
21		–	20[b]
22		1.9	83[b]
23		0.89	4.0
11		<0.001	0.007

(*continued on next page*)

Table 2 (*continued*)

Compound	R	Binding IC_{50}, μM[a]	K562 cell adhesion IC_{50}, μM[a]
24		0.003	0.081
25		0.002	0.047
26		0.021	0.61
27		0.008	0.046
28		0.002	0.031
29		0.18	0.40
30		0.11	0.51[b]
31		0.009	0.11
32		0.001	0.026
33		0.005	0.076
34		0.006	0.11

[a] In all tables, unless stated otherwise, numbers are a geometric mean of 2 or more values.
[b] $n = 1$ value.

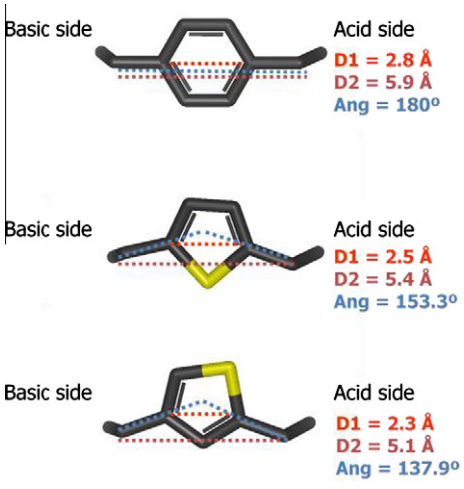


Figure 2. Geometric parameters of phenyl and thiophenes central cores.

Replacement of the phenyl core with a thiophene core was explored with the two possible thiophene isomers. From examination of the geometries, it was anticipated that the 2,5-thiophene would better position the basic chain than the 2,4-thiophene (Fig. 2). And indeed, compound **28** was much more potent than compound **29**. **28** was also slightly more potent than the corresponding phenyl analog **27**. Moreover, the benefit in potency which was observed with a phenyl core when moving from a carbon linker to an oxygen linker was not observed for 2,4 thiophene (**30** vs **29**). The thiophene core was key to improve the DMPK parameters in this series.

Eventually, azatyrosines were explored. With a carbon linker, the two isomers of azatyrosine **31** and **33** were within twofold equipotent with the tyrosine analog **27**. A larger drop off in potency was observed for an oxygen linked pyridines versus the phenyl analog **11**, with a preference for isomer **32** compared to **34**.

To assess the selectivity of our compounds against $\alpha_v\beta_3$, we developed cell adhesion assays that could differentiate selective from dual inhibitors.[14] The A375 cell adhesion assay with fibrinogen is reflecting binding mediated through $\alpha_v\beta_3$. Our series showed some inhibition in this assay but the level of potency was at least 10-fold lower than the Merck MK-0429 $\alpha_v\beta_3$ inhibitor.[15] The

Table 3
RGD integrin selectivity data[a]

Compound	A375 cell adhesion fibrinogen IC_{50}, μM[b]	A375 cell adhesion fibronectin IC_{50}, μM[b] without/with $\alpha_v\beta_3$ inhibitor (ratio)[c]
1	6.7	7.4/5.4 (1.4)
2	0.52	0.54/0.24 (2.2)
3	0.44	0.44/0.21 (2.1)
4	0.28	0.28/0.16 (1.7)
5	8.3	8.3/6.5 (1.3)
6	0.51	0.51/0.47 (1.1)
7	0.34	0.34/0.20 (1.7)
8	0.52	0.51/0.25 (2.0)
9	0.40	0.40/0.090 (4.5)
10	0.37	0.38/0.057 (6.7)
11	0.23	0.23/0.097 (2.3)
12	0.13	0.13/0.052 (2.6)
13	2.9	2.9/2.7 (1.1)
14	0.2	0.20/0.13 (1.5)
15	0.96	0.96/0.42 (2.3)
16	0.83	0.83/0.48 (1.8)
17	0.71	0.71/0.44 (1.6)
20	2.6	2.5/0.61 (4.2)
24	0.60	0.64/0.18 (3.6)
25	0.29	0.33/0.076 (4.3)
26	8.8	8.8/5.8 (1.5)
28	0.79	0.80/0.18 (4.4)
32	0.62	0.51/0.084 (6.1)
34	1.2	1.2/0.57 (2.1)
MK-0429[d]	0.0003	0.81/2.2 (0.4)

[a] All numbers are a geometric mean of 2 or more values.
[b] Assay performed in presence of Mg^{2+}.
[c] The Merck $\alpha_v\beta_3$ MK-0429 was used at 1 μM concentration.
[d] The Merck compound showed an IC_{50} of 4.6 μM in our K562 cell adhesion assay.

activity of our series for both $\alpha_5\beta_1$ and $\alpha_v\beta_3$ was confirmed using the A375 cell adhesion assay in presence of fibronectin without and with an $\alpha_v\beta_3$ inhibitor. Results in Table 3 illustrated that our series showed modest ratios (1.1–6.7) suggesting dual $\alpha_5\beta_1/\alpha_v\beta_3$ inhibition.

In conclusion, we have developed potent inhibitors of $\alpha_5\beta_1$ which also carry some activity against $\alpha_v\beta_3$. The in vivo properties of this zwitterionic series will be described in a forthcoming paper.

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14. To determine the ability of our compounds to inhibit $\alpha_v\beta_3$, a fibrinogen adhesion assay A375M cells was employed. A375M melanoma cells express a range of integrins including $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, however in the presence of the physiological cation Mg^{2+} binding to fibrinogen is mediated by $\alpha_v\beta_3$ integrin. This assay was validated with an α_v integrin blocking antibody (L230, active) and an $\alpha_5\beta_1$ integrin blocking antibody (M200, inactive).
A dual fibronectin adhesion assay was used to determine compound selectivity for $\alpha_5\beta_1$ over $\alpha_v\beta_3$. In the presence of Mg^{2+}, cell adhesion to fibronectin is mediated by both α_v and $\alpha_5\beta_1$ integrins. Inhibition of one integrin alone should not affect adhesion to fibronectin, as the other integrin can compensate. To eliminate adhesion via $\alpha_v\beta_3$ integrin, these assays were performed in the presence or absence of an $\alpha_v\beta_3$ inhibitor. The larger the shift in IC_{50} of cell adhesion to fibronectin from the absence to the presence of the $\alpha_v\beta_3$ inhibitor, the more $\alpha_5\beta_1$ selective the compound is.
15. The Merck compound MK-0429 demonstrated Proof of Concept in a phase II clinical trial against osteoporosis.