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SAR of *N*-phenyl piperidine based oral integrin α5β1 antagonists

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ABSTRACT

Recently, a new class of selective integrin α5β1inhibitors consisting of a heterocyclic based scaffold was published. Herein the SAR and pharmacokinetic profiles of *N*-phenyl piperidine derivatives are described.

Integrins are a family of transmembrane adhesion receptors that mediate cell contact to neighboring cells and proteins of the extracellular matrix.[1] They have been described to be involved in several pathological conditions including inflammatory diseases, autoimmune diseases, tumor progression and metastasis by regulating leukocyte trafficking, cell proliferation, migration, angiogenesis and lymphangiogenesis.[2–5] The therapeutic efficacy of integrin antagonists like cilengitide (αvβ3) and natalizumab (α4β1) has been shown for the treatment of glioblastoma and multiple sclerosis, respectively.[6,7]

Knockout studies of the fibronectin receptor α5β1 integrin and tumor angiogenesis studies with α5β1 integrin inhibitors have revealed a role in blood vessel formation for this integrin.[8,9] Furthermore, systemic treatment with an α5β1 integrin small molecule antagonist or an α5β1 integrin antibody reduced tumor growth in proof-of-concept studies for different tumor types.[10,11] Integrin α5β1 antagonists are therefore promising drug candidates for the therapy of diseases that involve angiogenic and proliferative processes.

Recently, the discovery of integrin α5β1 antagonists with low nanomolar affinities based on heterocyclic core structures (**2**) derived from a 3-hydroxypyrrolidine scaffold like in **1**[12] was reported (Fig. 1).[13] Herein, the SAR and optimization of one of these new scaffolds with an *N*-phenyl piperidine core is disclosed.

Compounds **3a–i** were synthesized according to the convergent synthesis strategy shown in Schemes 1–3. The three piperdines **6a–c** were obtained from alkylation of intermediate **5**[13] and subsequent deprotection of the piperidine nitrogen atom (Scheme 1).

The boronic acid derivatives **8** and **10** were synthesized from the corresponding commercially available starting materials (Scheme 2).

The key building blocks **11a–c** and (S)-**11a** were obtained by a copper mediated coupling reaction[14] of **6a–c** with either the racemic boronic acid **8**[15] or the optically pure potassium trifluoro borate **10**. After liberation of the primary amines, amide formation with different benzoic acids and saponification of the methyl ester gave the desired integrin antagonists **3a–i**.

Previously, the first piperidine based integrin α5β1 antagonist **3b** was reported[13] and was shown to have excellent integrin α5β1 affinity (2.3 nM). Selectivity against integrin αvβ3 was medium, whereas selectivities against integrins αvβ5 and αIIbβ3 were excellent (Table 1). Compound **3b** showed high total body clearance (CL = 70 ml/min/kg) after iv administration in rat. Therefore selectivity against integrin αvβ3 and the pharmacokinetic profile should be improved.

As previous reported,[12] the electron accepting or donating nature of substituents in the 4-position of the 2-aminopyridine has a great influence on integrin binding (Table 1). As expected, the electron donating 4-OMe derivative **3c**, had the highest integrin α5β1 binding activity in the ELISA and the cellular binding assay. Otherwise, the 4-H and 4-Me derivatives **3a** and **3b** showed similar IC_{50} values in the ELISA, whereas the cellular IC_{50} value is of **3c** is significantly lower. However, as the cellular binding assay represents the more functional assay it was considered to be more relevant one.[12]

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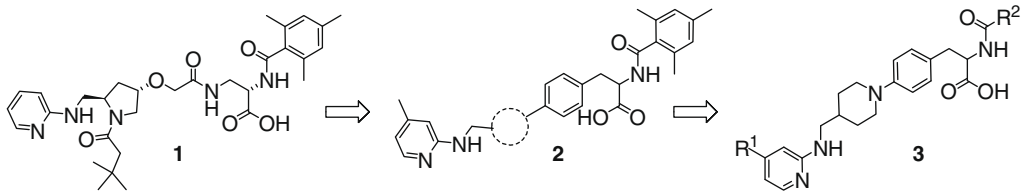
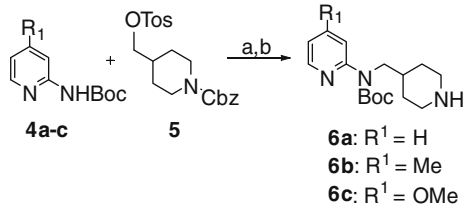


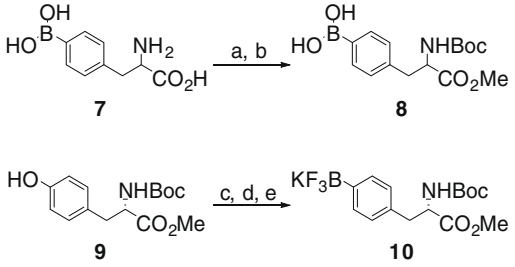
Figure 1. Generic structure **3** with an *N*-phenyl piperidine core was discovered during the optimization of the new integrin α5β1 antagonists type **2** which were based on the 3-hydroxypyrrolidine lead **1**. The dotted cycle in **2** represents heterocyclic five- or six-membered ring systems.

In contrast to the increasing integrin α5β1 binding affinity, an opposing trend was found for oral bioavailability and the in vitro permeability (Table 2). This observation can be rationalized by the different pK_a values caused by the increasing electron donating effect of the 4-H < 4-Me < 4-OMe substituents in **3a–c**. The different fractions of unprotonated base at physiological pH may explain the observed in vitro permeabilities as well as the oral bioavailabilities of **3a–c**.

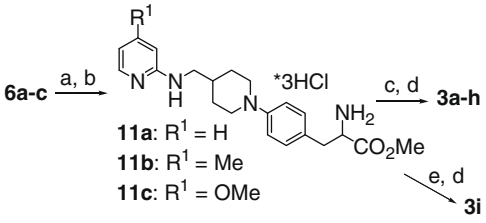
Furthermore, the same trend was found for the systemic clearance after iv administration and in the microsomal stability (MS rat). In favor of better pharmacokinetic properties, the unsubstituted 2-aminopyridine of derivative **3a** was therefore selected and kept fixed for further optimization.



Scheme 1. Reagents and conditions: (a) KHMDS, DMSO, 0 °C; (b) Pd/C, H_2, ethyl acetate, rt.

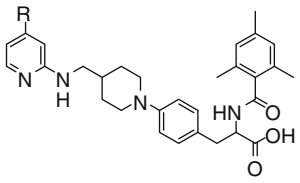


Scheme 2. Reagents and conditions: (a) HCl, MeOH, reflux; (b) Boc_2O, $NaHCO_3$, ^{t}BuOH; (c) Tf_2O, pyridine, DCM, −30 °C to rt; (d) $PdCl_2$(dppf), bis(pinacolato)diborane, KOAc, DMSO, 80 °C; (e) KHF_2, H_2O/MeOH, −5 °C.



Scheme 3. Reagents and conditions: (a) **8** or **10**, $Cu(OAc)_2$, DIPEA, DCE, 3 Å MS, 40 °C; (b) HCl, MeOH, reflux; (c) R^2CO_2H, HATU, DIPEA, DMF, rt; (d) LiOH, THF/H_2O, rt; (e) 2-(bromomethyl)benzoyl bromide, DIPEA, DCM, 0 °C to rt.

Table 1
IC_{50} values of **3a–c** for integrins α5β1, αvβ3, αvβ5, αIIbβ3 in competitive integrin binding assays



Compds	R	IC_{50}[a], nM				
		α5β1	αvβ3	αvβ5	αIIbβ3	cell
3a	H	2.1	642	15,000	40,200	198
3b	Me	2.3	84	10,700	23,400	69
3c	OMe	0.92	89	3540	34,400	34

[a] Values are means of three experiments.

It is well known that α-amino acid derived integrin α5β1 inhibitors can gain selectivity against αv-integrins by switching from the unsubstituted α-benzoylamide to mesitoylamide.[12,16–18]

As previously discussed, the selectivity for integrin αvβ3 for the *N*-phenyl piperidine scaffold had to be improved. For optimization the 2,6-dimethyl derivative **3d** was selected as starting point (Table 3). To increase selectivity a more bulky ethyl group was incorporated (**3e**). But both derivatives showed only medium stability to human microsomal degradation. To overcome this issue a 4-fluoro atom was introduced (**3f–g**), improving the microsomal stability. Specifically, the 2-ethyl-4-fluoro-6-methyl compound **3g** showed good integrin α5β1 affinities in both the ELISA (2.0 nM) and the cellular assay (76 nM) in combination with excellent selectivities and human microsomal stability.

Analysis of the various stereo isomers of **3g** (Table 4), indicates that stereo chemistry has a profound impact on the activity as well as the selectivity toward other integrins.

The (*S*)-enantiomer exhibited the highest binding activity and selectivity. The (*R*)-enantiomer was found to be less potent than the (*S*)-enantiomer or the racemic mixture and the relative selectivity toward other integrins dropped by two orders of magnitude.

As previously described, the 2,6-disubstituted benzamide moiety represents the key for selectivity of integrin α5β1 antagonists toward other integrins. To elucidate this, derivative **3i** with a plane isoindolin-1-one was synthesized and the integrin activities were compared with the more flexible benzamides **3h** and **3g** in the same position (Table 5).

The increasing activities for integrin α5β1 are associated with a decrease of integrin αvβ3 activities, which resulted in high integrin α5β1 selectivities. The calculated torsion angle[†] (Fig. 2) between the amide and phenyl planes correlates positively with these activities. On the other hand, no direct correlation of the integrin αIIbβ3

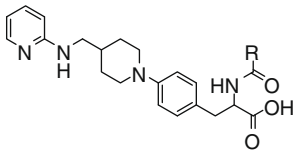
[†] For energy minimization the MM2 force field implemented in Chem3D Pro 10.0® (CambridgeSoft) was used.

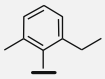
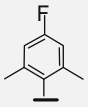
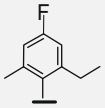
Table 2
In vitro and in vivo pharmacokinetic properties of **3a–c**

Compds	pK_a	P_{app}, 10^{-6} cm/s	Efflux ratio	CL[a], ml/min/kg	$t_{1/2}$[a], min	F[a], %	MS rat[b], %
3a	7.0	4.18	0.9	16	127	24	85
3b	7.5	1.42	2.0	70	66	16	77
3c	7.7	0.96	2.3	110	25	6	62

[a] The compounds were administered into two groups of male rats (4 animals in each group) at 1 mg/kg iv (group 1) and 10 mg/kg po (group 2). Eight blood samples were taken from each animal. The aliquots of the plasma samples were transferred, precipitated with methanol containing a structural analogue of the analyte as internal standard and analyzed by HPLC–MS/MS for any taken time point to determine time/concentration values. The pharmacokinetic parameters were calculated by using Winnonlin version 5.1.
[b] % Remaining after 1 h. The stability of the compounds at 1 μM in microsomal preparations was determined as described in Ref. 20. Microsomal preparations from different species were obtained from Tebu-bio, Offenbach, Germany.

Table 3
IC_{50} values of **3d–g** for integrins α5β1, αvβ3, αvβ5, αIIbβ3 in competitive integrin binding assays



Compds	R	IC_{50}[a] (nM)				hMS[b], %
		α5β1	αvβ3	αvβ5	αIIbβ3	
3d		1.6	239	1870	77,700	59
3e		2.2	648	11,500	87,200	52
3f		2.7	402	8290	105,000	69
3g		2.0	1050	11,700	71,700	105

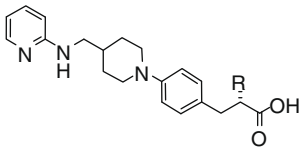
[a] Values are means of three experiments.
[b] % Remaining after 1 h, The stability of the compounds at 1 μM in microsomal preparations was determined as described in Ref. 20. Microsomal preparations from different species were obtained from Tebu-bio, Offenbach, Germany.

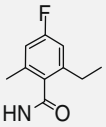
Table 4
IC_{50} values of the stereoisomers of **3g** for integrins α5β1, αvβ3, αvβ5, αIIbβ3 in competitive integrin binding assays

Compds	IC_{50}[a], nM				
	α5β1	αvβ3	αvβ5	αIIbβ3	Cell
3g	2.0	10,450	11,700	71,700	76
(*S*)-**3g**	0.90	614	12,400	121,000	21
(*R*)-**3g**	351	16,600	15,600	159,000	6912

[a] Values are means of three experiments.

Table 5
IC_{50} values of (*S*)-**3g** and **3h–i** for integrins α5β1, αvβ3, αvβ5, αIIbβ3 in competitive integrin binding assays



Compds	R	IC_{50}[a] (nM)				Torsion[b] (°)
		α5β1	αvβ3	αvβ5	αIIbβ3	
(*S*)-**3g**		0.90	614	12,400	121,000	57
3h		70	4.3	32.1	14,800	28
3i		234	13.4	3320	76,700	0

[a] Values are means of three experiments.
[b] Torsion angles between amid and phenyl plane (Fig. 2).

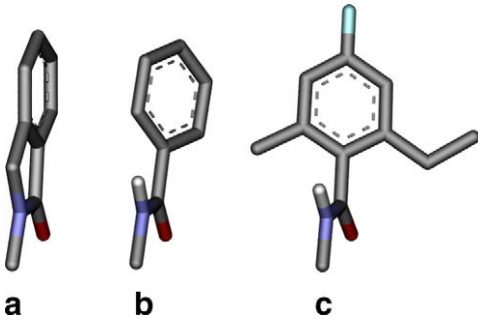


Figure 2. Torsion angles of *N*-methylamide and phenyl planes. (a) **3i** = 0°; (b) **3h** = 28°; (c) **3g** = 57°.

and αvβ5 binding and the torsion angles was found. It could be discussed that the missing NH-donor in **3i** is responsible for the low integrin α5β1 activity, but we assume that the twisted binding conformation of the 2,6-substituted benzamide of **3g** is directly correlated with the high activity on integrin α5β1 and the selectivity toward integrin αvβ3.

Compound (*S*)-**3g** was further characterized in male beagle dogs. Following an iv bolus injection of (*S*)-**3g** at 10 mg/kg, the systemic clearance was found to be 11 mL/min/kg, which corresponded to 37% of dog hepatic blood flow.[19] The mean value for elimination half-life ($t_{½}$) was 215 min while the bioavailability after oral administration of an aqueous solution was found to be 11%. The observed kinetics of (*S*)-**3g** represents a solid foundation for further investigations.

In summary, the SAR and pharmacokinetic properties of *N*-phenyl piperidine scaffolds as integrin antagonists are described. Modulating pharmacokinetic properties with the substitution of the 2-aminopyridine and selectivities by benzamide substitution pattern

led to compound (*S*)-**3g** with overall acceptable properties for further development.

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