Bioorganic & Medicinal Chemistry Letters 20 (2010) 380–382

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of orally available integrin α5β1 antagonists

Gunther Zischinsky, Frank Osterkamp, Doerte Vossmeyer, Grit Zahn, Dirk Scharn, Ariane Zwintscher, Roland Stragies *

Jerini AG, Invalidenstraße 130, Berlin D-10115, Germany

ARTICLE INFO

Article history:
Received 15 September 2009
Revised 15 October 2009
Accepted 16 October 2009
Available online 28 October 2009

Keywords:
Integrin α5β1
Antagonist
Oral bioavailability
Pharmacokinetic profile

ABSTRACT

Previous research within our laboratories identified the 3-hydroxypyrrolidine scaffold **1** as a new and selective integrin α5β1 inhibitor class which was designed for local administration. Herein the discovery of new orally available integrin α5β1 inhibitor scaffolds for potential systemic treatment is described.

Integrins are a family of multifunctional αβ heterodimeric transmembrane receptors that mediate cellular adhesion to adjacent cells and ECM proteins.[1] Integrins and their ligands are essential regulators of development, immune responses, leukocyte trafficking and homeostasis and have been shown to be involved in inflammatory, autoimmune or proliferative diseases.[1,2] In particular, an increasing number of integrins have been described to influence several aspects of tumor progression and metastasis, including cell survival and proliferation as well as angiogenesis and lymph-angiogenesis.[2–6] Specifically, integrins αvβ3, αvβ5, and α5β1 have been described to be involved in angiogenesis. However, in knockout studies, only the loss of integrin α5β1 could be directly correlated with defects in blood vessel development.[7] Additionally, integrin α5β1 has been shown to play a role in inflammation and fibrosis.[7–11] Collectively, these data have prompted interest for utilizing integrin α5β1 antagonists as potential drugs for the treatment of pathological angiogenic conditions such as tumors or age-related macular degeneration.[3,4,6]

Recently, a series of 3-hydroxypyrrolidine based integrin α5β1 antagonists (**1**) were reported to exhibit low-nanomolar affinity and excellent selectivity towards other integrin subtypes[12] (Table 1).

This inhibitor class was designed for ophthalmic indications thus resulting in low systemic exposure after elimination from the eye. While low systemic exposure is ideal for localized applications, effective treatment of systemic diseases such as cancer, inflammatory or fibrotic diseases will require integrin α5β1 antagonists having high systemic exposure and a convenient route of administration.

For the design of new selective integrin α5β1 antagonists, we aimed for reduction of molecular weight and rigidisation of compound **1** by elimination of the pyrrolidine amide residue and rigidisation of the hydroxyl acetamide part with a phenyl alanine

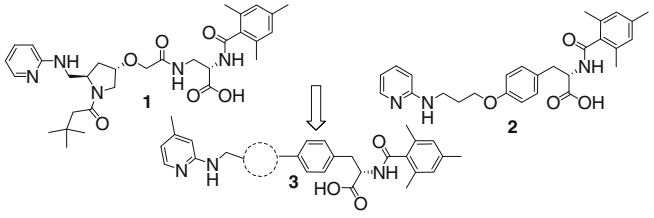


Figure 1. Combining structural features of compounds **1** and **2** affords of a new lead series represented by the generic structure **3**. The dashed circle in **3** represents aromatic and non-aromatic rings.

Table 1
IC_{50} values for inhibitors of integrins α5β1, αvβ3, αvβ5, αIIbβ3 in a competitive integrin binding assay[12]

Compds	IC_{50}[a] (nM)			
	α5β1	αvβ3	αvβ5	αIIbβ3
1	1.6	16,000	90,700	>50,000
2[13]	2.5	703	–	–

[a] Values are means of three experiments.

* Corresponding author. Correspondence should be addressed: Pericles Calias, Shire HGT, 125 Spring Street Lexington, MA 02421, USA. pcalias@shire.com. Tel.: +1 781 482 0701; fax: +1 781 482 2961.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.073

moiety like in **2** (Fig. 1).[13] As commonly accepted, the reduction of the number of rotatable bonds and molecular weight should result in molecules with improved pharmacokinetic profiles.[16]

All pharmacophoric key elements of **1** and **2** are still present in the newly designed compounds **3**. Therefore these bicyclic scaffolds should exhibit potentially high integrin binding affinity.

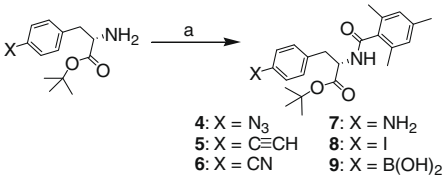
Different strategies were applied to construct the bicyclic ring system in compounds **3a**–**j**. Briefly, the phenylalanine derivatives **4**–**9** were prepared by the reaction of mesitoyl chloride with the corresponding, commercially available amino acid derivatives (Scheme 1). The resulting intermediates were then reacted in cyclo additions, carbon–carbon or nitrogen–carbon bond formation reactions, Schemes 2–4, respectively, to yield the bicyclic core motives **3a**–**j**.

Beginning with the amino acid derivatives **4**–**6**, the corresponding aldehydes **10**–**12** were obtained by cyclo addition reaction. The final compounds **3b**, **3c**, and **3e** were obtained by reductive amination and the removal of the protecting groups.

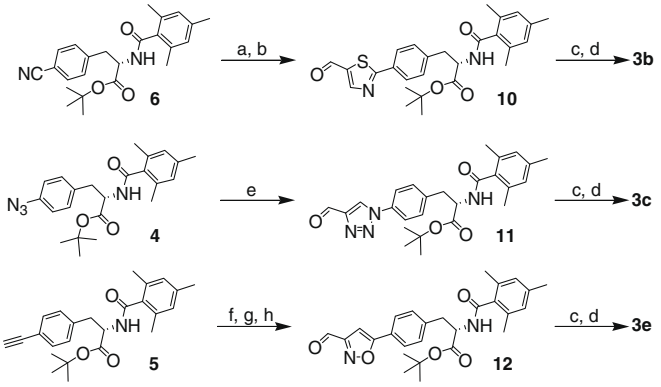
The synthesis of compounds **3d** and **3f**, utilized a Suzuki coupling[17] while the carbon–carbon bond formation in compound **3g** was achieved via diazonium salt formation.[18] The resulting aldehydes **13a**, **13b**, and **14** were transformed into the final compounds **3d**, **3f**, and **3g** by applying the reaction sequence used in the formation of **3b**, **3c**, and **3e**. In the preparation of compound **3a**, the precursor **16** was prepared via **15** from itaconic acid.[19] The introduction of the 4-methyl-pyridin-2-ylamine group was performed by alkylation followed by palladium catalyzed amidation[20] to give compound **3a** (Scheme 4).

For the synthesis of ***rac*-3h**, an Ulmann type reaction[21] was applied. The resulting biphenyl derivatives ***rac*-3i** and ***rac*-3j** were prepared by a Suzuki reaction of boronic acid ***rac*-9** and the corresponding aromatic bromides **23a** and **23b** (Scheme 4).

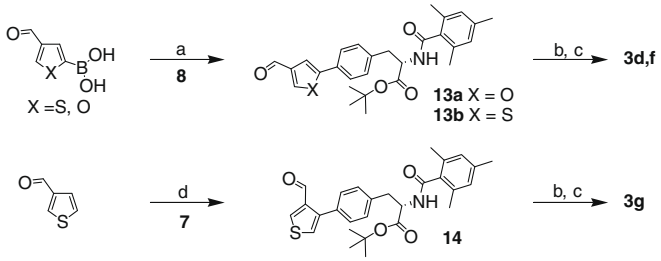
Pyrrolidinone derivative **3a** possess low-nanomolar affinity and more than two orders of magnitude higher selectivity towards integrin α5β1 as compared to the other selected integrins. In addition, the five-membered aromatic heterocycles in **3b**–**g** and the six-membered ring systems in ***rac*-3h**–**j** were shown to serve as suitable scaffolds in the current synthetic strategy (Table 2).



Scheme 1. Reagents and conditions: (a) mesitoyl chloride, DCM, NEt_3, 0 °C.



Scheme 2. Reagents and conditions: (a) H_2S, DIPEA, DMF, 50 °C; (b) 2-bromo-malonaldehyde, dioxane, 60 °C; (c) 4-methyl-pyridin-2-ylamine, $Ti(OiPr)_4$, $NaBH(OAc)_3$, DCE, rt; (d) TFA; (e) 3,3-diethoxy-propyne, $Cu(OAc)_2$, dioxane/H_2O, 10% HCl, rt; (f) chloro-hydroxyimino-acetic acid ethyl ester, NEt_3, DCM, 0 °C; (g) $NaBH_4$, LiCl, THF/EtOH, 0 °C; (h) Dess–Martin-periodinane, DCM, rt.



Scheme 3. Reagents and conditions: (a) **8**, $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O, 80 °C; (b) 4-methyl-pyridin-2-ylamine, $Ti(OiPr)_4$, $NaHB(OAc)_3$, DCE, rt; (c) TFA; (d) **7**, $NaNO_2$, HCl, H_2O, 0 °C, then thiophene-3-carbaldehyde, $CuCl_2$, dioxane, rt.

As discussed, integrin selectivity of the hydroxypyrrolidine based integrin α5β1 antagonists, that is, **1**, is mainly controlled by the amide of the 2-amino acid moiety.[12–15] Thus, the mesitoyl amide was used in all derivatives of **3** and, as predicted, resulted in excellent integrin selectivities of more than one order of magnitude.

All 1,3-substituted five-membered aromatic heterocycles showed good to excellent affinities for integrin α5β1, ranging from 1.5 nM (**3c**) to 79 nM (**3d**). However, the affinity of the 1,2-substituted derivative **3g** drops apparently due to the different geometric orientation of both substituents compared to the 1,3-substitution pattern.

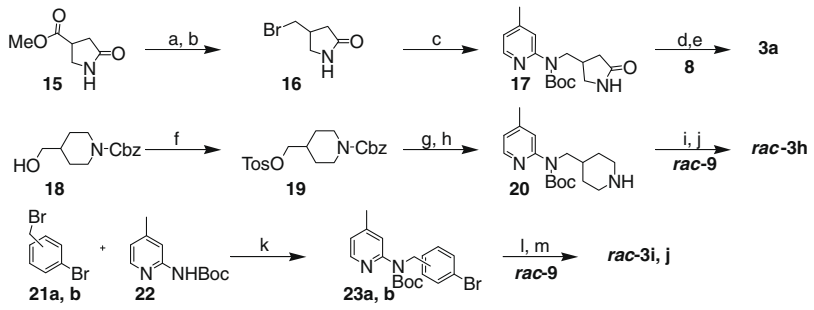
A comparison of the substitution vectors of planar five- and six-membered ring systems revealed that the 1′,3′-substitution of the five-membered ring orientated the 3′-vector between the 3- and 4-vectors of the 1,3- and 1,4-substituted six-membered ring (Fig. 2a). This orientation is supportive of the comparable affinities observed for the *meta*- and *para*-substituted biphenyl derivatives ***rac*-3i** and ***rac*-3j**.

However, the affinities of the five-membered ring derivatives **3a**, **3c**, and **3e** are significantly higher. Given that 1,3-substituted five-membered rings provide better scaffolds for integrin α5β1 antagonists than *meta*- or *para*-substituted six-membered rings, it is reasonable to assume the same three dimensional orientation of substituents can be achieved by means of a 1,4-substituted cyclohexane scaffold in the chair conformation (Fig. 2b). Indeed, the related piperidine based compound, ***rac*-3h**, proved to have high affinity for integrin α5β1 and therefore provides a new core structure.

Pharmacokinetic profile[†] of selected compounds show low systemic clearances (**3d** and **3e**) and long terminal half-lifes after iv administration in male Wistar rat (Table 3). Furthermore promising oral bioavailabilities were also observed for these compounds.

In summary, the discovery of a series of new heterocycle based integrin α5β1 antagonists is described. It was shown that the combination of 2-pyridyl amino methyl substituted heterocycles (compound **1**) with a phenylalanine moiety (compound **2**) results in a new series of potent integrin α5β1 scaffolds. In addition, it was shown that both aromatic and non-aromatic five- and six-mem-

[†] 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.



Scheme 4. Reagents and conditions: (a) $LiBH_4$, THF, rt; (b) CBr_4, PPh_3, ACN/DCM, 0 °C to rt; (c) 4-methyl-pyridin-2-yl-carbamic acid *tert*-butyl ester, NaH, DMF, 0 °C to rt; (d) **8**, $Pd(dba)_2CHCl_3$, xantphos, Cs_2CO_3, dioxane, 100 °C; (e) TFA; (f) Tos-Cl, NEt_3, DCM, 0 °C; (g) 4-methyl-pyridin-2-yl-carbamic acid *tert*-butyl ester, KHMDS, DMF, 0 °C to rt; (h) Pd/C H_2, MeOH; (i) ***rac*-9**, $Cu(OAc)_2$, NEt_3, DCM, rt; (j) concd HCl; (k) KHMDS, DMSO, 0 °C; (l) ***rac*-9**, $Pd(P^tBu_3)_2$, Cs_2CO_3, 1,4-dioxane, 100 °C; (m) TFA, DCM.

Table 2
IC_{50} values of derivatives of the generic structure **3** for integrins α5β1, αvβ3, αvβ5, αIIbβ3 in a competitive integrin binding assay[12]

Compd	Ring	IC_{50}[a] (nM)			
		α5β1	αvβ3	αvβ5	αIIbβ3
3a		1.6	319	1340	23k
3b		13	472	502	150k
3c		1.5	100	347	89k
3d		79	2423	2475	52k
3e		6.1	261	257	118k
3f		46	1734	3569	97k
3g		887	16k	17k	77k
***rac*-3h**		2.3	84	11k	23k
***rac*-3i**		65	2925	>20	165k
***rac*-3j**		87	890	>20k	180k

[a] Values are means of three experiments.

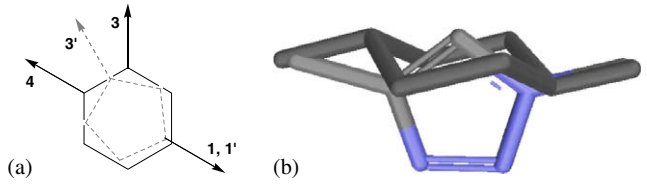


Figure 2. (a) Superimposing of 1,3-substituted five-membered ring (gray, dashed) with 1,3- and 1,4-substituted six-membered rings (black). (b) Superimposing of a 1,4-substituted triazole and a 1,4-substituted cyclohexane.

bered ring systems can be used to construct scaffolds with high affinity and selectivity as well as acceptable pharmacokinetic profiles.

Table 3
Pharmacokinetic data (male Wistar rat)

Compd	CL[a] (ml/min/kg)	$t_{1/2}$[a] (min)	F[b] (%)
3d	17	88	26
3e	9	138	11
***rac*-3h**	70	66	16

[a] iv, 1 mg/kg.
[b] po, 10 mg/kg.

Acknowledgments

We thank Pericles Calias and Brigitte Hoch for proofreading the manuscript and for their consent to handle any future correspondence. We also thank Edith Weigt, Corinna Mewes, Seike Gericke, Kerstin Mißbach, Frank Polster, Daniel Ohlendorf, and Thorsten Lanz for technical assistance.

References and notes

1. Hynes, R. O. *Cell* **1992**, *69*, 11.
2. Hynes, R. O. *Cell* **2002**, *110*, 673.
3. Dietrich, T.; Onderka, J.; Bock, F.; Kruse, F. E.; Vossmeyer, D.; Stragies, R.; Zahn, G.; Cursiefen, C. *Am. J. Pathol.* **2007**, *171*, 361.
4. Umeda, N.; Kachi, S.; Akiyama, H.; Zahn, G.; Vossmeyer, D.; Stragies, R.; Campochiaro, P. A. *Mol. Pharmacol.* **2006**, *696*, 1820.
5. Avraamides, C. J.; Garmy-Susini, B.; Varner, J. A. *Nat. Rev. Cancer* **2008**, *8*, 604.
6. Kim, S.; Bell, K.; Mousa, S. A.; Varner, J. A. *Am. J. Pathol.* **2000**, *1564*, 1345.
7. Yang, T. J.; Rayburn, H.; Hynes, R. O. *Development* **1993**, *119*, 1093.
8. Ishikawa, H.; Hirata, S.; Andoh, Y.; Kubo, H.; Nakagawa, N.; Nishibayashi, Y.; Mizuno, K. *Rheumatol. Int.* **1996**, *15*, 53.
9. Rinaldi, N.; Scharz-Eywill, M. *Z. Rheumatol.* **1999**, *58*, 333.
10. Thannickal, V. J.; Lee, D. Y.; White, E. S.; Zongbin, C.; Larios, J. M.; Chacon, R.; Horowitz, J. C.; Day, R. M.; Thomas, P. E. *J. Biol. Chem.* **2003**, *27814*, 12384.
11. Zhou, X.; Zhang, Y.; Zhang, J.; Zhu, H.; Zhou, X.; Du, W.; Zhang, X.; Chen, Q. *Chin. Med. J.* **2000**, *113*(3), 272.
12. Stragies, R.; Osterkamp, F.; Zischinsky, G.; Vossmeyer, D.; Kalkhof, H.; Reimer, U.; Zahn, G. *J. Med. Chem.* **2007**, *50*, 3786.
13. Heckmann, D.; Meyer, A.; Marinelli, L.; Zahn, G.; Stragies, R.; Kessler, H. *Angew. Chem.* **2007**, *119*, 3641.
14. Heckmann, D.; Meyer, A.; Laufer, B.; Zahn, G.; Stragies, R.; Kessler, H. *ChemBioChem* **2008**, *9*, 1.
15. Heckmann, D.; Laufer, B.; Marinelli, L.; Limongelli, V.; Novellino, E.; Zahn, G.; Stragies, R.; Kessler, H. *Angew. Chem.* **2009**, *121*, 4501.
16. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
17. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
18. Racane, L.; Tralic-Kulenovic, V.; Karminski-Zamola, G.; Fiser-Jakic, L. *Monatsh. Chem.* **1995**, *126*(12), 1375.
19. Felluga, F.; Pitacco, G.; Prodan, M.; Pricl, S.; Visintin, M.; Valentin, E. *Tetrahedron: Asymmetry* **2001**, *12*(23), 3241.
20. Hartwig, J. F.; Kawatsura, M.; Hauck, S. I.; Shaughnessy, K. H.; Alcazar-Roman, L. M. *J. Org. Chem.* **1999**, *64*, 5575.
21. Zhang, H.; Cai, Q.; Ma, D. *J. Org. Chem.* **2005**, *70*, 5164.