Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4553–4555
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and biochemical evaluation of novel $\alpha_v\beta_3$ integrin ligands

Juan Jose Marugan,* Kristin D. Haslow and Carl Crysler

3-Dimensional Pharmaceuticals, Inc, Eagleview Corporate Center, 665 Stockton Drive, Suite 104, Exton, PA 19341, USA

Received 9 April 2004; revised 8 June 2004; accepted 8 June 2004
Available online 3 July 2004

Abstract—Studies on integrin $\alpha_v\beta_3$ have implicated this receptor in a number of pathologies. In this article we describe some of our initial efforts to design small molecules $\alpha_v\beta_3$ ligands incorporating an indole core template and an oxyguanidine as basic ending. Synthesis, biochemical activity and pharmacological properties are analyzed.

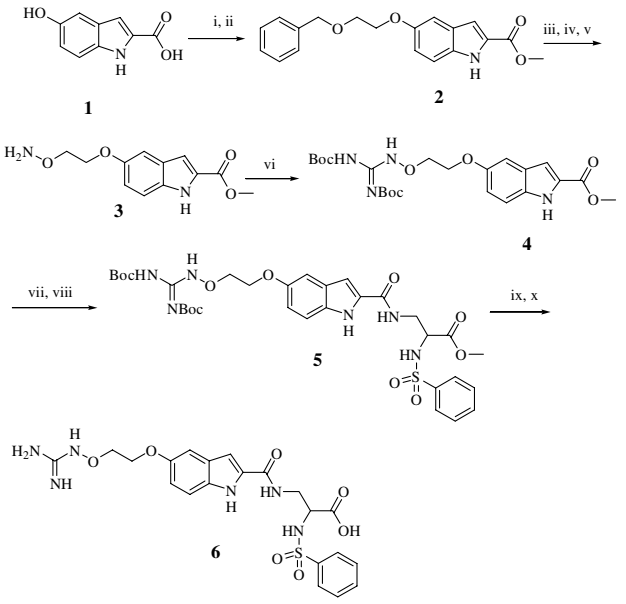
The integrins are a family of heterodimeric cell surface receptors that regulate the attachment of cells to extracellular matrix.[1] The dimerization of α and β glycoproteins subunits give rise to the heterodimeric receptors. Both integrin α and β subunits have a single large extracellular domain, one transmembrane region, and a short cytoplasmic tail without known enzymatic activity. The exception to this is the β4 subunit, which has an extended cytoplasmic domain containing four fibronectin (FN) type III-like domains. Integrins bind to a variety of extracellular ligands including other transmembrane proteins such as IgG-domain proteins in addition to the extracellular matrix (ECM). The binding of ligands can modulate a number of intracellular processes including activation of focal adhesion kinase (FAK), integrin-linked kinase (ILK), Abl kinase and reorganization of the actin cytoskeleton. The integrins also mediate inside-out signaling whereby cytoplasmic molecules such as protein kinase C can modulate the affinity of integrins for their ligands. Because of their ability to regulate cell mobility and cell–cell interaction there has been considerable work focused on modulating the activity of integrins over the last decade. Of particular interest to our research group was the vitronectin receptor $\alpha_v\beta_3$. The integrin has been implicated in osteoclast adhesion and in mobility of endothelial cells during vascular injury.[2] Thus, $\alpha_v\beta_3$ emerged as a potential therapeutic target for the treatment of osteoporosis, restenosis, ocular diseases, tumor-induced angiogenesis, metastasis formation, and sickle cell anemia disease.[2,3] A number of research groups have reported small molecule inhibitors of $\alpha_v\beta_3$[4] receptor. In this paper we describe our initial efforts in the design and synthesis of novel, nonpeptidic $\alpha_v\beta_3$ receptor antagonists. The selectivity of compounds toward $\alpha_5\beta_1$, $\alpha_1\beta_5$, and GP II_bIII_a was also evaluated.

Most of the potent $\alpha_v\beta_3$ inhibitors reported in the literature incorporate a RGD mimetic motif: (i) a basic group at the *N*-terminus, (ii) a carboxylic acid in the carboxy terminus, (iii) and a molecular core which can organize the molecule in the proper spatial geometry.

Although this RGD motif is essential for activity,[5] the zwitterionic nature of the pharmacaphore often becomes the 'Achilles' Heel' of the inhibitor. The highly charged compounds usually have high clearance rates and/or poor oral bioavailability. One potential way to address this problem is to choose a basic group with a relatively low pK_a (7–8). The oxyguanidine functional group ($pK_a = 7–7.5$) was chosen as a possible replacement for the more basic guanidine moiety as the means to a more oral bioavailable drugs with improved pharmacokinetic properties.[6] A second possible strategy to potentate the overall charge of the molecule is incorporate α-substituted amino acids at the acidic end.[7] The α-substituent, usually a sulfonamide or a carbamate, has been shown to increase the potency while modulating the acidity of these inhibitors. Based on these results, we designed and

Keywords: Integrin; $\alpha_v\beta_3$.
* Corresponding author. Tel.: +1-610-458-5264; fax: +1-610-458-8249; e-mail: jmaruga1@prdus.jnj.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.017



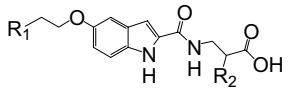
Scheme 1. Reagents and conditions: (i) (trimethylsilyl)diazomethane, MeOH, 98%; (ii) 2-benzyloxyethanol, DEAD, triphenylphosphine, THF, 99%; (iii) 10% Pd(C), MeOH, H_2, 100%; (iv) *N*-hydroxyphthalamide DEAD, triphenylphosphine, THF, 45%; (v) methylamine, THF, 96%; (vi) bis-Boc-pyrazole, THF, 97%; (vii) LiOH, MeOH–H_2O, 95%; (viii) 3-amino-2-benzenesulfonylamino-propionic acid methyl ester, DIEA, EDCI, 78%; (ix) LiOH, MeOH–H_2O, 97%; (x) TFA, CH_2Cl_2, 93%.

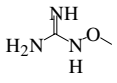
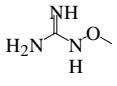
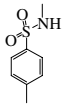
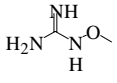
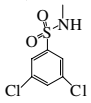
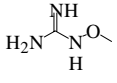
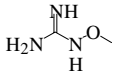
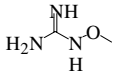
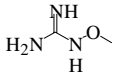
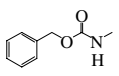
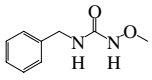
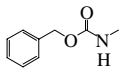
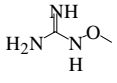
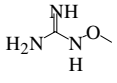
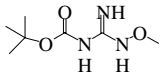
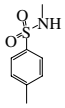
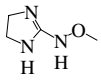
synthesized molecules bearing both an oxyguanidine at the basic ending and α-substitution at the carboxy terminus.

We looked at the 5-oxy-2-indole carboxylic amide as a suitable template for the synthesis of $\alpha_v\beta_3$ ligands and the synthesis of these molecules is shown in Scheme 1. The commercially available 5-hydroxy-2-indole carboxylic acid **1** was transformed to the corresponding methyl ester using (trimethylsilyl)diazomethane in MeOH. The methyl ester was coupled with 2-benzyloxyethanol via a Mitsunobu reaction to yield compound **2**. Deprotection of the benzylic alcohol of **2** was achieved by hydrogenation, followed by a Mitsunobu coupling with *N*-hydroxyphthalamide and subsequent deprotection with methylamine to give *O*-amine **3**. The oxyguanidine functional group was introduced by the reaction of compound **3** with bis-Boc-pyrazole to give **4**. This was hydrolyzed using lithium hydroxide and the free carboxylic acid was coupled with the previously synthesized 3-amino-2-benzenesulfonylamino-propionic acid methyl ester,[7b] using diisopropylethylamine as base and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as coupling reagent to give indole **5**. Cleavage of the ester, using lithium hydroxide as a base and deprotection of the Boc group with trifluoroacetic acid yielded the target compound **6**.

A series of modifications to the two termini was carried out as a means of generating a rapid SAR evaluation. The results from these modifications are listed in Table 1.

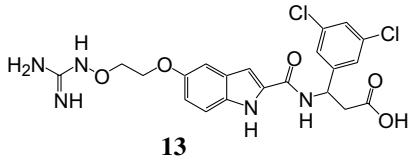
Table 1. Inhibition of $\alpha_v\beta_3$-vitronectin interaction



Compounds	R_1	R_2	$\alpha_v\beta_3$ IC_{50}[a] (nM)
1			3.4
2			9.0
3			6.1
4			10.5
5			0.4
6		NH_2	>20,000
7			7.2
8			>20,000
9			310
10			>20,000
11			130
12			100

[a] In vitro inhibition, as measured by ELISA, of $\alpha_v\beta_3$-vitronectin protein interactions.

Substitution α to the carboxy terminus was found to be essential to maintain activity (entry **6**). Aliphatic (entries **4** and **5**) or aromatic (entries **1**–**3**) sulfonamides or carbamates (entry **7**) gave the most potent inhibitors. Amide or benzyl substitution alpha to the acid resulted in a loss of all activity (entries **9** and **10**). With regard to substitution at the basic end, the free *O*-guanidinium provides better activity (entries **1** vs **12**). Mono-Boc protection and imidazolyl *O*-guanidinium analogues



13

Figure 1. Antagonist with substitution β to the carboxy terminus.

Table 2. Selectivity profile toward other integrins

	IC_{50} (nM)[a]			
Compounds	$\alpha_v\beta_3$	$\alpha_5\beta_1$	$\alpha_v\beta_5$	$\alpha_{IIb}\beta_{IIIa}$
1	3.4	ND	1000	13
3	6.1	>1000	ND	25
12	100	>20,000	>20,000	170

[a] In vitro inhibition, as measured by ELISA, of $\alpha_v\beta_3$-vitronectin, $\alpha_v\beta_5$-vitronectin, $\alpha_5\beta_1$-fibronectin, and $\alpha_{IIb}\beta_{IIIa}$-fibrinogen protein interactions.

retain some of the activity (entries **11** and **12**), but introduction of other functional groups such as the benzyl urea (entry **8**) results in the complete loss of activity.

Although substitutions alpha to the carboxy terminus are well tolerated, substitution β to the carboxy terminus had a negative effect on activity (compound **13**, $\alpha_v\beta_3$ IC_{50} >20 μM) (Fig. 1).

Several compounds were tested for their selectivity against other integrins, shown in Table 2. The potencies for the $\alpha_v\beta_3$ receptor are in the nM range, but the selectivity for $\alpha_{IIb}\beta_{IIIa}$ is less than 10.

Permeability and pharmacokinetic proprieties of this series was also determined. None of the compounds showed good oral bioavailability or desirable PK parameters. We attributed these negative results to the high number of heteroatoms contained in the molecules. In following papers, we will present another indole series, which address and resolve this problem.

Acknowledgements

We would like to thanks to Jen Kirpatrick for her help in the determination of some of the IC_{50} in Table 2, to Dr. Kevin Moriarty and Dr. Bruce Tomczuk for their help in the edition of this manuscript.

References and notes

1. Hynes, R. O. *Cell* **1992**, *69*, 11–25.
2. (a) Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157; (b) Engleman, V. W.; Nickols, G. A.; Ross, F. P.; Horton, M. A.; Griggs, D. W.; Settle, S. L.; Ruminski, P. G.; Teitelbaum, S. L. *J. Clin. Invest.* **1997**, *99*, 2284–2292.
3. (a) Noiri, E.; Gailit, J.; Sheth, D.; Magazine, H.; Gurrath, M.; Müller, G.; Kessler, H.; Goligorsky, M. S. *Kidney Int.* **1994**, *46*, 1050–1058; (b) Friedlander, M.; Theesfeld, C. L.; Sugita, M.; Fruttiger, M.; Thomas, M. A.; Chang, S.; Cheresh, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9764; (c) Yun, Z.; Menter, D. G.; Nicolson, G. L. *Cancer Res.* **1996**, *56*, 3103–3111; (d) Kaul, D. K.; Tsai, H. M.; Liu, X. D.; Nakada, M. T.; Nagel, R. L.; Coller, B. S. *Blood* **2000**, *95*, 368–374.
4. (a) Coleman, P. J.; Askew, B. C.; Hutchinson, J. H.; Whitman, D. B.; Perkins, J. J.; Hartman, G. D.; Rodan, G. A.; Leu, C.; Prueksaritanont, T.; Fernandez-Metzler, C.; Merkle, K. M.; Lynch, R.; Lynch, J. J.; Rodan, S. B.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2463–2465; (b) Smallheer, J. M.; Weigelt, C. A.; Woerner, F. J.; Wells, J. S.; Daneker, W. F.; Mousa, S. A.; Wexler, R. R.; Jadhav, P. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 383–387; (c) Zechel, C.; Backfisch, G.; Delzer, J.; Geneste, H.; Graef, C.; Hornberger, W.; Kling, A.; Lange, U. E. W.; Lauterbach, A.; Seitz, W.; Subkowski, T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 165–169; (d) Lange, U. E. W.; Backfisch, G.; Delzer, J.; Geneste, H.; Graef, C.; Hornberger, W.; Kling, A.; Lauterbach, A.; Subkowski, T.; Zechel, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1379–1382; (e) Corbett, J. W.; Graciani, N. R.; Mousa, S. A.; DeGrado, W. F. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1371–1376.
5. Duggan, M. E.; Fisher, J. E.; Gentile, M. A.; Hartman, G. D.; Hoffman, W. F.; Huff, J. R.; Ihle, N. C.; Krause, A. E.; Leu, T. C.; Nagy, R. M.; Perkins, J. J.; Rodan, G. A.; Rodan, S. B.; Wesolowski, G.; Whitman, D. B. *Abstracts of Papers*, 211th ACS National Meeting, New Orleans, LA, March 1996; MEDI 234.
6. Tomczuk, B.; Lu, T.; Soll, R. M.; Fedde, C.; Wang, A.; Murphy, L.; Crysler, C.; Dasgupta, M.; Eisennagel, S.; Spurlino, J.; Bone, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1495–1498.
7. (a) Pitts, W. J.; Wityak, J.; Smallheer, J. M.; Tobin, A. E.; Jetter, J. W.; Buynitsky, J. S.; Harlow, P. P.; Solomon, K. A.; Corjay, M. H.; Mousa, S. A.; Wexler, R. R.; Jadhav, P. K. *J. Med. Chem.* **2000**, *43*, 27–40; (b) Duggan, M. E.; Duong, L. T.; Fisher, J. E.; Hamill, T. G.; Hoffman, W. F.; Huff, J. R.; Ihle, N. C.; Leu, C.; Nagy, R. M.; Perkins, J. J.; Rodan, S. B.; Wesolowski, G.; Whitman, D. B.; Zartman, A. E.; Rodan, G. A.; Hartman, G. D. *J. Med. Chem.* **2000**, *43*, 27–40; (c) Manthey, C. L.; Lee, Y.; Wang, A.; Crysler, C.; Zhao, S.; Bone, R. F.; Soll, R. M.; Tomczuk, B. E. *Proc. Am. Assoc. Cancer Res.* **2001**, *42*(March 24–28), 368.