Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3380–3382

Arylamide derivatives as allosteric inhibitors of the integrin $\alpha_2\beta_1$/type I collagen interaction

Hang Yin,[a] Lars Ole Gerlach,[a] Meredith W. Miller,[a] David T. Moore,[a] Dahui Liu,[a] Gaston Vilaire,[b] Joel S. Bennett[b] and William F. DeGrado[a,c,*]

[a]*Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA*
[b]*Hematology-Oncology Division, Department of Medicine, Philadelphia, PA 19104, USA*
[c]*Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA*

Received 16 March 2006; accepted 5 April 2006
Available online 5 May 2006

Abstract—We herein report a group of allosteric inhibitors of integrin $\alpha_2\beta_1$ based on an arylamide scaffold. Compound **4** showed an IC_{50} of 4.80 μM in disrupting integrin I-domain/collagen binding in an ELISA. These arylamide compounds are able to block collagen binding to integrin $\alpha_2\beta_1$ on the platelet surface. Further we find that compound **4** recognizes a hydrophobic cleft on the side of the α_2 I-domain, suggesting an alternative targeting site for drug development.

Integrins are homologous heterodimeric proteins consisting of two trans-membrane subunits, α and β, that non-covalently interact.[1] Each αβ combination has its own binding specificity and signaling properties.[2] The $\alpha_2\beta_1$ integrin is a primary platelet adhesion receptor that can complex with collagen through the MIDAS motif within the α_2 I-domain.[3] Despite the significant therapeutic potential, only a few peptide-based agents have been reported to regulate the integrin $\alpha_2\beta_1$ functions by targeting the α_2 I-domain.[4] Herein, we report a group of synthetic inhibitors based on an arylamide scaffold identified from screening a focused small molecule library. These compounds bind to an allosteric site on the α_2 I-domain and inhibit the I-domain/collagen (type I) binding both in vitro and in whole cells.

Arylamide derivatives have been previously reported as a good template for membrane-binding proteins.[5] Other advantages of using these arylamide derivatives in drug development include: (1) they are prepared via straightforward modular synthesis, readily permitting modification of backbone and termini with different functional groups and (2) the rigid conformation enforced by the intra-molecular hydrogen binding lowers the entropy penalty upon binding to the receptors.[5a]

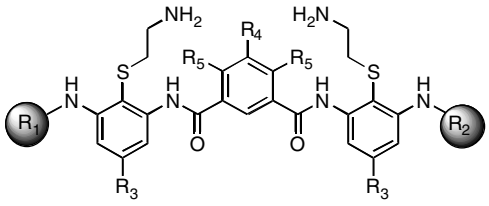
We used an enzyme-linked immunosorbent assay (ELISA) for fast initial screening to identify the lead compounds.[6] In this assay, soluble type I collagen was immobilized on a 96-well plate, and a recombinant GST-tagged α_2 I-domain construct was added, along with increasing concentrations of various arylamide derivatives. The amount of I-domain binding in the presence of the arylamide derivatives was measured by the activity of a peroxidase that was conjugated to an anti-GST antibody using a colorimetric reaction that generates absorbance at 405 nm.

The IC_{50} values of the arylamide derivatives are listed in Table 1. Compound **4**, with two flanking L-2-naphthylalanine (Nal) residues and *tert*-butyl groups on the center phenyl rings, potently disrupted the collagen/I-domain binding with an IC_{50} value of 4.8 μM. As a comparison, compound **1**, with D-2-Nal residues appended, showed about 4-fold weaker potency, suggesting the L-isomer provides better spatial complementarity. However, the *tert*-butyl groups appear to be not essential in the target recognition. In general, arylamides with aromatic, hydrophobic residues at both termini (**1**, **4**, **5**, **7**, **9**, and **12**) are more potent inhibitors, suggesting the target area on the α_2 I-domain has the same property. However, the limited solubility of these compounds precluded more detailed studies. Arylamide derivatives

Keywords: Integrin; Protein–protein interaction; Platelet adhesion; Allosteric inhibitor.
* Corresponding author. Tel.: +1 215 898 4590; fax: +1 215 573 7229; e-mail: wdegrado@mail.med.upenn.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.04.037

Table 1. Results of the ELISA and adhesion assays for the arylamide derivatives



Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)	
						ELISA	Adhesion
1	D-2-Nal[1]	D-2-Nal	*t*-Bu	H	H	>20	14.4
2	D-3-Pya[2]	D-3-Pya	*t*-Bu	H	H	>50	>100
3	3-Pya	H	H	H	H	>50	>100
4	L-2-Nal	L-2-Nal	*t*-Bu	H	H	4.80	3.40
5	D-2-Nal	D-2-Nal	H	H	H	19.7	16.9
6	D-2-Nal	H	H	H	H	>50	64.6
7	L-2-Nal	L-2-Nal	H	H	H	6.23	10.1
8	L-2-Nal	H	H	H	H	>50	85.3
9	D-Phe	D-Phe	H	$O(CH_2)_2NH_2$	H	>50	43.2
10	Gaba[3]	Gaba	*t*-Bu	H	H	>50	>100
11	D-Arg	D-Arg	*t*-Bu	H	H	>50	>100
12	D-Trp	D-Trp	*t*-Bu	H	H	>20	17.34
13	D-Ala	D-Ala	*t*-Bu	H	H	>50	>100
14	Gly	Gly	*t*-Bu	H	H	>50	>100
15	H	H	*t*-Bu	H	$O(CH_2)_2NH_2$	>50	>100
16	H	H	*t*-Bu	H	$O(CH_2)_2Gua$[4]	>50	>100

(1) Nal, naphthylalanine; (2) Pya, pyridinylalanine; (3) Gaba, gamma-amino butyric acid; (4) Gua, guanidium.

with small amino acid residues attached (**10**, **13**, and **14**) showed few inhibitory effects. Compounds with only one amino acid residue attached (**3**, **6**, and **8**) showed significantly lowered potency, indicating the necessity of the intact arylamide scaffold. It is also suggested that the total number of charges that these arylamide derivatives carry does not affect their binding to the α_2 I-domain to a large extent.

The inhibitory effects of these arylamide derivatives in disrupting collagen/integrin $\alpha_2\beta_1$ association in whole cells were tested using a previously described platelet adhesion assay.[7] The inhibitory effects of the arylamide derivatives were determined by measuring the number of gel-filtered human platelets bound to immobilized type I collagen on a 96-well plate. Consistent with the ELISA results, compound **4** showed the strongest inhibitory potency with an IC_{50} of 3.4 μM in disrupting collagen binding to intact platelets. This finding indicated that these arylamide derivatives are able to block type I collagen binding to integrin on the platelet surface (Supporting information Fig. 2).

Furthermore, the collagen-induced platelet aggregation can be blocked by compound **4**. Addition of soluble type I collagen to a suspension of washed platelets results in platelet aggregation caused by the activation of integrin $\alpha_2\beta_1$. We found this aggregation is blocked in the presence of 5.0 μM **4**, confirming that the collagen/integrin $\alpha_2\beta_1$ interaction is the target for these inhibitors.

Because amphiphilic compounds can cause cell lysis,[8] we examined the leakage potential of these arylamide derivatives in phospholipid vesicles. Compound **4** failed to induce a fluorescent dye, $Tb(DPA)_3$, to leak from phospholipid vesicles at lipid/compound ratio up to 10:1 (Supporting information Fig. 3), suggesting that these arylamide compounds did not significantly affect the cell membrane. Further toxicity tests for these compounds were conducted using a lactate dehydrogenase (LDH) release assay. Platelets release LDH when their plasma membrane is perturbed. In the presence of 30 μM of arylamide **4**, the level of LDH released by platelets is not significantly higher than the negative control.

In order to study the binding mode of these arylamide derivatives to integrin α_2 I-domain, we have conducted (^1H,^{15}N)-2D-HSQC NMR experiments. NMR samples containing ^{15}N-labeled I-domain protein (0.25 mM), in the presence of 0, 0.5, and 1.0 equivalence of arylamide derivative **4**, were prepared. Figure 1 shows the results of the HSQC experiments, with the amino acid residues that showed significant and moderate chemical shift changes upon addition of arylamide **4** colored in magenta and orange, respectively. Five amino acids (Lys294, Lys301, Y311, Ser316, and Asp317, labeled in Fig. 1) showed significant chemical shift changes upon addition of the arylamide derivatives and 12 other amino acids (Val208, Ala209, Thr246, Gln269, Asp273, Asn289, Lys298, Ser305, Ile306, Arg307, Phe313, and Ala319) showed moderate chemical shift changes. Noticeably, 12 out of these 17 affected residues are located around a hydrophobic cleft on one side of the α_2 I-domain, suggesting that arylamide **4** inhibits the collagen/α_2 I-domain association by targeting an allosteric site that is different from the collagen-binding MIDAS site.

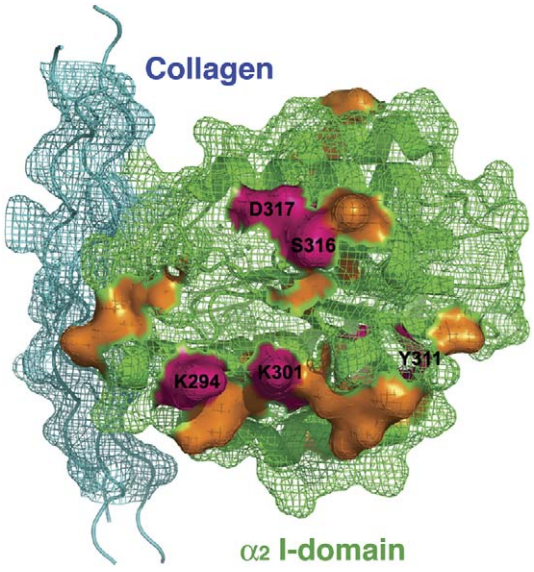


Figure 1. (^{1}H,^{15}N)-2D-HSQC NMR experiment results on I-domain/arylamide binding. The residues that showed significant and moderate chemical shift changes upon the addition of **4** (1.0 equiv) are shown in magenta and orange, respectively. The amino acid residues that showed significant chemical shift changes upon addition of **4** were labeled. The integrin α_2 I-domain is shown in green cartoon representation with mesh. The type I collagen triple-helix bundle ligand is shown in blue tubes.

Furthermore, this cleft overlaps with the α_7–β_6 loop, which plays an essential role in the integrin conformational change in the signaling pathway (Supporting information Fig. 5).[9] Interestingly, it has been recently reported that a marketed drug, lovastatin, complexes with the analogous α_L I-domain at a similar site.[10] This finding may suggest an alternative strategy to target the integrin I-domains and provide therapeutically useful agents. Along these lines, the compounds described here may serve as research tools or early leads for subsequent optimization.

Acknowledgments

This work was supported in part by NIH Grant HL62250. We thank Dr. Kathleen G. Valentine's generous help and discussion in the HSQC NMR experiments; Prof. Mark Kahn for helpful discussion. We also thank Dr. Gregory A. Caputo's help in the $Tb(DPA)_3$ release assay.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.037.

References and notes

1. Giancotti, F. G.; Ruoslahti, E. *Science* **1999**, *285*, 1028.
2. Arnaout, M. A.; Mahalingam, B.; Xiong, J. P. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 381.
3. (a) Emsley, J.; Knight, C. G.; Farndale, R. W.; Barnes, M. J.; Liddington, R. C. *Cell* **2000**, *191*, 47; (b) Auger, J. M.; Kuijpers, M. J.; Senis, Y. A.; Watson, S. P.; Heemskerk, J. W. M. *FASEB J.* **2005**, *19*, 825.
4. For examples, see: (a) Baronas-Lowell, D.; Lauer-Fields, J. L.; Fields, G. B. *J. Biol. Chem.* **2004**, *279*, 952; (b) Kim, J. K.; Xu, Y.; Xu, X.; Keene, D. R.; Gurusiddappa, S.; Liang, X.; Wary, K. K.; Höök, M. *J. Biol. Chem.* **2005**, *280*, 32512; (c) Sweeney, S. M.; DiLullo, G.; Slater, S. J.; Martinez, J.; Iozzo, R. V.; Lauer-Fields, J. L.; Fields, G. B.; San Antonio, J. D. *J. Biol. Chem.* **2003**, *278*, 30516; (d) Ivaska, J.; Käpylä, J.; Pentikäinen, O.; Hoffren, A. M.; Hermonen, J.; Huttunen, P.; Johnson, M.; Heino, J. *J. Biol. Chem.* **1999**, *274*, 3513.
5. (a) Yin, H.; Frederick, K. K.; Liu, D.; Wand, A. J.; DeGrado, W. F. *Org. Lett.* **2006**, *8*, 223; (b) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. *Proc. Natl. Aca. Sci. U.S.A.* **2002**, *99*, 5110; (c) Choi, S.; Clements, D. J.; Pophristic, V.; Ivanov, I.; Vemparala, S.; Bennett, J. S.; Klein, M. L.; Winkler, J. D.; DeGrado, W. F. *Angew. Chem., Int. Ed.* **2005**, *44*, 6685; (d) Liu, D.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; Winkler, J. D.; Klein, M. L.; DeGrado, W. F. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158.
6. Xu, Y.; Gurusiddappa, S.; Rich, R. L.; Owens, R. T.; Keene, D. R.; Mayne, R.; Höök, A.; Höök, M. *J. Biol. Chem.* **2000**, *275*, 38981.
7. Basani, R. B.; D'Andrea, G.; Mitra, N.; Vilaire, G.; Richberg, M.; Kowalska, M. A.; Bennett, J. S.; Poncz, M. *J. Biol. Chem.* **2001**, *276*, 13975.
8. Bechinger, B. *J. Membr. Biol.* **1997**, *156*, 197.
9. Takagi, J.; Springer, T. A. *Immunol. Rev.* **2002**, *186*, 141.
10. Weitz-Schmidt, G.; Welzenbach, K.; Dawson, J.; Kallen, J. *J. Biol. Chem.* **2004**, *279*, 46764.