Dehydro- β -proline Containing $\alpha_4\beta_1$ Integrin Antagonists:
Stereochemical Recognition in Ligand–Receptor InterplayAlessandra Tolomelli,^{*,†,||} Monica Baiula,^{*,‡,||} Angelo Viola,[†] Lucia Ferrazzano,[†] Luca Gentilucci,[†] Samantha Deianira Dattoli,[‡] Santi Spampinato,[‡] Eusebio Juaristi,[§] and Margarita Escudero[§][†]Department of Chemistry “G. Ciamician”, University of Bologna, Via Selmi 2, 40126 Bologna, Italy[‡]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy[§]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco 07360, México

S Supporting Information

ABSTRACT: A novel class of dehydro- β -proline-containing peptidomimetics, designed to be effective as $\alpha_4\beta_1$ integrin ligands, has been developed on the basis of the fundamental requirements for the interactions of these transmembrane receptors with bioactive ligands. Dehydro- β -proline ring has been synthesized through an original pathway, involving ring closing metathesis of a diallylamino derivative. The synthesized products showed to be effective and selective as $\alpha_4\beta_1$ integrin antagonists and displayed IC₅₀ values in the nanomolar range in cell adhesion inhibition assays and in VCAM-1-induced phosphorylation of extracellular-signal-regulated kinases. Significant activity was observed also toward the homologous integrin $\alpha_4\beta_7$, while they did not display any activity toward selected members of β_1 , β_2 , and β_3 families. A strong dependence on the stereochemistry of the heterocyclic central core could be observed. The great importance of $\alpha_4\beta_1$ integrin in chronic inflammatory and autoimmune diseases suggests a possible exploitation of these ligands as lead compounds for therapeutic tools development.

KEYWORDS: Small-molecule inhibitors, integrin ligands, peptidomimetics, enantiomers, inflammation

The importance of cell–cell and cell–matrix interactions in the regulation of cellular events has prompted the comprehension of the mechanisms regulating the activity of cell-surface molecules, as well as the synthesis of new ligands mimicking the recognition sequences present in extracellular matrix proteins.¹ α_4 -Integrins are noncovalent heterodimeric glycoprotein transmembrane receptors constitutively expressed on the surface of leukocytes,² which contribute to inflammatory reactions. In particular, integrin $\alpha_4\beta_1$ (VLA-4) plays a fundamental role in leukocyte trafficking, activation, and migration across the blood–endothelial barrier during inflammatory responses, through binding of its natural ligands, the vascular cell adhesion molecule-1 (VCAM-1, CD106), and the alternatively spliced portion of the type III connecting segment of fibronectin (FN).³ The second member of this subfamily, integrin $\alpha_4\beta_7$, is important in lymphocyte homing to mucosal tissue by adhering to the gut mucosal address cell adhesion molecule (MAdCAM), even if it also recognizes VCAM-1 and FN.^{4,5}

Dysregulation of the inflammatory reaction leads to the pathogenesis of chronic inflammations and autoimmune diseases such as asthma, rheumatoid arthritis, multiple sclerosis, and Crohn's disease.⁶ $\alpha_4\beta_7$ integrin contributes to the infiltration of leukocytes into the islets of Langerhans in type I diabetes and in demyelinating in multiple sclerosis, but its

most important role lies in the mediation of T lymphocyte migration into the gut in inflammatory bowel disease (IBD) subtypes of Crohn's disease.⁷

The pivotal role of $\alpha_4\beta_1$ integrin has been clearly demonstrated in tumor angiogenesis associated with chronic inflammation, suggesting that this condition may promote the angiogenetic switch in tumors.^{8–10} $\alpha_4\beta_1$ is also involved in the recruitment of progenitor cells, the multipotent cells derived from bone marrow stem cells, in the formation of new blood vessels.¹¹ Because of its overexpression in melanoma cells, $\alpha_4\beta_1$ may also be considered a marker for predicting metastatic risk.¹²

Upon FN or VCAM-1 binding, integrin $\alpha_4\beta_1$ forms clusters on the cell surface termed focal adhesions that act as links between the extracellular matrix and the actin cytoskeleton and activate focal adhesion kinase. This latter activates an integrin-induced signaling cascade that involves the extracellular regulated kinase (ERK), which, in turn, promotes cell proliferation and migration.¹³

On these bases, molecules able to interfere in $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ binding may represent useful tools for chronic

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inflammations, autoimmune diseases, and cancer therapy. Up to now, only the anti- α_4 monoclonal antibody Natalizumab¹⁴ and the anti- $\alpha_4\beta_7$ antibody Vedolizumab¹⁵ have reached the market. Natalizumab is currently applied for Crohn's disease¹⁶ and multiple sclerosis therapy, although it has been observed to induce in a few cases the development of progressive multifocal encephalopathy (PML) leading to patient death.¹⁷ Vedolizumab was recently approved for the treatment of ulcerative colitis and Crohn's disease.¹⁸

Aside monoclonal antibodies, a number of small molecules recognized as selective or dual ligands for $\alpha_4\beta_1$ and $\alpha_4\beta_7$ have been reported in the literature.¹⁹

Many of them are cyclic^{20,21} or linear peptides,^{22,23} designed starting from the LDV and IDS recognition sequences of $\alpha_4\beta_1$ with fibronectin and VCAM-1, respectively, or from the LDT recognition sequences of $\alpha_4\beta_7$ with MAdCAM, often elongated with particular capping moieties.²⁴ Because of the similarity of the tripeptide recognition sequences (LDV for CS-1 peptide of fibronectin vs LDT), small molecules designed starting from these fragments often shown dual affinity. MAdCAM specificity for $\alpha_4\beta_7$ is conferred by a protruding loop in its second domain, whose effect is quite complex to reproduce with small molecules.

Following this approach, tyrosine and phenylalanine derivatives showing great affinity for the receptor were discovered.^{25,26} Finally, the challenge to overcome peptide limitations was achieved with the preparation of bioactive peptidomimetic ligands.^{27,28} Anyway, a clear model for the design of potential ligands is still elusive. Although the crystal structure of the $\alpha_4\beta_1$ binding fragment of VCAM-1 is available,²⁹ 3D models,³⁰ in silico screening,^{31,32} and 3D QSAR studies³³ have been reported to understand the binding mode of natural or synthetic bioactive ligands to $\alpha_4\beta_1$. In general, fundamental features for effective ligand–receptor interaction are the presence of a carboxylate group, a donor of H-bond as an amide moiety in the central part of the molecule and a lipophilic chain mimicking the leucine side chain present in the LDV recognition sequence.²⁷ Moreover, the presence of 4([(N-2-methylphenyl)ureido]-phenylacetyl motif (PUPA) greatly enhances bioactivity, as observed for compound BIO1211 (Figure 1).³⁴

Following a very common trend in bioactive peptide research, proline derivatives^{35,36} and other five-membered heterocycles³⁷ have been used as central cores in the preparation of peptides and peptidomimetics designed to be $\alpha_4\beta_1$ ligands, affording excellent results of affinity. Recently, the term “privileged structure” has appeared frequently in the literature to define recurring structural elements that are likely to facilitate binding with biological targets, inducing ordered spatial disposition of side chains.³⁸ The cyclic structure of proline is known to give particular conformational rigidity to peptides, due to the reduced allowed rotations,³⁹ and for this reason, the use of proline and its analogues as conformational restraints or rigid cores in peptidomimetics has been extensively explored. Among the number of modified structures, β -proline has received great attention since the substitution of an α -amino acid with the corresponding β -analogue in key positions of a bioactive sequence represents a successful approach to obtain mimetics overcoming the low bioavailability limitations of natural peptides.^{40,41} Moreover, unsaturated amino acids have been also explored, and 3,4-dehydropoline (Dhp), the analogue of proline having a double bond between C β and C γ ,

Figure 1. General structure of the designed dehydro- β -proline containing $\alpha_4\beta_1$ ligands and of bioactive ligand BIO1211.

showed to induce changes in structures and biological properties of collagen, proteins, and peptides.⁴²

We have recently developed the synthesis of enantiopure 3,4-dehydro- β -proline, with the purpose to obtain useful scaffold for the synthesis of bioactive compounds.⁴³ By adding the proper pharmacophoric groups, these heterocycles may indeed be transformed into purported $\alpha_4\beta_1$ integrin ligands. The aim of this study is indeed the synthesis of peptidomimetics and the evaluation of their affinity/selectivity toward $\alpha_4\beta_1$ integrin compared to $\alpha_4\beta_7$ integrin and other integrin families.

To explore this possibility, two different structures have been designed, by changing the substitution pattern of the 3,4-dehydro- β -proline core (Figure 1). The two families of compounds have been synthesized starting from a common intermediate, the *N*-(*Z*)-allylamino ester **1**, whose preparation in enantiomerically pure form has been already reported by us.⁴³ The isopropyl and the cyclohexyl groups have been inserted on the heterocycle in order to mimic the lipophilic leucine side chain.

For the preparation of compounds belonging to the family **A** (Scheme 1), the allylamino moiety in (*Z*)-**1** was treated with 1-isocyanato-2-methylbenzene to introduce the phenylureido group and to protect the nitrogen in view of the following steps (70–90% yield). The ring closing metathesis of diallylamine (*Z*)-**2** was then performed to create the dehydro- β -proline ring. As already verified in our previous studies, Grubbs–Hoveida II generation catalyst is the better working catalyst for this reaction.

Compound **3**, obtained in 60–65% yield exclusively as *trans*-amide isomer, was then transformed into the corresponding free acid **4** by treatment with TFA in DCM at room temperature, which was obtained in almost quantitative yield. Two coupling reactions with HBTU/DIPEA, the first with 4-(aminomethyl)aniline and then the second between the obtained compound **5** with mono *tert*-butyl-malonate, afforded the ester **6** in 75% yield, which was finally transformed into the free acid **7** by treatment with TFA. The protocol was

Scheme 1. General Synthetic Route to $\alpha_4\beta_1$ Ligands: Class A^a

^aReactions and conditions: (a) 1-isocyanato-2-methylbenzene, DCM, r.t., 6 h, yield 70–90%; (b) Grubbs–Hoveida II catalyst (3% mol), MTBE, reflux, 3 h, yield 60–65%; (c) TFA, DCM, r.t., 12 h, yield >90%; (d) 4-(aminomethyl)aniline, HBTU, DIPEA, DCM, r.t., overnight; (e) mon-*tert*-butyl malonate, HBTU, DIPEA, DCM, r.t., 2 days, yield 75% (2 steps); (f) TFA, DCM, r.t., 12 h, yield >90%.

Scheme 2. General Synthetic Route to VLA-4 Ligands: Class B^a

^aReactions and conditions: (a) methyl malonyl chloride, TEA, DCM, r.t., 3 h, yield 90–95%; (b) Grubbs–Hoveida II catalyst (3% mol), MTBE, reflux, 3 h, yield 80–95%; (c) TFA, DCM, r.t., 12 h, yield >90%; (d) HBTU, DIPEA, DCM, 4-(aminomethyl)aniline, r.t., overnight, yield 75–85%; (e) 2-methylbenzene isocyanate, DCM, r.t., 6 h, yield 80–88%; (f) K₂CO₃, THF/H₂O, r.t., 2 h, yield >90%.

Table 1. Inhibition of Jurkat Cell Adhesion by Dehydro- β -proline-Containing Peptidomimetics

entry	compd	$\alpha_4\beta_1$ /VCAM-1 IC ₅₀ (nM) ^a
1	(S)-7	110 ± 9
2	(R)-7	250 ± 12
3	(S)-13a	>1000
4	(R)-13a	10 ± 3
5	(S)-13b	>1000
6	(R)-13b	62 ± 11

^aExperiments were conducted in quadruplicate and were repeated at least three times. BIO1211 (Figure 1, ref 34) was used as reference compound, inhibiting the adhesion with an IC₅₀ of 7.6 nM.

performed both on the (R) and (S) enantiomers separately, thus allowing to obtain enantiopure ligands (R)-7 and (S)-7.

For the preparation of peptidomimetic ligands belonging to the second family B (Scheme 2), the *N*-(*Z*)-allylamino ester 1a,b was protected at the nitrogen through coupling with methyl malonyl chloride to afford amide (*Z*)-8a,b in 90–95%

Table 2. Inhibition of Cell Adhesion through Alternative Integrins by Most Effective $\alpha_4\beta_1$ Antagonists, (R)-13a and (R)-13b

compd	IC ₅₀ (nM) ^a			
	$\alpha_4\beta_7$ /MadCam-1 ^b	$\alpha_5\beta_1$ /FN ^c	$\alpha_1\beta_2$ /ICAM-1 ^d	$\alpha_4\beta_3$ /FN ^c
(R)-13a	270 ± 33	>1000	>1000	>1000
(R)-13b	343 ± 26	>1000	>1000	>1000

^aExperiments were conducted in quadruplicate and were repeated at least three times. ^bPerformed on RPMI8866 cells. ^cPerformed on K-562 cells. ^dPerformed on Jurkat cells. ^ePerformed on SK-MEL-24 cells.

yield. Treatment of 8a,b with Grubbs–Hoveida catalyst allowed to obtain dehydro- β -proline 9a,b in yields ranging from 80% to 95%, as a *trans*/*cis* mixture of amide isomers depending on the alkyl substituent (80/20 for 9a; 70/30 for 9b). The spatial arrangement of the malonic chain for the two conformers was determined through ROESY experiments.⁴³ Removal of the *tert*-butyl ester without affecting the methyl malonate was obtained by treatment with TFA, to give 10a,b in almost

Figure 2. Compounds (R)-13a and (R)-13b prevent VCAM-1-induced phosphorylation of ERK1/2. Jurkat cells were serum-starved in RPMI-1640 containing 1% FBS for 16 h; cells were then preincubated with compounds (R)-13a (A) and (R)-13b (B) for 60 min in suspension. The cells were kept in suspension (Ctrl) or plated on VCAM-1 coated wells. After 60 min, cells were lysed, and lysates were analyzed by Western blot using an antibody directed against phosphorylated ERK1/2 (pERK1/2) or total ERK1/2 (tERK1/2). Representative Western blots show that control cells plated on VCAM-1 had a much stronger signal for pERK1/2 than control cells (Ctrl). Preincubation with compound (R)-13a caused a significant, concentration-dependent decrease in the amount of pERK1/2 in Jurkat cells, while (R)-13b reduced VCAM-1-induced ERK1/2 phosphorylation significantly only at the concentration 10⁻⁷ M. Densitometric analysis of the bands (mean $\pm$ SEM; $n = 6$); the amount of pERK1/2 is normalized to the tERK1/2. *** $p < 0.001$ versus Ctrl; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus VCAM-1 60' (Newman–Keuls test after ANOVA).

quantitative yield. Coupling with 4-(aminomethyl)aniline in the presence of HBTU and DIPEA afforded amide **11a,b** in 75–85% yield. Since the presence of PUPA was required to increase affinity toward VLA-4, the aniline moiety was then treated with 1-isocyanato-2-methylbenzene. Compound **12a,b**, obtained in 80–88% yield, was then treated with sodium carbonate to remove the methyl ester protection. Free acid **13a,b** was isolated in almost quantitative yield.

The protocol was performed starting from the enantiopure allylamines (R)-**1a** and (S)-**1a** having the isopropyl substituent and from the enantiopure allylamines (R)-**1b** and (S)-**1b** having the cyclohexyl side chain. The enantiopure ligands (R)-**7** and (S)-**7**, (R)-**13a** and (S)-**13a**, and (R)-**13b** and (S)-**13b**, belonging to both families A and B, were then evaluated for their affinity for $\alpha_4\beta_1$ integrin, by cell adhesion inhibition assays using Jurkat cells in the presence of VCAM-1 (Table 1), as previously described.^{41,44} For both compounds of the class A, enantiopure (R)-**7** and (S)-**7**, a moderate affinity could be observed since both values of IC₅₀ were in the 10⁻⁷ M range (Table 1, entries 1–2), thus suggesting that the class A designed structure is not optimal for ligand–receptor interaction, not depending on the configuration of the stereocenter on the dehydro- β -proline ring.

On the contrary, for compounds belonging to the class B, a strong dependence of the bioactivity on the ring substituent stereochemistry could be detected since both (S)-**13a** and (S)-**13b** (entries 3 and 5) turned out to be completely inactive, while the opposite (R)-**13a** and (R)-**13b** enantiomers, especially (R)-**13a**, displayed excellent affinity for integrin $\alpha_4\beta_1$ (entries 4 and 6). ROESY experiments⁴⁵ were performed in order to evaluate the conformations of **13a** and **13b**. Lack of significative signals, except for trivial ones, suggested an almost linear disposition of the molecules, as could be expected on the basis of structural restraints. This is in agreement with the preferred conformation reported for other active $\alpha_4\beta_1$ -integrin ligands.^{46,47} Since only one stereocenter is present in the

molecule, further computational minimization in solvent box would not add any useful explanation for the observed different recognition by the receptor of (R) and (S) enantiomers, giving of course the same result for both enantiomers.

In the absence of a reliable model for docking, the experimental evidence suggests that the spatial disposition of the alkyl group of the dehydro- β -proline ring in the (R) enantiomer should be more favorable than the one assumed by the same chain of the (S) enantiomer. Comparing bioactive molecule BIO1211 with **13a,b** (see Figure 1), we hypothesize that the alkyl group could mimic the lipophilic leucine or valine side chains.

The most bioactive compounds (R)-**13a** and (R)-**13b** were then examined for their integrin selectivity in cell-based adhesion assays involving different integrins.

As noted in Table 2, both compounds did not display any activity toward selected members of β_1 , β_2 , and β_3 families. As regards the homologous integrin $\alpha_4\beta_7$, (R)-**13a** and (R)-**13b** displayed significant activity; however, a noteworthy selectivity toward $\alpha_4\beta_1$ integrin can be highlighted.

Finally, we investigated the most effective compounds, (R)-**13a** and (R)-**13b**, on VCAM-1-induced phosphorylation of ERK1/2 in Jurkat cells.

It has been described that $\alpha_4\beta$ integrins activate focal adhesion kinase (FAK). Focal adhesion kinase can activate ERK via two pathways. First, FAK can recruit C3G and RAP1, which induces B-Raf activity and ERK activation. The second pathway involves the growth-factor-receptor-bound-2 and son-of-seven-less complex, which activates Ras–ERK.¹⁰ In agreement with this hypothesis, Brown et al.⁴⁸ have reported that VCAM-1 induces ERK phosphorylation in Jurkat cells expressing $\alpha_4\beta_1$ integrin, through the RAF family of serine/threonine kinases, as sorafenib, a selective B-Raf inhibitor prevents this effect.

Jurkat cells were serum-starved in RPMI-1640 containing 1% fetal bovine serum (FBS) for 16 h; thereafter, they were preincubated with compounds (R)-**13a** or (R)-**13b** for 60 min

in suspension and plated for 60 min on VCAM-1 or poly-L-lysine (used as nonspecific substrate). In cells exposed to VCAM-1, a significant, concentration-dependent increase in phosphorylated ERK1/2 was observed 60 min after VCAM-1 exposure (Figure 2). On the contrary, poly-L-lysine exposure was not able to induce any increment in ERK1/2 phosphorylation (data not shown). (R)-13a was more effective than (R)-13b in reducing VCAM-1-induced ERK1/2 phosphorylation (Figure 2). IC₅₀, obtained in a separate set of experiments in which Jurkat cells were exposed to different concentrations of (R)-13a or (R)-13b was 10 and 131 nM, respectively (see Supporting Informations).

Taken together, these results are in agreement with data of cell adhesion assays, thus confirming that (R)-13a is the most effective $\alpha_4\beta_1$ antagonist of the peptidomimetics here investigated capable to maintain a significant selectivity toward $\alpha_4\beta_7$ integrin.

In conclusion, a novel class of dehydro- β -proline-containing peptidomimetics, designed to be effective as $\alpha_4\beta_1$ integrin ligands, has been developed on the basis of the fundamental requirements for the interactions of these transmembrane receptors with bioactive ligands. To this purpose, dehydro- β -proline ring has been synthesized through an original pathway, involving ring closing metathesis of a diallylamino derivative. Appendages to the central core have been selected on the basis of previous experience and on the basis of information from the literature. The synthesized products have been tested in cell adhesion inhibition assays and in assays aimed to ascertain the effect of these purported $\alpha_4\beta_1$ antagonists. A specific compound, (R)-13a, resulted to be a good integrin ligand, showing IC₅₀ in the nanomolar range. A strong dependence on the stereochemistry of the heterocyclic central core could be observed, thus suggesting a preferred disposition of the lipophilic chain for (R) enantiomers. The great importance of $\alpha_4\beta_1$ integrin (VLA-4) in chronic inflammation, autoimmune diseases, and inflammation related to cancer suggests a possible exploitation of these ligands as lead compounds for therapeutic tools development.

■ ASSOCIATED CONTENT

■ Supporting Information

Complete characterization of compounds 2–13 and detailed experimental procedure for the synthesis and for the development of pharmacological assays. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00125.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

HBTU, *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)-uronium hexafluorophosphate; DIPEA, diisopropylethylamine; VCAM-1, vascular adhesion molecule 1; MAdCAM, mucosal address cell adhesion molecule; PUPA, 4([(N-2-methylphenyl)ureido]-phenylacetyl group

■ REFERENCES

- (1) Hynes, R. O. Integrins: bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673–687.
- (2) Postigo, A. A.; Teixidó, J.; Sánchez-Madrid, F. The $\alpha_4\beta_1$ /VCAM-1 adhesion pathway in physiology and disease. *Res. Immunol.* **1993**, *144* (9), 723–735.
- (3) Mould, P.; Komoriya, A.; Yamadall, K. M.; Humphries, M. J. The CSS Peptide Is a Second Site in the IIICS Region of Fibronectin Recognized by the Integrin $\alpha_4\beta_1$. *J. Biol. Chem.* **1991**, *266*, 3579–3585.
- (4) Postigo, A. A.; Sanchez-Mateos, P.; Lazarovits, A. I.; Sanchez-Madrid, F.; de Landazuri, M. O. $\alpha_4\beta_7$ integrin mediates B cell binding to fibronectin and vascular cell adhesion molecule-1. Expression and function of α_4 integrins on human B lymphocytes. *J. Immunol.* **1993**, *151*, 2471–2483.
- (5) Berlin, C.; Berg, E. L.; Briskin, M. J.; Andrew, D. P.; Kilshaw, P. J.; Holzmans, B.; Weissman, I. L.; Hamann, A.; Butcher, E. C. $\alpha_4\beta_7$ integrin mediates lymphocyte binding to the mucosal vascular address in MAdCAM-1. *Cell* **1993**, *74*, 185–195.
- (6) Hyun, Y. M.; Lefort, C. T.; Kim, M. Leukocyte integrins and their ligand interactions. *Immunol. Res.* **2009**, *45*, 195–208.
- (7) Meenan, J.; Spaans, J.; Gools, T. A.; Pals, S. T.; Tytgat, G. N.; van Deventer, S. J. Altered expression of $\alpha_4\beta_7$, a gut homing integrin, by circulating and mucosal T cells in colonic mucosal inflammation. *Gut* **1997**, *241*–246.
- (8) Coussens, L. M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867.
- (9) Garmy-Susini, B.; Jin, H.; Zhu, Y.; Sung, R. Y.; Hwang, R.; Varner, J. Integrin $\alpha_4\beta_1$ -VCAM-1-mediated adhesion between endothelial and mural cells is required for blood vessel maturation. *J. Clin. Invest.* **2005**, *115*, 1542–1551.
- (10) Shishido, S.; Böning, H.; Kim, Y. M. Role of integrin $\alpha_4\beta_1$ in drug resistance of leukemia. *Front. Oncol.* **2014**, *4* (99), 1–10.
- (11) Jin, H.; Aiyer, A.; Su, J.; Borgstrom, P.; Stupack, D.; Friedlander, M.; Varner, J. A homing mechanism for bone marrow-derived progenitor cell recruitment to the neovasculature. *J. Clin. Invest.* **2006**, *116*, 652–662.
- (12) Valcarcel, M.; Carrascal, T.; Crende, O.; Vidal-Vanaclocha, F. IL-18 Regulates Melanoma VLA-4 Integrin Activation through a Hierarchized Sequence of Inflammatory Factors. *J. Inv. Dermatol.* **2014**, *134*, 470–480.
- (13) Hood, J. D.; Cheres, D. A. Role of integrins in cell invasion and migration. *Nat. Rev. Cancer.* **2002**, *2*, 91–100.
- (14) McCormack, P. L. Natalizumab: a review of its use in the management of relapsing-remitting multiple sclerosis. *Drugs* **2013**, *73*, 1463–81.
- (15) Mitroulis, I.; Alexaki, V. I.; Kourtzelis, I.; Ziogas, A.; Hajishengallis, G.; Chavakis, T. Leukocyte integrins: Role in leukocyte re-cruitment and as therapeutic targets in inflammatory disease. *Pharmacol. Ther.* **2015**, *147*, 123–135.
- (16) Sakuraba, A.; Keyashian, K.; Correia, C.; Melek, J.; Cohen, R. D.; Hanauer, S. B.; Rubin, D. T. Natalizumab in Crohn's disease: results from a US tertiary inflammatory bowel disease center. *Inflamm. Bowel Dis.* **2013**, *19*, 621–626.

- (17) Kleinschmidt-DeMasters, B. K.; Tyler, K. L. Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. *New Engl. J. Med.* **2005**, *353*, 369–374.
- (18) Garnock-Jones, K. P. Vedolizumab: A Review of Its Use in Adult Patients with Moderately to Severely Active Ulcerative Colitis or Crohn's Disease. *Biodrugs* **2015**, *29*, 57–67.
- (19) Jackson, D. J. Alpha 4 Integrin antagonists. *Curr. Pharm. Design* **2002**, *8*, 1229–1253.
- (20) Tilley, J. W.; Chen, L.; Sidduri, A.; Fotouhi, N. The discovery of VLA-4 antagonists. *Curr. Top. Med. Chem.* **2004**, *4*, 1509–1523.
- (21) Boer, J.; Gottschling, D.; Schuster, A.; Semmrich, M.; Holzmann, B.; Kessler, H. Design and synthesis of potent and selective $\alpha_4\beta_7$ integrin antagonists. *J. Med. Chem.* **2001**, *44*, 2586–2592.
- (22) Huryn, D. M.; Konradi, A. W.; Ashwell, S.; Freedman, S. B.; Lombardo, L. J.; Pleiss, M. A.; Thorsett, E. D.; Yednock, T.; Kenedy, J. D. The identification and optimization of orally efficacious, small molecule VLA-4 antagonists. *Curr. Top. Med. Chem.* **2004**, *4*, 1473–1484.
- (23) Dubree, N. J.; Artis, D. R.; Castanedo, G.; Marsters, J.; Sutherland, D.; Caris, L.; Clark, K.; Keating, S. M.; Beresini, M. H.; Chiu, H.; Fong, S.; Lowman, H. B.; Skelton, N. J.; Jackson, D. Y. Selective $\alpha_4\beta_7$ integrin antagonists and their potential as antiinflammatory agents. *J. Med. Chem.* **2002**, *16*, 3451–3457.
- (24) Hagmann, W. K. The discovery and potential of N-sulfonylated dipeptide VLA-4 antagonists. *Curr. Top. Med. Chem.* **2004**, *4*, 1461–1471.
- (25) Xu, Y. Z.; Smith, J. L.; Semko, C. M.; Rossiter, K. I.; Fukuda, J. Y.; Dappen, M. S.; Quincy, D. A.; Konradi, A. W.; Mao, W.; Welch, B.; Dreyer, M. L.; Samant, B.; Zhang, H.; Lugar, J.; Liao, Z.; Henschel, C.; Petersen, E.; Vandever, C.; Shoemaker, M.; Wehner, N.; Mutter, L.; Shopp, G.; Krimm, M.; Chen, L.; Wipke, B.; Dofiles, L.; Gallagher, I.; Sauer, J. M.; Messersmith, E. K.; Pleiss, M. A.; Bard, F.; Yednock, T. A. Orally available and efficacious $\alpha_4\beta_1/\alpha_4\beta_7$ integrin inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4370–4373.
- (26) Castanedo, G. M.; Sailes, F. C.; Dubree, N. J.; Nicholas, J. B.; Caris, L.; Clark, K.; Keating, S. M.; Beresini, M. H.; Chiu, H.; Fong, S.; Marsters, J. C., Jr.; Jackson, D. Y.; Sutherland, D. P. Solid-phase synthesis of dual $\alpha_4\beta_1/\alpha_4\beta_7$ integrin antagonists: two scaffolds with overlapping pharmacophores. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2913–2917.
- (27) Gerard, E.; Meulle, A.; Feron, O.; Marchand-Brynaert, J. Diaryl urea LDV peptidomimetics as $\alpha_4\beta_1$ integrin antagonists: synthesis, adhesion inhibition and toxicity evaluation on CCRF-CEM cell line. *Med. Chem. Comm.* **2012**, *3*, 199–212.
- (28) Locardi, E.; Boer, J.; Modlinger, A.; Schuster, A.; Holzmann, B.; Kessler, H. Synthesis and Structure–Activity Relationship of Mannose-Based Peptidomimetics Selectively Blocking Integrin $\alpha_4\beta_7$ Binding to Mucosal Address in Cell Adhesion Molecule-1. *J. Med. Chem.* **2003**, *46*, 5752–5762.
- (29) Jones, E. Y.; Harlos, K.; Bottomley, M. J.; Robinson, R. C.; Driscoll, P. C.; Edwards, R. M.; Clements, J. M.; Dudgeon, T. J.; Stuart, D. I. Crystal structure of an integrin-binding fragment of vascular cell adhesion molecule-1 at 1.8 Å resolution. *Nature* **1995**, *376*, 539–544.
- (30) You, T. J.; Maxwell, D. S.; Kogan, T. P.; Chen, Q.; Li, J.; Kassir, J.; Holland, G. W.; Dixon, R. A. F. Small Molecule Agonist of Very Late Antigen-4 (VLA-4) Integrin Induces Progenitor Cell Adhesion. *Biophys. J.* **2002**, *82*, 447–457.
- (31) Thangapandian, S.; Shalini, J.; Sakthiah, S.; Lee, K. W. Discovery of Potential Integrin VLA-4 Antagonists Using Pharmacophore Modeling, Virtual Screening and Molecular Docking Studies. *Chem. Biol. Drug. Des.* **2011**, *78*, 289–300.
- (32) Singh, J.; van Vlijmen, H.; Liao, Y.; Lee, W.-C.; Cornebise, M.; Harris, M.; Shu, L.; Gill, A.; Cuevo, J. H.; Abraham, W. M.; Adams, S. P. Identification of Potent and Novel $\alpha_4\beta_1$ Antagonists Using in Silico Screening. *J. Med. Chem.* **2002**, *45*, 2988–2993.
- (33) Hutt, O. E.; Saubern, S.; Winkler, D. A. Modeling the molecular basis for $\alpha_4\beta_1$ integrin antagonism. *Bioorg. Med. Chem.* **2011**, *19*, 5903–5911.
- (34) Lin, K. C.; Ateeq, H. S.; Hsiung, S. H.; Chong, L. T.; Zimmermann, C. N.; Castro, A.; Lee, W. C.; Hammond, C. E.; Kalkunte, S.; Chen, L. L.; Pepinsky, R. B.; Leone, D. R.; Sprague, A. G.; Abraham, W. M.; Gill, A.; Lobb, R. R.; Adams, S. P. Selective, tight-binding inhibitors of integrin $\alpha_4\beta_1$ that inhibit allergic airway responses. *J. Med. Chem.* **1999**, *42*, 920–934.
- (35) Sattigeri, V. J.; Soni, A.; Dastidar, S. G.; Ray, A.; Ahmad, S.; Gupta, J. B.; Salman, M.; Soni, A.; Dastidar, S. G.; Ray, A.; Ahmad, S.; Gupta, J. B.; Salman, M. Proline ureas: synthesis and biological evaluation as VLA-4 antagonists. *Ind. J. Chem.* **2007**, *46B*, 2004–2020.
- (36) Setoguchi, M.; Imura, S.; Sugimoto, Y.; Yoneda, Y.; Chiba, J.; Watanabe, T.; Muro, F.; Iigo, Y.; Takayama, G.; Yokoyama, M.; Taira, T.; Aonuma, M.; Takashi, T.; Nakayama, A.; Machinaga, N. Identification of trans-4-[1-[[7-fluoro-2-(1-methyl-3-indolyl)-6-benzoxazolyl]acetyl]-(4S)-fluoro-(2S)-pyrrolidinylmethoxy]-cyclohexanecarboxylic acid as a potent, orally active VLA-4 antagonist. *Bioorg. Med. Chem.* **2012**, *20*, 1201–1212.
- (37) Soni, A.; Rehman, A.; Naik, K.; Dastidar, S.; Alam, M. S.; Ray, A.; Chaira, T.; Shah, V.; Palle, V. P.; Cliffe, I. A.; Sattigeri, V. J. Synthesis and evaluation of 4,5-dihydro-5-methylisoxazolin-5-carboxamide derivatives as VLA-4 antagonists. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1482–1485.
- (38) Müller, G. Medicinal chemistry of target family-directed masterkeys. *Drug Disc Today* **2003**, *8*, 681–691.
- (39) McDonald, D. Q.; Still, W. C. Molecular Mechanics Parameters and Conformational Free Energies of Proline-Containing Peptides. *J. Org. Chem.* **1996**, *61*, 1385–1391.
- (40) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. β -Peptides: From Structure to Function. *Chem. Rev.* **2001**, *101*, 3219–3232.
- (41) Dattoli, S. D.; De Marco, R.; Baiula, M.; Spampinato, S.; Greco, A.; Tolomelli, A.; Gentilucci, L. Synthesis and assay of retro- $\alpha_4\beta_1$ integrin-targeting motifs. *Eur. J. Med. Chem.* **2014**, *73*, 225–232.
- (42) Kang, Y. K.; Park, H. S. Conformational Preferences and Cis-Trans isomerization of L-3,4-Dehydropyrrolidine Residue. *Pept. Sci.* **2009**, *92*, 387–398.
- (43) Tolomelli, A.; Gentilucci, L.; Mosconi, E.; Viola, A.; Paradisi, E. A straightforward route to enantiopure 2-substituted-3,4-dehydro- β -proline via ring closing metathesis. *Amino Acids* **2011**, *41*, 575–586.
- (44) Qasem, A. R.; Bucolo, C.; Baiula, M.; Spartà, A.; Govoni, P.; Bedini, A.; Fasci, D.; Spampinato, S. Contribution of $\alpha_4\beta_1$ integrin to the antiallergic effect of levocabastine. *Biochem. Pharmacol.* **2008**, *76*, 751–62.
- (45) See supporting material.
- (46) Martins da Silva, J. H.; Dardenne, L. E.; Savino, W.; Caffarena, E. R. Analysis of $\alpha_4\beta_1$ integrin specific antagonists binding modes: structural insights by molecular docking, molecular dynamics and linear interaction energy method for free energy calculations. *J. Braz. Chem. Soc.* **2010**, *21*, 546–555.
- (47) Carlevaro, C. M.; Martins da Silva, J. H.; Savino, W.; Caffarena, E. R. Plausible binding mode of the active antagonist, MK-0617, determined by docking and free energy calculations. *J. Theor. Comp. Chem.* **2013**, *12*, 1250108–1/16.
- (48) Brown, W. S.; Khalili, J. S.; Rodriguez-Cruz, T. G.; Lizee, G.; McIntyre, B. W. B-Raf regulation of integrin $\alpha_4\beta_1$ -mediated resistance to shear stress through changes in cell spreading and cytoskeletal association in T cells. *J. Biol. Chem.* **2014**, *289*, 23141–23153.