Title

Dual antagonists of α5β1/αvβ1 integrin for airway hyperresponsiveness.

Journal

Bioorganic and Medicinal Chemistry Letters, 30(22)

Authors

Sundaram, Aparna
Chen, Chun
Isik Reed, Nilgun
et al.

Publication Date

2020-11-15

DOI

10.1016/j.bmcl.2020.127578

Published in final edited form as:

Bioorg Med Chem Lett. 2020 November 15; 30(22): 127578. doi:10.1016/j.bmcl.2020.127578.

Dual antagonists of α5β1/ αvβ1 integrin for airway hyperresponsiveness

Aparna Sundaram[a], **Chun Chen**[b], **Nilgun Isik Reed**[a], **Sean Liu**[c], **Seul Ki Yeon**[d], **Joel McIntosh**[d,1], **You-Zhi Tang**[d,2], **Hyunjun Yang**[d], **Marc Adler**[e], **Richard Beresis**[e], **Ian B. Seiple**[d], **Dean Sheppard**[a], **William F. DeGrado**[d], **Hyunil Jo**[d,*]

[a]School of Medicine, University of California San Francisco, San Francisco, CA 94158, USA

[b]Pliant Therapeutics, 260 Littlefield Ave, South San Francisco, CA 94080, USA

[c]Lung Biology Center, Department of Medicine, University of California San Francisco, San Francisco, CA 94158, USA

[d]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94158, USA

[e]ChemPartner, 280 Utah Avenue, South San Francisco, CA 94080, USA

Abstract

Inhibition of integrin alpha5beta1 emerges as a novel therapeutic option to block transmission of contractile forces during asthma attack. We designed and synthesized novel inhibitors of integrin alpha5beta1 by backbone replacement of known alphavbeta1 integrin inhibitors. These integrin alpha5beta1 inhibitors also retain the nanomolar potency against alphavbeta1 integrin, which shows promise for developing dual integrin alpha5beta1/alphavbeta1 inhibitor. Introduction of hydrophobic adamantane group significantly boosted the potency as well as selectivity over integrin alphavbeta3. We also demonstrated one of the inhibitors (**11**) reduced airway hyperresponsiveness in Ex vivo mouse tracheal ring assay. Results from this study will help guide further development of integrin alpha5beta1 inhibitors as potential novel asthma therapeutics.

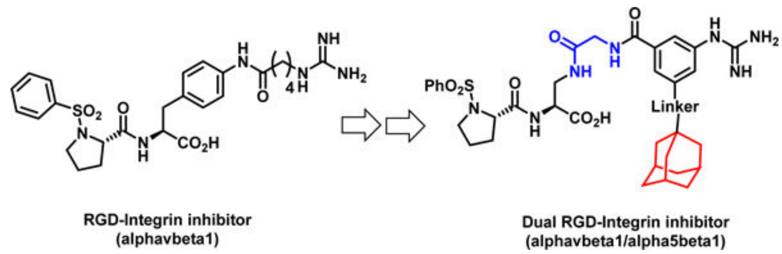
Graphical Abstract

[*]Corresponding author: Hyunil.Jo@ucsf.edu.
[1]Present address: Nurix Therapeutics, 1700 Owens Street, San Francisco, CA 94158, USA
[2]Present address: College of Veterinary Medicine, South China Agricultural University, Guangzhou, 510642, China

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127578.

Declaration of Competing Interest
W.F.D. and D. S. have an equity interest in Pliant Therapeutics which conducts work in a similar area of research. W.F.D. and D.S. are founders and scientific advisory board members and C.C. is an employee of Pliant Therapeutics.

Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Keywords

Integrin alpha5beta1; Integrin alphavbeta1; Integrin inhibitor; RGD integrin; asthma

---

Airway hyperresponsiveness (AHR) - excessive narrowing of airway in response to stimulus is the hallmark feature of asthma.[1] Most patients with asthma can benefit from anti-inflammatory agents and inhibitors of smooth muscle contraction to reduce AHR.[2] However, severe asthma still remains difficult to control and affects up to 10% of patients with asthma.[3] Although biologics targeting disease-relevant cytokines in the inflammation process have recently emerged as add-on therapies, this approach is hindered by targeting only a limited subset of asthma patients (T_2-high) as well as imposing a significant economic burden on the individual, family, and society.[4–5] Thus, there remains an urgent need to accelerate a novel therapeutic approaches to treat severe asthma.

Our groups have previously shown that reduction of AHR could be achieved by pharmacological inhibition of integrins.[6] Integrins are heterodimeric transmembrane proteins consisting of alpha and beta subunits that are involved in several critical cell processes including anchorage, migration, remodeling, and signaling.[7] They are the principal receptors used by cells to link the actin cytoskeleton with adjacent extracellular matrix (ECM) proteins, including fibronectin and collagens.[8] In airway tissue, this integrin-ECM interaction plays a crucial role in force transmission and airway smooth muscle (ASM) cell proliferation ultimately leading to exaggerated airway narrowing. We demonstrated that inhibition of the α5β1 integrin binding to fibronectin resulted in marked decrease of AHR in an ovalbumin-challenged mouse model of asthma. Our work also revealed that integrin inhibition has an additive effect when combined with currently available β-adrenergic agonists, which suggested the potential use of integrin inhibitors as adjuvant therapy to standard bronchodilators. However, the tool peptide (ATN-161)[9] used in our study suffered from low potency and exhibited low plasma stability. For this reason, we sought to identify novel small molecule inhibitors against the α5β1 integrin.

The α5β1 integrin has been previously proposed as an attractive therapeutic target in cancer therapy[10] due to its effects on inhibiting angiogenesis, and there are several small molecule inhibitors against the α5β1 integrin described in the literature.[11–14] In the initial activity profiling of these known inhibitors (**1**-**3**), we found they displayed poor selectivity against the αvβ3 integrin and micromolar potency for α5β1 integrin in our cell adhesion assay[15]. We next turned our attention to our αvβ1 integrin inhibitor series[16] and backbone replacement from phenylalanine with 2,3-diaminopropionic acid (DAP) was shown to influence the selectivity toward alpha5beta1 significantly (Figure 1). For example, **5** (DAP analogs of the selective αvβ1 integrin inhibitor **4**) exhibited sub-micromolar IC_{50} against α5β1 integrin while maintaining sub-nanomolar potency against αvβ1 integrin in the cell adhesion assay. Moreover, **5** still displayed excellent selectivity against αvβ3 integrin, which encouraged us to further investigate the DAP scaffold towards dual α5β1/αvβ1 integrin inhibitors. From dual mode of inhibition, it was anticipated that αvβ1 integrin inhibition could add benefit to AHR by reducing cytokine release in ASM.[17]

For design of a dual inhibitor, we synthesized a handful of analogs of **5** using conventional chemistry to explore the linker effect in DAP scaffold (Scheme 1). Thus, commercially available Boc-protected L-2,3-diaminopropionic acid methyl ester **6** was coupled with benzenesulfonyl-L-proline to provide amine **7** after deprotection of Boc protecting group under acidic condition. Reaction of the common intermediate amine **7** with Boc-protected ω-guanidino *p*-nitrophenylchloro carbamate yielded the urea series (**8–10**) after deprotection and hydrolysis. Similarly, coupling of amine **7** with N-Boc-protected carboxylic acids provided amides (**11–15**) after TFA removal of Boc group followed by mild hydrolysis with LiOH and HPLC purification.

8–15 were then tested against three integrins - α5β1, αvβ1, and αvβ3 for their potency and selectivity (Table 1). Among the compounds with simple alkyl linkers between DAP and the terminal phenylguanidine moiety, higher potency was observed for 6-atom linker (compound **5**). Increase of linker rigidity by introduction of phenyl ring further boosted the potency (compound **11–15**) toward α5β1 integrin. While amide linker for DAP (compound **10**) is preferred for α5β1 integrin inhibition, urea linker (compound **8**) showed much higher potency against αvβ1 integrin. Thus, presence of additional hydrogen bond donor in urea linker could fine tune the selectivity between α5β1 and αvβ1 integrin. On the other hand, a huge increase of potency against α5β1 integrin was observed in rigid *m*-phenyl guanidine compound **11** and **12**. Glycine-linked compound **11** is particularly promising for development of a dual inhibitor as it exhibits comparable potency for both α5β1 and αvβ1 integrin. It is also worth mentioning aza-glycine linker compound **12** favors α5β1 integrin as suggested by previous works.[11–12] While the potency was improved more than 10-fold in compound **11** or **12**, selectivity over αvβ3 integrin was not still satisfactory in either **11** or **12**. Surprisingly, a dramatic increase of selectivity over αvβ3 integrin was achieved by substituted *m*-phenylguanidine series (compound **13**-**15**). We chose an amide linker for phenyl ring substitution due to its facile synthesis and introduced additional alkyl chain to increase lipophilicity. Either hydrophobic alkyl amide substitution (**13**) or positively charged alkyl amide substitution (**14**) boosts the selectivity but did not increase the potency against α5β1 integrin. The best compound was **15** containing 1-adamantyl group to mask the amine. Compound **15** not only showed great selectivity over αvβ3 integrin but also exhibited excellent nanomolar potency for α5β1 and sub-nanomolar potency for αvβ1 integrin. Since all the amide compounds **13**-**15** shared the same feature in amide linker on the phenyl ring, we speculated that adamantane at the terminal position played a key role in improvement of potency.

In order to test if there is a specific binding site for adamantane in α5β1 integrin, we synthesized a series of adamantane analogs with varied length of alkyl chain and tested them in solid-phase binding assay (Table 2). A solid-phase binding assay was used to compare the binding affinity to different integrins without the influence of cellular factors affecting the cell adhesion assay. All the synthesized adamantane compounds **15-19** showed single-digit nanomolar IC_{50}s against both α5β1 and αvβ1 integrin. The linker length did not influence the potency and it was speculated that adamantane moiety induce non-specific binding. Furthermore, we also ruled out any specific hydrogen bonding patterns since reverse amide **18** or amide **15** showed comparable potency. Interestingly, the selectivity appears to decrease among αv integrins as the linker length increased.

Though we cannot exclude the possibility of integrin internalization pathway / degradation by hydrophobic adamantane for the increased potency,[18–19] it appears that combination of alkyl chain and adamantane moiety might increase integrin binding affinity through hydrophobic interaction. Hydrophobic interaction may also explain the superior selectivity over αvβ3 integrin. Computational docking study that showed hydrophobic moiety is not favored in αvβ3 integrin due to the presence of multiple polar residues (Tyr, Asp) in αvβ3 integrin (Figure 2). Further studies on the selectivity among different RGD integrins and different hydrophobic groups are currently underway in our laboratory. It is also remarkable that these adamantane-containing compounds **15–19** showed much improved selectivity (>100 fold) against α4β1 integrin while our previous potent αvβ1 inhibitor **4** demonstrated marginal selectivity.[20]

With potent dual inhibitors in hands, we also tested activity of compound **11** in an ex vivo ring contraction assay (Figure 3). This assay measures the force generated by mouse tracheal rings in response to increasing doses of the contractile agonist methacholine. The advantage to this functional assay is the ability to see the effect of integrin inhibition on a tissue level. We observed that treatment with the inhibitor caused a dose-dependent decrease in cytokine-enhanced contraction. It is also worth noting that inhibition of contraction was observed even in the concentration lower than IC_{50} in cell adhesion assay.

Unfortunately, our inhibitors showed suboptimal pharmacokinetic properties. For example, one of our promising compounds **15** showed limited solubility and poor permeability in Caco-2 cell assay (P_{app} (A-B/B-A, $x10^{-6}$ cm/s) = 0.37/0.27). Since airway smooth muscle resides under epithelial layer of the lung, compounds with low cell permeability are not favored for *in vivo* assessment in mouse mode. Further optimization to improve physicochemical and pharmacokinetic properties is in progress. In summary, highly potent dual inhibitors for α5β1/αvβ1 integrin based on DAP were identified. Excellent selectivity over αvβ3 integrin by boosting α5β1 potency was achieved using an adamantane moiety and we hope this study could provide additional insight to design better inhibitors against α5β1 and αvβ1 integrin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We (W.F.D. and D.S) would like to thank NIH (UH2HL123423) as well as UCCAI program (U54HL119893 CFDA NO.93.837/UCSF RAS Award# 122727) for support of this work. We are also grateful for generous support from ShangPharma Innovation-UCSF collaboration.

References

1. Postma DS; Kerstjens HA, Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998, 158 (5 Pt 3), S187–92. [PubMed: 9817744]
2. Bateman ED; Hurd SS; Barnes PJ; Bousquet J; Drazen JM; FitzGerald JM; Gibson P; Ohta K; O'Byrne P; Pedersen SE; Pizzichini E; Sullivan SD; Wenzel SE; Zar HJ, Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008, 31 (1), 143–78. [PubMed: 18166595]
3. Antonicelli L; Bucca C; Neri M; De Benedetto F; Sabbatani P; Bonifazi F; Eichler HG; Zhang Q; Yin DD, Asthma severity and medical resource utilisation. Eur Respir J 2004, 23 (5), 723–9. [PubMed: 15176687]
4. Barnes PJ, Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2018, 18 (7), 454–466. [PubMed: 29626211]
5. Eger KA; Bel EH, The emergence of new biologics for severe asthma. Curr Opin Pharmacol 2019, 46, 108–115. [PubMed: 31229937]
6. Sundaram A; Chen C; Khalifeh-Soltani A; Atakilit A; Ren X; Qiu W; Jo H; DeGrado W; Huang X; Sheppard D, Targeting integrin alpha5beta1 ameliorates severe airway hyperresponsiveness in experimental asthma. J Clin Invest 2017, 127 (1), 365–374. [PubMed: 27918306]
7. Bonnans C; Chou J; Werb Z, Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014, 15 (12), 786–801. [PubMed: 25415508]
8. Hynes RO, Integrins: bidirectional, allosteric signaling machines. Cell 2002, 110 (6), 673–87. [PubMed: 12297042]
9. Livant DL; Brabec RK; Pienta KJ; Allen DL; Kurachi K; Markwart S; Upadhyaya A, Anti-invasive, antitumorigenic, and antimetastatic activities of the PHSCN sequence in prostate carcinoma. Cancer Res 2000, 60 (2), 309–20. [PubMed: 10667582]
10. Kim S; Bell K; Mousa SA; Varner JA, Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. Am J Pathol 2000, 156 (4), 1345–62. [PubMed: 10751360]
11. Delouvrie B; Al-Kadhimi K; Arnould JC; Barry ST; Cross DA; Didelot M; Gavine PR; Germain H; Harris CS; Hughes AM; Jude DA; Kendrew J; Lambert-van der Brempt C; Lohmann JJ; Menard M; Mortlock AA; Pass M; Rooney C; Vautier M; Vincent JL; Warin N, Structure-activity relationship of a series of non peptidic RGD integrin antagonists targeting alpha5beta1: part 1. Bioorg Med Chem Lett 2012, 22 (12), 4111–6. [PubMed: 22575869]
12. Delouvrie B; Al-Kadhimi K; Arnould JC; Barry ST; Cross DA; Didelot M; Gavine PR; Germain H; Harris CS; Hughes AM; Jude DA; Kendrew J; Lambert-van der Brempt C; Lohmann JJ; Menard M; Mortlock AA; Pass M; Rooney C; Vautier M; Vincent JL; Warin N, Structure-activity relationship of a series of non peptidic RGD integrin antagonists targeting alpha5beta1: part 2. Bioorg Med Chem Lett 2012, 22 (12), 4117–21. [PubMed: 22572578]
13. Zischinsky G; Osterkamp F; Vossmeyer D; Zahn G; Scharn D; Zwintscher A; Stragies R, SAR of N-phenyl piperidine based oral integrin alpha5beta1 antagonists. Bioorg Med Chem Lett 2010, 20 (1), 65–8. [PubMed: 19959360]
14. Stragies R; Osterkamp F; Zischinsky G; Vossmeyer D; Kalkhof H; Reimer U; Zahn G, Design and synthesis of a new class of selective integrin alpha5beta1 antagonists. J Med Chem 2007, 50 (16), 3786–94. [PubMed: 17616113]
15. Reed NI; Jo H; Chen C; Tsujino K; Arnold TD; DeGrado WF; Sheppard D, The alphavbeta1 integrin plays a critical in vivo role in tissue fibrosis. Sci Transl Med 2015, 7 (288), 288ra79.

Author Manuscript

Author Manuscript

Author Manuscript

16. Reed NI; Tang YZ; McIntosh J; Wu Y; Molnar KS; Civitavecchia A; Sheppard D; DeGrado WF; Jo H, Exploring N-Arylsulfonyl-l-proline Scaffold as a Platform for Potent and Selective alphavbeta1 Integrin Inhibitors. ACS Med Chem Lett 2016, 7 (10), 902–907. [PubMed: 27774126]

17. Peng Q; Lai D; Nguyen TT; Chan V; Matsuda T; Hirst SJ, Multiple beta 1 integrins mediate enhancement of human airway smooth muscle cytokine secretion by fibronectin and type I collagen. J Immunol 2005, 174 (4), 2258–64. [PubMed: 15699160]

18. Procopiou PA; Anderson NA; Barrett J; Barrett TN; Crawford MHJ; Fallon BJ; Hancock AP; Le J; Lemma S; Marshall RP; Morrell J; Pritchard JM; Rowedder JE; Saklatvala P; Slack RJ; Sollis SL; Suckling CJ; Thorp LR; Vitulli G; Macdonald SJF, Discovery of (S)-3-(3-(3,5-Dimethyl-1 H-pyrazol-1-yl)phenyl)-4-((R)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic Acid, a Nonpeptidic alphavbeta6 Integrin Inhibitor for the Inhaled Treatment of Idiopathic Pulmonary Fibrosis. J Med Chem 2018, 61(18), 8417–8443. [PubMed: 30215258]

19. Kargbo RB, Selective Estrogen Receptor Degraders for the Potential Treatment of Cancer. ACS Med Chem Lett 2020, 11 (4), 412–413. [PubMed: 32292542]

20. Wilkinson AL; Barrett JW; Slack RJ, Pharmacological characterisation of a tool alphavbeta1 integrin small molecule RGD-mimetic inhibitor. Eur J Pharmacol 2019, 842, 239–247. [PubMed: 30389632]

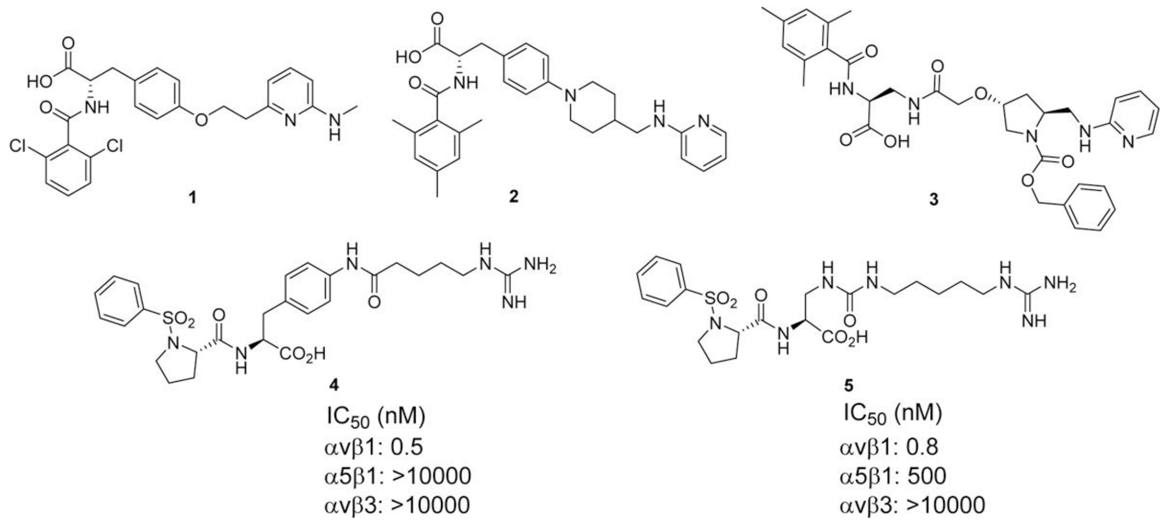


Figure 1.

Structures of α5β1 integrin inhibitors **1–3** and αvβ1 integrin inhibitors **4–5**

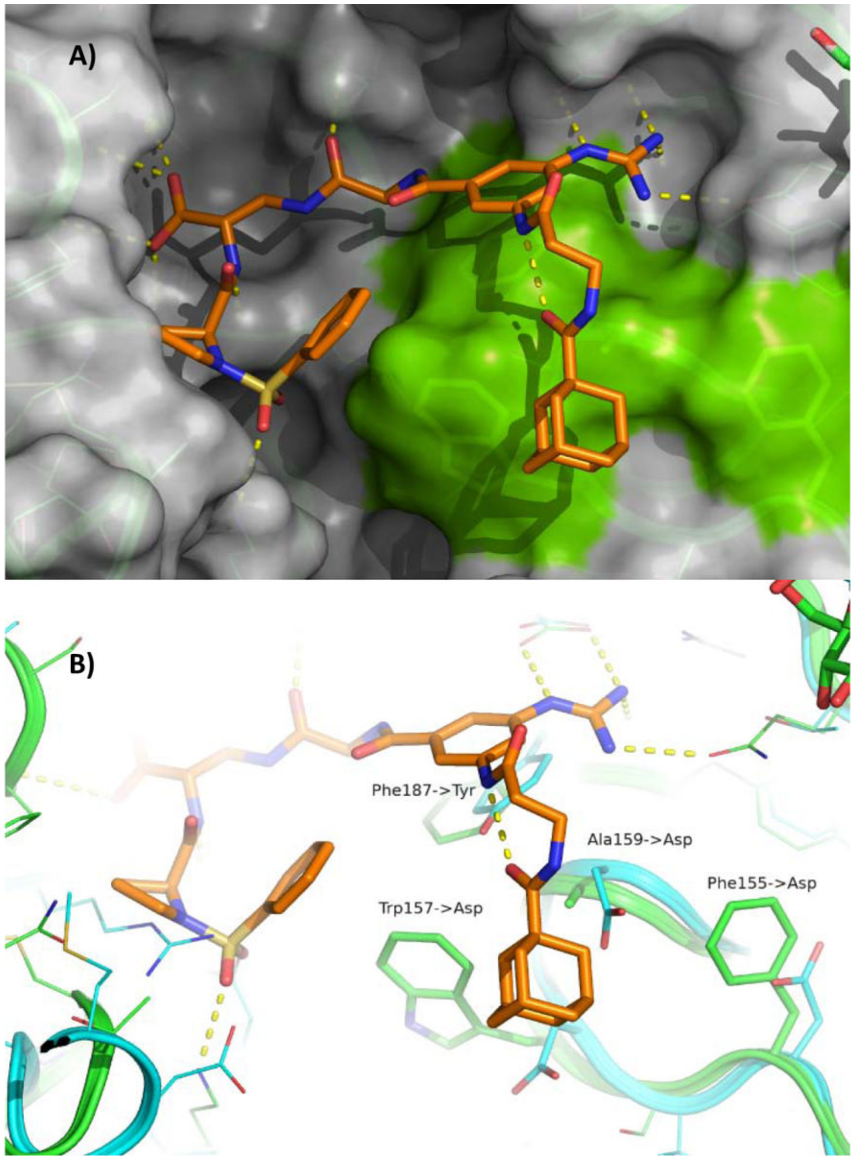


Figure 2. A model of 17 (orange) bound to α5β1.

The model is based on the published α5β1 structure 4wk0.pdb and has been minimized using MOE/CCG. A) Connelly surface of the model. The green patch shows selected hydrophobic side chains of α5: Phe155, Trp157, Ala159, & Phe187. B) Same α5β1 model (green) shown as above w/o surface. The cyan atoms represent the crystal structure of αvβ3, 3ije.pdb. Four hydrophilic substitutions are highlighted in the αv chain that attenuate the affinity for the adamantine group in **17**.

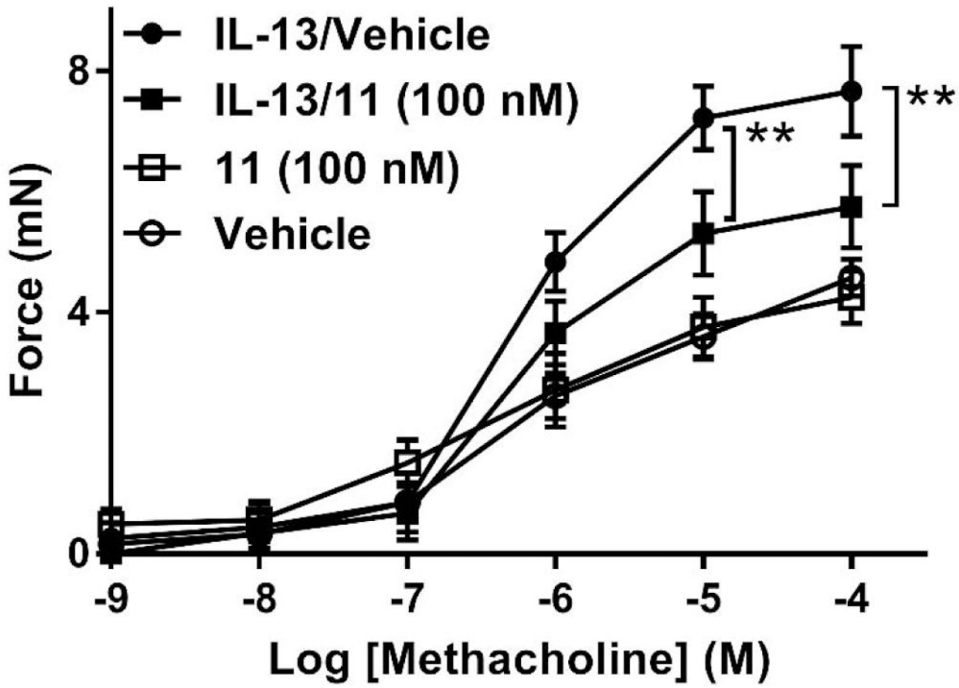
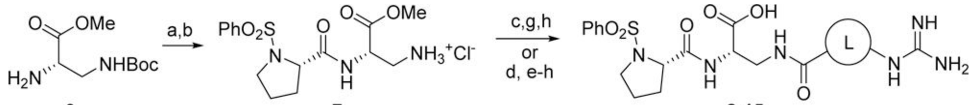


Figure 3. Mouse trachea ring contraction assay

Force exerted on WT mouse tracheal rings measured after incubation for 12 h with IL-13 (100 ng/mL), then 1 h with compound 11 or vehicle with a range of concentrations of methacholine. Negative controls without IL-13 treatment are also shown. n=3 rings per group. **P<0.01, repeated measures of variance.



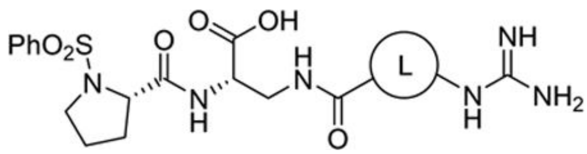
Scheme 1.

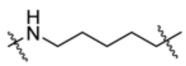
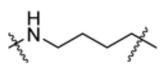
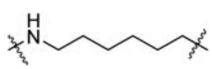
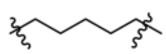
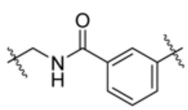
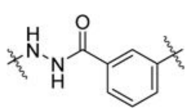
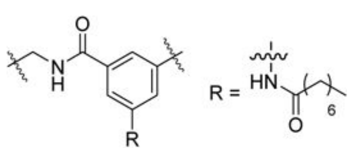
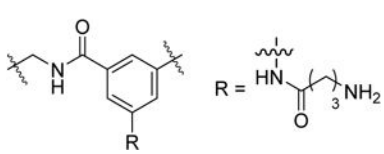
Synthesis of **8-15** *Reagents and Conditions:* a) N-benzenesulfonyl-L-proline, HCTU, DIPEA, b) 4M HCl in dioxane (70% for the two steps) c) $RNHCO_2PhNO_2$, DIPEA or $R'CO_2H$, HCTU, DIPEA, d) Boc-Gly, HCTU, DIPEA e) 4M HCl in dioxane f) $R''CO_2H$, HCTU, DIPEA g) TFA, DCM h) LiOH, THF-H_2O. See the Supplementary data for details of syntheses.

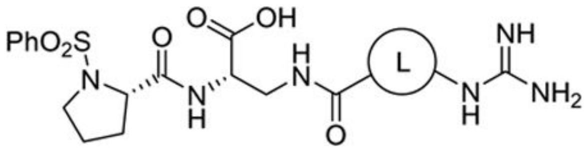
Table 1.

Cell adhesion assay for a5b1 integrin inhibitors

The measurement of cell adhesion was performed according to the published procedure.[15] SW480 plated on fibronectin (0.3 μg/ml) was used for α5β1 assay and CHOαv adhering to fibronectin (0.3 μg/ml) was used for αvβ1. For αvβ3, SW480 transfected with human β3 adhering to fibrinogen (1 μg/ml) was used. Data represent means ± S.D.; n = 3 or higher



ID	L	IC_{50} (nM) ± SD		
		α5β1	**αvβ1**	**αvβ3**
5		501±90	0.8±0.2	6,800±500
8		9,700±900	4±0.8	>100,000
9		900±150	0.9±0.2	15,000±930
10		830±20	316±80	10,000±790
11		40±18	32±8	1,100±200
12		50±20	158±20	6,500±900
13		110±24	Not tested	16,800±400
14		32±6	Not tested	56,000±2,000



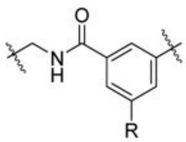
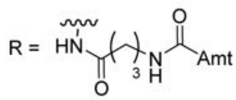
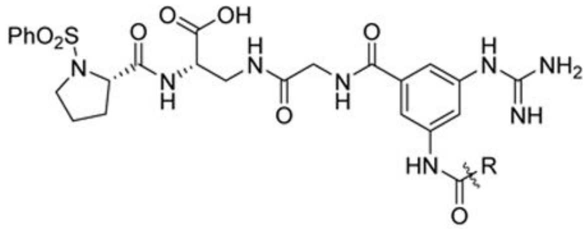
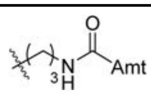
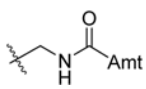
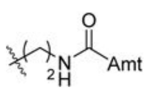
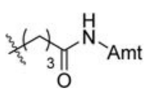
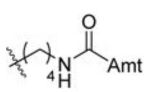
ID	L	IC$_{50}$ (nM) ± SD		
		α5β1	αvβ1	αvβ3
15	 	2.6±0.9	0.8±0.1	8,800±15

Table 2.

Solid phase binding assay of adamantane analogs 15–19.

See the Supplementary data for details. Data represent means ± S.D.; n = 3



		IC50 (nM) ± SD						
ID	**R**	**α5β1**	**αvβ1**	**αvβ3**	**αvβ5**	**αvβ6**	**αvβ8**	**α4β1**
15		0.8±0.2	0.14±0.01	>10000	3002±1240	2762±84	>10000	105±12
16		1.6±0.1	< 0.1	>10000	>10000	>10000	>10000	136±47
17		1.4±0.1	< 0.1	>10000	>10000	>10000	>10000	100±30
18		1.0±0.1	0.3±0.2	>10000	980±320	>10000	>10000	161±32
19		0.7±0.3	< 0.1	>10000	320±1.8	1867±56	6832±470	182±44