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Design and Synthesis of Potent and Selective Inhibitors of Integrin VLA-4

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Abstract—The synthesis and identification of a novel series of inhibitors of integrin VLA-4 are described. Their in vitro activity and selectivity against closely related integrins are also presented.

Antagonism of cell surface receptors belonging to the integrin superfamily is a new paradigm for drug discovery in transplantation and cancer, as well as in other diseases.[1] Integrin VLA-4 is an attractive therapeutic target: blockade of integrin VLA-4 by monoclonal antibodies (mAbs) has shown clear-cut efficacy in animal models of autoimmune disease, asthma, inflammation, and acute and chronic rejection of allogeneic grafts.[2,3] VLA-4 antagonists act to block leukocyte migration as well as leukocyte activation on the cell types thought to mediate these diseases. VLA-4 is expressed on memory T cells, memory B cells, NK cells, monocytes and macrophages. Although orally active antagonists of the platelet integrin receptor are in advanced development,[1d] a lack of similar progress is evident in the discovery of orally active low molecular weight (LMW) antagonists of the important leukocyte integrins such as VLA-4, despite their validation as targets by mAbs. Because of the importance of this integrin and its implication in various disease processes, efforts toward the design and synthesis of orally bioavailable LMW inhibitors have intensified in recent years.[4]

Herein, we describe the identification of potent and selective inhibitors of VLA-4 based on the known bisarylurea series[5] of LDV peptidomimetics. Although potent inhibition in the low nanomolar range was achieved with the progenitor members of the series, such as **1** and **2**,[5] concerns with their peptidic features led us to make various structural modifications (Scheme 1). We have successfully generated antagonists that retain nM potency, but are structurally simpler than the original leads.

Scheme 2 outlines the general approach to aza analogues **3**–**6**. Thus, *t*-butyl carbazate was reductively alkylated to give **15**. Coupling of **15** with the isocyanate **16**, which was derived from racemic ethyl 3-amino-3-phenylpropanoate, gave **17**. Deprotection of the *t*-BOC group, acylation with the bisarylurea acid **18**[5c] and hydrolysis of the ethyl ester finally resulted in the aza analogue **3**. Aza analogues **4** and **5** were similarly prepared from racemic ethyl 3-amino-4-hexenoate and ethyl valine, respectively. The aza analogue **6** was prepared as shown in Scheme 2. Sequential alkylation of *t*-butyl carbazate with ethyl bromoacetate and allyl bromide gave **19**. Removal of the *t*-BOC protecting group followed by coupling with *N*-*t*-BOC-L-leucine and deprotection afforded **20**. Acylation with the bisarylurea acid **18** and hydrolysis of the ester gave **6**.

The general route to β-aminoacid analogues (**7**–**10**) is illustrated by the synthesis of **7** in Scheme 3. Condensation of **18** with 1,1-dimethylethyl (3*R*)-3-amino-5-methylhexanoate followed by removal of the *t*-butyl ester with trifluoroacetic acid provided **21**. Standard coupling of the acid **21** with 1,1-dimethylethyl (3*S*)-3-

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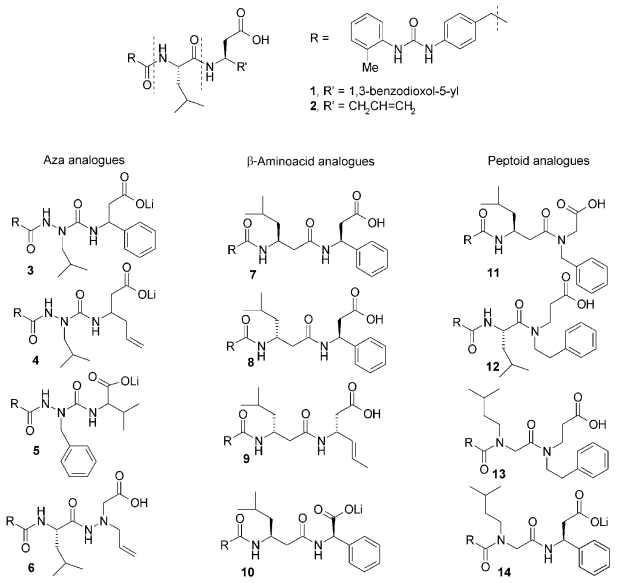
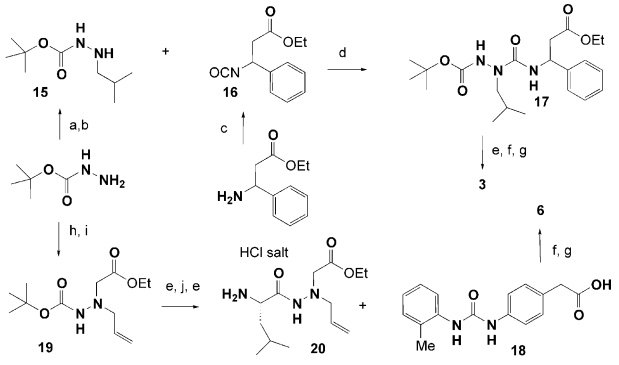
amino-3-phenylpropanoate and deprotection of the *t*-butyl ester gave **7**. The epimer **8** and other analogues (**9** and **10**) were analogously prepared in high yields.

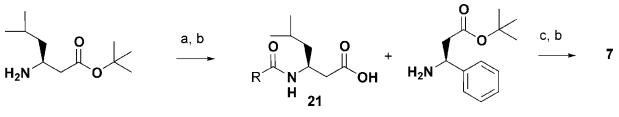
Peptoid analogue **11** was also prepared according to Scheme 3 by coupling *N*-benzylglycine ethyl ester with **21** (DIEA, EDC, CH_2Cl_2, rt, 48 h) followed by hydrolysis (LiOH, aq THF, rt, 2 h) and acidification. Other analogues were prepared as outlined in Scheme 4. Michael reaction of phenethylamine with ethyl acrylate in methanol at room temperature for 4 h cleanly gave



Scheme 1. Structural modifications of VLA-4 inhibitors.

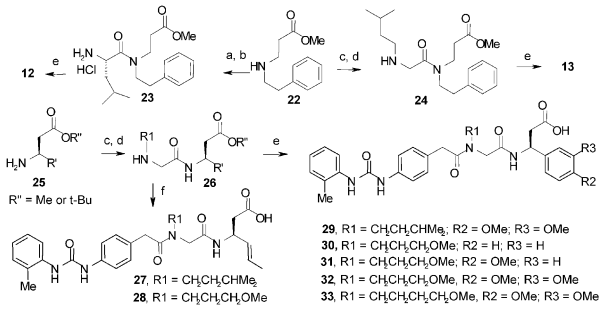


Scheme 2. (a) Isobutyraldehyde, THF, 24 h; (b) H_2, platinum/carbon, MeOH, rt, 12 h, 74%; (c) HCl gas, 0.5 h, then phosgene, rt, 12 h; (d) CH_2Cl_2, rt, 12 h, 54%; (e) HCl gas, Et_2O, 0 °C to rt, 1 h, quant. (f) **18**, DIEA, EDC, CH_2Cl_2, rt, 12 h, 34–57%; (g) LiOH, THF, H_2O, rt, 12 h, quant. (h) ethyl bromoacetate, Et_3N, DMF, rt, 12 h, 24%; (i) allyl bromide, Et_3N, DMF, rt, 12 h, 90%; (j) DIEA, EDC, CH_2Cl_2, rt, 12 h, 39%.



Scheme 3. (a) **18**, DIEA, EDC, CH_2Cl_2, rt, 12 h, 97%; (b) TFA, CH_2Cl_2, thioanisole, rt, 20 min, 90%; (c) DIEA, EDC, CH_2Cl_2, rt, 12 h, quant.

22, which was coupled with *N*-*t*-BOC-L-leucine hydroxysuccinate ester to give **23**, after removal of the *t*-BOC group. Acylation of **23** with bromoacetyl bromide and reaction with isoamylamine gave **24**. Acylation of **23** and **24** with **18** followed by ester hydrolysis gave **12** and **13**, respectively. Acylation of the β-aminoacid ester **25** with bromoacetyl bromide followed by alkylation with an amine gave **26**. Thus, compounds **14** and **27** were prepared in high yields from commercially available 1,1-dimethylethyl (3*S*)-3-amino-3-phenylpropanoate and 1,1-dimethylethyl (3*S*)-3-amino-hexenoate via the intermediate **26**. Analogues **28**–**33** were similarly synthesized from the ester of β-aminoacid **25**[6] in good yields.



Scheme 4. (a) *N*-*t*-BOC-L-leucine hydroxysuccinate ester, CH_2Cl_2, reflux, 8 h; (b) HCl gas, Et_2O, 0°C to rt, 1 h; (c) bromoacetyl bromide, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 86–97%; (d) the amine (isoamylamine or 3-methoxypropylamine or 4-methoxybutylamine), Et_3N, DMF, rt, 1 h, 70–82%; (e) **18**, DIEA, EDC, CH_2Cl_2, rt, 12 h, 67–97% then LiOH, THF, H_2O, rt, 2–3 h and HCl acidification, 95–99% (f) **18**, DIEA, EDC, CH_2Cl_2, rt, 12 h, 67–97% then TFA, CH_2Cl_2, rt, 2–3 h, 95–99%.

Compounds were evaluated for VLA-4 inhibitory activity in a cell adhesion assay[5c,7] that is based on the binding of fluorescently labeled Ramos cells to immobilized VCAM-1. IC_{50} values are listed in Table 1. The aza analogues of the leucine moiety, **3** (IC_{50} = 2900 nM) and **4** (IC_{50} = 5900 nM), were about 175 and 350 times less active than the known inhibitor **2** (IC_{50} = 16.5 nM), when tested side-by-side in the same assay. Replacement of the β-aminoacid moiety with an α-aminoacid such as

Table 1. In vitro VLA-4 cell adhesion inhibitory activity (VCAM-1/Ramos cells)[7]

Compd	IC_{50} (nM)[a]	Compd	IC_{50} (nM)[a]
1	3[b]	**12**	37,000
2	16.5	**13**	98
3	2900	**14**	29
4	5900	**27**	45
5	>500,000	**28**	15.4
6	11,000	**29**	20
7	7300	**30**	11.7
8	262,000	**31**	4.7
9	103,000	**32**	7.1
10	6400	**33**	1.3
11	247,000	**34**[c]	75,000

[a]Each IC_{50} value is an average of 2–5 determinations run in triplicate.
[b]Reported IC_{50} data.[5]
[c]Compound **34** is the ethyl ester of **31**.

Table 2. Selectivity of selected inhibitors against related integrins (IC_{50} values in nM)[a]

Integrin/ligand	**28**	**30**	**31**	**32**
VLA-4/VCAM-1	15.4	11.7	4.7	7.1
VLA-4/FN	7.9	14.4	36	13.4
VLA-4(mouse)/VCAM-1	7.7	21.7	51.9	27.3
VLA-4(rat)/VCAM-1	20.5	24.5	27.2	21.6
$\alpha_4\beta_7$/VCAM-1	> 100,000	> 100,000	6,140	> 100,000
VLA-5/FN	> 500,000	> 500,000	> 500,000	> 500,000
Mac-1 (mouse)/ICAM-1	> 500,000	> 500,000	> 500,000	> 500,000
$\alpha_V\beta_3 + \alpha_V\beta_5$/VN	59,000	> 100,000	> 100,000	> 100,000

[a]Values represent the average of at least four experiments with side-by-side comparisons.

valine as in **5** resulted in an inactive compound ($IC_{50} > 500{,}000$ nM). The aza analogue of the β-aminoacid moiety of **2** as in **6** ($IC_{50} = 10{,}100$ nM) was less potent in comparison with **3** and **4**. The β-aminoacid analogues such as **7**, **8**, and **9**, in which the α-leucine moiety of **2** was replaced with the corresponding β-leucine residue, also exhibited very poor activity. Compound **7** ($IC_{50} = 7300$ nM), which has the same absolute configuration of the (*S*)-leucine side chain as in **2**, however, was about 14- to 36-fold more active than the (*R*)-β-leucine containing compounds **8** ($IC_{50} = 262{,}000$ nM) and **9** ($IC_{50} = 103{,}000$ nM). An attempt to improve the activity of **7** by introducing the α-aminoacid phenylglycine to compensate for an extra carbon on the β-leucine moiety led to the synthesis of **10** ($IC_{50} = 6400$ nM), but no improvement in activity was observed. Further loss in activity was also observed when the β-phenylglycine unit of **7** was replaced with a peptoid mimic as in **11** ($IC_{50} = 247{,}000$ nM). A hybrid containing an α-leucine and a peptoid as in **12** ($IC_{50} = 37{,}000$ nM) also did not significantly improve potency. The peptoid–peptoid hybrid **13** ($IC_{50} = 98$ nM), however, showed more than a 370-fold improvement in comparison with **12**. A further improvement in potency was finally obtained with **14**, the leucine peptoid and the β-aminoacid hybrid, with an IC_{50} value of 29 nM. This simple inhibitor containing only one chiral center, which is one chiral center less than the known starting compounds such as **1** and **2**, became the lead for further optimization.

Earlier work on LDV peptidomimetics suggested that an ether oxygen atom in the leucine mimic chain of **14** could have a profound effect in increasing inhibitory activity.[8] In addition, known SAR[5] on analogues of **1** and **2** indicated that the phenyl chain on the β-phenylglycine moiety is not optimal. In order to demonstrate that the ether-containing chain is important for the potency improvement of **14** and to find a better replacement of the phenyl group, analogues **27**–**33** were prepared. There was no improvement in potency with the propenyl compound **27** ($IC_{50} = 45$ nM). A slight improvement in potency was observed with the 3,4-dimethoxyphenyl analogue of **14**, **29** ($IC_{50} = 20$ nM). The methoxypropyl replacement of the leucine mimic chain of **14** and **27** as in **30** ($IC_{50} = 11.7$ nM) and **28** ($IC_{50} = 15.4$ nM), respectively, also provided a slight improvement in potency. However, replacement of both of the phenyl and isoamyl moieties in **14** provided a significant (4- to 6-fold) improvement in potency as in the case of compound **31** ($IC_{50} = 4.7$ nM) and **32** ($IC_{50} = 7.1$ nM). The methoxybutyl analogue **33** ($IC_{50} = 1.3$ nM) showed a slight improvement in activity. The (*S*)-absolute configuration of the β-aminoacid chiral center is important for potency. For example, the opposite (*R*)-enantiomer ($IC_{50} = 630$ nM) of **28** was more than 40-fold less active than the (*S*)-isomer **28**. As expected from other LDV mimics, the corresponding ethyl ester of **31** (**34**) was inactive ($IC_{50} = 75{,}000$ nM).

Additional studies were carried out with highly potent compounds such as **28**, **30**, **31**, and **32** to assess their selectivity against other closely related integrins[5c,7] and the results are summarized in Table 2. VLA-4 binds either VCAM-1 or the CS-1 domain of fibronectin[9] (FN). Therefore, it was necessary to assess the ability of these compounds to block VLA-4 dependent cellular binding to FN as well as to VCAM-1. All compounds potently blocked VLA-4 binding to FN. Cross-reactivity with murine or rat VLA-4 was assessed using cell adhesion assays based on the binding of murine or rat cells to immobilized human VCAM-1. The compounds effectively block mouse and rat VLA-4-mediated cell binding to VCAM-1 so that these compounds could be used in rodent models of disease. Compound **31** blocked mouse VLA-4 about 11-fold less effectively than human VLA-4, suggesting that studies in the mouse may underestimate the activity of the compound in humans.

Selectivity of these compounds for VLA-4 against other integrins was demonstrated using cell adhesion assays for integrins $\alpha_4\beta_7$, VLA-5, $\alpha_V\beta_3$ and $\alpha_V\beta_5$, and Mac-1. They were found to be selective for integrin VLA-4. Compound **31** also weakly blocked integrin $\alpha_4\beta_7$ ($IC_{50} = 6140$ nM), but more than 1300-fold less effectively than integrin VLA-4 ($IC_{50} = 4.7$ nM). Thus, the selectivity of these compounds against the homologous integrin $\alpha_4\beta_7$ are far much better than the bisarylurea series of peptide analogues.[5c] Compound **28** reacted weakly with α_V-integrins, but was about 3800-fold more selective for VLA-4. Other compounds did not block integrin α4β7 or α_V-integrins at concentrations up to 100 μM. No inhibition of VLA-5- or Mac-1-dependent adhesion was observed with these compounds at concentrations up to 500 μM.

In conclusion, we have identified a new structural class of non-peptidic compounds as potent and selective inhibitors of VLA-4. The potent compounds reported herein provide not only useful leads for further development, but also an important tool for evaluating the role of VLA-4 blockade by LMW compounds in inflammation and immune responses in animal models. The results of their pharmacokinetic and pharmacological studies will be forthcoming.

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