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Isoxazolyl, Oxazolyl, and Thiazolylpropionic Acid Derivatives as Potent $\alpha_4\beta_1$ Integrin Antagonists

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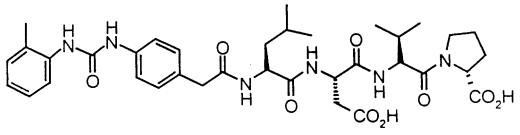
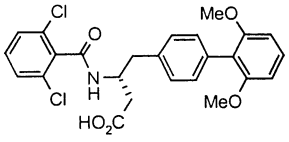
Abstract—A series of isoxazolyl, oxazolyl, and thiazolylpropionic acid derivatives derived from LDV was found to be a potent antagonist of the $\alpha_4\beta_1$ integrin. The synthesis and SAR leading up to 3-[3-(1-{2-[3-methoxy-4-(3-*o*-tolyl-ureido)-phenyl]-acetyl-amino}-3-methyl-butyl)-isoxazol-5-yl]-propionic acid (**22**) are reported. In an allergic mouse model, compound **22** was efficacious delivered systemically (58% inhib @ 10 mg/kg, sc) as well as by intra-tracheal instillation ($ED_{50} = 2$ μg/kg).

The integrin $\alpha_4\beta_1$ or very late antigen-4 (VLA-4) is constitutively expressed on the surface of all leukocytes (lymphocytes, monocytes, eosinophils, and basophils).[1] It binds to vascular cell adhesion molecule-1 (VCAM-1) on activated endothelial cells resulting in migration of cells out of the vasculature at sites of inflammation. In addition, once cells have reached the extravascular space, VLA-4 can bind to the CS-1 region on fibronectin resulting in enhanced leukocyte activation and release of inflammatory mediators.[2,3] Antibodies to α_4 have blocked leukocyte infiltration in a variety of animal models of human disease (asthma, multiple sclerosis, inflammatory bowel disease, etc.)[4–6] as well as in multiple sclerosis in man.[7] Small peptide and nonpeptide $\alpha_4\beta_1$ antagonists have similarly shown efficacy in disease models of inflammation (**BIO1211**[8] and **TR-14035**[9]). Thus, antagonism of $\alpha_4\beta_1$ represents a well validated target for the treatment of inflammatory disorders.

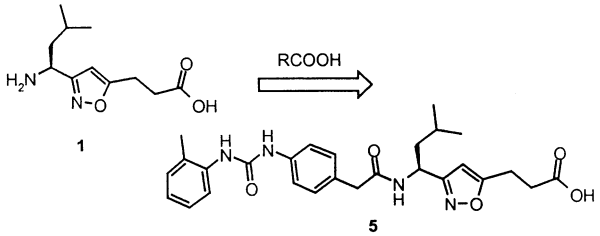
Humphries reported LDV to be the minimal essential binding sequence of the CS-1 region on fibronectin.[10] Subsequent reports by others have revealed potent $\alpha_4\beta_1$ antagonists by capping the amino terminus of LDV with substituted phenylacetyl groups, and capping the carboxy terminus with various amino acids (e.g., **BIO1211**).[11] In efforts to obtain an orally available $\alpha_4\beta_1$ antagonist we modified the LDV trimer by tying the aspartic acid moiety into an isoxazole ring and removing the valine group, thus reducing the number of metabolically labile amide bonds (**1**). The evaluation of several libraries in which the amino terminus of **1** was coupled with various carboxylic acids led us to the same diphenylurea substituent (**5**) that others have reported.[11]



BIO1211



TR-14035



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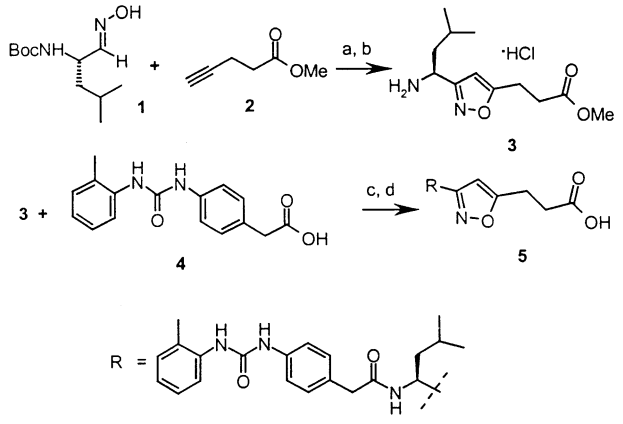
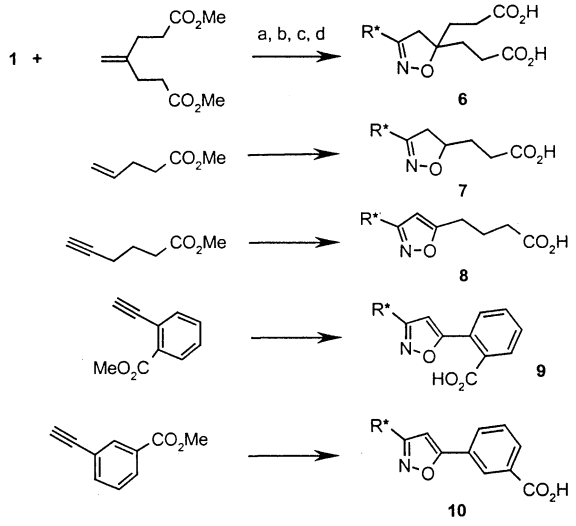
The preparation of isoxazole **5** began with the reaction of oxime **1**[12] with sodium hypochlorite[13] in the presence of the terminal alkyne **2** to form the appropriate isoxazole ring, which was subsequently deprotected with HCl in dioxane to give amine **3** as a hydrochloride salt (Scheme 1). EDCI coupling of **3** with carboxylic acid **4** followed by saponification with 1 N NaOH in *tert*-BuOH provided **5** in good yield. In a similar fashion, oxime **1** was coupled to various other olefins and alkynes to give compounds **6**–**10** (Scheme 2).

The synthesis of the oxazole and thiazole analogues of **5** is presented in Scheme 3. *N*-Cbz-L-leucine was coupled with 5-aminolevulinic acid methyl ester hydrochloride (**11**) in the presence of EDCI to provide the amide intermediate **12**, which was then treated with either phosphorus oxychloride[14] to give oxazole **13**, or Lawesson's reagent to give thiazole **15**. Compounds **13** and **15** were then converted to **14** and **16**, respectively, by Pd catalyzed hydrogenation to remove the Cbz group followed by EDCI coupling of the resulting amine with **4**, and subsequent saponification. Carboxylic acid **16** was converted to carboxamide **17** by reaction with isobutyl chloroformate followed by ammonia.[15]

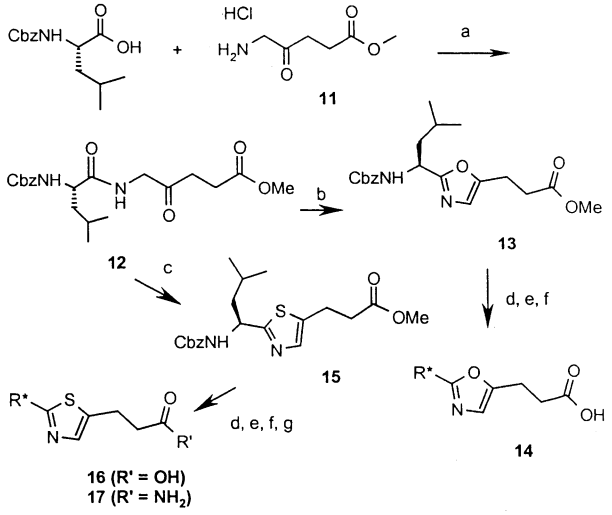
The preparation of thiazolylacetic acid **19** from **18**[16] was analogous to the synthesis of thiazolylpropionic acid **16** from **12**, and is presented in Scheme 4. The synthesis of the regioisomeric analogues of **16** and **19** (**21** and **20**, respectively) is presented in Scheme 5. The reaction of the thioamide of *N*-Cbz-L-leucine with ethyl 4-chloro-3-oxobutyrate in acetone followed by the addition of trifluoroacetic anhydride and pyridine[17] resulted in the desired thiazolyacetate precursor to **20**, but in low yield. Likewise, reaction with ethyl 5-chloro-4-oxopropionate in ethanol in the presence of potassium carbonate at reflux[18] gave the desired thiazolylpropionate precursor to **21** in 33% yield.



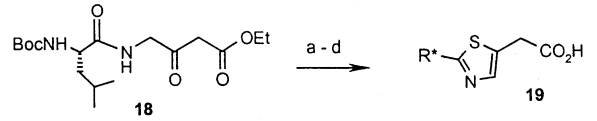
Scheme 1. (a) 5% NaOCl, TEA, CH_2Cl_2 (29%); (b) 4 M HCl in dioxane, rt, 1 h (99%); (c) HOBT, EDCI, DIEA, DMF, rt, 16 h; (d) 1:1 1 N NaOH, *tert*-BuOH.



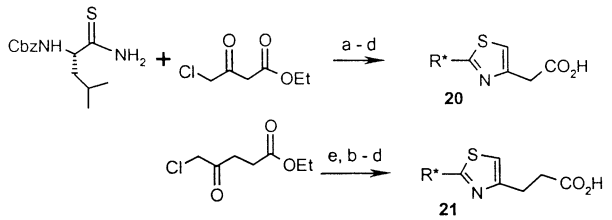
Scheme 2. (a) 5% NaOCl, TEA, CH_2Cl_2; (b) 4 M HCl in dioxane, rt, 1 h; (c) **4**, HOBT, EDCI, DIEA, DMF, rt, 16 h; (d) 1:1 1 N NaOH, *tert*-BuOH; * see Scheme 1 for definition of R.



Scheme 3. (a) HOBT, EDCI, DIEA, DMF, rt, 16 h; (b) $POCl_3$, toluene, reflux (35%); (c) Lawesson's reagent, toluene, reflux (41%); (d) H_2, Pd/C, EtOAc; (e) **4**, HOBT, EDCI, DIEA, DMF, rt, 16 h; (f) 1:1 1 N NaOH, *tert*-BuOH; (g) *N*-methylmorpholine, DME, isobutyl chloroformate, −10 °C, 15 min; then NH_3(g), 5 min; * see Scheme 1 for definition of R.



Scheme 4. (a) Lawesson's reagent, toluene, reflux; (b) 4 M HCl in dioxane, rt, 1 h; (c) **4**, HOBT, EDCI, DIEA, DMF, rt, 16 h; (d) 1:1 1 N NaOH, *tert*-BuOH; * see Scheme 1 for definition of R .



Scheme 5. (a) Acetone, rt, 4 h, then trifluoroacetic anhydride, pyridine, −10 °C, rt, 16 h (18%); (b) H_2, Pd/C, EtOAc; (c) **4**, HOBT, EDCI, DIEA, DMF, rt, 16 h; (d) 1:1 1 N NaOH, *tert*-BuOH; (e) K_2CO_3, EtOH, reflux, 5 days (33%); * see Scheme 1 for definition of R.

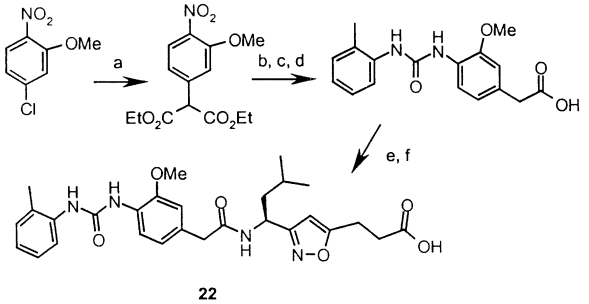
Table 1. In vitro potency of $\alpha_4\beta_1$ integrin antagonists

Compd	Receptor/ligand ELISA IC_{50} (nM)[a]	Jurkat cell IC_{50} (nM)[a]	Human eosinophil IC_{50} (nM)[a]
5	48±20 (9)	27±7 (13)	143±72 (6)
6	8 (2)	67 (2)	347±115 (3)
7	20 (1)	88 (1)	495±158 (3)
8	500 (1)	1800 (2)	nt
9	nt	na	nt
10	nt	na	nt
14	3±1 (3)	45±13 (8)	171±20 (3)
16	0.25±0.06 (4)	16±5 (5)	180±57 (8)
17	na	nt	nt
19	nt	2400 (1)	nt
20	800 (1)	nt	nt
21	800 (1)	2040 (1)	nt
22	10.3 (1)	0.69 (2)	1.0 (2)
BIO1211	2.6±1.0 (3)	4.3±2.0 (4)	97±47 (3)
TR-14035	nt	9.8 (1)	140±110 (3)

[a]Values±standard error are means of the number of experiments in parentheses (na = 0% inhib at 1 μM; nt = not tested).

The synthesis of **22**, an *ortho*-methoxyphenylurea analogue of **5**, is shown in Scheme 6. 4-Chloro-2-methoxynitrobenzene was added to a preformed mixture of sodium hydride and diethylmalonate in DMF to give the desired 4-nitro-3-methoxyphenylmalonate, which was reduced by means of Pd catalyzed hydrogenation. The resulting amine was treated with *o*-tolyl isocyanate followed by saponification, EDCI coupling with **3**, and final saponification to provide **22**.

Compounds were screened for VLA-4 antagonist activity in a cell-based assay in which calcein labeled Jurkat cells were allowed to bind to human recombinant sVCAM-1 in the presence of serial dilutions of test compounds. All active compounds were then profiled in a receptor/ligand ELISA assay to confirm that the compounds site of action is on VLA-4. In this assay, VLA-4 isolated from Jurkat cells was plated in 96-well plates and incubated with serial dilutions of compound together with the biotinylated CS-1 fragment of fibronectin. Binding was detected with streptavidin-alkaline phosphatase. Compounds that were active in both assays were then profiled in a human eosinophil cell-binding scintillation proximity assay (SPA). In this assay, eosinophils were freshly isolated from healthy donors, labeled with ^{3}H-arachidonic acid and incubated with VCAM-1 coated SPA beads in the presence of serial dilutions of compound.



Scheme 6. (a) Diethylmalonate, NaH, DMF, 90 °C, 16 h; (b) H_2, Pd/C, EtOAc; (c) *o*-tolyl isocyanate, dichloromethane, TEA; (d) NaOH, *tert*-BuOH, reflux; (e) **3**, HOBT, EDCI, DIEA, DMF, rt, 16 h; (f) NaOH, *tert*-BuOH.

As shown in Table 1, isoxazole **5** was found to be a potent $\alpha_4\beta_1$ antagonist in the ELISA (IC_{50} = 48 nM), Jurkat cell (IC_{50} = 27 nM), and human eosinophil (IC_{50} = 143 nM) assays. The isoxazole ring (**5**) could be replaced with other five-membered heterocyclic rings such as isoxazoline (**7**), isoxazole (**14**), and thiazole (**16**) without effecting the potency. Interestingly, isoxazolines **6** and **7** were equipotent, implying that one of the propionic acid groups in **6** is not involved with binding. The regiochemical placement of the propionic acid was found to be important, as can be seen with the greatly reduced potency of thiazolylpropionic acid **21**. Modification of the length of the propionic acid side chain was detrimental to potency (**8** and **19**). Compound **22**, a methoxy analogue of **5**, was found to be quite potent (IC_{50} = 1 nM) in both the Jurkat cell and human eosinophil assays. Moreover, **22** was found to be 100× more potent than both **BIO1211** and **TR-14035** against human eosinophils.

Compounds **5**, **16**, and **22** were evaluated in an allergic mouse model,[19] measuring the compound's effect on BALF eosinophil influx in response to antigen challenge.[20] Compound **22** was found to be efficacious following systemic administration (58% inhib @ 10 mg/kg, sc).[21] When dosed by intra-tracheal instillation, compounds **5**, **16**, and **22** had ED_{50}'s of 20, 100, and 2 μg/kg, respectively.[21] At similar doses, **BIO1211** was ineffective.

In conclusion, we have developed a novel series of $\alpha_4\beta_1$ integrin antagonists that were derived from LDV. Compound **22** is a representative analogue that has nanomolar potency in the human eosinophil assay, is stable in human plasma and is efficacious in an in vivo allergic mouse model. Future directions continue to focus on improving oral bioavailability.

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