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Influence of acid surrogates toward potency of VLA-4 antagonist

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Abstract—A series of VLA-4 antagonist were synthesized wherein carboxylic acid was replaced by various acid surrogates. The effect of these acid surrogates toward potency was evaluated in a binding assay. A number of acid surrogates were potent antagonist of VLA-4, albeit significantly less potent than the corresponding carboxylic acid. Heterocyclic acid surrogate, oxadiazolidinone **3**, demonstrated an improved pharmacokinetic property when dosed intravenously.

The adhesion molecule VLA-4 (very late activating antigen-4, α4β1, CD49d/CD29) is a member of the integrin family and is expressed on all circulating leukocytes except platelets.[1] VLA-4 is a receptor for vascular cell adhesion molecule-1 (VCAM-1), which is expressed on endothelial surface. This interaction is important for the activation, migration, and proliferation of leukocytes during normal and pathophysiological processes.[2] It has been shown that anti-VLA-4 antibodies or VLA-4 antagonists[3] inhibit leukocyte infiltration to extravascular tissue and prevent tissue damage in animal models of asthma,[4] multiple sclerosis (MS),[5] rheumatoid arthritis (RA), and inflammatory bowel disease (IBD).[6] In addition, a humanized monoclonal anti-α_4 antibody (Natalizumab) has shown efficacy for MS and Crohn's disease in clinical trials.[7] Hence, there is a significant interest in the development of small molecules VLA-4 antagonist for the treatment of these diseases.

To date, the small molecule VLA-4 antagonists reported in literature are primarily characterized by carboxylic acids (Fig. 1).[8] Removal of carboxylic acid moiety or replacement by alcohol leads to complete loss of activity. Furthermore, these carboxylic acids are often characterized by poor PK, high protein binding, and significant shifts in potency in the presence of human serum albumin. There are several examples in the literature from other targets, wherein acid surrogates have provided inhibitors with similar potency with improved PK properties.[9] In our laboratory, we undertook efforts to evaluate the effect of acid surrogates toward potency of VLA-4 antagonists.

The acid surrogates were chosen based on pK_a properties that were similar to carboxylic acids.[10] Oxadiazolidinone **3** was synthesized from the corresponding dipeptide carboxylic acid **1** by converting the acid to nitrile **2** under dehydrating conditions. The nitrile **2** was then allowed to react with hydroxylamine and subsequent cyclization with 2-ethylhexyl chloroformate to afford oxadiazolidinone **3** (Scheme 1). Tetrazole **4** was obtained by reaction of the nitrile intermediate with an azide.

The 3-hydroxyisooxazole acid surrogate **7** was synthesized from the corresponding dipeptide β-ketoester **6**.[11] The β-ketoester **6** was cyclized with hydroxyl amine which upon treatment with concentrated hydrochloric acid yielded compound **7** (Scheme 2). The β-ketoester was also condensed with hydrazine in ethanol to yield 3-pyrrazol **8**. In addition, *N*-trifluoroacetamide **9** and *N*-trifluoromethanesulfonamide **10** were synthesized by reaction of the corresponding amide with trifluoroacetic anhydride and trifluoromethanesulfonyl chloride, respectively.

The seldom used acid surrogate 1,2,4-oxadiazolidone-2,5-dione was easily incorporated into a α-amino acid

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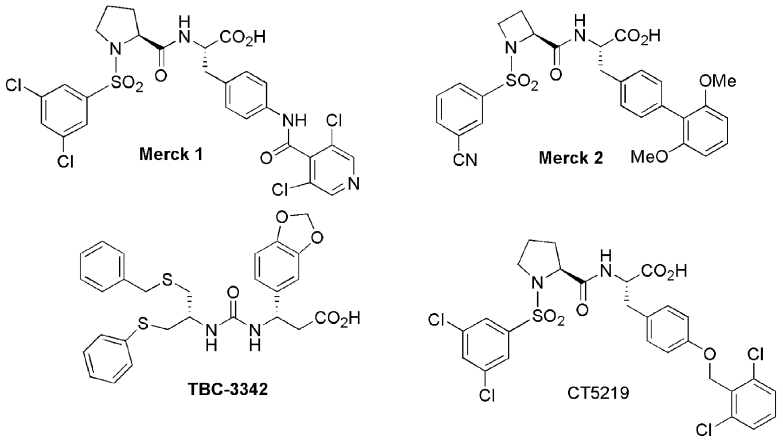
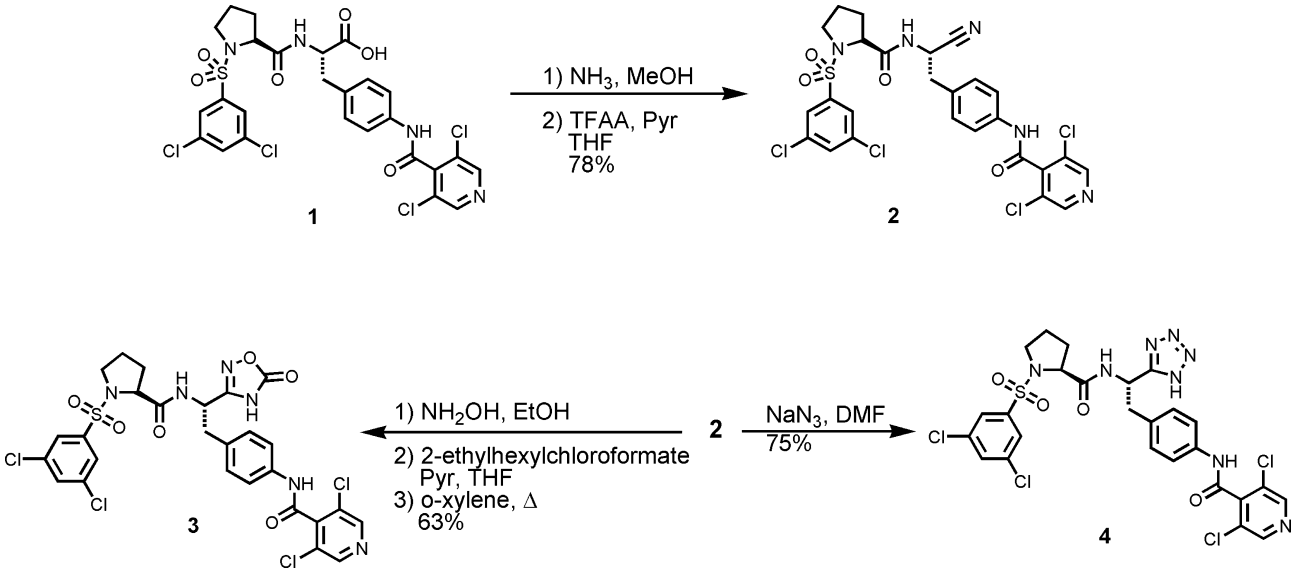
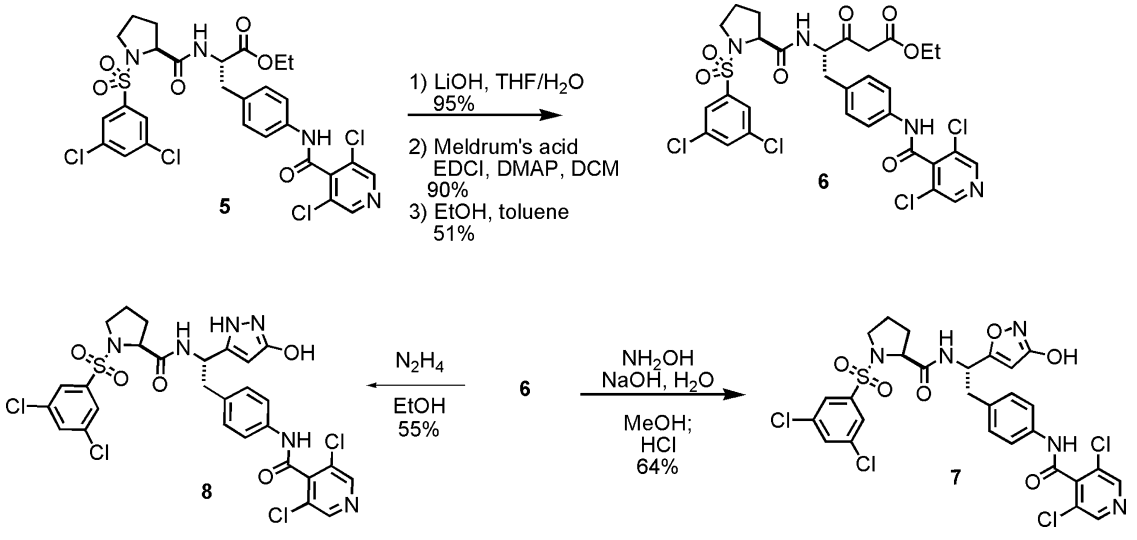


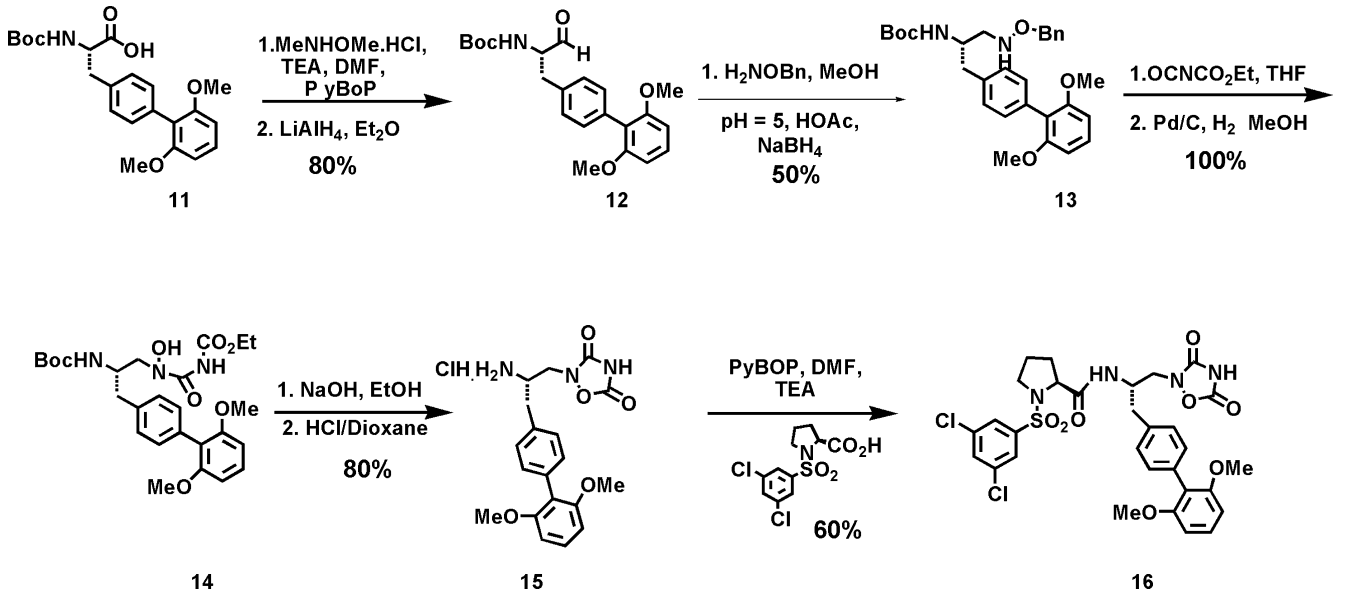
Figure 1. VLA-4 antagonist.



Scheme 1.

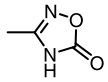
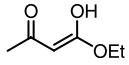
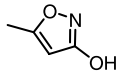
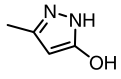
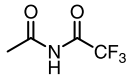
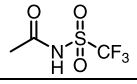


Scheme 2.



Scheme 3.

Table 1. Potency and relative off-rate for acid surrogates as inhibitors of VLA-4

Compound	R	Calc. pK_a[10]	BURJ (IC_{50nM})	ROR[a] % bound at 1 h
1		4.0	0.03	66
3		6.5	12	0
4		5.3	60	0
6		8.6	13	10
7		4.6	20	8
8		6.2	300	6
9		8.1	10	0
10		0.35	11	0

[a] ROR refers to the amount of compound bound to the receptor and measured as competition between binding ligand and the acid surrogates.

11 to yield **16** as described in literature[12] and is shown in Scheme 3.

The acid surrogates were evaluated as inhibitors using binding assay of the non-activated state of VLA-4 on Jurkat cells (BURJ assay).[13] A secondary assay measuring the relative off-rate under the non-activating state at 1 h was carried out to further distinguish them (Table 1). In general, acid surrogates **3**, **6**, **7**, **9**, and**10** were found to be potent, albeit significantly shifted compared to the parent carboxylic acid **1**. In the heterocyclic series, 1,2,4-oxadiazolidinone **3** and 3-hydroxyisoxoazole **7** were more potent than the most often used acid surrogate tetrazole **4**. In the acylic series, β-ketoester **6**, trifluoroacetamide **9**, and trifluoromethanesulfonamide **10** had potency similar to **3** and **7** but significantly less potent than **1**. The inhibitory potency of 1,2,4-oxadiazolidone-3,5-dione **16** (BURJ, IC_{50} = 6 μM) was found to be 3000-fold less than that of the corresponding carboxylic acid (BURJ, IC_{50} = 2 nM).

When compared to the parent carboxylic acid **1**, the acid surrogates demonstrated poor affinity for the receptor as evidenced by its relatively poor off-rate. The differences between the volume, dipole moment and polarizability of the carboxylic acid, and those of the acid surrogates may account for the differences in the binding affinity.

The heterocyclic acid surrogates were further evaluated for their pharmacokinetic properties in rats to determine

Table 2. PK of acid surrogates in rats

Compound	IV-AUC (μM h)	V_d (L/kg)	$t_{1/2}$ (h)[a]	Cl (ml/min/kg)	% F[b]	C_{max} (nM)[b]
1	0.68	0.57	0.72	38.2	n.d.	3.14
3	2.01	2.61	2.78	60	0.19	6.1
7	3.51	1.25	2.85	37.57	n.d.	n.d.

[a] Data from intravenous (i.v.) studies.

[b] Data from postoperative (p.o.) studies. nd: compound not detected, below the level of limit of quantification (<300 pM). The compounds were dissolved in 400 PEG/water (3:1) for i.v. and p.o. dosing.

whether they offer any advantage over the parent acid **1** (Table 2). When dosed intravenously at 5 mpk, they demonstrated an improved half-life and higher exposure compared to acid **1**. However, when dosed orally at 5 mpk, there was no significant improvement in oral bioavailability or in exposure.

In summary, a series of carboxylic acid surrogates in dipeptide series with moderate potency were identified, as VLA-4 antagonists. The oxadiazolidinone acid surrogate **3** demonstrated an improved pharmacokinetic property. These acid surrogates themselves represent novel structures and will be further evaluated in animal models of asthma and MS.

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