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Discovery of a potent, orally bioavailable pyrimidine VLA-4 antagonist effective in a sheep asthma model

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ABSTRACT

A series of *N*-(pyrimidin-4-yl)-phenylalanine VLA-4 antagonists is described. Optimization of substituents at the 2 and 5 positions of the pyrimidine ring gave **14**, a very potent VLA-4 inhibitor which is orally active in a sheep asthma model.

VLA-4 ($\alpha 4\beta 1$ integrin) is a cell surface receptor expressed on many leukocytes, including T- and B-lymphocytes, eosinophils, basophils, and mast cells.[1] It is well established that VLA-4 plays a key role in the recruitment of leukocytes from the bloodstream to inflamed or injured tissues via a process known as cell adhesion.[2] This process is mediated by the binding of VLA-4 to the vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells. VLA-4 also binds to the extracellular matrix protein fibronectin (FN). Inhibiting VLA-4/VCAM-1 and VLA-4/FN binding offers therapeutic opportunity for the treatment of inflammatory and autoimmune pathologies such as asthma, multiple sclerosis (MS), rheumatoid arthritis and Crohn's disease.[3] This approach has been validated with the approval of the humanized anti-α_4 antibody natalizumab for the treatment of relapsing–remitting MS[4] and Crohn's disease.[5] Since an antibody requires intravenous administration and has the potential of immunogenicity, an orally efficacious small molecule would serve as a valuable alternative therapeutic approach.

We have previously reported the discovery of dipeptide **1** (Fig. 1) as a potent antagonist of VLA-4. This compound demonstrated efficacy in a sheep asthma model by reducing early and late phase bronchoconstriction and carbachol induced airway hyperresponsiveness when dosed via inhalation.[6] Unfortunately, this class

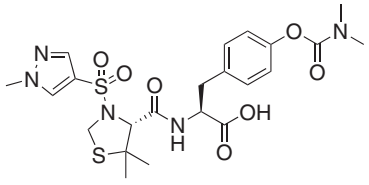

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Figure 1. Peptidic VLA-4 antagonist.

of peptidic acids demonstrated poor bioavailability resulting from low permeability and rapid biliary clearance. An ester prodrug approach was adopted to improve physical chemical characteristics of this class of compounds to impart oral efficacy.[7] In this Letter we describe our efforts to identify an orally efficacious small molecule antagonist of VLA-4 not dependent on a prodrug strategy.

Because all attempts to replace the C-terminal carboxylate with other functionality or a carboxylate isostere gave compounds of greatly reduced potency, attention was directed at the central carboxamide.[6] It was hypothesized that the carboxamide was a contributor to the poor pharmacokinetic profile in the dipeptide series so an appropriate isostere was desired. Benzamide **2** (Fig. 2) had been identified as a relatively potent antagonist as measured in an $\alpha 4\beta 1$ positive Jurkat cell-recombinant soluble VCAM-1 FACS assay.[6] Efforts in the dipeptide series had

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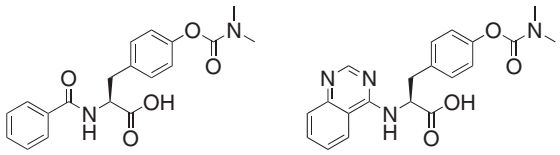


Figure 2. Benzamide and quinazoline VLA-4 antagonists.

demonstrated the importance of the amide carbonyl and *N*–H for potency. These results suggested N-arylated heterocycles could mimic the key structural and electronic features of the carboxamide. The near equivalent potency of quinazoline **3** with benzamide **2** confirmed replacement of the carboxamide could be achieved with an appropriately polarized heterocycle.[8]

Having success in replacing the carboxamide, we now focused to improve the potency of **3** by preparing variants of the quinazoline heterocycle including 5-arylated pyrimidines prepared according to Scheme 1. Reacting suitably protected L-tyrosine **4** with dimethylcarbamyl chloride followed by deprotection gave amino ester **5**, which was reacted with 4-chloro-5-iodopyrimidine to give **6**. This iodopyrimidine underwent Suzuki couplings with a variety of aryl boronic acids and subsequent treatment with formic acid gave desired acids **7a**–7**o**. Potency data and systemic exposure as determined from area under curve (AUC) quantitation for the 5-arylated pyrimidines is shown in Table 1.[9]

Compound **7o** was determined to be the most potent, but this compound exhibited poor exposure following oral dosing.

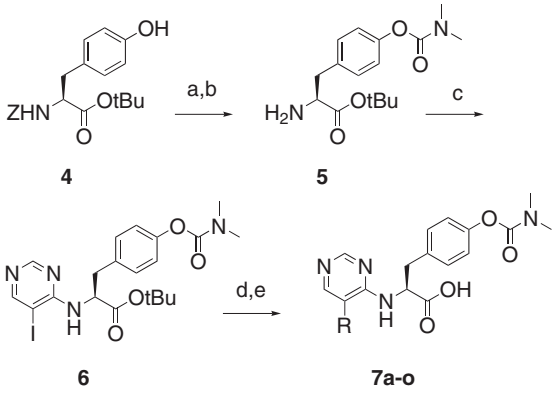
Efforts in the quinazoline series had revealed that 2-amino substitution increased potency with compound **8** containing *N*-methyl-*N*-cyclohexyl being optimal (Fig. 3).

This substitution was incorporated into **7b** via Scheme 2. The reaction of 2,4-dichloro-5-iodo-pyrimidine **9** with 1 equiv of **5** proceeded with complete regioselectivity to give **10**. Intermediate **10** was then treated with *N*-methyl-*N*-cyclohexylamine to give the iodopyrimidine which following Suzuki coupling with *o*-tolylboronic acid and ester deprotection gave **11a**.

Although the expected increase in potency was not seen, **11a** did demonstrate a good increase in exposure (Table 2). Since 2,4-diamino pyrimidines are known to be relatively basic (pK_a >7),[10] the AUC improvement may in part be explained by the zwitterionic nature of these compounds in the GI tract which facilitates oral absorption. A formal pharmacokinetic study of **11a** determined a bioavailability of 26% in the Sprague–Dawley (SD) rat with minimal biliary excretion (<3%) when dosed orally at 10 mg/kg. The favorable pharmacokinetic properties of this compound prompted a sheep asthma study in collaboration with Dr. William Abraham.[11] This study determined that **11a** prevented both the early and late phase response and carbachol induced hyperresponsiveness with a minimum effective oral dose of 10 mg/kg.

The efficacy demonstrated by **11a** inspired the preparation of a family of compounds which introduced a variety of amines at the 2-position of the pyrimidine to identify the amino substitution that retained the potency of **11a** and maximized exposure. Several compounds not only profiled with greater exposure but also higher potency including **11i** (Table 2).

Having discovered that simple diethylamino substitution at C-2 could impart good exposure as shown with **11i**, the analog of **7o** incorporating this moiety was prepared according Scheme 3. Treat-



Scheme 1. Reagents and conditions: (a) $ClCONMe_2$, Et_3N, DMAP, CH_2Cl_2; (b) Pd/C, H_2, EtOH; (c) 4-chloro-5-iodopyrimidine, DIEA, THF, reflux; (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DME, reflux; (e) formic acid.

Table 1
FACS potency and systemic exposure in Sprague–Dawley rat

Compd	R	FACS IC_{50} (nM)	AUC[a] (μg h/mL)
7a	Phenyl	300	n.t.[b]
7b	*o*-Tolyl	25	0.2
7c	2-Trifluoromethylphenyl	90	0.6
7d	2-Methoxyphenyl	16	0.2
7e	2-Fluorophenyl	18	0.6
7f	2,6-Difluorophenyl	27	0.6
7g	2,3-Dichlorophenyl	77	0.3
7h	2,4,6-Trimethylphenyl	100	<0.1
7i	3-Nitrophenyl	180	<0.1
7j	3-Pyridyl	130	n.t.
7k	4-Pyridyl	85	<0.1
7l	Thiophen-2-yl	16	0.3
7m	3-Methylthiophen-2-yl	5	0.6
7n	1*H*-Pyrrol-2-yl	75	n.t.
7o	3,5-Dimethylisoxazol-4-yl	<1	<0.1

[a] AUC = area under curve.
[b] Not tested.

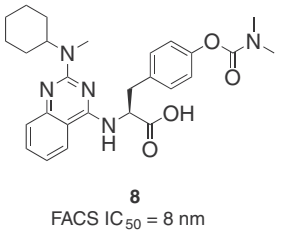
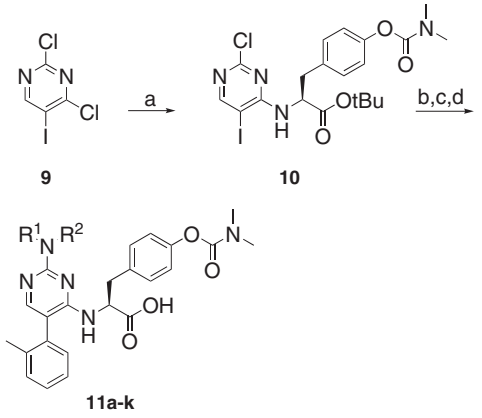


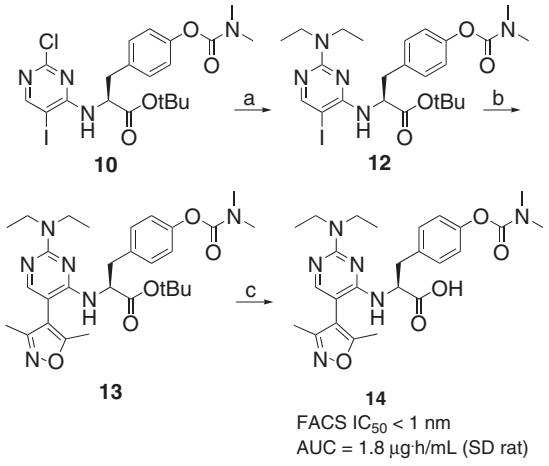
Figure 3. Quinazoline VLA-4 Antagonist.



Scheme 2. Reagents and conditions: (a) **5**, DIEA, IPA, 40 °C; (b) R^1R^2NH, IPA, 120 °C; (c) *o*-tolylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DME, reflux; (d) formic acid.

Table 2
FACS potency and systemic exposure in Sprague–Dawley rat

Compd	R^1	R^2	FACS IC_{50} (nM)	AUC (μg h/mL)
11a	Methyl	Cyclohexyl	20	0.9
11b	Methyl	Methyl	29	0.4
11c	Methyl	Isobutyl	7	0.3
11d	Methyl	Cyclopropyl	20	0.4
11e	Methyl	Cyclobutyl	10	1.8
11f	Methyl	(1-Methyl) piperidin-4-yl	68	<0.1
11g	Methyl	4-Chloro phenyl	107	5.7
11h	Ethyl	Methyl	5.3	0.7
11i	Ethyl	Ethyl	5.7	1.6
11j	Ethyl	Propyl	4.9	1.9
11k	Piperidin-1-yl		32	0.5



Scheme 3. Reagents and conditions: (a) Et_2NH, IPA, 120 °C; (b) 3,5-dimethyl-4-isoxazoleboronic acid, $Pd(PPh_3)_4$, K_3PO_4, DMF, 100 °C; (c) formic acid.

Table 3
Pharmacokinetic data for compound **14**

Species	*F*%	CL (mL/h/kg)	$T_{1/2}$ (h)	Vd (mL/kg)
SD rat[a]	19	170	1.4	346
Beagle dog[b]	59	404	0.9	524
Cynomolgus monkey[c]	16	808	1.1	1291

[a] Male rats dosed at 10 mg/kg iv and po.
[b] Male dogs dosed at 3 mg/kg iv and 10 mg/kg po.
[c] Male monkeys dosed at 3 mg/kg iv and 10 mg/kg po.

ing **10** with diethylamine gave iodopyrimidine **12** which underwent Suzuki coupling with 3,5-dimethyl-4-isoxazoleboronic acid to give ester **13**. Treatment of **13** with formic acid gave acid **14**.

Compound **14** retained the potency of **7o** but exhibited a vastly improved systemic exposure. Furthermore, this compound was found to be efficacious in the Abraham sheep model of asthma when dosed orally at 1 mg/kg while no effect was noted at 0.3 mg/kg. The pharmacokinetic profile of compound **14** in three species is summarized in Table 3. Notable is the acceptable oral availability and low clearance across species.

In conclusion, we have identified a class of pyrimidinyl VLA-4 antagonists that retain the potency of the peptidic series. Optimization of this class identified a bioavailable VLA-4 antagonist which achieved robust efficacy in a sheep asthma model when dosed as the parent carboxylic acid.

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9. Systemic exposure was determined as follows: Three fasted male Sprague–Dawley rats were administered test compound mixtures at a total dose of 10 mg/5 mL/kg orally. Individual test compounds typically varied from 1.1 to 2.0 mg/kg. Blood samples were collected at 4, 8, and 12 h post dose by intracardiac puncture. Separated acidified plasma was extracted with ethyl acetate, evaporated to dryness and reconstituted in the chromatographic mobile phase and subjected to LC/MS/MS analysis on a PE-Sciex API-365 triple quadurpole mass spectrometer. Quantitation was performed with PE-Sciex software (MacQuan v1.6).
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