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Identification of *N*-acyl 4-(5-pyrimidine-2,4-dionyl)phenylalanine derivatives and their orally active prodrug esters as dual-acting alpha4–beta1 and alpha4–beta7 receptor antagonists

Achyutharao Sidduri [a,*], Jefferson W. Tilley [a], Jianping Lou [a], Nadine Tare [b], Gary Cavallo [b], Karl Frank [c], Anjula Pamidimukkala [c], Duk Soon Choi [d], Louise Gerber [d], Aruna Railkar [d], Louis Renzetti [b]

[a] Discovery Chemistry, Pharmaceutical Research and Early Drug Development, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA
[b] Discovery Inflammation and Respiratory Diseases, Pharmaceutical Research and Early Drug Development, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA
[c] Drug Metabolism and Pharmacokinetics, Pharmaceutical Research and Early Drug Development, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA
[d] PARD, Pharmaceutical Research and Early Drug Development, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA

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ABSTRACT

N-Acyl 4-(5-pyrimidine-2,4-dionyl)phenylalanine derivatives of type **4** were designed to replace the 2,6-dichlorobenzoylamine portion of compound **1** in order to identify novel compounds with improved potency against α4-integrins. Several derivatives were identified as very potent dual-acting α4-integrin, α4β1 and α4β7 antagonists. Investigation of a limited number of prodrug esters led to the discovery of the ethyl ester prodrug **42**, which demonstrated good intestinal fluid stability and good permeability. Despite low solubility, **42** gave acceptable blood levels of **30** when dosed orally in non-human primates. Additionally, **42** had an overall excellent profile and was selected for clinical trials. Investigation of *N*-acyl 4-(5-pyrimidine-2,4-dionyl)phenylalanine derivatives led to the discovery of several very potent dual-acting α4-integrin antagonists. Ethyl ester prodrug 42 advanced to human clinical trials based on the excellent intestinal fluid stability, good permeability and superior efficacy in non-human primates.

Alpha4 integrins are expressed on a variety of leukocytes, including B-cells, T-cells, basophils and eosinophils, and are involved in the recruitment, activation and survival of these cell types.[1] Data supporting a role for alpha4 integrins in a number of inflammatory diseases, including asthma, rheumatoid arthritis, multiple sclerosis (MS), inflammatory bowel diseases, and atherosclerosis, has emerged and is summarized in recent reviews.[2] A humanized mouse anti-alpha4 antibody, Natalizumab, has been approved for the treatment of multiple sclerosis[3] and Crohn's disease.[4] Very recently a small molecule alpha4 integrin antagonist was shown to be effective for the treatment of MS,[5] fully validating alpha4 integrins as targets for human disease.

We previously reported that the potent, dual-acting antagonist of α4β1 and α4β7, **1**, (Fig. 1) is effective for the treatment of asthma in humans[6] and sought additional compounds that might have lower clearance and novel structures with superior potency for both alpha4 integrins, α4β1 and α4β7.

Inspired by reports of the alpha4 integrin antagonist activity of members of the biphenylalanine class such as **2**,[7] as well as the interesting, selective integrin antagonist activity of members of the pyridizinone family represented by **3**,[8] we report here the discovery of a new series of potent alpha4 integrin antagonists, *N*-acyl

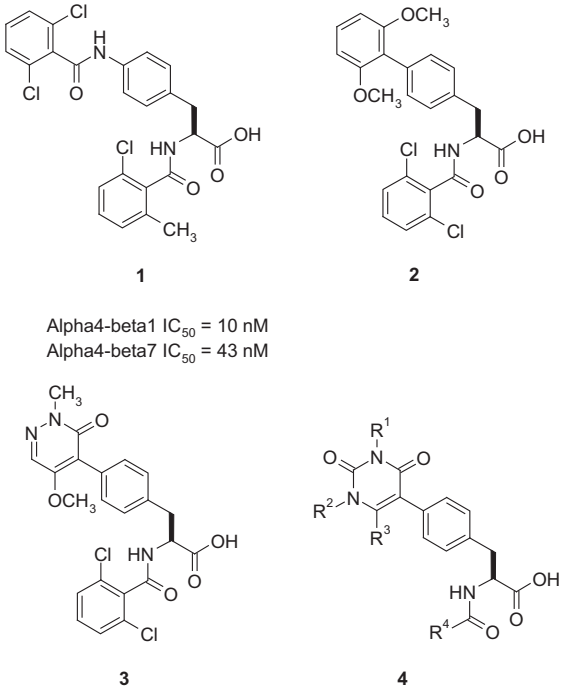

Figure 1. Structures of reported alpha4 integrin antagonists.

* Corresponding author. Tel.: +1 973 235 7143; fax: +1 973 235 7122.
E-mail address: sidduria@yahoo.com (A. Sidduri).

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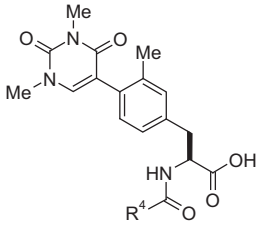
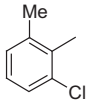
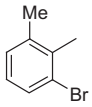
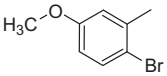
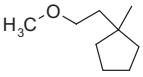
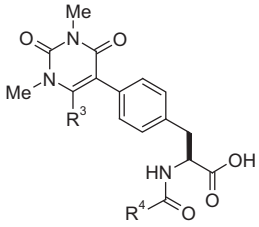
Table 1
SAR summary of R^4 modifications

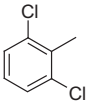
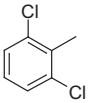
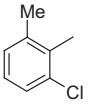
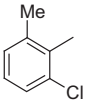
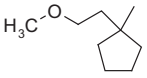
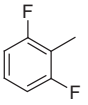
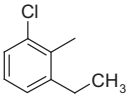
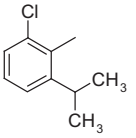
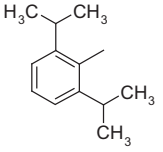


Compd	R^4	α4β1 Ramos cells/VCAM IC_{50} nM (Rel potency)[a]
21		81 (1.0)
22		25 (2.0)
23		255 (0.35)
24		64 (0.8)

[a] Rel potency refers to activity relative to the reference standard **1** run as a positive control in each binding experiment.

Table 2
SAR summary of R^3 and R^4 modifications



Compd	R^3	R^4	α4β1 Ramos cells/VCAM IC_{50} nM (Rel potency)	α4β7 RPMI cells/ MadCAM IC_{50} nM (Rel potency)
25	CH_3		8 (6.3)	35 (16)
26	CF_3		196 (0.46)	219 (1.3)
27	CH_3		35 (2.1)	58 (6.1)
28	CF_3		123 (0.1)	167 (2.4)
29	CH_3		287 (0.49)	324 (1.0)
30	CH_3		18 (3.2)	81 (3.3)
31	CH_3		87 (0.53)	151 (2.3)
32	CH_3		215 (0.13)	558 (1.0)
33	CH_3		1450 (0.04)	1970 (0.15)

4-(5-pyrimidine-2,4-dionyl)-phenylalanine derivatives **4** (Fig. 1) and their orally active prodrug esters.

The compounds reported in Tables 1–4 were prepared by a crucial palladium-catalyzed coupling reaction of the 4-iodophenylalanine derivatives **6** or **13** with an in situ generated organozinc intermediate (Schemes 1 and 2) that was derived from the 1, 3-disubstituted-5-iodo-pyrimidine-2,4-dione, the 1,3,6-trisubstituted-5-iodo-pyrimidine-2,4-dione (**5**) or the 1,3-disubstituted-6-trifluoromethyl-5-iodo-pyrimidine-2,4-dione (**16**) (Scheme 3).

The desired intermediate 5-iodo-pyrimidine-2,4-dione derivatives were prepared by alkylation or iodination reactions, as per literature procedures.[9] The iodide derivatives were then reacted with an activated zinc dust in dimethylacetamide at 70 °C to give the corresponding organozinc intermediates.[10] The coupling reaction of this organozinc intermediate with **6** proceeded smoothly in the presence of $Pd(dba)_2$ and trifurylphosphine (TFP) or tri-*ortho*-tolylphosphine in THF at 55 °C to afford the desired product in good yield. The remaining steps, BOC removal, benzoylation, and hydrolysis proceeded in a straightforward manner to obtain the target compounds (Scheme 1).

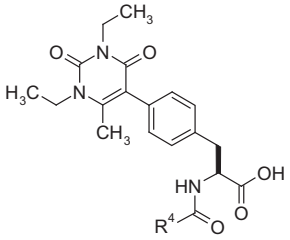
The synthesis of the 3-methyl-4-iodophenylalanine (**13**) and the corresponding target compounds **14** are shown in Scheme 2. Thus, 3-methyl-4-nitrobenzyl alcohol **9** was converted to the desired iodide **13** in six steps. The crucial step in this process was the chiral hydrogenation of olefin **11** to the (*S*)-phenylalanine **12** using a chiral rhodium catalyst, (+)-1,2-bis((2*S*,5*S*)-2,5-dimethylphospholano) benzene(cyclooctadiene)rhodium(I) trifluoromethanesulfonate ([[Rh(COD)(S,S)-(me)DuPHOS]$^+$TfO$^-$]) in methanol. Deprotection followed by diazotization under Sandmeyer reaction conditions gave the iodide **13**. Coupling of **13** with an in situ generated organozinc reagent (prepared from pyrimidinedione **5**) gave the expected 3,4-disubstituted phenylalanine derivative which was converted to the target compound **14** in three straight forward steps.

The 1,3-dimethyl-6-trifluoromethyl-5-iodopyrimidine-2,4-dione (**16**) was prepared in three steps from ethyl 3-amino-4,4,4-trifluorocrotonate and methyl isocyanate using a method previously described as shown in Scheme 3.[11]

The 2-chloro-6-alkyl substituted benzoic acid derivatives (**19**) were prepared following Scheme 4. The crucial step in this scheme was the displacement of an *ortho*-fluorine from *N*-(2-chloro-6-fluorobenzylidine)butylamine **18** with alkylmagnesium bromides. Deprotection of the imines, followed by oxidation gave the corresponding benzoic acids, **19**.

In general, alkyl ester prodrugs such as **20** were prepared by treatment of the carboxylic acids with the appropriate alkyl iodides or alkyl bromides in the presence of sodium bicarbonate in DMF as shown in Scheme 5.

Table 3
SAR summary of R^4 modifications with R^1 and R^2 as ethyl group



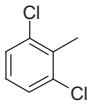
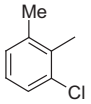
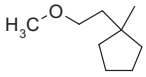
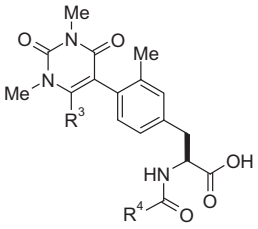
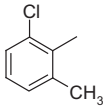
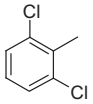
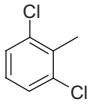
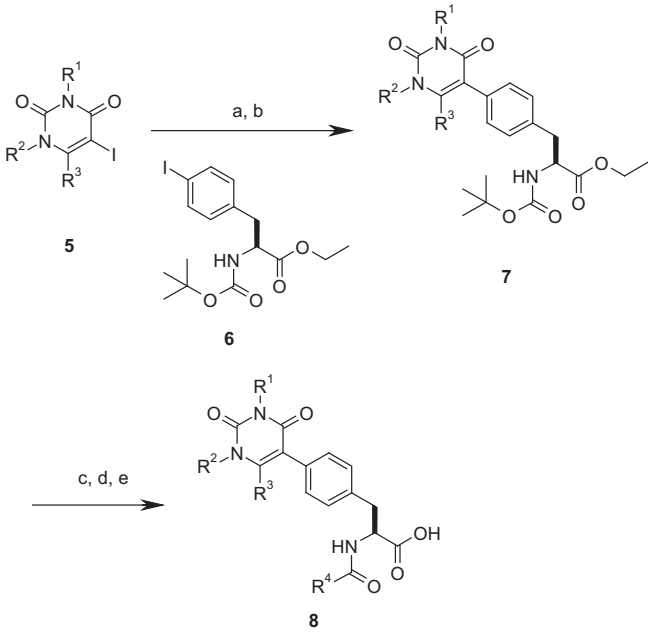
Compd	R^4	α4β1 Ramos cells/ VCAM IC_{50} nM (Rel potency)	α4β7 RPMI cells/ MadCAM IC_{50} nM (Rel potency)
34		63 (0.80)	124 (4.8)
35		296 (0.37)	164 (1.7)
36		382 (0.29)	565 (0.46)

Table 4
SAR summary of R^3 and R^4 modifications with 3-methyl-L-phenylalanine derivatives



Compd	R^3	R^4	α4β1 Ramos cells/ VCAM IC_{50} nM (Rel potency)	α4β7 RPMI cells/ MadCAM IC_{50} nM (Rel potency)
37	H		246 (0.14)	254 (1)
38	H		56 (0.68)	220 (1.9)
39	CH_3		10 (1.1)	168 (3.6)

The in vitro potency of the compounds listed in Tables 1–4 were assessed by determining the ability of serial dilutions to inhibit the binding of RAMOS cells (α4β1-specific binding) and RPMI 8866 cells (α4β7-specific binding) with recombinant human VCAM-1 used as a counter ligand for the RAMOS cell assay and human MAdCAM-1 for the RPMI 8866 cell assay. Cells were labeled with Calcein AM, a fluorescent dye, and then activated with a binding buffer containing Mn^{2+} to achieve maximum activation prior to the assay. Compound **1** was used as a positive control on each plate.[12] The



Scheme 1. Reagents and conditions: (a) Zn dust (activated using 10 mol % of 1,2-dibromoethane and 10 mol % of TMSCl in THF), DMAC, 70 °C, 3–15 h; (b) $Pd(dba)_2$, TFP or tri-*ortho*-tolylphosphine, THF, 50 °C, 15 h, 42–73%; (c) 4.0 N HCl in dioxane, dioxane, rt, 5 h, 91–99%; (d) R^4COOH, HBTU, DIPEA, DMF, rt, 15 h, 72–95% or R^4COCl, DIPEA, CH_2Cl_2, rt, 15 h, 80–93%; (e) 1.0 N NaOH, EtOH, rt, 5–15 h, 85–95%.

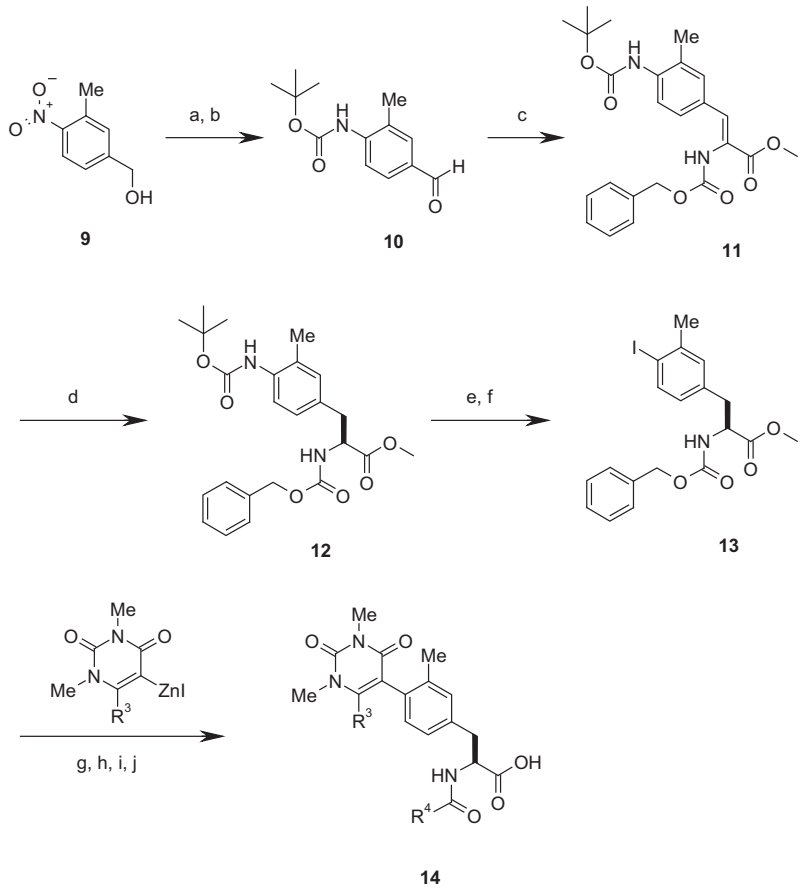
data in Tables 1–4 are reported both as the observed IC_{50} and as fold-potency relative to **1** since the activity of the cell lines varied over time with the number of passages.

We had previously proposed a binding model for **1** that included as α4β1 recognition elements a π-stacking interaction with the phenylalanine and attached electron deficient benzoyl moiety as well as a hydrogen bonding interaction with the phenylalanine 4-carboxamido group.[12] The first compounds prepared in the pyrimidinedione series incorporate those features. As shown in Table 1 they have similar potency in our α4β1 binding assay to **1**. The data also support the notion that in this series, in order to achieve high potency, the phenylalanine should bear a 2,6-disubstituted benzoyl functionality as seen with **21** and **22**.[13]

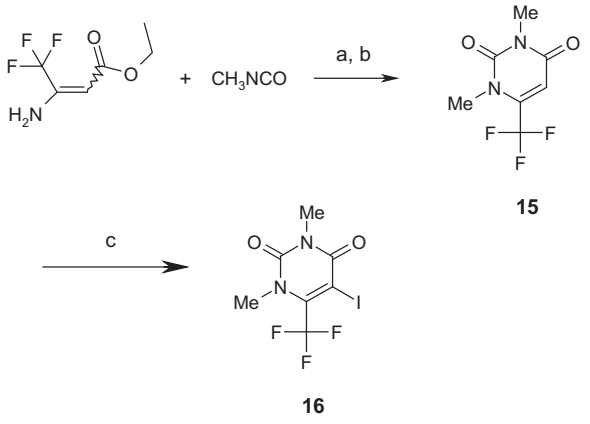
Drawing on the observation that 2′,6′-disubstitution on the distal aromatic ring is a requirement for high potency in **2**, we followed up with a series of analogues of **21** and **22** that included a substituent in the 6-position of the pyrimidinedione ring as shown in Table 2. We quickly made the following observations: first, addition of a 6-methyl substitution gave a 5- to 10-fold improvement in potency for both integrins; second, replacement of 6-methyl with 6-trifluoromethyl reduced potency drastically; third, the aroyl series again maintained a higher potency than the corresponding cycloalkanoyl series; and finally, a substitution bulkier than methyl or chloro at the 2- or 2,6-positions on the *N*-acyl aromatic ring gave a marked reduction in potency, which was consistent with our previous observations.[13]

Next, we decided to investigate the role of *N*-alkyl groups on the pyrimidinedione ring. It is clear from these examples (Table 3) that the *N*-methyl is preferred over the corresponding *N*-ethyl analogues, (**25** vs **34** and **27** vs **35**) suggesting that this hydrophobic pocket has very limited steric tolerance.

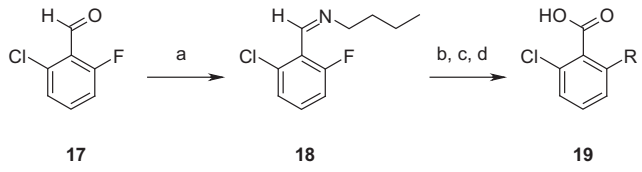
We presumed that the 6-methyl group on the pyrimidinedione ring is required to promote a preferred torsion angle between the phenylalanine aromatic ring and the pyrimidinedione. In this case, we thought that we could achieve a similar result through the introduction of a methyl group at the 3-position of the benzene



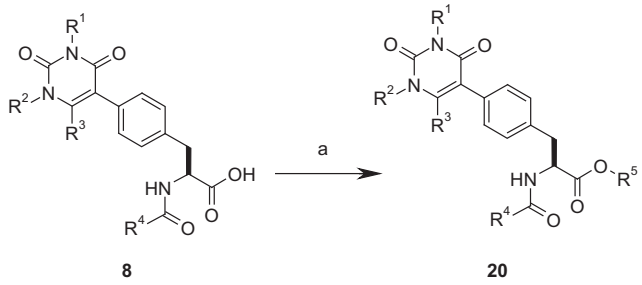
Scheme 2. Reagents and conditions: (a) Boc_2O, H_2, Pd/C, EtOAc, rt, 2 h, 76%; (b) MnO_2, CH_2Cl_2, 4 Å MS rt, 15 h, 77%; (c) *N*-(benzyloxycarbonyl)-α-phosphonoglycine methyl ester, tetramethylguanidine, CH_2Cl_2, −30 °C to rt, 15 h, 58%; (d) [[Rh(COD)(*S*,*S*)-(me)DuPHOS]$^+$TfO$^-$], H_2 (60 psi), MeOH, rt, 22 h, 55%; (e) 4.0 N HCl in dioxane, dioxane, rt, 2 h, 87%; (f) sodium nitrite, H_2SO_4, KI, H_2O, −10 °C to rt, 1 h, 64%; (g) $Pd(dba)_2$, tri-*ortho*-tolylphosphine, THF, 50 °C, 15 h, 30–69%; (h) 10% Pd/C, cyclohexene, EtOH, reflux, 20 min, 72–83%; (i) R^4COOH, HBTU, DIPEA, DMF, rt, 15 h, 70% or R^4COCl, DIPEA, CH_2Cl_2, rt, 15 h, 48–81%; (j) 1.0 N NaOH, EtOH, rt, 5–15 h, 41–93%.



Scheme 3. Reagents and conditions: (a) NaOMe, DMSO, mol. seives, 0 °C to rt, 15 h, 63%; (b) MeI, K_2CO_3, DME, reflux, 4 h, 83%; (c) NIS, CF_3COOH, $(CF_3CO)_2O$, reflux, 15 h, 66%.



Scheme 4. Reagents and conditions: (a) *n*-butylamine, heptane, rt, 3 h, 97%; (b) RMgBr, THF, 5–20 °C, 5 h; (c) 20% HCl in H_2O, 0 °C to rt, 15 h, 75–90% (in two steps); (d) monobasic sodium phosphate, H_2O_2, sodium chlorite, CH_3CN, H_2O, 0 °C, 15 h, 80–97%.



Scheme 5. Reagents and conditions: (a) R^5-I or R^5-Br, $NaHCO_3$, DMF, rt, 15 h, 49–77%.

ring. The data shown in Table 4 indicate that the ligand binding interactions are more complex and a 3-methyl group on the phenylalanine aromatic ring is not well tolerated in the presence or absence of substitution in the 6-position on the pyrimidinedione ring.

Among the potent α4β1 antagonists discovered in the present work, we focused on the 2′,6′ disubstituted derivatives **25**, **27** and **30** since these compounds had excellent potency against both α4β1 and α4β7 integrins and no safety flags. Based on our previous experience with phenylalanine derivatives, we realized that in order to achieve reasonable bioavailability, we would need to invoke a prodrug strategy.[6]

Table 5
Log*D*, Papp permeability and rat oral bioavailability of 3 parent compounds and their ester prodrugs

Parent/prodrug	Log*D*	Papp (nm/s)	*F* (%) (rat)
25	−2.02	13.3	1.7
40	2.01	183	6
27	−1.76	29	10
41	2.24	347	25
30	−2.36	7.7	ND
42	1.73	269	9
43	1.61	120	30

Thus, a relatively small number of prodrugs were prepared. Each was assessed for its log*D*, solubility, stability and permeability. As expected, the ester prodrugs have an improved log*D* and Papp permeability in comparison to their parent compounds (**25** vs **40**, **27** vs **41**, and **30** vs **42**, **43**) (Table 5). The top 4 candidates (Fig. 2) that emerged from these studies were profiled in rat PK studies (Table 5) using an aqueous suspension of the compound in a vehicle containing hydroxypropylcellulose (2% w/v) and Tween 80 (0.1% w/v). The improved permeability of the prodrugs compared to parent translated into improved PK profiles.

Compounds were discriminated based on their activity in the atopic non-human primate allergic asthma model. Animals were challenged with ascaris suum extract, to which they were allergic, 4 h after oral dosing with drug. After 48 h the bronchoalveolar lavage fluid was examined for the presence of inflammatory cells in comparison with vehicle-treated controls. Of the 3 ethyl ester prodrugs that were profiled in this study, compound **42** was the most effective (Fig. 3) in profoundly reducing the number of neutrophils, eosinophils, and lymphocytes in this model. Unfortunately, the acetal prodrug **43** formed inconsistent ratios of diastereomers that were difficult to separate, and thus **43** was not further pursued.

Based on the potency, solubility, stability, oral bioavailability and in vivo efficacy, the ethyl ester **42** was selected as a development candidate. The pharmacokinetic properties of **42** and its parent compound **30** in mouse, rat, dog and cynomolgus monkey are shown in the Tables 6 and 7. The clearance in mice, rats and monkeys were good although relatively high in dogs. As a result, the

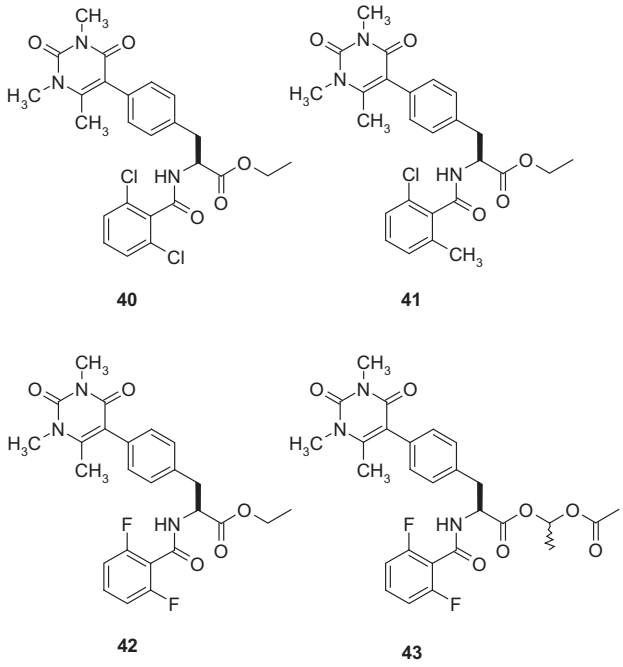


Figure 2. Structures of representative prodrug esters.

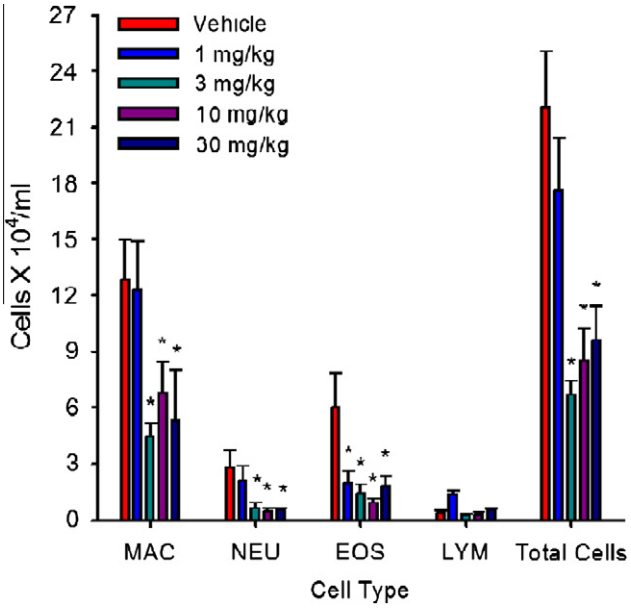


Figure 3. Allergen-induced inflammatory cell influx in the atopic non-human primate.

Table 6
Pharmacokinetic parameters of compound **30** following iv administration

	Dose (mg/kg)	$AUC_{0-8\ h}$(ng h/ml)	CL (ml/min/kg)	$T_{1/2}$ (h)	MRT (h)
Mouse	10	7894	21		0.26
Rat	10	41,532	4.8		0.61
Dog	5	2790	29.7	0.19	0.21
MK	10	22,161	6.88	1.46	0.25

Table 7
Pharmacokinetic parameters of compound **30** following po administration of **42**

	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-24\ h}$ (ng h/ml)	*F* (%)
Mouse	50	0.25	2988	8547	22
Rat	50	1.3	5287	18,097	9
Dog	50	1	471	1690	6
MK	30	2	337	3146	7

AUC was low in dogs. In mouse, the oral bioavailability was ~22%, however in the rats, dogs and monkeys it was low (<10%) using different formulation approaches or at higher doses.

Compound **42** was advanced to human clinical trials and formulations using reduced particle size provided the desired exposure.

In conclusion, *N*-acyl 4-(5-pyrimidine-2,4-dionyl)phenylalanine derivatives **4** were designed to replace the 2,6-dichlorobenzoylamine portion of compound **1** in order to identify a novel series with improved potency in both α4-integrins. Several derivatives were identified as very potent dual-acting α4-integrin antagonists from this discovery effort. Investigation of a limited number of prodrug esters led to the discovery of the ethyl prodrug **42** as a development candidate.

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