Synthesis and Biological Evaluation of Novel Pyridazinone-Based α_4 Integrin Receptor Antagonists

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A novel series of pyridazinone-functionalized phenylalanine analogues was prepared and evaluated for inhibition of cellular adhesion mediated by $\alpha_4\beta_1$/VCAM-1 and $\alpha_4\beta_7$/MAdCAM-1 interactions. Concise syntheses were developed and applied for exploration of structure−activity relationships pertaining to the pyridazinone ring as well as the *N*-acyl phenylalanine scaffold. Potent dual antagonists of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ were generated from an amide subseries; antagonists selective for $\alpha_4\beta_7$ were identified from urea and carbamate-based subseries. The pharmacokinetic properties of selected members of the series have been determined in rats and demonstrate that the use of ester prodrugs and alterations to the amide linkage can lead to improved oral bioavailability in this series. An $\alpha_4\beta_7$-selective member of the carbamate subseries (**36c**), upon oral admininstration, demonstrated in vivo efficacy in the mouse DSS colitis model.

Introduction

Integrins are heterodimeric transmembrane receptors that mediate cellular adhesion processes. The α_4 integrin family is composed of $\alpha_4\beta_1$ (also known as VLA-4) and $\alpha_4\beta_7$ integrins, which are expressed on the surface of leukocytes and are involved in their adhesion to the extracellular matrix, a key step in recruitment to sites of inflammation.[1,2] Vascular cell adhesion molecule-1 (VCAM-1) is expressed on endothelial cell surfaces and binds to $\alpha_4\beta_1$, facilitating extravasation of leukocytes through the endothelial cell layer. Integrin $\alpha_4\beta_7$ mediates leukocyte recruitment to the intestinal mucosa by binding mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on epithelial cell surfaces. The therapeutic potential of α_4 integrin antagonists has recently been demonstrated by Tysabri (natalizumab), a humanized monoclonal antibody that binds the integrin α_4 subunit, in the treatment of multiple sclerosis.[3] In November 2004, Tysabri received accelerated approval from the FDA,[4] but shortly thereafter it was voluntarily withdrawn from the United States market due to reports of rare but serious adverse events (progressive multifocal leukoencephalopathy, a demyelinating disease of the central nervous system).[5,6]

In addition to its therapeutic utility for the treatment of multiple sclerosis, Tysabri[7] (and MLN02, an $\alpha_4\beta_7$ integrin antibody from Millennium Pharmaceuticals[8]) has shown promise for treatment of Crohn's disease in clinical trials. A nonselective dual antibody such as Tysabri, active against both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, may have broader therapeutic utility but may also have more potential for side effects. Whereas selectively targeting either $\alpha_4\beta_1$ or $\alpha_4\beta_7$ may provide better safety profiles, one would expect more limited therapeutic utility. Presently, it is debatable which integrin ($\alpha_4\beta_1$ or $\alpha_4\beta_7$) is a better target for producing new safe and effective treatments. Targeting the tissue-specific or -selective, gut-homing integrin $\alpha_4\beta_7$ may represent a good therapeutic intervention for the treatment of Crohn's disease in light of recent developments with the dual antibody Tysabri. Anti-tumor necrosis factor-α (TNF-α) therapies, such as Remicade, have significantly improved the quality of life for many patients with Crohn's disease, but there are still patients who do not respond to the anti-TNF-α therapies. A combination of an anti-TNF-α antibody and an $\alpha_4\beta_7$ integrin antagonist may offer an improved modality for treating such unmet medical needs.

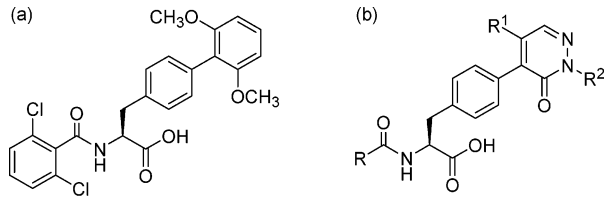

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Figure 1. Structures of (a) a representative antagonist of the *N*-acyl phenylalanine class (**1**) and (b) a generalized member of the pyridazinone series.

The discovery and development of orally active small-molecule α_4 integrin antagonists have been ongoing for some time. Although many small molecules have been identified to inhibit α_4 integrins with nanomolar potency and selectivity, there is still room for improvement in the discovery and development of antagonists with improved pharmacokinetic properties.[9] In addition, most of the known compounds are either dual antagonists against both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ or are selective for $\alpha_4\beta_1$ over $\alpha_4\beta_7$; few compounds are selective for $\alpha_4\beta_7$.[10] *N*-Acyl-4-arylphenylalanines constitute a major class of known α_4 integrin antagonists (e.g. **1**[11] (SB683698); Figure 1a). As a potential replacement for the substituted biphenyl group found in the majority of antagonists in this class, we became interested in 4-heteroaryl functionalized *N*-acyl phenylalanines.[12] We envisioned that a pyridazinone might serve as a bioisostere for the 2,6-dimethoxy-substituted phenyl ring that may be prone to metabolic demethylation (general structure of target antagonists; Figure 1b). In addition, the pyridazinone group can be easily

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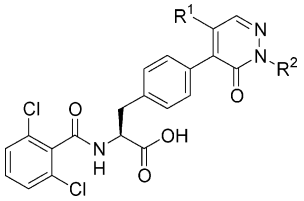
Table 1. Cellular Adhesion Assay Results for Benzamide Analogues[a,b]



cmpd	R^1	R^2	$\alpha_4\beta_1$/VCAM-1 IC_{50} (μM)	$\alpha_4\beta_7$/MAdCAM-1 IC_{50} (μM)
5[c]	OCH_3	CH_3	0.031 ± 0.008 (4)	0.003 ± 0.001 (6)
6	OCH_3	H	1.0 ± 0.25 (2)	0.041 ± 0.013 (2)
7	OCH_3	CH_2Ph	0.52	0.10 ± 0.004 (2)
8	OCH_3	Ph	3.9	0.018 ± 0.010 (2)
9	OCH_3	cyclohexyl	>5	0.33 ± 0.14 (2)
10	OCH_3	*t*-Bu	0.79	0.042 ± 0.005 (2)
11	OCH_3	CH_2CH_2OH	0.13 ± 0.026 (2)	0.002 ± 0.001 (5)
12	OCH_3	2-morpholin-4-yl-ethyl	0.36	0.002 ± 0.002 (3)
13	OH	CH_3	0.48 ± 0.061 (3)	0.049 ± 0.010 (7)
14	OEt	CH_3	0.17	0.002 ± 0.001 (3)
15	$OCHF_2$	CH_3	0.28 ± 0.066 (2)	0.008 ± 0.002 (4)
16	NH_2	CH_3	0.37	0.013 ± 0.009 (2)
17	pyrrolidin-1-yl	CH_3	0.11 ± 0.039 (3)	0.016 ± 0.013 (3)
18	morpholin-4-yl	CH_3	0.48 ± 0.14 (3)	0.003 ± 0.003 (2)
19	OCH_2CH_2OH	CH_3	0.30 ± 0.12 (3)	0.002 ± 0.001 (3)
20	2-morpholin-4-yl-ethoxy	CH_3	0.066 ± 0.013 (2)	0.003 ± 0.001 (2)
21	cyclohexyloxy	CH_3	0.15	0.011 ± 0.006 (3)

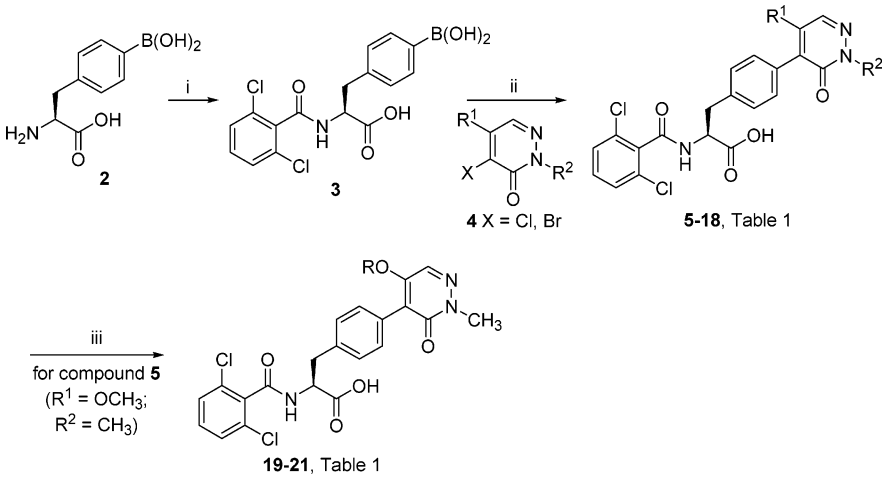
[a] Each IC_{50} is the mean of a duplicated run at six different concentrations. For repetitions (*n*), the IC_{50} is the mean ± SE with *n* in parentheses. [b] The IC_{50} of reference compound **1**[11] was 0.20 ± 0.046 μM in the $\alpha_4\beta_1$/VCAM-1 assay and 0.058 ± 0.010 μM in the $\alpha_4\beta_7$/MAdCAM-1 assay. [c] The enantiomer of compound **5** (prepared from 4-borono-D-phenylalanine) displayed reduced activity: IC_{50} ($\alpha_4\beta_1$) > 1 μM, IC_{50} ($\alpha_4\beta_7$) = 0.17 μM.

functionalized to provide additional receptor interactions for potency and selectivity improvements. Functionalization of the pyridazinone will affect the chemical and physical properties and may also change the pharmacokinetic profiles of the target molecules. We also considered that functionalization of the pyridazinone ring alone might not be sufficient to solve the poor pharmacokinetic properties observed with *N*-acyl phenylalanines since other parts of the target molecules may also be subject to metabolism. Our strategy was to modify the *N*-acyl region of the targeted molecules after the best pyridazinone was identified. Finally, the corresponding ester prodrugs would also be prepared for comparison with the parent acids. Our previously reported microwave-assisted Suzuki coupling with unprotected free amino acids[13] enabled us to synthesize a variety of functionalized, aryl-substituted pyridazinones. Since the initial member of this series (compound **5**, Table 1) proved to be a potent antagonist of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, a limited set of pyridazinone-functionalized phenylalanine analogues was explored further. In this report we describe in vitro cellular adhesion activity, selectivity profiles, pharmacokinetic properties, and in vivo activity of selected members of this new series.

Chemistry

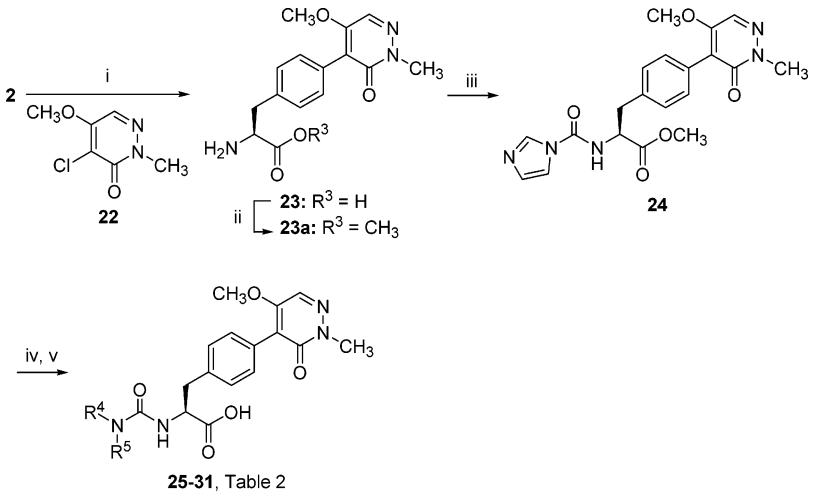
A series of 2,6-dichlorobenzoyl phenylalanines with varied substituents on the pyridazinone was initially prepared. The 2,6-dichlorophenylamide group, held constant in this portion of the study, was selected on the basis of its presence in a number of potent antagonists of the *N*-acyl phenylalanine class.[9,11] Acylation of 4-borono-L-phenylalanine **2** with 2,6-dichlorobenzoyl chloride and subsequent Suzuki coupling of the resulting amide **3** with 4-halopyridazinones **4** provided a convergent approach (Scheme 1) to the target analogues **5**−**18**.[14] The commercial

Scheme 1[a]



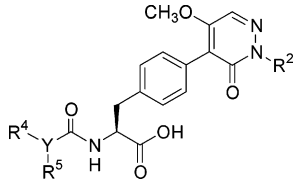
[a] Reagents and conditions: (i) 2,6-dichlorobenzoyl chloride, Na_2CO_3, H_2O, CH_3CN, 50 °C, 71%; (ii) $Pd(PPh_3)_2Cl_2$, Na_2CO_3, H_2O, CH_3CN, μW, 150 °C, 10 min or reflux in oil bath, 1 h, 10−62%; (iii) ROH, Na^0, 80 °C, 30−42%.

Scheme 2[a]



[a] Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$, Na_2CO_3, H_2O, CH_3CN, μW, 150 °C, 10 min, 55%; (ii) MeOH, $SOCl_2$, 66%; (iii) 1,1′-carbonyldiimidazole, THF, CH_2Cl_2, 81%; (iv) R^4R^5NH, CH_3CN, 23 °C (aliphatic amines) or μW, 130 °C, 10 min (anilines); (v) 2 N aq LiOH, 20−57% over two steps.

Table 2. Cellular Adhesion Assay Results for Urea and Carbamate Compounds[a]



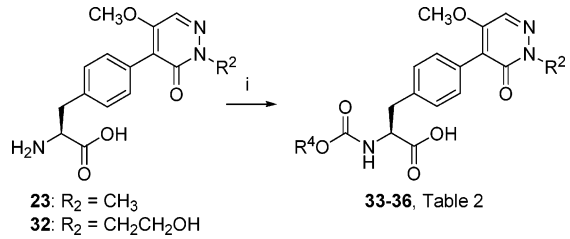
cmpd	R^2	R^4	R^5	Y	$\alpha_4\beta_1$/VCAM-1 IC_{50} (μM)	$\alpha_4\beta_7$/MAdCAM-1 IC_{50} (μM)
25	CH_3	Ph	CH_3	N	>5	0.063 ± 0.031 (5)
26	CH_3	Ph	*i*-Pr	N	>5	0.092 ± 0.015 (2)
27	CH_3	Ph	*n*-Pr	N	>5	0.35
28	CH_3	CH_3	CH_3	N	>5	1.5
29	CH_3	CH_2CH_2OH	CH_3	N	>5	0.094 ± 0.037 (5)
30	CH_3	*i*-Bu	CH_3	N	1.8	0.017 ± 0.009 (5)
31	CH_3				1.8	0.029 ± 0.001 (2)
33	CH_3	CH_3	−	O	>5	0.71
34	CH_3	CH_2Ph	−	O	>5	0.57
35	CH_3	*t*-Bu	−	O	>5	0.81 ± 0.30 (2)
36	CH_2CH_2OH	*t*-Bu	−	O	>5	0.11 ± 0.025 (3)

[a] As for Table 1.

availability and synthetic accessibility of substituted 4-halopyridazinones enabled preparation of analogues differentially substituted at the 3- and 5-positions. It was found that treatment of 5-methoxy pyridazinone **5** with sodium alkoxide nucleophiles resulted in substitution at the 5-position,[15] facilitating efficient preparation of additional analogues **19**−**21**.

A second focus of targeted structural modifications was replacement of the amide linkage with potential bioisosteres (urea and carbamate) to explore the selectivity profile and pharmacokinetic properties of the resulting compounds. The synthesis of antagonists based on a urea linkage is presented in Scheme 2. Unprotected amino acid **2** was coupled with 4-chloropyridazinone **22** under our previously reported microwave-assisted Suzuki conditions.[13] Following esterification, the corresponding amino ester intermediate **23a** was reacted with 1,1′-carbonyldiimidazole to afford carbamoyl imidazole **24**. This intermediate reacted smoothly with aliphatic amines at room temperature, or with anilines under microwave irradiation, to form ureas. Addition of aqueous base to the crude reaction mixtures provided acids **25**−**31** (Table 2). Carbamate analogues

Scheme 3[a]



[a] Reagents and conditions: (i) R^4OCOCl or Boc_2O, Na_2CO_3, H_2O, CH_3CN, 10−84%.

33−**36** were readily prepared from amino acid intermediates **23** and **32** by reaction with chloroformates or dialkyl dicarbonates (Scheme 3).

Ester prodrugs of several of the antagonists were prepared in order to assess their pharmacokinetic properties. These compounds were prepared from the corresponding carboxylic acids either by thionyl chloride- or bis(2-oxo-3-oxazolidinyl)-phosphinic chloride-mediated esterification, as detailed in the Experimental Section.

Table 3. Inhibition of Cellular Adhesion by Compound **5**[a]

leukocyte type (cell line)	endothelial cell line (method of stimulation)	adhesion nominally mediated by	IC_{50}, μM
human lymphocyte (Ramos)	HUVEC (TNF-α)	VCAM-1	0.043
human monocytes (U-937)	HUVEC (LPS)	ICAM-1/VCAM-1	0.014
human granulocytes (HL-60)	HUVEC (TNF-α)	ELAM-1	<0.005

[a] HUVEC: human umbilical vein endothelial cell. ICAM-1: intercellular adhesion molecule-1. ELAM-1: endothelial leukocyte adhesion molecule-1.

Table 4. Pharmacokinetic Properties of Selected Compounds in Rats[a]

cmpd	ester form	AUC[b] (μM-h)	$t_{1/2}$ (h)[c]	F (%)
5[d]	none (carboxylic acid)	0.5 ± 0.3	0.2 ± 0.05	1 ± 1
5a	methyl ester	1.7 ± 0.6	0.7 ± 0.01	8 ± 3
5b	ethyl ester	2.3 ± 0.7	nd	4 ± 1
5c	2-hydroxyethyl ester	3.3 ± 0.6	1.0	7 ± 1
30	none (carboxylic acid)	5.2 ± 2.9	0.9 ± 0.5	3 ± 2
30c	2-hydroxyethyl ester	47 ± 32	0.4 ± 0.3	14 ± 10
36	none (carboxylic acid)	1.6 ± 0.9	0.2 ± 0.03	1 ± 1
36c	2-hydroxyethyl ester	51 ± 13	1.1 ± 0.1	31 ± 11

[a] Averages from a group of four. Dosed at 30 mg/kg po (10% PEG 400 in PBS, pH 7.0−7.5), 3 mg/kg iv. In the case of ester prodrugs, data refer to measured concentrations of the parent carboxylic acids (quantitated with authentic samples of the acids as calibration standards); esters were not detected.[20] See Experimental Section for details. [b] Area under the plasma concentration−time curve following oral administration. [c] $t_{1/2}$ following iv administration; nd = not determined. [d] Clearance (Cl_p) values for carboxylic acids **5**, **30**, and **36** were 22, 7.8, and 6.0 mL/min/kg, respectively; volume of distribution (Vd_{ss}) values for **5**, **30**, and **36** were 0.40, 0.19, and 0.11 L/kg, respectively (iv administration).

Results and Discussion

In Vitro Activity. Compounds were assayed for their ability to inhibit the binding of human lymphocytes (Ramos) expressing $\alpha_4\beta_1$ to immobilized VCAM-1 and the binding of $\alpha_4\beta_7$-expressing K562 cells to immobilized MAdCAM-1. The results of the cellular adhesion assays are presented in Tables 1 and 2. Substitutions on the pyridazinone core were explored to determine their effects on potency, selectivity, and pharmacokinetic properties. 2-Methyl-5-methoxy pyridazinone **5** displayed activity against both $\alpha_4\beta_1$ (IC_{50} = 31 nM) and $\alpha_4\beta_7$ (IC_{50} = 3 nM). The *N*-desmethyl analogue **6** and *O*-desmethyl analogue **13** each had reduced potency. Replacement of the methyl group at the 2-position of the pyridazinone with larger hydrophobic groups (benzyl, phenyl, cyclohexyl, and *tert*-butyl; compounds **7**−**10**) generally decreased activity, particularly with respect to $\alpha_4\beta_1$, while certain polar groups (2-hydroxyethyl and 2-morpholinoethyl; compounds **11** and **12**) could be incorporated at this position with retention of $\alpha_4\beta_7$ activity. Substitution at the 5-position of the pyridazinone (R^1) with a variety of primary and secondary *O*-alkyl groups (compounds **14**, **15**, **19**−**21**) were well-tolerated in regard to inhibition of $\alpha_4\beta_7$-mediated cellular adhesion, but generally resulted in moderate decreases in $\alpha_4\beta_1$ activity. Similarly, substitution at the 5-position with amino and alkylamino groups resulted in analogues (**16**−**18**) that retained activity versus $\alpha_4\beta_7$, but displayed reduced inhibition of the $\alpha_4\beta_1$/VCAM-1 interaction.

Following this screen of modifications to the pyridazinone group, alternatives to the amide linkage were sought to explore the improvement of pharmacokinetic properties and selectivity for $\alpha_4\beta_7$ versus $\alpha_4\beta_1$. The majority of such analogues prepared incorporated the 2-methyl-5-methoxy pyridazinone found in the potent dual $\alpha_4\beta_1/\alpha_4\beta_7$ antagonist **5**. Table 2 summarizes the cellular inhibition results for urea and carbamate compounds, which were found to display selective inhibition of $\alpha_4\beta_7$/MAdCAM-1 based cellular adhesion compared to that mediated by $\alpha_4\beta_1$/VCAM-1. Double-digit nanomolar activity versus $\alpha_4\beta_7$ was achieved with a number of trisubstituted ureas, with the most active (**30**, R^4 = *i*-Bu, R^5 = CH_3) having an IC_{50} of 17 nM. It proved to be more difficult to optimize the carbamate series to achieve low nanomolar activity, and the most potent carbamate (**36**, R^4 = *t*-Bu, $\alpha_4\beta_7$ IC_{50} = 110 nM) required concomitant substitution of the pyridazinone 2-position with a 2-hydroxyethyl group, identified as a potent ligand in the earlier optimization of the amide series. The selectivity for $\alpha_4\beta_7$ observed with the ureas and carbamates might be explained by conformational changes when compared with the amides.

Since it has been shown that anti-α_4 antibodies can block synergistic effects between TNF-α and α_4 integrins,[16,17] we turned our attention to whether our compounds can block the adhesion of different types of cells induced by either TNF-α or lipopolysaccharide (LPS) in addition to the primary cellular assay that is activated by Mn^{2+}. Consequently, we studied the ability of compound **5** to block adhesion of human leukocytes to stimulated human endothelial cells.[18,19] Compound **5** was found to block the adhesion of human lymphocytes, monocytes, and granulocytes to stimulated human umbilical vein endothelial (HUVEC) cells with IC_{50} values in the low nanomolar range (Table 3). These results demonstrate that **5** blocks cellular adhesion in different cell types activated by Mn^{2+}, LPS, or the proinflammatory cytokine TNF-α. Considering the role of both TNF-α and α_4 integrins in many diseases, and the therapeutic effects of α_4 integrin antagonists (whether potentially separated from rare but fatal side effects) and anti-TNF-α antibodies, the combination of these two approaches may offer an improved treatment modality.

Pharmacokinetics. Pharmacokinetic (PK) data of selected compounds in rats are presented in Table 4. Compound **5** was found to have low bioavailability (F = 1%) and a low area under the plasma concentration−time curve (AUC = 0.5 μM−h) after oral dosing at 30 mg/kg. A few esters were selected as potential prodrugs of these antagonists.[21,22] Oral administration of methyl, ethyl, and 2-hydroxyethyl esters (**5a**, **5b**, and **5c**, respectively) of **5** resulted in enhanced plasma exposure of the parent carboxylic acid, with the latter ester (**5c**) providing the highest exposure (F = 7%, AUC = 3.3 μM−h). Alterations to the amide functionality also proved to have the potential to improve PK properties. Within the carbamate and urea subseries substantial improvements in oral bioavailability and systemic exposure level were achieved, provided that the compounds were dosed as 2-hydroxyethyl esters (urea **30c**: F = 14%, AUC = 47 μM−h; carbamate **36c**: F = 31%, AUC = 51 μM−h).

Dextran Sulfate Sodium (DSS) Colitis. With the discovery that the prodrug **36c** had considerably improved pharmacokinetics, the in vivo activity of this $\alpha_4\beta_7$-specific integrin

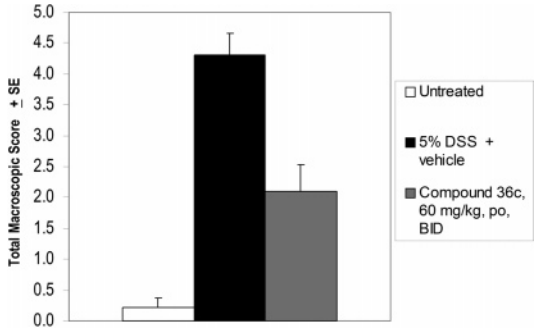


Figure 2. Prevention of DSS-induced colitis by compound **36c**. Mice were dosed with 60 mg/kg, bid, po, for 7 days. *$p < 0.05$ vs DSS + vehicle-treated. Macroscopic score was the combined scores from measurements of colon shrinkage, stool consistency, and colon damage.

antagonist in a mouse DSS colitis model was evaluated. After oral dosing at 60 mg/kg, bid, for 7 days, the macroscopic damage score was reduced by 54% (Figure 2). These data demonstrate that an $\alpha_4\beta_7$-specific integrin antagonist can effectively prevent the development of experimental DSS-induced colitis, presumably by preventing the recruitment of $\alpha_4\beta_7$ positive cells to the intestinal tract. Our results lend additional support to the concept that inhibition of integrin-dependent recruitment can have therapeutic value, consistent with data reported for monoclonal antibodies to α_4 integrins[7a] and for small molecule integrin antagonists.[23]

Conclusion

A novel series of integrin antagonists based on a pyridazinone-substituted phenylalanine scaffold has been discovered. Synthetic routes were developed that allow efficient preparation of analogues with varied substitutions on the pyridazinone ring, as well as compounds with an amide, urea, or carbamate linkage to the phenylalanine nitrogen. These compounds are potent antagonists of $\alpha_4\beta_1$/VCAM-1 and/or $\alpha_4\beta_7$/MAdCAM-1 mediated cellular adhesion whether activated by metal ion, LPS, or TNF-α. Dual antagonists were identified as well as selective $\alpha_4\beta_7$ antagonists that are very different from known α_4 antagonists (most of which are $\alpha_4\beta_1$ selective). The pharmacokinetic properties of selected members of the series have been determined in rats, leading to the finding that the use of ester prodrugs and bioisosteric replacement of the amide functionality has the potential to improve oral bioavailability and systemic exposure level. Our findings in a mouse colitis model offer the unique perspective that an integrin antagonist with specificity for $\alpha_4\beta_7$ integrin and with negligible activity against $\alpha_4\beta_1$ integrin can prevent development of experimental colitis. Further exploration of α_4 integrin receptor antagonists may provide new therapeutic interventions in diseases where the integrins play critical roles.

Experimental Section

General. ^{1}H NMR spectra were acquired on a Bruker 300-Avance (300 MHz) or Avance DMX-400 (400 MHz) spectrometer, with TMS as an internal standard; chemical shifts are expressed in parts per million (ppm, δ scale). Reverse-phase preparative HPLC purifications were performed using a Gilson system equipped with a YMC-Pack ODS-A column (100 × 30 mm; 5 μM, 120 Å) eluting at 32 mL/min with detection at 214 and 254 nm. Microwave-accelerated reactions were performed using either a CEM Discover or a Personal Chemistry Smith Synthesizer microwave instrument. LC-MS was performed on an Agilent 1100 series LC/MSD. Accurate mass determination was performed on a Micromass LCT time-of-flight mass spectrometer using electrospray ionization, with leucine enkephalin as an internal standard. Elemental analysis was conducted by Robertson Microlit Laboratories, Inc. Madison, NJ.

Where noted, compounds were determined to be >95% pure by analytical reverse-phase HPLC. The retention time (t_r) was determined using two of the following three HPLC systems. HPLC-1: Waters XTerra MS C_{18} column (2.5 μm, 2.1 × 30 mm), gradient 4−96% CH_3CN-H_2O, 0.05% HCO_2H over 3.5 min, hold 96% CH_3CN for 2 min, flow rate 0.5 mL/min, 190−360 nm PDA detection. HPLC-2: Supelco ABZ+PLUS column (3 μm, 2.1 × 50 mm), gradient 10−90% CH_3CN-H_2O, 0.05% TFA over 4 min, then to 100% CH_3CN over 0.1 min, hold at 100% CH_3CN for 1.9 min, flow rate 0.75 mL/min, detection at 220 and 254 nm; HPLC-3: Supelco ABZ+PLUS column (3 μm, 4.6 × 33 mm), gradient 4−95% CH_3CN-H_2O, 0.1% TFA over 8.5 min, hold at 95% CH_3CN for 0.3 min, flow rate 1.2 mL/min, detection at 220 and 254 nm.

4-Halopyridazinone precursors were commercially available or were prepared using published procedures.[24,25] In cases where preparation of the required halopyridazinone has not been reported, experimental methods are provided preceding the synthesis in which it was used.

Female Balb/c mice (Charles River Laboratories), 11−14 weeks of age, were used for dextran sulfate sodium (DSS) colitis studies. DSS (MW 36−44 kD) was purchased from ICN pharmaceuticals (Newport, CA).

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-(4-boronophenyl)-propionic Acid (3). 2,6-Dichlorobenzoyl chloride (1.47 mL, 10.2 mmol) was added to a mixture of 4-borono-L-phenylalanine (**2**, 2.04 g, 9.76 mmol) and Na_2CO_3 (2.07 g, 19.5 mmol) in CH_3CN-H_2O (1:1, 40 mL) at 50 °C. The resulting mixture was stirred at 50 °C for 1 h, then was cooled to 0 °C, and was acidified to pH 2 by addition of concentrated HCl (aq). The suspension was stirred at 0 °C for 30 min, and the precipitated solid was collected by vacuum filtration and was washed with water. The white solid was dried in a vacuum oven at 50 °C, affording compound **3** (2.65 g, 71%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.55 (d, 2H, J = 7.6 Hz), 7.28−7.40 (m, 5H), 4.95 (dd, 1H, J = 9.3, 4.7 Hz), 3.30 (dd, 1H, J = 13.9, 5.3 Hz), 3.03 (dd, 1H, J = 14.1, 9.4 Hz). MS m/z 382 $(MH)^+$.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (5). A 10 mL vial containing a magnetic stir bar was charged with boronic acid **3** (76 mg, 0.20 mmol), 4-chloro-5-methoxy-2-methyl-2*H*-pyridazin-3-one (**22**, 35 mg, 0.20 mmol), $Pd(PPh_3)_2Cl_2$ (7 mg, 0.01 mmol), 1.0 M sodium carbonate (0.50 mL, 0.50 mmol), and CH_3CN (0.50 mL). The vial was sealed, and the mixture was heated under microwave irradiation at 150 °C for 10 min. Acidification (with TFA) followed by reverse phase HPLC purification (20−40% CH_3CN-H_2O, 0.1% TFA) gave compound **5** as a white solid (57 mg, 58%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.17 (s, 1H), 7.38−7.33 (m, 7H), 4.99 (dd, 1H, J = 5.0, 9.4 Hz), 3.94 (s, 3H), 3.78 (s, 3H), 3.33 (dd, 1H, J = 5.1, 14.1 Hz), 3.08 (dd, 1H, J = 9.4, 14.1 Hz). MS m/z 476 $(MH)^+$. Anal. ($C_{22}H_{19}Cl_2N_3O_5 \cdot H_2O$) C, H, N, Cl.

Compounds **6**−**14** and **17**−**18** were prepared by the method given for compound **5**.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (6). Prepared from coupling of **3** (96 mg, 0.25 mmol) and 4-chloro-5-methoxy-2*H*-pyridazin-3-one. Yield: 29 mg (25%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (s, 1H), 7.41−7.33 (m, 7H), 4.97 (dd, 1H, J = 5.1, 9.3 Hz), 3.96 (s, 3H), 3.25 (dd, 1H, J = 5.1, 14.0 Hz), 3.07 (dd, 1H, J = 9.3, 14.0 Hz). MS m/z 462 $(MH)^+$. TOF HRMS calcd for $C_{21}H_{18}Cl_2N_3O_5$ $(MH)^+$ 462.0624; found 462.0605. HPLC-1, t_r = 2.82 min; HPLC-3, t_r = 3.61 min.

(*S*)-3-[4-(2-Benzyl-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(2,6-dichloro-benzoylamino)-propionic Acid (7). Prepared by coupling of **3** (76 mg, 0.20 mmol) and 2-benzyl-4-chloro-5-methoxy-2*H*-pyridazin-3-one. Yield: 52 mg (46%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (s, 1H), 7.35−7.27 (m, 12H), 5.34 (s, 2H), 4.97 (dd, 1H, J = 5.1, 9.2 Hz), 3.93 (s, 3H), 3.35 (dd, 1H, J = 5.0, 13.9 Hz), 3.08 (dd, 1H, J = 9.4, 13.9 Hz). MS m/z

552 $(MH)^+$. TOF HRMS calcd for $C_{28}H_{24}Cl_2N_3O_5$ $(MH)^+$ 552.1093; found 552.1094. Anal. ($C_{28}H_{23}Cl_2N_3O_5 \cdot 0.5H_2O$) C, H, N, Cl.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-3-oxo-2-phenyl-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (8). Prepared by coupling of **3** (76 mg, 0.20 mmol) and 4-chloro-5-methoxy-2-phenyl-2*H*-pyridazin-3-one. Yield: 46 mg (42%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.34 (s, 1H), 7.53−7.35 (m, 12H), 4.97 (dd, 1H, J = 5.1, 9.4 Hz), 4.01 (s, 3H), 3.35 (dd, 1H, J = 5.1, 14.0 Hz), 3.06 (dd, 1H, J = 9.3, 14.0 Hz). MS *m*/z 538 $(MH)^+$. TOF HRMS calcd for $C_{27}H_{22}Cl_2N_3O_5$ $(MH)^+$ 538.0937; found 538.0935. Anal. ($C_{27}H_{21}Cl_2N_3O_5 \cdot 0.5H_2O$) C, H, N, Cl.

(*S*)-3-[4-(2-Cyclohexyl-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(2,6-dichloro-benzoylamino)-propionic Acid (9). Prepared from coupling of **3** (40 mg, 0.10 mmol) and 4-chloro-2-cyclohexyl-5-methoxy-2*H*-pyridazin-3-one. Yield: 15 mg (27%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.22 (s, 1H), 7.36−7.33 (m, 7H), 4.99 (dd, 1H, J = 5.0, 9.3 Hz), 4.91 (m, 1H), 4.00 (s, 3H), 3.35 (dd, 1H, J = 5.0, 14.1 Hz), 3.07 (dd, 1H, J = 9.3, 14.1 Hz), 1.92−1.24 (m, 10H). MS *m*/z 544 $(MH)^+$. TOF HRMS calcd for $C_{27}H_{28}Cl_2N_3O_5$ $(MH)^+$ 544.1406; found 544.1391. HPLC-1, t_r = 3.63 min; HPLC-3, t_r = 5.46 min.

(*S*)-3-[4-(2-*tert*-Butyl-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(2,6-dichloro-benzoylamino)-propionic Acid (10). Prepared from coupling of **3** (76 mg, 0.20 mmol) and 2-*tert*-butyl-4-chloro-5-methoxy-2*H*-pyridazin-3-one. Yield: 16 mg (15%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.11 (s, 1H), 7.39−7.29 (m, 7H), 4.99 (dd, 1H, J = 5.0, 9.0 Hz), 3.92 (s, 3H), 3.31 (dd, 1H, J = 5.0, 14.0 Hz), 3.08 (dd, 1H, J = 9.1, 14.0 Hz), 1.66 (s, 9H). MS *m*/z 518 $(MH)^+$. TOF HRMS calcd for $C_{25}H_{24}Cl_2N_3O_5$ $(MH)^+$ 518.1250; found 518.1235. HPLC-1, t_r = 3.53 min; HPLC-3, t_r = 5.44 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-{4-[2-(2-hydroxy-ethyl)-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (11). Prepared from coupling of **3** (153 mg, 0.40 mmol) and 4-chloro-2-(2-hydroxy-ethyl)-5-methoxy-2*H*-pyridazin-3-one. Yield: 74 mg (35%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (s, 1H), 7.36−7.33 (m, 7H), 4.99 (dd, 1H, J = 5.0, 9.3 Hz), 4.32 (t, 2H, J = 5.7 Hz), 3.94 (s, 3H), 3.92 (t, 2H, J = 5.7 Hz), 3.32 (dd, 1H, J = 5.0, 14.1 Hz), 3.07 (dd, 1H, J = 9.3, 14.1 Hz). MS *m*/z 506 $(MH)^+$. TOF HRMS calcd for $C_{23}H_{22}Cl_2N_3O_6$ $(MH)^+$ 506.0886; found 506.0882. Anal. ($C_{23}H_{21}Cl_2N_3O_6 \cdot 0.25F_3CCO_2H$) C, H, N.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-{4-[5-methoxy-2-(2-morpholin-4-yl-ethyl)-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (12). Prepared from coupling of **3** (153 mg, 0.40 mmol) and 4-chloro-5-methoxy-2-(2-morpholin-4-yl-ethyl)-2*H*-pyridazin-3-one. Yield: 116 mg (42%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.28 (s, 1H), 7.42−7.33 (m, 7H), 4.98 (dd, 1H, J = 4.9, 9.6 Hz), 4.61 (t, 2H, J = 5.5 Hz), 3.98 (s, 3H), 3.90 (br, 4H), 3.64 (t, 2H, J = 5.5 Hz), 3.43 (br, 4H), 3.34 (dd, 1H, J = 4.9, 14.1 Hz), 3.06 (dd, 1H, J = 9.6, 14.1 Hz). MS *m*/z 575 $(MH)^+$. TOF HRMS calcd for $C_{27}H_{29}Cl_2N_4O_6$ $(MH)^+$ 575.1464; found 575.1464. HPLC-1, t_r = 2.52 min; HPLC-3, t_r = 3.18 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-hydroxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (13). Prepared from coupling of **3** (96 mg, 0.25 mmol) and 4-bromo-5-hydroxy-2-methyl-2*H*-pyridazin-3-one. Yield: 11 mg (10%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.77 (s, 1H), 7.35−7.44 (m, 7H), 4.98 (dd, 1H, J = 5.0, 8.6), 3.73 (s, 3H), 3.30 (obscured by CD_3OD signal), 3.12 (dd, 1H, J = 8.6, 14.0 Hz). MS *m*/z 462 $(MH)^+$. TOF HRMS calcd for $C_{21}H_{18}Cl_2N_3O_5$ $(MH)^+$ 462.0624; found 462.0613. HPLC-1, t_r = 2.75 min; HPLC-3, t_r = 3.90 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-ethoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (14). Prepared from coupling of **3** (76 mg, 0.20 mmol) and 4-chloro-5-ethoxy-2-methyl-2*H*-pyridazin-3-one. Yield: 44 mg (44%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.15 (s, 1H), 7.32−7.44 (m, 7H), 5.01 (dd, 1H, J = 9.3, 5.0 Hz), 4.27 (q, 2H, J = 7.0 Hz), 3.79 (s, 3H), 3.35 (dd, 1H, J = 14.4, 4.8 Hz), 3.10 (dd, 1H, J = 14.1, 9.4 Hz), 1.33 (t, 3H, J = 7.0 Hz). MS *m*/z 490 $(MH)^+$. TOF HRMS calcd for $C_{23}H_{22}Cl_2N_3O_5$ $(MH)^+$ 490.0937; found 490.0937. Anal. ($C_{23}H_{21}Cl_2N_3O_5 \cdot 0.5H_2O$) C, H, N, Cl.

4-Bromo-5-difluoromethoxy-2-methyl-2*H*-pyridazin-3-one. A pressure tube was charged sequentially with 4-bromo-5-hydroxy-2-methyl-2*H*-pyridazin-3-one[24] (1.29 g, 6.28 mmol), chlorodifluoroacetic acid sodium salt (1.15 g, 7.54 mmol), and NaOH (314 mg, 7.85 mmol). The vessel was purged with nitrogen, and DMF (3.0 mL) was added. The mixture was heated to 130 °C for 1 h, then was allowed to cool to 23 °C. The mixture was diluted with EtOAc (50 mL), and the resulting solution was washed with a saturated solution of NaCl (aq) (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated, to yield a tan solid which was purified by column chromatography (SiO_2, 50−70% EtOAc−hexanes), yielding a white solid (1.09 g, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (s, 1H), 6.70 (t, 1H, J_{HF} = 71.0 Hz), 3.86 (s, 3H). TOF HRMS calcd for $C_6H_6N_2O_2BrF_2$ $(MH)^+$ 254.9581; found 254.9582.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-difluoromethoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (15). To a mixture of **3** (370 mg, 0.968 mmol), 4-bromo-5-difluoromethoxy-2-methyl-2*H*-pyridazin-3-one (218 mg, 1.06 mmol) and $Pd(PPh_3)_2Cl_2$ (34 mg, 0.048 mmol) were sequentially added an aqueous solution of Na_2CO_3 (2 M, 2 mL, 4 mmol) and CH_3CN (2 mL). The resulting suspension was heated at reflux under N_2 for 1 h, then was allowed to cool to 23 °C. The mixture was partially concentrated to remove volatile solvent. The resulting mixture was diluted with half-saturated $NaHCO_3$ (aq) (20 mL) and was washed with Et_2O (20 mL). The combined aqueous extracts were cooled to 0 °C and were acidified to pH 2 by addition of 2 N aqueous HCl. The precipitated solid was collected by vacuum filtration and was purified by reverse-phase HPLC (35−55% CH_3CN-H_2O, 0.1% TFA), affording the title compound as a white powder (305 mg, 62%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.09 (s, 1H), 7.38−7.44 (m, 4H), 7.27−7.37 (m, 3H), 6.97 (t, 1H, J_{HF} = 72.2 Hz), 4.98 (dd, 1H, J = 9.3, 5.0 Hz), 3.81 (s, 3H), 3.33 (dd, 1H, J = 14.2, 5.1 Hz), 3.07 (dd, 1H, J = 14.1, 9.4 Hz). TOF HRMS calcd for $C_{22}H_{18}N_3O_5Cl_2F_2$ $(MH)^+$ 512.0592; found 512.0604. Anal. ($C_{22}H_{17}N_3O_5Cl_2F_2 \cdot 0.1F_3CCO_2H$) C, H, N.

(*S*)-3-[4-(5-Amino-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(2,6-dichloro-benzoylamino)-propionic Acid (16). Prepared by the method given above for compound **15** using 370 mg (0.968 mmol) of **3** and 5-amino-4-bromo-2-methyl-2*H*-pyridazin-3-one[25] (326 mg, 1.60 mmol). The crude product was a tan solid (599 mg, 90%). A purified sample was obtained by reverse-phase HPLC (20−40% CH_3CN-H_2O, 0.1% TFA). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.66 (s, 1H), 7.27−7.45 (m, 7 H), 4.98 (dd, 1H, J = 8.4, 5.6 Hz), 3.68 (s, 3H), 3.30 (dd, 1H, J = 13.9, 5.5 Hz), 3.13 (dd, 1H, J = 14.0, 8.4 Hz). TOF HRMS calcd for $C_{21}H_{19}N_4O_4Cl_2$ $(MH)^+$ 461.0783; found 461.0794. HPLC-1, t_r = 2.78 min; HPLC-2, t_r = 2.72 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(2-methyl-3-oxo-5-pyrrolidin-1-yl-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (17). Prepared from coupling of **3** (153 mg, 0.40 mmol) and 4-bromo-2-methyl-5-pyrrolidin-1-yl-2*H*-pyridazin-3-one. Yield: 31 mg (14%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.85 (s, 1H), 7.41−7.31 (m, 5H), 7.19 (d, 2H, J = 7.7 Hz), 4.92 (dd, 1H, J = 5.2, 8.4 Hz), 3.67 (s, 3H), 3.27 (dd, 1H, J = 5.2, 14.1 Hz), 3.13 (dd, 1H, J = 8.4, 14.1 Hz), 3.06 (br, 4H), 1.75 (br, 4H). MS *m*/z 515 $(MH)^+$. TOF HRMS calcd for $C_{25}H_{25}Cl_2N_4O_4$ $(MH)^+$ 515.1253; found 515.1252. HPLC-1, t_r = 3.15 min; HPLC-3, t_r = 4.56 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(2-methyl-5-morpholin-4-yl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (18). Prepared from coupling of **3** (153 mg, 0.40 mmol) and 4-chloro-2-methyl-5-morpholin-4-yl-2*H*-pyridazin-3-one. Yield: 117 mg (52%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.92 (s, 1H), 7.42−7.32 (m, 7H), 5.06 (dd, 1H, J = 5.5, 8.2 Hz), 3.71 (s, 3H), 3.68 (s, 3H), 3.52 (t, 4H, J = 4.6 Hz), 3.26 (dd, 1H, J = 5.5, 14.0 Hz), 3.14 (dd, 1H, J = 8.2, 14.0 Hz), 3.01 (t, 4H, J = 4.6 Hz). MS *m*/z 531 $(MH)^+$. TOF HRMS calcd for $C_{25}H_{25}Cl_2N_4O_5$ $(MH)^+$ 531.1202; found 531.1199. Anal. ($C_{25}H_{24}Cl_2N_4O_5 \cdot 0.3F_3CCO_2H$) C, H, N, Cl.

General Procedure for the Synthesis of Analogues 19−21 by Michael Addition/Elimination. A solution of an alkoxide nucleophile was generated by addition of sodium metal (25 mg, 1.08

mmol) to an alcohol (1 mL) at 23 °C. The mixture was stirred until solid metal was consumed. Compound **5** (100 mg, 0.20 mmol) was added, and the mixture was heated in an 85 °C oil bath for 2 h. The mixture was concentrated, and the residue was suspended in CH_3CN, acidified by addition of TFA, filtered, and purified by reverse-phase HPLC (CH_3CN-H_2O, 0.1% TFA).

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-{4-[5-(2-hydroxy-ethoxy)-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (19). Prepared from ethylene glycol. Yield: 32 mg (30%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.17 (s, 1H), 7.33−7.46 (m, 7H), 4.99 (dd, 1H, J = 9.0, 5.1 Hz), 4.24 (t, 2H, J = 4.7 Hz), 3.73−3.78 (m, 5H), 3.30−3.34 (m, obscured by CD_3OD signal), 3.09 (dd, 1H, J = 14.1, 9.0 Hz). TOF HRMS calcd for $C_{23}H_{22}N_3O_6Cl_2$ $(MH)^+$ 506.0886; found 506.0908. HPLC-1, t_r = 2.77 min; HPLC-2, t_r = 2.73 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-{4-[2-methyl-5-(2-morpholin-4-yl-ethoxy)-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (20). Prepared from 4-(2-hydroxyethyl)morpholine. Yield: 64 mg (42%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.16 (s, 1H), 7.33−7.44 (m, 7H), 4.95 (dd, 1H, J = 8.7, 5.4 Hz), 4.52−4.58 (m, 2H), 3.80 (s, 3H), 3.72−3.84 (br m, 4H), 3.42−3.52 (m, 2H), 3.33 (m, obscured by CD_3OD signal), 3.08−3.20 (m, 4 H), 3.09 (dd, 1H, J = 13.9, 8.8 Hz). TOF HRMS calcd for $C_{27}H_{29}N_4O_6Cl_2$ $(MH)^+$ 575.1464; found 575.1472. HPLC-1, t_r = 2.41 min; HPLC-2, t_r = 2.08 min.

(*S*)-3-[4-(5-Cyclohexyloxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(2,6-dichloro-benzoylamino)-propionic Acid (21). Prepared from cyclohexanol. Yield: 53 mg (46%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.12 (s, 1H), 7.34−7.43 (m, 7H), 4.99 (dd, 1H, J = 9.0, 5.1 Hz), 4.55−4.61 (m, 1H), 3.77 (s, 3H), 3.33 (m, obscured by CD_3OD signal), 3.10 (dd, 1H, J = 14.2, 9.2 Hz), 1.22−1.94 (m, 10 H). TOF HRMS calcd for $C_{27}H_{28}N_3O_5Cl_2$ $(MH)^+$ 544.1406; found 544.1417. Anal. ($C_{27}H_{27}N_3O_5Cl_2 \cdot 0.3\ F_3CCO_2H$) C, H, N.

(*S*)-2-Amino-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (23). A mixture of 4-borono-L-phenylalanine (**2**, 105 mg, 0.50 mmol), 4-chloro-5-methoxy-2-methyl-2*H*-pyridazin-3-one (**22**, 87 mg, 0.50 mmol) and $Pd(PPh_3)_2Cl_2$ (18 mg, 0.025 mmol) in 1.0 M sodium carbonate (1.0 mL, 1.0 mmol) and acetonitrile (1.0 mL) was heated under microwave irradiation at 150 °C for 10 min. The crude mixture, upon acidification with TFA, was purified by reverse phase HPLC (0−20% CH_3CN-H_2O, 0.1% TFA) to yield compound **23** as a white solid (TFA salt, 125 mg, 55%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (s, 1H), 7.42 (d, 2H, J = 8.3 Hz), 7.35 (d, 2H, J = 8.3 Hz), 4.24, (dd, 1H, J = 5.0, 8.3 Hz), 3.92 (s, 3H), 3.80 (s, 3H), 3.37 (dd, 1H, J = 5.0, 14.5 Hz), 3.14 (dd, 1H, J = 8.3, 14.5 Hz). MS m/z 304 $(MH)^+$. TOF HRMS calcd for $C_{15}H_{18}N_3O_4$ $(MH)^+$ 304.1297; found 304.1302. Anal. ($C_{15}H_{17}N_3O_4 \cdot 1.3F_3CCO_2H$) C, H, N.

(*S*)-2-Amino-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid Methyl Ester (23a). Compound **23** (TFA salt, 0.20 g, 0.48 mmol) was dissolved in MeOH (8 mL) and heated at reflux in the presence of $SOCl_2$ (0.2 mL) for 2 h. The solution was concentrated, and the resulting solid was treated with saturated $NaHCO_3$ (aq) and extracted with CH_2Cl_2 (3 × 2 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated, yielding ester **23a** as a clear gum (0.10 g, 66%). ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1H), 7.47 (d, 2H, J = 8.2 Hz), 7.24 (d, 2H, J = 8.2 Hz), 3.90 (s, 3H), 3.80 (s, 3H), 3.77 (dd, 1H, J = 5.0, 8.3 Hz), 3.73 (s, 3H), 3.15 (dd, 1H, J = 5.0, 13.6 Hz), 2.86 (dd, 1H, J = 8.3, 13.6 Hz). MS m/z 318 $(MH)^+$. TOF HRMS calcd for $C_{16}H_{20}N_3O_4$ $(MH)^+$ 318.1454; found 318.1454.

(*S*)-2-[(Imidazole-1-carbonyl)-amino]-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid Methyl Ester (24). To a solution of **23a** (336 mg, 1.06 mmol) in CH_2Cl_2-THF (5:1, 6 mL) was added 1,1′-carbonyldiimidazole (259 mg, 1.59 mmol). The resulting solution was stirred at 23 °C for 1 h. The mixture was concentrated, and the residue was purified by column chromatography (SiO_2, 2−10% $MeOH-CH_2Cl_2$). The title compound was obtained as a white solid (355 mg, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (s, 1H), 7.89 (s, 1H), 7.48 (d, 2H, J = 8.2 Hz), 7.17 (d, 2H, J = 8.2 Hz), 7.03 (s, 1H), 6.55 (d, 1H, J = 7.5 Hz), 4.91 (q, 1H, J = 6.3 Hz), 3.90 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.29 (d, 2H, J = 6.0 Hz). TOF HRMS calcd for $C_{20}H_{22}N_5O_5$ $(MH)^+$ 412.1621; found 412.1630.

General Procedure for Preparation of Ureas 25−27 from Anilines. To a solution of carbamoyl imidazole **24** (50 mg, 0.122 mmol) in acetonitrile (200 μL) was added the aniline (0.146 mmol). The mixture was heated by microwave irradiation (130 °C, 10 min). A 2 N aqueous solution of lithium hydroxide (200 μL) was added, and the resulting mixture was stirred at 23 °C for 9 h. The mixture was acidified by addition of TFA (100 μL), and the product was isolated by reverse-phase HPLC (CH_3CN-H_2O, 0.1% TFA).

(*S*)-3-[4-(5-Methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(3-methyl-3-phenyl-ureido)-propionic Acid (25). Prepared from 20 mg of **24** (0.049 mmol) and 6.3 μL of *N*-methylaniline (0.058 mmol). Yield: 5.5 mg (26%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.21 (s, 1H), 7.31−7.44 (m, 5H), 7.12−7.18 (m, 4H), 4.58 (dd, 1H, J = 7.9, 5.1 Hz), 3.97 (s, 3H), 3.81 (s, 3H), 3.17−3.21 (m, 4H), 3.00 (dd, 1H, J = 13.7, 7.9 Hz). TOF HRMS calcd for $C_{23}H_{25}N_4O_5$ $(MH)^+$ 437.1825; found 437.1839. Anal. ($C_{23}H_{24}N_4O_5$) C, H, N.

(*S*)-2-(3-Isopropyl-3-phenyl-ureido)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (26). Prepared from *N*-isopropylaniline. Yield: 23 mg (34%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.19 (s, 1H), 7.40−7.43 (m, 3H), 7.32 (d, 2H, J = 8.0 Hz), 7.04 (d, 2H, J = 5.9 Hz), 7.01 (d, 2H, J = 8.1 Hz), 4.70 (sept, 1H, J = 6.7 Hz), 4.53 (t, 1H, J = 6.2 Hz), 3.95 (s, 3H), 3.80 (s, 3H), 3.14 (dd, 1H, J = 13.8, 5.1 Hz), 2.94 (dd, 1H, J = 13.8, 7.4 Hz), 1.03 (d, 3H, J = 6.7 Hz), 1.02 (d, 3H, J = 6.6 Hz). TOF HRMS calcd for $C_{25}H_{29}N_4O_5$ $(MH)^+$ 465.2138; found 465.2122. Anal. ($C_{25}H_{28}N_4O_5 \cdot 0.8\ F_3CCO_2H$) C, H, N.

(*S*)-3-[4-(5-Methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-(3-phenyl-3-propyl-ureido)-propionic Acid (27). Prepared from *N*-*n*-propylaniline. Yield: 24 mg (35%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.19 (s, 1H), 7.29−7.43 (m, 5H), 7.06−7.12 (m, 4H), 4.55 (dd, 1H, J = 7.7, 5.2 Hz), 3.95 (s, 3H), 3.79 (s, 3H), 3.62 (dd, 1H, J = 14.0, 7.7 Hz), 3.53 (dd, 1H, J = 13.8, 7.6 Hz), 3.17 (dd, 1H, J = 13.8, 5.1 Hz), 2.97 (dd, 1H, J = 13.9, 7.7 Hz), 1.46 (sext, 2H, J = 7.4 Hz), 0.85 (t, 3H, J = 7.4 Hz). TOF HRMS calcd for $C_{25}H_{29}N_4O_5$ $(MH)^+$ 465.2138; found 465.2118. Anal. ($C_{25}H_{28}N_4O_5 \cdot 0.8F_3CCO_2H$) C, H, N.

General Procedure for Preparation of Ureas 28−31 from Aliphatic Amines. To a solution of carbamoyl imidazole **24** (40 mg, 0.097 mmol) in acetonitrile (200 μL) was added the amine (0.117 mmol). The resulting mixture was stirred at 23 °C for 19 h. A 2 N aqueous solution of lithium hydroxide (200 μL) was added, and the resulting mixture was stirred at 23 °C for 9 h. The mixture was diluted with H_2O (400 μL) and acidified by addition of TFA (100 μL), and the product was isolated by reverse-phase HPLC (CH_3CN-H_2O, 0.1% TFA).

(*S*)-2-(3,3-Dimethyl-ureido)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (28). Prepared from Me_2NH (2M in THF). Yield: 23.5 mg (51%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.17 (s, 1H), 7.38 (d, 2H, J = 8.1 Hz), 7.29 (d, 2H, J = 7.9 Hz), 4.53 (dd, 1H, J = 9.0, 4.8 Hz), 3.93 (s, 3H), 3.78 (s, 3H), 3.22 (dd, 1H, J = 13.8, 4.8 Hz), 3.06 (dd, 1H, J = 13.9, 8.9 Hz), 2.86 (s, 6H). TOF HRMS calcd for $C_{18}H_{23}N_4O_5$ $(MH)^+$ 375.1668; found 375.1676. Anal. ($C_{18}H_{22}N_4O_5 \cdot 0.9F_3CCO_2H$) C, H, N.

(*S*)-2-[3-(2-Hydroxy-ethyl)-3-methyl-ureido]-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (29). Prepared from *N*-methylethanolamine. HPLC gradient: 15−35%, CH_3CN-H_2O, 0.05% HCO_2H. Yield: 8.6 mg (20%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.18 (s, 1H), 7.38 (d, 2H, J = 8.3 Hz), 7.29 (d, 2H, J = 8.3 Hz), 4.53 (dd, 1H, J = 8.8, 4.9 Hz), 3.94 (s, 3H), 3.78 (s, 3H), 3.60 (t, 2H, J = 5.5 Hz), 3.33 (t, 2H, partially obscured by CD_3OD signal), 3.22 (dd, 1H, J = 14.0, 5.0 Hz), 3.05 (dd, 1H, J = 13.8, 8.8 Hz), 2.91 (s, 3H). TOF HRMS calcd for $C_{19}H_{25}N_4O_6$ $(MH)^+$ 405.1774; found 405.1790. Anal. ($C_{19}H_{24}N_4O_6 \cdot 0.8HCO_2H$) C, H, N.

(*S*)-2-(3-Isobutyl-3-methyl-ureido)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (30). Prepared from *N*-methylisobutylamine. Yield: 26.5 mg (55%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.17 (s, 1H), 7.38 (d, 2H, J = 8.3 Hz), 7.28 (d, 2H, J = 8.2 Hz), 4.56 (dd, 1H, J = 8.7, 5.0 Hz), 3.93 (s, 3H), 3.78 (s, 3H), 2.94−3.26 (m, 4H), 2.85 (s, 3H), 1.86−1.93 (m, 1H), 0.84 (d, 3H, J = 6.6 Hz), 0.83 (d, 3H, J = 6.7 Hz). TOF HRMS calcd for $C_{21}H_{29}N_4O_5$ $(MH)^+$ 417.2138; found 417.2128. Anal. ($C_{21}H_{28}N_4O_5 \cdot 0.7F_3CCO_2H$) C, H, N.

(*S*)-3-[4-(5-Methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-2-[(2-methyl-piperidine-1-carbonyl)-amino]-propionic Acid (31). Prepared from 2-methylpiperidine. Yield: 29 mg (56%). ^{1}H NMR (CD_3OD, 300 MHz, diastereomer ratio 1.1:1) δ 8.17 (s, 2H), 7.37 (d, 4H, J = 8.4 Hz), 7.28 (d, 4H, J = 7.0 Hz), 4.48−4.57 (m, 2H), 4.16−4.27 (br m, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.77 (s, 6H), 3.67−3.78 (m, 2H), 3.13−3.26 (m, 2H), 2.98−3.10 (m, 2H), 2.80−2.90 (m, 2H), 1.24−1.68 (m, 12H), 1.16 (d, 3H, J = 6.9 Hz), 1.08 (d, 3H, J = 6.9 Hz). TOF HRMS calcd for $C_{22}H_{29}N_4O_5$ $(MH)^+$ 429.2138; found 429.2144. Anal. ($C_{22}H_{28}N_4O_5 \cdot 0.9F_3CCO_2H$) C, H, N.

4,5-Dibromo-2-(2-hydroxy-ethyl)-2*H*-pyridazin-3-one. To a solution of mucobromic acid (38.68 g, 150 mmol) in EtOH (128 mL) at 5 °C was added 2-hydroxyethyl hydrazine (13.2 mL, 195 mmol) dropwise, maintaining an internal temperature below 10 °C during the addition. The resulting mixture was stirred at 5 °C for 1 h, then was allowed to warm to 23 °C, and finally was heated at reflux for 2 h. The mixture was concentrated, and the residue was purified by flash column chromatography (SiO_2, 50−75% EtOAc−hexanes), affording the title compound as a tan solid (22.93 g, 51%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.85 (s, 1H), 4.38 (t, 2H, J = 4.7 Hz), 4.04 (t, 2H, J = 5.1 Hz), 2.41 (br s, 1H). TOF HRMS calcd for $C_6H_7N_2O_2Br_2$ $(MH)^+$ 296.8874; found 296.8887.

4-Bromo-2-(2-hydroxy-ethyl)-5-methoxy-2*H*-pyridazin-3-one. Sodium methoxide (30 wt % in MeOH, 4.85 mL, 25.8 mmol) was added to an ice-cold solution of 4,5-dibromo-2-(2-hydroxy-ethyl)-2*H*-pyridazin-3-one (7.00 g, 23.5 mmol) in MeOH (40 mL). The mixture was allowed to warm to 23 °C with stirring overnight and was concentrated. The residual white solid was partitioned between CH_2Cl_2 (100 mL) and a saturated aqueous solution of NaCl (100 mL). A white solid precipated and was collected by vacuum filtration, affording the title compound (4.73 g, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (s, 1H), 4.43 (t, 2H, J = 4.9 Hz), 4.09 (s, 3H), 4.02 (app q, 2H, J = 5.2 Hz), 2.87 (t, 1H, J = 5.7 Hz). TOF HRMS calcd for $C_7H_{10}N_2O_3Br$ $(MH)^+$ 248.9875; found 248.9882.

(*S*)-2-Amino-3-{4-[2-(2-hydroxy-ethyl)-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (32). Prepared from 4-bromo-2-(2-hydroxy-ethyl)-5-methoxy-2*H*-pyridazin-3-one by the method given above for compound **23**. HPLC gradient: 5−15% CH_3CN-H_2O, 0.05% HCO_2H. Yield: 107 mg (HCO_2H salt, 57%). ^{1}H NMR (D_2O, 300 MHz) δ 8.16 (s, 1H), 7.27 (d, 2H, J = 8.5 Hz), 7.24 (d, 2H, J = 8.6 Hz), 4.22 (t, 2H, J = 5.4 Hz), 3.89 (dd, 1H, J = 7.9, 5.2 Hz), 3.83 (t, 2H, J = 5.5 Hz), 3.81 (s, 3H), 3.21 (dd, 1H, J = 14.5, 5.3 Hz), 3.03 (dd, 1H, J = 14.6, 8.0 Hz). TOF HRMS calcd for $C_{16}H_{20}N_3O_5$ $(MH)^+$ 334.1403; found 334.1415.

General Method for the Synthesis of Carbamates 33−36. Sodium bicarbonate (14 mg, 0.17 mmol) and an alkylchloroformate or dialkyl dicarbonate (0.17 mmol) were added in sequence to a solution of the amino acid (**23**, 26 mg, 0.058 mmol except as noted otherwise) in a mixture of acetonitrile (0.1 mL) and water (0.3 mL). The resulting suspension was stirred at 23 °C for 18 h. The reaction mixture was acidified to pH 2 by addition of trifluoroacetic acid (10 μL), and the product was isolated by reverse-phase HPLC (CH_3CN-H_2O, 0.1% TFA).

(*S*)-2-Methoxycarbonylamino-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (33). Prepared from methyl chloroformate. Yield: 9.9 mg (38%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.18 (s, 1H), 7.37 (d, 2H, J = 8.1 Hz), 7.28 (d, 2H, J = 8.1 Hz), 4.42 (dd, 1H, J = 9.0, 4.8 Hz), 3.93 (s, 3H), 3.77 (s, 3H), 3.60 (s, 3H), 3.21 (dd, 1H, J = 14.0, 4.7 Hz), 2.97 (dd, 1H, J = 14.0, 9.3 Hz). TOF HRMS calcd for $C_{17}H_{20}N_3O_6$ $(MH)^+$ 362.1352; found 362.1336. Anal. ($C_{17}H_{19}N_3O_6 \cdot 0.8F_3CCO_2H$) C, H, N.

(*S*)-2-Benzyloxycarbonylamino-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (34). Prepared from benzyl chloroformate. Yield: 3.1 mg (10%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.19 (s, 1H), 7.27−7.40 (m, 9H), 5.07 (s, 2H), 4.47 (dd, 1H, J = 9.1, 4.7 Hz), 3.93 (s, 3H), 3.80 (s, 3H), 3.25 (dd, 1H, J = 13.9, 4.7 Hz), 3.00 (dd, 1H, J = 13.9, 9.2 Hz). TOF HRMS calcd for $C_{23}H_{24}N_3O_6$ $(MH)^+$ 438.1665; found 438.1656. Anal. ($C_{23}H_{23}N_3O_6 \cdot 0.3F_3CCO_2H$) C, H, N.

(*S*)-2-*tert*-Butoxycarbonylamino-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid (35). Prepared from di-*tert*-butyl dicarbonate. HPLC gradient: 25−45% CH_3CN-H_2O, 0.05% HCO_2H. Yield: 11.5 mg (47%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 7.38 (d, 2H, J = 8.2 Hz), 7.28 (d, 2H, J = 8.2 Hz), 4.37 (dd, 1H, J = 9.0, 4.9 Hz), 3.93 (s, 3H), 3.78 (s, 3H), 3.19 (dd, 1H, J = 14.1, 4.9 Hz), 2.96 (dd, 1H, J = 14.0, 9.1 Hz), 1.40 (s, 9 H). TOF HRMS calcd for $C_{20}H_{26}N_3O_6$ $(MH)^+$ 404.1822; found 404.1819. Anal. ($C_{20}H_{25}N_3O_6 \cdot 0.4HCO_2H$) C, H, N.

(*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[2-(2-hydroxy-ethyl)-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid (36). Prepared from amino acid 32 (72 mg, 0.16 mmol) and di-*tert*-butyl dicarbonate. HPLC gradient: 25−45% CH_3CN-H_2O, 0.05% HCO_2H. Yield: 58 mg (84%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.22 (s, 1H), 7.40 (d, 2H, J = 8.1 Hz), 7.30 (d, 2H, J = 8.1 Hz), 4.39 (dd, 1H, J = 8.9, 4.7 Hz), 4.34 (t, 2H, J = 5.7 Hz), 3.96 (s, 3H), 3.94 (t, 2H, J = 5.8 Hz), 3.21 (dd, 1H, J = 13.9, 4.9 Hz), 2.97 (dd, 1H, J = 14.0, 9.2 Hz), 1.42 (s, 9H). TOF HRMS calcd for $C_{21}H_{28}N_3O_7$ $(MH)^+$ 434.1927; found 434.1935. Anal. ($C_{21}H_{27}N_3O_7$) C, H, N.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid Methyl Ester (5a). 2,6-Dichlorobenzoyl chloride (0.29 mL, 2.0 mmol) was added to a solution of **23a** (0.50 g, 1.6 mmol) and triethyamine (0.35 mL, 2.5 mmol) in dichloromethane (10 mL). The resulting mixture was stirred at 23 °C for 1 h, then was washed with $NaHCO_3$ (aq). The dichloromethane layer was concentrated, and the residue was purified by reverse-phase HPLC, affording compound **5a** (0.40 g, 51%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 7.86 (s, 1H), 7.46 (d, 2H, J = 8.2 Hz), 7.33−7.25 (m, 5H), 6.30 (d, 1H, J = 8.6 Hz), 5.25 (m, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.77 (s, 3H), 3.30 (m, 2H). MS m/z 490 $(MH)^+$. Anal. ($C_{23}H_{21}Cl_2N_3O_5$) C, H, N, Cl.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid Ethyl Ester (5b). Compound **5** (0.50 g, 1.0 mmol) was dissolved in EtOH (25 mL) and heated at reflux in the presence of $SOCl_2$ (2 mL) for 2 h. The solution was concentrated. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ (aq) and H_2O. The organic phase was dried ($MgSO_4$) and concentrated. The ester **5b** was purified by reverse-phase HPLC, yielding a white solid (0.32 g, 63%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (s, 1H), 7.44 (d, 2H, J = 8.2 Hz), 7.32−7.23 (m, 5H), 6.36 (d, 1H, J = 8.2 Hz), 5.22 (m, 1H), 4.24 (m, 2H), 3.89 (s, 3H), 3.80 (s, 3H), 3.30 (m, 2H), 1.27 (t, 3H, J = 7.1 Hz). MS m/z 504 $(MH)^+$. TOF HRMS calcd for $C_{24}H_{24}Cl_2N_3O_5$ $(MH)^+$ 504.1093; found 504.1071. HPLC-1, t_r = 3.30 min; HPLC-3, t_r = 4.70 min.

(*S*)-2-(2,6-Dichloro-benzoylamino)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid 2-Hydroxy-ethyl Ester (5c). Compound **5** (0.94 g, 1.9 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.59 g, 2.3 mmol), ethylene glycol (200 μL, 3.6 mmol), and *N*,*N*-diisopropylethylamine (1.0 mL, 5.7 mmol) were added to dichloromethane (3 mL). The reaction mixture was stirred overnight at 23 °C and then concentrated. The residue was subjected to column chromatography (SiO_2, EtOAc-heptanes gradient), providing a clear viscous oil. The oil was dissolved in MeOH−H_2O (1:1), frozen and lyophilized, yielding **5c** as a white powder (0.31 g, 31%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (s, 1H), 7.40−7.24 (m, 7H), 6.74 (d, 1H, J = 8.1 Hz), 5.23 (dd, 1H, J = 5.3, 6.8 Hz), 4.15 (m, 2H), 3.90 (s, 3H), 3.80 (s, 3H),

3.53 (t, 2H, $J = 4.4$ Hz), 3.47 (dd, 1H, $J = 5.3$, 13.6 Hz), 3.15 (dd, 1H, $J = 6.8$, 13.6 Hz). MS m/z 520 $(MH)^+$. TOF HRMS calcd for $C_{24}H_{24}Cl_2N_3O_6$ $(MH)^+$ 520.1042; found 520.1040. HPLC-1, $t_r = 2.96$ min; HPLC-3, $t_r = 3.87$ min.

(*S*)-2-(3-Isobutyl-3-methyl-ureido)-3-[4-(5-methoxy-2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-propionic Acid 2-Hydroxy-ethyl Ester (30c). Ethylene glycol (434 μL, 7.78 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (515 mg, 2.02 mmol), and triethylamine (564 μL, 4.05 mmol) were added in sequence to a solution of **30** (648 mg, 1.56 mmol) in CH_2Cl_2 (7.8 mL). The resulting mixture was stirred at 23 °C for 5 days. The mixture was concentrated, and the residue was purified by reverse-phase HPLC (20−40% CH_3CN-H_2O, 0.05% HCO_2H), yielding ester **30c** as a white powder (450 mg, 63%). ^{1}H NMR (CD_3OD, 300 MHz) 8.17 (s, 1H), 7.38 (d, 2H, $J = 8.2$ Hz), 7.28 (d, 2H, $J = 8.4$ Hz), 6.21 (d, 1H, $J = 7.9$ Hz), 4.52−4.60 (m, 1H), 4.11−4.24 (m, 2H), 3.93 (s, 3H), 3.77 (s, 3H), 3.68−3.72 (m, 2H), 2.96−3.25 (m, 4H), 2.86 (s, 3H), 1.80−1.94 (m, 1H), 0.84 (d, 3H, $J = 6.4$ Hz), 0.83 (d, 3H, $J = 6.7$ Hz). TOF HRMS calcd for $C_{23}H_{33}N_4O_6$ $(MH)^+$ 461.2400; found 461.2390. Anal. ($C_{23}H_{32}N_4O_6 \cdot 0.5H_2O$) C, H, N.

(*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[2-(2-hydroxy-ethyl)-5-methoxy-3-oxo-2,3-dihydro-pyridazin-4-yl]-phenyl}-propionic Acid 2-Hydroxy-ethyl Ester (36c). Prepared from compound **36** (574 mg, 1.32 mmol) by the method given for ester **30c** (reaction time 23 h). HPLC gradient: 20−40% CH_3CN-H_2O, 0.05% HCO_2H. Yield: 171 mg (27%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.22 (s, 1H), 7.40 (d, 2H, $J = 8.0$ Hz), 7.29 (d, 2H, $J = 8.1$ Hz), 6.98 (d, 1H, $J = 8.2$ Hz), 4.37−4.45 (br m, 1H), 4.34 (t, 2H, $J = 5.7$ Hz), 4.16−4.21 (br m, 2H), 3.96 (s, 3H), 3.94 (t, 2H, $J = 5.7$ Hz), 3.69−3.74 (br m), 3.20 (dd, 1H, $J = 13.8$, 5.4 Hz), 3.01 (dd, 1H, $J = 13.5$, 8.8 Hz), 1.42 (s, 9H). TOF HRMS calcd for $C_{23}H_{32}N_3O_8$ $(MH)^+$ 478.2189; found 478.2188. Anal. ($C_{23}H_{31}N_3O_8 \cdot 0.3H_2O$) C, H, N.

Ramos Cell Adhesion Assay ($\alpha_4\beta_1$ Mediated Adhesion/VCAM-1). Immulon 96-well plates (Dynex) were coated with 100 μL recombinant hVCAM-1 at 4.0 μg/mL in 0.05 M Na_2CO_3 buffer pH 9.0 overnight at 4 °C (R&D Systems). Plates were washed two times in PBS with 1% BSA and blocked for 1 h at room temperature in this buffer. PBS was removed and compounds to be tested (50 μL) were added at 2× concentration. Ramos cells (50 μL at 2 × 10^6/mL), labeled with 5 μM Calcein AM (Molecular Probes) for 1 h at 37 °C, were added to each well and allowed to adhere for 1 h at room temperature. Plates were washed 4 times in PBS + 1% BSA, and cells were lysed for 15 min in 100 μL of 1 M Tris pH 8.0 with 1% SDS. The plate was read at 485 nm excitation and 530 nm emission.

$\alpha_4\beta_7$-K562 Cell Adhesion Assay ($\alpha_4\beta_7$ Mediated Adhesion/MAdCAM-1). M2 anti-FLAG antibody coated 96-well plates (Sigma) were coated for 1 h at 4 °C with 2−8 μL/well recombinant FLAG-hMAdCAM-1 contained in 100 μL of Dulbecco's PBS, pH 7.4, with 1% BSA and 1 mM Mn^{2+} (PBS−BSA−Mn). Plates were washed once with PBS−BSA−Mn. Buffer was removed, and compounds to be tested (50 μL) were added at 2× concentration. Stably transfected K562 cells expressing human $\alpha_4\beta_7$ integrin (50 μL at 2 × 10^6/mL) that had been labeled with 100 μg/mL carboxymethyl fluorescein diacetate succinimidyl ester (CFDA-SE; Molecular Probes) for 15 min at 37 °C were added to each well and allowed to adhere for 1 h at room temperature. Plates were washed 4 times in PBS−BSA−Mn, and then cells were lysed for 2 min by addition of 100 μL of PBS without Ca^{2+} or Mg^{2+}, supplemented with 0.1 M NaOH. The plate was read on a 96-well fluorescent plate reader at 485 nm excitation and 530 nm emission.

Pharmacokinetic Assay. Rats were dosed intravenously (iv) at 3 mg/kg and by oral gavage at 30 mg/kg with tested compound. Blood samples (0.5−1.0 mL) were collected post dose into heparinized tubes and centrifuged for cell removal. Precisely 200 μL of plasma supernatant was then transferred to a clean vial, placed on dry ice, and subsequently stored in a −70 °C freezer prior to analysis. Plasma samples were prepared by adding 400 μL of acetonitrile containing internal standard to 200 μL of plasma to precipitate proteins. Samples were centrifuged, and supernatant was removed for analysis by LC-MS-MS. Calibration standards were prepared by adding appropriate volumes of stock solution directly into plasma and treated identically to collected plasma samples. Calibration standards were prepared in the range of 0.01 to 10 μM for quantitation. LC-MS-MS analysis was performed utilizing multiple reaction monitoring for detection of characteristic ions for each tested compound, additional related analytes, and internal standard. The limit of quantitation was 0.01 μM.

Dextran Sulfate Sodium Colitis Protocol. Details for this method have previously been reported.[26] In brief, mice ($n = 10$/treatment group) were provided with a solution of tap water containing 5% DSS ad libitum over a 7 day period. The DSS solution was changed daily and its consumption noted. During this same time, selected groups of test animals were administered a preparation of experimental antagonists. Mice were dosed by oral gavage either with vehicle (10% poly(ethylene glycol) 400 (Sigma-Aldrich; St. Louis, MO) in D5W, v/v) or with experimental compound starting on the day of induction with DSS and twice daily thereafter for 7 days. At the end of this period, the animals were euthanized and their colons dissected for further analysis. Among the parameters analyzed were length of the colon from the aboral end of the cecum to the anus, signs of diarrhea, and indications of gross inflammation. These data and observations were assigned a score, and the sums of the individual macroscopic indices were combined into a macroscopic score for each colon, where 0 = normal and 11 = maximally affected, as previously described.[26]

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Supporting Information Available: Table of elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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