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Synthesis and SAR of pyridazinone-substituted phenylalanine amide α_4 integrin antagonists

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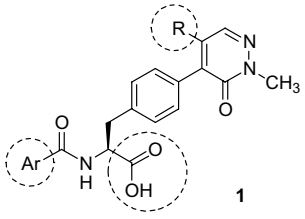
Abstract—Structural modification and cellular adhesion inhibition activities of pyridazinone-substituted phenylalanine amide α_4 integrin antagonists are described. Functionality requirements for the arylamide moiety and the carboxylic acid group were demonstrated. The study also revealed novel structure–activity relationships (SAR) for arylated pyridazinones. A correlation between bioavailability and permeability was also explored. A selected compound showed effectiveness in a mouse leukocytosis study.

Integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$ are heterodimeric transmembrane receptors from the α_4 integrin family, which are expressed on the surface of leukocytes. They play an essential role in leukocyte adhesion and trafficking. Undesired interactions of integrin $\alpha_4\beta_1$ with vascular cell adhesion molecule-1 (VCAM-1), and integrin $\alpha_4\beta_7$ with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), have been implicated in asthma, multiple sclerosis, ulcerative colitis, and Crohn's disease. Tysabri®, a humanized monoclonal antibody that targets α_4 integrin, has been approved for the treatment of multiple sclerosis.[1] Many small molecules, especially *N*-acyl 4-aryl phenylalanines, have been identified that inhibit the interaction of α_4 integrins with their receptors.[2] Recently, we discovered pyridazinone based α_4 integrin antagonists.[3] Cellular adhesion activity, pharmacokinetic (PK) properties, and in vivo activity of additional members of this series are presented herein.

In this study, structural modifications to three groups within the generalized lead antagonist **1** were targeted: the arylamide, the carboxylic acid, and the 5-position substituent on the pyridazinone. Variation of the arylamide group in **1** was accomplished as depicted in Scheme 1. Suzuki coupling of 4-boronophenylalanine (**2**) with 4-chloro-5-methoxy-2-methyl-2H-pyridazin-3-one under our previously reported conditions[3,4] yielded 4-pyridazinone-substituted phenylalanine **3**. Arylamides **5**–**7** in Table 1 were prepared by direct acylation of phenylalanine **3** with benzoyl chlorides. Arylamides **8**–**12** were obtained through esterification (to ester **4**), EDC-mediated coupling with carboxylic acids, and saponification. Thioamide analog **13** in Table 1 was prepared by refluxing amide **5** with Lawesson's reagent in toluene.



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Compounds in Table 2 contain alternatives to the carboxylic acid within **5**. Hydroxamic acid **14** and *N*-acylmethanesulfonamide **15** were prepared by acylation of hydroxylamine hydrochloride and methanesulfonamide, respectively, with parent acid **5** in the presence of EDC under standard coupling conditions. Alcohol **16** was

Keywords: Pyridazinone; Phenylalanine; α_4 Integrin antagonist.
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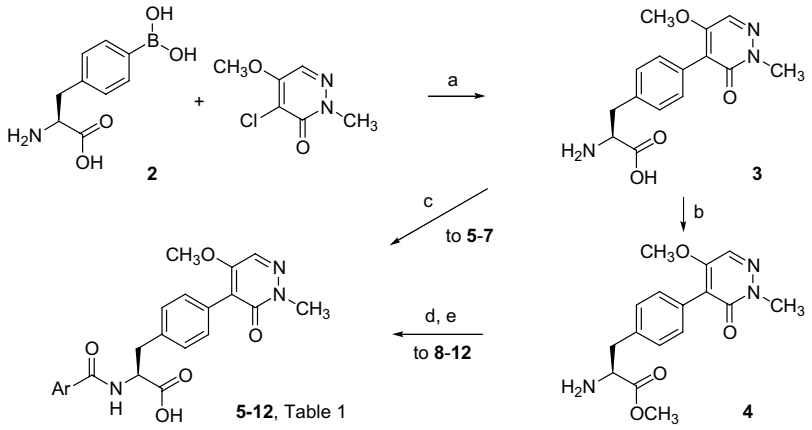
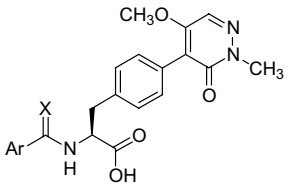
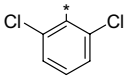
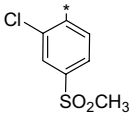
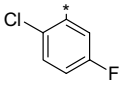
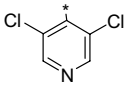
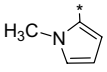
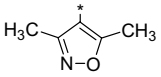
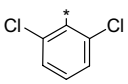


Scheme 1. (a) $Pd(PPh_3)_2Cl_2$, Na_2CO_3, H_2O/CH_3CN, reflux 2 h or μW 150 °C, 10 min; (b) CH_3OH, $SOCl_2$; (c) ArCOCl, Na_2CO_3, H_2O/CH_3CN; (d) $ArCO_2H$, EDC, HOBt, Et_3N, CH_2Cl_2; (e) LiOH, CH_3OH, H_2O.

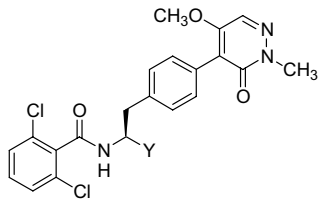
Table 1. Cellular adhesion assay results[a] for arylamides **5**–**13**



Compound	Ar	X	IC_{50} (μM)	
			$\alpha_4\beta_1$	$\alpha_4\beta_7$
5		o	0.031 ± 0.008 (4)	0.003 ± 0.001 (6)
6		o	0.31 ± 0.005 (2)	0.004 ± 0.002 (2)
7		o	1.2	0.019 ± 0.007 (2)
8		o	0.005 ± 0.001 (2)	0.018 ± 0.013 (5)
9		o	0.73 ± 0.043 (2)	0.035 ± 0.013 (6)
10		o	>5	1.4
11		o	>5	0.080 ± 0.024 (3)
12		o	0.79	0.25
13		s	0.35	0.23

[a] IC_{50}'s were determined from a six point titration, with each individual point being an average of two measurements at a particular antagonist concentration. IC_{50}'s are reported as value ± SEM, with *n* in parentheses.

Table 2. Cellular adhesion assay results for carboxylic acid alternatives

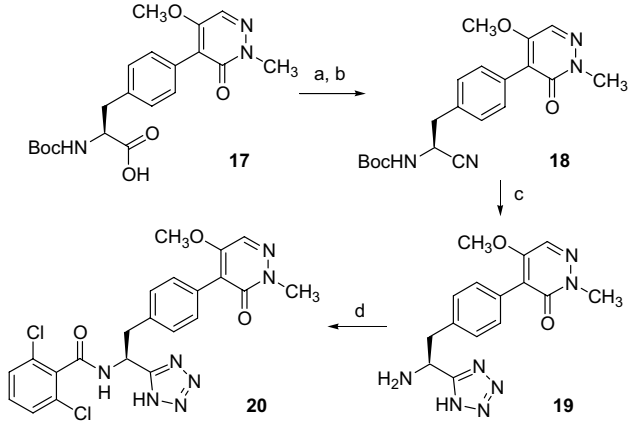


Compound	Y	IC_{50} (μM)	
		$\alpha_4\beta_1$	$\alpha_4\beta_7$
14	–CONHOH	>5	0.61
15	$-CONHSO_2CH_3$	>5	1.0
16	$-CH_2OH$	>5	0.23
20		2.1	0.089

prepared by reduction of the corresponding methyl ester with lithium borohydride. Tetrazole **20** was prepared by conversion of carboxylic acid **17**[3] to nitrile **18**, followed by conversion of **18** to unprotected tetrazole **19** and subsequent acylation, as shown in Scheme 2.

Synthesis of arylated pyridazinones **27**–**36** (Table 3) was accomplished through double Suzuki couplings (Scheme 3). 4,5-Dichloro-2-methyl-2H-pyridazin-3-one **25** was selectively mono-arylated to 5-aryl-4-chloro-2-methyl-2H-pyridazin-3-ones **26** under our previously developed conditions.[5] The second Suzuki arylation of compounds **26** with borono phenylalanine amide **24** furnished the targets. Azole substituted pyridazinones **37**–**40** were prepared by substitution of the methoxy group in **5** with an azole (sodium hydride, microwave heating).[6]

Table 1 lists in vitro activities of a selected set of arylamides in assays of $\alpha_4\beta_7$/MAdCAM-1 and $\alpha_4\beta_1$/VCAM-1 mediated cellular adhesion.[7] Compound **5**, containing the well-optimized 2,6-dichlorobenzamide moiety, demonstrated potent antagonist activity versus both $\alpha_4\beta_7$ and $\alpha_4\beta_1$. Compared to **5**, 2-chloro-4-methanesulfonylbenzamide **6** was an equally potent $\alpha_4\beta_7$ antagonist and was more selective with regard to $\alpha_4\beta_1$ inhibition.[8] 2-Chloro-5-fluorobenzamide **7** was fairly potent and selective for $\alpha_4\beta_7$. 3,5-Dichloro-isonicotinic amide **8** was a potent dual antagonist. Other heteroarylamides **9**–**12** were less potent. Thioamide analog **13** displayed only moderate activity, with sub-micromolar antagonism of both $\alpha_4\beta_7$ and $\alpha_4\beta_1$.



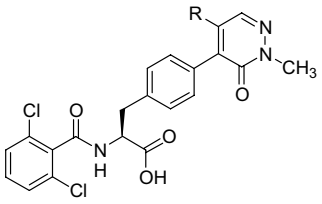
Scheme 2. (a) Boc_2O, NH_4HCO_3, pyridine, CH_3CN; (b) cyanuric chloride, DMF; (c) NaN_3, $ZnBr_2$ (5 equiv), *i*-PrOH, H_2O; (d) 2,6-dichlorobenzoyl chloride, Et_3N, CH_2Cl_2.

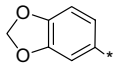
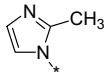
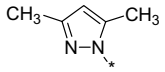
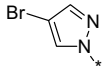
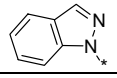
Efforts to replace the carboxylic acid group in **5** resulted, to varying degrees, in a reduction of potency (Table 2). Hydroxamic acid **14** and *N*-acyl-methanesulfonamide **15** produced only micromolar range activity against $\alpha_4\beta_7$. Alcohol **16**, surprisingly, displayed sub-micromolar activity against $\alpha_4\beta_7$. Tetrazole **20** provided double digit nM activity against $\alpha_4\beta_7$ and micromolar range activity against $\alpha_4\beta_1$. Although carboxylic acid group replacement did not generate the desired potency, it could potentially alter PK properties of this class of compounds.

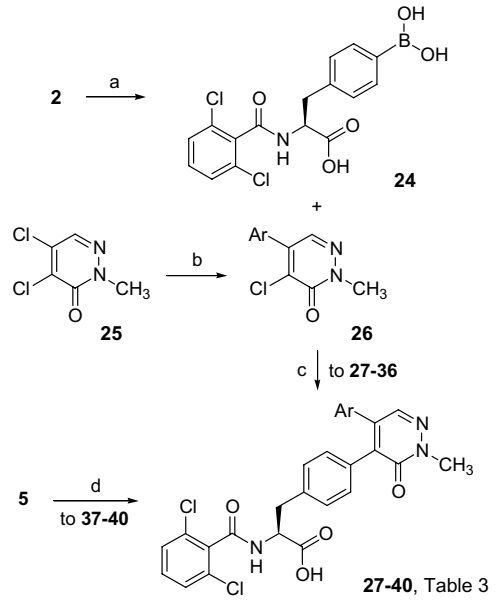
Previously reported structure–activity relationships within the *N*-acyl 4-aryl phenylalanine series suggested that *ortho* substitution of the terminal aryl ring, especially with methoxy group(s), was important in maintaining potency.[3,9] The methoxy groups, however, are potentially metabolically labile, which may contribute to the less than favorable PK properties associated with this series of compounds. When the methoxy group in pyridazinone **5** was removed, activity dropped significantly (compound **21**). Introduction of 5-methyl and -ethyl groups to the pyridazinone (**22** and **23**) resulted in partial recovery of potency versus $\alpha_4\beta_7$. Further enhancement of potency was achieved in arylated pyridazinones **27**–**40**. Substituted phenyls (**27**–**35**), as well as other heteroaryl groups (**36**–**40**), were well tolerated. The arylated compounds were more active in blocking $\alpha_4\beta_7$ than $\alpha_4\beta_1$-mediated cell adhesion.[10] Single to double digit nM potency versus $\alpha_4\beta_7$ and sub-μM to μM activity with respect to $\alpha_4\beta_1$ was generally observed. For example, 4-methanesulfonyl-phenyl analog **28** displayed an IC_{50} of 2 nM against $\alpha_4\beta_7$ and 150 nM against $\alpha_4\beta_1$; imidazole **37** exhibited IC_{50}'s of 7 and 790 nM, respectively. The successful extension of SAR into arylated pyridazinones provides a novel handle to potentially modify the PK properties of this class of compounds.

Pharmacokinetic data of selected compounds in rats are presented in Table 4. Compounds **5**, **6**, **7**, and **31**, each containing a carboxylic acid, showed almost no oral bioavailability. Prodrug hydroxyethyl ester **41** provided enhanced plasma levels of the parent carboxylic acid **7** (C_{max} = 19 μM, F = 5%).[11] Tetrazole **20** failed to improve PK properties. Alcohol **16**, however, offered modest improvements in systemic exposure level (C_{max} = 0.9 μM) and oral bioavailability (F = 8%). This compound also displayed an increased rate of cellular permeability (as indicated by the Caco-2 model), suggesting that increasing intestinal absorption may be important in improving oral bioavailability in the series.

Table 3. Cellular adhesion assay results for pyridazinone modifications



Compound	R	IC_{50} (μM)	
		$\alpha_4\beta_1$	$\alpha_4\beta_7$
21	H	>1	0.36
22	CH_3	3.2	0.078 ± 0.006 (2)
23	CH_3CH_2	>5	0.061
27	Ph	0.57 ± 0.075 (2)	0.021 ± 0.008 (2)
28	4-CH_3SO_2-Ph	0.15 ± 0.039 (2)	0.002 ± 0.001 (3)
29	4-NH_2CO-Ph	0.094 ± 0.019 (2)	0.003 ± 0.002 (4)
30	4-CN-Ph	0.74	0.023 ± 0.005 (3)
31	4-F-Ph	0.46 ± 0.047 (2)	0.019 ± 0.012 (2)
32	4-CH_3O-Ph	0.34 ± 0.082 (2)	0.013 ± 0.003 (2)
33	3-CF_3O-Ph	1.54	0.025 ± 0.009 (4)
34	3-CF_3-Ph	0.52	0.021 ± 0.009 (3)
35		0.40	0.005 ± 0.002 (4)
36		0.83 ± 0.026 (2)	0.069 ± 0.033 (2)
37		0.79 ± 0.019 (2)	0.007 ± 0.001 (2)
38		>5	0.024 ± 0.009 (2)
39		2.7	0.039 ± 0.008 (2)
40		0.51 ± 0.039 (2)	0.036 ± 0.017 (3)

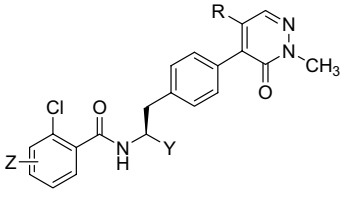


Scheme 3. (a) 2,6-$Cl_2C_6H_3COCl$, Na_2CO_3, H_2O/CH_3CN; (b) $Pd(PEt_3)_2Cl_2$, $ArB(OH)_2$, Na_2CO_3, H_2O/DMF; (c) $Pd(PPh_3)_2Cl_2$, Na_2CO_3, H_2O/CH_3CN, μW 150 °C, 10 min; (d) azole (5 equiv), NaH (5 equiv), THF, μW 100 °C, 10 min.

Compound **5** was selected for testing in a mouse leukocytosis study. The compound was dosed subcutaneously rather than orally to achieve a higher systemic drug level.[12] In vivo subcutaneous administration of **5** in naïve mice produced a dose-dependent elevation in circulating total leukocytes (Fig. 1). Circulating leukocytes were more than doubled over control at a 30 mg/kg dose, which reflects that inhibition of binding of $\alpha_4\beta_1$ to VCAM-1 and $\alpha_4\beta_7$ to MAdCAM-1 results in a blockage of the cell's extravasation.

In summary, convergent synthesis was applied to the SAR exploration of a pyridazinone-substituted phenyl-

Table 4. Pharmacokinetic properties of selected compounds in rats[a]



Compound	Z	Y	R	Caco-2 $P_{app}A \rightarrow B$ (10^{-6} cm/s)	C_{max} (μM)	F (%)
5	6-Cl	$-CO_2H$	OCH_3	0.13	0.1	1
6	4-CH_3SO_2	$-CO_2H$	OCH_3	—	0	0
7	5-F	$-CO_2H$	OCH_3	—	0	0
41	5-F	$-CO_2(CH_2)_2OH$	OCH_3	—	19	5
31	6-Cl	$-CO_2H$	4-F-Ph	0.16	0	0
16	6-Cl	$-CH_2OH$	OCH_3	2.5	0.9	8
20	6-Cl		OCH_3	0.02	0	0

[a] Dosed at 30 mg/kg po, 3 mg/kg iv.

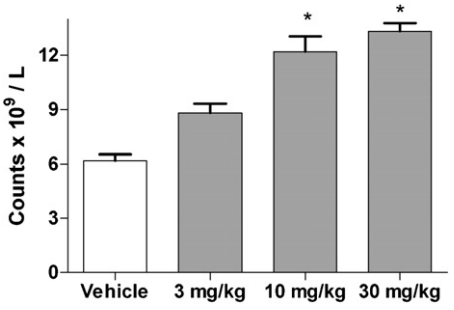


Figure 1. Effect on total leukocyte counts by compound **5** (sc, 1 h before sampling). $^{*}p < 0.05$.

alanine amide class of α_4 integrin antagonists. PK properties of this series of compounds were associated with their permeabilities. A selected compound (**5**) demonstrated dose-responsive effects on the elevation of circulating leukocytes in a mouse leukocytosis study. The results should serve as a guide for further investigation.

Acknowledgments

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References and notes

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4. Gong, Y.; He, W. *Org. Lett.* **2002**, *4*, 3803.
5. (a) Gong, Y.; He, W. *Heterocycles* **2004**, *62*, 851; (b) Boronic acids containing electron withdrawing groups increased the amount of diarylated byproducts.
6. In addition to unchanged starting material, a byproduct observed in these reactions was demethylation of the pyridazinone to the corresponding phenol.
7. A Ramos cell adhesion assay and a K562 cell adhesion assay were used to determine the inhibition of the adhesion of $\alpha_4\beta_1$+ cells to immobilized VCAM-1 and of $\alpha_4\beta_7$+ cells to immobilized MAdCAM-1. For details, see Ref. 3.
8. The 2-chloro-4-methanesulfonyl-benzoyl moiety was also reported recently. Lassoie, M.-A.; Broeders, F.; Collart, P.; Defrere, L.; de Laveleye-Defais, F.; Demaude, T.; Gassama, A.; Guillaumet, G.; Hayez, J.-C.; Kiss, L.; Knerr, L.; Nicolas, J.-M.; Norsikian, S.; Quere, L.; Routier, S.; Verbois, V.; Provins, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 142.
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