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N-(3-Phenylsulfonyl-3-piperidinoyl)-phenylalanine Derivatives as Potent, Selective VLA-4 Antagonists

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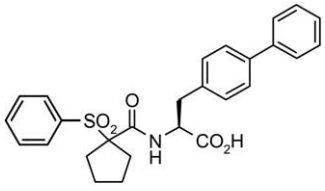
Abstract—The SAR of 1-sulfonyl-cyclopentyl carboxylic acid amides, ligands for the VLA-4 integrin, was investigated. This effort resulted in the identification of *N*-(3-phenylsulfonyl-3-piperidinoyl)-(L)-4-(2′,6′-dimethoxyphenyl)phenylalanine **52** as a potent, selective VLA-4 antagonist (IC_{50} = 90 pM). Expansion of the SAR demonstrated that this structural unit can be used to identify a diverse series of sub-nanomolar antagonists.

VLA-4 (Very Late Antigen-4, $\alpha_4\beta_1$, or CD49d/CD29) is a heterodimeric integrin expressed on all leukocytes.[1–3] Among its natural ligands, VCAM-1 (Vascular Cell Adhesion Molecule-1) is expressed on vascular endothelial cells in response to a number of proinflammatory cytokines. The VLA-4/VCAM-1 binding interaction has been shown to be critical for lymphocyte migration to extravascular spaces. Antibodies to VLA-4 or VCAM-1 are effective at limiting lymphocyte extravasation and have been shown to prevent tissue damage in several animal models of inflammatory disease. An anti-α_4 antibody and small molecule antagonists are currently in clinical trials.

Our initial lead in this area was the phenylsulfonyl cyclopentyl biphenylalanine (**1**, VLA-4 IC_{50} = 29 nM) identified through preparation of a library of biphenylalanine derivatives.[4] Our interest in this lead stemmed from its potency and especially its modest selectivity over $\alpha_4\beta_7$ (18% inh. @ 0.1 μM), since one of our program goals was to access compounds with reasonable selectivity for VLA-4 over $\alpha_4\beta_7$. Related efforts suggested that the incorporation of heterocycles might enhance both VLA-4 potency and selectivity over $\alpha_4\beta_7$.[5,6] Both solid- and solution-phase synthetic techniques led to the rapid optimization of **1**. We wish to report herein the discovery of a series of 3-phenylsulfonyl-piperidines as potent, selective VLA-4 antagonists (Table 1).



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Chemistry

Compounds were prepared via a variety of routes, as depicted in Scheme 1.[7] The biaryl amino acids (**A**, X = Ar) were assembled from a suitably protected 4-iodo-(L)-phenylalanine, typically by Suzuki methodology.[8] The 4-aminophenyl-amino acids (**A**, X = NR′R″) were prepared from the corresponding 4-amino-phenylalanine. Some examples were prepared by reacting the requisite amino acid, *t*-butyl ester with bromoacetic acid to give **B**. Following elaboration to the sulfonyl

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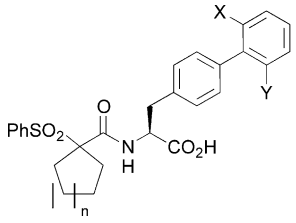
acetamide, a double substitution was used to install the remaining ring (typically either nucleophilic displacement of a dibromide or a diol under Mitsunobu conditions) leading to compounds **F**. This approach provided most of the carbocylic and many of the heterocyclic compounds, with various ring sizes ($n = 0$–2, Z = see Table 2).[9] Alternatively, the sulfonyl-cyclo-carboxylic acid **E** was assembled prior to peptide bond formation. In which case, either the 2-sulfonylacetic acid derivative **C** was elaborated to **E** by the methods described above; or the potassium enolate of a cyclic carboxylic acid ester **D** was sulfonylated (with a sulfonyl fluoride)[10] or sulfinylated (with a disulfide) then oxidized, both affording **E**. This latter method was used to prepare both the *N*-substituted-3-phenylsulfonyl-3-piperidine carboxylic acid derivatives and 3-phenylsulfonyl-tetrahydro-3-furoic acid. The methodology for the transformation of **B** to **F** was extended to solid-phase conditions (in parallel on a Quest Synthesizer™), using polystyrene-linked biphenylalanines (Scheme 2).

Biology

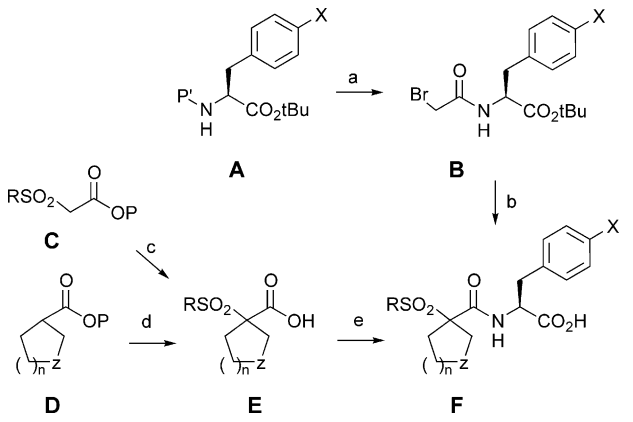
VLA-4 ($\alpha_4\beta_1$) binding affinity was determined in a radioligand binding assay by measurement of the reduction in binding of an ^{125}I-VCAM-Ig fusion protein to the VLA-4 receptor expressed on human Jurkat cells.[11] The equivalent assay was run using RPMI-8866 (a human B-cell line expressing $\alpha_4\beta_7$) as a counterscreen to determine the selectivity of our compounds for the $\alpha_4\beta_1$ integrin. In later experiments, an ^{125}I-MAdCAM-Ig fusion protein was used to determine $\alpha_4\beta_7$ binding affinity, since Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1) is the natural ligand for $\alpha_4\beta_7$. In all cases Mn^{2+} was included in the assay buffer; under these

Table 1. VLA-4 binding affinity of cycloalkyl series



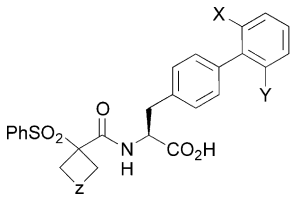
Compd	*n*	R	X,Y	VCAM $\alpha_4\beta_1$ IC_{50}, nM[a]	VCAM $\alpha_4\beta_7$ IC_{50}, nM[a]
1	1	Ph	H, H	28	18%
2	1	*p*-Me-Ph	H, H	28	2%
3	2	*p*-Me-Ph	H, H	53	0%
4	1	*p*-Br-Ph	H, H	23	20%
5	2	*p*-Br-Ph	H, H	44	19%
6	1	*o*-Br-Ph	H, H	105	8%
7	2	*o*-Br-Ph	H, H	45	13%
8	1	$PhCH_2$	H, H	81	−7%
9	2	$PhCH_2$	H, H	65	14%
10	1	*n*-octyl–	H, H	150	−12%
11	1	Cyclohexyl–	H, H	7	16%
12	2	Cyclohexyl–	H, H	14	24%
13	1	1-Naphthyl	H, H	735	7%
14	1	Ph	H, OMe	0.33	83
15	2	*p*-Me-Ph	H, OMe	1.5	91
16	2	*m*-Me-Ph	H, OMe	1.0	77
17	2	*o*-Me-Ph	H, OMe	2.7	149
18	2	*p*-Cl-Ph	H, OMe	1.1	53
19	1	*p*-F-Ph	H, OMe	0.27	40[b]
20	2	*p*-F-Ph	H, OMe	0.75	25
21	1	Et	H, OMe	0.53	58[b]
22	1	*t*-Bu	H, OMe	0.47	19
23	1	Cyclohexyl–	H, OMe	1.1	52
24	1	Cyclopentyl–	H, OMe	1.2	147[b]
25	1	2-N-Me-imidazolyl	H, OMe	0.21	4.0
26	1	PhNH–	H, OMe	15	521[b]
27	1	Et_2N–	H, OMe	0.97	54[b]
28	1	Ph	OMe, OMe	0.11	5.0[b]
29	2	Ph	OMe, OMe	0.19	13[b]
30	1	*p*-F-Ph	OMe, OMe	0.07	5[b]

[a]Values are means of three experiments, % inhibitions are at 100 nM.
[b]$\alpha_4\beta_7$ potency determined with ^{125}I-MADCAM fusion protein.



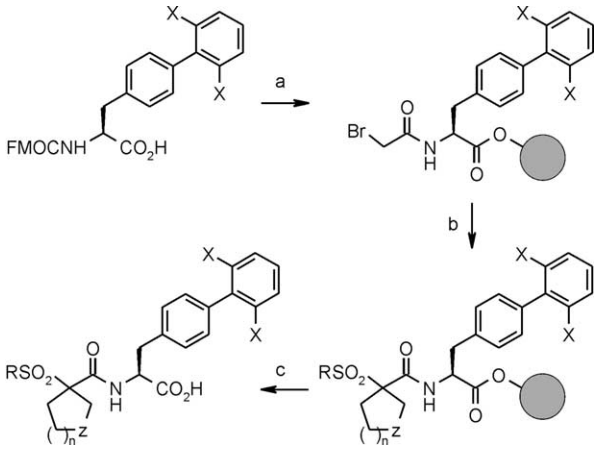
Scheme 1. (a) X = Ar, NHCOAr (prepared from X = I, OH or NH_2), $BrCH_2CO_2H$, 2-(dimethylamino)isopropyl chloride HCl, DMF; (b) RSH, diisopropylethylamine; *m*CPBA; $Br(CH_2)_{n+1}ZCH_2Br$, Cs_2CO_3 or $HO(CH_2)_{n+1}ZCH_2OH$, PMe_3, 1,1′-(azodicarbonyl)dipiperidine; TFA; (c) $Br(CH_2)_{n+1}ZCH_2Br$, Cs_2CO_3 or $HO(CH_2)_{n+1}ZCH_2OH$, PMe_3, 1,1′-(azodicarbonyl)dipiperidine; NaOMe; (d) KHMDS; RSO_2F or KHMDS, RSSR then *m*CPBA; NaOMe; (e) **A** (P′ = H), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophospate, 1-HOBt, diisopropylethylamine, DMF; TFA.

Table 2. VLA-4 binding affinity of other cycles



Compd	Z	X,Y	VCAM $\alpha_4\beta_1$ IC_{50}, nM[a]	MADCAM $\alpha_4\beta_7$ IC_{50}, nM[a]
14	$-(CH_2)_2-$	H, OMe	0.33	83[b]
28	$-(CH_2)_2-$	OMe, OMe	0.11	5.0
31	$-CH_2-$	H, OMe	1.2	16
32	$-CH_2-$	OMe, OMe	0.17	3.2
33	$-CH=CH-$	H, H	275	7%[b]
34	*o*,*o*–benzo	H, H	680	7335[b]
35	$-CH_2SCH_2-$	H, OMe	4.1	128
36	$-CH_2SOCH_2-$	H, OMe	4.4	318
37	$-CH_2SO_2CH_2-$	H, OMe	1.3	70
38	$-CH_2SCH_2-$	OMe, OMe	0.22	5.0
39	$-CH_2OCH_2-$	H, OMe	0.55	150
40	$-CH_2OCH_2-$	OMe, OMe	0.16	38
41	$-CH_2O-$	H, OMe	0.77	45
42	$-CH_2O-$	OMe, OMe	0.1	5.2
43	$-CH_2(N-Me)CH_2-$	H, OMe	3.0	1998
44	$-CH_2(N-Et)CH_2-$	H, OMe	31	1230
45	$-CH_2(N-^nBu)CH_2-$	H, OMe	51	9180
46	$-CH_2(N-^tBu)CH_2-$	H, OMe	56	9220
47	$-CH_2(N-Ph)CH_2-$	H, OMe	23	1260
48	$-CH_2(NH)CH_2-$	H, OMe	20	1900
49	$-CH_2(N-Me)CH_2-$	OMe, OMe	1.1	466
50	$-(CH_2)_2(NH)-$	H, OMe	0.48	622
51	$-(CH_2)_2(N-Me)-$	H, OMe	6.6	5790
52	$-(CH_2)_2(NH)-$	OMe, OMe	0.09	168

[a]Values are means of three experiments, % inhibitions are at 100 nM.
[b]$\alpha_4\beta_7$ potency determined with ^{125}I-VCAM-Ig fusion protein.



Scheme 2. (a) Wang resin, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMAP, CH_2Cl_2, THF; piperidine; DMA; $BrCH_2CO_2H$, 2-(dimethylamino)isopropyl chloride HCl, DMA; (b) RSH, diisopropylethylamine; *m*CPBA; $Br(CH_2)_{n+1}ZCH_2Br$, Cs_2CO_3 or $HO(CH_2)_{n+1}ZCH_2OH$, PMe_3, 1,1′-(azodicarbonyl)dipiperidine; (c) TFA.

conditions the integrin adopts a state resembling the activated one.

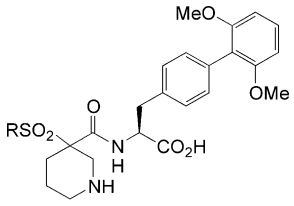
Results

Preliminary studies showed modification to both the sulfonyl substituent and the carbocycle was possible (**2** to **13**, Table 1). Substitution on the phenyl sulfonyl or modification to an alkyl sulfonyl group is tolerated, unless the group becomes large (e.g., *n*-octyl, **10** and naphthyl, **13**). The cyclohexyl sulfonyl (**11**) gave a modest improvement in potency. Expansion of the carbocycle from the cyclopentyl- to the cyclohexyl-system ($n=1$ to $n=2$) resulted in a modest loss of potency. Dramatic improvement in potency was accomplished with the incorporation of an *ortho*-methoxy substituent on the distal ring of the biphenylalanine (**14**–**27**). Addition of a second *ortho*-methoxy gave a further boost in potency (**28**–**30**). Thus a series of low- and sub-nanomolar inhibitors for $\alpha_4\beta_1$ were discovered, with roughly 10- to 100-fold specificity over the $\alpha_4\beta_7$ integrin.

A more extensive SAR focusing on the cycloalkyl moiety was then established (Table 2). The cyclobutyl system offered no advantage over the larger homologues (**31** and **32**). Unsaturation in the form of a cyclopentene was somewhat detrimental to potency (**33**), as was the benzo-fusion in analogue **34**.

In some cases, incorporation of a heteroatom in the cycle gave little improvement. The tetrahydro-thiopyrans (**35**–**38**), tetrahydropyrans (**39** and **40**), and tetrahydrofurans (**41** and **42**) had $\alpha_4\beta_1$ potencies and selectivity over $\alpha_4\beta_7$ comparable to the corresponding cycloalkanes (**14**, **28**, and **29**). The nitrogen-containing piperidine ring systems however gave significant improvement: the *N*-Me-4-piperidinoyl unit gave a compound (**43**) with over 650-fold selectivity for $\alpha_4\beta_1$ over $\alpha_4\beta_7$. Larger *N*-substituents were not tolerated (**44**–**47**). The unsubstituted 4-piperidinoyl system (**48**) had somewhat lower potency.

Table 3. VLA-4 binding affinity of biphenyl 3-piperidinoyl amides



Compd	R	VCAM $\alpha_4\beta_1$ IC_{50}, nM[a]	MADCAM $\alpha_4\beta_7 IC_{50}$, nM[a]
53	*p*-Br-Ph	0.19	94
54	*m*-Br-Ph	0.13	83
55	*o*-Br-Ph	0.15	96
56	*p*-(1-Pyrrolidinyl)-Ph	0.36	536
57	*o*-(1-Pyrrolidinyl)-Ph	0.39	388
58	$PhCH_2$	0.77	436
59	Me	0.19	21
60	2-Morpholinyl-ethyl–	0.67	257
61	$Me_2N(CH_2)_3NH(CH_2)_2$–	0.37	179
62	4-(N-Me)-imidazolyl	0.46	97
63	5-(N-Me)-imidazolyl	0.14	46

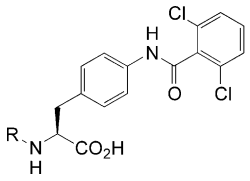
[a]Values are means of three experiments.

The isomeric 3-piperidinoyl system gave similar selectivities, with even better potency. In this series, the unsubstituted secondary amine was the preferred substitution (**50** cf. **51**). When combined with the potency-enhancing dimethoxy-substitution, a potent antagonist with around 1900-fold selectivity was discovered (**52**).

The SAR for the sulfonyl group in this 3-piperidinoyl series was then explored (Table 3). A range of substitutions were well tolerated. In particular, incorporation of various heteroatom-containing groups was possible (**60**–**63**), allowing modulation of physical properties of the series.

It was also ascertained that 3-phenylsulfonyl-3-piperidinoyl- and 4-phenylsulfonyl-4-tetrahydropyranoyl-

Table 4. VLA-4 binding affinity of the 3-piperidinoyl- and 4-tetrahydropyranoyl-containing structures into amides incorporating an alternative amino acid



Compd	R	VCAM $\alpha_4\beta_1$ IC_{50}, nM[a]	VCAM $\alpha_4\beta_7$ IC_{50}, nM[a]
64	*N*-3-phenylsulfonyl-3-piperidinoyl–	0.19	22
65	4-(phenylsulfonyl)tetrahydro-2*H*-pyran-4-carboxyl–	0.22	20

[a]Values are means of three experiments.

Table 5. Selected pharmacokinetic data (rat)

Compd	Cl_p mL/min/kg[a]	$t_{1/2}$ h[a]	F%[b]
40	133	0.8	3
52	46	0.5	1
64	78	0.3	0
65	48	0.6	1

[a]Following 1 mg/kg iv dose.
[b]Following 2 mg/kg po dose.

units could be successfully incorporated in other antagonist designs with different amino acid moieties. We have reported [6] that in other series *N*-(2,6-dichlorobenzoyl)aminophenylalanine[12] is a potency enhancing replacement of the biaryl-alanine unit. Both the 3-piperidinoyl- and the 4-tetrahydropyranoyl-containing *N*-(2,6-dichlorobenzoyl)-aminophenylalanines (**64** and **65**) were potent and selective compounds (Table 4).

The pharmacokinetic profiles in Sprague–Dawley rats of four representative compounds were determined (Table 5). The plasma clearance values following iv dosing are all high, and bioavailabilities following oral dosing all low.

Conclusions

Introduction of heteroatoms into the carbocycle of a 1-phenylsulfonyl-cyclopentyl-biphenylalanine lead, together with inclusion of 2′,6′-dimethoxy substituents on the distal biaryl ring, led to a significant increase in potency against VLA-4. The most interesting compounds discovered, the *N*-(3-phenylsulfonyl-3-piperidinoyl)- and the 4-(phenylsulfonyl)tetrahydro-2*H*-pyran-4-carboxyl derivatives (Table 2, **40** and **52**) possess significant selectivity for VLA-4 versus the related integrin $\alpha_4\beta_7$. Moreover, a favorable potency and selectivity profile was maintained following significant modification to these compounds. Thus this family of potent, structurally diverse VLA-4 antagonists has sufficient tolerance to modification to allow an optimization of other properties, including selectivity and pharmacokinetics. With respect to related series of VLA-4 antagonists,[5,6,8] this series offers greater selectivity relative to $\alpha_4\beta_7$, but still suffers the same poor pharmacokinetic profiles of high plasma clearance and generally low oral bioavailability.

Acknowledgements

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