Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 2051–2066

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and SAR of *N*-Benzoyl-L-Biphenylalanine Derivatives: Discovery of TR-14035, A Dual $\alpha_4\beta_7/\alpha_4\beta_1$ Integrin Antagonist†

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Received 26 September 2001; accepted 15 November 2001

Abstract—$\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins are key regulators of physiologic and pathologic responses in inflammation and autoimmune disease. The effectiveness of anti-integrin antibodies to attenuate a number of inflammatory/immune conditions provides a strong rationale to target integrins for drug development. Important advances have been made in identifying potent and selective candidates, peptides and peptidomimetics, for further development. Herein, we report the discovery of a series of novel *N*-benzoyl-L-biphenylalanine derivatives that are potent inhibitors of α4 integrins. The potency of the initial lead compound (**1**: IC_{50} $\alpha_4\beta_7/\alpha_4\beta_1 = 5/33$ μM) was optimized via sequential manipulation of substituents to generate low nM, orally bioavailable dual $\alpha_4\beta_1/\alpha_4\beta_7$ antagonists. The SAR also led to the identification of several subnanomolar antagonists (**134**, **142**, and **143**). Compound **81** (TR-14035; IC_{50} $\alpha_4\beta_7/\alpha_4\beta_1 = 7/87$ nM) has completed Phase I studies in Europe. The synthesis, SAR and biological evaluation of these compounds are described.

Introduction

The α4 integrins, $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ are heterodimeric cell surface proteins expressed on most leukocytes that are involved in cell–cell and cell–matrix interactions.[2] These leukocytes play a key role in inflammation and autoimmune diseases and $\alpha_4\beta_1$ is believed to be involved in the cell adhesion, migration and activation of these cell types at sites of inflammation.[3] Their central role in animal models of chronic inflammatory diseases, including asthma, rheumatoid arthritis and multiple sclerosis has been extensively documented using α4-specific monoclonal antibodies and inhibitory peptides.[4–8] The primary ligands for $\alpha_4\beta_1$ are the endothelial surface protein vascular cell adhesion molecule (VCAM-1)[9] and the alternatively spliced region of the extracellular matrix protein fibronectin connecting segment 1 (FN, CS-1).[10] Peptides and peptidomimetics derived from the connecting segment 1 sequence of FN have also been shown to block VCAM/VLA-4 interactions and to block allergen-induced airway responses in a sheep model of asthma.[11–13] The $\alpha_4\beta_7$ integrin is widely expressed on leukocyte subtypes, although its distribution is more restricted than that of $\alpha_4\beta_1$. In addition to the two ligands that are shared by both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ (VCAM-1 and CS-1), the ligand mucosal addressin cell adhesion molecule (MAdCAM) shows unique specificity for the integrin $\alpha_4\beta_7$. MAdCAM is highly expressed on Peyer's patch high endothelial venules, in mesenteric lymph nodes, gut lamina propria and mammary gland venules.[14,15] Integrin $\alpha_4\beta_7$ and MAdCAM have been shown to be important in regulating lymphocyte trafficking to normal intestine.[16,17] Using a panel of monoclonal antibodies, it was shown that the $\alpha_4\beta_7$ interaction with its three ligands involves distinct, but overlapping epitopes.[18]

Based on the promising integrin biology, these adhesion molecules have received considerable attention as drug targets. An intense effort by multiple biopharmaceutical

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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00021-4

companies has resulted in very rapid progress in small molecule development.[9,19] Although several highly potent compounds including a series of aryl diamide analogues of *p*-aminophenylalanine have been reported,[20] oral bioavailability was an issue with these early leads. In order to reduce the peptidic nature of these inhibitors the biphenylalanine scaffold was designed. Initial efforts in our laboratories led to the identification of compound **1** (unpublished results). Herein, we report the discovery of a series of potent *N*-benzoyl-L-biphenylalanine compounds (Fig. 1) that led to the first orally bioavailable small molecule α4 antagonist. Compound **81**, **TR-14035**, has completed phase 1 studies in Europe.

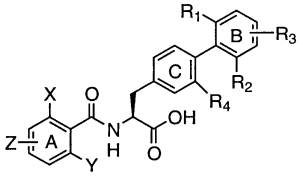
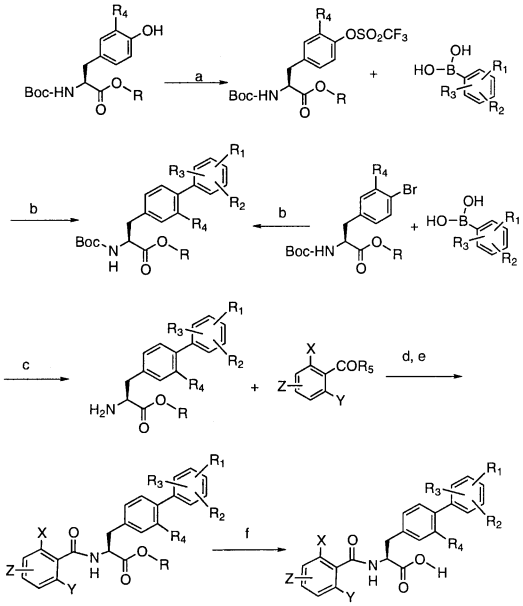
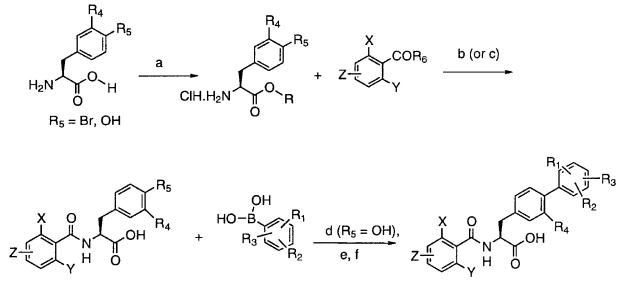


Figure 1.



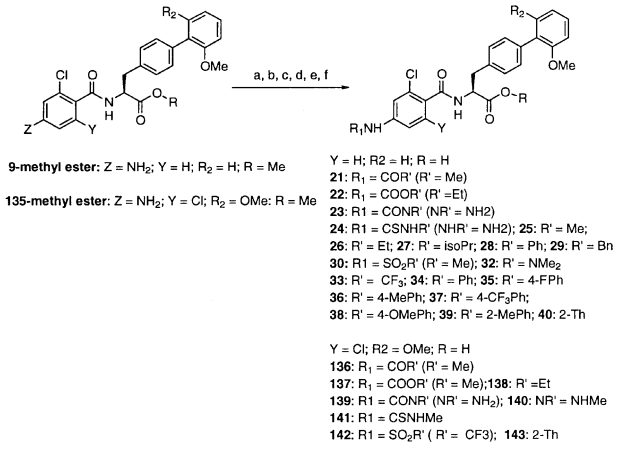
Scheme 1. Reagents: (a) $(CF_3SO_2)_2O$, pyridine; (b) K_2CO_3, $Pd(PPh_3)_4$, DME; (c) TFA; (d) EDC, HOBT (R_5 = OH); (e) DIEA, DCM (R_5 = Cl); (f) LiOH.



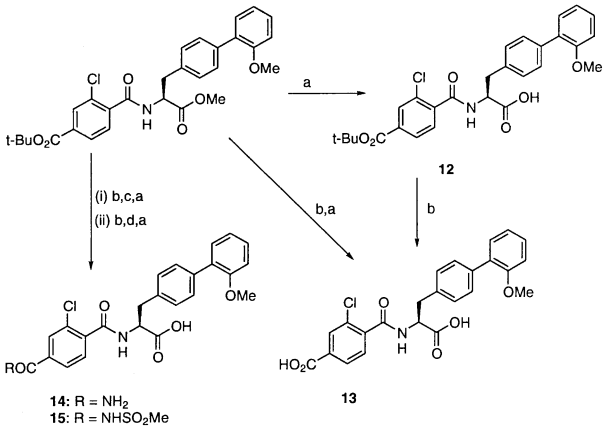
Scheme 2. Reagents: (a) ROH, HCl; (b) EDC, HOBT (R_6 = OH); (c) DIEA, DCM (R_6 = Cl); (d) $(CF_3SO_2)_2O$, pyridine; (e) K_2CO_3, $Pd(PPh_3)_4$, DME; (f) LiOH.

Chemistry

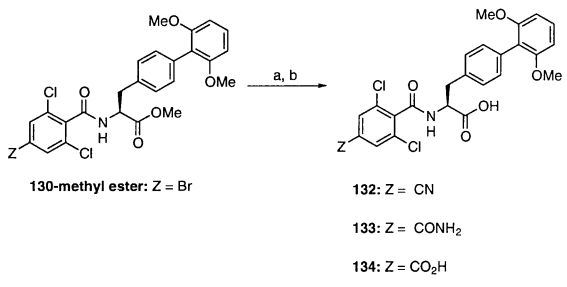
Schemes 1 and 2 were utilized for the synthesis of the *N*-benzoyl biphenyl scaffold. Schemes 3–9 illustrate reactions for functional group transformations for additional targets. For A-ring modified compounds, *N*-boc-tyrosine triflate methyl ester[21] or *N*-boc-*p*-BrPhe methyl ester was used as the starting material. These were reacted with the requisite boronic acids under Suzuki reaction[22,23] to give the desired *N*-boc-biphenylalanine derivatives. The boc group was removed and the amine was reacted with requisite benzoic acids (or chlorides) to give the coupled products, which were saponified to produce the



Scheme 3. Reagents: (a) R′COCl; (b) $(R'SO_2)_2O$, DIEA or $R'SO_2Cl$, DIEA; (c) R′NCO; (d) R′NCS; (e) R′OCOCl; (f) LiOH.



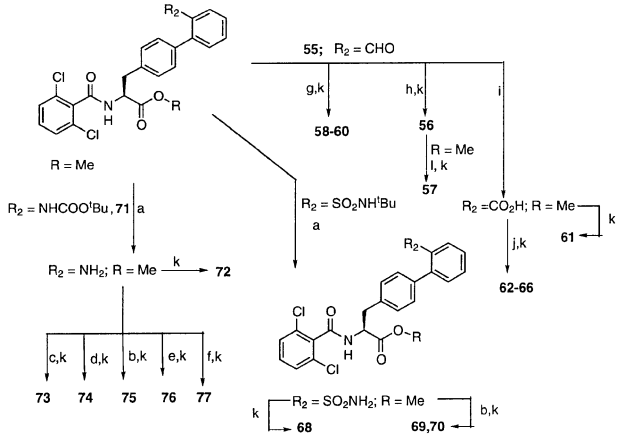
Scheme 4. Reagents: (a) LiOH; (b) TFA; (c) CDI, NH_4OH; (d) CDI, $MeSO_2NH_2$, DBU.



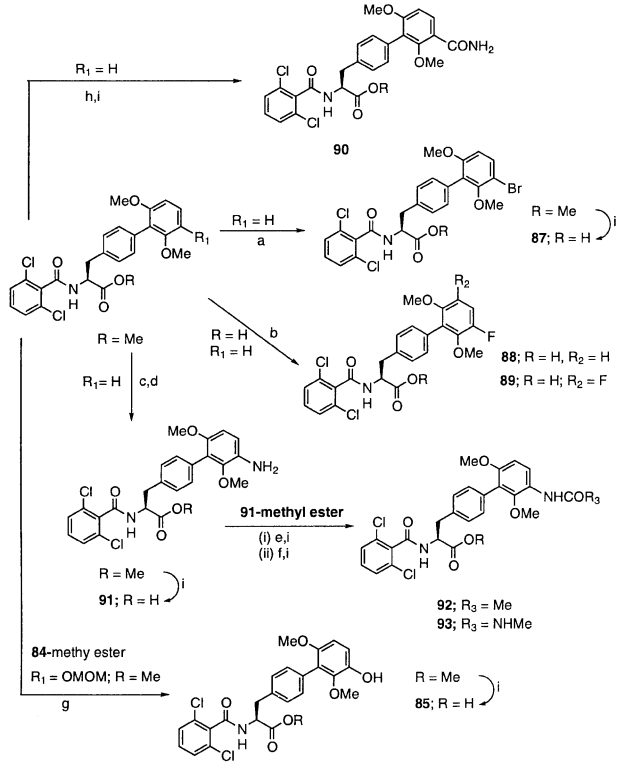
Scheme 5. Reagents: (a) KI, KCN, $Pd(PPh_3)_4$; (b) LiOH.

target compounds (Scheme 1). For B-ring modified compounds, the reaction sequences were reversed. Tyrosine or *p*-bromophenylalanine methyl ester was reacted with requisite benzoic acids (or chlorides) followed by Suzuki coupling of the resultant benzoyl derivatives with desired boronic acids. The resulting biphenyl compounds were saponified to yield the target acids (Scheme 2).

The amine derivatives (**20**–**43** and **136**–**143**) were prepared from the amine-ester via diverse transformations followed by saponification (Scheme 3).



Scheme 6. Reagents: (a) TFA; (b) R′COCl; (c) $ClSO_2NCO$; (d) $(RSO_2)_2O$, DIEA; (e) R′OCOCl, DIEA; (f) HCHO, $NaCNBH_4$; (g) RNH_2, $NaCNBH_4$; (h) $NaBH_4$; (i) $KMnO_4$; (j) CDI, R′R″N; (k) LiOH; (l) Cs_2CO_3, MeI.

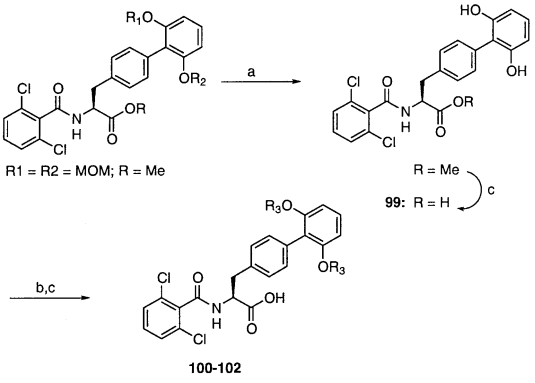


Scheme 7. Reagents: (a) $(Bu)_4NBr{\cdot}Br_2$; (b) tertrabutyl fluoropyridinium tetrafluoroborate; (c) HNO_3; (d) $Na_2S_2O_4$; (e) Ac_2O; (f) MeNCO; (g) HCl; (h) $ClSO_2NCO$; (i) LiOH.

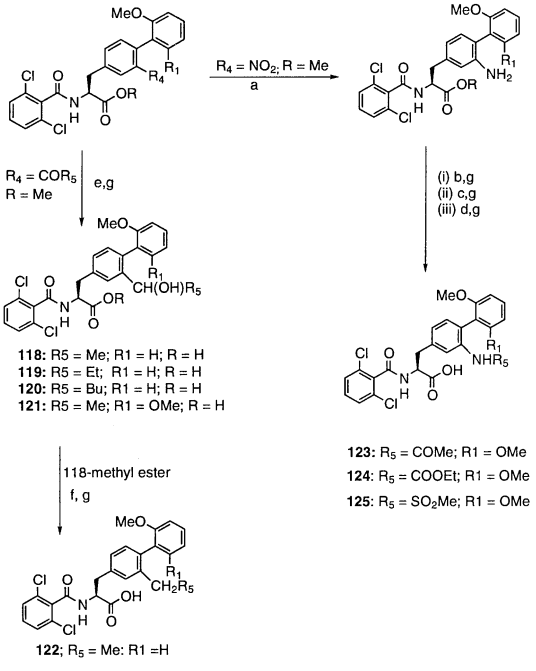
For the carboxylic acid derivatives (**14** and **15**), the differentially-protected diester was utilized (Scheme 4). The carboxylic acid analogues in the dichloro series (**133** and **134**) were prepared from the corresponding nitrile **132**, which in turn was synthesized from the bromide **130** by treatment with KCN[24] in the presence of $Pd(PPh_3)_4$ (Scheme 5).

Compounds **58**–**60** were prepared via reductive amination of **55** with requisite amines (Scheme 6). Additional functional group transformations in ring B (**62**–**66** and **68**–**77**) are also depicted in Scheme 6. The synthesis of **85** and **87**–**93** was accomplished from **81** methylester as outlined in Scheme 7.

Scheme 8 illustrates the synthesis of compounds **99**–**102**. The bis–MOM ether compound was deprotected with HCl to give the bis–OH compound, which was alkylated with the requisite alkyl halide in the presence of base to generate target compounds as methyl esters.



Scheme 8. Reagents: (a) HCl; (b) Cs_2CO_3, R_3X; (c) LiOH.



Scheme 9. Reagents: (a) H_2, Raney Ni; (b) Ac_2O, DIEA; (c) EtOCOCl, DIEA; (d) $MeSO_2Cl$; (e) $NaBH_4$; (f) Et_3SiH, $BF_3.Et_2O$; (g) LiOH.

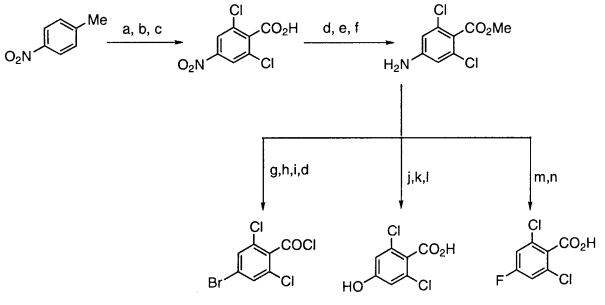
Saponification resulted in the desired acids. Similar procedures were utilized for compounds **94**, **96** and **97** starting from the 2-OMe, 6-MOM compound. Compounds in Table 7 (**104**–**115**) were prepared in a similar manner.

The synthesis of C-ring modified compounds is shown in Scheme 9. The hydroxyalkyl compounds **118**–**121** were prepared from the corresponding carbonyl compounds via $NaBH_4$ reduction. Compound **122** was prepared from **118** via reduction with $(Et)_3SiH$ and $BF_3{\cdot}Et_2O$. The amine derivatives **123**–**125** were prepared from the corresponding amine which was obtained from the nitro compound via reduction with Raney–Ni.

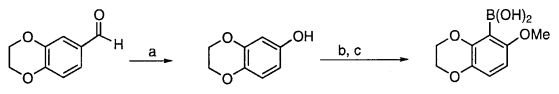
The synthesis of the requisite 2,6-di-chlorobenzoic acids (ring A) is shown in Scheme 10. 4-Amino-2,6-dichlorobenzoic acid was diazotized and subsequently converted to the corresponding 4-OH, 4-Br, and 4-F derivatives. Schemes 11 and 12 outline the synthesis of the starting boronic acids.

Biology

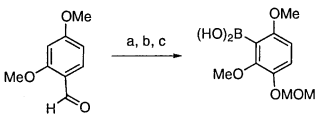
All compounds were tested for $\alpha_4\beta_7$ and $\alpha_4\beta_1$ activity in the RPMI and Jurkat cell adhesion assays (Tables 1–9). IC_{50} values were generated by nonlinear regression from titration curves of compounds from 10 doses and reported as the average of a minimum of two experiments as described in the Experimental. For the Jurkat-CS1 assay, the standard error of the mean was typically $<10\%$ for each experiment. For the RPMI assay, the IC_{50} values were normalized against an internal standard.



Scheme 10. Reagents: (a) Cl_2, $SbCl_3$; (b) $PhCO_2H$, Cu; (c) HNO_3, 160 °C; (d) $SOCl_2$; (e) MeOH; (f) $Na_2S_2O_4$; (g) HBr, $NaNO_2$; (h) Cu, heat; (i) LiOH; (j) HCl, $NaNO_2$; (k) H_2O, reflux; (l) NaOH, reflux; (m) HBF_4, heat; (n) TMSI.



Scheme 11. Reagents: (a) H_2O_2, H_2SO_4, MeOH; (b) K_2CO_3, MeI, Bu_4NI, DMF; (c) *n*-BuLi, $B(OMe)_3$.



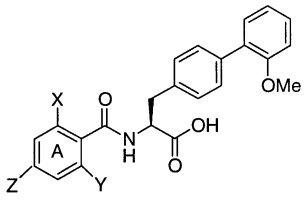
Scheme 12. Reagents: (a) H_2O_2, H_2SO_4; (b) MOMCl, K_2CO_3; (3) *n*-BuLi, $B(OMe)_3$.

Results and Discussion

Among the ring A substitutions studied in the des-methoxybiphenylalanine series (e.g., halogens, CN, OMe, SMe, NO_2, CO_2H, NH_2, and OCF_3), 2-halo compound showed the best potency (unpublished data, Tanabe Research laboratories). The SAR was extended in the 2-OMe series (Table 1). A halogen substitution in the ortho position enhanced $\alpha_4\beta_7$ potency by 7- to 9-fold (e.g., **2** and **3**) as seen with the des-methoxy series. The effects of additional substitution on the 2-chloro compound **2** were evaluated. Compound **9**, the 4-amine derivative, retained potency similar to **2**, whereas introduction of an acidic group at the 4-position increased potency. The di-acid **13** reached 15 and 200 nM potency for $\alpha_4\beta_7$ and $\alpha_4\beta_1$, respectively. In addition, a 6-halo substitution improved potency (**16**, $\alpha_4\beta_7/\alpha_4\beta_1$: 77/850 nM). The rank order of potency for 2,6-disubstitution was: 2,6-diCl $>$ 2-Cl,6-F $>$ 2,6-diF $=$ 2,6-diMe. In general, these compounds showed 10- to 60-fold selectivity for $\alpha_4\beta_7$ over $\alpha_4\beta_1$.

Several derivatives of **9** were synthesized in order to enhance its potency (Table 2). The acetamide **21,** carbamate **22**, urea **23** and thiourea **24** maintained a similar potency. Further exploration of a small series of thiourea derivatives resulted in the methyl urea **25** being the most potent and the benzyl urea **29** being the least potent. The methanesulfonamide **30** showed a 10-fold

Table 1. Inhibitory effects of the ring A modified compounds



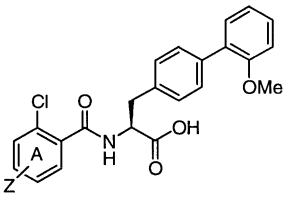
Compd	X	Y	Z	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
1	H	H	H	5.08	33
2	Cl	H	H	0.56	13
3	Br	H	H	0.77	16
4	CF_3	H	H	1.1	6.9
5	Cl	H	OMe	0.3	36
6	Cl	H	Cl	0.31	15
7	Cl	H	Br	1.4	64
8	Cl	H	NO_2	0.27	17
9	Cl	H	NH_2	0.31	15
10	Cl	H	SO_2Me	0.15	2.6
11	Cl	H	OH	0.14	9
12	Cl	H	CO_2^tBu	0.46	26
13	**Cl**	**H**	**CO_2H**	**0.015**	**0.20**
14	Cl	H	$CONH_2$	0.12	1.1
15	Cl	H	$CONHSO_2Me$	0.060	0.456
16	**Cl**	**Cl**	**H**	**0.077**	**0.85**
17	Cl	F	H	0.17	2.9
18	F	F	H	0.33	6.6
19	Me	Me	H	0.34	1.9

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

improvement in potency, whereas the corresponding *N*-methyl compound **31** showed a 5-fold reduction in potency implicating the need for an acidic proton for optimum potency. Compound **33**, a trifluoro derivative of **30**, showed improved $\alpha_4\beta_7$ potency (3-fold), but reduced $\alpha_4\beta_1$ potency (3-fold). Replacing the methyl group with a phenyl group in **30** produced **34** that approached a potency similar to **33**. The potency could not be enhanced further with additional substitution on the phenyl moiety. Both compounds **33** and **34** displayed improved $\alpha_4\beta_7$ selectivity by 246- and 271-fold, respectively. The 2-thienyl compound **40** showed even greater $\alpha_4\beta_7$ potency (11 nM) and selectivity (645-fold) relative to **34**. It should be noted that a negative charge at the 4-position improved both $\alpha_4\beta_7/\alpha_4\beta_1$ adhesion, whereas a large hydrophobe adjacent to the acidic moiety did not support $\alpha_4\beta_1$ adhesion (compare **13** to **34** and **40**, respectively). These data collectively implicate the divergence of $\alpha_4\beta_7/\alpha_4\beta_1$ SAR at this site. It is important to note that regioisomers of compound **33** lost potency in the order of 4- > 5- > 3- > 6-.

Having established the optimum substitutions for ring A, attention was turned to ring B. For ease of synthesis,

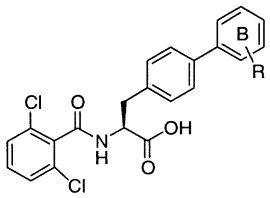
Table 2. Inhibitory effects of 4-aminoderivatives of the ring A



Compd	Z	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]	$\alpha_4\beta_1/\alpha_4\beta_7$
9	4-NH_2	0.31	15	48
20	4-NHMe	0.49	49	100
21	4-NHCOMe	0.33	5.7	17
22	4-NHCOOEt	0.20	9.6	48
23	4-$NHCONH_2$	0.14	2.4	17
24	4-$NHCSNH_2$	0.14	3.3	24
25	**4-NHCSNHMe**	**0.030**	**0.59**	**20**
26	4-NHCSNHEt	0.12	5.7	48
27	4-$NHCSNH^{iso}Pr$	0.32	15	39
28	4-NHCSNHPh	0.31	24	77
29	4-NHCSNHBn	1.5	27	15
30	**4-$NHSO_2Me$**	**0.041**	**1.3**	**32**
31	4-$NMeSO_2Me$	0.20	3.0	15
32	4-$NHSO_2NMe_2$	0.052	3.1	60
33	**4-$NHSO_2CF_3$**	**0.013**	**3.2**	**246**
34	**4-$NHSO_2Ph$**	**0.021**	**5.7**	**271**
35	4-$NHSO_2$(4-FPh)	0.049	11	224
36	4-$NHSO_2$(4-MePh)	0.073	9.8	134
37	4-$NHSO_2$(4-CF_3Ph)	0.15	26	173
38	4-$NHSO_2$(4-OMePh)	0.084	13	155
39	4-$NHSO_2$(2-MePh)	0.12	15	125
40	**4-$NHSO_2$(2-thienyl)**	**0.011**	**7.1**	**645**
41	3-$NHSO_2CF_3$	0.361	34	82
42	5-$NHSO_2CF_3$	0.060	4.9	82
43	6-$NHSO_2CF_3$	> 20	65	< 3

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically < 10% for each experiment.

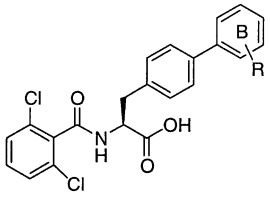
Table 3. Inhibitory effects of the ring B modified compounds



Compd	**R**	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
16	2-OMe	0.077	0.85
44	H	2.3	36
45	3-OMe	6.2	49
46	4-OMe	1.1	26
47	2-OCF_3	0.42	10.4
48	2-$O^{iso}Pr$	1.17	5.4
49	2-OBu	1.6	5.1
50	2-$O^{iso}Bu$	2.1	8.4
51	2-Me	0.75	11.0
52	2-CF_3	0.13	3.4
53	2-OH	0.33	1.9
54	2-CN	0.13	7.0
55	**2-CHO**	**0.16**	**3.7**
56	**2-CH_2OH**	**0.14**	**1.5**
57	**2-CH_2OMe**	**0.34**	**1.1**

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically < 10% for each experiment.

Table 4. Inhibitory effects of the ring B modified compounds

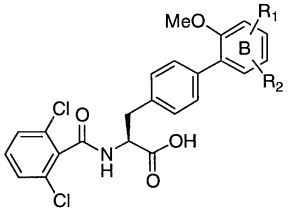


Compd	R	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
58	2-CH_2NH_2	0.30	1.7
59	2-CH_2NHPh	0.28	2.8
60	2-CH_2NHCH_2Ph	1.66	8.1
61	2-COOH	6.14	5.3
62	2-$CONH_2$	0.51	1.4
63	2-CONHMe	0.79	0.92
64	2-$CONMe_2$	0.79	1.0
65	2-CONHBu	1.44	2.4
66	2-$CONHCH_2Ph$	1.93	2.9
67	2-SO_2NH^tBu	0.15	0.55
68	2-SO_2NH_2	0.10	0.59
69	2-$SO_2NHCOPh$	0.35	3.1
70	2-$SO_2NHCOMe$	1.6	3.7
71	2-$NHCOO^tBu$	2.7	4.0
72	2-NH_2	0.45	0.94
73	2-$NHCONH_2$	0.40	1.9
74	2-$NHSO_2Me$	0.27	1.7
75	2-NHCOMe	0.78	0.88
76	2-NHCOOMe	0.57	1.3
77	2-NMe_2	0.20	0.72

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically < 10% for each experiment.

most of the SAR on ring B was carried out on the 2,6-dichlorobenzoyl compound **16**. Tables 3 and 4 list monosubstituted compounds. Moving the 2-OMe group around the ring resulted in a loss of potency implicating the importance of the twisted conformation of the biphenyl system. Extension of the 2-OMe group to higher alkyloxy and -OCF_3 groups also caused a loss in potency. Compound **51**, wherein the methoxy group was replaced with a methyl group, showed reduced potency (10- to 16-fold) implicating the need for a H-bond acceptor at this site. This loss was partially regained by substituting the methyl group with a trifluoromethyl group (**52**). Compounds **53–57** where other H-bond accepting moieties were incorporated at

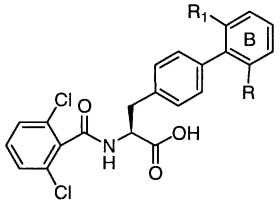
Table 5. Inhibitory effects of multisubstitution in the ring B



Compd	R_1	R_2	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
78	3-OMe	H	0.13	4.0
79	4-OMe	H	0.1	0.74
80	5-OMe	H	0.017	0.578
81	**6-OMe**	**H**	**0.007**	**0.087**
82	6-OMe	4-OMe	0.008	0.12
83	6-OMe	4-OH	0.017	0.108
84	6-OMe	3-OMOM	0.009	0.062
85	6-OMe	3-OH	0.042	0.208
86	6-OMe	3-OMe	0.010	0.046
87	6-OMe	3-Br	0.018	0.197
88	6-OMe	3-F	0.022	0.074
89	6-OMe	3,5-diF	0.071	0.313
90	6-OMe	3-$CONH_2$	0.010	0.029
91	6-OMe	3-NH_2	0.012	0.061
92	6-OMe	3-NHCOMe	0.020	0.036
93	6-OMe	3-NHCONHMe	0.020	0.029

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

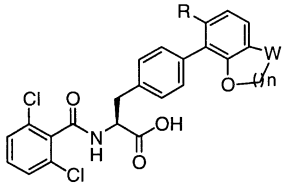
Table 6. Inhibitory effects of replacement of OMe group in the ring B



Compd	R	R_1	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
94	OMe	OEt	0.027	0.12
95	OMe	OH	0.031	0.56
96	OMe	$O(CH_2)_2F$	0.039	0.146
97	OMe	$O^{iso}Pr$	0.245	0.509
98	OEt	OEt	0.16	0.19
99	OH	OH	0.5	3.1
100	OCH_2CH_2OH	OCH_2CH_2OH	0. 282	0.545
101	OCH_2CF_3	OCH_2CF_3	0.676	2.9
102	OCH_2CN	OCH_2CN	0.055	0.183
103	Me	Me	1.34	31

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

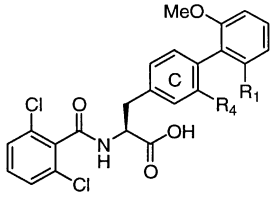
Table 7. Inhibitory effects of the ring B-fused bicyclic compounds



Compd	R	W	n	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
104	H	O	1	0.928	25
105	OMe	O	1	0.013	0.383
106	OCH_2OMe	O	1	0.068	1.3
107	OCH_2CN	O	1	0.069	2.3
108	OH	O	1	0.137	3.8
109	OCH_2CH_3	O	1	0.109	1.0
110	OCH_2CH_2OH	O	1	0.065	0.591
111	OCH_2CH_2OMe	O	1	0.45	0.735
112	$O(CH_2)_2NMe_2$	O	1	0.225	0.823
113	OMe	O	2	0.006	0.043
114	H	CH_2	1	0.233	13.6
115	H	CH_2	2	0.07	1.7

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

Table 8. Inhibitory effects of the ring C modified compounds



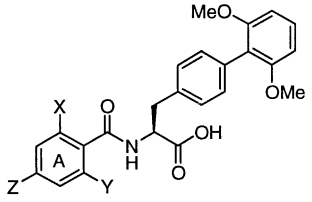
Compd	R_4	R_1	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]
16	H	H	0.077	0.85
81	H	OMe	0.007	0.087
116	OMe	H	0.040	0.46
117	COMe	H	0.17	0.5
118	CH(OH)Me	H	0.027	0.048
119	CH(OH)Et	H	0.047	0.071
120	CH(OH)Bu	H	0.155	0.253
121	CH(OH)Me	OMe	0.008	0.012
122	CH_2Me	H	0.21	0.68
123	NHCOMe	OMe	0.030	0.026
124	NHCOOEt	OMe	0.123	0.068
125	$NHSO_2Me$	OMe	0.010	0.022

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

the 2-position retained reasonable potencies. However, compounds with similar substitutions at the 3-position showed a significant reduction in potency (data not shown). Additional 2-substituted compounds with H-bond donating and accepting groups are listed in Table 4. Although the benzylamines **58** and **59** retained moderate $\alpha_4\beta_7$ potencies, the dibenzylamine **60** lost potency. A carboxylic acid group at this position was detrimental, whereas the carboxamides showed improved potency (compare **61** to **62–64**). The activity of the amides decreased with the increasing size of the amine. The sulfonamides **67** and **68** retained dual potencies similar to the methoxy compound **16**. A small series of amine derivatives (**72–77**) were evaluated that retained reasonably good potency for $\alpha_4\beta_7$. The SAR led to the hypothesis that a H-bond accepting group and a small hydrophobe around that region are minimum requirements for activity.

The prototype compound **16** was chosen for subsequent modification. As evident from Table 5, the introduction of a second methoxy group impacted on potency. A 10-fold enhancement in potency was observed with the 2,6-diOMe compound **81** (IC_{50} $\alpha_4\beta_7/\alpha_4\beta_1 = 7/87$ nM). This supported the initial hypothesis that the restricted rotation around the Phe–Phe bond was critical for optimum potency. 2,6-Disubstitution increased the rotational barrier between the phenyl groups resulting in a non-planar configuration of the phenyl groups. Similar potency enhancement was observed in sulfonylated proline derivatives.[25] Additional modifications were carried out with **81**. Incorporation of additional alkyloxy groups in the 4- and 3-position of **81** (**82** and **86**) maintained potency. By contrast, compounds **83** and **85**, 4- and 3-OH derivatives of **81**, lost potency. The fluorinated compounds (**88–89**) were synthesized to improve the physicochemical properties. Unfortunately, the potencies were not maintained with these analogues. The amine and its derivatives (**91–93**) retained excellent dual potency. Our next challenge was to find replacements for the metabolically labile methoxy groups of **81**. Table 6 lists compounds synthesized towards this end without much success. In general, the SAR paralleled the monomethoxy series (Table 3). Compounds **94**, **96**, and **97**, wherein one of the –OMe groups was replaced with higher alkyloxy groups, resulted in a loss of activity with the increasing size of the alkyl group. The potency loss was more significant in the di-alkyloxy compounds **98**, **100** and **101**. The electron withdrawing nature of the fluorines in **101** reduce the H-bonding potential of the ether oxygens compared to **98**. The loss of potency (0.16 to 0.676) further supports the role of H-bonding in this region of the molecule. The hydroxy compounds **95** and **99** showed reduced potency relative to **81**, as expected. Compound **103** lost potency similar to the monomethyl compound **51**.

Table 9. Representative derivatives of compound **81**



Compd	X	Y	Z	$\alpha_4\beta_7$-CS1 IC_{50}, μM[a,b]	$\alpha_4\beta_1$-CS1 IC_{50}, μM[a,c]	$\alpha_4\beta_1/\alpha_4\beta_7$
81	Cl	Cl	H	0.007	0.087	12
126	Br	Br	H	0.046	0.171	9
127	F	F	H	0.059	0.505	9
128	Cl	Me	H	0.018	0.158	9
129	Cl	Cl	F	0.023	0.098	4
130	Cl	Cl	Br	0.029	0.377	13
131	Cl	Cl	OH	0.018	0.044	2
132	Cl	Cl	CN	0.013	0.019	1
133	Cl	Cl	$CONH_2$	0.0025	0.012	5
134	**Cl**	**Cl**	**CO_2H**	**0.00033**	**0.003**	**10**
135	Cl	Cl	NH_2	0.007	0.17	24
136	Cl	Cl	NHCOMe	0.014	0.314	22
137	Cl	Cl	NHCOOMe	0.008	0.11	14
138	Cl	Cl	NHCOOEt	0.012	0.19	16
139	Cl	Cl	$NHCONH_2$	0.0075	0.083	11
140	Cl	Cl	NHCONHMe	0.0065	0.152	23
141	Cl	Cl	NHCSNHMe	0.025	0.125	5
142	**Cl**	**Cl**	**$NHSO_2CF_3$**	**0.0005**	**0.192**	**384**
143	**Cl**	**Cl**	**$NHSO_2$(2-thienyl)**	**0.00053**	**0.063**	**119**

[a]IC_{50} values were generated by nonlinear regression from titration curves of compounds from seven doses and reported as the average of a minimum of two experiments.
[b]IC_{50} values were normalized against an internal standard.
[c]Standard error of the mean was typically $<10\%$ for each experiment.

A small series of compounds were synthesized in the bicyclic system wherein the 2- and 3-methoxy groups were incorporated into the ring (Table 7). The parent compound **104** in the methylenedioxy series showed a 7-fold reduction in potency relative to **78**. Addition of a second -OMe group in the 6-position improved potency 71-fold. The resulting compound **105** retained $\alpha_4\beta_7$ potency similar to **86**; $\alpha_4\beta_1$ potency was 8-fold less, however. The replacement of the 6-methoxy group of **105** with other alkyloxy substituents caused a reduction in potency similar to that seen in the monocyclic series (Table 6). Compound **113,** an analogous compound in the ethylenedioxy series, gained potency similar to **81**. Similar trends were observed with compounds in the benzofuran and benzopyran series (**115** was more potent than **114**).

The final strategy was to modify the middle ring (ring C, Table 8). It was rationalized that if restricted rotation is important for activity, then addition of a substituent in the 3-position (*ortho* to ring B) should improve potency. Indeed, compound **116** improved $\alpha_4\beta_7$ potency $\sim$2-fold relative to **16.** This result encouraged us to evaluate the effects of additional H-bond donating and/or accepting groups at this position. The initial data indicated that a H-bond donating group might be preferred (compare **118** to **117**). The corresponding dimethoxy compound **121** showed enhanced potency ($\sim$4-fold) resulting in a balanced $\alpha_4\beta_7/\alpha_4\beta_1$ compound with IC_{50} values of 8 and 12 nM, respectively. The effects of higher alkyls on the hydroxyalkyl analogue **118** were also evaluated. Data from compounds **119** and **120** indicated that small alkyl groups were preferred at this site. The reduced potency associated with the deoxygenated analogue further supported the role of the H-bond donating moieties at this site (compare **118** to **122**). It is notable that compounds

118 and **121** are diastereoisomeric mixtures that were not resolved; therefore, the net effect of the hydroxyethyl at this position was to greatly enhance α4β1 potency relative to α4β7 potency. Of the three-amine derivatives (**123**–**125**), the sulfonamide compound **125** equalled the potency of **121**.

Additional analogues of **81** with variations on the A-ring are listed in Table 9. Di-Cl substitutions seems to be optimum for dual potency (compare **81** to **126**–**128**). Addition of a halogen at the 4-position (**129** and **130**) reduced potency 5- to 10-fold, whereas 4-OH and 4-CN (**131** and **132**) had very small effects. Compound **134**, the 4-CO_2H derivative, showed a 18-fold enhancement in potency reaching subnanomolar (0.33) and low nM (3.0) potency for $\alpha_4\beta_7$ and $\alpha_4\beta_1$ adhesion, respectively. The corresponding amide **133** also retained excellent potency relative to **81**. Also, a small series of 4-amine derivatives were evaluated. Of those, the two sulfonamides **142** and **143** showed improved potency resulting in subnanomolar compounds, as predicted from the SAR. The sulfonamides **142** and **143** were more selective for $\alpha_4\beta_7$ (384- and 119-fold, respectively) compared to the carboxylic acid **134** (10-fold). Thus, the pharmacophore requirements [2,6-diCl, 4-CO_2H in ring A, 2,6-diOMe in ring B and 3-CH(OH)Me in ring C] for best dual potency were established.

Conclusion

A novel series of small molecule non-peptide $\alpha_4\beta_7$ receptor antagonists has been discovered. The potency of the initial lead compound **1** (IC_{50} $\alpha_4\beta_7/\alpha_4\beta_1 = 5/33$ μM) was optimized to generate low nM compounds via SAR. Compound **81** (TR-14035), a dual active antagonist, showed a 1000-fold enhancement in potency (IC_{50} $\alpha_4\beta_7/\alpha_4\beta_1 = 7/87$ nM) compared to **1**. The SAR also led to three subnanomolar potency compounds, **134**, **142**, and **143**. Of those, the sulfonamide derivatives **142** and **143** showed 100- to 400-fold selectivity for $\alpha_4\beta_7$. All these compounds are fully selective at the tested concentrations with respect to $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$, and $\alpha_L\beta_2$ ($IC_{50} > 20$ μM). Compound **81** has been shown to be orally absorbed in several animal species (data not shown). Compound **81** was selected for clinical development and has finished Phase I testing in healthy volunteers in Europe. The pharmacological characterization of **81** (TR-14035) will be reported in a separate publication.

Experimental

^1H NMR spectra were recorded on a Bruker 300 MHz instrument in DMSO-d_6 (unless otherwise stated) with TMS as the internal standard. Mass spectra were obtained by electron spray method on the SCIEX API100. Medium pressure chromatography was conducted on a Biotage (4 × 15 cm, SiO_2) instrument. All flash chromatography was done with SiO_2. Melting points were uncorrected. Solutions in organic solvents were dried with anhydrous $MgSO_4$.

General procedures for compound synthesis

***N*-(2,6-Dichlorobenzoyl)-4-(2-methoxyphenyl)-L-phenylalanine (16).** To a mixture of 2-methoxybenzeneboronic acid (0.446 g, 2.94 mmol) and anhydrous K_2CO_3 (0.84 g, 6.1 mmol) in toluene/DMF (25 mL/2.5 mL) under N_2 was added a solution of *tert*-Boc-L-tyrosine(triflate)methyl ester[21] (1.0 g, 2.33 mmol) in 5 mL of toluene. The catalyst $Pd(PPh_3)_4$ (0.48 g, 0.42 mmol) was added and the mixture was heated at 80 °C for 24 h. The reaction mixture was cooled, filtered through Celite and evaporated to dryness. The residue was taken up in EtOAc and the solution washed with water. It was dried, evaporated, and the crude material was purified via flash chromatography (hexane/EtOAc, 3/1) to yield *N*-*tert*-butoxycarbonyl-4-(2-methoxyphenyl)-L-phenylalanine methyl ester (0.64 g) as a clear oil: MS *m*/*z* 386 ($M^+ + H$) and 408 ($M^+ + Na$). To a solution of the *tert*-Boc-derivative (2.97 g, 7.3 mmol) in CH_2Cl_2 (20 mL) was added TFA (20 mL) and the mixture stirred for 1.5 h. The solution was evaporated to dryness. The residue was re-dissolved in CH_2Cl_2 (20 mL) and the solution was evaporated. This process was repeated one more time and finally the residue was dried under high vacuum to yield the amine (2.93 g) as the TFA salt: MS *m*/*z* 286 ($M^+ + H$). To a solution of the above amine salt (2.3 g, 5.8 mmol) in CH_2Cl_2 (30 mL) containing DIEA (2.24 g, 3 mL, 17 mmol) at 0 °C was added a solution of 2,6-dichlorobenzoyl chloride (0.99 mL, 6.9 mmol) with stirring. The solution was warmed to room temperature and stirred for 24 h. The solution was washed with water, 1 N HCl, saturated $NaHCO_3$, brine, dried, filtered and concentrated. The residue was purified via flash chromatography (hexane/EtOAc, 4/1) to yield 1.64 g of *N*-(2,6-dichlorobenzoyl)-4-(2-methoxyphenyl)-L-phenylalanine methyl ester: MS *m*/*z* 458 ($M^+ + H$) and 471 ($M^+ + Na$). The above ester (0.1 g, 0.22 mmol) was dissolved in a mixture of THF/MeOH (5 mL/2 mL). A solution of LiOH (14 mg, 0.33 mmol) in water (2 mL) was added and the mixture stirred at room temperature for 3 h. The solution was evaporated and the residue was treated with water. The solution was adjusted to pH 2 and the organic material was extracted with EtOAc. The organic layer was washed with brine, dried and evaporated to yield 0.08 g of the titled compound: MS *m*/*z* 444 ($M^+ + H$) and 467 ($M^+ + Na$); mp 211 °C; ^1H NMR ($CDCl_3$) 3.32 (dd, 1H), 3.37 (dd, 1H), 3.78 (s, 3H), 5.2 (m, 1H), 6.3 (d, 1H), 6.88 (m, 2H), 7.20–7.38 (m, 7H), 7.42 (d, 2H). Anal. calcd for $C_{23}H_{19}Cl_2NO_4 0.3H_2O$ (449.72): C, 61.37, H, 4.36, N, 3.11; found: C, 61.08, H, 4.23, N, 3.09.

***N*-(2,6-Dichlorobenzoyl)-4-(2,6-dimethoxyphenyl)-L-phenylalanine (81).** HCl (g) was bubbled into a methanolic (100 mL) solution of (L) 4-bromo-phenylalanine (5 g) and the mixture was left overnight at room temperature. The solid was filtered off, washed with ether and air-dried to give 5.54 g of the methyl ester of (L) 4-bromophenylalanine, HCl salt: MS *m*/*z* 274 ($M^+ + H$) and 296 ($M^+ + Na$). Et_3N (8 mL, 56.7 mmol) was added to a suspension of the above methyl ester (5.55 g, 18.9 mmol) in CH_2Cl_2 (40 mL) at 0 °C. To this was added a solution of 2,6-dichlorobenzoyl chloride (3.52 mL, 25

mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried, filtered and concentrated to a syrup. The residue was purified by flash column chromatography (hexane/EtOAc, 2/1) to yield 3.9 g of *N*-(2,6-dichlorobenzoyl)-L 4-bromophenylalanine methyl ester: MS *m/z* 446 ($M^+ + H$) and 468 ($M^+ + Na$). This was used for the coupling reaction.

To a suspension of 2,6-dimethoxybenzeneboronic acid (0.3 g, 1.6 mmol) and K_2CO_3 (0.5 g, 3.6 mmol) in DME (10 mL) was added the above methyl ester (0.3 g, 0.7 mmol), $Pd(Ph_3P)_4$ (0.3 g, 0.26 mmol) and water (0.4 mL). The mixture was heated at 80 °C for 6 h. The reaction mixture was cooled, EtOAc and water were added to the reaction mixture and the phases separated. The EtOAc layer was dried, filtered and evaporated to provide a black gum. The gum was purified by flash column chromatography (hexane/EtOAc, 1/2) to give 0.2 g (59%) of the desired compound as a white solid: MS *m/z* 488 ($M^+ + H$); ^1H NMR ($CDCl_3$) 3.3 (dd, 2H), 3.7 (s, 6H), 3.8 (s, 3H), 5.2 (m, 1H), 6.4 (d, 1H), 6.6 (d, 2H), 7.2–7.3 (m, 8H). To a solution of the above methyl ester (0.1 g, 0.2 mmol) in THF (5 mL) was added aqueous LiOH (12 mg, 0.5 mmol in 0.5 mL water) and a few drops of MeOH. The resulting mixture was stirred at room temperature for 2 h. THF was removed, the residue dissolved in water and the solution neutralized with 10% citric acid to yield a white solid. The solid was filtered, washed with water and dried to provide 80 mg (85%) of the titled compound: MS *m/z* 474 ($M^+ + H$), 472 ($M^- H$); mp 243.6 °C (dec); ^1H NMR δ 2.9 (dd, 1H), 3.2 (dd, 1H) , 3.7 (s, 6H), 4.72 (m, 1H), 6.7 (d, 2H), 7.1–7.5 (m, 8H), 9.1 (d, 1H). Anal. calcd for $C_{24}H_{21}Cl_2NO_5$: C, 60.77; H, 4.46; N, 2.95; found: C, 60.63; H, 4.44; N, 2.82. Compound **81** was also prepared in a manner similar to **16**.

***N*-(4-Amino-2-chlorobenzoyl)-4-(2-methoxyphenyl)-L-phenylalanine (9).** To a solution of 4-(2-methoxyphenyl)-L-phenylalanine methyl ester, TFA salt (1.17 g, 2.78 mmol) in DMF (110 mL) containing DIEA (2.24 g, 0.48 mL, 5.57 mmol) at 0 °C was added a solution of 4-amino-2-chlorobenzoic acid (0.48 g, 2.78 mmol) with stirring. HOBT (0.64 g, 4.18 mmol) was added followed by EDC (0.53 g, 2.78 mmol) and the mixture was stirred overnight at room temperature. DMF was distilled and the residue was dissolved in EtOAc. The solution was washed with water, saturated $NaHCO_3$, brine, dried, filtered and concentrated. The residue was purified via flash chromatography (hexane/EtOAc, 1/1) to yield 0.85 g of the titled compound methyl ester as a white solid: MS *m/z* 439 ($M^+ + H$) and 461 ($M^+ + Na$). This was saponified to give 0.6 g of the titled acid: MS *m/z* 425 ($M^+ + H$) and 447 ($M^+ + Na$).

***N*-(2-Chloro-4-ureidobenzoyl)-4-(2-methoxyphenyl)-L-phenylalanine (23).** Chlorosulfonylisocyanate (189 μmol, 16.4 μL) was added to a solution of **9** methyl ester (55.2 mg, 126 μmol) in anhydrous MeCN (1 mL) and the mixture was stirred at room temperature under N_2 for 1 h. Saturated $NaHCO_3$ (40 mL) was added slowly and the mixture extracted with EtOAc (3 × 20 mL). The extracts were combined, dried, filtered and evaporated. The residue was purified by chromatotron using a gradient of $CHCl_3$/MeOH to give the sulfonylurea compound (43.0 mg, 61%) as a white solid: MS *m/z* 560 (M^-). The ester was saponified with 3 equiv of LiOH to yield the titled compound (24 mg, 87%) as a white solid: MS *m/z* 468 ($M^+ + H$). ^1H NMR (CD_3OD) δ 3.09 (dd, 1H), 3.37 (dd, 1H), 3.76 (s, 3H), 4.89 (dd, 1H), 6.97 (dt, 1H), 7.03 (dd, 1H), 7.22–7.42 (m, 6H), 7.60 (bs, 1H).

***N*-[2-Chloro-4-(3-methylthioureido)benzoyl]-4-(2-methoxyphenyl)-L-phenylalanine (25).** Methylisothiocyanate (628 μmol, 43 μL) was added to a solution of **9** methyl ester (55.1 mg, 126 μmol) in anhydrous DMF (1 mL) containing DIEA (126 μmol, 22 μL) and DMAP (cat.). The resulting mixture was then heated at 90 °C under N_2 for 1 day. The mixture was taken up with EtOAc (40 mL) and the solution was washed with 1 N HCl (3 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), water (1 × 20 mL), dried, filtered and evaporated. The residue was purified by chromatotron using CH_2Cl_2/MeOH (15/1) to give the desired thiourea (22.7 mg, 35%) as a white solid: MS *m/z* 512 ($M^+ + H$). This material was saponified to yield the titled compound (22.0 mg) as a white solid: MS *m/z* 496 ($M^+ + H$). ^1H NMR (CD_3OD) δ 3.02 (s, 1H), 3.09 (dd, 1H), 3.38 (dd, 1H), 3.76 (s, 3H), 4.90 (dd, 1H), 6.98 (dt, 1H), 7.04 (dd, 1H), 7.23–7.43 (m, 8H), 7.60 (d, 1H).

***N*-[2-Chloro-4-(methanesulfonylamino)benzoyl]-4-(2-methoxyphenyl)-L-phenyl alanine (30).** $MeSO_2Cl$ (24 μL, 306 μmol) was added to a solution of **9** methyl ester (56.0 mg, 128 μmol) in anhydrous CH_2Cl_2 (1 mL) containing DIEA (383 μmol, 66.6 μL). The resulting mixture was stirred at room temperature under N_2 for 3 h and taken up with CH_2Cl_2 (40 mL), washed with 1 N HCl (3 × 20 mL), water (20 mL), dried, filtered and evaporated. The residue was purified on a short silica plug using CH_2Cl_2 as eluent to yield 59.4 mg (78%) of a brownish solid: MS *m/z* 595 ($M^+ + H$). The ester was saponified to give the titled compound (43.4 mg, 86%) as a white solid: MS *m/z* 503 ($M^+ + H$). ^1H NMR (CD_3OD) 2.99 (s, 3H), 3.08 (dd, 1H), 3.38 (dd, 1H), 3.76 (s, 3H), 4.90 (dd, 1H), 6.98 (dt, 1H), 7.03 (dd, 1H), 7.17 (dd, 1H), 7.22–7.42 (m, 8H).

***N*-[2-Chloro-4-(trifluoromethanesulfonylamino)benzoyl]-4-(2-methoxyphenyl)-L-phenylalanine (33).** $(CF_3SO_2)_2O$ (2.91 mmol, 0.49 mL) was added dropwise to a solution of the compound **9** methyl ester (850 mg, 1.94 mmol) in anhydrous CH_2Cl_2 (20 mL) containing anhydrous pyridine (5.81 mmol, 0.47 mL) at 0 °C. The resulting mixture was stirred at room temperature under N_2 for 1 h. The reaction mixture was taken up with CH_2Cl_2 (80 mL) and the solution was washed with 1 N HCl (3 × 50 mL) followed by water (50 mL), dried, filtered and evaporated. The residue was purified by medium pressure chromatography (CH_2Cl_2/MeOH, 50/1) to afford a mixture of mono- and di-substituted sulfonamide (750 mg) as a brownish solid: TLC (CH_2Cl_2/MeOH, 20/1) R_f 0.05 for mono, 0.64 for di and 0.31 for starting material. 1 N LiOH (8 mL, 8 mmol) was added to a solution of the above mixture (750 mg) in THF/MeOH (47 mL/8 mL) and the resulting mixture stirred at room temperature

under N_2 for 2 h. The solution was concentrated and the residue was taken up with water (75 mL). The aqueous solution was acidified with 1 N HCl and extracted with EtOAc (3 × 40 mL). The extract was dried, filtered and evaporated. The residue was purified by HPLC on a C_{18} column with a flow rate of 90 mL/min with a gradient of CH_3CN in 0.1% aq TFA (35–60%) to yield the desired compound (388 mg, 36% for two steps) as a beige solid: MS *m*/*z* 557 (M^+ + H), 579 (M^+ + Na), 555 (M^-); mp 134.3 °C. ^{1}H NMR δ 2.96 (dd, 1H), 3.19 (dd, 1H), 3.74 (s, 3H), 4.62 (m, 1H), 7.00 (dt, 1H), 7.09 (dd, 1H), 7.22–7.41 (m, 10H), 8.85 (d, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-(2-carboxyphenyl)-L-phenylalanine (61).** The compound **55** methyl ester (104 mg, 228 μmol, prepared by following the Scheme 1) was dissolved in acetone (700 L) by warming to ~40 °C. A warm (40 °C) solution of $KMnO_4$ in a mixture of acetone/and water (900 μL/130 μL) was then added over a 1 h period and the resulting mixture was stirred at that temperature for an additional 2 h. The MnO_2 was filtered over Celite and the cake washed with acetone (5 mL). The filtrate was taken up with water (30 mL) and acidified to pH ~2 with 1N HCl. The solution was extracted with EtOAc (3 × 20 mL), the combined extract dried, filtered and evaporated. The residue was purified on a short silica plug using toluene followed by a gradient of toluene/EtOAc (20:1 to 3:1) to yield the desired acid ester (85.0 mg, 79%): MS *m*/*z* 472 (M^+ + H). The ester was saponified as usual to provide the desired dicarboxylic acid (34.1 mg, 96%) as a white solid: MS *m*/*z* 458 (M^+ + H). ^{1}H NMR (CD_3OD) 3.09 (dd, 1H), 3.33 (dd, 1H), 4.99 (dd, 1H), 7.24–7.44 (m, 9H), 7.53 (dt, 1H), 7.77 (dd, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-[2-(carbamoyl)phenyl]-L-phenylalanine (62).** Carbonyl diimidazole (223 μmol, 36.1 mg) was added to a solution of **61** methyl ester (111 μmol, 52.6 mg) in anhydrous THF (1 mL) and the mixture stirred at room temperature under N_2 for 2 h. NH_4OH (1.11 mmol, 135 μL of 29% in water) was added and the mixture stirred for an additional 22 h. The mixture was then taken up with EtOAc (40 mL) and washed with 1 N HCl (3 × 20 mL), saturated $NaHCO_3$ (3 × 20 mL) and brine (20 mL), dried, filtered and evaporated. The residue was purified on a short silica plug using toluene/EtOAc (1/1) to yield the desired amide ester (48.1 mg, 92%) as a white solid: MS *m*/*z* 471 (M^+ + H). The ester was saponified to provide the titled compound (41.6 mg, 89%) as a white solid: MS *m*/*z* 457 (M^+ + H). ^{1}H NMR (CD_3OD) 3.08 (dd, 1H), 3.32 (dd, 1H), 4.98 (dd, 1H), 7.33–7.54 (m, 11H).

***N*-(2,6-Dichlorobenzoyl)-4-[2-(N-benzylcarbamoyl)phenyl]-L-phenylalanine (66).** To a solution of **61** methyl ester (51.9 mg, 110 μmol) in anhydrous DMF (1 mL) was added successively EDC (132 μmol, 25.3 mg), HOBt (132 μmol, 20.2 mg), DIEA (165 μmol, 28.7 μL) and benzylamine (132 μmol, 14.4 μL). The resulting mixture was stirred at room temperature under N_2 for 20 h. EtOAc (40 mL) was added and the solution was washed with 1 N HCl (3 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), water (20 mL) and brine (20 mL). The organic layer was dried, filtered and evaporated. The residue was purified through a short silica plug using hexane/EtOAc (1/1) as eluent to yield the desired amide ester (59.3 mg, 96%) as a white solid: MS *m*/*z* 561 (M^+ + H). The ester was saponified to yield the titled compound (34.2 mg, 89%) as a white solid: MS *m*/*z* 547 (M^+ + H). ^{1}H NMR (CD_3OD) δ 3.07 (dd, 1H), 3.32 (dd, 1H), 4.32 (m, 2H), 4.98 (dd, 1H), 7.04–7.50 (m, 16H).

***N*-(2,6-Dichlorobenzoyl)-4-[2-(sulfamoyl)phenyl]-L-phenylalanine (68).** Anisole (20 μM) was added to a solution of *N* - (2,6 - dichlorobenzoyl) - 4 - [2 - (*N* - *tert* - butylsulfamoyl)phenyl]-L-phenylalanine methyl ester (**67**, 130 mg, 0.2 mmol) in TFA (2 mL) and the resulting solution stirred at room temperature for 6 h. TFA was removed under reduced pressure to yield 100 mg (84%) of the desired compound methyl ester: MS *m*/*z* 507 (M^+ + H). ^{1}H NMR δ 3.2 (dd, 1H), 3.3 (dd, 1H), 3.8 (s, 3H), 4.2 (s, 2H), 5.2 (m, 1H), 6.5 (d, 1H), 7.2–7.6 (m, 10H), 8.1 (d, 1H). The methyl ester (100 mg, 0.2 mmol) was saponified to provide 80 mg (83%) of the titled compound as a white solid: MS *m*/*z* 493 (M^+ + H) and 491 (M^-). ^{1}H NMR 3.0 (dd, 1H), 3.2 (dd, 1H), 4.7 (m, 1H), 7.1 (s, 2H), 7.2–7.6 (m, 10H), 8.0 (d, 1H), 9.1 (d, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-[2-(*N*-benzoylsulfamoyl)phenyl]-L-phenylalanine (69).** Benzoyl chloride (50 μL, 0.4 mmol) was added to a solution of **68** methyl ester (100 mg, 0.2 mmol) in anhydrous pyridine (5 mL) and the reaction stirred at room temperature under N_2 for 12 h. EtOAc and saturated $NaHCO_3$ were added to the reaction mixture and the layers were separated. The EtOAc phase was washed with 1 N HCl, dried, filtered and evaporated to give a gum which was purified by flash column chromatography (hexane/EtOAc, 2/1) to yield an oil. The ester was hydrolyzed to provide 80 mg (68%) of the desired acid as a white solid: MS *m*/*z* 595 (MH^-). ^{1}H NMR δ 3.0 (dd, 1H), 3.2 (dd, 1H), 4.7 (m, 1H), 7.2–7.7 (m, 15H), 8.2 (d, 1H), 9.2 (d, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-[2-(*N*,*N*-dimethylamino)phenyl]-L-phenylalanine (77).** Formalin (96 μL) and 1 N HCl (234 μL) was added to a solution of **72** methyl ester[23] (100 mg, 0.2 mmol) in EtOH (5 mL). $NaCNBH_3$ (36 mg, 0.6 mmol) was added and the resulting mixture was stirred for 0.5 h. A mixture of EtOH and 1 N HCl (1 mL each) were added dropwise and the reaction was stirred overnight. Additional HCl was added and the reaction stirred for 0.5 h. The mixture was neutralized with $NaHCO_3$ and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried, filtered and evaporated to give the desired ester as an oil. The ester was saponified to give 70 mg (68%) of the titled compound as a white solid: MS *m*/*z* 457 (M^+ + H) and 455 (M). ^{1}H NMR δ 2.5 (s, 6H), 3.0 (dd, 1H), 3.2 (dd, 1H), 3.5 (s, 3H), 4.7 (m, 1H), 7.0–7.5 (m, 11H), 9.1 (d, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-(2,6-dimethoxy-4-hydroxyphenyl)-L-phenylalanine (83).** 3,5-Dimethoxyphenol (1 g, 6.5 mmol) was dissolved in DMF (7 mL). To this solution was added chloro-*tert*-butyldiphenylsilane (2 mL) and imidazole (0.7 g) and the mixture was stirred overnight. Water was added and the mixture was extracted

with CH_2Cl_2. The organic phase was dried, filtered and concentrated to a solid. This was converted to the corresponding boronic acid for use in the coupling procedure (similar to **81**) to yield **83** methyl ester. Saponification gave the titled compound as a white solid: MS *m*/*z* 581 ($M^+ + H$). ^1H NMR δ 2.9 (dd, 1H), 3.2 (dd, 1H), 3.64 (s, 6H), 4.7 (m, 1H), 5.25 (s, 2H), 6.4 (s, 2H), 7.0–7.5 (m, 9H), 8.6 (d, 2H), 9.1 (d, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-[2,6-dimethoxy-3-(methoxymethoxy)phenyl]-L-phenyl alanine (84).** A solution of 2,4-dimethoxyphenol[26] (3.3 g, 21.4 mmol) in acetone (20 mL) was added to a suspension of anhydrous K_2CO_3 (3.55 g, 25.7 mmol) in acetone (10 mL) under N_2. Chloromethyl methyl ether (1.79 mL, 23.5 mmol) was then added dropwise and the mixture was stirred at room temperature for 18 h followed by heating at 50 °C for 24 h. Additional chloromethyl methyl ether (1.79 mL, 23.5 mmol) was added and the mixture was stirred for another day at 50 °C. The volatiles were evaporated and the residue taken up with water (75 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were dried, filtered and evaporated. The residue was purified by flash chromatography using hexane/EtOAc (20/1 to 10/1) to give the MOM-protected phenol (1.18 g, 28%) as a colorless oil. The oil was converted to the desired boronic acid and coupled in the usual manner to give **84** methyl ester. Saponification of the ester gave **84**: MS *m*/*z* 534 ($M^+ + H$); mp 156–157 °C.

***N*-(2,6-Dichlorobenzoyl)-4-(2,6-dimethoxy-3-hydroxyphenyl)-L-phenylalanine (85).** A solution of HCl in dioxane (4 M, 1 mL) was added to a solution of **84** methyl ester (165 mg, 0.3 mmol) in MeOH (5 mL) and the mixture was stirred at room temperature for 3 h. The solution was evaporated and the residue taken up with water (40 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried, filtered and evaporated. The residue was purified by preparative TLC (hexane/EtOAc, 3/1 to 1/1) to give the desired phenol methyl ester (145 mg, 96%) as a white solid. The ester was hydrolyzed as before to give 120 mg of **85**: MS *m*/*z* 490 ($M^+ + H$); mp 164–165 °C.

***N*-(2,6-Dichlorobenzoyl)-4-(3-bromo-2,6-dimethoxyphenyl)-L-phenylalanine (87).** Tetrabutylammonium tribromide (1.21 g, 2.51 mmol) was added to a solution of **81** methylester (1.01 g, 2.07 mmol) in CH_2Cl_2 (40 mL) under N_2 and the reaction stirred at room temperature overnight. Additional tetrabutylammonium tribromide (0.55 g, 1.14 mmol) was added and the mixture was stirred for 1 day. The reaction mixture was washed with water (25 mL) and the organic layer was dried, filtered and evaporated. The crude product was purified by flash chromatography using hexanes/EtOAc to give a light-yellow oil (1.17 g). The ester was hydrolyzed as usual to yield 1 g of the titled compound: MS *m*/*z* 555 ($M^+ + H$); mp 205–206 °C.

***N*-(2,6-Dichlorobenzoyl)-4-(3-fluoro-2,6-dimethoxyphenyl)-L-phenylalanine (88) and *N*-(2,6-dichlorobenzoyl)-4-(3,5-difluoro-2,6-dimethoxyphenyl)-L-phenylalanine (89).** To a solution of **81** (0.232 g, 0.475 mmol) in CH_3CN (10 mL) was added 2,6-dichloro-1-fluoropyridinium tetrafluoroborate (0.353 mg, 0.95 mmol) and the mixture was refluxed under N_2 for 24 h. Additional quantities of the above reagent (0.175 mg, 0.47 mmol) were added and the mixture was refluxed for 24 h. The mixture was concentrated, diluted with water and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, water, dried and evaporated. The residue was purified by chromatotron (hexane/EtOAc, 5/1 to 2/1) to give 0.109 g of the monofluoro- and 0.011 g of the difluoro- compounds, respectively. Upon saponification, 0.091 g of the acid **88** [MS *m*/*z* 492 ($M^+ + H$); mp 228–229 °C] and 0.008 g of the acid **89** [MS *m*/*z* 510 ($M^+ + H$); mp 201–202 °C] were obtained.

***N*-(2,6-Dichlorobenzoyl)-4-(3-carbamoyl-2,6-dimethoxyphenyl)-L-phenylalanine (90).** Chlorosulfonyl isocyanate (45 μL, 0.517 mmol) was added to a solution of **81** methyl ester (150 mg, 0.307 mmol) in MeCN (6 mL) under N_2. The mixture was stirred at room temperature for 2.5 h. The mixture was concentrated, 1 N HCl (8 mL) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was extracted with EtOAc (3 × 10 mL), and the extract was dried, filtered and evaporated. The residue was purified by preparative TLC using EtOAc to give the titled product methyl ester (156 mg, 96%). The ester was hydrolyzed as usual to yield 0.14 g of **90**: MS *m*/*z* 517($M^+ + H$); mp 227–228 °C.

***N*-(2,6-Dichlorobenzoyl)-4-(3-amino-2,6-dimethoxyphenyl)-L-phenylalanine (91).** HNO_3 (70%, 4 mL) was added to a solution of **81** methyl ester (1.59 g, 3.25 mmol) in THF (4 mL) under N_2 and the resulting mixture was stirred at 50 °C overnight. The reaction mixture was taken up in EtOAc (150 mL) and the solution washed with water (100 mL). The organic phase was dried, filtered and evaporated. The residue was dissolved in anhydrous MeOH (100 mL) and dry HCl (g) was bubbled through the mixture at 0 °C for a few min and the solution was stirred at room temperature overnight. The mixture was concentrated, the residue was taken up with EtOAc (150 mL) and the solution washed with 1 N HCl (100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried, filtered and evaporated. The crude product was then purified by flash chromatography (hexane/EtOAc) to give 1.1 g (58%) of the desired nitrated methyl ester. A solution of $Na_2S_2O_4$ (2.6 g, 14.9 mmol) in water (5 mL) was added dropwise to a solution of the above nitro-compound in EtOH (40 mL) and the reaction mixture was refluxed for 2 h and concentrated. The residue was taken up in EtOAc (100 mL) and the solution washed with brine (2 × 50 mL). The organic layer was dried, filtered and evaporated. The product was purified by preparative TLC (hexane/EtOAc) to give 0.31 g (30%) of the desired aniline. The ester was saponified as usual to give 0.24 g of the titled acid: MS *m*/*z* 489 ($M^+ + H$); mp 209–210 °C.

***N*-(2,6-Dichlorobenzoyl)-4-(2,6-dihydroxyphenyl)-L-phenylalanine (99).** To a solution of *N*-(2,6-dichlorobenzoyl)-4-[2,6-di(methoxymethoxy)phenyl]-L-phenylalanine ethyl ester (0.0766 mg, 0.3 mmol) in EtOH (5 mL)

was added a solution of HCl in dioxane (4 M, 1.2 mL) and the mixture was stirred at room temperature for 4 h. The solvents were evaporated and the residue taken up with ether and concentrated to yield a foam (61.6 mg): MS *m/z* 474 ($M^+ + H$), 472 ($M^- H$). Saponification gave 58.3 mg of **99**: MS *m/z* 446 ($M^+ + H$); mp 238 °C. ^1H NMR (400 MHZ) 2.93 (dd, 1H) , 3.12 (dd, 1H), 4.68 (m, 1H), 6.35 (d, 2H), 6.86 (t, 1H), 7.18 (d, 2H), 7.23 (d, 2H), 7.38–7.43 (m, 3H), 9.01 (s, 2H), 9.11(d, 1H), 12.72 (br s, 1H).

***N*-(2,6-Dichlorobenzoyl)-4-[2,6-di(2-hydroxyethoxy)phenyl]-L-phenylalanine (100).** A mixture of **99** ethyl ester (0.39 g, 0.82 mmol), 2-bromoethyl acetate (2.72 mL, 2.47 mmol) and anhydrous K_2CO_3 (1.6 g, 4.93 mmol) in DMF (3 mL) was maintained @ 90 °C under stirring for 4 h. The reaction mixture was cooled to room temperature and diluted with EtOAc and water (20 mL, each). The EtOAc layer was separated and the aqueous layer was extracted with additional EtOAc (3 × 20 mL). The combined extracts were dried, filtered and evaporated. The residue was purified by flash chromatography using hexane/EtOAc (1/1) to give 0.423 g (79.6%) of the desired ethyl ester: MS *m/z* 647 ($M^+ + H$). Saponification gave 0.298 g (85.8%) of **100**: MS *m/z* 534 ($M^+ + H$); mp 124–125 °C.

***N*-(2,6,-Dichlorobenzoyl)-3-methoxy-4-(2-methoxyphenyl)-L-phenylalanine (116).** This was prepared in a manner similar to **16** starting from 3-methoxy-L-tyrosine ethyl ester: MS *m/z* 474 ($M^+ + H$); mp 100–102 °C.

3-Acetyl-*N*-(2,6-dichlorobenzoyl)-4-(2-methoxyphenyl)-L-phenylalanine (117). This was prepared in a manner similar to **16** starting from 3-acetyl-L-tyrosine ethyl-ester:[27] MS *m/z* 486 ($M^+ + H$); mp 87–89 °C.

***N*-(2,6-Dichlorobenzoyl)-3-(1-hydroxyethyl)-4-(2-methoxyphenyl)-L-phenylalanine (118).** $NaBH_4$ (12 mg, 0.31 mmol) was added to a solution of **117** ethyl ester (0.1 g, 0.21 mmol) in MeOH (3 mL) and the mixture was stirred at room temperature for 2 h. The reaction was quenched with 1 N HCl and extracted with CH_2Cl_2. The extract was washed successively with 1 N HCl and brine, dried and concentrated. Chromatography of the residue (hexane/EtOAc, 3/1) gave 45 mg of the desired ester: MS *m/z* 516 ($M^+ + H$). Saponification gave 28 mg of the titled acid as a white solid: MS *m/z* 488 ($M^+ + H$); mp 103–105 °C.

***N*-(2,6,-Dichlorobenzoyl)-3-ethyl-4-(2-methoxyphenyl)-L-phenylalanine (122).** To a solution of **118** ethyl ester (0.23g, 0.446 mmol) in CH_3CN (10 mL) at 0 °C was added Et_3SiH (0.156 mg, 1.34 mmol) followed by $BF_3{\cdot}Et_2O$ (0.064 mg, 0.445 mmol) and the mixture was warmed to room temperature. The reaction was stirred for 1 h and quenched with MeOH/water. The mixture was diluted with CH_2Cl_2 and the layers separated. The aqueous solution was extracted with additional CH_2Cl_2 and the organic layers were combined. The combined solution was dried, evaporated and the residue purified by chromatotron (hexanes/EtOAc, 2/1) to give 0.117 mg of the desired ester. Saponification of the ester gave 0.1 g of **122**: MS *m/z* 472 ($M^+ + H$); mp 105–107 °C.

3-*N*-Acetylamino-*N*-(2,6-dichlorobenzoyl)-4-(2,6-dimethoxyphenyl)-L-phenylalanine (123). Raney-Ni (100 mg) was added to a degassed solution of *N*-(2,6,-dichlorobenzoyl)-3-nitro-4-(2,6-dimethoxyphenyl)-L-phenylalanine methyl ester (1.07 g, 2.01 mmol) in MeOH (15 mL) and H_2 gas was bubbled through the mixture for 15 min. Stirring under H_2 was continued for 6 h. The mixture was filtered through Celite and the filter pack was washed with MeOH. MeOH was removed and the residue was purified via chromatography (hexane to hexane/EtOAc, 1/1 gradient elution) to provide the desired aniline (845 mg, 84%) as an off white solid: MS *m/z* 503 ($M^+ + H$). Ac_2O (45 μL, 0.472 mmol) was added to a solution of the above aniline (119 mg, 0.236 mmol) in CH_2Cl_2 (1 mL) and pyridine (57 μL, 0.708 mmol) and the reaction was stirred at room temperature for 18 h. The solvent was removed under reduced pressure. Chromatography of the residue (hexane to EtOAc gradient elution) provided **123** methyl ester (127 mg, 99%) as a white solid: MS *m/z* 545 ($M^+ + H$). The methyl ester was hydrolyzed to give the titled acid (98 mg, 80%) as a white solid: MS *m/z* 531 ($M^+ + H$); mp 142–144 °C.

***N*-(4-Carboxy-2,6-dichlorobenzoyl)-4-(2,6-dimethoxyphenyl)-L-phenylalanine (134).** $Pd(PPh_3)_4$ (0.204 mg, 2.64 mmol) was added, under Ar, to a mixture of *N*-(4-bromo-2,6-dichlorobenzoyl)-4-(2,6-dimethoxyphenyl)-L-phenylalanine methyl ester (**130** methyl ester, 0.5 g, 0.88 mmol), KI (0.366 g, 2.2 mmol), KCN (0.172 mg, 2.64 mmol) in HMPA (5 mL) and the mixture was heated @ 100 °C overnight.[24] The mixture was allowed to cool and diluted with EtOAc (75 mL). The solution was washed with saturated LiCl (15 mL) followed by brine (50 mL), dried and evaporated. The residue was purified via chromatotron using hexane/EtOAc (10:1 to 2:1) to yield 0.240 mg of *N*-(4-cyano-2,6-dichlorobenzoyl)-4-(2,6-dimethoxyphenyl)-L-phenylalanine methyl ester as a yellow solid: MS *m/z* 513 ($M^+ + H$). LiOH (2 mL) was added to a mixture of the above cyano compound (0.237 mg, 0.46 mmol) in THF (2 mL) and the mixture stirred overnight. The mixture was concentrated, taken up with water and acidified to pH 2. The precipitated solid was filtered and purified via HPLC to give 0.162 mg of the titled compound as a white solid: MS *m/z* 518 ($M^+ + H$); mp 294 °C. The corresponding carboxamido compound, *N*-(4-Aminocarbonyl-2,6-dichlorobenzoyl)-4-(2,6-dimethoxy phenyl)-L-phenylalanine (**133,** 31 mg) was obtained as a side product: MS *m/z* 517 ($M^+ + H$); mp 266.7 °C.

3-Methoxy-L-tyrosine. 3,4-Dihydroxy-L-phenylalanine methyl ester was prepared by bubbling HCl (g) into a solution of the corresponding acid (10 g) in methanol (100 mL). *tert*-Boc-anhydride (12.1 g, 55.84 mmol) was added to a solution of the ester in THF (250 mL) and DIEA (35.4 mL, 203 mmol). The mixture was warmed for 5 min and stirred for 1 h at room temperature. THF was removed and the residue was partitioned between water and EtOAc. The organic layer was separated, washed with 1 N HCl, brine and dried ($MgSO_4$) and stripped. The residue was purified by chromatography (Biotage, hexane/EtOAc, 1/1) to yield the desired *tert*-boc-methylester derivative (13.4 g): MS *m/z* 312 ($M^+ + H$) and 334 ($M^+ + Na$). 2,6-Dichlorobenzyl chloride

(1.73 g, 8.83 mmol), was added with stirring to a suspension of the above phenylalanine derivative (2.5 g, 8.03 mmol), K_2CO_3 (2.22 g, 16.06 mmol) and *n*-Bu_4NI (0.297 g, 0.803 mmol) in DMF (15 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. The mixture was diluted with water (~20 mL) and the solution was extracted with ether (30 mL). The organic layer was separated and the aqueous phase was extracted with additional ether (2 × 30 mL). The combined ether extract was dried, filtered and the solution was stripped to a light yellow oil. The oil was purified by chromatography (Biotage, hexane/CH_2Cl_2/EtOAc. 5/5/1) to yield three products: *tert*-Boc-3,4-di-*O*-(2,6-dichloro)benzyl [2.0 g; MS *m*/*z* 630 (M^+ + H)], *tert*-Boc-4-hydroxy, 3-*O*-(2,6-dichloro)benzyl [0.39 g; MS *m*/*z* 470 (M^+ + H)] and *tert*-Boc-3-hydroxy-4-*O*-(2,6-dichloro)benzyl [0.45. g; MS *m*/*z* 470 (M^+ + 1)] phenylalanine derivatives, respectively. CH_3I (0.072 mL, 1.15 mmol) was added with stirring to a suspension of above *tert*-boc-3-hydroxy-4-*O*-(2,6-dichlorobenzyl)phenylalanine methyl ester (0.45 g, 0.96 mmol), K_2CO_3 (0.199 g, 1.44 mmol) and *n*-Bu_4NI (0.035 g, 0.096 mmol) in DMF (4.0 mL) and the reaction mixture was stirred overnight at room temperature. DMF was removed and the residue was partitioned between water (3.0 mL) and EtOAc (20 mL). The organic layer was separated and the aqueous phase was extracted with additional EtOAc (2×, 20 mL). The combined EtOAc extract was dried, filtered and concentrated to a colorless oil. The oil was purified via chromatography (Chromatotron, 2 mm rotor, hexane/CH_2Cl_2/EtOAc, 3/3/1) to yield 0.396 g of the desired product as a yellow gum: MS *m*/*z* 484 (M^+ + H). Hydrogen was bubbled with stirring to a suspension of the above methyl ester (0.39 g, 0.81 mmol) containing 10% Pd/C (wet, degussa,0.05 g) in MeOH (10 mL) for 24 h. The catalyst was filtered over Celite and the filtrate was stripped to a solid which was purified via chromatography (Chromatotron, 1 mm rotor, CH_2Cl_2/CH_3OH, 10/1) to yield 0.21 g of the titled compound as a white solid: MS *m*/*z* 348 (M^+ + Na). This material was used to prepare **116** in a manner similar to **16**.

Synthesis of boronic acids: prototypic procedures

2,6 - Dimethoxybenzeneboronic acid. 1,3 - Dimethoxybenzene (4 g, 0.029 mol) was dissolved in freshly distilled THF (10 mL). This solution was cooled to −78 °C and *n*-BuLi (24 mL, 1.6 M solution in hexanes) was added dropwise to the cold solution. The reaction mixture was stirred at −78 °C for 1 h, then warmed to room temperature and stirred for an additional 1 h. The resulting mixture was cooled again to −78 °C and $(MeO)_3B$ (6.7 mL, 0.06 mol) was added. The resulting mixture was allowed to warm to room temperature and stirred overnight. Water (10 mL) was added and the solution was stirred for 0.5 h, then acidified to pH 4 with acetic acid and extracted with EtOAc (50 mL × 3). The combined ether layers were dried, filtered and evaporated to give 2,6-dimethoxybenzeneboronic acid as a gum that was used without further purification.

6-Methoxy-1,4-benzodioxan-5-boronic acid. 1,4-Benzodioxan-6-carboxaldehyde (5.20 g, 31.65 mmol), was dissolved in MeOH (60 mL) containing concd H_2SO_4 (0.6 mL). An aqueous solution of 30% H_2O_2 (4.7 mL, ~41.1 mmol) was added to the reaction mixture over 5 min at 0 °C.[28] The mixture was warmed to room temperature and stirred for an additional 18 h. The solvent was removed under high vacuum and the residue taken up in H_2O (30 mL) and extracted with CH_2Cl_2. The combined organic layers were dried, filtered and the solvent evaporated. Chromatography of the residue (hexane to hexane/EtOAc 3/1 gradient elution) provided 6-hydroxy-1,4-benzodioxan (3.85 g, 80%) as a colorless oil: MS *m*/*z* 153 (M^+ + H). CH_3I (2.3 mL, 37.75 mmol) was added to a mixture of 6-hydroxy-1,4-benzodioxan (3.83 g, 25.17 mmol), K_2CO_3 (7.0 g, 50.34 mmol) and *n*-Bu_4NI (186 mg, 0.50 mmol) in DMF (10 mL) and the mixture was stirred under N_2 at room temperature for 24 h. The resulting slurry was filtered and the filter pack was rinsed with EtOAc (3 × 15 mL). The EtOAc solution was washed with brine (15 mL), dried and concentrated. Chromatography of the residue (hexane to hexane/EtOAc 4/1 gradient elution) provided 6-methoxy-1,4-benzodioxan (3.25 g, 78%) as a colorless oil: MS *m*/*z* 167 (M^+ + H).

The corresponding boronic acid was prepared by the procedure described above.

5-Methoxy-1,3-benzopyran-4-boronic acid. This was prepared from sesamol in a similar manner.

2,3-Dihydrobenzo[*b*]furan-7-boronic acid and 3,4-dihydro-2*H*-benzopyran-8-boronic acids. These were prepared from the corresponding bromides[28,29] using standard procedures.

Synthesis of benzoic acid intermediates

Methyl 4-amino-2,6-dichlorobenzoate. Thionyl chloride (40 mL) was added to a solution of 2,6-dichloro-4-nitrobenzoic acid[30] (12.8 g, 54 mmol) in anhydrous CH_2Cl_2 (60 mL) and the resulting mixture was refluxed for 19 h. The reaction mixture was allowed to cool to room temperature and evaporated. Additional CH_2Cl_2 (10 mL) was added and the solution was evaporated again. MeOH (100 mL) was added slowly to the residue and the mixture was refluxed for 17 h. The mixture was allowed to cool to room temperature and was then placed in an ice-bath. The precipitated product was collected by filtration to give a white solid (10.8 g, 80%). Methyl 2,6-dichloro-4-nitrobenzoate (10.8 g, 43.4 mmol), thus obtained, was slurried in EtOH (250 mL) and a solution of $Na_2S_2O_4$ (45 g, 220 mmol) in water (100 mL) was added dropwise. The mixture was refluxed for 2 h and stirred at room temperature overnight. It was filtered and concentrated to a white solid which was dissolved in 1 N HCl (250 mL) and stirred for 2 h. The mixture was neutralized with 10% NaOH and extracted with EtOAc (3 × 75 mL). The combined organic layers were dried, filtered, evaporated and the resulting residue was re-crystallized using EtOAc and hexane to give methyl 4-amino-2,6-dichlorobenzoate as a yellowish solid (7.48 g, 78%).

2,6-Dichloro-4-fluorobenzoic acid. Methyl 4-amino-2, 6-dichlorobenzoate (0.5 g, 2.27 mmol) was suspended in

15% HCl (10 mL). The suspension was stirred for 30 min and cooled to 0–5 °C. Diazotization was done by portionwise addition of $NaNO_2$ (188 mg, 2.73 mmol). After each addition, the flask was stoppered and stirred for ~5 min. The mixture was then stirred for 30 min at that temperature and pre-cooled HBF_4 (0.46 mL, 2.5 mmol) was added rapidly. Stirring was continued for 30 min. The tetrafluoroborate salt was filtered and washed successively with cold water (10 mL), MeOH (10 mL) and ether (10 mL). The solid was then dried over concd H_2SO_4 in a vacuum desiccator for a few days. The dry tetrafluoroborate salt was heated with a Bunsen burner until it started to decompose. It was then heated as necessary until the entire solid was melted. The fluoroboric acid fumes were collected over water via a distilling apparatus. The product was recovered with Et_2O from the reaction flask, as well as the distilling apparatus and the receiving flask. The ether was evaporated and the crude product was purified by preparative TLC. The compound was loaded with CH_2Cl_2 and eluted using hexane/EtOAc (50/1 to 20/1) to give methyl 2,6-dichloro-4-fluorobenzoate (241 mg, 48%). TMSI (0.2 mL, 1.4 mmol) was added to a solution of the above ester (157 mg, 0.7 mmol) in CCl_4 (5 mL). The mixture was then stirred under N_2 at 50 °C for 2 days. Water (5 mL) was added and the mixture was stirred for 1 h. HCl (1 N, 25 mL) was then added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried, filtered and evaporated. The residue was purified on a short silica plug using a $CHCl_3$/MeOH gradient to give the desired 2,6-dichloro-4-fluorobenzoic acid (85 mg, 58%).

2,6-Dichloro-4-hydroxybenzoic acid. Methyl 4-amino-2,6-dichlorobenzoate (0.5 g, 2.27 mmol) was diazotized with $NaNO_2$ (188 mg, 2.73 mmol) as described above. The reaction mixture was stirred for 30 min at 0–5 °C and added dropwise to boiling water (50 mL). The resulting solution was refluxed for 2 h, allowed to cool to room temperature and extracted with EtOAc (3 × 50 mL). The extract was dried, filtered and evaporated. The crude product was then purified twice by preparative TLC using CH_2Cl_2 as the solvent to give the desired ester as a dark orange oil which solidified on standing (275 mg, 55%). NaOH (1 N, 3.6 mL) was added to a solution of the above ester (265 mg, 1.2 mmol) in THF/MeOH (25 mL, 6/1) and the mixture was refluxed for 1 day. Additional NaOH (1 N, 3.6 mL) was added and the mixture was refluxed for an additional 24 h. The mixture was evaporated and the residue was taken up with water (50 mL). The aqueous solution was acidified with 1 N HCl and extracted with EtOAc/MeOH (95/5, 3 × 50 mL). The extract was dried, filtered and evaporated to give the desired acid as a brownish solid (248 mg). This material was used for the coupling reaction.

4-Bromo-2,6-dichlorobenzoyl chloride. Methyl 4-amino-2,6-dichlorobenzoate (1.00 g, 4.54 mmol) was suspended in 40% aq HBr and the mixture was cooled to 0–5 °C. $NaNO_2$ (376 mg, 5.45 mmol) was added in small portions. After each addition, the flask was stoppered and stirred for ~5 min. Copper powder (100 mg, 1.6 mmol) was added and the mixture was slowly warmed to 100 °C. The flask was temporarily taken off of the oil bath when nitrogen evolution was too strong. The mixture was stirred at 100 °C for an additional 30 min., diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were dried, filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc, 50/1) to give the desired bromo ester as a pale yellow oil (1.07 g, 83%). LiOH (1 M, 7.47 mL) was added to a solution of the above ester (1.06 g, 3.73 mmol) in THF/MeOH (50 mL, 6/1), and the reaction mixture was refluxed for 1 day. The mixture was evaporated, taken up with water (50 mL) and acidified with 1 N HCl. The aqueous solution was extracted with EtOAc (3 × 50 mL), dried, filtered and evaporated to give 4-bromo-2,6-dichlorobenzoic acid (0.94 g, 99%) as a pale-yellow solid. The above acid (0.93 g, 3.45 mmol) was dissolved in CH_2Cl_2 (20 mL), thionyl chloride (2.51 mL, 34.5 mmol) was added and the mixture was refluxed for 5 h. The mixture was then evaporated, co-evaporated with CH_2Cl_2 (2 × 10 mL), dried under vacuum and used for the coupling reaction.

2-Chloro-4-*N*-methylaminobenzoic acid. A solution of *tert*-Boc-anhydride (1.39 g, 6.41 mmol) in dioxane (15 mL) was added with stirring to a solution of 4-amino-2-chloro-benzoic acid (1.0 g, 5.83 mmol) in NaOH (1 N, 12.82 mL, 12.8 mmol) at 0 °C. The reaction mixture was warmed up to room temperature and stirred overnight. Dioxane was distilled, water was added to the residue and the solution was extracted with ether. The aqueous solution was acidified and the precipitate was filtered, washed with 1 N HCl, water and dried under vacuum to yield 2.7 g of the desired *N*-Boc acid: MS *m*/*z* 294 (M^+ + Na). NaOMe (0.22 g, 4.1 mmol) was added, under N_2, to a solution of the above acid (0.52 g, 1.85 mmol) in DMF (7 mL). The mixture was cooled in ice and MeI (0.5 L, 7.5 mmol) was added. After stirring for 6 h, DMF was removed and the residue was partitioned between EtOAc and water. The EtOAc layer was separated and the aqueous layer was extracted with additional EtOAC. The combined EtOAc extract was washed with water, dried, filtered, and evaporated. Flash chromatography of the residue (hexane/EtOAc, 1/1) provided 0.27 g of 2-chloro-4-*N*-(boc)methylaminobenzoic acid methyl ester: MS *m*/*z* 322 (M^+ + Na). TFA (3 mL) was added to a solution of the above Boc-ester (0.21 g) in CH_2Cl_2 (10 mL) and the solution was stirred for 2 h. Workup similar to that described for **16** gave 0.13 g of 2-chloro-4-*N*-methylaminobenzoic acid methyl ester: MS *m*/*z* 200 (M^+ + H). The methyl ester was hydrolyzed to give 81 mg of the titled acid that was used as is for the coupling reaction: MS *m*/*z* 185 (M^+ + H).

2-Chloro-4-(*N*-methylsulfonyl)methylaminobenzoic acid. $MeSO_2Cl$ (0.4 mL, 5 mmol) was added under N_2 to a solution of 2-chloro-4-*N*-methylamino benzoic acid methyl ester (0.25 g, 1.25 mmol) in CH_2Cl_2 (20 mL) containing pyridine (0.4 mL, 5 mmol). The mixture was refluxed for 4 h. Additional CH_2Cl_2 (40 mL) was added and the solution was washed successively with 1 N HCl (3 × 20 mL) and water. It was dried, filtered and evaporated. The residue was purified via chromatography (hexane/EtOAc, 3/1 to 1/1) to give 0.26 g of the desired

methyl ester: MS m/z 278 ($M^+ + H$), 300 ($M^+ + Na$). The methyl ester was hydrolyzed to give 85 mg of the titled acid that was used as is for the coupling reaction : MS m/z 264 ($M^+ + H$).

Adhesion assay

Tissue culture reagents were purchased from Irvine Scientific (Irvine, CA, USA). The B cell lymphoblastoma cell line RPMI8866 was a gift from Dr. John Wilkens (University of Manitoba, Winnipeg, Canada). The Jurkat T lymphoblastoid cell line was purchased from ATCC (Rockville, MD, USA). Both were grown as a suspension culture in RPMI 1640 media, 10% FCS, 2 mM glutamine, 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate at 37°C and 5% CO_2. Heat shock fraction V of bovine serum albumin (BSA) was purchased from Boehringer Mannehim (Indianapolis, IN, USA). Human serum albumin (HSA) was purchased from Intergen (Purchase, NY, USA). Chicken ovalbumin (OV) and 3-(2-pyridyldithio)propionic acid *N*-hydroxysuccinimide ester (SPDP) were purchased from Sigma.

Adhesion assays have been detailed elsewhere.[31] Microtiter plates were coated with 20 μg/mL HSA for 2 h at room temperature, washed once with PBS and derivatized with 10 μg/mL SPDP for 1 h. After washing, CS-1 (or sCS-1) derived peptide solution (100 mL at 100 μg/mL) was added to the wells and allowed to crosslink to the plates overnight at 4 °C. Non-reacted sites were blocked with 100 μL of 1% OV in PBS for 1 h at 37 °C. RPMI8866 cells were suspended in Dulbecco's modified Eagle's medium with 0.25% OV at a density of 2.5×10^6/mL and incubated for ~1 h at 37 °C with varying concentrations of antagonists on peptide-coated plates. Following washing [EL404 plate washer (Bio-Tek Instruments, VT, USA)], bound cells were quantified by measuring endogenous *N*-acetyl-hexosaminidase activity by reading the optical density at 405 nm using the enzyme substrate *p*-nitrophenol-*N*-acetyl-β-D-glucoseaminide.[32] IC_{50} values were generated by nonlinear regression from titration curves of antagonists from seven doses and reported as the average of a minimum of two experiments. Since experimental variability was noted with respect to the IC_{50} of the internal standard [(1*S*-*cis*)-*N*-[(3-carboxy-2,2,3-trimethylcyclopentyl)-carbonyl]-*O*-[(2,6-dichlorophenyl)methyl]-L-tyrosine] a normalization procedure was done using the global mean value [$IC_{50} = 0.224 \pm 0.17$ μM ($N = 19$)] of the internal standard. For the Jurkat cell adhesion assay, OV was replaced with 0.25% HSA for both blocking and adhesion buffers. Standard error of the mean for the Jurkat cell adhesion assay was typically <10% for each experiment and no normalization was needed.

Acknowledgements

The authors thank Dr. Kenneth Locke for critical review of the manuscript. We also express our gratitude to Tanabe Seiyaku Co. Ltd., Japan for their support in this project.

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