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Solid-Phase Synthesis of Dual $\alpha_4\beta_1/\alpha_4\beta_7$ Integrin Antagonists: Two Scaffolds with Overlapping Pharmacophores

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Abstract—Two structural classes of dual $\alpha_4\beta_1/\alpha_4\beta_7$ integrin antagonists were investigated via solid-phase parallel synthesis. Using an acylated amino acid backbone, lead compounds containing biphenylalanine or tyrosine carbamate scaffolds were optimized for inhibition of $\alpha_4\beta_1$/VCAM and $\alpha_4\beta_7$/MAdCAM. A comparison of the structure–activity relationships in the inhibition of the $\alpha_4\beta_7$/MAdCAM interaction for substituted amines employed in both scaffolds suggests a similar binding mode for the compounds.

Integrins are heterodimeric proteins that when displayed on leukocytes, mediate their recruitment to sites of inflammation in a tissue specific manner.[1] The binding of integrins to surface expressed endothelial proteins initiates cell-cell contacts which eventually lead to the extravasation of the leukocyte into the tissue. It has been proposed that improper control of this process can result in a large variety of disease states that are particular to the expression of the molecules involved. Interaction of the integrin $\alpha_4\beta_7$ with MAdCAM[2] (mucosal addressin cell adhesion molecule) has been implicated in uclerative colitis and inflammatory bowel disease.[2a,3] The interaction between $\alpha_4\beta_1$ and VCAM[4] (vascular cell adhesion molecule) is thought to contribute to asthma, multiple sclerosis, and other autoimmune diseases.[5] Inhibition of these protein–protein interactions significantly effects animal models of disease.[2b,6] This biological connection to disease has increased the interest in the development of small molecule antagonists for these integrins.[7]

Previous efforts identified cyclic pentapeptide **1** as a potent inhibitor of both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ (IC_{50} 10 and 2 nM,[7b] respectively). The SAR (structure–activity relationship) of cyclic peptides like **1** demonstrated the importance of the carboxylate, the tyrosine at the N-terminus, and a hydrophobic amino acid in the fourth position. During the course of our research to identify potent inhibitors of $\alpha_4\beta_7$ antagonists, parallel synthesis was used to further investigate two leads, biphenylalanine **2** (IC_{50} $\alpha_4\beta_7 = 25$ nM) that was designed to satisfy the tyrosine binding site in **1**, and carbamate **3** (IC_{50} $\alpha_4\beta_7 = 72$ nM). Compounds **2** and **3** represent ideal scaffolds for solid-phase synthesis and good candidates for a library-based approach, given the limited availability of structural information about the target. Synthetic routes were designed such that they would explore the contributions of the benzylamino substituents in the biphenylalanine series and the nitrogen substituents found in the tyrosine carbamates. Compounds were then tested for inhibition of the $\alpha_4\beta_7$/MAdCAM interaction in a protein based ELISA. As a measure of selectivity for $\alpha_4\beta_7$, inhibition of the related $\alpha_4\beta_1$/VCAM interaction was also tested.

Biphenyl compound **2** and its analogues were prepared via the DCC coupling of Fmoc-4-iodophenylalanine **4** onto Wang resin (Scheme 1).[8] Following Fmoc deprotection, 2-chlorobenzoic acid was coupled using standard amide bond forming conditions to yield substituted iodophenylalanine **5**. Suzuki couplings[9] were performed using formylarylboronic acids, $PdCl_2(dppf)_2$

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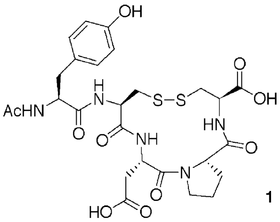

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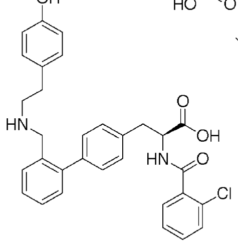

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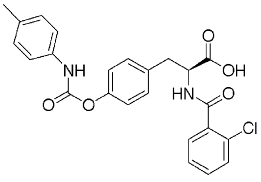


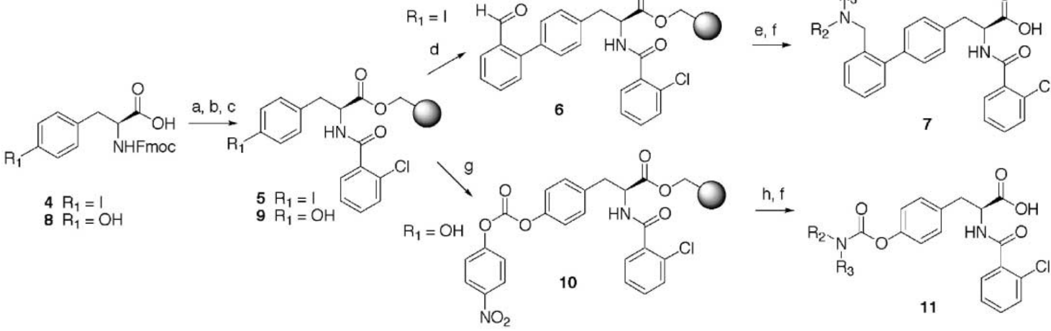
3

and $N(iPr)_2Et$ at 80 °C resulting in resin bound biphenyl **6**. Initial attempts using carbonate as the base in THF/H_2O resulted in low yields, attributed to hydrolysis of the compounds from the resin. The aldehyde was elaborated with mono- and disubstituted amines via reductive amination, followed by a TFA cleavage, yielding the desired aminomethylbiphenylalanines **7**. The compounds were purified by reversed-phase chromatography to a > 95% purity and their molecular composition was confirmed via electrospray mass spectroscopy prior to assay.

Analogues of carbamate **3** were synthesized by coupling Fmoc-tyrosine **8** to Wang resin (Scheme 1).[10] Reaction time and stoichiometry was controlled to limit any polymerization involving the unprotected phenol. The coupled amino acid was deprotected and acylated using 2-chlorobenzoic acid to yield **9**. The phenol was then activated with *p*-nitrophenylchloroformate to give the reactive carbonate **10**. The reaction vessel was drained and the resin was treated immediately with various amines. Resin cleavage gave carbamate **11**, which was purified and analyzed as previously mentioned.

To explore the contribution of the phenol in compound **2**, a library of over 100 compounds was prepared altering the position and the nature of the R group on the ring and compared in protein based ELISAs for $\alpha_4\beta_7$ and $\alpha_4\beta_1$.[11] Representatives from this library highlighting key elements of the SAR are presented in Table 1. Movement of the 4-hydroxylphenethylamino group from the *ortho* position in **2** to the *para* position **12b** resulted in a significant decrease in potency in both assays. Consequently, further analogue synthesis focused on the *ortho* position. Contrary to the SAR in the YCDPC peptide **1**,[7b] where the removal of the phenol oxygen results in a 500-fold loss in potency, **12c** only showed a 2 fold reduction in potency. This, and the comparison of **2** with **12d**, demonstrates that the activity is not likely due to the phenol. *ortho*-Methoxy compound **12e** (also reported in ref 7g) demonstrates the importance of the benzylamine functionality for potency in the ELISA. This observation along with the SAR suggesting trisubstituted amines to be more potent than their disubstituted counterparts, prompted the synthesis of the trisubstituted benzylamino compounds **12f** and **g**. In contrast to the selectivity for $\alpha_4\beta_1$ observed in *ortho*-methoxy compound **12e**, compounds **12f** and **g** showed a slight preference towards inhibiting $\alpha_4\beta_7$ and were an improvement from phenol **2** in overall size and complexity.

After the discovery of carbamate leads **3** and **13a** (Table 2), a library of 80 compounds was prepared to probe the nature of the amine portion of the carbamate. Neither the removal of the methyl group from the aryl ring in **3** (**13b**) nor changes in the substitution pattern and functionality about the ring improved activity (data not shown). Improvement over the allyl amine **13a** was also difficult to achieve with a variety of substitutions (**13c**–**13e**). However, the methylpiperizyl carbamate, **13f**, demonstrated that cyclic trisubstituted amines are preferred in this position in both assays. The *N*,*N*-dimethyl carbamate **13g** also showed acceptable activity in both assays. The parallel approach used in the preparation of the compounds along with an understanding of the SAR helped to rapidly improve the potency of the lead molecules in both scaffolds. The compounds **12** and **13**,



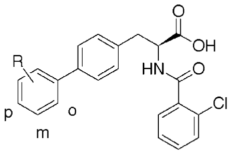
Scheme 1. (a) Wang resin, DIC, DMAP, DCM; (b) piperidine DMF; (c) 2-chlorobenzoic acid, HOBT, HBTU, $N(iPr)_2Et$, DMF; (d) $CHOPhB(OH)_2$, $PhCl_2(dppf)_2$, $N(iPr)_2Et$, NMP, 80 °C; (e) R_2R_3NH, $Na(OAc)_3BH$, 1% AcOH, DMF; (f) TFA/DCM/H_2O/Et_3SiH, 90/5/2.5/2.5; (g) NMM, pNO_2-PhOCOCl, DCM; (h) R_1R_2NH, DCM; biphenylalanines were 50–90% pure prior to HPLC, 10–30% yield after HPLC. Tyrosine carbamates were 70–95% pure prior to HPLC, 10–30% yield after HPLC.

f and **g** have reasonable activities in both protein assays and warrant further biological study.

The convergence to disubstituted and cyclic amines in both scaffolds pointed to the intriguing possibility that these two classes of compounds may present the nitrogen substituents to the same location on the protein. This potential overlap was further investigated through a panel of compounds, prepared using the same amine inputs in both series. The similarity of the SAR for the $\alpha_4\beta_7$/MAdCAM interaction resulting from this set of compounds was remarkable (Table 3). A general trend shows a size dependence, with larger substituents leading to poor activities. In several cases, the IC_{50} values are nearly identical for both structures (amines **1**, **5**, **7**, and **10–12**) and, interesting trends exist in cases where they differ slightly. When the distal piperizine nitrogen contains a large group connected with a methylene (amines **2** and **6**), the biphenylalanine tends to be the preferred scaffold. However, for amines **8** through **10,** the tyrosine carbamate series is two times more potent, and the distal piperizine nitrogen is either acylated or a urethane. These same trends were not as apparent in the $\alpha_4\beta_1$/VCAM assay where the carbamate scaffold generally resulted in a much lower IC_{50} values for the amines (data not shown, about 10-fold). Overall, the same trends in potency were observed.

Computational chemistry was used to investigate how these two scaffolds may commonly orient key functional groups. The energy of rotation for the dimethylamino carbamate C–O–C=O torsion was determined quantum mechanically (B3LYP/G-311G*). From the energy minimum (0°), the energy increases almost linearly by 12.3 kcal/mol to a maximum at 180°. The predicted high energy cost was surprising and went against original hypothesis that placed the nitrogens of both scaffolds in proximity with a carbamate C–O–C=O torsional angle of around 130°. To explore other possible overlaps, the two dimethylamine molecules (**12g** and **13g**) were both subjected to 10,000 steps of a Monte Carlo Multiple Minimum (MCMM) search using the MMFF force field and the GB/SA water solvent model. The carbons of the carboxylate, amide and 4-position of the benzoyl were constrained by harmonic potentials, as were both nitrogens, both methyls and the chlorine. The energy required to maintain the overlap of the two structures verses the ground state conformation was used to evaluate likely binding conformations. One of three conformations (9.0 kcal/mol above the sum of the energies

Table 1. IC_{50} data for biphenylalanine compounds in $\alpha_4\beta_1$/VCAM and $\alpha_4\beta_7$/MAdCAM ELISA assays



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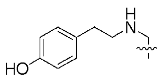
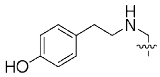
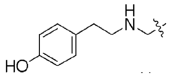
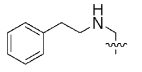
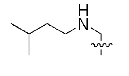
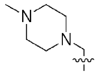
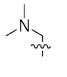
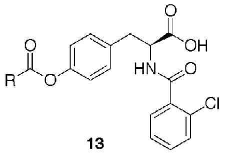
Compd	R	Substitution	$\alpha_4\beta_7$ IC_{50} (nM)	$\alpha_4\beta_1$ IC_{50} (nM)
2		o	25.0	25.3
12a		m	53	48
12b		p	240	237
12c		o	47.7	61.3
12d		o	50.3	82.1
12e	MeO-	o	736	51.1
12f		o	10	25
12g		o	11.3	16.8

Table 2. IC_{50} data for tyrosine carbamate compounds in $\alpha_4\beta_1$/VCAM and $\alpha_4\beta_7$/MAdCAM ELISA assays



13

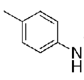
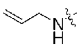
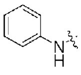
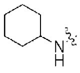
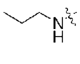
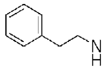
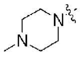
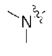
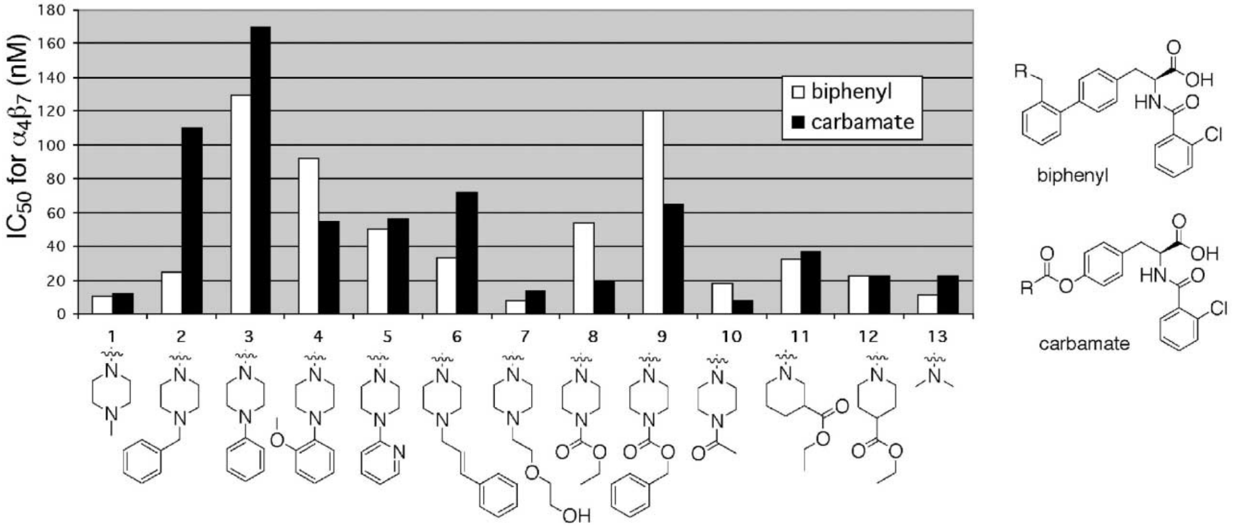
Compd	R	$\alpha_4\beta_7$ IC_{50} (nM)	$\alpha_4\beta_1$ IC_{50} (nM)
3		72.2	454
13a		55.9	34.9
13b		1320	604
13c		734	165
13d		39.6	19.8
13e		170	18.0
13f		12	1.1
13g		22	9.3

Table 3. IC_{50} data for both biphenylalanine compounds (white bars) and tyrosine carbamate compounds (black bars) in $\alpha_4\beta_7$/MAdCAM ELISA assays using identical amines in both scaffolds



of the isolated molecules) that satisfied the constraints, and also maintained the general conformation of the phenylalanine ring, is shown in Figure 1. The necessity of the carbonyl of the carbamate to rotate out of plane with the ring is further supported by the observation that urea analogues, which are indicated by MMFF optimizations to have a carbonyl in plane with the aryl ring, show a 100-fold loss in potency.

This pharmacophore overlap leads to a better understanding of the binding modes of these classes of α4 integrin antagonists. A combinatorial approach helped to accelerate the SAR of these types of inhibitors and facilitated the initial observation of the two-scaffold, one-pharmacophore model. Further synthesis guided by computational input may result in other structural analogues that maintain potency against the integrins.

Figure 1. Overlap of **12g** (teal) and **13g** (fuchsia).

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8. A general procedure for biphenyl synthesis is as follows. Compounds were prepared using either the Quest 210 workstation or a 48 well Robbins Block with the rotating oven. 153 mg of Fmoc-4-iodophenylalanine (0.30 mmol) was preactivated with 47 μL of DIC (0.30 mmol) and 4 mg of DMAP (0.03 mmol) in 2 mL of dichloromethane (DCM) for 10 min prior to addition to 100 mg of Wang resin (1.0 mmol/g) and then agitated for 4 h. The resin was drained and rinsed three times each with MeOH, DCM, DMF, and again with DCM (this wash was repeated after all steps except the final cleavage). The Fmoc group was removed in 10 min using a 25% piperidine soln. in DMF. A 2 mL solution of 2-chlorobenzoic acid, HOBT, HBTU, and N(*i*Pr)$_2$Et each at a 0.25 M concentration in DMF was prepared and agitated with the resin for 16 h. The resin was treated with a 2 mL solution of boronic acid, $PdCl_2(dppf)_2$ and N(*i*Pr)$_2$Et each at a 0.25 M concentration and agitated with the resin for 16 h at 80° C. Compounds containing an aldehyde were treated with a soln. of 0.25 M amine and $Na(OAc)_3BH$ in 3 mL of DMF with 1% AcOH. Products were cleaved from the resin for 1 h in a mixture of 90% TFA, 5% dichloromethane, 2.5% triethyl silane and 2.5% water.

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10. A general synthesis of the carbamates is as follows. Compounds were prepared using either the Quest 210 workstation or a 48-well Robbins Block with the rotating oven. Compound **10** was prepared using the same steps in reference 8 with the substitution of 121 mg of Fmoc-tyrosine (0.15 mmol). The resin was treated with 1.5 mL solution of 0.5 M N(*i*Pr)$_2$Et in DCM and then a 1.5 mL solution of 0.5 M *p*-nitrophenylchloroformate in DCM. After 1 h of agitation the resin was drained and then quickly treated (no wash) with a 0.25 M solution of amine in DCM and agitated for 4 h. Cleavage from the resin was performed as described in ref 8.

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