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Identification of Unique VLA-4 Antagonists from a Combinatorial Library

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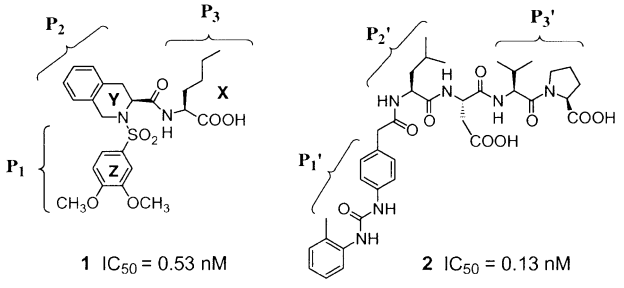
Abstract—A combinatorial library of 28 pools of 180 compounds (345 diastereomers) was designed and prepared in support of the delineation of the SAR of two prototypical VLA-4 antagonists. Deconvolution of the active pools led to the identification of three novel series of VLA-4 antagonists with low nanomolar potencies. © 2002 Published by Elsevier Science Ltd.

VLA-4 (very late antigen-4, CD49d/CD29 or $\alpha_4\beta_1$) is a member of the superfamily of transmembrane glycoprotein integrins made up of α- and β-heterodimers expressed on all leukocytes except platelets.[1] The ligands for VLA-4 include vascular cell adhesion molecule-1 (VCAM-1) and the CS-1 domain of fibronectin. Expression of VCAM-1 on vascular endothelial cells is induced by inflammatory cytokines. Interaction of VCAM-1 with circulating leukocytes results in leukocyte adhesion and migration into extravascular tissues.[2] Intervention into this process has the potential for treating chronic inflammatory disorders associated with cell adhesion and extravasation, including asthma, multiple sclerosis, and inflammatory bowel disease.[3]

Through screening an unrelated capped dipeptide combinatorial library, a selective inhibitor **1** of VLA-4 was identified (**1**).[4] This compound and others developed from it are characterized by the presence of a lipophilic P_3 α-amino acid unit, a P_2 prolyl or related cyclic amine derivative core, and a P_1 sulfonamide cap (Fig. 1). Potent VLA-4 antagonists containing a diaryl urea moiety, such as **2**, may also be divided into three P_1'–P_3' regions: a critical urea containing P_1' unit is connected via an α-amino acid at P_2' to a β-amino acid at P_3'.[5] (The P_1–P_3 and P_1'–P_3' regions of **1** and **2** are not intended to represent the same binding interactions with VLA-4.) The pharmacokinetic properties of these and related compounds are poor, having low oral bioavailability and high plasma clearance rates. We wished to identify novel antagonists that may have advantages over these two series. Reduction of the peptide character of the series through the introduction of non-natural amino acids, modification of the functional groups incorporated within the structure and/or alteration of the physical properties of the analogues may improve their pharmacological and pharmacokinetic characteristics.

Both **1** and **2** are constructed of sulfonylated and acylated units and are amenible to rapid-solution or solid-phase synthesis provided the individual synthons are readily available.[6] The potential ease of synthesis of analogues related to **1** and **2** and the presence of a carboxylic acid for use as a linking group suggested that solid-phase combinatorial synthesis of a library of related structures might identify novel VLA-4 antagonists.



1 IC_{50} = 0.53 nM **2** IC_{50} = 0.13 nM

Figure 1. Potent inhibitors of VLA-4.

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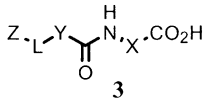
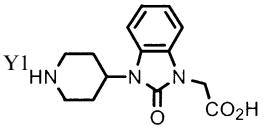
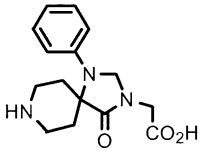
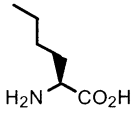
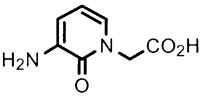
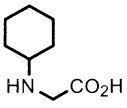
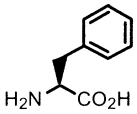
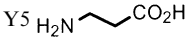
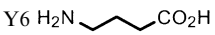
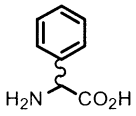
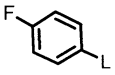
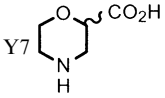
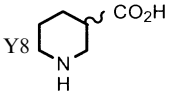
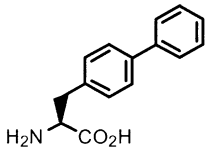
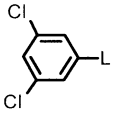
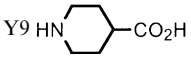
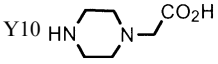
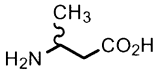
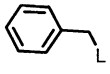
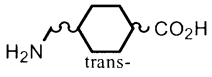
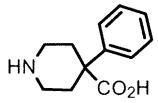
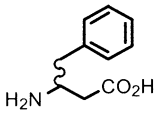
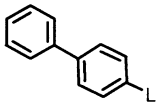
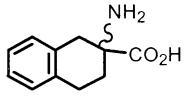
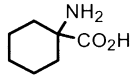
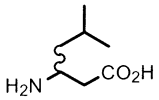
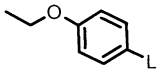
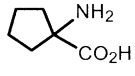
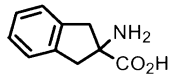
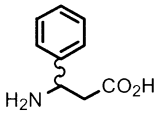
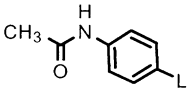
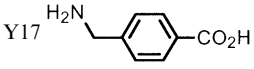
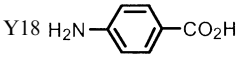
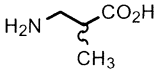
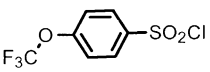
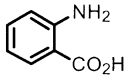
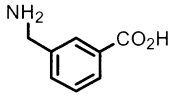
As discussed above, **1** and **2** may be divided into three regions. Ten alkyl and aryl P_1 (Z) pharmacophores incorporating hydrogen bond accepting and donating substituents were chosen (see **3**, Table 1). The linking groups (L) had to be readily synthesized sulfonyl, urea and amide groups. Twenty-three P_2 amino acids (including racemates) (Y) were identified that could position P_3 and P_1 in varying conformations or positions. Investigation of α- and β-amino acids as P_3 units was of considerable interest as **1** and **2** had demonstrated that

Table 1. Positional subunits of a VLA-4 combinatorial library **3**



3

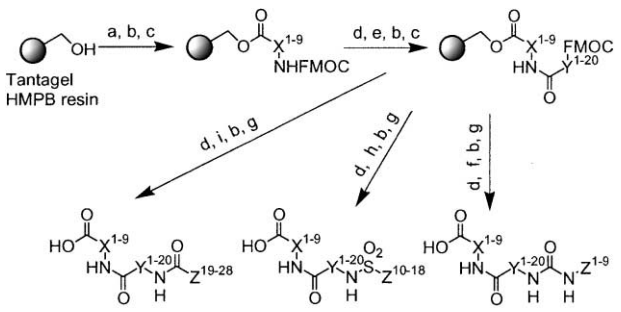
Z + L subunits (28)	Y subunits (23)		X subunits (15)
Ureas, sulfonamide amide L = –NCO, $–SO_2Cl$, $–CO_2H$			
Z1, 10, 19	Y1 	Y2 	X1 
Z2, 11, 20 	Y3 	Y4 	X2 
Z3, 12, 21 	Y5 	Y6 	X3 
Z4, 13, 22 	Y7 	Y8 	X4 
Z5, 14, 23 	Y9 	Y10 	X5 
Z6, 15, 24 	Y11 	Y12 	X6 
Z7, —, 25 	Y13 	Y14 	X7 
Z8, 16, 26 	Y15 	Y16 	X8 
Z9, 17, 27 	Y17 	Y18 	X9 
Z18 	Y19 	Y20 	
Z28			

both were acceptable pharmacophores. We had previously determined that the (*S*)-configuration was preferred at P_3 in the case of α-amino acids.[4] The ease of potential racemization of phenyl glycine and our lack of knowledge of the preferred absolute configuration in the β-amino acids prompted us to use racemic amino acids in these cases.

The synthetic method used to prepare the library is outlined in Scheme 1.[7] Nine *N*-FMOC protected amino acids (X) were coupled to Tantagel-HMPB resin. Aliquots of the individual resins were archived (X^{1-9}) and equal amounts (loading was approximately the same) were mixed and split into 21 polyethylene syringe tubes fitted with frits. Treatment with piperidine was followed by washing and coupling with 20 *N*-FMOC protected Y groups. After coupling was complete (determined by Kaiser free amine test), the resins were washed and an aliquot of each was archived ($X^{1-9}Y^{1-20}$). The balance of the resins were mixed and split into 30 tubes, treated with piperidine and washed as before and then reacted with isocyanates, sulfonyl chlorides and activated carboxylic acids. The resins were washed thoroughly and then treated with 10% TFA in methylene chloride, filtered and the filtrate was concentrated in vacuo to provide the individual mixtures of products. The products were weighed and dissolved in DMSO to provide 10 mM solutions based on the average molecular weights of the products. The solutions were assayed in a competitive radioligand binding assay of ^{125}I-VCAM-Ig to Jurkat cells expressing VLA-4.[4]

The binding of mixtures distinguished by the 28 Z components to Jurkat cells is shown in Figure 2. The data illustrates the sulfonamide linker is generally more potent than either the urea or amide linkers, excepting the known urea pharmacophore Z^{28}. The four most potent mixtures were titrated to give the IC_{50}'s indicated. Mixtures Z^{14} and Z^{28} were deconvoluted to identify potential novel P_2 scaffolds along with their SARs. To this end, the separate archived resins $X^{1-9}Y^{1-20}$ were derivatized individually with Z^{14} and Z^{28}, washed and cleaved to give forty samples of mixtures of nine compounds containing a varied number of potential stereoisomers.[7]

Figure 3 illustrates the VLA-4 binding affinity of each pool of nine compounds (15 stereoisomers) of X^{1-9} derivatized with Z^{14} and Z^{28}. Several Y groups: Y^4, Y^5, Y^6, Y^8, Y^{12}, and Y^{15} acylated with the urea Z^{28} were potent mixtures of ligands. Only three 3,5-dichlorophenylsulfonamides were potent ligands—Y^4, Y^{12}, and Y^{14}. We decided to complete the deconvolution of all the potent ($IC_{50} < 1$ μM) sulfonylated pools $X^{1-9}Y^4Z^{14}$, $X^{1-9}Y^{12}Z^{14}$, and $X^{1-9}Y^{17}Z^{14}$ as their physical and chemical properties might be more likely to provide unique new lead. Two urea pools were also examined to compare with their sulfonylated congener: $X^{1-9}Y^4Z^{28}$ and $X^{1-9}Y^{12}Z^{28}$. The linear spacers Y^5 and Y^6 belong to the same structure class as the spirocycloalkyl scaffolds Y^{14} and Y^{15}. Archived resin of the individual X groups was coupled with the appropriate Y and Z groups to prepare 45 compounds.



Scheme 1. (a) FMOC–NH–X^n–CO_2H, EDC, DMAP cat CH_2Cl_2; (b) wash 2 × reaction solvent, 2 × CH_2Cl_2, MeOH, CH_2Cl_2, ether, CH_2Cl_2, ether; (c) archive, mix and split; (d) 2 × 20% piperidine/DMF, wash 2 × DMF; (e) FMOC–N–Y–CO_2H, HBTU, HOBt, DMF, DIEA; (f) ZNCO, CH_2Cl_2/THF; (g) 10% TFA/CH_2Cl_2; (h) ZSO_2Cl, CH_2Cl_2/THF; (i) Z^nCO_2H, HBTU, HOBt, DIEA, DMF.

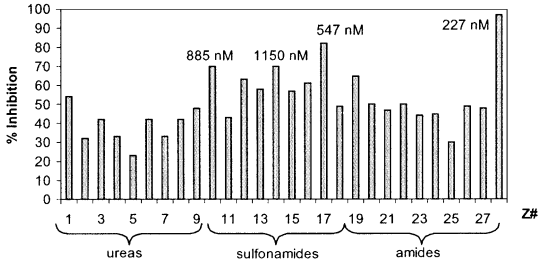


Figure 2. Inhibition of VLA-4 by combinatorial library $X^{1-9}Y^{1-20}Z^{1-28}$.

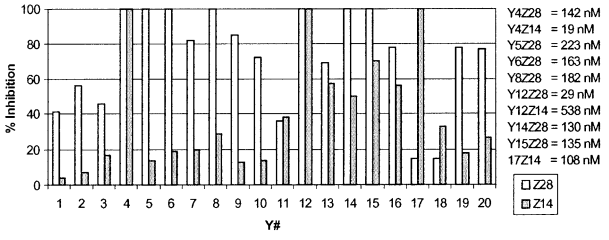


Figure 3. Inhibition of VLA-4 by first deconvolution $X^{1-9}Y^{1-20}Z^{14,28}$.

The potencies of the individual components, the mixture prepared in the Y group deconvolution above and a reconstituted mixture prepared by recombining the individual components (except for $Z^{28}Y^4X^{1-9}$) are illustrated in 3-D format in Figure 4. In all cases the potency of the original mixture and reconstituted mixture was approximately the same suggesting that the sample prepared from a mixture of different resins provided the same mixture of products as formed by combining the individual components. The first three rows illustrate the potencies of the sulphonamide series. Unfortunately, the IC_{50}'s measured for the mixtures $Z^{14}Y^4X^{1-9}$ and $Z^{14}Y^{12}X^{1-9}$ reflected a series of moderate individual potencies rather than a highly potent single analogue. In contrast, deconvolution of $Z^{14}Y^{17}X^{1-9}$ illustrated how a single potent analogue $Z^{14}Y^{17}X^4$ (**4**) could be identified in a mixture of non-potent analogues (note: blank spaces, data were not determined). Both urea based series $Z^{28}Y^{12}X^{1-9}$ and $Z^{28}Y^4X^{1-9}$ had not been previously reported. Comparison of the SAR of the two series illustrates that in the case of $Z^{28}Y^4$, β-amino acids

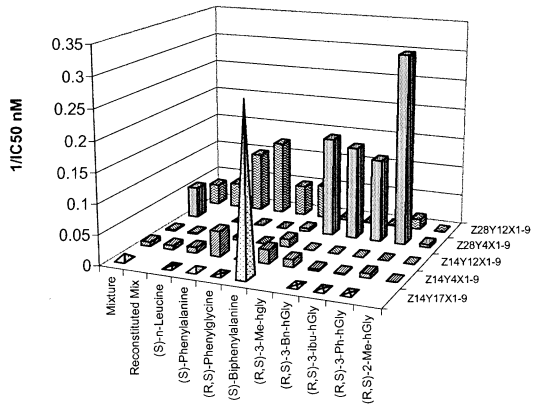
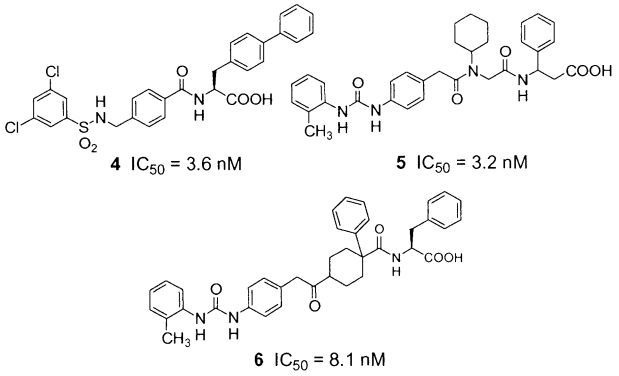


Figure 4. Inhibition of VLA-4 by second deconvolution $X^{1-9}Y^{4,12,17}Z^{14,28}$.

are preferred at P_3. The most potent example in this series being $Z^{28}Y^{4}X^{8}$ (**5**). In contrast, α-amino acids are preferred at P_3 in the series $Z^{28}Y^{12}$; the most potent analogue being $Z^{28}Y^{12}X^{2}$ (**6**). Unfortunately, no compound from this study afforded any pharmacokinetic advantages over **1** or **2**.



In summary, we have prepared a combinatorial library of 28 pools each containing 180 compounds and 345 stereoisomers for a total of 9660 individual compounds. Evaluation of the binding affinity of these pools to the VLA-4 receptor demonstrated the previously described urea Z^{28} to act as a potent VLA-4 pharmacophore. Aryl sulphonamides were also found to be potent P_1 pharmacophores. The experiment demonstrates the applicability of using complex mixtures as a means of identification of new lead structures. However, the relatively poor potency of the members of pools $Z^{14}Y^{4}X^{1-9}$ and $Z^{14}Y^{12}X^{1-9}$ also illustrates the potential pitfall of interesting pool potency relecting the sum of a group of relatively modestly active compounds.[8]

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7. General method: To a suspension of Tantagel-HMPBOH swollen in 50% THF/CH_2Cl_2 was added DMAP followed by a solution of $FMOCX^{1-9}COOH$ and EDC dissolved in 50% THF/CH_2Cl_2). The mixture was agitated overnight, filtered, and resubmitted to coupling. The mixture was filtered and washed thoroughly and the resin was dried in vacuo. The resin was suspended in 70% CH_2Cl_2/DMF, gently stirred for 2 h. The suspension was transferred to polyethylene tubes fitted with scinters. The solvent was drained and washed once with DMF. The resin was treated with 20% piperidine/DMF for 20 min and washed with DMF and drained. A solution of $FMOCY^{1-20}COOH$ was individually dissolved in DMF and was treated with a solution of HBTU, HOBt and DIPEA in DMF. The resulting solutions were added to the resin suspensions and agitated for 4 h. The tubes were drained and washed and evaluated for unreacted amine. Y^{11}, Y^{14} and Y^{21} were resubmitted to the acylation for 4 h and washed as above. The resins were suspended in 70% CH_2Cl_2/DMF and transferred from each tube to a silanized flask (the balance of each was archived), the resin was stirred, diluted and split into filtered tubes. Each tube was treated with piperidine/DMF and washed as above and was then treated with the appropriate reagent for Z^{1-30} derivitization. The urea Z units were prepared by addition of isocyanate to a suspension of resin in 50% CH_2Cl_2/THF (Z9 was added as the *p*-nitrochloroformate). The sulfonamides were prepared by addition of sulfonyl chloride and DIEA in 50% CH_2Cl_2/THF and the acids by HBTU/HOBt/DIEA coupling in DMF. The mixtures were agitated over night, washed and evaluated by Kaiser test. The mixtures were then thoroughly washed, treated with 10% TFA/CH_2Cl_2 and the combined filtrates were concentrated in vacuo to provide the mixture of products. The mixture was dissolved in DMF to give a ~10 mM solution (based on calculated average MW). Deconvolution of the library was achieved by application of the reaction conditions to the archived resins.
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