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Cyclic Thioether Peptide Mimetics as VCAM–VLA-4 Antagonists

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Abstract—Selective substitution of a sulfur atom by carbon in a highly potent 13-membered cyclic disulfide was accomplished by intramolecular displacement of a bromide. The potency of the resulting thioethers in the VCAM/VLA-4 assay was dependant on ring size and the position of the sulfur atom.

Vascular cell adhesion molecule-1 (VCAM-1), is expressed on activated, but not resting, endothelium. The principal receptor for VCAM-1, the integrin very late antigen 4 (VLA-4, $\alpha_4\beta_1$), is expressed on many lymphocytes including circulating eosinophils, basophils, and monocytes, but not neutrophils. Antibodies to either protein are effective at inhibiting leukocyte infiltration and preventing tissue damage in animal models of inflammation, including asthma and arthritis.[1,2] Peptides derived from the connecting segment 1 (CS1) sequence of fibronectin have also been shown to block the VCAM–VLA4 interaction and to block allergen induced airway responses in a sheep model of asthma.[3,4] Thus, we were interested in discovering orally active VCAM–VLA-4 antagonists that might be useful for the treatment of asthma or rheumatoid arthritis.

In the accompanying paper,[5] we described the design of a class of spirocyclic disulfides, typified by **1**, which are potent inhibitors of the VLA-4/VCAM interaction. Although these molecules are relatively small (MW <600), they are still somewhat peptidic in nature and contain a disulfide bond which may result in potential metabolic liabilities. The group at Genentech has demonstrated in a related series that the N-terminal tyrosine may be replaced by a more drug like moiety without any loss in potency.[6] Indeed, replacement of tyrosine in 1 with (±)-*trans*-*N*-acetyl-3-(4-hydroxyphenyl) proline resulted in the potent antagonist **2a** (manuscript in prep.).

We hypothesized that replacement of one of the sulfur atoms in the cyclic disulfide with a carbon atom, together with the N-terminal modification of the cyclic core would reduce the metabolic liabilities that may be associated with a structure such as **1**, while retaining the desired biological activity. We also reasoned that since the geometry of the disulfide may be different from that of a thioether, the overall ring size may also affect the activity. Molecular modeling suggested that carbon replacement would have minimal impact on the N- and C-terminal vectors as well as the position of the cyclopentyl moiety. We were thus encouraged to prepare the thioether analogues **2b**, to determine their effect on the VCAM/VLA-4 interaction.

Results and Discussions

The target compounds were prepared by an intramolecular bromide displacement by a thiolate. Two of the proposed analogues were prepared according to Scheme 1. The starting protected cysteine or homocysteine, was coupled to the azido acid **3** under standard conditions to give the corresponding azides **4a,b**. Reduction with trimethylphosphine afforded the corresponding amines. Protection of the amines and purification were necessary for the success of the next two steps. The Boc groups were removed and the resulting amines were treated with benzyloxy homoserine lactone **5** in pyridine at 80 °C to afford the alcohols **6a,b**. Conversion of the alcohols to bromides, followed by deprotection of the thiols and intramolecular displacement afforded the cyclic thioethers in relatively poor yield (12–15%) for the three steps. The low yield is at least partially due to the relatively poor conversion of the alcohols to the bromides (around 50% yields), but is mainly due to an inefficient intramolecular cyclization. The yield of the bromide displacement could not be improved even

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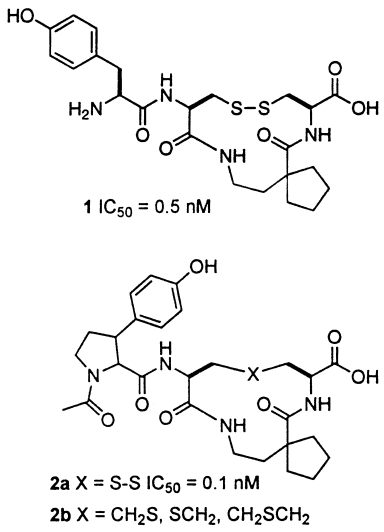
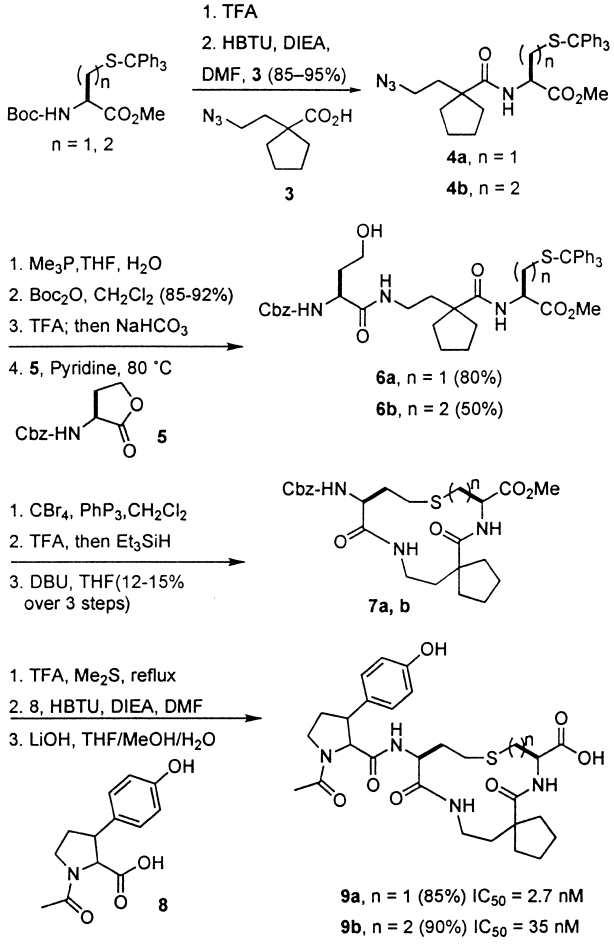


Figure 1.

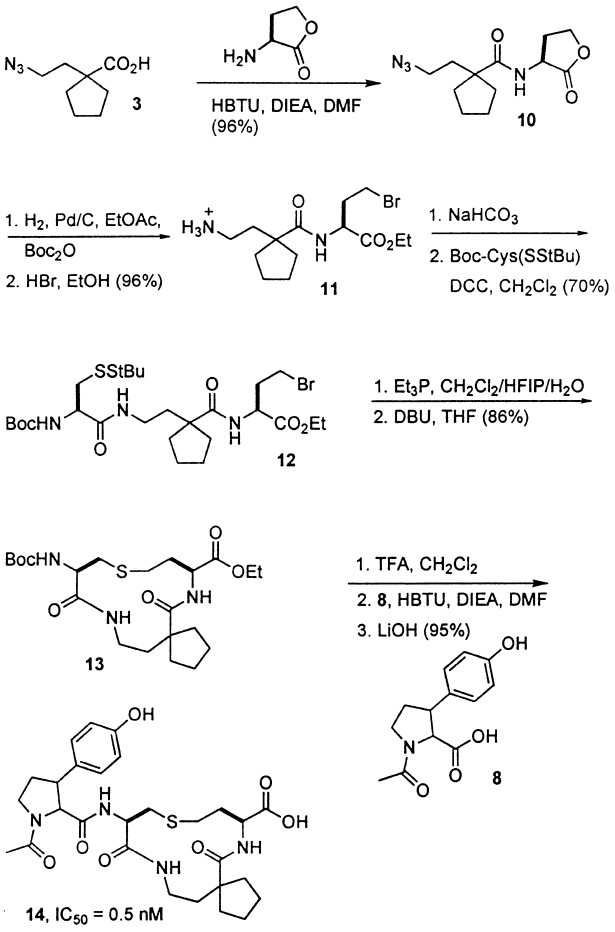
under high dilution conditions. The carbobenzyloxy group was then removed cleanly in refluxing TFA/dimethylsulfide, and the cyclic thioether core was coupled with (±)-*trans*-*N*-acetyl-3-(4-hydroxyphenyl)proline **8**[6] under standard conditions. Final deprotection with LiOH afforded **9a** and **9b.**

The third analogue was prepared using a similar intramolecular cyclization, as indicated in Scheme 2. The azido acid **3** was coupled with homoserine lactone under standard conditions to give the lactone **10**. The azide was reduced with hydrogen over Pd/C and protected in situ with Boc_2O to facilitate isolation. Hydrogen bromide gas was bubbled through an ethanolic solution of the resulting lactone at 0 °C, followed by warming to room temperature, to give the aminobromide **11,** in excellent yield. The amine was coupled with Boc(*t*-butylthio)cysteine using standard DCC coupling to afford **12**. The *t*-butylthio protecting group was removed with triethylphosphine, and the resulting bromothiol was cyclized directly in the presence of DBU to give the core cyclic derivative **13** in excellent yield (86%). Unlike the two examples above, the intramolecular bromide displacement is quite facile and occurs cleanly in 1 h, even at 10 mM concentration. Removal of the Boc group, followed by coupling with (±)-*trans*-*N*-acetyl-3-(4-hydroxyphenyl) proline (**8**), and final deprotection with LiOH afforded the cyclic thioether **14** (95% yield over three steps).

Compounds were assayed for VLA-4 antagonist activity using a solid-phase, dual antibody ELISA in which VLA-4 derived from Ramos cells was allowed to compete for bound recombinant human VCAM in the presence of serial dilutions of test compound. VLA-4 bound to VCAM-1 was detected by a complex of anti-β1 anti-

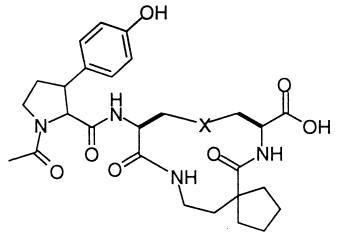


Scheme 1.



Scheme 2.

Table 1. Activity of cyclic thioethers VCAM–VLA-4 assays



		Solid phase assay	Ramos cell assay
No.	X	IC_{50} (nM)	IC_{50} (nM)
2a	S-S	0.1	1.5
9a	CH_2S	2.7	220
9b	CH,SCH_2	35	—
14	SCH_2	0.5	20

body and HRP-conjugated anti-mouse IgG: chromogenic substrate (K-Blue).[7] A secondary, cell based assay was also run in which fluorescently labeled Ramos cells were allowed to compete for immobilized VCAM. As the data summarized in Table 1 indicate, increasing the ring size by one atom had a detrimental effect on potency. Similarly decreasing the ring size by one carbon resulted in a significant loss in potency (unpublished result). In contrast, replacement of a single sulfur atom by carbon, while maintaining the ring size resulted in only a relatively modest decrease in potency. More interestingly, there was a significant difference between the effects of substitution of the N-terminal sulfur atom and the C-terminal sulfur. In fact, the thioether **14** was at least 5-fold more potent than its positional isomer **9a,** while only 4–5-fold less potent than the parent disulfide in the ELISA assay. Although the differences are more significant in the cell-based assay, a similar trend is observed. It is possible that the C-terminal sulfur atom makes a key hydrophobic contact with the receptor, and consequently, its replacement with a carbon atom results in a suboptimal interaction. In conclusion, the substitution of the disulfide with a thioether and replacement of the N-terminal tyrosine was found to be well tolerated, resulting in a very potent relatively non-peptidic small molecule antagonist of VLA-4.

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