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TETRAPEPTIDE DERIVED INHIBITORS OF COMPLEXATION OF A CLASS II MHC: THE PEPTIDE BACKBONE IS NOT INVIOLATE

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Abstract: Major histocompatabilty (MHC) proteins rely heavily on peptide backbone recognition for ligation. Nonetheless, modifications to the polyamide backbone of a tetrapeptide ligand can be made without abrogating binding.

'Collateral damage' and 'friendly fire' incidents are unpalatable hallmarks of armed conflict. The operations of the immune system are no exception. Infectious disease pathologies are often the work of an unfocussed cytokine response, while autoimmune conditions are the result of inappropriate targeting of self-components. It would be a short-tenured general indeed whose response to a 'friendly fire' incident was to withhold weaponry from the entire army, yet this is the level at which current immunosuppressive autoimmune therapies operate. Selective immunosuppression obviously represents a preferable strategy.

The critical role of MHC proteins in the development of an immune response is widely appreciated.[2] Heterodimeric class II MHC forms non-covalent complexes with ≥10-mer peptides derived from extracellular foreign protein and these complexes are ultimately displayed on the surface of professional antigen presenting cells (APC) where they interact with receptors on the surface of cognate T-cells *via* the T-cell receptor (TCR). This MHC/peptide/TCR mediated interaction between APC and T-cell is central to the cytokine controlled proliferation characteristic of an amplified yet directed immune response. Certain evidence suggests that the causative event in autoimmunogenic disease states (and/or an on-going, exacerbating feature) is the aberrant presentation of autoantigenic (self) peptides by a subset of MHC class II alleles.[3] What is clear is that there is an epidemiologic connection between susceptibility to certain autoimmune conditions and one's pattern of MHC class II expression. For example, expression of the class II alleles designated DR4 ([DRA1*0101, DRB1*0401], [DRA1*0101, DRB1*0404]) or DR1 (DRA1*0101, DRB1*0101) is associated with increased risk of rheumatoid arthritis.[4] We have been interested in the possibility of generating a non-peptide capable of inhibiting the MHC class II/autoantigen association in a manner selective for only the implicated alleles. Untargeted class II alleles would remain fully functional. Such a strategy might provide effective protection from autoantigenic events without global immunosuppression.[5]

The smallest natural ligands for MHC class II are 10-mer peptides. However, we have recently reported the development of a tetrapeptide (EtOCO-FRNvaL-NH_2, **1** [mapped herein as EtOCO-$P_1P_2P_3P_4$-NH_2]) that inhibits the binding of full length peptide to purified DRB1*0101 with nanomolar IC_{50}.[6,7] There are conceptual,

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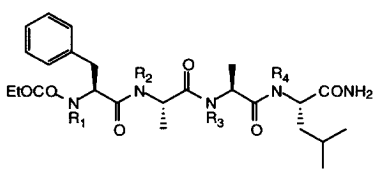
structural and experimental reasons that lead one to suspect that MHC class II is unusually reliant on peptide backbone interactions for high affinity ligation. Each of us must manage all foreign protein using our quota of just six MHC class II alleles. Consequently, each class II heterodimer is required to accept a large array of peptide ligands of limited sequence similarity (in contrast to customary receptor specificity).[8] Class II functions as a rather indiscriminate peptide recognition device. Under such circumstances it would be conceptually logical for recognition to be weighted toward the common denominator, the regular array of peptide bonds. Structurally, X-ray determination of a complex of haemaglutinin $HA_{307-319}$ with DR1 reveals an extensive network of backbone H-bonds along the entire 13-mer, while only one side chain is bound in a classically distinct pocket.[9] Experimentally, simultaneous replacement of 11 out of 13 residues by alanine does not impair binding affinity of a full length peptide (and indeed one of the remaining non-Ala residues is simply a solubility device that has no site preference).[10] For these reasons, the transformation of a peptide lead such as **1** into an appropriately non-peptidic ligand might be expected to be an unusual challenge. No non-peptide ligands for MHC proteins are known.[11,12,13] In this and the following communication we report our attempts to distance **1** from the peptide manifold.[14]

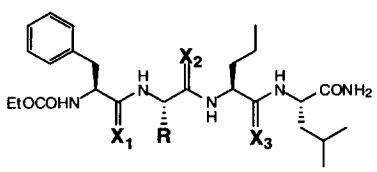
Initially, we evaluated the sensitivity of the tetrapeptide to backbone alteration in a very simplistic way, by preparing a series of N-methylated analogs[6] and reduced peptide bond analogs. N-Methylation was generally detrimental to the IC_{50}s for competitive binding with a biotinylated full length peptide ligand to DR1 (Figure 1).[15] The only leniency lay with N-methylation of the P3 residue (**5**) where binding was unaffected (**5** relative to **2**). Reduction of any one of the peptide bonds caused a dramatic increase (>1000-fold) in the IC_{50}s Figure 2.[16]

Figure 1. N-Methyl Peptides (IC_{50} 20')[15]



(**2**)	R_1-R_4 = H	0.27 μM
(**3**)	R_1 = Me, R_2-R_4 = H	1.9 μM
(**4**)	R_2 = Me, R_1 R_3 R_4 = H	12 μM
(**5**)	R_3 = Me, R_1 R_2 R_4 = H	0.32 μM
(**6**)	R_4 = Me, R_1-R_3 = H	88 μM

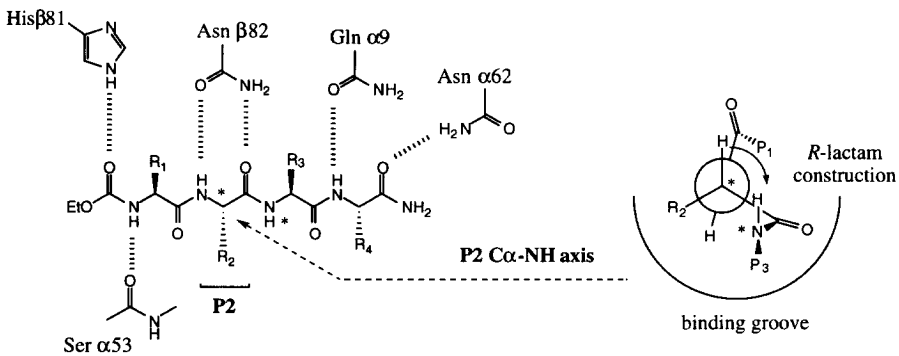
Figure 2. Ψ(CH_2NH) Peptides (IC_{50} 20')[15]



(**1**)	P_2=Arg	X_1 X_2 X_3 = O	0.001 μM
(**7**)	P_2=Arg	X_1 = H/H, X_2 X_3 = O	3.0 μM
(**8**)	P_2=Arg	X_3 = H/H, X_1 X_2 = O	1.2 μM
(**9**)	P_2=Val	X_1-X_3 = O	0.001 μM
(**10**)	P_2=Val	X_2 = H/H, X_1 X_3 = O	45 μM

Peptide SAR for **1** had suggested that it binds to the class II protein in the same manner as full length epitopes and in the $HA_{309-312}$ frame (i.e., FRNvaL corresponding to YVKQ of $HA_{307-319}$ PKYVKQNTLKLAT).[7] The N-methylation data support that view as they can be rationalized with reference to the DR1/$HA_{307-319}$ co-crystal structure. That structure reveals that in the YVKQ region only the K_{311} NH (that would correspond to R_3 in Figure 1) does not make a H-bond contact with DR1 and is directed out of the binding groove.[9] The ψ(CH_2NH) surrogate data is an indication that direct application of such simplistic devices is unlikely to provide viable solutions to the peptide bond problem in this case. The carbonyl oxygens of Y_{309} [*cf.* X_1 in Figure 2] and K_{311} [*cf.*

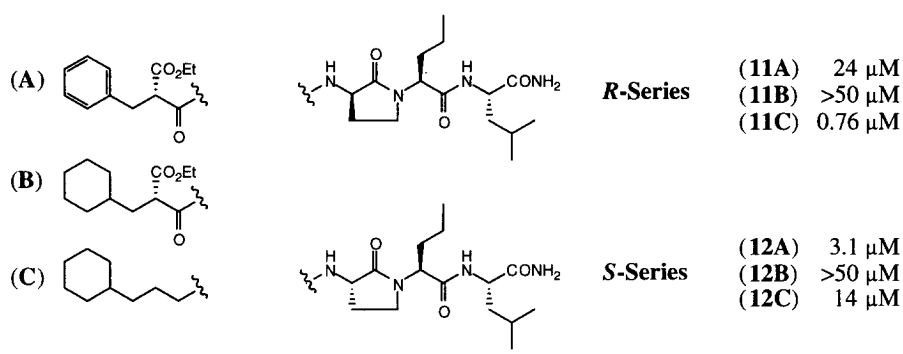
Figure 3. Putative mode of binding of EtOCO-FRNvaL-NH_2 (1) to DR1 Based on the $HA_{307-319}$/DR1 Co-Crystal[9]



X_3 in Figure 2] do not make H-bonding contacts with DR1, yet cannot be replaced by simple methylenes, no doubt a reflection of the divergent conformational preference. Clearly more sophisticated methods of masking the peptidic nature of **1** were required.

Our on-going SAR studies[17] suggested that the putative diad of H-bonds linking Asnβ82 with P2-Arg was a critical contributor to the binding of **1** to DR1. This is easy to rationalize in terms of the model, as it would be the central bidentate backbone mooring for the peptide (Figure 3). Inspection of the co-crystal structure suggested that an *R*-lactam construction around P2-P3 might serve as a viable mask for this key component (i.e., a backbone-to-backbone constraint). The *R*-configuration would conform to the local configuration of $HA_{310-311}$, the spine of the lactam would be directed out of the binding groove rather than abut the protein and the requisite N-alkylation (*cf.* **5** above) should be acceptable (Newman projection in Figure 3).

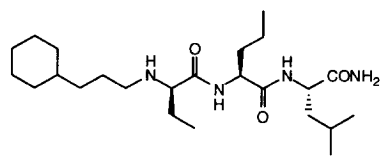
Figure 4. Simple γ-Lactam Constraints (IC_{50} 20')[15]



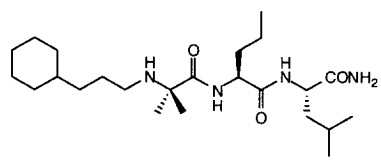
Our first targets were the direct γ-lactam analogs **11A&B**, **12A&B** (Figure 4).[18] These simple 'Freidinger lactams' were prepared through closure of appropriate Met-Nva dipeptide precursors according to precedented

chemistry.[19] Initial results (Figure 4) were disappointing in that all four were markedly worse inhibitors than related acyclic peptides (e.g., IC_{50}/20' for EtOCO-FANvaL-NH_2 was 8 nM and EtOCO-FGNvaL-NH_2 was 66 nM) and, in fact, what rump activity remained was resident in the *S*-lactam (**12A**).[20] The introduction of the lactam could propagate changes in conformational preference beyond the immediate vicinity of the lactam. We reasoned that it might be advantageous to maximize the conformational flexibility at the key P1 residue to facilitate 'self-correction'. In fact, our first attempt to implement this by replacing the EtOCO-cyclohexylalanine residue (**11B** & **12B**) with a simple 3-cyclohexylpropyl residue (prepared by reductive amination of the corresponding 'tripeptidic' lactam amine)[21] was successful in generating a good inhibitor in the *R*-lactam series (**11C**, 760 nM). The corresponding *S*-lactam was only a weak inhibitor (**12C**, 14 μM).

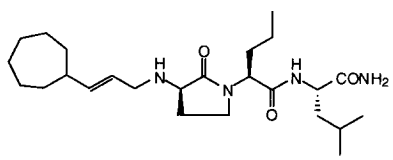
Figure 5. (IC_{50} 20')[15]



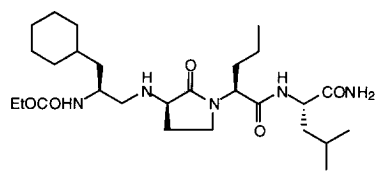
(**13**) >50 μM



(**14**) 0.26 μM



(**15**) 0.34 μM



(**16**) >50 μM

Two acyclic controls for the *R*-lactam suggested that the lactam was playing the role envisaged for it. Tripeptide **13** (the direct acyclic analog of **11C**, IC_{50} >50 μM) implied that an unconstrained P2-*R*-centre is entirely unacceptable. However, tripeptide **14** (IC_{50} 0.26 μM) implied that the *R*-site *could* be occupied if the ligand is driven into the crystallographic conformation, wherein it is projected out of the binding groove (i.e. Newman projection in Figure 3). Presumably the presence of the *S*-site residue provides such driving force in the case of P2 disubstitution. Although we found indications that modest refinement of the P1 surrogate was possible, these involved simple remoulding of the hydrophobicity (e.g., **15**) and attempts to recapture specific interactions proved unsuccessful (e.g., H-bonds to Serα53 and/or Hisβ81 *via* the P1 carbamate, **16**, IC_{50} >50 μM). Since our purpose was to prune the peptide scaffold as severely as possible this was not discouraging.

Clearly then viable inhibitors can be generated that encompass significant alterations in peptide backbone structure. This particular design was made with due consideration to the peptide contact points as defined by the peptide/class II co-crystal structure. If the design hypothesis is correct, we have also made use of increased hydrophobic contact in the P1 binding pocket, the only such pocket available. We were encouraged to try and

refine these inhibitors but were not disavowed of the belief that one cannot be too structurally cavalier. The following report details our ensuing efforts.

References and Notes

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11. To date, reports of unnatural ligands for MHC class II have described small (<13-mer) peptide ligands (e.g., Hammer, J.; Belunis, C.; Bolin, D.; Papadopoulos, J.; Walsky, R.; Higelin, J.; Danho, W.; Sinigaglia, F., Nagy, Z. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4456 and our own work[7]), substantially peptidic ligands (e.g., Smith, A. B.; Benowitz, A. B.; Guzman, M. C.; Sprengler, P. A.; Hirschmann, R.; Schweiger, E. J.; Bolin, D. R.; Nagy, Z.; Campbell, R. M.; Cox, D. C.; Olson, G. L. *J. Am. Chem. Soc.* **1998**, *120*, 12704) or weak retro-inverso peptides (e.g., Howard, S. C.; Zacheis, M. L.; Bono, C. P.; Welply, J. K.; Hanson, G. J.; Vuletich, J. L.; Bedell, L. J.; Summers, N. L.; Schwartz, B. D.; Woulfe, S. L. *Protein Pept. Lett.* **1997**, *4*, 63).
12. There are a number of reports of the binding of modified peptides to MHC class I. The least peptidic of these retain four unaltered peptide residues and appear to be rather weak ligands (Bianco, A.; Brock, C.; Zabel, C.; Walk, T.; Walden, P.; Jung, G. *J. Biol. Chem.*, **1998**, *273*, 28759).
13. Distantly related though non-MHC encoded proteins are capable of presenting non-peptides (e.g., hCD1b & lipid antigen; Sieling, P.A.; Chaterjee, D.; Porcelli, S. A.; Prigozy, T. I.; Mazzaccaro, R. J.; Soriano, T.; Bloom, B. R.; Brenner, M. B.; Kronenberg, M.; Brennan, P. J.; Modlin, R. L. *Science* **1995**, *269*, 227). The X-ray structure of mCD1d1 highlights the global structural similarity of CD1 to MHC proteins, but also clearly differentiates it in terms of ligand binding characteristics (Zeng, Z.-H.; Castano, A. R.; Segelke, B. W.; Stura, E. A.; Peterson, P. A.; Wilson, I. A. *Science* **1997**, *277*, 339).
14. Early accounts of some of our findings in this area were presented at the 25th National Medicinal Chemistry Symposium, Ann Arbor, MI, June 1996 and at the 14th International Medicinal Chemistry Symposium, Maastricht, Netherlands, September 1996.
15. A fluorescence readout assay was employed that measures the ability of test compounds to inhibit binding of a biotinylated rat myelin basic protein 13-mer peptide ($RMBP_{90\text{-}102}$) to purified DRB1*0101. A description of the protocol is given in reference 10. IC_{50}s were time dependent. We usually recorded the IC_{50}s at 20' and 5 h (typically IC_{50} @ 5 h = 5-10xIC_{50} @ 20'). Herein we report only the 20' IC_{50} for simplicity.
16. The P2 ψ (CH_2NH) surrogate was compared in the FVNvaL series rather than the FRNvaL series of lead **1** because of chemical diffculties associated with attempting to use arginal derivatives.

17. Certain data presented herein, certain unpublished data and also Adams, A. D.; Yuen, W.; Jones, A. B.; Acton, J. A. presented at the 25th National Medicinal Chemistry Symposium, Ann Arbor, MI, June 1996.
18. We chose both P1 Phe and P1 cyclohexylalanine (Cha) as targets since we knew from peptide SAR[7] that the Phe⇒Cha transition generally afforded a ~20-fold boost in potency.
19. Friedinger, R. M.; Schwenk Perlow, D.; Veber, D. F. *J. Org. Chem.* **1988**, *31*, 1369.
20. We also prepared a series of analagous δ-lactams and saw a similar feature. The δ-lactams were consistently weaker inhibitors than the corresponding γ-lactams.
21. Replacement of the P1 residue in all-*L* tripeptides by 3-(cyclohexyl)propanoyl or 3-(cyclohexyl)propyl was also modestly successful - Rusiecki, V.; Warne, S.; Tolman, R.; Wicker, L.; Schwartz, C.; Nichols, E.; Hermes, J. presented at 20th IUPAC Symposium on the Chemistry of Natural Products, Chicago, Sept. 1996). See also Hanson, G. J.; Vuletich, J. L.; Bedell, L. J.; Bono, C. P.; Howard, S. C.; Welply, J. K., Woulfe, S. L.; Zacheis, M. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1931.