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TETRAPEPTIDE DERIVED INHIBITORS OF COMPLEXATION OF A CLASS II MHC: FULLY UNNATURAL LIGANDS

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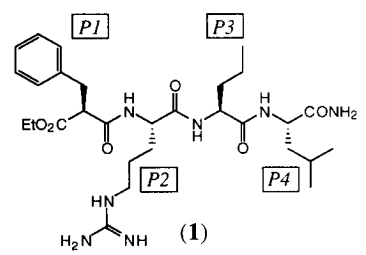
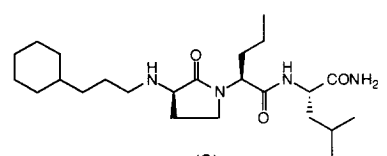
Abstract: Tetrapeptide derived major histocompatability (MHC) II ligands have been developed that contain no unadulterated peptide bonds. These are the 'least peptidic' ligands for any MHC protein yet reported.

The preceding report described the initial modifications of a tetrapeptide ligand (**1**) for an MHC class II protein.[2] The success of that enterprise served as a demonstration, at least to us, that masking of the peptidic nature of MHC ligands was feasible even when these modifications were situated in the heart of the binding contact region. This suggested that it might be possible to generate a peptide derived inhibitor wherein every peptide bond had been altered. In general, this is not necessary in order to create effective, bioavailable molecules.[3] However, this is an unusually rigourous application, as the site of competition is the endosome. Not only does access to this compartment require penetration of two membranes (pseudopeptide targets have frequently been cell surface receptors), but more significantly the endosome is precisely the organelle where endogenous and exogenous peptide is degraded.[4] Consequently we felt that the peptidic nature of an inhibitor would need to be minimized if the molecule were to have a useful lifetime at the endogenous site of action.



(**1**)



(**2**)

We had shown that lactam **2** (compound **11C** in the preceding report) inhibited the binding of full length peptide to purified DRB1*0101 with an IC_{50} of 760 nM. We sought to improve on this while simultaneously modifying the remaining natural peptide bond. As envisaged from the founding design hypothesis,[2] the *R*-lactam of **2** forms a bridge from peptide backbone to peptide backbone and ought to be lacking a P2 side chain contribution. We therefore sought to re-install that residue in the form of additional substitution at the lactam 3-position. For this we developed a synthetic scheme capable of generating a versatile 3-allyl intermediate to facilitate late stage P2 side-chain variation.[6] Initially however, to satisfy ourselves that the design might have some practical merit, we prepared independently all four diastereomers of the P2-P3 unit (**3-6**) bearing a simple propyl side chain from reduction of the corresponding allyl residue. As shown (Table 1) the addition of the propyl residue to the *R*-lactam/*S*-Nva series provided a five-fold increase in potency (**3***vs***2**). A similar potency improvement was also observed for the *S,S*-isomer (**4**) relative to its unsubstituted parent (**7**).

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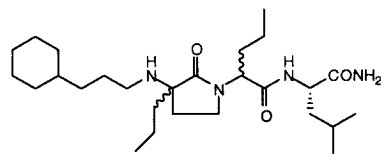
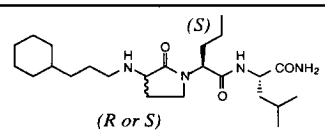
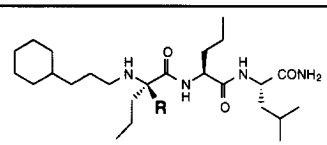
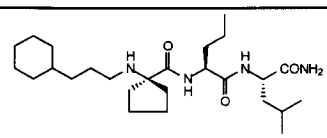
While **4** remains an order of magnitude weaker an inhibitor than **3**, the fact that potentiation is observed for both the **3/2** and **4/7** pairs undermines any claim that the success of **3** can be viewed as validating the design hypothesis. However, neither of the P3 (*R*)-Nva diastereomers (**5**,**6**) showed any inhibitory activity. Design veracity aside, the *R*-propyl-lactam (**3**, 150 nM) compares quite favourably to the corresponding acyclic tripeptides (**8**, 25 nM and **9**, 100 nM) and appears quite respectable for such a conformationally significant and potentially proteolytically protective alteration.[7] In a similar vein, it is interesting that an alternate restrictive cyclization in the context of these P2 disubstituted analogs is more damaging (**10**).

Table 1. α-Disubstituted Lactams

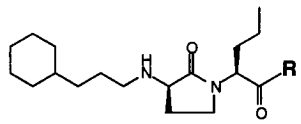


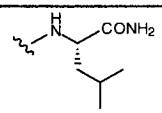
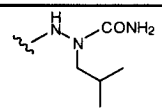
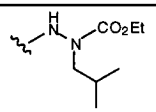
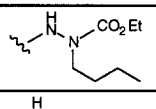
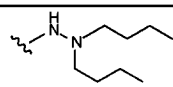
Compound	Configuration of lactam 3-position	Configuration of P3 α centre	$IC_{50}/\mu M$[5]
3	R	S	0.15
4	S	S	1.8
5	R	R	>50
6	S	R	>50
2 (*R,S*) **7** (*S,S*)			0.76 14
8 R = H **9 R = Me**			0.025 0.10
10			1.26

In the course of evaluating other peptide bond modifications in the context of the tetrapeptide we had found that certain α-aza amino acid derivatives were viable P4 residues. α-Aza amino acids are capable of confering proteolytic stabilty to a parent peptide, though this may not be universally true.[8,9] When applied to lactam **2**, the direct α-aza amino acid replacement totally abrogated inhibition (compound **11**, Table 2). This had also been our experience in the case of tetrapeptides. As in those cases however, replacing the terminal primary urea (**11**) with a terminal ethyl carbamate (**12**) restored some activity. A simple ^{n}Bu for ^{i}Bu isomer (**13**) completely recovered the inhibitory activity of the parent lactam analog (**2**). Simpler hydrazides were not effective (e.g., **14**) and may

reflect the necessity of maintaining the H-bonding contact of the carbonyl oxygen with Asnα62 of the class II protein (see preceding report).

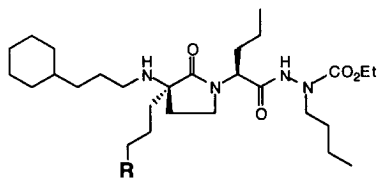
Table 2. α-Aza Amino Acid Hybrids



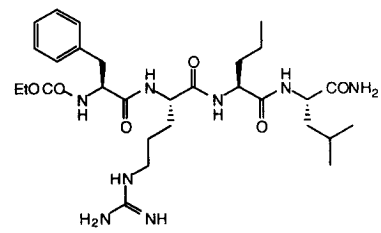
Compound	R	$IC_{50}/\mu M^5$
2		0.76
11		>50
12		4.7
13		0.78
14		>50

Subsequently we were able to effectively incorporate this same α-aza carbamate into the 3-disubstituted lactams, generating our most advanced series of inhibitors. Thus compound **15**, containing the simple P2 propyl side chain, was similarly unaffected by such modification of the terminal residue (compare **15** to **3**). At this point we also utilized the allyl functional handle installed in the precursors (Scheme 1) to generate further natural (e.g., **16**) and unnatural (e.g., **17**) P2 residue side chains.[6] This included the direct P2-arginine counterpart to the lead tetrapeptide **1** (i.e., **16**). Such polymorphisms had little impact on the IC_{50} although they are of potential benefit from the point of view of modifying the physical properties of the compounds. However, the important point is that we had been able to generate relatively potent inhibitors wherein each peptide bond has been modified, albeit presumably with varying degrees of proteolytic robustness.

These inhibitors were designed with reference to the $HA_{307-319}$/DR1 co-crystal structure on the assumption that the tetrapeptide binds analogously at the $HA_{309-312}$ (YVKQ) site. Many of the SAR features suggest that the design rationale is correct. On the other hand MHC class II proteins are likely to be quite flexible[10] and some of



	IC_{50} 20' [5]
(**15**) R = -H	0.24 μM
(**16**) R = -NHC(=NH)NH_2	0.21 μM
(**17**) R = -OH	0.16 μM



(**1**)

our SAR correlations are not without imperfections. Consequently a claim of successful rational design would be premature and indeed unecessary as we had *in practice* achieved our aim of finding acceptable surrogates for every peptide bond. While **15, 16** and **17** retain the indelible stamp of the peptide lead, they are substantially reconditioned relative to *bona fide* peptides and, although a rather subjective descriptor, could be described fairly as the 'least peptidic' inhibitors of MHC proteins yet reported.

References and Notes

1. e-mail : brian_jones@merck.com

2. Jones, A. B.; Acton, J. J.; Rivetna, M. N.; Cummings, R. T.; Nichols, E. A.; Schwartz, C. D.; Wicker, L. S.; Hermes, J. D. *Bioorg. Med. Chem. Lett.* **1998**, preceding report.

3. Humphrey, M. J.; Ringrose, P. S. *Drug Metab.Rev.* **1986**, *17*, 283.

4. A full accounting of intra-endosomal proteases is not available. Members of the cathepsin family have been implicated in antigen processing but are clearly not the only players (e.g., Deussing, J.; Roth, W.; Saftig P.; Ploegh, H. L.; Villadangos, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4516). It is reasonable to assume that almost any unguarded peptide bond would provide a weak link in a putative inhibitor.

5. 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. See referral in preceding report.[2] IC_{50}s were time dependent. We usually recorded the IC_{50}s at 20' and 5 h (typically IC_{50} @ 5 h = 5-10 x IC_{50} @ 20'). Herein we report only the 20' IC_{50} for simplicity.

6. Acton, J. J.; Jones, A. B. *Tetrahedron Lett.* **1996**, *37*, 4319.

7. A formal assessment of the value of such lactams in conferring proteolytic stability appears lacking. However such a study may be redundant as this property is likely to vary from case to case. One cannot assume improved stability, even where improved *in vivo* properties are observed. However it seems unlikely that such a modification would *increase* susceptibility.

8. Dutta, A. S.; Giles, M. B. *J. Chem. Soc. Perkin 1*, **1976**, 244

9. Janda, K. D.; Han, H. *J. Am. Chem. Soc.* **1996**, *118*, 2539 and references therein.

10. There is evidence for discrete steps during the peptide binding/displacement process that may involve class II conformational changes (Sadegh-Nasserl, S.; Stern, L. J.; Wiley, D. C.; Germain, R. N. *Nature* **1994**, *370*, 647, and references therein).