Macrocyclic Peptide Optimisation

Integrating Computational Approaches with Biophysical Data

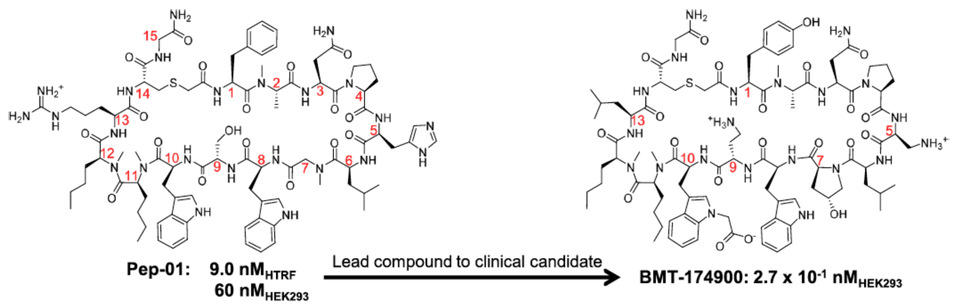
Ajay N. Jain[1], Alexander C. Brueckner[2] , Christine Jorge[2], Ann E. Cleves[1], Purnima Khandelwal[2], Janet Caceres Cortes[2], Luciano Mueller[2]

[1]BioPharmics Division, Optibrium Ltd, Cambridge, United Kingdom, [2]Bristol-Myres Squibb Company, Princeton, NJ, USA, ***email: info@optibrium.com***

Introduction

Using the integrated set of computational methods within the BioPharmics™ Platform, macrocycles can be effectively modelled for lead optimisation. Here, we present a retrospective case study involving optimisation of a macrocyclic inhibitor of the PD-1/PD-L1 interface that was discovered through mRNA library screening. We demonstrate acceleration of lead optimisation based on NMR data on the lead compound and a single crystal structure of the target protein using a combination of deep conformational search, molecular docking, and careful estimation of bound ligand strain. Additionally, a ligand-based approach for predicting bound poses was also effective in prioritising lead-compound analogues.

Thousands of analogues were synthesised and tested to develop the clinical candidate, **BMT-174900**, a macrocyclic peptide inhibitor of PD-L1/PD-1 interaction, starting from the lead compound **Pep-01**.



Objective

Develop an efficient approach to advance a lead macrocyclic peptide to a clinical candidate – faster, cheaper, and with accuracy.

This was achieved by integrating biophysical data from the lead peptide with practical, efficient computational methods within the BioPharmics™ suite, in collaboration with BMS, to prioritise compounds for synthesis.

Dataset and Methods

72 macrocyclic peptides, including the lead and optimised peptides, from the BMS patent disclosure[1], each with associated IC_{50} values.

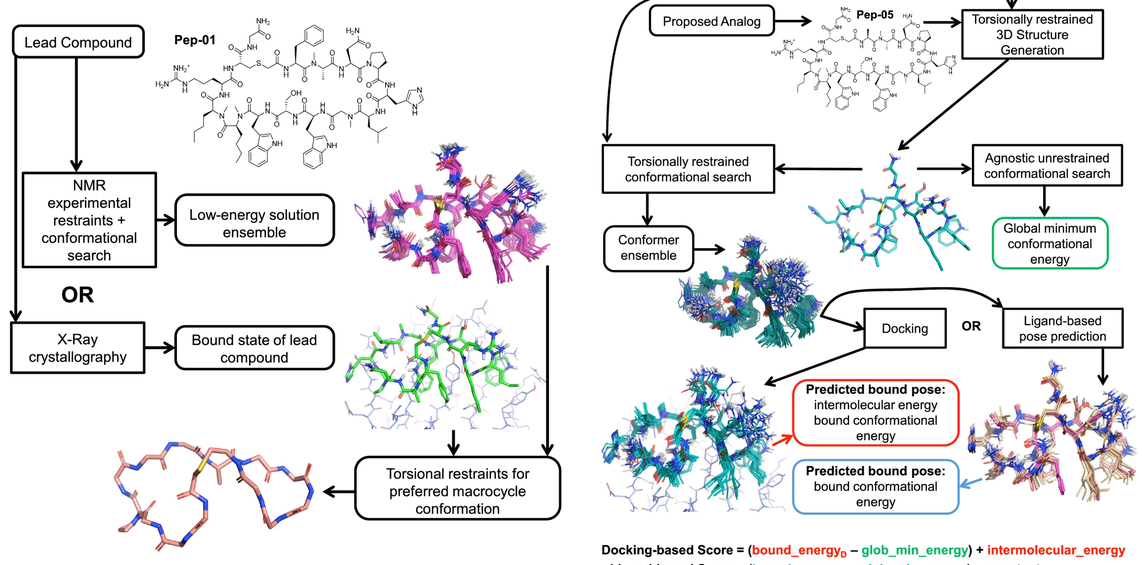
NMR restraints - 50 proton-proton distance restraints, 115 involving chemically equivalent protons, and 6 torsional restraints (1 omega, 5 psi).

ForceGen™ - **Template-free**, **force-field-based** conformational search with NMR-restraints[2] using the **fgen_deep** approach for enhanced sampling

Surflex-Dock™ - **Flexible-ligand**, **ensemble** docking leveraging prior bound ligand **knowledge** for accurate pose prediction[3].

eSim™ - Electrostatic-field and surface-shape similarity-based poses[4].

Workflow for compound ranking:



Docking-based Score = (bound_energy$_D$ – glob_min_energy) + intermolecular_energy
Ligand-based Score = (bound_energy$_L$ – glob_min_energy) + constant

Docking/Ligand-based ranking helps prioritise analogues

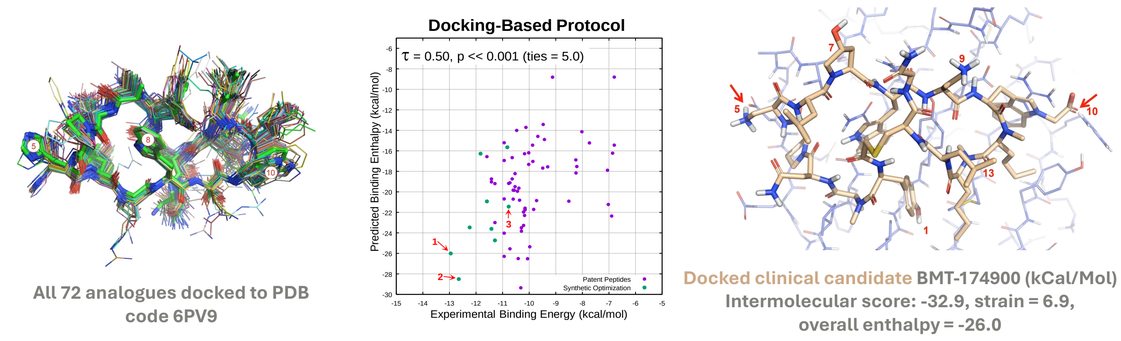


Figure 2: Docked poses of all analogues superimposed on the bound pose of Pep-01 (green) (*top-left*) and the predicted bound pose for the clinical candidate BMT-174900 (*top-right*). Optimal ligand-based alignment of BMT-174900 to Pep-01 for pose prediction (*bottom-left*) compared to its docked pose (*bottom-right*). Experimental vs predicted binding energies show synergy and both protocols correctly rank optimised peptides (green). The points 1, 2, and 3 correspond to three of the optimised peptides, BMT-174900, BMT- 153099, and BMT-139699, respectively (*middle*).

Ligand strain is a dominant predictive component

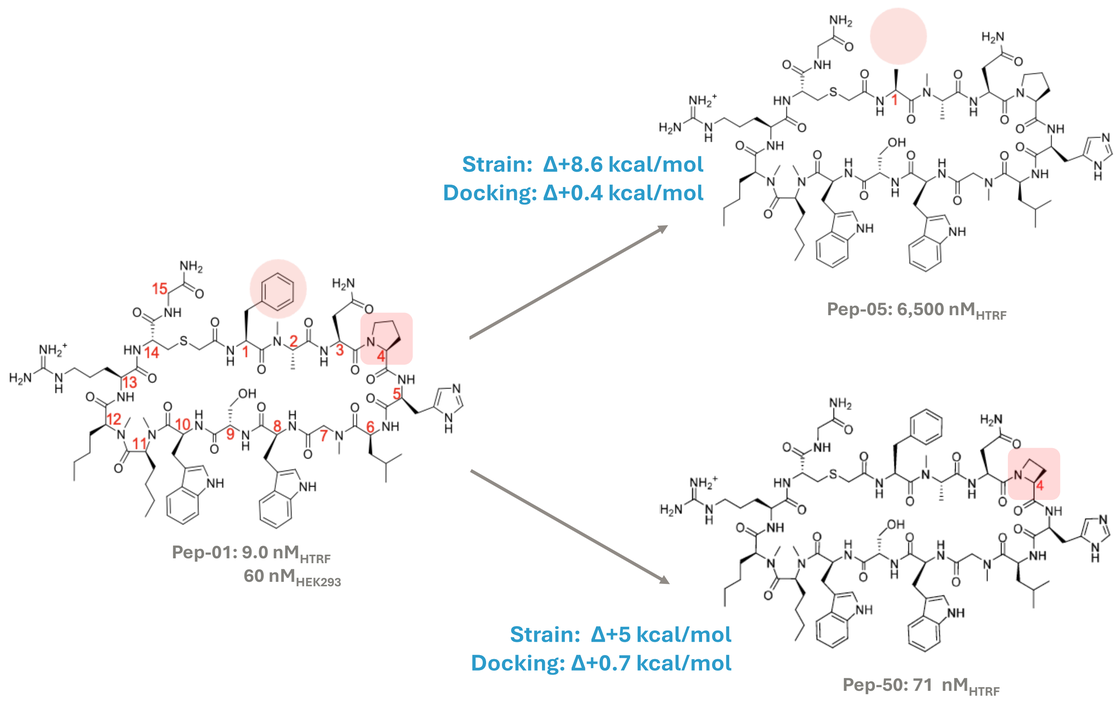


Figure 3: Substituting phenylalanine with alanine at position 1 in Pep-05 reduces activity by 3 log units despite a minor loss in intermolecular binding energy (<0.5 kcal/mol). However, this change increases macrocycle strain by ~8 kcal/mol. Conformational "locking" with rigid substituents significantly impacts strain. Similarly, deleting a single methylene from proline residue at position 4 minimally affects the interaction footprint (0.7 kcal/mol) but increases strain by 5 kcal/mol.

Results

NMR restraints guide biologically relevant conformations

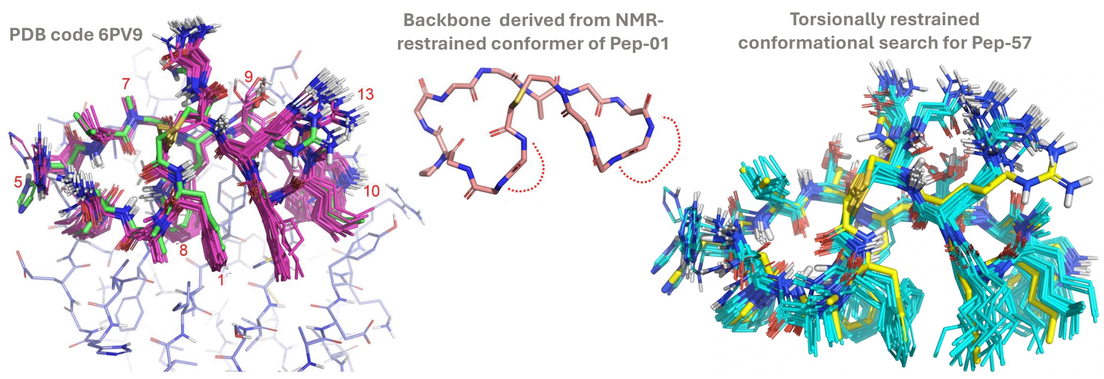


Figure 1: Lowest energy non-redundant conformers from NMR-restrained search of Pep-01 (*magenta*) superposed on its crystallographic pose (green) *(left)*. The backbone derived from the lowest energy conformer (*middle*). Low energy conformers of Pep-57 (cyan), superimposed on its crystallographic pose (yellow) (*right*). Deposited structures were re-fitted using xGen™ to obtain best fits to the X-ray density, ensuring accurate comparisons.

Conclusion

3D molecular modeling accelerates macrocycle lead optimisation with three key elements:

1. A fast, rigorous and template-free method for macrocycle conformational search
2. Effective use of biophysical data to constrain the conformational search and identify bioactive conformations
3. An accurate model of ligand strain; small ligand modifications can cause large changes in strain, significantly impacting binding energy

References

[1] Miller MM, Mapelli C, Allen MP, *et al* US Patent 9:308

[2] Jain AN, Brueckner AC, Cleves AE, *et al* (2023) *J Med Chem* 66(3)

[3] Cleves AE, Jain AN (2015) *J Comput Aided Mol Des*, 29

[4] Cleves AE, Johnson SR, Jain AN (2019) *J Comput Aided Mol Des* 33(10)

This work: Jain AN, Brueckner, AC, Jorge, C, *et al* (2023) *J Comput Aided Mol Des* 37

Learn more on our webpage