# SUPPLEMENTAL MATERIAL FOR:

# Structure-based exploration of cyclic dipeptide chitinase inhibitors.

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## Table I: Details of data collection and structure refinement

Values in parenthesis are for the highest resolution shell. Crystals were of space group $P2_12_12_1$, and were cryo-cooled to 100K. All measured data were included in refinement.

| | Cyclo-(L-Arg-L-Pro) | Cyclo-(Gly-L-Pro) | Cyclo-(L-His-L-Pro) | Cyclo-(L-Tyr-L-Pro) |
|---|---|---|---|---|
| Resolution | 25.00-1.85 (1.92-1.85) | 25.00-2.10 (2.18-2.10) | 25.00-1.90 (1.97-1.90) | 25.00-1.85 (1.92-1.85) |
| Observed reflections | 274989 (16960) | 187319 (18640) | 291104 (24886) | 330099 (23615) |
| Unique reflections | 88471 (6949) | 58162 (5816) | 85317 (8166) | 89533 (7965) |
| Redundancy | 3.1 (2.4) | 3.2 (3.2) | 3.4 (3.0) | 3.7 (3.0) |
| I/$\sigma$I | 18.9 (7.0) | 12.3 (5.3) | 8.5 (2.3) | 20.9 (8.3) |
| Completeness | 95.5 (76.1) | 89.7 (90.9) | 99.4 (96.8) | 97.6 (88.1) |
| $R_{merge}$ | 0.047 (0.175) | 0.072 (0.262) | 0.079 (0.445) | 0.035 (0.159) |
| $R_{cryst}$, $R_{free}$ | 0.186, 0.213 | 0.200, 0.250 | 0.193, 0.223 | 0.170, 0.202 |
| RMSD bond ideality (Å) | 0.012 | 0.096 | 0.011 | 0.011 |
| RMSD angle ideality ($^o$) | 1.58 | 1.45 | 1.53 | 1.63 |
| RMSD $B$ restraints (Å$^2$) | 1.51 | 1.45 | 1.37 | 1.48 |
| $<B_{protein}>$ (Å$^2$) | 27.2 | 26.9 | 27.5 | 20.7 |
| $<B_{ligand}>$ (Å$^2$) | 27.6 | 23.4 | 39.1 | 25.0 |

## Table II: Inhibitor hydrogen bonds.

Hydrogen bonds were calculated with WHAT IF [1] using the HB2 algorithm [2], which assigns a score between 0 (no hydrogen bond) and 1 (optimal hydrogen bond) based on hydrogen bond geometry (HB2 column). A cut-off of 0.4 was applied to the HB2 score and a distance cut off of 3.5 Å was applied to the D-A distance to exclude weak hydrogen bonds. Hydrogen bonding type was assigned to ligand atoms using PRODRG [3, 4].

| Atom | | Protein/water/glycerol | D-A | HB2 |
|---|---|---|---|---|
| Pro O | CI-4 | Tyr 214, O$\eta$ | 2.7 | 0.63 |
| | Cyclo-(L-Arg-L-Pro) | Trp 97, N | 3.0 | 0.61 |
| | Cyclo-(Gly-L-Pro) | Trp 97, N | 2.7 | 0.62 |
| | Cyclo-(L-His-L-Pro) | Trp 97, N; $H_2O$ | 3.0; 2.9 | 0.60; 0.57 |
| | Cyclo-(L-Tyr-L-Pro) | Trp 97, N | 2.9 | 0.67 |
| Non-Pro O | CI-4 | Trp 97, N | 2.7 | 0.71 |
| | Cyclo-(L-Arg-L-Pro) | Tyr 214, O$\eta$ | 2.6 | 0.69 |
| | Cyclo-(Gly-L-Pro) | Tyr 214, O$\eta$ | 2.8 | 0.74 |
| | Cyclo-(L-His-L-Pro) | Tyr 214, O$\eta$ | 2.7 | 0.75 |
| | Cyclo-(L-Tyr-L-Pro) | Tyr 214, O$\eta$ | 2.6 | 0.71 |
| Non-Pro N | CI-4 | $H_2O$ | 2.7 | 0.81 |
| | Cyclo-(L-Arg-L-Pro) | Glycerol O | 2.7 | 0.52 |
| | Cyclo-(Gly-L-Pro) | Glycerol O | 2.6 | 0.45 |
| | Cyclo-(L-His-L-Pro) | $H_2O$ | 2.8 | 0.83 |
| | Cyclo-(L-Tyr-L-Pro) | $H_2O$ | 2.9 | 0.75 |
| Arg N$\varepsilon$ | CI-4 | Glycerol O | 2.9 | 0.65 |
| | Cyclo-(L-Arg-L-Pro) | - | - | - |
| His N$\delta$1 | Cyclo-(L-His-L-Pro) | Asp215 O$\delta$2 | 2.5 | 0.42 |
| His N$\varepsilon$2 | Cyclo-(L-His-L-Pro) | Glu144 O$\varepsilon$1 | 2.5 | 0.71 |
| Tyr O$\eta$ | Cyclo-(L-Tyr-L-Pro) | Tyr 98, O$\eta$ | 2.7 | 0.75 |

## Table III: Details of cyclic dipeptide conformations.

The values for $\phi$, $\psi$ backbone dihedral angles and the $\chi$1,2 side chain dihedral angles (in degrees) are listed. Optimum side torsion angles were taken from the O rotamer database, which has been compiled using the most common side chain conformations in 140 high-resolution, low $R_{cryst}$ structures [5]. Deviations from the nearest rotamer ($\Delta_{ideal}$) are listed.

| | $\phi$ | $\psi$ | $\chi_1$ ($\Delta_{ideal}$) | $\chi_2$ ($\Delta_{ideal}$) |
|---|---|---|---|---|
| **CI4** | | | | |
| L-Arginine | 30 | -24 | -94 (-34) | -155 (15) |
| D-Proline | 38 | -32 | | |
| **cyclo(L-Arg-L-Pro)** | | | | |
| L-Arginine | -36 | 37 | -74 (-14) | -170 (0) |
| L-Proline | -35 | 37 | | |
| **cyclo(Gly-L-Pro)** | | | | |
| Glycine | -35 | 34 | | |
| L-Proline | -33 | 32 | | |
| **cyclo(L-His-L-Pro)** | | | | |
| L-Histidine | -11 | 14 | 64 (-6) | -35 (35) |
| L-Proline | -21 | 24 | | |
| **cyclo(L-Tyr-L-Pro)** | | | | |
| L-Tyrosine | -39 | 40 | -74 (-14) | 85 (-5) |
| L-Proline | -42 | 43 | | |

# References


[1] G. Vriend, WHAT IF: a molecular modeling and drug design program, *J. Mol. Graph.*, **8**, 52-56 (1990).

[2] R. W. W. Hooft, C. Sander, and G. Vriend, Positioning hydrogen atoms by optimizing hydrogen-bond networks in protein structures, *Proteins*, **26**, 363-376 (1996).

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[4] A. W. Schuettelkopf and D. M. F. van Aalten, PRODRG: a tool for high-throughput crystallography of, *Acta Cryst.*, **D60**, 1355-1363 (2004).

[5] S. C. Lovell, J. M. Word, J. S. Richardson, and D. C. Richardson, The penultimate rotamer library, *Proteins*, **40**, 389-408 (2000).