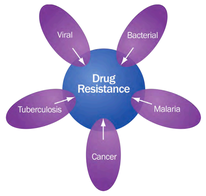
Investigation of The Role of Macrocyclization in HCV Protease Inhibitor MK-5172's Drug Resistance Profile

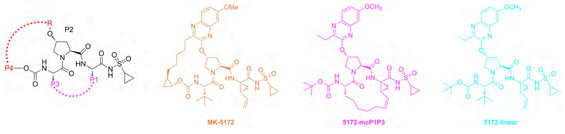
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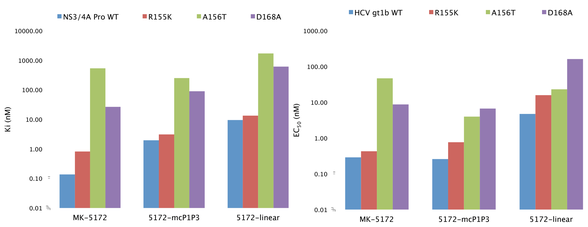
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- **Background:** Structure guided drug design has been a powerful tool for the discovery and development of inhibitors to the viral HCV NS3/4A protease. The NS3/4A protease inhibitors (PIs) have benefited from extensive optimizations in the common P4 capping, P3-P1' peptidomimetic scaffold and various macrocyclization states. However, neither structural nor thermodynamic information have been presented evaluating the benefits of different protease macrocyclization states in the context of resistance.



MK-5172 is extremely potent against the wildtype (WT) enzyme, while subverting resistance to R155K and D168A due to packing against the protease catalytic triad, this inhibitor is susceptible to A156T due to a strong steric clash



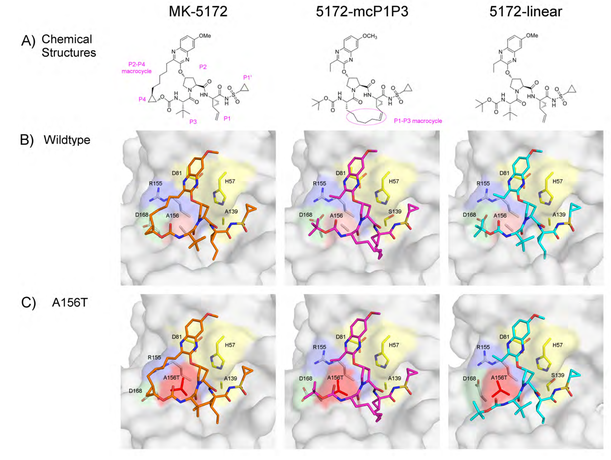
Here, we present structural and biophysical evidence detailing the role of macrocyclization of HCV PIs in drug resistance.

- **Methods:** A combination of standard protein crystallographic, molecular dynamic, kinetic and thermodynamics methods were applied to the protease domain of HCV NS3/4A WT and A156T resistance variant

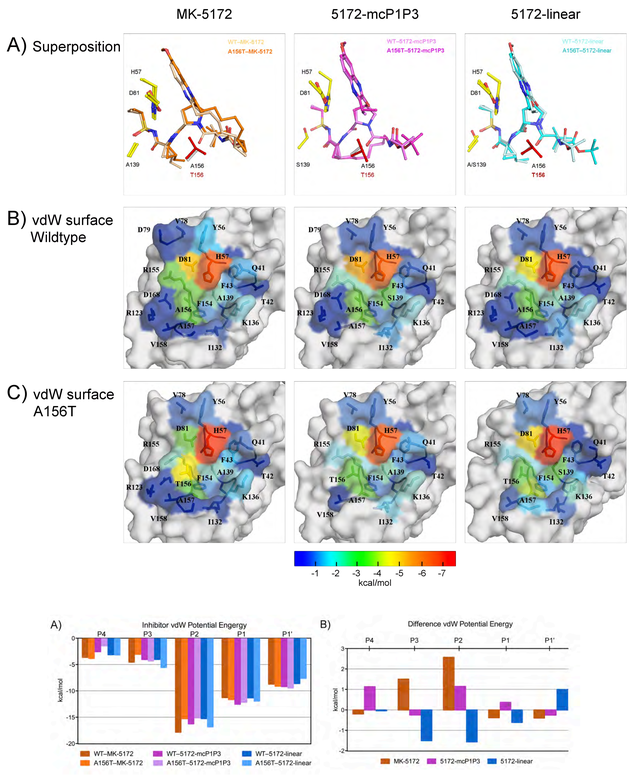
	5172-mcP1P3		5172-linear	
Complexes	WT	A156T	WT	A156T
PDB Code				
Resolution (A)	2.3	2.3	1.6	2.3
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
Twin Law	-	-	-	-k,-h,-l
Twin Fraction (%)	-	-	-	0.1
Molecules in AU	1	1	1	4
Cell Dimensions				
a	55.2	54.9	54.71	65.4
b	58.5	58.4	58.42	63.2
c	60.1	60.0	59.96	92.3
β (°)	90	90	90	91.6
Completeness (%)	99.2	99.9	99	99.9
Measured Reflections	38720	39511	156955	129154
Unique Reflections	8793	9070	23941	31801
Average I/σ	9	9.1	9.1	18.4
Redundancy	4.4	4.1	6.6	4.1
R_{sym} (%)	6.7 (18.1)	6.8 (20)	7.9 (32.9)	7.2 (11.3)
RMSD in				
Bonds (A)	0.005	0.011	0.014	0.015
Angles (°)	1.3	1.3	1.7	1.7
R_{ractor} (%)	15.2	17.3	16.3	20.0
R_{free} (%)	19.9	21.6	19.7	25.4

a, AU, asymmetric unit.
b, $R_{sym} = \Sigma|I - \langle I\rangle|/\Sigma I$, where I = observed intensity, <I> = average intensity over symmetry equivalent.
c, values in parentheses are for the highest resolution shell.
d, RMSD, root mean square deviation.
e, $R_{factor} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
f, R_{free} was calculated from 5% of reflections, chosen randomly, which were omitted from the refinement process.

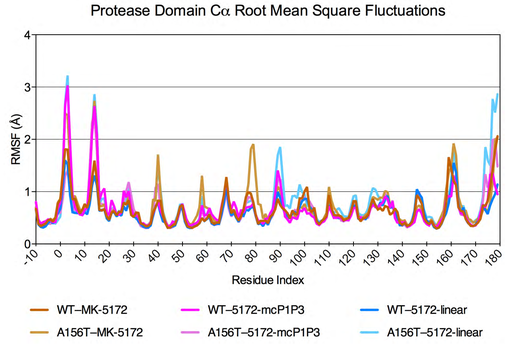
- **1_ Does the presence or location of macrocyclization affect MK-5172's binding mode?** MK-5172 and its analogues have a similar biding mode in the static structure.



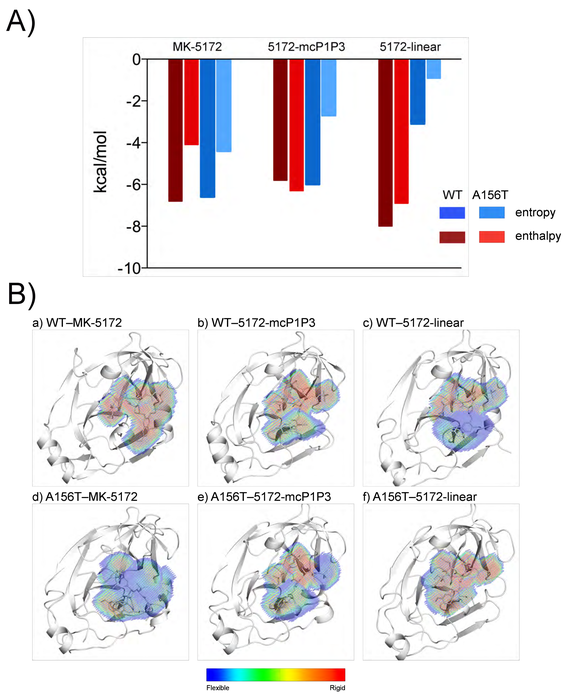
- **2_Does the presence or location of macrocyclization affect MK-5172's packing?** Subtle changes in inhibitor-protease contacts occur at the catalytic aspartate and histidine, the S2 pocket as well as the inhibitor's P2 and P4 moieties.



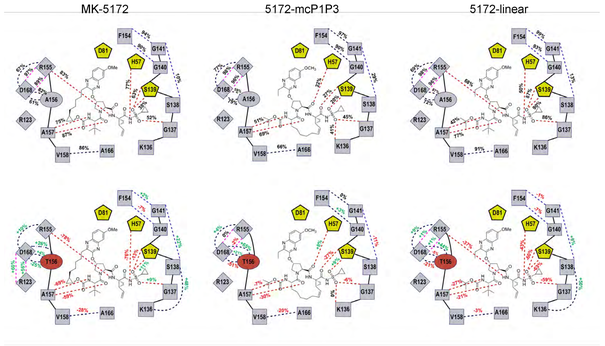
- **3_ How do the different binding modes affect the overall protein dynamics?** 100 ns Molecular dynamic simulations revealed extensive backbone deviations through the protease.



- **4_ How does macrocyclization affect the dynamic flexibility of the inhibitors?** Macrocyclization differentially alters the dynamic flexibility of the inhibitors (B). Enthalpy-entropy balance of inhibitor binding is shifted in the absence of a macrocycle (A).



- **5_ How are the hydrogen bonding patterns affected by the drug resistance mutation A156T?** The Conserved D168-R155 hydrogen bond is lost in the A156T-MK-5172 complex. In the context of the mutant structures, the P1P3 analogue maintains its context better.



- **Conclusions:** We have determined high-resolution crystal structures of linear and P1-P3 macrocyclic analogs of MK-5172 bound to WT and A156T protease, and compared these structures, their dynamics from molecular dynamics simulations and the experimental binding thermodynamics to the parent compound. We find that the "unique" binding mode of MK-5172 is conserved even when the P2-P4 macrocycle is removed or replaced with a P1-P3 macrocycle. While beneficial to decreasing the entropic penalty associated with binding, the constraint exerted by the P2-P4 macrocycle prevents efficient rearrangement to accommodate the A156T mutation, a deficit alleviated in the linear and P1-P3 analogs. Design of macrocyclic inhibitors to NS3/4A need to achieve the best balance by exerting optimal conformational constraint to enhancing potency versus allowing adaptability to be robust against resistance mutations.

Acknowledgement

This poster was supported by the National Institute of General Medical Sciences and National Institute of Allergy and Infectious Diseases (F31-GM103259, RO1-AI085051) and the Biomedical Science Career Program HOPE Scholarship