Probing contacts of inhibitor locked in transition states in the catalytic triad of DENV2 type serine protease and its mutants by NMR spectroscopy.

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Detailed structural knowledge of enzyme-inhibitor complexes trapped in intermediate state is the key for fundamental understanding of mechanism of reactions taken place in enzymes. Solution nuclear magnetic resonance (NMR) uniquely allows to study an active site of an enzyme interconverting between different tautomers. It was demonstrated on serotype II Dengue virus NS2B:NS3pro serine protease in complex with high-affinity ligands containing either trifluoromethyl ketone or boronic groups in the C-terminal of a tetra-peptide. In this study ^{1}H, ^{19}F and ^{15}N NMR spectroscopy has been used to probe the interaction contacts of inhibitors locked in transition states. A low barrier hydrogen bond (LBHB) is present in both complexes. Monitoring ^{19}F resonances, it was demonstrated that the access of bulk water to the active site is limited. Moreover there were no bound water found in proximity of the active site manifesting the most favourable condition for formation of LBHB hydrogen bonds in catalytical triad.
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