

Supplementary Information

Hydroxychloroquine Inhibits Zika Virus NS2B-NS3 Protease

Ankur Kumar,[†] Brooke Liang,^{‡,§} Murali Aarthy,[⊥] Sanjeev Kumar Singh,[⊥] Neha Garg,^{†,#}

Indira U. Mysorekar,^{‡,§,||} and Rajanish Giri*,^{†,#}

[†]Indian Institute of Technology Mandi, Mandi 175005, Himachal Pradesh, India; [‡]Department of Obstetrics and Gynecology, [§]Center for Reproductive Health Sciences, and ^{||}Department of Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110, United States; [⊥]Department of Bioinformatics, Computer Aided Drug Design and Molecular Modeling Laboratory, Alagappa University, Science Block, Karaikudi 630003, Tamil Nadu, India; [#]BioX Center, Indian Institute of Technology Mandi, Mandi 175005, Himachal Pradesh, India

Figure S1. Molecular interaction diagram of NS2B-NS3 protease, 3D view. Interacting amino acid residues at the active site of NS2B-NS3 protease, with A) Mitoxantrone, B) Miglustat, C) Nadolol, D) Carteolol, and E) Pindolol. The diagram shows molecular interactions between the drug molecules and amino acid residues of the protease complex. Drug molecules (grey in colour) form H-bonds (yellow dashed lines), Pi-Pi interactions (cyan dashed lines), Pi-cation interactions (green dashed lines), and salt bridges (pink dashed lines) with NS2B-NS3 protease. [Asterisks (*) represent residues belonging to NS2B co-factor]

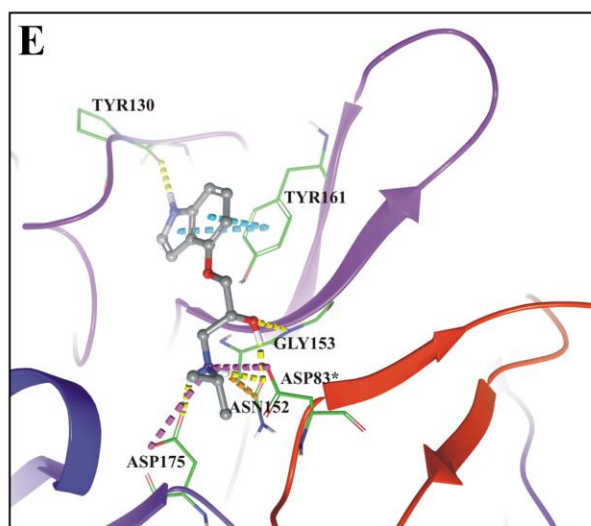
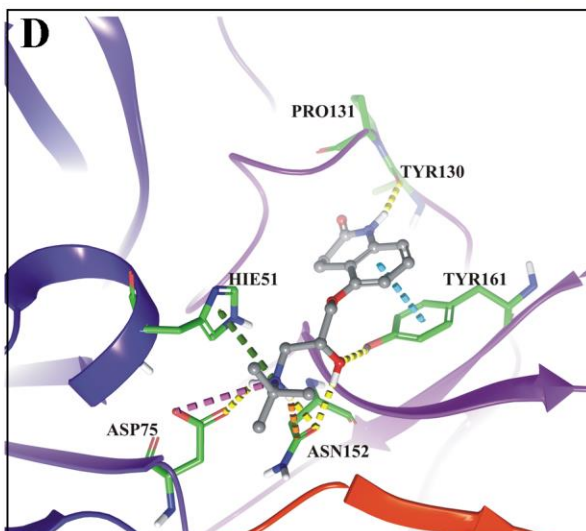
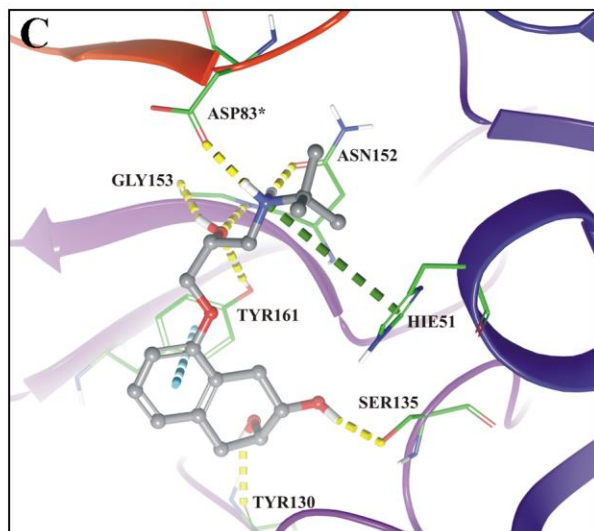
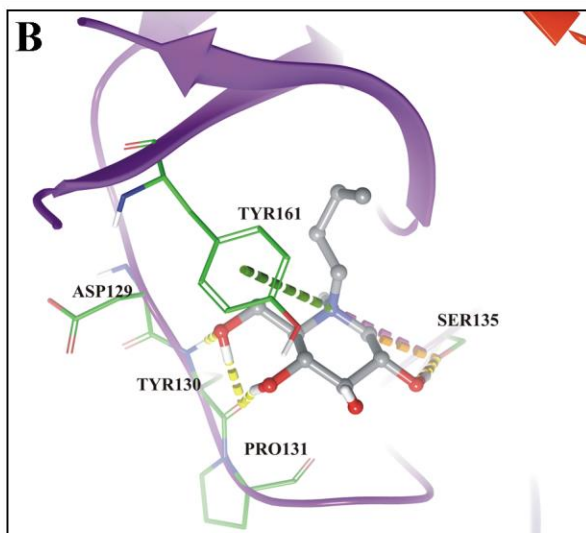
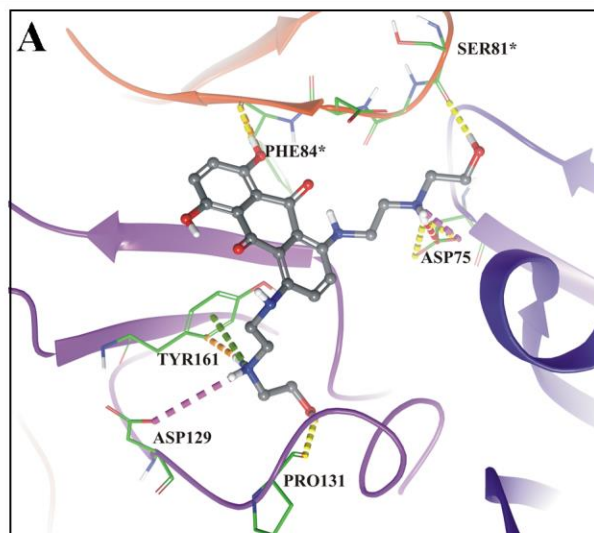
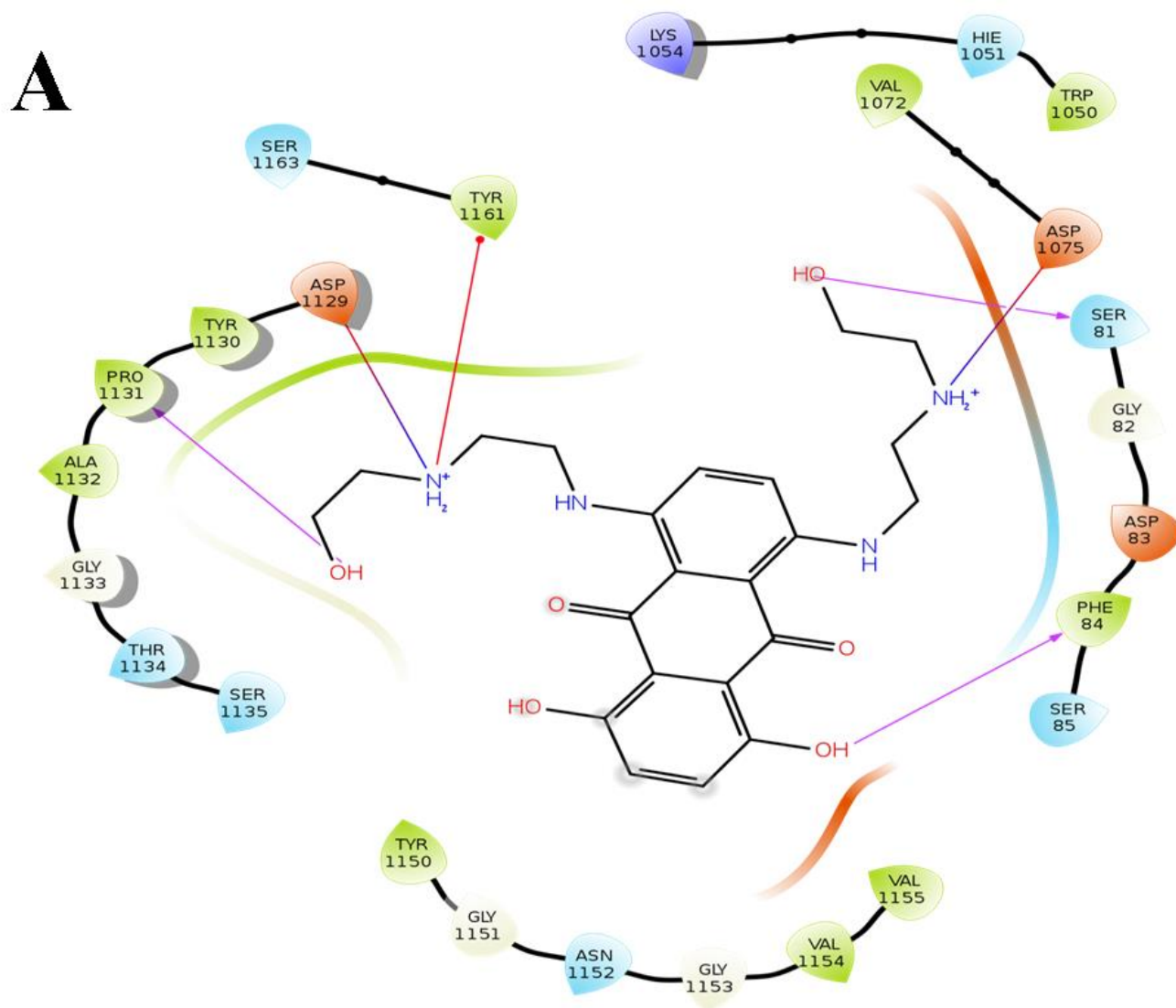
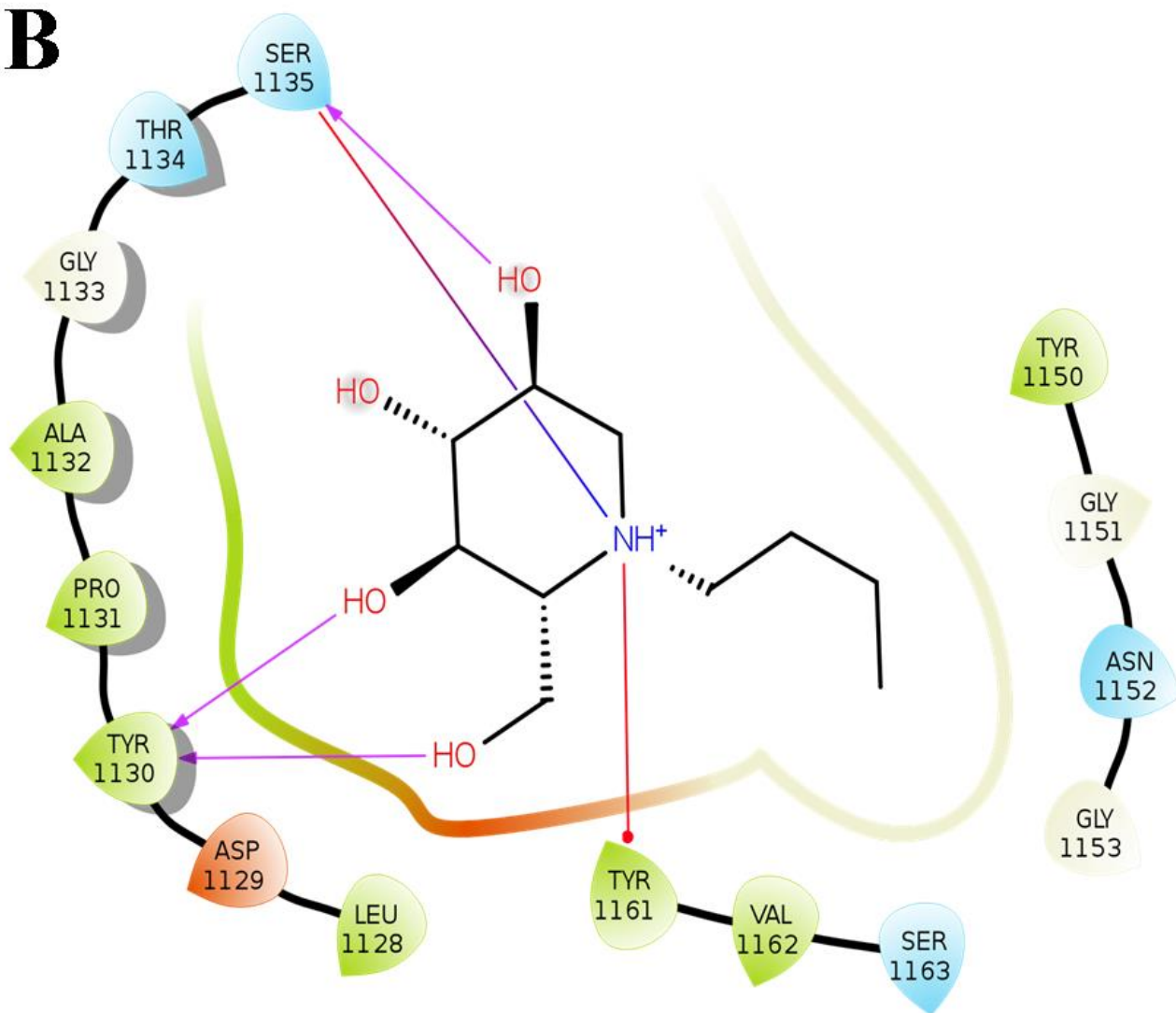


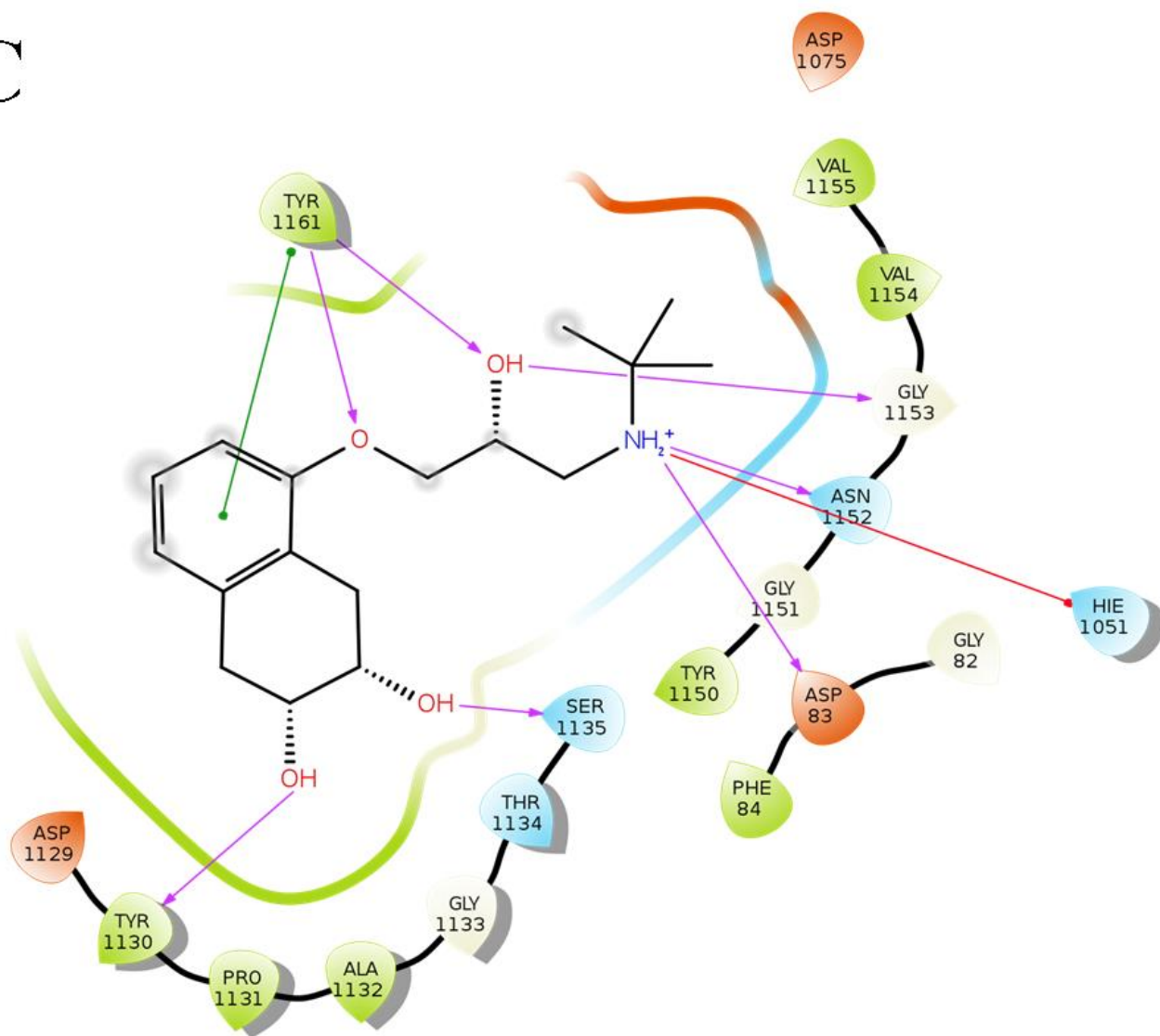
Figure S2. Molecular interaction diagram of NS2B-NS3 protease, 2D view. Interactions between amino acids at the active site of NS2B-NS3 protease and A) Mitoxantrone, B) Miglustat, C) Nadolol, D) Carteolol, and E) Pindolol. The drug molecules form H-bonds (pink arrows), Pi-Pi interactions (green lines), Pi-cation interactions (red lines), salt bridges (blue-red lines) and hydrophobic interactions with the NS2B-NS3 protease. [To differentiate the residue numbers between NS2B and NS3 protease, residues 49-87 of NS2B are denoted 49-87 while residues 15-167 of NS3 protease are denoted 1015 – 1167]

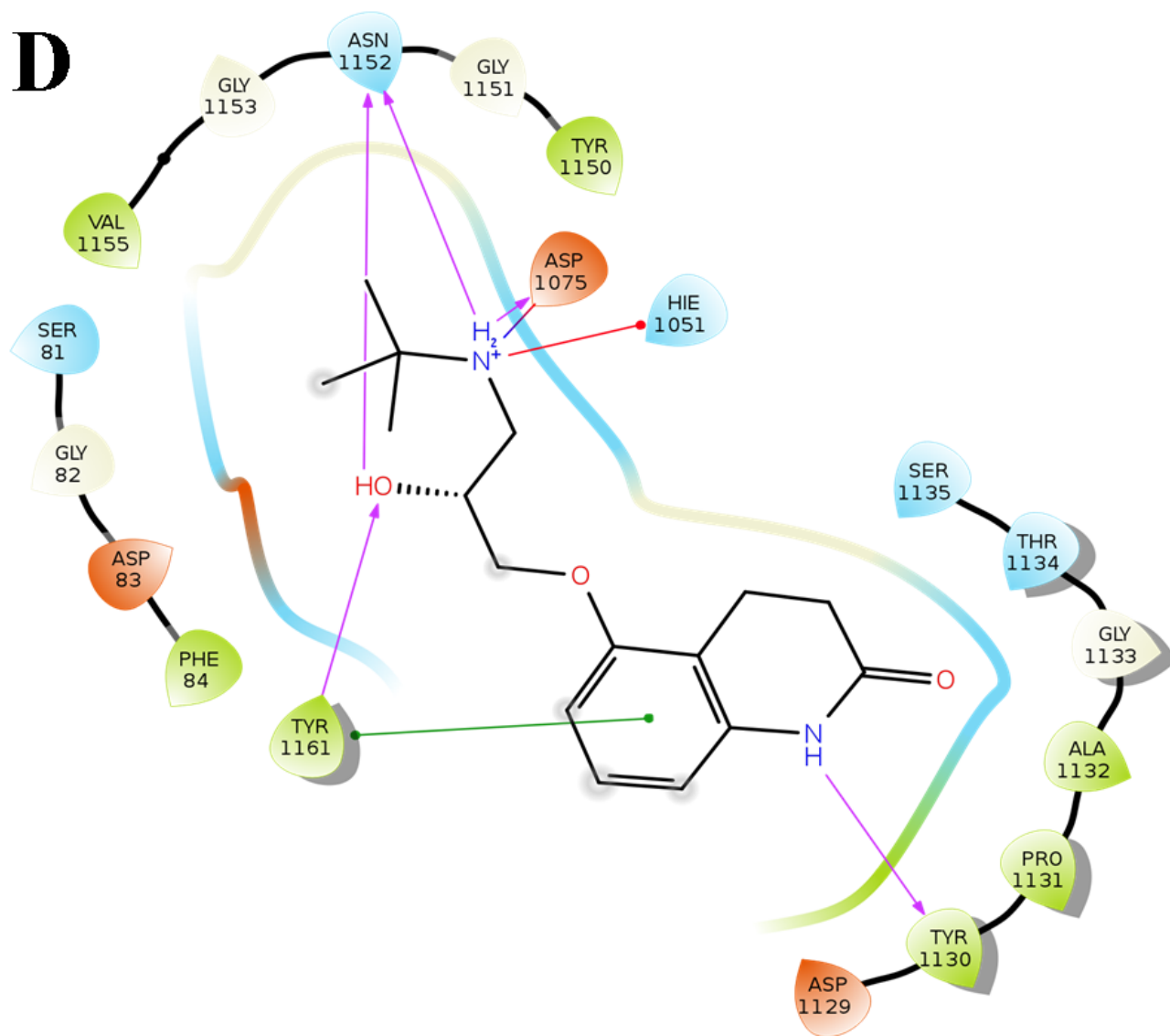


B



C



D

E

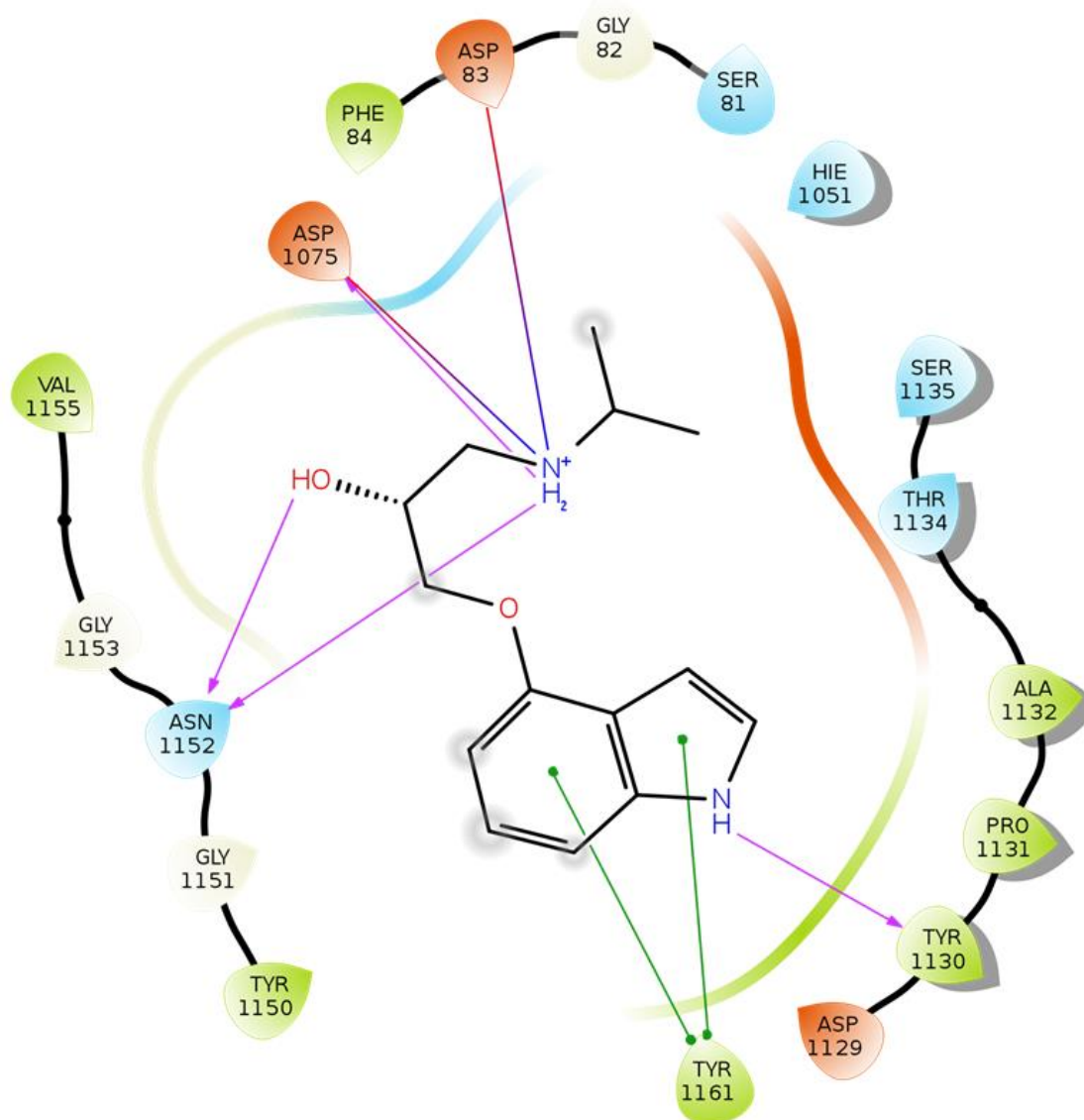
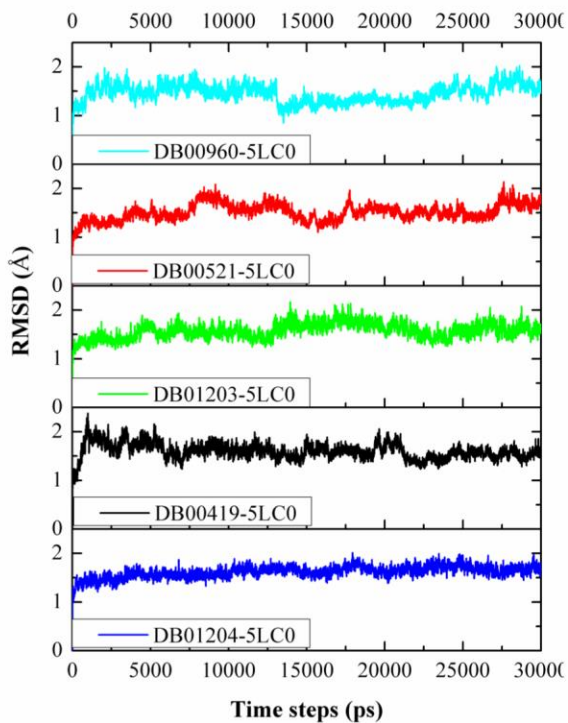
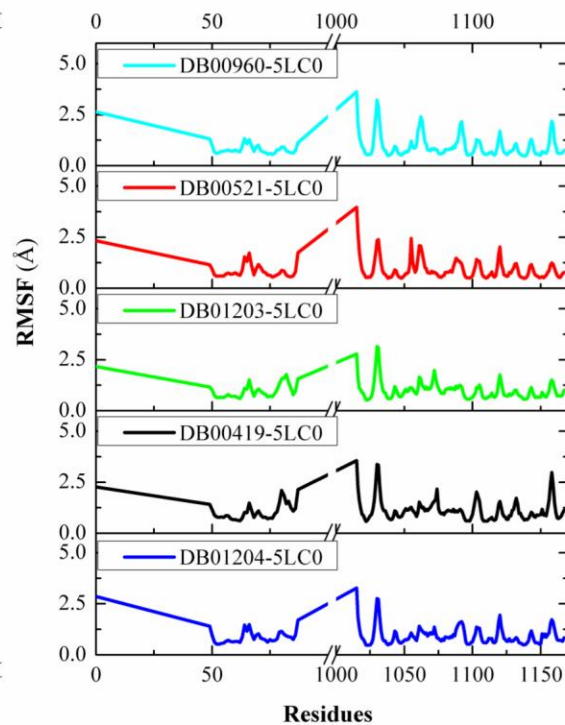


Figure S3. Molecular dynamics simulation of the NS2B-NS3 protease with drug molecules. Plots A), B), C), and D) represent the RMSD, RMSF, radius of gyration and hydrogen bonds analysis respectively. Each plot shows the analysis of NS2B-NS3 protease (ID: 5LC0) in association with pindolol (DB0960), carteolol (DB00521), nadolol (DB01203), miglustat (DB00419), and mitoxantrone (DB01204). ps: picosecond. [To differentiate the residue numbers between NS2B and NS3 protease, residues 49-87 of NS2B are denoted 49-87 while residues 15-167 of NS3 protease are denoted 1015 – 1167]

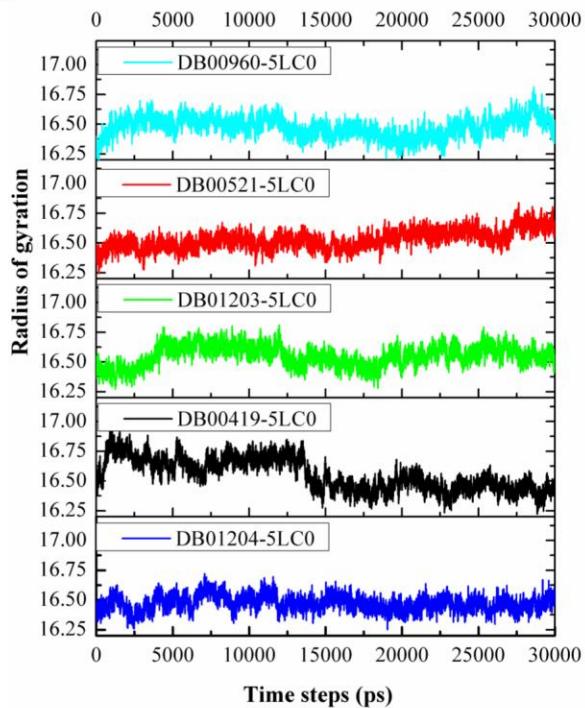
A



B



C



D

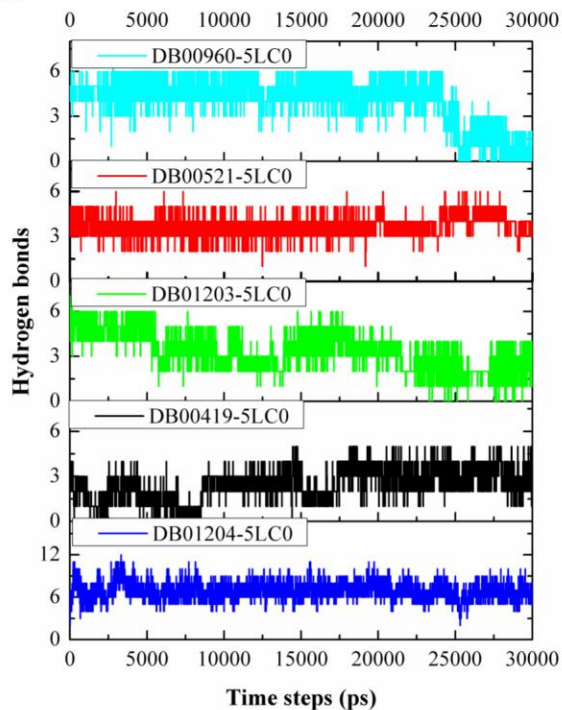


Table S1. A summary of induced fit docking (IFD) results of top hit FDA approved drugs. Top six compounds from FDA-approved drug library, showing the docking score and interacting amino acid residues of NS2B-NS3 protease.

Compound name	Drug Bank ID	Docking score (kcal/mol)	Glide energy (kcal/mol)	Glide emodel (kcal/mol)	Interacting amino acid residues		
					H-Bond	Pi interaction	Salt bridge
Mitoxantrone	DB01204	-12.785	-66.749	-91.380	Ser81*, Phe84*, Asp75, Pro131	Pi-Pi: Tyr161, Pi-cation: Tyr161	Asp129, Asp75
Hydroxychloroquine	DB01611	-10.725	-56.832	-74.079	Asp 83*, Tyr 130, Asn152, Gly151	Pi-Pi: Tyr161, Pi-Cation: Tyr161	Asp129
Miglustat	DB00419	-10.086	-34.145	-39.879	Tyr130, Ser135	Pi-Pi: Tyr161	Ser135
Nadolol	DB01203	-9.922	-51.931	-73.118	Asp83*, Asn152, Gly153, Tyr161, Ser135, Tyr130	Pi-Pi: Tyr161, Pi-Cation: Hie51	
Carteolol	DB00521	-9.783	-47.585	-67.859	Asp75, Asn152, Tyr161, Tyr 130	Pi-Pi: Tyr161, Pi-Cation: Hie51	Asp75
Pindolol	DB00960	-9.613	-45.338	-64.355	Asp75, Tyr130, Asn152, Gly153	Pi-Pi: Tyr161	Asp83*, Asp75

[Asterisk (*) for residues that belong to NS2B co-factor]