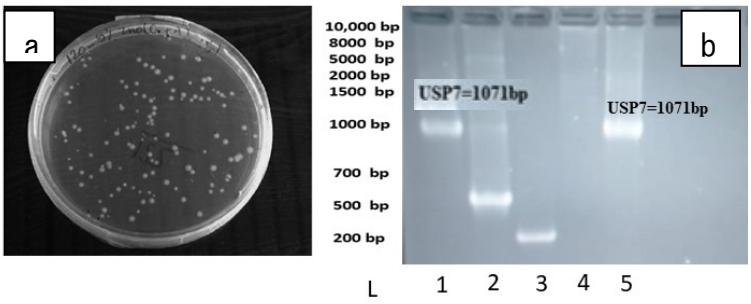
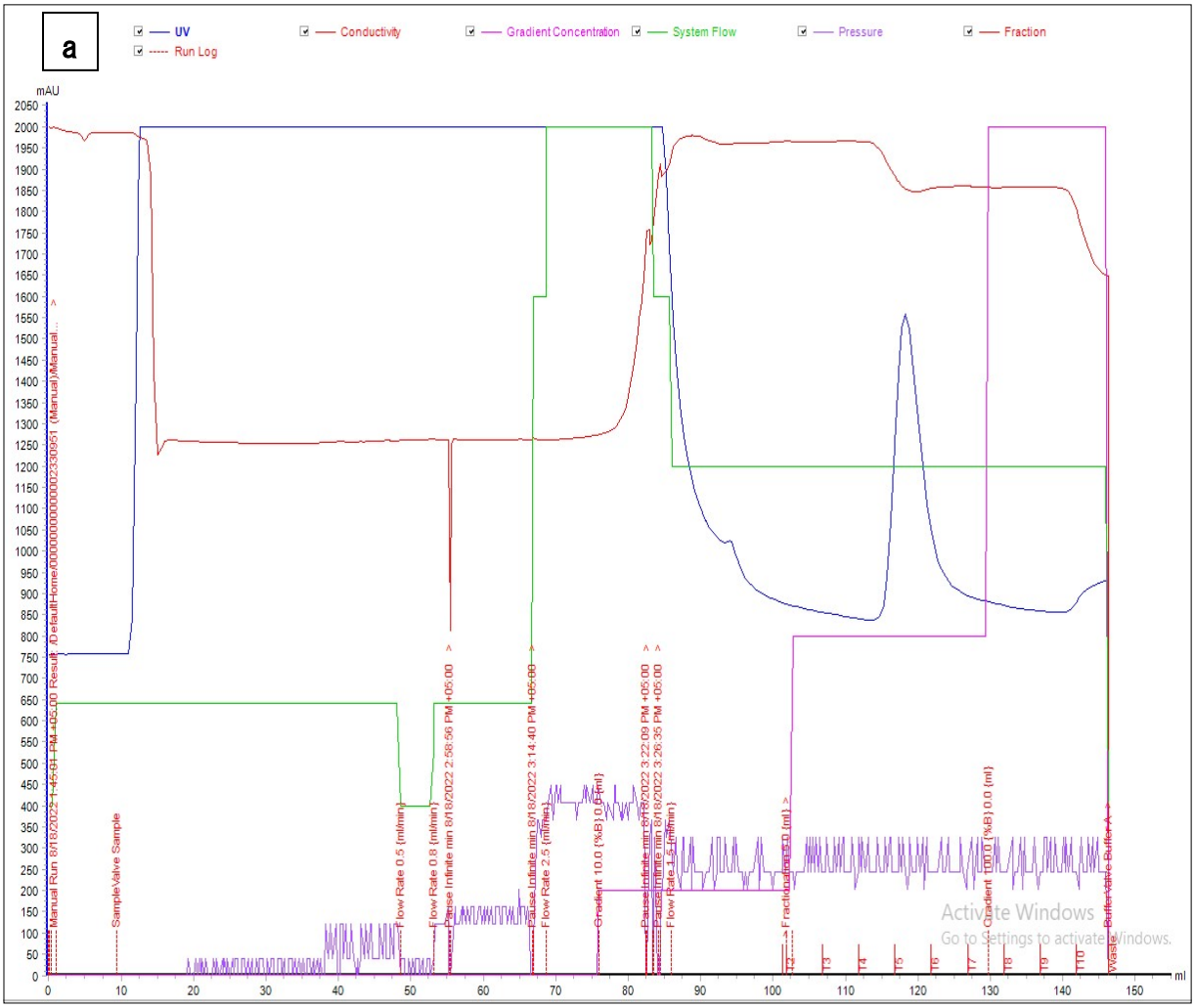
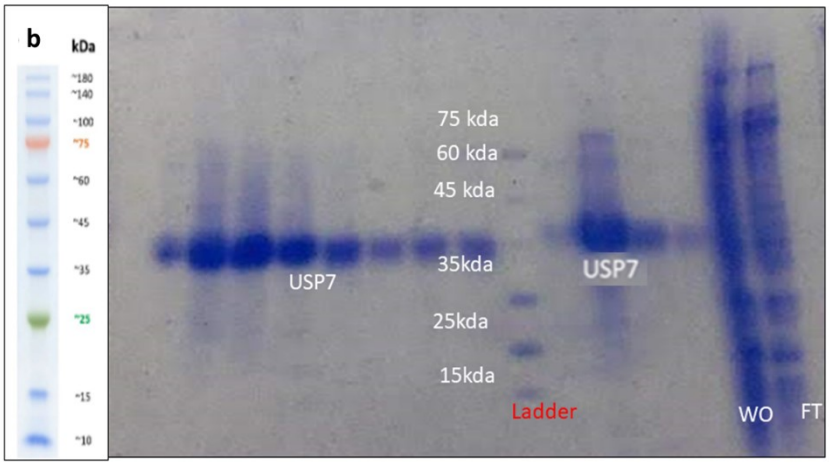
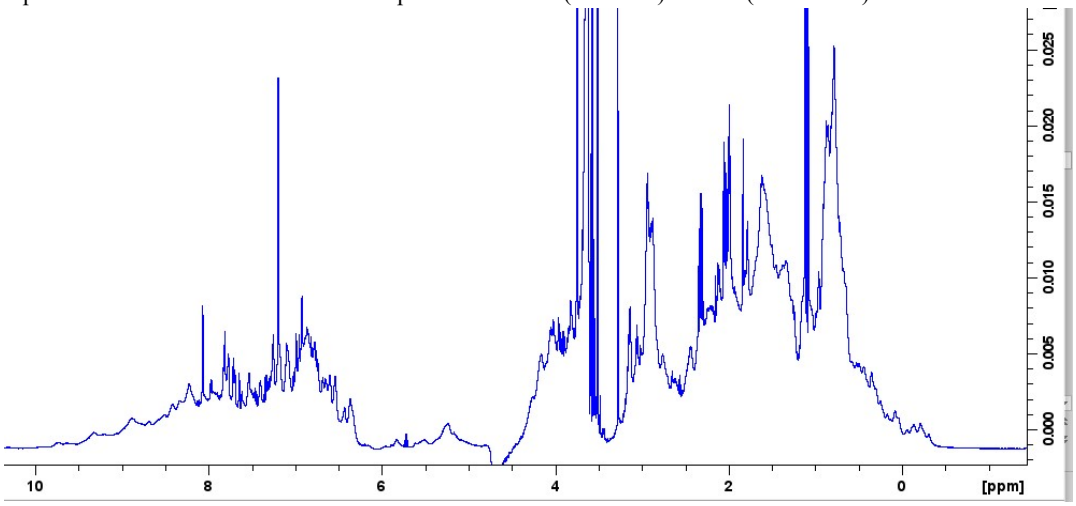


S1 Fig. Agar Plates showing the successful transformation and Colony PCR Gel (a). Colonies for USP7-CD plasmid (b) The 1% agarose gel analysis for USP7-CD, L indicates the ladder, lane 1 and 5 are for gene of USP7-CD.

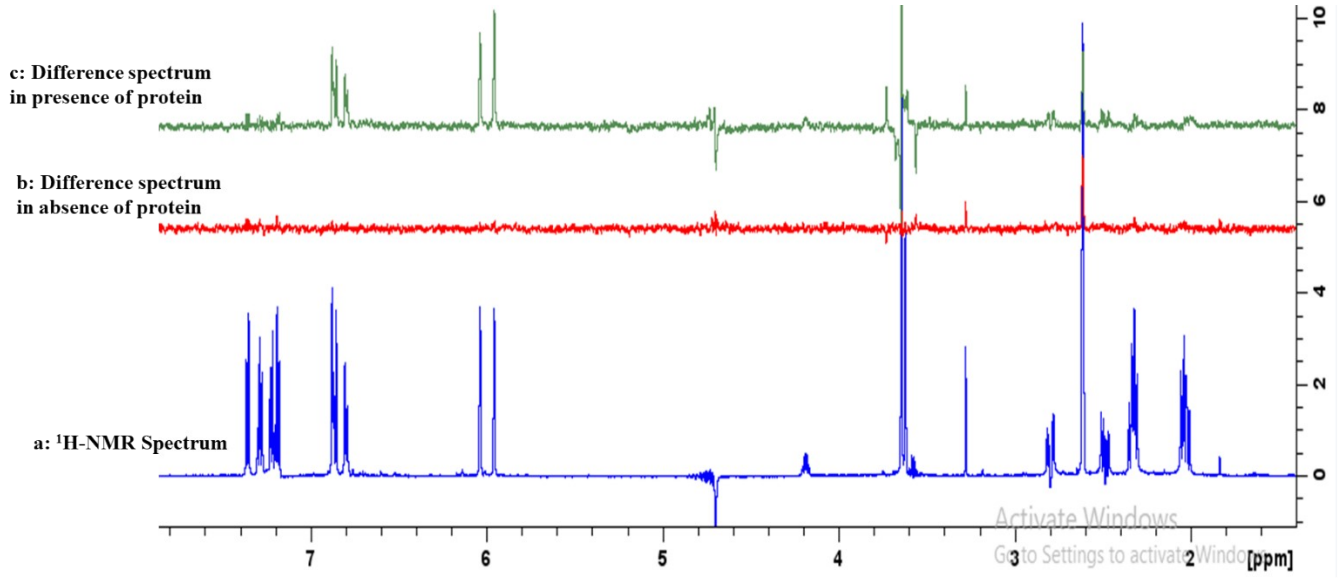




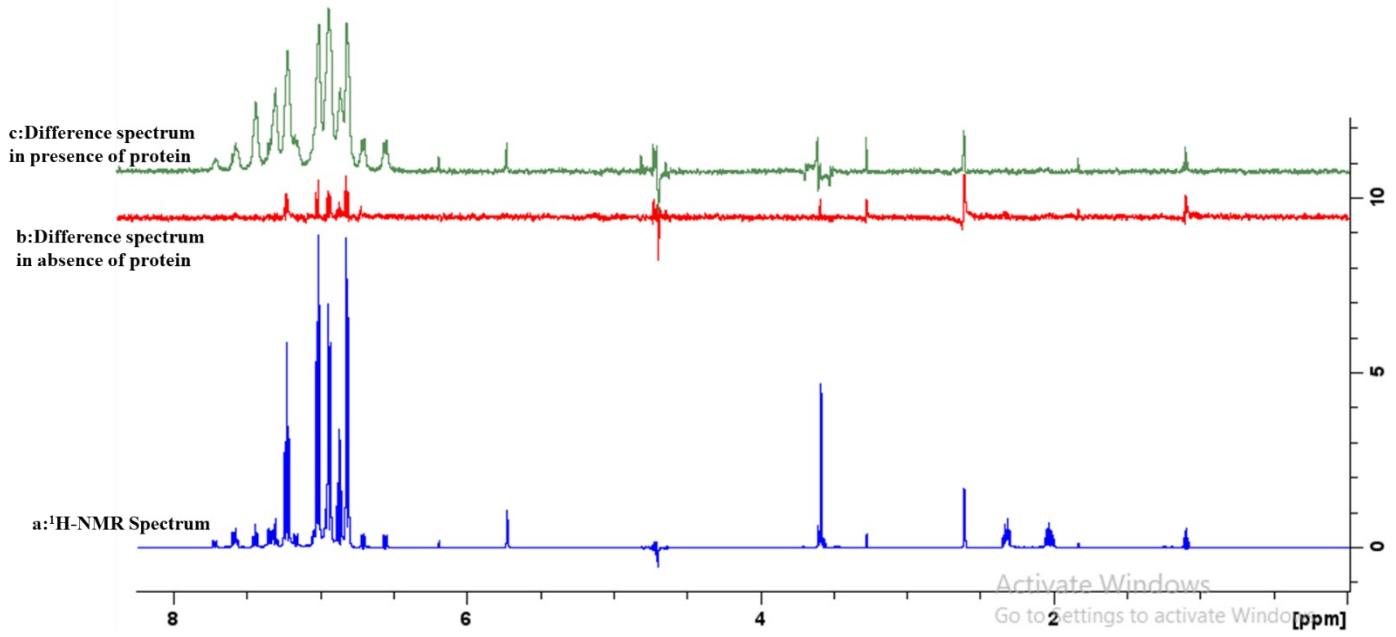
S2 Fig. The FPLC-and SDS-PAGE profile for USP proteins (a) Chromatogram represents the peak from the 0-90ml for an unbound protein. The protein eluted at 140ml represents the target protein (USP7-CD) highlighted by circle (b) The SDS-PAGE analysis of purified protein fractions of the USP7-CD. The fractions before the ladder represents the USP7 protein and fractions after the ladder represents the WO (wash out) and FT (flow-throw).



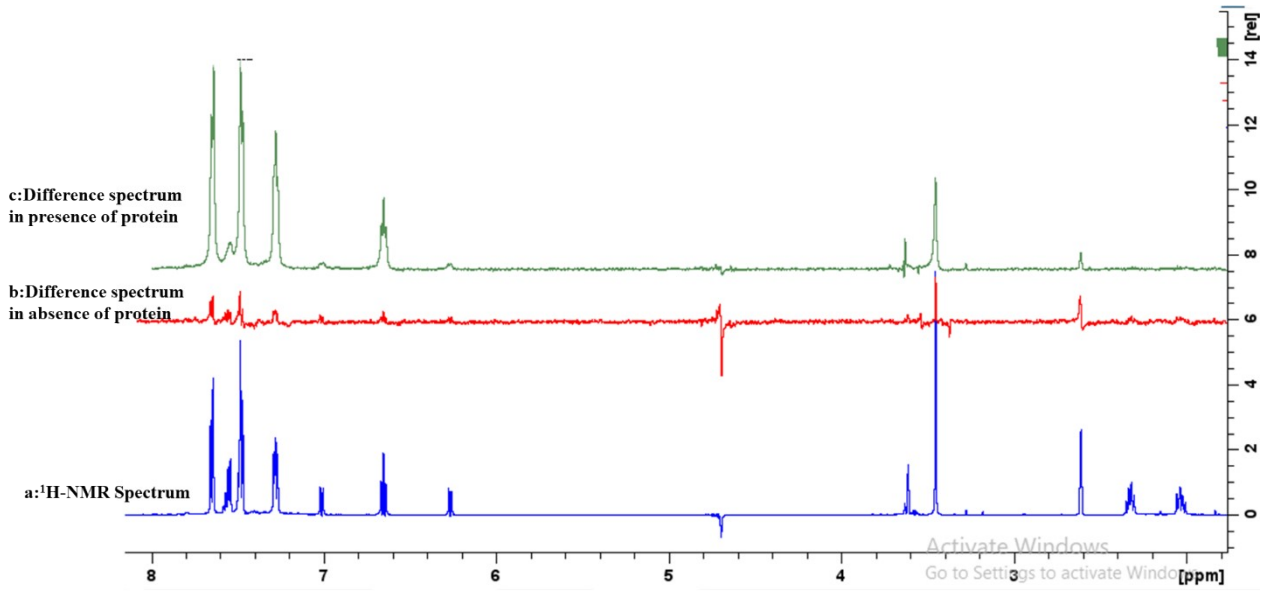
S3 Fig. The ^{1}H-NMR Spectra for USP7-CD



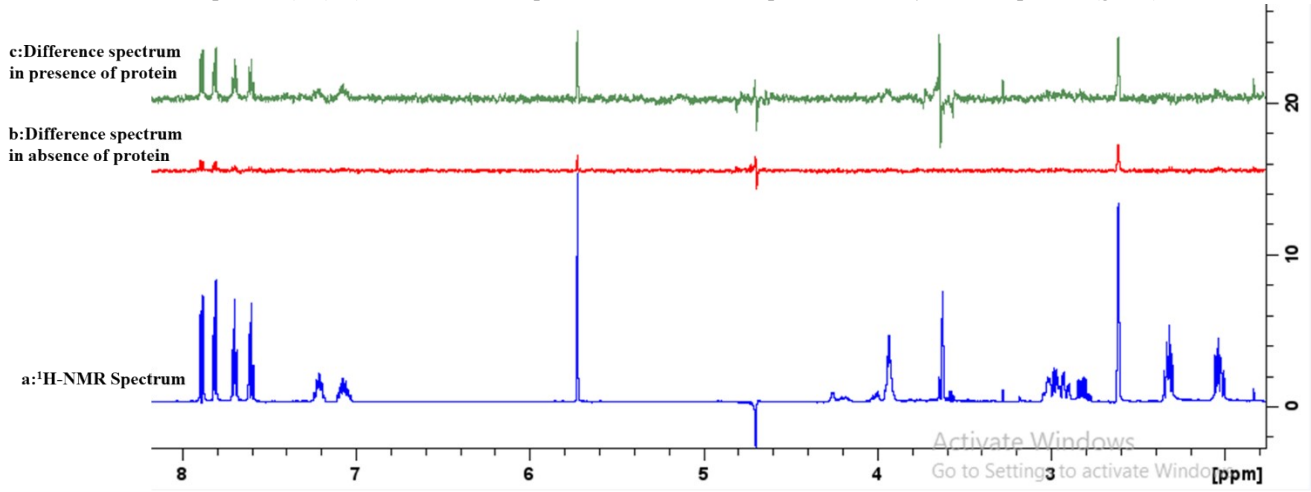
S4 Fig. NMR Analysis of Mixture 1. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



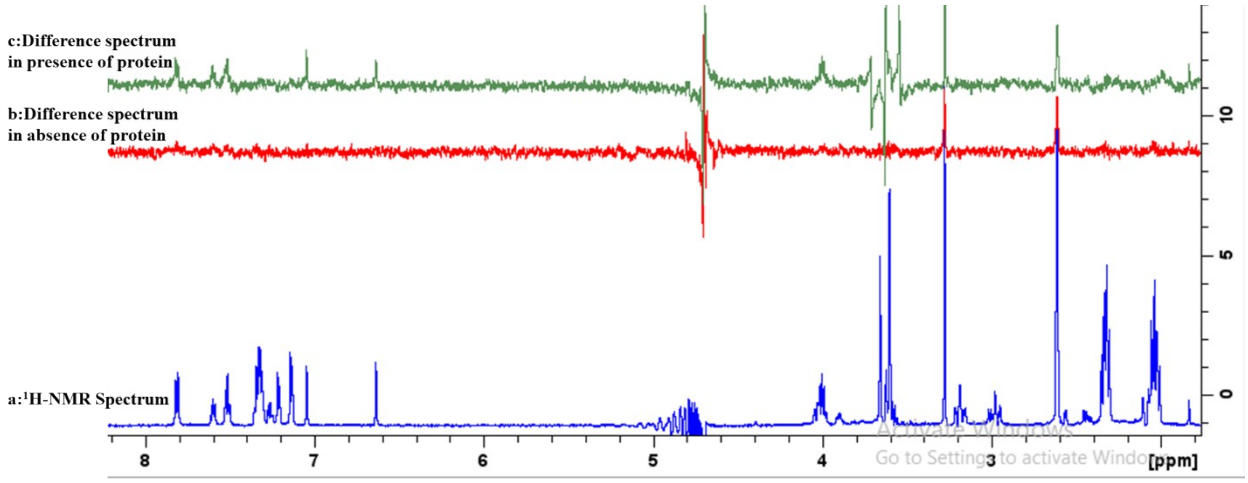
S5 Fig. STD-NMR Analysis of Mixture 4. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



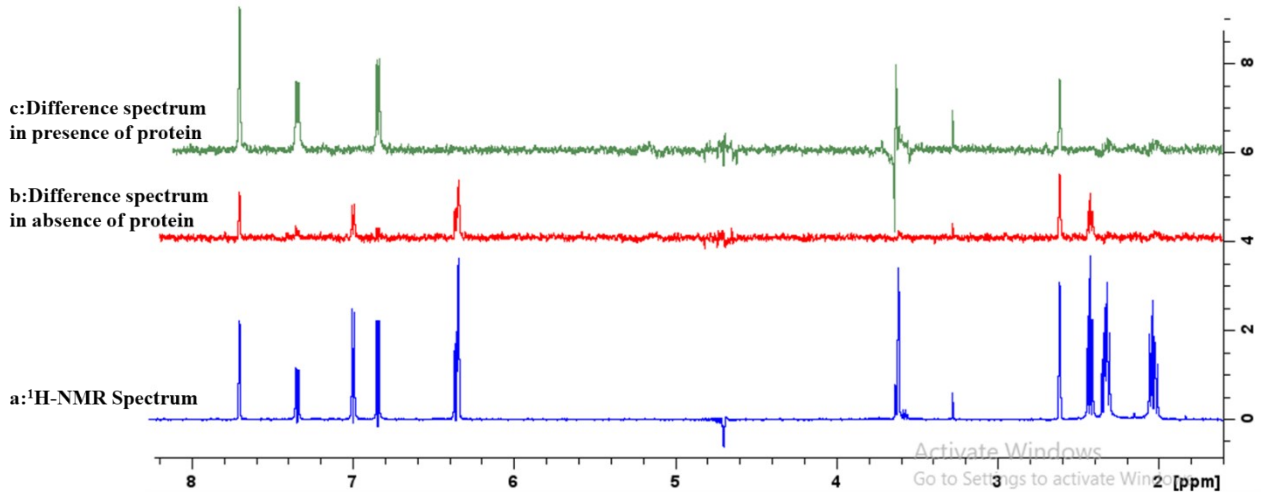
S6 Fig. STD-NMR Analysis of Mixture 7. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



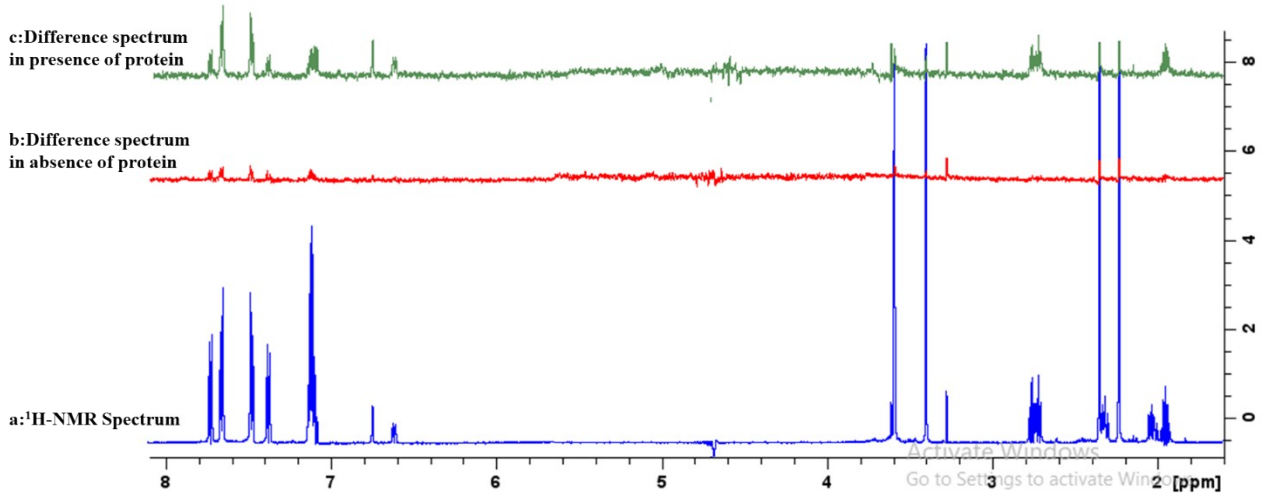
S7 Fig. STD-NMR Analysis of Mixture 9. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



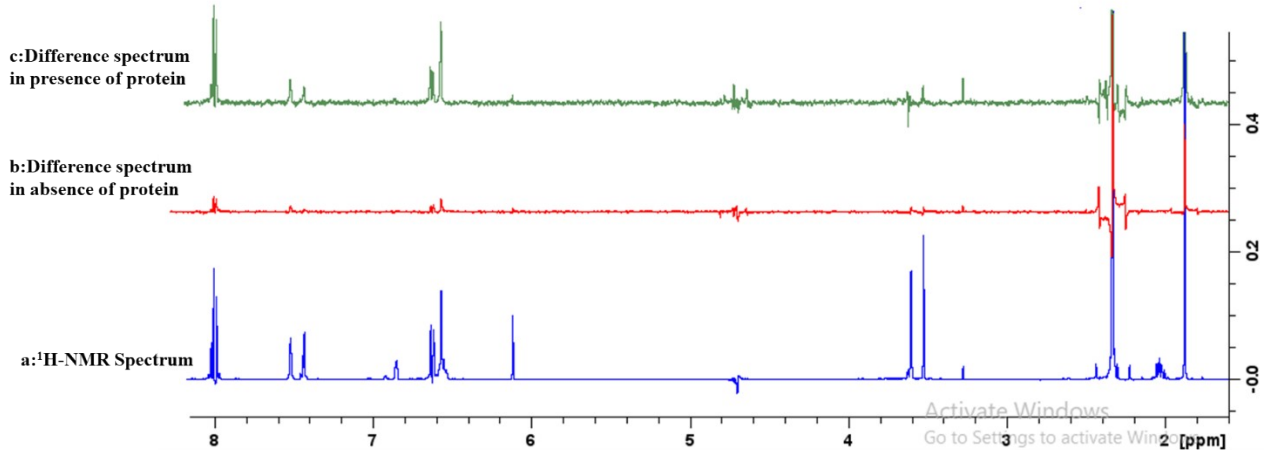
S8 Fig. STD-NMR Analysis of Mixture 11. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



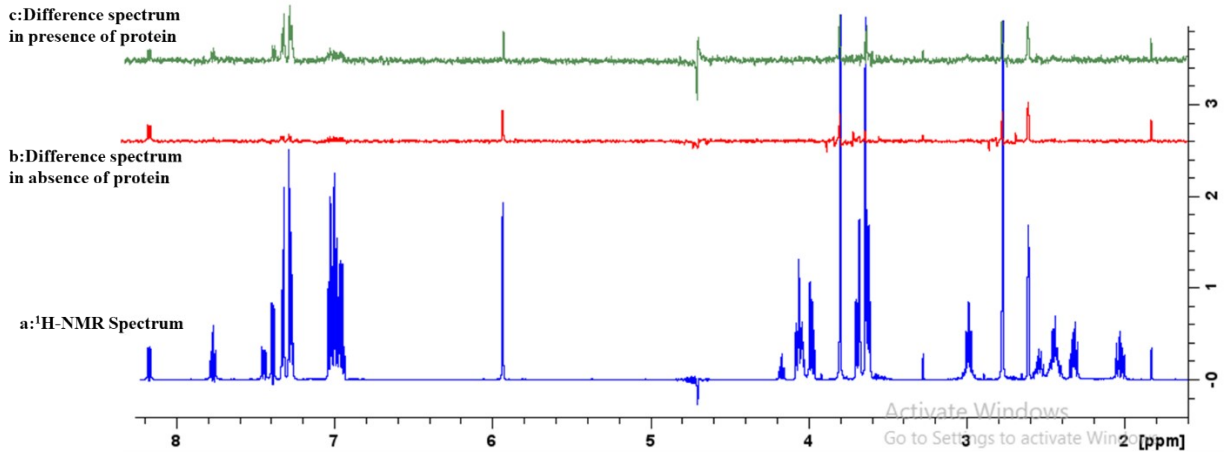
S9 Fig. STD-NMR Analysis of Mixture 14. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



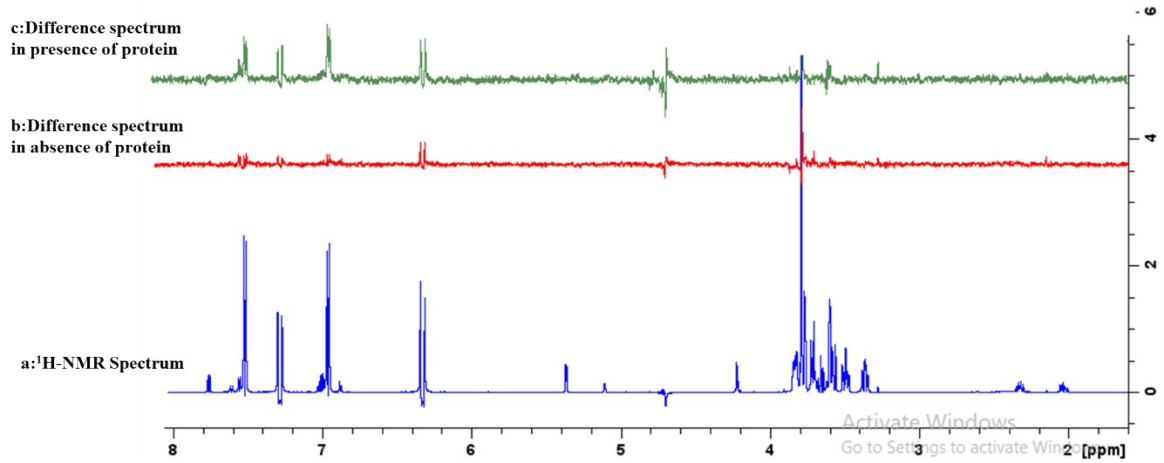
S10 Fig. STD-NMR Analysis of Mixture 17. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



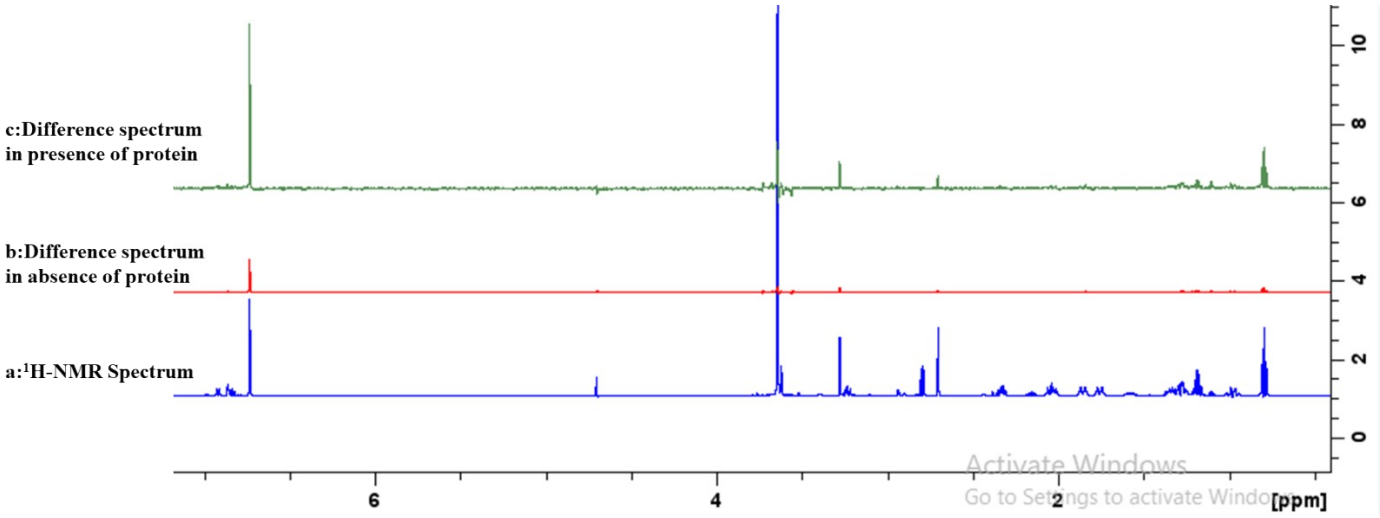
S11 Fig. STD-NMR Analysis of Mixture 26. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



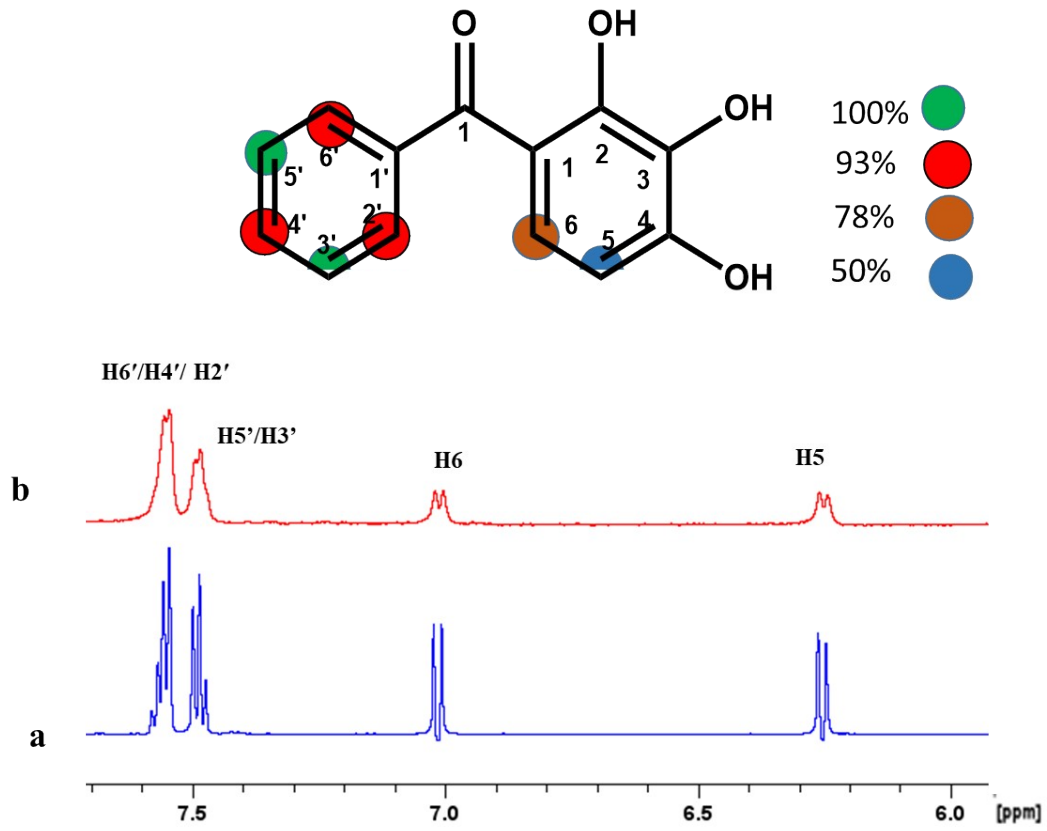
S12 Fig. STD-NMR Analysis of Mixture 29. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



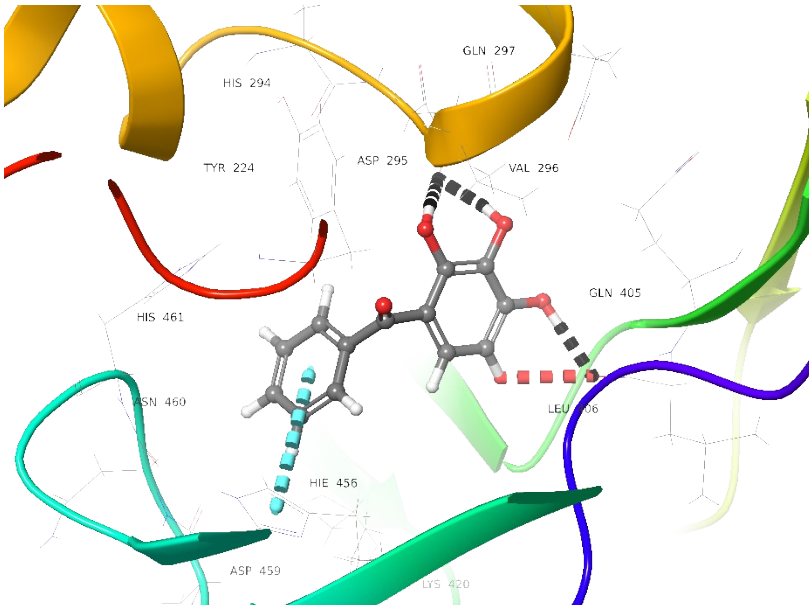
S13 Fig. STD-NMR Analysis of Mixture 32. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



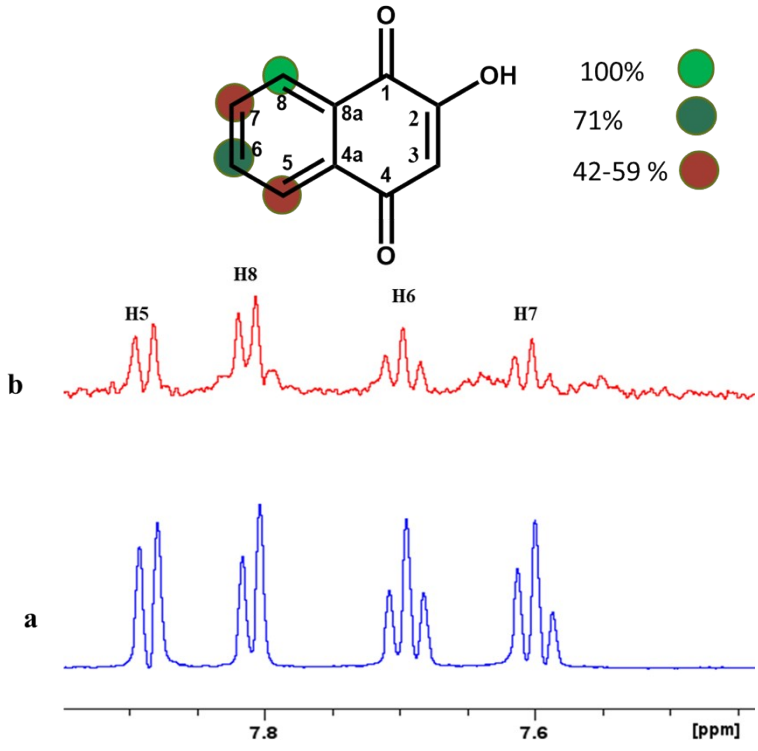
S14 Fig. STD-NMR Analysis of Mixture 38. a) ^{1}H spectrum (blue) of the mixture, b) STD reference spectrum recorded in the absence of protein (red), c) STD difference spectrum recorded in the presence of 10 µM USP7 protein (green).



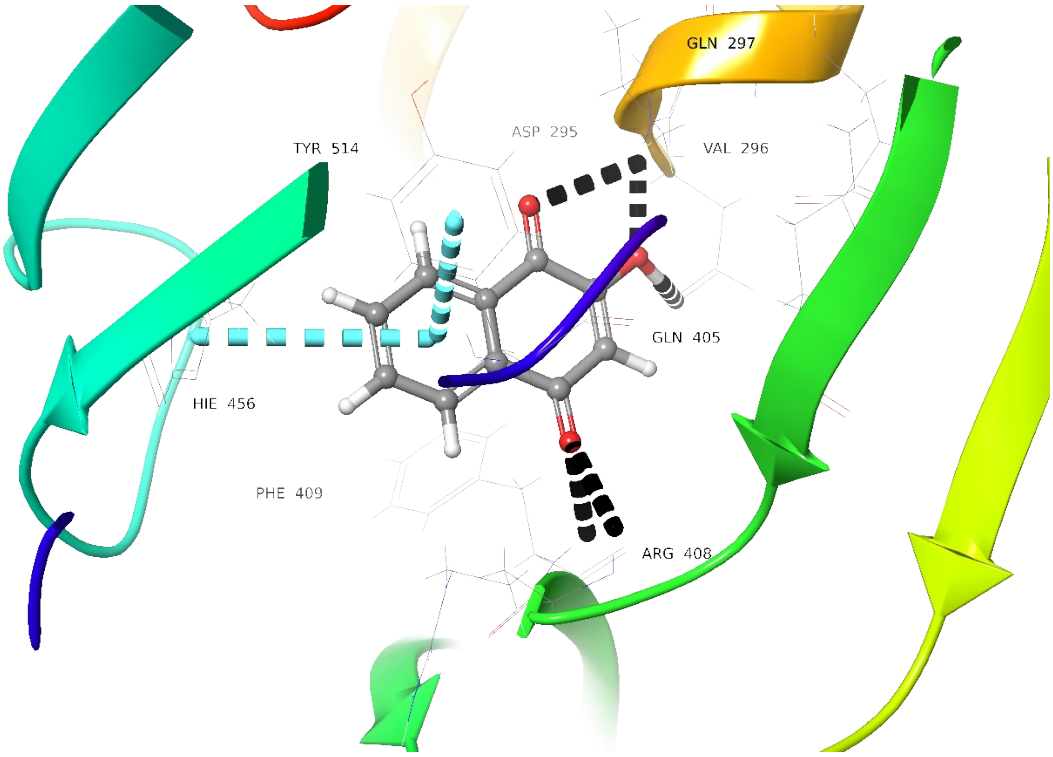
S15a Fig. STD-NMR Analysis of Compound 3 with USP7-CD. a) The ^{1}H NMR of compound **3** (blue) and b) STD-difference spectrum of compound **3** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to proton H3′/H5′ is represented with different color codes.



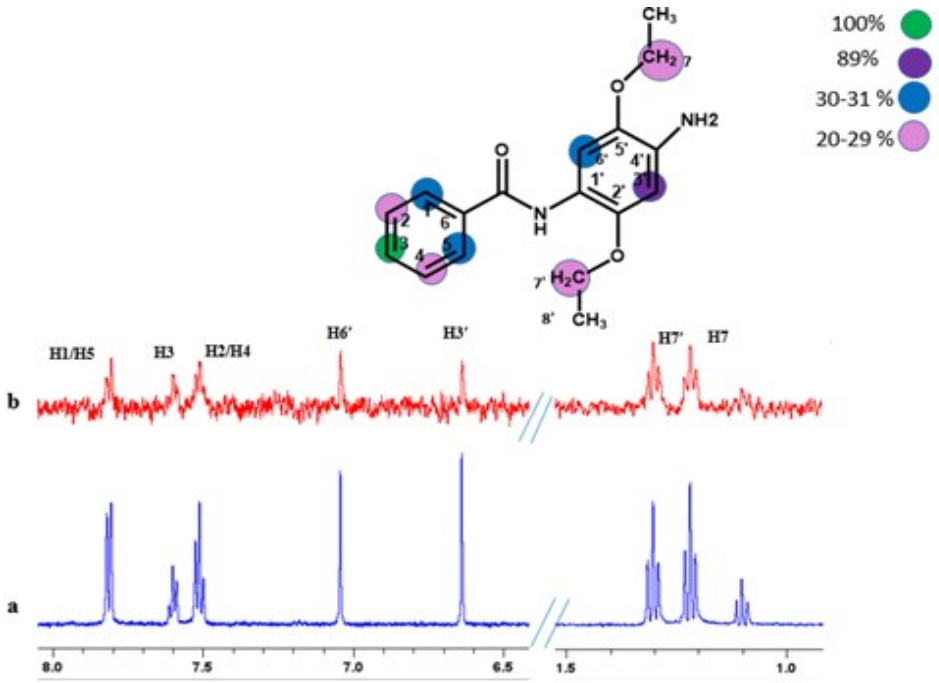
S15b Fig. Ribbon Representation of USP7-CD with Compound 3: The hydrogen bonds (black color) are shown with Asp295, and Leu406, while aromatic hydrogen bond (red color) with Leu406. The unsubstituted ring showed π-π stacking interactions (blue color) with His456.



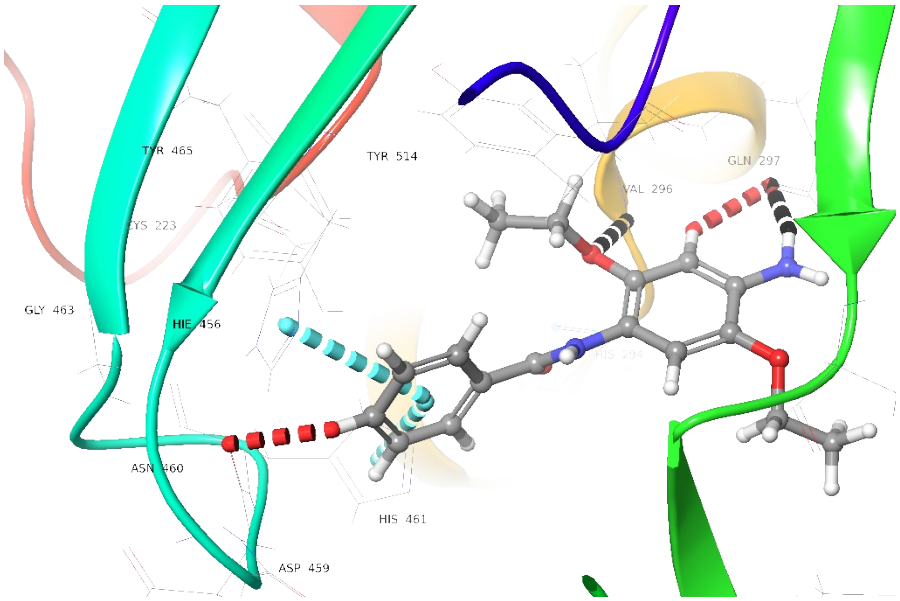
S16a Fig. STD-NMR Analysis of Compound 4 with USP7-CD a) ^{1}H NMR spectrum (blue) of compound **4** b) STD difference spectrum of compound **4** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H6 is represented with different color codes.



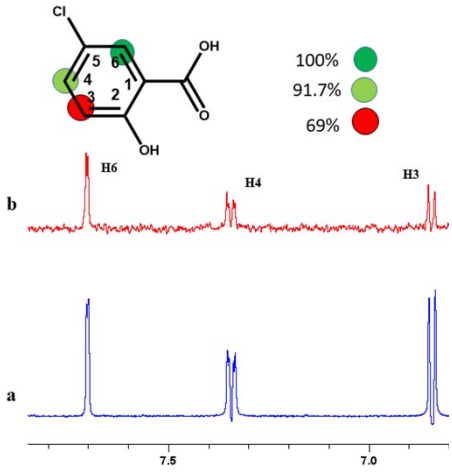
S16b Fig. Ribbon Representation of USP7-CD with Compound 4: The hydrogen bonds (black color) are shown with Asp295, Val296, Arg408, and Phe409. The aromatic ring showed two π-π stacking interactions (blue color) with His461, and Tyr514.



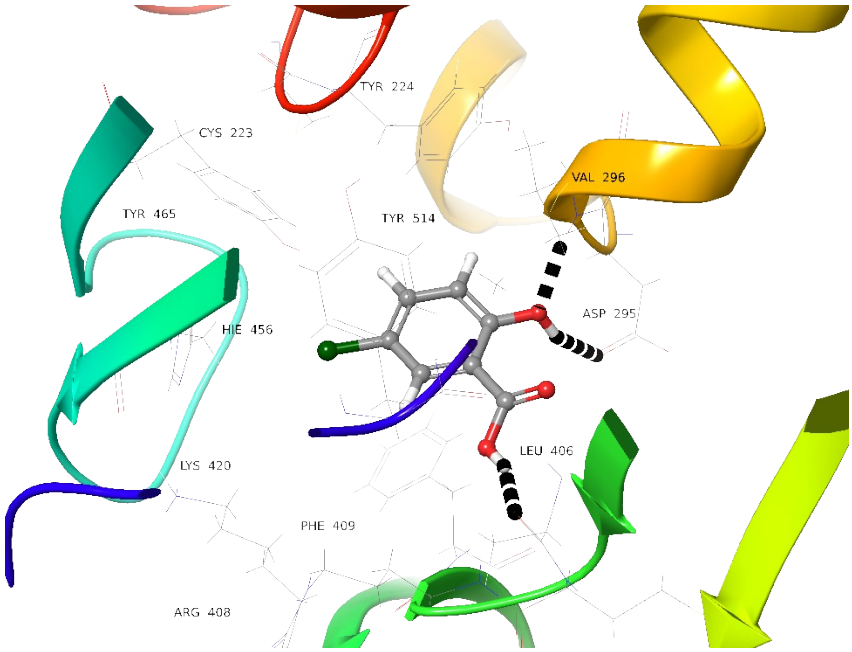
S17a Fig. STD-NMR Analysis of Compound 5 with USP7-CD. a) ^{1}H NMR spectrum of compound **5** (blue) b) STD difference spectrum of compound **5** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H3 is represented ith different color codes.



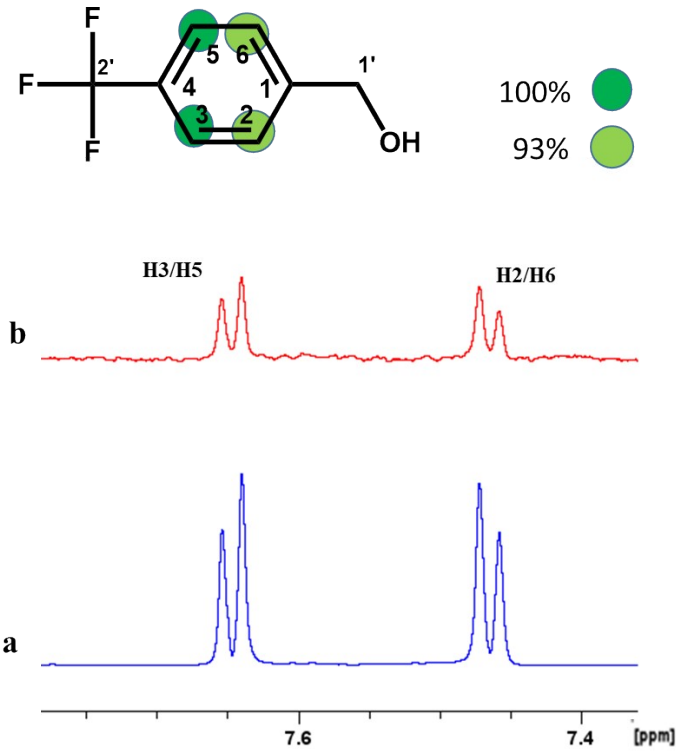
S17b Fig. Ribbon Representation of USP7-CD with Compound 5: The hydrogen bonds (black color) are shown with Val296, and Gln297, while aromatic hydrogen bond (red color) with Asn465, and Gln297. The unsubstituted ring showed two π-π stacking interactions (blue color) with His461, and His456.



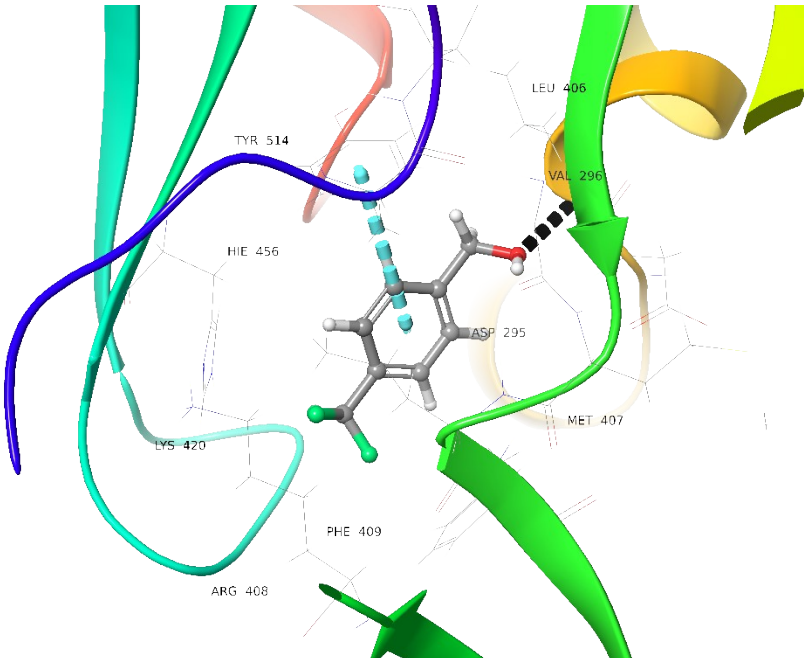
S18a Fig. STD-NMR Analysis of Compound 6 with USP7-CD. a) ^{1}H NMR spectrum of compound **6** (blue) b) STD difference spectrum of compound **6** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H6 is represented with different color codes.



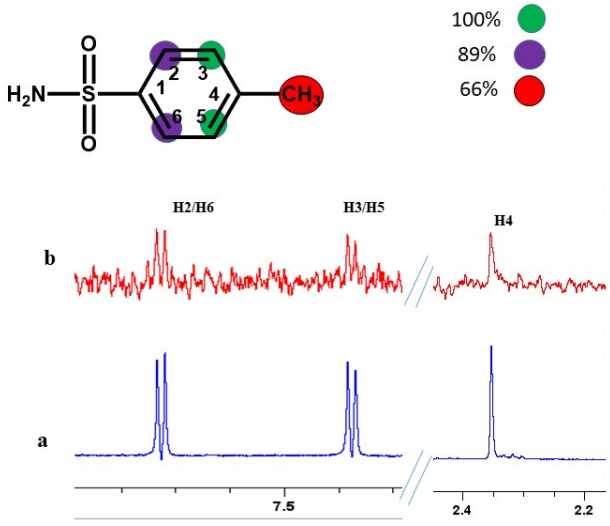
S18b Fig. Ribbon Representation of USP7-CD with Compound 6: The hydrogen bonds (black color) are shown with Val296, Asp295, and Leu406.



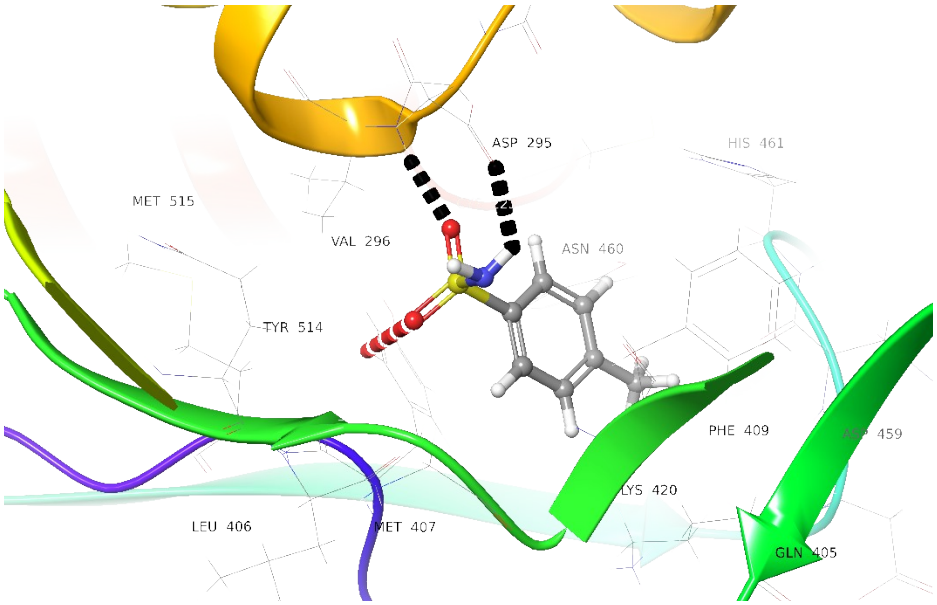
S19a Fig. STD-NMR Analysis of compound 7 with USP7-CD. a) ^{1}H NMR spectrum of compound **7** (blue) b) STD difference spectrum of compound **7** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H1′is represented with different color codes.



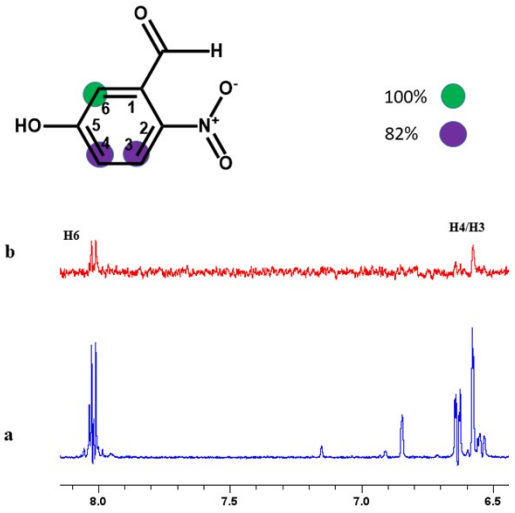
S19b Fig. Ribbon Representation of USP7-CD with Compound 7: The hydrogen bonds (black color) are shown with Val296. The aromatic ring showed two π-π stacking interactions (blue color) with Tyr514.



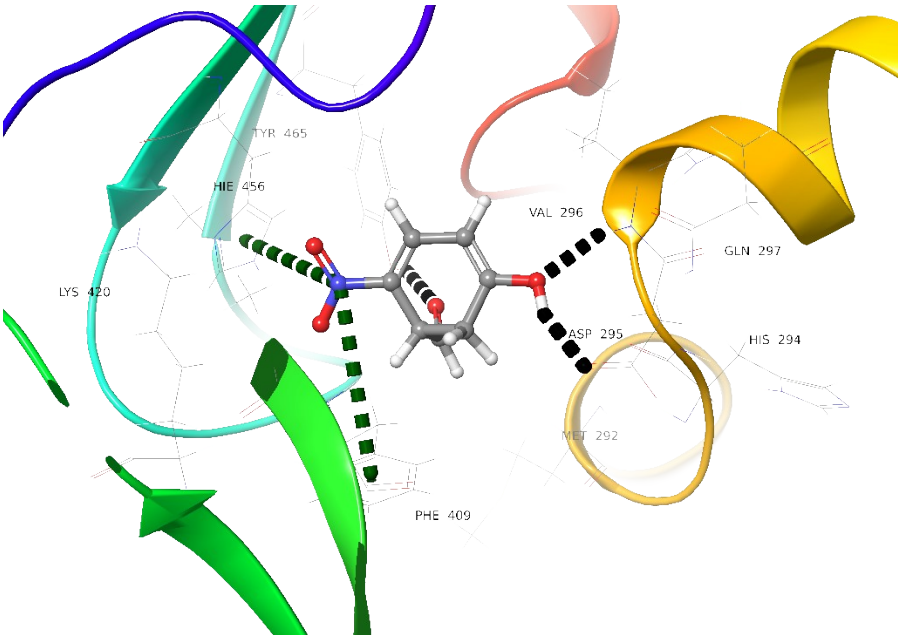
S20a Fig. STD-NMR Analysis of Compound 8 with USP7-CD. a) ^{1}H NMR spectrum of compound **8** (blue) b) STD difference spectrum of compound **8** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H3/H5 is represented with different color codes.



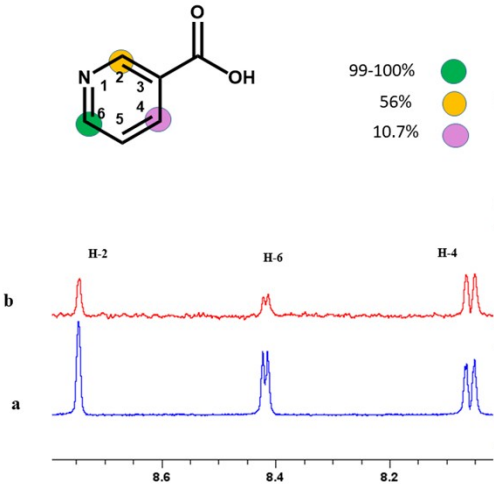
S20b Fig. Ribbon Representation of USP7-CD with Compound 8: The hydrogen bonds (black color) are shown with Val296, and Asp295, while aromatic hydrogen bond (red color) with Tyr514.



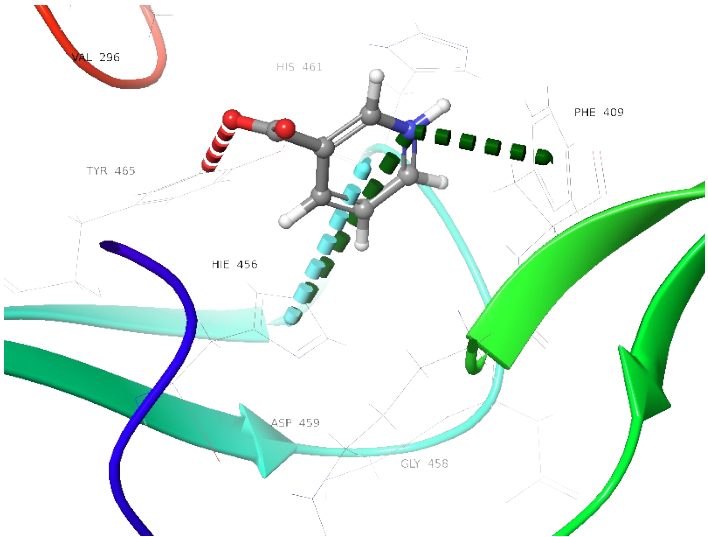
S21a Fig. STD-NMR Analysis of Compound 9 with USP7-CD. a) ^{1}H NMR spectrum of compound **9** (blue) b) STD difference spectrum of compound **9** recorded in the presence of 10 µM USP7 protein (red). Relative saturation of other protons normalized with reference to H6 is represented with different color codes.



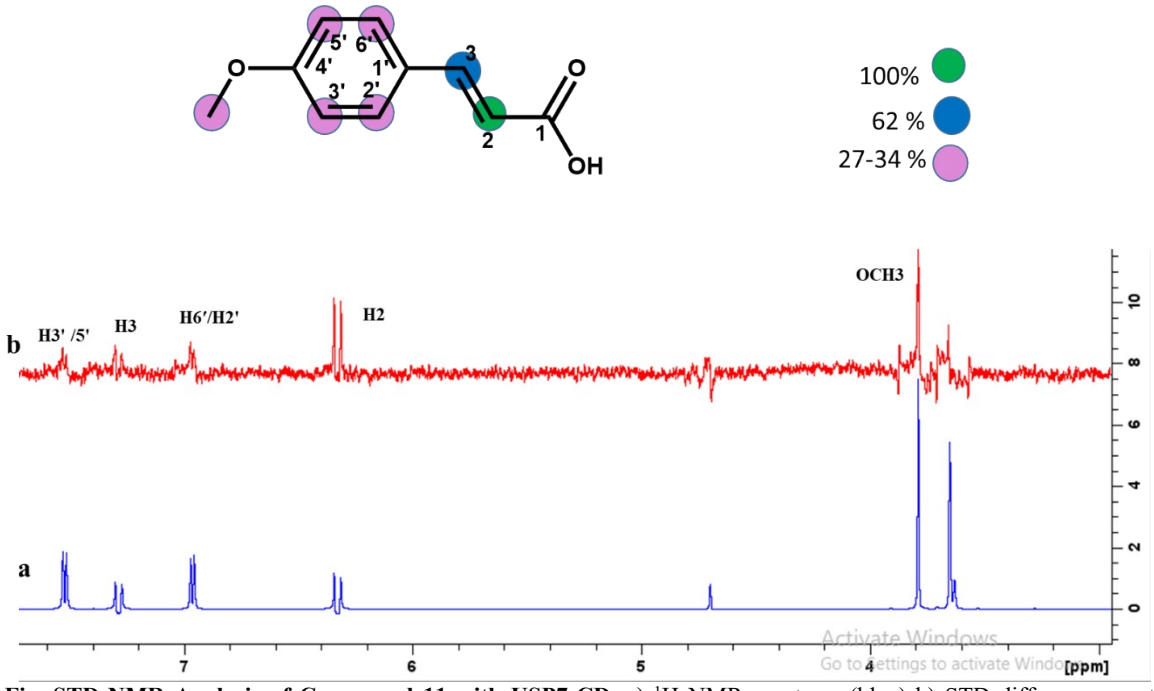
S21b Fig. Ribbon Representation of USP7-CD with Compound 9: The hydrogen bonds (black color) are shown with Val296, Asp295, and Tyr465. The aromatic ring showed two π-cationic interactions (green color) with Phe409, and His456.



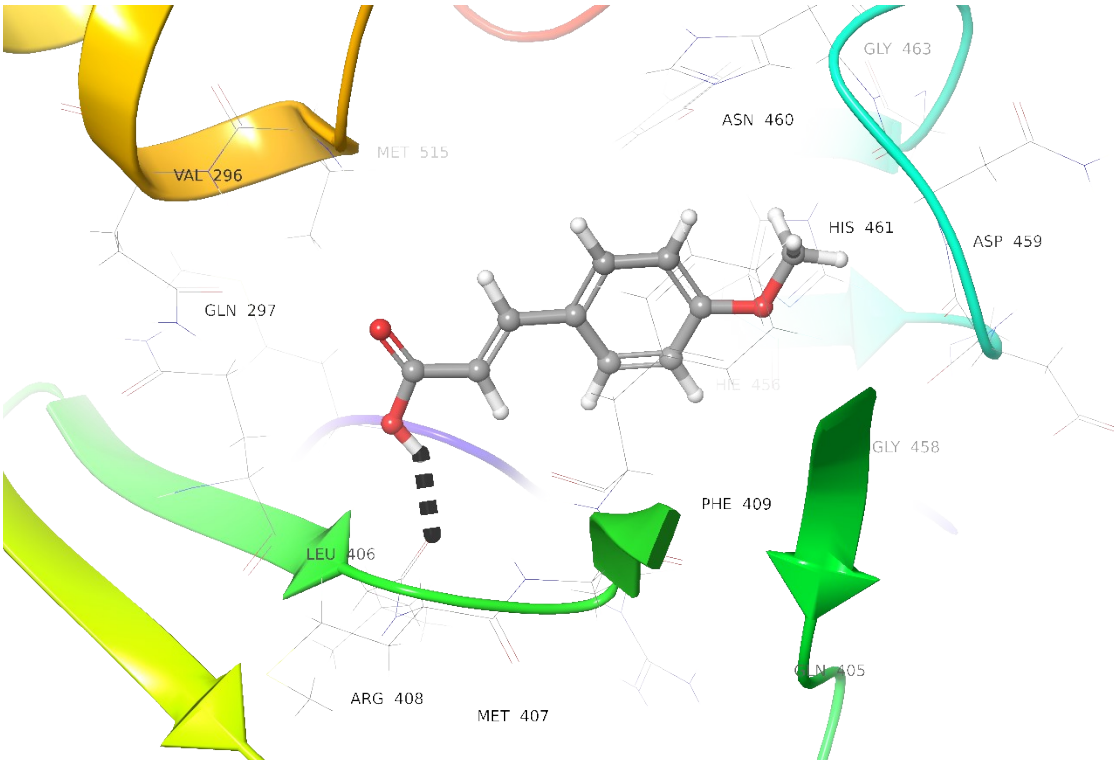
S22a Fig. STD-NMR Analysis of Compound 10 with USP7-CD. a) ^{1}H NMR spectrum of compound **10** (blue) b) STD difference spectrum of compound **10** recorded in the presence of 10 μM USP7 protein (red). Relative saturation of protons normalized with reference to H6 is represented with different color codes.



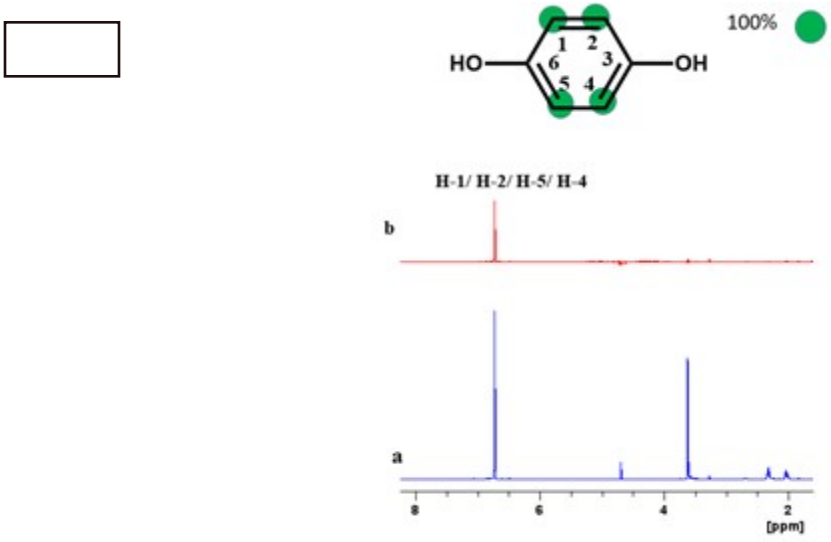
S22b Fig. Ribbon Representation of USP7-CD with Compound 10: The aromatic hydrogen bond (red color) are shown with Tyr 465, the nitrogen showed π-cationic interactions (green color) with Phe409, and His456, the aromatic ring showed π- π interactions (blue color) with His456.



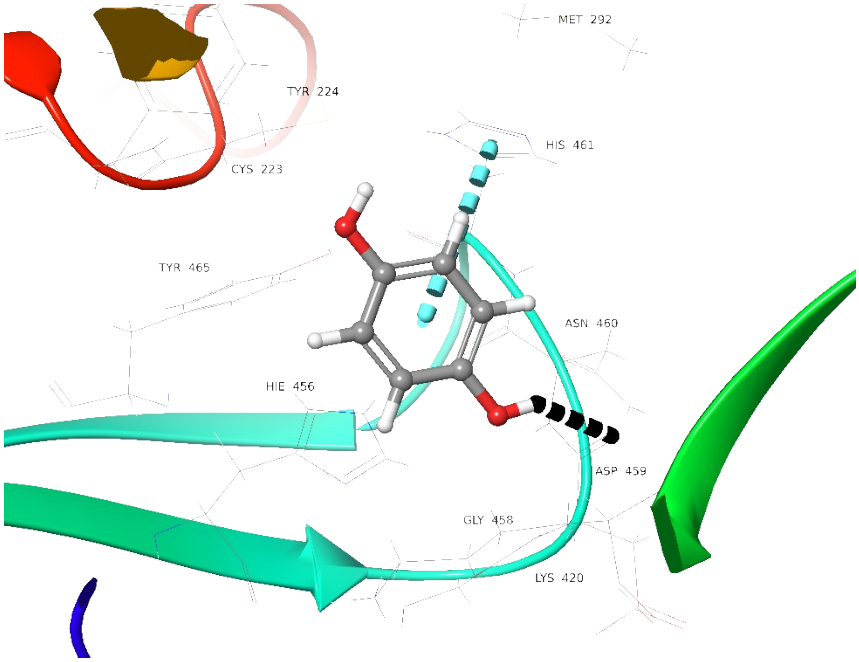
S23a Fig. STD-NMR Analysis of Compound 11 with USP7-CD. a) ^{1}H NMR spectrum (blue) b) STD difference spectrum recorded in the presence of 10 µM USP7 protein (red). Relative saturation of protons normalized with reference to H6 is represented with different color codes.



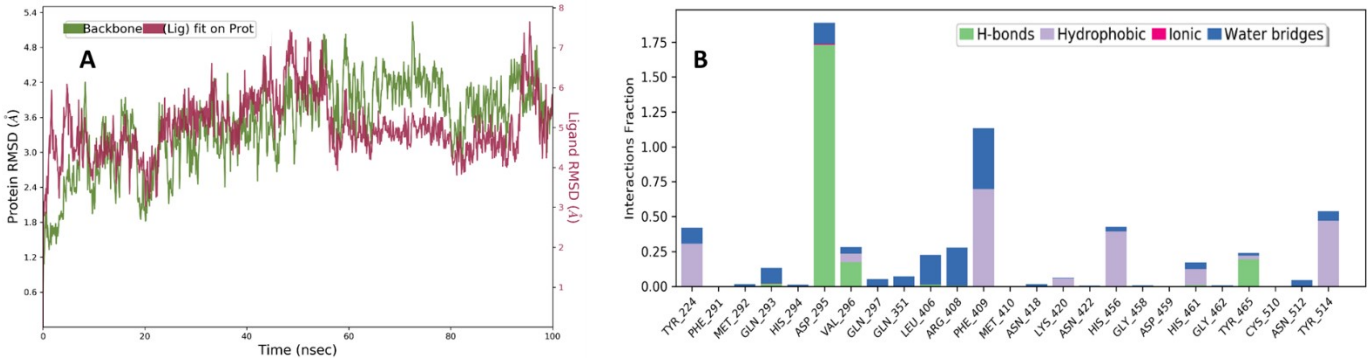
S23b Fig. Ribbon Representation of USP7-CD with Compound 11: The hydrogen bond (black color) is shown with Leu406.



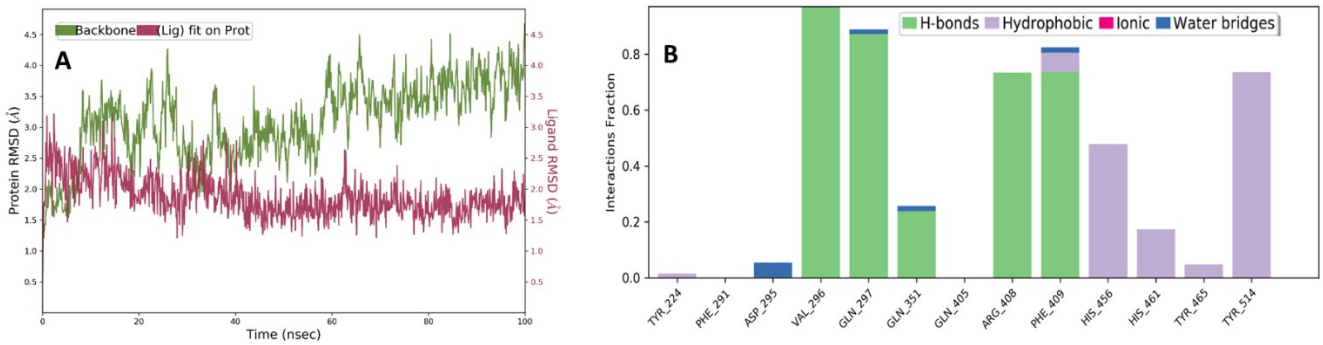
S24a Fig. STD-NMR Analysis of Compound 12 with USP7-CD. a) ^{1}H-NMR spectra of the compound **12** (blue), b) STD difference spectrum compound **12** recorded in the presence of 10 µM USP7 protein (red).



S24b Fig. Ribbon Representation of USP7-CD with Compound 12: The hydrogen bonds (black color) is shown with Asp459, while π-π stacking interactions (blue color) with His461.

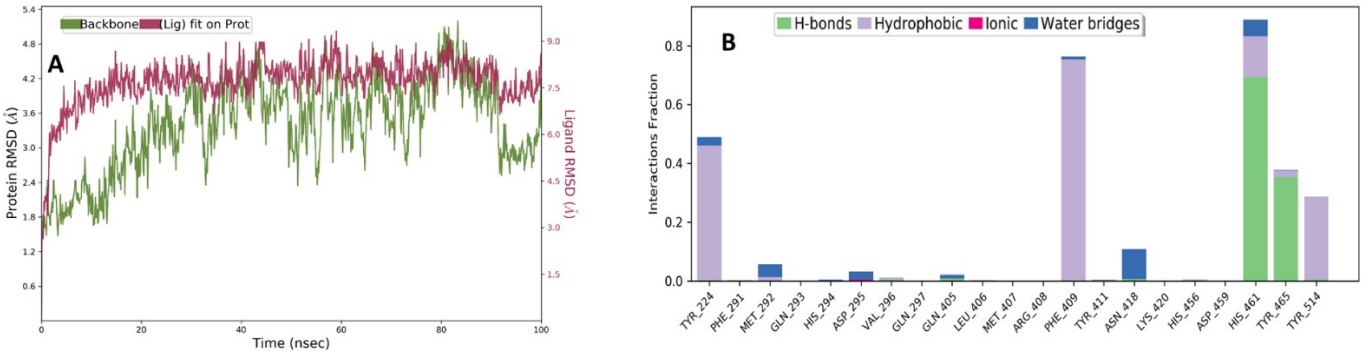


S25 Fig. RMSD plots of USP7 (green color), and compound **3** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **3.**

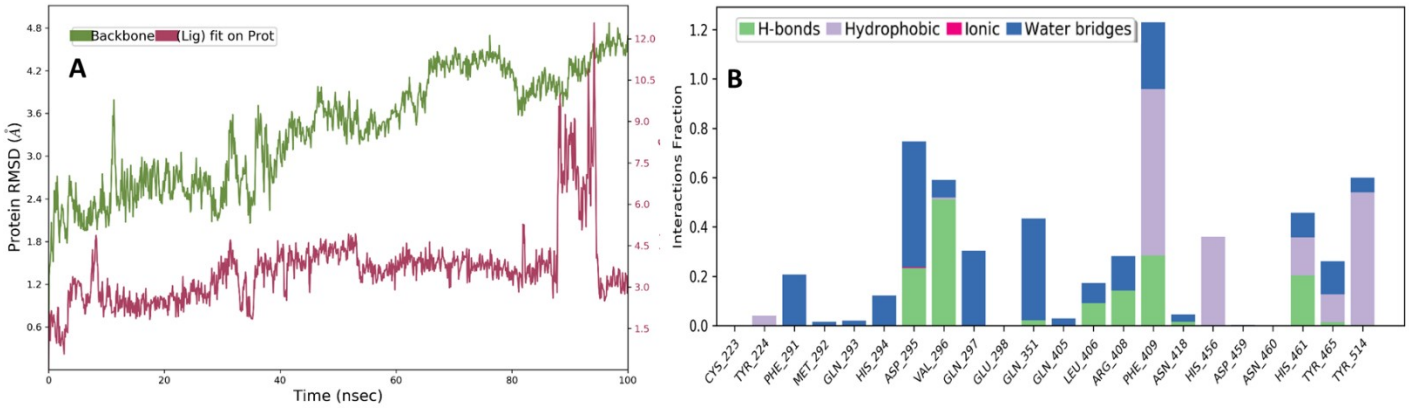


S26 Fig. RMSD plots of USP7 (green color), and compound **4** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7

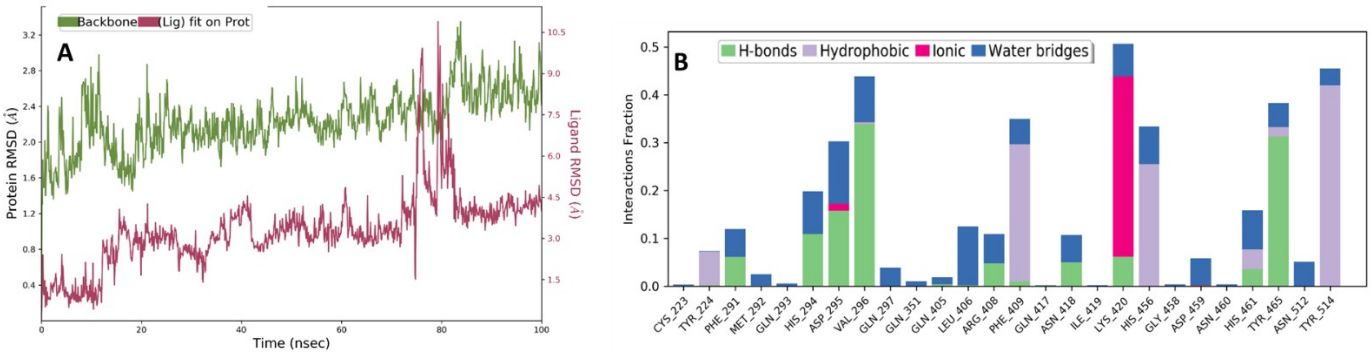
and compound **4.**



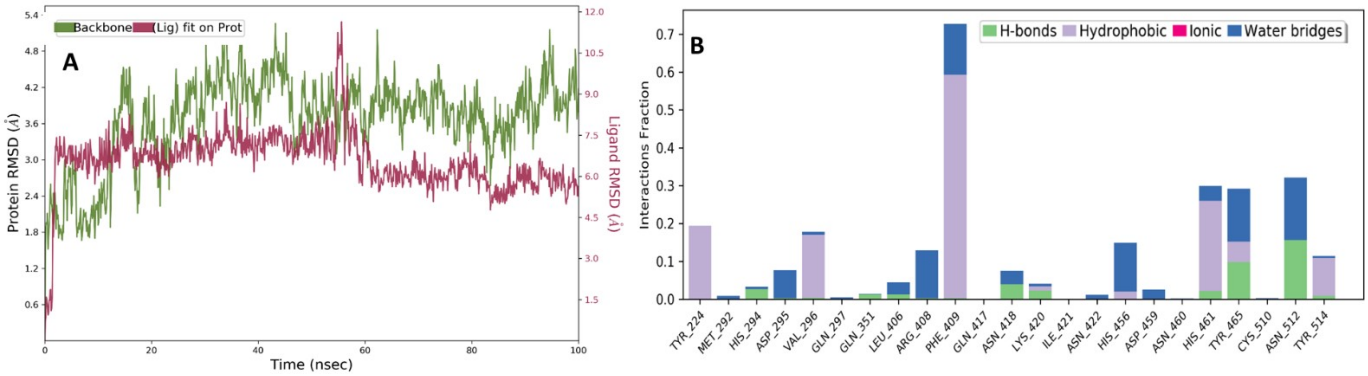
S27 Fig. RMSD plots of USP7 (green color), and compound **5** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **5**.



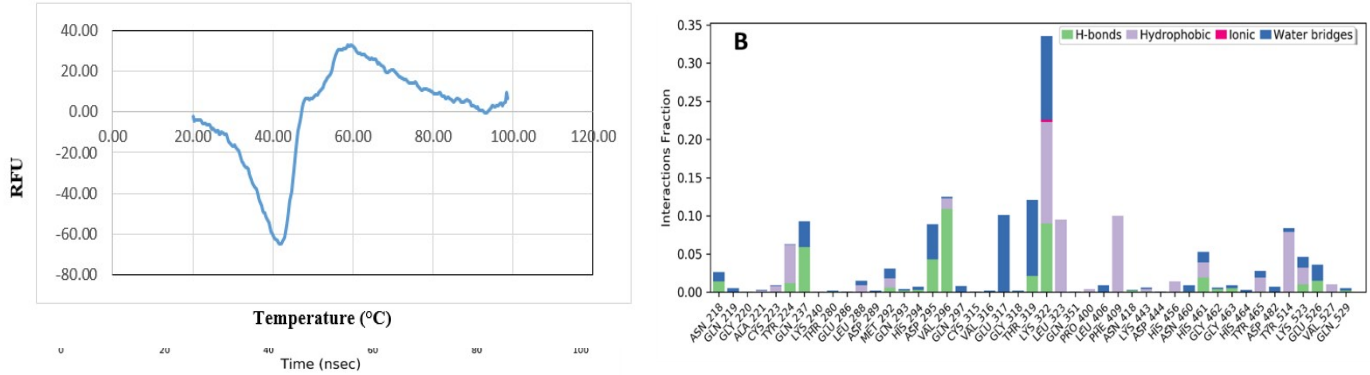
S28 Fig. RMSD plots of USP7 (green color), and compound **6** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **6**.



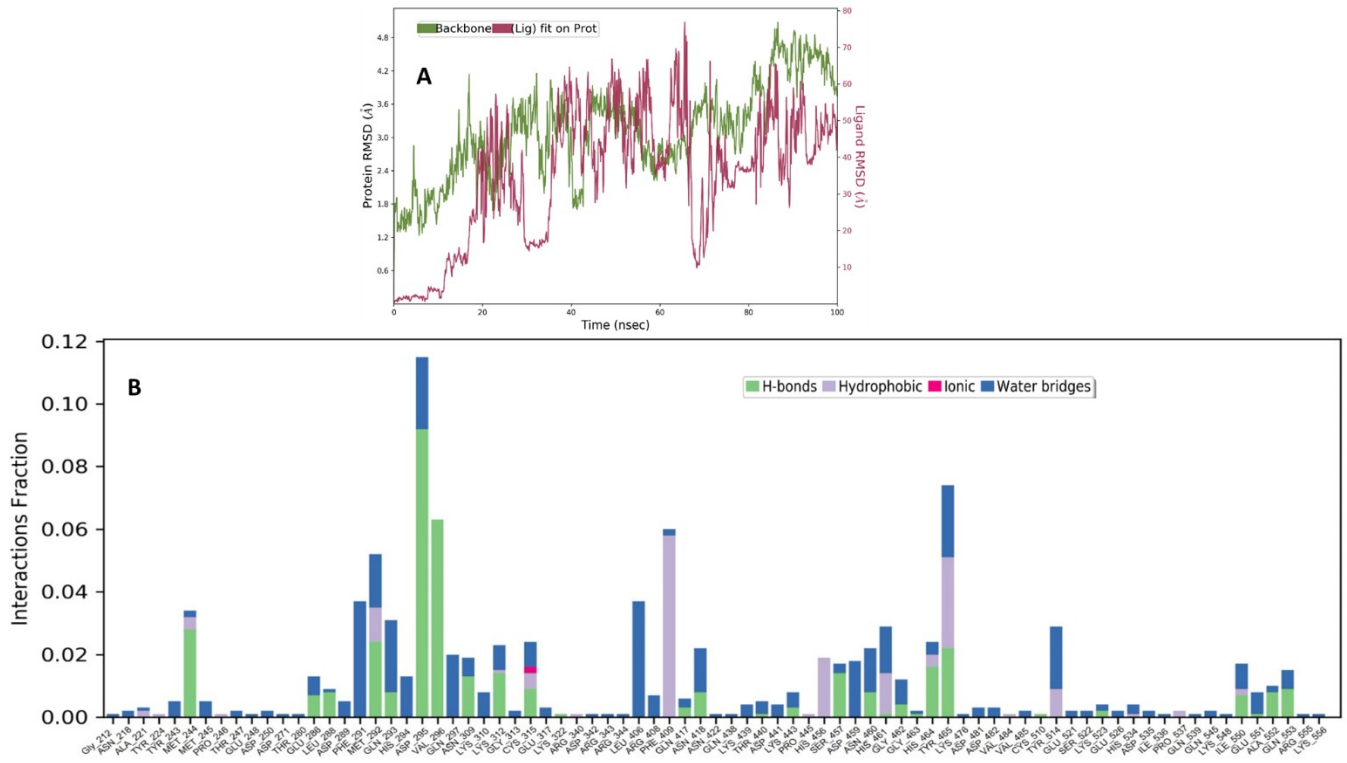
S29 Fig. RMSD plots of USP7 (green color), and compound **9** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **9**.



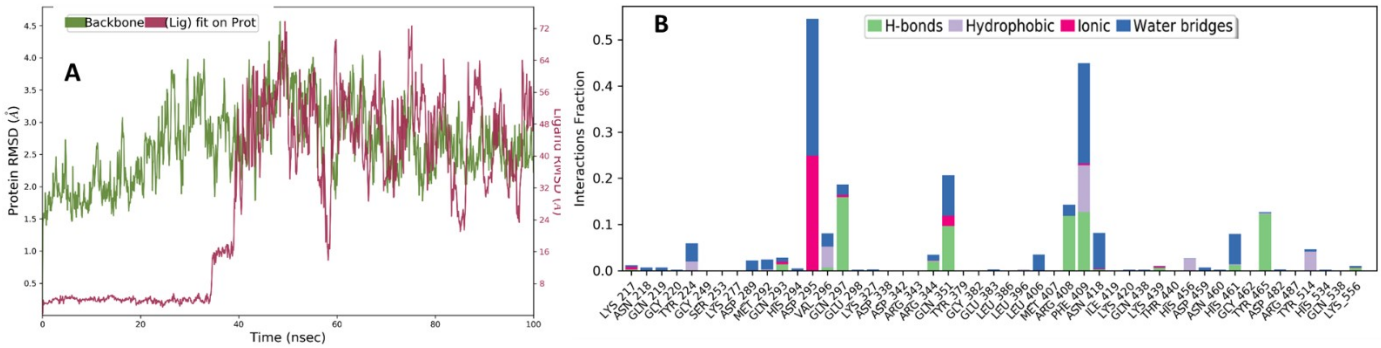
S30 Fig. RMSD plots of USP7 (green color), and compound **11** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **11**.



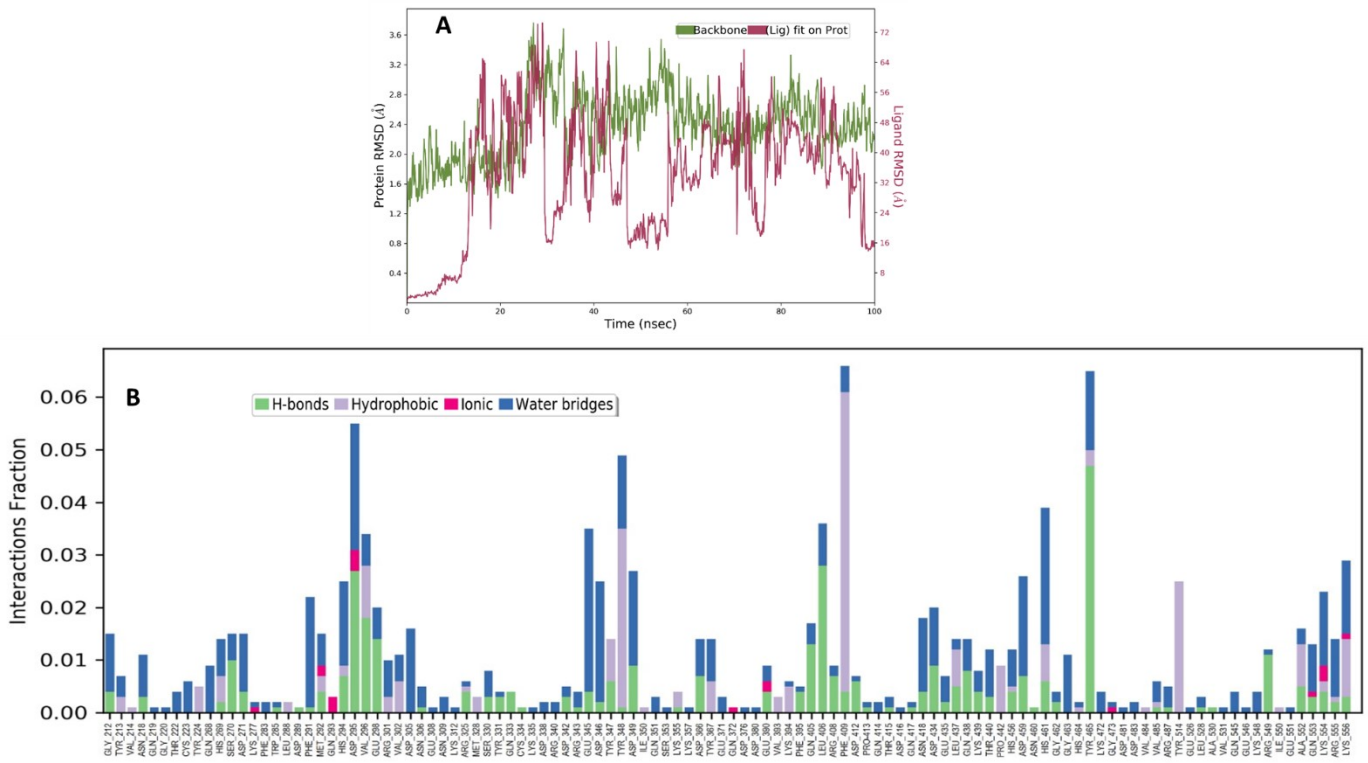
S31 Fig. RMSD plots of USP7 (green color), and compound **7** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **7**.



S32 Fig. RMSD plots of USP7 (green color), and compound **8** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **8**.

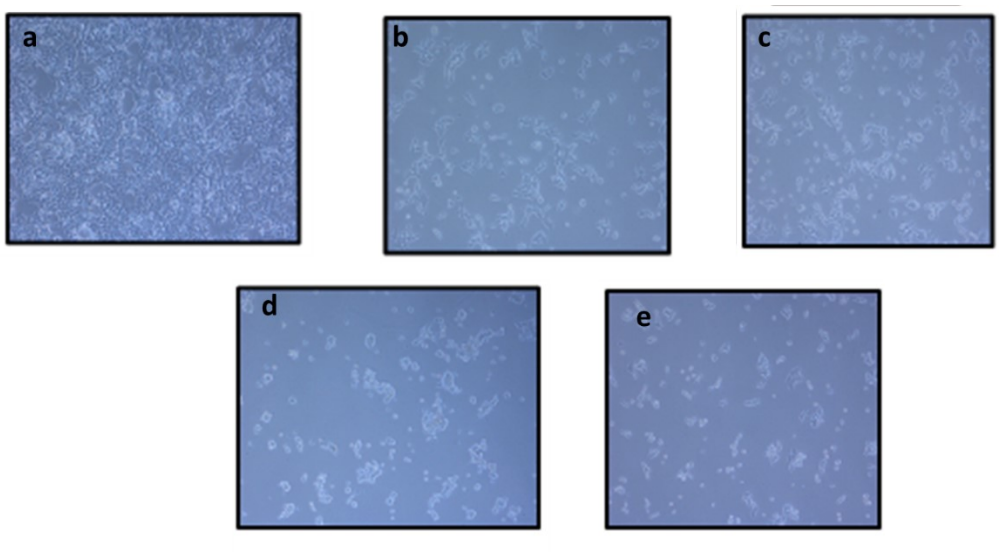


S33 Fig. RMSD plots of USP7 (green color), and compound **10** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **10**.

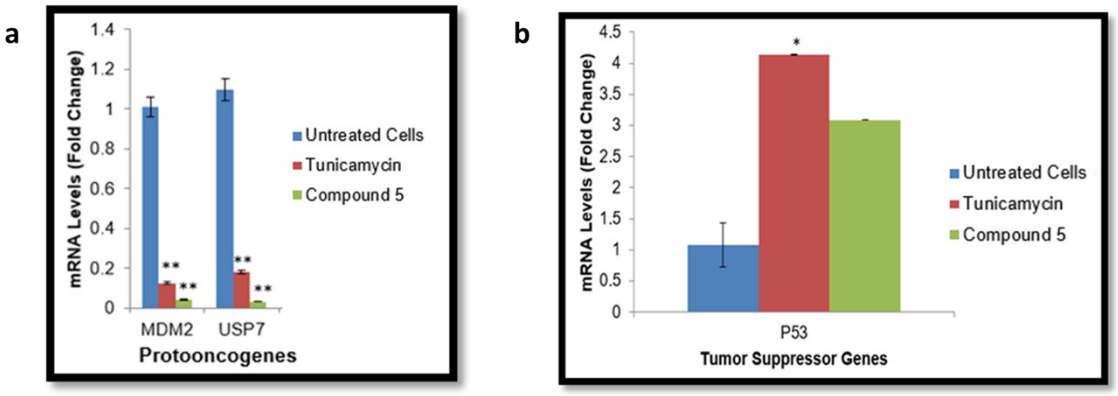


S34 Fig. RMSD plots of USP7 (green color), and compound **12** (red color) indicating the evolution of protein-ligand complex for 100 nsec, (B) Histogram of the fraction of time for which non-covalent interactions were retained between the residues of USP7 and compound **12**.

S35 Fig. The Thermal Shift Profile of USP Protein. The blue line in the figure represents the thermal profile of the protein when subjected to a temperature range from 20 to 99°C indicating a Tm of 45 °C for USP7-CD.

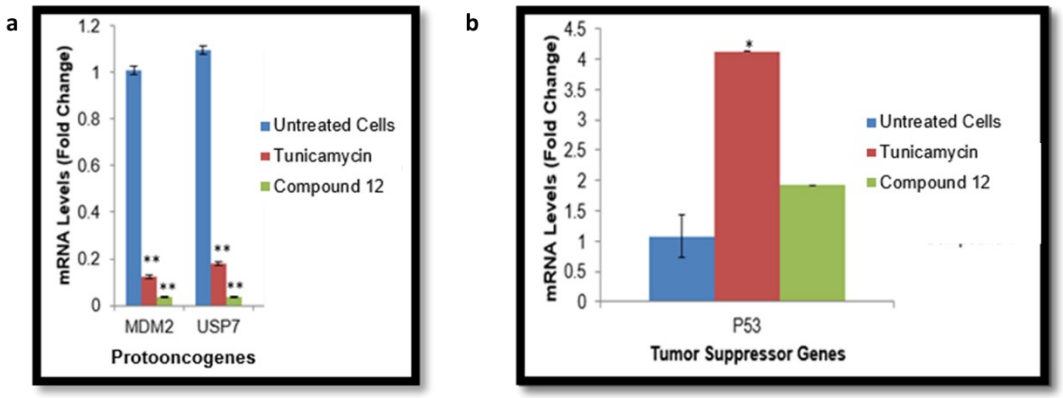


S36 Fig. **Morphology of HCT-116 cells. a**) shows control cells, **b**) compound **2** treated cells, **c**) compound **3** treated cells, **d**) compound **5** treated cells, and **e)** compound **12** treated cells.



S37 Fig. Effect of compound 5 on the mRNA expression of proto-oncogene and tumor suppressor gene. a) mRNA expression of

proto-oncogenes USP7 and MDM2 (b) mRNA expression of tumor-suppressive gene p53 (*$P \leq 0.05$, and **$P \leq 0.01$).



S38 Fig. Effect of compound 12 on the expression of proto-oncogene and tumor suppressor gene. a) mRNA expression of proto-oncogenes USP7 and MDM2 (b) mRNA expression of tumor-suppressive gene p53 (*$P \leq 0.05$, and **$P \leq 0.01$).