Supplementary Data

Table-S1: PCR reaction mixture for gene isolation.

Component	Volume in µL (Concentration)
Nuclease Free Water	44.5
PCR Buffer (10 x)	7.5 (1x)
dNTPs	15 (100 µM)
Primer Forward	3 (0.4 µM)
Primer Reverse	3 (0.4 µM)
MRSA252 DNA	1 (65 ng)
Pfu Polymerase	1 (5 Units)
Final Volume	75

Table-S2: Thermal conditions for gene isolation PCR

Steps	Temperature (˚C)	Time (sec)	No. of Cycle/s
Initial denaturation	95	120	1
Denaturation	94	30	35
Annealing	53-55	30	
Extension	72	30	
Final Extension	72	300	1
End/Hold	4	∞	

Table-S3: Reaction mixtures for double digestion of pET25b (+) and urease subunit γ gene

Component	pET25b	Ure G
NFW	31.36 µL	19.5 µL
NEB 2.1 (10X)	5 µL	5 µL
Vector/ Insert (1µg)	12.64 µL	24.5 µL
NdeI (20000 U/mL)	0.5 µL	0.5 µL
HindIII (20000 U/mL)	0.5 µL	0.5 µL
Final volume	50 µL	50 µL

Table-S4: Reaction mixture for ligation of digested vector and urease subunit γ gene

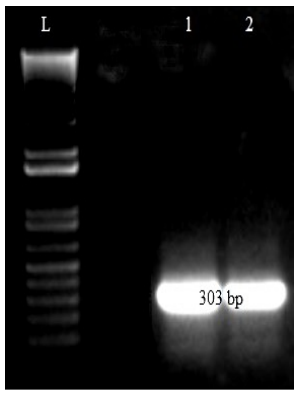
Component	Volume (Concentration)
Nuclease Free Water	Up to 20 µL
Ligation Buffer (5X)	4 µL (1X)
Restricted Vector	1 molar ratio
Restricted Insert	5 molar ratio
T4 DNA Ligase	1 µL (1 Units)

Table-S5: Reaction mixture for colony PCR

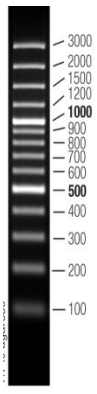
Component	Volume in µL (Concentration)
Nuclease free water	8
PCR master mix (2X)	10 (1X)
T7 promoter primer (10 µM)	1 (0.4 µM)
Gene reverse primer (10 µM)	1 (0.4 µM)
Transformed cultures	0.1

Table-S6: Thermal cycle conditions for colony PCR

Steps	Temperature (˚C)	Time (sec)	No. of Cycle/s
Initial denaturation	95	120	1
Denaturation	94	30	35
Annealing	45	30	
Extension	72	30	
Final Extension	72	300	1
End/Hold	4	∞	

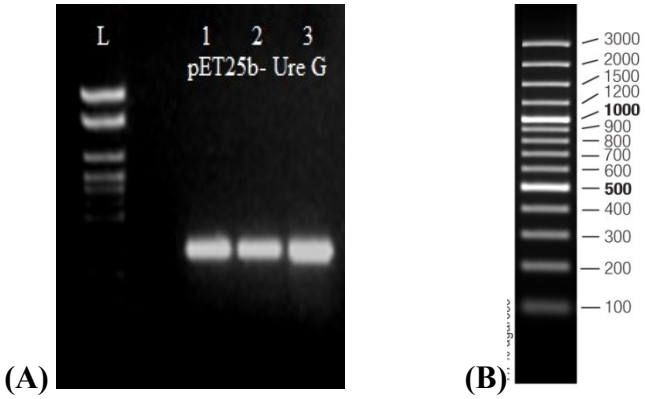


(A)



(B)

Figure-S1: (A) Agarose gels of gene isolation PCR for gene corresponding to urease subunit γ. L represent the DNA ladder and numbers represents the wells containing PCR product. (B) DNA ladder profile.



(A) (B)

Figure-S2: (A) Colony PCR screening of recombinant clones for the successfully cloned gene urease subunit γ. L represents molecular size DNA ladder and numbers represents the screened colonies. (B) represents the molecular DNA ladder profile.

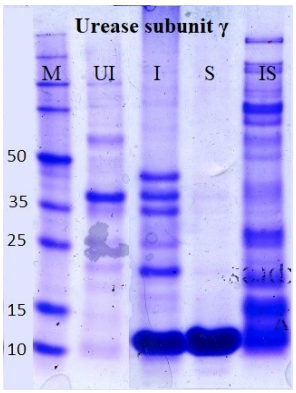


Figure-S3: Analysis of gene expression on SDS PAGE. M represents protein molecular weight marker, Whereas UI= un-induced, I= induced, S= soluble and IS= insoluble fractions. Urease subunit γ were successfully expressed in *E. coli* BL21DE3 cells.

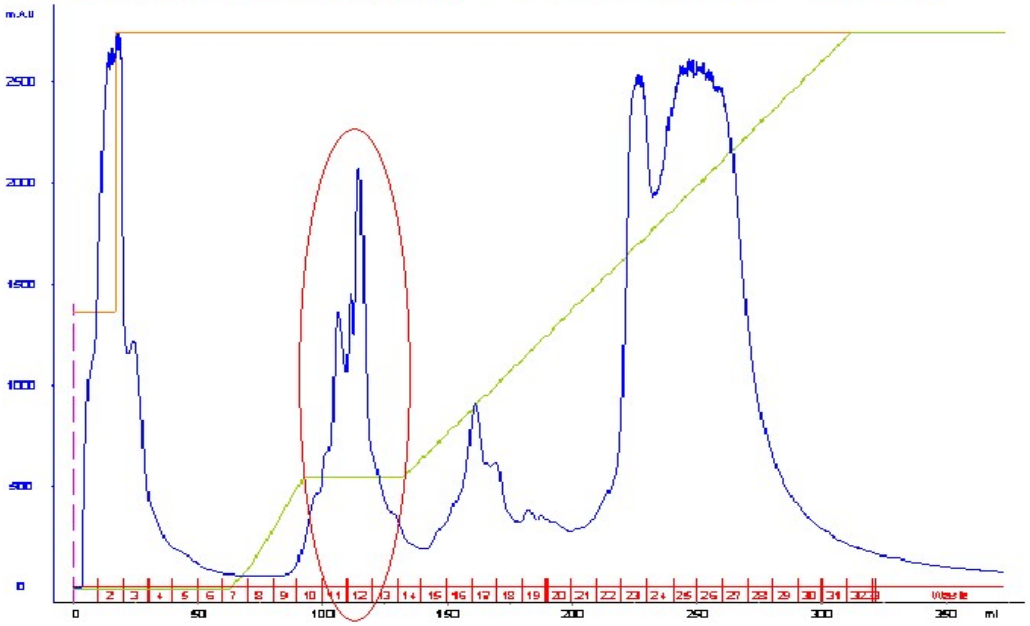


Figure-S4: An-ion exchange chromatogram of urease γ. Unbound fractions are 1 to 4, whereas elution starts from fraction 10 till 33. Urease γ was eluted in fraction 11 and 12 at 17% gradient of elution buffer.

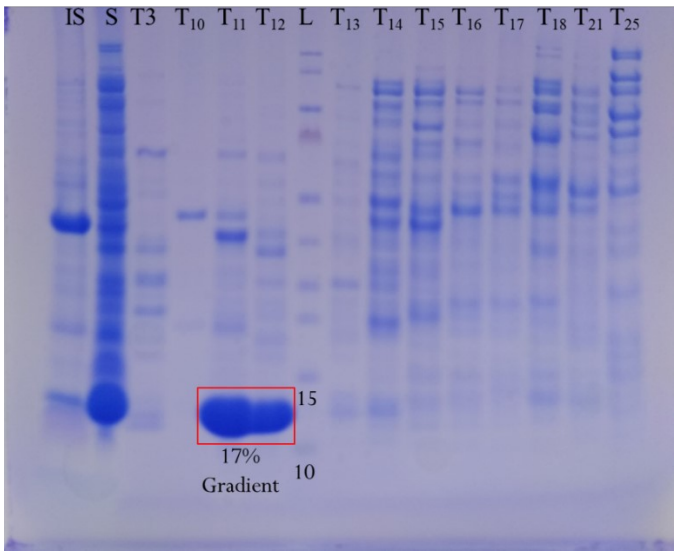
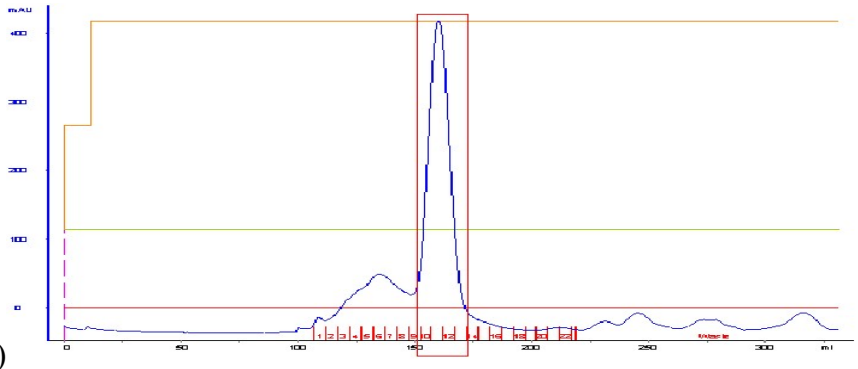


Figure-S5: SDS PAGE analysis of anion exchange. Fractions 11 and 12 contains partially purified Urease γ.

(A)



(B)

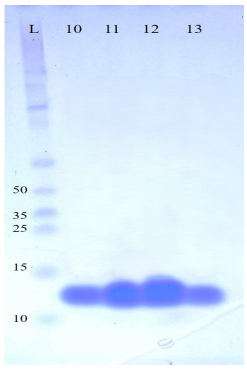


Figure-S6: Size exclusion chromatogram(A) and SDS PAGE analysis (B) of UreA. Fractions 10 to 13 contained eluted protein. Elution peak was obtained at 161 mL.

Table-S7: Urease subunit gamma (MRSA252) sequence similarity with Jack bean urease

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
sp|P07374|UREA_CANEN Urease OS=Canavalia ensiformis OX=3823 PE=1 SV=3		102	122	99%	6e-31	49.00%	840	Query_6184523

Table-S8: List of drugs for STD NMR

S. No.	Drug Code	Drug Name	Mixture No.
1.	DB155	Nicotinamide	01
2.	DB156	Suxamethonium HCl	
3.	DB157	Thiamine HCl (Vitamin B1)	
4.	DB158	Topiramate	
5.	DB163	Doxofylline	
6.	DB164	Drotaverine hydrochloride	02
7.	DB172	Citric Acid	

8.	DB173	Clidinium bromide	
9.	DB179	Sitagliptin.PO4.H2O	
10.	DB184	Amikacin sulphate	
11.	DB188	Dorzolamide HCl	03
12.	DB196	Phenylephrine HCl	
13.	DB197	Ribavirin	
14.	DB211	Yohimbine	
15.	DB240	Isoniazid	
16.	DB244	Pyrazinamide	04
17.	DB250	Gentamicin Sulphate	
18.	DB252	Histamin dihydrochloride	
19.	DB258	Serotonin HCl	
20.	DB273	Solifenacin Succinate	
21.	DB275	Ceftriaxone Sodium	05
22.	DB277	Pefloxacin Mesylate	
23.	DB281	Tobramycin	
24.	DB286	Cefepime-L-Arginine	
25.	DB294	Mannitol	
26.	DB302	Guaifenesin	06
27.	DB308	Sulfanilamide	
28.	DB310	Nicorandil	
29.	DB316	Lisinopril	
30.	DB332	Clindamycin Phosphate	
31.	DB334	Cetylpyridinium Chloride	07
32.	DB339	Hydoxychloroquine Sulphate	
33.	DB343	Dexamethasone Sodium Phospahte	
34.	DB346	Doxycycline	
35.	DB347	Hydralazine Hydrochloride	
36.	DB358	Ivabradine HCl	08
37.	DB359	Glucosamine HCl	
38.	DB362	Ketamine	
39.	DB364	Ephedrine	
40.	DB367	Lactose Anhydrous	
41.	DB379	Sodium Cyclamate	09
42.	DB381	Sodium Tartrate	
43.	DB382	Bambuterol HCl	
44.	DB385	Eperisone HCl	
45.	DB400	Penicillin G Sodium	
46.	DB298	Fusidic Acid	10
47.	DB318	Clomifene Citrate	
48.	DB373	Promethazine Teoclate	
49.	DB402	Sulbactam Sodium	
50.	DB408	Benzoyl Metronidazole	
51.	DB008	Captopril	11
52.	DB020	Lidocaine Hydrochloride monohydrate	
53.	DB029	Paracetamol (Acetaminophen)	
54.	DB035	Sodium valproate	
55.	DB038	Tramadol Hydrochloride	

56.	DB071	Metformin HCl	12
57.	DB075	Nicotinic Acid	
58.	DB077	D-Penicillamine	
59.	DB082	Tranexamic acid	
60.	DB084	Epinephrine Bitartrate/Adrenaline Bitartrate	
61.	DB088	Cefradine	13
62.	DB104	Lysine Hydrochloride	
63.	DB107	Salbutamol Sulfate	
64.	DB110	Bupropion Hydrochloride	
65.	DB120	Levetiracetam	
66.	DB135	Lincomycin Hydrochloride Monohydrate	14
67.	DB137	Metronidazole	
68.	DB142	Aminophylline	
69.	DB146	Pyridoxine HCl (Vit. B6)	
70.	DB170	Aspartame	
71.	DB177	Pregabalin	15
72.	DB183	Alendronate sodium	
73.	DB190	Hydroquinone	
74.	DB210	Tenofovir disoproxil	
75.	DB214	Caffeine	
76.	DB232	Ranitidine Hydrochloride	16
77.	DB236	Ascorbic acid	
78.	DB239	Ethambutol 2HCl	
79.	DB248	Colchicine	
80.	DB295	Deferiprone	
81.	DB313	Lidocaine HCl	17
82.	DB328	Tinidazole	
83.	DB330	Levamisole HCl	
84.	DB340	Chlorpheniramine Maleate	
85.	DB366	Chondroitin Sulphate Sodium	
86.	DB368	Lactose Monohydrate	18
87.	DB369	Lactulose	
88.	DB371	Monosodium Glutamate	
89.	DB380	Sodium Saccharin	
90.	DB392	L-Arginine	
91.	DB393	L-Citrulline	19
92.	DB394	L-Ornithine	
93.	DB410	Methamphetamine	
94.	DB416	Salicin	
95.	DB424	Thiamine B1 Mononitrate	
96.	DB479	Ibandronate Sodium	20
97.	DB480	L-Phenylalanine	
98.	DB481	Ceftriaxone	
99.	DB492	Rasagiline Mesylate	
100.	DB496	Sucralose	

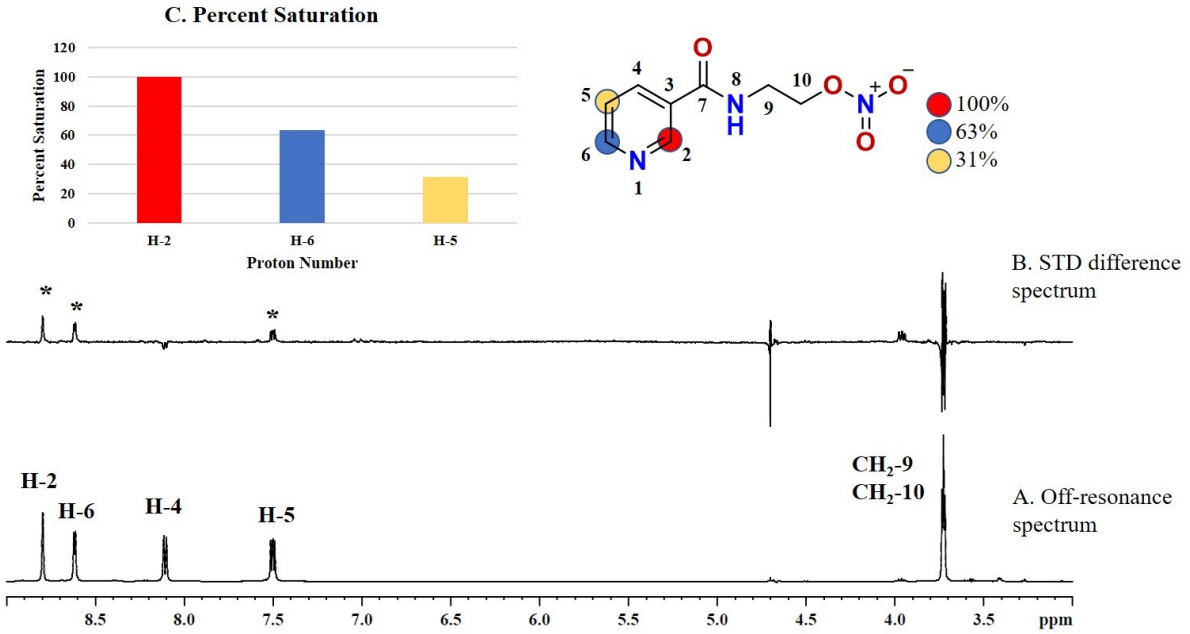


Figure-S7: STD-NMR Spectra of nicorandil (**5**): A. Reference spectrum of the drug. B. STD difference spectrum of nicorandil with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

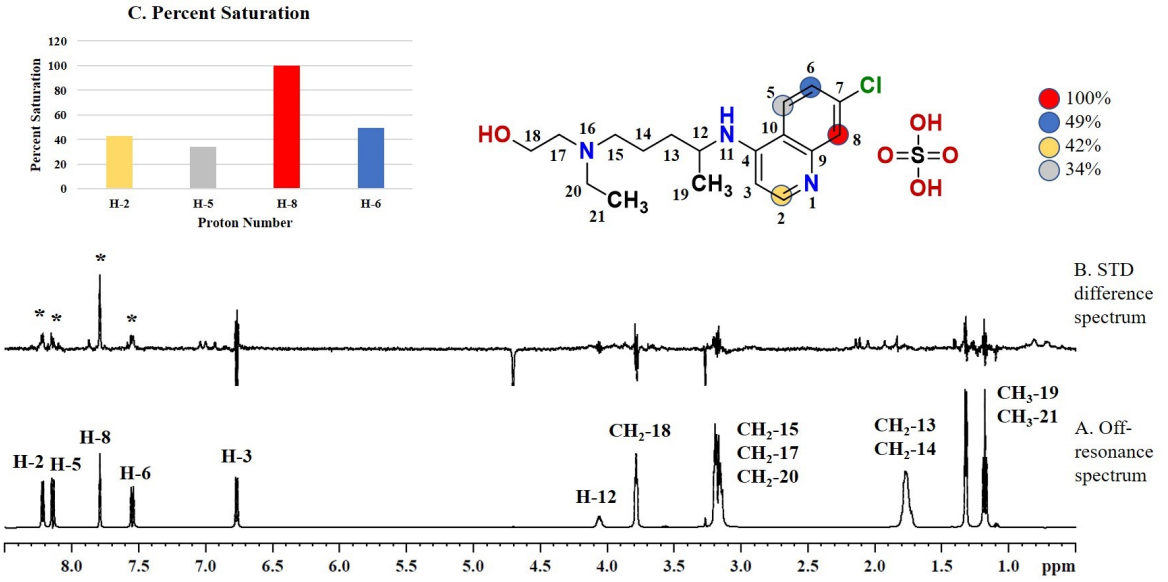


Figure-S8: STD-NMR Spectra of hydroxychloroquine sulphate (**6**): A. Reference spectrum of the drug. B. STD difference spectrum of hydroxychloroquine sulphate with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

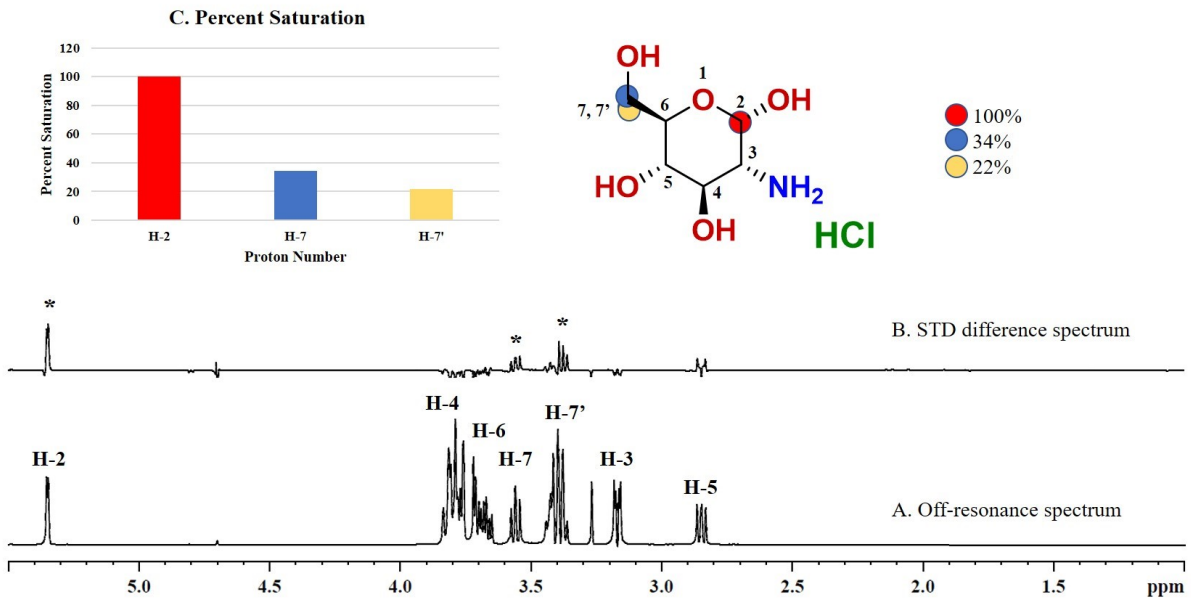


Figure-S9: STD-NMR Spectra of glucosamine HCl (**7**): A. Reference spectrum of the drug. B. STD difference spectrum of gluosamine HCl with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

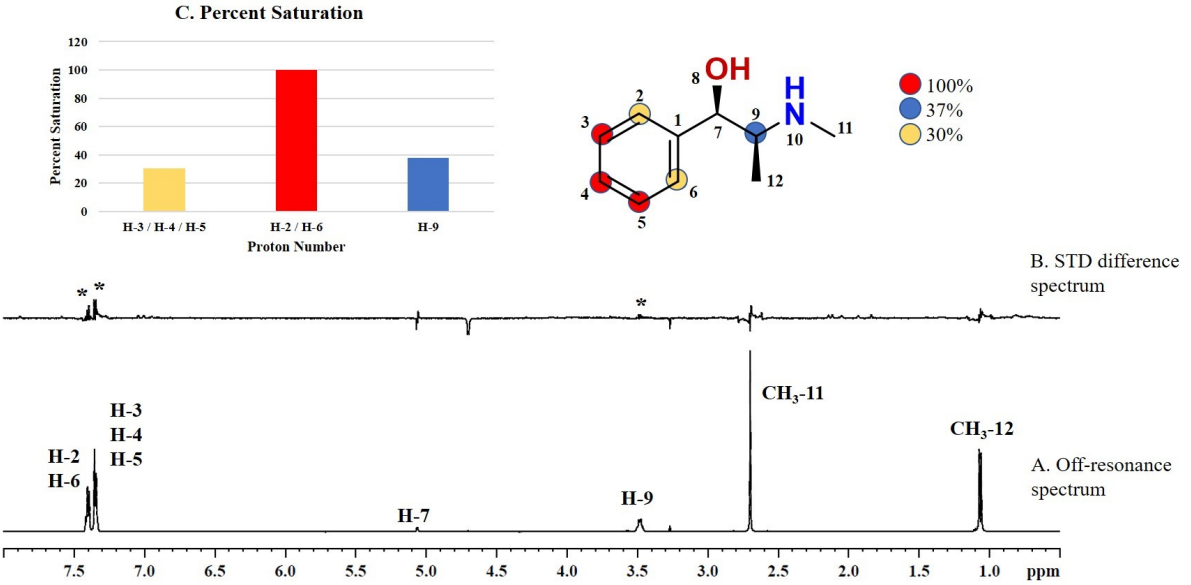


Figure-S10: STD-NMR Spectra of ephedrine (**8**): A. Reference spectrum of the drug. B. STD difference spectrum of ephedrine with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

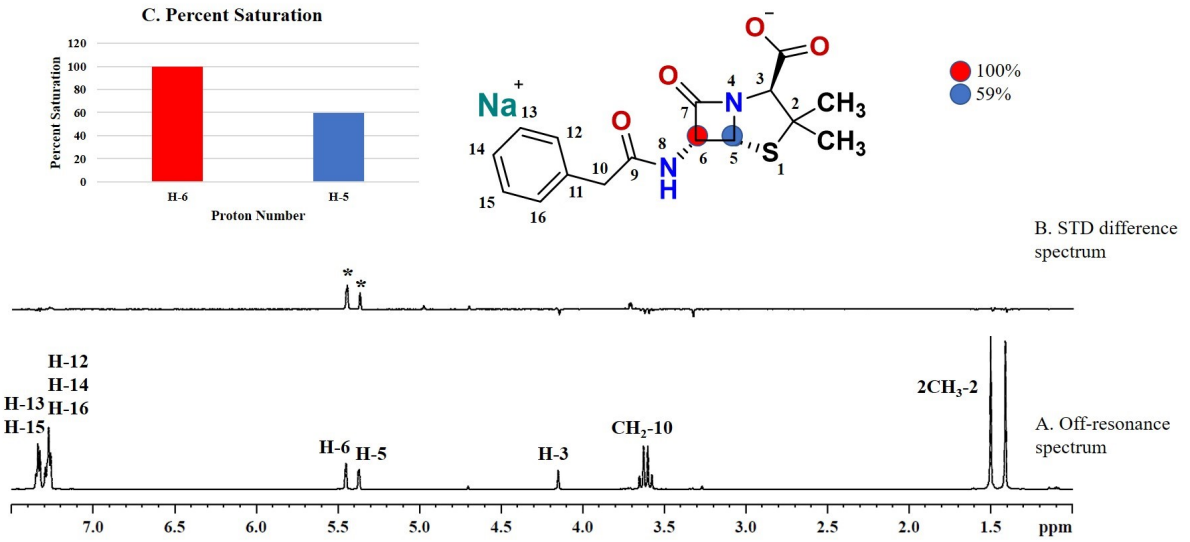


Figure-S11: STD-NMR Spectra of penicillin G sodium (**9**): A. Reference spectrum of the drug. B. STD difference spectrum of penicillin G sodium with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

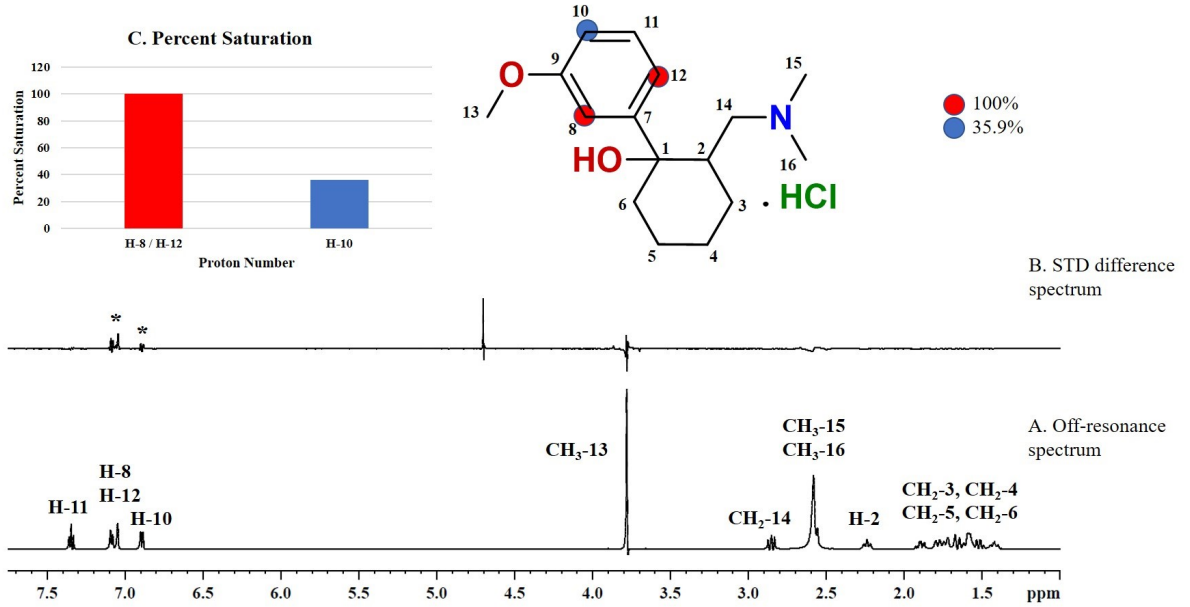


Figure-S12: STD-NMR Spectra of tramadol HCl (**10**): A. Reference spectrum of the drug. B. STD difference spectrum of tramadol HCl with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

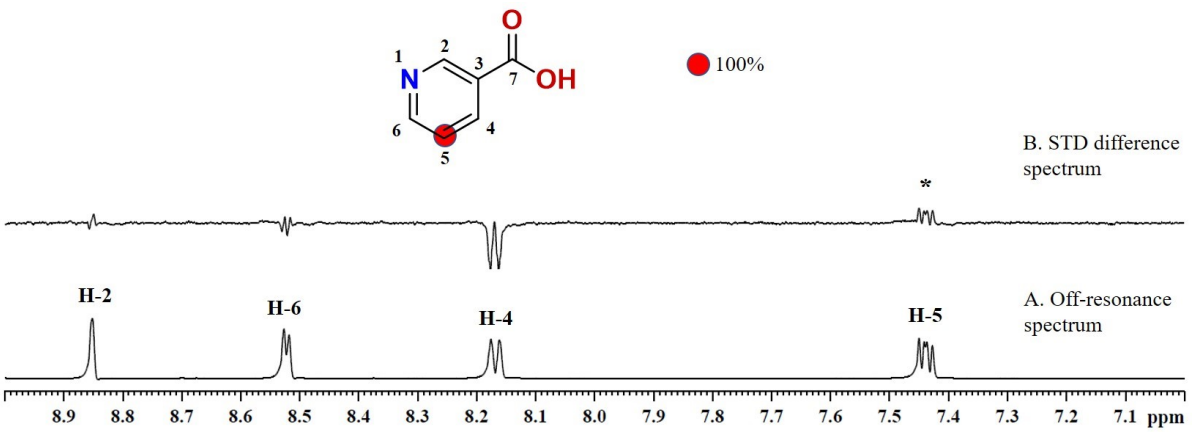


Figure-S13: STD-NMR Spectra of nicotinic acid (**11**): A. Reference spectrum of the drug. B. STD difference spectrum of nicotinic acid with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*').

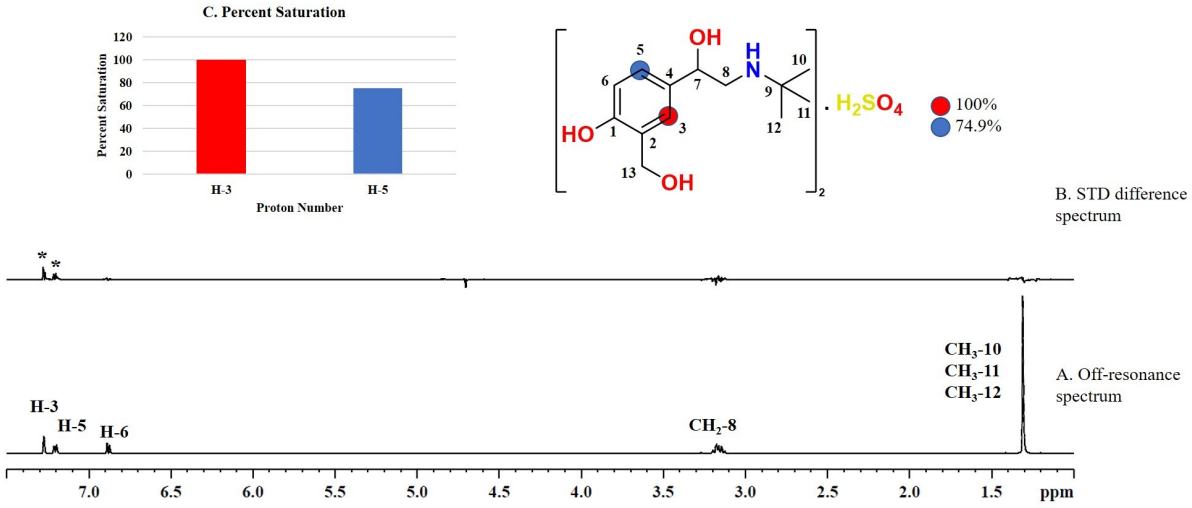


Figure-S14: STD-NMR Spectra of salbutamol sulfate (**12**): A. Reference spectrum of the drug. B. STD difference spectrum of salbutamol sulfate with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

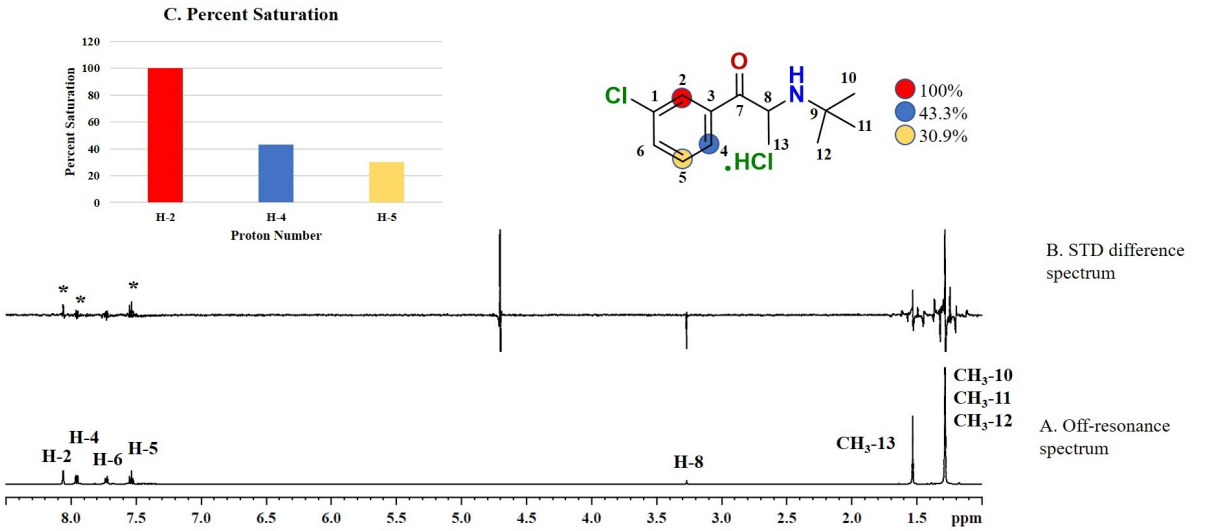


Figure-S15: STD-NMR Spectra of bupropion HCl (**13**): A. Reference spectrum of the drug. B. STD difference spectrum of bupropion HCl with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

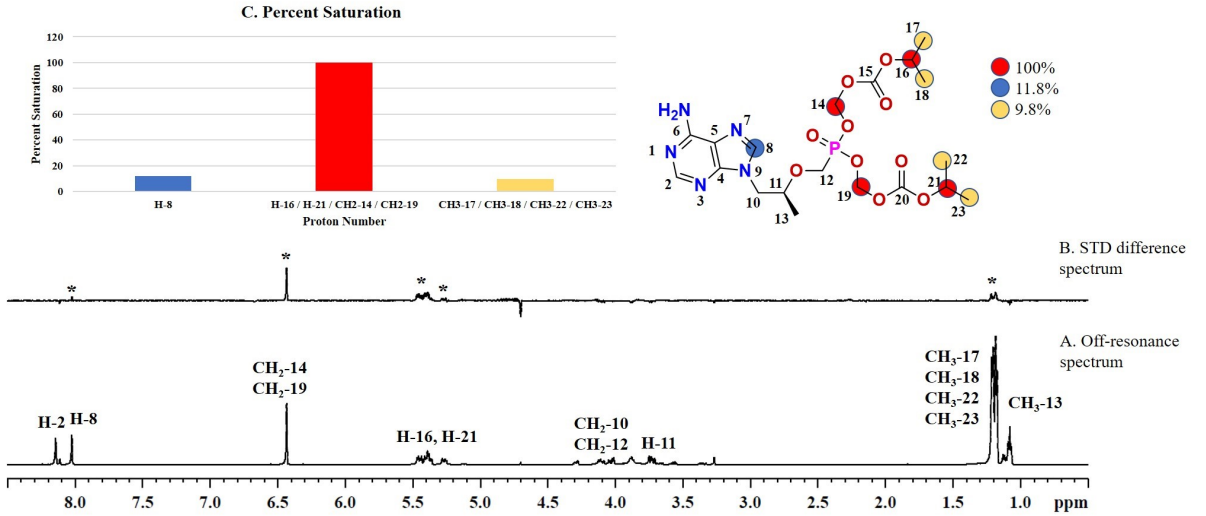


Figure-S16: STD-NMR Spectra of tenofovir disoproxil (**14**): A. Reference spectrum of the drug. B. STD difference spectrum of tenofovir disoproxil with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

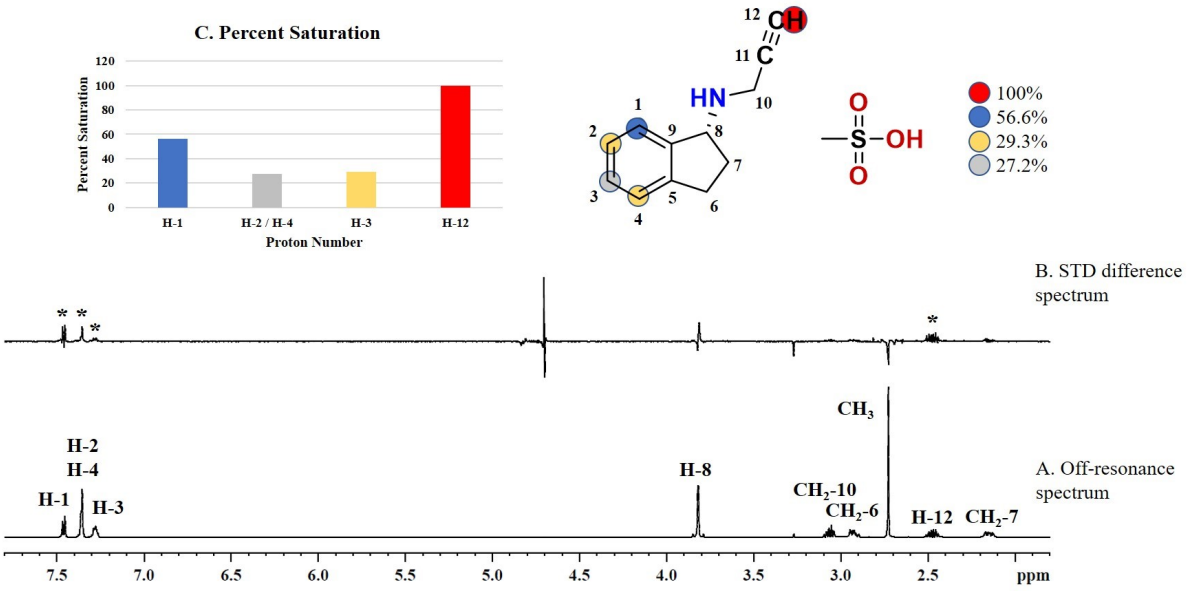


Figure-S17: STD-NMR Spectra of rasagiline mesylate (**15**): A. Reference spectrum of the drug. B. STD difference spectrum of Rasagiline Mesylate with urease subunit gamma, indicating the interacting protons of drug (Ligand protons interacting with protein are marked with '*'). C. Graphical representation of percent saturation of ligands proton receiving from protein.

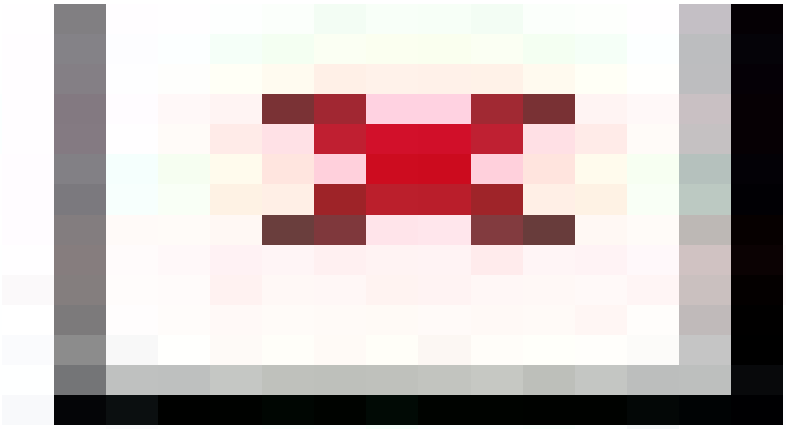


Figure-S18: Docked pose of nicotinamide (**1**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black).

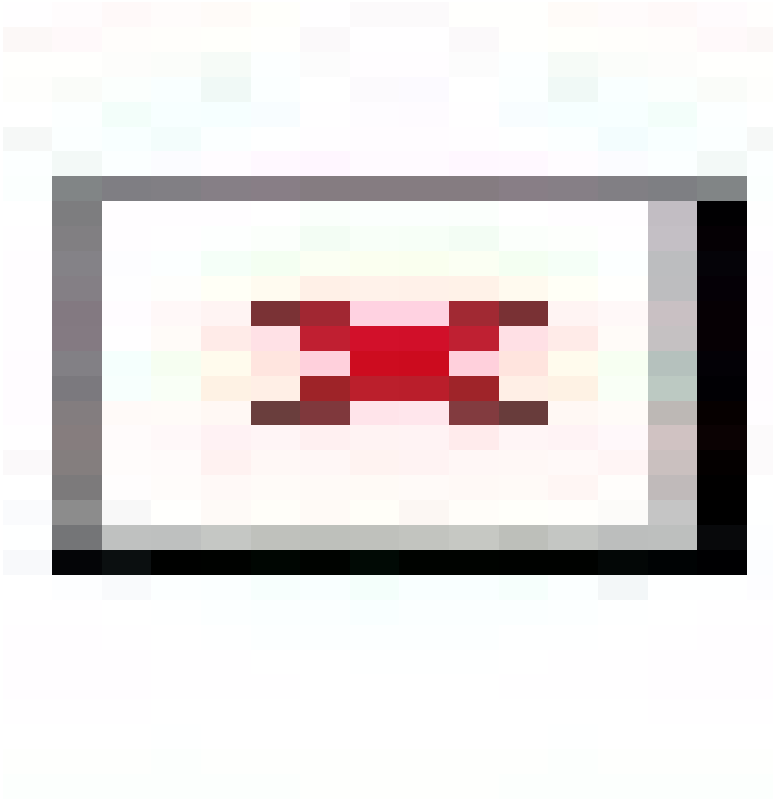


Figure-S19: Docked pose of drotaverine hydrochloride (**2**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing aromatic hydrogen bonding (light blue).

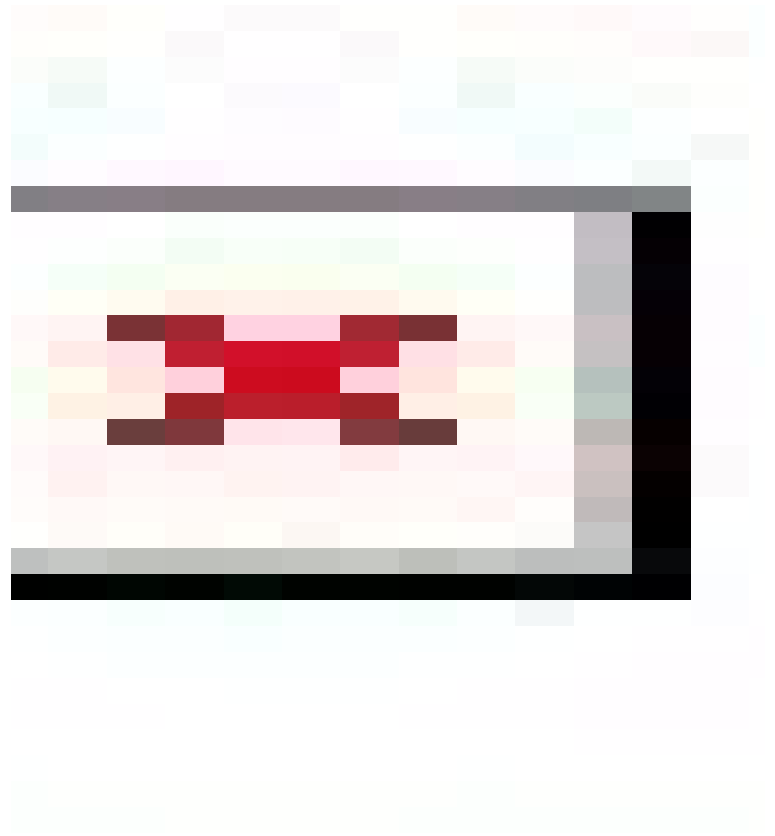


Figure-S20: Docked pose of sulfanilamide (**4**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black).

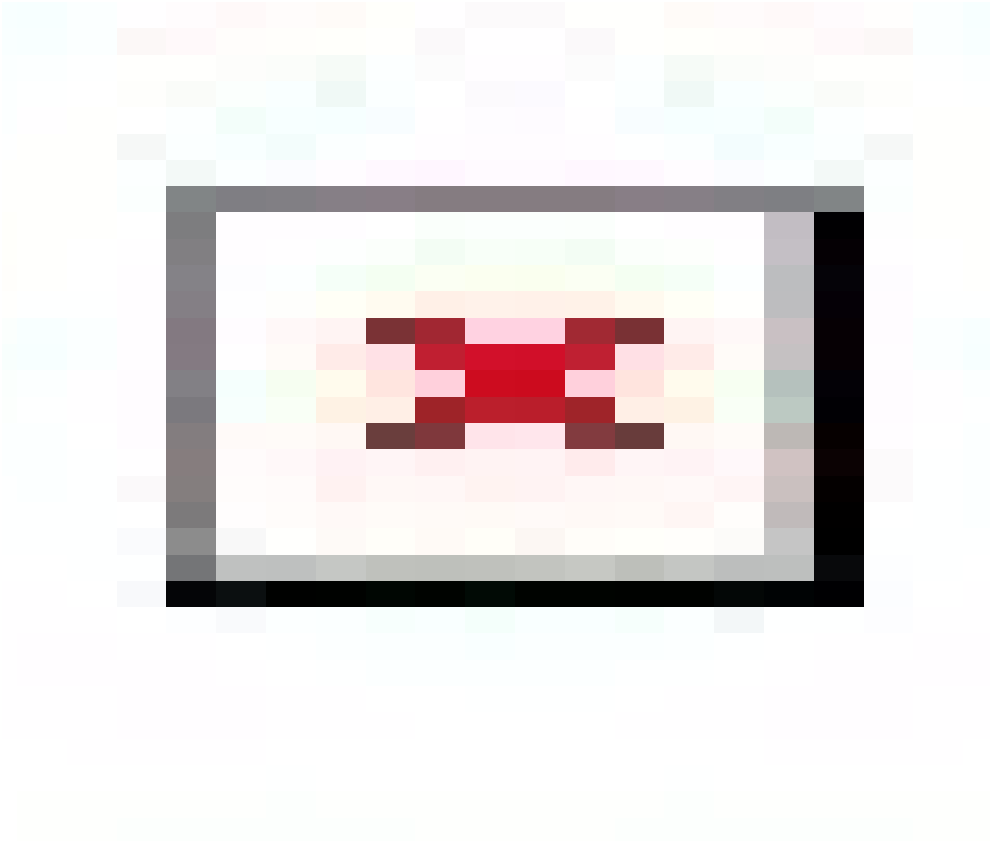


Figure-S21: Docked pose of nicorandil (**5**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black) and salt bridges (pink).

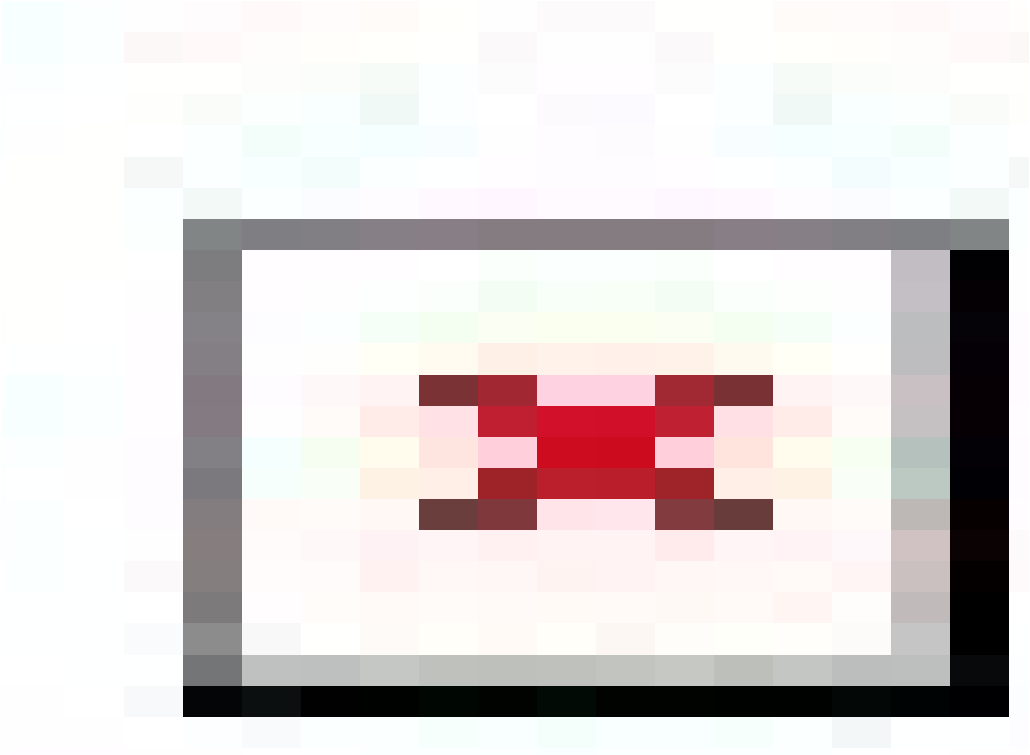


Figure-S22: Docked pose of hydroxychloroquine sulphate (**6**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black), aromatic hydrogen bonding (light blue), and salt bridge (pink).

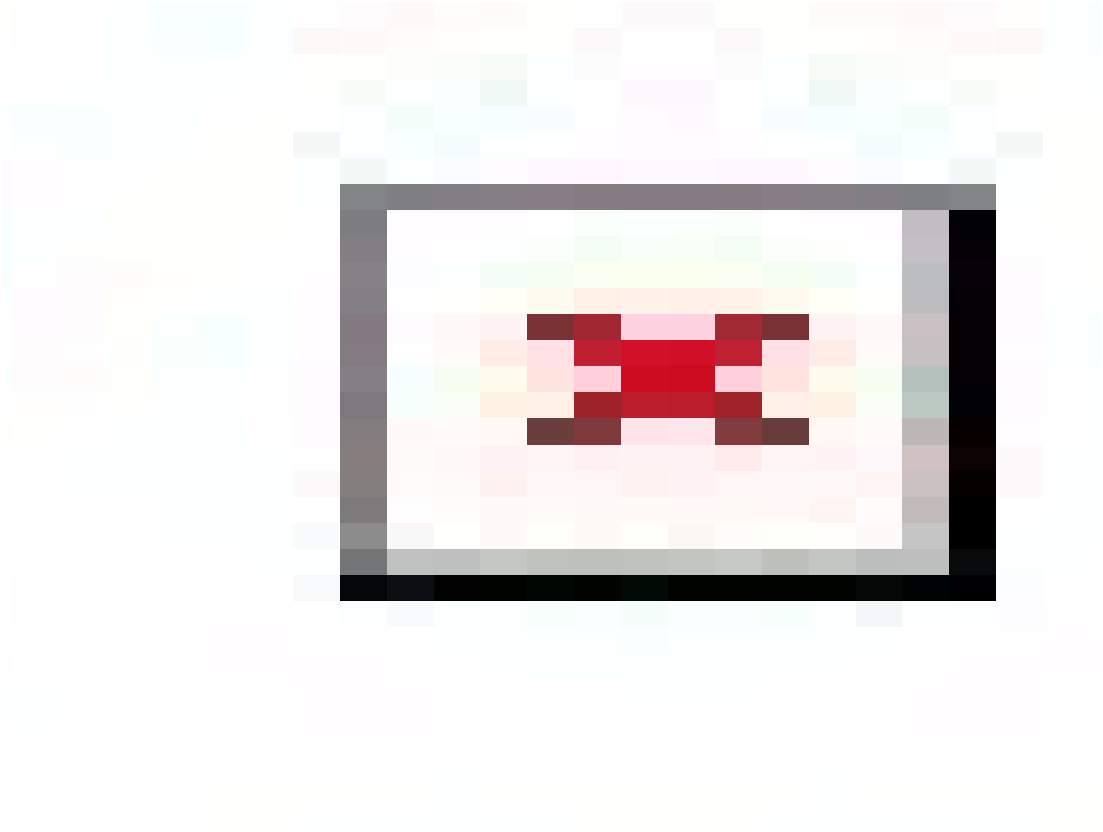


Figure-S23: Docked pose of glucosamine hydrochloride (**7**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black).

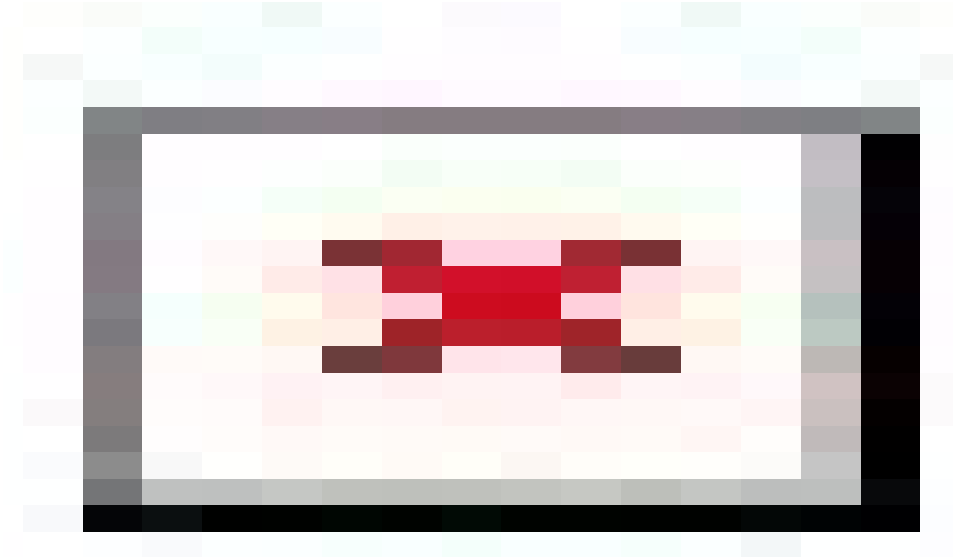


Figure-S24: Docked pose of penicillin G sodium (**9**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black), π- π stacking (red), and salt bridge (pink).

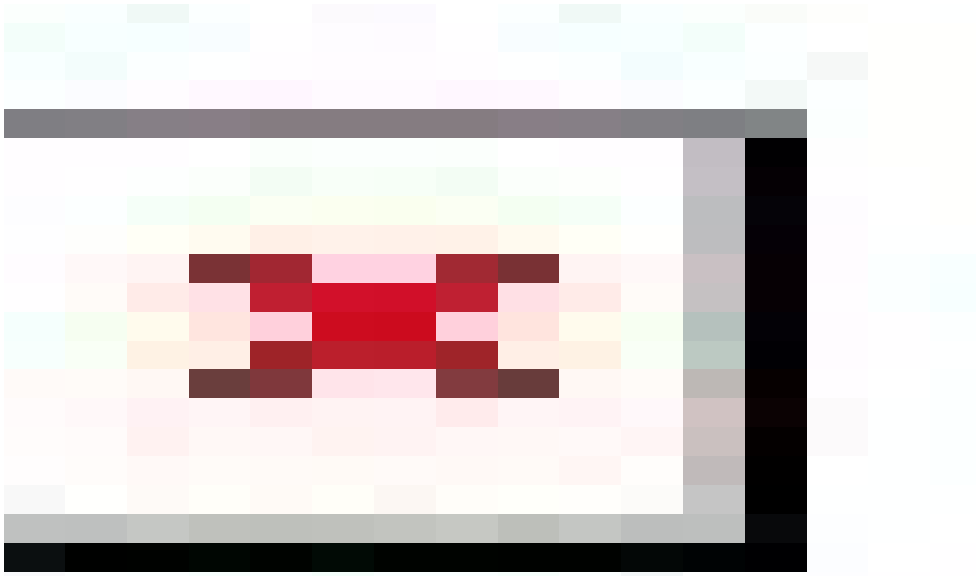


Figure-S25: Docked pose of tramadol hydrochloride (**10**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing π-π stacking (blue).

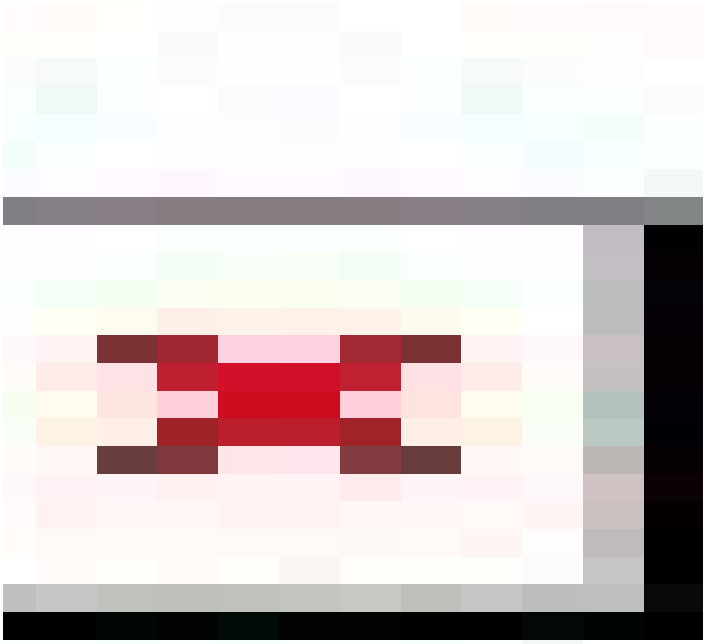


Figure-S26: Docked pose of nicotinic acid (**11**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black), salt bridge (pink), and aromatic hydrogen bond (light blue).

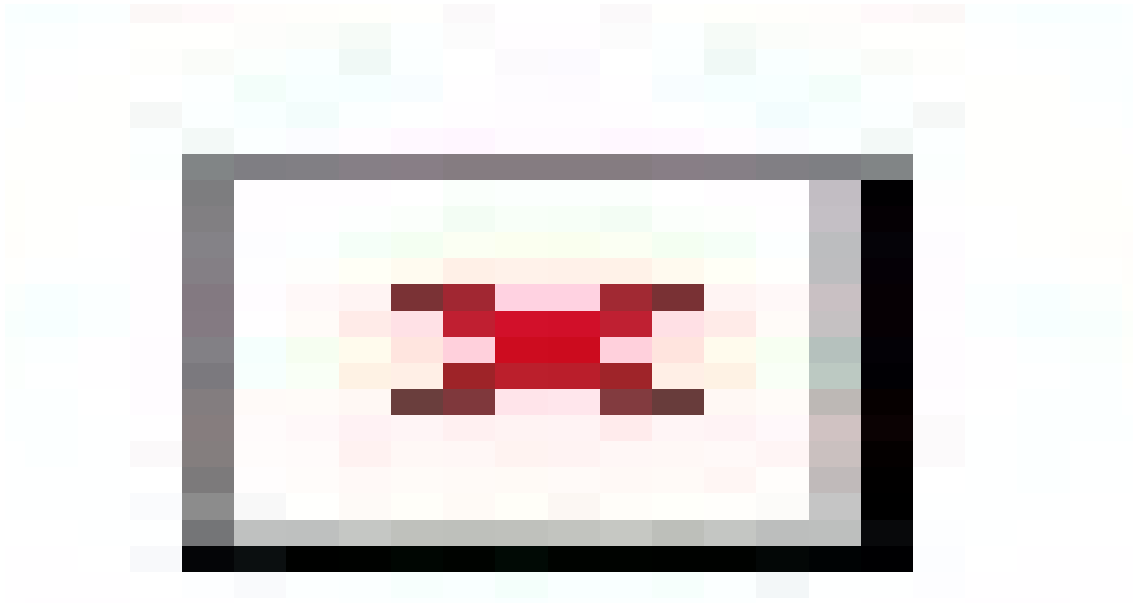


Figure-S27: Docked pose of salbutamol sulfate (**12**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing hydrogen bonding (black), and salt bridge (pink).

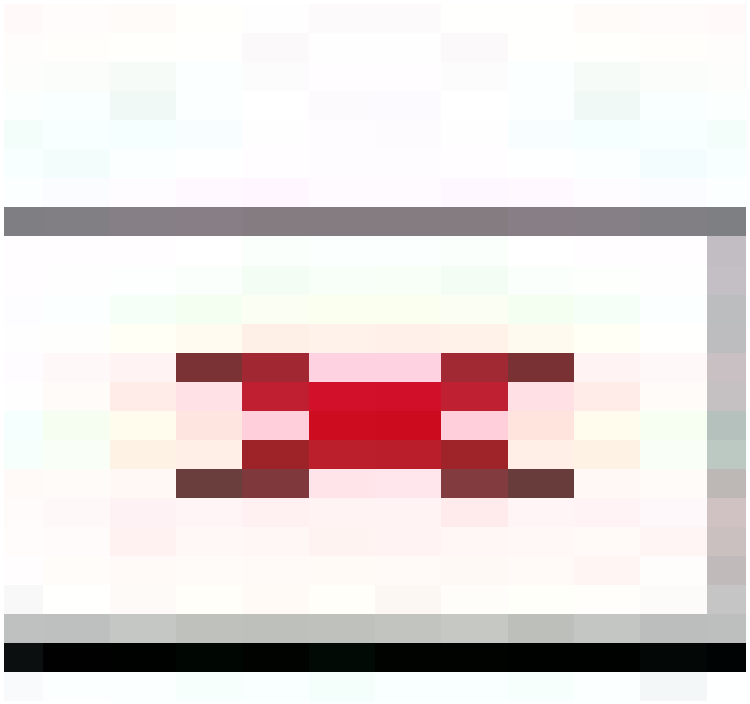


Figure-S28: Docked pose of bupropion hydrochloride (**13**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing halogen bonding (golden).

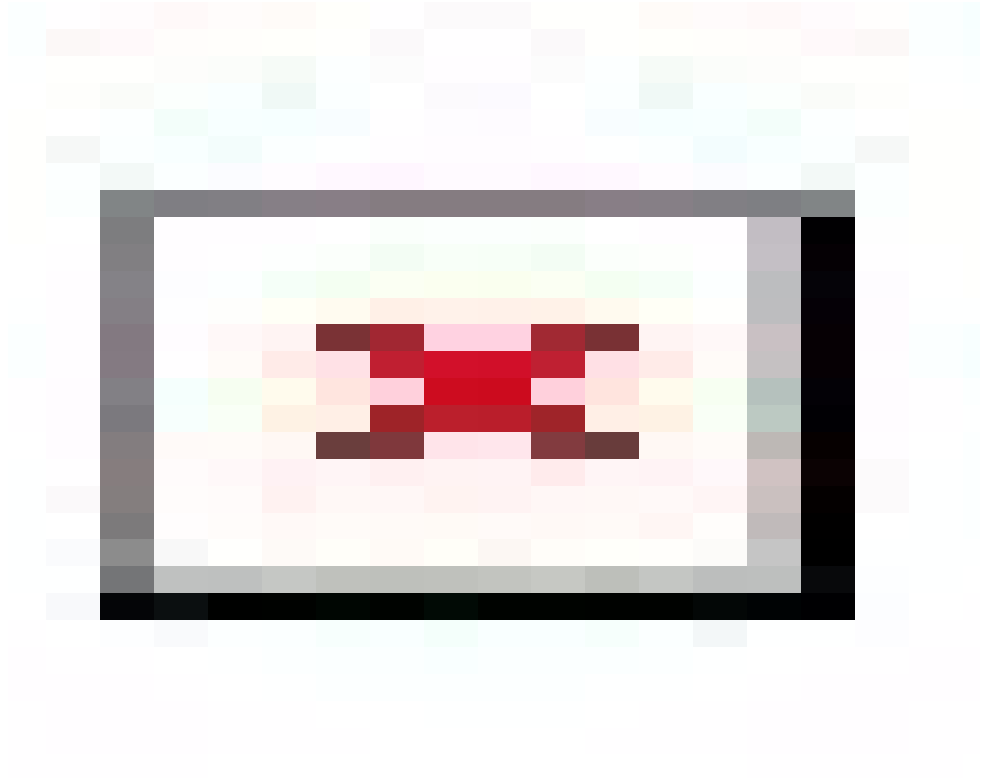


Figure-S29: Docked pose of tenofovir disoproxil (**14**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation.

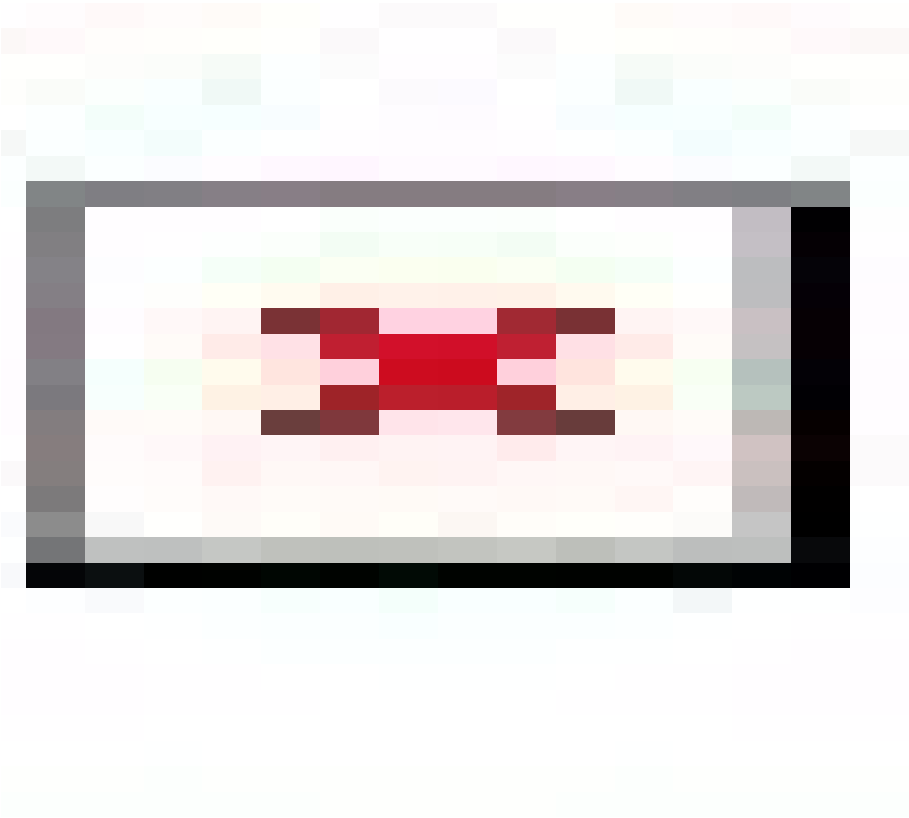


Figure-S30: Docked pose of rasagiline mesylate (**15**) with urease subunit gamma. Protein-ligand interaction depicted in 3D representation (dotted lines), representing π-cationic interaction (blue), aromatic hydrogen bonding (light blue), and π-π stacking (red).

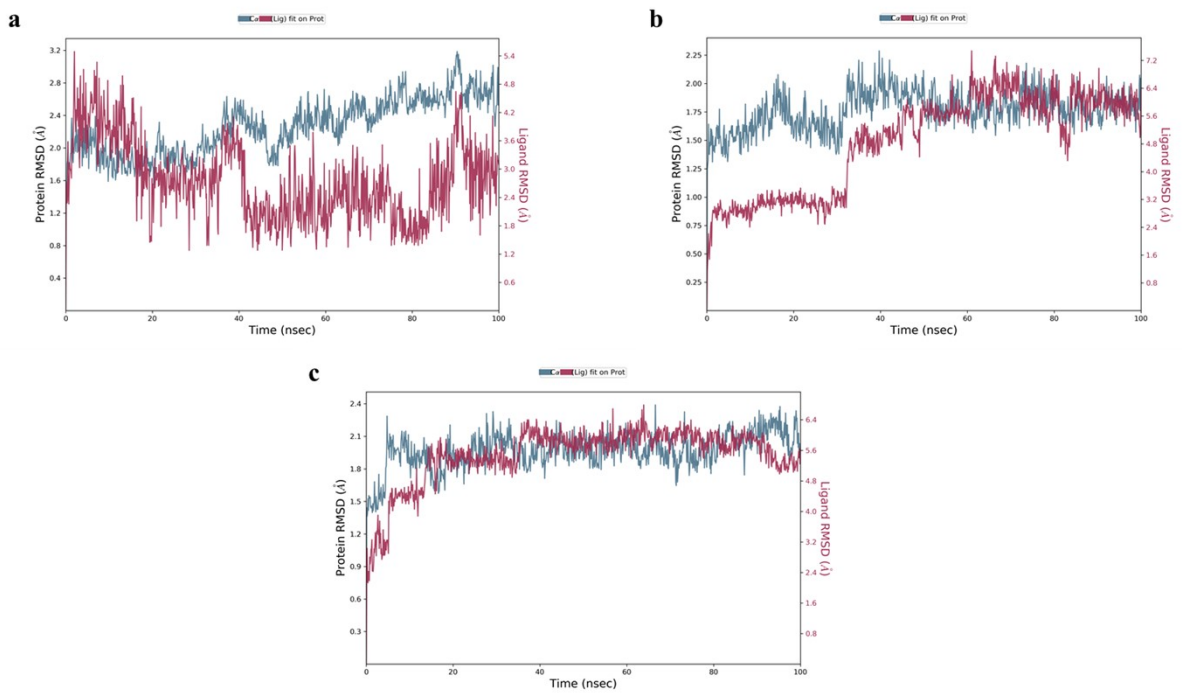


Figure-S31: RMSD plots of drotaverine HCl (**a**), penicillin G sodium (**b**), and tenofovir disoproxil (**c**) with UreA protein.

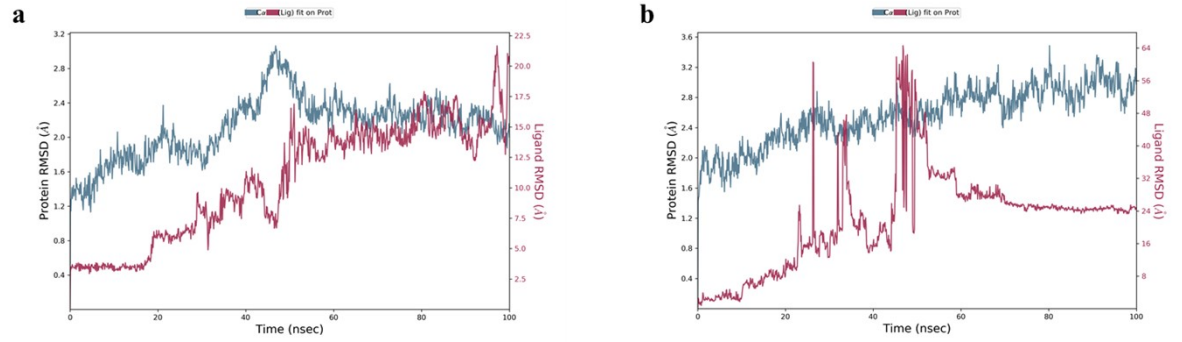


Figure-S32: RMSD plots of hydroxychloroquine sulphate (**a**) and ephedrine (**b**) with UreA protein.

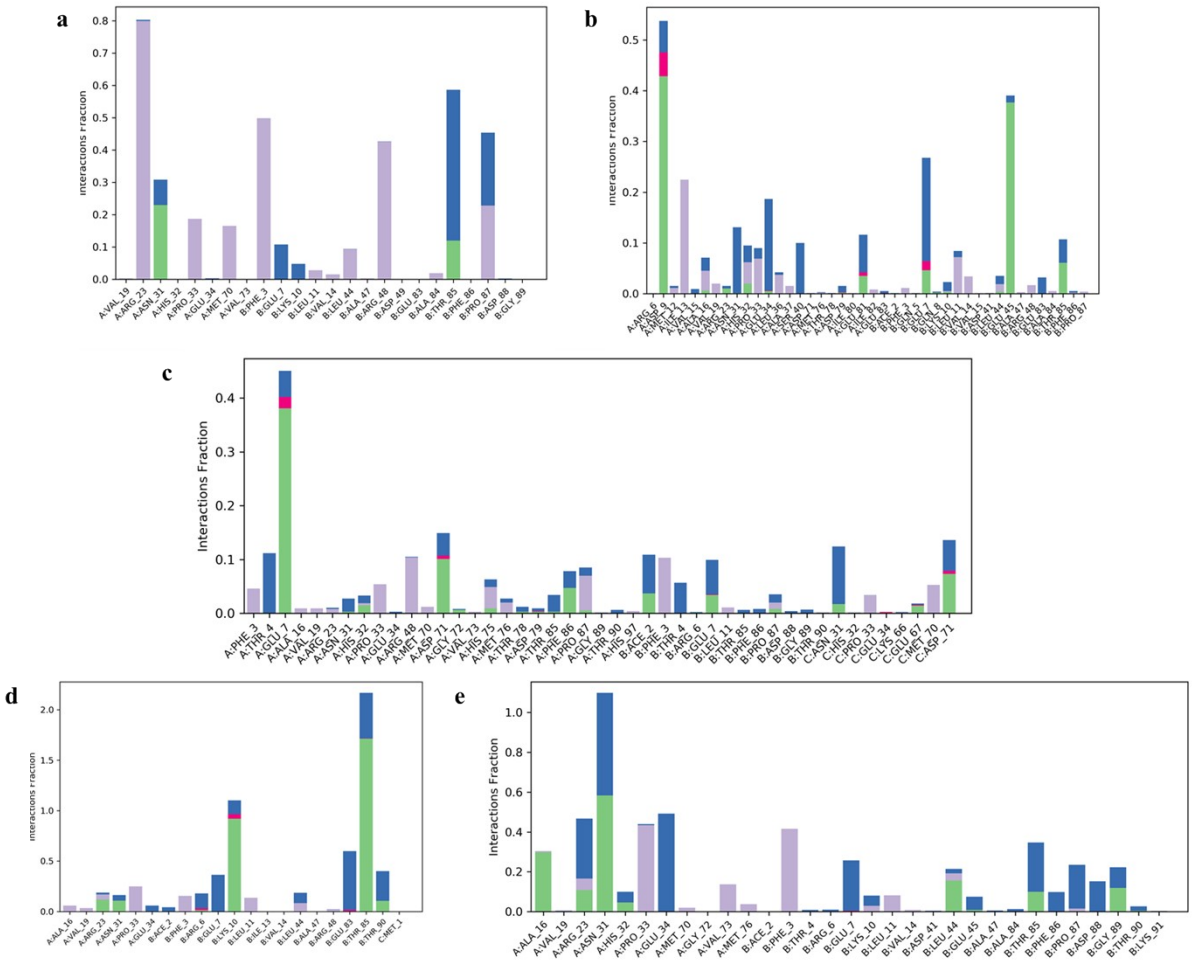


Figure-S33: Histogram of drotaverine HCl (**a**), hydroxychloroquine sulpate (**b**), ephedrine (**c**), penicillin G sodium (**d**), and tenofovir disoproxil (**e**) interactions with UreA protein binding site residues.

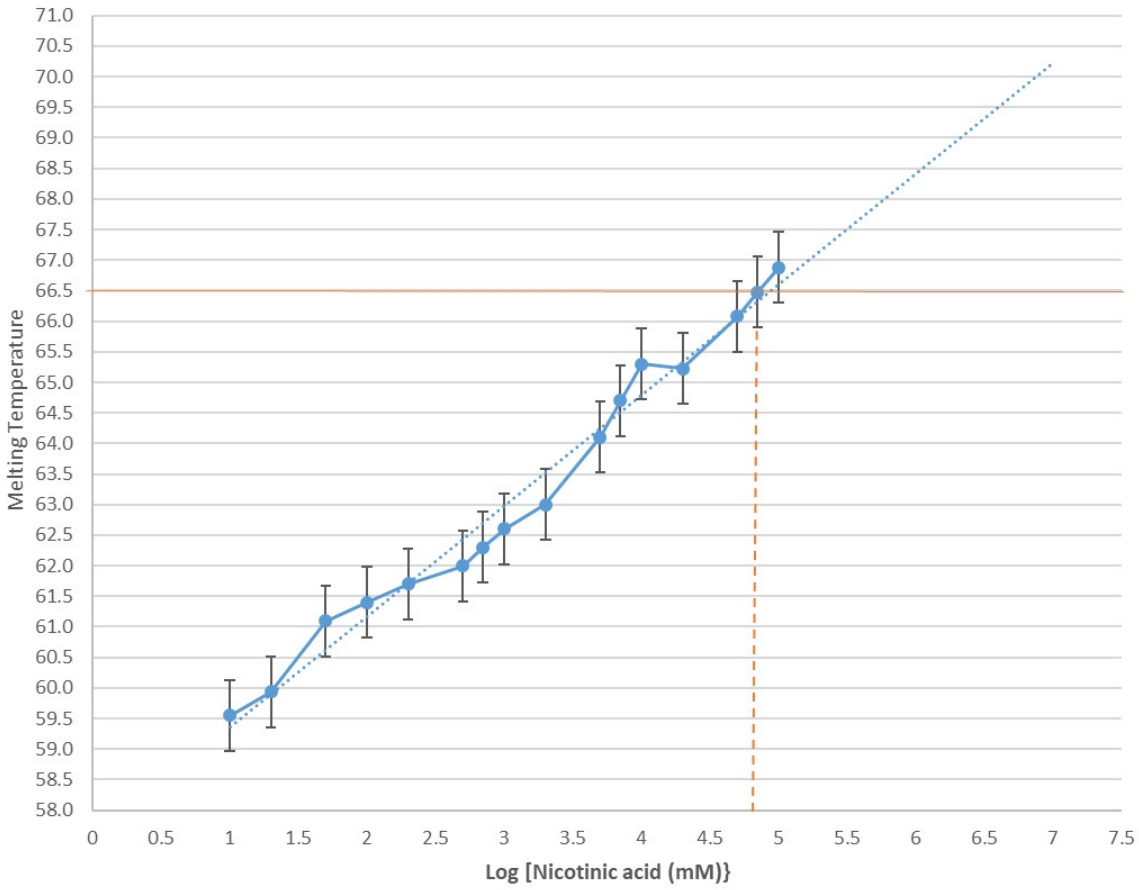


Figure-S34: The variation in *T*m with increasing concentration of nicotinic acid (blue solid line). The blue dashed line represents the linear extrapolation of the curve at higher concentrations and the solid orange line represent Tm_0, *i.e.* the *T*m of protein in the absence of ligand. These two lines intercept at the log of the K_d value.