Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for:

Computational Identification of the Binding Mechanism of Triple Reputake Inhibitor Amitifadine for the Treatment of Major Depressive Disorder

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Supplementary (SI) Tables

Table S1. List of TRIs in clinical trial for MDD treatment with structure available.

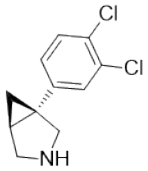
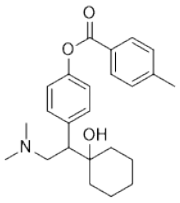
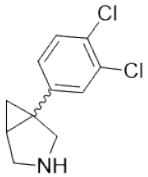
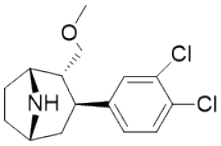
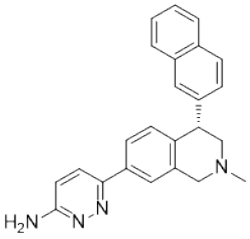
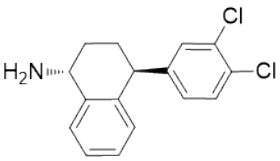
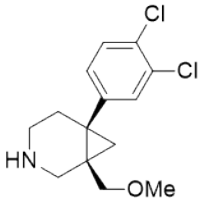
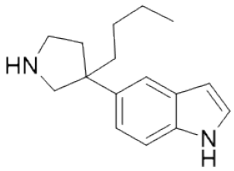
Drug	Structure	Activity			Company	Highest Clinical Phase
		SERT	NET	DAT		
Amitifadine		12	23	96	DOV Pharma ceutical; Euthymics Bioscience	Phase 3
Ansofaxine		723	763	491	Luye Pharma	Phase 2
DOV 216,303		14	20	78	DOV Pharmaceutical	Phase 2
GSK372475		10	2	10	NeuroSearch; Saniona	Phase 2
Liafensine		1.1	8.8	5.7	AMRI Global; Bristol-Myers Squibb	Phase 2
SEP-225289		12	4	2	Sepracor; Sunovion	Phase 2
GSK1360707		9.2	8.1	8	GSK	Phase 1
RG-7166		16	9	90	Roche	Phase 1

Table S2. The calculated binding free energies of studied amitifadine binding to hSERT, hNET and hDAT (Energy is in kcal/mol).

Targets	Trajectories	ΔE_{ele}	ΔE_{vdW}	$\Delta E_{pol,PB}$	$\Delta E_{nonpol,PB}$	$\Delta G_{calc,PB}$[a]		$\Delta\Delta G_{calc,PB}$[b]	$\Delta E_{pol,GB}$	$\Delta E_{nonpol,GB}$	$\Delta G_{calc,GB}$[a]		$\Delta\Delta G_{calc,GB}$[b]
hSERT	**100~150ns**	**-32.36**	**-36.68**	**25.99**	**-3.92**	**-46.97**		**0**	**32.24**	**-3.85**	**-40.65**		**0**
	200~250ns	-34.16	-36.91	26.00	-3.88	-48.95			33.67	-3.78	-41.19		
	200~250ns	-34.88	-36.81	27.04	-3.90	-48.55	-48.32	0	34.43	-3.85	-41.11	-41.22	0
	200~250ns	-33.85	-36.28	26.58	-3.89	-47.45			32.66	-3.88	-41.35		
hNET	**100~150ns**	**-44.90**	**-32.74**	**35.37**	**-3.83**	**-46.10**		**0.87**	**43.06**	**-3.92**	**-38.50**		**2.15**
	200~250ns	-42.67	-32.98	34.66	-3.93	-44.91			41.27	-3.94	-38.32		
	200~250ns	-43.15	-31.72	36.87	-4.00	-42.01	-43.34	4.98	41.94	-3.92	-36.86	-37.73	3.49
	200~250ns	-45.46	-32.62	38.90	-3.93	-43.10			44.04	-3.96	-38.00		
hDAT	**100~150ns**	**-67.26**	**-32.54**	**59.43**	**-3.78**	**-44.15**		**2.82**	**65.02**	**-3.82**	**-38.59**		**2.06**
	200~250ns	-67.04	-33.09	57.32	-3.73	-46.54			-65.47	-3.81	-38.47		
	200~250ns	-67.26	-32.54	59.43	-3.78	-44.15	-45.35	2.97	65.02	-3.82	-38.59	-38.74	2.63
	200~250ns	-64.83	-34.40	57.65	-3.78	-45.36			63.85	-3.78	-39.16		

[a] Calculated binding energy by MM/GB(PB)SA method in this work.
[b] Binding free energy variation were calculated using amitifadine-hSERT complex as a reference.

Table S3. Comparison of homology models (hSERT, hNET and hDAT) and crystal structures (hSERT and dDAT).

	hSERT(dDAT) [a]				hNET(dDAT) [a]				hDAT(dDAT) [a]			
	Whole structure		S1 binding site [b]		Whole structure		S1 binding site [b]		Whole structure		S1 binding site [b]	
	Identity	RMSD	Identity	RMSD	Identity	RMSD	Identity	RMSD	Identity	RMSD	Identity	RMSD
hSERT (crystal structure) [c]	--	8.06	--	2.50	54%	--	62%	--	52%	--	57%	--
hNET (hSERT) [d]	54%	--	62%	--	--	6.62	--	1.26	71%	--	85%	--
hDAT (hSERT) [d]	52%	--	57%	--	71%	--	85%	--	--	5.24	--	1.31
dDAT(crystal structure) [e]	56%	--	67%	--	61%	--	76%	--	55%	--	69%	--

[a] Homology model of hSERT, hNET and hDAT using the dDAT crystal structure[1] as a template.
[b] The S1 binding site is primary surrounded by TM1, 3, 6, 8 and 10 regions (see in Figure S3, S10 and S11).
[c] Crystal structure of hSERT[2].
[c] Homology model of hNET and hDAT using the hSERT crystal structure[2] as a template.
[d] Crystal structure of dDAT[1].

Table S4. The energy contributions of hot spot residues for amitifadine binding in hSERT, hNET and hDAT (Energies are in kcal/mol).

Number[a]	Amitifadine-hSERT			Amitifadine-hNET			Amitifadine-hDAT		
	Residue	$\Delta G^{per-residue}_{calc,GB}$	$\Delta G^{per-residue}_{calc,PB}$	Residue	$\Delta G^{per-residue}_{calc,GB}$	$\Delta G^{per-residue}_{calc,PB}$	Residue	$\Delta G^{per-residue}_{calc,GB}$	$\Delta G^{per-residue}_{calc,PB}$
19	Tyr95	-4.30	-6.13	Phe72	-3.79	-5.97	Phe76	-3.22	-5.19
22	Asp98	-3.04	-12.38	Asp75	-2.87	-14.23	Asp79	-3.69	-13.57
93	Ala169	-0.60	-0.87	Ala145	-0.63	-0.39	Ser149	-0.53	-0.52
96	Ile172	-1.63	-0.84	Val148	-1.55	-0.91	Val152	-1.02	-0.9
100	Tyr176	-1.71	-0.01	Tyr152	-1.66	-1.27	Tyr156	-1.47	-0.35
265	Phe335	-0.30	0.03	Phe317	-1.00	-2.47	Phe320	-1.02	-2.15
266	Ser336	-1.03	-1.93	Ser318	-0.54	-2.03	Ser321	-1.12	-2
271	Phe341	-1.93	-0.88	Phe323	-1.90	-1.8	Phe326	-2.28	-1.99
368	Ser438	-1.01	-0.03	Ser419	-1.43	-0.56	Ser422	-1.98	-0.15
369	Thr439	-1.31	-1.26	Ser420	-1.00	-1.57	Ala423	-0.37	-0.52
372	Gly442	-0.87	0.44	Gly423	-0.61	0.47	Gly426	-0.55	-0.18

[a] The number used in the hierarchical clustering analysis.

Table S5. The energy change ($\Delta\Delta G^{per-residue}_{calc,GB}$) of corresponding residues in S1 site of hSERT, hNET and hDAT contribute to amitifadine binding (Energies are in kcal/mol)

hSERT-hNET	$\Delta\Delta G^{per-residue}_{calc,GB}$	hSERT-hDAT	$\Delta\Delta G^{per-residue}_{calc,GB}$	hDAT-hNET	$\Delta\Delta G^{per-residue}_{calc,GB}$
Tyr95-Phe72	-0.51	Tyr95-Phe76	-1.08	Phe76-Phe72	0.57
Asp98-Asp75	-0.06	Asp98-Asp79	-0.07	Asp79-Asp75	0.01
Ala96-Ala73	-0.17	Ala96-Ala77	0.65	Ala77-Ala73	-0.82
Glu136-Glu113	-0.01	Glu136-Glu117	-0.01	Glu117-Glu113	0.00
Ala169-Ala145	0.03	Ala169-Ser149	-0.07	Ser149-Ala145	0.10
Ile172-Val148	-0.08	Ile172-Val152	-0.61	Val152-Val148	0.53
Ala173-Gly149	-0.09	Ala173-Gly153	-0.20	Gly153-Gly149	0.11
Tyr176-Tyr152	-0.05	Tyr176-Tyr156	-0.24	Tyr156-Tyr152	0.19
Phe335-Phe317	0.70	Phe335-Phe320	0.72	Phe320-Phe317	-0.02
Ser336-Ser318	-0.49	Ser336-Ser321	0.09	Ser321-Ser318	-0.58
Leu337-Leu319	-0.19	Leu337-Leu322	-0.10	Leu322-Leu319	-0.09
Gly338-Gly320	-0.23	Gly338-Gly323	-0.18	Gly323-Gly320	-0.05
Pro339-Ala321	-0.11	Pro339-Val324	-0.09	Val324-Ala321	-0.02
Phe341-Phe323	-0.03	Phe341-Phe326	0.35	Phe326-Phe323	-0.38
Val343-Val325	0.00	Val343-Val328	-0.01	Val328-Val325	0.01
Asp437-Asp418	-0.02	Asp437-Asp421	0.10	Asp421-Asp418	-0.12
Ser438-Ser419	0.42	Ser438-Ser422	0.97	Ser422-Ser419	-0.55
Thr439-Ser420	-0.31	Thr439-Ala423	-0.94	Ala423-Ser420	0.63
Gly442-Gly423	-0.26	Gly442-Gly426	-0.32	Gly426-Gly423	0.06
Leu443-Met424	-0.20	Leu443-Met427	-0.34	Met427-Met424	0.14

Table S6. The calculated binding energy changes of amitifadine-hSERT complexes before and after hNET-like or hDAT-like hSERT by *in silico* single and multiple mutagenesis studies (ΔG is in kcal/mol)

Target	Mutations	Calculated value						Experimental value
		ΔE_{ele}	ΔE_{vdW}	$\Delta G_{pol,GB}$	$\Delta G_{nonpol,GB}$	ΔG_{calc}	$\Delta\Delta G_{calc,GB}$[a]	$\Delta\Delta G_{exp}$[d]
hNET-like	Y95F	-25.38	-34.59	24.97	-3.87	-38.87	1.78	2.15
	I172V	-26.59	-33.88	25.98	-3.81	-38.30	2.35	
	T439S	-27.58	-33.91	26.63	-3.78	-38.64	2.01	
	Y95F-I172V-T439S	-30.65	-33.39	30.17	-3.86	-37.72	2.93	
hDAT-like	Y95F	-25.38	-34.59	24.97	-3.87	-38.87	1.78	2.06
	A169S	-26.71	-34.14	26.25	-3.86	-38.46	2.19	
	I172V	-26.59	-33.88	25.98	-3.81	-38.30	2.35	
	T439A	-32.34	-34.79	24.75	-3.88	-38.35	2.30	
	Y95F-I172V-A169S-T439S	-31.23	-32.16	29.62	-3.74	-37.51	3.14	

[a] $\Delta\Delta G_{cal} = \Delta G_{mutation} - \Delta G_{wild\ type}$ (the $\Delta G_{wild\ type}$ of complex was listed in **Table 2** and **SI, Table S2**).

[b] $\Delta\Delta G_{exp}$ were derived from the FC_{exp} by the equation $\Delta\Delta G_{exp} = RTln(FC_{exp})$.

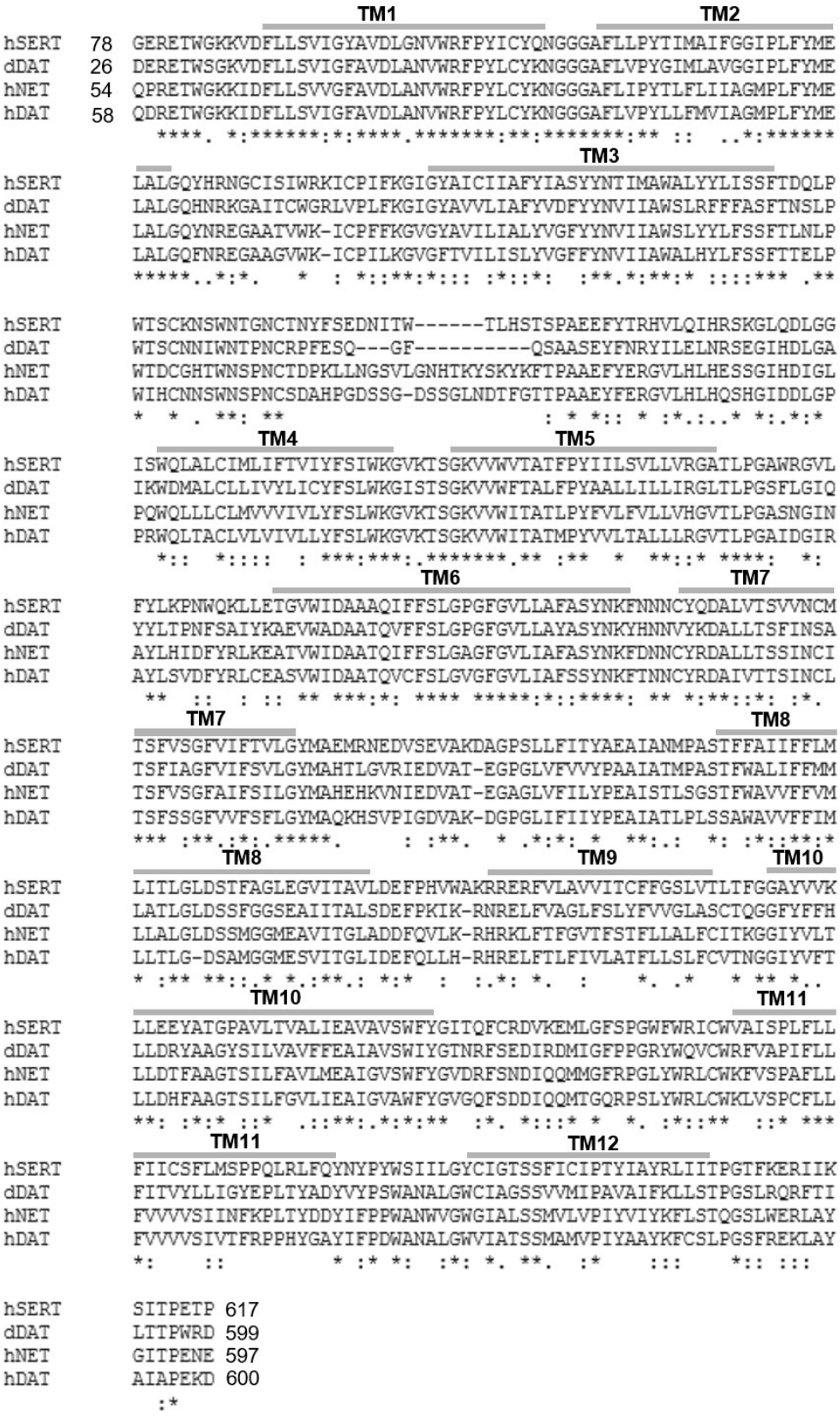


Figure S1. Sequence alignment of hSERT (from Glu78 to Pro617), hNET (from Gln54 to Glu597) and dDAT (from Glu26 to Asp599) using ClustalW2 program[3]. The twelve transmembrane (TM1 to TM12) alpha helices are labeled above the sequence. Stars refer to the identical residues, the double filled periods refer to the conservative substitutions and the filled periods refer to the variable conservative substitutions.

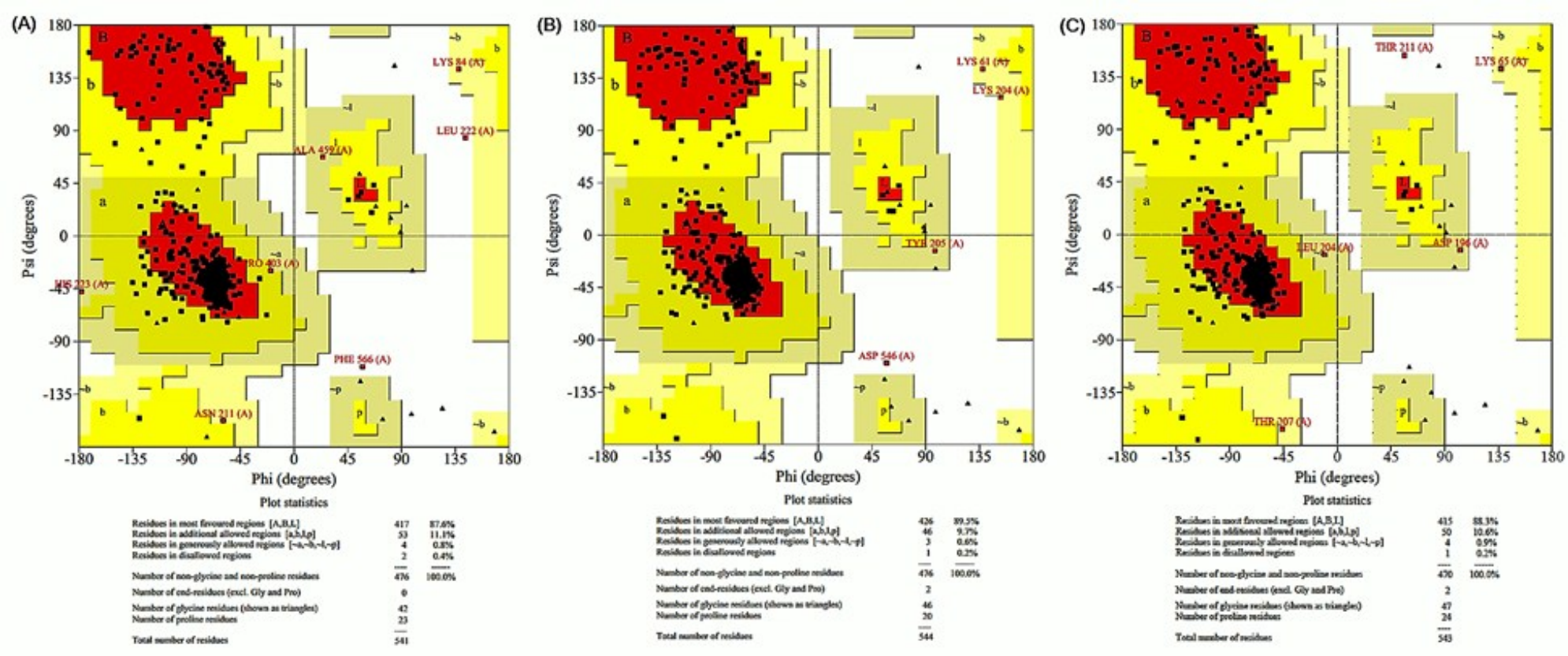


Figure S2. Ramachandran plot of the (A) hSERT, (B) hNET and (C) hDAT models. 99.6%, 99.8% and 99.8% amino acids in the hSERT, hNET and hDAT models locate in the allowed zone.

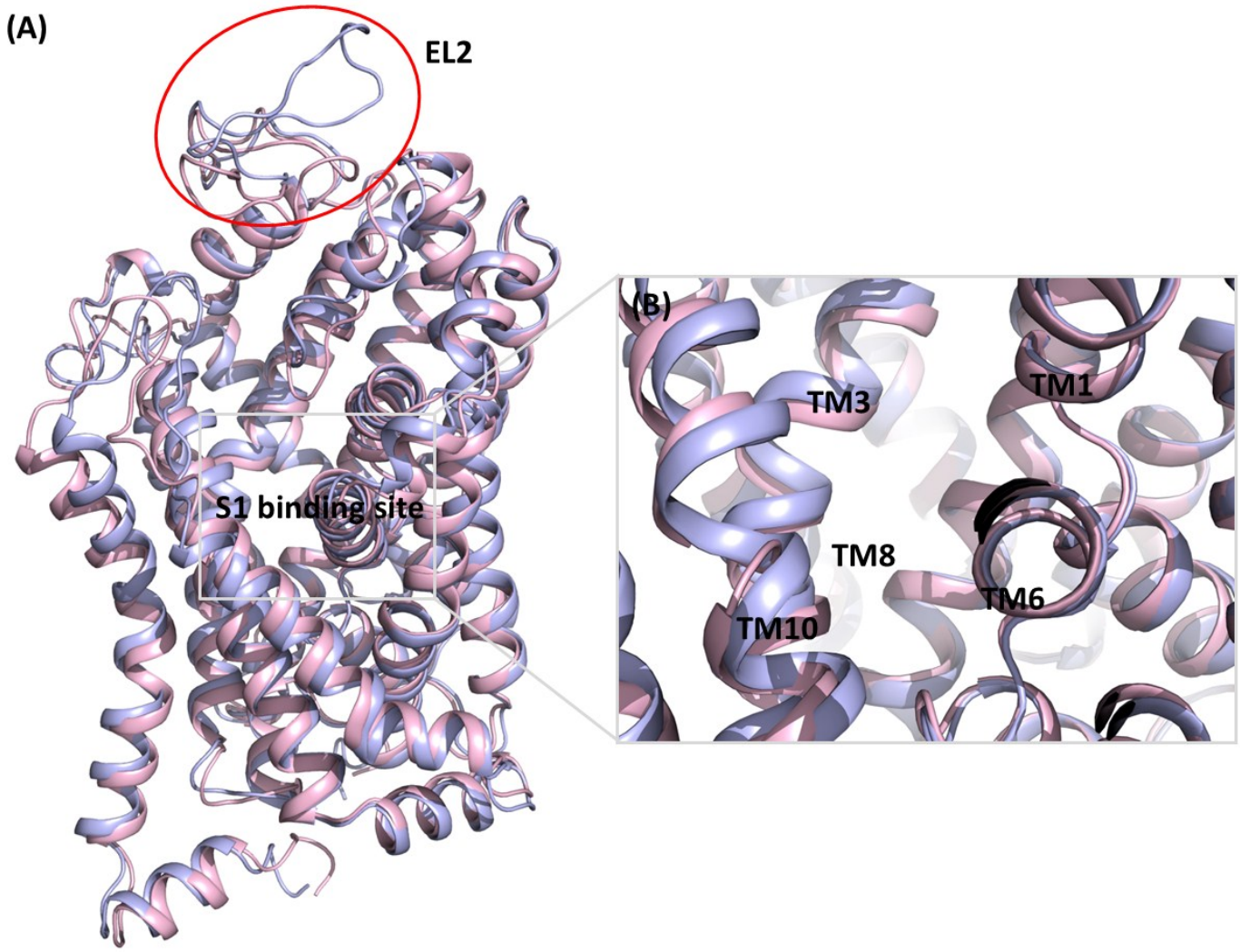


Figure S3. Structural superimposition between the crystal structure of hSERT (PDB code 5I6Z) and the homology model on the basis of the dDAT crystal structure (PDB code 4M48) as a template. The crystal and homology model structures of hSERT are shown in cartoon representation in light blue and light pink, respectively. The proteins were visualized using PyMOL[4].

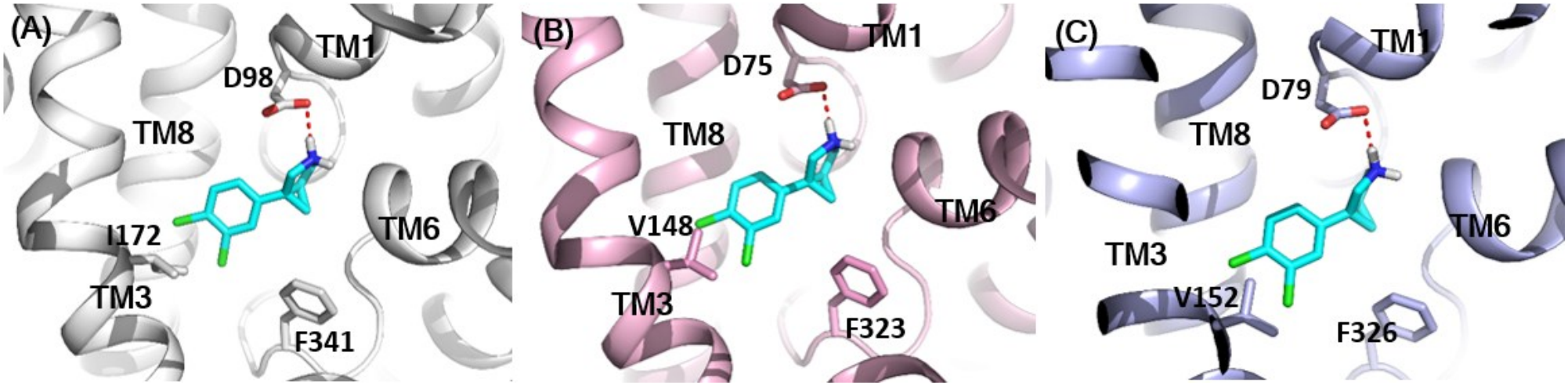


Figure S4. Docking poses of amitifadine in the binding pocket of the modeled (A) hSERT, (B) hNET and hDAT. The hSERT (gray) and hNET (light pink) and hDAT (light blue) were shown in ribbon representation. Amitifadine was shown as cyan stick. The protein-ligand complexes were visualized using PyMOL[4].

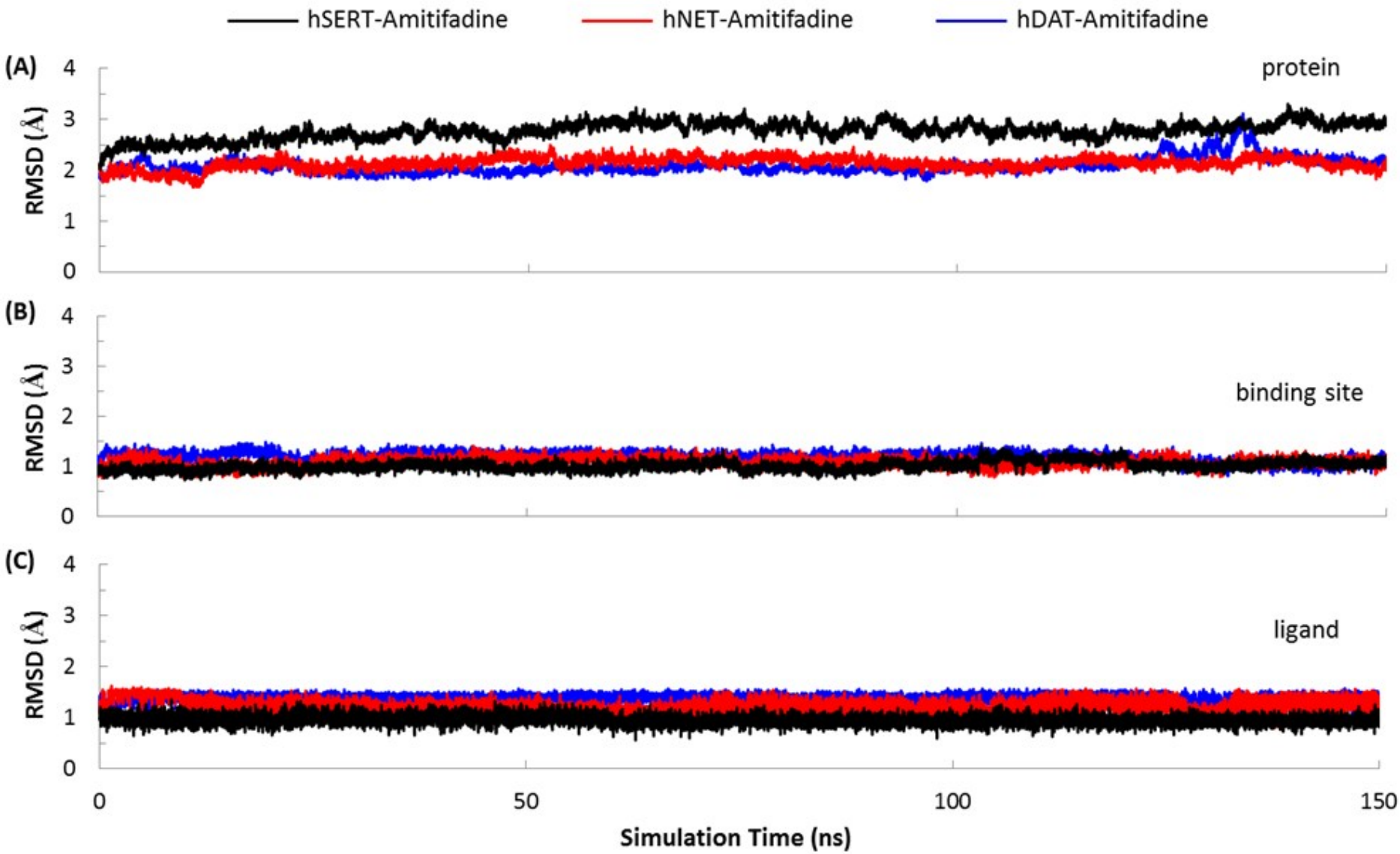


Figure S5. RMSD of the protein backbone atoms and ligand heavy atoms as a function of time during MD simulations.

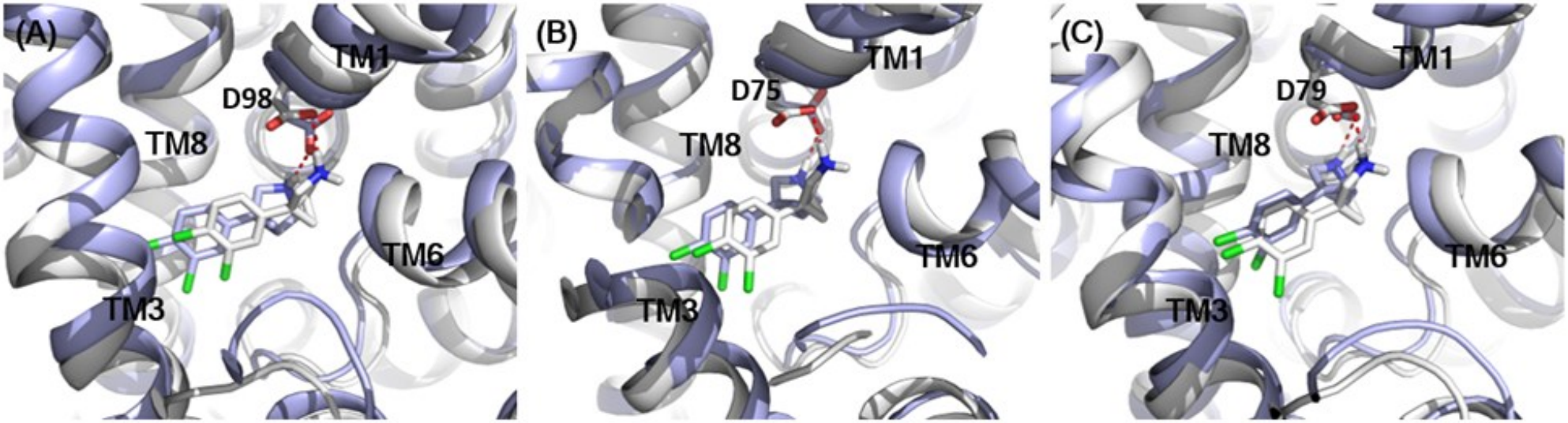


Figure S6. Structural superimposition between the docking poses (gray) and MD results (light brown) of amitifadine in (A) hSERT, (B) hNET and (C) hDAT. The protein-ligand complexes were visualized using PyMOL[4].

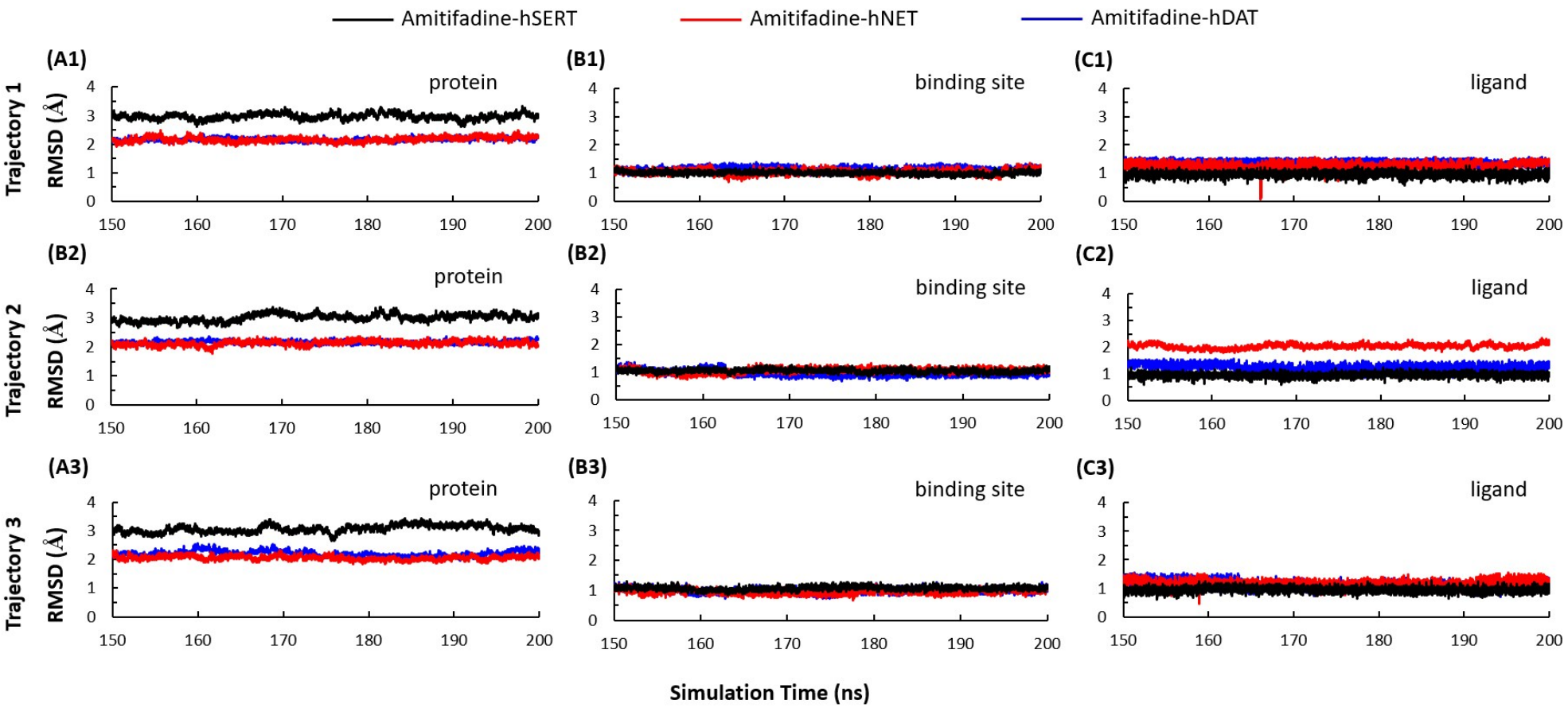


Figure S7. RMSD of the protein backbone atoms and ligand heavy atoms as a function of time during additional 50 ns MD simulations.

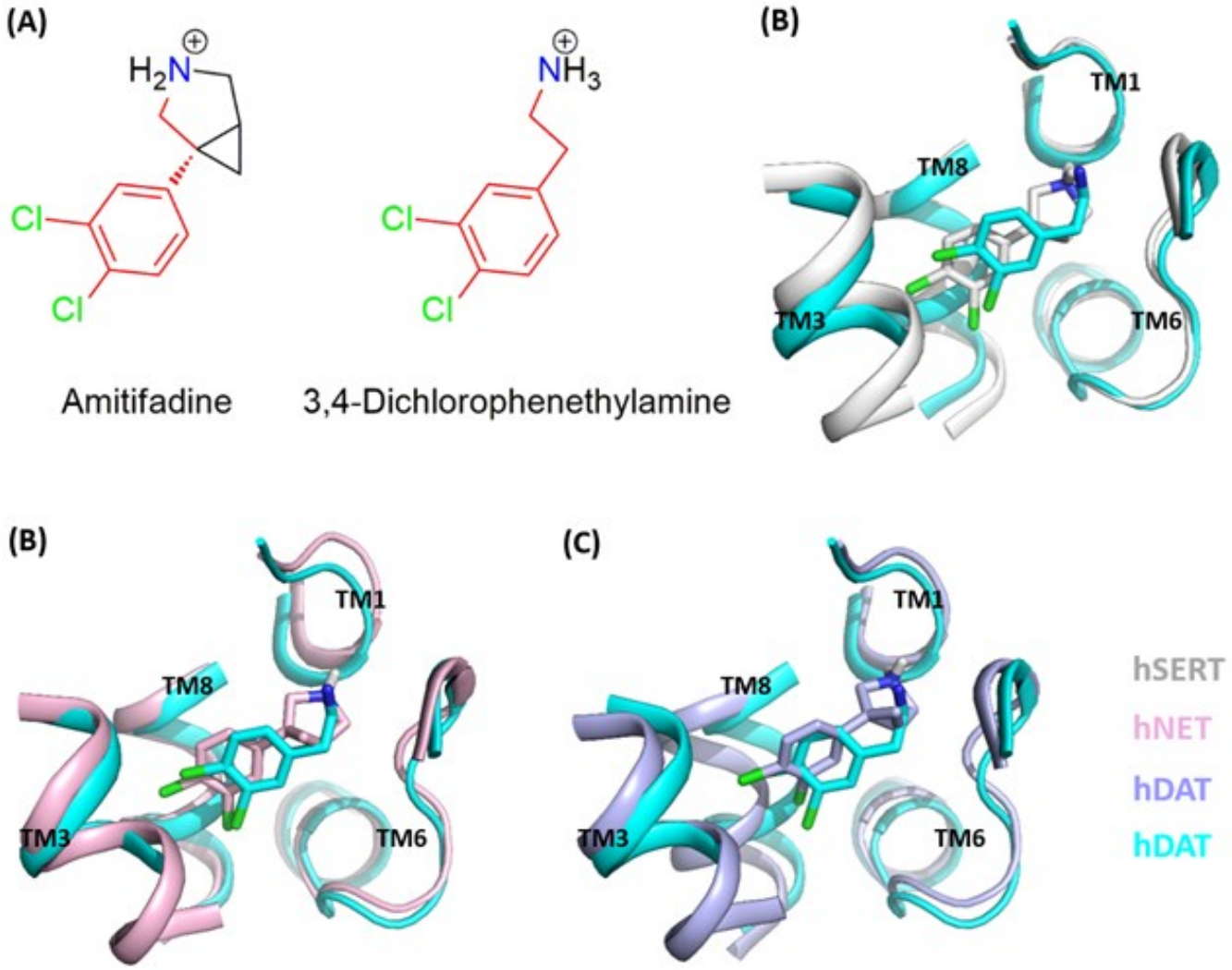


Figure S8. (A) Structures of amitifadine and 3,4-dichlorophenethylamine. Colors highlight the common features. (B-C) The overlay of representative snapshots of hSERT (gray), hNET (light pink) and hDAT (light blue) bound with amitifadine onto the crystal structure of dDAT in complex with 3,4-dichlorophenethylamine. The proteins are shown as carton. The TM regions are labeled as discussed in the text. The protein-ligand complexes were visualized using PyMOL[4].

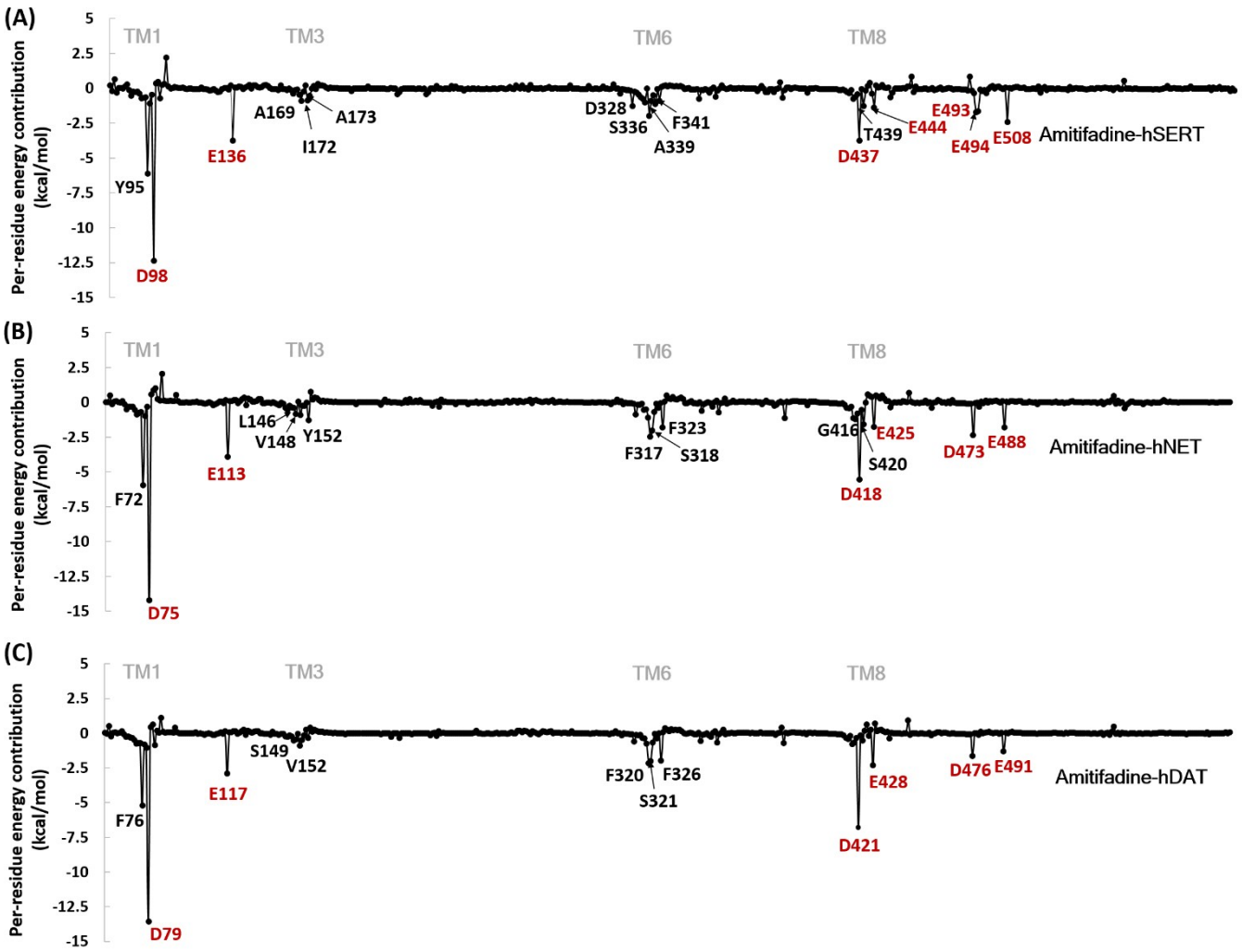


Figure S9. Per-residue contributions (MM/PBSA) of binding energies of the three studied protein-ligand complexes.

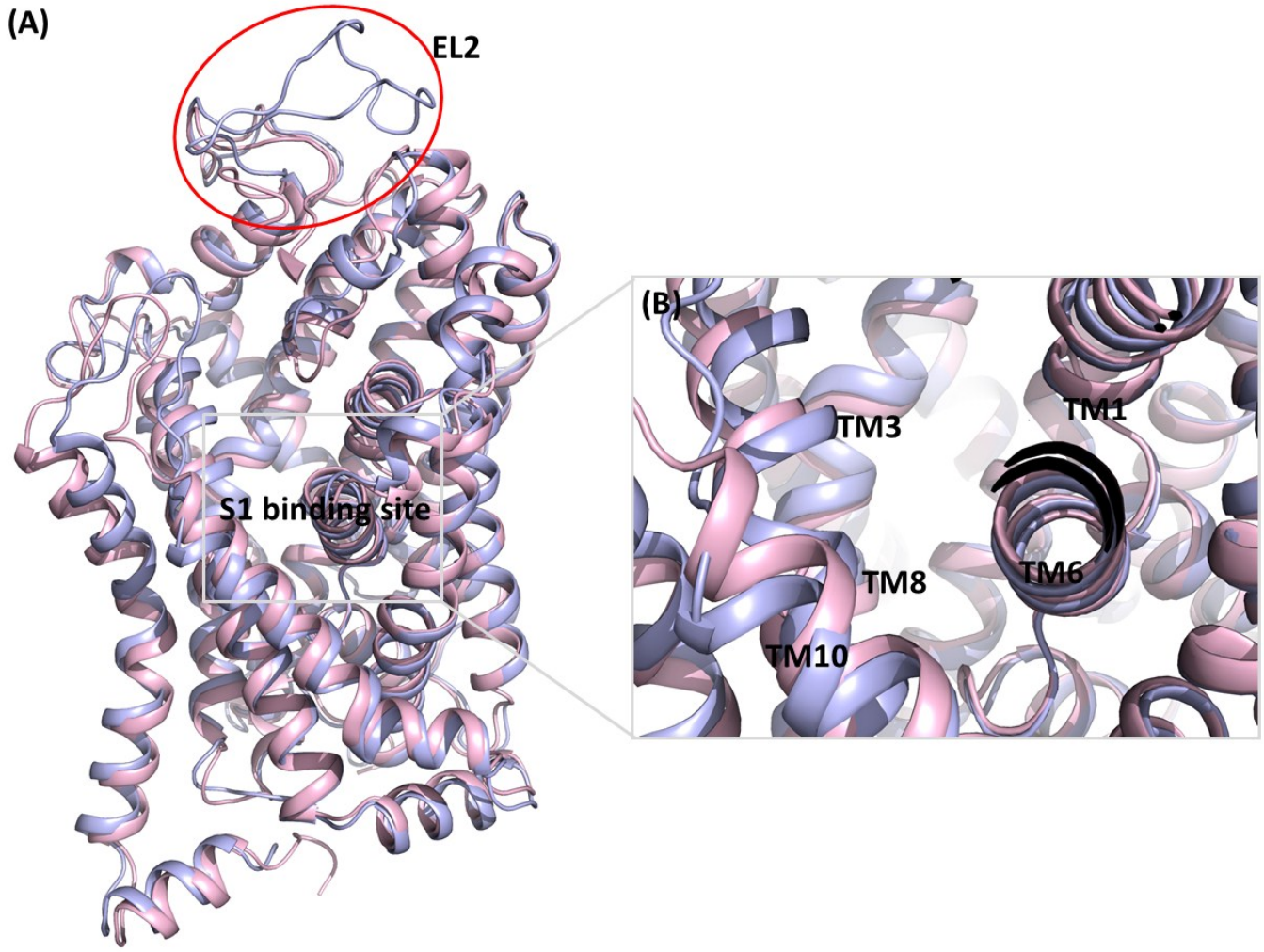


Figure S10. Structural superimposition between the homology model structures of hNET on the basis of the hSERT (PDB code 5I6Z) and dDAT (PDB code 4M48) crystal structured as the template. The two homology model structures of hNET are shown in cartoon representation in light blue and light pink, respectively. The proteins were visualized using PyMOL[4].

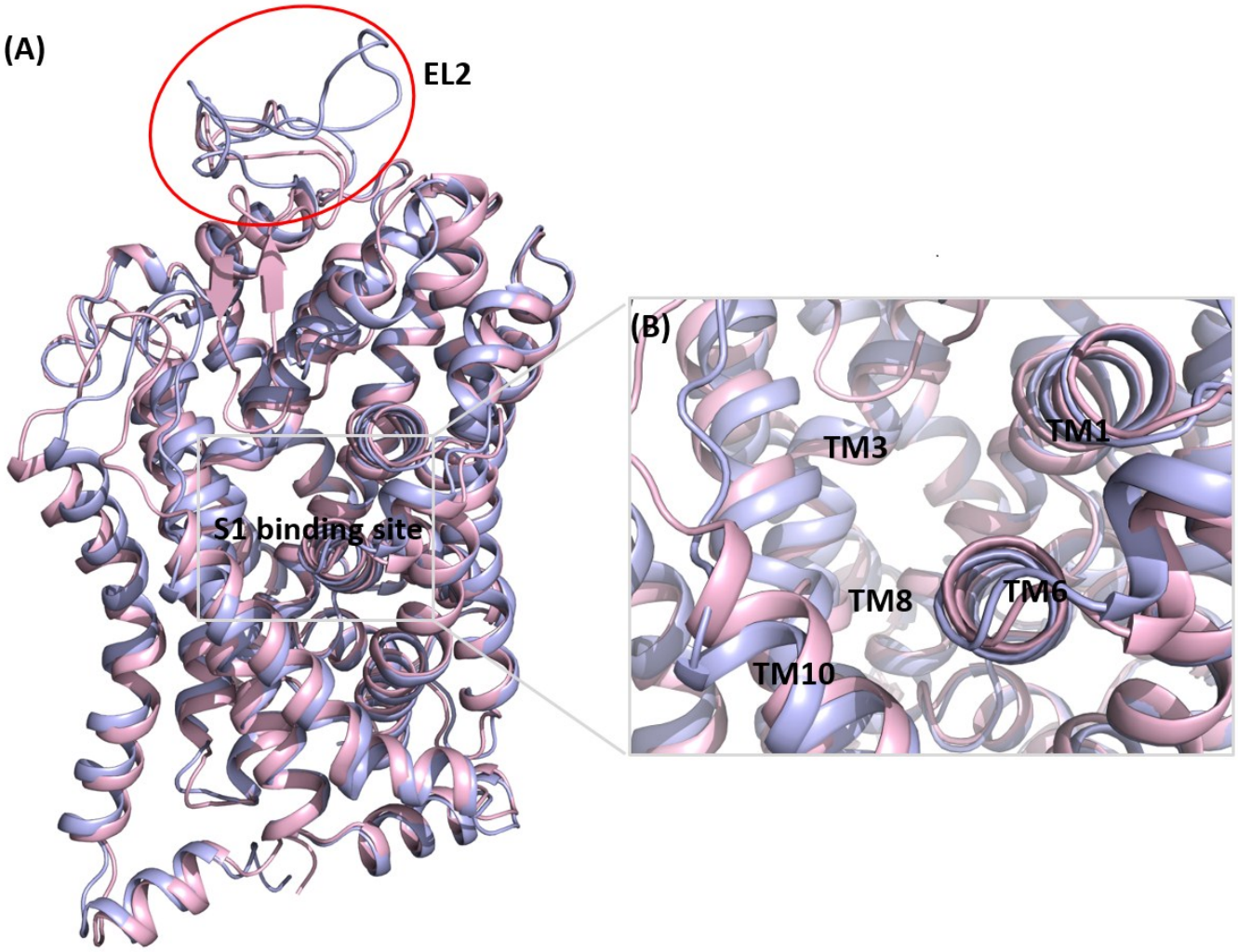


Figure S11. Structural superimposition between the homology model structures of hDAT on the basis of the hSERT (PDB code 5I6Z) and dDAT (PDB code 4M48) crystal structured as the template. The two homology model structures of hNET are shown in cartoon representation in light blue and light pink, respectively. The proteins were visualized using PyMOL[4].

References

1. A. Penmatsa, K. H. Wang and E. Gouaux, *Nature*, 2013, **503**, 85-90.
2. J. A. Coleman, E. M. Green and E. Gouaux, *Nature*, 2016, **532**, 334-339.
3. M. A. Larkin, G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson and D. G. Higgins, *Bioinformatics*, 2007, **23**, 2947-2948.
4. PyMOL Molecular Graphics System v. 1.3, Schrödinger, LLC.