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Supporting Information For

Predicted structures and dynamics for agonists and antagonists bound to serotonin 5-HT2B and 5-HT2C receptors

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Table S1. Predicted transmembrane (TM) region by three methods and hydrophobic centers by two methods. The hydrophobic center by peak method displayed in bold face, while area center was underlined in the sequence.

TM#	Method	TM region	Seq.	Peak	Area
TM1	Predict1	L H W A A L L I L M V I I **P** T I G G N T L V I L A V S L E	29	13.67	13.35
	Predict2	N K L H W A A L L I L M V I **I** P T I G G N T L V I L A V S L E	31	15.00	14.66
	Predict3	L H W A A L L I L M V I **I** P T I G G N T L V I L A V S L E	29	13.50	12.92
TM2	Predict1	T N Y F L M S L A V A D L **L** V G L F V M P I A L L T I M F E A M	32	13.67	19.34
	Predict2	T N Y F L M S L A V A D L L V G L F V M P I **A** L L T I M F E A M	32	23.50	19.89
	Predict3	A T N Y F L M S L A V A D L **L** V G L F V M P I A L L T I M F E A M	33	15.00	18.51
TM3	Predict1	P A W L F L D V L F **S** T A S I M H L C A I S V D	24	11.33	9.35
	Predict2	L C P A W L F L D V L F **S** T A S I M H L C A I	23	13.50	9.85
	Predict3	C P A W L F L D V L F **S** T A S I M H L C A I S V D	25	12.50	11.77
TM4	Predict1	T A F I K I T V V W L I **S** I G I A I P V P I K G	24	12.67	12.49
	Predict2	A F I K I T V V W L I S **I** G I A I P V P I K G	23	13.00	11.83
	Predict3	A F I K I T V V W L I S **I** G I A I P V P I K G I	24	12.83	12.32
TM5	Predict1	F G D F M L F G S L A A F F **T** P L A I M I V T Y F L T I H	29	14.67	16.07
	Predict2	M L F G S L A A F F **T** P L A I M I V T Y F L T I H	25	11.50	12.53
	Predict3	F M L F G S L A A F **F** T P L A I M I V T Y F L T I H	26	11.00	12.79
TM6	Predict1	L G I V F F L F L L M **W** C P F F I T N I T L V L C D	26	12.33	12.94
	Predict2	L G I V F F L F L L M W C **P** F F I T N I T L V L C D	25	14.00	12.60
	Predict3	G I V F F L F L L M **W** C P F F I T N I T L V L C D	25	11.00	12.06
TM7	Predict1	Q M L L E I F V W I G Y V S **S** G V N P L V Y T L F N K	27	14.67	12.69
	Predict2	Q M L L E I F V W I G Y V S **S** G V N P L V Y T L F N K	27	15.00	12.46
	Predict3	Q M L L E I F V W I G Y V **S** S G V N P L V Y T L F N K	27	14.50	12.15

Table S2. The experiment binding affinity and predicted scoring energy (kcal/mol) of several 5-HT2B agonists at the human 5-HT2B receptor

Charge

Compound	Pki	UnifiedCav	PartialSol	Total
SNF	7.76	-70.87	-18.70	24.72
DesMeNF	7.26	-70.76	-14.04	58.60
RNF	7.21	-69.26	-13.78	26.51
EthylNF	6.11	-64.69	-12.58	36.16

Neutral

Compound	Pki	UnifiedCav	PartialSol	Total
SNF	7.76	-25.87	-21.88	-31.26
RNF	7.21	-25.55	-18.50	-31.14
DesMeNF	7.26	-25.42	-18.13	-5.18
EthylNF	6.11	-22.95	-18.97	-20.09

- UnifiedCav: unified caivity E, PartialSol: partial solvation E, Total: Total E

Table S3. The 10 most stable 7-helix conformations for the human 5-HT2B receptor from the BiHelix analysis. Here the reference angle of zero = [0, 0, 0, 0, 0, 0, 0] in bold face is final MembEnsemb conformations, which corresponds to {-60, -60, 0, 60, -60, 60, 0} referenced to the frog rhodopsin template. InterHB is the sum of hydrogen bond energies between various helices. BiHelE (S3) is the sum of the helix-pairwise interaction energies from the BiHelix analysis.

#	H1	H2	H3	H4	H5	H6	H7	InterHB	BiHelE
1	0	0	0	330	0	0	0	-59.1	245.5
2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**-54.2**	**249.7**
3	0	0	0	330	0	60	90	-69.3	262.7
4	0	0	0	0	0	60	90	-64.4	267.0
5	0	0	0	330	0	90	120	-64.4	273.0
6	0	0	0	0	0	90	120	-59.5	277.2
7	0	0	0	330	0	60	120	-58.7	286.4
8	0	0	0	330	0	30	120	-41.9	288.4
9	0	0	0	0	0	60	120	-53.8	290.6
10	0	0	0	0	0	30	120	-37.0	292.7

Table S4. Cavity analysis of SB-206533 **1** and **2** at human 5-HT2B and 2C receptors. The residues was ordered by the non-bonding energy difference (2C -2B).

2 (pKi, 2B: 7.3)			**2 (pKi, 2C: 5.4)**			**Diff. 2C-2B**	**1 (2B: 7.6)**	**1 (2C: 7.9)**
Res	**#**	**NonBond**	**Res**	**#**	**NonBond**		**NonBond**	**NonBond**
SUM		**-47.53**	**SUM**		**-36.06**	**11.47**	**-42.48**	**-42.28**
LEU	**132**	**-2.35**	**ILE**	**132**	**6.12**	**8.46**	**-2.48**	**-0.84**
ASN	278	-2.40	ASN	276	-1.54	0.86	-2.19	-0.47
PHE	226	-2.05	PHE	224	-1.38	0.68	-1.02	-2.39
THR	277	-1.33	THR	275	-0.66	0.67	-1.17	
VAL	136	-5.34	VAL	136	-4.91	0.43	-3.78	-5.44
ALA	225	-1.09	ALA	223	-0.68	0.41	-0.48	-1.12
MET	270	-0.40				0.40	-0.06	
PHE	275	-0.38				0.38		
LEU	137	-0.38				0.38		
LEU	**281**	**-0.56**	**SER**	**279**	**-0.23**	**0.33**	**-0.67**	**0.00**
MET	**218**	**-0.32**	**VAL**	**216**		**0.32**	**-0.35**	**-0.28**
LEU	219	-0.28				0.28	-0.27	
LEU	**223**	**-0.26**	**PHE**	**221**		**0.26**	**-0.20**	**-0.30**
ILE	143	-0.51	ILE	143	-0.26	0.25	-0.28	-0.31
SER	222	-1.70	SER	220	-1.49	0.21	-2.03	-2.92
PHE	267	-0.59	PHE	265	-0.42	0.17	-0.52	-0.09
ILE	276	-0.16	ILE	274		0.16		-0.10
SER	306	-0.15				0.15		
ILE	302	-0.12				0.12	-0.10	
PHE	**220**	**-0.07**	**ILE**	**218**	**0.00**	**0.07**	**0.00**	**-0.08**
SER	*139*	*-6.34*	*SER*	*139*	-6.28	*0.06*	*-5.67*	*-6.95*
ALA	**224**	**-0.05**	**VAL**	**222**		**0.05**	**0.00**	**-0.14**
CYS	272	-0.03				0.03	-0.03	
LEU	**269**	**-0.03**	**ILE**	**267**	**0.00**	**0.03**	**-0.03**	**0.00**
PHE	274	-5.95	PHE	272	-5.93	0.02	-4.43	-5.80
LYS	**211**	**-0.01**				**0.01**	**-0.06**	
ILE	**298**	**0.00**				**0.00**	**-0.02**	
TRP	131	-0.32	TRP	131	-0.32	**0.00**	-0.37	-0.44
ILE	**186**	**0.00**	**VAL**	**186**	**0.00**	**0.00**	**-0.10**	**-1.27**
ALA	**187**	**0.00**	**SER**	**187**	**0.00**	**0.00**	**-0.12**	**-0.58**
ASP	216						-0.38	
SER	307	-0.85	SER	306	-0.86	0.00	-0.77	
MET	**294**	**0.01**				**-0.01**		
VAL	107	-0.08	VAL	107	-0.10	-0.02	-0.10	
LEU	295	-0.23	LEU	294	-0.26	-0.03	-0.26	
PHE	265	0.05				-0.05		

PHE	138	-0.70	PHE	138	-0.76	-0.06	-0.63	
TYR	304	-1.59	TYR	303	-1.65	-0.06	-1.35	-0.53
THR	140	-1.16	THR	140	-1.32	-0.17	-0.96	-2.12
VAL	**190**	**-0.51**	**ILE**	**190**	-0.72	**-0.20**	**-0.76**	**-1.24**
GLY	303	-0.87	GLY	302	-1.10	-0.23	-0.73	-0.31
TRP	271	-0.84	TRP	269	-1.14	-0.30	-1.42	-1.77
VAL	300	-1.72	VAL	299	-2.19	-0.48	-1.83	-0.09
LEU	296	-1.05	LEU	295	-1.59	-0.54	-1.21	-0.69
ASP	135	-2.87	ASP	135	-3.63	-0.75	-3.81	-3.50
PHE	299	-1.95	PHE	298	-2.76	-0.82	-1.87	-1.54
			GLY	219				-0.68
			PRO	191				-0.07
			PRO	271				-0.11
			VAL	304				-0.06
			LEU	278				-0.05
			ASN	296				-0.04
			TRP	300				-0.03
			ILE	226				0.01

Table S5. Cavity analysis of PRX-08066 **3**, at the human 5-HT2B and 2C receptors. The residues was ordered by the nonbonding energy difference (2C – 2B).

3 (FLIPR: 3.2 nM, Ki, 5HT2B: 30 nM)			**3 (HT2C FLIPR selectivity: > 3,200)**			**Diff. 2C-2B**
Res	**#**	**NonBond**	**Res**	**#**	**NonBond**	
SUM		**-42.25**	**SUM**		**-17.98**	**20.44**
PHE	365	-1.12	PHE	353	3.44	4.56
VAL	366	-1.68	VAL	354	2.71	4.39
PHE	138	-0.53	PHE	137	3.21	3.75
TYR	370	-2.10	TYR	358	1.04	3.14
PHE	340	-3.90	PHE	327	-2.33	1.57
THR	343	-1.41	THR	330	0.15	1.56
LEU	**132**	**-3.76**	**ILE**	**131**	**-2.43**	**1.33**
LEU	**347**	**-2.49**	**SER**	**334**	**-1.60**	**0.90**
VAL	**190**	**-0.20**	**ILE**	**189**	**0.42**	**0.62**
VAL	**103**	**-0.80**	**VAL**	**102**	**-0.24**	**0.56**
TRP	131	-1.23	TRP	130	-0.74	0.49
SER	373	0.21	SER	361	0.57	0.36
LEU	362	-1.88	LEU	350	-1.55	0.33
SER	139	-2.74	SER	138	-2.60	0.14
TRP	337	-0.73	TRP	324	-0.60	0.13
VAL	107	-0.50	VAL	106	-0.39	0.11
VAL	348	-0.41	VAL	335	-0.34	0.07
PHE	333	-0.78	PHE	320	-0.74	0.04
SER	142	-0.07	SER	141	-0.12	-0.04
SER	222	-0.83	SER	219	-0.88	-0.04
MET	**218**	**-0.50**	**VAL**	**215**	**-0.57**	**-0.07**
APP	*135*	*-7.52*	*APP*	*134*	*-7.70*	-0.19
VAL	136	0.70	VAL	135	0.45	-0.25
			MET	107	-0.31	-0.31
			VAL	208	-0.47	-0.47
THR	**210**	**-0.32**	**ASN**	**210**	**-0.97**	**-0.65**
ASN	*344*	*-2.67*	*ASN*	*331*	*-3.44*	-0.77
LEU	361	-1.16	LEU	349	-1.95	-0.78
LYN	**211**	**-2.56**				
PRO	191	-0.57				
GLP	**212**	**-0.30**				
MET	336	-0.23				
SER	**372**	**-0.17**				

- The residues in the same line for 5-HT2B and 2C receptor have the same Ballestreros numbers .
- The residues that are variable between 2B and 2C are displayed in bold face.

Table S6. The ensemble docking results of the human 5-HT2B receptor for agonist, HT and antagonist, SB1, in charged and neutral system

Charge system

HT

Rank	TM rotation	UnifiedCav	FullSol	PartialSol	LocalCav	Total	Interaction	ProteinE
1	0, 0, 0, 0, 0, 0, 0	**-53.54**	-5.50	-33.07	-53.24	**-45.86**	-12.69	**-33.17**
5	0, 0, 0, 0, 0, 0, -30	-52.58	-11.11	**-34.14**	-53.02	-23.45	**-20.97**	-2.48
2	0, 0, 0, -30, 0, 0, 0	-53.26	**-13.27**	-33.00	**-53.59**	6.88	-12.40	19.28
4	0, 0, 0, 0, 0, 30, -30	-44.59	-6.63	-24.29	-43.46	24.25	-9.11	33.36
3	0, 0, 0, 0, 0, 30, 0	-44.46	-3.06	-22.67	-43.40	59.44	-7.67	67.11

SB1

Rank	TM rotation	UnifiedCav	FullSol	PartialSol	LocalCav	Total	Interaction	ProteinE
5	0, 0, 0, 0, 0, 0, -30	-35.70	-33.63	-51.31	-34.89	**112.27**	2.53	**109.75**
1	0, 0, 0, 0, 0, 0, 0	-40.80	-43.25	-50.79	-39.66	114.87	4.30	110.57
4	0, 0, 0, 0, 0, 30, -30	-39.61	-32.05	-50.55	-37.59	148.64	5.16	143.48
2	0, 0, 0, -30, 0, 0, 0	**-44.45**	**-46.70**	**-52.74**	**-43.83**	176.82	**1.43**	175.39
3	0, 0, 0, 0, 0, 30, 0	-36.08	-23.84	-43.65	-35.18	220.11	9.75	210.36

Neutral system

HT

Rank	TM rotation	UnifiedCav	FullSol	PartialSol	LocalCav	Total	Interaction	ProteinE
1	0, 0, 0, 0, 0, 0, 0	-7.21	84.69	93.85	-7.35	**-67.32**	4.24	**-71.57**
5	0, 0, 0, 0, 0, 0, -30	-2.12	104.71	118.87	-2.74	-25.27	10.98	-36.25
2	0, 0, 0, -30, 0, 0, 0	-10.66	**79.27**	**89.91**	-10.14	-15.57	**0.54**	-16.12
3	0, 0, 0, 0, 0, 30, 0	-11.39	109.72	115.67	-11.32	16.63	12.38	4.25
4	0, 0, 0, 0, 0, 30, -30	**-17.92**	99.51	105.48	**-18.24**	11.49	5.68	5.81

SB1

Rank	TM rotation	UnifiedCav	FullSol	PartialSol	LocalCav	Total	Interaction	ProteinE
5	0, 0, 0, 0, 0, 0, -30	-35.78	-41.99	-48.60	-34.80	**49.99**	5.13	**44.86**
1	0, 0, 0, 0, 0, 0, 0	-40.90	-47.29	-48.54	-39.85	54.66	6.42	48.24
4	0, 0, 0, 0, 0, 30, -30	-39.11	-40.44	-47.05	-37.11	76.63	8.17	68.45
2	0, 0, 0, -30, 0, 0, 0	**-44.25**	**-48.96**	**-50.28**	**-43.71**	120.75	**3.74**	117.01
3	0, 0, 0, 0, 0, 30, 0	-37.05	-37.10	-42.97	-36.23	126.73	10.32	116.41

- Rank number is from CombiHelix result.
- Final complexes were ordered by total energy (kcals/mol).
- UnifiedCav: unified caivity E, PartialSol: partial solvation E, Total: Total E

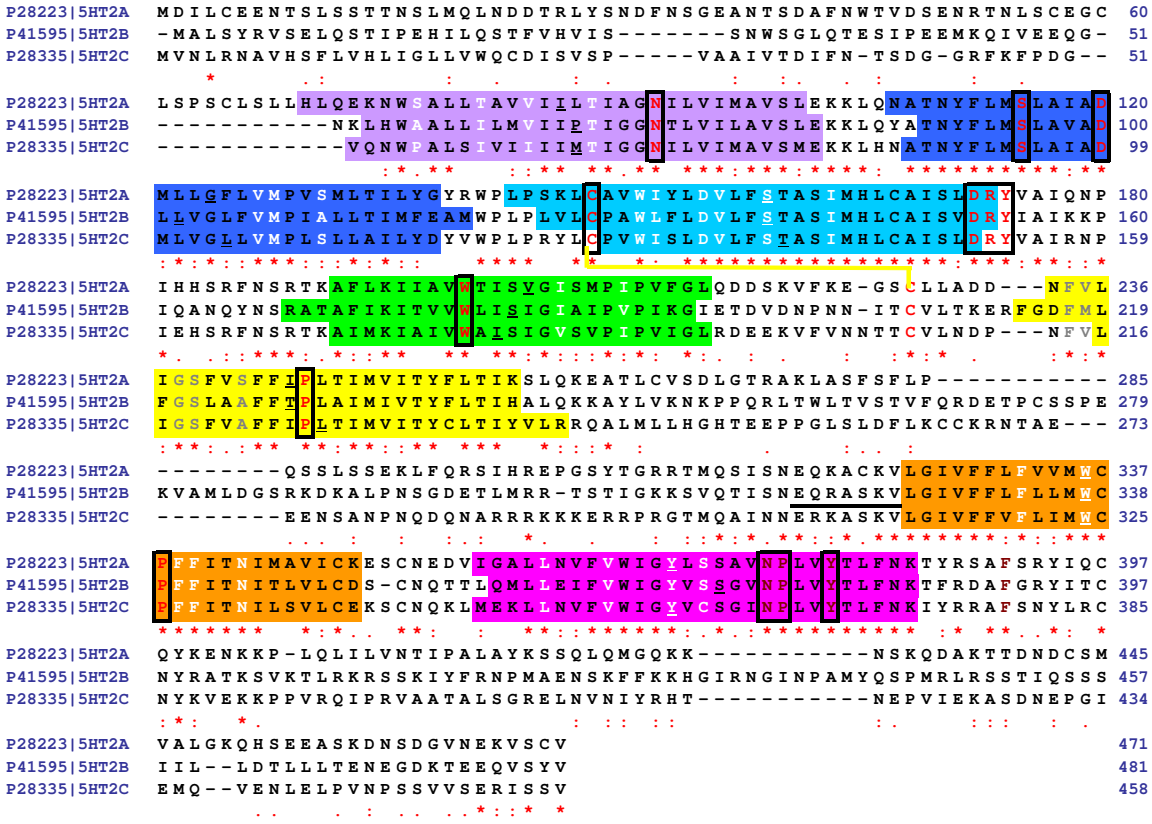

Fig. S1. TMPredict multiple alignments of three 5-HT2 receptors, 2A, 2B and 2C. The transmembrane (TM) helix predicted by TMPredict program are shown in different colors (TM1 in purple, TM2 in blue, TM3 in cyan, TM4 in green, TM5 in yellow, TM6 in orange, TM7 in red). Highly conserved residues in Family A receptors (N1.50, S2.45, D2.50, C3.25, D3.49, R3.50, Y3.51, W4.50, P5.50, P6.50, N7.49, P7.50, and Y7.53) are displayed in box. The residues in white are important amino acids from cavity analysis of the bound ligands. The % sequence identities are 38% for 2A:2B, 48% for 2A:2C, and 39% for 2B:2C.

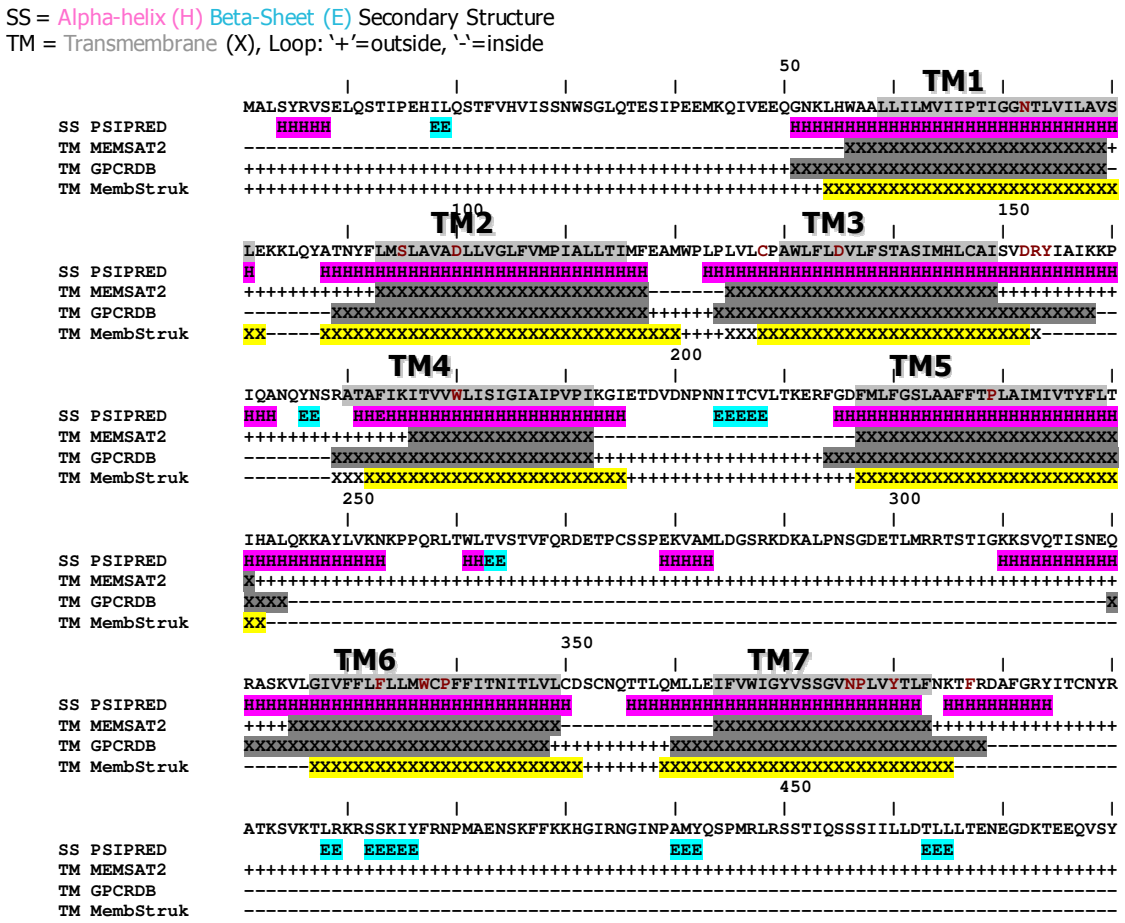

Fig. S2. The prediction of secondary structure for the human 5-HT2B receptor.

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TM1       LHWAALLILMVIIPTIGGNTLVILAVSLE(29) 13.67
TM2       TNYFLMSLAVADLLVGLFVMPIALLTIMFEAM(32) 13.67
TM3      LVLCPAWLFLDVLFSTASIMHLCAISVDRYIAIK(34) 15.33
TM4      RATAFIKITVVWLISIGIAIPVPIKG(26) 14.67
TM5      FGDFMLFGSLAAFFTPLAIMIVTYFLTIH(29) 14.67
TM6 EQRASKVLGIVFFLFLLMWCPFFITNITLVLCD(33) 19.33
TM7      QMLLEIFVWIGYVSSGVNPLVYTLFNK(27) 14.67
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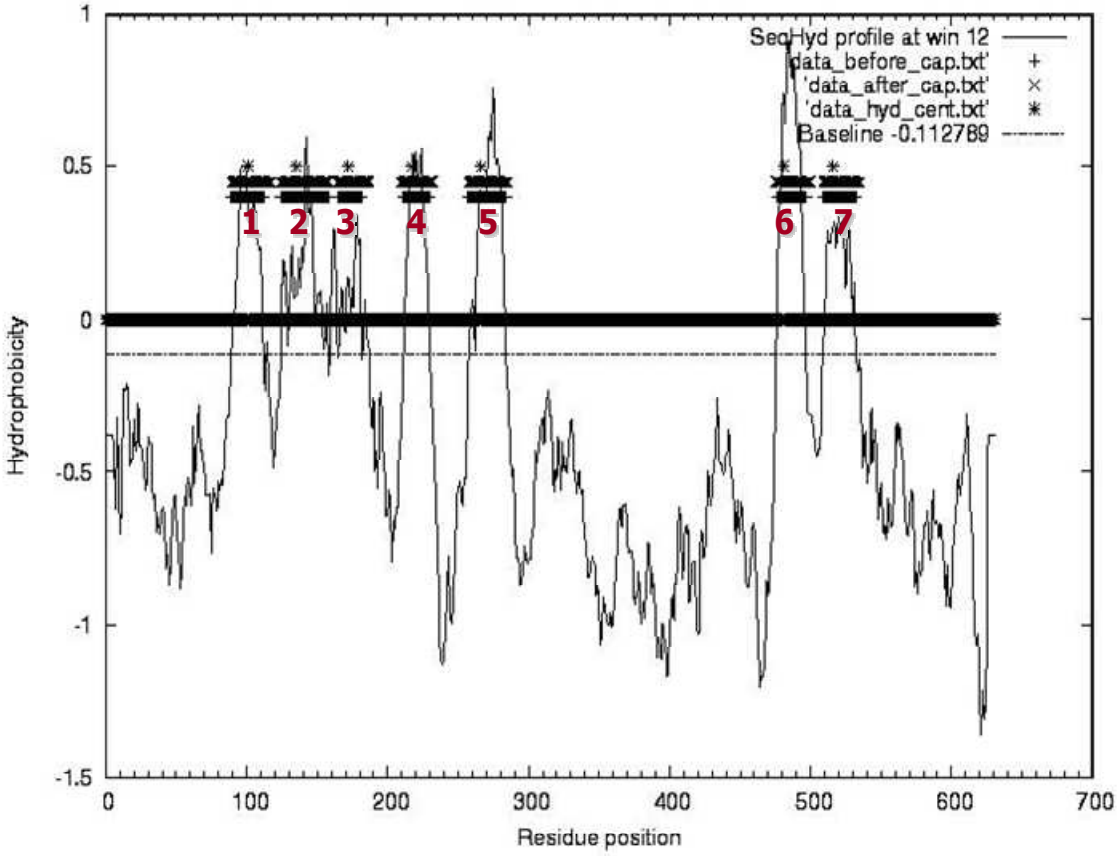


Fig. S3. The sequence of seven transmembrane (TM) regions (Top) and its hydropathy plot (Bottom) of human 5-HT2B receptor predicted by TMPred program. Hydrophobic centers by the peak method in underline were calculated. Highly conserved residues in each TM were shown in red.

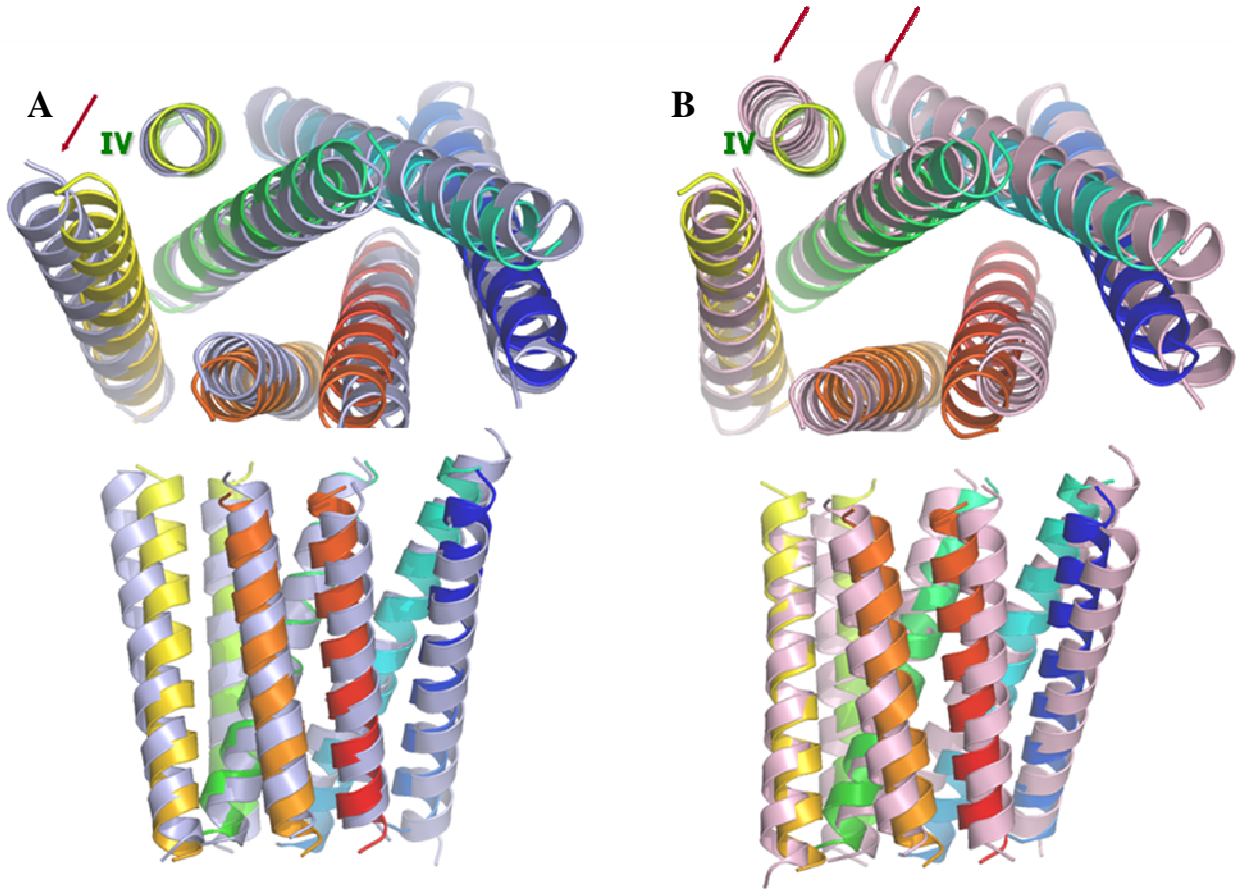


Fig. S4. Superimposition of two different templates with the frog rhodopsin[19] template in seven different colors (TM1: blue, TM2: cyan, TM3: green, TM4: light green, TM5: yellow, TM6: orange, TM7: red). The seven helices of human 5-HT2B receptor were generated using different templates of (A) mouse Mas-related gene (Mrg) C11 (mMrgC11)[19] in light blue and (B) human CCR1 (hCCR1) Chemokine receptor[20] in pink. Compared to the structure generated by the frog rhodopsin template, the RMSD of the 5-HT2B structure generated by mMrgC11 and hCCR1 receptor templates showed 3.44 and 3.98 Å, respectively. Major structural deviations are shown by arrows.

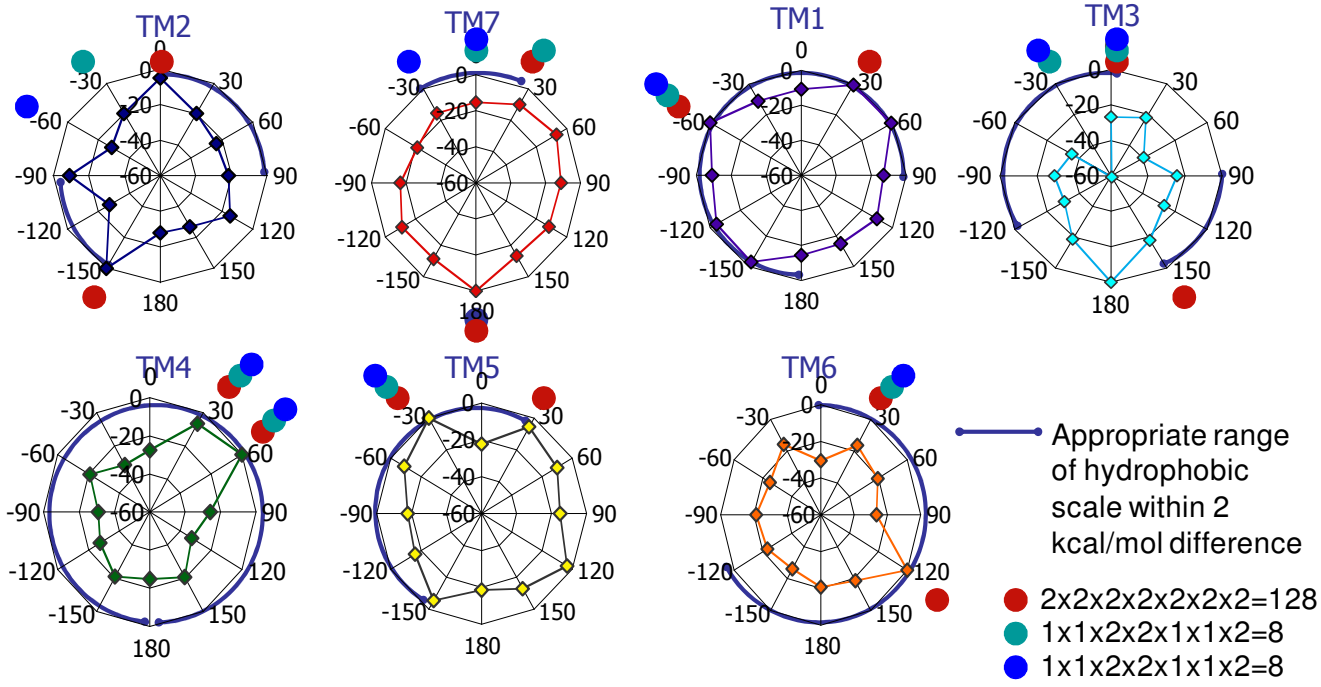


Fig. S5. Interhelical interaction energies of MembScream. E-scream energy of each transmembrane (TM) was calculated and plotted radically outward in kcal/mol. In the plot of Scream E, 0 is the lowest Scream E, and others are the relative energy compared with the lowest one. Energetically preferred angles of the hHT2B receptor at each TM were shown in red, green and blue at the first, second and third round, respectively. Those angles were considered within 2 kcal/mol relative energy differences of hydrophobic penalty.

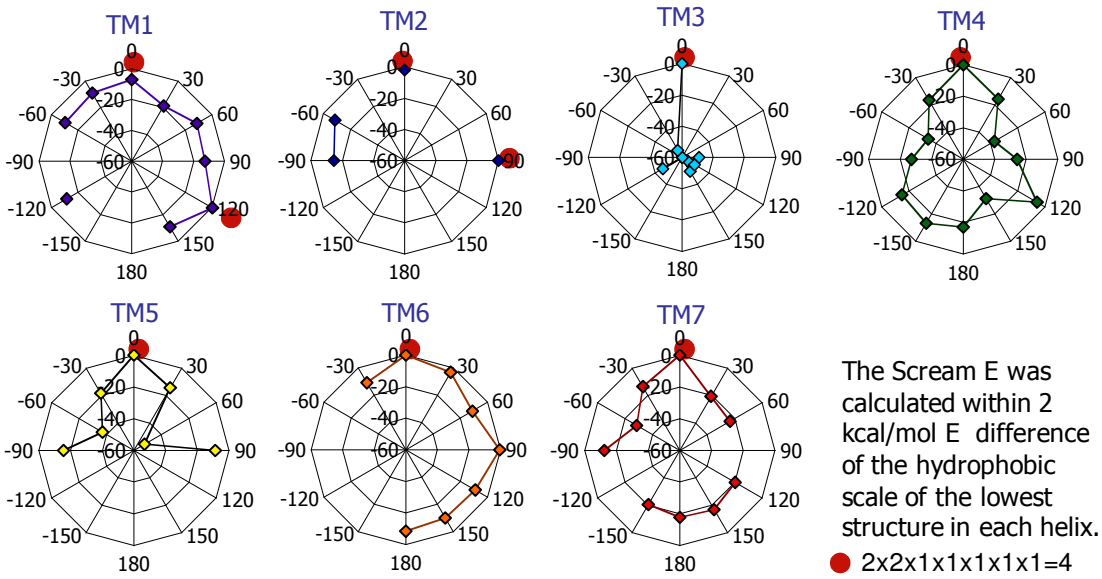


Fig. S6. Interhelical interaction energies of MembScream at the third round.

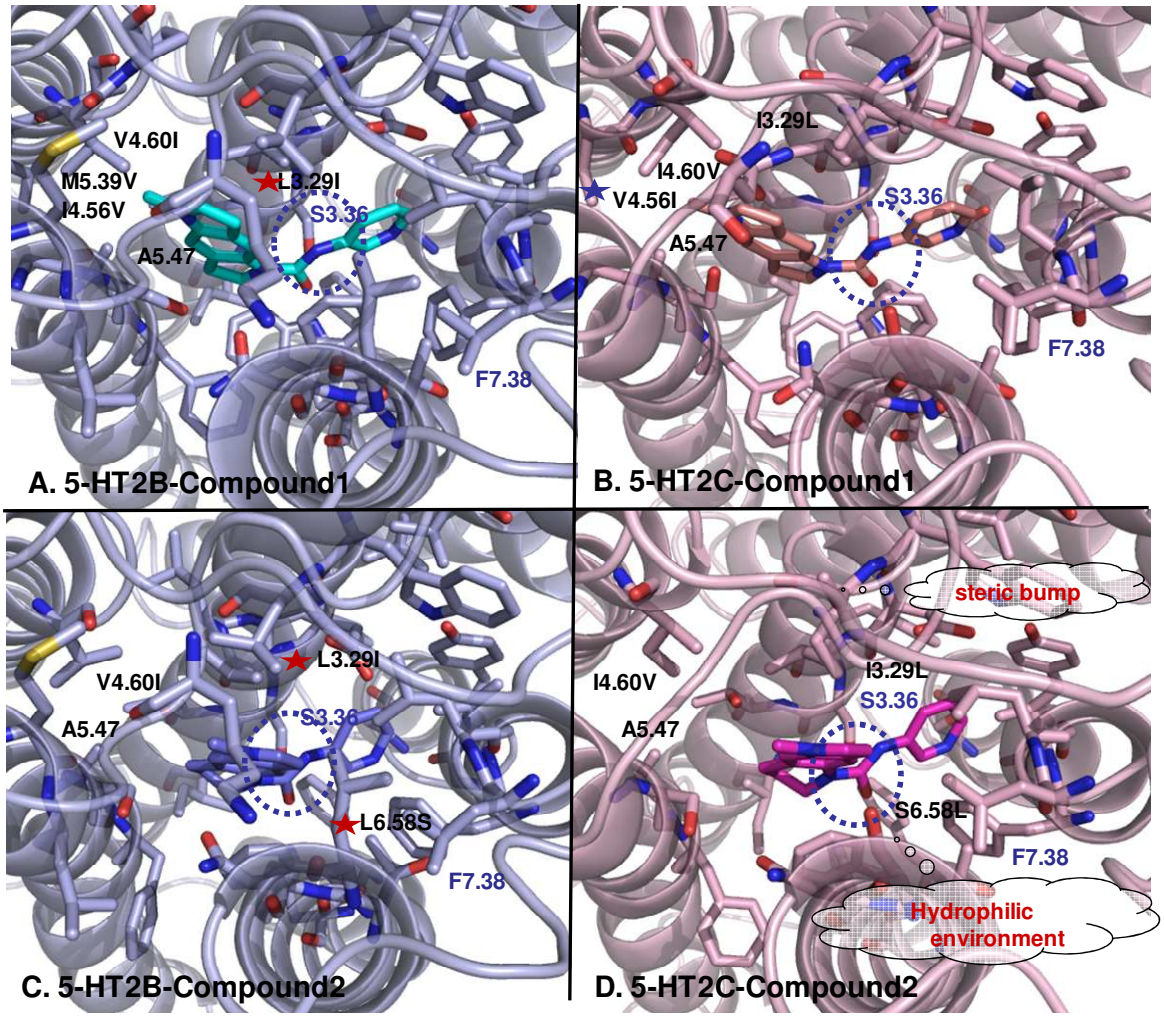


Fig. S7. The complexes of the nonselective 5-HT2B/2C receptor antagonist (SB-206533 **1**) and the selective 5-HT2B receptor antagonist (2) at the human 5-HT2B and 2C receptors. A) 5-HT2B/ SB-206533 **1**, B) 5-HT2C/ SB-206533 **1**, C) 5-HT2B/ **2**, and D) 5-HT2C/ **2**. The reduced interaction of **2** at the 5-HT2C receptors is predicted. Terminal methyl group has unfavorable van der Waals (vdW) interactions with I132 (3.29) at the 5-HT2C receptor, compared with the favorable vdW interaction of L132 (3.29) at the 5-HT2B receptor, leading to +8.46 kcal/mol differential binding at the 5-HT2C receptor.

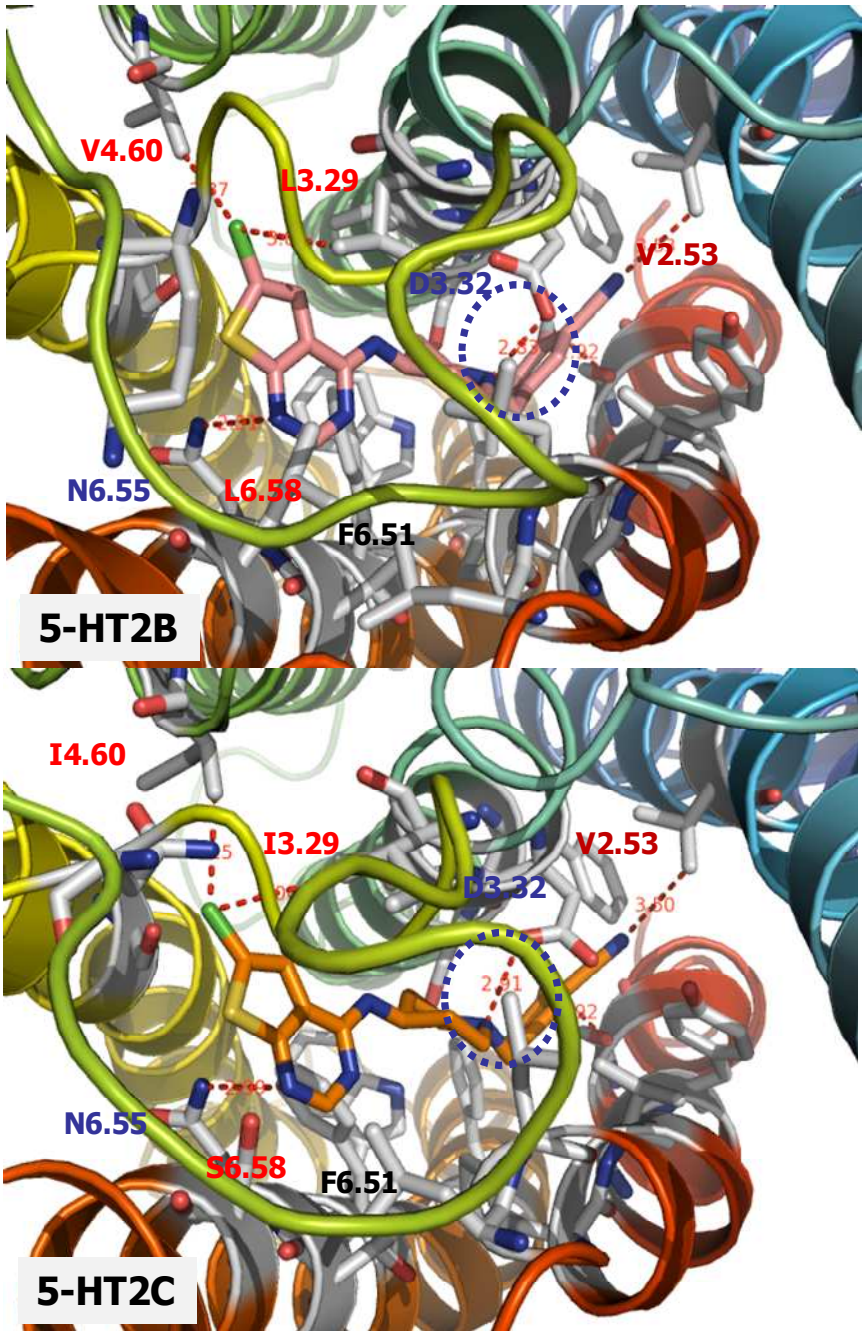


Fig. S8. The binding mode of highly selective 5-HT2B receptor antagonist PRX-08066 **3** at the human 5-HT2B (top) and 2C (bottom) receptors. Salt-bridge interactions in circle were shown at conserved D3.32 with protonated nitrogen in pyridine ring. H-bondings were displayed dotted red line with their distances.

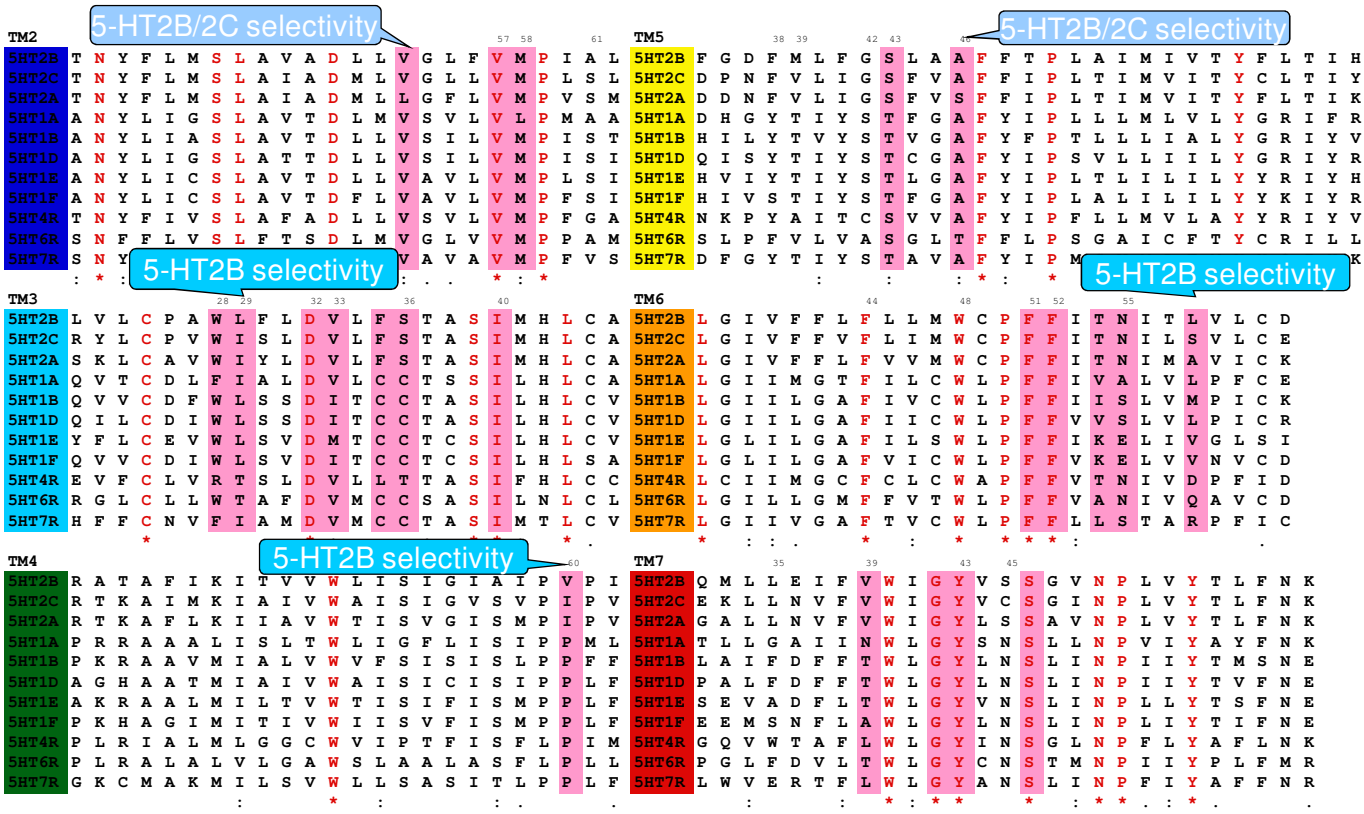
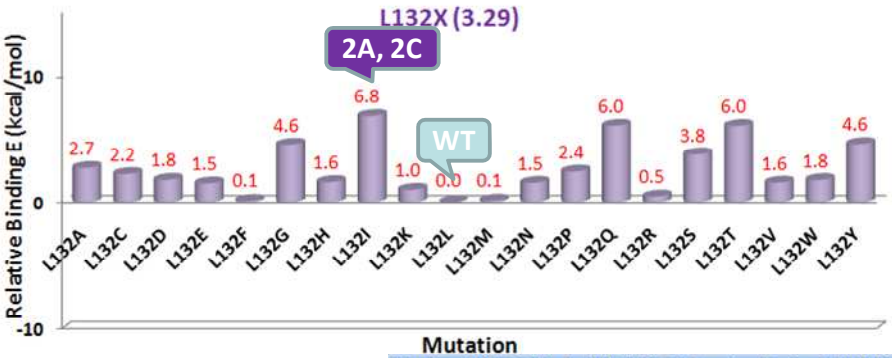
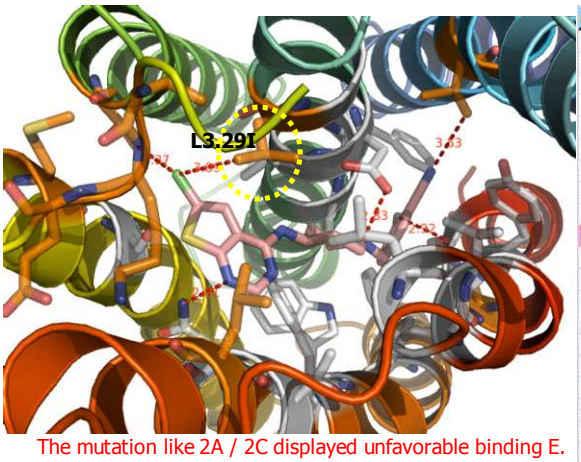


Fig. S9. Multiple alignments of 11 serotonin receptors.





The mutation like 2A / 2C displayed unfavorable binding E.

Mutation	UnifiedCavity	PartialDelphi	Total	LocalCavity	FullDelphi	Interaction	RelativeE
L132A	-35.23	-52.53	-23.59	-36.16	-41.85	-33.59	2.72
L132C	-35.71	-53.15	-41.64	-36.64	-42.70	-34.20	2.24
L132D	-36.17	-53.46	-59.96	-37.10	-41.96	-34.49	1.78
L132E	-36.46	-53.76	-55.75	-37.38	-42.77	-34.80	1.50
L132F	-37.83	-55.20	-24.31	-38.76	-45.10	-36.23	0.12
L132G	-33.40	-51.84	-24.56	-35.45	-40.07	-32.89	4.55
L132H	-36.35	-53.96	-23.49	-37.28	-44.59	-34.98	1.60
L132I	-31.13	-52.41	-12.27	-32.06	-43.17	-33.30	6.82
L132K	-36.96	-54.21	-40.54	-37.88	-43.68	-35.26	0.99
L132L	-37.95	-54.67	-30.41	-38.09	-44.68	-35.72	0.00
L132M	-37.81	-55.24	-40.51	-38.73	-44.69	-36.25	0.14
L132N	-36.41	-53.65	-55.70	-37.34	-42.51	-34.71	1.54
L132P	-35.52	-53.21	4.62	-36.45	-42.96	-34.31	2.43
L132Q	-31.90	-52.77	-54.65	-32.83	-46.86	-33.20	6.05
L132R	-37.49	-54.82	-132.85	-38.42	-44.06	-35.86	0.46
L132S	-34.17	-51.70	-21.33	-35.10	-40.55	-32.75	3.79
L132T	-31.92	-50.49	-42.27	-32.84	-40.87	-31.34	6.03
L132V	-36.39	-53.77	-25.26	-37.32	-43.27	-34.82	1.56
L132W	-36.19	-54.53	-19.18	-37.11	-44.41	-35.24	1.77
L132Y	-33.35	-53.35	-27.82	-34.28	-44.27	-34.23	4.60

Fig. S10. The mutation study of subtype selective residue L132X (3.29) from the cavity analysis of the complex of PRX-08066 **3/** 5-HT2B receptor in neutral system