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An ensemble docking-based virtual screening according to different TRPV1 pore states toward identifying phytochemical activators

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Table A.1

The details of 21 selected PDBs for the TRPV1 structure.

	PDB ID	Name	Method/Resolution		PDB ID	Name	Method/Resolution
1	7LQZ	Structure of squirrel TRPV1 in complex with RTX	ELECTRON MICROSCOPY 3.41	**12**	7L2W	cryo-EM structure of RTX-bound minimal TRPV1 with NMDG at state a	ELECTRON MICROSCOPY 3.16
2	7LR0	Structure of squirrel TRPV1 in complex with capsaicin	ELECTRON MICROSCOPY 3.81	**13**	7L2V	cryo-EM structure of RTX-bound minimal TRPV1 with NMDG at state b	ELECTRON MICROSCOPY 3.64
3	3J5R	Reconstruction of TRPV1 ion channel in complex with capsaicin by single particle cryo-microscopy	ELECTRON MICROSCOPY 4.2	**14**	7L2U	cryo-EM structure of DkTx-bound minimal TRPV1 in open state	ELECTRON MICROSCOPY 3.47
4	7LPB	Cryo-EM structure of full-length TRPV1 with capsaicin at 25 degrees Celsius	ELECTRON MICROSCOPY 3.54	**15**	7L2T	cryo-EM structure of DkTx-bound minimal TRPV1 in partial open state	ELECTRON MICROSCOPY 3.08
5	7L2N	Cryo-EM structure of RTX-bound full-length TRPV1 in C1 state	ELECTRON MICROSCOPY 3.09	**16**	7L2M	Cryo-EM structure of DkTx/RTX-bound full-length TRPV1	ELECTRON MICROSCOPY 3.84
6	7MZE	Cryo-EM structure of minimal TRPV1 with 2 bound RTX in opposite pockets	ELECTRON MICROSCOPY 3.42	**17**	7L2L	Cryo-EM structure of RTX-bound full-length TRPV1 in O1 state	ELECTRON MICROSCOPY 3.42
7	7MZD	Cryo-EM structure of minimal TRPV1 with RTX bound in C2 state	ELECTRON MICROSCOPY 2.90	**18**	5IS0	Structure of TRPV1 in complex with capsazepine, determined in lipid nanodisc	ELECTRON MICROSCOPY 3.43
8	7MZC	Cryo-EM structure of minimal TRPV1 with RTX bound in C1 state	ELECTRON MICROSCOPY 3.03	**19**	5IRX	Structure of TRPV1 in complex with DkTx and RTX, determined in lipid nanodisc	ELECTRON MICROSCOPY 2.95
9	7MZ	Cryo-EM structure of minimal TRPV1 with 2	ELECTRON MICROSCOPY	**20**	7L2R	Cryo-EM structure of DkTx-bound minimal	ELECTRON MICROSCOPY

	A	bound RTX in adjacent pockets	3.46
10	7MZ5	Cryo-EM structure of RTX-bound full-length TRPV1 in C2 state	ELECTRON MICROSCOPY 2.76
11	7L2X	cryo-EM structure of RTX-bound minimal TRPV1 with NMDG at state c	ELECTRON MICROSCOPY 3.26
		TRPV1 at the pre-open state	3.30
21	3J5P	Structure of TRPV1 ion channel determined by single particle electron cryo-microscopy	ELECTRON MICROSCOPY 3.27

Table A.2

CHEMBL IDs of active compounds of TRPV1 target that were selected from CHEMBL database (https://www.ebi.ac.uk/chembl/).

CHEMBL1761696	CHEMBL1092853	CHEMBL1214342	CHEMBL457188	CHEMBL408581	CHEMBL404165
CHEMBL456162	CHEMBL456778	CHEMBL526827	CHEMBL3393837	CHEMBL226723	CHEMBL375681
CHEMBL1807875	CHEMBL1807877	CHEMBL255420	CHEMBL229430	CHEMBL214989	CHEMBL516769
CHEMBL2177429	CHEMBL1092852	CHEMBL498666	CHEMBL190874	CHEMBL511391	CHEMBL3935231
CHEMBL457003	CHEMBL1761693	CHEMBL2312050	CHEMBL3671846	CHEMBL3682255	CHEMBL1761695
CHEMBL195115	CHEMBL214796	CHEMBL258054	CHEMBL258265	CHEMBL457842	CHEMBL226672
CHEMBL1173756	CHEMBL258056	CHEMBL497650	CHEMBL1093132	CHEMBL1779676	CHEMBL1173690
CHEMBL436638	CHEMBL517566	CHEMBL456128	CHEMBL446258	CHEMBL456152	CHEMBL1173242
CHEMBL3426347	CHEMBL3644131	CHEMBL3907261	CHEMBL402637	CHEMBL458058	CHEMBL456571
CHEMBL1089119	CHEMBL1779687	CHEMBL3644136	CHEMBL102073	CHEMBL195875	CHEMBL419351
CHEMBL231518	CHEMBL3426378	CHEMBL3426376	CHEMBL258117	CHEMBL323134	CHEMBL456570
CHEMBL101751	CHEMBL214331	CHEMBL254866	CHEMBL465990	CHEMBL460373	CHEMBL1761691
CHEMBL1684275	CHEMBL3326581	CHEMBL3682254	CHEMBL3956803	CHEMBL3961217	CHEMBL2024668
CHEMBL1761702	CHEMBL1807883	CHEMBL1172953	CHEMBL231206	CHEMBL387996	CHEMBL2088399
CHEMBL1779839	CHEMBL1083396	CHEMBL1779665	CHEMBL3644129	CHEMBL226724	CHEMBL3644137
CHEMBL427266	CHEMBL1807882	CHEMBL191247	CHEMBL513942	CHEMBL1214402	CHEMBL1089824
CHEMBL1807878	CHEMBL1669532	CHEMBL104028	CHEMBL1761700	CHEMBL3959317	CHEMBL3326569

Table A.3

The obtained results for molecular docking for phytochemicals of capsicum, cinnamon, and ginger on three MD clusters of the TRPV1 receptor.

Name	**PubChem ID**	#	**cluster-1** (kcal/mol)	**cluster-2** (kcal/mol)	**cluster-3** (kcal/mol)
Capsicum					
Homodihydrocapsaicin II	387095179	1	-6.5	**-7**	**-7.3**
17-Hydroxycapsaicin	129835627	2	-6.6	**-7.7**	-6.5
N-Vanillyl-9-methyl-6-decenamide	71448975	3	-6.5	-6.3	**-7.8**
Homocapsaicin II	11674147	4	**-7**	**-7.7**	**-7**
Capsiate	9839519	5	-6.4	-6.6	**-7.7**
Nordihydrocapsiate	9817607	6	-6.4	-6.3	-6.4
Homocapsaicin	6442566	7	-6.9	**-7.2**	**-7.9**
Scopoletin	5280460	8	**-7**	-6.4	-6.2
Homodihydrocapsaicin	3084336	9	-6.4	**-7.3**	**-7**
Capsaicin	1548943	10	**-7.3**	**-7**	**-7.8**
n-Vanillyldecanamide	169252	11	-6.8	-6.8	-6.9
Nordihydrocapsaicin	168836	12	-6.9	-6.6	-6.8
Dihydrocapsaicin	107982	13	-6.8	-6.9	**-7.2**
Vanillylamine	70966	14	-5.6	-5	-6.2
4-Hydroxy-3-methoxyphenylacetone	17262	15	-6.4	-5.8	-5.8
Homovanillyl alcohol	16928	16	-6.1	-5.5	-5
N-(3-(Aminomethyl)benzyl)acetamidine	1433	17	-6.3	-5.8	-5.3
Nonivamide	2998	18	-6.6	-6.5	-6.4
Cinnamon					
Benzaldehyde (Vanillin azine)	135414383	1	**-7.3**	-6.7	**-8.8**
3,5,6-Trimethyleugenol	45266889	2	-6.8	-5.9	-5.5
2-Hydroxycinnamaldehyde	5318169	3	-6.1	-5.6	-5.4
Caffeic acid phenethyl ester	5281787	4	**-7.3**	-6.8	**-8.5**
Sinapaldehyde	5280802	5	-6.1	-6.2	-4.6

4-Hydroxy-3-methoxycinnamaldehyde	5280536	6	-6.3	-5.8	-6
cis-Isoeugenol	1549041	7	-6	-5.7	-5.1
Caffeic acid	689043	8	-6.8	-6.2	-4.9
Sinapic acid	637775	9	-6.6	-6.5	-5.4
4-Hydroxycinnamic acid	637542	10	-6.8	-6.1	-6.3
2-Hydroxycinnamic acid	637540	11	**-7**	-6.3	-6
Phenol, 2-methoxy(2-propenyl)-	596373	12	-5.9	-5.5	-5.7
Cinnamic acid	444539	13	-6.4	-6.1	-5.3
1,3,6-tri-o-galloylglucose	250395	14	**-9.8**	**-8.9**	**-7.6**
4-Allyl-2,6-dimethoxyphenol	226486	15	-6	-5.6	-4.2
Cinnamophilin	169883	16	**-7.4**	**-7.4**	**-9**
3-Hydroxy-4-methoxybenzoic acid	12575	17	-6.3	-5.5	-4.9
3-O-Methyldopa	9307	18	-6.6	-6.2	-5
Cianidanol	9064	19	**-7.9**	**-8.4**	**-9**
3,4-Dihydroxybenzaldehyde	8768	20	-5.9	-5.1	-4.4
2-Methoxybenzaldehyde	8658	21	-5.5	-4.9	-4.4
Vanillic acid	8468	22	-6.3	-5.6	-4.4
Acetyleugenol	7136	23	-6.5	-6	-5.2
Eugenol	3314	24	-6	-5.8	-5.8
Homovanillic acid	1738	25	-6.5	-5.6	-5.7
Guaiacol	460	26	-5.4	-4.7	-4.2
Gallic acid	370	27	-6.4	-5.5	-5.1
Salicylic acid	338	28	-6.1	-5.4	-5.3
4-Hydroxybenzaldehyde	126	29	-5.7	-4.8	-5.2
3,4-Dihydroxybenzoic acid	72	30	-6.2	-5.5	-5.9
Ginger					
10-Gingerdiol	101572265	1	-6.5	**-7.1**	-5.2
[12]-Shogaol	85080428	2	-6.4	-5.7	-6.9
[10]-Gingerdione	14440539	3	-6.6	-6.9	-5.4
Gingerdiol	11369949	4	-6.3	-6.6	-5.8
6-Dihydroparadol	10378937	5	-6.3	-5.4	-5.8

[6]-Dehydrogingerdione	9796015	6	-6.6	-6.1	-5.9
[4]-Shogaol	9794897	7	-6.7	-6.3	-6
[10]-Shogaol	6442612	8	-6.9	-6.5	**-7.7**
[8]-Shogaol	6442560	9	-6.8	-6.9	**-7.4**
[4]-Gingerdiol 3,5-diacetate	5318274	10	**-7**	-6.6	-5
5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)octan-3-one	5317596	11	-6.3	-6.5	-5.5
[6]-Gingerdiol 3,5-diacetate	5317587	12	-6.9	-6.9	-5.4
Shogaol	5281794	13	-6.3	-6.6	-6.6
Gingerenone A	5281775	14	**-7.4**	**-7.5**	-6.1
Curcumin	969516	15	**-7.6**	**-7.9**	**-7.7**
Ferulic acid	445858	16	-6.6	-6.2	-5.6
Gingerol	442793	17	-6.5	-6.5	-6.6
(10)-Gingerol	168115	18	-6.5	-6.6	-5.5
(8)-Gingerol	168114	19	**-7.2**	-5.9	-6.1
[6]-Gingerdione	162952	20	-6.8	-6.2	-5.8
6-Gingesulfonic acid	126890	21	**-7.2**	-6.2	-4.8
Dibenzylbutyrolactone	99938	22	**-8.6**	**-7.6**	-6.8
Paradol	94378	23	-6.2	-6.4	-6.6
4-Ethyl-2-methoxyphenol	62465	24	-5.8	-5.5	-5
Zingerone	31211	25	-6.6	-6.1	-5.7
3-(4-Hydroxy-3-methoxyphenyl)propionic acid	14340	26	-6.6	-6	-5.2
Syringic acid	10742	27	-6.1	-5.9	-5.4
Acetovanillone	2214	28	-6.1	-5.7	-4.8
Vanillin	1183	29	-5.8	-5.2	-4.3
2-Methoxy-4-vinylphenol	332	30	-5.8	-5.5	-4.7
4-Hydroxybenzoic acid	135	31	-5.9	-5.1	-4.6

Table A.4

Physicochemical Appraisal and ADMET profile of all phytochemicals.

Pubchem ID	#	MW	XLogP	Hydrogen Don/Acc	Human intestinal absorption	Total clearance	Max tolerated	Ames test	LD50	LOAEL
Capsicum										
6442566	7	319.4	4.1	2/3	89.731	1.327	0.434	no	2.087	1.865

Cinnamon										
9064	19	290.27	0.4	5/6	68.829	0.183	0.438	no	2.428	2.5
Ginger										
969516	15	368.4	3.2	2/6	82.19	-0.002	0.081	no	1.833	2.228

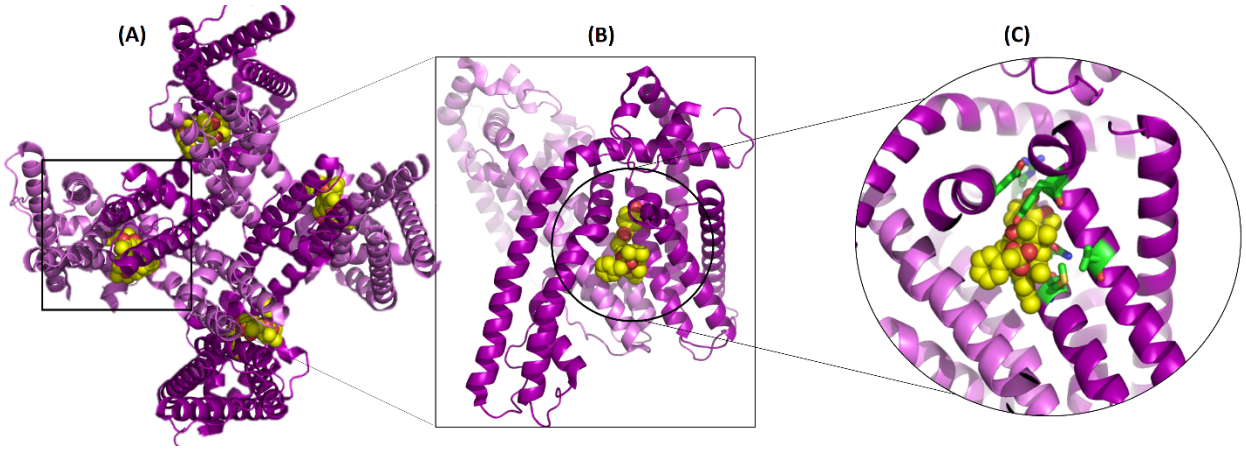

Figure A.1. Binding site of TRPV1 in complex with RTX from different views. (A) Top view focusing on the position of RTX binding mode between two neighboring subunits. (B) Side view showing only two adjacent subunits. (C) Amino acids in vanilloid binding pocket of TRPV1. S1-S4 domains flank and interact with S5-P-S6 pore modules from the adjacent subunit. Protein is shown by purple cartoon model. RTX is depicted by yellow sphere model. Amino acids are shown by green thick line.

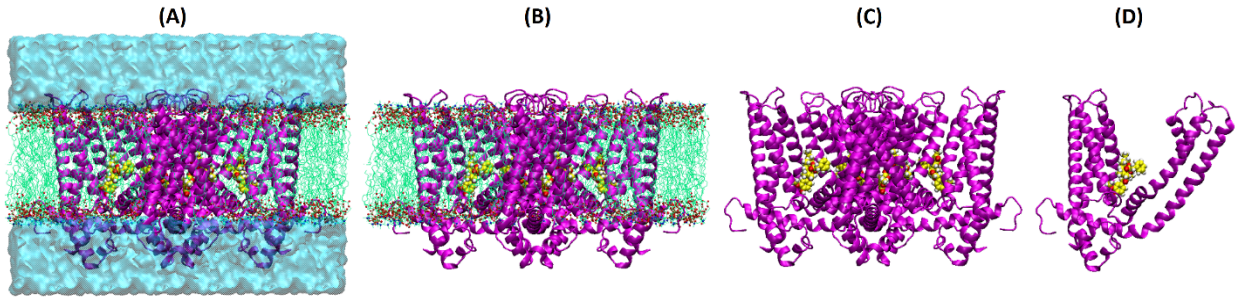

Figure A.2. Different views of the initial structure for MD simulation to extract the snapshots for ensemble docking. (A) Protein molecule embedded in POPC bilayer which solvated in water environment, (B) the orientation and position of protein compared to lipid, (C) the orientation of ligand molecules in all four subunits of TRPV1, and (D) only one subunit in complex with ligand. 7MZC is PDB code of protein and all ligands are RTX activator. Protein is represented by magenta carton structure and RTX ligands are shown by yellow sphere model. The water environment is shown by transparent light green and DOPC lipid by line model.

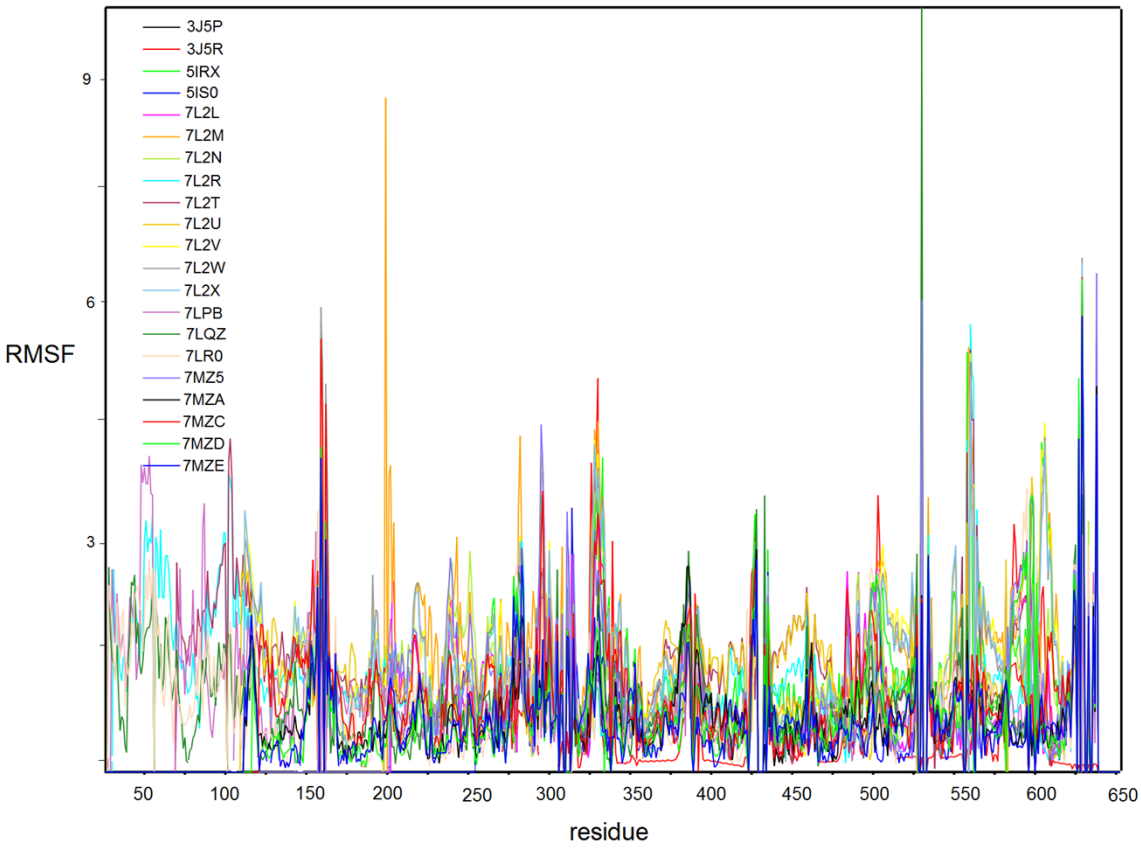

Figure A.3. Root-mean-square fluctuations for 21 TRPV1 structures.

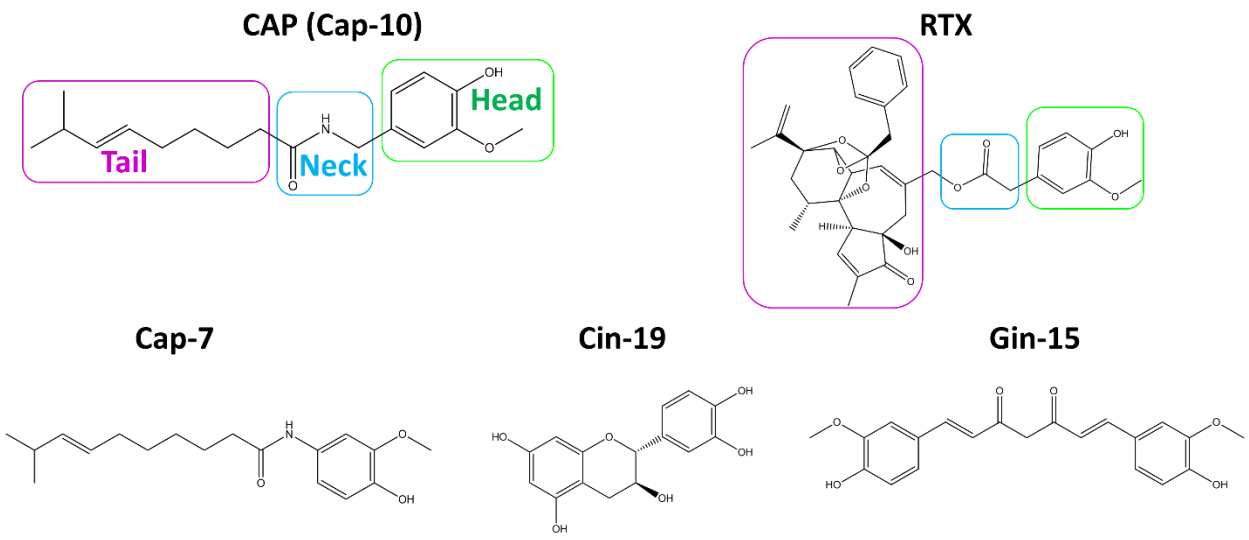

Figure A.4. The chemical structure of the three top-screened ligands and the structure of CAP and RTX activators.