The quercetin metabolite 4-methylcatechol causes vasodilation via voltage-gated potassium (K_V) channels

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Supplementary data

10 pages

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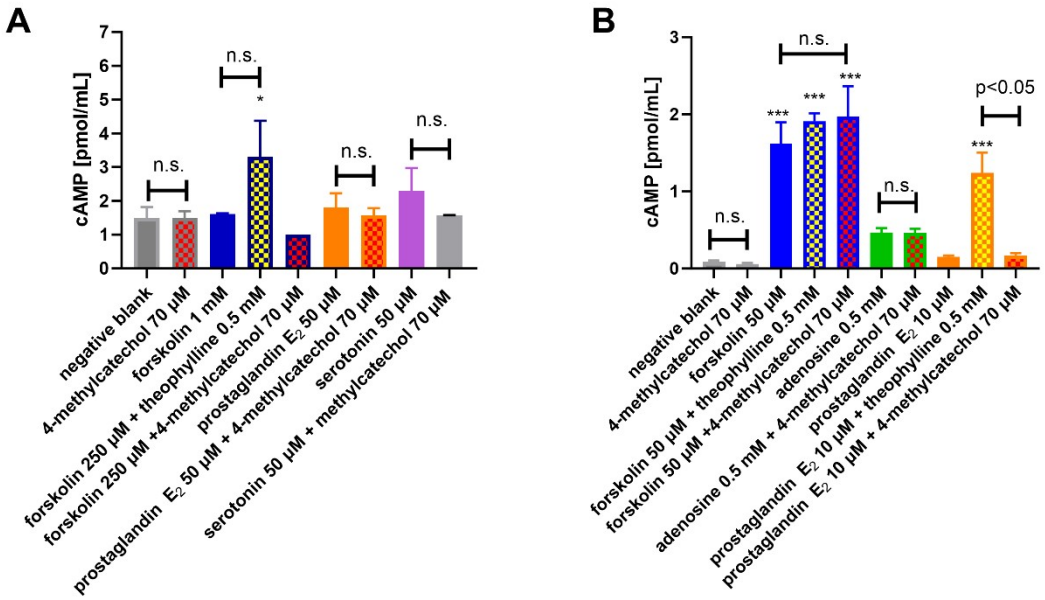

Fig. S1. Impact of 4-methylcatechol on cAMP levels. A: rat smooth muscle cells A-10 and **B**: human platelets. Data are shown as mean ± SEM from at least 4 measurements. * $p<0.05$ *vs.* negative blank, *** $p<0.001$ *vs.* negative blank.

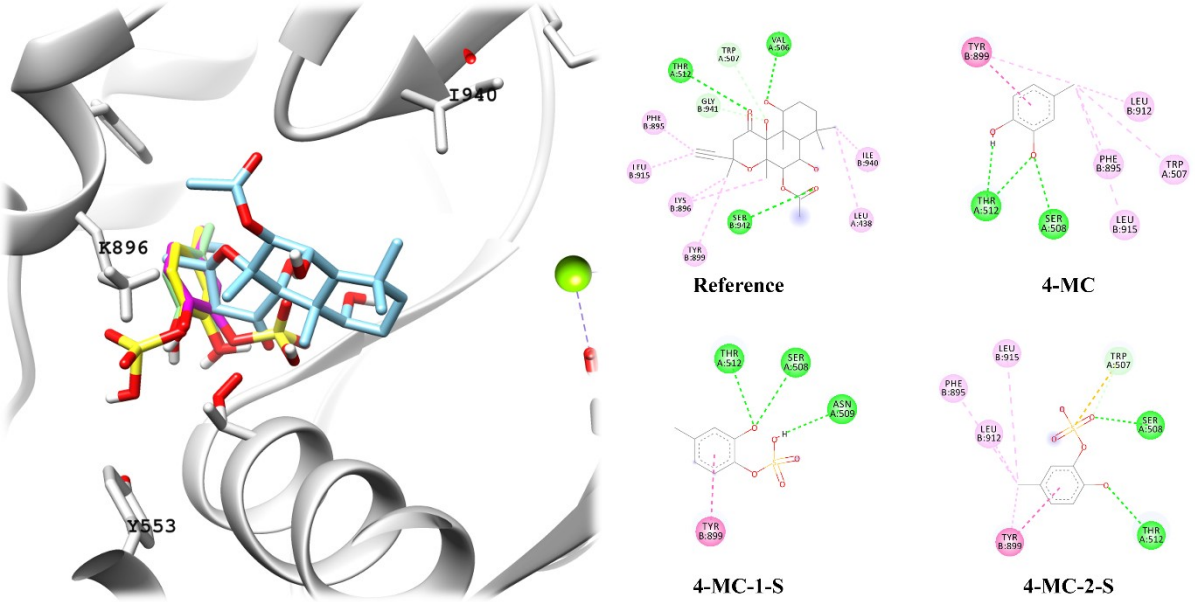

Fig. S2. Docking pose of forskolin/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of adenylate cyclase (AC, PDB ID: 1CJK) and 2D representation of molecular interactions between ligands and adenylyl cyclase.

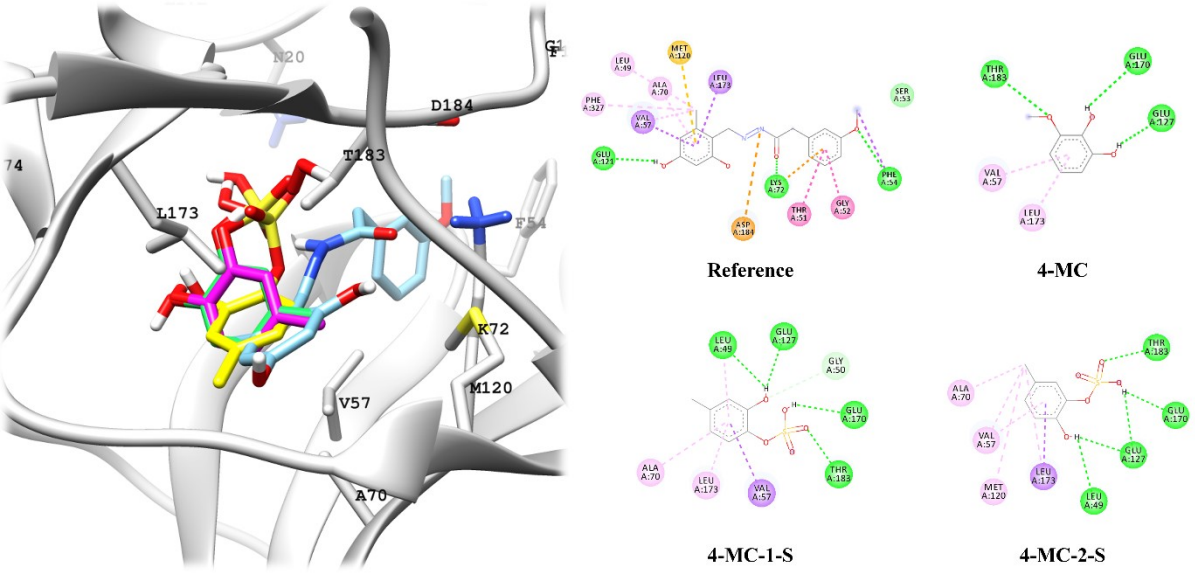

Fig. S3. Docking pose of the activator S69/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of human protein kinase A (PKA, PDB ID: 3POO) and 2D representation of molecular interactions between ligands and protein kinase A.

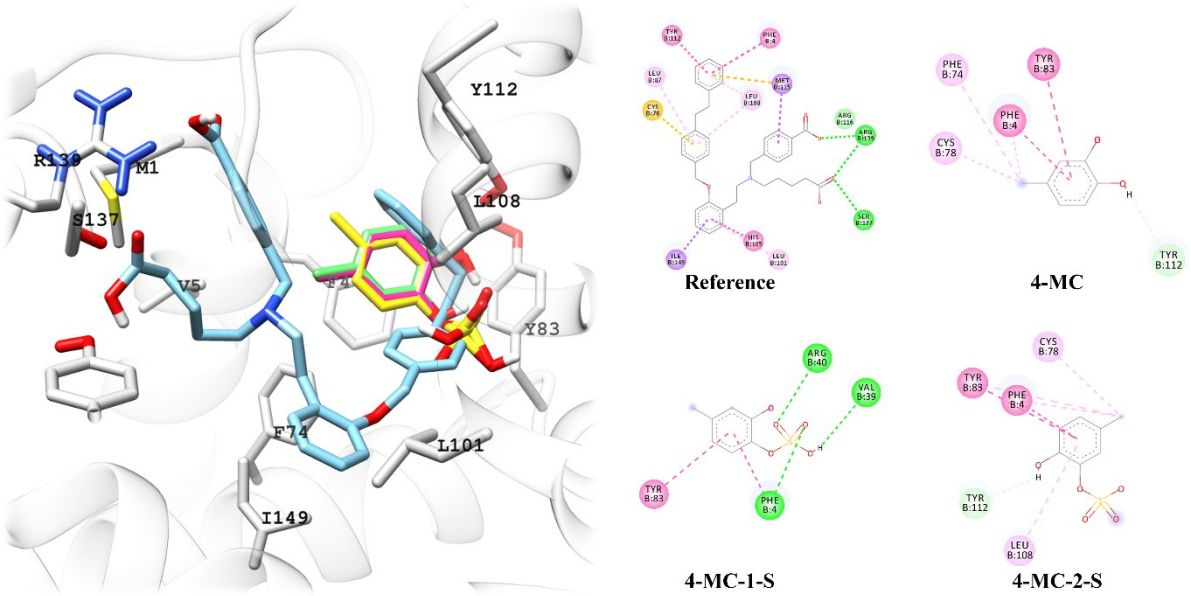

Fig. S4. Docking pose of cinaciguat/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of human soluble guanylate cyclase (sGC, PDB ID: 7D9T) and 2D representation of molecular interactions between ligands and sGC.

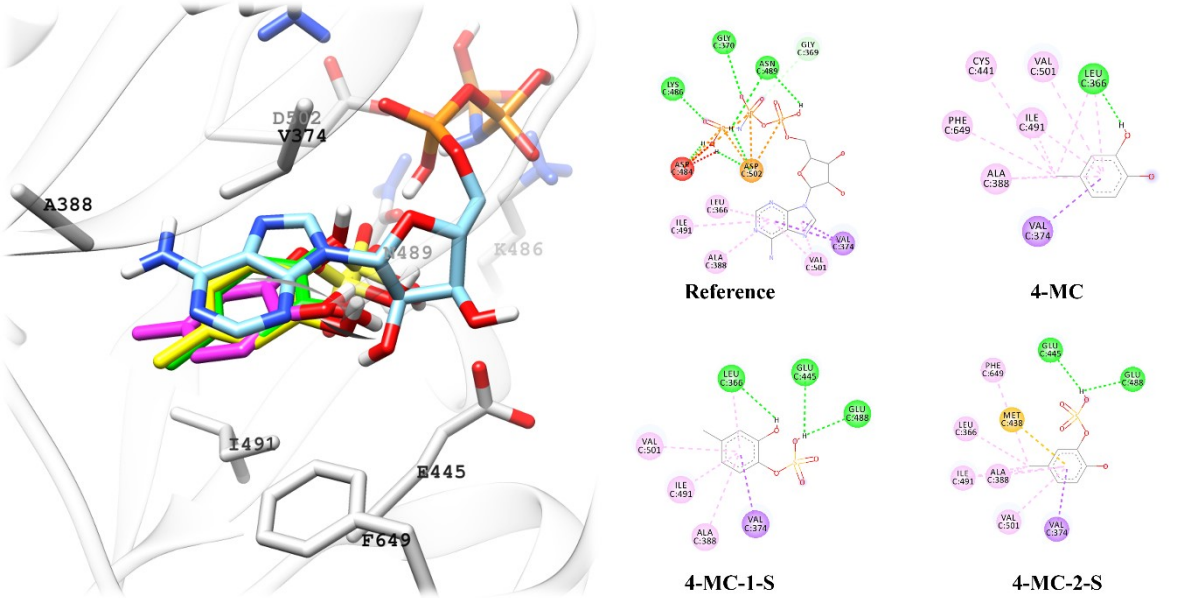

Fig. S5. Docking pose of the activator ANP/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of human protein kinase G (PKG)-Iα (PDB ID: 6C0T) and 2D representation of molecular interactions between ligands and PKGIα.

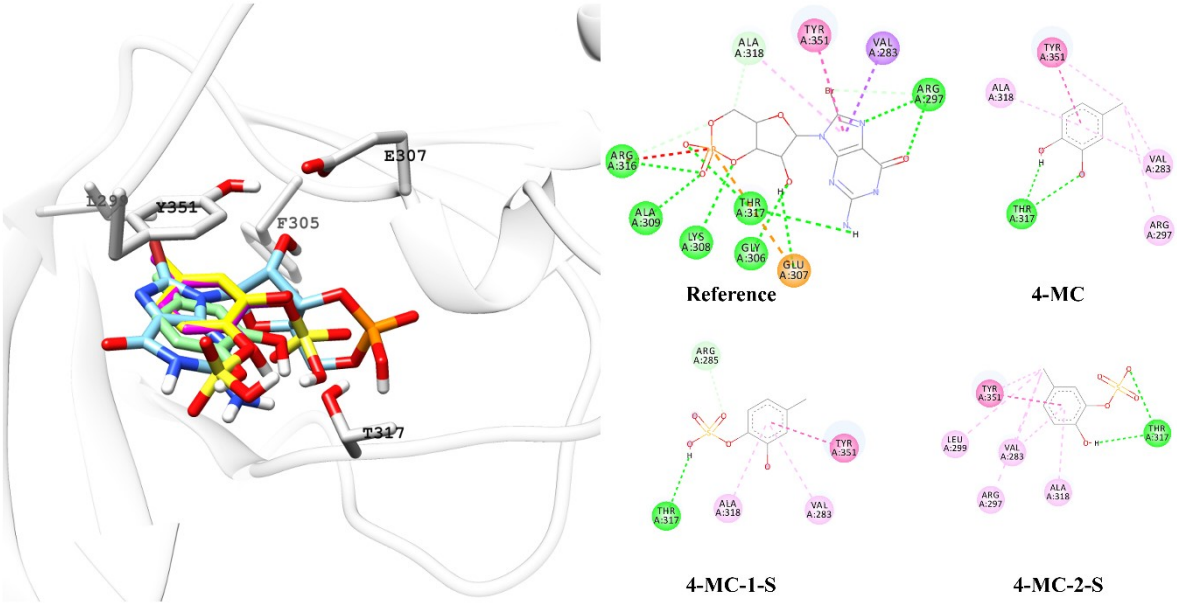

Fig. S6. Docking pose of the activator 8-Br-cGMP/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of human protein kinase G (PKG)-Iβ (PDB ID: 5JAX) and 2D representation of molecular interactions between ligands and PKGIβ.

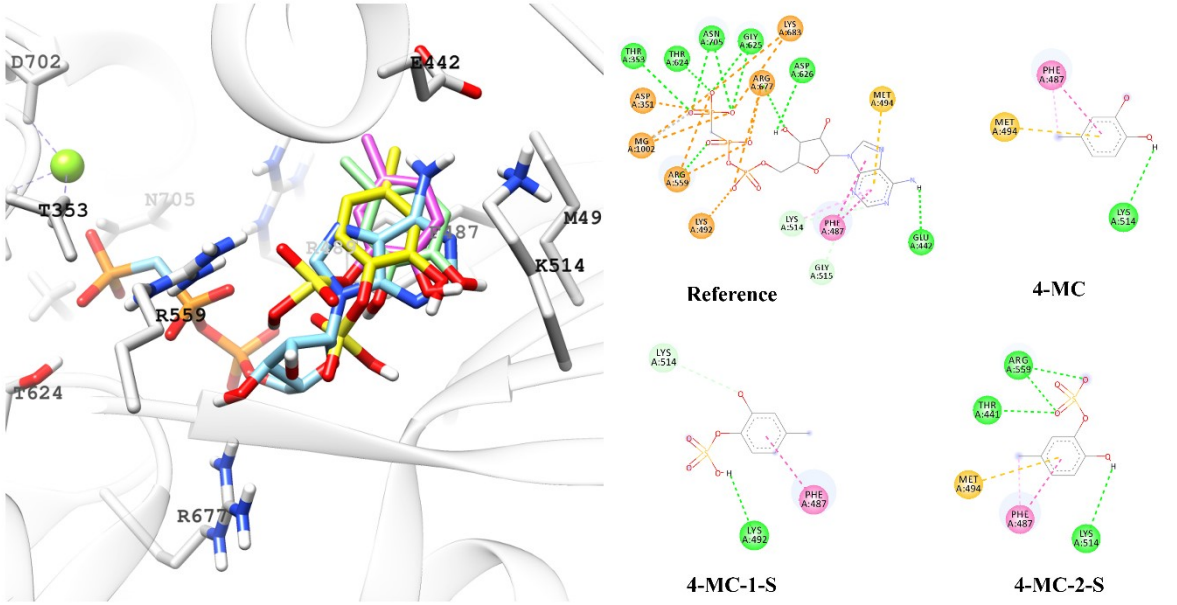

Fig. S7. Docking pose of the activator AMP-PCP/reference (turquoise); 4-methylcatechol (4-MC, green); 4-methylcatechol-1-sulfate (4-MC-1-S, yellow); 4-methylcatechol-2-sulfate (4-MC-2-S, purple) in the ligand binding domain of human sarco/endoplasmic calcium ATPase (SERCA, PDB ID: 6JJU) and 2D representation of molecular interactions between ligands and SERCA

Table S1. Docking scores and ligand efficiency (LE) of reference, 4-methylcatechol (4-MC), 4-methylcatechol-1-sulfate (4-MC-1S) and 4-methylcatechol-2-sulfate (4-MC-2S) for each target protein.

PDB ID	Target protein	Docking score (kcal/mol)				Ligand efficiency (docking score/heavy atom)			
		Reference	4-MC	4-MC-1-S	4-MC-2-S	Reference	4-MC	4-MC-1-S	4-MC-2-S
7D9T	sGC	-10.7	-5.3	-5.9	-5.9	0.25	0.59	0.45	0.45
6C0T	PKGIα	-8.2	-6.5	-6.6	-6.5	0.31	0.72	0.51	0.50
5JAX	PKGIβ	-11.6	-5.8	-5.7	-7.0	0.48	0.64	0.44	0.54
3POO	PKA	-9.3	-5.9	-6.8	-6.8	0.40	0.66	0.52	0.52
7TCI	K_V7.1	-8.8	-6.0	-6.0	-6.4	0.28	0.67	0.46	0.49
7CR1	K_V7.2	-8.1	-6.1	-5.9	-6.3	0.37	0.68	0.45	0.48
7BYM	K_V7.4	-7.6	-6.0	-5.8	-6.1	0.35	0.67	0.45	0.47
1CJK	AC	-9.7	-5.6	-6.2	-6.3	0.33	0.62	0.48	0.48
6JJU	SERCA	-9.7	-5.5	-6.0	-6.5	0.31	0.61	0.46	0.50

PDB ID, Protein Data Bank Identifiers; sGC, soluble guanylate cyclase; PKGIα, protein kinase G isoform Iα; PKGIβ, protein kinase G isoform Iβ; PKA, protein kinase A; K_V, voltage-gated potassium channel; AC, adenylate cyclase; SERCA, sarco/endoplasmic reticulum calcium-ATPase.

Table S2. Intermolecular interactions of reference ligands (RL) and 4-MC with target proteins.

Ligand	Conventional hydrogen bonds	Carbon hydrogen bond	Pi-Pi Stacked	Pi-Sulfur	Pi-Alkyl	Pi-sigma	Pi-Pi T-Shaped	Other interactions
soluble Guanylate Cyclase (sGC)								
cinaciguat (RL)	SER137 (2.96 Å) ARG139 (3.05 Å) ARG139 (2.87 Å)		TYR112 (3.87 Å)	CYS78 (4.20 Å) MET115 (4.93 Å)	LEU101 (5.10 Å) LEU87 (5.41 Å) LEU108 (5.40 Å) LEU108 (5.19 Å)	MET115 (3.73 Å) ILE149 (3.72 Å)	PHE4 (5.15 Å) HIS105 (4.90 Å)	
4-MC		TYR112 (2.90 Å)	TYR83 (3.72 Å)		PHE4 (4.43 Å) PHE74 (5.09 Å)		TYR83 (5.28 Å)	Alkyl: CYS78 (4.13 Å)
4-MC-1-S	PHE4 (3.07 Å) ARG40 (2.62 Å) VAL39 (2.89 Å)		PHE4 (3.75 Å)				TYR83 (5.19 Å)	
4-MC-2-S		TYR112 (2.51 Å)	PHE4 (4.25 Å)		PHE4 (4.29 Å) TYR83 (4.36 Å) LEU108 (5.44 Å)		TYR83 (5.19 Å)	Alkyl: CYS78 (4.16 Å)
Protein kinase G (PKGIα)								
ANP (RL)	GLY370 (3.12 Å) LYS486 (2.31 Å) ASN489 (2.84 Å) ASN489 (2.43 Å) ASP502 (2.11 Å) ASP484 (2.94 Å) ASP502 (1.82 Å)	GLY369 (3.79 Å)			VAL501 (4.65 Å) LEU366 (4.76 Å) ALA388 (4.58 Å) ILE491 (5.11 Å) VAL501 (5.31 Å)	VAL374 (3.90 Å) VAL374 (3.52 Å) VAL374 (3.48 Å)		Electrostatic: ASP502 (3.97 Å) ASP484 (5.34 Å) ASP502 (3.76 Å) ASP484 (2.49 Å ASP502 (2.82 Å)
4-MC	LEU366 (2.67 Å)				PHE649 (5.42 Å) LEU366 (5.10 Å) ALA388 (5.12 Å) ILE491 (4.87 Å) VAL501 (5.03 Å)	VAL374 (3.90 Å)		Alkyl: LEU366 (4.56 Å) CYS441 (4.89 Å) ILE491 (4.33 Å)
4-MC-1-S	GLU445 (2.54 Å) GLU488 (2.20 Å) LEU366 (2.70 Å)				LEU366 (4.93 Å) ALA388 (4.95 Å) ILE491 (4.84 Å) VAL501 (5.14 Å)	VAL374 (3.85 Å)		
4-MC-2-S	GLU445 (2.33 Å) GLU488 (2.27 Å)			MET438 (5.62 Å)	PHE649 (5.03 Å) ALA388 (4.56 Å) ILE491 (4.84 Å) VAL501 (4.49 Å)	VAL374 (3.92 Å)		Alkyl: ALA388 (4.20 Å) LEU366 (4.21 Å)

Ligand	Conventional hydrogen bonds	Carbon hydrogen bond	Pi-Pi Stacked	Pi-Sulfur	Pi-Alkyl	Pi-sigma	Pi-Pi T-Shaped	Other interactions
Protein kinase G (PKGIβ)								
8-Br-cGMP (RL)	ARG297 (2.34 Å) ARG297 (2.40 Å) GLY306 (2.05 Å) LYS308 (2.59 Å) ALA309 (2.14 Å) ARG316 (2.20 Å) THR317 (2.03 Å) THR317 (1.86 Å) THR317 (1.88 Å) GLU307 (2.08 Å)	ARG297 (3.68 Å) ARG316 (3.58 Å) ARG316 (3.51 Å) ALA318 (3.47 Å)	TYR351 (3.75 Å)		ALA318 (4.98 Å)	VAL283 (3.80 Å)		Electrostatic: Glu307 (5.48 Å)
4-MC	THR317 (2.49 Å) THR317 (2.32 Å)		TYR351 (3.98 Å)		TYR351 (4.39 Å) VAL283 (4.99 Å) ALA318 (4.18 Å)			Alkyl: VAL283 (3.74 Å) ARG297 (5.07 Å)
4-MC-1-S	THR317 (2.70 Å)	ARG285 (3.34 Å)	TYR351 (3.91 Å)		VAL283 (4.66 Å) ALA318 (4.69 Å)			
4-MC-2-S	THR317 (2.42 Å) THR317 (2.28 Å) THR317 (2.40 Å)		TYR351 (3.97 Å)		TYR351 (4.78 Å) VAL283 (4.66 Å) ALA318 (4.63 Å)			Alkyl: VAL 283 (3.94 Å) ARG297 (4.98 Å) LEU299 (4.40 Å)
Voltage-gated potassium channels (Kv7.1)								
ML277 (RL)	GLY262 (3.09 Å) PHE325 (2.08 Å)		PHE325 (5.44 Å)	TRP238 (5.53 Å) PHE329 (5.03 Å)	LEU261 (4.95 Å) LEU 252 (5.21 Å)	PHE265 (3.87 Å)	PHE265 (5.02 Å) TRP238 (5.30 Å) PHE329 (4.73 Å)	
4-MC	SER328 (2.60 Å) SER328 (2.27 Å) PHE330 (2.17 Å)				VAL324 (5.29 Å); LEU256 (5.27 Å); PRO333 (3.91 Å)	PHE330 (3.70 Å)		Alkyl: LEU256 (5.00 Å)
4-MC-1-S	SER328 (2.15 Å) PHE330 (2.73 Å)				VAL324 (5.42 Å) LEU256 (5.05 Å) PRO333 (3.94 Å)	VAL324 (3.63 Å)		
4-MC-2-S	LEU252 (2.59 Å) VAL324 (2.03 Å)				VAL324 (5.27 Å) LEU256 (5.08 Å) PRO333 (4.04 Å)	PHE330 (3.64 Å)		

Ligand	Conventional hydrogen bonds	Carbon hydrogen bond	Pi-Pi Stacked	Pi-Sulfur	Pi-Alkyl	Pi-sigma	Pi-Pi T-Shaped	Other interactions
Voltage-gated potassium channels (Kv7.2)								
retigabine (RL)	TRP236 (3.35 Å) PHE305 (2.31 Å) SER303 (2.48 Å) LEU299 (2.11 Å) SER303 (2.91 Å)	SER 303 (3.12 Å); PHE305 (2.45 Å)	TRP236 (4.72 Å); GLY239 (4.79 Å)		PRO308 (4.76 Å); LEU299 (4.84 Å)	PHE304 (3.84 Å)	TRP236 (5.12 Å)	LEU299 (4.73 Å)
4-MC	PHE240 (2.98 Å) ALA235 (2.70 Å) TRP236 (2.58 Å)		PHE305 (3.86 Å)		PHE305 (4.80 Å) LEU299 (5.28 Å)			
4-MC-1-S	TRP236 (2.60 Å) ALA235 (2.80 Å)	GLY239 (3.78 Å)	PHE305 (4.05 Å)		LEU299 (4.90 Å)		TRP236 (4.05 Å)	
4-MC-2-S	TRP236 (2.47 Å) TRP236 (2.51 Å) ALA235 (2.75 Å)		PHE305 (3.87 Å)		PHE305 (4.89 Å) PRO308 (5.45 Å) LEU299 (5.36 Å)		TRP236 (5.11 Å)	Alkyl: LEU299 (4.73 Å)
Voltage-gated potassium channels (Kv7.4)								
retigabine (RL)	TRP242 (2.22 Å) PHE311 (2.97 Å) LEU305 (2.80 Å SER309 2.49 Å)	TRP242 (3.61 Å)	TRP242 (4.42 Å)		PRO314 (4.95 Å) LEU305 (4.79 Å)	LEU305 (3.82 Å) PHE310 (3.71 Å)	PHE246 (5.21 Å)	
4-MC	PHE246 (2.90 Å) ALA241 (2.72 Å)		PHE311 (4.18 Å) TRP242 (4.75 Å)		TRP242 (5.29 Å) PRO314 (5.29 Å) LEU305 (5.29 Å)			Alkyl: PRO314 (3.90 Å)
4-MC-1-S	PHE246 (2.84 Å) TRP242 (2.47 Å)		PHE311 (4.56 Å)		LEU305 (4.53 Å)		TRP242 (4.81 Å)	
4-MC-2-S	PHE246 (2.85 Å) TRP242 (2.64 Å)		PHE311 (4.11 Å)		TRP242 (5.28 Å) PRO314 (5.36 Å) LEU305 (5.27 Å)		TRP242 (4.81 Å)	Alkyl: PRO314 (4.00 Å)
adenylyl cyclase (AC)								
forskolin (RL)	THR512 (3.12 Å) SER942 (3.29 Å) VAL506 (3.02 Å)	TRP507 (3.71 Å) GLY941 (3.72 Å)			PHE895 (4.63 Å) TYR899 (4.26 Å) LEU438 (5.00 Å) ILE940 (5.13 Å)			Alkyl: LEU915 (5.21 Å) LYS896 (4.79 Å) LYS896 (4.58 Å)
4-MC	SER508 (3.34 Å) THR512 (2.24 Å) THR512 (2.10 Å)		TYR899 (3.87 Å)		TRP507 (5.05 Å) PHE895 (4.85 Å) PHE895 (4.36 Å)			Alkyl: LEU912 (4.55 Å) LEU912 (5.30 Å)
4-MC-1-S	SER508 (2.71 Å) THR512 (2.49 Å) ASN509 2.17 Å)		TYR899 (3.98 Å)					
4-MC-2-S	SER508 (2.30 Å) THR512 (2.63 Å)	TRP507 (2.98 Å)	TYR899 (4.05 Å)	TRP507 (5.80 Å)	PHE895 (4.75 Å) TYR899 (4.07 Å)			Alkyl: LEU912 4.46 Å) LEU915 (5.40 Å)

(continued)

Ligand	Conventional hydrogen bonds	Carbon hydrogen bond	Pi-Pi Stacked	Pi-Sulfur	Pi-Alkyl	Pi-sigma	Pi-Pi T-Shaped	Other interactions
sarco/endoplasmic reticulum calcium ATPase (SERCA)								
AMP-PCP (RL)	THR353 (3.05 Å) ARG559 (3.23 Å) THR624 (3.01 Å) GLY625 (3.26 Å) GLY625 (3.11 Å) ARG677 (3.14 Å) ASN705 (3.02 Å) ASN705 (3.22 Å) ASP626 (3.03 Å) GLU442 (2.26 Å)	GLY515 (3.38 Å) LYS514 (3.36 Å)	PHE487 (4.07 Å) PHE487 (4.45 Å)	MET494 (5.50 Å)	LYS514 (4.98 Å)			Salt bridge: ARG559 (3.38 Å) LYS683 (3.37 Å) Electrostatic: LYS492 (4.30 Å) ARG559 (5.49 Å) ARG677 (4.45 Å) ARG677 (4.70 Å) LYS683 (4.62 Å) ASP351 (3.75 Å)
4-MC	LYS 514 (2.77 Å)		PHE487 (3.75 Å)	MET494 (5.13 Å)	PHE487 (4.37 Å)			
4-MC-1-S	LYS492 (2.57 Å)	LYS514 (3.73 Å)	PHE487 (4.06 Å)					
4-MC-2-S	THR441 (2.34 Å) ARG559 (2.27 Å) ARG559 (2.04 Å) ARG559 (2.41 Å) LYS514 (3.02 Å)		PHE487 (3.73 Å)	MET494 (5.23 Å)	PHE487 (4.67 Å)			
Protein Kinase A (PKA)								
S69 (RL)	PHE54 (3.25 Å) LYS72 (3.07 Å) GLU121 (2.73 Å)			MET120 (5.92 Å)	PHE327 (5.00 Å) ALA70 (3.92 Å) LYS722 (4.84 Å)	VAL57 (3.97 Å) LYS72 (3.54 Å) LEU173 (3.40 Å) PHE54 (3.96 Å)		Electrostatic: ASP184 (5.09 Å) Pi-cation: LYS72 (4.09 Å) Amide Pi-stacked: THR51 (4.00 Å) GLY52 (4.50 Å)
4-MC	GLU127 (2.66 Å) GLU170 (2.99 Å) LEU49 (2.71 Å)				VAL57 (4.55 Å)		LEU173 (3.97 Å)	Alkyl: ALA70 (4.36 Å) VAL57 (4.33 Å) MET120 (4.93 Å) LEU173 (5.11 Å)
4-MC-1-S	THR183 (2.03 Å) GLU170 (2.04 Å) LEU49 (2.93 Å) GLU127 (2.84 Å)	GLY50 (3.44 Å)			LEU49 (5.38 Å) ALA70 (5.21 Å) LEU173 (4.78 Å)	VAL57 (3.94 Å)		
4-MC-2-S	THR183 (2.30 Å) GLU127 (2.74 Å) GLU170 (2.32 Å) LEU49 (2.87 Å) GLU127 (2.66 Å)				VAL57 (4.50 Å)	LEU173 (3.87 Å)		Alkyl: ALA70 (4.09 Å) VAL57 (4.32 Å) MET120 (4.77 Å) LEU173 (5.23 Å)