SUPPLEMENTARY INFORMATION

Article

Monoamine Oxidase Inhibition by Major Tanshinones from *Salvia miltiorrhiza* and Selective Muscarinic Acetylcholine M_4 Receptor Antagonism by Tanshinone I

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Table S1. In vitro pharmacology: Cellular and nuclear receptor functional assays.

Assay	Source	Stimulus	Incubation	Measured Component	Detection Method	References
M_1 (h) (agonist effect)	Human recombinant CHO cells	None (100 nM acetylcholine for control)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[33]
M_1 (h) (antagonist effect)	Human recombinant CHO cells	Acetylcholine (10 nM)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[33]
M_2 (h) (agonist effect)	Human recombinant CHO cells	None (3 μM acetylcholine for control)	10 min 37 °C	cAMP	HTRF	[34]
M_2 (h) (antagonist effect)	Human recombinant CHO cells	Acetylcholine (300 nM)	10 min 37 °C	cAMP	HTRF	[34]
M_3 (h) (agonist effect)	Human recombinant CHO cells	None (1 μM acetylcholine for control)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[33]
M_3 (h) (antagonist effect)	Human recombinant CHO cells	Acetylcholine (100 nM)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[33]
M_4 (h) (agonist effect)	Human recombinant CHO cells	None (1μM acetylcholine for control)	10 min 37 °C	cAMP	HTRF	[35]
M_4 (h) (antagonist effect)	Human recombinant CHO cells	Acetylcholine (100 nM)	10 min 37 °C	cAMP	HTRF	[35]
M_5 (h) (agonist effect)	Human recombinant RBL cells	None (0.624 μM acetylcholine for control)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[36]
M_5 (h) (antagonist effect)	Human recombinant RBL cells	Acetylcholine (10 nM)	RT	Intracellular $[Ca^{2+}]$	Fluorimetry	[36]

HTRF: homogeneous time-resolved fluorescence; RT: room temperature

Table S2. Binding site residues and docking scores of tanshinone I, tanshinone IIA, cryptotanshinone and the reference ligands in human monoamine oxidases (hMAO-A and hMAO-B).

Ligand	Binding Energy (kcal/mol)[a]	Interacting residue[b]		
		H-bond	Hydrophobic	Electrostatic
		hMAO-A		
Tanshinone I (catalytic inhibitor)	-10.06	FAD600	Pi-sigma: Ile335, Tyr407, FAD600, Phe208 Pi-Pi Stacked: Tyr407, FAD600, Ile335 Alky: Ile335 Pi-Alkyl: Tyr444, FAD600, Ile180, Ile35, Leu337	–
Tanshinone I (allosteric inhibitor)	-7.87	Thr205	Pi-sigma: His488 Pi-Pi T-shaped: Phe112, Trp128 Alkyl: Arg129 Pi-Alkyl: Tyr121, Tyr124, Arg129	Pi-Anion: Glu492
Tanshinone IIA (catalytic inhibitor)	-9.91	–	Pi-sigma: Ile335, Tyr407 Pi-Pi T-shaped: Phe208 Alkyl: Cys323, Ile335, Ile325 Pi-Alkyl: Tyr69, Phe208, Phe352, FAD600, Leu337, Ile180, Ile335	–

Tanshinone IIA (allosteric inhibitor)	-7.3	Thr205	Pi-sigma: His488 Pi-Pi T-shaped: Phe112, Tyr124, Trp128 Alkyl: Arg129 Pi-Alkyl: Tyr121, Tyr124, Arg129	Pi-Anion: Glu492
Cryptotanshinone (catalytic inhibitor)	-10.07	–	Pi-sigma: Tyr407 Pi-Pi T-shaped: Phe208 Alkyl: Val210, Cys323, Leu337 Pi-Alkyl: Tyr69, Phe352, Tyr407, FAD600, Ile180, Ile335	–
Cryptotanshinone (allosteric inhibitor)	-8.67	His488	Pi-Pi T-shaped: Phe112, Trp128 Alkyl: Val115 Pi-Alkyl: Tyr121, Tyr124, His488	Pi-Anion: Glu492
HRM[c]	-6.72	Asn181	Pi-Sigma: Tyr444 Pi-Pi Stacked: Tyr407 Alkyl: Ile180 Pi-Alkyl: Phe208, Tyr407, FAD600, Ile180, Ile335	–
		hMAO-B		

Tanshinone I (catalytic inhibitor)	-9.87	Cys172	Pi-Sigma: Tyr435 Pi-Pi Stacked: Tyr398 Pi-Pi T-shaped: Tyr326 Alkyl: Leu171, Ile199 Pi-Alkyl: Tyr398, FAD600, Leu171, CYs172, Ile198, Ile199	–
Tanshinone I (allosteric inhibitor)	-7.5	Arg172, Thr196	Pi-Sigma: Ile477, THr479 Amide-Pi Stacked: GLy194, THr195 Alkyl: Ile477 Pi-Alkyl: Arg120	Pi-Anion: Asp123, Glu483
Tanshinone IIA (catalytic inhibitor)	-10.29	Cys172	Pi-Sigma: Leu171, Ile199 Alkyl: Ile199, Ile316, Pro104, Leu164, Leu171 Pi-Alkyl: Phe103, Trp119, Ile316, Ile199	–
Tanshinone IIA (inhibitor)	-7.98		P-Pi Stacked: Phe103, Tyr112, Trp119 Alkyl: Trp119, Arg120, Val106 Pi-Alkyl: Phe103, Tyr112	Pi-Anion: Glu483

Cryptotanshinone (catalytic inhibitor)	-9.89	Tyr326	Pi-Sigma: Leu171 Pi-Pi T-shaped: Tyr326 Alkyl: Leu171, Cys172, Ile198, Leu164, Leu167, Ile316 Pi-Alkyl: Tyr326, Tyr398, Ile199, Leu171	–
Deprenyl[d]	-6.32	Leu171	Pi-Sigma: Leu171 Pi-Pi T-shaped: Tyr326 Pi-Alkyl: Phe343, Tyr398, FAD600, Ile199	–

[a]Estimated free binding energy of the ligand–receptor complex. [b]The number of hydrogen bonds and all amino acid residues from the enzyme–inhibitor complex were determined with the AutoDock 4.2 program. [c]7-Methoxy-1-methyl-9H-pyrido [3,4-b]indole. [d]Reference ligand.

Table S3. Binding site residues and docking scores of tanshinone I and the reference ligands in human M_4 muscarinic acetylcholine receptor (hM_4R).

Ligand	Binding Energy (kcal/mol)[a]	Interacting residue[b]		
		H-bond	Hydrophobic	Electrostatic and others
Tanshinone I	-8.77	–	Pi-Sigma: Tyr439, Tyr443 Pi-Pi stacked: Tyr113, Tyr439 Pi-Alkyl: Ala200, Ala203, Cys442 Alkyl: Ala200, Ala203, Cys442	–
Acetylcholine[c] (Agonist)	-3.92	Ser116	Pi-Sigma: Tyr439, Tyr433	Attractive charge: Asp112 Pi-Cation: Tyr439
Tiotropium[c] (Inverse agonist)	-10.12	Asn417	Pi-Sigma: Tyr439 Pi-Alkyl: Ala200, Val420, Ala203	Attractive charge: Asp112 Pi-Cation: Tyr439 Pi-Sulfur: Trp164

[a]Estimated free binding energy of the ligand–receptor complex. [b]The number of hydrogen bonds and all amino acid residues from the enzyme–inhibitor complex were determined with the AutoDock 4.2 program. [c]Reference ligands.

References [in the main text]

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