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Supporting Information

Taspine: Bioactivity-Guided Isolation and Molecular Ligand-Target Insight of a Potent Acetylcholinesterase Inhibitor from *Magnolia* x *soulangiana*

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Table S1. AChE Inhibitory Activity of some Magnoliaceae Extracts and of Galanthamine [a]

plant species	organ	extract	concentration	inhibition in %
Liriodendron tulipifera	bark	CH_2Cl_2	1 mg/mL	32.11 ± 9.10**
L. tulipifera	bark	MeOH	1 mg/mL	15.22 ± 8.03*
L. tulipifera	leaf	CH_2Cl_2	1 mg/mL	no activity
L. tulipifera	leaf	MeOH	1 mg/mL	no activity
Magnolia stellata	bark	CH_2Cl_2	1 mg/mL	10.27 ± 5.37
M. stellata	bark	MeOH	1 mg/mL	55.64 ± 6.52***
M. stellata	leaf	CH_2Cl_2	1 mg/mL	58.19 ± 17.89**
M. stellata	leaf	MeOH	1 mg/mL	29.46 ± 11.95*
Magnolia x *soulangiana*	bark	CH_2Cl_2	1 mg/mL	56.13 ± 2.50***
M. x *soulangiana*	bark	MeOH	1 mg/mL	84.69 ± 2.66***
M. x *soulangiana*	leaf	CH_2Cl_2	1 mg/mL	74.83 ± 4.00***
M. x *soulangiana*	leaf	MeOH	1 mg/mL	89.40 ± 2.66***
M. x *soulangiana*	leaf	non-alkaloid MeOH fraction	100 µg/mL	no activity
M. x *soulangiana*	leaf	alkaloid MeOH fraction	100 µg/mL	98.09 ± 0.66***
galanthamine			100 µM	97.66 ± 1.82***

[a] Statistical analysis: data are means ± SD; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, Student's test of absorption data after 30 min in comparison with medium control, $n = 4$.

Evaluation of Binding Orientations of Taspine (1) and Tacrine in AChE.

1W6R: The PDB entry 1W6R is a co-crystallization product of *Tc*AChE with a positivized galanthamine derivative. In the non-protonated state, taspine (**1**) is observed in multiple high ranked docking poses, the dimethylethyl side chain is not involved in specific interactions with the enzyme. In the protonated state however, taspine (**1**) nicely interacts with Glu199. In 1W6R docking, the protonated form of tacrine either forms a hydrogen bond with Glu199 or π-stacks with Trp84. Due to the conformation of the binding pocket, no concomitant interaction with both amino acid residues – as observed in BChE docking – can be established. All solutions for the non-protonated form of tacrine nicely interact with Trp84.

Both the protonated and non-protonated form of galanthamine are observed in docking positions identical to the binding mode of the co-crystallized galanthamine-derivative. However, GoldScore overestimates the affinity of galanthamine by ranking it much higher than tacrine and taspine (**1**).

1B41: Interestingly, in *h*AChE, only the neutral form of tacrine is docked to interact with Glu202. The protonated form is held in position by a bifurcated hydrogen bond with Tyr337 and the backbone oxygen of Gly82. Both forms approximately π-stack with Trp86 but not in an ideal geometry.

The top-ranked solution of taspine (**1**) stretches its positively ionized side chain to the catalytic amino acid Ser203. Its aromatic core positions itself between Trp86 and Tyr337. Due to the geometry of the binding pocket, no ideal sandwich-like π-π stacking complex can be formed. Ser125 interacts with one of the methoxy hydrogens attached to the aromatic core structure. The hydroxyl group of Tyr337 is not involved in any interaction with taspine (**1**). In the non-protonated state, taspine (**1**) is oriented in a similar position as in the protonated state. However, the positively ionized side chain has a less well-defined interaction pattern and changes its conformation from solution to solution. GoldScore

successfully predicts that taspine (**1**) and galanthamine will be similarly active in the human enzyme while tacrine is ranked with a higher affinity than both of them.

1ACJ: The PDB entry 1ACJ shows tacrine bound to *Tc*AChE. Due to the flat, aromatic structure of the ligand, Phe330 slightly reorients itself so that a sandwich-like π-stacking complex is formed between Trp84, tacrine, and Phe330. Tacrine was docked in both neutral and protonated states into this binding site. The best ranked docking pose for the neutral ligand was identical to the binding mode observed in X-ray crystallography. The protonated form was also π-stacked between Trp84 and Phe330, but in a reversed orientation forming a hydrogen bond with the backbone oxygen of His440. Obviously, the crystallization conditions did not favour the protonation of tacrine, so the ligand was crystallized in its neutral form. Docking of taspine (**1**) returned a preferred orientation that was identical for most protonated and neutral solutions. Identical to tacrine, taspine (**1**) is observed in π-π-stacking with Trp84 and Phe330. The flexible dimethylaminoethyl side chain interacts with Ser200/His440 of the catalytically active site.

Docking of Tacrine and Taspine (1) into the *h*BChE Active Site

Figure S1. Tacrine docked into *h*BChE (PDB entry 1P0M) visualized with LigandScout;[29] left without interactions, right with visualized interactions (violet, aromatic stacking; green, hydrogen bond donors); **A**: non-protonated form; **B**: protonated form

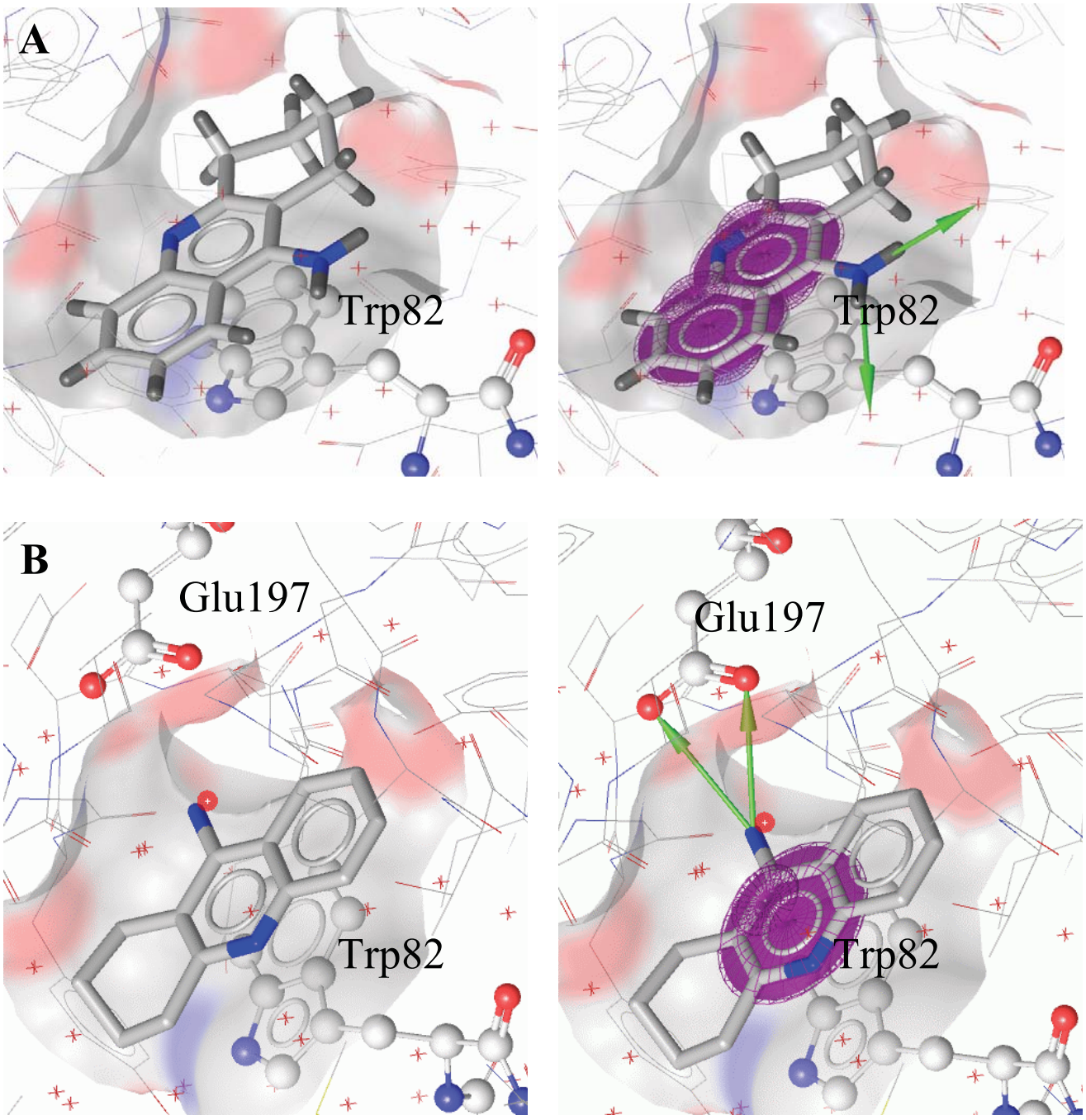

Figure S2. Taspine (**1**) docked into *h*BChE (PDB entry 1P0M) visualized with LigandScout[29] in two distinct docking orientations

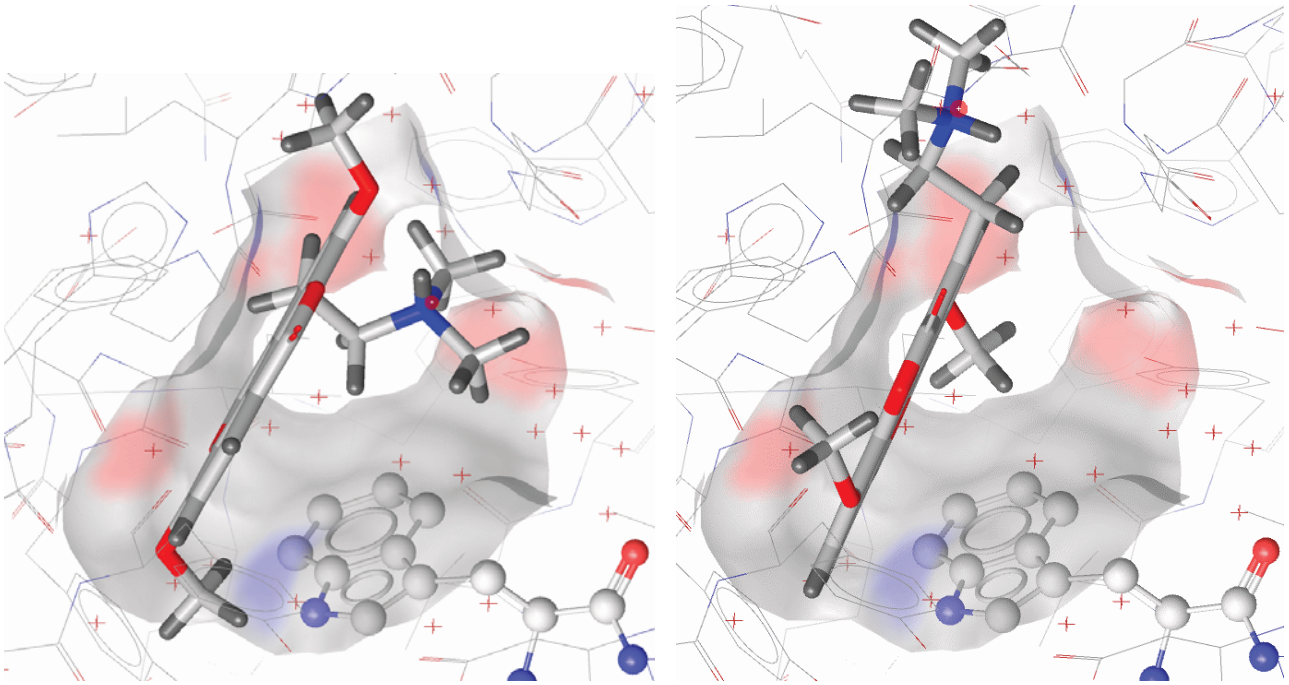

Table S2. NMR Data and HMBC Correlations for Taspine (**1**) (300 MHz, $CDCl_3$, δ in ppm, *J* in Hz)

position	δ_H (*J*)	δ_C	HMBC
1		144.4	
2	7.16 s	116.5	C-1, C-3, C-3a, C-10, C-10a, C-10b, C-1'
3		150.9	
3a		136.7	
5		158.7	
5a		111.5	
6	8.16 d (8.7)	126.9	C-5, C-5a, C-7, C-8, C-10c
7	7.28 d (8.7)	113.6	C-5, C-5a, C-8, C-8a
8		151.2	
8a		137.9	
10		157.7	
10a		109.2	
10b		119.1	
10c		118.4	
1'	3.48 m [a]	33.0	C-1, C-2, C-10a, C-2'
2'	2.63 m [a]	60.3	C-1, N-CH_3, C-1'
C_3-O-CH_3	4.09 s	56.5 [b]	C-3 or C-8
C_8-O-CH_3	4.09 s	56.5 [b]	C-3 or C-8
N-CH_3 (1)	2.36 s	45.2	C-2', N-CH_3
N-CH_3 (2)	2.36 s	45.2	C-2', N-CH_3

[a] Signal partly obscured. [b] Signals may be interchanged

Table S3. NMR Data and HMBC Correlations for (-)-Asimilobine (**2**) (300 MHz, $CDCl_3$, δ in ppm, *J* in Hz)

position	δ_H (*J*)	δ_C	HMBC
1		142.9	
2		148.5	
3	6.73 s	114.5	C-1, C-2, C-4, C-6a, C-11c
3a		128.6	
4	3.13 m	27.4	C-3a
4	2.76 m	27.4	C-3, C-3a, C-11c
5	3.54 m	42.4	C-3a, C-4, C-6a
5	3.09 m	42.4	C-3a, C-4, C-6a
6 (NH)	2.01 s		
6a	3.97 dd (13.2, 4.8)	53.1	C-3a, C-7, C-7a, C-11c
7	3.04 dd (13.2, 4.8)	35.9	C-3a, C-6a, C-7a, C-11a, C-11c
7	2.95 dd (13.2, 1.0)	35.9	C-3a, C-6a, C-7a, C-11a, C-11c
7a		134.6	
8	7.19-7.35 m (7.5, 2.7, 1.5) [a]	127.4-127.9 [a]	C-7, C-7a
9	7.19-7.35 m (7.5, 2.7, 1.0) [a]	127.4-127.9 [a]	C-7a, C-11
10	7.19-7.35 dd (7.8, 1.5) [a]	127.4-127.9 [a]	C-8, C-11a
11	8.29 dd (7.8, 1.0)	127.2	C-7a, C-9, C-10, C-11b, C-11c
11a		131.2	
11b		135.1	
11c		125.5	
OCH_3	3.59 s	60.2	C-1
C_2-OH	5.30 br s		

[a] Signals overlap.