Supplementary Materials

Acetylcholinesterase as a Target for Heliotridine-type Alkaloids Isolated from Plants: A Computational Study

Contents

1.Detailed description of molecular docking calculations.

Calculations were carried out on a PC running at 3.4 GHz on an AMD Ryzen 9 5950X 16 core (32 threads) processor with 32 GB RAM and 1 TB hard disk with Windows 10 Home 64-bit as an operating system. The ligands were built using PC Model version 10 (Serena Software, Bloomington, IN 47402e3076) and minimized using the force field MMX. The minimized molecules were saved in pdb extension. The AutoDock Tools (ADT) package version 1.5.6rc3 [8] was applied to generate the docking input files used for the docking calculations. The different crystallographic structures of AChE were from the Protein Data Bank (PDB; http://www.pdb.org/). The structure of *Torpedo californica* AChE complexed with 12-amino-3-chloro-6,7,10,11-tetrahydro-5,9-dimethyl-7,11-methanocycloocta[b] quinolin-5-ium (6G1V) with a resolution of 1.82 Å [9] and the structure of *Drosophila melanogaster* AChE in complex with tacrine derivative 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine (6XYU) with a resolution of 2.51 Å [10] were determined by X-ray crystallography. The structures were modified as follows: the ligand and all of the crystallization water molecules were removed, with the file saved in pdb extension. All hydrogen atoms were added using AutoDock Tools (ADT), and the Gasteigere Marsili charges were calculated, with the resulting file saved in pdbqt extension. Rotatable bonds were defined for each minimized ligand molecule. For the docking calculation two different approach were used: AutoDock Vina 1.2.3 (in the future text reported as Vina), [11] which employs a genetic algorithm and Protein-Ligand-ANTSystem (PLANTS) which uses a class of stochastic optimization algorithms called ant colony optimization (ACO) [12]. For Vina calculation a grid box of 14 x 14 x 14 Å in the x, y and z directions was created, with spacing of 1.00 Å and centred at x=3.697, y=-4.587, z=20.236 for 6G1V structure, whereas for 6XYU structure a same grid box was created centered at X=32.844, y=67.762, z=9.588. Further parameters were set as follows: exhaustiveness of the local search, 100; number of conformations to calculate, 10. To validate the goodness of the calculation, the original ligands were re-docked and visual inspection of the data showed a very tight overlap for both original ligands. The results are expressed as the energy associated to each ligand-enzyme complex in terms of the Gibbs free energy values.

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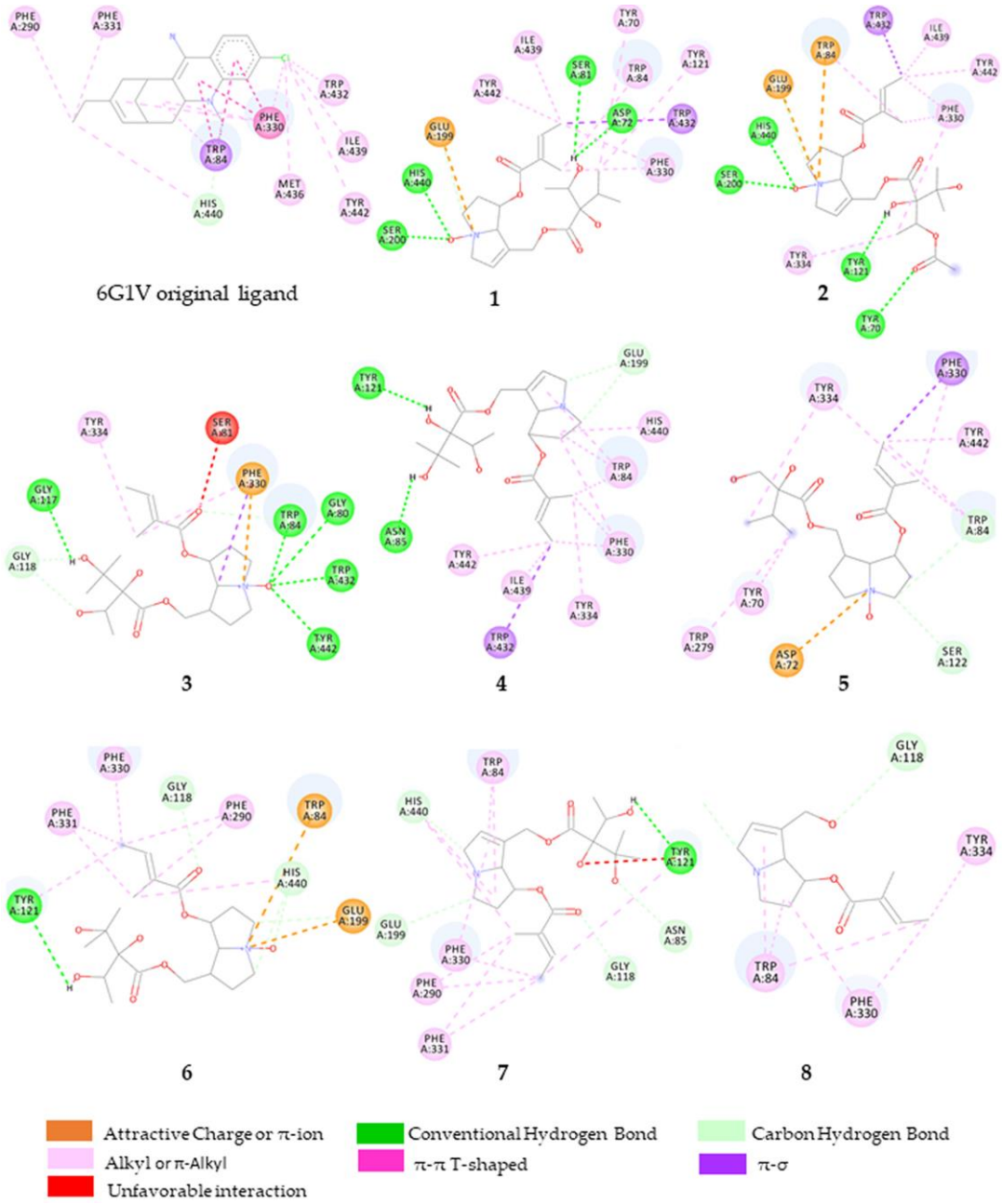


Figure S1. Bidimensional representations for the interactions of metabolites **1–8** and original ligand 12-amino-3-chloro-6,7,10,11-tetrahydro-5,9-dimethyl-7,11-methanocycloocta[b]quinolin-5-ium with *Torpedo californica* AChE (6G1V) according to AutoDock Vina calculations, with indications of the type of interactions

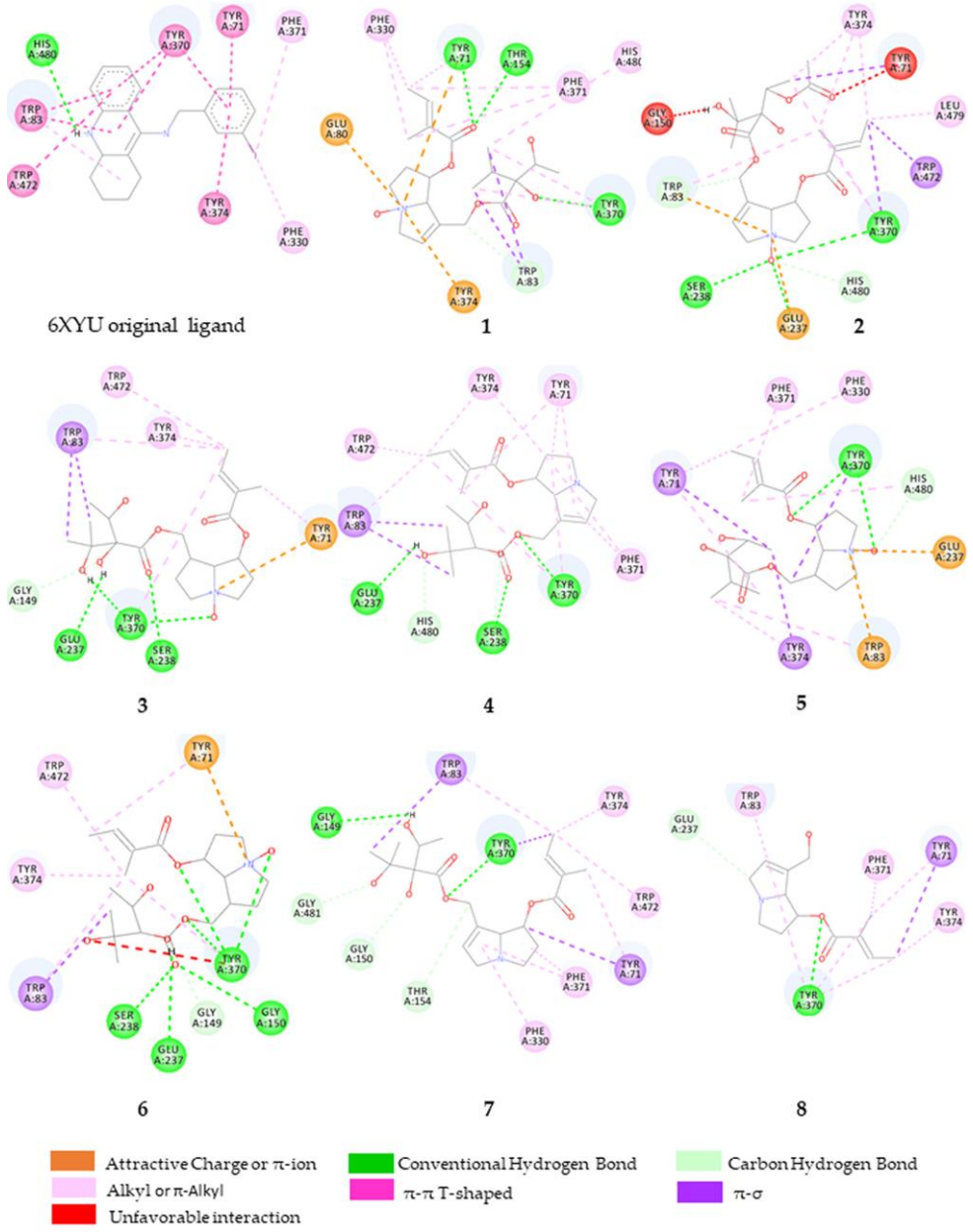
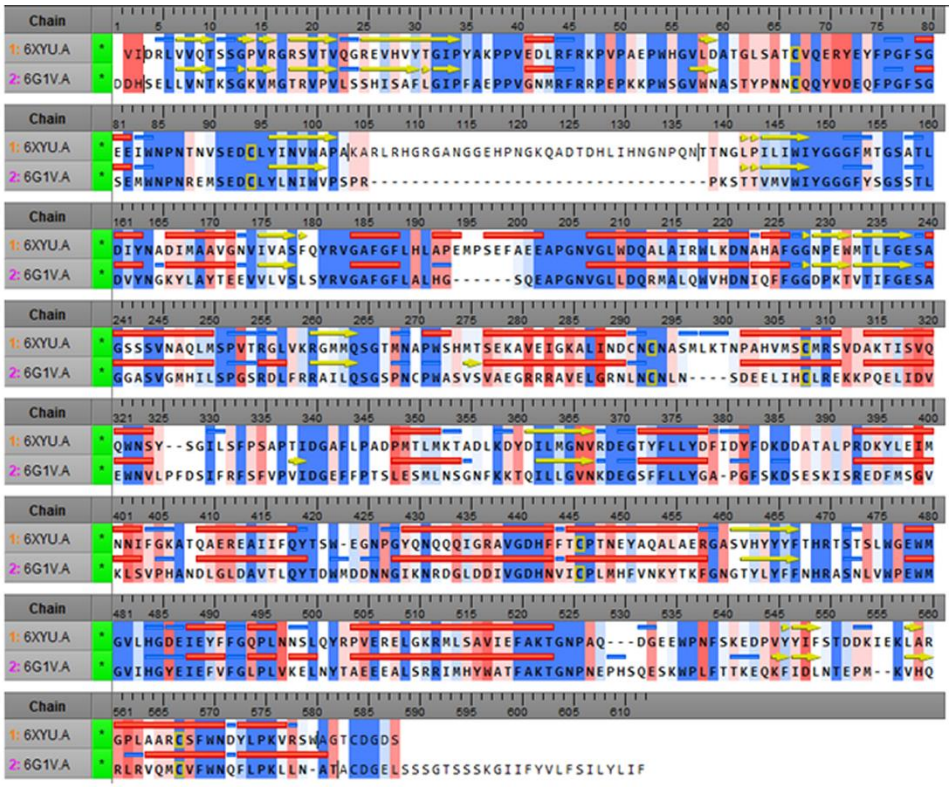
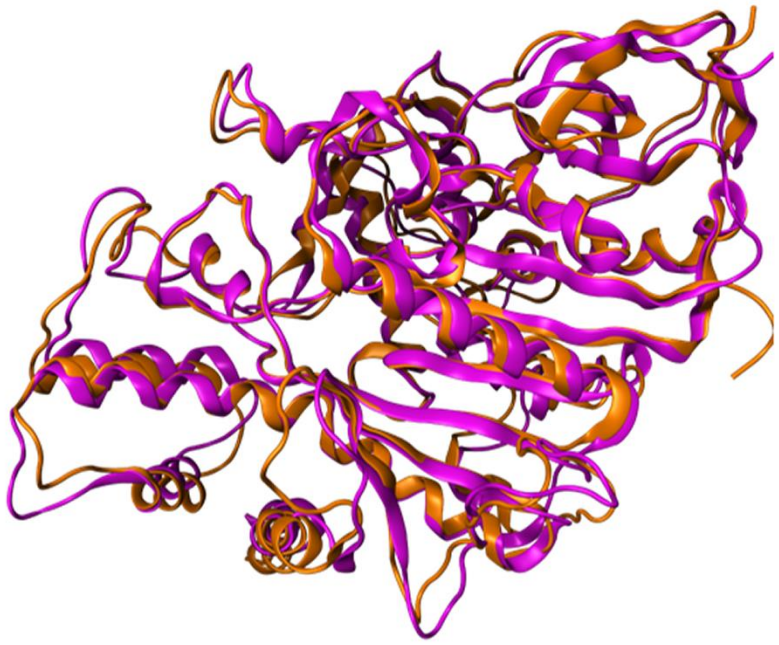


Figure S2. Bidimensional representations for the interactions of metabolites **1**–**8** and original ligand 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine with *Drosofila melanogaster* AChE (6XYU) according to the AutoDock Vina calculations, with indications of the type of interactions. In the structures **4** and **6** the oxygen of an OH group looks like overlapped to carbon of ester linked to the hydroxylated chain.



(a)



(b)

Figure S3. (a) Alignment of structures of AChE sequences from *Torpedo californica* (6G1V) and *Drosophila melanogaster* (6XYU): (a) the colors represent residues similarity, with the same aminoacid residues indicated in blu; (b) 3D-view of overlapped 6G1V (magenta) and 6XYU(orange).

Table S1. Data by ADME/toxicity prediction for compounds **1–8**.

Compound	1	2	3	4	5	6	7	8
				Human ADME				
Human oral bioavailability	-	-	-	-	-	-	-	-
Blood Brain Barrier	-	-	-	-	-	-	-	+
Human Intestinal Absorption	-	-	-	+	-	-	+	+
				Human Toxicity				
Eye corrosion	-	-	-	-	-	-	-	-
Eye irritation	-	-	-	-	-	-	-	-
Skin corrosion	-	-	-	-	-	-	-	-
Skin irritation	-	-	-	-	-	-	-	-
Skin sensitisation	-	-	-	-	-	-	-	-
Carcinogenicity	-	-	-	-	-	-	-	-
Ames mutagenesis	-	+	+	+	-	+	+	-
Acute Oral Toxicity (LD_{50} in mol/Kg)	3.627	3.349	3.386	4.020	3.627	3.386	4.020	3.350
				Eco-toxicity				
Water solubility (LogS)	-2.325	-2.325	-2.271	-1.599	-2.325	-2.271	-1.599	-1.206
Biodegradation	-	-	-	-	-	-	-	-
Crustacea aquatic toxicity	-	+	-	-	-	-	-	-
Fish aquatic toxicity	+	+	+	-	+	+	-	-
Honey bee toxicity	-	-	-	-	-	-	-	-
Tetrahymena pyriformis ($pIGC_{50}$ in μg/L)	0.380	0.502	0.058	-0.015	0.380	0.058	-0.015	0.422
Avian toxicity	-	-	-	-	-	-	-	-