Supporting information

Bis-isoquinolinium and bis-pyridinium acetylcholinesterase inhibitors: in vitro screening of probes for novel selective insecticides

Veronika Hrabcova[a,b], Jan Korabecny[b,c], Brigita Manyova[b,d], Lenka Matouskova[b,d], Tomas Kucera[b], Rafael Dolezal[a,c], Kamil Musilek[a,b,c], Lukas Gorecki[b,c], Eugenie Nepovimova[b,c], Kamil Kuca[a,b,c], Daniel Jun[b,c*]

[a] *University Hradec Kralove, Faculty of Science, Department of Biology, Rokitanskeho 62, 50003 Hradec Kralove, Czech Republic*

[b] *University of Defence, Faculty of Military Health Sciences, Department of Toxicology and Military Pharmacy, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic*

[c] *University Hospital, Biomedical Research Centre, Sokolska 581, 500 05 Hradec Kralove, Czech Republic*

[d] *Charles University, Faculty Of Pharmacy, Department of Pharmacology and Toxicology, Akademika Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic*

* Corresponding author: Daniel Jun; address: University of Defence, Faculty of Military Health Sciences, Department of Toxicology and Military Pharmacy, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic.; E-mail: daniel.jun@unob.cz; phone: 00 420 973 255 193

1. *Homogenate preparation from Musca domestica*

The housefly larvae were bought at specialized store. Larvae were kept in the dark under the room temperature until the adult house flies hatched out. Adult flies were frozen and keep under -80 °C at least for two weeks. 30 fly's heads were homogenized at 4 °C in 0.1 M phosphate buffer, pH 7.4, 0.1% (w/v) Triton X-100 by using Bandelin Sonoplus 2070. The homogenates containing AChE from *Musca domestica* (*Md*AChE) were centrifuged for 15 minutes (4 °C, 5000 RPM). The supernatants were filtrated through 0.2 µm PVDF filters and used for studying acetylcholine inhibitors.

2. Preparation of AChE from human blood

Haemoglobine-free erythrocyte ghosts were prepared according to Worek et al.[1] with minor modifications. Heparinised human blood was centrifuged for 20 minutes (4 °C, 5000 RPM) by Hettich Universal 320R centrifuge. The plasma was separated, buffy coat was removed and erythrocytes were washed three times by 0.1 M phosphate buffer, pH 7.4. Washed erythrocytes were centrifuged 5 minutes at 5000 RMP at 4 °C. Then, the packed erythrocytes were hemolyzed by dilution in the 0.01 M phosphate buffer, pH 7.4 and the process was accelerated by storage at -80 °C. Haemoglobine was removed by process of dilution and ultracentrifugation. Erythrocytes were washed two times with 0.01 mM phosphate buffer, pH 7.4 and centrifuged for 30 minutes (4 °C, 20 800 RPM) by Beckman Coulter, Optima L-90K centrifuge. Then, the sediment was resunspended by 0.1 M phosphate buffer, pH 7.4 and centrifuged for the last time under the constant conditions. The haemoglobine-free erythrocyte ghosts containing AChE were collected and storage at -80 °C.

3. Biological evaluation of AChE activity

In order to investigate the selectivity of AChE inhibitors to the insect enzyme we used modified Ellman protocol.[2,3] The assay mixture (200 µl) contained 1 mM acetylthiocholine iodide as substrate and 0.25 mM DTNB as chromogen in 0.1 M phosphate buffer (pH 7.4). The activity was determined by measuring of the increase in absorbance at 412 nm at 37°C in 2 min intervals using Multi-mode microplate reader Synergy 2 (Vermont, USA).

4. Inhibition and selectivity to insect AChE

The IC50 values were expressed as a concentration of compound required for 50% reduction in cholinesterase activity. Homogenate from *Musca domestica* fly heads was used as a source of the insect AChE and the heaemoglobine-free erythrocyte ghosts as a source of human AChE. Assays were handled parallel on human and insect enzyme. Assayed solutions of target compounds (10^{-3} – 10^{-10} M) were preincubated at 96-well plate for 5 min at 37°C. Each concentration was assayed

in triplicate. Software Microsoft Excel (Redmont, WA, USA) and GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA) were used for the statistical data evaluation. Selectivity index (SI) was calculated as a ratio of IC50 value of human AChE/IC50 value of fly AChE.

5. In silico studies

Two structures of AChE were gained from RCSB Protein Data Bank – PDB ID: 4EY7 (crystal structure of *h*AChE) and 2HCP (theoretical model of Greenbug AChE provided by Pang, Y.P.; *Schizaphis graminum*, *Sg*AChE).[4] Structure of *Md*AChE was built by homology modeling. The model was based on the sequence ID Q7YWJ9 from UniProt database. The 3D structure of *Drosophila melanogaster* AChE was chosen as the template (PDB ID 1DX4, X-ray structure, resolution 2.7 Å, complex with tacrine derivative 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine, overall sequence identity 88.32 %).[5] The model was built by Swiss-model software (ProMod3 v. 1.0.0; QMQE 0,74, QMEAN4), inspected (one Ramachandran outlier outside of binding cavity) and found suitable for docking.[6–8] All receptor structures were prepared by DockPrep function of UCSF Chimera (version 1.4) and converted to pdbqt-files by AutodockTools (v. 1.5.6).[9,10] Flexible residues selection was based on previous experience (*h*AChE) or spherical region around the binding cavity.[11–13] Three-dimensional structures of ligands were built by Open Babel (v. 2.3.1), minimized by Avogadro (v 1.1.0) and converted to pdbqt-file format by AutodockTools.[14] The docking calculations were done by Autodock Vina (v. 1.1.2) with the exhaustiveness of 8.[15] Calculation was repeated ten times for each ligand and receptor and the best-scored result was selected for manual inspection. The visualization of enzyme-ligand interactions was prepared using 1.5.0.4 (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC, Mannheim, Germany). 2D diagrams were created with Discovery Studio 2016 Client.

Notes and references

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