Computational discovery and experimental verification of tyrosine kinase inhibitor pazopanib for the reverse of memory and cognitive deficits in rat model of neurodegeneration

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Electronic Supporting Information (ESI)

ESI Materials and Methods

Parameters for the two dimensional screening of approved drugs

The preparation and parameters of the virtual screening is set as follows: the initial preparation was performed by the AutoDock Tools (ADT). The side chains and termini protons of AchE (PDB ID: 1EVE) were prepared according to the protonation states at pH 7.0. The grid size is 80×80×80 and the spacing value is 0.375 Å. The following parameters were applied during the screening: the population size is 100; the energy evaluation is 100,000 per run with the maximum number of generations of 27,000; the number of docking runs was 10000. The other parameters were set at default values.

Protocol for rodent model of neurodegeneration induced by quinolinic acid (QA)

Animals were placed in a stereotaxic frame under chloral hydrate anesthesia (350mg/kg body weight). The nucleus basalis of Meynert (NBM) of the animals were

located by the stereotaxic atlas. The skull was opened using a sterile surgical drill and 2μl PBS solution (pH 7.4) containing 120nmol quinolinic acid (QA; Sigma, Shanghai) was injected into the left NBM (bregma coordinates AP:B-1.4mm, ML:±2.4mm, DV:-7.5mm) using a 2μl Hamilton microsyringe coupled to an automatic microinjector at a speed of 0.2 μl/min. The injection was applied to the right NBM of the animals following the same protocol. The control group animals received an injection of 2μl of the vehicle PBS solution. The animals were maintained until recuperating from anesthesia after suture.

Protocol for Morris water maze task and probe trial test

The testing platform was hidden below the opaque water surface but accessible for the rats. The learning of the fixed platform location was then analyzed in consecutive training trials and probe trials. The water maze consisted of a dark gray pool filled with opaque water. The circular platform of 8cm in diameter was placed 1.5cm below the opaque water surface and in the middle of one quadrant (southeast) of the pool. Animals were placed into the water facing the edge of the pool at one of four arbitrary starting positions. Animals were allowed to swim until they found the platform. The animals which failed to locate the platform for 90s were guided to the platform and allowed to rest for 10s. The animals were subjected to two trials per day for 4 days with a video system monitoring and recording the percentage of time spent in the various quadrants. On the next day, the platform was removed and each rat was allowed to explore in the water pool for 90s. The swimming time and the swimming distance in the quadrant where the platform was located were calculated.

Parameters for long-timescale molecular dynamics simulation

Crystallographic structure of AchE was obtained from the PDB bank (PDB code: 1EVE). Molecular docking of pazopanib and other molecules was performed following the standard protocol. The starting structure of MD simulation was obtained from the best binding mode of docking. The Gaussian 03 package was used to calculate the electrostatic potential of pazopanib at the HF/6-31G* level via the RESP fitting technique. The whole complex was neutralized by appropriate Na^+ counterions and was immersed in an explicit periodic boundary conditions box of TIP3P water molecules with a margin of at least 10 Å from any solute atoms. One 1000 ns and three 300 ns production molecular dynamics simulations were performed with different random seed values at constant pressure (1 atm) and temperature (300 K) using PMEMD.CUDA enabled NVIDIA graphics processing units (GPUs) implemented with Amber 10(42). All the other simulations were performed on a high-performance DELL T7500 workstation with 24 core 2.66GHz processors running Linux operating system. All the molecular graphics were displayed and prepared using the free Pymol educational version.

ESI Figures and Figure Legends

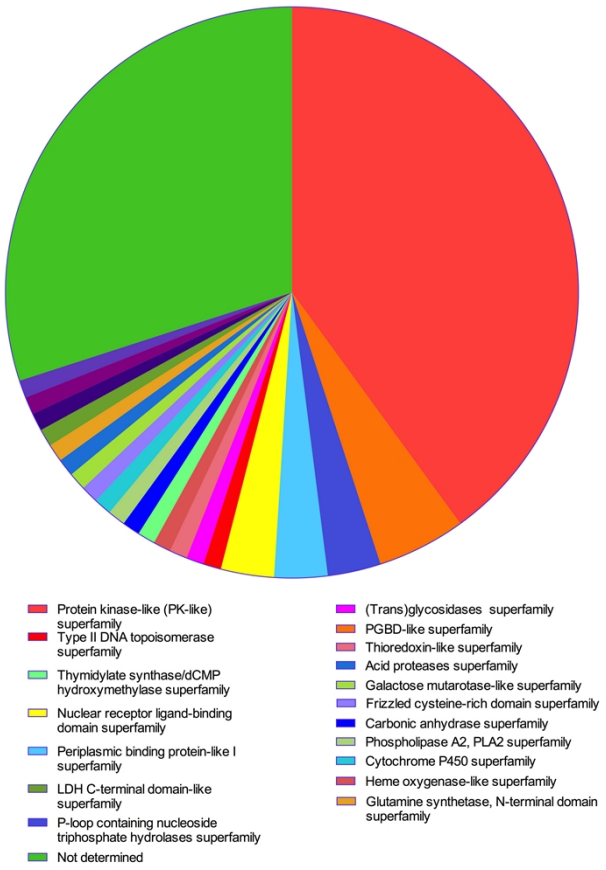

Figure S1. Protein superfamily information of top 100 hits by local binding sites alignment with AchE.

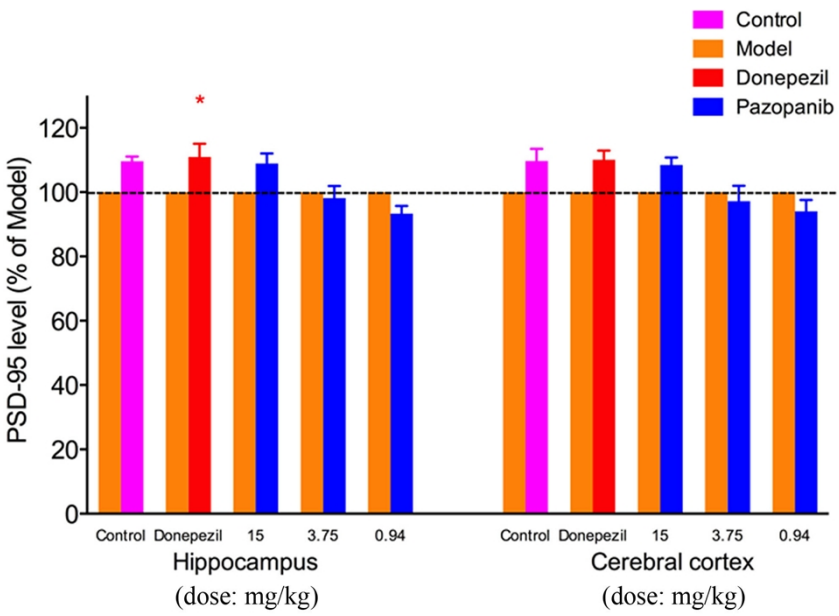

Figure S2. Effect of pazopanib on the expression of PSD-95 in the hippocampus and the cerebral cortex of rat model. The intensity of each protein band was quantified by densitometry using the Quantity One software (Bio-Rad, Hercules, CA, USA), and then corrected with the corresponding β-actin level. Results were expressed as mean ± SEM (n=6) and analyzed with one-way ANOVA, followed by Dunnett's post test for multiple comparisons. ###P <0.001 vs control group; ***P <0.001 vs. model group.

ESI Dataset Captions

Dataset S1. Alignment results of AchE with 1105 crystallographic structures of 377 FDA-approved drug targets retrieved from the PDB bank. The results of TM-align were highlighted in orange color.

Dataset S2. Superfamily and domain information of top 100 targets aligned with AchE.

Dataset S3. Top 50 hits ranked by the score of EON_ET_Combo as the sum of 3D similarity and electrostatics similarity. The three dimensional shape comparison between donepezil and 1385 FDA-approved drugs was performed by the ROCS program and the comparison of electrostatics properties was performed by EON program implemented within OpenEye (Rapid Overlay of Chemical Structures, version 3.1, OpenEye Scientific Software).

Dataset S4. The 13 purchased TKIs (Tyrosine Kinase Inhibitors) used for *in vitro* AchE enzymatic assay.