

## ABSTRACT

The current research was aimed at finding peripheral anionic site (PAS) binding acetylcholinesterase (AChE) inhibitors to be used in the treatment of Alzheimer's disease (AD). The crystal structure of human acetylcholinesterase (hAChE) in complex with fasciculin II (2.76 Å) (PDB ID: 1b41) was found to be the most suitable protein for identifying PAS binding ligands in docking studies and was used for virtual screening of about 830 unknown ligands. Twenty-one compounds with a lowest binding energy ranging from -8.7 kcal/mol to -5.6 kcal/mol were selected for testing for AChE inhibitory activity. Four of these compounds were found to inhibit AChE with IC<sub>50</sub> values in the range 1–15 µM and two also gave significant inhibition of self-induced amyloid beta (Aβ) aggregation at final concentration of 10 µM. A series of 27 2'-hydroxy-and 2'-methoxy chalcones were also tested and 8 of these compounds were found to inhibit AChE with IC<sub>50</sub> values in the range 40–350 µM. Four of them also inhibited self-induced Aβ aggregation at 10 µM. Docking studies showed that all but one of the active compounds appear to be PAS-binding ligands. In summary, we have identified some promising PAS-binding anticholinesterase compounds which show high inhibitory activity towards AChE and self-induced Aβ aggregation. Further studies are required to investigate the effect of these compounds towards AChE-induced Aβ peptide aggregation and determine the inhibition mode of these compounds.

## ABSTRAK

Penyelidikan ini telah dijalankan atas tujuan mencari perencat bahagian ‘peripheral’ enzim acetylcholinesterase yang boleh mengubati penyakit Alzheimer’s disease (AD). Melalui kerja ‘dock’, struktur kristal enzim acetylcholinesterase daripada manusia yang berkompleks dengan fasciculin II (2.76 Å) (PDB ID: 1b41) telah didapati sebagai protein yang paling sesuai untuk mencari perencat bahagian ‘peripheral’ enzim acetylcholinesterase dan telah digunakan untuk kajian virtual 830 ligan tidak diketahui. Dua puluh satu ligan dengan tenaga dalam lingkungan -8.7 kcal/mol kepada 5.6 kcal/mol telah dipilih untuk diuji didalam ujian perencatan enzim acetylcholinesterase. Empat ligan daripada kumpulan ini telah didapati merencat enzim acetylcholinesterase dengan nilai  $IC_{50}$  dalam lingkungan 1–15  $\mu$ M dan dua daripadanya telah memberi perencatan signifikan didalam ujian sendiri-teraruh pengagregatan ‘amyloid beta’ ( $A\beta$ ) pada kepekatan akhir 10  $\mu$ M. Sebanyak 27 chalcone yang tergolong dalam siri 2'-hydroxy-dan 2'-methoxy juga telah diuji dan didapati lapan daripadanya merencat enzim acetylcholinesterase dengan nilai  $IC_{50}$  dalam lingkungan 40–350  $\mu$ M. Empat daripadanya juga telah didapati merencat sendiri-teraruh pengagregatan  $A\beta$  pada kepekatan 10  $\mu$ M. Kerja ‘dock’ telah menunjukkan bahawa kesemua tetapi salah satu ligan aktif adalah ligan perencat bahagian ‘peripheral’. Secara kesimpulannya, kami telah menjumpai segelintir perencat bahagian ‘peripheral’ enzim acetylcholinesterase berpotensi yang telah menunjukkan perencatan yang tinggi terhadap enzim acetylcholinesterase dan sendiri-teraruh pengagregatan  $A\beta$ . Kajian lanjut merangkumi pengkajian kesan ligan-ligan ini terhadap acetylcholinesterase-teraruh pengagregatan  $A\beta$  serta penentuan jenis perencatan ligan-ligan ini.

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## LIST OF ABBREVIATIONS

ABS	Absorbance
AD	Alzheimer's disease
AChE	Acetylcholinesterase
ACL	Acetylcholine
ACLTHIO	Acetylthiocholine
AMB	Ambenonium
APP	Amyloid precursor protein
ATChI	Acetylthiocholine iodide
A $\beta$	Amyloid beta
BW284C51	1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide
CAS	Catalytic site
CR	Cluster rank
DECA	Decamethonium
DECI	Decidium
DNA	Deoxyribonucleic acid
DONE	Donepezil
DTNB	5, 5'-dithiobis[2-nitrobenzoic acid]
EDR	Edrophonium
FAS	Fasciculin
FAS-II	Fasciculin-II
GALLMN	Gallamine
(-)-GLNTM	(-)-Galantamine
HUP A	Huperzine A
HUP B	Huperzine B
(-)-HUP X	(-)-Huprine x

PROP	Propidium
TAC	Tacrine
$\Delta^9$ THC	Tetrahydrocannabinol
TUBO	Tubocurarine

## UNIT OF MEASUREMENTS

kcal/mol	Kilocalorie per mole
M	Molar
mg	Milligram
min	Minute
mL	Milliliter
$\mu$ L	Microliter
mM	Millimolar
nM	Nanomolar
s	Second
$\mu$ M	Micromolar
$^{\circ}$ C	Degree celsius

## AMINO ACIDS

Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
His	Histidine
Leu	Leucine
Phe	Phenylalanine
Ser	Serine
Thr	Threonine
Tyr	Tyrosine