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Virtual Screening of Acetylcholinesterase Inhibitors

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1. Introduction

Alzheimer's disease (AD) is a progressive, neuro-degenerative disease, which is clinically characterized by loss of memory and progressive deficits in different cognitive domains. Widespread epidemic, long-term treatment and high medical costs have made AD a major public health problem.

AD is associated with low in vivo level of acetylcholinesterase. The consistent neuropathologic hallmark of the disorder is a massive deposit of aggregated protein degradation products, amyloid- β ($A\beta$) plaques and neurofibrillary tangles (Haviv et al., 2005). Even if the primary cause of AD is still speculative, $A\beta$ aggregates are thought to be mainly responsible for the pathogenesis of the disease (Hardy & Selkoe, 2002). In recent years, significant research has been devoted to the role of free radical formation, oxidative cell damage, and inflammation in the pathogenesis of AD, providing new promising targets and validated animal models (Capsoni et al., 2000).

The major marketed drugs for the symptomatic treatment of AD are acetylcholinesterase (AChE) inhibitors, that is, tacrine (TC) (Davis & Powchik, 1995), donepezil (Bryson & Benfield, 1997), rivastigmine (Gabelli, 2003), and galantamine (Sramek et al., 2000) which inhibit AChE activity to promote an increase in the concentration and the duration of acetylcholine in the brain. However, they do not address the etiology of the disease.

The crystal structure of Torpedo California AChE (TcAChE) revealed that its active site lies at the bottom of a deep and narrow gorge (20 Å), named the "active site gorge" or "aromatic gorge" (Axelsen, 1994), the schematic can be seen in the Fig. 1. At the active site, the catalytic triad Ser200-His440-Glu327 is responsible for hydrolyzing the ester bond in ACh. At the "anionic" subsite of the active site (historically termed the "catalytic anionic site", CAS), consisting of Trp84, Tyr130, Gly199, His441 and His444 amino acid residues, adjacent to the catalytic triad, the indole side chain of the conserved residue Trp84 makes a cation- π interaction with the quaternary amino group of ACh. (Ma, 1997) A second aromatic residue, Phe330, is also involved in the recognition of ligands. The conserved residue Trp279 is the major component of a second binding site, named the peripheral "anionic" site (PAS), consisting of Tyr70, ASP72, Tyr121, Trp279 and Tyr334 amino acid residues, 14 Å from the active site, near the top of the gorge. (Harel, 1993) The oxyanion hole formed by the peptidic amino groups of Gly118, Gly119, Ala201 amino acid residues is another important functional unit in the esteratic subsite (Wiesner et al., 2007).

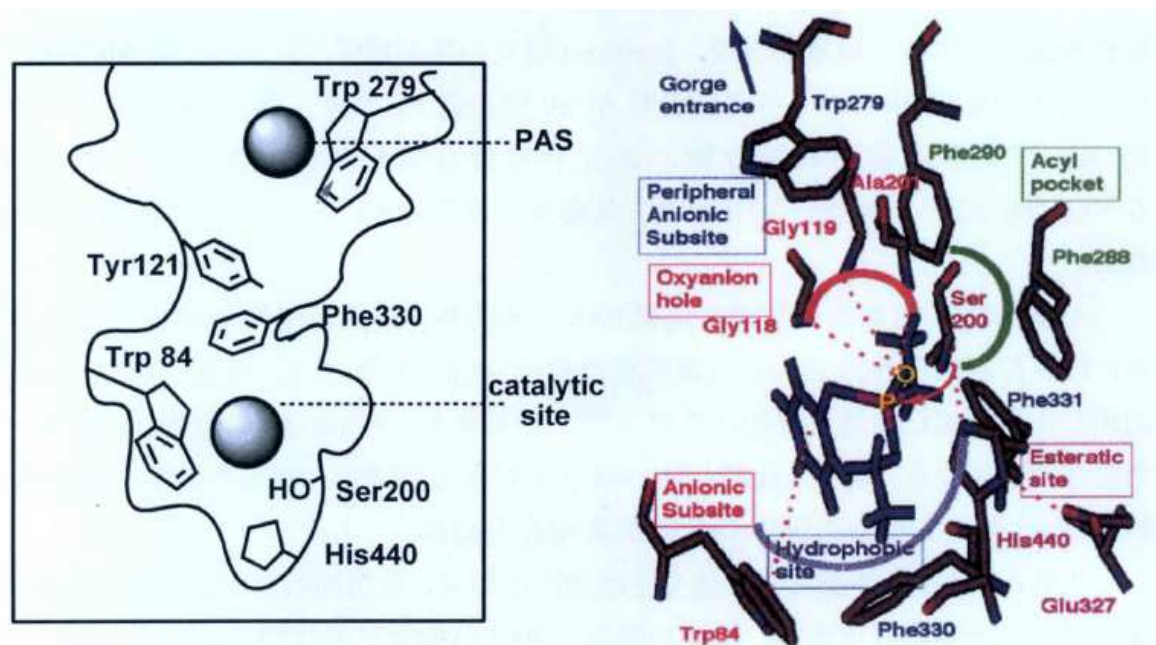


Fig. 1. The active site of AChE

Recently the peripheral anionic site (PAS) of AChE has been indicated to be involved in A β peptide aggregation and formed a steady compound with A β . On the basis of these premises, AChE inhibitors, which may alleviate cognitive deficits and behave as disease-modifying agents by inhibiting the β -amyloid (A β) peptide aggregation through binding to both catalytic and peripheral sites of the enzyme are becoming a new field for AD therapy.

Traditional medicine *Corydalis caca* has been embodied by Chinese Pharmacopoeia with the function of promoting blood circulation, relieving the pain and strengthening immunity. Ma et al. had extracted and isolated eight kinds of Tetrahydroisoquinoline alkaloids from the herb *Corydalis*, including Corydaline (Ma, 2008). The rigid structure containing the unit of Tetrahydroisoquinoline was shown in Fig. 2 (a). Adseren et al. found the methanol extract of *Corydalis caca* showing a potential inhibitory effects on the AChE activity, especially Corydaline, which is not sensible to BChE ($IC_{50} > 100 \mu M$) has the highest activity ($IC_{50} < 15 \mu M$) and selectivity among the alkaloids isolated (Adseren et al., 2007). Because of its well-established biological properties, a type of cordalis alkaloid corydaline, which has demonstrated moderate inhibition to AChE, was used as a lead compound to obtain the action mechanism of corydaline to AChE and screen a series of its open ring derivatives by means of molecular docking and virtual screen.

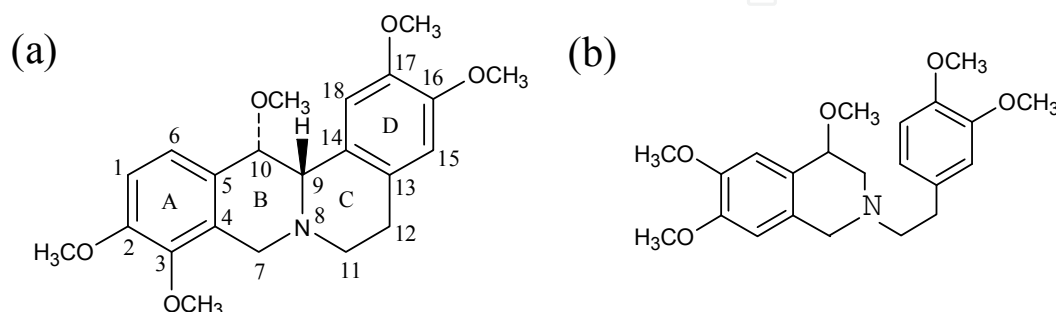


Fig. 2. Structure of Corydaline (a) and compound 7(b)

Molecular docking method (GOLD) was used by us to investigate the binding mode of corydaline with acetylcholinesterase and to screen a series of open ring derivatives with different carbon linkages and different substituent groups. Molecular Dynamics research has been done by us between the Corydaline and TcAChE with the open conformation, semi-open conformation and close conformation respectively. The best result was obtained by GOLD when corydaline was bound to the enzyme catalytic site in the open conformation. The conformation model in Fig. 5(a) indicates that phenyl ring A interacts with the phenyl group of Tyr 334 via a classic parallel π - π accumulation and that the positively charged nitrogen atom interacts with the phenyl group of Phe 330 in the hydrophobic site by the cation- π effect. Phenyl D that penetrate to the catalytic position at the bottom of the active pocket, interacts with Trp 84 via π - π effect.

Based on our former research, Yuren Jiang, et al. designed and screened a series of compounds, in which, the virtual molecule 7 was synthesized and assayed the inhibitory activity for AChE ($IC_{50}=473.3 \text{ nM} \cdot \text{L}^{-1}$) (Yuren et al., 2009). The structure of virtual molecule 7 was shown in the Fig. 2 (b). Virtual screening showed that the scores of most derivatives were higher than that of corydaline, and the open ring substances with the highest scores were mainly derived from those substituted by phenoxy groups and those with 2- to 7- carbon linkages.

On account of the chemical structure of Corydaline is consisted of rigid fused heterocycle, The length of molecule was too short to reach both the catalytic anionic site and the peripheral "anionic" site, We decided to use Corydaline as lead compound. In the reserves of Protons turn nitrogen, The C ring of Corydaline was opened in order to get derivatives that were based on the Tetrahydroisoquinoline structure. The virtual ligands with the respective substituent groups of -OH, -OCH₃, benzyl ect. and different length with 2- to 7- linkages were designed from alkaloids of Corydaline as potential acetylcholinesterase inhibitors. Such derivatives are able to interact with both catalytic anionic site and PAS in the TcAChE. Such design may increase the flexibility and affinity of the virtual ligands to interact with both central catalytic site and peripheral site in the TcAChE more favourably. The designed analogs of tetrahydroisoquinoline derivatives as showed in Fig. 3.

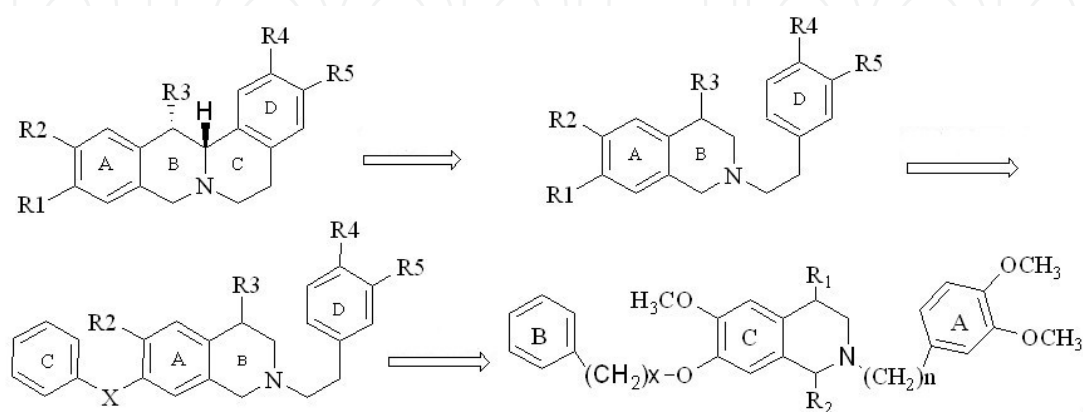


Fig. 3. The designed analogs of tetrahydroisoquinoline derivatives

2. Materials and methods

A comparison of several docking methods was carried out (Xie et al., 2006). With the purpose of investigating which docking method was more suitable for the AChE inhibitors, and the results indicated that GOLD (Jones et al., 1997) reproduced the X-ray determined conformations of known AChE inhibitors better than others. Therefore, GOLD version 3.0.1 (Jones et al., 1997) was employed to probe into the binding mode between AChE and its inhibitors.

2.1 Acquisition of the AChE conformation

The crystal structure of the complex with hAChE and ligand has not been reported yet. However, Account for the sequence identity of hAChE and TcAChE is as high as 50 percent (Wiesner et al., 2007), as the Fig. 4 shows, TcAChE that obtained from the Protein Data Bank (Berman et al., 2000), can be chosen as protein acceptor. The process of the AChE conformation is to import the crystal structure of TcAChE (1EVE) to SYBYL 7.3 (Tripos Inc, 2006), delete the ligand and water molecule in the complex by using the Built/Edit module. Then add the hydrogen atom and AMBER charges to all amino acid residues by using the Biopolymer module. Save the conformation as the format of MOL2 for docking preparation.

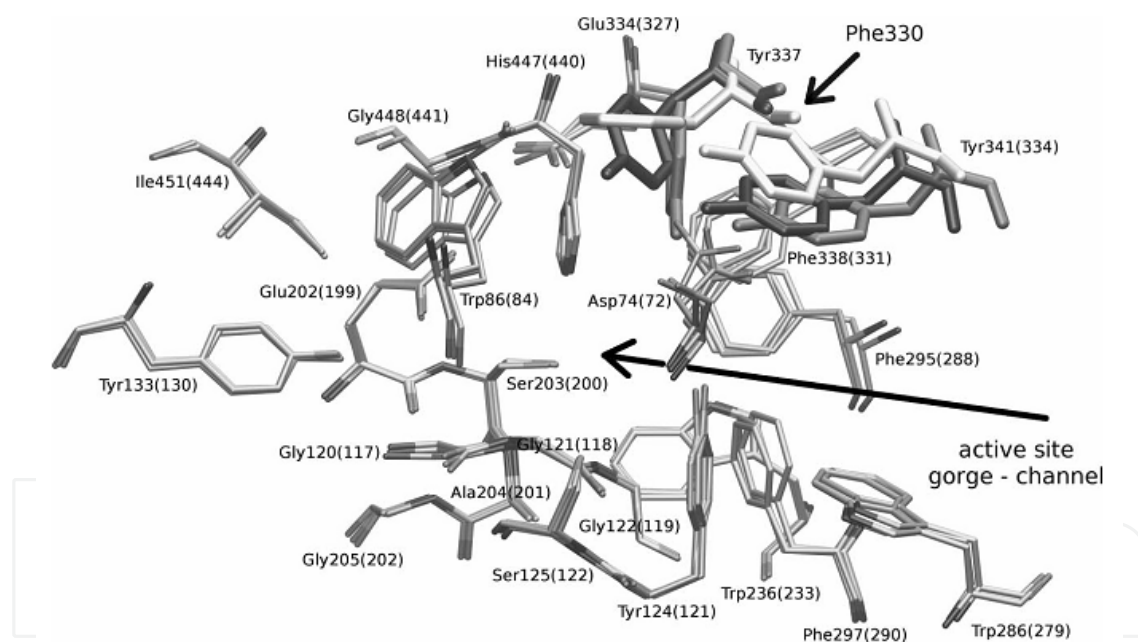


Fig. 4. 3-D alignment of human 1B41, mouse 1N5M and torpedo 1EA5 structures

2.2 Acquisition and optimization of the ligands

Corydaline and the open ring derivatives which have been designed were sketched in the Build/Edit module of SYBYL7.3. The type of the atoms and bonds in the ligands were modified to make sure they could be well- distinguished by the docking software. Then add the hydrogen atom to the ligands by using the Built/Edit module, and in the Calculation module, the charge of Gasteiger-Hukel was added to the ligands. Tripos molecular force field algorithm was chosen to modify the ligands by 3000 steps molecular mechanics

optimization, Powell was chosen as iterative algorithm, and the energy gradient termination condition of the iteration was set to 0.005 kJ/(mol · nm). The key interaction truncation value was set to 1.0 nm, dielectric constant was set to 1, and other parameters were for default. Save the ligand conformations as the format of MOL2 for docking preparation.

2.3 Molecule docking and virtual screening

The active site was defined as all the residues within 10 Å from the original ligand molecule. The default parameters of genetic algorithms (GA) were applied to search the reasonable binding conformation of these flexible ligands. The maximum number of GA runs was set to 600 for each compound. Early termination was allowed if the root mean square deviation (RMSD) of top 3 solutions are within 0.15 nm. The GOLDScore fitness function was used to evaluate the docking conformations and only energetically favourable conformations were selected for further analysis. These docked conformations were saved in MOL2 format.

2.4 Image display and data analysis

The ligand that got the highest Fitness Score in MD and the TcAChE conformation were imported into Silver at the same time, “show hydrogen bond” option was chosen to display the hydrogen bonds, “show close contacts” option was chosen to display the distance between the close atoms, and “Measure” option was chosen to calculate Hydrogen bonding Angle as well as the distance and Angle of hydrophobic interaction.

3. Results and discussion

Virtual screening showed that the scores of most derivatives were higher than that of corydaline, and the 10 open ring substances with the highest scores were mainly derived from those substituted by benzyl, methoxyl, hydroxymethyl groups, ect, and those with 2- to 7- carbon linkages. The main interactions between the ligands and the acceptor were hydrophobic interaction and hydrogen bond. The Rank and Fitness Score of the top 10 tetrahydroisoquinoline derivatives can be seen from the Table 1, in which, the Fitness score is a comprehensive evaluation of Van der Waals' force as well as hydrogen bond, considering both intramolecular and intermolecular interaction. The higher score the ligand got, represented the better affinity, the smaller IC₅₀ and the stronger inhibitory activity. The results of molecule docking and interaction analysis showed that: all of the derivatives listed in Table 1 could interact with multi-active sites in TcAChE. In instance, the compound 8a could bind with both catalytic site and PAS at the same time, that as shown in the Fig. 5(b).

Molecular modelling obtained by GOLD suggests that, in the compound 8a, the phenyl group A of the derivatives buried within the core of the enzyme binds with the catalytic site via face-to-face π -stacking interaction (distance: 3.155 Å) with Trp84. The phenyl group B reaches the peripheral site on the surface of the enzyme by face-to-face π -stacking interaction (distance: 3.225 Å) with Trp279. The protonated nitrogen atom of the tetrahydroisoquinoline moiety interacts with the phenyl group of Phe330 by cation- π interaction (distance: 4.021 Å). The phenyl group of the tetrahydroisoquinoline moiety interacts with Tyr334 by a classic parallel π - π accumulation (distance: 3.818 Å). The distance between the phenyl group A and B is 13.225 Å, which is similar to the distance that had been reported between the Trp84 and Trp279 (Harel et al., 1993).

According to the comparison of the binding mode between the Compound 8a and Corydaline, The increase has been found in both length and flexibility among the screened ligands, which guaranteed their interaction with more active sites in TcAChE, and got higher Fitness Scores and inhibitory activities.

Comp	x	n	R1	R2	Fitness score
8a	1	1	OCH ₃	H	75.41
8b	1	2	OCH ₃	H	74.63
8c	1	1	H	CH ₂ OH	72.62
8d	1	3	OCH ₃	H	72.35
8e	2	1	H	CH ₂ OH	71.47
8f	2	1	OCH ₂ CH ₃	CH ₂ OH	71.06
8g	2	1	OCH ₃	CH ₂ OH	70.86
8h	1	2	H	CH ₂ OH	70.39
8i	1	2	H	H	69.40
8j	1	1	H	H	67.62
donepezil	-	-	-	-	68.53
Corydaline	-	-	-	-	56.26

Table 1. Rank and Fitness Score of the top 10 tetrahydroisoquinoline derivatives

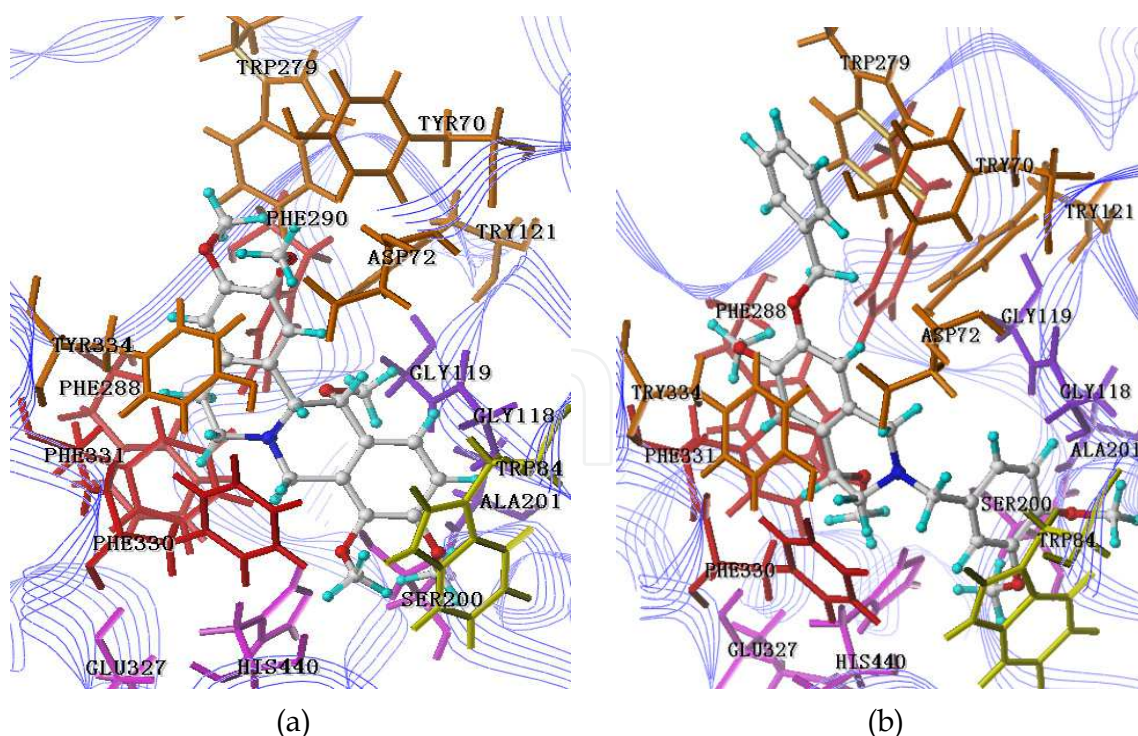


Fig. 5. Binding mode of Corydaline (a) and compound 7(b) (ball and stick) on TcAChE (stick). The active site was marked in purple, and the peripheral "anionic" site was marked in orange.

4. Conclusion

On basis of the former research, Corydaline was used as lead compound. In the reserves of Protons turn nitrogen, the C ring of Corydaline was opened in order to get a series of derivatives. The virtual ligands with the respective substituent groups of -OH, -OCH₃, benzyl etc, and different length with 2- to 7- linkages were designed from alkaloids of Corydaline as potential acetylcholinesterase inhibitors. Such derivatives are able to interact with both active site and PAS in the TcAChE. Such design may increase the flexibility and affinity of the virtual ligands to interact with both central catalytic site and peripheral site in the TcAChE more favourably.

Molecular modelling obtained by GOLD suggests that by means of virtual screening, the compounds can bind with both catalytic anionic site and PAS in the TcAChE. The modes of binding were cation- π interaction and hydrophobic interaction. The mode was significant for the further optimization of Corydaline as well as the design of the novel following drugs.

5. Acknowledgment

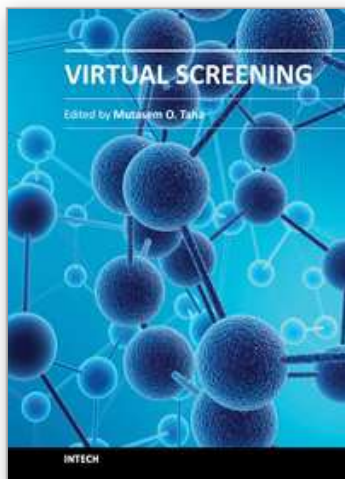
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