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Discovery of new acetylcholinesterase inhibitors with small core structures through shape-based virtual screening

Yao Chen [a,d], Zong-liang Liu [e], Ting-ming Fu [a,d], Wei Li [a], Xiao-li Xu [b,c,*], Hao-peng Sun [b,c,*]

[a] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210038, China
[b] Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China
[c] Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China
[d] Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, China
[e] School of Pharmacy, Yantai University, Yantai 264005, China

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ABSTRACT

Targeting acetylcholinesterase (AChE) using small molecule inhibitors is considered to be the most successful therapeutic strategy in the treatment of Alzheimer's disease (AD). Herein we present a shape-based virtual screening to identify new cores for the designing of AChE inhibitors. Ten active hits are identified and the most active hit, **5169-0032** and **T5369186**, showed comparable AChE inhibitory activity to tacrine. Prediction of physicochemical properties and ADME/T risk indicates their potential in druggability and safety. The two compounds provide new core and can serve as a promising fragment to design potent AChE inhibitors.

Alzheimer's disease (AD) is an age-related and progressive neurological disease leading to impairment in memory, language skills, judgment and orientation.[1] It accounts for nearly 70% of adult dementia.[2] Worldwidely, it is estimated that 40 million people suffer from AD. Worst of all, the average age of people that suffer from this disease increases, indicating the prevalence of AD will rise significantly in the next several decades.[3] Although the etiology of AD is not fully understood till now, common hallmarks, such as cholinergic dysfunction,[4] amyloid-β (Aβ) deposits[5] and τ-protein aggregation[6] are considered to play important roles in the pathophysiology of AD. Recently, with a deepening understanding of the mechanisms leading to neurodegeneration, numerous other pathogenic factors have been revealed, including oxidative stress, neuroinflammation, excitotoxicity, calcium impairment, mitochondrial dysfunction, et al.[7] These factors provides novel insights into the therapeutic strategy of AD, which may lead to efficacious drugs in future.

Although many mechanisms have been reported, there are only two classes of drugs currently available for AD treatment. One is acetylcholinesterase inhibitor (AChEI), including tacrine, donepezil, huprine, rivastigmine, galantamine, with the aim to increase acetylcholine (ACh) concentration in cholinergic synaptic clefts. The other is *N*-methyl-D-aspartate receptor (NMDAR) antagonist memantine.[8] Unfortunately, the effectiveness of AChEIs has been proved to be palliative because they are not able to delay or prevent the progression of AD.[9,10] Traditional approach such as single-target-molecule can generally only offer limited and transient benefits, therefore, it is not preferred in the treatment of AD.

Major research efforts in recent years have been devoted to the discovery and development of compounds that bind simultaneously to both the catalytic anionic site (CAS)[11,12] and the peripheral anionic site (PAS)[13,14] of AChE. This 'multi-target-directed ligands' (MTDLs) strategy is believed to enhance the inhibitory potency of AChEIs. Among all the AChEIs that have used clinically, tacrine is the first generation inhibitor. Although its clinical usage is limited by strong hepatotoxicity, it indeed shows superiority in the designing of MTDLs for several reasons: (1) the very simple structure of tacrine results in a very potent AChE inhibitory effect, indicating an ideal ligand efficiency (LE) which is a key parameter in drug design; (2) our[15–18] and others' previously efforts have proved that tacrine can endure drastically chemical optimization while retains the activity; (3) compared to other naturally originated compounds such as huprine and galantamine, the synthetic route of tacrine is much more simple, which makes it more suitable for further development. As a result, tacrine can serve as ideal template in the discovery of novel scaffold with high LE in inhibiting AChE.

* Corresponding authors. Tel.: +86 25 8586 3169.
E-mail addresses: xuxiao_li@126.com (X.-l. Xu), sunhaopeng@cpu.edu.cn (H.-p. Sun).

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To identify AChEIs with new chemical core, here we report a computational-based method that combines shape-based modeling, virtual screening, molecular docking, and prediction of physicochemical and absorption, distribution, metabolism, excretion/toxicity (ADME/T) properties (Fig. 1A).

Rapid Overlay of Chemical Structures (ROCS) is a highly efficient shape comparison application which is based on the principle that molecules will form similar shape if their volumes overlay well. Gaussian function is applied in the program to represent the molecular volume.[19] Herein, tacrine was used as the template to generate the ROCS model, in which the molecular shape of tacrine was displayed in gray shadow (Fig. 1B). The model contained hydrophobes derived from the core of tacrine. The amino group supplied the hydrogen-bond acceptor and donor feature, while the N atom on pyridine gave another hydrogen-bond acceptor.

After the generation of the model, it was applied in the virtual screening of commercial compound libraries including Chemdiv, Enamine and Specs. The shape similarity between the screened compounds and tacrine was evaluated by the combo score method, which was consisted of the shape Tanimoto coefficient and the score retrieved from the ROCS color force field, which stand for the structural complementarity between the template and the screened molecules. Briefly, the ROCS color force field describes one molecule by the spatial arrangement of chemical features including six types: hydrogen-bond donors, hydrogen-bond acceptors, hydrophobes, anions, cations, and rings. The combo score ranges from 0 to 2, the higher the score is, the more similar of a given compound is to tacrine.

Finally, 30 hits were retained and purchased from Topscience cooperation. The inhibitory rate of these compounds (at 10 μM) against *Electrophorus electricus* AChE (eeAChE) and BuChE from equine serum were tested following Ellman's method (the data of all the tested compounds were listed in Supplementary data).[20] Ten of them that exhibited over 80.0% inhibition were subsequently evaluated for the IC_{50}, while tacrine was used as positive control (Table 1 and Fig. 2). The results proved that these ten hits effectively inhibited the activity of AChE and BuChE. The best compound **5169-0032** showed even more potent activity than tacrine, with the IC_{50} of 0.03 ± 0.01 on AChE. Other hits such as **AE-641/00355019**, **T5369186**, **8003-7752** and **4817-2931** also showed comparable activity to tacrine, with the IC_{50} of 0.26 ± 0.05, 0.32 ± 0.12, 0.17 ± 0.01 and 0.30 ± 0.08 on AChE, respectively. Most of the hits have similar molecular weight (MW) to tacrine, indicating their potential in further structural modification without losing the activity.

Among all the hits, two 4-aminoquinoline compounds, **AG-687/25019010** and **T5369186,** attracted our attention. The chemical core of the two compounds was obviously different from that in tacrine. Additionally, **AG-687/25019010** exhibited good selectivity on AChE compared to BuChE.

Table 1
The inhibitory activity of the hits from virtual screening

Compound	MW	AChE IC_{50}[a] (μM)	BuChE IC_{50} (μM)
AG-687/25019010	267	0.69 ± 0.37	30.68 ± 1.44
AH-357/02177012	275	0.74 ± 0.25	2.25 ± 0.44
AE-641/00355019	204	0.26 ± 0.05	0.11 ± 0.02
AG-690/36482004	240	7.19 ± 1.43	36.15 ± 2.80
T5369186	200	0.32 ± 0.12	0.46 ± 0.09
8003-7752	188	0.17 ± 0.01	0.07 ± 0.01
3647-2435	213	1.15 ± 0.17	15.94 ± 1.51
D724-1018	214	7.41 ± 2.12	60.91 ± 56.15
5169-0032	260	0.03 ± 0.01	0.33 ± 0.13
4817-2931	320	0.30 ± 0.08	8.92 ± 2.45
Tacrine	198	0.05 ± 0.01	0.82 ± 0.12

[a] The inductivity of the compound is calculated compared to the blank control, and data are presented as mean ± SEM of five separate experiments.

To gain information on the mechanism of inhibition, we selected two compounds for kinetic studies of AChE inhibition by using Lineweaver–Burk plots, which were reciprocal rates versus reciprocal substrate concentrations for the different inhibitor concentrations resulting from the substrate–velocity curves for ChEs (Fig. 3). One was the most potent compound **5169-0032** and the other was **T5369186** that had different core from tacrine. Both the two compounds exhibited a mixed-type inhibition of AChE, for the plot showed both increased slopes (decreased V_{max}) and intercepts (higher K_m) when the concentration of the inhibitors were increased, indicating that the compounds may bind to both CAS and PAS site.

Next, the binding modes of **T5369186** and **5169-0032** to both CAS (Fig. 4A and B) and PAS (Fig. 4C and D) of AChE were predicted by using molecular docking method. The binding conformation suggested that the two compounds can insert into both the CAS and PAS of AChE, and this can support the mixed-type inhibition from kinetic study. In detail, **T5369186** formed strong π–π interactions with Phe330, Tyr334, Trp84 and His440 of the CAS of AChE. The methyl group contacted with Tyr334 and Trp84 through hydrophobic contacts. Additionally, the amino group formed a H-bond with His440, which was considered as a critical member of the catalytic triad of AChE. **5169-0032** also contacted with CAS of AChE mainly through hydrophobic and π–π stacking interactions, including Trp84, Tyr121 and Phe330. A H-bond between pyridine N of **5169-0032** and hydroxyl group of Tyr121 was observed, which improved the binding affinity of this compound to AChE.

For PAS of AChE, **T5369186** and **5169-0032** showed a similar binding pattern by inserting into the narrow groove consisted by Tyr70 and Trp279. Strong π–π stacking interactions were observed

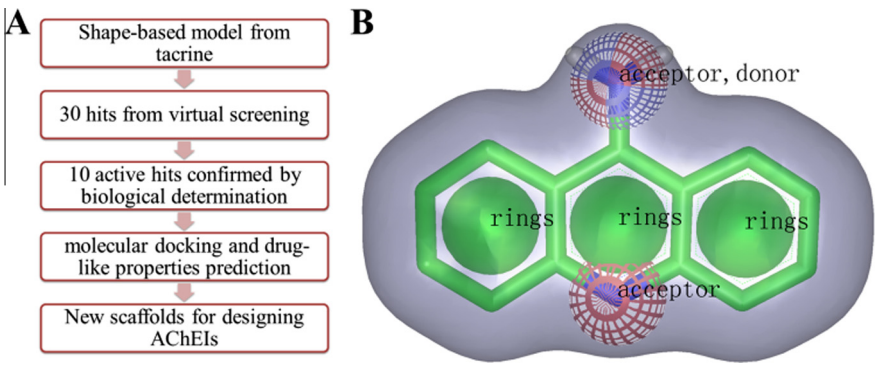

Figure 1. (A) Shape-based model generated from tacrine. (B) Identification of new AChEIs based on the shape-based model.