Accepted Manuscript

Design, synthesis, evaluation and molecular modeling study of 4-*N*-phenylaminoquinolines for Alzheimer disease treatment

Jun Zhu, Li-Ning Wang, Rong Cai, Shang-Qi Geng, Ya-Fei Dong, Yu-Ming Liu

PII: S0960-894X(19)30205-7
DOI: https://doi.org/10.1016/j.bmcl.2019.03.050
Reference: BMCL 26364

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 1 January 2019
Revised Date: 22 March 2019
Accepted Date: 30 March 2019

Please cite this article as: Zhu, J., Wang, L-N., Cai, R., Geng, S-Q., Dong, Y-F., Liu, Y-M., Design, synthesis, evaluation and molecular modeling study of 4-*N*-phenylaminoquinolines for Alzheimer disease treatment, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.03.050

Design, synthesis, evaluation and molecular modeling study of 4-*N*-phenylaminoquinolines for Alzheimer disease treatment

Jun Zhu[a], Li-Ning Wang[b], Rong Cai[a], Shang-Qi Geng[a], Ya-Fei Dong[a], Yu-Ming Liu[a,*]

[a] *Department of Pharmacy Engineering, Tianjin University of Technology, Tianjin 300384, PR China*

[b] *College of Traditional Chinese Medicine, Tianjin Univerisity of Traditional Chinese Medicine, Tianjin 300193, PR China*

* Corresponding author at: Department of Pharmacy Engineering, Tianjin University of Technology, Tianjin 300384, PR China.

E-mail address: coumarin1968@hotmail.com (Y.M. Liu).

Keywords:
Acetylcholinesterase inhibitors
Butyrylcholinesterase inhibitors
Alzheimer's disease
4-*N*-phenylaminoquinoline
Molecular modeling

ABSTRACT

Dual binding site acetylcholinesterase (AChE) inhibitors and butyrylcholinesterase (BChE) inhibitors have recently emerged as two classes of new anti-Alzheimer agents to positively modify the disease's course. In this work, a new series of 4-*N*-phenylaminoquinolines was synthesized and evaluated for their abilitites to inhibit AChE and BChE. Compound **11b** showed significant inhibitory activities on AChE and BChE with IC_{50} values of 0.86 and 2.65 μM, respectively, a lot better than that of reference drug galanthamine. Furthermore, docking study showed that compound **11b** interacted simultaneously not only with active and peripheral sites of AChE, but also with all five regions of BChE active site. These findings suggest that these derivatives could be regarded as promising starting points for further drug discovery developments.

Alzheimer's disease (AD), as a neurodegenerative disease, accounts for about 50% to 75% of the type of dementia and has seriously threatened the health of old people. With the acceleration of the aging process of the world population, the incidence of AD increases year by year, and it is estimated that the number of AD patients worldwide will exceed 100 million by 2050.[1,2] Now there is a huge market demand for AD therapeutic drugs, but there are few available drugs on the market. Therefore, it is of great realistic and social significance to accelerate the search for more effective AD drugs.

The well-known cognitive impairments observed in AD patients, mainly in memory and language, are due, among other causes, to β-amyloid (Aβ) aggregation, tau hyperphosphorylation, acetylcholine (ACh) deficiency, inflammation, and oxidative stress.[2]

Currently AChE inhibitors are the primary anti-AD drugs used in clinic, including selective AChE inhibitors donepezil and galantamine. These AChE inhibitors mainly act on the catalytic active site (CAS) at the bottom of AChE, which can relieve symptoms of mild to moderate AD patients. Nevertheless, the recent studies found that the peripheral active site (PAS), at the entrance to the AChE gorge active site, is proven to have a close relationship with both the hydrolysis of acetylcholine and the neurotoxic cascade of AD through Aβ aggregation induced by AChE,[3,4] and that dual interaction with two binding sites (CAS and PAS) leads to much better AChE inhibitory activity.[5,6] Therefore, dual binding site AChE inhibitors have currently been recognized as a new strategy to increase inhibitory activity through the enhancement of number of drug-target interactions and to prevent from Aβ aggregation.[5,6,7]

The multifaceted factors of the AD led to the development of multitarget-directed ligands (MTDLs) to act as agents for the treatment of this disease. In recent years the pivotal role played by butyrylcholinesterase (BChE), the sister enzyme of AChE, has gained increasing interest. It was found that BChE was distributed in the brain and could also degrade the neurotransmitter ACh. There was strong evidence that BChE compensated the loss of neuronal AChE in progressed AD and took over the function

of AChE when AChE production was insufficient or its activity was inhibited.[4,8] In fact, the level of AChE drops down to 90 % in advanced AD compared to the healthy brain, while the level of BChE is about doubled and tends to increase continuously.[9] This is the main reason why AD patients are resistant to AChE inhibitors. In addition, butyrylcholinesterase-knockout reduced brain deposition of Aβ in Alzheimer mouse model;[10] while BChE inhibitors could significantly reduce the formation of Aβ and Aβ precursor protein-α (APP)[11] and ameliorated cognitive dysfunction.[9] What has the extreme advantage is that BChE inhibitors have no notable deleterious physiological effects, as confirmed by more than 30 years of clinical use of the antiasthmatic drug bambuterol,[12] a prodrug of the β_2-adrenergic agonist terbutaline, which also effectively and selectively inhibits BChE, and especially haven't any significant peripheral side effects associated with BChE inhibition.[12–15] Therefore BChE is an important drug target for AD in the later stage.[15-17] Now rivastigmine is the sole powerful inhibitor of cholinesterase (ChE, id est BChE and AChE) used in the treatment of AD, which could be attributed to the long-term neglect of the role of BChE. But rivastigmine is strongly bound only with the CAS of AChE, and does not interact with the PAS of AChE. [18]

Quinoline derivatives and their analogues exhibited a variety of pharmacological activities, such as anti-tumor, anti-malaria, bacteriostatic and ChE inhibitory,[19,20] and tacrine was before used as an AChE inhibitor in the treatment of AD.[21] Recently some 4-*N*-phenylaminoquinoline analogues were synthesized and evaluated as AChE inhibitors.[22,23,24] The previous investigations in our laboratory suggested that a 4-*N*-phenylaminoquinoline derivative with pyridinium salt side chain, lead compound **1** (**Fig. 1**), had more potent effects on inhibiting AChE and BChE (IC_{50} values equal to 0.92 μM and 14.20 μM, respectively) than galanthamine.[25]

As part of our ongoing project, compound **1** was chosen for a more detailed investigation and was subjected to structural modifications to further evaluate the structure-activity relationship (SAR) of this series of compounds. Our design was based on bioisosteric replacement of pyridinium salt of compound **1** with *N*-methylbenzylamine fragment to produce new compounds, which was inspired by this

pharmacophoric unit with attractive ChE inhibition.[26–31] Furthermore, different substituents were now introduced at the 4-*N*-phenyl ring to investigate their effects on ChE inhibition.

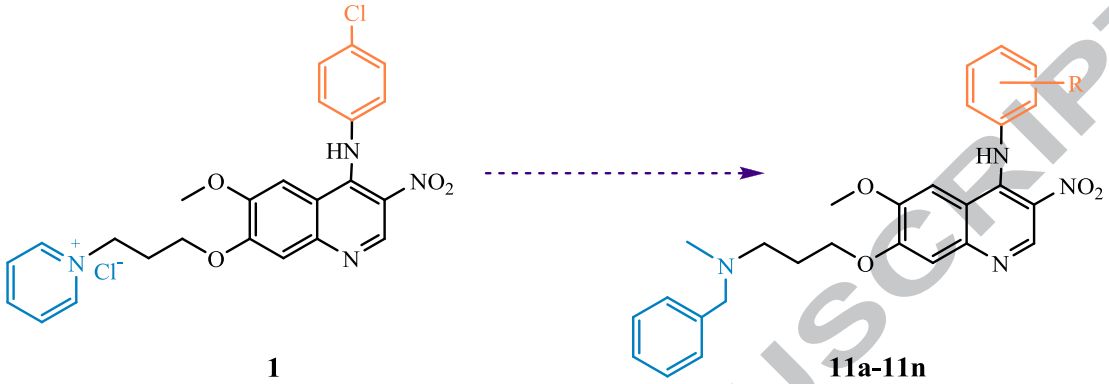
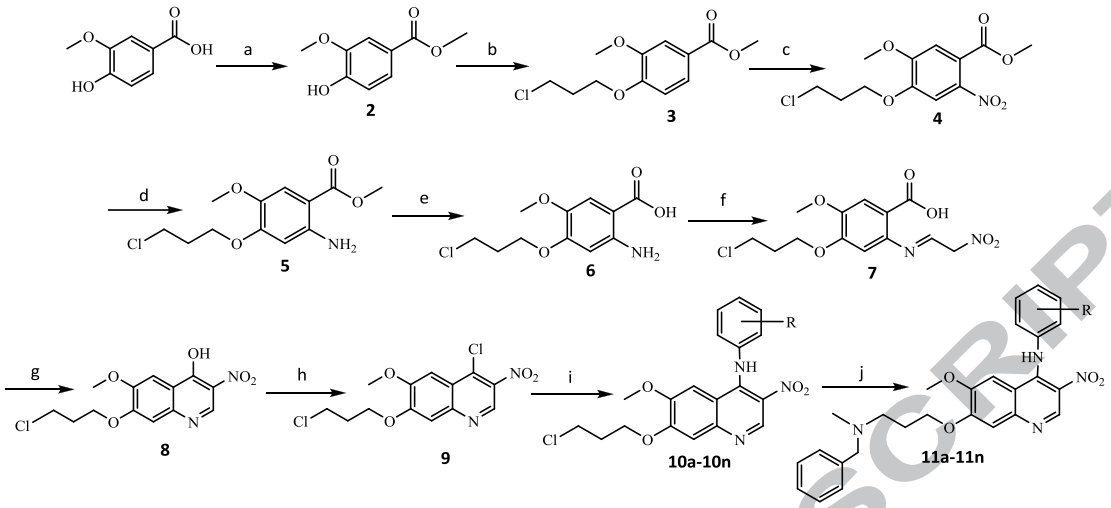


Fig. 1. Design strategy leading to compounds **11a–11n.**

According to **Scheme 1**, compounds **11a–11n** were synthesized starting from the commercially available material vanillic acid, which was esterificated using methanol to obtain compound **2**. Compound **2** was alkylated with 1-bromo-3-chloropropane in acetonitrile under basic condition to provide compound **3**, which was converted to nitro compound **4** using fuming nitric acid as nitration reagent in dichloromethane for 5 h with 86.25% yield. Compound **4** was reduced using iron powder and catalytic amounts of ammonium chloride in ethanol to obtain compound **5**, which was further hydrolyzed with NaOH to provide compound **6.** Next, intermediate **7** was formed by the reaction of compound **6** and 2-nitroacetaldoxime, which was prepared beforehand from nitromethane in the presence of NaOH.[32] Compound **7** was cyclized with acetic anhydride to yield compound **8**, which was subsequently treated with phosphorus oxychloride to afford compound **9**. Compound **9** was further reacted with the corresponding aniline, affording compounds **10a–10n**. Then, final compounds **11a–11n** were achieved upon the substitution of compound **10a–10n** with excessive *N*-methylbenzylamine, respectively. All target compounds were purified by column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and HR-ESI-MS (Supplementary material).



Scheme 1. Synthesis of compounds **11a**–**11n**. Reagents and conditions: (a) MeOH, HCl, 70°C, 12 h; (b) $Br(CH_2)_3Cl$, K_2CO_3, CH_3CN, 70°C, 10 h; (c) fuming HNO_3, CH_2Cl_2, room temperature, 5 h; (d) Fe, NH_4Cl, EtOH, reflux; (e) 5 % NaOH, EtOH, 50°C, 10 h; (f) $HON=CHCH_2NO_2$, HCl, room temperature, 18 h; (g) KOAc, Ac_2O, 15 min, reflux; (h) $POCl_3$, 70°C, 10 h; (i) corresponding aniline, isopropanol, 90°C, 8 h; (j) *N*-methylbenzylamine, CH_3CN, 10 h, reflux.

The inhibitory potencies of compounds **11a–11n** toward AChE and BChE were evaluated using Ellman's assay.[33] For comparison purposes, the IC_{50} values of lead compound **1** and the reference drug galanthamine were also considered. As shown in **Table 1**, all the tested compounds **11a–11n** turned out to successfully inhibit AChE. Among them, compounds **11a–11e**, **11g**, **11j**, **11l** and **11m** with IC_{50} values within a very close range (from 0.65 to 1.88 μM), showed comparable inhibitory activity as those of compound **1** (IC_{50} = 0.92 μM) and galanthamine (IC_{50} = 1.28 μM). Generally, the order of the inhibitory potency of these derivatives bearing different substituents was as followed: hydroxyl group > chloro group > methoxy group > methyl group > trifluoromethyl group, which implied that carrying electron-drawing substituents would have an adverse impact on their activities. On the other hand, based on the substituted position in the 4-*N*-phenyl ring, the order of inhibitory potency against AChE was: *Meta* > *Ortho* > *Para*. Interestingly, compound **11a** was the most potent AChE inhibitor, with IC_{50} value of 0.65 μM, which seemed that small aromatic moiety in quinoline scaffold would be optimal for AChE inhibitory activity.

In terms of inhibitory activity against BChE, all derivatives showed IC_{50} values higher

than those obtained for AChE inhibition. Therefore, as also seen with lead compound **1**, all the tested compounds **11a–11n** acted as selective AChE inhibitors, with selectivity index ranging from 1.10–31.75. Similarly, all *Para*-substituted derivatives in the 4-*N*-phenyl ring were clearly less potent against BChE than *Meta*-substituted or *Ortho*-substituted ones, however, polysubstitution (such as compound **11e**) seemed to be very detrimental to their activities. Notably, compound **11b**, as the most active BChE inhibitor, was 5-fold more potent than compound **1**, and 9-fold more potent than the reference drug galanthamine.

Table 1

Inhibition of cholinesterases activity and selectivity index (SI) of compounds.

Compound	R	AChE IC_{50} (μM)[a]	BChE IC_{50} (μM)[a]	SI[b]
1		0.92 ± 0.05[c]	14.20 ± 0.96[c]	15.43
11a	H	0.65 ± 0.01	10.11 ± 1.76	15.55
11b	2-Cl	0.86 ± 0.03	2.65 ± 0.52	3.08
11c	3-Cl	0.83 ± 0.02	15.17 ± 2.23	18.27
11d	4-Cl	1.88 ± 0.04	17.45 ± 2.77	9.28
11e	2,4-di-Cl	1.85 ± 0.09	58.74 ± 5.15	31.75
11f	2-CH_3	10.13 ± 1.29	29.71 ± 1.13	2.93
11g	3-CH_3	1.86 ± 0.01	2.78 ± 0.67	1.49
11h	4-CH_3	11.84 ± 1.65	73.59 ± 4.57	6.22
11i	2-OCH_3	7.90 ± 1.64	8.67 ± 0.45	1.10
11j	3-OCH_3	1.72 ± 0.01	9.19 ± 0.36	5.34
11k	4-OCH_3	10.87 ± 1.44	50.37 ± 1.82	4.63
11l	3-OH	0.71 ± 0.05	10.77 ± 1.25	15.16
11m	4-OH	0.74 ± 0.01	17.38 ± 2.49	23.48
11n	3-CF_3	7.78 ± 0.74	55.41 ± 3.46	7.12
Galanthamine		1.28 ± 0.01	24.41 ± 2.01	19.07

[a] IC_{50} values are at least from three independent experiments and are expressed as the means ± SD.

[b] SI for AChE = IC_{50} BChE/IC_{50} AChE.

[c] Data from Ref. 25.

Even though a somewhat lower AChE inhibitory activity compared with the most potent AChE inhibitor **11a**, compound **11b** was endowed with its highest BChE inhibitory potency. To clarify the interaction mode in the active sites of AChE, molecular docking simulations (Supplementary material) were performed for compounds **11b** using the CDOCKER in Discovery Studio 3.0 software (**Fig. 2** and **Fig.**

3). The crystal complex of AChE with galanthamine (PDB: 1DX6) was selected for the docking study. *N*-methylbenzylamine fragment of compound **11b** interacted with the CAS through π-π-stacked interaction with Trp84. Additionally, compound **11b** displayed a π-alkyl interaction between the 4-*N*-phenyl ring and the sec-butyl moiety of Ile287, which was adjacent to acyl binding site Phe288. Furthermore, compound **11b** was involved in a series of interactions with the majority of amino acid residues in the PAS: *ortho*-chloro group at the 4-*N*-phenyl ring established a π-alkyl interaction with Trp279 and an alkyl interaction with Leu282; nitro group formed a π-cation and a π-anion interactions both with Trp279; quinoline moiety made a carbon hydrogen bond with Tyr70 and three π-π-stacked interactions with Trp279 and Tyr121; and the propyl ether fragment attached to quinoline skeleton showed a carbon hydrogen bond with Asp72. This is known to be an important feature for the inhibition of AChE-induced Aβ aggregation.[34]

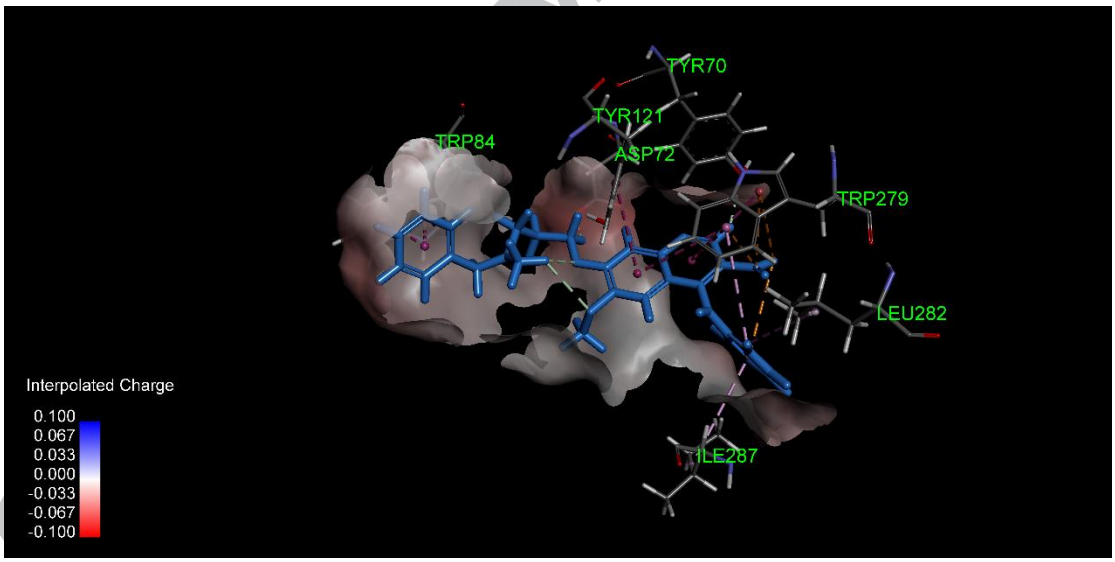


Fig. 2. 3D binding mode of compound **11b** with AChE.

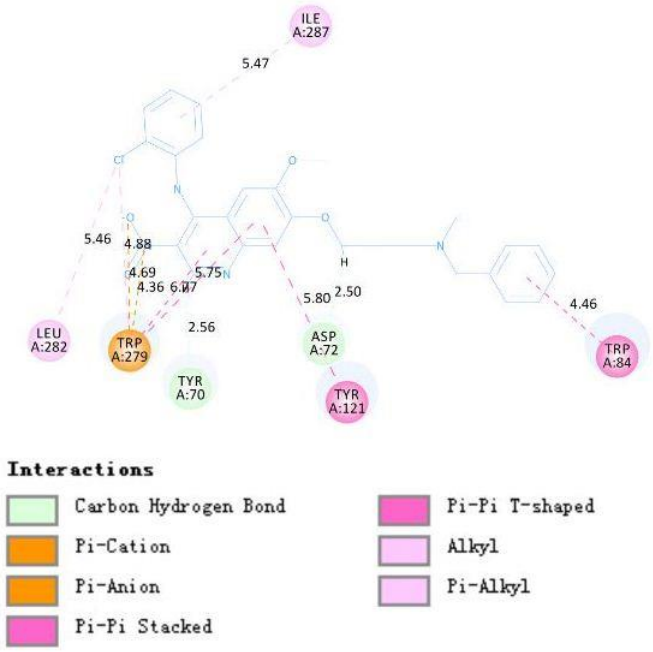


Fig. 3. 2D diagram of the ligand-protein interaction for compound **11b** with AChE.

The interaction of compound **11b** with BChE (PDB code: 4BDS) was also carried out (Supplementary material). The modeling results (**Fig. 4** and **Fig. 5**) suggested that compound **11b** was bound to the residues Trp82 from the choline binding site, Gly117 from the oxyanion hole, Phe329 from the acyl pocket, and His438 from catalytic triad, but also bound to Ser287 residue and two PAS amino acid residues such as Asp70 and Pro285, via one hydrogen bond, three charge interactions, and eight hydrophobic interactions, which interacted with all five regions of BChE active site.[35,36] Significantly, the presence of *ortho*-chloro group at the 4-*N*-phenyl ring was important for BChE inhibition, as the chlorine atom was stabilized by the formation of two π–alkyl interactions both with the electron density of the imidazole ring of His438 and the benzene ring of Trp82. Instead, the lower inhibitory potency of compounds **11c** and **11d** compared with compound **11b** may be ascribed to less favorable specific interactions in the binding site, which separately contain *Meta*-chloro group or *Para*-chloro group at the 4-*N*-phenyl ring.

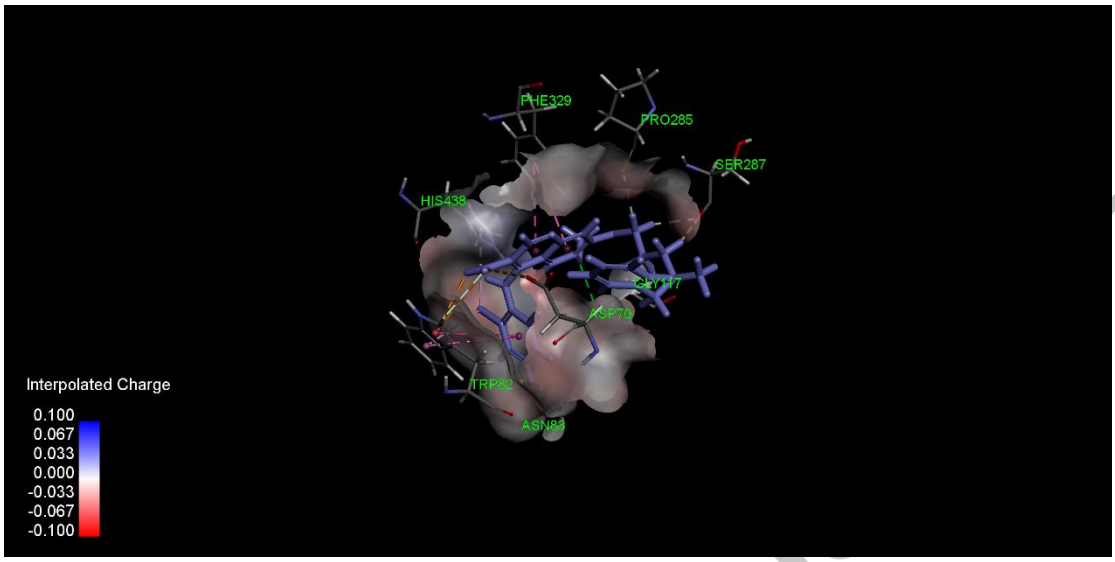


Fig. 4. 3D binding mode of compound **11b** with BChE.

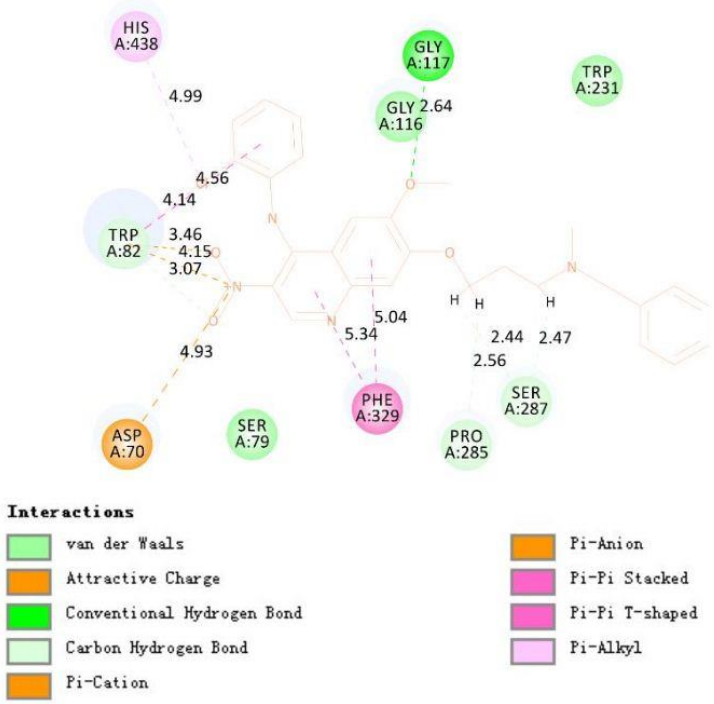


Fig. 5. 2D diagram of the ligand-protein interaction for compound **11b** with BChE.

As a potential compound for treatment of AD, lipophilicity (log*P*) was thought as an important physical chemistry parameter to valuate or predict the ability to cross blood brain barrier (BBB). It was reported that the log*P* with appropriate central nervous system (CNS) penetration was within the range of 2–5.[37] After calculated using ChemDraw Ultra 11.0, the log*P* value of active compound **11b** was 4.58, which

indicated that the active compounds had sufficient lipophilicity to pass the BBB in vivo. Polar surface area (PSA) is another important predictor for BBB. Generally, drugs aimed at the CNS tends to have lower PSA than 95 $Å^2$.[37] After assessed using Discovery Studio 2017 R2, the PSA value for compound **11b** was 88.107, which also supported its ability to penetrate the BBB. As p-glycoprotein is also closely related to BBB penetration, it is now underway in our laboratory to evaluate whether compound **11b** is a substrate or an inhibitor of P-glycoprotein.

In summary, a new series of 4-*N*-phenylaminoquinoline derivatives was designed, synthesized, and evaluated in vitro for their ability to inhibit AChE and BChE. Biological assays demonstrated that compound **11b** is a dual AChE and BChE inhibitor with IC_{50} values (0.86 and 2.65 μM), which are a lot better than that of the commercially available drug galanthamine. Molecular modelling studies suggested not only the ability of the *N*-methylbenzylamine fragment of **11b** to establish interactions with the CAS residue Trp84 but also the ability to establish interactions with the majority of amino acid residues in the PAS, leading to the speculation of a possible reduction of AChE-induced Aβ aggregation. On the other hand, as treatment with a selective AChE inhibitor reduced AChE activity, BChE could compensate for the lack of AChE in the late stages of AD. So more importantly, compound **11b**, being also highly active on BChE, could shift to inhibiting this enzyme during the progression of the disease. Finally, the comprehensive analysis of SARs indicated that compound **11g** also showed rather better activities simultaneously on both AChE and BChE than the rest. Further studies on structural optimization and Aβ aggregation inhibition about these derivatives are still in progress.

Acknowledgment

This work was supported by the Training Project of Innovation Team of Colleges and Universities in Tianjin (TD13-5020).

References

1. Dighe SN, Deora GS, Mora EDl, et al. Discovery and structure-activity relationships of a

highly selective butyrylcholinesterase inhibitor by structure-based virtual screening. *J Med Chem*. 2016;59(16):7683−7689.

2. Sawatzky E, Wehle S, Kling B, et al. Discovery of highly selective and nanomolar carbamate-based butyrylcholinesterase inhibitors by rational investigation into their inhibition mode. *J Med Chem.* 2016;59(5):2067−2082.
3. Basiri A, Xiao M, McCarthy A, et al. Design and synthesis of new piperidone grafted acetylcholinesterase inhibitors. *Bioorg Med Chem Lett.* 2017;27(2):228−231.
4. Decroocq C, Stauffert F, Pamlard O, et al. Iminosugars as a new class of cholinesterase inhibitors. *Bioorg Med Chem Lett*. 2015;25(4):830−833.
5. Najafi Z, Mahdavi M, Saeedi M, et al. Novel tacrine-1,2,3-triazole hybrids: In vitro, in vivo biological evaluation and docking study of cholinesterase inhibitors. *Eur J Med Chem*. 2017;125:1200−1212.
6. Sang Z, Wang K, Wang H, et al. Design, synthesis and biological evaluation of phthalimide-alkylamine derivatives as balanced multifunctional cholinesterase and monoamine oxidase-B inhibitors for the treatment of Alzheimer's disease. *Bioorg Med Chem Lett.* 2017;27(22):5053−5059.
7. Almeida JR, Figueiro M, Almeida WP, et al. Discovery of novel dual acetylcholinesterase inhibitors with antifibrillogenic activity related to Alzheimer's disease. *Future Med Chem*. 2018;10(9):1037−1053.
8. Wang L, Wang Y, Tian Y, et al. Design, synthesis, biological evaluation, and molecular modeling studies of chalcone-rivastigmine hybrids as cholinesterase inhibitors. *Bioorg Med Chem*. 2017;25(1):360−371.
9. Furukawa-Hibi Y, Alkam T, Nitta A, *et al*. Butyrylcholinesterase inhibitors ameliorate cognitive dysfunction induced by amyloid-β peptide in mice. *Behav Brain Res.* 2011;225(1):222−229.
10. Reid GA, Darvesh S. Butyrylcholinesterase-knockout reduces brain deposition of fibrillar β-amyloid in an Alzheimer mouse model. *Neuroscience*. 2015;298:424−435.
11. Greig NH, Utsuki T, Ingram DK, et al. Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β-amyloid peptide in rodent. *Proc Natl Acad Sci USA*. 2005;102(47): 17213−17218.
12. Wu J, Tian Y, Wang S, et al. Design, synthesis and biological evaluation of bambuterol analogues as novel inhibitors of butyrylcholinesterase. *Eur J Med Chem.* 2017;126:61−71.
13. Makhaeva GF, Boltneva NP, Lushchekina SV, et al. Synthesis, molecular docking and biological evaluation of N,N-disubstituted 2-aminothiazolines as a new class of butyrylcholinesterase and carboxylesterase inhibitors. *Bioorg Med Chem*. 2016;24(5):1050−1062.
14. Brus B, Košak U, Turk S, et al. Discovery, biological evaluation, and crystal structure of a novel nanomolar selective butyrylcholinesterase inhibitor. *J Med Chem.* 2014;57(19):8167−8179.
15. Rahim F, Javed MT, Ullah H, et al. Synthesis, molecular docking, acetylcholinesterase and butyrylcholinesterase inhibitory potential of thiazole analogs as new inhibitors for Alzheimer disease. *Bioorg Med.* 2015;62:106−116.
16. Si W, Zhang T, Zhang L, et al. Design, synthesis and bioactivity of novel phthalimide derivatives as acetylcholinesterase inhibitors. *Bioorg Med Chem Lett*. 2016;26(9):2380−2382.

17. Park B, Nam JH, Kim JH, et al. 3,4-Dihydroquinazoline derivatives inhibit the activities of cholinesterase enzymes. *Bioorg Med Chem Lett.* 2017;27(5):1179−1185.
18. Shaikh S, Verma A, Siddiqui S, et al. Current acetylcholinesterase-inhibitors: a neuroinformatics perspective. *CNS Neurol Disord – Dr*. 2014;13(3):391−401.
19. Wang L, Esteban G, Ojima M, et al. Donepezil + propargylamine + 8-hydroxyquinoline hybrids as new multifunctional metal-chelators, ChE and MAO inhibitors for the potential treatment of Alzheimer's disease. *Eur J Med Chem.* 2014;80:543−561.
20. Wang XQ, Xia CL, Chen SB, et al. Design, synthesis, and biological evaluation of 2-arylethenylquinoline derivatives as multifunctional agents for the treatment of Alzheimer's disease. *Eur J Med Chem.* 2015;89: 349−361.
21. Boulebd H, Ismaili L, Martin H, et al. New (benz)imidazolopyridino tacrines as nonhepatotoxic, cholinesterase inhibitors for Alzheimer disease. *Future Med Chem*. 2017;9(8):723–729.
22. Go ML, Ngiam TL, Lim LY. Anticholinesterase activity of 4-aminoquinolines related to amodiaquine and their respective N-oxides. *Eur J Med Chem.* 1986;21(1):81−85.
23. Najafi Z, Saeedi M, Mahdavi M, et al. Design and synthesis of novel anti-Alzheimer's agents: Acridine-chromenone and quinoline-chromenone hybrids. *Bioorg Chem.* 2016;67:84−94.
24. Mantoani SP, Chierrito TPC, Vilela AFL, et al. Novel triazole-quinoline derivatives as selective dual binding site acetylcholinesterase inhibitors. *Molecules.* 2016;21(2):193/1−12.
25. Liu Y, Tian L, Hu D, et al. Syntheses and anti-cholinesterase activity of 4-N-phenylaminoquinoline derivatives. *Chem J Chinese Universities.* 2017;38(3):392−397.
26. Rizzo S, Bartolini M, Ceccarini L, et al. Targeting Alzheimer's disease: novel indanone hybrids bearing a pharmacophoric fragment of AP2238. *Bioorg Med Chem*. 2010;18(5):1749–1760.
27. Belluti F, Bartolini M, Bottegoni G, et al. Benzophenone-based derivatives: a novel series of potent and selective dual inhibitors of acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation. *Eur J Med Chem*. 2011;46:1682−1693.
28. Guzior N, Bajda M, Rakoczy J, et al. Isoindoline-1,3-dione derivatives targeting cholinesterases: design, synthesis and biological evaluation of potential anti-Alzheimer's agents. *Bioorg Med Chem*. 2015;23(7): 1629–1637.
29. Estrada M, Herrera-Arozamena C, Pérez C, et al. New cinnamic–N-benzylpiperidine and cinnamic–N,N-dibenzyl(N-methyl)amine hybrids as Alzheimer-directed multitarget drugs with antioxidant, cholinergic, neuroprotective and neurogenic properties. *Eur J Med Chem*. 2016;121:376−386.
30. Catto M, Pisani L, Leonetti F, et al. Design, synthesis and biological evaluation of coumarin alkylamines as potent and selective dual binding site inhibitors of acetylcholinesterase. *Bioorg Med Chem.* 2013;21(1):146–152.
31. Sang Z, Qiang X, Li Y, et al. Design, synthesis and evaluation of scutellarein-*O*-acetamidoalkylbenzylamines as potential multifunctional agents for the treatment of Alzheimer's disease. *Eur J Med Chem.* 2017;135: 307−323.
32. Kokatla HP, Yoo E, Salunke DB, et al. Toll-like receptor-8 agonistic activities in C2, C4, and C8 modified thiazolo[4,5-c]quinolones. *Org Biomol Chem.* 2013;11(7):1179–1198.
33. Evaluation of AChE & BuChE inhibition: Stock solutions of compounds (10 mM) were prepared in DMSO and diluted with pH 8.0 phosphate buffer. AChE (EC 3.1.1.7, from electric

eel), BChE (EC 3.1.1.8, from equine serum), Acetylthiocholine iodide, butyrylthiocholine iodide, and 5,5'-dithiobis-(2-nitrobenzoic)acid (DNTB) were purchased from Sigma-Aldrich. To determine the percent of inhibition, 20 µl of the test compound, 20 µl of enzyme solution (0.05 U/ml) and 10 µl of DTNB 10 mM were mixed in 140 µl sodium phosphate buffer 0.1 M (pH 8). After 15 min of incubation at 25°C, 20 µl substrate (7.5 mM) was added. Afterward, absorption was measured using BioTek Epoch microplate reader at 412 nm. Each concentration was analyzed in triplicate. The percentage of inhibition was calculated by comparing the rate of hydrolysis with or without an inhibitor. IC50 values were determined with Origin 8.0 software.

34. Rampa A, Montanari S, Pruccoli L, et al. Chalcone-based carbamates for Alzheimer's disease treatment. *Future Med Chem*. 2017;9(8):749–764.
35. Shen YF, Chen GH, Lin SH, Lin G. Molecular modeling and docking calculations of 4-acyloxy-biphenyl-4'-N-butylcarbamates as potential inhibitors of human butyrylcholinesterase. *Can J Chem*. 2016;94(1):72–77.
36. Brus B, Košak U, Turk S, et al. Discovery, biological evaluation, and crystal structure of a novel nanomolar selective butyrylcholinesterase inhibitor. *J Med Chem*. 2014;57(19):8167−8179.
37. Jain P, Wadhwa PK, Rohilla S, et al. Rational design, synthesis and in vitro evaluation of allylidene hydrazinecarboximidamide derivatives as BACE-1 inhibitors. *Bioorg Med Chem Lett*. 2016;26(1):33−37.

Design, synthesis, evaluation and molecular modeling study of 4-*N*-phenylaminoquinolines for Alzheimer disease treatment

Jun Zhu, Li-Ning Wang, Rong Cai, Shang-Qi Geng, Ya-Fei Dong, Yu-Ming Liu