HHS Public Access

Author manuscript

Chem Biol Interact. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Chem Biol Interact. 2019 August 01; 308: 372–376. doi:10.1016/j.cbi.2019.05.051.

Structure-based virtual screening leading to discovery of highly selective butyrylcholinesterase inhibitors with solanaceous alkaloid scaffolds

Shuo Zhou[1,2], **Yaxia Yuan**[1,2], **Fang Zheng**[1,2,*], and **Chang-Guo Zhan**[1,2,*]

[1]Molecular Modeling and Biopharmaceutical Center, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536

[2]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536

Abstract

According to recent research advance, it is interesting to identify new, potent and selective inhibitors of human butyrylcholinesterase (BChE) for therapeutic treatment of both the Alzheimer's disease (AD) and heroin abuse. In this study, we carried out a structure-based virtual screening followed by *in vitro* activity assays, with the goal to identify new inhibitors that are selective for BChE over acetylcholinesterase (AChE). As a result, a set of new, selective inhibitors of human BChE were identified from natural products with solanaceous alkaloid scaffolds. The most active one of the natural products (compound **1**) identified has an IC_{50} of 16.8 nM against BChE. It has been demonstrated that the desirable selectivity of these inhibitors for BChE over AChE is mainly controlled by three key residues in the active site cavity, *i.e.* residues Q119, A277, and A328 in BChE *versus* the respective residues Y124, W286, and Y337 in AChE. Based on this structural insight, future rational design of new, potent and selective BChE inhibitors may focus on these key structural differences in the active site cavity.

Introduction

Cholinesterases, including both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are among the well-known targets for treatment of Alzheimer's disease (AD), the most serious neurodegenerative disease that has affected about 30 million people [1]. In fact, the first four drugs that have ever been approved by the FDA for the AD treatment are

*To whom correspondence may be addressed. fzhen2@uky.edu (F.Z.); zhan@uky.edu (C.-G.Z.). **Correspondence to:** Chang-Guo Zhan, Ph.D., *Director*, Molecular Modeling and Biopharmaceutical Center (MMBC), *Director*, Chemoinformatics and Drug Design Core of CPRI, *University Research Professor, Endowed College of Pharmacy Professor in Pharmaceutical Sciences, Professor*, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536, Phone: 859-323-3943, FAX: 859-257-7585, zhan@uky.edu.

Additional Information

Competing Financial Interests statement: The authors declare that there is no conflict of interest for this work.

cholinesterase inhibitors that either inhibit both AChE and BChE (tacrine, doneperzil, and rivastigmine) or selectively inhibit AChE (galantamine). However, studies reported in recent years have revealed that BChE might be a better target compared to AChE for the AD treatment [2–4]. In other words, a BChE-selective inhibitor might be more promising than an AChE inhibitor for the AD treatment [2–14].

In addition, in our recently reported study [15], we proposed and validated a new therapeutic strategy for heroin toxicity treatment by using a selective BChE inhibitor to block heroin activation. It has been demonstrated that a selective BChE inhibitor can be used to significantly attenuate the heroin-induced toxicity and physiological effects [15]. Hence, it is interesting to develop potent and selective BChE inhibitors in drug development for therapeutic treatment of both the AD and heroin abuse.

In fact, a variety of cholinesterase inhibitors have been reported in literature, with various scaffolds. Most of reported cholinesterase inhibitors are either not sufficiently potent for BChE [16–27] or almost equally potent for both AChE and BChE [25, 28–41]. For some other BChE inhibitors reported, the selectivity is unknown (not tested at all) [42, 43]. Few types of cholinesterase inhibitors have promising potency and selectivity for BChE over AChE [12, 17, 25, 38, 44–56]. Some BChE inhibitors were also identified through virtual screening methods but either their activity was mediocre [57] or their selectivity over AChE was not reported [58, 59]. It is highly desired to identify new, potent and selective BChE inhibitors as options for further drug development.

Here we report the identification of a set of new, selective BChE inhibitors from natural products with solanaceous alkaloid scaffolds through structure-based virtual screening and *in vitro* activity assays.

Materials and methods

Structure-based virtual screening.

Our virtual screening was performed on the Development Therapeutics Program (DTP) Release 4 compound library including ~265,000 compounds available at the National Cancer Institute (NCI) (https://cactus.nci.nih.gov/download/nci/) by using the X-ray crystal structures of human BChE (PDB entry 4BDS) [60] and AChE (PDB entry 4EY5) [61]. During the virtual screening, Autodock Vina 1.1.2 software [62] was used to search for the optimum binding conformation for each compound in the NCI compound library. To minimize the searching area, a 15 Å×15 Å×15 Å box containing the active site of BChE or AChE was chosen as the target binding site. The protein was set rigid and all the water molecules in the original crystal structure were removed prior to molecular docking. The default settings of the Autodock Vina was used and no other parameters were modified. The compounds were then ranked by their binding free energies (docking score) with BChE. Within the top-ranked compounds, only the compounds predicted to have a positive binding free energy with AChE will be selected and ordered from the NIH DTP program. All the binding modes were then investigated and visualized using the PyMol [63].

In vitro activity tests.

The top compounds selected from the virtual screening were first ordered from the NCI DTP program and assayed for their inhibitory activity against BChE. The most compromising compounds (**1** to **3**) were also ordered from Sigma-Aldrich (St Louis, MO) for comparison and verification. Both wild-type AChE and BChE were expressed and purified based on our previous reported protocol [64]. For activity determination, we followed the original Ellman's protocol, as described in detail in our previous report [64]. The concentration of substrate butyrylthiocholine for BChE (or acetylthiocholine for AChE) was at 10 μM. The tested compounds were first dissolved in DMSO and the final DMSO concentration was at 1%.

Results and discussion

Based on the structure-based virtual screening, we selected a set of 10 compounds (**1** to **10** depicted in Figure 1) that were predicted to bind with BChE only, without binding with AChE. All these compounds are natural products with solanaceous alkaloid scaffolds. The binding free energies calculated for AChE binding with compounds **1** to **10** ranged from +4.5 kcal/mol to +17 kcal/mol; the positive binding free energy means that the free energy of the protein-ligand complex is higher than the total free energy of the separated protein and ligand. This is because BChE has a relatively larger active site cavity than AChE such that these larger molecules can only fit the BChE active site cavity, whereas the AChE active site cavity is not large enough to accommodate these molecules (see below for the detailed binding structures).

Next, the computationally selected compounds **1** to **10** (ordered from the NCI DTP) were assayed for their inhibitory activity against human BChE. They were assayed first for their inhibitory activity at a concentration of 5 μM. As seen in Table 1, these compounds at 5 μM inhibited the BChE activity by 8-100%. The top-3 compounds (**1** to **3**) inhibited BChE by at least 95%. The most active compounds (**1** to **3**) were tested further for the dose-dependent inhibition in order to determine their IC_{50} values (see Figure 2 and Table 1) against BChE. As seen in Table 1, we obtained IC_{50} = 16.8 nM, 346 nM, and 391 nM for compounds **1** to **3**, respectively. Similar results were also obtained from the use of compounds **1** to **3** ordered from Sigma-Aldrich (St Louis, MO).

Interestingly, according to our detailed literature search, compounds **1** to **3** were tested for their inhibitory activity against AChE by Roddick *et al.* [65], and they demonstrated that none of these compounds (**1** to **3**) at a high concentration of 100 μM had significant inhibition against AChE. According to our own assays in this study, at a concentration of 5 μM, compounds **1** to **3** from Sigma-Aldrich (St Louis, MO) inhibited the AChE activity by only about 10%, 19%, and 2%, respectively (see Table 1). To the best of our knowledge, we have not found any report of testing these compounds against BChE. Taking all of these experimental data together, we can conclude that compounds **1** to **3** are indeed selective inhibitors of BChE, which is consistent with the aforementioned prediction from computational screening.

Depicted in Figure 3 are the docked binding structures of BChE with the most active compounds (**1** to **3**) identified. According to the docked binding structures with BChE, the inhibitor (compound **1** or **2** or **3**) stays in a mainly hydrophobic environment, but having a favorable hydrogen bond (HB) between the hydroxyl group of the inhibitor and the backbone oxygen of amino-acid residue H438. The HB with compound **1** is the strongest (with the shortest O···H distance of 1.956 Å), explaining why compound **1** is the most potent inhibitor of BChE with IC_{50} = 16.8 nM within these three inhibitors. With these three inhibitors, their order of the HB strengths (see Figure 2) is consistent with their order of the IC_{50} values.

Panels B, D, and F of Figure 3 also show why the AChE active site cavity cannot accommodate any of these compounds. Specifically, panels A, C, and E show the docked favorable binding structures of BChE with compounds **1**, **2**, and **3**, respectively. The corresponding unfavorable interactions of the compounds with AChE (after BChE is replaced with AChE) are depicted in panels B, D, and F, respectively. For the major differences between BChE and AChE in the protein-ligand interactions, residues Q119, A277, and A328 in BChE are replaced with Y124, W286, and Y337, respectively, in AChE. In the X-ray crystal structures of AChE-inhibitor complexes [66, 67], these residues (Y124, W286, and Y337) also played important roles in AChE binding with the inhibitors through hydrophobic interactions. Possible clash with any of these residues is expected to greatly impair the binding of the compound with AChE. According to our molecular modeling studies (see Figure 3), the compound (**1** or **2** or **3**) has clash with the side chains of Y124, W286, and Y337 in AChE, explaining why these compounds are selective for BChE over AChE for their inhibitory activities.

Conclusion

Through combined structure-based virtual screening and *in vitro* activity assays, we have successfully identified a set of new, selective inhibitors of human BChE from natural products with solanaceous alkaloid scaffolds. The most potent BChE inhibitor (compound **1**) identified has an IC_{50} of 16.8 nM against BChE. These interesting outcomes suggest that natural products may be used as a promising resource in our future search of potent and selective inhibitors of BChE for BChE-based drug discovery.

Notably, the selectivity of these compounds for BChE over AChE is mainly controlled by three key residues in the active site cavity, *i.e.* residues Q119, A277, and A328 in BChE *versus* the respective residues Y124, W286, and Y337 in AChE (all with relatively larger side chains). In light of this structural insight, it is interesting to focus on these key structural differences in future rational design of new, potent and selective BChE inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health (NIH grants UH2/UH3 DA041115, R01 DA035552, R01 DA032910, R01 DA013930, and R01 DA025100) and the National Science Foundation (NSF grant CHE-1111761).

References

[1]. Dolgin E, How to defeat dementia, Nature News, 539 (2016) 156.

[2]. Chatonnet A, Lockridge O, Comparison of butyrylcholinesterase and acetylcholinesterase, Biochem J, 260 (1989) 625–634. [PubMed: 2669736]

[3]. Li B, Duysen EG, Carlson M, Lockridge O, The butyrylcholinesterase knockout mouse as a model for human butyrylcholinesterase deficiency, Journal of Pharmacology and Experimental Therapeutics, 324 (2008) 1146–1154. [PubMed: 18056867]

[4]. Manoharan I, Boopathy R, Darvesh S, Lockridge O, A medical health report on individuals with silent butyrylcholinesterase in the Vysya community of India, Clin Chim Acta, 378 (2007) 128–135. [PubMed: 17182021]

[5]. Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry T, Lee B, Ingram DK, Lahiri DK, A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase, Curr Med Res Opin, 17 (2001) 159–165. [PubMed: 11900310]

[6]. Mushtaq G, Greig NH, Khan JA, Kamal MA, Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus, CNS Neurol Disord Drug Targets, 13 (2014) 1432–1439. [PubMed: 25345511]

[7]. Giacobini E, Cholinergic function and Alzheimer's disease, International Journal of Geriatric Psychiatry, 18 (2003) S1–S5. [PubMed: 12973744]

[8]. Ciro A, Park J, Burkhard G, Yan N, Geula C, Biochemical differentiation of cholinesterases from normal and Alzheimer's disease cortex, Current Alzheimer Research, 9 (2012) 138–143.

[9]. Greig NH, Utsuki T, Ingram DK, Wang Y, Pepeu G, Scali C, Yu Q-S, Mamczarz J, Holloway HW, Giordano T, Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β-amyloid peptide in rodent, Proceedings of the National Academy of Sciences of the United States of America, 102 (2005) 17213–17218. [PubMed: 16275899]

[10]. PERRY EK, Perry R, Blessed G, Tomlinson B, Changes in brain cholinesterases in senile dementia of Alzheimer type, Neuropathology and applied neurobiology, 4 (1978) 273–277. [PubMed: 703927]

[11]. Davies P, Maloney A, Selective loss of central cholinergic neurons in Alzheimer's disease, The Lancet, 308 (1976) 1403.

[12]. Kosak U, Brus B, Knez D, Sink R, Zakelj S, Trontelj J, Pislar A, Slenc J, Gobec M, Zivin M, Tratnjek L, Perse M, Salat K, Podkowa A, Filipek B, Nachon F, Brazzolotto X, Wieckowska A, Malawska B, Stojan J, Rascan IM, Kos J, Coquelle N, Colletier JP, Gobec S, Development of an in-vivo active reversible butyrylcholinesterase inhibitor, Sci Rep, 6 (2016) 39495. [PubMed: 28000737]

[13]. Furukawa-Hibi Y, Alkam T, Nitta A, Matsuyama A, Mizoguchi H, Suzuki K, Moussaoui S, Yu Q-S, Greig NH, Nagai T, Butyrylcholinesterase inhibitors ameliorate cognitive dysfunction induced by amyloid-β peptide in mice, Behavioural brain research, 225 (2011) 222–229. [PubMed: 21820013]

[14]. Hartmann J, Kiewert C, Duysen EG, Lockridge O, Greig NH, Klein J, Excessive hippocampal acetylcholine levels in acetylcholinesterase □ deficient mice are moderated by butyrylcholinesterase activity, Journal of neurochemistry, 100 (2007) 1421–1429. [PubMed: 17212694]

[15]. Zhang T, Zheng X, Kim K, Zheng F, Zhan C-G, Attenuate heroin's toxicity and physiological effects by blocking its activation, Cell Chem. Biol (submitted), (2018).

[16]. Schott Y, Decker M, Rommelspacher H, Lehmann J, 6-Hydroxy- and 6-methoxy-beta-carbolines as acetyl- and butyrylcholinesterase inhibitors, Bioorganic & medicinal chemistry letters, 16 (2006) 5840–5843. [PubMed: 16945529]

[17]. Decker M, Novel inhibitors of acetyl- and butyrylcholinesterase derived from the alkaloids dehydroevodiamine and rutaecarpine, European journal of medicinal chemistry, 40 (2005) 305–313. [PubMed: 15725500]

[18]. Wadkins RM, Hyatt JL, Wei X, Yoon KJ, Wierdl M, Edwards CC, Morton CL, Obenauer JC, Damodaran K, Beroza P, Danks MK, Potter PM, Identification and characterization of novel benzil (diphenylethane-1,2-dione) analogues as inhibitors of mammalian carboxylesterases, J Med Chem, 48 (2005) 2906–2915. [PubMed: 15828829]

[19]. Hatfield MJ, Tsurkan LG, Hyatt JL, Edwards CC, Lemoff A, Jeffries C, Yan B, Potter PM, Modulation of esterified drug metabolism by tanshinones from Salvia miltiorrhiza ("Danshen"), J Nat Prod, 76 (2013) 36–44. [PubMed: 23286284]

[20]. Leader H, Smejkal RM, Payne CS, Padilla FN, Doctor BP, Gordon RK, Chiang PK, Binary antidotes for organophosphate poisoning: aprophen analogues that are both antimuscarinics and carbamates, J Med Chem, 32 (1989) 1522–1528. [PubMed: 2738887]

[21]. Hicks LD, Hyatt JL, Moak T, Edwards CC, Tsurkan L, Wierdl M, Ferreira AM, Wadkins RM, Potter PM, Analysis of the inhibition of mammalian carboxylesterases by novel fluorobenzoins and fluorobenzils, Bioorg Med Chem, 15 (2007) 3801–3817. [PubMed: 17399985]

[22]. Zaheer-Ul-Haq ZU, Wellenzohn B, Liedl KR, Rode BM, Molecular docking studies of natural cholinesterase-inhibiting steroidal alkaloids from Sarcococca saligna, J Med Chem, 46 (2003) 5087–5090. [PubMed: 14584959]

[23]. Hyatt JL, Moak T, Hatfield MJ, Tsurkan L, Edwards CC, Wierdl M, Danks MK, Wadkins RM, Potter PM, Selective inhibition of carboxylesterases by isatins, indole-2,3-diones, J Med Chem, 50 (2007) 1876–1885. [PubMed: 17378546]

[24]. Decker M, Krauth F, Lehmann J, Novel tricyclic quinazolinimines and related tetracyclic nitrogen bridgehead compounds as cholinesterase inhibitors with selectivity towards butyrylcholinesterase, Bioorg Med Chem, 14 (2006) 1966–1977. [PubMed: 16289855]

[25]. Savini L, Campiani G, Gaeta A, Pellerano C, Fattorusso C, Chiasserini L, Fedorko JM, Saxena A, Novel and potent tacrine-related hetero- and homobivalent ligands for acetylcholinesterase and butyrylcholinesterase, Bioorganic & medicinal chemistry letters, 11 (2001) 1779–1782. [PubMed: 11425559]

[26]. Darvesh S, Pottie IR, Darvesh KV, McDonald RS, Walsh R, Conrad S, Penwell A, Mataija D, Martin E, Differential binding of phenothiazine urea derivatives to wild-type human cholinesterases and butyrylcholinesterase mutants, Bioorg Med Chem, 18 (2010) 2232–2244. [PubMed: 20181484]

[27]. Hyatt JL, Wadkins RM, Tsurkan L, Hicks LD, Hatfield MJ, Edwards CC, Ross CR, 2nd, S.A. Cantalupo, G. Crundwell, M.K. Danks, R.K. Guy, P.M. Potter, Planarity and constraint of the carbonyl groups in 1,2-diones are determinants for selective inhibition of human carboxylesterase 1, J Med Chem, 50 (2007) 5727–5734. [PubMed: 17941623]

[28]. Savini L, Gaeta A, Fattorusso C, Catalanotti B, Campiani G, Chiasserini L, Pellerano C, Novellino E, McKissic D, Saxena A, Specific targeting of acetylcholinesterase and butyrylcholinesterase recognition sites. Rational design of novel, selective, and highly potent cholinesterase inhibitors, J Med Chem, 46 (2003) 1–4. [PubMed: 12502352]

[29]. Fang L, Appenroth D, Decker M, Kiehntopf M, Roegler C, Deufel T, Fleck C, Peng S, Zhang Y, Lehmann J, Synthesis and biological evaluation of NO-donor-tacrine hybrids as hepatoprotective anti-Alzheimer drug candidates, J Med Chem, 51 (2008) 713–716. [PubMed: 18232655]

[30]. Decker M, Homobivalent quinazolinimines as novel nanomolar inhibitors of cholinesterases with dirigible selectivity toward butyrylcholinesterase, J Med Chem, 49 (2006) 5411–5413. [PubMed: 16942014]

[31]. Butini S, Guarino E, Campiani G, Brindisi M, Coccone SS, Fiorini I, Novellino E, Belinskaya T, Saxena A, Gemma S, Tacrine based human cholinesterase inhibitors: synthesis of peptidic-tethered derivatives and their effect on potency and selectivity, Bioorganic & medicinal chemistry letters, 18 (2008) 5213–5216. [PubMed: 18786825]

[32]. Gemma S, Gabellieri E, Huleatt P, Fattorusso C, Borriello M, Catalanotti B, Butini S, De Angelis M, Novellino E, Nacci V, Belinskaya T, Saxena A, Campiani G, Discovery of huperzine A-tacrine hybrids as potent inhibitors of human cholinesterases targeting their midgorge recognition sites, J Med Chem, 49 (2006) 3421–3425. [PubMed: 16722663]

[33]. Chen WS, Cocolas GH, Cavallito CJ, Chai KJ, Potent reversible anticholinesterase agents. Bis- and mono-N-substituted benzoquinolinium halides, J Med Chem, 20 (1977) 1617–1623. [PubMed: 592327]

[34]. Sun Q, Peng DY, Yang SG, Zhu XL, Yang WC, Yang GF, Syntheses of coumarin-tacrine hybrids as dual-site acetylcholinesterase inhibitors and their activity against butylcholinesterase, Abeta aggregation, and beta-secretase, Bioorg Med Chem, 22 (2014) 4784–4791. [PubMed: 25088549]

[35]. Peng DY, Sun Q, Zhu XL, Lin HY, Chen Q, Yu NX, Yang WC, Yang GF, Design, synthesis, and bioevaluation of benzamides: novel acetylcholinesterase inhibitors with multifunctions on butylcholinesterase, Abeta aggregation, and beta-secretase, Bioorg Med Chem, 20 (2012) 6739–6750. [PubMed: 23041347]

[36]. Lin G, Tsai HJ, Tsai YH, Cage amines as the stopper inhibitors of cholinesterases, Bioorganic & medicinal chemistry letters, 13 (2003) 2887–2890. [PubMed: 14611850]

[37]. Atta ur R, Khalid A, Sultana N, Ghayur MN, Mesaik MA, Khan MR, Gilani AH, Choudhary MI, New natural cholinesterase inhibiting and calcium channel blocking quinoline alkaloids, J Enzyme Inhib Med Chem, 21 (2006) 703–710. [PubMed: 17252943]

[38]. Darvesh S, Darvesh KV, McDonald RS, Mataija D, Walsh R, Mothana S, Lockridge O, Martin E, Carbamates with differential mechanism of inhibition toward acetylcholinesterase and butyrylcholinesterase, J Med Chem, 51 (2008) 4200–4212. [PubMed: 18570368]

[39]. Raza R, Saeed A, Arif M, Mahmood S, Muddassar M, Raza A, Iqbal J, Synthesis and biological evaluation of 3-thiazolocoumarinyl Schiff-base derivatives as cholinesterase inhibitors, Chem Biol Drug Des, 80 (2012) 605–615. [PubMed: 22726458]

[40]. Musilek K, Roder J, Komloova M, Holas O, Hrabinova M, Pohanka M, Dohnal V, Opletalova V, Kuca K, Jung YS, Preparation, in vitro screening and molecular modelling of symmetrical 4-tert-butylpyridinium cholinesterase inhibitors--analogues of SAD-128, Bioorganic & medicinal chemistry letters, 21 (2011) 150–154. [PubMed: 21144749]

[41]. Butini S, Campiani G, Borriello M, Gemma S, Panico A, Persico M, Catalanotti B, Ros S, Brindisi M, Agnusdei M, Fiorini I, Nacci V, Novellino E, Belinskaya T, Saxena A, Fattorusso C, Exploiting protein fluctuations at the active-site gorge of human cholinesterases: further optimization of the design strategy to develop extremely potent inhibitors, J Med Chem, 51 (2008) 3154–3170. [PubMed: 18479118]

[42]. Lin G, Chen GH, Ho HC, Conformationally restricted carbamate inhibitors of horse serum butyrylcholinesterase, Bioorganic & medicinal chemistry letters, 8 (1998) 2747–2750. [PubMed: 9873615]

[43]. Kalir A, Teomy S, Amir A, Fuchs P, Lee SA, Holsztynska EJ, Rocki W, Domino EF, N-allyl analogues of phencyclidine: chemical synthesis and pharmacological properties, J Med Chem, 27 (1984) 1267–1271. [PubMed: 6481761]

[44]. Luo W, Yu QS, Zhan M, Parrish D, Deschamps JR, Kulkarni SS, Holloway HW, Alley GM, Lahiri DK, Brossi A, Greig NH, Novel anticholinesterases based on the molecular skeletons of furobenzofuran and methanobenzodioxepine, J Med Chem, 48 (2005) 986–994. [PubMed: 15715468]

[45]. Sawatzky E, Wehle S, Kling B, Wendrich J, Bringmann G, Sotriffer CA, Heilmann J, Decker M, Discovery of Highly Selective and Nanomolar Carbamate-Based Butyrylcholinesterase Inhibitors by Rational Investigation into Their Inhibition Mode, J Med Chem, 59 (2016) 2067–2082. [PubMed: 26886849]

[46]. Wang J, Wang ZM, Li XM, Li F, Wu JJ, Kong LY, Wang XB, Synthesis and evaluation of multi-target-directed ligands for the treatment of Alzheimer's disease based on the fusion of donepezil and melatonin, Bioorg Med Chem, 24 (2016) 4324–4338. [PubMed: 27460699]

[47]. Arbiser JL, Kraeft SK, van Leeuwen R, Hurwitz SJ, Selig M, Dickersin GR, Flint A, Byers R, Chen LB, Clioquinol-zinc chelate: a candidate causative agent of subacute myelo-optic neuropathy, Mol Med, 4 (1998) 665–670. [PubMed: 9848083]

[48]. Prati F, Bergamini C, Fato R, Soukup O, Korabecny J, Andrisano V, Bartolini M, Bolognesi ML, Novel 8-Hydroxyquinoline Derivatives as Multitarget Compounds for the Treatment of Alzheimer's Disease, ChemMedChem, 11 (2016) 1284–1295. [PubMed: 26880501]

[49]. Makhaeva GF, Boltneva NP, Lushchekina SV, Serebryakova OG, Stupina TS, Terentiev AA, Serkov IV, Proshin AN, Bachurin SO, Richardson RJ, Synthesis, molecular docking and biological evaluation of N,N-disubstituted 2-aminothiazolines as a new class of butyrylcholinesterase and carboxylesterase inhibitors, Bioorg Med Chem, 24 (2016) 1050–1062. [PubMed: 26827140]

[50]. Carlier PR, Han YF, Chow ES, Li CP, Wang H, Lieu TX, Wong HS, Pang YP, Evaluation of short-tether bis-THA AChE inhibitors. A further test of the dual binding site hypothesis, Bioorg Med Chem, 7 (1999) 351–357. [PubMed: 10218828]

[51]. Lan J-S, Xie S-S, Li S-Y, Pan L-F, Wang X-B, Kong L-Y, Design, synthesis and evaluation of novel tacrine-^-carboline) hybrids as multifunctional agents for the treatment of Alzheimer's disease, Bioorganic & medicinal chemistry, 22 (2014) 6089–6104. [PubMed: 25282654]

[52]. Tunek A, Svensson LA, Bambuterol, a carbamate ester prodrug of terbutaline, as inhibitor of cholinesterases in human blood, Drug Metab Dispos, 16 (1988) 759–764. [PubMed: 2906603]

[53]. Sitar DS, Clinical pharmacokinetics of bambuterol, Clin Pharmacokinet, 31 (1996) 246–256. [PubMed: 8896942]

[54]. Pistolozzi M, Du H, Wei H, Tan W, Stereoselective inhibition of human butyrylcholinesterase by the enantiomers of bambuterol and their intermediates, Drug Metab Dispos, 43 (2015) 344–352. [PubMed: 25504505]

[55]. Gulçin İ, Abbasova M, Taslimi P, Huyut Z, Safarova L, Sujayev A, Farzaliyev V, Beydemir ş, Alwasel SH, Supuran CT, Synthesis and biological evaluation of aminomethyl and alkoxymethyl derivatives as carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 32 (2017) 1174–1182. [PubMed: 28891347]

[56]. Kosak U, Brus B, Knez D, Zakelj S, Trontelj J, Pislar A, Sink R, Jukic M, Zivin M, Podkowa A, The magic of crystal structure-based inhibitor optimization: development of a butyrylcholinesterase inhibitor with picomolar affinity and in vivo activity, Journal of medicinal chemistry, 61 (2017) 119–139. [PubMed: 29227101]

[57]. Dighe SN, Deora GS, De la Mora E, Nachon F, Chan S, Parat M-O, Brazzolotto X, Ross BP, Discovery and Structure-Activity Relationships of a Highly Selective Butyrylcholinesterase Inhibitor by Structure-Based Virtual Screening, Journal of medicinal chemistry, 59 (2016) 7683–7689. [PubMed: 27405689]

[58]. Sakkiah S, Lee KW, Pharmacophore-based virtual screening and density functional theory approach to identifying novel butyrylcholinesterase inhibitors, Acta Pharmacologica Sinica, 33 (2012) 964. [PubMed: 22684028]

[59]. Lešnik S, Štular T, Brus B, Knez D, Gobec S, Janežič D.a., Konc J, LiSiCA: a software for ligand-based virtual screening and its application for the discovery of butyrylcholinesterase inhibitors, Journal of chemical information and modeling, 55 (2015) 1521–1528. [PubMed: 26158767]

[60]. Nachon F, Carletti E, Ronco C, Trovaslet M, Nicolet Y, Jean L, Renard P-Y, Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl-and butyryl-cholinesterase, Biochemical Journal, 453 (2013) 393–399. [PubMed: 23679855]

[61]. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height JJ, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, Journal of medicinal chemistry, 55 (2012) 10282–10286. [PubMed: 23035744]

[62]. Trott O, Olson AJ, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J Comput Chem, 31 (2010) 455–461. [PubMed: 19499576]

[63]. Schrödinger L, The PyMOL Molecular Graphics System, Version 2.0, (2018).

[64]. Hou S, Xue L, Yang W, Fang L, Zheng F, Zhan C-G, Substrate selectivity of high-activity mutants of human butyrylcholinesterase, Org. Biomol. Chem, 11 (2013) 7477–7485. [PubMed: 24077614]

[65]. Roddick JG, The acetylcholinesterase-inhibitory activity of steroidal glycoalkaloids and their aglycones, Phytochemistry, 28 (1989) 2631–2634.

[66]. Harel M, Hyatt JL, Brumshtein B, Morton CL, Yoon KJP, Wadkins RM, Silman I, Sussman JL, Potter PM, The crystal structure of the complex of the anticancer prodrug 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) with Torpedo californica acetylcholinesterase provides a molecular explanation for its cholinergic action, Molecular pharmacology, 67 (2005) 1874–1881. [PubMed: 15772291]

[67]. Dvir H, Jiang H, Wong D, Harel M, Chetrit M, He X, Jin G, Yu G, Tang X, Silman I, X-ray structures of Torpedo californica acetylcholinesterase complexed with (+)-huperzine A and (−)-huperzine B: structural evidence for an active site rearrangement, Biochemistry, 41 (2002) 10810–10818. [PubMed: 12196020]

Research Highlights

- Virtual screening using 3D structures of both BChE and AChE is effective.
- Highly selective BChE inhibitors have been identified from natural products.
- The compounds with a solanaceous alkaloid scaffold can bind with BChE only.
- Three key residues are mainly responsible for the extremely high selectivity.

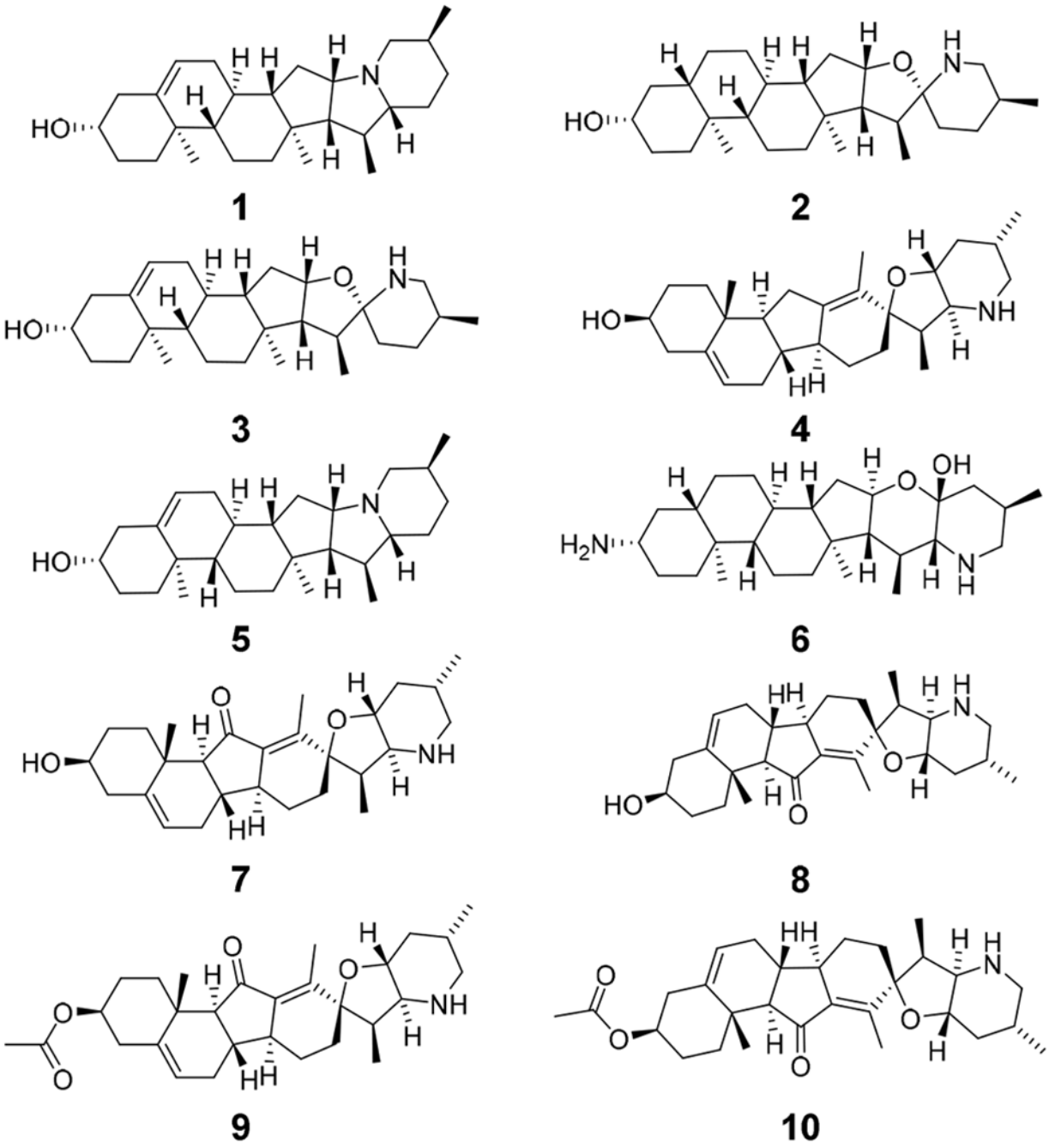

Figure 1.
Molecular structures of the 10 compounds with solanaceous alkaloid scaffolds selected through virtual screening

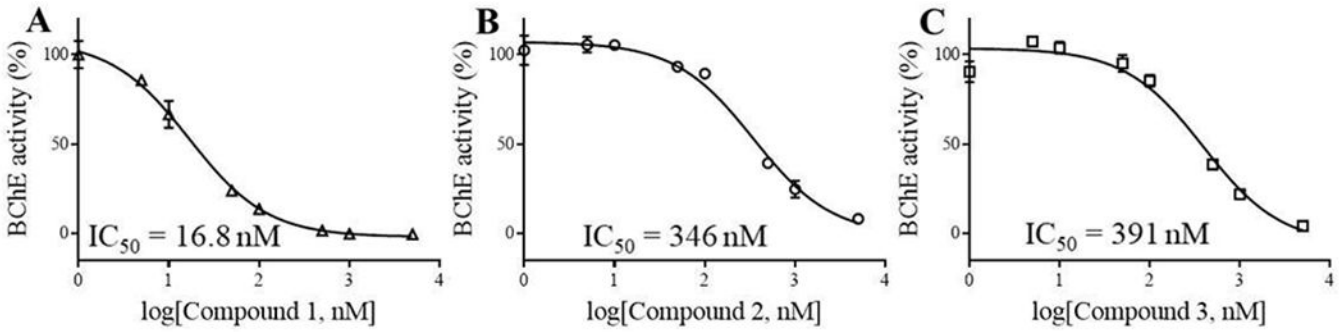

Figure 2.

Dose-dependent inhibition of human BChE by compounds **1** (A), **2** (B), and **3** (C).

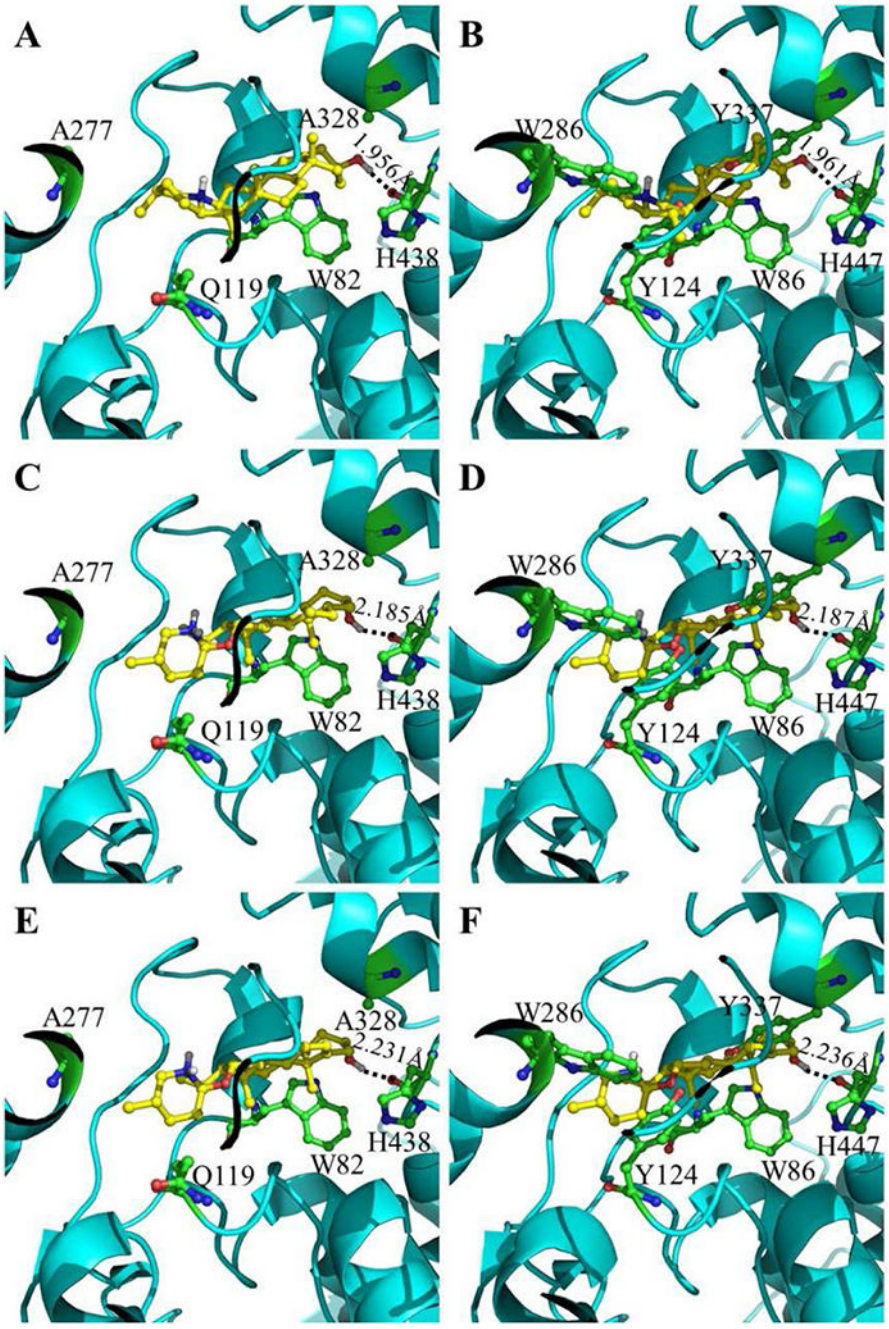

Figure 3.

Modeled interactions of BChE and AChE with compounds **1** to **3**. (A) Favorable binding structure of compound **1** with BChE. (B) Unfavorable interaction of compound **1** with AChE after BChE in panel A is replaced with AChE. (C) Favorable binding structure of compound **2** with BChE. (D) Unfavorable interaction of compound **2** with AChE after BChE in panel C is replaced with AChE. (E) Favorable binding structure of compound **3** with BChE. (F)

Unfavorable interaction of compound 3 with AChE after BChE in panel E is replaced with AChE.

Table 1.

Inhibitory activity of computationally selected solanaceous alkaloids (compounds **1** to **3**) against human BChE

Compound ID	NCI ID	Conventional Name	Inhibition (%) of BChE at 5 μM	Inhibition (%) of AChE at 5 μM	IC_{50} against BChE (nM)
1	76025	Solanidine	100	10	16.8
2	27592	Tomatidine	95	19	346
3	35543	Solasodine	98	2	391
4	734950		44		
5	76026		39		
6	152144		24		
7	23898		22		
8	7520		18		
9	117607		14		
10	117612		8.3		