



In Silico Design, Molecular Docking and ADME/Toxicity Profiling of 3-Substituted 3,4-Dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide Derivatives as Potential Acetylcholinesterase Inhibitors for Alzheimer's Disease

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ABSTRACT :

Introduction: Alzheimer's disease (AD) is characterised by cholinergic deficit, and inhibition of acetylcholinesterase (AChE) remains a mainstay of symptomatic therapy. The 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide scaffold has been explored mainly for AMPA-receptor modulation, and its potential against AChE has not been examined systematically. The present study evaluated five 3-substituted derivatives (CPD 1–5; phenyl, 4-chlorophenyl, 2-phenylethenyl, 4-hydroxy-3-methoxyphenyl and furan-2-yl) *in silico*.

Materials and Methods: Molecular docking against human AChE (PDB ID: 4EY7) was performed with AutoDock Vina in PyRx and the complexes were analysed in BIOVIA Discovery Studio Visualizer. Physicochemical properties, pharmacokinetics and drug-likeness were predicted with SwissADME, and toxicity risks with OSIRIS Property Explorer.

Results and Discussion: Predicted binding affinities ranged from –8.6 to –10.8 kcal/mol. CPD 3 (2-phenylethenyl) ranked highest (–10.8 kcal/mol), forming π - π contacts with Trp86, Tyr337 and Trp286, hydrogen bonds with Arg296, Phe295 and Tyr124, and π -sulfur contacts with Phe338 and Phe297, thereby contacting residues of both the catalytic and peripheral anionic sites. CPD 5 (furan-2-yl) showed the highest ligand efficiency (0.58 kcal/mol per heavy atom). All five compounds showed zero Lipinski violations, high predicted gastrointestinal absorption and no PAINS or Brenk alerts, and no mutagenic, tumorigenic, irritant or reproductive-toxicity risk was flagged by OSIRIS. CPD 1–3 were additionally predicted to cross the blood–brain barrier.

Conclusion: These computational findings identify CPD 3 as the most promising lead for synthesis and enzymatic validation; they are predictions and do not establish inhibitory activity.

Keywords: *acetylcholinesterase, Alzheimer's disease, benzothiadiazine 1,1-dioxide, molecular docking, AutoDock Vina, SwissADME, OSIRIS.*

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia in older adults. Loss of basal forebrain cholinergic neurons and the resulting deficit in cholinergic neurotransmission underlie much of the early memory impairment, a view formalised as the cholinergic hypothesis [1-3]. Acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine and galantamine raise synaptic acetylcholine and provide modest symptomatic benefit, but their efficacy is limited and dose-related adverse effects are common, which sustains interest in new chemotypes [4,5].

Human AChE possesses a narrow gorge of roughly 20 Å lined with aromatic residues. At its base lies the catalytic anionic site (CAS), containing the Ser203–His447–Glu334 triad and the choline-binding Trp86; near the entrance lies the peripheral anionic site (PAS), formed by Trp286, Tyr72, Tyr124, Asp74 and Tyr341 [6-8]. The PAS has also been implicated in AChE-promoted aggregation of amyloid- β , so ligands able to contact both sites are of particular interest [9]. The crystal structure of human AChE in complex with donepezil (PDB ID: 4EY7) provides a high-quality template for structure-based work [10].

The 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide nucleus is a cyclic sulfonamide that combines hydrogen-bond donor (N–H) and acceptor (S=O) functions with a fused aromatic ring. It has been studied principally as a template for positive allosteric modulators of AMPA/kainate receptors [11-13], and 3-substituted members are accessible by condensation of 2-aminobenzenesulfonamide with aldehydes [14,15]. Related sulfur-containing benzo-fused heterocycles have shown AChE inhibition [16], yet the benzothiadiazine dioxide scaffold has not been systematically evaluated against this enzyme.

Molecular docking and early ADME/toxicity filtering allow candidates to be prioritised before synthesis, reducing cost and attrition [17-19]. Here we report a purely computational evaluation of five 3-substituted benzothiadiazine 1,1-dioxide derivatives: their predicted binding mode and affinity towards

human AChE, their physicochemical and pharmacokinetic profile, and their predicted toxicity risks, with the aim of identifying a lead for experimental follow-up.

MATERIALS AND METHODS

Ligand design and preparation

Five derivatives were designed by varying the C-3 substituent of the 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide core, using aryl groups corresponding to benzaldehyde, 4-chlorobenzaldehyde, cinnamaldehyde, vanillin and furfural (Table 1). Structures were drawn in ChemSketch, energy-minimised in Chem3D, and converted to PDBQT format in PyRx after minimisation with the Universal Force Field (UFF) [20].

Target preparation

The crystal structure of human AChE (PDB ID: 4EY7) was retrieved from the RCSB Protein Data Bank [10]. Water molecules, ions and co-crystallised heteroatoms were removed, polar hydrogens and Gasteiger charges were added, and the receptor was saved as a PDBQT macromolecule in PyRx.

Molecular docking and interaction analysis

Docking was carried out with AutoDock Vina [21] as implemented in PyRx [20]. The grid box was centred on the active-site gorge ($x = -13.2324$, $y = -43.1299$, and $z = 30.3805$ Å, with dimensions of $28.9740 \times 24.7203 \times 27.4155$ Å). The lowest-energy pose of each ligand (binding affinity in kcal/mol) was selected for analysis. Complexes were visualised in BIOVIA Discovery Studio Visualizer, which was used to generate 2D interaction diagrams and to identify hydrogen bonds, π -interactions and van der Waals contacts. Ligand efficiency (LE) was calculated as $LE = -(\text{docking score})/N$, where N is the number of non-hydrogen atoms [22].

Physicochemical, pharmacokinetic and drug-likeness prediction

Molecular weight, rotatable bonds, hydrogen-bond donors/acceptors, topological polar surface area (TPSA), consensus lipophilicity (LogP), aqueous solubility (ESOL), gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeation and P-glycoprotein (P-gp) substrate status were predicted with SwissADME [23], whose GI/BBB predictions derive from the BOILED-Egg model [24]. Drug-likeness was assessed with the Lipinski [25], Ghose [26], Veber [27], Egan [28] and Muegge [29] filters and the bioavailability score [30]. Medicinal-chemistry alerts (PAINS [31], Brenk [32]) and synthetic accessibility [33] were also recorded.

Toxicity prediction

Mutagenic, tumorigenic, irritant and reproductive-toxicity risks, together with cLogP, solubility, drug-likeness and overall drug-score, were predicted with the OSIRIS Property Explorer [34]. Fragment-based alerts are displayed in red (high risk), yellow (moderate risk) or green (no risk).

RESULTS AND DISCUSSION

Designed compounds

The five derivatives share the benzothiadiazine 1,1-dioxide core and differ only at C-3 (Fig 1, Table 1). Molecular weights range from 250.27 to 306.34 g/mol (17–21 heavy atoms).

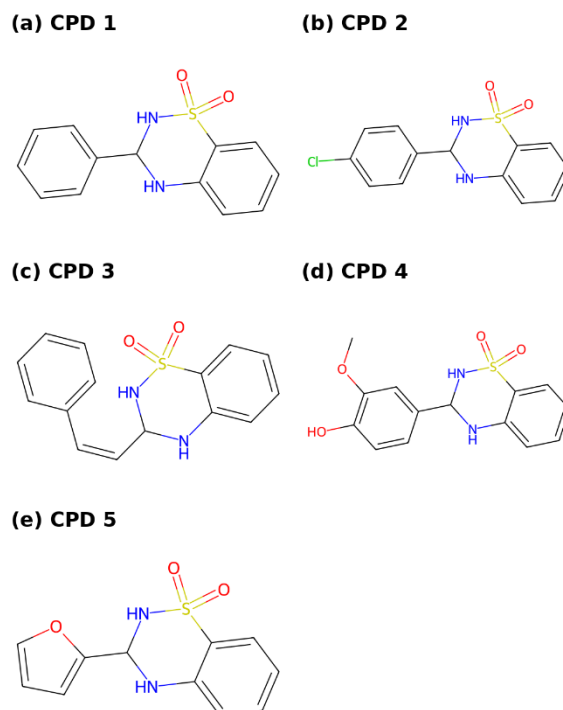


Fig 1: Structures of the designed derivatives CPD 1–5. CPD 3 is shown as modelled for docking (Z-alkene).

Table 1: Designed 3-substituted 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides

ID	C-3 substituent	Formula	MW (g/mol)	Heavy atoms
CPD 1	Phenyl	C ₁₃ H ₁₂ N ₂ O ₂ S	260.31	18
CPD 2	4-Chlorophenyl	C ₁₃ H ₁₁ ClN ₂ O ₂ S	294.76	19
CPD 3	2-Phenylethenyl	C ₁₅ H ₁₄ N ₂ O ₂ S	286.35	20
CPD 4	4-Hydroxy-3-methoxyphenyl	C ₁₄ H ₁₄ N ₂ O ₄ S	306.34	21
CPD 5	Furan-2-yl	C ₁₁ H ₁₀ N ₂ O ₃ S	250.27	17

Physicochemical properties, pharmacokinetics and drug-likeness

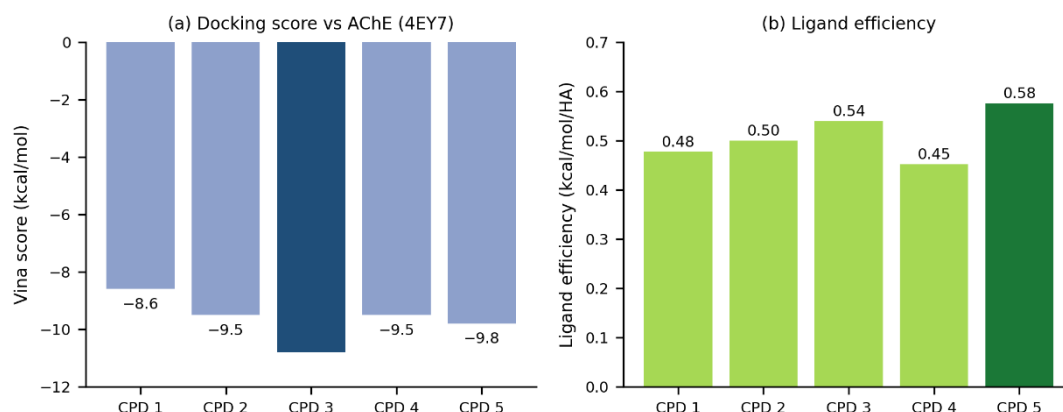
SwissADME predictions are summarised in Table 2. All compounds satisfied the Lipinski, Ghose, Veber, Egan and Muegge filters with zero Lipinski violations, and all had a bioavailability score of 0.55. Consensus LogP ranged from 1.02 to 2.44 and TPSA from 66.58 to 96.04 Å², and all compounds were classified as soluble by the ESOL model (LogS –2.67 to –3.90). GI absorption was predicted to be high for all five. CPD 1, 2 and 3 were predicted to be BBB-permeant and not P-gp substrates, whereas CPD 4 and CPD 5 were predicted not to cross the BBB, and CPD 4 was predicted to be a P-gp substrate. No PAINS or Brenk alerts were raised, all compounds were lead-like, and synthetic accessibility scores were between 3.29 and 3.71.

Table 2: SwissADME predictions for CPD 1–5

Parameter	CPD 1	CPD 2	CPD 3	CPD 4	CPD 5
MW (g/mol)	260.31	294.76	286.35	306.34	250.27
Rotatable bonds	1	1	2	2	1
HBA / HBD	3 / 2	3 / 2	3 / 2	5 / 3	4 / 2
TPSA (Å ²)	66.58	66.58	66.58	96.04	79.72
Consensus LogP	1.83	2.44	2.30	1.33	1.02
LogS (ESOL)	–3.31	–3.90	–3.76	–3.22	–2.67
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	No	No
P-gp substrate	No	No	No	Yes	No
Lipinski violations	0	0	0	0	0
Bioavailability score	0.55	0.55	0.55	0.55	0.55
PAINS / Brenk alerts	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Synthetic accessibility	3.31	3.29	3.71	3.40	3.45

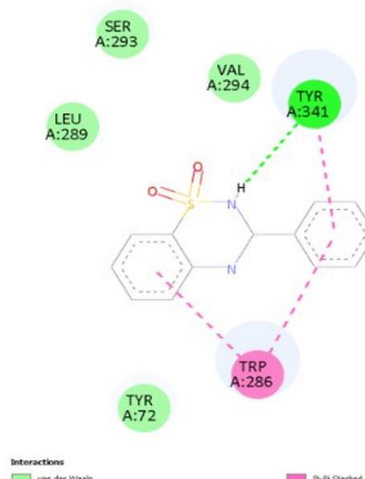
Molecular docking against AChE

All five derivatives docked within the AChE gorge with predicted binding affinities between –8.6 and –10.8 kcal/mol (Table 3, Fig 2). CPD 3 gave the most favourable score (–10.8 kcal/mol), followed by CPD 5 (–9.8), CPD 2 and CPD 4 (both –9.5) and CPD 1 (–8.6). When normalised for size, CPD 5 had the highest ligand efficiency (0.58), followed by CPD 3 (0.54).

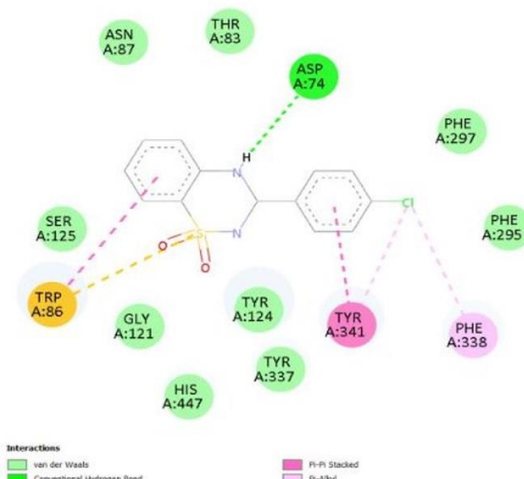
**Fig 2: (a) Predicted AutoDock Vina binding affinities and (b) ligand efficiencies of CPD 1–5 against human AChE (PDB ID: 4EY7).**

The interaction patterns (Fig 3, Table 3) differed between compounds. CPD 3 formed the richest network: π - π interactions with Trp86 (CAS), Tyr337 and Trp286 (PAS); hydrogen bonds from the sulfonyl oxygens to Arg296 and Phe295 and from the ring N-H to Tyr124; π -sulfur contacts with Phe338 and Phe297; a carbon-hydrogen bond with Val294; and van der Waals contacts with His447, Tyr341 and Tyr72. CPD 1 was anchored mainly through a hydrogen bond to Tyr341 and π - π stacking with Trp286, without contact with Trp86. CPD 2 formed a hydrogen bond with Asp74, π - π stacking with Tyr341, a π -sulfur contact with Trp86 and a π -alkyl contact between the chlorine-bearing ring and Phe338. CPD 4 made π - π contacts with Tyr124, Tyr341 and Trp86 and a π -alkyl contact with Leu130, but its phenolic hydroxyl was involved in an unfavourable donor-donor interaction with Gly120. CPD 5 formed a hydrogen-bond interaction with Trp86 and a π - π interaction with Asp74, contributing to its favourable binding within the AChE active-site gorge.

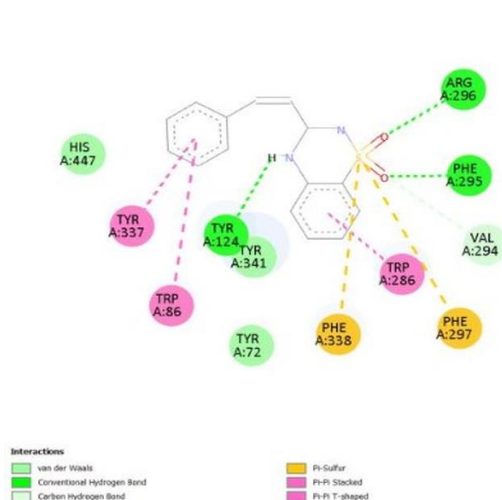
(a) CPD 1



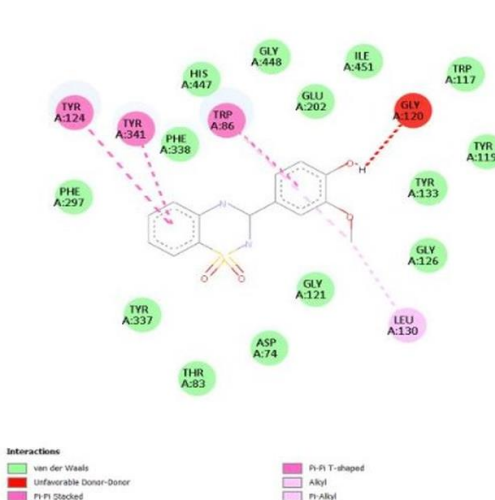
(b) CPD 2



(c) CPD 3



(d) CPD 4



(e) CPD 5

Fig 3: 2D protein–ligand interaction diagrams (BIOVIA Discovery Studio) of (a) CPD 1, (b) CPD 2, (c) CPD 3, (d) CPD 4 and (e) CPD 5 in the AChE active-site gorge. Green: van der Waals/hydrogen bond; pink: π - π ; orange: π -sulfur; light pink: π -alkyl; red: unfavourable donor–donor.

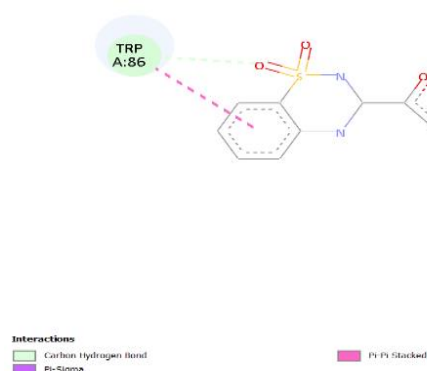


Table 3: Docking scores, ligand efficiency and key interactions with AChE (4EY7)

ID	Score (kcal/mol)	LE	Key interactions
CPD 1	−8.6	0.48	H-bond: Tyr341. π - π : Trp286. vdW: Tyr72, Tyr124, Leu289, Ser293, Val294, Phe295, Phe297, Phe338.
CPD 2	−9.5	0.50	H-bond: Asp74. π - π : Tyr341. π -sulfur: Trp86. π -alkyl: Phe338. vdW: Thr83, Asn87, Ser125, Gly121, Tyr124, Tyr337, His447, Phe295, Phe297.
CPD 3	−10.8	0.54	H-bond: Arg296, Phe295, Tyr124. π - π : Trp86, Tyr337, Trp286. π -sulfur: Phe338, Phe297. C–H bond: Val294. vdW: His447, Tyr341, Tyr72.
CPD 4	−9.5	0.45	π - π : Tyr124, Tyr341, Trp86. π -alkyl: Leu130. Unfavourable donor–donor: Gly120. vdW: Phe297, Phe338, His447, Tyr337, Gly121, Asp74, Thr83, Glu202, Gly448, Ile451, Trp117, Tyr119, Tyr133, Gly126.
CPD 5	−9.8	0.58	H-bond: Trp86. π - π : Asp74.

Toxicity prediction

OSIRIS predicted no mutagenic, tumorigenic, irritant or reproductive-toxicity risk for any compound (all indicators green; Table 4). Drug-scores were 0.82–0.93, with CPD 3 lowest (0.82) because of its lower drug-likeness value (1.16).

Table 4: OSIRIS Property Explorer predictions for CPD 1–5

Parameter	CPD 1	CPD 2	CPD 3	CPD 4	CPD 5
Mutagenic	None	None	None	None	None
Tumorigenic	None	None	None	None	None
Irritant	None	None	None	None	None
Reproductive	None	None	None	None	None
cLogP	1.41	2.02	2.38	0.99	0.60
Solubility (logS)	−2.59	−3.32	−3.24	−2.31	−2.27
TPSA ($\text{\AA}^2$)	66.58	66.58	66.58	96.04	79.72
Drug-likeness	2.30	3.19	1.16	2.85	2.34
Drug-score	0.92	0.92	0.82	0.92	0.93

Discussion

Docking placed all five derivatives in the AChE gorge, where the aromatic lining (Trp86, Tyr124, Trp286, Tyr337, Tyr341, Phe338) provided repeated π - π and π -sulfur contacts. The sulfonamide group was a recurrent interaction element, accepting hydrogen bonds from Arg296 and Phe295 in CPD 3 and engaging Trp86 through a π -sulfur contact in CPD 2, which supports its use as a pharmacophoric anchor.

Structure–activity trends can be read from the C-3 substituent. Relative to the unsubstituted phenyl compound CPD 1 (−8.6), the 4-chloro derivative CPD 2 gained 0.9 kcal/mol, consistent with the additional π -sulfur and π -alkyl contacts. The 2-phenylethenyl group of CPD 3 gained 2.2 kcal/mol: the extended conjugated linker allows the terminal phenyl ring to stack against Trp86 and Tyr337 while the benzo ring stacks against Trp286, so the molecule contacts residues of both CAS and PAS, a binding mode comparable in principle to that of dual-site inhibitors such as donepezil [10]. Contact with both sites is relevant to AD because the PAS is implicated in amyloid- β aggregation [9]. The polar vanillyl group of CPD 4 did not improve affinity (−9.5), and its phenolic hydroxyl was placed in an unfavourable donor–donor arrangement with Gly120. The small furan ring of CPD 5 achieved −9.8 kcal/mol with only 17 heavy atoms and therefore the highest ligand efficiency, making it an attractive fragment-like starting point. Scores did not track lipophilicity: CPD 3 outscores the more lipophilic CPD 2.

For a central-nervous-system target, brain penetration matters as much as affinity. CPD 1–3 combined high predicted GI absorption, BBB permeation, absence of P-gp efflux and TPSA values of 66.58 $\text{\AA}^2$, well below the roughly 90 $\text{\AA}^2$ guideline commonly associated with CNS drugs [35]. CPD 4 (TPSA 96.04 $\text{\AA}^2$, P-gp substrate) and CPD 5 were predicted not to cross the BBB, which would limit their use as central AChEs despite good docking scores. Taking affinity, binding mode, ADME and toxicity together, CPD 3 is the most promising candidate; its slightly lower OSIRIS drug-score (0.82) arises from the drug-likeness term and does not reflect a toxicity flag.

Several limitations apply. Vina scores are empirical estimates with an error of about 2 kcal/mol, so differences below about 1 kcal/mol (CPD 2, 4 and 5) should not be over-interpreted [18,19]. Donepezil showed favourable binding within the AChE active-site gorge and maintained interactions with important aromatic and hydrophobic residues involved in ligand recognition. In particular, interactions involving Trp86, Trp286, Tyr337, and Phe338 contributed to stabilization of the ligand within the binding gorge. The observed binding orientation was consistent with the known ability of donepezil to occupy both the catalytic active-site region and the peripheral anionic site (PAS) of AChE. These interactions provided a suitable reference binding profile for comparison with the synthesized benzothiadiazine derivatives. SwissADME and OSIRIS are model-based predictions. Binding poses and affinities therefore require confirmation by dynamics simulations and enzyme-inhibition assays, which for these derivatives will be reported separately.

CONCLUSION

Five 3-substituted benzothiadiazine 1,1-dioxide derivatives were evaluated *in silico* as AChE inhibitors. All showed favourable predicted binding to human AChE (–8.6 to –10.8 kcal/mol), full compliance with drug-likeness filters, high predicted GI absorption, no structural alerts and no OSIRIS toxicity flags. CPD 3 (3-(2-phenylethenyl) derivative) combined the best docking score (–10.8 kcal/mol), contacts with both catalytic and peripheral sites and a CNS-compatible predicted profile, and is proposed as the lead for synthesis, molecular dynamics and enzymatic validation. These results are computational predictions and do not by themselves demonstrate AChE inhibition.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCE

1. Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp Neurol*. 2000;163(2):495-529.
2. Mesulam M. The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn Mem*. 2004;11(1):43-49.
3. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: targeting the cholinergic system. *Curr Neuropharmacol*. 2016;14(1):101-115.
4. Schneider LS. A critical review of cholinesterase inhibitors as a treatment modality in Alzheimer's disease. *Dialogues Clin Neurosci*. 2000;2(2):111-128.
5. Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr Neuropharmacol*. 2013;11(3):315-335.
6. Harel M, Schalk I, Ehret-Sabatier L, Bouet F, Goeldner M, Hirth C, et al. Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proc Natl Acad Sci U S A*. 1993;90(19):9031-9035.
7. Ordentlich A, Barak D, Kronman C, Ariel N, Segall Y, Velan B, et al. Contribution of aromatic moieties of tyrosine 133 and of the anionic subsite tryptophan 86 to catalytic efficiency and allosteric modulation of acetylcholinesterase. *J Biol Chem*. 1993;268(23):17083-17095.
8. Barak D, Kronman C, Ordentlich A, Ariel N, Bromberg A, Marcus D, et al. Acetylcholinesterase peripheral anionic site degeneracy conferred by amino acid arrays sharing a common core. *J Biol Chem*. 1994;269(9):6296-6305.
9. Inestrosa NC, Alvarez A, Pérez CA, Moreno RD, Vicente M, Linker C, et al. Acetylcholinesterase accelerates assembly of amyloid- β -peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron*. 1996;16(4):881-891.
10. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, et al. Structures of human acetylcholinesterase in complex with pharmacologically active ligands. *J Med Chem*. 2012;55(22):10282-10286.
11. Chhabra S, Shah K. The novel scaffold 1,2,4-benzothiadiazine-1,1-dioxide: a review. *Med Chem Res*. 2021;30:15-30.
12. Braghiroli D, Puia G, Cannazza G, Tait A, Parenti C, Losi G, et al. Synthesis of 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide derivatives as potential allosteric modulators of AMPA/kainate receptors. *J Med Chem*. 2002;45(11):2355-2357.
13. Larsen AP, Francotte P, Frydenvang K, Tapken D, Goffin E, Fraikin P, et al. Synthesis and pharmacology of mono-, di-, and trialkyl-substituted 7-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides combined with X-ray structure analysis to understand the unexpected structure-activity relationship at AMPA receptors. *ACS Chem Neurosci*. 2016;7(3):378-392.
14. Wright JB. New syntheses of 6- and 7-substituted 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides. *J Org Chem*. 1965;30(11):3960-3963.
15. Pennamuthirayan A, Rengan R. Nickel pincer complexes catalyzed sustainable synthesis of 3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxides via acceptorless dehydrogenative coupling of primary alcohols. *J Org Chem*. 2024;89(4):2494-2504.
16. Haji Ali S, Osmaniye D, Sağlık BN, Levent S, Özkay Y, Kaplancıklı ZA. Design, synthesis, and evaluation of novel 2H-benzo[b][1,4]thiazin-3(4H)-one derivatives as new acetylcholinesterase inhibitors. *Molecules*. 2022;27(7):2121. doi:10.3390/molecules27072121.
17. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des*. 2011;7(2):146-157.
18. Grinter SZ, Zou X. Challenges, applications, and recent advances of protein-ligand docking in structure-based drug design. *Molecules*. 2014;19(7):10150-10176.
19. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov*. 2004;3(11):935-949.
20. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods Mol Biol*. 2015;1263:243-250.
21. 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*. 2010;31(2):455-461.
22. Hopkins AL, Groom CR, Alex A. Ligand efficiency: a useful metric for lead selection. *Drug Discov Today*. 2004;9(10):430-431.

23. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7:42717. doi:10.1038/srep42717.
24. Daina A, Zoete V. A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*. 2016;11(11):1117-1121. doi:10.1002/cmdc.201600182.
25. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001;46(1-3):3-26.
26. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J Comb Chem*. 1999;1(1):55-68.
27. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem*. 2002;45(12):2615-2623.
28. Egan WJ, Merz KM, Baldwin JJ. Prediction of drug absorption using multivariate statistics. *J Med Chem*. 2000;43(21):3867-3877.
29. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. *J Med Chem*. 2001;44(9):1841-1846.
30. Martin YC. A bioavailability score. *J Med Chem*. 2005;48(9):3164-3170.
31. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J Med Chem*. 2010;53(7):2719-2740.
32. Brenk R, Schipani A, James D, Krasowski A, Gilbert IH, Frearson J, et al. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. *ChemMedChem*. 2008;3(3):435-444.
33. Ertl P, Schuffenhauer A. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. *J Cheminform*. 2009;1:8.
34. Sander T. OSIRIS Property Explorer. Allschwil: Idorsia Pharmaceuticals Ltd (formerly Actelion Pharmaceuticals Ltd); Organic Chemistry Portal.
35. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*. 2005;2(4):541-553.