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DESIGN OF ACETYLCHOLINESTERASE INHIBITORS FOR ALZHEIMER'S DISEASE

Jiawei Ren [1]

[1] Lake Forest Academy, Illinois, USA

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Abstract

Introduction

Alzheimer's disease is a worldwide progressive neurodegenerative disease. In the past two decades, acetylcholinesterase (AChE) inhibitors have been the most popular medication in mitigating Alzheimer's disease symptoms. However, all common AChE inhibitors have side effects, and traditional inhibitor discovery is a very expensive and timely process. Replacing the traditional workflow with computational experiments, this research selected novel candidate AChE inhibitors for future drug development.

Methods

18 compounds were retrieved after virtually screening for pharmacophore structures affinitive to AChE. After compounds' interaction simulations with the AChE, their inhibition efficiencies were ranked based on Gibb's free energy of. Then the research further evaluated the compounds' oral bioavailability and ability to across the blood brain barrier by comparing their properties with the Lipinski's rule of 5.

Results

Overall, 17 out of 18 compounds passed the Lipinski's rule of 5, qualifying good absorption and permeation. While compound ZINC04713297 featured the best inhibition efficiency (−9.37kcal/mol), both ZINC92926669 and ZINC08756522 required the second lowest reaction energy (−9.20kcal/mol). ZINC92926669 stood out among the two in the absorption assessment for its stability and portability in the blood stream.

Conclusion

This research discovered 17 novel AChE inhibitors through the workflow combining virtual screening, interaction simulation and absorption assessment. It highlighted compound ZINC04713297 and ZIN92926669, which served as a starting point for development of novel acetylcholinesterase inhibitors for Alzheimer's disease.

Keywords: *Alzheimer Disease, Acetylcholinesterase, Drug Discovery, Computational Molecular Biology*

Introduction

Alzheimer's disease (AD), a neurodegenerative disease, is the most common cause of dementia (Breijyeh, Z. and R. Karaman, 2020). There are currently 55 million people worldwide suffering from dementia. The

number is projected to double every 20 years, reaching 78million by 2030 (Yiannopoulou, K.G. and Papageorgiou S.G., 2020).The most popular current medicine for AD is acetylcholinesterase (AChE) inhibitor (Marucci, G., et al. 2021). By hindering the activity of AChE, this class of drug maintains a healthy level of the neurotransmitter acetylcholine at synapses (Sharma, K. 20190).

Most physicians would recommend AChE inhibitors donepezil, galantamine and rivastigmine as the first-line drug to cope with mild to moderate AD symptoms (Birks, J. 2006). However, all of them posted side effects such as nausea, vomiting and insomnia (in *Liver Tox: 2012;* Birks, J.S. and Harvey R.J., 2018; Hager, K., et al., 2014).

Considering the limitations of popular medicines, personalized drugs are brought to attention. Some current researches begin to design novel AChE inhibitor. Research in 2021 used virtual screening to test on 1220 galantamine derivatives (GAL–L-Ar) for anti-AChE activities (Stavrakov, G., et al., 2016). However, more drug options are still needed for doctors to match the drug with patients' physical characters. To meet the need for more candidate AChE inhibitors, this research virtually screened for compounds with high affinity to AChE as a start point for novel drug development.

Methods

Virtual screening

The virtual screening process aimed to find drugs that mimicked binding structures of AChE antibodies. These structures allowed drugs to have a high affinity to AChE.

PocketQuery

Fab410 is an AChE antibody, one of the largest peptide inhibitors targeting the peripheral site of AChE (Bourne, Y., et al., 2013). The entire antibody-protein complex has total six chains, and all antibodies constitute of a heavy chain and a light chain. This experiment chose to study two interactions: **a.** the interaction of AChE's chain A with antibody's light Chain E, **b.** the interaction between AChE's chain A with heavy Chain F (Charles A. Janeway, J., Paul Travers, Mark Walport, and Mark J. Shlomchik, 2001).

I entered the PDB code of the Fab410-BfAChE complex, 4QWW, on PocketQuery (http://pocketquery.csb.pitt.edu/). Clicked "search" to run the search for binding clusters with high affinity to AChE. Pocke tQuery yielded Fab410's residue clusters at the interface of the interaction. The interactive clusters were ranked by scores, based on a support vector machine (SVM) classifier (Koes, D.R. and C.J., 2012).

Pharmacophore Screening

I uploaded three highest ranking clusters each from chain E and chain F onto ZINCPharmer. ZINCPharmer (http://zincpharmer.csb.pitt.edu) found matching compound hits that shared similar structure with the cluster. For each residue clusters, three compounds that had the highest RMSD (similarity scores) were chosen for further study.

Docking Experiment

SwissDock (http://www.swissdock.ch) calculated the Gibbs free energy of interaction between compounds and AChE. On the "Submit Docking" page, I selected "targets" by uploading the structure file for 3LII. Then ligands were entered by either using their identifiers from PDB or their structure files (Grosdidier, A., Zoete, V., and Michielin, O., 2011). Entering the job name and email, clicked "submit" to run the experiment. After approximately twelve hours, results were sent via email. I ranked the compounds' inhibition efficiencies based on their interactions' Gibbs free energy. A negative value of energy of interaction indicated that the reaction was spontaneous. Therefore, the lower the value was, the more likely a compound could inhibit AChE.

Absorption Assessment

In order to qualify as applicable drugs, the compounds had to meet specific features — Lipinski's Rule of 5 (Nogara, P.A., et al., 2015). This set of rules included restrictions on number of hydrogen bond acceptor, number of hydrogen bond donor, iLOGP, and molecular weight.

Swiss ADME

To prepare the input from Swiss ADME, SMILES format of the eighteen compounds were copied from ZINC database. By pasting

them on the Swiss ADME page and clicking "run", the program generated a range of compound data. The compound couldn't have more than ten H-bond acceptors, more than five H-bond donors, a iLOGP value greater than five, or the molecular weight bigger than five hundred gram per mole. Any compounds that violated the rules were eliminated.

Results

Virtual Screening

PocketQuery

Fab410 was a non-competitive AChE antibody that had promise in further research (Bourne, Y., Renault, L. and Marchot P., 2015). After submitting the PDB code for Fab410-BfAChE complex on Pocket Query, three clusters with the highest score each were chosen from chain E and chain F. Size represented the number of amino acid residues in the cluster, and the distance embodied the longest distance in Angstroms between the centroids of any two residues in the cluster. Overall, clusters from chain E earned a better score than clusters of chain F (Table 1).

Table 1. *Chain, size, distance and score of the six clusters*

Name	Chain	Size	Distance	Score
Cluster1	E	2	7.3511	0.981743
Cluster2	E	3	7.3511	0.981391
Cluster3	E	2	9.9599	0.979564
Cluster4	F	1	0	0.969102
Cluster5	F	2	11.1823	0.95847
Cluster6	F	2	5.8989	0.956513

Finding Matching Hits

The six clusters were sent to ZINCPharmer. The system searched for structurally similar compounds for each cluster. The compounds were given a value of RMSD (root mean square deviation) — a larger value indicated a more significant deviation from the original cluster structure (Koes, D. R. and Camacho, C. J., 2011). The best three compound with the lowest RMSD were selected for each cluster (Figure1) (Table 2).

Figure1. *Structures of the compounds*

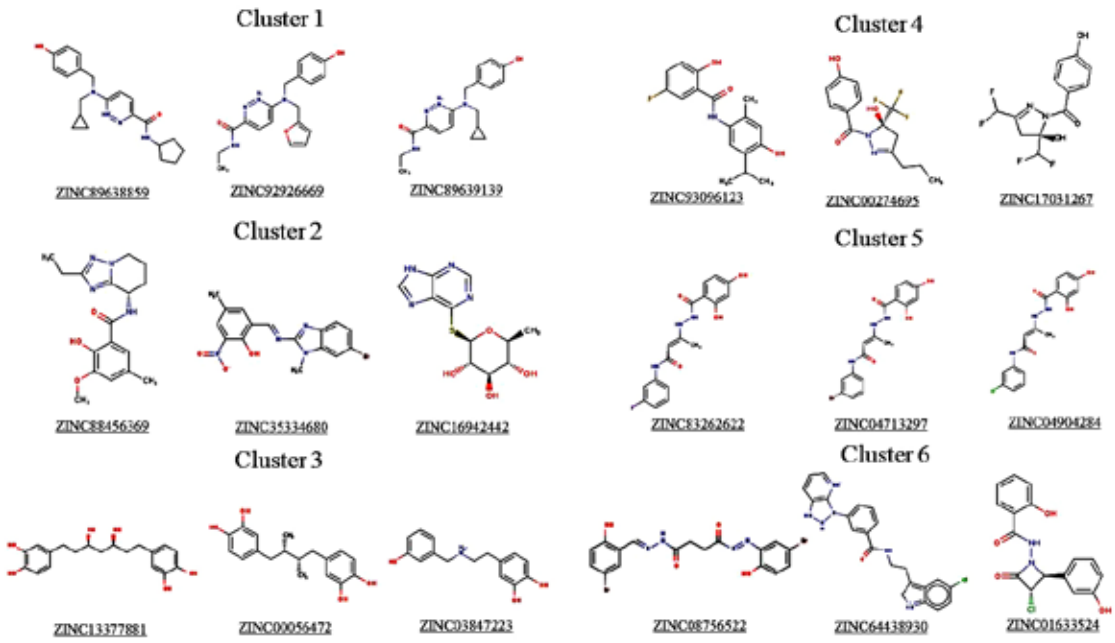

Table 2. *Chain, cluster and RMSD value of compounds*

Chain	Cluster	Compound	RMSD
		ZINC89638859	0.465
	Cluster 1	ZINC92926669	0.480
		ZINC89639139	0.480
		ZINC88456369	0.337
Chain E	Cluster 2	ZINC35334680	0.398
		ZINC16942442	0.453
		ZINC13377881	0.234
	Cluster 3	ZINC00056472	0.247
		ZINC03847223	0.456
		ZINC93096123	0.268
	Cluster 4	ZINC00274695	0.272
		ZINC17031267	0.272
		ZINC83262622	0.315
Chain F	Cluster 5	ZINC04713297	0.359
		ZINC04904284	0.402
		ZINC08756522	0.242
	Cluster 6	ZINC64438930	0.289
		ZINC01633524	0.338

Docking Experiment

The docking algorithm worked by first generating 5000 to 15000 binding models. The binding energy were later evaluated, and those that had the most favorable energies were selected to the file shown as results (Grosdidier, A., Zoete, V., and Michielin, O., 2011). Every compound's lowest Gibbs free energy of reaction were sorted into the table (Table 3). The lowest ΔG ranged from –9.37kcal/mol of ZINC04713297 to –7.36 kcal/mol of ZINC64438930. The compounds were ranked based on the estimated ΔG indicating the spontaneity of its interaction with AChE.

Table 3. *Estimated ΔG and rank of the interactions between compounds and AChE*

Cluster	Compound	Estimated ΔG (kcal/mol)	Rank
	ZINC89638859	– 7.69	14
Cluster 1	ZINC92926669	– 9.20	2
	ZINC89639139	– 8.13	7
	ZINC88456369	– 7.65	16
Cluster 2	ZINC35334680	– 9.19	4
	ZINC16942442	– 8.90	5
	ZINC13377881	– 7.64	17
Cluster 3	ZINC00056472	– 7.72	13
	ZINC03847223	– 8.68	6
	ZINC93096123	– 7.75	12
Cluster 4	ZINC00274695	– 7.88	9
	ZINC17031267	– 7.80	11

Cluster	Compound	Estimated ΔG (kcal/mol)	Rank
Cluster 5	ZINC83262622	– 7.89	8
	ZINC04713297	– 9.37	1
	ZINC04904284	– 7.82	10
Cluster 6	ZINC08756522	– 9.20	2
	ZINC64438930	– 7.36	18
	ZINC01633524	– 7.67	15

Absorption Assessment

Swiss ADME

According to the Lipinski's Rule of 5, a compound would have a poor absorption or permeation if it had more than ten H-bond acceptors, more than five H-bond donors, a iLOGP value greater than five, or the molecular weight bigger than five hundred gram per mole (Lipinski, C.A., et al., 2001). Based on the data acquired from SwissADME (Table 4), all of the compounds passed Lipinski's Rule of 5, except ZINC13377881 of cluster 3. Therefore, 17 out of 18 compounds qualified the assessment with good absorption and ability to cross blood brain barrier.

Table 4. *Number of H-bond acceptors, donors, Log Po/w, and molecular weight of compounds*

Cluster	Compound	Num. H-bond acceptors	Num. H-bond donors	Log Po/w (iLOGP)	Molecular weight
Cluster 1	ZINC89638859	3	3	3.40	367.46 g/mol
	ZINC92926669	4	3	3.03	353.40 g/mol
	ZINC89639139	3	3	2.34	327.40 g/mol
Cluster 2	ZINC88456369	5	2	2.87	330.38 g/mol
	ZINC35334680	5	1	2.25	389.20 g/mol
	ZINC16942442	7	4	1.01	298.32 g/mol
Cluster 3	ZINC13377881	6	6	2.13	348.39 g/mol
	ZINC00056472	4	4	2.44	302.36 g/mol
	ZINC03847223	3	4	2.06	260.31 g/mol
Cluster 4	ZINC93096123	4	3	3.09	303.33 g/mol
	ZINC00274695	7	2	2.32	316.28 g/mol
	ZINC17031267	8	2	1.91	306.21 g/mol
Cluster 5	ZINC83262622	4	5	2.16	453.23 g/mol
	ZINC04713297	4	5	2.21	406.23 g/mol
	ZINC04904284	4	5	2.08	361.78 g/mol
Cluster 6	ZINC08756522	7	3	3.03	498.13 g/mol
	ZINC64438930	2	5	3.02	420.89 g/mol
	ZINC01633524	4	3	1.51	332.74 g/mol

Discussion

Virtual screening and docking experiment

AChE antibody was screened to locate structures highly affinitive to AChE. Then, a searching tool discovered compounds similar to those structures. Results from ZINCPharmer showed that compounds mimicking cluster 4 had the lowest RMSD. Although this group of compounds were most structur-

ally similar to the original cluster, they only ranked at the 9th, 11th, and 12th in the docking experiment. ZINC04713297 (–9.37kcal/mol) with a higher RMSD score showed the best inhibition efficiency, followed by ZINC92926669 (–9.20kcal/mol) and ZINC08756522 (–9.20kcal/mol). Therefore, duplicating the original cluster structure didn't guarantee the best inhibition. A slight deviation might lead to a better effect.

Absorption assessment

Last part of the research was to test the absorption and permeation of the candidate AChE inhibitors using SwissADME. Compounds' properties had to pass the Lipinski's Rule of 5 by having no more than five H-bond donors, no more than ten H-bond acceptors, Log Po/w value smaller than five, and molecular weight less than 500g/mol. Number of H-bond donor and acceptors correlate to reactivity of the compounds. If a compound had too many reacting groups, it could interact with other molecules in the blood before reaching the brain. Log Po/w and molecular weight couldn't be too high either. A high Log Po/w value would lead to poor solubility in water and fast metabolism rate. A heavy molecular weight would render a compound harder to be transported through the blood stream. While Lipinski's rule of 5 wasn't a hard cutoff line for absorption and permeation, it was a good standard to compare with. For the results, ZINC13377881 had six H-bond donors, making it potentially overly reactive. The rest seventeen compounds passed the Lipinski's Rule of 5, which made them qualified with good absorption and ability to cross the blood brain barrier.

Best candidate compounds

The docking experiment highlighted ZINC04713297, ZINC92926669 and ZINC08756522 as the three best compounds at inhibiting AChE. Since the virtual screening didn't aim for a specific pharmacophore structure, there wasn't an established mechanism that contributed to the high inhibition efficiencies of all three compounds. However, possible interactions included hydrophobic interactions, hydrogen bonding, and ionic bonds. In addition, the carbon oxygen double bonds in all three compounds resembled the acetyl group, which might facilitate the binding to the anionic site of acetylcholinesterase.

While both ZINC92926669 and ZINC08756522 had the same Gibb's free energy, ZINC08756522 weighed 498.13 g/mol, which almost violated the Lipinski's rule of 5. This left our focus on ZINC04713297 and ZINC92926669. Compared to ZINC04713297 which had the lowest Gibb's free energy of –9.37kcal/mol, ZINC92926669 featured a better absorption with lower molecular weight (353.40 g/mol) and only 3 H-bond donors. It's not reasonable to decide on the best compound without the context. ZINC92926669 had a more comprehensive performance with good absorption and inhibition. But once the compound reaches the target site, ZINC04713297 would inhibit AChE more spontaneously. Therefore, ZINC04713297 and ZINC92926669 were the two best compounds discovered from this research

Workflow evaluation

This study established the workflow combining binding site identification, virtual screening, docking experiment, and ADME assessment to identify compounds that inhibit the acetylcholinesterase. The research introduced candidate compounds as a starting point for production of effective drug in Alzheimer's Disease therapy. All the experiment were on computer, which didn't involve real chemicals. Therefore, there were no exposures to dangerous compounds. In addition, all the compound data and interactions simulations were online. So, the research required neither purchasing nor shipping of any molecules, which saved both money and time. However, due to the lack of real experiments, the actual inhibition efficiency, properties and toxicity of the candidate drugs required future studies. The next step of this research would be testing compounds through in vivo study, particularly reaction with AChE for inhibition efficiency and trial on mice for toxicity study.

Conclusion

Through this virtual screening, seventeen compounds were chosen as potential therapeutics for Alzheimer's Diseases. Overall, ZINC04713297 and ZINC92926669 from

heavy chain F of AChE antibody stood out for their outstanding inhibition efficiencies. While ZINC04713297 exhibits a more spontaneous inhibition, ZINC92926669 featured better absorption and permeation. This research recognized the necessity of testing the compounds properties in person. Before going into future drug development, candidate compounds had to be reacted with AChE for actual inhibition efficiency. It's also crucial conduct in vivo studies to research on the effect of drugs on living organism and their toxicities.

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Contact: ren.jiawei@outlook.com