In-Silico Analysis of ADME, Target Specificity, and Non-Covalent Interactions for Acetylcholinesterase Inhibition by a Modified Galantamine

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**Abstract**

Galantamine is an acetylcholinesterase inhibitor that is commonly prescribed to increase acetylcholine levels in patients with Alzheimer’s disease. In this study theoretical chemical modifications to galantamine were conceived, and the new ligand was analyzed through several in-silico tools to predict ADME, target specificity, and non-covalent interactions with acetylcholinesterase. The proposed modification of galantamine includes a 4-carbon chain with an aldehyde at the end extended from the azepine tertiary nitrogen of galantamine into the peripheral anionic site of acetylcholinesterase. In-silico analysis suggested similar ADME and druglike properties, somewhat reduced specificity, and an increase in both hydrogen bonding and hydrophobic interactions with amino acid residues of acetylcholinesterase compared to the precursor molecule.

**Introduction**

Acetylcholine (ACh) is a major neurotransmitter in both the central and peripheral nervous systems. It plays an inhibitory role in cardiac muscle and an excitatory role in all other known pathways (Colović et al., 2013). Unlike many other neurotransmitters, ACh is not taken back up into the synaptic button, but is degraded by the enzyme acetylcholinesterase (AChE).

AChE is a hydrolase with very high catalytic capability, able to perform hydrolysis on about 25,000 molecules of ACh per second (Colović et al., 2013). Two molecules of ACh bind to AChE as depicted in Figure 1.

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**KEY**

**AChE Carbon Atoms:** Grey

**Oxygen Atoms:** Red

**Nitrogen Atoms:** Blue

**Hydrogen Bonds: **

**Salt Bridges: **

**Water Bridges: **

**Pi-Cation Interactions: **

**Hydrophobic Interactions: **

**FIGURE 1: Two ACh molecules (lime green) and an acetate (cyan) in the AChE active site. Both ACh 545 and 546 form hydrogen bonds with TYR124. ACh 545 has two pi-cation interactions with TRP286 while ACh 546 has one pi-cation interaction with TRP86(Labeled but not pictured). A salt bridge is formed between ACh 546 and GLU202. ACh 546 has hydrophobic interactions with PHE338 and TYR337.**

The active site of AChE is composed of two subsites (Dvir et al., 2010). The esteratic site (ES) contains a catalytic triad of S203A, H447, and E334 and is the main site of catalytic activity. In Figure 1, ACH546 is bound to the ES. Adjacent to the ES is the anionic site (AS), to which ACH545 is bound in Figure 1 through pi-cation interactions. The AS is important for achieving the proper orientation of acetylcholine in the ES, and also serves as a binding site for many inhibitors (Bajda et al., 2013). Adjacent to the AS, and sitting at the bottom of a large “gorge,” is the peripheral anionic site (PAS). The PAS has been shown to cause substrate inhibition at higher concentrations and may be a transient binding site in the catalytic pathway (Dvir et al., 2010).

Low concentrations of acetylcholine are characteristic of Alzheimer’s disease. Thus, acetylcholinesterase inhibitors are commonly prescribed to increase the acetylcholine concentration and, ultimately, cognitive function in patients with Alzheimer’s disease (Dvir et al., 2010). Galantamine is one such inhibitor, which binds to the AChE active site as depicted in Figure 2.

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**KEY**

**AChE Carbon Atoms:** Tan

**Oxygen Atoms:** Red

**Nitrogen Atoms:** Blue

**Hydrogen Bonds: **

**Salt Bridges: **

**Water Bridges: **

**Pi-Cation Interactions: **

**Hydrophobic Interactions: **

**Figure 2: Galantamine (lime) bound to the AChE active site. A hydrogen bond occurs between galantamine and SER200. Six hydrophobic interactions occur with the ligand, three of which are with TRP84, while the other three are with PHE290, PHE331, and TYR121. Three water bridges occur (dashed white lines, water molecules not shown).**

Galantamine binds to the anionic site and inhibits AChE in a reversible competitive manner (Colović et al., 2013). Galantamine binds to AChE through a hydrogen bond with SER200, and several water bridges with other residues in the anionic site. The side of the molecule that is oriented towards the PAS is involved in several hydrophobic interactions.

In this study in-silico methods were used to attempt to develop a lead for a modification that could increase the effectiveness galantamine as an inhibitor of AChE either through a predicted increase in its ADME, target specificity, or non-covalent ligand-enzyme interactions with ACh. The observation was made that the methyl attached to the azepine tertiary nitrogen of galantamine was oriented towards an area of the PAS with little interaction with the ligand and multiple potential hydrogen bond donors. It was hypothesized the extension of a hydrogen bond acceptor into this area could result in an increase in hydrogen bonding between the ligand and enzyme, thus increasing affinity of the ligand for the enzyme and perhaps improving clinical value. A proposed drug lead (PDL, Figure 3) was generated and analyzed in-silico that was predicted to have increased non-covalent interaction with AChE.

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| --- | --- |
| **Galantamine** | **Proposed Drug Lead** |
|  |  |

**Figure 3: Lewis Structure of galantamine and a proposed drug lead containing the addition of an aldehyde to a 4-carbon chain extended from the azepine tertiary nitrogen of galantamine**

**Methods**

*Chemical Editing/SMILES Generation*

The canonical SMILES for galantamine available from PubChem was imported to the PubChem Sketcher, cited at the end of this report. After making the desired changes to the molecule, a new SMILES was generated from the PubChem Sketcher.

*ADME Prediction*

The SMILES for the new molecule, as well as galantamine was uploaded to the SwissADME online tool, cited at the end of this report. Key factors related to the druglikeness and ADME of the new molecule were compared to galantamine.

*Target Specificity Prediction*

The predicted specificity of the new molecule, as well as galantamine, was generated using SwissTargetPrediction, cited at the end of this report.

*Docking the Ligand/Visualization of Enzyme-Ligand Interactions*

Docking of galantamine to AChE was visualized using the enzyme-ligand complex’s PDB code, 1DX6, on ChimeraX and protein-ligand interactions were predicted using the Protein Ligand Interaction Profiler, both of which are cited at the end of this report. Docking of the new molecule was done by conversion of the PubChem generated SMILES to a PDB format and taking the top predicted result from the SwissDock program, cited at the end of this report.

**Results**

Comparison of galantamine and the PDL using SwissADME predicted the PDL to have a 9.0% greater LogP (lipophilicity), a 3.1% greater LogS (solubility), 5.5% greater synthetic accessibility, and no significant change in cytochrome inhibition, GI absorption, blood brain barrier permeability, and bioavailability score (Table 1). In addition, both molecules were determined to be P-gp substrate and have zero Lipinski violations (Table 1).

The boiled egg plot generated for galantamine and the PDL places the PDL in a similar location to galantamine, with both molecules displaying GI absorption and BBB permeability (Figure 4).

For the target specificity of the PDL, targeting probability for acetylcholinesterase was shown to be less than that of galantamine, but higher than any other biological target except butylcholinesterase for which galantamine and the PDL both have significant targeting probability (Figure 5).

Profiling the interactions between each ligand with AChE indicated the extended aldehyde forms a hydrogen bond with ASP72A for the PDL while the carbon chain to which the aldehyde is attached has a hydrophobic interaction with ASP72A (Figure 6). In addition to the new hydrogen bond formed in the PAS, 5 hydrogen bonds are predicted where water bridges are predicted in galantamine binding, but orientation of residues in the anionic site remained largely unchanged when visualized in ChimeraX.

|  |  |  |  |
| --- | --- | --- | --- |
| Molecule | Galantamine | PDL | Percent Difference |
| LogP | 1.91 | 2.09 | 9.0 |
| LogS | -2.93 | -2.84 | 3.1 |
| Inhibited Cytochromes | CYP2D6 | CYP2D6 | n/a |
| GI Absorption | High | High | n/a |
| BBB Permeant | Yes | Yes | n/a |
| P-gp substrate | Yes | Yes | n/a |
| Lipinski Violations | 0 | 0 | n/a |
| Bioavailability | 0.55 | 0.55 | 0 |
| Synthetic Accessibility | 4.57 | 4.83 | 5.5 |

**Table 1: Comparison of the properties of galantamine and the proposed drug lead (PDL) generated from SwissADME**

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Galantamine

ProposedDrug Lead

**Figure 4: Boiled egg plot featuring galantamine and a proposed drug lead extending an aldehyde from the nitrogen-containing cycloheptane in galantamine. The white portion of the plot signifies gastrointestinal absorptivity, yellow indicates blood-brain barrier permeability.**

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| --- |
| **PDL** |
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| **Galantamine** |
| Table  Description automatically generated with medium confidence |

**Figure 5: Comparison of the top 5 predicted targets for the proposed drug lead (PDL, top) and galantamine (bottom) generated from SwissTargetPrediction**

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**PDL**

**Galantamine**

**Figure 6: Comparison of enzyme-ligand interactions with acetylcholinesterase for galantamine (left) versus proposed drug lead (PDL, right) with tabulated comparison of non-covalent interactions**

**Discussion**

Overall, the PDL retained most of the druglike properties of galantamine (Table 1). The LogP was 9.0% greater for the PDL, indicating greater lipophilicity. This is no surprise, as the modification to the molecule (Figure 3) is a side chain that is quite lipophilic. The LogS also increased, by 3.1%, indicating increased solubility. Increased solubility was a little more surprising since the side chain addition was mostly nonpolar, but the aldehyde at the end of the chain appears to have had a greater predicted effect in increasing polarity and solubility than the hydrophobic chain it is attached to decreased solubility. The PDL was an inhibitor of CYP2D6 which is not ideal, but given that galantamine inhibits this same cytochrome, it seems likely that the PDL will have no greater toxicity as a result of this than its precursor. Both molecules were predicted to have high GI absorbance, BBB permeability, and bioavailability (Figure 4, Table 1). Ultimately, there was nothing in the ADME predictions that signaled the PDL to possess any problematic properties that were not already present in the precursor.

Target specificity is the one area investigated where the PDL was predicted to be significantly inferior to its precursor (Figure 5). Still, by understanding the principles that guide the predictive algorithm of SwissTargetPrediction this is not terribly surprising. The target prediction model employed by SwissTargetPrediction utilizes a “similarity principle” which compares your molecule to other molecules already known to bind a particular biological target. Thus, the predicted specificity for acetylcholinesterase of the PDL was likely due to its similarities to galantamine and its decrease in specificity compared to its precursor was due to its dissimilarities to galantamine. This highlights an important limitation in the predictive targeting software, as an attempt to make a ligand for a specific target that is very different from any currently known ligand for that target will result in low targeting prediction regardless of what specificity may result in-vitro or in-vivo. What is perhaps a more important takeaway from the target prediction is that no new targets of high probability appeared, which means the PDL did not bear high resemblance to any molecules known to bind any undesired targets. That said, the addition of an aldehyde with little steric hindrance in its immediate surroundings does seem likely to react with other targets.

The objective underlying the modification made to galantamine was to create a hydrogen bond between the ligand and the PAS. Analysis of the ligand docked in-silico predicts this was accomplished as the aldehyde formed a hydrogen bond with ASP72A (Figure 6). The side chain that contains the aldehyde also has a hydrophobic interaction with that same residue. The docking of galantamine predicts the formation of several water bridges in the AS while docking of the PDL predicts direct hydrogen bonds. It is unclear if this is a result of lack of water molecules in the AChE docking PDB file used, but the orientation of AChE residues in this area appears unaltered compared to galantamine docking and thus this seems a less pertinent development than the new interactions achieved by the extension of the ligand into the PAS.

**Conclusion**

The in-silico analysis of this modification indicates that it is likely possible to modify galantamine to achieve bonds in the PAS while retaining interactions in the AS, as well as the druglike character of the molecule. An aldehyde at this site was shown to have the highest specificity out of various hydrogen bond acceptors initially tested. Yet, as discussed previously, this likely only indicates addition of an aldehyde left the molecule more similar to its precursor than other additions. The key takeaway from these results in that the molecule is likely to interact with the PAS and likely to retain druglike character. Were the concept of modifying galantamine to achieve both AS and PAS bonding to be explored further in the future it would be useful to perform in-vitro or in-vivo testing of not only the PDL explored here but also other functional groups attached to a side chain of the same length that retain favorable ADME character. Another alteration that could take this modification a step further would be to increase the hydrophobicity of the added side chain which currently has minimal hydrophobic interaction with the PAS. Addition of hydrophobic group on this chain prior to the aldehyde may not only create further interference with the functionality of AChE but increase specificity of the drug by creating more steric hindrance around the aldehyde oriented towards the PAS, assuming this could be done so in a way that still allows the aldehyde to form a hydrogen bond with a residue of the PAS.

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