Chapter 11
Summary and conclusions

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The cholinergic hypothesis proposes that AD is caused by the loss of the cholinergic tract, and it is therapeutically relevant for the symptomatic management of mild to moderate AD. The inhibition of AChE in the brain results in a sufficient increase in ACh levels in synapses, which assist in learning, cognition, and memory formation. Besides AChE, BChE is also responsible for the process. An increase in BChE to AChE ratio from 1:5 to 11:1 with the reduction in AChE levels due to cholinergic neuronal loss is observed with the progression of the disease. The BChE inhibitors were designed due to the absence of classical cholinergic side effects observed with the available AChE inhibitors, which was advantageous. ML was employed for the identification of BChE inhibitors from the data available on Bindingdb database. It resulted in the development of a Pybiomed descriptor-based GB model that predicted *N-phenyl-4-(phenylsulfonamido) benzamide* (**30**) as a BChE inhibitor. Numerous substitutions to the identified compound resulted in 36 derivatives. Compounds *N-(2-chlorophenyl)-4-(phenylsulfonamido) benzamide* (**34**) and *N-(2-bromophenyl)-4-(phenylsulfonamido) benzamide* (**37**) were the most active and selective BChE inhibitors with IC_{50} values of 61.32 ± 7.21, 42.64 ± 2.175 nM and Ki values 124.01 and 147.52 nM, respectively. The compounds were non-toxic and BBB permeable with a mixed-type mode of BChE inhibition. The *ortho*-position on the *phenyl* ring, connected through an amide bond, was crucial for the inhibition. The compounds **34** and **37** produced tight binding with BChE and the sulfonamide group played a crucial role. Further, the treatment with compounds **34** and **37** led to an improvement in scopolamine-induced amnesia in the rats. The compounds were further modified by using a scaffold hopping approach. Compound **I,** obtained from our previous study, was an effective inhibitor and displayed inhibition of both ChE. The *para-aminobenzoic acid* of compound **34** was replaced by *phenylglycine* (from compound **I**). It resulted in a series of 22 derivatives of *N-phenyl-2-phenyl-2-(phenylsulfonamido) acetamide* (**26**). Compounds

30 and **33**, the *ortho-chloro* and *ortho-bromo* derivatives were the most active inhibitors with selectively inhibiting the BChE enzyme. Compounds *N-(2-chlorophnyl)-2-phenyl-2-(phenylsulfonamido) acetamide* (**30**) and *N-(2-bromophenyl)-2-phenyl-2-(phenylsulfonamido) acetamide* (**33**) had IC_{50} values 7.331 ± 0.946, 10.964 ± 0.936 µM and K_i values 29.44 and 56.23 µM, respectively, against BChE. The compounds were found to be BBB permeable and with no toxicity at a concentration of the IC_{50} values. The *in vivo* administration of these compounds reduced the effect of scopolamine treatment on learning and memory when evaluated on scopolamine-induced amnesia models in rats. These identified compounds could be considered pharmacological candidates for the treatment of AD.

The *in silico* studies were conducted to identify the inhibitors and learn their binding mechanism. In another study, AChE inhibitors were identified using a variety of SBDD based computational methods. ZINC000013719534, ZINC000035551243 and ZINC000035596918 produced stable complexes with the AChE enzyme and have the potential for AChE inhibition. The α7 Nicotinic Acetylcholine Receptors (nAChR) plays a crucial role in cognition and is linked to AD. AVL-3288 is an allosteric modulator of the α7-nAChR and increases the peak current generated by ACh binding. In preclinical trials, the molecule showed promising outcomes. Hence, the computational tools were employed to identify the binding site of the ligand on α7- nAChR. The a1 binding modes of the ligand at the agonist sub-pocket had higher binding affinity than t1 and v1 modes which was observed in various *in silico* studies. The results indicated that the probable binding site for AVL-3288 would be the agonist sub-pocket.

AChE from *Electrophorus electricus* is commonly used enzyme for the *in vitro* screening of AChE inhibitors, instead of human AChE. Both organisms share a high degree of homology in the sequence. However, no 3D protein model for ecAChE is available. Hence, a validated protein model for ecAChE was developed through homology

modelling, which was followed by development of docking protocol. However, the native Autodock SF performed poorly with correlation coefficient of 0.013 between pIC_{50} and predicted binding energies of AChE inhibitors. The developed RF-based regression model has improved the correlation coefficient of 0.94 on training dataset and provided output in the form of IC_{50} value. Further, the developed binary and multiclass classification models displayed AUC of 0.837 and 0.87, respectively, which was better than the AUC of 0.574 obtained in case of Autodock SF. These models would be helpful in the prediction of the accurate category of the inhibition.

Horse BChE is also widely used enzyme for the *in vitro* screening of BChE inhibitors and shares a high degree of sequence similarity with human BChE. However, no 3D structure for the protein is available. A homology model and docking protocol for horse BChE was developed and validated. The Autodock SF displayed AUC of 0.404 for ROC curve, while the developed extra tree-based classifier for identification BChE inhibitors displayed much higher AUC of about 0.911. Further, the two regression models, i.e., RF and extra-tree regressors were developed and had a correlation coefficient of 0.964 and 1, respectively, which was far better than the Autodock SF of about 0.013 between predicted binding energy and log IC_{50}. These two customised SF for ecAChE and horse BChE were developed in the form of python libraries and distributed through the website as protein-ligand scoring functions (PLSF) (https://www.drugdesign.in/tools/plsf).

AD is a multifactorial disease with various therapeutic targets viz. AChE, BChE, BACE1, GSK-3β, MAO-B and N_2B subunit of NMDA. Finally, a series of ML models, based on binary classification algorithms, were developed on the inhibitor datasets and validated. The models that performed well in terms of accuracy, precision, recall and F1-score were selected. These selected models were made available to the users through a web application named AlzLeads and were deployed on the website for identification of anti-AD compounds (https://www.drugdesign.in/tools/alzleads).