Chapter 3

Objective and Plan of Work

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3.1. Objectives

The study aims to contribute in the development of effective therapeutic strategies for AD through a comprehensive, multi-pronged research approach integrating computational modelling, ML, and MTDL strategies. The specific objectives of the study are as follows:

- To identify novel compounds targeting neuroinflammatory pathways involved in the pathogenesis of AD using advanced computational drug discovery approaches, including both SBDD and LBDD.
- To develop ML-based virtual screening tools for efficient and accurate prediction of potential inhibitors against key neuroinflammatory targets. The models are designed to streamline the drug discovery pipeline and serve as accessible tools for the broader scientific community.
- To rationally design, synthesize, and evaluate novel small-molecule compounds capable of modulating multiple pathological targets in AD, particularly AChE and BACE1, through a combination of *in vitro* and *in vivo* studies and assessing the multi-target activity, BBB permeability, antioxidant effects, and neuroprotective potential of novel molecules.

Through the above objectives, the study seeks to address key challenges in AD drug development and contribute novel therapeutic candidates and methodologies to the field.

3.2. Plan of work

3.2.1. Computational identification of potential DAPK1 inhibitors for targeting neuroinflammation in Alzheimer's disease

- Utilization of an integrated SBDD-LBDD approach to identify potential DAPK1 inhibitors.
- Development and validation of pharmacophore hypotheses.
- Virtual screening of the Zinc15 compound library using the selected pharmacophore models.
- Applying drug-likeness and PAINS filtering to refine the identified hits from the virtual screening process.
- High throughput virtual screening and molecular docking studies to assess binding energy and ligand-target interactions of the selected compounds.
- Analysis of the predicted toxicity profiles of the selected compounds to ensure safety for potential therapeutic applications.
- Extensive molecular dynamics simulations on the top identified hits to evaluate stability under simulated conditions.
- Binding free energy calculations of the selected protein-ligand complexes.

3.2.2. Computational screening of coumarin derivatives as NLRP3 inflammasome inhibitors for Alzheimer's disease

- Compilation of a comprehensive dataset of unique coumarin derivatives for structure-based virtual screening.
- Screening for drug-like compounds that are PAINS-free and have the potential to permeate the BBB.

- Rigorous molecular docking studies to evaluate the binding affinities and interactions of coumarin derivatives with the active site of the NLRP3 inflammasome.
- Assessment of predicted toxicity profiles to evaluate suitability for therapeutic development.
- Molecular dynamics simulations and analysis of the results to gain insights into the stability and dynamics of the protein-ligand complexes.
- Comparison of the protein-ligand complexes of identified lead compounds with a standard known NLRP3 inhibitor.
- Binding free energy analysis for the selected protein-ligand complexes.

3.2.3. Development of machine learning-based KNIME workflow to predict COX-2 inhibitors

- Leveraging of the existing datasets of COX-2 inhibitors, supplemented by experimental activity data, to facilitate model development.
- Comprehensive extraction of molecular descriptors spanning 0D to 2D, alongside diverse molecular fingerprints, including Morgan, AtomPair, Avalon, MACCS, RDKit, and Pattern fingerprints.
- Implementation and systematic comparison of various machine learning algorithms, such as Logistic Regression, K-Nearest Neighbors, Decision Tree, Random Forest, and Extreme Gradient Boosting, to optimize predictive performance.
- Construction of both basic classifiers and ensemble models employing consensus-based methodologies to enhance robustness and predictive accuracy.
- Assessment of model performance using key evaluation metrics, including accuracy, precision, recall, MCC, and F1 score.

- Structural analysis of predicted active and inactive compounds, providing insights into the molecular features driving the classification outcomes.

3.2.4. Development of machine learning-based KNIME workflow to predict selective COX-2 inhibitors

- Construction of a dedicated virtual screening tool for COX-1 inhibitors by applying machine learning algorithms to existing datasets of COX-1 inhibitors, leveraging molecular descriptors and fingerprint features.
- Integration of the COX-1 and COX-2 virtual screening models into a unified computational platform to facilitate simultaneous analysis.
- Virtual screening of candidate compounds for COX-2 inhibition using the COX-2 screening tool, followed by a secondary screening through the COX-1 model.
- Exclusion of compounds predicted to exhibit inhibitory activity against COX-1, thus refining the selection to compounds predicted to selectively inhibit COX-2 while minimizing COX-1 binding affinity.

3.2.5. Development of multi-targeted biphenyl sulfonamide derivatives for the treatment of Alzheimer's disease

- Identification of dual AChE and BACE1 inhibitors through a molecular hybridization approach.
- Synthesis and structural characterization of the derivatives using FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.
- *In silico* analysis through molecular docking.
- Assessment of *in vitro* inhibitory activity against AChE and BuChE, along with enzyme kinetic studies.
- Evaluation of *in vitro* inhibitory activity against BACE1.

- Evaluation of *in vitro* BBB permeability using the PAMPA assay.
- *In vitro* Aβ-aggregation inhibition and antioxidant potential evaluations.
- *In vivo* testing of the compounds in a scopolamine-induced amnesia model in rats, with assessments of behavioural and neurochemical parameters.

- *In vivo* efficacy testing in an $A\beta_{1-42}$ induced intracerebroventricular rat model, to assess behavioural and neurochemical parameters.

3.2.6. Development of multi-targeted N-phenylbenzenesulfonamide derivatives for the treatment of Alzheimer's disease

- Identification of dual AChE and BACE1 inhibitors using lead optimization technique.
- Synthesis and characterization of derivatives via FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

- *In silico* analysis through molecular docking.
- Assessment of *in vitro* inhibitory activity against AChE and BuChE, and perform enzyme kinetic studies.
- Evaluation of *in vitro* inhibitory activity against BACE1.
- Evaluation of BBB permeability by PAMPA assay.
- *In vivo* efficacy testing in scopolamine-induced amnesia rat model to assess behavioural and neurochemical parameters.
- *In vivo* efficacy testing in an $A\beta_{1-42}$ Induced intracerebroventricular rat model, to assess behavioural and neurochemical parameters.