Chapter 2
Literature Review

Chapter 2

Literature Review

2.1. CADD in drug discovery

CADD has become an indispensable component of modern drug discovery, offering efficient, cost-effective, and rational strategies for identifying and optimising bioactive compounds. CADD techniques are generally categorised into two primary approaches: SBDD and LBDD. More recently, integrated strategies combining SBDD and LBDD have gained prominence for leveraging the advantages of both methodologies. Each of these approaches provides distinct contributions to various stages of the drug discovery pipeline, from hit identification to lead optimisation and pharmacokinetic profiling.

2.1.1. SBDD

SBDD relies on the 3D structure of the target protein, obtained through X-ray crystallography, cryo-electron microscopy, or NMR spectroscopy, to rationally design compounds that bind with high affinity and specificity. The most widely used techniques in SBDD include molecular docking, pharmacophore modelling, and structure-based virtual screening (SBVS).

A notable example of SBDD success was demonstrated in EphB4 tyrosine kinase inhibitor discovery, where fragment-based high-throughput docking followed by molecular dynamics simulations led to the identification of a novel chemical class. The approach improved inhibitory potency from 8.4 μM to 160 nM through structure-guided optimisation, with the binding mode validated by crystal structure determination [111]. Similarly, Jumper *et al.* developed AlphaFold which significantly enhanced the reliability of structural models for previously uncharacterized proteins, thereby expanding the scope

of SBDD to proteins lacking experimental structures [112]. Another significant contribution of SBDD was made in the discovery of Mpro inhibitors against SARS-CoV-2. Dai *et al.* utilized a crystal structure of the viral main protease (Mpro) to design α-ketoamide inhibitors. The structure-guided optimization led to the development of a potent inhibitor with high in vitro and *in vivo* activity [113]. This highlights the pivotal role of SBDD in antiviral drug discovery, particularly during emergent pandemics. Moreover, SBDD has facilitated the design of allosteric inhibitors. In their work on SHP2 phosphatase, Chen *et al.* used crystal structures to identify and optimize inhibitors that bind to an allosteric site rather than the active site, offering a new therapeutic strategy for targeting proteins that are difficult to modulate via classical orthosteric inhibition [114].

2.1.2. LBDD

In contrast, LBDD is employed when structural information about the target protein is limited or unavailable. This approach utilizes information from known ligands, such as their chemical structures, physicochemical properties, and biological activities, to predict novel compounds with similar profiles. Common techniques include QSAR modelling, pharmacophore modelling, and ML-based predictive models.

For instance, a stepwise-multiple linear regression (SW-MLR) was used by Akbari *et al.* to develop linear QSAR model for prediction of COX-2 inhibitory activity of 4-dihydropyridine and 5-oxo-1,4,5,6,7,8-hexahydroquinoline derivatives [115]. Beirami *et al.* developed docking-based 3D-QSAR (CoMFA-RG and CoMSIA) models on hydroquinoline and thiazinan-4-one derivatives as selective COX-2 inhibitors and utilized those to design new as selective COX-2 inhibitors [116]. Similarly, Kadioglu *et al.* utilized ligand-based virtual screening to identify novel inhibitors of RNA-dependent RNA polymerase (RdRp) for SARS-CoV-2. Their work used pharmacophore mapping

and similarity screening based on known RdRp inhibitors, leading to several candidates with promising antiviral activity [117].

Recent developments in ML have further strengthened LBDD. For instance, Cherkasov *et al.* reviewed the integration of ML in QSAR modelling and highlighted its superior performance in handling nonlinear relationships, thereby increasing predictive accuracy [118]. Mazumdar *et al.* utilized three ensemble algorithms (i.e., XGBoost, Random Forest and Extra-Tree classifiers) and a deep neural network to build predictive models to evaluate the BBB permeability of compounds [119]. Another BBB permeability prediction model by Aftab *et al.* used convolutional neural networks (CNNs), recurrent neural networks (RNNs), and ordinary differential equations (ODEs) by training the optimized deep learning model on LightBBB and B3DB databases [120].

2.1.3. Integrated SBDD-LBDD approaches

Recognizing the complementary strengths of SBDD and LBDD, several studies have successfully integrated these strategies to enhance drug discovery outcomes. Hybrid approaches can leverage structural information when available, while still utilizing ligand data to refine and prioritize compounds.

For example, Alturki *et al.* employed SBDD and LBDD approaches to identify selective Ketohexokinase inhibitors for the treatment of fructose-related metabolic disorders [121]. Another illustrative study by Yin *et al.* employed a hybrid approach to identify novel inhibitors of JAK2. Structure-based docking was used to assess binding poses, while ligand-based fingerprint similarity and QSAR modelling were applied to prioritize molecules. This two-tiered system led to the identification of several nanomolar inhibitors with excellent selectivity profiles [122]. In a recent example, Zhang *et al.* developed a deep learning framework combining 3D convolutional neural networks (CNNs) and molecular fingerprints to evaluate compound-target binding affinities. The approach

integrated spatial information from protein-ligand complexes with ligand-centric features, yielding significantly improved predictive performance over single-strategy models [123]. Integrated methods have also been applied in drug repurposing. Mottin *et al.* combined ligand-based similarity screening with structure-based docking to identify potential inhibitors of the Zika virus protease from a library of approved drugs. Their integrative workflow allowed rapid triaging of repurposing candidates with favourable binding modes and ADMET properties [124].

CADD continues to evolve as a central tool in drug discovery, with SBDD, LBDD, and their integration offering distinct and complementary benefits. SBDD enables rational design based on protein structures, while LBDD leverages existing ligand information to predict novel bioactives. Integrated approaches combine the strengths of both to enhance prediction accuracy, efficiency, and success rates. As computational power, structural data availability, and ML algorithms advance, the integration of SBDD and LBDD is expected to play an increasingly critical role in delivering novel therapeutics across a range of therapeutic areas.

2.2. Machine learning in drug discovery

ML has emerged as a transformative tool in drug discovery, offering a wide range of advantages across various stages of the development pipeline. Its integration into the drug discovery process has demonstrated the potential to accelerate timelines, reduce costs, and improve overall success rates.ML enables the automated prioritization of high-value experiments, thereby enhancing predictive modelling and accelerating the identification of active compounds in drug discovery workflows [125]. By automating repetitive tasks related to data processing and analysis, ML significantly expedites what has traditionally been a time-intensive and resource-heavy process. ML applications span multiple phases of drug discovery, including target identification, hit-to-lead optimization, and clinical

outcome prediction [126]. A major advantage of ML lies in its capacity to process large-scale datasets and uncover complex patterns that may not be immediately evident to human researchers. ML algorithms are widely applied in both structure-based and ligand-based virtual screening, de novo drug design, prediction of physicochemical and pharmacokinetic properties, and drug repurposing [127]. Moreover, ML has shown promise in facilitating the development of drug formulations by enabling the rapid exploration of novel materials and innovative strategies [128]. machine learning holds the potential to transform the drug discovery process by enhancing its efficiency, cost-effectiveness, and precision. Through the use of simulations, it can minimize reliance on extensive clinical trials and allow for a more comprehensive investigation of molecular candidates without the need for physical experimentation [129]. As ML continues to advance, it is expected to play an increasingly crucial role in expediting drug discovery and development, ultimately benefiting patients and public.

ML has emerged as a transformative technology in drug discovery, offering significant advantages across multiple stages of the process. The integration of ML techniques has demonstrated potential to accelerate drug development, reduce costs, and improve success rates. In the early stages of drug discovery, ML has enhanced target identification and validation efforts. For example, BANDIT, a Bayesian ML model, demonstrated approximately 90% accuracy in predicting drug-binding targets by integrating diverse datasets [130]. Additionally, ML models are utilized to predict protein-protein interactions, which are critical for identifying viable therapeutic targets [131]. During lead discovery and optimization, ML significantly improves virtual screening and molecular design strategies. Predictive modelling allows researchers to prioritize candidates with higher probabilities of success, thereby reducing the need for exhaustive experimental testing [132]. Cutting-edge ML methods, such as reinforcement learning

and generative adversarial networks (GANs), have also been employed for the de novo generation of molecules with desired pharmacological profiles [133, 134]. ML models are further applied to predict pharmacokinetic and pharmacodynamic (PK/PD) properties, including ADMET parameters. These predictions assist in the early detection of liabilities and inform lead optimization strategies [135, 136]. In the context of clinical development, ML contributes to improving patient recruitment, stratification, and outcome prediction, thereby potentially lowering trial failure rates and expediting progression through clinical phases [137]. Drug repurposing also benefits from ML by leveraging existing data to identify new therapeutic applications for approved drugs, shortening development timelines and reducing associated costs [138]. Furthermore, ML is increasingly used in chemical synthesis prediction, both for retrosynthetic route planning and forward reaction prediction, streamlining synthetic chemistry efforts in drug development [139].

While ML offers numerous advantages, challenges remain, including the need for high-quality, diverse datasets and the interpretability of complex ML models. As these challenges are addressed and ML techniques continue to evolve, the impact on drug discovery is expected to grow, potentially leading to more efficient and effective therapeutic development.

2.2.1. Applications of supervised learning in drug discovery and development

Supervised learning (SL) methods have been widely applied in drug discovery and development, spanning various processes from molecular property prediction to genomic exploration. The applications of SL in this field are diverse and impactful, contributing to the acceleration and improvement of drug development processes.

In evaluating the performance of these SL models, two key metrics are commonly used:

- **Accuracy:** This straightforward metric is defined as the ratio of correctly predicted instances to the total instances in the dataset. It provides a simple measure of overall model performance.
- **Area under the curve (AUC):** Derived from the Receiver Operating Characteristic (ROC) curve, AUC plots the True Positive Rate (TPR) against the False Positive Rate (FPR) at various thresholds. AUC values range from 0 to 1, with 0.5 indicating random guessing, 1 representing perfect separation of positive and negative instances, and 0 indicating a completely incorrect model.

These metrics help researchers assess the effectiveness of SL models in drug discovery applications, enabling comparison and optimization of different approaches [140, 141].

2.2.1.1. Molecular properties prediction

Supervised ML models have demonstrated significant utility in predicting molecular properties crucial for assessing the viability of chemical compounds as therapeutic agents. Recent studies have showcased the effectiveness of various ML techniques in this domain. For example, Tayyebi *et al.* employed Random Forest (RF) algorithms to predict aqueous solubility of chemicals using molecular descriptors and Morgan fingerprints [142]. Similarly, Ahmad *et al.* developed SolPredictor, a residual graph neural network (RGNN) model, for enhanced solubility prediction accuracy [143]. Marchetti *et al.* developed classification models using LR, SVM, and RF to predict the functional effects of allosteric ligands on the Hsp90 molecular chaperone [144]. Several studies have applied ML techniques to various aspects of drug discovery. Zhang *et al.* explored eight ML methods for accelerating Acetyl-CoA Carboxylases inhibitors discovery [145]. Feinberg *et al.* designed PotentialNet, a graph neural network (GNN) for predicting protein-ligand binding affinity [146]. Wang *et al.* created AdvProp, combining graph-based and sequence-based ML methods for molecular property prediction [147]. Lane *et*

al. applied ML algorithms to predict bioactivity, toxicity, and target interactions using the ChEMBL database [148]. Ashraf *et al.* developed models to predict drug potency against SARS-CoV-2 3CLpro protein [149]. Wallach *et al.* introduced AtomNet, a convolutional neural network (CNN) for bioactivity prediction in structure-based drug discovery [150]. Aly and Alotaibi explored LSTM networks for predicting properties of modified Gedunin [151]. These studies underscore the growing importance of supervised ML techniques in molecular property prediction, offering efficient and accurate methods for assessing compound viability in drug discovery and development processes.

2.2.1.2. Pharmacogenomics

Recent advancements in supervised ML have significantly impacted pharmacogenomics, which studies how genes influence individual drug responses. Several studies have demonstrated the effectiveness of ML in this field. For instance, Ikonnikova *et al.* used CatBoost to predict aspirin resistance in ischemic stroke patients [152]. Athreya *et al.* employed Random Forest to forecast SSRI efficacy in major depressive disorder (MDD) [153]. Tang *et al.* compared various ML algorithms to predict stable tacrolimus doses in renal transplant patients [154]. Verhaeghe *et al.* developed CatBoost and Gaussian methods to predict piperacillin plasma concentrations in critically ill patients [155]. Fu *et al.* created ML models for individualized sertraline dosing in adolescents with depression [156]. Pandi *et al.* proposed ML methods for classifying pharmacogenomic variants, focusing on rare and novel variants [157]. Lin *et al.* used neural networks and ensemble approaches to predict antidepressant treatment responses based on genetic and clinical data [158]. Sheu *et al.* utilized ML techniques to predict patient responses to various antidepressant classes using electronic health records [159]. Common ML models in pharmacogenomics include CatBoost, Random Forest, Artificial Neural Networks, AdaBoost, and XGBoost. Performance metrics such as AUC, accuracy, precision,

sensitivity, and specificity are used to evaluate these models. The studies utilize both open and proprietary databases, highlighting the importance of comprehensive datasets in developing robust predictive models for personalized medicine.

2.2.1.3. Biomarker identification

Supervised ML techniques have been widely applied in biomarker identification for various diseases, particularly in cancer research. The methods have shown significant potential in classifying patients based on specific biomarkers and predicting treatment responses. For example, Xie *et al.* used ML algorithms for early lung cancer diagnosis in Chinese patients [160]. Tabl *et al.* employed Random Forest (RF), Support Vector Machines (SVM), and Naive Bayes for breast cancer gene biomarker identification [161]. Ghosh *et al.* applied RF for metabolomic biomarker identification in lung cancer [162]. Zhang and Liu utilized RF for biomarker gene identification across 12 cancer types [163]. In case of neurological disorders, Gutiérrez-Gómez *et al.* used SVM for schizophrenia biomarker identification [164]. Salvatore *et al.* applied SVM for Alzheimer's Disease biomarker identification using MRI data [165]. Furthermore, Hajjo *et al.* used ML models to identify MRI biomarkers for cancer diagnosis and prognosis [166] and Rehman *et al.* employed SVM and RF for validating microRNAs as breast cancer biomarkers [167]. Common ML algorithms used include RF, SVM, Naive Bayes, and Decision Trees. These models often incorporate feature selection methods to enhance interpretability and identify key biomarkers. The studies utilize diverse data sources, including gene expression profiles, metabolomic data, and medical imaging, to ensure comprehensive model training. The high performance of these ML models demonstrates their potential for reliable predictions in biomarker identification, contributing to advancements in personalized medicine and improved patient outcomes.

2.2.1.4. Pharmacokinetic and pharmacodynamic

Recent studies have demonstrated the significant potential of supervised ML in advancing pharmacokinetic and pharmacodynamic (PK/PD) modelling. ML techniques, such as Random Forest and Support Vector Machines, have shown promising results in predicting drug pharmacokinetics and optimizing dosing strategies. For instance, An AI-PBPK platform integrates machine learning and mechanism-based models to predict PK/PD outcomes in early drug discovery, enabling alignment of drug PK profiles with desired PD effects [168]. An example of ML application in PK/PD modelling is the prediction of rifampicin pharmacokinetics. Researchers trained various ML algorithms, including linear regressions, Gradient Boosting Machines, XGBoost, and Random Forest, to predict plasma concentration-time series and area under the concentration-versus-time curve for rifampicin [169]. This approach demonstrates how ML can accelerate PK analysis compared to traditional pharmacometric methods, while still providing valuable insights for drug development and personalized medicine. Another example is the use of XGBoost combined with population pharmacokinetics to improve individual prediction of vancomycin clearance in adult patients [170]. This method showed improved stability and accuracy in predicting vancomycin clearance compared to population pharmacokinetics alone, potentially optimizing initial dosing for more effective and safer drug therapy. Zhang *et al.* successfully applied ML to predict tacrolimus pharmacokinetics in kidney transplant patients [171]. The integration of ML with mechanistic modelling approaches has led to improved model predictions and decision-making in drug development. Deep learning methods, including reinforcement learning, have been explored for personalized drug dosing, demonstrating the potential of ML in tailoring treatments to individual patients [172]. These advancements in ML-driven PK/PD modelling are accelerating drug discovery and development processes, enhancing

the accuracy of drug response predictions, and contributing to the advancement of personalized medicine. As ML techniques continue to evolve, they are expected to play an increasingly crucial role in improving healthcare outcomes through more efficient and personalized drug therapies [173, 174].

2.2.1.5. Prediction of chemical synthesis and retrosynthetic pathway planning

Recent studies have demonstrated the significant potential of supervised ML in chemical synthesis prediction and retrosynthesis planning. Coley *et al.* showcased the effectiveness of graph neural networks (GNNs) in predicting chemical reaction outcomes by encoding molecular structures as graphs [175]. Schwaller *et al.* introduced ReLeaSE, a deep learning model utilizing generative adversarial networks (GANs) for retrosynthesis planning, which generates diverse and synthetically feasible pathways [176]. A Bayesian optimization algorithm (BOA) was developed to optimize enzyme-catalysed reactions, outperforming traditional methods. It improved turnover numbers by up to 80% compared to response surface methodology and 360% versus previous BOAs, while optimizing enzyme activity and selectivity simultaneously [177]. Gao *et al.* trained a neural network model on 10 million reactions predicts suitable catalysts, solvents, reagents, and temperatures for organic reactions with high accuracy, demonstrating ML's potential to enhance computer-assisted synthetic planning [178]. These studies highlight ML's capacity to accelerate the discovery of new chemical compounds, optimize synthetic routes, and enhance reaction efficiency. By leveraging large datasets of chemical reactions and molecular structures, ML models provide valuable insights for advancing drug discovery and materials science.

2.2.2. Applications of unsupervised learning in drug discovery

Unsupervised learning has emerged as a powerful approach in drug discovery, enabling the analysis of complex and high-dimensional datasets without the need for labelled

inputs. By uncovering hidden structures, patterns, and relationships in chemical and biological data, unsupervised methods facilitate various stages of drug development, from target identification and compound clustering to drug repurposing and de novo design.

One of the primary applications of unsupervised learning in drug discovery is chemical space exploration and compound clustering. Clustering algorithms, such as k-means, hierarchical clustering, and DBSCAN, are routinely employed to group molecules based on structural similarity or physicochemical descriptors. These methods have proven instrumental in lead prioritization and scaffold hopping, allowing researchers to focus on diverse chemical series with potential biological activity. For instance, hierarchical clustering of chemical fingerprints has been used to organize large compound libraries, improving hit identification by enabling the selection of representative structures from each cluster [179]. Dimensionality reduction techniques, including PCA, t-SNE, and uniform manifold approximation and projection (UMAP), are also widely used to visualize and interpret the underlying structure of high-dimensional molecular datasets. These methods help identify clusters of structurally or functionally related compounds and can guide library design and virtual screening efforts [180]. In drug repurposing, unsupervised approaches such as similarity-based clustering and network-based integration of heterogeneous data sources (e.g., gene expression, drug-target interactions, side effect profiles) have been employed to uncover novel indications for existing drugs. For example, integrative clustering of drugs based on transcriptomic and chemical similarity has led to the identification of repositioning candidates, with experimental validation supporting the predictions [181]. Latent variable models, such as variational autoencoders (VAEs), represent another key application area. The deep learning-based unsupervised models compress molecular representations into a lower-dimensional latent space, capturing essential chemical features. Such representations have been used in

generative tasks to design novel compounds with desired properties and to score compounds based on their drug-likeness or similarity to known actives [182]. Self-organizing maps (SOMs) have been employed for visualization and classification of molecular datasets, enabling the identification of structurally novel compounds with similar pharmacological profiles. SOMs have also supported scaffold hopping and activity landscape modelling in ligand-based drug design [183].

In summary, unsupervised learning methods provide critical insights into complex datasets, enabling more informed decision-making in drug discovery pipelines. Their ability to detect latent patterns, reduce dimensionality, and cluster high-dimensional data renders them indispensable tools in modern cheminformatics and pharmaceutical research.

2.3. MTDLs for AD

AD is a multifactorial neurodegenerative disorder characterized by multiple pathological pathways, including cholinergic deficit, Aβ aggregation, tau hyperphosphorylation, oxidative stress, and neuroinflammation. The traditional "one-drug-one-target" approach has limited therapeutic efficacy in such complex diseases. Consequently, the MTDL strategy has emerged as a promising alternative for simultaneously modulating multiple targets involved in AD pathogenesis. Several MTDLs have been developed by combining pharmacophores targeting key proteins such as AChE, β-secretase (BACE1), monoamine oxidase-B (MAO-B), and metal chelation sites.

2.3.1. Molecular hybridization-based MTDLs

The molecular hybridization approach involves the rational design of a single molecule by combining two or more pharmacophoric moieties to achieve multi-target activity. These hybrids aim to inhibit AChE to restore cholinergic function inhibit BACE1 to

reduce Aβ production, and provide favourable BBB permeability and neuroprotective profiles.

Recent studies have reported MTDLs with dual AChE/BACE1 inhibitory activity and improved selectivity. For instance, Viayna *et al.* have synthesized novel heterodimers consisting of a unit of racemic or enantiopure huprine Y or X and a donepezil-related 5,6-dimethoxy-2-[(4-piperidi-nyl)methyl]indane moiety connected through a short oligomethylene linker. The heterodimers were observed to be potent *h*AChE inhibitors and moderately potent inhibitors of *h*BChE, AChE-induced and self-induced Aβ aggregation, and BACE1. The most potent compound in the series reached *h*AChE and *h*BACE1 IC_{50} values of 2.61 ± 0.2 nM and 11.0 ± 0.59 μM, respectively [184]. Another novel series of donepezil analogues was developed by Gabra and Abdel-Raziq, through the incorporation of amide linkers that facilitated hydrogen bonding with the BACE1 catalytic site, while retaining high affinity for AChE. Among the analogues, 1-Benzoyl-N-(3,4-dimethoxybenzyl)piperidine-4-carboxamide demonstrated potent dual inhibitory activity (*h*AChE IC_{50} 4.11 nM and BACE1 IC_{50} 18.3 nM), significantly outperforming donepezil itself [185]. A series of halogenated chalcones and flavonols were developed as hybrid molecules targeting AChE, BChE, and BACE1. Chalcones (E)-1-(4-Bromo-2-hydroxy-5-iodophenyl)-3-phenylpropenone and (E)-3-(4-Fluorophenyl)-1-(2-hydroxy-5-iodo-4-methoxyphenyl)prop-2-en-1-one showed strong BChE (IC_{50} = 0.12–0.23 μM) and BACE1 inhibition (IC_{50} = 0.19–0.26 μM), while flavonols 7-fluoro-2-(4-fluorophenyl)-3-hydroxy-6-iodo-4H-chromen-4-one and 2-(4-chlorophenyl)-7-fluoro-3-hydroxy-6-iodo-4H-chromen-4-one were more selective for AChE (IC_{50} = 0.28–0.31 μM). Compound 7-bromo-3-hydroxy-6-iodo-2-(4-methoxyphenyl)-4H-chromen-4-one also exhibited potent BChE and BACE1 inhibition. Kinetic studies confirmed mixed-type inhibition, and molecular docking indicated strong interactions with CAS and PAS of

AChE/BChE and the active site of BACE1, validating their potential as hybrid MTDLs for AD [186]. A notable example of molecular hybridization in MTDL design is the development of ferulic acid-based 1,3,4-oxadiazole hybrids, which were synthesized and evaluated for their multi-target inhibitory potential against AChE, BChE, and BACE1. Among them, 2-Methoxy-4-(2-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)vinyl)phenol exhibited high potency against AChE (IC_{50} = 0.068 μM), and also demonstrated balanced inhibition of BChE and BACE1 (IC_{50} = 0.218 μM and 0.255 μM, respectively) [187]. In a continued effort to design MTDLs for the treatment of AD, the same group developed a novel series of 2-pyridylpiperazine and 5-phenyl-1,3,4-oxadiazole molecular hybrids. The compounds were systematically evaluated for their inhibitory activities against key AD targets. 2-(2,4-Difluorophenyl)-5-(4-(pyridin-2-yl)piperazin-1-yl)-1,3,4-oxadiazole, bearing a 2,4-difluoro substitution on the phenyl ring, showed the most promising profile, with potent inhibition of *h*AChE (IC_{50} = 0.054 μM), *h*BChE (IC_{50} = 0.787 μM), and *h*BACE1 (IC_{50} = 0.098 μM) [188]. These studies exemplify the potential of hybrid scaffolds to provide multifunctional action against key enzymes implicated in AD pathogenesis.

2.3.2. Lead optimization-based MTDLs

Several MTDLs have undergone systematic lead optimization to enhance their dual inhibitory activity against AChE and BACE1. Modifications aimed at improving potency, selectivity, metabolic stability, and BBB permeability have been extensively explored. Structural refinements, such as optimization of linker length and flexibility, strategic substitution on aromatic rings, and fine-tuning of hydrogen bond donors and acceptors, have led to improved binding affinities and pharmacological profiles.

For example, quinazoline-based MTDLs were optimized to improve dual inhibition of AChE and BACE1. Through structural modifications, 4-(4-Benzylpiperazin-1-yl)-6,7-

dimethoxyquinazoline emerged as the most potent derivative, showing IC_{50} values of 0.283 μM for *h*AChE and 0.231 μM for hBACE1, with minimal activity against hBChE (IC_{50} > 10 μM). It also inhibited Aβ aggregation and displaced propidium iodide from the AChE peripheral site. *In vivo* studies in rat and drosophila AD models demonstrated improved cognition and reduced Aβ and BACE1 levels. Notably, BBB permeability of said compound (0.977 μg/mL at 20 mg/kg) [189] was significantly enhanced as compared to the lead compound 4-(4-Benzylpiperazin-1-yl)-2-phenylquinazoline (0.054 μg/mL) [186], indicating successful lead optimization. In another study, Piazzi *et al.* designed and synthesized a dual binding site AChE inhibitor, AP2243, that could simultaneously contact both the CAS and PAS of AChE with an IC_{50} of 18.3 nM [190]. The group later optimized the lead by introducing a halophenylalkylamidic function on the scaffold of AP2243 to obtain potent BACE1 inhibitors while retaining the AChE inhibitory activity. This lead to the identification of compounds N-[2-(3-{4-[(Benzylethylamino)methyl]phenyl}-2-oxo-2H-chromen-6-yloxy)ethyl]-2-(3,5-difluorophenyl)acetamide [*h*AChE IC_{50} 0.551 ± 0.049 μM, BACE1 IC_{50} 0.149 ± 0.002 μM], N-[2-(3-{4-[(Benzylethylamino)methyl]phenyl}-2-oxo-2H-chromen-7-yloxy)ethyl]-2-(3,5-difluorophenyl)acetamide [*h*AChE IC_{50} 0.181 ± 0.010 μM, BACE1 IC_{50} 0.150 ± 0.007 μM], and N-[2-(3-{4-[(Benzylethylamino)methyl]phenyl}-2-oxo-2H-chromen-7-yloxy)ethyl]-3-(3,4-difluorophenyl)propionamide [*h*AChE IC_{50} 0.327 ± 0.034 μM, BACE1 IC_{50} 0.114 ± 0.007 μM] as promising MTDLs with sub-micromolar affinity [191].

2.4. Sulfonamides

Sulfur-containing drugs play a pivotal role in pharmaceutical chemistry due to the unique chemical and biological properties of sulfur. Sulfur's ability to form multiple bonds, spanning oxidation states from –2 to +6, enables the creation of diverse functional groups

with defined molecular geometries. Functional groups, such as sulfides, sulfoxides, sulfones, sulfonamides, and sulfur-based heterocycles, have been extensively utilized in drug design to develop therapeutics with varied mechanisms of action and target specificities. Sulfur's larger atomic size, more diffuse orbitals, and lower electronegativity compared to oxygen confer distinct bioisosteric and pharmacophoric properties that enhance potency, metabolic stability, and target binding in small-molecule drugs.

Historically, sulfur-containing compounds have demonstrated their versatility across therapeutic classes, exemplified by the discovery of sulfa drugs like Prontosil in 1932. These compounds remain integral to the development of antivirals, antibacterials, antitumor agents, antifungals, and antiparasitic drugs. Notably, sulfur is present in approximately 25% of the top 200 small-molecule pharmaceuticals as of 2020 and has been a key component in nearly 30 FDA-approved drugs between 2017 and 2021. Additionally, sulfur-containing natural products (e.g., penicillin) highlight its significance in medicinal chemistry. Despite synthetic challenges associated with sulfur chemistry, advancements in catalytic methods and green chemistry approaches continue to address these limitations, further expanding its potential in drug discovery [192].

2.4.1. Sulfonamides as anti-Alzheimer agents

Recent studies have demonstrated the potential of sulfonamide derivatives as effective AChE inhibitors for AD treatment. Various research groups have synthesized novel sulfonamide-containing compounds with promising AChE inhibitory activities. Bag *et al.* synthesized novel cyclic and long chain aliphatic sulphonamides, among which 3-(4-benzylpiperazin-1-yl)-N-(2-(naphthalen-1-ylamino)ethyl) propane-1-sulfonamide produced 88% inhibition of AChE at 2 μM and 43% inhibition of Aβ-assembly in the Ab fibrillogenesis assay [193]. Rayala *et al.* reported the synthesis of quinoline containing

biphenyl sulfonamide compounds as multifunctional novel therapeutic agents for AD with the lead compound exhibiting promising AChE (IC_{50}: 4.28 ± 0.15 μM), BuChE (IC_{50}: 1.32 ± 0.02 μM), and MMP-2 (IC_{50}: 18.24 ± 1.62 nM) inhibitory potential [194]. Taha *et al.* synthesized indole-based sulfonamide derivatives that showed AChE inhibitory potentials comparable with donepezil [195]. Khan *et al.* reported the synthesis of thiazole based thiadiazole bearing sulfonamide analogues. The lead compound N-[5-(4,5-dimethyl-1,3-thiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,5-difluorobenzene-1-sulfonamide displayed the most promising IC_{50} = 2.50 ± 0.20 nM and 3.30 ± 0.50 nM for AChE and BuChE, respectively, due to the presence of two electronegative fluorine (F) atoms [196]. Nevyhoštěná *et al.* performed a multistep synthesis of nine novel compounds with sulfonamide and amide functional groups as potential anti-AD drugs, where the entire series was reportedly more active in inhibiting AChE than rivastigmine [197]. Khan *et al.* reported the synthesis of a novel hybrid series of thiazole-based sulfonamide analogues as AChE and BuChE inhibitors, where 3,5-Dichlro-2-hydroxy-N′-(4-(3-nitrophenyl)thiazol-2-yl)benzenesulfonohydrazide was the most potent one for both AchE and BuChE with IC50 values = 0.10 ± 0.05 μM and 0.20 ± 0.050 μM, respectively [198]. The lead compound Diethyl 2,6-Dimethyl-4-(4-(methylsulfonamido)phenyl)-1,4-dihydropyridine-3,5-dicarboxylate from the novel class of compounds synthesised by Dakhlaoui *et al.* through the combination of sulfonamide moieties and the 1,4-dihydropyridine scaffold reportedly has an *Ee*AChE inhibition IC_{50} of 12.6 ± 1.2 μM [199]. A series of new dopamine analogues featuring urea and sulfonamide functional groups was synthesized by Gök *et al.* using 3,4-dimethoxyphenethylamine as the starting material. The compound 2-[2-(3,3-Dimethylureido)ethyl]-4,5-dimethoxy-N,N-dimethylbenzenesulfonamide had the lowest AChE IC_{50} of this series, i.e., 298 ± 43 μM [200]. Kurban *et al.* designed and synthesised

a series of novel sulfonamide derivatives bearing an aryl sulfonate moiety and 2-(((4-Sulfamoylphenethyl)imino)methyl)phenyl benzenesulfonate (K_i= 12.90 ± 0.29 nM) was reported to be the most active inhibitors against AChE [201]. (E)-N-(4-{[1-oxo-3,4-dihydronaphthalen-2(1H)-ylidene]methyl}phenyl)methanesulphonamide (K_i= 87.38 ± 7.63 nM) synthesised by Güleç *et al.* is also a strong inhibitor of AChE [202]. The 1,2,4-triazine-sulfonamide hybrid 3-Sulfenyl-2,4-dinitrophenyl-1,2,4-triazine, which contains an ortho-, para-dinitrophenyl group, exhibited notable biological activity at the AChE binding site, with an IC_{50} of 1.677 μM and a K_i of 0.560 μM [203]. These studies collectively demonstrate that sulfonamide-based compounds are promising candidates for AChE inhibition in AD treatment. The diverse structural modifications and hybrid approaches in sulfonamide synthesis offer a rich avenue for developing potent and selective AChE inhibitors, warranting further investigation in the pursuit of effective AD therapeutics.