Chapter 3

# Recent Advances in Computational Modeling of BACE1 Inhibitors as Anti-Alzheimer Agents

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## Abstract

A growing number of people worldwide are being affected by aging-associated neurodegenerative illnesses, the most prevalent of which, Alzheimer's, is defined by progressive neuronal death and synaptic loss in the human brain and can be brought on by both genetic and environmental risk factors. The beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is the major beta secretase for the generation of amyloid-β peptides in the neurons, which – according to the amyloid hypothesis – results in the formation of amyloid plaques. Therefore, in order to avert the accumulation of beta-amyloid and (per the amyloid hypothesis) delay or prevent the progression of Alzheimer's disease, the creation of BACE1 small-molecule inhibitors consists of one of the principal pharmaceutical routes. Using cComputer-aAided dDrug dDesign, inhibitors for the BACE1 biomolecular target connected to Alzheimer's disease have been effectively created. In this chapter, the

recent developments in the computational modeling search of novel BACE1 inhibitors are discussed.


## Keyw~~-W~~ords

Alzheimer's disease

C~~c~~omputer-aided drug design

β-secretase

C~~c~~omputational structure-based design

M~~m~~olecular docking

M~~m~~olecular dynamics

C~~c~~omputational ligand-based design

QSAR

C~~c~~heminformatics

M~~m~~achine learning


## 1. Introduction

One of the main causes of dementia in the elderly is Alzheimer'~~-~~s disease (AD), an irreversible, progressive neurodegenerative brain ailment for which there is presently no therapy [1, 2]. Millions of individuals throughout the world are suffering from AD, which has severe effects on both the patients and their relatives. According to estimations from the Alzheimer's association, approximately 6.5 million Americans at the age of 65 and above are living with AD today [3], and recent global estimates including persons with AD dementia, prodromal AD, and preclinical AD reported a prevalence of 22% of all people above 50 years [4]. Families, health~~-~~care systems, and society as a whole are severely financially burdened by the care and support of AD patients. Therefore, many research efforts have been made in recent decades to understand the origins of AD so that safe and effective pharmaceutical agents can be developed [1].

Despite the extensive funding and research invested into AD or other forms of dementia, the underlying causes are still unclear, but the evidences point to a combination of risk factors including the person'~~-~~s lifestyle, genetics, and

environmental factors such as exposure to toxins and pollution [5, 6]. Moreover, the lack of effective medical treatments and inadequate diagnostic technologies is expected to further amplify AD's negative societal impact. Thus, understanding the molecular pathogenesis of AD is essential for the creation of better diagnostic and treatment methods [5].

AD is a neurodegenerative disorder of the central nervous system (CNS) [7, 8], associated with abnormal amyloid-β (Aβ) metabolism [9], hyperphosphorylation of tTubulin-associated unit (Tau) [10, 11], oxidative stress [12, 13], reactive glial [14], microglial changes [15], and other pathological abnormalities [7, 8]. Even though substantial improvements in research have led to new understandings of AD'-s pathogenesis, the disease'-s molecular pathways are complicated and poorly understood [16]. The focus of research for the creation of AD treatments is on the Aβ and Tau pathways that result in amyloid plaques and neurofibrillary tangles (NFTs), respectively [17].

The transmembrane enzyme β-secretase or β-site APP cleaving enzyme I (BACE1) is involved in the amyloidogenic pathway [18], and its inhibition is being regarded as a possible treatment method for the development of AD drugs [19]. BACE1 was first identified in 1999 [20]. It is a membrane-anchored aspartic protease ubiquitously expressed in the neurons, with a higher activity in the Golgi apparatus, trans-Golgi network (TGN), secretory vesicles and endosomes [20–22], and optimal enzymatic activity in an acidic pH [23]. Chromosomal localization studies facilitated the identification of BACE1's homologue, BACE2 [24]. BACE1 is involved in the rate-limiting step of the cleavage process of the amyloid precursor protein (APP), which results in the generation of the neurotoxic amyloid (Aβ) protein after BACE1 completes its function. Insoluble Aβ aggregates that are formed cause plaque buildup and neurodegeneration (Fig. 1) [25]. Therefore, inhibition of APP proteolysis by BACE1 to decrease the concentration of neurotoxic Aβ -peptides is considered one of the most important therapeutic approaches for AD, making BACE1 an alluring target [2, 19, 20, 26].

Effective clinical aspartic protease inhibitors were initially discovered for other therapeutic targets, like the treatment of hypertension and the human immunodeficiency virus (HIV) [27]. This development, coupled with the elucidation of BACE1 first crystal structure in 2000 [28], supplied vital data for using structure-based (SB) drug design and at first appeared to simplify the overall procedure for creation of BACE1 inhibitors. Unfortunately, this expectation was quickly unrealized for several reasons, mainly due to the reduced binding efficacy of the candidate compounds in BACE1's catalytic pocket, their selectivity toward~~s~~ other aspartic proteases and not meeting the strict requirements for CNS penetration [26]. Despite these obstacles, significant progress has been achieved recently in the development of BACE1 small-~~-~~molecule inhibitors that exhibit improved pharmacokinetic (PK) and blood-brain barrier (BBB) permeation profiles. Milestones in the development of small-~~-~~molecule BACE1 inhibitors [2, 19, 26] many of which reached late stages of clinical trials include the identification of acyl guanidine-~~-~~based compounds [29], followed by the discovery of aminothiazine- and aminooxazoline-~~-~~based compounds [30] and the fluorinated iminothiadiazinane dioxide-based compounds best represented by Merck's verubecestat (MK-8931) [31]. The latter was the most advanced BACE1 inhibitor for a long time before it was discontinued due to an unsatisfactory risk/benefit ratio after two significant phase III trials, a fate followed by other BACE1 inhibitors that showed great potential in early development stages [32]. Because of the high failure rate of drug candidates in clinical trials [33], more extensive efforts in the creation of novel small-~~-~~molecule inhibitors are necessary [34]. The intricacy of the molecular pathways involved in the course of AD renders the discovery of small-~~-~~molecule BACE1 inhibitors a difficult and time-consuming process [35]. This is an issue hampering all aspects of AD treatment. For example, aducanumab (branded as A~~a~~duhelm) [36] is the first new drug approved by the U~~.~~S~~.~~ Food and Drug Administration for Alzheimer's disease in 18 years [37].

In this regard, c~~C~~omputer-a~~A~~ided d~~D~~rug d~~D~~esign (CADD) techniques are particularly effective tools for the rational selection of hit compounds and hit-to-lead optimization. CADD includes ligand-based design methods like quantitative structure-activity relationships (QSAR), descriptor-based QSAR, and pharmacophore

mapping as well as computational structure-based design methods like molecular docking and dynamics [38]. In order to fully comprehend and make predictions on the binding mechanisms and energetics of small-molecule BACE1 inhibitors, CADD harnesses the power of computers, mathematics, and statistical mechanics [39]. Several examples of successful CADD application with respect to AD can be found in literature [40, 41]. In this chapter, the structure and function of BACE1 as a druggable target involved in AD is discussed, along with several recent CADD application examples for the development of BACE1 inhibitors and modulators as anti-Alzheimer agents.

## 2. BACE1: Structure and Aactive Ssite Ccharacteristics

More than 300 BACE1 crystal structures with and without an inhibitor in the active site have been resolved [42]. BACE1 comprises 501 amino acids [43] having a large two-lobe catalytic domain, the crystal structure of which (385 residues) was first determined in 2000 (PDB code: 1FKN) [28] and has the typical conserved folding of aspartic proteases [44] (Fig. 2). The catalytic domain is characterized by a substrate binding site, namely, the "cleft," located centrally between the N-terminal lobe (residues 1–150) and C-terminal lobes (residues 151–385), which features two catalytic aspartate amino acids, namely, Asp32 and Asp228 [45]. A combination of molecular docking and mMolecular dDynamics (MD) simulations was integral in the elucidation of the catalytic dyad protonation state [46, 47]. Like other aspartic proteases, a water molecule bridges the catalytic residues in the BACE1 apo structure [48]. The BACE1 active site can be further subdivided into a total of eleven 11 subsites that offer an increased number of available interactions, mainly with peptidic substrates, as three 3 of these subsites are rarely occupied by small molecular inhibitors [42]. The substrate binding site is sensitive to pH changes, which are correlated with conformational changes that affect its activity [49].

The substrate binding "cleft" is shielded by a long, flexible β-hairpin loop (residues 67–77) that is often referred to as the "flap," which is positioned at the N-terminal lobe and controls substrate access through a conformational change (Fig. 3) [45, 49]. A rich network of hydrogen bonds in the BACE1's flap region stabilizes a flap-open

apo structure conformation [50], and when a substrate is bound, it assumes a flap-closed or a flap-open conformation, depending on the substrate characteristics [29]. Other important residues involved in substrate binding include Leu30, Tyr71, and Phe108 located near the flap region [42]. The remarkable flexibility exhibited by BACE1 has also been supported by MD simulation studies [51].

# 3. CADD of Anti-Alzheimer BACE1 Inhibitors: Techniques and Recent Developments

This section will summarize recent CADD developments in search for new BACE1 inhibitors. Historically, the breakthrough in BACE1 inhibitor development is considered the discovery of OM99-2 and OM00-3 substrate-based inhibitors (Fig. 4) [52, 53]; the elucidation of their binding mode with the active site of the enzyme was a turning point in future inhibitor design [54]. Since then, CADD techniques have been extensively employed and for a comprehensive review of past efforts (the interested reader is referred to previous works [19, 39]). In the following sections, recent studies on CADD design of novel BACE1 inhibitors are presented, roughly organized according to the main computational methods used.

## 3.1. Virtual Screening–Based Approaches

### 3.1.1. Molecular Docking-Molecular Simulations

Virtual screening (VS), molecular docking and MD simulations combined with molecular mechanics-generalized born surface area (MM-GBSA) [55, 56] calculations are important tools in CADD studies for the research of BACE1 inhibitors. Briefly, VS is a computational technique complementary to high-throughput screening (HTS) whose primary objective is to facilitate the quick and affordable evaluation of large databases to screen lead compounds for the discovery of new drugs. [40, 57]. Molecular docking calculations are used for the prediction of the most favorable interaction of small-molecules (ligands) within a protein (receptor) binding site by scanning the conformational space using a scoring function that uses a semi-empirical free energy force field to rank and discriminate the bound conformations [58]. The force field is a mathematical expression describing the potential energy of a system of atoms or molecules as the sum of individual classical

potential energy terms accounting for the bonded (bonds, bond angles, and dihedral angles) and non-bonded interactions (van der Waals and electrostatic), designed to reproduce the behavior of the system [59]. Classical force fields are commonly utilized in MD simulations that are applied in large atomic and molecular systems to calculate and predict various macroscopic properties using statistical mechanics. Configurations of a molecular system are generated sequentially by integrating Newton's laws of motion, and as a result, trajectories are obtained in the phase space, which determine the change in the positions and velocities of the atoms/molecules of the system with time. Statistical analysis of the resulting trajectories allows the calculation of a series of characteristic structural and thermodynamic properties of a system. [59]. MD trajectory analysis with methods like the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) and MM-GBSA allows for the estimation of relative protein-ligand binding affinities by decomposing the binding free energy into individual energy terms accounting for the gas phase molecular mechanics energy, the polar and non-polar contributions to the solvation free energy and the conformational entropy [55, 56].

As an application example of the aforementioned methods, nNotopterol (a furan coumarin from *Notopterygium incisum*, Fig. 5) was identified from VS to possess simultaneous inhibitory activity towards both BACE1 ($IC_{50}$: 26.01 µM) and glycogen synthase kinase-3 beta (GSK3β) ($IC_{50}$: 1 µM) [60]. Molecular docking with biological evaluations of cardamonin, pinocembrin, and pinostrobin (Fig. 5) – i.e., *Boesenbergia rotunda* flavonoids – showed cardamonin as the strongest BACE1 inhibitor (cardamonin, pinocembrin, and pinostrobin $IC_{50}$ values of 4.35 ± 0.38, 27.01 ± 2.12, and 28.44 ± 1.96 µM, respectively), while none of the other tested compounds exhibited binding in BACE1's active site, illustrating non-competitive inhibitory activity for all three compounds [61]. Another molecular docking and experimental investigation identified three synthesized flavone derivatives exhibiting significant biological effects on both acetylcholinesterase (AChE) and BACE1 [62]. It was also observed that the three compounds with the lowest half maximal inhibitory concentration ($IC_{50}$) (~~namely~~ viz., B3, 3.98; D5, 1.66; and D6, 1.58 µM) on BACE1 are in correlation with the calculated docking scores [62].

Molecular docking calculations along with kinetic studies were employed to investigate sulforaphane (an isothiocyanate found in cruciferous vegetables, Fig. 5) against BACE1 [63]. Results revealed that ~~not only~~ sulforaphane activity not only was six~~-~~fold in potency ($IC_{50}$ value of 2.80 ± 0.19 µM) compared to well-known positive controls resveratrol and quercetin ($IC_{50}$ 18.10 ± 0.03 and 15.04 ± 0.87 µM, respectively)~~,~~ but also displayed selective and non~~-~~competitive BACE1 inhibitory activity by developing van der Waals interactions with other BACE1 binding sites [63]. In a molecular docking, MD simulation, liquid chromatography, and high-resolution electrospray mass spectrometry study, *Morus macroura* was investigated as a food supplement for AD management [64]. It was found that among 29 phytochemicals, resveratrol together with chrysin exhibited comparable inhibitory activities toward~~s~~ BACE1 ($IC_{50}$ 16.78 ± 0.9 and 21.34 ± 1.1 µM, respectively) [64].

A molecular docking-based VS with structure-based (SB) and ligand-based (LB) pharmacophore model protocol employed to design and screen several drug-like compound databases~~,~~ identified 13 novel hit BACE1 inhibitors (peptidomimetic, indole, coumarin, piperazine, piperidine, and 1,3,5 triazine-derivatives, Fig. 5), further subjected to molecular docking, in silico filtering and biochemical experiments to predict their blood-brain barrier (BBB) crossing potential [65]. The most promising BACE1 hit detected (~~namely~~ viz., compound 11) showed an $IC_{50}$ value of 15 µM [65]. In another molecular docking and in vivo study, it was revealed that melatonin and its derivatives displayed potent inhibitory activity for BACE1 [66]. Recently, molecular docking MD simulations and post-MD analyses showed one of these melatonin derivatives to exhibit strong allosteric BACE1 binding and considerable stability at eight different subsites [67].

More advanced MD methods such as m~~M~~ultiple r~~R~~eplica (MR) accelerated molecular dynamics (aMD) [68, 69] simulations combined with principal component analysis (PCA) [70] were used to investigate the effect of BACE1 disulfide bonds (SSBs) on the binding of three inhibitors (~~namely~~ viz., 3KO, 3KT, and 779), showing that SSB breaking impacts their binding modes as well as the structural flexibility and dynamics of inhibitor-BACE1 complexes [71]. A combination of MR Gaussian aMD (MR-GaMD) [72, 73] simulations and the MM-GBSA calculations showed a strong pH-

dependent protonation effect on the structural flexibility, correlated motions, dynamic behavior, and binding energetics of CS9 [74], C6U [75], and 6WE [76] inhibitors on BACE1, following the experimental $IC_{50}$ value trends [77].

BACE1 and BACE2 are structurally very similar [78]; hence designing BACE1 inhibitors with high selectivity is a particularly demanding and laborious task. Recently [79], a possible BACE1 inhibitor, namely, C28 [80], was reported to exhibit greater selectivity towards BACE1 than BACE2 compared to inhibitors Lanabecestat (AZD3293) [81] and AZD3839 [82]. To this end, classical (cMD), accelerated MD (aMD) simulations, and free energy calculations revealed similar binding affinities of AZD3293 to BACE1 and BACE2 and higher binding affinity of AZD3839 and C28 to BACE1 than BACE2. The underlying mechanisms of these findings are attributed to differences in electrostatic interactions patterns associated with different energy barriers, hence aiding the rational design of more potent BACE1 selective inhibitors [79]. Another molecular docking and molecular dynamic (MD) simulation study of several potential BACE1 and BACE2 ligands revealed that 8 (55E, 6Z2, 6Z5, BRW, F1B, GVP, IQ6, and X37) showed favorable binding towards both, with 6Z5 having the best binding potential [83]. Molecular docking and MD simulation studies combined with clustering analysis and phylogenetic studies identified 11-oxotigogenin (Fig. 5) as the most promising inhibitor against BACE1 [84].

Compounds from *Cajanus cajan* and *Citrus reticulata* plants were investigated for their BACE1 and AChE inhibitory properties using in silico methods to identify genistin, naphthalen-2-yl-acetic acid, 6-hydroxy-6-methyl-cyclodecyl ester, and vitexin (Fig. 5) as potent binders that also passed the oral druggability test, while naphthalen-2-yl-acetic acid and 6-hydroxy-6-methyl-cyclodecyl ester exhibited BBB permeation [85].

Molecular docking, MD simulations, and free energy calculations on the daidzin, genistin, mangiferin, puerarin, and tuberosin phytochemicals (Fig. 5) from *Pueraria tuberosa* showed that they are all potent binders within the BACE1 active site [86]. Molecular docking calculations predicted the binding modes of b-sitosterol and stigmasterol in the BACE1 binding site, with b-sitosterol being more favorable

compared to stigmasterol. Othman et al. [87] reported docking, absolute binding free energy calculations, and MD simulations on seven amide alkaloids, namely, N-trans-feruloyl-3-methoxytyramine (1), N-trans-feruloyltyramine (2), S-(−)-N-trans-feruloyl normetanephrine (3), S-(−)-N-trans-feruloyloctopamine (4), R-(+)-N-trans-feruloyloctopamine (5), N-trans-caffeoyltyramine (6), and S-(−)-3-(4-hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)-methoxyethyl]acrylamide (7), from halophytic plants *Bassia~~.~~ indica* and *Agathophora~~.~~ alopecuroides*, with compounds 1, 2, and 7 displaying strong BACE1 inhibition ($IC_{50}$ < 6 mg $mL^{-1}$) [87].

The inhibitory activities of 83 endophyte~~s~~-derived compounds and standard BACE1 inhibitors were assessed by means of molecular docking, MD simulations, druglikeness, a~~A~~bsorption-~~–~~d~~D~~istribution-m~~–M~~etabolism-e~~–E~~xcretion-t~~T~~oxicity (ADMET) and BBB properties, showing the remarkable inhibitory activity of asperflavin, ascomfurans C, camptothecin~~,e~~ and corynesidone A against BACE1, with corynesidone A being safe and able to transverse the BBB [88]. In order to prioritize candidates for BACE1 inhibitors, do Bomfim et al. [89] performed a hierarchical VS by pharmacophore model and molecular docking against 216,833 molecules contained in several databases. Four molecules were finally selected and evaluated for mutagenic potential, tested against the descriptors on Lipinski's Ro5 [90] and Veber rules [91], and were subjected to MD simulations to finally identify ZINC01589617 as a potential candidate for biological tests [89]. A docking study of more than 4000 naturally occurring compounds in the Vietnamese plants (VIETHERB) database combined with steered MD (SMD) simulations to show that myricetin, quercetin, and hydroxysafflor are remarkably better binders than the 23I BACE1 inhibitor [92]. SB docking screening approaches, Lipinski Ro5, and ADMET predictions along with MD simulations and MM-GBSA calculations were employed for a pharmacological activity analysis of 876 bioactive *Medhya R~~r~~asayana* plant compounds, to finally identify convolidine and N-(4-hydroxybutyl) phthalimide (Fig. 5) as potential BACE1 inhibitors [93]. A series of newly synthesized 6-chloro-N'-(substituted benzylidene)nicotinohydrazide were investigated experimentally and in silico to identify a single compound (~~namely~~ viz., P5) exhibiting high inhibition rates with $IC_{50}$ values of 0.205 ± 0.008 µM and 0.027 ± 0.001 µM against BACE1 and Aβ AChE,

respectively, also exhibiting high BBB permeation [94]. Molecular docking studies identified curcumin (Fig. 5), a natural flavonoid with potent antioxidant and anti-aging properties as a candidate BACE1 inhibitor [95]. Finally, Gupta et al. [96] performed VS, molecular docking, MD simulations, and binding free energy calculations to find five potential BACE1 inhibitors (B1, $C_{21}H_{23}N_5O_2$; B2, $C_{21}H_{23}N_5O$; B3, $C_{22}H_{30}FN_3O_3$; B4, $C_{19}H_{21}FN_4O_2$; and B5, $C_{22}H_{26}N_4O$) from the Asinex chemical library database [96].

### 3.1.2. Ligand- and Structure-Based Design; Cheminformatics

Another important CADD technique for the production of small-molecule inhibitors particularly when a receptor is not accessible is ligand-based (LB) design, as accurately described in previously published review articles [39, 97]. Because of the plethora of BACE1 crystal structures, hybrid SB virtual screening techniques including both SB and LB design were developed for discovering possible BACE1 inhibitors [98]. The application of QSAR approaches has previously resulted in the successful development of structure-activity relationship models with potential uses in binding affinity predictions of prospective BACE1 inhibitors [99]. Recently, QSAR models combined with molecular docking, MD simulations, MM-GBSA calculations, virtual screening, and pharmacophore modeling led to the discovery of natural compounds as BACE1 inhibitors that were screened for anti-amyloidogenic activity [100]. Iwaloye et al. [101] performed VS of ~33,000 natural compounds from the NPASS database [102] based on molecular docking, ADME/TOX, and QSAR analysis to identify four natural compounds (NPC469686, NPC262328, NPC29763, and NPC86744) as novel potential BACE1 inhibitors [101]. A five descriptor QSAR model was developed to finally screen five flavonols (isorhamnetin, syringetin, galangin, tamarixetin, rhamnetin) and two flavanonols (dihydromyricetin, taxifolin) (Fig. 5) natural compounds as potent BACE1 inhibitors [103]. Another QSAR-based VS study on a 26,467 food compounds database coupled with MD simulations and MM-GBSA calculations led to the identification of 4-(3,4-dihydroxyphenyl)-2-hydroxy-1H-phenalen-1-one as a hit BACE1 inhibitor [104]. 3D-pharmacophore, 2D-QSAR, and molecular docking in silico models were employed for the VS of a library containing more than three million curcumin and flavonoid derivatives, to finally identify 47

substances (~~two~~ 2 curcumins and 45 flavonoids) with remarkable predicted pIC50 values against AChE and BACE~~-~~1 (from 4.24 to ~~-~~5.11 and from 4.52 to ~~-~~10.27, respectively), as confirmed by in vitro assays [105].

A LB pharmacophore design produced a 64 molecule ensemble with BACE1 inhibitory activity, to finally select ~~three~~ 3 molecules (~~namely~~ viz., F3S4-m, F2S4-m, and F2S4-p) after molecular docking calculations, cheminformatics analyses, and in silico predicted toxicity screening for synthesis and evaluation, with the former ~~two~~ 2 displaying BACE1 inhibition ($IC_{50}$ 15.97 and 8.38 µM, respectively) [106]. In another study, a toxicity evaluation with the admetSAR online database [107, 108] along with molecular docking calculations and ADME prediction~~,~~ indicated q~~Q~~uercetin as a possible BACE1 inhibitor [109].

A new robust ligand-based predictive model for BACE1 inhibitory data was developed, validated according to Organization for Economic Co-operation and Development (OECD) principles as part of the Enalos Chemoinformatics Cloud Platform [110]. The online interface of the service has been deliberately developed for simplicity and user-friendliness, allowing individuals with no informatics background to readily utilize the BACE LB models and profit from the provided forecasts and outcomes, thus allowing the interested user to submit and virtually screen one or several compounds (Fig. 6) [110, 111]. Three different options are available for submitting a structure that include the following: (i) d~~D~~rawing a structure with the available sketcher –~~;~~ compounds can be easily generated and modified to create a set of structures that can be first visualized and then submitted;~~,~~ (ii) s~~S~~ubmitting the SMILES notation for one or many compounds at the form available;~~,~~ and (iii) s~~S~~ubmitting an .sdf file including a batch of compounds (Fig. 6). After importing the structures with one of the options described, the BACE workflow will run after the submit button is pressed. When structures are submitted, the results page will appear, which includes a class prediction for each of the structures submitted, and an indication of whether this prediction can be considered as reliable or not, based on the domain of applicability. This web service dedicated to the proposed model can easily facilitate the virtual screening of new structures that fall within its domain of applicability. In general, the Enalos Cloud Platform [112]

currently hosts 23 predictive models released as web services for a broad spectrum of material design and development, drug discovery, virtual screening of chemical substances, nanosafety, and safe-by-design (nano)materials applications (Fig. 7) and is being utilized by several EU research projects. As it has already been pointed out, the development of a predictive model might turn out unusable unless it is delivered as a user-friendly tool to ensure sustainability. Based on this, the platform is an easy-to-use portal where web -services are arranged by categories of use (chemoinformatics, nanoinformatics, image analysis, exposure, and biokinetics models) and projects so that users can quickly explore and choose the tool of their preference, easily use the models, and benefit from the produced predictions and results (Fig. 7).

### 3.1.3. Machine Learning

Modern machine learning (ML), the foundation of artificial intelligence (AI), has had a significant influence on all branches of research, including chemistry [113, 114]. Compared to classic computational approaches, new ML methods based on deep neural networks and representation learning tend to deliver superior prediction quality [113]. In this context, Singh et al. [115] performed a classification analysis on 3536 different BACE1 inhibitors taken from the bBinding DB database by extracting two types of descriptors, namely, molecular property (Mordred) and fingerprints (PubChem, MACCS, and KRFP), on the basis of which ML algorithms like Naïve Bayesian (NB), nearest known neighbors (kNN), support vector machine (SVM), random forest (RF), and gradient-boosted algorithms (XGB) were applied to develop classification models. The BACE1 inhibitors were divided into 11 subgroups, and their structural properties were studied, identifying important fragments shared between active and inactive BACE1 drugs, thus developing a model for building and virtual screening [115].

In a recent example of combining ML and deep learning (DL) approaches with VS for the identification of potential BACE1 small--molecule inhibitors, a dataset containing 57 AChE and 53 BACE1 N-benzyl piperidine derivatives was used for multi-target directed ligand-based 2D-QSAR model development using five different classes of

molecular descriptors, namely, structural (Cl element counts, number of rotatable bonds), electro-topological (number of oxygen atoms connected with one double bond), electronic (induced dipole moment along the Z_axis), and spatial (extent of molecule shadows) for BACE1 [116]. Linear, genetic function approximation (GFA);~~,~~ nonlinear, SVM; and artificial neural network (ANN) ML methods were used to show that these molecular descriptors should be further utilized in the rational design of multi-targeted anti-lead Alzheimer'~~'~~s compounds [116], highlighting the strength and great promise that this approach holds in the future of BACE1 inhibitor discovery.

ML methods are commonly utilized to analyze chemical compound ligand activity toward putative target proteins like BACE1. The exploration of highly selective ligands is critical for the creation of novel pharmaceuticals with improved safety. The lack of data on true negative compound-protein interactions (i.e., molecules that do not bind to relevant proteins) is key to building such predictive models for inhibitor efficacy [117]. To address this challenge, a graph convolution neural network (GCNN) approach was suggested by Miyazaki et al. [117], aiming to thoroughly investigate natural compounds targeting BACE1 with reduced off-target effect toward cathepsin D. Results unveiled significantly different BACE1 and cathepsin D ligand distributions on the density map, which is likely to hasten the search for new candidates for highly selective AD therapeutics [117]. Lastly, based on multiple-property optimization via gradient descent in the latent space, a generative network complex (GNC) was developed to automatically generate many novel BACE1 inhibitors, as well as thousands alternatives to commercially available pharmaceuticals, including c~~C~~eritinib, r~~R~~ibociclib, a~~A~~calabrutinib, i~~I~~delalisib, d~~D~~abrafenib, m~~M~~acimorelin, e~~E~~nzalutamide, and p~~P~~anobinostat [118].

## 3.2. Other T~~t~~echnique-B~~–b~~ased M~~m~~ethods

### 3.2.1. Quantum M~~m~~echanical A~~a~~pproaches

Quantum mechanical (QM) approaches comprise ab initio, semi-empirical, empirical, and d~~D~~ensity f~~F~~unctional t~~T~~heory (DFT) methods [59, 119–122] and have been extensively employed in the elucidation of small peptide BACE1 modulators as well as in binding energy predictions of potential inhibitors [123]. The estimation of

binding affinities with high accuracy is very important; hence QM approaches are growing in popularity in computational drug design and development. Briefly, QM methods aim to approximate the wave function and determine the electronic structure of a molecule or polyatomic system by solving the electronic Hamiltonian of the Schrödinger equation. DFT methods use the electron density instead of the wave function that describes the system under study to calculate its electronic energy [121]. QM methods allow the accurate calculation of many molecular properties such as equilibrium structures, vibrational frequencies, dipole moments, binding free energies, reaction paths, etc. but are computationally very expensive and time-consuming and are therefore limited to molecular systems comprising a small number of atoms. Recently, DFT coupled with molecular docking calculations were used with in vitro and in vivo experiments to investigate BACE1 inhibition by 3150 phytochemicals from almost 25 different plants [124]. These calculations together with ADMET studies revealed seven phytochemicals (shinflavanone, glabrolide, glabrol, and prenyllicoflavone A from *Glycyrrhiza glabra*, macleanine from *Huperzia serrata*, 3a-dihydro-cadambine from *Uncaria rhynchophylla*, and valvalerelactone B from *Valeriana officinalis*) having high BACE1 inhibitory activity [124]. In another study, two potent BACE1 inhibitors, namely, AM-6494 [125], a newly reported potent BACE1 inhibitor picked for preclinical considerations, and Umibecestat (CNP-520) [126, 127], recently discontinued at human trials, were investigated using DFT and our own N-layered integrated molecular orbital and molecular mechanics (ONIOM) [128] calculations to illustrate AM-6494 as more favorable towards BACE1 inhibition ($\Delta G_{bind}$ = −62.849 kcal mol$^{-1}$) than CNP-520 ($\Delta G_{bind}$ = −33.463 kcal mol$^{-1}$), with the calculated binding free energy reproducing the in vivo inhibition trends ($IC_{50}$ values of 0.4 and 11 nM, respectively) [129]. Gnanaraj et al. [130] investigated karanjin – a furanoflavonoid isolated primarily from *Pongamia pinnata* – with extensive docking, MD simulations, frontier molecular orbitals (FMOs), and DFT calculations, using the Lipinski's rule of five (Ro5) [90] and ADMET to show that it could be considered as a suitable therapeutic lead [130]. Lastly, a nonlinear optical (NLO) responses computational investigation of the active phytochemicals of the *Clitoria ternatea* at

the B3LYP/6-311G++(d, p) level of theory coupled with docking and MD simulations identified the kaempferol glycoside CClitorin as the most active and inhibiting compound towards BACE1 [131].

# 4. Future Outlook

Alzheimer's disease has unmet medical requirements, particularly in terms of disease-modifying medicines. For many years, the role of Aβ amyloid in AD pathogenesis has been central to drug discovery efforts, leading to a substantial number of drugs targeting BACE1. Unfortunately, the majority of these drugs have failed in clinical trials [43] for reasons that are being actively investigated. It has long been hypothesized — and is now being thoroughly explored — that multitarget medication therapy is probably preferable to single-target therapy for treating complicated neurological illnesses like AD. It is inevitable to adopt new strategies for the CADD of new drugs against AD considering the associated factor range responsible for the initiation, progression, and severity of the disease. However, this increases the complexity of their pathophysiology and is linked to the ineffectiveness of the available therapeutic tools [39]. In a recent example, molecular docking, MD simulations, artificial neural networks, and multilinear regression models were employed for the virtual screening of 20,397 small compounds (MW < 600) extracted from the ZINC database [132]. Three potential multifunctional drug candidates acting simultaneously toward AChE, SERT, BACE1, and GSK3β protein targets were proposed [132].

Another promising aspect involves CADD design for drug delivery systems (DDS) in order to ensure improved effectiveness and minimal adverse effects of novel BACE1 pharmaceuticals. In this regard, highly porous inorganic materials such as metal-organic frameworks (MOFs) are currently being investigated as DDS candidates for the administration of various pharmaceuticals, owing to their simple preparation, regulated release, and organ-targeting advantages [133]. For example, recent studies showed that magnolol – a phenolic natural product – exhibits neuroprotective properties through degradation of Aβ-amyloid plaques in PC-12 cells [134] and prevention of behavioral impairments and neuropathological findings

in transgenic mice models (TgCRDN8) [135]. The inhibitory activity, bioavailability, and BBB properties of magnolol against BACE1 and $AlCl_3$ were significantly enhanced by the use of UiO-66(Zr) MOF as determined by in silico molecular docking, MD simulation studies, and extensive in vitro evaluations, to confer that MOFs are promising DDS platforms for poorly bioavailable drugs [136].

It has already been discussed in this chapter's introduction that environmental neurotoxicants have been linked to neurodegenerative diseases including Parkinson's and Alzheimer's and is currently another intriguing field of scientific research. BACE1 comprises a significant pathogenic target of environmentally induced neurotoxicity, with neurotoxicants such as metals, pesticides, herbicides, fungicides, polyfluoroalkyl compounds, heterocyclic aromatic amines, advanced glycation end-products, and acrolein being prime examples that can modulate BACE1 [137]. In this category, per- and poly-fluoroalkyl substances (PFASs) are present in a wide variety of industrial and consumer applications, the majority of which having unknown hazardous potential in terms of bioactivity, bioaccumulation, and toxicity. Several studies reported on neurotoxicity of PFAS and particularly on their effects on neurotransmission [138]. In this respect, it was recently attempted to develop a powerful ML-based QSAR model for the prediction of 4730 PFASs bioactivity from the OECD report [139], where a total of 6 different data sets including BACE1 [140] were utilized for the construction of the PFAS-specific data [141].

Overall, future CADD developments in the field of BACE1 inhibitors are extremely promising, paving the way in providing innovative means and improved understanding for the generation of a diversified and expanded arsenal of pharmaceuticals against Alzheimer's disease.

# 5. Conclusions

Alzheimer's disease is a neurodegenerative illness causing neuronal death and synaptic degeneration in the human brain. The Aβ pathway, which leads to amyloid plaques, is considered one of the primary biological pathways for the development of Alzheimer's therapeutics. The BACE1 protein is a key center target at the heart of

the amyloid pathway. The principal BACE1 structural features and functions that render it an attractive target for computer-aided drug design methodologies aimed at the production of novel small-molecule inhibitors were presented. Recent examples of state-of-the-art CADD techniques and approaches employed in the development of BACE1 inhibitors were also summarized.

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Fig. 1 Representation BACE1 function in the amyloidogenic pathway. BACE1 cleaves APP to form Aβ peptides. (Figure reproduced with permission from Ref. [25])

Fig. 2 The *Homo sapiens* three-dimensional BACE1 structure [28]. The N- (cyan) and C- (lime) terminal lobes, the "flap" (blue), the bound ligand (brown), and the catalytic dyad of Asp32/Asp228 (red) are illustrated. (Figure was prepared by means of VMD [142]).

Fig. 3 Superimposed BACE1 crystal structures with (green, PDB ID: 3TPP) and without (orange, PDB ID: 3TPJ) a bound inhibitor (grey) reveal the “flap” conformational change. The catalytic aspartate residues are also shown (red).

Fig. 4 Chemical structures of the OM99-2 and OM00-3 inhibitors.

Fig. 5 Selected structures of potential small-molecule BACE1 inhibitors discussed in this chapter.

Fig. 6 Screenshot of the Enalos Chemoinformatics Cloud Platform input page for the BACE ligand-based predictive model [111].

Fig. 7 The integrated in silico Enalos Cloud Platform toolset of innovative chem-, bio-, and& nano- informatics models and services [112].