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**REVIEW ARTICLE**

# Potential Therapeutic Approaches to Alzheimer's Disease By Bioinformatics, Cheminformatics And Predicted Adme-Tox Tools

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**Abstract:** ***Background:*** Alzheimer's disease (AD) is considered a severe, irreversible and progressive neurodegenerative disorder. Currently, the pharmacological management of AD is based on a few clinically approved acethylcholinesterase (AChE) and N-methyl-D-aspartate (NMDA) receptor ligands, with unclear molecular mechanisms and severe side effects.

***Methods:*** Here, we reviewed the most recent bioinformatics, cheminformatics (SAR, drug design, molecular docking, friendly databases, ADME-Tox) and experimental data on relevant structure-biological activity relationships and molecular mechanisms of some natural and synthetic compounds with possible anti-AD effects (inhibitors of AChE, NMDA receptors, beta-secretase, amyloid beta (Aβ), redox metals) or acting on multiple AD targets at once. We considered: (i) *in silico* supported by experimental studies regarding the pharmacological potential of natural compounds as resveratrol, natural alkaloids, flavonoids isolated from various plants and donepezil, galantamine, rivastagmine and memantine derivatives, (ii) the most important pharmacokinetic descriptors of natural compounds in comparison with donepezil, memantine and galantamine.

***Results:*** *In silico* and experimental methods applied to synthetic compounds led to the identification of new AChE inhibitors, NMDA antagonists, multipotent hybrids targeting different AD processes and metal-organic compounds acting as Aβ inhibitors. Natural compounds appear as multipotent agents, acting on several AD pathways: cholinesterases, NMDA receptors, secretases or Aβ, but their efficiency *in vivo* and their correct dosage should be determined.

***Conclusion:*** Bioinformatics, cheminformatics and ADME-Tox methods can be very helpful in the quest for an effective anti-AD treatment, allowing the identification of novel drugs, enhancing the druggability of molecular targets and providing a deeper understanding of AD pathological mechanisms.

**Keywords:** Alzheimer's disease, bioinformatics, cheminformatics, synthetic and natural compounds, QSAR, docking.

## 1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder extremely prevalent in the world population, as ~47 million people are suffering from the disease and the number is expected to increase by 62% in 2030 due to the global aging of populations [1]. Also, it was estimated that the number of people affected by dementia will reach ~115 million by 2050 [2]. According to the World Alzheimer Report, 818 billion dollars were allocated worldwide in 2015 to support AD patients [2]. Concerning AD etiology, some high-impact risk factors were identified as genetics [3-6], age [7-9], cardiovascular [10, 11], obesity [12, 13] or lifestyle [12].

In the discussion about neurodegenerative disorders, it is necessary to mention the epigenetic point of view that is significantly relevant to neuroscience, as it refers to brain development and neuronal differentiation, as well as to more dynamic processes related to cognition [14-17].

An interesting review was published by Pena-Bautista *et al.* [15] on AD physiopathological mechanisms relevant to the improvement of early diagnosis and to the development of potent treatments based on omics-based biomarkers. The paper reviewed the most recent advances in metabolomics/lipidomics, epigenomics and proteomics applied to early AD diagnosis. The main research lines are represented by the evaluation of: (i) metabolites resulted from lipids, amino

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acids and neurotransmitters metabolisms, cholesterol biosynthesis, Krebs and urea cycles; (b) some microRNAs and proteins (microglobulins, interleukins) related to a common network with amyloid precursor protein and tau [15]. Stoccoro *et al.* [17] also reported promising results of studies performed on peripheral blood DNA that could provide early biomarkers in AD.

Cerebrospinal fluid biomarkers are broadly investigated in AD and are applied in clinical practice. Cerebrospinal fluid biomarkers like amyloid beta (Aβ), total tau and phosphorylated tau in AD condition reflect the extent of neuronal damage, and may be used as quantitative traits for genetic analyses [18]. The study of de Matos *et al.* highlighted five genes involved in AD pathogenesis, namely APOE, LOC100129500, PVRL2, SNAR-I and TOMM40 [18]. Recent investigations identified some loci, namely INPP5D, CD2AP and CASS4 that mediate AD susceptibility and are high-incidence risk factors in AD [18-20]. Additionally, apolipoprotein E [APOE4 allele] was identified as a high-incidence risk factor in AD [21, 22].

The molecular mechanisms that lead to the very complex symptoms of AD are not fully elucidated, but some "AD hypotheses" were postulated: cholinergic, Tau, glutamatergic, amyloid cascade or oxidative stress [23, 24]. Many years ago, it was postulated that the deficit of cholinergic neurons is involved in AD symptoms [25-27]. Today, scientists continue to pursue this hypothesis, as important research is focused on acetylcholine (ACh) synthesis and its biological functions in the brain during AD [28, 29]. It is widely known that acetylcholinesterase (AChE) plays an important role in memory and learning [30]. Increasing the level of ACh by applying AChE inhibitors represents a suitable way in AD therapeutic approach [31, 32].

Tau hypothesis [33-35] postulates that the excessive or abnormal phosphorylation of Tau protein and its transformation into PHF-Tau (paired helical filament) and NFT'-Tau (neurofibrillary tangles) precedes AD. A study performed by Merlini *et al.* [36] showed that pathological cerebrovascular remodeling is an early-onset Braak-tau related process occurring independently of amyloid-related angiopathy or AD condition and having the potential to contribute to downstream amyloid-induced vascular effects seen in AD.

N-metyl-d-aspartate (NMDA) receptors hypothesis [23] postulates that the hyperactivation of NMDA receptors in AD condition: (i) enhances the influx of calcium ion, leading to the production of free radicals that further contribute to neuronal death; (ii) the increase in calcium, sodium and chloride levels as a result of NMDA glutamate receptors hyperactivation was associated with excessive depolarization of the postsynaptic membrane, the onset of neurodegenerative processes and cell death [23].

The amyloid cascade hypothesis of AD suggests that, in AD, abnormalities occur during the secretion of the amyloid precursor protein (APP), leading to an unbalance between production and clearance of Aβ [23, 37].

A different interesting AD hypothesis refers to the Metabolism-Centric pathogenesis of AD. Kang *et al.* [38] considered that AD is "as a kind of metabolic disease", suggesting that insulin, adiponectin and antioxidants may be considered AD therapeutic targets. They identified that patients with AD presented reduced insulin signal transductions in the brain and showed that intranasal insulin injections are beneficial in AD treatment. In addition, the reduction of adiponectin in patients with obesity induces metabolic dysfunctions both in the body and in the brain, leading to AD pathogenesis [38].

The oxidative stress hypothesis [23, 38, 39] postulates that oxidative stress can represent a risk in AD. In their paper, Prasad *et al.* [39] described the possible pathways by which the oxidative stress and chronic inflammation are some of the earliest defects that promote AD. It was mentioned that up-regulated microRNAs induced neurodegeneration by: (i) decreasing the levels of a nuclear transcriptional factor-2 (Nrf2), (ii) reducing the levels of α-secretase ADM10; and (iii) reducing the levels of phosphatases. Instead, the down-regulated microRNAs induced neurodegeneration by: (i) increasing the levels of β-secretase, (ii) increasing the levels of tau kinase; (iii) elevating the levels of tau proteins; or (iv) increasing the levels of nuclear factor-kappaB (NF-kB) [39]. The exact connection between protein damage and neuronal death is still unknown. Recently, autophagy was mentioned as a possible AD mechanism [40, 41].

Rare forms of early-onset familial AD (EO-FAD) are induced by gene mutations, especially in APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN 2) genes [42-44]. Approximately, 300 mutations occurring in PSEN1 or PSEN2 have been reported in the Dementia Mutation Database [6, 45]. The majority of these mutations were observed in PSEN1 and over 230 mutations were reported as pathogenic in Alzforum database [7, 45].

Presenilin proteins present a critical involvement in EO-FAD development by intramembrane cleavage of APP and the generation of Aβ [44]. Structurally, PSEN1 protein contains nine transmembrane (TM) domains, connected by hydrophilic loop regions. Being a member of γ- secretase complex, PSEN1 works as a catalytic subunit of aspartyl protease, involved in the cleavage of C99 residue in APP protein into Aβ peptide. It was shown that PSEN1 mutations lead to the reduction of Aβ production [44, 46]. PSEN1 contains five native cysteine residues, and all of these can be replaced with serine to form a cysteine-less PSEN1 that retains the ability to assemble into an active γ-secretase complex [47].

An interesting experimental and *in silico* mutagenesis study on PSEN1 [45] showed that: (i) Trp165Cys mutation is located in TM-III region that is conserved between PSEN1/PSEN2, (ii) as proved by *in vitro* studies, PSEN1 Trp165Cys could result in amyloid metabolism disturbance, (iii) PSEN1 p.Trp165Cys may be commonly associated with EO-FAD. All these findings support the hypothesis that presenilins may be used to identify the relatives at risk that may be potential candidates for clinical trials.

The presence of redox metals like iron, copper or zinc and also of aluminum or manganite is considered another hypothesis for AD progression. This hypothesis was extensively studied in the last years by considering the molecular level interactions of metal ions like iron [48, 49], copper (I)

[50, 51] or zinc [51] with the Aβ peptide leading to reactive oxygen species (ROS) production. Telling *et al.* [48] identified the presence of iron biomineral deposits in the cortical tissue and provided the evidence that Aβ-induced chemical reduction of iron could occur in *vivo*. Using advanced X-ray microscopy techniques at sub-micron resolution, the authors investigated the specific role of iron in amyloid deposition and AD pathology and they established the relationships between iron biochemistry and AD pathology in intact cortex. The study results supported a strong correlation of amyloid plaque morphology with iron and the formation of an iron-amyloid complex [48].

Atrián-Blasco *et al.* [51] mentioned that copper and zinc ions are able to induce amyloid-related diseases, including AD, by modifying the aggregation pathways of Aβ peptides. Cu(i), Cu(ii) or Zn(ii) coordination by Aβ has been extensively studied in the last years, but resulting heterobimetallic Aβ complexes are still poorly characterized. Lam *et al.* [49] mentioned that ferroxidase activity is altered in various biological fluids in neurodegenerative diseases, but the sources contributing to the altered activity are uncertain.

A hypothesis that gained increasing attention during the years is the mitochondrial cascade hypothesis [52-55]. Mitochondria in the brain of AD patients present altered functions and turnover: decreased membrane potential, ATP levels, cytochrome c oxidase and other metabolic enzyme levels and increased oxidative stress, production of ROS or mitochondrial fission [54, 56]. The mitochondrial cascade hypothesis was discussed in conjunction with amyloid hypothesis. In a recent extensive review, Swerdlow [54] presented evidence on the existence of both an independent AD mitochondrial dysfunction (primary cascade) that triggers the amyloid cascade by altering APP expression, processing and accumulation and Aβ-induced mitochondrial dysfunctions (secondary cascade). The author assumes the possibility that the two cascades co-exist. He argues in favor of the primary mitochondrial cascade by taking into account that changes in mitochondrial function also occur in other tissues outside the brain, temporally preceding changes in Aβ homeostasis and the deposition of Aβ plaques under specific bioenergetic conditions that can be induced by mitochondria dysfunction [54]. The primary mitochondrial cascade hypothesis shifts the attention from Aβ and tau as determinants of AD condition. It considers Aβ as a marker of brain aging not limited to AD patients, estimating that AD therapy by Aβ removal would be effective to the extent that brain damage is mediated by Aβ [53]. The hypothesis opens a new therapy direction involving the prevention of age-related mitochondrial dysfunctions [54] and pharmacological modulation of cell bioenergetic pathways and mitochondrial functions [54, 57].

## 2. BRIEF OVERVIEW OF BIOINFORMATICS AND CHEMINFORMATICS TOOLS APPLIED TO ALZHEIMER MANAGEMENT

### 2.1. Bioinformatics Tools

The study of AD molecular mechanisms and the prediction of natural and synthetic compounds anti-AD therapeutic effects could benefit from the usage of bioinformatics and cheminformatics methods. A brief description of some bioinformatics and cheminformatics tools that are widely used in various pathologies, including AD, are presented below.

Bioinformatics studies are performed by using information deposited in databases. Some databases are freely available, like UniProt [58], Protein Data Bank (PDB) [59], Expasy [60], NCBI Gene [61] or AlzGene [62]. These comprise protein sequences and functional information, many entries being derived from genome sequencing projects. The experimental data is doubled by a wealth of information on protein biological functions derived from the research literature.

UniProt database [58] contains useful information on proteins and their encoding gene names, their functions, enzyme-specific information, such as catalytic activity, cofactors and catalytic residues homology, subcellular location, protein-protein interactions, *etc*. The UniProt query on AD leads to the identification of almost 620 entries. We accessed presenilin-1 (Uniprot code P49768 [63]) and presenilin-2 (Uniprot code P49810 [64]), two proteins strongly involved in the induction of AD. Their homology is presented in Fig. **1**.

Also, we addressed the possible role of natural and mutant presenilin variants in AD. We obtained interesting results on the mutant variants of presenilin 1 (P49768), in which case we identified several mutation positions, some of them with unknown pathological significance (*e.g.* 177F → L [65], 35 R → Q [65], 79A → V [65]), but some of them with proved connection with AD, like 113 L → P involved in frontal dementia [66], 214 H → Y, a probable disease-associated mutation founded in a patient with dementia [67] or 436P → Q that partially abolishes gamma-secretase activity [68]. In the case of presenilin 2 (P49810), the mutations 148 V → I were identified to be involved in late-onset of AD [69], or 239 M → V, identified just in AD Italian patients [70].

Protein Data Bank [59] is a freely accessible crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The deposited data is typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy. In PDB we found almost 187 structural entries of proteins involved in AD. At this moment, the newest entries present the structure of a mutant amyloid protein precursor inhibitor (T11V/M17R/I18F/F34V) in an interaction with a human serine protease (mesotrypsin) (PDB code 6GFI) [71] or the structure of C-terminal modified Tau peptide-hybrid 4.2e-I with 14-3-3sigma (PDB code 6FAU) [72]. Also, a wealth of structural information is available on beta-secretase 1 (BACE 1) inhibitors, that are very attractive for AD treatment (more than 300 entries, *e.g.* structures 6EJ3 (Fig. **2**), 6EJ2 [73]).

Another important database for AD studies is AlzGene database [62]. It contains extensive information on AD genes, meta-analyses and AD treatments. It provides VCPA, SNP/Indel Variant Calling Pipeline and data management tools that are useful for the analysis of whole genome and exome sequencing (WGS/WES) for Alzheimer's Disease Sequencing Project [74].

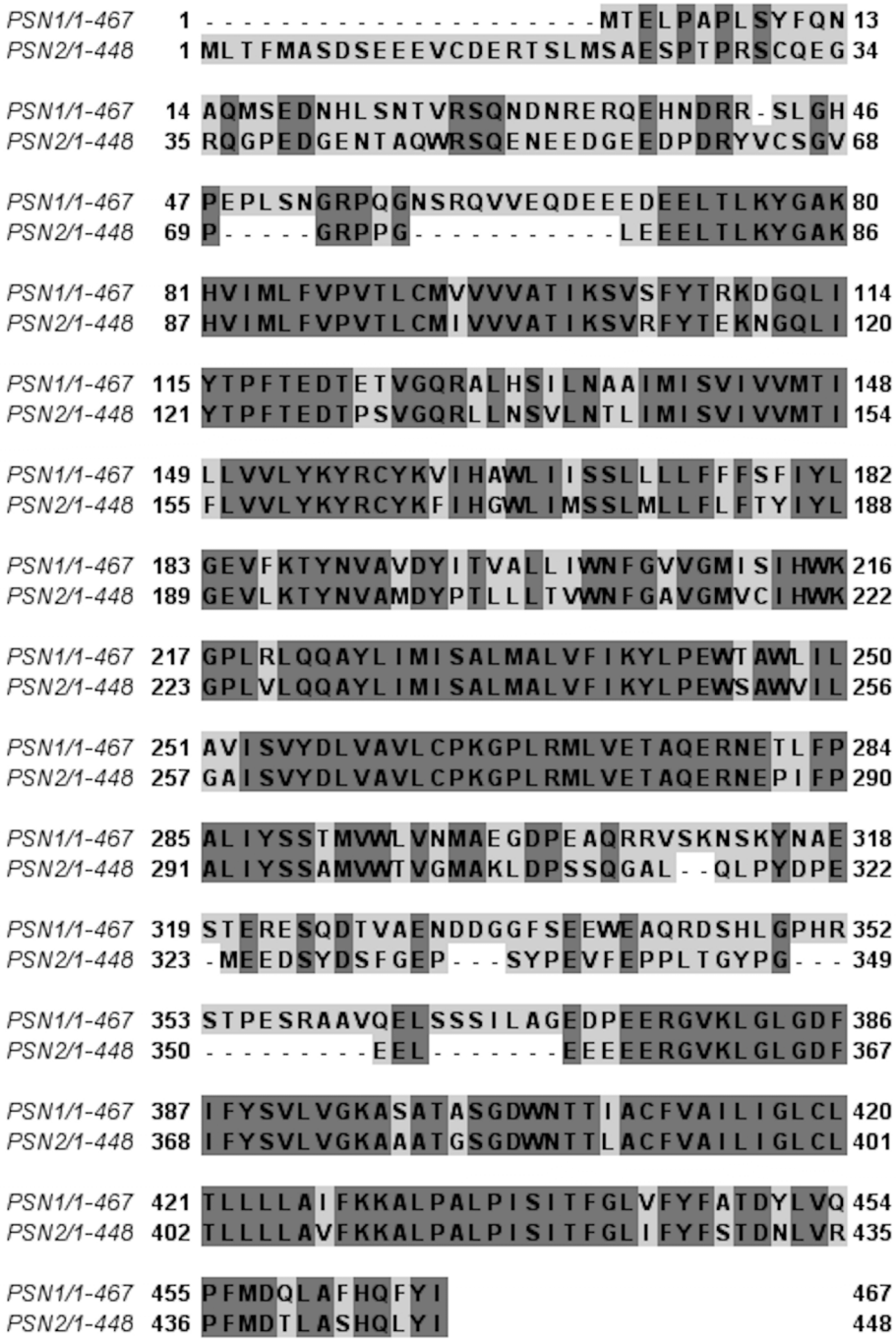


**Fig. (1).** Sequence alignment between human presenilin-1 (PSN1 - Uniprot code P49768 [63]) and presenilin-2 (PSN2 – Uniprot code P49810 [64]). The conservation of residues is marked with grey (high) to white (no conservation). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fang *et al.* [75], by simultaneously using real-time PCR and bioinformatics tools, identified genes associated with the cellular processes closely related to AD progression. Rao *et al.* [76] used bioinformatics tools to identify almost 74 genes strongly involved in AD. The authors retrieved the encoded functional protein sequences in FASTA format from NCBI (National Center for Biotechnology Information) [61] and determined their role based on ClustalW multiple sequence alignments. In addition, they constructed a phylogenetic tree using the functional protein sequences. This study reported once more that the genetic components of AD are more important in comparison to environmental, metabolic and age related factors [76].

In 2012, Sun *et al.* [77] performed a bioinformatics analysis of anti-AD herb ingredients. Results showed that the herbs might treat AD symptomatically or might modify the disease progression through inflammation-associated pathways, cancer-associated pathways, diabetes-associated pathways, *etc*., that are closely related to AD. In their research,

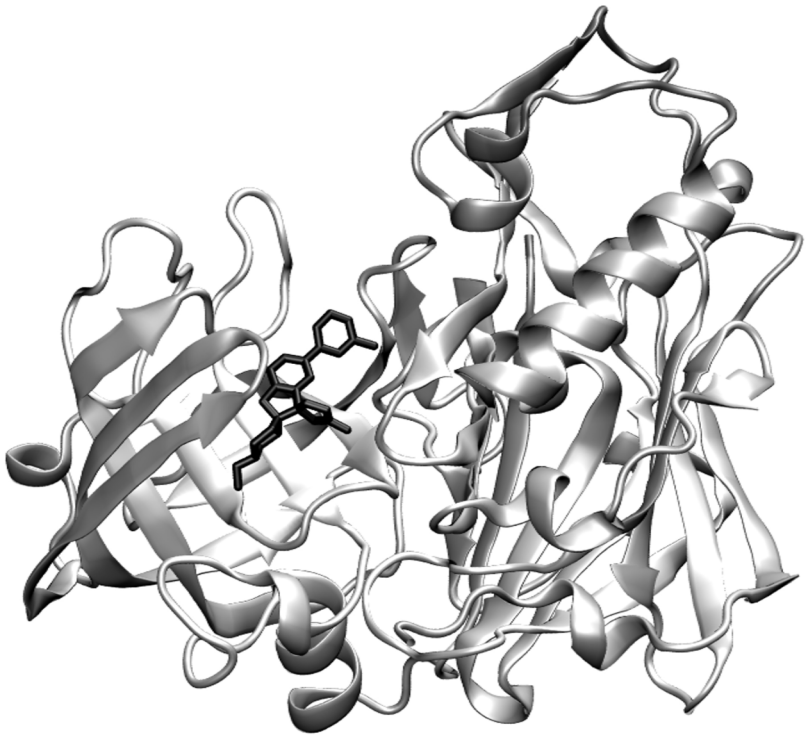

**Fig. (2).** Crystal structure of BACE1 (silver backbone) in complex with compound 23 (black licorice) according to 6EJ3 structure [73]. Compound 23 is a molecule from a series of 47 compounds that were cocrystalized and analyzed in their BACE1 inhibitory activity [73]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

authors accessed two complex databases, namely the Therapeutic Targets Database (TTD) and Online Mendelian Inheritance in Man (OMIM) database. They reported that [77]: (i) five successful therapeutic targets were collected from TTD: AChE, 5-hydroxytryptamine receptor 2A (5-HT2A), cholinesterase (BChE), 5-hydroxytryptamine receptor 1A (5-HT1A) and Glutamate [NMDA] receptor and (ii) four AD disease genes were identified in OMIM: amyloid precursor protein (APP), presenilin 1 (PSEN1), presenilin 2 (PSEN2) and Apolipoprotein E (APOE). Their corresponding proteins were obtained according to Uniprot database.

When addressing the pharmacokinetic and pharmacodynamic features of anti AD drugs, relevant information can be found in databases like DrugBank [78] or FooDB (http://foodb.ca/). Regarding ADME-TOX bioinformatics tools, some useful databases are Tox-DATABASE [79], pkCSM platform [80, 81] or ProTox-II [82].

DrugBank [78] contains entries on drug properties, functional and bioeconomic information on large sets of small compounds. Both bioinformatics and cheminformatics tools collect the detailed information regarding features of drugs (*i.e*. chemical, pharmacological, pharmaceutical, economical, *etc*) with comprehensive drug target information (*i.e*. sequence, structure, and molecular pathway).

FooDB (http://foodb.ca/) is a freely available, open-access database containing chemical structures represented by micro and macronutrients found in common unprocessed foods. Users are able to browse information from FooDB based on the food source, name, descriptors or function. From our experience, FooDB is the largest comprehensive resource on the chemistry and biology of food constituents. Each chemical entry from FooDB represented by natural compounds contains more than 100 data fields covering detailed compositional, biochemical and physiological information.

The traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) [83], operating under a Open Database License, comprises information on 499 Chinese herbs in terms of their active ingredients, molecular targets and associated diseases. TCMSP can be used to identify drug-target and drug-disease networks involving compounds from Chinese plants. Additionally, each compound is characterized by twelve ADME properties, including human oral bioavailability, half-life, drug-likeness, Caco-2 permeability, blood-brain-barrier (BBB) permeability and Lipinski's rule of five, all of which can help the screening of compounds promising for drug discovery and development.

ADME-Tox database generally comprises ADME-Tox information (adsorption, distribution, metabolism, excretion and toxicity), critical for drugs, but more importantly for *de novo* chemicals and herbal compounds. pkCSM database [80, 81] uses the SMILES structure file of molecules to predict descriptors like: Absorption - intestinal absorption, Caco-2 permeability, water solubility, P-glycoprotein substrate, skin permeability, Distribution – state volume of distribution, BBB permeability, CNS permeability, Fraction unbound, Metabolism –cytochrome substrates; Excretion-renal OCT2 substrate and total clearance and Toxicity- Rat LD50, AMES toxicity and echo-toxicity.

### 2.2. Cheminformatics Tools

Quantitative structure-activity relationship (QSAR) is a very useful tool in medical chemistry when investigating the interactions between synthetic/natural compounds and their targets, such as enzymes or membrane receptors. This method is especially useful when the fast prediction of "uncharacterized" natural compounds biological activities is required.

In the background, all QSAR methods consider that the chemical structures of compounds determine their biological activities and each modification of their chemical structure changes their biological activity. QSAR models are created depending on each program. For instance, in 2D QSAR, the biological activity of compounds is calculated as follows:

Biological Activity (log1/experimental features of compounds) = const + $(c_1 \bullet d_1) + (c_2 \bullet d_2) + (c_3 \bullet d_3) + ...$,

where the parameters $d_1$-$d_n$ represent molecular features (*e.g.* atom and bound counts, molecular weight, sum of atomic properties topological descriptors) of each chemical compound and the coefficients $c_1$ through $c_n$ are evaluated as fitting the variation in the parameters and the biological activity [84].

Basically, QSAR methods consider different types of probe atoms, manners of calculating the electronic interactions and force fields [85]. Alignment based 3D-QSAR-CoMFA (Comparative Molecular Field Analyses) considers steric (Lennard-Jones) and electrostatic (Coulombic) force fields to predict the interactions between compounds and macromolecular targets [86], while 3D-QSAR-CoMSIA (Comparative Molecular Similarity Indices Analysis) requires steric, electrostatic, hydrophobic and atom hydrogen donor/acceptors electronic fields [86]. Non-alignment 3D-QSAR ALMOND considers many atom probes such as, hydrogen bond acceptor (carboxyl-O::)/donor (nitrogen in cationic state as atom probe), electrostatic (water as atoms probe), hydrophobicity, ions (natrium, potassium, calcium, *etc*), individually or in different combinations [87].

Other important cheminformatics tools are represented by the evaluation "drug-likeness" features, given by Lipinski's "rule of five" and Veber rules. Lipinski's "rule of five" is an important criterion for the identification of chemical compounds likely to preset pharmaceutical activity into biological systems. According to this rule, the drug-likeness of a chemical compound is given by the following features: (i) its chemical structure presents no more than 5 hydrogen bond donors and no more than 10 hydrogen bond acceptors, (ii) its molecular mass is less than 500 Daltons, (iii) octanol-water partition coefficient log *P* is not greater than 5 [88]. Starting from Lipinski rules, Benet *et al.* [89] mentioned the Biopharmaceutics Drug Disposition Classification System (BDDCS) of drug ability. In this respect, cheminformatics software as Molecular Operating Environment-MOE (Chemical Computing Group) or VolSurf+ (Molecular Discovery), Biovia Discovery Studio (Dassault Systems, Biovia) are helpful.

A "me-too" compound or "follow-on" drug is a chemical compound that usually has a very similar structure with a known active pharmaceutical compound (namely parent drug), but comprises a very active chemical part, leading to higher biological activity versus parent drug. In some respects, the "me-too" compound may present a different pharmacokinetics profile relative to the parent drug, but uses the same molecular mechanism as the parent drug and is used for the same therapeutic purpose as the parent drug [90]. Besides "me-too" compounds, the "me-better" compounds (also called best-in-class) [91] represent leader compounds, with improved activity, selectivity and potency over original compounds [90].

"Follow-on" drugs benefit patients and the health care system in several ways [92]: (i) reduction of health care costs by providing price competition, (ii) providing therapeutic improvements for some patients, especially in the pharmacogenomics context; (iii) providing superior or more flexible dosage and administration; (iv) "perhaps most important, a follow-on drug, which typically has a longer remaining patent life than pioneers, motivate and support research that applies to an entire therapeutic class" [92]. Becker *et al*. [93] mentioned the importance of "follow-on" compounds in psychiatric disorders, with a special mention in AD.

Another important method in drug design is represented by Fragment-Based Lead Discovery. It is used for finding lead compounds during the drug discovery process [94]. Basically, the method identifies the small chemical fragments that may bind only weakly to the biological target, and then growing them or combining them to produce a lead with a higher affinity. This method was applied with real success in AD treatment development, in designing BACE-1, Aβ or tau protein inhibitors [95, 96]. Also, another structure-based drug design method, namely *de novo* design of new ligands, was used for the design of anti-AD drugs [97].

The understanding of binding interactions between any proteins or membrane receptors and their small ligands plays a key role in the rationalization of affinity and selectivity of the ligands [98]. In this respect, the fragment molecular orbital method (FMO) can compute very large molecular systems with thousands of atoms using *ab initio* quantum-chemical wave functions. The critical advantage of FMO is that it can reveal atomistic details about the individual contributions and chemical nature of each residue and water molecule toward ligand binding, which would otherwise be difficult to detect without the usage of quantum mechanics approaches [98].

Shinzato *et al.* [99] reported *ab initio* molecular simulations applied to curcumin derivatives used in AD therapy. The authors proposed novel curcumin derivatives as potent inhibitors against Aβ aggregation and investigated their binding properties to these peptides, using protein-ligand docking and *ab initio* fragment molecular orbital methods. They showed that a curcumin derivative in which $COH_3$ group of the aromatic ring is replaced by OH strongly binds to Aβ and can be a potent inhibitor against Aβ aggregation.

Also, semi-empirical complex optimization can be considered a useful tool in AD drug design and analyses [100]. Moreover, this method was implemented in several computa-

tional chemistry softwares, such as MOPAC, GAMESS, Amber, Spartan, HyperChem, and AMPAC [100].

Molecular docking methods can be used for predicting the binding sites of compounds, ranking docking poses based on ligand-receptor binding affinities, quantitative predictions of binding energetics or investigation of ligand-receptor interactions that stabilize the complex [101]. The development of docking methods started with the rigid-body treatment of both ligand and receptor and aimed at identifying geometric fits between the shapes, following the assumption that a ligand should fit in the receptor binding pocket as a key in a lock [102]. As the understanding of protein-ligand interactions shifted to the induced fit theory, molecular docking methods evolved to the flexible treatment of only the ligand while the receptor was rigid and the flexible treatment of both ligand and target [103]. Rigid body docking protocols identify conformations of the docked ligand based on surface complementarity. The space of the docked conformations is systematically explored using different approaches like fast Fourier transform (FFT) correlation approach, computer vision concepts, Boolean operations or genetic algorithms [104]. The FFT based algorithms can be accelerated by performing: (i) 3D grid-based searches, using spherical harmonic decompositions (FRODOCK algorithm [105]), atomic contact energies that account for the desolvation energy (RDOCK algorithm [106]) or electrostatic corrections (ZDOCK algorithm [107]); (ii) sampling of rotational and translational space using spherical polar corrections (Hex program [108]). In the flexible docking approaches, flexible ligands are freely docked to a rigid binding site (the approach used in most flexible docking programs) or the receptor can change its binding site conformation to accommodate ligands (GOLD program [109]) [104]. Different methods are used in flexible docking programs, some of them being Monte Carlo (AutoDock Tools [110]), swarm-based docking methods like ant colony optimization method (implemented in PLANTS [111]), genetic algorithms (GOLD program [109]), incremental construction approaches (FLEXX program [112]) or systematic search techniques (Glide program [113]).

Analysis of the interactions between the docked molecule and the receptor (hydrogen bonds, hydrophobic contacts, salt bridges, water bridges, halogen bonds, *etc*) is useful in characterizing the binding process and in the design of novel ligands with desirable binding properties. Such interactions can be visualized and characterized using 2D interaction diagram tools such as LIGPLOT [114], PoseView [115], MOE, LeView [116], PLIP [117].

In order to exemplify the results that can be obtained by molecular docking and the analysis of non-covalent contacts, in Fig. **3A** we present a pose of compound 3 isolated from *C. obtusifolia* docked at AChE binding site in a mixed inhibition mode (interaction energy = −9.06 Kcal/mol) [118]. The interaction involved AChE residues from both peripheral anionic site (PAS) like Tyr70 and catalytic anionic site (CAS): Asn85, Ser122, Glu199, and His440. Residues that interact with the compound are labeled on the figure. In Fig. **3B**, we present the 2D interaction diagram of compound 3 bound in mixed inhibition mode to AChE. Hydrogen bonds are labeled on the Figure, as well as the interatomic distances. Also, the hydrophobic interactions are highlighted and involved residues Val71, Asp72, Gln74, Trp84, Gly117, Gly118, Tyr121, Ser200, Phe290, Phe330, Phe331, Tyr334, and Gly441 [118].

Even if docking methods have evolved over time, there are still challenges like accurate description of binding energetics, treatment of water molecules from the binding pocket or modeling the flexibility of the receptor and of the binding pockets [119]. These issues can be tackled by molecular dynamics (MD) simulations. In this context, MD simulations can be used to: (i) explore the flexibility of protein targets over time and to identify cryptic binding sites, (ii) investigate the free energy and binding kinetics, (iii) guide ligand optimization by describing the main interactions that contribute to ligand binding, (iv) assessment of water stability in binding pockets [119, 120]. MD simulations can be of great assistance in investigating the pathological mechanisms of AD and other amyloid-related pathologies [120]. Since it is difficult to characterize the structure and dynamics of amyloid-like deposits only by experimental techniques, MD simulations could be used for investigating protein misfolding and aggregation mechanisms and to predict the effect of different conditions (pH, temperature, mutations) on these processes [120].

Investigating the interactions between ligands and protein targets by molecular docking or by MD require the three-dimensional structures of targets and ligands, as well as knowledge on the putative binding sites of ligands. If the structure of a protein target is not available in PDB [59], it can be modeled using homology modeling methods. These methods are based on the fact that the spatial arrangement of a protein is more conserved than its amino acids sequence and small and medium changes in the amino acids sequence result in small conformational changes [121]. Thus, a structural model of the target protein is built using as template a similar (homologous) protein with a known crystal structure. The quality of the resulting model depends on the degree of homology with the structure used as template [122]. Different methods can be used for model building [123], like rigid-body assembly (implemented in SwissModel web server [124]), segment matching [125], spatial restraint (implemented in Modeller software [126]) or artificial evolution (implemented in Nest program [127]). In addition, databases of homology models were developed, such as ModBase [128] that currently comprises over 6 million unique sequences modeled and over 37 million models. Homology models can be further used for identifying binding sites, searching for ligands active at a binding site, modeling substrate specificity, novel ligands design or performing other computational experiments. Extensive reviews on these applications are given in [123, 129].

Putative ligand binding sites can be identified using software that search for the cavities on the surface of a protein that are likely to accommodate ligands. There is a multitude of cavity detection software based on different algorithms, some of them being: Fpocket (based on Voronoi tesselation) [130], DogSiteScorer (based on support vector machine) [131], GHECOM (uses mathematical morphology) [132], LIGSITEcsc (uses the Connolly surface and the degree of conservation) [133], Q-SiteFinder (an energy-based

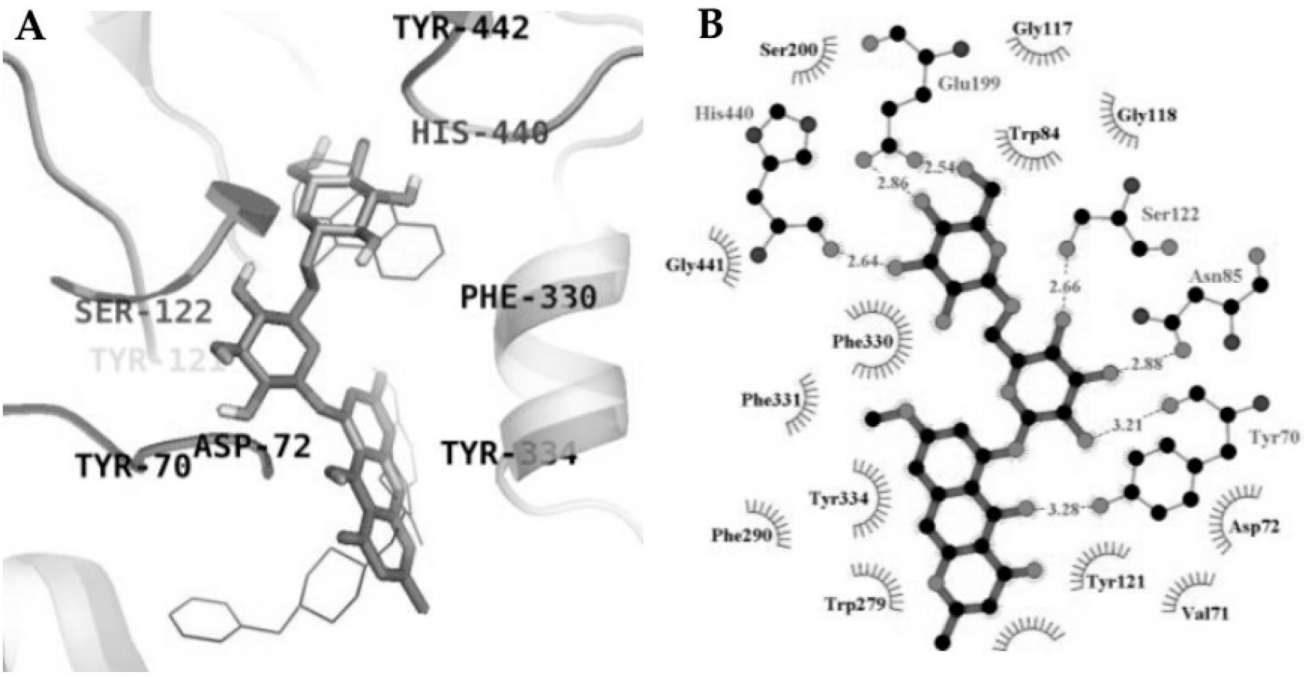


**Fig. (3). A**. Docking pose of compound 3 docked to AChE. The ligand is represented with bonds and the standard ligands tacrine (close to His440) and donepezil (close to Tyr334) are represented with lines. **B**. The 2D interaction diagram of compound 3 bound to AChE in mixed inhibition mode. Residues that interact through hydrogen bonds are represented explicitly. Hydrogen bonds are pictured as dashed lines and their length is labeled on the figure. Hydrophobic interactions are highlighted and residues participating are only labeled in the figure [118]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

method) [134], POCKET (grid-based detection algorithm) [135].

## 3. STRUCTURAL-BIOLOGICAL FUNCTION OF COMPOUNDS PROMISING TO AD MANAGEMENT

The usage of bioinformatics and cheminformatics methods is one of the first steps to be undertaken in the modern drug discovery and development pipeline process that usually involves: identification of disease targets, lead identification and optimization, pre-clinical, followed by clinical trial testing, approval and circulation on the market [136]. The first step in drug discovery involves the identification of proteins or genes that can be targeted by drugs in order to modulate their activity in the context of a certain disease. A target is valid if its modulation can result in a desired clinical effect [136]. Lead identification and optimization is a drug discovery/development phase involving *in silico* methods like high throughput screening, SAR, QSAR, molecular docking, ADMET properties calculation that results in the identification of compounds with promising biological effects on the selected targets. Nevertheless, these selected compounds must go a long way to becoming approved drugs. They go next through pre-clinical testing (*in vitro* on cell cultures and in animal models) in order to validate their biological effects predicted computationally. Best performing compounds go into the development process represented by clinical trials and approval procedures [136]. This review is focused mainly on the *in silico* identification and optimization of drugs acting on different AD molecular targets. When available, we also present pre-clinical experimental evidence supporting the computational predictions.

The identification of valid drug targets is especially difficult in the case of AD due to its complex pathophysiology pathways. As presented in Section 1, several hypotheses exist in AD development and progression, each of them opening a therapeutic strategy for AD management. AD treatment approaches can be divided into two directions: (i) treatment preventing or delaying the disease onset and progression, leading to limitation of or even repairing neuronal damage and (ii) the symptomatic treatment aiming to conserve cognitive functions, behavior and the ability of individuals to perform daily tasks [137]. Currently approved AD medication falls in the symptomatic treatment direction and involves cholinergic inhibitors (donepezil, galantamine, rivastigmine) or NMDA antagonists (memantine). Other therapeutic agents and strategies could be modulation of secretases, inhibition of Aβ aggregation, removal/degradation of amyloid plaques, inhibition of tau hyperphosphorylation and aggregation, stabilization of microtubules, antiamyloid/anti-Tau immunotherapy [138], usage of miRNAs [139], antioxidant therapies [137], hypothalamic-pituitary-adrenal axis (stress axis) modulation by targeting the glucocorticoid receptors [140], increase anti-AD agents BBB permeability by using nanotherapeutic strategies [141], *etc*.

In the following subsections, we present some compounds (synthetic parent compounds, their derivatives and natural compounds) that were analyzed by bioinformatics and cheminformatics methods aiming to predict their biological effects in relationship to particular molecular targets (colinesterases, NMDA receptor, secretases, amyloid) and predictive ADME-Tox methods predicting their pharmacokinetic features. In the case of synthetic compounds, we

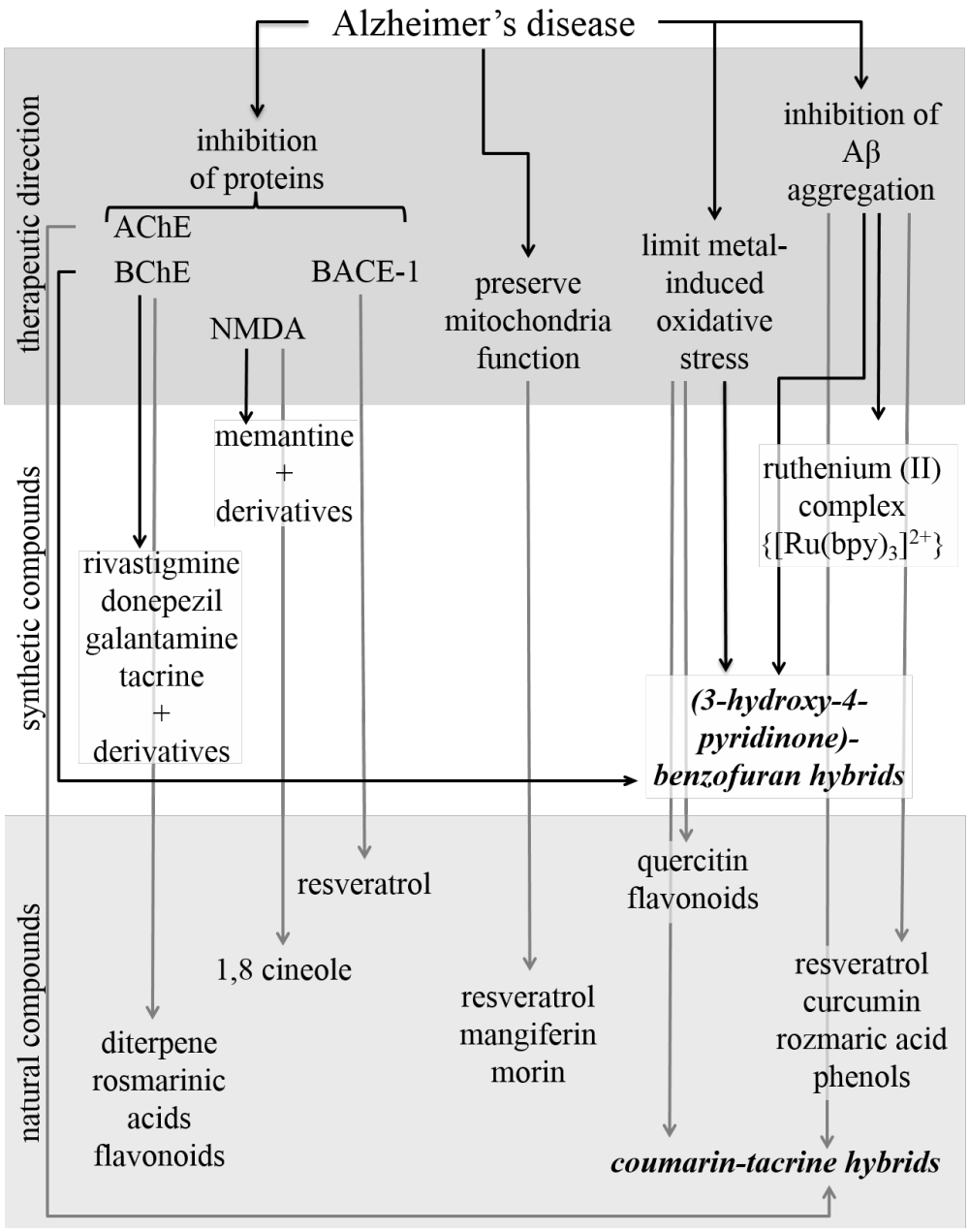


**Fig. (4).** The therapeutic directions discussed in the current section with examples of synthetic and natural compounds acting on the targets involved in each direction. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

identified *in silico* studies presenting not only new compounds acting as potent cholinesterase inhibitors or NMDA antagonists, but also new multitarget compounds with cholinesterase (ChE) and Aβ inhibitory activities, antioxidant and neuroprotective effects. In the section discussing natural compounds, we present the molecules that were proposed to have beneficial effects on AD by *in silico* methods. In spite of the neuroprotective effects of natural compounds, their analysis by *in silico* methods is limited by the lack of experimental studies pointing out to their exact molecular targets. Thus, we discuss their predicted effectiveness in relation to known targets: cholinesterases, NMDA receptors, secretases or amyloid. In Fig. **4**, we provide a schematic representation of the therapeutic directions discussed in the current section, proving examples of synthetic and natural compounds acting on the targets involved in each direction.

### 3.1. Synthetic Compounds

#### *3.1.1. ChEs Inhibitors*

Currently, three cholinesterase inhibitors: rivastigmine, donepezil and galantamine are available on the market and are used as a first symptomatic treatment for the neurocognitive decline caused by AD. The long-term efficiency of this treatment is under debate, as it does not guarantee clinical effects over time and it has side effects (nausea, anorexia, vomiting and diarrhea) that put at risk the frail patients [142].

A very interesting paper was published by Ali *et al.* [143] on the adverse effects of cholinergic inhibitors in AD, in agreement with two pharmacovigilance databases, namely Food and Drug Administration Adverse Event Reporting System (FAERS) and Canada Vigilance Adverse Reaction Database (CVARD). In this paper, the important items evaluated with respect to each adverse event were the frequency of the event and the reporting odds ratios (ROR) [143]. A total of 9877 reports were extracted from the FAERS and 2247 reports were extracted from CVARD databases. Briefly, FAERS database reports death, sudden death, sudden cardiac death, cardiac death, brain death, accidental death and apparent death as different adverse events, while the CVARD indicates the term 'death' for any fatal outcome. The evaluation showed that: (i) there is a disproportionately higher frequency of reports of death as an adverse effect of rivastigmine in comparison with the other AChE inhibiting drugs (the FAERS (ROR = 3.42; CI95% = 2.94–3.98; P<0.0001) and CVARD (ROR = 3.67; CI95% = 1.92–7.00; P = 0.001)) and (ii) reporting odds ratios from FAERS indicates that donepezil is highly associated with reports of rhabdomyolysis [143].

Given these shortcomings of currently approved ChE inhibitors, new inhibitors of AChE or both AChE and butyrylcholine esterase (BChE) have been developed in recent years [144]. Bajda *et al.* [145] used ZINC database (free data set of commercially available compounds, serving to virtual screening) for identifying new cholinesterase inhibitors. The latest version of the database (ZINC 12) contains over 35 million compounds and it is provided by the Shoichet Laboratory from the University of California. By bioinformatics and cheminfiormatics tools (molecular modeling, virtual screening of pharmacophore and molecular docking), the authors succeed to report a set of 88 compounds as possible cholinergic inhibitors, the most active compounds being 1-[4-(1H-indol-3-ylmethyl)piperazin-1-yl]-2-phenoxyethanone 2 (50.1% inhibition against BChE) and 2-[(1-benzylpiperidine-4- yl)amino]-1-phenylethanol 4 (79.5% inhibition against BChE), in comparison with donepezil [145]. Borges *et al.* [146] combined virtual screening methods with molecular dynamics and docking in order to identify new AChE inhibitors. By using ZINC database and 3D similarity searches, seven new structures were selected as potential hAChE inhibitors.

Donepezil is the most potent reversible AChE inhibitor that was initially approved in 1996 for Alzheimer's dementia treatment, with a large perspective also to be used in other types of dementia, but is not recommended for long term treatment [147]. Recently, a few interesting studies combining *in silico* and experimental methods applied on donepezil derivatives were reported [148-153]. Correa-Basurto *et al.* [148] used a set of 84 N-aryl derivatives for developing powerful QSAR models that postulated the significant involvement of molecular descriptors such as the "bond lengths of CAr–N and N–CO bonds, molecular electrostatic potential, and the frontier orbital energies" into ChE's inhibition. QSAR equations and statistic validation of QSAR models are represented in Table **1**.

Donepezil fluorinat derivative was investigated computationally in its anti-Alzheimer efficiency [153]. The most important molecular features (structural stability, molecular polarity, solubility, hydrogen bonding, partial charge and count of aromatic rings aromatic) were taken into account by using computational techniques, such as molecular docking, DFT, natural bond orbital (NBO) and atoms in molecules (AIM) theories. It was reported that fluorination substitution changed donepezil stability, altering its solubility and molecular polarities. AChE inhibitory capacity of donepezil derivatives was shown to be enhanced or decreased by the fluorine substitution position.

### *3.1.2. Multipotent Hybrid Compounds with ChE Inhibitory Activities*

The efficiency of ChE inhibitors in AD complex conditions can be enhanced by derivation with moieties exerting other desirable effects, thus resulting in multitarget lead compounds. Bautista-Aguilera *et al.* [149] studied the biological activity of donepezil-pyridyl hybrids (DPH) on AChE, BChE and momoamine oxidase (MAO) active sites

**Table 1. Molecular descriptors involved in the inhibitory activity of donepezil-like derivatives expressed as: molecular volume ($V_M$), water/octanol coefficient ($logP_m$), molar refractivity (MR), Bond lengths ($C_{AR}$-N,N-CO), polarizability (α), atomic charge of nitrogen atom using the Mulliken analysis ( $CA_{MLK}$) molecular electrostatic potential (MEP), reactivity parameter (X, χ,ω), Energy of the frontier molecular orbitals ($E_{HOMO;LUMO}$) and Dipole moment (μ) and QSAR statistic parameters expressed as: coefficient correlation $R^2$, adjusted determination coefficient $R^2_{adj}$, Standard Deviation SD, Fisher Criterion F [148].**

| Equation | Statistical Validation |
|---|---|
| $125.6397–0.00707* V_M + 0.3168* logP_M + 0.1624*MR – 0.4763*\alpha – 10.1741* CA_{MLK} + 1.7706* MEP – 27.9435* C_{Ar}–N – 63.1731* N–CO$ | $R^2 = 0.7327$, $R^2_{adj} = 0.5544$, SD = 0.1677, F = 4.1, $q^2_{cv} = 0.7327$, $q^2_{lmo} = 0.8698$, $r^2_{m(test)} = 0.7046$ |
| $5497–0.0178* V_M + 0.4336* MR – 1.0032*\alpha – 7.5257 MEP + 22.5256* \chi$ | $R^2 = 0.4909$, $R^2_{adj} = 0.3212$, SD = 0.4532, F = 2.8, $q^2_{cv} = 0.4909$, $q^2_{lmo} = 0.5704$, $r^2_{m(testÞ)} = 0.4418$ |
| $4.4128–14.0321* E_{HOMO} – 1.5212* logP_H + 0.4101* MR – 1.1650*\alpha – 6.45775* MEP$ | $R^2 = 0.7629$, $R^2_{adj} = 0.6839$, SD = 0.3095, F = 9.7, $q^2_{cv} = 0.7629$, $q^2_{lmo} = 0.8211$, $r^2_{m(test)} = 0.7516$ |
| $946.0467 – 25.1090* E_{LUMO} + 0.0065* V_M 2.2561* logP_H – 0.3313*\mu + 67.4759* CA_{MLK} + 46.8473 ESP – 3.5601 MEP+ 98.9023* \chi + 109.1875* \omega – 32.0204* C_{Ar}–N$ | $R^2 = 0.9672$, $R^2_{adj} = 0.9343$, SD = 0.1523, F = 29.5, $q^2_{cv} = 0.9672$, $q^2_{lmo} = 0.9124$, $r^2_{m(test)} = 0.9660$ |

by using experimental (spectrophotometric, fluorometric methods) and *in silico* (QSAR, molecular docking, ADMET) methods. The study results showed that DPH14 may be considered as the most potent compound, with AChE inhibitory activity equal to donepezil, but with an enhanced BChE inhibitory activity and with the ability to irreversibly inhibit MAO B enzyme.

Mishra *et al.* [152] performed a complex study in order to develop new donepezil multitarget derivatives starting from "(E)-5,6-dimethoxy-2-(4-(4-33 substituted piperazine-1-yl)benzylidene)-2,3-dihydro-1H-inden-1-ones". In this study, a combination of *in silico* (molecular modeling, molecular dynamics and docking methods) and experimental (1HNMR, 13CNMR, mass spectroscopy) methods were used in order to synthesize, characterize and evaluate the biological activity of compounds for their AChE inhibition and Aβ disaggregation potential, antioxidant and neuroprotective activities. Results showed that: (i) among all, compounds IP-13 and IP-15 appeared as the most active and selective AChE inhibitors versus donepezil, (ii) IP-9, IP-13 and IP-15 present enhanced anti Aβ effects in comparison to curcumin, (iii) derivatives IP-9, IP-13 and IP-15 significantly reduced $H_2O_2$ induced oxidative stress in SH-SY5Y cells. Furthermore, these derivatives exert a potential neuroprotective effect against $H_2O_2$ and Aβ induced cytotoxicity in SH-SY5Y cell line [152]. The chemical structures of IP-9, IP-13 and IP-15 derivatives are presented below (Fig. **5**).

In order to understand the binding mode and the interaction of the most active compounds with Aβ1-42, a docking protocol has been performed using the X-ray crystal structure of human Aβ1-42 (PDB ID: 1YT). Molecular dynamics simulation studies of compound IP-15 revealed its efficient binding with AChE and Aβ and its consistent interaction with the essential active sites during 40 ns of simulation. Besides, the potential of IP-9, IP-13 and IP-15 against Aβ1–42 induced neurodegeneration in SH-SY5Y neuroblastoma cells was addressed using a cell viability assay. Results showed that IP-9, IP-13 and IP-15 exhibited neuroprotective effects (IP-9 and IP-13 presented the highest protective capability at 10 μM). The results demonstrated that these newly synthesized compounds did not display cytotoxic effects on the cells in the range of tested concentrations [152].

Hiremathad *et al*. [154] reported a series of (3-hydroxy-4-pyridinone)-benzofuran hybrids that mimic the donepezil drug, which was studied as potential multitargeting agents for AD acting on different pathophysiological targets of AD: AChE inhibition, metal chelation, radical scavenging and inhibition of Aβ aggregation. The compounds had lower AChE inhibitory activities in comparison to donepezil, the closest similarity being obtained for the O-benzyl-hydroxypyridinone hybrids containing a 2-methylene linker. Free-hydroxypyridinone hybrids had the highest activities in terms of metal chelation, radical scavenging and inhibition of Aβ aggregation. It is interesting that all compounds presented drug-likeness properties and had neuroprotective effects on neuronal cells in AD conditions.

Dias *et al.* evaluated a series of novel feruloyl-donepezil hybrids as potential multitarget drugs for the treatment of AD [155]. In their work, a series of 12a-n feruloyl-donepezil

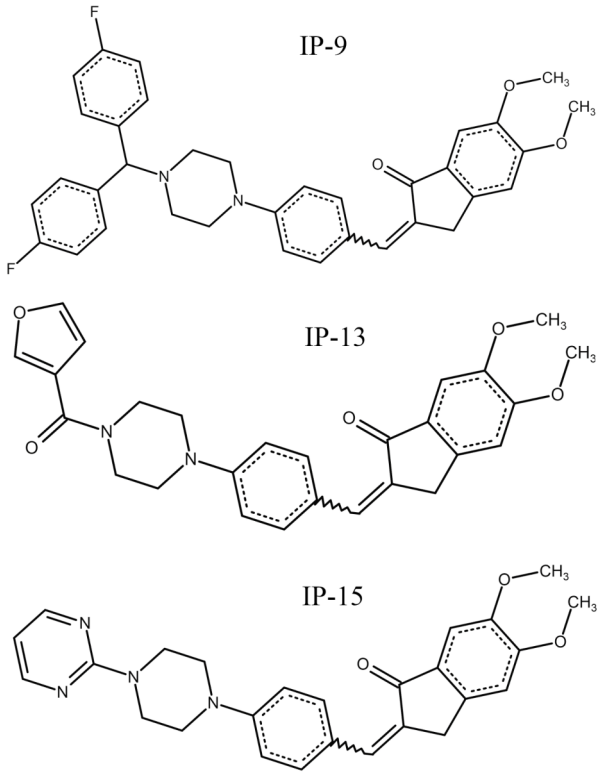


**Fig. (5).** IP-9, IP-13 and IP-15 donepezil derivatives that presented enhanced AChE inhibitory, Aβ disaggregation and antioxidant activity in comparison to their parent compound – donepezil [152].

hybrids were designed, synthesized and evaluated as AChE inhibitors and also as antioxidant compounds. The results revealed that compounds 12a-c were the most potent AChE inhibitors, highlighting 12a with IC50 = 0.46 mM. All compounds presented moderate antioxidant properties. Very interesting, compounds 12a-c presented: (i) significant *in vivo* anti-inflammatory activity proved on mice paw edema, (ii) metal chelator activity for $Cu^{2+}$ and $Fe^{2+}$, and (iii) neuroprotection of human neuronal cells against oxidative damage. [155]

Dias Viegas *et al*. [156] tested the anti-AD activity of several N-benzyl-piperidine-aryl-acylhydrazones hybrid derivatives called 4 a-o. Their hybridization approach involved combining the following chemical structures in a single molecule: (i) the pharmacophoric N-benzyl-piperidine subunit of donepezil, (ii) the substituted hydroxy-piperidine fragment of LASSBio-767 (AChE inhibitor) and (iii) an acylhydrazone linker that was also used into a number of synthetic aryl- and aryl-acylhydrazone derivatives that had significant AChE inhibitory and anti-inflammatory activities. *In vitro* and *in vivo* results showed that: (i) compounds 4c, 4d, 4g and 4j presented the best AChE inhibitory activities; (ii) compounds 4c and 4g presented anti-inflammatory activity against amyloid beta oligomer; (iii) compound 4c also showed the best *in vitro* and *in vivo* neuroprotective effects against Aβ oligomer. Molecular docking results revealed that compound 4c showed a similar binding mode to donepezil and presented suitable ADME-Tox profiles [156].

Bellozi *et al.* [157] synthesized and evaluated the antioxidant, anti-inflammatory and neuroprotective properties of a series of N-benzyl-piperidine-aryl-acylhydrazone derivatives. Four very potent compounds were identified, namely PQM-56, PQM-57, PQM-67 and PQM-75, with very good AChE inhibitory activity and also anti-inflammatory and neuroprotective effects. Compounds PQM-56 and PQM-67 displayed the best profile of memory recovery, representing potential drug candidates for AD treatment [157].

Galantamine, an alkaloid isolated from the bulbs and flowers of *Galanthus caucasicus*, *Galanthus woronowii,* is currently considered the most valuable drug for AD treatment. There are a few studies [158-160] that combined *in silico* with experimental methods in order to design and test the efficiency of galantamine derivatives in modulating AChE activity and in preventing AChE induced aggregation of Aβ peptides. The latter feature can be achieved by inhibiting AChE PAS that is involved in accelerating amyloid deposition [161].

Atanasova *et al.* [158] designed and synthesized a series of 41 galantamine derivatives with indole moiety in the side chain and tested these compounds in their AChE inhibitory activities. Study results showed that all newly synthesized derivatives presented AChE inhibitory activities between 11 and 95 folds, all of them being more active than galantamine at AChE active site. The study concluded that there is a good correlation between docking results (GoldScores) and *in vivo* tests results of galantamine derivatives. The chemical structure of newly synthesized compounds allows the concomitant targeting of both AChE binding sites. The galantamine moiety binds the CAS, while the indole moiety can bind in the PAS or in its proximity, preventing the deposition of Aβ on the enzyme.

Stavrakov *et al.* [159] used docking-based predictions to identify 20 novel galantamine derivatives, with alkylamide spacers of different lengths ending with aromatic fragments. Their results showed that among the tested terminal aromatic fragments, the phenethyl substituent is the most suitable for binding in PAS. Supplementary, critical molecular features like molecular mass, logP, number of hydrogen bond donors and acceptors were calculated for the considered molecules using ACD/logD v.9.08 (Advanced Chemistry Development, Inc.). The BBB permeability was predicted by the BBB Predictor [162]. According to this database, compounds are classified into compounds that can cross the blood-brain barrier (BBB+) or do not cross (BBB-). Study results showed that: (i) the heptylamide spacer is long enough to bridge the galantamine moiety bound in the catalytic site and the aromatic fragments interacting with PAS, (ii) the phenethyl substituent is the most suitable for binding in PAS, (iii) the presence of a methyl carboxylate group in close proximity to the aromatic fragment is unfavorable for the binding conformation.

Rivastigmine is frequently used as an efficient treatment against AD. Wang *et al.* [163] reported a series of 13 novel chalcone-rivastigmine hybrids that were designed, synthesized, and tested *in vitro* for their inhibitory activities against human AChE and BChE. In this study, hybrid compounds were obtained through interactions between rivastigmine with precursors of flavonoids and isoflavonoids, namely chalcones (trans-1,3-diphenyl-2-propen-1-ones, 1). The chalcones were chosen based on their antioxidant, anticancer, anti-inflammatory, antimalarial, antifungal, antilipidemic, antiviral and anti-amyloid activities. The authors used a combination of *in silico* methods like drug design, SAR analysis, molecular docking, molecular dynamic and predictive ADMET with experimental procedures involving the synthesis, Ellman assay, kinetic characterization, *in vitro* cell viability assays, antioxidation activity assays to determine the anti-AChE and BChE activities of compounds. Results showed that the most potent inhibitor, namely compound 3: (i) inhibits BChE with IC50 values (0.87 and 0.36 μM), which are comparable or slightly better than rivastigmine, (ii) has negligible cytotoxicity on SH-SY5Y cell line in comparison with rivastigmine, (iii) has good predicted ADMET features, namely a good absorption, low solubility, low permeability of BBB and does not present hepatotoxicity, (iv) blocks the formation of ROS in SH-SY5Y cells.

Babitha *et al.* [164] reported the anti-AD activity of two hybrid compounds formed by rivastigmine-fluoxetine and coumarin-tacrine. The authors reported that p-chlorophenyl substituted rivastigmine-fluoxetine and –OCH3 substitute Coumarin–Tacrine hybrids demonstrated a superior pharmacological profile. For these compounds, the molecular docking and predicted ADME-Tox were performed.

New candidates for AD treatment were identified [165] as 7-methoxytacrine-p-anisidine hybrid compounds that target both cholinesterases and the amyloid cascades. Korabecny *et al.* [165] used 7-methoxytacrine (7-MEOTA), a less toxic derivative of tacrine, an AChE inhibitor previously used in AD that was removed from the market due to its he-

patotoxicity. 7-MEOTA was conjugated through thiourea or urea moieties alkyl tether with p-anisidine, a compound that decreases the intracellular accumulation of APP. The inhibitory potencies of 7-MEOTA-p-anisidine heterodimers (synthesized compounds 9–22) on human AChE and BChE were determined experimentally by a modified Ellman method and were compared to those of tacrine and 7-MEOTA. A higher AChE inhibitory activity was associated with compounds with longer methylene tethers, with either thiourea (compounds 14, 15) or urea (compound 19) moieties. The binding of compounds to AChE investigated by molecular docking showed that, in spite of the differences in the linker region of compounds, their orientation and interactions in the active site of AChE are determinants for their effects. In the case of BChE, synthesized compounds (9-22) are less effective than tacrine, but more potent than 7-MEOTA. In thiourea subset, the length of the linker is inversely correlated with BChE inhibition, the most potent compound being the shortest analog (compound 9). An opposite trend was identified in the urea subset, the longer derivative (compound 22) being the most active. Results showed that a thiourea derivative (compound 9) is the most effective inhibitor of human BChE, while a urea derivative (compound 9) is the most effective AChE inhibitor. Kinetic analyses showed that the identified AChE inhibitors perform a non-competitive AChE inhibition, suggesting that AChE PAS is involved in the interaction. This type of interaction, also supported by molecular docking, suggests an additional effect of compounds in preventing AChE promoted Aβ aggregation [165].

#### *3.1.3. NMDA Antagonists*

Memantine is a non-competitive NMDA antagonist, as the steady activation of NMDA receptors by the excitatory amino acid glutamate has been considered to have a meaningful role in AD symptomatology [166]. At present, only a few *in silico* studies on memantine or memantine-like compounds were published. Recently, Takahashi *et al.* [167] published an interesting paper on nitro-memantine, a memantine derivative that provides a dual-site, hypoxia-regulated antagonism on the NMDA receptor. The study results showed that: (i) nitro-memantine can down-regulate excessive NMDA receptor activity at a point when memantine alone would lose effectiveness, (ii) it might be expected that the nitro group would target the NMDA receptor only during periods of open-channel block by the aminoadamantane moiety, *i.e*., in the presence of agonists; (iii) nitro-memantine spares even more synaptic activity while antagonizing extrasynaptic activity to an even greater degree than memantine. These may account for the increased efficacy of the new compound and bodes well for its clinical tolerability in human testing.

#### *3.1.4. Other Anti Aβ Molecular Hybrids*

New approaches in prospective AD treatment are based on the application of hybrid molecules connecting synthetic drugs or nanoparticles. Very recently, Son *et al.* [168] reported that small, nontoxic fragments using ruthenium (II) complex $\{[Ru(bpy)_3]^{2+}\}$ work as highly sensitive, biocompatible and photoresponsive anti-Aβ agents. Here, we predicted the pharmacokinetic features of ruthenium (II) complex $\{[Ru(bpy)_3]^{2+}\}$ by using the ADME-Tox databases like pkCSM database [80] and ProTox-II [82] and the most significant features are presented in Table **2**. These predictions suggest that ruthenium (II) complex $\{[Ru(bpy)_3]^{2+}\}$ is not toxic for liver or skin, does not induce mutagenesis, is not cytotoxic and does not trigger stress response pathways, predictions in agreement with the experimental results of Son *et al.* [168].

### 3.2. Natural Compounds

Currently, the limited potential of pharmacological therapy in AD management led to an increasing interest in the identification of compounds from natural products that could be helpful in managing AD. This direction is especially interesting since, during the last decade, various pharmaceutical active compounds extracted from plants had neuroprotective effects in phenotypic screening assays reflecting AD neurotoxicity pathways [169] and there is clinical evidence that the usage of adjuvant plant based therapies like Chinese herbal medicine has additive beneficial effects in the treatment of AD [170]. Research in this direction is encouraged by the existence of exhaustive natural compounds databases and analysis platforms like Traditional Chinese Medicine Integrated Database (TCMID) [171], Traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) [83], FooDB, Medicinal plant Activities, Phytochemical and Structural Database (MAPS) [172], Phytochemica platform [173], *etc*. Their usage would allow the identification of natural compounds effective on diverse AD targets and of new scaffolds to be used in the design of anti-AD medication. Also, such investigations would help in understanding the mechanism of action behind effective phytotherapies as Traditional Chinese Medicine and harness it to the benefit of AD patients.

Sun *et al.* [77] published a critical review on bioinformatics and cheminformatics methods applied on various herbals with applicability in AD treatment. By an extensive text mining on the literature from PubMed and the clinical trial database (www.Clinicaltrials.gov), the authors identified *Ginkgo biloba*, *Huperzia serrata*, *Melissa officinalis* and *Salvia officinale* as the top in AD treatment research. The investigation conducted by inquiring several databases in order to identify the active compounds from these plants that were experimentally linked to AD, their molecular targets and the relevance of the targets for AD and other disorders from a network perspective led to the conclusion that herbs could have beneficial effects on AD symptoms and could inhibit pathways closely related to AD like downregulation of intracellular $Ca^{2+}$ homeostasis, inflammation, cancer or diabetes [77]. *In silico* combined with experimental methods were applied in order to highlight the therapeutic effects of natural compounds extracted from *Mentha spicata*, *Salvia oficinalis* or *Perovskia atriplicifolia Benth* and *Salvia glutinosa L.* in AD treatments [174-179].

In the following, we reviewed *in silico* studies focusing on the potential benefits of natural products in AD treatment due to their interaction with AChE, BChE, BACE, NMDA receptors, or with other AD targets such as metal ions that induce oxidative stress, mitochondria or amyloid.

**Table 2. Predicted pharmacokinetics features of ruthenium (II) complex {$[Ru(bpy)_3]^{2+}$} obtained by inquiring the bioinformatics databases pkCSM database [80] and ProTox-II [82].**

| Pharmacokinetics Features | pkCSM Database | ProTox-II |
|---|---|---|
| Intestinal absorption (human) | Good | - |
| BBB permeability | Good | - |
| CNS permeability | Low | - |
| Organ Toxicity | | |
| Hepatotoxicity | No | No |
| Skin Sensitisation | No | - |
| Toxicity end points | | |
| Mutagenity | No | No |
| Carcinogenicity | - | No |
| Immunotoxicity | - | No |
| Citotoxicity | - | No |
| Stress response pathway | | |
| Nuclear factor-like2/antioxidant responsive element | - | No |
| Heat shock factor response element | - | No |
| Mitochondrial Membrane Potential | - | No |
| Phosphoprotein (Tumor Suppressor) p53 | - | No |

### *3.2.1. ChE Inhibitors*

Ferlemi *et al.* [176] published a paper regarding the anxiolytic and AChE inhibitory activity of rosemary tea at mice by pharmacophore alignment and molecular docking. Natural compounds from rosemary are able to penetrate the BBB and can be used as AChE inhibitors. A similar study was performed by Senol *et al.* [178] that used molecular docking, QSAR and ELISA assays to prove that diterpene and rosmarinic acids present different inhibitory activities on BChE and AChE. Results revealed that rosmarinic acid is a good BChE inhibitor versus galantamine, but it is a weak AChE inhibitor. All of the tested compounds could be good precursor models for BChE-inhibiting molecules. In particular, 15,16-dihydrotanshinone could be more promising since it can display dual inhibition toward both enzymes.

Very recently, Kuppusamy *et al.* [177] identified that some commercially available flavonoids (diosmin, silibinin, scopoletin, taxifolin and tricetin) are good candidates as inhibitors of AChE enzyme by *in silico* and *in vitro* studies. Flavonoids and their related compounds are a group of natural products that exhibit various biological and pharmacological activities like antibacterial, antiviral, antioxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, antimutagenic and several enzymes inhibitory effects. Flavonoids can be promising remedies to alleviate AD symptoms by inhibiting AChE and thereby increasing ACh levels. In this study, authors combined bioinformatics and cheminformatics programs such as Python 2.7, Molecular graphics laboratory (MGL) tools and AutoDock 4.2, Discovery studio visualizer 2.5.5, Chemsketch, molecular docking with the chemical synthesis of compounds and *in vitro* testing of AChE and BChE inhibition. From these flavonoid compounds, scopoletin was found to be the most potent and specific inhibitor of ChEs with IC50 values of 10.18±0.68 μM. The molecular docking results reinforced that scopoletin (binding energy =-6.95 kcal/mol) and diosmin (binding energy =-6.03 kcal/mol) are good candidates for AChE inhibition. These results are confirmed by experimental results in the case of scopoletin (inhibitory constant = 36.86 microM) and diosmin (inhibitory constant = 8.36 microM), while tricetin (binding energy =-4.27kcal/mol; inhibitory constant = 739.02 mM) and taxifolin (binding energy =-5.22kcal/mol; inhibitory constant = 150.32 mM) appeared as week AChE inhibitors. The study results are optimistic with respect to the usage of flavonoids as AChE inhibitors, the *in silico* AChE inhibitory activities of the compounds being in the following order: scopoletin > diosmin > silibinin > taxifolin > tricetin > donepezil. *In vitro* AChE inhibitory assay confirmed these results, including that scopoletin is more potent than the standard, donepezil.

### *3.2.2. NMDA Antagonists*

Recently, Avram *et al.* [174] published a computational study aiming to identify more potent lead phytopharmaceutical compounds for AD treatment, considering the natural compounds isolated from *Mentha spicata L. subsp. spicata* essential oil. In this paper, complex structure - activity relationship (SAR) models were generated in order to predict the biological activities of 14 natural compounds from *Mentha*

**Table 3. Biological activity expressed as IC50 at AChE and NMDA receptor of anti-AD conventional drugs and natural compounds extracted from *Mentha spicata* essential oil [174].**

| *Mentha spicata extract oil* | | | |
|---|---|---|---|
| **No** | **Constituents** | **IC50 AChE (microM)** | **IC50 NMDA** |
| 1 | Limonene | 195 | |
| 2 | 1.8-cineole | 41 | |
| 3 | Linalool L | 200 | |
| 4 | Pulegone | 136 | |
| 5 | Piperitenone | 110 | |
| 6 | Piperitenone oxide | 64 | |
| **Conventional anti-AD drugs** | | | |
| 7 | donepezil | 16 | |
| 8 | galantamine | 300 | |
| 9 | memenatine | 4360 | 1.24 |

*spicata L. subsp. spicata* in their interaction with active sites of AChE and NMDA receptor. The molecular features of natural and synthetic compounds (memantine, donepezil an galantamine) were compared. The names and biological activities of synthetic and natural compounds expressed as IC50 against AChE are presented in Table **3**.

Avram *et al.* [174] reported the molecular features relevant for the biological activity of natural compounds isolated from *M. spicata* essential oil, such as rotatable bonds, hydrophobicity, energy of molecular orbitals, solvent accessible area and their subdivided. Results predict that one of the main compounds of *M. spicata* essential oil, namely 1,8 cineole, could be a NMDA antagonist in a similar manner with memantine, while it presents a weak inhibitory activity on AChE, in respect of: (i) hidrophobicity (logP 1,8 - cineole =2.95, logP memantine =2.81, logP donepezil =4.11), (ii) the energy of LUMO (eLUMO 1,8 - cineole =3.01 eV, eLUMO memantine =3.35 eV, eLUMO donepezil = - 0.35 eV) and (iii) the solvent accessible surface areas over all hydrophobic (SA_H 1,8 - cineole = 350 $Å^2$, SA_H memantine = 358 $Å^2$, SA_H donepezil = 655 $Å^2$) or polar atoms (SA_P 1,8 - cineole = 4 $Å^2$, SA_P memantine =10 $Å^2$, SA_P donepezil =44.62 $Å^2$) [174].

### *3.2.3. BACE Inhibitors*

Resveratrol is a natural compound from red wine. Due to the health benefits of resveratrol and resveratrol derivatives, these are considered for alternate therapy or prevention in several disorders such as, cancer, inflammation, brain disease and disorders, *etc*. Koukoulitsa *et al.* [175] performed a biological and computational evaluation of the inhibitory activities of resveratrol and its derivatives on beta secretase (BACE-1) by molecular docking and molecular dynamics methods. Results showed that resveratrol derivatives 3, 5, 10 and 11 have higher inhibitory activities in comparison with resveratrol.

A very interesting computational study on the pharmacokinetic and pharmacodynamics features of resveratrol was performed by Udrea *et al.* [81]. In this study, ADME-Tox features of resveratrol were presented in depression, as secondary pathology in AD. The results are presented in Table **4**. The ADMET study was run in pkCSM platform [80], the SMILES structure of the drugs and natural compounds were retrieved from the Drug Bank database [77].

### *3.2.4. Anti-inflammatory and Antioxidant Compounds*

Another important natural compound in AD is ursolic acid. Recently, Duda Seiman *et al.* [180] addressed the anti-inflammatory features of ursolic acids by computational and experimental methods. Besides, ADME-Tox features of ursolic acids were evaluated in comparison to quercetin. The authors identified that both quercetin and ursolic acid comply with Lipinski and Veber rules, implying that both compounds can be considered drug-like. Also, the bioavailability of compounds is very good, ursolic acid presenting a higher bioavailability than quercetin. The predictive ADME-Tox investigations showed that: (i) Ursolic acid presents a predicted value of 1.287, which indicates a good Caco-2 permeability, while Caco-2 permeability of quercetin is low (-0.162); (ii) Both molecules present a high intestinal absorption; (iii) ursolic acid (-0.174) has a medium predicted BBB permeability; (iv) ursolic acid has a CNS permeability value of -1.118, indicating that it penetrates; (v) ursolic acid present no AMES toxicity, AMES toxicity test being an indicative if a compound is mutagenic or not [180].

*Caesalpinia crista* is a medicinal plant known for its antimicrobial, anti-inflammatory and anti-oxidant properties. A recent study [181] evaluated the neuroprotective effects of methanolic extracts of *Caesalpinia crista* on aluminium-induced neurodegeneration in rats. Its co-administration significantly ameliorated the aluminium-dependent cognitive impairment, AChE hyperactivity and oxidative stress in the hippocampus and in the frontal cortex of the rat brain.

**Table 4.** ***In silico*** **ADME-Tox predicted values for natural compounds: resveratrol, melatonin, linalool and linalyl acetate compared with antidepressant fluoxetine [81].**

| Model | Predicted Values for | | | | | Unit |
|---|---|---|---|---|---|---|
| | Resveratrol | Melatonin | Linalyl Acetate | Linalool | Fluoxetine | |
| Intestinal Absorption (human) | 90.935 | 94.164 | 95.275 | 93.163 | 91.813 | % Absorbed (>30%=poorly absorbed) |
| VDss (human) | 0.296 | 0.082 | 0.069 | 0.152 | 1.058 | log L/kg (low -0.15 >logVDss> 0. 45 high) |
| Fraction unbound (human) | 0.166 | 0.289 | 0.423 | 0.484 | 0.039 | Fu (higher fraction unbound more efficient drug) |
| BBB permeability | -0.048 | -0.076 | 0.516 | 0.598 | 0.505 | log BBB (poorly cross -1 >logBBB >0.3 crosses) |
| Total Clearance | 0.076 | 0.735 | 1.627 | 0.446 | 0.68 | log ml/min/kg |
| Rat (LD50) | 2.529 | 2.159 | 1.729 | 1.704 | 2.849 | mol/kg |
| Hepatotoxicity | No | No | No | No | Yes | Yes/No |

Jomova *et al.* [182] studied the role of the flavonoid quercetin as a promising potent anti-copper and antioxidant agent and obtained interesting results: (i) copper(II)-quercetin is more efficient in radical scavenging in comparison to quercetin alone; (ii) the formation of copper(II)-quercetin complexes significantly suppressed the formation of hydroxyl radicals in the Cu(II) catalyzed Fenton reaction; (iii) quercetin has a protective effect against DNA damage, but only at higher stoichiometric ratios relative to copper.

The effect of natural products on mitochondria was reviewed by Gibellini *et al.* [183], showing that natural products influence all mitochondrial functions: (i) quercetin, resveratrol, curcumin, (−)epigallocatechin gallate (EGCG), (−)epicatechin gallate, genistein, or biochanin inhibit F0F1 ATPase; (ii) curcumin, resveratrol and quercetin, the more extensively studied products, exhibit dose-dependent and tissue-dependent effects on the oxidative phosphorylation process; (iii) resveratrol has antioxidant effects in mitochondria by decreasing ROS, modulating the expression of proteins and increasing the expression of ROS scavenging enzymes; (iv) curcumin is an efficient antioxidant that prevents mitochondrial dysfunctions and mitochondrial-induced apoptosis; (v) the effects of curcumin, quercetin and resveratrol on mitochondria biogenesis are quite controversial, being influenced by animal model, experimental design or dosage [183]. Mangiferin and morin were also identified as powerful neuroprotective antioxidants that limit mitochondrial damage in Aβ oligomer-treated neurons [184]. Their identified effects on mitochondria were the prevention of cytochrome c release in cytosol and preservation of mitochondria membrane potential [184]. The quest for natural compounds with applicability in diseases associated with mitochondrial dysfunctions takes into account different sources, like traditional Chinese medicines. A screening study of potential mitochondrial-targeting compounds of these medicines through an efficient mitochondria-based centrifugal ultrafiltration/liquid chromatography/mass spectrometry led to the identification of 12 novel compounds that can target mitochondria, from which the ability of 6 compounds to affect mitochondrial function was proven *in vitro* [185].

### *3.2.5. Anti Aβ Compounds*

Natural compounds were also proved to be effective amyloid inhibitors. Velander *et al.* [186] conducted a database search for natural compounds acting as amyloid inhibitors, resulting a list of 72 compounds comprising 44 phenolic compounds: "16 flavonoids, 4 anthraquinones, 13 alkaloids (including 3 indoles, 3 pyridines, and 2 porphyrins), terpenes, and steroids" [186], from which we can mention resveratrol, curcumin, oleuropein, oleocanthal, EGCG, caffeic acid, rosmarinic acid, cinnamaldehyde, genistein. These compounds may bind Aβ fibrils in different ways (covalent, non-covalent, charge-charge). Molecular modeling techniques were used to address the binding of curcumin and EGCG to amyloid and tau fibrils. Results on the molecular docking of curcumin to a fragment ("steric zipper" hexapeptide) of $A\beta_{1-40}$ led to the identification of a "binding and destabilization" effect, as curcumin binding produces a perturbation of β-sheets in Aβ spine [187]. A similar mechanism was revealed by molecular dynamics (MD) simulation of curcumin bound to tau hexapeptide VQIVYK [188]. The simulation was performed starting from the crystal structure of curcumin bound to four tau hexapeptides that pictures curcumin located in a β-sheet pocket formed by tau fragments [189]. MD showed that curcumin disrupts the order of fibrils by breaking the interaction between tau chains [188]. MD also helped characterize a different mechanism by which curcumin prevents high order amyloid aggregation, namely curcumin molecules self-assemble to form a nucleation site that binds and stabilizes small amylin assemblies which become unavailable for further aggregation [190]. MD simulations of EGCG bound to amylin dimers showed that EGCG alters the cross-beta amyloid conformation, leading to the formation of conformations comprising mostly coil structures [191]. Velander *et al* [186] reviewed experimental data and molecular modeling studies on the binding types and

interaction mechanisms (including mediated by redox metals copper, zinc, iron, aluminum) of phenolic compounds with AD fibrils (amyloid, tau), providing their atomic-level analysis, which is an important starting point for the design of potent Aβ inhibitors.

#### *3.2.6. Multipotent Hybrid Compounds*

Coumarins are found in a large variety of sources, like fruits, nuts, coffee, tea, vegetables or fruits. Depending on their core structure and substituents, these exhibit many effects that could be desirable in AD condition, including antioxidant, ROS scavenging, anti-inflammatory, cholinesterases inhibition of CNS stimulation [192]. In a recent review, Stefanachi *et al.* [192] presented coumarins as a scaffold of great interest for the development of novel derivatives and therapeutic multitarget agents useful in complex pathologies, such as neurodegenerative diseases. Hamulakova *et al.* [193] reported a series of coumarin–tacrine hybrid compounds as therapeutic agents with multiple effects: (ii) antioxidant and copper-chelating activity, (ii) DNA protection, (iii) modulation of cholinergic activity and (iv) inhibition of amyloid aggregation. The efficiency of these compounds is based on their chemical structure. While the presence of tacrine segment assures the cholinesterase inhibitory activity of compounds, the antioxidant properties of these compounds rely on the interaction of hybrids with Cu(II) that involves the reduction of Cu(II) to Cu(I) species, which prevents the Cu(II) mediated decomposition of hydrogen peroxide [193].

## CONCLUSION

The main problem that we are facing in developing a cure for AD is its highly complex pathophysiology. Therefore, a combination of therapeutic methods is more likely required in order to hopefully cure or at least maintain the cognitive state of the patients. Current AD medication has proven ineffective on the long-term progression of the disease; therefore a great effort is put into the identification of novel compounds effective in inhibiting AD neurotoxic pathways. Bioinformatics, cheminformatics and ADME-Tox methods can be of great assistance in this quest, as they allow efficient management of the wealth of information existing on drugs and natural compounds, their molecular targets, the association of targets with diseases, the interactions between targets in the context of cellular networks, *etc*. Based on the analysis of this data, they allow the prediction of compounds biological activities and pharmacokinetic features, resulting in the identification of promising compounds for the treatment of AD. Nevertheless, the predictions must be pre-clinically and afterward clinically validated in order to establish their effects in the complex AD condition.

Here we listed a series of synthetic chemicals and natural compounds extracted from plants with inhibitory action on AD molecular targets and pathways, thus showing potential beneficial effects that could be harnessed in relieving AD symptomatology and modifying disease progression. Our focus was on the compounds and their derivatives that were analyzed by bioinformatics, cheminformatics and predictive ADME-Tox methods. Wherever available, we present experimental studies validating their effects on molecular targets, cell cultures and animal models.

In the case of synthetic compounds, we identified *in silico* combined with experimental studies presenting new molecules acting mostly as cholinesterase inhibitors and only a few NMDA antagonists. Furthermore, we presented studies on synthetic multipotent hybrids targeting at the same time ChEs and other AD processes (Aβ deposition, oxidative stress, *etc*) and metal-organic compounds acting as very potent, nontoxic Aβ inhibitors.

Regarding the neuroprotective effects of natural compounds, their analysis by *in silico* methods is limited by the lack of experimental studies pointing to their exact AD molecular targets. Most of the studies that we reviewed take into account the inhibitory effect of natural compounds on cholinesterases, NMDA receptors, secretases or amyloid. The usage of natural compounds in AD has great potential, as clinical trials showed that a plant-based therapy can be adjuvant to the classic AD therapy. Natural compounds appeared as multipotent agents, acting on several AD pathways. In the future, more effort should be directed toward a coherent characterization of their *in vivo* efficacy in AD condition and in the identification of their optimal dosages in order to prevent side effects. Also, attention should be given to therapeutic approaches involving hybrid molecules connecting synthetic drugs, natural compounds or nanoparticles.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| Aβ | = | Acetylcholine |
| ACD | = | Advanced Chemistry Development |
| ACh | = | amyloid beta |
| AChE | = | Acetylcholinesterase |
| AD | = | Alzheimer's disease |
| ADMET | = | absorption, distribution, metabolism, and excretion – toxicity |
| APOE | = | apolipoprotein E |
| APP | = | amyloid precursor protein |
| BACE | = | beta-secretase |
| BBB | = | blood-brain-barrier |
| BChE | = | butyryl-cholinesterase |
| BDDCS | = | Biopharmaceutics Drug Disposition Classification System |
| CAS | = | catalytic anionic site |
| CVARD | = | Canada Vigilance Adverse Reaction Database |
| EO-FAD | = | early-onset familial Alzheimer's disease |
| FAERS | = | Food and Drug Administration Adverse Event Reporting System |
| MEOTA | = | Methoxytacrine |
| MGL | = | Molecular graphics laboratory |
| MOE | = | Molecular Operating Environment |
| NCBI | = | National Center for Biotechnology Information |

| | | |
|---|---|---|
| NF-kB | = | nuclear factor-kappaB |
| NFT | = | neurofibrillary tangles |
| NMDA | = | N-metil-D-aspartat |
| NRf2 | = | nuclear transcriptional factor-2 |
| OMIM | = | Online Mendelian Inheritance in Man |
| PAS | = | peripheral anionic site |
| PCR | = | Polymerase chain reaction |
| PDB | = | protein databank |
| PSEN1 | = | presenilin 1 |
| PSEN2 | = | presenilin 2 |
| QSAR | = | quantitative structure–activity relationship |
| ROR | = | reporting odds ratios |
| SAR | = | structure - activity relationship |
| TM | = | transmembrane |
| TTD | = | Therapeutic Targets Database |
| WGS/WES | = | whole genome and exome sequencing |

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## CONFLICT OF INTEREST

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