Current Neuropharmacology, 2022, *20*, 857-885

REVIEW ARTICLE

Computer Aided Drug Design Methodologies with Natural Products in the Drug Research Against Alzheimer's Disease

Natália Ferreira de Sousa[1], Luciana Scotti[1,2,*], Érika Paiva de Moura[1], Mayara dos Santos Maia[1], Gabriela Cristina Soares Rodrigues[1], Herbert Igor Rodrigues de Medeiros[1], Simone Mendes Lopes[1] and Marcus Tullius Scotti[2]

[1]*Postgraduate Program of Natural and Synthetic Bioactive Products (PgPNSB), Health Sciences Center, Federal University of Paraíba, João Pessoa-PB, Brazil;* [2]*Lauro Wanderley University Hospital (HULW), Health Sciences Center, Federal University of Paraíba, João Pessoa-PB, Brazil*

ARTICLE HISTORY

Received: June 09, 2021
Revised: August 19, 2021
Accepted: August 26, 2021

DOI:
10.2174/1570159X19666211005145952

Abstract: Natural products are compounds isolated from plants that provide a variety of lead structures for the development of new drugs by the pharmaceutical industry. The interest in these substances increases because of their beneficial effects on human health. Alzheimer's disease (AD) affects occur in about 80% of individuals aged 65 years. AD, the most common cause of dementia in elderly people, is characterized by progressive neurodegenerative alterations, as decrease of cholinergic impulse, increased toxic effects caused by reactive oxygen species and the inflammatory process that the amyloid plaque participates. *In silico* studies is relevant in the process of drug discovery; through technological advances in the areas of structural characterization of molecules, computational science and molecular biology have contributed to the planning of new drugs used against neurodegenerative diseases. Considering the social impairment caused by an increased incidence of disease and that there is no chemotherapy treatment effective against AD; several compounds are studied. In the researches for effective neuroprotectants as potential treatments for Alzheimer's disease, natural products have been extensively studied in various AD models. This study aims to carry out a literature review with articles that address the *in silico* studies of natural products aimed at potential drugs against Alzheimer's disease (AD) in the period from 2015 to 2021.

Keywords: Alzheimer's disease, amyloid plaque, cholinergic impulse, *in silico* studies, natural products, oxigen-reactive species (ROS).

1. INTRODUCTION

Natural products are chemical compounds resulting from the evolutive process of plants, marine organisms and fungi; in which many such chemicals have been optimized under the selective forces of coevolution of the organisms producing them with their predators. These natural compounds are used by humans since ancient times for treatment and cure of several diseases. The use of these compounds has been the single most successful strategy in the discovery of novel medicines, and many medical breakthroughs are based on natural products. Half of the top 20 best-selling drugs are natural chemical compounds and their total sales amounted to $16 billion. These numbers show that natural chemicals can be considered pre-optimized to be potentially bioactive and therefore to possess "Drug-Like Properties" [1-3].

In silico methods or studies of CADD (Computer Aided Drug Design) are increasingly being used, both in industry and universities. They are the representation and manipulation of three-dimensional (3D) molecular structures, calculation of descriptors and molecular properties of these dependent structures, model construction and other tools that encompass the computational assistance in the research of drugs. Therefore, it involves the understanding of the molecular interaction of qualitative and quantitative points of view. The analysis of the molecular structure of the given system allows relevant information to be extracted to predict the potential of bioactive compounds [4].

Theoretical studies have aided in the process of drug discovery [5]. Technological advances in the areas of structural characterization, computational science and molecular biology have contributed to the planning of new molecules to become faster and doable [6, 7].

Many chemoinformatics studies exist, which show that a large fraction of natural products are especially "drug-like" or at least "lead-like" with structural and physicochemical properties rendering them potential drugs or leads [8, 9]. Some authors have even suggested a "natural product-likeness score" as a means to filter large chemical databases to find new active entities suitable for testing [10]. The use

*Address correspondence to this author at the Health Sciences Center, Chemioinformatic Laboratory, Federal University of Paraíba, Paraíba, Brazil; E-mail: luciana.scotti@gmail.com

1875-6190/22 $65.00+.00

of natural products has been the subject of increasing interest in phytochemistry, biochemistry and other fields of research at the chemistry-biology-ecosystems interface [11].

Alzheimer's disease was discovered in 1907 by the german neuropathologist, Alois Alzheimer. One of his patients presented memory loss, behavioral disorders, and cognitive deficits. On biopsy, extensive brain damage was observed, with loss of neurons and synapses, mainly in the hippocampus and neocortex association regions; senile plaques, and neuron tangles, called neurofibrillary tangles and spindles. Hippocampal atrophy is the starting point of Alzheimer's disease pathogenesis [12-15].

Alzheimer's affects about 80% of individuals aged 65 years; dementia only occurs in a small percentage of individuals at this age, yet the prevalence of dementia in Alzheimer's increases to 25% in individuals aged 80 years [16]. Alzheimer's disease (AD) is the most common cause of dementia in elderly people, and it is characterized by progressive neurodegenerative alterations, with 35.6 million people living with dementia worldwide in 2010, a projection of 65.7 million by 2030, and one million new cases per year until 2050 [17-20].

Current treatment strategies against the AD are: γ-, β- secretase inhibitors, aβ vaccination, cu-zn chelators, cholesterol lowering drugs, statins, non-steroidal anti-inflammatory drugs (NSAIDS) [19]. Also, neurotrophins (NTS) can be used in the treatment of AD because neuronal death is prevented by endogenous neurotrophic factors (NFS) [21]. Several treatments have introduced drugs that act to increase cholinergic brain function by stimulating cholinergic receptors [22], favoring the development of acetylcholine and preventing the degradation of cholinesterase and anti-inflammatory enzymes [23]. Existing drugs for the treatment of AD are currently concentrated on donepezil, galantamine, rivastigmine as acetylcholinesterase inhibitors, while memantine (Fig. **1**) acts as a non-competitive inhibitor of *n*-methyl-*d*-aspartate (NMDA) [24]. However, the treatments available against AD are limited; they don't cure neither prevent the death of neuronal cells; they act only in disease symptoms improving the quality of life for the patient. Therefore, these diseases do not only affect the patient and their family [16].

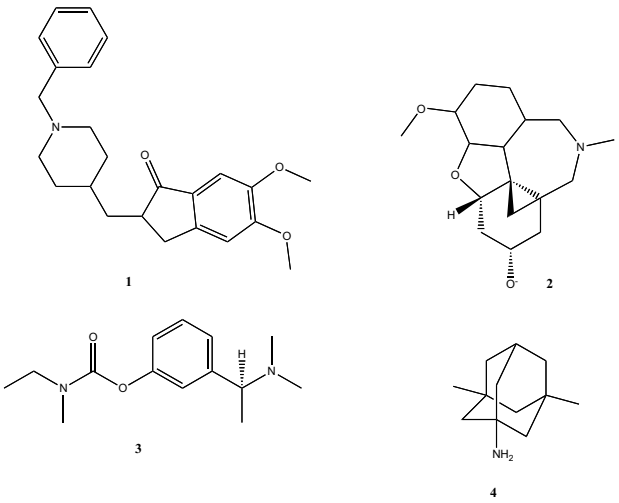

Fig. (1). Drugs used to treat Alzheimer's disease. **1**) Donepezil, **2**) Galantamine, **3**) Rivastigmine, **4**) Memantine.

Drug design has acted as a tool in the field of medicinal chemistry, bringing to light chemical manipulation in the development of lead compounds, producing highly active compounds with minimal side effects [25]. In conjunction with drug development are studies involving *in silico* methods that seek to understand existing properties in the structure of compounds, as well as promote compounds with low or no toxicity value [26].

Studies in this area include studies involving quantitative structure-activity relationship (QSAR) methods, which are based on the use of a dataset library using numerous statistical techniques [26, 27]. On the other hand, the existence of the molecular modeling technique promotes the evaluation of the interactions between a ligand and its receptor [28, 29]. These tools have been widely inserted into drug development as they require less time and cost to rank better profiled compounds without harming lead discovery [30].

In scientific literature, many reviews report studies using natural products as potential drug candidates for the treatment of Alzheimer's disease with computational studies [20, 31, 32]. Choi *et al.*, (2015) [33] report many marine compounds effective in experimental models of neurologic disorders, since 1997. Khan *et al.*, (2013) [34] reported molecular docking study in 4-acetoxy-plakinamine b (4apb), a marine natural product with inhibitory effect against acetylcholinesterase (ACHE). Guzior *et al.*, (2015) [35] defends multitargets mechanisms against AD and report 696 multifunctional natural and synthetic substances found in the last three years, between them the natural flavonoids, alkaloids and coumarins compounds.

Thus, the present work aims to carry out a literature review with articles that address the *In silico* studies of natural products aimed at potential drugs against alzheimer's disease (AD) in the period from 2015 to 2021.

2. NATURAL PRODUCTS AND DERIVATIVES ANALYZED THROUGH *IN SILICO* METHODOLOGIES AGAINST AD

2.1. Polyphenols and Derivatives - Flavonoids

Polyphenols represent a class of secondary metabolites from plants that can be divided into flavonoids and non-flavonoids [36]. These phenolic compounds have structural diversity and are characterized by hydroxylated aromatic rings [37]. They have potential in food preservation and therapeutic use; thus their effects on human health and in various diseases (hypertension, diabetes, cancer, among others) are studied [38]. The most obvious and easy to understand action of these compounds is the reduction of cell damage caused by free radicals, observed in AD [39, 40]. Thus, several scientific studies report the benefic activities of the polyphenols against AD [41, 42].

In the review by Lakey-Beitia and Collaborators (2015) [43], the authors developed a hypothesis on how polyphenols can modulate APP processing, prevent fibrils and Aβ aggregation in AD based in examples of other scientific studies. As examples, it was observed in research that water extract from *Caesalpinia crista* leaves containing polyphenols prevented Aβ aggregation from monomers and disintegrated preformed Aβ fibril; *Ginkgo biloba* extract inhibited the

formation of Aβ oligomers; and extracts prepared from the medicinal herb *Paeonia suffruticosa* inhibited Aβ fibril formation and also de-stabilized the preformed amyloid fibril [44, 45].

A polyphenolic xanthone derived from *Garcinia mangostana* Linn, the α-Mangostin (Fig. **2**), was studied by Wang and Collaborators (2012) [46]. This compound is used in several studies [47, 48] and has many activities, such as antimalarial [49], membrane-protective [50], antimicrobial [51] and antiviral [52]. Wang and colleagues performed the molecular docking of α-Mangostin with Aβ and observed that xanthone exhibited binding energy of -68.76 kcal/mol. Regarding the interactions with the amino acids of the protein, α-Mangostin formed hydrogen bonds with Lys16 and Asp23 and interacted with Glu22. The results obtained by the authors suggest that α-M has neuroprotective effects similar to curcumin [53].

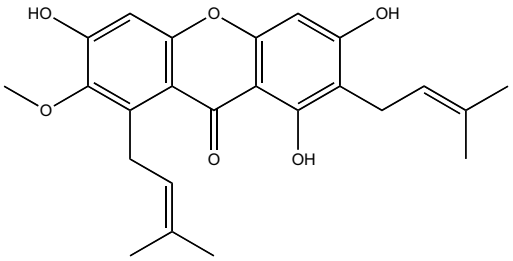

Fig. (2). 2D Chemical structure of α-Mangostin [46]. Source: Wang and Collaborators (2012).

Tau proteins are the major constituents of intraneuronal and glial fibrillar lesions of AD, causing dementia and increasing the neurodegenerative process. Madeswaran and Collaborators (2015) [54] studied the tau protein kinase I inhibitory activity of 6 flavonoids through molecular docking. The investigated compounds were acacatechin, catechin, galangin, scopoletin, silbinin and memantine. Parameters like molecular formula, molecular weight, aromatic carbons, rotatable bonds and number of torsions were calculated and evaluated according to the Lipinski's rule. Silbinin and galangin showed the lowest energies (-7.1 and -6.5 kcal/mol, respectively), having a greater affinity with tau protein kinase I (PDB ID: 1J1C) compared to the memantine control (-5.9 kcal/mol). Thus, these two flavonoids can be active against the enzyme, and further studies are needed to verify their potential in the treatment of AD.

CDK5 is an enzyme that belongs to the cyclin-dependent serine/threonine kinase family, and that regulates neuronal activity and increases GABAergic neurotransmission. Research reports that this enzyme can impair neuronal plasticity and that its inhibitors prevent pathological phosphorylation of tau and consequently neurofibrillary pathology [55-57]. Thus, CDK5 is studied as a new target for drugs against AD. In the study of Shresta and Collaborators (2013) [58], the six flavonoids hovetrichoside C, sulfuretin, aureusidin, ureusidin-6-O-b-D-glucopyranoside, cupressuflavone and quercetin-3-O-b-D-galactopyranoside were isolated from the indigenous medicinal shrub *Rhus parviflora* (Anacardiaceae). The substances showed to be CDK inhibitors through nonradiometric *in vitro* CDK5/p25 inhibitory activity. Then, the compounds were submitted to molecular docking with CDK5/p25 (PDB ID: 1UNL). All flavonoids formed good interactions with the receptor through the hydrogen bond with active site residues of Cys83 and Gln130. Among the studied compounds, it is believed that aureusidin (Fig. **3**) is a potential neuroprotective drug candidate due to the good results demonstrated through *in silico* (docking energy of -8.73 kcal/mol) and *in vitro* analysis (IC_{50} values of 3.50 μM in nonradiometric and 4.81 μM in radiometric assays).

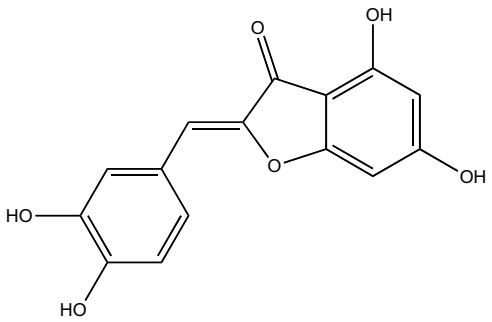

Fig. (3). 2D Chemical structure of aureusidin [58]. Source: Shresta and Collaborators (2013).

Changes in the cholinergic system occur majorly in AD. There are two cholinesterases that play important roles in cholinergic regulation: AChE, which is found in the blood, neural synapses and hydrolyzes the acetylcholine; and BuChE, which is found in the liver and hydrolyzes the butyrylcoline [59-61]. Acetylcholine (Ach) can be degraded by the two types of cholinesterases and inhibition of both enzymes by a dual inhibitor should result in higher levels of ACh in the brain and provide greater clinical efficacy. Researches for AChE inhibitors include tetrahydroaminoacridine (tacrine), physostigmine, velnacrine, rivastigmine and donepezil [62-64]. Kuppusamy and Collaborators (2017) [65] investigated the inhibitory activity of commercially available flavonoids against AChE using *in silico* and *in vitro* analyzes. The *in silico* study used by the authors was molecular docking, with the objective of verifying the binding energy, intermolecular energy, the inhibition constant and rootmean square deviation (RMSD) of the starting and the final docked conformation of flavonoid against AChE enzyme (PDB ID: 5HCU). It was noted that all the flavonoids analyzed (diosmin, silibinin, taxifolin, tricetin) showed excellent docking results against AChE compared to the standard donepezil ligand. While the binding energy of the flavonoids varied from -6.93 kcal/mol to - 4.27 kcal/mol, the binding energy of the standard ligand was -3.87 kcal/mol. Regarding intermolecular energy, flavonoids exhibited values that ranged from -8.72 kcal/mol to - 6.06 kcal/mol and the standard ligand showed a value of -5.46 kcal/ mol. As the inhibition constant is directly proportional to binding energy, flavonoids showed good results in this parameter, which ranged from 8.37 μM to 739.02 μM. Therefore, their inhibition constant was less than that of the standard ligand (994.14 μM). It was also observed that the flavonoids had excellent results for the RMSD (26.46 to 35.19) compared to the standard ligand (42.19). In addition, when verifying the interactions of flavonoids with protein amino acids, the authors predicted that these compounds can act as reversible AChE inhibitors.

Amyloid plaques in the brain represent one of the major pathological hallmarks of AD [22]. The major protein component of the plaques is the amyloid β-peptide (Aβ), which is a peptide with 39-43 amino acids. It is produced from sequential cleavage of APP by β-secretase, known as BACE-1 and by γ-secretase. Aβ deposition generates ROS, which is involved in Alzheimer's inflammatory and neurodegenerative pathology [18, 21, 31, 66]. Therefore, Aβ and BACE-1 are potential targets for AD [67, 68]. Espargaró and Collaborators (2017) [69] used molecular docking and molecular dynamics techniques to verify the anti-Aβ aggregation activity of several flavonoids. Among them, the flavonoids apigenin and quercetin (Fig. **4**) showed potent inhibitory activity against Aβ aggregation. The docking results showed that these flavonoids had a binding affinity with site 1 of the Aβ fibril (PDB ID: 2BEG), which is delimited by the Leu17, Phe19, Gly38, and Val40 residues. Furthermore, it was observed that the values of binding energies of apigenin (-8.7 kcal/mol) and quercetin (-8.8 kcal/mol) for 2BEG were lower than the energy value of the Thioflavin T control (-8.4 kcal/mol). Regarding the dynamics analysis, the results indicated that apigenin underwent a slight structural rearrangement at the beginning of the simulation and then remained stable at site 1, due to the hydrogen bonds with Leu17B, Val18D, Leu17E, and Val39E. In addition, this flavonoid and quercetin were visualized interacting with residues Phe20A, Ala21A, Gly37C, Phe20B, Gly37C, Gly38B and Gly73C from site 2 of 2BEG through hydrogen bonds. Therefore, the *in silico* simulations carried out by the authors suggest that the 2-phenyl-3,4-dihydro-2H-1-benzopyran scaffold can interact with Aβ42 fibrils, preventing them from being elongated and consequently reducing the polymerization of Aβ42.

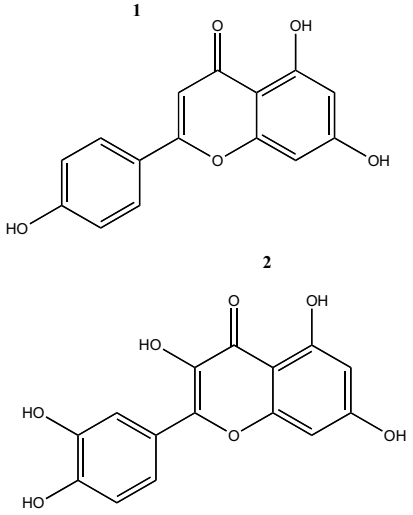

Fig. (4). 2D Chemical structures of apigenin (**1**) and quercetin (**2**) [69]. Source: Espargaró and Collaborators (2017).

Chakraborty and Collaborators (2014) [70] built QSAR models to investigate the anti-amyloidogenic activity of polyphenols. The QSAR analysis demonstrated that hydrophobic interactions are responsible for the recognition of amyloid fibrils by polyphenols, thus, it can be concluded that potent compounds interact within the core hydrophobic region of the fibril and interrupt hydrophobic interactions, destabilizing the fibril structure. In addition, it was noted that inhibitors with highly flexible substituents and highly branched substituents have a greater ability to destabilize amyloid fibrils. The derived model has been used by the authors to screen a small in-house developed phytochemicals database comprising 200 compounds. Through virtual screening, 59 anti-amyloidogenic phytochemicals were obtained. Therefore, the study by Chakraborty and collaborators clarified the mechanism of action of polyphenols against amyloid fibrils and the substituting groups that allow an improvement in activity, in addition to providing a strategy for the discovery of potent compounds against Aβ fibrils.

Shahinozzaman and Collaborators (2018) [71] verified the effects of prenylated flavonoids from *Okinawa propolis* against several pathologies, including AD. The authors performed the molecular docking of the following flavonoids against AChE (PDB ID: 4EY7): nymphaeol-A (NA), nymphaeol-B (NB), nymphaeol-C (NC), isonymphaeol-B (INB), and 3′- geranyl-naringenin (GN). The results indicated that the lowest binding energy was demonstrated by NA (-11.5 kcal/mol) followed by GN (-11.3 kcal/mol) and NB (-11.2 kcal/mol). In contrast, NC and INB exhibited equal bonding energy values (-11.0 kcal/mol). It was reported that all compounds tested interacted with the AChE choline-binding site residue known as Trp 86, through hydrophobic interactions (π - π stacked, π - σ, or π - alkyl bond formation). The flavonoids that showed the highest binding affinities (NA and GN) interacted with residues close to the protein's catalytic site and showed distinct interactions. When comparing the interactions of the two compounds, it was noted that NA bonded with AChE only through hydrophobic interactions, while GN formed two hydrogen bonds with the backbone residues Gln 291 and Tyr 124. The authors concluded that the binding energies, interactions and binding modes of the compounds with AChE suggest that they could delay the catabolism of ACh in the synaptic cleft.

Ogidigo and Collaborators (2021) [72] identified the compounds present in *Bryophyllum pinnatum* flavonoid-rich fraction (BPFRF) and analyzed its anticholinesterase activity through *in vitro* and *in silico* studies. The flavonoids carlinoside, luteolin-7-glucoside, luteolin, quercetin and isorhamnetin were found in BPFRF, which exhibited potent activity against AChE and BuChE *in vitro*, with IC_{50} values of 22,283 ± 0.27 mg/mL and 33,437 ± 1.46 mg/mL, respectively. Thus, the authors performed a study of molecular docking with the constituents of BPFRF at the active site of AChE (PDB ID: 4EY7) and BuChE (PDB ID: 1POM). They noted that the constituents of BPFRF showed favorable binding energies against the two enzymes, which ranged from -14.9 to -8.9 kcal/mol for AChE and -12.7 to -7.4 kcal/mol for BuChE. Luteolin-7-glucoside exhibited the highest binding affinity with AChE and interacted with residues from the enzyme's active site, either through hydrogen bonds (Tyr341, Phe295) or through pi - pi stacking (Trp286 and Tyr341). Quercetin and the rivastigmine control were also visualized interacting with the active protein site. Regarding BuChE, carlinoside exhibited the best binding energy among the flavonoids (-12.7 kcal/mol), while rivastigmine showed a value of -6.9 kcal/ mol. In addition, it was observed that carlinoside, quercetin and rivastigmine interacted with the residues

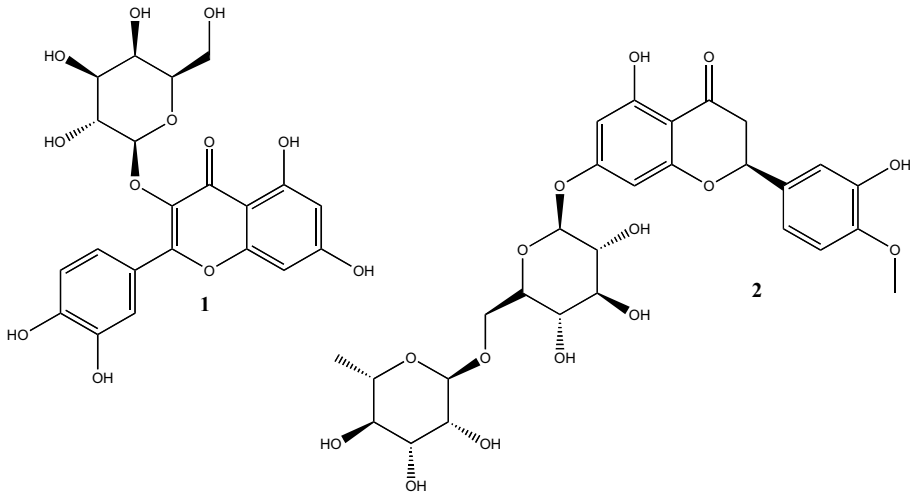

Fig. (5). 2D Chemical structures of hyperoside (**1**) and hesperidin (**2**) [73]. Source: Baltacci and Collaborators (2021).

of the BuChE catalytic site (Ser198, His438). Thus, the *in vitro* and *in silico* analyzes carried out by the authors suggest that BPFRF flavonoids may have inhibitory effects against cholinesterases.

Baltaci and Collaborators (2021) [73] determined the chemical composition and inhibitory activity of methanol extracts isolated from *Onosma gracilis* (Trautv.) and *O. orthodoxa* (Boiss. & Heldr.) against various enzymes. Among the compounds identified, two flavonoids were discovered in the extracts in large quantities: hyperoside and hesperidin (Fig. **5**). The *in vitro* study carried out by the authors demonstrated that the inhibitory activity of *O. gracilis* extract against AChE and BuChE was similar (2.57 mg GALAEs/g). However, this extract showed greater activity compared to *O. orthodoxa*, which resulted in 2.40 for AChE and 2.21 mg GALAEs/g for BuChE. Thus, the authors performed a molecular docking analysis to determine the contribution of hyperoside and hesperidin to the enzyme inhibitory activity. The docking results indicated that the binding affinities of hyperoside and hesperidin with cholinesterases were high, in which hyperoside exhibited binding energy values of -7.22 kcal/mol for AChE and -12.78 kcal/mol for BuChE, while hesperidin demonstrated values of -8.76 kcal/mol for AChE and -11.86 kcal/mol for BuChE. Therefore, it is believed that these compounds are effective inhibitory agents against cholinesterases.

Pitchai and Collaborators (2020) [74] have identified the flavonoid trans-tephrostachin from *Tephrosia purpurea* and synthesized this compound (**4a**) and its derivatives (**4b** to **4e**) in order to research new molecules with possible anti-AD activity. In the assay that verified anti-AChE activity, compounds 4a, 4b, 4c, 4d and 4e demonstrated excellent results with IC_{50} values of 35.0, 35.6,10.6, 10.3, and 28.1μM, respectively. Thus, the authors performed molecular docking and molecular dynamics simulations to analyze the binding affinity of compounds with AChE (PDB ID: 4PQE). In the docking study, it was found that trans-tephrostachin derivatives had a high binding affinity with the enzyme, in which the binding energies ranged from -7.3 to -6.9 kcal/mol. In the dynamics analysis, it was noted that the minimum distance between trans-tephrostachin derivatives and AChE is less than the distance of AChE-galantamine and AChE-donepezil complexes. Thus, AChE-trans-tephrostachin derivatives complexes showed better stability due to the strong bonds between the molecules. Therefore, trans-tephrostachin and its derivatives may be good candidates for research on drugs against AD.

Llorent-Martínez and Collaboartors (2017) [75] detected that flavonoids were the most abundant natural products in the extracts of *Lathyrus czeczottianus* and *L. nissolia* and investigated their antioxidant and anti-cholinesterase properties. *In vitro* tests showed that *L. czeczottianus* had more potent antioxidant effects than *L. nissolia*; however, the latter species exhibited strong inhibitory activity against cholinesterases. Among the flavonoids found in the extracts, the compounds vitexin and isoschaftoside (Fig. **6**) were selected for molecular docking with AChE (PDB ID: 4X3C) and BuChE (PDB ID: 4BDS). The results showed that vitexin had binding energies of -8.30 kcal/mol for AChE and -11.24 kcal/mol for BuChE, while isoschaftoside exhibited values of -11.05 kcal/mol for AChE and -10.08 kcal/mol for BuChE. Regarding interactions with protein amino acids, vitexin formed hydrogen bonds with AChE residues (Ser286, Arg289, Asp72, Tyr334 and Tyr70) and with BuChE residues (Asp70, Gln197 and Thr120). Isoschaftoside also demonstrated hydrogen bonds with AChE residues (Asp285, Ser286 and Tyr121) and with BuChE residues (Ser198, Ser 287, Glu197, and Leu286). Based on the results obtained by the authors, vitexin and isoschaftoside are promising flavonoids in the treatment of AD.

Tran and Collaborators (2020) [76] synthesized flavone derivatives and researched their inhibitory activity against two important targets of AD: AChE and BACE-1. The results of *in vitro* tests demonstrated that the compounds exhibited IC_{50} values of 340.09-25.51 μM for AChE and 70.79-1.58 μM for BACE-1. It was observed that the most promising synthesized molecules were B3, D5 and D6, as they showed the highest bioactivities against the two enzymes. Thus, a molecular docking study was conducted to explain the experimental results. *In silico* analysis showed that all flavone derivatives successfully anchored into the binding pockets of AChE and BACE-1, resulting in binding energies ranging from -10.36 to -27.06 kJ/mol for AChE and from -8.29 to -22.07 kJ/mol for BACE-1. Regarding the

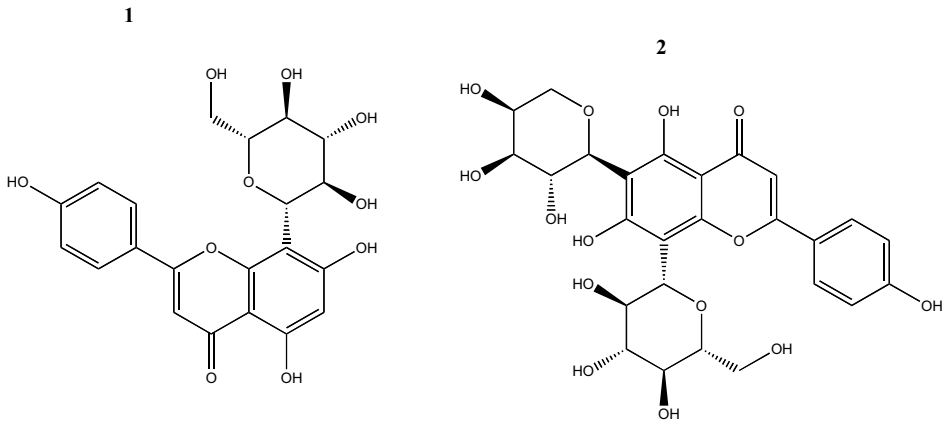

Fig. (6). 2D Chemical structures of vitexin (**1**) and isoschaftoside (**2**) [75]. Source: Llorent-Martínez and Collaboartors (2017).

molecules that showed the greatest potential *in vitro*, it was noted that B3 formed an important hydrogen bond with the Ser200 residue from AChE and van der Waals interactions with His440. When anchored with BACE-1, this compound interacted with Asp32, Asp228 and Gly230 residues. The compounds D5 and D6 formed strong hydrogen bonds with Thr232 (D5: length 2.69 Å; D6: length 2.49 Å) from BACE-1, which explained the high inhibitory activities of these compounds against the enzyme. Thus, the flavone derivatives B3, D5 and D6 showed good results against both enzymes through *in vitro* and *in silico* studies and consequently could be useful in future studies.

Ribaudo and Collaborators (2019) [77] reported semi-synthetic isoflavones obtained from the derivatization of flavonoids from *Maclura pomifera*, which were analyzed against BACE-1 using *in vitro* and *in silico* assays. The human recombinant BACE-1 inhibition assay demonstrated that compounds 7, 8 and 13 were the most active against the enzyme. During *in silico* analyzes, they showed good results: they demonstrated positive scores in the prediction of permeation in the BBB, ranging from 0.06 to 0.105; in molecular docking, all compounds were able to interact selectively and strongly with the active site of BACE-1. In addition, compounds 7, 8 and 13 were found to exhibit higher binding affinity with the enzyme than the oxazine-based compound, which has been co-crystalized with BACE-1 (PDB ID: 5CLM). Thus, these molecules showed a good correlation between their biological properties and computational predictions, indicating that they are possibly active against BACE-1.

El-Hawary and Collaborators (2021) [78] found that phlorizin, a dihydrochalcone isolated from *Malus domestica*, has neuroprotective effects (Fig. **7**). According to its results, phlorizin has antioxidant potential: it exhibits an IC_{50} value of 5.14 mg/mL in the DPPH assay, and increased GSH levels and reduced GSSG and MDA levels in the dexamethasone/scopolamine treated rats. In the *in vitro* assay against BACE-1, phlorizin inhibited the enzyme with an IC_{50} of 1.18 µg/mL, demonstrating an inhibitory activity comparable to one of the strongest BACE-1 inhibitors, the trihydroxychalcone. In the molecular docking analysis, phlorizin fitted well in the binding site of the enzyme and showed binding energy of -16.21 kcal/mol, similar to the energy value of the co-crystallized ligand (-17.47 kcal/mol). In addition, the dihydrochalcone showed a mode of binding with the enzyme that allowed most of the reported interactions with amino acid residues from the binding site (Thr 231, Asp228, Asp32 and Arg235). Therefore, all the results obtained by the authors suggest that phlorizin has the potential to suppress or delay the onset of AD.

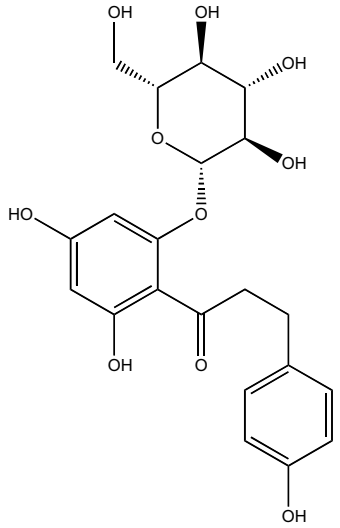

Fig. (7). 2D Chemical structure of phlorizin [78]. Source: El-Hawary and Collaborators (2021).

2.2. Lignans

Lignans are a large group of natural phenol species that are widespread within the plant kingdom and are formed between two units of phenylpropane with a different degree of oxidation in the side chain and a different substitution pattern in the aromatic portions [79]. Lignans are characterized by two C_6C_3 units and are linked by a β, β' link [80].

A complex study involving several *in silico* tools carried out by Maia and Collaborators (2020) [81] sought to identify lignans with multitarget potential for enzymes that are directly or indirectly involved in the oxidative pathway for the treatment of AD. For this, the researchers used virtual screening methods based on the ligand and structure, that is; through the quantitative structure-activity relationship (QSAR) and molecular docking, respectively. Eight targets were selected, they are: c-Jun N-terminal kinase 3 (JNK-3),

Fig. (8). 2D Chemical structures of austrobailignan (**1**), anolignano c (**2**), 7-epi-virolina (**3**), 6 - [(2R, 3R, 4R, 5R) -3,4- dimetil-5- (3, 4,5-trimetoxifenil) oxolan-2-il] -4-metoxi- 1,3-benzodioxol (**4**), ococimosina (**5**) e mappiodoinina b (**6**) [81]. Source: Dos Santos and Collaborators (2020).

protein tyrosine phosphatase 1B (PTP1B), nicotinamide adenine dinucleotide phosphate oxidase 1 (NOX1), NADPH quinone oxidoreductase 1 (NQO1), phosphodiesterase 5 (PDE5), nuclear factor erythroid 2-related factor 2 (Nrf2), cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (*i*NOS) and then were subjected to a combined approach of the two virtual screening methods. Of 159 lignans used in the study, several potentially active compounds were identified: three compounds with probabilities of activity ranging from 50% to 61% for JNK-3; 43 compounds with probabilities of 52-72% for PTP1B; 57 compounds with odds of 51%-72% for PDE5; 27 compounds with probabilities between 50% and 61% for COX-2; 27 compounds with probabilities between 50% and 81% for *i*NOS; 111 compounds with probabilities ranging from 50% to 64% have been identified for Nrf2; nine compounds with probabilities ranging from 51% to 78% have been identified for NOX1, and 156 compounds have been selected, with probabilities ranging from 27% to 100%, for NQO1. It was also possible to identify 139 potentially active molecules for two to five enzyme targets from the whole set analyzed. After these analyzes, an evaluation of the properties of absorption, distribution, metabolism and toxicity (ADMET) were also carried out. Of all 139 lignans that were considered to be potentially active and multitarget, 92 showed good absorption, bioavailability and solubility, ranging from soluble to moderately soluble and six lignans showed no toxicity for the analyzed parameters. Therefore, the compounds austrobailignan (06), anolignan c (11), 7-epi-viroline (19), 6 - [(2R, 3R, 4R, 5R) -3,4-dimethyl-5- (3, 4, 5-trimethoxyphenyl)oxolan-2-yl]-4-methoxy-1,3-benzodioxol (64), ococymosin (116) and mappiodoinin b (135) were considered potentially active against several enzymes that may be involved in the pathogenesis of AD and can confer neuroprotective effects, with low toxicity (Fig. **8**).

Hu *et al.* [82] isolated four stereoisomers of Schisandrin B (Sch B) from the fruits of *Schisandra chinensis* and tested against the enzyme glycogen synthase kinase-3β (GSK-3β), which is a key enzyme in hyperphosphorylation of tau proteins and is a promising therapeutic target in AD. Initially, they performed molecular coupling and based on the results; they performed experimental tests. For this, the chemical structures of the test compounds were sketched in Chemdraw Ultra (7.0) and saved in Mol2 format. The X-ray crystal structure of GSK-3β (PDB ID: 1UV5), was obtained from Protein Data Bank (PDB) (http: // www. Wwpdb.org). The sybyl 6.9.1 software was used to prepare the protein and a small minimization was performed to release the internal tension. CDOCKER was used for molecular docking, a Docking module, according to all parameters defined as the default mode. After coupling, *in vitro* and *in vivo* tests against GSK-3β were performed. The results showed that the data obtained from the Docking corroborated with the results of the experimental tests. It was verified that the stereoisomers of Sch B, (+) - 1, (-) - 1, (+) - 2 and (-) - 2, are potent inhibitors of GSK-3β in an orthosteric binding mode, with values of IC_{50} of 340, 290, 80 and 70 nM, respectively. Connection energy and IC_{50} values can be viewed in Table **1**. Segundo Liu and Collaborators (2019) [83], lignans and phenylpropanoids are increasingly attracting attention to the discovery of useful agents to inhibit Aβ aggregation. Therefore, these researchers isolated two pairs of new

Table 1. Binding energy values and inhibitory activities of target compounds against GSK-3β [82].

Compound	GSK-3β Inhibition (10 μM)	IC_{50} (nM)	Docking Score (cal/mol)
(+)-**1**	89.23	341.32 ± 12.3	-12.34
(-)-**1**	87.34	290.89 ± 15.7	-14.58
(+)-**2**	90.23	80.67 ± 5.5	-39.45
(-)-**2**	92.12	73.22 ± 4.2	-42.12
Bio	-	25.49 ± 3.11	-5.32

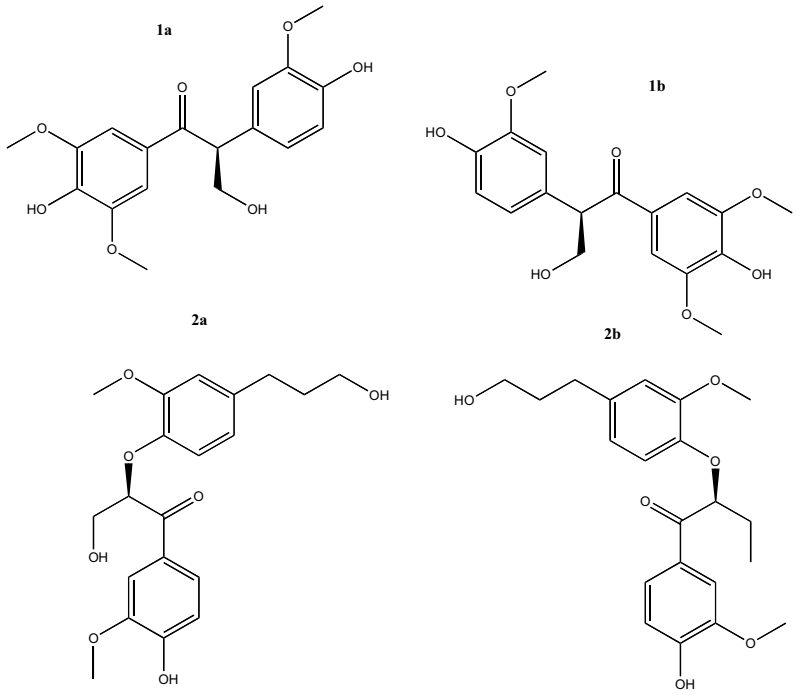

Fig. (9). Chemical structures of compounds **1a/1b-2a/2b**. Source: Liu and Collaborators (2019).

enantiomeric lignans and phenylpropanoids from the seeds of *Prunus tomentosa* (Fig. **9**). In addition, the inhibitory activity on Aβ aggregation of all pure optical compounds was tested by thioflavin T (ThT) assay. The isolates showed more potent inhibitory activity than the positive control with an inhibitory rate of 73.89 ± 3.41% (1a); 78.69 ± 1.50% (1b); 63.25 ± 2.68% (2a); and 67.13 ± 0.90% (2c) at 20 μM, respectively. In addition, the inhibition profiles were explained by studies of dynamics (MD) and molecular docking. The MD simulations were performed with the GROMACS 5.0 package using the CHARMM36 force field and docking was performed using the Molegro Virtual Docker 4.0 program. Both methodologies used the protein code Aβ1-42 (PDB: 1IYT). The results of *in silico* studies showed that the coupling of the compounds with the active site of the protein was strong, presenting several hydrogen bonds, among them: Tyr10, Gln15, Glu11 and Gly9, Leu17, Val18. However, the link with the amino acid Gln15 suggests that it is a key active residue for isolates against Aβ1-42 self-induced aggregation.

Another study with activities *in silico*, *in vitro* and *in vivo* using lignan was carried out by Somani and Collaborators (2017) [84]. In that study, cubebine, a dibenzylbutyrolactone lignan, was isolated from *Piper cubeba* and investigated for its inhibitory activity against AChE. The in silico results showed that lignan was able to bind well within the catalytic site of the enzyme showing π-π stacking with His 440, hydrogen bonding with Gly119, Gly118 and Tyr121. This linker also showed hydrophobic interactions with Trp84, Phe330 at the hill binding site, Tyr 121 at a peripheral binding site and Phe 290 at the hill binding site. Cubebin slip score (-10.51) indicated the stability of the enzymatic complex cubebin. These data guided the experimental tests that confirmed the results *in silico*, where Cubebin inhibited the AChE enzyme in an assay with an IC_{50} value of 992 μM.

Abouelela and Collaborators (2020) [85], isolated four flavanolignans, ceibapentains A (**1**) and B (**2**) and cinchonains Ia (**3**) and Ib (**4**), from an ethyl acetate extract from the aerial part of *Ceiba pentandra* (L) (Bombacaceae). Compounds 1-4 were tested for their anti-Alzheimer's activity by assessing their inhibitory effect on amyloid β42 aggregation. The results revealed that cinconain Ia (3) had a greater inhibitory effect (91%) than the standard (70%). Compounds **1**, **2** and **4** exhibited moderate activity, with inhibition rates of

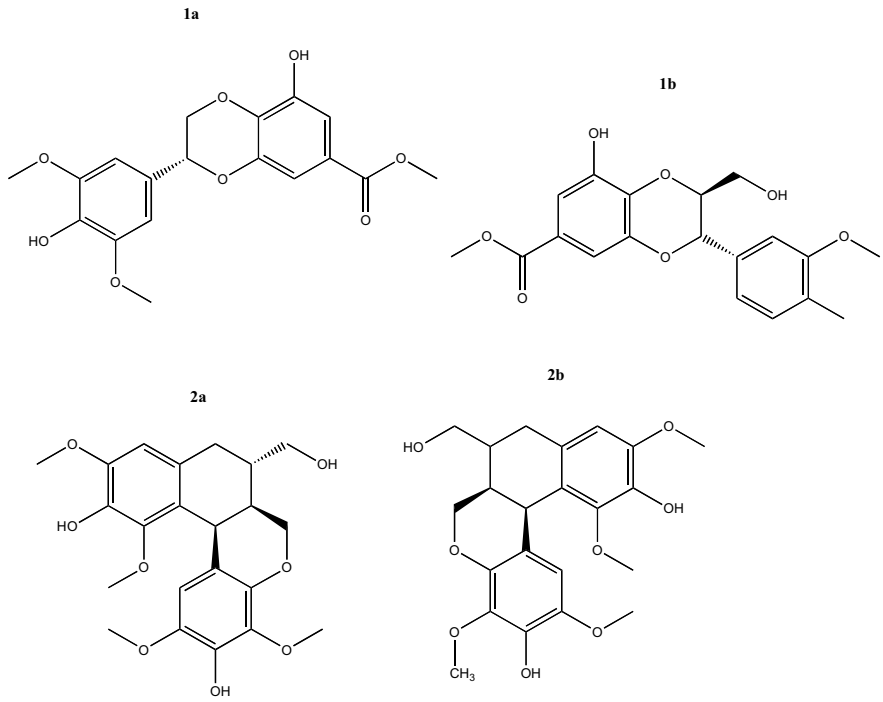

Fig. (10). The structures of compounds **1a/1b and 2a/2b**. Source: Wang and Collaborators (2017).

43%, 47% and 58%, respectively. A molecular docking study was used to investigate the mode of attachment of the β1-40 amyloid peptide fibril structure. For this, Aβ-peptide 1-40 (PDB ID: 1BA4) was used using the molecular operating environment 2008.10 (MOE). Compound **3** showed a maximum bond score of -12.3 kcal / mol involving two hydrogen bonds between the hydroxy group 3 phen-phenolic with Lys16 and Gly9, confirming the experimental results.

Acetylcholinesterase (AChE) inhibitors are the most promising drugs currently available for the treatment of AD. That is why, Hung and Collaborators (2015) [86] performed a screening based on the isolation of the methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. The bioassay led to the isolation of a new lignan glycoside, licocernuaside A, and fourteen known compounds. The docking results for the complexes with AChE or BChE revealed that some inhibitors have positioned themselves stably in several pocket / catalytic domains of the residues in these enzymes.

Two new pairs of rare enantiomeric lignans, named 8 ', 9'-dinor-3', 7-epoxy-8,4'-oxineolignans (7S, 8S) - and (7R, 8R) -pithecellobiumin A (1a / 1b) and a pair of 2',9'-epoxy-arylnaphthalenes named (7R, 8R, 8'R) - and (7S, 8S, 8'S) - pithecellobiumin B (2a/2b) were isolated from *Pithecellobium clypearia* Benth leaves by Wang and Collaborators (2017) [87]. The inhibitory activity on Aβ aggregation of all compounds was tested by ThT assay. It was observed that enantiomeric inhibitors 1a (62.1%) and 1b (81.6%) exhibited different degrees of anti-Aβ aggregation activity. However, 2a (65.4%) and 2b (68.4%) had a similar rate of inhibition. The different inhibition profiles were explained by studies of molecular dynamics and docking. For this, the X-ray crystal structure code of the Aβ42 protein was 1IYT. The dynamics were performed with the GROMACS 5.0 package using the CHARMM36 force field. The fitting studies were performed using the Molegro Virtual Docker 4.40 program and the water molecules in the PDB file were removed. The results of in silico studies revealed that compound 1b (1a: 69.6, 1b: 74.5 kcal / mol) has a greater binding affinity for Aβ42 with the most negative binding energy. Both compounds 1a / 1b, 2a / 2b (Fig. **10**) formed hydrogen bonds with Gln15 and Lys16, respectively. In addition, they were also displayed similarly with binding energy, such as 61.8 and 62.5 kcal / mol, respectively. Thus, the inhibitory activity was clearly explained by the comparison of the fitting models and further proved that compound 1b showed optical selectivity in the aggregated inhibition of Aβ42.

According to Song and Collaborators (2014) [88], ent-sauchinona is a polyphenolic compound found in plants belonging to the lignan family. Due to its anti-inflammatory property, the researchers in this study sought to investigate whether ent-sauchinone could have anti-amyloidogenic effects by inhibiting the NF-κB pathways. The results showed that ent-sauchinone (1, 5 and 10 μM) effectively decreased lipopolysaccharide (LPS) - (1 μg / ml) induced inflammatory responses by reducing the generations of ROS and NO and expressions of iNOS and COX-2 in cultured astrocytes and microglial BV-2 cells. In addition, it was able to inhibit LPS-induced amyloidogenesis by inhibiting β-secretase and β-secretase activity. NF- κB amyloid and STAT3, critical transcriptional factors that regulate not only inflammation, but also amyloidogenesis, were also inhibited in a concentration in a dependent manner by ent-Sauchinone. A docking study using the Autodock VINA program on the STAT3 protein (PDB ID: 3CWG) was carried out. And the result showed that ent-Sauchinone binds in a STAT3 binding pocket where three domains of the DNA binding domain (DBD), the binding domain, and the coiled-coil (CCD) domain come together. The compound managed to interact with amino acids Gln247, Ala250, Cys251, Ile258, Arg325, Pro333 and Pro336.

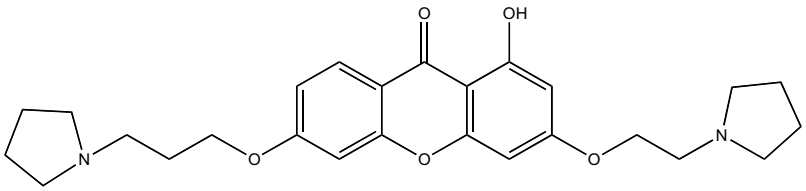

Fig. (11). Structure 2d of compound 2a. **Source:** Kou and Collaborators (2020).

2.3. Coumarins, Chromones and Xanthones

Coumarins are oxygenated heterocyclic lactones, called benzopyranones, which basically consist of a pyran ring attached to a benzene with the pyrone carbonyl specifically located in position 2, comprising an important class of secondary metabolites that are widely distributed in vegetables, as well as in fungi and bacteria [89, 90] already thechromones are classified as coumarin isomers and are also oxygenated heterocyclic compounds that may come from natural or synthetic routes, and its structure consists of the fusion of a benzene ring with a pyran-4-one ring [91, 92].

The xanthones, in turn, are also oxygenated heterocyclic compounds that can be of natural origin (present in plants, fungi, ferns, lichens) or synthetic; however, its basic structure is dibenzo-γ-pyro or, as it is known, 9H-xanten-9-one, formed in plants by the combination of the chiquimate and acetate pathways [93, 94]. In view of their respective molecular architectures and easy modification, coumarins, chromones and xanthones have been the target of several studies, presenting numerous pharmacological properties [89, 90, 93, 95].

In view of this fact, Kou and Collaborators (2020) [96] analyzed and synthesized a series of xanthone derivatives as multifunctional ligands against Alzheimer's disease (AD). For this, the authors designed and synthesized a series of xanthone derivatives and evaluated their biological potentials *in silico* and *in vitro*, to mention: cholinesterase inhibition activities (ChE), metal chelating properties and antioxidant activity. In addition, the authors used kinetic and molecular docking studies to better understand the enzymatic inhibition of the compounds.

In view of the results, the synthesized compounds were 4, named 2a-2d and their respective structures were confirmed by spectroscopic methods. In view of *in vitro* analyzes, it was shown that all xanthone derivatives exhibited excellent antioxidant activity (since they showed greater antioxidant activity than vitamin C) and good metal chelating properties, as well as exhibiting selective inhibitory activities against acetylcholinesterase (AChE), specifically compound 2a (Fig. **11**), which showed a greater inhibitory activity against AChE, with an IC_{50} of 0.328 μM, which was comparable to the thorax drug (IC_{50} of 0.207μM).

In addition, through kinetic and molecular docking studies, the authors concluded that compound 2a may be able to bind to both the catalytically active site (CAS) and the peripheral anionic site (PAS) of AChE and it was demonstrated that inhibition of acetylcholinesterase was related to tertiary amines, since the order of inhibitory potency of these compounds containing different substituents was as follows: pyrrole alkyl > piperidinyl 1 > dimethylamino > morpholinyl, which implies that carrying electrons with design substituents would have an adverse impact on their activities. Moreover, the authors emphasize that these xanthone derivatives are potential ligands directed to multiple targets for further development for the treatment of AD.

In the studies by Cruz and Collaborators (2017) [97], the synthesis and evaluation of xanthone and flavone derivatives was carried out as antioxidants, chelators and with AChE inhibitory activity, using *in silico* and *in vitro* methods. In this sense, 11 compounds (**1-3**, **6-9**, **11-14**) were synthesized, both characterized by spectroscopic methods, the xanthone derivative being called compound 3 and the flavone derivative called compound 14 (Fig. **12)**, demonstrated potential inhibitors against AChE, with an IC_{50} of 49.08 μM for compound 3 and 69.34 μM for compound 14. In addition, both demonstrated antioxidant properties, exercising dual activity.

In molecular docking studies, the xanthone derivative (3) had a score of -9.4 kcal/mol^{-1} and the flavone derivative (14) showed -10 kcal/mol^{-1}, good results, if comparable to the results of the control drugs used (-5.7 to -12.1 kcal/mol^{-1}). Given the results, the authors justify the importance of such as motivators to identify new xanthone and flavone derivatives as dual anti-Alzheimer agents with AChE inhibitory and antioxidant activities.

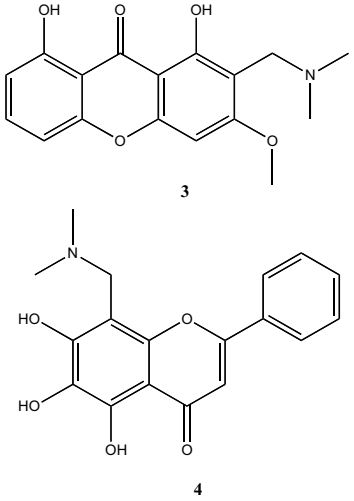

Fig. (12). 2d structure of the xanthone derivative (3) and flavone (14). **Source:** Cruz and Collaborators (2017).

Yang and Collaborators (2020) [98] synthesized 4 xanthone derivatives and evaluated their potential AChE inhibitors and their metal chelating and antioxidant capacities against Alzheimer's disease (AD). In view of the results, most compounds showed potential inhibitory, antioxidant and metal chelating properties of acetylcholinesterase (AChE) and butylcholinesterase (BuChE). Among them, compound 2 (Fig. **13**) showed a greater ability to inhibit AChE and high selectivity for AChE (IC_{50} = 2.403 μM for

AChE and IC_{50} = 31.221 μM for BuChE), in addition, it proved to be a good antioxidant (IC_{50} = 2.662 μM).

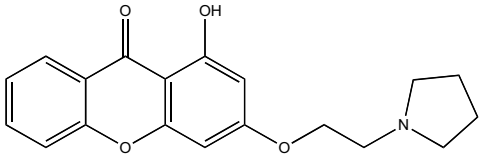

Fig. (13). Structure 2d of the xanthone derivative, compound 2. Source: Yang and Collaborators (2020).

In relation to and studios of enzymatic kinetics, it was demonstrated that this compound was a mixed type inhibitor, which could interact simultaneously with the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of AChE. Still, the authors point out that its copper complex showed more significant inhibitory activity for AChE (IC_{50} = 0.934 μM) and antioxidant activity (IC_{50} = 1.064 μM). Additionally, molecular docking studies were performed, and compound 2 showed a higher enzymatic affinity, with the energy of -41.3323 kcal/mol^{-1}, further justifying the significant results *in vitro*.

Still, the authors analyzed the prediction of penetration of the blood-brain barrier (BBB) and through the results, it was demonstrated that all compounds could penetrate the BBB. Results that together were motivating for compound 2 to be a promising AChE inhibitor, with metal chelating capacity and antioxidant capacity for future studies.

Singla and Piplani (2016) [99] synthesized and evaluated (*in silico, in vitro* and *in vivo*) a series of 15 new coumarin hybrids in which the coumarin moiety was linked to different heterocyclic amines substituted by an appropriate ligand as potential AChE inhibitors. The target compounds were named 15a-i, 16a-d and 17a-b, with compound 15a (Fig. **14)** being considered the most promising, exhibiting a greater *in vitro* inhibitory activity of AChE (IC_{50} = 2.42 μM) against standard drug donepezil (IC_{50} = 1.82 μM).

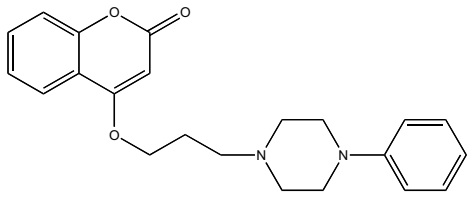

Fig. (14). 2d structure of the coumarin derivative (15a). Source: Singla and Piplani (2016).

In view of the *in silico* analyses, molecular docking studies were carried out to assess their potential as acetylcholinesterase double bond inhibitors for the treatment of cognitive dysfunction caused by increased acetylcholine hydrolysis and oxidative stress induced by scopolamine. Thus, the molecular docking study of compound 15a, indicated that it interacted with all the crucial amino acids present in the AChE catalytic active site (CAS), mid-gorge and PAS through hydrophobic interactions, Van der Waal and π - π stacking, resulting in greater AChE inhibitory potency compared to the other 14 analogues in the series, which justified the promising results. Specifically, the compound showed significant binding interactions with both the enzyme's Trp86 and Trp286 binding pockets.

In a more detailed analysis, the authors pointed out that the phenylpiperazine fragment of compound 15a was entered into the canyon of the AChE enzyme, resulting in stacking interactions π - π parallel with the catalytic site of the amino acids Trp86 and His447, thus, adopting a shape similar to a sandwich. In addition, the coumarin portion interacted through aromatic interactions π - π with the indole and phenyl rings of Trp286 and Tyr72, which were located at the peripheral anionic site. Thus, the results for compound 15a, express its potential as a drug candidate against AD.

He and Collaborators (2018) [100] designed, synthesized and evaluated by *in silico, in vitro* and *in vivo* methods 28 coumarin-dithiocarbamate hybrids as multitargetal agents for the treatment of Alzheimer's disease. Motivated by a previous project, the authors justified that the portion coumarin was chosen to inhibit MAO-B and interact with the PAS of AChE due to its aromatic character, whereas the portion dithiocarbamate_was used for binding to AchE CAS, while a flexible ligand was used to connect these two fragments, which could allow the projected compounds to act simultaneously in the CAS and in the AChE PAS.

The compounds called 7a-n and 8a-n were subjected to test *in vitro* and *in silico*, and given the results, most showed potent and clearly selective inhibition for AChE and MAO-B, with compound 8f being the most potent inhibition for AChE with IC_{50} values of 0.0068 μM and 0.0089 μM for Electric Eel AchE and Human AchE, respectively. Additionally, compound 8g (Fig. **15**) was identified as the most potent inhibitor for human MAO-B, and also a good and balanced inhibitor for human AChE and hMAO-B (0.114 μM for AChE; 0.101 μM for MAO-B).

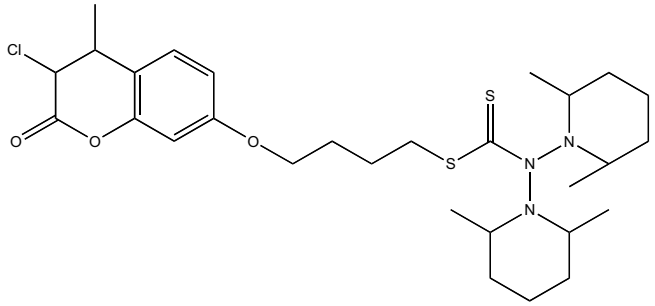

Fig. (15). 2d structure of the coumarin derivative (8g). Source: He and Collaborators (2018).

The kinetic and molecular modeling studios revealed that compound 8g was a double binding site inhibitor for AChE and a competitive inhibitor for MAO-B. Still, other studies have indicated that 8g could penetrate BBB and show no toxicity in SH-SY5Y neuroblastoma cells. Moreover, the authors point out that 8g did not exhibit any acute toxicity in mice at doses up to 2500 mg/kg and could reverse the cognitive dysfunction of mice with AD induced by scopolamine. As a result, these results highlighted 8g as a potential multitarget agent for the treatment of AD and offered a starting point for the design of new AChE/MAO-B multitarget inhibitors based on dithiocarbamate.

Baruah and Collaborators (2019) [95] motivated by the promising activity of chromones as AChE antagonists, analyzed through *in silico* and *in vitro* methods, 2 substituted

chromones, one containing a methyl group and a primary amine in its chromone skeleton (7-amino-2-methylchromone) called of AMC, and the other containing a tertiary amine (3-cyanochromone), called CyC, as shown in Fig. (**16**).

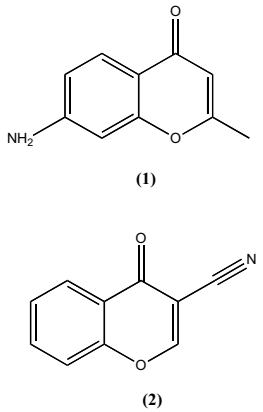

Fig. (16). 2d structure of 7-amino-2-methylchromone (AMC) (**1**) and 3-cyanochromone (CyC) (**2**). Source: Baruah and Collaborators (2019).

Substituted chromones were acquired commercially by the company Sigma Aldrich and the colorimetric enzymatic assays and fluorescence measurements were performed using the Ellman method, with some modifications to obtain the experimental results, which were later corroborated by studies of molecular docking and simulation. With this, the substituted chromones exhibited strong inhibition activities against AChE, with CyC (IC_{50} = 85.12 nM) acting as a better inhibitor than AMC (IC_{50} = 103.09 nM); however, both presented IC_{50} values in the range of the FDA-approved cholinergics, for example, donepezil (IC_{50} = 74.13 nM).

Through the analyzes *in vitro* and *in silico,* the authors report that the inhibition was of a non-competitive type, which was observed in both cases with the binding of the compounds close to the peripheral anionic site (PAS) of the enzyme. The docking score was -35.02 kJ/mol for CyC and -33.37 kJ/mol for AMC. Still, the authors stressed that having a planar nitrile group in CyC compared to hybridized sp3 substituents in AMC, facilitated the stacking interactions in the first, justifying its greater inhibitory efficacy.

In addition, a significant decrease in the inhibition potency of CyC (~ 32%) was observed compared to AMC (~ 5%) when the experiments were carried out in a modulator medium containing human serum albumin (HSA) instead of pure aqueous buffer. This can be justified by the fact that when HSA was used as a modulating medium, the reason behind the reduced inhibitory efficacy of the compounds can be attributed to the confiscation of chromones by HSA. This sequestration, in turn, would result in a decrease in the unbound fraction of the drug to bind to the enzyme. Thus, greater affinity for HSA, as also verified by calculation of molecular docking, results in a greater reduction of the inhibitory potency of CyC in HSA.

With this, this study confirms the importance of a meticulous substitution in the molecular architecture of the chromones to promote the maximum potency of inhibition, considering its use as drug for AD.

Liu and Collaborators (2015) [101] planned, synthesized and evaluated a series of chromone-2-carboxamido-alkylbenzylamines. Specifically, the compounds were evaluated for their biological activities, including AChE and BuChE inhibition, enzyme inhibition kinetics, antioxidant activities, biometal chelation and effects on Aβ aggregation. The results showed that the majority exhibited good multifunctional activities. However highlighted, compound 49 (Fig. **17)** exhibited excellent inhibitory potency for rat AChE (IC_{50} of 0.07 μM) compared to the control used donepezil (IC_{50} of 0.015 μM). However, it showed a weaker inhibition for rat BuChE, with an IC_{50} of 51.50 μM compared to donepezil (IC_{50} of 20.70 μM).

In view of these results, the authors subjected compound 49 to kinetic and molecular docking, in order to better understand its mechanism of action. Thus, the results showed that the compound had mixed-type inhibition, as it was able to bind to the catalytic active site (CAS) as well as to the peripheral anionic site (PAS) of AChE. The docking results showed that the compound interacted with several amino acid residues from AChE, justifying such inhibitory potency.

Regarding the inhibitory activity of Aβ aggregation self-induced and induced by $Cu2^{+}$, compounds 50 and 49 showed excellent activities, with a percentage of inhibition of 63.0% and 59.2% for self-induced Aβ and 55.6% and 48.3%, for that induced by $Cu2^{+}$. In addition, compound 49 has also been shown to have a good property of acting as a selective metal chelator, moderate antioxidant activity, activities that, together, made compound 49 a promising multifunctional agent for the treatment of AD.

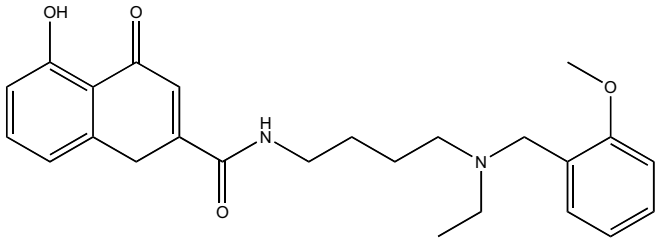

Fig. (17). 2d structure of compound **49**. Source: Liu and Collaborators (2015).

2.4. Terpenoids

These compounds are formed by two main precursors are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) and are sub-classified by their structure: as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes. Despite the diversity of classes, plenty of compounds and several beneficial effects attributed to these natural products [102, 103], including against AD [104-108], but little is done in rational planning.

Bidon-Chanal and collaborators (2013) [109] reported the isolation of furanosesquiterpene palinurin, a marine natural product. The authors observed its inhibitory effect on glycogen synthase kinase 3b (GSK-3b). This enzyme is a potential target studied against Alzheimer's disease [110], neurodegenerative disorders [111], myocardial ischemia [112] and cancer [113]. Through molecular docking, the authors identified two pockets in the receptor, on C-terminal part and one

in the N-terminal lobe, which is a potential allosteric binding site. The inhibitor activity of the compound occurs with binding to that site.

The application of an in silico approach in the discovery of new inhibitors against Alzheimer's disease (AD) is reported by Dash and Collaborators (2019) [114] that carried out the study of the aerial part of *Geophila repens* (L.) I.M. Johnst (Rubiaceae). In this research, the authors isolated the Pentilcurcumenate terpene (Fig. **18**) from the G. repens hydro-alcohol extract (GRHA), which was identified by spectroscopic data. In the absence of an experimentally resolved structure for mouse butyrylcholinesterase (BChE), the authors performed homology modeling to generate a useful structure for the design of new inhibitors using the MODELLER 9.14 software.

The tool High performance thin layer chromatography (HPTLC) method was applied in the bioautographic detection of pentylcurcumene that has successfully demonstrated its anticholinesterase activities. Pentylcurcumene demonstrated anticholinesterase activities, with IC 50 of the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) of 73.12 ± 0.56 and 97.65 ± 0.46 µg / ml, respectively. Cellular antioxidant tests, such as DPPH, oxygen radical absorption capacity (ORAC), and cell-based erythrocyte antioxidant protection (CAP-e) assays showed that pentylcurcumene showed markedly different degrees of dose-dependent antioxidant activities. Molecular docking simulations using the module in Schrodinger's molecular modeling software for pentylcurcumene (ligand) and enzymes (proteins) exhibited ligand binding at active sites of acetylcholinesterase (AChE; human / rat) and butyrylcholinesterase (BChE; human / rat) efficiently and also predicted the hydrophobic interaction of the drug for different amino acid residues within proteins.

The authors concluded that HPTLC is an improved and advanced tool that resulted in a successful implementation of the bioautography detection method of Pentylcurcumene in G. repens for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities. The results of the antioxidant study and the support of the molecular docking analysis state that pentylcurcumene could be a potential first-line cholinesterase inhibitor for Alzheimer's disease.

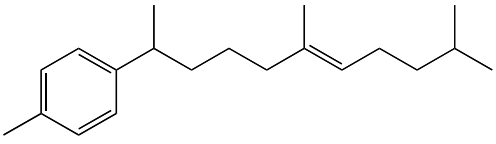

Fig. (18). 2D chemical structure of Pentylcurcumene [114]. **Source:** Dash and Collaborators (2019).

Liu and collaborators (2018) [115] state that Alzheimer's disease (AD) is closely related to neuroinflammation and the continuous search for new nitric oxide (NO) inhibiting substances as anti-neuroinflammatory agents, isolated two new sesquiterpenes and ten known terpenes of Inula flowers Japonica (Asteraceae), its structures were established based on extensive analysis of spectroscopic data from Nuclear magnetic resonance spectroscopy (NMR) and Mass spectrometry (MS), as well as calculated and experimental electronic circular dichroism (ECD) spectra. Among these isolates, a new sesquiterpene was identified with a rare fused tricyclic skeleton, and a second one with a 1.10-dry-eudesmane skeleton.

The authors sought to examine the antineuroinflammatory effect by inhibiting the release of NO in murine microglial induced by LPS Cells BV-2. The possible NO inhibition mechanism was investigated by applying molecular docking calculations using the AutoDock Tools (ADT 1.5.6) software using the hybrid Lamarckian Genetic Algorithm (LGA). The three-dimensional (3D) crystalline structure of iNOS (PDB code 3E6T) was obtained from Protein Data Bank. Molecular docking results showed that compounds 2-4 and 11 had strong NO inhibitory effects with good affinities with the iNOS protein.

The authors concluded that bioactive compounds from I. japonica flowers may be useful for the development of anti-neuroinflammatory and anti-neurodegenerative agents for inflammation and other related disorders.

Usuki e and collaborators (2020) [116] state that *Ginkgo biloba* extracts that have trilactone terpenes (TTLs) as the main active components, such as bilobalide (BB) and ginkgolides (Fig. **19**) are used as a potential treatment for Alzheimer's disease. The authors sought to verify how the modification of the lactone portions of BB would affect their biological activities in a series of assays, including proliferating cell activity, neuroprotective effects against Aβ peptides (1-40) and neurite growth effects in PC12 neuronal cells.

The authors' methodology covered the extraction and isolation of Ginkgo biloba extracts and the synthesis of four derivatives from BB isolated from Ginkgo biloba leaves; they were BB-diether 2, diAc-isoBB 3, diBrBz-isoBB 4 and diBn-BB- aldehyde 5. Structure-Activity Relationship (SAR) tools were applied to ginkgolide, which produced biologically more potent species by modifying the lactone portions. Derivatives with lactone groups exhibited biological activity similar to native BB, while those that lacked lactone portions showed less neurite growth effects.

The authors concluded that the results suggested that the lactone moieties of BB play an important role in exerting neurite outgrowth effects on PC12 cells. In addition, the functionalization of hydroxyl groups appeared to play a role, although more detailed studies are needed to clarify these effects.

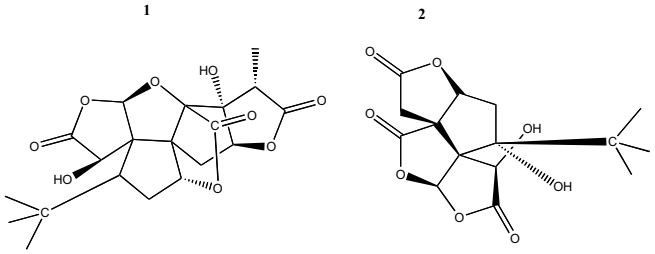

Fig. (19). 2D chemical structure of ginkgolide (**1**) and bilobalide (**2**) [116]. Source: Usuki e and collaborators (2020).

The study developed by Agatonovic-Kustrin and collaborators (2020) [117] applied quantitative structure activity relationship tools (QSARs) to predict the penetration capacity of the skin, blood and blood-brain barrier (BBB) of 119 essential oil terpenoids (OE) used in aromatherapy.

For this, the authors submitted the structures of each molecule were modeled and optimized for minimum energy conformation using the standard geometry minimization procedure MM2 (complete molecular mechanics) in Molecular Modeling Pro 6.1. (ChemSW, Fairfield, CA, USA). 91 2D and 3D structural descriptors were generated for each molecule, which described their physical-chemical properties. Two predictive QSAR models of nonlinear modeling artificial neural network (ANN) approach were developed using StatisticaVR Neural Networks version 7 (StatSoft Inc., Tulsa). One model was built based on experimentally measured skin permeability for 162 molecules and the other on BBB permeability for 138 molecules.

The authors concluded that the developed QSAR Models confirm that the components of EOs penetrate through the skin and throughout the BBB. Some descriptors, such as logP (lipophilicity), size and molecular shape, were found to dominate the QSAR model for the BBB. The main disadvantage of the new ligands aimed at multiple targets for the treatment of Alzheimer's disease is the size of the molecules, requiring compounds with more appropriate physicochemical properties.

Karolina and collaborators (2017) [118] analyzed antioxidant activity and the potential to increase the acetylcholine level of 18 selected terpenoids for possible acetylcholinesterase inhibitory activity. The methodology used consisted of the application of Marston (chromatographic assay) and Ellman (spectrophotometric assay) methods. The three-dimensional structures of the ligands were prepared and optimized using Spartan 10 V.1.1.0 (Wavefunction, Inc. Irvine, CA, USA). The authors submitted the 18 terpenes and galantamine (positive control compound) to molecular docking simulations with human acetylcholinesterase (huAChE) obtained from the protein database (PDB ID: 4EY6) using AutoDock (V.4.0) and Molegro Virtual Docker (MVD, V.5.0.0., MolegroApS Aarhus, Denmark) to describe the interaction between terpenes and AChE. The terpenes analyzed were also evaluated for their cytotoxic properties against two normal cell lines using the MTT assay.

The authors concluded that of the terpenes analyzed, carvone, pulegone and γ-terpinene have desirable AChE inhibitory activity. In addition, all terpenes were characterized by potent antioxidant activity and low cytotoxicity, with carvone, pulegone and γ-terpinene being promising candidates for the development of ligands targeting multiple targets.

Haghaei and Collaborators (2020) [119] addressed the kinetic and thermodynamic study of the interaction of Beta-Boswellic acid (BBA) that has beneficial effects in neurodegenerative diseases with the Tau protein investigated by surface plasmon resonance and molecular modeling methods. In the methodology, the authors applied surface plasmon resonance (SPR) method and molecular modeling for the investigation of BBA interaction with the Tau protein (PDB: 5O3L, https://www.rcsb.org/structure/5O3L) using the Gold software. The Goldscore scoring function was performed using the ChemScore score function method was used for molecular docking studies. The results were visualized using LigandScout (http: // www.inteligand.com/ ligandscout/) and Pymol software for the best conformation of BBA in the studied connection site. The results showed that BBA forms a stable complex with Tau (KD = 8.45 × 10-7 M) at 298 K. The molecular modeling analysis showed a hydrophobic interaction between BBA and HVPGGG filament segment of R2 and R4 repeated domains of Tau.

The authors concluded that the association and dissociation of the BBA-Tau complex were accompanied by an entropic activation barrier, however, the positive enthalpy and entropy changes revealed that the hydrophobic bond is the main force involved in the interaction.

The authors Xu and Collaborators (2020) [120] evaluated the 50 active compounds that were divided into 6 categories: 23 terpenes, 6 fatty acids, 4 diphenylpeptanes, 4 sterols, 3 flavonoids, and 10 others present in *Alpinia oxyphylla* Miq. and 164 putative targets were collected and identified with 251 target proteins associated with Alzheimer's disease (AD) clinically tested using network pharmacological approaches. In the methodology, the authors submitted the candidate compounds to the criteria of absorption, distribution, metabolism, excretion and toxicity (ADMET), such as oral bioavailability, drug similarity assessment and blood-brain barrier. AutoDockTools-1.5.6, Pymol 2.3 and Discovery Client Studio 4.5 to identify and analyze the interactions between the compound and the target proteins. The 3D chemical structures of the compounds were obtained from PubChem and minimized energy used in ChemBioDraw 3D. In the Pymol 2.3 and UCSF Chimera 1.14rc software, the proteins obtained from Protein Data Bank (http: //www.pdb.org/) were optimized.

The results showed that 12 compounds had a good score in the ADMET criterion and 18 compounds had a moderate score. Ninety-nine putative terpene target proteins were selected based on their integration score and after enrichment analyzes of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways terpenes were identified as those that play a regulatory role key in diseases of the nervous system, such as AD.

The authors concluded that *A. oxyphylla*, mainly terpenes, seem to have neuroprotective effects in the regulation of the synthesis, release and transmission of neurotransmitters, as well as in the formation and plasticity of dendritic spines and synapses in the nervous system that contributes to the development of new drugs for the treatment of Alzheimer's disease (AD).

The authors Awasthi and Collaborators (2018) [121] evaluated one hundred terpenoids for their inhibitory potential against acetylcholinesterase (AChE) enzyme and beta amyloid (Aβ) using the molecular docking approach. For this, the authors submitted the hundred selected terpenoids with neuroprotective properties to molecular docking with the targets, AChE and Aβ peptide using Autodock 4.2 software applying the Lamarckian genetic algorithm to calculate the binding energy and the inhibitory constant of the ligands with respect to their targets. The pharmacokinetic profile of the terpenoids was evaluated using ADMET properties using DS, in addition aqueous solubility, penetration of the blood-brain barrier, CYP2D6 binding, human intestinal absorption, plasma protein binding (PPB) and hepatotoxicity have been described. They were also subjected to analysis of the Lipinski rule of five using the DS, such as the properties of

molecular weight and partition coefficient (AlogP). The best performing terpenoids were selected to be investigated for 25 nanoseconds (ns) of MD simulations of GROMOS96 43a1 forcefield using GROMACS 4.5.5 package that used the protein docking positions with their respective ligands.

The authors' results regarding the mapping of the AChE active site residues involved demonstrated that the best embedded terpenoid, Nimbolide (Fig. **20)**, revealed 17 interactions with active site residues: Tyr72, Asp74, Leu76, Tyr124, Trp286, His287, Leu289, Gln291, Glu292, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338 and Tyr341 among which Ser293 and Phe295 were found to be involved in hydrogen bonds. Likewise, the mapping of the Aβ enzyme active site residues involved in nimbolide binding revealed four active site residues, namely Val12, Lys16, Phe19 and Phe20, among which Val12 exhibited pi-alkyl interaction while Lys16, Phe19 and Phe20 were involved in van der Waals interactions.

The authors concluded that a triterpene, nimbolide, was considered the most potent and safe inhibitor for AChE and Aβ compared to its respective known drugs / inhibitors. Furthermore, the docking results were similar to those of molecular dynamics simulation of nimbolide complexes with both targets, and that these data are the basis for clinical investigations of nimbolide as a drug against AD.

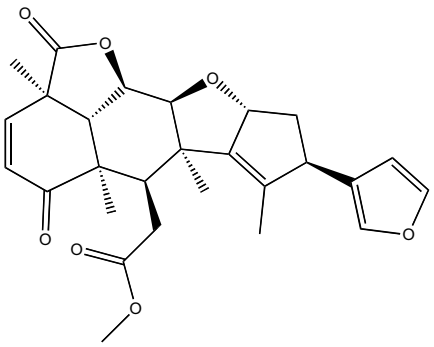

Fig. (20). 2D chemical structure of nimbolide. Source: Awasthi and Collaborators (2018).

Similarly, the authors Koirala and Collaborators (2017) [122] investigated the potential of lupane triterpenoid isolated from *Pueraria lobata* roots against the cleavage enzyme of the amyloid precursor protein b-site 1 (BACE1). The methodology consisted of applying a systematic study of 70% of the ethanolic extract of the root of P. lobata that was applied to identify its BACE1 inhibitory potential and the BACE1 inhibitory potential of two lupane terpenoids. Moreover, the application of molecular docking calculations using the AutoDock 4.2 software to assess the structure of the enzyme-inhibitor complex, in which lupeol and lupenone were tested for BACE1 inhibition. The 3D structures of BACE1 (2WJO) were purchased from the RCSB Protein Data Bank and the 3D structures of lupeol and lupenone obtained from Pubchem Compound (NCBI).

The authors' results showed the inhibitory potential of the 70% ethanolic extract of P. lobata against BACE1 (IC50 = 80.35 mg / mL), lupeol and lupenone were subsequently isolated and exhibited notable or moderate inhibitory activity of BACE1 with IC_{50} values. 5.12 and 62.98 mmol/L, respectively, compared to the positive quercetin control (IC_{50} = 21.28 mmol/L). The analysis of the docking simulations revealed that the hydroxyl group of lupeol formed two hydrogen bonds with ASP32 (catalytic aspartic residue) and SER35 residues of BACE1 with the binding energy of (-8.2 kcal / mol), while the ketone group of lupenone did not form any hydrogen bonds with BACE1, confirming less binding affinity.

The authors concluded that the molecular mechanism of lupane triterpenoids is exhibited via BACE1 inhibition, indicating that lupeol (Fig. **21**), in particular can be used as a useful therapeutic and preventive agent to mitigate Alzheimer's disease.

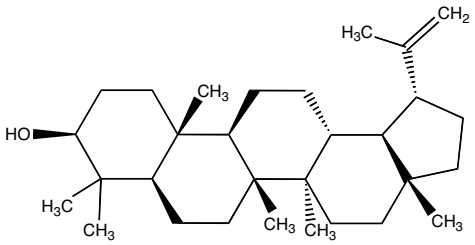

Fig. (21). 2D chemical structure of lupeol. Source: Koirala and Collaborators (2017).

2.5. Alkaloids

In the search for new drugs, several methodologies can be applied, such as the isolation of chemical compounds from natural plants, and the class of alkaloids presents several compounds, such as terpenes, from vegetable plants, which present great demand in popular medicine, that arouse scientific study from virtual screening, using computational tools, aiming at the application of molecular modeling, highlighting an advancement in the search for new drugs, relating the structure-activity study of chemical compounds.

Mathew and Collaborators (2019) [123] studied natural alkaloids, from antienzyme properties, investigating the action of some compounds, such as alkaloids, coumarins, chalcones, donepezyl-propargylamine conjugates, homoisoflavonoids, resveratrol analogues, hydrazones, and pyrazolines, with inhibitory action of monoamine oxidases and cholinesterases and/or adenosine A2AA receptors. In addition, the steroidal/triterpenoidal, quino-lizidine, isoquinoline and indole classes, distributed mainly within the Buxaceae, Amaryllidaceae and Lycopodiaceae classes have investigated the interaction of the molecules with amino acid residues in the action of enzymes.

The study from human cholinesterases (hAChE and hBChE) by the alkaloids protoberberine (+)-talictricavine (1) and (+)-canadine (2), was developed in order to obtain new drugs effective against Alzheimer's disease, in addition to determining a mechanism of AChEI inhibition [124-126].

Investigations of AChEIs associated with the study of computational methods, aimed at drug discovery assisting by computational modeling techniques [127]. Modeling techniques enable the discovery of new AChEIs with higher pharmacokinetic efficacy and lower toxicity [128].

Chlebek and Collaborators (2019) [126] proved through their research that the protoberine alkaloids (+)-talictricavine (1) and (+)-canadine (2) (Fig. **22**) have selective hAChE inhibitory action, from acomplination tests, it was possible to identify the binding positions on hAChE, and proving that these alkaloids are potential in the study of new drugs to inhibit Alzheimer's disease.

According to Cortes and Collaborators (2017) [129] inhibiting the enzymatic action of AChE is key in the treatment of Alzheimer's disease (AD), and AChEA inhibitors are the main drugs. Therefore, the alkaloids of the Amaryllidaceae family have different structural types that comprise the AChE inhibition activity, characterizing the Amaryllidaceae as an important group of alkaloids in the treatment of AD.

Cortes and Collaborators (2017) [129] determined the inhibition of AChE and BChE from seven extracts of Amaryllidaceae plants, obtaining the best results for the alkaloids of Eucharis bonplandii, as they present high level of inhibition (IC50 = 0.72 ± 0.05 μg/mL) against human recombinant AChE (hAChE). For human serum BChE (hBChE), the bulb and leaf extracts of Crinum jagus showed higher inhibition (IC50 = 8.51 ± 0.56 μg/mL and 11.04 ± 1.21 μg/mL). Galantamine is a natural product extracted from plants of the Amaryllidaceae family [129-131]. Therefore, Amaryllidaceae show good inhibitions of cholinesterases and are promising for the development of drugs against Alzheimer's disease.

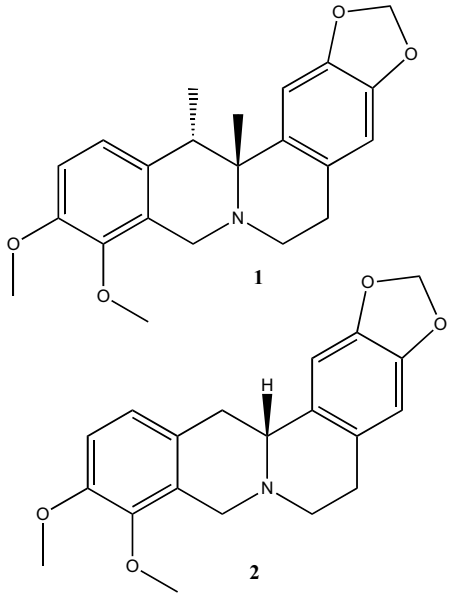

Fig. (22). Structures of protoberine (+)-talictyltricavine (**1**) and (+)-canadine (**2**) alkaloids. Source: Chlebek and Collaborators (2019).

Alkaloids extracted from the Amaryllidaceae family are highlighted in research, as they show anticholinergic activity [132]. According to Pinho and Collaborators (2013) [133], these alkaloids usually have nitrogen atoms present in a cyclic ring, giving them nitrogen characteristic. Other compounds, such as, quinones, stilbenes, flavanoids, flavones, isoflavones, xanthones and monoterpenes, also have similar characteristics, as they have anticholinesterase activity. Therefore, they add the efforts in search of other alkaloids from Amaryllidaceae family to analyze the interaction between human recombinant enzyme rhAChE from in silico study using molecular modeling.

Toledo and Collaborators (2021) [134] evaluated the role of the alkaloid boldine (Fig. **23**) in the attenuation of synaptic failure and mitochondrial dysregulation in Alzheimer's cell models. The methodology included the synthesis of the studied alkaloid, as well as the performance of aggregation assays in hippocampal neurons, mitochondrial analysis and immunohistochemical assays. The in silico methodology addressed the realization of molecular docking simulations, the protein used was obtained from the Protein Data Bank (PDB) and corresponded to the monomer Aβ1-42 (PDB ID: 1IYT). Protein-ligand anchoring was performed using Glide with high precision (XP) configuration. The complexes were analyzed using the docking score provided by Glide and the calculation of the MM-GBSA 1Gbind using Prime (Schrödinger, LLC, New York, NY). All images shown were created with PyMOL (Schrödinger, LLC, New York, NY).

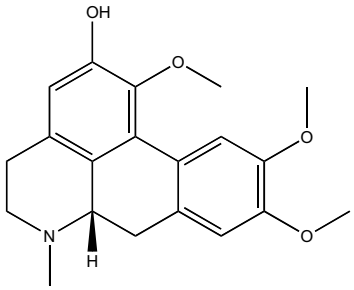

Fig. (23) Structure of compound Boldine. Source: Toledo and Collaborators (2013).

Molecular docking simulations showed that boldine interacted with residues VAL 12, HIS 13, LYS 16, LEU 17 and PHE 20. A Pi-Pi stacking interaction was established with HIS 13 and a hydrogen bond with LYS 16. Interaction of boldine and the C-terminal region, a docking score of -1.427 was obtained and the data show that boldine interacted with ALA 30, ILE 31, GLY 33, LEU 34 and GLY 37, establishing hydrogen bonds with ALA 30. For interaction with the complete structure, a fit score of -3.373 was obtained, and we found that boldine interacted with several residues, namely ALA 17, PHE 20, ALA 21, VAL 24, GLY 25, LYS 28, LEU 34, MET 35. Boldine also interacted with Aβ fibers (2BEG), with the highest score of -4.596.

In general, it was noticeable that boldine interacts with Aβ in silico affecting its aggregation and protecting hippocampal neurons from AβO-induced synaptic failures. Boldina also normalized changes in intracellular Ca^{2+} levels associated with mitochondria or endoplasmic reticulum in HT22 cells treated with AβO. Furthermore, boldine completely rescued the decrease in mitochondrial membrane potential (19m) and the increase in mitochondrial reactive oxygen species, and induced reduction of attenuated AβO in mitochondrial respiration in hippocampal HT22 cells. Thus, it is concluded that boldine provides neuroprotection in AD models by both direct interactions with Aβ and preventing

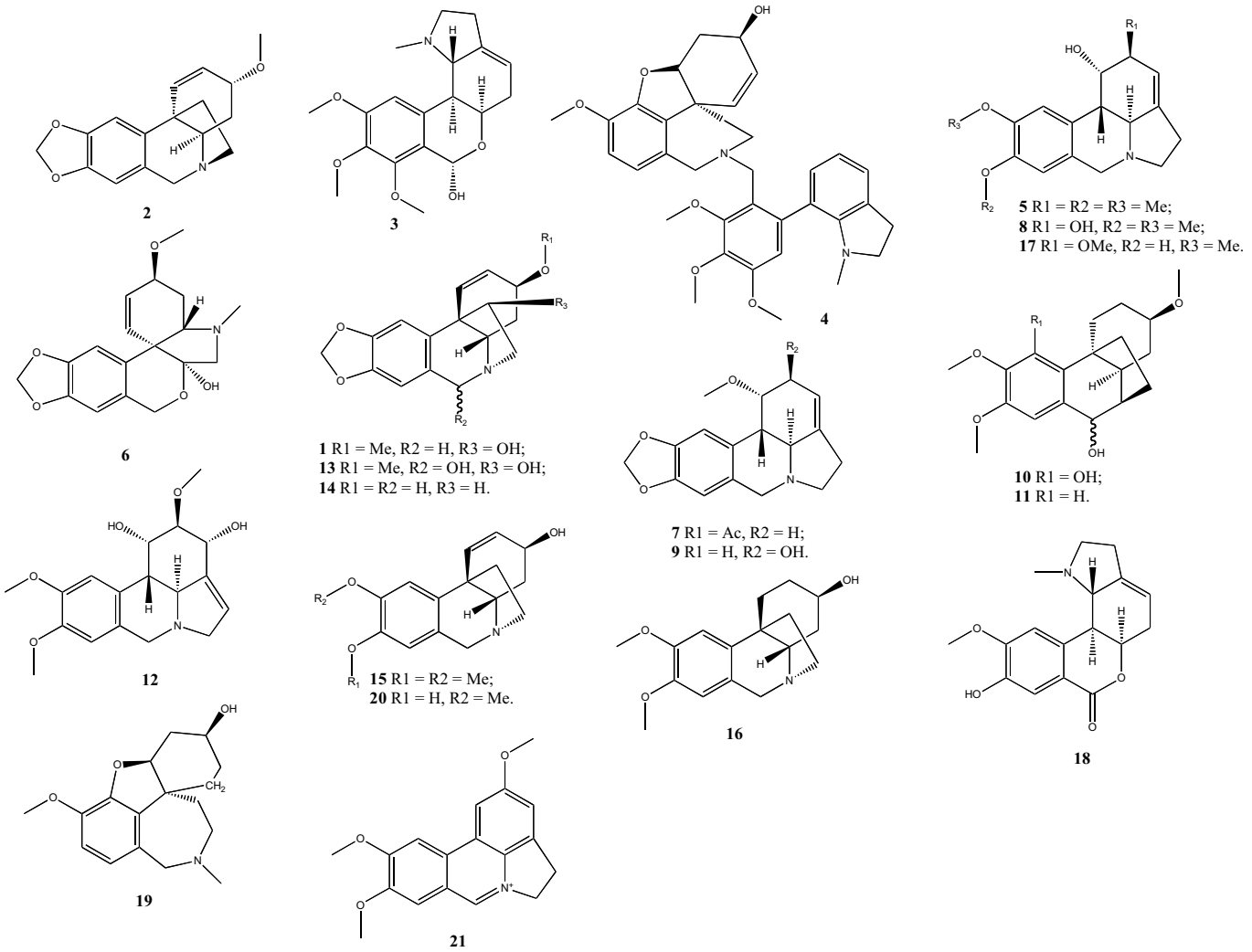

Fig. (24) Structure of compounds: (**1**) Buphanisine, (**2**) Nerinine, (**3**) Narcieliine, (**4**) Galanthine, (**5**) Tazettine, (**6**) Haemanthamine, (**7**) 1-*O*-acetylcaranine, (**8**) Methylpseudolycorine, (**9**) Lycorine, (**10**) 6-hydroxyhippeastidine, (**11**) 10-deoxy-6-hydroxyhippeastidine, (**12**) Narcissidine, (**13**) Haemanthidine, (**14**) Vittatine, (**15**) Maritine, (**16**) Dihydromaritine, (**17**) 9-*O*-demethylgalanthine, (**18**) 9-*O*-demethylhomolycorine, (**19**) Lycoramine, (**20**) 8-*O*-demethylmaritidine, (**21**) Tortuosine. Source: Kohelová and Collaborators (2021).

oxidative stress and mitochondrial dysfunction. Additional studies are needed to assess the effect of boldine on Aβ-induced cognitive and behavioral deficits *in vivo*.

In another study, Kohelová and Collaborators (2021) [135], performed the isolation, structural elucidation and biological activity of 20 plant alkaloids *Zephirantes citrina* from the Amaryllidaceae family called narcieliine (Fig. **24)**. The compounds isolated in a sufficient amount were evaluated *in vitro* against the inhibition of the enzymes acetylcholinesterase (AChE; E.C. 3.1.1.7), butyrylcholinesterase (BuChE; EC.3.1.1.8) and prolyl oligopeptidase (POP; E.C. 3.4.21.26).

The computational studies included the realization of molecular docking simulations that were carried out in conjunction with molecular dynamics analysis, in order to find the ideal pose. The 3D binder structures were built by OpenBabel, v. 2.3.2 and optimized by Avo gadro, v. 1.2.0.3 using GAFF force fields. The enzyme used corresponded to Acetylcholinesterase and was obtained from the Protein Data Bank (PDB) with ID: 4EY6. The molecular dynamics simulation was performed by Gromacs, v. 2018. The AChE-ligand complex was solvated in a water box and neutralized by the addition of Na^+ and Cl^- to a concentration of 10 nM. First, the system energy was minimized (maximum force <1000.0 kJ/mol/nm), and the 100 ps isothermal isochoric NVT and 100 ps isothermal-isobaric NPT equilibria were made. Then, the 10 ns molecular dynamics simulation was done at 310 K. The results were visualized by the PyMOL v software. 2.0.6 Schrodinger, LLC, Mannheim, Germany.

Molecular docking simulations demonstrated that compound 3 expands the catalytic anionic site (CAS) of hAChE entirely, with several apparent interactions. The "galanthamine" nucleus of 3 is centrally housed, demonstrating: (i) π-π displaced stacking with Y337 (5.5 Å), (ii) hydrogen bonds

between the oxygen atom of the 2,3-dihydrofuran portion of 3 and a water molecule (2.1 Å) and the hydroxyl group of Y337 (2.0 Å), and (iii) a hydrogen web bonds between the hydroxyl group of 3 and the carboxyl portion of E202 (1.6 Å), and the backbone amides of G120 (2.3 Å) and G121 (2.6 UM); the last two amino acids form an enzyme oxyanion hole [58]. In addition to the "galantamine" region of 3, the linker is surrounded by several amino acid residues (eg, W86, A127, G126, F338), establishing hydrophobic interactions.

The biological activity showed IC50 values of 18.7 ± 2.3 μM and 1.34 ± 0.31 μM, respectively, proving that the isolated heterodimeric alkaloid narcelliin exhibited promising biological activities related to the potential treatment of neurodegenerative diseases. Thus, the structural details of this compound can be used as a hit for the development and structural optimization of dual cholinesterase and inhibitors.

In another article, Innock and Collaborators (2021) [136] carried out a similar survey, where they aimed to carry out investigations *in sílico* and *in vitro* anti-AChE of the chemical constituents of the plant Mytragyna speciosa, these being 27 alkaloids and 37 flavonoids, the main compounds under study being Mitragynine oxindole B (MITOB) and Mitragynine (MIT) (Fig. **25**).

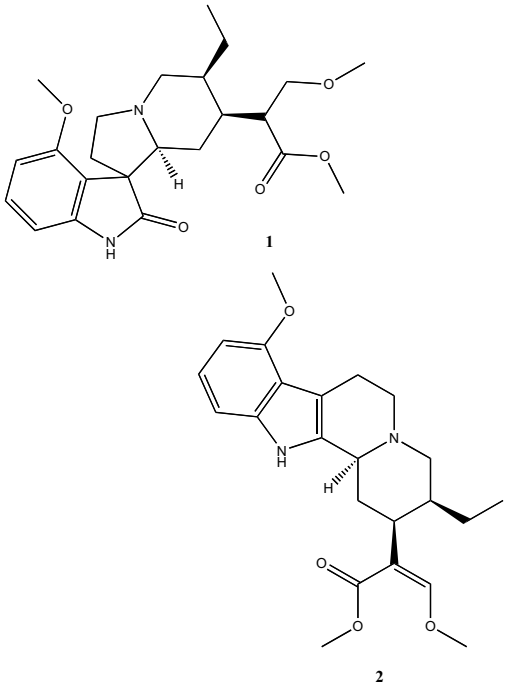

Fig. (25) Structure of compounds: Mitragynine oxindole B (MITOB) (1) e o Mitragynine (MIT) (2). Source: Innock and Collaborators (2021).

Molecular docking simulations were performed by Autodock 4.2 software, and the atomic partial charges of the protein were identified by the Gasteiger-Marsilli method implemented by AutoDock Tolls. The drugs used as control corresponded to Galantamine, Donepezil and Rivastigmine. The molecular dynamics simulations were calculated using the AMBER 14 software. In addition, the compounds under study were isolated and tested against the inhibition of the AChE enzyme activity.

The fitting results showed that almost all alkaloid compounds exhibited better binding affinity than flavonoid compounds. Mitraginine oxidol B (MITOB), an indole alkaloid, from M.Speciosa (kratom), had a superior binding affinity of - 11.52 kcal/mol against AChE. Furthermore, mitragynine (MIT) exhibited good binding affinity against AChE (-10.81 kcal/mol). In order to investigate the stability of the anchored MIT-AChE and MITOB-AChE complexes, the MD simulation was performed using the AMBER 14 computational package. Free binding energies (ΔG) based on MM-PBSA suggested that MIT and MITOB have similar binding affinity for Target AChE. Although only a strong hydrogen bond with E202 at the catalytic anionic site was detected with MIT, many more residues in the AChE active site could have contributed to this alkaloid bond. Since MIT is the main constituent alkaloid of *M. Speciosa* leaves, this compound was isolated for in vitro studies of anti-AChE activity. From the experiment, MIT alone had an inhibitory effect with an IC50 value of 3.57. This discovery suggested that the MIT compound is able to inhibit AChE function, and therefore could serve as a compound for therapeutic treatment of Alzheimer's disease.

Aiming at the combination of prospecting *in sílico* with biological testing, Biradar and Collaborators (2020) [137] evaluated the mechanism of action of the plant's constituents *Erytrina variegata* L. against scopolamine-induced memory impairment in rats.

Initially, three models, namely, Morris Water Maze (MWM), Elevated Plus Maze (EPM) and Passive Avoidance Paradigm (PA) were used to elucidate the memory function. Then, the level of biomarkers, *i.e.*, acetylcholinesterase enzyme, reduced glutathione and lipid peroxidation level were measured in brain tissue. Subsequently, the main bioactive phytoconstituents targeting potential protein targets and pathways were identified through gene cluster enrichment analysis and network pharmacology. Finally, the interaction between bioactive phytoconstituents and their respective targets was confirmed by molecular docking analysis, and the simulation was performed in Auto-Dock 4.0 software and interactions were evaluated in Discovery Studio Visualizer 2019v. The proteins under study were obtained from the PDB with ID corresponding to: 4PQE and 1J1C, respectively. The PubChem chemical database was used to retrieve the 3D structure of the phytoconstituents and energy was minimized using Marvin Sketch applying the mmff 94 force field

The results showed that the phytocomplex as a whole without class distinction attenuated the memory impairment induced by scopolamine; however, in the molecular docking analysis, the protagonism was due to the flavonoids and alkaloids, since Alpinumisoflavone, Auricularin and Eryvarin B showed the highest scores and binding affinity with MAPT, *i.e.* -8.8, -8.9 and -8.8, kcal/mol respectively. Among these, Alpinumisoflavone scored the highest hydrogen bond interaction, namely five HBI with MAPT protein. Donepezil, a proven ACHE inhibitor, together with the phytoconstituent Glucoerysodine had higher scores with ACHE, *i.e.,* 8.5 and -8.0, respectively. Furthermore, MWM, EPM and PA activity showed that scopolamine administration increased escape latency time (ELT), transfer latency (TL) and step latency

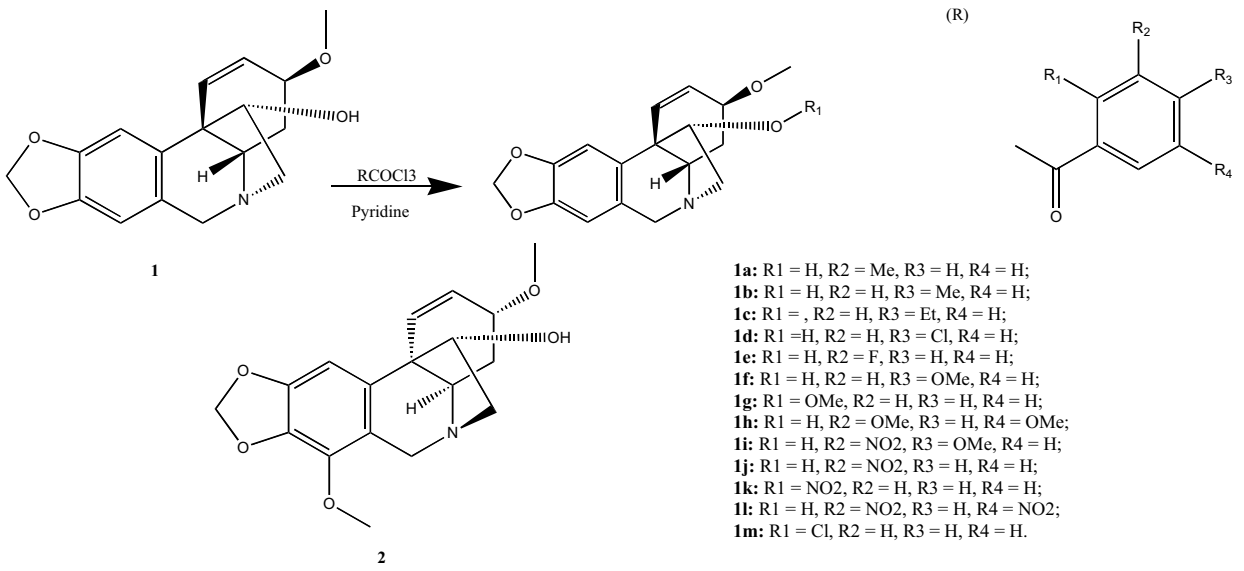

Fig. (26) Synthetic procedure for **1a-1m** from haemanthamine (**1**) and the structure of ambeline (**2**). Source: Perinová and Collaborators (2020).

(STL), respectively on day 0, 7 , 14 and 21, while treatment with E. variegata was able to significantly reverse ELT, TL and STL activity. The decreased level of acetylcholinesterase (AChE) and the level of MDA in treated animals reflected the increased memory and was considered comparable to the clinically proven drug, *i.e.* donepezil. Sixty compounds were identified in the *E. variegata* bark, among which twenty-two compounds are predicted to modulate potential targets involved in Alzheimer's disease and considered bioactive. Furthermore, the coupling study revealed that Alpinumisoflavone, Auriculatin, Osajin and Scandenone have the highest binding affinity with the Tau protein, while Donepezil and Glucoerysodin with the enzyme acetylcholinesterase.

The authors concluded that chronic treatment with *Erythrina variegata* improves scopolamine-induced memory impairment by restoring cholinergic system function in the hippocampus and cerebral cortex through inhibition of peroxidation in the brain.

Another strategy used was the application of organic synthesis to obtain alkaloids with potential action in Alzheimer's Disease. The research was developed by Perinová and Collaborators (2020) [138], which performed the aromatic functionalization of alkaloids haemanthamine from the Amaryllidaceae family (Fig. **26**), as well as the evaluation *in silico* and *in vitro* of its activity against Alzheimer's. Twelve compounds were produced and all analogues were explored for their inhibitory potential against cholinesterases. In order to reveal the availability of the CNS, its potential to permeate through the blood-brain barrier was also tracked (BBB; PAMPA-BBB method) and the logBB values for the selected derivatives were calculated. The data *in vitro* are supported by docking studies that describe their orientation in the active sites of hAChE and hBuChE, together with enzymatic kinetic analysis. Molecular docking simulations were performed in AutoDock Vina software, being selected in the PDB the crystal structure of AChE complexed with galanthamine (PDB ID: 4EY6) and in BuChE, the BuChE-tacrine model was chosen (PDB ID: 4BDS).

The results demonstrated that the strongest hAChE inhibition capacity was demonstrated by the 11-O- (2-nitrobenzoyl) - (1j), 11-O-(3-nitrobenzoyl) - (1k) and 11-O-(2 -chlorobenzoyl) - (1m) of hemantamine with IC50 values of 9.9 ± 0.5 μM for 1j, 4.0 ± 0.3 μM for 1k and 9.9 ± 0.7 μM for 1m, respectively, of this thus, it is clear that the most prominent inhibition of hAChE was associated with the presence of a nitro group on the benzoyl ring, with position 3 highlighted for the nitro group on the benzoyl ring. For the inhibition of HBuChE, the presence of the methoxy group in the benzoyl ring seems to be crucial since the most active derivatives have this functionality in position 2. Within the benzoyl-hemantamine derivatives that generate methyl in the aromatic; the best results were achieved for the derivatives reported in the previous study, where 11-O- (2-methylbenzoyl) hemantamine showed a non-selective profile for both cholinesterases on the micromolar scale (IC50 for hAChE = 18 ± 1.3 μM, and for hBuChE 6.6 ± 1.2 μM).

Generally speaking, in the test *in vitro,* the results obtained for the new set of hemantamine derivatives indicated that its inhibiting potency for both enzymes did not surpass the inhibition efficacy of the galantamine and serine patterns. On the other hand, the structural types hemantamine and crinin represent interesting building blocks for further development in the field of AD research.

The molecular docking results showed that for the hAChE complexes, 1g, 1j and 1m, all revealed a very similar topology arrangement with the ligands accommodated in Dor's catalytic active site. Common observable characteristics can be summarized as follows i) parallel π-π contact

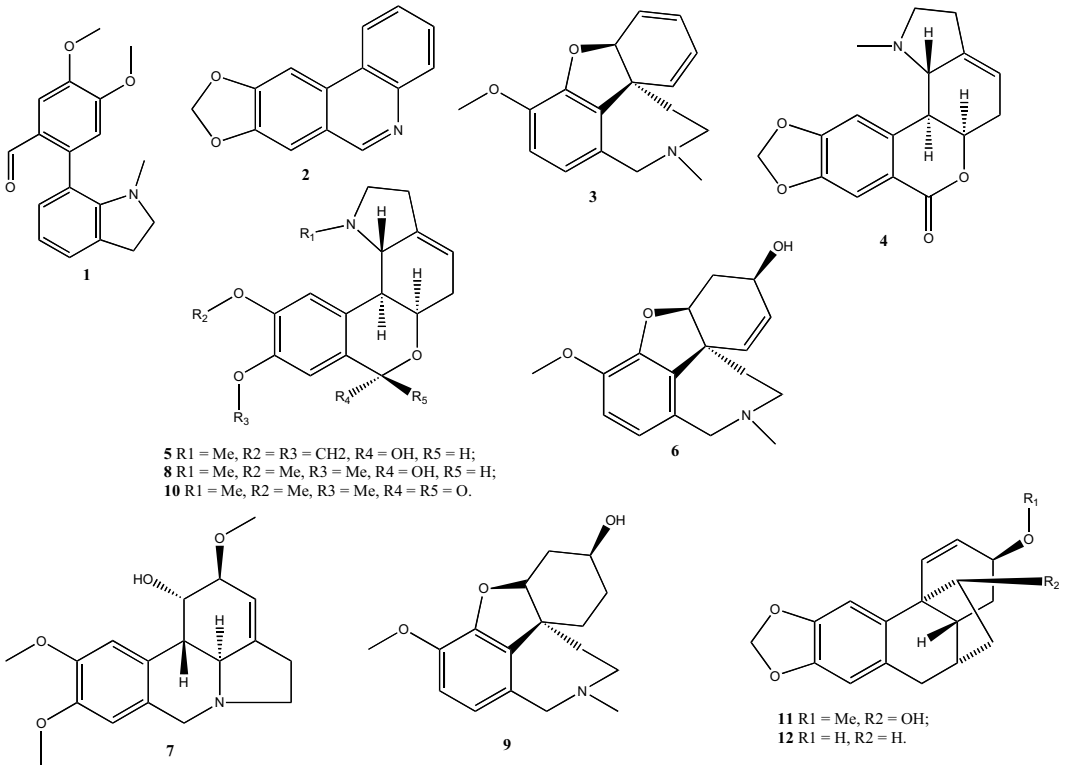

Fig. (27) Structure of compounds of the plant *Narcissus pseudonarcissus* belonging to the Family Amarilidaceae. Source: Al Mamun and Collaborators (2020).

between Trp86 and the ring-attached substituted phenyl and ii) β-crine accommodation in the vicinity of various aromatic residues such as Tyr341, Phe338, Tyr337, Tyr124 and Phe297 forming different hydrophobic interactions. Some differences can be seen for 1j, where the nitro group established a favorable web of hydrogen bonds with Tyr133, Glu202 and Ser203. In contrast to other derivatives, this can be considered a critical aspect for the high in vitro potential of 1j. The chlorine atom in 1m strengthened the hydrophobic contact with Trp86, which is lacking in the 1g-AChE complex and may explain the lower inhibitory potency of 1g. In silico results for hBuChE showed that all ligands were found buried deep in the throat of the hBuChE cavity. Topological analysis revealed that compound 1j adopted a completely different method compared to molecule 1g and 1m. This can be seen by superimposing all the ligands on the active sites of hBuChE. Concomitantly, all ligands demonstrated i) π-π in the form of T interaction between the substituted phenyl appendix and Phe329, and ii) with the ester group being implicated in hydrogen bonding with the residue of the catalytic triad Ser198. The nitro group at 1j is anchored to the Gln119 oxyanion hole residue by a hydrogen bond that distorts the β-crinane scaffold into a less energetically favorable conformation and may be blamed for its low activity. *in vitro*.

Overall, the authors concluded that hemantamine may represent a versatile framework that generates potential drugs for the treatment of Alzheimer's.

Al Mamun and collaborators (2020) [139] evaluated Belladin-type alkaloids against the inhibition of the enzyme butylcholinesterase; these were extracted from the plant *Narcissus pseudonarcissus* and belong to the Amarilidaceae family. The study used 20 alkaloids called Carltonine A-C (Fig. **27**).

The constituents of the study were isolated and characterized and after these steps, molecular docking simulations were performed, using two enzymes hAChE and hBuChE obtained in the PDB with ID's: ID: 4EY6 (crystal structure of hAChE) and 4BDS (crystal structure of hBuChE)). All receiver structures were prepared by UCSF Chimera's DockPrep function and converted to pdbqt files by AutodockTools. The selection of flexible residues was based on previous experience with either hAChE, hBuChE or the spherical region around the binding cavity. Three-dimensional binder structures were built by Open Babel, minimized by Avogadro and converted to pdbqt file format by AutodockTools. Fitting calculations were performed by Autodock Vina with exhaustiveness of 8. Visualization of enzyme-ligand interactions were prepared using the PyMOL Molecular Graphics System.

In general, the authors concluded that the phytochemical study of the alkaloid extract of Narcissus pseudonarcissus cv. Carlton resulted in the isolation of thirteen previously described AAs, and three new belladine-type AAs, termed carltonin A - C. Their structures were elucidated using a combination of NMR and MS analysis. Compounds were isolated in sufficient quantity and were screened for their potential to inhibit hAChE, hBuChE and POP. The inhibitory activity was significant and selective for hBuChE being demonstrated by the just-described alkaloids Carltonin A (**13**) and Carltonin B (**14**) with IC_{50} values of 0.91 ± 0.02 μM and 0.031 ± 0.001 μM, respectively. The in vitro results were justified by computational studies predicting plausible binding modes of

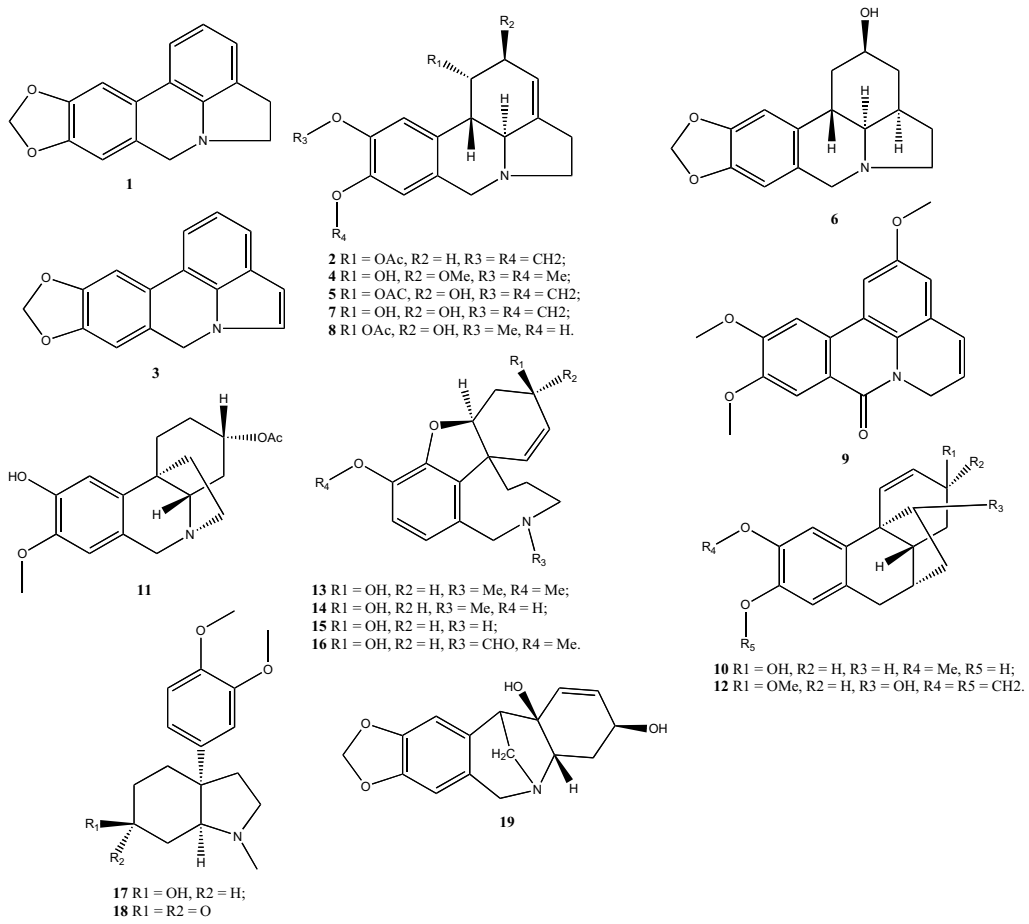

Fig. (28) Alkaloids identified in Ecuadorian *Phaedramassa* herb by gas chromatography coupled to mass spectrometry (GC-MS) and docking studies. Source: Moreno and Collaborators (2020).

compounds 13 and 14 in the active site of hBuChE, proving that the new compounds exerted an interesting biological profile that deserves further optimization.

Amarylidaceae species were also the aim of the researcher's study Moreno and Collaborators (2020) [140]. The authors evaluated the inhibitory potential of the plant's chemical constituents *Phaedranassa* Herb. of species from Ecuador.

The methodology consisted of identifying 19 alkaloids from the plant (Fig. **28**) by CG-MS, which were tested by inhibiting the enzymes acetylcholinesterase and butyrylcholinesterase. Docking analyzes were performed using the AutoDock v.4.2 programs, using the active site of two different enzymes obtained from the Protein Data Bank (PDB), being the ID's: (1DX6) and (4BDS).

The results showed that thirty-three compounds were detected, and nineteen known alkaloids were identified by GC-MS in five different species of *Phaedranassa* Herb. of Ecuador. Galantamine-type alkaloids were detected in all samples, with the highest concentration in *P. cuencana*. Thus, it was noticed that the compounds extracted from all species under study showed activity against AChE and BuChE *in vitro*, and *P. cuencana* and *P. dubia* proved to be the most active against AChE and BuChE, respectively, while the in silico results indicated that cantabricine is highly inhibitory against both cholinesterases. It was noticeable that this study is unprecedented and reported the alkaloid profile and the biological activities of *P. cuencana, P. glauciflora* and *P. tunguraguae,* thus supporting the role of Amaryllidaceae Species as a source of alkaloids with potential application for the palliative treatment of Alzheimer's Disease.

Induction of neuronal plasticity through the action on the GSK3β enzyme by meridianins and lignarenone B (Fig. **29**) was the objective of the work developed by Llorent-Martinez and Collaborators (2020) [75].

The methodology comprised the testing of substances against the enzyme through the percentage of inhibition, while the computational studies carried out included the performance of molecular docking simulations, with the structure obtained from the Protein Data Bank with ID: 6B8J and the procedure performed in the software Itzamna. Fpocket software, a protein pocket prediction algorithm was used to identify different cavities on the surface of GSK3β. In addition, molecular dynamics simulations were performed with the software NAMD version 2.1, and the visual inspection for each trajectory and the occupation analysis of hydrogen bonds (HBs) was performed using the Visual Molecular Dynamics (VMD) software. Thermodynamics (temperature, potential, kinetic and total energy) and structural (radius of rotation (Rg), root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analysis were performed

using GROMACS simulation package and ADMETer, a software tool containing supper vector regression (SVR) and supper vector machine (SVM) predictive machine learning (ML). ADMET models were used to evaluate ADMET properties (LogS, LogP, Caco2, barrier blood brain (BBB), plasma protein binding (PPB), P-glycoprotein (Pgp), human ether-a-go-go gene hERG) of meridianin AG and lignarenone B.

In sílico studies demonstrated that MD docking post-processing allowed the observation of induced adjustment events, as mentioned above, and the existence or magnitude of these events can be measured in different ways. RMSF analysis revealed that when meridianin G and lignarenone B are bound to the substrate pocket, a further fluctuation at residue 66 can be observed in relation to the ATP cavity, where this fluctuation is not observed in binding either of the compounds analyzed. Another pattern detected is the fact that when lignarenone B is bound to either pocket, the fluctuation of GSK3β is generally greater than when meridianins bind, taking into account that the induced adjustment may only involve small conformational changes in the overall protein, and this may be related to the structure of lignarenone B.

PK studies pointed out that, as LogP values are less than 5, the compounds have adequate hydrophobicity and permeability behavior. However, to become drugs that penetrate the central nervous system (CNS), the molecules should have a LogP around 2.

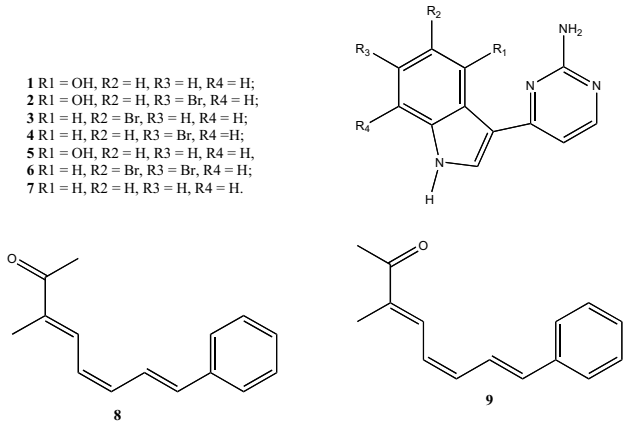

Fig. (29) Structure of meridianins A-G (1-Meridianin A, 2-Meridianin B, 3-Meridianin C, 4-Meridianin D, 5-Meridianin E, 6-Meridianin F, 7-Meridianin G) and lignarenones A-B (8-Lignarenone A; 9-Lignarenone B). **Source:** Llorach-Pares and Collaborators (2020).

Another possible candidate therapeutic method for the treatment of pathologies such as AD may involve GSK3β.

Kashyap and collaborators (2020) [141] carried out a research with the alkaloids ajmalicin and reserpine (Fig. **30**), which are indole alkaloids as multi-targeted ligands towards factors implicated in Alzheimer's disease. The methodology consisted of isolating the compounds and evaluating the inhibition of acetylcholinesterase and butyrylcholinesterase enzymes. Molecular docking simulations were performed with selected targets (Aβ42, AChE, BuChE, BACE-1, and MAO-B) using the Autodock 4.2 software; the proteins were obtained from the Protein Data Bank (PDB) with ID's: (1YIT for Aβ42, 4PQE for AChE, 2J4C for BuChE, 4D8C for BACE-1 and 1S2Q for MAO-B). ADMET analysis was performed using Drulito software (www.niper.gov.in/pi_dev_tools/DruLiToWeb / DruLiTo_index.html) to study the optimal pharmacokinetic profile of RES and AJM for Drug Development. Two filters were used for screening; Lipinski's rule and blood-brain barrier.

The results demonstrate that molecular docking analysis clearly indicates that RES and AJM are excellent multi-target ligands capable of inhibiting the aggregation of Aβ42 and key enzymes implicated in AD, such as AChE, BuChE, BACE-1 and MAO-B. RES and AJM strongly interacted with key Aβ42 residues with very low binding energy. AJM interacted with the residue from the MAO-B catalytic site through hydrogen bonding while the RES interacted with the comparatively weak hydrophobic environment, suggesting that AJM is a better inhibitor of MAO-B than RES. RES forms hydrogen to bond with the key residue (Asp23) and interact hydrophobically with Lys 28 of Aβ42, internally bound in the steric zipper composed of KLVFFA (residues 16-21). In addition, the RES was also oriented to cover the hydrophobic core residues (Leu17, Val18, Phe19 and Phe20) of Aβ42, stabilizing the advancing complex. The Aβ42 assembly requires Asp23 from one monomer to form a hydrogen bond with Lys28 from another monomer, so completing RES can potentially inhibit Aβ42 aggregation. In addition, RES gave a binding score comparable to the positive control compound (tannic acid). AJM interacts most with the hydrophobic core within the steric zipper through hydrophobic interactions. It also linked to Asp23 with a hydrogen bond, thus covering the residue (Asp23) necessary for oligomerization and inhibiting the Aβ42 aggregation process.

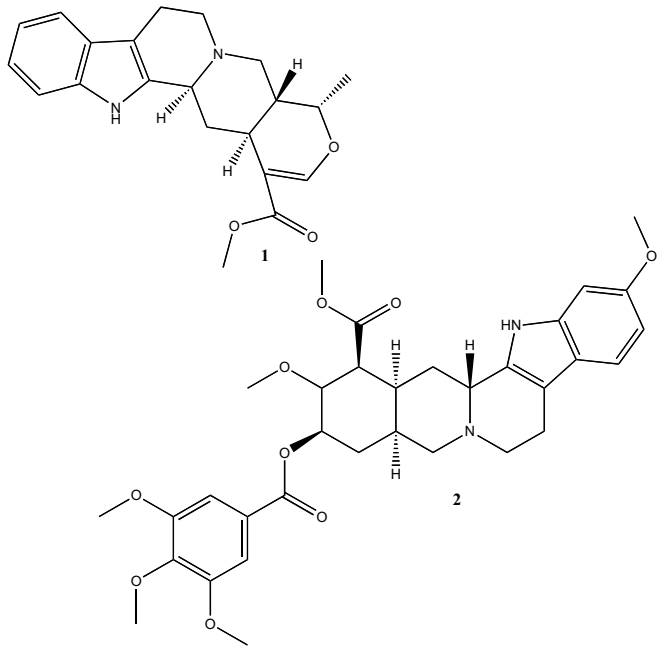

Fig. (30) Structure of compounds Ajmalicine (**1**) and reserpine (**2**). **Source:** Kashyap and Collaborators (2020).

Regarding the pharmacokinetic analysis for ADMET, identified phytoconstituents showed that AJM proves to be a more ideal drug molecule, as it obeys Lipinski's rule of five, along with an adequate profile to cross the blood-brain barrier (BBB).

Enzyme inhibition assays demonstrated that reserpine is a more potent dual cholinesterase inhibitor than ajmalicin (IC50 values of 1.7 μM (AChE) and 2.8 μM (BuChE)). The anti-aggregation activity of reserpine (68%) was more than that of ajmalicin (56%). Both compounds demonstrated neuroprotective activity against Aβ42 (92%) and H_2O_2 (93%) induced toxicity in PC12 cells against controls. Thus, the authors concluded that the RES and AJM compounds can act as ligands targeting multiple targets and can be used to develop a new compound that can be used against multiple targets Aβ42, AChE, BuChE, MAO-B, BACE-1 and ROS that are implicated in AD and therefore help in relieving symptoms and having a disease-modifying effect in AD.

In another article, Almeida and Collaborators (2020) [142] carried out an evaluation in order to know the inhibition of the production of the toxic beta amyloid peptide by isoquinolinic alkaloids from the Amaryllidaceae and Fabaceae families (Fig. **31**) in an experimental model of *Caenorhabditis elegans*. The methodology comprised the performance of inhibition assays of the acetylcholinesterase enzyme activity in a nematode model. The in silico assays, on the other hand, comprised simulations of molecular docking with the enzyme acetylcholinesterase, using the drug galantamine as an inhibitor.

The results showed that in in vitro tests, acetylcholinesterase inhibition was observed for the first time for alkaloids Erythrine; however, Lycorine was the most active being in the 24-46 μg/ml range, while lycorine and tazetin showed IC 50 of 24.55 and 27.07 μg/ml respectively. The anchoring simulation contributed to understanding this potential by showing a hydrophobic interaction between acetylcholinesterase and licorine at the amino acid residue TRP 84, as well as hydrogen bonds with TRY 121 and ASP 72.

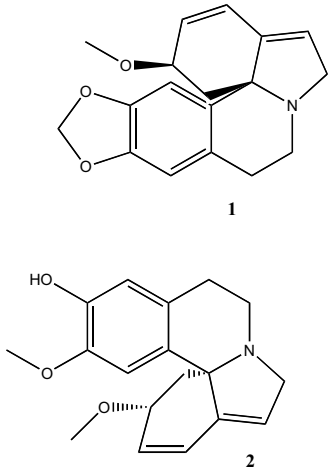

Fig. (31) Structure of compounds Erythraline (1) and Erysodine (2). **Source:** Ameida and Collaborators (2020).

Thus, it was possible to conclude that isoquinolinic alkaloids from Amaryllidaceae and Fabaceae increased the mean time of paralysis in transgenic CL2006 *C. elegans,* which is recognized as an alternative model for AD investigation *in vivo*. The biological potential of Erythrine alkaloids is less explored than Amaryllidaceae alkaloids, although both belong to the isoquinoline group and share structural similarities. Erythralin and erysodine showed the greatest potential in delaying Ab1-42 - induced paralysis in *C. elegans* (*in vivo*) compared to Memantine, a drug currently used in AD therapy. These results suggest that the *in vivo* attenuation potential of A b 1-42 - the induced paralysis observed for both alkaloids was not associated with their ability to inhibit AChE.

CONCLUSION

Alzheimer's is characterized as chronic, extremely serious and requires special attention when looking for new therapeutic alternatives, as these are diseases of extreme complexity and severity.

The inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine, is the main pharmacological strategy against AD investigated by molecular docking, using natural products as ligands. In addition, it is clear that the classes of flavonoids and terpenes were the most prevalent classes in the studies, as well as that the most discussed methodology was related to molecular docking simulations, absent the elaboration of prediction models and the realization of a more thorough screening. However, it was noticeable that when used, the *in silico* studies were accurate and provided reliable and reliable results with the experimental validation carried out later.

Thus, the importance of *in silico* studies in research and drug discovery for Alzheimer's diseases is emphasized, from the planning of the experimental methodology used in obtaining it, as well as in the selection of compounds with greater potential, in order to understand the mechanism of interaction involved and the pharmacokinetic and pharmacodynamic characters.

LIST OF ABBREVIATIONS

ACh	=	Acetylcholine
AChE	=	Acetylcholinesterase
AD	=	Alzheimer's disease
ADMET	=	Absorption, distribution, metabolism, excretion and Toxicity
ANN	=	Artificial Neural Network
APP	=	Amyloid precursor protein
Aβ	=	Amyloid β
BACE-1	=	β-site APP cleaving enzyme 1
BBB	=	Blood-brain barrier
BPFRF	=	Flavonoid-rich fraction
BuChE	=	Butyrylcholinesterase
CADD	=	Computer Aided Drug Design
CAP-e	=	Cell-Based Erythrocyte Antioxidant Protection
CAS	=	Catalytically Active Site

CAT	=	Choline acetyltransferase enzyme
CCD	=	Coiled-coil domain
CDK5	=	Cyclin-dependent kinase 5
COX-2	=	Cycloxygenase 2
DBD	=	DNA binding domain
DMAPP	=	Dimethylallyl Pyrophosphate
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
ECD	=	Electronic Circular Dichroism
GSH	=	Reduced glutathione
GSK-3β	=	Glycogen synthase kinase-3β
GSSG	=	Oxidized glutathione
HPTLC	=	High Performance Thin Layer Chromatography
IC_{50}	=	Half maximal inhibitory concentration
ID	=	Identifier
*i*NOS	=	Inducible nitric oxide synthase
IPP	=	Isopentenyl Pyrophosphate
JNK-3	=	c-Jun N-terminal kinase 3
LGA	=	Lamarckian Genetic Algorithm
LPS	=	Lipopolysaccharide
MD	=	Molecular dynamics
MDA	=	Malondialdehyde
MIT	=	Mitragynine
MITOB	=	Mitragynine Oxindole B
MS	=	Mass Spectrometry
NFS	=	Neurotrophic Factors
NMDA	=	*n*-methyl-*d*-aspartate
NMR	=	Nuclear Magnetic Resonance Spectroscopy
NO	=	Nitric Oxide
NSAIDS	=	Non-Steroidal Anti-Inflammatory Drugs
NOX1	=	Nicotinamide adenine dinucleotide phosphate oxidase 1
NQO1	=	NADPH quinone oxidoreductase 1
Nrf2	=	Nuclear factor erythroid 2-related factor 2
NTS	=	Neurotrophins
OE	=	Essential Oil
ORAC	=	Oxygen Radical Absorption Capacity
PAS	=	Peripheral Anionic Site
PDB	=	Protein Data Bank
PDE5	=	Phosphodiesterase 5
PTP1B	=	Protein tyrosine phosphatase 1B
QSAR	=	Quantitative structure-activity relationship
ROS	=	Reactive oxygen species
Sch B	=	Schisandrin B
SPR	=	Surface Plasmon Resonance
TTLs	=	Trilactone Terpenes
2D	=	Two-dimensional
3D	=	Three-Dimensional

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The study was supported by the Coordination of Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The present work was carried out with the support of the Coordination of Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

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