Oxidative Stress, Mitochondrial Abnormalities and Proteins Deposition: Multitarget Approaches in Alzheimer's disease

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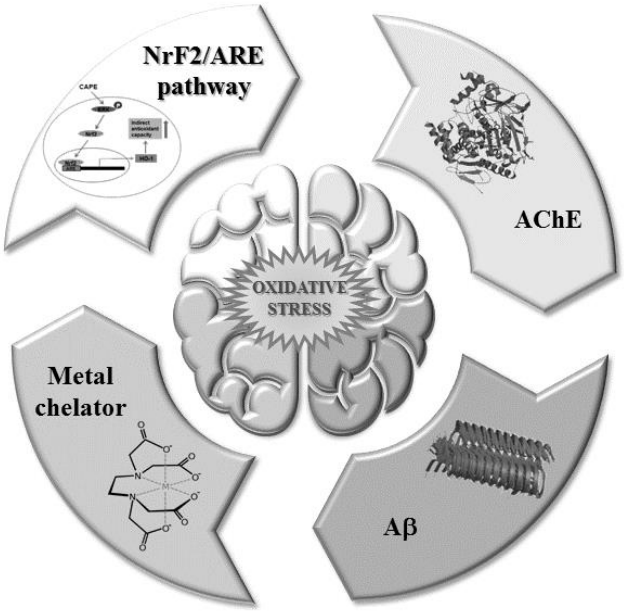
Graphical Abstract: The existing linkage between oxidative stress and the hallmarks of AD represents a challenge in developing multitarget drugs by addressing simultaneously ROS production, metal accumulation and protein deposition.



Abstract: Alzheimer diseases (AD) is a multifactorial pathology characterized by a complex etiology. The hallmarks of AD, such as Aβ deposits in senile plaque and neurofibrillary tangles (NFT), are strongly intertwined with reactive oxygen species (ROS) production and oxidative stress (OS), which are considered the common effectors of the cascade of degenerative events. An increasing body of evidence reveals that both mitochondrial abnormalities and metal accumulations synergistically act as major producers of ROS, thus contributing to neuronal toxicity. Consequently, the detrimental role of ROS production together with the neurodegenerative events involved in AD has been widely investigated as new potential therapeutic strategies. This review will concisely summarize the link between OS and the hallmarks of AD, emphasizing on their strong correlation with neurodegenerative events and elucidating the pivotal role of ROS in AD pathology. Furthermore, through this review, we will provide a short account of some of the efforts, challenges and opportunities in developing multitarget drugs by addressing ROS production, metal accumulation and protein depositions.

Keywords: Alzheimer diseases, Oxidative stress, Multitarget-ligand, Neurodegeneration, AD therapy, Hybrid scaffold.

1.Introduction

Oxidative stress (OS) was redefined by Sies and Jones as "the result of a mismatch between the excessive formation of reactive oxygen or nitrogen species (ROS, RNS) and limited antioxidant defenses that occurs constantly in every cell during the biochemical changes implicated in the metabolism"[1]. The human brain is one of the most metabolically active organs in the body, utilizing about 20% of the body's total basal oxygen and subsequently generating relatively high level of ROS [2]. Under physiological conditions, mitochondria produce ROS such as superoxide anion ($O_2^{-}\cdot$), hydroxyl radical (OH·) and hydrogen peroxide (H_2O_2), as well as RNS, such as nitric oxide (NO·) and peroxynitrite ($ONOO^{-}$). Consistently, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and other proteins with chelating properties and constituting the antioxidant system, minimize the harmful effects of these reactive species inactivating free radicals.

Recent findings recognized that the deregulation of the redox-state actively participates to the Alzheimer's disease (AD) and triggers several cellular pathways which contribute to both the onset and the progression of this neurodegenerative disease. Therefore, nowadays, the OS should be considered a key player in AD as well as in many other forms of dementia[3].

Currently, many experimental evidences highlight an intimate linkage between OS and cellular events that contribute to neuronal damage such as β-amyloid generation, neurofibrillary tangles (NFTs) formation, neuroinflammation and aberrant mitochondrial activity. All these components represent the "constituent elements" of a vicious cycle of events, in which OS plays a fundamental role (Fig. 1). Consequently, the simultaneous regulation of OS, together with the inhibition of other AD hallmarks, could lead to the development of more than one compelling therapy than the already existing ones.

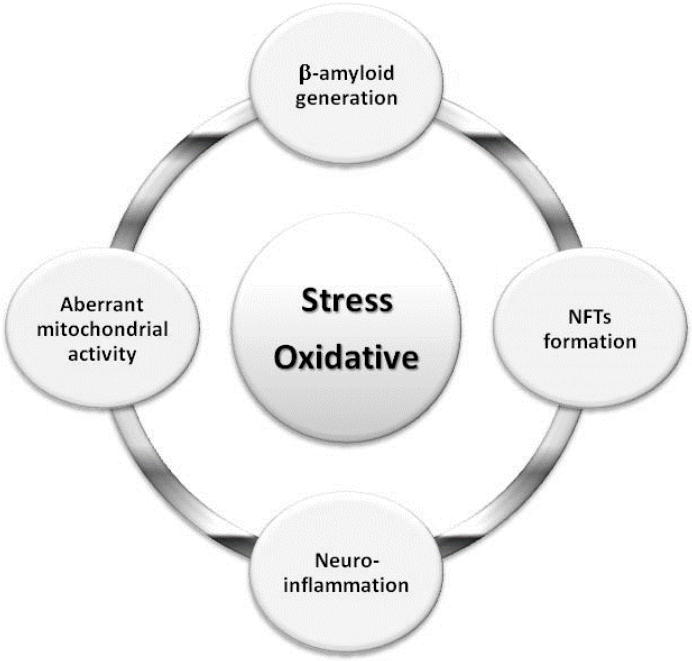


Figure1. Schematic representation of the AD targets associated withOS.

This review focuses on the recent advances in the search for innovative anti-AD therapeutic strategies designed taking into account the existing interactions between the "vicious cycle components" and OS. Moreover, medicinal chemistry approaches targeting these interactions will be deeply discussed as examples of possible innovative therapeutic strategies to treat AD.

2. Amyloid β-peptide and OS

As it has already been mentioned, AD has a complex, multifactorial etiology but until now, the putative mechanisms involved in the pathology are not completely clarified. Among the different postulated "hypotheses", the amyloid one assumes that extracellular amyloid beta (Aβ) deposits are the fundamental causes of the disease[4].

Amyloid β-peptide (Aβ) is constituted by a 40–42 amino acids[5, 6] and it is considered as the major component of senile plaques, occurring in AD. Aβ is generated from amyloid precursor protein (APP), a type I transmembrane protein which was found in several tissues. APP seems to be involved in cell growth, neurite outgrowth, cell adhesion, cell signaling and cell survival[7]. It undergoes a proteolytic cleavage through two different ways: the non-amyloidogenic and the amyloidogenic pathways.

The amyloidogenic pathway is responsible for the formation of neurotoxic Aβ fragment, the major hallmark of AD. In this pathway APP is cleaved by β-secretase 1 (BACE1), a type I transmembrane aspartyl protease which has been found in elevated amount in AD, as two products: a soluble residue APPsβ (β-secretase-cleaved soluble APP) and C terminal fragment (CTFβ). In turn, the CTFβ is cleaved by γ-secretase thus releasing Aβ fragment. The action of γ-secretase occurs at multiple sites of Aβ (from ζ to γ), and leads to the formation of Aβ fragments ranging from 37 to 46 amino acids in length. Among them, the neurotoxic Aβ(1-42) tends to form oligomers and aggregates. During the progression of AD, the level of Aβ(1-40) seems to be constant in cerebrospinal fluid (CSF), on the contrary, the Aβ(1-42) level decreases in CSF but increases in senile plaques[8]. This effect is probably due to a deficient efflux of Aβ(1-42). Recent

evidence revealed that the higher damage in CNS is principally due to the aggregation of Aβ(1-42) in oligomers rather than plaques formation[9].

Although Aβ accumulation is considered a hallmark of AD, it is still unclear if the OS is the trigger or the consequence of Aβ deposition in brain.

Therefore, evidence of both the Aβ-induced OS hypothesis and OS-induced Aβ accumulation have been proposed. Implications of metionine-35 (Met-35) residue of Aβ(1-42) in the ROS generation have been largely studied. It seems that Aβ(1-42) oligomers are able to insert themselves into the lipid bilayer forming the alpha-helices. During this process Met-35 is easily oxidized thus obtaining the Met sulfuranyl free radical ($MetS^+$) which is, in turn, stabilized by the hydrophobic environment of the lipid bilayer[10]. $MetS^+$ starts a series of free radical chain reactions, generating lipid peroxidation products and oxidative modified membrane proteins[11]. This hypothesis has been confirmed by different in *invitro* studies: for instance, the replacement of the critical aminoacids needed for a correct spatial displacement of Aβ(1-42) nullifies the toxic effect of Aβ(1-42)[12, 13], thus showing the detrimental role of Met-35 in the Aβ-induced neurotoxicity. Also in *in vivo* studies performed in AD mice have shown that the mutation of the Met-35 residue to Leu produced the loss of OS in brain[14]. These studies seem to confirm the hypothesis that Met-35 of Aβ(1-42) plays an important role in OS.

However, there are alternative hypotheses that explain the relationship between Aβ(1-42) and OS in which the possible role of OS is highlighted as a causal factor and not as an effect induced by the Aβ-production. Indeed, *post-mortem* studies showed that OS appears in the early phase of AD, and it declines when beta amyloid begins to accumulate in the CNS, whereas, in transgenic AD models, the oxidation of proteins and lipids seems to anticipate the deposition of Aβ(1-42)[15]. Further cell based studies indicated that the OS provokes an overexpression of APP and an increase of BACE activity, thus improving the production of Aβ(1-42)[16]. In opposition with the hypothesis discussed above, Sinha et al. have recently proposed that Aβ(1-42) could have an antioxidant action, principally linked to its metal-chelating property[17]. According to this study, it has been suggested that the accumulation of Aβ(1-42) could represent a cellular defense against the OS and metal dyshomeostasis, even if all of their toxic properties are still maintained.

Although the exact mechanism linking Aβ(1-42) to OS is not clear yet, their important role in both the onset and evolution of AD has urged several medicinal chemists to design new multifunctional compounds that target simultaneously Aβ(1-42) and OS. Unfortunately, none of them has been approved by FDA [18, 19], further prompting the research toward the development of multi-target entities as an effective strategy for the treatment of complex and multifactorial pathologies such as AD.

One of the initial attempts in designing AD multitarget directed ligand (MTDL) was carried out by Lenhart et al., that designed bivalent multifunctional Aβ oligomerization inhibitors (BMAOIs) targeting the small Aβ oligomers (AβOs), OS and the membrane microdomains of neuronal cells, namely cell membrane/lipid rafts (CM/LR), which are rich in cholesterol and sphingolipids. CM/LR were found efficient in accelerating the bind of Aβ with the cell membrane thus promoting the AβOs formation[20-22]. BMAOIs were obtained by the combination of curcumin, one of the most renowned phytochemical that owns well-known antioxidant, anti-inflammatory and anti-Aβ properties[23], with cholesterol, which exerts the function to anchor CM/LR in mammalian cells. A series of BMAOIs with different spacers were synthetized; in particular, compound **1** which possesses a 21-atom-spacer in C-4 position of curcumin, showed to potently reduce the intracellular level of AβOs in both MC65 (a human neuroblastoma cell line) and ML60 (a line of Chinese hamster ovary that specifically produce high levels of extracellular AβOs cell lines). Moreover, compound **1** displayed antioxidant properties comparable to curcumin and showed to cross the blood brain barrier (BBB) in Caco-2 cell line.(Fig. 2)

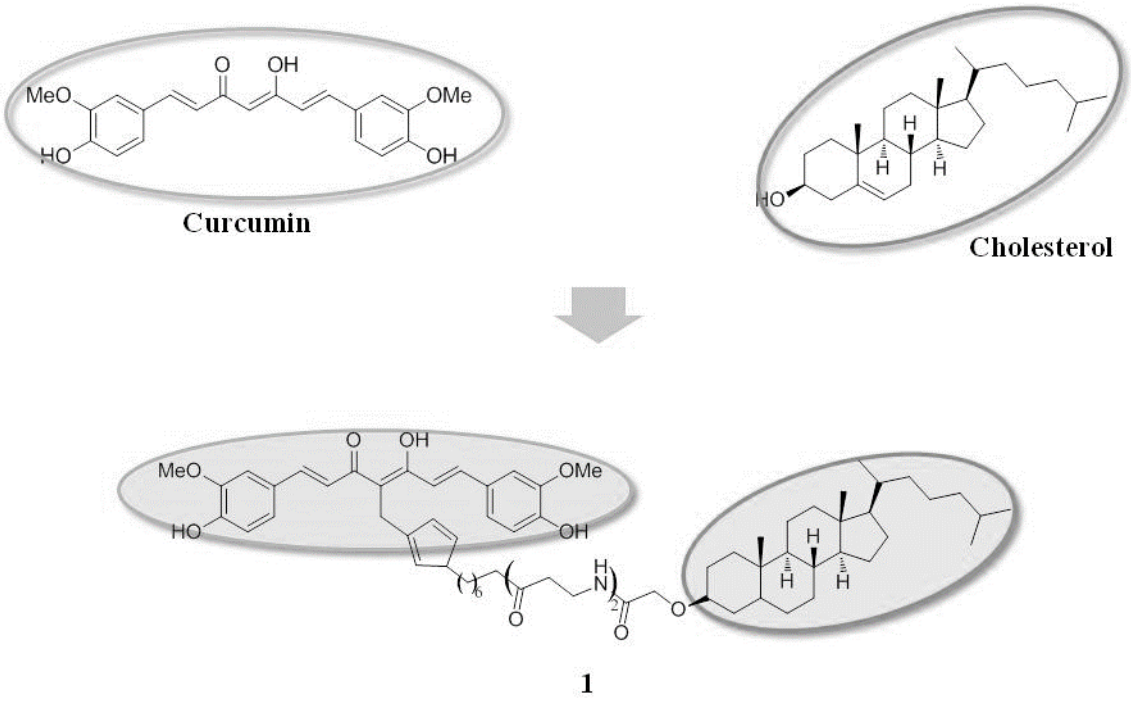


Figure2. Design strategy of compound **1**

Another class of MTDL was synthesized starting from the styrylbenzene scaffold. The new compounds interacted with Aβ thus reducing its aggregation and, in the meantime, acted as free radical scavengers[24]. The most interesting compounds were obtained by the combination of bis-styrylbenzene scaffold of DF-9, previously synthetized from the same group[25], and resveratrol. Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural polyphenolic compound highly concentrated in grape skin and red wine; this phytochemical is known to possess a strong antioxidant behaviour due to the presence of phenolic units [26, 27], but it is also able to inhibit Aβ aggregation in *in vitro* models [28] thanks to its diaryl conjugated structure. Among the hybrids designed with this strategy, compound **2** (Fig. 3) displayed a good Aβ binding affinity (Kd = 5.3±0.3 nM), a good capability to cross the BBB and also the ability to bind with Aβ plaques in *in vivo* assays. On the whole, this compound could be considered as a lead molecule for the early Aβ detection. However, because of the limited free radical scavenging properties, a medicinal chemistry optimization is needed for future possible applications in AD therapy.

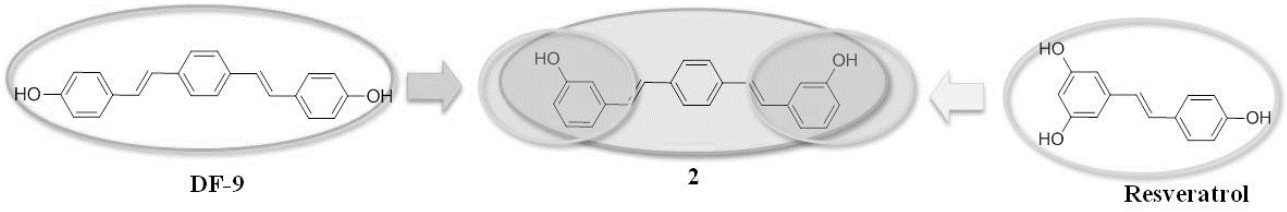


Figure 3. Design strategy of compound **2**.

Recently, a new drug candidate, memoquin (MQ), has been identified as a promising anti-AD drug candidate mainly for its exquisite multitarget profile [29]. MQ is a quinone-bearing polyamine compound. It has been rationally designed and synthesized by replacing the linear alkyl chain of caproctamine with a radical scavenger function, in a deliberate attempt to find an MTDL able to interfere with different key target points of AD neurodegeneration [30](Fig. 4).

Memoquin has already been shown to decrease plaque number and morphology in *in vivo* models and displayed a significant capability to reduce the Aβ aggregation in *in vitro* assay. MQ proved to inhibit, in a concentration-dependent manner, BACE1, one of the two enzymes involved in the APP amyloidogenic cleavage (IC_{50}=108 nM). Moreover, in SH-SY5Y neuroblastoma cells, MQ reduced AChE-mediated Aβ aggregation [31] and displayed a dose-dependent neuroprotective effect against Aβ(1–42) oligomers-induced neurotoxicity[32].

As far as the antioxidant properties are concerned, MQ demonstrated to act as a substrate for the NADPH quinine oxidoreductase 1 (NQO1) with Kmof 12.7 μM. This enzyme catalizes the reduction of the MQ-quinone moiety into the hydroquinone form which is the real antioxidant specie [29]. This mechanism of action has been also confirmed in cellular assays performed in SH-SY5Y cell line, where MQ showed to neutralize the formation of free radicals and ROS.

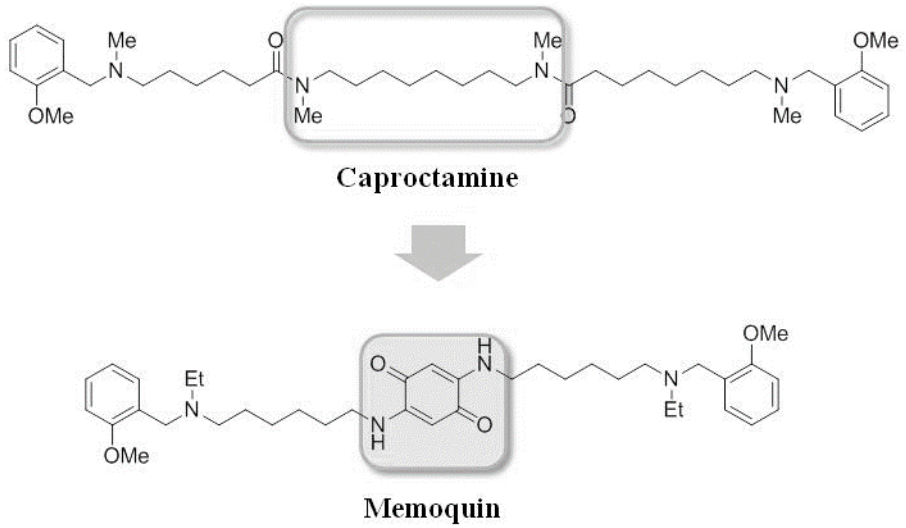


Figure4. Design strategy of Memoquin.

In the last decade, it is emerged that the cerebrovascular function plays a detrimental role in AD pathogenesis and, more importantly, that cerebral hypoperfusion should represent a primary trigger for neuronal dysfunction that lead to Aβ-accumulation in brain and to a reduced Aβ clearance[33]. The worsening of the neurovascular dysfunction seems to be strongly related to the loss of functionality of specific receptors and/or to the reduction of carrier-mediated transport activity across the BBB [33, 34]. Many receptors regulate the transport equilibrium of Aβ across the BBB and among these, the receptor for advanced glycation end products (RAGE; also known as AGeR) and the low-density lipoprotein receptor-related protein 1 (LRP-1) [35]. Many experimental evidences showed an increased RAGE expression at the BBB in models of Alzheimer's disease [36]. In addition to that, in order to mediate the flux of Aβ across the BBB, RAGE promotes synaptic dysfunction as well as neuroinflammation and OS, following the activation of specific mediators such as the nuclear transcription factor kB (NF-kB). Consequently, the blockade of Aβ–RAGE signaling in AD could represent a promising strategy to control Aβ accumulation and thus Aβ-mediated injuries. Indeed, PF-04494700 (Azeliragon), a small-molecule inhibitor of the RAGE recently developed by Pfizer, has completed a phase II clinical trial aimed at evaluating the efficacy and safety in subjects with mild to moderate Alzheimer's disease

(ClinicalTrials.gov Identifier: NCT00566397) [37]. Previously, *in vitro* studies showed that Azeliragon inhibits amyloid plaque formation, reduces the size of pre-existing plaques, and decreases the behavioral effects due to toxicity of Aβ-deposition [19]. More interestingly, the effects induced by the RAGE inhibitor are the blockade of Aβ-deposition, but above all, the reversal of symptoms of amyloidosis [19, 38]. Further studies of Galasko et al. proved that PF-04494700 (after ten weeks of treatment) is safe and well-tolerated in patients with mild to moderate AD, thus indicating the feasibility of a wider long-term clinical trial[39].

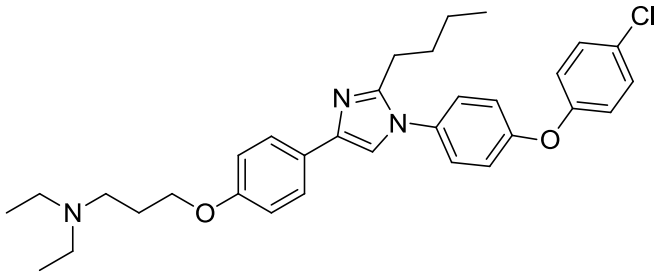


Figure5. Structure of PF-04494700.

3. NFT and OS

AD is pathologically characterized by the presence of extracellular senile plaques, constituted by a core of Aβ, and intracellular neurofibrillary tangles (NFTs).

NFTs are formed by an aggregation of paired helical filaments (PHF), which are made up of phosphorylate tau protein. The physiological function of tau is to maintain the assembly and the stability of microtubules: when tau is hyperphosphorylated, a disassembly of microtubules occurs, compromising their correct action and consequently causing a decrease of the axonal and dendritic transport. Tau filaments accumulate in dystrophic neuritis as fine neuropil threads or as bundles of PHF in neuronal bodies, constituting the NFTs that lead to the formation of extracellular tangles. The formation of tangles could be due to release in the brain of antibodies against different phosphorylation sites of tau[40].

It is still debated whether NFTs play a primary or a peripheral role in AD, however the NFT density seems to be linked, at least in part, to the neuronal loss and to the level of dementia. Many studies revealed that neuronal death it is not directly induced by NFT formation, but it is mediated by OS, which is closely linked to the NFT formation. Indeed, OS activates some kinases such as glycogen synthase kinase-3β (GSK-3β)[41] and mitogen-activated protein kinases (MAPKs)[42] that phosphorylate tau protein. Phosphorylated tau undergoes oxidative modification and aggregates into fibrils because of a high presence of lysine-serine–proline (KSP) domains[43].

Recently, the NFT formation has been suggested as a "compensatory response" to OS. Indeed, as observed for Aβ, the antioxidant role of NFT could be due to the capability to form complexes with free metals. In particular, iron and copper bind to phosphorylated tau protein whereas iron ions tightly interact with NFT [44, 45].

In various cell cultures, invertebrate and mammalian models of AD increasing GSK3 activity leads to the hyperphosphorylation of tau, increased Aβ generation and deficits in learning and memory accompanied with neurodegeneration[46]. There are numerous studies showing that abnormal increases in the level and activity of GSK-3β induces neuronal cell death, paired with helical filament tau formation and neurite retraction. The strong link between NFT and OS prompted researchers to target all these hallmarks of AD simultaneously.

Recent years have witnessed the development of an increasing number of novel GSK-3β inhibitors, many of which are ATP-competitive. Within this class, SB-415286 , an anilinomaleimide, developed by Glaxo Smith Kline, showed the ability to inhibit GSK-3β with an IC_{50} value in the nanomolar range [47]. The compound acutely reduces cellular GSK-3 activity as assessed by activation of glycogen synthase. Moreover, SB-415286 shows antioxidant properties, which may explain the cytoprotective effect against H_2O_2 damage [48]. In the light of these findings, the use of dual antioxidant GSK-3 inhibitors could be considered one of the most promising approaches for treatment of AD (Fig. 6).

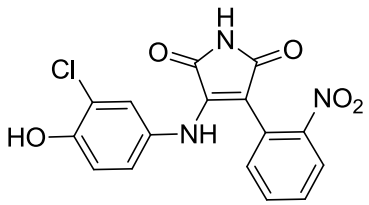


Figure6. Structure of SB-415286.

Several studies suggest that over-expressed tau (normal and/or hyperphosphorylated) has also been found to impair axonal transport and abnormal distribution of mitochondria in AD neurons[49]. Given that, one of the primary targets of pathological forms of tau may be mitochondria. Many proposed therapies target mitochondrial ROS production and cellular oxidative stress which result from malfunctioning mitochondria and compromise cell viability.

One Mitochondria-Targeted Antioxidants (MTAs) termed mitoQ (mitoquinol, or mitoquinone, or a mixture of these redox forms) is produced by conjugation of the lipophilic triphenylphosphonium cation (TPP^+) to coenzyme Q (Fig. 7)[50]. TPP^+ is a lipophilic cation that enables the molecule to enter and accumulate inside the mitochondria as a result of the electrochemical gradient. Coenzyme Q plays an integral role in Complex II of the mitochondrial respiratory chain. By positive charge, mitoQ accumulates in mitochondria and it has been used to modulate ROS in the mitochondrial matrix [51]. It has been reported that mitoQ also exerts protective effects on cells by reducing free radicals, decreasing oxidative damage and maintaining mitochondrial functions[52].

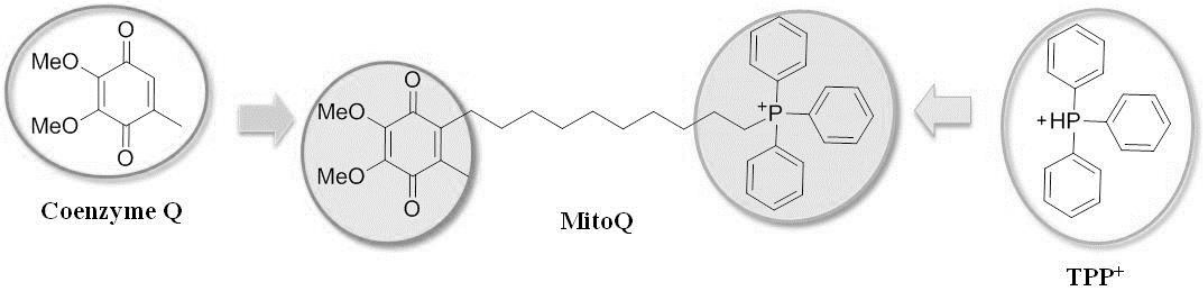


Figure7. Design strategy of MitoQ.

4. Cholinesterase and OS

The systematic biochemical investigation of the brain, between the late 1960s and early 1970s, established that a loss of cholinergic function in the CNS contributes to the cognitive decline associated with AD [53]. A support for this perspective came when Perry et al. reported a substantial neocortical deficits of choline acetyltransferase (ChAT), the

enzyme responsible for the synthesis of acetylcholine (ACh)[54]. The "cholinergic hypothesis" was postulated on this basis. According to this hypothesis, the degeneration of cholinergic neurons in the basal forebrain is associated with the loss of cholinergic neurotransmission in the cerebral cortex as well as in other cerebral areas and these events significantly contributed to the deterioration in cognitive function of AD patients[55].

In the mammalian brain, the most prominent enzyme involved in acetylcholine hydrolysis is acetylcholinesterase (AChE). AChE has two binding sites: a catalytic active site (CAS), located at the bottom of a deep narrow gorge lined with several aromatic amino acid residues, and a peripheral anionic site (PAS) uncovered at the entrance. The PAS is involved in a secondary non-cholinergic function that includes the promotion of Aβ formation associated with deposition of senile plaques/neurofibrillary tangles in the brain of AD afflicted individuals.

Additionally, butyrylcholinesterase (BuChE) can also hydrolyse acetylcholine in the brain and may play a critical role in cholinergic transmission[56], so the inhibition of this enzyme leads to an increase in the acetylcholine concentration of the synaptic cleft and it is thus expected to enhance cholinergic transmission and ameliorate cholinergic deficit[57].

Despite outstanding efforts to develop innovative therapies for Alzheimer's disease, acetylcholinesterase inhibitors (AChEIs) still continue to represent the most important class of compounds for the treatment of mild and moderate forms of AD [58]. These drugs have proved themselves useful in maintaining cognitive and functional abilities in most AD patients. Therefore, many compounds endowed with both acetylcholinesterase inhibitory activity and antioxidant properties have been synthesized in order to produce additional benefits preventing Aβ mediated oxidative damage in AD patients [59, 60]. A wide plethora of new hybrid drugs have been recently published [61-64].

Many examples include multi-target compounds of tacrine, the first AChEI approved for the treatment of AD. In order to combine its potent AChE inhibition with other pharmacological properties, such as antioxidant and metal chelating activities, this drug was appropriately conjugated with many other pharmacological moieties.

Melchiorre's research group developed Lipocrine (Fig. 8), a multitarget compound in which lipoic acid, a natural antioxidant, was combined with tacrine. In *in vitro* tests, lipocrine turned out to have better AChE and BuChE inhibitory activity than tacrine (IC_{50} = 0.253±0.016 nM and 10.8±2.5 nM, vs IC_{50} = 424±21nM and 45.8±3.0 nM respectively) [60].

Moreover, as highlighted from kinetic analyses, this compound resulted to be a mixed AChEI type, able to bind both catalytic (CAS) and peripheral (PAS) sites of AChE. Results obtained in *in vitro* models showed that lipocrine induced a significant inhibition of AChE-induced Aβ aggregation. As regard the antioxidant properties, lipocrine was able to counteract the ROS production (64% inhibition at 50 μM) more effectively than lipoic acid[65]. Altogether the pharmacological multitarget profile of lipocrine leads to consider it as an attractive multicomponent drug for the treatment of AD[66].

Another molecule, obtained through the combination of two molecules of tacrine, namely bis(7)-tacrine, exhibited a higher AChE inhibition potency and a better pharmacological profile than native compound; with the aim of improving the antioxidant profile, bis(7)-tacrine was subjected to chemical manipulations in order to improve its biological profile as radical scavenger[67]. In particular, the alkyl chain of bis(7)-tacrine was replaced by cystamine (Fig. 8), a well-known molecule endowed with antioxidant and neuroprotective properties[68].

The cystamine-tacrine dimer [69] showed a pharmacological profile comparable to bis(7)-tacrine, as regard the inhibition of both cholinesterases (AChE IC_{50}= 4.4 ± 1.7μM and BuChE IC_{50}= 6.7 ± 1.6 μM) and the inhibition of the Aβ aggregation. Unlike bis(7)-tacrine, cystamine-tacrine dimer displayed antioxidant activity due to the contribute of disulfide bridge. Two different pathways PI3K/Akt and ERK1/2 seem to be linked to the neuroprotective effects. As regards the pathway PI3K/Akt, it is able to protect neurons from apoptosis, and inhibits the Aβ-induced neurotoxicity.

Moreover, it was demonstrated that cystamine-tacrine dimer can phosphorylate also ERK1/2, demonstrating a different mechanism of action in comparison with bis(7)-tacrine that does not show any effects on Akt or ERK1/2 kinases[70].

Recently, Pi and collaborators developed tacrine–ferulic acid hybrids (TAFA)[71], and tacrine-caffeic acid hybrids (TACA)[72] as potential AD therapeutics. Enzyme kinetic studies of both series of compounds showed that the new molecules were able to simultaneously interact with the catalytic site and the peripheral anionic site (PAS) of the AChE [71].

Among these compounds, T6FA (Fig. 8), inhibited the AChE-induced $A\beta_{1-40}$ aggregation by 50.27% and 20.23% at 100 μM and 50 μM, while the effects of native drugs alone (FA and tacrine) on Aβ aggregation were lower or not detectable at such concentrations. This multifunctional dimer protects HT22 cells from glutamate-induced cytotoxicity and decreases the level of ROS induced by glutamate. Besides, T6FA dramatically provokes the Nrf2 nuclear translocation, and the activation of the ARE-dependent transcription. All results suggest that T6FA can be an useful tool for cognitive impairment and may be considered as a promising multifunctional drug candidate for neurodegenerative diseases[71].

Subsequently, in order to widen the pharmacological profile of the compounds TAFA and TACA, a series of TAFA and TACA was synthesized [72, 73], in which the linker was replaced by the 1,3-diamino-2-propanol chain.

Within this series, compound **3** (Fig. 8) displayed a good ability to inhibit the $A\beta_{1-42}$ self-aggregation, with percentage value of 53%, even if it exhibited modest BACE1 and AChE inhibitor activities. Moreover, tacrine–caffeic acid hybrid, showed antioxidant properties with scavenging percentage values ranging from 60.87% to 90.36% and comparable to caffeic acid (44.10% and 90.27% respectively). Compound **3** also proved to chelate copper ions in UV–vis spectrometry thus delineating a multi-functional AChEI-antioxidant-chelating profile [73].

Given the growing interest on natural-based drugs, recently, Santos [74]explored a set of hybrid compounds obtained by the conjunction of the tacrine moiety with S-propargylcysteine. This combination was aimed at improving both the cholinergic system and neuroprotective capacity. The two main molecular moieties have been combined through linkers that were able to ensure a dual binding interaction with CAS and PAS of AChE.

An analysis of the results reveals that Tacrine–SPRC(Fig. 8) showed an AChE inhibitory activity with IC_{50} values of 1.21 μM. Moreover this molecule displayed a combined role in both preventing Aβ-induced toxicity and reducing H_2O_2-induced oxidative stress in a cellular context. Even if the scavenger activity assessed by the DPPH method resulted to be quite low, a significant neuroprotective activity was observed in *in vitro* assays. This apparent mismatch could be explained, at least in part, by the capability of this compound to react with sulphydryl groups of antioxidant enzymes such as the intracellular glutathione (GSH). Finally, this multitarget compound showed to be able to chelate Cu(II) (pCu = 7.14–7.5) throughout the diamine-linker within the two main molecular units [75]. The most promising results were achieved by Tacrine–SPRC that could be suggested as potential lead compound toward AD therapy[74].

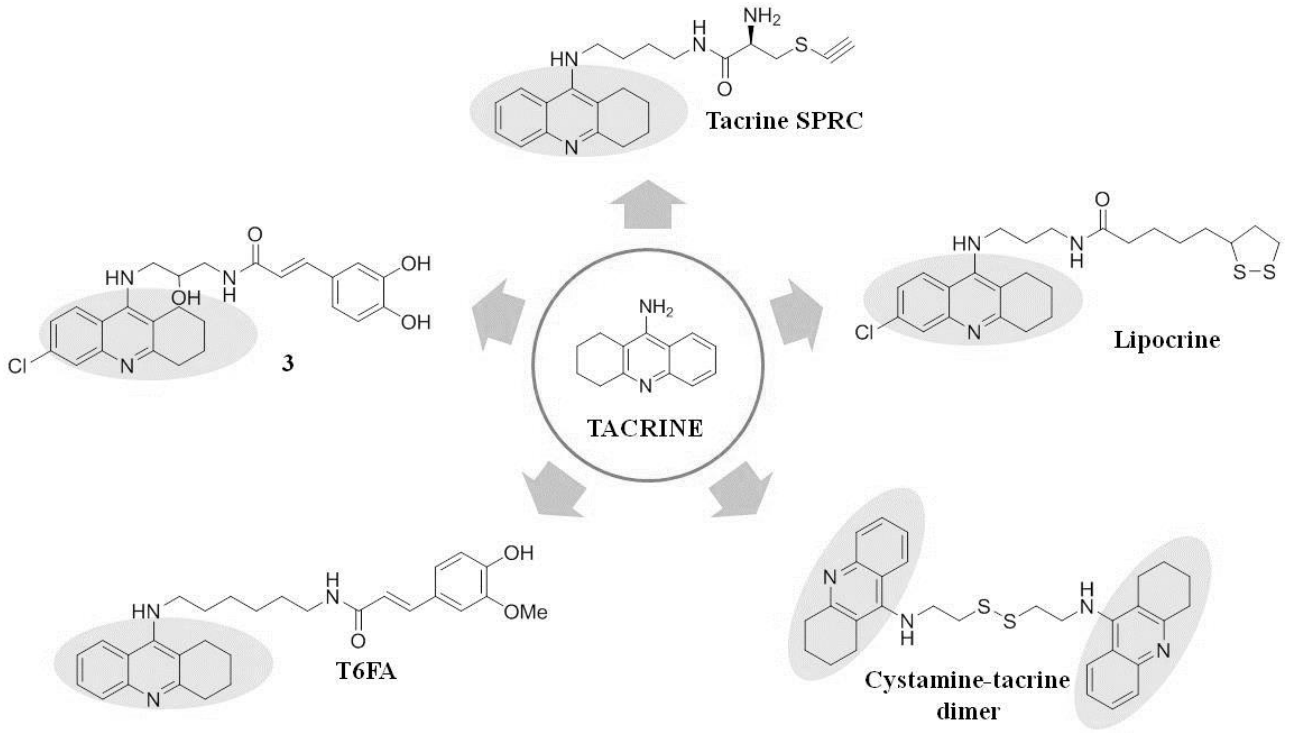


Figure8. Design strategy of multitarget tacrine derivatives.

Evidence indicates that BuChE might be a co-regulator of the activity of the neurotransmitter Ach [76]. In a more recent study, structural scaffold of donepezil has been used as a lead-structure to design multitarget-directed ligands. Donepezil is the second drug approved by the U.S. Food and Drug Administration for the treatment of mild to moderate AD and shows a greater affinity for AChE than for BuChE. In particular, this observation prompted Benchekroun [77] to develop multifunctional molecules able to restore acetylcholine levels and reduce oxidative stress. These compounds were designed starting from benzylpiperidine-scaffold of donepezil and ferulic acid (Fig. 9). Among the synthesized hybrids, DFAH showed a strong antioxidant potency (8.71) which resulted to be higher than that of ferulic acid (3.74). DFAH displayed also a good BuChE inhibitory activity displaying to be 198-fold more potent than donepezil (IC_{50} = 10.39 ± 0.48 nM vs 2057 ± 290 nM). In addition, the new compound revealed to be able to trap toxic radical species, preventing the neuronal damages strictly related to the oxidative stress [77]. On the whole, the multitarget profile of DFAH points out that also this compound could be further investigated as a potential multifunctional neuroprotective agent for the treatment of AD.

Similarly, a novel series of molecules, obtained by fusing the cholinesterase inhibitor donepezil and the antioxidant ebselen, were described as potential multi-modal agents to manage the AD [78]. Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a lipid-soluble cyclic selenenamide that has been extensively studied as the GPx mimic (Fig. 9). It protects cells by catalyzing the reduction of peroxides with glutathione. Besides, a recent study, indicates that this molecule is able to inhibit iron-induced tau phosphorylation[79]. Within the new series of derivatives, SE-518 turned out to be the most potent AChE inhibitor (IC_{50}=0.097 μM for AChE) with a moderate BuChE inhibitory activity (IC_{50}=1.586 μM). Moreover, it also proved to scavenge H_2O_2 and peroxynitrite and to have additional glutathione peroxidase-like activity, without any toxicity in mice at a dose of 2000 mg/kg. Further permeability studies performed in *in vitro* models indicated that SE-518 is able to penetrate the central nervous system thus highlighting that also this

kind of conjunction could be successfully accomplished for the discovery of new lead compounds for the treatment of AD.

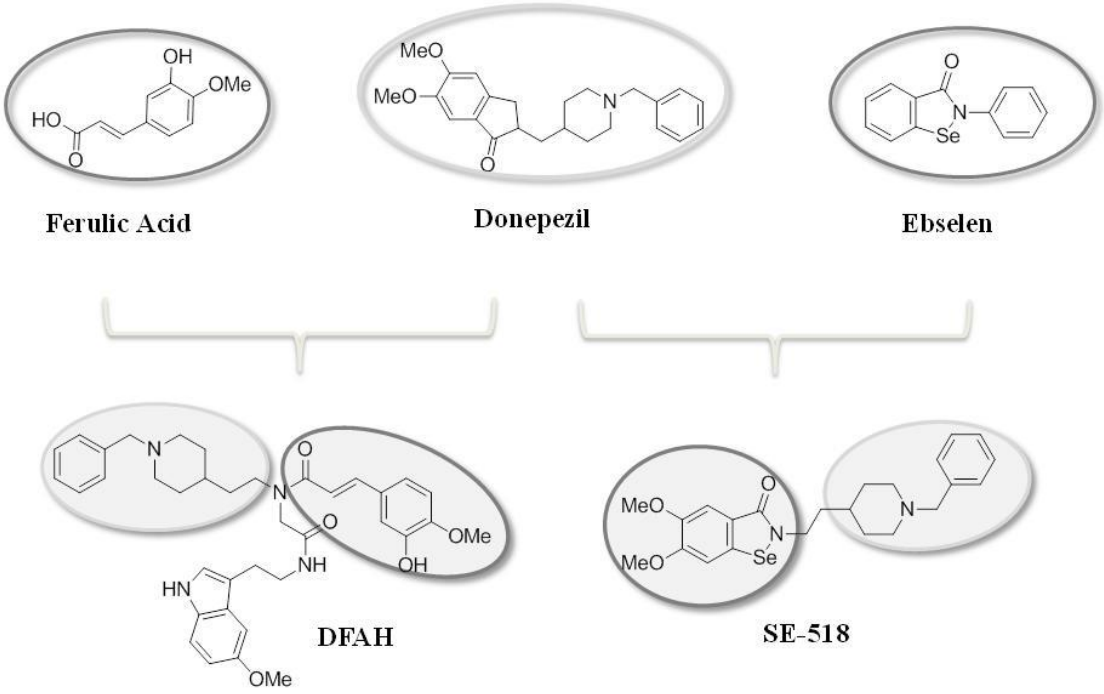


Figure9.MTDL strategy of Donepezil derivatives.

5. NrF2/ARE and OS

As a part of a delicate equilibrium, since ROS induces to oxidative damage, also the decrease in antioxidant defenses contributes to worsen the neurodegeneration. Several lines of evidence suggest that a transcriptional increase of the activity of the Nrf2/ARE pathway leads to an increase of antioxidant defenses throughout the transcription of anti-inflammatory and antioxidant genes. Consistently, this pathway may represent a compelling target to halt or prevent the onset of neurodegenerative diseases such as AD and Parkinson's diseases [80, 81].

Nuclear factor E2-related factor 2 (Nrf2) is a master regulator that induces the expression of a variety of cytoprotective and detoxificant genes including antioxidant enzymes, anti-inflammatory mediators and several transcription factors involved in mitochondrial biogenesis. Nrf2 bound to its inhibitor kelch-like ECH-associated protein (Keap1) in the cytosol thus preventing the Nrf2 translocation into the nucleus.

OS is able to disrupt the complex Nrf2-Keap1 thus inducing the translocation of Nrf2 into the nucleus and then promoting the activation of the Antioxidant Response Element (ARE), a promoter region of genes encoding various antioxidants and detoxifying enzymes. Nrf2 also antagonizes the transcription factor Nuclear Factor-κB (NF-κB), which regulates the expression of inflammatory genes, assuming also a key role against neuroinflammation [82]. Moreover, Nrf2 is considered a stress-sensing genetic transcription factor since its expression generally declines with age. Suh et al showed a marked age –related loss both in total and nuclear Nrf2 levels**,** suggesting therefore an attenuation in Nrf2-dependent gene transcription [83]. In addition to that, the release of Nrf2 from keap1 is significantly impaired in AD.

This effect could be related to the reduction observed in the frontal cortex of AD patients of both p62 gene expression and cytoplasmic p62 protein levels [84]. P62 could bind to the Keap1 thus hindering the interaction of Keap1 with Nrf2 transcription factor, which, in turn, induces the stress responsive cellular defence genes. Nrf2 also stimulates the expression of p62 protein with a positive feedback loop, between the expression of p62 protein and Nrf2-mediated stress responses. Therefore, a decrease in the level of p62 protein can perturb the signalling pathways of Nrf2, thus increasing OS [85]. Moreover, the lack of p62 expression induces an aggravation of tau pathology in neurons by increasing GSK3β activity as well as by decreasing the synaptic function with loss of working memory and neuronal apoptosis [86].

Besides its role in regulating cellular anti-oxidative response, Nrf2 pathway shows also anti-inflammatory activity [87] thus representing a new therapeutic approach for suppressing or reducing the inflammatory stress. Therefore, disruption of Nrf2/ARE pathway may contribute to induce or worsen the main hallmarks of neurodegeneration such as oxidative stress, misfolded proteins aggregation, excitotoxicity and inflammation.

A wide range of dietary phytochemicals showed to induce the expression of enzymes involved in cellular antioxidant defence. In particular natural compounds such as sulphorafane, resveratrol, allyl sulfides, epigallocatechin gallate and curcumin, proved to activate the Nrf2 pathway and to produce neuroprotective effects [88]. It has to be underlined that these molecules possessed an intricate polypharmacology, including anti-amyloidogenic, anti-oxidative, and anti-inflammatory properties [23]. Unfortunately, phytochemicals display a poor bioavailability, thus hindering their application in therapy. Several ongoing studies aim at looking for new strategies to improve the ADME profile of these molecular entities[89-91], in order to use them as multitarget therapeutics for the treatment of neurodegenerative diseases.

Sulforaphane [1-isothiocyanato-(4*R*,*S*)-(methylsulfinyl)butane] (SFN) (Fig. 10) is an isothiocyanate derivative found in broccoli and other cruciferous vegetables. SFN directly interacts with Keap1 by covalent binding to the thiol groups of the reactive cysteine residues, used by the protein as sensors of the intracellular redox state. SFN showed to increase Nrf2 and several ARE-dependent antioxidant enzymes, such as Glutathione Reductase (GR), GPX, glutaredoxin (GLRX), thioredoxin (TX), thioredoxin reductase (TR), Heme oxygenase 1 (HO1), and NQO1, in different cell systems [92]. In 2013 Kim et al demonstrated that administration of SFN ameliorated cognitive function of Aβ-induced AD acute mouse models[93]. Although the exact mechanism of action of SFN in AD has not been established yet, the authors suggest that SFN can be of help in cognitive impairment and may defend the brain from amyloidogenic damages, indicating that antioxidants, such as SFN, can be a complementary yet promising strategy to approach AD treatment.

Following those premises, in 2015 Egea et al. synthetized compound ITH12674, a hybrid molecule designed to exert a dual drug-prodrug mechanism of action, which combine SFN and melatonin structures[94] (Fig. 10). Melatonin is a neuro-hormone synthesized in the pineal gland. Its expression decreases with aging. Melatonin is already in use in AD therapy for its chrono-biological properties that make it capable of correcting the circadian rhythm disorders observed in AD patients. Moreover, it has been widely reported as a potent antioxidant and scavenger compound, also able to induce antifibrillogenic effects in both *in vitro* and *in vivo* models[95]. ITH12674 collects the melatonin and SFN features in one molecule with an improved neuroprotective profile compared with that of the parent compounds. ITH12674 exerted a concentration-dependent protective effect in cortical neurons subjected to OS, also decreasing mitochondrial ROS production and increasing GSH levels in cortical neurons. In addition to that, this molecule was able to enhance the Nrf2–ARE response in transfected HEK293T cells and to protect organotypic cultures of hippocampal slices from stress by increasing the expression of HO-1 and reducing free radical production [94].

Likewise, the same group synthetized compound **4** [96], a multi-target hybrid structure derived from the conjunction of melatonin and ethyl cinnamate (Fig. 10). Cinnamate esters are moderate Nrf2 inducers, while the corresponding free cinnamic acids proved to be inactive. Interestingly, the Nrf2-induction potency of cinnamate esters is influenced by the position of the substituents on the aromatic ring. Among the series of new synthesized hybrid-molecules, compound **4** showed a free radicals scavenger activity four-fold more potent than melatonin and a significant Nrf2 induction effect with the best antioxidant activity, maybe related to its electrophilic character. Compound **4** displayed high neuroprotective potency in an OS model too. Also this effect resulted higher than the parent compound melatonin in two different models of neurodegeneration associated with AD and brain ischemia [96].

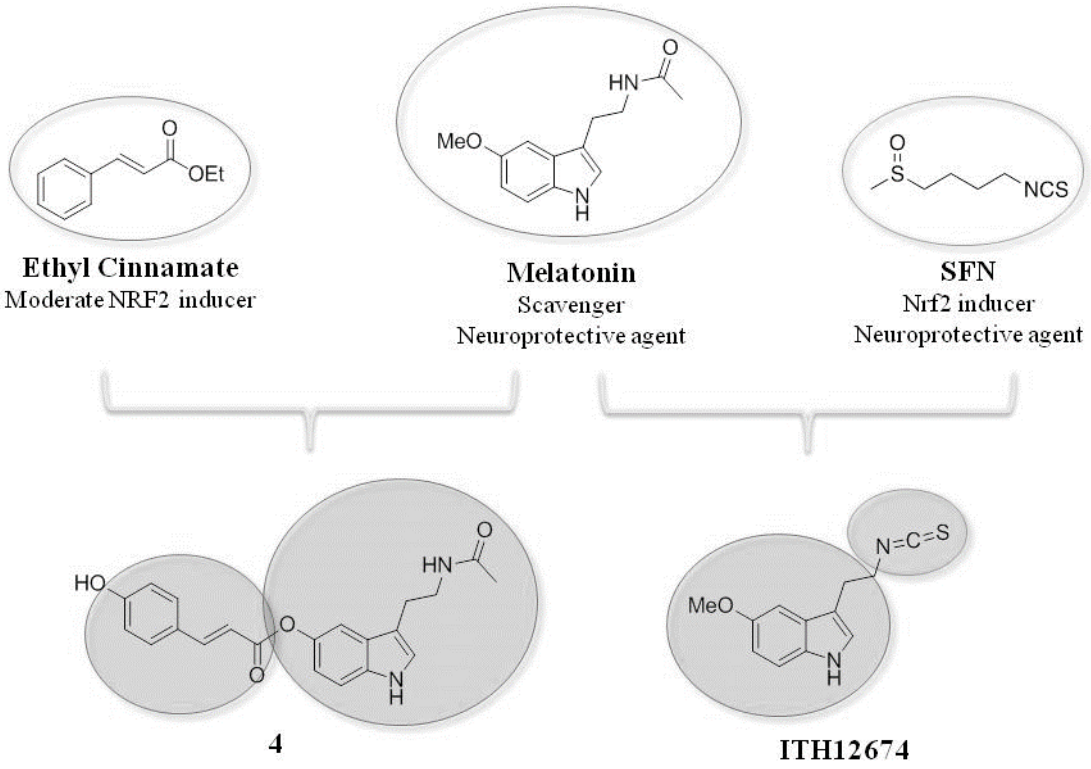


Figure10. Structures of hybrids obtained by the conjunction between Ethyl cinnamate and Melatonin (Compound **4**) and Melatonin and SFN (**ITH2674**).

6. Metal chelating and OS

Disruption of metal homeostasis is included among the major pathological features playing important roles in the progress of AD. Iron (Fe), copper (Cu), and zinc (Zn) are the triad of transition elements involved in the metal hypothesis. Brains affected by AD suffer from metallostasis, or fatigue of metal trafficking, which leads to redistribution of metals into inappropriate compartments [97].

Levels of Fe, Cu, and Zn were found enriched in AD due to a dysfunction of the corresponding regulatory system.While reporting of zinc and copper levels in AD-affected brains has been inconsistent until now, iron accumulation occurs in AD patient's cortex and not in cerebellum [98, 99], consistently with the anatomical profile of such neurodegeneration. All three metals bind Aβ and accelerate its aggregation into insoluble precipitates thus increasing metal sequestration into amyloid deposits. Additionally, Zn bound to Aβ may hinder the proteolytic cleavage site preventing its degradation

by matrix metalloproteases. Moreover, these metals are also involved in tau pathology: indeed, both Cu and Fe(II) metals induce tau hyperphosphorylation [100], while Zn modifies tau conformation and induces both fibrils formation and aggregation of tau-protein[101]. Interferences of metals with APP processing and function were also found. Furthermore, investigations on the Cu–Aβ complex lead to speculate that the complex-induced toxicity may involve the inhibition on human cytochrome *c* oxidase and the production of H_2O_2 via a catalytic cycle [97].

All these processes contribute to generate OS damage, making the design of specific multitarget-compounds endowed of metal chelating properties and anti-OS activity a challenging issue to restore metal homeostasis and reduce OS in AD brains. Since Cu exhibits the greatest implication with dyshomeostasis in amyloid plaques and increasing of Aβ deposition, Cu is the main metal studied for this purpose. On the contrary, despite the confirmed role of Fe in AD pathogenesis, the number of iron chelator-based drugs, is limited[97].

Clearly, being AD a multifactorial disease, the design of new molecular entities, which combine chelating scaffolds with functional moieties able to hit other important disease targets, is a synthetic strategy widely applied in the design of novel combined metal chelating-antioxidant compounds. For instance GS(HQ)H is a hybrid molecule linking the scaffolds of 8-hydroxyquinoline (8HQ) and GSH [102] (Fig. 11). The molecule joins the metal chelating function of 8HQ, which is the most common nucleus used for the development of metal chelating multi-target anti-AD drugs, and the antioxidant effect of GSH. GS(HQ)H displays a good cellular neuroprotective effect and antioxidant properties against H_2O_2 insult and against 8HQ-induced OS. The new molecule exhibits affinity for Cu(II) and Zn(II) comparable to that of 8HQ. Moreover, GS(HQ)H seems to be able to reach the BBB, where it displays a good capability in removing Cu(II) and Zn(II) from the Aβ-peptide *in vivo*, without causing any depletion of these metal ions [102].

Besides, 8HQ scaffold was also fused with resveratrol molecular scaffold to obtain hybrids endowed with Aβ interacting, anti-OS and chelating activities [103]. The resulting set of 8HQ-resveratrol hybrid molecules exhibited excellent MTDL properties such as the inhibition of self-induced and copper(II)-induced Aβ aggregation, as well as potential antioxidant and biometals chelation properties. Within the series, derivative (*E*)-5-(4-hydroxystyryl)quinoline-8-ol **5** (Fig. 11) possessed the best profile: **5** was able to disassemble the well-structured Aβ fibrils generated by self- and Cu(II)-induced Aβ aggregation. Compound **5** revealed also to exert a control on Cu(I/II)-triggered hydroxyl radical production by halting Cu redox cycling via metal complexation. Furthermore, **5** also demonstrated a potent oxygen radical absorbance capacity. Notably, **5** showed to pass the BBB and did not show acute toxicity *in vivo* at doses of up to 2000 mg kg^{-1}[103].

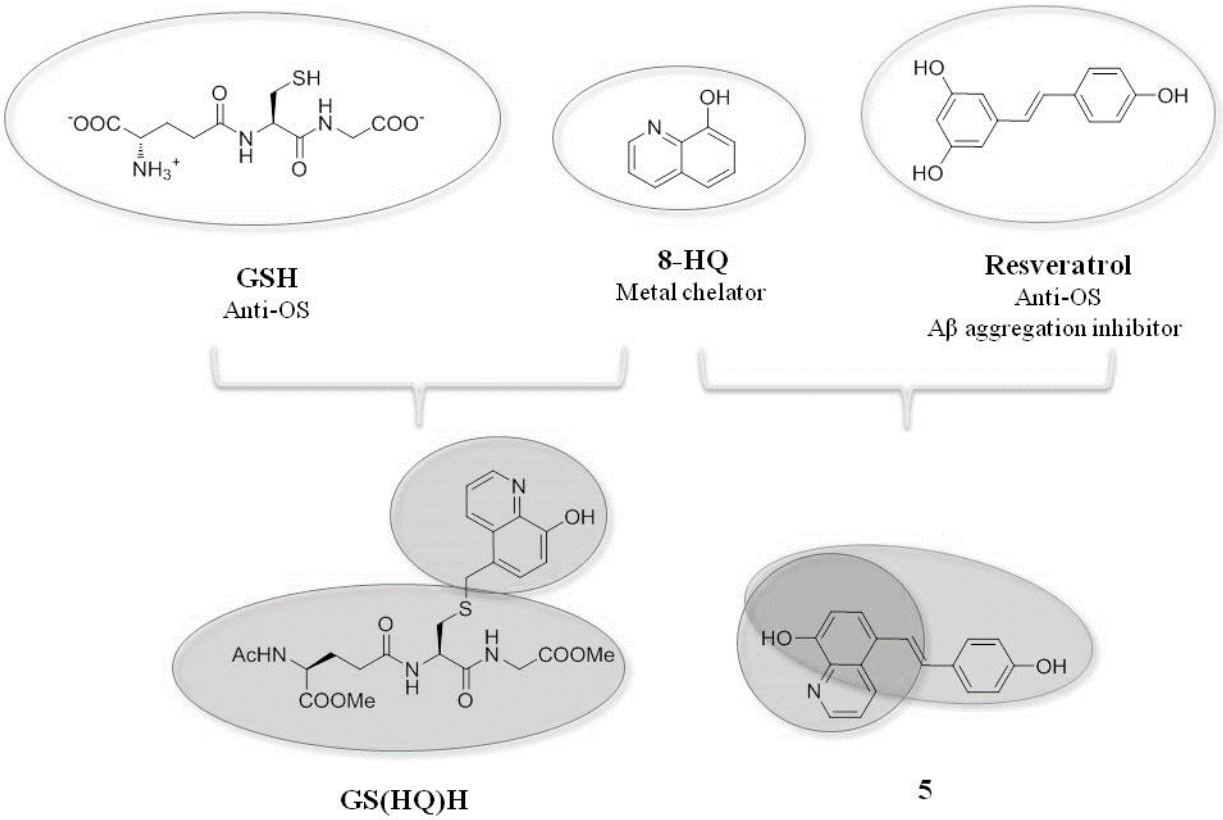


Figure11. Structures of hybrids obtained by the conjunction between GSH and 8-HQ (**GS(HQ)H**) and 8-HQ and Resveratrol (Compound **5**).

7. Inflammation and OS

Considerable evidence gained over the past decade has supported the conclusion that both a prominent activation of inflammatory processes and the innate immune response are associated with AD pathology [104, 105]. The major players involved in the inflammatory process are microglia, astrocytes, macrophages and lymphocytes; all of them have many critical roles in the release of inflammatory mediators such as cytokines, chemokines, neurotransmitters and ROS. The release of mediators leads to recruitment of monocytes and lymphocytes through the BBB as well as activation of additional microglia, inducing further release of other inflammatory factors. In addition, both the microglia and astrocytes have been shown to generate Aβ that seems to play a key role in driving microglia activation and the neuroinflammatory response[106].

Glial cells, which constitute a source and a target for free radical species, as well as contribute to neuroinflammatory processes, promote and aggravate the oxidative damage and hamper neuronal viability[107]. Since oxidative stress and neuroinflammation are strongly correlated, it is not surprising to know that, among the strategies being followed to design new potential anti-AD drugs, the combination of traditional anti-inflammatory and antioxidant molecules is also pursued.

Non-steroidal anti-inflammatory drugs (NSAIDs) including the over-the-counter (OTC) drugs ibuprofen and naproxen, shown to reduce Alzheimer's disease risk [108]. Moreover, NSAIDs proved (i) to alter the conformation of Aβ peptides, thus exerting an anti-aggregating activity, and (ii) to induce the expression of amyloid-binding proteins[109]; despite countless benefits, chronic use of NSAIDs is limited by their gastric toxicity.

On this basis, more recently, Cacciatore et al.[110]explored a series of novel multitarget-NSAIDs derivatives obtained by the conjunction of (S)-naproxen or (R)-flurbiprofen with (R)-lipoic acid (LA), through alkylene diamine linkers. This combination was designed to increase the brain permeability and to reduce the gastric toxicity commonly associated to the use of traditional anti-inflammatory drugs. Among the new synthesized compounds, containing the linker between amino ethylene (R)-flurbiprofen and LA, AL7 (Fig. 12), demonstrated to possess the most favorable chemical stability and thus it was selected for further investigations on two different cell lines, THP-1 (leukemic monocytes) and U937 (lymphoblast lung from human), to evaluate the induced attenuation of the damage; results showed a marked increase of cellular vitality after 48h in U937 cells at the concentrations of 0.1 and 1μM. ROS production was measured using 2`,7`-dichlorofluorescein (DCF), as a fluorescent probe. After pretreatment with the new compound, the ROS production induced by phorbol 12-miristate 13-acetate (PMA), in THP-1 cells (leukemic monocytes), was significantly reduced after 24h (0.1 μM) and after 48h (1 μM) (44.82% and 35.50%, respectively). Moreover, the new molecule also reduced the Aβ -induced ROS production at a dose of 1μM in both cell lines. Finally, the modulation of the expression of COX-2, IL-1 and TNF-α were observed, thus indicating that AL7 is also endowed of anti-inflammatory properties. These results reveal that AL7 could represent a potential lead-multifunctional-NSAID worthy of further evaluation in suitable models of AD.

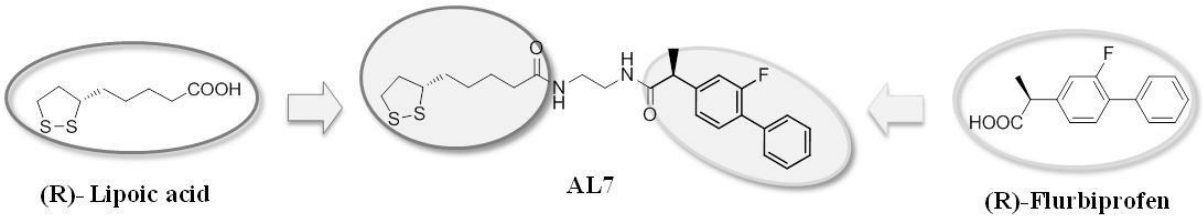


Figure 12. Design strategy of **AL7.**

It is widely recognized that NSAIDs can regulate gene expression via the interaction with a class of nuclear receptor superfamily members, namely Peroxisome Proliferator-Activated Receptors (PPARs) [111]. Three different PPAR genes (PPARα, PPARβ also called δ, and PPARγ) have been identified, each one with splicing variants. PPARγ, mainly expressed in microglia and astrocytes, has been shown to play essential roles in energetic metabolism, adipocyte differentiation, insulin sensitization and tumor suppression. The PPAR-γ activation induces a decrease in neuronal death preventing both oxidative and inflammatory processes implicated in cerebral injury. To date, extensive and compelling studies indicate that PPAR-γ ligands inhibit the production of proinflammatory cytokines, including IL-1β, TNF-α and IL-6, in human monocytes and arrest the differentiation of monocytes into activated macrophages[112-114].

A collected body of evidence shows that PPARγ activation can lead to suppression of cellular APP levels. Recently, Wang et al identified astragaloside IV (AS-IV) as a natural PPARγ agonist. The authors proved that the treatment of cultured SH-SY5Y cells with this selective PPARγ ligand increases the activity of PPARγ and inhibits BACE1 resulting in a significant decrease of Aβ level [115]. Globally, the multi-functional effects induced by PPARγ activation prove to positively influence the pathology of Alzheimer's disease [116]. Their ability to ameliorate the inflammatory status of the AD brain (by decreasing the production of proinflammatory molecules), and to reduce the amyloidogenic Aβ-production, underlines the beneficial effects of PPARγ agonists for future AD therapy.

Nowadays, PPARγ agonists are extensively investigated as therapeutic agents. The benefits in AD are addressed to their ability to regulate the transcription of a wide variety of oxidative, inflammatory, fibrotic, and neuronal survival genes,

although also transcription-independent effects may be involved [117]. Indeed, it has been reported that PPARγ agonists promote the amyloid clearance by affecting the overexpression of Apolipoprotein E (ApoE), a lipoprotein, mainly found in liver and brain and able to enhance both the degradation and phagocytosis of Aβ in the microglia and astrocytes [118].

Thiazolidinediones (TZDs) (Fig. 13) belong to the class of PPARγ agonists exhibit several pharmacological activities that prove to be beneficial for the treatment of neurodegenerative diseases. Besides the inhibition of neuroinflammation, TZDs demonstrate to enhance synaptic plasticity, ameliorate mitochondrial function and reduce tau hyperphosphorylation [119]. TZDs were introduced in the late 1990s as a class of oral antidiabetic drugs for the treatment of type 2 diabetes. Within this class, pioglitazone (Actos, Takeda Pharmaceuticals North America, Inc.) and rosiglitazone (Avandia, GlaxoSmithKline), are now in clinical trials for the treatment of AD. Rosiglitazone and pioglitazone, proved to relieve neurodegeneration and reduce cognitive decline associated with AD [120, 121]. In particular pioglitazone is undergoing Phase III of clinical trials (ClinicalTrials.gov Identifier:NCT02284906) for the treatment of mild cognitive impairment due to Alzheimer's Disease [122] whereas a phase III study for rosiglitazone has been completed in 2009, but the results haven't been published yet (ClinicalTrials.gov Identifier:NCT00348140)[123].

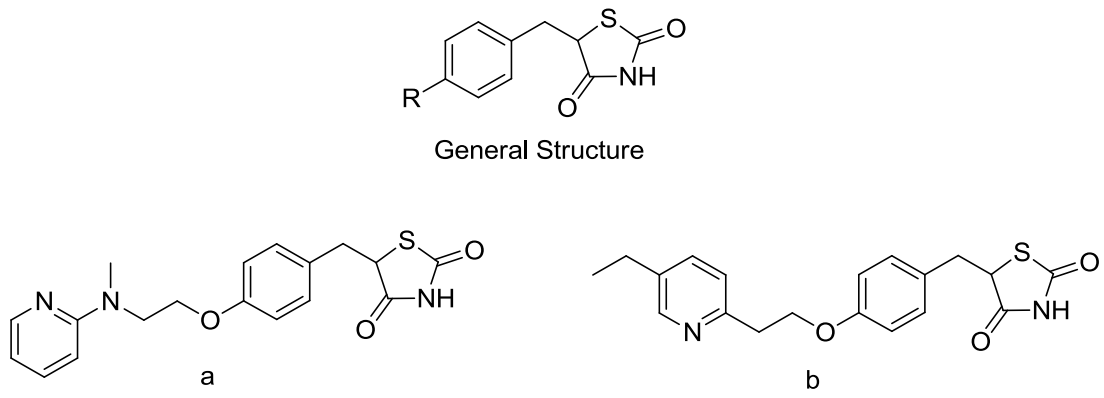


Figure 13. Structure of thiazolidinediones:Rosiglitazone (a) and Pioglitazone (b).

8. Nature-based ligands as a source to design multitarget drugs

Nature has been always considered an inexhaustible source of bioactive substances with beneficial and/or healing properties. Several important drugs that have revolutionized treatment of serious diseases were born from natural products and/or from their pharmacophores. Many recent reviews have provided important insights on this current issue [124-126]. In particular, Newman and Cragg have recently published an interesting review in which a detailed analysis of the key role played by natural products in the drug discovery process has been highlighted [127].

Since natural products often already possess established pharmacological activities, including off-targets effects, the concept to use Nature as primary resource to find "lead-scaffolds" for the synthesis of new drugs is strongly pursued. Therefore, the identification of "nature-based scaffolds" suitable for the development of new multitarget drugs, is widely investigated as a possible starting point to design new treatments for several diseases, including AD. Herein, we briefly discuss the main phytochemicals and the corresponding pharmacological effects assessed in *in vitro* or *in vivo* AD-models. (Table 1)

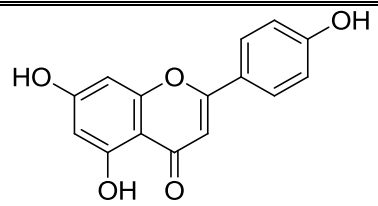
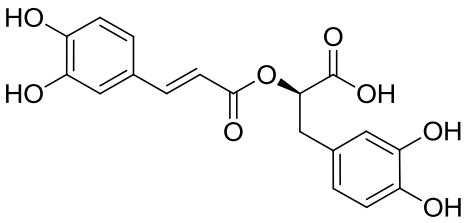
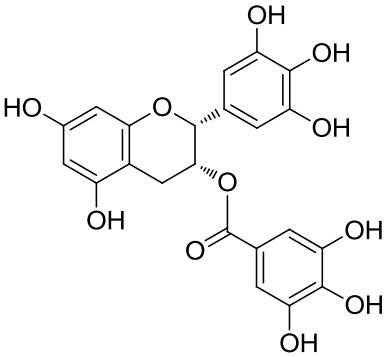
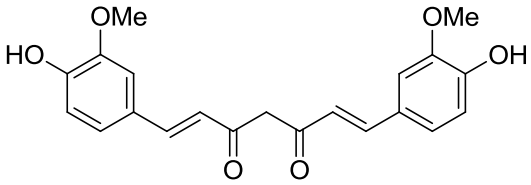
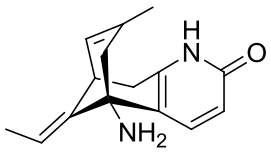
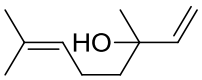
Structure	Phytochemicals	Effects
	Apigenin	Block of Aβ aggregation
	Rosmarinic acid	Protection of PC12 cells from Aβ-induced neurotoxicity
	(−)-Epigallocatechin-3-gallate	Metal chelator Anti-inflammatory
	Curcumin	Inhibition of Aβ insult
	Huperzine A	NMDA receptor modulator Neuroprotective
	Linalool	Inhibition of acetylcholinesterase *in vitro*

Table **1**. Effects of natural products on AD.

Among the well-known natural compounds widely studied in several diseases, the flavones are largely known to bind to the benzodiazepine site on the GABA-A receptor resulting in sedation, anxiolytic or anti-convulsive effects[128]. Among them, Apigenin (4',5,7-trihydroxyflavone) is emerging as a potential lead scaffold for the development of anti - AD drugs. This polyphenol has been found in high amounts in the ligulate flowers of the chamomile plant and it resulted to be scarcely toxic and non-mutagenic and exhibited well-established anti-inflammatory, antioxidant, and

anticarcinogenic activities. A great body of evidence shows that it strongly decreases the levels of IL-6 and TNF-α in activated in murine microglia Interferon gamma (IFN-γ) through its effect on phosphorylation of STAT1[129]. Anti-inflammatory effect of Apigenin seems to occur via the reduction of the expression of iNOS and COX-2 in both microglial and macrophage mouse cells[130]. Besides the protection against neuroinflammation, Apigenin promotes an increase of GSH levels and reveals to be a good free radical scavenger. Both properties cooperate to the significant neuroprotective effect of this molecule, against the oxidative stress. Moreover, another study performed by Zhao et al. proved that the neuroprotective effect of Apigenin could be also explained by the significant decreasing of amyloid deposition via a down-regulation of BACE1[131]. Latterly, Hang et al. have shown that the antioxidant properties of Apigenin were dependent on activation of Nrf2 signaling [132]. The intrinsic pharmacological properties of Apigenin allow us to consider this molecule a suitable and promising natural scaffold for the development of new MTDL for AD therapy.

Another important phytochemical which proved to protect neuronal cells from Aβ-induced neurotoxicity is the Rosmarinic acid (RA)[133]. RA, the major constituent of sage, is an ester of caffeic acid (CA) and 3,4-dihydroxyphenyllactic acid and it is one of the most important and well known natural antioxidant compounds, which exhibits neuroprotective effects in different models of neuroinflammation and neurodegeneration[134, 135]. The compelling results obtained by several studies performed in *in vitro* models of AD for both sage and RA, prompt researchers to further investigate this spice for the treatment of AD and eventually to explore the possible use of RA as a therapeutic approach in AD.

The polyphenolic compound (−)-epigallocatechin-3-gallate (EGCG) is the major catechin found in green tea; the pharmacological effects induced by this phytochemical have the potential to modify several human pathologies, including neurodegenerative ones. EGCG was demonstrated to be a powerful hydrogen-donating antioxidant, but also a free radical scavenger of ROS and RNS *in vitro*. The antioxidant effects of EGCG are addressed to the presence of phenolic moieties that are sensitive to oxidation, generating quinone species [136]. It has also been demonstrated that EGCG exerts an antioxidant effect by activating the Nrf2 signaling pathway, which initiates the transcription of a series of cytoprotective genes [137]. EGCG demonstrated also a significant efficacy in restoring β-amyloid-induced behavioral imbalance in rats, as well as the memory and coordination abilities. Treatments with the polyphenol are able to restore altered mitochondrial membrane potential, mitochondrial respiratory rates and ROS production or ATP levels [138]. Moreover, it should be noticed that also the decline induced by EGCG in MAO activity could provide protection against oxidative neurodegeneration.

As previously discussed, the pivotal role of iron in neurodegeneration was already established and recent studies explored the EGCG activity in chelating the Fe^{2+}ion, demonstrating the neuro-restorative activity and Fe^{2+}-chelating properties[139] of the tea polyphenol. In hippocampal neurons, the examined natural compound exerted a protective effect against ischemic insult, but it also decreased the $A\beta_{1-42}$-induced neurotoxicity of EGCG [140]. Then, EGCG has proved to possess such a wide range of biological effects (neuropathological, neurochemical, and cognitive benefits) to be considered a promising compound as well as a safe and effective therapeutic agent against AD. Nowadays, EGCG has completed a phase II/III clinical trial aimed at evaluating the efficacy and safety in the Early Stage of Alzheimer´s Disease (ClinicalTrials.gov Identifier: NCT00951834)[141].

Curcumin (1,7-bis [4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) or diarylheptanoid is a bioactive polyphenol found in the rhizomes of *Curcuma longa L.* Curcumin like other antioxidants, it protects brain cells and their mitochondria against amyloid beta-induced toxicity and also inhibiting the formation of abnormal proteins [142]. This natural compound also possesses an inhibitory effect against the expression of enzymes such as COX-2 and inducible nitric oxide synthase (iNOS), thus causing a marked reduction in the levels of prostaglandin E2 (PGE2) and NO. A very recent experimental study showed that the curcumin attenuates oxidative stress by modulating Nrf2-Keap1 pathway and then leading to increases in the expression and activity of HO-1[143]. In addition to that, curcumin is a potent inducer of HO-1 in vascular endothelial cells. The pre-treatment with tin protophorphyrin IX, an inhibitor of HO-1 activity, proved to completely abolish the curcumin-mediated cytoprotection against oxidative stress [144]. Moreover, it reduces levels of the astrocyte marker glial fibrillary acidic protein (GFAP) and prevents $A\beta_{42}$ oligomers formation and toxicity.

Several studies in AD animal models have evaluated the effects of these multiple mechanisms on learning and memory. Curcumin supplements administration, even after the occurrence of Alzheimer's-like symptoms, results in less mistakes on memory-dependent tasks, and improved performance on mazes. Both results show positive effects on functions of both reasoning and memory [145]. Today, Phase II clinical trials studies are ongoing in order to define the efficacy and the safety of high-bioavailability curcumin formulation (Longvida) in AD patients.(ClinicalTrials.gov Identifier: NCT01001637)[146].

Huperzine A (HupA) is a sesquiterpene alkaloid found in the *Huperzia Serrata*, a plant widely used in the traditional Chinese medicine for centuries [147]. HupA acts as a potent, highly specific and reversible inhibitor of acetylcholinesterase (AChEI). It crosses the blood–brain barrier easily. Ever since its isolation in 1986, Huperzine A, progressed as the most-promising and widely-studied reversible AChE inhibitor and it showed to be efficient drug candidate with potential anticholinesterase effects. Indeed, it has been licensed as anti-AD drug in China. The AChE inhibitory activity, pharmacology and the detailed properties of Huperzine A let to consider such a molecule as a promising agent for treating dementia (including AD)[148, 149]. Several clinical trials suggested that HupA exerts positive beneficial effects in AD patients without any severe adverse effects [150, 151]. Good pharmacokinetic profile with rapid absorption, wide distribution in body, good BBB value and low to moderate rate of elimination make the Huperzine A more attractive than other drugs [151]. HupA also shows a high bioavailability after oral administration, ability to penetrate into the CNS, long duration of action and minimal side effects. Administration of HupA significantly prevents the decrease caused by Aβ in mitochondrial active respiration and ATP synthesis. Moreover, evidence highlight that huperzine is capable to reverse or attenuate cognitive deficits in several animal models. In order to assess the safety and efficacy of the HupA in the treatment of AD, a phase II clinical trial has been performed and completed in 2008. No results have been published yet (ClinicalTrials.gov Identifier: NCT00083590)[152].

Linalool, a natural compound of coriander, has been reported to have anti-inflammatory effects mediated by TNF-α, IL-1β, NO and PGE2. The coriander possesses strong antioxidant properties related to its ability to decrease the stimulation of lipid peroxidation and protein oxidation. In *in vitro* tests, Linalool showed to inhibit with a good efficacy AChE[153]. Moreover, Zheng et al. reported that linalool exerts anti-inflammatory effects through the induction of Nrf2 nuclear translocation and the expression of HO-1.[154] An *in vivo* study performed on aged mice and transgenic model of AD (3xTg-AD) showed that a chronic treatment with linalool induced a significant reduction of Aβ-deposition, and in the levels of the pro-inflammatory markers p38 MAPK, NOS2, COX2 and IL-1β [155, 156]. All these data suggest

that linalool is able to reverse the histopathological hallmarks of AD and to restore cognitive functions via an anti-inflammatory effect.

9. Conclusion

In the last years, the development of ligands endowed of a polypharmacological profile is a strategy highly pursued in medicinal chemistry field. Several pathologies characterized by an intricate etiology such as cancer, cardiovascular and neurodegenerative diseases have witnessed the failure of numerous clinical trials. These failures are probably due to the lack of efficacy of mono-targeted therapy and this is the first reason why the attention of medicinal chemists has been directed toward the multitarget strategy. As concerns neurodegenerative diseases, and in particular AD, several phase III trials failed to reach the clinical endpoints probably because of the complexity, not yet completely clarified, of the pathology itself. Therefore, the design of innovative therapies is going rapidly toward the searching for new molecules able to regulate the equilibrium between oxidative stress, and the hallmarks of AD such as Aβ-aggregation, NFT-tangles, metal disomeostasis and neuroinflammation. To date, significant progresses in this field have been done, even if the complexity of this pathology still represents the main drawback to find successful multitarget therapies to be further investigated in clinical trials. Nutraceuticals play an increasingly important role in the treatment of various pathological states and consequently recent trends highlight that bioactive molecules found in functional foods and supplements play a key therapeutic role in several diseases. Since Nature is an almost endless source of bioactive compounds, it could be interesting to use it also as primary resource of bioactive moieties or "Lead-Nature-based-Scaffolds" (LNSs) for the synthesis of new multitarget neuroprotective drugs. Future directions in the comprehension of the existing links between OS and the hallmarks of AD as well as their correlation with neurodegenerative events, will be a smart challenge to fine-tune the multitarget strategy for the development of new effective therapeutics for AD.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

REFERENCES

Primary Sources

Secondary Sources

Uncategorized References

[1] Sies, H., Oxidative stress: introductory remarks. *Oxidative stress,* **1985**, 1-8.

[2] Shulman, R.G.; Rothman, D.L.; Behar, K.L.; Hyder, F., Energetic basis of brain activity: implications for neuroimaging. *Trends in neurosciences,* **2004**, *27*, (8), 489-495.

[3] Huang, W.J.; Zhang, X.; Chen, W.W., Role of oxidative stress in Alzheimer's disease (Review). *Biomedical reports,* **2016**, *4*, (5), 519-522.

[4] Hardy, J.; Allsop, D., Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in pharmacological sciences,* **1991**, *12*, 383-388.
[5] Kang, J.; Lemaire, H.-G.; Unterbeck, A.; Salbaum, J.M.; Masters, C.L.; Grzeschik, K.-H.; Multhaup, G.; Beyreuther, K.; Müller-Hill, B., The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. **1987**.
[6] Tanzi, R.E.; McClatchey, A.I.; Lamperti, E.D.; Villa-Komaroff, L.; Gusella, J.F.; Neve, R.L., Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. **1988**.
[7] Billnitzer, A.J.; Barskaya, I.; Yin, C.; Perez, R.G., APP independent and dependent effects on neurite outgrowth are modulated by the receptor associated protein (RAP). *Journal of neurochemistry,* **2013**, *124*, (1), 123-132.
[8] Tamaoka, A., [Characterization of amyloid beta protein species in the plasma, cerebrospinal fluid and brains of patients with Alzheimer's disease]. *Nihon Ronen Igakkai zasshi. Japanese journal of geriatrics,* **1998**, *35*, (4), 273-277.
[9] Glabe, C.C. In *Alzheimer's Disease*; Springer, **2005**, pp 167-177.
[10] Butterfield, D.A.; Kanski, J., Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid β-peptide 1–42. *Peptides,* **2002**, *23*, (7), 1299-1309.
[11] Butterfield, D.A.; Sultana, R., Methionine-35 of aβ (1–42): importance for oxidative stress in Alzheimer disease. *Journal of amino acids,* **2011**, *2011*.
[12] Kanski, J.; Aksenova, M.; Schöneich, C.; Butterfield, D.A., Substitution of isoleucine-31 by helical-breaking proline abolishes oxidative stress and neurotoxic properties of Alzheimer's amyloid β-peptide (1–42). *Free Radical Biology and Medicine,* **2002**, *32*, (11), 1205-1211.
[13] Kanski, J.; Aksenova, M.; Butterfield, D.A., The hydrophobic environment of Met35 of Alzheimer's Aβ (1-42) is important for the neurotoxic and oxidative properties of the peptide. *Neurotoxicity research,* **2002**, *4*, (3), 219-223.
[14] Butterfield, D.A.; Galvan, V.; Lange, M.B.; Tang, H.; Sowell, R.A.; Spilman, P.; Fombonne, J.; Gorostiza, O.; Zhang, J.; Sultana, R., In vivo oxidative stress in brain of Alzheimer disease transgenic mice: requirement for methionine 35 in amyloid β-peptide of APP. *Free Radical Biology and Medicine,* **2010**, *48*, (1), 136-144.
[15] Nunomura, A.; Perry, G.; Aliev, G.; Hirai, K.; Takeda, A.; Balraj, E.K.; Jones, P.K.; Ghanbari, H.; Wataya, T.; Shimohama, S., Oxidative damage is the earliest event in Alzheimer disease. *Journal of Neuropathology & Experimental Neurology,* **2001**, *60*, (8), 759-767.
[16] Tong, Y.; Zhou, W.; Fung, V.; Christensen, M.; Qing, H.; Sun, X.; Song, W., Oxidative stress potentiates BACE1 gene expression and Aβ generation. *Journal of neural transmission,* **2005**, *112*, (3), 455-469.
[17] Sinha, M.; Bhowmick, P.; Banerjee, A.; Chakrabarti, S., Antioxidant role of amyloid β protein in cell-free and biological systems: implication for the pathogenesis of Alzheimerdisease. *Free Radical Biology and Medicine,* **2013**, *56*, 184-192.
[18] Panza, F.; Solfrizzi, V.; Frisardi, V.; Imbimbo, B.P.; Capurso, C.; D'Introno, A.; Colacicco, A.M.; Seripa, D.; Vendemiale, G.; Capurso, A., Beyond the neurotransmitter-focused approach in treating Alzheimer's disease: drugs targeting β-amyloid and tau protein. *Aging clinical and experimental research,* **2009**, *21*, (6), 386-406.
[19] Sabbagh, M.N., Drug development for Alzheimer's disease: Where are we now and where are we headed? *The American Journal of Geriatric Pharmacotherapy,* **2009**, *7*, (3), 167-185.
[20] Lenhart, J.A.; Ling, X.; Gandhi, R.; Guo, T.L.; Gerk, P.M.; Brunzell, D.H.; Zhang, S., "Clicked" Bivalent Ligands Containing Curcumin and Cholesterol As Multifunctional Aβ Oligomerization Inhibitors: Design, Synthesis, and Biological Characterization. *Journal of medicinal chemistry,* **2010**, *53*, (16), 6198-6209.
[21] Cordy, J.M.; Cordy, J.M.; Hooper, N.M.; Turner, A.J., The involvement of lipid rafts in Alzheimer's disease (Review). *Molecular membrane biology,* **2006**, *23*, (1), 111-122.
[22] Kim, S.I.; Yi, J.S.; Ko, Y.G., Amyloid β oligomerization is induced by brain lipid rafts. *Journal of cellular biochemistry,* **2006**, *99*, (3), 878-889.

[23] Kim, J.; Lee, H.J.; Lee, K.W., Naturally occurring phytochemicals for the prevention of Alzheimer's disease. *Journal of neurochemistry,* **2010**, *112*, (6), 1415-1430.
[24] Flaherty, D.P.; Kiyota, T.; Dong, Y.; Ikezu, T.; Vennerstrom, J.L., Phenolic bis-styrylbenzenes as β-amyloid binding ligands and free radical scavengers. *Journal of medicinal chemistry,* **2010**, *53*, (22), 7992-7999.
[25] Flaherty, D.P.; Walsh, S.M.; Kiyota, T.; Dong, Y.; Ikezu, T.; Vennerstrom, J.L., Polyfluorinated bis-styrylbenzene β-amyloid plaque binding ligands. *Journal of medicinal chemistry,* **2007**, *50*, (20), 4986-4992.
[26] Frémont, L., Biological effects of resveratrol. *Life sciences,* **2000**, *66*, (8), 663-673.
[27] Ourique, G.M.; Pês, T.S.; Saccol, E.M.H.; Finamor, I.A.; Glanzner, W.G.; Baldisserotto, B.; Pavanato, M.A.; Gonçalves, P.B.D.; Barreto, K.P., Resveratrol prevents oxidative damage and loss of sperm motility induced by long-term treatment with valproic acid in Wistar rats. *Experimental and Toxicologic Pathology*.
[28] Regitz, C.; Fitzenberger, E.; Mahn, F.L.; Dußling, L.M.; Wenzel, U., Resveratrol reduces amyloid-beta (Aβ1–42)-induced paralysis through targeting proteostasis in an Alzheimer model of Caenorhabditis elegans. *European journal of nutrition,* **2016**, *55*, (2), 741-747.
[29] Cavalli, A.; Bolognesi, M.L.; Capsoni, S.; Andrisano, V.; Bartolini, M.; Margotti, E.; Cattaneo, A.; Recanatini, M.; Melchiorre, C., A small molecule targeting the multifactorial nature of Alzheimer's disease. *Angewandte Chemie International Edition,* **2007**, *46*, (20), 3689-3692.
[30] Bolognesi, M.L.; Cavalli, A.; Melchiorre, C., Memoquin: a multi-target–directed ligand as an innovative therapeutic opportunity for Alzheimer's disease. *Neurotherapeutics,* **2009**, *6*, (1), 152-162.
[31] Capurro, V.; Busquet, P.; Lopes, J.P.; Bertorelli, R.; Tarozzo, G.; Bolognesi, M.L.; Piomelli, D.; Reggiani, A.; Cavalli, A., Pharmacological characterization of memoquin, a multi-target compound for the treatment of Alzheimer's disease. *PLoS One,* **2013**, *8*, (2), e56870.
[32] Bolognesi, M.L.; Bartolini, M.; Tarozzi, A.; Morroni, F.; Lizzi, F.; Milelli, A.; Minarini, A.; Rosini, M.; Hrelia, P.; Andrisano, V., Multitargeted drugs discovery: balancing anti-amyloid and anticholinesterase capacity in a single chemical entity. *Bioorganic & medicinal chemistry letters,* **2011**, *21*, (9), 2655-2658.
[33] Zlokovic, B.V., Neurodegeneration and the neurovascular unit. *Nature medicine,* **2010**, *16*, (12), 1370-1371.
[34] Deane, R.; Du Yan, S.; Submamaryan, R.K.; LaRue, B.; Jovanovic, S.; Hogg, E.; Welch, D.; Manness, L.; Lin, C.; Yu, J., RAGE mediates amyloid-β peptide transport across the blood-brain barrier and accumulation in brain. *Nature medicine,* **2003**, *9*, (7), 907-913.
[35] Zlokovic, B.V., Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nature reviews. Neuroscience,* **2011**, *12*, (12), 723-738.
[36] Yan, S.D.; Chen, X.; Fu, J.; Chen, M.; Zhu, H.; Roher, A.; Slattery, T.; Zhao, L.; Nagashima, M.; Morser, J.; Migheli, A.; Nawroth, P.; Stern, D.; Schmidt, A.M., RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature,* **1996**, *382*, (6593), 685-691.
[37] Burstein, A.H.; Grimes, I.; Galasko, D.R.; Aisen, P.S.; Sabbagh, M.; Mjalli, A.M., Effect of TTP488 in patients with mild to moderate Alzheimer's disease. *BMC neurology,* **2014**, *14*, (1), 1.
[38] Mjalli, A.; Andrews, R.; Shen, J.; Rothlein, R.; Google Patents, **2005**.
[39] Galasko, D.; Bell, J.; Mancuso, J.Y.; Kupiec, J.W.; Sabbagh, M.N.; van Dyck, C.; Thomas, R.G.; Aisen, P.S., Clinical trial of an inhibitor of RAGE-Abeta interactions in Alzheimer disease. *Neurology,* **2014**, *82*, (17), 1536-1542.
[40] Liu, Q.; Lee, H.-g.; Honda, K.; Siedlak, S.L.; Harris, P.L.; Cash, A.D.; Zhu, X.; Avila, J.; Nunomura, A.; Takeda, A., Tau modifiers as therapeutic targets for Alzheimer's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* **2005**, *1739*, (2), 211-215.
[41] Rojo, A.I.; Sagarra, M.R.d.; Cuadrado, A., GSK-3β down-regulates the transcription factor Nrf2 after oxidant damage: relevance to exposure of neuronal cells to oxidative stress. *Journal of neurochemistry,* **2008**, *105*, (1), 192-202.
[42] Perluigi, M.; Barone, E.; Di Domenico, F.; Butterfield, D., Aberrant protein phosphorylation in Alzheimer disease brain disturbs pro-survival and cell death pathways. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* **2016**, *1862*, (10), 1871-1882.

[43] Lee, H.-g.; Perry, G.; Moreira, P.I.; Garrett, M.R.; Liu, Q.; Zhu, X.; Takeda, A.; Nunomura, A.; Smith, M.A., Tau phosphorylation in Alzheimer's disease: pathogen or protector? *Trends in molecular medicine,* **2005**, *11*, (4), 164-169.
[44] Wataya, T.; Nunomura, A.; Smith, M.A.; Siedlak, S.L.; Harris, P.L.; Shimohama, S.; Szweda, L.I.; Kaminski, M.A.; Avila, J.; Price, D.L., High molecular weight neurofilament proteins are physiological substrates of adduction by the lipid peroxidation product hydroxynonenal. *Journal of Biological Chemistry,* **2002**, *277*, (7), 4644-4648.
[45] Liu, Z.; Li, T.; Li, P.; Wei, N.; Zhao, Z.; Liang, H.; Ji, X.; Chen, W.; Xue, M.; Wei, J., The ambiguous relationship of oxidative stress, tau hyperphosphorylation, and autophagy dysfunction in Alzheimer's disease. *Oxidative medicine and cellular longevity,* **2015**, *2015*.
[46] Crespo-Biel, N.; Theunis, C.; Van Leuven, F., Protein tau: prime cause of synaptic and neuronal degeneration in Alzheimer's disease. *International Journal of Alzheimer's Disease,* **2012**, *2012*.
[47] Cross, D.A.; Culbert, A.A.; Chalmers, K.A.; Facci, L.; Skaper, S.D.; Reith, A.D., Selective small-molecule inhibitors of glycogen synthase kinase-3 activity protect primary neurones from death. *Journal of neurochemistry,* **2001**, *77*, (1), 94-102.
[48] Pizarro, J.G.; Yeste-Velasco, M.; Rimbau, V.; Casadesús, G.; Smith, M.A.; Pallàs, M.; Folch, J.; Camins, A., Neuroprotective effects of SB-415286 on hydrogen peroxide-induced cell death in B65 rat neuroblastoma cells and neurons. *International Journal of Developmental Neuroscience,* **2008**, *26*, (3), 269-276.
[49] Brandt, R.; Hundelt, M.; Shahani, N., Tau alteration and neuronal degeneration in tauopathies: mechanisms and models. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* **2005**, *1739*, (2), 331-354.
[50] Murphy, M.P.; Smith, R.A., Drug delivery to mitochondria: the key to mitochondrial medicine. *Advanced drug delivery reviews,* **2000**, *41*, (2), 235-250.
[51] Echtay, K.S.; Murphy, M.P.; Smith, R.A.; Talbot, D.A.; Brand, M.D., Superoxide activates mitochondrial uncoupling protein 2 from the matrix side Studies using targeted antioxidants. *Journal of Biological Chemistry,* **2002**, *277*, (49), 47129-47135.
[52] Testai, L.; Rapposelli, S.; Martelli, A.; Breschi, M.; Calderone, V., Mitochondrial potassium channels as pharmacological target for cardioprotective drugs. *Medicinal research reviews,* **2015**, *35*, (3), 520-553.
[53] Bartus, R.T.; Dean, R.r.; Beer, B.; Lippa, A.S., The cholinergic hypothesis of geriatric memory dysfunction. *Science,* **1982**, *217*, (4558), 408-414.
[54] Perry, E.K.; Gibson, P.H.; Blessed, G.; Perry, R.H.; Tomlinson, B.E., Neurotransmitter enzyme abnormalities in senile dementia: Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *Journal of the neurological sciences,* **1977**, *34*, (2), 247-265.
[55] Bartus, R.T., On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Experimental neurology,* **2000**, *163*, (2), 495-529.
[56] Mesulam, M.; Guillozet, A.; Shaw, P.; Quinn, B., Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. *Neurobiology of disease,* **2002**, *9*, (1), 88-93.
[57] Lockridge, O., Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacology & therapeutics,* **2015**, *148*, 34-46.
[58] León, R.; Garcia, A.G.; Marco-Contelles, J., Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease. *Medicinal research reviews,* **2013**, *33*, (1), 139-189.
[59] Zafrilla, P.; Mulero, J.; Xandri, J.; Santo, E.; Caravaca, G.; Morillas, J., Oxidative stress in Alzheimer patients in different stages of the disease. *Current medicinal chemistry,* **2006**, *13*, (9), 1075-1083.
[60] Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M.L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C., Rational approach to discover multipotent anti-Alzheimer drugs. *Journal of medicinal chemistry,* **2005**, *48*, (2), 360-363.
[61] Bolognesi, M.L.; Matera, R.; Minarini, A.; Rosini, M.; Melchiorre, C., Alzheimer's disease: new approaches to drug discovery. *Current opinion in chemical biology,* **2009**, *13*, (3), 303-308.
[62] Marco-Contelles, J.; León, R.; de los Ríos, C.; Guglietta, A.; Terencio, J.; López, M.G.; García, A.G.; Villarroya, M., Novel multipotent tacrine-dihydropyridine hybrids with improved acetylcholinesterase

inhibitory and neuroprotective activities as potential drugs for the treatment of Alzheimer's disease. *Journal of medicinal chemistry,* **2006**, *49*, (26), 7607-7610.
[63] Estrada, M.; Herrera-Arozamena, C.; Pérez, C.; Viña, D.; Romero, A.; Morales-García, J.A.; Pérez-Castillo, A.; Rodríguez-Franco, M.I., New cinnamic–N-benzylpiperidine and cinnamic–N, N-dibenzyl (N-methyl) amine hybrids as Alzheimer-directed multitarget drugs with antioxidant, cholinergic, neuroprotective and neurogenic properties. *European journal of medicinal chemistry,* **2016**, *121*, 376-386.
[64] Wang, Y.; Wang, H.; Chen, H.-z., AChE Inhibition-based Multi-target-directed Ligands, a Novel Pharmacological Approach for the Symptomatic and Disease-modifying Therapy of Alzheimer's Disease. *Current neuropharmacology,* **2016**, *14*, (4), 364-375.
[65] Biewenga, G.P.; Haenen, G.R.; Bast, A., The pharmacology of the antioxidant lipoic acid. *General Pharmacology: The Vascular System,* **1997**, *29*, (3), 315-331.
[66] Castro, A.; Martinez, A., Targeting beta-amyloid pathogenesis through acetylcholinesterase inhibitors. *Current pharmaceutical design,* **2006**, *12*, (33), 4377-4387.
[67] Pang, Y.-P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S., Highly Potent, Selective, and Low Cost Bis-tetrahydroaminacrine Inhibitors of Acetylcholinesterase Steps toward Novel Drugs for Treating Alzheimer's Disease. *Journal of Biological Chemistry,* **1996**, *271*, (39), 23646-23649.
[68] Kessler, A.; Biasibetti, M.; da Silva Melo, D.A.; Wajner, M.; Dutra-Filho, C.S.; de Souza Wyse, Â.T.; Wannmacher, C.M.D., Antioxidant effect of cysteamine in brain cortex of young rats. *Neurochemical research,* **2008**, *33*, (5), 737-744.
[69] Minarini, A.; Milelli, A.; Tumiatti, V.; Rosini, M.; Simoni, E.; Bolognesi, M.; Andrisano, V.; Bartolini, M.; Motori, E.; Angeloni, C., Cystamine-tacrine dimer: a new multi-target-directed ligand as potential therapeutic agent for Alzheimer's disease treatment. *Neuropharmacology,* **2012**, *62*, (2), 997-1003.
[70] Romero, A.; Cacabelos, R.; Oset-Gasque, M.J.; Samadi, A.; Marco-Contelles, J., Novel tacrine-related drugs as potential candidates for the treatment of Alzheimer's disease. *Bioorganic & medicinal chemistry letters,* **2013**, *23*, (7), 1916-1922.
[71] Pi, R.; Mao, X.; Chao, X.; Cheng, Z.; Liu, M.; Duan, X.; Ye, M.; Chen, X.; Mei, Z.; Liu, P., Tacrine-6-ferulic acid, a novel multifunctional dimer, inhibits amyloid-β-mediated Alzheimer's disease-associated pathogenesis in vitro and in vivo. *PLoS One,* **2012**, *7*, (2), e31921.
[72] Chao, X.; He, X.; Yang, Y.; Zhou, X.; Jin, M.; Liu, S.; Cheng, Z.; Liu, P.; Wang, Y.; Yu, J.; Tan, Y.; Huang, Y.; Qin, J.; Rapposelli, S.; Pi, R., Design, synthesis and pharmacological evaluation of novel tacrine-caffeic acid hybrids as multi-targeted compounds against Alzheimer's disease. *Bioorganic and Medicinal Chemistry Letters,* **2012**, *22*, (20), 6498-6502.
[73] Digiacomo, M.; Chen, Z.; Wang, S.; Lapucci, A.; Macchia, M.; Yang, X.; Chu, J.; Han, Y.; Pi, R.; Rapposelli, S., Synthesis and pharmacological evaluation of multifunctional tacrine derivatives against several disease pathways of AD. *Bioorganic & Medicinal Chemistry Letters,* **2015**, *25*, (4), 807-810.
[74] Keri, R.S.; Quintanova, C.; Chaves, S.; Silva, D.F.; Cardoso, S.M.; Santos, M.A., New Tacrine Hybrids with Natural-Based Cysteine Derivatives as Multitargeted Drugs for Potential Treatment of Alzheimer's Disease. *Chemical biology & drug design,* **2016**, *87*, (1), 101-111.
[75] Quintanova, C.; Keri, R.S.; Chaves, S.; Santos, M.A., Copper (II) complexation of tacrine hybrids with potential anti-neurodegenerative roles. *Journal of inorganic biochemistry,* **2015**, *151*, 58-66.
[76] Darvesh, S.; Hopkins, D.A.; Geula, C., Neurobiology of butyrylcholinesterase. *Nature Reviews Neuroscience,* **2003**, *4*, (2), 131-138.
[77] Benchekroun, M.; Ismaili, L.; Pudlo, M.; Luzet, V.; Gharbi, T.; Refouvelet, B.; Marco-Contelles, J., Donepezil-ferulic acid hybrids as anti-Alzheimer drugs. *Future medicinal chemistry,* **2015**, *7*, (1), 15-21.
[78] Luo, Z.; Sheng, J.; Sun, Y.; Lu, C.; Yan, J.; Liu, A.; Luo, H.-b.; Huang, L.; Li, X., Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. *Journal of medicinal chemistry,* **2013**, *56*, (22), 9089-9099.
[79] Xie, L.; Zheng, W.; Xin, N.; Xie, J.-W.; Wang, T.; Wang, Z.-Y., Ebselen inhibits iron-induced tau phosphorylation by attenuating DMT1 up-regulation and cellular iron uptake. *Neurochemistry international,* **2012**, *61*, (3), 334-340.

[80] Ramsey, C.P.; Glass, C.A.; Montgomery, M.B.; Lindl, K.A.; Ritson, G.P.; Chia, L.A.; Hamilton, R.L.; Chu, C.T.; Jordan-Sciutto, K.L., Expression of Nrf2 in neurodegenerative diseases. *Journal of Neuropathology & Experimental Neurology,* **2007**, *66*, (1), 75-85.
[81] Huang, W.Y.; Chao, X.J.; Ouyang, Y.; Liu, A.M.; He, X.X.; Chen, M.H.; Wang, L.H.; Liu, J.; Yu, S.W.; Rapposelli, S., Tacrine-6-Ferulic Acid, a Novel Multifunctional Dimer Against Alzheimer's Disease, Prevents Oxidative Stress-Induced Neuronal Death Through Activating Nrf2/ARE/HO-1 Pathway in HT22 Cells. *CNS neuroscience & therapeutics,* **2012**, *18*, (11), 950-951.
[82] Jin, W.; Wang, H.; Yan, W.; Xu, L.; Wang, X.; Zhao, X.; Yang, X.; Chen, G.; Ji, Y., Disruption of Nrf2 enhances upregulation of nuclear factor-B activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury. *Mediators of inflammation,* **2009**, *2008*.
[83] Suh, J.H.; Shenvi, S.V.; Dixon, B.M.; Liu, H.; Jaiswal, A.K.; Liu, R.-M.; Hagen, T.M., Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proceedings of the National Academy of Sciences of the United States of America,* **2004**, *101*, (10), 3381-3386.
[84] Du, Y.; Wooten, M.C.; Gearing, M.; Wooten, M.W., Age-associated oxidative damage to the p62 promoter: implications for Alzheimer disease. *Free Radical Biology and Medicine,* **2009**, *46*, (4), 492-501.
[85] Salminen, A.; Kaarniranta, K.; Haapasalo, A.; Hiltunen, M.; Soininen, H.; Alafuzoff, I., Emerging role of p62/sequestosome-1 in the pathogenesis of Alzheimer's disease. *Progress in neurobiology,* **2012**, *96*, (1), 87-95.
[86] Ramesh Babu, J.; Lamar Seibenhener, M.; Peng, J.; Strom, A.L.; Kemppainen, R.; Cox, N.; Zhu, H.; Wooten, M.C.; Diaz-Meco, M.T.; Moscat, J., Genetic inactivation of p62 leads to accumulation of hyperphosphorylated tau and neurodegeneration. *Journal of neurochemistry,* **2008**, *106*, (1), 107-120.
[87] Thimmulappa, R.K.; Scollick, C.; Traore, K.; Yates, M.; Trush, M.A.; Liby, K.T.; Sporn, M.B.; Yamamoto, M.; Kensler, T.W.; Biswal, S., Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-Imidazolide. *Biochemical and biophysical research communications,* **2006**, *351*, (4), 883-889.
[88] Surh, Y.-J.; Kundu, J.K.; Na, H.-K., Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta medica,* **2008**, *74*, (13), 1526-1539.
[89] Smoliga, J.M.; Blanchard, O., Enhancing the delivery of resveratrol in humans: if low bioavailability is the problem, what is the solution? *Molecules,* **2014**, *19*, (11), 17154-17172.
[90] Calani, L.; Ounnas, F.; Salen, P.; Demeilliers, C.; Bresciani, L.; Scazzina, F.; Brighenti, F.; Melegari, C.; Crozier, A.; de Lorgeril, M., Bioavailability and metabolism of hydroxycinnamates in rats fed with durum wheat aleurone fractions. *Food & function,* **2014**, *5*, (8), 1738-1746.
[91] Aqil, F.; Munagala, R.; Jeyabalan, J.; Vadhanam, M.V., Bioavailability of phytochemicals and its enhancement by drug delivery systems. *Cancer letters,* **2013**, *334*, (1), 133-141.
[92] Tarozzi, A.; Angeloni, C.; Malaguti, M.; Morroni, F.; Hrelia, S.; Hrelia, P., Sulforaphane as a potential protective phytochemical against neurodegenerative diseases. *Oxidative medicine and cellular longevity,* **2013**, *2013*.
[93] Kim, H.V.; Kim, H.Y.; Ehrlich, H.Y.; Choi, S.Y.; Kim, D.J.; Kim, Y., Amelioration of Alzheimer's disease by neuroprotective effect of sulforaphane in animal model. *Amyloid,* **2013**, *20*, (1), 7-12.
[94] Egea, J.; Buendia, I.; Parada, E.; Navarro, E.; Rada, P.; Cuadrado, A.; López, M.G.; García, A.G.; León, R., Melatonin–sulforaphane hybrid ITH12674 induces neuroprotection in oxidative stress conditions by a 'drug–prodrug'mechanism of action. *British journal of pharmacology,* **2015**, *172*, (7), 1807-1821.
[95] P Cardinali, D.; M Furio, A.; I Brusco, L., Clinical aspects of melatonin intervention in Alzheimer's disease progression. *Current Neuropharmacology,* **2010**, *8*, (3), 218-227.
[96] Buendia, I.; Navarro, E.; Michalska, P.; Gameiro, I.; Egea, J.; Abril, S.; López, A.; González-Lafuente, L.; López, M.G.; León, R., New melatonin-cinnamate hybrids as multi-target drugs for neurodegenerative diseases: Nrf2-induction, antioxidant effect and neuroprotection. *Future medicinal chemistry,* **2015**, *7*, (15), 1961-1969.
[97] Ayton, S.; Lei, P.; Bush, A.I., Metallostasis in Alzheimer's disease. *Free Radical Biology and Medicine,* **2013**, *62*, 76-89.

[98] Duce, J.A.; Tsatsanis, A.; Cater, M.A.; James, S.A.; Robb, E.; Wikhe, K.; Leong, S.L.; Perez, K.; Johanssen, T.; Greenough, M.A., Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell,* **2010**, *142*, (6), 857-867.
[99] Andrási, E.; Farkas, É.; Scheibler, H.; Réffy, A.; Bezúr, L., Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. *Archives of gerontology and geriatrics,* **1995**, *21*, (1), 89-97.
[100] Cristóvão, J.S.; Santos, R.; Gomes, C.M., Metals and Neuronal Metal Binding Proteins Implicated in Alzheimer's Disease. *Oxidative medicine and cellular longevity,* **2016**, *2016*.
[101] Sun, X.Y.; Wei, Y.P.; Xiong, Y.; Wang, X.C.; Xie, A.J.; Wang, X.L.; Yang, Y.; Wang, Q.; Lu, Y.M.; Liu, R.; Wang, J.Z., Synaptic released zinc promotes tau hyperphosphorylation by inhibition of protein phosphatase 2A (PP2A). *J Biol Chem,* **2012**, *287*, (14), 11174-11182.
[102] Cacciatore, I.; Cornacchia, C.; Fornasari, E.; Baldassarre, L.; Pinnen, F.; Sozio, P.; Di Stefano, A.; Marinelli, L.; Dean, A.; Fulle, S., A glutathione derivative with chelating and in vitro neuroprotective activities: synthesis, physicochemical properties, and biological evaluation. *ChemMedChem,* **2013**, *8*, (11), 1818-1829.
[103] Mao, F.; Yan, J.; Li, J.; Jia, X.; Miao, H.; Sun, Y.; Huang, L.; Li, X., New multi-target-directed small molecules against Alzheimer's disease: a combination of resveratrol and clioquinol. *Organic & biomolecular chemistry,* **2014**, *12*, (31), 5936-5944.
[104] Glass, C.K.; Saijo, K.; Winner, B.; Marchetto, M.C.; Gage, F.H., Mechanisms Underlying Inflammation in Neurodegeneration. *Cell,* **2010**, *140*, (6), 918-934.
[105] Citron, M., Alzheimer's disease: strategies for disease modification. *Nature reviews Drug discovery,* **2010**, *9*, (5), 387-398.
[106] Doens, D.; Fernández, P.L., Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *Journal of neuroinflammation,* **2014**, *11*, (1), 1.
[107] Agostinho, P.; A Cunha, R.; Oliveira, C., Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Current pharmaceutical design,* **2010**, *16*, (25), 2766-2778.
[108] Weggen, S.; Eriksen, J.L.; Das, P.; Sagi, S.A.; Wang, R.; Pietrzik, C.U.; Findlay, K.A.; Smith, T.E.; Murphy, M.P.; Bulter, T., A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature,* **2001**, *414*, (6860), 212-216.
[109] Gasparini, L.; Rusconi, L.; Xu, H.; Del Soldato, P.; Ongini, E., Modulation of β-amyloid metabolism by non-steroidal anti-inflammatory drugs in neuronal cell cultures. *Journal of neurochemistry,* **2004**, *88*, (2), 337-348.
[110] Cacciatore, I.; Marinelli, L.; Fornasari, E.; Cerasa, L.S.; Eusepi, P.; Türkez, H.; Pomilio, C.; Reale, M.; D'Angelo, C.; Costantini, E., Novel NSAID-Derived Drugs for the Potential Treatment of Alzheimer's Disease. *International Journal of Molecular Sciences,* **2016**, *17*, (7), 1035.
[111] Lehmann, J.M.; Lenhard, J.M.; Oliver, B.B.; Ringold, G.M.; Kliewer, S.A., Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *Journal of Biological Chemistry,* **1997**, *272*, (6), 3406-3410.
[112] Yang, X.Y.; Wang, L.H.; Farrar, W.L., A Role for PPAR. *PPAR research,* **2008**, *2008*.
[113] d'Abramo, C.; Massone, S.; Zingg, J.-M.; Pizzuti, A.; Marambaud, P.; Dalla Piccola, B.; Azzi, A.; Marinari, U.M.; Pronzato, M.A.; Ricciarelli, R., Role of peroxisome proliferator-activated receptor γ in amyloid precursor protein processing and amyloid β-mediated cell death. *Biochemical Journal,* **2005**, *391*, (3), 693-698.
[114] Széles, L.; Töröcsik, D.; Nagy, L., PPARγ in immunity and inflammation: cell types and diseases. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* **2007**, *1771*, (8), 1014-1030.
[115] Wang, X.; Wang, Y.; Hu, J.-P.; Yu, S.; Li, B.-K.; Cui, Y.; Ren, L.; Zhang, L.-D., Astragaloside IV, a Natural PPARγ Agonist, Reduces Aβ Production in Alzheimer's Disease Through Inhibition of BACE1. *Molecular neurobiology,* **2016**, 1-11.
[116] Heneka, M.T.; Reyes-Irisarri, E.; Hüll, M.; Kummer, M.P., Impact and Therapeutic Potential of PPARs in Alzheimer's Disease. *Current Neuropharmacology,* **2011**, *9*, (4), 643-650.
[117] Pérez, M.J.; Quintanilla, R.A., Therapeutic Actions of the Thiazolidinediones in Alzheimer's Disease. *PPAR research,* **2015**, *2015*.

[118] Mandrekar-Colucci, S.; Karlo, J.C.; Landreth, G.E., Mechanisms underlying the rapid peroxisome proliferator-activated receptor-γ-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. *The Journal of neuroscience,* **2012**, *32*, (30), 10117-10128.
[119] Cho, D.H.; Lee, E.J.; Kwon, K.J.; Shin, C.Y.; Song, K.H.; Park, J.H.; Jo, I.; Han, S.H., Troglitazone, a thiazolidinedione, decreases tau phosphorylation through the inhibition of cyclin-dependent kinase 5 activity in SH-SY5Y neuroblastoma cells and primary neurons. *Journal of neurochemistry,* **2013**, *126*, (5), 685-695.
[120] Watson, G.S.; Cholerton, B.A.; Reger, M.A.; Baker, L.D.; Plymate, S.R.; Asthana, S.; Fishel, M.A.; Kulstad, J.J.; Green, P.S.; Cook, D.G., Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *The American journal of geriatric psychiatry,* **2005**, *13*, (11), 950-958.
[121] Hanyu, H.; Sato, T.; Kiuchi, A.; Sakurai, H.; Iwamoto, T., Pioglitazone improved cognition in a pilot study on patients with Alzheimer's disease and mild cognitive impairment with diabetes mellitus. *Journal of the American Geriatrics Society,* **2009**, *57*, (1), 177-179.
[122] Cummings, J.; Morstorf, T.; Lee, G., Alzheimer's drug-development pipeline: 2016. *Alzheimer's & Dementia: Translational Research & Clinical Interventions,* **2016**.
[123] Revill, P.; Moral, M.; Prous, J., Impaired insulin signaling and the pathogenesis of Alzheimer's disease. *Drugs of Today,* **2006**, *42*, (12), 785-790.
[124] Harvey, A.L.; Edrada-Ebel, R.; Quinn, R.J., The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery,* **2015**, *14*, (2), 111-129.
[125] Dias, D.A.; Urban, S.; Roessner, U., A historical overview of natural products in drug discovery. *Metabolites,* **2012**, *2*, (2), 303-336.
[126] Patridge, E.; Gareiss, P.; Kinch, M.S.; Hoyer, D., An analysis of FDA-approved drugs: natural products and their derivatives. *Drug discovery today,* **2016**, *21*, (2), 204-207.
[127] Newman, D.J.; Cragg, G.M., Natural products as sources of new drugs from 1981 to 2014. *Journal of natural products,* **2016**, *79*, (3), 629-661.
[128] Jäger, A.K.; Saaby, L., Flavonoids and the CNS. *Molecules,* **2011**, *16*, (2), 1471-1485.
[129] Rezai-Zadeh, K.; Ehrhart, J.; Bai, Y.; Sanberg, P.R.; Bickford, P.; Tan, J.; Shytle, R.D., Apigenin and luteolin modulate microglial activation via inhibition of STAT1-induced CD40 expression. *Journal of neuroinflammation,* **2008**, *5*, (1), 1.
[130] Ha, S.K.; Lee, P.; Park, J.A.; Oh, H.R.; Lee, S.Y.; Park, J.-H.; Lee, E.H.; Ryu, J.H.; Lee, K.R.; Kim, S.Y., Apigenin inhibits the production of NO and PGE 2 in microglia and inhibits neuronal cell death in a middle cerebral artery occlusion-induced focal ischemia mice model. *Neurochemistry international,* **2008**, *52*, (4), 878-886.
[131] Zhao, L.; Wang, J.-L.; Liu, R.; Li, X.-X.; Li, J.-F.; Zhang, L., Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. *Molecules,* **2013**, *18*, (8), 9949-9965.
[132] Xu, X.; Li, M.; Chen, W.; Yu, H.; Yang, Y.; Hang, L., Apigenin Attenuates Oxidative Injury in ARPE-19 Cells thorough Activation of Nrf2 Pathway. *Oxidative Medicine and Cellular Longevity,* **2016**, *2016*.
[133] Iuvone, T.; De Filippis, D.; Esposito, G.; D'Amico, A.; Izzo, A.A., The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-β peptide-induced neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics,* **2006**, *317*, (3), 1143-1149.
[134] Luan, H.; Kan, Z.; Xu, Y.; Lv, C.; Jiang, W., Rosmarinic acid protects against experimental diabetes with cerebral ischemia: relation to inflammation response. *Journal of Neuroinflammation,* **2013**, *10*, (1), 1-10.
[135] Ghaffari, H.; Venkataramana, M.; Jalali Ghassam, B.; Chandra Nayaka, S.; Nataraju, A.; Geetha, N.P.; Prakash, H.S., Rosmarinic acid mediated neuroprotective effects against H2O2-induced neuronal cell damage in N2A cells. *Life sciences,* **2014**, *113*, (1-2), 7-13.
[136] Mandel, S.; Weinreb, O.; Amit, T.; Youdim, M.B., Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *Journal of neurochemistry,* **2004**, *88*, (6), 1555-1569.

[137] Shin, J.-H.; Jeon, H.-J.; Park, J.; Chang, M.-S., Epigallocatechin-3-gallate prevents oxidative stress-induced cellular senescence in human mesenchymal stem cells via Nrf2. *International Journal of Molecular Medicine,* **2016**, *38*, (4), 1075-1082.
[138] Davinelli, S.; Sapere, N.; Zella, D.; Bracale, R.; Intrieri, M.; Scapagnini, G., Pleiotropic protective effects of phytochemicals in Alzheimer's disease. *Oxidative medicine and cellular longevity,* **2012**, *2012*.
[139] Perron, N.R.; Hodges, J.N.; Jenkins, M.; Brumaghim, J.L., Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorganic Chemistry,* **2008**, *47*, (14), 6153-6161.
[140] Choi, Y.-T.; Jung, C.-H.; Lee, S.-R.; Bae, J.-H.; Baek, W.-K.; Suh, M.-H.; Park, J.; Park, C.-W.; Suh, S.-I., The green tea polyphenol (−)-epigallocatechin gallate attenuates β-amyloid-induced neurotoxicity in cultured hippocampal neurons. *Life sciences,* **2001**, *70*, (5), 603-614.
[141] Andrich, K.; Bieschke, J. In *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*; Springer, **2015**, pp 139-161.
[142] Huang, H.-C.; Xu, K.; Jiang, Z.-F., Curcumin-mediated neuroprotection against amyloid-β-induced mitochondrial dysfunction involves the inhibition of GSK-3β. *Journal of Alzheimer's Disease,* **2012**, *32*, (4), 981-996.
[143] Soetikno, V.; Sari, F.R.; Lakshmanan, A.P.; Arumugam, S.; Harima, M.; Suzuki, K.; Kawachi, H.; Watanabe, K., Curcumin alleviates oxidative stress, inflammation, and renal fibrosis in remnant kidney through the Nrf2–keap1 pathway. *Molecular nutrition & food research,* **2013**, *57*, (9), 1649-1659.
[144] Motterlini, R.; Foresti, R.; Bassi, R.; Green, C.J., Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radical Biology and Medicine,* **2000**, *28*, (8), 1303-1312.
[145] Ahmed, T.; Enam, S.; Gilani, A., Curcuminoids enhance memory in an amyloid-infused rat model of Alzheimer's disease. *Neuroscience,* **2010**, *169*, (3), 1296-1306.
[146] Brondino, N.; Re, S.; Boldrini, A.; Cuccomarino, A.; Lanati, N.; Barale, F.; Politi, P., Curcumin as a therapeutic agent in dementia: a mini systematic review of human studies. *The Scientific World Journal,* **2014**, *2014*.
[147] Sun, Z.-K.; Yang, H.-Q.; Chen, S.-D., Traditional Chinese medicine: a promising candidate for the treatment of Alzheimer's disease. *Translational Neurodegeneration,* **2013**, *2*, 6-6.
[148] Kozikowski, A.P.; Tueckmantel, W., Chemistry, pharmacology, and clinical efficacy of the Chinese nootropic agent huperzine A. *Accounts of chemical research,* **1999**, *32*, (8), 641-650.
[149] Zhang, H.Y.; Tang, X.C., Neuroprotective effects of huperzine A: new therapeutic targets for neurodegenerative disease. *Trends in Pharmacological Sciences,* **2006**, *27*, (12), 619-625.
[150] Little, J.T.; Walsh, S.; Aisen, P.S., An update on huperzine A as a treatment for Alzheimer's disease. *Expert opinion on investigational drugs,* **2008**, *17*, (2), 209-215.
[151] Qian, B.-C.; Wang, M.; Zhou, Z.-F.; Chen, K.; Zhou, R.-R.; Chen, G.-S., Pharmacokinetics of tablet huperzine A in six volunteers. *Zhongguo yao li xue bao= Acta pharmacologica Sinica,* **1995**, *16*, (5), 396-398.
[152] Zhang, H.Y.; Zheng, C.Y.; Yan, H.; Wang, Z.F.; Tang, L.L.; Gao, X.; Tang, X.C., Potential therapeutic targets of huperzine A for Alzheimer's disease and vascular dementia. *Chemico-Biological Interactions,* **2008**, *175*, (1), 396-402.
[153] Loizzo, M.R.; Menichini, F.; Tundis, R.; Bonesi, M.; Conforti, F.; Nadjafi, F.; Statti, G.A.; Frega, N.G.; Menichini, F., In vitro biological activity of Salvia leriifolia Benth essential oil relevant to the treatment of Alzheimer's disease. *Journal of Oleo science,* **2009**, *58*, (8), 443-446.
[154] Li, Y.; Lv, O.; Zhou, F.; Li, Q.; Wu, Z.; Zheng, Y., Linalool inhibits LPS-induced inflammation in BV2 microglia cells by activating Nrf2. *Neurochemical research,* **2015**, *40*, (7), 1520-1525.
[155] Cioanca, O.; Hritcu, L.; Mihasan, M.; Hancianu, M., Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid β (1–42) rat model of Alzheimer's disease. *Physiology & behavior,* **2013**, *120*, 193-202.
[156] Sabogal-Guaqueta, A.M.; Osorio, E.; Cardona-Gomez, G.P., Linalool reverses neuropathological and behavioral impairments in old triple transgenic Alzheimer's mice. *Neuropharmacology,* **2016**, *102*, 111-120.