Tackling Alzheimer's Disease with Existing Drugs:A Promising Strategy for Bypassing Obstacles

Tackling Alzheimer's Disease with Existing Drugs: A Promising Strategy for Bypassing Obstacles

Angela Rampa,* Silvia Gobbi, Federica Belluti, Alessandra Bisi*

Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy

Corresponding authors:

Angela Rampa Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy Tel/Fax: (+39-051)-20199710. Email angela.rampa@unibo.it

Alessandra Bisi Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy Tel/Fax: (+39-051)-20199710. Email alessandra.bisi@unibo.it

ABSTRACT

The unmet need for the development of effective drugs to treat Alzheimer's disease has been steadily growing, representing a major challenge in drug discovery. In this context, drug repurposing, namely the identification of novel therapeutic indications for approved or investigational compounds, can be seen as an attractive attempt to obtain new medications reducing both the time and the economic burden usually required for research and development programs. In the last years, several classes of drugs have evidenced promising beneficial effects in neurodegenerative diseases, and for some of them preliminary clinical trials have been started. This review aims to illustrate some of the most recent examples of drugs reprofiled for Alzheimer's disease, considering not only the finding of new uses for existing drugs, but also the new hypotheses on disease pathogenesis, that could promote previously unconsidered therapeutic regimens. Moreover, some examples of structural modifications performed on existing drugs in order to obtain multifunctional compounds will also be described.

KEYWORDS: Drug repurposing; drug reprofiling; multitarget drug, Alzheimer's Disease, antibiotics, chelating agents, antidiabetics.

1. INTRODUCTION

In medicinal chemistry, the target-based drug discovery approach, developed during the nineties, aimed to obtain small molecules able to interact with a single desired biological target [1]. In this context, the interaction of drugs with unrelated targets was sometimes reported as responsible for unwanted side effects, and was referred to as drug promiscuity, with a negative connotation [2,3]. However, from the beginning of this century, a rapid expansion of the concept of multitarget drug emerged [4], mainly related to the treatment of complex diseases involving multiple factors in their physiopathology [5], and drug promiscuity started to acquire a more positive meaning. In fact, the ability of a single drug to simultaneously interact with different targets involved in the progression of a disease could result in higher efficacy, even if unwanted off-target actions may also emerge, leading to undesirable side effects. It is also well acknowledged that most of the marketed small-molecule drugs revealed promiscuous properties in following studies or while in use, even if not intentionally designed to this aim. In recent years, due to the ever-increasing cost of drug development and thanks to the deeper understanding of the potential benefits of drug promiscuity, the opportunity to exploit the off-target effects of a small-molecule drug gradually emerged [6]. Indeed, the discovery and development process leading to a new-marketed drug is extremely expensive and time consuming and in particular, for compounds acting on CNS, further hurdles, such as the need to bypass the blood brain barrier (BBB) and the complexity in setting up suitable animal models, require additional considerations [7]. Drug repurposing (or drug repositioning, drug reprofiling) is thus a current strategy in drug discovery, and can be defined as the identification of new therapeutic indications for existing compounds, either approved and marketed for a different purpose, or stopped in the latest phases of clinical trials. This approach holds the advantage to reduce the time and the economic burden required for drug discovery, lead optimization and ADME properties evaluation, since the repurposed compounds have already been properly profiled regarding safety and pharmacokinetic [5]. As previously mentioned, drugs targeting CNS have additional obstacles to overcome, making research in this area even more complex.

Neurodegenerative diseases (NDs) are age-dependent disorders with unrelated pathophysiology mechanisms and still largely unknown origins. Due to the progressive increase in the average age of global population, their incidence is

expecting to dramatically multiply in the next 20 years, with a strong social impact and a huge financial and emotional stress on patients and caregivers [8]. Among NDs, dementias are the main responsible for the burden of these pathologies, and Alzheimer's disease (AD) represent the leading illness in industrialized countries [9]. As evidence of the challenging issue for research and development of effective drugs, only four non disease-modifying compounds have been approved for treating AD during the last 20 years. In this discouraging framework, the repurposing approach seems particularly promising, and an increasing number of prescribed drugs have recently been subjected to screening programs to find new therapeutic options. Consequently, several interesting reviews on this topic appeared in the literature in the last years [7, 10, 11], and the aim of this paper is to give an overview of the most recent data regarding both the drug repurposing strategy and the coupling of existing therapeutics in a multitarget perspective, applied to AD.

2. ALZHEIMER'S DISEASE PATHOGENESIS

AD shares with other NDs, such as Parkinson's, amyotrophic lateral sclerosis, prion and Huntington's diseases, a common molecular mechanism, involving the formation of misfolded protein aggregates in well-defined brain areas as trigger for neuronal cell death. Collectively, these pathologies have been labelled conformational diseases. Regarding AD, the deposition of beta-amyloid (Aβ) protein into plaques and the formation of neurofibrillary tangles (NFTs), abnormal aggregates composed by hyperphosphorylated tau protein, have been recognized as the main hallmarks of the disease. In detail, one of the etiopathological pathways involved in AD progression is represented by the aggregation of Aβ peptide in a process initially defined as "amyloid cascade" [12]. In fact, in a neuropathological situation, the formation of Aβ peptides is a multistep process starting with the proteolytic sequential cleavage of the Amyloid Precursor Protein (APP), an integral transmembrane protein located in different cell types, including neurons, by β-secretase (BACE 1) and γ-secretase enzymes, yielding Aβ (1-40) or Aβ (1-42) peptides [13]. The amyloid aggregates formation starts with a self-association of Aβ monomers in their soluble native state and, moving through a number of intermediate species endowed with different size and toxic properties, ends with the formation of fibrillary amyloid plaques. In recent years, *in vitro* studies labelled the pre-fibrillary oligomer forms, instead of the mature fibrils, as the actual neurotoxic species and the primary pathogenic agents in AD, and proved the ability of Aβ peptides to catalyse the formation of NFTs [14]. This significant linking between the two main hallmarks of AD endorse the impact of the amyloid theory in the pathogenesis of the disease. Notwithstanding, the actual mechanism underlying AD pathology is far from being identified and is still controversial: undoubtedly, we are faced with a complex, multifactorial process in which, beside a recognized progressive

cholinergic decline, neuroinflammation, oxidative stress and mitochondrial dysfunction also play a pivotal role. In this intricate picture, the possibility for an existing drug to affect one or more of these pathways may not be so unlikely, and could be a valuable approach, given the urgent need of new therapeutic options and the inability to find them. To date, the main therapeutic options for AD treatment are still confined to the cholinesterases (ChEs) inhibitors, such as donepezil, rivastigmine or galantamine, and to an *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine, merely able to act as palliative treatments, leading to a temporary restoration of the cholinergic transmission [12].

3. ANTIMICROBIALS IN AD

The option to cure AD with antimicrobials can be regarded as a particular case of drug repositioning. Indeed, since the early 1990s, the virologist Ruth Itzhaki proposed a possible link between NDs and microbial infections [15], and later in 2016 an increasing number of scientists presented evidence for a causal role of pathogens in AD [16, 17]. The so-called 'microbial hypothesis' suggests the role of chronic microbial infections, involving both viruses and bacteria, in AD onset, maybe triggering systemic inflammation processes, ultimately resulting in a progressive cognitive decline in aged people. The pathogens that have been proposed as most likely candidates include human herpesviruses and bacteria, such as *Helicobacter pylori*, *Porphyromonas gingivalis*, *Chlamydia pneumoniae* and several types of *Spirochetes*. These microorganisms could reach the CNS compartment directly, by crossing the BBB, or exploiting both the oral-olfactory cavity and the gastrointestinal mucosa pathway, reaching the systemic circulation and then invading the brain, causing the onset of an inflammatory process leading to neuronal damage. Indeed, a number of experimental evidences indicate a correlation between herpes simplex virus (HSV) infection and chronic periodontitis and increased risk of developing AD in old age [18].

To complicate the picture, a pivotal role of gut microbiota in brain development has recently been underlined, and the existence of a microbiota-gut-brain axis suggests that bacteria usually resident in the gut may also affect brain functions [19]. Thus, a significant role of gut microbiota alterations in AD cannot be overlooked, since a link between gut bacteria and immune activation, resulting from a damaged enteric barrier, has also been reported, with the induction of a systemic inflammatory response able to impair the BBB integrity thus promoting neurodegeneration [20]. In this context, Aβ deposition, or better Aβ oligomerization, acquires a completely different role in AD pathogenesis, acting as a "protection" against the microbial attack. Recent studies have highlighted the antimicrobial properties of Aβ, and this renewed physiological protective role have led to the 'Antimicrobial Protection Hypothesis' of AD, in which the overproduction

of this peptide in AD brain would appear as a tentative to fight microbial infections, by entrapping and neutralize pathogens [21]. Unfortunately, this helpful effect would terminate with the persistence of the infection and the progression of the inflammation state, gradually becoming detrimental.

In this context, the use of antibiotics and antivirals in AD treatment can be seen as a factual therapeutic option rather than the repositioning of pre-existing drugs.

3.1 Antibiotics and AD

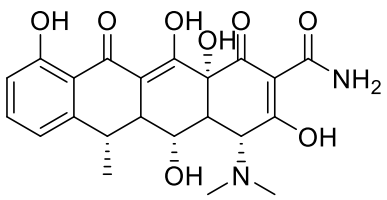
Several *in vivo* studies, both in mouse models of AD and in patients in an advanced stage of the disease, agree that long-term antibiotic treatments considerably increase survival, maybe reducing Aβ plaque deposition and mitigating the neuro-inflammatory state. Actually, beside their anti-bacterial activity, a number of ancillary properties are recognized to antibiotics, among which anti-inflammatory, anti-aggregating and antioxidant properties, which could be exploited for treating neurological disorders [22]. An appreciable multitarget behavior has been reported for tetracyclines, an antibiotic class widely prescribed, reasonably safe and able to cross the BBB [23, 24]. In particular, doxycycline (DOX) and minocycline (Figure 1) proved to significantly reduce the aggregation of Aβ (1-42) and to disaggregate the amyloid fibrils. Moreover, an increased susceptibility of the aggregates to protease activity was reported, which could be due to a different pattern of Aβ aggregation induced by the tetracycline itself, also leading to lower toxicity [25]. Further studies in a cell model of AD demonstrated that DOX induces the formation of non-toxic, amorphous and soluble low molecular weight Aβ aggregates, though not being able to counteract the neurotoxicity of pre-aggregated oligomers [26]. A recent study by Gautieri *et al*. attempted to investigate the molecular mechanism behind this destabilization of Aβ fibrils using a series of molecular dynamics simulations, starting from two different Aβ42 fibril structures (PDB id 2MXU and PDB id 5OQV), acquired from solid-state NMR data and cryo-EM, respectively. This study proved the ability of DOX to stably bind amyloid fibrils, particularly with specific hydrophobic amino acids in a definite core of the fibril. Moreover, thanks to the stable binding of this tetracycline with the 2MXU polymorph, three main binding sites were identified within the protein, corresponding with regions previously targeted by unrelated anti aggregating compounds. Unfortunately, in the 5OQV fibril a precise identification of binding sites was not possible. This study validated the experimental observations of the anti-amyloidogenic properties of DOX, and shed light on the molecular mechanism underlying the destabilization of the fibrillary structure induced by this drug, promoting a more accessible structure-based drug design for AD treatment [27]. As pointed out, tetracyclines can be considered as multipotent drugs, also showing anti-inflammatory effects, anti-oxidative and anti-apoptotic activities, which could remarkably contribute to their therapeutic potential. Minocycline, a

second-generation tetracycline antibiotic, proved to reduce the formation of tau aggregates, probably through a reduction in caspase-3 activation and a consequent decrease in aggregation of caspase-3-cleaved tau fragments [28]. Moreover, minocycline is also able to efficiently cross the BBB and to exert anti-inflammatory and neuroprotective properties directly in the CNS, by inhibiting microglial activation and reducing the release of pro-inflammatory mediators, such as TNF-α and IL-1β. A further intriguing feature of this antibiotic is its ability to improve the symptoms related to depression, the most common neuropsychiatric disorder associated with AD. In a recent paper, Amani *et al*. highlighted the relevance of TNF-α and IL-1β in the expression of depression-related symptoms induced by intracerebroventricular administration of Aβ (1–42), suggesting a potential beneficial impact of minocycline in AD-associated depression [29]. A two-year multicenter double-blind placebo-controlled study of minocycline (Minocycline in Alzheimer's Disease Efficacy, MADE) has recently been performed, recruiting 480 patients with early AD in the United Kingdom, aimed to establish the actual efficacy of this drug in reducing the rate of cognitive decline and its tolerability. Unfortunately, the outcomes were disappointing, showing no clinically significant improvement in the intellectual and functional skills in patients with moderate AD [30].

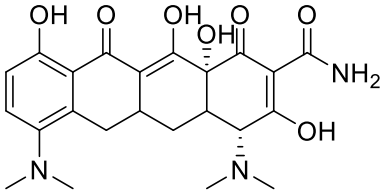
Promising results were also obtained following the administration of the third-generation cephalosporin ceftriaxone (CEF, Figure 1), which showed neuroprotective activity and proved to significantly recover neurological deficiencies in different AD animal models. These results may be due to the modulation of the genetic expression of enzymes involved in Aβ metabolism, reducing the mRNA expression of genes responsible for Aβ production (mainly *BACE 1*) and upregulating genes encoding for Aβ metabolizing enzymes [31]. Moreover, CEF proved to reduce oxidative stress and to restore impaired ChE levels [32]. In addition, the ability to downregulate tau protein and to upregulate the expression of GLT-1, a pre-synaptic glutamate transporter responsible for the clearance of glutamate in the brain, was also observed in CEF-treated animal models of AD [33]. Taken together, these data suggest a potential beneficial effect of this drug for the treatment of neurological disorders, and further studies and randomized clinical trials in humans are recommended to validate its real neuroprotective potential [34].

Besides tetracyclines and cephalosporins, other antimicrobials have been studied for their potential as anti-AD candidates. Among macrolide antibiotics, azithromycin and erythromycin (Figure 1) were able to reduce the brain levels of Aβ peptides, maybe through the decrease of APP levels. In particular, a pilot study in the transgenic (Tg) CRND8 mouse model of AD showed more than 50 % reduction in Aβ (1-42) levels following the treatment with erythromycin in the drinking water for 3 months [35]. Clarithromicin (Figure 1) is another macrolide antibiotic that has been used in a triple

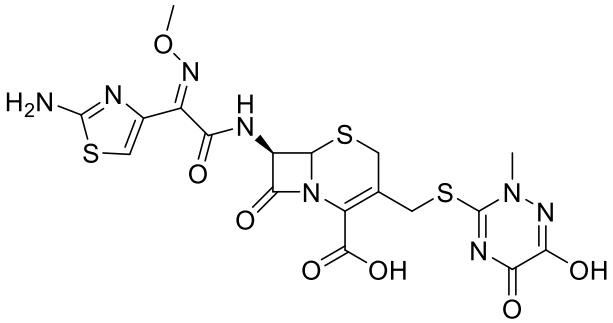
therapy with omeprazole and amoxicillin to eradicate *Helicobacter pylori* infections in a study involving 56 AD patients. After two years treatment, the subgroup of patients successfully treated also showed a significant improvement of cognitive performance [36].



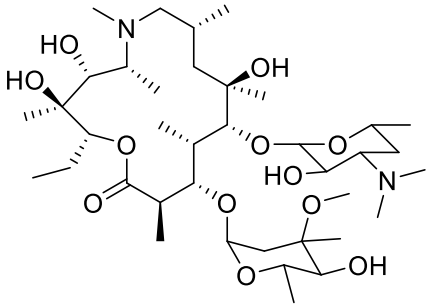
Doxycycline



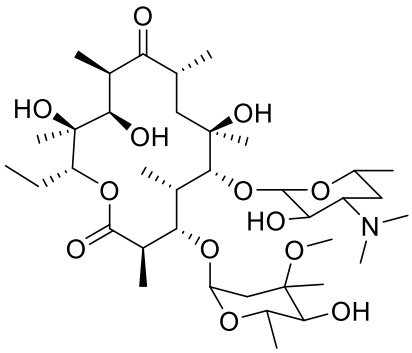
Minocycline



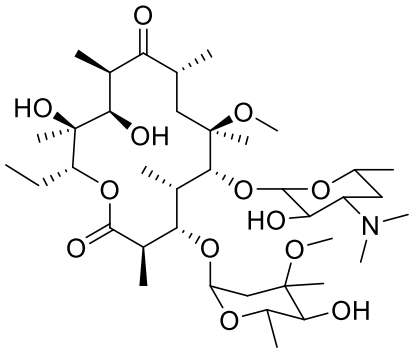
Ceftriaxone



Azithromycin



Erithromycin



Clarythromycin

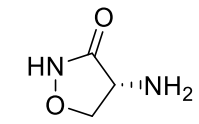
Figure 1. Antibiotics repurposed for AD.

3.2 Antimycobacterials and AD

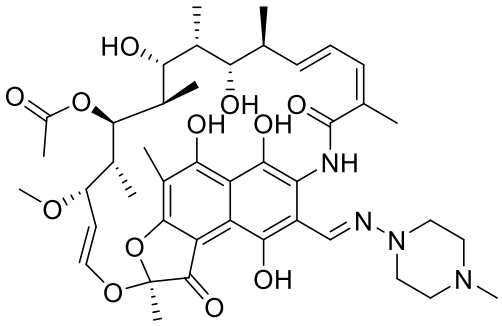
Glutamate transporters, such as GLP-1, play a pivotal role in ensuring the clearance of the excess of glutamate from AD brain [37]. Indeed, glutamatergic mechanisms are involved in learning and memory processes: the NMDA receptors play a crucial role in neuroplasticity of the human brain through a mechanism called Long Term Potentiation (LTP). D-

Cycloserine (DCS, Figure 2), approved for human use in tuberculosis therapy since 1950s, also proved to target the glycine-binding site of the NMDA receptor, acting as selective partial NMDA agonist [38]. *In vivo*, it showed a dose-dependent behavior, acting as an agonist at low doses but as antagonist with increasing doses, probably due to a different receptor subtype selectivity. This ability to modulate the activity of the NMDA receptor gave the rationale for studying the effects of DCS on a number of neurological diseases. Regarding AD, the first studies gave controversial results, likely related to the daily administration of the drug, resulting in a down-regulation of the receptor. On the contrary, in a 4-week, double-blind, placebo-controlled study in 17 AD patients, the administration of DCS once a week produced significant cognitive improvement [39]. Interestingly, Chaturvedi *et al*. recently reported that DCS significantly reduced the toxicity of Aβ (1-42) aggregates, probably through a direct interaction with specific residues of the peptide, resulting in the formation of stable complexes DCS/Aβ (1-42), able to prevent the development of toxic species [40].

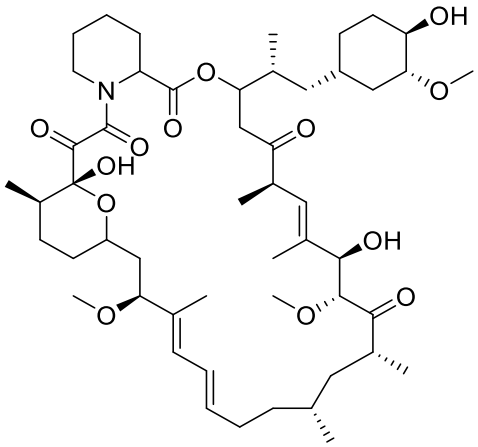
Rifampicin (Figure 2) is a macrocyclic antibiotic used to combat tuberculosis and leprosy. It is able to easily cross the BBB and showed a potent Aβ (1-42) antiaggregating activity, with a consistent reduction of Aβ oligomers accumulation at 100 μM concentration. Remarkably, rifampicin proved to prevent oligomerization not only of Aβ, but also of tau and α-synuclein, leading to speculate on a possible common mechanism underlying the formation of these toxic aggregates [41]. As recently reported by Espargaró *et al*. [42], this feature deserves particular attention, since the ability of a single molecule to inhibit the aggregation of multiple unrelated proteins could represent a valuable therapeutic strategy to cope with different conformational diseases. In addition, due to the synergistic interplay between Aβ, tau and α-synuclein in accelerating mutual pathology [41], it could be highly convenient to switch from the development of a specific anti aggregation agent to a pan-inhibitor, capable of acting on more than one toxic species. In this respect, rifampicin may represent a validated lead compound. Unfortunately, preformed fibrils in amyloid plaques seem not to be affected by this drug. Its neuroprotective effect may be rather due to the inhibition of the oligomerization process, related to its ability to directly bind to oligomers, preventing their interaction with the neuronal membrane and the resulting membrane damage [43]. Moreover, the radical-scavenging properties and the ability to halt microglia activation of rifampicin suggest a strong potential for this ready-to-use therapeutic in treating AD [44]. *In vivo* studies on mouse models of AD confirmed the ability of this drug to hamper the progression of the disease, by reducing the accumulation of both Aβ and tau oligomers and improving cognitive function [41]. However, the hepatotoxicity of this agent could significantly limit its long-term use, especially in elderly patients undergoing polytherapy, and this issue should be carefully considered.



D-Cycloserin



Rifampicin



Rapamycin

Figure 2. Antimycobacterial and antifungal drugs repurposed for AD.

3.3 Antifungals and AD

Rapamycin (Figure 2), also known as Sirolimus, is a macrolide antibiotic developed in the middle of 70's as antifungal agent. Later, it showed to efficiently act as immunosuppressive and was approved by the FDA in 1999 to be used in association with other drugs as antirejection compound in transplanted patients [45]. This derivative was found to bind to a specific protein, Target of Rapamycin (TOR), a serine/threonine kinase involved in several processes, among which longevity emerged as the most promising [46]. In particular, it was hypothesized, and later probed on mice, that rapamycin might increase lifespan in mammals, acting as mTOR inhibitor. In a study performed in 2015 by Richardson and co-workers, it was found that this macrolide appreciably prevented memory loss in two different transgenic AD mouse models, mainly when administered at an early stage of memory impairment. Besides, a significant reduction of Aβ and tau aggregation and of microglia activation was also observed, likely related to the rapamycin induction of autophagy through mTOR signalling pathway. A further advantage in the use of this drug is the well-known safety profile, associated

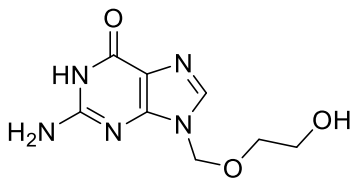
with its use for a long time in other diseases [47]. Taken together, these data seem to indicate rapamycin as a promising multipotent compound deserving a deeper evaluation.

3.4 Gingipain Inhibitors

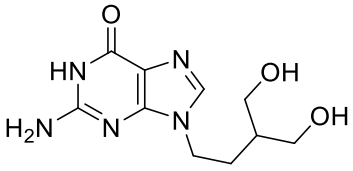
Porphyromonas gingivalis is a nonmotile, Gram-negative bacterium found in human oral microbiota and involved in the development of chronic periodontitis (CP), a chronic inflammatory condition induced by the significant accumulation of dental plaque. CP has been identified as a prominent risk factor in several systemic diseases, among which AD and increased systemic inflammation, and *P. gingivalis* has been repeatedly identified in the brain of AD patients, supporting the hypothesis of its involvement in AD pathogenesis [48]. Its virulence is mediated by the production of toxic cysteine proteases (gingipains), essential for the bacterium survival and reported as lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). Recently, a number of inhibitors targeting gingipains have been reported for treating periodontitis and one of them, COR388, was also evaluated for its ability to reduce neuroinflammation and Aβ (1-42) production, giving promising results [49].

3.5 Antivirals and AD

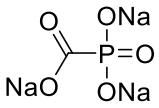
A possible contribution of viral infections in AD development cannot be excluded: accumulating evidence correlates HSV infections with increased risk of AD onset [50]. The *herpesviruses* are a family of DNA viruses able to remain latent in humans and inducing recurring infections. Among them, herpes simplex virus type 1 (HHV-1), human herpesvirus 6A (HHV6A) and human herpesvirus 7 (HHV7) are considered the most involved in AD-related cognitive decline [51]. Consequently, it is not surprising that antiviral drugs have been reprofiled for their anti-AD properties. An *in vitro* study by Wozniak *et al*. with acyclovir (Figure 3), a nucleoside analogue interfering with viral DNA replication, proved the ability of the drug to consistently reduce Aβ (1-42) and hyperphosphorylated tau accumulation in a dose-dependent manner. The decrease in Aβ (1-42) formation was later attributed to reduced viral replication [52]. Similar results were also obtained in an AD cell model with penciclovir, another guanosine analogue antiviral, and with foscarnet, a DNA polymerase inhibitor (Figure 3) [52].



Acyclovir



Penciclovir



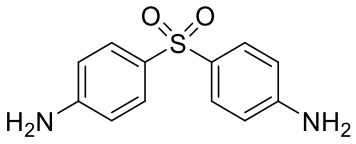
Foscarnet

Figure 3. Antivirals studied for AD.

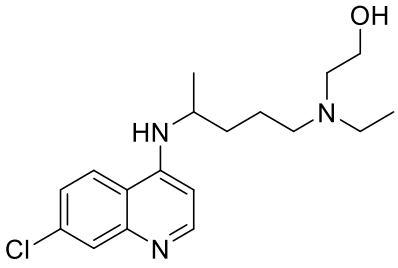
3.6 Miscellanea

Several drugs used to fight human parasitosis have recently been revalued to find new therapeutic options for AD. Dapsone (Figure 4), an anti-leprosy antibiotic, was deeply evaluated in order to establish its ability to reduce senile plaques deposition in AD patients, giving conflicting and inconclusive results [53].

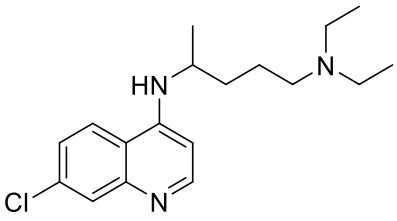
A similar behaviour was reported for chloroquine and hydroxychloroquine (Figure 4), well-known antimalarial drugs, endowed with a number of attractive properties in AD perspective, mainly anti-inflammatory and immunomodulating. Unfortunately, a double blind, multicenter trial on patients with early AD failed to give the expected results [54].



Dapsone



Hydroxychloroquine



Chloroquine

Figure 4. Antiparasitic drugs evaluated for AD.

4. CHELATING AGENTS

Clioquinol (CLQ, Figure 5), a quinoline derivative developed at the end of 1800 as antiseptic agent, was later used worldwide to treat fungal and protozoan infections. The proposed mechanism of action involved its ability to chelate metals, and metal dyshomeostasis in the human brain has long been recognized as one of the feasible triggers of AD development. Moreover, the recently proposed concept of metallostasis, a dysfunction in metal redistribution at the CNS level, mainly involving the transition metals Fe, Cu and Zn, makes chelating property particularly attractive [55]. Indeed, CLQ and its second generation derivative PBT2 were reported to inhibit plaque formation in a mouse AD model [56] and several structural modifications to these compounds were reported, aimed at increasing their therapeutic potential. In this respect, CLQ appears as a synthetically accessible pharmacophore fragment, suitable to be exploited in a multitarget perspective. In fact, due to the complex pathophysiology of AD and the dynamic interactive network of components that underpin the disorder, usually monotherapy may not be capable of inducing significant outcomes. Combined therapies targeting more than one aspect of the disease can be a useful approach, and, as seen in the abovementioned examples, several marketed drugs showed the ability to act with different interrelated mechanisms. It is also true that the multitarget-directed ligand strategy, i.e. the purposed design of a hybrid molecule composed by linked selected pharmacophores able to interact with different targets, may represent a valuable strategy to interface with AD [4, 5]. Following this approach, a number of drug candidates emerged, among which hybrid molecules obtained linking the CLQ pharmacophore moiety to selected fragments found in inhibitors of key enzymes involved in AD pathogenesis, such as ChEs and phosphodiesterase (PDE) 4D. The second enzyme, belonging to the PDE4 family, has been implicated in LTP and memory consolidation [57]. The hybrid compounds, designed by connecting the hydroxyquinoline moiety of CLQ to selected portions of tacrine and donepezil as ChEs inhibitors and to moracin, rolipram and roflumilast as PDE4D inhibitors [58], were reported as promising multitarget-directed metal-chelating agents.

In detail, linking the tacrine scaffold to CLQ allowed to obtain IQM-622 (Figure 5), a CNS permeating compound capable of controlling several pathological processes involved in AD, i.e. increasing patient cognition due to ChEs inhibition, while reducing plaque formation and protecting neurons from oxidative damage thanks to the CLQ ability to capture free radicals and to chelate metals [59]. Moreover, a further *in vivo* study suggested that the neuroprotective effects of IQM-622 was also due to ChE unrelated mechanisms, involving the modulation of Aβ clearance in AD brain [60].

Regarding CLQ-donepezil hybrids, some of them (**1-3**, Figure 5) proved to effectively act as multifunctional compounds *in vitro*, inhibiting butyrylcholinesterase and showing (derivative **2**) also anti-aggregating (59% inhibition at 50 μM), metal chelating and antioxidant properties. Moreover, parallel artificial membrane permeability assay (PAMPA) data predicted their high passive BBB permeability [61].

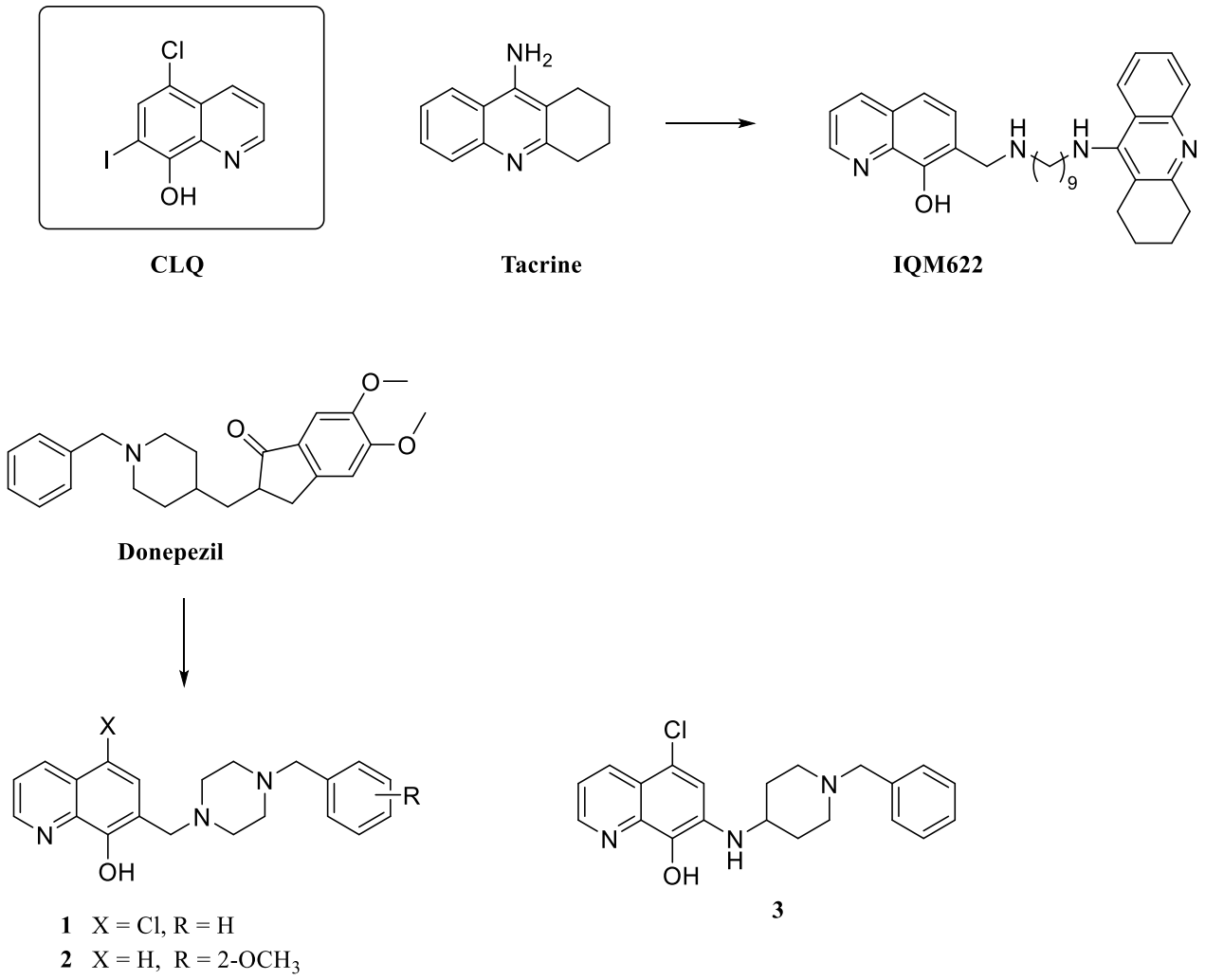


Figure 5. CLQ and ChEs inhibitors hybrids.

On the other hand, Wang *et al.* reported a series of derivatives in which CLQ was merged with moracin M, a natural product isolated from the root bark of *Morus alba L.* and endowed with a number of biological activities, namely anti-inflammatory, antioxidant and PDE4D inhibitory. Some of the studied compounds showed an excellent multipotent profile, and the most promising of the series, WBQ5187 (Figure 6), proved to easily penetrate the BBB, inhibit Aβ

aggregation (67.5% inhibition at 5 μM and Aβ (1−42) at 25 μM) and PDE4D activity (IC_{50} = 0.32 μM) and exert antioxidant effects [62]. This compound was also able to disaggregate self- or metal-induced Aβ aggregates, and further *in vivo* studies in APP/PS1 transgenic AD mice revealed the capability to improve cognitive and memory impairment and alleviate Aβ pathology and gliosis in the brain, after oral administration [63]. In continuing its studies, the same research group designed a series of compounds fusing key structural fragments of CLQ and other PDE4D inhibitors, namely rolipram and roflumilast. Indeed, several studies have underlined the positive outcomes obtained by these PDE4 inhibitors in facilitating memory performance and improving cognitive deficits in a APP/PS1 transgenic mouse model of AD. The results once again showed for the new compound **4** a remarkable multitarget profile, summarized in Figure 6 [64].

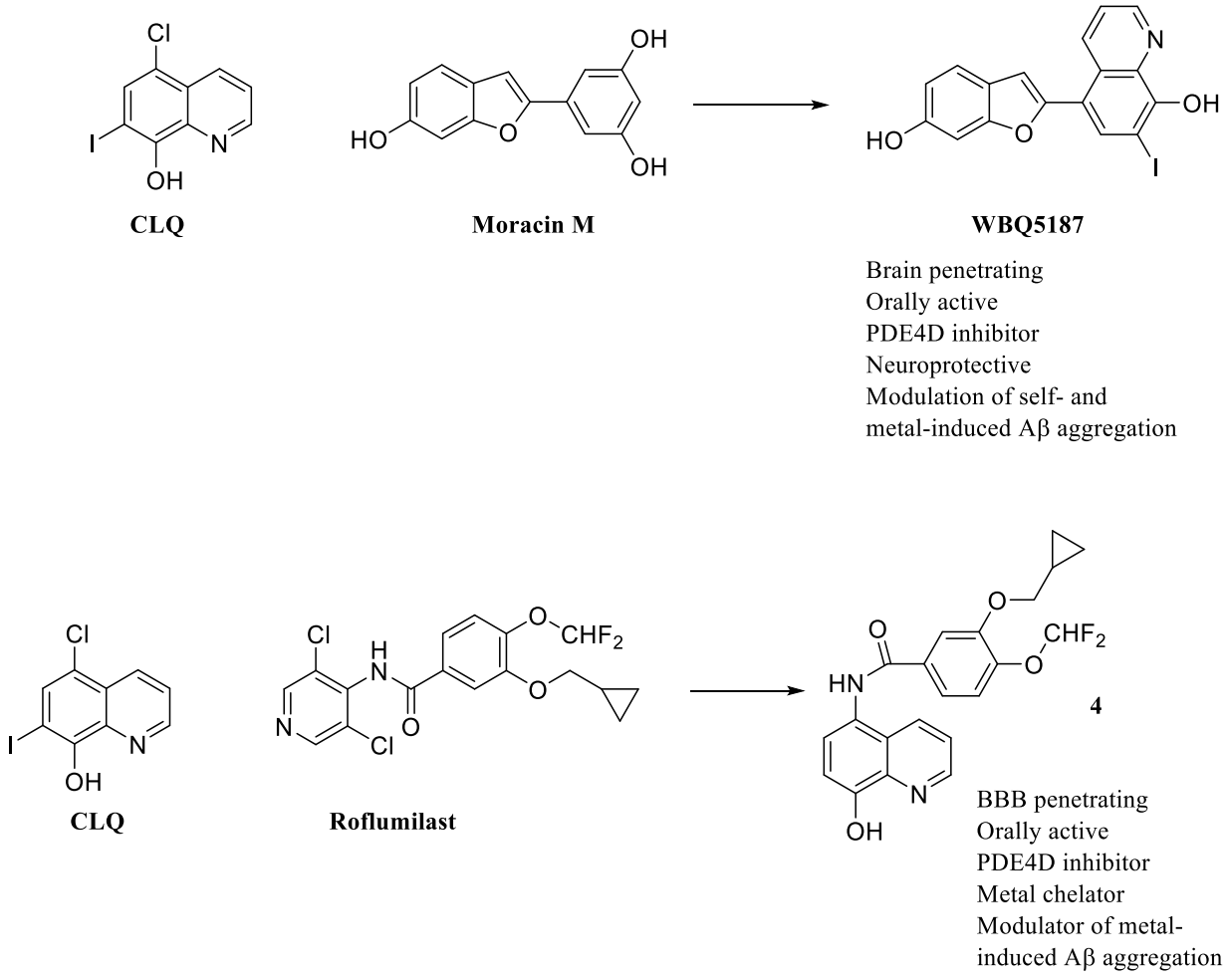


Figure 6. Hybrids obtained by merging CLQ and PDE4D inhibitors.

In a different approach, the CLQ pharmacophore fragment was connected with well-known antioxidant compounds, namely resveratrol and epigallocatechin gallate (EGCG), in order to obtain hybrid multifunctional modulators able to

exert protective effects against Aβ toxicity (Figure 7). By exploiting the fusing strategy, a series of resveratrol-CLQ hybrids was designed and synthesized, among which compound **5** exhibited both inhibition of self-induced Aβ aggregation (IC_{50} = 8.5 µM) and disassembling properties towards the preformed Aβ fibrils (88.7% disaggregation at 25 µM). This compound was also reported to be endowed with good metal-chelating ability, very potent antioxidant activity, good BBB penetration together with low toxicity [65]. Derivatives **6-8**, hybrids of CLQ and EGCG, showed antioxidant effects, inhibition of Aβ (1-42) aggregation (ranging from 50 to 90% at 50 µM) and quenching of ROS formation and **6** was reported as a promising lead compound for further modifications[66].

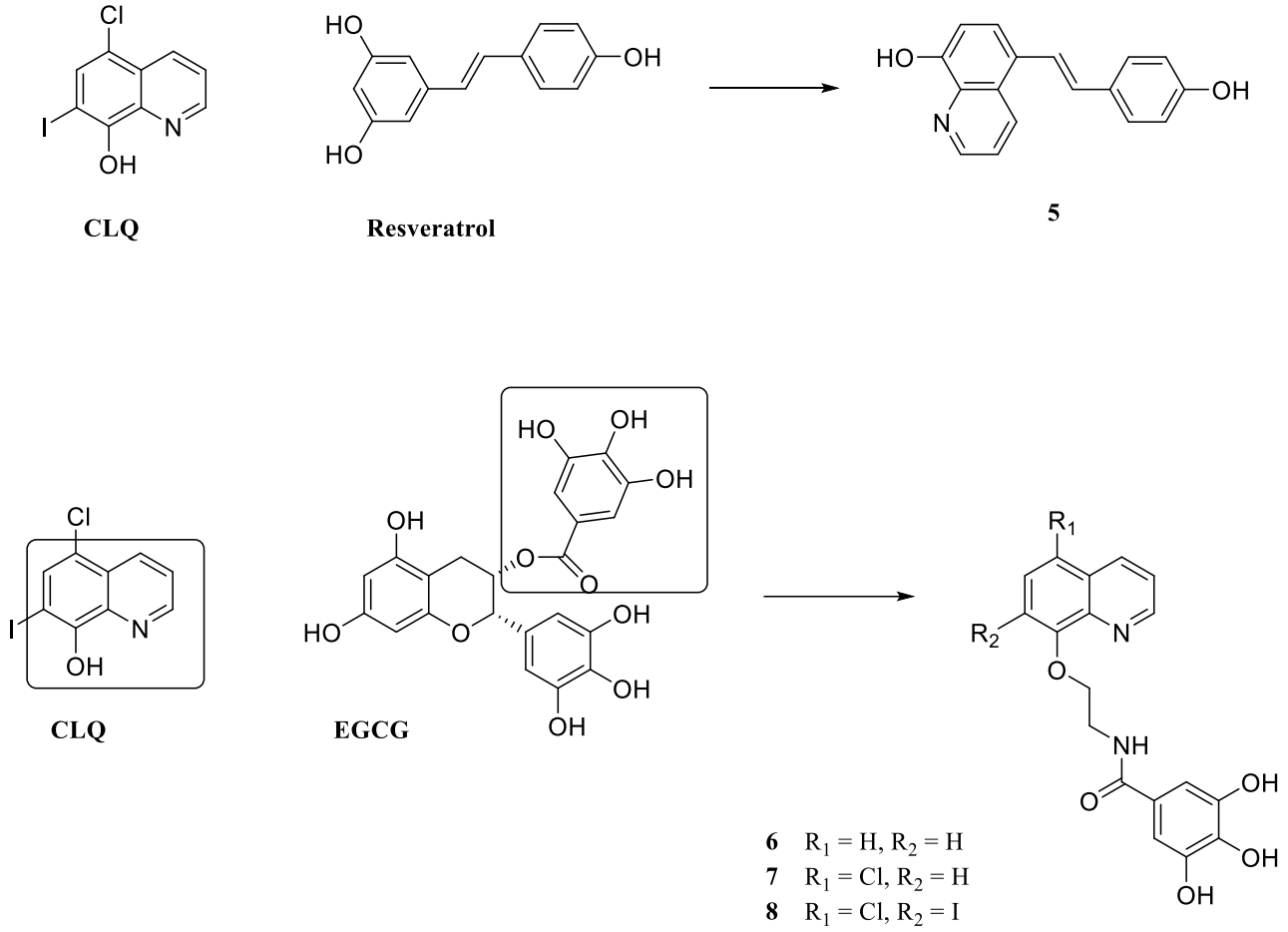


Figure 7. CLQ and antioxidants hybrids.

Besides CLQ and its hydroxyquinoline framework, catechol moiety also possesses chelating properties, suitable to be engaged in multitarget drug design. Tolcapone and entacapone (Figure 8), catechol-*O*-methyltransferase (COMT) inhibitors, are currently approved to treat Parkinson disease and proved to inhibit α synuclein and Aβ aggregation [67].

More recently, a study from Rao and coworkers demonstrated that tolcapone can also prevent the aggregation of tau-derived hexapeptide AcPHF6, a model peptide widely used to investigate tau-aggregation process [68]. These results led to the identification of the nitrocatechol (3,4-dihydroxy-5-nitrophenyl) pharmacophore fragment, successfully exploited attempting to obtain promising anti-AD drug candidates. Notably, appreciable anti-aggregating activity was also observed for the naturally occurring caffeic acid and its derivative caffeic acid phenethyl ester, pinpointing the catechol moiety, as a key feature for this activity [69]. A series of tolcapone and entacapone related compounds bearing the nitrocatechol moiety was then suitably designed and evaluated for the capability to interfere with the aggregation of AcPHF6 tau-derived peptide, showing promising results (Figure 8). In particular, for some of the reported compounds (**9-12**) a peculiar ability to chelate Cu^{2+} ions, whose involvement in tau aggregation is widely recognized, was observed. Moreover, the presence of an α-cyano group, a carboxamide function and a bulky benzyl or phenethyl substituent were recognized as beneficial structural features for an effective anti-aggregating activity (inhibition of 90% at 50 μM) [70]. These data validate the nitrocatechol fragment as a new attractive scaffold for the design of drugs against tauopathies.

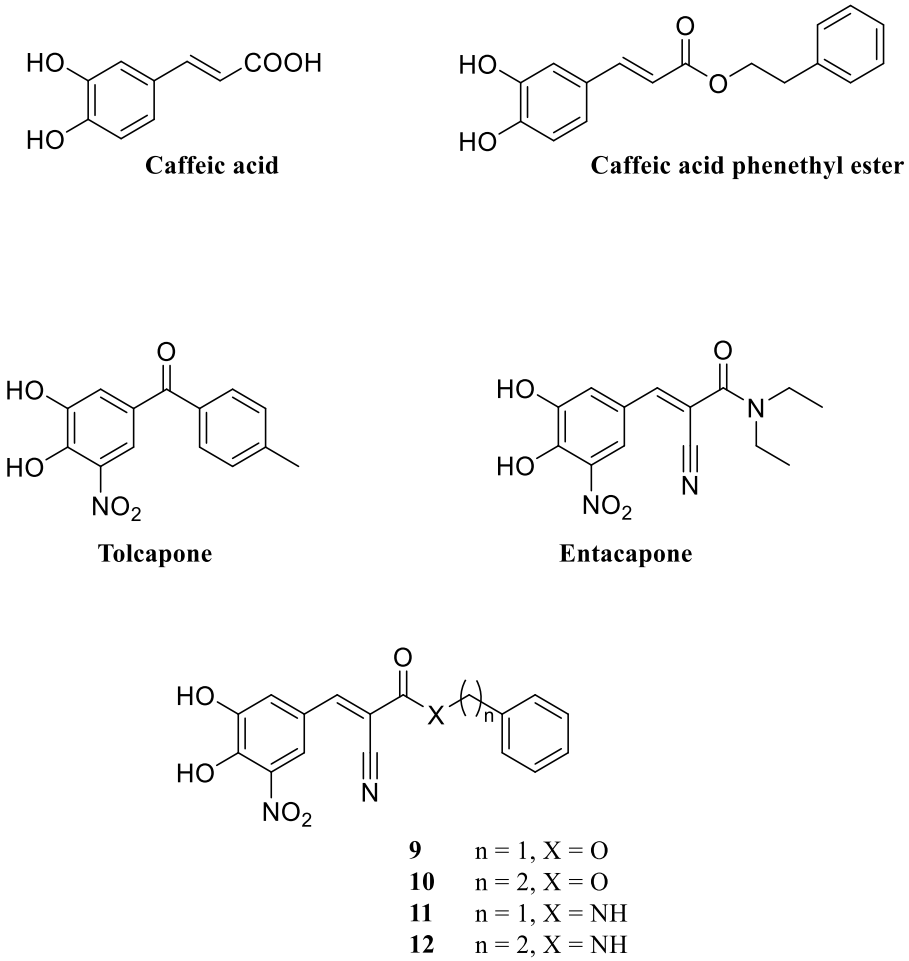


Figure 8. Nitro-catechol as promising privileged structure for antiaggregating properties.

A more detailed knowledge of the mechanism of CLQ and other chelating agents (with particular attention to copper chelating agents) in NDs has been reported in a comprehensive and exhaustive review recently published on this journal [71].

5. ANTIDIABETIC DRUGS

Although the causes of sporadic AD remain under discussion, evidence shows that, among other factors, altered insulin-signaling pathways are early events in disease pathogenesis. Indeed, insulin signaling impairment has been increasingly associated with cognitive decline and increased risk of dementia [72]. Moreover, it is becoming clear that diabetes is a risk factor for AD, and that these two diseases are connected by several common molecular and cellular processes, being insulin signaling one of the main links. Indeed, insulin sensitivity is decreased with aging, and with increasing life

expectancy the prevalence of diabetes, particularly type 2 diabetes (T2D), and dementia are globally rising [73]. A number of studies have documented the pivotal role played by insulin in humans. Besides glucose metabolism, this hormone has been involved in mitochondrial function and in cognitive performance, making brain insulin signaling of ever-increasing interest in neurological research. In particular, a reduction in neuronal Aβ peptides deposition, mainly due to an insulin modulation in APP metabolism, was reported, even if the precise molecular mechanism still remains largely unknow. Clearly, these findings enriched the scope for the repurposing of antidiabetic drugs in AD management, aiming also to elucidate the cellular pathways involved in the intersection between AD and diabetes, that could be of the outmost importance to find future therapeutic options for both diseases [72]. In fact, only few and controversial studies were reported dealing with the relationship between antidiabetic medications and dementia. Some of them were performed on metformin (MET, Figure 9) and showed that this widely prescribed antidiabetic drug not only reduced insulin levels and the risk of metabolic syndrome, but also improved insulin sensitivity, suggesting its potential in exerting protective effects on cognitive function, and therefore the ability to decrease the risk of dementia [73] (this also applies to the use of sulphonylureas [74], Figure 9). Unfortunately, some researchers suggest a potentially harmful consequence of its use in monotherapy in elderly diabetic patients. Indeed, MET also affects APP metabolism increasing the generation of Aβ peptides through upregulation of BACE-1 in various cell models. These potentially deleterious effects of MET may be avoided by using a combination of MET and insulin. Notably, the combination may result in a beneficial effect both in treating T2D and in mitigating AD progression [75].

The thiazolidinediones (TZDs), as rosiglitazone and pioglitazone (Figure 9), are synthetic ligands that activate the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and are used to treat T2D for their insulin sensitization effect. It has been suggested that both compounds have a role in regulating different aspects of AD, such as Aβ synthesis, inflammation, and lipid homeostasis. Moreover, these compounds were also found to be involved in mitochondrial activity restoration, a further beneficial effect in NDs, often featuring mitochondrial impairment.

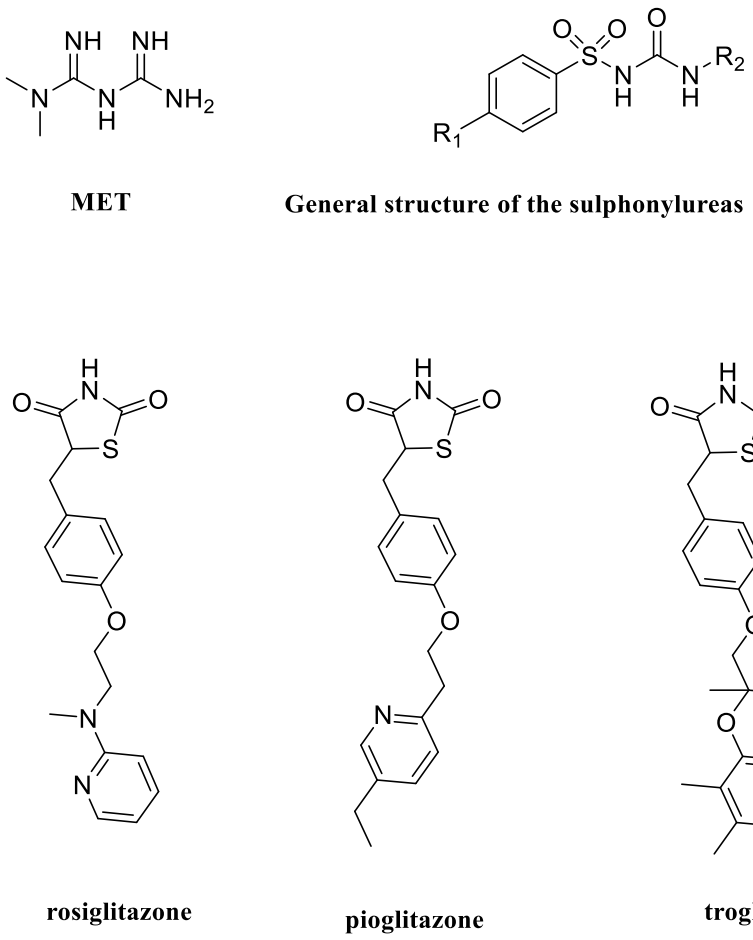


Figure 9. Antidiabetic drugs for AD.

TZDs held a promising therapeutic potential against AD, as supported by preclinical studies [76, 77], although their efficacy has not yet been conclusively demonstrated in clinical trials. Indeed, while in a phase II clinical trial rosiglitazone demonstrated encouraging therapeutic effects, a subsequent phase III trial failed to show any evidence of drug efficacy in the whole AD patient population enrolled in the study [78]. To explain this failure, the authors hypothesized that effective levels of rosiglitazone may not reach the target tissues in patient's brain, being this drug a substrate of P-glycoprotein (P-gp), a major drug efflux transporter present at the BBB compartment. During neuroinflammation, common condition in AD patients, an overexpression of P-gp is detected, leading to a considerable reduction in brain exposure to the drug [78]. Anyway, rosiglitazone has now been withdrawn from the market for diabetic patients as it carries an increased risk of cardiovascular events. Pioglitazone, which has been shown to be cardiovascular safe, is the sole alternative PPARγ agonist available on the market and it is currently undergoing clinical trials for the treatment of AD, with contradictory outcomes.

Poor brain penetration remains the main obstacle to its development for AD cure, and a recent study has speculated, given the structural close similarity with rosiglitazone, that P-gp could again be involved in the low brain penetration of pioglitazone. This study has then validated, with *in vivo* experiments, that P-gp inhibition significantly increases pioglitazone brain penetration [79]. The same authors also investigated the stereoselectivity requirements of pioglitazone uptake in the brain. In mice, the administration of (+)-pioglitazone, instead of the racemic mixture, may significantly increase brain exposure to the drug, suggesting a stereoselective interaction of P-gp with the drug. Therefore, the administration of the more BBB permeating (+) enantiomer could potentially encourage its development for AD treatment [79]. The opposite trend was observed in plasma, and this can result in fewer side effects or drug-induced toxicity in peripheral tissue organs. Taken together, these pharmacokinetic and target-delivery abilities render (+)-pioglitazone a very promising candidate for AD treatment.

Other authors found that pioglitazone inhibits cyclin-dependent kinase 5 (Cdk5) activity by decreasing p35 protein (the specific activator of Cdk5) level. It is known that aberrant activation of Cdk5 is associated with the pathogenesis of several NDs, including AD. Furthermore, blockade of Cdk5 activity by pioglitazone reverses the synaptic dysfunctions and improves spatial memory in AD mouse models [80]. These findings collectively reinforce the potential therapeutic use of this anti-diabetes drug for AD cure. In addition, other researchers reported that long-term treatment with pioglitazone could exert several beneficial effects, among which the greatly reduced hyperphosphorylated tau deposit in hippocampal region [81]. However, the molecular mechanisms underlying this effect are not fully understood: a recent study clearly demonstrated that TZDs, including pioglitazone, rosiglitazone and troglitazone (a parent TZD drug, withdrawn from the market due to liver toxicity, Figure 9) decreased tau-Thr231 phosphorylation in a PPARγ-independent manner, increasing p35 degradation that, as previously reported, induces the repression of Cdk5 activity. These outcomes offer a possible molecular mechanism to explain the improvement of memory deficits and the reduction in NFT deposition observed in AD mouse and cell models [82].

A very recent study dealt with intranasal (IN) nano lipid carriers (NLC) of pioglitazone for its targeted transport to the brain. The *in vitro* drug delivery study showed a sustained release of drug from the NLC. The formulation was found to significantly improve the penetration of pioglitazone across the nasal mucosa *ex vivo* and the subsequently performed toxicity studies confirmed the safety of the formulation for *in vivo* administration. Direct nose to brain transport of the drug was observed from the IN-NLC that significantly improved the concentration of the drug reaching the brain *in vivo*, promoting the repurposing capability of pioglitazone in AD management [83].

Not surprisingly, the opportunity to exploit the multitarget approach was evaluated in this context as well, and a recent study examined the potential of a combination of a putative neuroprotective hormone (leptin) with an antidiabetic drug able to regulate the inflammatory response (pioglitazone) [84]. Leptin is a polypeptide hormone, primarily secreted by adipocytes, that exerts its main biological function in the brain. Recent studies on different Tg animal AD models proved a potential neuroprotective effect of leptin, leading to a decrease in Aβ levels and in intracellular tau phosphorylation both *in vitro* and *in vivo*. Moreover, leptin was also able to modulate LTP and improve memory in rodent experimental models, indicating a promising role in avoiding cognitive decline. This study showed that this combination significantly reduced Aβ levels and hippocampus-dependent spatial memory deficits, alleviated synaptic alterations, and reduced glial response, altering the expression of genes involved in inflammation and oxidative stress. These results support the initial hypothesis that the neuroprotective effect of leptin in combination with the anti-inflammatory action of pioglitazone could appreciably attenuate the pathologic features that characterize AD [84].

Apolipoprotein E (ApoE) is the principal apolipoprotein in the brain, where it acts as a scaffold for HDL-like particles and facilitates the trafficking of lipids throughout the CNS. ApoE also modulates Aβ deposition and clearance in an isoform-dependent manner. Its production is transcriptionally regulated by liver X receptors (LXRs), type II nuclear receptors that function as cholesterol sensors. A recent study investigated a combination therapy of LXR and PPARγ agonists in an AD mouse model and demonstrated that this combination elicits biochemical and behavioral improvements, being able to affect a further increase in LXR target gene production and effectively reducing inflammatory markers. Importantly, combination treatment acts more effectively than individual agonist treatments in improving several biochemical markers and alleviating cognitive impairments in AD mice, with an additional reduction of side effects [85].

6. CARDIOVASCULAR DRUGS

It has been widely recognized that a multifaceted interplay between cardiovascular diseases (CVDs) and the development of cognitive impairment exists, mainly involving hypertension on the one hand and dementia on the other [86]. Not surprisingly, researchers' attention has focused on anti-hypertensive therapeutic regimens as possible modifiers of cognitive impairment.

6.1 Renin angiotensin system modulators

Over the last decade, several studies have pointed towards the renin angiotensin system (RAS) as the main linker between CVDs and AD, as a number of RAS components were altered in AD patients, in particular the key peptide angiotensin II (ANG II) and its derivative angiotensin III (ANG III) [87]. Typically, the RAS cascade starts with the enzyme renin that is responsible for degrading angiotensinogen to the decapeptide angiotensin 1 (ANG I), that in turn is converted into the vasoconstrictor octapeptide ANG II by angiotensin converting enzyme (ACE). This system is finely tuned by the simultaneous activation of the ANG II type 1 receptor (AT1R) responsible for vasoconstriction and primary signalling pathway in RAS, and of the ANG II type 2 receptor (AT2R), mediating vasodilation [88]. Moreover, ANG II can also be metabolized to ANG III by aminopeptidase A (APA) and in turn to ANG IV by aminopeptidase N (APN), both acting on AT1Rs and mediating vasoconstrictive effects [89]. Notably, ANG IV, acting also through the ANG II type IV receptor (AT4R), also called Insulin Regulated Aminopeptidase receptor (IRAP), has been shown to enhance neuronal and cognitive functions [90]. A number of drugs have been marketed in order to correct a dysregulation of this system helping the management of hypertension, either by reducing ANG II production and by preventing its actions on AT1R.

The brain possesses a paracrine RAS, independent from, but interacting with, the peripheral RAS and holds precursors and enzymes required for the biosynthesis of ANG II and its metabolites [91]. It is widely recognized that defects of this system can be noticed in AD, in particular upregulation of ANG II, ANG III and AT1Rs and reduced activity of ACE and APN. These observations gave rise to the angiotensin hypothesis in AD, focusing on the role of RAS in the brain. Indeed, several studies demonstrated the involvement of ANG II in different pathways affecting AD: this peptide proved to reduce acetylcholine release and to stimulate Aβ production and tau phosphorylation when intracerebroventricularly injected to non-transgenic rodents. Moreover, RAS over-activation in AD may modulate neuroinflammation, since ANG II could exert pro- and anti-inflammatory effects, also contributing to BBB maintenance and to calcium signalling. On the other hand, a reduction in APN activity could determine the accumulation of ANG III and a reduction in ANG IV production, whose interaction with AT4Rs proved to improve learning and memory [91].

Taken together, these data suggest that targeting ANG II could not only positively impact on AD pathological mechanisms mediated by Aβ and tau, but also promote cholinergic pathway, and RAS-interfering drugs may thus complement existing therapeutic options [87]. Fortunately, the availability of a plethora of already tested medicines acting on this system that could be repurposed for AD makes this approach easily feasible. Initially, attention was focused on ACE inhibitors (ACEIs), mainly due to their wide diffusion as anti-hypertensive agents. Unfortunately, not all ACEIs were able to cross the BBB, giving rise to conflicting results. These drugs were then categorized in two different groups, the Centrally acting

ACEIs (C-ACEIs), including among others captopril, ramipril, fosinopril, lisinopril, and Non-Centrally acting (NC-ACEIs), such as enalapril, cilazapril, imidapril [92]. Notwithstanding, the results of a study involving C-ACEIs were similarly unconvincing, indicating an improvement in cognitive performance only for a short time. In this picture, captopril emerged as the most promising among C-ACEIs, being endowed of ancillary properties favourable in AD managing. Indeed, treatment of Tg2576 AD mice with captopril (Figure 10) resulted in a decrease of ROS levels and reduced hippocampal amyloidogenic processing of APP [93]. Moreover, a recent study of Asraf and co-workers pinpointed an anti-inflammatory behaviour of captopril, proving that this ACEI can decrease LPS-induced nitric oxide (NO) release from primary mixed glial cells, also regulating a number of mediators involved in microglia activation. In addition, an IN administration of captopril decreased Aβ burden in treated mice, suggesting a neuroprotective role of captopril in AD [94].

More recently, angiotensin receptor blockers (ARBs), widely used to treat hypertension and metabolic disorders, were deeply evaluated in AD managing perspective and generally proved to be superior to ACEIs. Several studies reported positive outcomes with losartan, telmisartan and candesartan in rodent AD models [95-97], and in human clinical trials superior improvement of cognitive loss with respect to ACEIs and other antihypertensive drugs was observed. ARBs were endowed with a pleiotropic biological profile, being not only AT1Rs antagonists, but also PPARγ agonists. This dualistic behaviour appears of great interest in managing neuroinflammation, being AT1R associated with inflammation, and PPARγ exerting anti-inflammatory activity. In this respect, telmisartan (Figure 10) showed the most interesting profile, being a strong AT1R ligand and an efficient PPARγ activator. Moreover, it easily crosses the BBB following oral administration and exerts remarkable anti-inflammatory effects in *in vivo* AD mouse models [98]. A recent study by Wang *et al.* demonstrated that telmisartan was also able to ameliorate Aβ-Oligomers (AβO)-induced microglial inflammation via PPARγ/PTEN pathway, providing the first explanation of the molecular mechanism responsible for the protective effects against microglia inflammation induced by AβO [99].

Interestingly, ARBs treatment has been suggested to induce an increase in ANG IV formation [100], which has been involved in learning and memory amelioration through its interaction with AT4R. To validate this hypothesis, Royea *et al*. evaluated, in a transgenic mouse model of AD, the ability of divalinal, an AT4R antagonist, to impact on the cognitive improvements induced by the ARB losartan (Figure 10). The results proved that blocking AT4Rs effectively countered the beneficial effects of losartan on different central functions, such as cerebrovascular function, neuroinflammation and

spatial learning and memory. These interesting findings reinforce the hypothesis of the involvement of the ANG IV/AT4R cascade in the cognitive and cerebrovascular dysfunctions observed in AD [101].

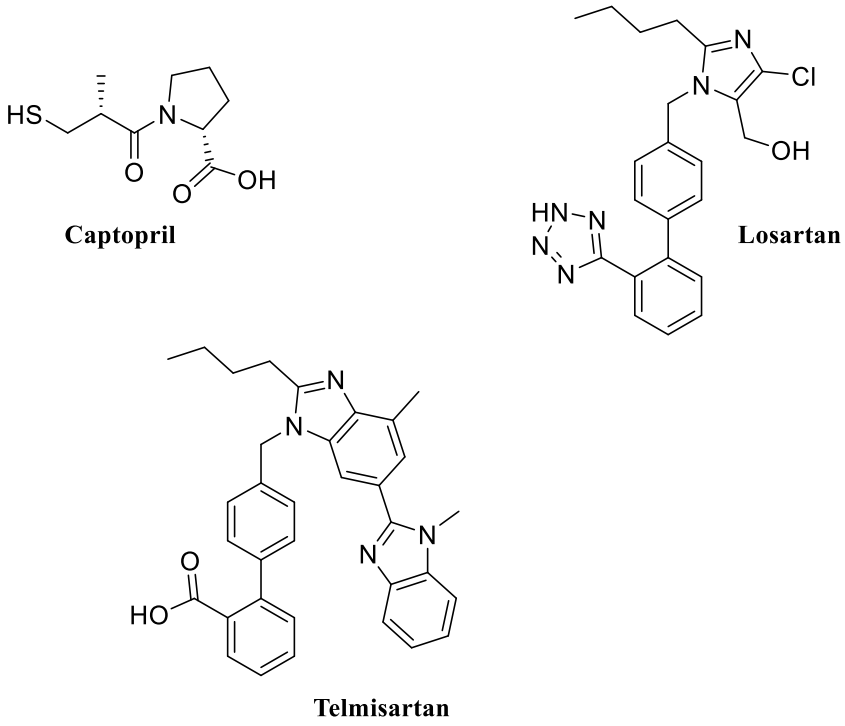


Figure 10. ACE inhibitors and ARBs repurposed for AD.

6.2 Calcium channel blockers

During the evaluation of hypertension as a possible risk factor for AD, it was perceived that certain antihypertensive drugs may exert protective effects independently from their ability to reduce high blood pressure. Epidemiological studies were conducted, particularly with different calcium channel blockers (CCBs), aimed at determining their potential usefulness in AD treatment. Verapamil (Figure 11) was initially identified from a library of approved drugs as autophagy inducer, thus facilitating the degradation of cytoplasmic proteins prone to aggregate and involved in NDs, including α-synuclein and tau [102]. Unfortunately, verapamil is unable to cross the BBB, and then the attention switched to 1,4-dihydropyridines (DHPs), and studies were performed to validate a possible repurposing of this class of antihypertensives in AD. Appreciable results were obtained with amlodipine, nitrendipine and nilvadipine (Figure 11), allowing to establish that the protection against AD could not be explained on the basis of their typical mechanisms of action [6]. Moreover, the observed anti-AD effects of certain DHPs could not be generically attributed to all DHPs, being some of them (i.e. nifedipine) detrimental in AD patients. Experimental studies revealed that nilvadipine did not act in AD with a mechanism

involving its CCB activity, but rather by inducing Aβ lowering effects [103]. Notably, unlike CCB effects, its anti-AD properties were independent of the stereochemistry and included, beside Aβ-lowering, Aβ-BBB clearing, anti-inflammatory activity and inhibition of aberrant tau hyperphosphorylation. Nilvadipine was also capable of increasing regional cerebral blood flow and reducing the expression of BACE 1 enzyme and of proinflammatory mediators that contribute to neuronal damage [104]. Following these observations, a large-scale Phase III investigator-driven clinical trial (NILVAD) was performed. This study was conducted on 511 patients in nine European countries, and was a 78-week randomized, placebo-controlled and double-blind trial. The results, published in 2018, unfortunately showed no overall effect in the selected population covering mild to moderate AD [105]. Nevertheless, these discouraging results need to be carefully evaluated, and some issue should be considered, among which the low dosage employed, the lack of biomarkers to assess the actual diagnosis of AD and finally the timing of the intervention in the course of AD. The failure of anti-Aβ treatments may be due to a late intervention on the disease, when a significant neuronal damage was already established [105].

Recently, Siddiqi and co-workers published a study on felodipine (Figure 11), a BBB penetrating DHP, to evaluate its autophagy-inducer potential on an *in vivo* mouse model of tauopathy, and its potential application to multiple NDs. Felodipine, when administrated in mice at concentrations similar to those applied in humans, was able to induce autophagy in mouse brain, leading to a reduction in neurotoxic proteins levels. These results underline the potential of this drug for treating different NDs sharing the accumulation of misfolded proteins [102].

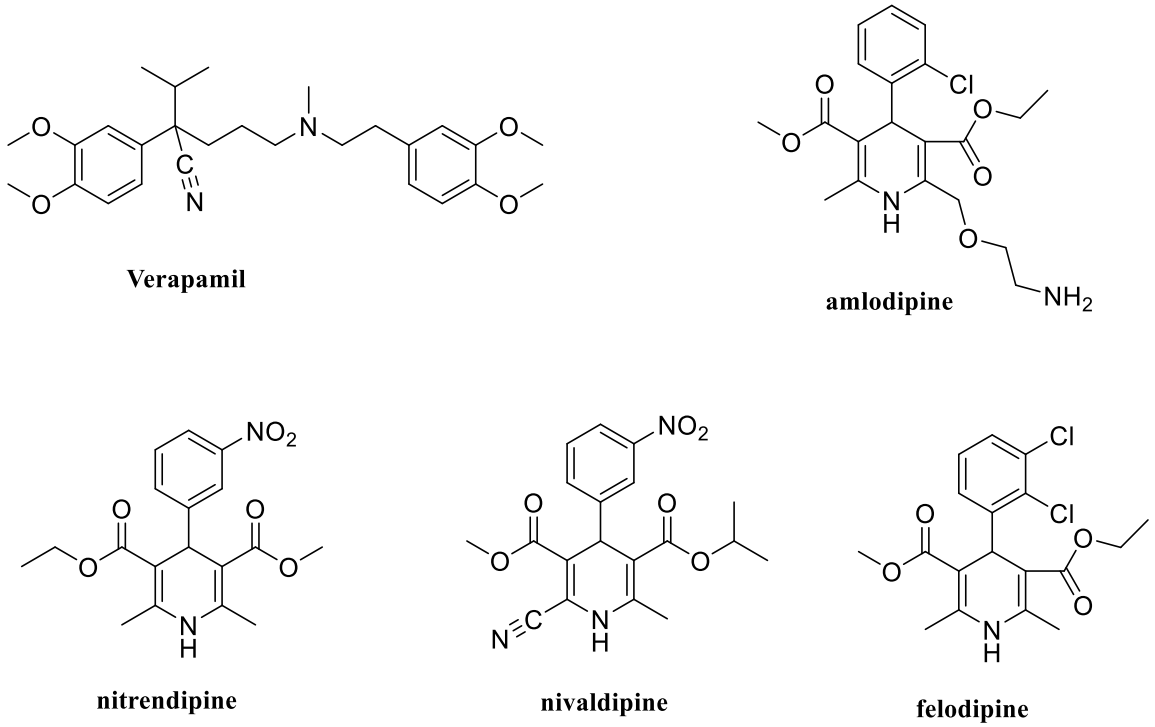


Figure 11. CCBs repurposed for AD.

7. ANTICANCER DRUGS

Cell cycle can be deemed as a sequence of events usually leading to cellular division, occurring in four phases and consisting of complex networked mechanisms and regulatory checkpoints aimed at preserving its proficiency. Cancer cells keep dividing with uncontrolled cell cycles, and an aberrant cell cycle is also observed in post-mortem human and/or animal studies of dying neurons in a series of neurological diseases, including AD [106]. Quiescent cells, such as mature neurons, exist in silent G0 phase and were generally thought to be incapable of re-entering cell cycle. In AD, and in cancer too, an erroneous recruitment of quiescent G0 neurons into G1 phase and beyond occurs, finally leading to neuronal death. Pathological evidence, in the form of aberrant cell cycle markers and regulatory proteins, suggests that this cell cycle re-entry is an early event in AD, which precedes the formation of Aβ plaques and NFTs. Notably, the precise origins of mitotic dysfunction in AD are not fully understood, even if genetic, inflammatory and oxidative defects could induce the pathways from which the altered mitotic signalling could arise [107]. Thus, the link between cancer and NDs in re-entering cell cycle may allow a re-profiling of anticancer drugs acting on this target for AD cure, protecting mature neurons from death. Remarkably, being the cell cycle re-entry an early event in the disease progression, a therapeutic intervention at this stage could be effective in reversing or preventing symptoms.

Src inhibitors target the Src kinase family causing cell cycle arrest. Indeed, abnormal Src activation has been reported in NDs, such as AD. Moreover, Src inhibitors (PP2, AZD0530, Figure 12) have been tested for the treatment of AD and other neurological diseases [106, 108].

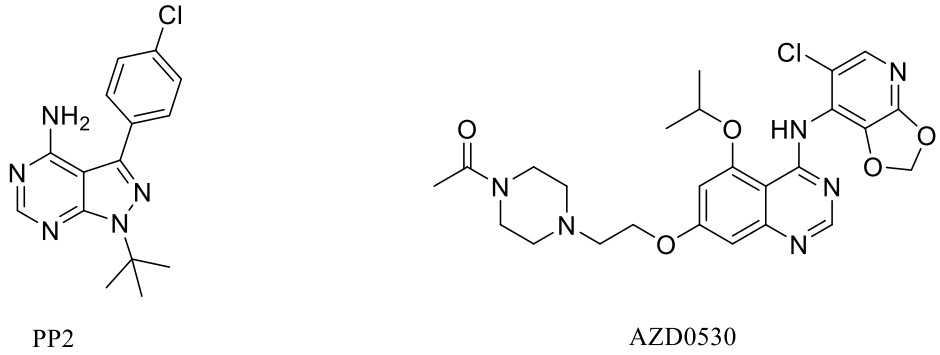


Figure 12. Src inhibitors for AD.

Microtubule stabilizing agents are among the most clinically useful chemotherapeutic drugs, acting by stabilizing microtubules and inhibiting cell division [109, 110]. Neurons are particularly susceptible to microtubule defects, and deregulation of the microtubule cytoskeleton occurs in a range of NDs, making the efforts to reposition microtubule-targeting chemotherapeutic agents for these treatments of particular interest [111]. Currently, taxol (paclitaxel, Figure 13) is a first-line chemotherapy anticancer agent for the treatment of breast and ovarian cancers. Study of paclitaxel binding to microtubules revealed that it displaced tau protein from microtubules, suggesting that this compound and tau share a common binding site and microtubule stabilization mechanism [112]. One major drawback for the potential use of paclitaxel in the treatment of NDs is its limited bioavailability in brain, mainly due to its difficulties in BBB crossing. In addition, the compounds of the taxane family are good substrates for P-gp transporters, which rapidly exports them from cells into the bloodstream, reducing brain bioavailability and contributing to drug resistance. To overcome these hurdles, experimental delivery approaches to the brain by exploiting taxol conjugation or taxol-loaded nanoparticles are in progress.

Cabazitaxel (Jevtana, XRP-6258, Figure 13) is a second-generation microtubule-binding drug from the taxane family. It received approval by the FDA in 2010 for the treatment of refractory metastatic prostate cancer [113]. Like classical taxanes, cabazitaxel disrupts cell cycle by its effects on the microtubule cytoskeleton. Remarkably, this effect can be obtained with a dosage not damaging surrounding primary neurons and astrocytes [114], suggesting for this drug the ability to treat microtubule disorders in brain, in addition to tumors, with lower doses.

TPI-287 is a third generation taxane (Figure 13) and a semisynthetic derivative of abeo-taxane, widely used in cancer therapy and now in Phase I clinical trials for the treatment of mild to moderate symptoms of AD (NCT 01966666).

The Epothilones are 16-membered macrocyclic lactones isolated from the soil dwelling bacteria *Sorangium cellulosum* [115]. Like taxol, they bind and stabilize the microtubule polymers with the consequence of arresting the cell cycle. They are water-soluble and, most importantly, poor substrates of the P-gp transport pump, thereby ensuring good brain bioavailability. Since their first identification, several epothilone analogs have been synthesized, resulting in higher anti-tumor efficacy compared to the original natural compounds epothilones A and B [115]. Analogs of epothilone B, such as epothilone D (Figure 13), has proven beneficial for the amelioration of tau-related pathologies in experimental animal models, by stabilizing microtubule polymers and restoring microtubule dynamics. Now, epotylone D is undergoing clinical trials in patients with mild AD, hoping that this molecule, or its analogues, can provide beneficial treatment.

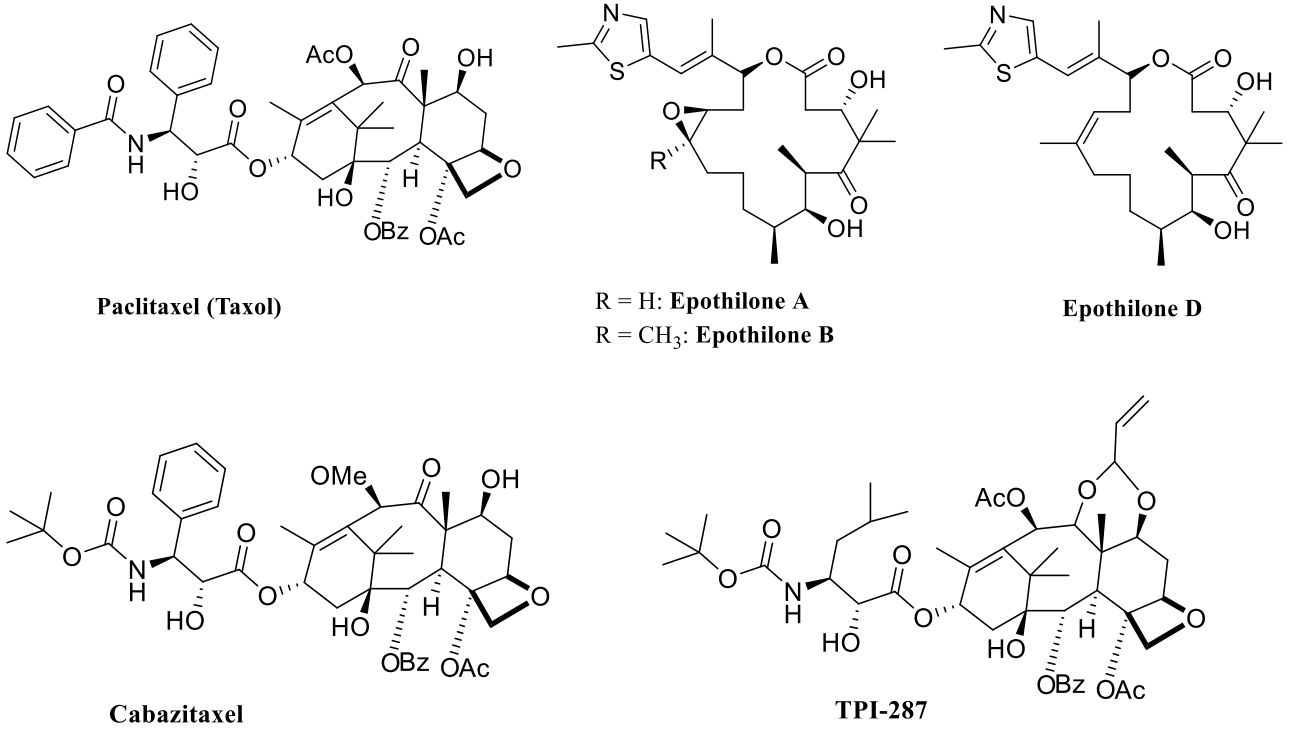


Figure 13. Microtubule stabilizing agents for AD.

The attempt to reposition anticancer drugs for NDs treatment requires extreme attention, representing the cognitive decline a relevant side effect often observed in patients that experienced antitumor therapy. Undoubtedly, a deeper

understanding of the precise mechanisms and differences underpinning the cell cycle of both neuronal and cancer cells is advisable to start a repurposing program of cancer drugs for NDs.

8. CONCLUSION

Drug repurposing is a promising approach allowing the identification of new and fast access points for therapeutic regimens of complex and multifaceted pathologies such as AD. This strategy, based on the repositioning of molecules that have already undergone all the steps of drug development, offers the advantage for new treatments to reach the clinic with reduced timelines, this being a clear benefit for diseases that dramatically need effective pharmacological interventions. In the last years, a number of drugs have been proposed to be repurposed for AD, thanks both to the new hypotheses progressively conceived in order to explain new experimental findings and to the discovery of the involvement of new targets that could be modulated by already employed medicines. Notably, further challenges in repositioning of drugs for NDs are related to the need of BBB crossing and the difficulty in creating appropriate animal models to validate the repositioning strategy and/or to support indications obtained, for instance, via phenotypic screening or computational studies [116]

This review reported some of the most intriguing classes of drugs whose reprofiling for AD has been proposed, together with structural features and modifications performed to overcome the most common issues for CNS-acting drugs, namely poor pharmacokinetics or low brain penetration. At present, CCBs and ARBs seem to be the most promising agents, but also (+) pioglitazone formulations, GLP1 analogues and minocycline showed appreciable *in vivo* data, although additional studies are required to start clinical trials, in order to reduce the likelihood of failures, often occurring in AD research. Moreover, an in-depth evaluation of the mechanism of action of the potential repurposed drug is of pivotal importance, in order to establish which could be the appropriate stage of the disease to start the new therapeutic regimen. Despite the often disappointing clinical trial results, these drugs deserve further investigations, holding validated scaffolds that could serve as a starting point for chemical modifications, possibly leading to new and more effective compounds.

9. LIST OF ABBREVIATIONS

CNS = Central Nervous System

BBB = Blood Brain Barrier

ND = Neurological Disease

AD	=	Alzheimer's Disease
Aβ	=	Beta-Amyloid
APP	=	Amyloid Precursor Protein
BACE	=	Beta-site Amyloid precursor protein Cleaving Enzyme 1 (beta secretase)
NFT	=	NeuroFibrillary Tangles
ChE	=	Cholinesterase
NMDA	=	N-Methyl D-Aspartate
HSV	=	HerpeS Virus
Tg	=	TransGenic
DOX	=	Doxycycline
CEF	=	Ceftriaxone
LTP	=	Long-Term Potentiation
DCS	=	D- Cycloserine
TOR	=	Target Of Rapamycin
CP	=	Chronic Periodontitis
CLQ	=	Clioquinol
PDE	=	PhosphoDiEsterase
PAMPA	=	Parallel Artificial Membrane Permeability Assay
T2D	=	Type 2 Diabetes
MET	=	Metformin
TZD	=	TiaZolidineDione
PPAR	=	Peroxisome Proliferator-Activated Receptor
P-gp	=	P-Glycoprotein
IN	=	IntraNasal
NLC	=	NanoLipid Carriers
LXR	=	Liver X Receptor
ApoE	=	Apolipoprotein E
CVD	=	CardioVascular Disease

RAS = Renin-Angiotensin System

ACE = Angiotensin Converting Enzyme

ATR = AngioTensin Receptor

APA = AminoPeptidase A

APN = AminoPeptidase N

IRAP = Insulin Regulated AminoPeptidase

ACEI = ACE Inhibitor

ARB = Angiotensin Receptor Blockers

CCB = Calcium Channel Blockers

DHP = Dihydropyridines

10. CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise

11. REFERENCES

[1] Frank, S.D. Target-based drug discovery: is something wrong? *Drug Discov. Today*, **2005**, *10*(2), 139–147.

[2] Yicheng, M.; Baowei, Y. Rational application of drug promiscuity in medicinal chemistry. *Fut. Med. Chem.*, **2018**, *10*(15),1835–1851.

[3] Chartier, M.; Morency, L.P.; Zylber, M.I.; Najmanovich, R.J. Large-scale detection of drug off-targets: hypotheses for drug repurposing and understanding side-effects. *BMC Pharmacol. Toxicol.*, **2017**, *18*, 18.

[4] Morphy, R.; Rankovic, Z. Designed mutiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.*, **2005**, *48*(21), 6523–6543.

[5] Ramsay, R.R.; Popovic-Nikolic, M.R.; Nikolic, K.; Uliassi, E.; Bolognesi, M.L. A perspective on multi-target drug discovery and design for complex diseases. *Clin. Transl. Med.*, **2018**, *7*, 3.

[6] Ashburn, T.T.; Thor, K.B. Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.*, **2004**, *3*(8), 673–683.

[7] Parsons, C.G. CNS repurposing-potential new uses for old drugs: examples of screens for Alzheimer's disease, Parkinson's disease and spasticity. *Neuropharmacology*, **2019**, *147*, 4-10.

[8] Prince, M.; Comas-Herrera A.; Knapp, M.; Guerchet, M; Karagiannidou, M. World Alzheimer Report 2016: Improving healthcare for people living with dementia: Coverage, quality and costs now and in the future. *Alzheimer's Disease International*, **2017**.

[9] Alzheimer's Association. 2019 Alzheimer's disease facts and figures. *Alzheimers Dement*., **2019**,*15*(3), 321- 387.

[10] Corbett, A.; Pickett, J.; Burns, A.; Corcoran, J.; Dunnett, S.B.; Edison, P.; Hagan, J.J.; Holmes, C.; Jones, E.; Katona, C.; Kearns, I.; Kehoe, P.; Mudher, A.; Passmore, A.; Shepherd, N.; Walsh, F.; Ballard, C. Drug repositioning for Alzheimer's disease. *Nat. Rev. Drug Disc.*, **2012**, 11(11), 833-846.

[11] Durães, F.; Pinto, M.; Sousa, E. Old drugs as new treatments for neurodegenerative diseases. *Pharmaceuticals*, **2018**, *11*, 44-65.

[12] Folch, J.; Petrov, D.; Ettcheto, M.; Abad, S.; Sánchez-López, E.; García, M.L.; Olloquequi, J.; Beas-Zarate, C.; Auladell, C.; Camins, A. Current research therapeutic strategies for Alzheimer's disease treatment. *Neural Plast.*, **2016**, Article ID 8501693.

[13] Willem, M.; Lammich, S.; Haass, C., Function, regulation and therapeutic properties of beta-secretase (BACE1). *Semin. Cell Dev. Biol.*, **2009**, *20* (2), 175-182.

[14] Hardy, J.; Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, **2002**, *297*(5580), 353-356.

[15] Jamieson, G.A.; Maitland, N.J.; Wilcock, G.K.; Craske, J.; Itzhaki, R.F. Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains. *J. Med. Virol*.**, 1991**, *33*, 224-227.

[16] Itzhaki, R.F.; Lathe, R.; Balin, B.J.; Ball, M.J.; Bearer, E.L.; Braak, H.; Bullido, M.J.; Carter, C.; Clerici, M.; Cosby, S.L.; Del Tredici, K.; Field, H.; Fulop, T.; Grassi, C.; Sue, W.; Griffin, T.; Haas, J.; Hudson, A.P.; Kamer, A.R.; Kell, D.B.; Licastro, F.; Letenneur, L.; Lovheim, H.; Mancuso, R.; Miklossy, J.; Otth, C.; Palamara, A.T.; Perry, G.; Preston, C.; Pretorius, E.; Strandberg, T.; Tabet, N.; Taylor-Robinson, S.D.; Whittum-Hudson, J.A. Microbes and Alzheimer's disease. *J. Alzheimer's Dis*., **2016**, *51*, 979–984.

[17] Itzhaki, R.F. A Turning point in Alzheimer's disease: microbes matter. *J. Alzheimer's Dis.*, **2019**, *72*, 977–980.

[18] Panza, F.; Lozupone, M.; Solfrizzi, V.; Watling, M.; Imbimbo, B.P. Time to test antibacterial therapy in Alzheimer's disease. *Brain*, **2019**, *142*, 2905–2929.

[19] Angelucci, F.; Cechova, K.; Amlerova, J.; Hort, J. Antibiotics, gut microbiota, and Alzheimer's disease. *J. Neuroinflamm.*, **2019**, *16*, 108-118.

[20] Zhuang, Z.; Shen, L.; Li, W.; Fu, X.; Zeng, F.; Gui, L.; Lu, Y.; Cai, M.; Zhu, C.; Tan, Y.; Zheng, P.; Li, H.; Zhu, J.; Zhou, H.; Bu, H.; Wang, Y. Gut Microbiota is Altered in Patients with Alzheimer's Disease. *J. Alzheimer's Dis.*, **2018**, *63*, 1337–1346.

[21] Moir, R.D.; Lathe, R.; Tanzi, R.E. The antimicrobial protection hypothesis of Alzheimer's disease. *Alzheimers Dement.*, **2018**, *14*, 1602–1614.

[22] Socias, S.B.; González-Lizárraga, F.; Avila, C.L.; Vera, C.; Acuña, L.; Sepulveda-Diaz, J.E.; Del-Bel, E.; Raisman-Vozari, R.; Chehin, R.N. Exploiting the therapeutic potential of ready-to-use drugs: repurposing antibiotics against amyloid aggregation in neurodegenerative diseases. *Prog. Neurobiol.*, **2018**, *162*, 17-36.

[23] Santa-Cecília, F.V.; Leite, C.A.; Del-Bel, E.; Raisman-Vozari, R. The neuroprotective effect of doxycycline on neurodegenerative diseases. *Neurotox. Res.*, **2019**, *35*, 981–986.

[24] Forloni, G.; Colombo, L.; Girola, L.; Tagliavini, F.; Salmona, M. Anti-amyloidogenic activity of tetracyclines: studies in vitro. *FEBS Lett.*, **2001**, *487*(3), 404-440.

[25] Diomede, L.; Salmona, M. Tetracycline and its analogues protect *Caenorhabditis elegans* from β amyloid-induced toxicity by targeting oligomers. *Neurobiol. Dis.*, **2010**, *40*(2), 424-431.

[26] Costa, R.; Speretta, E.; Crowther, D.C.; Cardoso, I. Testing the therapeutic potential of doxycycline in a *Drosophila melanogaster* model of Alzheimer disease. *J. Biol. Chem*., **2011**, *286*, 41647–41655.

[27] Gautieri, A.; Beeg, M.; Gobbi, M.; Rigoldi, F.; Colombo L.; Salmona, M. The anti-amyloidogenic action of doxycycline: a molecular dynamics study on the interaction with Aβ42. *Int. J. Mol. Sci.*, **2019**, *20*, 4641-4653.

[28] Noble, W.; Garwood, C.J.; Hanger, D.P. Minocycline as a potential therapeutic agent in neurodegenerative disorders characterized by protein misfolding. *Prion*, **2009**, 3, 78-83.

[29] Amani, M.; Shokouhi, G.; Salari, A. Minocycline prevents the development of depression-like behavior and hippocampal inflammation in a rat model of Alzheimer's disease. *Psychopharmacology*, **2019**, *236*, 1281–1292.

[30] Howard, R.; Zubko, O.; Bradley, R.; Harper, E.; Pank, L.; O'Brien, J.; Fox, C.; Tabet, N.; Livingston, G.; Bentham, P.; McShane, R.; Burns, A.; Ritchie, C.; Reeves, S.; Lovestone, S.; Ballard, C.; Noble, W.; Nilforooshan, R.; Wilcock, G.; Gray, R. Minocycline in Alzheimer disease efficacy (MADE) Trialist group. Minocycline at 2 different dosages vs placebo for patients with mild Alzheimer disease: a randomized clinical trial. *JAMA Neurol*., **2020**, *77*(2), 164-174.

[31] Tikhonova, M.A.; Amstislavskaya, T.G.; Belichenko, V.M.; Fedoseeva, L.A.; Kovalenko, S.P.; Pisareva, E.E.; Avdeeva, A.S.; Kolosova, N.G.; Belyaev, N.D.; Aftanas, L.I. Modulation of the expression of genes related to the system of amyloid-beta metabolism in the brain as a novel mechanism of ceftriaxone neuroprotective properties. *BMC Neurosci.*, **2018**, *19*(Suppl. 1), 13.

[32] Akina, S.; Thati, M.; Puchchakayala, G. Neuroprotective effect of ceftriaxone and selegiline on scopolamine induced cognitive impairment in mice. *Adv. Biol. Res.*, **2013**, *7*, 266–275.

[33] Zumkehr, J.; Rodriguez-Ortiz, C.J.; Cheng, D.; Kieu, Z.; Wai, T.; Hawkins, C.; Kilian, J.; Lim, S.L.; Medeiros, R.; Kitazawa, M. Ceftriaxone ameliorates tau pathology and cognitive decline via restoration of glial glutamate transporter in a mouse model of Alzheimer's disease. *Neurobiol. Aging*, **2015**, *36*, 2260–2271.

[34] Tai, C.H.; Bellesi, M.; Chen, A.C.; Lin, C.L.; Li, H.H.; Lin, P.J.; Liao, W.C.; Hung, C.S.; Schwarting, R. K.; Ho, Y.J. A new avenue for treating neuronal diseases: ceftriaxone, an old antibiotic demonstrating behavioral neuronal effects. *Behav. Brain Res*., **2019**, *64*, 149-156.

[35] Tucker, S.; Ahl, M.; Bush A.; Westaway, D.; Huang, X.; Rogers, J.T. Pilot study of the reducing effect on amyloidosis in vivo by three FDA pre-approved drugs via the Alzheimer's APP 5' untranslated region. *Curr. Alzheimer Res*., **2005**, *2*, 249–254.

[36] Kountouras, J.; Boziki, M.; Gavalas, E.; Zavos, C.; Deretzi, G.; Grigoriadis, N.; Tsolaki, M.; Chatzopoulos, D.; Katsinelos, P.; Tzilves, D.; Zabouri, A.; Michailidou, I. Increased cerebrospinal fluid Helicobacter pylori antibody in Alzheimer's disease. *Int. J. Neurosci.*, **2009**, *119*, 765–777.

[37] Scofield, M.D.; Kalivas, P.W. Astrocytic dysfunction and addiction: consequences of impaired glutamate homeostasis. *Neuroscientist*, **2014**, *20*, 610–622.

[38] Peyrovian, B.; Rosenblat, J.D.; Pan, Z.H.; Iacobucci, M.; Brietzke, E.; McIntyre, R.S. The glycine site of NMDA receptors: a target for cognitive enhancement in psychiatric disorders *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **2019**, *92*, 387-404.

[39] Tsai, G.E.; Falk, W.E.; Gunther, J, Coyle, J.T. Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. *Am. J. Psychiatry*, **1999**, *156*, 467–469.

[40] Chaturvedi, S.K.; Zaidi, N.; Alam, P.; Khan, J.M.; Qadeer, A.; Siddique, I.A.; Asmat, S.; Zaidi, Y.; Khan, R.H. Unraveling comparative anti-amyloidogenic behavior of pyrazinamide and D-Cycloserine: a mechanistic biophysical insight. *PLoS One*, **2015**, *10* (8) e0136528.

[41] Umeda, T.; Ono, K.; Sakai, A.; Yamashita, M.; Mizuguchi, M.; Klein, W.L.; Yamada, M.; Mori, M.; Tomiyama, T. Rifampicin is a candidate preventive medicine against amyloid-β and tau oligomers. *Brain*, **2016**, *139*, 1568–1586.

[42] Espargaró, A.; Pont, C.; Gamez, P.; Muñoz-Torrero, D.; Sabate, R. Amyloid pan-inhibitors: one family of compounds to cope with all conformational diseases. *ACS Chem. Neurosci*., **2019**, *10* (3), 1311–1317.

[43] Tomiyama, T.; Asano, S.; Suwa, Y.; Morita, T.; Kataoka, K.; Mori, H.; Endo, N. Rifampicin prevents the aggregation and neurotoxicity of amyloid β protein *in vitro*. *Biochem. Biophys. Res. Commun*., **1994**, *204* (1), 76–83.

[44] Tomiyama, T.; Shoij, A.; Kataoka, K.; Suwa, Y.; Asano, S.; Kaneko, H.; Endo, N. Inhibition of amyloid beta protein aggregation and neurotoxicity by rifampicin. Its possible function as a hydroxyl radical scavenger. *J. Biol. Chem*., **1996**, *271* (12), 6839–6844.

[45] Camardo, J.; The Rapamune era of immunosuppression 2003: the journey from the laboratory to clinical transplantation. *Transpl. Proc.*, **2003**, *35*, 18S–24S.

[46] Brown, E.J.; Albers, M.W.; Shin, T.B.; Ichikawa, K.; Keith, C.T.; Lane, W.S.; Shreibers, S.L. A mammalian protein targeted G1-arresting Rapamycin-receptor complex. *Nature*, **1994**, *369*, 756–758.

[47] Richardson, A.; Galvan, V.; Lin, A.L.; Oddo, S. How longevity research can lead to therapies for Alzheimer's disease: The rapamycin story. *Exp. Gerontol*., **2015**, *68*, 51–58.

[48] Kaye, E.K.; Valencia, A.; Baba, N.; Spiro III, A.; Dietrich, T.; Garcia, R. Tooth loss and periodontal disease predict poor cognitive function in older men. *J. Am. Geriatr. Soc.*, **2010**, *58*, 713–718.

[49] Dominy, S.S.; Lynch, C.; Ermini, F.; Benedyk, M.; Marczyk, A.; Konradi, A.; Nguyen, M.; Haditsch, U.; Raha, D.; Griffin, C.; Holsinger, L.J.; Arastu-Kapur, S.; Kaba, S.; Lee, A.; Ryder, M.I.; Potempa, B.; Mydel, P.; Hellvard, A.; Adamowicz, K.; Hasturk, H.; Walker, G.D.; Reynolds, E.C.; Faull, R.L. M.; Curtis, M.A.; Dragunow, M.; Potempa, J. *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.*, **2019**, *5*, eaau3333.

[50] Qin, Q.S.; Li, Y. Herpesviral infections and antimicrobial protection for Alzheimer's disease: implications for prevention and treatment. *J. Med. Virol.*, **2018**, *91* (8), 1368–1377.

[51] Allnutt, M.A.; Johnson, K.; Bennett, D.A.; Connor, S.M.; Troncoso, J.C.; Pletnikova, O.; Albert, M.S.; Resnick, S.M.; Scholz, S.W.; De Jager, P.L.; Jacobson, S. Human Herpesvirus 6 detection in Alzheimer's disease cases and controls across multiple cohorts. *Neuron*, **2020**, *105* (6) 1027–1035.e2.

[52] Wozniak, M.A.; Frost, A.L.; Preston, C.M.; Itzhaki, R.F. Antivirals reduce the formation of key Alzheimer's disease molecules in cell cultures acutely infected with *Herpes simplex* virus type 1. *PLoS One*, **2011**, *6*, e25152.

[53] Kimura, T.; Goto, M. Existence of senile plaques in the brains of elderly leprosy patients. *Lancet*, **1993**, *342*, 1364.

[54] Van Gool, W.A.; Weinstein, H.C.; Scheltens, P.K.; Walstra, G.J. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. *Lancet*, **2001**, *358*, 455–460.

[55] Zatta, P.; Drago, D.; Bolognin, S.; Sensi S.L. Alzheimer's disease, metal ions and metal homeostatic therapy. *Trends Pharmacol. Sci.*, **2009**, *30*, 346–355.

[56] Grossi, C.; Francese, S.; Casini, A.; Rosi, M.C.; Luccarini, I.; Fiorentini, A.; Gabbiani, C.; Messori, L.; Moneti, G.; Casamenti, F. Clioquinol decreases amyloid-beta burden and reduces working memory impairment in a transgenic mouse model of Alzheimer's disease. *J. Alzheimers Dis.*, **2009**, *17*, 423–440.

[57] Garcia-Osta, A.; Cuadrado-Tejedor, M.; Garcia-Barroso, C.; Oyarzabal, J.; Franco, R. Phosphodiesterases as therapeutic targets for Alzheimer's disease. *ACS Chem. Neurosci*., **2012**, *3*, 832−844.

[58] Chen, S.K.; Zhao, P.; Shao, Y.X.; Li, Z.; Zhang, C.; Liu, P.; He, X.; Luo, H.B.; Hu, X. Moracin M from *Morus alba L*. is a natural phosphodiesterase-4 inhibitor. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 3261−3264.

[59] Fernandez-Bachiller, M.I.; Perez, C.; Gonzalez-Munoz, G.C.; Conde, S.; Lopez, M.G.; Villarroya, M.; García, A.C.; Rodríguez-Franco, M.I. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. *J. Med. Chem.*, **2010**, *53*, 4927–4937.

[60] Antequera, D.; Bolosa, M.; Spuch, C.; Pascual, C.; Ferrer, I.; Fernandez-Bachiller, M.I.; Rodríguez-Franco, M.I.; Carro, E. Effects of a tacrine-8-hydroxyquinoline hybrid (IQM-622) on Aβ accumulation and cell death: Involvement in hippocampal neuronal loss in Alzheimer's disease. *Neurobiol. Dis*., **2012**, *46*, 682–691.

[61] Prati, F.; Bergamini, C.; Fato, R.; Soukup, O.; Korabecny, J.; Andrisano, V.; Bartolini, M.; Bolognesi, M.L. Novel 8-hydroxyquinoline derivatives as multitarget compounds for the treatment of Alzheimer's disease. *ChemMedChem*, **2016**, *11*, 1284–1295.

[62] Wang, Z.; Wang, Y.; Wang, B.; Li, W.; Huang, L.; Li, X. Design, synthesis, and evaluation of orally available clioquinol-moracin M hybrids as multitarget-directed ligands for cognitive improvement in a rat model of neurodegeneration in Alzheimer's disease. *J. Med. Chem.*, **2015**, *58*, 8616–8637.

[63] Wang, Z.; Cao, M.; Xiang, H.; Wang, W.; Feng, X.; Yang, X. WBQ5187, a multitarget directed agent, ameliorates cognitive impairment in a transgenic mouse model of Alzheimer's disease and modulates cerebral β Amyloid, gliosis, cAMP levels, and neurodegeneration. *ACS Chem. Neurosci.*, **2019**, *10*, 4787–4799.

[64] Hu, J.; Pan, T.; An, B.; Li, Z.; Li, X.; Huang, L. Synthesis and evaluation of clioquinol-rolipram/roflumilast hybrids as multitarget-directed ligands for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2019**, *163*, 512–526.

[65] Mao, F.; Yan, J.; Li, J.; Ji, L.; Miao, H.; Sun, Y.; Huang, L.; Li, X. New multi-target-directed small molecules against Alzheimer's disease: a combination of resveratrol and clioquinol. *Org. Biomol. Chem*., **2014**, *12*, 5936–5944.

[66] Rajasekhar, K.; Mehta, K.; Govindaraju, T. Hybrid multifunctional modulators inhibit multifaceted Aβ toxicity and prevent mitochondrial damage. *ACS Chem. Neurosci.*, **2018**, *9*, 1432–1440.

[67] Di Giovanni, S.D.; Eleuteri, S.; Paleologou, K.E.; Yin, G.; Zweckstetter, M.; Carrupt, P.A.; Lashuel, H.A. Entacapone and tolcapone, two catechol-o-methyltransferase inhibitors, block fibril formation of α-synuclein and β-amyloid and protect against amyloid-induced toxicity. *J. Biol. Chem.*, **2010**, *285*, 14941−14954.

[68] Mohamed, T.; Hoang, T.; Jelokhani-Niaraki, M.; Rao, P.P.N. Tau-derived-hexapeptide ^{306}VQIVYK^{311} aggregation inhibitors: nitrocatechol moiety as a pharmacophore in drug design. *ACS Chem. Neurosci.*, **2013**, *4*, 1559–1570.

[69] Bastianetto, S.; Krantic, S.; Quirion. R. Polyphenols as potential inhibitors of amyloid aggregation and toxicity: possible significance to Alzheimer's disease. *Mini-Rev. Med. Chem.*, **2008**, *8* (5), 429–435.

[70] Tiago, S.; Mohamed, T.; Shakeri, A.; Rao, P.P.N.; Soares da Silva, P.; Remiao, F.; Borges, F. Repurposing nitrocatechols: 5-Nitro-α-cyanocarboxamide derivatives of caffeic acid and caffeic acid phenethyl ester effectively inhibit aggregation of tau-derived hexapeptide AcPHF6. *Eur. J. Med. Chem.*, **2019**, *167*, 146–152.

[71] Lanza, V.; Milardi, D.; Di Natale, G.; Pappalardo, G. Repurposing of Copper(II)-chelating Drugs for the Treatment of Neurodegenerative Diseases. *Curr. Med. Chem*., **2018**, *25*, 525-539.

[72] Candeias, E.; Duarte, A.I.; Carvalho, C.; Correia, S.C.; Cardoso, S.; Santos, R.X.; Placido, A.I.; Perry, G.; Moreira. P.I.; Critical review. The impairment of insulin signaling in Alzheimer's disease. *IUBMB Life*, **2012**, *64* (12), 951–957.

[73] Hsu, C.C.; Wahlqvist, M.L.; Lee, M.S.; Tsai, H.N. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J. Alzheimers Dis.*, **2011**, *24*, 485–493.

[74] Cheng, C.; Lin, C.; Tsai, Y.W.; Tsai, C.J.; Chou, P.H.; Lan, T.H. Type 2 diabetes and antidiabetic medications in relation to dementia diagnosis. *J. Gerontol. A. Biol. Sci. Med. Sci.*, **2014**, *69* (10), 1299–1305.

[75] Chena, Y.; Zhou, K.; Wang, R.; Liu, Y.; Kwak, Y.D.; Ma, T.; Thompson, R.C.; Zhao, Y.; Smith, L.; Gasparini, L.; Luo, Z.; Xu, H.; Liao, F.F. Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. *PNAS*, **2009**, *106*, 3907–3912.

[76] Escribano, L.; Simón, A-M.; Gimeno, E.; Cuadrado-Tejedor, M.; López de Maturana, R.; García-Osta, A.; Ricobaraza, A.; Pérez-Mediavilla, A.; Del Río, J.; Frechilla, D. Rosiglitazone rescues memory impairment in Alzheimer's transgenic mice: mechanisms involving a reduced amyloid and tau pathology. *Neuropsychopharmacol.*, **2010**, *35*, 1593–1604.

[77] Papadopoulos, P.; Rosa-Neto, P.; Rochford, J.; Hamel, E. Pioglitazone improves reversal learning and exerts mixed cerebrovascular effects in a mouse model of Alzheimer's disease with combined amyloid-beta and cerebrovascular pathology. *PloS One*, **2013**, *8*, e68612.

[78] Gold, M. Alderton, C.; Zvartau-Hind, M.; Egginton, S.; Saunders, A.M.; Irizarry, M.; Craft, S.; Landreth, G.; Linnamägi, Ü.; Sawchak, S. Rosiglitazone monotherapy in mild-to-moderate Alzheimer's disease: results from a randomized, double-blind, placebo-controlled phase III study. *Dement. Geriatr. Cogn. Disord*., **2010**, *30*, 131–146.

[79] Chang, K.L.; Pee, H.N.; Yang, S.; Ho, P.C. Influence of drug transporters and stereoselectivity on the brain penetration of pioglitazone as a potential medicine against Alzheimer's disease. *Sci. Rep.*, **2015**, 5, 9000.

[80] Chen, J.; Li, S.; Sun, W.; Li, J. Anti-diabetes drug pioglitazone ameliorates synaptic defects in AD transgenic mice by inhibiting cyclin-dependent kinase 5 activity. *PLoS One*, **2015**, *10* (4), e0123864.

[81] Searcy J.L.; Phelps J.T.; Pancani T.; Kadish, I.; Popovic, J.; Anderson, K.L.; Beckett, T.L.; Murphy, M.P.; Chen, K-C.; Blalock, E.M.; Landfield, P.W.; Porter, N.M.; Thibaulta, O. Long-term pioglitazone treatment improves learning and attenuates pathological markers in a mouse model of Alzheimer's disease. *J. Alzheimers Dis.*, **2012**, *30*, 943–961.

[82] 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. *J. Neurochem.*, **2013**, *126*, 685–695.

[83] Jojo, G.M.; Kuppusamy, G.; De, A.; Karri, V.V.S. N.R. Formulation and optimization of intranasal nanolipid carriers of pioglitazone for the repurposing in Alzheimer's disease using Box-Behnken design. *Drug Dev. Ind. Pharm.*, **2019**, *45* (7), 1061–1072.

[84] Fernandez-Martos, C.M.; Atkinson, R. A.K.; Chuah, M.I.; King, A.E.; Vickers J.C. Combination treatment with leptin and pioglitazone in a mouse model of Alzheimer's disease. *Alzheimer's & Dementia: TRCI*, **2017**, *3*, 92–106.

[85] Skerrett, R.; Pellegrino, M.P.; Casali, B.T.; Taraboanta, L.; Landreth, G.E. Combined liver X receptor/peroxisome proliferatoractivated receptor γ agonist treatment reduces amyloid β levels and improves behavior in amyloid precursor protein/presenilin 1 mice. *J. Biol. Chem.*, **2015**, *290*, (35), 21591–21602.

[86] Whitmer, R.; Sidney, S.; Selby, J.; Johnston, S.C.; Yaffe, K. Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology*, **2005**, *64*, 277–281.

[87] Kehoe, P.G. The coming of age of the angiotensin hypothesis in Alzheimer's disease: progress toward disease prevention and treatment? *J. Alzheimers Dis.*, **2018**, *62*, 1443–1466.

[88] Kanaide, H.; Ichiki, T.; Nishimura, J.; Hirano, K. Cellular mechanism of vasoconstriction induced by angiotensin II: It remains to be determined. *Circ. Res.*, **2003**, *93*, 1015–1017.

[89] Reaux, A.; Iturrioz, X.; Vazeux, G.; Fournie-Zaluski, M.C.; David, C.; Roques, B.P.; Corvol, P.; Llorens-Cortes, C. Aminopeptidase A, which generates one of the main effector peptides of the brain renin-angiotensin system, angiotensin III, has a key role in central control of arterial blood pressure. *Biochem. Soc. Trans.*, **2000**, *28*, 435–440.

[90] Dupont, A.G.; Yang, R.; Smolders, I.; Vanderheyden, P. IRAP and AT(1) receptor mediated effects of angiotensin IV. *J. Intern. Med.*, **2009**, *265*, 401-403.

[91] Ganten, D.; Marquez-Julio, A.; Granger, P.; Hayduk, K.; Karsunky, K.P.; Boucher, R.; Genest, J. Renin in dog brain. *Am. J. Physiol*., **1971**, *221*, 1733–1737.

[92] Fazal, K.; Perera, G.; Khondoker, M.; Howard, R.; Stewart, R. Associations of centrally acting ACE inhibitors with cognitive decline and survival in Alzheimer's disease. *B. J. Psych. Open*, **2017**, *3*, 158–164.

[93] Kaur, P; Muthuraman, A.; Kaur, M. The implications of angiotensin-converting enzymes and their modulators in neurodegenerative disorders: current and future perspectives. *ACS Chem. Neurosci.*, **2015**, *6* (4), 508–521.

[94] Asraf, K.; Torika, N.; Apte, R.N.; Fleisher-Berkovich, S. Microglial activation is modulated by captopril: *in Vitro* and *in Vivo* studies. *Front. Cell. Neurosci*., **2018**, 12, 116.

[95] Ongali, B.; Nicolakakis, N.; Tong, X.K.; Aboulkassim, T.; Papadopoulos, P.; Rosa-Neto, P.; Lecrux, C.; Imboden, H.; Hamel, E. Angiotensin II type 1 receptor blocker losartan prevents and rescues cerebrovascular, neuropathological and cognitive deficits in an Alzheimer's disease model. *Neurobiol. Dis.*, **2014**, *68*, 126–136.

[96] Shindo, T.; Takasaki, K.; Uchida, K.; Onimura, R.; Kubota, K.; Uchida, N. Irie, K.; Katsurabayashi, S.; Mishima, K.; Nishimura, R.; Fujiwara, M.; Iwasaki, K. Ameliorative effects of telmisartan on the inflammatory response and impaired spatial memory in a rat model of Alzheimer's disease incorporating additional cerebrovascular disease factors. *Biol. Pharm. Bull.*, **2012**, *35* (12), 2141–2147.

[97] Villapol, S.; Yaszemski, A.K.; Logan, T.T.; Sanchez-Lemus, E.; Saavedra, J.M.; Symes, A.J. Candesartan, an angiotensin II AT(1)-receptor blocker and PPAR-gamma agonist, reduces lesion volume and improves motor and memory function after traumatic brain injury in mice. *Neuropsychopharmacology*, **2012**, *37* (13), 2817–2829.

[98] Villapol, S.; Saavedra J.M. Neuroprotective effects of angiotensin receptor blockers. *Am. J. Hypertens.*, **2015**, *28* (3) 289–299.

[99] Wang, Z.F.; Li, J.; Ma, C.; Huang, C.; Li, Z.Q. Telmisartan ameliorates Aβ oligomer-induced inflammation via PPARγ/PTEN pathway in BV2 microglial cells *Biochem. Pharmacol.*, **2020**, *171*, 113674.

[100] Braszko, J.J.; Walesiuk, A.; Wielgat, P. Cognitive effects attributed to angiotensin II may result from its conversion to angiotensin IV. *J. Renin Angiotensin Aldosterone Syst.*, **2006**, *7*, 168–174.

[101] Royea, J.; Tong, Z.X.K.; Hamel E. Angiotensin IV receptors mediate the cognitive and cerebrovascular benefits of losartan in a mouse model of Alzheimer's disease. *J. Neurosci.*, **2017**, *37* (22), 5562–5573.

[102] Farah H. Siddiqi, F.H.; Menzies, F.M.; Lopez, A.; Stamatakou, E.; Karabiyik, C.; Ureshino, R.; Ricketts, T.; Jimenez-Sanchez, M.; Esteban, M.A.; Lai, L.; Tortorella, M.D.; Luo, Z.; Liu, H.; Metzakopian, E.; Fernandes, H.J.R.; Bassett, A.; Karran, E.; Miller, B.L.; Fleming, A.; Rubinsztein, D.C. Felodipine induces autophagy in mouse brains with pharmacokinetics amenable to repurposing. *Nat. Commun.*, **2019**, *10*, 1817.

[103] Paris, D. Bachmeier, C.; Patel, N.; Quadros, A.; Volmar, C-H.; Laporte, V.; Ganey, J.; Beaulieu-Abdelahad, D.; Ait-Ghezala, G.; Crawford, F.; Mullan, M.J. Selective antihypertensive dihydropyridines lower Aβ accumulation by targeting both the production and the clearance of Aβ across the blood–brain barrier. *Mol. Med*., **2011**, *17*, 149–162.

[104] McCarthy, H.; Kennelly, S.; Crawford, F.; Mullan, M.; Cregg, F.;. Lawlor, B.A. Repurposing nilvadipine for treatment of dementia: an overview. *Drugs Fut*., **2017**, *42*(5), 281–284.

[105] Lawlor, B.; Segurado, R.; Kennelly, S.; Olde Rikkert, M.G.M.; Howard, R.; Pasquier, F.; Borjesson-Hanson, A.; Tsolaki, M.; Lucca, U.; Molloy, D.W.; Coen, R.; Riepe, M.W.; Kalman, J.; Kenny, R.A.; Cregg, F.; O'Dwyer, S.; Walsh, C.; Adams, J.; Banzi, R.; Breuilh, L.; Daly, L.; Hendrix, S.; Aisen, P.; Gaynor, S.; Sheikhi, A.; Taekema, D.G.; Verhey, F.R.; Nemni, R.; Nobili, F.; Franceschi, M.; Frisoni, G.; Zanetti, O.; Konsta, A.; Anastasios, O.; Nenopoulou, S.; Tsolaki-Tagaraki, F.; Pakaski, M.; Dereeper, O.; de la Sayette, V.; Sénéchal, O.; Lavenu, I.; Devendeville, A.; Calais, G.; Crawford, F.; Mullan, M., for the NILVAD Study Group. Nilvadipine in mild to moderate Alzheimer disease: A randomised controlled trial. *PLoS Med*., **2018**, *15*(9): e1002660.

[106] Liu, D.; Sharp, F.R.; Van, K.C.; Ander, B.P.; Ghiasvand, R.; Zhan, X.; Stamova, B.; Jickling, G.C.; Lyeth, B.G. Inhibition of Src family kinases protects hippocampal neurons and improves cognitive function after traumatic brain injury. *J. Neurotrauma*, **2014**, *31*, 1268–1276.

[107] Bonda, D.J.; Lee, H-pil, Kudo, W.; Zhu, X.; Smith, M.A.; Lee, H-gon. Pathological implications of cell cycle re-entry in Alzheimer disease. *Expert Rev. Mol. Med*., **2010**, Jun 29, *12*, e19.

[108] Nygaard, H.B.; van Dyck, C.H.; Strittmatter, S.M. Fyn kinase inhibition as a novel therapy for Alzheimer's disease. *Alzheimers Res. Ther*., **2014**, *6*,8.

[109] Wani, M.C.; Taylor, H.L.; Wall, M.E.; Coggon, P.; McPhail, A.T. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Am. Chem. Soc.*, **1971**, *93*, 2325–2327.

[110] Schiff, P.B.; Horwitz, S.B. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. U.S.A.*, **1980**, *77*, 1561–1565.

[111] Varidaki, A.; Hong, Y.; Coffey, E.T. Repositioning Microtubule Stabilizing Drugs for Brain Disorders. *Front. Cell Neurosci.*, **2018**, *12*, 226.

[112] Kar, S.; Fan, J.; Smith, M.J.; Goedert, M.; Amos, L.A. Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. *EMBO J.*, **2003**, *22*, 70–77.

[113] Abidi, A. Cabazitaxel: a novel taxane for metastatic castration–resistant prostate cancer–current implications and future prospects. *J. Pharmacol. Pharmacother.*, **2013**, *4*, 230–237.

[114] Ghoochani, A.; Hatipoglu Majernik, G.; Sehm, T.; Wach, S.; Buchfelder, M.; Taubert, H., Eyupoglu, I.Y.; Savaskan, N. Cabazitaxel operates anti–metastatic and cytotoxic via apoptosis induction and stalls brain tumor angiogenesis. *Oncotarget*, **2016**, *7*, 38306–38318.

[115] Hoefle, G.P.D.; Bedorf, N.D.; Gerth, K.D.; Reichenbach, H.P.D. Epothilone, deren herstellungsverfahren sowie sie enthaltende mittel epothilones, their manufacturing processes as well as medium containing them. *Google Patents, PCT/EP1997006442. Braunschweig: WIPO*, **1993**.

[116] Paranjpe, M.D.; Taubes, A.; Sirota, M. Insights into computational drug repurposing for neurodegenerative disease. *Trends Pharmacol. Sci*., **2019**, *40*(8), 565-576.