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REVIEW ARTICLE

From combinations to multitarget-directed ligands: A continuum in Alzheimer's disease polypharmacology

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Abstract

The continued drug discovery failures in complex neurodegenerative diseases, including Alzheimer's disease (AD), has raised questions about the classical paradigm "one-drug, one-target, one-disease." In parallel, the ever-increasing awareness of the multiplicity of the underlying pathways has led to the affirmation of polypharmacological approaches. Polypharmacology, which broadly embodies the use of pharmaceutical agents acting on multiple targets, seems to be the best way to restore the complex diseased network and to provide disease-modifying effects in AD. In this review, our aim is to provide a roadmap into a world that is still only partly explored and that should be seen as a continuum of pharmacological opportunities, from drug combinations to multitarget-directed ligands (both codrugs and hybrids). Each modality has unique features that can be effectively exploited by medicinal chemists. We argue that understanding their advantages and drawbacks is very helpful in choosing a proper approach and developing successful AD multitarget drug-discovery endeavors. We also briefly dwell on (co)target validation, an aspect that is quite often neglected, but critical for an efficient clinical translation. We substantiate our discussion with instructive examples taken from the recent literature. Our wish is that, in spite of the specter of the high attrition rates, best researchers preferring to enter, stay, and progress in the

field would help grow the sector and develop AD polypharmacology to full potential.

KEYWORDS
drug combinations, hybrid compounds, MTDLs, multitarget drug discovery, polypharmacology

1 | INTRODUCTION

The concept of "magic bullets"—drugs with exquisite specificity for their target—is an old and venerable theme in medicinal chemistry and drug discovery.[1]

Introduced by Paul Ehrlich for the development of antibacterial agents, it was based on the idea that it could be possible to specifically kill bacteria, without harming the human body. Still it remains indisputable that a targeted therapy, with its specificity toward cancer cells, while sparing toxicity to the healthy ones, is an asset in cancer treatment.

However, the idea of a magic bullet seems not similarly winning for the treatment of complex neurodegenerative diseases.

Recent research into ground-breaking network pharmacology[2] has significantly shifted drug discovery paradigms for many neurodegenerative disease categories, including Alzheimer's disease (AD). From this perspective, neurodegeneration is the result of the systemic breakdown of brain physiological networks. As robustness and redundancy are typical features of such diseased networks, it is unlikely that a magic bullet targeting specifically one check point can restore the perturbed situation. Conversely, the simultaneous modulation of several targets through a concerted intervention, that is, polypharmacology, seems essential to achieve the desired therapeutic effect.[3]

In 2008, attracted by the potential of polypharmacology to combat neurodegeneration, we proposed the development of single molecules that is able to simultaneously modulate multiple targets responsible for the complex neurodegeneration cascade.[4] We coined for them the term, *multitarget-directed ligands* (MTDLs), as we wanted to bring out the fact that this definition "more completely describes those compounds that are effective in treating complex diseases because of their ability to interact with the multiple targets thought to be responsible for the disease pathogenesis."[4]

Looking back, it really has been a remarkable decade for polypharmacology[5-9] and MTDLs,[10-13] and we believe that is only going to continue for the next ones.

However, how to effectively develop novel MTDLs and successfully bring them to AD patients and their families in need, remains a fundamental challenge. As we and others[14] have already realized, AD multitarget drug discovery (MTDD) combines the hurdles of an extremely challenging area, such as central nervous system (CNS),[15] with those of novel pharmaceutical tools, such are MTDLs.

Indeed, notwithstanding massive investments in basic and translational research by government and nonprofit organizations worldwide,[16] pharmaceutical companies and investors continue to view AD drug discovery as a risky area. In the last 5 years, pharmaceutical companies have cut their programs by half or even pulled out of research pertaining to AD due to continued clinical failures.[17] As the latest of a long series of setbacks, in July 2019, Novartis decided to discontinue investigation of beta-secretase 1 (BACE-1) inhibitor CNP520 in two phase II/III trials.[17]

With a double aim of pushing the community to remain in the field and stimulating a more structured and efficient approach to AD polypharmacology, this paper touches on two aspects that we consider critical to the overall success. The first one relates to a very early decision, that is, how to select the right target combination.

Second, we try to offer some clues to navigate the polypharmacology chemical space and choose the molecular option that is better suited to a given program.

2 | POLYPHARMACOLOGY: COMPLEX PHARMACOLOGY FOR COMPLEX DISEASES

Polypharmacology comes from the Greek prefix "poly," which means "many," and pharmacology. So, by definition, polypharmacology is the design or use of pharmaceutical agents that act on multiple targets or disease pathways.[18] Two key approaches are included under the broad heading of "pharmaceutical agents": (a) multiple drugs binding to multiple targets (ie, drug combinations) and (b) one drug binding to multiple targets (ie, MTDLs) (Figure 1).[19,20]

A strong argument in favor of complex pharmacology strategies for the treatment of complex neurodegenerative diseases is based on the fact that these diseases are multifactorial, that is, they are caused by multiple factors, being possibly genetic, environmental, endogenous, or even having more than one factor operating at the same time.[21] This creates a simple but indisputable background to the idea of effectively attacking them through the modulation of more than one molecular target, that is, a polypharmacological approach.[4] Additionally, the simultaneous modulation of multiple targets by one or more chemical entities is correlated to the concept of lower doses usage.[8] In a multifactorial pathologic condition, the inhibition of one pathway is normally compensated by higher activation of other pathways. This may lead to a resistance phenotype and result in a higher dose usage, which, in turn, may increase the risk of side effects, as a consequence of off-target modulation.[7]

AD is the most important complex neurodegenerative disease. It is the most common form of dementia and it is being estimated that by 2050, more than 115 million people worldwide will develop AD.[22] Being multifactorial, it is not strange that several different hypotheses have attempted to explain its causes. Briefly, they can be listed as: (a) genetic—genetic factors are estimated to play a role in at least 80% of AD cases[23]; (b) cholinergic—related to

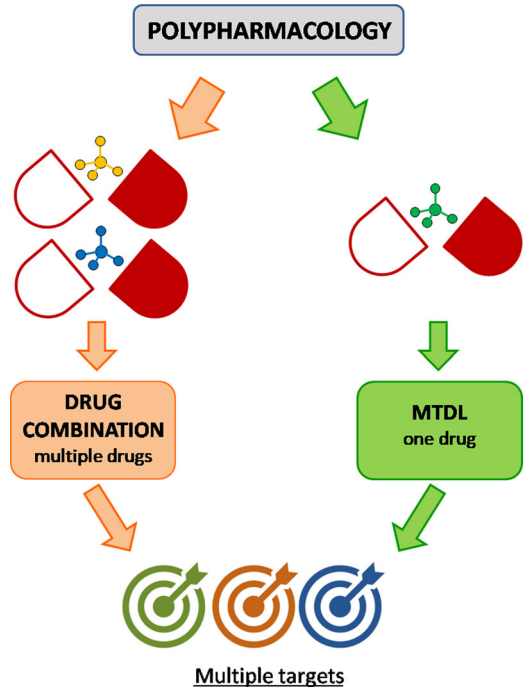

FIGURE 1 Main clinical scenarios for polypharmacology [Color figure can be viewed at wileyonlinelibrary.com]

dysfunction of acetylcholine (ACh) receptor system in neurons[24,25]; (c) amyloid—neurodegeneration results from the accumulation of oligomeric, fibrillar amyloid beta (Aβ) peptides,[26,27] (d) tau—neurodegeneration is a consequence of the abnormal phosphorylation of tau protein and formation of neurofibrillary tangles[28-30]; (e) neuroinflammation—immune response driven by micro- and astroglia contributes to disease progression[31]; (f) oxidative stress—excess of free radicals is involved in AD neuronal death.[32,33]

Notwithstanding the clear multifactorial nature of AD, the available drugs are single-target small molecules that act by inhibiting acetylcholinesterase (AChE) or blocking the *N*-methyl-D-aspartate receptor (NMDAR). Despite the advancements toward pathological mechanism elucidation, the available therapeutic arsenal is not effective, treating just symptoms and not stopping disease progression. This is a clear indication of the necessity of searching for new drugs by exploiting polypharmacological approaches.

3 | MULTITARGET VALIDATION FROM A POLYPHARMACOLOGY PERSPECTIVE

In this *network* era,[1] polypharmacology programs are of extreme interest for the pharmaceutical community. However, there hangs the specter of the high attrition rates experienced by the AD sector.[17] Therefore, a great clarity around the aims and tasks associated with each project,[34] together with a high level of specialized expertise, is needed.

One of the most challenging and crucial steps in the development of a new drug for AD is target identification/validation.[35] In a polypharmacology context, we should consider validated those target pairs which, when investigated in consistent and reliable in vitro and in vivo models, demonstrate additive or synergistic effects resulting from a concomitant modulation. Thus, selection of a given target pair should be a consequence of an intense investigation of the signaling pathways of each target and how these signaling pathways are interrelated. All this should always aim to avoid redundancy, antagonism, or suppression effects.[36-38] Moreover, there is one important additional issue to be considered, that is, neurotoxicity as a consequence of a chronic treatment in AD elderly patients.

It thus follows that the choice of targets to be combined should always be guided by rational, mechanistic considerations. This, in addition to the multitude of potential AD drug targets,[39] necessitates systematic and efficient methods. Clearly, as for any pharmaceutical agent, polypharmacology should be directed against the most important pathological processes.[35] In this respect, the current availability of huge amounts of trans-omic biomedical data, and our increasing ability to utilize them, might allow to assess the most relevant targets.[40,41] In the specific case of a polypharmacological project, once putative targets have been identified, we need a network pharmacology understanding of the pathways in which they are involved, and their reciprocal link to neurodegeneration. In silico modeling that addresses how the targets are "networked" (collective arrangement, connections, and interactions) in the neurodegeneration cascade is fundamental to obtain clues regarding this issue. Currently, network models are not only providing useful information to analyze the interconnection of pathways and targets, but also their relation with chemical compound networks.[42] This topic, albeit particularly relevant, is outside the scope of this article. Therefore, interested readers are referred to recent articles[43,44] for further discussion.

As an alternative to the in silico modeling, purposely addressed experimental studies can effectively demonstrate networked mechanisms of action, synergistic or additive activity, and acceptable safety at clinically achievable drug concentrations.[45] In a simplified way, the following flowchart consisting of four basic sequential steps can be envisaged (Figure 2): (a) selection of selective and possibly nearly equipotent chemical probes for each target under investigation; (b) cytotoxicity evaluation of the chemical probes in combination; (c) demonstration of additive or synergistic effects in cell models recapitulating AD pathology; (d) confirmation of the effects observed in cells by using reliable in vivo models.

If at least the first three steps are successfully performed, significant superiority of the drug combination compared with the single agents has been demonstrated and the target pair can be considered validated for a de novo polypharmacological approach. From a polypharmacology perspective, the cell-based systems are a perfect

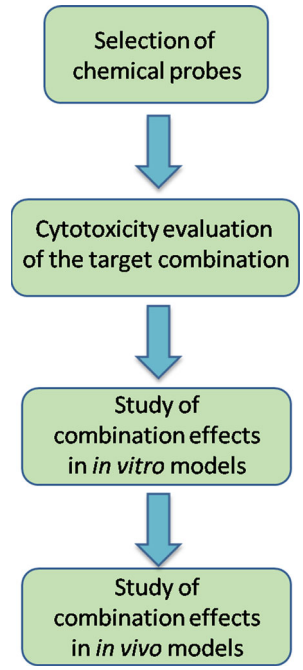

FIGURE 2 Multitarget validation process [Color figure can be viewed at wileyonlinelibrary.com]

compromise between isolated proteins and in vivo screening: they maintain a reasonable experimental efficiency while preserving critical molecular pathway interactions.[3]

To date, there is a number, albeit limited, of MTDLs in which drug combination studies have preceded their development, and whose design has been inspired by rigorous target validation studies (vide infra).

4 | POLYPHARMACOLOGY MEDCHEM TOOLBOX: DRUG COMBINATIONS AND MTDLS FOR AD

As discussed above, a successful (co)target validation opens two possible polypharmacology scenarios (Figure 1) to drug developers,[20] namely drug combinations or MTDLs. However, we should point out that such a rigid classification that distinctly separates polypharmacology based on multiple (combinations) or single (MTDLs) active pharmaceutical ingredients (APIs) may result in an oversimplification. Indeed, various options of both drug combinations and MTDLs exist. In the following, selected examples of different applications of combinations and MTDLs will be discussed, together with critical reflections on their pro and cons related to the peculiar AD context.

4.1 | Polypharmacology by drug combinations

Under the umbrella term of "combination"—that is, two or more monotherapies combined in a therapeutic regimen—two different therapeutic modalities are included: (a) drug cocktail (b) fixed-dose combination. Drug cocktail is a combination of different dosage forms, each one containing a different API, while fixed-dose combination refers to a single-dosage form containing multiple APIs (Figure 3).[20]

Both modalities are used in the clinical practice. Notably, in 2014, the Food and Drug Administration (FDA) approved a fixed-dose combination of AChE inhibitor (AChEI) donepezil (**1**) and NMDAR blocker memantine (**2**) for the symptomatic treatment of moderate-to-severe AD (Figure 4).[46] Potential advantages include a simplified treatment

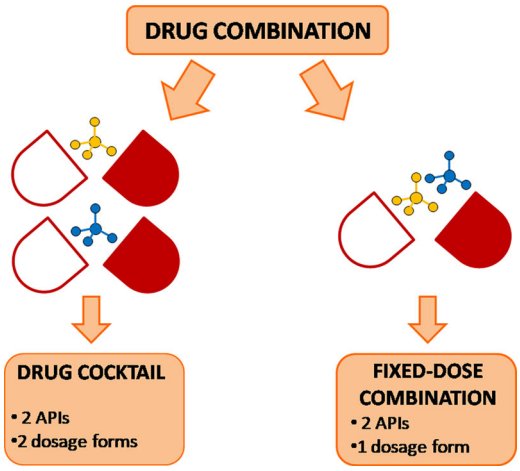

FIGURE 3 Strategies to obtain a polypharmacological approach by drug combinations (drug cocktail and fixed-dose combination). API, active pharmaceutical ingredient [Color figure can be viewed at wileyonlinelibrary.com]

regimen, reduction in pill burden, and the ability to sprinkle the capsule onto soft foods. Patients who may particularly benefit are those with significant dysphagia, a history of poor compliance, or limited caregiver interaction. However, available evidence that these advantages would increase treatment adherence and persistence is contradictory.[47]

From a drug discovery perspective, polypharmacological approaches based on drug combinations have proven more successful in terms of clinical translation with respect to MTDLs. Indeed, after providing experimental evidence of the additive or synergistic effects of two existing drugs by appropriate in vitro and in vivo models, the transition to the clinical phases results faster compared with that needed for MTDLs, which are completely new chemical entities. In addition, drug cocktails have the considerable advantage of permitting to adjust the dose regimen, thus allowing a personalized medicine approach.[48] This not the case for both MTDLs and fixed-dose combinations, which, in turn, account for a simplified therapeutic regimen. On the other side, combinations show an inherently higher risk of drug-drug interactions (DDIs) and of toxic effects. Both are critical issues in AD: (a) adherence to a complex therapy is unlikely for forgetful AD patients and their caregivers; (b) coexistence of chronic disease, polypharmacy, and impaired organ functions makes geriatric population particularly susceptible to DDIs.[49]

These considerations, together with the increased recognition of complexity and the positive experience with other similarly complex diseases (cancer and human immunodeficiency virus-1), has led to believe that combination therapies may prove always more successful in AD.[50] Thus, drug development landscape for combination therapy is becoming increasingly crowded with both symptomatic and disease-modifying applications.[51] Examples of current trials are shared, with a focus on the polypharmacological rationale for their development.

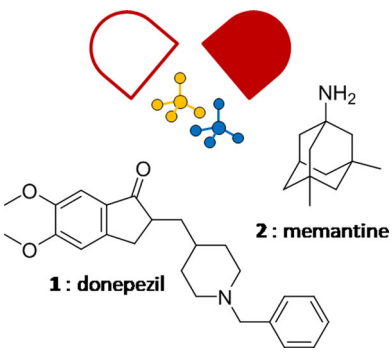

FIGURE 4 Fixed-dose combination of donepezil (**1**) and memantine (**2**) approved by FDA [Color figure can be viewed at wileyonlinelibrary.com]

4.1.1 | Cromolyn-ibuprofen drug combination to target neuroinflammation in AD

Neuroinflammation is one of the major AD pathological processes and a drug discovery research priority.[52] Although at the moment there are no drugs able to effectively modulate this process, an interesting phase III clinical trial (NCT02547818) is currently ongoing. It aims to repurpose cromolyn (**3**) and ibuprofen (**4**) (Figure 5A), two drugs in the market for the treatment of asthma and inflammatory conditions, respectively. They are coadministered as two different dosage forms[53]: cromolyn as inhaled powder, while ibuprofen as oral tablet.[53,54] Both drugs had been already investigated separately in vivo for their potential to combat neuroinflammation in AD: **3** had shown effects on neuroinflammation by stabilizing mast cell membranes and inhibiting microglia activation,[55] and on Aβ levels by directly interacting with Aβ oligomers.[56] On the other side, **4**, a nonsteroidal anti-inflammatory drug (NSAID) and a cyclooxygenase (COX) inhibitor, prevents COX-mediated prostaglandin E2 responses in synapses, reduces microglia overactivation and Aβ deposition, restoring Aβ phagocytosis is by microglia.[57,58] This is a case where well-investigated profiles of the involved drugs helped to quickly switch from the preclinical to the clinical phase. An efficacy study in in vivo AD mouse model evidenced better effects of the drug combination compared with cromolyn and ibuprofen alone.[59] The study showed the promotion of Aβ42 uptake in microglia by **3** and its superior activity, in combination with **4**, in reducing Aβ level thanks to the induction of a neuroprotective microglia activation state favoring Aβ phagocytosis.

All these pieces of evidence supported a fast progress to the clinical development setting. Indeed, phase I clinical trial has confirmed the safety of the drug combination and positive pharmacokinetic studies in healthy elderly volunteers, which has allowed rapid transition to phase III.[60]

4.1.2 | Quercetin-dasatinib combination to target senescent cells in AD

Quercetin (**5**) and dasatinib (**6**) (Figure 5B) cotreatment is an example of a senolytic drug combination repurposing, currently in a phase I clinical trial (NCT04063124).[61] Senolytic therapies are those that selectively eliminate senescent cells by transiently disabling prosurvival networks, to produce rejuvenation and prevent or attenuate age-associated diseases.[62] Both (**5** and **6**) are already marked for other indications. **6** is a thiazole-based Src/Abl

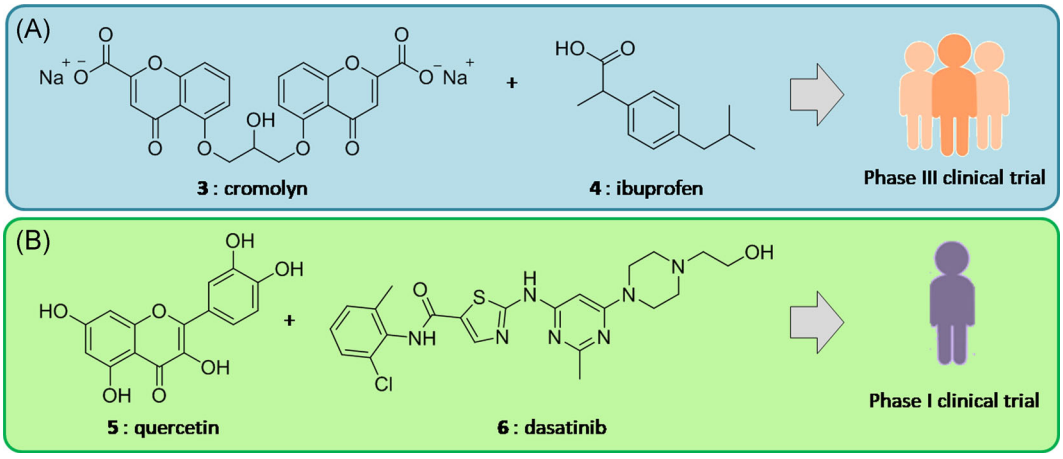

FIGURE 5 Examples of drug combinations investigated in Alzheimer's disease clinical trials. A) Phase III clinical trial: combination of cromolyn and ibuprofen; B) Phase I clinical trial: combination of quercetin and dasatinib [Color figure can be viewed at wileyonlinelibrary.com]

kinase inhibitor approved by the FDA for myelogenous leukemia.[63] On the other side, **5** is a potent natural antioxidant used as a dietary supplement for metabolic and inflammatory disorders, as well as AD.[64] This combination has already been investigated for its effect on decreasing senescent cell burden in different chronic illnesses, such as diabetes, chronic kidney disease, and osteoarthritis.[65] Successful repurposing in AD is supported by in vivo studies in amyloid precursor protein (APP)/PS1 mutant transgenic mouse models. The combination seems to act by the selective removal of senescent cells from the Aβ plaque environment, leading to a reduction of pro-inflammatory cytokine secretion and a decrease of Aβ concentration, with an overall amelioration of cognitive disturbances.[66] This is a demonstration of the reciprocal involvement of Src/Abl kinase and oxidative stress in AD pathology network. Thus, this clinical trial is a "proof-of-concept" of dasatinib-quercetin cotreatment, with the goal of understanding whether it can effectively reach the brain in humans.

4.1.3 | AChE inhibitors investigated in AD clinical trials in combination with other drugs

Most of the trials for new polypharmacological approaches in AD are conducted in patients already receiving cholinesterase inhibitors (ChEIs), memantine or both, and are thus new types of add-on treatments. By definition, in an add-on therapy clinical trial, a new agent is compared with placebo in patients who are already receiving treatment with a background therapy.

In January 2020, Theranexus successfully completed a phase I clinical trial (NCT03698695) aimed to evaluate safety, pharmacodynamics, and pharmacokinetics of THN201, a drug combination of two oral drugs, mefloquine (**7**) and donepezil (**1**) (Figure 6A).[67] **1** is an AChEI currently used as the first-line treatment for AD.[68] Mefloquine is a drug registered for malaria chemoprophylaxis, but its neurological side effects have suggested its repurposing for CNS diseases.[69] Particularly, at low dose, mefloquine has been shown to modulate neuron-glia interface, by inhibiting the gap junction channels expressed in neurons and glial cells and providing cytoplasmic continuity and direct communication between neighboring cells.[70] The early results of this clinical trial[71] revealed an extension of the pharmacological profile of THN201 compared with donepezil monotherapy in healthy male volunteers, after impairment by a scopolamine challenge. This extension is consistently reflected in a higher mnemonic fluidity observed during cognitive tests and a greater power in the electroencephalography gamma band related to cognitive activity. To note, other studies about mefloquine potential in AD, have demonstrated its AChE and butyrylcholinesterase (BChE) inhibitory activities, attributable to its quinoline ring, a well-known ChE pharmacophoric function.[72]

Masitinib (**8**) (Figure 6B) is an oral and selective inhibitor of Fyn, a tyrosine kinase involved in the survival, migration, and activity of mast cells. **8** has been extensively investigated for its potential effects against cancer, inflammatory diseases, inflammatory bowel disease, asthma, and mastocytosis.[73] In recent years, a new potential application of **8** in AD has emerged. Peptide Aβ42 seems to promote mast cells degranulation and a consequent generalized inflammatory response.[73] Thus, in the ongoing phase III clinical trial (NCT01872598) masitinib is repositioned to treat mild-to-moderate AD, in combination with AChEIs (donepezil (**1**), rivastigmine (**9**), or galantamine (**10**)) standard therapy (Figure 6B).[74] Additionally, Fyn is upregulated in AD, and Fyn kinase inhibition by **8** can modulate different pathological pathways, such as tau phosphorylation and neurofibrillary tangles formation. Apparently, in this case, the drug combination reached the clinical phase without reported evidence of a strong in vivo validation of the synergic or additive effects involved.[75]

Another example of combinations of AChEIs and other drugs is NCT00940589,[76] a clinical trial that has completed phase II. It provides prolonged-release melatonin (PRM) administration to AD patients with and without insomnia comorbidity. In this clinical trial, PRM is administrated in combination with an AChEI (**1**, **9**, **10**) with and without memantine (**2**) (Figure 6B). The idea of the drug combination came from the observed relation between circadian rhythm dysfunction and neurocognitive disorders, a phenomenon that has been observed as consequence of the decreased circulating melatonin (**11**) level (Figure 6B).[76,77] Melatonin combines both chronobiotic and cytoprotective properties. As a chronobiotic, melatonin can modify phase and amplitude of the circadian sleep-

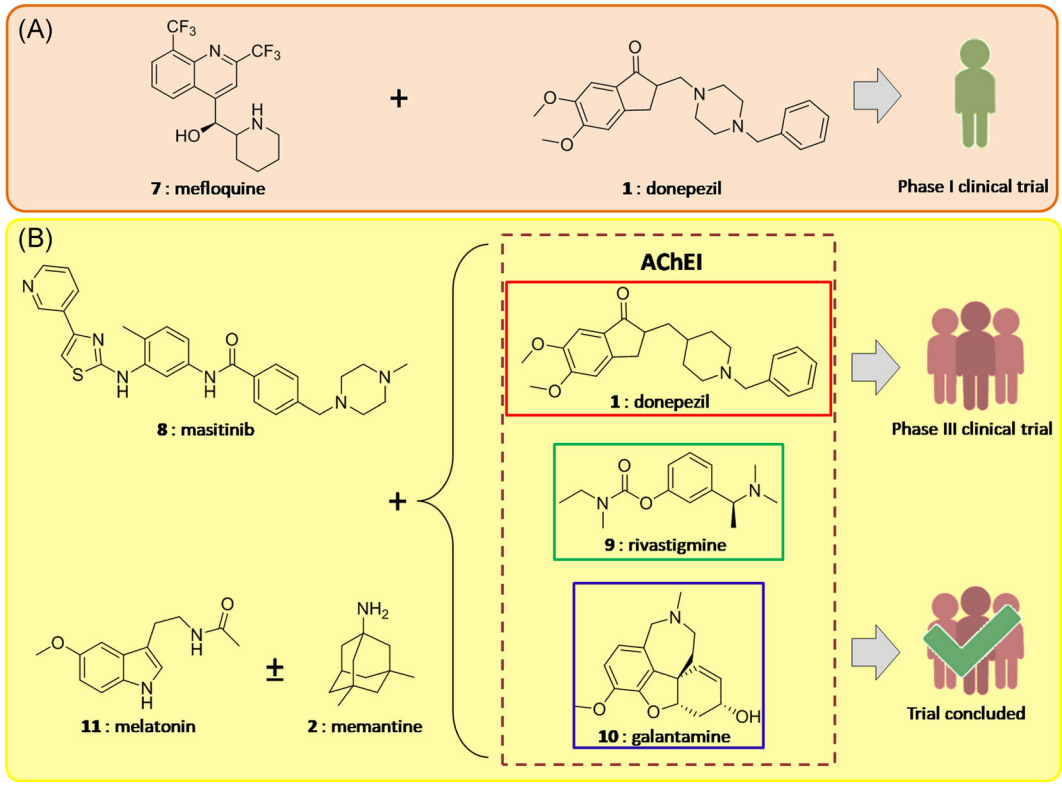

FIGURE 6 Examples of acetylcholinesterase inhibitor (AChEI)-based combinations investigated in clinical trials. A) Phase I clinical trial: combination of mefloquine and donepezil ; B) Pase III clinical trial: combination of masitinib and AChE inhibitors; concluded clinical trial: combination of melatonin, memantine and AChE inhibitors [Color figure can be viewed at wileyonlinelibrary.com]

wake rhythm. As a cytoprotective molecule, melatonin reverses inflammatory damage, scavenges free radicals, and facilitates the immune response.[78] Melatonin in AD has been validated by in vivo and in vitro studies demonstrating also a beneficial effect in reducing Aβ generation and deposition.[79] In light of this, the potential therapeutic use of a memantine-melatonin drug combination has been studied in a double transgenic APP/PS1 mouse model with severe amyloid pathology.[80] The results showed restoration of the episodic memory and an efficient reduction of Aβ aggregates and biomarkers of neuroinflammation, when compared with memantine or melatonin alone.[80] According to what was observed in preclinical studies, clinical trial results have underlined an amelioration of disease progression in patients treated with PRM compared with placebo. Although longer study duration is required to further validate these results, combination of PRM and AChEIs, with or without memantine, has emerged as a safe way to improve cognitive functions and to control sleep disturbances in mild-to-moderate AD patients.[81]

4.2 | Polypharmacology by MTDLs

As discussed, in addition to combinations, another polypharmacological option is based on MTDLs. These are single molecular entities in single-dosage forms, which, in turn, can be divided into two main classes—codrugs and hybrids (Figure 7).[82]

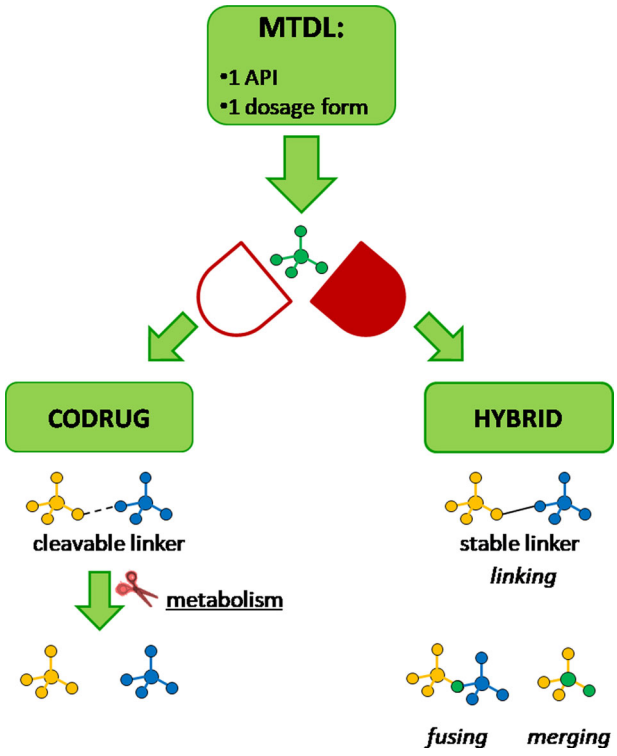

FIGURE 7 Strategies to obtain a polypharmacological approach by -s (codrugs and hybrids). API, active pharmaceutical ingredient; MTDL, multitarget-directed ligand [Color figure can be viewed at wileyonlinelibrary.com]

Codrugs consist in two synergistic drugs chemically linked together to mainly improve the drug delivery properties of one or both drugs.[83] This process can provide a high level of selectivity because the two molecules are rendered essentially inactive by the covalent linking.[84] In fact, in codrugs the two starting chemical entities are combined via a cleavable linker, which, only after enzymatic biotransformation, allows their release and their individual biological effects against multiple pathways, in the same target cells and at the same time.[84] Thus, the polypharmacological effect that can be produced by the simultaneous delivery of the starting chemical entities is unique and cannot be replicated by administering multiple drugs in combinations.[84]

In contrast, hybrids are constituted by two diverse drugs or their respective pharmacophores joined via a permanent bond. As hybrids do not undergo enzymatic cleavage, they exert a dual effect acting simultaneously on two biological targets as a single chemical entity.

Of note, codrugs are the only feasible option when the two starting drugs do not share common functionalities and thus no structural amalgamation to provide a molecular chimera is possible.

The outcomes of drug combinations in preclinical or clinical studies, besides providing a strong validation of target networking, might be a source of inspiration for MTDLs de novo design. This is not trivial as, despite the tremendous therapeutic potential of MTDLs, their rational discovery and further development still represents a formidable challenge. Thus, starting from already validated chemical entities and targets would streamline the entire drug discovery pipeline.

So far, the development of MTDLs for AD has been mostly pursued in academia, where in vivo efficacy studies may often be cost-prohibitive and limited by ethical constraints. As a result, the majority of papers on MTDLs only report in vitro activity data, sometimes with inadequate investigation, of their multitarget mechanism of action. For this reason, in the following sections, we will highlight the examples of MTDLs (codrugs and hybrids) developed in the last 5 years reporting in vivo "proof-of-concept."

4.2.1 | Polypharmacology by MTDLs: Examples of codrugs investigated in in vivo models

Nowadays, there are no examples of ongoing clinical trials involving codrugs for AD, but some promising in vivo results suggest that AD drug development might evolve in this direction. In this section, selected examples of rationally designed codrugs, that have undergone both in vitro and vivo validation, will be discussed.

Ibuprofen-lipoic acid conjugates as codrugs with potential neuroprotective activity in AD

Sozio et al,[85] following the hypothesis that proposes the use of NSAIDs and antioxidants as a neuroprotective therapy against AD, designed and synthesized a small library of ibuprofen-(*R*)-α-lipoic acid codrugs (**13**-**15**) (Figure 8). Ibuprofen (**4**), as other NSAIDs, has demonstrated in in vivo studies the capability to delay the onset of AD.[86] In particular, crucial factors in the efficiency of NSAIDs as neuroprotective drugs are their high percentage of binding to the plasma proteins and low distribution volume, as well as blood-brain barrier (BBB) penetration.[87] In contrast, (*R*)-α-lipoic acid (**12**) (Figure 8), already in the market as dietary supplement, has been proposed as a lead structure for AD drug discovery[88] and studied in several clinical trials (NCT01058941, NCT00090402, NCT00117403). **12** is a dithiol that binds lysine residues of mitochondrial α-keto acid dehydrogenase complex. It can easily cross the BBB and accumulate in all neuronal cell types. Anyway, the active form of lipoic acid is its reduced form, the dihydrolipoic acid, which is produced in mitochondria. Dihydrolipoic acid activates choline acetyltransferase, chelates redox-active transition metals, increases the amount of the reduced form of glutathione, and downregulates the redox-sensitive inflammatory processes.[89] Thus, the goal of this codrug approach was to target ibuprofen and lipoic acid directly to the neurons, by modulating the pharmacokinetic properties of the parent drugs. **13** to **15** have been synthesized by the interposition of different-length linkers to form metabolically cleavable bonds. In detail, the formation of amide bonds among different diamine linkers, (2, 4, and 6 carbon atoms), and the acidic groups of the two starting compounds (**4**, **12**) have been exploited. In this way, thanks to the masking of the carboxylic groups and the respective negative charges, the BBB permeability should be improved compared with **12** and **4**.[85] To prove that, the authors investigated **13** to **15** in vitro stability in rat and human plasma and brain homogenates. The results highlighted that even if all the codrugs showed a more rapid hydrolysis in brain tissue than in plasma, **13** has a good stability in both conditions. Moreover, in vitro assays showed better free radical-scavenging properties for **13** to **15** than **4** and **12** tested alone.[85] The most promising compound **13** was

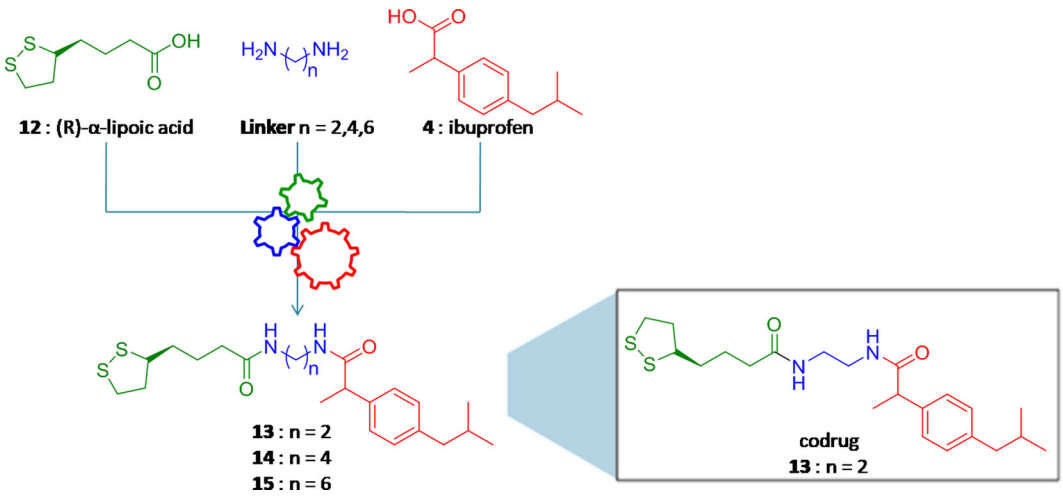

FIGURE 8 Design of (*R*)-α-lipoic acid-ibuprofen codrugs (**13**-**15**) [Color figure can be viewed at wileyonlinelibrary.com]

then investigated in in vivo rat model, where the positive in vitro results were confirmed.[85] Moreover, **13** was studied in a rat model of AD, following injection of Aβ40 peptide. After treatment with codrug **13**, Aβ40 peptide accumulation was reduced in cerebral cortex in a more significant way than after treatment with **4** or **12** alone.[90]

Tacrine-silybin codrug shows neuroprotective and hepatoprotective effects against AD

Tacrine (**16**) was the first AChEI registered for AD, but was withdrawn from the market due to its hepatotoxic side effects. In spite of that, thanks to its low-molecular weight and high synthetic accessibility, **16** has been widely exploited as a starting fragment of hybrids able to combine AChE inhibition with other additional effects and endowed with reduced hepatotoxicity.[91]

Following a rationale design, Chen et al developed codrugs between tacrine and silybin B (**17**). **17** is one of the main components of the *Carduus marianus* extract (Figure 9), proposed as an anti-inflammatory and anticancer agent. It is considered an especially interesting scaffold for AD drug discovery, thanks to its neuroprotective effects.[92] In details, the designed codrug **20** is a hybrid between **17**, aminohexamethylene-tacrine (**19**), and a molecule of succinic acid (**18**) (Figure 9).[93] In the final structure, **17** and **18** are linked by a labile ester bond, while **18** and **19** by a more stable amide bond. By exploiting the different bond stability, the AChE-inhibiting fragment (**19**) can be released, penetrating the BBB, and acting at the brain level. Although no evidence of additive/synergistic/less toxic effects has been provided a priori by in vitro/in vivo combination studies, the biological and pharmacological properties of **20** have been appropriately compared with an equimolar mixture of **16** and **17**, with

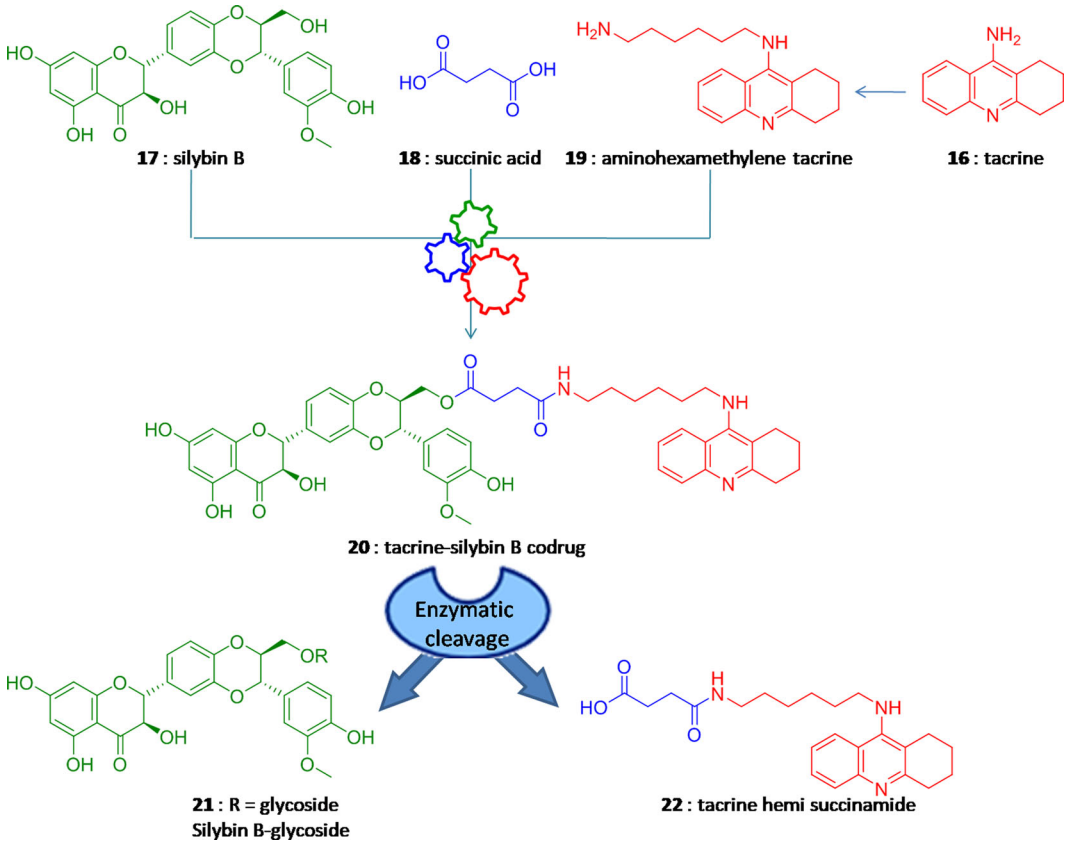

FIGURE 9 Design of tacrine-silybin codrug **20** and its in vivo metabolism [Color figure can be viewed at wileyonlinelibrary.com]

the aim to demonstrate whether the linking strategy performed could provide polypharmacological advantages.[93] It was observed that **20** is less potent as AChEI and BChE inhibitor than **16**, but it showed no neurotoxic effects on hippocampal cell line, while displaying neuroprotective effects against glutamate-induced oxidative stress, not observed with the drug combination. Importantly, both **16** and **17** coadministration and **20**, showed in vivo a hepatoprotective effect and an improvement of the cognitive impairment.[93] The following in vitro study developed by the same group reported the presumable codrugs' metabolism.[94] The experiment showed, by microsomal incubation assay with human liver microsomes that **20** was rapidly cleaved by cellular unspecific esterases and widely metabolized to silybin-glycosides (**21**) and tacrine hemisuccinamide (**22**). This has been proposed as the possible active principle in vivo.[94] In particular, an evaluation of **22** in hematopoietic stem cells-based assay, not only confirmed the in vivo low toxicity, but also suggested a crucial role of the hemisuccinamide linker, being nontoxic and probably critical in conferring hepatoprotective properties.

Ibuprofen-glutathione codrug as potential therapeutic agent for treating AD

As previously mentioned, NSAIDs might protect against AD by targeting underlying neuroinflammation.[95] Furthermore, supplementation of physiological antioxidants and free radical scavengers can be another therapeutic strategy in AD. Glutathione (**23**) is one of the most prevalent antioxidants in the brain and is able to protect against reactive oxygen species (ROS), redox metal ions, reactive lipid peroxidation products, and other electrophiles that could be dangerous for cell viability.[96] Pinnen et al[97] designed and synthesized an ibuprofen-glutathione codrug (**25**) by linking a prodrug form of **23**, that is, glutathione dimethyl ester (**24**) and ibuprofen (**4**) (Figure 10). To obtain an in vivo cleavable conjugate, the authors exploited the insertion of an amide bond between the amine group of glutamine of **24** and the carboxylic group of **4**. The aim was to obtain a targeted delivery of the two starting molecules directly to neurons, where oxidative stress and inflammatory processes occur. In vitro evaluation of **25** highlighted a good stability in human plasma and showed a free radical-scavenging activity similar to **23**, in a time- and concentration-dependent way. In light of this, **25** was administered to rats to evaluate its ability to reverse the neuronal damage provoked by intracerebroventricular infusion of Aβ40 peptide.[97] The data obtained by behavioral tests of long-term spatial memory demonstrated that animals treated with **25** performed better than those treated with **4** and **23** singularly. Moreover, histochemical studies revealed that Aβ protein was less expressed in cerebral cortex of mice treated with **25** than in mice treated with **4**. In this way, the authors could successfully conclude that **15** has an improved effect compared with **4** and **23**.[97] It is a pity that they did not proceed further in attempting to demonstrate the advantage of their codrug in comparison with the coadministration of **4** and **23**.

4.2.2 | Polypharmacology by MTDLs: Examples of hybrids investigated in in vivo models or studied in clinical trials

Hybrids represent a major promising tool in the field of drug discovery for complex neurodegenerative diseases.[98-102] Their versatility has already spawned a great deal of literature and it is envisaged that this area will achieve even greater prominence in the future. In the polypharmacology context, hybrids can be considered as the evolution of a continuum, starting from the combination of multiple drugs with synergic effects, to single chemical entities releasing two drugs in vivo, as codrugs, and single chemical entities modulating two targets simultaneously.

As a general rule, an effective and structured hybrid drug development process has to be driven by the proof of better performances of the combination when compared with the efficacy of the single starting compounds and by exploiting structural similarity whenever possible. As first enunciated by Morphy and Rankovic[19] in 2005, hybrids can be obtained by *linking*, *fusing*, or *merging* strategies (Figure 7). *Linked* hybrids, unlike codrugs, exploit a metabolically stable linker. Conversely, hybrids obtained by *fusing* or *merging* strategies, due to the lack of a linker, have lower molecular weight when compared with both *linked* hybrids and codrugs. Clearly, molecular weight is a physicochemical parameter fundamental for CNS penetration and for the overall profile of drugs against

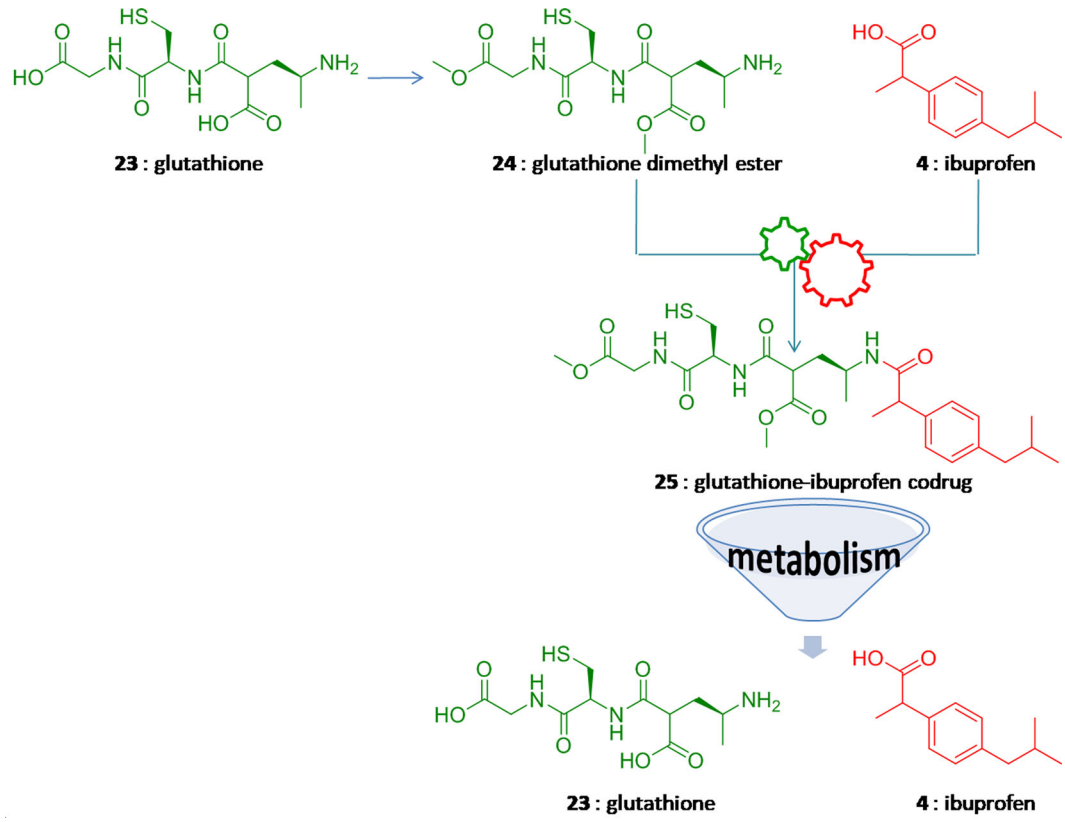

FIGURE 10 Design of ibuprofen-glutathione codrug **25** and its in vivo metabolism [Color figure can be viewed at wileyonlinelibrary.com]

neurodegenerative diseases.[103,104] Furthermore, the single-molecule pharmacokinetic profile of hybrids, from administration to secretion, may allow to avoid DDI, that occur more frequently in AD patients, because of the coexistence of chronic disease, therapies, and impaired organ functions.[49] In addition, differently from codrugs, which only have a single pharmacokinetic in the distribution phase, end up being hydrolyzed and released as two chemical entities; hybrids may avoid DDI also in the elimination phase. During elimination from the body, in fact, drugs can undergo many interactions as a consequence of mechanisms of competition and blockage at active tubular secretion level, if two or more drugs use the same transport system.[105] In the same way as codrugs, hybrids could be cost effective due to simpler pharmacodynamic and pharmacokinetic property evaluation, as the profile results from a single chemical entity, rather than from a combination of two drugs.

There is a plethora of literature highlighting the potential of hybrids in AD treatment. However, in the following discussion, we will intently focus on those examples where drug design was based on solid cotarget validation studies (eg, donecopride, memagal, and CM-414) or where the polypharmacology profile has received in vivo "proof-of-concept."

Combining AChE inhibitory and 5-HT$_4$ receptor agonist activities as potential treatment for AD

It is broadly accepted that AD etiopathology is complex and multifactorial. Activation of serotonin 5-HT$_4$ receptor (5-HT$_4$R) and blockage of 5-HT$_6$ receptor (5-HT$_6$R) have been reported to enhance ACh release, suggesting that modulation of serotoninergic system could efficiently restore the cholinergic neurotransmission deficit observed in AD. Furthermore, 5-HT$_4$ receptor (5-HT$_4$R) agonists are able to promote the nonamyloidogenic cleavage of the APP

and to favor the production of the neurotrophic protein sAPPα.[106] Rochais et al, designed and synthesized the novel hybrid donecopride (**27**), conceived as a structural amalgamation between 5-HT_4R agonist RS67333 (**26**) and donepezil (**1**) (Figure 11).[107,108] As mentioned above, the most coherent way to start a new MTDL project is to assess the potential synergic effect by evaluating the corresponding combination. In this case, **26** (0.1 mg/kg) and **1** (0.3 mg/kg), when co-administrated at subactive doses, resulted in improved memory performances in mice. In light of the observed synergistic effect and thanks to a close structural similarity of the involved molecules, a new series of highly *merged* hybrids was proposed, selecting donecopride as the best-performing compound.[109,110] Successfully, **27** displayed nanomolar dual-binding site AChE inhibitory effects and partial 5-HT_4R agonist activity.

Following the development of donecopride, pharmacomodulation of its structure has led to a series of novel derivatives obtained by replacement of the benzene ring by an indole residue. This substitution has been envisaged to increase the interaction of the ligand with the peripheral anionic site of AChE.[111] The selected compound **28** eventually resulted in an increased inhibition of Aβ aggregation in addition to a potent nanomolar inhibition of AChE and affinity for the σ1 receptor, associated with a lower affinity for the 5-HT_4R. Those preliminary data were also supported by in vivo assays.[105]

Combining AChE inhibitory activity and NMDAR antagonism as potential treatment for AD

In 2012, Simoni et al started a drug combination study of the NMDAR antagonist memantine (**2**) and the AChEI galantamine (**10**) (Figure 11A). This study showed how the two drugs were able to reverse NMDA toxicity alone or in combination. In the latter case, they resulted in a significant synergistic effect, even at subactive concentrations.[112] Interestingly, **10** has been proposed to possess a dual mechanism of action: in addition to AChE inhibition, it can also enhance synaptic NMDAR activity. It was thus inferred that, thanks to the additive activity on the same pathways, combination of **2** and **10** might offer a promising therapeutic strategy for AD treatment. This provided a solid background to the development of a small library of hybrids, obtained by the combination of memantine and galantamine moieties through stable linkers.[113] Memagal (**29**), with a six-carbon linker, and some of the reported hybrids were nanomolar inhibitors of AChE and displayed micromolar affinities for NMDAR. Furthermore, they exhibited remarkable neuroprotective profiles at a cellular level. Among the series, shorter tethered ARN14140 (**30**) showed the best compromise between pharmacological and pharmacokinetic properties. **30** was then selected

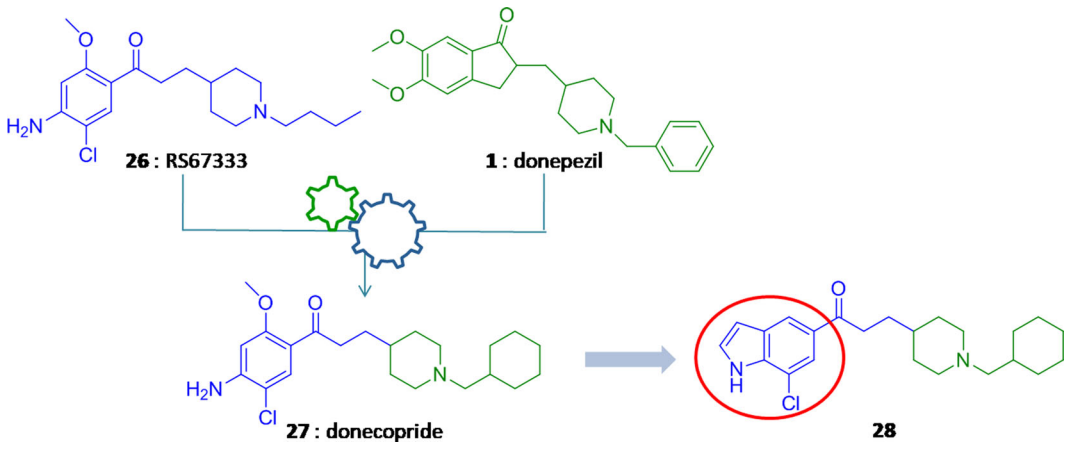

FIGURE 11 Design of donecopride (**27**) and its derivative **28** [Color figure can be viewed at wileyonlinelibrary.com]

for in vivo studies in mice treated with the amyloidogenic fragment 25 to 35 of the Aβ peptide, in which it demonstrated its role in preventing the cognitive impairment and its neuroprotective potential (Figure 12A).[114]

Combining histone deacetylases and phosphodiesterase-5 inhibitor activities against AD

Another successful example, whose design follows the flowchart of Figure 2, is related to the development of compound CM-414 (**33**). **33** is a first-in-class dual inhibitor of phosphodiesterase-5 (PDE5) and histone deacetylases (HDAC), recently proposed as a potential therapeutic tool for AD.[115,116] Inhibition of PDE5 increases cyclic guanosine monophosphate level, significantly decreased in AD patients by direct and/or indirect activation of cAMP-response element binding protein (CREB) and by favoring the inactive form of glycogen synthase kinase 3 (GSK3β), thus decreasing the levels of phosphorylated tau.[117] HDACs are epigenetic modulators that deacetylate lysine residues in histone and nonhistone substrates and their inhibition has attracted much interest for the treatment of neurodegenerative disorders, as several isoforms seem to be implicated in AD memory-related dysfunction: HDAC2 is a nuclear isoform that reduce transcription of CREB-regulated genes involved in learning and memory, HDAC6 targets α-tubulin facilitating the amelioration of tau pathogenesis.[118] Before commencing any design and synthetic efforts of new hybrids, the beneficial synergistic effects obtainable by concomitant HDAC and PDE5 inhibition was evaluated. Combination of sildenafil (**31**), a PDE5 inhibitor, and of vorinostat (**32**), a pan-HDAC inhibitor (Figure 12B), showed, in fact, significant synergy in inducing histone acetylation, which was also confirmed by in vivo models. The development of **33**, whose design was inspired by the pharmacophoric combination of sildenafil and vorinostat, provides an excellent case study in MTDD. A rigorous optimization strategy based on adequate cellular functional responses, an acceptable therapeutic window and the ability to cross the BBB was pursued to obtain a proper tool compound for in vivo testing in AD models. Chronic treatment of Tg2576 mice with **33** diminished brain Aβ and tau levels, increased the inactive form of GSK3β, and reversed the cognitive deficits.[119]

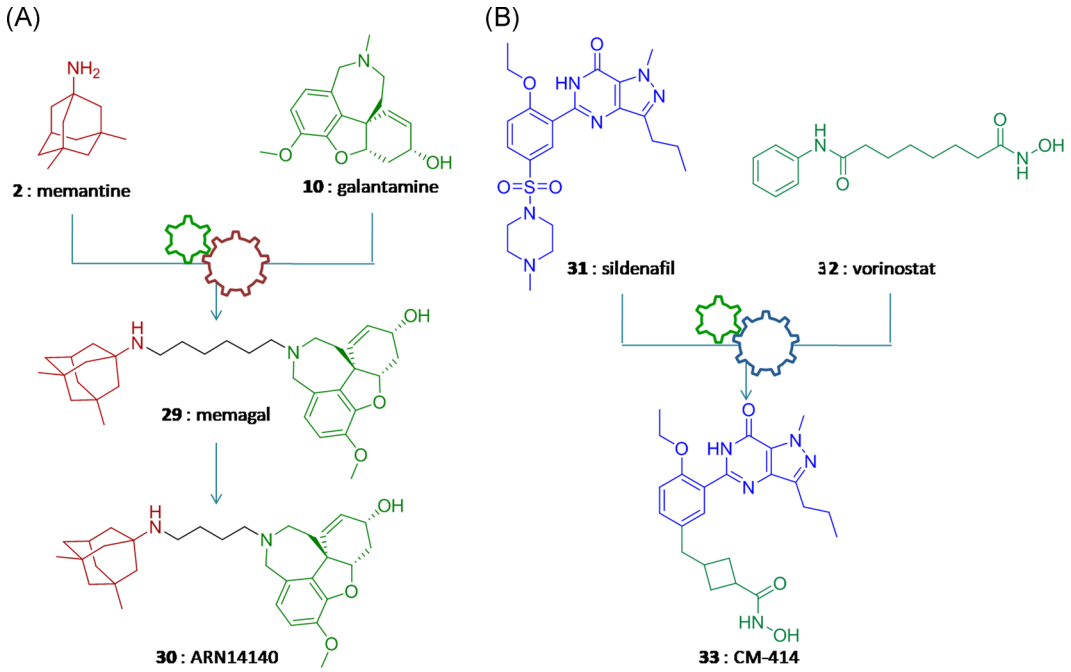

FIGURE 12 A) Design of memagal (**29**), ARN14140 (**30**); B) Design of CM-414 (**33**) [Color figure can be viewed at wileyonlinelibrary.com]

4.2.3 | Hybrids investigated in in vivo models

Apart from the previously reported examples, the development of hybrids is not always proceeded by the combination study of the parent single-target compounds. Although this may be seen as a lack of background validation and upstream demonstration of additive or synergistic effects, clearly it can be compensated by downstream in vivo efficacy studies.

On this basis, herein, we will discuss some interesting examples of hybrids, developed in the last 5 years, reporting in vivo evaluation as MTDD "proof-of-concept."

Combining cholinesterase and MAO inhibition with histamine H_3 receptor antagonism

In 2017, Bautista-Aguilera et al[120] rationally modified the structure of hybrid ASS234 (**34**), able to inhibit AChE, both isoforms of monoamine oxidases A and B (MAO A/B), and to reduce the production of ROS, with the aim of fitting a pharmacophore of histamine H_3 receptor (H_3R) antagonists (Figure 13A). Involvement of H_3R in the cognitive process is based on the fact that blocking of central H_3R induces the release of procognitive neurotransmitters, including ACh.[121] To avoid the adverse effects associated with imidazole-containing H_3R antagonists as in ciproxifan (**35**), imidazole was replaced with a piperidine, connected via a (propyloxy)phenyl linker (Figure 13A). The developed hybrids successfully combined in a single molecule the inhibitory properties against cholinesterase (ChE) and MAO A/B enzymes, alongside H_3R affinity. Among those, contilisant (**36**) showed the best overall multitarget profile in terms of well-balanced activities, drug-likeness properties, as well as antioxidant and neuroprotective effects. Compared with **34**, **36** was more potent for MAO A/B. In vivo efficacy studies showed that administration of **36** at 1 mg/kg is able to restore the cognitive deficit in lipopolysaccharide (LPS)-treated mice.[120]

Combining H_3 receptor antagonism, calcium channels blockade with additional cholinesterase inhibition

A further proof of the interesting role of H_3R in cognitive impairments is pitolisant (**37**), an H_3R antagonist registered for narcolepsy and with an expanded evaluation for the treatment of neurologic diseases, such as Parkinson's disease and epilepsy.[122] Conversely, 1,4-dihydropyridine (1,4-DHP) is the core fragment of well-known calcium channel antagonists (Figure 13B), which have reached phase III AD clinical trial.[123] Indeed, increased

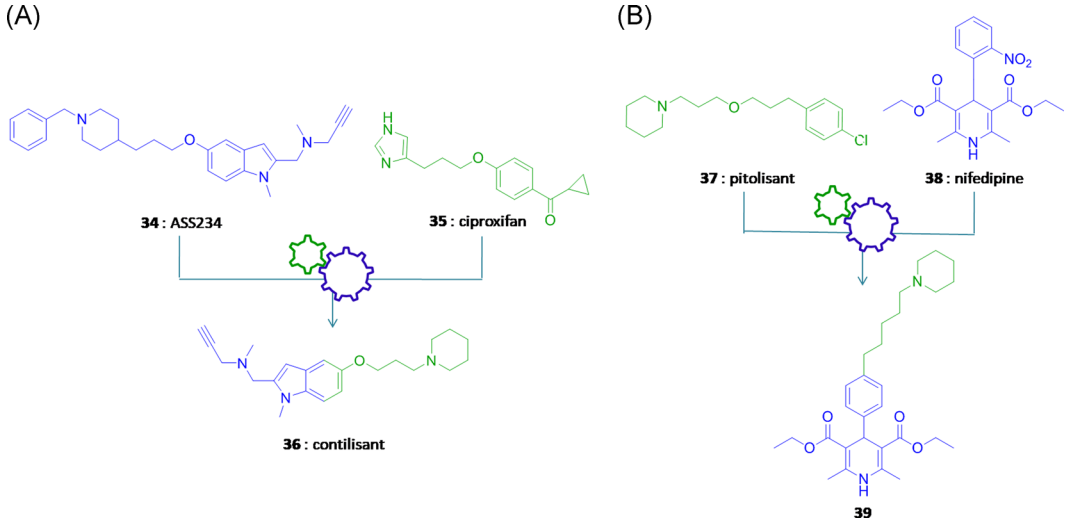

FIGURE 13 A) Design of contilisant (**36**); B) Design of hybrid **39** [Color figure can be viewed at wileyonlinelibrary.com]

cytosolic calcium level is involved in the pathogenesis of AD, by facilitating Aβ formation and activating the apoptotic cascade through the mitochondria.[124] Starting from these promising therapeutic targets, a new family of hybrids was designed by the incorporation of the typical cycloalkylamine H_3R antagonist motif of **37** (Figure 13B) and **36** (Figure 13A), onto a 1,4-DHP, by means of convenient linkers.[125] The hybrids were investigated for their calcium channels blockade activity, affinity toward H_3R, their ChE inhibition, and antioxidant activity. Some of them were more potent calcium channel blockers than nifedipine (**38**), while showing concomitant affinity for H_3R. From in vivo results, **39** was identified as a promising lead molecule thanks to its ability to restore cognitive impairment induced by LPS. In addition, contrary to **38**, **39** did not show in vivo a significant vasorelaxant effect.[125]

Design of quinoline-indole hybrids to promote neuroregeneration

In AD patients' brains, higher concentrations of biometals in Aβ plaque deposits support the role of metal dyshomeostasis in AD pathogenesis.[126] Therefore, the use of appropriate metal-chelating agents to inhibit the production or accumulation of Aβ plaques has been proposed.[126] In the last few years, several hybrids have been developed as metal-chelating agents reporting preclinical and clinical promising evaluations. Among those, 8-hydroxyquinoline (**40**) represents a key scaffold, thanks to its moderate chelating properties and suitable capability to extract metals from Aβ aggregates.[127] Promotion of adult hippocampal neurogenesis with small molecules was identified as an effective therapeutic strategy to address long-term neurodegeneration. Interestingly, among those small molecules, indole-based melatonin-*N*-benzylamine hybrids, have been proven to effectively promote the development of neural stem cells into neuronal phenotypes.[128] Taking into account these information, Wang et al designed a new family of hybrids by merging quinoline and indole (**41**) scaffolds (Figure 14A). In vitro evaluation revealed that all the hybrids had antioxidant effects, biometal chelation activity, Aβ aggregation modulation, neurotrophic, and neuroprotective properties. Furthermore, in vivo assays demonstrated hippocampal cell proliferation in living adult mice, after compound administration. Favorable drug-like properties (ie, microsomal metabolic stability, pharmacokinetic profile, and oral bioavailability) were also demonstrated for selected compound **42** in vivo. Pharmacodynamics studies revealed that chronic oral administration of **42** substantially ameliorated the cognitive and spatial memory deficits in APP/PS1 AD mice and noticeably reduced overall cerebral Aβ deposits.[128]

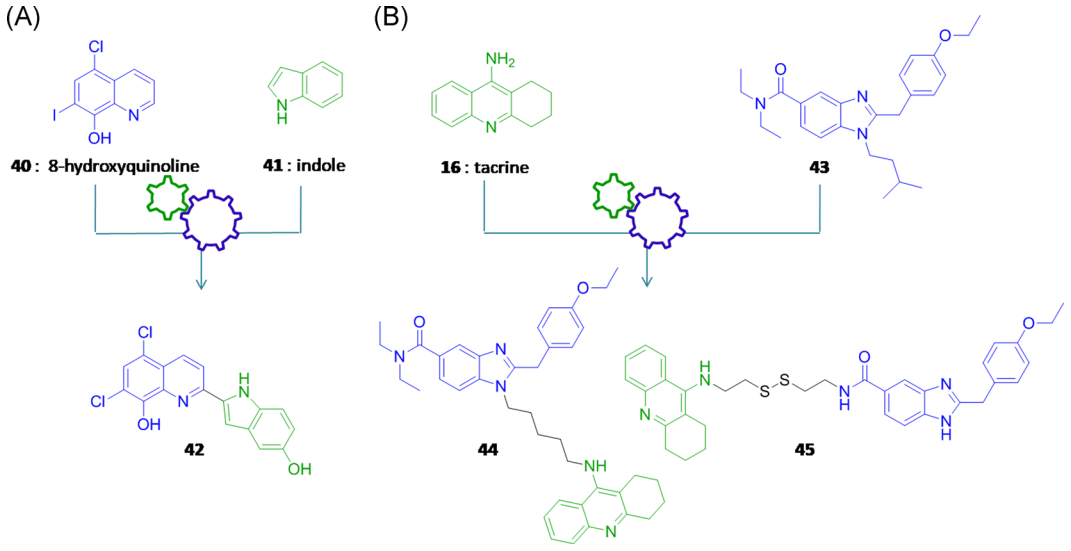

FIGURE 14 A) Design of hybrid **42**; Design of hybrids **44** and **45** [Color figure can be viewed at wileyonlinelibrary.com]

Dual-acting ChE-cannabinoid receptor 2 hybrids with pronounced neuroprotective property

Over the last decades, the field of tacrine (**16**)-based MTDLs has grown enormously, encouraged by the results obtained from the first rationally designed hybrid, the bis(7)-tacrine.[129,130] In 2019, Dolles et al[131] designed and developed a series of hybrids combining tacrine (**16**) and a benzimidazole-based human cannabinoid receptor subtype 2 (hCB2R) agonist (**43**), which was previously reported. The endocannabinoid system has been widely investigated for its role in neurodegeneration, inhibition of inflammatory mediator release, and suppression of microglia activation.[132,133] The hybrids were designed through a linking strategy (Figure 14B), combining the benzimidazole unit of **43** and the scaffold of **16**, to yield higher affinity at both targets. Defining linker composition (eg, polyethylene glycol, carbon chain, cysteamine) and its suitable connecting position, resulted crucial steps. Overall, in vitro assays showed higher inhibition of ChE by the hybrids compared with **16**. Different from **16**, all the tested hybrids showed effect in reducing Aβ aggregation. Conversely, despite the significantly lower hCB2R affinity compared with **43**, the hybrids displayed an immunomodulatory effect similar to the parent molecule (**43**). The idea of incorporating a disulfide into the linker to introduce neuroprotection was investigated in an HT22 cell assay and both tested compounds, **45** and the sulfur-free analog **44**, showed neuroprotection against glutamate-induced oxidative stress. Eventually, in vivo efficacy of the tested hybrids resulted significantly higher (0.1 mg/kg), than for the parent molecules **16** and **43**. Keeping in mind the hepatotoxicity of tacrine, it is extremely relevant that the developed hybrids showed no hepatotoxicity effect at 3 mg/kg dose.[134]

Novel tacrine-tryptophan hybrids as potential treatment for AD

Another recent example belonging to this highly investigated area is the report of a series of tacrine-tryptophan hybrids. Studies on patients with AD have shown an inverse correlation between L-tryptophan (L-Trp, **46**) intake and learning impairment and Aβ accumulation (Figure 15A). Additionally, considering the key involvement of L-Trp residues in Aβ misfolding processes, this molecular framework possesses strong potential for the development of targeted anti-amyloid agents.[135] Based on previously synthesized 1,4-naphthoquinon-2-yl-L-tryptophan[136] and tacrine-naphthoquinone hybrids,[137] a novel family of tryptophan-tacrine hybrids has been synthesized. Most of the hybrids exhibited moderate inhibitory activity against neuronal nitric oxide synthase and good ability to inhibit Aβ42 self- and AChE-induced aggregation. In comparison with the previously reported derivatives, the tacrine-tryptophan hybrids showed excellent balanced ChE inhibition, with potentially greater clinical efficacy and fewer side effects. The lead compound **47**, featuring an hexamethylene linker, was selected for in vivo behavioral studies. By using scopolamine-induced cognitive deficit rat model, **47** confirmed its procognitive potential.[138]

Novel hybrids designed by combination of ChE inhibitors and 5-HT$_6$ receptor antagonist

Recent clinical trials in patients with moderate AD, have shown a superior effect of the combination therapy of donepezil (**1**) and 5-HT$_6$R antagonist idalopirdine over monotherapy with donepezil.[139] In accordance with our view, this has been taken as a solid starting point for the design of new MTDLs by Więckowska et al. In fact, a novel class of hybrids that combines the 5-HT$_6$R antagonist **48** (Figure 15B) with ChE inhibitor scaffolds (**1** or **16**), has been recently synthesized and evaluated in vivo.[140] Tacrine (**16**) and donepezil moieties were linked to **48** by flexible alkyl spacers of different lengths. It is worth to note that the unique combination of pharmacophores **16** and **48**, not only preserved their high affinity for the selected biological targets, but also improved the activity against AChE compared with **16**. In addition, selected hybrid **49** also possessed the highest affinity on 5-HT$_6$R.

4.2.4 | Hybrids investigated in clinical trials

A continued lack of success has given rise to skepticism about the development of traditional single-target drugs able to significantly modify AD progression. Suggestions to abandon the "one-drug, one-target, one-disease"

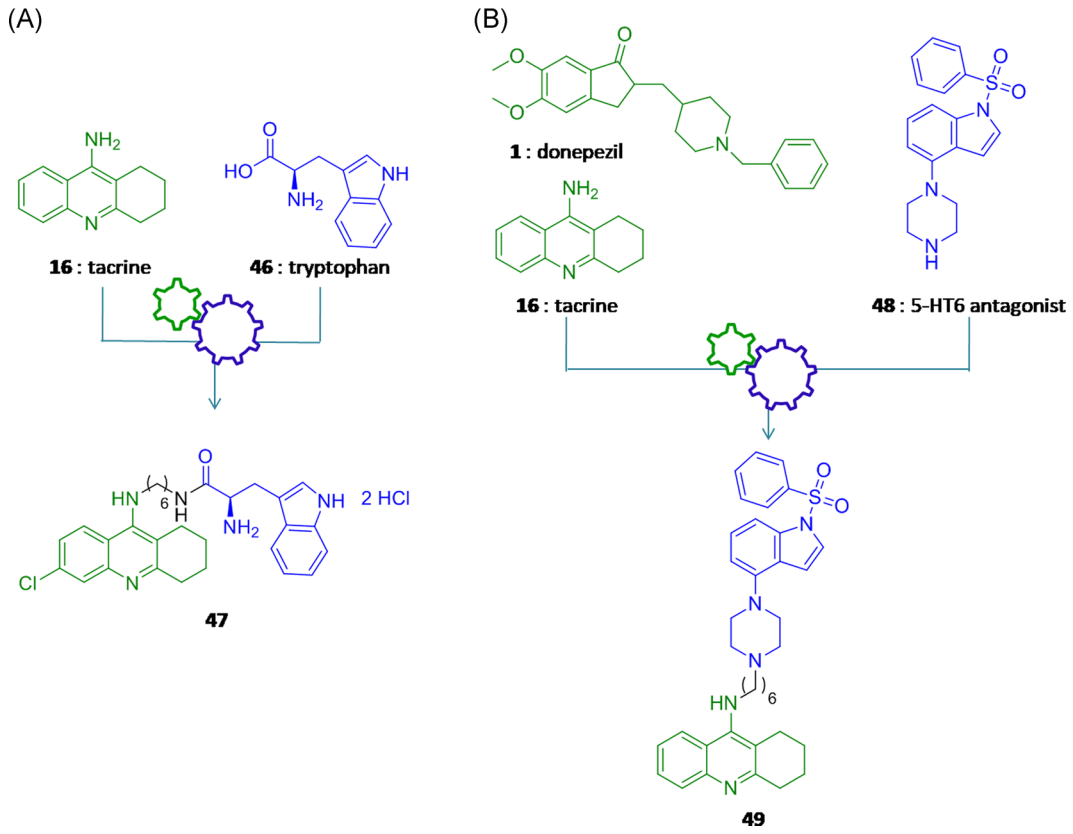

FIGURE 15 A) Design of hybrid **47**; B) Design of hybrid **49** [Color figure can be viewed at wileyonlinelibrary.com]

paradigm, have led to focus on the preclinical development of combination therapies and polypharmacological approaches. This trend is mirrored in the currently ongoing clinical trials, often based on drugs with multitarget profiles or drug combinations.[17]

We will discuss some recent examples of investigational drugs with a multitarget profile of action, even though some of them were not rationally designed and meant to be "truly" hybrids. We believe that these examples provide further evidence that hitting several targets may represent a promising prospective therapy against neurodegeneration.

Ladostigil, the most notorious example of a hybrid reaching clinical phases

The most notorious example of a hybrid that reached clinical trial is ladostigil (**50**) (Figure 16A). The structure of **50** results from the rational merging of a large portion of the structures of the MAO B inhibitor rasagiline and the AChEI rivastigmine (**9**), thereby leading to a low-molecular-weight hybrid compound **50**, which combines the neuroprotective effects of MAO inhibition and AChEI activity in a single molecule, and is intended for the treatment of AD comorbidity with extrapyramidal disorders and depression.[141] **50**'s safety and potential efficacy was assessed in a 3-year, randomized, double-blind, placebo-controlled phase II clinical trial (NCT01429623) in patients with mild cognitive impairment. It was safe and well-tolerated, but, unfortunately, it did not delay the progression of dementia.[142] Even though **50** did not meet the primary endpoint, its development has the clear merit of demonstrating the successful clinical translation of a rational MTDD approach against neurodegeneration.[4]

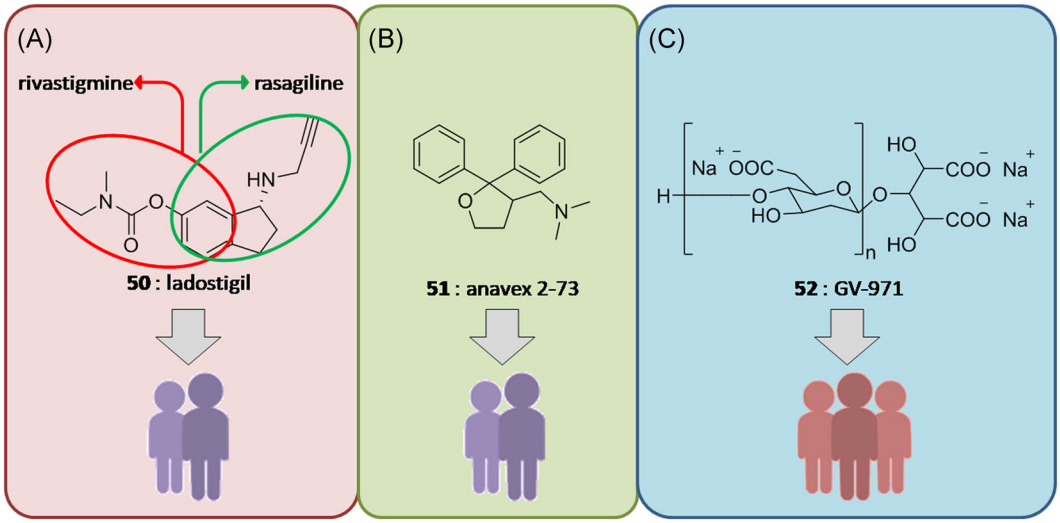

FIGURE 16 Examples of hybrids with multitarget profiles investigated in clinical trials A) **50**; B) **51**; C) **52** [Color figure can be viewed at wileyonlinelibrary.com]

Anavex 2-73, a promising drug for cognitive decline in patients with mild-to-moderate AD

Anavex 2-73 (**51**) (Figure 16B) is a small molecule, which is able to bind muscarinic and σ1 receptors with affinities in the low micromolar range. In in vivo studies, **51** has been shown effective in reversing scopolamine-induced long- and short-term memory deficits. These observations are in line with the pharmacological profile mediated by muscarinic and σ1 receptors; M1 muscarinic agonists are known to reverse the scopolamine-induced amnesia, whereas σ1 activation is involved in long-term memory processes.[135] Furthermore, stimulation of the M1 receptor blocks BACE-1, resulting in a decrease of Aβ peptide production. Even though **51** was not rationally designed as a proper hybrid, its dual cholinergic/σ1 activity, low active dose range, and long duration of action reinforce its therapeutic potential in AD.[143,144] In addition, it has been demonstrated that **51** may also target protein misfolding by modulating GSK3β, inhibit oxidative stress and mitochondrial dysfunction, and reduce inflammation and cellular stress.[136] Compound **51** has proven to be safe and well-tolerated throughout a phase II clinical study, which showed significant association between the dosage of **51** and cognitive and functional improvements.[145] The IIb/III phase (NCT03790709) is currently ongoing to evaluate the effects of **51** on cognition and function after 48 weeks of daily treatment.[146]

GV-971, an algae-based drug, successfully completed clinical trials in China

GV-971 (**52**) (Figure 16C) is the first drug registered for AD since 2003. Conditionally approved by China's regulators, it has caused controversy and skepticism among the scientific community. The FDA approval, however, will wait until significant results on two measures of cognitive ability will be collected in further multicenter international phase III clinical trial, starting in early 2020.[147] Although the mechanism of action of GV-971 remains unclear, a mechanistic link between gut microbiota dysbiosis and neuroinflammation in AD progression has been provided. In AD mouse models, alteration of gut microbiota composition leads to the peripheral accumulation of phenylalanine and isoleucine, which stimulates the differentiation and proliferation of pro-inflammatory T-helper 1 cells (Th1). This facilitates peripheral Th1 immune cells' cell entry into the brain, with subsequent M1 microglia activation and toxic neuroinflammatory response. **52**, sodium oligomannate derived from marine algae, has been shown to suppress gut dysbiosis and the associated phenylalanine/isoleucine accumulation, to harness neuroinflammation and reverse cognition impairment.[148] The same authors have also shown preclinical evidence of a multitarget mechanism of action for **52**, which should not only act by reversal of neuroinflammation, but also on Aβ plaques, neurofibrillary tangles, mitochondrial function, and cholinergic disruption.[149] In 2018, **52** completed

phase III clinical trial in China (NCT03715114) showing a statistically significant improved cognitive function in mild-to-moderate AD patients, as early as week 4 and further each follow-up assessment visit.[150]

5 | CONCLUSIONS

Much has been said about the challenges facing the pharmaceutical industry, as a result of the global AD epidemics and the high rate of failure in drug development, to the point where the industry and a few of its major players have pulled down.

Polypharmacology might have the potential to unlock a golden age of AD drug discovery, where medicinal chemists could play an even more important role than ever before. We anticipate that discovering and developing an innovative, first-in-class anti-AD MTDL has the potential to offer patients significant benefits as well as add substantially to the developer's bottom-line, as these medicines might become the new standard of AD care. Thus, it is our responsibility to make the most out of the opportunity currently offered by polypharmacology, based on all the different tools available (ie, drug combinations, codrugs, and hybrids) and the ever-increasing number of examples reported in the literature.

In this respect, a more structured approach to MTDD is needed. As discussed, target validation is a step that crucially determines the final outcome. We understand that approaching a rigorous target validation in a network perspective is not always feasible, especially in academic settings. As an easy way out, we foresee that one consistent starting point may come from drug combinations evaluated in ongoing clinical trials. Taking inspiration from trials based on robust evidence of synergic properties of the involved targets would build a strong rationale for a new MTDL project. This might allow to skip a phase, which is cumbersome and time-consuming and boost MTDD in academia and small biotechnology companies.

Clearly, another unprecedented opportunity is offered by computational tools being able to analyze and extract big data and take full advantage of the large amounts of information collected, ranging from genetic and molecular “omics” to the clinical phenotypes of AD patients. In addition, the fact that multiple polypharmacology options exist, each with peculiar therapeutic profiles, is a solid background. We are positive that an expanded polypharmacology armamentarium together with the recent big data opportunities may help to find the right tools for the right patient and contribute to win the battle against dementia.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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