Multi-Target Directed Drugs: A Modern Approach for Design of New Drugs for the treatment of Alzheimer's Disease

Kris Simone Tranches Dias[a,b] and Claudio Viegas, Jr.[a,b,*]

aLFQM - Laboratório de Fitoquímica e Química Medicinal, Institute of Chemistry, Federal University of Alfenas, 37130-000, Brazil; bPrograma de Pós-Graduação em Química, Federal University of Alfenas, 37130-000, Brazil

Abstract: Alzheimer's disease (AD) is a complex neurodegenerative disorder with a multi-faceted pathogenesis. So far, the therapeutic paradigm "one-compound-one-target" has failed and despite enormous efforts to elucidate the pathophysiology of AD, the disease is still incurable.

The multiple factors involved in AD include amyloid aggregation to form insoluble neurotoxic plaques of Aβ, hyperphosphorylation of tau protein, oxidative stress, calcium imbalance, mitochondrial dysfunction and deterioration of synaptic transmission. These factors together, accentuate changes in the CNS homeostasis, starting a complex process of interconnected physiological damage, leading to cognitive and memory impairment and neuronal death.

A recent approach for the rational design of new drug candidates, also called multitarget-directed ligand (MTDL) approach, has gained increasing attention by many research groups, which have developed a variety of hybrid compounds acting simultaneously on diverse biological targets. This review aims to show some recent advances and examples of the exploitation of MTDL approach in the rational design of novel drug candidate prototypes for the treatment of AD.

Keywords: Alzheimer's disease, multi-target directed drugs, neurodegenerative disorders, rational drug design.

1. INTRODUCTION

Alzheimer's disease (AD) is one of the biggest challenges of current research for new drugs [1-3]. In addition to cancer, diabetes, rheumatoid arthritis and other chronic inflammatory and neurodegenerative diseases, AD is characterized by multiple factors involving physiological, biochemical, chemical mediators operating concurrently with, caused by the same or different pathways [4, 5].

In recent years, advances in biochemistry, neuro-pharmacology and other biological fields have been responsible for new insights in the high complexity multifactorial pathophysiological hallmarks of AD. Thus, the development of innovative and effective multi-target directed drugs for AD represents a new paradigm in the discovery and drug design. Until then, the question that guided the planning of a new drug was "What is the best target for the treatment or prevention of a disease?" which supported, since the decade of 90, the origin of the reductionist strategy. By this concept, a drug should have its action limited to individual genes, a single protein or enzyme and a specific ligand to a target, following the current paradigm "one gene, one target, one drug" [1, 4, 6].

The limitations imposed by the low therapeutic efficacy against most of these diseases, coupled with the apparent reduction in the approval of new bioactive chemical entities, despite significant investments in the Pharmaceutical Industry, have reinforced the need to look up new strategies for planning and discovery of more effective and safer drugs.

In this context, three therapeutic strategies are possible: the first would be the use in combination of multiple active ingredients, like the associations of natural ingredients used for centuries in Traditional Chinese Medicine; or cocktails of drugs, such as used in the treatment of the acquired immunodeficiency syndrome (AIDS) and cancer. Another alternative would be a combination of more than one active ingredient in the same formulation. In a third and more recent approach, it would be a single molecule assembling structural requirements to allow molecular recognition of more than one target simultaneously, featuring a multi-target drug [4, 5]. When multiple targets related to the same pathology are associated with distinct biochemical cascades, the active ligand can be also called as a symbiotic compound [4].

2. SOCIO-ECONOMIC IMPACT AND PREVALENCE OF ALZHEIMER'S DISEASE

With the increase in average life expectancy worldwide, neurodegenerative diseases such as AD have attracted great attention, especially in more developed countries. As a progressive, disabling and incurable disease, patients die within average of 10 years after its installation [7]. The age is its main risk factor, with a prevalence of 0.7% among individuals 60-65 years of age and about 40% in the age groups above 90 years [8, 9]. In 2012, about 5.4 million of American people were carriers of AD. Despite the very

*Address correspondence to this author at the LFQM - Laboratório de Fitoquímica e Química Medicinal, Institute of Chemistry, Federal University of Alfenas, 37130-000, Brazil; Tel: +55 35 3299 1466; E-mails: cvjviegas@gmail.com or claudio.viegas@unifal-mg.edu.br

imprecise estimates available, it is believed that by 2050, over 16 million Americans will have AD, with an annual cost that could exceeds U$ 1.1 trillion [11, 12]. Other estimates point to a global epidemic of AD, reaching 26 million people by 2050, if a cure is not yet discovered and available [10, 14].

The increase in life quality, as a natural consequence of recent advances in medicine and technological development, has been reflected directly in the increased longevity worldwide. The United Nations (UN) believes that the "age of aging" began in 1975 and this trend is expected to extend until 2025 [14, 15]. According to the Brazilian Institute of Geography and Statistics, Europe occupies the 1st position in the proportion of the population above 60 years (19.8%), followed by North America (16.3%) [16].

3. MULTIFACTORIAL ASPECTS OF ALZHEIMER'S DISEASE

AD is a degenerative disorder of the central nervous system (CNS) and is one of the most common dementia among the population above age 65. AD is characterized by a progressive loss of memory, cognition, motor and functional capacity, gradually undermining social behavior and ability to perform routine tasks such as feeding, personal hygiene and interpersonal relationships. Although the etiology of AD is not completely understood, it is well established that operation of multiple interconnected factors are related to the installation, development and evolution of the disease [13].

Apparently, physiological deregulation in some brain regions, which the origin remains not entirely understood, is responsible for an overproduction of amyloid peptide (Aβ). Then, β- e γ-secretase enzymes abnormally cleave Aβ, producing insoluble fragments of 39-43 amino acid residues. Fragments Aβ$_{1-42}$, even at low concentrations, seem to be more prone to oligomerization and formation of insoluble neurotoxic aggregates, the so-called amyloid plaques, and are the main therapeutic target advocated by the "Amyloid Hypothesis" [5, 6, 17].

A secondary event due to neurotoxicity of amyloid plaques is the hyperphosphorylation of the neuronal microtubule constitutive tau protein. This abnormal phosphorylation of tau leads to structural collapse of microtubules and the consequent release of tau protein fragments, which take the form of insoluble fibrillar coiled, depositing intracellular as neurofibrillary tangles [6, 12, 18-20].

These two biochemical factors acting together lead to accentuate changes in the CNS homeostasis and function, causing numerous cellular processes and organelles activation, acting as starting points of a complex process of interconnected physiological damage, also leading to neuronal death [19]. Although most studies have emphasized the neurotoxicity of amyloid plaques, recent evidences indicate that increasing the concentration of soluble amyloid oligomers generated by overproduction of Aβ can be also responsible for increasing neuronal injury [6, 21].

Mitochondria are the major intracellular targets of soluble Aβ oligomers (sAβ). In excess, sAβ eventually interfere with normal function of mitochondria, causing overproduction of reactive oxygen species (ROS), inhibition of cellular respiration, ATP production and damage in mitochondrial structure [21, 22]. It seems that, the deleterious effects of sAβ the mitochondria are the result of changes in homeostasis and intracellular Ca^{2+} signaling, as evidenced by the induction of massive influx of Ca^{+2} in cultured neurons, causing its concentration increasing in mitochondria and neuronal apoptosis [21]. Accumulation of calcium in mitochondria causes opening of the mitochondrial permeability transition pore (mPTP), a wide mitochondrial membrane channel that allows the passage of large molecules bidirectional uncontrolled, resulting in disintegration of organelles and functional structure [21].

Recent studies have shown that the dephosphorylation of neurofibrillary tangles of tau protein was able to restore its ability to bind to microtubules neurons, indicating that the kinetic mechanisms that regulate the phosphorylation/dephosphorylation process are altered in AD [23]. The nature of the protein kinases, phosphatases and tau sites involved in these lesions was recently unveiled, suggesting that activation of protein phosphatase fosfoseril or fosfotreonil (PP-2a) or inhibition of glycogen synthase-kinase 3β (GSK-3β) protein as cyclin-dependent kinase 5 (CDK5) may be required for inhibiting the degeneration caused by neurofibrils in AD [23].

In recent years, many other hypotheses have been proposed to explain the complexity and multifactorial pathogenesis of AD, including oxidative stress, disruption of homeostasis by metal ions and neuroinflammation [19, 24, 25]. Currently, oxidative stress is considered one of the major causative factors of AD, unifying a number of other sequential or individual pathophysiologic events. Oxidative damage in the brain of AD patients is a result of excessive production of free radicals induced by Aβ, functional alteration in mitochondria, inadequacy in energy supplement, production of inflammatory mediators and alteration of antioxidant defenses [26, 28]. Modulation of cellular oxidative processes is closely related to the redox properties of some metals. Change in concentration of such ions can lead to oxidative stress and increased ROS production. Cupper (Cu^{2+}) and zinc (Zn^{2+}) and other metal ions influence the processes of protein aggregation, a critical step in many neurodegenerative diseases. In the case of AD, amyloid precursor protein (APP) and Aβ are able to form complexes with and reduce Cu^{2+}, which forms a high affinity complex with Aβ, promoting their aggregation.

Furthermore, *in vitro* studies showed that Aβ neurotoxicity is dependent on the catalytic generation of H$_2$O$_2$, which is enhanced by the presence of Aβ-Cu^{+2} complexes. In addition, Cu^{2+}, Zn^{2+} and Fe^{3+} are present in amyloid plaques in brains with AD, which can be dissolved by the action of metal chelating substances [6, 20, 29].

The production of Aβ also depends on the bioavailability of cholesterol in nerve cells, since the activity balance of the α- and β-secretases is related to the lipid composition of cells. High concentrations of cholesterol into the cells lead to an increase in amyloidogenic APP process by β-secretase, whereas at lower levels, cholesterol metabolism stimulates

the increasing the physiological α-secretase action on APP. The hypothesis that the control of plasmatic cholesterol levels would be beneficial for treating AD has been demonstrated by using anticholesterolemic drugs, such as statins, which act as HMG-CoA reductase inhibitors [20].

Finally, deposition of Aβ fragments and neurofibrils, coupled with the uncontrolled production of ROS, are crucial for the installation of a neuroinflammatory process, with the same complexity observed in peripheral tissues. The scope and relevance of this process in the establishment and development of chronic DA have been demonstrated in several recent studies in the literature [30-33]. Among all the brain cells, microglia appears to have fundamental importance in CNS inflammation associated with AD. These cells could be activated by Aβ and modulate the production of cytokines, chemokines and neurotoxins that are highly neurotoxic, contributing to neuronal degeneration [19, 30-33].

Given the variety of factors associated with the onset, progress and severity of AD, increasing their degree of pathophysiological complexity, and associated to the inefficiency of the current therapeutic arsenal available, it becomes unavoidable to adopt a new concept for the rational design of new drugs against DA. In this context, drug candidate prototypes with dual mode of action were the first attempts to look up ligands recognized by more than one molecular target or more than one site on the same macromolecular target. Currently, a new strategy of multi-target directed ligands is gaining special attention in the scientific community, which has been seeking in molecular hybridization, a tool for designing new molecular patterns. These molecular hybrids could lead to the identification of new bioactive chemical entities with selectively affinity for multiple targets, preferably in different biochemical cascades. Therefore, these innovative ligands could play a singular role in the advance of a broadly and more efficient therapy, and perhaps, in the cure of AD.

4. RECENT MULTI-TARGET DIRECTED DRUG CANDIDATES DESIGNED FOR THE TREATMENT OF AD

This new approach, considering multifunctional drugs or ligands directed at multiple targets associated with the same disease (symbiotic drugs), has gained special importance and introducing a new thinking approach in the design of new drug candidates for AD. Molecular hybridization of pharmacophoric subunits of different bioactive molecules, is the main tool for structural planning, and have provided the discovery of numerous ligands with multiple properties, including antioxidant, neuroprotective, metal chelation, anti-inflammatory, anti-Aβ aggregation, and cholinesterase and secretase inhibitory activities. Therefore, a set of other potential therapeutic targets have been studied for simultaneous intervention, seeking for a more efficacy in relieve the symptoms and slowing the progression of AD, and why not, its definitive control and cure [34-38].

Since 2005, the literature has shown several results from applying this innovative approach of drug design. Drugs such as donepezil, tacrine and rivastigmine [39, 40] have been used as structural models for molecular hybridization

with bioactive synthetic and natural products such as curcumine [41, 42], berberine [43, 44], 8-hydroxyquinolines [45], among others in the search for new chemical entities with multiple bioactive properties useful for the treatment of AD.

As one example of this strategy, berberine (**1**) was used as a basic skeleton for the construction of a series of hybrid compounds with molecular subunits that include melatonin (**2**) and ferulic acid (**3**, Fig. **1**). The aiming was to obtain new derivatives with antioxidant properties and anti-Aβ aggregation, but with reduced inhibitory potency of acetylcholinesterase (AChE). The hybrid compound hidroquine **4** demonstrated the best ability for suppression of Aβ aggregation, plus excellent antioxidant effect and reasonable ability to inhibit acetylcholinesterase (AChE) and butirylcholinesterase (BuChE). In addition, derivative **5** (Fig. **1**) was identified as a potent inhibitor of AChE, with strong antioxidant properties, confirming the multi-target potential of these substances in comparison to berberine, the original prototype [44].

Memoquin (**8**, Fig. **2**), a 1,4-benzoquinone-poliamine hybrid compound, was reported by Cavalli and co-workers as a promising drug prototype for AD. The structural design of compound **8** was based on a polyamine core, derived from coproctamine (**9**), a dimeric agent with anticholinesterasic and antimuscarinic. Aiming to add to these properties, the ability to neutralize ROS (reactive oxygen species) and neuroprotection, authors proposed the insertion of a 1,4-benzoquinone, from coenzyme Q10 (CoQ10), a fragment with a recognized potent mitochondrial antioxidant property and a protector of the hippocampus neurons against Aβ(1–40)–induced neurotoxicity. The inner polymethylene chain was replaced by the benzoquinone nucleus. The insertion of the benzoquinone nucleus contributes to increase the neuroprotective activity, because a hydrophobic and planar π system is generated, which is able in principle to bind Aβ [42, 47, 48, 50].

Memoquin (**8**) exhibited ability to inhibit AChE (IC_{50}= 1.55 nM), almost 15-fold more potent than the reference compound (coproctamine, **9**). Memoquin (**8**) also inhibited AChE-induced $Aβ_{1-40}$ aggregation with an IC_{50} of 28.3 μM and the self-induced $Aβ_{1-42}$ aggregation with an IC_{50} of 5.93 μM. In addition, compound **8** is also capable to inhibit BACE-1 (β secretase) activity in range nanomolar concentrations (IC_{50}= 108 nM) and showed antioxidant properties, reducing the formation of free radicals by 44.1%. It was demonstrated that the 1,4-benzoquinone moiety can be reduced *in vivo* to the 1,4-dihydroquinone form, increasing its antioxidant potential and scavenging properties. Finally, *in vivo* assays showed the potential of memoquin to restore the cholinergic deficit and successfully reduce Aβ expression and accumulation, also reducing hyperphosphorylation of tau protein [48, 49].

Considering all these multi-target profile of memoquin (**8**), Bolognesi and co-workers reported in 2009 the design of new hybrid lipoic acid-memoquin (**11**) derivatives inspired on the memoquin structure and lipoic acid structure which is a potent antioxidant. In addition to the observed biological properties of memoquin, compound **12** also exhibited a good
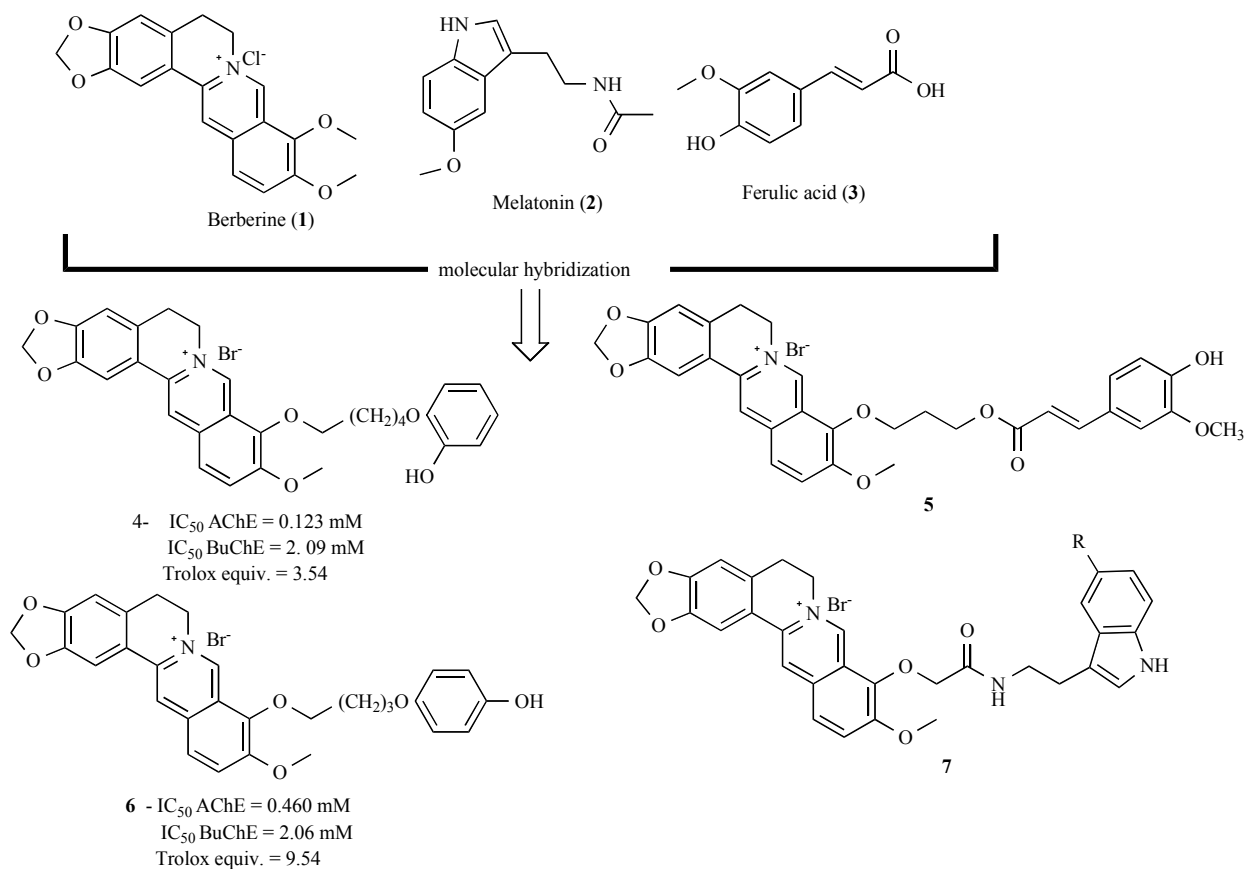
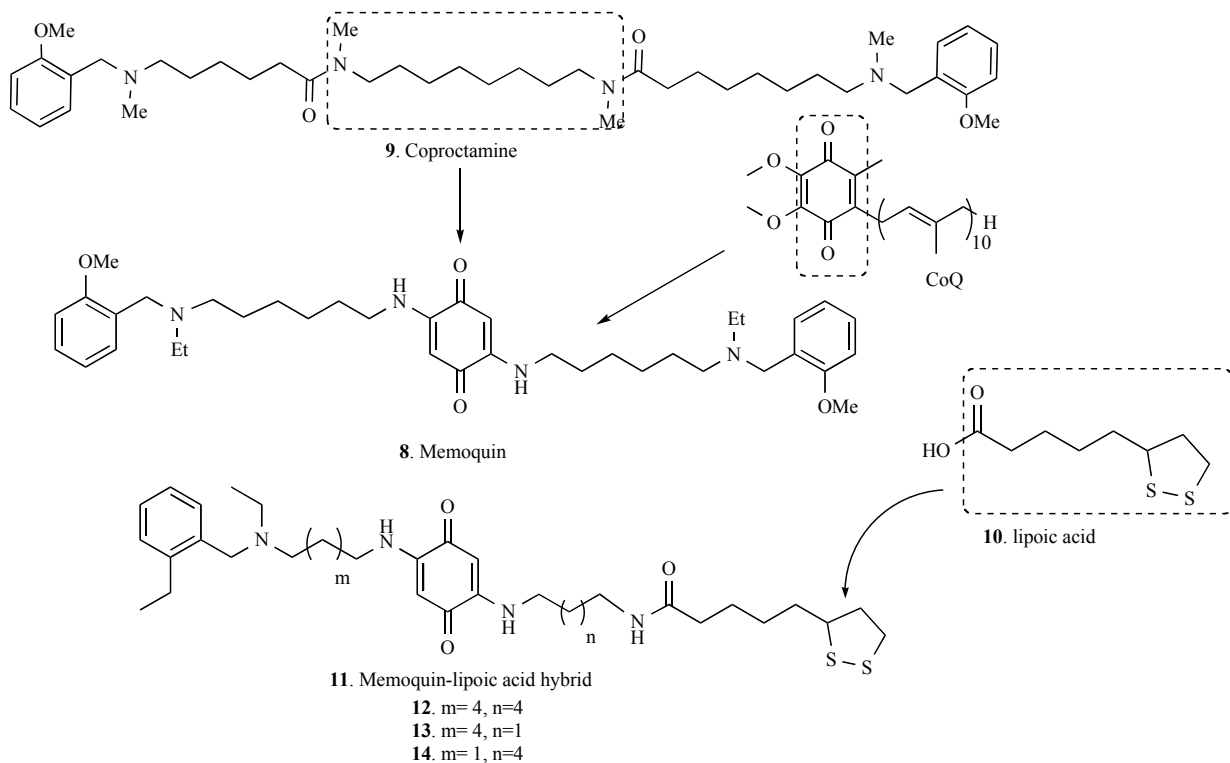**Fig. (1).** Series of hybrid derivatives (**4-7**) structurally designed from berberine (**1**).

Fig. (2). Design strategy for memoquin (**8**) and their derivatives (**12-14**).

neuroprotective effect against oxidative stress, greater than the reference control lipoic acid (**10**) [49].

Curcumine (**15**, Fig. **3**) is the major constituent of *Curcuma longa*, a plant species used in Indian Traditional Medicine, with Aβ-aggregation inhibitory properties have been used as a structural model for molecular to generate new analogs of memoquin with enhanced neuroprotective AChE and BuChE inhibitory properties. Among the novel memoquin hybrid derivatives, compounds **16** and **17** (Fig. **3**) showed the best multiple-target action profile with selective inhibitory activities of AChE and BuChE, with modulatory effects on the amyloid aggregation and inhibition of neurofibrils formation [46].

Tacrine (**19**, Fig. **4**) is the first approved ChEs inhibitor by the FDA for the treatment of AD. However, due to some undesirable side effects this drug have its therapeutic use restricted and, therefore, the search for more effective and secure tacrine derivatives is still of interest. In recent years, many studies have focused on the combining the potent

ChEs inhibitory properties of tacrine with additional biological properties to search for new MTDLs.

Based on this strategy, Contelles and co-workers synthesized a new series of 4H-pyrano[2,3-b]quinolone derivatives (**20**), planned by molecular hybridization of tacrine fused to a benzylamino-3-pyridyl ring moiety, aiming to obtain new AChEIs with an innovative molecular scaffold and to explore its pharmacophoric contributions to additional neuroprotective properties and modulatory effects on voltage-dependent Ca^{+2} channels. In general, the new tacrine-hybrid compounds showed to be better selective for AChE inhibition than tacrine. It seems that the substitution at C-20 position of the 3-pyridyl ring system (compounds **22, 23, 24**) was deleterious for AChE inhibition as observed for derivative **21** (X= H). In contrast, compounds **22-24** showed a good neuroprotective profile, as the derivative **23** exhibiting the best neuroprotective effect, with only 46% of cell-death suppression [51].
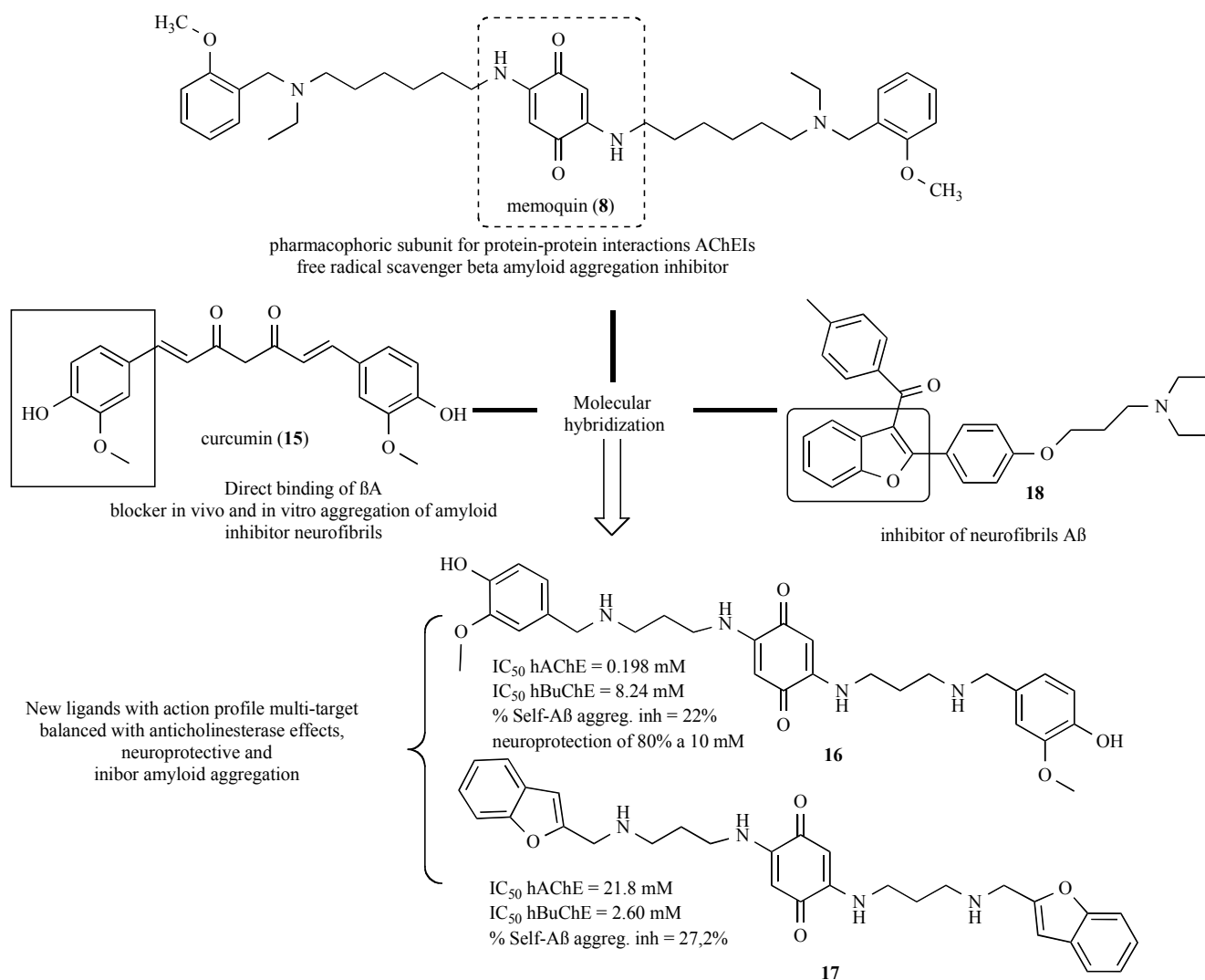

Fig. (3). Compounds **16** and **17** designed as new analogues of memoquin (**8**) with neuroprotective properties, anti-amyloid, and selective AChE and BuChE inhibitory activities.
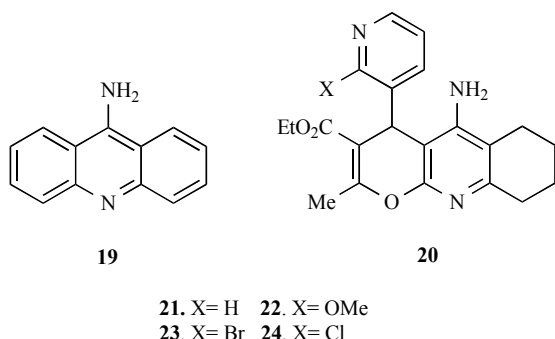
Fig. (4). Chemical structure of tacrine (**19**) and tacrine-hybrid derivatives (**21-24**).

The dimer bis-(7)-tacrine (**25**, Fig. **5**), was one the first homodimers reported in the literature with increased AChE affinity, exhibiting a 1000-fold higher inhibitory potency than tacrine. This best inhibitory profile is due to a dual simultaneously interaction with active and peripheral sites of AChE. Further studies, demonstrated that this compound was also capable to inhibit the AChE-induced Aβ aggregation with an IC$_{50}$ of 41.7 μM. Because of this symbiotic profile, compound **25** has been described as an important structural model for rational design of novel MTDLs [52].

In 2007, Bolognesi and co-workers proposed modifications on the structure of bis-(7)-tacrine (**25**) with an insertion of a spacer subunit with functional groups capable to of chelate metals, which are involved in the degenerative process. For this purpose, compounds **26** (BW284c51) and ambenonium (**27**) were selected as model prototypes due to their

cholinesterase inhibitory activity and their singular structural feature, carrying carbonyl and oxalamide functionalities with could lead to a the desired metal chelation property (Fig. **5**). The multifunctional compounds maintained a potent AChEIs in the nanomolar range, showed additional inhibitory effect of AChE-induced amyloid-β aggregation. The results showed the ability for chelation of Cu^{2+} and Fe^{3+} ions and suggest that these compounds might act against AD by a chelation mechanism [40].

Minarini and co-workers also explored the structure of bis-(7)-tacrine (**25**), in an attempt to obtain the hybrid derivative cystamine bis-(7)-tacrine (**31**), with an insertion of the cystamine moiety **32** as a linker between two acrydine subunits of the bis-tacrine framework of **25** (Fig. **6**). Cystamine (**31**) have shown important biological properties such as antioxidant and neuroprotective. This study revealed the hybrid dimer cystamine-tacrine **31** was able to inhibit AChE (IC$_{50}$= 5.04 nM), BuChE (IC$_{50}$= 4.23 nM), self-Aβ aggregation (IC$_{50}$= 24.2 μM) and AChE-induced Aβ aggregation (52,6%) in the same range of the reference compound, with additional neuroprotective effect on SH-SY5Y cell line against H$_2$O$_2$-induced oxidative injury, with low toxicity [52].

Fang and co-workers reported in 2008 the synthesis and pharmacologic evaluation of a new series of tacrine-ferulic acid hybrid derivatives (**33**) (Fig. **7**). They planned a new structural pattern by the connection of ferulic acid (**34**) to the structure of tacrine (**18**) *via* an alkylenediamine as a linker side chain. This approach was used aiming to obtain new multi-target hybrid compounds which could conjugate the AChE inhibitory activity originating from the tacrine
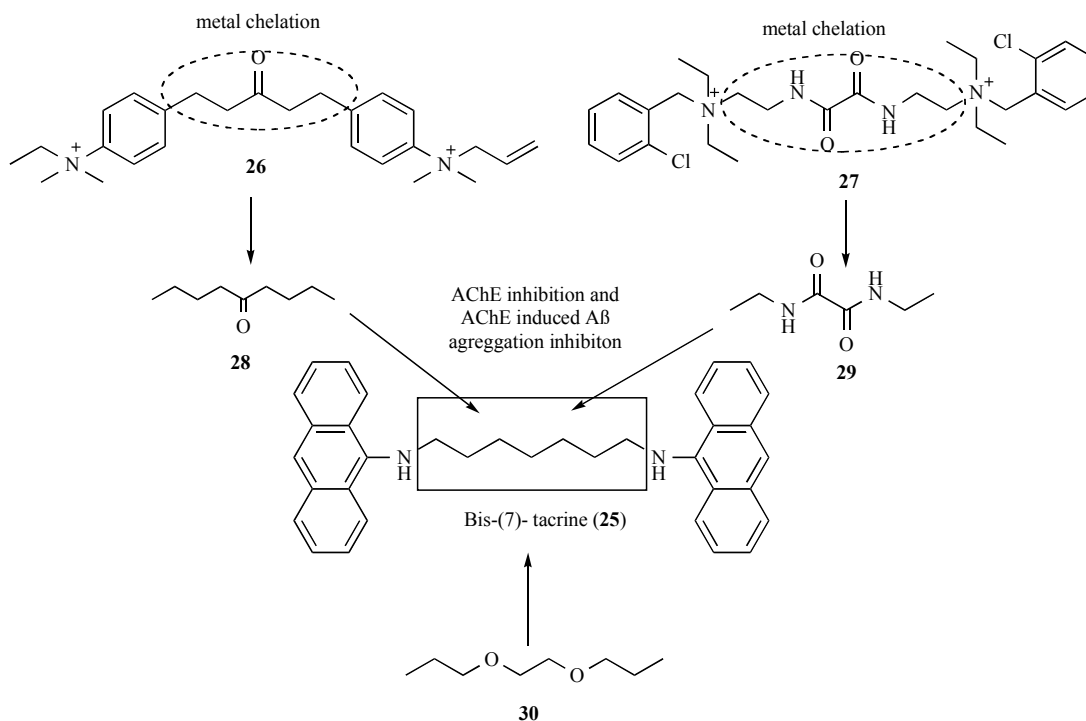

Fig. (5). Design of the new series of derivatives of bis-(7)-tacrine (**25**) with improved activities for inhibition of AChE inhibition and AChE-induced amyloid-β aggregation, and ion metal chelation.
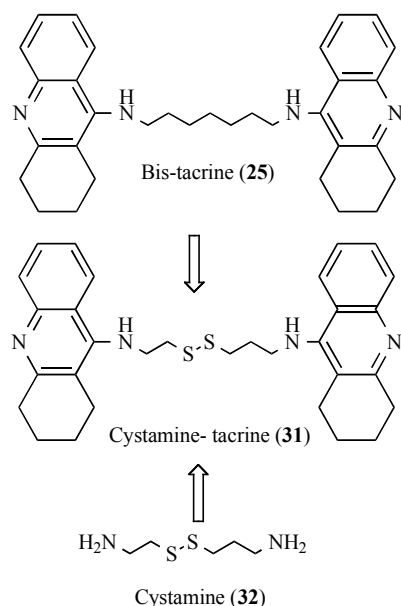
Fig. (6). Design of the multipotent cystamine-tacrine dimer (**31**) by molecular hybridization of bis-(7)-tacrine (**25**) and cystamine (**32**).
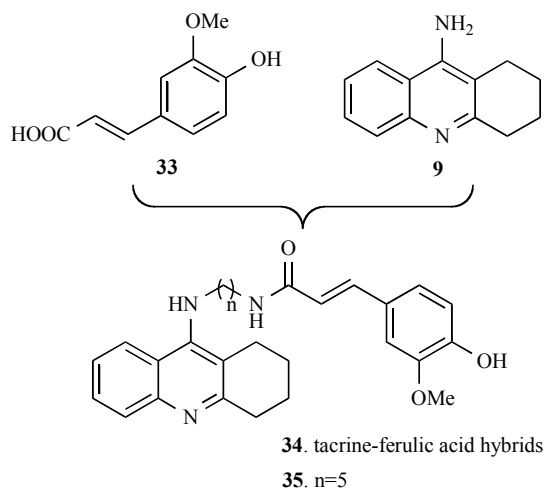

Fig. (7). Design of the new series of hybrid tacrine-ferulic acid (**34**) derivatives with dual antioxidant and IAChE properties.

template and the antioxidant activity from the ferulic acid moiety. The compounds showed lower antioxidant activity than ferulic acid, but all hybrids tested showed moderate to good antioxidant activity. All compounds effectively inhibited AChE in *in vitro* assays. Particularly, compounds **35** showed a 10-fold higher AChE inhibitory activity (IC$_{50}$= 4.4 nM) than tacrine. Enzymatic kinetics studies suggest that this compound possess high affinity binding to the peripheral anionic site (PAS) of AChE and, therefore, could inhibit Aβ-peptide fibril formation [53].

In another approach, Rosini and co-workers also used tacrine as a model for the planning of a new series of hybrid compounds (**36-39**) based on the structural feature of carvedilol (**35**). This compound had been identified by

endows a significant neuroprotective efficacy, probably related to its modulatory action as a low-affinity antagonist of N-methyl-D-aspartate (NMDAR). Thus, carbazole moiety of **35** was elected as a probable structural pharmacophoric subunit responsible by its antioxidant properties and efficient inhibitory activity of Aβ-fibril formation (Fig. **8**). Biological evaluation revealed that all compounds showed effective AChE-inhibiting activity in a nanomolar range, being more potent than tacrine. Particularly, compounds **36-39** showed an adequate biological profile for multi-target drugs, inhibiting AChE activity and also capable to block *in vitro* Aβ self-aggregation, AChE-mediated Aβ-aggregation, NMDARs antagonistic effect and to reduce cerebral oxidative stress [54].

In another approach, tacripyrines (**40**) were designed by combining tacrine with a calcium antagonist such as nimodipine (**41**) (Fig. **9**). In this study, the AChE inhibitory activity of the nimodipine–tacrine hybrids **40** is potentiated, with IC$_{50}$ in the nanomolar range for AChE. Besides acting as an inhibitor of AChE, these compounds also showed significant neuroprotective effects. In particular, compound **42** (IC$_{50}$= 105 ±15 nM), proved to be moderately active in models AChE-induced and Aβ-self aggregation models (30 and 35% inhibition, respectively). Further kinetic studies and molecular modeling data suggest that compound **42** could interact with the PAS of the AChE, exerting its inhibitory properties by noncompetitive mode. Additionally, the most of the compounds showed moderate Ca^{2+} channel blocking effect and therefore the compounds are neuroprotective agents [55].

A novel series of tacrine–caffeic acid hybrids (**43**) were designed by combining the structure of caffeic acid (**44**) and tacrine (**19**) (Fig. **10**) as multitarget-directed ligands against Alzheimer's disease. *In vitro* studies showed that most of the target molecules exhibited significant antioxidant activities on oxygen radical absorbance capacity method (ORAC). The hybrid molecules showed to be good metal chelators and also exhibited higher antioxidant capacity than the caffeic acid with values of 2.75−9.37 µM. In particular, compound **45** exhibited the greatest ability in inhibition of self- or AChE-induced β-amyloid$_{1-40}$ aggregation, as well as showed potent neuroprotective effects against H$_2$O$_2$- and glutamate-induced cell death, with low toxicity in HT22 cells. Further molecular modeling and enzymatic kinetics studies clarified that these inhibitors may act in two binding sites of AChE, which could explain the inhibitory effect exerted over Aβ-aggregation [56].

In another work, Wang and collaborators reported a series of tacrine-based hybrid compounds, containing an additional pharmacophoric subunit 4-phenyl-2-aminothiazole with different spacer subunits. The pharmacophoric groupment 4-phenyl-2-aminothiazole was elected based on data form literature that suggest this functionality as responsible for inhibitory effects on tau protein aggregation and on Aβ self-aggregation, neuroprotective and anti-inflammatory.

All 10 compounds of the series of phenylthiazole–tacrine hybrids (**47**, Fig. **11**) were potent inhibitors of cholinesterases with IC$_{50}$ values ranging from 5.78 ± 0.05 to 7.14 ± 0.01 nM for AChE, and from 5.75 ± 0.03 to 10.35 ± 0.15 nM for
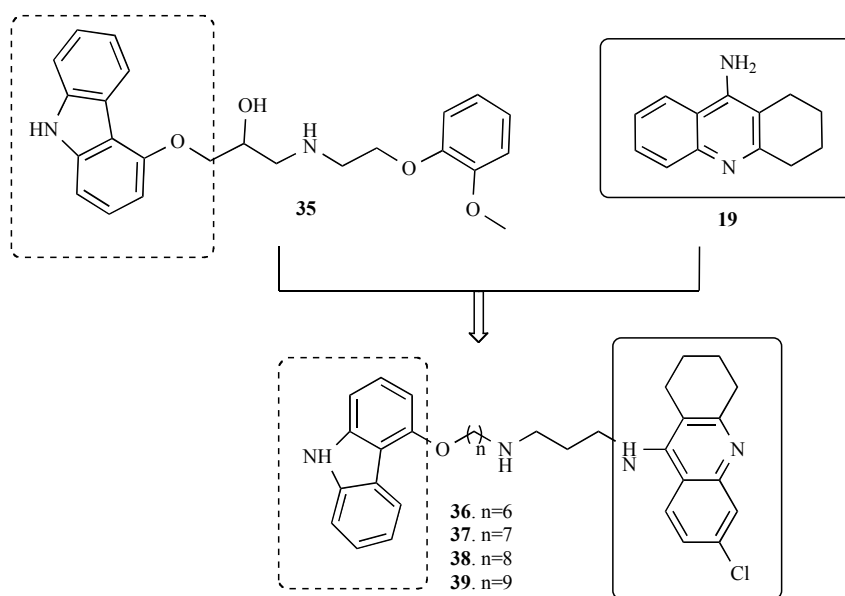
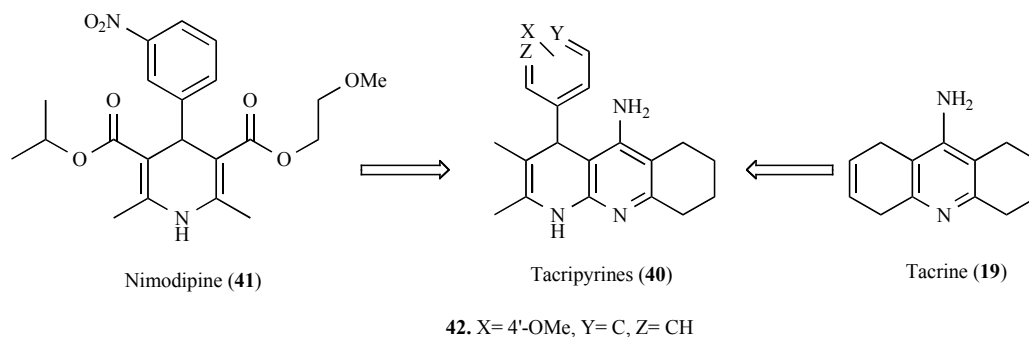**Fig. (8).** Chemical structure of carvelodiol (**35**) and their active derivatives **36-39**.

Fig. (9). Structure of tacripyrines (**40**) and the most active compound **42** designed by molecular hybridization of Tacrine (**19**) and Nimodipine (**41**).

BuChE. Moreover, a structure-activity relationship study was conducted to study the influence on the type of middle linker and substitutions at 40-position of 4-phenyl-2-aminothiazole. The results were indicative that the length of middle linker affected the AChE inhibitory potency. Compound **48**, with the largest linker subunit exhibited showed the greater potency in AChE inhibition (IC$_{50}$= 7.14 ± 0.01 nM). Data from kinetic studies have shown that this compound is a mixed-type inhibitor and could bind simultaneously at the catalytic and the peripheral sites of AChE. Most of phenylthiazole–tacrine hybrids showed a good inhibitory potency on Aβ$_{1-42}$ self-aggregation, although the activity was lower when compared with subunit 4-phenyl-2-aminothiazole. Additionally, compound **48** displayed a blockade effect on Ca^{2+} overload in the primary cultured cortical neurons [57].

According to the studies of Simoni and co-workers, a series of hybrid derivatives of galanthamine (**49**), an AChE inhibitors and memantine (**50**), a noncompetitive NMDA receptor antagonist, showed potent AChE inhibitory activities with multiple neuroprotective effects *in vitro* (Fig. **12**). Some of these hybrid compounds were also capable to prevent

glutamate-induced neurotoxicity by moderately blocking glutamate receptor NMDA subtype and neuronal cell
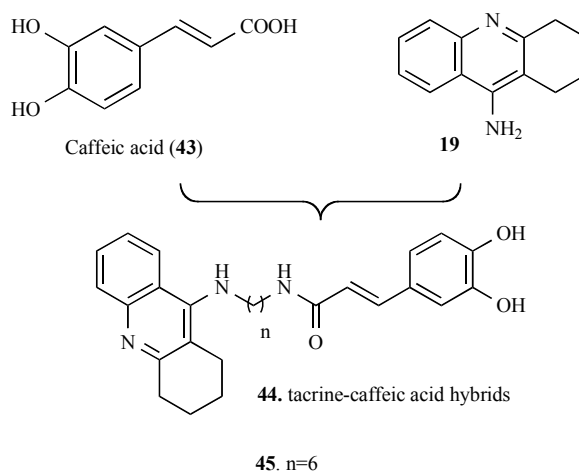

Fig. (10). Chemical structure of caffeic acid (**43**) and their hybrid derivatives **44** and **45**.
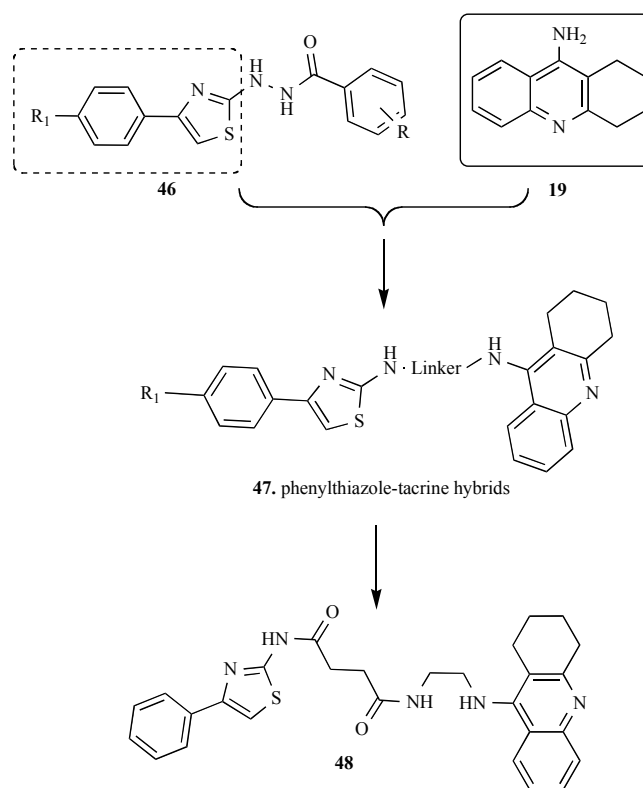
Fig. (11). Design of the phenylthiazole–tacrine hybrid compounds **47** and structure of the most active derivative **48**.

death *via* NMDA receptor-dependent effect at nanomolar concentrations (e.g., memagal (**52**), $IC_{50} = 0.28$ nM). These molecules were the first examples reported of dual AChE/NR2B drug prototype candidates [58].

A new series of 30 bivalent β-carboline derivatives (**53**) was synthesized by Rook and co-workers based on previous studies that disclosed monovalent β-carbolines as potent dual AChE/BuChE inhibitors (Fig. **13**). During an investigative study for the ability of these β-carboline derivatives to interact with multiple targets, the researchers also found that several compounds were also potent NMDA receptor blockers, in addition to the potent inhibitory properties of both AChE/BuChE enzymes. A structure-activity study revealed that the inhibitory activity of quaternary N9-bivalent β-carbolines was increased compared to their monovalent analogues. Compound **55** showed the higher AChE inhibitory activity ($IC_{50}= 0.5$ nM) and proved to be a potent inhibitor of the transient induced-glutamate Ca^{+2} with an $IC_{50}= 1.4$ μM, which is 4-fold more potent than memantine ($IC_{50}= 5\ 5.6$ μM) in the same assay conditions [59].

In another approach for drug design of MDTLs, Rizzo and co-workers reported a new series of hybrid molecules based on the structure of the AChE inhibitor *N*-Methyl-*N*-benzylamine (**56**), and compound SKF-64346 (**57**), a benzofuran derivative with good inhibitory properties of Aβ-fibril formation, using a heptyloxy moiety as a linker subunit. All compounds evaluated have shown to be good AChE and Aβ-fibril formation inhibitors, reducing the Aβ neurotoxicity. Compound **58** showed an IC_{50} of 1.82 μM in
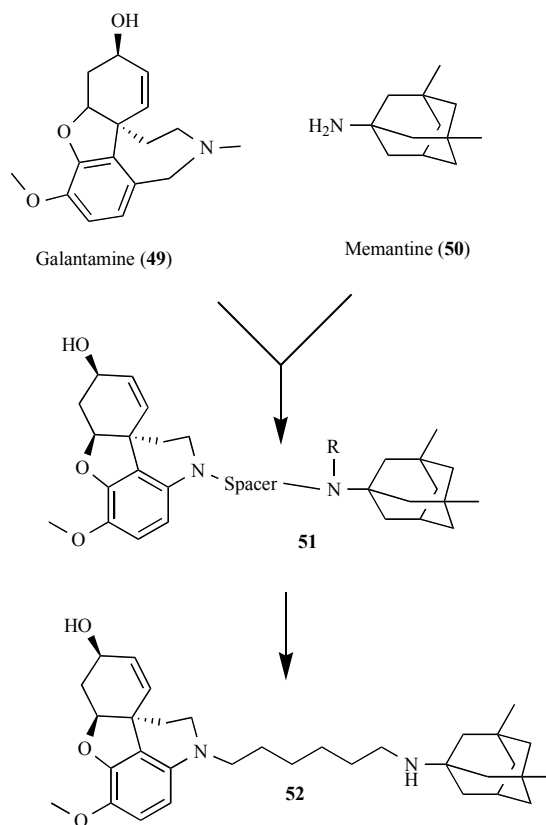

Fig. (12). Chemical structure of galanthamine-memantine hybrid compounds **51** with dual AChE/NR2B effect.
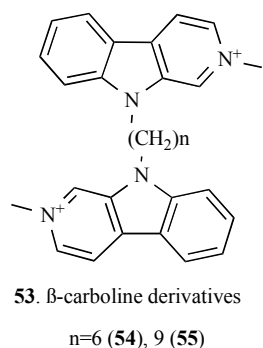
53. ß-carboline derivatives

n=6 (**54**), 9 (**55**)

Fig. (13). Chemical structure of new bivalent β-carboline derivatives **54** and **55**.

BuChE inhibition, being 5.7-fold more selective toward BuChE than AChE (IC$_{50}$ =10.5 μM). Furthermore, this compound was also able to inhibit Aβ self-aggregation with an IC$_{50}$ of 13 μM and showed a neuroprotective effect of 63% at 30 mM [60, 61]. Some years later, aiming to improve its pharmacological profile the researchers modified the original structure of compound **58** by variation of the spacer subunit between the 2-arylbenzofuran and the *N*-methyl-*N*-benzylamine moieties. They proposed a variation in the length of the spacer subunit, modification in the substituents in 3 position

of the benzofuran scaffold and change of the *N*-methyl-*N*-benzylamine heptyloxy side chain from the *para* to *meta* position (Fig. **14**) [61]. These structural modifications led to a new series **59** that exhibited an increased in activity for most of the modified compounds.

Among the most active compounds of the series **59**, compounds **60** and **61** exhibited the best BuChE inhibitory properties, with carbamate **62** also showing higher dual inhibitory activity of AChE and BuChE in comparison to rivastigmine. In addition, compounds **63** and **64** also showed significant *in vitro* inhibitory activities on Aβ-fibril formation up to 81% (Fig. **15**) [61, 62].

In another work, Piazza and co-workers also searching for multifunctional drug candidates for AD planned a series dual of inhibitors of human AChE (hAChE) and β-secretase (BACE-1). The molecular design was based in a dual binding site AChE inhibitor, AP2238 (**65**), which is able to simultaneously interact with both the central and the peripheral anionic sites of AChE. It was synthesized a series of compound **65** derivatives by replacing the methoxy substituents of the coumarin moiety by an amidic chain subunit aiming to extend the activity to BACE-1 (Fig. **16**). The positions 6 or 7 of the coumarin moiety were also replaced by a dihalophenyl acid subunit, considering that this moiety emerged as a leitmotif in different BACE-1 inhibitors
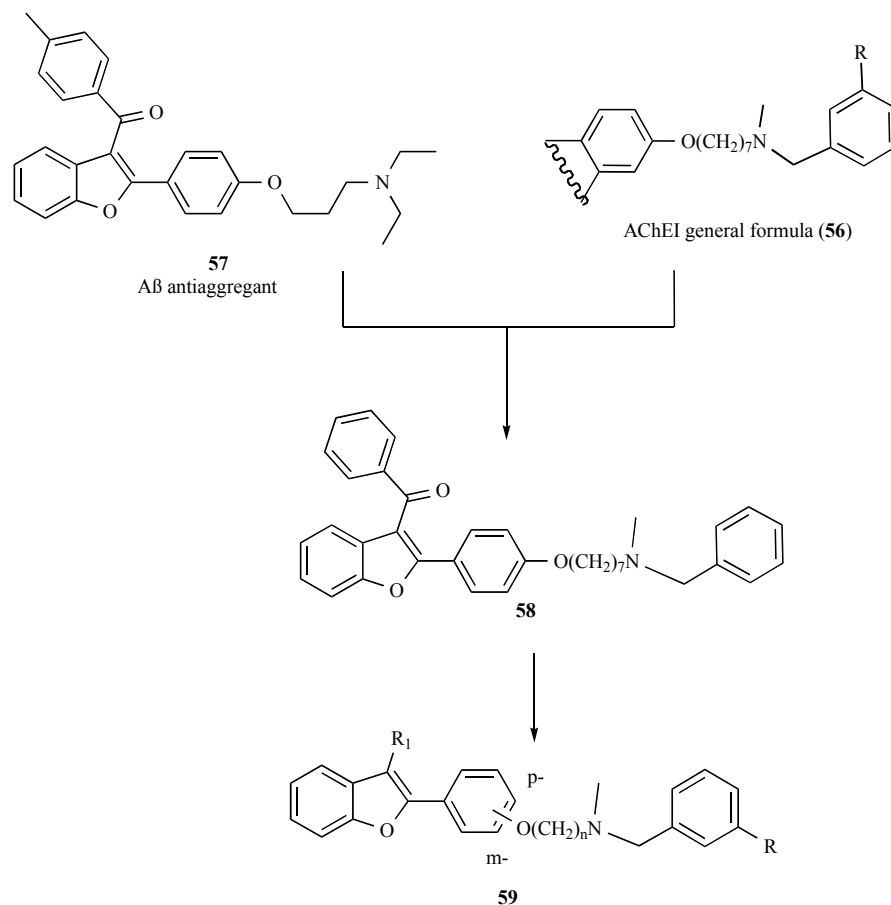

57
Aß antiaggregant

AChEI general formula (**56**)

58

59

Fig. (14). Design of compound **58** by molecular hybridization of SKF-64346 (**57**) and *N*-Methyl-*N*-benzylamines (**56**) and its molecular derivative series **59**.
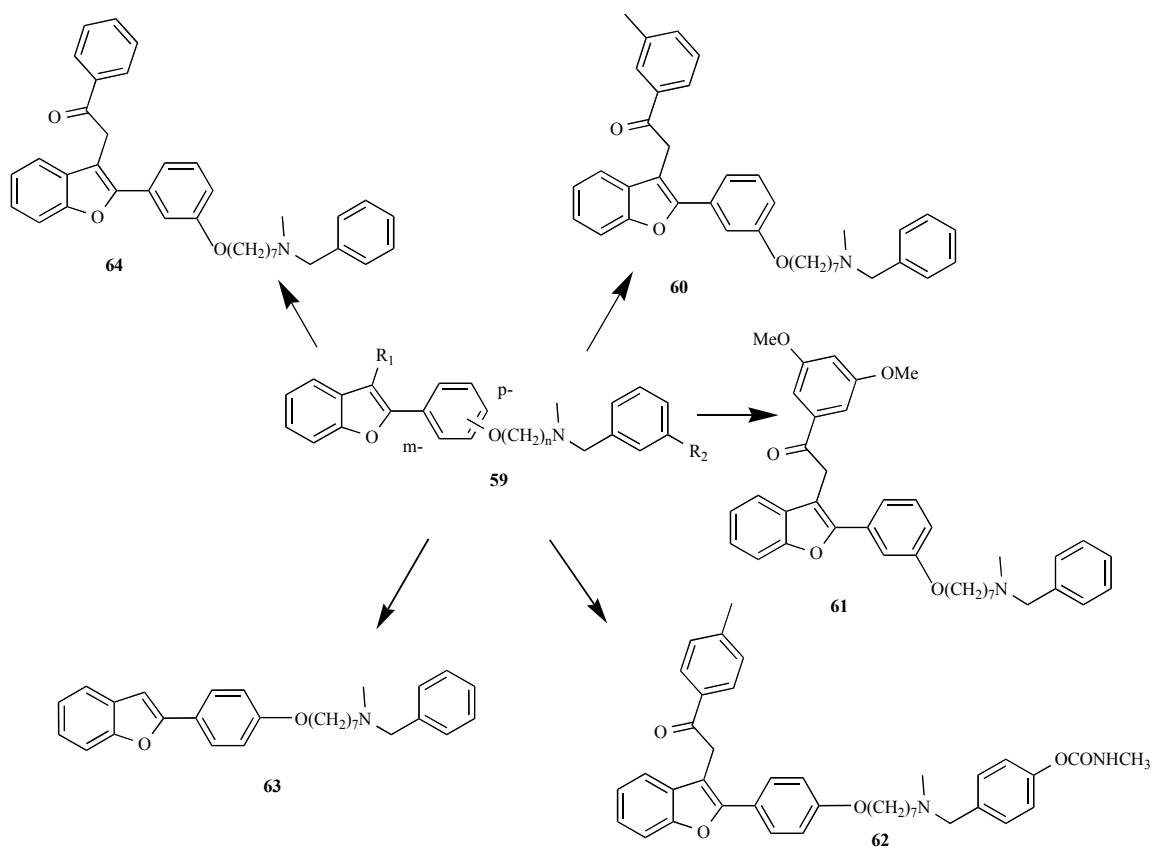
Fig. (15). Chemical structures of the improved derivatives **60-64** designed by molecular optimization of the hybrid prototype **59**.
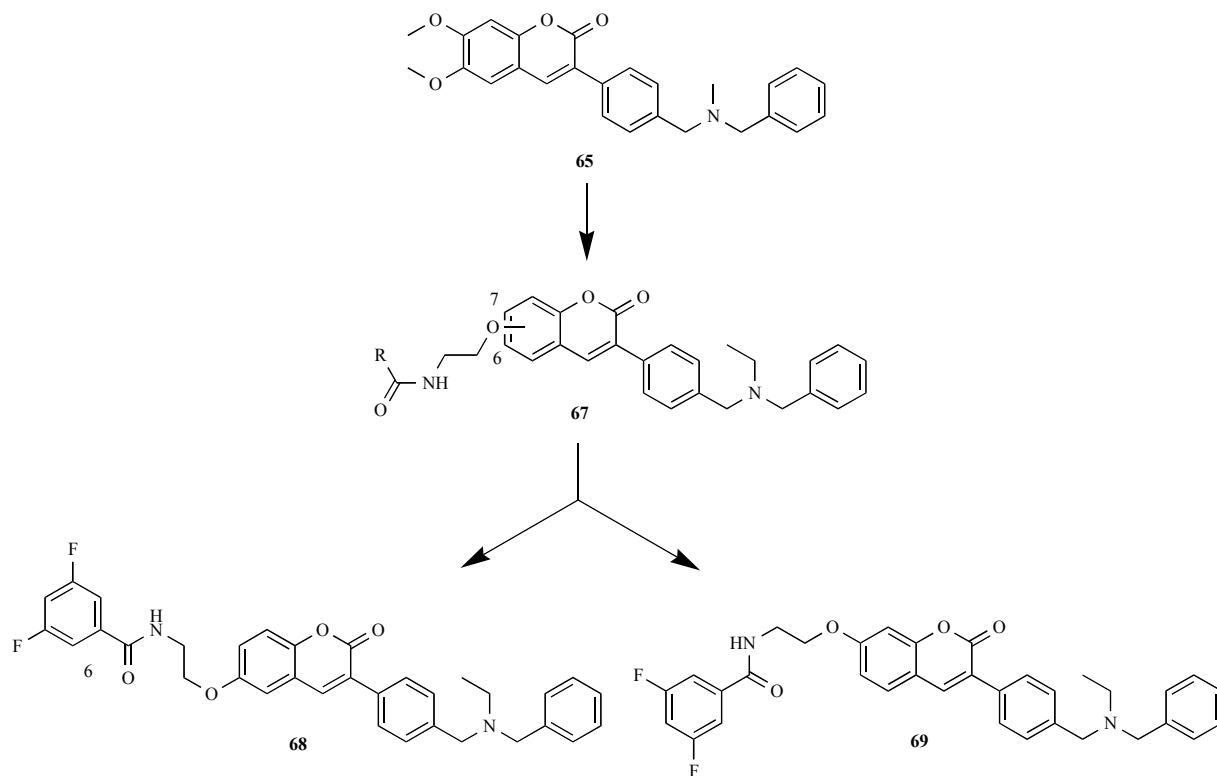

Fig. (16). Chemical structure of compounds **68** and **69**, designed as dual hybrid inhibitors of hAChE and BACE-1 based on the AChE inhibitor AP2238 (**65**).
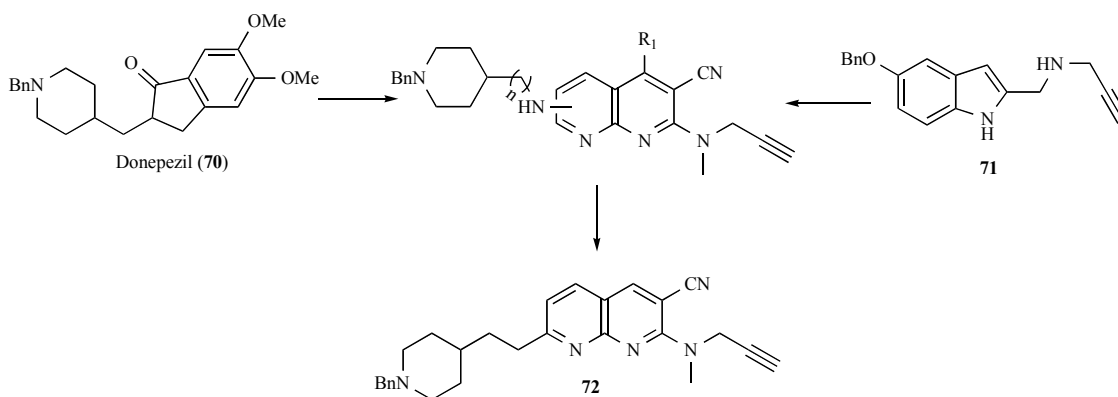
Fig. (17). Chemical structure of the most active dual AChE and MAO-A inhibitor designed by molecular hybridization of donepezil and PF9601N (**72**).

reported in the literature. All compounds were potent inhibitors, particularly compounds **67** (IC$_{50}$ $_{hAChE}$ = 0.551 µM, (IC$_{50}$ $_{BACE-1}$= 0.149 µM), and **65** (IC$_{50}$ $_{hAChE}$= 0.181 µM, IC$_{50}$ $_{BACE-1}$= 0.150 µM) that exhibited the best inhibitory profile in the target series. Biological data clearly indicated that the introduction of a halophenyl alkylamidic subunit on the scaffold of **65** allowed obtaining more potent BACE-1 inhibitors, and the presence of substituents in the positions 6 or 7 on the coumarin nucleus seems to equally contribute to the relative binding affinity of this molecular series [62].

A new family of multitarget molecules able to interact with both AChE and BuChE, as well as with monoamine oxidase (MAO) A and B, has been synthesized by Samadi and collaborators. Rational structural pattern of these compounds considered conjunctive approach by combination of benzylpiperidine and *N*-propargylamine moieties present in the AChE inhibitor donepezil (**70**) and the MAO inhibitor PF9601N (**71**), respectively, connected by a central pyridine or naphthyridine ring system (Fig. **17**). The most promising derivative **72** showed a potent inhibitory activity of AChE (IC$_{50}$= 37 nM), but less potent than donepezil. This compound also showed a moderate inhibitory potency of MAO-A (IC$_{50}$= 41 µM). Moreover, molecular modeling showed that these inhibitors probably act in two binding sites of AChE, and that the length of the spacer subunit is of particular relevance in the modulation of AChE inhibition, being crucial for the dual interaction of these molecules with both CAS and PAS sites the enzyme [63].
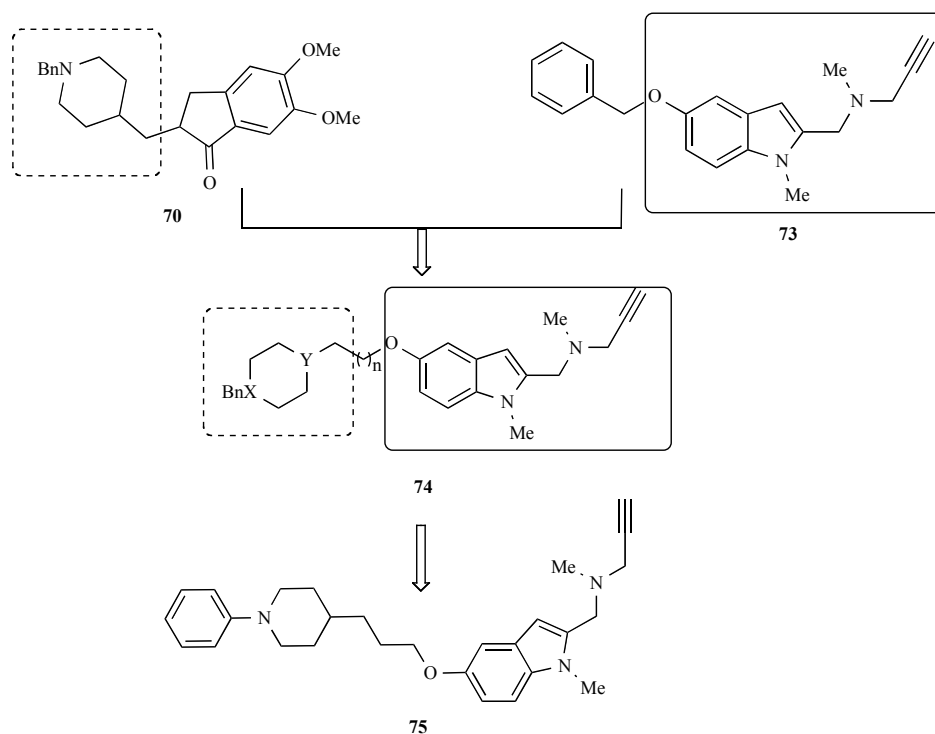

Fig. (18). Chemical structure of the lead-compound **75** designed as a MDTL ligand for ChEs and MAO-A and B.
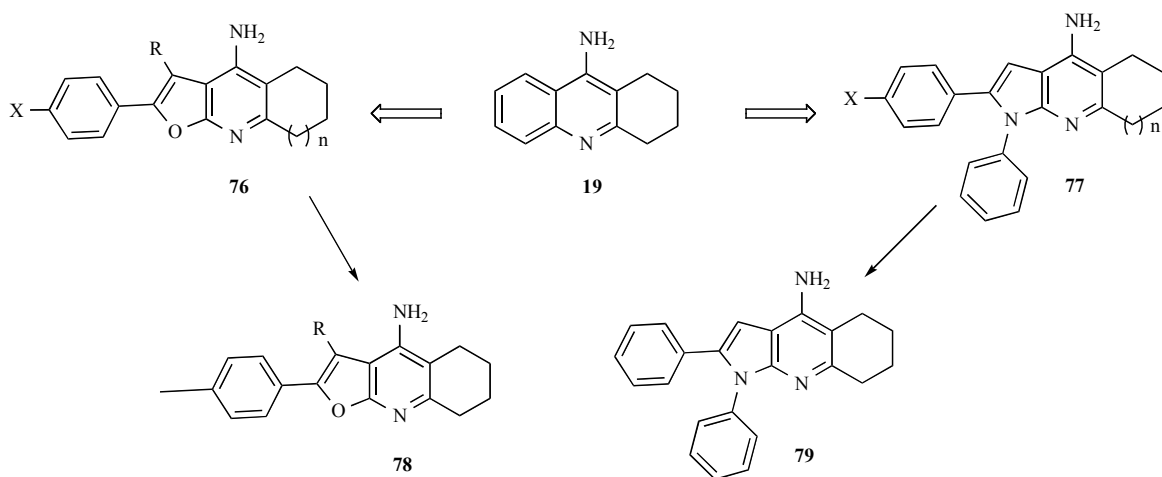
Fig. (19). Chemical structure of the compound **79**, the lead-molecule form the pyrrolo[2,3-b] quinolin-4-amine series.

In 2011, another recent publication by Bolea and collaborators also reported the synthesis and pharmacological evaluation of a new family of multifunctional molecules capable to interact simultaneously with the AD-related enzymes AChE, BuChE, MAO-A and MAO-B. The structural architecture of the series **74** (Fig. **18**) was based on the molecular hybridization of the benzylpiperidine moiety of donepezil (**70**) and the indolyl-propapylamino subunit present in the structure of the MAO inhibitor N-[(5benzyloxy-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine (**73**), connected a central oxy-methylene chain [64]. Compound **75** was identified from series **74** as the most promising ligand, showing a good selectivity and high potency in the inhibition of MAO-A with an $IC_{50} = 5.2$ nM ($IC_{50 \text{ MAO-B}} = 43.0$ nM), but less potent and with poor selectivity for the AChE ($IC_{50} = 0.35$ μM) and BuChE ($IC_{50} = 0.46$ μM). Further molecular modeling and enzymatic kinetics studies clarified that these inhibitors act in two binding sites of AChE, which could explain the inhibitory effect over Aβ-self-induced (47.8 ± 2.1 %) and AChE-dependent aggregation (32.4 ± 7 %) exerted compound **75** [64].

Based on the remarkable capacity of tacrine to interact in the active site of AChE, Martins and collaborators proposed a new series of tacrine-derived analogues by the insertion of a furo[2,3-b]quinolin-4-amine (**76**) and pyrrolo[2,3-b]quinolin-4-amine (**77**) subunits (Fig. **19**). Pharmacological results revealed that the furanotacrines and pyrrolotacrines showed selective inhibition of BuChE, in a micromolar range, but these compounds have lower potency than tacrine. The lead-compound **79**, exhibited the best inhibitory profile with $IC_{50}= 0.61$ nM and $IC_{50 \text{ BuChE}}= 0.074$ nM, for AChE from *Electrophorus electricus* and BuChE from equine serum, with significant neuroprotective effects against Aβ-induced toxicity in concentrations below 300 nM. Molecular modelling studies have corroborate that the hybrid derivatives containing pyrrole or furan ring subunits interacts with AChE and BuChE and the presence of a phenyl ring at the position 1 of the pyrrole ring is beneficial for both AChE and BuChE inhibition [65].

Taking the structure of donepezil (**70**) as a model, Huang and co-workers reported in 2012 a new family of derivatives

based on the structure of benzylidene-indanone (**80**) pharmacophoric subunit of **70** with the insertion of set of substituted benzene ring systems (**81**) (Fig. **20**). *In vitro* evaluation showed that most of the compounds were potent inhibitors of MAO-B (IC_{50} of 7.5−40.5 μM), besides antioxidant (ORAC-FL value of 2.75−9.37 μM) and metal chelator properties. In addition, they showed a great ability for inhibition self-induced β-amyloid aggregation (10.5−80.1%, 20 μM). Compound **82**, was identified as the most potent inhibitor agent of $A\beta_{1-42}$ aggregation (80.1%) and MAO-B ($IC_{50} = 7.5$ μM). Moreover, compound **82** proved to be an excellent antioxidant and metal chelator agent, being able to inhibit Cu^{2+}-induced $A\beta_{1-42}$ aggregation and disassembling the Aβ fibrils [66]. Thus compound **82** is one of the most current example from literature of a genuine MDTL drug prototype candidate for AD treatment.

Resveratrol (**83**), a potent phenolic natural plant metabolite, with remarkable antioxidant and anti-inflammatory properties, have been recently described as an inhibitor of
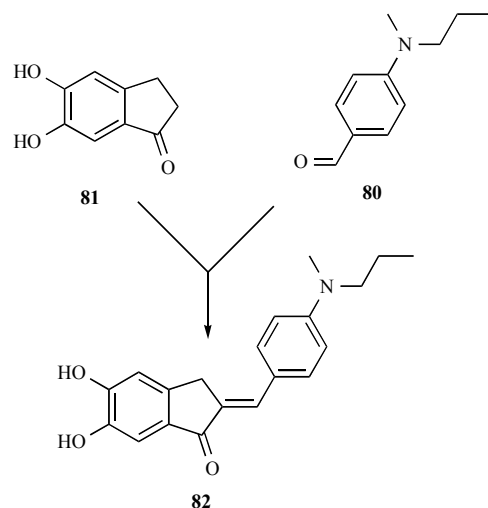

Fig. (20). Chemical structure of compound **72**, a substituted-benzylidene indanone derivative with a wide range of multi-activity profile.

Aβ aggregation. Thus, its molecular scaffold has been also explored for assembly of innovative MTDLs with antioxidant, anti-inflammatory and inhibitory of Aβ aggregation properties. Compounds **86** and **87** were the lead-compounds identified among a series of resveratrol-derived molecular hybrids (**85**), planned by the combination of the catechol pharmacophore of **83** with the well-known metal chelator clioquinol (**84**) (Fig. **21**). These two hybrid-compounds showed high potency in the inhibition of self-induced Aβ aggregation (**86**, IC_{50} = 7.56 μM and **87**, IC_{50} = 6.51 μM) in comparison to resveratrol (IC_{50} = 15.11 μM). Furthermore, antioxidant assays performed by the ORAC method, showed the ability of compounds **86** and **87** in decreasing the generation of ROS species in 4.72 and 4.70 trolox equivalent, respectively. These compounds were also capable to disassembling the highly structured Aβ fibrils generated by self- and Cu (II)-induced Aβ aggregation and exhibited significant inhibitory effect on MAO-A and MAO-B with a moderate AChE inhibition and low neurotoxicity [67].
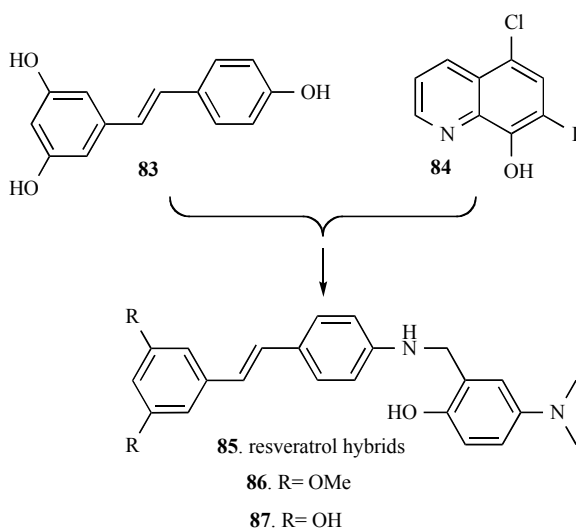

Fig. (21). Chemical structures of the very active MTDL resveratrol-derived compounds **73** and **74**.

5. CONCLUSION

The development of drugs for a more effective treatment, and hopefully curing, AD is still a challenge for Medicinal Chemistry. The more recent insights about the pathophysiology of AD have proven a complex inter-connected variety of deleterious events, with multiple pathways involving in its etiology and a variety of factors that are associated with the installation, progress and severity of AD. This complex multifactorial aspect of AD and other neuronal disorders have supported the new paradigm of MTDLs (or one-compound-multi-targets strategy) as a more rational strategy approach to the development of new drug candidates, than the one-target-one drug concept. The low efficacy of the therapeutic approach currently employed could be, in part attributed to this reductionist concept, and it becomes inevitable to adopt a new concept for the rational design of new drugs against AD. Therefore, a drug for the treatment of AD will only be effective if modulate multiple targets

simultaneously involved in the disease. In the last decade efforts have being employed in the design and search for new biological chemical entities capable to direct simultaneously with different molecular targets involved in the pathogenesis of AD and many new significantly active and promise molecules have been discovered. Consequently, new drug prototype candidates have being identified and they are the starting point of a revolutionary new way of thinking drug design and some drug candidates are under pre-clinical evaluation. Despite none of these MTDL drug candidates have reached clinical phase of development, we hope that in the next few years the system biology approach could effectively contribute to the Medicinal Chemists in the discovery of innovative chemical entities able to understand and effectively modify the course of this devastating neurodegenerative disorder.

CONFLICT OF INTEREST

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

ABBREVIATIONS

AChE	=	acetylcholinesterase
Aβ	=	amyloid-beta
BACE 1	=	beta secretase 1
BuChE	=	butirylcholinesterase
CNS	=	central nervous system
DA	=	doença de Alzheimer
IAChEs	=	acetilcolinesterase inhibitor
IC_{50}	=	minimum inhibitory concentration of 50%
MAO A	=	monoamine oxidase A
MAO B	=	monoamine oxidase B
MTDLs	=	multitarget-directed ligand
NMDARs	=	N-methyl-D-aspartate
PAS	=	peripheral anionic site
ROS	=	reactive oxygen species
UN	=	United Nations

REFERENCES

[1]   Bolognesi, M. L.; Matera, R.; Minarini, A.; Rosini, M.; Melchiorre, C. Alzheimer's disease: new approaches to drug discovery. *Curr. Opin. Chem. Biol.* **2009**, *13*, 303-308. doi.org/10.1016/j.cbpa.2009.04.619

[2]   Youdim, M. B. H.; Buccafusco, J. J. Multi- functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.* **2005**, *26*, 27-35. doi:10.1016/j.tips.2004.11.007

[3]   Alzheimer's Association. Alzheimer's Disease Facts and Figures. *Alzheimer's Dementia* **2011**, *7*, 2-68. doi:10.1016/j.jalz.2011.02. 004

[4]   Barreiro, E. J.; Fraga, C. A. M. New Insights for Multifactorial Disease Therapy: The Challenge of the Symbiotic Drugs. *Curr. Drug Ther.* **2008**, *3*, 1-13. **DOI**: 10.2174/157488508783331225

[5]   Zhang, H-Y. One-compound-multiple-targets strategy to combat Alzheimer's disease. *FEBS Lett.* **2005**, *579*, 5260-5264. doi.org/ 10.1016/j.febslet.2005.09.006

[6]   Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Inhibition of Acetylcholinesterase, β-Amyloid Aggregation, and NMDA Receptors in Alzheimer's Disease: A Promising Direction for the Multi-target-Directed Ligands Gold Rush. *J. Med. Chem.* **2008**, *51*, 4381-4384. DOI: 10.1021/ jm800577j

[7]   Moller, H. J.; Graeber, M. B. The case described by Alois Alzheimer in 1911. *Eur. Arch. Psychiatry Clin. Neurosci.* **1998**, *248*, 111-122. 111doi.org/10.1007/s004060050027

[8]   Site of the Brazilian Institute of Geography and Statistics. Available in: http://biblioteca.ibge.gov.br/visualizacao/periodicos/93/cd_2010_ caracteristicas_populacao_domicilios.pdf. [Accessed on: August, 2013]. Available in:<http://www.ibge.gov.br/home/presidencia/noticias/ noticia_visualiza.php?id_noticia=1722&id_pagina=1>. Accessed: 08/ 2012

[9]   Site of Alzheimer Med. Available in: http://www.alzheimermed. com.br/conceitos/aspectos-socioeconomicos. [Accessed on: August, 2013]

[10]   Alzheimer's Association. Alzheimer's Disease Facts and Figures. *Alzheimer's Dementia,* **2011**, *7*, 2.

[11]   Brookmeyer, R.; Johnson, E.; Ziegler-Graham, K.; Arrighi, H. M. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.* **2007**, *3*, 186-191. doi:10.1016/j.jalz.2007.04.381

[12]   Alzheimers Association. Available in: http://www.alz.org/ downloads/Facts_Figures_2012.pdf. [Accessed on: August, 2012].

[13]   Piau, A.; Nourhashémi, F.; Hein, C.; Caillaud, C.; Vellas, B. Progress in the development of new drugs in Alzheimer's disease. *J. Nutr. Health Aging* **2011**, *15*, 45-57. doi.org/10.1007/s12603-011-0012-x

[14]   Site of World Health Organization. Population ageing: a public health challenge. Available in: https://apps.who.int/inf-fs/en/fact135. html. [Accessed on: August, **2013**].

[15]   Kalache, A.; Keller, I. Ageing in developing countries. In: Tallis, R. Increasing longevity: medical, social and political implications. Royal College Of Physicians of London: London, **1998**, pp. 69-80.

[16]   Site of the Brazilian Institute of Geography and Statistics. Available in: http://www.ibge.gov.br/home/estatistica/populacao/ censo2000/populacao/censo2000_populacao.pdf. [Accessed on: August, 2013].

[17]   Chen, S. Y.; Chen, Y.; Li, Y. P.; Chen, S. H.; Tan, J. H.; Ou, T. M.; Gu, L. Q.; Huang, Z. S. Design, synthesis, and biological evaluation of curcumin analogues as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg. Med. Chem.* **2011**, *19*, 5596-5604. doi.org/10.1016/j.bmc.2011.07.033

[18]   Ray, B.; Lahiri, D. K. Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including curcumin**.** *Curr. Opin. Pharmacol.* **2009**, *9*, 434-444.

[19]   Viegas, F. P. D.; Simões, M. C. R.; Rocha, M. D.; Castelli, M. R.; Moreira, M. S.; Viegas Junior, C. Doença de Alzheimer: Caracterização, Evolução e Implicações do Processo Neuro-inflamatório. *Rev. Virtual Quim.* **2011**, *3*, 286-306. ISSN 1984-6835.

[20]   Schmitt, B.; Bernhardt, T.; Moeller, H-J.; Heuser, I.; Frölich, L. Combination Therapy in Alzheimer's disease. *CNS Drugs* **2004**, *18*, 827-844. doi.org/10.2165/00023210-200418130-00001

[21]   Starkov, A. A.; Beal, F. M. Portal to Alzheimer's disease. *Nat. Med.* **2008**, *14*, 1020-1021.

[22]   Reddy, P. H.; Beal, M. F. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends Mol. Med.* **2008**, *14*, 45-53. doi. org/10.1016/j.molmed.2007.12.002

[23]   Wang, J. Z.; Grundke-Iqbal, I.; Iqbal, K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration.

[24]   Blennow, K.; de Leon, M. J.; Zetterberg, H. Plasma Aβ in Alzheimer's disease—up or down? *Lancet* **2006**, *368*, 387-388.

[25]   da Rocha, M. D.; Viegas, F. P. D.; Campos, H. C.; Nicastro, P. C.; Fossaluzza, P. C.; Fraga, C. A. M.; Barreiro, E. J.; Viegas Jr., C. The Role of Natural Products in the Discovery of New Drug Candidates for the Treatment Neurodegenerative Disorders II: Alzheimers Disease. *CNS Neurol Disorders - Drug Targets* **2011**, *10*, 251-270. DOI: 10.2174/187152711794480429

[26]   Liu, Q.; Xie, F.; Rolston, R.; Moreira, P. I.; Nunomura, A.; Zhu, X.; Smith, M. A.; Perry, G. Prevention and treatment of Alzheimer disease and aging: antioxidants**.** *Mini Rev. Med. Chem.* **2007**, *7*, 171-180. **DOI**: 10.2174/138955707779802552

[27]   Azzi, A. Oxidative stress: A dead end or a laboratory hypothesis? *Biochem. Biophys. Res. Comm.* **2007**, *362*, 230-232. DOI: 10.1016/ j.bbrc.2007.07.124

[28]   Legg, K. Neurodegenerative diseases: An alternative path to reduce neuroinflammation. *Nat. Rev. Drug Disc.* **2011**, *10*, 901-910. doi:10. 1038/nrd3607

[29]   Gaggelli, E.; Kozlowski, H.; Valensin, G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis) *Chem. Rev.* **2006**, *106*, 1995-2044.

[30]   Akiyama, H.; Barger, S.; Barnum, S.; Bradt, B.; Bauer, J.; Cole, G. M.; Cooper, N. R.; Eikelenboom, P.; Emmerling, M.; Fiebich, B. L.; Finch, C. E.; Frautschy, S.; Griffin, W. S. T.; Hampel, H.; Hull, M.; Landreth, G.; Lue, L-F.; Mrak, R.; Mackenzie, I. R.; McGeer, P. L.; O'Banion, M. K.; Pachter, J.; Pasinetti, G.; Plata-Salaman, C.; Rogers, J.; Rydel, R.; Shen, Y.; Streit, W.; Strohmeyer, R.; Tooyoma, I.; Van Muiswinkel, F. L.; Veerhuis, R.; Walker, D.; Webster, S.; Wegrzyniak, B.; Wenk, G.; Wyss-Coray, T. Inflammation and Alzheimer's disease. *Neurobiol. Aging* **2000**, *21*, 383-421. doi.org/10.1016/S0197-4580(00)00124-X

[31]   Heneka, M. T.; O'Banion, M. K. Inflammatory processes in Alzheimer's disease. *J. Neuroimmunol.* **2007**, *184*, 69-91. doi:10. 1016/j.jneuroim.2006.11.017

[32]   Giunta, B.; Fernandez, F.; Nikolic, W. V.; Obregon, D.; Rrapo, E.; Town, T.; Tan, J. J. Inflammaging as a prodrome to Alzheimer's disease. *Neuroinflammation* **2008**, *5*, 51-66. doi:10.1186/1742-2094-5-51

[33]   Kamal, M. A.; Greig, N. H.; Reale, M. Anti-Inflammatory Properties of Acetylcholinesterase Inhibitors Administred in Alzheimer's Disease *Anti-inflamm. Anti-Allergy Med. Chem.* **2009**, *8*, 85-100. **DOI**: 10.2174/187152309787580810

[34]   Mangialasche, F.; Solomon, A.; Winblad, B.; Mecocci, P.; Kivipelto, M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* **2010**, *9*, 702-716. doi.org/10.1016/S1474-4422(10) 70119-8

[35]   Youdim, M.B.H.; Buccafusco, J. J. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.* **2005**, 26, 27-35. doi.org/10.1016/ j.tips.2004.11.007

[36]   Bolognesi, M.L.; Matera, R.; Minarini, A.; Rosini, M.; Melchiorre, C. Alzheimer's disease: new approaches to drug discovery. *Curr. Op. Chem. Biol.* **2009**, *13*, 303-308. doi.org/10.1016/j.cbpa.2009. 04.619

[37]   Piau, A.; Nourhashemmi, F.; Hein, C.; Caillaud, C.; Vellas, B. Progress in the development of new drugs in Alzheimer's disease, *J. Nut. Health Aging* **2010**, *15*, 45-57. doi.org/10.1007/s12603-011-0012-x

[38]   Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-Directed Ligands To Combat Neurodegenerative Diseases. *J. Med. Chem.* **2008**, *51*, 347. doi.org/10.1021/jm7009364

[39]   Samadi, A.; Valderas, C.; de los Ríos, C.; Bastida, A.; Chioua, M.; González-Lafuente, L.; Colmena, I.; Gandía, L.; Romero, A.; del Barrio, L.; Martín-de-Saavedra, M. D.; López, M.G.; Villarroya, M.; Contelles, J. M. Cholinergic and neuroprotective drugs for the treatment of Alzheimer and neuronal vascular diseases. II. Synthesis, biological assessment, and molecular modelling of new tacrine analogues from highly substituted 2-aminopyridine-3-

carbonitriles. *Bioorg. Med. Chem.* **2011**, *19*, 122-133. doi.org/10.1016/
j.bmc.2010.11.040

[40]   Bolognesi, M. L.; Cavalli, A.; Luca Valgimigli, L.; Bartolini, M.;
Rosini, M.; Andrisano, V.; Recantini, M.; Melchiorre, C. Multi-
Target-Directed Drug Design Strategy: From a Dual Binding Site
Acetylcholinesterase Inhibitor to a Trifunctional Compound against
Alzheimer's Disease. *J. Med. Chem.* **2007**, *50*, 6446-6449.

[41]   Chen, S-Y.; Chen, Y.; Li, Y-P.; Chen, S-H.; Tan, J-H.; Ou, T-M.;
Gu, L-Q.; Huang, Z-S. Design, synthesis, and biological evaluation
of curcumin analogues as multifunctional agents for the treatment
of Alzheimer's disease. *Bioorg. Med. Chem.* **2011***, 19*, 5596-5604.
doi.org/10.1016/j.bmc.2011.07.033

[42]   Cavalli A, Bolognesi ML, Capsoni S, Andrisano V, Bartolini M,
Margotti E, Cattaneo A, Recanatini M, Melchiorre C. A small
molecule targeting the multifactorial nature of Alzheimer's disease.
Angew Chem. Int. **2007**, *46*, 3689-3692. DOI: 10.1002/anie.200700256

[43]   Shan, W-J.; Huang, L.; Zhou, Q.; Meng, F-C.; Li, X-S. Synthesis,
biological evaluation of 9-N-substituted berberine derivatives
as multi-functional agents of antioxidant, inhibitors of
acetylcholinesterase, butyrylcholinesterase and amyloid-β aggregation.
Eur. J. Med. Chem. **2011**, *46*, 5885-5893. doi:10.1016/j.ejmech.
2011.09.051.

[44]   Jiang, H.; Wang, X.; Huang, L.; Luo, Z.; Su, T.; Ding, K.; Li, X.
Benzenediol-berberine hybrids: Multifunctional agents for
Alzheimer's disease. *Bioorg. Med. Chem.* **2011**, *19*, 7228-7235.
doi:10.1016/j.bmc.2011.09.040

[45]   Fernandez-Bachiller, M. I.; Pérez, C.; González-Muñoz, G. C.;
Conde, S.; López, M. G.; Villarroya, M.; Garcia, A. G.; Rodriguez-
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. DOI: 10.1021/
jm100329q

[46]   Bolognesi, M. L.; Bartolini, M.; Tarozzi, A.; Morroni, F.; Lizzi, F.;
Milelli, A.; Minarini, A.; Rosini, M.; Hrelia, P.; Andrisano, V.;
Melchiorre, C. Multitargeted drugs discovery: Balancing anti-
amyloid and anticholinesterase capacity in a single chemical entity.
Bioorg. Med. Chem. Lett. **2011**, *21*, 2655-2658. doi.org/10.1016/
j.bmcl.2010.12.093

[47]   Bolognesi, M. L.; Bartolini, M.; Rosini, M.; Andrisano, V.
Melchiorre, C. Structure-activity relationships of memoquin:
Influence of the chain chirality in the multi-target mechanism of
action. *Bioorg. Med. Chem. Lett.* **2009***, 19,* 4312-4315. doi.org/
10.1016/j.bmcl.2009.05.087

[48]   Bolognesi, M. L.; Cavalli, A.; Melchiorre, C. Memoquin: a multi-
target-directed ligand as an innovative therapeutic opportunity for
Alzheimer's disease. *Neurotherapeutics* **2009**, *6*, 152-162.

[49]   Bolognesi, M. L.; Cavalli, A.; Bergamini, C.; Fato, R.; Lenaz, G.;
Rosini, M.; Bartolini, M.; Andrisano, V.; Melchiorre, C. Toward a
rational design of multitarget-directed antioxidants: merging
memoquin and lipoic acid molecular frameworks. *J. Med. Chem.*
2009, *52*, 7883-7886. **DOI:** 10.1021/jm901123n

[50]   Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.;
Melchiorre, C. Propidium-based polyamine ligands as potent
inhibitors of acetylcholinesterase and acetylcholinesterase-induced
amyloid-beta aggregation. *J. Med. Chem.* **2005***, 48,* 24-27.
doi.org/10.1021/jm049156q

[51]   Contelles, J. M.; Leo, R.; Lopez, M. G.; Garcı́a, A. G.; Villarroya,
M. Synthesis and biological evaluation of new 4H-pyrano[2,3-
b]quinolone derivatives that block acetylcholinesterase and cell
calcium signals, and cause neuroprotection against calcium overload
and free radicals. *Eur. J. Med. Chem.* **2006**, *41*, 1464-1469.

[52]   Minarini, A.; Milelli, A.; Tumiatti, V.; Rosini, M.; Simoni, E.;
Bolognesi a, M. L.; Andrisano, V.; Bartolini, M.; Motori, E.;
Angeloni, C.; Hrelia, S. Cystamine-tacrine dimer: A new multi-
target-directed ligand as potential therapeutic agent for Alzheimer's
disease treatment. *Neuropharmacology* **2012**, *62*, 997-1003. doi.org/
10.1016/j.neuropharm.2011.10.007

[53]   Fang, L.; Kraus, B.; Lehmann, J.; Heilmann, J.; Zhang, Y.; Decker,
M. Design and synthesis of tacrine-ferulic acid hybrids as multi-
potent anti-Alzheimer drug candidates. *Bioorg. Med. Chem. Lett.*
2008, *18*, 2905-2909. doi.org/10.1016/j.bmcl.2008.03.073

[54]   Rosini, M.; Simoni,E.; Bartolini, M.; Cavalli, A.; Ceccarini, L.;
Pascu, N.; McClymont, D. W.; Tarozzi, A.; Bolognesi,M.L.; Minarini,
A.; Tumiatti, V.; Andrisano, V.; Mellor, I. R.; Melchiorre, C.
Inhibition of Acetylcholinesterase, β-Amyloid Aggregation, and
NMDA Receptors in Alzheimer's Disease: A Promising Direction
for the Multi-target-Directed Ligands Gold Rush. *J. Med. Chem.*
2008, *51*, 4381-4384. doi.org/10.1021/jm800577j

[55]   Contelles, J. M.; Leo´n, L.; de los Rı̀os, C.; Samadi, A.;
Bartolini, M.; Andrisano, V.; Huertas, O.; Barril, X.; Luque, F. J.;
Rodrıguez-Franco, M. I.; Lopez, B. Lopez, M. B.; Garcıa, A.G.;
Carreiras, M.C.; Villarroya, M. Tacripyrines, the First Tacrine-
Dihydropyridine Hybrids, as Multitarget-Directed Ligands for the
Treatment of Alzheimer's Disease. *J. Med. Chem.* **2009**, *52*, 2724-
2732. doi.org/10.1021/jm801292b

[56]   Chao, X.; He, X.; Yang, Y.; Zhou, X.; Jin, M.; Liu, S.; Cheng, Z.;
Liu, P.; Wang, Y.; Yu, J.; Tan, Y.; Huang, Y.; Qin, J.; Rapposelli,
S.; Pi, R. Design, synthesis and pharmacological evaluation of
novel tacrine-caffeic acid hybrids as multi-targeted compounds
against Alzheimer's disease. *Bioog. Med. Chem. Lett.*, **2012**, *22*,
6498-6502. doi.org/10.1016/j.bmcl.2012.08.036

[57]   Wang, Y.; Wang, F.; Yu, J. P.; Jiang, F. C.; Guan, X. L.; Wang, C.
M.; Li, L.; Cao, H.; Li, M. X.; Chen, J. G. Novel multipotent
phenylthiazole-tacrine hybrids for the inhibition of cholinesterase
activity, b-amyloid aggregation and Ca^{2+} overload. *Bioorg. Med.
Chem.* **2012**, *20*, 6513-6522. doi.org/10.1016/j.bmc.2012.08.040

[58]   Simoni, E.; Daniele, S.; Bottegoni, G.; Pizzirani, D.; Trincavelli,
M.L.; Goldoni, L.; Tarozzo, G. Reggiani, A.; Martini, C.; Piomelli,
D.; Melchiorre, C.; Rosini, M.; Cavalli, A. Combining Galantamine
and Memantine in Multitargeted, New Chemical Entities
Potentially Useful in Alzheimer's Disease. *J. Med. Chem.* **2012**, *55*,
9708-9721. doi.org/10.1021/jm3009458

[59]   Rook, Y.; Schmidtke, K. U.; Gaube, F.; Schepmann, D.; Wunsch,
B.; Heilmann, J.; Lehmann, J.; Winckler, T. Bivalent β-Carbolines
as Potential Multitarget Anti-Alzheimer Agents. *J. Med. Chem.*
2010, *53*, 3611-3617. doi.org/10.1021/jm1000024

[60]   Rizzo, S.; Rivie`re, C.; Piazzi, L.; Bisi, A.; Gobbi, S.; Bartolini, M.;
Andrisano, V.; Morroni, F.; Tarozzi, A.; Monti, J. P.; Rampa, A.
Benzofuran-Based Hybrid Compounds for the Inhibition of
Cholinesterase Activity, β Amyloid Aggregation, and Aβ
Neurotoxicity. *J. Med. Chem.* **2008**, *51*, 2883-2886. doi.org/10.1021/
jm8002747

[61]   Rizzo, S.; Tarozzi, A.; Bartolini, M.; Costa, G.; Bisi, A.; Gobbi,
S.; Belluti, F.; Ligresti, A.; Allarà, M.; Monti, J. P.; Andrisano, V.;
Di Marzo, V.; Hrelia, P.; Rampa, A. 2-Arylbenzofuran-based
molecules as multipotent Alzheimer's disease modifying agentes.
Eur. J. Med. Chem. **2012**, *58*, 519-532. doi.org/10.1016/j.ejmech.
2012.10.045

[62]   Piazzi, L.; Cavalli, A.; Colizzi, F.; Belluti, F.; Bartolini, M.;
Mancini, F.; Recanatini, M.; Andrisano, V.; Rampa, A. Multi-target-
directed coumarin derivatives: hAChE and BACE1 inhibitors as
potential anti-Alzheimer compounds. *Bioorg. Med. Chem. Lett.*
2008, *18*, 423-426. doi.org/10.1016/j.bmcl.2007.09.100

[63]   Samadi, A.; Chioua, M.; Bolea, I.; de los Ríos, C.; Iriepa, I.;
Moraleda, I.; Bastida, A.; Esteban, G.; Unzeta, M.; Gálvez, E.;
Contelles, J. M. Synthesis, biological assessment and molecular
modeling of new multipotente MAO and cholinesterase inhibitors
as potential drugs for the treatment of Alzheimer's disease. *Eur. J.
Med. Chem.* **2011**, *46,* 4665-4668. doi.org/10.1016/j.ejmech.2011.
05.048

[64]   Bolea, I.; Jiménez, J. J.; de los Ríos, C.; Chioua, M.; Pouplana, R.;
Luque, J.; Unzeta, M.; Contelles, J. M.; Samadi, A. Synthesis,
Biological Evaluation, and Molecular Modeling of Donepezil and
N-[(5-(Benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-
2-yn-1-amine Hybrids as New Multipotent Cholinesterase/
Monoamine Oxidase Inhibitors for the Treatment of Alzheimer's
Disease. *J. Med. Chem.* **2011**, *54*, 8251-8270. doi.org/10.1021/
jm200853t

[65]   Martins, C.; Carreiras, M. C.; León, R. de los Ríos, C.; Bartolini,
M.; Andrisano, V.; Iriepa, I.; Moraleda, I.; Gálvez, E.; García, M.;
Egea, J.; Samadi, A.; Chioua, M.; Contelles, J. M. Synthesis and
biological assessment of diversely substituted furo[2,3-b]quinolin-
4-amine and pyrrolo[2,3-b]quinolin-4-amine derivatives, as novel

tacrine analogues. *Eur. J. Med. Chem.* **2011**, *46,* 6119-6130. doi.org/10.1016/j.ejmech.2011.09.038

[66]   Minarini, A.; Milelli, A.; Tumiatti, V.; Rosini, M.; Simoni, E.; Bolognesi, M.E.; Andrisano, V.; Bartolini, M.; Motori, E.; Angeloni, C.; Hrelia, S. Multitarget-Directed Benzylideneindanone Derivatives: Anti-β-Amyloid (Aβ) Aggregation, Antioxidant, Metal Chelation, and Monoamine Oxidase B (MAO-B) Inhibition

Properties against Alzheimer's Disease. *Neuropharmacology* **2012**, *62*, 997-1003. doi.org/10.1016/j.neuropharm.2011.10.007

[67]   Lu, C.; Guo, Y.; Yan, J.; Luo, Z.; Luo, H. B.; Yan, M.; Huang, L.; Li, X. Design, Synthesis, and Evaluation of Multitarget-Directed Resveratrol Derivatives for the Treatment of Alzheimer's Disease. *J. Med. Chem.* **2013**, *56*, 5843-5859. doi.org/10.1021/jm400567s