Non-Cholinergic Pharmacotherapy Approaches to the Future Treatment of Alzheimer's Disease

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Abstract: Research on the molecular basis of Alzheimer's disease has elucidated pathogenic pathways from which a range of rational pharmacological interventions has emerged. The most promising strategies involve approaches to retarding, halting or preventing the formation or accumulation of beta amyloid plaques and neurofibrillary tangles. Other therapeutic approaches include those acting via excitatory amino acid receptors, limiting the oxidative stress and inflammatory response associated with dementia, molecules with nerve growth factor like activity. In the present article these and the other recent advances in the neurobiology and pharmacotherapy of AD will be reviewed.

1. INTRODUCTION

Alzheimer's disease (AD) is a multifaceted progressive disorder characterised by a slow, progressive decline in cognitive function and behaviour. No single factor has arisen as a distinguishing and sole cause of AD; it is rather likely that AD may be a many-faceted syndrome that arises as a consequence of neuronal impairment caused by many distinct biological aberrations [1]. The biological mechanism underlying the formation of AD is complex, as several factors contribute to the neuropathology of the disease. Research on the molecular basis of AD has elucidated pathogenic pathways from which a range of rational pharmacological interventions has emerged [2-4]. The past two decades have witnessed a considerable research effort directed towards discovering the cause of AD with the ultimate aim of developing safe and effective pharmacological treatments [5-9]. Besides the acetylcholinesterase inhibitors currently in use [10], the most promising strategies involves approaches to retarding, halting or preventing the formation or accumulation of beta amyloid (Aβ) plaques and neurofibrillary tangles, the two hystopathological hallmarks of AD. Other therapeutic approaches directed towards neurotransmitter substitution include those acting via excitatory amino acid receptors, such as ampakines or NMDA antagonists. Many other compounds which target other aspects of the disease, such as reducing neuronal damage, limiting the oxidative stress and inflammatory response associated with dementia, molecules with nerve growth factor like activity, estrogens therapy [11] or immune response [12, 13] are currently being proposed or utilized in disease prevention trials [14].

In this article, these and the other recent advances in the neurobiology and pharmacotherapy of AD will be reviewed.

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2. THERAPEUTIC APPROACHES TO THE TREATMENT OF AD RELATED TO THE Aβ-PEPTIDE

A great effort has been devoted to understand the genesis and pathology of AD and, although many factors and the relationships among them have been discovered, many other essential points remain unknown. The so called β-amyloid hypothesis points out the Aβ plaques and their neurotoxicity as the origin of the cascade of biochemical changes that leads the patients to the cognitive and behavioural impairments associated with AD. Modulating the chain of events from its production by cleavage of the amyloid precursor protein (APP) to the deposition as senile plaques or even the clearance of already formed plaques offers several reasonable approaches to develop a future treatment of AD [15-16].

2.1 Production of β-Amyloid

Aβ, the main component of the senile plaques, is derived by the proteolytic cleavage of APP [17], a large transmembrane protein with neurotrophic functions [18], although not clearly known. The main pathway of APP processing, about 90% [19], is the proteolysis catalysed by α-secretase, the non-amyloidogenic route. The second processing pathway is the N- and C-terminal cleavage of APP by β- and γ-secretase respectively. The resulting molecules of these two proteolytic steps are the central fragments of APP $A\beta_{40}$ and $A\beta_{42}$. $A\beta_{40}$ is the more abundant species of the whole Aβ formed, about 90%. However $A\beta_{42}$, though a minor component, aggregates easily and quickly into amyloid fibrils while $A\beta_{40}$ does it in a far more slow way.

Two general approaches arise with the ultimate goal of limiting the presence of Aβ. The first approach may be undertaken by enhancing the activity of α-secretase, inhibiting β- and γ-secretases or avoiding the aggregation of the amyloid into fibrils. Nowadays, immunisation with $A\beta_{42}$ or some suitable fragment is the most promising

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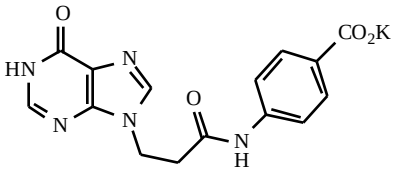
strategy of the second approach, but it will be discussed somewhere else in this issue.

2.2 α-Secretase Activity Enhancers

α-Secretase is a membrane-bound enzyme that hydrolyses APP roughly in the middle of the Aβ sequence between the β- and γ-secretases cleaving sites. As a result, there is not any possibility of either $A\beta_{40}$ or $A\beta_{42}$ to be formed throughout this route and an upregulation of its activity could be a potentially useful approach. Unfortunately, very little is known about α-secretase and there are reasonable concerns that an increase of its activity might cause undesirable complications.



1 AIT-082

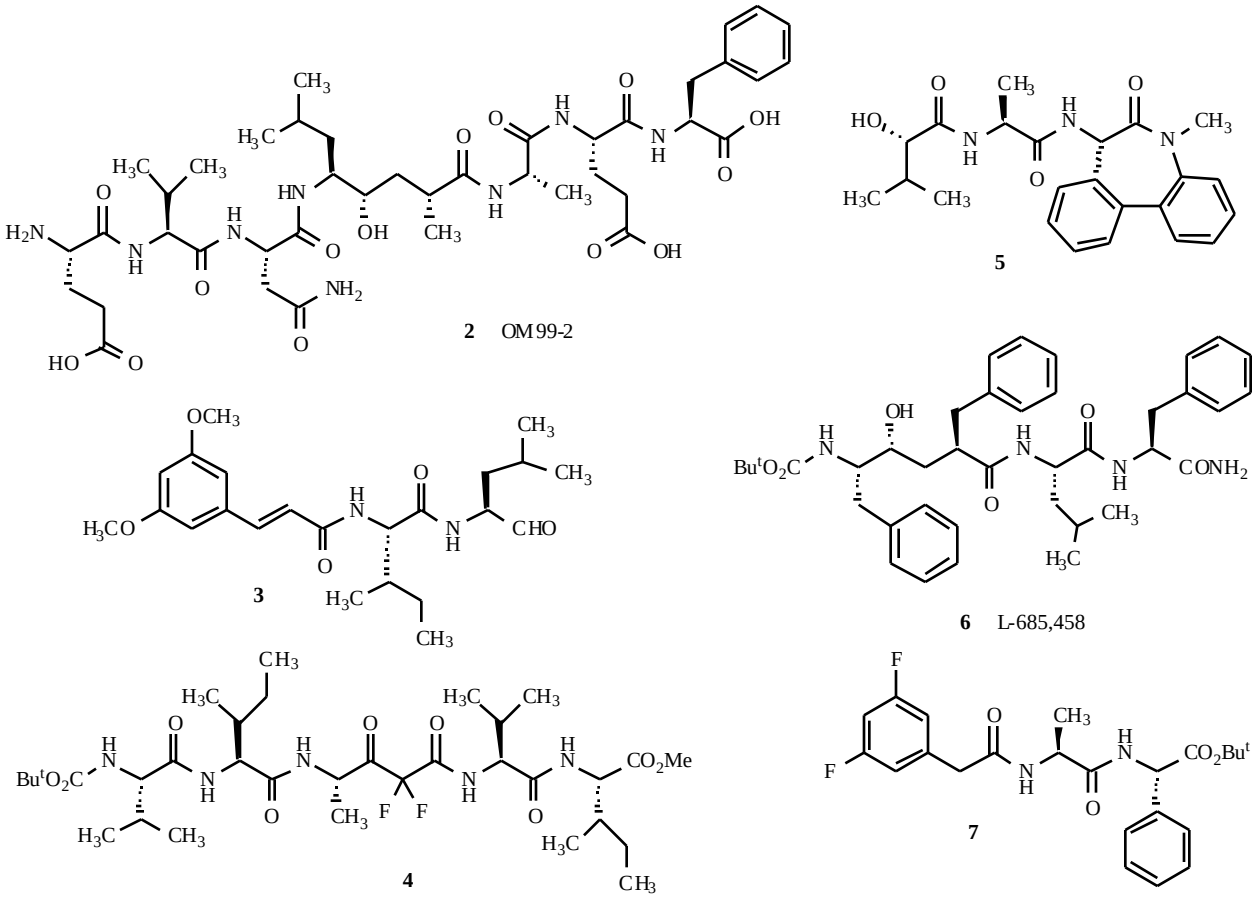
Fig. (1). Enhancer of α-secretase activity.

AIT-082 (Neotrofin™, Fig. **1**) is a neurotrophic and memory-enhancing new drug [20], currently subjected to Phase II clinical trials. It has also been described that AIT-082 (**1**) may have some stimulating influence on the α-secretase activity as high levels of APPsα were found in cells treated with the drug [21]. However, it is still not clear if that extra-released APPsα is due to an increased activity of the enzyme or to the neurotrophic properties of AIT-082.

2.3 β- and γ-Secretase Inhibitors

The production of Aβ from APP requires two cleavage steps. β-Secretase (BACE: beta-site cleaving enzyme) cleaves the extracellular moiety of APP in a quite specific site. β-secretase is a membrane-bonded aspartyl protease [22], recently identified by four independent groups, three of them [23-25] following genetic techniques and the fourth one by the classical method of biochemical purification from human brain tissue [26]. It has also been presented [27] the crystal structure of β-secretase complexed with the inhibitor OM99-2 (Fig. **2**). The discovering of a β-secretase homologue (BACE-2) that likely cleaves within the Aβ sequence [28] indicates that its generation is a complicated process. At present, there are no data about clinical trials of any β-secretase inhibitors although some peptidomimetic compounds have been described [29] as potent inhibitors, such as the above mentioned OM99-2 (**2**) in which a Leu-Ala bond is substituted by a hydroxyethylene transition-state isostere. The approach of limiting the Aβ secretion by inhibition of β-secretase is a highly important target for the research labs but, in any case, the effects of a long-term suppression of secretase are still unknown.

γ-Secretase cleaves the C-terminal portion of APP in the transmembrane region of the molecule. This unusual region



2 OM99-2 **5** **3** **6** L-685,458 **4** **7**

Fig. (2). β- and γ-Secretase inhibitors.

to carry out a hydrolytic reaction probably imposes a complex mechanism with other molecules involved. γ-Secretase has proven to be even more elusive than its relatives α- and β-secretases as it is not identified yet. Presenilins (PSs), particularly PS-1, have been associated with the γ-secretase activity [30, 31] and there are strong evidence that PSs contain the catalytic component of γ-secretase [32].

As in the case of β-secretase, there are no data of γ-secretase inhibitors subjected to clinical trials and, furthermore, the potential side effects of γ-secretase inhibition are still unknown. However, potent inhibitors have been identified (Fig. **2**). The peptidyl aldehyde bearing a cinnamyl residue **3** (IC_{50} = 9.6 μM) was obtained using combinatorial techniques [33]. The substrate-based difluoroketone **4** derives from the APP sequence at the γ-cleavage site and inhibits $Aβ_{40}$ secretion at 25 μM [34]. Benzodiazepine derivatives such as **5** have recently been patented as inhibitors of γ-secretase [35]. Using directed screening in cell culture and *in vitro* assays of APP processing, the identification of L-685,458 as a specific inhibitor of γ-secretase, with a similar potency toward $Aβ_{40}$ and $Aβ_{42}$ (IC_{50} = 17 nM) has been reported [36]. L-685,458 (**6**) contains a hydroxyethylene dipeptide isostere, which suggests that it mimics the transition state at the catalytic site. Dovey *et al*. have recently reported [37] an effective reduction of Aβ levels by inhibiting the γ-secretase activity without affecting protein secretion. Brain levels of Aβ were reduced in a dose-dependent manner within 3 hours when *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*butyl-ester **7** (Fig. 2) was orally administered to mice transgenic for human APP. As far as our knowledge, this work reports the first demonstration of reduction of brain Aβ *in vivo*.

2.4 Inhibitors of Aβ Aggregation

The inhibition of the oligomerisation of Aβ as a way to avoid its subsequent arrangement into fibrils and plaques appears as an attractive target for small-molecules therapy. The mechanism of toxicity of oligomers and fibrils still remain unidentified, but a small handful of molecules have been described to inhibit Aβ aggregation. Researchers of the University of Lancaster synthesised a group of benzofuran derivatives with inhibitory properties in order to study the mechanism of Aβ aggregation [38]. Compound **8** was effective at μM concentrations (IC_{50} = 56 μM against 11μM synthetic $Aβ_{40}$). Rhodanine derivatives **9** and **10** were reported [39, 40] to have an IC_{50} of 1.5 and 0.3 μM respectively in assays for inhibition of self-seeded amyloid fibril growth (Fig. **3**).

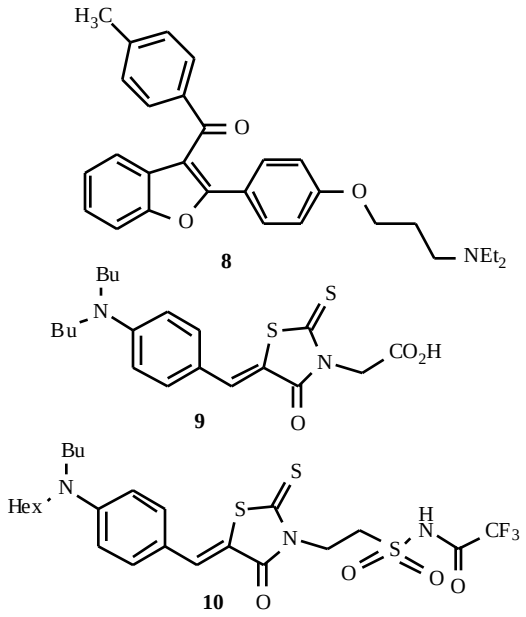


Fig. (3). Inhibitors of Aβ aggregation.

The involvement of acetylcholinesterase (AChE), a cholinergic enzyme, in the Aβ aggregation [41] is recently shown. Peripheral and dual binding site AChE inhibitors may represent a new therapeutical option enhancing the cognitive deficiency and avoiding the Aβ aggregation [42].

It is quite doubtful that the inhibitors of β- or γ-secretases could reach any clinical application in a reasonable term because altering the processing of APP may effectively prevent deposition of Aβ but might raise the uncontrolled presence of APP fragments with potentially harmful effects. Nowadays, they are reduced to the useful role of tools to understand the mechanism of APP processing and its part within the whole Alzheimer's pathology.

3. TAU PROTEIN AS TARGET FOR DEVELOPING NEW ANTI-ALZHEIMER DRUGS

Neurofibrillary tangles (NFTs) of paired helical filaments (PHFs) are one of the neuropathological hallmarks of AD and abnormally hyperphosphorylated microtubule-associated protein tau is the major protein subunit of PHF [43, 44]. Unlike normal tau which promotes microtubule assembly and stabilizes the structure of microtubules [45] the abnormally hyperphosphorylated tau from AD brain sequesters normal microtubule-associated proteins [46] and causes inhibition and disruption of microtubules *in vitro* [47].

Increased understanding of the physiology and pathophysiology of tau has opened up the possibility of targeting this molecule for therapeutic purposes in AD and related disorders [48]. Two principal lines of investigation have been pursued: preventing the hyperphosphorylation of tau molecules [49] and preventing the aggregation of tau molecules into the PHFs [50].

3.1 Preventing Tau Hyperphosphorylation

All six tau isoforms are abnormally hyperphosphorylated at least at 21 sites in AD and about half of tau sites are serine/threonine followed by proline [51]. There is some evidence to suggest that hyperphosphorylation, which is most probably the result of an imbalance of tau kinase and phosphatase activities in the affected neurons [52], is an early event in the development of neurofibrillary pathology. Thus, the blockade of this step may be a prime target at which to interrupt the pathogenic cascade [53].

Although many protein kinases are known to phosphorylate tau *in vitro*, the *in vivo* players contributing to the hyperphosphorylation of tau remains elusive. *In vitro* and cell culture studies have shown that GSK-3β [54] and cdk5/p25 [55] are likely to be the major protein kinases that phosphorylate tau [56].

The search for tau protein kinase inhibitors is an active field, although until the moment few compounds are known with this inhibitory enzymatic property. Lithium behaves as a specific inhibitor of GSK-3β *in vitro* and in intact cells [57] and also significantly protects cultured neurons from Aβ-induced cell death [58], but its high IC_{50} value prevents it from therapeutical use. Initially, some substituted propanones (**11**) (Fig. **4**) were reported to inhibit the formation of abnormally phosphorylated paired helical filament epitopes [59], and the development of feasible *in vitro* model for screening compounds [60] leads to the identification of some purine derivatives (**12**) as the first GSK-3β inhibitors [61].

The widely used protein kinase C inhibitors bisindolylmaleimides I (**13**) and IX (**14**) have recently been reported as potent ATP-competitive GSK-3β inhibitors [62]. More simple chemical structures (**15**) with a common maleimide core has recently been identified by high troughput screening as GSK-3β inhibitors [63]. SAR studies showed that the maleimide NH is essential for binding to the enzyme, while additional pharmacological studies revealed the involvement of these new inhibitors in neuroprotection [64] and glycogen metabolism [65]. Small thiadiazolidinones (TDZD) derivatives (**16**) are the first ATP-non competitive GSK-3β inhibitors reported to date. Their structural key features have been established and an hypothetical GSK-3β binding mode has been proposed [66]. TDZD do not show inhibition on others several kinases as PKA, PKC, CK-2 and cdc-2, and studies in whole cell have demonstrated the tau phosphorylation decrease in the presence of these new inhibitors [67].

The inhibition of tau phosphorylating protein kinase cdk5 also prevents the Aβ-induced neuronal death [68] which represents an opportunity to exploit the potential of the cyclin-dependent kinases inhibitors in neurology. The marine sponge constituent hymenialdisine (**17**) [69], the bis-indole indirubin (**18**) [70] or the new benzazepinone class, called paullones (**19**) [71] have shown potent inhibition on cdk5/p25. All these inhibitors act by competing with ATP for binding at the catalytic site, presenting a flat heterocycle ring system in their chemical structure that occupies the purine binding pocket on the enzyme [72]. Recently, it is showed that many, but not all, reported CDK inhibitors are powerful inhibitors of GSK-3β. Indirubins [73] and paullones [74] constitute the first examples of low nanomolar inhibitors of GSK-3β. To which extent these compounds have implications in the study and treatment of neurodegenerative disorders remains to be determined.

Recently, cholinergic compounds such as M1 muscarinic agonist have been shown as potential disease-modifying

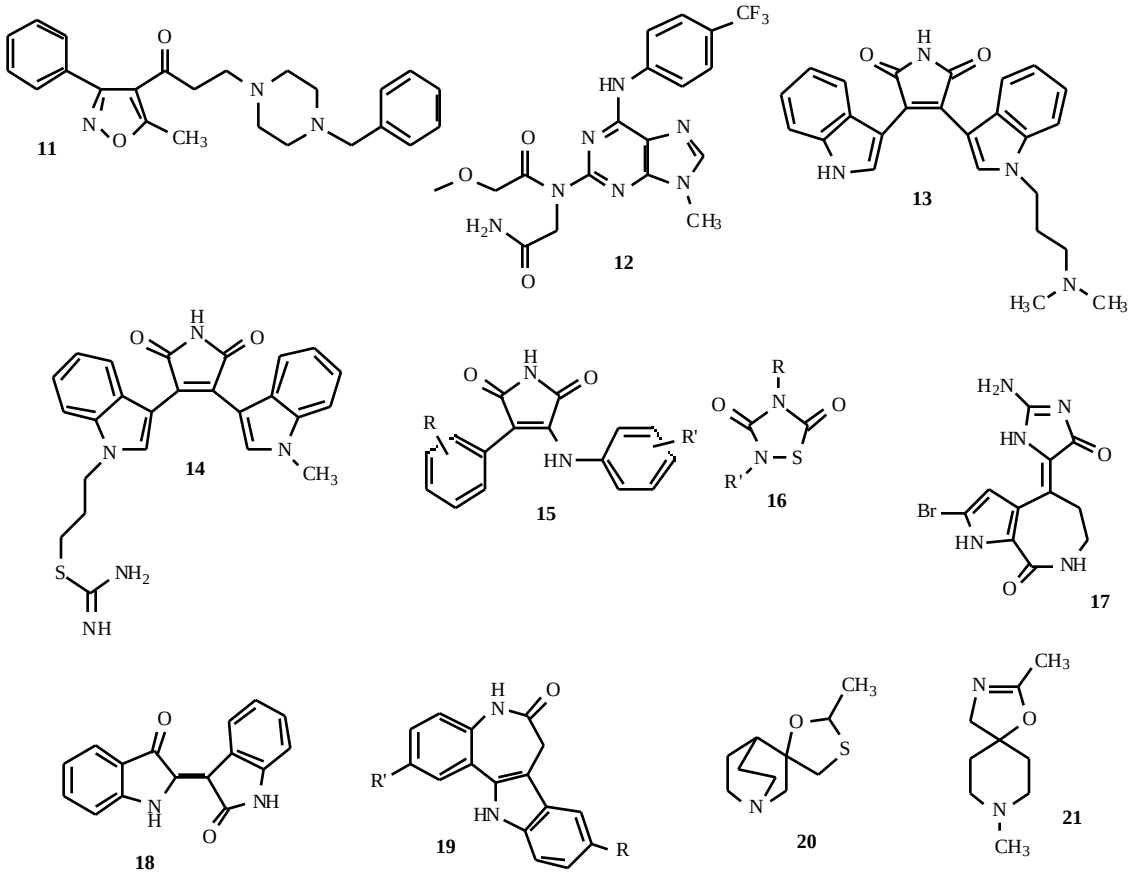


Fig. (4). GSK-3β and cdk5/p25 inhibitors.

agents in AD promoting the nonamyloidogenic APP processing pathways and decrease tau protein hyperphosphorylation [75]. Some of them, including AF102B (**20**) and AF150 (**21**) reduce tau phosphorylation via GSK-3β inhibition [76]. This unique property of M1 agonist to alter different aspects of AD pathogenesis can represent the most remarkable, yet unexplored, clinical value of such compounds [77].

It is worthmentioning that phosphorylation of tau is a dynamic event in which the addition and removal of phosphate groups is in constant flux. Thus, an imbalance between kinase and phosphatase activity can lead to an hyperphosphorylated environment. In fact, protein phosphatase PP-2A and PP-1 are decreased in AD brain [78]. It is suggested that PP-1 upregulates the activities of GSK-3β, cdk5 and cdc2 [79] while PP-2A upregulates the cytosolic calmoduline kinase CaM-KII activity and the phosphorylation of tau at Ser262/356 [80]. Increase in tau phosphatase activity is a promising, yet theoretical, approach to inhibit neurofibrillary degeneration and thereby the diseases characterized by this lesion. Molecular agents which regulate these events, and ultimately decrease the degree of tau phosphorylation, are being thoughfully pursued.

3.2 Preventing Tau Aggregation

Two main features are observed in tau pathology: microtubule destabilization and tau polymerization. For the first feature, the abnormal phosphorylation of tau could play a direct role, whereas for tau aggregation it does not appear to influence it in a direct way [81, 82]. Development of compounds that could be used to facilitate the proteolytic degradation of tau aggregates and prevent the further propagation of tau capture in AD might represent other new therapeutical approach to the treatment of taupathies.

In this way the selective inhibition of tau aggregation by diaminophenotiazine (**22**) (Fig. **5**) was reported, but toxicological and pharmacokinetic data are still unavailable [83]. The side groups added to the phenothiazine nucleus to achieve neuroleptic activity (e.g., chlorpromazine) abolished inhibitory activity. Likewise, the aminoacridine tacrine, was inactive.

There are reasons to assume that selective inhibitors of cathepsin D, a protease which is elevated in AD vulnerable neurons and cleave tau at neutral pH, could be useful in regulating the formation of the precursors to neurofibrillary tangles [84]. Potent nonpeptidic cathepsin D inhibitors (**23-25**) developed using combinatorial chemistry blocked production of hyperphosphorylated tau fragments in a dose-dependent fashion [85]. These results support the hypothesis that cathepsin D links lysosomal dysfunction to the etiology of AD and suggest a new approach to the treatment of disease [86].

Whereas there is no doubt that neurofibrillary degeneration is pivotally involved in the pathogenesis of AD and other taupathies, recently it has been demonstrated that the abnormal hyperphosphorylation of tau is critically involved in this process [87]. So, inhibitors of the

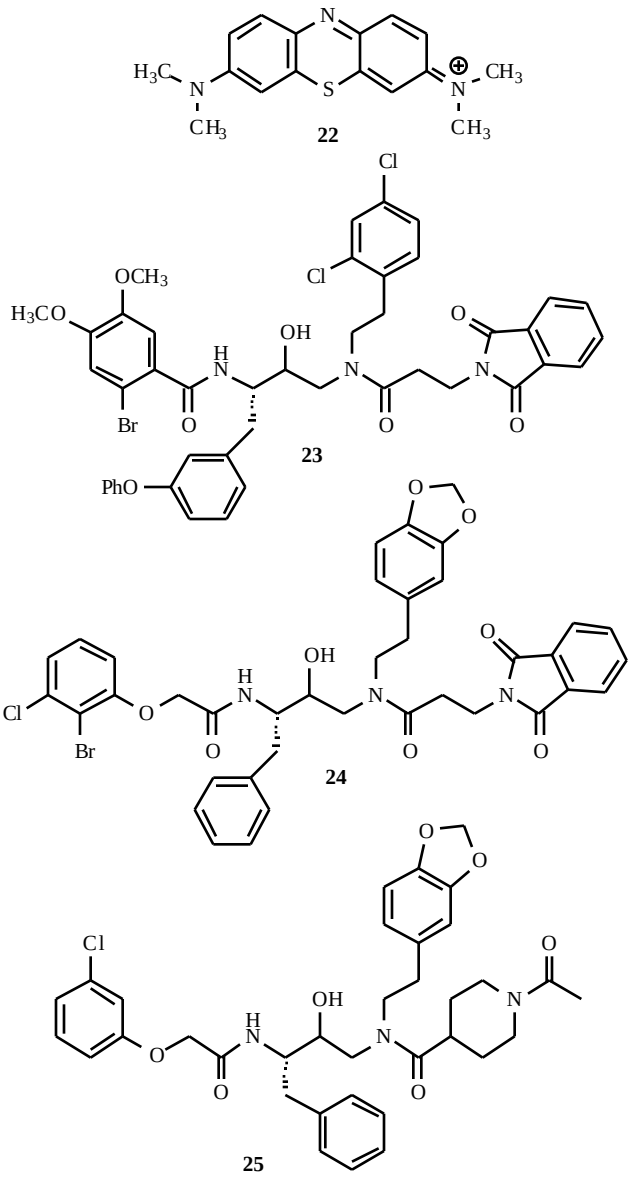


Fig. (5). Inhibitors of tau aggregation.

implicated kinases, such as mentioned above, might arrest these neurodegenerative diseases. Whereas whether these compounds will translate into efficacious clinical treatment is a question to be answered by further investigations.

4. OXIDATIVE STRESS AS BASIS FOR DEVELOPING ANTI-DEMENTIA DRUGS

Besides the histopathological changes in AD mentioned before there is also evidence that the brain tissue in patients with AD is exposed to oxidative stress conditions during course of the disease [88]. The vulnerability of central nervous system is associated to a number of factors, which may promote oxidative damage in AD, such as the excessive oxygen utilisation and high unsaturated lipid content [89]. Under normal conditions, damage by reactive oxygen species (ROS) is kept in check by an efficient antioxidant cascade, including enzymatic and non-enzymatic entities [90]. However, during degenerative process the imbalance between

the production of ROS and the cellular antioxidant defenses leads to critical failure of biological functions and ultimately cell death [91].

Oxidative stress is manifested in numerous ways, including alterations in the levels of anti-oxidant metalloenzymes such as copper-zinc superoxide dismutase (CuZnSOD) and manganase superoxide dismutase (MnSOD), which convert superoxide to O_2 and H_2O_2. [92] Protein damage, ranging from advanced glycation end products (AGEs) [93], to lipid peroxidation [94] and/or by direct oxidation of protein side chains are markers typically associated with attacks to free radicals [95]. Moreover, other sources of oxidative stress in AD also are perturbations in metal homeostasis [96] such as iron, cooper, zinc, and aluminium, capable of catalysing reactions that produce free radicals [97].

One of the major source of ROS are formed in mitochondrial oxidative metabolism and the fact that mitochondrial dysfunction is associated to degenerative disorders through a variety of different pathways is gaining increasing support [98]. In this regard, reductions in mitochondrial electron transport and mutations in cytochrome c oxidase genes have been linked in AD [99]. Moreover, associated to mithocondrial dysfunction, calcium homeostasis can be related to the production of free radicals [100].

On the other hand, microglia are immune system cells that exhibit phagocytic activity. Their activation leads to massive production of a number of inflammatory factors including inflammatory cytokines [101], ROS and nitrogen species [102] and thereby contributing to the oxidative damage [103]. Therefore oxidative stress and inflammatory cascade, working in concert [104], are important in the pathogenetic cascade of neurodegeneration in AD [105], offering new strategies for drug development [106].

Aβ is central to the pathology of AD. In the context of oxidative stress it could be classified as other important source associated to induce oxidative damage, because it produces neurotoxic effects [107], directly by inducing more free radicals, or indirectly by activating microglia [108]. Oxidative stress has also been implicated in the other hallmark of AD, neurofibrillary tangles, by the observation that oxidation of *tau* at certain residues is required for PHF formation *in vitro* [109].

Taking into account the multiple sources of oxidative stress, previously discussed, several pharmacological opportunities for influencing the disease can be suggested [110]. It is possible to distinguish several types of possible therapeutics agents according to their pharmacological point of attack. These approaches include antioxidants and anti-inflammatory drugs [111].

4.1 Antioxidant Agents

Based on their mechanism of action, antioxidants can be classified into:

(i) *Radical scavengers*, the agents directly interacting with free radicals before these oxygen or nitrogen species damage cell constituents. These include vitamins E (**26**) and C (**27**), Ginkgo biloba (flavonoids, terpenoids), selegeline (**28**), melatonin (**29**), idebenone (**30**), exifone (**31**), and estrogens (17β-estradiol) (**32**), Fig. **6** [112]. Some of them (vitamin E, selegeline and Ginkgo biloba extract (Egb 761) have been used for therapeutic purposes in different fields and now they have also been considered in clinical studies of AD producing beneficial results [113-115]. On the other hand, since lipophilic phenols also display neuroprotective effects, alkyl ethers of 17β-estradiol (**33**) have shown higher dose-dependent neuroprotection *in vitro* against stress oxidative [116]. Recently the neuroprotective effect of non-esteroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals has been reported [117].

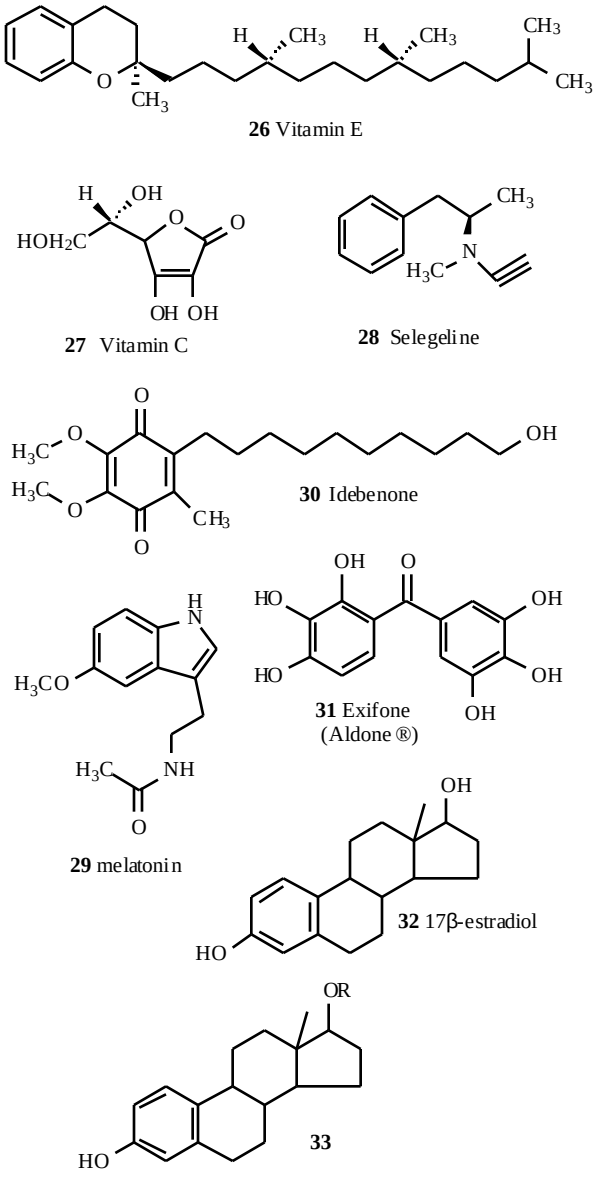


Fig. (6). Radical scavengers.

(ii) *Antioxidants*, which are able to prevent or decrease the production of free radicals by use of specific neuropharmacological properties.

Among others, *chelating agents* such as desferrioxamine (**34**) and deferiprone (**35**), which inhibits the catalytic action of iron [118]. Due to the important role of redox-active metals in the initial production of ROS, this approach may be effective in controlling oxidative stress. *AGE-inhibitors*, as tenilsetam (**36**), are able to inhibit covalent protein or protein linking by sugars or sugar-derived oxidation products. Although this strategy might represent valuable treatment option, no convincing preclinical and clinical studies have been performed Fig (**7**) [119]. Membrane lipid peroxidation inhibitors as lazabemide (**37**), a potent monoamino oxidase B inhibitor, can inhibit the propagation of free radicals by partitioning into the membrane hydrocarbon core [120].

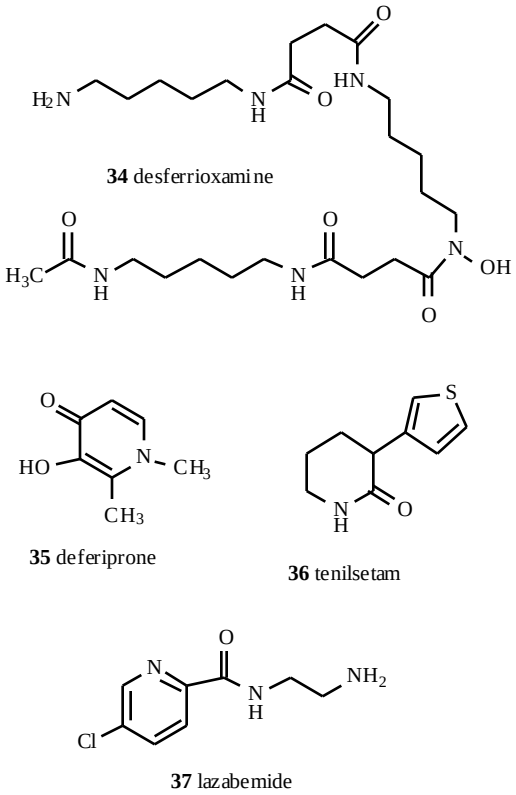


Fig. (7). Chelating agents and AGE inhibitors.

4.2 Anti-inflammatory Drugs

Anti-inflammatory agents attenuat microglia radical and cytokine production [121]. In this context, nonsteroidal anti-inflammatory (NSAID) drugs, such as indometacin (**38**), naproxen (**39**) or ibuprofen (**40**), have also been shown to have a useful effect in countering AD [122]. It is known that NSAIDs affect the cyclooxygenase (COX) activity, when involved in neurodegenerative processes [123, 124]. In addition to the development of selective COX inhibitors, i.e. celecoxib (**41**) and rofecoxib (**42**), it has led to renewed interest in the therapeutic potential of NAIDs in AD (Fig. **8)** [125].

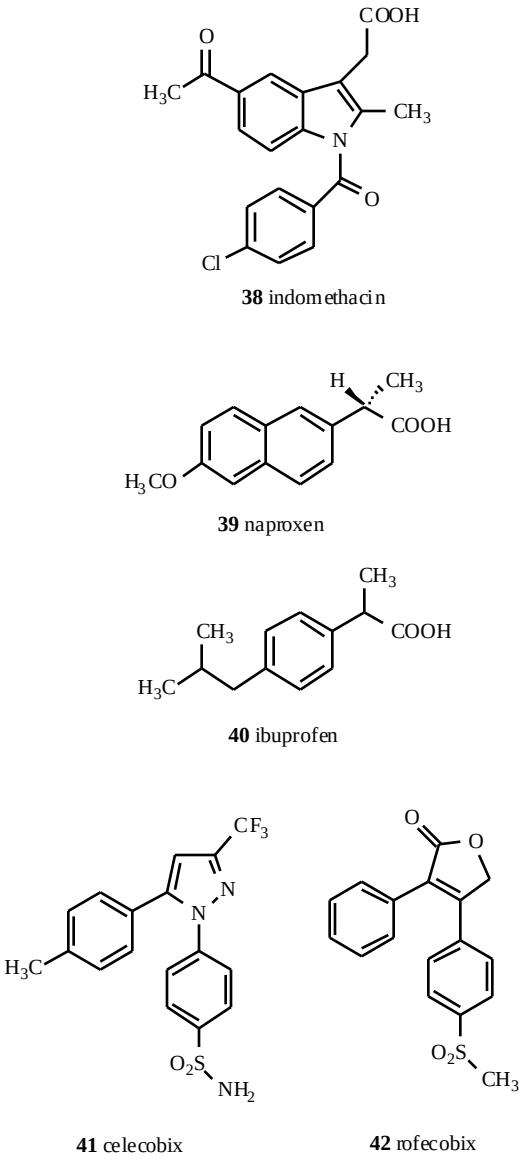
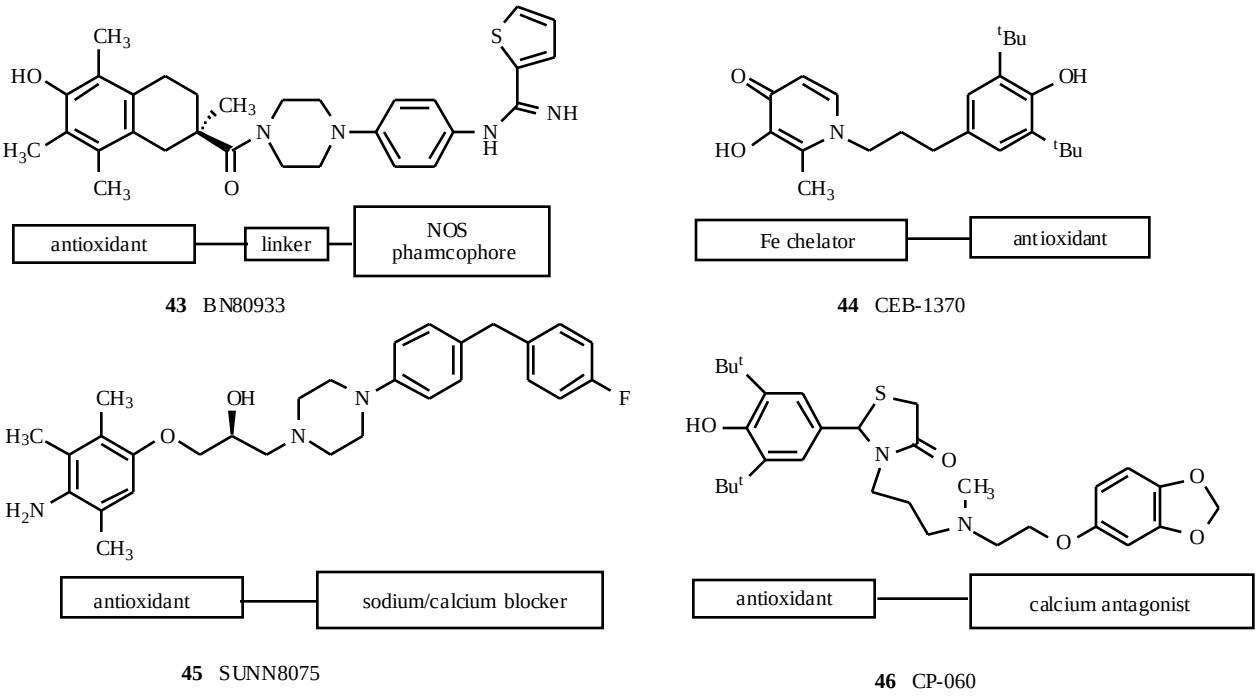


Fig. (8). Anti-inflammatory agents.

4.3 Combined-activity Compouds

The understanding of the complex process that involves AD as well as other neurodegenerative disorders has encouraged a growing trend to produce neuroprotective drugs with more than one mechanism of action. Hence, BN 80933 (**43**) combines activity against neuronal nitric oxide synthase together with lipid peroxidation inhibition [126]. CEB-1370 (**44**) incorporates in its structure antioxidant counterparts and Fe chelators components showing superior neuroprotection action compared to the dual administration of known radical scavenger and Fe chelator [127]. Additionally SUNN8075 (**45**) appears as a promising dual sodium and calcium blocker which exhibits antioxidant activity [128]. In this context, the thiazolidinone derivative, CP-060 (**46**) appears as a potent calcium antagonist with antioxidant activity (Fig. **9**) [129].

These are some examples that indicate how different pharmacological approaches may break the cycles of oxidative stress and neurodegeneration, offering new



43 BN80933

44 CEB-1370

45 SUNN8075

46 CP-060

Fig. (9). Combined activity compounds.

opportunities as the potential targets for therapy, for the treatment of AD. However, further characterization of oxidative mechanism is essential to improve the efficacy of the treatment [130].

5. EXCITATORY AMINO ACID AGONISTS AND ANTAGONISTS

L-glutamic acid (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), playing both physiological and pathological roles, and binding with two types of specific receptors called ionotropics and metabotropics. To date, three types of ionotropic receptors have been discovered: *N*-methyl-D-aspartate (NMDA), kainic acid (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) [131], that are involved in the control of the fast (AMPA) and slow (NMDA) component of excitatory postsynaptic currents. The metabotropic glutamate receptors, that can be divided into eight different subtypes $mGlu_1$-$mGlu_8$, are GTP-binding proteins, constituted by seven transmembrane spanning regions (7TM) that modulate second messenger systems [132].

At normal membrane potentials glutamate activates only AMPA receptors, since there is insufficient glutamate to activate metabotropic receptors, and NMDA-receptor channels are blocked by Mg^{2+}. After a learning stimulus, an increased level of glutamate is released from presynaptic neuron that activates metabotropic receptors and displaces Mg^{2+} at the NMDA channel. Then, the combined binding of glutamate and glycine to their respective sites on the NMDA receptor complex opens the cation channel, producing an increase in intracellular calcium, that in turn activates protein kinase C (PKC) and nitric oxide synthase (NOS) [133]. Then, PKC phosphorylates AMPA receptors, causing facilitation of the transmitter action in the postsynaptic cell, and nitric oxide diffuses across the synapse contributing to the enhancement of presynaptic neurotransmitter release [134].

On the basis of the above findings, AMPA modulators, NMDA partial agonists, and NMDA non-competitive antagonists have emerged as valuable drugs in the treatment of AD and related memory impairs [135].

5.1 AMPA Receptor Modulators

In this regard, the positive modulators of AMPA receptors (ampakines), such as the benzo[1,2,4]thiadiazine-1,1-dioxide derivatives (**47**-**49**) and the benzoylpiperidine derivatives (**50**-**52**) (Fig. **10**), showed to have beneficial therapeutic effects in animal as well as in human behavioral experiments [136, 137]. In addition, some ampakines have found to increase brain-derived neurotropic factor (BDNF) mRNA and protein levels in cultured rat enthorhinal/hipppocampal slices, suggesting the possibility of using these molecules to regulate neurotrophin levels in aged brain [138].

Preclinical and human studies with ampalex (**51**) have shown that this compound produces a dose-dependent improvement of a number of aspects of cognitive functions including attention, problem solving, memory and verbal learning, both in elderly [139] and in young people [140]. In addition, additive and synergistic interactions between **51** and antipsychotic drugs have been found, suggesting that positive modulators of cortical glutamatergic systems may be useful adjuncts in treating schizophrenic patients [141]. Currently **51** is under clinical trials, alone in AD patients

[142], and in combination with clozapine in schizophrenia patients [143].

Very recently, two novel and selective AMPA receptor modulators with biarylpropylsulfonamide structure (**53, 54)** (Fig. **10**), have been developed and tested *in vitro* and *in vivo* experiments [144]. Both molecules selectively enhance glutamate-evoked currents through AMPA receptor/channels of isolated neurons [145] and through recombinant homomeric human AMPA receptor ion channels [146], with considerably greater potency and efficacy than did the previously known AMPA agonists. The *in vivo* experiments have showed that both compounds cross the blood-brain barrier to affect directly the responses at central AMPA receptors [147]. Like other ampakines, in primary neuron culture (**53, 54**) evoke a time- and concentration-dependent increase in mRNA encoding brain-derived neurotrophic factor (BDNF), and thus they can be considered as neuroprotectant drugs [148].

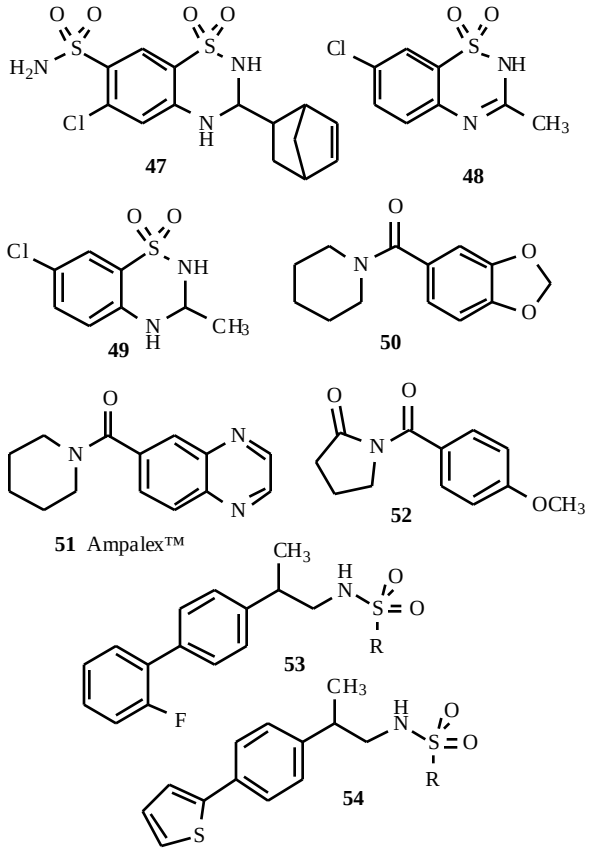


Fig. (10). AMPA receptor modulators.

5.2 NMDA Receptor Modulators

D-Cycloserine (DCS) (**55**) is a partial agonist at the glycine recognition site of the NMDA receptor complex (Fig. **11**), that has been found to facilitate activation of NMDA receptors in membranes from inferior parietal cortex of patients with AD [149]. In experiments with rodents and mammalians under different memory-impairment paradigms, DCS reversed working memory failures (eg, in the water maze) and improved certain forms of memory formation (eg, visual recognition) [150, 151]. However, an early clinical trial with AD patients, employing daily oral doses of DCS from 25 to 500 mg during two weeks, revealed no significant or consistent effect on Mini Mental State Examination scores (MMSE) [152]. In more recent studies either lower doses of DCS (15 mg/day) during more time (10 weeks) [153], or higher doses of DCS (100 mg/day) [154], produced significant improvements in the cognitive subscale of the AD Assessment Scale (improvement of 3.0 points).

Memantine (**56**) is a moderate-affinity, non-competitive antagonist of NMDA receptors, with strong voltage dependency and rapid blocking/unblocking kinetics [155]. It was registered in Germany for a variety of CNS-indications in 1978, although its therapeutic mechanism of action at NMDA receptor level was only discovered ten years later [156]. In contrast with other NMDA antagonists, memantine is devoid of side effects at therapeutic concentrations as has been demonstrated in several clinical trials for a variety of CNS disorders (stroke, CNS trauma, Parkinson's disease, amyotrophic lateral sclerosis, epilepsy) [157]. For example, in patients with severe dementia (49% of the Alzheimer type and 51% of the vascular type) memantine (10 mg/day, 12 weeks) produced an overall clinical improvement in about 70% of the patients, independent of the etiology of dementia, reducing their care dependence and without adverse drug reactions [158].

At the biochemical level, the mode of action of memantine has received diverse explanations [159, 160], and it has been found that it could also act preventing β-amyloid excitotoxicity in AD [161]. A recent study has suggested that the clinical combination of memantine with a reversible AChE inhibitor (tacrine, donepezil or galanthamine) may be more beneficial than independent drugs. This combination could both slow down the progression of the AD, by providing neuroprotection from glutamate and β-amyloid, as well as enhancing daily cognitive performance, by increasing the function of cholinergic neurons [162]. Memantine is expected to be approved for AD in USA by the year 2002.

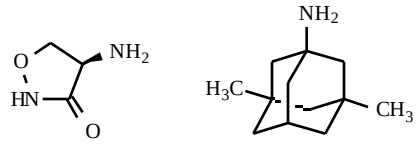


Fig. (11). NMDA receptor modulators.

6. NEUROTROPHIC COMPOUNDS

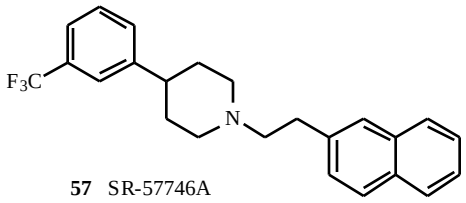
The ability of neurotrophic factors, such as the neurotrophins NGF, BDNF, NT-3 and NT-4/5, to regulate neuronal survival and adult nervous system plasticity suggests the use of these molecules to treat neurodegeneration [163]. Since human NGF does not cross the blood-brain barrier and is easily metabolized when administered peripherally, substances that increase the

synthesis of NGF or potentiate its effects are being tested (Fig. **12**).

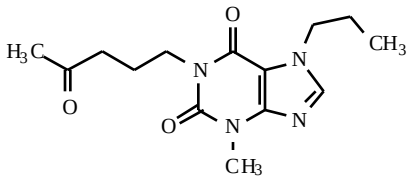
The orally active 5-HT_{1A} receptor agonist SR-57746A (**57**), in addition to its antidepressant properties, is known to have protective effects in central and peripheral models of neurodegenerative disorders in rodents and primates [164]. In several types of neuronal cells, SR-57746A has demonstrated its capability of promoting the synthesis of NGF and of increasing the outgrowth and the number of neurites [165, 166]. Besides its neurotrophic properties, SR-57746A also prevents the deficits in short-term memory induced by the intraseptal infusion of β-amyloid in rats [167], suggesting to have also neuroprotective effects as well. Currently clinical trials for amyotrophic lateral sclerosis is under phase III (ALS) and in phase IIb for AD.

In addition to its known properties as free-radical scavenger, idebenone (**30**) (see Fig. **6**) also induces nerve growth factor in the brain, improving learning and memory in basal forebrain-lesioned rats [168]. Now it is in phase II clinical trial for AD.

Since in the nervous system purine nucleosides and their metabolic products xanthine and hypoxanthine mediate both neurotransmission and trophic effects, some purine derivatives have been tested as memory-enhancing agents in AD [169]. Two of these, Neotrofin (AIT-082) (**1**) (see Fig. **1**) and propentofylline (**58**), easily cross the blood-brain barrier and have trophic effects *in vivo*, stimulating neurite outgrowth and the production of adenosine and neurotrophins from astrocytes [170, 171]. Recent findings provide evidence that propentofylline can also prevent the cell death induced by nerve growth factor withdrawal and β-amyloid toxicity [172]. Currently, both molecules have passed to phase III of clinical trials for AD [173, 174] and propentofylline is also in phase III for vascular dementia [175].



57 SR-57746A



58 Propentofylline

Fig. (12). Neurotrophic compounds.

CONCLUSION

AD is the idiopathic progressive loss of cognitive function over a period of several years. The risk of late onset dementia increases significantly with each decade of life so much so that half of the population over the age of 80 is vulnerable to this disease. Over the past few years, molecular biological research has considerably deepened our understanding of the pathophysiological basis of AD dementia. This makes it possible to identify points of attack for rational drug treatment of the disease as the Aβ, tau protein, oxidative stress, NMDA receptors, etc. Besides these kind of disease-modifying agents, other therapeutic approaches such as anti-sense technology [176], gene therapy [177, 178] or apoptosis modulators [179] are in development for the treatment of central nervous system acquired chronic diseases. The arrival of all these new treatments for AD has also provided the catalyst for the widespread development of diagnostic methods [180, 181] to enable proper assessment of patients with dementing disorders, offering a promise for meaningful therapeutic outcome.

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