

Medicinal Chemistry Approaches for the Treatment and Prevention of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is the most common form of dementia, which is characterised by progressive deterioration of memory and higher cortical functions that ultimately result in total degradation of intellectual and mental activities. Modern strategies in the search of new therapeutic approaches are based on the morphological and biochemical characteristics of AD, and focused on following directions: agents that compensate the hypofunction of cholinergic system, agents that interfere with the metabolism of beta-amyloid peptide, agents that protect nerve cells from toxic metabolites formed in neurodegenerative processes, agents that activate other neurotransmitter systems that indirectly compensate for the deficit of cholinergic functions, agents that affect the process of the formation of neurofibrillary tangles, anti-inflammatory agents that prevent the negative response of nerve cells to the pathological process. The goal of the present review is the validation and an analysis from the point of view of medicinal chemistry of the principles of the directed search of drugs for the treatment and prevention of AD and related neurodegenerative disorders. It is based on systematization of the data on biochemical and structural similarities in the interaction between physiologically active compounds and their biological targets related to the development of such pathologies. The main emphasis is on cholinomimetic, anti-amyloid and anti-metabolic agents, using the data that were published during the last 3 to 4 years, as well as the results of clinical trials presented on corresponding websites. © 2002 Wiley Periodicals, Inc. *Med Res Rev*, 23, No. 1, 48–88, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/med.10026

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1. INTRODUCTION

Alzheimer's disease (AD) is one of the major health problems in the economically developed countries along with cardiovascular disorders and cancer. It is characterised by progressive

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deterioration of memory and higher cortical functions that ultimately result in total degradation of intellectual and mental activities. AD is the most common form of dementia. It affects up to 10% of people over the age of 65 and 30–35% or more of those over age of 85 years.^{1,2} It is estimated that more than 800 thousand and 360 thousands new cases of dementia are developing each year among European and American population, respectively.^{3,4} Currently, AD affects approximately 20 million people all over the world and imposes an annual economic burden of about US \$ 100 billion.²

The annual market for an effective medication for AD in the USA only is estimated to be between US \$ 2.0 and 8.0 billion a year.² Currently, there are only a few therapeutic drugs that are in the market for the treatment of AD. The main pharmacological effect of most of the agents is to improve the cognitive functions decreased in AD due to hypofunction of cholinergic neurotransmitter system. Meanwhile, new approaches, which are based on the concepts of molecular mechanisms of the development of AD, are very promising, since they allow an intelligent design of drugs that combine properties of both cognition-stimulating agents and neuroprotective agents.⁵

The key neuropathological characteristics of AD are: senile plaques (SP), which are associated with beta-amyloid peptide, neurofibrillary tangles (NFT), and the loss of neurons in the hippocampus and nucleus basalis of Meynart.^{6–8} Neurotransmitter specificity of AD is characterised by a pronounced degradation of cholinergic system and by disturbances of functions in other neurotransmitter systems, such as glutamatergic and serotonergic systems.^{9,10}

Novel strategies in the search of new therapeutic approaches are based on the morphological and biochemical characteristics of AD, and focused on following directions:

- agents that compensate the hypofunction of cholinergic system;
- agents that interfere with the metabolism of beta-amyloid peptide;
- agents that protect nerve cells from toxic metabolites formed in neurodegenerative processes;
- agents that activate other neurotransmitter systems, which indirectly compensate for the deficit of cholinergic functions;
- agents that affect the process of the formation of NFT;
- anti-inflammatory agents that prevent the negative response of nerve cells to the pathological process.

The goal of the present work is a validation and an analysis from the point of view of medicinal chemistry of the principles of the directed search of drugs for the treatment and prevention of AD and related neurodegenerative disorders. It is based on systematisation of the data on biochemical and structural similarities in the interaction between physiologically active compounds and their biological targets related to the development of such pathologies. The main emphasis is on cholinomimetic, anti-amyloid and anti-metabolic agents, using the data, which were published during the last 3–4 years, as well as the results of clinical trials presented on corresponding websites (in particular, at Alzheimer Research Forum web site: <http://www.alzforum.org>).

2. CHOLINOMIMETIC AGENTS FOR AD TREATMENT

The cholinergic hypothesis of AD pathogenesis was formulated about 20 years ago.^{11,12} It was based on the following experimental data: (i) a significant deficit of markers of cholinergic neurons observed in limbic and temporal-parietal neocortical areas in the brain of AD patients, (ii) a significant cognition-stimulating effect of many cholinomimetic compounds, and (iii) a partial compensation of hypofunction of cholinergic system from intracerebral transplantation of foetal cholinergic cells. According to this hypothesis, a series of approaches for treatment of AD primarily by improvement of cognitive functions of the patients is proposed. These approaches are focused on the compensation of the deficit of the activity of the cholinergic neurotransmitter system in central nervous system (CNS) (Fig. 1).

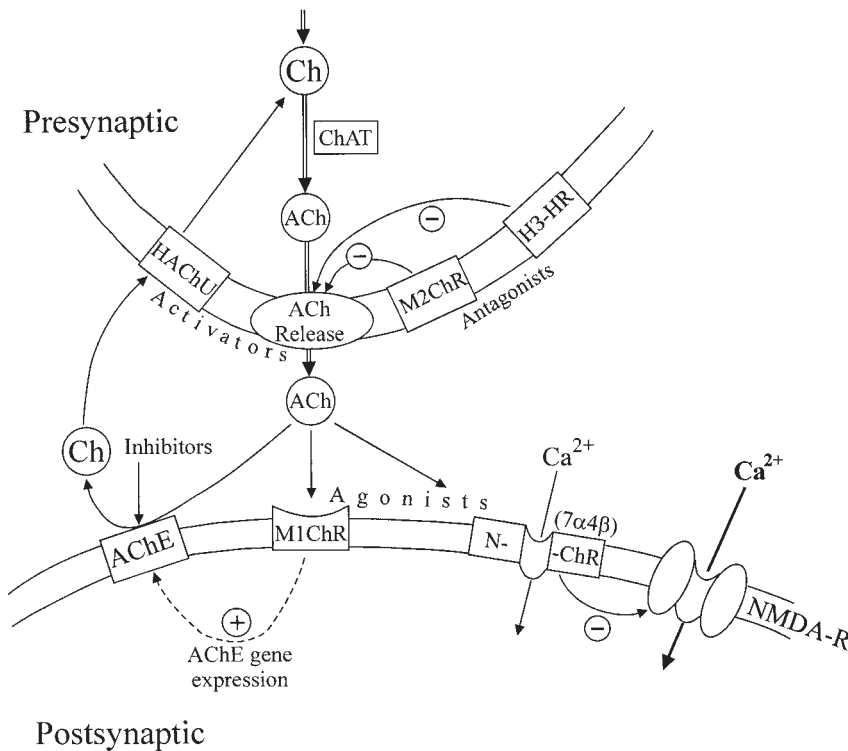


Figure 1. The main targets of cholinomimetic therapy of Alzheimer's disease. Abbreviations: Ach, acetylcholine; ACh release, acetylcholine release system; AChE, acetylcholinesterase; Ch, choline; H₃-HR, H₃-subtype histamine receptors; H3HR, high-affinity choline uptake system; M1ChR, M2ChR, muscarinic subtypes 1 and 2 (respectively) cholinergic receptors; N-ChR (7α4β), nicotinic 7α4β-subtype cholinergic receptors; NMDA-R, N-methyl-D-aspartate receptor.

A. Acetylcholinesterase (AChE) Inhibitors

Historically, cholinesterase inhibitors (ChEI) are the first and the most developed group of drugs proposed for AD treatment. According to the classical concepts of the impact of ChEI on neurotransmission, the main effect of ChEI is thought to be associated with the increase of both the duration of action and concentration of acetylcholine (ACh) neurotransmitter in the synaptic cleft that result in a potentiation of the activation of cholinergic receptors decreased in AD-type pathology.^{13,14} However, the magnitude of ChEI effect depends on the integrity of presynaptic neurons. Apparently, it will be reduced at late stages of the disease when a significant decrease in the number of terminals of cholinergic neurons is observed.

The first generation of ChEI, such as *Physostigmine*, *Tacrine (Cognex)* and *Amiridine (NIK-247)*, exhibited a low organospecificity and an unselective inhibition of AChE, also inhibiting another enzyme of this group, butyrylcholinesterase (BuChE).² *Amiridine* and *Tacrine* also block potassium channels and, to a lesser extent, sodium channels. These inhibitors have a certain muscarine-like cholinomimetic activity. The clinical efficacy of these drugs is moderate (e.g., *Tacrine* improved memory and cognitive functions only in 20–30% of AD patients) and develops slowly.¹⁵ These compounds have pronounced side effects, such as sedation and hepatotoxicity that significantly limit their use.¹⁶ On the other hand, since hepatotoxicity and a sedative effect of *Amiridine* are less pronounced than those of *Tacrine*, the former appears to be more promising for the prolonged therapy.¹⁷

One of the most popular approaches in the improvement of acceptability of cholinesterase (ChE) inhibitors is to decrease the selectivity of the AChE inhibition with respect to BuChE in order to

minimise their peripheral toxicity. These principles were taken into account in the development of the second generation of ChEIs.¹⁸ These newer ChEIs, which exhibit a pronounced selectivity towards AChE, include *Aricept (Donepezil)*¹⁹ developed by Pfizer and Eisai, *Galanthamine*²⁰ and *Eptastigmine*²¹ produced by Janssen and Mediolanum, respectively. Another approach was successfully introduced by Novartis for the drug *Exelon (Rivastigmine, ENA 713)*. The focus of the company was to design compounds with pronounced regioselectivity that provided a selective inhibition of the brain AChE rather than an inhibition of peripheral forms of cholinesterases.²² The new medications have significantly lesser side effects compared to *Tacrine*. These medications are especially effective in the early to moderate stages of AD. A development of ChEI with the optimal pharmacokinetics may be considered as an alternative to the above strategy. It can be realised in practice by creating so-called “pro-drugs,” which slowly release an active component into the blood, thus, resulting in a “soft” long-term inhibition of ChE. An example of such a drug is *Metrifonate*, a pro-form of dimethyl-2,2-dichlorovinylphosphate, developed by Bayer.²³ Another example is a new form of *Physostigmine*, which has slow pharmacokinetics for the release of the active component (so-called “sustained-release”), developed by Synaton Forest Laboratories.

In addition to the synthetic ChEIs, a plant alkaloid *Huperzine A* derived from a traditional Chinese herbal remedy for fever²⁴ has also been tested. The compound exhibits an anti-cholinesterase activity and is now under investigation as a potential medication for the therapy of AD.

Though the second generation of ChEIs differs in their specificity, they have a similar range of side effects. This can lead to a conclusion that the selectivity of these compounds to ChE inhibition does not affect their acceptability. Evidently, the development of optimal ChEIs drugs is limited primarily by the target of their effect. Toxic and depressive side effects of these compounds result from their activity as AChE inhibitors.²⁵

Recent results indicate that in addition to the “classical” cholinomimetic effect of ChEIs, which is exhibited via the stimulation of cognitive functions due to the compensation of the cholinodeficit in CNS, these compounds can impact amyloidose processes in AD.^{26,27} As a rule, this effect is associated with an additional activation of the cholinergic receptors caused by the inhibition of AChE. These receptors, in particular, muscarinic cholinoreceptors, participate in the processing of the amyloid precursor protein (APP) that initiates a cascade of neurodegenerative processes in brain (it will be discussed below). In addition, AChE can affect a transformation of the beta-amyloid peptide into toxic aggregates.²⁸ Similar considerations were expressed about the role of BuChE in creating compact forms of the amyloid plaques in the brain of AD patients.²⁹ Thus, in addition to the direct cholinomimetic action, inhibition of ChE can lead to a certain neuroprotective effect, which is very important for the development of new types of medicines for AD treatment.³⁰

The structural formulas of some of the described ChEI are presented in Figure 2.

B. Ligands of Muscarinic Cholinergic Receptors (mChR)

Originally, the major stimulus in the search for anti-AD drugs in a series of mChR ligands was the same as in the case of ChEI, namely, the necessity to compensate for AD deficit of cholinergic innervation. Later, it was shown that muscarinic cholinoreceptors could regulate APP processing³¹ that defines another aspect of mChR agonists in the early stages of AD. The mechanism and selectivity of such a neuroprotective effect of mChR agonists may be described as follows. All choline receptors are known to act via G-proteins. While M1, M3 and M5 mChR subtypes stimulate a hydrolysis of phosphatidylinositol via the activation of phospholipase C (PLC), M2 and M4 ChR subtypes participate in the inhibition of the adenylate cyclase activity. The receptors connected with G-proteins on neuron membranes regulate the APP processing.³² An activation of M1 subtype of ChR can lead to the PLC-catalysed hydrolysis of phosphatidylinositol with the formation of diacylglycerol. Then, diacylglycerol activates protein kinase C (PKC) providing a phosphorylation of APP. This triggers APP processing with a secretion of the corresponding form of the amyloid peptide. Thus, the

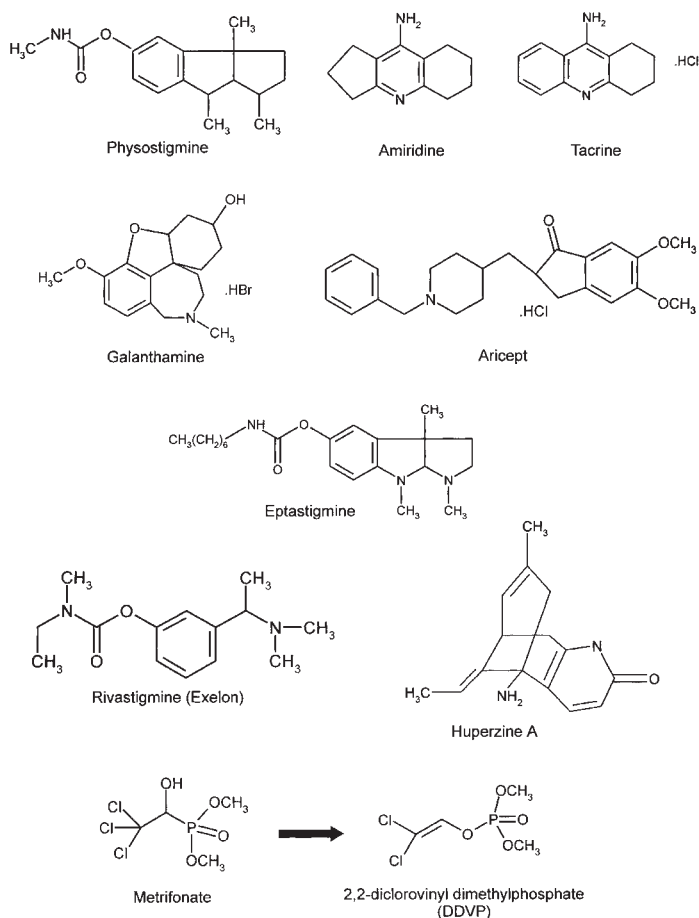


Figure 2. Structures of some AChE inhibitors proposed for AD treatment.

phosphorylation of APP activated via mChR increases a secretion of a trophic form of the amyloid and, what is especially important, leads to a decrease of the beta form of the amyloid. The hypoactivity of mChR or a disbalance in functioning of the mChR/G-protein couple leads to the deactivation of PLC, disturbance of APP phosphorylation and an increase of the yield of the pathological form of the amyloid protein.³³

A relatively low therapeutic effect of mChR ligands observed in early studies of cognition-stimulating properties of complete agonists of mChR was attributed to their low bioaccessibility and various side effects. At present, it is believed that side effects of complete agonists of mChR resulted from a stimulation of peripheral M2 and M3 subtypes of mChR. Taking together considerations on the protective effect of mChR ligands, specific agonists of M1 subtype of mChRs, that dominate in CNS, appear to be the most promising along with compounds that exhibit properties of both agonists of M1 and antagonists of M2 subtypes of ChR.³⁴

Agonists of mChR positively differ from ChEIs, since their cholinimimetic effect does not vary with the extent of degradation of presynaptic terminals of cholinergic neurons. It should be also noted that mChR agonists affect noncognitive symptoms of AD stronger than ChEI.

The efficacy of mChR agonists is limited mainly due to the fact that these receptors participate in the activation of the expression of AChE gene. This event results in the formation of a negative feedback in the cholinergic neurotransmitter transmission.³⁵

The agent *Xanomeline* (LY 246708) should be particularly mentioned as one of the potential anti-dementia drugs that was studied among a broad spectrum of mChR ligands. It is a selective agonist of M1 and M4 mChR subtypes.³⁶ A Phase II clinical trial of “skin patch” formula of this compound is now under way; “oral formula” was discontinued due to a negative effect on the gastrointestinal tract.

A number of efficient mChR ligands, that demonstrated pronounced cognition-enhancing properties in animal models of AD, failed to improve cognition and/or revealed side effects in clinical trials in AD patients. Among these compounds there are quinuclidine derivatives, such as agent *AF102B* and its analogue *SB 202026* (*Sabcomeline* or *Memric*),³⁷ that are partial agonists of M1 subtype of mChR, and agent *Lu25-109*, that has M1-agonist/M2-antagonist properties.³⁸ According to the Internet data, further development of these compounds has been suspended.

Structures of these compounds are presented in Figure 3.

C. Ligands of Nicotinic Cholinoreceptors

Nicotinic cholinoreceptors (nChRs), a subtype of cholinoreceptors, are directly connected with chemically regulated ion channels and are very important for cognitive processes in brain.³⁹ Brain nChRs are classified as alpha (comprising at least six subtypes, namely, $\alpha 2$ - $\alpha 7$) and beta ($\beta 2$ - $\beta 4$) subunits. Neuronal nChRs are usually composed of two alpha and three beta subunits producing a channel-forming five-component complex. The only exception is an $\alpha 7$ subtype of nChRs that participates in the regulation of calcium homeostasis in neurons. It was shown that both $\alpha 7$ and $\alpha 4 \beta 2$ nicotinic acetylcholine receptor subtypes are involved in the formation of working memory.⁴⁰ According to the data of autoradiography and positron emission tomography (PET), AD patients are characterised by a significant decrease of a number of nChR binding sites. This number varies in

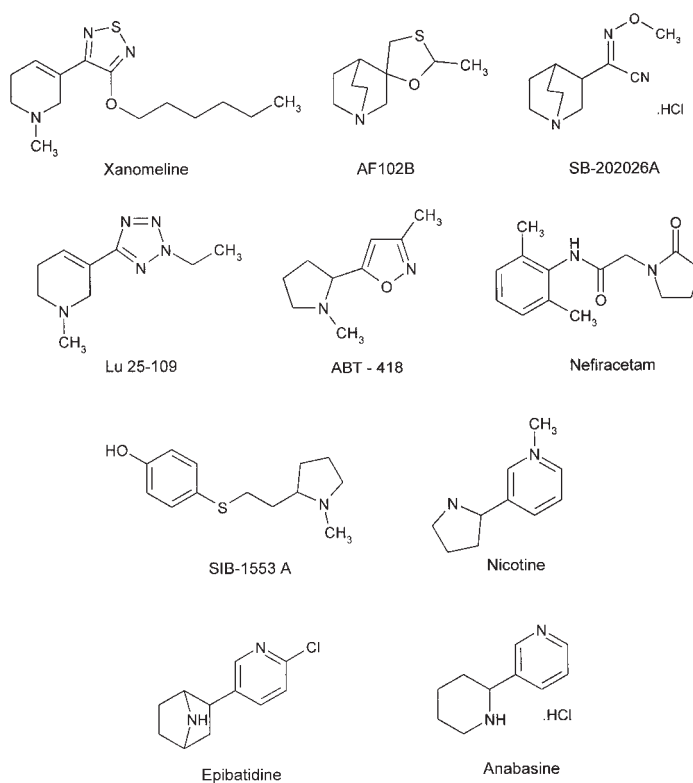


Figure 3. Structures of some cholinergic receptors ligands proposed for AD treatment.

different regions of the brain by up to 70% in the cerebral cortex. It is important that the decrease of a nChR content is observed at early stages of AD, possibly, before the development of irreversible processes in the brain.⁴¹ Although the inter-relationship between the function of nChR and the development of AD is known for a long time, a strategy for the search of medicines for the treatment of AD in a series of nChR ligands has been developed only recently.⁴² In particular, a chronic effect of nChR agonists was found to result in the upregulation of $\alpha 7$ and $\alpha 4\beta 2$ subtypes of nicotinic receptors, that gives ground for directed search of efficient neuroprotectors in a series of agonists of these receptors subtypes. Indeed, in cell culture experiments it was shown that activation of $\alpha 7$ and $\alpha 4\beta 2$ nChR subtypes can prevent neurons from both a toxic effect of beta-amyloid and a glutamate excitotoxicity.⁴³ Number of selective agonists of $\alpha 7$ nChR exhibited pronounced cognition-stimulating properties in animal models of the AD-type dementia.

However, nowadays only a few medicines developed on the basis of nChR ligands are currently suggested for the treatment of AD. One of them is a selective agonist of $\alpha 4\beta 2$ nChR *ABT418* (Abbot Laboratories),⁴⁴ which was tested in Phase II clinical trials on AD patients. A series of its analogues (*A-98284*, *A-85380*, *A-84543*) are currently under investigation. A compound *Nefiracetam* (*Translon*)⁴⁵ has been registered as a medicine for AD treatment. Its cognition-stimulating effect may be associated with an activation of nChR, a stimulation of choline-acetyltransferase, and a short- and long-term suppression of ACh-induced currents observed at submicromolar and micromolar concentrations, respectively. A well-known nChR agonist *Anabasin*, the alkaloid *Epibatine* extracted from skin of poisonous South American frog, and their numerous derivatives and analogues, such as newly synthesised selective nChR agonist *SIB1553A*,⁴⁶ were also suggested as potential medicines for the AD treatment. According to cell culture data, such ChEI as Tacrine and Aricept as well as certain estrogens decrease the toxicity of beta-amyloid in *in vitro* experiments via the nChR activation mechanism. Structures of several of the nChR ligands mentioned above are presented in Figure 3.

The principal limitation that may arise in the development of therapeutic approaches using ligands of nChR is connected with the fast desensitisation of these receptors to the effect of their agonists.

D. Compounds Affecting Synthesis and Release of Acetylcholine (ACh)

A search for compounds that may stimulate the synthesis and the release of acetylcholine is an important approach to the development of cholinomimetic drugs compensating for the deficit of the cholinergic system. These compounds may be classified into three groups based on their mechanism.

1. Biochemical Precursors of ACh

The cholinomimetic effect of such compounds is based on the increase in the amount of the ACh precursor choline. Compounds that have such a cognition-stimulating mechanism include exogenous choline, lecithine, and phosphatidylcholine. The best known medicine in this group is *Gliatilin* produced by Italfarmaco (or *Brezal* manufactured by Sandoz).⁴⁷ The compound ALCAR (acetyl-L-carnitine manufactured by Sigma-Tau Pharm) that is now in Phase III clinical trials⁴⁸ is also regarded sometimes as a drug of the same group. However, acetyl-L-carnitine is not considered as a proper precursor of ACh. It acts as a weak agonist of ChR and (what is more important) normalises lipid metabolism and stabilises the mitochondrial function.⁴⁹

2. Stimulators of Acetylcholine Release

The release of acetylcholine may be stimulated via different mechanisms. One example is a medicine *Linopirdine* (*DuP 996*), which is now in Phase III clinical trials. The mechanism of the ACh release with *Linopirdine* was demonstrated to be associated with either a selective inhibition of M-type potassium channels on presynaptic terminals of cholinergic neurons or a nonselective inhibition of

certain potential-regulated calcium-dependent channels, which was observed at micromolar or high concentrations of the drug, respectively.⁵⁰ Another mechanism of the stimulation of the ACh release is realised via antagonists of H₃ histamine receptors (H₃-HRs). H₃-HRs, a subtypes of HRs, are localised on presynaptic terminals of histaminergic and nonhistaminergic neurons in the central and peripheral nervous system. Several selective ligands of H₃-HR in a series of 4-substituted imidazol derivatives, such as a H₃-HR antagonist *Clobenpropit* and a H₃-HR inverse agonist *Thioperamide* developed by Glaxo Wellcome, as well as the compound *GT-2016* (Gliatech) demonstrated high cognition-stimulating properties in an animal model of dementia.^{51,52} Similar results were also obtained for other derivatives of imidazol, such as *Iodoproxyfan* and *UCL-1390* (both developed by INSERM)⁵³ as well as the compound *AQ-0145* developed jointly by Green Cross and Tohoku University (Japan).⁵⁴

A stimulation of the ACh release via the increase of the presynaptic uptake of endogenous choline, formed due to the AChE-catalysed enzymatic degradation of ACh, is considered to be an alternative to the receptor-regulated ACh release. The compound *MKC-231* (Mitsubishi Chemical)⁵⁵ should be noted as one of the most active medicines of this class. It is an activator of a system of the high-affinity choline uptake, the key component of ACh resynthesis. In independent studies it was also shown that *MKC-231* protects against a glutamate neurotoxicity by suppressing the NO formation triggered by Ca⁽²⁺⁾-influx.⁵⁶

Structures of compounds discussed in this section are presented in Figure 4.

3. PHARMACOLOGICAL APPROACHES TO AD THERAPY BASED ON CONCEPTS OF BETA-AMYLOID EFFECT ON AD PATHOGENESIS

Among numerous assumptions on the ethiopathogenesis of AD, a hypothesis about the key role of beta-amyloid peptide (beta-amyloid, Aβ) in the development of neurodegenerative processes in AD deserves a special attention.⁵⁷ An enormous amount of *de facto* data has been accumulated during the

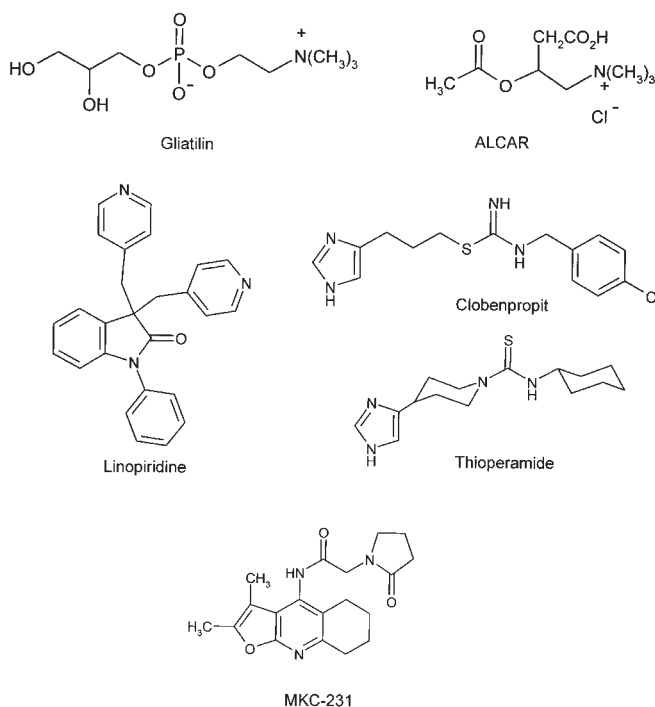


Figure 4. Structures of compounds affecting synthesis and release of ACh.

past decade on various aspects of the beta-amyloid cascade. Although many questions are not yet answered, the anti-amyloid strategy in the search for new medicines and approaches for the AD treatment is considered as one of the most promising ones.^{58,59}

According to the up-to-date concepts of AD pathogenesis, the pathological form of the amyloid peptide (AP) produced from amyloid protein precursor (APP) may play the key role in the development of neurodegenerative processes in the brain. Under “normal conditions”, AP is secreted mainly in a soluble form (sAPP α) as a result of the effect of α -proteases. It is believed that it has various trophic functions. Changes in the mechanism of APP cleavage caused by ageing or mutations of the APP gene result in a decrease of the sAPP α content and in the production of the beta-form (AP β) of the peptide due to the effect of β - and γ -proteases. Beta-amyloid can be produced in several forms containing a different amount of amino acid residues (AP β 40, AP β 42, AP β 43). A further aggregation of AP β leads to the formation of insoluble fibrils (compact forms) that participate in the formation of SP. While both soluble and aggregated forms of AP β 42 and AP β 43 peptides are considered to have toxic effects on neurons, the AP β 40 peptide may exhibit toxic properties only in its aggregated state.⁶⁰

Following mechanisms of the beta-amyloid induced neurodegeneration are usually emphasised: a disruption of calcium homeostasis, oxidative stress, a potentiation of the toxicity of excitatory amino acids, and an induction of apoptosis.

According to proposed mechanisms of the formation and the effect of AP β , a number of approaches to the prevention and correction of the cell neurodegeneration caused by AP β are considered. The main groups of prospective and already applied compounds for the treatment of AD-type dementia are systematised below with respect to their participation in the amyloid cascade.

A. Inhibition of the Formation of AP β

The step of the formation of AP β from the protein precursor APP is probably one of the most attractive targets to block the development of a cascade of neurodegenerative processes. As mentioned above, processing of APP is regulated by the mChR activation via protein kinases. The expression of APP is stimulated by such endogenous factors as cytokines, some neurotrophic factors (BFGF, EGF), estrogens, and by stress (trauma, focal ischemia, etc.).⁵⁸ The formation of AP β proceeds via two stages, first, via the intracellular proteolysis affected by β -secretases that results in the production of the amino-terminal AP β residue, and, then, via the extracellular cleavage by γ -secretases at a C-terminal of the AP β sequence. Both the nature and properties of true β - and γ -proteases, which are responsible for the cleavage of AP β from the protein precursor, have been unclear until recently. Late in 1999, the first data on the identification of the β -secretase participating in APP processing have been published.⁶¹ This information may be used for the directed search for specific blockers of the cleavage of APP. Details on γ -secretase are still awaited. Various known proteases, such as calpain, cathepsin D, were suggested to be its prototypes. During the last few years, it was revealed that the protein presenilin-1 (PS-1) plays an important role in the γ -secretase activity. The conclusion at this point is that either PS-1 is γ -secretase itself or it works as a specific cofactor for γ -secretase, or it plays a broader role in facilitating the trafficking of γ -secretase or membrane substrates of γ -secretase to the appropriate degradation compartment.^{62,63}

During the past few years, a large number of results have been published concerning compounds affecting APP processing. However, the mechanism of this regulation is significantly different. For example, *Monensin* and *Brefeldin A* seem to decrease the formation of AP β by a destabilisation of the pH gradient and/or by a vesicular transport. Obviously, such a nonselectivity significantly decreases the probability of the development of effective medicines based on these agents. *Bafilomycin A* and its analogues are of great interest, since they very effectively (EC₅₀ = 50 nM) and selectively block the formation of AP β by an indirect inhibition of the β -secretase activity. This indirect inhibition results from the prevention of the lysosomal acidification because of blocking the V-type of acetyltransferase.⁶⁴ Another mechanism that leads to a decrease in the formation of AP β is described

for peptides *Leupeptine* and *E-64*. These peptides stabilise the C-terminal fragment of AP β by a direct or indirect inhibition of the γ -secretase activity. Recently, experiments with transgenic mice demonstrated for the first time that a reduction of brain AP β *in vivo* results from a selective inhibition of γ -secretase.⁶⁵ It was shown that a synthetic derivative of the oligopeptide *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine, t-butyl ester, (*DAPT*) effectively reduced the brain level of AP β in a dose-dependent manner within 3 hr.

A stimulation of the normal cleavage of APP resulting in the formation of the trophic sAPP α may be considered as an interesting alternative to the inhibition of the pathological processing of APP discussed above. Thus, stimulation of 1 α metabotropic glutamate receptors increases the formation of sAPP α , and may be used as a rational strategy to prevent the development of AP β -induced pathology of AD type.⁶⁶

During the past few years, it was revealed that the cholesterol metabolism is associated with the susceptibility to the AD. It was shown that among patients with different types of dementia, those who were treated with lovastatin and pravastatin, two statin drugs that are widely used to decrease a cholesterol level, had lower prevalence of AD.⁶⁷ It is known that statins act via the inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate. The latter reaction is an early and rate-limiting step in the biosynthesis of cholesterol. It is hypothesised that a reduction in the level of cholesterol triggered by statins may alter the APP metabolism, and, thus, may reduce the production of AP β .⁶⁸ It was also shown that statins have an immunomodulatory effect.⁶⁹ They block the ability of a cytokine interferon-gamma (IFN-gamma) to activate T-cells. Therefore, statins may have a neuroprotective effect, which would be based on their ability to decrease inflammation and to reduce a lipoprotein oxidation and a generation of ROS. Currently, a statin agent *Lipitor* (*Atorvastatins*) is in the Phase II clinical trial for the therapy of AD.⁷⁰ During the last year, it was shown a possibility to reduce the cerebral AP β level *in vivo* with an FDA-approved drug *Simvastatin*, which is currently used to lower serum cholesterol.⁷¹

Structural formulas of selected compounds that display an effect discussed in this chapter are presented in Figure 5.

B. β -Sheet Breakers

Neurodegenerative properties of AP β may be only observed when its soluble form is transformed into an aggregate. Hence, compounds that slow down or reverse the aggregation of AP β may be considered as potential anti-AD medicines with a neuroprotective effect. It is proposed that a fibrilization of AP β is based on its ability to form β -folded oligomeric structures.⁷² Therefore, a directed search for compounds that may prevent the formation of β -folded aggregates (so-called “ β -sheet breaker”) is quite natural. There are several synthetic peptides, modified analogues of AP β that display the described properties. These include 5-residue peptide *KLVEE* (AP β fragment with amino acid sequence 16–20)⁷³ and its modified analogues *LPFFD*, *LPYFD*, *RDLPFYVPID*.⁷⁴ It was emphasised that the main disadvantage of the use of these compounds in clinical practice was their weak penetration through the blood–brain barrier (BBB), as well as potential side effects, such as various allergic reactions. Recently, the low-molecular weight analogues of these peptides have been synthesised. They displayed strong AP β anti-aggregation properties and a protective effect against the amyloid fibrils formation.⁷⁵ A number of synthetic glycosaminoglycans and endogenous proteoglycans, other types of high-molecular weight compounds, may also decrease an aggregation and deposition of AP β fibrils in the brain.

For example, the glycoprotein *Laminin*, which is expressed in brain tissues as a result of trauma, and some of its derivatives reduce the neurotoxicity of AP β by inhibiting a formation and by inducing a disaggregation of β -fibrils.⁷⁶ Similar effect is also found for the antibiotic *Rifampicin*, which is used for the treatment of tuberculosis and leprosy, and for certain tetracycline derivatives. It has been

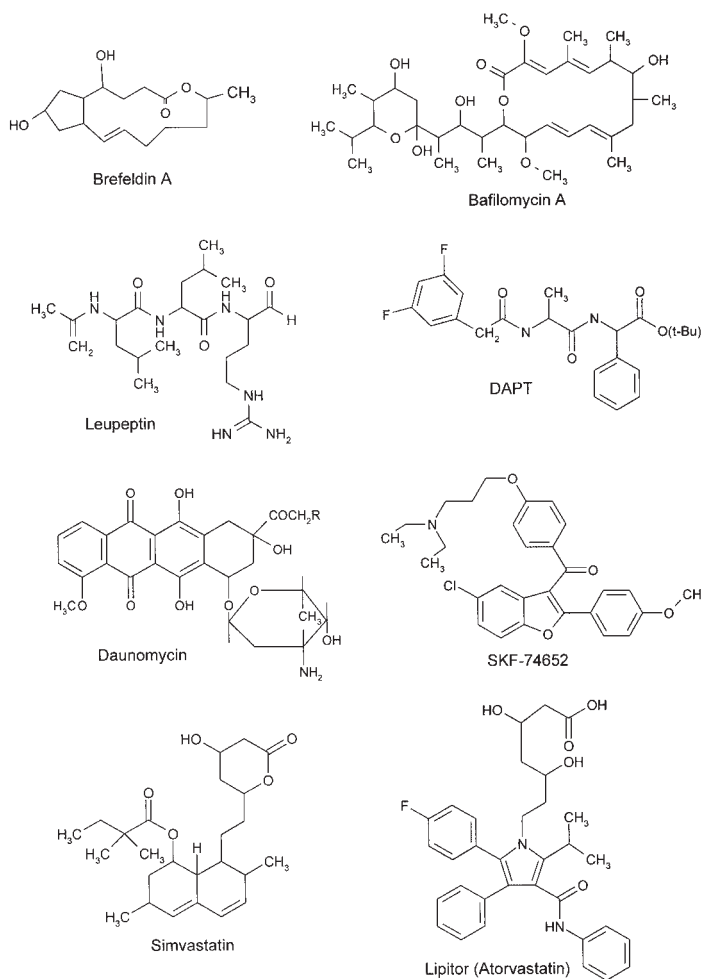


Figure 5. Structures of compounds affecting APP processing and β -Sheet breakers.

shown that *Daunomycin* (*Rubomycin*) binds to AP β and, thus, blocks the formation of amyloid fibrils. Molecular docking suggests that all these compounds may form a similar three-dimensional pharmacophore, thus may participate in the interaction with AP β .⁷⁷ The ability to block an amyloidosis was revealed recently for a series of newly synthesised benzofuranes,⁷⁸ in particular, for the agent *SKF 74652*.

Recently, it was confirmed that ChE enzymes participate in the aggregation of AP β . It was shown that AChE accelerated the transition of the soluble AP β into fibrillar complexes. The interaction of AP β with peripheral centres of AChE binding was very significant. It was interesting that the resulting AChE-AP β complex exhibited its own neurotoxic properties. The complex with AP β 40 was found to be the most toxic.⁷⁹ This is a new aspect in the therapeutic effect of AChE inhibitors. The neuroprotective effect of AChEIs results from their ability to block the interaction of the AChE enzyme with AP β .

Mechanisms of endogenous regulation of the formation of amyloid fibrils, especially the function of apolipoprotein E (apoE) and apolipoprotein J (apoJ) in this process, are of special interest. There are several isoforms of the apoE protein, such as E2, E3 and E4. This protein significantly affects the metabolism of lipids and lipoproteins. Allele ϵ 4 of the *APOE* gene was revealed to be one of the most important genetic risk factors for AD. The ability of apoE4 to increase the fibrillogenesis of AP β and

to decrease the removal of A β from the intracellular space is considered as one of the possible mechanisms of the initiation of neurodegeneration by this protein.^{80,81} Recent data suggest that the apoJ protein, which stimulates the accumulation of the trophic form of amyloid sAPP α in CNS, also has a protective function.⁸² The possibility to influence the apoE/apoJ-regulated process of the formation and deposition of amyloid fibrils in the brain appears to be a very attractive prospective in the development of a new approach to prevent AD, but is as yet untapped.

Another approach in the correction of Alzheimer-type pathologies is associated with using antibodies against A β (ABAP β). These antibodies are already used as possible markers for the diagnostics of AD. However, therapeutic features of immune methods of the AD correction have been revealed only recently. It was found that the immunisation of transgenic PDAPP mice, which have increased levels in both A β 42 production and deposition of the amyloid plaques, with A β 42 amyloid prevented a deposition of the amyloid plaques in young animals and significantly decreased the amount of the plaques in older animals.⁸³ The immunisation with A β results in increasing in the formation of ABAP β . These antibodies “bind” and “take out” endogenous A β , the main component of the amyloid depositions, that results in the destabilisation and destruction of SP. The effect of antibodies on the prevention of the toxic effect of A β was demonstrated later in the experiment with artificial antibodies IgM508 against fragments of APP protein. It was also shown that such antibodies might block the formation of insoluble A β aggregates and dissolve amyloid fibrils that are already formed.⁸⁴ In late 2000, two papers by Canadian⁸⁵ and American⁸⁶ scientists were published simultaneously in “Nature”. Using different transgenic mice with mutations of the APP gene or both APP and presenilin-1 genes, it was demonstrated that the immunisation of animals with A β peptide protected them from the memory decline and the deposition of amyloid plaques. In addition, it was emphasised that the immunisation did not result in a decrease of A β level in the brain.

A synthetic form of A β 42 AN-1792 (AIP-001)⁸⁷ proposed by Elan Corporation and American Home Products was under investigation in Phase IIa clinical trials. However, recently four cases of inflammation of the central nervous system were reported in clinical trial in France on 97 patients, who were given the vaccine. This report suggests that the vaccine may produce unwanted side effects. Elan has reported that it will suspend dosing in Phase IIa trials until the cause of the inflammation is determined, although the study will continue.⁸⁸

Thus, as it was shown in the laboratory experiments on animal models, the deposition of amyloid formations, the most typical pharmacological features of AD, may be prevented and even reversed successfully.

C. Agents That Prevent Neurotoxic Effect of A β

Despite the lack of details on molecular mechanisms of neurodegenerative effect of A β in *in vivo* experiments, the following mechanisms of its neurotoxicity are proposed:

- increase of toxicity of excitatory amino acids (EAA);
- disruption of cell calcium homeostasis;
- initiation of lipid peroxidation (LP) and generation of reactive oxygen species (ROS);
- induction of apoptosis;
- energy depletion of cells.

Since all these processes are closely interrelated, most of the compounds that will be discussed in this section affect the whole spectrum of the neurotoxicity of A β .

1. Agents That Block Neurotoxic Effect of EAA

The following data justify the role of the CNS glutamatergic system in the ethiopathogenesis of AD: (i) a significant decrease in the amount of glutamate receptors (GluR) in the hippocampus in AD;

(ii) the important role of glutamate receptors (in particular, NMDA and AMPA subtypes of receptors) in learning and memory; (iii) the ability of endogenous glutamate and other agonists of GluR to induce a neurotoxic effect (so-called “excitotoxicity”) resulted in neuronal cell death.

The role of excitotoxicity in the ethiopathogenesis of neurodegenerative disorders is thoroughly studied and described in literature.⁸⁹ Three phases of “acute” excitotoxicity are recognised:

1. Initial depolarisation of the neuronal membrane via AMPA and/or Kainate subtypes of glutamate receptors, that results in an increased influx of sodium ions (through AMPA and potential-regulated sodium channels), calcium ions, and water molecules into the cell. This causes so-called “osmotic swelling” of the cell and a dissociation of magnesium ions that block NMDA receptors. This phase is known as a “calcium-independent process”.
2. Hyperactivation of NMDA receptors, which is accompanied by an increased influx of calcium ions into the cell and an increase in the calcium concentration ($[Ca^{2+}]_c$) in cytosol by several orders of magnitude. This causes the activation of certain intercellular enzyme systems (proteases, nucleases, lipases), that triggers a cascade of degenerative processes and, ultimately, a cell lysis. This phase strongly depends on the presence of calcium ions.
3. Exocytosis of cells that leads to the release of a high amount of endogenous glutamate. This results in a sharp increase in the concentration of extracellular glutamate and in an additional hyperactivation of the glutamate receptors that amplifies neurodegenerative reactions in the cell (phase 1 and phase 2).

In addition to the “classic” mechanism of the excitotoxicity, a “slow” or “metabolic” excitotoxicity is also described. This type of neurodegeneration is observed at a normal (non-increased) concentration of glutamate when the cellular energy status is low. In this case, a disruption of the mitochondrial function and of the production of ATP triggers pathological processes. A decrease in the activity of ATP-dependent enzymes Na^+/K^+ dependent ATPases, which maintain the membrane potential of the cell, causes a depolarization of the cell membrane (observed even at normal concentration of EAA). As a result, magnesium ions dissociate from NMDA receptors, and a massive influx of calcium ions into the neuron initiates a cascade of intracellular neurodegenerative reactions. The above mechanism is typical for long-term neurodegenerative disorders.⁹⁰

In the early nineties, experiments on cell culture demonstrated the ability of $AP\beta$ to potentiate the neurotoxicity of EAA and to destabilise the calcium homeostasis in the cell.⁹¹ Based on these data, it has been proposed that antagonists of AMPA/Kainate receptors and antagonists of NMDA receptors that block the glutamate-induced calcium influx may prevent the neurotoxicity induced by $AP\beta$. On the other hand, as mentioned above, the amount of the glutamate receptors is significantly decreased in the brain of patients suffering from Alzheimer’s disease and is in a reverse correlation with the progression of the disease.⁹² From this standpoint it was suggested that agonists of GluR may compensate for a deficit of the glutamatergic innervation in striatum and hippocampus, and, thus, may improve cognitive functions. However, GluR-agonists also may aggravate the development of excitotoxicity as well. Despite such a dualism in the neurologic effects of GluR ligands, a glutamatergic system is considered now as a very promising target for the new effective drugs both: cognition enhancers and neuroprotectors.⁹³ To minimise the risk of negative side effects and to optimise combination of cognition enhancing and neuroprotective properties the following mechanism-based approaches for development efficient therapeutics for AD treatment in series of GluR ligands have been proposed:

1. Compounds demonstrating properties of both antagonists of NMDA receptors and agonists of AMPA receptors. There are promising data on the treatment of AD with an agent *Akanitol Memantine* (Merz). *Memantine* is a low-affinity non-competitive antagonist of NMDA receptors. It is also an agonist of the AMPA receptors. Recently published results on clinical trials show a

positive response to a therapy with *Memantine* in 73% of patients with a moderate to severe Alzheimer type dementia.⁹⁴ No neurotoxic effects typical for nonspecific antagonists of the NMDA receptor or side effects observed for cholinomimetic drugs were reported for this drug. *Memantine* is approved for the clinical application in a number of European countries including Russia. However, some of clinicians are very cautious about using *Memantine* on patients, because it targets the phencyclidine receptoric site. This site is associated with strong psychomimetic side effects of a model compound *Dizocilpine (MK-801)*.⁹⁵

2. Compounds that indirectly stimulate the activation of other than NMDA receptors,⁹⁶ for example, AMPA receptors.⁹⁷ A group of so-called “ampakines” represents an important group of the compounds in this category.⁹⁸ Ampakines act at the specific site of the AMPA receptor. They decrease the desensitisation of the receptor by the positive modulation of the AMPA receptor response to agonist application. It was shown that an agent *CX516* significantly improved so-called “delayed recall,” one of the parameters of cognitive functions, in trials on elderly patients.⁹⁹ Derivatives of this agent, including the agent *Ampalex* produced by Cortex, Inc., displayed even higher cognition-stimulating properties in laboratory animals compared to *CX516*.⁹⁸ Some derivatives of thiourea, which are highly effective as cognition enhancing agents in animal models of AD, probably act via a similar mechanism.¹⁰⁰
3. Compounds that act at the glycine site of the NMDA receptor. The agent *D-Cycloserine* (Searle), a partial agonist of a glycine B site, showed a strong cognition-enhancing effect in animal models of dementia.¹⁰¹ It is under investigation as a potential medicine for the therapy of AD.

In addition to the strategic approaches described above, there are several agents that target glutamate system and show promising results in pre-clinical studies. Information on these agents is summarised below.

- The agent *Sabeluzole (R58735 or Reminyl)* produced by Janssen Pharmaceuticals) improved learning skills and memory function in laboratory animals and in healthy volunteers. The neuroprotective effect of this antagonist of GluR is associated with its recently revealed ability to block the glutamate-induced calcium influx and to stabilise the cytoskeleton of nerve cells.¹⁰² However, clinical trials with *Sabeluzole* have been suspended recently.
- A therapeutic drug *Dimebon*, which was in the clinical practice as an anti-histamine agent in Russia, demonstrated a pronounced anti-NMDA (ED₅₀ = 42 mg/kg i.p.) effect and anti-calcium (IC₅₀ = 57 μM) effect. *Dimebon* is also a reversible inhibitor of AChE and BuChE (IC₅₀ values are 42 and 7.9 μM, respectively). A pronounced cognition-stimulating effect of the drug was also observed in a neurotoxicological model of AD.¹⁰³ A small scale clinical trial with *Dimebon* conducted on Alzheimer's patients supported results of pre-clinical studies. *Dimebon* is currently in the development as a new therapy for the treatment of AD.¹⁰⁴
- The natural glycoside *Gastrodin* was extracted from a plant *Gastrodia elate* that was used in Chinese medicine. This agent blocked the glutamate-induced calcium uptake in SY5Y culture, and exhibited memory-enhancing properties in animal model of dementia.¹⁰⁵ *Gastrodin* is recommended to be tested as a neuroprotector on patients who suffer from AD and vascular dementia.
- *Taurine* protected against glutamate toxicity by blocking the glutamate-induced calcium uptake.¹⁰⁶ Interestingly, that β-alanine, a close analogue of *Taurine*, has the opposite effect. It increases a vulnerability of neurons towards the neurotoxic effects of EAA and APβ, and, thus, may be considered as an antagonist of the protective effect of *Taurine*.

Structures of selected compounds mentioned above are presented in Figure 6.

Ligands of metabotropic glutamate receptors (mGluR) are the focus of interest of a new approach in the search of therapies for the treatment of neurodegenerative disorders, including AD.

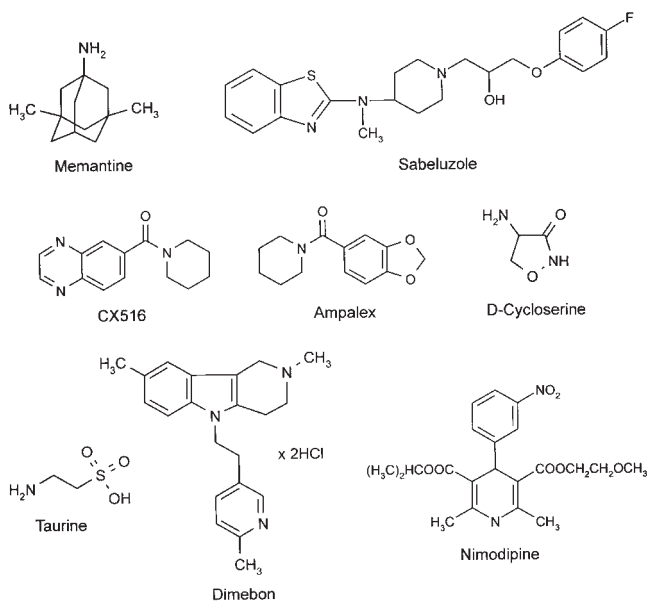


Figure 6. Structures of some glutamate receptors ligands and calcium channel blockers proposed for AD treatment.

In contrast to the NMDA and AMPA/Kainate receptors, mGluR are not directly associated with ion channels. These receptors are involved in the regulation of the intracellular calcium via G-proteins and a system of secondary messengers. Three groups of metabotropic glutamate receptors are recognised. Group I (mGluR subtypes 1 and 5) stimulate hydrolysis of phosphoinositide (PI) that ultimately leads to the release of Ca^{2+} ions from intracellular pools. Group II (mGluR subtypes 2 and 3) and group III (mGluR subtypes 4 and 6–8) are involved in the inhibition of adenylatecyclase that results in a decrease in cyclic AMP content. It is believed that the activation of the group I mGluR results in an increase of the neuronal excitation, while the activation of the group II and group III tends to decrease neuronal excitation.¹⁰⁷ All three groups are highly heterogeneous and vary significantly by their localisation in presynaptic and postsynaptic membranes in different regions of the brain. This may explain why ligands of mGluRs display both neuroprotective and neurotoxic properties. Nevertheless, certain preliminary conclusions on general properties of mGluR ligands may be made based on the massive experimental data published during the last years.

1. Agonists of mGluR group I potentiate excitotoxicity. However, they also stimulate processing of APP that results in the formation of the secreted form sAPP α . Antagonists of the group I of mGluR are usually neuroprotectors.
2. Agonists of the group II of mGluR usually display neuroprotective properties. However, this depends significantly on the synaptic localisation of their target receptor.
3. Agonists of the group III of mGluR display pronounced neuroprotective properties.

In conclusion of this section, according to the original hypothesis by J. W. Olney, who is the author of the theory of excitotoxicity, AD is a result of the hypofunction of NMDA receptors. Therefore, a new strategy in the search for effective therapies of AD should be focused on compounds that block the development of the hypofunction of these receptors.¹⁰⁸

2. Agents That Block Calcium Toxicity of AP β

The calcium hypothesis of ageing and dementia shares many common features with the glutamate theory of AD. A key element in both theories is the cytotoxic effect of the “hypernormal”

concentration of calcium that is caused by a disturbance in the metabolism of calcium ions in the nerve cell.^{109,110} Recently, it was shown that a mutation in the gene encoding the protein PS-1 is the main genetic factor that determines changes in a regulation of the calcium homeostasis by endoplasmic reticulum in response to various stimuli.^{111,112} In ageing, a role of the calcium-induced cell death increases because of a significant loss in the content of calcium-binding proteins, for example, calbindin-D_{28K}, in basal cholinergic neurons and also because of the reduction of calcium-buffer properties of mitochondria. These aspects will be discussed in the section “Approaches Associated With Prevention of Apoptosis and Normalisation of Mitochondrial Functions (Mitochondrial Protectors).”

A proposed mechanism of the neurotoxicity of AP β , that directly involves a function of calcium channels associated with the glutamate receptors, has been already discussed above. The following section of present review will be focused on the mechanism of the effect of AP β on voltage-sensitive calcium channels (VSCC). Although the calcium-mediated neurotoxicity has been studied for a long time, details of a molecular mechanism remain unknown. Two phases in the development of the AP β toxicity are recognised. First, an early Ca²⁺-independent phase, which may be developed within several hours and, second, the late Ca²⁺-dependent phase, which lasts for several days.¹¹³ A neurotoxicity induced by the AP β (25–35) fragment is effectively prevented by blockers of L-type calcium channels, but not by the blockers of N-type or P/Q-type channels. This prevention is associated with the level of ROS in cells. It was shown that radical scavengers prevented both the accumulation of ROS and the potential-dependent Ca²⁺ uptake. Specific blockers of VSCC, however, have an effect only on the Ca²⁺ uptake. They did not change the level of ROS in neural cells.¹¹⁴ It is possible that the early Ca²⁺-independent phase of the toxicity of AP β that is monitored by a decrease in the redox activity of intracellular systems is associated with the initiation of LP and accumulation of ROS. This causes disturbances in the function of membrane, such as a depolarization of VSCC and a calcium influx into the cell. The latter event is calcium dependent and is considered as the late phase in the toxicity of AP β . Variations in the calcium permeability of the glutamate receptor-channel complexes may accompany these events. An alternative hypothesis to the theory of the effect of AP β on the “endogenous” calcium channels, is a hypothesis about the ability of AP β to form new (“exogenous”) calcium-permeable channels and, thus, to cause a calcium-induced cell death. The only evidence in favour of this mechanism was so far collected from *in vitro* experiments on the artificial bilayered lipid membranes.^{115,116} However, a theoretical simulation of the structure of these AP β -induced calcium channels supports the possibility of their formation in real membranes.¹¹⁷

Thus, there is a firm evidence that various approaches that aim to stabilise the calcium homeostasis in the neuron have a positive effect on both age-dependent neuronal cell death and on the specific neurodegenerative effect of AP β . Therefore, the anti-calcium strategy to prevent a neurotoxicity of AP β is justified as a promising approach in the search of neuroprotectors for the therapy of AD.

The following group of biologically active compounds may be considered as promising agents for preventing the AP β -induced calcium toxicity:

- Blockers of L-type calcium channels, for example, agent *Nimodipine*, which has neuroprotective and cognition-stimulating properties as demonstrated in pre-clinical studies and in a small scale clinical trial.¹¹⁸
- Antagonists of ryanodine receptors that are responsible for the release of calcium ions from intracellular pools and, thus, are critical for the regulation of the level of intraneuronal calcium.¹¹⁹

3. Anti-Oxidant Strategy in the Treatment and Prevention of AD

Close correlation between AD and the accumulation of ROS in CNS cells has been known for a long time. It forms a backbone of the oxidative stress theory of AD, and provides a basis for anti-

oxidant strategy in the therapy of AD.¹²⁰ The role of ROS in AD is thoroughly analysed in numerous publications (e.g., for recent review see Behl¹²¹). In the present article, only key aspects of how ROS contribute in the development of the Alzheimer's neuropathology will be analysed to highlight the role of anti-oxidant agents in the therapy of the disease. Numerous studies illustrate that ROS are generated as a result of either a direct effect of AP β on cells in CNS or an activation of microglial cells in regions where amyloid plaques and NFT are formed. A recently published hypothesis suggests that in AD and some other ND, ROS are directly produced from aggregated proteins, in particular, AP β fibrils in case of AD.¹²² A spectrum of the produced ROS varies from oxygen radical metabolites, such as peroxide and nitroxyl radicals, to a variety of products of the lipid peroxidation (LP). Intracellular calcium has a significant effect on the production of ROS. It activates a number of metabolic reactions in mitochondria that result in the formation of O₂⁻ radicals. When present as a complex with calmodulin, calcium also activates NO-synthase, which generates NO[•] radicals. A product of these radicals, a peroxynitrite ion NO₃⁻, may initiate LP and a degradation of intracellular proteins and nucleic acids. It is known also that iron ions are important in the formation of hydroxyl radicals.

Nerve cells have endogenous system that protects them from excessive ROS levels. This system essentially determines the effect of the oxidative stress on the cell and on the entire organism. The key elements of the system are the enzymes, such as glutathione peroxidase (GTP) and catalase, which detoxify ROS. The function of these enzymes is highly regulated by a nuclear transcription factor NF- κ B.

A schematic illustration of the correlation between generation of ROS and a homeostasis of intracellular calcium influenced by AP β is presented in Figure 7.

The analysis of the potential effect of neuroprotectors against the oxidative stress in AD leads to alternative approaches, namely, application of "external" antioxidants which block ROS effect, and/or stimulation of the intracellular ("endogenous") anti-oxidant systems. General approaches

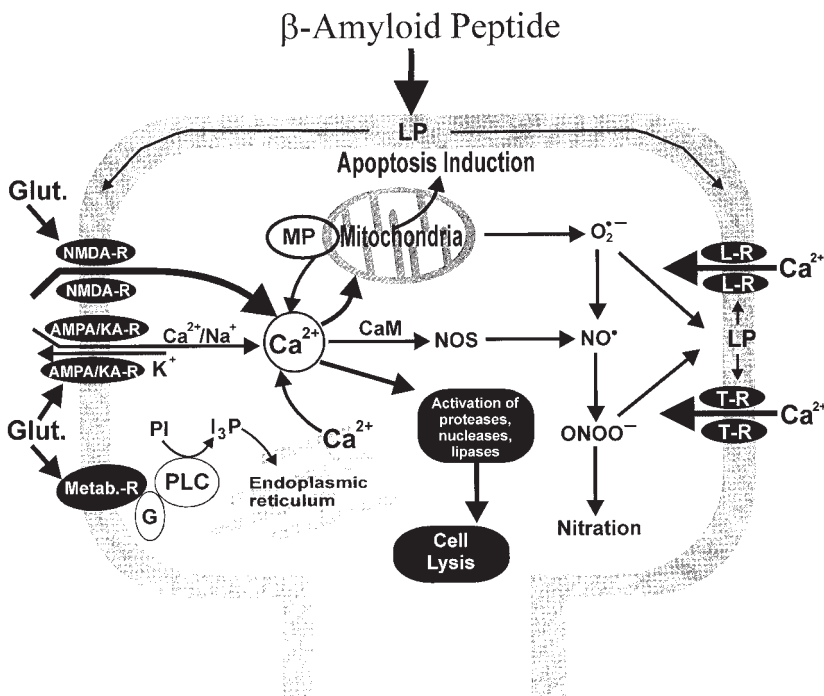


Figure 7. Schematic representation of AP β influence on intracellular calcium homeostasis, ROS formation and cell degenerative processes.

and certain specific drugs reviewed in this article are suggested for prevention and treatment of AD, in particular, for protection against the oxidative stress stimulated by the pathogenic peptide A β .

D. Approaches Based on Application of the “External” Anti-Oxidants

There are some general structural requirements for a compound to be an effective anti-oxidant. These are a presence of a functional group that may accept a radical or a free electron, and a presence of a highly conjugated molecular fragment, which is capable of delocalising a molecular charge with the formation of a stable chemically low active product. An additional requirement is the ability of an anti-oxidant molecule to penetrate the BBB, since AD is a pathology of the brain.

An analysis of the correlation between the structure of the compound and its anti-oxidant activity helps to reveal several basic groups of compounds that satisfy these requirements. One of these groups is a group of aromatic phenols with lipophilic substitutes.¹²³ *Vitamin E*, *steroid hormones* and their synthetic analogues represent this group.¹²⁴ The hydroxyl group of these compounds reacts with free radicals that results in the formation of sufficiently stable metabolites. The hydroxyl group is subsequently regenerated in the reaction with ascorbic acid (vitamin C).

Schematic presentation of these processes is illustrated in Figure 8

Another group is represented by hydrophobic derivatives of indol, such as endogenous hormone *Melatonin* and its derivatives that also display strong anti-oxidants properties.¹²⁵

VITAMIN E AND ITS FUNCTIONAL ANALOGUES

It is generally agreed that cytoprotective properties of α -*tocopherol*, a predominant form of the *Vitamin E*, are realized via anti-oxidant mechanism. Neuroprotective properties of the *Vitamin E* were studied in various experimental models of AD.¹²⁶ There is some evidence that *Vitamin E* has anti-apoptotic properties in addition to its “pure” anti-oxidant effect that increases its neuroprotective and gerantoprotective potential. Unlike its synthetic analogue *Idebenone*, *Vitamin E* has been successfully tested in clinical trials on AD patients. It was revealed that *Vitamin E* might slow down the development of AD in patients with a moderate AD.¹²⁷ Among extremely broad spectrum of anti-oxidants described in literature, the following natural and synthetic compounds should be mentioned as promising agents for their clinical application in the therapy of some types of dementia:

- Extract of *ginkgo biloba* (*Egb 761*): The neuroprotective effect of this medicine is associated mainly with its anti-oxidant activity. It is proposed that *Egb 761* neutralises NO \cdot radicals and inhibits NO-induced activity of protein kinase C.¹²⁹ It was also shown that *Egb 761* displays moderate cognition-enhancing and neuro-reparative properties in cell and animal models of neurodegeneration.¹²⁸

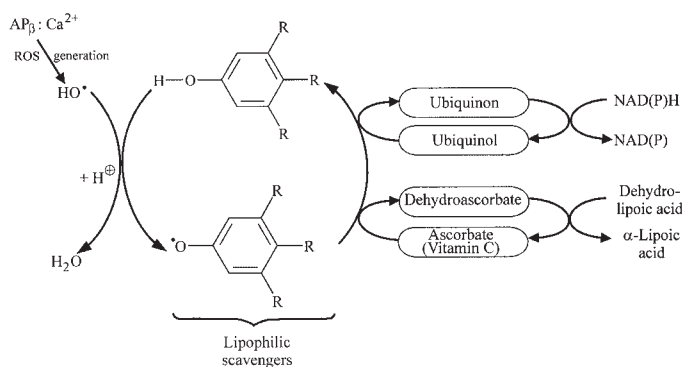


Figure 8. Radical scavenger action and regeneration of lipophilic aromatic phenols (vitamin E, steroid hormones, etc.).

- Herbal tripterine *Celastrol*: Anti-oxidant properties of this agent exceed those of α -tocopherol by the factor of 15. *Celastrol* also displays an anti-inflammatory activity. This drug significantly stimulates learning, memory, and a psychomotor activity of experimental animals with a model dementia. *Celastrol* was recommended for clinical trials on AD patients.¹³⁰
- *Raxofelast (IRFI0016)* and *MDL-74180DA*: These synthetic analogues of the *Vitamin E* are patented as effective anti-oxidants are now in preclinical studies.¹³¹

STEROID HORMONES (SH) AND THEIR ANALOGUES

Earlier it was revealed that hormonal dysfunction progresses along with AD and ageing. In particular, it was found that a decrease in the level of estrogens correlates with an increased risk of the development of AD.¹³² Nevertheless, the study of exact mechanisms of the effect of SH on the stability of nerve cells against the degeneration caused by different AD pathogenetic factors, including AP β , remains a key priority in the field.

It is known that a regulative effect of SH on nervous cells may involve several alternative mechanisms. According to the first (classic) mechanism, SH interact with specific hormonal receptors. This results in the activation of receptors. Then activated receptors are translocated into the nucleus, where they act as transcription factors in the regulation of the gene expression.¹³³ Recently, it was found that some of SH may be *de novo* synthesised in nerve cells and, then, modulate a neurotransmission via a direct interaction with the neurotransmitter receptors. This type of SH, for example, 17 β -estradiol, were named “neuroactive steroids”.¹³⁴ As mentioned above, an alternative mechanism of the protective effect of SH on nerve cells is associated with pronounced anti-oxidant properties of these compounds. Thus, the diversity of possible mechanisms of the effect of SH on nervous cells the homeostasis of nervous cells should be taken into an account in the analysis of the neuroprotective properties of these compounds in the development of AD type pathology.

Estradiol and its derivatives were historically the first and the most actively studied neuro-protectors of the SH.¹³⁵ It was shown in a number of publications that 17 β -*estradiol* (17 β E), its isomer 17 α -*estradiol* (17 α E), and series of their derivatives may effectively block the intracellular accumulation of ROS and, thus, may prevent neurons against the toxic effect of factors that initiate the oxidative stress. In experiments on a nervous cell culture it was shown that in addition to its anti-oxidant effect, 17 β E was effective in reducing the formation of AP β 40 and AP β 42 from APP and in blocking the neurotoxicity of AP β (25-35).¹³⁶

The most promising derivatives and analogues of estrogens are listed below:

1. Transdermal form of 17 β E (*Estraderm*) developed by Novartis. This compound significantly improves cognitive functions in women at menopause.
2. Synthetic analogues of 17 α E, such as agents *J811* and *J861* developed by JenaPharm. These drugs display a strong anti-oxidant activity in experiments *in vitro* (in this connection they were named “scavestrogens”),¹³⁷ and an ability to stimulate cognitive functions on a neurotoxicological animal model of AD.¹³⁸ Neuroprotective properties of these compounds are probably not associated with their effect on transcription processes.
3. A number of newly synthesised conjugated estrogens that display stronger anti-oxidant properties than 17 β E.¹³⁹

Although recent studies demonstrated a low efficacy of the estrogen replacement therapy of mild to moderate AD,¹⁴⁰ estrogen is currently on Phase III clinical trials as a potential neuroprotector that may delay the onset or the risk of AD in postmenopausal women.

In recent years, androgens, another group of SH, are becoming a centre of attention as potential anti-oxidants and neuroprotective agents. It was shown on neuronal cultures that *Testosterone* promoted a formation of the secreted form of sAPP α and decreases the production of the pathogenic form of AP β .¹⁴¹ Additional input to neuroprotective effects of androgens could be related to their

anti-apoptotic activity mediated by the androgen receptor¹⁴² as well as to prevent (in contrast to estrogen) the phosphorylation of the microtubule-associated protein.¹⁴³

Structures of some anti-oxidants mentioned above are presented in Figure 9.

MELATONIN AND ITS ANALOGUES

One of the promising approaches in the development of new preventive therapies of AD is a design of agents based on analogues and derivatives of melatonin. *Melatonin* (or *N*-acetyl-5-methoxytryptamine) is an endogenous hormone, which is produced from *N*-acetyl-serotonin (Fig. 10) mainly in the pineal gland. The secreted hormone could easily penetrate BBB and interact with different targets in the brain. The main function of *Melatonin* is a regulation of the body's sleep-awake cycle (circadian rhythms). During the past few years numerous studies have demonstrated that *Melatonin* displays a pronounced protective effect against the oxidative stress of various aetiologies in CNS.¹⁴⁴ Because of its chemical structure, *Melatonin* may interact with oxygen and hydroxyl radicals to form a non-toxic *N*¹-acetyl-*N*⁵-phormyl-5-metoxo-kynuramine, which is easily metabolised. *Melatonin* also interacts with peroxynitrite ROS with the formation of hydroxylated low-toxicity products (predominantly 6-hydroxymelatonin).¹⁴⁵ It is shown in experiments on cell cultures and on animals, that *Melatonin* has a complex neuroprotective effect that includes both a specific anti-amyloid component and a non-specific gerantoprotective effect because of its strong radical-blocking properties. The properties of *Melatonin*, in addition to its direct anti-oxidant effect with respect to ROS, include a regulation of processing of APP, inhibition of the formation of amyloid fibrils by blocking the creation of β -folded aggregates, protection of nerve cells against the EAA excitotoxicity and a toxicity of AP β , and

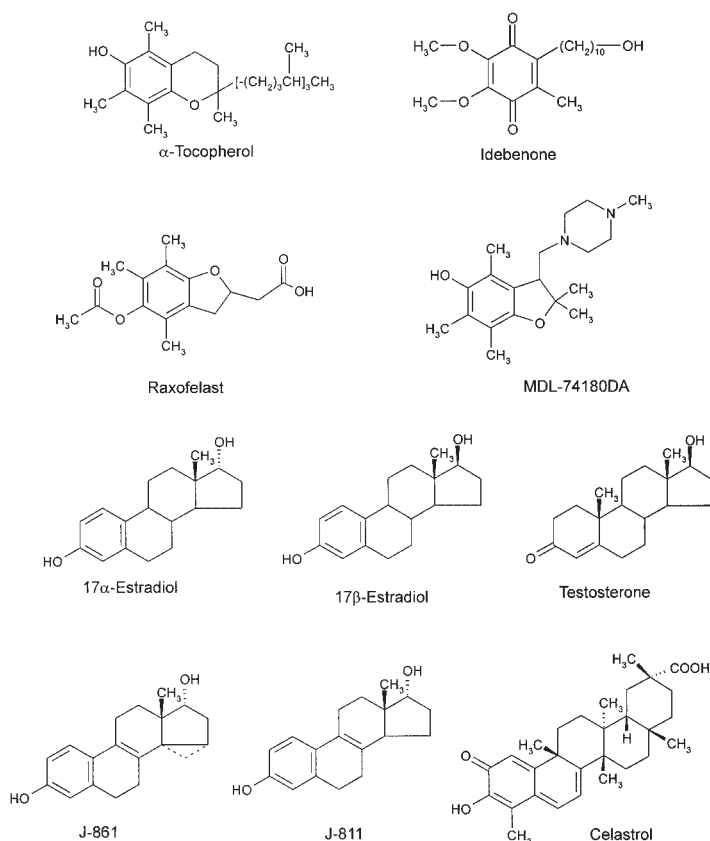


Figure 9. Structures of some anti-oxidants proposed for AD treatment.

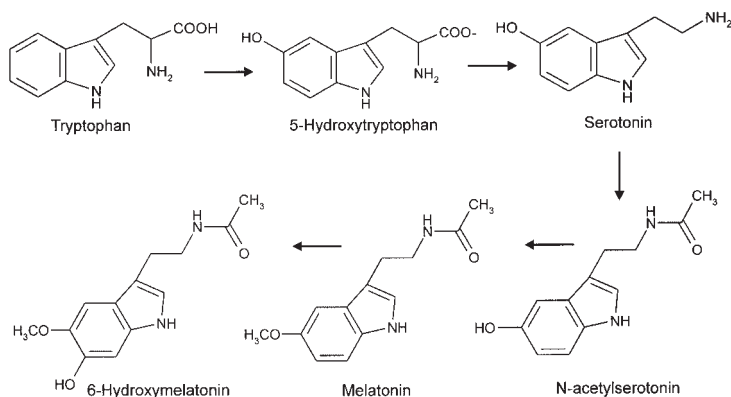


Figure 10. Melatonin biosynthesis pathway.

anti-apoptotic effect.¹⁴⁶ These protective functions together with the fact that the level of *Melatonin* is decreasing with ageing and in AD, suggest that this endogenous bioregulator has an important “preventive” function against the pathogenesis of AD. It is also important that *Melatonin* easily penetrates through BBB and practically lacks of side effects in single or systematic applications. *Melatonin* is registered in the USA and most of the European countries as a food additive. Now the Alzheimer’s Disease Co-operative Study (ADCS) funded and led by the National Institute on Ageing (NIA) is seeking people with diagnosed AD to participate in a clinical trial to study the efficacy of melatonin as a treatment of sleep disturbances in AD.

It should be emphasised, however, that a mild neuroprotective effect of *Melatonin*, which is a very attractive feature for the preventive therapy of age-related dementia can make problems for its efficient use in the therapy of moderate and severe AD. Therefore, a search for potential agents among analogues of *Melatonin* that would have more pronounced neuroprotective and cognition-stimulating properties remains a very actual problem.

Melatonin precursors, such as *N-acetylserotonin* (*NAS*) and its analogues, are a focus of a particular interest. It was found that *NAS* effectively inhibited LP and demonstrated more pronounced radical-scavenger properties^{147,148} and a higher anti-amyloid activity¹⁴⁹ than *Melatonin*. While being a more effective agent, *NAS* has all positive pharmacokinetic and toxicological characteristics of *Melatonin*.

There are a lot of data in literature on synthetic structural analogues of *Melatonin* and *NAS*. Indol-3-propionic acid,¹⁵⁰ that have a high therapeutic potential, and an agent *Dimebon*, a structurally rigid analogue of *Melatonin*, described in preceding section are only a few examples of this group of compounds.

E. Approaches Based on the Activation of the Endogenous Factors That Protect Against the Oxidative Stress

An important role in the regulation of endogenous mechanisms of nervous cells protection against the oxidative stress is attributed to the nuclear transcription factor NF- κ B. Initially, this transcription factor was characterised as a stimulator of the expression of gene encoding the κ -component of immunoglobulins in B-type lymphocytes. It was shown recently that cells respond to the ROS-induced oxidative stress by increasing the formation of NF- κ B. However, the function (neuroprotective or neurotoxic) of this factor and molecular mechanisms of its effect on the intracellular anti-oxidant system are still debated.¹⁵¹ The level of NF- κ B was found to be higher in the clone of PC12 cells, which are stable to the neurotoxicity induced by AP β , compared to “normal” PC12 cells that are susceptible to the degenerative effect of AP β . A suppression of NF- κ B activity by certain compounds, such as glucorticoids, or a stimulation of the expression factor I κ B α , that inhibits

NF- κ B, results in a decreased cell stability against AP β . The stability of nerve cells against AP β is associated with a high intracellular level of NF- κ B. A nuclear translocation of NF- κ B results in the induction of genes responsible for the synthesis of proteins protecting cells against the neurotoxic effect of AP β .¹⁵² Enzymes catalase and glutathione peroxidase are potential candidates to be these proteins, since their content is elevated in cells resistant to AP β . Recently it was shown that NF- κ B plays a crucial role in the NMDA-receptor mediated neuroprotection.¹⁵³ Thus, the stimulation of NF- κ B may result in an increase of the nerve cell viability, at least with the respect to the amyloid peptide AP β , and, thus, may be considered as an alternative anti-oxidant strategy.

However, it should be emphasised again that in other models of the oxidative stress stimulation of the formation of NF- κ B by ROS and a correlation between the level of this factor and the ROS content are considered as the indicators of the NF- κ B destructive potential.

F. Approaches Associated With Prevention of Apoptosis and Normalisation of Mitochondrial Functions (Mitochondrial Protectors)

Apoptosis, or a programmed cell death, is one of the fundamental self-regulating mechanisms of the growth and development of an organism. The phenomenon is observed for cells of different organs and tissues and comprises a wide spectrum of intra- and intercellular reactions. Apoptosis has a significant effect on the development of various ND. A regulation of apoptosis in nerve cells is a promising but not yet attained approach to the pharmacology of neuropathologies.¹⁵⁴ Since apoptotic processes may be initiated and regulated by an enormous number of different endogenous and exogenous factors, it is rather difficult to influence these factors specifically in certain cell groups. It is known that, apoptosis may be initiated by certain neurotransmitters (EAA, dopamine), modulators of protein phosphorylation, neurotropic factors, external stimuli (such as ionising irradiation), by a decrease in the extracellular concentration of potassium ions or by an increase in intracellular calcium, etc. Triggers (e.g., Apaf-1), "amplifiers" (cytochrome *c*), activators (proteins of Bax, Bad, Diva, and DP5 groups) and inhibitors (Bcl-2 and Bcl-xL families of proteins) are recognised as endogenous regulators of apoptosis. The activation of a group of cysteine proteases, so-called the caspases that directly initiate the process of DNA fragmentation is believed to be one of the key steps in apoptosis.¹⁵⁵ The apoptotic processes can be subdivided into three phases. In the first (premitochondrial) phase, proapoptotic signals are formed and activated. The second (mitochondrial) phase is characterised by a release of effectors of apoptosis from mitochondria. In the third (postmitochondrial) phase, the effect of the activated caspases and nucleases is realised in the formation of the morphological pattern of apoptosis.

Comprehensive description of all existing mechanisms of the development and treatment of apoptosis is not a purpose of this review. Our goal is rather a brief analysis of up-to-date hypotheses and approaches on attenuating of apoptosis specifically related to AD.

The fact that apoptosis takes place in AD is believed to be well established. There are several alternative hypotheses on specific mechanisms of the development of apoptosis and on its role in the pathogenesis of AD, that are based on literature data.^{156,157}

Inherited forms of AD that are developed in early age are associated with specific chromosomal mutations. There were found mutations in chromosomes 1 and 14 encoding the formation of mutant forms of preseniline proteins PS2 and PS1, and mutations in chromosome 21 encoding the synthesis of APP. On the other hand, it was found in cell culture experiments that cells with higher levels of the PS2 and PS1 mutant forms are much more vulnerable to apoptosis than cells with normal forms of the presenilins. The same effect was observed for cells with a decreased content of PS1 that result from the inhibiting effect of the proapoptotic factors p53 and p21. It was demonstrated that a cleavage of the mutant forms of PS1 and PS2 by the caspase-3 proteases takes place in sites that are different from cleavage sites of "normal" presenilins.¹⁵⁸ These results suggested that, unlike normal presenilins, which exert trophic functions in cells, their mutant forms PS1 and PS2 undergo an alternative proteolysis that is affected by the caspases-3. This increases a vulnerability of nerve cells to apoptotic

processes. The hypothesis may initiate a development of the new therapeutic approach to alter apoptosis in AD by blocking the caspase-catalysed cleavage of presenilin mutant forms.

An alternative to this mechanism is a hypothesis on the main role of the mutant presenilins in the stimulation of the formation of pathogenic forms of AP β , such as AP β 40, AP β 42, and AP β 43.¹⁵⁹ The increase in the level of pathogenic forms of AP β results in the accumulation of calcium in a cell, and then, in a calcium-induced emission of cytochrome *c* from the mitochondria, which stimulates a transformation of caspases into their active forms. Thus, a specificity of apoptosis in AD depends exclusively on the specificity of the initiating factor namely, beta-amyloid peptide. Then apoptosis follows the “classic” scenario. According to this hypothesis, a potential therapeutic anti-AD effect may be expected for drugs that have an effect on the major steps of apoptosis.¹⁶⁰ The anti-apoptotic effect of estrogens is an example. Estrogens strongly stimulate the expression of Bcl-xL protein and inhibit an intracellular proteolysis by caspases that correlates well with an anti-amyloid effect of estrogens observed in a hippocampus cell culture.¹⁶¹

Recently it was found, that mitochondria and associated with mitochondria regulatory peptide factors play an important role in the development and regulation of apoptosis.^{162,163} Although molecular mechanisms of apoptosis and the role of specific mitochondrial systems in the apoptotic cascade are not fully understood, there are several key steps that are known to be important in the development of apoptosis. A regulation of apoptosis by agents that target these steps appear to be one of the promising approaches to design new neuroprotective drugs.

Mitochondrial permeability transition pores (megapores, MP), which provide exit out of mitochondria for both calcium ions and for compounds with the molecular weight of up to 1.5 kDa, are important features in the mitochondrial part of the apoptotic cascade. It is assumed that MP represent a multiprotein complex located at mitochondrial membrane contact sites and includes components of both outer and inner membranes.¹⁶⁴ The outer membrane part of MP is formed by voltage-dependent anion channel, so-called “porin,” anti-apoptotic proteins of Bcl-2 family and, probably, a peripheral benzodiazepine receptor. The inner part of MP contains an adenine nucleotide translocator (ANT) interacting with proapoptotic proteins of Bax family. Enzymes creatine kinase and hexokinase are also likely to participate in the regulation of the MP properties. In the “normal” (low-conductive) state at a physiological concentration of calcium in cytosol ($[Ca^{2+}]_c$), opening and closing of MP is reversible and is regulated by the membrane potential. This function supports a calcium homeostasis in a cell. According to the proposed mechanism of this process, the influx of Ca^{2+} into mitochondria is accompanied by the exit of protons that increases the pH value in a matrix resulting in opening of MP. This also results in a collapse of both a mitochondrial proton gradient and a membrane potential, Ca^{2+} re-uptake through MP and an acidification of the matrix. The latter event causes closing of MP. The function of the mitochondrial respiratory chain restores the proton gradient that allows calcium ions to re-enter into mitochondria, etc. Under these conditions mitochondria play the role of a reversible buffer for “excessive” calcium.

Recently D.G. Nicholls proposed a hypothesis of mitochondria-connected mechanism of excitotoxic effect of glutamate.¹⁶⁵ It seems that this mechanism might be also extended to explain a AP β -induced neurotoxicity. According to this hypothesis, the increased Ca^{2+} -influx into the cytoplasm (through NMDA-receptor channels, for example) leads to accumulation Ca^{2+} in mitochondria and initiates massive generation of ROS, which induce degradation of Ca^{2+} -ATPase. This process, in its turn, decreases the ability of the cell membrane to remove the calcium ions and leads to further increase of calcium concentration in mitochondria. At some critical mitochondrial Ca^{2+} loading the depolarization of mitochondrial membranes and irreversible opening of MP are observed. These changes are accompanied by exit of calcium ions and macromolecular compounds, in particular, caspases activators, cytochrome *c* and Apaf-1 factors, from the mitochondria. This process is considered to predetermine the death of the cell.¹⁶⁶

Analysis of mechanisms of MP functioning leads to a somewhat paradoxical conclusion. Inhibition of Ca^{2+} uptake by mitochondria and/or MP blocking may prevent cells against the

development of apoptosis (and necrosis) in the presence of such pathological factors, as $AP\beta$, excitotoxins, oxidants, etc. This suggestion was recently supported by Stout and co-authors.¹⁶⁷ These authors demonstrated that a Ca^{2+} uptake by mitochondria is required for the EAA neurotoxicity and may be successfully blocked by such protonophors and uncouplers of the oxidative phosphorylation, as, for example, phenylhydrazon derivative *FCCP* (Fig. 11).

Megapores may be also influenced by a regulation of one of their components, in particular, ANT. It was shown that a combined action of a proapoptotic peptide Bax and ANT stimulates an increase of the MP permeability and the cell death.¹⁶⁸ In this case, interaction of ANT with *Bongrekate* and *Cyclosporin A*, which support MP in its closed state, prevents Bax-induced apoptosis, while atractyloside, which interacts with ANT via a site that supports MP in its open state, potentiates Bax-induced apoptosis. There is also a potential to regulate apoptosis via a modulation of the interaction of mitochondria with anti-apoptotic protein Bcl-2. This was recently demonstrated with an agent *Dantrolen*, used in clinical practice as a muscle relaxant, which displayed an antiapoptotic protective effect (Fig. 11).¹⁶⁹

It should be noted that the threshold for calcium-induced opening of MP in brain mitochondria decreases with ageing.¹⁷⁰ This emphasises the importance of the search for effective MP blockers that may be a new type of neuroprotectors for the prevention and treatment of different age-related ND associated with the mitochondria-mediated neuronal death.

The earlier described agent *ALCAR* (acetyl-L-carnitine HCl, Phase III clinical trial) improves the efficiency of the mitochondrial/energy production and stabilises cell membranes in CNS cells.⁴⁹ The anti-AD therapeutic potential of *ALCAR* is associated with its effect on mitochondria.

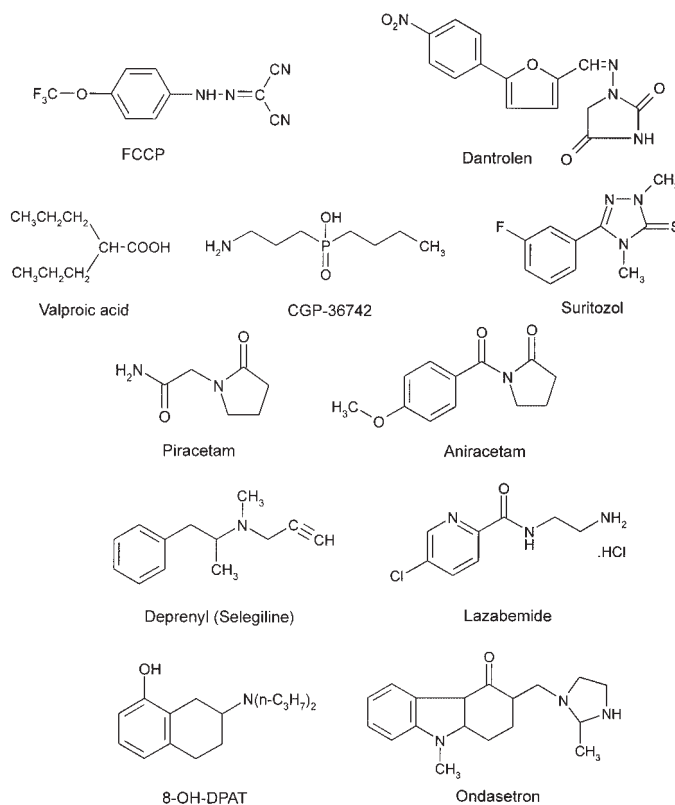


Figure 11. Structures of some compounds affecting mitochondrial functions and ligands of GABA and monoaminergic systems as potential anti-Alzheimer's agents.

4. COMPOUNDS THAT ACT ON GABA- AND MONOAMINERGIC NEUROTRANSMITTER SYSTEMS

As it was mentioned above, the AD progression leads to the degradation of cholinergic neurons and decrease in the activity of cholinergic and glutamatergic neurotransmitter systems. Agents that follow the mechanism of the ‘direct compensation’ of functions of cholinergic and glutamatergic systems have been described in the previous sections. In addition to those, there is a broad range of compounds described in literature that may compensate for a deficit of the depleted neurotransmitters and display pronounced neuroprotective and cognition-stimulating properties by activating other neurotransmitter systems, in particular, GABA and monoaminergic systems.

Therapeutic agents that follow this “alternative” mechanism are listed below.

A. Compounds Acting on GABAergic System

- *Valproate* (*Depakote*, *Depakene*, *valproic acid* or *sodium valproate*) is a GABA-enhancing agent, well-known as an efficient anticonvulsant. Its neuroprotective effect is associated with its ability to attenuate AP β neurotoxicity, Ca²⁺ deregulation and a cytoskeletal pathology.¹⁷¹ Currently *Valproate* is in Phase III clinical trial.¹⁷²
- *CGP-36742* (Ciba-Geiger) is an antagonist of GABA-B receptors. It displays pronounced cognition-stimulating properties in AD models. A possible mechanism of its cognition-enhancing activity may be associated with an indirect stimulation of NMDA receptors.¹⁷³
- *Suritazol* (*MD 26479*) produced by Hoechst-Marion Roussel is an inverse agonist of GABA-A receptors at the benzodiazepine site. This agent was studied in clinical trials on AD patients¹⁷⁴ and showed pronounced cognition-enhancing properties. However, according to Internet information presented on site www.alzforum.org the trials are temporarily suspended.

Because of the ability of *nootropic compounds*, such as *Aniracetam* and *Piracetam*, to enhance cognitive functions in animal models of dementia, these agents may also be suggested as potential drugs for the treatment of AD.¹⁷⁵ The mechanism of neuro-stimulating effects of these compounds is likely to be associated with their effect on the GABAergic system. However, their efficacy in the improvement of cognitive functions in AD patient is quite controversial.^{176,177}

B. Effectors of Monoaminergic Systems

- *Deprenyl* (*L-deprenyl*, *Selegiline*, *Eldepryl*): This propargylamine derivative is a selective irreversible inhibitor of monoamine oxidase B (MAO-B). *Deprenyl* is used in clinical practice as an anti-parkinson drug. During the past decade, controversial data were obtained about *Deprenyl* in AD treatment. It was found that *Deprenyl* improved memory and cognitive functions of experimental animals. In addition to the inhibition of MAO-B, *Deprenyl*, also protected cells against the toxic effect of AP β and blocked the NO formation (possibly by inhibition of NO synthase).¹⁷⁸ It was also shown that in double blind placebo controlled clinical trial, *Deprenyl* in combination with vitamin E slowed down the development of AD.¹⁷⁹
- *Lazabemide*, that combines properties of both a MAO-B inhibitor and an anti-oxidant, has been proposed as a potential agent for the prevention of neurodegenerative processes in AD-type dementia.¹⁸⁰

Ligands of Serotonin (5HT) Receptors

- An agent *8-OH-DPAT*, a selective agonist of the 5-HT_{1A} subtype of serotonin receptors, protects cells against an excitotoxic effect of NMDA¹⁸¹ and stimulates an increase of extracellular acetylcholine in the brain (in experimental animal models).¹⁸²

- *Ondansetron*, a selective antagonist of a 5-HT_{1A} subtype of serotonin receptors, demonstrated the ability to enhance both learning skills and memory in the animal model of scopolamin-induced amnesia. A cognition-stimulating effect of *Ondansetron* is probably associated with its capacity to potentiate the release of ACh in the hippocampus and cortex. However, according to recent results of double-blind clinical trials, a therapeutic effect of *Ondansetron* is insignificant.¹⁸³

Structures of selected compounds mentioned in this section are presented in Figure 11.

5. AGENTS THAT STIMULATE NEUROTROPHIC EFFECTS AND STABILISE CELL MEMBRANES

The following compounds that have a neuroprotective and cognition-stimulating activity act via complex mechanisms, predominantly, via a stimulation of neurotrophic functions in CNS (Fig. 12):

- *Propentofylline* (HWA 285) produced by Hoechst Marion Roussel. This compound has a broad spectrum of neuroprotective and cognition-stimulating effects. The main effect of *Propentofylline* is the inhibition of the adenosine re-uptake system. This results in the accumulation of adenosine in CNS and a consequent activation of adenosine receptors of A₁ type and A₂ type that block the EAA release. It was shown that *Propentofylline* stabilised an intracellular level of cAMP and cGMP by the inhibition of the corresponding phosphodiesterases. It also stimulated the formation of a nerve growth factor in the CNS and suppressed the activation of microglia in degenerative processes. The latter results in a decrease in the level of ROS and endogenous cytotoxic factors, such as cytokines.¹⁸⁴ Further development of propentofylline, however, was discontinued after Phase IIIb clinical trial.
- *Citicoline* (*Cytidine 5'-diphosphocholine*, *CDPcholine*), an endogenous intermediate in the biosynthesis of structural membrane phospholipids and brain acetylcholine, was extensively used for the treatment of neurodegenerative disorders associated with cerebrovascular pathology, head trauma, stroke, etc. During the past few years, it was shown that citicoline may improve memory via its neurotrophic effect. It may also regulate a blood circulation in brain vessels and stabilise mitochondria functions by the membrane stabilization.¹⁸⁵ Clinical trials demonstrated a positive influence of *Citicoline* on cognitive functions in elderly people.¹⁸⁶
- A herbal agent *Anapsos* is extracted from *Filices Polypodium Leucomotos*. *Anapsos* improves cognitive functions, a cerebral circulation of the blood and a brain bioelectric activity in patients with senile dementia. It is important that positive effects of *Anapsos* are well pronounced in patients with moderate form of AD-type dementia.¹⁸⁷

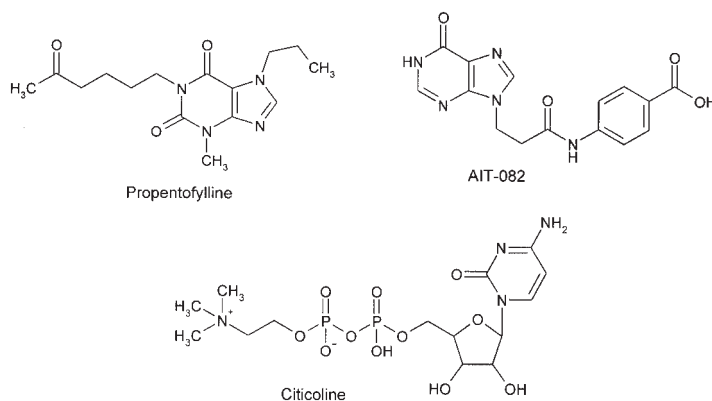


Figure 12. Structures of some compounds with neurotrophic activity proposed for AD treatment.

- *AIT-082 (NeoTrofin)* manufactured by NeoTherapeutics, Inc.: It acts at the site of heme oxygenase to generate carbon monoxide and by activation of guanylyl cyclase induces a cascade of biochemical reactions through the second messenger system leading to the production of mRNA for neurotrophins.¹⁸⁸ *AIT-082* is currently in Phase IIb/III clinical trials.
- *Cerebrolyzin*: This medicine contains a complex mixture of amino acids (approximately 85%) and peptides extracted from animal brains. In clinical practice it is used for the improvement of the cerebral circulation of blood and for the stimulation of the brain. *Cerebrolyzin* has a complex effect on CNS.¹⁸⁹ It displays nootropic, neuromodulating and neurotrophic properties. It may also regulate a metabolic process in nerve cells. Recent clinical trials performed with *Cerebrolyzin* on patients with AD and vascular dementia showed that a therapeutic effect of the drug depended both on the duration of the therapy and the severity of the disease. The most pronounced therapeutic effect for *Cerebrolyzin* was achieved in its long-term therapy. Experiments *in vitro* demonstrate that *Cerebrolyzin* inhibits calcium-dependent protease calpain II, which participates in the AD proteolysis of a microtubule-associated protein MAP2. *Cerebrolyzin* also displays anti-excitotoxic and anti-oxidant properties. Experimental data on *Cerebrolyzin* suggest that the drug has both cognition-stimulating and neuroprotective effects. *Cerebrolyzin* is currently in Phase II/III clinical trials in Europe and North America.^{190,191}

6. INHIBITORS OF NEUROFIBRILLARY FORMATIONS

In addition to amyloid plaques, NFT are the most important pathomorphological characteristics of the AD.¹⁹² NFT are composed of pairs of filaments formed by specific microtubule-associated tau proteins (MAP). Tau proteins in AD undergo post-translational modifications, such as hyperphosphorylation, glycosylation, etc. A disturbance in phosphorylation and dephosphorylation of tau proteins that are catalysed by phosphorylases, and phosphatases result in the hyperphosphorylation of MAP. Pathologically modified tau proteins induce a destruction of “normal” microtubules, that are responsible for the intracellular transport, and, ultimately, cause a retrograde degeneration of neurons. While the exact mechanism of the formation and effect of NFT in AD is unknown, there are numerous data that demonstrate that the hyperphosphorylation of MAP is the key element in this pathology.^{193,194} Therefore, development of compounds, decreasing and stabilising the normal level of the phosphorylated tau proteins, may be considered as a promising strategy in the search for anti-AD drugs.¹⁹⁵

There are two alternative approaches to slow down the NFT formation in AD: (i) by inhibiting the tau-phosphorylase activity and (ii) by stimulating the tau-phosphatase activity. The latter approach was indirectly supported when it was found that inhibitors of phosphatase-1, -2A and -2B induce a hyperphosphorylation of tau protein in the rat brain.¹⁹⁶

Despite obvious advantages of agents that target NFT, there is very limited information in literature on specific compounds, which may effectively and selectively influence the pathological formation of NFT. As was mentioned above, the drug *Cerebrolyzin* may inhibit calcium-dependent protease calpain II, that catalyses a pathological proteolysis of MAP2 in AD.¹⁹⁷ An inhibition of the enzyme glycogen synthase kinase-3, which catalyses a tau protein phosphorylation, by *lithium salts* is another example of the directed regulation of the NFT formation with potential prospective for the treatment of AD.¹⁹⁸

7. ANTI-INFLAMMATORY AGENTS IN THE THERAPY OF AD

It is known that neurodegenerative changes in the brain of AD patients are accompanied by inflammatory reactions of CNS. Inflammatory processes in AD brain include an acute phase caused

by the effect of cytokines IL-1 and IL-6, an activation of a complement cascade, which results in the production of anaphilotoxins, a formation of an active membrane complex, and an activation of microglia. Extremely potent biologically active mediators of inflammation are eicosanoids (prostaglandins and leukotrienes), generated from the arachidonic acid by following main groups of enzymes: cyclo-oxygenases (COXs), or prostaglandin endoperoxide H synthase and lipoxygenases (LOXs). A number of recent reviews provide a detailed analysis of these processes.^{199–201} It has not been revealed yet, whether neuroinflammatory processes in the brain may cause the AD pathology or whether they represent a non-specific response of an organism to pathologies (β -amyloidosis, NFT formation). However, there are several possible scenarios of the development of the inflammatory cascade in AD. Cytokines IL-1 and IL-6, mediators of neuroinflammatory processes, may be neurotoxic and may potentiate a neurodegenerative effect of β -amyloid. Activated microglia may secrete endogenous neurotoxic factors. A membrane-active complex formed in neurites in AD brain may destroy membranes of neural cells. The inflammatory complement cascade may initiate the formation of ROS and oxidative stress in CNS. In the frame of the present review there is no possibility to analyze all the spectrum of therapeutic approaches based on the inflammatory concepts of AD. Below we will briefly characterise only selected agents described in literature, whose therapeutic potential in AD is associated with their anti-inflammatory properties. Structures of these compounds are presented in Figure 13.

Non-steroidal anti-inflammatory drugs (NSAIDs) decrease the risk of developing AD as shown in epidemiological studies.²⁰² *Ibuprofen* (Advil, Motrin, Nuprin) is the first in a series of NSAIDs suggested for the therapy of AD.²⁰³ As well as for other NSAIDs, the anti-inflammatory effect of *Ibuprofen* is associated predominantly with the non-selective inhibition of COXs, the key enzymes in

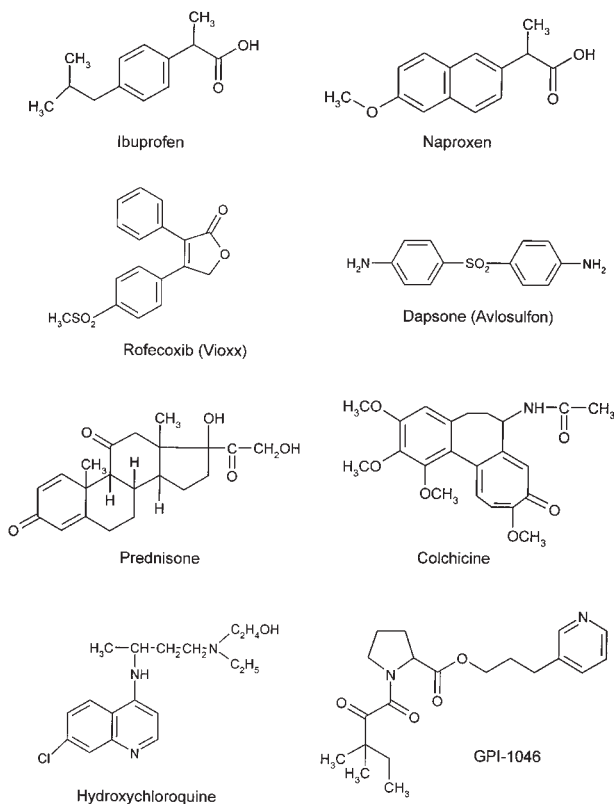


Figure 13. Structures of some anti-inflammatory agents, proposed for AD treatment.

the cascade of prostaglandins synthesis. *Ibuprofen* is currently in Phase III clinical trials. *Naproxen* (Aleve, Anaprox, Naprosyn) is another example of NSAIDs proposed to slow down the development of AD. In 1994, it was approved by FDA as an “over the counter” NSAIDs. A wide application of these medicines, however, may be limited because of the risk of side effects, such as ulcerogenic activity, that are observed for most of NSAIDs. This risk is higher with a long-term application of the medicines. From these standpoints the focused search for *selective inhibitors of cyclooxygenase-2* (COX-2) seems much more promising. This approach is based on the observations that inducible form of cyclooxygenase (the enzyme catalysing synthesis of key prostaglandin-PGH₂ in the prostaglandins synthesis cascade)—COX-2, located in neocortex and hippocampus, participates in the regulation of brain synaptic function and its expression is increased in AD.²⁰⁴ The different distribution patterns of COX-1 (constitutive form of the enzyme) and COX-2 in AD could implicate that these enzymes are involved in different cellular processes in the pathogenesis of AD. It gives ground to propose that selective inhibition COX-2 while sparing COX-1 represent a new attractive therapeutic approach for AD treatment.²⁰⁵ According to data presented at the Alzheimer Research Forum website (www.alzforum.org) agent *Rofecoxib* (*Vioxx*) (Merck & Co), selective inhibitor of COX-2 approved by FDA in 1999 for relief of the signs and symptoms of osteoarthritis, management of acute pain and treatment of primary dysmenorrhea is under investigation now in Phase II clinical trials in AD patients.²⁰⁶

It should be noted that according to recent findings NSAIDs could directly affect amyloid pathology in the brain by reducing AP β 42 peptide levels independently of COX activity and that this AP β 42-lowering activity could be optimised to selectively target the pathogenic AP β 42 species.²⁰⁷

Another approach based on the stabilisation of the level of eicosanoids in the AD brain by the inhibition of 5-lipoxygenase (5LOX), an enzyme, which expression is upregulated in ageing or AD, was proposed recently.²⁰⁸

Among other groups of anti-inflammatory agents the following compounds could be mentioned as promising ones:

- *Dapsone* (*Avlosulfon*) (Immune Network, Inc., Phase II)—An anti-infective agent used to treat leprosy. Earlier studies indicated a lower prevalence of Alzheimer Disease in elderly leprosy patients. This observation suggested that the anti-inflammatory effect of *Dapsone* may slow down the progression of AD. However, an *in vitro* study of the potential neuroprotective effect of anti-leprosy drugs, such as dapsone, rifampicin, clofazimine, minomycin and ofloxacin, against the AP β neurotoxicity did not reveal any distinctive effect.²⁰⁹
- *Prednisone*, a synthetic glucocorticoid, is a dehydrogenated derivative of the endogenous hormone of adrenal cortex cortisone. For a long time *Prednisone* was actively used as an anti-inflammatory drug. At present it is almost completely superseded by its more effective analogue *Prednisolone*, which structure contains a hydroxyl group at the carbon atom C₁₁ instead of ketogroup. Pilot clinical trials organised by NIA (USA) demonstrated a low efficacy of *Prednisone* in the treatment of AD patient.²¹⁰ In general, a neurotoxic side-effect of glucocorticoids on the hippocampus, which was observed in experiments on animals, may be a problem in the wide application of these compounds.
- *Colchicine*. Colchicine itself displays neurodegenerative properties. It disrupts the assembly and disassembly of microtubules. Nevertheless, because of its anti-amyloid activity and the ability to block microglial inflammation in AD-type pathologies this compound was proposed as a drug for the therapy of AD. Since primary clinical trials with colchicine on AD patients demonstrated only a moderate efficacy, its clinical application is currently being questioned.²¹¹
- *Hydroxychloroquine* is a well-known anti-malarial drug. The ability of this compound to decrease the AP β level in plasma suggested that it has a possible lysosomal activity providing a normal processing of APP. Clinical trials with this medicine (both alone and in combination

with colchicine) on a limited number of AD patients have been recently initiated in the Netherlands.²¹²

The possibility of using immunophilin-binding agents, such as natural immunosuppressive agent *Cyclosporin A*, agent *FK506* and their low molecular-weight analogues, for the prevention of the development of neurodegenerative disorders have been recently discussed in literature. It was shown, that a treatment of model animals with the compound *GPI-1046*, that structurally simulates a receptor-binding domain of *FK506*, stimulated a partial recovery of central cholinergic systems. This effect may hold a promise for ameliorating cholinergic deficit present in AD. It is believed that a possible therapeutic effect of this group of agents may be associated with the intracellular complex between immunosuppressants and immunophilins that is formed *in vivo*.²¹³

8. CONCLUSIONS

A brief analysis of literature on potential mechanisms of the development of the AD-type pathology illustrates that a complex character of the pathology suggests a broad spectrum of approaches

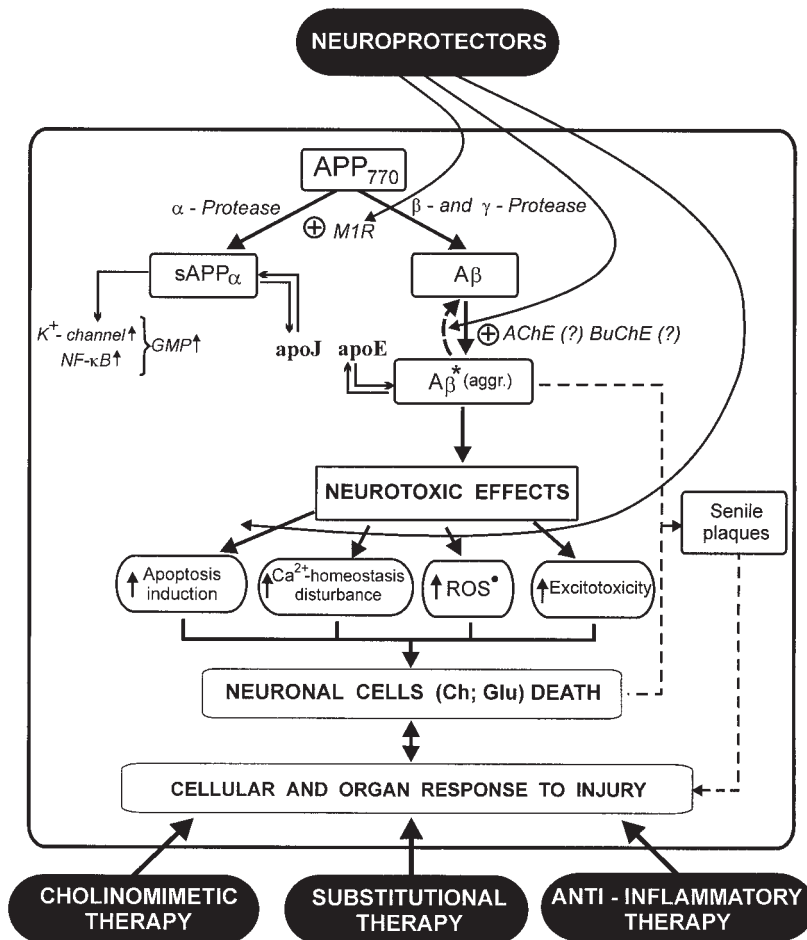


Figure 14. Main strategic lines for the correction of and protection from the $A\beta$ -induced neurodegeneration.

for its pharmacological correction. Indeed, a development of this neurodegenerative disorder is characterised by a participation of various intra- and extracellular biological systems, which may be pharmacologically affected by potential therapeutic drugs. In addition to the direct effect on the AD pathogenesis, the pathology may be corrected by the activation of compensatory mechanisms in CNS, which significantly extends a range of methods of the AD therapy. A scheme presented in Figure 14 illustrates main strategic approaches for the correction of the AD pathology. According to this formalised concept, potential drugs may be classified by their pharmacological effect into the following groups: protectors that block neuropathology progression; agents that compensate (directly or indirectly) for a deficit of functions of degenerated neuronal systems, and, medicines (immune vaccines and, probably, trophic factors), which may stimulate a functional rehabilitation of damaged nerve cells. In reality therapeutic drugs may have, of course, a simultaneous effect on several biological targets. From the other hand, biological targets of the drug also participate in the regulation of a whole complex of interrelated processes (for example, mChR participating both in transmission of neuronal signals and APP processing). A combination of these events results in the formation of specific neuropharmacological properties for each therapeutic agent.

Unfortunately, a broad prospective for the design of medicines for the prevention and treatment of ND, primarily AD, does not correspond to the progress in this field. This may be explained by the following reasons:

1. Insufficient data about intimate molecular and genetic mechanisms of the pathology, which significantly decreases the “yield” of countless synthetic and screening projects.
2. The absence of convenient and adequate models that have a high “predictive” ability for the selection of the most promising compounds.

In this respect, the application of modern computational approaches, based on the analysis of quantitative structure–activity relationships for the focused design of novel efficient neuroprotectors and cognition-enhancers for the therapy of AD appears to be especially promising.

ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
AD	Alzheimer’s disease
ABAP β	antibodies against AP β
AP β	beta-amyloid peptide
AP β 40, AP β 42, AP β 43, AP β (25–35)	different forms of beta-amyloid peptide, containing 40, 42, 43 amino acids, or its fragment, containing the sequence of amino acid residues from 25 th to 35 th residue
AMPA	α -amino-3-hydroxy-5methylisoxazole-4-propionic acid
ANT	adenine nucleotide translocator
APP	amyloid precursor protein
sAPP α	secreted, soluble form of amyloid peptide
ATP	adenosine triphosphate
BBB	blood–brain barrier
BuChE	butyrylcholinesterase
[Ca ⁽²⁺⁾] _c	concentrations of calcium in cytosol
Ca ⁽²⁺⁾ -ATPase	Ca ⁽²⁺⁾ -dependent APT synthase
ChE	cholinesterase
(A)ChEI	(acetyl)cholinesterase inhibitors

(m/n)ChR	(muscarinic/nicotinic) cholinergic receptors
cAMP	cyclo-adenosine monophosphate
cGMP	cyclo-guanosine monophosphate
COX	cyclooxygenase
EAA	excitatory amino acids
ED50	dose of compound, which produces 50% of its maximal physiological effect (or dose of compound which produce special physiological effect in 50% of experimental animals)
17 α E	17 α -estradiol
17 β E	17 β -estradiol
GABA	gamma-aminobutyric acid
(m)GluR	(metabolic) glutamate receptors
GTP	glutathione peroxidase
H ₃ -HR	H ₃ -subtype histamine receptors
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HT	Hydroxytryptamine (serotonin)
IC50	concentration of compound, which inhibit tested biological activity on 50%
IL-1, IL-6	interleukin I and 6 types
5LOX	5-lipoxygenase
LP	lipid peroxidation
MAO	monoamine oxidase
MAP	microtubule-associated tau proteins
MP	mitochondrial permeability transition pores or megapores
ND	neurodegenerative diseases
NFT	neurofibrillary tangles
NF- κ B	nuclear transcription factor κ B
NMDA (R)	<i>N</i> -methyl-D-aspartate (receptor)
PGH-synthase	prostaglandin endoperoxide H ₂ synthase
PS-1	presenilin-1
PS-2	presenilin-2
ROS	radical oxygen species
SH	steroid hormones
SP	senile plaques
VSCC	voltage-sensitive calcium channels

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