

Cholinergic Drugs in Pharmacotherapy of Alzheimer's Disease

P. Camps* and D. Muñoz-Torrero*

Laboratori de Química Farmacèutica, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, E-08028, Barcelona, Spain

Abstract: The cholinergic hypothesis of Alzheimer's disease has spurred the development of numerous structural classes of compounds with different pharmacological profiles aimed at increasing central cholinergic neurotransmission, thus providing a symptomatic treatment for this disease. Indeed, the only drugs currently approved for the treatment of Alzheimer's disease are cholinomimetics with the pharmacological profile of acetylcholinesterase inhibitors. Recent evidence of a potential disease modifying role of acetylcholinesterase inhibitors and M₁ muscarinic agonists have led to a revival of this approach, which might be considered as more than a symptomatic treatment.

1. INTRODUCTION

Alzheimer's disease (AD) is a slow progressive neurodegenerative disorder, clinically characterized by a noticeable cognitive decline defined by a loss of memory and learning ability, together with a reduced ability to perform basic activities of daily living [1], and a diverse array of neuropsychiatric symptoms such as apathy, verbal and physical agitation, irritability, anxiety, depression, delusions and hallucinations [1]. Taking into account the increase in life expectancy, the fact that the incidence of AD increases with advancing age, and the devastating effects of this illness, nowadays AD represents a major public health problem and will presumably be the most important pathology of the XXI century in the developed countries. Important efforts have been made in the last two decades in order to determine the etiopathogenesis of AD, and to carry out its early diagnosis and therapeutic control [2,3].

2. THERAPEUTIC STRATEGIES

In spite of the multifactorial nature of AD [4], most treatment strategies have been directed to two main targets: the β -amyloid peptide (A β) and the cholinergic neurotransmission. Therefore, there are two main approaches for the treatment of AD.

The first approach is to prevent the neurodegenerative changes that ultimately cause irreversible damage to the brain. A β is the main component of the extraneuronal senile plaques, one of the main neurohistopathological signs of AD. A β derives from the proteolytic processing of the β -amyloid precursor protein (APP), which can take place through two alternative pathways which are mutually exclusive [5]. The primary pathway results from the cleavage

of the protein by an α -secretase, resulting in the generation of a soluble APP fragment (APPs) that displays neurotrophic and neuroprotective properties. Alternatively, successive cleavages of APP by β - and γ -secretases result in the generation of the A β peptide. While trace amounts of A β have been detected as part of the normal cellular metabolism of APP, an increase in the production of the peptide and its subsequent deposition as insoluble amyloid plaques may represent the key pathological event that triggers the disease process [5]. Therefore, any manipulation that diminishes or prevents the generation or deposition of A β may be a potential therapeutic strategy, which could serve to slow down the rate of progression of the disease and prevent further neuronal cell losses. Although recently developed strategies based on immunization with A β [6], use of α - and γ -secretase inhibitors [7] or amyloid aggregation inhibitors [8] show a promise for the treatment and prevention of AD, these approaches are still in their infancy and no clinical experience is available at present.

The second main approach is to slow the decline of neuronal degeneration and to treat the symptoms of the disease by repletion of several deficient neurotransmitters. Among different neurotransmitter deficits occurring in AD, reproducible cholinergic deficits are consistently reported [9-10], which appear early in the disease process, and correlate well with the degree of dementia [11]. The deficit of the neurotransmitter acetylcholine (ACh) is secondary to a selective degeneration of cholinergic neurons that originate in the basal forebrain and project to the cortex and hippocampus. As a consequence, losses in all known presynaptic cholinergic markers, such as choline acetyltransferase, the rate limiting enzyme for ACh synthesis, ACh levels, acetylcholinesterase (AChE), the enzyme responsible for the degradation of ACh, as well as presynaptic M₂ muscarinic and nicotinic receptors have been repeatedly found in the cerebral cortex and hippocampus [12], usually in post-mortem brain examination, while postsynaptic M₁ muscarinic receptors are relatively preserved in AD [13]. This selective cholinergic neurodegeneration forms the basis for the so-called cholinergic hypothesis of AD [14-16], that postulates that many of the cognitive, functional and behavioral symptoms experienced by patients

*Address correspondence to this author at the Laboratori de Química Farmacèutica, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, E-08028, Barcelona, Spain. Phone: int. code + 34 + 93-402-4536 / int. code + 34 + 93-402-4542; FAX: int. code + 34 + 93-403-5941; E.mail: camps@farmacia.far.ub.es / dmunoz@farmacia.far.ub.es

with AD result from a deficiency in neurotransmitter ACh, and thus in cholinergic neurotransmission. Pharmacological and lesion studies in animals support the involvement of central cholinergic systems in learning and memory. Anticholinergic substances such as the muscarinic antagonist scopolamine are known to induce a marked deterioration in short-term memory, similar to that observed in the first stage of AD, which can be reversed by administration of cholinomimetics such as the centrally active AChE inhibitor physostigmine. The cholinergic hypothesis of AD has provided the rationale for the current major therapeutic approach to AD: enhancement or restoration of central cholinergic function may significantly improve the cognitive impairments present in AD. Currently, the only FDA-approved AD therapies are a group of indirect cholinomimetics which enhance cholinergic function by inhibiting ACh degradation.

Different treatment strategies aimed at enhancing cholinergic neurotransmission have been attempted for the symptomatic treatment of AD. Cholinergic drugs can act presynaptically or synaptically, essentially by increasing the release or the bioavailability of ACh at the synaptic cleft, or postsynaptically through direct stimulation of muscarinic receptors. In this paper, we offer an overview of the structure and pharmacological profile of the different classes of drugs which have been developed for restoring central cholinergic tone, highlighting the new strategies or novel compounds which are under preclinical or clinical development.

3. PRESYNAPTIC CHOLINERGIC DRUGS

One of the first attempts to treat AD was directed to increase the synthesis of ACh by supplying ACh precursors such as lecithin and choline with generally disappointing results [9]. Consequently, this approach has, for the most part, been abandoned. Alternatively, cholinergic

neurotransmission can be enhanced by increasing the release of the remaining presynaptic neurotransmitter in the brain or by increasing the uptake of choline into the presynaptic neuron in order to increase ACh synthesis.

3.1. Acetylcholine Release Enhancers

Several distinct pharmacologic mechanisms result in the enhancement of ACh release in the central nervous system (CNS).

Potassium Channel Blockers [Fig. (1)]

On the one hand, ACh release is modulated by voltage-gated ion channels. Among the voltage-gated ion channel modulators, potassium channel blockers have been the most studied ACh release enhancers. In spite of the memory-enhancing effects in animal models of cognition displayed by the initial prototypes of this class of compounds, 4-aminopyridine and linopirdine (DuP 996), their development was halted by lack of efficacy in clinical trials with AD patients [17,18], probably due to their short and variable half-life and poor blood-brain barrier (BBB) penetration [19]. Different structural modifications around these lead compounds have been carried out in order to develop a second generation of ACh release enhancers which can overcome these limitations. Replacement of the 2-indolinone core of linopirdine with the tricyclic anthrone core and additional introduction of a fluorine atom *ortho* to the nitrogen in the pyridylmethyl pendant groups has led to the more lipophilic XE991 and to the *N*-oxidation resistant DMP 543, with improved *in vitro* and *in vivo* potency [20,21]. Particularly interesting is DMP 543, which is currently undergoing clinical development [18,22] on the basis of its 10-20-fold higher potency, 4-fold longer half-life and 6-fold greater brain penetration in comparison with linopirdine. Recently, some hybrid compounds have been

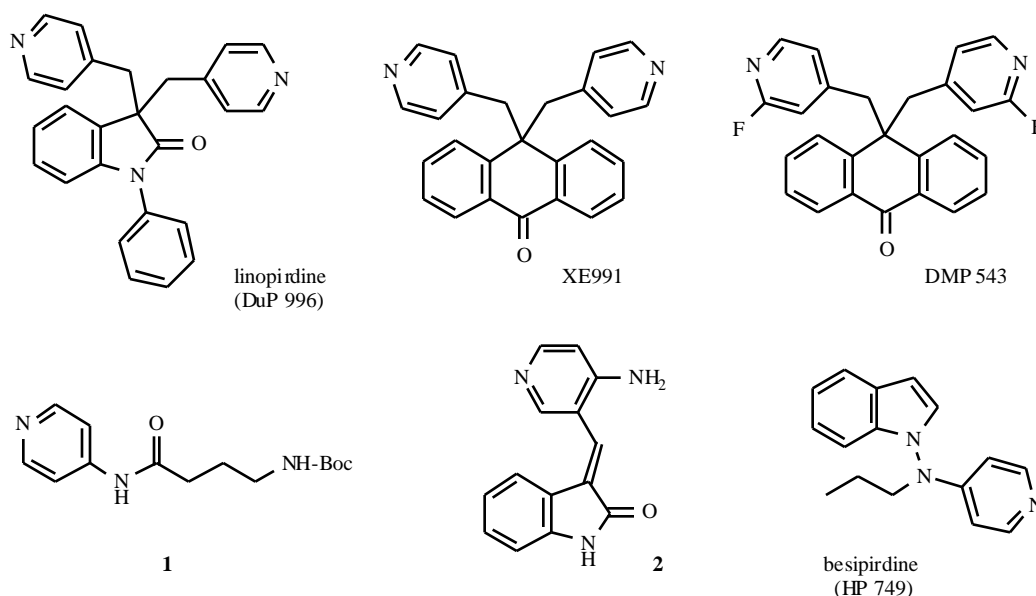


Fig. (1). Potassium channel blockers.

designed to improve the pharmacokinetic profile of the lead compounds, by connecting a 4-aminopyridine subunit to a 4-aminobutyric acid (GABA) chain in order to improve the diffusion into the brain (compound **1**) or to the 2-indolinone subunit of linopirdine (compound **2**), showing potent anti-amnesic activity compared to piracetam [23]. Besipirdine (HP 749) emerged from a program for the synthesis of arylaminopyridines as more lipophilic analogues of 4-aminopyridine [24]. Although interactions of besipirdine with potassium channels have been described, recent evidence points to an interaction with voltage-dependent sodium channels [25]. Besipirdine reverses memory deficits in animal behavioral models [26], and interestingly, it displays a unique combination of adrenergic and cholinomimetic properties, which has supported its development, being currently in advanced clinical trials [24]. Given the fact that multiple biochemical deficits are associated with AD, compounds able to modulate neurotransmission by multiple biochemical mechanisms may find a great utility in this disease. Second generation drugs such as besipirdine and also XE991 and DMP 543, which also enhance the release of dopamine and aspartic acid [20] as well, may provide such a therapeutic benefit to patients with AD. Moreover, with the more recent availability of cloned potassium channel subunits which play a critical role in regulating neuronal excitability in the nervous system, more potent and selective compounds useful for treatment of AD are likely to be forthcoming [27].

*M*₂ muscarinic antagonists. [Fig. (2)]

ACh release in the CNS is also modulated by negative feedback *via* presynaptic *M*₂ muscarinic receptors in cholinergic terminals, whose blockade by selective

antagonists should provide another means of increasing ACh release, thus restoring the cholinergic tone in AD at least at the stage where these receptors are not completely lost [28]. Several pyridobenzodiazepinones and dibenzodiazepinones such as BIBN 99 and DIBD have been designed around the initial low lipophilic tricyclic prototypes AF-DX 116 [29] and AQ-RA 741 [30], by structural modifications of the side chain directed to increase lipophilicity and consequently their penetration into the CNS, thus minimizing peripheral actions on *M*₂ receptors in gastrointestinal and cardiac tissues [31,32]. Moreover, these novel compounds are endowed with improved *M*₂ versus *M*₁ selectivity, that might avoid counteraction of their presynaptic action on ACh release by acting on postsynaptic muscarinic receptors [33,34]. While these compounds are still in an early stage of development, they seem to have the pharmacokinetic and subtype selectivity properties that make them possible candidates for AD treatment. From a synthetic program aimed to develop atropine-related derivatives endowed with more intensive antinociceptive and anti-amnesic activities than those recently reported for atropine [35], PG-9 emerged as novel *M*₂ muscarinic antagonist with central anti-amnesic and antinociceptive effects in mice and rats, and good side effect profile [36]. SCH-57790 is the prototype of another structural class of selective *M*₂ muscarinic antagonists [37]. Although it was shown to increase ACh levels *in vivo* after oral administration and was effective in animal models of cognition [38], the presence of the chemically labile benzylic cyano group precluded its development as a clinical candidate. Pharmacomodulation of this lead structure has led to more interesting compounds such as SCH-217443 [39] and SCH-72788 [40], with a higher *M*₂/*M*₁ affinity ratio (623 and 84, respectively), which seem sufficient to inhibit *M*₂ autoreceptor function without blocking *M*₁ activity, thus

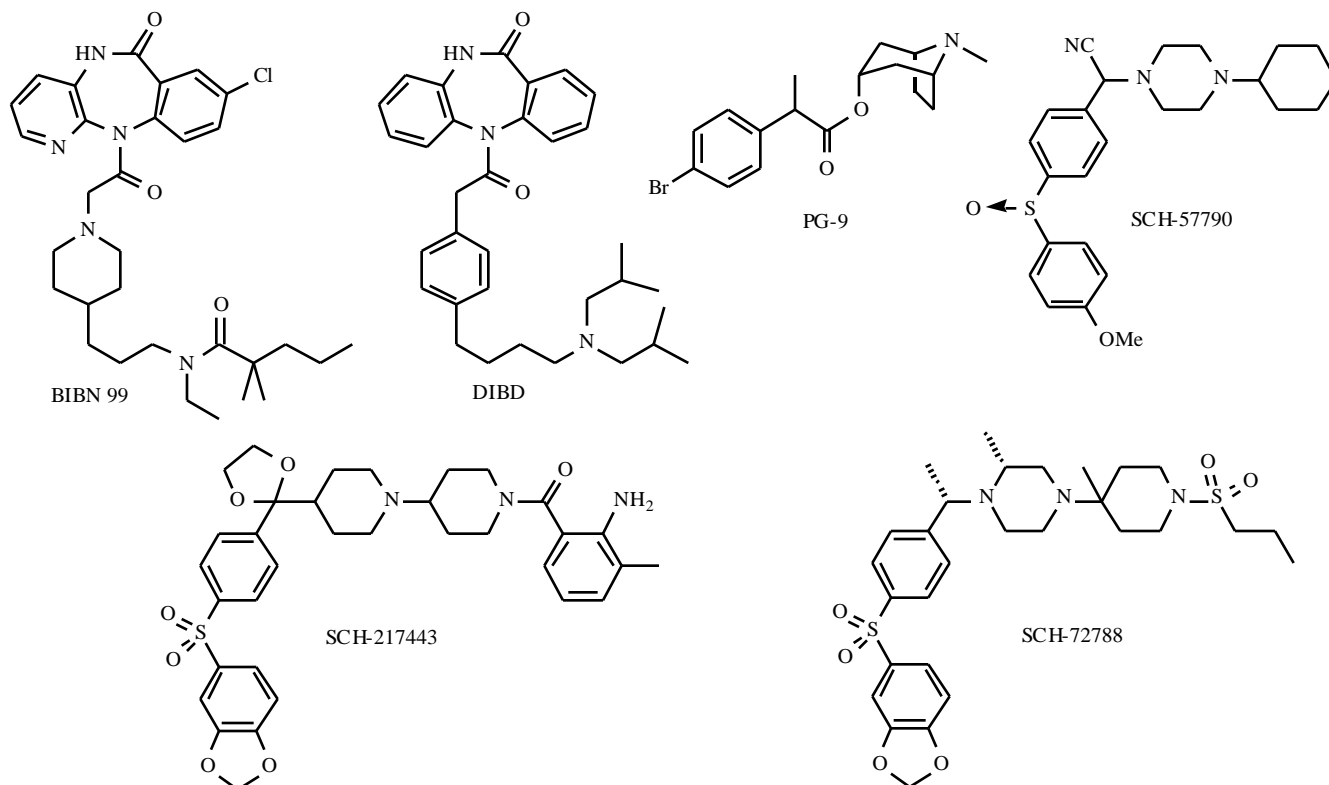


Fig. (2). *M*₂ muscarinic antagonists.

preventing side effects produced by non-selective muscarinic antagonists such as scopolamine. These novel potent and selective M_2 muscarinic antagonists have been chosen for further evaluation as potential drug candidates for AD treatment.

Nicotinic Agonists. [Fig. (3)]

Nicotinic receptors are located on presynaptic cholinergic terminals, and nicotinic agonists increase ACh release resulting in a feed-forward effect on cholinergic transmission. Nicotine itself, which stimulates all known nicotinic receptor subtypes to some degree, has been reported to be beneficial for memory in human and animal tests. However, the occurrence of side-effects at higher doses, such as anxiety, adverse mood changes, nausea and vomiting, have apparently delayed intensive research efforts to develop nicotinic agonists which would restore central cholinergic tone [41]. In the past few years, however, thanks to progress in molecular biology, physiology and pharmacology of central nicotinic receptors [42], the possibility that nicotinic stimulation may have beneficial effects in AD and other neuropsychiatric disorders has been recognised. Of the many nicotinic receptor subtypes that are expressed in the mammalian brain, $\alpha_4\beta_2$ and α_7 subtypes are the most prominent ones [43], the former having the greatest relevancy to AD [44]. Recently, it started an intensive search for novel selective nicotinic agonists, designed by modification of either the pyridine or the pyrrolidine ring of nicotine [44-46], that could possess a better therapeutic index, improved pharmacokinetics, and higher degree of efficacy in AD patients. ABT-418, an isoxazole analogue of (-)-nicotine, was the first novel selective nicotinic agonist tested in human patients. It is a potent agonist at the $\alpha_4\beta_2$ nicotinic receptor subtype [47], which in preclinical tests was shown to be 3- to 10-fold more potent than (-)-nicotine in memory enhancement and in anxiolytic test paradigms, with an improved side effect profile [47,48]. GTS-21 (DMXB A)

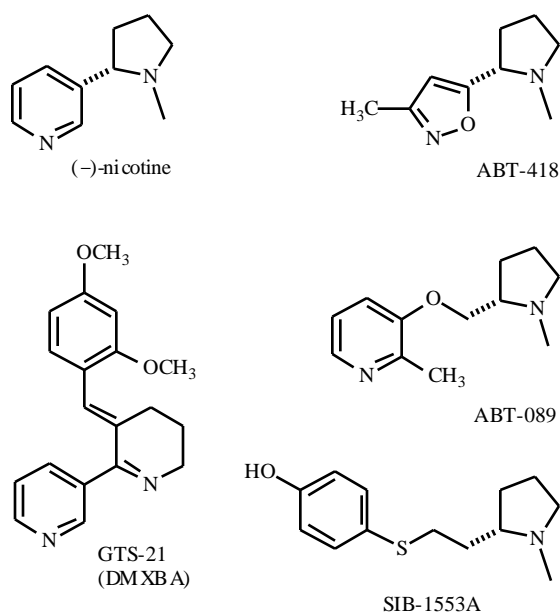


Fig. (3). Nicotinic agonists.

the first selective α_7 nicotinic agonist to be developed as a drug candidate for AD. It displays neuroprotective activity and much lower toxicity than (-)-nicotine [49]. In phase I clinical tests it improved memory and attention, and it showed to be safe after oral administration of large doses [49]. More recently, novel potent and selective nicotinic agonists such as ABT-089 [50] and SIB-1553A [51] are shown to be more efficacious and better tolerated than (-)-nicotine in preclinical tests, exhibiting also a better oral bioavailability than ABT-418 and GTS-21 [52] and a marked increase in cortical and hippocampal levels of ACh and other neurotransmitters relevant for cognitive processes [53], respectively. Although these new selective nicotinic agonists are unlikely to be totally free of nicotine-like adverse effects such as dizziness and nausea [44], their overall pharmacological profile supports their potential as drug candidates for the treatment of AD.

3.2. High Affinity Choline Uptake Enhancers

High affinity choline uptake (HACHU) is a regulatory step in ACh synthesis, and as other presynaptic cholinergic markers, its levels are decreased in AD [54]. Therefore, the increase of the uptake of choline into the presynaptic neuron constitutes another approach to activate the presynaptic cholinergic function and improve central cholinergic tone.

In preclinical tests, Z-4105 [Fig. (4)] enhanced HACHU after acute treatment in hippocampus, without affecting the levels of other neurotransmitters and displaying low toxicity after oral administration [55]. Starting from 4-aminopyridine, which is known to increase HACHU, as a lead compound, a series of 4-acylaminopyridine derivatives was synthesized [56], among which the compound MKC-231 [Fig. (4)] emerged as a potent HACHU enhancer, with *in vivo* activity and low acute toxicity in rats [56]. This compound is currently undergoing clinical trials.

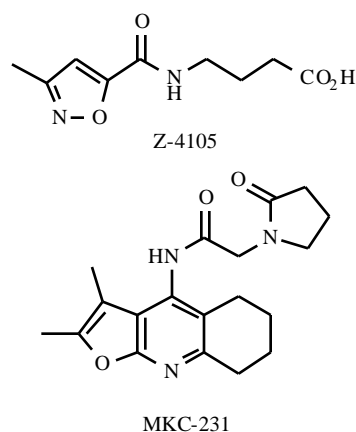


Fig. (4). High-affinity choline uptake enhancers.

4. SYNAPTIC CHOLINERGIC DRUGS

Two types of cholinesterases, AChE and butyrylcholinesterase (BChE), are present in a wide variety of tissues. Cholinesterase in the brain is predominantly

AChE, which hydrolyzes ACh to choline and acetate, thereby terminating the effect of this neurotransmitter at cholinergic synapses. AChE is therefore the target of cholinesterase inhibitors used for the cholinergic pharmacotherapy of AD, since its inhibition leads to an increase in the bioavailability of ACh at the synaptic cleft, and consequently to an increased stimulation of postsynaptic muscarinic receptors, thus improving cholinergic neurotransmission, while neither of the cholinesterases in peripheral tissues (AChE and BChE) is a target for treatment of AD, since their inhibition would cause side effects.

Acetylcholinesterase inhibitors (AChEIs) are assumed to take advantage of the relative preservation of postsynaptic muscarinic receptors in AD. However, this approach is limited, in principle, to patients who have intact and functionally active presynaptic neurons that are capable of synthesizing and releasing ACh. Therefore, AChEIs may be most useful in the early stages of AD and lose effectiveness over time. The efficacy of AChEIs may be further limited by activation of presynaptic M_2 muscarinic autoreceptors by ACh, leading to an inhibition of further presynaptic release of this neurotransmitter, thus counteracting their effects. In spite of these drawbacks, AChEIs constitute, to date, the most effective approach to treat the cognitive symptoms of AD [57-59]. They have shown clear therapeutic utility on both cognitive performance, as well as on the quality of life in these patients [59]. Indeed, the only drugs currently approved for the treatment of AD are AChEIs (i.e. tacrine, donepezil, rivastigmine and galantamine). A large amount of AChEIs has been developed, which differ among themselves in selectivity for AChE and BChE, mechanism of inhibition and reversibility. On the basis of their mechanism of inhibition and structure, AChEIs can be divided in several groups.

4.1. Pseudo-irreversible AChEIs

This class of AChEIs includes a group of carbamates [Fig (5)] which form a carbamoylated complex with the serine residue of the catalytic triad of AChE, that is hydrolyzed at a

slower rate than the acylated form resulting from the interaction with the substrate ACh. The prototype of this class of compounds is physostigmine, which was the first AChEI to be clinically studied for the treatment of AD. In spite of the first encouraging results, its potential use for AD was discarded following the completion of phase III clinical studies, where a lack of efficacy was shown, resulting from its short half-life, variable bioavailability, and narrow therapeutic index. In order to improve the *in vivo* profile of physostigmine, while retaining its *in vitro* potency, a number of more lipophilic analogues have been designed. Eptastigmine is a second generation AChEI carbamate, with lower toxicity and longer duration of action. Although, in phase III clinical trials, it has shown to be efficacious [60], its clinical utility could be limited by the occurrence of neutropenia and aplastic anemia in some patients [61]. Quilostigmine (NXX-066) is another potent and long-acting second generation AChEI carbamate [62], which in phase I studies has shown a good side effect profile. The miotine derivative rivastigmine (SDZ-ENA-713, Exelon[®]) is less potent than physostigmine *ex vivo* and *in vitro* and also inhibits BChE. However, its superior global pharmacological profile, including a good combination of brain selectivity, long-lasting *in vivo* activity, good tolerability and neuroprotective properties [57,63], led to its approval for the treatment of AD by the European Union in 1998 and by the US FDA in 2000. A third generation of AChEI carbamates which combine long duration of action and selectivity for AChE *versus* BChE inhibition has recently been developed. Phenserine and tolserine display long-lasting action, high selectivity for AChE *versus* BChE (75-200-fold) and also high brain *versus* plasma selectivity (10-24-fold) [64,65], which is reflected in an unusually wide therapeutic window and high potency to improve memory and cognition in preclinical animal studies [66]. Phenserine is entering phase I clinical trials. Ro 46-5934 is another novel potent and selective AChEI, with M_2 muscarinic antagonist activity, leading to a higher efficacy in increasing extracellular levels of ACh [67]. CHF2819 is a novel, orally active geneserine derivative with long-lasting AChE inhibitory activity, which produces a concomitant increase in the levels of ACh and serotonin in rat hippocampus, which

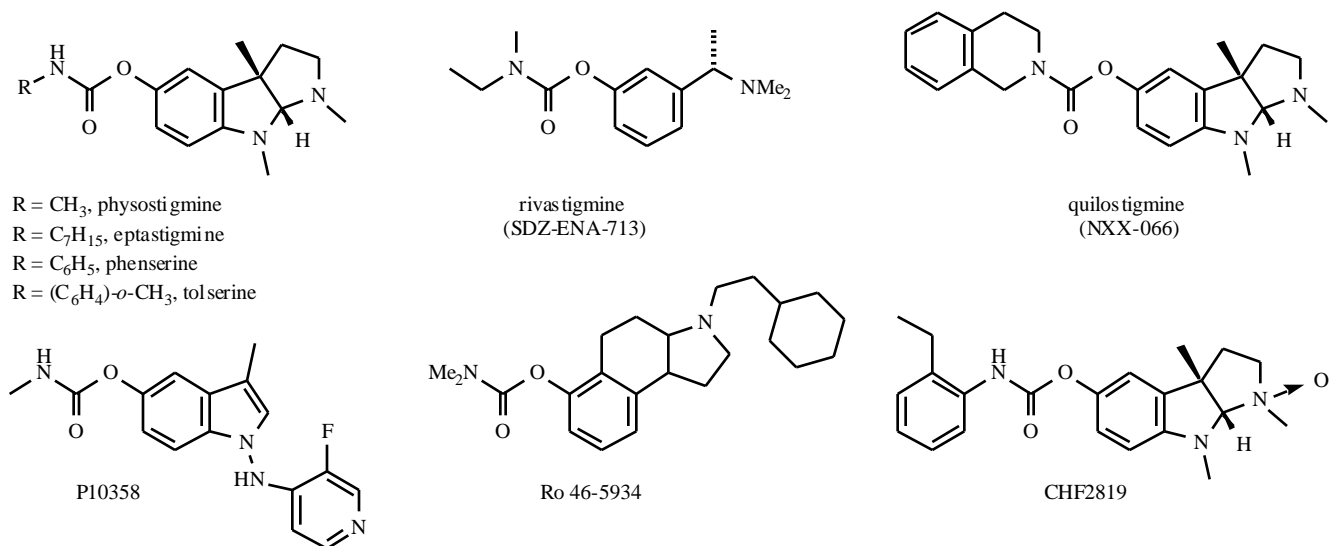


Fig. (5). Pseudo-irreversible acetylcholinesterase inhibitor carbamates.

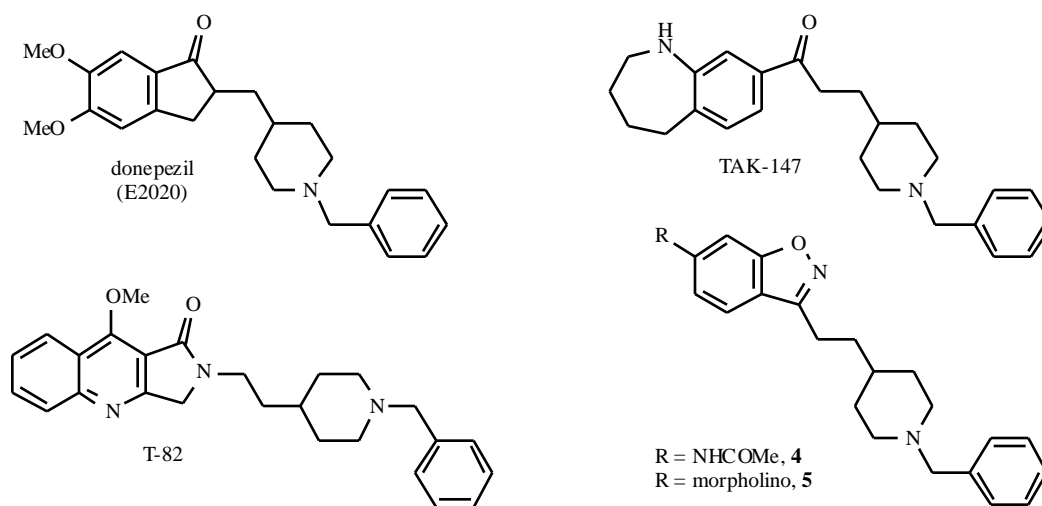


Fig. (9). Reversible acetylcholinesterase inhibitor *N*-benzylpiperidines.

suronacrine, and 7-methoxytacrine were active in animal models of cognition [75-77], showing a lower acute toxicity. However, occurrence of hepatotoxicity in some patients treated with velnacrine [78] has precluded further development. Amiridine (NIK-247) showed well-defined therapeutic benefits and safety in initial clinical studies [79], and is currently undergoing phase III clinical evaluation in Japan. Introduction of halogen atoms at positions 6 or 8 of tacrine is known to have a positive effect on AChE inhibitory activity [80]. One of such derivatives, SM-10888 is nearly equipotent to tacrine and 2-4 times more potent than amiridine and velnacrine, and has started clinical trials in Japan for the treatment of AD [81]. CI-1002 is another derivative bearing halogen atoms at positions equivalent to positions 6 and 8 of tacrine. While this compound is nearly equipotent to tacrine [82], recent studies suggest that it might be more effective than tacrine in maintaining ACh in the synaptic cleft [83].

N-Benzylpiperidines. [Fig. (9)]

Donepezil (Aricept[®]), the prototype of this structural class, was the second FDA-approved drug for the treatment of mild to moderate AD in 1996. It is a potent, long-acting and highly selective AChEI, exhibiting an affinity for AChE 1250 times greater than for BChE, and also exhibiting brain versus plasma selectivity *in vivo* [84,85]. Its superior pharmacological profile, including high efficacy, safety profile and brain selectivity, has spurred the development of other *N*-benzylpiperidine derivatives. TAK-147, although less potent than donepezil, has shown beneficial effects in animal models of cognition, without eliciting significant side effects [86], and is currently undergoing phase II clinical testing in Japan. T-82 is another potent AChEI, which additionally seems to have antagonistic activity on 5-HT₃ receptors, what could lead to an enhanced release of ACh from presynaptic cholinergic terminals, resulting in a synergistic effect with AChE inhibition [87]. Some *N*-benzylpiperidine derivatives in which the indanone moiety of donepezil has been replaced by different heterocyclic systems have been recently described [88,89]. Among these compounds, bioisosteric *N*-benzylpiperidine benzisoxazoles such as **4** and **5** displayed

higher potency and selectivity than donepezil and were effective in animal models of cognition [89].

Alkaloids. [Fig. (10)]

Galantamine (Reminyl[®]) is a tertiary amine alkaloid isolated from Amaryllidaceae (*Galanthus woronowi*, the Caucasian snowdrop), that has been recently approved in several countries for the symptomatic treatment of AD [90]. It is a reversible, competitive inhibitor of AChE with relatively less BChE activity, and also an allosteric modulator of nicotinic receptors *in vitro*, enhancing the response of nicotinic receptors to ACh [91], which results in an increase in the release of ACh and other neurotransmitters, additional to the increase in ACh bioavailability by inhibition of AChE. Although not clinically proven useful yet, this dual action of galantamine makes it an intriguing prospect for the treatment of AD. Several galantamine analogues have been developed [92], among which the 6-ester derivatives P11012 and P11149 are the most promising ones. These compounds are in fact prodrugs, which after oral administration are quickly hydrolyzed *in vivo* to 6-demethylgalantamine, which is 10-fold more potent and 6-fold more selective than galantamine. (-)-Huperzine A, an alkaloid isolated from the Chinese medicinal herb *Huperzia serrata*, is a very potent, selective and long-acting AChEI with low toxicity, which enhances cognitive function in

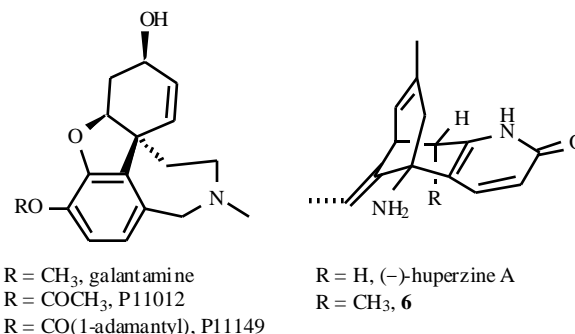


Fig. (10). Reversible acetylcholinesterase inhibitor alkaloids.

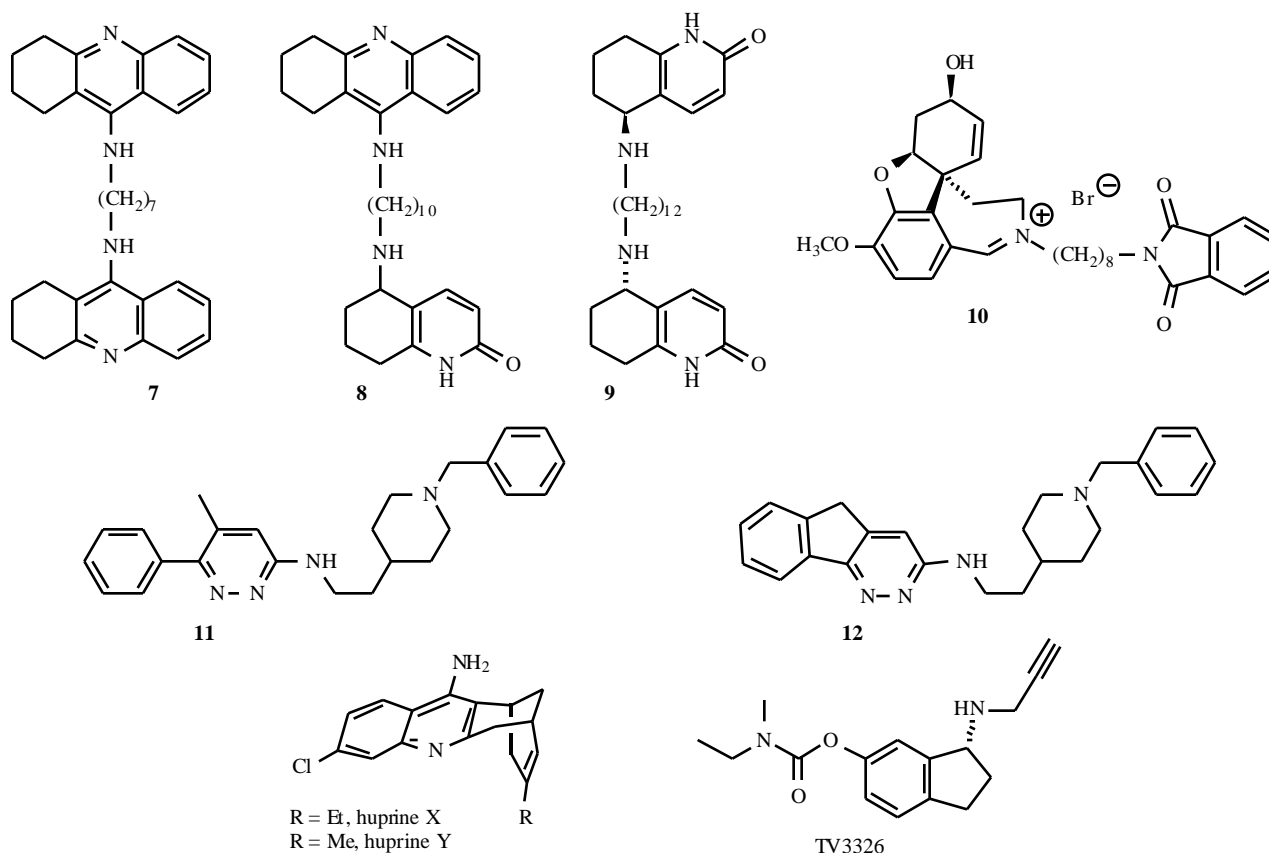


Fig. (11). Dimeric or hybrid acetylcholinesterase inhibitors.

animals and human, and exhibits a neuroprotective action on hippocampal and cortical neurons [93,94]. (–)-Huperzine A is undergoing clinical trials in AD patients in China, and has been recently marketed in USA as a dietary supplement. As a promising lead compound, much efforts have been devoted to the synthesis of huperzine A analogues [93,94]. However, the sole analogues which can rival the activity of the lead compound are the 10-methyl substituted analogue **6** which is 8-fold more potent than (±)-huperzine A and a 10-spirocyclopropyl analogue which is equipotent to (–)-huperzine A.

Recently very interesting novel AChEIs have been designed by enlargement of known lead structures either by duplication of the parent drug or by association in the same molecule of structural fragments of different lead compounds [Fig. (11)]. Three different goals have been pursued with these conjunctive approaches.

On the one hand, bivalency is an effective strategy for improving drug potency and selectivity, when multiple recognition sites for the same substrate exist. In this sense, important efforts have been made to develop new AChE inhibitors of increased affinity, potency and selectivity, able to bind simultaneously to the two known binding sites of AChE, namely the catalytic and the peripheral sites. Thus, several homo- or hetero-dimers, such as compounds **7-10**, containing units of tacrine, a key fragment of huperzine A or galantamine linked by an oligomethylene chain with a suitable length to locate both components at the most

appropriate distance for interaction with both binding sites, have been recently synthesized [95-99]. Although all of these bis-ligands are clearly more potent than the parent compounds, but the most promising of them is compound **7**, which is 149-fold more potent than tacrine and has recently shown to be effective in animal models of cognition [100] and in protection against oxidative damage [101]. Compounds **11** and **12**, which combine the 3-amino-6-phenylpyridazine moiety of minaprine, an antidepressant with weak AChE inhibitory activity, with the *N*-benzylpiperidine moiety of donepezil also seem to interact with both binding sites of the enzyme, and are more potent and selective than tacrine [102,103].

On the other hand, some tacrine–huperzine A hybrids (huprines) were designed by combination of the 4-aminoquinoline substructure of tacrine with the carbobicyclic substructure of (–)-huperzine A, with the idea of increasing their binding to the active site of AChE, since the binding sites of tacrine and (–)-huperzine A are close and partially overlap. Huprine X and huprine Y are the most promising compounds of this novel class of AChEIs as drug candidates for the treatment of AD [104,105]. These compounds act as tight-binding reversible AChEIs, being more than 400-fold more potent than tacrine and 492- and 777-fold, more potent toward AChE than toward BChE. They are able to cross the BBB, and bind to human AChE with an inhibition constant K_I around 30 pM, indicating that they bind to the enzyme with one of the highest affinities yet reported. The affinity of these compounds for human AChE is around 1200-fold

higher than that of tacrine, 180-fold higher than that of (-)-huperzine A and 40-fold higher than that of donepezil. Further development of huprine X and huprine Y is awaiting partner.

A third used strategy is based on the design of compounds able to interact with two binding sites, but belonging to two different biological targets. TV3326 can be considered as a hybrid compound which combines the phenyl *N*-ethyl-*N*-methylcarbamate moiety of rivastigmine with the *N*-propargyl-(1*R*)-aminoindan structure of rasagiline, a potent selective inhibitor of MAO-B. Indeed, TV3326 possesses both AChE and monoamine oxidase (MAO-A and MAO-B) inhibitory activity, which results in a unique combination of pharmacological activities including cognitive enhancing, neuroprotective and antidepressant properties. TV3326 is currently under development for the treatment of AD [106].

While convincing efficacy data for other classes of cholinomimetics are lacking at present, AChEIs have proven to be the most effective class of medication for short term (6 to 12 months) improving cognitive function and activities of daily living. However, overall results are usually modest, affecting only one third of treated patients [107], and cholinergically mediated gastrointestinal side-effects, although characteristically mild in severity and short-lived, are frequent. Inhibition of BChE was initially thought to be related to the occurrence of peripheral side-effects, but non-selective and selective AChEIs produce qualitatively similar adverse effects that, therefore, do not appear to result from inhibition of BChE [108]. In spite of these drawbacks, the interest for AChEIs has been renewed due to the discovery of additional benefits of these drugs beyond improving intellectual functions, such as the decrease and amelioration of the neuropsychiatric symptoms of AD, especially apathy and visual hallucinations [1]. More interestingly, recent evidence suggests that both muscarinic agonists and AChEIs may actually modify disease progression [57,107]. Activation of M₁ muscarinic receptors can stimulate

secretion of APPs *via* the β -secretase pathway, with consequent reduction in A β release, what suggests that cholinomimetics can prevent the formation of amyloid plaques and promote normal processing of APP. To the extent that cholinergic therapies may have effects beyond the short-term symptomatic improvement of cognition or function, and may modify disease progression, their potential importance for delaying the onset or modifying clinical progression is evident, although this remains to be demonstrated in extensive clinical trials. Meanwhile, as newer potential non-cholinergic therapeutics are still early in clinical development, AChEIs are likely to be actively used for the next several years.

5. POSTSYNAPTIC CHOLINERGIC DRUGS

Postsynaptic M₁ muscarinic receptors are essentially spared from degeneration or even up-regulated in AD [109]. While AChEIs depend on ACh release from presynaptic neurons that are progressively lost during the course of the disease, M₁ muscarinic agonists might be effective in the treatment of AD, regardless of the extent of degeneration of presynaptic cholinergic projections to cortex and hippocampus. For this reason, M₁ muscarinic agonists have been proposed to represent a more rational treatment for AD than AChEIs [41,110-112]. Moreover, the recent evidence that M₁ agonists could display a modifying disease role through stimulation of the non-amyloidogenic β -secretase pathway of APP, regulation of tau phosphorylation and induction of neurotrophic responses, has reinforced the development of these compounds [111].

Early clinical studies with first-generation muscarinic agonists, such as arecoline [Fig. (12)], oxotremorine [Fig. (15)], pilocarpine [Fig. (15)] and RS86 [Fig. (13)], were very disappointing due to their low potency, short-term duration of action, poor oral bioavailability and occurrence of significant peripheral cholinergic side-effects due to insufficient receptor subtype specificity [113]. To overcome

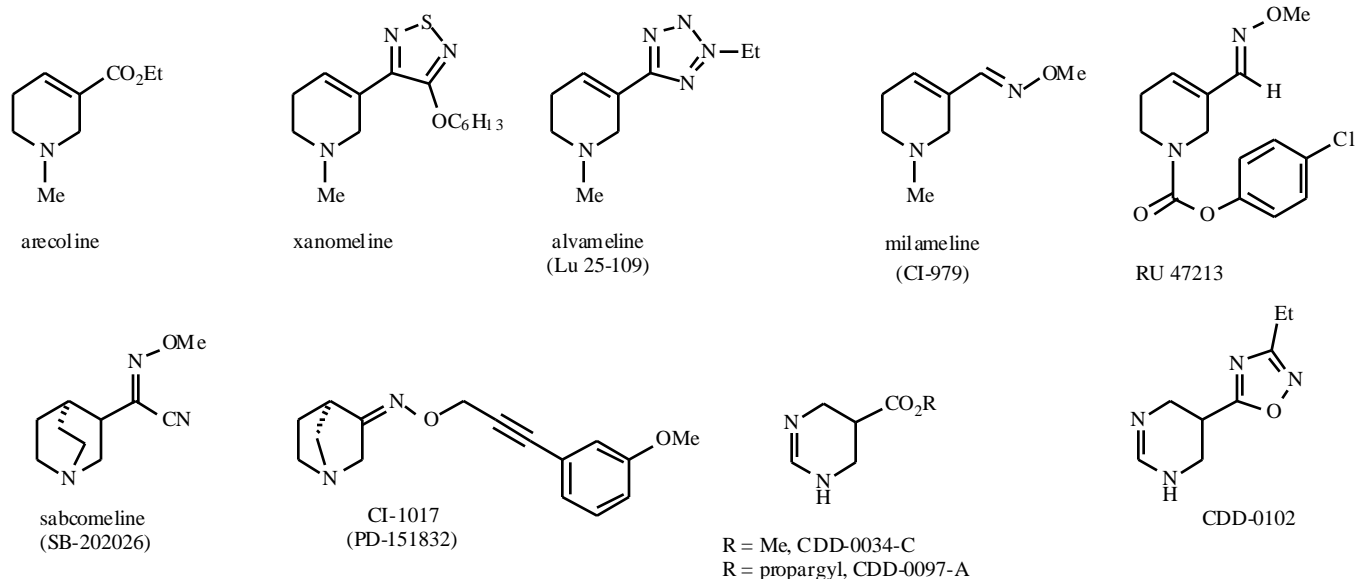


Fig. (12). Arecoline-analogue muscarinic agonists.

these limitations, second generation agonists with improved pharmacokinetics, BBB penetration and M_1 selectivity have been designed. However, attaining receptor subtype selectivity among muscarinic agonists has proven to be an illusive goal, probably due to the high degree of homology between the five known muscarinic receptor subtypes (M_1 - M_5) [114]. An alternative approach is based on the use of partial agonists to confer functional selectivity [115], even in the absence of significant differences in affinity with respect to receptor subtypes, taking advantage of the high M_1 muscarinic receptor reserved in brain and low levels of ACh. Many muscarinic agonists have been designed, mostly by pharmacomodulation of first-generation agents or ACh itself.

Several muscarinic agonists have been designed around arecoline or conformationally rigid azabicyclic analogues, by replacement of the metabolically labile ester function either with bioisosteric five-membered heterocyclic rings or with an oxime ether functionality [Fig. (12)], for improving both metabolic stability and muscarinic receptor subtype selectivity. Initially developed derivatives such as the M_1 / M_4 preferring agonist xanomeline [116], the M_1 partial agonist and M_2 / M_3 antagonist alvaneline (Lu 25-109) [117,118], the nonselective muscarinic agonist milaneline (CI-979) [119], and the functionally selective partial M_1 agonist sabcomeline (SB-202026) [120,121] failed to show statistically significant efficacy and / or were associated with a high incidence of gastrointestinal side effects in phase II or phase III clinical trials, that led to discontinuation of their development. Other recently developed arecoline derivatives are still under preclinical or clinical evaluation. RU 47213 is a prodrug under development for treatment of AD, whose carbamate function is hydrolyzed *in vivo* to form the tetrahydropyridine oxime RU 35963, a nonselective muscarinic agonist [122]. After oral administration, RU 47213 seems superior to arecoline in terms of potency, central selectivity and duration of action, and is also active in animal models of cognition, without eliciting significant cholinergic side effects [122]. CI-1017 (PD-151832) is a functionally selective M_1 muscarinic agonist, which emerged from a program directed to the synthesis of muscarinic agonists longer/larger than the classical ones with the aim of increasing subtype selectivity by ensuring maximum contact between the agonist and the internal surface of the binding cavity [113]. CI-1017 was well tolerated in phase I clinical trials and is entering phase II studies [123]. CDD-0034-C, an analogue of arecoline in which the tetrahydropyridine ring is replaced with a tetrahydropyrimidine ring, emerged from a program directed to the synthesis of amidines as suitable bioisosteres of the ammonium group of ACh or arecoline (in its protonated form) [124]. The moderate affinity for central muscarinic receptors displayed by CDD-0034-C was increased either by introduction of larger alkyl substituents or by bioisosteric replacement of the methyl ester group with a five-membered heterocyclic ring. Thus, compounds CDD-0097-A and CDD-0102 display relatively high affinity, functional selectivity for M_1 versus M_3 receptors, high central bioavailability and a limited side effect profile [125]. Indeed recent studies have shown that CDD-0102 improves memory function in animals, exhibiting good oral bioavailability and low toxicity, that supports further development [126].

The potent agonists muscarine and *cis*-dioxolane [Fig. (13)] are quaternary ammonium compounds which do not penetrate BBB. The related spirocyclic piperidine succinimide RS86 and the quinuclidine dioxolane analogue AF-30 are known agonists of moderate potency [127], which have been used as lead compounds for the design of novel spiropiperidines and spiroquinuclidines with therapeutic potential for AD. One of such compounds, the dioxolane analogue SDZ 210-086, is a potent, centrally active muscarinic agonist with good bioavailability. However, elevation of liver transaminases at the expected therapeutic dose level precluded further clinical studies [127]. YM-796 is a muscarinic M_1 agonist with 3.5-fold selectivity for M_1 over M_2 receptors, which improves learning in animal models of cognition [128] and is currently in phase II clinical trials for AD. Cevimeline (AF102B), AF150(S) and AF267B (the AF series) are other well studied spirocyclic compounds, which are considered rigid analogues of ACh. This rigidity might limit their ability to adapt to minor differences in receptor structure, thereby providing selectivity toward a limited population of receptor subtype [129]. Indeed, these compounds are M_1 functionally selective agonists which restore memory and learning deficits in several models of cognition with relatively few side-effects and a high safety margin [129]. Development of cevimeline for AD was, however, discontinued due to a lack of efficacy and the high incidence of cholinergic side-effects.

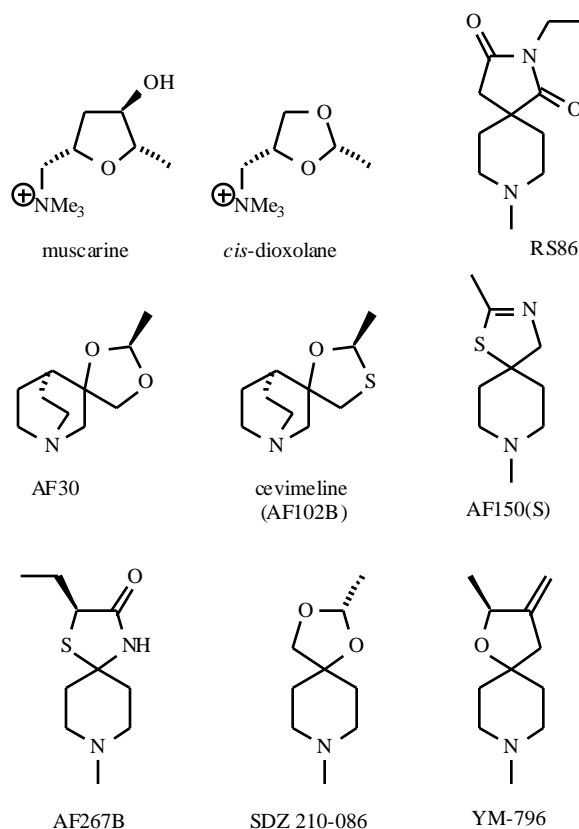


Fig. (13). Spiropiperidines and spiroquinuclidines with muscarinic activity.

Talsaclidine (WAL 2014) [Fig. (14)] can be considered as a derivative of the rigid ACh analogue aceclidine. Talsaclidine is a functionally preferential M_1 agonist with

full intrinsic activity and less pronounced effects at M_2 and M_3 receptors. Because of this favourable receptor profile and its pharmacokinetic properties [130], talsaclidine was expected to cause fewer and less severe side-effects than other muscarinic agonists. Indeed, in safety clinical trials, talsaclidine was well-tolerated in both healthy volunteers and AD patients. It is currently undergoing phase III clinical trials for AD treatment, although it seems not to show convincing any improvement in cognitive functions [130].

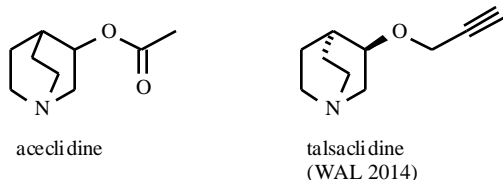


Fig. (14). Rigid analogues of acetylcholine with muscarinic activity.

Several analogues of the classical muscarinic agonists oxotremorine and pilocarpine have been developed [Fig. (15)]. Oxotremorine analogue UH5 has been reported to be somewhat subtype selective muscarinic agonist [131]. Oxime **13**, an analogue of UH5, is a novel potent and M_1 selective partial muscarinic agonist, with a 5-fold selectivity for M_1 over M_2 muscarinic receptors [131]. Compound **14** is another recently developed oxotremorine analogue with selective affinity for brain M_1 receptors compared to heart M_2 receptors [132]. The pilocarpine isostere thiopilocarpine (SDZ-ENS 163) exhibits an unusual receptor profile of postsynaptic M_1 agonist as well as presynaptic M_2 antagonist, which makes it an ideal drug for augmenting central cholinergic transmission. It is currently in clinical trials for AD [133].

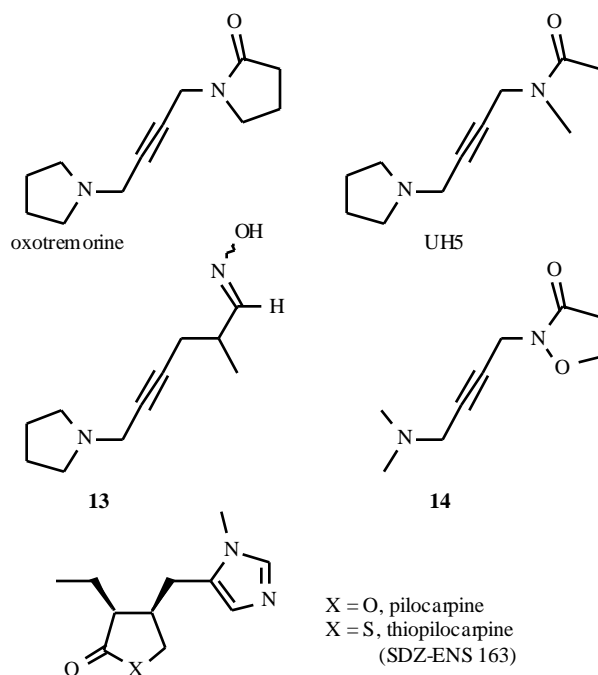


Fig. (15). Oxotremorine- and pilocarpine-analogue muscarinic agonists.

Although M_1 muscarinic agonists seem to improve the psychiatric symptoms usually present in AD patients [41], clinical results have so far not been satisfactory due to a doubtful efficacy of cognitive function and a high incidence of muscarinic-related side-effects, as a consequence of their usually modest selectivity. A possible explanation of the failure of these agents, where AChEIs are relatively effective, is that inhibition of AChE and subsequent elevation of ACh levels enhances not only muscarinic but also nicotinic stimulation. It has also been suggested that the limited efficacy of muscarinic agonists may be due to dysfunctional receptor response mechanisms in AD [110]. It is not clear if sufficient M_1 receptor reserve exists in brain areas of AD patients, which regulate cognitive function to compensate for these partial reductions in muscarinic receptor function.

As already mentioned, activation of M_1 muscarinic receptors accelerates APPs secretion and inhibits A formation. This has been evidenced in numerous studies *in vitro*, *in vivo*, and in AD patients as well as, with different muscarinic agonists including xanomeline [134], alvamine [112], milamine [112], sabcomeline [112], CI-1017 [123], cevimeline and AF150(S) [129,135], and talsaclidine [136]. However, only large, long-term phase III studies in humans will allow to assess the speculated potential of these agents to modify AD.

6. FUTURE PROSPECTS

The general acceptance of the cholinergic hypothesis of AD has experienced some ups and downs due to the modest effects displayed by AChEIs and the failure of the other classes of cholinomimetics. However, the finding that APP processing may be under cholinergic control, and therefore the assumption that cholinergic pharmacotherapy might have more than a symptomatic role in the management of AD, has boosted a recent revival of this strategy.

While AChEIs are likely to be actively used for the indefinite future as the only available efficacious treatment for AD, future trends in cholinergic pharmacotherapy of AD seem to address, on the one hand, the development of allosteric modulators of both muscarinic [110,137] and nicotine [91] receptors, as a way to achieve better subtype selectivity and to prevent compensatory processes such as receptor desensitization or downregulation of expression, which are induced by agonists.

On the other hand, treatment of AD with a single drug is probably not a realistic option due to the complicated nature of the disease. In this sense, a combination therapy of AChEIs with muscarinic or nicotinic agonists, or with non-cholinergic agents such as estrogens, antioxidants or anti-inflammatory agents will be a future alternative to the present monotherapy [10,49,107,111]. Moreover, combination therapies could be directed to the manipulation of several neurotransmitters which are deficient in AD patients and are also involved in the various components of memory and cognitive ability. Recent evidence suggests that combined cholinergic-monoaminergic therapies are markedly effective in restoring some aspects of cortical functioning [138-141], and a combination therapy of AChEIs with the NMDA

receptor antagonist memantine has been suggested to result in both functional improvement and neuroprotection from glutamate [142]. Thus, a more thorough investigation of the potential benefits of this polypharmaceutical approach in AD seems warranted.

LIST OF ABBREVIATIONS

A	=	-Amyloid peptide
ACh	=	Acetylcholine
AChE	=	Acetylcholinesterase
AChEI	=	Acetylcholinesterase inhibitor
AD	=	Alzheimer's disease
APP	=	-Amyloid precursor protein
APPs	=	Soluble APP fragment
BBB	=	Blood-brain barrier
BChE	=	Butyrylcholinesterase
CNS	=	Central nervous system
FDA	=	Food and Drug Administration
GABA	=	-Aminobutyric acid
HACHU	=	High-affinity choline uptake

REFERENCES

- Cummings, J.L.; Askin-Edgar, S. *CNS Drugs*, **2000**, *13*, 385.
- Cutler, N.R.; Sramek, J.J. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **2001**, *25*, 27.
- Gauthier, S. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **2001**, *25*, 73.
- Cacabelos, R.; Alvarez, A.; Lombardi, V.; Fernández-Novoa, L.; Corzo, L.; Pérez, P.; Laredo, M.; Pichel, V.; Hernández, A.; Varela, M.; Figueroa, J.; Prous, J., Jr.; Windisch, M.; Vigo, C. *Drugs Today*, **2000**, *36*, 415.
- Coughlan, C.M.; Breen, K.C. *Pharmacol. Therapeut.*, **2000**, *86*, 111.
- Schenk, D.; Barbour, R.; Dunn, W.; Gordon, G.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Liao, Z.; Lieberburg, I.; Motter, R.; Mutter, L.; Soriano, F.; Shopp, G.; Vasquez, N.; Vandevent, C.; Walker, S.; Wogulis, M.; Yednock, T.; Games, D.; Seubert, P. *Nature*, **1999**, *400*, 173.
- Wolfe, M.S. *J. Med. Chem.*, **2001**, *44*, 2039.
- Talaga, P. *Mini Rev. Med. Chem.*, **2001**, *1*, 175.
- Cacabelos, R.; Nordberg, A.; Caamaño, J.; Franco-Maside, A.; Fernández-Novoa, L.; Gómez, M.J.; Alvarez, X.A.; Takeda, M.; Prous, J.; Nishimura, T.; Winblad, B. *Drugs Today*, **1994**, *30*, 295.
- Farlow, M.R.; Evans, R.M. *Neurology*, **1998**, *51* (Suppl. 1), S36.
- Francis, P.T.; Palmer, A.M.; Snape, M.; Wilcock, G.K. *J. Neurol. Neurosurg. Psychiatry*, **1999**, *66*, 137.
- Cooper, J.R.; Bloom, F.E.; Roth, R.H. In *The Biochemical Basis of Neuropharmacology*; Oxford University Press, New York, **1991**, p. 190.
- Young, A.B.; Penney, J.B., Jr. In *Alzheimer Disease*; Terry, R.D.; Katzman R.; Bick, K.L. Eds: Raven Press, New York, **1994**, p. 293.
- Perry, E.K.; Perry, R.H.; Blessed, G.; Tomlinson, B.E. *Lancet*, **1977**, 189.
- Bartus, R.T.; Dean III, R.L.; Beer, B.; Lippa, A.S. *Science*, **1982**, *217*, 408.
- Whitehouse, P.J.; Price, D.L.; Clark, A.W., Coyle, J.T.; DeLong, M.R. *Ann. Neurol.*, **1981**, *10*, 122.
- Davidson, M.; Zemishlany, Z.; Mohs, R.C.; Horvath, T.B.; Powchik, P.; Blass, J.P.; Davis, K.L. *Biol. Psychiatry*, **1988**, *23*, 485.
- Pesti, J.A.; Chorvat, R.J.; Huhn, G.F. *Chem. Innovation*, **2000**, *30*, 28.
- Pieniaszek, H.J., Jr.; Fiske, W.D.; Saxton, T.D.; Kim, Y.S.; Garner, D.M.; Xilinas, M.; Martz, R. *J. Clin. Pharmacol.*, **1995**, *35*, 22.
- Zaczek, R.; Chorvat, R.J.; Saye, J.A.; Pierdomenico, M.E.; Maciag, C.M.; Logue, A.R.; Fisher, B.N.; Rominger, D.H.; Earl, R.A. *J. Pharmacol. Exp. Ther.*, **1998**, *285*, 724.
- Earl, R.A.; Zaczek, R.; Teleha, C.A.; Fisher, B.N.; Maciag, C.M.; Marynowski, M.E.; Logue, A.R.; Tam, S.W.; Tinker, W.J.; Huang, S.-M.; Chorvat, R.J. *J. Med. Chem.*, **1998**, *41*, 4615.
- Pieniaszek, H.J., Jr.; Garner, D.M.; Klingerman, C.A.; Kornhauser, D.M. *J. Clin. Pharmacol.*, **1997**, *37*, 867.
- Andreani, A.; Leoni, A.; Locatelli, A.; Morigi, R.; Rimbaldi, M.; Pietra, C.; Villetti, G. *Eur. J. Med. Chem.*, **2000**, *35*, 77.
- Klein, J.T.; Davis, L.; Olsen, G.E.; Wong, G.S.; Huger, F.P.; Smith, C.P.; Petko, W.W.; Cornfeldt, M.; Wilker, J.C.; Blitzer, R.D.; Landau, E.; Haroutunian, V.; Martin, L.L.; Effland, R.C. *J. Med. Chem.*, **1996**, *39*, 570.
- Tang, L.; Smith, C.P.; Huger, F.P.; Kongsamut, S. *Br. J. Pharmacol.*, **1995**, *116*, 2468.
- Santucci, A.S.; Haroutunian, V.; Davis, K.L. *Clin. Neuropharmacol.*, **1991**, *14* (Suppl. 1), S1.
- Coghlan, M.J.; Carroll, W.A.; Gopalakrishnan, M. *J. Med. Chem.*, **2001**, *44*, 1627.

- [28] Stillman, M.J.; Shukitt-Hale B.; Galli, R.L.; Levy, A.; Lieberman, H.R. *Brain Res. Bull.*, **1996**, *41*, 221.
- [29] Giachetti, A.; Micheletti, R.; Montagna, E. *Life Sci.*, **1986**, *38*, 1663.
- [30] Doods, H.; Entzeroth, M.; Mayer, N. *Eur. J. Pharmacol.*, **1991**, *192*, 147.
- [31] Cohen, V.I.; Jin, B.; McRee, R.C.; Boulay, S.F.; Cohen, E.I.; Sood, V.K.; Zeeberg, B.R.; Reba, R.C. *Brain Res.*, **2000**, *861*, 305.
- [32] Cohen, V.I.; Jin, B.; Gitler, M.S.; de la Cruz, R.A.; Boulay, S.F.; Sood, V.K.; Zeeberg, B.R.; Reba, R.C. *Eur. J. Med. Chem.*, **1995**, *30*, 61.
- [33] Doods, H.; Entzeroth, M.; Ziegler, H.; Schiavi, G.; Engel, W.; Mihm, G.; Rudolf, K.; Eberlein, W. *Eur. J. Pharmacol.*, **1993**, *242*, 23.
- [34] Quirion, R.; Wilson, A.; Rowe, W.; Aubert, I.; Richard, J.; Doods, H.; Parent, A.; White, N.; Meaney, M.J. *J. Neurosci.*, **1995**, *15*, 1455.
- [35] Ghelardini, C.; Malmberg-Aiello, P.; Giotti, A.; Malcangio, M.; Bartolini, A. *Br. J. Pharmacol.*, **1990**, *101*, 49.
- [36] Ghelardini, C.; Galeotti, N.; Romanelli, M.N.; Gualtieri, F.; Bartolini, A. *CNS Drug Rev.*, **2000**, *6*, 63.
- [37] Kozlowski, J.A.; Lowe, D.B.; Guzik, H.S.; Zhou, G.; Ruperto, V.B.; Duffy, R.A.; McQuade, R.; Crosby, G., Jr.; Taylor, L.A.; Billard, W.; Binch, H., III; Lachowicz, J.E. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2255.
- [38] Lachowicz, J.E.; Lowe, D.; Duffy, R.A.; Ruperto, V.; Taylor, L.A.; Guzik, H.; Brown, J.; Berger, J.G.; Tice, M.; McQuade, R.; Kozlowski, J.; Clader, J.; Strader, C.D.; Murgolo, N. *Life Sci.*, **1999**, *64*, 535.
- [39] Greenlee, W.; Clader, J.; Asberom, T.; McCombie, S.; Ford, J.; Guzik, H.; Kozlowski, J.; Li, S.; Liu, C.; Lowe, D.; Vice, S.; Zhao, H.; Zhou, G.; Billard, W.; Binch, H.; Crosby, R.; Duffy, R.; Lachowicz, J.; Coffin, V.; Watkins, R.; Ruperto, V.; Strader, C.; Taylor, L.; Cox, K. *Il Farmaco*, **2001**, *56*, 247.
- [40] Lachowicz, J.E.; Duffy, R.A.; Ruperto, V.; Kozlowski, J.; Zhou, G.; Clader, J.; Billard, W.; Binch, H., III; Crosby, G.; Cohen-Williams, M.; Strader, C.D.; Coffin, V. *Life Sci.*, **2001**, *68*, 2585.
- [41] Gualtieri, F. *Pharmaceutica Acta Helv.*, **2000**, *74*, 85.
- [42] Chavez-Noriega, L.E.; Crona, J.H.; Washburn, M.S.; Urrutia, A.; Elliott, K.J.; Johnson, E.C. *J. Pharmacol. Exp. Ther.*, **1997**, *280*, 346.
- [43] Lindstrom, J. *Mol. Neurobiol.*, **1997**, *15*, 193.
- [44] Newhouse, P.A.; Potter, A.; Kelton, M.; Corwin, J. *Biol. Psychiatry*, **2001**, *49*, 268.
- [45] Rusted, J.M.; Newhouse, P.A.; Levin, E.D. *Behav. Brain Res.*, **2000**, *113*, 121.
- [46] Glennon, R.A.; Dukat, M. *Pharmaceutica Acta Helv.*, **2000**, *74*, 103.
- [47] Decker, M.W.; Brioni, J.D.; Sullivan, J.P.; Buckley, M.J.; Radek, R.J.; Raskiewicz, J.L.; Kang, C.H.; Kim, D.J.B.; Giardina, W.J.; Wasicak, J.T.; Garvey, D.S.; Williams, M.; Arneric, S.P. *J. Pharmacol. Exp. Ther.*, **1994**, *270*, 319.
- [48] Decker, M.W.; Curzon, P.; Brioni, J.D.; Arneric, S.P. *Eur. J. Pharmacol.*, **1994**, *261*, 217.
- [49] Kem, W.R. *Behav. Brain Res.*, **2000**, *113*, 169.
- [50] Lin, N.-H.; Gunn, D.E.; Ryther, K.B.; Garvey, D.S.; Donnelly-Roberts, D.L.; Decker, M.W.; Brioni, J.D.; Buckley, M.J.; Rodrigues, A.D.; Marsh, K.G.; Anderson, D.J.; Buccafusco, J.J.; Prendergast, M.A.; Sullivan, J.P.; Williams, M.; Arneric, S.P.; Holladay, M.W. *J. Med. Chem.*, **1997**, *40*, 385.
- [51] Vernier, J.-M.; El-Abdellaoui, H.; Holsenback, H.; Cosford, N.D.P.; Bleicher, L.; Barker, G.; Bontempi, B.; Chavez-Noriega, L.; Menzaghi, F.; Rao, T.S.; Reid, R.; Saccaan, A.I.; Suto, C.; Washburn, M.; Lloyd, G.K.; McDonald, I.A. *J. Med. Chem.*, **1999**, *42*, 1684.
- [52] Decker, M.W.; Bannon, A.W.; Curzon, P.; Gunther, K.L.; Brioni, J.D.; Holladay, M.W.; Lin, N.-H.; Li, Y.; Daanen, J.F.; Buccafusco, J.J.; Prendergast, M.A.; Jackson, W.J.; Arneric, S.P. *J. Pharmacol. Exp. Ther.*, **1997**, *283*, 247.
- [53] Lloyd, G.K.; Menzaghi, F.; Bontempi, B.; Suto, C.; Siegel, R.; Akong, M.; Stauderman, K.; Velicelebi, G.; Johnson, E.; Harpold, M.M.; Rao, T.S.; Saccaan, A.I.; Chavez-Noriega, L.E.; Washburn, M.S.; Vernier, J.M.; Cosford, N.D.P.; McDonald, L.A. *Life Sci.*, **1998**, *62*, 1601.
- [54] Rylett, R.J.; Ball, J.M.; Colhoun, E.H. *Brain Res.*, **1983**, *289*, 169.
- [55] Ricciardi, S.; Bisiani, C.; Camisasca, C.; Fusi, R.; Ornaghi, F.; Pastoris, R.; Scatturin, M.; Masotto, C. *J. Drug Dev.*, **1994**, *6*, 159.
- [56] Chaki, H.; Yamabe, H.; Sugano, M.; Morita, S.; Bessho, T.; Tabata, R.; Saito, K.-I.; Egawa, M.; Tobe, A.; Morinaka, Y. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1495.
- [57] Schneider, L.S. *Curr. Opin. CPNS Invest. Drugs*, **2000**, *2*, 427.
- [58] Giacobini, E. *Neurochem. Int.*, **1998**, *32*, 413.
- [59] Brufani, M.; Filocamo, L.; Lappa, S.; Maggi, A. *Drugs Future*, **1997**, *22*, 397.
- [60] Imbimbo, B.P. *CNS Drugs*, **2001**, *15*, 375.
- [61] Imbimbo, B.P.; Martelli, P.; Troetel, W.M.; Lucchelli, F.; Lucca, U.; Thal, L.J. *Neurology*, **1999**, *52*, 700.
- [62] Snape, M.F.; Misra, A.; Murray, T.K.; De Souza, R.J.; Williams, J.L.; Cross, A.J.; Green, A.R. *Neuropharmacology*, **1999**, *38*, 181.
- [63] Miguel-Hidalgo, J.J. *Curr. Opin. CPNS Invest. Drugs*, **2000**, *2*, 438.
- [64] Greig, N.H.; De Micheli, E.; Holloway, H.W.; Yu, Q.-S.; Utsuki, T.; Perry, T.A.; Brossi, A.; Ingram, D.K.;

- Deutsch, J.; Lahiri, D.K.; Soncrant, T.T. *Acta Neurol. Scand.*, **2000**, *102*, 74.
- [65] Kamal, M.A.; Greig, N.H.; Alhomida, A.S.; Al-Jafari, A.A. *Biochem. Pharmacol.*, **2000**, *60*, 561.
- [66] Patel, N.; Spangler, E.L.; Greig, N.H.; Yu, Q.S.; Ingram, D.K.; Meyer, R.C. *Neuroreport*, **1998**, *9*, 171.
- [67] Borroni, E.; Damsma, G.; Giovacchini, C.; Mutel, V.; Jakob-Rötne, R.; Da Prada, M. *Biochem. Soc. Trans.*, **1994**, *22*, 755.
- [68] Trabace, L.; Cassano, T.; Steardo, L.; Pietra, C.; Villetti, G.; Kendrick, K.M.; Cuomo, V. *J. Pharmacol. Exp. Ther.*, **2000**, *294*, 187.
- [69] Smith, C.P.; Bores, G.M.; Petko, W.; Li, M.; Selk, D.E.; Rush, D.K.; Camacho, F.; Winslow, J.T.; Fishkin, R.; Cunningham, D.M.; Brooks, K.M.; Roehr, J.; Hartman, H.B.; Davis, L.; Vargas, H.M. *J. Pharmacol. Exp. Ther.*, **1997**, *280*, 710.
- [70] Ormrod, D.; Spencer, C. *CNS Drugs*, **2000**, *13*, 443.
- [71] Nair, H.K.; Lee, K.; Quinn, D.M. *J. Am. Chem. Soc.*, **1993**, *115*, 9939.
- [72] Zhu, X.-D.; Giacobini, E.; Hornsperger, J.-M. *Eur. J. Pharmacol.*, **1995**, *276*, 93.
- [73] Gualtieri, F.; Deu, S.; Manetti, D.; Romanelli, M.N. *Farmacologie*, **1995**, *50*, 489.
- [74] Davis, M. *New Engl. J. Med.*, **1992**, *327*, 1253.
- [75] Shutske, G.M.; Pierrat, F.A.; Cornfeldt, M.L.; Szewczak, M.R.; Huger, F.P.; Bores, G.M.; Haroutunian, V.; Davis, K.L. *J. Med. Chem.*, **1988**, *31*, 1278.
- [76] Shutske, G.M.; Pierrat, F.A.; Kapples, K.J.; Cornfeldt, M.L.; Szewczak, M.R.; Huger, F.P.; Bores, G.M.; Haroutunian, V.; Davis, K.L. *J. Med. Chem.*, **1989**, *32*, 1805.
- [77] Dejmek, L. *Drugs Future*, **1990**, *15*, 126.
- [78] Murphy, M.F.; Hardiman, S.T.; Nash, R.J.; Huff, F.J.; Demkovich, J.J.; Dobson, C.; Knappe, U.E. *Ann. N. Y. Acad. Sci.*, **1991**, *640*, 253.
- [79] Yoshida, S.; Suzuki, N. *Eur. J. Pharmacol.*, **1993**, *250*, 117.
- [80] Gregor, V.E.; Emmerling, M.R.; Lee, C.; Moore, C.J. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 861.
- [81] Anonymous. *Drugs Future*, **1991**, *16*, 33.
- [82] Emmerling, M.R.; Gregor, V.E.; Schwarz, R.D.; Scholten, J.D.; Callahan, M.J.; Lee, C.; Moore, C.J.; Raby, C.; Lipinsky, W.J.; Davis, R.E. *Mol. Neurobiol.*, **1994**, *9*, 93.
- [83] Ros, E.; Aleu, J.; Marsal, J.; Solsona, C. *Eur. J. Pharmacol.*, **2000**, *390*, 7.
- [84] Sugimoto, H.; Yamanishi, Y.; Iimura, Y.; Kawakami, Y. *Curr. Med. Chem.*, **2000**, *7*, 303.
- [85] Kosasa, T.; Kuriya, Y.; Matsui, K.; Yamanishi, Y. *Eur. J. Pharmacol.*, **2000**, *389*, 173.
- [86] Ishihara, Y.; Goto, G.; Miyamoto, M. *Curr. Med. Chem.*, **2000**, *7*, 341.
- [87] Mucke, H.A.M.; Castaner, J. *Drugs Future*, **1998**, *23*, 1075.
- [88] Martinez, A.; Fernandez, E.; Castro, A.; Conde, S.; Rodriguez-Franco, I.; Baños, J.E.; Badia, A. *Eur. J. Med. Chem.*, **2000**, *35*, 913.
- [89] Villalobos, A.; Blake, J.F.; Biggers, C.K.; Butler, T.W.; Chapin, D.S.; Chen, Y.L.; Ives, J.L.; Jones, S.B.; Liston, D.R.; Nagel, A.A.; Nason, D.M.; Nielsen, J.A.; Shalaby, I.A.; White, W.F. *J. Med. Chem.*, **1994**, *37*, 2721.
- [90] Sramek, J.J.; Frackiewicz, E.J.; Cutler, N.R. *Exp. Opin. Invest. Drugs*, **2000**, *9*, 2393.
- [91] Maelicke, A.; Samochocki, M.; Jostock, R.; Fehrenbacher, A.; Ludwig, J.; Alburquerque, E.X.; Zerlin, M. *Biol. Psychiatry*, **2001**, *49*, 279.
- [92] Bores, G.M.; Kosley, R.W., Jr. *Drugs Future*, **1996**, *21*, 621.
- [93] Kozikowski, A.P.; Tückmantel, W. *Acc. Chem. Res.*, **1999**, *32*, 641.
- [94] Bai, D.L.; Tang, X.C.; He, X.C. *Curr. Med. Chem.*, **2000**, *7*, 355.
- [95] Carlier, P.R.; Han, Y.F.; Chow, E.S.-H.; Li, C.P.-L.; Wang, H.; Lieu, T.X.; Wong, H.S.; Pang, Y.-P. *Bioorg. Med. Chem.*, **1999**, *7*, 351.
- [96] Carlier, P.R.; Chow, E.S.-H.; Han, Y.; Liu, J.; El Yazal, J.; Pang, Y.-P. *J. Med. Chem.*, **1999**, *42*, 4225.
- [97] Carlier, P.R.; Du, D.-M.; Han, Y.; Liu, J.; Pang, Y.-P. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2335.
- [98] Carlier, P.R.; Du, D.-M.; Han, Y.-F.; Liu, J.; Perola, E.; Williams, I.D.; Pang, Y.-P. *Angew. Chem., Int. Ed. Engl.*, **2000**, *39*, 1775.
- [99] Mary, A.; Renko, D.Z.; Guillou, C.; Thal, C. *Bioorg. Med. Chem.*, **1998**, *6*, 1835.
- [100] Liu, J.; Ho, W.-L.; Lee, N.T.-K.; Carlier, P.R.; Pang, Y.-P.; Han, Y.-F. *Neurosci. Lett.*, **2000**, *282*, 165.
- [101] Xiao, X.Q.; Lee, N.T.-K.; Carlier, P.R.; Pang, Y.-P.; Han, Y.F. *Neurosci. Lett.*, **2000**, *290*, 197.
- [102] Contreras, J.-M.; Rival, Y.M.; Chayer, S.; Bourguignon, J.-J.; Wermuth, C.G. *J. Med. Chem.*, **1999**, *42*, 730.
- [103] Contreras, J.-M.; Parrot, I.; Sippl, W.; Rival, Y.M.; Wermuth, C.G. *J. Med. Chem.*, **2001**, *44*, in press.
- [104] Camps, P.; Muñoz-Torrero, D. *Mini Rev. Med. Chem.*, **2001**, *1*, 163.
- [105] Ros, E.; Aleu, J.; Gómez de Aranda, I.; Muñoz-Torrero, D.; Camps, P.; Badia, A.; Marsal, J.; Solsona, C. *Eur. J. Pharmacol.*, **2001**, *421*, 77.
- [106] Weinstock, M.; Goren, T.; Youdim, M.B.H. *Drug Dev. Res.*, **2000**, *50*, 216.
- [107] Giacobini, E. *Neurochem. Res.*, **2000**, *25*, 1185.

- [108] Weinstock, M. *CNS Drugs*, **1999**, *12*, 307.
- [109] Harrison, P.J.; Barton, A.J.L.; Najlerahim, A.; McDonald, B.; Pearson, R.C.A. *Mol. Brain Res.*, **1991**, *9*, 15.
- [110] Felder, C.C.; Bymaster, F.P.; Ward, J.; DeLapp, N. *J. Med. Chem.*, **2000**, *43*, 4333.
- [111] Korczyn, A.D. *Exp. Opin. Invest. Drugs*, **2000**, *9*, 2259.
- [112] Fisher, A. *CNS Drugs*, **1999**, *12*, 197.
- [113] Schwarz, R.D.; Callahan, M.J.; Davis, R.E.; Jaen, J.C.; Teclé, H. *Drug Dev. Res.*, **1997**, *40*, 133.
- [114] Hulme, E.C.; Birdsall, N.J.M.; Buckley, N.J. *Ann. Rev. Pharmacol. Toxicol.*, **1990**, *30*, 633.
- [115] Ringdahl, B. *Mol. Pharmacol.*, **1987**, *31*, 351.
- [116] Bymaster, F.P.; Whitesitt, C.A.; Shannon, H.E.; DeLapp, N.; Ward, J.S.; Calligaro, D.O.; Shipley, L.A.; Buelkesam J.L.; Bodick, N.C.; Farde, L.; Sheardown, M.J.; Olesen, P.H.; Hansen, K.T.; Suzdak, P.P.; Swedberg, M.D.B.; Sauerberg, P.; Mitch, C.H. *Drug Dev. Res.*, **1997**, *40*, 158.
- [117] Meier, E.; Frederiksen, K.; Nielsen, M.; Lembol, H.L.; Pedersen, H.; Hyttel, J. *Drug Dev. Res.*, **1997**, *40*, 1.
- [118] Thal, L.J.; Forrest, M.; Loft, H.; Mengel, H. *Neurology*, **2000**, *54*, 421.
- [119] Sedman, A.J.; Bockbrader, H.; Schwarz, R.D. *Life Sci.*, **1995**, *56*, 877.
- [120] Watson, J.M.; Hunter, A.J.; Brown, A.M.; Middlemiss, D.N. *Eur. J. Pharmacol.*, **1999**, *370*, 69.
- [121] Bromidge, S.M.; Brown, F.; Cassidy, F.; Clark, M.S.G.; Dabbs, S.; Hadley, M.S.; Hawkins, J.; Loudon, J.M.; Naylor, C.B.; Orlek, B.S.; Riley, G.J. *J. Med. Chem.*, **1997**, *40*, 4265.
- [122] M'Harzi, M.; Willig, F.; Gieules, C.; Palou, A.-M.; Oberlander, C.; Barzaghi, F. *Pharmacol. Biochem. Behav.*, **1997**, *56*, 663.
- [123] Teclé, H.; Schwarz, R.D.; Barrett, S.D.; Callahan, M.J.; Caprathe, B.W.; Davis, R.E.; Doyle, P.; Emmerling, M.; Lauffer, D.J.; Mirzadegan, T.; Moreland, D.W.; Lipiniski, W.; Nelson, C.; Raby, C.; Spencer, C.; Spiegel, K.; Thomas, A.J.; Jaen, J.C. *Pharmaceutica Acta Helv.*, **2000**, *74*, 141.
- [124] Messer, W.S., Jr.; Dunbar, P.G.; Rho, T.; Periyasamy, S.; Ngur, D.; Ellerbrock, B.R.; Bohnett, M.; Ryan, K.; Durant, G.J.; Hoss, W. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 781.
- [125] Messer, W.S., Jr.; Abuh, Y.F.; Liu, Y.; Periyasamy, S.; Ngur, D.O.; Edgar, M.A.N.; El-Assadi, A.A.; Sbeih, S.; Dunbar, P.G.; Roknich, S.; Rho, T.; Fang, Z.; Ojo, B.; Zhang, H.; Huzl, J.J., III; Nagy, P.I. *J. Med. Chem.*, **1997**, *40*, 1230.
- [126] Messer, W.S., Jr.; Rajeswaran, W.G.; Cao, Y.; Zhang, H.-J.; El-Assadi, A.A.; Dockery, C.; Liske, J.; O'Brien, J.; Williams, F.E.; Huang, X.-P.; Wroblewski, M.E.; Nagy, P.I.; Peseckis, S.M. *Pharmaceutica Acta Helv.*, **2000**, *74*, 135.
- [127] Shapiro, G.; Floersheim, P.; Amstutz, R.; Boddeke, H.; Bolliger, G.; Cottens, S.; Enz, A.; Gmelin, G.; Gull, P.; Supavilai, P. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 815.
- [128] Yamaguchi, T.; Suzuki, M.; Yamamoto, M. *Brain Res.*, **1995**, *669*, 107.
- [129] Fisher, A. *Jpn. J. Pharmacol.*, **2000**, *84*, 101.
- [130] Wienrich, M.; Meier, D.; Ensinger, H.A.; Gaida, W.; Raschig, A.; Walland, A.; Hammer, R. *Life Sci.*, **2001**, *68*, 2593.
- [131] Sanders, K.B.; Thomas, A.J.; Pavia, M.R.; Davis, R.E.; Coughenour, L.L.; Myers, S.L.; Fisher, S.; Moos, W.H. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 803.
- [132] Conti, P.; Dallanocce, C.; De Amici, M.; De Micheli, C.; Ebert, B. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1033.
- [133] Brass, E.P.; Polinsky, R.; Sramek, J.J.; Moore, M.; Jones, D.; Veroff, A.E.; Wardle, T.S.; Cutler, N.R. *J. Clin. Psychopharmacol.*, **1995**, *15*, 58.
- [134] DeLapp, N.; Wu, S.; Belagaje, R.; Johnstone, E.; Little, S.; Shannon, H.; Bymaster, F.; Calligaro, D.; Mitch, C.; Whitesitt, C.; Ward, J.; Sheardown, M.; Fink-Jensen, A.; Jeppesen, L.; Thomsen, C.; Sauerberg, P. *Biochem. Biophys. Res. Commun.*, **1998**, *244*, 156.
- [135] Beach, T.G.; Walker, D.G.; Potter, P.E.; Sue, L.I.; Fisher, A. *Brain Res.*, **2001**, *905*, 220.
- [136] Müller, D.M.; Mendla, K.; Farber, S.A.; Nitsch, R.M. *Life Sci.*, **1997**, *60*, 985.
- [137] Ellis, J. *Drug Dev. Res.*, **1997**, *40*, 193.
- [138] Buccafusco, J.J.; Terry, A.V., Jr. *J. Pharmacol. Exp. Ther.*, **2000**, *295*, 438.
- [139] Dringenberg, H.C. *Behav. Brain Res.*, **2000**, *115*, 235.
- [140] Benzi, G.; Moretti, A. *Eur. J. Pharmacol.*, **1998**, *346*, 1.
- [141] Dringenberg, H.C.; Diavolitsis, P.; Noseworthy, P.A. *Neurobiol. Aging*, **2000**, *21*, 135.
- [142] Wenk, G.L.; Quack, G.; Moebius, H.-J.; Danysz, W. *Life Sci.*, **2000**, *66*, 1079.