Peripheral and Dual Binding Site Acetylcholinesterase Inhibitors: Implications in treatment of Alzheimer's Disease

Ana Castro and Ana Martinez*

Instituto de Química Médica (C.S.I.C.), Juan de la Cierva, 3, 28006 Madrid (Spain)

Abstract: Recently advances in understanding the molecular basis of Alzheimer's disease have led to the consideration of the relationship between cholinergic inhibitors and amyloid deposition as a new hypothesis for the future rational design of effective anti-Alzheimer drugs. In the present review, the non-cholinergic functions of acetylcholinesterase (AChE) and the therapeutic potential of peripheral and dual binding site AChE inhibitors in delaying the neurodegenerative process will be discussed.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is one of the most common causes of mental deterioration in elderly people, accounting for about 50-60 % of the overall cases of dementia among persons over 65 years of age [1]. Brain regions that are associated with higher mental functions, particularly the neocortex and hippocampus, are those most affected by the characteristic pathology of AD [2]. This includes the extracellular deposits of β-amyloid (derived from amyloid precursor protein, APP) in senile plaques [3, 4], intracellular formation of neurofibrillary tangles (containing an abnormally phosphorylated form of a microtubule associated protein, tau) [5, 6], and the loss of neuronal synapsis and pyramidal neurons [7]. These changes result in the development of the typical symptomology of AD characterized by gross and progressive impairments of cognitive function and often accompanied by behavioral disturbances such as aggression, depression and wandering [8].

The past two decades have witnessed a considerable research effort directed towards discovering the cause of AD with the ultimate hope of developing safe and effective pharmacological treatments [9]. Nowadays, research in the knowledge of the pathogenic cascade that characterizes AD has provided a robust framework for new therapeutic intervention targets [10]. As a consequence, there are multiple hypotheses around which therapeutic agents can be developed, including drugs that interfere with the synthesis, deposition and aggregation of β-amyloid protein [11], or with the hyperphosphorylation of tau protein [12]. In addition, transmitter replacement therapies [13], anti-inflammatory agents [14], antioxidants approach [15, 16], molecules with nerve growth factor like activity [17], estrogens therapy [18] or immune response [19, 20] are currently being proposed or utilized in disease prevention trials [21, 22].

Nevertheless, current treatment approaches in this disease continue being primarily symptomatic [23], with the major therapeutic strategy based on the cholinergic hypothesis [24] and specifically on acetylcholinesterase (AChE) inhibition [25]. The successful development of these compounds was based on a well-accepted theory that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission [26]. This link between cholinergic dysfunction in the basal-cortical system and cognitive deficits has focused scientific efforts on developing tools to elucidate the neurobiological role of the cholinergic system in cognition and to elucidate therapeutic interventions in the disorder [27]. As result, over last decade, the cholinergic hypothesis of AD has launched on the market various cholinergic drugs primarily AChE inhibitors as tacrine [28], donepezil [29] or rivastigmine [30], (Fig. **1**), indicated modest improvement in the cognitive function of Alzheimer's patients.

The three dimensional structure of AChE, as determined by x-ray crystallography, revealed that its active site can apparently be reached only through a deep and narrow

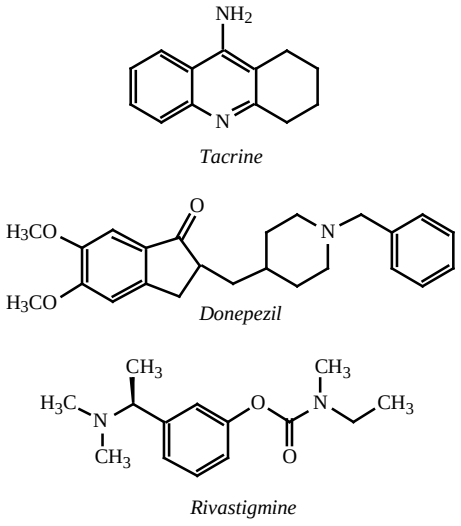

Fig. (1). AChE inhibitors in current use for the treatment of Alzheimer's disease.

*Address correspondence to this author at the Instituto de Química Médica (C.S.I.C.), Juan de la Cierva, 3, 28006 Madrid (Spain)

1389-5575/01 $20.00+.00

"catalytic gorge" [31]. Inhibitors of AChE act on two target sites on the enzyme, the active site and the peripheral site. Inhibitors directed to the active site prevent the binding of a substrate molecule, or its hydrolysis, either by occupying the site with a high affinity (tacrine) [32] or by reacting irreversibly with the catalytic serine (organophosphates and carbamates) [33]. The peripheral site consists of a less well-defined area, located at the entrance of the catalytic gorge. Inhibitors that bind to that site include small molecules, such as propidium [34, 35] and peptide toxins as fasciculins [36]. Bis-quaternary inhibitors as decamethonium [37], simultaneously bind to the active and peripheral sites, thus occupying the entire catalytic gorge.

Recently, although the correlation between plaques, tangles and dementia is not absolute, advances in understanding the molecular basis of AD have led to consideration of new hypotheses which take into account both cholinergic deficits and neuropathologic lesions [38-40]. In the present review, we summarize the non-cholinergic functions of AChE and the potential of peripheral and dual binding site acetylcholinesterase inhibitors in delaying the neurodegenerative process of AD.

2. NON-CHOLINERGIC FUNCTIONS OF AChE: RELATIONSHIPS BETWEEN AChE AND β-AMYLOID

Parallel to the development of antidementia drugs, research efforts have been focused, among others, on the therapeutic potential of AChE inhibitors to slow the disorder progression. This fact was based on a range of evidence, which showed that AChE has secondary non-cholinergic functions [41, 42].

It is well established that the function of AChE at cholinergic synapses is to terminate cholinergic neurotransmission, however, AChE is expressed in tissues that are not directly innervated by cholinergic nerves [43]. Recent results indicate that AChE has an extrasynaptic, noncholinergic role during neural development [44]. Abnormal expression of AChE and BChE has been detected around the amyloid plaques and neurofibrillary tangles in the brains of patients with AD which has implications for pathogenesis and for therapeutic strategies of AD [45].

New evidence shows that AChE may have a direct role in neuronal differentiation [46]. Transient expression of AChE in the brain during embryogenesis suggests that AChE may function in the regulation of neurite outgrowth [47, 48] and in the development of axon tracts [49]. Additionally, the role of AChE in cell adhesion have been studied [50]. The results indicate that AChE promotes neurite outgrowth in neuroblastoma cell line through a cell adhesive role [51]. Moreover, recent studies have shown that the peripheral anionic site of the AChE is involved in the neurotrophic activity of the enzyme [52] and conclude that the adhesion function of AChE is located at the peripheral anionic site [53]. This finding has implications, not only for our understanding of neural development and its disorders, but also for the treatment of neuroblastoma, the leukemias, and especially for Alzheimer's disease [54].

As it has been previously mentioned, senile plaques are one of pathological hallmarks in AD in which their main component is βA peptide. This is found as an aggregated poorly soluble form. In contrast soluble βA is identified normally circulating in human body fluids. Structural studies of βA showed that synthetic peptides containing the sequences 1-40 and 1-42 of βA can adopt two major conformational states in solution: an amyloidogenic conformer (βA ac) with a high content of β-sheet and partly resistant to proteases and a non-amyloidogenic conformer (βA nac) with a random coil conformation or α-helix and protease-sensitive. AChE colocalizes with βA peptide deposits present in the brain of Alzheimer's patients [55]. It is postulated that AChE binds to a βA nac form acting as a "pathological chaperone" and inducing a conformational transition from βA nac into βA ac in vitro and therefore to amyloid fibrils [56]. AChE directly promotes the assembly of βA peptide into amyloid fibrils forming stable βA-AChE complexes [57]. These complexes are able to change the biochemical and pharmacological properties of the enzyme and cause an increase in the neurotoxicity of the βA fibrils [58, 59]. Moreover, the interaction between these two molecules to form the complex was confirmed by crosslinking experiments [60]. Different studies concerned to the establishment of the binding site of AChE on Aβ have suggested that hydrophobic interactions may play a role in the stabilization of the βA-AChE complex probably due to specific binding to peripheral sites [61].

Considering the non-cholinergic aspects of the cholinergic enzyme AChE, their relationship to Alzheimer's hallmarks and the role of the peripheral site of AChE in all these functions, an attractive target for the design of new antidementia drugs emerged. Peripheral or dual site inhibitors of AChE may simultaneously alleviate the cognitive deficit in Alzheimer's patients and what it is more important, avoid the assembly of beta-amyloid which represents a new way to delay the neurodegenerative process.

3. PERIPHERAL AND DUAL BINDING SITE AChE INHIBITORS

As revealed by the crystallographic structure of AChE and their inhibitors complexes, the AChE active site contains a catalytic triad (Ser 200, His 440, Glu 327) located at the bottom of a deep and narrow gorge, lined with aromatic residues and a subsite, including Trp 84, located near the bottom of the cavity. Trp 84 has been identified as the binding site of the quaternary group of acetylcholine, decamethonium and edrophonium [31]. In addition, Trp 279 at the peripheral site, located at the opening of the gorge, is involved in the binding of the second quaternary group of decamethonium being responsible for the adhesion function of the enzyme [53].

These residues (Trp 84 and 279) have been the basis of the design of a new generation of AChE inhibitors. Thus, ligands able to interact simultaneously with active and peripheral sites could implicate several advantages over the known inhibitors. On one hand, they should improve greatly the inhibitory potency and on the other hand they should be involved in neurotrophic activity.

The first reported compounds following this idea have been bis-tetrahydroaminoacridine (bis-THA) derivatives [62]. Pang and co-workers designed and synthesized bifunctional analogues of THA using computer modeling of ligand docking with the target protein. The strategy was to connect two THA molecules with an alkylene chain spaced to allow simultaneous binding at the catalytic and peripheral sites. The best results were found with heptylene-linked THA dimer (**1**), 149-fold more potent and 250-fold more selective for AChE inhibition than THA, (Fig. **2**). These studies suggest that a low affinity THA peripheral site exits in AChE which may be responsible for the noncholinergic biological effects observed after the treatment with tacrine [63, 64]. Complementary studies were carried out in order to define the mimimun length of the linker between the two tacrine moieties that allow to bind simultaneously at both sites of the enzyme [65]. Additionally, in an effort to further delineate structural requirements for optimal binding to the AChE peripheral site, dimer AChE inhibitors containing a single THA moiety have been synthesized [66]. Calculations on these THA heterodimers, such as **2**, confirm the importance of ligand hydrophobicity for effective cation-π interaction with the peripheral AChE site residues. It is worthmentioning that further pharmacological studies on bis(7)-tacrine **1** have provided evidence for their potential usage in the prevention and treatment of Alzheimer's disease [67-69].

Recently, others bis-interacting ligands in the galanthamine series have been reported [70, 71]. Derivatives **3** and **4** have been described to be highly potent inhibitors of AChE, (Fig. **2**). These examples confirmed that AChE inhibitors, which can simultaneously interact with catalytic and peripheral site, show enhanced potencies compared to compounds which interact with a single site.

De novo design techniques have been applied to new dual binding site acetylcholinesterase inhibitors. The chemical structure of the theoretical designed imidazoles **5** allows the simultaneous interaction with the catalytic and the peripheral anionic site. The binding constants of the candidate molecules were calculated and exhibited lower interaction energies than those of known inhibitors such as THA or huperzine A [72]. These new ligands should more effectively bind to AChE than the evaluated inhibitors.

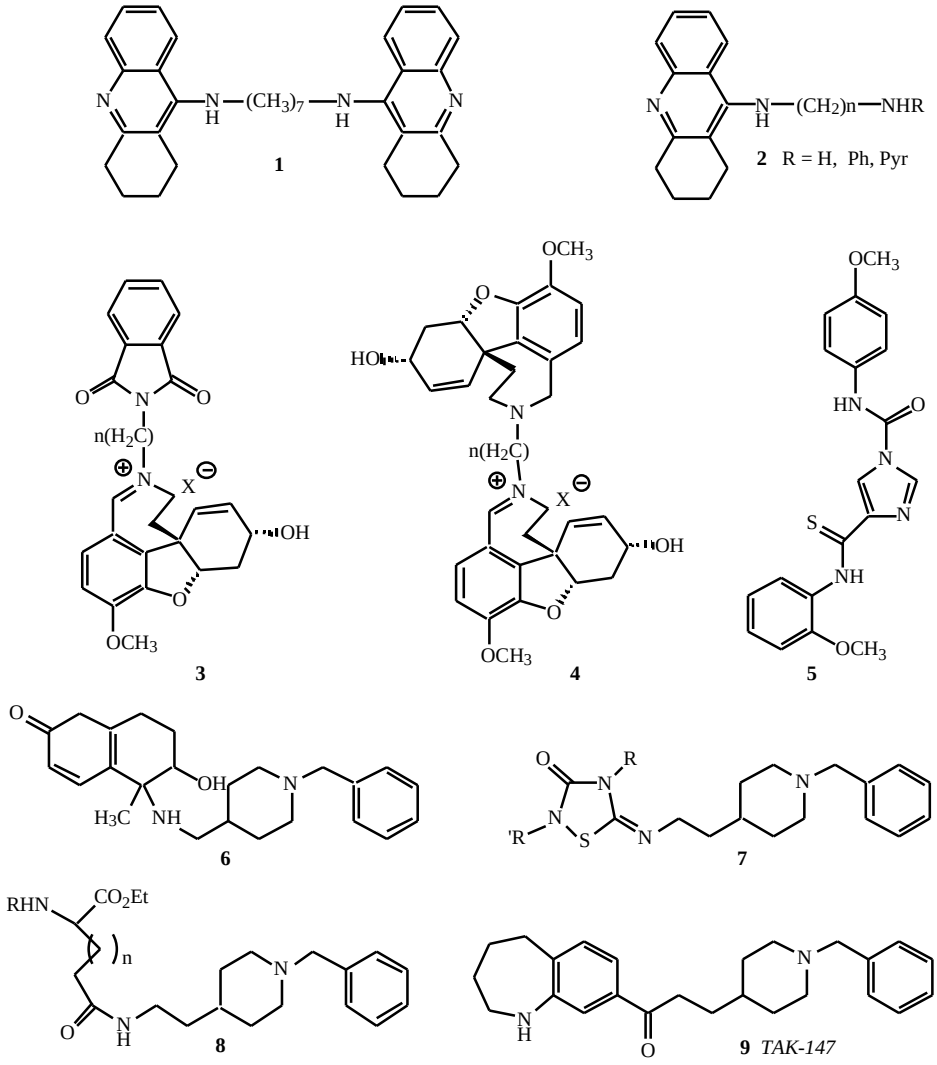

Fig. (2). Dual binding site AChE inhibitors.

As the adhesion function of AChE is located in the peripheral site where Trp279 is situated [53, 54], an inhibitor blockage of this amino acid forming a cation-π or an π−π interaction should inhibit the noncholinergic enzymatic function. This is the case of donepezil, (Fig. **1**), a commercially available drug for Alzheimer's disease. The first molecular modeling work on its binding mode [73, 74] revealed an interaction of the indadone moiety with Trp279 whilst the benzylpiperidine fragment interacts in the catalytic site with the Trp84. This topography has been confirmed with the recent X-ray resolution of the complex donepezil-AChE, providing new clues for further design of new dual binding site inhibitors [75]. This is the case of the huperzine A-E2020 combined compounds (**6**) [76], 1,2,4-thiadiazolidinone (**7**) [77], amino acids (**8**) [78] or benzazepine derivatives (**9**) [79] in which the N-benzylpiperidine fragments is conserved, (Fig. **2**). In all cases, the molecular modeling studies revealed interactions both with amino acids located in the active and peripheral site of AChE as Trp84 and Trp279. So, cholinergic and noncholinergic responses should be expected after the treatment with these drugs. The neurotrophic activity on central cholinergic neurons of TAK-147 at concentrations where it inhibits AChE activity has been recently reported [80]. Therefore, TAK-147 and potentially peripheral and dual binding site AChE inhibitors, are expected not only to ameliorate the clinical symptoms in Alzheimer's disease via AChE inhibition but to prevent or slow the progression of the disease via their neurotrophic action.

Recently, some heterocyclic compounds have been designed as AChE inhibitors able to interact with peripheral anionic site of the enzyme. This is the case of 4,4'-bipyridine (**10**) [81], the substituted phenyl carbamates (**11**) [82] or the indenoquinolinylamine (**12**) [83]. The inhibition mechanism in the three cases was determined from kinetic studies. Thus, the compounds are characterized as peripheral anionic site-directed inhibitors of AChE, (Fig. **3**). Huprine X (**13**), a hybrid that combines the carbocyclic substructure of huperzine A with the 4-aminoquinoline substructure of tacrine, has one of the highest affinities for AChE yet reported [84]. The location of its binding site on enzyme was probed in competition studies with the peripheral site inhibitor propidium and the acylation site inhibitor edrophonium. Results indicated that huprine X binds to the enzyme acylation site in the active site gorge but interferes slightly with the binding of peripheral site ligands [85].

CONCLUSIONS

AChE directly promotes the assembly of βA peptide into amyloid fibrils forming stable βA- AChE complexes. These amyloid fibrils are neurotoxic. The adhesion function of AChE to βA is located in the peripheral site through Trp 279. This fact together with the existence of peripheral and dual binding site AChE inhibitors able to interact with Trp 279 suppose a novel approach for drug design that are opening new tactics for developing anti-dementia drugs. These novel therapeutic agents could interfere simultaneously with the cholinergic deficit and with the synthesis, deposition and aggregation of βA protein *via* AChE peripheral site inhibition [86]. Therefore, in a near future this strategy could provide a single effective therapy for the devastating Alzheimer's disease being both symptomatic, facilitating the memory process, and preventive, exerting a neuroprotective effect.

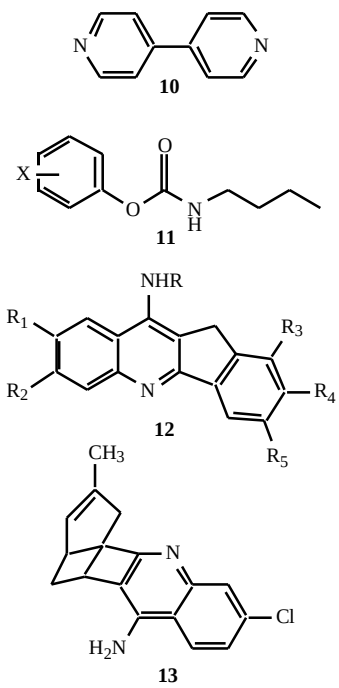

Fig. (3). Peripheral AChE inhibitors.

ACKNOWLEDGEMENTS

Financial support from CICYT (project no. SAF 99/98) is acknowledged.

REFERENCES

[1] Bennett, D.A. Alheimer's disease. Part I. *Dis. Mon.*, **2000**, *46*, 657-665.

[2] Shinosaki, K.; Nishikawa, T.; Takeda, M. *Psychiatry Clin. Neurosci.*, **2000**, *54*, 611-620.

[3] Neve, R.L.; McPhie, D.L.; Chen, Y. *Brain Res.*, **2000**, *886*, 54-66.

[4] Bayer, T.A.; Wirths, O.; Majtenyi, K.; Hartmann, T.; Multhaup, G.; Beyreuther, K.; Czech, C. *Brain Pathol.*, **2001**, *11*, 1-11.

[5] Crowther, R.A.; Goedert, M. *J. Struct. Biol.*, **2000**, *130*, 271-279.

[6] Iqbal, K.; Alonso, A.D.; Gondal, J.A.; Gong, C.X.; Haque, N.; Khatoon, S.; Sengupta, A.; Wang, J.Z.; Grundke-Iqbal, I. *J. Neural Transm. Suppl.*, **2000**, *59*, 213-222.

[7] Terry, R.D. J. *Neuropathol. Exp. Neurol.*, **2000**, *59*, 1118-1119.

[8] Cummings, J.L. *Neurobiol. Aging*, **2000**, *21*, 845-861.

[9] Grundman, M.; Corey-Bloom, J., Thal, L.J. *J. Neural Transm. Suppl.*, **1998**, *53*, 255-275.

[10] Grundman, M.; Thal, L.J. **2000,** *18*, 807-828.

[11] Harkany, T.; Abraham, I.; Konya, C.; Nyakas, C.; Zarandi, M.; Penke, B.; Luiten, P.G. *Rev. Neurosci.*, **2000**, *11*, 329-382.

[12] Castro, A.; Martinez, A. *Exp. Opin. Ther. Patents*, **2000**, *10*, 1519-1527.

[13] Mohr, E.; Mendis, T.; Rusk, I.N.; Grimes, J.D. *J. Psychiatry Neurosci.*, **1994**, *19*, 17-23.

[14] McGeer, P.L.; McGeer, E.G.; Yasojima, K. *J. Neural Transm. Suppl.*, **2000,** *59*, 53-57.

[15] Smith, M.A.; Rottkamp, C.A.; Nunomura, A.; Raina, A.K., Perry, G. *Biochim. Biophys. Acta*, **2000**, *1502*, 139-144.

[16] Rottkamp, C.A.; Nunomura, A.; Raina, A.K.; Sayre, L.M.; Perry, G.; Smith, M.A. *Alzheimer Dis. Assoc. Disord.*, **2000**, *14 Suppl 1*, S62-66.

[17] Nabeshima, T.; Yamada, K. *Alheimer Dis. Assoc. Disord.*, **2000**, *14 Suppl 1*, S39-46.

[18] Granholm, A.C.. *Expert Opin. Investig. Drugs*, **2000,** *9*, 685-694.

[19] Chorsky, R.L.; Yaghmai, F.; Hill, W.D.; Stopa, E.G. *Med. Hypotheses*, **2001**, *56*, 124-127.

[20] Frenkel, D.; Katz, O.; Solomon, B. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 11455-11459.

[21] Sramek, J.J.; Cutler, N.R. *Expert Opin. Investig. Drugs*, **2000**, *9*, 899-915.

[22] Issa, A.M.; Keyserlingk, E.W. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2000**, *24*, 1229-1249.

[23] Cacabelos, R.; Alvarez, A.; Lombardi, V.; Fernandez-Novoa, L.; Corzo, L.; Perez, P.; Laredo, M.; Pichel, V.; Hernandez, A.; Varela, M.; Figueroa, J.; Prous, J.; Windisch, M.; Vigo, C. *Drugs Today*, **2000**, *36*, 415-499.

[24] Francis, P.T.; Palmer, A.M.; Snape, M.; Wilcock, G.K. *J. Neurol. Neurosurg. Psychiatry*, **1999**, *66*, 137-147.

[25] Taylor, P. *Neurology*, **1998**, *51*, S30-35.

[26] Bartus, R.T. *Exp. Neurol.*, **2000**, *163*, 495-529.

[27] VanDenBerg, C.M.; Kazmi, Y.; Jann, M.W. *Drugs Aging*, **2000**, *16*, 123-138.

[28] Kurz, A. *J. Neural. Transm. Suppl.*, **1998**, *54*, 295-299.

[29] Dooley, M.; Lamb, H.M. *Drugs Aging*, **2000**, *16*, 199-226.

[30] Gottwald, M.D.; Rozanski, R.I. *Expert Opin. Investig. Drugs*, **1999**, *8*, 1673-1682.

[31] Sussman, J.L.; Harel, M.; Silman, I. *Chem. Biol. Interact.*, **1993**, *87*, 187-197.

[32] Wlodek, S.T.; Antosiewicz, J.; McCammon, J.A.; Stratsma, T.P.; Gilson, M.K.; Briggs, J.M.; Humblet, C.; Sussman, J.L. *Biopolymers*, **1996**, *38*, 109-117.

[33] Bartolucci, C.; Perola, E.; Cellai, L.; Brufani, M.; Lamba, D. *Biochem.*, **1999**, *38*, 5714-5719.

[34] Szegletes, T.; Mallender, W.D.; Thomas, P.J.; Rosenberry, T.L. *Biochem.*, **1999**, *38*, 122-133.

[35] Rosenberry, T.L.; Mallender, W.D.; Thomas, P.J.; Szegletes, T. *Chem. Biol. Interact.*, **1999**, *119-120*, 85-97.

[36] Harel, M.; Kleywegt, G.J.; Ravelli, R.B.; Silman, I.; Sussman, J.L. *Structure*, **1995**, *3*, 1355-1366.

[37] Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P.H.; Silman, I.; Sussman, J.L. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 9031-9035.

[38] Lahiri, D.K.; Farlow, M.R.; Nurnberger, J.I.; Greig, N.H. *Ann. N. Y. Acad. Sci.*, **1997**, *826*, 416-421.

[39] Roberson, M.R.; Harrell, L.E. *Brain Res. Brain Res. Rev.*, **1997**, *25*, 50-69.

[40] Windish, M. *J. Neural Transm. Suppl.*, **2000**, *59*, 301-313.

[41] Balasubramanian, A.S.; Bhanumathy, C.D. *FASEB J.*, **1993**, *7*, 1354-1358.

[42] Blasina, M.F.; Faria, A.C.; Gardino, P.F.; Hokoc, J.N.; Almeida, O.M.; Mello, F.G.; Arruti, C.; Dajas, F. *Cell Tissue Res.*, **2000**, *299*, 173-184.

[43] Grisaru, D.; Sternfeld, M.; Eldor, A.; Glick, D.; Soreq, H. *Eur. J. Biochem.*, **1996**, *264*, 672-686.

[44] Bigbee, J.W.; Sharma, K.V.; Gupta, J.J.; Dupree, J.L. *Environ. Health Perspect.*, **1999**, *107 Supl1*, 81-87.

[45] Small, D.H.; Michaelson, S.; Sberna, G. *Neurochem Int.*, **1996,** *28*, 453-483.

[46] Brimijoin, S.; Koenigsberger, C. *Environ. Health Perspect.*, **1999**, *107 Supl.1*, 59-64.

[47] Grifman, M.; Galyam, N.; Seidman, S.; Soreq, H. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 13935-13940.

[48] Bigbee, J.W.; Sharma, K.V.; Chan, E.L.; Bogler, O. *Brain Res.*, **2000**, *861*, 354-362.

[49] Anderson, R.B.; Key, B. *Int. J. Dev. Neurosci.*, **1999**, *17*, 787-793.

[50] Johnson, G.; Moore, S.W. *Int. J. Dev. Neurosci.*, **2000**, *18*, 781-790.

[51] Sharma, K.V.; Bigbee, J.W. *J. Neurosci. Res.*, **1998**, *53*, 454-464.

[52] Muñoz, F.J.; Aldunate, R.; Inestrosa, N.C. *Neuroreport*, **1999,** *10*, 3621-3625.

[53] Johnson, G.; Moore, S.W. *Biochem. Biophys. Res. Commun.*, **1999**, *258*, 758-762.

[54] Johnson, G.; Moore, S.W. *Appl. Biochem. Biotechnol.*, **2000**, *83*, 131-144.

[55] Inestrosa, N.C.; Alarcon, R. *J. Physiol. Paris*, **1998**, *92*, 341-344.

[56] Alvarez, A.; Bronfman, F.; Perez, C.A.; Vicente, M.; Garrido, J.; Inestrosa, N.C. *Neurosci. Lett.*, **1995**, *201*, 49-52.

[57] Alvarez, A.; Opazo, C.; Alarcon, R.; Garrido, J.; Inestrosa, N.C. *J. Mol. Biol.*, **1997**, *272*, 348-361.

[58] Alvarez, A.; Alarcon, R.; Opazo, C.; Campos, E.; Muñoz, F.J.; Calderon, F.H.; Dajas, F.; Gentry, M.K.; Doctor, B.P.; DeMello, F.G.; Inestrosa, N.C. *J. Neurosci.*, **1998**, *18*, 3213-3223.

[59] Muñoz, F.J.; Inestrosa, N.C. *FEBS Lett.*, **1999**, *450*, 205-209.

[60] Opazo, C.; Inestrosa, N.C. *Mol. Chem. Neuropathol.*, **1998**, *33*, 39-49.

[61] Inestrosa, N.C.; Alvarez, A.; Perez, C.A.; Moreno, R.D.; Vicente, M.; Linker, C.; Casanueva, O.I.; Soto, C.; Garrido, J. *Neuron*, **1996**, *16*, 881-891.

[62] Pang, Y.P; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.*, **1996**, *271*, 23646-23649.

[63] DeFerrari, G.V.; VonBernhardi, R.; Calderon, F.H.; Luza, S.C.; Inestrosa, N.C. *J. Neurosci. Res.*, **1998**, *52*, 435-444.

[64] Lahiri, D.K.; Farlow, M.R.; Sambamurti, K. *Brain Res. Mol. Brain Res.*, **1998**, *62*, 131-140.

[65] Carlier, P.R.; Han, Y.F.; Chow, E.S.; Li, C.P.; Lieu, T.X.; Wong, H.S.; Pang, Y.P. *Bioorg. Med. Chem.*, **1999,** *7*, 351-357.

[66] Carlier, P.R.; Chow, E.S.; Han, Y.; Liu, J.; Yazal, J.; Pang, Y.P. *J. Med. Chem.*, **1999**, *42*, 4225-4231.

[67] Li, C.Y.; Wang, H.; Xue, H.; Carlier, P.R.; Hui, K.M.; Pang, Y.P.; Li, Z.W.; Han, Y.F. *Neuroreport*, **1999**, *10*, 795-800.

[68] Wang, H.; Carlier, P.R.; Ho, W.L.; Wu, D.C.; Lee, N.T.; Li, C.P.; Pang, Y.P.; Han, Y.F. *Neuroreport*, **1999**, *10*, 789-793.

[69] Xiao, X.Q.; Lee, N.T.; Carlier, P.R.; Pang, Y.; Han, Y.F. *Neurosci. Lett.*, **2000**, *290*, 197-200.

[70] Mary, A.; Renko, D.Z.; Guillou, C.; Thal, C. *Bioorg. Med. Chem.*, **1998**, *6*, 1835-1850.

[71] Guillou, C.; Mary, A.; Renko, D.Z.; Gras, E.; Thal, C. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 637-639.

[72] Castro, A.; Richards, W.G.; Lyne, P.D. *Med. Chem. Res.*, **1999**, *9*, 98-107.

[73] Pang, Y.P.; Kozikowski, A.P. *J. Comput. Aided Mol. Des.*, **1994**, *8*, 683-693.

[74] Inoue, A.; Kawai, T.; Wakita, M.; Iimura, Y.; Sugimoto, H.; Kawakami, Y. *J. Med. Chem.*, **1996**, *39*, 4460-4470.

[75] Kryger, G.; Silman, S.; Sussman, J.L. *Structure*, **1999**, *7*, 297-307.

[76] Zeng, F.; Jiang, H.; Zhai, Y.; Zhang, H.; Chen, K.; Ji, R. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 3279-3284.

[77] Martinez, A.; Fernandez, E.; Castro, A.; Conde, S.; Rodriguez-Franco, M.I.; Baños, J.E.; Badia, A. *Eur. J. Med. Chem.*, **2000,** *35*, 913-922.

[78] Martinez, A.; Lanot, C.; Perez, C.; Castro, A.; Lopez-Serrano, P.; Conde S. *Bioorg. Med. Chem.*, **2000**, *8*, 731-738.

[79] Ishihara, Y.; Hirai, K.; Miyamoto, M.; Goto, G. *J. Med. Chem.*, **1994,** *37*, 2292-2299.

[80] Ishihara, Y.; Goto, G.; Miyamoto, M. *Curr. Med. Chem.*, **2000**, *7*, 341-354.

[81] Simeon-Rudolf, V.; Kovarik, Z.; Radic, Z.; Reiner, E. *Chem. Biol. Interact.*, **1999**, *119-120*, 119-128.

[82] Lin, G.; Lai, C.Y.; Liao, W.C. *Bioorg. Med. Chem.*, **1999**, *7*, 2683-2689.

[83] Rampa, A.; Bisi, A.; Belluti, F.; Gobbi, S.; Valenti, P.; Andrisano, V.; Cavrini, V.; Cavalli, A.; Recanatini, M. *Bioorg. Med. Chem.*, **2000**, *8*, 497-506.

[84] Camps, P.; ElAchab, R.; Morral, J.; Muõz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.*, **2000**, *43*, 4657-4666.

[85] Camps, P.; Cusack, B.; Mallender, W.D.; ElAchab, R.; Morral, J.; Muõz-Torrero, D.; Rosenberry, T.L. *Mol. Pharmacol.*, **2000**, *57*, 409-417.

[86] Pakaski, M.; Rakonczay, Z.; Kasa, P. *Neurochem. Int.*, **2001**, *38*, 219-226.