European Journal of Medicinal Chemistry 139 (2017) 773–791

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

From dual binding site acetylcholinesterase inhibitors to allosteric modulators: A new avenue for disease-modifying drugs in Alzheimer's disease

Talita P.C. Chierrito [a], Susimaire Pedersoli-Mantoani [a], Carlos Roca [b], Carlos Requena [b], Victor Sebastian-Perez [b], Willian O. Castillo [c], Natalia C.S. Moreira [c], Concepción Pérez [e], Elza T. Sakamoto-Hojo [c, d], Catarina S. Takahashi [c, d], Jesús Jiménez-Barbero [b, f], F. Javier Cañada [b], Nuria E. Campillo [b], Ana Martinez [b, **], Ivone Carvalho [a, *]

[a] School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Avenida do Café, s/n, 14040-903, Ribeirão Preto, SP, Brazil
[b] IPSBB Unit, Centro de Investigaciones Biologicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain
[c] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirão Preto, SP, Brazil
[d] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo, Avenida Bandeirantes, 3900, 14040-900, Ribeirão Preto, SP, Brazil
[e] Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006, Madrid, Spain
[f] CIC BioGUNE, Parque Tecnologico de Bizkaia, Edif. 801A, 48160, Derio-Bizkaia, Bilbao, Spain

ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form
21 August 2017
Accepted 22 August 2017
Available online 26 August 2017

Keywords:
Acetylcholinesterase inhibitors
Allosteric modulators
Alzheimer's disease

ABSTRACT

The lack of an effective treatment for Alzheimer' disease (AD), an increasing prevalence and severe neurodegenerative pathology boost medicinal chemists to look for new drugs. Currently, only acethylcholinesterase (AChE) inhibitors and glutamate antagonist have been approved to the palliative treatment of AD. Although they have a short-term symptomatic benefits, their clinical use have revealed important non-cholinergic functions for AChE such its chaperone role in beta-amyloid toxicity. We propose here the design, synthesis and evaluation of non-toxic dual binding site AChEIs by hybridization of indanone and quinoline heterocyclic scaffolds. Unexpectely, we have found a potent allosteric modulator of AChE able to target cholinergic and non-cholinergic functions by fixing a specific AChE conformation, confirmed by STD-NMR and molecular modeling studies. Furthermore the promising biological data obtained on human neuroblastoma SH-SY5Y cell assays for the new allosteric hybrid **14**, led us to propose it as a valuable pharmacological tool for the study of non-cholinergic functions of AChE, and as a new important lead for novel disease modifying agents against AD.

1. Introduction

Firstly described as an "unusual disease of the cerebral cortex" in 1906 [1], Alzheimer disease (AD) is a neurodegenerative disorder characterized by a slow, silent and progressive damage of the human brain that remains uncured and fatal. The first signals of AD are memory failure and cognitive impairment, which progresses to memory loss and behavioral changes [2,3]. The underlying mechanisms include cholinergic transmission decreasing, abnormal processes as beta-amyloid (Aβ) deposits and neurofibrillary tangles due hyperphosphorilation of tau protein. In 2015, it was estimated that there were 46.8 million people worldwide with dementia, affecting mainly those over the age 65. This number will reach around 131.5 million in 2050 [4].

The treatments for mild-moderate AD are based on drugs that boost the levels of acetylcholine by inhibiting acetylcholinesterase (AChE), such as donepezil, galantamine, rivastigmine and tacrine, the latter no longer prescribed due its hepatotoxicity, as well as the NMDA receptor antagonist, memantine, for severe AD. Although acetylcholinesterase inhibitors (AChEIs) are not able to halt the progress of the disease, these drugs improve the quality of life for patients and caregivers [5]. Furthermore, evidence has indicated

* Corresponding author.
** Corresponding author.
E-mail addresses: ana.martinez@csic.es (A. Martinez), carronal@usp.br (I. Carvalho).

http://dx.doi.org/10.1016/j.ejmech.2017.08.051
0223-5234/

that some of these acetylcholinesterase inhibitors also have non-cholinergic functions on the pathogenesis of AD [6].

The symptomatic short-term benefits of AChEIs led to new therapeutic strategies focused on amplifying the cholinergic activity at M-1 muscarinic and α-7 nicotinic receptors, as illustrated by cevimeline and encenicline (EVP-6124), respectively [7]. Also of upmost importance is the modulation of enzymes involved in the proteolytic cleavage of the amyloid precursor protein (APP) owing to the A$\beta_{(1\text{-}42)}$ metabolite neurotoxicity in senile plaques formation and soluble oligomeric Aβ deposits [8]. In this regard, the inhibition of BACE-1 (β-secretase) has been highly explored, leading to verubecestat (MK8931) [9] in clinical trials. In addition, the anti-neuroinflammatory effects of minocycline have established the relationship between the reduction of cytokines/chemokines and microgliosis in AD patient's brain and neuroprotection [10]. Taking into account the critical role played by abnormal post translational modifications on tau protein in the neuropathogenesis of AD, the microtubule-stabilizing agent davunetide was developed, but it failed in Phase II trial in mild to moderate AD patients [11]. On the other hand, methylene blue [12] and its reduced form, leuco-methylthioninium [11], showed high tau aggregation inhibition and affinity for hippocampal cells, being the second generation of methylthioninium dye (TRx0237) in advanced clinical trials due to its improved bioavailability [11].

Finally, anti-Aβ immunotherapy involving anti-Aβ monoclonal antibodies (solanezumab, gantenerumab, and aducanumab) [13] and active vaccines for antibody-induced tau clearance, such as AADvac1, are under investigation for AD treatment [11], but at the moment none of them reached the market [14].

The link between several targets, such as Aβ soluble oligomers in the activation of GSK-3β, promoting tau-phosphorylation [8,15] and oxidative stress through ROS production [16,17], along with decreased density of nicotinic acetylcholine receptors (nAChR) [18] may entail multitargeting drug development approaches [19]. In fact, the amyloid peptide aggregation induced by the peripheral site (PAS) of AChE [20] led to the synergistic dual binding site AChEI, providing an interesting therapeutic strategy to increase the acetylcholine synapses level (active site-CAS) and modulate Aβ aggregation [21–25]. Thus, based on evidence that both *N*-benzyl piperidine group and indanone moiety of donepezil bind to AChE through CAS and PAS sites, respectively [26], novel indanone-based derivatives containing either *N*-benzylpyridinium [27] or phenylpiperazine cores [28] were described with moderate to high inhibition of AChE and Aβ-aggregation, anti-oxidant activity and neuroprotection. Besides this multifunctional AChE inhibition, further improvement of anti-oxidant and anti-inflammatory activities, metal-quelating properties and MAO inhibition were achieved for hybrids that just preserve the *N*-benzyl piperidine function of donepezil, but have the indanone core replaced by cinnamoyl [29], feruloyl [30] or 8-hydroxyl-quinolinyl groups [31]. Furthermore, tacrine derivatives containing the acridine core are reported as potent inhibitors of AChE, in particular, the known tacrine-donepezil hybrids conserving the indanone moiety (I) [32], or the phtalimide bioisoster (II) [33], and benzylpiperidine/benzylpiperazine units (III, IV) [34,35], related to donepezil structure (Fig. 1).

The high activity of compounds I-IV and the DNA intercalating and highly mutagenic properties of acridine group [36–38] led us to pursue a different hybridization strategy for the synthesis of novel potential dual binding site AChEIs by connecting a simplified and non-toxic quinoline moiety [39], rather than acridine, to indanone core, mimicking both donepezil to interact at peripheral site and tacrine at active site (Fig. 2). In spite of the significant inhibition of *h*AChE (IC_{50} 73 nM), metal chelation, and self-induced, *h*AChE-induced and Cu^{2+}-induced $A\beta_{1\text{-}42}$ aggregation achieved by donepezil hybrids containing 2-methyl-pyridine rather than 8-hydroxy-quinoline cores [40], our previous docking studies had shown the promising profile of 4-amino-quinoline nucleolusmoiety due to potential interactions with Trp86, His440 and Glu199 residues at the AChE active site [41].

To establish whether this chemical modification should impact on the binding mode of AChE, we have synthetized this new compound, conducted cholinesterase kinetic assays, showing experimentally that this compound acts as dual binding site AChE inhibitor. As a proof of concept, we also used SH-SY5Y cell lines to assess the potential of the hybrid to reverse the neurocytotoxicity induced by A$\beta_{(1\text{-}42)}$ peptide (clonogenic assay). Unexpectely, one of the intermediates compound in the hybrid synthesis has shown a very interesting enzymatic profile. We have found a potent allosteric modulator of AChE able to target cholinergic and non-cholinergic functions by fixing a specific AChE conformation, confirmed by STD-NMR and molecular modeling studies.

2. Results and discussion

2.1. Synthesis

A convergent synthesis strategy was pursued to prepare the intermediate piperidinyl-chloroquinoline (**3**) by reductive amination between 4-chloro-quinolinyl-2-carbaldehyde (**4**) and hidroxymethyl-piperidine (**5**) [42]. Both precursors were obtained straightforwardly by some classical reactions, for instance, ethyl isonipecotate (**7**) was reduced to the alcohol compound **5** (90% of yields) after treatment with $LiAlH_4$ in THF [43]. In spite of the 2-methyl-quinoline (**6**) being commercially available, a modified Friedländer synthesis was explored to obtain the quinoline core, after a cyclization reaction between the cheaper commercial *o*-aminobenzoic acid and acetone in the presence of phosphoryl chloride, to afford the desired compound **6** (40% yield) [44]. The methyl group of **6** was oxidized with SeO_2 and the auxiliary reagent *tert*-butylhydroperoxide to afford the aldehyde **4** in 63% of yields [45]. Thus, the condensation product **3**, obtained in 65% yield, submitted to mild oxidation using Dess-Martin reagent gave the aldehyde **8** (36%) [46]. Despite the straight route to obtain the final amino product **15** by replacing the aromatic chlorine atom of intermediate **8** by azide group giving compound **9** (48%) [47] and, then, condensation with indanone **10** to afford product **11** [48], several attempts to reduce the azide group of **11** under hydrogenation conditions were not successful due to formation of complex mixture. For this reason, an alternative strategy was pursued condensing the chlorine aldehyde **8** and indanone **10** in the presence of sodium ethoxide, followed by E1cB elimination reaction to give the E-isomer of α,β-unsaturated chloride, compound **12** (86% yields) [48]. In order to establish the double bound geometry of this enone intermediate, G-BIRD$_{R,X}$-CPMG-HSQMBC experiments were carried out [49,50] to measure heteronuclear spin-spin coupling constant C-H ($^3J_{CH}$) between olefinic hydrogen and the carbonyl carbon. According to Karplus equation, vicinal coupling constants present a strong correlation with the dihedral angle (θ) enabling correlation of the $^3J_{CH}$ value with E or Z (θ = 0° or 180°, respectively, for *H*-C-C-*C* correlation) stereochemistry of the double bond [51,52]. Thus, in this case, the $^3J_{CH}$ = 5.9 Hz was observed in the NMR experiment (compound **12**), which corresponds to θ around 0°, confirming the exclusive presence of the expected E isomer, due to the more favorable anti elimination in the E1cB reaction, giving rise to a more stable E-isomer (Support information S-2) [53].

The α,β-unsaturated double bond of compound **12** was reduced after the treatment with $NaBH_4$ and pyridine, yielding compound **13** (57%) [54]. Finally, starting from either compounds **12** and **13** was possible to replace the chlorine atom to the amine group by