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Synthesis, biological assessment, and molecular modeling of racemic 7-aryl-9,10,11,12-tetrahydro-7*H*-benzo[7,8]chromeno[2,3-*b*]quinolin-8-amines as potential drugs for the treatment of Alzheimer's disease

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ABSTRACT

The synthesis, pharmacological analysis and molecular modeling of the readily available racemic tacrine analogs **21**–**30**, bearing the 7-aryl-9,10,11,12-tetrahydro-7*H*-benzo[7,8]chromeno[2,3-*b*]quinolin-8-amine heterocyclic ring system (**II**), prepared by Friedländer reaction of 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carbonitriles (**11**–**20**) with cyclohexanone, are described in this paper. Molecules **21**–**30** are potent and selective inhibitors of hAChE, in the low micromolar range, one of the most potent inhibitors, 4-(8-amino-9,10,11,12-tetrahydro-7*H*-benzo[7,8]chromeno[2,3-*b*]quinolin-7-yl)-2-methoxyphenol (**25**), showing a IC_{50} (hAChE) = 0.33 ± 0.04 μM. Kinetic studies of compound **25** proved that this compound is a mixed type inhibitor for *Ee*AChE (K_i = 81 nM). Accordingly, molecular modeling of inhibitor **25** showed that both enantiomers have two major predicted binding modes at the active and at the peripheral anionic sites of AChE. Inhibitor **25** has an excellent antioxidant profile as determined in the ORAC experiment (1.47 ± 0.10 Trolox equiv). Inhibitors **26**–**28** and **30** are permeable to BBB as determined in the PAMPA assay. Compared to tacrine, selected compounds **26**–**28** and **30** showed less hepatic toxicity in HepG2 cells. Moreover, cell viability-related studies in cortical neurons in primary cultures show that compounds **26**–**28** and **30** (0.1–50 μM) have significant neuroprotective effects against mitochondrial chain blockers-induced cell death, and, unlike tacrine, are not neurotoxic at concentrations lower than 50 μM. It is worth highlighting that compound **27** has the best neuroprotective properties out of all assayed compounds and shows no neurotoxicity. To sum up, these tacrine analogs can be considered as attractive multipotent therapeutic molecules on pharmacological receptors playing key roles in the progress of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative process characterized by a progressive loss of memory, along with other cognitive impairments [1]. Although the etiology of AD is still poorly understood, several factors such as amyloid-β (Aβ) deposits [2], τ-protein aggregation, and deficits of acetylcholine (ACh) [3] are thought to play significant roles in the pathology of the disease [4]. The cholinergic theory [1] suggests that the selective loss of cholinergic neurons in AD results in low levels of ACh in specific regions of the brain that mediate learning and memory functions [5]. Consequently, acetylcholinesterase (AChE) inhibitors such as tacrine, donepezil, rivastigmine, and galanthamine, are known to

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improve AD symptoms by inhibiting AChE, i.e. the enzyme responsible for the hydrolysis of ACh, thereby rising the levels of ACh in the synaptic cleft. As a result, they have been approved for commercial use [6]. Recently, a renewed interest for AChE inhibitors has been stimulated by the potential role of AChE in accelerating the formation of amyloid fibrils in the brain and forming stable complexes with Aβ [7]. This role involves the peripheral anionic binding site (PAS) of AChE [8]. The multifactorial nature of AD supports new therapeutic strategies [9–14], such as NO-donor-tacrine-hybrids [15], dual inhibitors of MAO/AChE [16,17], serotonin transporters [18], ChE inhibitors with antioxidant and neuroprotective properties [19], gallamine–tacrine hybrids binding at ChE/M_2 receptors [20], and AChE plus τ-hyperphosphorylation regulators [21]. On the other hand, Ca^{2+} overload is the main factor that augments Aβ formation [22], and favors mitochondrial disruption, which leads to the activation of the apoptotic cascade and cell death [23]. Nimodipine, a neuronal L-type Ca^{2+} channel blocker, protects neurons from death evoked by focal cerebral ischemia [24].

In this context, some years ago we embarked on a long-term research project aimed at the synthesis of a series of multipotent compounds designed to target AChE inhibition and neuronal Ca^{2+} modulation [25]. We have therefore synthesized and evaluated a number of hybrid derivatives such as ethyl 5-amino-4-(4-fluorophenyl)-2-methyl-6,7,8,9-tetrahydrobenzo[*b*][1,8]naphthyridine-3-carboxylate (**A**) and ethyl 5-amino-4-(4-methoxyphenyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrano[2,3-*b*]quinoline-3-carboxylate (**B**), which combine the tetrahydroaminoquinoline moiety present in tacrine (Chart 1) with a pyridine or a 4*H*-pyran ring system, a substitution pattern similar to that found in the isosteric 1,4-dihydropyridines [25]. Compounds **A** and **B** were less potent as AChEIs than tacrine, but they blocked voltage-dependent Ca^{2+} channels (38% and 50% inhibition, respectively) [25].

More recently, we have reported the chemistry and pharmacology of **RL2**/**101** (Chart 1), a potent and selective AChE *vs* BuChE mixed-type inhibitor, which binds preferentially at the PAS of AChE, interfering with the pro-aggregation Aβ effect of hAChE, and thus being a mild inhibitor of Aβ self aggregation, as well as a potent Ca^{2+}antagonist that permeates the blood brain barrier (BBB), and displays neuroprotective and antioxidant properties [26].

In this context, with these precedents from our laboratory in mind, and in order to validate the proposed binding mode [25,26] and modify the structure of the previous active compounds **A**, **B** and **RL2**/**101**, looking for more equipotent AChE *vs* BuChE inhibitors with higher antioxidant and neuroprotective and lower toxicity properties, we have recently reported the synthesis, the biological evaluation, and molecular modeling of a number of racemic 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines of type (**I**) (Chart 2) [27]. During the course of this project, Li [28] and Perumal [29] communicated the synthesis of compounds of type **I**, but no biological activity has been described for them. From our work [27], compound 4-(13-amino-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-14-yl)phenol (**A4**) (Chart 2) emerged as a selective, potent and mixed type *Ee*AChE inhibitor ($IC_{50} = 7 \pm 2$ nM; K_i of 5.00 nM), 4-fold more active than tacrine; however, compound **A4** was unable to displace propidium iodide, suggesting that this inhibitor does not strongly bind to the PAS of AChE, an observation confirmed later by docking, molecular dynamics simulations, and MM-GBSA calculations [27].

Based on these results, we have carried out the synthesis and pharmacological evaluation of a number of related, racemic, isomeric tacrine analogs bearing the 7-aryl-9,10,11,12-tetrahydro-7*H*-benzo[7,8]chromeno[2,3-*b*]quinolin-8-amine structure (**II**) (Chart 2). We hypothesized that by changing the location of the fused benzene ring A [compare compounds of type **I** with those of type **II** (Chart 2)] consequences in the AChE binding should arise and, consequently, in the inhibition power of these new inhibitors.

Accordingly, in this work we report the synthesis [30], studies on the inhibition of hAChE/hBuChE, the antioxidant activities determined using the ORAC method, and the BBB of compounds **21**–**30**. In addition, we have deepened into the hepatotoxicity and neuroprotective properties of selected derivatives **26**–**28** and **30** (Scheme 1). Overall, from the ChE inhibition studies, confirmed by

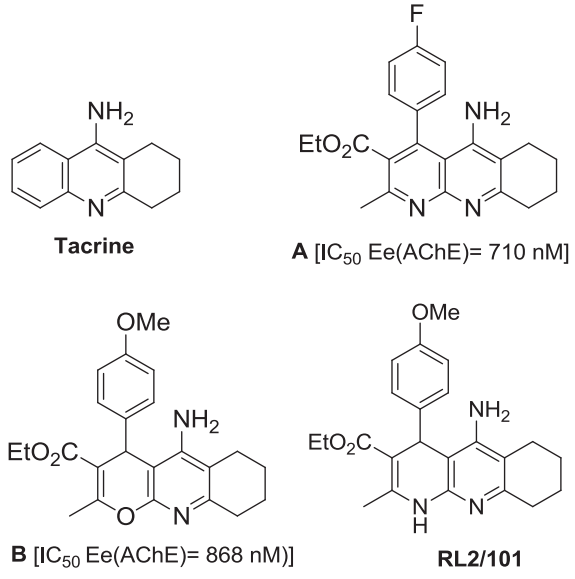

Chart 1. Structure of tacrine, ethyl 5-amino-4-(4-fluorophenyl) 2-methyl-6,7,8,9-tetrahydrobenzo[*b*][1,8]naphthyridine-3-carboxylate (**A**) [25], ethyl 5-amino-4-(4-methyoxyphenyl)-2-methyl-6,7,8,9-tetrahydro 4*H*-pyrano[2,3-*b*]quinoline-3-carboxylate (**B**) [25], and ethyl 5-amino-4-(4-methoxphenyl)-2-methyl-1,4,6,7,8,9-hexahydrobenzo[*b*][1,8] naphthridine-3-carboxylate (**RL2**/**101**) [26].

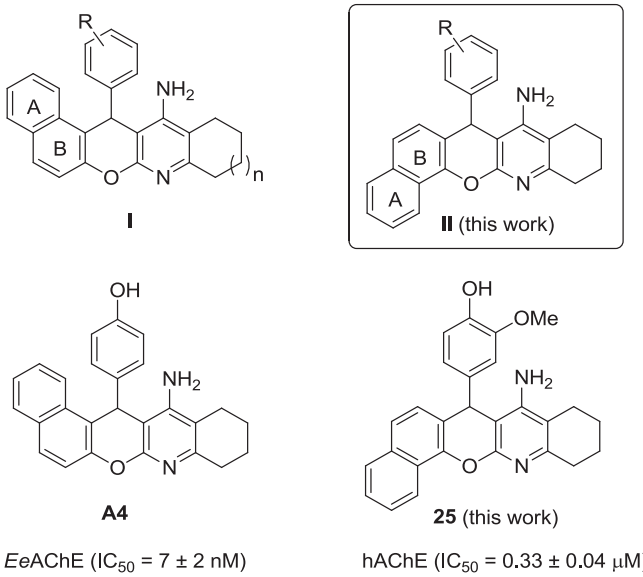

Chart 2. Structures of the previously investigated tacrine analogs **I**, and the most potent AChE inhibitor in this series (**A4**) [27], the target 7-aryl-9,10,11,12-tetrahydro-7*H*-benzo[7,8]chromeno[2,3-*b*]quinolin-8-amines (**II**), and compound **25**, described in this work.