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In silico, NMR and pharmacological evaluation of an hydroxyoxindole cholinesterase inhibitor

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A B S T R A C T

From a screening study of various potential inhibitors for cholinesterases (ChEs), compound (*rac*)-**1** (4-((3-hydroxy-2-oxo-3-phenylindolin-1-yl) methyl) piperidin-1-ium chloride) showed an IC_{50} of 18 μM for butyrylcholinesterase (BuChE). Herein we present a toxicological and pharmacological evaluation of (*rac*)-**1** to determine its potential for use as an alternative ChE inhibitor for the treatment of Alzheimer's disease. The strategy adopted included *in vivo* and *ex vivo* studies with mouse models, Molecular Modelling and Saturation Transfer Difference (STD) NMR studies.

Preliminary molecular docking studies were conducted with both (*R*) and (*S*)-**1** with acetylcholinesterase (AChE) and BuChE, prior to advancing to the mouse model, and indeed favorable interactions were observed, with (*R*)-**1** showing the best binding with AChE and (*S*)-**1** with BuChE. STD-NMR studies were used to successfully validate these results. Toxicological studies were also conducted using the *Artemia salina* model, with donepezil as reference. It was found that in the *in vivo* mouse studies that (*rac*)-**1** presented a slightly better inhibition of AChE (0.096 μmol.min^{-1}.mg^{-1}) than donepezil (0.112 μmol.min^{-1}.mg^{-1}) and the same level of inhibition for BuChE as donepezil (0.014 μmol.min^{-1}.mg^{-1}).

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and the most prevalent cause of dementia among the elderly.[1,2] This irreversible neurological disorder is characterized by memory and cognitive impairment, behavioral and learning deficits.[3,4] Neuropathologically, AD is portrayed by the presence of amyloid β (Aβ) plaques and neurofibrillary tangles (NFT) of a phosphorylated form of protein tau. The formation of Aβ plaques and NFTs leads to neurodegeneration and loss of cholinergic neurons in the basal forebrain with consequent reduction of acetylcholine (ACh) levels in the hippocampus and cortex of the brain.[5] Both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) degrade ACh, this is more prevalent for BuChE in the latter stages of the disease. In order to increase ACh levels, the use of reversible inhibitors of the enzyme acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) has been one of the prime strategies for controlling the symptoms of AD.[6,7] There is no cure for this disease. Nowadays, only three drugs are commercially available as AChE inhibitors for the treatment of AD: galantamine, rivastigmine and donepezil (Fig. 1) and which have different draw-backs.[8] Given this situation, it is imperative that new and less expensive AChE inhibitors become available for AD treatment.

The hydroxyoxindole scaffold is present in a number of molecules that exhibit diverse pharmacological profiles, that includes anti-cancer, anti-HIV, antioxidant, antibacterial, antidiabetic, AChE/BuChE inhibition, kinase inhibition, vasopressin antagonism, progesterone antagonism, anti-leishmanial, β3 adrenergic receptor antagonism, analgesic, spermicidal, phosphatase inhibition, neuroprotection, and *N*-methyl-D-aspartate (NMDA) blocker activities.[9]

We have previously reported the design and synthesis of a new family of tertiary 3-hydroxy- and 3-alkoxyoxindole derivatives which presented good AChE and BuChE inhibitory activities.[10] Some of the key hits ((*rac*)-**1** to (*rac*)-**4**) that were identified are shown in Fig. 2 with their IC_{50} values. Our interest then moved to evaluating these compounds in an animal model, and thus the issue of bioavailability was critical. We found that (*rac*)-**1** (4-((3-hydroxy-2-oxo-3-phenylindolin-1-yl) methyl) piperidin-1-ium chloride) (Fig. 2) gave promising inhibition of BuChE (18 μM) (under incubation conditions). In addition, only after

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