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Novel tacrine/acridine anticholinesterase inhibitors with piperazine and thiourea linkers

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ABSTRACT

A new series of substituted tacrine/acridine and tacrine/tacrine dimers with aliphatic or alkylene-thiourea linkers was synthesized and the potential of these compounds as novel human acetylcholinesterase (*h*AChE) and human butyrylcholinesterase (*h*BChE) inhibitors with nanomolar inhibition activity was evaluated. The most potent AChE inhibitor was found to be homodimeric tacrine derivative **14a**, which demonstrated an IC_{50} value of 2 nM; this value indicates an activity rate which is 250-times higher than that of tacrine **1** and 7500-times higher than 7-MEOTA **15**, the compounds which were used as standards in the study. IC_{50} values of derivatives **1**, **9**, **10**, **14b** and **15** were compared with the dissociation constants of the enzyme-inhibitor complex, K_{i1}, and the enzyme-substrate-inhibitor complex, K_{i2}, for. A dual binding site is presumed for the synthesized compounds which possess two tacrines or tacrine and acridine as terminal moieties show evidence of dual site binding. DFT calculations of theoretical desolvation free energies, $\Delta\Delta G_{theor}$, and docking studies elucidate these suggestions in more detail.

1. Introduction

Alzheimer's disease (AD) is a common neurological disorder which is characterized by progressive memory loss and a wide range of cognitive impairments. The ever increasing spread of this disorder is already placing huge strains on the economies and health systems of modern aging societies. Intensive research over the last three decades has confirmed that the pathophysiology of AD has a multifactorial nature. Despite the huge effort and resources invested in the development of drugs which could treat AD, the most widely used palliative treatment is still based on the well established cholinergic process in which the concentration of acetylcholine (ACh) neurotransmitters in the brain and central nervous system (CNS) is increased using cholinesterases inhibitors (ChEIs) [3–5]; an overview of cholinesterase inhibitors and their mode of action have been summarized in reviews [1,2]. In addition to acetylcholinesterase (AChE) which hydrolyzes about 80% of acetylcholine in the healthy brain, attention has also focused on butyrylcholinesterase (BChE), whereas the function of this enzym is less clearly defined due to its ability to hydrolyze ACh and other esters [6–10].

Various anti-AChE agents such as tacrine **1** (Fig. 1), donepezil, rivastigmine, galantamine, and N-methyl-D-aspartate (NMDA) receptor antagonist, memantine have shown to cause a slight improvement in cognitive and memory disorders [5,11]. Crystallographic studies have demonstrated that these anti-AChE agents all bind to AChE within the catalytic active site (CAS) in a 20 Å deep gorge, while the larger donepezil compound also binds at a peripheral anionic binding site (PAS) at the rim of the AChE gorge. The peripheral anionic centre of AChE has also been proven to take

Abbreviations: AD, Alzheimer's disease; ACh, acetylcholine; CNS, central nervous system; ChE, cholinesterase; ChEI, cholinesterase inhibitor; *h*AChE, human acetylcholinesterase; *h*BChE, human butyrylcholinesterase; THA, 1,2,3,4-tetrahydroacridine; NMDA, *N*-methyl-D-aspartate; CAS, catalytic active site; PAS, peripheral anionic site; APP, amyloid precursor protein; Aβ, amyloid β-protein; E, enzyme; S, substrate; I, inhibitor; DFT, density functional theory; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ATChCl, acetylthiocholine chloride; PBS, phosphate buffered saline.

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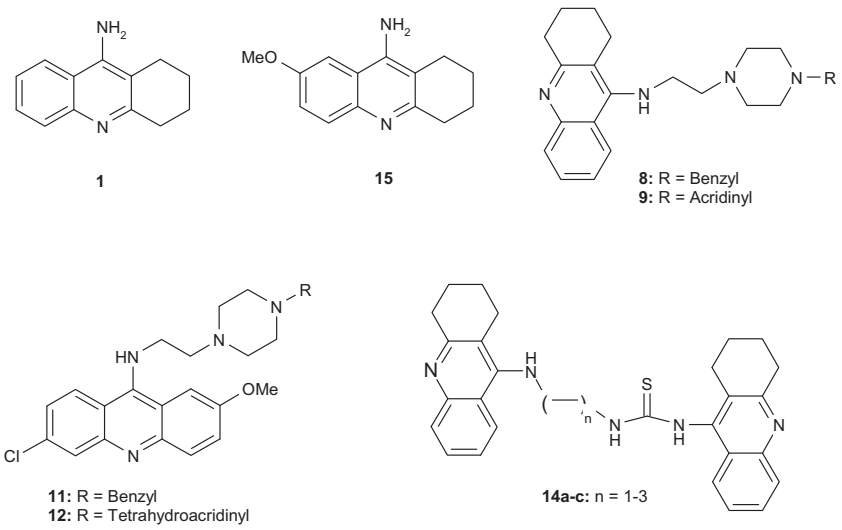

Fig. 1. Structure of tacrine **1**, homo and hetero tacrine/acridine hybrids **8, 9**, **11**, **12**, **14a–c**, and 7-MEOTA **15**.

part in amyloid-β peptide aggregation into neuric plagues and neurofibrilary tangles [12,13], effects which are important hallmarks of AD and key pathological events which trigger neurodegenerative processes in AD.

Several papers have emphasized the importance of heteroatoms in the linkers, in particular nitrogens or amide moieties, which form additional cation-π or H-bond interactions of inhibitors with aminoacids lining the enzyme gorge. This optimization of the linker's structure [14] has gradually shifted the observed anti-ChE activity to the attractive sub-nanomolar or even picomolar range [14–16]. The development of a hydrazide linker for tacrine-derived heterodimers has led to the practical application of these agents in the inhibition of cholinesterases, the bivalent binding to nicotinic and muscarinic acetylcholine receptors, as well as the histochemical imaging of acetylcholinesterase and amyloid-β [17].

As an integration of the above approaches and in a continuation of our previous study concerning the development of new AChE inhibitors [18–21], this paper is devoted to the synthesis of tacrine-piperazine/acridine hybrids as novel ChEIs with aliphatic or alkylene-thiourea linkers, and an evaluation of the AChE/BChE inhibition evaluation of these derivatives combined with binding mode studies using quantum chemistry and a molecular docking study. Piperazine was used in combination with benzyl as a substitute for the well-known piperidine-benzyl moiety of donepezil in an attempt to examine the effect of a second protonable tertiary nitrogen inside the piperazine ring. Additionally, a little explored thiourea compound [22,23] connected to an alkylenediamine spacer of variable length was included as the linker in order to examine its role in inhibition.

2. Experimental methods

2.1. Materials

All chemicals and solvents were obtained commercially and used without purification. Human erythrocytal AChE, human plasmatic BChE, 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB] were purchased from Sigma-Aldrich, St. Louis, MO, USA. Thin-layer chromatography was performed on Macherey-Nagel Alugram ®Sil G/UV254 plates and spots were visualized with UV light. Column chromatography was performed using silica gel from Merck (0.063–0.040 mm).

2.2. Chemistry

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury Plus NMR spectrometer in $CDCl_3$ or DMSO-D_6 with tetrametylsilane as an internal standard. Chemical shifts, δ, are expressed in parts per million (ppm), and coupling constants, J, in Hz. Melting points were recorded on a Boetius hotplate apparatus and are uncorrected. Yields refer to isolated pure products. CHN analysis was performed on a CHN analyzer Perkin-Elmer 2400. The obtained values matched the calculated ones within a range of ±0.3%. A multichannel Sunrise spectrophotometer (Tecan, Salzburg, Austria) and 96-well polystyrene photometric microplates (Nunc, Rockilde, Denmark) were used to measure the anticholinesterase activity.

2.3. Preparation of tacrine/acridine dimers

The reaction course of the synthesis of derivatives **7–9** is depicted in Scheme 1, derivatives **11** and **12** in Scheme 2, and derivatives **14a–c** in Scheme 3. Schemes 1–3 are given in Supplementary material. Four starting compounds, 9-chloro-1,2,3,4-tetrahydroacridine (**2**) [24], 9-chloroacridine (**3**) [25], 6,9- dichloro-2-methoxyacridine (**4**) [26], and 9-isothiocyanato-1,2,3,4-tetrahydroacridine (**5**) [24], were employed in the synthesis of the studied derivatives. Reaction conditions and compound characterisation are given in the Supplementary material.

2.4. Measurement of AChE and BChE activity

A multichannel Sunrise spectrophotometer was used to measure anticholinesterase activity. The potency was expressed in IC_{50} values which represent an inhibitor concentration which reduces cholinesterase activity by 50%. A previously optimized procedure [27] was slightly adapted to estimate the anticholinergic properties, and 96-well polystyrene photometric microplates were used. AChE and BChE were suspended in the phosphate buffer (pH 7.4) up to the final activity of 0.002 U/μL. Cholinesterase (5 μL), a freshly mixed solution of 5,5′-dithiobis(2- nitrobenzoic acid) in the phosphate buffer (0.4 mg/mL, 40 μL), and a given concentration of the inhibitor (10^{-3}–10^{-10} M) dissolved in iso-propanol were injected into each well. The enzymatic reaction was initiated with acetylthiocholine chloride (ATChCl) in the phosphate buffer (1 mM, 20 μL). The absorbance was measured at 412 nm after 5 min incubation upon a vibrating microplate.