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Original article

Indolinone-based acetylcholinesterase inhibitors: Synthesis, biological activity and molecular modeling

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ABSTRACT

A series of indolinone-based compounds bearing benzylpyridinium moiety was designed as dual-binding inhibitors of acetylcholinesterase (AChE). The target compounds **3a**–**u** were synthesized by condensation of oxindole and pyridin-4-carbalehyde, and subsequent *N*-benzylation. The anti-cholinesterase activity evaluation of synthesized compounds revealed that most of them had very potent inhibitory activity against AChE, superior to standard drug donepezil. Particularly, 2-chlorobenzyl derivative **3c** was the most potent compound against AChE with IC_{50} value of 0.44 nM, being 32-fold more potent than donepezil. Also, most of compounds were more potent than standard drug donepezil against butyrylcholinesterase (BuChE). Docking study revealed that the hydrophobic aromatic part (indoline) of representative compound **3c** binds to the PAS and the *N*-benzylpyridinium residue binds to the CAS of AChE.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline in memory, learning and cognitive functions. Currently, it is estimated that AD affects about 36 million people worldwide and expecting to reach 66 million by 2030 [1]. AD is involved with a loss of presynaptic cholinergic function in the areas of the brain related to memory and learning. This neurological disorder is also associated with the presence of amyloid β-peptide (Aβ) deposits and neurofibrillary tangles in the brain [2]. The enhancement of cholinergic neurotransmission by preserving acetylcholine (ACh) levels would be an effective way to overcome the occurrence, symptoms and progression of AD [3,4]. Accordingly, the inhibition of acetylcholinesterase (AChE) which is responsible for the metabolic breakdown of ACh has been regarded as one of the most promising approaches [5]. Therefore, anti-AChE drugs such as donepezil, rivastigmine, galantamine and tacrine, were developed for treatment of AD [6]. Among the anti-AChE drugs, tacrine is associated with hepatotoxicity thus it is rarely used [7]. On the other hand, donepezil and rivastigmine which are commonly used in the early-to-moderate stages of AD often present adverse effects and are not completely effective [8]. However, clinical trial studies revealed that galantamine shows promising pharmacological profile and clinically relevant neuroprotective effects in AD [9]. Therefore, design of more effective anti-AChE drugs with low side effects and better pharmacokinetics properties is an urgent need in the field of AD pharmacotherapy.

Previous studies on the structure and function of AChE revealed that this enzyme has two binding sites; catalytic anionic site (CAS) and peripheral anionic site (PAS) [10]. It was proposed that PAS could promote the deposition and aggregation of Aβ in the brain [11]. Accordingly, the multi-binding inhibitors which can inhibit catalytic activity of AChE and perturb the self-assembly of Aβ could be more effective agents for the management of AD [12]. For example, the dual-binding mode of donepezil with AChE has been demonstrated by X-ray crystallography and docking studies. While the hydrophobic aromatic part (5,6-dimethoxyindan-1-one) of

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donepezil binds to the PAS, the *N*-benzyl piperidine residue binds to the CAS of AChE [13,14]. Accordingly, it was found that the presence of functionalized amine group such as benzyl piperidine, benzylamino, phenylpiperazine, and anilino moieties contribute to inhibitor activity by interacting with the catalytic site of the AChE. On the other hand, a ligand that is rich in aromatic groups, may engage to favorable stacking interactions with PAS [15]. Numbers of aromatic and heteroaromatic rings were found in AChE inhibitors as PAS binding scaffolds [16,17]. Recently, we introduced benzofuranone-based AChE-inhibitors containing benzylpyridinium moiety (Fig. 1) [18a]. In continuation of our previous efforts in order to find new AChE inhibitors [18], in this work we describe indolinone-based compounds bearing benzylpyridinium moiety as dual-binding inhibitors of AChE.

2. Results and discussion

2.1. Chemistry

The oxindole derivatives **3a**–**u** were synthesized via the route outlined in Scheme 1. In the first step, (*E*)-3-(pyridin-4-ylmethylene)indolin-2-one (**2**) were synthesized using oxindole (**1**) and pyridin-4-carbalehyde in the presence of *p*-toluenesulfonic acid (PTSA) as a catalyst. Several acid catalysts and solvents were screened for this reaction but the best results were obtained in the presence of PTSA in refluxing toluene. In this reaction, the (*E*)-isomer was the major product which further crystallized from acetonitrile to obtain the pure (*E*)-**2**. The chemical shift of the vinylic proton could be used to assign the configuration of product. In the (*E*)-geometry of compound **2**, the vinylic proton would be shifted downfield due to deshielding effect of carbonyl group [19]. According to the literature reports, the vinylic hydrogen in (*E*)-isomers is appeared at 7.6–8.0 ppm [20]. The target compounds **3a**–**u** were easily prepared by the reaction of proper benzyl bromide or chloride with compound (*E*)-**2**. Accordingly, the reaction mixture was stirred in dry acetonitrile without catalyst, at 60–70 °C for 6–24 h. On cooling, the product was precipitated as a solid which was separated, washed with diethyl ether or *n*-hexane and recrystallized from ethanol–water. The ^{1}H NMR data of final compounds **3** revealed that the (*E*)-geometry of compounds have been preserved based on the downfield chemical shifts of vinilic proton ($\delta > 7.9$ ppm).

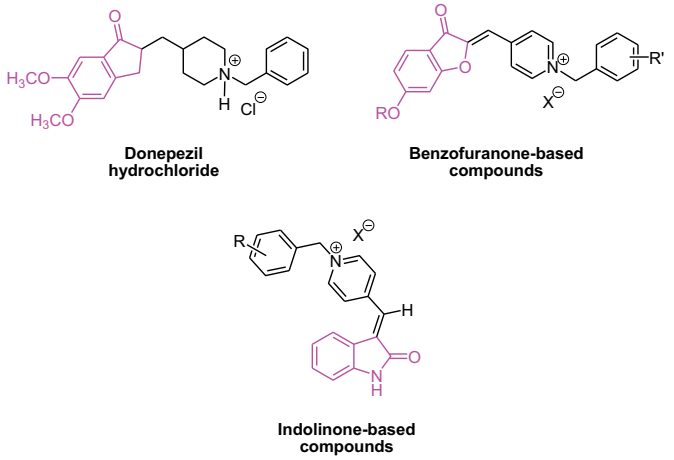
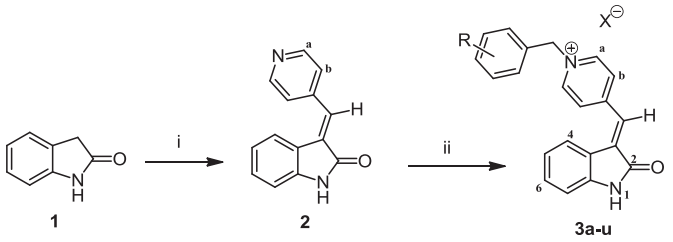


Fig. 1. Structures of donepezil hydrochloride as a well-known anti-AChE drug, benzofuranone-based compounds reported as AChE-inhibitors and indolinone-based compound as newly designed AChE-inhibitors.



Scheme 1. Synthesis of (*E*)-1-benzyl-4-((2-oxoindolin-3-ylidene)methyl)pyridinium halide derivatives **3a**–**u**. Reagents and conditions: (i) pyridine-4-carboxaldehyde, PTSA, toluene, reflux (ii) benzyl halide derivatives, acetonitrile, 60–70 °C.

2.2. Inhibitory activity against AChE and BuChE

The IC_{50} values of test compounds against AChE reveled that compounds **3b**, **3c**, **3e**, **3g**, **3i**–**m** showed very potent inhibitory activity (IC_{50} values = 0.44–12.8 nM) superior to standard drug donepezil. Among them, 2-chlorobenzyl derivative **3c** was the most potent compound against AChE, with IC_{50} value of 0.44 nM. This compounds was about 32-fold more potent than donepezil. Moreover, 2-fluoro and 2-bromo analogs (compounds **3b** and **3e**, respectively) with IC_{50} values $\leq$1.46 nM showed high activity against AChE. The comparison of un-substituted compound **3a** with *ortho*- or *meta*-substituted analogs **3b**–**m** demonstrated that introduction of halo, methyl and methoxy group at 2- or 3-position of *N*-benzyl pendent residue significantly improved the anti-AChE activity. The 2-chloro substituent had the most impact on the AChE inhibition of designed compounds. In contrast, introduction of different substituents on the *para*-position of benzyl group diminished the inhibitory activity against AChE (**3n**–**r** vs. **3a**). As shown by compound **3r**, the 4-nitro group more significantly decreased the activity. Interestingly, the insertion of second chlorine atom on *ortho* or *meta* positions of 4-chlorobenzyl derivative **3o** resulted in more potent compounds **3s** and **3t**. While, in the case of 2- or 3-chlorobenzyl derivatives (compounds **3c** or **3i**, respectively), introduction of second halogen decreased the anti-AChE activity as observed with compounds **3g**, **3h**, **3s** and **3t**. The displacement of halogen atom (Br, Cl and F) on benzyl group dramatically affects the anti-AChE activity. The order of activity was as follow: 2-halo > 3-halo > 4-halo.

The observed IC_{50} values of target compounds against BuChE revealed that all compounds with the exception of **3q** and **3r** were more potent than standard drug donepezil. The anti-BuChE activity of the most potent compound **3d** was 6 times higher than that of donepezil. Most of substituted benzyl compounds were more potent than unsubstituted analog **3a** against BuChE. These results showed that substitution on benzyl group had often positive effect on anti-BuChE activity. The highest activity was observed with 2-methyl analog. However, 3-fluoro, 4-methoxy and 4-nitro substituents decreased the inhibitory activity against BuChE.

As calculated in Table 1, the most active compound against AChE (compound **3c**) showed very high selectivity for this enzyme (SI = 3113). Moreover, other potent compounds **3b** and **3e** had high selectivity for AChE (SI > 842).

2.3. Docking studies

In order to gain functional and structural insight into the binding mode of the compounds, molecular docking simulation was performed using Autodock Vina software. To confirm the