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Synthesis and cholinesterase inhibitory activity of new 2-benzofuran carboxamide-benzylpyridinum salts

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This paper is dedicated to the memory of the late Professor Abbas Shafiee at Tehran University of Medical Sciences.

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ABSTRACT

A series of benzofuran-2-carboxamide-*N*-benzyl pyridinium halide derivatives (**6a-o**) are synthesized as new cholinesterase inhibitors. The synthetic pathway involves the reaction of salicylaldehyde derivatives and ethyl bromoacetate, followed by hydrolysis and amidation with 3- and 4-picolyl amine. Subsequently, *N*-((pyridin-4-yl) methyl) benzofuran-2-carboxamide and substituted *N*-((pyridin-3-yl) methyl) benzofuran-2-carboxamides reacts with benzyl halides to afford target compounds (**6a-o**). The chemical structures of all derivatives were confirmed by spectroscopic methods. The studies reveal that some of the synthesized compounds are potent butyrylcholinesterase inhibitors with IC_{50} values in the range of 0.054–2.7 μM. In addition, good inhibitory effects on Aβ self-aggregation are observed for **6h** and **6k** (33.1 and 46.4% at 100 μM, respectively).

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease, classified as the fourth leading cause of illness and death in people at old ages in developed countries. Scientists predict that the number of people, suffering from this disease will be nearly tripled by 2050 [1]. Alzheimer is a progressive disorder, characterized by loss of cognitive abilities, severe behavioral disorders and ultimately death. Low levels of acetylcholine (ACh) [2,3], β-amyloid (Aβ) deposits [4], metal-ion imbalance [5,6] and many other factors have been considered as important pathogenesis in AD [7–9].

The cognitive impairment in AD patients is due to the loss of cholinergic neurons and subsequently reduction in acetylcholine levels in the specific regions of the brain [10]. Therefore, according to cholinergic hypothesis, the reduced levels of acetylcholine in the brain have been introduced as the most important target among the treatment strategies [11].

The structures of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are very similar, expressing 65% identity in amino acid sequence [12]. The inhibition of acetylcholinesterase and butyrylcholinesterase enzymes led to the alleviation of the Alzheimer's symptoms, including cognitive level and short-term memory, by restoring ACh level. The increased levels of BuChE at the later stages of AD in the hippocampus and temporal cortex highlights the importance of selective BuChE inhibitors in regulating the ACh level in cholinergic neurons. This fact was also proved by Mesulam's experiments with nullizygous and wild-type mice, exhibiting the constitutive function of BuChE in the hydrolysis of ACh in the normal brain [13]. Relied on multifactorial nature of AD, the synchronous inhibition, which is achieved by AChE and BChE inhibitors may be beneficial for AD treatment [14–16].

Another crucial hypothesis for AD suggested that the aggregation and accumulation of β-amyloid peptide in a brain could lead to toxic fibrils and consequently neuronal cell death [17–20]. Many strategies are currently being assessed for preventing the formation of amyloid and toxic oligomers. In this regard, several classes of small molecules

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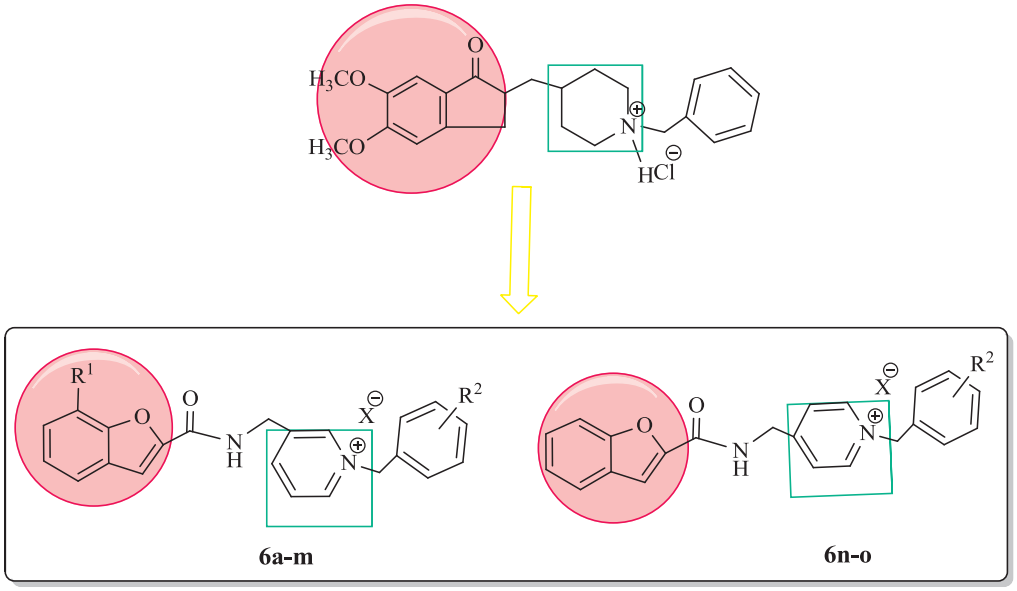

Fig. 1. Structure of donepezil hydrochloride as a ChE inhibitor and designed compounds **6a-o** as selective ChE inhibitors.

have been reported in the literature, capable of preventing or reversing fibrillization [21,22], while none of them are successful to be moved to clinical phase.

Therefore, design and synthesis of new compounds with dual inhibitory activity may be promising in AD treatment [23]. Benzofurans are one of the most significant heterocyclic categories with wide range of bioactivities [24]. In addition, benzofuran-2-carboxamide derivatives are also known as antitumor [25–28], antimicrobial [29], anti-hyperlipidemic [30], AChE inhibitor [31], antibacterial [32] and suppressing agent in allergic rhinitis [33].

Recently, various benzofuran-based compounds were reported as potent acetylcholinesterase inhibitors. Considering our experience in the synthesis of bioactive heterocyclic compounds [34–40], herein we report the synthesis and cholinesterase inhibitory activity of a series of derivatives bearing *N*-benzylpyridinium halides and benzofuran backbone which providing interactions with catalytic active site (CAS) and peripheral anionic site (PAS) of AChE, respectively (Fig. 1) [41,42]. It is expected that the intrinsic dual mechanism of action could be resulted in the efficient anticholinesterase agent discoveries.

2. Results and discussion

2.1. Chemistry

The synthetic pathway towards benzofuran-2-carboxamide bearing pyridinium moiety is shown in Scheme 1. The condensation reaction between salicylaldehyde derivatives **1a-b** and ethyl bromoacetate in the presence of K_2CO_3 afforded ethyl benzofuran-2-carboxylates [43]. Subsequent hydrolysis in aqueous ethanol/KOH afforded **3a-b** [44]. The amidation reaction of **3a-b** with 3-(methylamino) pyridine or 4-(methylamino) pyridine in the presence of hydroxybenzotriazole (HOBt) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC.HCl) as coupling agents resulted in **4**, **5a-b** [45]. The *N*-benzylation of the latter compounds with appropriate benzyl halides in acetonitrile at reflux temperature resulted in desired products **6a-6o** in good yields [46].

The presence of electron-donating groups including Me or OMe at the *ortho*, *meta* or *para* positions of benzyl moiety decreased the reaction yield. However, no distinguishing influence on the isolated yields was observed in the presence of electron-donating and electron-withdrawing groups.

2.2. Cholinesterase activity evaluation

Inhibitory activities of **6a-o** series of compounds towards AChE and BChE were measured by modified Ellman's protocol [47]. The results are summarized in Table 1 and are reported as IC_{50} and the percent of inhibition at 56 μM for target compounds (Table 1).

None of the compounds exhibit higher AChE inhibition compared to donepezil, but all of them except **6j** are better BChE inhibitors. Among the target compounds, **6k** shows the best inhibitory activity against AChE and **6h** is more potent than donepezil against BChE, exhibiting IC_{50} value of 0.054 μM, which is 100 times stronger than positive control.

As depicted in Table 1, in both series, the *ortho* and *meta* substituted derivatives exhibit better inhibition towards AChE and BChE than the *para* substituted ones. The presence of strong electron withdrawing groups at *para* position in **6e** decreases the inhibitory activity against AChE in 3-pyridinium series. As shown in Table 1, the presence of methoxy group at 7-position of benzofuran leads to the reduced butyrylcholinesterase inhibitory activity (**6a** vs. **6i**). The 4-pyridinium series display the weakest inhibitory activity against both AChE and BChE compared to 3-pyridinium series.

To predict BBB penetration and intestinal absorption the admetSAR server was used [48]. Calculated logP and tPSA were retrieved from chembiodraw ultra 14.0 (PerkinElmer). Based on the obtained data from the server all compounds were predicted as CNS active with satisfactory probability (Table 2).

2.3. Docking studies

Docking studies were carried out to provide insight into the binding mode of the synthesized compounds **6h** and **6k**. In order to validate the docking reliability, root-mean-square distance (RMSD) values of 0.774 Å and 0.615 Å were obtained between bounded ligands and the re-docked ligands tacrine and donepezil, which shows the high reliability of the GOLD method to reproduce the known binding mode.

According to the docking results, the best docked pose of molecule **6h** shows a hydrogen bond between oxygen atom of benzofuran and Ser198. The positively charged nitrogen induces the π-cation interactions with aromatic residues Phe329 and Trp82. Moreover, the benzyl pyridinium section of **6h** involves in the π-π interaction with Tyr332 (Fig. 2).

Amongst the synthetic compounds, **6k** (IC_{50} = 2.1 μM) with