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2-Phenylbenzofuran derivatives as butyrylcholinesterase inhibitors: Synthesis, biological activity and molecular modeling

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ABSTRACT

A series of 2-phenylbenzofurans compounds was designed, synthesized and evaluated as cholinesterase inhibitors. The biological assay experiments showed that most of the compounds displayed a clearly selective inhibition for butyrylcholinesterase (BChE), while a weak or no effect towards acetylcholinesterase (AChE) was detected. Among these benzofuran derivatives, compound **16** exhibited the highest BChE inhibition with an IC_{50} value of 30.3 μM. This compound was found to be a mixed-type inhibitor as determined by kinetic analysis. Moreover, molecular dynamics simulations revealed that compound **16** binds to both the catalytic anionic site (CAS) and peripheral anionic site (PAS) of BChE and it displayed the best interaction energy value, in agreement with our experimental data.

Alzheimer's disease (AD) is an irreversible and progressive brain disorder, which is characterized by progressive memory loss and a wide range of cognitive impairments.[1] Although the precise cause of AD is not completely known, there are some factors that have been described to play a significant role in the pathogenesis of AD, such as: deficit of acetylcholine (ACh), presence of amyloid-β deposits, τ-protein aggregation, oxidative stress and metal ions imbalance. Among these distinct research approaches, the cholinergic hypothesis has been examined more extensively. In fact, low levels of ACh appear to be a critical element in the development of cognitive and neurodegenerative disorders in AD patients.[2]

Accordingly, one strategy in AD treatment is to restore the levels of ACh by inhibiting acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), which are mainly responsible for ACh hydrolysis. These enzymes belong to the superfamily of α/β-hydrolase fold proteins and they are able to hydrolyze ACh with different efficiencies.[3] They are encoded by two distinct human genes and display 65% homology in their amino acid sequences. These two proteins also show a great similarity in both their tertiary structure and their overall architecture of active sites.[4,5] Both AChE and BChE have indeed a primarily hydrophobic active gorge into which ACh diffuses and is cleaved.

Ligand binding specificity between the two enzymes has been related to differences in the residue's structural arrangement, which lead to the active site located near the bottom of a deep and narrow gorge (Fig. 1, Table S1 in Supplementary material). The gorge is characterized by (a) a peripheral site at the entrance, (b) an oxyanion hole, (c) a choline-binding site located within the entrance, and (d) the active site constituted by an acyl pocket buried near the catalytic triad.

In fact, structural analysis had revealed that these enzymes have two major substrate-binding sites. One is a peripheral anionic site (PAS) at the entrance of the gorge, acting as a regulatory site; the other is the catalytic anionic site (CAS), which is located in the bottom of the gorge and it is assigned to a Ser-His-Glu catalytic triad.[6]

AChE and BChE appear to be simultaneously active in the synaptic hydrolysis of ACh, terminating its neurotransmitter action and co-regulating levels of ACh.[7] AChE has a very high catalytic efficiency for ACh hydrolysis and it is mainly found in cholinergic synapses, while BChE has lower efficiency and it is widely

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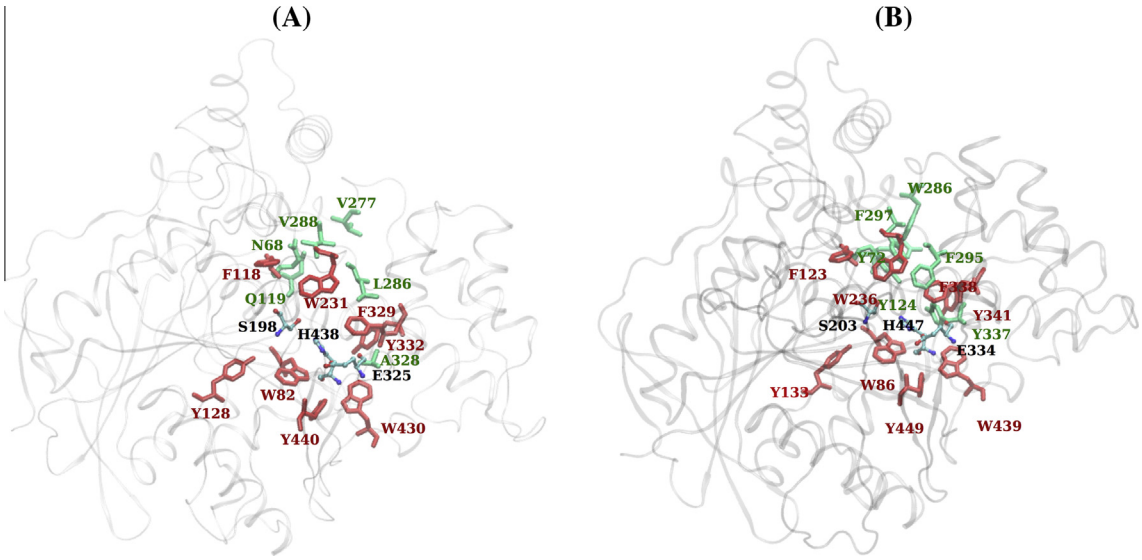


Figure 1. Cartoon representation for the enzymes under investigation. (A) *Equine serum* BChE (B) *Electrophorus electricus* AChE. The residues lining the gorge of the two enzymes are shown. The conserved residues between the two are shown in red (licorice representation) and non-conserved in green. The catalytic triad residues are shown as ball-stick representation.

distributed in tissues and plasma. In a healthy brain, AChE predominates and BChE is considered to play a minor role in regulating ACh levels. On the other hand, BChE activity increases in the temporal cortex and hippocampus during the development of AD, while at the same time AChE activity decreases.[8]

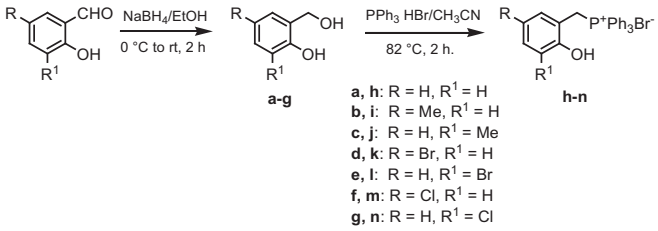
Since AD is characterized by a forebrain cholinergic neuron loss and a progressive decline in ACh, a possible therapeutic strategy involves the use of cholinesterase (ChE) inhibitors to restore the neurotransmitter levels and thus retard AD symptoms.[9–11]

These inhibitory molecules may act by binding the CAS site (competitive mechanism) or PAS (non-competitive mechanism) or may exert a dual binding enzyme inhibition—acting as mixed-type inhibitors.[12] Moreover, since the oxidative stress may be a risk factor for the initiation and progression of AD drugs, both antioxidant and inhibitory actions might be useful for either the prevention or the treatment of AD.

Benzofuran scaffold (oxygen heterocycle) is a common moiety found in many biologically active natural and synthetic products. Therefore, it represents a very important pharmacophore in drug discovery.[13] It is present in many medicinally important compounds which show biological activity, including anticancer and anti-inflammatory properties.[14,15] Benzofuran scaffold has drawn considerable attention over the last few years due to its profound physiological and chemotherapeutic properties.[16] Some benzofuran derivatives are also known as monoamine oxidase and 5-lipoxygenase inhibitors, antagonists of the angiotensin II receptor, blood coagulation factor Xa inhibitors, ligands of adenosine A_1 receptor, etc.[13,17] Recent studies have also investigated their inhibitory activity towards AChE.[18–21]

In this study, a series of 2-phenylbezonfurans was synthesized and their inhibitory activity towards the ChE was investigated. To better understand the enzyme inhibition mechanisms, in relation to the substituents and their positions in the presented compounds, molecular modeling studies were also performed.

Compounds **1**–**16** were efficiently synthesized by an intramolecular Wittig reaction (Schemes 1 and 2). The desired Wittig reagent was readily prepared from the conveniently substituted *ortho*-hydroxybenzyl alcohol **a**–**g** and triphenylphosphine hydrobromide (Scheme 1).[22–31] The key step for the formation of the benzofuran moiety was achieved by an intramolecular reaction between *ortho*-hydroxybenzyltriphosphonium salts **h**–**n** and the



Scheme 1.

appropriate benzoyl chlorides (Scheme 2) (Supplementary material).[30,32–39]

Hydrolysis of the methoxy groups of compound **2**–**8** was performed by treatment with hydrogen iodide in acetic acid/acetic anhydride, to gave the corresponding hydroxy derivatives **10**–**16**.[30,33,40–42] We report the ^{1}H NMR, ^{13}C NMR and mass spectrometry analysis (Supplementary material Figs. S1–S3) of our more active compound **16**.

The AChE and BChE inhibitory activity[43] of all synthetized compounds was firstly evaluated at compound concentration of 100 μM (Supplementary material). As observed, only compounds **9** and **11** exerted a very weak inhibitory activity towards AChE, while all the compounds, except compounds **2** and **10**, inhibited BChE enzymatic activity with a varying efficiency (Table 1). In particular, compounds **12**, **14** and **16** showed the highest inhibition percentages and the lowest IC_{50} values.

Compound **16** was found to be the best BChE inhibitor with an IC_{50} value of 30.3 μM. Thus, it has been chosen for the kinetic studies. We investigated the kinetic behavior of BChE at different concentrations of inhibitor and substrate (BTCI). In the presence of compound **16**, the Lineweaver–Burk plots showed that this compound was a mixed-type inhibitor, since increasing the concentration of compound **16** resulted in a family of straight lines which intersected in the second quadrant (Fig. 2). In this case, the inhibitor can bind not only with the free enzyme but also with the enzyme–substrate complex. The equilibrium constants for binding with the free enzyme (K_I) and with the enzyme–substrate complex (K_{IS}) were obtained either from the slope or the V_{max} values (y-intercepts) plotted against inhibitor concentration, respectively.