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Research paper

Potential of aryl–urea–benzofuranylthiazoles hybrids as multitasking agents in Alzheimer's disease

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ABSTRACT

New benzofuranylthiazole derivatives containing the aryl–urea moiety were synthesized and evaluated *in vitro* as dual acetylcholinesterase (AChE)-butyrylcholinesterase (BuChE) inhibitors. In addition, the cupric reducing antioxidant capacities (CUPRAC) and ABTS cation radical scavenging abilities of the synthesized compounds were assayed. The result showed that all the synthesized compounds exhibited inhibitory activity on both AChE and BuChE with 1-(4-(5-bromobenzofuran-2-yl)thiazol-2-yl)-3-(2-fluorophenyl)urea (**e25**, IC_{50} value of 3.85 μM) and 1-(4-iodophenyl)-3-(4-(5-nitrobenzofuran-2-yl) thiazol-2-yl)urea (**e38**, IC_{50} value of 2.03 μM) as the strongest inhibitors against AChE and BuChE, respectively. Compound **e38** was 8.5-fold more potent than galanthamine. The selectivity index of **e25** and **e38** was 2.40 and 0.37 against AChE and BuChE, respectively. Compound **e2**, **e4** and **e11** (IC_{50} = 0.2, 0.5 and 1.13 μM, respectively) showed a better ABTS cation radical scavenging ability than the standard quercetin (IC_{50} = 1.18 μM). Best poses of compounds **e38** on BuChE and **e25** on AChE indicate that the thiazole ring and the amidic moiety are important sites of interaction with both ChEs. In addition, the benzofuran ring and phenyl ring are anchored to the side chains of both enzymes by π–π(pi–pi) interactions.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease with symptoms of memory loss, cognition defect and behavioural impairment [1–3]. The classical hypothesis of AD, named "cholinergic hypothesis", suggests that acetylcholinesterase inhibitors (AChEI) could increase ACh levels in AD patients through the inhibition of AChE and, therefore, relieve some symptoms experienced by AD patients [4,5]. AD is probably associated with multifaceted etiologies and pathogenic phenomena. In any case, oxidative stress can be considered the causative unifying factor [6].

Cholinergic system is the earliest and most profoundly affected neurotransmitter system in AD, with substantial loss of the forebrain, cortex, and hippocampus. Ach and the above mentioned brain regions are critical in the acquisition, processing, and storage of memories and have supported the use of cholinomimetics in the treatment of AD [7]. It is well known that two forms of cholinesterases coexist ubiquitously throughout the body, i.e., acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8). Among its functions, AChE regulates the impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter ACh. BuChE, also known as pseudocholinesterase, is primarily localized in plasma, liver, and muscle tissues.

The pharmacological role of BuChE was not yet completely understood but it is supposed that it may have a compensatory role in the modulation of the hydrolysis of ACh in brains causing degenerative changes. Consequently, BuChE may be a target for increasing the cholinergic tone in AD patients [8,9].

Based on these findings, many efforts have been made in the search for potent AChE inhibitors, and a large number of naturally

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occurring and synthetic AChE inhibitors such as galantamine, huperzine A, physostigmine, ambenonium and tacrine have already been reported (Fig. 1) [10–13].

Crystallographic structure of AChE from *Torpedo californica* [14] shows three main binding sites, namely (a) the catalytic triad at the bottom of active site including Ser200, His440 and Glu327; (b) the catalytic anionic site (CAS) at the vicinity of the catalytic triad consisting of Trp84, Tyr130, Gly199, His441 and His444; (c) peripheral anionic site (PAS) at the gorge rim comprising Tyr70, Asp72, Tyr121, Trp279 and Tyr334 [15,16]. Inhibition of AChE can be accomplished by three different ways depending on the nature of the interaction of the inhibitor with the enzyme binding site [17]. Irreversible inhibitors, such as organophosphates, form a strong covalent bond with the serine residue in the catalytic triad. Pseudo-irreversible inhibitors, as carbamates, lead to formation of a carbamylated serine into the catalytic triad that is slowly hydrolysed to regenerate the active enzyme. Reversible inhibitors give a transient non-covalent binding through electrostatic interactions with the active and/or peripheral sites. The reversible inhibitors may be classified as (a) active-site inhibitors directed toward the catalytic anionic sub-site at the bottom of the gorge, (b) peripheral anionic site inhibitors which bind at the entrance to the gorge, or (c) elongated gorge-spanning inhibitors which bridge the two sites [17].

Recently, some works on AChE inhibitors with a benzofuran moiety have been reported [18,19]. Amide or imide-based AChE inhibitors have also been reported [20,21] with both these functionalities acting as hydrogen bond donors towards oxygen or nitrogen lone pairs of Tyr72, Tyr124, Tyr203 and Tyr337 of the enzyme. Amidic or imidic fragments interact with the catalytic triad Ser203-Glu334-His447 of the active binding site. Hydrogen bonds with Tyr70 and His447 were also reported for compounds having urea, carbamate or sulphonamide moieties as spacers [22–25].

Dibenzofuran and tricyclic tacrine-ferrulic acid derivatives as multifunctional anti-Alzheimer agents were also previously reported by Fang and co-workers [26,27]. Tricyclic and heterocyclic rings derivatives, such as tacrine, quinolizidinyl, piperidine and indolinone derivatives give strong parallel π–π (pi–pi) stacking with residues at CAS [28–33]. An additional π–π (pi–pi) stacking interaction was also observed between the furan ring and Phe330 [18,19]. Moreover, it has been reported that thiazolo-triazin derivatives form a hydrogen bond with Tyr124 and π–π interaction with Trp286 [34]. Docking into the BuChE gorge, which is larger than that of AChE, showed that heterocyclic rings give (i) a cation-π interaction with Trp82 and Trp430 and also (i) hydrophobic and/or π–π (pi–pi) stacking interactions with Phe118 and Trp231 [3,24].

Overall, heterocyclic and aromatic rings can have strong parallel π–π stacking with residues at the CAS of the enzyme whereas the urea moiety might contribute to inhibitor activity by additional interactions.

On the basis of the above reported evidences a series of 38 novel urea substituted benzofuran derivatives (**e1**–**e38**), including the thiazole ring as an additional spacer, was designed and synthesized (Fig. 2). AChE/BuChE inhibition and antioxidant properties were evaluated. Structure–activity relationships are described and rationalized by docking studies.

2. Results and discussion

2.1. Chemistry

The synthetic procedures are depicted in Scheme 1. 2-Acetyl benzofuran derivatives **b1-b3** were prepared as previously reported [35]. 1-(1-benzofuran-2-yl)-2-bromoethanone derivatives (**c1**–**c3**) were prepared by brominating of 2-acetyl benzofuran using molecular bromine in chloroform. The reaction of **c1**–**c3** with thiourea in ethanol gave 4-(1-benzofuran-2-yl)-1,3-thiazol-2-amines (**d1**–**d3**). These compounds were reacted with arylisocyanates in THF to get the final products (**e1**–**e38**) at high yields.

All the new compounds were characterized through ^{1}H NMR, ^{13}C NMR, IR, MS and elemental analysis. Infrared spectra for **e1**–**e38** show absorptions between 3500 and 3000 cm^{-1} related to N–H stretching, absorptions at 1650-1700 cm^{-1} from the urea carbonyl moiety stretching and absorptions at 1550 cm^{-1} for the thiazole C=N moiety stretching. Furthermore, absorptions among 3111 cm^{-1} and 2950 cm^{-1} indicated C–H stretching for the thiazole and furan rings, respectively. In case of ^{1}H NMR spectra, the resonance for the hydrogen attached to the amide nitrogen was between 8.20 and 11.50 ppm. Signals for aromatic protons were observed between 6.50 and 8.52 ppm and those for the proton of thiazole and furan ring were detected around 7.10 and 7.50 ppm as a singlet. Regarding ^{13}C NMR spectra, carbon atoms of urea carbonyl, benzofuran ring (C_2 and C_8) and thiazole ring (C_3) were observed between 150.7 and 161.4 ppm.

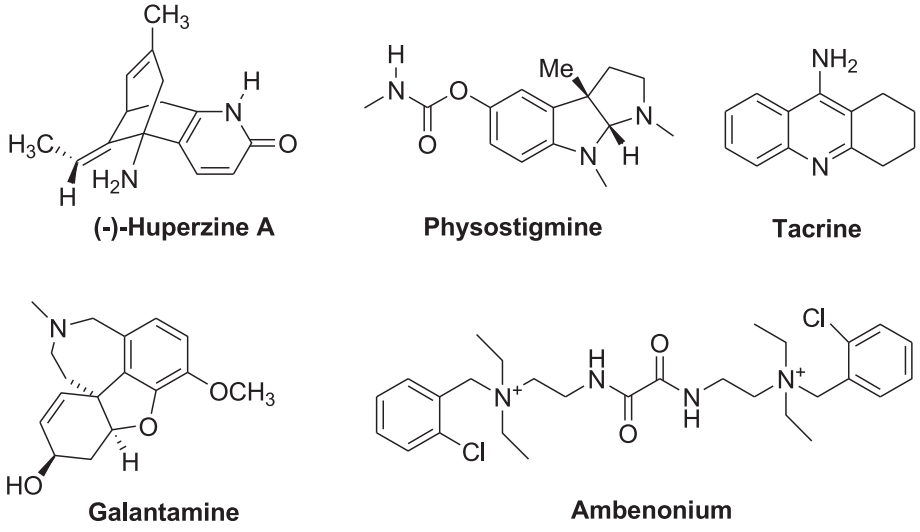

Fig. 1. Structures of well-known cholinesterase inhibitors.