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Design and synthesis of some new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring and the investigation of their inhibitory potential on in-vitro acetylcholinesterase and butyrylcholinesterase

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ABSTRACT

A series of new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring were designed, synthesized and evaluated for their ability to inhibit both cholinesterase enzymes. In addition, a series of carboxamide and propanamide derivatives bearing biphenyl instead of phenylpyridazine were also synthesized to examine the inhibitory effect of pyridazine moiety on both cholinesterase enzymes. The inhibitory activity results revealed that compounds **5b, 5f, 5h, 5j, 5l** pyridazine-3-carboxamide derivative, exhibited selective acetylcholinesterase (AChE) inhibition with IC_{50} values ranging from 0.11 to 2.69 μM. Among them, compound **5h** was the most active one (IC_{50} = 0.11 μM) without cytotoxic effect at its effective concentration against AChE. Additionally, pyridazine-3-carboxamide derivative **5d** (IC_{50} for AChE = 0.16 μM and IC_{50} for BChE = 9.80 μM) and biphenyl-4-carboxamide derivative **6d** (IC_{50} for AChE = 0.59 μM and IC_{50} for BChE = 1.48 μM) displayed dual cholinesterase inhibitory activity. Besides, active compounds were also tested for their ability to inhibit Aβ aggregation. Theoretical physicochemical properties of the compounds were calculated by using Molinspiration Program as well. The Lineweaver-Burk plot and docking study showed that compound **5 h** targeted both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE.

1. Introduction

With respect to the increase in the average life expectancy, Alzheimer Disease (AD), the most common form of age-related dementia, has become a major threat to the population over the age of 65 during the past several decades. According to the 2015 report of Alzheimer Disease International, the number of patients with dementia is about 46.8 million, and it is expected to increase up to 131.5 million by 2050. Besides, it has been reported that AD is responsible for about more than 50% of dementia cases and is the third leading cause of death for the high-income countries following ischemic heart disease and stroke [1,2]. Hence, this situation indicates that AD is a serious health problem.

Although the first AD case was described by Alois Alzheimer in 1906, the precise cause of AD is still unclear due to its complex and multifactorial nature. However, several pathophysiological elements such as amyloid-β (Aβ) aggregates, hyperphosphorylated tau-protein tangles, acetylcholine (ACh) deficiency and oxidative stress are identified to be related to the pathophysiological processes of the disease. Based on these, several hypotheses have been put forward to explain the cause of AD [3–5]. Among them, amyloid β and tau hypotheses have been investigated extensively, however; the drug candidates targeting them could not come into use due to their severe adverse effects or inadequate clinic results [6,7]. On the other hand, cholinergic hypothesis, the most studied one, continues to be attractive for drug development studies [3–5]. Accordingly, clinical symptoms such as difficulty in recalling recent events and other cognitive impairments are the consequence of reduced cholinergic transmission, leading to the

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suggestion that all attempts to increase cholinergic transmission might improve the cognitive functions. In this connection, the strategy for enhancing cholinergic neurotransmission via the inhibition of cholinesterase enzymes, responsible for synaptic cleavage of acetylcholine, has become an important means in the treatment of AD [8–11]. Indeed, there are just three approved drugs called donepezil, galantamine, and rivastigmine, which are cholinesterase inhibitors, and there is one drug called memantine, which is *N*-methyl-D-aspartic acid (NMDA) receptor antagonist [3–5]. Although this validates the importance of cholinesterase inhibition for the treatment of AD at one hand, it is obvious that the number of these drugs to be used in the treatment of this illness is quite limited.

Acetylcholine is a neurotransmitter of the peripheral and central nervous system (CNS). It provides neural conduction at the neuromuscular junctions in the peripheral nervous system and mainly controls the memory and learning process in the CNS. Two enzymes that break down the acetylcholine to terminate cholinergic transmission are the acetylcholinesterase (AChE) (EC 3.1.1.7) and butyrylcholinesterase (BChE) (EC 3.1.1.8) enzymes. AChE is mainly synthesized in the cholinergic nervous system and muscles, while BChE is primarily synthesized in the liver, and is the dominant cholinesterase in the plasma. Although both can hydrolyze acetylcholine, their kinetics and selectivity for substrate acetylcholine are different [12,13].

Regarding the current drugs for the treatment of AD, although they differ each other with respect to the organization of their active sites, both AChE and BChE are the potential targets to be focused on. The active site of AChE consists of two primary binding sites, called the catalytic active site (CAS) and the peripheral anionic site (PAS). CAS is located at the bottom of enzyme active-site gorge and reveals the catalytic activity of the enzyme. On the other hand, PAS is located at the entrance of the active site-gorge and enhances the catalytic activity of the enzyme by directing acetylcholine towards the active site [14]. However, recent studies have reported that PAS also has a non-catalytic function in such a way that it can interact with Aβ peptide and cause Aβ plaque formation by triggering Aβ aggregation and these plaques mediate neurodegeneration observed in AD. Thus, the design of the compounds having the ability to bind the both sites of the enzyme may provide both cholinergic enhancement and Aβ aggregation inhibition [15]. Among the three AChEIs drugs, only donepezil has dual binding sites inhibition for AChE. The general structure of BChE enzyme is highly similar to the AChE. However, most of the aromatic residues in the AChE active site replaces with the aliphatic amino acids in the BChE enzyme, which results in BChE active pocket being about 200 $Å^3$ larger in volume. The increase in volume allows binding of the inhibitors to the BChE active pocket in alternative conformations [16]. Moreover, in healthy individuals, the primary enzyme responsible for the acetylcholine hydrolysis in CNS is the AChE. However, as the disease progresses, levels of AChE decrease while the levels of BChE increase [17,18]. Therefore, simultaneous inhibition of both enzymes should provide additional benefits in the treatment of AD.

Taking into account the aspects stated above, within this research, it was aimed to design novel pyridazine derivatives and corresponding biphenyl derivatives that have the potential to inhibit cholinesterase enzymes.

For many years, our research group has focused on pyridazine derivatives associated with different biological activities [19–22]. For the starting point of our studies, we have employed minaprine, a known pyridazine derivative antidepressant drug. As classical to some of the CNS acting agents, minaprine can interact with various neuroreceptors and it is a weak inhibitor of AChE (IC_{50} = 85 μM) [23]. Considering the structure of minaprine, it is apparent to observe how it also mimics the structure of acetylcholine (Fig. 1). Therefore, a pyridazine derivative, minaprine, is a good tool to design novel molecules that have higher potential to inhibit cholinesterase enzymes. Furthermore, we preferred the bioisosteric replacement of the ester group of acetylcholine with an amide with the aim of improving the hydrolytic stabilities of designed compounds. As a result, on the basis of these findings and as a continuation of our research for new cholinesterase inhibitors [19,24], we have designed 6-(substitutedphenyl)pyridazine-3-carboxamide and 6-(substitutedphenyl)pyridazine-3-yl propanamide derivatives along with the [1,1′-biphenyl]-4-carboxamide and ([1,1′-biphenyl]-4-yl)propanamide derivatives employing the bioisosteric replacement of acetylcholine in minaprine structure and remembering the Aryl-Spacer-Tertiary amine pharmacophore, which also exist in minaprine, and within the current cholinesterase inhibitor drugs (Fig. 1). Subsequently, the synthesized compounds were screened for their ability to inhibit both cholinesterase enzymes. Additionally, Aβ aggregation inhibition, cytotoxicity and molecular docking studies were also performed for selected compounds.

2. Result and discussion

2.1. Chemistry

The synthetic scheme for the synthesis of the compounds are shown in Schemes 1–4. Initially, 6-chloro-*N*-(2-substitutedethyl)pyridazine-3-carboxamide intermediates **(1–4)** were prepared by commercially available 6-chloropyridazine-3-carboxylic acid and appropriate ethylamine derivatives. It is important to note that the intermediates **1** to **4** have also been originally synthesized compounds within this research. Next, the Suzuki cross-coupling reaction of the prepared intermediate with suitable phenylboronic acid derivative afforded original corresponding *N*-(2-substitutedethyl)-6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl) pyridazine-3-carboxamide derivative **(5a–l)**. Compounds **(6a–d),** bearing biphenyl core, were synthesized by the reaction of commercially available [1,1′-biphenyl]-4-carboxylic acid and appropriate ethylamine derivative in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP). To obtain 3-substituted-*N*-[6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-yl] propanamide derivatives **(13a-i)**, we synthesized both 6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-amine intermediates **(7–9)** and 3-substitutedpropanoic acid intermediates **(10–12)** as starting materials. Intermediates **7–9** were prepared by a previously reported method via the Suzuki cross-coupling reaction using commercially available 6-phenylpyridazin-3-amine and suitable phenylboronic acid derivative [25]. On the other hand, Michael addition reaction of appropriate secondary amine derivative and methyl acrylate afforded esters which were further hydrolyzed with NaOH to yield intermediates **10–12**. Then, compound **13a-i** were synthesized by the reaction of obtained intermediates in the presence of EDC and DMAP. Finally, the treatment of commercially available [1,1′-biphenyl]-4-amine and 3-chloropropionyl chloride gave the intermediate **14**, which was treated with secondary amine derivatives to give corresponding *N*-([1,1′-biphenyl]-4-yl)-3-substitutedpropanamide derivatives **(15a-c)**.

Each of the title compounds synthesized are original except for compounds **6d** and **15b**. **6d** has CAS number 756780–04-8 but there are no registered reference and experimental data for this compound on SciFinder. **15b** is registered with CAS number 191168–70-4 and there is only one reference about this compound [26]. The chemical structures of newly synthesized compounds were verified by ^{1}H NMR, ^{13}C NMR, HRMS and elemental analysis. The ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses data of the compounds are consistent with the proposed structures. Chemical shifts of protons as explained in details under the experimental section.

2.2. Cholinesterase inhibitory activities

The inhibitory activities of the synthesized compounds on AChE (from electric eel) and BChE (from equine serum) were determined by the modified Ellman's method using donepezil and galantamine (10 μM) as the reference compounds. First, the percent inhibitions of