Bioorganic Chemistry 72 (2017) 208–214

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Novel multi-targeted agents for Alzheimer's disease: Synthesis, biological evaluation, and molecular modeling of novel 2-[4-(4-substitutedpiperazin-1-yl)phenyl]benzimidazoles

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ARTICLE INFO

Article history:
Received 3 April 2017
Revised 28 April 2017
Accepted 29 April 2017
Available online 2 May 2017

Keywords:
Benzimidazole
Alzheimer's disease
Butyrylcholinesterase
Aβ aggregation
Neuroprotection

ABSTRACT

The present study describes the synthesis, pharmacological evaluation (BChE/AChE inhibition, Aβ antiaggregation, and neuroprotective effects), and molecular modeling studies of novel 2-[4-(4-substitutedpiperazin-1-yl)phenyl]benzimidazole derivatives. The alkyl-substituted derivatives exhibited selective inhibition on BChE with varying efficiency. Compounds **3b** and **3d** were found to be the most potent inhibitors of BChE with IC_{50} values of 5.18 and 5.22 μM, respectively. The kinetic studies revealed that **3b** is a partial non-competitive BChE inhibitor. Molecular modeling studies also showed that the alkyl-substituted derivatives were able to reach the catalytic anionic site of the BChE. The compounds with an inhibitory effect on BChE were subsequently screened for their Aβ antiaggregating and neuroprotective activities. Compounds **3a** and **3b** exerted a potential neuroprotective effect against H_2O_2 and Aβ-induced cytotoxicity in SH-SY5Y cells. Collectively, **3b** was found as the most promising compound for the development of multi-target directed ligands against Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a multifactorial, neurodegenerative disease that will affect an estimated 66 million people by 2030 [1]. Evidence suggests that AD is associated with complex interactions between amyloid precursor protein, amyloid β (Aβ), tau, α-synuclein, and apoE4, resulting in synaptic depression, aberrant network activity, impaired cellular homeostasis, and neuronal loss [2]. Given the complexity of the disease process, effective treatments for AD have proved elusive. Cholinergic neurotransmission has been the most studied target, since the 1970s [3,4].

The cholinergic hypothesis states that reduced acetylcholine transmission, altered choline uptake, and insufficient expression of cholinergic receptors contribute to the cognitive decline seen in AD patients [3]. Acetylcholine is predominantly hydrolyzed by acetylcholinesterase in mammalian CNS. However, studies in AChE-knockout mice revealed the presence of a compensation mechanism by kin enzyme butrylycholinesterase [5].

Today, cholinesterase inhibitors are widely used to rectify cholinergic transmission in the treatment of AD. The FDA has approved four AChE inhibitors, tacrine, donepezil, galantamine, and rivastigmine. Donepezil is the most widely used and leads the AD market [6]. Although AChE is still the primary target for new drugs designed to treat AD, recent evidence has highlighted the importance of BChE on fibril formation, which also leads to ACh degradation [7–10].

Pathogenic Aβ peptide fibrillation, another neuropathological hallmark of AD, is purported to induce sequential events leading to neuronal death. These include tau protein phosphorylation, neurofibrillary tangle formation, mitochondrial dysfunction, neuroinflammation, and apoptosis [11–13]. The results of clinical trials with new drug candidates targeting Aβ showed that monotherapeutic approaches were not sufficient to improve the clinical outcomes of AD [12,14,15].

The acceptance of the multifactorial nature of AD has led to the development of a new treatment approach using Multi-Target Directed Ligands (MTDLs). Novel AD drug candidates with cholinesterase inhibitory, Aβ lowering, and neuroprotective activities are designed using the MTDL paradigm [16–18]. Benzimidazoles with diverse biological activities [19–21] may be a viable starting point for AD. Moreover, many researchers demonstrated that benzimidazoles (Fig. 1A) have significant ChE activity [22–24]. Besides, it was shown that some benzimidazole derivatives (Fig. 1B) are useful as amyloid imaging probes due to their high binding affinity to Aβ aggregates and high uptake into the brain [25,26]. Their ChE

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http://dx.doi.org/10.1016/j.bioorg.2017.04.018
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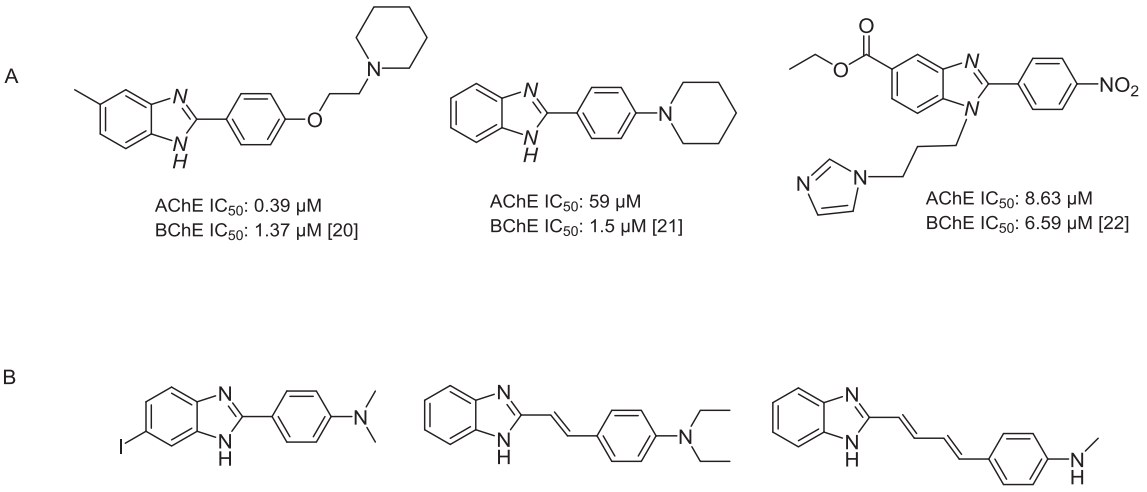


Fig. 1. Benzimidazoles (A) with ChE inhibitory activity (B) with high binding affinity to Aβ aggregates.

inhibitor activity and high binding affinity to Aβ make them an interesting scaffold for the development novel MTDLs against AD.

In the light of these considerations, the present study is focused on the design, synthesis, and biological evaluation of some 2-[4-(4-substitutedpiperazin-1-yl)phenyl]benzimidazoles to create novel MTDLs by combining BChE and Aβ aggregation inhibitory activities in one neuroprotective structure.

2. Results and discussion

2.1. Chemistry

A series of 2-[4-(4-substitutedpiperazin-1-yl)phenyl]benzimidazole derivatives **3a-h** were synthesized as outlined in Fig. 2. The synthesis of **3a** and **3c** were previously reported in the literature [27,28]. The starting compounds, 4-(4-substitutedpiperazin-1-yl) benzaldehydes (1a-d), were obtained using 4-fluorobenzaldehyde and appropriate piperazines in accordance with previously published method [29]. The sodium hydroxy[4-(4-substitutedpiperazin-1-yl)phenyl]methanesulfonate salts (**2a-d**) were gained by the reaction of 4-(4-substitutedpiperazin-1-yl)benzaldehydes (**1a-d**) with sodium bisulfide. The target compounds (**3a-h**) were achieved with 48–78% yield by treating appropriate o-phenylenediamines with corresponding salts [30]. The structures of the compounds (except **3a** and **3c**) were characterized by spectral methods (IR, $^1H/^{13}C$ NMR, ESI–MS) and elemental analysis.

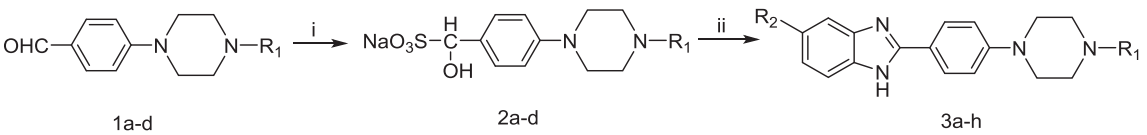
In the IR spectra, the target compounds showed absorption bands around 1610 cm^{-1} due to —C=N— stretching. In the ^1H NMR spectra of the compounds, NH protons of some derivatives appeared at around 13.0 ppm. The signals of the methylene protons belonging to the piperazine ring resonated as two peaks at around 2.5 and 3.2 ppm. The protons on the benzimidazole ring of **3b** and **3d** appeared as two multiple peaks at around 7.1 (4-H and 7-H) and 7.5 (5-H and 6-H) ppm where as those of **3e-h** observed at around 6.7 (6-H), 7.0 (4-H) and 7.4 (7-H) ppm. In the ^{13}C NMR spectra, the signals of methylene carbons of piperazine ring appeared at around 47 and 52 ppm. In the ESI-MS spectra, the signals of protonated $[M+H]^+$ and sodiated $[M+Na]^+$ molecular ions of all compounds were observed. Elemental analysis was in agreement with the suggested chemical structures of the synthesized compounds.

2.2. Biological activity

2.2.1. Cholinesterase inhibitory activity

The target compounds were tested for their potential to inhibit human recombinant AChE and equine BChE enzymes by the Ellman method. The IC_{50} values and selectivity indices of **3a-h**, as well as the reference drug donepezil, are summarized in Table 1.

Among the compounds, phenyl substituted derivatives, **3c** and **3g**, showed no inhibitory activity against either ChE. Alkyl substituted derivatives **3a**, **3b**, **3d-f**, and **3h** were more potent in inhibiting BChE (IC_{50}: 5.18–20.21 μM) as compared to AChE (IC_{50}: 34.85–229.30 μM). Furthermore, ethyl (**3b**) and benzyl (**3d**) substituted derivatives were found to be the most active BChE inhibitors with IC_{50} values of 5.18 and 5.22 μM, respectively. In addition, **3d** demonstrated the highest selectivity for BChE over AChE (18-fold). Meanwhile, replacement of benzyl group by phenyl led to a dramatic decrease in activity (IC_{50} > 100 μM for both ChEs). A comparison of the inhibitory activities highlights that the inhibitory potency of the compounds toward both ChEs is negatively affected by the substitution of the methoxy group at position 6 of the benzimidazole nucleus.



R_1 = CH_3, C_2H_5, C_6H_5, $C_6H_5CH_2$
R_2 = H, OCH_3

Fig. 2. Synthesis of the target compounds. Reagents and conditions: (i) $NaHSO_3$, 1 h, stirred in ethanol-water mixture, (ii) appropriate o-phenylenediamine, 4–6 h, refluxed in DMF.