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Research paper

Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes

Begüm Nurpelin Sağlık [a, b], Sinem Ilgın [c], Yusuf Özkay [a, b, *]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey
[b] Doping and Narcotic Compounds Analysis Laboratory, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey
[c] Department of Toxicology, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

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ABSTRACT

Donepezil (DNP), an acetylcholinesterase (AChE) inhibitor, is one of the most preferred choices in Alzheimer diseases (AD) therapy. In the present study, 38 new DNP analogues were synthesized. Structures of the synthesized compounds (**1**–**38**) were elucidated by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic methods and elemental analysis. Inhibitory potential of the compounds on cholinesterase enzymes was investigated. None of the compounds displayed significant activity on butyrylcholinesterase (BChE) enzyme. On the other hand, compounds **26**–**29** indicated important inhibitory activity on AChE enzyme. Kinetic studies were performed in order to observe the effects of the most active compounds on substrate-enzyme relationship. Cytotoxicity studies and theoretical calculation of pharmacokinetic properties were also carried out to get an information about toxicity and pharmacokinetic profiles of the compounds. The compounds **26**–**29** were found to be nontoxic at their effective concentrations against AChE. A good pharmacokinetic profile was predicted for these compounds. Docking studies were performed for the most active compounds **26**–**29** and interaction modes with enzyme active sites were determined. Docking studies revealed that there is a strong interaction between the active sites of AChE enzyme and analyzed compounds.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative process, characterized by age-related loss of memory [1]. Language disorders [2], visual deficiency [3], orientation disorders [4], shortage of decision-making, management function [5] and memory impairment [6] are the primary symptoms of the disease. Its etiology has not been enlightened yet. It is thought that accumulation of β amyloid in the senile plaques [7], neurofibrillary tangle composed by hyperphosphorylation of tau protein [8], loss of cholinergic activity in certain part of brain [9], cerebrovascular disorders [10], oxidative stress [11], functional loss of neuron and synapse [12] and absence of acetylcholine (ACh) [13] are responsible for the pathophysiology of AD [14]. Although numerous therapeutic approaches have been offered, only non-competitive *N*-methyl-D-aspartate receptor antagonist, memantine, and acetylcholinesterase (AChE) inhibitors, tacrine, donepezil (DNP), rivastigmine and galantamine have been approved by the European and United States regulatory authorities [15,16]. Among these drugs, DNP is the most preferred AChE inhibitor because it gives the most positive response in AD treatment. Furthermore, DNP has some advantages as blood-brain barrier permeability, non-hepatotoxicity, the least side efficacy and usage once-daily [17].

Cholinergic hypothesis reveals that there is a loss in cholinergic activity because of the decreased level of Ach, which is hydrolyzed by cholinesterase enzymes in synaptic gap [18,19]. There are two types of cholinesterase enzymes in the central nervous system (CNS): AChE and butyrylcholinesterase (BChE) [20]. These two isoenzymes are responsible for hydrolyzing ACh. Although AChE has more hydrolytic activity than BChE, it is reported that BChE plays a key role in the regulation of AChE activity [21]. Thus, AChE inhibitors are preferred in the treatment of AD to keep up ACh normal levels in the CNS and to eliminate the symptoms of disease.

According to the X-ray crystallographic structure of AChE (PDB ID:4EY7), two main binding sites has been determined: the catalytic anionic site (CAS) including Ser203, Glu334, His447, Trp86,

* Corresponding author. Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470, Eskişehir, Turkey.
E-mail address: yozkay@anadolu.edu.tr (Y. Özkay).

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Tyr130, Tyr133, Tyr337, Phe338 and the peripheral anionic site (PAS) consisting of Tyr72, Asp74, Tyr124, Tyr341, Trp286 [22–25]. It has been reported that DNP interacts with both CAS and PAS and thus it is situated in the gorge concordantly owing to the feature of dual binding site (DBS) [26–28]. Analyses of binding modes of the DNP indicate that the benzyl moiety is in a π- π interaction with the indole of Trp86 in the CAS. The formation of hydrogen bond between the oxygen atom of the carbonyl group in the 1-indanone and the amino group of Phe338 is a very significant interaction in terms of binding to the active site. The 1-indanone constitutes a π-π interaction with the indole of Trp286 in the PAS region. The piperidine has a position in the gorge to interact with Tyr337 and Tyr341 by doing a hydrogen bond. It also set up a van der Waals interaction with amino acids in both CAS and PAS [29–31].

As stated above, receptor-ligand interactions and mechanism of molecular recognition of the DNP have been clarified well. Observed data clearly indicate that both 1-indanone and piperidine on the chemical structure of DNP are responsible for inhibition of the AChE. Therefore, there are too many studies including anticholinesterase activity evaluation of piperidine and/or 1-indanone compounds [32–40]. There are also many reports about cholinesterase inhibitory potential of novel compounds, including bioisosteric replacement of piperidine with another basic center like piperazine, pyrrolidine etc. [41–47]. In the view of chemical structure of DNP, it has been reported that new AChE inhibitor candidates should bear a core ring system that interacts with PAS, a basic center that binds to CAS and a linker as –O–, CH_2, CONH, CONH(CH_2)n, etc. between the core ring system and basic center [48–50]. For example, a strong AChE inhibitor BYYT-25, containing an indanone core ring, an oxygen linker and a 4-(pyrrolydin-1-ylmethyl)phenyl basic center, has been synthesized as a result of described chemical requirements [49].

Based on the pharmacological profile of DNP and the information about its molecular interaction with AChE, in the present study, a novel series of DNP analogues was synthesized (Fig. 1) to investigate their inhibitory potency against cholinesterase enzymes. Thus, it is aimed to gain new cholinesterase inhibitors with enhanced biological activity.

2. Result and discussion

2.1. Chemistry

The compounds **1**–**14** and **15**–**38** were synthesized as shown in Scheme 1 and Scheme 2. Initially, 2-chloro-*N*-arylacetamide derivatives were prepared via the acetylation reaction using chloroacetyl chloride. In the second step, substitution reaction between 5,6-dimethoxy-2-[(piperidin-4-yl)-methyl]-2,3-dihydro-1*H*-inden-1-one and 2-chloro-*N*-arylacetamide derivatives gave the compounds **1**–**14**. The reaction of 4-fluorobenzaldehyde and appropriate secondary amine afforded 4-substitutedbenzaldehyde derivatives, which were treated with corresponding 2,3-dihydro-1*H*-inden-1-one to gain compounds **15**–**38** by Claisen-Schmidt condensation.

The structure of the newly synthesized compounds was elucidated by IR, ^{1}H NMR, ^{13}C NMR, HRMS spectroscopic methods (Electronic Supporting Information) and elemental analysis.

2.2. Anticholinesterase activity assay

All synthesized compounds were assessed as the AChE and BChE inhibitors by using in vitro modified Ellman's spectrophotometric method [51]. The assay was performed in two steps. First of all, the compounds **1**–**38** were tested at 10^{-3} and 10^{-4} M concentrations. Second step was performed by using 10^{-5}-10^{-9} M concentrations of the selected compounds that indicate more than 50% inhibitory activity at initial concentrations.

Table 1 presents the anticholinesterase activity of compounds **1**–**38** at initial concentrations. None of the compounds displayed remarkable inhibitor activity on BChE enzyme. Even the most effective compound **17** could show the inhibition of 19.23% against BChE at 10^{-3} M concentration. It was noted that all synthesized compounds showed more potent inhibition profile on AChE rather than BChE. The compounds **1**–**14** displayed lower inhibition against

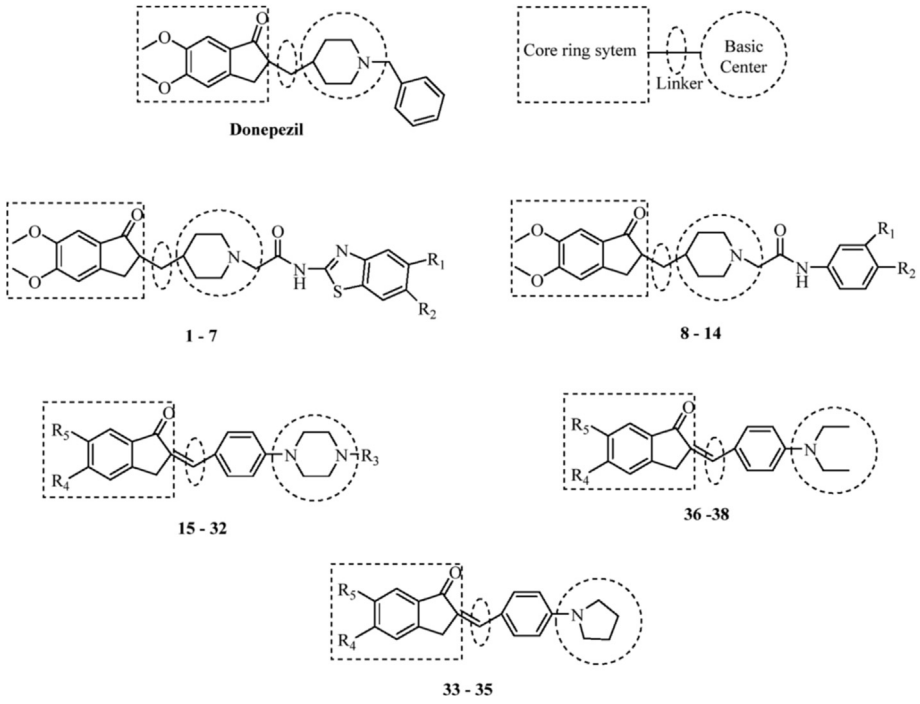

Fig. 1. Structures of donepezil and synthesized compounds **1**–**38**.