SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF NEW BENZIMIDAZOLE-PIPERAZINE DERIVATIVES AS ANTICHOLINESTERASE AGENTS

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Abstract - Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disease characterized clinically by memory and cognitive deficits. It is primarily seen in the elderly population and is the most common cause of dementia. The most important pathological features of Alzheimer's disease are β-amyloid (Aβ) extracellular plaques, intracellular neurofibrillary tangles (NFT) resulting from excessive phosphorylation of tau protein, and loss of cholinergic neurons in the basal forebrain. Inadequate cholinergic transmission plays an important role in the development of cognitive, functional and behavioral symptoms in Alzheimer's disease. Therefore, treatments are generally planned to increase the function of the cholinergic system either with receptor agonist or acetylcholinesterase inhibitors (AChEI). The aim of this study was to investigate the inhibition of eight new benzimidazole-piperazine derivatives (2a-2h) on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme activity, which are likely to be used in the treatment of Alzeheimer's disease (AD). The structures of synthesized compounds were confirmed via ^{1}H and ^{13}C NMR spectroscopic methods. Anticholinesterase enzyme inhibition assay of the compounds was determined spectrophotometrically by the Ellman method. None of the compounds in the series showed remarkable activity on BChE enzyme. In terms of AChE enzyme inhibition potency, compound 2g was found as the most active derivative in the series with IC_{50} value of 0.2118±0.008 μM.

Keywords - Alzheimer' Disease, Amyloid Plaques, Cholinergic Hypothesis, Benzimidazole, Piperazine

I. INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia seen in advanced age. It is characterized by progressive deterioration in cognitive functions. In the clinic, despite the exact criteria of AD, the diagnosis is made with the exclusion of secondary causes and other dementia. The prevalence of Alzheimer's disease increasing day by day due to the increase in the share of the elderly population in the general population due to developing health and living conditions, which is an important biopsychosocial problem nowadays, is limited and early diagnosis and effective treatment possibilities are very limited [1].

Nowadays, amyloid plaques and neurofibrillary tangles are considered the main neuropathological hallmarks of Alzheimer's Disease (AD). It is a neurodegenerative disease and it is the most common form of irreversible dementia. Symptoms of the disease include memory loss, confusion, impaired judgment, personality changes, disorientation, and loss of language skills. Alzheimer's patients get worse over time; hence it is a fatal disease [2].

Several hypotheses are suggested to explain the cause of AD. These hypotheses are cholinergic hypothesis, amyloid cascade hypothesis and the amyloid cascade/ neuroinflammation hypothesis.
Second hypothesis is the amyloid cascade hypothesis. It has received much support and has been under investigation for many years [3]. According to this hypothesis, AD is driven by two processes: extracellular deposition of amyloid beta (Aβ) and intracellular accumulation of tau protein. Both these compounds are insoluble. Aβ is the main component of senile plaques and tau is the component of neurofibrillary tangles (NFT). Aβ deposition is specific for AD and is thought to be primary deposit and deposits of Aβ are responsible for causing tau phosphorylation and NFT formation (secondary deposits) leading to neuronal death and dementia [4].
The amyloid cascade/neuroinflammation hypothesis is a modified and extended version of the amyloid cascade hypothesis. In this version, microglial (M) cells enter the cascade and play a crucial role in the disease process.

According to neuroinflammation hypothesis, acute-phase proteins are elevated in serum [5,6] and deposited in senile plaques (SPs); microglial cells accumulate around SPs; and complement components are present in SPs. These activated microglial cells produce potentially neurotoxic substances, such as reactive oxygen and nitrogen species, cytokines and other inflammatory mediators, that trigger the neurodegenerative changes [7,8].
The oldest hypothesis which is proposed by the scientists is cholinergic hypothesis and most currently available drug therapies is based on this hypothesis. It postulates that AD is caused by reduced synthesis of the neurotransmitter acetylcholine. So, AChE has turned out to be the most suitable therapeutic target for symptomatic change in AD because of the cholinergic deficiency is a reliable and early finding in AD. Inhibition of AChE was thought to be achievable as a

restorative target because of demonstrated adequacy of inhibition of AChE as a treatment for myasthenia gravis (MG) demonstrating that the methodology was possible.

Some benzimidazole derivatives have been shown to be useful as amyloid imaging probes because of their high binding affinity to Aß aggregates and high uptake into the brain [6]. Also, benzimidazole scaffold is the ring isoster of indanone pharmacophore of donepezil which is one of the most important AChE inhibitors [8]. Benzimidazoles with different biological activities were thought to be an appropriate starting point for AD.

Prompted from the observations above we synthesized a new series of benzimidazole-piperazine derivatives and investigated their anticholinesterase activities so as to obtain new biologically active compounds.

II. DETAILS EXPERIMENTAL

A. Chemistry

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). The reactions and the purities of the compounds were observed by thin layer chromatography (TLC) on silica gel 60 F254aluminum sheets obtained from Merck (Darmstadt, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (J) were reported as Hertz.

1) General procedure for 4-(4-phenyl/(4-methoxyphenyl)piperazin-1-yl)benzaldehyde derivatives (1a-1b)

A mixture of 4-fluorobenzaldehyde (20 mmol, 2.14 mL) and 1-phenylpiperazine/ 1-(4-methoxyphenyl)piperazine derivative (40 mmol) in dimethylformamide (DMF) (10 mL) was refluxed for 48 h in the presence of potassium carbonate (20 mmol, 2.76 g). The raw product was precipitated by pouring into ice water. The residue was filtered, washed with water, dried and recrystallized from ethanol.

2) General synthesis procedure for 2-(4-(4-phenyl/(4-methoxyphenyl)piperazin-1-yl)phenyl)-5-substitued-1H-benzoimidazole derivatives (2a -2h)

4-(4-phenyl/(4-methoxyphenyl)piperazin-1-yl)benzaldehyde derivatives (1a-1b) (30 mmol), sodium disulfite (30 mmol) and DMF (5 mL) were added into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300, Graz, Austria). The reaction mixture, was heated under conditions of 240 °C and 10 bar for 5 min. The mixture was cooled down, 5-substituted-1,2-phenylenediamine (28 mmol) was added and then reaction mixture was kept under the same reaction conditions in microwave reactor. After cooling, the mixture was poured into iced-water, precipitated product was washed with water, dried and recrystallized from ethanol.

3) 5-fluoro-2-(4-(4-phenylpiperazin-1-yl)phenyl)-1 H-benzo[d]imidazole (2c)

Yield: 84 %, 1H-NMR (300 MHz, DMSO-d6): δ = 3.42-3.45 (4H, m, piperazine), 3.22-3.33 (4H, m, piperazine), 6.81 (1H, d, J = 7.05 Hz, Benzimidazole C-H), 7.27 (2H, d, J=7.10 Hz, 1,4-disubstituebenzene), 7.50 (1H, d, J = 7.18 Hz, Benzimidazole C-H), 8.04 (2H, d, J=7.28 Hz, 1,4-disubstituebenzene), 12.74 (1H, s, Benzimidazole N-H). 13C-NMR (75 MHz, DMSO-d6): δ = 47.77, 48.63, 97.71, 98.05, 104.05, 104.40, 109.60, 110.50, 115.15, 115.50, 116.18, 119.67, 120.29, 122.39, 128.60, 129.46, 130.39, 151.33, 152.27, 157.33, 160.42 (Fig. 1 and 2).

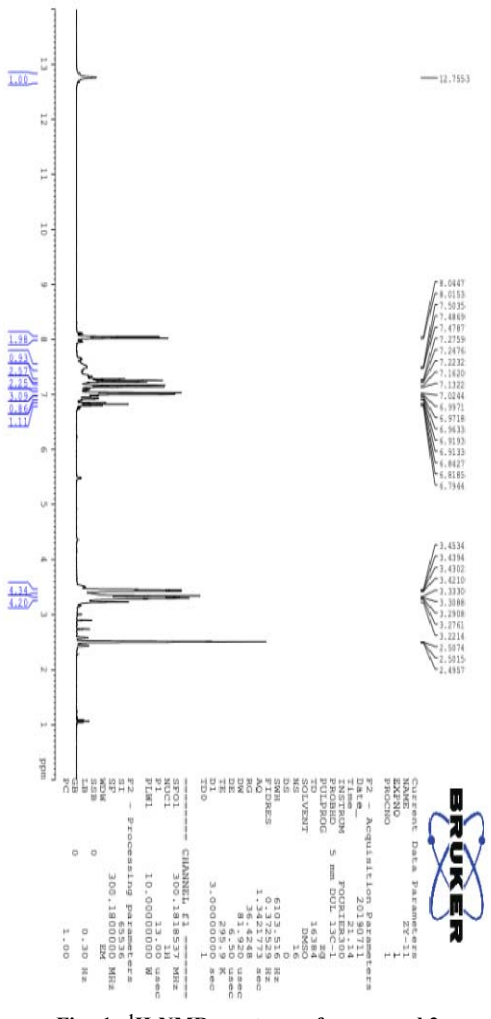


Fig. 1. ^{1}H-NMR spectrum of compound 2c.

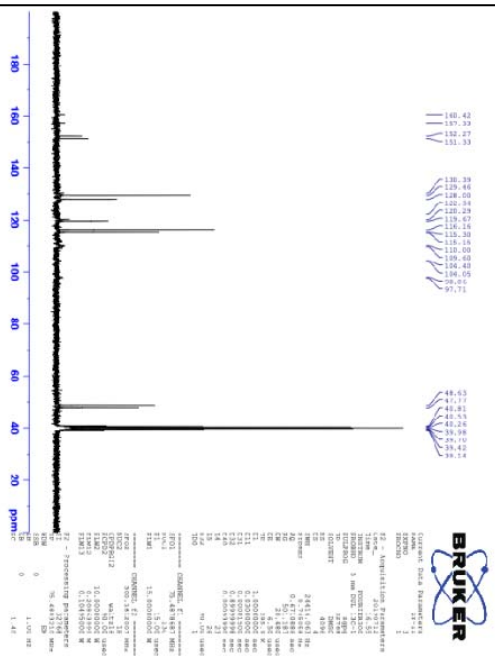


Fig. 2. ^{13}C-NMR spectrum of compound 2c.

B. Anticholinesterase Activity Assay

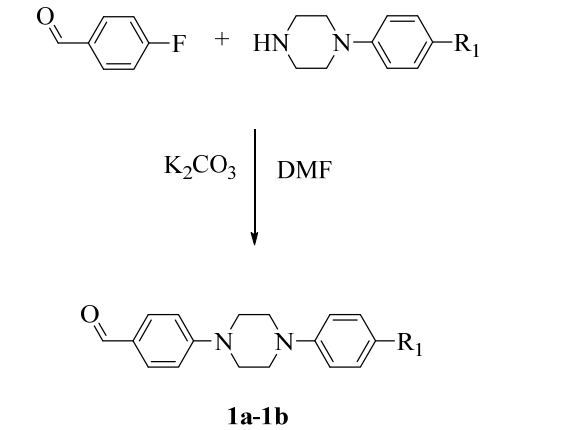
Modified Ellman's spectrometric method [9] was used for searching the inhibitory affinity of the obtained compounds (2a-2h) on AChE and BChE enzymes as described in our previous studies [10-14]. Donepezil and tacrine were included in the enzyme activity assay as reference drugs. The robotics system, Biotek Precision XS (Winooski, VT, USA), was used for all pipetting procedures in the enzyme inhibition assay. The measurements of percent inhibition at 412 nm were carried out by using a microplate reader, BioTek-Synergy H1 microplate reader (Winooski, VT, USA).

The inhibitory activities of the compounds against AChE and BChE were determined in 96-well plates. Initially, two concentrations (10^{-3} and 10^{-4} M) of the synthesized compounds by using 2% DMSO were used in the first step of enzyme inhibition assay and inhibition potencies were measured. At the second step, the further concentrations (10^{-5}-10^{-9} M) of selected compounds, which showed more than 50% inhibition, were prepared and inhibition assays were performed. The IC_{50} values of the selected compounds were performed as previously demonstrated. Each concentration was analyzed in quadruplicate.

III. RESULTS AND DISCUSSION

A. Chemistry

The compounds 2a-2h were synthesized as presented in Scheme 1. Firstly, 4-(4-phenyl/(4-methoxyphenyl) piperazin-1-yl)benzaldehyde derivatives (1a-1b) were synthesized by a reaction of 4-fluorobenzladehyde and piperazine derivatives. At last, final compounds 2-(4-(4-phenyl/(4-methoxyphenyl)piperazin-1-yl)phenyl)-5-substitued-1H-benzoimidazole derivatives (2a-2h) were prepared through the reaction of 4-(4-phenyl/(4-methoxy phenyl)piperazin-1-yl)benzaldehyde derivatives (1a-1b) and 5-substituted-1,2-phenylenediamine in DMF. The synthesized compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR and ^{13}C-NMR).



Scheme 1. Synthesis way of the target compounds 2a-2h.

Compounds	R_1	R_2
2a	-H	$-CH_3$
2b	-H	$-CF_3$
2c	-H	-F
2d	-H	-CI
2e	$-OCH_3$	$-CH_3$
2f	$-OCH_3$	$-CF_3$
2g	$-OCH_3$	-F
2h	$-OCH_3$	-CI

Table 1. R groups of the target compounds 2a-2h.

B. Anticholinesterase Activity Assay

The synthesized compounds, which are bearing benzimidazole-piperazine moieties, and donepezil and tacrine, which are used as reference drugs, was subjected to modified Ellman method in order to

determine their anticholinesterase activity. For first step of enzyme inhibition assay, related compounds were prepared at 10^{-3} M and 10^{-4} M concentrations. Table 2 presents the percent ChE inhibition of all tested compounds.

Table 2 express that there is no significant enzyme activity of all synthesized compounds about BChE enzyme. On the other hand, it can be seen that all tested compounds show higher AChE inhibition potency than BChE. At the 10^{-3} M concentration, compounds 2e, 2f, 2g and 2h could display more than 50% activity, whereas only compound 2g could show more than 50% enzyme inhibition. So, the second step of enzyme inhibition assay was performed for only compound 2g by preparing its further concentrations (ranging from 10^{-5} M to 10^{-9} M concentrations). This compound showed AChE enzyme inhibition profile with IC_{50} value of 0.2118±0.008 µM. Thus, compound 2g was found as the most active derivative in the series. But, when compared this IC_{50} value with the IC_{50} value of donepezil (0.0287±0.005 µM), compound 2g was not as effective as donepezil on AChE enzyme. This finding was promising in terms of modifying the chemical structures of synthesized compounds in further studies.

Compounds	AChE % Inhibition		BChE % Inhibition	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
2a	37.18 ±0.78	20.15 ±0.56	26.10 ±0.71	18.15 ±0.50
2b	41.08 ±0.79	22.50 ±0.61	23.71 ±0.52	20.39 ±0.47
2c	41.75 ±0.88	35.63 ±0.70	22.74 ±0.61	18.29 ±0.42
2d	34.20 ±0.90	26.55 ±0.71	21.44 ±0.63	15.79 ±0.50
2e	57.83 ±0.97	41.19 ±0.70	30.29 ±0.54	21.63 ±0.50
2f	59.62 ±0.90	42.49 ±0.81	31.88 ±0.66	20.46 ±0.59
2g	87.55 ±1.13	80.29 ±1.05	38.18 ±0.90	23.55 ±0.69
2h	62.89 ±0.88	44.28 ±0.71	35.75 ±0.72	26.79 ±0.65
Donepezil	99.37 ±1.16	98.64 ±1.09	-	-
Tacrine	-	-	98.25 ±1.05	96.10 ±1.16

Table 2. % Inhibition of all compounds 2a–h against AChE and BChE enzymes.

IV. CONCLUSIONS

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid-beta (Aβ) peptides and the formation of neurofibrillary tangles in the brain, accompanied by neuronal loss and inflammation, decreased cognitive functions and dementia. Based on the cholinergic hypothesis, the most successful approach to treat Alzheimer's disease is to restore the level of acetylcholine (ACh). The cholinergic hypothesis is the only theory that explains the nature of Alzheimer's disease and is still accepted. In addition to this, almost all medicines currently used to relieve the symptomatic effects of moderate to severe Alzheimer species are based on this hypothesis. In this study eight new benzimidazole-piperazine derivatives (2a-2h) were designed and synthesized to investigate their ChE enzymes inhibition potencies. All of the synthesized compounds showed enzyme inhibition selectivity against AChE enzyme. Compounds 2g was found as the most potent derivative in the series.

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