SYNTHESIS OF NEW PIPERIDINE-HYDRAZIDE DERIVATIVES AND EVALUATION OF THEIR IN VITRO ENZYME ACTIVITY

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Abstract - Alzheimer's disease (AD) has an insidious onset and its progression is characterized by memory dysfunction and cognitive disturbances, such as problems recalling familiar names and objects and/or behavioral changes. Loss of short-term memory and impaired visuospatial orientation are typical early symptoms of the disease. As AD progresses there is a general decline of multiple cognitive functions related to daily activities. Ultimately patients become very dependent on caregivers. The lack of treatment has led researchers to work on this subject. The aim of this study was to investigate the inhibition of three new piperidine-hydrazide derivatives (3a-3c) 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 significant activity on BChE enzyme. In terms of AChE enzyme inhibition potency, compound 3a was found as the most active derivative in the series with IC_{50} value of 0.1856±0.006 μM.

Keywords - Alzheimer' Disease, in Vitro Enzyme Inhibition, Piperidine, Hydrazide

I. INTRODUCTION

AD is characterized by neuropathological lesions, referred to as amyloid plaques (APs) and neurofibrillary tangles (NFTs), which along the last quarter of the previous century were found to contain amyloid-beta peptide (Aβ) and microtubule-associated protein-tau, respectively [1]. Accumulated evidence clearly indicates that the former is the result of years long amyloid deposition, which is a pre-clinical phenomenon and culminates as the first objective symptoms appear [2]. Whereas, the latter seems to be the main drive of neurodegeneration. Hence the progressive amnestic disorder of the pre-dementia stage or prodromal AD [3].
Warning signs of AD are difficulties in speaking, writing, planning, understanding visual images, and performing familiar tasks. Changes in personality follow the changes in memory. When a patient has these characteristic signs, diagnosis can be confirmed by cellular pathology and cognitive tests. Because AD cannot be identified by a single test, a medical history and physical examination are also necessary for diagnosis. Reductions in brain size because of the loss of neurons and synapses and clearly visible amyloid plaques confirm the presence of AD.

Unfortunately, AD is incurable now. Available treatments retard the progress of the disease by increasing the concentration of acetylcholine (ACh). One of the significant features of AD is the reduction of acetylcholine by the death of cholinergic neurons [4]. Donepezil, galantamine and rivastigmine are cholinesterase inhibitors that have been used as drugs for AD. In addition to showing no effect in delaying the onset of AD, these drugs have side effects that include vomiting, muscle cramps, bradycardia, and decreased appetite [5].

Piperidine and hydrazide moieties are important pharmacophore groups in medicinal chemistry. For treatment of AD, these groups are generally could be selected and used in the studies.

Prompted from the observations above we synthesized a new series of piperidine-hydrazide 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 ethyl 2-(4-benzylpiperidin-1-yl)acetate (1)
A mixture of 4-benzylpiperdine (30 mmol), ethyl 2-chloroacetate (30 mmol), and potassium carbonate (30 mmol) was refluxed in acetone for 10 h. After

completion of the reaction, the acetone was evaporated, and the precipitated product was washed with deionized water, dried, and recrystallized from EtOH.

2) General synthesis procedure for 2-(4-benzylpiperidin-1-yl)acetohydrazide (2)

Ethyl 2-(4-benzylpiperidin-1-yl)acetate (1) (20 mmol) and an excess of hydrazine hydrate (10 mL) were stirred in EtOH for 8 h. After completion of the reaction, the precipitated product was filtered, washed with cold EtOH, dried, and recrystallized from EtOH.

3) 2-(4-Benzylpiperidin-1-yl)-N'-(4-substituebenzylidene)acetohydrazide (3a-3c)

2-(4-benzylpiperidin-1-yl)acetohydrazide (2) (1.2 mmol) an appropriate substitute benzaldehyde derivatives (1.2 mmol), and a catalytic quantity of acetic acid (0.1 mL) were refluxed in EtOH for 2 h. The mixture was cooled, and the precipitated product was filtered, dried, and recrystallized from EtOH.

4) 2-(4-benzylpiperidin-1-yl)-N'-(4-ethoxybenzylidene)acetohydrazide (3a)

Yield: 75 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.30-1.36 (5H, m), 1.50-1.54 (2H, m), 1.97-2.11 (3H, m), 2.78-2.82 (3H, m), 2.99 (2H, s, -CH_2-), 4.03-4.08 (4H, m), 6.98 (2H, d, J=8.7 Hz, Ar-H), 7.14-7.17 (3H, m, Ar-H), 7.26 (2H, d, J=7.2 Hz, Ar-H), 7.59 (2H, d, J=8.8 Hz, Ar-H), 8.24 (1H, s, Ar-H), 10.91 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 15.05, 32.01, 32.27, 37.40, 42.86, 53.97, 58.15, 61.59, 63.68, 115.15, 126.21, 128.60, 129.05, 129.44, 140.78, 147.39, 160.48, 166.11, 171.23 (Fig. 1 and 2).

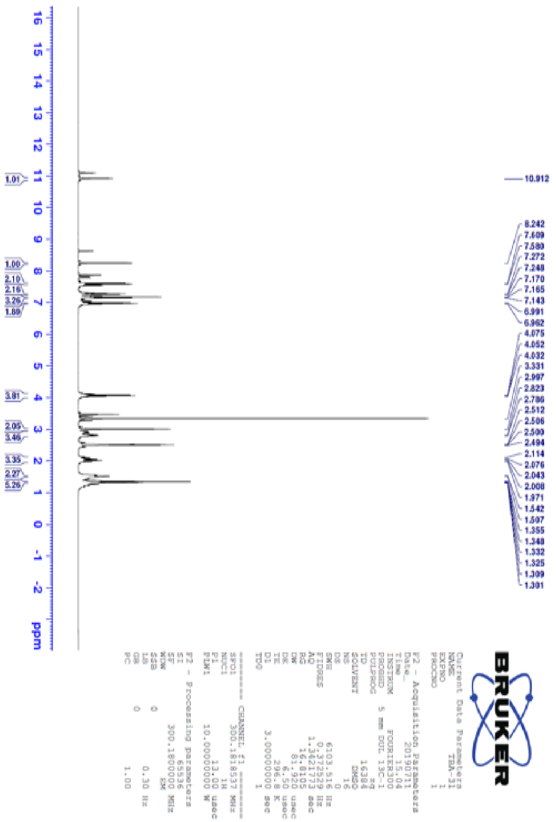


Fig. 1. ^{1}H-NMR spectrum of compound 3a.

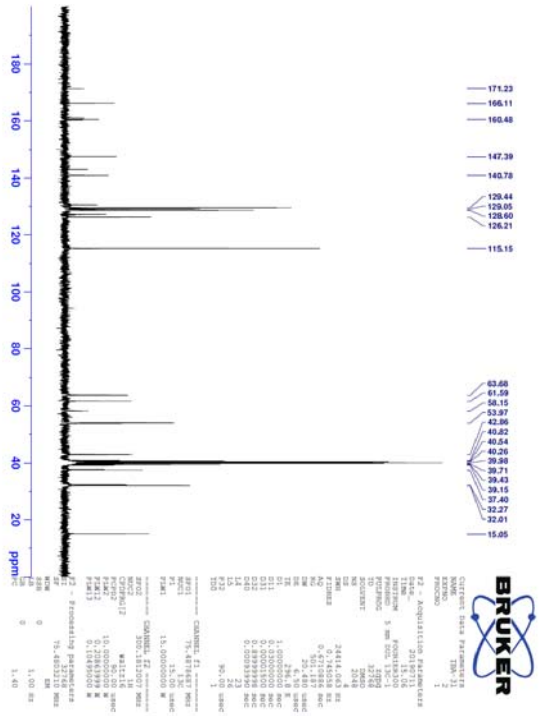


Fig. 2. ^{13}C-NMR spectrum of compound 3a.

B. Anticholinesterase Activity Assay

Modified Ellman's spectrometric method [6] was used for searching the inhibitory affinity of the obtained compounds (3a-3c) on AChE and BChE enzymes as described in our previous studies [7-11]. 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).

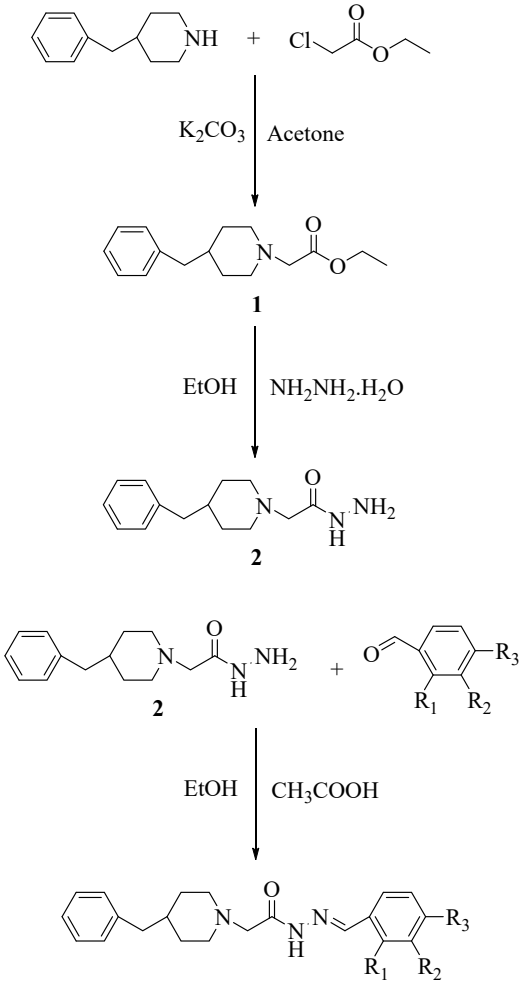
The inhibitory activities of the compounds against AChE and BChE were determined in 96-well plates. Initially, two concentrations (10^{-3} and 10^{-4} M) of the synthesized compounds by using 2% DMSO were used in the first step of enzyme inhibition assay and inhibition potencies were measured. At the 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 3a-3c were synthesized as presented in Scheme 1. Firstly, ethyl 2-(4-benzylpiperidin-1-yl)acetate (1) was synthesized by a reaction of 4-benzylpiperdine and ethyl 2-chloroacetate. Then, this product was treated with

hydrazine hydrate to gain 2-(4-benzylpiperidin-1-yl)acetohydrazide (2). At last, final compounds 2-(4-benzylpiperidin-1-yl)-N'-(4-substitue benzylidene)acetohydrazide (3a-3c) were prepared through the reaction of 2-(4-benzylpiperidin-1-yl)acetohydrazide (2) and substitute benzaldehyde derivatives in EtOH. 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 3a-3c.

Compounds	R_1	R_2	R_3
3a	-H	-H	$-OC_2H_5$
3b	$-OCH_3$	$-OCH_3$	-H
3c	$-OCH_3$	-H	$-OCH_3$

Table 1. R groups of the target compounds 3a-3c.

B. Anticholinesterase Activity Assay

The synthesized compounds, which are bearing piperidine-hydrazide 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.

According to Table 2, unfortunately, none of the compounds showed remarkable activity on 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 3a and 3c could display more than 50% activity, whereas only compound 3a could show more than 50% enzyme inhibition. So, only compound 3a could pass the second step of enzyme inhibition assay by using 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 0.1856±0.006 μM. Compound 3a 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 3a was not as effective as donepezil on AChE enzyme.

When compared all synthesized compounds structurally, compound 3a has only one substitution (ethoxy moiety) at 4th position of phenyl ring. The other compounds have functional groups at 2nd-3rd of 2nd-4th positions of phenyl ring. So, it can be understood that the 2nd and 3rd positions of phenyl ring effect the enzyme inhibition profile negatively by creating steric barrier. All these 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
3a	85.19 ±0.88	78.21 ±0.76	28.11 ±0.63	25.15 ±0.44
3b	48.23 ±0.59	33.51 ±0.50	21.59 ±0.42	17.85 ±0.40
3c	62.57 ±0.72	35.28 ±0.66	24.98 ±0.58	20.61 ±0.49
Donepezil	99.37 ±1.16	98.64 ±1.09	-	-
Tacrine	-	-	98.25 ±1.05	96.10 ±1.16

Table 2. % Inhibition of all compounds 3a–3c against AChE and BChE enzymes.

IV. CONCLUSIONS

Alzheimer's disease (AD), is the most common type of dementia. It causes failure of intellectual functions and recent memory, which are characteristic features of AD. When a patient has these signs, diagnosis can be confirmed by cellular pathology and cognitive tests. Reductions in brain size, because of the loss of neurons and synapses, and clearly visible amyloid plaques confirm the presence of AD. Also, it is accepted that cholinergic hypothesis is the main

treatment approach of AD. Therefore, in this study, the aim was to develop new cholinesterase inhibitor candidates. So, three new piperidine-hydrazide derivatives (3a-3c) were designed and synthesized to investigate their ChE enzymes inhibition potencies. All of the synthesized compounds showed enzyme inhibition selectivity against AChE enzyme. Compounds 3a was found as the most potent derivative in the series.

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