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Cholinesterases Inhibitory Activity of 1*H*-benzimidazole Derivatives

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes brain cells to waste away and die. The most common strategy for the treatment of AD is the inhibition of acetyl/butyrylcholinesterase (AChE and BChE) enzymes. Benzimidazole derivatives are important heterocyclic bioactive agents. In this study, *in vitro* inhibitory potential of 12 previously synthesized benzimidazoles was evaluated against acetyl/butyrylcholinesterases. Results showed that some derivatives have moderate AChE (IC_{50} = 1.01-1.19 mM) and BChE (IC_{50} = 1.1-1.87 mM) inhibitory activity. Findings could be helpful in the design and development of new effective anti-AD drugs with benzimidazole core.

Keywords: benzimidazole; Alzheimer's disease; acetylcholinesterase; butyrylcholinesterase.

1. Introduction

Benzimidazole scaffold is an important nitrogen-containing heterocycle in organic and medicinal chemistry, which is found in numerous bioactive compounds [1, 2]. These heterocyclic compounds are highly biologically active class of heterocyclic compounds with several biological and pharmacological activities such as antibacterial [3-5], antifungal [6, 7], anthelmintic [8], anticancer [9], antiulcer [10], α-glucosidase inhibition [11, 12], anti-Alzheimer's [13, 14], antiviral [15], antihistamine [16], antihypertensive [17], anti-inflammatory [18], and anti-HIV [19, 20]. Moreover, these compounds have been used as organic ligands toward transition metals with various natural molecules such as vitamin B12, its derivatives, and a variety of metalloproteins (Figure 1) [21, 22]. The benzimidazole scaffold is found in many commercial drugs such as Nocodazole (anticancer), Triclabendazole (anthelmintic), Tiabendazole (antifungal and antiparasitic), Omeprazole (proton pump inhibitor), Flubendazole (anthelmintic), and Maribavir (antiviral) (Figure 2). In addition to the biological activities of benzimidazole compounds, they have been used as corrosion inhibitors [23, 24], dyes [4], and fluorescence reagents [25, 26]. Therefore, the synthesis of compounds with the benzimidazole core has attracted considerable attention from organic and medicinal chemists.

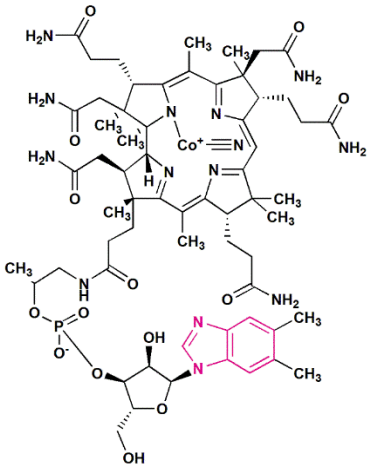


Figure 1. Vitamin B12.

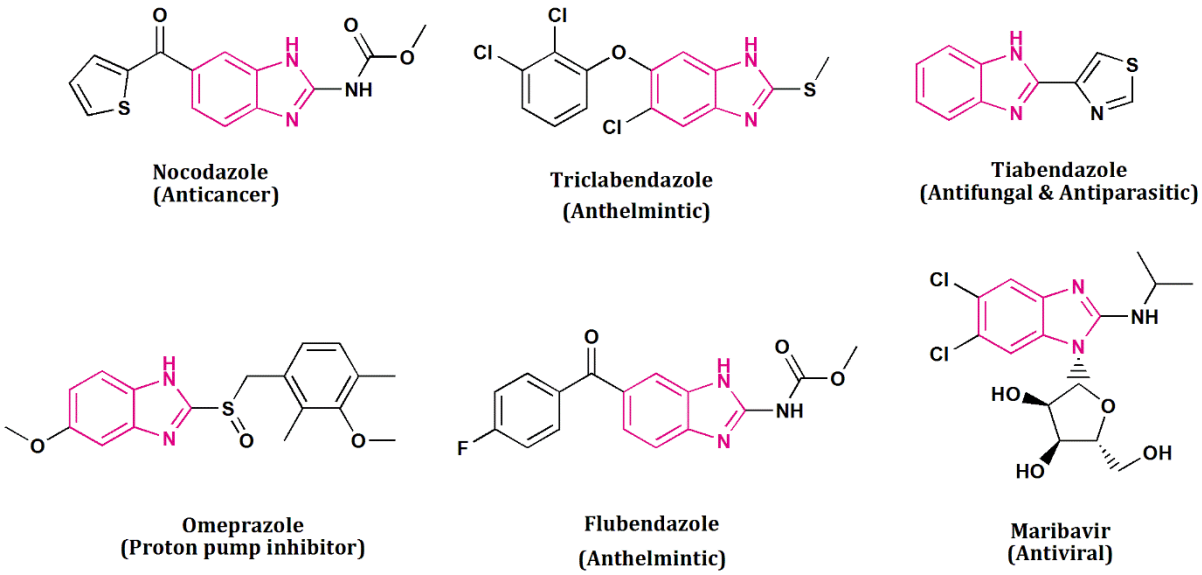
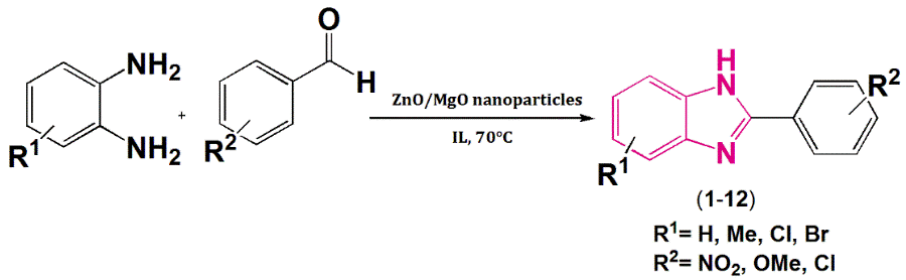


Figure 2. Benzimidazole core in some important drugs.

Alzheimer's disease (AD), also referred to simply as Alzheimer's, is a chronic neurodegenerative disorder and the most common cause of dementia [27]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), known as cholinesterase enzymes, are present in the body. One of the successful strategies for the treatment of AD is the inhibition of cholinesterase enzymes [28]. Up to now, Alzheimer's has no cure, and the available treatments cannot stop the disorder; they can just slow the dementia symptoms [29]. So, there is still considerable demand for design, synthesis, and discovery of effective anti-Alzheimer's agents. Several reports showed the cholinesterases inhibitory potential of benzimidazole compounds such as ricobendazole, thiabendazole, albendazole, and oxfendazole [30]. In a recent study, the tested drugs showed a significant inhibitory effect on AChE and BChE. Ricobendazole (IC_{50} = 123.02 nM) and thiabendazole (IC_{50} = 64.26 nM) were the best inhibitors of AChE and BChE, respectively.

The evaluation of anti-Alzheimer's property of our previously synthesized benzimidazole derivatives (1-12) was the aim of the present study (Scheme 1) [11]. For this purpose, the acetyl/butyrylcholinesterase inhibitory activity of these compounds was tested. The findings of this work could be useful for future studies about the design, synthesis, and discovery of effective anti-Alzheimer's drugs containing benzimidazole moiety.



Scheme 1. Synthesis procedure of 1*H*-benzimidazoles.

2. Materials and Methods

2.1. Chemistry.

Acetylcholinesterase, butyrylcholinesterase, 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB), acetylthiocholine iodide, and galantamine were purchased from Merck (Germany) and used without further purification. UV/Vis based *in vitro* assays were performed on a BioTek microplate reader (USA).

2.2. Benzimidazole derivatives.

Benzimidazole derivatives (**1-12**) which used in this study have been synthesized previously through a green chemistry method [11]. In brief, benzaldehyde derivatives and *o*-phenylenediamine mixed together in the presence of ionic liquid and ZnO/MgO nanoparticles. The reaction mixture was stirred at 70˚C for the required time. The final pure 1*H*-benzimidazoles were obtained after the workup of the reaction mixture and column chromatography.

2.3. Enzymatic assays.

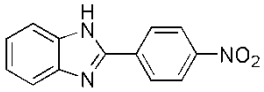
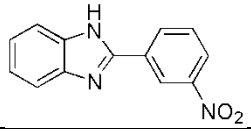
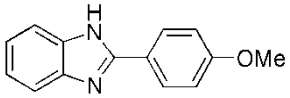
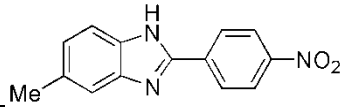
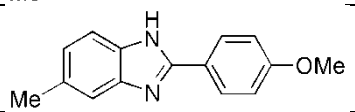
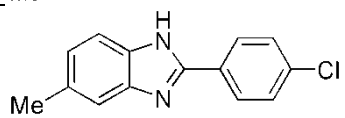
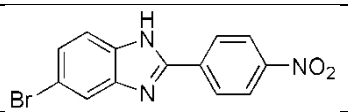
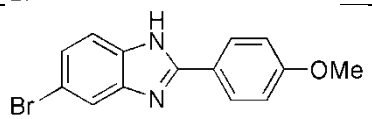
The cholinesterase inhibitory potential of benzimidazole compounds was evaluated using a previously reported approach [31]. Briefly, the benzimidazole solution (20 µL) with known concentration was mixed with AChE/BChE solution (20 µL, 0.5 unit/mL). Phosphate buffer (40 µL, 0.1 mM, pH = 8.0) and DTNB (20 µL, 0.2 M) were added to the reaction mixture in a 96-well microplate and incubated for 15 min at 25˚C. The reaction was started with the addition of acetylthiocholine iodide/butyrylthiocholine chloride solution (10 µL, 0.2 M). By the formation of the yellow 5-thio-2-nitrobenzoate anion, the activity level of the enzymes could be determined. For this, the absorbance of sample solution and blank were recorded at 412 nm after 10 min incubation at 25˚C. The absorbance of the blank was subtracted from that of the sample, and the cholinesterase inhibitory activity was expressed as IC_{50} values. Galantamine was used as a reference drug.

3. Results and Discussion

One of the aging health problems is Alzheimer's disease (AD). Inhibition of cholinesterase enzymes is one of the common effective AD treatment approaches. In this study, the inhibition potential of 12 synthetic benzimidazole analogs (Table 1) was evaluated against AChE and BChE using the spectroscopic method, and the results were expressed as IC_{50} values. As seen in Table 2, AChE could be inhibited by compounds 2, 3, 4, 6, 8, and 12 with IC_{50} values ranging from 1.01 to 1.25 mM. Also, compounds 1, 2, 3, 4, 6, 8, 10, and 12 showed the

inhibitory activity against BChE (IC_{50} = 1.20-1.87 mM). Obtained activities were close to each other. On the other hand, there is no significant difference between the cholinesterase inhibitory power of the most active compound and the less active one. Moreover, the selective inhibitory effect against AChE/BChE was not observed for the tested benzimidazoles. The inhibitory activities of benzimidazole derivatives are moderate in comparison with galantamine as a standard drug. The interpretation of the relationship between the obtained results and the structure of benzimidazoles is not possible because of the similarity of results. For further investigations and the discovery of structure-activity relationships, more diverse benzimidazoles should be synthesized and checked for AChE/BChE inhibitory capacity. 1*H*-benzimidazole derivatives were synthesized and evaluated for their eeAChE (electric eel acetylcholinesterase), hAChE (recombinant human enzyme), and BChE inhibitory potential by Alpan *et al*. [32]. Findings showed that most of the tested compounds were active against eeAChE (IC_{50} = 0.58-17.33 μM), hAChE (IC_{50} = 0.39-50.98 μM), and BChE (IC_{50} = 1.37-8.52 μM). Tacrin as a standard drug could inhibit eeAChE, hAChE, and BChE with IC_{50} values of 0.075, 0.52, and 0.0098 μM, respectively. Just a few compounds were selective against AChE/BChE. Moreover, the inhibitory activity of the benzimidazoles against AChE was more than BChE. Recently, a series of hydrazone derivatives bearing imidazole (5 compounds) and benzimidazole (5 compounds) nucleus were designed, synthesized, and investigated for AChE inhibitory activity [33]. Results showed that all of the imidazole-hydrazone derivatives were weak inhibitors of AChE (IC_{50} values > 200 μM). Meanwhile, good to moderate AChE inhibitory activity was observed for benzimidazole-hydrazones (IC_{50} = 11.8-61.8 μM). In this work, the IC_{50} value of 8.9 μM was obtained for galantamine as a standard drug.

Table 1. The structure and name of the previously synthesized 1*H*-benzimidazoles [11].

Compound	Structure	Name
1		2-(4-nitrophenyl)-1*H*-benzo[d]imidazole
2		2-(3-nitrophenyl)-1*H*-benzo[d]imidazole
3		2-(4-methoxyphenyl)-1*H*-benzo[d]imidazole
4		5-methyl-2-(4-nitrophenyl)-1*H*-benzo[d]imidazole
5		2-(4-methoxyphenyl)-5-methyl-1*H*-benzo[d]imidazole
6		2-(4-chlorophenyl)-5-methyl-1*H*-benzo[d]imidazole
7		5-bromo-2-(4-nitrophenyl)-1*H*-benzo[d]imidazole
8		5-bromo-2-(4-methoxyphenyl)-1*H*-benzo[d]imidazole

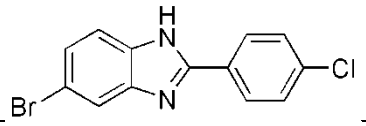
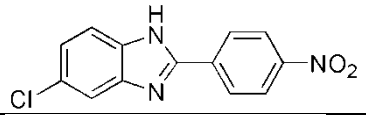
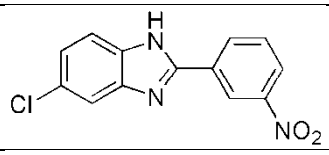
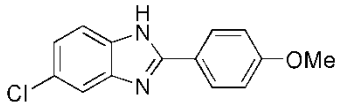
Compound	Structure	Name
9		5-bromo-2-(4-chlorophenyl)-1*H*-benzo[d]imidazole
10		5-chloro-2-(4-nitrophenyl)-1*H*-benzo[d]imidazole
11		5-chloro-2-(3-nitrophenyl)-1*H*-benzo[d]imidazole
12		5-chloro-2-(4-methoxyphenyl)-1*H*-benzo[d]imidazole

Table 2. Acetyl/butyrylcholinesterase inhibitory activity of benzimidazole compounds (IC_{50} mM).

Compound	AChE	BChE
1	na [a]	1.10 ± 0.08
2	1.19 ± 0.04 [b]	1.87 ± 0.03
3	1.02 ± 0.01	1.22 ± 0.02
4	1.01 ± 0.04	1.20 ± 0.06
5	na	na
6	1.05 ± 0.06	1.42 ± 0.07
7	na	na
8	1.25 ± 0.04	1.58 ± 0.05
9	na	na
10	na	1.44 ± 0.03
11	na	na
12	1.01 ± 0.01	1.27 ± 0.03
Galantamine [c]	0.01 ± 0.001	0.02 ± 0.001

[a] IC_{50} not achieved in tested concentration (2 mM); [b] values expressed are means ± S.D.; [c] standard drug

4. Conclusions

The activity of some benzimidazole compounds in inhibition of cholinesterases was investigated here. Compared with the standard drug galantamine, moderate cholinesterase inhibitory activity was determined for some derivatives. Results suggest that benzimidazole derivatives studied at the present work could be considered for further investigation for designing new derivatives for effective neuroprotective therapeutics.

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Conflicts of Interest

The authors declare no conflict of interest.

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