Acetylcholine Esterase Inhibitory Potential of Some Benzimidazole Derivatives

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ABSTRACT
*This study was undertaken to evaluate anticholinesterase activity of some benzimidazole derivatives due to their biological importance. Previously synthesized 14 benzimidazole compounds were used in biological studies. Inhibitory activities of related compounds on Acetylcholine esterase (AChE, E.C.3.1.1.7 from Electric Eel) enzyme have been determined by a modified Ellman's colorimetric method. Compound **4f** was found as the most active derivative with 69.26%, 51.23% and 38.22% inhibition profiles at 1 mM, 0.1 mM and 0.01 mM concentrations, respectively. It was determined that the substituents increasing liphophilicty of the compounds have an influence on biological activity.*

Keywods: Benzimidazole, anticholinesterase activity, liphophilicity

INTRODUCTION

Alzheimer's disease (AD) is the most common reason of dementia among the elderly. As average duration of life increases, the number of AD cases worldwide is expected to rise from 35 million today to more than 115 million by 2050. Molecular studies of the brain at the initial phases of memory loss related with AD have been problematic in humans (Ashe and Zahs, 2010).

AD patients display episodic memory deficiencies, judgment insufficiencies, confusion, altered mood, and motor impairments at later periods (McKhann et al.,1984). Although no cure exists for AD, there are two types of drugs currently being used for treatment of AD. The NMDA receptor antagonist memantine prevents glutamate excitotoxicity caused by over activation of the NMDA receptor (Chen et al., 1992). Although memantine can slow the cognitive failure in patients with moderate to severe AD, it neither reverses nor prevents cognitive deficiencies and offers no apparent advantage to patients with mild to moderate AD. The second class of AD therapeutics are the cholinesterase inhibitors (ChEIs), which elevate acetylcholine (ACh) levels (Cuadra et al., 1994), which partially offsets the loss of cholinergic input to the cerebral cortex that occurs in AD (Whitehouse et al., 1981). ChEIs are effective for mild to moderate AD, unlike memantine, with clinical trials reporting slower rates of cognitive failure, as well as functional improvements (Rogers et al., 1998; Rösler et al., 1999; Raskind et al., 2000). Hence, ChEIs are the first choice for treatment of cognitive, functional and behavioral symptoms of AD.

Benzimidazole compounds have an essential role in medical area due to various pharmacological effects as antimicrobial, antiviral, antidiabetic, anticancer, amti-HIV, antihypertensive, antiulcerative, antioxidant and anticonvulsant (Walia et al., 2011). Furthermore, some studies about antichlolinesterase effects of benzimidazole derivatives have been reported (Bacharaju et al., 2012; Alpan et al., 2013;Yoon et al., 2013a; Yoon et al., 2013b).

Prompted from above observations, we performed an activity study by using previously synthesized benzimidazole compounds to investigate their Acetylcholine esterase inhibitory potencies (Özkay et al., 2011)

MATERIALS AND METHODS

Chemistry

Test compounds were synthesized at four steps. Synthetic pathway was outlined in Scheme 1. In first reaction step, Schiff base of 2-aminobenzimidazole and 4-formylbenzoic acid methyl ester was prepared (**1**). In second step, compound 1 was reduced by $NaBH_4$ to obtain compound **2**, which was reacted with hydrazine hydrate to gain hydrazide compound (**3**). In final step, reaction between compound **3** and corresponding benzaldehyde derivatives gave target benzimidazole derivatives (**4a**-**4n**). Detailed synthesis procedures and structure confirmations of the benzimidazole derivatives were reported in our previous study (Özkay et al., 2011). Some physicochemical properties of the compounds were presented in Table 1.

Pharmacology

AChE Inhibition

AChE inhibition potency of the compounds **4a-4n** were investigated by modified method of Ellman (Ellman et al., 1961). This spectrophotometric method is based on the reaction of released thiocholine to give a colored product with a chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and Donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine and acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a Thermo microplate spectrophotometer (Multiskan GO, Massachusetts, USA). Cholinesterase activity of the compounds (**4a-4n**) was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control.

Enzymatic assay

AChE inhibition of the compounds **4a-4n** were investigated by modified Ellman's test (Ellman et al., 1961; Perry et al., 2000). Donepezil hydrochloride was used as a positive control (Table 2). Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2% DMSO at 0.1 and 1 mM concentrations were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 oC for 5 min. The reaction was started by adding DTNB) (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

Inhibition % = $[(A_C\text{-}A_B) – (A_I\text{-}A_B)] / (A_C\text{-}A_B) \times 100$

Where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Student's t- test was used for all statistical calculations. Data were expressed as Mean ± SD inactive in culture medium.

RESULTS AND DISCUSSION

In the present study, anticholinesterase activity of previously synthesized benzimidazole derivatives was investigated (Özkay et al., 2011). The anticholinesterase effects of the compounds (**4a-4n**) were determined by modified Ellman's colorimetric assay (Ellman et al., 1961) (Table 2). In the series, compound **4f** that bear methoxy substituent was determined as the most active compound with 69.26%, 51.23% and 38.22% inhibition potencies at 1 mM, 0.1 mM and 0.01 mM concentrations, respectively. In addition compound **4g** carrying ethoxy substituent showed 64.70% and 49.42% inhibition at 1 mM and 0.1 mM concentrations. The IC_{50} values were calculated as 0.091 mM and 0.134 mM for the compounds **4f** and **4g**. The inhibition percentages of the compounds **4a**-**4e**, **4h**-**4n** were lower than 50% at 1 mM concentration and thus enzyme inhibitory potency of these compounds were not determined in further concentrations. Standard drug Donepezil was studied at lower concentrations in order to determine IC_{50} value and it was calculated as 0.054 µM. None of the compounds showed comparable activity with reference drug.

CONCLUSION

Consuquently, a series of benzimidazole derivatives were observed for their AChE inhibitory potency. In respect of the activity test results, compound **4f** displayed the best inhibitory activity with a value of IC_{50}=0.091 mM. Compound **4g** (IC_{50}=0.134 mM) displayed moderate enzyme inhibition. The other compounds in the series did not show adequate biological activity. None of the tested compounds displayed biological activity as much as standard drug Donepezil. It was determined that the substituents as methoxy and ethoxy, which increase liphophilicty of the compounds have an influence on biological activity.

REFERENCES

Ashe K. H. and Zahs K. R. (2010) Probing the biology of Alzheimer's disease in mice. Neuron, 66, 631-645.

Alpan A. S., Parlar S., Carlino L., Tarikogullari A.H. and Alptüzün V. (2013) Synthesis, biological activity and molecular modeling studies on 1H-benzimidazole derivatives as acetylcholinesterase inhibitors. Bioorganic and Medicinal Chemistry, 21, 4928–4937.

Bacharaju K, Jambula S. R., Sivan S., Jyostnatangeda S. and Manga V. (2012) Design, synthesis, molecular docking and biological evaluation of new dithiocarbamates substituted benzimidazole and chalcones as possible chemotherapeutic agents. Bioorganic and Medicinal Chemistry Letters, 22, 3274-3277.

Chen H. S., Pellegrini JW, Aggarwal SK, Lei SZ, Warach S, Jensen F. E and Lipton S. A. (1992) Openchannel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptormediated neurotoxicity. Journal of Neuroscience 12, 4427-4436.

Cuadra G., Summers, K. and Giacobini, E. (1994) Cholinesterase inhibitor effects on neurotransmitters in rat cortex in vivo. Journal of Pharmacology and Experimental Therapeutics, 270, 27-284.

Ellman G. L., Courtney K. D., Andres V. and Feather-Stone R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7, 88-95.

McKhann G., Drachman D., Folstein M., Katzman R., Price D. and Stadlan E. M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer"s Disease. Neurology, 34, 939-944.

Özkay Y., Tunalı Y., Karaca H. and Işıkdağ I. (2011) Antimicrobial activity of a new series of benzimidazole derivatives. Archives of Pharmacal Research, 34, 1427-1435.

Perry N. S. L., Houghton P. J., Theobald A. E., Jenner P. and Perry E.K. (2000) In-vitro inhibition of human erythrocyte acetylcholine esterase by Salvia lavandulae folia essential oil and constituent terpenes. Journal of Pharmacy and Pharmacology, 52, 895-902.

Raskind M. A., Peskind E. R., Wessel T. and Yuan, W. (2000) Galantamine in AD: A 6-month randomized, placebocontrolled trial with a 6-month extension. The Galantamine USA-1 Study Group. Neurology, 54, 2261-2268.

Rogers S. L., Farlow M. R., Doody R. S., Mohs R., and Friedhoff L.T. (1998) A 24-week, double blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. Neurology, 50, 136-145.

Rösler M., Anand R., Cicin-Sain A., Gauthier S., Agid Y., Dal-Bianco P., Stähelin H. B., Hartman R., Gharabawi M. (1999) Efficacy and safety of rivastigmine in patients with Alzheimer's disease: International randomised controlled trial. British Medical Journal, 318, 633-638.

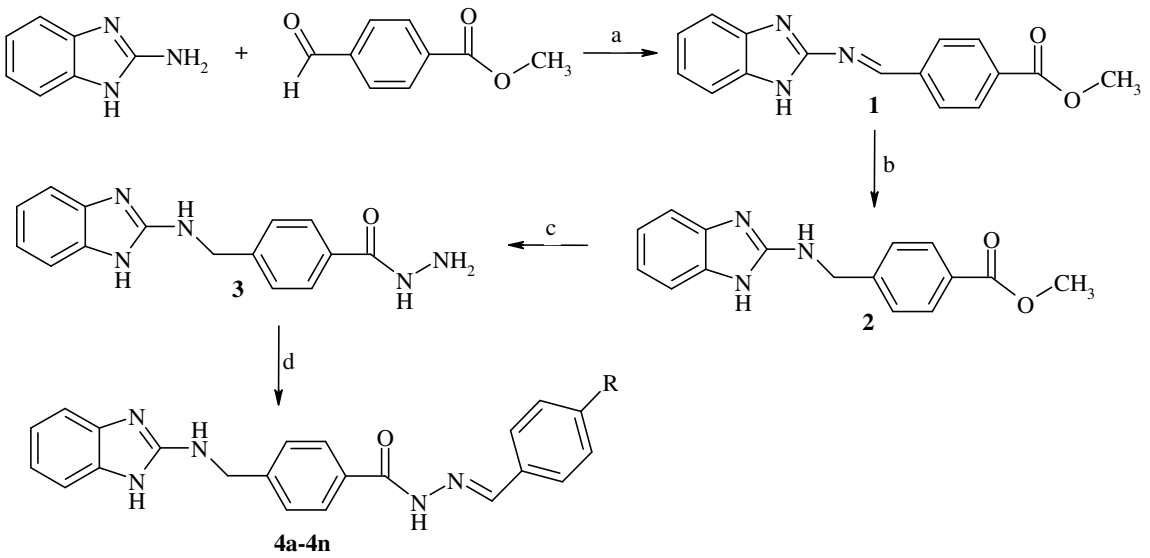
Walia R., Hedaitullah M. D., Naaz S. F., Iqbal K. and Lamba H. S. (2011) Benzimidazole derivatives-an overview. International Journal of Pharmaceutical Chemistry Research. 1, 565-574.

Whitehouse P. J., Price D. L., Clark A. W., Coyle J. T. and DeLong M.R. (1981) Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Annals of Neurology, 10, 122-126.

Yoon Y. K., Ali M. A., Wei A. C., Choon T. S., Khaw K. Y., Murugaiyah V., Osman H. and Masand V. H. (2013a) Synthesis, characterization, and molecular docking analysis of novel benzimidazole derivatives as cholinesterase inhibitors. Bioorganic Chemistry 49, 33-39.

Yoon Y. K, Ali M. A., Wei A. C., Choon T. S and Kumar R. S. (2013b) Drug Design: An efficient and facile synthesis of novel polar benzimidazoles of biological interests, Drug Design, 3, 110.

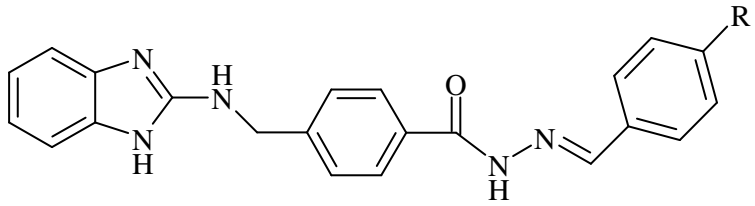
Scheme 1. Synthesis of benimidazole derivatives.



R: -H, -N(CH_3)$_2$, -N(C_2H_5)$_2$, -OH, -CH_3, -OCH_3, -OC_2H_5, -Cl, -Br, -F, -NO_2, -CF_3, CN, COOH.

Reagents and conditions: **a:** EtOH/benzene (5:1), catalytic amount CH_3COOH, reflux 6 h; **b:** $NaBH_4$, *i*-PrOH, reflux 2 h; **c:** 80% $NH_2NH_2.H_2O$, EtOH, reflux 12 h; **d:** corresponding 4-substitutedbenzaldehyde, catalytic amount CH_3COOH, n-ButOH, reflux 3 h.

Table 1. Some physicochemical properties of the compounds



Comp.	R	Mol. Formula	Mol. Weight (g/mol)
4a	-H	$C_{22}H_{19}N_5O$	369.43
4b	-N(CH_3)$_2$	$C_{24}H_{24}N_6O$	412.50
4c	-N(C_2H_5)$_2$	$C_{26}H_{28}N_6O$	440.55
4d	-OH	$C_{21}H_{15}ClN_4O$	385.43
4e	-CH_3	$C_{23}H_{21}N_5O$	383.46
4f	-OCH_3	$C_{23}H_{21}N_5O_2$	399.46
4g	-OC_2H_5	$C_{24}H_{23}N_5O_2$	413.48
4h	-Cl	$C_{22}H_{18}ClN_5O$	403.87
4i	-Br	$C_{22}H_{18}BrN_5O$	448.33
4j	-F	$C_{22}H_{18}FN_5O$	387.42
4k	-NO_2	$C_{22}H_{18}N_6O_3$	414.43
4l	-CF_3	$C_{23}H_{18}F_3N_5O$	437.43
4m	-CN	$C_{23}H_{18}N_6O$	394.44
4n	-COOH	$C_{23}H_{19}N_5O_3$	413.44

Table 2. % AChE inbition of the compounds and IC_{50} values

Comp.	AChE Inhibition (%)			
	1 mM	0.1 mM	0.01 mM	IC_{50} (mM)
4a	42.74±3.18**	ND	ND	> 1
4b	28.85±1.76**	ND	ND	> 1
4c	36.28±2.32**	ND	ND	> 1
4d	14.51±1.09**	ND	ND	> 1
4e	23.91±2.12**	ND	ND	> 1
4f	69.26±3.29**	51.23±2.42**	38.22±0.95**	0.091±0.003
4g	64.85±4.45**	49.42±2.48**	ND	0.134±0.027
4h	39.17±1.43**	ND	ND	> 1
4i	12.26±0.93**	ND	ND	> 1
4j	38.30±2.84**	ND	ND	> 1
4k	31.07±1.81**	ND	ND	> 1
4l	34.51±1.31**	ND	ND	> 1
4m	22.66±1.22**	ND	ND	> 1
4n	36.35±2.81**	ND	ND	> 1
Donepezil	98.42±2.98	96.08±3.45	87.49±2.77	0.054±0.002 (μM)

ND: Not determined. **p< 0.01 (unpaired Student's t test between test compound and Donepezil)