

Studies on Hydrazone–Hydrazones Derivatives As Acetylcholinesterase Inhibitors

Usama Abu Mohsen¹, Bedia Koçyiğit-Kaymakçioğlu², Emine Elçin Oruç-Emre³,
Zafer Asım Kaplancıklı⁴, Sevim Rollas²

¹Al-Azhar University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Gaza, Palestine.

²Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Haydarpaşa, İstanbul - Turkey

³Gaziantep University, Faculty of Arts and Sciences, Pharmacy, Department of Chemistry, Gaziantep - Turkey

⁴Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir - Turkey

Yazışma Adresi / Address reprint requests to: Bedia Koçyiğit Kaymakçioğlu
Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Haydarpaşa 34668, İstanbul - Turkey
Elektronik posta adresi / E-mail address: bkaymakciolu@marmara.edu.tr
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ÖZET

Asetilkolin esteraz inhibitörü olan hidrazid hidrazonlar üzerinde çalışmalar

Amaç: On beş adet hidrazid-hidrazon türevi sentezlenmiş ve asetilkolinesteraz enzimini (AChE) inhibe etme yetenekleri Ellman'ın modifiye spektrofotometrik yöntemi ile değerlendirilmiştir.

Yöntem: Anti-asetilkolinesteraz aktivite tayini Ellman'ın modifiye edilmiş spektrofotometrik yöntemi kullanılarak yapılmıştır. Bu spektrofotometrik yöntem bir kromojenik reaktif olan 5,5-ditiyo-bis-(2-nitrobenzoik asit) ile salınan tiyokolinin renkli bir ürün vermesi esasına dayanır.

Bulgular: Test edilen bileşikler arasında, 4-fluorobenzoik asit [(4-metoksifenil) metilen] hidrazid(6) ve 2-[(fluorobenzoil) hidrazono]-1,3-dihidro-indol-3-on (15), referans ilaç donepezil ($IC_{50}=0.054\pm 0.002\mu M$) ile kıyaslandığında kayda değer anti-AChE aktivite göstermiştir.

Sonuç: Anti-AChE aktivite sonuçları, p-metoksifenil sübstitüenti taşıyan bileşik 6 ve 1,3-dihidro-indol-3-on sübstitüenti taşıyan bileşik 15'in en aktif bileşikler olduğunu göstermiştir. Aktivite sonuçlarından, hidrazid-hidrazon yapısı üzerinde hacimli grupların bulunmasının anti-AChE aktiviteye olumlu yönde katkıda bulunduğu görülmektedir.

Anahtar sözcükler: Hidrazid, hidrazon, anti-asetilkolinesteraz aktivite

ABSTRACT

Studies on hydrazone–hydrazones derivatives as acetylcholinesterase inhibitors

Objective: Fifteen hydrazone-hydrazone derivatives were synthesized and evaluated for their ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method.

Methods: Anti-acetylcholinesterase activity was evaluated by using a modification of Ellman's spectrophotometric method. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5,5-dithio-bis-(2-nitrobenzoic acid).

Results: Among the tested compounds, 4-fluorobenzoic acid [(4-methoxyphenyl) methylene] hydrazide (6) and 2-[(fluorobenzoil) hydrazono]-1,3-dihydro-indol-3-one (15), showed noteworthy anti-AChE activity when compared to standard drug donepezil ($IC_{50}=0.054\pm 0.002\mu M$).

Conclusion: The anti-AChE activity screening indicated that among the tested compounds, 6 with p-methoxyphenyl substitution and 15 with 1,3-dihydro-indol-3-one substitution represent the most active compounds. Based on the activity results, it appears that bulky groups on the hydrazone-hydrazone moiety have made good contribution to the anti-AChE activity.

Key words: Hydrazone, hydrazone, anti-acetylcholinesterase activity

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative brain disorder characterized by loss of memory, mood changes, and problems with communication and reasoning. AD is described by loss of cholinergic neurons and synaptic markers in cerebral cortex and in certain sub-cortical regions (1,2). Firstly, Alzheimer's disease was reported in 1907 by the German neurologist Alois Alzheimer (3). Researchs in the last two decades have correlated Alzheimer's disease with acetylcholine deficiency (4). Tacrine was the first of the AChE

inhibitors approved for the AD treatment in 1993, but its use has been abandoned because of a high incidence of side effects including hepatotoxicity (4).

The use of enzymes-inhibition theory in the diagnosis of disease and synthesis of new drugs is one of the important benefits derived from the intensive research in medicine. The human body is composed of a wide variety of components, and has developed complex enzymatic inhibition mechanisms to alter the progress of many disease by drugs molecules (4,5). Since the cholinergic therapy may alter the symptoms and progress of AD by stopping any

decrease in acetylcholine level through inhibition of acetylcholinesterase enzyme, therefore a strategy for the treatment of AD is focused on acetylcholinesterase enzyme. Two ChEs are identified clinically in humans: acetylcholinesterase (AChE) and butyryl cholinesterase (BuChE). Both of them are present in cholinergic synapses, central nervous system (CNS), parasympathic synapses in the periphery, and in the neuro muscular junction. AChE is selective for ACh hydrolysis, while BuChE hydrolyses acetylcholine and other choline esters and as regarded its as a non-specific cholin esterase (5–11). Medications currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines

Agents (EMA) to treat the cognitive manifestations of AD and to improve life quality of the patients are: donepezil, rivastigmine and galantamine as reversible AChE inhibitors, and memantine as a NMDA receptor antagonist (12). However these AChE inhibitors are known to have side effects such as hepatotoxicity, short half life and gastrointestinal tract excitement (13). Therefore the investigation on searching for new and better AChE

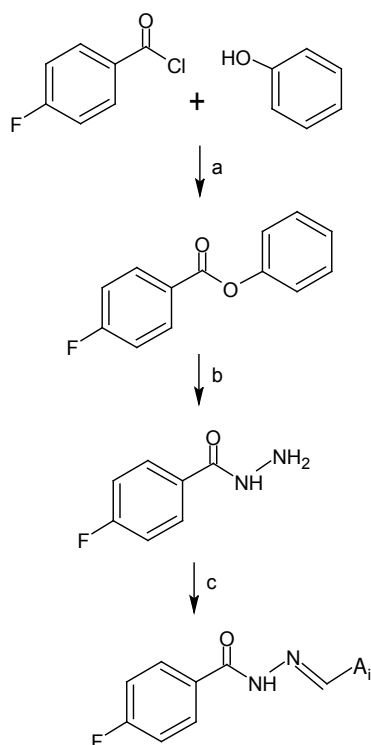
inhibitors is still of great interest.

Since that hydrazide-hydrazone moiety plays an important role for anticholinesterase activity (14-21), in the present study, prompted by these observations, we synthesized hydrazide-hydrazone derivatives as AChE inhibitors.

MATERIALS AND METHODS

Synthesis of Test Compounds

General procedures for the preparation of target compounds 1-15 are described in Scheme 1. The 4-fluorobenzoyl chloride was first reacted with phenol in alkaline medium, to give the corresponding ester 1 in very good yield (85%). This ester was then converted almost quantitatively to the hydrazide after treatment with hydrazine hydrate in dry methanol. The reaction of the hydrazide with aldehydes and ketones in ethanol afforded the corresponding substituted hydrazides 1-15 (Table 1). Physicochemical and spectroscopic characterization of all compounds have been previously described (22,23).



Scheme 1: Synthetic pathway for compounds 1-15

Reagents and conditions: (a) NaOH; (b) NH_2NH_2 , CH_3OH ; (c) RCHO, $\text{C}_2\text{H}_5\text{OH}$
 A_i : Substituted phenyl / thiophenyl, furanyl, pyrrolyl, isatine

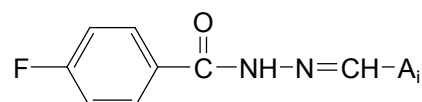
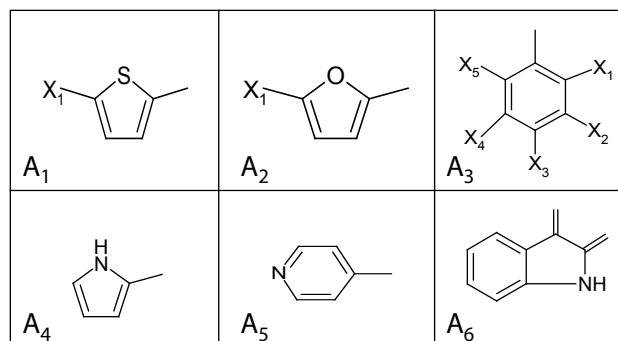


Table 1: The synthesized hydrazide-hydrazone derivatives

Comp.	A_i	X_i	Comp.	A_i	X_i
1	A_3	$X_3=\text{H}$	9	A_3	$X_2=\text{OCH}_3$; $X_3=\text{OH}$
2	A_3	$X_3=\text{Br}$	10	A_3	$X_2=\text{O}(\text{C}_2\text{H}_5)$; $X_3=\text{OH}$
3	A_3	$X_3=\text{Cl}$	11	A_1	-
4	A_3	$X_3=\text{F}$	12	A_2	-
5	A_3	$X_3=\text{CH}_3$	13	A_4	-
6	A_3	$X_3=\text{OCH}_3$	14	A_5	-
7	A_3	$X_3=\text{N}(\text{CH}_3)_2$	15	A_6	-
8	A_3	$X_1=\text{OH}$			



Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (21) in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured 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 dihydrogenphosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV-Vis spectrophotometer. Cholinesterase activity of the compounds (1-15) 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 (Table 2) (25).

Table 2: AChE inhibition (%) of the tested compounds and their IC₅₀ values

Comp.	AChE Inhibition (%)		
	1 mM	0.1 mM	IC ₅₀ (mM)
1	43,38±1,26	42,68±0,80	> 1
2	9,81±1,46	9,44±3,02	> 1
3	9,39±1,77	8,13±2,25	> 1
4	9,34±1,44	6,29±0,92	> 1
5	12,87±1,26	11,69±0,70	> 1
6	46,08±0,93	42,85±0,45	> 1
7	44,73±3,06	43,43±1,16	> 1
8	8,84±3,02	7,24±0,92	> 1
9	40,72±0,46	36,63±2,89	> 1
10	8,83±1,64	6,77±0,92	> 1
11	41,91±1,32	40,12±1,32	> 1
12	43,64±0,93	42,88±1,7	> 1
13	40,28±1,22	40,23±1,02	> 1
14	40,29±1,08	40,23±1,96	> 1
15	52,38±2,05	40,61±0,24	> 1
Donepezil	99,01±4,89	95,52±5,01	0,054±0,002(µM)

IC₅₀: The half maximal inhibitory concentration

Enzymatic assay

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 a concentration range of 10⁻¹-10⁻⁶mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25°C 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:

$$\text{Inhibition \%} = (A_c - A_i) / A_c \times 100$$

Where AI is the absorbance in the presence of the inhibitor, AC is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. Data were expressed as Mean±SD.

RESULTS AND DISCUSSION

It was reported that hydrazone derivatives show anti-acetylcholinesterase (AChE) activity (15,16,26,27). According to this information, the anti-AChE activity of the compounds (1-15) were determined by modified Ellman's spectrophotometric method (Table 2). Among these (1-15) compounds, compound 15 with 1,3-dihydro-indol-3-one substitution and compound 6 with p-methoxyphenyl substitution represent the most active compounds. Thus, inhibition percentages are 52,38 and 40,61% at 1 and 0.1 mM concentrations for compound 15 and 46,08 and 42,85% at 1 and 0.1 mM concentrations for compound 6. The IC₅₀ values could not be well defined in all compounds. Compound 1 bearing phenyl moiety, compound 7 bearing 4-(N,N-dimethyl- amino) phenyl moiety, and compound 12 bearing 2-furanyl group exhibited anticholin-esterase activity with nearly 43% inhibition value. Compound 9, 11, 13 and 14 showed moderate activity with the inhibition percentages about 41%. The other compounds 2, 3, 4, 5, 8 and 10 showed relatively weak activity and the inhibition values were found to be less than 12,87%. Standard drug Donepezil was studied at lower concentrations for the purpose of finding IC₅₀ value and it was determined as

0.054 μM . None of the compounds showed comparable activity with Donepezil and there were no significant anti-AChE activity and this is contrary to expectations.

CONCLUSION

In conclusion, a series of hydrazide-hydrazone derivatives have been synthesized and screened for

their anti-AChE activity. The anti-AChE activity screening indicated that among the tested compounds, 15 with 1,3-dihydro-indol-3-one substitution and 6 with p-methoxyphenyl substitution represent the most active compounds. Based on the activity results, it appears that bulky groups on the hydrazide-hydrazone moiety have made good contribution to the anti-AChE activity.

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