MÜSBED 2014;4(1):38-42
DOI: 10.5455/musbed.20140101090010

Araştırma / Original Paper

Study on Thiazolyl-Hydrazone Derivatives as Acetylcholinesterase Inhibitors

Yusuf Özkay[1], Leyla Yurttaş[1], Usama Abu Mohsen[2], Belgin Sever[1], Weiam Hussein[1], Ömer Öztürk[1], Begüm Nurpelin Sağlık[1], Ulviye Acar[1], Özge Nurhan Erdoğan[1], Ayşen Pekbağ[1], Zafer Asım Kaplancıklı[1,3]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir - Turkey
[2]Al-Azhar University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Gaza - Palestine
[3]Anadolu University, Graduate School of Health Sciences, Department of Pharmaceutical Chemistry, Eskişehir - Turkey

Yazışma Adresi / Address reprint requests to: Zafer Asım Kaplancıklı
Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir - Turkey
Elektronik posta adresi / E-mail address: zakaplan@anadolu.edu.tr
Kabul tarihi / Date of acceptance: 1 Ocak 2014 / January 1, 2014

ÖZET

Asetilkolinesteraz inhibitörü olarak tiyazolil-hidrazon türevleri üzerine çalışma

Amaç: Bu çalışmada, tiyazolün bazı hidrazon türevlerinin sentezlerinin ve antikolinesteraz aktivitelerinin araştırılması amaçlandı.
Yöntem: Pirol-2-karboksaldehidler tiyosemikarbazit ile etanol içinde direkt olarak reaksiyona tabi tutuldu ve oluşan tiyosemikarbazon, α-bromoasetofenon türevleri ile (Hantzsch reaction) kondanse edilerek, 1-sübstitüe pirol-2-karboksaldehid (4-(4-sübstitüe fenil)-1,3-tiyazol-2-il)hidrazon türevlerini verdi. Bileşiklerin kimyasal yapıları, IR, 1H-NMR ve FAB+-MS spektral verileri ve elementel analiz verileri ile aydınlatıldı. Modifiye edilmiş Ellman spektrofotometrik metodu kullanılarak, elde edilen tüm bileşiklerin asetilkolinesteraz (AChE) inhibisyonları incelendi.
Bulgular: Bileşik 1, AChE üzerinde %64.10 (1 mM) and 33.00 (0.1 mM) inhibisyon oranları ve IC50=0.59 mM değeri ile en aktif antikolinesteraz molekül olarak belirlenmiştir.
Sonuçlar: Fenil halkası üzerinde para konumundan ve pirol halkasının 1. konumundan sübstitüsyonlar antikolinesteraz etkiyi negatif yönde etkilemiştir.
Anahtar sözcükler: Tiyazol, hidrazon, pirol, antikolinesteraz aktivite

ABSTRACT

Study on thiazolyl-hydrazone derivatives as acetylcholinesterase inhibitors

Objective: In this study we aimed to synthesize some hydrazone derivatives of thiazole and to evaluate their anticholinesterase activities.
Method: Pyrrole-2-carboxaldehydes were reacted directly with thiosemicarbazide in ethanol and then obtained thiosemicarbazones were condensed with α-bromoacetophenone derivatives (Hantzsch reaction) to give 1-substituted pyrrole-2-carboxaldehyde (4-(4-substituted phenyl)-1,3-thiazol-2-yl) hydrazones. The structures of the obtained compounds were elucidated by using IR, 1H-NMR and FAB+-MS spectral data and elemental analyses results. In the pharmacological studies, anticholinesterase activities of these compounds have been evaluated by using modified Ellman's spectrophotometric method.
Results: The compound (1) can be identified as the most active anticholinesterase molecule due to its inhibitory effect on acetylcholinesterase with inhibition percentages of 64.10 (1 mM) and 33.00 (0.1 mM) % and also IC50 value of 0.59 mM.
Conclusion: The substitutions on phenyl ring at para position and at first position of pyrrole ring have negatively affected anticholinesterase activity.
Key words: Thiazole, hydrazone, pyrrole, anticholinesterase activity

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, a serious brain disorder that impacts daily living through memory loss and cognitive changes. Although not all memory loss indicates AD, one in ten people over 65 years of age, and over half of those over 85 have AD. Currently, it affects about 4.5 million Americans and close to 27 million people worldwide. AD destroys neurons in the cortex and limbic structures of the brain, areas that are responsible for learning, memory, behavior, emotion, and reasoning in humans. The devastating effects of AD include memory loss, dementia, and ultimately death (1-3). AD is mainly characterized by the pre-synaptic decrease of

acetylcholine (ACh) due to damage of cholinergic neurons in some special parts of the brain such as hippocampus and cortex (cholinergic hypothesis) (4,5). Concerning the cholinergic hypothesis one of the rational and effective approaches to treat the AD's symptoms, is raising the ACh through inhibition of acetylcholinesterase (AChE) that is responsible for hydrolysis of ACh in presynaptic areas (6,7).

In the last few decades, the chemistry of thiazoles and their fused heterocyclic derivatives has received considerable attention due to their synthetic and biological importance. Compounds bearing thiazole moiety have been reported to exhibit a wide spectrum of biological effects including anticholinesterase activity (8-14).

The literature survey reveals that, hydrazone group plays an important role for anticholinesterase activity (15,16).

Some researchers also carried out considerable research for novel cholinesterase inhibitors bearing pyrrole moiety previously (17-19).

In previous study (20), we identified the synthesis of title compounds and antimicrobial activity profile. In extending our work, motivated by the above data, we synthesized 1-substituted pyrrole-2-carboxaldehyde (4-(4-substituted phenyl)-1,3-thiazol-2-yl)) hydrazones (1–14) again, confirmed the structures of the compounds and focused on their potential acetylcholinesterase inhibitor activities.

MATERIALS AND METHODS

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co. All melting points (m.p.) were determined by Electrothermal 9100 digital melting point apparatus and were uncorrected. Spectroscopic data were recorded with the following instruments: 1HNMR, Bruker 400 MHz spectrometer; MS-FAB, VG Quattro Mass spectrometer and elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA).

General procedure for synthesis of the compounds

Preparation of 1-substituted pyrrol-2-carbaldehyde thiosemicarbazones (A, B)

Thiosemicarbazide (5 mmol) and 2-formylpyrrole or N-methyl-2-formylpyrrole (5 mmol) was refluxed for 3 h in ethanol (40 mL). The resulting mixture was cooled, filtered

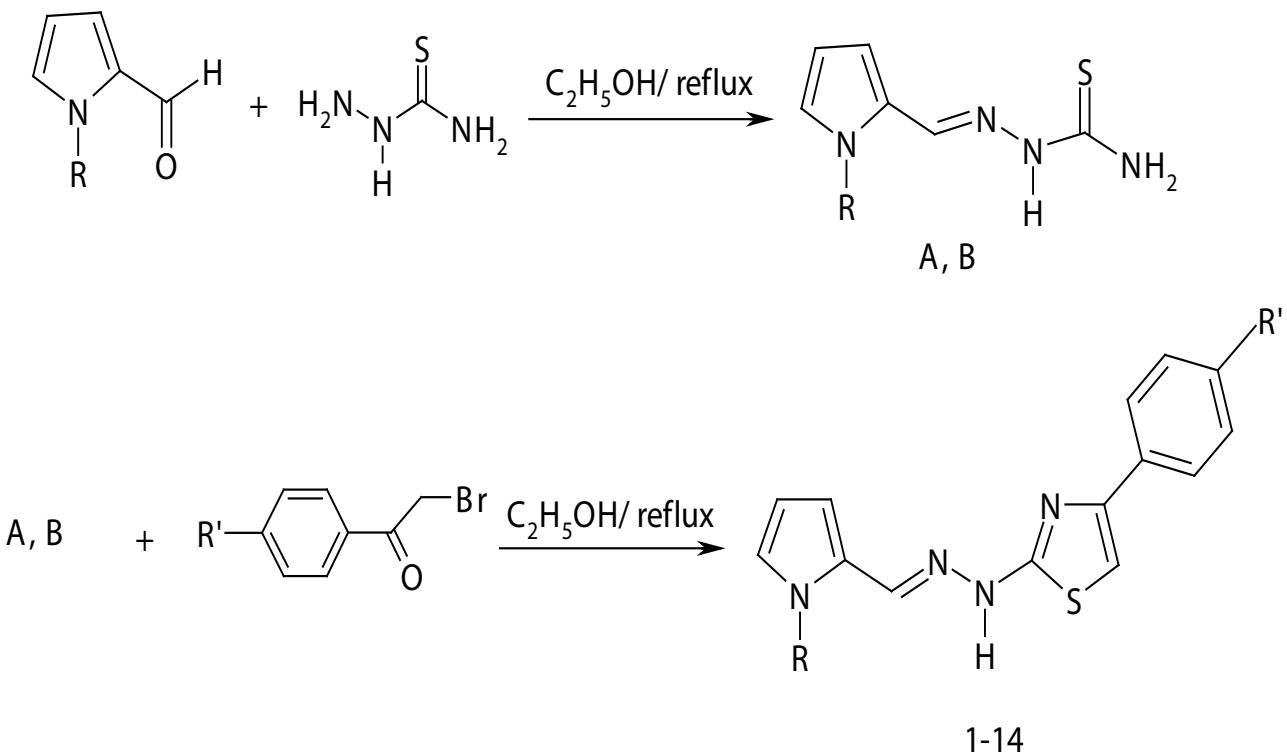

Figure 1: The synthetic protocol of the compounds

and recrystallized from ethanol (21).

Preparation of 1-substituted pyrrole-2-carboxaldehyde (4-(4-substituted phenyl)-1,3-thiazol-2-yl)) hydrazones (1–14)

A or B (0.3 mmol) and α-bromoacetophenone (0.3 mmol) were stirred at room temperature in ethanol (10 mL). The precipitate was filtered and recrystallized from ethanol to give title compounds (Table 1) (Figure 1) (20).

Pyrrole-2-carboxaldehyde (4- phenyl-1,3-thiazol-2-yl) hydrazone (1):

IR (KBr) ν_{max}(cm^{-1}): 3375-3110 (N-H), 3045 (aromatic C-H), 1621-1470 (C=C and C=N). 1H NMR (DMSO-d6) δ ppm: 6.10 (brs, 1H, pyrrole-H), 6.55 (brs, 1H, pyrrole-H), 6.95 (brs, 1H, pyrrole-H), 7.35 (s, 1H, C_5-H thiazole), 7.40–7.49 (m, 3H, Ar-H), 7.79–7.85 (m, 2H, Ar-H), 8.10 (s, 1H, -CH=N-N), 11.30 (s, 1H, pyrrole N-H), 11.85 (brs, 1H, C=N-NH). For $C_{14}H_{12}N_4S$ calculated: 62.66 % C, 4.51 % H, 20.88 % N; found: 62.62 % C, 4.50 % H, 20.92 % N.

MS (FAB) $[M+1]^+$: m/z 269.

Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (22) in order to evaluate their potency to inhibit the AChE. The 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

Table 1: Some characteristics of the compounds

Comp.	R	R′	Mp (°C)	Yield %	Mol. Formula
1	H	H	125	85	$C_{14}H_{12}N_4S$
2	H	CH_3	157	80	$C_{15}H_{14}N_4S$
3	H	OCH_3	155	80	$C_{15}H_{14}N_4OS$
4	H	Br	171	75	$C_{14}H_{11}BrN_4S$
5	H	Cl	200	82	$C_{14}H_{11}ClN_4S$
6	H	F	165	90	$C_{14}H_{11}FN_4S$
7	H	NO_2	201	82	$C_{14}H_{11}N_5O_2S$
8	CH_3	H	224	70	$C_{15}H_{14}N_4S$
9	CH_3	CH_3	183	75	$C_{16}H_{16}N_4S$
10	CH_3	OCH_3	210	75	$C_{16}H_{16}N_4OS$
11	CH_3	Br	214	70	$C_{15}H_{13}BrN_4S$
12	CH_3	Cl	202	85	$C_{15}H_{13}ClN_4S$
13	CH_3	F	222	73	$C_{15}H_{13}FN_4S$
14	CH_3	NO_2	199	72	$C_{15}H_{13}N_5O_2S$

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

Comp.	AChE Inhibition (%) 1 mM	0.1 mM	IC_{50} (mM)
1	64.10±4.13**	33.00±2.32**	0.59±3.64
2	ND	ND	ND
3	ND	3.05±2.36**	> 1
4	ND	4.22±3.52**	> 1
5	ND	ND	ND
6	ND	ND	ND
7	ND	ND	ND
8	17.23±4.56**	12.93±3.45**	> 1
9	19.74±5.42**	15.14±3.47**	> 1
10	18.05±2.54**	22.52±4.87**	> 1
11	17.41±3.69**	11.89±2.78**	> 1
12	ND	ND	ND
13	ND	ND	ND
14	ND	ND	ND
Donepezil	99.01±4.89	95.52±5.01	0.054±0.002 (μM)

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

(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 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (1-14) was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (Table 2) (23).

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 0.1 and 1 mM concentrations 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:

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 were 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.

RESULTS AND DISCUSSION

In this work, we synthesized fourteen thiazolyl-hydrazone derivatives, which had been synthesized previously in order to check their antimicrobial activity as described in the introduction section. Structure elucidation of those compounds was explained in detail in the referenced literature and these data were used to check the characteristics of the compounds synthesized again in this study (20). It was observed that, all the spectral data were in agreement with the spectral data of the previous study. IR, ^{1}H-NMR and FAB$^+$-MS spectral data and elemental analyses results were given only for a sample compound (1).

The anticholinesterase effects of the compounds (1-14) were determined by modified Ellman's spectrophotometric method (Table 2). Among these compounds (1-14), compound 1 including non-substituted phenyl moiety in its structure was found as the most active compound with inhibition values of 64.10 and 33.00% at 1 and 0.1 mM concentrations, respectively. The IC_{50} value was calculated as 0.59 mM only for this compound. The inhibition percentages were not determined for compounds 2-7, 12-14 and these compounds were evaluated as inactive at two tested concentrations. Compound 10 bearing 1-methyl pyrrole and 4-methoxy phenyl moieties exhibited moderate anticholinesterase activity with 18.05 and 22.52% inhibition values. The other compounds 8, 9, 11 showed relatively weak activity and the inhibition values were found less than 19.74%. 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 significant anticholinesterase activity contrary to expectations.

CONCLUSION

In conclusion, a series of thiazolyl-hydrazone derivatives were synthesized and studied for their anticholinesterase activities. According to the activity test results, compound 1 exhibited the highest anticholinestrerase activity with a value of IC_{50}=0.59 mM and none of the compounds showed activity as much as standard drug Donepezil. The results indicate that the substitutions on phenyl ring at para position and at first position of pyrrole ring have negatively affected anticholinesterase activity.

REFERENCES

1. Aderinwale GO, Ernst HW, Mousa SA. Current therapies and new strategies for the management of Alzheimer's Disease. Am J Alzheimers Dis Other Demen. 2012;25: 414-424.

2. Dipiro J, Talbert RL, Yee GC, Matzke GR, Wells BG. Alzheimer's disease. In: Jennifer Dea, eds. Pharmacotherapy: A Pathophysiology Approach. New York: NY McGraw-Hill; 2005.

3. Salloway S, Correia S. Alzheimer disease: time to improve its diagnosis and treatment. Cleve Clin J Med. 2009;76(1): 49-58.

4. Terry Jr. AV, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther. 2003;306: 821-827.

5. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev. 2001; 81: 741-766.

6. Giacobini E. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol Res. 2004; 5: 433-440.

7. Asadipour A, Alipour M, Jafari M, Khoobi M, Emami S, Nadri H, Sakhteman A, Moradi A, Sheibani V, Moghadam FH, Shafiee A, Foroumadi A. Novel coumarin-3-carboxamides bearing N-benzylpiperidine moiety as potent acetylcholinesterase inhibitors. Eur J Med Chem. 2013; 70: 623-630.

8. Siddiqui N, Arshad MF, Ahsan W, Alam MS. Thiazoles: a valuable insight into the recent advances and biological activities. Int J Pharm Sci Drug Res. 2009; 1: 136-143.

9. Mustafa SM, Naira VA, Chittoorb JP, Krishnapillaic S. Synthesis of 1,2,4-triazoles and thiazoles from thiosemicarbazide and its derivatives. Mini-Rev Org Chem. 2004;1: 375-385.

10. Andreani A, Burnelli S, Granaiola M, Guardigli M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Rizzoli M, Varoli L, Roda A. Chemiluminescent high-throughput microassay applied to imidazo [2,1-b]thiazole derivatives as potential acetylcholinesterase and butyrylcholinesterase inhibitors. Eur J Med Chem. 2008;43: 657-661.

11. Sengupta AK, Garg M. New 5-arylamino-2[N-(2'-mercaptoacetylamino-4'-arylthiazolo)]thiadiazoles(III): as AChE inhibitors. J Indian Chem Soc. 1980;57: 1241-1243.

12. Shaw FH, Bentley GA. The pharmacology of some new anticholinesterases. Aust J Exp Biol Med Sci. 1953;31: 573–576.

13. Matsunaga Y, Tanaka T, Yoshinaga K, Ueki S, Hori Y, Eta R. Acotiamide hydrochloride (Z-338), a new selective acetylcholinesterase inhibitor, enhances gastric motility without prolonging QT interval in dogs: comparison with cisapride, itopride, and mosapride. J Pharmacol Exp Ther. 2011;336: 791-800.

14. Alspach JD, Ingraham LL. Inhibition of acetylcholinesterase by thiamine. A structure-function study. J Med Chem. 1977; 20: 161-164.

15. Utku S, Gökçe M, Orhan I, Sahin MF. Synthesis of novel 6-substituted 3(2H)-pyridazinone-2-acetyl-2-(substituted/-nonsubstituted benzal)hydrazone derivatives and acetylcholinesterase and butyrylcholinesterase inhibitory activities in vitro. Arznei-forschung. 2011; 61(1): 1-7.

16. Alptüzün V, Prinz M, Hörr V, Scheiber J, Radacki K, Fallarero A, Vuorela P, Engels B, Braunschweig H, Erciyas E, Holzgrabe U. Interaction of (benzylidene-hydrazono)-1,4-dihydropyridines with beta-amyloid, acetylcholine, and butyrylcholine esterases. Bioorg Med Chem. 2010; 18(5): 2049-2059.

17. Sangnoi Y, Sakulkeo O, Yuenyongsawad S, Kanjana-opas A, Ingkaninan K, Plubrukarn A, Suwanborirux K. Acetylcholinesterase-inhibiting activity of pyrrole derivatives from a novel marine gliding bacterium, Rapidithrix thailandica. Mar Drugs. 2008; 6: 578-586.

18. Ribeiro TS, Prates A, Alves SR, Oliveira-Silva JJ, Riehlc CAS, Figueroa-Villar JD. The effect of neutral oximes on the reactivation of human acetylcholinesterase inhibited with paraoxon. J Braz Chem Soc. 23; 2012: 1216-1225.

19. Andreani A, Cavalli A, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Recanatini M, Roda A. Synthesis and screening for anticholinesterase activity of (1-benzyl- 4-oxopiperidin-3-ylidine) methylindoles and pyrroles related to Donepezil. J Med Chem. 2001; 44: 4011-4014.

20. Yurttaş L, Özkay Y, Kaplancıklı ZA, Tunalı Y, Karaca H. Synthesis and antimicrobial activity of some new hydrazone-bridged thiazole-pyrrole derivatives. J Enzym Inhib Med Chem. 2013; 28(4): 830-835.

21. Anderson FE, Duca CJ, Scudi JV. Some heterocyclic thiosemicarbazones. J Am Chem Soc. 1951; 73: 4967-4968.

22. Perry NSL, Houghton PJ, Theobald AE, Jenner P, Perry EK. In-vitro inhibition of human erythrocyte acetylcholine esterase by Salvia lavandulae folia essential oil and constituent terpenes. J Pharm Pharmacol. 2000; 52: 895-902.

23. Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88-95.