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Araştırma / Original Paper

Biological Evaluation of Some Tetrazole Derivatives as Cholinesterase Inhibitors

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ÖZET

Bazı tetrazol türevlerinin kolinesteraz inhibitörü olarak biyolojik açıdan değerlendirilmesi

Amaç: Bu çalışmada, ondört farklı 2-[(1-metil-1H-tetrazol-5-il) tiyo]-1-(sübstitüe fenil) etanon (1–14) türevinin sentezleri ve antikolinesteraz aktivitelerinin araştırılması amaçlandı.
Yöntem: 1-(Sübstitüe fenil)-2-[(1-metil-1H-tetrazol-5-il) tiyo] etanon (1–14) türevi bileşikler, 1-metil-1H-tetrazol-5-tiyol ile bazı fenaçil bromür türevlerinin reaksiyonuyla sentezlenmiştir. Bileşiklerin kimyasal yapıları, IR, ^{1}H-NMR, ^{13}C-NMR ve FAB^{+}-MS spektral verileri ve elementel analiz verileri ile aydınlatılmıştır. Tüm türevlerin asetilkolinesteraz enzimini (AChE) inhibisyon yetenekleri modifiye Ellman spektrofotometrik metodu kullanılarak değerlendirilmiştir.
Bulgular: Bileşik 2 ve 3 AChE üzerinde %29.56 ve %24.38 inhibisyon oranları ile en aktif bileşikler olarak bulunmuştur.
Sonuç: Bileşiklerden fenil artığı üzerinde 3-konumunda elektron verici metil ve kloro sübstitüentleri içerenler en yüksek antikolinesteraz aktivite göstermişlerdir.
Anahtar sözcükler: Tetrazol, kolinesteraz inhibitörleri

ABSTRACT

Biological evaluation of some tetrazole derivatives as cholinesterase inhibitors

Objectives: In this study, we aimed to synthesize fourteen different 2-[(1-methyl-1H-tetrazole-5-yl) thio]-1-(substituted phenyl) ethanone derivatives (1–14) and to investigate their anticholinesterase activities.
Method: 1-(Substituted phenyl)-2-[(1-methyl-1H-tetrazol-5-yl) thio]ethanone compounds were synthesized by reacting 1-methyl-1H-tetrazol-5-thiol with some phenacyl bromide derivatives. The structures of the obtained compounds were elucidated using IR, ^{1}H-NMR, ^{13}C-NMR and FAB^{+}-MS spectral data and elemental analyses results. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method.
Results: The compound 2 and 3 were found as the most active compounds due to their inhibitory effect on AChE with inhibition percentages of 29.56 and 24.38%.
Conclusion: The compounds with electron donating substituents methyl and chloro at the third position of phenyl residue have exhibited the highest anticholinesterase activity.
Key words: Tetrazole, cholinesterase inhibitors

INTRODUCTION

Alzheimer's Disease (AD) is the most common single cause of dementia in the ageing society. Traditionally thought of as an untreatable degenerative condition, recent advances in drug therapy have challenged this view. The disease is characterized by an insidious decline in cognitive and non-cognitive function. Classically, short and long-term memory is impaired while language skills, concentration and attention are often affected. This results in impaired ability to learn and retain new skills as well as the loss of existing ones. Non-cognitive function is the global term used to describe problems such as depression, agitation, personality changes, delusions and hallucinations. These factors have a significant impact on patient behaviour and a very real impact on the quality of life for both patients and caregivers (1). The development of acetylcholinesterase (AChE) inhibitor drugs followed the finding that the cholinergic pathways in the cerebral cortex and basal forebrain are compromised in AD (2) and the resultant cholinergic deficit contributes to the cognitive impairment of these patients (3). Although many believe this 'cholinergic hypothesis' to be important, others feel it represents a less significant component of the disease process (4). Many

other neurotransmitters are affected in AD, and the relative importance of each in relation to clinical findings has not been fully elucidated.

The medicinal, biochemical and pharmacological properties of tetrazole compounds support a vast progress in their chemistry over the last years (5-7). Tetrazoles have a wide variety of applications as pharmaceutical products and explosives, in cholinesterase inhibitor activity (8).

It is known that the tetrazole ring can serve as a bioisosteric moiety of carboxylic group in biologically active molecules, because both groups possess comparable acidity and size. The tetrazole moiety, however, was confirmed to be superior in resisting metabolic degradation (9-12).

We identified the synthesis of title compounds and anticandidal activity and cytotoxicity profile previously (13). The title compounds are also accomplished in this work and the target compounds are evaluated regarding their ability to inhibit acetylcholinesterase.

MATERIALS AND METHODS

Chemistry

General procedure for the synthesis of the compounds (1-14)

2-[(1-Methyl-1H-tetrazol-5-yl)thio]-1-(substituted phenyl) ethanones (1-14) were synthesized by reacting 1-methyl-1H-tetrazole-5-thiol with some substituted phenacyl bromide derivatives. Physicochemical and spectroscopic characterization of the title compounds (1-14) have been previously described (13). The analysis results of the compound 1 are as follows:

2-[(1-Methyl-1H-tetrazol-5-yl)thio]-1-(2-chlorophenyl) ethanone (compound 1)

IR (KBr) $_{vmax}$ (cm^{-1}): 1672 (C=O), 1597 (C=N). ^{1}H NMR (500 MHz, DMSO-d6): δ 4.01 (3H, s, NCH_3), 4.97 (2H, s, CH_2), 7.50-7.73 (1H, m, Ar-H), 7.61 (2H, d, J=3.7 Hz, Ar-H), 7.86 (1H, d, J=7.6 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d6): δ 34.85, 43.86, 128.70, 131.33, 131.54, 131.95, 134.43, 137.52, 154.48, 196.14. MS (FAB) $[M+1]^+$: m/z 269. For $C_{10}H_9ClN_4OS$ calculated: 44.70 % C, 3.38 % H, 20.85 % N; found 44.74 % C, 3.40 % H, 20.83 % N.

Pharmacology

AChE inhibition

All compounds were subjected to a slightly modified method of Ellman's test (14) 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 dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatin and acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu

Table 1: AChE inbition (%) of the compounds and IC_{50} values

Comp.	R	AChE Inhibition (%) 1 mM	0.1 mM	IC_{50} (mM)
1	2-Cl	11.64±4.13	11.51±2.32	> 1
2	3-CH_3	29.56±2.54	14.53±3.47	> 1
3	3-Cl	24.38±4.87	12.96±2.37	> 1
4	3-NO_2	11.77±2.54	10.07±3.14	> 1
5	4-CH_3	17.71±3.69	4.69±2.78	> 1
6	4-OCH_3	14.59±2.35	ND	> 1
7	4-Cl	21.8±2.54	ND	> 1
8	4-F	ND	ND	ND
9	4-Br	ND	ND	ND
10	4-NO_2	ND	ND	ND
11	2,4-diCH_3	21.07±3.25	ND	> 1
12	2,4-diCl	ND	ND	ND
13	2,5-diCl	ND	ND	ND
14	3,4-diCl	ND	ND	ND
Donepezil		99.01±4.89	95.52±5.01	0.054±0.002 (μM)

ND: Not determined

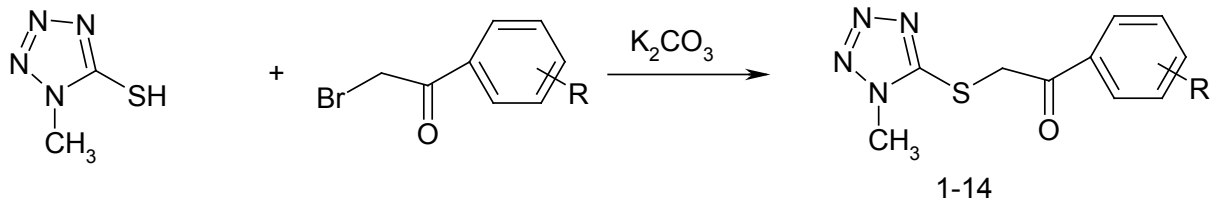

Figure 1: The synthetic protocol of the compounds

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, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (Table 1) (15).

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 0.1 and 1 mM 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 DTN (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 % = $(A_C–A_I) / A_C$ x 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. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as mean ± SD.

RESULTS AND DISCUSSION

The anticholinesterase effects of the 1-(substituted phenyl)-2-[(1-methyl-1H-tetrazol-5-yl)thio] ethanone compounds (1-14) were determined by modified Ellman's spectrophotometric method (Table 1). Among these compounds (1-14), compound 2 with 3-methyl phenyl substitution and compound 3 with 3-chloro phenyl substitution were found as the most active compounds. The inhibition percentages were calculated as 29.56% and 14.53% at 1 and 0.1 mM concentrations for compound 2 and the inhibition percentages were calculated 24.38% and 12.96% at 1 and 0.1 mM concentrations for compound 3. The inhibition percentages were not determined for compounds 8-10, 12-14 and these compounds were evaluated as inactive at two tested concentrations. Compound 7 bearing 4-chloro phenyl moiety and compound 11 bearing 2,4-dimethyl phenyl moiety exhibited anticholinesterase activity with nearly 21% inhibition value. Compound 5 showed moderate activity with the inhibition percentages 17.71% and 4.69% at 1 and 0.1 mM concentrations. The other compounds 1 and 4 showed relatively weak activity and the inhibition values were found less than 11.77%. Standard drug Donepezil was studied at lower concentrations for the purpose of finding IC_{50} 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, fourteen 2 -[(1-methyl-1H-tetrazol-5-yl) thio]-1-(substituted phenyl) ethanone compounds were synthesized and screened for their anticholinesterase activity. Compounds did not show obvious anticholinesterase activity, but compound 2 with 3-methyl phenyl substitution and 3 with 3-chloro phenyl substitution attracted attention among the others.

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