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ARAŞTIRMA / RESEARCH

Synthesis of new piperazine compounds and investigation of their anticholinesterase effects

Yeni piperazin bileşiklerinin sentezi ve antikolinesteraz etkilerinin araştırılması

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Abstract

Purpose: The aim of present study is to evaluate anticholinesterase activities of some new piperazine compounds.

Materials and Methods: Ten new piperazine derivatives were synthesized. Structure elucidation of the synthesized compounds was performed by spectroscopic medhods. Inhibitory activities of these compounds on acetylcholine esterase (AChE) and butrylcholine esterase (BChE) enzymes have been determined by Ellman's colorimetric assay. Enzyme kinetic studies were performed for the most active compound 2b by using Lineweaver–Burk plots.

Results: The compound 2b including chloro substituent was found as the most active derivative with 82.95 %, 66.93 % and 42.63 % inhibition rates at 1 mM, 0.1 mM and 0.01 mM concentrations, respectively. Non-competitive type of inhibition was determined for compound 2b as a result of enzyme kinetic studies.

Conclusion: None of the compounds showed activity as much as standard drug donepezil. However, compound 2b and 2c displayed a promising and selective inhibitory activity against AChE.

Key words: Piperazine, AChE, BChE, inhibition.

Öz

Amaç: Bu çalışmada bazı yeni piperazin bileşiklerinin antikolinesteraz etkinliklerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: On yeni piperazin türevi sentezlenmiştir. Sentezlenen bileşiklerin yapı aydınlatmaları spektroskopik yöntemler ile gerçekleştirilmiştir. Bu bileşiklerin asetilkolin esteraz (AChE) ve butirilkolin esteraz enzimleri üzerindeki etkileri, Elmann kolorimetrik metodu ile belirlenmiştir. En etkili 2b bileşiği için Lineweaver–Burk grafiği kullanılarak enzim kinetik çalışmaları gerçekleştirilmiştir.

Bulgular: Klor değişken grubu içeren 2b kodlu bileşiğin 1mM, 0.1 mM and 0.01 mM konsantrasyonlarda sırasıyla % 82.95, % 66.93 ve %42.63 inhibisyon oranları ile en etkili türev olduğu belirlenmiştir. Enzim kinetik çalışmaları sonucunda 2b kodlu bileşiğin non-kompetitif tip inhibisyon gösterdiği tespit edilmiştir.

Sonuç: Hiçbir bileşik standart ilaçlar donepezil ve takrin kadar etki göstermemiştir. Ancak 2b ve 2c kodlu bileşikler AChE enzimine karşı seçici ve umut verici düzeyde inhibisyon sergilemiştir.

Anahtar kelimeler: Piperazin, AChE, BChE, inhibisyon.

INTRODUCTION

Alzheimer's disease (AD) as an age-related neurodegenerative disorder that damages memory and cognition of the patient. AD is the most common and the most prevalent reason of dementia which occurs with ageing. It is responsible for 50% cases of dementia in elderly patients over 65 years of age. AD is a progressive disease that affects to perform ability of daily activities as communication, discernment and learning[1-5]. Decrease in the functional capacity results in death, approximately 8-10 years after the beginning of the symptoms[6].

A dysfunction of acetylcholine (ACh) containing neurons in some areas of the brain such as cortex and hippocampus is related to the deficits in memory and cognitive function in AD. The synaptic cholinergic transmission is regulated by acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes that rapidly

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hydrolyse acetylcholine (ACh)[7]. AChE shows more hydrolytic activity than BChE does. Hence, AChE inhibitors are preferred in the to prolong the duration of action of acetylcholine (ACh) and render symptomatic relief in AD[8-12]. The use of AChE inhibitors as anti-Alzheimer drugs has beneficial effects on cognitive, functional and behavioral symptoms of the disease[2,13]. Present therapies for AD mainly focus on the use of FDA accepted AChE inhibitors, i.e. donepezil, rivastigmine, galantamine, tacrine. These medications are counted as solely symptomatic. Thus there is a need to find more efficient agents to stop the disease progression[14].

Piperazine ring possesses two tertiary nitrogen elements that act as proton acceptor. Thus, nitrogen elements convert to quaternary form and can interact with anionic site of AChE by electrostatic attraction. Due to this property of piperazine it is usually sited into chemical structure of new inhibitor candidates of AChE[15-21].

In recent studies, the importance of piperazine ring system has been emphasised. For instance, it was found out that the existence of benzyl piperazine moiety in the compounds provide inhibitor effect thanks to interaction with AChE's catalytic site[22]. In another assay, it was indicated that piperazine derivatives were more effective than other heterocyclic compounds on AChE activity[23].

In consideration of above referred observations we synthesized a new series of piperazine derivatives and investigated their BuChE and AChE inhibitory activity in order to acquire new biologically active compounds.

MATERIALS AND METHODS

The chemicals used in the syntheses were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). 1H NMR and 13C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d6, respectively.

HRMS studies were performed on Shimadzu LCMS-IT-TOF system (Shimadzu, Tokyo, Japan). The purities of compounds were checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

Preparation of 4'-[4-[2-(dimethylamino) ethyl]-piperazine-1-yl]acetophenone (1)

4-Fluoroacetophenone (10 mmol, 1,214 mL), K2CO3 (10 mmol, 1,38 g), 1-[2-(dimethylamino)ethyl]piperzine (20 mmol), and DMF (5 mL) were added into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 200 ∘C and 10 bars for 15 min. After the control of reaction by TLC, the mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

General synthesis procedure for 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-(4-substitutedphenyl)prop-2-en-1-one derivatives (2a-2l)

The compound 1 (10 mmol), appropriate benzaldehyde derivative (10 mmol) and potassium hydroxide (10 mmol) in methanol (10 mL) was stirred at room temperature for 12 h. After TLC screening, the resulting solid was filtered, washed with water, dried, and recrystallized from ethanol. Isolated compounds were subjected to spectral analyses for structure conformation. The following spectral data for 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-phenyl prop-2-en-1-one (2a) was observed and presented as an example: 1H-NMR (300 MHz, DMSO-d6): δ = 2.13 (6H, s, -CH3), 2.35-2.37 (2H, m, -CH2-), 2.40-2.43 (2H, m, -CH2-), 2.53 (4H, br. s, piperazine -CH2-), 3.32 (4H, br. s, piperazine -CH2-), 7.00 (2H, d, J= 9.00 Hz, 1,4-disubstituted benzene -CH-), 7.43-7.45 (3H, m, monosubstituted benzene -CH-), 7.66 (1H, d, J= 15.60 Hz, ethylene -CH-), 7.84-7.87 (2H, m, monosubstituted benzene -CH-), 7.91 (1H, d, J= 15.60 Hz, ethylene -CH-), 8.05 (2H, d, J= 9.00 Hz, 1,4-disubstituted benzene). 13C-NMR (75 MHz, DMSO-d6): δ = 46.03, 46.98, 53.25, 56.24, 57.12, 113.55, 122.67, 127.44, 129.10, 129.33, 130.64, 131.07, 135.48, 142.58, 154.37, 186.88. HRMS (m/z): [M+H]+ calcd for C23H29N3O: 364.2383; found 364.2393.

AChE and BuChE inhibitory activity

Acetylcholinesterase (AChE, E.C.3.1.1.7, from electric eel), butyrylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), 5,5'-dithiobis-(2-nitrobenzoic acide) (DTNB), donepezil

hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Acetylthiocholine iodide (ATC) and butyrylthiocholine iodide (BTC) were obtained from Fluka (Germany). All pipetting processes were performed using a Biotek Precision XS robotic system (USA). Measurements of the percentage inhibition were carried out at 412 nm by using a BioTek-Synergy H1 microplate reader (USA). The inhibitory activities of the compounds against AChE and BChE were determined in 96-well plates by modified Ellman's method24 using donepezil as a reference drug. The synthesized compounds (2a-2l) were prepared at three concentrations (1 mM, 0.01 mM and 0.001 mM) using 2% DMSO and inhibition potencies were measured.

Enzymatic assay

The final volume of a well was 210 µL consisting of 140 µL phosphate buffer (0.1 M, pH=8), 20 µL inhibitor solution, 20 µL enzyme solution (2.5 U/mL), 20 µL DTNB (0.01 M) and 10 µL substrate solution (0.075 M ATC or BTC). First of all, the solutions of inhibitor, enzyme and DTNB were added to phosphate buffer and incubated at 25°C for 15 min.

After the incubation, the substrate (ATC or BTC) was added to the enzyme-inhibitor mixture. The production of the yellow anion (5-thio-2-nitrobenzoic acid) was recorded for 5 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor was processed. Control and inhibitor readings were corrected with blank-readings.

Enzyme kinetics

Kinetics studies were performed by using Ellman's method[24]. The most active compounds 2b was tested at 100 µM, 50 µM and 25 µM concentrations. The solution of phosphate buffer (0.1 M, pH=8, 140 µL/well), inhibitor (20 µL/well), enzyme (2.5 U/mL, 20 µL/well) and DTNB (0.01 mM, 20 µL/well) were added to the wells and incubated at 25°C for 15 min.

After incubation period, the solutions including various concentrations (600, 300, 150, 75, 37.5 and 18.75 µM) of substrate (ATC) (10 µL/well) were added. The increase of the absorbance was recorded for 5 min at 412 nm. A parallel experiment was carried out without inhibitor.

Statistical analysis

In both enzymatic assay and kinetic studies, each concentration was analysed in quadruplicate. The inhibition rate (%) was calculated by the following equation:

Inhibition % = [(AC-AB) – (AI-AB)] / (AC-AB) 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. Data were expressed as mean ± standard deviation (SD). In the enzyme kinetics the results were analysed as Lineweaver-Burk plots. The Ki value of compound 2b were calculated from second plot with a common intercept on the x-axis (corresponding to −Ki). Microsoft Excel 2013 were used for the statistical and graphical evaluations.

RESULTS

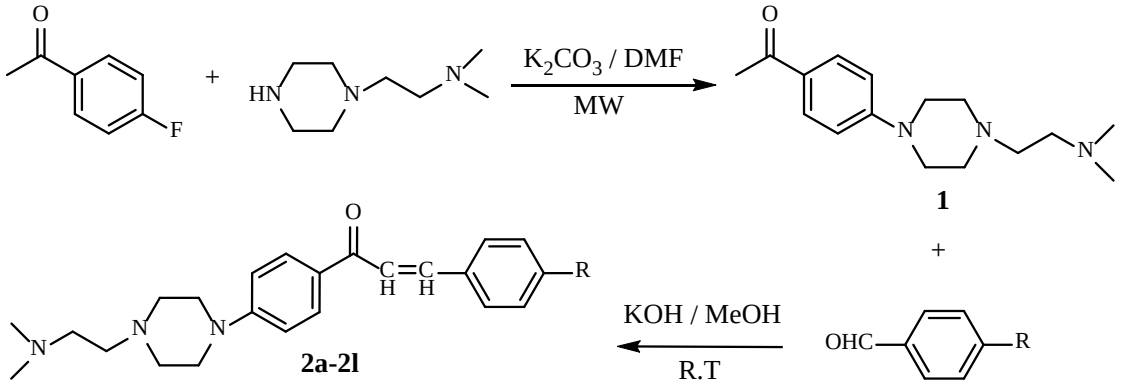
In this work, ten novel 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-(4-substitutedphenyl)prop-2-en-1-one derivatives (2a-2l) derivatives were synthesized as outlined in the Scheme 1. Structure confirmations of the obtained compounds were performed by spectroscopic analyses.

The anticholinesterase effects of the compounds 2a-2l against AChE and BChE enyzmes were determined by Ellman's spectrophotometric method[24] (Table 1). Among these compounds, compound 2b including chloro substituent was found as the most active compound with 82.95 %, 66.93 % and 42.63 % inhibition rates at 1 mM, 0.1 mM and 0.01 mM concentrations, respectively. Also compound 2c containing fluoro substituent showed 79.65 % 58.95 %, 38.26 % inhibition at concentrations of 1 mM, 0.1 mM and 0.01 mM, respectively. The compounds 2j, 2g and 2i indicated moderate AChE inhibitory activity with 66.82-67.46% inhibition values at 1 mM concentration. On the other hand, all compounds found to be inactive against BChE.

The mechanism of AChE inhibition was investigated via enzyme kinetics by using the Ellman's spectrophotometric method[24]. Linear Lineweaver-Burk graphics were used to observe the type of inhibition. Further, we analyzed the enzyme

kinetics by recording substrate-velocity curves in the absence and presence of the most potent compound 2b (0.1 mM, 0.05 mM and 0.025 mM). In each case, initial velocity measurements were obtained at different substrate (ATC) concentrations ranging from 600 μM to 18.75 μM.

The Ki (intercept on the x-axis) value of compound 2b was calculated from the secondary plot of the 1/V versus concentrations of compounds. The graphical analysis of steady-state inhibition data for compound 2b is shown in Figure 1. It has been determined that compound 2b is a non-competitive inhibitor of AChE. Ki value for compound 2b was calculated as 0.225 mM, which is about 100 fold higher than reported Ki value (2.3 μM) of donepezil[25].



Scheme 1. Synthesis way for 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-(4-substitutedphenyl)prop-2-en-1-one derivatives (2a-2l).

Table 1. Anticholinesterase activity and selectivity of the compounds (2a-2l) towards AChE and BChE.

Compound	R	AChE Inhibition %			BChE Inhibition %			Selectivity
		10^{-3} M	10^{-4} M	10^{-5} M	10^{-3} M	10^{-4} M	10^{-5} M	
2a	-H	46.49 ±1.53	18.98 ±0.76	8.74 ±0.52	26.05 ±1.32	9.08 ±0.27	4.24 ±0.16	AChE
2b	-Cl	82.95 ±3.36	66.93 ±2.25	42.63 ±1.83	8.09 ±0.47	7.63 ±0.19	5.91 ±0.31	AChE
2c	-F	79.65 ±4.08	58.95 ±2.11	38.26 ±1.66	8.47 ±0.221.	3.45 ±0.13	1.25 ±0.07	AChE
2d	-Br	18.43 ±1.19	4.75 ±0.33	2.65 ±0.16	15.10±0.87	7.55 ±0.43	1.85 ±0.12	AChE
2e	$-CF_3$	30.67 ±0.94	16.23 ±0.98	9.52 ±0.66	22.32 ±1.14	13.35 ±0.17	6.21 ±0.09	AChE
2f	$-CH_3$	41.12 ±1.24	17.28 ±1.14	10.75 ±1.23	29.67 ±1.83	14.55 ±0.70	8.61 ±0.21	AChE
2g	$-OCH_3$	67.46 ±2.65	37.34 ±1.53	19.47 ±1.31	38.09 ±1.12	16.98 ±0.83	9.14 ±0.66	AChE
2h	$-OC_2H_5$	34.96 ±1.65	16.99 ±0.88	9.90 ±0.74	22.94 ±1.07	11.27 ±0.65	4.23 ±0.45	AChE
2i	$-NO_2$	67.24 ±1.25	48.63±1.14	28.64 ±0.82	47.44 ±1.21	16.15 ±0.27	10.08 ±0.63	AChE
2j	-CN	66.82±2.16	44.94 ±1.27	22.45 ±0.80	30.09 ±1.18	18.67 ±0.77	13.95 ±0.49	AChE
Donepezil	-	99.52 ±3.68	98.46 ±2.89	96.24 ±3.29	78.87 ±1.72	69.70 ±1.49	56.65 ±1.88	AChE

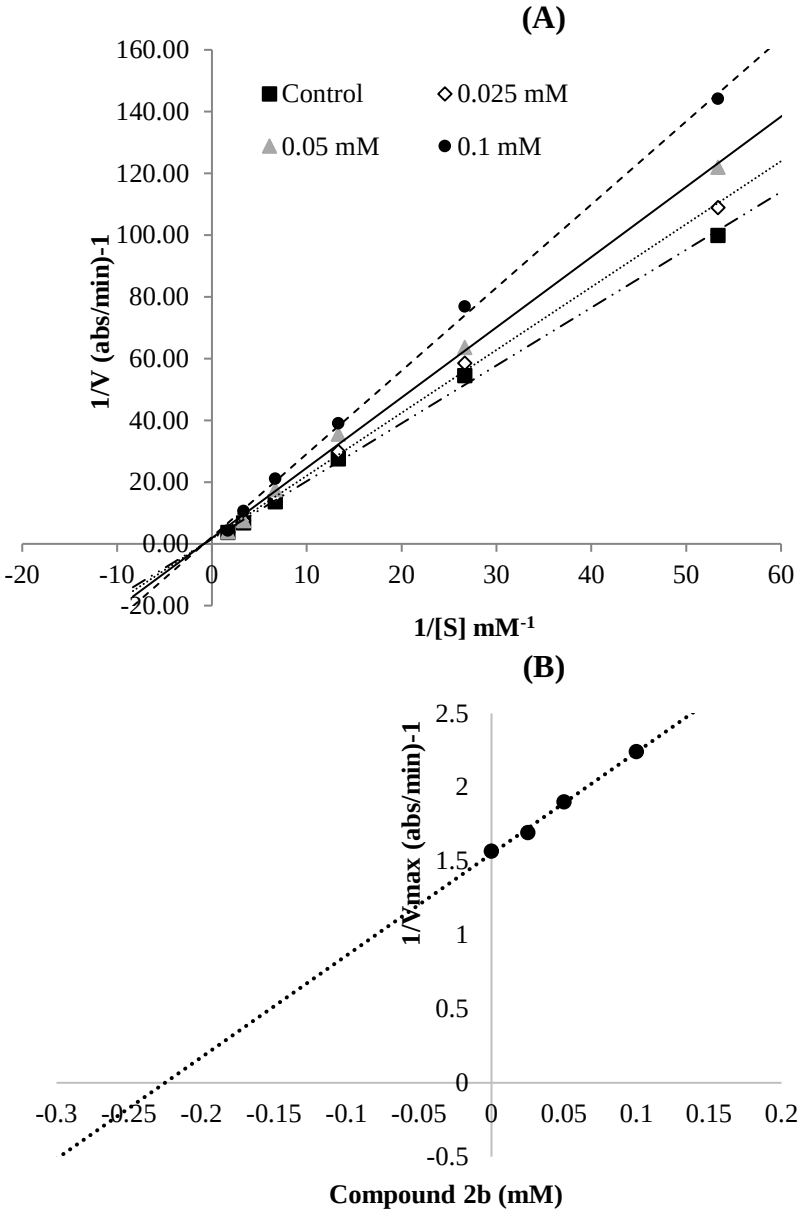


Figure 1. (A): Lineweaver-Burk plot for the inhibition of AChE (E.C.3.1.1.7, from electric eel) by compound 2b at different concentrations of substrate (ATC). Inhibitor concentrations are shown at the left. V_{max} values for 0.1 mM, 0.05 mM, 0.025 mM and control were calculated as 0.45, 0.53, 0.255, 0.59 and 0.64 (abs/min). K_m value of the non-competitive inhibition; 1.20 (mM). (B): Secondary plot for calculation of steady-state inhibition constant (K_i) of compound 2b. K_i was calculated as 0.225 mM.

DISCUSSION

Enzyme inhibition studies revealed that none of the compounds were as active as donepezil, used as a reference agent. Besides, it was determined that synthesized compounds have selectivity towards AChE enzyme. This finding may be explained with structural similarity between synthesized compounds

and donepezil, which is also selective inhibitor of AChE. It has been reported that AChE inhibitors contain a basic centre, a core ring system and a linker such as -O-, -CH2-, -CONH-, -CONH(CH2)n- etc. between core ring and basic centres26-28. Chemical structures donepezil fits well to such definition. It contains 1-indanone as a core ring, methylene as a linker and piperidine as a basic centre. Similarly, synthesized compounds (2a-2l) contain phenyl, ethylene and dimethylamino moieties as a core ring, linker and basic centre respectively. However, apart from donepezil in the synthesized compounds there is a second aromatic system (4-substitutedbenzylidene), which probably causes a decrease in enzyme inhibition, Therefore, it can be suggested for further studies that synthesis of new compounds, which do not include second aromatic ring may have more potent against AChE. On the other, hand, promising AChE inhibition potencies of compounds 2b and 2c indicated the positive contribution of chloro and fluoro substituents to pharmacological activity. Thus, incorporation of these substituents in the chemical structures of new compounds may cause beneficial contribution to AChE inhibition.

In the current work, enzyme kinetics of compound 2b was also investigated. The Lineweaver–Burk plot introduces the inhibition type as mixed-type, competitive or non-competitive. In the mix-typed inhibition, the lines cross neither x- nor y-axis at the same point. Competitive inhibitors possess the same intercept on y-axis but there are diverse slopes and intercepts on x-axis between the two data sets. Non-competitive inhibition has plots with the same intercept on x-axis but there are different slopes and intercepts on y-axis18, which is observed in Figure 1. Therefore, this pattern indicates that the mechanism of AChE inhibition of 2b is non-competitive, explaining that the inhibitor can bind to either the free enzyme or the enzyme–substrate complex.

In conclusion, a new series of 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-(4-substitutedphenyl)prop-2-en-1-one derivatives (2a-2l) derivatives were examined for their anticholinesterase activities. None of the compounds showed inhibitory activity as much as standard drug donepezil. However, compound 2b and 2c displayed a promising and selective inhibitory activity against AChE. Thus, results of the present study not only direct us to synthesize new compounds but also have an impact on researchers to obtain similar derivatives that may have an higher inhibition potency against AChE.

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