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# Synthesis and Biological Evaluation of Some New Benzimidazole Derivatives Bearing Dithiocarbamate Moiety as Potential Cholinesterase Inhibitors

## Ditiyiokarbamat Yapısı İçeren Yeni Bazı Benzimidazol Türevlerinin Sentezi ve Potansiyel Kolinesteraz İnhibitörleri olarak Biyolojik Etki Çalışmaları

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### ABSTRACT

**Purpose:**The synthesis of eight new benzimidazole derivatives bearing dithiocarbamate moiety and subsequently their anticholinesterase activity evaluations were aimed in this present study.

**Material and Methods:** 2-Bromoacetylbenzimidazole react with dithiocarbamate salt derivatives and the resulted compounds were elucidated by 1H NMR, mass spectral data, and elemental analyses. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) in vitro by using a modification of Ellman's spectrophotometric method.

**Results:**The new compound 1-methyl-2{2'[(N,Ndimethylaminothiocarbamoylthio)-acetyl]}benzimidazole (2b) can be identified as promising anticholinesterase agent in vitro due to its moderate inhibition effect, which is about 37%, when compared with standard substance Donepezil under the same experimental conditions.

**Conclusion:**In comparing with the reference standard, among the synthesized eight new compounds the only compound namely 1-methyl-2{2'[(N,Ndimethylaminothiocarbamoyl-thio)-acetyl]}benzimidazole (2b) has a promising anticholinesterase activity.

**Key Words:**Benzimidazole, Dithiocarbamate, Anticholinesterase Activity.

### ÖZET

**Amaç:** Bu çalışmada, ditiyokarbamat yapısı içeren benzimidazol türevlerinden sekiz yeni madde sentezlenmiş olup, in vitro koşullarda antikolinesteraz aktivitelerinin değerlendirilmesi amaçlanmıştır.

**Materyal ve Metod:** 2-Bromoasetilbenzimidazol ve ditiyokarbamat türevlerinin reaksiyonu sonucu sentezlenen bileşiklerin yapıları 1H-NMR, kütle spektral verileri ayrıca elemental analiz ile aydınlatılmıştır. Ayrıca yapıları tayin edilmiş bileşiklerin Elmann's spektrofotometrik yöntemiyle asetilkolin esteraz etkileri değerlendirilmiştir.

**Bulgular:** Tüm bileşikler arasında 1-metil-2{2'[N,Ndimetilaminokarbamoyl-tio)asetil]}benzimidazol (2b) maddesi kayda değer in vitro antikolinesteraz aktivite göstermiştir. Referans madde olarak kullanılan Donepezil ile aynı koşullarda kıyaslandığında, 2b maddesi %37 oranında inhibe ettiği görülmüştür.

**Sonuç:** Sentezlenen sekiz yeni bileşikten sadece (1-metil-2{2'[N,Ndimetilaminokarbamoyl-tio)asetil]}benzimidazol (2b) maddesi Donepezil ile kıyaslandığında, kayda değer in vitro antikolinesteraz aktiviteye sahip olduğu tespit edilmiştir.

**Anahtar Kelimeler:** Benzimidazol, Ditiokarbamat, Antikolinesteraz Aktivite.

## INTRODUCTION

Alzheimer's is the most common form of dementia, a general term for memory loss and it is serious enough to interfere with daily life. Alzheimer's disease affecting about 10% and the majority of people with Alzheimer's are 65 and over in age[1]. Thus it was proposed that degeneration of cholinergic neurons and the associated loss of cholinergic neurotransmission level contributed significantly to the deterioration in cognitive function seen in patients with Alzheimer's disease[2,3]. There are many other mechanisms including oxidative stress, inflammation and apoptosis, results in neuron loss, which affects acetylcholine (ACh) release. It becomes more difficult to maintain nerve impulses and the transmission of information at low neurotransmitter levels[4,5]. In addition to cholinergic dysfunction, other theories strongly correlates between dementia and β-amyloid deposition, oxidative stress and inflammation have been investigated in the etiology of AD[6].So, the treatment strategies in AD and a fundamental goal, is to treat the cholinergic dysfunction and this is become possible therapeutic approaches. Two different cholinesterase (ChE) enzymes are present in the human brain: acetylcholinesterase, and butrylcholinesterase. AChE is present at cholinergic nerve terminals whereas BuChE is associated with glial cells or neurons. Although AChE comprises 90% of the total ChE in the temporal cortex of normal brain and mediates the inactivation of most synaptic ACh, there is increasing recognition that BuChE may also be involved in hydrolysis of ACh and play an important role in AD[7].Everyone is looking for new treatments to alter the course of the disease and improve the quality of life for people with AD and the most well-known class is carbamates as a powerful anticholinesterase drugs. Rivastigmine possesses a carbamate moiety that resembles the ester linkage of acetylcholine. It is one of the most widely used anticholinesterase agents for the treatment of Alzheimer's disease[8-16]. Since dithiocarbamates are important pharmacophores due to their lipophilic property, which is critical for the delivery of central nervous system drugs to their site of action through the blood-brain barrier they become an important moiety in drugs which are using for the same purpose. There are lots of drug trials happening all the time to look for new medications, which might help in the treatment of Alzheimer's disease. Currently, dithiocarbamates extensively studied due to the fact that new and effective compounds can be obtained by the bioisosteric replacement of a carbamate with a dithiocarbamate moiety[17–21]. In addition it cannot overlook that benzimidazole ring processes a remarkably anticholinesterase activity[22-25]

In this present study, we have synthesized new dithiocarbamates using 2-bromoacetylbenzimidazoles for the acetylcholine esterase inhibition evaluation.

## MATERIAL and METHOD

All melting points (m.p.) of the synthesized compounds were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-UK), which are given in Table 1. The purity of the compounds were routinely checked by thin layer chromatography (TLC) using silica gel (60G, Merck, Darmstadt-Germany). Spectroscopic data were recorded by the following instruments 1H-NMR: Bruker 250 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO-d6 using TMS as internal standard; and MS data were obtained by MS-FAB: VG Quattro Mass spectrometer (Agilent, Minnesota, USA).

**Table1. Some characteristics of the compounds**

| Compound | Ring | R | Yield (%) | M.P. ($^0$C) | Molecular formula | Molecular weight |
|---|---|---|---|---|---|---|
| **2a** | Thiomorpholinyl | H | 65 | 105 | $C_{15}H_{17}N_3OS_3$ | 351,51 |
| **2b** | - | dimethylamino | 70 | 93 | $C_{13}H_{15}N_3OS_2$ | 293,41 |
| **2c** | Pyrrolidinyl | H | 62 | 90 | $C_{15}H_{17}N_3OS_2$ | 319,45 |
| **2d** | piperidinyl | H | 58 | 92 | $C_{16}H_{19}N_3OS_2$ | 333,48 |
| **2e** | Piperidinyl | 4-methyl | 45 | 98 | $C_{17}H_{21}N_3OS_2$ | 347,50 |
| **2f** | Piperidinyl | 3-benzyl | 54 | 87 | $C_{23}H_{25}N_3OS_2$ | 423,60 |
| **2g** | Piperazinyl | 4-fluorophenyl | 60 | 83 | $C_{21}H_{21}FN_4OS_2$ | 428,55 |
| **2h** | Piperazinyl | 4-nitrophenyl | 57 | 126 | $C_{21}H21N_5O_3S_2$ | 455,56 |

**Chemistry**

General procedure for the synthesis of the compounds:

*Sodium salts of N, N-disubstituted dithiocarbamic acids* **(1a–h)**

Sodium hydroxide (10 mmol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (10 mmol) the mixture was cooled in an ice bath and carbon disulfide (100 mmol) was added drop wise with stirring. The reaction mixture was stirred for 1 h at room temperature. The products were afforded by filtration and washed with diethyl ether 26.

*Dithiocarbamate derivatives* **(2a–h):**

A mixture of 1-methyl-2-(2'bromoacetyl)benzimidazole (0.01 mol) and appropriate sodium salts of N,N-disubstituted dithiocarbamic acids (0.01 mol) was treated with acetone (15 mL) at room temperature for 6 h. The solvent was evaporated, the resulting solid was washed with water, and recrystallized from ethanol.

*1-Methyl-2-[(4-thiomorpholine)thiocarbamoylthio)acetyl]benzimidazole **(2a):***

1H NMR (250 MHz) δ (ppm) (DMSO-d6): 3,52(3H, s, CH3) 2.62 (4H, t, C3 and C5 protons of thiomorpholine), 4.20-4.50 (4H, two brs, C2 and C6 protons of thiomorpholine), 4.70 (2H, s, COCH2), 7.20 - 7.48 (4H, m, aromatic protons). MS (FAB); m/z: 352 [M+1]

**Biological Evaluation**

**AChE Inhibition**

All compounds were subjected to a modified method of Ellman's test 27 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, 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 (2a-2h) were 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 28.

**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 10-1-10-6 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 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 % = (AC–AI) / AC 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

The target compounds were prepared by two step reactions as shown in Scheme1.Various Sodium salts of N,N-disubstituted dithiocarbamic acids were reacted with 2(2'bromoacetyl)benzimidazole deravatives in acetone to yield the titled compounds **(2a-2g),** respectively. The yields of individual compounds in this series ranged from 45 % to 70 %. In the 1H-NMR spectra protons were recorded at estimated areas. The mass spectra of the compound showed [M+1] peaks, in agreement with their molecular weight.

The anticholinesterase effects of the compounds **(2a-2h)** were determined by modified Ellman's spectrophotometric method. The IC50 values could not be defined of the tested compounds and were reported by comparing with standard drug Donepezil. % AChE inhibition values of the tested compounds and control reference substance are summarized in Table 2.

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

| Comp. | AChE Inhibition (%) | | |
|---|---|---|---|
| | 1 mM | 0.1 mM | $IC_{50}$ (mM) |
| **2a** | 15,69±0,48 | 11,98±2,32 | > 1 |
| **2b** | 37,08±2,03 | 27,53±2,83 | > 1 |
| **2c** | 5,01±1,29 | 2,44±2,06 | > 1 |
| **2d** | 6,31±1.98 | 2,35±2,56 | > 1 |
| **2e** | 3,91±3,60 | 2,96±3,07 | > 1 |
| **f** | 15,66±0,45 | 11,04±2,34 | > 1 |
| **2g** | 21,80±2,54 | 9,39±3,05 | > 1 |
| **2h** | 4.72±2,56 | 2.91±2,22 | > 1 |
| **Donepezil** | 99,01±4,89 | 95,52±5.01 | 0.054±0.002 (µM) |

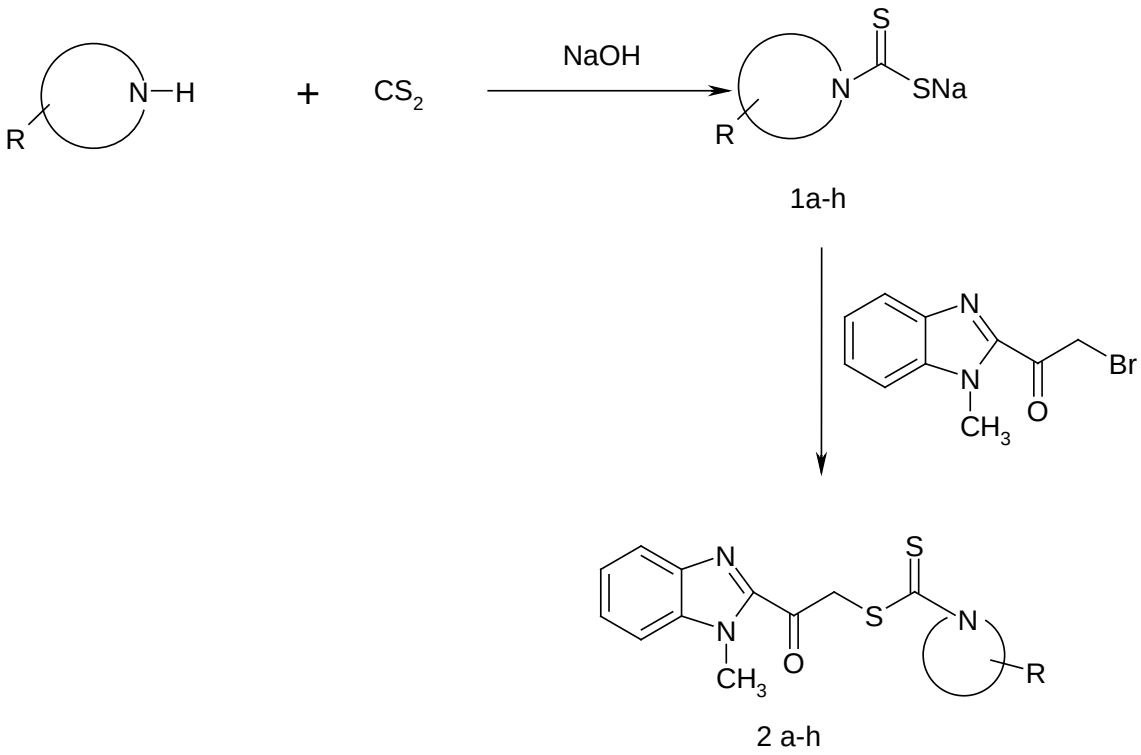
## DISCUSSION

Among these compounds **(2a-2h),** a compound **2b** with dimethylamino substitution and compound **2g** with 4-fluoro phenyl piperidine substitution were found as the most active compounds. The inhibition percentages were calculated and they are **37.08** and **27.53** % at 1 and 0.1 mM concentrations for compound **2b** and 21.80 and 9.39 % at 1 and 0.1 mM concentrations for compound **2g**. The IC50 values could not be defined well in all compounds. However, inhibition percentages was not be determined for compound **2c.** Compound **2a** bearing thiomorpholinyl moiety

and compound **2f** bearing 3-benzylpiperidine moiety exhibited anticholinesterase activity with nearly 16 % inhibition value. The other compounds **2c, 2d, 2e** and **2h** showed relatively weak activity and the inhibiton values were found to be less than **6.31** %. A standard drug Donepezil was studied at lower concentrations for the purpose of finding the inhibition percentage and it was determined as **95%.** None of the synthesized compounds showed comparable anticholinesterase activity with Donepezil in contrary to our expectations.

## CONCLUSION

In this study we report the original synthesis, structural elucidation and anticholinesterase activities of eight new benzimidazole derivatives bearing dithiocarbamate moiety. The structure elucidation of the synthesized compound are fully supported by spectroscopic data. Additionally, all synthesized compounds were evaluated for their anticholinesterase activity. All compounds were showed anticholinesterase activity but the compound 2b which include aliphatic amine substituent showed the significant anticholinesterase activity. This outcome confirms that aliphatic amine substituent may has a considerable influence on anticholinesterase activity.




**Scheme 1**. The general synthesis route towards 2a-h

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