ANADOLU UNIVERSITY

European Journal of Life Sciences 2023; 2: 71-77

Research Article

Screening of new morpholine dithiocarbamate derivatives bearing benzimidazole moiety for anticholinesterase potential

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https://doi.org/10.55971/EJLS.1328405

Received: 17.07.2023
Accepted: 23.08.2023
Available online: 30.10.2023

ABSTRACT

Alzheimer's disease (AD) is basically associated with disturbances of cholinesterase metabolism which result in acetylcholine deficiency. Target of acetylcholinesterase (AChE) inhibitors used in symptomatic therapy of disease is to increase of ACh levels. Consequently, cholinesterase inhibitors were developed to increase acetylcholine is to inhibit AChE and butrylcholinesterase (BuChE). Studies demonstrate the clinical importance of dual inhibitors that inhibit not only the acetylcholinesterase enzyme but also the butyrylcholinesterase enzyme.

In recent years, benzimidazoles have attracted particular interest owing to their anticolinesterase activity. In this manner, we have synthesized benzimidazole and morpholine including compounds (**2a-i**). Final compounds were achieved with the reaction of (benzimidazol-2-yl) methyl morpholine-4-carbodithioate and α-bromoacetophenone derivatives in acetone at room temperature with stirring. Inhibition effects of novel morpholine dithiocarbamates (**2a-i**) were tested on AChE and BuChE. Compound **2d** demonstrated dual inhibitory activity on AChE and BuChE (78±1,56, 70,71±1,53, respectively), with the lowest cytotoxicity to normal cell line.

Keywords: Acetylcholinesterase, butrylcholinesterase, Alzheimer, benzimidazole

1. INTRODUCTION

Alzheimer's-type dementia (ATD) is characterized by behavioural disturbances and mood changes associated with progressive cognitive and memory loss. Extracellular deposition of the Aβ peptide in senile plaques is the main marker of the disease. Aβ can trigger neuronal cell death via oxidative stress [1]. Loss of neuron has been linked to a deficiency in brain, neuromediator acetylcholine. With less acetylcholine, it becomes more diffucult to maintain transmission of information and nerve signals between neurons. Consequently, AChE inhibitors (AChEIs) were developed to increase acetylcholine by inhibiting the enzyme AChE [2]. AChEIs including tacrin (hepatotoxic in 25% patients), donepezil, rivastigmine and galantamin has been approved for the treatment of moderate to severe Alzheimer's Disease (AD) [3]. Data obtained from clinical trials showed that rivastigmine 6-12 mg per day produces improvements in cognition, daily activities, and global evaluation rating in patients with mild to moderate AD. Effects of rivastigmine are dose dependent [4,5]. Rivastigmine is a pseudoirreversible, second-generation carbamate-based, noncompetitive AChE and BuChE inhibitor with equal potency. Both of enzymes are responsible for hydrolysis of ACh [6,7]. Two different cholinesterases exist in mammals: AChE, which can selectively hydrolyze ACh, and BuChE, which can hydrolyze both ACh and other

choline esters [2,8,9]. The peptidase or protease of the butyrylcholinesterase enzyme activity has a role in the pathological processes of AD. BuChE causes the production of amyloid protein and diffusion of the protein into ß-amyloid plaques in Alzheimer's disease [10]. Therefore, researchers working on this subject focused on the discovery of new dual cholinesterase inhibitor molecules to enhance cholinergic functions.

Benzimidazole scaffold is an important pharmacophore that has been extensively utilized as a drug in medicinal chemistry for years due to its high affinity towards various enzymes and receptors [11]. Among them, 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazoles have a particular interest as a result of studies which we have been reported before with satisfactory anticancer activity results [12,13]. Additionally, benzimidazoles have a broad range of pharmacological activities. In recent years, they have attracted particular interest due to their anticolinesterase activity [13-16]. Additionally, gefitinib, a recent morpholine carrying anticancer drug, is expected to play a significant role in designing and synthesizing new drugs [17].

In this present study, novel morpholine dithiocarbamate derivatives (**2a-i**) bearing 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazole moiety were synthesized and their potential anticholinesterase effects and cytotoxic properties against NIH/3T3 cells were investigated.

2. MATERIALS AND METHODS

2.1. Chemicals and Equipments

The chemicals and solvents used in the study were purchased from commercial suppliers. Electrothermal 9300 digital melting point apparatus (Essex, UK) Melting points (m.p.) was used for melting point detection. Spectroscopic analysis was realized with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan), NMR, Bruker 500 spectrometer (Billerica, MA, USA) in DMSO-d_6; M+1 peaks were detected by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA, USA) and Elemental analyses were worked on an elemental analyser (Perkin Elmer, Norwalk, CT, USA).

2.2. General procedure for the preparation of (1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (1)

2-(Chloromethyl)benzimidazole (0.05 mol) and potassium salt of morpholine dithiocarbamate (0.052 mol) were stirred in acetone for 5h. The reaction mixture was treated with excess water and precipitated raw intermediate product was filtered, later it was crystallised from ethanol [18].

2.3. General procedure for the synthesis of [1-(2-oxo-2-(4-substitutedphenyl)ethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate derivatives (2a-i)

The yellow intermediate product (**1**) was reacted with appropriate α-bromoacetophenone derivatives with the presence of potassium carbonate in acetone. After the reaction mixture was stirred at room temperature for 3 hours, it was collapsed with water. The products (**2a-i**) were given by filtration and then crytallisation from ethanol.

2.4.1. [1-(2-Oxo-2-phenylethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate (2a)

Yield : 67%. M.P. 167-169 °C. IR (cm^{-1}) : 1680 (C=O), 1595-1425 (C=C, C=N), 1269-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.57 (brs, 4H, NCH_2), 3.83 (brs, 2H, OCH_2), 4.11 (brs, 2H, OCH_2), 4.89 (s, 2H, SCH_2), 6.10 (s, 2H, $COCH_2$), 7.21-7.23 (m, 3H, Ar-H), 7.50-7.52 (m, 1H, Ar-H), 7.63-7.66 (m, 3H, Ar-H), 7.77 (t, 1H, J:7.5 Hz, Ar-H), 8.13 (d, 1H, J:7.0 Hz, Ar-H). $C_{21}H_{21}N_3O_2S_2$ calculated: (%) C 61.29, H 5.14, N 10.21; found: (%) C 61.35, H 5.18, N 10.35. MS $[M+1]^+$: *m/z* 412.

2.4.2. [1-(2-Oxo-2-(4-methylphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2b)

Yield : 62%. M.P. 105-108 °C. IR (cm^{-1}) : 1684 (C=O), 1604-1423 (C=C, C=N), 1230-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 2.82 (s, 3H, CH_3), 3.59 (brs, 4H, NCH_2), 3.84 (brs, 2H,

OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.11 (s, 2H, $COCH_2$), 7.24-7.25 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.68-7.71 (m, 3H, Ar-H), 7.95 (t, 1H, J:7.5 Hz, Ar-H), 8.12 (d, 1H, J:7.0 Hz, Ar-H). $C_{22}H_{23}N_3O_2S_2$ calculated: (%) C 62.09, H 5.45, N 9.87; found: (%) C 62.15, H 5.34, N 9.95. MS $[M+1]^+$: *m/z* 426.

2.4.3. [1-(2-Oxo-2-(4-methoxyphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2c)

Yield : 68%. M.P. 179-181 °C. IR (cm^{-1}) : 1674 (C=O), 1597-1420 (C=C, C=N), 1265-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 4.13 (brs, 2H, OCH_2), 4.86 (s, 2H, SCH_2), 6.02 (s, 2H, $COCH_2$), 7.16 (d, 2H, J: 9.0 Hz, Ar-H), 7.20-7.22 (m, 2H, Ar-H), 7.47-7.49 (m, 2H, Ar-H), 7.61-7.63 (m, 1H, Ar-H), 8.09 (d, 1H, J:9.0 Hz, Ar-H). $C_{22}H_{23}N_3O_3S_2$ calculated: (%) C 59.84, H 5.25, N 9.52; found: (%) C 59.89, H 5.33, N 9.47. MS $[M+1]^+$: *m/z* 442.

2.4.4. [1-(2-Oxo-2-(4-chlorophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2d)

Yield : 69%. M.P. 165-170 °C. IR (cm^{-1}) : 1683 (C=O), 1589-1424 (C=C, C=N), 1269-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.12 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.21-7.23 (m, 2H, Ar-H), 7.50-7.52 (m, 1H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.73 (d, 2H, J:8.5 Hz, Ar-H), 8.13 (d, 2H, J:8.5 Hz, Ar-H). $C_{21}H_{20}ClN_3O_2S_2$ calculated: (%) C 56.55, H 4.52, N 9.42; found: (%) C 56.61, H 4.39, N 9.46. MS $[M+1]^+$: *m/z* 446.

2.4.5. [1-(2-Oxo-2-(4-florophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2e)

Yield : 68%. M.P. 198-201 °C. IR (cm^{-1}) : 1681 (C=O), 1593-1426 (C=C, C=N), 1254-989 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.21-7.22 (m, 2H, Ar-H), 7.48-7.52 (m, 1H, Ar-H), 7.62-7.64 (m, 3H, Ar-H), 8.20-8.22 (m, 2H, Ar-H). $C_{21}H_{20}FN_3O_2S_2$ calculated: (%) C 58.72, H 4.69, N 9.78; found: (%) C 58.66, H 4.75, N 9.71. MS $[M+1]^+$: *m/z* 430.

2.4.6. [1-(2-Oxo-2-(4-nitrophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2f)

Yield : 63%. M.P. 135-140 °C. IR (cm^{-1}) : 1679 (C=O), 1599-1423 (C=C, C=N), 1267-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.88 (brs, 2H, OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.22-7.28 (m, 2H, Ar-H), 7.49-7.54 (m, 3H, Ar-H), 7.63-7.65 (m, 1H, Ar-H), 8.21-8.24 (m, 2H, Ar-H). $C_{21}H_{20}N_4O_4S_2$ calculated: (%) C 55.25, H 4.42, N 12.27; found: (%) C 55.32, H 4.56, N 12.35. MS $[M+1]^+$: *m/z* 457.

2.4.7. [1-(2-Oxo-2-(3-methoxyphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2g)

Yield : 70%. M.P. 143-148 °C. IR (cm^{-1}) : 1677 (C=O), 1597-1422 (C=C, C=N), 1259-993 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.84 (brs, 2H, OCH_2), 3.97 (s, 3H, OCH_3), 4.13 (brs, 2H, OCH_2), 4.87 (s, 2H, SCH_2), 6.11 (s, 2H, $COCH_2$), 7.16 (d, 1H, J: 9.0 Hz, Ar-H), 7.22-7.24 (m, 1H, Ar-H), 7.53-7.55 (m, 1H, Ar-H), 7.58-7.60 (m, 1H, Ar-H), 7.72 (t, 1H, J:7.5 Hz, Ar-H), 7.87 (d, 1H, J:7.5 Hz, Ar-H), 8.06 (d, 1H, J:7.5 Hz, Ar-H), 8.14 (s, 1H, Ar-H). $C_{22}H_{23}N_3O_3S_2$ calculated: (%) C 59.84, H 5.25, N 9.52; found: (%) C 59.85, H 5.36, N 9.49. MS $[M+1]^+$: *m/z* 442.

2.4.8. [1-(2-Oxo-2-(3-chlorophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2h)

Yield : 75%. M.P. 102 °C (decomp). IR (cm^{-1}) : 1682 (C=O), 1572-1421 (C=C, C=N), 1255-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.12 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.12 (s, 2H, $COCH_2$), 7.20-7.22 (m, 2H, Ar-H), 7.52-7.53 (m, 1H, Ar-H), 7.61-7.63 (m, 1H, Ar-H), 7.68 (t, 1H, J:7.5 Hz, Ar-H), 7.84 (d, 1H, J:7.5 Hz, Ar-H), 8.05 (d, 1H,

J:7.5 Hz, Ar-H), 8.15 (s, 1H, Ar-H). $C_{21}H_{20}ClN_3O_2S_2$ calculated: (%) C 56.55, H 4.52, N 9.42; found: (%) C 56.64, H 4.57, N 9.49. MS $[M+1]^+$: *m/z* 446.

2.4.9. [1-(2-Oxo-2-(3-florophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2i)

Yield : 78%. M.P. 165-169 °C. IR (cm^{-1}) : 1680 (C=O), 1593-1423 (C=C, C=N), 1257-989 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.87 (brs, 2H, OCH_2), 4.15 (brs, 2H, OCH_2), 4.89 (s, 2H, SCH_2), 6.14 (s, 2H, $COCH_2$), 7.24-7.27 (m, 2H, Ar-H), 7.54-7.56 (m, 1H, Ar-H), 7.63-7.68 (m, 1H, Ar-H), 7.74 (t, 1H, J:7.5 Hz, Ar-H), 7.92 (d, 1H, J:7.5 Hz, Ar-H), 8.10 (d, 1H, J:7.5 Hz, Ar-H), 8.22 (s, 1H, Ar-H). $C_{21}H_{20}FN_3O_2S_2$ calculated: (%) C 58.72, H 4.69, N 9.78; found: (%) C 58.62, H 4.76, N 9.76. MS $[M+1]^+$: *m/z* 430.

2.5. Biochemistry

2.5.1. Determination of AChE and BuChE inhibitory potency

A modified Ellman's assay was used for evaluation of their ChE inhibitory activities [19]. Experiment was done in triplicate.

The inhibition (percent) of AChE or BuChE was calculated using the following equation.

$$I\ (\%) = 100-(OD_{sample} / OD_{control}) \times 100$$

2.5.2. Determination of cytotoxicity

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used to determine the cytotoxic effects of compounds **2a-i** on NIH/3T3 cells [20, 21].

3. RESULTS AND DISCUSSION

3.1. Chemistry

Target molecules (**2a-i**) were synthesized in two steps. Two starting materials, 2-(chloromethyl) benzimidazole and potassium salt of morpholine *N*-dithiocarbamic acid, were synthesized according to the previously reported literature [13,22]. The obtained intermediate product (**1**) was reacted in acetone to give [1-(2-aryl-2-oxoethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate (**2a-i**). IR, ^{1}H-NMR and MS spectroscopic analysis and elemental analysis were performed to confirm the structures of the compounds. In the IR spectra of the compounds, bands at 1684-1674, 1604-1420 and 1269-987 cm^{-1} were detected for C=O; C=C, C=N and C-O, C-N bonds, respectively. In the ^{1}H-NMR spectra of the compounds, peaks belong to piperazine ring were observed at 3.57-4.15 ppm range. Singlet peaks were determined for methylene protons at 4.86-4.89 and 6.02-6.14 ppm for S-CH_2 and $COCH_2$, respectively. All other aromatic protons were observed at estimated fields of the spectrum. MS spectroscopic data and elemental analysis data were confirmed the structures of the molecules with satisfactory results.

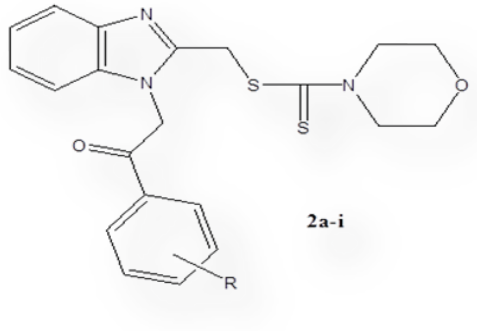
3.2. Evaluation of ChE inhibitory activity and cytotoxicity results

A colorimetric assay was employed to assess the inhibition effects of compounds **2a-i** on ChEs (Table 1). While compounds **2e** and **2d** displayed the highest inhibitory activity on AChE, compounds **2c** and **2d** showed the highest inhibition rates on BuChE, respectively In addition, compounds **2d** and **2e** exhibited the lowest cytotoxicity against normal (NIH/3T3) cells.

In recent years, results showing the anticholinesterase effect potential of various dithiocarbamate derivatives have been obtained and the importance of carbamate derivatives in the development of new drugs has been emphasized [23, 24].

When the structure-activity relationships of the compounds are examined, it is seen that the two derivatives containing 4-methyl (**2b**) and 4-chloro (**2d**) substituents exhibit high inhibition potential on both enzymes. In addition, compound **2e** bearing 4-fluoro substituent and compound **2c** including 4-methoxy substituent showed the highest inhibitory potential against AChE and BuChE, respectively. In the compounds, higher anticholinesterase activity was determined in the para-substituted derivatives compared to the meta-substituted derivatives.

Table 1. Cholinesterases inhibition (%) and cytotoxicity



Compound	AChE% inhibition (80 µg/mL)	BuChE %inhibition (80 µg/mL)	IC_{50}b
2a	33,93±0,11	29,69±1,01	69,0±5,29
2b	64,69±0,47	65,35±3,44	125,0±22,91
2c	15,06±2,30	80,51±1,58	125,67±8,14
2d	78±1,56	70,71±1,53	>200
2e	82,26±1,41	30,68±1,25	188,33±16,07
2f	33,9±4,06	49,95±1,12	128,33±20,21
2g	11,11±0,08	---	>200
2h	47,44±3,10	35,28±0,66	65,67±9,29
2i	57,76±1,08	27,50±2,60	68,33±15,28
Donepezil IC_{50}a	$3,76x10^{-3}$±$0,18x10^{-3}$	1,48±0,44	nt

---: not active; nt: non tested
a: The half maximal inhibitory concentration of the compounds to inhibit 50% of the indicated enzymes
b: The half maximal inhibitory concentration of the compounds to inhibit 50% of the mouse fibroblast cells (NIH/3T3)

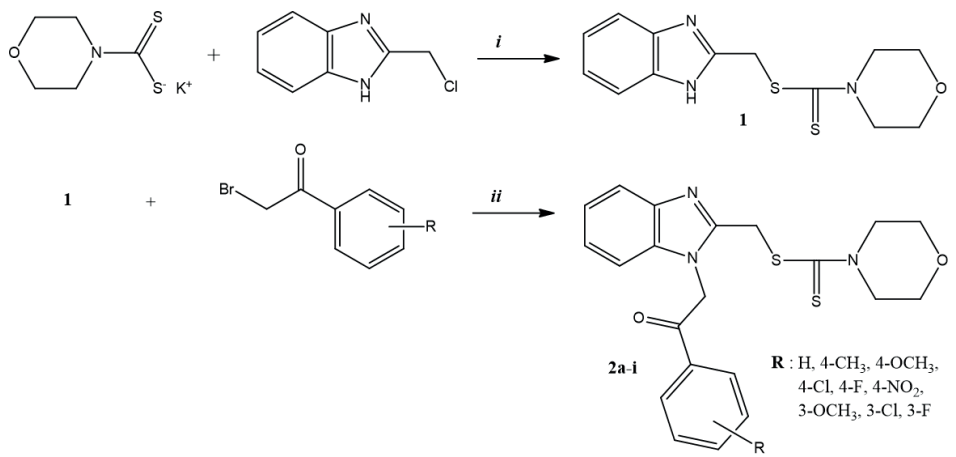


Figure 1. The synthesis of the compounds (**2a-2i**). Reagents and conditions : ***i*** : acetone, r.t., 5h; ***ii*** : K_2CO_3, acetone, 3h.

In addition, the lowest cytotoxicity against NIH/3T3 cell line was observed in the meta-substituted derivatives.

4. CONCLUSION

Compound **2d** may be a good drug candidate with either it's dual inhibitory effect on cholinesterases enzymes or with the lowest cytotoxicity to normal cell lines. This work could represent inhibition potential of morpholine dithiocarbamate derivatives bearing benzimidazole moiety on AChE and BuChE enzyme activity.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: HET, GAÇ, LY; Design: HET, GAÇ, LY; Supervision: HET, GAÇ, LY; Materials: HET, GAÇ, LY; Data Collection and/or Processing: HET, GAÇ, LY; Analysis and/or Interpretation: HET, GAÇ, LY; Literature Search: HET; Writing: HET; Critical Reviews: HET, GAÇ, LY.

Source of funding

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1404S116.

Conflict of interest

The authors declared that there is no conflict of interest.

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