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Original Article

BIOLOGICAL EVALUTION OF THIAZOLE DERIVATIVES BEARING DITHIOCARBAMATE MOIETY AS POTENTIAL CHOLINESTERASE INHIBITORS

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ABSTRACT

Objective: This study aimed to synthesize some thiazole derivatives bearing dithiocarbamic acid esters and to evaluate their anticholinesterase activity.

Methods: 2-Chloro-N-[4-(2-methyl-4-thiazolyl)phenyl]acetamide was stirred with appropriate sodium salts of dithiocarbamic acids in acetone. The resulted compounds were elucidated using IR, ^{1}H-NMR, and FAB$^+$-MS spectral data. Each derivative was evaluated for its ability to inhibit acetyl cholinesterase (AChE) in vitro by using a modification of Ellman's spectophotometric method.

Results: Two of the synthesized compounds **(6b,6c)** can be identified as promising anticholinesterase agent due to their inhibitory effect on ACEH with IC_{50} value of 86.34±1.31, 91.74±1.43 respectively when compared with standard substance Donepezil (IC_{50} =0.054±0.002µM) under the same experimental conditions.

Conclusion: dimethylaminoethyl/propyl substitution on Piperazine residue have a crucial influence on anticholinesterase activity.

Keywords: Thiazole, Dithiocarbamate, Cholinesterase inhibitors.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia (a general term for memory loss). It is serious enough to interfere with daily life. AD affects about 10% of the population and the majority of people with AD are 65 in age and above [1]. Thus, it was proposed that degeneration of cholinergic neurons and the association loss of cholinergic neurotransmission level contribute significantly to the deterioration in cognitive function seen in patients with Alzheimer's disease[2,3]. There are many other mechanisms such as oxidative stress, inflammation and apoptosis may result in neuron loss, and affect 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 correlate between dementia and β-amyloid deposition, oxidative stress and inflammation have been investigated in the etiology of AD [6].

So, the treatment strategies of AD and the fundamental goals, are to treat cholinergic dysfunction and to become possible therapeutic approaches. There are two different cholinesterase (ChE) enzymes in the human brain: acetylcholinesterase (AChE), and butrylcholinesterase (BuChE). 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]. So, researchers are looking for new treatments to alter the course of the disease and improve the quality of life for people with AD. The most well-known class is carbamate as a powerful anti cholinesterase 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 AD[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 the same purpose. Lots of drug trials are happening all the time to look for new medications, which might help in the treatment of AD. Currently, dithiocarbamates are extensively studied due to the fact that they are new and effective compounds and can be obtained by bio isosteric replacement of a carbamate with a dithiocarbamate moiety [17–21]. In addition, it cannot overlook that the thiazole ring processes a remarkably anticholinesterase activity [22-25].

Piperazine ring plays an important role for antiacetylcholinesterase activity [26-29]. On the basis of these findings and in the continuation of our ongoing research program, synthesis and investigation of acetylcholinesterase inhibitor activity of the 2-[[4-(2-Methyl-4-thiazolyl) phenyl] amino]-2-oxoethyl 4-substituted piperazine-1-carbodithioate derivatives (6a-6i*)* were reported in this study.

MATERIALS AND METHODS

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO). All melting points, (m. p.) Were determined by Electrothermal 9100 digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H-NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), [13]C-NMR Bruker DPX 125 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-d_6 using TMS as internal standard; M+1 peaks were determined by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA, USA).

General procedure for synthesis of compounds 2-[[4-(2-Methyl-4-thiazolyl) phenyl] amino]-2-oxoethyl 4-substitutedpi perazine-1-carbodithioate (6a-6i)

Compound **(5)** 2-Chloro-N-[4-(2-methyl-4-thiazolyl) phenyl] acetamide (0.001 mol) was stirred with appropriate sodium salts of dithiocarbamic acids (0.0011 mol) in acetone for 3 h. The precipitated product was filtered and washed with water.

2-[[4-(2-Methyl-4-thiazolyl) phenyl] amino]-2-oxoethyl 4-(2-hydroxyethyl) piperazine-1-carbodithioate (6a)

Yield: 82%. M. p. 157-158 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3312 (amide N-H), 1679 (amide C=O), 1310-1018 (C-N and C-O). ^{1}H NMR (500 MHz,

DMSO-d_6) δ 2.44 (t, *J*=6.10 Hz, 4H, piperazine CH_2), 2.70 (s, 3H, CH_3), 3.53 (q, 2H, *J*: 8.55 Hz, CH_2OH, 3.95 (brs, 2H, piperazine CH_2), 4.22 (brs, 2H, piperazine CH_2), 4.29 (s, 2H, CH_2S), 4.52 (t, *J*=5.20 Hz, 1H, OH), 7.66 (d, *J*=8.70 Hz, 2H, Ar-H), 7.79 (s, 1H, thiazole C_5-H), 7.88 (d, *J*=8.65 Hz, 2H, Ar-H), 10.39 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 39.87, 49.80, 52.51, 58.46, 59.46, 112.46, 119.14, 126.39, 129.39, 138.63, 153.52, 165.32, 165.36, 194.23. MS (ES^+): m/z 437.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-[2-(N,N-dimethylamino)ethyl]piperazine-1-carbodithioate (6b)

Yield: 78%. M. p. 110-112 °C. IR (KBr) ν_{max} (cm^{-1}): 3289 (amide N-H), 1676 (amide C=O), 1352-1018 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.35 (t, 2H, *J*=6.67 Hz, CH_2), 2.43 (t, 2H, *J*=6.70 Hz, CH_2), 2.44 (t, *J*=6.20 Hz, 4H, piperazine CH_2), 2.71 (s, 3H, CH_3), 3.41 (s, 6H, $N(CH_3)_2$), 3.94 (brs, 2H, piperazine CH_2), 4.20 (brs, 2H, piperazine CH_2), 4.28 (s, 2H, CH_2S), 7.65 (d, *J*=8.65 Hz, 2H, Ar-H), 7.80 (s, 1H, thiazole C_5-H), 7.88 (d, 2H, *J*=8.65 Hz, Ar-H), 10.39 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 39.88, 45.46, 49.76, 52.43, 55.0, 56.53, 112.46, 119.12, 126.39, 129.39, 138.63, 153.52, 165.29, 165.34, 194.23. MS (ES^+): m/z 464.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-[3-(N,N-dimethylamino)propyl]piperazine-1-carbodithioate (6c)

Yield: 81%. M: p. 153-154 °C. IR (KBr) ν_{max} (cm^{-1}): 3290 (amide N-H), 1677 (amide C=O), 1337-1025 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53-1.58 (m, 2H, CH_2), 2.20 (t, 2H, *J*=7.15 Hz, CH_2), 2.32 (t, 2H, *J*=7.35 Hz, CH_2), 2.45 (brs, 4H, piperazine CH_2), 2.71 (s, 3H, CH_3), 3.40 (s, 6H, $N(CH_3)_2$), 3.95 (brs, 2H, piperazine CH_2), 4.21 (brs, 2H, piperazine CH_2), 4.28 (s, 2H, CH_2S), 7.65 (d, *J*=8.60 Hz, 2H, Ar-H), 7.80 (s, 1H, thiazole C_5-H), 7.88 (d, *J*=8.55 Hz, 2H, Ar-H), 10.39 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 24.43, 41.29, 45.17, 51.12, 52.17, 55.33, 57.13, 112.45, 119.12, 126.39, 129.39, 138.64, 153.53, 165.30, 165.33, 194.23. MS (ES^+): m/z 478.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-cyclo hexylpiperazine-1-carbodithioate (6d)

Yield: 85%. M. p. 194-196 °C. IR (KBr) ν_{max} (cm^{-1}): 3298 (amide N-H), 1678 (amide C=O), 1356-1023 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.06-1.23 (m, 5H, cyclohexyl CH_2), 1.56-1.76 (m, 5H, cyclohexyl CH_2), 2.27-2.29 (m, 1H, cyclohexyl CH_2), 2.58 (brs, 4H, piperazine CH_2), 2.71 (s, 3H, CH_3), 3.92 (brs, 2H, piperazine CH_2), 4.19 (brs, 2H, piperazine CH_2), 4.27 (s, 2H, CH_2S), 7.65 (d, *J*=8.50 Hz, 2H, Ar-H), 7.80 (s, 1H, thiazole C_5-H), 7.88 (d, *J*=8.50 Hz, 2H, Ar-H), 10.39 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 25.20, 25.77, 28.25, 39.88, 39.96, 40.05, 41.22, 62.23, 112.46, 119.10, 126.38, 129.38, 138.63, 153.53, 165.34, 194.02. MS (ES^+): m/z 475.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-phenyl piperazine-1-carbodithioate (6e)

Yield: 84%. M. p. 190-193 °C. IR (KBr) ν_{max} (cm^{-1}): 3299 (amide N-H), 1674 (amide C=O), 1356-1014 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 3.26 (brs, 4H, piperazine CH_2), 4.13 (brs, 2H, piperazine CH_2), 4.29 (s, 2H, CH_2S), 4.37 (brs, 2H, piperazine CH_2), 6.82 (t, 1H, *J*=7.28 Hz, Ar-H), 6.96 (d, 2H, *J*=8.10 Hz, Ar-H), 7.25 (t, 2H, *J*=7.98 Hz, Ar-H), 7.66 (d, *J*=8.70 Hz, 2H, Ar-H), 7.81 (s, 1H, thiazole C_5-H), 7.88 (d, *J*=8.65 Hz, 2H, Ar-H), 10.42 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 39.89, 39.97, 40.06, 41.27, 47.56, 112.49, 115.45, 119.13, 119.26, 126.40, 129.03, 129.41, 138.63, 149.99, 153.52, 165.27, 165.36, 194.02. MS (ES^+): m/z 469.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(4-fluorophenyl)piperazine-1-carbodithioate (6f)

Yield: 78%. M. p. 188-189 °C. IR (KBr) ν_{max} (cm^{-1}): 3315 (amide N-H), 1672 (amide C=O), 1367-1010 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 3.21 (brs, 4H, piperazine CH_2), 4.12 (brs, 2H, piperazine CH_2), 4.32 (s, 2H, CH_2S), 4.36 (brs, 2H, piperazine CH_2), 7.44 (2H, d, *J*=8.02 Hz, Ar-H), 7.66 (d, *J*=8.70 Hz, 2H, Ar-H), 7.81 (s, 1H, thiazole C_5-H), 7.89 (d, *J*=8.60 Hz, 2H, Ar-H), 8.12 (2H, d, *J*=8.12 Hz, Ar-H), 10.42 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 39.88, 39.96, 40.05, 41.29, 48.47, 50.74, 112.48, 115.32, 115.49, 117.39, 117.45, 117.77, 119.14, 126.40, 129.41, 138.63, 146.91, 165.26, 165.36, 194.65. MS (ES^+): m/z 487.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate (6g)

Yield: 75%. M. p. 210-212 °C. IR (KBr) ν_{max} (cm^{-1}): 3305 (amide N-H), 1671 (amide C=O), 1345-1018 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 3.73 (brs, 4H, piperazine CH_2), 4.17 (brs, 2H, piperazine CH_2), 4.33 (s, 2H, CH_2S), 4.37 (brs, 2H, piperazine CH_2), 6.94 (d, 2H, *J*=9.50 Hz, Ar-H), 7.66 (d, 2H, *J*=8.70 Hz, Ar-H), 7.81 (s, 1H, thiazole C_5-H), 7.88 (d, *J*=8.60 Hz, 2H, Ar-H), 8.10 (d, 2H, *J*=9.35 Hz, Ar-H), 10.42 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.89, 39.88, 39.96, 41.21, 44.64, 111.79, 112.49, 119.13, 125.76, 126.40, 129.41, 138.62, 153.51, 153.65, 165.22, 165.36, 194.72. MS (ES^+): m/z 514.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(4-methoxyphenyl)piperazine-1-carbodithioate (6h)

Yield: 80%. M. p. 195-196 °C. IR (KBr) ν_{max} (cm^{-1}): 3325 (amide N-H), 1675 (amide C=O), 1363-1016 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 3.15 (s, 3H, OCH_3), 3.69 (brs, 4H, piperazine CH_2), 4.11 (brs, 2H, piperazine CH_2), 4.32 (s, 2H, CH_2S), 4.37 (brs, 2H, piperazine CH_2), 6.85 (d, 2H, *J*=9.05 Hz, Ar-H), 6.93 (d, 2H, *J*=9.05 Hz, Ar-H), 7.67 (d, 2H, *J*=8.70 Hz, Ar-H), 7.81 (s, 1H, thiazole C_5-H), 7.89 (d, *J*=8.65 Hz, 2H, Ar-H), 10.42 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.90, 39.89, 39.97, 49.32, 55.15, 112.48, 114.31, 117.88, 119.14, 126.41, 129.41, 138.64, 144.37, 153.38, 153.53, 165.28, 165.36, 194.72. MS (ES^+): m/z 499.

2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(4-methylbenzyl)piperazine-1-carbodithioate (6i)

Yield: 82%. M. p. 140-141 °C. IR (KBr) ν_{max} (cm^{-1}): 3318 (amide N-H), 1674 (amide C=O), 1355-1024 (C-N and C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.28 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 3.42 (s, 2H, CH_2), 3.46 (brs, 4H, piperazine CH_2), 3.95 (brs, 2H, piperazine CH_2), 4.22 (brs, 2H, piperazine CH_2), 4.29 (s, 2H, CH_2S), 7.11 (d, 2H, *J*=8.10 Hz, Ar-H), 7.18 (d, 2H, *J*=7.90 Hz, Ar-H), 7.67 (d, 2H, *J*=8.70 Hz, Ar-H), 7.80 (s, 1H, thiazole C_5-H), 7.89 (d, *J*=8.65 Hz, 2H, Ar-H), 10.42 (s, 1H, N-H). [13]C NMR (125 MHz, DMSO-d_6) δ 18.90, 20.69, 41.35, 49.79, 51.82, 61.02, 112.46, 119.14, 126.40, 128.79, 128.92, 129.41, 134.30, 136.16, 138.65, 153.54, 165.30, 165.33, 194.29. MS (ES^+): m/z 497.

Pharmacology

AChE inhibition

All compounds were subjected to a slightly modified method of Ellman's test [30] 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 (**6a-i**) 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 [31] (Table-1).

Enzymatic assay

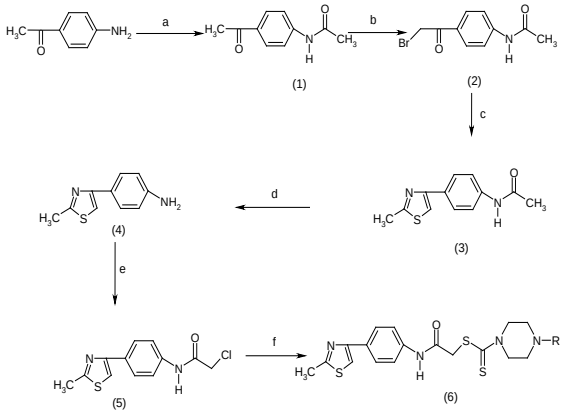
Enzyme solutions were prepared in the gelatin solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2% DMSO at a concentration range of 10^{-1}-10^{-6} mM was added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction 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 buffers, 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 A_I is the absorbance in the presence of the inhibitor, A_C 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 was expressed as Mean ± SD.

RESULTS AND DISCUSSION

2-[[4-(2-methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(substituted) piperazine-1-carbodithioate derivatives were synthesized in a similar way in our earlier study [32]. The thiazole ring was synthesized with a well-known reaction between haloketones(*N*-[4-(2-bromoacetyl) phenyl]acetamide-(2) and thioamide (thioacetamide) called Hantzsch reaction. Then, acetylated compound (2-chloro-*N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide-(5) was reacted with carbon disulphide, sodium hydroxide and appropriate secondary amines to give final compounds (6a-i).



Scheme 1: synthesis of the compounds (6a-i). Reagents and conditions: a. acetyl chloride, TEA, THF, 0-5 ^{o}C; b. Br_2, AcOH; c. thioacetamide, EtOH, r. t. d. 10 % HCl, EtOH, reflux; e. chloroacetyl chloride, TEA, THF, r. t.; f. appropriate sodium salts of *N,N*-disubstituted dithiocarbamic acids, K_2CO_3, acetone, reflux

The structures of the synthesized compounds were elucidated by spectral data. In the IR spectra of the compounds characteristic stretching bands for C=O and N-H groups were observed at 1671-1679 cm^{-1} and at 3289-3325 cm^{-1}, respectively. In the ^{1}H-NMR spectra of the compounds, methyl protons at the second position of the thiazole ring and N-H protons belonging to amide moiety were observed at about 2.70-2.71 ppm and 10.39-10.42 ppm. In aromatic region C_5-H of the thiazole ring was observed at about 7.79-7.81 ppm as singlet peaks. Protons of the-CH_2 group linked to sulphur atom were assigned at 4.27-4.33 ppm as singlets and protons of the piperazine ring were seen at the range of 2.44 ppm and 4.22 ppm as broad singlets, commonly. In the [13]C-NMR spectra of the compounds, characteristic signals were determined at about 18.90 and 194.65 ppm belonging to CH_3 and C=S carbons. Peaks which were seen at about 39-61 ppm assigned for piperazine carbons. The mass spectra of the compounds showed (M+1) peaks in agreement with their molecular weight.

Anticholinesterase activity

In the present study, some thiazole based piperazinecarbodithionic acid ester derivatives were tested for their AChE inhibitory activities by slightly modified Ellman's assay. Initially, all compounds were tested for their inhibition potency against AChE at a single dose of 100 µM. Then the compounds **6b** and **6c** showing greater than 50 % enzyme inhibition were assayed at 10-0.001 µM concentration ranges and IC_{50} values were calculated. Donepezil was used as a control agent. Anticholinesterase inhibitory activity of the synthesized compounds is presented in Table 1.

As seen in the table 1, the compounds 6b and 6c were the most active derivatives in the series with a IC_{50} of 86.34 µM, 91.74 respectively. These compounds showed very closed inhibition potency to donepezil at the concentrations of 100 and 10 µM. The compounds were the other derivatives which showed higher inhibition potency than 50% at 100 µM concentrations. However, these compounds had low inhibitory activity at further concentrations. Structure activity relationships of compounds clearly showed that aliphatic side chain located fourth position of the piperazine moiety enhances the biological activity since all of the active compounds bear dimethylamino ethyl or dimethylamino propyl groups. On the other hand phenyl or cyclohexyl substituted piperazine containing compounds did not showed notable enzyme inhibitory activity. Among the aliphatic side chain carrying compounds, the compounds 6b substituted with dimethylamino ethyl, displayed higher activity than the dimethylamino propyl substituted compounds 6c. This result suggests that increase of the carbon number in the aliphatic side chain causes an activity loss. Similarity between acetylcholine and dimethylaminoethyl side chain may explain the increasing enzyme inhibitory activity of the compounds 6b.

Table 1: Ach E Inhibition potency of compounds Thiazole-dithiocarbamate 6(a-i)

Compound	100	10	1	0.1	0.01	0.001	IC_{50} (µM)*
6a	51.03±8.42	ND*	ND	ND	ND	ND	ND
6b	89.70±6.37	48.95±6.91	23.42±2.28	11.74±1.43	9.76±0.72	5.41±0.86	86.34±1.31
6c	72.13±9.26	39.95±1.45	20.68±0.93	10.23±1.08	9.34±0.18	2.46±0.10	91.74±1.43
6d	50.21±1.86	ND	ND	ND	ND	ND	ND
6e	57.44±1.97	ND	ND	ND	ND	ND	ND
6f	50.77±1.87	ND	ND	ND	ND	ND	ND
6g	51.96±1.65	ND	ND	ND	ND	ND	ND
6h	52.87±2.77	ND	ND	ND	ND	ND	ND
6i	50.54±1.88	ND	ND	ND	ND	ND	ND
Donepezil	99.00±0.28	96.93±0.28	79.99±1.66	23.17±1.34	18.16±0.78	12.24±0.11	0.054±0.002

*ND: Not determined, *IC_{50}:50 % inhibitory concentration (means + SD of three independent experiments) of AChE

CONFLICT OF INTERESTS

The author reports no conflicts of interest.

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