STUDIES ON BENZIMIDAZOLE DITHIOCARBAMATE DERIVATIVES AS ACETYCHOLINESTERASE INHIBITORS

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ABSTRACT

In the current work some benzimidazole derivatives bearing dithiocarbamate were synthesized in order to investigated for their anticholinesterase activity. The structures of the obtained final compounds (3a-j) were confirmed by spectral data (1H-NMR, and FAB+MS) and elemental analysis. Each derivative was evaluated for its ability to inhibit acetyl cholinesterase (AChE) using a modification of Ellman’s spectrophotometric method. Compounds 3b, 3c were found to be the most active against anticholinesterase agents due to their inhibitory effect when compared with Donepezil (IC50 =0.054±0.002μM) as a reference drug.

KEYWORDS: benzimidazole, dithiocarbamate, cholinesterase inhibitors.

INTRODUCTION

It is well-known two different cholinesterase (ChE) enzymes are present in the human brain: acetylcholinesterase (ACHE), and butrylcholinesterase (BuChE). AChE is a terminator enzyme of nerve impulse transmission, 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 [1]. Inhibition of AChE evolves a strategy for the treatment of several diseases as Alzheimer’s disease (AD), senile dementia, myasthenia gravis, ataxia, and Parkinson’s disease [2]. 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\(^3\)\[^{11}\]. 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\(^12\)\[^{16}\). In addition it cannot overlook that benzimidazole ring processes a remarkably anticholinesterase activity \(^17\)\[^{20}\). Piperazine ring plays an important role for antiacetylcholinesterase activity \(^21\)\[^{24}\). 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-((1-Methyl-1\(H\)-benzimidazol-2-yl)amino)-2-oxoethyl4-substitutedpiperazine-1-carbodithioate (3a-3j) derivatives were reported in this study.

**EXPERIMENTAL**

All reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and were uncorrected. 1H-NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Billerica, USA). Mass spectra were recorded on a VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin-Elmer, Norwalk, USA). The TLC was performed on Kieselgel 60 F254 (Merck) layer using petroleum ether:ethyl acetate (3:1 v/v) as eluents.

**General procedure for the synthesis of the compounds**

2-Chloro-N-(1-methyl-1\(H\)-benzimidazol-2-yl)acetamide (1)

Chloroacetyl chloride (0.1 mol) was added dropwise with stirring to a mixture of 1-methyl-1\(H\)-benzimidazol-2-amine (0.1 mol) and triethylamine (0.1 mol) in THF (50 mL) at 0-5 °C. The solvent was evaporated under reduced pressure. The residue was washed with water to remove triethylamine hydrochloride and crystallized from ethanol \(^25\).
Sodium salts of N, N-disubstituted dithiocarbamic acids (2)
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].

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-substitutedpiperazine-1-carbodithioate (3a-3j)
A mixture of 2-chloro-N-(1-methyl-1H-benzimidazol-2-yl)acetamide (1) (2 mmol) and appropriate sodium salt of N,N-disubstituted dithiocarbamic acid (2) (2 mmol) in acetone (10 mL) was stirred at room temperature for 8 hours and filtered. The residue was washed with water and crystallized from ethanol.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(2-hydroxyethyl) piperazine-1-carbodithioate (3a)
82% yield; mp176. 1H-NMR (500 MHz, DMSO-d6): 2.44 (2H, t, J = 6.5 Hz, 6.0 Hz), 3.32-3.34 (4H, m), 3.53 (2H, t, J = 6.0 Hz), 3.60 (3H, s), 3.97-4.26 (7H, m), 7.19-7.26 (2H, m), 7.44-7.49 (2H, m). MS (ESI) (m/z): [M+1]+ 394. Anal. Calcd. for C17H23N5O2S2: C, 51.89; H, 5.89; N, 17.80; Found: C, 51.90; H, 5.88; N, 17.80.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(2-(dimethylamino)ethyl) piperazine-1-carbodithioate (3b)

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(3-(dimethylamino)propyl) piperazine-1-carbodithioate (3c)
67% yield; mp95. 1H-NMR (500 MHz, DMSO-d6): 1.53-1.59 (2H, m), 2.10 (6H, s), 2.22 (2H, t, J = 7.5 Hz, 7.0 Hz), 2.32 (2H, t, J = 7.5 Hz), 2.45-2.46 (4H, m), 3.63 (3H, s), 3.97-4.26 (6H, m), 7.19-7.26 (2H, m), 7.45-7.50 (2H, m). MS (ESI) (m/z): [M+1]+ 435. Anal. Calcd. for C20H30N6OS2: C, 55.27; H, 6.96; N, 19.34; Found: C, 55.26; H, 6.95; N, 19.36.
2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(pyrimidin-2-yl) piperazine-1-carbodithioate (3d)

68% yield; mp213. 1H-NMR (500 MHz, DMSO-d6): 3.60 (3H, s), 3.90-4.31 (8H, m), 6.70 (1H, t, J= 5.0 Hz, 4.5 Hz), 7.19-7.27 (2H, m), 7.45 (1H, d, J= 7.5 Hz), 7.50 (1H, d, J= 7.5 Hz), 8.41 (2H, d, J= 5.0 Hz). MS (ESI) (m/z): [M+1]+ 428. Anal. Calcd. for C19H21N7OS2: C, 53.38; H, 4.95; N, 22.93; Found: C, 53.39; H, 4.94; N, 22.94.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-phenylpiperazine-1-carbodithioate (3e)

78% yield; mp175. 1H-NMR (500 MHz, DMSO-d6): 3.31-3.33 (4H, m), 3.60 (3H, s), 4.10-4.40 (6H, m), 6.83 (1H, t, J= 7.5 Hz, 7.0 Hz), 6.97 (2H, d, J= 8.0 Hz), 7.21-7.27 (4H, m), 7.45-7.50 (2H, m). MS (ESI) (m/z): [M+1]+ 426. Anal. Calcd. for C21H23N5OS2: C, 59.27; H, 5.45; N, 16.46; Found: C, 59.26; H, 5.44; N, 16.48.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(4-fluorophenyl) piperazine-1-carbodithioate (3f)

65% yield; mp179. 1H-NMR (500 MHz, DMSO-d6): 3.31-3.34 (4H, m), 3.60 (3H, s), 4.14-4.37 (6H, m), 6.96-7.00 (2H, m), 7.05-7.11 (2H, m), 7.19-7.27 (2H, m), 7.45 (1H, d, J= 7.5 Hz), 7.50 (1H, d, J= 7.5 Hz). MS (ESI) (m/z): [M+1]+ 444. Anal. Calcd. for C21H22FN5OS2: C, 56.86; H, 5.00; N, 15.79; Found: C, 56.85; H, 5.02; N, 15.77.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(4-nitrophenyl) piperazine-1-carbodithioate (3g)

77% yield; mp255. 1H-NMR (500 MHz, DMSO-d6): 3.60 (3H, s), 3.67-3.77 (4H, m), 4.21-4.31 (6H, m), 6.96 (2H, d, J= 9.5 Hz), 7.20-7.27 (2H, m), 7.45 (1H, d, J= 7.5 Hz), 7.50 (1H, d, J= 7.5 Hz), 8.10 (1H, d, J= 9.5 Hz). MS (ESI) (m/z): [M+1]+ 471. Anal. Calcd. for C21H22N6O3S2: C, 53.60; H, 4.71; N, 15.37; Found: C, 53.59; H, 4.70; N, 15.78.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl-4-(4-methoxyphenyl) piperazine-1-carbodithioate (3h)

70% yield; mp185. 1H-NMR (500 MHz, DMSO-d6): 3.12-3.18 (4H, m), 3.60 (3H, s), 3.70 (3H, s), 6.85-6.87 (2H, m), 6.94-6.96 (2H, m), 7.20-7.29 (2H, m), 7.44-7.50 (2H, m). MS (ESI) (m/z): [M+1]+ 456. Anal. Calcd. for C22H25N5O2S2: C, 58.00; H, 5.53; N, 15.37; Found: C, 58.02; H, 5.51; N, 15.36.
2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl4-benzylpiperazine-1-carbodithioate (3i)
75% yield; mp186. 1H-NMR (500 MHz, DMSO-d6): 2.46-2.48 (4H, m), 3.52-3.53 (2H, m), 3.66 (3H, s), 4.17-4.24 (6H, m), 7.08-7.13 (2H, m), 7.26-7.41 (7H, m). MS (ESI) (m/z): [M+1]+ 440. Anal. Calcd. for C22H25N5OS2: C, 60.11; H, 5.73; N, 15.93; Found: C, 60.10; H, 5.72; N, 15.92.

2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl 4-(4-methylbenzyl) piperazine-1-carbodithioate (3j)
71% yield; mp169. 1H-NMR (500 MHz, DMSO-d6): 2.29 (3H, s), 2.44-2.46 (4H, m), 3.48-3.50 (2H, m), 3.60 (3H, s), 4.26-4.30 (3H, m), 7.14 (2H, d, J= 8.0 Hz), 7.17-7.25 (4H, m), 7.44 (1H, d, J= 7.5 Hz), 7.49 (1H, d, J= 7.5 Hz). MS (ESI) (m/z): [M+1]+ 454. Anal. Calcd. for C23H27N5OS2: C, 60.90; H, 6.00; N, 15.44; Found: C, 60.91; H, 6.01; N, 15.45.

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 (3a-3j) 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 AND DISCUSSION

The synthesis of compounds 3a-j followed the general pathway outlined in Scheme 1. Initially, 2-chloro-N-(1-methyl-1H-benzimidazol-2-yl)acetamide (1) was obtained by the reaction of 1-methyl-1H-benzimidazol-2-amine with chloroacetyl chloride in the presence of triethylamine. N 2-((1-methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl 4-Substituted piperazine-1-carbodithioate derivatives (3a-3j) were synthesized via the treatment of 2-chloro-N-(1-methyl-1H-benzimidazol-2-yl)acetamide (1) with appropriate sodium salts of N,N-disubstituted dithiocarbamic acids(2). The structure elucidations of the compounds (3a-3j) were performed with 1H-NMR, MS-FAB+ spectral data and elemental analyses. In the 500 MHz 1H-NMR spectra protons were recorded at the estimated areas. The mass spectra of the compounds showed [M+1] peaks, in agreement with their molecular weight. Elemental analysis results for C,H and N elements were satisfactory within ± 0.4% calculated values of the compounds.

The anticholinesterase effects of the compounds (3a-3j) were determined by modified Ellman’s spectrophotometric method (Table 1). Among these compounds (3a-3j), compounds 3b and 3c can be identified as promising anticholinesterase agents due to their inhibitory effect on AChE with IC50 value of 74.42±4.29and 88.35± 6.88 μM respectively when compared with Donepezil (IC50 =0.054±0.002 μM). Structure activity relationships of the compounds clearly showed that dimethylaminoethyl/propyl substitution on Piperazine residue increased the compound activity. However the highest activity was obtained from the compound bearing a similar carbon chain to that of ACHE.
Table 1. % AChE inhibition of the compounds and IC50 values AChE inhibition (%)

<table>
<thead>
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<th>Compound</th>
<th>100 (mM)</th>
<th>1 (mM)</th>
<th>0.01 (mM)</th>
<th>IC50 (mM)*</th>
</tr>
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<tbody>
<tr>
<td>3a</td>
<td>29.10±2.39</td>
<td>ND*</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b</td>
<td>93.03±0.56</td>
<td>60.88±1.02</td>
<td>22.70±0.89</td>
<td>74.42±4.29</td>
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<tr>
<td>3c</td>
<td>64.24±1.57</td>
<td>50.00±1.43</td>
<td>25.74±1.69</td>
<td>88.35±6.88</td>
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<tr>
<td>3d</td>
<td>14.16±3.18</td>
<td>ND</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3e</td>
<td>6.78±2.33</td>
<td>60.88±1.02</td>
<td>22.70±0.89</td>
<td>74.42±4.29</td>
</tr>
<tr>
<td>3f</td>
<td>10.72±1.17</td>
<td>ND</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3g</td>
<td>19.13±4.48</td>
<td>ND</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3h</td>
<td>10.63±1.98</td>
<td>ND</td>
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</tr>
<tr>
<td>3i</td>
<td>17.45±4.01</td>
<td>ND</td>
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<tr>
<td>3j</td>
<td>10.60±3.39</td>
<td>ND</td>
<td>ND</td>
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<td>Donepezil</td>
<td>96.82±5.09</td>
<td>76.96±5.01</td>
<td>36.42±4.28</td>
<td>0.054±0.002</td>
</tr>
</tbody>
</table>

*ND: Not determined

*IC50: 50 % inhibitory concentration (means + SD of three independent experiments) of AChE

Scheme(1). Synthesis of the compounds. Reagents and reaction conditions; i: ClCH2COCl, Et3N, THF, r.t.; ii: CS2, NaOH, EtOH, ice bath and then r.t. 1 h; iii: K2CO3, Acetone, r.t.

CONCLUSION

Some 2-((1-Methyl-1H-benzimidazol-2-yl)amino)-2-oxoethyl4-substitutedpiperazine-1-carbdithioate derivatives were synthesized. According to the activity results, all the final compounds were screened for anticholinesterase activity. None of the compounds shows prominent anticholinesterase activity.
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