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RESEARCH ARTICLE

Synthesis and biological evaluation of some thiazole derivatives as new cholinesterase inhibitors

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Abstract
In the present study, some thiazole derivatives were synthesized via the ring closure reaction of 1-[2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetyl]thiosemicarbazide with various phenacyl bromides. The chemical structures of the compounds were elucidated by ^{1}H NMR, ^{13}C NMR and mass spectral data and elemental analyses. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) using a modification of Ellman's spectrophotometric method. The compounds were also investigated for their cytotoxic properties using MTT assay. The most potent AChE inhibitor was found as compound **4e** ($IC_{50} = 25.5 \pm 2.12$ µg/mL) followed by compounds **4i** ($IC_{50} = 38.50 \pm 2.12$ µg/mL), **4c** ($IC_{50} = 58.42 \pm 3.14$ µg/mL) and **4g** ($IC_{50} = 68 \pm 2.12$ µg/mL) when compared with eserine ($IC_{50} = 0.025 \pm 0.01$ µg/mL). Effective compounds on AChE exhibited weak inhibition on BuChE ($IC_{50} > 80$ µg/mL). MTT assay indicated that the cytotoxic dose ($IC_{50} = 71.67 \pm 7.63$ µg/mL) of compound **4e** was higher than its effective dose.

Keywords: Thiazole, hydrazide, anticholinesterase activity, cytotoxicity

Introduction

Drug design through enzyme inhibition has gained great importance as a promising approach for the rational discovery of new drugs due to the increasing need to discover more selective and potent inhibitors in an effort to increase the therapeutic benefit to patients[1,2].

Cholinesterase inhibitors (ChEIs) have attracted a great deal of interest among researchers owing to their importance in the treatment of myasthenia gravis, glaucoma and Alzheimer's disease[1-3].

Two cholinesterases are present in humans: acetylcholinesterase (AChE), which selectively hydrolyses acetylcholine, and butyrylcholinesterase (BuChE), which is a non-specific cholinesterase. The main difference between two types of cholinesterase is the respective preferences for substrates: the former hydrolyses acetylcholine more quickly; the latter hydrolyses butyrylcholine more quickly. The main function of AChE is the termination of cholinergic neurotransmission, but the function of BuChE is not so clear[1,4].

Acetylcholinesterase inhibitors (AChEIs) exert their therapeutic action by inhibiting AChE, which results in the enhancement of cholinergic action[2]. In particular, AChEIs play a leading role in the first-line treatment for symptoms of Alzheimer's disease, which is the most common age-related neurodegenerative disorder[5-9].

In the last few decades, the chemistry of thiazoles and their fused heterocyclic derivatives has received considerable attention due to their synthetic and biological importance. Compounds bearing thiazole moiety have been reported to exhibit a wide spectrum of biological effects including anticholinesterase activity[10-16].

Acotiamide hydrochloride (acotiamide; N-[2-[bis(1-methylethyl)amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]thiazole-4-carboxamide monohydrochloride trihydrate, Z-338) has been reported

to be a new selective AChEI for the treatment of functional dyspepsia in clinical studies[15].

In a previous work, the inhibition of AChE by thiamine and its derivatives was investigated and structure-activity relationship study was also carried out to identify structural features that are associated with the inhibitory potency of these compounds[16].

Some researchers also carried out considerable research for novel cholinesterase inhibitors bearing amide and hydrazide moieties previously[17].

On the basis of these findings, we became interested in biological evaluation of thiazoles as anticholinesterase agents. Herein, we described the synthesis of novel thiazole derivatives bearing hydrazide moiety and focused on their anticholinesterase effects on AChE and BuChE. The compounds were also investigated for their cytotoxic properties.

Methods

Chemistry

All reagents were purchased from commercial suppliers and used without further purification. Melting points (m.p.) were determined on a Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker 400 MHz spectrometer (Bruker, Billerica, MA, USA). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker 100 MHz spectrometer (Bruker, Billerica, MA, USA). Chemical shifts were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. 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, CT, USA).

General procedure for the synthesis of the compounds
Ethyl 2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetate (1)
A mixture of benzo[d]thiazol-2(3H)-one (0.1 mol) and ethyl chloroacetate (0.1 mol) in the presence of potassium carbonate (0.1 mol) in acetone (50 mL) was refluxed for 10 h. The reaction mixture was poured into 100 mL of ice–water mixture, filtered and washed with water[18].

2-(2-Oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (2)
A mixture of the ester (**1**) (0.09 mol) and hydrazine hydrate (0.18 mol) in ethanol (50 mL) was stirred at room temperature for 3 h and then filtered[18].

1-[2-(2-Oxobenzo[d]thiazol-3(2H)-yl)acetyl] thiosemicarbazide (3)
A mixture of the hydrazide (**2**) (0.05 mol), potassium thiocyanate (0.1 mol), conc. HCl (20 mL) in water (40 mL) was refluxed for 3 h. After cooling, the resulting solid was

collected by filtration, washed with water, dried, and recrystallized from ethanol.

2-(2-Oxobenzo[d]thiazol-3(2H)-yl)-N′-(4-phenylthiazol-2-yl) acetohydrazide derivatives (4a–j)
A mixture of 1-[2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetyl]thiosemicarbazide (**3**) (0.001 mol) and appropriate phenacyl bromide (0.001 mol) was refluxed in ethanol (15 mL) for 3 h. After cooling, the resulting solid was collected by filtration, dried, and recrystallized from ethanol.

N′-(4-phenylthiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl) acetohydrazide (4a)
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 4.77 (2H, s), 7.10–7.45 (6H, m), 7.68 (2H, m), 7.84 (2H, m), 9.64 (1H, brs), 10.75 (1H, s).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 43.22 (CH$_2$), 103.4 (CH), 111.3 (CH), 121.2 (C), 122.8 (CH), 123.3 (CH), 125.6 (2CH), 126.5 (CH), 127.5 (CH), 128.5 (2CH), 134.5 (C), 137.0 (C), 150.4 (C), 166.3 (C), 169.1 (C), 171.5 (C).

For C$_{18}$H$_{14}$N$_4$O$_2$S$_2$, calculated: C, 56.53; H, 3.69; N, 14.65; found: C, 56.52; H, 3.71; N, 14.61.

MS (ES): [M + 1]$^+$: 383

N′-(4-p-tolylthiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl) acetohydrazide (4b)
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.32 (3H, s), 4.77 (2H, s), 7.10–7.40 (5H, m), 7.68 (2H, m), 7.72 (2H, m), 9.60 (1H, brs), 10.73 (1H, s).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 20.77 (CH$_3$), 43.21 (CH$_2$), 102.5 (CH), 111.4 (CH), 121.2 (C), 122.9 (CH), 123.3 (CH), 125.5 (2CH), 126.5 (CH), 129.1 (2CH), 131.9 (C), 136.8 (C), 137.0 (C), 150.5 (C), 166.3 (C), 169.1 (C), 171.5 (C).

For C$_{19}$H$_{16}$N$_4$O$_2$S$_2$, calculated: C, 57.56; H, 4.07; N, 14.13; found: C, 57.55; H, 4.05; N, 14.15.

MS (ES): [M + 1]$^+$: 397

N′-(4-(4-chlorophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4c)
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 4.78 (2H, s), 7.15–7.40 (3H, m), 7.46 (2H, m), 7.68 (2H, m), 7.85 (2H, m), 9.68 (1H, brs), 10.76 (1H, s).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 43.22 (CH$_2$), 104.2 (CH), 111.3 (CH), 121.2 (C), 122.9 (CH), 123.3 (CH), 126.5 (CH), 127.2 (2CH), 128.6 (2CH), 131.9 (C), 133.4 (C), 137.0 (C), 149.2 (C), 166.3 (C), 169.1 (C), 171.7 (C).

For C$_{18}$H$_{13}$ClN$_4$O$_2$S$_2$, calculated: C, 51.86; H, 3.14; N, 13.44; found: C, 51.85; H, 3.15; N, 13.40.

MS (ES): [M + 1]$^+$: 418

N′-(4-(4-bromophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4d)
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 4.77 (2H, s), 7.15–7.30 (3H, m), 7.36 (2H, m), 7.55–7.70 (2H, m), 7.78 (2H, m), 9.66 (1H, brs), 10.74 (1H, s).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 43.22 (CH$_2$), 104.3 (CH), 111.3 (CH), 120.5 (C), 121.2 (C), 122.9 (CH), 123.3

(CH), 126.5 (CH), 127.6 (2CH), 131.5 (2CH), 133.7 (C), 137.0 (C), 149.3 (C), 166.3 (C), 169.2 (C), 171.7 (C).

For $C_{18}H_{13}BrN_4O_2S_2$, calculated: C, 46.86; H, 2.84; N, 12.14; found: C, 46.85; H, 2.80; N, 12.15.

MS (ES): [M + 1]+: 462

N′-(4-(4-nitrophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4e)

1H NMR (400 MHz, DMSO-d6): δ 4.78 (2H, s), 7.15–7.40 (3H, m), 7.66 (2H, s), 8.10 (2H, m), 8.27 (2H, m), 9.80 (1H, brs), 10.81 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.22 (CH2), 108.3 (CH), 111.3 (CH), 121.2 (C), 122.9 (CH), 123.3 (CH), 124.0 (2CH), 126.4 (2CH), 126.5 (CH), 137.0 (C), 140.5 (C), 146.2 (C), 148.4 (C), 166.4 (C), 169.2 (C), 172.0 (C).

For $C_{18}H_{13}N_5O_4S_2$, calculated: C, 50.58; H, 3.07; N, 16.38; found: C, 50.55; H, 3.10; N, 16.35.

MS (ES): [M + 1]+: 428

N′-(4-(3-chlorophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4f)

1H NMR (400 MHz, DMSO-d6): δ 4.77 (2H, s), 7.0–8.0 (9H, m), 9.69 (1H, s), 10.75 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.21 (CH2), 105.0 (CH), 111.3 (CH), 121.2 (C), 122.9 (CH), 123.3 (CH), 124.1 (CH), 125.2 (CH), 126.5 (CH), 127.2 (CH), 130.5 (CH), 133.4 (C), 136.5 (C), 137.0 (C), 148.8 (C), 166.3 (C), 169.2 (C), 171.6 (C).

For $C_{18}H_{13}ClN_4O_2S_2$, calculated: C, 51.86; H, 3.14; N, 13.44; found: C, 51.85; H, 3.16; N, 13.42.

MS (ES): [M + 1]+: 418

N′-(4-(3-nitrophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4g)

1H NMR (400 MHz, DMSO-d6): δ 4.78 (2H, s), 7.05–7.40 (3H, m), 7.61 (1H, s), 7.70 (2H, m), 8.14 (1H, dd, J = 8.1, 2.3 Hz), 8.27 (1H, d, J = 7.9 Hz), 8.64 (1H, t, J = 2.0 Hz), 9.78 (1H, brs), 10.80 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.21 (CH2), 106.2 (CH), 111.3 (CH), 119.9 (CH), 121.2 (C), 122.1 (CH), 122.9 (CH), 123.3 (CH), 126.5 (CH), 130.2 (CH), 131.6 (CH), 136.0 (C), 137.0 (C), 148.1 (C), 148.3 (C), 166.4 (C), 169.1 (C), 172.1 (C).

For $C_{18}H_{13}N_5O_4S_2$, calculated: C, 50.58; H, 3.07; N, 16.38; found: C, 50.59; H, 3.09; N, 16.34.

MS (ES): [M + 1]+: 428

N′-(4-(3,4-dichlorophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4h)

1H NMR (400 MHz, DMSO-d6): δ 4.77 (2H, s), 7.0–7.4 (3H, m), 7.49 (1H, s), 7.62–7.67 (2H, m), 7.80 (1H, dd, J = 8.5, 2.0 Hz), 8.06 (1H, d, J = 2.0 Hz), 9.70 (1H, brs), 10.75 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.23 (CH2), 105.7 (CH), 111.3 (CH), 121.2 (C), 122.9 (CH), 123.3 (CH), 125.6 (CH), 126.5 (CH), 127.1 (CH), 129.7 (C), 130.8 (CH), 131.4 (C), 135.0 (C), 137.0 (C), 147.8 (C), 166.3 (C), 169.1 (C), 171.7 (C).

For $C_{18}H_{12}Cl_2N_4O_2S_2$, calculated: C, 47.90; H, 2.68; N, 12.41; found: C, 47.92; H, 2.70; N, 12.38.

MS (ES): [M + 1]+: 452

N′-(4-(2,4-dichlorophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4i)

1H NMR (400 MHz, DMSO-d6): δ 4.77 (2H, s), 7.05–7.35 (3H, m), 7.37 (1H, s), 7.48 (1H, dd, J = 8.5, 2.2 Hz), 7.63–7.70 (2H, m), 7.88 (1H, d, J = 8.5 Hz), 9.68 (1H, brs), 10.75 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.22 (CH2), 109.0 (CH), 111.3 (CH), 121.2 (C), 122.8 (CH), 123.3 (CH), 126.5 (CH), 127.4 (CH), 129.7 (CH), 131.4 (C), 131.9 (C), 132.3 (CH), 132.4 (C), 137.0 (C), 145.7 (C), 166.3 (C), 169.1 (C), 170.7 (C).

For $C_{18}H_{12}Cl_2N_4O_2S_2$, calculated: C, 47.90; H, 2.68; N, 12.41; found: C, 47.89; H, 2.71; N, 12.39.

MS (ES): [M + 1]+: 452

N′-(4-(2,5-dichlorophenyl)thiazol-2-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetohydrazide (4j)

1H NMR (400 MHz, DMSO-d6): δ 4.77 (2H, s), 7.15–7.32 (3H, m), 7.40 (1H, dd, J = 8.6, 2.7 Hz), 7.47 (1H, s), 7.55 (1H, d, J = 8.6 Hz), 7.66 (1H, dd, J = 7.8, 1.2 Hz), 7.92 (1H, d, J = 2.7 Hz), 9.71 (1H, brs), 10.76 (1H, s).

13C NMR (100 MHz, DMSO-d6): δ 43.21 (CH2), 109.8 (CH), 111.3 (CH), 121.2 (C), 122.8 (CH), 123.3 (CH), 126.4 (CH), 128.5 (CH), 129.1 (C), 130.3 (CH), 131.8 (C), 132.1 (CH), 134.3 (C), 137.0 (C), 145.3 (C), 166.3 (C), 169.1 (C), 170.7 (C).

For $C_{18}H_{12}Cl_2N_4O_2S_2$, calculated: C, 47.90; H, 2.68; N, 12.41; found: C, 47.88; H, 2.70; N, 12.38.

MS (ES): [M + 1]+: 452

AChE and BuChE inhibitory activity

AChE and BuChE inhibitory activity was determined by Ellman's method with minor modifications (Electric eel AChE enzyme was used instead of bovine AChE enzyme and buffer was added 2.4 mL instead of 3 mL)[19]. Compounds **4a–j** were dissolved in DMSO and tested at final concentration range 5–80 μg/mL. Twenty micro litre of enzyme (AChE or BuChE, 1 U/mL), 10 μL sample added to 2.4 mL buffer, the mixture was incubated at 37°C for 15 min. After 15 min incubation, 50 μL of 0.01 M 5,5′-dithio-bis(2-nitrobenzoic acid) and 20 μL of 75 mM acetylthiocholine iodide or 25 mM butyrylthiocholine iodide were added, and the final mixture was incubated at room temperature for 30 min. Blank was prepared using 10 μL of DMSO instead of the test sample with all other procedures similar to those used in the case of the sample mixture. Absorbances were measured at 412 nm and 37°C using polystyrol cuvets with spectrophotometer (Shimadzu, UV-1700). Experiment was done in triplicate. Data are expressed as mean ± standard deviation (SD).

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

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

Toxicity

The level of cellular MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) reduction was quantified as previously described in the literature with small modifications as represented below in detail[20,21].

Cell culture and drug treatment

NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Life Technologies, UK), 100 IU/mL penicillin (Gibco, Paisley, Scotland) and 100 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in DMSO (Sigma-Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability).

MTT assay for cytotoxicity of the compounds

The MTT assay is widely used as a measure of cytotoxicity. After 24 h of preincubation, the tested compounds were added to give final concentration in the range 0.5–500 μg/mL and the cells were incubated for 24 h. At the end of this period, MTT was dissolved in phosphate buffered saline at 5 mg/mL and filtered to sterilize. At the time indicated above, stock MTT solution (20 μL/200 μL medium) was added to all wells of assay, and the plates were incubated for 4 h at 37°C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 μL DMSO (instead of acid-isopropanol) to each well and after a few minutes at room temperature to ensure that all crystals were dissolved, absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). Each concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

Results and discussion

Initially, ethyl 2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetate (**1**) was synthesized via the reaction of benzo[d]thiazol-2(3H)-one with ethyl chloroacetate in the presence of potassium carbonate. Then, this ester (**1**) was converted to the corresponding hydrazide derivative (**2**).

1-[2-(2-Oxobenzo[d]thiazol-3(2H)-yl)acetyl]thiosemicarbazide (**3**) was obtained by the reaction of the hydrazide derivative (**2**) with potassium thiocyanate and conc. HCl. The ring closure of compound **3** with phenacyl bromides gave the target compounds (**4a–j**). These reactions are summarized in Scheme 1 and some properties of the compounds are given in Table 1. The structures of these compounds (**4a–j**) were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral data and elemental analyses.

The anticholinesterase effects of the compounds (**4a–j**) on AChE and BuChE were determined by a modification of Ellman's spectrophotometric method (Table 2).

Among these compounds (**4a–j**), compound **4e** can be identified as the most promising anticholinesterase agent due to its inhibitory effect on AChE with an IC_{50} value of 25.5±2.12 μg/mL when compared with eserine ($IC_{50}=0.025±0.01$ μg/mL). In our previous study, we also reported that galantamine exhibited its inhibitory effect on AChE with an IC_{50} value of 0.28±0.04 μg/mL[22]. Compound **4e** also showed the highest AChE inhibitory effect when compared with galantamine. Although compounds **4e** and **4g** possess nitro substituent on phenyl ring, they show different levels of anticholinesterase activity. The
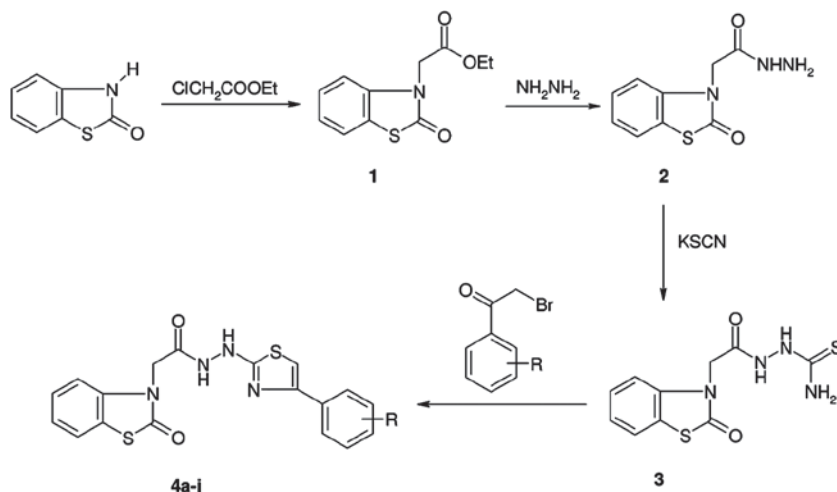
Scheme 1. The synthetic protocol of the title compounds (**4a–j**).

Table 1. Some properties of the synthesized compounds (**4a–j**).

Compound	R	Yield (%)	m.p. (°C)	Molecular formula	Molecular weight
4a	H	64	227	$C_{18}H_{14}N_4O_2S_2$	382.46
4b	p-CH$_3$	67	224	$C_{19}H_{16}N_4O_2S_2$	396.49
4c	p-Cl	75	239	$C_{18}H_{13}ClN_4O_2S_2$	416.90
4d	p-Br	69	255	$C_{18}H_{13}BrN_4O_2S_2$	461.36
4e	p-NO$_2$	89	252	$C_{18}H_{13}N_5O_4S_2$	427.46
4f	m-Cl	68	229	$C_{18}H_{13}ClN_4O_2S_2$	416.90
4g	m-NO$_2$	87	256	$C_{18}H_{13}N_5O_4S_2$	427.46
4h	3,4-Cl	72	240	$C_{18}H_{12}Cl_2N_4O_2S_2$	451.35
4i	2,4-Cl	71	231	$C_{18}H_{12}Cl_2N_4O_2S_2$	451.35
4j	2,5-Cl	70	235	$C_{18}H_{12}Cl_2N_4O_2S_2$	451.35

m.p., melting point.

Table 2. The anticholinesterase activities of the compounds (**4a–j**) as IC$_{50}$ values (μg/mL).

	AChE		BuChE	
Compound	80 μg/mL (% inhibition)	IC$_{50}$ μg/mL	80 μg/mL (% inhibition)	IC$_{50}$ μg/mL
4a	NA	NA	32.95 ± 1.78	>80
4b	NA	NA	20.51 ± 0.77	>80
4c	67.02 ± 2.52	58.42 ± 3.14	6.04 ± 3.17	>80
4d	4.74 ± 0.84	>80	NA	NA
4e	93.30 ± 2.30	25.5 ± 2.12	24.78 ± 3.75	>80
4f	6.38 ± 1.49	>80	12.60 ± 1.62	>80
4g	59.12 ± 3.54	68 ± 2.12	5.85 ± 0.53	>80
4h	20.93 ± 2.09	>80	14.04 ± 3.15	>80
4i	84.90 ± 2.67	38.50 ± 2.12	18.75 ± 3.58	>80
4j	10.53 ± 2.16	>80	NA	NA
Eserine	–	0.025 ± 0.01		0.05 ± 0.02

NA, not active.

former bearing p-nitro group exhibits the inhibitory effect on AChE with an IC$_{50}$ value of 25.5 ± 2.12 μg/mL, whereas the latter bearing m-nitro group exhibits the inhibitory effect on AChE with an IC$_{50}$ value of 68 ± 2.12 μg/mL. Compounds **4e** and **4g** also exhibit different levels of cytotoxicity with IC$_{50}$ values of 71.67 ± 7.63 and 4.93 ± 0.11 μg/mL, respectively. These observations indicate that the position of nitro substituent on phenyl ring has a crucial influence on anticholinesterase activity and cytotoxicity. It is apparent that there is a positive correlation between anticholinesterase activity and p-nitro group. Compound **4i** carrying 2,4-dichloro group on phenyl ring exhibits the inhibitory effect on AChE with an IC$_{50}$ value of 38.50 ± 2.12 μg/mL, whilst compound **4c** bearing p-chloro group on phenyl ring exhibits the inhibitory effect on AChE with an IC$_{50}$ value of 58.42 ± 3.14 μg/mL. Compounds **4d**, **4f**, **4h**, **4j** exhibit weak inhibition on AChE (IC$_{50}$ > 80 μg/mL), whereas compounds **4a** and **4b** are inactive.

Compounds **4a**, **4b**, **4c**, **4e**, **4f**, **4g**, **4h**, **4i** showed weak inhibitory effects on BuChE (IC$_{50}$ > 80 μg/mL), whilst compounds **4d** and **4j** were inactive.

The compounds were also evaluated for their cytotoxic properties using MTT assay (Table 3). The biological

Table 3. *In vitro* cytotoxicity of the compounds (**4a–j**).

Compound	IC$_{50}$ (μg/mL)[a]
4a	52.67 ± 23.69
4b	18.50 ± 1.32
4c	46.67 ± 2.88
4d	75.00 ± 21.79
4e	71.67 ± 7.63
4f	42.67 ± 6.43
4g	4.93 ± 0.11
4h	28.33 ± 5.77
4i	52.33 ± 7.50
4j	103.33 ± 20.81

[a]Cytotoxicity of compounds to mouse fibroblast (NIH/3T3) cell line. Incubation for 24 h. IC$_{50}$ is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± standard deviation of triplicate determinations.

study indicated that compound **4g** possessed the highest cytotoxicity, whereas compound **4j** exhibited the lowest cytotoxicity against mouse fibroblast (NIH/3T3) cell line among the title compounds.

Conclusion

In the present paper, we synthesized a series of thiazole derivatives and evaluated their anticholinesterase effects and cytotoxicity.

The biological results indicate that functional groups on the phenyl ring have a considerable influence on anticholinesterase activity and toxicity. In particular, compound **4e** is the most promising AChEI due to its inhibitory effect on AChE with an IC$_{50}$ value of 25.5 ± 2.12 μg/mL. In addition, the cytotoxic dose (IC$_{50} = 71.67 \pm 7.63$ μg/mL) of compound **4e** is higher than its effective dose.

Declaration of interest

The authors report no conflicts of interest.

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