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RESEARCH ARTICLE

Design, synthesis and evaluation of new thiazole-piperazines as acetylcholinesterase inhibitors

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Abstract

In this study, some new 2-(4-substituted piperazine-1-yl)-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide derivatives were synthesized. The synthesized compounds were screened for their anticholinesterase activity on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes by *in vitro* Ellman's method. The structural elucidation of the compounds was performed by using IR, ¹H-NMR, ¹³C-NMR and FAB⁺-MS spectral data and elemental analyses results. Biological assays revealed that at 0.1 μM concentration, the most active compounds against AChE were **5n**, **5o** and **5p** that indicated 96.44, 99.83 and 89.70% inhibition rates, respectively. Besides, IC₅₀ value of the compound **5o** was determined as 0.011 μM, whereas IC₅₀ value of standard drug donepezil was 0.054 μM. The synthesized compounds did not show any notable inhibitory activity against BChE.

Keywords: Anticholinesterase activity, Ellman's method, thiazole, piperazine

Introduction

Acetylcholine (ACh) acts as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system and as a preganglionic and a postganglionic transmitter in the parasympathetic nervous system of vertebrates and invertebrates^{1,2}. Acetylcholinesterase (AChE) is a terminator enzyme of nerve impulse transmission at the cholinergic synapses by quick hydrolysis of ACh to choline and acetate. Inhibition of AChE evolves a strategy for the treatment of several diseases as Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease³. AD is one form of senile dementia which occurs due to various neuropathological conditions such as senile plaques and neurofibrillary tangles. It is the most common dementias that affects half of the population aged 85 years^{4,5} and seventh main cause of life lost affecting 5.3 million people over the world. In AD, growing numbers of nerve cells degenerate and die along with loss in synapse through which information flows from and to the brain. As a result cognitive impairment and dementia occur⁶. The neuropathology of AD is generally characterized by the presence of numerous amyloid β-peptide (Aβ) plaques, neurofibrillary tangles (NFT), and degeneration

or atrophy of the basal forebrain cholinergic neurons. The loss of basal forebrain cholinergic cells results in an important reduction in ACh level, which plays an important role in the cognitive impairment associated with AD⁷.

Disruption of cholinergic transmission in AD was proved in many clinical and neuropathological studies^{8,9}. As well as deficit of ACh, the loss of presynaptic M₂ muscarinic and nicotinic receptors has also been found¹⁰. There are also evidences of an interaction between AChE and Aβ, which is participated in plaques and plays an essential role in AD pathophysiology. AChE constitutes a stable complex with senile plaque components and may even enhance the aggregation of Aβ peptides and amyloid formation. The neurotoxicity of amyloid components may be risen up by the presence of AChE^{11,12}. In contrast to the overall decrease of AChE in AD brains, at least in its later stages, the local concentration of AChE around the plaques increases as these lesions occur¹³. In addition to hydrolysing ACh, AChE may be also involved in other functions such as cell proliferation, differentiation, and responses to various damaging factors including stress and amyloid formation^{11,14}. Hence, for the most part, inhibitors of AChE, enhancing the ACh concentration in

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the brain, have been introduced to the market for treating mild-to-moderate AD.

Consistent with reported studies, cholinesterase inhibitors contain a basic centre, a core ring system and a linker such as $-O-$, CH_2 , $CONH$, $CONH(CH_2)_n$ etc. between core ring and basic centres¹⁵⁻¹⁷. For example, chemical structures of AChE inhibitors donepezil and BYYT-25 fit well to such definition. These agents contain an indan as a core ring, methylene or oxygen as a linker and piperidine or pyrrolidine cyclic amines as a basic centre. In the light of above structural motif, we designed a new series of thiazole-piperazine derivatives (Figure 1). The reason for the choice of thiazole and piperazine moieties was their AChE inhibitory potency. Namely, thiazole based biomolecule thiamine (vitamin B1) and its analogues were described as AChE inhibitors¹⁸. Acotiamide, new thiazole based drug that enhances ACh release in the enteric nervous system via muscarinic receptor antagonism and AChE inhibition was discovered¹⁹. Besides, in previous studies, cholinesterase

activity of some thiazole²⁰⁻²² and piperazine²³⁻²⁵ derivatives was reported. As a result, we synthesized thiazole-piperazine derivatives so as to investigate their cholinesterase inhibitory potency, which will enable to study *in vivo* pharmacological activity against AD on animal models.

Materials and methods

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co. All melting points (m.p.) were determined by Electrothermal 9100 digital melting point apparatus and were uncorrected. Spectroscopic data were recorded with the following instruments: ¹H-NMR, Bruker 400 MHz spectrometer; ¹³C-NMR, Bruker 100 MHz spectrometer; MS-FAB, VG Quattro Mass spectrometer and elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser. Some characteristics of the compounds are given in Table 1.

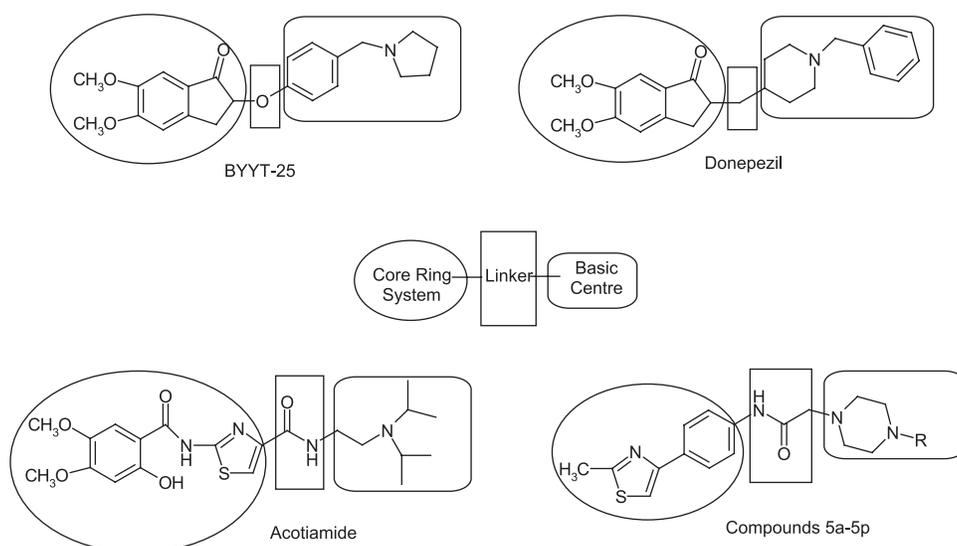


Figure 1. Structural motifs of AChE inhibitors BYYT-25, Donepezil, Acotiamide, and the synthesized compounds **5a-5p**.

Table 1. Some physicochemical characteristics of the synthesized compounds.

Compound	R	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
5a	Methyl	70	119	C ₁₇ H ₂₂ N ₄ OS	330
5b	Ethyl	72	90	C ₁₈ H ₂₄ N ₄ OS	344
5c	Cyclohexyl	75	128	C ₂₂ H ₃₀ N ₄ OS	398
5d	Phenyl	80	160	C ₂₂ H ₂₄ N ₄ OS	392
5e	4-Methylphenyl	81	172-175	C ₂₃ H ₂₆ N ₄ OS	406
5f	4-Methoxyphenyl	73	180	C ₂₃ H ₂₆ N ₄ O ₂ S	422
5g	4-Chlorophenyl	75	176	C ₂₂ H ₂₃ ClN ₄ OS	426.5
5h	4-Fluorophenyl	80	227	C ₂₂ H ₂₃ FN ₄ OS	410
5i	4-Nitrophenyl	76	283-286	C ₂₂ H ₂₃ N ₅ O ₃ S	437
5j	3-Methylphenyl	72	190	C ₂₃ H ₂₆ N ₄ OS	406
5k	3-Methoxyphenyl	74	143	C ₂₃ H ₂₆ N ₄ O ₂ S	422
5l	2-Methylphenyl	75	142	C ₂₃ H ₂₆ N ₄ OS	406
5m	2-Methoxyphenyl	70	168	C ₂₃ H ₂₆ N ₄ O ₂ S	422
5n	2-Pyridyl	71	167	C ₂₁ H ₂₃ N ₅ OS	393
5o	Benzyl	82	140	C ₂₃ H ₂₆ N ₄ OS	406
5p	2-Furoyl	76	146	C ₂₁ H ₂₂ N ₄ O ₃ S	410

Synthesis of the compounds

N-[4-(2-Bromoacetyl)phenyl]acetamide (1)

4'-aminoacetophenone (0.05 mol, 6.75 g) and triethylamine (0.06 mol, 8.34 mL) were dissolved in THF (100 mL) with a constant stirring at 0–5°C. Acetyl chloride (0.06 mol, 4.78 mL) was added dropwise to this solution gradually. The reaction mixture thus obtained was further agitated for 1 h at room temperature. After evaporation of solvent the residue was filtered and washed with water and dried. Then the obtained *N*-(4-acetylphenyl)acetamide (0.04, 7.08 g mol) was brominated in 30 mL acetic acid with the presence of 0.05 mol (2.58 mL) bromine and 0.5 mL HBr to give *N*-[4-(2-bromoacetyl)phenyl]acetamide (1) in 86% yield.

N-[4-(2-Methyl-4-thiazolyl)phenyl]acetamide (2)

N-[4-(2-bromoacetyl)phenyl]acetamide (0.03 mol, 7.68 g) (1) and thioacetamide (0.03 mol, 2.25 g) were stirred in ethanol at room temperature for 48 h. The precipitated solid was filtered, dried and recrystallized from ethanol to afford title compound in 78% yield.

4-(2-Methyl-4-thiazolyl)aniline (3)

N-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (0.025 mol, 5.8 g) (2) was refluxed in ethanol (15 mL) with 1 N HCl solution (10 mL). The reaction was monitored by TLC. When the reaction was completed, the mixture was poured into ice water, neutralized with 10% NaOH solution and then filtered to give 4-(2-Methyl-4-thiazolyl)aniline (3) in 92% yield.

2-Chloro-*N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (4)

Chloroacetyl chloride (0.025 mol, 2 mL) was added dropwise over 15 min to a magnetically stirred solution of the 4-(2-Methyl-4-thiazolyl)aniline (0.022 mol, 4.18 g) (3) and triethylamine (0.025 mol, 3.48 mL) in dry THF (15 mL). The reaction was monitored by TLC. After the reaction was completed, the solvent was evaporated under reduced pressure. Water was added to wash the resulting solid, the mixture was filtered, dried and recrystallized from ethanol to afford compound 4 in 83% yield.

General procedure for 2-(4-substituted piperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide derivatives (5a–5p)

A mixture of compound 4 (0.001 mol, 0.266 g), appropriate piperazine derivative (0.0011 mol) and K_2CO_3 (0.001 mol, 0.138 g) in acetone (15 mL) was refluxed for 2 h. After cooling, the solvent was evaporated until dryness. The residue was treated with 25 mL of water. Solidified product was filtered, washed with water and recrystallized from ethanol to give the 5a–5p.

2-(4-Methylpiperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5a)

IR (KBr) ν_{\max} (cm⁻¹): 3282 (amide N-H), 3042 (aromatic C-H), 2978 (aliphatic C-H), 1679 (amide C=O), 1584–1411 (C=C and C=N), 1309–1018 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.14 (3H, s, N-CH₃), 2.35–2.48 (8H, m, piperazine C-H), 2.67 (3H, s, C-CH₃), 3.09 (2H, s, CO-CH₂), 7.66 (2H, d, *J* = 9.2 Hz, Ar-H), 7.77 (1H, s, thiazole C₅-H), 7.84 (2H, d, *J* = 8.8 Hz, Ar-H), 9.74 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.59 (CH₃), 46.43 (CH₃), 53.39 (2CH₂), 55.23 (2CH₂), 62.49 (CH₂), 113.15 (CH), 120.13 (2CH), 127.01 (2CH), 130.15 (C), 138.92 (C), 154.27 (C), 166.02 (C), 168.98 (C).

For C₁₇H₂₂N₄O₂ calculated: 61.79% C, 6.71% H, 16.95% N; found: 61.75% C, 6.70% H, 16.91% N.

MS (FAB) [M + 1]⁺: *m/z* 331.

2-(4-Ethylpiperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5b)

IR (KBr) ν_{\max} (cm⁻¹): 3280 (amide N-H), 3054 (aromatic C-H), 2940 (aliphatic C-H), 1680 (amide C=O), 1594–1401 (C=C and C=N), 1280–1019 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 0.99 (3H, t, *J* = 7.2, 7.2 Hz, CH₂-CH₃), 2.32 (2H, q, *J* = 7.2, 14.2 Hz, CH₂-CH₃), 2.42–2.53 (8H, m, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.12 (2H, s, CO-CH₂), 7.69 (2H, d, *J* = 8.4 Hz, Ar-H), 7.81 (1H, s, thiazole C₅-H), 7.86 (2H, d, *J* = 8.8 Hz, Ar-H), 9.76 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 16.81 (CH₃), 19.59 (CH₃), 28.02 (CH₂), 53.86 (2CH₂), 55.47 (2CH₂), 63.06 (CH₂), 113.23 (CH), 120.47 (2CH), 127.08 (2CH), 130.76 (C), 139.02 (C), 154.47 (C), 166.16 (C), 169.00 (C).

For C₁₈H₂₄N₄O₂ calculated: 62.76% C, 7.02% H, 16.26% N; found: 62.74% C, 7.01% H, 16.25% N.

MS (FAB) [M + 1]⁺: *m/z* 345.

2-(4-Cyclohexylpiperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5c)

IR (KBr) ν_{\max} (cm⁻¹): 3288 (amide N-H), 3056 (aromatic C-H), 2991 (aliphatic C-H), 1675 (amide C=O), 1568–1407 (C=C and C=N), 1311–1020 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 1.16–1.24 (5H, m, cyclohexyl C-H), 1.59–1.86 (5H, m, cyclohexyl C-H), 2.22–2.26 (1H, m, cyclohexyl C-H), 2.51–2.62 (8H, m, piperazine C-H), 2.73 (3H, s, C-CH₃), 3.09 (2H, s, CO-CH₂), 7.22 (1H, s, thiazole C₅-H), 7.59 (2H, d, *J* = 8.4 Hz, Ar-H), 7.81 (2H, d, *J* = 8.8 Hz, Ar-H), 9.18 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.52 (CH₃), 26.04 (2CH₂), 26.49 (CH₂), 29.20 (2CH₂), 49.33 (2CH₂), 54.23 (2CH₂), 62.24 (CH₂), 63.62 (CH), 111.81 (CH), 119.72 (2CH), 127.16 (2CH), 130.77 (C), 137.61 (C), 154.82 (C), 166.08 (C), 168.72 (C).

For C₂₂H₃₀N₄O₂ calculated: 66.30% C, 7.59% H, 14.06% N; found: 66.32% C, 7.52% H, 14.02% N.

MS (FAB) [M + 1]⁺: *m/z* 399.

2-(4-Phenylpiperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5d)

IR (KBr) ν_{\max} (cm⁻¹): 3284 (amide N-H), 3053 (aromatic C-H), 2958 (aliphatic C-H), 1676 (amide C=O), 1596–1412 (C=C and C=N), 1302–1025 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.67–2.69 (4H, m, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.20–3.22 (6H, m,

piperazine C-H and CO-CH₂), 6.78 (1H, t, *J* = 7.2, 7.2, Ar-H), 6.94 (2H, d, *J* = 7.6 Hz, Ar-H), 7.21 (2H, t, *J* = 8, 8 Hz, Ar-H), 7.71 (2H, d, *J* = 8.8 Hz, Ar-H), 7.81 (1H, s, thiazole C₅-H), 7.88 (2H, d, *J* = 8.8 Hz, Ar-H), 9.85 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.60 (CH₃), 53.52 (2CH₂), 53.65 (2CH₂), 62.48 (CH₂), 113.18 (CH), 116.12 (2CH), 119.02 (CH), 120.78 (2CH), 127.49 (2CH), 129.14 (2CH), 130.15 (C), 138.92 (C), 151.28 (C), 154.27 (C), 166.22 (C), 167.95 (C).

For C₂₂H₂₄N₄O₂S calculated: 67.32% C, 6.16% H, 14.27% N; found: 67.30% C, 6.14% H, 14.26% N.

MS (FAB) [M + 1]⁺: *m/z* 393.

2-[4-(4-Methylphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5e)

IR (KBr) ν_{\max} (cm⁻¹): 3285 (amide N-H), 3012 (aromatic C-H), 2963 (aliphatic C-H), 1678 (amide C=O), 1595–1413 (C=C and C=N), 1294–1026 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.18 (3H, s, C-CH₃), 2.64–2.66 (4H, m, piperazine C-H), 2.69 (3H, s, C-CH₃), 3.12–3.14 (4H, m, piperazine C-H), 3.18 (2H, s, CO-CH₂), 6.83 (2H, d, *J* = 8.8 Hz, Ar-H), 7.00 (2H, d, *J* = 8 Hz, Ar-H), 7.69 (2H, d, *J* = 8, 4 Hz, Ar-H), 7.80 (1H, s, thiazole C₅-H), 7.86 (2H, d, *J* = 8.8 Hz, Ar-H), 9.82 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.60 (CH₃), 21.33 (CH₃), 53.47 (2CH₂), 55.84 (2CH₂), 62.73 (CH₂), 113.39 (CH), 120.56 (2CH), 121.56 (2CH), 127.73 (2CH), 130.73 (C), 132.48 (2CH), 138.92 (C), 139.46 (C), 141.63 (C), 154.54 (C), 166.79 (C), 168.78 (C).

For C₂₃H₂₆N₄O₂S calculated: 67.95% C, 6.45% H, 13.78% N; found: 67.93% C, 6.47% H, 13.74% N.

MS (FAB) [M + 1]⁺: *m/z* 407.

2-[4-(4-Methoxyphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5f)

IR (KBr) ν_{\max} (cm⁻¹): 3282 (amide N-H), 3050 (aromatic C-H), 2943 (aliphatic C-H), 1679 (amide C=O), 1585–1414 (C=C and C=N), 1305–1018 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.76 (3H, s, C-CH₃), 2.80 (4H, t, *J* = 4.8, 4.8 Hz, piperazine C-H), 3.17 (4H, t, *J* = 4.8, 4.8 Hz, piperazine C-H), 3.21 (2H, s, CO-CH₂), 3.78 (3H, s, O-CH₃), 6.86 (2H, d, *J* = 8.4 Hz, Ar-H), 6.92 (2H, d, *J* = 8.8 Hz, Ar-H), 7.25 (1H, s, thiazole C₅-H), 7.64 (2H, d, *J* = 8, 8 Hz, Ar-H), 7.85 (2H, d, *J* = 8.4 Hz, Ar-H), 9.20 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.32 (CH₃), 50.91 (2CH₂), 53.66 (2CH₂), 55.56 (CH₃), 62.02 (CH₂), 111.61 (CH), 114.51 (2CH), 118.35 (2CH), 119.48 (2CH), 126.98 (2CH), 130.67 (C), 137.32 (C), 145 (C), 154.08 (C), 154.60 (C), 165.85 (C), 168.14 (C).

For C₂₃H₂₆N₄O₂S calculated: 65.38% C, 6.20% H, 13.26% N; found: 65.34% C, 6.23% H, 13.24% N.

MS (FAB) [M + 1]⁺: *m/z* 423.

2-[4-(4-Chlorophenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5g)

IR (KBr) ν_{\max} (cm⁻¹): 3279 (amide N-H), 3052 (aromatic C-H), 2947 (aliphatic C-H), 1673 (amide C=O), 1579–1445 (C=C and C=N), 1289–1030 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.67 (4H, t, *J* = 4.4, 4.8 Hz, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.21 (4H, t, *J* = 4.4, 4.4 Hz, piperazine C-H), 3.35 (2H, s, CO-CH₂), 6.95 (2H, d, *J* = 8.8 Hz, Ar-H), 7.23 (2H, d, *J* = 8.8 Hz, Ar-H), 7.72 (2H, t, *J* = 9.2 Hz, Ar-H), 7.81 (1H, s, thiazole C₅-H), 7.88 (2H, d, *J* = 9.2 Hz, Ar-H), 9.85 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.59 (CH₃), 52.47 (2CH₂), 54.88 (2CH₂), 62.73 (CH₂), 113.77 (CH), 120.14 (2CH), 121.58 (2CH), 127.56 (2CH), 128.49 (C), 130.23 (C), 132.69 (2CH), 138.86 (C), 152.63 (C), 154.16 (C), 166.45 (C), 168.89 (C).

For C₂₂H₂₃ClN₄O₂S calculated: 61.89% C, 5.43% H, 13.12% N; found: 61.84% C, 5.41% H, 13.00% N.

MS (FAB) [M + 1]⁺: *m/z* 427.5.

2-[4-(4-Florophenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5h)

IR (KBr) ν_{\max} (cm⁻¹): 3277 (amide N-H), 3044 (aromatic C-H), 2973 (aliphatic C-H), 1680 (amide C=O), 1589–1410 (C=C and C=N), 1296–1012 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.68 (4H, t, *J* = 4.4, 4.8 Hz, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.16 (4H, t, *J* = 4.4, 4.4 Hz, piperazine C-H), 3.21 (2H, s, CO-CH₂), 6.92–7.07 (4H, m, Ar-H), 7.71 (2H, d, *J* = 9.2 Hz, Ar-H), 7.82 (1H, s, thiazole C₅-H), 7.88 (2H, d, *J* = 8.8 Hz, Ar-H), 9.85 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.54 (CH₃), 52.47 (2CH₂), 54.49 (2CH₂), 62.79 (CH₂), 113.25 (CH), 115.62 (2CH), 117.83 (2CH), 120.88 (2CH), 127.83 (2CH), 130.45 (C), 138.86 (C), 147.80 (C), 154.16 (C), 158.32 (C), 166.45 (C), 168.89 (C).

For C₂₂H₂₃FN₄O₂S calculated: 64.37% C, 5.65% H, 13.65% N; found: 64.40% C, 5.61% H, 13.62% N.

MS (FAB) [M + 1]⁺: *m/z* 411.

2-[4-(4-Nitrophenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5i)

IR (KBr) ν_{\max} (cm⁻¹): 3278 (amide N-H), 3020 (aromatic C-H), 2943 (aliphatic C-H), 1677 (amide C=O), 1598–1477 (C=C and C=N), 1311–1045 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.68 (4H, t, *J* = 4.4, 4.8 Hz, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.34 (2H, s, CO-CH₂), 3.55 (4H, t, *J* = 5.2, 4.4 Hz, piperazine C-H), 7.04 (2H, d, *J* = 8.8 Hz, Ar-H), 7.71 (2H, d, *J* = 8.8 Hz, Ar-H), 7.81 (1H, s, thiazole C₅-H), 7.88 (2H, d, *J* = 8.4 Hz, Ar-H), 8.06 (2H, d, *J* = 9.2 Hz, Ar-H), 9.88 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.61 (CH₃), 53.96 (2CH₂), 55.88 (2CH₂), 62.49 (CH₂), 112.14 (2CH), 113.29 (CH), 120.83 (2CH), 127.25 (2CH), 127.93 (2CH), 130.89 (C), 138.92 (C), 139.52 (C), 154.21 (C), 156.19 (C), 166.56 (C), 168.78 (C).

For C₂₂H₂₃N₅O₃S calculated: 60.39% C, 5.30% H, 16.01% N; found: 60.36% C, 5.32% H, 16.03% N.

MS (FAB) [M + 1]⁺: *m/z* 438.

2-[4-(3-Methylphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5j)

IR (KBr) ν_{\max} (cm⁻¹): 3282 (amide N-H), 3041 (aromatic C-H), 2963 (aliphatic C-H), 1683 (amide C=O), 1589–1427 (C=C and C=N), 1311–1040 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.33 (3H, s, C-CH₃), 2.76 (3H, s, C-CH₃), 2.80 (4H, t, *J* = 4.8, 4.8 Hz, piperazine C-H), 3.21 (2H, s, CO-CH₂), 3.26 (4H, t, *J* = 4.8, 4.8 Hz, piperazine C-H), 6.71–6.77 (3H, m, Ar-H), 7.17 (1H, t, *J* = 7.6, 7.6 Hz, Ar-H), 7.25 (1H, s, thiazole C₅-H), 7.63 (2H, t, *J* = 9.2 Hz, Ar-H), 7.85 (2H, d, *J* = 8.4 Hz, Ar-H), 9.20 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 19.33 (CH₃), 21.77 (CH₃), 49.59 (2CH₂), 53.58 (2CH₂), 62.04 (CH₂), 111.65 (CH), 113.40 (CH), 117.13 (CH), 119.47 (2CH), 121.09 (CH), 127.01 (2CH), 129.04 (C), 130.69 (C), 137.29 (CH) 138.92 (C), 151.05 (C), 154.60 (C), 165.85 (C), 168.10 (C).

For C₂₃H₂₆N₄O₂S calculated: 67.95% C, 6.45% H, 13.78% N; found: 67.91% C, 6.48% H, 13.72% N.

MS (FAB) [M + 1]⁺: *m/z* 407.

2-[4-(3-Methoxyphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5k)

IR (KBr) ν_{max} (cm⁻¹): 3281 (amide N-H), 3043 (aromatic C-H), 2967 (aliphatic C-H), 1681 (amide C=O), 1578–1419 (C=C and C=N), 1289–1013 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.66 (4H, t, *J* = 4.8, 4.8, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.20–3.22 (4H, m, piperazine C-H), 3.32 (2H, s, CO-CH₂), 3.71 (3H, s, O-CH₃), 6.35–6.54 (3H, m, Ar-H), 7.11 (1H, t, *J* = 8, 8.2 Hz) 7.71 (2H, d, *J* = 9.2 Hz, Ar-H), 7.81 (1H, s, thiazole C₅-H), 7.87 (2H, d, *J* = 8.8 Hz, Ar-H), 9.84 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 19.53 (CH₃), 53.42 (2CH₂), 55.64 (2CH₂), 55.90 (CH₃), 62.88 (CH₂), 103.50 (CH), 106.12 (CH), 109.45 (CH), 113.79 (CH), 120.46 (2CH), 127.09 (2CH), 130.14 (CH), 130.18 (C), 138.89 (C), 150.11 (C), 154.17 (C), 160.15 (C), 166.78 (C), 168.49 (C).

For C₂₃H₂₆N₄O₂S calculated: 65.38% C, 6.20% H, 13.26% N; found: 65.37% C, 6.21% H, 13.25% N.

MS (FAB) [M + 1]⁺: *m/z* 423.

2-[4-(2-Methylphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5l)

IR (KBr) ν_{max} (cm⁻¹): 3280 (amide N-H), 3046 (aromatic C-H), 2974 (aliphatic C-H), 1679 (amide C=O), 1599–1402 (C=C and C=N), 1299–1004 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.31 (3H, s, C-CH₃), 2.76 (3H, s, C-CH₃), 2.80 (4H, t, *J* = 4.8, 4.8 Hz, piperazine C-H), 3.01 (4H, t, *J* = 4.4, 4 Hz, piperazine C-H), 3.23 (2H, s, CO-CH₂), 6.99–7.07 (2H, m, Ar-H), 7.19 (2H, t, *J* = 6, 6 Hz, Ar-H), 7.25 (1H, s, thiazole C₅-H), 7.65 (2H, d, *J* = 8 Hz, Ar-H), 7.86 (2H, d, *J* = 8 Hz, Ar-H), 9.26 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 18.08 (CH₃), 19.54 (CH₃), 52.20 (2CH₂), 54.30 (2CH₂), 62.34 (CH₂), 111.87 (CH), 119.258 (2CH), 119.78 (CH), 123.72 (CH), 126.84 (CH), 127.24 (2CH), 130.89 (C), 131.41 (CH), 132.94 (C), 137.50 (CH), 151.27 (C), 154.83 (C), 166.14 (C), 168.61 (C).

For C₂₃H₂₆N₄O₂S calculated: 67.95% C, 6.45% H, 13.78% N; found: 67.94% C, 6.49% H, 13.72% N.

MS (FAB) [M + 1]⁺: *m/z* 407.

2-[4-(2-Methoxyphenyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5m)

IR (KBr) ν_{max} (cm⁻¹): 3281 (amide N-H), 3054 (aromatic C-H), 2976 (aliphatic C-H), 1680 (amide C=O), 1596–1414 (C=C and C=N), 1301–1019 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.66–2.70 (4H, m, piperazine C-H), 2.71 (3H, s, C-CH₃), 3.04 (4H, brs, piperazine C-H), 3.32 (2H, s, CO-CH₂), 3.77 (3H, s, O-CH₃), 6.85–6.95 (4H, m, Ar-H), 7.72 (2H, d, *J* = 8.4 Hz, Ar-H), 7.86 (1H, s, thiazole C₅-H), 7.88 (2H, d, *J* = 8 Hz, Ar-H), 9.85 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 19.66 (CH₃), 53.79 (2CH₂), 55.90 (2CH₂), 56.45 (CH₃), 62.02 (CH₂), 112.54 (CH), 113.56 (CH), 118.75 (CH), 120.79 (2CH), 122.64 (CH), 124.86 (CH), 127.89 (2CH), 130.79 (C), 139.52 (C), 142.14 (C), 151.46 (C), 154.17 (C), 166.52 (C), 168.99 (C).

For C₂₃H₂₆N₄O₂S calculated: 65.38% C, 6.20% H, 13.26% N; found: 65.36% C, 6.21% H, 13.26% N.

MS (FAB) [M + 1]⁺: *m/z* 423.

2-[4-(2-Pyridyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5n)

IR (KBr) ν_{max} (cm⁻¹): 3278 (amide N-H), 3044 (aromatic C-H), 2978 (aliphatic C-H), 1678 (amide C=O), 1595–1407 (C=C and C=N), 1307–1005 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.73–2.75 (4H, m, piperazine C-H), 2.76 (3H, s, C-CH₃), 3.21 (2H, s, CO-CH₂), 3.63 (4H, t, *J* = 5.2, 4.8 Hz piperazine C-H), 6.65–6.68 (2H, m, Ar-H), 7.26 (1H, s, thiazole C₅-H), 7.50 (1H, t, *J* = 7.6, 4.8 Hz, Ar-H), 7.63 (2H, d, *J* = 8.4 Hz, Ar-H), 7.85 (2H, d, *J* = 8.8 Hz, Ar-H), 8.21 (1H, d, *J* = 5.2 Hz, Ar-H), 9.85 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 19.32 (CH₃), 45.52 (2CH₂), 53.34 (2CH₂), 62.13 (CH₂), 107.26 (CH), 111.62 (CH), 113.81 (CH), 127.01 (2CH), 130.71 (C), 137.28 (CH), 137.57 (C), 148.01 (CH), 154.59 (C), 159.32 (C), 165.85 (C), 168.98 (C).

For C₂₁H₂₃N₅O₂S calculated: 64.10% C, 5.89% H, 17.80% N; found: 64.14% C, 5.87% H, 17.83% N.

MS (FAB) [M + 1]⁺: *m/z* 394.

2-[4-(2-Benzyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5o)

IR (KBr) ν_{max} (cm⁻¹): 3276 (amide N-H), 3023 (aromatic C-H), 2986 (aliphatic C-H), 1676 (amide C=O), 1598–1421 (C=C and C=N), 1301–1027 (C-N and C-O).

^1H NMR (400 MHz, DMSO-*d*₆): 2.50–2.65 (8H, brs, piperazine C-H), 2.77 (3H, s, C-CH₃), 3.14 (2H, s, CO-CH₂), 3.55 (2H, s, N-CH₂-C), 7.25–7.34 (6H, m, thiazole C₅-H and Ar-H), 7.62 (2H, d, *J* = 9.2 Hz, Ar-H), 7.85 (2H, d, *J* = 9.2 Hz, Ar-H), 9.21 (1H, s, N-H).

^{13}C NMR (100 MHz, DMSO-*d*₆): 19.59 (CH₃), 53.48 (2CH₂), 53.80 (2CH₂), 62.22 (CH₂), 63.17 (CH₂), 111.82 (CH), 119.72 (2CH), 127.21 (2CH), 127.43 (CH), 128.54 (2CH), 129.37 (2CH), 130.81 (C), 137.59 (CH), 138.14 (C), 154.86 (C), 166.11 (C), 168.67 (C).

For C₂₃H₂₆N₄O₂S calculated: 67.95% C, 6.45% H, 13.78% N; found: 67.93% C, 6.41% H, 13.74% N.

MS (FAB) [M + 1]⁺: *m/z* 407.

2-[4-(2-Furoyl)piperazine-1-yl]-N-[4-(2-methylthiazol-4-yl)phenyl]acetamide (5p)

IR (KBr) ν_{\max} (cm⁻¹): 3288 (amide N-H), 3056 (aromatic C-H), 2969 (aliphatic C-H), 1679 (amide C=O), 1599–1427 (C=C and C=N), 1280–1020 (C-N and C-O).

¹H NMR (400 MHz, DMSO-*d*₆): 2.71 (4H, t, *J* = 4.4, 4.8 Hz, piperazine C-H), 2.77 (3H, s, C-CH₃), 3.21 (2H, s, CO-CH₂), 3.92 (4H, brs, piperazine C-H), 6.49–6.50 (1H, m, furan C₄-H), 7.05 (1H, d, *J* = 3.6 Hz, furan C₃-H), 7.27 (1H, s, thiazole C₅-H), 7.49 (1H, s, furan C₅-H), 7.63 (2H, d, *J* = 8.4 Hz, Ar-H), 7.87 (2H, d, *J* = 8.8 Hz, Ar-H), 9.06 (1H, s, N-H).

¹³C NMR (100 MHz, DMSO-*d*₆): 19.56 (CH₃), 53.83 (2CH₂), 55.31 (2CH₂), 62.30 (CH₂), 113.66 (CH), 111.93 (CH), 117.13 (CH), 119.81 (2CH), 127.27 (2CH), 131.09 (C), 137.34 (C), 144.05 (CH), 148.01 (C), 154.27 (C), 159.34 (C), 166.02 (C), 168.98 (C).

For C₂₁H₂₂N₄O₃S calculated: 61.44% C, 5.40% H, 13.65% N; found: 61.40% C, 5.43% H, 13.68% N.

MS (FAB) [M + 1]⁺: *m/z* 411.

AChE/BChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test²⁶ in order to evaluate their potency to inhibit AChE and BuChE. 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), BChE, (E.C. 3.1.1.8, from horse serum, 1000 units) and donepezil hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) and butyrylthiocholine iodide (BTC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV-Vis spectrophotometer.

Cholinesterase activity of the compounds (**5a–5p**) was measured in 100 mM phosphate buffer (pH 8.0) at 25°C, using ATC and BTC (75 mM) as substrates. In both cases, DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control²⁷.

Enzymatic assay

Enzyme solutions were prepared in gelatine solution (1%), at a concentration of 2.5 units/mL. AChE or BChE solution (50 μL) 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 was 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:

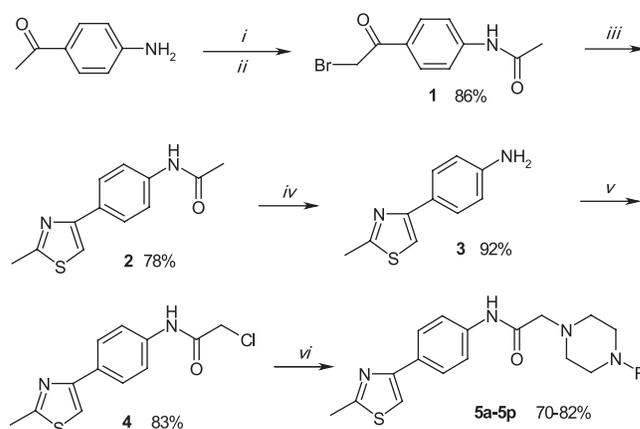
$$\text{Inhibition \%} = (A_C - A_I) / A_C \times 100$$

where *A*_I is the absorbance in the presence of the inhibitor, *A*_C is the absorbance of the control and *A*_B is the absorbance of blank reading. Both of the values were corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data was expressed as Mean ± SD.

Results and discussion

Chemistry

In the present study, some 2-(4-substituted piperazin-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide derivatives (**5a–5p**) were synthesized. Target compounds were obtained at five steps. Initially, 4-aminoacetophenone was acetylated with acetyl chloride to obtain *N*-(4-acetylphenyl)acetamide. Thus, amine group was protected in further ring closure step. *N*-(4-acetylphenyl)acetamide was brominated to afford *N*-[4-(2-bromoacetyl)phenyl]acetamide (**1**). Reaction of compound **1** with thioacetamide gave the *N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (**2**), which was deacetylated in 10% HCl solution to 4-(2-methyl-4-thiazolyl)aniline (**3**) in the next reaction step. 2-Chloro-*N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (**4**) was obtained via acetylation of compound **3** with chloroacetyl chloride. At the final reaction step, corresponding *N*-substituted piperazines were reacted with compound **4** to achieve 2-(4-substituted piperazine-1-yl)-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide derivatives (**5a–5p**). Synthetic protocol for the compounds is outlined in Scheme 1. Some characteristics of the synthesized compounds are presented in Table 1.



Scheme 1. Synthesis of the compounds (**5a–5p**). Reagents: (i) acetyl chloride, TEA, THF, 0–5°C; (ii) Br₂, AcOH; (iii) thioacetamide, EtOH, r.t.; (iv) 1 N HCl, EtOH, reflux; (v) chloroacetyl chloride, TEA, THF, r.t.; (vi) appropriate 4-substituted piperazine, K₂CO₃, acetone, reflux.

Structure elucidations of the final compounds were performed with IR, ^1H NMR and FAB-MS spectroscopic methods and elemental analysis. Characteristic stretching absorption of C=O groups were observed at 1683–1673 cm^{-1} . The stretching absorption at about 3288–3276, 1599–1402 and 1311–1004 cm^{-1} were recorded for N-H bonds, C=C and C=N double bonds, and C-O and C-N bonds, respectively. In the ^1H NMR spectra, all of the aromatic and aliphatic protons were observed at estimated areas. N-H and CH protons of the acetamide moiety gave peaks at about δ 9.88–9.06 and 3.35–3.09 ppm as singlet, respectively. The C_5 -H proton of the thiazole was observed as a singlet at δ 7.86–7.22. Methyl protons on the thiazole ring gave a singlet at δ 2.77–2.18 ppm. Protons of piperazine ring were resonated at δ 3.92–2.35 largely as two different triplets. Two doublets belonging to aromatic protons of 1,4-disubstituted phenyl ring were observed at δ 7.88–7.59 ppm. In the ^{13}C NMR spectra, the signal of characteristic carbonyl carbon was seen at about δ 166 ppm. Methylene (CH_2) carbon, which was bonded to carbonyl was seen at about δ 62.02–63.06 ppm. Methyl carbon on the thiazole ring was appeared at about δ 19.32–19.66 ppm. The carbons of the piperazine were seen at δ 45.52–55.84 ppm as two different peaks. $M + 1$ peaks in MS spectra were in agreement with the calculated molecular weight of the target compounds. Elemental analysis results for C, H and N elements were satisfactory within $\pm 0.4\%$ calculated values of the compounds.

Enzymatic activity

Synthesized compounds were assayed by Ellman's method so as to investigate their inhibitory activity against AChE and BChE. Results are given in Table 2. Test compounds were found to be inactive on BChE. On the other hand, they inhibited AChE to different extents. IC_{50}

values of the compounds **5d–5i**, **5k**, and **5l** could not be calculated due to their poor inhibition potency, which did not exceed the 50% even at the highest concentration (100 μM). However, the compounds **5a–5c**, and **5m** showed IC_{50} values in the range of 6.34–8.42 μM . Besides, the compounds **5n**, **5o**, and **5p** indicated significant AChE inhibitory activity, which is comparable with that of donepezil. The compound **5n**, containing 2-pyridyl moiety at 4th position of piperazine ring, displayed similar inhibitory potency with the reference drug. Furthermore, IC_{50} (0.11 μM) of 4-benzylpiperazine fragment bearing compound **5o** was five-fold lower than that of donepezil.

Observed results prompted us to explore a relationship between AChE inhibitory activity and chemical structures of the compounds. The compounds **5d–5m**, which carry *N*-(substituted-phenyl)piperazines as variable side groups showed poorest AChE inhibitory potency in the series. In these compounds, substitution of phenyl ring on different positions with methyl, methoxy, chloro or nitro groups did not influence the enzymatic activity. The reason of ineffectiveness of the compounds **5d–5m** may be related with electron withdrawing character of the phenyl ring, which decreases electron density on the piperazine moiety. In a previous study, electron donating effect was reported as the most important factor on the benzyl benzene ring, suggesting a role in regulating the protonation equilibrium at the benzylic nitrogen of the piperazine skeleton [24]. This report may be useful for explanation of inhibition potency differences of the compounds (**5a–5p**) against AChE. Contrary the phenyl substituent, aliphatic side groups at 4th position of piperazine as methyl, ethyl, cyclohexyl and benzyl enhance the AChE inhibitory activity to different extents. In addition, *N*-substitution of piperazine with heteroaryls as 2-pyridyl or 2-furoyl also increases the biological activity. However, similar to phenyl ring, heteroaryl moieties

Table 2. Percentage AChE and BChE inhibition of the compounds and IC_{50} values.

Comp.	AChE Inhibition (%)				BChE Inhibition (%)		
	100 μM	1 μM	0.01 μM	IC_{50} (μM)	100 μM	1 μM	IC_{50} (μM)
5a	69.42 \pm 5.09	41.26 \pm 2.14	8.52 \pm 0.18	7.12 \pm 0.64	29.32 \pm 3.41	6.13 \pm 1.53	>100
5b	58.79 \pm 6.44	38.24 \pm 4.04	14.65 \pm 1.26	11.74 \pm 1.43	14.22 \pm 2.16	4.81 \pm 0.94	>100
5c	72.13 \pm 9.26	40.29 \pm 3.49	10.23 \pm 0.96	6.34 \pm 1.31	25.54 \pm 4.32	8.75 \pm 2.62	>100
5d	38.17 \pm 4.21	19.24 \pm 2.61	6.17 \pm 1.11	>100	18.36 \pm 0.87	4.43 \pm 0.82	>100
5e	21.49 \pm 3.22	16.42 \pm 1.28	6.31 \pm 1.34	>100	13.21 \pm 1.63	8.62 \pm 1.43	>100
5f	43.15 \pm 5.89	22.70 \pm 0.89	9.67 \pm 0.72	>100	18.37 \pm 1.44	3.56 \pm 0.45	>100
5g	39.71 \pm 5.27	18.47 \pm 1.67	8.29 \pm 2.07	>100	21.51 \pm 2.96	6.31 \pm 0.97	>100
5h	42.63 \pm 2.64	19.06 \pm 2.16	5.23 \pm 0.49	>100	16.23 \pm 1.84	7.14 \pm 0.68	>100
5i	30.48 \pm 4.25	14.28 \pm 1.81	6.41 \pm 1.34	>100	9.29 \pm 2.45	2.24 \pm 1.24	>100
5j	58.36 \pm 7.34	27.42 \pm 2.28	13.96 \pm 2.16	88.35 \pm 6.88	16.33 \pm 1.47	7.24 \pm 1.16	>100
5k	34.24 \pm 2.51	11.74 \pm 4.16	4.89 \pm 1.44	>100	12.81 \pm 1.61	5.41 \pm 0.82	>100
5l	28.53 \pm 4.81	12.24 \pm 3.91	5.47 \pm 0.28	>100	27.67 \pm 2.12	8.03 \pm 1.84	>100
5m	63.21 \pm 7.26	39.53 \pm 4.21	13.28 \pm 1.55	8.42 \pm 1.23	31.48 \pm 4.93	13.07 \pm 0.56	>100
5n	96.44 \pm 5.21	84.96 \pm 7.63	41.13 \pm 3.47	0.051 \pm 0.002	42.09 \pm 5.16	18.89 \pm 2.61	>100
5o	99.83 \pm 4.42	87.70 \pm 6.75	49.22 \pm 4.57	0.011 \pm 0.001	46.35 \pm 5.08	21.12 \pm 1.15	>100
5p	89.70 \pm 6.37	63.61 \pm 4.19	29.16 \pm 1.84	0.27 \pm 0.001	39.27 \pm 1.19	14.69 \pm 0.76	>100
Donepezil	96.82 \pm 5.09	78.16 \pm 5.43	36.26 \pm 4.28	0.054 \pm 0.002	71.56 \pm 6.14	42.83 \pm 3.23	7.18 \pm 0.67

decrease the electron density of piperazine ring, but there is a significant difference between the inhibitory activity of the compounds **5d-5m** and **5n** and **5p**, which contain N-phenyl substituted piperazine derivatives and N-heteroaryl substituted piperazine derivatives, respectively. Thus, some other factors to electron donating or withdrawing characteristics of substituents need to be sought. In this case, polarizability and hydrogen bonding ability distances between phenyl and heteroaryl side groups may be suggested for the reason of enzymatic activity differences. Due to increasing polarizability and hydrogen bonding capability, interaction between AChE may be higher for N-heteroaryl substituted piperazine containing compounds **5n** and **5p** than the N-phenyl substituted piperazine bearing compounds (**5d-5m**). As a result of higher interaction, inhibitory activity enhancement may be occurred for the compounds **5n** and **5p**.

Declaration of interest

The authors report no declaration of interest.

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