Turkish Journal of Chemistry
http://journals.tubitak.gov.tr/chem/
Research Article

Turk J Chem
(2013) 37: 262 – 270

doi:10.3906/kim-1207-67

Synthesis and evaluation of acetylcholineesterase inhibitory potential and antioxidant activity of benzothiazine derivatives

Durre SHAHWAR,* Uzma SANA, Naeem AHMAD
Research Lab. II, Department of Chemistry, Government College University, Lahore-54000, Pakistan

Received: 28.07.2012 • **Accepted:** 31.01.2013 • **Published Online:** 17.04.2013 • **Printed:** 13.05.2013

Abstract: The aim of the present study was to synthesize and characterize benzothiazine derivatives prepared by using 2-aminothiophenol and saccharine and in vitro screen their enzyme inhibition and antioxidant potential. Nine different derivatives were prepared and their structures were confirmed by spectral studies (EIMS, ^{1}H and ^{13}C NMR). Enzyme inhibition potential of the synthesized compounds was evaluated by Ellman's method, while antioxidant activity was determined by DPPH and FRAP assays. All the derivatives showed remarkable activity against acetylcholine esterase with IC_{50} values in the range of 35.9–122.0 μM. All other derivatives, except compound **4a**, were inactive towards DPPH radical. The results of the FRAP assay showed significant reducing potential of compounds **1**, **2**, **2a**, and **4a**.

Key words: Acetylcholine esterase, benzothiazine, DPPH, FRAP

1. Introduction

Acetylcholinesterase (AChE) catalyzes hydrolysis of the neurotransmitter acetylcholine, which results in the termination of the nerve impulse in cholinergic synapse, and consequently induces several neurological disorders such as Alzheimer disease (AD) and Parkinson disease.[1] AChE inhibitors are the most effective approach to treat the cognitive symptoms of AD and other possible therapeutic applications in the treatment of Parkinson disease, senile dementia, and ataxia, among others.[2] Inhibition of AChE increases the neurotransmitters in the synaptic cleft and results in a positive cognitive effect in AD patients.[3] Acetylcholine acts as principle neurotransmitter in the central and peripheral nervous system; it plays a role in the transformation of information between a neuron and its adjacent cells (synaps).[4] AChE inhibitors such as tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of AD. However, due to their short half-lives or unfavorable side effects these drugs are known to have limitations for clinical use.[5]

Free radicals are implicated in oxidative stress reactions, which can damage cells and tissues and cause disorders in the immune system resulting in cancer, aging, and cardiovascular and neurodegenerative diseases.[6] Antioxidants, therefore, play an important role in disease prevention and health maintenance. To this extent, there is an increasing role of free radical mediated damage in human disease etiology.[7–9] Due to several side effects associated with the already available antioxidants and drugs for AD, there is a need for more selective and potent drugs.

Previous studies of the enzyme inhibition activity of benzothiazines indicated a very good binding affinity of these compounds towards cyclooxygenases that possess 2 active sites. Acetylcholine esterase also comprises

*Correspondence: drdshahwar@yahoo.com

2 active sites, an anionic subsite that binds with the quaternary amine of acetylcholine and an esteratic subsite where acetylcholine is hydrolyzed. We prepared various derivatives of benzothiazines to evaluate the binding affinity of these compounds towards the active sites of acetylcholine esterase. For future studies we propose docking calculations and superimposition of the best molecules with the co-crystallized ligand in the active site of AChE.

Benzothiazine derivatives have pronounced importance in pharmaceutical chemistry and organic synthesis. Many of these derivatives have been reported to possess a wide range of biological activities such as antibiotic, anticancer, antiviral, antifungal, antimicrobial, and antiparkinson properties.[10] Other studies on benzothiazines derivatives indicate that they possess antipyretic and analgesic activities higher than aspirin.[11,12] Studies of the structure–activity relationship of benzothiazine derivatives reveal that changing the structure of the substituent group commonly results in a change in its bioactivity. Substitution of the hydroxy group in benzothiazine derivatives enhances the effectiveness of these compounds against AD, acute neurodegenerative disorders, Pick disease, epilepsy, and ischemia.[13] Since modifications in basic structures cause changes in compound strength and can lead to the synthesis of many biologically active analogues, the present study was designed to synthesize 1,2- and 1,4-substituted benzothiazine derivatives that possess interesting biological activities.[14]

2. Materials and methods

2.1. Chemicals and instruments

All the chemicals and reagents used in the synthesis were purchased from Merck (Germany) and Panreac (Spain). The reactions were monitored by TLC using pre-coated silica gel cards. Infrared spectra were recorded as KBr disks using a PerkinElmer 735B infrared spectrophotometer. EI mass spectrometry was carried out using a MAT 312 instrument. ^{1}H NMR and ^{13}C NMR samples were prepared in CD_3OD containing TMS as an internal standard and spectra were recorded at 400 MHz and 100 MHz respectively using a Bruker Avance spectrometer. Folin–Ciocalteu (FC) reagent, DPPH, butylated hydroxy toluene (BHT), acetylthiocholine iodide, and 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) were purchased from Sigma (St. Louis, MO, USA), while erythrocytes (acetylcholine esterase) were obtained from the Biochemistry Lab, Mayo Hospital, Lahore.

2.2. Preparation of benzothiazine derivatives

2*H*-1,4-Benzothiazine-2, 3(4*H*)-dione (1): 1.07 mL (0.01 mol) of 2-aminothiophenol and 1.26 g (0.01 mol) of oxalic acid were refluxed for 2 h in 5 mL of xylene. The yellow precipitates obtained were filtered, dried, and recrystallized in methanol (mp = 180 °C, 65% yield).

Ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate (2): 15.2 mL (0.1 mol) of diethylmalonate and 10.8 mL (0.1 mol) of 2-aminothiophenol were taken in a 50-mL round bottom flask and refluxed for 1.5 h. The reaction was monitored with the help of TLC n-hexane and ethyl acetate (1:1). After the completion of the reaction, the precipitates were filtered, washed with distilled water, and dried. Recrystallization in methanol yielded yellow needle-like crystals (mp = 86 °C, 53% yield).[15]

Ethyl 4-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate (2a): 0.24 g (0.001 mol) of compound **2**, 5 mL of acetone, and 0.4 mL of 20% sodium hydroxide solution were taken in a round bottom flask. The reaction mixture was stirred for 5 min and then 2.9 mL of DMS was added, followed by refluxing for 30 min. The reaction was monitored with the help of TLC by using the solvent system ethyl acetate and chloroform (1:1). After the completion of the reaction, water was added and the organic layer extracted with

ethyl acetate. The solvent was evaporated and the precipitates obtained were recrystallized in ethyl acetate (mp = 130 °C, 64% yield).[16]

Ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxide (2b): 0.47 g (0.002 mol) of compound **2** was mixed with 40 mL of 50% glacial acetic acid, the mixture was stirred, then 0.31 g (0.002 mol) of potassium permanganate was added dropwise, and the stirring continued for 30 min. The progress of the reaction was monitored with the help of TLC by using the solvents n-hexane and ethyl acetate (1:1). After the completion of the reaction, sodium thiosulfate was added. The precipitates formed were filtered, washed with distilled water, and dried (mp = 160 °C, 60% yield).[17]

N-cyclohexyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine-2-carboxamide: 0.23 mL (0.002 mol) of cyclohexylamine was mixed with 0.44 g (0.002 mol) of compound **2** in 5 mL of ethanol. The reaction mixture was refluxed for 1 h and was monitored with the help of TLC by using n-hexane and ethyl acetate (1:1). The crude solid separated was filtered, washed with distilled water, and recrystallized in ethyl acetate (mp = 140 °C, 64% yield).[18]

3-Phenyl-2*H*-1,4-benzothiazine (3): 1.9 g (0.01 mol) of bromoacetophenone was mixed with 1.07 mL (0.01 mol) of 2-aminothiophenol, followed by 2 mL of triethylamine in 5 mL of ethanol. The reaction mixture was refluxed for 1 h. The precipitates formed were filtered, washed with water, dried, and recrystallized in methanol (mp = 185 °C, 53% yield).[19]

3-Phenyl-2*H*-1,4-benzothiazine 1,1-dioxide (3a): 0.24 g (2.1 mol) of **3** was mixed with 40 mL of glacial acetic acid. The mixture was stirred for 5 min, then 4.2 mmol of $KMnO_4$ was added dropwise, and the stirring was continued for 30 min. The progress of the reaction was monitored with the help of TLC by using ethyl acetate and n-hexane (1:1). After the completion of the reaction, 20% solution of sodium thiosulfate was added, and the precipitates formed were filtered, dried, and recrystallized in EtOAc (mp = 150 °C, 66% yield).[17]

2-(2-Oxo-2-phenylethyl)-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (4): 0.05 mol (10.2 g) of sodium saccharine was mixed with 10 g of bromoacetophenone in 30 mL of DMF. The reaction mixture was refluxed for 3 h and monitored with the help of TLC by using n-hexane and ethyl acetate (1:1) (mp = 120 °C, 68% yield).[20]

(4-Hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)(phenyl)methanone (4a): Sodium metal (0.14 mol, 3.3 g) was dissolved in 50 mL of methanol in a 250-mL round bottom flask. The white precipitates formed on evaporation were mixed with 15 mL of DMF. This solution was added to cold solution of **4** with stirring. After complete addition, stirring was continued for 30 min. The reaction was monitored with the help of TLC by using the solvent system ethyl acetate and n-hexane (1:1). After the completion of the reaction, the reaction mixture was poured into an ice cold solution of 50 mL of HCl (10%). The aqueous layer was extracted with ethyl acetate (mp = 140 °C, 55% yield).[20]

(4-Hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)(phenyl)methanone (4b): 82 g of compound **7** was mixed with 12.5 m of acetone and 2.2 mL of sodium hydroxide. The mixture was stirred for 5 min and then 1.5 mL of DMS was added, followed by refluxing for 30 min. The reaction was monitored with the help of TLC by using the solvent system ethyl acetate and chloroform (1:1). After the completion of the

reaction, water was added and the organic layer extracted with ethyl acetate. The solvent was evaporated and the crude solid was recrystallized in methanol (mp = 176 °C, 68% yield).[20]

2.3. In vitro AChE inhibition assay

The inhibitory potential of synthetic compounds against acetylcholine esterase (AChE) was measured by spectrophotometer.[21] The reaction mixture was composed of 1.5 mL of (100 mM) Tris buffer (pH 7.8), 30 μL of DTNB, 100 μL (1 mg/mL) of sample, and 30 μL of acetyl cholinesterase solution (erythrocytes). The reaction mixture was incubated for 20 min at 25 °C, and then 30 μL of the substrate solution (acetylthiocholine) was added. At 412 nm hydrolysis of acetylthiocholine was measured over 30 min. The assay was conducted in triplicate. The inhibitory potential was calculated as follows:

$$\% \text{ inhibition } = \frac{E-S}{E} \times 100$$

where E is the activity of the enzyme without test sample and S is the activity of enzyme with test sample.

2.4. Determination of antioxidant activity

2.4.1. Scavenging assay of DPPH free radical

The radical scavenging activity of the synthesized compounds was determined by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical.[22,23] DPPH solution was prepared by adding 0.0025 g/mL to methanol. Then 200 μL of sample (1 mg/mL) was taken in a test tube containing 0.1 mL of DPPH solution, kept in darkness for 30 min, and absorbance was noted at 517 nm. The scavenging of free radical was calculated using the following formula:

$$\% \text{ inhibition of DPPH } = \frac{A-B}{A} \times 100$$

where A is the optical density of blank and B is the optical density of sample.

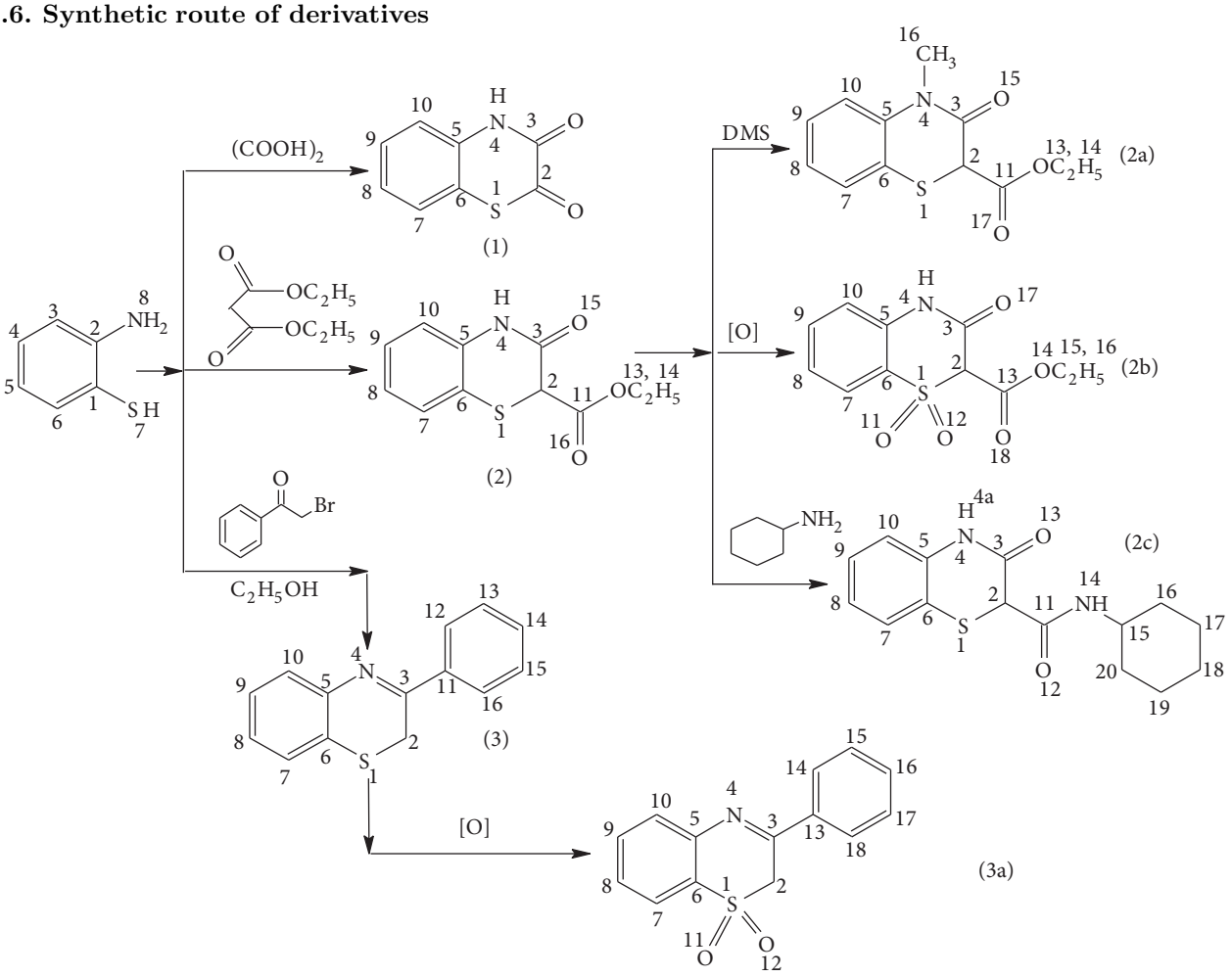
2.4.2. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power assay of the prepared compounds was carried out according to the method described by Benzi and Stain.[24] The FRAP reagent was composed of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine), and 20 mM $FeCl_3.6H_2O$ solution. FRAP reagent (150 μL) was mixed with sample (50 μL) and absorbance was read at 595 nm after 15 min. The results are expressed in μM equivalent to $FeSO_4.7H_2O$ by calculating from the calibration curve.

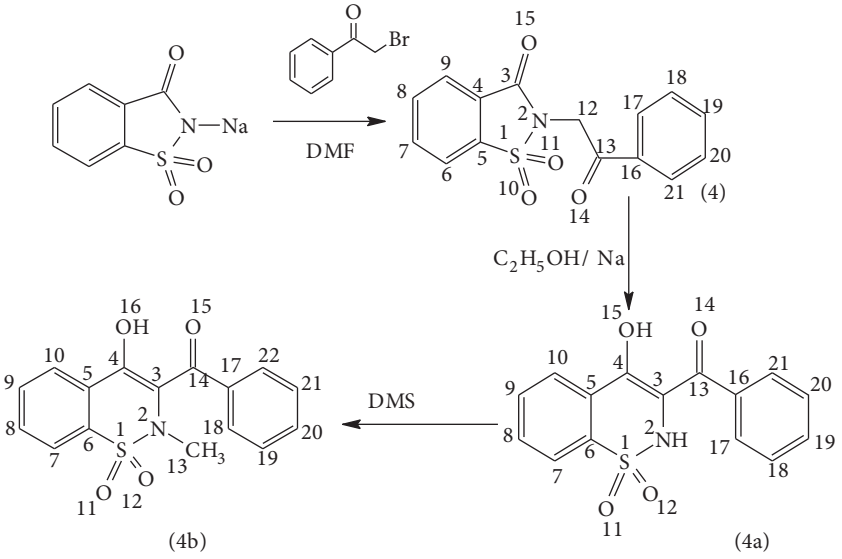
2.5. Statistical analysis

The experiments were repeated 3 times and data are presented as ±SD using MS Excel 2007 software.

2.6. Synthetic route of derivatives



Scheme 1. Synthesis of 1,4-benzothiazine derivatives.



Scheme 2. Synthesis of 1,2-benzothiazine derivatives.

2.7. Spectral values of benzothiazines

2*H*-1,4-benzothiazine-2,3(4*H*)-dione (1): IR (KBr, cm^{-1}) υ_{max}: 3220 (N-H), 1630 (C=O), 1234 (C=C). EIMS m/z (Int. rel., %): 179 (M+, 1), 84 (99), 56 (12), 41 (30). ^{1}H NMR (CD_3OD, 400 MHz) δ: 11.65 (1H, m, H-2), 7.33–7.60 (4H, m, H-7, H-8, H-9, H-10). ^{13}C NMR (CD_3OD, 100 MHz) δ: 184.01 (C-2), 171.08 (C-3), 147.37 (C-5) 121.1 (C-6), 128.6 (C-7), 124.5 (C-8), 136.4 (C-9), 118.3 (C-10).

Ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate (2): IR (KBr, cm^{-1}) υ_{max}: 3274 (N-H), 1720 (ester C=O), 1160 (C-O), 1480 (C=C). EIMS m/z (Int. rel., %): 43 (80), 57 (60), 71 (100), 95 (50), 123 (49). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.3–7.5 (4H, m, H-7, H-8, H-9, H-10), 4.9 (1H, m H-2), 3.54 (3H, m, H-16), 4.42 (2H, m, H-13), 1.22 (3H, m, H-14). ^{13}C NMR (CD_3OD, 100 MHz) δ: 48.7 (C-2), 162.77 (C-3), 147.2 (C-5), 122.8 (C-6), 128.2 (C-7), 123.3 (C-8), 135.8 (C-9), 117.12 (C-10), 170.9 (C-11), 61.4 (C-13), 13.9 (C-14).

Ethyl 4-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate (2a): IR (KBr, cm^{-1}) υ_{max}: 1340 (C-N). EIMS m/z (Int. rel., %): 196 (99), 169 (25), 133 (49), 105 (70), 76 (75), 50 (35). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.0–7.62 (4H, m, H-7, H-8, H-9, H-10), 5.03 (1H, s, H-2), 4.42 (2H, m, H-13), 1.23 (3H, m, H-14), 3.54 (3H, m, H-16). ^{13}C NMR (CD_3OD, 100 MHz) δ: 47.15 (C-2), 161.7 (C-3), 145.3 (C-8), 124.8 (C-9), 116.8 (C-10), 170.7 (C-11), 61.4 (C-13), 13.9 (C-14), 27.5 (C-16).

Ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxide (2b): IR (KBr, cm^{-1}) υ_{max}: 1280 (O=S=O). EIMS m/z (Int. rel., %): 226 (15), 211 (100), 119 (35), 91 (40), 39 (30). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.4–7.8 (4H, m, H-7, H-8, H-9, H-10), 5.2 (1H, s, H-2), 10.97 (H-4), 4.31 (2H, m, H-15), 1.24 (3H, m, H-16). ^{13}C NMR (CD_3OD, 100 MHz) δ: 68.3 (C-2), 156.4 (C-3), 146.7 (C-5), 127.3 (C-6), 126.8 (C-7), 124.7 (C-8), 141.1 (C-9), 117.8 (C-10), 164.1 (C-13), 61.8 (C-15), 13.9 (C-16).

N-cyclohexyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine-2-carboxamide (2c): IR (KBr, cm^{-1}) υ_{max}: 2900 (C-H), 1460 (CH_2 str.). EIMS m/z (Int. rel., %): 125 (100), 108 (5), 93 (30), 80 (49), 53 (10). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.2–7.5 (4H, m, H-7, H-8, H-9, H-10), 5.06 (1H, s, H-2), 9.26 (1H, m, H-4), 4.62 (1H, m, H-15), 0.2–0.6 (4H, m, H-16, H-20), 1.27–1.72 (6H, m, H-17, H-18, H-19). ^{13}C NMR (CD_3OD, 100 MHz) δ: 56.7 (C-2), 164.4 (C-3), 147.2 (C-5), 127.7 (C-6), 128.1 (C-7), 123.2 (C-8), 136.1 (C-9), 117.0 (C-10), 167.7 (C-11), 41.3 (C-15), 32.7 (C-16), 26.3 (C-17), 25.5 (C-18), 26.3 (C-19), 32.4 (C-20).

3-Phenyl-2*H*-1,4-benzothiazine (3): IR (KBr, cm^{-1}) υ_{max}: 1640 (C=N). EIMS m/z (Int. rel., %): 211 (100), 108 (33), 69 (20), 207 (30). ^{1}H NMR (CD_3OD, 400 MHz) δ: 4.5–4.6 (2H, m, H-2), 7.2–7.44 (4H, m, H-7, H-8, H-9, H-10), 7.4–7.9 (5H, m, H-12, H-13, H-14, H-15, H-16). ^{13}C NMR (CD_3OD, 100 MHz) δ: 25.5 (C-2), 159.5 (C-3), 143.9 (C-5), 125.7 (C-6), 127.2 (C-7), 128.8 (C-8), 129.2 (C-9), 123.4 (C-10), 126.9 (C-11), 129.8 (C-12), 129.6 (C-13), 131.9 (C-14), 129.6 C-15), 129.8 (C-16).

3-Phenyl-2*H*-1,4-benzothiazine 1,1-dioxide (3a): IR (KBr, cm^{-1}) υ_{max}: 1275 (O=S=O), 1640 (C=N). EIMS m/z (Int. rel., %): 257 (5), 215 (12.5), 198 (100), 167 (20), 43 (30). ^{1}H NMR (CD_3OD, 400 MHz) δ: 4.0–4.5 (2H, m, H-2), 7.3–7.8 (4H, m, H-7, H-8, H-9, H-10), 7.6–8.02 (5H, m, H-14, H-15, H-16, H-17, H-18). ^{13}C NMR (CD_3OD, 100 MHz) δ: 134.8 (C-2), 146.9 (C-3), 155.6 (C-5), 52.7 (C-6), 126.6 (C-7), 126.9 (C-8), 134.8 (C-9), 123.4 (C-10), 127.6 (C-13), 132.5 (C-14), 130.3 (C-15), 131.9 (C-16), 130.34 (C-17), 132.5 (C-18).

2-(2-Oxo-2-phenylethyl)-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (4): IR (KBr, cm^{-1}) υ_{max}:

1270 (O=S=O), 1760 (C=O). EIMS m/z (Int. rel., %): 210 (10), 118 (99), 91 (70), 65 (30). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.8–8.5 (4H, m, H-6, H-7, H-8, H-9), 5.33 (2H, m, H-12), 7.7–8.06 (5H, m, H-17, H-18, H-19, H-20, H-21). ^{13}C NMR (CD_3OD, 100 MHz) δ: 162.3 (C-3), 125.4 (C-4), 136.3 (C-5), 121.5 (C-6), 134.3 (C-7), 135.5 (C-8), 128.6 (C-9), 43.6 (C-12), 195.5 (C-13), 135.6 (C-16), 129.3 (C-17), 125.8 (C-18), 131.7 (C-19), 125.6 (C-20), 129.3 (C-21).

(4-Hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)(phenyl)methanone (4a): IR (KBr, cm^{-1}) υ_{max}: 3300 (OH), 3260 (N-H), 1700 (C=O). EIMS m/z (Int. rel., %): 301 (5), 118 (100), 91 (60), 65 (35). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.39 (2H, m, H-2, H-15), 7.7–8.00 (4H, m, H-7, H-8, H-9, H-10), 7.4–7.78 (1H, m, H-17, H-18, H-19, H-20, H-21). ^{13}C NMR (CD_3OD, 100 MHz) δ: 107.4 (C-3), 155.8 (C-4), 130.1 (C-5), 137.7 (C-6), 125.4 (C-7), 131.4 (C-8), 132.7 (C-9), 126.8 (C-10), 185.5 (C-13), 138.1 (C-16), 129.9 (C-17), 128.7 (C-18), 131.7 (C-19), 128.7 (C-20), 129.9 (C-21).

(4-Hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)(phenyl)methanone (4b): IR (KBr, cm^{-1}) υ_{max}: 3300 (OH), 1360 (C-N-), 1320 (O=S=O), 1695 (C=O) 1420 (C=C). EIMS m/z (Int. rel., %): 315 (12), 158 (90), 130 (65), 50 (35). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.74–7.98 (4H, m, H-7, H-8, H-9, H-10), 2.98 (3H, s, H-13), 12.8 (1H, m, H-16), 7.5–8.18 (5H, m, H-18, H-19, H-20, H-21, H-22). ^{13}C NMR (CD_3OD, 100 MHz) δ: 73.6 (C-3), 59.4 (C-4), 138.5 (C-5), 142.5 (C-6), 126.7 (C-7), 137.4 (C-8), 133.7 (C-9), 128.7 (C-10), 32.2 (C-13), 186.7 (C-14), 138.8 (C-17), 131.4 (C-18), 129.2 (C-19), 129.3 (C-20), 129.2 (C-21), 131.4 (C-22).

3. Results and discussion

Compounds **1** (2*H*-1, 4-benzothiazine-2, 3(4*H*)-dione) and **2** (ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate) were obtained through condensation followed by cyclization of 2-aminothiophenol with oxalic acid and ethyl malonate respectively in fairly good yield. N-methylation of **2** with DMS in alkaline medium gave ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxide (**2a**). Sulfooxidation of compound **2** with $KMnO_4$ in glacial acetic acid yielded ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxide

Table. Results of AChE inhibitory potential and antioxidant activity of benzothiazine derivatives.

Sample	AChE		DPPH		FRAP μM equivalent to $FeSO_4.7H_2O$
	% inhibition	IC_{50} (μM)	% inhibition	IC_{50} (μM)	
1	73.0 ± 1.5	62.1 ± 1.3	–	–	765 ± 1.2
2	83.3 ± 1.5	48.3 ± 1.1	–	–	720 ± 1.1
2a	81.2 ± 1.4	40.1 ± 0.8	–	–	700 ± 1.5
2b	65.6 ± 1.1	71.1 ± 1.0	–	–	163 ± 1.3
2c	62.4 ± 2.4	60.1 ± 1.5	–	–	156 ± 1.1
3	59.6 ± 1.8	122.0 ± 2.5	–	–	259 ± 1.4
3a	58.0 ± 1.5	69.1 ± 1.1	–	–	105 ± 1.2
4	70.2 ± 1.9	35.9 ± 1.0	–	–	78 ± 1.0
4a	63.6 ± 2.5	66.4 ± 1.5	87.6 ± 2.5	316.5± 3.5	886 ± 1.2
4b	72.6 ± 2.0	50.2 ± 1.3	–	–	153 ± 1.3
galanthamine	94.1 ± 2.4	2.7 ± 1.5	–	–	–
gallic acid	–	–	93.2 ± 0.9	5.2 ± 0.8	–

– = not calculated. All assays were performed in triplicate and SD (±) was calculated using MS Excel 2007 software.

(**2b**). Condensation of cyclohexylamine with compound **2** resulted in N-cyclohexyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine-2-carboxamide (**2c**). Reacting 2-aminothiophenol with bromophenone resulted in a quantitative yield of 3-phenyl-2*H*-1,4-benzothiazine (**3**), which on sulfooxidation with $KMnO_4$ yielded 3-phenyl-2*H*-1,4-benzothiazine 1,1-dioxide (**3a**). The reactions of 2-aminothiophenol are shown in Scheme 1.

Synthesis of 1,2 benzothiazine derivatives was carried out using saccharine as the starting material (Scheme 2). Refluxing of sodium saccharine and bromoacetophenone in DMF yielded 2-(2-oxo-2-phenylethyl)-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (**4**). Compound **4** was transformed into **4a** (4-hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3yl) (phenyl)methanone) through ring opening followed by intramolecular cyclization on treatment with cold sodium methoxide. (4-Hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)(phenyl) methanone (**4b**) was synthesized using dimethylsulfate in cold alkaline solution.

All the synthesized compounds were identified through spectral analysis (EIMS, ^{1}H and ^{13}C NMR). The ^{1}H NMR spectrum of compound **1** exhibited a typical pattern of aromatic protons for 1,2 disubstituted benzene at δ 7.33–7.60 as a multiplet. The presence of carbonyl groups was confirmed by 2 downfield signals at δ 184.01 (C-2) and 171.08 (C-3) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of compound **2** was characterized by the presence of a downfield singlet at δ 4.9, assigned to H-2. Typical signals of a CH_2-CH_3 group appeared at δ 4.42 (2H, q, H-13) and 1.24 (3H, m, H-14). The signal at δ 170.9 (C-11) in the ^{13}C NMR spectrum indicated ester moiety in the molecule. The structure of compound **2a** was confirmed by the presence of an additional singlet of the N-CH_3 group at δ 3.54. Compound **2b** showed stretching of the O=S=O group at 1280 cm^{-1} in the IR spectrum, which confirmed sulfooxidation in the molecule. Moreover, in the NMR spectrum, signals of H-2 and C-2 appeared in the downfield region at δ 5.2 and δ 68, which was in accordance with the sulfooxoide group in the neighborhood. The ^{1}H NMR spectrum of compound **2c** was characterized by the presence of methylene hydrogens at δ 5.06. The ^{13}C NMR spectrum exhibited a signal for the amide carbon at δ 167.7 (C-11). The ^{1}H NMR spectrum of compound **3** was characterized by 2 distinct pairs of 2H multiplets at δ 7.4–7.43 (H-12, H-16) and δ 7.9–7.92 (H-12-H-16), while a 1H signal at δ 7.6 was assigned to H-14. The signal at δ 4.67 was assigned to H-2. Compound **3a** was identified by the appearance of stretching of the O=S=O group at 1275 cm^{-1} in the IR spectrum, while the 2H signal of H-2 was further shifted downfield to δ 4.67. The IR spectrum of compound **4a** was characterized by the presence of broad peaks at 3300 and 3260 cm^{-1} due to OH and NH groups respectively and a strong signal of C=O at 1700 cm^{-1}. The ^{1}H NMR spectrum of **4b** exhibited an additional peak of methyl protons at δ 2.98, which confirmed N-methylation in compound **4a**. Acetylcholine esterase activity of the product (**2**) obtained after condensation of 2-aminothiophenol with diethyl malonate was most significant (% inhibition = 83.33 $\pm$ 3.51, IC_{50} = 48.3 μM). Further derivatives of this compound with N-cyclohexylamine and sulfooxidation showed a decrease in activity (**2c**, IC_{50} = 60.1 μM: **2b**, IC_{50} = 70.1 μM), while the N-methyl derivative of **2** showed similar results to the parent compound (**2a**, IC_{50} = 40.1 μM). Similar results were obtained for compound **4b** (IC_{50} = 50.2 μM), formed through cyclization followed by N-methylation of **4** (IC_{50} = 35.9 μM). These results suggested that the nitrogen of amide functionality does not interfere in the binding with AChE. Benzothiazine derivatives have shown a diverse range of biological activities,[12] but this is the first report of AChE inhibition activity of these compounds. Antioxidant activity of the synthesized compounds was measured using DPPH and FRAP assays. All compounds were inactive in the DPPH assay except compound **4a.** Ferric reducing ability of the derivatives as determined in the FRAP assay showed the highest reducing potential in compound **4a** (886 μM equivalent to $FeSO_4.7H_2O$). It has been reported in the literature that electron donating substituents enhance

antioxidant activity.[24,25] Therefore, the significant antioxidant potential of **4a** can be attributed to the presence of a hydroxyl group at C-4. Except compounds **1**, **2**, and **2a**, all compounds were moderately active in the FRAP assay (Table 1).

4. Conclusions

Benzothiazines exhibit a wide range of biological properties due to their unique structure; therefore, synthesis of benzothiazines is an area of current interest. In the present study, 9 different benzothiazine derivatives were prepared and identified by spectral analysis. The compounds were screened for their acetylecholine esterase and antioxidant activities and showed significant enzyme inhibition and moderate reducing properties. Among all the synthesized derivatives only compound **4a** exhibited radical scavenging potential. In conclusion, we have identified a series of benzothiazine derivatives as AChE inhibitors. Among the 1,4 derivatives, compound **2** displayed the most significant enzyme inhibition activity. Structurally related compounds to derivative **2** could be promising candidates for further research into AChE inhibitors.

References

1. Katzung, B. G. *Basic and Clinical Pharmacology*, McGraw-Hill, New York, 8th Ed., pp. 75–91, 2001.
2. Atta-ur-Rahman; Wahab, A. T.; Nawaz, S. A.; Choudhary, M. I. *Chem. Pharm. Bull.* **2004**, *52*, 802–806.
3. Kolari, D.; Wimmerova, L.; Kadek, R. *Phytopharmacol.* **2010**, *1*, 7–11.
4. Campbell, N. A.; Reece, J. B. *Biology*, Pearson, San Francisco, CA, 6th Ed., p. 1037, 2002.
5. Sung, S. Y.; Kang, S. Y.; Lee, K. Y.; Park, M. J.; Kim, J. H.; Park, J. H.; Kim, Y. C.; Kim, J.; Kim, Y. C. *Biol. Pharm. Bull.* **2002**, *25*, 125–127.
6. Priyadarsini, R.; Tamilarasi, K.; Karunambigai, A.; Gayathri, D. S. *Plant Arch.* **2010**, *10*, 815–818.
7. Ghous, T.; Aziz, N.; Abid, A.; Rasheed, A.; Iqbal, M. *J. Chem. Soc. Pak.* **2010**, *32*, 814–818.
8. Suresh, C.; Rao, J. V.; Jayaveera, K. N.; Reddy, G. J. *Int. J. Pharm. Biol. Sci.* **2011**, *1*, 409–413.
9. Gautam, N.; Ajmera, N.; Gupta, S.; Gautam, D. C. *Eur. J. Chem.* **2012**, *3*, 106–111.
10. Ramamoorthy, P.; Bono, A. *J. Eng. Sci. Tech.* **2007**, *2*, 70–80.
11. Khoobia, M.; Ramazania, A.; Foroumadib, A. R.; Hamadic, Z.; Hojjatia, Z.; Shafieeb, A. *J. Iran. Chem. Soc.* **2011**, *8*, 1036–1042.
12. Ajani, O. O. *Arch. De Pharmaz.* **2012**, *345*, 841–851.
13. Rathore, B. S.; Kumar, M. *Res. Chem. Intermed.* **2006**, *32*, 647–651.
14. Maleki, B.; Salehabadi, H. *Eur. J. Chem.* **2010**, *1*, 377–380.
15. Sheibani, H.; Islami, M. R.; Hassanpour, A.; Saidi, K. *Phos. Sulp. and Silic.* **2008**, *183*, 13–20.
16. Kalpana, G.; Singh, R. B.; Rajni, G.; Vandana, G.; Kumar, M. *Hetero. Commun.* **2003**, *9*, 120–124.
17. Gautam, N.; Gautam, D. C.; Gupta, R. R. *Hetero. Commun. 1997*, *3*, 130–134.
18. Zia-ur-Rehman, M.; Choudary, J. M.; Ahmad, S. *Bull. Korean Chem. Soc.* **2005**, *26*, 1771.
19. Deshmukh, M. B.; Mulik, A. R.; Desai, S. D. *E- J. Chem.* **2004**, *1*, 206–210.
20. Siddique, W. A.; Ahmad, S.; Ullah, I.; Malik, A. *J. Chem. Soc. Pak.* **2006**, *28*, 583.
21. Shahwar, D.; Raza, M. A.; Rehman, S. U.; Khan, T. *Asian J. Chem.* **2011**, *23*, 1783–1785.
22. Shahwar, D.; Raza, M. A.; Mirza, A. S. M.; Abbasi, M. A.; Ahmad, V. U. *J. Chem. Soc. Pak.* **2010**, *32*, 357–362.
23. Benzie, I. F.; Strain, J. J. *Methd. Enzymol.* **1999**, *299*, 15–27.
24. Eiserich, J. P.; Shibamoto, T. *J. Agric. Food Chem.* **1994**, *42*, 1060–1063.
25. Simic, A.; Manojlovic, D. S.; Egan, D.; Todorovic, M. *Molecules* **2007**, *12*, 2327–2340.