Synthesis, characterization and biological screening of *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2′′-sulfanyl acetamide

Sabahat Zahra Siddiqui[1*], Aziz-ur-Rehman[2], Muhammad Athar Abbasi[1], Nadia Abbas[1], Khalid Mohammed Khan[2], Muhammad Ashraf[3] and Syeda Abida Ejaz[4]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
[3]Department Biochemistry and Biotechnology, [4]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract: A series of new *N*-substituted derivatives of 5-benzyl-1, 3, 4-oxadiazole-2yl-2′′-sulfanyl acetamide (6a-n) were synthesized in three phases. The first phase involved the sequentially converting phenyl acetic acid into ester, hydrazide and finally cyclized in the presence of CS_2 to afford 5-benzyl-1, 3, 4-oxadiazole-2-thiol. In the second phase *N*-substituted-2-bromoacetamides were prepared by reacting substituted amines with bromoacetyl bromide in basic media. In the third phase, 5-benzyl-1,3,4-oxadiazole-2-thiol was stirred with *N*-substituted-2-bromoacetamides in the presence of *N,N*-dimethyl formamide (DMF) and sodium hydride (NaH) to get the target compounds. Spectral techniques were used to confirm the structures of synthesized compounds. Synthesized compounds were screened against butyrylcho linesterase (BChE), acetylcholinesterase (AChE), and lipoxygenase enzymes (LOX) and were found to be relatively more active against acetylcholinesterase.

Keywords: Phenyl acetic acid, 5-benzyl-1,3,4-oxadiazole-2-thiol, *N*-substituted 2-bromoacetamide, acetylcholinesterase, ^{1}H-NMR and EI-MS.

INTRODUCTION

Oxadiazoles belong to the group of heterocyclic compounds. For last two decades, oxadiazoles have been made a core of concentration due to their wide range of biological activities. Some of these compounds have exhibited antibacterial, anticancer and anticonvulsant activities. They are also being used to fight infections involving AIDS. 2,5-disubstituted-1,3,4-oxadiazoles exhibit important biological activities like anti-depressive (Piala and Yale, 1964), anti-convulsive (Almasirad *et al.,* 2004), analgesic (Angilini *et al.,* 1969), herbicidal (Kennedy and summers, 1981), muscle relaxant (Yale and Losee, 1966), pesticidal (Ram and Vlietinck, 1988), anti-malarial (Bahadur and Pandey, 1980), antitumor, anti-HCV activity (Rostom *et al.,* 2003), anti-inflammatory, as well as insecticidal activity (Boschelli *et al.,* 1993; Cansız *et al.,* 2004; Koparır *et al.,* 2005; Hemavathi *et al.,* 2011 and Kumar, 2011). Literature survey revealed that slight alteration in the structure of substituted 1,3,4-oxadiazole can lead to quantitative as well as qualitative changes in the biological activity (Amir *etal*., 2011).

Butyrylcholinesterase (BChE, EC 3.1.1.8) and Acetylcholinesterase (AChE, EC 3.1.1.7) consist of family of enzymes which includes serine hydrolases. The diverse specificities for the substrates and inhibitors for these enzymes are due to the differences in amino acid residues of the active sites of BChE and AChE. These enzymes are responsible for the termination of acetylcholine at cholinergic synapses (Cygler *et al.,* 1993 and Tougu, 2001). The major function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine as a result of which of the nerve impulse is terminated in cholinergic synapses. It is reported that BChE is present in comparatively lower amount in the normal age linked dementia of brains than in Alzheimer's plaques. H_1 and H_2 receptor antagonists possess AChE inhibitory activities. Cholinesterase inhibitors raise the quantity of acetylcholine available for neuronal and neuromuscular transmission through their ability to reversibly or irreversibly (Bertaccini, 1982 and Gauthier, 2001). Hence, it is considered imperative to search for new cholinesterase inhibitors and ongoing plan to bring in new drug candidates for the treatment of Alzheimer's disease and other related diseases.

The present research work consists of two phases, the first phase describes the preparation of 2-bromo-*N*-acetamide derivatives by vigorous shaking of different substituted amines with bromoacetyl bromide in basic medium (5% Na_2CO_3) and the second phase explains the synthesis of 5-benzyl-1,3,4-oxadiazole-2-thiol by refluxing hydrazide of phenylacetic acid with carbon disulfide in ethanol using potassium hydroxide as a base for 4hrs. Further the 2,5-disubsituted oxadiazole derivatives were synthesized in the presence of DMF and NaH.

**Corresponding author:* e-mail: atrabbasi@yahoo.com

MATERIALS AND METHODS

Chemical and instrumentation

All the chemicals and solvents utilized were of analytical grade and were purchased from authorized dealers of Merck and Fluka, Pakistan. Open capillary tubes were used to determine melting points of all the synthesized compounds and were found to be uncorrected. Melting point determination was performed on Griffin and George melting point apparatus. The purity of compounds was tested by thin layer chromatography technique on G-25-UV254on aluminum foil precoated silica gel plates using *n*-hexane and ethylacetate (75:25) as mobile phase. The I.R. spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). NMR spectra were recorded in MeOD on Bruker spectrometer operating at 300 MHz. Chemical shifts are given in ppm.

Synthesis of Phenylacetic acid ester (ethyl phenylacetate) (2)

(13.6 g; 0.1 moles) of Phenylacetic acid (1) was taken in a round bottom flask of 250mL capacity, fitted with a reflux condenser. (18.4 mL; 0.4 moles) of ethyl alcohol (absolute) and (4.9 mL; 0.05 moles) of concentrated sulphuric acid was further added in the reaction flask and reaction contents were stirred and refluxed for 3 hours. The completion of reaction was monitored by TLC method (*n*-hexane 75: ethyl acetate 25). The reaction mixture after completion was neutralized with 10% Na_2CO_3 solution to remove unreacted free acid and to obtain pure ester. The ester was purified by solvent extraction of neutralized reaction mixture with diethyl ether (20 mL×4) in a separating funnel. Ethereal layer was separated while the lower aqueous layer was discarded. Pure ester of phenylacetic acid (ethyl phenyl acetate) was obtained by distilling diethyl ether using rotary evaporator.

Synthesis of hydrazide of phenylacetic acid (3)

Ethyl phenylacetate (15 mL; 0.3 moles) and (10mL) methanol was taken in a 100mL round bottom flask. The reaction contents were cooled to 0-5^{o}C.Hydrazine hydrate (80% 15 ml; 0.3 moles) was added drop wise in reaction mixture which was further stirred at 0^{o}-5^{o}C for 60 min. Methanol was evaporated slowly at moderate temperature, after completion of reaction. Washing of crude precipitate of Phenylacetic acid hydrazide with *n*-hexane afforded pure product. TLC was performed to check the purity of product in (*n*-hexane 60: ethyl acetate 40).

Synthesis of 5-benzyl-1,3,4-oxadiazole-2-thiol (4)

In a 250mL round bottom flask (15.0 g, 0.1 moles) of Phenylacetic acid hydrazide (3) was dissolved in (25mL) of ethanol (absolute) (16.8g; 0.3 moles) of KOH was added further and contents of reaction were heated and stirred till partial dissolution of potassium hydroxide in alcohol. Carbon disulfide (CS_2) (8mL; 0.1 moles) was then slowly added to the reaction mixture. The reaction mixture was refluxed for 4hrs along with stirring. Evolution of hydrogen sulfide (H_2S) gas was observed during the reaction. After the completion, the reaction mixture was acidified to pH 3 with dilute HCl. Yellow precipitate of oxadiazole (5-benzyl-1,3,4-oxadiazole-2-thiol**)** thus obtained were filtered, washed with distilled water and dried in air. After recrystallization, pure 5-benzyl-1, 3, 4-oxadiazole-2-thiol was obtained from ethyl alcohol. Purity of the product was tested by TLC method (*n*-hexane 60: ethyl acetate 40) which showed a single spot.

General procedure for the synthesis of substituted 2-bromo-N-acetamide derivatives (5a-n)

Various substituted amines (0.005 moles) were taken in an iodine flask containing 10 mL of distilled water and 5% Na_2CO_3 solution. Bromoacetyl bromide (0.005 moles) was further poured drop wise in the iodine flask containing different substituted amine in mildly basic medium. The iodine flask was vigorously shaken (manually) till the formation of solid precipitates of substituted of 2-bromo-*N*-acetamide derivatives. Product was filtered and washed with distilled water, then dried in air. Purity was confirmed by TLC (*n*-hexane 70: ethyl acetate 30).

General procedure for the synthesis of S-substituted derivatives of 5-benzyl-1, 3, 4-oxadiazole-2-thiol (6a-n)

5-benzyl-1,3,4-oxadiazole-2-thiol (0.001 moles) was dissolved in DMF (*N,N*-dimethyl formamide) (10mL) in a 50mL RB flask. Lithium hydride (0.002 moles) was added to the reaction mixture and was stirred at room temperature for 20 min. 2-bromo-*N*-acetamide derivatives (0.001 moles) were further added to the reaction flask and the solution was stirred and slowly heated for three additional hours. Progress of the reaction was monitored by TLC. *S*-Substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol were purified by using solvent extraction technique. Diethyl ether was utilized as organic media in a weakly basic medium (5% Na_2CO_3). Distillation of ethereal layer afforded pure product. Confirmation of purity was done by TLC (n-hexane 75: ethyl acetate 25).

ENZYME INHIBITION ASSAYS

Cholinesterase assay

The AChE and BChE inhibition activity was performed according to the method described by Ellman *et al.* (1961) with minor modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50 mM Na_2HPO_4, pH 7.7. 10 µL test compound (0.5 mM $well^{-1}$) was added, followed by the addition of 10 µL (0.005 AChE and 0.5 unit BChE unit $well^{-1}$, Sigma Inc.) enzyme. The contents were mixed

and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM well^{-1} substrate (acetylthiocholine iodide or butyrylthiocholine chloride), followed by the addition of 10 µL DTNB (0.5 mM well^{-1}). After 15 min of incubation at 37ºC absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as a positive control. The percent inhibition was calculated by the help of following equation. Inhibition (%) = (Abs of control – Abs of test comp/Abs of control) x 100. IC_{50} values were calculated with the help of EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

IC_{50} values were determined by serial dilution of the compounds from 0.5 mM to 0.25, 0.125, 0.0625, 0.03125, 0.015625 mM. IC_{50} value was calculated from the graph, the concentration at which the enzyme inhibition was 50%. Values are mean of 3 independent experiments.

Lipoxygenase assay

Lipoxygenase (LOX) activity was assayed according to the method (Tappel, 1953; Evans, 1987; Baylac and Racine 2003) with small modifications. A total volume of 200 µL lipoxygenase assay mixture contained 150 µL sodium phosphate buffers (pH 8.0, 100 mM), 10 µL test compound and 15 µL purified lipoxygenase enzyme (600 units well^{-1}, Sigma Inc.). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 minutes at 25°C. 25µL of the substrate solution were added to initiate the reaction. The change in absorbance value was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were carried out in triplicates. The positive and negative controls were included in the assay. Baicalin (0.5 mM well^{-1}) was used as a positive control. The percentage inhibition and IC_{50} values were calculated as mentioned above.

STATISTICAL ANALYSIS

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± SEM.

Spectral characterization of the synthesized compounds

5-Benzyl-1,3,4-oxadiazole-2-thiol (4)

Yellowish crystals, yield: 87%, M.P: 130ºC. Molecular formula $C_9H_8N_2OS$; Mol. Wt. 192; IR (KBr): ν_{max}: 3058 (C-H stretching of aromatic ring), 1540 (C=C stretching of aromatic ring), 1590 (C=N stretching of oxadiazole ring); ^{1}H-NMR: (300 MHz, CD_3OD δ/ppm): 7.46 (m, 2H, H-2′, H-6′), δ 7.34 (m, 2H, ArH-3′, H-5′), 7.26 (m, 1H, H-4′), 4.02 (s, 2H, CH_2-7); EIMS: *m/z* 192 (28%) $[M]^+$, 117 (100%; loss of benzyl cyanide cation fragment), 91 (35%; loss of tropylium ion fragment).

2′-Bromo-N-(2-methoxyphenyl)acetamide (5a)

Greenish granules; yield: 87%; M.P: 76.8ºC. Molecular formula $C_9H_{10}BrNO_2$; Mol. Wt. 244;IR (KBr, cm^{-1}): ν_{max}: 3035 (C-H stretching of aromatic ring), 1562 (C=C stretching of aromatic ring), 1633/* (C=O stretching of amide); ^{1}H-NMR (300 MHz, CD_3OD, δ / ppm): 7.99 (dd, *J* = 1.2, 7.6 Hz, H-6), 7.11 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-5), 6.88 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-4), 7.01 (dd, *J* = 1.0, 7.6 Hz, H-3), 3.87 (s, 3H, CH_3O-2), 3.88 (s, 2H, H-2′); EIMS: *m/z* 244 (42%) $[M]^+$, 229 (48%), 213 (35%), 122 (65%), 107 (53%).

2′-Bromo-N-(2-ethoxyphenyl)acetamide (5b)

Purple gummy; yield: 89%. Molecular formula $C_{10}H_{12}BrNO_2$; Mol. Wt. 258; IR (KBr, cm^{-1}): ν_{max}: 3032 (C-H stretching of aromatic ring), 1561 (C=C stretching of aromatic ring), 1631 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 8.00 (dd, *J* = 1.2, 7.6 Hz, H-6), 7.09 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-5), 6.90 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-4), 7.01 (dd, *J* = 1.0, 7.6 Hz, H-3), 4.12 (s, 2H, H-7), 1.43(t, *J* = 7.0 Hz, 3H, CH_3-2), 4.16 (q, *J* = 7.0 Hz, 2H, CH_2-2), 4.10 (s, 2H, H-2′); EIMS: *m/z* 258 (38%) $[M]^+$, 243 (42%), 229 (31%), 213 (58%), 136 (42%), 121 (62%).

2′-Bromo-N-phenylacetamide (5c)

Orange brown semisolid, yield: 79%; Molecular formula C_8H_8BrNO; Mol. Wt. 214; IR (KBr): ν_{max}: 3023 (C-H stretching of aromatic ring), 1532 (C=C stretching of aromatic ring), 1585 (C=N stretching of oxadiazole ring); 1638 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.12-7.19 (m, 5H, H-2 to H-6), 3.62 (s, 2H, H-2′);EIMS: *m/z* 214 (34%) $[M]^+$,120(46%), 92 (58%).

2′-Bromo-N-benzylacetamide (5d)

Offwhite granules; yield: 78%, M.P: 152.5ºC. Molecular formula $C_9H_{10}BrNO$; Mol. Wt. 228; (KBr, cm^{-1}): ν_{max}: 3038 (C-H stretching of aromatic ring), 1570 (C=C stretching of aromatic ring), 1640 (C=O stretching of amide);^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.21-7.34 (m, 5H, H-2 to H-6), 4.38 (s, 2H, H-2′); EIMS: *m/z* 228 (42%) $[M]^+$, 134 (34%), 106 (42%), 91 (51%).

2′-Bromo-N-(2-ethylphenyl)acetamide (5e)

Yellow powder; yield: 74%, M.P: 65.3ºC. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3023 (C-H stretching of aromatic ring), 1552 (C=C stretching of aromatic ring), 1637 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.15-7.29 (m, 5H, H-2 to H-6), 3.41 (t, *J* = 7.5 Hz, CH_2-8), 2.79 (t, *J* = 7.5 Hz, CH_2-7), 3.77 (s, 2H, H-2′), 3.44 (s, 2H, H-2′); EIMS: *m/z* 242 (26%) $[M]^+$, 148 (34%), 120 (49%), 91 (68%).

2′-Bromo-N-(2-methylphenyl)acetamide (5f)

Dark brown gummy material, yield: 77%; M.P: 115.8ºC.

Molecular formula $C_9H_{10}BrNO_2$; Mol. Wt. 228; IR (KBr): ν_{max}: 3024 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1634 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.08 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-5), 6.92 (ddd, *J* = 1.2, 7.6, 8.0 Hz, H-4), 7.02 (dd, *J* = 1.0, 7.6 Hz, H-3), 4.10 (s, 2H, H-2′), 2.23 (s, 3H, -CH_3-2); EIMS: *m/z* 228 (34%) $[M]^+$, 134 (14%), 106 (22%), 91 (48%).

2′-Bromo-N-(3-methylphenyl)acetamide (5g)

Dark brown gummy material, yield: 81%; M.P: 102.8°C. Molecular formula $C_9H_{10}BrNO_2$; Mol. Wt. 228; IR (KBr): ν_{max}: 3024 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1634 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.11 (1H, *t, J*=7.5 Hz, H-5), 7.09 (1H, *dd, J* = 8.1, 0.9 Hz, H-6), 6.76 (1H, *d, J* = 1.5 Hz, H-2), 6.52 (1H, *dd, J*=8.1, 1.5Hz, H-4), 3.85 (s, 2H, H-2′), 2.23 (s, 3H, -CH_3-3); EIMS: *m/z* 228 (34%); EIMS: *m/z* 228 (19%) $[M]^+$, 134 (54%). 106 (20%), 91 (100%).

2′-Bromo-N-(4-methylphenyl)acetamide (5h)

Dark brown powder, yield: 88%, M.P:106.8°C. Molecular formula $C_9H_{10}BrNO_2$; Mol. Wt. 228; IR (KBr, cm^{-1}): ν_{max}: 3012 (C-H stretching of aromatic ring), 1548 (C=C stretching of aromatic ring), 1631 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.43 (d, *J* = 8.1 Hz, 2H, H-3 & H-5), 7.14 (d, *J* = 8.1 Hz, 2H, H-2 & H-6), 3.94 (s, 2H, H-2′), 2.29 (s, 3H, CH_3-4); EIMS: *m/z* 228 (34%) $[M]^+$, 134 (40%), 106 (28%), 91 (100%).

2′-Bromo-N-(2,3-dimethylphenyl)acetamide (5i)

Light yellowish crystals, yield: 87%, M.P: 126.7°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3040 (C-H stretching of aromatic ring), 1553 (C=C stretching of aromatic ring), 1628 (C=O stretching of amide);^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.05-7.09 (m, 3H, H-4 to H-6), 4.00 (s, 2H, H-2′), 2.28 (s, 3H, CH_3-2), 2.14 (s, 3H, CH_3-3); EIMS: *m/z* 242 (27%) $[M]^+$, 148 (35%), 120 (43%), 105 (53%).

2′-Bromo-N-(2,4-dimethylphenyl)acetamide (5j)

White powder, yield: 88%, M.P: 79.4°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3035 (C-H stretching of aromatic ring), 1562 (C=C stretching of aromatic ring), 1633 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.18 (d, *J* = 8.0 Hz, 1H, H-6), 7.04 (dd, *J* = 8.0, 1.5 Hz, 1H, H-5), 6.98 (d, *J* = 1.5 Hz, 1H, H-3), 4.00(s, 2H, H-2′), 2.28 (s, 3H, CH_3-2), 2.20 (s, 3H, CH_3-4); EIMS: *m/z* 242 (32%) $[M]^+$, 148 (39%), 120 (48%), 105 (65%).

2′-Bromo-N-(2,5-dimethylphenyl)acetamide (5k)

Light greenish powder, yield: 88%, M.P: 133.6°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3012 (C-H stretching of aromatic ring), 1543 (C=C stretching of aromatic ring), 1622 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 6.80-7.23 (m, 5H, H-3 to H-5), 4.01 (s, 2H, H-2′), 3.72 (s, 2H, H-2″), 2.21 (s, 6H, CH_3-2, CH_3-5); EIMS: *m/z* 242 (24%) $[M]^+$, 148 (32%), 120 (48%), 105 (48%).

2′-Bromo-N-(2,6-dimethylphenyl)acetamide (5l)

White powder, yield: 89%, M.P: 161.8°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3018 (C-H stretching of aromatic ring), 1532 (C=C stretching of aromatic ring), 1628 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.24 (dd, *J* = 7.8, 2.1 Hz, 1H, H-4), 7.15 (d, *J* = 2.1 Hz, 1H, H-5), 6.95 (d, *J* = 7.8 Hz, 1H, H-3), 4.01 (s, 2H, H-2′), 2.28 (s, 3H, CH_3-6), 2.20 (s, 3H, CH_3-2); EIMS: *m/z* 242 (34%) $[M]^+$, 148 (40%), 120 (54%), 105 (45%).

2′-Bromo-N-(3,4-dimethylphenyl)acetamide (5m)

Light purple powder, yield: 89%, M.P: 87.5°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3023 (C-H stretching of aromatic ring), 1545 (C=C stretching of aromatic ring), 1642 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.30 (d, *J* = 2.1 Hz, 1H, H-2), 7.25 (dd, *J* = 8.1, 2.1Hz, 1H, H-6), 7.04 (d, *J* = 8.1 Hz, 1H, H-5), 4.84 (s, 2H, H-2′), 2.22 (s, 3H, CH_3-3), 2.20 (s, 3H, CH_3-4); EIMS: *m/z* 242 (35%) $[M]^+$, 148 (28%), 120 (34%), 105 (52%).

2′-Bromo-N-(3,5-dimethylphenyl)acetamide (5n)

Cream powder, yield: 86%, M.P: 138°C. Molecular formula $C_{10}H_{12}BrNO$; Mol. Wt. 242; IR (KBr, cm^{-1}): ν_{max}: 3036 (C-H stretching of aromatic ring), 1543 (C=C stretching of aromatic ring), 1635 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.17 (s, 2H, H-2 & H-6), 6.77 (s, 1H, H-4), 3.93(s, 2H, H-2′), 2.26 (s, 6H, CH_3-3, CH_3-5); EIMS: *m/z* 242 (34%) $[M]^+$, 148 (30%), 120 (45%), 105 (60%).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴ methoxyphenyl)-2-sulfanyl acetamide (6a)

Light golden brown material, yield: 89%; Molecular formula $C_{18}H_{17}N_3O_3S$; Mol. Wt. 355; IR (KBr): ν_{max}: 3011 (C-H stretching of aromatic ring), 1538 (C=C stretching of aromatic ring), 1592 (C=N stretching of oxadiazole ring); 1640 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.86 (dd, *J*=1.2, 7.6 Hz, H-6‴), 7.28-7.33 (m, 5H, H-2′ to H-6′), 7.01 (ddd, *J*=1.2, 7.6, 8.0 Hz, H-5‴), 6.88 (ddd, *J*=1.2, 7.6, 8.0 Hz, H-4‴), 6.99 (dd, *J*=1.0, 7.6 Hz, H-3‴), 4.11 (s, 2H, H-7′), 3.84 (s, 3H, CH_3O-2‴), 3.88 (s, 2H, H-2″); EIMS: *m/z* 355 (24%) $[M]^+$, 324 (30%), 233 (42%), 159 (100%), 117 (42%; loss of benzyl cyanide cation fragment),91 (35%;loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴-ethoxyphenyl)-2-sulfanyl acetamide (6b)

Orange brown semisolid, yield: 79%; Molecular formula

$C_{19}H_{19}N_3O_3S$; Mol. Wt. 369; IR (KBr): ν_{max}: 3023 (C-H stretching of aromatic ring), 1532 (C=C stretching of aromatic ring), 1585 (C=N stretching of oxadiazole ring); 1638 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.84 (dd, J = 1.2, 7.6 Hz, H-6‴), 7.30-7.34 (m, 5H, H-2′ to H-6′), 7.00 (ddd, J = 1.2, 7.6, 8.0 Hz, H-5‴), 6.91 (ddd, J = 1.2, 7.6, 8.0 Hz, H-4‴), 6.96 (dd, J = 1.0, 7.6 Hz, H-3‴), 4.12 (s, 2H, H-7′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-2‴), 4.08 (q, J = 7.0 Hz, 2H, CH_2-2‴), 3.62 (s, 2H, H-2″); EIMS: *m/z* 369 (24%) $[M]^+$, 324 (30%), 121 (48%), 159 (100%), 117 (39%; loss of benzyl cyanide cation fragment), 91 (48%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-phenyl-2-sulfanyl acetamide (6c)

Orange brown semisolid, yield: 79%; Molecular formula $C_{17}H_{15}N_3O_2S$; Mol. Wt. 325; IR (KBr): ν_{max}: 3021 (C-H stretching of aromatic ring), 1530 (C=C stretching of aromatic ring), 1582 (C=N stretching of oxadiazole ring); 1636 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.32-7.35 (m, 5H, H-2′ to H-6′), 7.00-7.15 (m, 5H, H-2‴ to H-6‴), 4.12 (s, 2H, H-7′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-2‴), 4.08 (q, J = 7.0 Hz, 2H, CH_2-2‴), 3.62 (s, 2H, H-2″); EIMS: *m/z* 325 (14%) $[M]^+$, 121 (48%), 159 (100%), 117 (39%; loss of benzyl cyanide cation fragment), 91 (48%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(phenylmethyl)-2-sulfanyl acetamide (6d)

Reddish brown greasy material, yield: 89%; Molecular formula $C_{18}H_{17}N_3O_2S$; Mol. Wt. 339; IR (KBr): ν_{max}: 3018 (C-H stretching of aromatic ring), 1528 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1641 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.21-7.50 (m, 10H, H-2′ to H-6′ & H-2‴ to H-6‴), 4.39 (s, 2H, CH_2-7‴), 4.01 (s, 2H, H-7′), 3.61 (s, 2H, H-2″); EIMS: *m/z* 339 (32%) $[M]^+$, 159 (100%), 134 (32%), 117 (24%; loss of benzyl cyanide cation fragment), 91 (51%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2-phenylethyl)-2-sulfanyl acetamide (6e)

Yellow gummy material, yield: 81%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3018 (C-H stretching of aromatic ring), 1528 (C=C stretching of aromatic ring), 1592 (C=N stretching of oxadiazole ring); 1642 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.17-7.44 (m, 10H, H-2′ to H-6′ & H-2‴ to H-6‴), 3.44 (t, J = 7.5 Hz, CH_2-8‴), 3.79 (t, J = 7.5 Hz, CH_2-7‴), 4.01 (s, 2H, H-7′), 3.61 (s, 2H, H-2″); EIMS: *m/z* 353 (35%) $[M]^+$, 159 (100%), 117 (46%; loss of benzyl cyanide cation fragment), 120 (23%), 105 (28%), 91 (39%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴-methylphenyl)-2-sulfanyl acetamide (6f)

Dark brown gummy material, yield: 89%; Molecular formula $C_{18}H_{17}N_3O_2S$; Mol. Wt. 339; IR (KBr): ν_{max}: 3024 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1634 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.32-7.34 (m, 5H, H-2′ to H-6′), 7.08 (ddd, J = 1.2, 7.6, 8.0 Hz, H-5‴), 6.92 (ddd, J = 1.2, 7.6, 8.0 Hz, H-4‴), 7.02 (dd, J = 1.0, 7.6 Hz, H-3‴), 4.10 (s, 2H, H-7′), 3.85 (s, 2H, H-2″), 2.15 (s, 3H, -CH_3-2‴); EIMS: *m/z* 339 (12%) $[M]^+$, 159 (100%), 117 (34%; loss of benzyl cyanide cation fragment), 106 (12%), 91 (71%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(3‴-methylphenyl)-2-sulfanyl acetamide (6g)

Dark brown gummy material, yield: 89%; Molecular formula $C_{18}H_{17}N_3O_2S$; Mol. Wt. 339; IR (KBr): ν_{max}: 3024 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1634 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.29-7.31 (m, 5H, H-2′ to H-6′), 7.11 (1H, *t, J* = 7.5 Hz, H-5‴), 7.09 (1H, *dd, J* = 8.1, 0.9 Hz, H-6‴), 6.76 (1H, *d, J* = 1.5 Hz, H-2‴), 6.52 (1H, *dd, J* = 8.1, 1.5Hz, H-4‴), 4.10 (s, 2H, H-7′), 3.85 (s, 2H, H-2″), 2.23 (s, 3H, -CH_3-3‴); EIMS: *m/z* 339 (19%) $[M]^+$, 159 (100%), 117 (22%; loss of benzyl cyanide cation fragment), 106 (37%), 91 (49%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(4‴-methylphenyl)-2-sulfanyl acetamide (6h)

Dark brown gummy material, yield: 89%; Molecular formula $C_{18}H_{17}N_3O_2S$; Mol. Wt. 339; IR (KBr): ν_{max}: 3024 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1634 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.54 (d, J = 8.1 Hz, 2H, H-3‴ & H-5‴), 7.17 (d, J = 8.1 Hz, 2H, H-2‴ & H-6‴), 7.31-7.33 (m, 5H, H-2′ to H-6′), 4.10 (s, 2H, H-7′), 3.85 (s, 2H, H-2″), 2.09 (s, 3H, -CH_3-2‴); EIMS: *m/z* 339 (29%) $[M]^+$, 159 (100%), 117 (41%; loss of benzyl cyanide cation fragment), 106 (29%), 91 (53%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴,3‴-dimethylphenyl)-2-sulfanyl acetamide (6i)

Brown semi solid, yield: 89%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3012 (C-H stretching of aromatic ring), 1542 (C=C stretching of aromatic ring), 1595 (C=N stretching of oxadiazole ring); 1629 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.24-7.36 (m, 5H, H-2′ to H-6′), 6.97 (d, J = 8.0 Hz, 1H, H-4‴), 7.04 (t, J = 7.5 Hz, 1H, H-5‴), 7.08 (d, J = 8.5 Hz, 1H, H-6‴), 4.09 (s, 2H, H-7′), 3.62 (s, 2H, H-2″), 2.27 (s, 3H, CH_3-2″), 2.13 (s, 3H, CH_3-3″);

EIMS: *m/z* 353 (23%) $[M]^+$, 159 (100%), 117 (49%; loss of benzyl cyanide cation fragment), 105 (36%), 91 (58%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴,4‴-dimethylphenyl)-2-sulfanyl acetamide (6j)

Reddish brown semi solid, yield: 85%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3027 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1582 (C=N stretching of oxadiazole ring); 1641 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.26-7.34 (m, 5H, H-2′ to H-6′), 7.37 (d, J = 8.0 Hz, 1H, H-6‴), 6.85 (dd, J = 8.0, 1.5 Hz, 1H, H-5‴), 7.01 (d, J = 1.5 Hz, 1H, H-3‴), 4.09 (s, 2H, H-7′), 3.62 (s, 2H, H-2″), 2.19 (s, 3H, CH_3-2″), 2.26 (s, 3H, CH_3-4″); EIMS: *m/z* 353 (18%) $[M]^+$, 159 (100%), 117 (51%; loss of benzyl cyanide cation fragment), 105 (27%), 91 (61%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴, 5‴-dimethylphenyl)-2-sulfanyl acetamide (6k)

Orange brown greasy liquid, yield: 81%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3013 (C-H stretching of aromatic ring), 1531 (C=C stretching of aromatic ring), 1593 (C=N stretching of oxadiazole ring); 1626 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.32-7.37 (m, 5H, H-2′ to H-6′), 7.03 (d, J = 8.0 Hz, 1H, H-5‴), 7.25 (dd, J = 8.0, 1.5 Hz, 1H, H-6‴), 7.30 (d, J = 1.5 Hz, 1H, H-2‴), 4.10 (s, 2H, H-7′), 3.72 (s, 2H, H-2″), 2.22 (s, 3H, CH_3-2″), 2.10 (s, 3H, CH_3-5″); EIMS: *m/z* 353 (23%) $[M]^+$, 159 (100%), 117 (53%; loss of benzyl cyanide cation fragment), 105 (35%), 91 (55%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴,6‴-dimethylphenyl)-2-sulfanyl acetamide (6l)

Golden brown greasy material, yield: 85%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3031 (C-H stretching of aromatic ring), 1525 (C=C stretching of aromatic ring), 1589 (C=N stretching of oxadiazole ring); 1639 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.27-7.333 (m, 5H, H-2′ to H-6′), 7.04-7.13 (m, 3H, H-3‴ to H-5‴), 4.06 (s, 2H, H-7′), 3.61 (s, 2H, H-2″), 2.28 (s, 6H, CH_3-2‴ & CH_3-6‴); EIMS: *m/z* 353 (29%) $[M]^+$, 159 (100%), 117 (49%; loss of benzyl cyanide cation fragment), 105 (31%), 91 (59%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(3‴,4‴-dimethylphenyl)-2-sulfanyl acetamide (6m)

Brown greasy material, yield: 78%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3025 (C-H stretching of aromatic ring), 1537 (C=C stretching of aromatic ring), 1593 (C=N stretching of oxadiazole ring); 1645 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.27-7.30 (m, 5H, H-2′ to H-6′), 7.04 (d, J = 8.0 Hz, 1H, H-5‴), 7.32 (dd, J = 8.0, 1.5 Hz, 1H, H-6‴), 7.37 (d, J = 1.5 Hz, 1H, H-2‴), 4.11 (s, 2H, H-7′), 3.84 (s, 2H, H-2″), 2.27 (s, 3H, CH_3-3″), 2.18 (s, 3H, CH_3-5″); EIMS: *m/z* 353 (21%) $[M]^+$, 159 (100%), 117 (43%; loss of benzyl cyanide cation fragment), 105 (39%), 91 (53%; loss of tropylium ion fragment).

5-Benzyl-1,3,4-oxadiazole-2yl-N-(3‴,5‴-dimethylphenyl)-2-sulfanyl acetamide (6n)

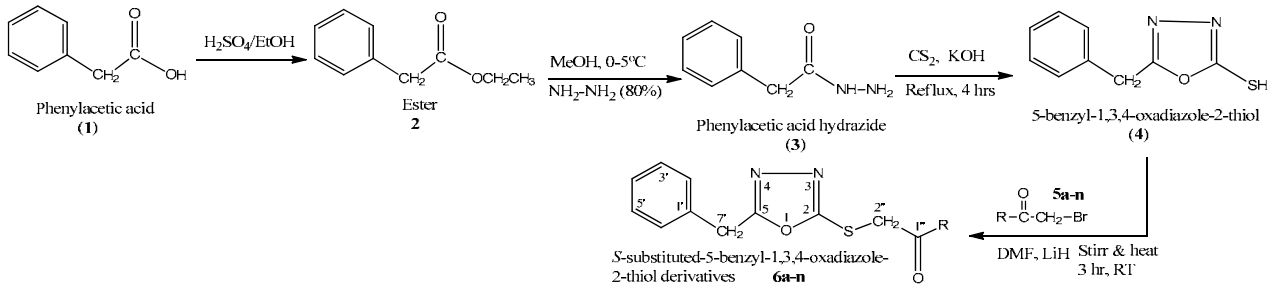
Dark brown gummy material, yield: 76%; Molecular formula $C_{19}H_{19}N_3O_2S$; Mol. Wt. 353; IR (KBr): ν_{max}: 3033 (C-H stretching of aromatic ring), 1543 (C=C stretching of aromatic ring), 1597 (C=N stretching of oxadiazole ring); 1647 (C=O stretching of amide); ^{1}H-NMR: (300 MHz, CD_3OD, δ / ppm): 7.13-7.33 (m, 5H, H-2′ to H-6′), 7.05 (s, 2H, H-2‴, H-6‴), 6.65 (s, 1H, H-4‴), 4.11 (s, 2H, H-7′), 3.86 (s, 2H, H-2″), 2.25 (s, 6H, CH_3-3‴ & CH_3-5‴); EIMS: *m/z* 353 (35%) $[M]^+$, 159 (100%), 117 (41%; loss of benzyl cyanide cation fragment), 105 (53%), 91 (65%; loss of tropylium ion fragment).

RESULTS

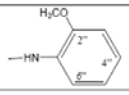
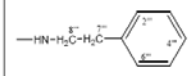
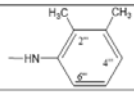
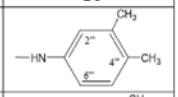
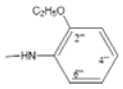
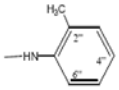
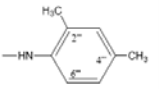
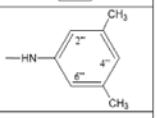
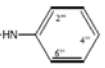
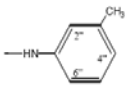
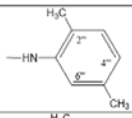
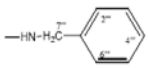
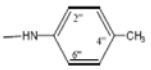
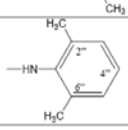
We report herein the synthesis of some new *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulf any l acetamide. These synthesized compounds were evaluated for their enzyme inhibition activities. The reaction sequence leading to the preparation of the desired heterocyclic compounds are outlined in scheme-1. 5-benzyl-1,3,4-oxadiazole-2-thiol was prepared by converting phenyl acetic acid (1) to its corresponding ester 2 which on further reaction with hydrazine hydrate yielded acid hydrazide 3. The hydrazide was cyclized to 5-benzyl-1,3,4-oxadiazole-2-thiol (4) with potassium hydroxide and carbon disulfide. A series of *N*-substituted 2-bromoacetmaide derivatives were also synthesized by manually shaking equimolar ratios of bromoacetyl bromide with different substituted amines in basic media (pH 8-9). These *N*-substituted 2-bromoacetmaide derivatives were reacted further with 5-benzyl-1,3,4-oxadiazole-2-thiol (4) in NaH and DMF media which yielded *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulfanyl acetamide in 70-89% yield as outlined in Scheme 1. (Aziz-ur-Rehman *et al.,* 2012).
Complete conversion was achieved within 30 to 90 min by stirring. The products were isolated by adding cold water in the reaction mixture and filtering off the precipitated solid. In some cases, compound was taken out through solvent extraction method by chloroform/ ethyl acetate. The structure of the parent compound and its derivatives were confirmed by IR, ^{1}H-NMR, and mass spectral data as described in experimental section.

Enzyme inhibition studies

The results of *in vitro* enzyme inhibition activity of the synthesized compounds against butyrylcholinesterase,



Scheme 1: Synthesis of *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulfanyl acetamide

Compounds	-R	Compounds	-R	Compounds	-R	Compounds	-R
5a, 6a		5e, 6e		5i, 6i		5m, 6m	
5b, 6b		5f, 6f		5j, 6j		5n, 6n	
5c, 6c		5g, 6g		5k, 6k			
5d, 6d		5h, 6h		5l, 6l			

acetylcholinesterase, and lipoxygenase enzymes are shown in table 1.

DISCUSSION

Compound 6a was synthesized as light golden brown material. The molecular formula $C_{18}H_{17}N_3O_3S$ was established by EI-MS showing molecular ion peak at *m/z* 355. The IR spectrum showed absorption bands at 3011 cm^{-1}, 1538 cm^{-1}, 1592 cm^{-1} and 1640 cm^{-1} which were assigned to C-H (aromatic ring stretching), C=C stretching of aromatic ring), C=N (stretching of oxadiazole ring) and C=O (stretching of amide) respectively. The EI-MS gave a characteristic peak at *m/z* 117 and 91 which were attributed to the loss of benzyl cyanide cation and tropylium ion fragment respectively. In the aromatic region of the ^{1}H-NMR spectrum signals appeared at δ 7.28-7.33 (m, 5H, H-2′ to H-6′) which were assigned to the mono substituted aromatic ring and at δ 7.86 (dd, J = 1.2, 7.6 Hz, H-6‴), 7.01 (ddd, J = 1.2, 7.6, 8.0 Hz, H-5‴), 6.88 (ddd, J = 1.2, 7.6, 8.0 Hz, H-4‴) and 6.99 (dd, J = 1.0, 7.6 Hz, H-3‴) were assigned to disubtituted aromatic ring. In the aliphatic region of the ^{1}H-NMR spectrum, three singlet signal appeared at 3.84 (s, 3H, CH_3O-2‴), 4.11 (s, 2H, CH_2-7′) and 3.88 (s, 2H, CH_2-2″) indicated the presence of one methoxy and two methylene groups present in the molecule. According to above cumulative evidences, the structure of 6a was assigned as 5-benzyl-1,3,4-oxadiazole-2yl-N-(2‴-methoxyphenyl)-2-sulfanyl acetamide. Similarly, the structures of other compounds were characterized by IR, ^{1}H-NMR and mass spectral data as described in experimental section.

Enzyme inhibition activity

The screening of *N*-substituted-2-bromoacetamide (5a-n) compounds against butyrylcholinesterase (BChE), acety lcholinesterase (AChE), and lipoxygenase (LOX) enzymes revealed that these molecules exhibited good inhibitory potential against acetyl cholinesterase and butyryl cholinesterase as it was evident from their IC_{50} values (table1). It is obvious from the Table1 that compound 5m was found to be a promising inhibitor against butyrylcholinesterase enzyme having IC_{50} value of 23.11±0.17 µmoles relative to Eserine, a reference standard with IC_{50} value of 0.04±0.001 µmoles. The screening against acetylcholinesterase enzyme exposed that the compounds 5k, 5d and 5l exhibited excellent inhibitory potential having IC_{50} 17.11±0.03, 17.91±0.06 and 26.22±0.34 µmoles as compared to standard.

However, some compounds (table1) showed weak inhibition against lipoxygenase enzyme but all other compounds remained inactive. When the synthesized compounds of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulfanyl acetamide (6a-n) were screened against these enzymes it was disclosed that these molecules showed good inhibitory potential against acetylcholinesterase enzyme as it was evident from their IC_{50} values (table 2).

Table 1: Biological activities of *N*-substituted bromoacetamides

Compound No.	BChE			AChE			LOX		
	Conc. (mM)	Inhibition (%)	IC_{50} (µmol.)	Conc. (mM)	Inhibition (%)	IC_{50} (µmol.)	Conc. (mM)	Inhibition (%)	IC_{50} (µmol.)
5a	0.5	65.91±0.08	307.81±0.08	0.5	66.45±0.17	227.41±0.11	0.5	66.92±0.04	217.21±0.11
5b	0.5	66.33±0.01	219.11±0.04	0.5	66.45±0.12	187.11±0.33	0.5	56.30±0.14	426.61±0.82
5c	0.5	72.18±0.83	220.41±0.81	0.5	63.83±0.32	210.11±0.72	0.5	52.43±0.76	425.12±0.13
5d	0.5	54.07±0.02	445.61±0.05	0.5	82.79±0.02	17.91±0.06	0.5	58.80±0.44	405.71±0.06
5e	0.05	46.65±0.11	-	0.05	76.86±0.34	38.71±0.02	0.05	23.85±0.52	-
5f	0.5	71.18±0.85	225.41±0.89	0.5	67.83±0.37	212.11±0.71	0.5	57.43±0.71	427.12±0.13
5g	0.5	65.34±0.04	315.91±0.05	0.5	57.81±0.34	342.41±0.08	0.5	55.06±0.07	415.61±0.04
5h	0.5	56.78±0.56	369.51±0.78	0.5	69.02±0.25	204.61±0.27	0.5	52.43±0.21	432.12±0.02
5i	0.5	62.91±0.71	139.31±0.07	0.5	73.50±0.11	40.61±0.82	0.5	53.93±0.14	-
5j	0.5	63.77±0.56	214.41±0.06	0.5	73.70±0.66	41.25±0.87	0.5	52.81±0.36	-
5k	0.5	65.06±0.28	328.21±0.17	0.5	84.25±0.31	17.11±0.03	0.5	59.68±0.52	412.31±0.82
5l	0.5	60.77±0.07	380.51±0.68	0.5	66.25±0.85	26.22±0.34	0.5	60.30±0.71	196.11±0.08
5m	0.5	80.74±0.78	23.11±0.17	0.5	85.43±0.71	46.81±0.71	0.5	35.96±0.22	-
5n	0.5	74.75±0.68	142.61±0.08	0.5	85.76±0.11	45.31±0.33	0.5	41.57±0.08	-
Control	Eserine		0.04±0.001	Eserine		0.85±0.001 µM	Baicalein		22.4±1.3 µM

Table 2: Biological activities of *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulfanyl acetamide

Sample Code	BChE			AChE			LOX		
	Conc. (mg/ml)	Inhibition (%)	IC_{50} (µg/ml)	Conc. (mg/ml)	Inhibition (%)	IC_{50} (µg/ml.)	Conc. (mg/ml)	Inhibition (%)	IC_{50} (µg/ml.)
6a	0.5	61.06±0.03	394.11±0.17	0.5	94.86±0.02	37.21±0.06	0.5	34.83±0.78	-
6b	0.5	56.78±0.15	385.81±0.96	0.5	93.94±0.41	26.71±0.06	0.5	39.08±0.56	-
6c	0.1	30.33±0.87	-	0.1	92.35±0.14	39.91±0.14	0.1	45.03±0.22	-
6d	0.1	58.80±0.89	347.41±0.89	0.1	93.28±0.11	24.61±0.62	0.1	23.09±0.71	-
6e	0.5	54.93±0.06	444.81±0.36	0.5	87.74±0.01	39.31±0.34	0.5	45.66±0.87	-
6f	0.1	78.68±0.52	264.21±0.56	0.1	94.40±0.24	98.61±0.03	0.1	20.19±0.54	-
6g	0.1	35.76±0.09	-	0.1	89.85±0.47	74.71±0.65	0.1	7.02±0.86	-
6h	0.1	45.46±0.17	-	0.1	85.89±0.24	129.61±0.05	0.1	32.78±0.68	-
6i	0.1	45.19±0.22	-	0.1	93.25±0.22	107.91±0.04	0.1	19.01±0.45	-
6j	0.1	74.46±0.26	382.21±0.28	0.1	90.44±0.15	70.84±0.05	0.1	6.38±0.85	-
6k	0.5	32.52±0.65	-	0.5	89.85±0.24	23.51±0.03	0.5	47.07±0.09	-
6l	0.5	57.77±0.28	372.71±0.25	0.5	92.02±0.06	17.5±0.11	0.5	80.02±0.52	157.21±0.07
6m	0.5	28.20±0.89	-	0.5	96.11±0.52	42.41±0.13	0.5	38.39±0.09	-
6n	0.1	80.42±0.31	194.82±0.22	0.1	94.13±0.52	75.81±0.34	0.1	21.56±0.24	-
Control	Eserine		0.04±0.001 µM	Eserine		0.85±0.001 µM	Baicalein		22.4±1.3 µM

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).
LOX = Lipoxygenase, AChE = Acetyl cholinesterase, BChE = Butyryl cholinesterase.

It was apparent from table2 that compounds 6l and 6d were promising inhibitors against acetylcholinesterase enzyme having IC_{50} value of 17.5±0.11 and 24.61±0.62 µmoles respectively, relative to Eserine, a reference standard with IC_{50} value of 0.85±0.001 µmoles. The test against butyrylcholinesterase enzyme exposed that some compounds showed moderate activity. However, only one compound 6l (table 2) showed weak inhibition against lipoxygenase enzyme but all other compounds remained inactive.

CONCLUSION

The proposed structures of the synthesized compounds are well supported by spectroscopic data. From the enzyme inhibition data (table1 and 2), it might be concluded that the synthesized compounds 6a-n have talented activity against acetylcholinesterase enzyme as it was evident from their IC_{50} values, relative to the standard used. Some compounds showed moderate inhibition activity against butyrylcholinesterase enzyme and only one compound showed weak activity against lipoxygenase enzyme but all others were stayed inactive. Hence, on the basis of aforesaid results, these synthesized derivatives provide an overall indispensable basis to introduce new drug candidates for the treatment of Alzheimer's disease and other associated diseases. These entrants can also be helpful for the treatment of a variety

of disorders such as inflammation, bronchial asthma, autoimmune diseases and cancer.

REFERENCES

Aziz-ur-Rehman, Siddiqui SZ, Abbasi MA, Abbas N, Khan KM, Shahid M, Mahmood Y, Akhtar MN and Lajis NH (2012). Synthesis, antibacterial screening and hemolytic activity of *S*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol. *Int. J. Pharm. Pharm. Sci.,* **4**: 676-680.

Aziz-ur-Rehman, Nafeesa K, Abbasi MA, Khalid H, Khan KM, Ashraf M, Ahmad I and Ijaz SA (2012). Synthesis, spectral characterization and biological activity of *S*-substituted derivatives of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-thiol. *Asian J. Pharm. Hea. Sci*., **2**(3): 370-376.

Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A and Shafiee A (2004). Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. *Bioorg. Med. Chem Lett.,* **14**: 6057-6059.

Amir M, saifullah K and Akhter W (2011). Design, synthesis and pharmacological evaluation of 1,3,4-oxadiazole derivatives of aryl acetic acid as anti-inflammatory and analgesic agents. *Ind. J. Chem*., **50**B: 1107-1111.

Angilini I, Angilini L and sparacc F (1969). 2-phenyl-5-chloro alkyl-1,3,4-oxadiazoles, British patent, 196, 9 1:161, 801: *Chem. Abstract.*, **71**: 112936.

Bahadur S and Pandey KK (1980). Synthesis of para-alkyl-(2-benzimidazolyl)-methyl-minobenzoates and corresponding hydrazides as possible anti-malarial agents. *J. Ind. Chem. Soc.,* **57**: 447-451.

Baylac S and Racine P (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *Int. J. of Aromatherap.*, **13**: 138-142.

Bertaccini G (1982). Substance P. Handbook of Experimental Pharmacology. *Springer, Berlin.,* **59**: 85-105.

Boschelli DH, Connor DT, Bornemeier DA, Dyer RD, Kennedy JA, Kuipers PJ, Okonkwo GC, Svhrier DJ and Wright CD (1993). 1,3,4-oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole analogs of the fenamates – *in vitro* inhibition of cyclooxygenase and 5-lipoxygenase activities. *J. Med. Chem.,* **36**: 1802-1810.

Cansız A, Koparır M and Demirdag A (2004). Synthesis of some new 4,5-substituted-4H-1,2,4-triazole-3-thiol derivatives. *Molecules,* **9**: 204-212.

Cygler M, Schrag JD, Sussman J, Harel LM, Silman I and Gentry MK (1993). Between sequence conservation and three dimentional structure in a large family of esterases, lipases and related proteins. *Protein Sci*., **2**: 366-382.

Ellman GL, Courtney KD, Andres V and Featherstone RM (1961).calorimetric determination of acetyl-cholinesterase activity. *Bio. Pharm.,* **7**: 88-95.

Evans AT (1987). Actions of cannabis constituents on enzymes of arachidonate metabolism: Anti-inflammatory potential. *Bio Pharm.,* **36**: 2035-2037.

Gauthier S (2001). Cholinergic adverse effects of cholinesterase inhibitors in Alzheimer's disease. *Drug & Aging,* **18**: 853:862.

Hemavathi SN, Vishu kumar BK and Lokanatha RKM (2011). Synthesis and biological screening of some new 2, 5-disubstituted 1,3,4-oxadiazoles. *Int. J. Pharm. Pharmaceut. Sci.*, **3**(4): 110-114.

Kennedy D.A and summers LA (1981). Chemical constitution and activity of Herbicides. *J. Heterocyclic Chem.,* **18**(2) 409-410.

Koparır M, Çetin A and Cansız, A (2005). 5-Furan-2yl[1,3,4]oxadiazole-2-thiol,5-furan-2yl-4H [1,2,4] triazole-3-thiol and their thiol-thione tautomerism. *Molecules,* **10**: 475-480.

Kumar S (2011). Synthesis and biological activity of 5-Substituted-2-amino-1,3,4- oxadiazole derivatives. *Turk. J. Chem.*, **35**: 99-108.

Piala JJ and Yale HL (1964). 2-amino-5-aryl-1,3,4-oxadiazoles US Patent 3141022,1964. *Chem. Abstr*., **61**: 8317.

Ram V J and Vlietinck AJ (1988). Chemotherapeutical agents .7. Synthesis and pesticidal activities of sulfides and sulfones derived from bis[4-aryl-1,2,4-triazoline-5-thione-3-yl]alkane and 5-phenyl-1,3,4-oxadiazole-2-thione. *J. Heterocycl. Chem.,* **25**: 253-256.

Rostom SAF, Shalaby MA and EL-Demellawy MA (2003) Polysubstituted pyrazoles, part 5. Synthesis of new 1-(4-chlorophenyl)-4-hydroxy-1h-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents. *Eur. J. Med. Chem.*, **38**: 959-974.

Tappel AL (1953). The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. *Arch. Biochem. Biophys.*, **44**(2): 378-395.

Tougu V (2001). Acetylcholinesterase: Mechanism of catalysis and Inhibition. *Curr. Med. Chem.,* **1**: 155-170.

Yale HL and Losee K (1966). 2-amino 5-substituted 1, 3, 4-oxadiazoles and 5-imino substituted-1,3,4-oxadiazolines. A group of novel muscle relaxants. *J. Med. Chem.,* **9**: 478-483.