Synthesis, characterization and biological screening of 5-substituted-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide

Aziz-ur-Rehman[1]*, Ambreen Fatima[1], Nadia Abbas[1], Muhammad Athar Abbasi[1], Khalid Mohammed Khan[2], Muhammad Ashraf[3], Irshad Ahmad[4] 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 of Biochemistry and Biotechnology; [4]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract: In the current study, a series of 5-substituted-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide was synthesized by converting variously substituted/unsubstituted aromatic organic acids successively into the corresponding esters, hydrazides and then 5-substituted-1,3,4-oxadiazole-2-thiols. Finally the target compounds were obtained by stirring 5-substituted-1,3,4-oxadiazole-2-thiols with *N*-(2-methoxy-5-chlorophenyl)-2-bromoacetamide in the presence of *N,N*-dimethyl formamide (DMF) and sodium hydride (NaH). The structures of the synthesized compounds were confirmed based on ^{1}H-NMR, IR and mass spectral data. The synthesized compounds were screened against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase enzymes (LOX) and were found to be relatively more active against acetyl cholinesterase.

Keywords: Aromatic acids; oxadiazoles; acetylcholinesterase; ^{1}H-NMR and EI-MS.

INTRODUCTION

1,3,4-oxadiazoles belong to the group of compounds that have been made a center of attention for last two decades due to their broad range of biological activities. Some of them exhibit anticonvulsant, antibacterial, anticancer activities and are used to fight infections involving AIDS. 2,5-disubstituted-1,3,4-oxadiazoles have been reported to have significant activities like antidepressive (Piala *et al*., 1964), anticonvulsive (Almasirad *et al*., 2004), analgesic (Angilini *et al*., 1969), herbicidal (Kennedy *et al*., 1981), muscle relaxant (Yale *et al*., 1966), pesticidal (Ram *et al*., 1988), anti-malarial agents (Bahadur *et al*., 1980), antitumor as well as anti-HCV agents (Rostom *et al*., 2003). Due to the appealing biological activity of 2,5-disubstituted 1,3,4-oxadiazole, an extensive concentration has been focused on this group. The significance of these compounds lies in the fact that they can be successfully utilized as antibacterial, analgesic, anti-inflammatory, anticancer, anti-HIV agent, anti-tubercular and insecticidal agents (Boschelli *et al*., 1993; Cansız *et al*., 2004; Koparır *et al*., 2005; Hemavathi *et al*., 2011 and Kumar, 2011). Literature survey revealed that minor modification in the structure of substituted 1,3,4-oxadiazole can lead to quantitative as well as qualitative changes in the biological activity.

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) consist of an enzymes family which includes serine hydrolases. The diverse specificities for the substrates and inhibitors for these enzymes are due to the dissimilarity in amino acid remains of the active sites of AChE and BChE. Actually the system of enzyme is responsible for the termination of acetylcholine at cholinergic synapses. These are key components of cholinergic brain synapses and neuromuscular junctions. The central function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses (Cygler *et al*., 1993 and Tougu, 2001). It has been found that BChE is present in appreciably higher quantity in Alzheimer's plaques than in the normal age linked dementia of brains. 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. Hence, the search for new cholinesterase inhibitors is considered an important and ongoing strategy to introduce new drug candidates for the treatment of Alzheimer's disease and other related diseases (Bertaccini 1982 and Gauthier 2001).

This paper reports the synthesis and biological screening of some new 5-substituted-1,3,4-oxadiazole-2-sulfanyl acetamide with the objective of having lesser toxicity and enhanced activity of the synthesized compounds. In continuation of our previous work (Rehman *et al*., 2012), the synthesis was carried out through the intermolecular cyclization of different substituted/unsubstituted organic

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

aromatic acid hydrazides to the corresponding 5-substituted-1,3,4-oxadiazoles-2-thioles and finally to 5-substituted-1,3,4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide.

MATERIALS AND METHODS

General

Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus by open capillary tube and were uncorrected. Purity was checked on thin layer chromatography (TLC) on pre-coated silica gel G-25-UV_{254} plates with different solvent systems using ethyl acetate and n-hexane giving single spot. Detection was accepted at 254 nm, and by Ce_2SO_4 reagent. The I.R. spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^{1}H-NBMR spectra were recorded in $CDCl_3/CD_3OD$ on a Bruker spectrometers operating at 400 MHz. Chemical shifts are given in ppm. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system.

Procedure for the preparation of ethyl-4-methoxy benzoate (1a)

4-Methoxy benzoic acid (**Ra**; 1g) was taken in round bottom flask fitted with reflux condenser. Absolute C_2H_5OH (4ml) and conc. H_2SO_4 (1/2ml) was also added in the flask and reaction mixture was refluxed for 90 mints. The reaction completion was established by thin layer chromatography (TLC). After completion, reaction mixture was poured into the separating funnel that contained 40 ml distilled water. Diethyl ether (15ml) was added to the separating funnel and mixture was neutralized by using conc. Na_2CO_3 solution until all free acid was removed. The solution was shaken and stand for some time, two layers were formed i.e. lower layer was aqueous and upper layer was ether that contained required ester ethyl-4-methoxy benzoate. Lower aqueous layer was discarded and upper layer was taken in distillation flask. Diethyl ether was distilled off and the transparent ester, ethyl-4-methoxy benzoate (**1a**) was obtained from the flask.

Procedure for the preparation of 4-Methoxy benzoic acid hydrazide (2a)

Reaction mixture of ethyl-4-methoxy benzoate (**1a**; 0.01mol, 1.64 ml) and methanol (10ml) was added in the round bottom flask and mixture was cooled to 0-5^{o}C. Hydrazine hydrate (80% 2ml) was added drop wise in the mixture and then this solution was stirred for 40 minutes at temperature of 0-5^{o}C. Reaction completion was checked by TLC. After reaction completion, precipitate was formed by adding cold distilled water, filtered and washed with distilled water to get compound **2a**.

Procedure for the preparation of 5-(4'-methoxyphenyl)-1, 3, 4-oxadiazole-2-thiol (3a)

Acid hydrazide (**2a**; 1.5 g, 0.01 mol) was dissolved in absolute C_2H_5OH (10 mL) in a 250 ml RB flask. Carbon disulfide (1.89 ml, 0.03 mol) was then added to the flask followed by the addition of excess KOH (1.12 g, 0.02 mol) in water (5 mL). The mixture was refluxed for 6 hours along with proper stirring. Color of solution was changed; initially it was yellow which turned to brownish yellow with the progress of reaction. H_2S gas was evolved during the reflux. The reaction completion was checked by TLC. The mixture was diluted with distilled H_2O (30 mL) and acidified with dilute HCl to pH 2-3. It was then filtered, washed with distilled water and re-crysallized from ethanol.

Procedure for the preparation of N-(2-methoxy-5-chlorophenyl)-2'-bromoacetamide (4)

2-methoxy-5-chloroaniline (1g, 0.1 mmol) was taken in an iodine flask (100 mL), then water (10ml) and sodium carbonate solution was added to maintain pH 9. Small amount methanol was also added to dissolve it followed by the addition of bromoacetyl bromide (0.1 mmol) to the mixture. The mixture was shaken vigorously till precipitation formed. Precipitate was filtered, rinsed with distilled H_2O, dry and used in further reaction. Yield: 85%; recrystallization from EtOH; Molecular formula $C_9H_9BrClNO_2$; Mol. Wt. 278. IR (KBr, cm^{-1}): v_{max}: 3039 (Ar-H), 1566 (C=C of aromatic ring), 1638 (C=O), ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 9.01 (br.s., N-H), 8.52 (d, J = 2.1Hz, 1H, H-6), 7.01 (dd, J = 8.5, 2.1 Hz, H-4$'''$), 6.79 (d, J = 8.5 Hz, H-3$'''$), 4.15 (s, 2H, H-2$'$), 3.91 (s, 3H, H_3CO-2); EIMS: m/z 278 (44%) $[M]^+$, 184 (32%), 156 (52%), 141 (36%).

General procedure for the synthesis of 5-benzyl-1,3,4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide (5a)

The calculated amount of 5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-thiol (**3a**; 0.1 mmol) was taken in a round bottomed flask (50 mL), then *N,N*-dimethyl formamide (DMF) (10 mL) was added to dissolve it followed by the addition of sodium hydride (0.1 mmol) to the mixture. The mixture was stirred for 30 minutes at room temperature and then added *N*-(2-methoxy-5-chlorophenyl)-2'-bromoacetamide to the mixture and the solution was further stirred for three hours. The reaction progress was examined via TLC till single spot. After completion of reaction, distilled water was added in the flask and the product was obtained by solvent extraction method.

Acetylcholinesterase Assay

The activity of AChE inhibition was carried out according to the process (Ellman *et al*., 1961) with slight alteration. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50

mM and ph 7.7. Ten µL test compound (0.5 mM well^{-1}) was added, followed by the addition of 10 µL (0.005 unit well^{-1}) 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), 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.

$$\text{Inhibition } (\%) = \frac{Control - Test}{Control} \times 100$$

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase Assay

The BChE inhibition activity was performed according to the method (Ellman *et al*., 1961) with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, $Na_2H\ PO_4$ buffer, 50 mM and pH 7.7. Ten µL test compound 0.5 mM well^{-1} was added followed by the addition of 10 µL (0.5 unit well^{-1}) BChE (Sigma Inc.). The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM well^{-1} substrate (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 positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition } (\%) = \frac{Control - Test}{Control} \times 100$$

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Lipoxygenase Assay

Lipoxygenase (LOX) activity was assayed according to the method (Tappel 1953; Evans, 1987 and Baylac *et al*., 2003) with small modifications. A total volume of 200 µL lipoxygenase assay mixture contained 150 µL sodium phosphate buffers (100 mM, pH 8.0), 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. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were performed 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 (%) was calculated by formula given below.

$$\text{Inhibition } (\%) = \frac{Control - Test}{Control} \times 100$$

Where

Control =Total enzyme activity without inhibitor

Test = Activity in the presence of test compound

IC_{50} values were calculated using EZ–Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).

STATISTICAL ANALYSIS

All the measurements were completed in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are offered as mean ± sem.

5-(4′-methoxyphenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5a)

Colour light purple; yield: 80%; recrystallization from EtOH; m.p. 84-88 ^oC; Molecular formula $C_{18}H_{16}ClN_3O_4S$; Mol. Wt. 405. IR (KBr, cm^{-1}): v_{max}: 3035 (Ar-H), 1562 (C=C of aromatic ring), 1633 (C=O), 1517 (C=N); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 9.36 (br.s., N-H), 8.37(d, J = 2.1Hz, 1H, H-6‴), 7.93 (d, J = 8.5Hz, H-2′, H-6′), 6.99 (d, J = 8.5 Hz, H-3′, H-5′), 6.96 (dd, J = 8.5, 2.1 Hz, H-4‴), 6.71 (d, J = 8.5 Hz, H-3‴), 4.06 (s, 2H, H-2″), 3.84 (s, 3H, H_3CO-2‴), 3.86 (s, 3H, H_3CO-4′); EIMS: m/z 405 (30%) $[M]^+$, 107 (56%; loss of methoxy phenyl cation fragment), 133 (48%; loss of methoxy phenyl cyanide cation fragment), 156 (100%), 249 (65%).

5-(2′-methylphenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5b)

Color reddish; yield: 64%; recrystallization from EtOH; m.p. 80-84 ^oC; Molecular formula $C_{18}H_{16}ClN_3O_3S$; Mol. Wt. 389. IR (KBr, cm^{-1}): v_{max}: 3029 (Ar-H), 1552 (C=C of aromatic ring), 1630 (C=O), 1521 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.60 (br.s., N-H), 8.32 (d, J = 2.1Hz, 1H, H-6‴), 6.71 (dd, J = 8.5, 2.1 Hz, 1H, H-3'), 7.84 (t, J = 8.5 1H, H-5′), 7.37-7.33 (m, 2H, H-4′ & H-6′), 6.95 (dd, J = 8.5, 2.1 Hz, H-4‴), 6.78 (d, J = 8.5 Hz, H-3‴), 4.07 (s, 2H, H-2″), 3.92 (s, 3H, H_3CO-2‴), 2.71 (s, 3H, CH_3-2′); EIMS: m/z 389 (42%) $[M]^+$, 358 (48%), 233 (65%), 117 (42%; loss of methyl phenyl cyanide cation fragment), 91 (52%; loss of methyl phenyl cation fragment).

5-(2′-chlorophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5c)

Color grey; yield: 72%; recrystallization from EtOH; m.p. 116-120^oC; Molecular formula $C_{17}H_{13}Cl_2N_3O_3S$; Mol. Wt. 410. IR (KBr, cm^{-1}): v_{max}: 3025 (Ar-H), 1549 (C=C of aromatic ring), 1628 (C=O), 1519 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.62 (br.s., N-H), 8.28 (d, J = 2.1 Hz, 1H, H-6‴), 7.95 (dd, J = 8.5, 2.1 Hz, 1H, H-6'), 7.51 (d, J = 8.5 1H, H-3′), 7.45-7.35 (m, 2H, H-4′ & H-5′), 6.99

(dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.82 (d, J = 8.5 Hz, 1H, H-3‴), 4.01 (s, 2H, H-2″), 3.92 (s, 3H, H_3CO-2‴); EIMS: m/z 389 (42%) $[M]^+$, 410 (48%), 379 (35%), 253 (65%), 137 (53%; loss of chloro phenyl cyanide cation fragment), 111 (35%; loss of chloro phenyl cation fragment).

5-(3′-chlorophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5d)

Color white; yield: 72%; recrystallization from EtOH; m.p. 156-160 °C; Molecular formula $C_{17}H_{13}Cl_2N_3O_3S$; Mol. Wt. 410. IR (KBr, cm^{-1}): ν_{max}: 3025 (Ar-H), 1549 (C=C of aromatic ring), 1628 (C=O), 1519 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.26 (br.s., N-H), 8.29 (d, J = 2.1 Hz, 1H, H-6‴), 7.98 (dd, J = 8.5, 2.1 Hz, 1H, H-6′), 7.52 (d, J = 2.1 Hz, 1H, H-2′), 7.96 (t, J = 8.5 Hz, 1H, H-5′), 7.49 (dd, J = 8.5, 2.1 Hz, 1H, H-4′), 6.99 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.88 (dd, J = 8.5 Hz, 1H, H-3‴), 3.92 (s, 2H, H-2″), 3.83 (s, 3H, H_3CO-2‴); EIMS: m/z 389 (42%) $[M]^+$, 410 (48%), 379 (35%), 253 (65%), 137 (53%; loss of chloro phenyl cyanide cation fragment), 111 (35%; loss of chloro phenyl cation fragment).

5-(4'-chlorophenyl)-1,3,4-oxadiazole-2yl-N-(2'''-methoxy-5'''-chlorophenyl)-2''-sulfanyl acetamide (5e)

Color grey; yield: 65%; recrystallization from EtOH; m.p. 130-134 °C; Molecular formula $C_{17}H_{13}Cl_2N_3O_3S$; Mol. Wt. 410. IR (KBr, cm^{-1}): ν_{max}: 3025 (Ar-H), 1546 (C=C of aromatic ring), 1626 (C=O), 1519 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.28 (br.s., N-H), 8.36 (d, J = 2.1 Hz, 1H, H-6'''), 7.91 (d, J = 8.5 Hz, 2H, H-3' & H-5'), 7.49 (d, J = 8.5 Hz, 1H, H-2' & H-6'), 7.00 (dd, J = 8.5, 2.1 Hz, 1H, H-4'''), 6.72 (d, J = 8.5 Hz, 4.08 (s, 2H, H-2''), 3.85 (s, 3H, H_3CO-2'''); EIMS: m/z 389 (46%) $[M]^+$, 410 (58%), 253 (60%), 137 (42%; loss of chloro phenyl cyanide cation fragment), 111 (38%; loss of chloro phenyl cation fragment).

5-(2′-nitrorophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5f)

Color off white; yield: 70%; recrystallization from EtOH; m.p. 150-154 °C; Molecular formula $C_{17}H_{13}ClN_4O_5S$; Mol. Wt. 420. IR (KBr, cm^{-1}): ν_{max}: 3017 (Ar-H), 1539 (C=C of aromatic ring), 1621 (C=O), 1516 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.21 (br.s., N-H), 9.25 (d, J = 2.1 Hz, 1H, H-6‴), 8.08 (dd, J = 8.5, 2.1 Hz, 1H, H-6'), 7.62 (d, J = 8.5 1H, H-3′), 7.47-7.38 (m, 1H, H-4′ & H-5′), 7.01 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.75 (d, J = 8.5 Hz, 1H, H-3‴), 4.10 (s, 2H, H-2″), 3.91 (s, 3H, H_3CO-2‴); EIMS: m/z 420 (46%) $[M]^+$, 389 (51%), 264 (65%), 148 (46%; loss of nitro phenyl cyanide cation fragment), 122 (40%; loss of nitro phenyl cation fragment).

5-(3′-nitrorophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5g)

Color light brown; yield: 65%; recrystallization from EtOH; m.p. 75-79 °C; Molecular formula $C_{17}H_{13}ClN_4O_5S$; Mol. Wt. 420. IR (KBr, cm^{-1}): ν_{max}: 3022 (Ar-H), 1542 (C=C of aromatic ring), 1623 (C=O), 1519 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.29 (br.s., N-H), 9.20 (d, J = 2.1 Hz, 1H, H-6‴), 8.40 (dd, J = 8.5, 2.1 Hz, 1H, H-6′), 8.38 (d, J = 2.1 Hz, 1H, H-2′), 7.72 (t, J = 8.5 Hz, 1H, H-5′), 8.31 (dd, J = 8.5, 2.1 Hz, 1H, H-4′), 7.00 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.76 (d, J = 8.5 Hz, 1H, H-3‴), 4.12 (s, 2H, H-2″), 3.87 (s, 3H, H_3CO-2‴); EIMS: m/z 420 (46%) $[M]^+$, 389 (51%), 264 (65%), 148 (46%; loss of nitro phenyl cyanide cation fragment), 122 (40%; loss of nitro phenyl cation fragment).

5-(4′-nitrorophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5h)

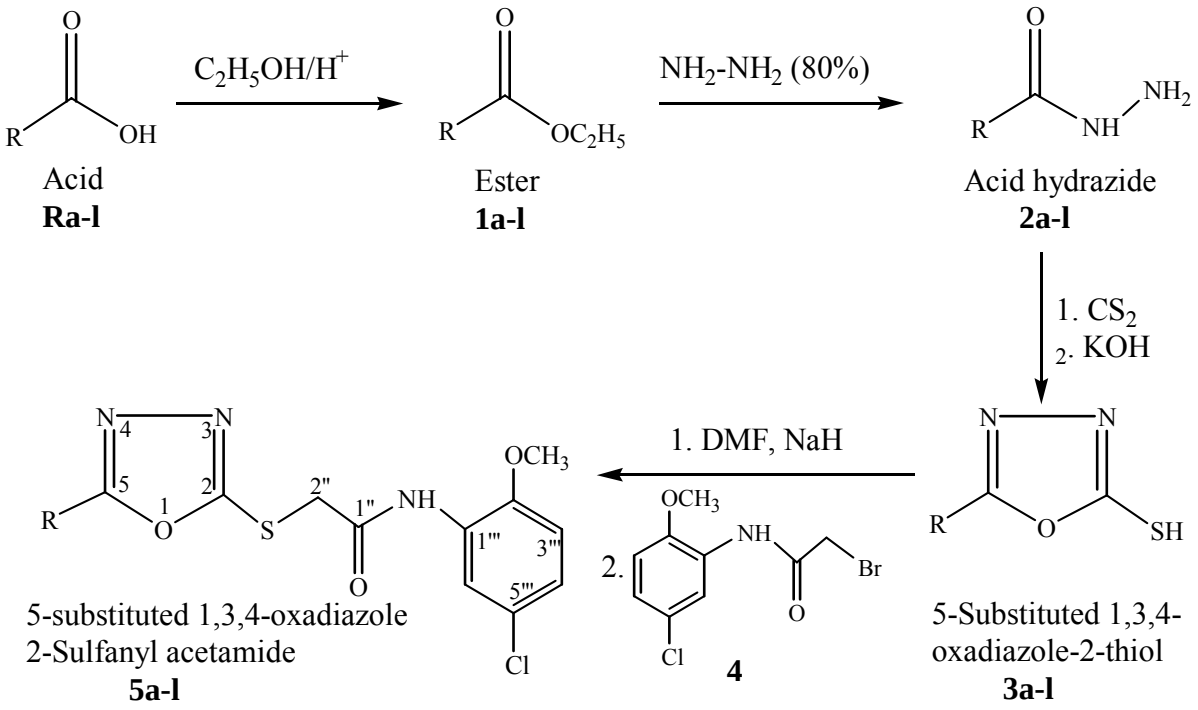
Color light yellow; yield: 65%; recrystallization from EtOH; m.p. 121-125°C; Molecular formula $C_{17}H_{13}ClN_4O_5S$; Mol. Wt. 420. IR (KBr, cm^{-1}): ν_{max}: 3022 (Ar-H), 1542 (C=C of aromatic ring), 1623 (C=O), 1519 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.21 (br.s., N-H), 7.91(d, J = 8.5 Hz, 1H, H-3′ & H-5′), 8.27 (d, J = 2.1 Hz, 1H, H-6‴), 7.44 (d, J = 8.5 Hz, 1H, H-2′ & H-6′), 6.99 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.82 (d, J = 8.5 Hz, 1H, H-3‴), 4.12 (s, 2H, H-2″), 3.92 (s, 3H, H_3CO-2‴); EIMS: m/z 420 (46%) $[M]^+$, 389 (51%), 264 (65%), 148 (46%; loss of nitro phenyl cyanide cation fragment), 122 (40%; loss of nitro phenyl cation fragment),

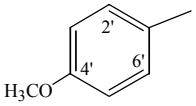
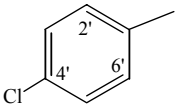
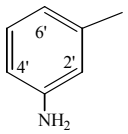
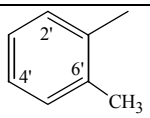
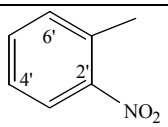
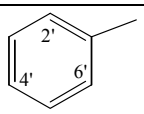
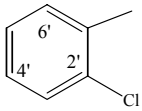
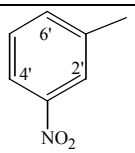
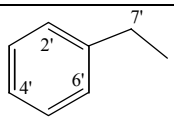
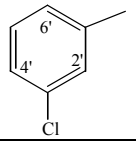
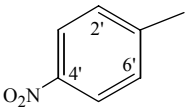
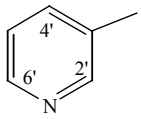
5-(3′-aminophenyl)-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5i)

Color dark brown; yield: 65%; recrystallization from EtOH; m.p. 101-105 °C; Molecular formula $C_{17}H_{15}ClN_4O_3S$; Mol. Wt. 390. IR (KBr, cm^{-1}): ν_{max}: 3025 (Ar-H), 3210 (N-H), 1544 (C=C of aromatic ring), 1628 (C=O), 1523 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.20 (br.s., N-H), 8.29 (d, J = 2.1 Hz, 1H, H-6‴), 7.97 (dd, J = 8.5, 2.1 Hz, 1H, H-6′), 7.48 (d, J = 2.1 Hz, 1H, H-2′), 7.95 (t, J = 8.5 Hz, 1H, H-5′), 7.47 (dd, J = 8.5, 2.1 Hz, 1H, H-4′), 6.98 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.82 (dd, J = 8.5 Hz, 1H, H-3‴), 4.01 (s, 2H, H-2″), 3.95 (s, 3H, H_3CO-2‴); EIMS: m/z 390 (46%) $[M]^+$, 359 (51%), 234 (65%), 118 (46%; loss of amino phenyl cyanide cation fragment), 92 (40%; loss of amino phenyl cation fragment).

5-phenyl-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5j)

Color off white; yield: 65%; recrystallization from EtOH; m.p. 92-96°C; Molecular formula $C_{17}H_{13}ClN_3O_3S$; Mol. Wt. 375. IR (KBr, cm^{-1}): ν_{max}: 3029 (Ar-H), 1546 (C=C of aromatic ring), 1628 (C=O), 1514 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.21 (br.s., N-H), 8.16(d, J = 2.1Hz, 1H, H-6‴), 7.24-7.35 (m, 5H, H-2′ to H-6′), 3.89 (s, 3H, H_3CO-2‴), 4.11 (s, 2H, H-2″), 6.93 (dd, J = 8.5, 2.1 Hz, H-4‴), 6.76 (d, J = 8.5 Hz, H-3‴); EIMS: m/z 420 (46%) $[M]^+$, 375 (45%), 344 (51%), 219 (56%), 101 (35%; loss of phenyl cyanide cation fragment), 77 (38%; loss of phenyl cation fragment).



Compound	R	Compound	R	Compound	R
5a		5e		5i	
5b		5f		5j	
5c		5g		5k	
5d		5h		5l	

Scheme 1: Synthesis of 5-substitued-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide.

5-Benzyl-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5k)

Color off white; yield: 65%; recrystallization from EtOH; m.p. 102-106 °C; Molecular formula $C_{18}H_{16}ClN_3O_3S$; Mol. Wt. 389. IR (KBr, cm^{-1}): ν_{max}: 3024 (Ar-H), 1546 (C=C of aromatic ring), 1621 (C=O), 1516 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.11 (br.s., N-H), 8.15 (d, J = 2.1Hz, 1H, H-6‴), 7.28-7.36 (m, 5H, H-2′ to H-6′), 3.91 (s, 3H, H_3CO-2‴), 3.84 (s, 2H, H-7′), 4.11 (s, 2H, H-2″), 6.96 (dd, J = 8.5, 2.1 Hz, H-4‴), 6.71 (d, J = 8.5 Hz, H-3‴); EIMS: m/z 389 (45%) $[M]^+$, 358 (55%), 233 (60%), 117 (42%; loss of benzyl cyanide cation fragment), 91 (35%; loss of tropylium ion fragment).

5-pyridinyl-1,3,4-oxadiazole-2yl-N-(2‴-methoxy-5‴-chlorophenyl)-2″-sulfanyl acetamide (5l)

Color grey; yield: 65%; recrystallization from EtOH; m.p. 165-169 °C; Molecular formula $C_{16}H_{13}ClN_4O_3S$; Mol. Wt. 376. IR (KBr, cm^{-1}): ν_{max}: 3024 (Ar-H), 1546 (C=C of aromatic ring), 1621 (C=O), 1516 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 9.02 (br.s., N-H), 8.80 (d, J = 2.1 Hz, 1H, H-2′), 8.60 (dd, J = 8.5, 2.1 Hz, 1H, H-6'), 8.21

(d, J = 2.1Hz, 1H, H-6‴), 7.44(m, 2H, H-5′, H-4′), 7.03 (dd, J = 8.5, 2.1 Hz, 1H, H-4‴), 6.79 (d, J = 8.5 Hz, 1H, H-3‴), 3.94 (s, 2H, H-2″), 3.86 (s, 3H, H_3CO-2‴); EIMS: m/z 376 (56%) $[M]^+$, 345 (45%), 220 (54%), 104 (35%; loss of pyridinyl cyanide cation fragment), 78 (31%; loss of pyridinyl cation fragment).

RESULTS

Our objective was to synthesize some new 5-substituted-1,3,4-oxadiazole-2-sulfanyl acetamide compounds having lesser toxicity and enhanced activity. Keeping that objective in mind, we synthesized 5-substituted-1,3,4-oxadiazole-2-sulfanyl acetamide in excellent yield and having good biological activities. The synthesis was carried out through the intermolecular cyclization of different substituted/unsubstituted organic aromatic acid hydrazides to the analogous of 5-substituted-1,3,4-oxadiazoles-2-thioles and finally a series of 5-substituted-1,3,4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide (5a-l) after the reaction with N-(2-methoxy-5-chlorophenyl)-2'-bromoacetamide (**3**) in DMF (*N*,*N*-dimethylformamide) using sodium hydride (NaH) as the base as represented in Scheme-1. Complete conversion was achieved within 2 to 4 hours by simple stirring at room temperature. The product was isolated by adding cold water in the reaction mixture and subsequently, it was taken out through solvent extraction method by chloroform/ ethyl acetate. The structures of these synthesized compounds were ascertained by ^{1}H-NMR (table 1), IR and mass spectral data as illustrated in experimental section.

Enzyme Inhibition Studies

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

Table 1: ^{1}H-NMR data of 5-substitued-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide **5a-l.**

Comp.	^{1}H-NMR (300 MHz, MeOD) δ (ppm), Multiplicity, *J* (Hz)											Other protons
	H-2′	H-3′	H-4′	H-5′	H-6′	H-3‴	H-4‴	H-6‴	MeO-2‴	CH_2-2″	N-H	
5a	7.93 1H, d 8.5	6.99 1H, d, 8.5	-	6.99 1H, d, 8.5	7.93 1H, d, 8.5	6.71 1H, d 8.5	6.96 1H, dd 8.5,2.1	8.37 1H, d 2.1	3.84 s, 3H	4.06 s, 2H	9.36 br.s.	3.86 s, 3H, MeO-4′
5b	-	6.71 1H, dd 8.5, 2.1	7.37-7.33 1H, m	7.84 1H, t 8.5	7.37-7.33 1H, m	6.78 1H, d 8.5	6.95 1H, dd 8.5,2.1	8.32 1H, d 2.1	3.92 s, 3H	4.07 s, 2H	8.60 br.s.	2.71 s, 3H, Me-2′
5c	-	7.51 1H, d 8.5	7.45-7.35 1H, m	7.45-7.35 1H, m	7.95 1H, dd 8.5, 2.1	6.82 1H, d 8.5	6.99 1H, dd 8.5,2.1	8.28 1H, d 2.1	3.92 s, 3H	4.01 s, 2H	8.62 br.s.	-
5d	7.52 1H, d 2.1		7.49 1H, dd 8.5, 2.1	7.96 1H, t 8.5	7.98 1H, dd 8.5, 2.1	6.88 1H, d 8.5	6.99 1H, dd 8.5,2.1	8.29 1H, d 2.1	3.83 s, 3H	3.92 s, 2H	9.26 br.s.	-
5e	7.49 1H, d 8.5	7.91 1H, d 8.5		7.91 1H, d 8.5	7.49 1H, d 8.5	6.72 1H, d 8.5	7.00 1H, dd 8.5,2.1	8.36 1H, d 2.1	3.85 s, 3H	4.08 s, 2H	9.28 br.s.	-
5f	-	7.62 1H, d 8.5	7.47-7.38 1H, m	7.47-7.38 1H, m	8.08 1H, dd 8.5, 2.1	6.75 1H, d 8.5	7.01 1H, dd 8.5,2.1	9.25 1H, d 2.1	3.91 s, 3H	4.10 s, 2H	9.21 br.s.	-
5g	8.38 1H, d 2.1	-	8.31 1H, dd 8.5, 2.1	7.72 1H, t 8.5	8.40 1H, dd 8.5, 2.1	6.76 1H, d 8.5	7.00 1H, dd 8.5,2.1	9.20 1H, d 2.1	3.87 s, 3H	4.12 s, 2H	9.29 br.s.	-
5h	7.44 1H, d 8.5	7.91 1H, d 8.5	-	7.91 1H, d 8.5	7.44 1H, d 8.5	6.82 1H, d 8.5	6.99 1H, dd 8.5,2.1	8.27 1H, d 2.1	3.92 s, 3H	4.12 s, 2H	9.21 br.s.	-
5i	7.48 1H, d 2.1	-	7.47 1H, dd 8.5, 2.1	7.95 1H, t 8.5	7.97 1H, dd 8.5, 2.1	6.82 1H, d 8.5	6.98 1H, dd 8.5,2.1	8.29 1H, d 2.1	3.95 s, 3H	4.01 s, 2H	9.20 br.s.	-
5j	7.24-7.35 1H, m	7.24-7.35 1H, m	7.24-7.35 1H, m	7.24-7.35 1H, m	7.24-7.35 1H, m	6.76 1H, d 8.5	6.93 1H, dd 8.5,2.1	8.16 1H, d 2.1	3.89 s, 3H	4.11 s, 2H	9.21 br.s.	-
5k	7.28-7.36 1H, m	7.28-7.36 1H, m	7.28-7.36 1H, m	7.28-7.36 1H, m	7.28-7.36 1H, m	6.71 1H, d 8.5	6.96 1H, dd 8.5,2.1	8.15 1H, d 2.1	3.91 s, 3H	4.11 s, 2H	9.11 br.s.	3.84 s, 2H, H-7′
5l	8.80 1H, d 2.1	-	7.44 1H, m	7.44 1H, m	8.60 1H, dd 8.5, 2.1	6.79 1H, d 8.5	7.03 1H, dd 8.5,2.1	8.21 1H, d 2.1	3.86 s, 3H	3.94 s, 2H	9.02 br.s.	-

butyrylcholinesterase and lipoxygenase are presented in table 2.

DISCUSSION

Compound **5a** was synthesized as dark brown beads. The molecular formula $C_{18}H_{16}ClN_3O_4S$ was established with the help of EI-MS showing molecular ion peak at m/z 405 and by counting the number of protons in ^{1}H-NMR spectrum. In the IR spectrum, the absorption bands appeared at 3035 cm^{-1}, 1562 cm^{-1} and 1645 cm^{-1} which were assigned to C-H (aromatic), C=C (aromatic) and C=O (amide) respectively. The IR spectrum also showed peaks in 1600-1620 cm^{-1} for (C=N-N=C) stretching and 1063-1073 cm^{-1} for (C-O-C) stretching in compounds, which confirmed the presence of 1,3,4-oxadiazoles ring. The EI-MS also gave a distinct peak at m/z 107 after the removal of methoxyphenyl cation group and at m/z 133 after the loss of methoxy phenyl cyanide cation. In its ^{1}H-NMR spectrum (Table-1), the signals in aromatic region appeared at δ 8.37(d, J = 2.1Hz, 1H, H-6$'''$), 6.96 (dd, J = 8.5, 2.1 Hz, H-4$'''$), 6.71 (d, J = 8.5 Hz, H-3$'''$) which were assigned to the protons of tri-substituted ring and the signals appearing at δ 7.93 (d, J = 8.5Hz, H-2$'$, H-6$'$) and 6.99 (d, J = 8.5 Hz, H-3$'$, H-5$'$) were typical for the protons of the para di-substituted aromatic ring. In the aliphatic region of ^{1}H-NMR spectrum three singlet appeared at δ 3.84 (s, 3H, H_3CO-2$'''$), 3.86 (s, 3H, H_3CO-4$'$) and 4.06 (s, 2H, H-2$''$) which were assigned to the protons of two methoxy groups and one methylene group respectively, present in the molecule. On the basis of above cumulative evidences the structure of 5a was allocated as 5-(4$'$-methoxy phenyl)-1,3,4-oxadiazole-2yl-N-(2$'''$-methoxy-5$'''$-chlorophenyl)-2$''$-sulfanyl acetamide which is a new 2,5-disubstituted 1,3,4-oxadiazole. Similarly on the basis of spectral evidences from IR, EI-MS and ^{1}H-NMR (table-1), the structures of other derivatives of 2,5-disubstituted 1,3,4-oxadiazole were elucidated as described in experimental section.

The screening of these 5-substituted-1,3,4-oxadiazole-2-sulfanyl acetamide derivatives against enzymes revealed that they exhibited appropriate inhibitory potential against acetylcholinesterase (AChE) as it was evident from their IC_{50} values. It is obvious from table-2 that compounds **5b, 5f** and **5k** showed reasonably good inhibiting activity against acetylcholinesterase having IC_{50} value of 34.61 ± 0.62, 40.21 ± 0.25 and 45.11 ± 0.22 µmol/L respectively, relative to Eserine, a reference standard with IC_{50} value of 0.04±0.001 µmol/L, probably due to the ortho substitution of methyl and nitro group on the aromatic ring respectively in these molecules. The screening against butyrylcholinesterase enzyme exposed that only one compound, 5-benzyl-1,3,4-oxadiazole-2yl-N-(2$'''$-methoxy -5$'''$-chlorophenyl)-2$''$-sulfanyl acetamide (5f) exhibited excellent inhibitory potential having IC_{50} 33.31±0.52 µmol/L, however the compounds **5b, 5c, 5e, 5h** and **5i** were remained inactive but all other compounds showed weak activity. Similarly, lipoxygenase enzyme screening revealed that all the 5-substituted-1,3,4-oxadiazole-2-sulfanyl acetamide compounds showed moderate activity except **5b** and **5k** compounds which were inactive against this enzyme.

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Table 2: Bioactivity studies of 5-substitued-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide 5a-l.

C. No.	LOX			AChE			BChE		
	Conc./ well (mM)	Inhibition (%)	IC_{50} µM	Conc./ well (mM)	Inhibition (%)	IC_{50} µM	Conc./ well (mM)	Inhibition (%)	IC_{50} µM
5a	0.5	50.79±0.31	398.91±0.34	0.25	63.82±0.31	139.11±0.41	0.5	58.07±0.31	409.31±0.08
5b	0.25	44.46±0.41	Nil	0.25	87.50±0.14	34.61±0.62	0.25	25.60±0.19	-
5c	0.25	76.86±0.34	166.11±0.24	0.25	84.65±0.22	75.81±0.04	0.5	45.78±0.45	-
5d	0.5	64.56±0.34	356.11±0.18	0.25	74.01±0.71	132.51±0.66	0.5	64.46±0.24	273.21±0.11
5e	0.25	68.94±0.61	179.11±0.62	0.25	69.85±0.35	165.61±0.32	0.5	52.08±0.51	386.91±0.23
5f	0.5	69.67±0.13	354.91±0.25	0.5	69.08±0.52	40.21±0.25	0.5	67.95±0.44	375.11±0.35
5g	0.5	95.13±0.87	184.31±0.53	0.5	71.93±0.01	66.71±0.92	0.5	71.72±0.59	264.11±0.65
5h	0.25	60.54±0.25	192.21±0.87	0.25	54.57±0.05	213.25±0.07	0.25	39.16±0.05	-
5i	0.25	56.45±0.22	384.91±0.28	0.25	57.57±0.34	192.91±0.14	0.25	37.63±0.22	-
5j	0.5	70.16±0.19	260.81±0.34	0.5	59.10±0.36	360.81±0.25	0.5	60.09±0.11	425.91±0.24
5k	0.25	47.50±0.09	Nil	0.125	78.73±0.59	45.11±0.22	0.25	92.84±0.09	33.31±0.52
5l	0.25	77.71±0.59	144.43±0.18	0.25	66.12±0.73	67.91±0.41	0.5	63.82±0.59	328.71±0.57
Control		Baicalein	22.4±1.3		Eserine	0.04±0.001		Eserine	0.85±0.001

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.

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