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Synthesis, Characterization and Pharmacological Evaluation of *N*-Substituted Derivatives of 5-(4-Nitrophenyl)-1,3,4-oxadiazole-2yl-2''-sulphanyl Acetamide

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A series of new *N*-substituted 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl-2''-sulphanyl acetamides was synthesized and enzyme inhibiting activity was screened for all these chemical entities. Structural characterization of all these compounds was made by IR, EI-MS and ^{1}H NMR. All derivatives demonstrated different extent of activity for lipoxygenase (LOX), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Compounds **6j** exhibited excellent lipoxygenase inhibitory potential but against acetylcholinesterase the inhibitory potential is not appreciable and it is inactive against butyrylcholinesterase. Similarly compound **6e** have good inhibitory potential against lipoxygenase with IC_{50} value 50.41 ± 0.12 µM while for acetylcholinesterase IC_{50} value is 174.21 ± 0.11 µM and **6e** does not show any inhibitory potential for butyrylcholinesterase. Compound **6o** showed good inhibition against acetylcholinesterase and butyrylcholinesterase but for lipoxygenase its inhibitory activity is the least.

Key Words: 4-Nitrobenzoic acids, Oxadiazoles, Cholinesterase, Lipoxygenase, ^{1}H NMR and EI-MS.

INTRODUCTION

Most of the drugs used for the treatment of Alzheimer's disease (AD) are acetylcholinesterase inhibitors. Acetylcholinesterase causes the hydrolysis of acetyl choline in the synaptic cleft, so acetyl cholinesterase inhibitors ensure the availability of acetylcholine in the synaptic cleft by decreasing the activity of acetylcholinesterase and help to enhance the cholinergic neurotransmission[1]. Lipoxygenase enzymes contain iron in their structural frame work and are involved in dioxygenation of lipids, consisting of poly unsaturated fatty acids. Lipoxygenases perform a key role in the synthesis of leukotrienes, which are responsible for pathophysiology of different allergic diseases. Lipoxygenase inhibitors are required to cure these allergic and inflammatory diseases[2]. Butyryl cholinesterase is a sister enzyme of acetyl cholinesterase. It is also known as pseudocholinesterase[3]. Butyrylcholinesterase is toxicologically and pharmacologically very important[4].

The aim of our present research work was to synthesize new and pharmacologically active compounds to meet the needs of pharmacy due to increased resistance against present antibacterial, antiviral, fungicidal and pesticidal drugs. Oxadiazole analogues are pharmacologically very active due to their antiinflammatory, antituberculosis, anticonvulsant, antianalgesic, antidepressant and anti HIV activities[5-7]. The significance of these compounds lies in the fact that they can be successfully utilized as antibacterial, analgesic, antiinflammatory, anticancer, anti HIV agent, antitubercular and insecticidal agents[8-10]. 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. Biologically and pharmacologically most important class of oxadiazoles is 1,3,4-oxadiazole-2-thiol. In view of the published information and in the continuation of our previous work[11], we now report the synthesis of *N*-substituted 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl- 2''-sulphanyl acetamides and screening out their biological activities.

EXPERIMENTAL

Chemicals were purchased from Sigma Aldrich and Alfa Aesar (Germany). By using open capillary tube method melting points were taken on Griffin and George melting point apparatus. Melting points were uncorrected. By using thin layer chromatography and *n*-hexane and ethyl acetate as mobile phase purity of the synthesized compounds was detected at 364 nm. By using KBr, IR peaks were recorded on a Jasco-320-A

spectrophotometer. ^{1}H NMR signals were recorded at 500 MHz in mixture of $CDCl_3$ and CD_3OD using Bruker spectrometers, chemical shift values are mentioned in ppm unit. EIMS signals are recorded by utilizing a JMS-HX-110 spectrometer.

Synthesis of ethyl-4-nitro benzoate (2): 4-Nitrobenzoic acid (**1**) (5g, 0.0295 mol.) was refluxed with absolute ethanol (20 mL) and conc. H_2SO_4 (2.5 mL) in round bottom flask fitted with condenser for 1 h. Thin layer chromatography was used to check the completion of reaction. Reaction mixture was poured in 50 mL distilled water in separating funnel, after the indication of completion of reaction by TLC. Diethyl ether (45 mL) was used to extract the product by vigorous shaking in separating funnel and addition of conc. sodium carbonate afforded the neutralization of reaction mixture. Two layers were separated by allowing the solution to stand for some time. To avoid contaminants upper organic layer containing ethyl-4-nitrobenzoate was collected from the neck of separating funnel. Lemon coloured ester was collected by evaporating diethyl ether.

Preparation of 4-nitro benzohydrazide (3): Synthesis of 4-nitrobenzohydrazide was afforded by allowing ethyl 4-nitrobenzoate (2 g, 0.01 mol), on complete dissolution, to react with hydrazine hydrate (7.90 mL, 0.03 mol.) in methanol (15 mL) by vigorous stirring at room temperature for 3 h in a round bottom flask. Thin layer chromatography was used to check the completion of reaction. Precipitates of product were quenched by addition of distilled water and filtered washed. Recrystallization was brought about by using methanol.

Synthesis of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-thiol (4): Compound (**3**) (4 g, 0.022 mol) to carbon disulphide (1.32 mL, 0.022 mol) in the presence of potassium hydroxide (2.46 g, 0.044 mol) and ethanol (40 mL) as solvent in 250 mL round bottom flask, reaction assembly was set to reflux reaction contents for 5 h with continuous stirring. By using TLC reaction coordinates were monitored after every hour. On completion reaction mixture was acidified and pH was set 2.0-3.0 then treated with distilled water to quench the synthesized product that was filtered and washed with distilled water. Recrystallization was done by using methanol.

General procedure for the synthesis of S-substituted 5-(4-nitrophenyl)-1,3,4-oxadiazole derivatives (6a-o): Compound (**4**), (0.1 g, 0.0045 mol.) was allowed to react with equimolar ratios of aliphatic/ aromatic substituted bromo-acetamide (**5a-o**) by dissolving the first in DMF (5-10 mL) by stirring at room temperature in 100 mL round bottom flask in the presence of NaH (0.002 g), that acts as a strong base. The time span for different aliphatic/aromatic substituted bromoacetamide varies from 2-3 h. Thin layer chromatography by using ethyl acetate and *n*-hexane as a mobile phase was carried out to check the reaction completion. Distilled water was added in the reaction mixture to separate the precipitates. Precipitates were washed, dried and subjected for spectral analysis.

Characterization of the synthesized compounds

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2-methoxyphenyl)acetamide (6a): Lemon yellow colour; yield: 73 %; m.p. 110-112 ºC; m.f.: $C_{17}H_{14}N_4O_5S$; m.w. 386; IR (KBr, ν_{max}, cm^{-1}): 3327 (N-H, stretching), 3067 (C-H, str. of aromatic ring), 1649 (C=O str.), 1547 (C=C, aromatic str.), 1653 (C=N, str. of oxadiazole ring), 1275, 1053 (C-O-C bond str.), 641 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.23 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.16 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.10 (d, *J* = 7.8 Hz, 1H, H-6'''), 7.06 (br.t, *J* = 7.8 Hz, 1H, H-5'''), 6.97 (d, *J* = 8.4 Hz, 1H, H-3'''), 6.86 (ddd, *J* = 8.4, 1.5 Hz, 1H, H-4'''), 4.31 (s, 2H, H-2''), 3.85 (s, 3H, OCH_3-2'''); EIMS (m/z): 386 (17 %)[M^+], 264 (21 %), 164 (100 %), 150 (37 %), 148 (71 %), 122 (56 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(3-methoxyphenyl)acetamide (6b): Creamy white colour; yield: 60 %; m.p. 130-132 ºC; m.f.: $C_{17}H_{14}N_4O_5S$; m.w. 386; IR (KBr, ν_{max}, cm^{-1}): 3343 (N-H, stretching), 3079 (C-H, str. of aromatic ring), 1663 (C=O str.), 1557 (C=C, aromatic str.), 1671 (C=N, str. of oxadiazole ring), 1281, 1061 (C-O-C bond str.), 637 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.24 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.17 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.29 (d, *J* = 1.8 Hz, 1H, H-2'''), 7.21 (t, *J* = 8.4 Hz, 1H, H-5'''), 7.08 (dd, *J* = 8.4, 1.8 Hz, 1H, H-6'''), 6.70 (dd, *J* = 8.4, 1.8 Hz, 1H, H-4'''), 4.28 (s, 2H, H-2''), 3.77 (s, 3H, OCH_3-3'''); EIMS (m/z): 386 (20 %) [M^+], 264 (24 %), 164 (100 %), 150 (41 %), 148 (68 %), 122 (49 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2-methylphenyl)acetamide (6c): Lemon yellow colour; yield: 75 %; m.p. 42-44 ºC; m.f.: $C_{17}H_{14}N_4O_4S$; m.w. 370; IR (KBr, ν_{max}, cm^{-1}): 3325 (N-H, stretching), 3033 (C-H, str. of aromatic ring), 1610 (C=O str.), 1521 (C=C, aromatic str.), 1630 (C=N, str. of oxadiazole ring), 1235, 1065 (C-O-C bond str.), 618 (C-S bond str.); ^{1}H NMR (DMSO, 500 MHz): δ (ppm) 14.95 (br. s, 1H, -NH), 8.24 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.12 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.37 (d, *J* = 7.5 Hz, 1H, H-6'''), 7.21 (d, *J* = 7.5 Hz, 1H, H-3'''), 7.15 (br.t, *J* = 7.5 Hz, 1H, H-5'''), 7.09 (ddd, *J* = 6.5, 1.0 Hz, 1H, H-4'''), 4.41 (s, 2H, H-2''), 2.19 (s, 3H, CH_3-2'''); EIMS (m/z): 370 (20 %)[M^+], 222 (41 %), 148 (100 %), 122 (35 %), 91 (75 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(3-methylphenyl)acetamide (6d): Lemon yellow colour; yield: 70 %; m.p. 42-44 ºC; m.f.: $C_{17}H_{14}N_4O_4S$; m.w. 370; IR (KBr, ν_{max}, cm^{-1}): 3341 (N-H, stretching), 3075 (C-H, str. of aromatic ring), 1669 (C=O str.), 1563 (C=C, aromatic str.), 1667 (C=N, str. of oxadiazole ring), 1279, 1058 (C-O-C bond str.), 631 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.38 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.21 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 8.37 (d, *J* = 3.0 Hz, 1H, H-2'''), 8.12 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.17 (t, *J* = 8.0 Hz, 1H, H-5'''), 6.88 (d, *J* = 8.0 Hz, 1H, H-4'''), 4.37 (s, 2H, H-2''), 2.26 (s, 3H, CH_3-3'''); EIMS (m/z): 370 (10 %)[M^+], 222 (32 %), 148 (75 %), 122 (39 %), 91 (100 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(4-methylphenyl)acetamide (6e): Off-white colour; yield: 67 %; m.p. 42-44 ºC; m.f.: $C_{17}H_{14}N_4O_4S$; m.w. 370; IR (KBr, ν_{max}, cm^{-1}): 3345 (N-H, stretching), 3060 (C-H, str. of aromatic ring), 1630 (C=O str.), 1537 (C=C, aromatic str.), 1645 (C=N, str. of oxadiazole ring), 1255, 1090 (C-O-C bond str.), 625 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.23 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.17 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.44 (d, *J* = 7.1 Hz, 2H, H-2''' and H-6'''), 7.14 (d, *J* = 7.1 Hz, 2H, H-3''' and H-5'''), 4.27 (s, 2H, H-2''), 2.29 (s, 3H, CH3-4'''); EIMS (m/z): 370 (12 %)[M^+], 222 (17 %), 148 (100%), 122 (37 %), 91 (71 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2,4-dimethylphenyl)acetamide (6f): Lemon yellow colour; yield: 63 %; m.p. 160-162 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, ν_{max}, cm^{-1}): 3339 (N-H, stretching), 3085 (C-H, str. of aromatic ring), 1655 (C=O str.), 1560 (C=C, aromatic str.), 1667 (C=N, str. of oxadiazole ring), 1287, 1065 (C-O-C bond str.), 637 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.26 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.17 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.23 (d, *J* = 8.1 Hz, 1H, H-6"'), 7.03 (s, 1H, H-3"'), 6.99 (d, *J* = 8.1 Hz, 1H, H-5"'), 4.30 (s, 2H, H-2"), 2.27 (s, 3H, CH_3-2"'), 2.20 (s, 3H, CH_3-4"'); EIMS (m/z): 384 (12 %) [M^+], 264 (17 %), 148 (100 %), 122 (37 %), 120 (71 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2,5-dimethylphenyl)acetamide (6g): Mustard colour; yield: 65 %; m.p. 180-182 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, ν_{max}, cm^{-1}): 3315 (N-H, stretching), 3065 (C-H, str. of aromatic ring), 1676 (C=O str.), 1547 (C=C, aromatic str.), 1640 (C=N, str. of oxadiazole ring), 1265, 1043 (C-O-C bond str.), 642 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.25 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.15 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.18 (d, *J* = 1.5 Hz, 1H, H-6"'), 7.08 (dd, *J* = 8.1, 1.5 Hz, 1H, H-4"'), 6.92 (d, *J* = 8.1 Hz, 1H, H-3"'), 4.30 (s, 2H, H-2"), 2.18 (s, 3H, CH_3-2"'), 2.16 (s, 3H, CH_3-5"'); EIMS (m/z): 384 (17 %) [M^+], 264 (22 %), 148 (100 %), 122 (31 %), 120 (67 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2,6-dimethylphenyl)acetamide (6h): Creamy white colour; yield: 66 %; m.p. 160-162 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, ν_{max}, cm^{-1}): 3315 (N-H, stretching), 3067 (C-H, str. of aromatic ring), 1676 (C=O str.), 1538 (C=C, aromatic str.), 1658 (C=N, str. of oxadiazole ring), 1283, 1047 (C-O-C bond str.), 643 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.27 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.17 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.08-7.06 (m, 3H, H-3"' to H-5"'), 4.35 (s, 2H, H-2"), 2.20 (s, 6H, CH_3-2"' and CH_3-6"'); EIMS (m/z): 384 (16 %)[M^+], 264 (19 %), 148 (100 %), 122 (41 %), 120 (73 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(3,4-dimethylphenyl)acetamide (6i): Buff colour; yield: 71 %; m.p. 145-147 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, n_{max}, cm^{-1}): 3313 (N-H, stretching), 3076 (C-H, str. of aromatic ring), 1648 (C=O str.), 1567 (C=C, aromatic str.), 1673 (C=N, str. of oxadiazole ring), 1279, 1059 (C-O-C bond str.), 642 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.23 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.17 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.29 (d, *J* = 2.4 Hz, 1H, H-2"'), 7.26 (dd, *J* = 8.1, 2.4 Hz, 1H, H-6"'), 7.04 (d, *J* = 8.1 Hz, 1H, H-5"'), 4.26 (s, 2H, H-2"), 2.23 (s, 3H, CH_3-4"'), 2.21 (s, 3H, CH_3-3"'); EIMS (m/z): 384 (15 %) [M^+], 264 (27 %), 148 (100 %), 122 (41 %), 120 (69 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(3,5-dimethylphenyl)acetamide (6j): Yellow colour; yield: 69 %; m.p. 163-165 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, ν_{max}, cm^{-1}): 3317 (N-H, stretching), 3063 (C-H, str. of aromatic ring), 1654 (C=O str.), 1558 (C=C, aromatic str.), 1661 (C=N, str. of oxadiazole ring), 1291, 1069 (C-O-C bond str.), 633 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.40 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.23 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.96 (s, 2H, H-2"' to H-6"'), 7.18 (s, 1H, H-4"'), 4.26 (s, 2H, H-2"), 2.26 (s, 6H, CH_3-3"' and CH_3-5"'); EIMS (m/z): 384 (18 %) [M^+], 264 (21 %), 148 (100 %), 122 (31 %), 120 (73 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-benzylacetamide (6k): Lemon yellow colour; yield: 67 %; m.p. 150-152 ºC; m.f.: $C_{17}H_{14}N_4O_4S$; m.w. 370; IR (KBr, ν_{max}, cm^{-1}): 3343 (N-H, stretching), 3053 (C-H, str. of aromatic ring), 1663 (C=O str.), 1573 (C=C, aromatic str.), 1677 (C=N, str. of oxadiazole ring), 1273, 1058 (C-O-C bond str.), 653 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.41 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.16 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.29-7.06 (m, 5H, H-2"' to H-6"'), 4.28 (s, 2H, H-2"), 3.76 (s, 2H, H-7"'); EIMS (m/z): 370 (15 %) [M^+], 264 (24 %), 148 (100 %), 134 (45 %), 122 (69 %), 106 (47 %), 91 (73 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2-phenylethyl)acetamide (6l): Light yellow colour; yield: 59 %; m.p. 156-158 ºC; m.f.: $C_{18}H_{16}N_4O_4S$; m.w. 384; IR (KBr, ν_{max}, cm^{-1}): 3351 (N-H, stretching), 3047 (C-H, str. of aromatic ring), 1660 (C=O str.), 1576 (C=C, aromatic str.), 1671 (C=N, str. of oxadiazole ring), 1279, 1069 (C-O-C bond str.), 647 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.41 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.24 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.27-7.12 (m, 5H, H-2"' to H-6"'), 4.03 (s, 2H, H-2"), 3.44 (t, *J* = 7.2 Hz, 2H, H-8"'), 2.72 (t, *J* = 7.2 Hz, 2H, H-7"'); EIMS (m/z): 384 (19 %) [M^+], 264 (21 %), 162 (81 %), 148 (100 %), 122 (67 %), 120 (43 %), 105 (67 %), 91 (77 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2-hydroxyphenyl)acetamide (6m): Coffee colour; yield: 68 %; m.p. 90-92 ºC; m.f.: $C_{16}H_{12}N_4O_5S$; m.w. 372; IR (KBr, ν_{max}, cm^{-1}): 3351 (N-H, stretching), 3047 (C-H, str. of aromatic ring), 1660 (C=O str.), 1576 (C=C, aromatic str.), 1671 (C=N, str. of oxadiazole ring), 1279, 1069 (C-O-C bond str.), 647 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.41 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.18 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 7.80 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6"'), 7.00 (ddd, *J* = 8.1, 1.5 Hz, 1H, H-4"'), 6.85 (dd, *J* = 8.1, 1.5 Hz, 1H, H-3"'), 6.79 (ddd, *J* = 8.1, 1.5 Hz, 1H, H-5"'), 4.33 (s, 2H, H-2"); EIMS (m/z): 372 (11 %) [M^+], 264 (23 %), 222 (76 %), 150 (45 %), 148 (79 %), 136 (56 %), 108 (91 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2,4-dinitrophenyl)acetamide (6n): Lemon yellow colour; yield: 61 %; m.p. 160-162 ºC; m.f.: $C_{16}H_{10}N_6O_8S$; m.w. 446; IR (KBr, ν_{max}, cm^{-1}): 3356 (N-H, stretching), 3041 (C-H, str. of aromatic ring), 1657 (C=O str.), 1569 (C=C, aromatic str.), 1659 (C=N, str. of oxadiazole ring), 1283, 1071 (C-O-C bond str.), 635 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.98 (d, *J* = 2.7 Hz, 1H, H-3"'), 8.82 (d, *J* = 9.6 Hz, 1H, H-6"'), 8.69 (d, *J* = 9.0 Hz, 2H, H-3' and H-5'), 8.53 (dd, *J* = 9.6, 2.4 Hz, 1H, H-5"'),8.39 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 4.16 (s, 2H, H-2"); EIMS (m/z): 446 (17 %) [M^+], 264 (21 %), 224 (100 %), 210 (37 %), 182 (71 %), 148 (41 %), 122 (56 %).

2-[{5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-cyclohexylacetamide (6o): Buff colour; yield: 58 %; m.p. 140-142 ºC; m.f.: $C_{16}H_{18}N_4O_4S$; m.w. 362; IR (KBr, ν_{max}, cm^{-1}): 3357 (N-H, stretching), 3035 (C-H, str. of aromatic ring), 1653 (C=O str.), 1583 (C=C, aromatic str.), 1657 (C=N, str. of oxadiazole ring), 1263, 1057 (C-O-C bond str.), 653 (C-S bond str.); ^{1}H NMR (MeOD, 300 MHz): δ (ppm) 8.25 (d, *J* = 9.0

Hz, 2H, H-3' and H-5'), 8.18 (d, *J* = 9.0 Hz, 2H, H-2' and H-6'), 4.06 (s, 2H, H-2"), 3.61 (m, 1H, H-1"'), 1.87-1.71 (m, 4H, H-2"' and H-6"'), 1.64-1.60 (m, 2H, H-4"'), 1.36-1.17 (m, 4H, H-3"' and H-5"'); EIMS (m/z): 362 (17 %) [M^+], 264 (26 %), 148 (100 %), 140 (49 %), 126 (71 %), 122 (46 %), 98 (77 %), 83 (75 %).

Enzyme inhibition essays

Acetylcholinesterase and butyrylcholinesterase assay: The AChE and BChE inhibition activity was performed according to the method[12] with slight modifications. 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 (Sigma, USA). The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37 ºC. 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 37 ºC 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

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

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

Lipoxygenase assay: Lipoxygenase (LOX) activity was assayed addording to the reported method[13-15] with slight modifications. A total volume of 200 µL lipoxygenase assay mixture contained 150 µL sodium phosphate buffer (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 preincubated for 10 min at 25 ºC. 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.

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

where Control = total enzyme activity without inhibitor, Test = activity in the presence of test compound.

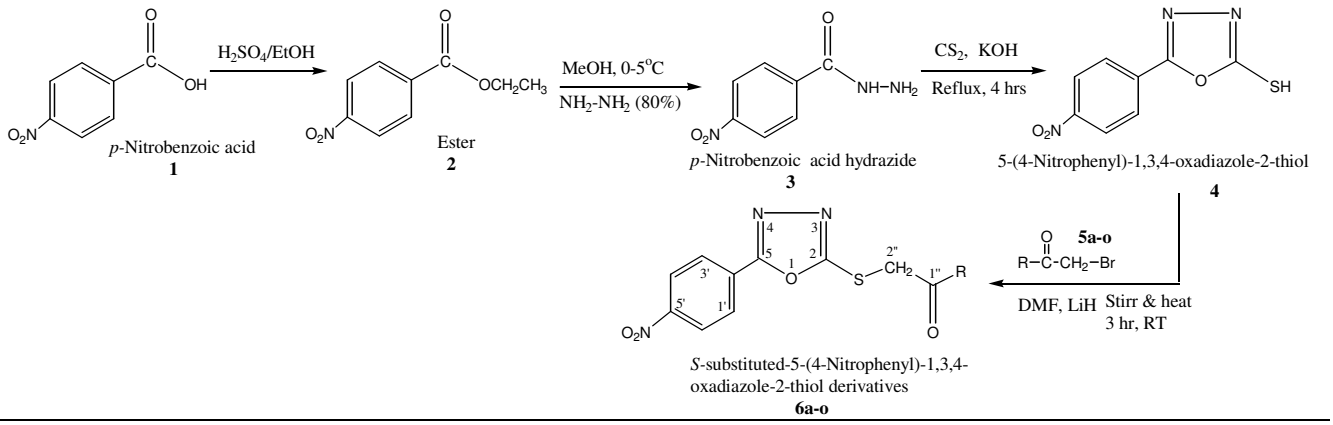
IC_{50} values was calculated using EZ-Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).

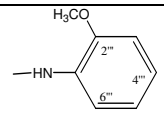
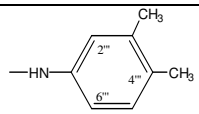
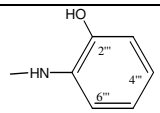
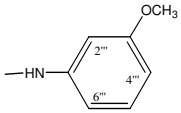
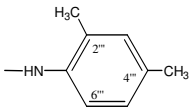
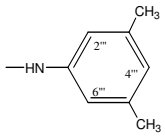
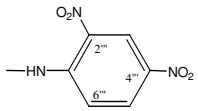
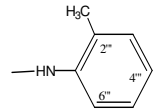
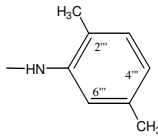
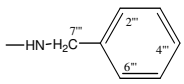
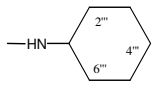
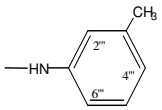
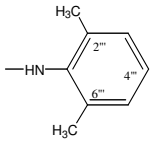
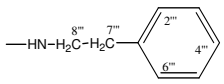
Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

RESULTS AND DISCUSSION

The aim of the present research work was to synthesize new compounds that have great potential as lipoxygenase, AChE and BChE inhibitors. The need of hours is to introduce pharmacologically active drugs to help pharmacy against the increasing resistance for existing drugs. Keeping that objective in mind, we synthesized *N*-substituted 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl-2"-sulphanyl acetamides in excellent yield and having good biological activities. The synthesis was carried out through the intermolecular cyclization of 4-nitrobenzoic acid hydrazide to 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-thiol and finally a series of *N*-substituted 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl-2"-sulphanyl acetamides (**6a-o**) after the reaction with *N*-aryl/alkyl-substituted 2-bromoacetamide (**5a-o**) in DMF using sodium hydride as a base as represented in **Scheme-I**. Complete conversion was achieved within 2-4 h 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. All the derivatives (**6a-o**) were characterized by IR, ^{1}H NMR and EI-MS analysis. IR spectra revealed characteristic peaks at 3357-3313 cm^{-1} for N-H str. 1676-1610 for C=O str. of amide group, 1677-1630 for C=N str. of oxadiazole ring. ^{1}H NMR data gave peaks for -NH at 14.95 ppm and -CH_2 of acetamide group appeared at 4.03-4.41 ppm as singlet having integration of two protons. Compound (**6a**) was obtained as lemon yellow colour solid with m.f. $C_{17}H_{14}N_4O_5S$, that is confirmed by EI-MS and molecular ion peak appeared at 386. In IR spectrum characteristic peaks appeared at 3327 (N-H, stretching), 3067 (C-H, str. of aromatic ring), 1649 (C=O str.), 1547 (C=C, aromatic str.), 1653 (C=N, str. of oxadiazole ring), 1275, 1053 (C-O-C bond str.), 641 (C-S bond str.), 1267 cm^{-1} (C=S, stretching) confirming the presence of oxadiazole ring. In ^{1}H NMR spectrum signals of aromatic proton appeared at δ 8.23 (br.d, *J* = 9.0 Hz, 2H, H-3' and H-5') and 8.16 (br.d, *J* = 9.0 Hz, 2H, H-2' and H-6') typical for para di-substituted aromatic ring other aromatic ring showed peaks at 7.10 (d, *J* = 7.8 Hz, 1H, H-6"'), 7.06 (br.t, *J* = 7.8 Hz, 1H, H-5"'), 6.97 (d, *J* = 8.4 Hz, 1H, H-3"'), 6.86 (ddd, *J* = 8.4, 1.5 Hz, 1H, H-4"') for four different protons of *ortho* substituted ring. In ^{1}H NMR spectrum signals appeared in the aliphatic region at 4.31 (s, 2H, H-2"), 3.85 (s, 3H, OCH_3-2"') which specified -CH_2 group of acetamide moiety and methoxy group present on the benzene ring. On the basis of these spectral evidences, the structure was determined as 2-[{5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(2-methoxyphenyl)acetamide. 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.

Pharmacology: All the compounds were screened against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX) enzymes and results are mentioned in Table-1. Compounds 2-[{5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(3,5-dimethyl phenyl)acetamide (**6j**) exhibit excellent inhibitory potential against lipoxygenase with IC_{50} value 46.51 ± 0.02 µmol/L relative to Baicalein, a reference standard with IC_{50} value of 22.4 ± 1.3 µmol/L but against acetylcholinesterase the inhibitory potential is not appreciable and it is inactive against butyrylcholinesterase. Compound 2-[{5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl}-



Comp.	-R	Comp.	-R	Comp.	-R	Comp.	-R
6a		**6e**		**6i**		**6m**	
6b		**6f**		**6j**		**6n**	
6c		**6g**		**6k**		**6o**	
6d		**6h**		**6l**		**–**	–

Scheme-I: Synthesis of N-substituted derivatives of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2yl-2"-sulfanyl acetamide

TABLE-1
BIOACTIVITY STUDIES OF N-SUBSTITUTED DERIVATIVES OF
5-(4-NITROPHENYL)-1,3,4-OXADIAZOLE-2YL-2"-SULFANYL ACETAMIDE

Sample code	AChE		BChE		LOX	
	Inhibition (%) conc./well (0.5 mM)	IC_{50} (µM)	Inhibition (%) conc./well (0.5 mM)	IC_{50} (µM)	Inhibition (%) conc./well (0.5 mM)	IC_{50} (µM)
6a	55.11 ± 0.64	<400	28.62 ± 0.51	–	41.76 ± 0.58	–
6b	76.09 ± 0.82	147.81 ± 0.15	34.92 ± 0.37	–	60.85 ± 0.66	248.51 ± 0.14
6c	66.79 ± 0.15	198.51 ± 0.01	62.41 ± 0.47	281.31 ± 0.11	64.83 ± 0.58	215.41 ± 0.21
6d	65.33 ± 0.72	205.31 ± 0.11	40.19 ± 0.22	–	54.07 ± 0.25	<400
6e	67.52 ± 0.63	174.21 ± 0.11	36.88 ± 0.48	–	95.83 ± 0.22	50.41 ± 0.12
6f	79.74 ± 0.09	112.11 ± 0.05	46.23 ± 0.54	–	70.16 ± 0.11	147.91 ± 0.15
6g	77.55 ± 0.14	134.21 ± 0.12	53.21 ± 0.17	<400	39.59 ± 0.87	–
6h	77.37 ± 0.59	128.11 ± 0.14	73.06 ± 0.55	182.31 ± 0.07	68.61 ± 0.69	183.25 ± 0.13
6i	68.80 ± 0.37	134.71 ± 0.11	51.76 ± 0.22	<300	69.51 ± 0.81	98.61 ± 0.11
6j	57.12 ± 0.17	<400	18.18 ± 0.11	–	96.91 ± 0.69	46.51 ± 0.02
6k	67.88 ± 0.15	150.11 ± 0.15	57.23 ± 0.91	<400	45.25 ± 0.18	–
6l	74.45 ± 0.11	158.21 ± 0.14	67.56 ± 0.08	231.71 ± 0.06	14.15 ± 0.19	–
6m	40.33 ± 0.55	–	51.03 ± 0.39	<400	38.18 ± 0.56	–
6n	53.65 ± 0.31	<400	18.90 ± 0.25	–	58.43 ± 0.15	285.21 ± 0.25
6o	91.06 ± 0.23	98.11 ± 0.14	94.51 ± 0.22	81.21 ± 0.15	51.26 ± 0.35	<400
Control	Eserine 91.29 ± 1.17	0.04 ± 0.001	Eserine 82.82 ± 1.09	0.85 ± 0.0001	Baicalein 93.79 ± 1.27	22.4 ± 1.3

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). AChE = Acetylcholinesterase. BChE = Butyrylcholinesterase. LOX = Lipoxygenase.

sulphanyl]-*N*-cyclohexylacetamide (**6o**) showed good inhibition against AChE and BChE but for lipoxygenase its inhibitory activity is not mentionable and IC_{50} values for AChE and BChE are 98.11 ± 0.14 and 81.21 ± 0.15 µmol/L, respectively. Similarly the compound 2-[{5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl}sulphanyl]-*N*-(4-methylphenyl)acetamide (**6e**) revealed good inhibitory potential against lipoxygenase enzyme with IC_{50} value of 50.41 ± 0.12 µmol/L while for AChE IC_{50} value is 174.21 ± 0.11 µmol/L and **6e** does not show any inhibitory potential for BChE.

Conclusion

The proposed structures of the synthesized compounds are well supported by spectroscopic data. From the enzyme inhibition data (Table-1), it is concluded that acetamide derivatives of oxadiazoles compounds showed moderate to weak activity against these enzymes which was evident from their IC_{50} values, relative to the standards used. Hence, on the basis of aforesaid results, it was generally concluded that these derivatives seem relatively more suitable drug candidates for the treatment of Alzheimer's disease and other associated diseases.

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