# Synthesis of biologically active *O*-substituted derivatives of 1-[(3, 5-dichloro-2-hydroxyphenyl)sulfonyl]piperidine

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**Abstract**: In the present work, a series of 2-*O*-substituted derivatives of 1-[(3,5-dichloro-2-hydroxy phenyl) sulfonyl]piperidine (5a-j) were synthesized. These derivatives were geared up by the coupling of 3,5-dichloro-2-hydroxy benzenesulfonyl chloride (1) with piperidine (2) under dynamic pH control in aqueous media to form parent compound 1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidine (3), followed by the substitution at oxygen atom with different electrophiles (4a-j) in the presence of sodium hydride (NaH) and dimethyl formamide (DMF) to give a series of *O*-substituted derivatives of sulfonamides bearing piperidine nucleus 5a-j. The synthesized *O*-substituted sulfonamides were spectrally characterized. The bioactivity of all the synthesized compounds were evaluated against lipoxygenase (LOX), acetylcholinesterae (AChE) and butyrylcholinesterase (BChE) enzymes and found to be having talented activity against butyrylcholinesterase enzyme.

**Keywords**: Piperidine, 3,5-dichloro-2-hydroxybenzenesulfonyl chloride, enzyme inhibition activity, $^1$H-NMR, EI-MS.

## INTRODUCTION

Several thousand of piperidine compounds have been cited in clinical and preclinical studies. Besides the novel structural features, these sulfonamides also exhibit an extensive range of biological activities. The piperidine nucleus is a ubiquitous structural feature in several pharmacologically active compounds, for example berberine and hydrastine which are natural medicinally important alkaloids (Sanchez-Sancho *et al.,* 1998; Nithiya *et al.,* 2011; Adger *et al.,* 1996 and Scopes *et al.,* 1992). There is loads of piperidine containing compounds which possess remarkable biological and medicinal properties (Daly *et al.,* 1986 and Fodor *et al.,* 1985). Piperidine and Pyrrolidine nucleus containing compounds showed their appreciable effect on plasma glucose level (Campfield *et al.,* 1995), Insulin normalization, cure of cocaine abuse (Kozikowski *et al.,* 1998).

Piperidine is also active as local anesthetics, such as mepivacaine, ropivacaine, and bupivacaine are extensively used in clinical practice (Brau *et al.,* 2000 and Bolzani *et al.,* 1995). Piperidine derivatives are found to attain pharmacological activity and form a vital part of the molecular structures of important drugs such as raloxifene and minoxidil. Selective inhibition of a number of enzymes has rendered piperidine alkaloids as important paraphernalia in the study of biochemical pathways (Gulluoglu *et al.,* 2007). Sulfonamides are in use as restorative agents from many years. Very first use of sulfonamides was as antibacterial agent, but their uses have unmitigated to treat other diseases. Sulfonamides are also prominent for enzyme inhibition such as carbonic anhydrase, cysteine protease, HIV protease and cyclooxygenase (Supuran *et al.,* 2003). Moreover; sulfonamides have extensive potential to other therapeutic applications i.e. in cancer chemotherapy, hypoglyceamia, and diuretics (Supuran *et al.,* 2004).

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcho linesterase (BChE, EC 3.1.1.8) belong to an enzymes family which comprises serine hydrolases. The various specificities for the substrates and inhibitors for these enzymes are due to the dissimilarity in amino acid residues of the active sites of AChE and BChE. In fact the system of enzyme is responsible for the termination of acetylcholine at cholinergic synapses. These are key components of cholinergic brain synapses and neuromu scular junctions. The main function of both cholinesterase enzymes is to catalyze the hydrolysis of the neurotran smitter acetylcholine and termination of the nerve impulse (Cygler *et al.,* 1993 and Tougu 2001). It has been studied that BChE is present in elevated extent 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. Therefore, the exploration for new cholinesterase inhibitors is considered an essential and

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constant strategy to launch new drug candidates for the treatment of Alzheimer's disease and other connected diseases (Gauthier 2001 and Bertaccini 1982).

The present research work is a successful effort to synthesize such novel compounds exhibiting diverse and improved pharmacological potential. We have synthesized the sulfonamides of piperidine nucleus to fuse two biological energetic classes with an objective to search new contenders of drugs having significantly enhanced activity and could be helpful in controlling many degenerative diseases.

## MATERIALS AND METHODS

### *General*

Melting points of all synthesized compounds were recorded on a Griffin-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 solvent systems using EtOAc and *n*-hexane giving single spot. Detection was carried out at 254 nm, and by ceric sulphate reagent. 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 a Bruker spectrometers operating at 300 MHz. Chemical shifts are given in ppm. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system. Piperidine, 3,5-dichloro-2-hydroxybenzenesulfonyl chloride, bromoacetyl bromide, substituted/unsubstituted aromatic amines of Merck, Alfha Aesar were purchased from local suppliers. All the other used solvents were of analytical grade.

### *Procedure for the synthesis of 1-[(3,5-dichloro-2-hydroxyphenyl sulfonyl]piperidine (3) in aqueous medium*

Piperidine (2) (20.0 mmol; 2.00 mL) was suspended in 100 mL water and the pH was maintained at 9.0 to 10.0 by adding basic aqueous solution of a $Na_2CO_3$ at 0-5°C. Then, 3,5-dichloro-2-hydroxy benzenesulfonyl chloride (1) (20.0 mmol; 5.16 mL) was added in the reaction mass slowly over 10-15-min. After complete addition of compound 1, the temperature of the reaction mixture was permitted to rise to room temperature. The reaction mixture was stirred and monitored with TLC for the completion of reaction. Then conc. HCl (around 4 mL) was added slowly to adjust the pH to 2.0. The reaction mixture was reserved at RT for 15 minutes; white solid was filtered and washed with distilled water to afford the title compound 3 on drying.

### *General procedure for the synthesis of compounds 5a-j*

To a solution of compound 3 (0.20g, 0.68 mmol) in *N,N*-dimethyl formamide (5 mL) sodium hydride (0.01 g, 0.42 mmol) was added at room temperature and stirred for 15 min. The corresponding electrophiles (0.68 mmol) was added into the reaction mixture and stirred for 30-40 min. The reaction mass was then monitored by TLC. After complete renovation, the reaction mixture was quenched with ice water (200 mL). The obtained solid was filtered, washed with distilled water and dried to yield the corresponding *O*-substituted derivatives of 1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidine 5a–j.

## ENZYME INHIBITION ASSAYS

### *Acetylcholinesterase Assay*

The AChE inhibition activity was performed according to the method (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 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

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

$IC_{50}$ values were designed 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, $Na_2HPO_4$ 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 $well^{-1}$. Absorbance was measured at 405 nm after 15 min of incubation at 37ºC, 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.

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

$IC_{50}$ values were designed 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 slight 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:

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

Where Control = Total enzyme activity without inhibitor
Test = Activity in the presence of test compound
$IC_{50}$ values 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.

***Spectral characterization of the synthesized compounds***

***1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidine (3)***

White powder, Yield 93.67%, m.p.196-198 °C. Molecular formula: $C_{11}H_{13}Cl_2O_3S$; Mol. Wt. 296g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3018 (C-H stretching of aromatic ring), 2945 (-$CH_2$- stretching), 1529 (C=C aromatic stretching), 3350 (O-H-stretching), 1323 (-$SO_2$- stretching), $^1$H-NMR (300 MHz, MeOD δ / ppm): 7.36 (1H, d, *J* = 1.5 Hz, H-6'), 7.35 (1H, d, *J* = 1.5 Hz, H-4'), 2.96 (2H, t, *J* = 5.4 Hz, $H_{eq}$-2 & $H_{eq}$-6), 2.47 (2H, t, *J* = 5.4 Hz, $H_{ax}$-2 & $H_{ax}$-6), 1.61 (2H, m, $CH_2$-4), 1.44 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 296 (13%) $[M]^+$, 232 (29%), 225 (47%), 212 (100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-benzylacetamide (5a)***

Off-white shiny crystals, Yield 80.00%, m.p.73-75°C. Molecular formula: $C_{20}H_{22}Cl_2N_2O_4S$; Mol. Wt. 457g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3431 (N-H stretching), 3018 (C-H stretching of aromatic ring), 2945 (-$CH_2$- stretching), 1529 (C=C aromatic stretching), 1323 (-$SO_2$- stretching), 1268 (Ar-O stretching), 1058 (C-O stretching), $^1$H-NMR (300 MHz, MeOD *δ* / ppm): 7.76 (1H, d, *J* = 1.8 Hz, H-6'), 7.74 (1H, d, *J* = 1.8 Hz, H-4'), 7.31-7.24 (5H, m, Ar-2'''to 6'''), 4. 39 (2H, s, $CH_2$-7'''), 4.36 (2H, s, O-$CH_2$), 3.19 (2H, t, *J* = 5.4 Hz, $H_{eq}$-2 & $H_{eq}$-6), 2.67 (2H, t, *J* = 5.4 Hz, $H_{ax}$-2 & $H_{ax}$-6), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 457 (12%) $[M]^+$, 393 (21%), 375 (35%), 366 (48%), 325 (100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2,3-dimethyl)phenylacetamide (5b)***

Light yellow crystal, Yield 80.50%, m.p.110-112°C. Molecular formula: $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3440 (N-H stretching), 3032 (C-H stretching of aromatic ring), 2941 (-$CH_2$- stretching), 1523 (C=C aromatic ring stretching), 1318 (-$SO_2$- stretching), 1266 (Ar-O stretching), 1059 (C-O stretching), $^1$H-NMR (300 MHz, MeOD *δ* / ppm): 7.87 (1H, d, *J* = 1.8 Hz, H-6'), 7.79 (1H, d, *J* = 1.8 Hz, H-4'), 7.52-7.46 (3H, m, Ar-4''' to 6'''), 4.36 (2H, s, O-$CH_2$), 3.25 (2H, t, *J* = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, *J* = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.83 (6H, s, $CH_3$-2'''&, $CH_3$-3'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (18%) $[M]^+$, 323 (28%), 387 (37%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2,4-dimethyl)phenylacetamide (5c)***

Mustard crystals, Yield 82.51%, m.p.112-114 °C. Molecular formula: $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3438 (N-H stretching), 3029 (C-H stretching of aromatic ring), 2939 (-$CH_2$- stretching), 1521 (C=C aromatic ring stretching), 1315 (-$SO_2$- stretching), 1269 (Ar-O stretching), 1057 (C-O stretching), $^1$H-NMR (300 MHz, MeOD *δ* / ppm): 8.544 (1H, *s*,H-3''' ), 7.87 (1H, d, *J* = 1.8 Hz, H-6'), 7.78 (1H, d, *J* = 1.8 Hz, H-4'), 7.28 (1H, d, *J* = 6 Hz, H-6'''),7.08 (1H, dd, *J* = 6, 1.5 Hz, H-5'''), 4.77 (2H, s, O-$CH_2$), 3.25 (2H, t, *J* = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, *J* = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.83 (6H, s, $CH_3$-2'''&, $CH_3$-4'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (9%) $[M]^+$, 323 (33%), 387 (45%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2,5-dimethyl)phenylacetamide (5d)***

Brown powder, Yield 83.56%, m.p.108-110 °C. Molecular formula $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3448 (N-H stretching), 3029 (C-H aromatic ring stretching), 2935 (-$CH_2$- stretching), 1519 (C=C stretching of aromatic ring), 1313 (-$SO_2$- stretching), 1267 (Ar-O stretching), 1055 (C-O stretching), $^1$H-NMR (300 MHz, MeOD *δ* / ppm): 8.544 (1H, s,H-6''' ), 7.87 (1H, d, *J* = 1.8 Hz, H-6'), 7.78 (1H, d, *J* = 1.8 Hz, H-4'), 7.28 (1H, d, *J* = 6 Hz, H-3'''),7.08 (1H, dd, *J* = 6, 1.5 Hz, H-4'''), 4.77 (2H, s, O-$CH_2$), 3.25 (2H, t, *J* = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, *J* = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.83 (6H, s, $CH_3$-2'''&, $CH_3$-5'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (14%) $[M]^+$, 323 (25%), 387 (39%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2,6-dimethyl)phenylacetamide (5e)***

Light green sticky solid, Yield 83.71%, Molecular formula $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. IR (KBr, cm$^{-1}$): $\nu_{max}$: 3441 (N-H stretching), 3033 (C-H aromatic ring stretching), 2942 (-$CH_2$- stretching), 1521 (C=C aromatic stretching), 1319 (-$SO_2$ stretching), 1261 (Ar-O

stretching), 1047 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 7.88 (1H, d, $J$ = 1.8 Hz, H-6'), 7.11-7.08 (3H, m, H-3''' to 5'''), 4.81 (2H, s, O-$CH_2$), 3.27 (2H, t, $J$ = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, $J$ = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.56 (6H, s, $CH_3$-2'''&, $CH_3$-6'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (20%) $[M]^+$, 323 (35%), 387 (40%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(3,4-dimethyl)phenylacetamide (5f)***

Mustard sticky solid, Yield 83.31%. Molecular formula $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. 3437 (N-H stretching), 3028 (C-H aromatic ring stretching), 2937 (-$CH_2$- stretching), 1520 (C=C stretching of aromatic ring), 1313 (-$SO_2$ stretching), 1260 (Ar-O stretching), 1050 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 7.56 (1H, d, $J$ = 2.1 Hz, H-6'), 7.52 (1H, d, $J$ = 2.1 Hz, H-4'), 7.32 (1H, d, $J$ = 1.5, H-2'''), 7.26 (1H, dd, $J$ = 6, 1.5 Hz, H-6'''),7.06 (1H, d, $J$ = 6 Hz, H-5''' ), 4.07 (2H, s, O-$CH_2$), 3.27 (2H, t, $J$ = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, $J$ = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.23 (3H, s, $CH_3$-3'''), 2.56 (3H, s, $CH_3$-4''') 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (18%) $[M]^+$, 323 (29%), 387 (39%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(3,5-dimethyl)phenylacetamide (5g)***

Buff powder, Yield 81.14%, m.p.166-168$^o$C. Molecular formula $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt. 471g. IR (KBr, $cm^{-1}$): $\nu_{max}$: 3441 (N-H stretching), 3021 (C-H aromatic ring stretching), 2931 (-$CH_2$- stretching), 1511 (C=C stretching of aromatic ring), 1311 (-$SO_2$ stretching), 1253 (Ar-O stretching), 1042 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 7.86 (1H, d, $J$ = 2.1 Hz, H-6'), 7.79 (1H, d, $J$ = 2.1 Hz, H-4'), 7.14 (1H, s, H-4''' ), 7.02 (3H, s, H2'''-6'''), 4.47 (2H, s, O-$CH_2$), 3.27 (2H, t, $J$ = 3.9 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.19 (2H, t, $J$ = 3.9 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.34 (6H, s, $CH_3$-3''' & $CH_3$-5'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (17%) $[M]^+$, 323 (27%), 387 (45%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2-phenyl)ethylacetamide (5h)***

Mustard sticky solid, Yield 83.24%, Molecular formula $C_{21}H_{24}Cl_2N_2O_4S$; Mol. Wt.471g. IR (KBr, $cm^{-1}$): $\nu_{max}$: 3437 (N-H stretching), 3023 (C-H stretching of aromatic ring), 2932 (-$CH_2$- stretching), 1521 (C=C aromatic ring stretching), 1327 (-$SO_2$- stretching), 1254 (Ar-O stretching), 1043 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 7.82 (1H, d, $J$ =2.1 Hz, H-6'), 7.75 (1H, d, $J$ =2.1 Hz, H-4'), 7.14 (1H, s, H-4''' ), 7.27-7.20 (5H, m, Ar-2''' to 6'''), 3.58 (2H, s, O-$CH_2$), 3.49 (2H, t, $J$ =5.4, $CH_2$-7'''), 3.37 (2H, t, $J$ = 4.5 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.14 (2H, t, $J$ = 4.5 Hz, $H_{ax}$-2 & $H_{ax}$-6), 2.83 (2H, t, $CH_2$-8'''), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 471 (19%) $[M]^+$,394 (30%), 323 (27%), 293(100%).

***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(2-hydroxy)phenylacetamide (5i)***

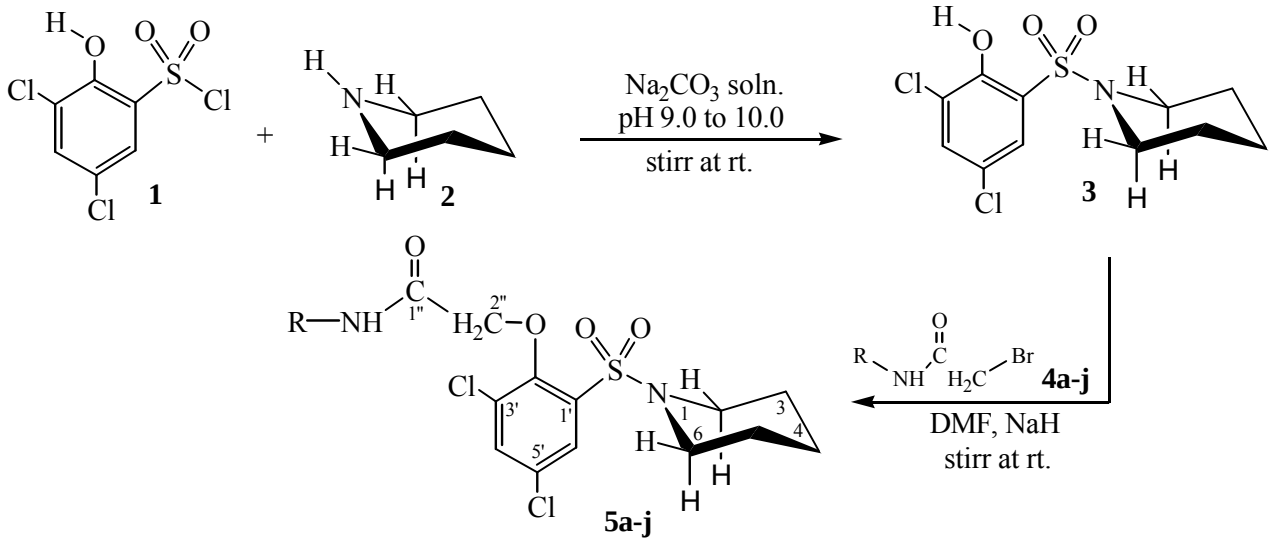
Brown sticky solid, Yield 84.43%, Molecular formula $C_{19}H_{20}Cl_2N_2O_5S$; Mol. Wt. 459 g. IR (KBr, $cm^{-1}$): $\nu_{max}$: 3437 (N-H stretching), 3023 (C-H aromatic ring stretching), 2932 (-$CH_2$- stretching), 1521 (C=C stretching of aromatic ring), 1327 (-$SO_2$- stretching), 1250 (Ar-O stretching), 1040 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 8.12 (1H, dd, $J$ = 6, 1.2 Hz, H-6'''), 7.87 (1H, d, $J$ = 1.8 Hz, H-6'), 7.76 (1H, d, $J$ = 1.8 Hz, H-4'), 6.92 (2H, m, H-4''', 5''' ), 6.81 (1H, dd, $J$ = 7.5, 0.9 Hz, H-3'''), 4.75 (2H, s, O-$CH_2$), 3.22 (2H, t, $J$ = 4.5 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.14 (2H, t, $J$ = 4.5 Hz, $H_{ax}$-2 & $H_{ax}$-6), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 459 (8%) $[M]^+$, 311 (15%), 375 (43%), 144 (100).

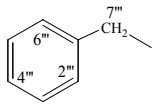
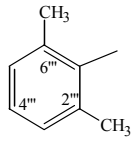
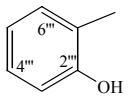
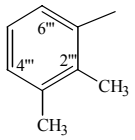
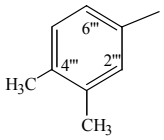
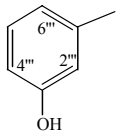
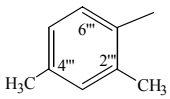
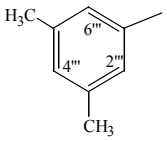
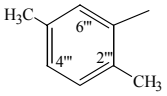
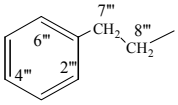
***2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-N-(3-hydroxy)phenylacetamide (5j)***

Buff powder, Yield 86.34%, m.p.90-92$^o$C, IR (KBr, $cm^{-1}$): $\nu_{max}$: 3437 (N-H stretching), 3023 (C-H aromatic ring stretching), 2932 (-$CH_2$- stretching), 1521 (C=C stretching of aromatic ring), 1327 (-$SO_2$- stretching), 1255 (Ar-O stretching), 1045 (C-O stretching), $^1$H-NMR (300 MHz, MeOD $\delta$ / ppm): 7.85 (1H, d, $J$ = 1.8 Hz, H-6'), , 7.79 (1H, d, $J$ = 1.8 Hz, H-4'), 7.20 (1H, t, $J$ = 1.8 Hz, H-2'''), 7.14 (1H, t, $J$ = 6 Hz, H-5'''), 6.99 (1H, dd, $J$ = 6.6, 0.6 Hz, H-6'''), 6.58 (1H, dd, $J$ = 6.3, 1.8 Hz, H-4'''), 4.75 (2H, s, O-$CH_2$ -2''), 3.22 (2H, t, $J$ = 4.5 Hz, $H_{eq}$-2 & $H_{eq}$-6), 3.14 (2H, t, $J$ = 4.5 Hz, $H_{ax}$-2 & $H_{ax}$-6), 1.61 (2H, m, $CH_2$-4), 1.45 (4H, m, $CH_2$-3 & $CH_2$-5). EIMS: *m/z* 459 (12%) $[M]^+$,311 (28%), 375 (37%), 144 (100).

## RESULTS

In the undertaken research, heterocyclic *O*-substituted derivative of sulfonamides bearing piperidine nucleus were synthesized. The parent compound 1-[(3,5-dichloro-2-hydroxyphenyl) sulfonyl]piperidine (3), was prepared by the coupling of 5-dichloro-2-hydroxybenzenesulfonyl chloride (1) with piperidine (2) under dynamic pH control in aqueous media (Deng *et al*., 2006 and Jafarpour *et al*., 2011). Further, the reaction of 3 with different electrophiles yielded a series of *O*-substituted derivatives of 1-[(3,5-dichloro-2-hydroxyphenyl) sulfonyl]piperidine (5a-j) as represented in Scheme 1. Synthesis of all derivatives 5a-j was performed in DMF (*N*,*N*-dimethylformamide) and sodium hydride (NaH) as the base. Complete conversion was achieved within 30 to 70 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 *O*-substituted derivatives were confirmed by spectral data as described in experimental section.




| Compound | R | Compound | R | Compound | R |
|---|---|---|---|---|---|
| **5a** |  | **5e** |  | **5i** |  |
| **5b** |  | **5f** |  | **5j** |  |
| **5c** |  | **5g** |  | | |
| **5d** |  | **5h** |  | | |

**Scheme 1**: Synthetic scheme of 2-*O*- Substituted Derivatives of 1-[(3,5-dichloro-2-hydroxyphenyl) sulfonyl]piperidine

***Enzyme inhibition studies***

The results of *in vitro* enzyme inhibition activity of the synthesized compounds against acetylcholinesterase, butyrylcholinesterase and lipoxygenase enzymes are presented in table 1.

## DISCUSSION

Parent compound 3 was synthesized as white powder. The molecular formula $C_{11}H_{13}Cl_2O_3S$ was established by molecular ion peak at *m/z* 296 in EI-MS and by counting the number of protons in its $^1$H-NMR spectrum. The Infrared spectrum showed absorption bands at 3018 $cm^{-1}$, 1529 $cm^{-1}$, 3350 $cm^{-1}$ and 1323 $cm^{-1}$ which were assigned to C-H (aromatic stretching), C=C (stretching of aromatic ring), O-H (stretching of hydroxyl group) and -$SO_2$ (stretching of sulfonyl group) respectively. The EI-MS gave characteristic peaks at *m/z* 232 and 212 which were attributed to the loss of $SO_2$ (sulfonyl) and piperidinyl groups respectively. In the aromatic region of the $^1$H-NMR spectrum, signals appeared at $\delta$ 7.36 (d, $J$ = 1.5 Hz, 1H, H-6') and 7.35 (d, $J$ = 1.5 Hz, 1H, H-4') which were assigned to the tetrasubstituted benzenesulfonyl ring. In the aliphatic region of the $^1$H-NMR spectrum, signals appeared at $\delta$ 2.96 (t, $J$ = 5.4 Hz, 2H, $H_{axial}$-2 & $H_{axial}$-6), 2.47 (t, $J$ = 5.4 Hz, 2H, $H_{eq}$-2 & $H_{eq}$-6), 1.61 (*m,* 2H, $CH_2$-4) and 1.44 (m, 4H, $CH_2$-3 & $CH_2$-5) which indicated the presence of piperidine nucleus in the molecule. On the basis of mentioned cumulative evidences, the structure of 3 was assigned 1-[(3,5-dichloro-2-hydroxyphenyl)

**Table 1**: Bioactivity studies of 2-*O*- Substituted Derivatives of 1-[(3,5-dichloro-2-hydroxyphenyl) sulfonyl] piperidine

| Sample Code | AChE | | BChE | | LOX | |
|---|---|---|---|---|---|---|
| | Inhibition (%) Conc./well (0.5 mM) | $IC_{50}$ (µmol.) | Inhibition (%) Conc./well (0.5 mM) | Inhibition (%) Conc./well (0.5 mM) | $IC_{50}$ (µmol.) | Inhibition (%) Conc./well (0.5 mM) |
| 3 | 79.53±0.25 | 152.41±0.06 | 82.78±0.34 | 81.91±0.22 | 30.99±0.49 | - |
| 5a | 79.32±0.31 | 134.71±0.07 | 82.39±0.11 | 148.71±0.24 | 20.54±0.21 | - |
| 5b | 63.33±0.45 | 199.51±0.06 | 66.72±0.18 | 202.41±0.34 | 45.389±0.63 | - |
| 5c | 61.41±0.25 | 244.61±0.55 | 78.69±0.61 | 128.51±0.11 | 46.13±0.29 | - |
| 5d | 71.43±0.29 | 143.91±0.22 | 76.83±0.51 | 145.81±0.38 | 89.32±0.56 | 137.21±0.25 |
| 5e | 62.90±0.66 | 223.71±0.78 | 95.21±0.28 | 36.41±0.02 | 54.34±0.18 | <400 |
| 5f | 34.97±0.11 | - | 84.86±0.85 | 104.71±0.39 | 86.85±0.21 | 143.41±0.28 |
| 5g | 65.88±0.35 | 150.61±0.29 | 67.41±0.27 | 196.61±0.54 | 30.99±0.11 | - |
| 5h | 87.42±0.64 | 63.61±0.55 | 95.21±0.71 | 49.31±0.61 | 33.22±0.45 | - |
| 5i | 84.01±0.82 | 78.81±0.39 | 90.42±0.18 | 48.11±0.24 | 80.99±0.42 | - |
| 5j | 50.32±0.33 | <500 | 94.90±0.71 | 46.81±0.82 | 37.56±0.65 | - |
| Control | Eserine 91.29±1.17 | 0.04±0.001 | Eserine 82.82±1.09 | 0.85±0.001 | 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).
LOX = Lipoxygenase, AChE = Acetylcholinesterase, BChE = Butyrylcholinesterase.

sulfonyl] piperidine. Similarly, the structures of other compounds were characterized by $^1$H-NMR, IR and mass spectral data as described in experimental section.

*Enzyme inhibition activity*

The screening of these synthesized compounds against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX) enzymes revealed that these molecules exhibited good inhibitory potential against butyrylcholinesterase as it was evident from their $IC_{50}$ values. The results are depicted in Table-1.It is clearly evident from results in table 1 that the compounds 2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-*N*-(2,6-dimethyl)phenylacetamide (**5e**), 2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl)phenoxy]-*N*-(3-hydroxy) phenylacetamide (**5j**) and 2-[2,4-dichloro-6-(piperidin-1-ylsulfonyl) phenoxy]-*N*-(2-hydroxy)phenylacetamide (5i) were found to be promising inhibitors against butyrylcholinesterase enzyme having $IC_{50}$ values of 36.41±0.02, 46.81±0.82 and 48.11±0.24 µmoles/L respectively, relative to Eserine, a reference standard with $IC_{50}$ value of 0.85±0.001 µmoles, probably due to the *O*-substitution of electrophiles containing *N*-(2,3-dimethyl) phenylacetamide, *N*-(3-hydroxy)phenylacetamide and *N*-(2-hydroxy) phenyl cetamides groups respectively in these molecules. The screening against acetylcho linesterase enzyme exposed that the two compounds 5h and **5i** exhibited good inhibitory potential having $IC_{50}$ values of 63.61±0.55 and 78.81±0.39 µmoles/L as compared to standard but all others showed moderate activity. However, only two compounds (table 1) showed weak inhibition but all other remained inactive against lipoxygenase enzyme.

## CONCLUSION

The projected structures of the synthesized compounds are well supported by spectroscopic data. From the enzyme inhibition data (table 1), it might be concluded that the compounds have moderate to talented activity against acetylcholinesterase, butyrylcholinesterase enzymes as it was evident from their $IC_{50}$ values, relative to the standard used. Only two compounds showed inhibition activity against lipoxygenase enzyme but all others stayed inactive. Hence, on the basis of aforesaid results, these synthesized derivatives provide an overall indispensable basis to introduce newfangled drugs for the cure of Alzheimer's disease and other associated diseases. These entrants can also be helpful for the healing a variety of disorders such as autoimmune diseases, bronchial asthma, inflammation, and cancer.

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