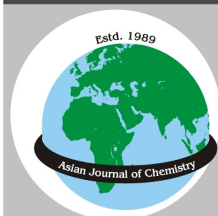

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Synthesis, Structural Analysis and Pharmacological Screening of Chlorinated Sulfonamides

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In present work, a facile and environmentally benign series of chlorinated sulfonamides was synthesized and screened against different enzymes. These were geared up by the coupling of 4-chlorobenzenesulfonyl chloride **(1)** with different substituted aromatic amines (**2a-l**) under dynamic pH control in aqueous media to form various chlorinated sulfonamides (**3a-l**). The synthesized chlorinated sulfonamides were spectrally characterized like ^{1}H NMR, IR and EI-MS. The bioactivity of all the synthesized compounds were evaluated against urease, butyrylcholinesterase (BChE) and lipoxygenase (LOX) enzymes and found to be having talented activity against butyrylcholinesterase enzyme.

Key Words: 4-Chlorobenzenesulfonyl chloride, Substituted aromatic amine, Enzyme inhibition activity.

INTRODUCTION

The sulfonamide (-SO_2-NH-) moiety occurs in numerous biologically active compounds, which comprise antimicrobial drugs, carbonic anhydrase inhibitors, antitumour drugs, insulin-releasing sulfonamides and number of other biological activities. Sulfonamides are the most extensively used antibacterial agents in the world, mainly because of their low cost, low toxicity and excellent activity against common bacterial diseases. The synergetic action of sulfonamides with trimethoprim has brought about enormous resurgence of sulfonamide usage everywhere over the last decade. Sulfonamides have a great importance in medicinal chemistry and constitute the largest class of antimicrobial agents. Sulfonamides including the protease inhibitor and antiretroviral fosamprenavir, the non-steroidal antiinflammatory drug celecoxib and sumatriptan, which has been used to treat migraine headaches, have found widespread use as pharmaceuticals[1-4].

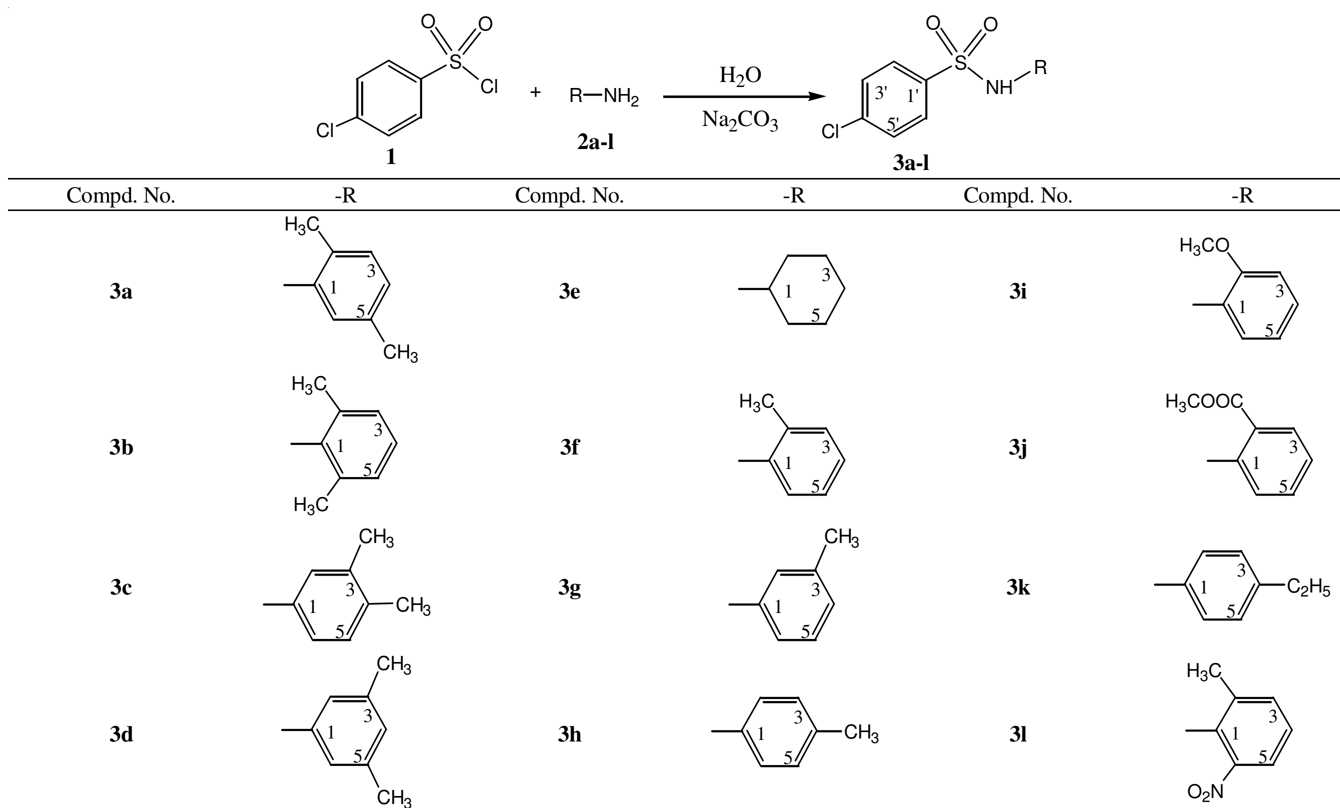
Butyryl cholinesterase (BChE, EC 3.1.1.8) consist of a family of enzyme which include serine hydrolases. It has been found that butyrylcholinesterase inhibition is an effective tool for the treatment of alzheimer disease and related dementias. Butyryl cholinesterase is found in significantly higher quantities in Alzheimer's plaques than in plaques of normal age-related non-demented brains. Butyryl cholinesterase is produced in the liver and enriches blood circulation. In addition, it is also present in adipose tissue, intestine, smooth muscle cells, white matter of the brain and many other tissues[5-7].

Lipoxygenases (EC 1.13.11.12) constitute a family of non-haem iron containing dioxygenases that are widely distributed in animals and plants. These are involved in arachidonic acid metabolism, generating various biologically active lipids that play important roles in inflammation. Thrombosis and tumor angiogenesis, the formation of new capillary vessels from pre-existing ones, underpins a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer. Lipoxygenases are, therefore, potential targets for rational drug design and discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer and autoimmune diseases[8-10].

The present research work is a successful effort to synthesize such type compounds exhibiting diverse and improved pharmacological potential. In view of the published information and in the continuation of our previous work[11-14], we now report the synthesis of chlorinated sulfonamides and screening out their biological activities.

EXPERIMENTAL

Melting points of all synthesized compounds were recorded on a Griffin-George melting point apparatus by open



Scheme-I: Outline for the synthesis of sulfonamides from 4-chlorobenzenesulfonyl chloride

capillary tube and were uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) with solvent systems using EtOAc and *n*-hexane on aluminum sheets precoated with silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; E-Merck). Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying with ceric sulfate solution (with heating). The IR spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^{1}H NMR spectra were recorded in CD_3OD on a Bruker spectrometers operating at 500 MHz. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer. 4-Chloro-benzenesulfonyl chloride and substituted aromatic amines were purchased from Merck and Alfa Aeser through local suppliers. All the other used solvents were of analytical grade.

General procedure for the synthesis of different sulfonamides (3a-l): Amines (1.0 mmol: **2a-l**) were suspended in 100 mL water and the pH 9-10 was maintained by adding basic aqueous solution of Na_2CO_3 (10 %). Then, 4-chloro benzenesulfonyl chloride (1.0 mmol; **1**) was added in the reaction mass slowly over 10-15-min. After complete addition of compound **1**, the reaction mixture was stirred and monitored with TLC (*n*-hexane: EtOAc; 70:30) 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 room temperature for 15 min; white solid was filtered and washed with distilled water to afford the corresponding compound (**3a-l**) on drying (**Scheme-I**).

Characterization of the synthesized compounds

***N*-(2,5-Dimethylphenyl)-4-chlorobenzene sulfonamide (3a):** Light pink amorphous solid; yield: 92 %; m.p. 100-102 ºC; m.f.: $C_{14}H_{14}NO_2SCl$; m.w. 295 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3379 (N-H), 3058 (Ar-H), 1531 (Ar C=C), 1409 ($-SO_2-$), 1138 (C-N); ^{1}H NMR (500 MHz, CD_3OD, ppm): δ 7.62 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.49 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.99 (d, *J* = 7.5 Hz, 1H, H-3), 6.92 (d, *J* = 8.0 Hz, 1H, H-4), 6.85 (s, 1H, H-6), 4.83 (s, H-N), 2.19 (s, 3H, CH_3-2), 1.93 (s, 3H, CH_3-5); EIMS: *m/z* 297 [M + 2]$^+$, 295 [M]$^+$, 231 [M-SO_2]$^+$, 120 [$C_8H_{10}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(2,6-Dimethylphenyl)-4-chlorobenzene sulfonamide (3b):** Pinkish amorphous solid; yield: 90 %; m.p. 101-103 ºC; m.f.: $C_{14}H_{14}NO_2SCl$; m.w.: 295 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3378 (N-H), 3057 (Ar-H), 1530 (Ar C=C), 1408 ($-SO_2-$), 1137 (C-N); ^{1}H NMR (500 MHz, CD_3OD, ppm): δ 7.67 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.52 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.98-7.07 (m, 3H, H-3 to H-5), 4.87 (s, H-N), 2.00 (s, 6H, CH_3-2, CH_3-6); EIMS: *m/z* 297 [M + 2]$^+$, 295 [M]$^+$, 231 [M-SO_2]$^+$, 120 [$C_8H_{10}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(3,4-Dimethylphenyl)-4-chlorobenzene sulfonamide (3c):** Off-white amorphous solid; yield: 89 %; m.p. 104-106 ºC; m.f.: $C_{14}H_{14}NO_2SCl$; m.w.: 295 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 3059 (Ar-H), 1532 (Ar C=C), 1412 ($-SO_2-$), 1141 (C-N); ^{1}H NMR (500 MHz, CD_3OD, ppm): δ 7.66 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.45 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.95 (d, *J* = 13.0 Hz, 1H, H-6), 6.82 (br s, 1H, H-2), 6.77 (dd, *J* = 10.0, 1.0 Hz, 1H, H-5), 4.87 (s, H-N), 2.14 (s, 6H, CH_3-3, CH_3-4); EIMS: *m/z* 297 [M + 2]$^+$, 295 [M]$^+$, 231 [M-SO_2]$^+$, 120 [$C_8H_{10}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(3,5-Dimethylphenyl)-4-chlorobenzene sulfonamide (3d):** White amorphous solid; yield: 89 %; m.p. 106-108 ºC; m.f.: $C_{14}H_{14}NO_2SCl$; m.w.: 295 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3383 (N-H), 3054 (Ar-H), 1531 (Ar C=C), 1413 (-SO$_2$-), 1138 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.68 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.71 (s, 2H, H-2, H-6), 6.69 (s, 1H, H-4), 4.86 (s, H-N), 2.17 (s, 6H, CH$_3$-3, CH$_3$-5); EIMS: *m/z* 297 [M + 2]$^+$, 295 [M]$^+$, 231 [M-SO$_2$]$^+$, 120 [$C_8H_{10}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-Cyclohexyl-4-chlorobenzene sulfonamide (3e):** White amorphous solid; yield: 89 %; m.p. 86-88 ºC; m.f.: $C_{12}H_{15}NO_2SCl$; m.w.: 272 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3376 (N-H), 3056 (Ar-H), 1526 (Ar C=C), 1416 (-SO$_2$-), 1146 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.82 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.55 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 1.51-1.67 (m, 4H, H-2, H-6), 1.14-1.27 (m, 6H, H-3 to H-5), 4.86 (s, H-N); EIMS: *m/z* 274 [M + 2]$^+$, 272 [M]$^+$, 208 [M-SO$_2$]$^+$, 175 [M-$C_6H_{11}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(2-Methylphenyl)-4-chlorobenzene sulfonamide (3f):** Pinkish white amorphous solid; Yield: 86 %; m.p. 96-98 ºC; m.f.: $C_{13}H_{12}NO_2SCl$; m.w.: 281 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3384 (N-H), 3057 (Ar-H), 1534 (Ar C=C), 1414 (-SO$_2$-), 1144 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.63 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.48 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.00-7.11 (m, 4H, H-3 to H-6), 4.86 (s, H-N), 2.01 (s, 3H, CH$_3$-2); EIMS: *m/z* 283 [M + 2]$^+$, 281 [M]$^+$, 217 [M-SO$_2$]$^+$, 175 [M-C_7H_8N]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(3-Methylphenyl)-4-chlorobenzene sulfonamide (3g):** Purple amorphous solid; yield: 85 %; m.p. 98-100 ºC; m.f.: $C_{13}H_{12}NO_2SCl$; m.w.: 281 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3385 (N-H), 3053 (Ar-H), 1533 (Ar C=C), 1413 (-SO$_2$-), 1143 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.68 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.09 (d, *J* = 7.5 Hz, 1H, H-6), 7.05 (s, 1H, H-2), 6.85-6.89 (m, 2H, H-4, H-5), 4.86 (s, H-N), 2.22 (s, 3H, CH$_3$-3); EIMS: *m/z* 283 [M + 2]$^+$, 281 [M]$^+$, 217 [M-SO$_2$]$^+$, 175 [M-C_7H_8N]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(4-Methylphenyl)-4-chlorobenzene sulfonamide (3h):** White amorphous solid; yield: 89 %; m.p. 98-100 ºC; m.f.: $C_{13}H_{12}NO_2SCl$; m.w.: 281 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3386 (N-H), 3054 (Ar-H), 1532 (Ar C=C), 1412 (-SO$_2$-), 1142 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.65 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.02 (d, *J* = 13.5 Hz, 2H, H-2, H-6), 6.93 (d, *J* = 14.0 Hz, 2H, H-3, H-5), 4.86 (s, H-N), 2.23 (s, 3H, CH$_3$-4); EIMS: *m/z* 283 [M + 2]$^+$, 281 [M]$^+$, 217 [M-SO$_2$]$^+$, 175 [M-C_7H_8N]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(2-Methoxyphenyl)-4-chlorobenzene sulfonamide (3i):** Light pink amorphous solid; yield: 89 %; m.p. 82-84 ºC; m.f.: $C_{13}H_{12}NO_3SCl$; m.w.: 297 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3375 (N-H), 3055 (Ar-H), 1531 (Ar C=C), 1411 (-SO$_2$-), 1141 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.65 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.44 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.40 (dd, *J* = 13.5, 2.5 Hz, 1H, H-6), 7.11 (ddd, *J* = 13.5, 3.0 Hz, 1H, H-4), 6.88 (ddd, *J* = 10.5, 2.5 Hz, 1H, H-5), 6.81 (dd, *J* = 13.5, 1.5 Hz, 1H, H-3), 4.86 (s, H-N), 3.50 (s, 3H, CH$_3$O-2); EIMS: *m/z* 299 [M + 2]$^+$, 297 [M]$^+$, 233 [M-SO$_2$]$^+$, 175 [M-C_7H_8NO]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(2-(Methoxycarbonyl)phenyl)-4-chlorobenzene sulfonamide (3j):** White amorphous solid; Yield: 93 %; m.p. 78-80 ºC; m.f.: $C_{14}H_{12}NO_4SCl$; m.w.: 325 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3383 (N-H), 3053 (Ar-H), 1527 (Ar C=C), 1407 (-SO$_2$-), 1137 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.91 (dd, *J* = 11.0, 2.5 Hz, 1H, H-3), 7.74 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.66 (d, *J* = 13.5 Hz, 1H, H-6), 7.54 (ddd, *J* = 12.0, 2.5 Hz, 1H, H-4), 7.48 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.15 (ddd, *J* = 11.5, 1.5 Hz, 1H, H-5), 4.86 (s, H-N), 3.85 (s, 3H, CH$_3$-2''); EIMS: *m/z* 327 [M + 2]$^+$, 325 [M]$^+$, 261 [M-SO$_2$]$^+$, 175 [M-$C_8H_8NO_2$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(4-Ethylphenyl)-4-chlorobenzene sulfonamide (3k):** Light pink amorphous solid; yield: 88 %; m.p. 112-114 ºC; m.f.: $C_{14}H_{14}NO_2SCl$; m.w.: 295 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3390 (N-H), 3059 (Ar-H), 1532 (Ar C=C), 1412 (-SO$_2$-), 1142 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 7.66 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.05 (d, *J* = 14.0 Hz, 2H, H-2, H-6), 6.96 (d, *J* = 14.0 Hz, 2H, H-3, H-5), 4.86 (s, H-N), 2.54 (q, *J* = 11.5 Hz, 2H, H-1''), 1.15 (t, *J* = 11.5 Hz, 3H, CH$_3$-2''); EIMS: *m/z* 297 [M + 2]$^+$, 295 [M]$^+$, 231 [M-SO$_2$]$^+$, 120 [$C_8H_{10}N$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

***N*-(2-Methyl-6-nitrophenyl)-4-chlorobenzenesulfonamide (3l):** Orange amorphous solid; yield: 89 %; m.p. 58-60 ºC; m.f.: $C_{13}H_{11}N_2O_4SCl$; m..: 326 g mol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3371 (N-H), 3052 (Ar-H), 1533 (Ar C=C), 1413 (-SO$_2$-), 1143 (C-N); ^{1}H NMR (500 MHz, CD$_3$OD, ppm): δ 8.06 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.93 (d, *J* = 14.5 Hz, 1H, H-5), 7.73 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.29 (d, *J* = 15.0 Hz, 1H, H-3), 6.57 (t, *J* = 15.0 Hz, 1H, H-4), 4.88 (s, H-N), 2.23 (s, 3H, CH$_3$-2); EIMS: *m/z* 328 [M + 2]$^+$, 326 [M]$^+$, 262 [M-SO$_2$]$^+$, 175 [M-$C_7H_7N_2O_2$]$^+$, 111 [C_6H_4Cl]$^+$, 76 [C_6H_4]$^+$.

Enzyme inhibition essays

Urease inhibition assay: The enzyme assay is the modified form of the commonly known Berthelot assay[15]. A total volume of 85 µL assay mixture contained 10 µL of phosphate buffer of pH 7 in each well in the 96-well plate followed by the addition of 10 µL of sample solution and 25 µL of enzyme solution (0.135 units). Contents were pre-incubated at 37 ºC for 5 min. Then, 40 µL of urea stock solution (20 mM) was added to each well and incubation continued at 37 ºC for further 10 min. After given time, 115 µL phenol hypochlorite reagents were added in each well (freshly prepared by mixing 45 µL phenol reagent with 70 µL of alkali reagent). For colour development, incubation was done at 37 ºC for another 10 min. Absorbance was measured at 625 nm using the 96-well plate reader Synergy HT BioTek, USA). The percentage enzyme inhibition was calculated by the following formula: Inhibition (%) = (Abs of control – Abs of test comp/Abs of control) × 100. IC_{50} values were designed using EZ-Fit enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

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

Butyrylcholinesterse assay

The BChE inhibition activity was performed according to the reported method[16] 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}, followed by the addition of 10 µL (0. 5 unit well^{-1}) BChE. 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 (butyrylthiocholine bromide), 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. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as a positive control. The percentage inhibition and IC_{50} values were calculated as mentioned above.

Lipoxygenase assay: Lipoxygenase activity was assayed according to the reported method[17-19] but with slight modifications. A total volume of 200 µL assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15 µL purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 min at 25 ºC. The reaction was initiated by the addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalein (0.5 mM well^{-1}) was used as a positive control. The percentage inhibition and IC_{50} values were calculated as mentioned above.

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± sem.

RESULTS AND DISCUSSION

In present research, a series of chlorinated sulfonamides were synthesized and further, all were subjected to a battery of biological assay to record their potential. These were prepared by the coupling of 4-chlorobenzenesulfonyl chloride **(1)** with different substituted aromatic amines **(2a-l)** under dynamic pH control in aqueous media. Complete conversion was achieved within 30-70 min by stirring. The chlorinated sulfonamide products (**3a-l**) were isolated by adding cold water in the reaction mixture and filtering off the precipitated solid. The structures of all the compounds were confirmed by spectral data. The compound **3a** was synthesized as light pink amorphous powder and the yield was 92 %. The molecular formula $C_{14}H_{14}NO_2SCl$ was established by EI-MS showing molecular ion peak 295 and also by counting the number of protons in ^{1}H NMR spectrum. The IR spectrum uncovered the occurrence of a sulfonyl group (1409 cm^{-1}) and -NH- group (3379 cm^{-1}) in the molecule. The EI-MS presented a distinct peak at *m/z* 231 due to the removal of sulfonyl group. In the aromatic section of ^{1}H NMR spectrum, the signals appeared at δ 7.62 (d, *J* = 9.0 Hz, 2H, H-2', H-6') and 7.49 (d, *J* = 8.5 Hz, 2H, H-3', H-5') were allocated to the protons of *para* disubstituted ring

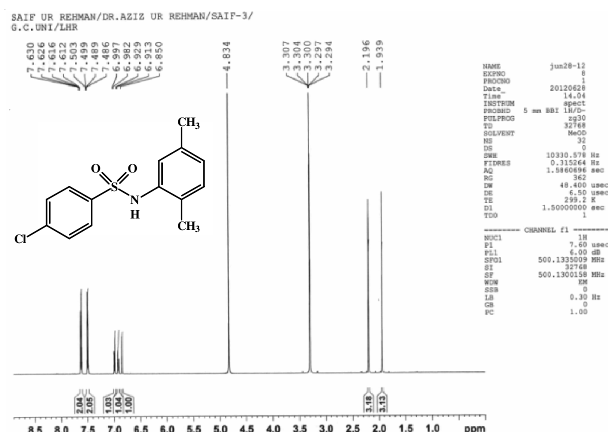


Fig. 1. ^{1}H NMR spectrum of *N*-(2,5-dimethylphenyl)-4-chlorobenzenesulfonamide (**3a**)

with chloro and sulfonyl groups. The signals appeared at δ 6.99 (d, *J* = 7.5 Hz, 1H, H-3), 6.92 (d, *J* = 8.0 Hz, 1H, H-4) and 6.85 (s, 1H, H-6) were allotted to the protons of other trisubstituted ring. In the aliphatic section of ^{1}H NMR spectrum, the signals became visible at δ 4.83 (s, H-N), 2.19 (s, 3H, CH_3-2) and 1.93 (s, 3H, CH_3-5) indicating the presence of one proton attached to nitrogen and two methyl groups in the molecule. On the basis of above mentioned cumulative evidences, the structure of compound **(3a)** was named as *N*-(2,5-dimethylphenyl)-4-chlorobenzenesulfonamide and its ^{1}H NMR spectrum was given in Fig. 1. The mass fragmentation pattern of *N*-(2,5-dimethylphenyl)-4-chlorobenzene sulfonamide **(3a)** was clearly described in Fig. 2. Similarly, the structures of other compounds **(3b-l)** were characterized by ^{1}H NMR, IR and mass spectral data as described in experimental section.

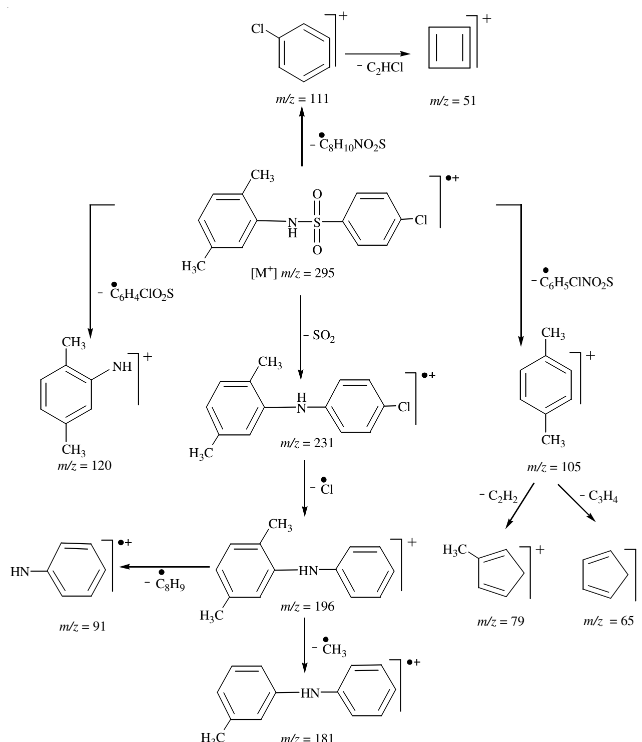


Fig. 2. Mass fragmentation pattern of *N*-(2,5-dimethylphenyl)-4-chlorobenzene sulfonamide

TABLE-1
PHARMACOLOGICAL SCREENING OF VARIOUS CHLORINATED SULFONAMIDES

Compound No.	Urease		BChE		LOX	
	Inhibition (%) at 0.5 mM	IC_{50} (μmol)	Inhibition (%) at 0.5 mM	IC_{50} (μmol)	Inhibition (%) at 0.5 mM	IC_{50} (μmol)
3a	45.67 ± 0.19	–	93.42 ± 0.98	48.91 ± 0.34	73.53 ± 0.47	183.21 ± 0.31
3b	40.06 ± 0.51	–	85.84 ± 0.68	79.61 ± 0.11	64.86 ± 0.51	78.52 ± 0.07
3c	70.87 ± 0.39	124.72 ± 0.04	98.51 ± 0.11	41.21 ± 0.05	54.87 ± 0.25	>400
3d	83.80 ± 0.48	78.06 ± 0.09	93.54 ± 0.22	49.61 ± 0.17	39.71 ± 0.55	–
3e	80.09 ± 0.28	112.06 ± 0.11	45.27 ± 0.57	–	70.05 ± 0.13	197.61 ± 0.19
3f	60.41 ± 0.49	258.09 ± 0.05	94.58 ± 0.64	51.22 ± 0.09	74.97 ± 0.17	132.41 ± 0.19
3g	55.05 ± 0.71	>300	91.24 ± 0.14	62.21 ± 0.43	68.71 ± 0.71	261.41 ± 0.14
3h	82.88 ± 0.49	118.08 ± 0.07	43.59 ± 0.81	–	87.24 ± 0.11	93.41 ± 0.12
3i	69.66 ± 1.39	159.3 ± 0.09	33.78 ± 0.78	–	64.02 ± 0.34	>400
3j	43.50 ± 1.19	–	69.85 ± 0.22	104.21 ± 0.14	61.63 ± 0.88	>400
3k	54.07 ± 1.29	>300	98.57 ± 0.55	43.59 ± 0.55	57.76 ± 0.43	>400
3l	46.14 ± 1.17	–	34.81 ± 0.61	–	65.82 ± 0.14	>400
Control	Thiourea	21.28 ± 0.11	Eserine	0.85 ± 0.0001	Baicalein	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). BChE = Butyrylcholinesterase, LOX = Lipoxygenase.

Enzyme inhibition activity: The screening of these synthesized compounds against urease, 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 that the compounds, *N*-(2,5-dimethylphenyl)-4-chlorobenzene sulfonamide **(3a)**, *N*-(3,4-dimethylphenyl)-4-chlorobenzene sulfonamide **(3c)**, *N*-(3,5-dimethylphenyl)-4-chlorobenzene sulfonamide **(3d)**, *N*-(2-methylphenyl)-4-chlorobenzene sulfonamide **(3f)** and *N*-(4-ethylphenyl)-4-chlorobenzene sulfonamide **(3k)** were the most effective inhibitors for butyrylcholinesterase having IC_{50} values of 48.91 ± 0.34, 41.21 ± 0.05, 49.61 ± 0.17, 51.22 ± 0.09 and 43.59 ± 0.55 μmol, respectively, relative to eserine, a reference standard with IC_{50} value of 0.85 ± 0.0001 μmol. These compounds can further be exploited and their derivatives could be synthesized to get closer to IC_{50} values of the standard eserine. In this way, the compounds could be potential target in the drug discovery and drug development program. The screening against urease enzyme revealed that *N*-(3,5-dimethylphenyl)-4-chlorobenzene sulfonamide **(3d)** was found to be the most potent inhibitors for urease having IC_{50} values of 78.06 ± 0.09 μmoles, respectively, relative to thiourea, a reference standard with IC_{50} value of 21.28 ± 0.11 μmoles. Similarly, the screening against lipoxygenase enzyme exposed that the compounds, *N*-(2,6-dimethylphenyl)-4-chlorobenzene sulfonamide **(3b)** and *N*-(4-methylphenyl-4-chlorobenzene sulfonamide **(3h)** were the most efficient inhibitors for lipoxygenase having IC_{50} values of 78.52 ± 0.07 and 93.41 ± 0.12 μmoles, respectively, relative to baicalein, a reference standard with IC_{50} value of 22.4 ± 1.3 μmol. These enhanced activities might be due to the alkyl substitution of aromatic amine which is probably more complimentary for the inhibition of enzymes.

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 chlorinated sulfonamides 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 compounds can further be exploited and their derivatives could be synthesized to get closer to IC_{50} values of the standard.

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