Synthesis, spectral characterization and enzyme inhibition Studies of different chlorinated sulfonamides

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Abstract: Sulfonamides are adherent to a biologically dynamic category of compounds and are under consideration of many organic synthetic researches to synthesize pharmacologically important compounds. In this demonstrated research work, a benignant series of chlorinated sulfonamides were synthesized and screened against different enzymes. These various chlorinated sulfonamides (**3a-i**) were set up by pairing of different substituted anilines (**2a-i**) with 4-chlorobenzenesulfonyl chloride (**1**) under basic pH in an aqueous media. The structures of the synthesized chlorinated sulfonamides were furnished by ^{1}H-NMR, IR & EI-MS. The different enzymes used for the evaluation of bioactivity of all the synthesized compounds were urease, butyrylcholinesterase (BChE) and lipoxygenase (LOX). All the compounds exhibited good inhibitory activities against these enzymes but the strong activity was shown against BChE and hence can be employed for discovery of 'lead' compounds against Alzheimer's disease (AD).

Keywords: Substituted anilines, 4-chlorobenzenesulfonyl chloride, enzyme inhibition, Urease, LOX, BChE.

INTRODUCTION

Sulfonamides bear -SO_2NH- group and are present in many pharmacologically dynamic compounds. Sulfonamides are believed to be a crucial moiety of medicines, extensively used as many agents (Aziz-ur-Rehman *et al.*, 2011). This moiety has been a part of pharmaceutically important compounds being widely used as carbonic anhydrase inhibitors; anticancer, anti-inflammatory, antiviral agents, anti-microbial drugs, insulin-releasing sulfonamides; saluretics, anti-hydrod agents, anti-tumor drugs and also for a number of other biological activities. These are antibacterial agents, extensively used due to less prices, less toxic effects and brilliant activity (Alsughayer *et al.*, 2011; Baskin and Wang, 2002; Kumar *et al.*, 2010; Ozbek *et al.*, 2007; Shi *et al.*, 2009). The *p*-aminobenzoic acid (PABA) is required by the bacteria in the body for the production of folic acid. Sulfonamides are analogous to PABA in structure and so inhibit its conversion into folic acid. Thus sulfonamides finally suppress the synthesis of purine and DNA (Aziz-ur-Rehman *et al.*, 2012a, b, c).

Butyrylcholinesterase (BChE, EC 3.1.1.8) comprises a family of enzymes such as serine hydrolases. BChE inhibition is an efficient source for the intervention of Alzheimer's disease (AD) and related disorders. Significantly higher quantities of BChE are found in Alzheimer's plaques rather than in plaques of normal age-related non-demented brains. BChE enriches blood circulation and is present in adipose tissue, intestine, smooth muscle cells, white matter of the brain and many other tissues (Ahmad *et al.*, 2005; Cygler *et al.*, 1993; Tougu, 2001; Ye *et al.*, 2002).

Urease (EC 3.5.1.5) hydrolyses urea into ammonia presents a critical role in many pathogenic processes in living organisms including humans & animals. It has a major role in the cause of kidney stone, peptic ulceration and pyelonephritis. Its role is also very prognosticating in urinary catheter urolithiasis, incrustation and hepatic encephalopathy (Lodhi *et al.*, 2006; Lodhi *et al.*, 2007; Mobley *et al.*, 1995, 1988; Samtoy *et al.*, 1980).

Lipoxygenase (LOX, EC 1.13.11.12) belongs to a class of non-haem iron containing dioxygenases, widely distributed in animals and plants. These are involved in arachidonic acid metabolism and generation of various biologically active lipids, which possess an important role in inflammation. These have also a role in thrombosis & tumor angiogenesis, organization of newfangled capillary vessels, corroborate a large number of physiological functions and take part in the maturation of many pathological circumstances such as cancer and arthritis. Therefore, LOXs are likely objectives for noetic medicine design and also for the uncovering of inhibitors based on mechanism, for the ailment of a number of disorders such as inflammation, bronchial asthma, autoimmune diseases and cancer (Abbasi *et al.*, 2005; Alitonou *et al.*, 2006; Byrum *et al.*, 1997; Clapp *et al.*, 1985; Jensen *et al.*, 1992; Kemal *et al.*, 1987; Steinhilber, 1999).

In continuation of our previous work of chlorinated sulfonamides (Aziz-ur-Rehman *et al.*, 2012b), the presented research work was a fruitful attempt to extend

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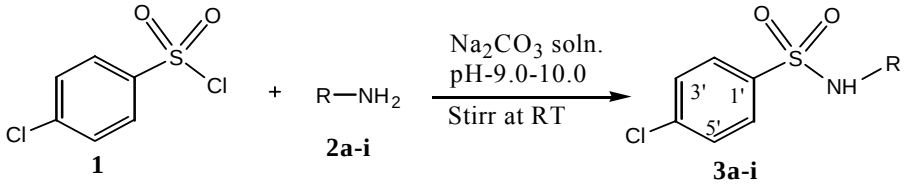

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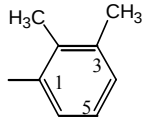
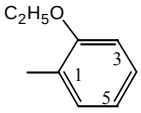
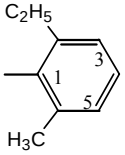
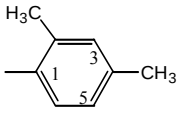
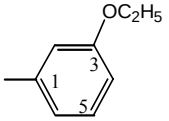
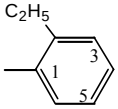
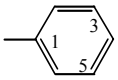
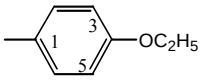
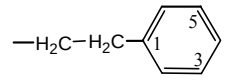
3a		3d		3g	
3b		3e		3h	
3c		3f		3i	

Scheme 1: Outline for the synthesis of various chlorinated sulfonamides

the biological activity results of the synthesized derivatives of chlorinated sulfonamides against certain enzymes and hence to introduce pharmacologically important compounds. The last work, on such molecules by our group, reported these compounds for their antibacterial and anti-fungal activities and the further synthesis of the chlorinated sulfonamides was performed with an aim to prepare new competitors of drug having prominent activity for the discovery of drugs against AD.

MATERIALS AND METHODS

General

4-Chlorobenzenesulfonyl chloride and substituted anilines were purchased from Sigma Aldrich (**2a** & **2b**) and Alfa Aeser (**1** & **2c-i**) through local suppliers and were used without further purification. All the other used solvents were of analytical grade. Purity of synthesized compounds was assured by thin layer chromatography (TLC) applying ethyl acetate & *n*-hexane as solvent systems. TLC plates were purchased from local supplier. TLC plates were visualized under UV at 254 nm and also by spraying with ceric sulfate solution. The I.R. spectra were recorded in potassium bromide pellet method on a Jasco-320-A spectrophotometer with wave number in cm^{-1}. Melting points of all the synthesized compounds were recorded by open capillary tube, on a Griffin-George melting point apparatus and were also uncorrected. ^{1}H-NMR spectra were recorded in MeOD on a Bruker spectrometers operating at 500 MHz. The chemical shift values are reported in ppm (*d*) units taking TMS as reference, and the coupling constants (*J*) are in Hz. Mass spectra (EI MS) were recorded on a JMS-HX-110 spectrometer.

General procedure for the synthesis of different chlorinated sulfonamides 3a-i

Substituted anilines (0.001 mol: **2a-i**) were dispersed in 250 mL RB flask containing 100 mL water under the pH of 9.0 to 10.0, maintained by 10% aqueous solution of Na_2CO_3. 4-Chlorobenzenesulfonyl chloride (0.001 mol; 1) was added in the basic solution gradually over 10-15-min keeping the pH of solution 9.0 to 10.0. After that the reaction contents were kept on stirring for 3-5 hours. The reaction completion was monitored by TLC (*n*-hexane: EtOAc; 70:30) and 3-4 mL conc. hydrochloric acid was poured to attain the pH of 2.0-3.0. The reaction mixture was kept at RT for 10-15 min; the solid precipitates were filtered and washed with distilled water to yield the corresponding compounds (**3a-i**) on drying. Recrystallization was carried out from methanol.

Butyrylcholinesterase assay

The BChE inhibition activity was executed as by the reported Ellman *et al.,* (1961) but with some differences. Total volume of 100µL of reaction mixture was prepared having 60µL sodium hydrogen phosphate buffer (50 mM with pH 7.7). 10µL compound (to be tested) with concentration of 0.5 mM $well^{-1}$ and 10µL enzyme with concentration of 0.005 unit $well^{-1}$ were added. The reaction mixture was thoroughly mixed and pre-incubated for 10 min at 37°C and pre-read at 405 nm. The reaction process was inducted by the accession of 10µL butyrylthiocholine bromide (substrate) with concentration of 0.5 mM $well^{-1}$ and 10µL DTNB with concentration of 0.5mM $well^{-1}$. Absorbance was measured at 405 nm after incubation for 30 min at 37°C. Synergy HT (BioTek, USA) 96-well plate reader was employed in these experiments. All these experiments were accomplished

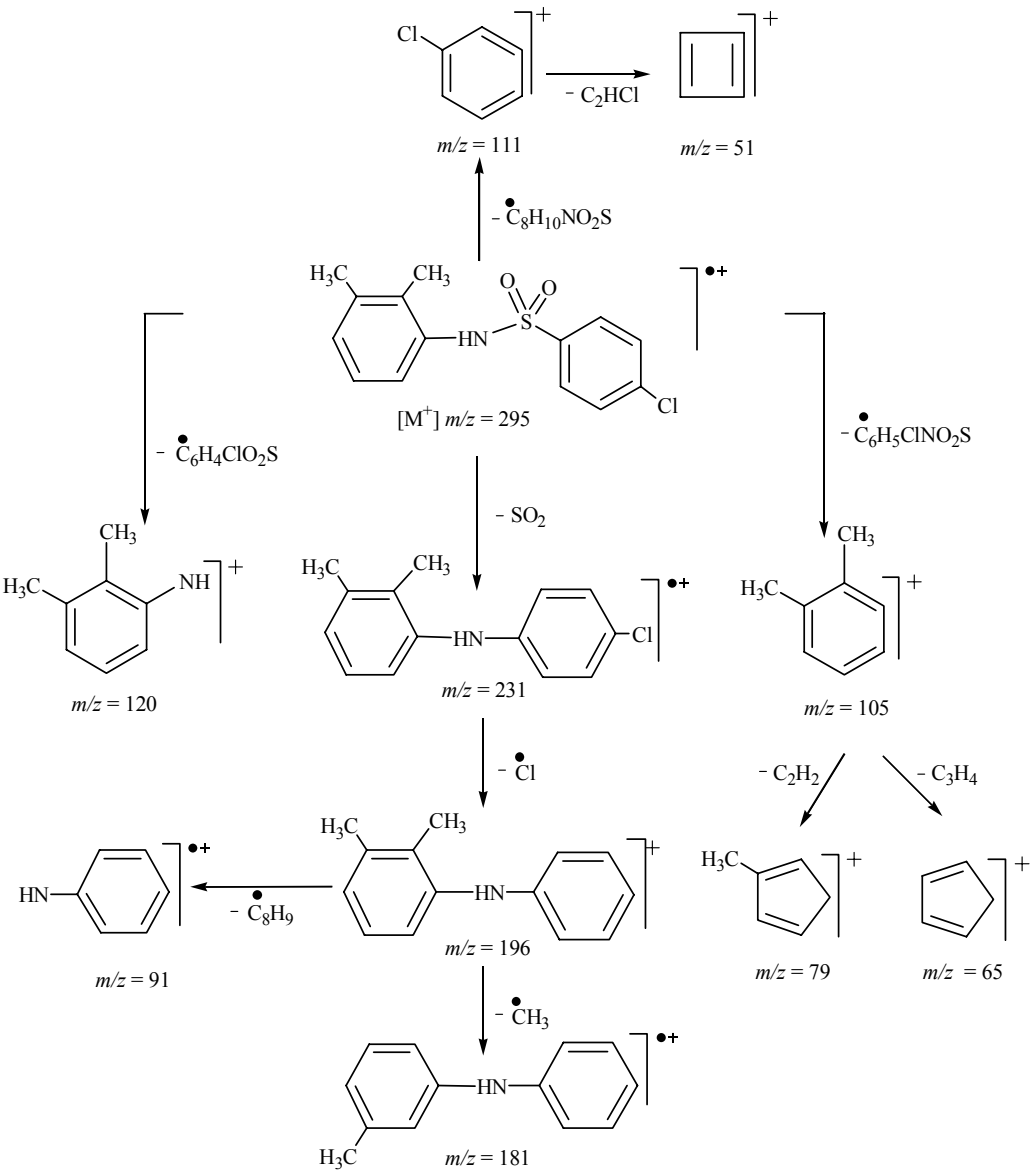


Fig. 1: Mass Fragmentation pattern of *N*-(2,3-dimethylphenyl)-4-chlorobenzenesulfonamide (3a)

with the corresponding controls in threefold. Eserine with concentration of 0.5mM well^{-1}, was utilized as a positive control. The percentage inhibition and IC_{50} values were calculated as:

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

where

Control = Total enzyme activity without inhibitor

Test = Activity in mien of test compound

IC_{50} values (concentration of compound at which 50% enzyme is inhibited) of compound were computed by EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). IC_{50} values were calculated (as mean of three independent experiments) from the graph by dilution of compounds to different concentrations.

Urease inhibition assay

This enzyme assay is the customized form of the commonly known Berthelot assay (Jensen *et al.*, 1992; Mobley *et al.*, 1988). The assay mixture of 85µL is prepared containing 10µL of phosphate buffer of pH 7.0 (in each well in the 96-well plate), 10µL of sample solution and 25µL of enzyme solution (0.135 units). Contents were pre-incubated at 37ºC for 5 minutes. 40µL of urea stock solution (20 mM) was added to each well with incubation for 10 min at 37ºC. It is followed by the addition of 115µL phenol hypochlorite reagents (freshly prepared by mixing 45µL phenol with 70 µL of alkali) per well. For color development, incubation was carried out for further 10 min at 37ºC. Absorbance was measured at 625 nm. The percentage enzyme inhibition and IC_{50}

Table 1: Enzyme inhibition studies of various chlorinated sulfonamides.

Compd. No.	BChE		Urease		LOX	
	Inhibition (%) at 0.5 mM	IC_{50} µmoles/L	Inhibition (%) at 0.5 mM	IC_{50} µmoles/L	Inhibition (%) at 0.5 mM	IC_{50} µmoles/L
3a	95.21±0.82	40.21±0.31	81.56±0.32	98.09±0.05	57.76±0.17	>400
3b	75.53±0.38	98.71±0.18	72.94±0.52	109.01±0.03	81.23±0.62	51.21±0.41
3c	90.21±0.14	63.21±0.61	74.70±0.86	157.7±0.08	72.56±0.33	62.51±0.07
3d	92.47±0.98	58.61±0.77	69.66±0.73	212.91±0.04	86.76±0.38	94.21±0.04
3e	97.43±0.35	45.21±0.32	63.36±1.69	269.21±0.13	87.61±0.34	91.21±0.11
3f	66.21±0.68	142.31±0.18	64.36±0.63	271.08±0.02	80.87±0.77	124.71±0.33
3g	87.58±0.17	75.89±0.11	70.71±0.93	238.33±0.04	67.03±0.19	275.61±0.18
3h	97.87±0.47	44.71±0.07	66.82±0.59	265.08±0.08	50.78±0.78	>400
3i	86.97±0.91	79.68±0.08	83.94±1.31	113.09±0.04	84.61±0.87	98.61±0.17
Control	Eserine	0.85±0.0001	Thiourea	21.28±0.11	Baicalein	22.4±1.3

values were calculated by the same procedure as mentioned above.

Lipoxygenase assay

LOX activity was determined using the already mentioned procedure (Baylac *et al.*, 2003; Bertaccini, 1982; Clapp *et al.*, 1985) but with a little change. 200 µL assay mixture was prepared containing 150 µL Na_3PO_4 buffer with concentration of 100 mM and pH of 8.0, 10 µL compound (to be tested) and 15 µL enzyme. The reaction contents were pre-incubated for duration of 10 min at a temperature of 25°C. After homogeneous mixing and pre-reading at 234 nm the process was inducted by the accession of 25µL substrate. The absorbance change was noticed at 234 nm after 6 min. All reactions were executed in threefold corresponding to the controls. Baicalein at 0.5 mM $well^{-1}$, was used as a positive control. The percentage inhibition and IC_{50} values were calculated as described 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.

Spectral data

N-(2,3-Dimethylphenyl)-4'-chlorobenzenesulfonamide (3a)

Light pink amorphous solid; Yield: 89%; M. P. 103-105 °C; Mol. formula: $C_{14}H_{14}ClNO_2S$; Mol. Weight: 295 $gmol^{-1}$; IR (KBr): $\square_{max}$ (cm^{-1}): 3380 (N-H), 3056 (Ar-H), 1530 (Ar C=C), 1410 (-SO_2-), 1140 (C-N), 710 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): *δ* 7.61 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.49 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.02 (d, *J* = 7.5 Hz, 1H, H-6), 6.93 (t, *J* = 7.5 Hz, 1H, H-5), 6.76 (d, *J* = 8.0 Hz, 1H, H-4), 4.83 (s, H-N), 2.20 (s, 3H, CH_3-2), 1.97 (s, 3H, CH_3-3); EIMS: *m/z* 297 $[M+2]^+$, 295 $[M]^+$, 231 $[M-SO_2]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(2,4-Dimethylphenyl)-4'-chlorobenzenesulfonamide (3b)

Light brown amorphous solid; Yield: 87%; M. P. 105-107 °C; Mol. formula: $C_{14}H_{14}ClNO_2S$; Mol. Weight: 295 $gmol^{-1}$; IR (KBr): $\square_{max}$ (cm^{-1}): 3381 (N-H), 3057 (Ar-H), 1529 (Ar C=C), 1411 (-SO_2-), 1141 (C-N), 711 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.61 (d, *J* = 8.5 Hz, 2H, H-2', H-6'), 7.48 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 6.94 (brs, 1H, H-6), 6.87 (dd, *J* = 6.0, 1.5 Hz, 1H, H-5), 6.86 (s, 1H, H-3), 4.83 (s, H-N), 2.23 (s, 3H, CH_3-2), 1.97 (s, 3H, 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 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-Phenyl-4'-chlorobenzenesulfonamide (3c)

White amorphous solid; Yield: 88%; M. P. 80-82 °C; Mol. formula: $C_{12}H_{10}ClNO_2S$; Mol. Weight: 267 $gmol^{-1}$; IR (KBr): $\square_{max}$ (cm^{-1}): 3377 (N-H), 3055 (Ar-H), 1534 (Ar C=C), 1414 (-SO_2-), 1143 (C-N), 708 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.69 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.22 (d, *J* = 10.0 Hz, 2H, H-2, H-6), 7.04-7.08 (m, 3H, H-3 to H-5), 4.87 (s, H-N); EIMS: *m/z* 269 $[M+2]^+$, 267 $[M]^+$, 203 $[M-SO_2]^+$, 175 $[M-C_6H_6N]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(2-Ethoxyphenyl)-4'-chlorobenzenesulfonamide (3d)

Purple amorphous solid; Yield: 89%; M. P. 110-112 °C; Mol. formula: $C_{14}H_{14}ClNO_3S$; Mol. Weight: 311 $gmol^{-1}$; IR (KBr): $\square_{max}$ (cm^{-1}): 3387 (N-H), 3057 (Ar-H), 1530 (Ar C=C), 1410 (-SO_2-), 1140 (C-N), 713 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.61 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.43 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.39 (dd, *J* = 13.5, 2.5 Hz, 1H, H-6), 7.10 (dt, *J* = 13.5, 3.0 Hz, 1H, H-4), 6.88 (dt, *J* = 10.5, 2.5 Hz, 1H, H-5), 6.79 (dd, *J* = 13.5, 1.5 Hz, 1H, H-3), 4.86 (s, H-N), 3.73 (q, *J* = 11.5 Hz, 2H, H-1"), 1.14 (t, *J* = 11.5 Hz, 3H, CH_3-2"); EIMS: *m/z* 313 $[M+2]^+$, 311 $[M]^+$, 247 $[M-SO_2]^+$, 175 $[M-C_8H_{10}NO]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(3-Ethoxyphenyl)-4'-chlorobenzenesulfonamide (3e)
Gray amorphous solid; Yield: 84%; M. P. 112-114 ^{o}C; Mol. formula: $C_{14}H_{14}ClNO_3S$; Mol. Weight: 311 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3388 (N-H), 3059 (Ar-H), 1528 (Ar C=C), 1408 (-SO_2-), 1138 (C-N), 714 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.72 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.48 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.08 (t, *J* = 13.0 Hz, 1H, H-5), 6.59-6.65 (m, 3H, H-2, H-4, H-6), 4.86 (s, H-N), 3.93 (q, *J* = 11.5 Hz, 2H, H-1"), 1.32 (t, *J* = 11.5 Hz, 3H, CH_3-2"); EIMS: *m/z* 313 $[M+2]^+$, 311 $[M]^+$, 247 $[M-SO_2]^+$, 175 $[M-C_8H_{10}NO]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(4-Ethoxyphenyl)-4'-chlorobenzenesulfonamide (3f)
Purple amorphous solid; Yield: 90%; M. P. 114-116 ^{o}C; Mol. formula: $C_{14}H_{14}ClNO_3S$; Mol. Weight: 311 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3374 (N-H), 3058 (Ar-H), 1529 (Ar C=C), 1409 (-SO_2-), 1139 (C-N), 712 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.62 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.93 (d, *J* = 14.5 Hz, 2H, H-2, H-6), 6.75 (d, *J* = 15.0 Hz, 2H, H-3, H-5), 4.86 (s, H-N), 3.94 (q, *J* = 11.5, 2H, H-1"), 1.32 (t, *J* = 11.5, 3H, CH_3-2"); EIMS: *m/z* 313 $[M+2]^+$, 311 $[M]^+$, 247 $[M-SO_2]^+$, 175 $[M-C_8H_{10}NO]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(2-Ethyl-6-methylphenyl)-4'-chlorobenzene sulfonamide (3g)
White amorphous solid; Yield: 87%; M. P. 235-237 ^{o}C; Mol. formula: $C_{15}H_{16}ClNO_2S$; Mol. Weight: 309 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3389 (N-H), 3058 (Ar-H), 1526 (Ar C=C), 1406 (-SO_2-), 1136 (C-N), 709 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.68 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.53 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.98-7.14 (m, 3H, H-3 to H-5), 4.86 (s, H-N), 2.46 (q, *J* = 11.5 Hz, 2H, H-1"), 1.97 (s, 3H, CH_3-6), 1.02 (t, *J* = 11.5, 3H, CH_3-2"); EIMS: *m/z* 311 $[M+2]^+$, 309 $[M]^+$, 245 $[M-SO_2]^+$, 175 $[M-C_9H_{12}N]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(2-Ethylphenyl)-4'-chlorobenzenesulfonamide (3h)
Light pink amorphous solid; Yield: 89%; M.P. 108-110 ^{o}C; Mol. formula: $C_{14}H_{14}ClNO_2S$; Mol. Weight: 295 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3372 (N-H), 3057 (Ar-H), 1531 (Ar C=C), 1411 (-SO_2-), 1141 (C-N), 714 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.64 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.50 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.18 (dd, *J* = 10.0, 3.0 Hz, 1H, H-6), 7.14 (dt, *J* = 11.0, 2.0 Hz, 1H, H-5), 7.04 (dt, *J* = 11.5, 3.0 Hz, 1H, H-4), 6.95 (dd, *J* = 13.0, 3.0 Hz, 1H, H-3), 4.86 (s, H-N), 2.48 (q, *J* = 11.5 Hz, 2H, H-1"), 1.01 (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 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

N-(2-Phenylethyl)-4'-chlorobenzenesulfonamide (3i)
Light green amorphous solid; Yield: 89%; M. P. 78-80 ^{o}C; Mol. formula: $C_{14}H_{14}ClNO_2S$; Mol. Weight: 295 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3391 (N-H), 3057 (Ar-H), 1529 (Ar C=C), 1423 ((-SO_2-), 1145 (C-N), 715 (C-Cl); ^{1}H-NMR (500 MHz, MeOD, ppm): δ 7.75 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.52 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.09-7.22 (m, 5H, H-2 to H-6), 4.86 (s, H-N), 3.09 (t, *J* = 11.5, 2H, H-8), 2.71 (t, *J* = 11.5, 2H, H-7); EIMS: *m/z* 297 $[M+2]^+$, 295 $[M]^+$, 231 $[M-SO_2]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$, 51 $[C_4H_3]^+$.

RESULTS

In the presented research work, a number of chlorinated sulfonamides were synthesized by mixing of 4-chlorobenzenesulfonyl chloride (**1**) with different substituted anilines (**2a-i**) under a basic pH in aqueous media and screened against urease, butyrylcholinesterase and lipoxygenase enzymes. The maximum yields of products, **3a-i**, were obtained within 3-5 hours by continuous stirring at RT. The products were isolated by filtration after the addition of concentrated HCl (to make pH of solution 2.0 to 3.0) and washing of precipitates was carried out with cold distilled water (Aziz-ur-Rehman *et al.*, 2012). The structures of all the compounds were elucidated from spectral data.

Enzyme inhibition studies (in vitro)
The results of enzyme inhibition activity of the synthesized compounds against urease, butyryl-cholinesterase and lipoxygenase enzymes are presented in table 1.

DISCUSSION

The compound **3a** was synthesized as light pink amorphous powder with yield of 89% and melting point of 103-105 ^{o}C. The molecular formula $C_{14}H_{14}ClNO_2S$ was instituted by EIMS having molecular ion peak at *m/z* 295 and also by counting the number of protons in ^{1}H-NMR spectrum. The IR spectrum revealed the presence of a sulfonyl group (1410 cm^{-1}) and -NH- group (3380 cm^{-1}) in the synthesized compound. The EI-MS presented a distinguishable peak at *m/z* 231 after the separation of sulfonyl group (SO_2). The other prominent peaks were mentioned in the spectral data. In the aromatic section of ^{1}H–NMR spectrum, the signals appearing at *δ* 7.61 (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 1,4-disubstituted ring with chloro and sulfonyl groups. The signals appearing at *δ* 7.02 (d, *J* = 7.5 Hz, 1H, H-6), 6.93 (t, *J* = 7.5 Hz, 1H, H-5) and 6.76 (d, *J* = 8.0 Hz, 1H, H-4) were allotted to three protons of other tri-substituted aniline ring. In the aliphatic section of ^{1}H-NMR spectrum, the signals became visible at *δ* 4.83 (s, H-N), 2.20 (s, 3H, CH_3-2) and 1.97 (s, 3H, CH_3-3) indicating the presence of one proton attached to nitrogen of sulfonamide group and two methyl groups in the molecule at second and third position of substituted aniline. On the basis of all these

evidences, the structure of compound **3a** was named as *N*-(2,3-Dimethylphenyl)-4-chlorobenzenesulfonamide.

The mass fragmentation pattern of *N*-(2,3-Dimethylphenyl)-4-chlorobenzenesulfonamide (**3a**) is clearly described in fig. 1. Similarly, the structures of other compounds (**3b-i**) were characterized by ^{1}H-NMR, IR and EI-MS as described in spectral data section.

Enzyme inhibition activity

The screening of these synthesized compounds against butyryl cholinesterase (BChE), urease and lipoxygenase (LOX) enzymes exposed good inhibitory potential against all the three enzymes especially butyryl cholinesterase enzyme as it was clear from their IC_{50} values (table 1). Inhibition study for butyryl cholinesterase enzyme showed that all the synthesized compounds showed good activity against it but *N*-(2,3-Dimethylphenyl)-4-chlorobenzenesulfonamide (**3a**), *N*-(3-Ethoxyphenyl)-4-chlorobenzenesulfonamide (**3e**) and *N*-(2-Ethylphenyl)-4-chlorobenzene sulfonamide (**3h**) were the most effective inhibitors having IC_{50} values of 40.21±0.31, 45.21±0.32 and 44.71±0.07 µmoles/L respectively, with respect to eserine, a reference standard with IC_{50} value of 0.85±0.0001 µmoles/L. The effective inhibitory results of these compounds were probably due to the presence of alkyl and alkoxy groups adjacent to the amino group of the aniline ring. These compounds can further be tapped in the form of derivatives to obtain good inhibitory results and might be potential target in the drug development program. The screening against urease enzyme revealed that the compound *N*-(2,3-Dimethylphenyl)-4-chlorobenzenesulfonamide (**3a**) was found to be the most potent inhibitors for urease having IC_{50} values of 98.09±0.05 µmoles/L, with respect to thiourea, a reference standard with IC_{50} value of 21.28±0.11 µmoles/L. The beneficial inhibition activity of this compound was likely due to the presence of alkyl groups at *ortho* and *meta* position of aniline. Its derivatives can be further produced for good inhibition activities. Against lipoxygenase enzyme, the two compounds, *N*-(2,4-Dimethylphenyl)-4-chlorobenzenesulfonamide (**3b**) and *N*-Phenyl-4-chlorobenzenesulfonamide (**3c**) were the most efficient having IC_{50} values of 51.21±0.41 and 62.51±0.07 µmoles/L respectively, with respect to baicalein, a reference standard with IC_{50} value of 22.4±1.3 µmoles/L. The enhanced activity might be due to the alkyl substitution of aromatic amine, which is probably more complimentary for the inhibition of lipoxygenase enzyme.

CONCLUSION

All the molecules were synthesized in awesome amounts and structurally corroborated through spectral data analysis. The screening of these molecules against different enzymes rendered them valuable inhibitors of BChE enzyme, which has a great role in AD. So the most active molecules can be evaluated for *in vivo* activity and these might be valuable for the drug discovery program in pharmacological industries.

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