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Synthesis and Biological Screening of *N*-(Dimethylphenyl Substituted)-*N*-Ethyl/Benzyl-4-Chlorobenzenesulfonamide Derivatives

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ABSTRACT
In the undertaken research, a number of *N*-dimethylphenyl substituted derivatives of *N*-ethyl/benzyl-4-chlorobenzenesulfonamide, (**6a-f** & **7a-f**) was synthesized and also estimated for their biological potential. The reaction of 4-chlorobenzenesulfonyl chloride with different dimethyl substituted phenyl amine (**2a-f**) in the presence of basic aqueous media yielded *N*-[(Dimethyl substituted) phenyl]-4-chlorobenzenesulfonamides (**3a-f**). The targeted compounds **6a-f** & **7a-f** were synthesized by the reaction of compounds **3a-f** with electrophiles, ethyl bromide/benzyl chloride in the presence of base NaH and polar aprotic solvent (DMF). The structures of the compounds were elucidated through ^{1}H-NMR, IR and mass spectral data. The synthesized compounds (**6a-f** & **7a-f**) were screened against Gram-negative & Gram-positive bacteria and also subjected for enzyme inhibition potential against lipoxygenase and chymotrypsin enzymes and almost all the compounds exhibited moderate to good activities.

Keywords: 4-Chlorobenzenesulfonyl chloride, Spectral analysis, Antibacterial activity, Enzyme inhibition activity.

1. INTRODUCTION
The sulfonamides have a great significance in medicinal chemistry because of various biological activities[1-4] such as antibacterial[5], hypoglycemic[6], diuretic[7], anticarbonic anhydrase, antithyroid *in vitro* and *vivo*, antiinflammatory[8,9], anticancer, antihypertensive[10] and anticonvulsing activities; and potential herbicidal properties for agricultural applications[11]. Although new methodologies have been introduced yet the formal synthesis by stirring of amino compounds with sulfonyl halides is still in practice[12]. Environmentally benign sulfonamides have been synthesized at room temperature in H_2O under pH control with sodium carbonate[13,14].

In continuation of our previous research work[17,18] to synthesize variety of potent sulfonamide derivatives and evaluation of their biological activity. The present research work was an effort to synthesize *N*-dimethylphenyl substituted derivatives of *N*-ethyl/benzyl-4-chlorobenzenesulfonamide. Further, *in vitro* antibacterial screening of all the synthesized compounds against clinically isolated two gram-positive bacteria (*Bacillus subtilis & Staphylococcus aureus*) and four gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae & Salmonella typhi*). Furthermore, they were also subjected for enzyme inhibition activity using standard protocol[19, 20]. It was observed that different *N*-substitution notably influenced the antibacterial and enzyme inhibition potential from moderate to excellent level.

2. RESULTS AND DISCUSSION

2.1 *Chemistry*
The *N*-dimethylphenyl substituted sulfonamides **6a-f** & **7a-f** were synthesized according to the procedure sketched in **(Scheme 1)** and different phenyl group substitutions are mentioned in **(Table-1)**. The general reaction conditions and the structural characterization are described in experimental section.

The presented research work is an attempt to explore new series of biological active compounds which may be helpful in drug development. The parent molecules, *N*-[(Dimethyl substituted)phenyl]-4-chlorobenzenesulfonamide **(3a-f)** were synthesized by the coupling of dimethyl substituted phenyl amine **(2a-f)** with 4-chlorobenzenesulfonyl chlorides **(1)** under dynamic pH control of basic aqueous medium of sodium carbonate. The products were obtained on acidification using dilute. HCl dropwise and avoid excess of acid that can decrement the yield. The parent molecules were further derivatized by coupling with electrophiles, ethyl bromide/benzyl chloride (**4** & **5**) to synthesize the target compounds, **6a-f** & **7a-f**, in the presence of NaH as an activator in polar aprotic solvent of *N*,*N*-dimethylformamide (DMF). Reaction completion was attained within 2-3 hours by stirring at room temperature (30 °C). The products were isolated by filtration after the addition of cold distilled water. The structure of all the synthesized compounds was affirmed by spectral data as presented in experimental section.

Compound **6a** was synthesized as white amorphous solid with 87% yield and melting point 112-114 ^{o}C. The molecular formula $C_{16}H_{18}ClNSO_2$ was established by molecular ion peak at *m/z* 323 in EI-MS and also by counting the number of protons in its ^{1}H-NMR spectrum. The Infrared spectrum showed absorption bands at 3058 cm^{-1}, 1532 cm^{-1} and 1415 cm^{-1} because of C-H (aromatic stretching), C=C (aromatic stretching) and -SO_2 (stretching of sulfonyl group) respectively. The EI-MS spectrum gave characteristic peaks at *m/z* 175, 120 and 111 which were ascribed to

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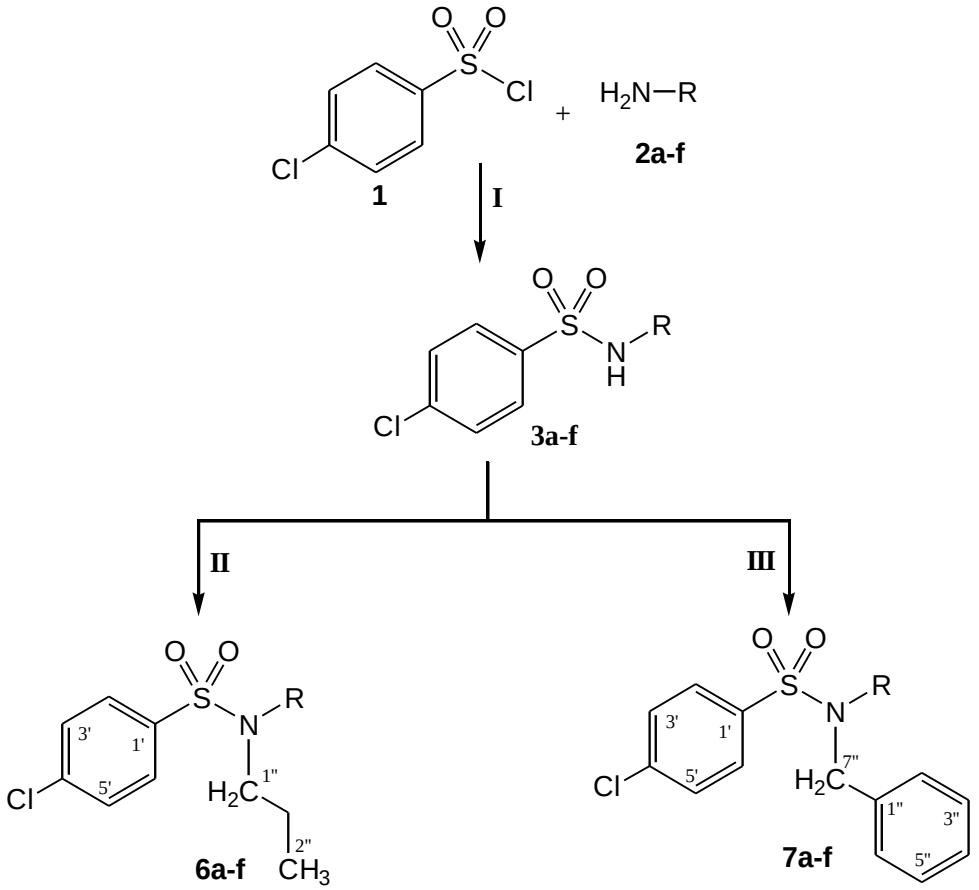
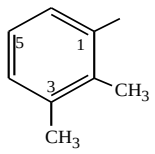
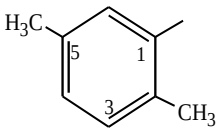
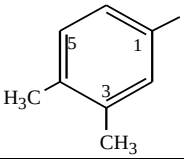
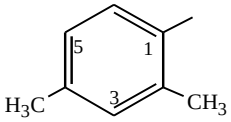
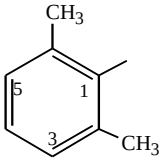
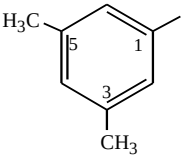
the loss of chlorinated phenylsulfonyl cation, dimethyl phenyl amine cation and chlorophenyl fragments respectively. In the ^{1}H-NMR spectrum, the signals resonating at δ 7.62 as doublet with *J* coupling of 9.0 Hz and 7.47 as doublet with *J* coupling of 8.5 Hz each integrated for two protons, confirmed the presence of 4-chlorobenzenesulfonyl group. Signals appeared at 7.05 (d, *J* = 7.5 Hz, 1H, H-6), 6.96 (t, *J* = 7.5 Hz, 1H, H-5), 6.79 (d, *J* = 8.0 Hz, 1H, H-4), 2.23 (s, 3H, CH_3-2) and 1.99 (s, 3H, CH_3-3) affirmed the presence of 2,3-dimethylphenyl group attached at nitrogen of sulfonamide linkage. A triplet and a quartet appeared at 0.88 and 3.36 respectively with coupling constant of 7.5 Hz corresponding to ethyl group substituted at nitrogen of sulfamoyl group confirmed the electrophilic substitution. All the spectral data corroborated the structure of **6a** as *N*-(2,3-Dimethylphenyl)-*N*-ethyl-4-chlorobenzene sulfonamide. The mass fragmentation pattern of *N*-(2, 3-Dimethylphenyl)-*N*-ethyl-4-chlorobenzenesulfonamide **(7a)** is sketched in **(Figure-1)**. In the same way, the structures of all the synthesized compounds were confirmed by ^{1}H-NMR, IR and mass spectral data.



Scheme-1: Outline for the synthesis of *N*-[(Dimethyl substituted)phenyl]-*N*-ethyl/benzyl-4-chlorobenzenesulfonamide.

Reagents & conditions: (I) H2O/5% Na2CO3 soln./pH 9-10/stirring at room temperature (II) Ethyl bromide (4) DMF/NaH/stirring at RT for 2-3 hours. (III) Benzyl chloride (5) DMF/NaH/stirring at RT for 2-3 hours.

Different disubstituted aryl groups.

Compd.	R	Compd.	R	Compd.	R
6a, 7a		**6c, 7c**		**6e,7e**	
6b, 7b		**6d, 7d**		**6f, 7f**	

2.2 *Antibacterial activity*

The results of antibacterial study of the synthesized compounds (**6a-f** & **7a-f**) with MIC values are tabulated in **(Table-1)**. The compounds of series were found to be relatively more active against two gram-positive (*Bacillus*

subtilis and Staphylococcus aureus) and four gram-negative *(Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Salmonella typhi*) bacterial strains as compared to enzyme inhibition activity. Compound **6a** was found to be active against two bacterial strains; *E. coli* & *P. aeruginosa* with MIC values of 9.31±1.87 & 10.51±1.34 as compared to reference standard drug ciprofloxacin having MIC values 8.06±1.07 & 8.48±1.91 respectively. Compound **6b** was found to be active against four bacterial strains i.e., *S. typhi*, *P. aeruginosa, B. subtilis* and *S. auerus* with MIC values of 10.01±1.91, 9.34±2.11, 12.73±1.95 and 10.08±1.34 respectively, as compared to ciprofloxacin standard. Against *P. aeruginosa*, the sequence of activity was **6f>6b>6a>6d>6c** from the maximum to lowest but lower antibacterial activity than the standard compound used in this assay. In general the activity exposed by the synthesized compounds was good as supported by their MIC values. Such kind of compounds can further be exploited and their analogous could be synthesized to acquire closer MIC values of the standard, Ciprofloxacin. In this approach, the compounds could be potential target in the drug development program.

Table-1: Antibacterial activity (MIC) of the tested compounds.

Compound	MIC					
	Gram-negative bacteria			Gram-positive bacteria		
	S.typhi (-)	***E.coli (-)***	***K.pneumoniae (-)***	***P.aeruginosa (-)***	***B.subtilis (+)***	***S.auerus (+)***
6a	14.43±1.25	9.31±1.87	18.41±1.09	10.51±1.34	12.51±2.27	14.76±1.09
6b	10.01±1.91	11.36±2.21	14.67±0.66	9.34±2.11	12.73±1.95	10.08±1.34
6c	14.56±0.31	14.56±0.09	14.44±1.88	10.83±2.98	13.02±0.73	10.88±1.48
6d	14.17±2.92	10.13±1.91	-	10.66±0.22	12.11±1.17	14.72±0.06
6e	13.17±1.36	12.67±0.45	13.54±1.17	12.35±1.25	14.67±0.04	9.57 ±0.31
6f	12.25±2.08	10.82±1.47	10.52±1.80	9.25±1.76	11.92±1.83	10.37±1.80
7a	-	-	-	13.21±1.94	14.86±2.09	-
7b	-	-	-	-	-	-
7c	15.11±1.04	-	-	16.10±1.22	-	-
7d	13.75±1.61	15.04±2.88	16.86±0.75	11.45±1.42	13.21±0.07	12.10±0.65
7e	13.28±0.48	15.03±1.33	12.99±2.03	16.32±2.31	13.63±0.62	14.11±1.54
7f	17.66±0.78	13.97±0.63	16.81±2.61	11.21±2.10	12.89±1.73	14.13±1.52
Ciprofloxacin	9.27±1.58	8.06±1.07	8.51±0.14	8.48±1.91	9.04±1.86	8.95±1.33

NOTE: Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/ well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software.

2.3 *Enzyme inhibition activity*

The screening of the synthesized compounds (insert compounds number or codes) against lipoxygenase (LOX) enzymes and chymotrypsin activity **(Table-2)** revealed that these molecules exhibited good inhibitory potential only against lipoxygenase enzyme as it was evident from their IC_{50} values. The compound, **7b** was the efficient inhibitor for lipoxygenase enzyme having IC_{50} values of 103.56±1.35 µmoles, relative to Baicalein, a reference standard with IC_{50} value of 22.4±1.3 µmoles probably due to the presence of 2,4-dimethylphenyl group in the molecule. All the compounds were not promising inhibitors against chymotrypsin enzyme. Such type of compounds can further be explored by changing the substitution on the aromatic ring to get closer IC_{50} values of the standard, Baicalein so that they could be possible target in the drug expansion program.

Table-2: Enzyme inhibition potential of synthesized derivatives

Compound	Conc. (mM)	LOX		Chymotrypsin	
		% Inhibition	**IC_{50}**	**% Inhibition**	**IC_{50}**
6a	0.5	24.71±1.02	-	56.40±0.04	>400
6b	0.5	92.83±1.04	187.52±1.07	42.12±0.14	-
6c	0.5	77.50±1.26	163.28±1.32	36.51±0.11	-
6d	0.5	18.60±1.21	-	22.91±0.11	-
6e	0.5	68.80±1.06	185.93±1.28	50.47±0.08	>400
6f	0.5	10.55±1.29	-	57.56±0.10	>400
7a	0.25	65.17±1.21	135.45±1.18	59.30±0.07	>400
7b	0.25	91.83±1.05	103.56±1.35	14±98.09	-
7c	0.25	18.44±1.16	-	40.12±0.09	-
7d	0.25	43.56±1.14	-	43.14±0.12	-
7e	0.25	36.53±1.21	-	53.14±0.02	>400
7f	0.25	57.78±1.18	159.63±1.11	54.88±0.05	>400
Baicalein	**0.5**	**93.79±1.27**	**22.4±1.3**		
Chymostatin	**0.5**			**93.50±0.91**	**8.24±0.11**

3. CONCLUSION

All the compounds were synthesized in good yield and purity. The structures were elucidated through spectral analysis and were supported by the biological screening. The results of biological analysis, obtained as MIC and IC_{50} values rendered the synthesized compounds as moderate inhibitors for enzymes but relatively better antibacterial agents.

4. EXPERIMENTAL

4.1*General*

Thin layer chromatography (TLC) was utilized to detect the purity of the synthesized compounds using ethyl acetate and *n*-hexane as mobile phase and visualized by UV lamp at 254 nm. Griffin-George melting point apparatus was used to record the melting points of all derivatives by open capillary tube method and were uncorrected. Jasco-320-A spectrophotometer (wave number in cm^{-1}) was utilized for I.R. spectra by KBr pellet method. ^{1}H-NMR spectra were recorded in CD_3OD on a Bruker spectrometers at 500 MHz using tetramethylsilane as reference standard and chemical shift mentioned in δ-values while the coupling constants (J) is given in Hz. JMS-HX-110 spectrometer was used to record the mass spectra (EIMS). All the compounds were of Merck and Alfa Aesar purchased from local suppliers. The solvents used, were of analytical grade.

4.2 *Procedure for the synthesis of N-[(Dimethyl substituted)phenyl]-4-chlorobenzenesulfonamide (3a-f)*

Dimethyl substituted phenyl amine (**2a-f,** 0.01 mol) was taken in 100 mL round bottomed flask (RB) and (20%) Na_2CO_3 solution was added to maintain pH around 9-10. After 30 min stirring at room temperature, 4-chlorobenzenesulfonyl chlorides (**1**, 0.01 mol) was introduced into the flask. The reaction contents were allowed to stir for 2-3 hours and progress of reaction was monitored by TLC time to time till the single spot. On reaction completion dil. HCl was added drop by drop along with continuous shaking to bring the pH to 2-3. The precipitates of *N*-[(Dimethylsubstituted)phenyl]-4-chlorobenzenesulfonamide **(3a-f)** formed were then filtered, washed with distilled water and dried to follow the further reaction.

4.3 *Procedure for the synthesis of N-[(Dimethyl substituted) phenyl]-N-*ethyl/benzyl-*4-chlorobenzenesulfonamide* (6a-f & 7a-f):

Compounds **3a-f** (0.01 mol) was dissolved in 10 mL *N*, *N*-dimethyl formamide (DMF) in a 100 mL RB flask. Sodium hydride (0.01 mol) was added to the reaction flask and set to stir for half an hour at room temperature (RT). The electrophile, ethyl bromide/benzyl chloride (**4/5**; 0.01 mol) was added to reaction mixture and set to stir for further 4-5 hours to synthesize the target compounds **6a-f** & **7a-f**. After the completion of reaction, the contents were quenched with ice cold distilled water along with vigorous shaking. The precipitates formed were left for 10-15 minutes undisturbed and then filtered, washed with distilled water and dried to acquire yield and analysis of the target compounds **6a-f** and **7a-f.**

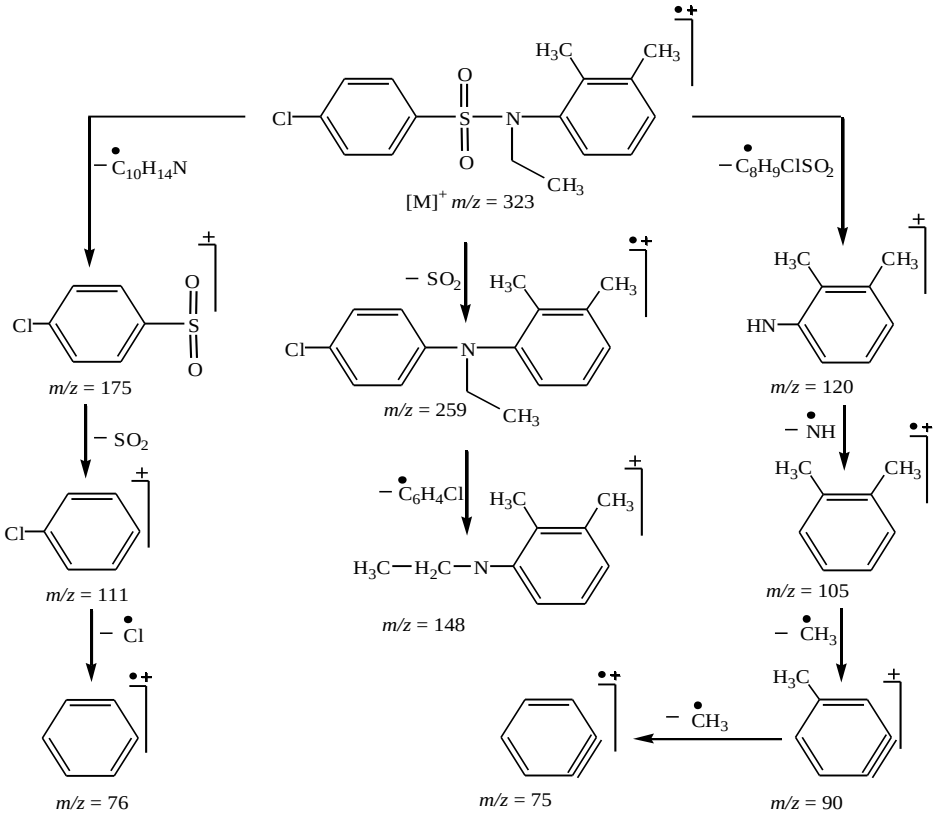


Fig-1: Proposed Mass fragmentation pattern of compound **6a.**

4.3.1 ***N-(2, 3-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6a)***

White amorphous solid; Yield: 87%; M. P. 112-114 ^{o}C; Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3058 (Ar-H), 1532 (Ar C=C), 1415 (-SO_2-), 1144 (C-N), 567 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.62 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.05 (d, *J* = 7.5 Hz, 1H, H-6), 6.96 (t, *J* = 7.5 Hz, 1H, H-5), 6.79 (d, *J* = 8.0 Hz, 1H, H-4), 3.39 (q, *J* = 7.5 Hz, 2H, H-1''), 2.23 (s, 3H, CH_3-2), 1.99 (s, 3H, CH_3-3), 0.88 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (7.1 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.9 %), 175 $[C_6H_4ClSO_2]^{+}$ (2.6 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.4 %), 111 $[C_6H_4Cl]^{+}$ (33.2 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (22.3 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.5 %), 76 $[C_6H_4]^{\bullet+}$ (5.4 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (26.9 %).

4.3.2 ***N-(2,4-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6b)***

Cream White amorphous solid; Yield: 85%; M. P. 108-110 ^{o}C; Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3058 (Ar-H), 1524 (Ar C=C), 1417 (-SO_2-), 1144 (C-N), 557 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.63 (d, *J* = 8.5 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 6.95 (br. s, 1H, H-6), 6.86 (dd, *J* = 6.0,1.5 Hz, 1H, H-5), 6.85 (s, 1H, H-3), 3.37 (q, *J* = 7.5 Hz, 2H, H-1''), 2.22 (s, 3H, CH_3-2), 1.96 (s, 3H, CH_3-4), 0.89 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (6.5 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.2 %), 175 $[C_6H_4ClSO_2]^{+}$ (4.5 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.1 %), 111 $[C_6H_4Cl]^{+}$ (31.8 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (20.9 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.2 %), 76 $[C_6H_4]^{\bullet+}$ (4.7 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (24.2 %).

4.3.3 ***N-(2,5-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6c)***

Cream White amorphous solid; Yield: 90%; M. P. 104-106 ^{o}C; Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3057 (Ar-H), 1532 (Ar C=C), 1407 (-SO_2-), 1135 (C-N), 551 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.61 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.97 (d, *J* = 7.5 Hz, 1H, H-3), 6.95 (d, *J* = 8.0 Hz, 1H, H-4), 6.83 (s, 1H, H-6), 3.37 (q, *J* = 7.5 Hz, 2H, H-1''), 2.18 (s, 3H, CH_3-2), 1.92 (s, 3H, CH_3-5), 0.87 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (6.7 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.5 %), 175 $[C_6H_4ClSO_2]^{+}$ (4.7 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.8 %), 111 $[C_6H_4Cl]^{+}$ (29.8 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (27.9 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.3 %), 76 $[C_6H_4]^{\bullet+}$ (4.3 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (24.5 %).

4.3.4 ***N-(2,6-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6d)***

White amorphous solid; Yield: 85%; M. P. 114-116 ^{o}C Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3059 (Ar-H), 1532 (Ar C=C), 1406 (-SO_2-), 1138 (C-N), 555 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.66 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.53 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.09-6.97 (m, 3H, H-3 to H-5), 3.36 (q, *J* = 7.5 Hz, 2H, H-1''), 2.02 (s, 6H, CH_3-2, CH_3-6), 0.85 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (6.8 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.7 %), 175 $[C_6H_4ClSO_2]^{+}$ (4.2 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.7 %), 111 $[C_6H_4Cl]^{+}$ (31.3 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (21.8 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.4 %), 76 $[C_6H_4]^{\bullet+}$ (4.5 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (24.5 %).

4.3.5 ***N-(3,4-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6e)***

White amorphous solid; Yield: 86%; M. P. 118-120 ^{o}C Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3056 (Ar-H), 1533 (Ar C=C), 1415 (-SO_2-), 1143 (C-N), 565 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, 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'), 6.97 (d, *J* = 13.0 Hz, 1H, H-6), 6.83 (br. s, 1H, H-2), 6.79 (dd, *J* = 10.0, 1.0 Hz, 1H, H-5), 3.35 (q, *J* = 7.5 Hz, 2H, H-1''), 2.15 (s, 6H, CH_3-3, CH_3-4), 0.86 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (6.5 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.2 %), 175 $[C_6H_4ClSO_2]^{+}$ (4.5 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.1 %), 111 $[C_6H_4Cl]^{+}$ (31.8 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (20.9 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.2 %), 76 $[C_6H_4]^{\bullet+}$ (4.7 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (24.2 %).

4.3.6 ***N-(3,5-Dimethylphenyl)-N-ethyl-4-chlorobenzenesulfonamide (6f)***

White amorphous solid; Yield: 87%; M. P. 108-110 ^{o}C; Mol. formula: $C_{16}H_{18}ClNSO_2$; Mol. Wt.: 323; IR (KBr, ν_{max}/cm^{-1}): 3055 (Ar-H), 1534 (Ar C=C), 1412 (-SO_2-), 1136 (C-N), 565 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.68 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.44 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 6.74 (s, 2H, H-2, H-6), 6.67 (s, 1H, H-4), 3.38 (q, *J* = 7.5 Hz, 2H, H-1''), 2.15 (s, 6H, CH_3-3, CH_3-5), 0.87 (t, *J* = 7.5 Hz, 2H, H-2''); EIMS (*m/z*): 323 $[M]^{\bullet+}$ (7.0 %), 259 $[M\text{-}SO_2]^{\bullet+}$ (3.7 %), 175 $[C_6H_4ClSO_2]^{+}$ (5.1 %), 148 $[M\text{-}C_6H_4ClSO_2]^{+}$ (100 %), 120 $[M\text{-}C_8H_8ClSO_2]^{+}$ (3.9 %), 111 $[C_6H_4Cl]^{+}$ (31.6 %), 105 $[M\text{-}C_8H_9ClNSO_2]^{\bullet+}$ (20.7 %), 90 $[M\text{-}C_9H_{12}ClNSO_2]^{+}$ (1.9 %), 76 $[C_6H_4]^{\bullet+}$ (4.9 %), 75$[M\text{-}C_{10}H_{15}ClNSO_2]^{\bullet+}$ (24.8 %).

4.3.7*N-(2,3-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7a)*

White crystalline solid; Yield: 83%; M. P. 118-120 ^{o}C; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3054 (Ar-H), 1533 (Ar C=C), 1415(-SO_2-), 1147 (C-N), 563 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.63 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.43 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.19-7.16 (m, 5H, H-2'' to H-6''), 7.05 (d, *J* = 7.5 Hz, 1H, H-6), 6.96 (t, *J* = 7.5 Hz, 1H, H-5), 6.78 (d, *J* = 8.0 Hz, 1H, H-4), 4.74 (s, 2H, H-7''), 2.23 (s, 3H, CH_3-2),

1.95 (s, 3H, CH_3-3); EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.1 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.6 %), 175 $[C_6H_4ClSO_2]^+$ (6.1 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.6 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.0 %), 111 $[C_6H_4Cl]^+$ (35.9 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.1 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.8 %), 76 $[C_6H_4]^{\bullet+}$ (9.6 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.1 %).

4.3.8 *N-(2,4-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7b)*

White amorphous solid; Yield: 82%; M. P. 116-118 ^oC; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3059 (Ar-H), 1527 (Ar C=C), 1414 (-SO_2-), 1147 (C-N), 553 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.65 (d, *J* = 8.5 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 7.23-7.19 (m, 5H, H-2" to H-6"), 6.95 (br. s, 1H, H-6), 6.85 (dd, *J* = 6.0,1.5 Hz, 1H, H-5), 6.84 (s, 1H, H-3), 4.69 (s, 2H, H-7"), 2.24 (s, 3H, CH_3-2), 1.98 (s, 3H, CH_3-4); EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.3 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.9 %), 175 $[C_6H_4ClSO_2]^+$ (6.7 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.9 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.4 %), 111 $[C_6H_4Cl]^+$ (35.1 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.7 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.2 %), 76 $[C_6H_4]^{\bullet+}$ (9.1 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.3 %).

4.3.9 *N-(2,5-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7c)*

White amorphous solid; Yield: 86%; M. P. 108-110 ^oC; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3059 (Ar-H), 1532 (Ar C=C), 1410 (-SO_2-), 1139 (C-N), 553 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, 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'), 7.25-7.21 (m, 5H, H-2" to H-6"), 6.98 (d, *J* = 7.5 Hz, 1H, H-3), 6.91 (d, *J* = 8.0 Hz, 1H, H-4), 6.84 (s, 1H, H-6), 4.67 (s, 2H, H-7"), 2.17 (s, 3H, CH_3-2), 1.91 (s, 3H, CH_3-5); EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.3 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.3 %), 175 $[C_6H_4ClSO_2]^+$ (6.3 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.2 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.3 %), 111 $[C_6H_4Cl]^+$ (35.7 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.5 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.6 %), 76 $[C_6H_4]^{\bullet+}$ (9.4 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.4 %).

4.3.10 *N-(2,6-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7d)*

Light pink amorphous solid; Yield: 88%; M. P. 124-126 ^oC; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3055 (Ar-H), 1528 (Ar C=C), 1412 (-SO_2-), 1138 (C-N), 555 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.66 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.51 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.21-7.16 (m, 5H, H-2" to H-6"), 7.06-6.96 (m, 3H, H-3 to H-5), 4.67 (s, 2H, H-7"), 2.03 (s, 6H, CH_3-2, CH_3-6) ; EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.6 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.3 %), 175 $[C_6H_4ClSO_2]^+$ (6.3 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.5 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.1 %), 111 $[C_6H_4Cl]^+$ (35.2 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.3 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.6 %), 76 $[C_6H_4]^{\bullet+}$ (9.4 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.6 %).

4.3.11 *N-(3,4-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7e)*

Cream White amorphous solid; Yield: 87%; M. P. 114-116 ^oC; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3057 (Ar-H), 1533 (Ar C=C), 1413 (-SO_2-), 1145 (C-N), 565 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.67 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.22-7.17 (m, 5H, H-2" to H-6"), 6.97 (d, *J* = 13.0 Hz, 1H, H-6), 6.84 (br. s, 1H, H-2), 6.79 (dd, *J* = 10.0, 1.0 Hz, 1H, H-5), 4.71 (s, 2H, H-7"), 2.15 (s, 6H, CH_3-3, CH_3-4); EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.4 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.5 %), 175 $[C_6H_4ClSO_2]^+$ (6.2 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.7 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.3 %), 111 $[C_6H_4Cl]^+$ (35.7 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.7 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.5 %), 76 $[C_6H_4]^{\bullet+}$ (9.3 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.4 %).

4.3.12 *N-(3,5-Dimethylphenyl)-N-benzyl-4-chlorobenzenesulfonamide (7f)*

White amorphous solid; Yield: 85%; M. P. 112-114 ^oC; Mol. formula: $C_{21}H_{20}ClNSO_2$; Mol. Wt.: 385; IR (KBr, ν_{max}/cm^{-1}): 3056 (Ar-H), 1533 (Ar C=C), 1415 (-SO_2-), 1139 (C-N), 565 (C-Cl); ^{1}H-NMR (500 MHz, CD_3OD, ppm): δ 7.69 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 8.5 Hz, 2H, H-3', H-5'), 7.18-7.13 (m, 5H, H-2" to H-6"), 6.73 (s, 2H, H-2, H-6), 6.67 (s, 1H, H-4), 4.69 (s, 2H, H-7"), 2.15 (s, 6H, CH_3-3, CH_3-5); EIMS (*m/z*): 385 $[M]^{\bullet+}$ (22.7 %), 321 $[M\text{-}SO_2]^{\bullet+}$ (1.2 %), 175 $[C_6H_4ClSO_2]^+$ (6.4 %), 210 $[M\text{-}C_6H_4ClSO_2]^+$ (43.2 %), 120 $[M\text{-}C_{13}H_{10}ClSO_2]^+$ (1.5 %), 111 $[C_6H_4Cl]^+$ (35.7 %), 105 $[M\text{-}C_{13}H_{11}ClNSO_2]^{\bullet+}$ (34.5 %), 90 $[M\text{-}C_{14}H_{14}ClNSO_2]^+$ (24.6 %), 76 $[C_6H_4]^{\bullet+}$ (9.2 %), 75$[M\text{-}C_{15}H_{17}ClNSO_2]^{\bullet+}$ (31.9 %).

4.4 *Antibacterial activity*

The antibacterial activity method was based on the principle that microbial cell number or microbial growth was directly related to the log phase of growth with increase in absorbance of broth medium[19-20]. The clinically isolated two gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and four gram-negative (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi*) were stored on stock culture agar medium. 20 µg test samples with dilution by suited solvents and 180 µL overnight maintained fresh bacterial cultures with suited dilution with fresh nutrient broth were mixed. The initial absorbance was crucially between 0.12-0.19 at 540 nm. The incubation was processed at 37 ^oC for 16-24 hrs with lid on the micro plate. The absorbance was measured at 540 nm using micro plate reader before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\textbf{Inhibition } \% = \frac{\textbf{Control} - \textbf{Test}}{\textbf{Control}} \times \textbf{100}$$

where Control = Absorbance in control with bacterial culture
Test = Absorbance in test sample

Results are mean of triplicate (n=3, ± sem). Ciprofloxacin was taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software, and data was expressed as MIC.

4.5 *Lipoxygenase assay*

Lipoxygenase activity was assayed according to the reported method[21-23] 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 ^{0}C. 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.

4.6 *α-Chymotrypsin assay*

α-Chymotrypsin inhibition assay was carried out according to the reported method[24-26]. A total volume of 100 µL reaction mixture contained 60 µL of 50 mM Tris-HCl buffer (pH 7.6), 10 µL of 0.5 mM test compound and 15 µL (0.9 units) of enzyme (Sigma, USA) prepared in the above buffer. The contents were o mixed, preincubated for 15 min at 37 C and preread at 410 nm. The reaction was initiated by the addition of 15 µL of 1.3 mM substrate, N-succinyl phenylalanine-p-nitroanilide (Sigma,USA). Absorbance was measured at 410 nm using Synergy HT microplate reader after 30-60 min when absorbance values of uninhibited enzyme assay reached 0.7-0.9. The positive and negative controls were included. Chymostatin (0.5 mM well^{-1}) was used as a positive control. All experiments were carried out in triplicate. The percentage inhibition and IC_{50} values were calculated as mentioned above.

5. STATISTICAL ANALYSIS

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

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