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Enzyme Inhibition Studies on *N*-Substituted Sulfonamides Derived from *m*-phenetidine

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ABSTRACT
Organic synthesis of various compounds followed by biological activities is the going on methodology in the world for pharmacological evaluation. The undertaken research is the synthesis of *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide **(3)** through condensation reaction of *m*-phenetidine **(1)** and 4-methylbenzenesulfonyl chloride **(2)** using basic aqueous media of sodium carbonate. Further, the synthesized compound **3** was reacted with different alkyl/aralkyl halides **(4a-j)** using DMF as aprotic polar solvent and NaH as a base to yield **5a-j** compounds. The synthesized molecules were characterized from their spectral data. The synthesized compounds were evaluated against cholinesterase (AChE and BChE), lipoxygenase (LOX), urease, chymotrypsin and tyrosinase enzymes; and found to be the moderate inhibitor against tyrosinase enzyme.

Keywords: *m*-Phenetidine, 4-methylbenzenesulfonyl chloride, sulfonamide, anti-enzymatic activity.

1. INTRODUCTION
The molecules possessing sulfamoyl group are known as sulfonamides. This group is owned by a number of biologically active compounds[1,2]. The action of sulfonamides is in folic acid pathway is because of resemblance with PABA[3]. Sulfonamides find their application in biological systems such as, anticancer, anticonvulsant, antidiuretics, anti-inflammatory, carbonic anhydrase inhibitors and HIV protease inhibitors[4]. Because of bacteriostatic activity, these are utilized for the ailment of urinary-tract infection[5].

Tyrosinase (EC 1.14.18.1) performs multiple functions in humans. Tyrosinase overproduction causes hyperpigmentation, ocular retinitis pigmentosa; accelerates the induction of catecholamine quinone derivatives by its oxidase activity; over expression of tyrosinase results in increased intracellular dopamine contents in association with the formation of melanin pigments in neuronal stomata which causes apoptotic cell death. Tyrosinase constitutes the neuro-coloring pigments in human brain stimulating intropin toxicity as well as neuro-retrogression related to Parkinsonism. Undesirable browning of fruits due to enzymatic action & unusual darkening of skin in humans have pepped up the researchers to sought out potent tyrosinase enzyme inhibitors[6,7].

The literature survey has shown that the variation in structure of a molecule affects the biological activity[8-12]. This prompted us to inaugurate various *N*-substituted sulfonamides using *m*-phenetidine and their screening against different enzymes. The biological activity results showed these compounds as overall moderate active and so may be considerable in drug designing for the pharmacological industries.

2. RESULTS AND DISCUSSION

2.1 *Chemistry*
New *N*-alkyl/aralkyl derivatives of *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide were prepared accordingly the protocol outlined in scheme-1. The general procedures with conditions and spectral characterization are depicted in experimental section.

Our objective was to synthesize some new *N*-substituted sulfonamides and to find out their enzyme inhibition activity. We synthesized different sulfonamides in moderate to excellent yields. Parent compound *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide **(3)**, was prepared by the coupling of *m*-phenetidine (**1**) with 4-methylbenzenesulfonyl chloride (**2**) in an aqueous basic medium with pH control. Further, the compound **3** on reaction with various alkyl/aralkyl halides afforded a number of *N*-alkyl/aralkyl substituted compounds derived from *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (**5a-j)**, sketched in scheme-1. Synthesis of **5a-j** was progressed in polar aprotic solvent, DMF (*N*,*N*-dimethylformamide) and a weak base sodium hydride (NaH). After a stirring of 1-2 hours, reaction completion was accomplished. Ice cold water was added for the isolation of products which were filtered off but solvent extraction through chloroform/ethyl acetate was also used in some cases. Spectral data depicted in experimental section was the source of structure elucidation of the all synthesized molecules.

The parent compound **3** was obtained as a brown crystalline solid having melting point 182-184 $^{\circ}$C. The molecular formula $C_{15}H_{17}NO_3S$ was accomplished through EI-MS and integration of peaks in the ^{1}H-NMR spectrum. The IR spectrum showed absorption bands at 3625, 3078, 2909, 1425 and 1241 cm^{-1} for bond stretching of N-H, C-H (aromatic), C-H (alkyl group), C=C (aromatic) and -SO_2 respectively. In EI-MS, molecular ion peak at *m/z* 291 and

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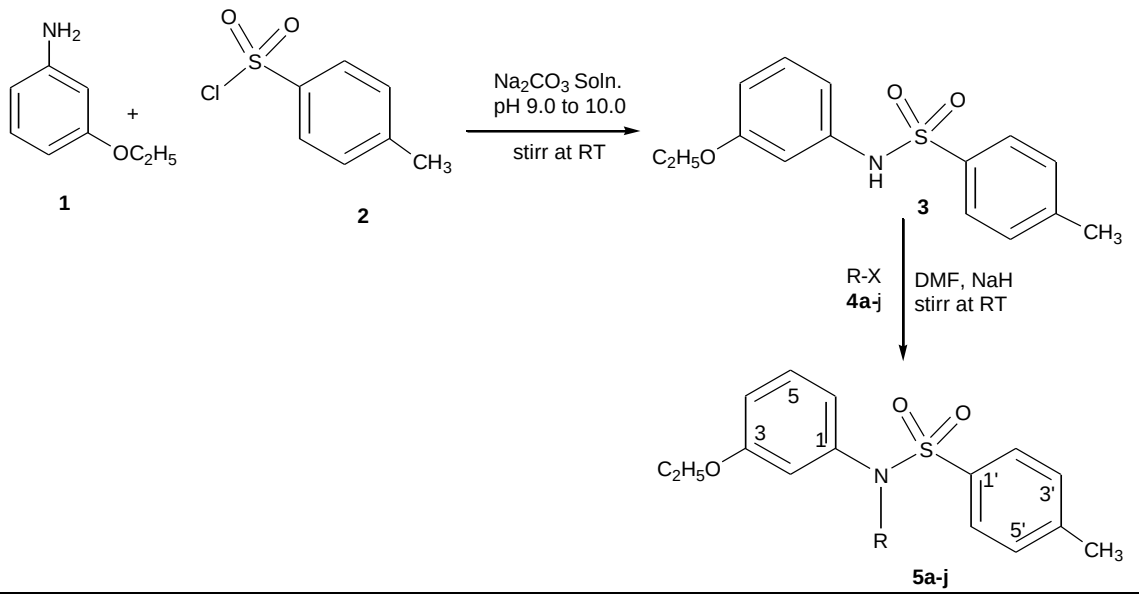
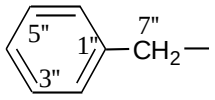
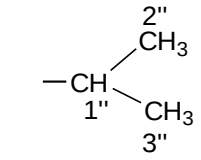
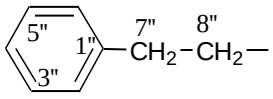
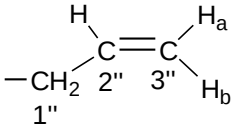
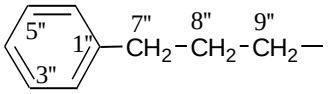


Compound	R	Compound	R
5a	—CH$_3$ (1")	**5f**	—CH$_2$—CH$_2$—CH$_2$—CH$_3$ (1" 2" 3" 4")
5b	—CH$_2$—CH$_3$ (1" 2")	**5g**	—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_3$ (1" 2" 3" 4" 5")
5c	—CH$_2$—CH$_2$—Br (1" 2")	**5h**	
5d		**5i**	
5e		**5j**	

Scheme-1: Synthetic scheme of *N*-substituted derivatives of *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide **(3)**

two distinct peaks at *m/z* 227 and 91 because of release of sulfonyl group and tropyllium ion respectively. In ^{1}H-NMR spectrum, signals resonating at δ 7.64 (d, J = 8.0 Hz, 2H, H-2' & H-6') and 7.21 (d, J = 8.0 Hz, 2H, H-3' & H-5') confirmed the presence of *p*-substituted toluenesulfonyl ring, the signals resonating at δ 7.07 (t, J = 8.0 Hz, 1H, H-5), 6.66 (s, 1H, H-2), 6.59 (dd, J = 8.4, 1.6 Hz, 1H, H-6) and 6.56 (d, J = 8.4 Hz, 1H, H-4) corroborated the *m*-substituted aniline ring, the two signals at δ 3.93 (q, J = 6.8 Hz, 2H, $CH_3\underline{CH_2}O$-3) and 1.34 (t, J = 6.9 Hz, 3H, $\underline{CH_3}CH_2O$-3) affirmed ethoxy group and a singlet at δ 2.35 (s, 3H, CH_3-4') resonated for the methyl group. All of these manifests assigned structure of **3** as *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide. The structure was also confirmed from its crystal data[13]. The mass fragmentation pattern of *N*-benzyl-*N*-(3-ethoxyphenyl)-4-methylbenzene sulfonamide **(5h)** was clearly outlined in fig-1. Likewise, all the synthesized compounds were well supported by spectral data depicted in experimental section.

2.2 *Enzyme inhibition activity*

The results of IC_{50} values of enzyme inhibition study of the synthesized compounds are presented in Table-I and Table-II. The most of the synthesized compounds showed inhibition potential against tyrosinase enzyme and a few were active against the remaining ones.

Against AChE enzyme, only two compounds **3** and **5f** were minimum active with IC_{50} values of 132.51±0.12 and 175.31±0.22 µM relative to the reference standard, eserine, with IC_{50} value of 0.04±0.0001 µM. Among the synthesized compounds, only **3** and **5j** were moderately active against BChE enzyme with IC_{50} values of 145.21±0.12 and 181.52±0.14 µM relative to eserine with IC_{50} value of 0.85±0.0001 µM. The compound **5g** showed IC_{50} value of 187.91±0.61 µM relative to baicalein with IC_{50} value of 22.4±1.3 µM.

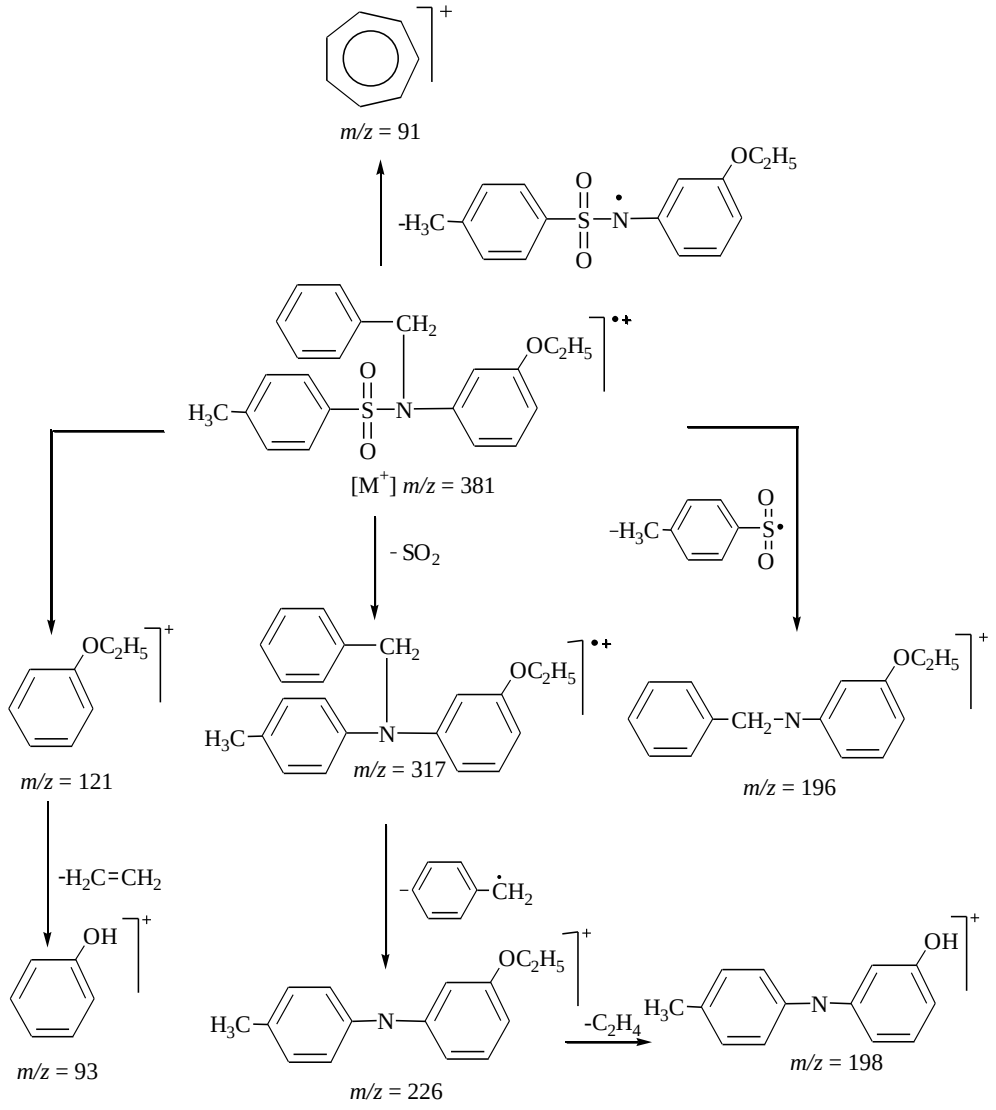


Fig-1: Mass Fragmentation pattern of *N*-benzyl-*N*-(3-ethoxyphenyl)-4-methylbenzene sulfonamide **(5h).**

Table-I: Enzyme inhibition against AChE, BChE and LOX

Compound	AChE		BChE		LOX	
	Inhibition (%) at 0.5 mM	IC_{50} µM	Inhibition (%) at 0.5 mM	IC_{50} µM	Inhibition (%) at 0.5 mM	IC_{50} µM
3	78.85±0.22	132.51±0.12	77.09±0.32	145.21±0.12	40.12±0.25	-
5b	47.34±0.18	-	55.69±0.11	>400	50.87±0.17	>400
5d	48.08±0.34	-	31.44±0.33	-	43.46±0.38	>400
5f	72.78±0.18	175.31±0.22	35.35±0.11	-	12.94±0.16	-
5g	19.97±0.35	-	49.67±0.36	-	65.84±0.82	187.91±0.61
5h	29.59±0.19	-	40.47±0.15	-	48.26±0.63	-
5i	38.91±0.25	-	37.29±0.35	-	46.95±0.82	-
5c	14.51±0.23	-	38.81±0.22	-	46.81±0.16	-
5j	26.33±0.11	-	72.74±0.54	181.52±0.14	22.97±0.42	-
5e	26.04±0.52	-	50.17±0.56	>300	53.92±0.88	>300
Control	**Eserine 91.29±1.17**	**0.04±0.0001**	**Eserine 82.82±1.09**	**0.85±0.0001**	**Baicalein 93.79±1.27**	**22.4±1.3**

Note: AChE = Acetylcholinesterase, BChE = Butyrylcholinesterase, LOX = Lipoxygenase.

The synthesized compounds **5h** and **5j** showed IC_{50} values of 229.12±0.16 and 24.4±0.19 µM relative to thiourea with IC_{50} value of 21.50±0.22 µM against the urease enzyme. The compound **5j** showed good activity because of presence of unsubstituted aralkyl group with long aliphatic chain. This long aliphatic chain increases the chances of the π-π interactions of the aromatic ring. The enzyme Chymotrypsin was inhibited by **5b**, **5d**, **5f** and **5g** with IC_{50} values of 63.2±0.12, 91.0±0.19, 94.5±0.21 & 108.2±0.05 µM with respect to Chymostatin having IC_{50} value of 8.24±0.11 µM. The synthesized compounds, **3**, **5b**, **5d**, **5f**, **5h** and **5j** showed inhibition potential against tyrosinase enzyme relative to kojic acid with IC_{50} value of 6.04±0.11 µM. Among these six compounds **3** and **5b** showed the relatively better

inhibition potential with IC_{50} values of 52.84±0.12 and 26.61±0.14 µM respectively relative to reference standard. An overview shows that this series of synthesized compounds showed moderately low activity against the series of enzymes used for the biological evaluation.

Table-II: Enzyme inhibition against urease, chymotripsin and tyrosinase

Compound	Anti-urease activity		Anti-chymotrypsin activity		Anti-tyrosinase activity	
	Inhibition (%) at 0.5 mM	**IC_{50} µM**	**Inhibition (%) at 0.5 mM**	**IC_{50} µM**	**Inhibition (%) at 0.5 mM**	**IC_{50} µM**
3	34.64±0.66	>500	30.74±0.51	>500	89.54±0.26	52.84±0.12
5b	20.39±0.21	-	82.38±0.30	63.2±0.12	98.12±0.21	26.61±0.14
5d	27.40±0.52	-	78.34±0.82	91.0±0.19	79.96±0.52	146.71±0.11
5f	23.57±0.31	-	76.65±0.78	94.5±0.21	76.60±0.31	141.88±0.22
5g	55.67±0.41	>300	83.44±0.39	108.2±0.05	56.91±0.41	>300
5h	62.59±0.63	229.12±0.16	33.46±0.21	>500	78.01±0.63	142.88±0.16
5i	54.61±0.76	>300	14.79±0.34	-	57.27±0.76	>300
5c	29.70±0.51	-	15.56±0.95	-	57.62±0.51	>300
5j	89.92±0.32	24.4±0.19	21.07±1.12	-	66.31±0.12	169.14±0.14
5e	3.75±0.41	-	33.07±0.62	>500	50.21±0.41	>300
Control	**98.61±0.77**	**21.50±0.22**	**92.60±0.96**	**8.24±0.11**	**93.50±0.91**	**6.04±0.11**

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).

3. CONCLUSION

The synthesized molecules are corroborated by spectral data. The evaluation of enzyme inhibition activity (Table-1 and II), it is obvious that these compounds possess sulfamyl group and was considered to be active against different enzymes. This evaluation of enzyme inhibition showed that the synthesized compounds remained relatively less active against the six enzymes taken into account for the study.

4. MATERIALS AND METHODS

4.1 *General*

Melting points of solids (powdered or crystalline) were recorded on a Griffin-George melting point apparatus by open capillary tube. Purity of the compounds was checked by thin layer chromatography (TLC) using EtOAc and *n*-hexane (solvent system) on aluminum sheets precoated with silica gel 60 F_{254} under UV at 254 and also ceric sulfate solution (with heating). The I.R. spectra were recorded in potassium bromide pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometers operating at 300 & 400 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-Methylbenzenesulfonyl chloride, *m*-phenetidine and alkyl/aralkyl halides were purchased from Merck and Alfa Aeser and solvents of analytical grade through local suppliers.

4.2 *Procedure for the synthesis of N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (3):*

m-Phenetidine (1.0 mmol: **l**) was dispersed in 100 mL water in a RB flask and the pH 9.0-10.0 was strictly maintained by using basic aqueous solution of Na_2CO_3 (10%). Then, 4-methylbenzenesulfonyl chloride (1.0 mmol; **2**) was added gradually in parts. The reaction mixture was stirred and monitored with TLC (*n*-hexane: EtOAc; 70:30) till the end of reaction. Dilute HCl (2.0-3.0 mL) was poured slowly along with shaking till the pH to 2.0-3.0. The reaction mixture was kept on RT for 5.0-10.0 minutes; brown crystalline precipitates were filtered, washed with distilled water and dried off to afford the product **3**. Brown crystalline solid; Yield: 62%; M.P.: 182-184 ^oC; Mol. Formula: $C_{15}H_{17}NO_3S$; Mol. Weight: 291; IR (KBr, cm^{-1}) ν_{max}: 3625 (N-H stretching), 3078 (C-H str. of aromatic ring), 2909 (C-H str. of alkyl group), 1425 (C=C aromatic str.), 1241 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.64 (d, J = 8.0 Hz, 2H, H-2' & H-6'), 7.21 (d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.07 (t, J = 8.0 Hz, 1H, H-5), 6.66 (s, 1H, H-2), 6.59 (dd, J = 8.4, 1.6 Hz, 1H, H-6), 6.56 (d, J = 8.4 Hz, 1H, H-4), 3.93 (q, J = 6.8 Hz, 2H, $CH_3\underline{CH_2}O$-3), 2.35 (s, 3H, CH_3-4'), 1.34 (t, J = 6.9 Hz, 3H, $\underline{CH_3}CH_2O$-3); EIMS (*m/z*): 291 $[M^+]$, 227 $[M\text{-}SO_2]^+$, 199 $[C_{13}H_{13}NO]^+$, 108 $[C_6H_6NO]^+$, 91 $[C_7H_7]^+$.

4.3 *General procedure for the synthesis of N-alkyl/aralkyl substituted-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5a-j):*

Compound **3** (0.56 mol, 0.20 g) was dissolved in DMF (5.0-10.0 mL) in a round bottom flask followed by sodium hydride (0.01g, 0.42 mmol) at RT. The reaction mixture was kept on stirring for 15-30 min and then the corresponding electrophiles (**4a-j**; 0.56 mol) were added into the mixture. The reaction mixture was further stirred along with monitoring through TLC. After complete reaction, 200 mL cold water was added. The received precipitates were

filtered, washed with water and dried off to yield the *N*-substituted derivatives of *N*-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (**5a-j**).

4.3.1 ***N-Methyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5a):***
Oily liquid; Yield: 80%; Mol. Formula: $C_{16}H_{19}NO_3S$; Mol. Weight: 305; IR (KBr, cm^{-1}) ν_{max}: 3072 (C-H str. of aromatic ring), 2906 (C-H str. of alkyl group), 1429 (C=C aromatic str.), 1239 (S=O str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 7.43 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.21 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.13 (t, *J* = 8.1 Hz, 1H, H-5), 6.77 (dd, *J* = 8.4, 2.4 Hz, 1H, H-6), 6.69 (t, *J* = 2.4 Hz, 1H, H-2), 6.59 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 3.96 (q, *J* = 6.9 Hz, 2H, $CH_3\underline{CH_2}O$-3), 3.12 (s, 3H, CH_3-1"), 2.39 (s, 3H, CH_3-4'), 1.36 (t, *J* = 6.9 Hz, 3H, $\underline{CH_3}CH_2O$-3); EIMS (*m/z*): 305 $[M]^+$, 241 $[M\text{-}SO_2]^+$, 226 $[C_{15}H_{16}NO]^+$, 122 $[C_7H_8NO]^+$, 91 $[C_7H_7]^+$.

4.3.2 ***N-Ethyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5b):***
Light yellow oily liquid; Yield: 60%; Mol. Formula: $C_{17}H_{21}NO_3S$; Mol. Weight: 319; IR (KBr, cm^{-1}) ν_{max}: 3069 (C-H str. of aromatic ring), 2912 (C-H str. of alkyl group), 1434 (C=C aromatic str.), 1228 (S=O str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 7.48 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.22 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.15 (t, *J* = 8.1 Hz, 1H, H-5), 6.80 (dd, *J* = 8.4, 2.4 Hz, 1H, H-6), 6.62 (t, *J* = 2.4 Hz, 1H, H-2), 6.53 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 3.94 (q, *J* = 6.9 Hz, 2H, $CH_3\underline{CH_2}O$-3), 3.54 (q, *J* = 6.6 Hz, 2H, H-1"), 2.39 (s, 3H, CH_3-4'), 1.36 (t, *J* = 6.9 Hz, 3H, $\underline{CH_3}CH_2O$-3), 1.04 (t, *J* = 6.6 Hz, 3H, CH_3-2"); EIMS (*m/z*): 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 227 $[C_{15}H_{17}NO]^+$, 164 $[C_{10}H_{14}NO]^+$, 136 $[C_8H_{18}NO]^+$, 91 $[C_7H_7]^+$.

4.3.3 ***N-(2-Bromoethyl)-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5c):***
Pinkish white sticky solid; Yield: 76%; Mol. Formula: $C_{17}H_{20}BrNO_3S$; Mol. Weight: 397; IR (KBr, cm^{-1}) ν_{max}: 3077 (C-H str. of aromatic ring), 2915 (C-H str. of alkyl group), 1431 (C=C aromatic str.), 1226 (S=O str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 7.49 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.23 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.16 (t, *J* = 8.0 Hz, 1H, H-5), 6.82 (dd, *J* = 8.4, 2.4 Hz, 1H, H-6), 6.64 (t, *J* = 2.4 Hz, 1H, H-2), 6.54 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 3.94 (q, *J* = 6.8 Hz, 2H, $CH_3\underline{CH_2}O$-3), 3.83 (t, *J* = 7.2 Hz, 2H, H-1"), 3.36 (t, *J* = 7.2 Hz, 2H, H-2"), 2.40 (s, 3H, CH_3-4'), 1.36 (t, *J* = 6.8 Hz, 3H, $\underline{CH_3}CH_2O$-3); EIMS (*m/z*): 399 $[M+2]^+$, 397 $[M]^+$, 333 $[M\text{-}SO_2]^+$, 306 $[C_{15}H_{16}BrNO]^+$, 243 $[C_{10}H_{13}BrNO]^+$, 215 $[C_8H_9BrNO]^+$, 91 $[C_7H_7]^+$.

4.3.4 ***N-(Iso-propyl)-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5d):***
Light pink amorphous solid; Yield: 60%; M.P.: 80 ^oC; Mol. Formula: $C_{18}H_{23}NO_3S$; Mol. Weight: 333; IR (KBr, cm^{-1}) ν_{max}: 3025 (C-H aromatic str.), 2902 (alkyl group C-H str.), 1562 (C=C aromatic str.), 1395 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.62 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.23 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.18 (t, *J* = 8.1 Hz, 1H, H-5), 6.87 (dd, *J* = 8.4, 2.4 Hz, 1H, H-6), 6.58 (t, *J* = 2.4 Hz, 1H, H-2), 6.56 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 4.54 (sep, *J* = 6.6 Hz, 1H, H-1"), 3.94 (q, *J* = 6.9 Hz, 2H, $CH_3\underline{CH_2}O$-3), 2.40 (s, 3H, CH_3-4'), 1.36 (t, *J* = 6.9 Hz, 3H, $\underline{CH_3}CH_2O$-3); 1.03 (d, *J* = 6.6 Hz, 6H, CH_3-2" & CH_3-3"); EIMS (*m/z*): 333 $[M]^+$, 269 $[M\text{-}SO_2]^+$, 241 $[C_{16}H_{19}NO]^+$, 178 $[C_{11}H_{16}NO]^+$, 91 $[C_7H_7]^+$.

4.3.5 ***N-Allyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5e):***
Yellowish brown sticky solid; Yield: 72%; Mol. Formula: $C_{18}H_{21}NO_3S$; Mol. Weight: 331; IR (KBr, cm^{-1}) ν_{max}: 3069 (C-H str. of aromatic ring), 2921 (C-H str. of alkyl group), 1644 (C=C aliphatic str.), 1428 (C=C aromatic stretching), 1223 (S=O str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 7.49 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.23 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.13 (t, *J* = 8.0 Hz, 1H, H-5), 6.78 (dd, *J* = 8.4, 2.0 Hz, 1H, H-6), 6.62 (br.s, 1H, H-2), 6.54 (d, *J* = 8.0 Hz, 1H, H-4), 5.75-5.68 (m, 2H, H-2"), 5.17 (dd, *J* =17.0, 1.2 Hz, 1H, H_b-3"), 5.13 (dd, *J* = 10.0, 1.2 Hz, 1H, H_a-3"), 4.12 (d, *J* = 6.4 Hz, 2H, H-1"), 3.93 (q, *J* = 7.2 Hz, 2H, $CH_3\underline{CH_2}O$-3), 2.40 (s, 3H, CH_3-4'), 1.35 (t, *J* = 6.8 Hz, 3H, $\underline{CH_3}CH_2O$-3); EIMS (*m/z*): 331 $[M]^+$, 267 $[M\text{-}SO_2]^+$, 239 $[C_{16}H_{17}NO]^+$, 176 $[C_{11}H_{14}NO]^+$, 148 $[C_9H_{10}NO]^+$, 91 $[C_7H_7]^+$.

4.3.6 ***N-Butyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5f):***
Yellow sticky solid; Yield: 67%; Mol. Formula: $C_{19}H_{25}NO_3S$; Mol. Weight: 347; IR (KBr, cm^{-1}) ν_{max}: 3068 (C-H str. of aromatic ring), 2911 (C-H str. of alkyl group), 1427 (C=C aromatic str.), 1219 (S=O str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.21 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.14 (t, *J* = 8.0 Hz, 1H, H-5), 6.79 (dd, *J* = 8.4, 2.0 Hz, 1H, H-6), 6.61 (br.s, 1H, H-2), 6.54 (d, *J* = 8.4 Hz, 1H, H-4), 3.94 (q, *J* = 6.8 Hz, 2H, $CH_3\underline{CH_2}O$-3), 3.46 (t, *J* = 7.2 Hz, 2H, H-1"), 2.39 (s, 3H, CH_3-4'), 1.34-1.29 (m, 4H, H-2" & H-3"), 1.36 (t, *J* = 6.8 Hz, 3H, $\underline{CH_3}CH_2O$-3), 0.83 (t, *J* = 7.2 Hz, 3H, H-4"); EIMS (*m/z*): 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 255 $[C_{17}H_{21}NO]^+$, 192 $[C_{12}H_{18}NO]^+$, 164 $[C_{10}H_{14}NO]^+$, 91 $[C_7H_7]^+$.

4.3.7 ***N-Pentyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5g):***
Light yellow amorphous solid; Yield: 57%; M.P.: 70 ^{o}C; Mol. Formula: $C_{20}H_{27}NO_3S$; Mol. Weight: 361; IR (KBr, cm^{-1}) ν_{max}: 3064 (C-H str. of aromatic ring), 2909 (C-H str. of alkyl group), 1423 (C=C aromatic str.), 1217 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.21 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.14 (t, *J* = 8.0 Hz, 1H, H-5), 6.79 (dd, *J* = 8.4, 2.0 Hz, 1H, H-6), 6.61 (t, *J* = 2.4 Hz, 1H, H-2), 6.54 (dd, *J* = 8.4, 2.0 Hz, 1H, H-4), 3.94 (q, *J* = 6.8 Hz, 2H, $CH_3\underline{CH_2}$O-3), 3.45 (t, *J* = 7.2 Hz, 2H, H-1"), 2.39 (s, 3H, CH_3-4'), 1.36 (t, *J* = 6.8 Hz, 3H, $\underline{CH_3}CH_2$O-3), 1.31-1.19 (m, 4H, H-2" to H-4"), 0.81 (t, *J* = 7.2 Hz, 3H, H-5"); EIMS (*m/z*): 361 $[M]^+$, 297 $[M\text{-}SO_2]^+$, 267 $[C_{18}H_{23}NO]^+$, 206 $[C_{13}H_{20}NO]^+$, 176 $[C_{11}H_{16}NO]^+$, 91 $[C_7H_7]^+$.

4.3.8 ***N-Benzyl-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5h):***
White amorphous solid; Yield: 75%; M.P.: 90 ^{o}C; Mol. Formula: $C_{22}H_{23}NO_3S$; Mol. Weight: 381; IR (KBr, cm^{-1}) ν_{max}: 3073 (C-H str. of aromatic ring), 2912 (C-H str. of alkyl group), 1429 (C=C aromatic str.), 1218 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.54 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.26 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.21-7.17 (m, 5H, H-2" to H-6"), 7.05 (t, *J* = 8.1 Hz, 1H, H-5), 6.70 (dd, *J* = 8.1, 2.4 Hz, 1H, H-6), 6.54 (t, *J* = 2.4 Hz, 1H, H-2), 6.50 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 4.67 (s, 2H, H-7"), 3.85 (q, *J* = 6.9 Hz, 2H, $CH_3\underline{CH_2}$O-3), 2.42 (s, 3H, CH_3-4'), 1.30 (t, *J* = 6.9 Hz, 3H, $\underline{CH_3}CH_2$O-3); EIMS (*m/z*): 381 $[M]^+$, 317 $[M\text{-}SO_2]^+$, 226 $[C_{15}H_{16}NO]^+$, 198 $[C_{13}H_{12}NO]^+$, 121 $[C_8H_9O]^+$, 91 $[C_7H_7]^+$.

4.3.9 ***N-(2-Phenylethyl)-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5i):***
White amorphous solid; Yield: 60%; Mol. Formula: $C_{23}H_{25}NO_3S$; Mol. Weight: 395; IR (KBr, cm^{-1}) ν_{max}: 3071 (C-H str. of aromatic ring), 2915 (C-H str. of alkyl group), 1424 (C=C aromatic str.), 1221 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.44 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.22 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.20-7.16 (m, 5H, H-2" to H-6"), 7.10 (t, *J* = 8.1 Hz, 1H, H-5), 6.82 (dd, *J* = 8.1, 2.4 Hz, 1H, H-6), 6.62 (t, *J* = 2.4 Hz, 1H, H-2), 6.55 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 3.94 (q, *J* = 7.2 Hz, 2H, $CH_3\underline{CH_2}$O-3), 3.71 (s, 2H, H-8"), 2.76 (s, 2H, H-7"), 2.38 (s, 3H, CH_3-4'), 1.37 (t, *J* = 6.9 Hz, 3H, $\underline{CH_3}CH_2$O-3); EIMS (*m/z*): 395 $[M]^+$, 331 $[M\text{-}SO_2]^+$, 303 $[C_{21}H_{21}NO]^+$, 240 $[C_{16}H_{18}NO]^+$, 212 $[C_{14}H_{14}NO]^+$, 91 $[C_7H_7]^+$.

4.3.10 ***N-(3-Phenylpropyl)-N-(3-ethoxyphenyl)-4-methylbenzenesulfonamide (5j):***
Sticky solid; Yield: 72%; Mol. Formula: $C_{24}H_{27}NO_3S$; Mol. Weight: 409; IR (KBr, cm^{-1}) ν_{max}: 3069 (C-H str. of aromatic ring), 2918 (C-H str. of alkyl group), 1432 (C=C aromatic str.), 1226 (S=O str); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 7.64 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.20 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.19-7.14 (m, 5H, H-2" to H-6"), 7.07 (t, *J* = 8.4 Hz, 1H, H-5), 6.81 (dd, *J* = 8.4, 2.4 Hz, 1H, H-6), 6.66 (t, *J* = 2.4 Hz, 1H, H-2), 6.54 (dd, *J* = 8.8, 2.0 Hz, 1H, H-4), 3.93 (q, *J* = 6.8 Hz, 2H, $CH_3\underline{CH_2}$O-3), 3.56 (t, *J* = 6.8 Hz, 2H, H-9"), 3.31 (t, *J* = 6.8 Hz, 2H, H-7"), 2.40 (s, 3H, CH_3-4'), 1.95 (qui, *J* = 6.8 Hz, 2H, H-8"), 1.34 (t, *J* = 6.8 Hz, 3H, $CH_3\underline{CH_2}$O-3); EIMS (*m/z*): 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 317 $[C_{22}H_{23}NO]^+$, 254 $[C_{17}H_{20}NO]^+$, 226 $[C_{15}H_{16}NO]^+$, 91 $[C_7H_7]^+$.

5. ENZYME INHIBITION ASSAYS

All the enzyme inhibition assays were carried out according to the reported methods[14-19].

5.1 *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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