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Synthesis and Screening of Some New *N*-Substituted Derivatives of *N*-(4-Methylpyridin-2-yl)benzenesulfonamides as Potential Antibacterial Agents

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ABSTRACT
The two step synthesis of a series of *N*-substituted derivatives of *N*-(4-Methylpyridin-2-yl)benzenesulfonamide with potential antibacterial activity, has been reported. First step includes the synthesis of *N*-(4-Methylpyridin-2-yl)benzenesulfonamide (**3**) by reaction of 2-Amino-4-methylpyridine (**1**) and Benzenesulfonyl chloride (**2**) in a slightly basic aqueous medium. The molecule **3** was converted to *N*-Alkyl/aralkyl-*N*-(4-methylpyridin-2-yl)benzenesulfonamide derivatives, **5a-f**, on treatment with alkyl/aralkyl halides, **4a-f**, using lithium hydride as activator in *N*,*N*-dimethylformamide. The synthesized molecules were well corroborated by ^{1}H-NMR, IR and EI-MS spectral data and evaluated for antibacterial activity against four gram-negative and two gram-positive bacteria. The evaluation results rendered these compounds as moderately good inhibitors and may be employed as therapeutic agent for certain inflammatory ailments.

Keywords: 2-Amino-4-methylpyridine, Antibacterial activity, Sulfonamides, ^{1}H-NMR and EI-MS.

1. INTRODUCTION
The sulfonamides (-SO_2-NH-) have great importance in the medicinal chemistry[1], with different biological activities[2-4]. The compounds of this class possess the anti-bacterial[5], hypoglycemic[6], diuretic[7], anti-carbonic anhydrase, anti-thyroid (*in vitro* and *vivo*), anti-inflammatory[8,9], anti-cancer, anti-hypertensive[10] and anti-convulsing activities along with potential herbicidal properties for agricultural applications[11]. Although large efforts have been attempted for the development of new methodologies for sulfonamide synthesis yet the conventional preparation by stirring of amino compounds and sulfonyl halides is still employed because of reaction simplicity and high reactivity[12]. Recently water has attracted considerable attention as a desired solvent for chemical reactions because of cost, safety and environmental benefits. Environmentally benign sulfonamides have been synthesized at room temperature in water under pH control with Na_2CO_3[13,14]. The action of sulfonamides is based on their structural resemblance with 4-Aminobenzoic acid (PABA). This structural feature inhibits the formation of folic acid and hence the production of purines for DNA[15,16].

The search for new efficient methodologies for synthesis of sulfonamides under mild conditions is under consideration by organic chemists. With reference of our ongoing research projects[17,18] in search of new therapeutic agents with low toxicity and high potential, we have reported here the facile synthesis of various *N*-alkyl/aralkyl substituted sulfonamides using 2-Amino-4-methylpyridine, a heterocyclic compound, as precursor and also their antibacterial activity. The heterocyclic compounds have been known to possess a broad spectrum of biological activities such as heterocyclic sulfonamide[18,19] and 1,3,4-Oxadiazole[20]. All the synthesized molecules remained moderately good inhibitors as evident from their MIC (Minimum Inhibitory Concentration) values, discussed in results and discussion section.

2. RESULTS AND DISCUSSION
The benign synthesis of *N*-(4-Methylpyridin-2-yl)benzenesulfonamide (**3**) and its *N*-substituted derivatives was reported (scheme-1) with an aim to inaugurate some new potential antibacterial molecules. The results rendered these molecules better antibacterial inhibitors and hence showed that the minute changes in the structure had a great effect on biological properties of molecules. The reaction conditions and reagents are depicted in experimental section.

2.1 *Chemistry*
The parent molecule, *N*-(4-Methylpyridin-2-yl)benzenesulfonamide (**3**) was synthesized by gearing up 2-Amino-4-methylpyridine (**1**) with Benzenesulfonyl chloride (**2**) at room temperature in an aqueous medium. The excellent yield of product was accomplished by continuous stirring for 3-4 hours. The precipitated product was collected by filtration after the addition of the hydrochloric acid. The acidic medium is necessary at the time of filtration to bounce back the salt form of sulfonamide into acidic. Although acid is mandatory yet excess of it should be avoided because of decrement in the yield. The parent product **3** was utilized to synthesize a series of *N*-Alkyl/aralkyl-*N*-(4-methylpyridin-2-yl)benzenesulfonamide, **5a-f**, by its reaction with alkyl/aralkyl halides in a polar aprotic solvent using LiH as an activator. The final products were obtained through solvent extraction or filtration after addition of a weak base. The base was added to wash out the unreacted parent sulfonamide as salt.

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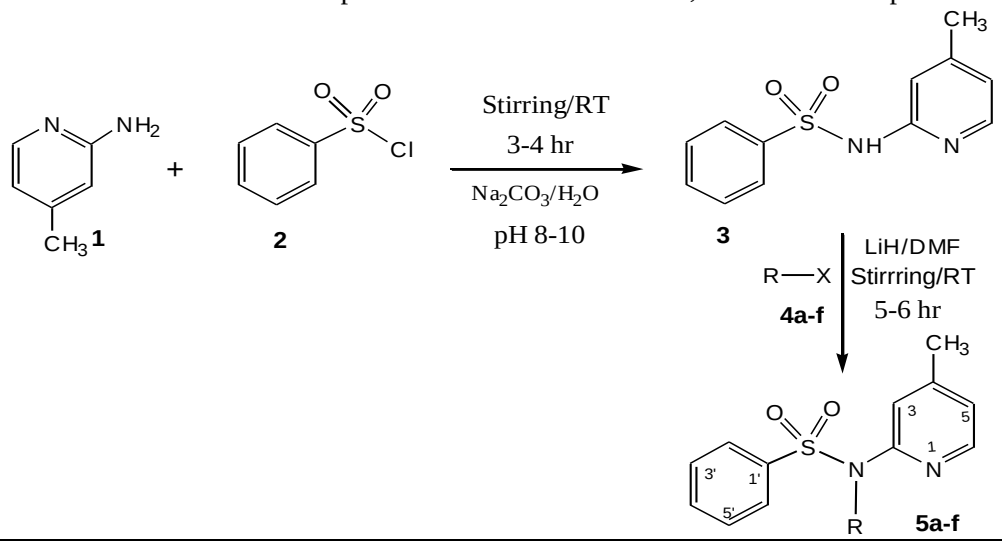
The sulfonamide **3** was obtained as white amorphous powder with 86% yield and melting point of 150-152°C. The EI-MS showed the $[M]^+$ ion peak at *m/z* 248 corresponding to the molecular formula, $C_{12}H_{12}N_2O_2S$. The molecular formula was also supported by counting the number of protons in its ^{1}H-NMR spectrum. One *ortho/meta* coupled doublet of doublets (dd pattern) appearing in its ^{1}H-NMR spectrum at δ 7.94 ppm (for H-2' & H-6'), one *ortho* coupled triplet at δ 7.55 ppm (for H-3' & H-5') and a multiplet resonating in the range of δ 7.80-7.83 ppm (for H-4') were the characteristics signals of benzenesulfonyl moiety. The three signals in aromatic region appearing as singlet at δ 7.14 ppm (for H-3), doublet at δ 7.09 ppm (for H-5) and doublet at δ 8.01 ppm (for H-6) confirmed the presence of 2,4-disubstituted pyridine ring. In aliphatic region, a singlet at δ 2.40 ppm (CH_3-4) was due to methyl protons attached at 4th position of pyridine ring. The IR and EI-MS data, expressed in experimental section, of this molecule well supported the above inferred structural units. On the basis of these collective evidences, the structure of **3** was assigned as *N*-(4-Methylpyridin-2-yl)benzenesulfonamide. The mass fragmentation pattern of *N*-(4-Bromobenzyl)-*N*-(4-methylpyridin-2-yl)benzenesulfonamide (**5d**) was sketched in figure 1. Likewise the structures of other synthesized molecules were corroborated on the basis of spectral evidences of ^{1}H-NMR, IR and EI-MS spectra.



Compd.	-R	Compd.	-R
5a	—CH$_2$—CH$_3$ (1", 2")	**5d**	7", H$_2$C, 1", 3", 5", Br
5b	—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_3$ (1"–5")	**5e**	7", H$_2$C, H$_2$C, 8", 1", 3", 5"
5c	—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_3$ (1"–7")	**5f**	9", H$_2$C, H$_2$C, 7", H$_2$C, 8", 1", 3", 5"

Scheme-1: Synthesis of *N*-Alkyl/aralkyl-*N*-(4-methylpyridin-2-yl)benzenesulfonamide, **5a-f**

2.2 *Antibacterial activity (In vitro)*

The screening of the parent compound **3** and the synthesized derivatives **5a-f** against gram-negative and gram-positive bacteria demonstrated that all showed better activity against both, as evident from the MIC values (table 2). Among these molecules, *N*-Heptyl-*N*-(4-methylpyridin-2-yl)benzenesulfonamide (**5c**) was found to be the most active inhibitor against all the gram negative and gram positive bacterial strains, showing almost the same activity as that of the reference standard, ciprofloxacin. The better inhibitory action of this molecule was the most probably due to the presence of long straight chain aliphatic group in the parent compound. It was further assessed from the MIC values that substitution of long aliphatic chains on this nucleus would be beneficial for the pharmaceutical industries regarding antibacterial activity. All the compounds executed almost the 50% inhibitory action against all the bacterial strains with some exceptions, relative to the reference standard. *K. pneumonae* was inhibited by all the molecules up to same extent as that by the reference standard. The %age inhibition and MIC values of the other synthesized molecules relative to ciprofloxacin are shown in table 1 & 2.

3. CONCLUSION

A series of molecules were synthesized in better yields in a facile and benign way to evaluate their antibacterial activity. All the synthesized molecules of this series were assessed for their antibacterial activity and found to be

moderately better inhibitors relative to the reference standard, ciprofloxacin. The purpose of evaluation was to introduce new molecules being better inhibitors than the existing drugs. The compound **5c** with long aliphatic chain remained the better inhibitor because of long straight chain aliphatic group present in the molecule. Thus it may be concluded that straight chain long aliphatic groups linked at sulfamoyl nitrogen of this synthesized nucleus are better inhibitors of bacterial strains. This may assist the drug industries for further research on these compounds to evaluate their toxicity and affectivity to other microbes.

Table 1: %age inhibition values of antibacterial activity of *N*-substituted derivatives of **3**

Compound	%age inhibition					
	S. typhi (-)	***E. coli (-)***	***K. pneumonae (-)***	***P. aeroginosa (-)***	***B. subtilis (+)***	***S. aureus (+)***
3	62.89±2.58	72.33±1.33	67.33±1.65	71.30±2.22	67.06±1.39	77.09±0.45
5a	55.67±3.77	66.39±0.50	65.06±2.33	60.00±1.99	62.27±2.68	76.50±0.50
5b	53.25±3.45	59.94±3.28	60.91±3.77	54.57±0.87	59.69±0.10	70.68±3.14
5c	75.10±3.04	69.78±1.00	77.73±4.00	76.90±0.92	63.87±0.15	80.95±0.23
5d	56.86±2.32	52.39±0.61	51.88±1.31	60.33±0.87	54.74±1.34	68.36±2.82
5e	54.69±0.67	61.94±3.28	58.07±4.43	57.01±3.60	59.74±1.29	68.95±3.50
5f	66.19±3.51	61.17±1.17	57.84±0.23	58.86±2.60	61.91±2.11	71.14±1.86
Ciprofloxacin	**89.71±1.43**	**88.76±1.94**	**91.32±0.54**	**88.95±2.05**	**90.10±0.77**	**90.00±1.23**

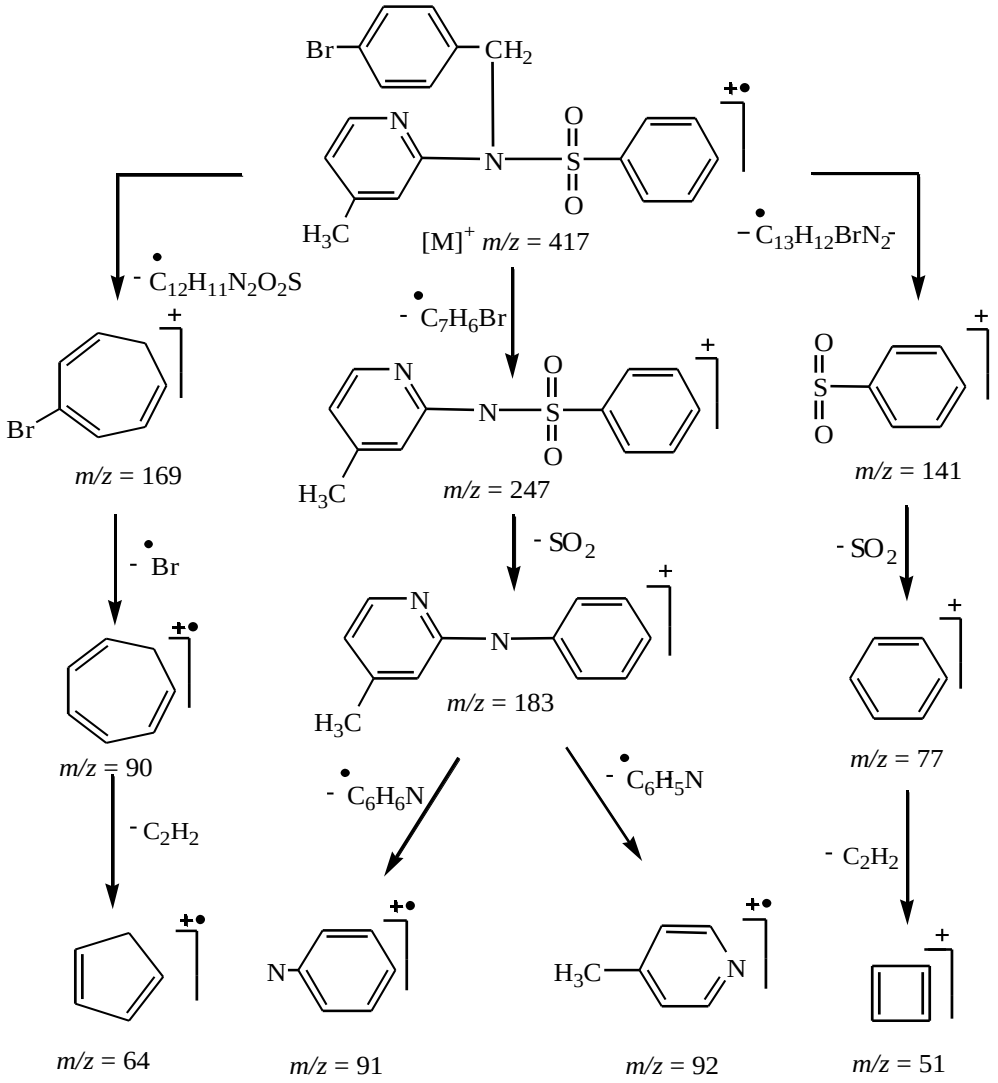


Fig-1: Mass fragmentation pattern of *N*-(4-Bromobenzyl)-*N*-(4-Methylpyridin-2-yl)benzenesulfonamide (**5d**)

4. EXPERIMENTAL WORK

4.1 *General*

TLC (Thin Layer Chromatography) for purity of compounds (using ethyl acetate and *n*-hexane) was developed on the pre-coated silica gel G-25-UV_{254} plates with visualization under 254 nm, and by ceric sulphate reagent. The melting

points were recorded on a Griffin & George melting point apparatus by open capillary tube and were uncorrected. ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker spectrometer using methanol-d_1. Chemical shifts are given on the δ-scale with TMS as internal reference. The abbreviations used in ^{1}H-NMR spectral interpretation are s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, qui = quintet and m = multiplet. The IR spectra were recorded in KBr pellet on a Jasco-320-A spectrophotometer. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system.

Table 2: MIC values of antibacterial activity of *N*-substituted derivatives of **3**

Compound	MIC (µg/mL)					
	S. typhi (-)	***E. coli (-)***	***K. pneumonae (-)***	***P. aeroginosa (-)***	***B. subtilis (+)***	***S. aureus (+)***
3	14.92±3.02	15.99±0.14	11.72±2.68	14.99±1.81	12.08±3.15	14.06±1.17
5a	17.23±2.08	15.30±0.29	11.25±3.32	17.68±1.63	12.06±3.05	10.03±2.70
5b	18.43±0.88	16.98±2.43	11.52±0.68	18.95±1.16	12.77±2.00	14.77±4.50
5c	10.85±1.05	11.45±3.56	9.24±2.87	11.56±2.98	11.06±1.04	10.25±2.16
5d	17.13±4.47	19.15±1.21	14.80±1.21	17.72±3.05	11.59±1.21	13.87±4.00
5e	18.15±1.15	17.30±3.80	14.81±3.85	18.46±3.00	12.40±1.17	13.68±1.62
5f	16.38±0.92	17.16±2.85	15.26±2.17	18.37±3.13	13.44±2.40	16.45±3.65
Ciprofloxacin	**9.66±1.08**	**9.27±0.58**	**8.34±1.50**	**9.61±2.08**	**9.61±2.08**	**9.20±2.31**

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

4.2 *Procedure for the synthesis of N-(4-Methylpyridin-2-yl)benzenesulfonamide (3)*

2-Amino-4-methylpyridine (**1**; 0.02 mol) was suspended in 50 mL distilled water in a 250 mL round bottom flask and then equimolar Benzenesulfonyl chloride (**2**) was added gradually. The pH of the reaction mixture was maintained at 8-10 by aqueous sodium carbonate solution or solid Na_2CO_3. The reaction mixture was continuously stirred for 3-4 hours and reaction completion was monitored with TLC. After single spot on TLC, concentrated HCl was poured into reaction contents gradually up to pH of 3-5. The precipitated product was collected by filtration, washed with distilled water and dried to get the title compound. Recrystallization was executed from methanol to yield pure *N*-(4-Methylpyridin-2-yl)benzenesulfonamide (**3**). White amorphous powder; Yield: 86%; M.P.: 150-152 ^{o}C; Mol. Formula: $C_{12}H_{12}N_2O_2S$; Mol. Mass: 248 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3452 (N-H), 3060 (Aromatic C-H), 1640 (Aromatic C=C), 1605 (Aromatic C=N), 1337 (S=O); ^{1}H-NMR (CD_3OD, 300 MHz, δ/ppm): 8.01 (d, J = 7.2 Hz, 1H, H-6), 7.94 (dd, J = 8.7, 1.2 Hz, 2H, H-2' & H-6'), 7.83-7.80 (m, 1H, H-4'), 7.55 (t, J = 7.8 Hz, 2H, H-3' & H-5'), 7.14 (s, 1H, H-3), 7.09 (d, J = 7.2 Hz, 1H, H-5), 2.40 (s, 3H, CH_3-4); EIMS (*m/z*): 248 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 92 $[C_6H_6N]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.3 *General procedure for the synthesis of N-alkyl/aralkyl substituted sulfonamides (5a-f)*

The molecule **3** (0.8 mmol) was homogenized in DMF (10.0 mL) followed by the addition of activator, lithium hydride (0.5 mmol). The mixture was stirred for 40-45 min and then alkyl/aralkyl halides (0.8 mmol) were added. Stirring was continued for further 5-6 hours. The completion of reaction was developed by TLC. Ice cold distilled water was added to the reaction mixture and kept still for 5-10 min. The formed products were isolated from the reaction mixture after basifying it (pH = 9-10), through solvent extraction using chloroform as most of the products were liquid and sometimes through filtration. The products were further processed for spectral analysis.

4.3.1 *N-Ethyl-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5a)*

Pale yellow liquid; Yield: 79%; Mol. Formula: $C_{14}H_{16}N_2O_2S$; Mol. Mass: 276 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3070 (Aromatic C-H), 1645 (Aromatic C=C), 1602 (Aromatic C=N), 1338 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 7.98 (d, J = 7.2 Hz, 1H, H-6), 7.91 (dd, J = 8.1, 1.5 Hz, 2H, H-2' & H-6'), 7.84-7.79 (m, 1H, H-4'), 7.57 (t, J = 7.5 Hz, 2H, H-3' & H-5'), 7.18 (s, 1H, H-3), 7.11 (d, J = 7.2 Hz, 1H, H-5), 3.20 (q, J = 6.9 Hz, 2H, H-1"), 2.40 (s, 3H, CH_3-4), 0.95 (t, J = 6.9 Hz, 3H, CH_3-2"); EIMS (*m/z*): 276 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 92 $[C_6H_6N]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$, 29 $[C_2H_5]^+$.

4.3.2 *N-(Pentan-1-yl)-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5b)*

Orange brown liquid; Yield: 74%; Mol. Formula: $C_{17}H_{22}N_2O_2S$; Mol. Mass: 318 g/mol; IR (KBr, ν_{max}, cm^{-1}): 2958 (Aromatic C-H), 1643 (Aromatic C=C), 1550 (Aromatic C=N), 1338 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 8.02 (d, J = 7.5 Hz, 1H, H-6), 7.91 (d, J = 7.5 Hz, 2H, H-2' & H-6'), 7.85-7.80 (m, 1H, H-4'), 7.61 (t, J = 7.5 Hz, 2H, H-3' & H-5'), 7.17 (s, 1H, H-3), 7.13 (d, J = 7.5 Hz, 1H, H-5), 3.34 (t, J = 6.9 Hz, 2H, H-1"), 2.39 (s, 3H, CH_3-4), 1.95

(qui, *J* = 6.9 Hz, 2H, H-2"), 1.45-1.39 (m, 4H, H-3" & H-4"), 0.98 (t, *J* = 6.9 Hz, 3H, CH_3-5"); EIMS (*m/z*): 318 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 92 $[C_6H_6N]^+$, 77 $[C_6H_5]^+$, 71 $[C_5H_{11}]^+$, 66 $[C_5H_6]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.3.3 *N-(Heptan-1-yl)-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5c)*

Brown sticky solid; Yield: 76%; Mol. Formula: $C_{19}H_{26}N_2O_2S$; Mol. Mass: 346 g/mol; IR (KBr, ν_{max}, cm^{-1}): 2963 (Aromatic C-H), 1647 (Aromatic C=C), 1548 (Aromatic C=N), 1339 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 8.01 (d, *J* = 7.2 Hz, 1H, H-6), 7.93 (d, *J* = 7.5 Hz, 2H, H-2' & H-6'), 7.89-7.84 (m, 1H, H-4'), 7.66 (t, *J* = 7.5 Hz, 2H, H-3' & H-5'), 7.15 (s, 1H, H-3), 7.08 (d, *J* = 7.5 Hz, 1H, H-5), 3.28 (t, *J* = 6.9 Hz, 2H, H-1"), 2.40 (s, 3H, CH_3-4), 1.95-1.85 (m, 6H, H-2" to H-4"), 1.57-1.49 (m, 4H, H-5" & H-6"), 0.99 (t, *J* = 6.9 Hz, 3H, CH_3-7"); EIMS (*m/z*): 346 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 92 $[C_6H_6N]^+$, 85 $[C_6H_{13}]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 57 $[C_4H_9]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.3.4 *N-(4-Bromobenzyl)-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5d)*

Brown amorphous powder; Yield: 79%; M.P.: 122 ^oC; Mol. Formula: $C_{19}H_{17}BrN_2O_2S$; Mol. Mass: 417 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3060 (Aromatic C-H), 1640 (Aromatic C=C), 1605 (Aromatic C=N), 1330 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 7.96 (d, *J* = 7.5 Hz, 1H, H-6), 7.59 (dd, *J* = 8.4, 1.2 Hz, 2H, H-2' & H-6'), 7.50-7.44 (m, 1H, H-4'), 7.41 (t, *J* = 8.7 Hz, 2H, H-3' & H-5'), 7.37 (d, *J* = 9.6 Hz, 2H, H-3" & H-5"), 7.36 (s, 1H, H-3), 7.12 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 6.65 (dd, *J* = 7.2, 1.5 Hz, 1H, H-5), 5.32 (s, 2H, H-7"), 2.28 (s, 3H, CH_3-4); EIMS (*m/z*): 417 $[M]^+$, 170 $[C_7H_6Br]^+$, 144 $[C_5H_4Br]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 92 $[C_6H_6N]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 65 $[C_5H_5]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.3.5 *N-(2-Phenylethyl)-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5e)*

Orange amorphous powder; Yield: 69%; M.P.: 146 ^oC; Mol. Formula: $C_{20}H_{20}N_2O_2S$; Mol. Mass: 352 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3065 (Aromatic C-H), 1643 (Aromatic C=C), 1604 (Aromatic C=N), 1332 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 7.94 (dd, *J* = 8.1, 2.1 Hz, 2H, H-2' & H-6'), 7.56-7.51 (m, 1H, H-4'), 7.43 (d, *J* = 7.2 Hz, 1H, H-6), 7.34 (s, 1H, H-3), 7.17 (t, *J* = 7.5 Hz, 2H, H-3' & H-5'), 7.09-7.01 (m, 5H, H-2" to H-6"), 6.42 (dd, *J* = 7.2, 1.5 Hz, 1H, H-5), 4.38 (t, *J* = 7.2 Hz, 2H, H-8"), 3.05 (t, *J* = 6.9 Hz, 2H, H-7"), 2.24 (s, 3H, CH_3-4); EIMS (*m/z*): 352 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 107 $[C_6H_7N_2]^+$, 105 $[C_8H_9]^+$, 92 $[C_6H_6N]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 65 $[C_5H_5]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.3.6 *N-(3-Phenylpropyl)-N-(4-Methylpyridin-2-yl)benzenesulfonamide (5f)*

Orange brown liquid; Yield: 67%; Mol. Formula: $C_{21}H_{22}N_2O_2S$; Mol. Mass: 366 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3058 (Aromatic C-H), 1637 (Aromatic C=C), 1600 (Aromatic C=N), 1333 (S=O); ^{1}H-NMR (MeOD, 300 MHz, δ/ppm): 7.93 (dd, *J* = 7.8, 1.5 Hz, 2H, H-2' & H-6'), 7.61-7.55 (m, 1H, H-4'), 7.44 (d, *J* = 7.5 Hz, 1H, H-6), 7.31 (s, 1H, H-3), 7.15 (t, *J* = 7.2 Hz, 2H, H-3' & H-5'), 7.11-7.06 (m, 5H, H-2" to H-6"), 6.69 (d, *J* = 7.2 Hz, 1H, H-5), 4.31 (t, *J* = 6.9 Hz, 2H, H-9"), 3.10 (t, *J* = 6.9 Hz, 2H, H-7"), 2.28 (s, 3H, CH_3-4), 2.13 (qui, *J* = 7.2 Hz, 2H, H-8"); EIMS (*m/z*): 366 $[M]^+$, 141 $[C_6H_5SO_2]^+$, 119 $[C_9H_{11}]^+$, 107 $[C_6H_7N_2]^+$, 105 $[C_8H_9]^+$, 92 $[C_6H_6N]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 66 $[C_5H_6]^+$, 65 $[C_5H_5]^+$, 52 $[C_3H_2N]^+$, 51 $[C_4H_3]^+$.

4.4 *Antibacterial activity*

The antimicrobial activity was determined following the principle that increased absorbance of broth medium is directly related to log phase of growth and was performed in sterile 96-wells microplates under aseptic conditions[21,22]. Four gram-negative (*Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonae* and *Pseudomonas aeroginosa*) and two gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) were included in the study and were maintained on stock culture agar medium. The test samples (with suitable solvents and dilutions) 20 µg/well and 180 µL fresh bacterial cultures (with suitable dilution by fresh nutrient broth) was poured into wells to make a volume of 200 µL. The initial absorbance of the culture was kept 0.12-0.19 at 540 nm. The absorbance was measured at 540 nm using microplate reader, before and after incubation at 37 ^oC for 16-24 hours with lid on the microplate. The difference was related to bacterial growth. The percent inhibition was calculated using the formula,

$$\text{Inhibition (\%)} = (X - Y) / X \times 100$$

where, X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n=3, ± SEM). Ciprofloxacin was taken as reference 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.

4.4.1 *Statistical Analysis*

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

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