N-Sulfonated Derivatives of 2,3-Xylidine as Suitable Antibacterial Agents

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(Received on 23rd September 2014, accepted in revised form 3rd January 2015)

Summary: The objective of the current research effort was to unravel the antibacterial potential of some newly synthesized sulfonamides. 2,3-Dimethylaniline (2,3-xylidine, **1**) was reacted with different sulfonyl chlorides **(2a-g)** under dynamic control of pH (9-10) in aqueous alkaline medium to generate a series of *N*-sulfonated derivatives of 2,3-xylidine (**3a-g)**. The plausible structures of the synthesized derivatives were corroborated by contemporary spectral techniques e.g. IR, ^{1}H-NMR and EIMS. Moreover, *N*-sulfonated derivatives were screened against different gram negative and positive bacterial strains to evaluate their inhibitory potential. They depicted good to moderate inhibitory potential, especially, *N*-(2,3-dimethylphenyl)methanesulfonamide (**3a**), *N*-(2,3-dimethylphenyl)-4-methylbenzenesulfonamide (**3b**), *N*-(2,3-dimethylphenyl)-4-bromobenzenesulfonamide (**3d**) and *N*-(2,3-dimethylphenyl)naphthalene-2-yl-sulfonamide (**3f**) were amongst the potent inhibitors as compared to standard; Ciprofloxacin.

Key Words: 2,3-Dimethylaniline, *N*-Sulfonated Derivatives, Anti-bacterial Potential, ^{1}H-NMR, EIMS.

Introduction

Sulfonamides bearing –SO_2NH linkage are being utilized as anti-bacterial agents since their discovery about 70 years ago. They initiated a fascinating era for the development of chemotherapeutic agents and the use of sulfa drugs has increased to treat broad spectrum of microbial diseases [1-5]. Many antibiotics e.g. Ampicillin (Fig. 1), Carbenicillin (Fig. 2), Streptomycin (Fig. 3) etc. have been discovered to treat diseases caused by pathogens like bacteria, fungi, virus and ricketteseas [6].

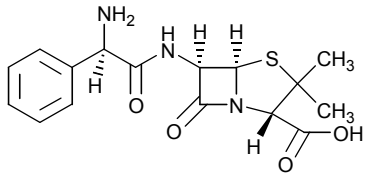

Fig. 1: Structure of Ampicillin.

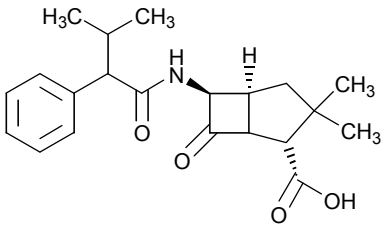

Fig. 2: Structure of Carbenicillin.

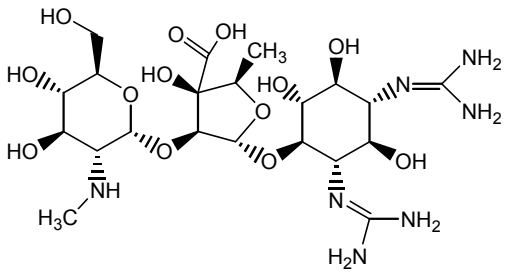

Fig. 3: Structure of Streptomycin.

E.coli strains are resistant to sulfonamides because they contain sulfonamide-resistant dihydropteroate synthase [7]. Sulfonamide molecules are not only widely used as anti-bacterial agents in the pharmaceutical industries but also have broad applications as anti-convulsant [8-11], anti-migraine [12], diuretic [13], hypoglycemic [14-15], caspase [16] and antiviral agents [1]. Recently, sulfonamides are also introduced as protease inhibitor and therefore they are also effective as anti-cancer [17-19], anti-inflammatory [20] and HIV agents [21-22]. In addition, several benzene sulfonamides have been reported to have anti-parasitic activity [23]. Moreover, several quinoline derivatives combined with sulfonamide moiety have shown significant effect as anti-cancer agents [24]. Today, sulfonamide-trimethoprim (Fig. 4) combinations are extensively used to treat opportunistic infections in

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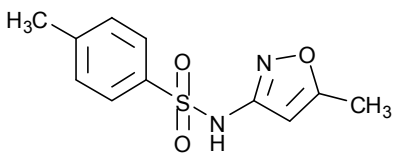
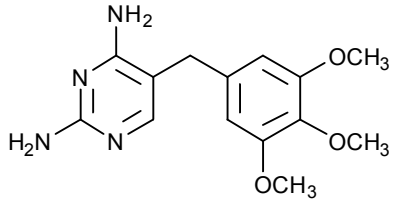
the patients facing AIDS along with urinary tract infection and burn therapy [7].



(a)



(b)

Fig. 4: Structures of Sulfamethoxazole (a) and Trimethoprim (b).

Pazopanib; a benzene sulfonamide derivative (Fig. 5) recently been proved an effective drug for the clinical use [25].

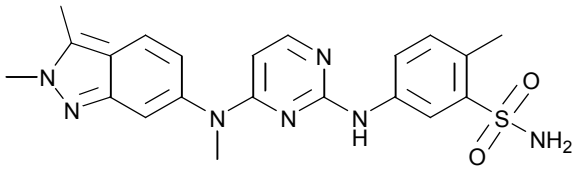


Fig. 5: Structures of Pazopanib.

Here by, the incredible pharmacological importance of sulfonamides stimulated us to document the synthesis and bioactivity of *N*-sulfonated derivatives of 2,3-xylidine by reaction with various alkyl/arenylsulfonyl chlorides. The results revealed that the sulfonamide moieties enhanced anti-bacterial activity against gram-positive and gram-negative bacteria [26]. In light of the above mentioned facts and in hope to obtain some novel compounds with significant anti-bacterial potential, we have reported a convenient strategy for the synthesis of *N*-sulfonated derivatives of 2,3-xylidine in the aqueous alkaline medium. The structures of synthesized compounds were categorized by FT-IR, ^{1}H-NMR and EIMS techniques. To some extent our effort endured fruitful as almost all of the molecules depicted good inhibitory action against almost all bacterial strains.

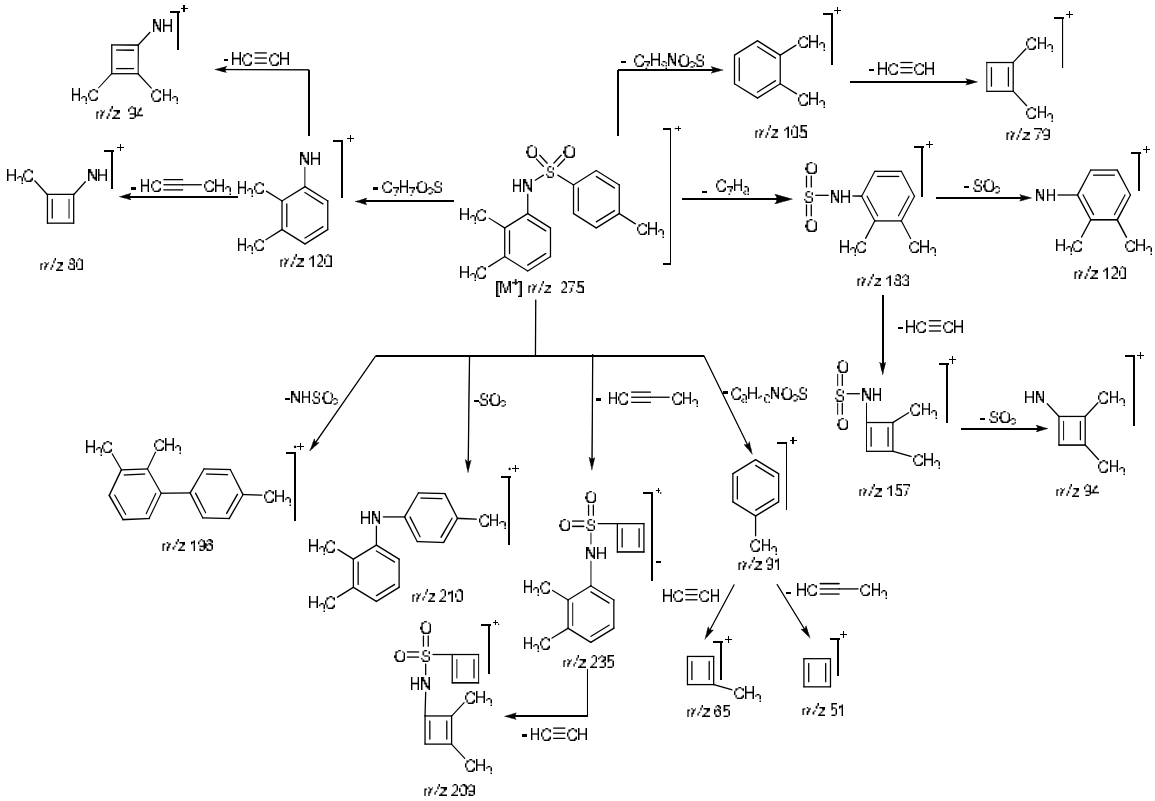


Fig. 6: Mass fragmentation pattern of *N*-(2,3-Dimethylphenyl)-4-methylbenzenesulfonamide (3b).

Experimental

General

The chemicals used in the research work were procured from Sigma Aldrich/Fluka. Progress of reaction was monitored by Thin Layer Chromatography using pre-coated silica gel G-25-UV254. UV light was used as visualizing agent. The purity was confirmed using different percentages of *n*-hexane and ethyl acetate. The solvents used were of analytical grade and were used as such. Melting points were determined with the help of Gallenkemp melting point apparatus using open capillary tube and were found to be uncorrected. The spectra of FT-IR were recorded on a Jasco-320-A spectrophotometer in KBr disc and the wave number was in cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker spectrometer operating at 400MHz and $CDCl_3$ was used as solvent. Chemical shifts (δ) were in ppm and coupling constant (J) are given in Hz. Mass spectrum was recorded on a Finnigan MAT-312 instrument with a data system.

Synthesis of N-substituted alkyl/arenyl Sulfonamides **(3a-g)**

Equimolar quantities of 2,3-dimethylaniline (0.004 mol, 0.5ml; **1**) and different sulfonyl chlorides (0.004 mol, **2a-g**) was suspended in 25ml distilled water in a 250 ml round-bottomed flask. The pH of the reaction mixture was maintained at 9.0-10.0 with aqueous Na_2CO_3. The reaction mixture was stirred for 2-3 hours at room temperature and progress of the reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the products were precipitated using dilute HCl at pH 2.0. The precipitates were filtrated, washed with the distilled water and air-dried to achieve *N*-sulfonated molecules **(3a-g)**. Moreover, the products were re-crystallized in methanol for further purification.

Spectral Characterization

N-(2,3-Dimethylphenyl)methanesulfonamide **(3a)**

Tea pink powder, yield 60%, m.p. 92-93˚C, Molecular formula: $C_9H_{13}NO_2S$, Molecular mass: 199 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3044 (N-H), 3047 (Ar C-H), 1470 (Ar C=C), 1155 (C-N), 1325 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.04-6.99 (m, 3H, H-4, H-5 & H-6), 3.07 (s, 3H, CH_3-1''), 2.18 (s, 3H, CH_3-2'), 1.92 (s, 3H, CH_3-1'); EIMS: *m/z* 201 $[M+2]^{\cdot+}$, 199 $[M]^{\cdot+}$, 135 $[M\text{-}SO_2]^{\cdot+}$, 120$[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 94 $[C_4HNH(CH_3)_2]^+$, 91 $[C_6H_4CH_3]^+$, 79 $[CH_3SO_2+2]^+$, 77 $[CH_3SO_2]^+$.

N-(2,3-Dimethylphenyl)-4-methylbenzenesulfonamide **(3b)**

Creamy white powder, yield 85%, m.p. 142-143˚C, Molecular formula: $C_{15}H_{17}NO_2S$, Molecular mass: 275 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3281 (N-H), 2991 (Ar C-H), 1470 (Ar C=C), 1155 (C-N), 1325 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.57 (d, J = 8.4 Hz, 2H, H-2'' & H-6'') , 7.20 (d, J = 8.4 Hz, 2H, H-3'' & H-5''), 7.02-6.97 (m, 3H, H-4, H-5 & H-6), 2.38 (s, 3H, CH_3-1'''), 2.19 (s, 3H, CH_3-2'), 1.94 (s, 3H, CH_3-1'); EIMS: *m/z* 277 $[M+2]^{\cdot+}$, 275 $[M]^{\cdot+}$, 210 $[M\text{-}SO_2]^{\cdot+}$, 196 $[C_6H_4CH_3C_6H_3(CH_3)_2]^{\cdot+}$, 186 $[C_6H_3(CH_3)_2NHSO_2+2]^+$, 184 $[C_6H_3(CH_3)_2NHSO_2]^+$, 157 $[C_4NH_2(CH_3)_2]^+$, 156 $[C_6H_4CH_3SO_2+2]^+$, 154 $[C_6H_4CH_3SO_2]^+$, 120$[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 94 $[C_4NH_2(CH_3)_2]^+$, 91 $[C_6H_4CH_3]^+$, 79 $[C_4H_2NHCH_3]^+$, 77 $[C_6H_5]^+$, 65 $[C_4H_2CH_3]^+$, 51 $[C_4H_3]^+$.

N-(2,3-Dimethylphenyl)-4-chlorobenzenesulfonamide **(3c)**

Light pink powder, yield 78%, m.p. 105-106˚C, Molecular formula: $C_{14}H_{14}ClNO_2S$, Molecular mass: 295 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3244 (N-H), 2969 (Ar C-H), 1469 (Ar C=C), 1193 (C-N), 1397 (S=O), 676 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.62 (d, J = 8.4 Hz, 2H, H-2'' & H-6''), 7.39 (d, J = 8.4 Hz, 2H, H-3''& H-5''), 7.01-6.98 (m, 3H, H-4, H-5 & H-6), 2.21 (s, 3H, CH_3-2'), 1.95 (s, 3H, CH_3-1'); EIMS: *m/z* 299 $[M+4]^{\cdot+}$, 297 $[M+2]^{\cdot+}$, 295 $[M]^{\cdot+}$, 232 $[M\text{-}SO_2+2]^{\cdot+}$, 230 $[M\text{-}SO_2]^{\cdot+}$, 218 $[C_6H_4ClC_6H_3(CH_3)_2+2]^{\cdot+}$, 216 $[C_6H_4ClC_6H_3(CH_3)_2]^{\cdot+}$, 178 $[C_6H_4ClSO_2+4]^+$, 176 $[C_6H_4ClSO_2+2]^+$, 174 $[C_6H_4ClSO_2]^+$, 120 $[C_6H_3NH(CH_3)_2]^+$, 113 $[C_6H_4Cl+2]^+$, 111 $[C_6H_4Cl]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 94 $[C_4HNH(CH_3)_2]^+$, 79 $[C_4H_2NHCH_3]^+$, 77 $[C_6H_5]^+$.

N-(2,3-Dimethylphenyl)-4-bromobenzenesulfonamide **(3d)**

Creamy white powder, yield 60%, m.p. 132-133˚C, Molecular formula: $C_{14}H_{14}BrNO_2S$, Molecular mass: 339 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3200 (N-H), 2787 (Ar C-H), 1463 (Ar C=C), 1147 (C-N), 1389 (S=O), 586 (C-Br); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.59 (d, J = 8.4 Hz, 2H, H-2'' & H-6''), 7.23 (d, J = 8.3 Hz, 2H, H-3''& H-5''), 7.05-6.99 (m, 3H, H-4, H-5 & H-6), 2.16 (s, 3H, CH_3-2'), 1.93 (s, 3H, CH_3-1'); EIMS: *m/z* 343 $[M+4]^{\cdot+}$, 341 $[M+2]^{\cdot+}$, 339 $[M]^{\cdot+}$, 278 $[M\text{-}SO_2+2]^{\cdot+}$, 276 $[M\text{-}SO_2]^{\cdot+}$, 261 $[C_6H_4BrC_6H_3(CH_3)_2+2]^{\cdot+}$, 259 $[C_6H_4BrC_6H_3(CH_3)_2]^{\cdot+}$, 222 $[C_6H_4BrSO_2+4]^+$, 220 $[C_6H_4BrSO_2+2]^+$, 218

$[C_6H_4BrSO_2]^+$, 154 $[C_6H_4Br+2]^+$, 152 $[C_6H_4Br]^+$, 131 $[C_4H_2Br+2]^+$, 129 $[C_4H_2Br]^+$, 120 $[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 94 $[C_4HNH(CH_3)_2]^+$, 79 $[C_4H_2NHCH_3]^+$, 77 $[C_6H_5]^+$.

N-(2,3-Dimethylphenyl)-4-acetamidobenzenesulfonamide **(3e)**

Light pink powder, yield 78%, m.p. 213-214˚C, Molecular formula: $C_{16}H_{18}N_2O_3S$, Molecular mass: 318 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3241 (N-H), 3016 (Ar C-H), 1528 (Ar C=C), 1153 (C-N), 1370 (S=O), 1695 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.78 (d, J = 8.0 Hz, 2H, H-2"& H-6"), 7.66 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 7.03-6.99 (m, 3H, H-4, H-5 & H-6), 2.23 (s, 3H, -CO$\underline{CH_3}$), 2.17 (s, 3H, CH_3-2'), 1.92 (s, 3H, CH_3-1'); EIMS: *m/z* 320 $[M+2]^{\cdot+}$, 318 $[M]^{\cdot+}$, 254 $[M\text{-}SO_2]^{\cdot+}$, 261 $[C_6H_4NHCOCH_3C_6H_3(CH_3)_2]^{\cdot+}$, 200 $[C_6H_4NHCOCH_3SO_2+2]^+$, 198 $[C_6H_4NHCOCH_3SO_2]^+$, 134 $[C_6H_4NHCOCH_3]^+$, 120 $[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 94 $[C_4HNH(CH_3)_2]^+$, 91 $[C_6H_4NH]^+$, 79 $[C_4H_2NHCH_3]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

N-(2,3-Dimethylphenyl)naphthalene-2-yl-sulfonamide **(3f)**

Off white powder, yield 93%, m.p. 158-159˚C, Molecular formula: $C_{18}H_{17}NO_2S$, Molecular mass: 311 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3285 (N-H), 3012 (Ar C-H), 1474 (Ar C=C), 1128 (C-N), 1394 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.35 (br s, 1H, H-1") , 7.97 (d, J = 8.8 Hz, 1H, H-8"), 7.95 (d, J = 8.8 Hz 1H, H-3"), 7.91 (d, J = 8.4 Hz, 1H, H-4"), 7.79 (dd, J = 8.4, 2.0 Hz, 1H, H-5"), 7.66-7.60 (m, 2H, H-6"& H-7"), 7.04-6.95 (m, 3H, H-4, H-5 & H-6), 2.18 (s, 3H, CH_3-2'), 1.92 (s, 3H, CH_3-1'); EIMS: *m/z* 313 $[M+2]^{\cdot+}$, 311 $[M]^{\cdot+}$, 246 $[M\text{-}SO_2]^{\cdot+}$, 232 $[C_{10}H_7C_6H_3(CH_3)_2]^{\cdot+}$, 192 $[C_{10}H_7SO_2+2]^+$, 190 $[C_{10}H_7SO_2]^+$, 127 $[C_{10}H_7]^+$, 120 $[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 102 $[C_8H_5]^+$, 94 $[C_4HNH(CH_3)_2]^+$, 79 $[C_4H_2NHCH_3]^+$, 77 $[C_6H_5]^+$.

N-(2,3-Dimethylphenyl)camphor-10-yl-sulfonamide **(3g)**

Brown sticky, yield 29%, Molecular formula: $C_{18}H_{25}NO_3S$, Molecular mass: 335 $gmol^{-1}$; IR (KBr, υ_{max} (cm^{-1})): 3068 (N-H), 3076 (Ar C-H), 1605 (Ar C=C), 1128 (C-N), 1384 (S=O), 1221 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.04-6.96 (m, 3H, H-4, H-5 & H-6), 3.35 (s, 2H, H-10"), 2.42-2.39 (m, 2H, H-3"), 2.20-2.14 (m, 2H, H-6"), 2.12 (s, 3H, CH_3-2'), 2.11-2.03 (m, 2H, H-4"), 1.95 (s, 3H, CH_3-1'), 1.36-1.33 (m, 1H, H-5"), 0.95 (s, 6H, CH_3-8" & CH_3-9"); EIMS: *m/z* 337 $[M+2]^{\cdot+}$, 335 $[M]^{\cdot+}$, 271 $[M\text{-}SO_2]^{\cdot+}$, 256$[C_{18}H_{24}O]^{\cdot+}$, 217 $[C_{10}H_{15}O_3S+2]^+$, 215 $[C_{10}H_{15}O_3S]^+$, 120$[C_6H_3NH(CH_3)_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_6H_4CH_3]^+$, 79 $[C_4H_2NHCH_3]^+$.

Antibacterial Assay

MICs were defined as the lowest concentrations of the compounds which inhibit the growth of microorganisms [27]. The antibacterial activity was performed in sterile 96-wells microplates under aseptic environment. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in a log phase of growth which results in increased absorbance of broth medium [28]. Three Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi)* and two Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus)* were included in the study. The organisms were maintained on stock culture agar medium. The test samples with suitable solvents and dilutions were pipetted into wells (20µg$well^{-1}$). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µl). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µl. The incubation was done at 37°C for 16-24 hours with the lid on the microplate. The absorbance was measured at 540 nm using microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\% \text{ age Inhibition} = \frac{X-Y}{X} \times 100$$

Where:

X = Absorbance in control with bacterial culture
Y = Absorbance in the test sample

Ciprofloxacin was used as reference standard.

Statistical Analysis

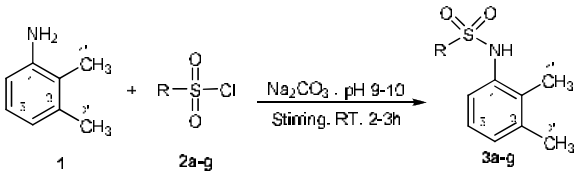
The measurements involved in this script were completed in triplicate and the statistical analysis was performed by Microsoft Excel 2003. Moreover, the results were offered as mean ± SEM.

Results and Discussion

Chemistry

N-sulfonated derivatives of 2,3-xylidine were synthesized by reacting 2,3-dimethylaniline

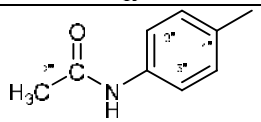
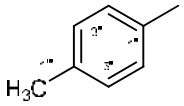
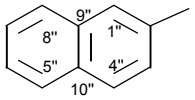
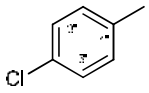
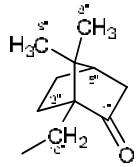
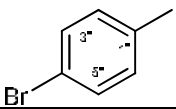
with different sulfonyl chlorides in aqueous alkaline medium according to protocol illustrated in Scheme-1, Table-1. The reaction procedure and the conditions are elaborated in the experimental section and substantiation of the synthesized compounds was done using different spectral techniques i.e. IR, ^{1}H-NMR and EIMS. Further, these molecules were screened against various gram negative and positive bacterial strains aiming to introduce potent anti-bacterial agents who might be beneficial in pharmacology.



Scheme-1: Synthesis of *N*-sulfonated derivatives of 2,3-xylidine **3a-g**.

The nucleophilic attack of nitrogen atom of 2,3-dimethyl aniline (**1**) on electrophilic sulfur atom of sulfonyl chlorides, **2a-g**, rendered the formation of various *N*-sulfonated derivatives **3a-g** in the presence of alkaline aqueous Na_2CO_3 medium at pH 10 which yielded water-soluble sodium salts of sulfonamides. Finally, sodium salts of sulfonamides were precipitated as water-insoluble sulfonamides by aqueous hydrochloric acid e.g. 2,3-dimethylaniline (**1**) was reacted with *p*-toluenesulfonylchloride (**2b**) to yield *N*-(2,3-dimethylphenyl)-4-methylbenzenesulfonamide (**3b**) in aqueous alkaline medium under stirring for 2-3 hours. The product was obtained by the acidifying with dilute hydrochloric acid as creamy white powder having molecular formula $C_{15}H_{17}NO_2S$, which was affirmed by counting the number of protons using integration curves in its ^{1}H-NMR spectrum and molecular ion peak in EI-MS which appeared at *m/z* 275 and also supported the predicted formula. The IR spectrum affirmed the presence of functionalities present in the molecules e.g. absorption bands at 3281, 2991, 1470, 1155, 1325 for (N-H), (Ar C-H), (Ar C=C), (C-N), (S=O) groups respectively. In the aromatic region of ^{1}H-NMR spectrum, A_2B_2 spin type system was observed as two *diortho*-coupled doublets, one at δ 7.57 (d, J = 8.4 Hz, 2H, H-2" & H-6") and other at δ 7.20 (d, J = 8.4 Hz, 2H, H-3" & H-5") confirming the 1,4-substitution of the phenyl ring. A multiplet resonated at δ 7.02 - 6.97 for 3H's of the second phenyl ring. In the aliphatic region, three singlets were observed, one at δ 2.38 (s, 3H, CH_3-1'"), other at δ 2.19 (s, 3H, CH_3-2') for the dimethyl group and finally at δ 1.94 (s, 3H-1') for methyl group of toluene moiety confirming the union of 2,3-dimethylaniline (**1**) with *p*-toluenesulfonylchloride (**2**). On the basis of above collective evidences, the projected structure of **3b** was confirmed as *N*-(2,3-dimethylpheyl)-4-methylbenzenesulfonamide.
Similarly, the other synthesized molecules **(3a-g)** were affirmed in an analogous manner.

Table-1: Different R-groups in *N*-sulfonated derivatives, 3a-g.

Compd.	R	Compd.	R
3a		**3e**	
3b		**3f**	
3c		**3g**	
3d			

Antibacterial Activity (*In vitro*)

The antibacterial results manifestly exhibited that *N*-sulfonated derivatives of 2,3-xylidine possess a comprehensive range of activity against different bacterial strains. The results of the synthesized compounds are demonstrated in (Table-2 and Table-3) as %age inhibition and MIC values, respectively. The synthesized compounds were found to be potentially active against five different bacterial strains i.e. *Salmonella typhi (-), E.coli (-), Pseudomonas aeruginosa (-)*, *Bacillus subtilis (+) and Staphylococcus aureus (+)* e.g. *N*-(2,3-dimethylphenly)methanesulfonamide **(3a)** and *N*-(2,3-dimethylphenyl)-4-methylbenzenesulfonamide **(3b)** were found to be most active inhibitors against *Salmonella typhi (-)* having IC_{50} values of 8.24±0.62 µg/mL and 8.31±0.45 µg/mL respectively relative to standard Ciprofloxacin having IC_{50} (7.83±0.78 µg/mL). The improved activity may be attributed to the presence of methyl groups. The halogenated arenyl moiety in *N*-(2,3-dimethylphenyl)-4-bromobenzenesulfonamide **(3d)** and *N*-(2,3-dimethylphenyl)-4-chlorobenzenesulfonamide **(3c)** showed least inhibitory values as evident from their MIC values.

Against *E. coli (-) N*-(2,3-dimethylphenyl)-4-bromobenzenesulfonamide **(3d)** showed the most active inhibition as compared to the rest of the series having IC_{50} value of 8.6±0.73 µg/mL relative to the reference standard ciprofloxacin (8.0±0.12 µg/mL). Antibacterial results against *Pseudomonas aeruginosa (-)* showed that *N*-(2,3-dimethylphenyl)-4-methylbenzenesulfonamide **(3b)** showed the excellent activity with IC_{50} value of 9.28±0.85 µg/mL relative to the standard ciprofloxacin having IC_{50} value of 7.9±0.89 µg/mL. Against *Bacillus subtilis (+) N*-(2,3-dimethylphenyl)-2-napthalenesulfonamide **(3f)** showed relatively good inhibition activity as compared to the rest of the sulfonamides having IC_{50} value of 8.16±0.70 µg/mL relative to ciprofloxacin (7.22±0.67 µg/mL). Studies against *Staphylococcus aureus (+) N*-(2,3-dimethylphenyl)-2-napthalenesulfonamide **(3f)** and *N*-(2,3-dimethyl phenyl)-4-chlorobenzenesulfonamide **(3c)** showed effective inhibition having IC_{50} values of 8.17±0.38 and 8.54±0.66 µg/mL, respectively, relative to the reference standard ciprofloxacin (7.00±0.54 µg/mL).

Conclusion

N-sulfonated derivatives of 2,3-xylidine were obtained in good yields by reaction of 2,3-xylidine with different sulfonyl chlorides in alkaline media. The proposed structures of *N*-sulfonated molecules were in concordance with the spectral data. The synthesized sulfonamides depicted noticeable inhibition action against five different bacterial strains especially by compounds *N*-(2,3-dimethylphenly)methanesulfonamide (**3a**), *N*-(2,3-dimethylphenyl)-4-methylbenzenesulfonamide **(3b)**, *N*-(2,3-dimethylphenyl)-4-bromobenzenesulfonamide (**3d**) and *N*-(2,3-dimethylphenyl)-2-napthalenesulfonamide (**3f**) exhibited potent inhibition which was evident from their IC_{50} values, relative to the standard ciprofloxacin. It can be contemplated that the synthesized sulfonamides bearing methane, 4-methylbenzene, 4-bromobenzene and 2-naphthyl moieties seem to be suitable drug candidates for the treatment of various diseases.

Table-2: Antibacterial activity (% age inhibition) of sulfonamides**, 3a-g.**

Codes	%age Inhibition				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*B. subtilis (+)*	*S. aureus(+)*
3a	**64.2±1.92**	**60.1±0.10**	**72.3±0.87**	**69.7±1.00**	**59.3±0.50**
3b	**78.6±0.56**	**73.1±0.61**	**80.1±0.25**	**75.7±1.00**	**79.2±0.81**
3c	**79.3±0.38**	**77.2±0.54**	**85.1±0.06**	**82.8±0.57**	**86.0±0.63**
3d	**82.1±0.13**	**77.8±0.95**	**85.1±0.45**	**59.5±0.60**	**84.3±0.30**
3e	**56.5±0.25**	**64.7±0.85**	**84.5±0.00**	**70.8±0.90**	**78.6±1.07**
3f	**64.7±0.32**	**70.2±0.73**	**78.0±0.20**	**84.7±0.65**	**80.0±0.53**
3g	**61.9±0.40**	**67.4±0.67**	**77.2±0.31**	**62.8±0.50**	**78.7±0.58**
Ciprofloxacin	**92.5±0.14**	**93.0±0.05**	**93.4±0.18**	**92.7±0.45**	**91.6±0.22**

Table-3: Antibacterial activity (MIC) of sulfonamides, **3a-g**.

Codes	MIC				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*B. subtilis (+)*	*S. aureus(+)*
3a	**8.24±0.62**	**11.7±0.59**	**10.4±0.59**	**14.3±1.08**	**14.2±0.67**
3b	**8.31±0.45**	**11.4±0.35**	**9.28±0.85**	**11.6±0.82**	**9.09±0.34**
3c	**10.8±0.93**	**11.5±0.96**	**11.4±0.95**	**9.47±1.90**	**8.54±0.66**
3d	**11.3±0.98**	**8.61±0.73**	**10.6±0.41**	**14.0±0.86**	**8.79±0.64**
3e	**10.2±0.37**	**12.4±0.75**	**10.2±0.84**	**11.7±0.94**	**9.09±0.48**
3f	**9.4±0.69**	**10.2±0.80**	**12.7±0.83**	**8.16 ±0.70**	**8.17±0.38**
3g	**9.72±0.63**	**10.1±0.34**	**11.3±0.85**	**9.77±0.92**	**10.9±0.93**
Ciprofloxacin	**7.83±0.78**	**8.01±0.12**	**7.98±0.89**	**7.22±0.67**	**7.00±0.54**

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

References

1. S. T. Won, P. Chandra and T. S. Shim, Simultaneous detection of antibacterial sulfonamides in a microfluidic device with amperometry. *Biosens. Bioelectron*., **39**, 204 (2013).
2. M. A. Abbasi, Aziz-ur-Rehman, M. Z. Qureshi, F. M. Khan, K. M. Khan M. Ashraf and I. Afzal, Synthesis of brominated 2-phenitidine derivatives as a valuable inhibitors of cholinestersaes for the treatment of Alzheimer's disease, *Braz. J. Pharm. Sci.*, **49**, 127 (2013).
3. E. E. Connor, Sulfonamide Antibiotics, *Prim. Care Update Ob. Gyn*., **5**, 32 (1998).
4. A. Alsughayer, A. A. Elassar, S. Mustafa and F. Al Sagheer, Synthesis, structure analysis and antibacterial activity of new potent sulfonamide derivatives, *J. Biomat. Nanobiotech.*, **2**, 144 (2011).
5. X. Liang, Z. Wang, C. Wang, K. Wen, T. Mi, J. Zhang and S. Zhang, A proof of concept receptor-based assay for sulfonamides, *Anal. Biochem.*, **438**, 110 (2013).
6. P. Patel, J. Pillai, N. Darji, P. Patel and B. Patel, Design, synthesis and characterization of novel molecules comprising benzothiazole and sulphonamide linked to substituted aryl group via azo link as potent antimicrobial agents, *Int. J. Drug Res.Tech.*, **2**, 289 (2012).
7. M. M. J. Al-Mudhafar, M. Q. Abdulkadir, A. A. Muhammed, A. M. Hussain and F. A. Al-hilli, Synthesis and microbiological study of new sulfonamides, *Int. J. Comp. Pharm.*, **6**, 02 (2012).
8. W. R. Chegwidden, N. D. Carter and Y. H. Edwards, *The Carbonic Anhydrases*, New Horizon, Birkauser, Basel, 2000, p. 175 (2004).
9. M. F. Sugrue, The pharmacology of antiglaucoma drugs, *Pharmacol. Ther*., **43**, 91 (1989).
10. C. T. Supuran and A. Scozzafava, Carbonic anhydrase inhibitors, *Curr. Med. Chem. Immunol. Endocr. Metab. Agents*, **1**, 61 (2001).
11. I. Nishmori, D. Vullo, A. Innocenti, A. Scozzafra and C. T. Supuran, Carbonic anhydrase inhibitors: inhibition of the transmembrane isozyme XIV with sulfonamides, *Bioorg. Med. Chem. Lett.,* **15**, 3828 (2005).
12. A. Kleemann, J. Engel, B. Kutscher and D. Reichert, *Pharmaceutical substances, synthesis, patents and applications, Stuggart Guide*, Oxford press, Thieme, p. 3967 (1999).
13. T. H. Maren, Relations between structure and biological activity of sulfonamides, *Annu. Rev. Pharmacol. Toxicol.,* **16**, 309 (1976).
14. M.E. Wolff, *Sulfonamides and sulfones, Burger's Medicinal Chemistry and Drug Discovery*; *Therapeutic agents*, J. Wiley and Sons, p. 527 (1997).
15. A. E. Boyd, III Sulfonylurea receptors, ion channels, and fruit flies, *Diabetes* **37**, 847 (1988).
16. W. Chu, J. Rothfuss, A. Auignon, C. Zeng, D. Zhou, R. S. Hotchkiss and R. H. Mach, Isatin sulfonamide analogs containing a Michael addition acceptor: a new class of caspase 3/7 inhibitors, *J. Med. Chem.*, **50** 3751 (2007).
17. T. Owa and T. Nagasu, Novel sulphonamide derivatives for the treatment of cancer, *Expert Opin. Ther. Pat*., **10**, 1725 (2000).
18. C.T. Supuran and A. Scozzafava, Carbonic-anhydrase inhibitors and their therapeutic potential, *Expert Opin. Ther. Pat*., **10**, 575 (200).
19. J. C. Medina, D. Roche, B. Shan, R. M. Learned, W. P. Frankmoelle, D. L. Clark, T. Rosen and J. C. Jeven, Novel halogenated sulfonamides inhibit the growth of multidrug resistant MCF-7/ADR cancer cells, *Bioorg. Med. Chem. Lett.*, **9**, 1843 (1999).
20. M. T. Vazquez, G. Rosoll and M.D. Pujol, Synthesis and anti-inflammatory activity of rac-2-(2,3-dihydro-1,4-benzodioxin)propionic acid and its R and S enantiomers, *Eur. J. Med. Chem.*, **32**, 529 (1997).
21. M. Mirian, S. Sadeghi, H. aliabadi, A. Zarghi, P. Tabaraki, M. Tavallace and O. Badrass, Synthesis and Cytotoxic Evaluation of Some Novel Sulfonamide Derivatives Against a Few Human Cancer Cells, *Iran. J. Pharm. Res.*, **10**, 741 (2011).
22. M. A. Abbasi, A. Saeed, Aziz-ur-Rehman, K. M. Khan, M. Ashraf and S. A. Ejaz, Synthesis of brominated 2-phenitidine derivatives as valuable inhibitors of cholinestersaes for the treatment of Alzheimer's disease, *Iran. J. Pharm. Res.*, **13**, 87 (2014).
23. P. Bilbao-Ramos, C. Galiana-Rosello, M. A. Dea-Ayuela, M. Gonzalez-Alvarez, C. Vega, M. Rolon, J. Perez-Serrano, F. Balos-Fernandez and M. E. Gonzalez-Rosende, Nuclease activity and ultrastructural effects of new sulfonamides with anti-leishmanial and trypanocidal activities, *Parasitol. Int.*, **61**, 604 (2012).
24. M. S. Al-Said, M. M. Ghorab, M. S. Al-Dosari and M. M. Hamed, Synthesis and in vitro anticancer evaluation of some novel hexahydroquinoline derivatives having a benzenesulfonamide moiety, *Eur. J. Med. Chem.*, **46**, 201 (2011).
25. J. Y. Winum, A. Maresca, F. Carta, A. Scozzafava and C. T. Supuran, Polypharmacology of sulfonamides: pazopanib, a

multitargeted receptortyrosine kinase inhibitor in clinical use, potently inhibits several mammalian carbonic anhydrases, *Chem. Comm.*, **48**, 8177 (2012).

26. H. Jeon, N. H. Jo, K. H. Yoo, J. H. Choi, H. Cho, J. H. Cho and C. H. Oh, Synthesis and antibacterial evaluation of 1B-methyl-2-[5-(1-methoxyimino-2-substitutes sulfonamides ethyl)pyrrolidin-3-ylthio]carbapenems and their related compounds, *Eur. J. Med. Chem*., **42**, 358 (2007).
27. U. O. Ozdemir, P. Guvene, E. Sahin and F. Hamurcu, Synthesis, characterization and antibacterial activity of new sulfonamide derivatives and their nickel(II), cobalt(II) complexes. *Inorg. Chim. Acta.,* **362**, 2613 (2008).
28. M. Kaspady, V. K. Narayanaswamy, M. Raju and G. K. Rao, Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioesters, *Lett. Drug Des. Discov.*, **6**, 21 (2009).