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Original Research Article

Synthesis, Characterization, Antibacterial, α-Glucosidase Inhibition and Hemolytic Studies on Some New N-(2,3-Dimethylphenyl)benzenesulfonamide Derivatives

Muhammad A Abbasi[1]*, Mudassar Islam[1], Aziz-ur-Rehman[1], Shahid Rasool[1], Kaniz Rubab[1], Ghulam Hussain[1], Irshad Ahmad[2], Muhammad Ashraf[3], Muhammad Shahid[4] and Syed Adnan Ali Shah[5,6]

[1]Department of Chemistry, Government College University, Lahore-54000, [2]Department of Pharmacy, [3]Department of, Chemistry, The Islamia University of Bahawalpur ,Bahawalpur-63100, [4]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan, [5]Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam-42300, [6]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

For correspondence:* ***Email:* *atrabbasi@yahoo.com, abbasi@gcu.edu.pk;* ***Tel:*** *(+92)-42-111000010 ext 266.*

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Abstract

Purpose: *To synthesize a series of new N-(2,3-dimethylphenyl)benzenesulfonamide derivatives with pharmacological analysis.*
Methods: *N-(2,3-Dimethylphenyl)benzenesulfonamide (3) was synthesized by the reaction between 2,3-dimethylaniline (1) and benzenesulfonyl chloride (2) in aqueous basic medium. Compound 3 was further treated with various alkyl/aralakyl halides (4a-m) to yield new compounds, 5a-m, in a weak basic aprotic polar organic medium. The proposed structures of synthesized compounds were confirmed using proton-nuclear magnetic resonance (1H-NMR), infra-red spectroscopy (IR) and electron impact mass spectrometry (EIMS). The synthesized compounds were screened for in vitro antibacterial, anti-enzymatic and hemolytic activities using standard procedures.*
Results: *All the synthesized compounds showed moderate to high activity against Gram-positive and Gram-negative bacterial strains. The molecules 5g and 5j exhibited good inhibition of α-glucosidase enzyme with half-maximal inhibitory concentration (IC_{50}) of 59.53 ± 0.01 and 55.31 ± 0.01 µmoles/L, respectively, relative to acarbose with IC_{50} of 38.25 ± 0.12 µmoles/L. All the compounds exhibited cytotoxicity levels ranging from 27.20 ± 0.24 to 5.20 ± 0.41 %, relative to Triton X-100.*
Conclusion: *Compound 5f is the most potent antibacterial while 5j is the best α-glucosidase inhibitor; 5e showed the least cytotoxicity.*

Keywords: *2,3-Dimethylaniline, Antibacterial activity, Anti-enzymatic activity, α-Glucosidase inhibitor, Hemolytic activity, Sulfonamides*

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INTRODUCTION

Sulfonamides exhibit numerous pharmacological activities [1-3] and sulfonamide antibiotics are among the most common instigators of allergic or hypersensitivity reactions [4-6]. α-Glucosidase (α-D-glucosideglucohydrolase, EC 3.2.1.20)hydrolyzes the 1,4-glycosidic linkage from the non-reducing end of the α-glucosides [7-9].Its inhibitors retard the liberation of D-glucose resulting in delay glucose absorption [10]. The hemolysis takes place due to the stroke

of the microbial products and resident parasites on the red blood corpuscles membranes [11,12]. Our group has synthesized many antibacterial and anti-enzymatic potent molecules [13-16] and a series of new compounds was synthesized in the present study to explore their pharmacological behavior.

EXPERIMENTAL

General

The chemicals solvents were of commercial grade and were used without purification. Reaction progress and product purity was monitored by pre-coated TLC silica gel G-25-UV254 plates with different solvent systems using ethyl acetate and n-hexane. Melting points were noted on Gallenkamp apparatus by open capillary tube and were uncorrected. FTIR spectra were recorded on MIDAC M 2000 spectrometer. 1H-NMR spectra were recorded in $CDCl_3$ on Bruker (400 MHz). Mass spectra (EI-MS) were measured on Finnigan MAT-312 instruments.

Procedure for the synthesis of N-(2,3-dimethylphenyl)benzenesulfonamide (3)

An equimolar mixture of 2,3-dimethylaniline (**1**, 0.02 mol) was dispersed in 25 mL waterfollowed by the addition ofbenzenesulfonyl chloride (**2**, 0.02 mol). The pH of the suspension was maintained at 9-10 during the whole reaction by adding Na_2CO_3 at RT. The reaction solution was stirred for 3 h. After complete reaction, the concentrated HCl (2-3 mL) was added slowly to adjust the pH to 2. Product formation was confirmed by TLC. The reaction mixture was kept still for 3-5 min. The off-white precipitates were collected by filtration, washed with distilled water and dried to acquire the compound **3**.

General procedure for the synthesis of N-alkyl/aralakyl substituted sulfonamides (5a-m)

The calculated amount of **3** (0.7 mmol; 0.2 g) was taken in a round bottom flask (50 mL), then N,N-dimethyl formamide (DMF) was added to dissolve it followed by the addition of lithium hydride LiH (0.004 g). The mixture was stirred for 45 min at RT and then alkyl/aralakyl halides (**4a-m**; 0.7 mmol) were added to the mixture. The solution was further stirred for 3-4 h. The progress of reaction was monitored by TLC. The product was precipitated by adding cold water, filtered, washed with distilled water and dried.

Evaluation of antibacterial activity

The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments by the methods of Kaspady *et al* [17] and Yang *et al* [18].

α-Glucosidase assay

The α-glucosidase inhibition activity was performed according to the method of Chapdilaine *et al* [10].

Determination of hemolytic activity

Hemolytic activity was studied by the reported method of Powell *et al* [11,19]. 3.0 mL fresh heparin added human blood obtained from volunteers after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. The study protocol was approved by the institutional ethical committee (approval no. DGS/8786-89, dated 09-03-2015), University of Agriculture, Faisalabad, Pakistan and was conducted in accordance with 1964 Declaration of Helsinki and its subsequent amendments [20].

Data analysis

All the measurements were performed in triplicate and statistical analysis was carried out by Microsoft Excel 2010. The results are presented as mean ± SEM with CL 80 % (for antibacterial), 85 % (for α-glucosidase) and 95 % (for hemolytic analysis). MIC (minimum inhibitory concentration and IC_{50} (50 % inhibitory concentration) were calculated using EZ-Fitz software (Perrella Scientific Inc. Amherst, USA).

RESULTS

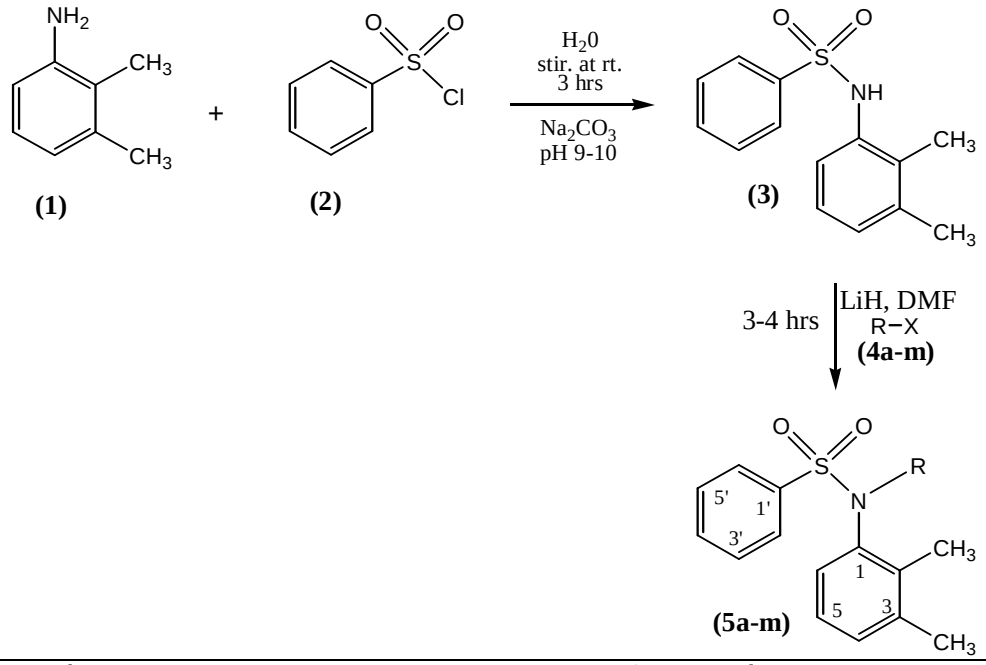
The new N-substituted derivatives of N-(2,3-dimethylphenyl)benzenesulfonamide were synthesized according to the protocol sketched in Scheme-1.

The antibacterial activities are given in Table-1 while inhibition against α-glucosidase enzyme and hemolytic activity are tabulated in Table 2.

Spectral characteristics of synthesized compounds (3, 5a-m)

N-(2,3-Dimethylphenyl)benzenesulfonamide (3)

Yield: 98 %; off-white solid; mp. 118-119 °C; Molecular formula: $C_{14}H_{15}NO_2S$; Molecular



Compound	R	Compound	R
5a	$-CH_2-CH_3$ (1" 2")	**5h**	$-CH_2$ (7") – Ph (1" 3" 5")
5b	$-CH_2-CH_2-CH_3$ (1" 2" 3")	**5i**	Cl – Ph (1" 3" 5") – CH_2 (7")
5c	$-CH(CH_3)(CH_3)$ (1" 2" 3")	**5j**	$-CH_2$ (7") – Ph (1" 3" 5") – Cl
5d	$-CH_2-CH_2-CH_2-CH_3$ (1" 2" 3" 4")	**5k**	$-CH_2$ (7") – Ph (1" 3" 5") – Br
5e	$-CH(CH_3)(4")-CH_2-CH_3$ (1" 2" 3")	**5l**	$-H_2C$ (8") $-CH_2$ (7") – Ph (1" 3" 5")
5f	$-CH_2-CH_2-CH_2-CH_2-CH_3$ (1" 2" 3" 4" 5")	**5m**	$-CH_2$ (1") $-C$ (2") $=C$ (3") with H_a, H_b
5g	$-CH(5")(CH_3)-CH_2-CH_2-CH_3$ (1" 2" 3" 4")		

Scheme-1: *N*-alkyl/aralakyl substituted derivatives of **3**

weight: 261; Analysis. Calcd: C, 64.32; H, 5.74; N, 5.36; S, 12.25. Found: C, 64.03; H, 6.40; N, 5.52; S, 11.73;IR (cm^{-1}) u_{max}:3208(N-H), 3042 (Ar C-H), 2924(Alkane C-H), 1583(Ar C=C), 1324 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.69 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.53 (1H, *t*, *J* = 7.6 Hz, H-4'), 7.41 (2H, *t*, *J* = 7.6 Hz, H-3', H-5'), 7.13 (1H, *d*, *J* = 7.6Hz, H-6), 6.96 (1H, *t*, *J* = 7.6Hz, H-5), 6.46 (1H, *d*, *J* = 8.4Hz, H-4), 2.18 (3H, *s*, CH_3-2), 1.91 (3H, *s*, CH_3-3); EI-MS (*m/z*): 261 (M^+, 28.2%), 141, 120 (BP, 100%), 105, 77, 51.

N-(2,3-Dimethylphenyl)-N-ethylbenzenesulfonamide (5a)

Yield: 99 %; light mustard solid; mp. 85.8 °C; Molecular formula: $C_{16}H_{19}NO_2S$; Molecular weight: 289; Analysis. Calcd: C, 66.43; H, 6.56; N, 4.84; S, 11.07. Found: C, 64.90; H, 6.29; N,

4.52; S, 11.02;IR (cm^{-1}) u_{max}:3065 (Ar C-H), 2934 (Alkane C-H), 1583 (Ar C=C), 1341 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.67 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.55 (1H, *t*, *J* = 7.6Hz, H-4'), 7.46 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.08 (1H, *d*, *J* = 7.6Hz, H-6), 6.92 (1H, *t*, *J* = 7.6Hz, H-5), 6.39 (1H, *d*, *J* = 8.0Hz, 1H, H-4), 3.86-3.77 (1H, *m*, H_a-1''), 3.31-3.23 (1H, *m*, H_b-1''), 2.28 (3H, *s*, CH_3-2), 2.26 (3H, *s*, CH_3-3), 1.02 (3H, *t*, *J* = 7.2Hz, CH_3-2''); EI-MS (*m/z*): 289 (M^+, 9.3%), 148 (BP, 100%), 141, 120, 105, 77, 51.

_N_-(2,3-Dimethylphenyl)-_N_-propan-1-ylbenzenesulfonamide (5b)

Yield: 99 %; light pink solid; mp. 79 °C; Molecular formula: $C_{17}H_{21}NO_2S$; Molecular weight: 303; Analysis. Calcd: C, 67.32; H, 6.93; N, 4.62; S, 10.56. Found: C, 66.35; H, 6.70; N, 4.61; S, 10.57; IR (cm^{-1}) u_{max}:3071 (Ar C-H), 2978 (Alkane C-H), 1586 (Ar C=C), 1336 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.66 (2H, *d*, *J* = 7.2Hz, H-2', H-6'), 7.56 (1H, *t*, *J* = 7.6Hz, H-4'), 7.46 (2H, *t*, *J* = 7.6Hz, 2H, H-3', H-5'), 7.08 (1H, *d*, *J* = 7.6Hz, H-6), 6.91 (1H, *t*, *J* = 7.6Hz, H-5), 6.89 (1H, *d*, *J* = 8.0Hz, H-4), 3.69-3.62 (1H, *m*, H_a-1''), 3.18-3.12 (1H, *m*, H_b-1''), 2.27 (3H, *s*, CH_3-2), 2.25 (3H, *s*, CH_3-3), 1.53-1.33 (2H, *m*, H-2''), 0.83 (3H, *t*, *J* = 7.2Hz, CH_3-3''); EI-MS (*m/z*): 303 (M^+, 12.0%), 141, 120 (BP, 100%), 105, 77, 51.

N-(2,3-Dimethylphenyl)-N-(1-methylethyl) benzenesulfonamide (5c)

Yield: 53 %; light pink solid; mp. 103.1 °C; Molecular formula: $C_{17}H_{21}NO_2S$; Molecular weight: 303; Analysis. Calcd: C, 67.32; H, 6.93; N, 4.62; S, 10.56. Found: C, 66.56; H, 6.69; N, 4.37; S, 10.70; IR (cm^{-1}) u_{max}:3066 (Ar C-H), 2977 (Alkane C-H), 1584 (Ar C=C), 1335 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.72 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.53 (1H, *t*, *J* = 7.6Hz, H-4'), 7.44 (2H, *t*, *J* = 7.2Hz, H-3', H-5'), 7.13 (1H, *d*, *J* = 7.6Hz, H-6), 6.96 (1H, *t*, *J* = 7.6Hz, H-5), 6.56 (1H, *d*, *J* = 7.9Hz, H-4), 4.63-4.56 (1H, *m*, H-1''), 2.28 (3H, *s*, CH_3-2), 2.23 (3H, *s*, CH_3-3), 1.02 (3H, *d*, *J* = 6.4Hz, CH_3-2''), 0.92 (3H, *d*, *J* = 6.4Hz, CH_3-3''); EI-MS (*m/z*): 303 (M^+, 33.4%), 141, 120 (BP, 100%), 105, 77, 51.

N-(2,3-Dimethylphenyl)-N-butan-1-ylbenzenesulfonamide (5d)

Yield: 98 %; light pink solid; mp. 54.7 °C; Molecular formula: $C_{18}H_{23}NO_2S$; Molecular weight: 317; Analysis. Calcd: C, 68.13; H, 7.25; N, 4.41; S, 10.09. Found: C, 66.91; H, 6.87; N, 4.09; S, 10.18; IR (cm^{-1}) u_{max}:3070 (Ar C-H), 2955 (Alkane C-H), 1583 (Ar C=C), 1335 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.65 (2H, *d*, *J* = 7.2Hz, 2H, H-2', H-6'), 7.56 (1H, *t*, *J* = 7.6Hz, H-4'), 7.45 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.08 (1H, *d*, *J* = 7.2Hz, H-6), 6.91 (1H, *t*, *J* = 7.6Hz, H-5), 6.39 (1H, *d*, *J* = 8.0Hz, H-4), 3.74-3.67 (1H, *m*, H_a-1''), 3.20-3.13 (1H, *m*, H_b-1''), 2.28 (3H, *s*, CH_3-2), 2.25 (3H, *s*, CH_3-3), 1.52-1.41 (2H, *m*, H-2''), 1.30-1.26 (2H, *m*, H-3''), 0.92 (3H, *t*, *J* = 7.2Hz, CH_3-4''); EI-MS (*m/z*): 317 (M^+, 22.5%), 141 (BP, 100%), 120, 105, 77, 57, 51.

N-(2,3-Dimethylphenyl)-N-(1-methylpropyl) benzenesulfonamide(5e)

Yield: 41 %; pinkish white solid; m.p. 92.6 °C; Molecular formula: $C_{18}H_{23}NO_2S$; Molecular weight: 317; Analysis. Calcd: C, 68.13; H, 7.25; N, 4.41; S, 10.09. Found: C, 66.65; H, 6.88; N, 4.64; S, 10.34;IR (cm^{-1}) u_{max}: 3062 (Ar C-H), 2973 (Alkane C-H), 1585 (Ar C=C), 1337 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.71 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.53 (1H, *t*, *J* = 7.2Hz, H-4'), 7.44 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.12 (1H, *d*, *J* = 7.2Hz, H-6), 6.95 (1H, *t*, *J* = 7.6Hz, H-5), 6.54 (1H, *d*, *J* = 8.0Hz, H-4), 4.29-4.23 (1H, *m*, H-1''), 2.26 (3H, *s*, CH_3-2), 2.23 (3H, *s*, CH_3-3), 1.01 (3H, *d*, *J* = 6.8 Hz,H-4''), 0.90-0.85 (2H, *m*, H-2''), 0.74 (3H, *t*, *J* = 7.2Hz, CH_3-3''); EI-MS (*m/z*): 317 (M^+, 16.7%),147 (BP, 100%), 141, 120, 105, 77, 57, 51.

N-(2,3-Dimethylphenyl)-N-pentylbenzenesulfonamide (5f)

Yield: 93 %; pinkish white solid; mp. 72.3 °C; Molecular formula: $C_{19}H_{25}NO_2S$; Molecular weight: 331; Analysis. Calcd: C, 68.88; H, 7.55; N, 4.22; S, 9.66. Found: C, 68.26; H, 7.16; N, 4.52; S, 9.95;IR (cm^{-1}) u_{max}: 3069 (Ar C-H), 2959 (Alkane C-H), 1585(Ar C=C), 1332 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.65 (2H, *d*, *J* = 7.2Hz, H-2', H-6'), 7.55 (1H, *t*, *J* = 7.6Hz, H-4'), 7.45 (2H, *t*, *J* = 7.2Hz, H-3', H-5'), 7.08 (1H, *d*, *J* = 7.2Hz, H-6), 6.91 (1H, *t*, *J* = 7.6Hz, H-5), 6.39 (1H, *d*, *J* = 8.0Hz, H-4), 3.73-3.65 (1H, *m*, H_a-1''), 3.20-3.13 (1H, *m*, H_b-1''), 2.28 (3H, *s*, CH_3-2), 2.25 (3H, *s*, CH_3-3), 1.51-1.45 (2H, *m*, H-2''), 1.26-1.20 (4H, *m*, H-3'', H-4''), 0.81 (3H, *t*, *J* = 6.8Hz, CH_3-5''); EI-MS (*m/z*): 331 (M^+, 34.1%), 141, 120, 105, 77 (BP, 100%), 71, 51.

N-(2,3-Dimethylphenyl)-N-(1-methylbutyl) benzenesulfonamide (5g)

Yield: 89 %; light brownish liquid (extracted with chloroform); Molecular formula: $C_{19}H_{25}NO_2S$; Molecular weight: 331; Analysis. Calcd: C, 68.88; H, 7.55; N, 4.22; S, 9.66. Found: C, 68.23; H, 7.46; N, 4.19; S, 8.92;IR (cm^{-1}) u_{max}: 3070 (Ar C-H), 2936 (Alkane C-H), 1580 (Ar C=C), 1341

(S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.71 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.53 (1H, *t*, *J* = 7.2Hz, H-4'), 7.44 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.12 (1H, *d*, *J* = 7.2Hz, H-6), 6.95 (1H, *t*, *J* = 7.6Hz, H-5), 6.64 (1H, *d*, *J* = 7.6Hz, H-4), 4.36-4.33 (1H, *m*, H-1''), 1.32-1.23 (2H, *m*, H-2''), 1.15-1.12 (2H, *m*, H-3''), 1.01 (3H, *d*, *J* = 6.8Hz, CH_3-5''), 0.77 (3H, *t*, *J* = 7.2Hz, CH_3-4''); EI-MS (*m/z*): 331 (M^+, 1.1%), 141, 120, 105, 77 (BP, 100%), 71, 56, 51.

N-(2,3-Dimethylphenyl)-N-benzylbenzene-sulfonamide (5h)

Yield: 88 %; shiny pinkish white solid; mp. 80.8 °C; Molecular formula: $C_{21}H_{21}NO_2S$; Molecular weight: 351; Analysis. Calcd: C, 71.79; H, 5.98; N, 3.98; S, 9.11. Found: C, 70.32; H, 5.72; N, 3.66; S, 9.26;IR (cm^{-1}) u_{max}: 3054 (Ar C-H), 2927 (Alkane C-H), 1585 (Ar C=C), 1330 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.71 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.59 (1H, *t*, *J* = 7.6Hz, H-4'), 7.48 (2H, *t*, *J* = 7.2Hz, H-3', H-5'), 7.18-7.09 (5H, *m*, H-2'' to H-6''), 7.01 (1H, *d*, *J* = 7.6Hz, H-6), 6.88 (1H, *t*, *J* = 7.6, H-5), 6.41 (1H, *d*, *J* = 8.0Hz, H-4), 4.96 (1H, *d*, *J* = 13.6 Hz, H_a-7''), 4.24 (1H, *d*, *J* = 13.6 Hz, H_b-7''), 2.14 (3H, *s*, CH_3-2), 1.88 (3H, *s*, CH_3-3); EI-MS (*m/z*): 351 (M^+, 13.4%), 141, 120, 105, 91 (BP, 100%), 77, 51.

N-(2,3-Dimethylphenyl)-N-(2-chlorobenzyl) benzenesulfonamide (5i)

Yield: 98 %; pinkish white solid; mp. 109 °C; Molecular formula: $C_{21}H_{20}ClNO_2S$; Molecular weight: 385; Analysis. Calcd: C, 65.45; H, 5.19; N, 3.63; S, 8.31. Found: C, 65.01; H, 5.38; N, 3.80; S, 7.71;IR (cm^{-1}) u_{max}: 3063 (Ar C-H), 2927 (Alkane C-H), 1582 (Ar C=C), 1342 (S=O), 576 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.72 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.60 (1H, *t*, *J* = 7.6Hz, H-4'), 7.49 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.33 (1H, *dd*, *J* = 7.2, 3.2 Hz, H-3''), 7.19 (1H, *dd*, *J* = 6.4, 2.8Hz, H-6''), 7.13-7.09 (2H, *m*, H-4'', H-5''), 7.00 (1H, *d*, *J* = 7.6 Hz, H-6), 6.87 (1H, *t*, *J* = 7.6 Hz, H-5), 6.52 (1H, *d*, *J* = 7.6Hz, H-4), 5.08 (1H, *d*, *J* = 14.0 Hz, H_a-7''), 4.53 (1H, *d*, *J* = 14.0 Hz, H_b-7''), 2.13 (3H, *s*, CH_3-2), 1.91 (3H, *s*, CH_3-3); EI-MS (*m/z*): 387 ($[M+2]^+$, 6.1%), 385 (M^+, 17.9%), 141, 120, 125 (BP, 100%), 105, 90, 77, 51.

N-(2,3-Dimethylphenyl)-N-(4-chlorobenzyl) benzenesulfonamide (5j)

Yield: 50 %; pinkish white solid; mp. 104.8 °C; Molecular formula: $C_{21}H_{20}ClNO_2S$; Molecular weight: 385; Analysis. Calcd for: C, 65.45; H, 5.19; N, 3.63; S, 8.31. Found: C, 64.86; H, 5.45; N, 3.35; S, 8.18; IR (cm^{-1}) u_{max}:3066 (Ar C-H), 2923 (Alkane C-H), 1584 (Ar C=C), 1342 (S=O), 581 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.69 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.59 (1H, *t*, *J* = 7.2Hz, H-4'), 7.48 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.15 (2H, *d*, *J* = 8.4 Hz, H-3'', H-5''), 7.05 (2H, *d*, *J* = 8.4Hz, H-2'', H-6''), 7.02 (1H, *d*, *J* = 8.0 Hz, H-6), 6.88 (1H, *t*, *J* = 7.6 Hz, H-5), 6.39 (1H, *d*, *J* = 7.6Hz, H-4), 4.91 (1H, *d*, *J* = 13.6 Hz, H_a-7''), 4.21 (1H, *d*, *J* = 13.6 Hz, H_b-7''), 2.15 (3H, *s*, CH_3-2), 1.89 (3H, *s*, CH_3-3); EI-MS (*m/z*): 387 ($[M+2]^+$, 5.2%), 385 (M^+, 15.4%), 141, 120, 125 (BP, 100%), 105, 90, 77, 51.

N-(2,3-Dimethylphenyl)-N-(4-bromobenzyl) benzenesulfonamide (5k)

Yield: 98 %; off-white solid; mp. 106.3 °C; Molecular formula: $C_{21}H_{20}BrNO_2S$; Molecular weight: 430; Analysis. Calcd: C, 58.60; H, 4.65; N, 3.25; S, 7.44. Found: C, 58.19; H, 4.72; N, 3.41; S, 7.12;IR (cm^{-1}) u_{max}:3065 (Ar C-H), 2924 (Alkane C-H), 1586 (Ar C=C), 1342 (S=O), 585 (C-Br); ^{1}H-NMR (400 MHz, $CDCl_3$, δ inppm): 7.69 (2H, *d*, *J* = 7.2Hz, H-2', H-6'), 7.59 (1H, *t*, *J* = 7.6Hz, H-4'), 7.48 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.30 (2H, *d*, *J* = 8.4 Hz, H-3'', H-5''), 7.02 (1H, *d*, *J* = 8.0 Hz, H-6), 6.99 (2H, *d*, *J* = 8.4Hz, H-2'', H-6''), 6.88 (1H, *t*, *J* = 7.6 Hz, H-5), 6.39 (1H, *d*, *J* = 8.0Hz, H-4), 4.89 (1H, *d*, *J* = 13.6 Hz, H_a-7''), 4.20 (1H, *d*, *J* = 14.0 Hz, H_b-7''), 2.16 (3H, *s*, CH_3-2), 1.90 (3H, *s*, CH_3-3); EI-MS (*m/z*): 432 ($[M+2]^+$, 7.8%), 430 (M^+, 8.2%), 141, 120, 169, 105, 90, 77 (BP, 100%), 51.

N-(2,3-Dimethylphenyl)-N-(phenylethyl)ben-zenesulfonamide (5l)

Yield: 89 %; pinkish white solid; mp. 103.5 °C; Molecular formula: $C_{22}H_{23}NO_2S$; Molecular weight: 365; Analysis. Calcd for: C, 72.32; H, 6.30; N, 3.83; S, 8.76. Found: C, 71.71; H, 6.12; N, 3.95; S, 8.90; IR (cm^{-1}) u_{max}:3064 (Ar C-H), 2922 (Alkane C-H), 1582 (Ar C=C), 1342 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ inppm): 7.64 (2H, *d*, *J* = 8.0Hz, H-2', H-6'), 7.54 (1H, *t*, *J* = 7.2Hz, H-4'), 7.44 (2H, *t*, *J* = 8.0Hz, H-3', H-5'), 7.22 (2H, *d*, *J* = 7.6 Hz, H-3'', H-5''), 7.17 (1H, *t*, *J* = 6.8 Hz, H-4''), 7.11 (1H, *d*, *J* = 7.2 Hz, H-6), 7.06 (2H, *d*, *J* = 7.2Hz, H-2'', H-6''), 6.95 (1H, *t*, *J* = 7.6 Hz, H-5), 6.47 (1H, *d*, *J* = 8.0Hz, H-4), 4.00-3.93 (1H, *m*, H_a-8''), 3.46-3.36 (1H, *m*, H_b-8''), 2.88-2.82 (1H, *m*, H_a-7''), 2.68-2.62 (1H, *m*, H_b-7''), 2.29 (3H, *s*, CH_3-2), 2.21 (3H, *s*, CH_3-3); EI-MS (*m/z*): 365 (M^+, 25.8%), 141, 120, 105, 104, 77 (BP, 100%), 51.

N-(2,3-Dimethylphenyl)-N-allylbenzenesulfonamide (5m)

Yield: 88 %; brownish white solid; mp. 60.5 °C; Molecular formula: $C_{17}H_{19}NO_2S$; Molecular weight: 301; Analysis. Calcd: C, 67.77; H, 6.31; N, 4.65; S, 10.63. Found: C, 66.83; H, 6.08; N, 4.30; S, 10.72;IR (cm^{-1}) u_{max}: 3063(Ar C-H), 2946 (Alkane C-H), 1580 (Ar C=C), 1705 (Alkene C=C), 1341 (S=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 7.68 (2H, *d*, *J* = 7.6Hz, H-2', H-6'), 7.56 (1H, *t*, *J* = 7.2Hz, H-4'), 7.47 (2H, *t*, *J* = 7.6Hz, H-3', H-5'), 7.07 (1H, *d*, *J* = 7.6 Hz, H-6), 6.90 (1H, *t*, *J* = 7.6 Hz, H-5), 6.39 (1H, *d*, *J* = 7.6Hz, H-4), 5.76-5.69 (1H, *m*, H-2''), 4.97 (1H, *dd*, *J* = 6.4, 2.8 Hz, H_b-3''), 4.30 (1H, *dd*, *J* = 7.6, 2.8 Hz, H_a-3''), 3.87 (2H, *d*, *J* = 6.4 Hz, H-1''), 2.26 (3H, *s*, CH_3-2), 2.23 (3H, *s*, CH_3-3); EI-MS (*m/z*): 301 (M^+, 20.9%), 141, 120, 105, 77 (BP, 100%), 51.

Antibacterial activity

The synthesized compounds were evaluated against two Gram-positive and four Gram-negative bacterial strains using ampicillin and ciprofloxacin as standards. (Table 1). The compounds showed moderate to significant activity against bacterial strains except compounds, **5a**, **5d** and **5e**. The compound **3** was active against one strain of gram-positive bacteria only. The preliminary screening showed that compound, **5c**, **5f**, **5g**, **5i**, **5l** and **5m** were the most active ones against gram-positive and compounds, **5b**, **5f**, **5j** and **5k** were more active against gram-negative bacterial strains.

Anti-enzymatic activity

The screening against α-glucosidase enzyme demonstrated that the most of compounds were active with good inhibitory potential except **5a**, **5b**, **5c** and **3** (Table-2). The molecules **5g** and **5j** were found to be excellent inhibitors with their IC_{50} values of 59.53 ± 0.008 and 55.31 ± 0.01 µmoles/L respectively, relative to acarbose a reference standard with IC_{50} value 38.25 ± 0.12µmoles/L.

Hemolytic activity

The compounds **5a**, **5b**, **5d**, **5h** and **5j** exhibited cytotoxic activity with percent lysis of 27.2 ± 0.24, 22.4 ± 0.21, 21.7 ± 0.68, 18.2 ± 0.56 and 18.9 ± 0.23 respectively, which was much lower than the positive control triton X-100 (0.1 % v/v) which has percent lysis value of 100.The compound **5e** was the least cytotoxic in this series with percent lysis of 5.2 ± 0.41.

DISCUSSION

Sulfonamide **3** was obtained as off-white powder. Although its structure has been established by the x-ray crystallography [21] but more structural details are presented here for the first time. Its molecular formula, $C_{14}H_{15}NO_2S$, was determined through CHNS analysis with percentage of elements as C: 64.03 %; H: 6.40 %; N: 5.52 % and S: 11.73 %.

Table 1: MIC of synthesized compounds

Compound	MIC					
	***B. subtilis* (+)**	***S. aureus* (+)**	***E. coli* (-)**	***S. sonnei* (-)**	***S. typhi* (-)**	***P. aeruginosa* (-)**
3	16.11±0.19	-	-	-	-	-
5a	-	-	-	-	-	-
5b	11.46±0.22	-	12.11±0.27	19.83±0.04	16.56±0.31	11.7 ±0.32
5c	14.28±0.24	11.36 ±0.20	16.23±0.14	-	13.01±0.18	11.06±0.70
5d	-	-	-	-	-	-
5e	-	-	-	-	-	-
5f	17.40±0.28	12.51 ±0.31	11.36±0.00	15.04±0.33	13.17±0.18	13.34±0.12
5g	18.52±0.00	19.33 ±0.32	16.13±0.57	12.62±0.19	-	-
5h	-	15.52 ±0.09	19.00±0.18	-	16.33±0.28	14.40±1.23
5i	10.78±0.09	13.47 ±0.52	18.86±0.01	-	-	12.20±0.31
5j	12.52±0.73	-	17.36±0.18	11.86±0.12	12.33±0.15	13.22±0.50
5k	-	10.78 ±0.46	16.54±0.38	16.43±0.21	10.89±0.11	13.98±0.90
5l	17.43±0.24	12.52 ±0.11	15.36±0.28	18.09±0.83	-	14.52±0.24
5m	10.10±0.29	17.43 ±0.13	-	-	-	14.55±0.02
Ampicillin	**13.92±0.29**	**11±0.01**	**11.32±0.13**	**13.11±0.11**	**12.78±0.21**	**16.86±0.31**
Ciprofloxacin	**8.36±0.12**	**9.42±0.11**	**8.21±0.02**	**15.34±0.22**	**7.59±0.11**	**11.03±0.10**

The molecular formula was also supported by EI–MS, showing a $[M]^+$ ion peak at m/z 261 and by the integration curves in its ^{1}H-NMR spectrum indicating the number of protons for each peak. The signals in ^{1}H-NMR spectrum, appearing at δ 7.69 (d, J = 7.6Hz, 2H, H-2', H-6'), 7.53 (t, J = 7.6 Hz, 1H, H-4') and 7.41 (t, J = 7.6 Hz, 2H, H-3', H-5') were typical for the protons of the benzenesulfonyl group present in the molecule. Furthermore, the signals resonating at δ 7.13 (d, J = 7.6Hz, 1H, H-6), 6.96 (t, J = 7.6Hz, 1H, H-5), 6.46 (d, J = 6.39Hz, 1H, H-4), 2.18 (s, 3H, CH3-2) and 1.91 (s, 3H, CH3-3) were assigned to the protons of substituted aniline ring. FT-IR supported the structure by absorption bands at 3208 (N-H), 3042 (Ar C-H), 2924 (Alkane C-H), 1583 (Ar C=C) and 1324 (S=O) cm^{-1}. On the basis of above accumulative manifests, the structure of **3** was confirmed as N-(2,3-dimethylphenyl)benzenesulfonamide. Similarly, the structures of other compounds, **5a-m**, were characterized.

The antibacterial activity results declared the compound **5f** bearing aliphatic straight chain with moderate length at nitrogen atom was the most active molecule against all the bacterial strains used in this study. The compounds with sulfamoyl moiety have been introduced as valuable biologically active compounds and in accordance with our previous findings [13-16], the overview of synthesized compounds hereby showed that N-substitution of sulfamoyl group with aliphatic chains and halogenated aromatic compounds presented best activity because of best fit to active site of enzyme and better π-π interactions. The two molecules, **5g** and **5j**, showed good activity against α-glucosidase probably because of branched long alkyl chain in **5g** and p-substituted halogenated aralkyl group in **5j**, presented better binding interactions with the active site of enzyme. The compound **5e** was the least cytotoxic in this series but it was inferred that all the compounds can be used as possible therapeutic agents.

CONCLUSION

All the compounds have been structurally identified by spectral data and screened for antibacterial, α-glucosidase and hemolytic analysis. The synthesized compounds exhibit cytotoxicity and antibacterial potentials.

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