

Synthesis and Antibacterial Activity of *N,N*-Diethylamide Bearing Benzenesulfonamide Derivatives

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Authors' contributions

This work was carried out in collaboration between the authors. Author OOA carried out the synthesis and wrote the first draft. Author OBF designed the scheme and the protocol for synthetic pathway. Author FW managed the analysis of the study and spectroscopic evaluation. Author JOE did the collation of the data and editing of the write-up. Author ZS carried out all the antibacterial screening. All authors read and approved the final manuscript.

Research Article

Received 24th December 2012
Accepted 3rd January 2013
Published 25th January 2013

ABSTRACT

Sulfonamides are known to represent a class of medicinally important compounds which are extensively used as antibacterial agents. Hence, a series of new *N,N*-diethyl amide bearing sulfonamides (2a-k) were synthesized via amidation of easily prepared benzenesulfonamide precursors (1a-k). The chemical structures of all synthesized compounds were substantiated using spectroscopic means such as IR, Mass spectra and ¹H-NMR as well as analytical data. The antimicrobial activity of these compounds along with streptomycin, was investigated on *Escherichia coli* and *Staphylococcus aureus*. The

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results showed that this skeletal framework exhibited marked potency as antibacterial agents. The most active antibacterial agent against both targeted organisms was *N,N*-diethyl-1-(phenylsulfonyl) piperidine-2-carboxamide (2b).

Keywords: 2-(Phenylsulfonamido) acetic acid; inhibition zone; *N,N*-diethylamide; antibacterial study; streptomycin.

1. INTRODUCTION

The pharmacological applications of sulfonamides have been proven to attract continuous attention since earlier discovery of sulfanilamide [1]. The chemical properties of sulfonamides have recently shown them to be highly efficient synthons in the preparation of various valuable biologically active compounds [2]. In view of the versatile utilization of such scaffolds as ligands, various researchers have attempted and embarked upon designing and synthesizing various novel metal based templates [3]. Sulfonamides inhibit the multiplication of bacteria by acting as competitive inhibitors of *p*-aminobenzoic acid (PABA) in the folic acid metabolism cycle [4]. They are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases [5]. They have been reported to possess, among others, antimicrobial [6], analgesic [7], antiinflammatory [8], anti-HIV [9], anticancer [10], anticonvulsant [11], antiviral [12], antitumoral [13], antibacterial [14], antiplatelet aggregation [15] and antimalarial [16] properties.

The sulfonamide of paramount importance in this study is benzenesulfonamide which is an integral part of many drugs and drug-like scaffolds [17,18]. Many derivatives of benzenesulfonamide have been explored as important starting materials and reactive intermediates in various organic syntheses [19]. For example, 2-hydroxyalkylbenzene sulfonamides have been reported as the important starting materials produced in large quantities. The upsurge of widespread multi-drug resistance microorganisms and emergence of new diseases have been reported as a major threat to human health [20]. In view of this occurrence of microorganisms' resistance to drugs currently in use and emergence of new diseases, there is a continuous need for the synthesis of new organic compounds as potential antimicrobial agents. Another motivation behind synthesis of targeted sulfonamide was based on the earlier report that disubstituted amides are more biologically active than the non-substituted counterparts [21]. Therefore, incorporation of amide group into the benzenesulfonamide was carried out in order to vary or boost the antibacterial activity of such templates. Thus, it is conceivable to develop a series of *N,N*-diethyl amide bearing benzenesulfonamides by highly expeditious amidation technique with the aim of investigating their antibacterial properties.

2. MATERIALS AND METHODS

2.1 General Conditions

The ¹H-NMR spectra were recorded in either CDCl₃ or DMSO-d₆ on NMR Bruker DPX 400 spectrometer operating at 400 MHz. Tetramethyl silane (TMS) was used as internal standard with the deuterium signal of the solvent as the lock and chemical shifts δ recorded in ppm. The melting points were determined on XT-4 Digital Binocular Microscope melting point apparatus manufactured by Beijing Technical Instrument Co. Ltd. and were uncorrected. IR

spectra were run on Varian Excalibur HE 3100 FT-IR Spectrometer while the Mass Spectra were obtained using Waters GCT Premier Spectrometer. The elemental analyses (C, H, N) of the compounds were performed using Flash EA 1112 Elemental Analyzer.

In addition, the pH was monitored and confirmed during acidification by using Portable pH Meter Model PHB4. All drying were conducted at reduced pressure with DHG-9023A Vacuum Oven. The reaction progress was monitored with TLC using CHCl₃/CH₃OH (9:1) solvent system and the developed plates were visualized under UV lamp and/or in iodine tank where necessary. Column chromatographic purifications were carried out on the products using CHCl₃/CH₃OH (9:1) solvent system and Merck silica gel F (Mesh 200-300) as the mobile and stationary phase respectively. Organic solutions were dried over anhydrous Na₂SO₄ and concentrated with a RE-2000B Buchi Rotary Evaporator at reduced pressure. At all stage of the experiments, the synthetic protocols were effected in bone dried solvents under nitrogen atmosphere in dried glassware which were wiped with stream flow of nitrogen gas prior to use. Other reagents were used directly after ascertaining the purity condition.

2.2 Synthesis

2.2.1 General procedure for synthesis of benzenesulfonamides (1a-k)

To a solution of amino acid (25.00 mmol) was added Na₂CO₃ (5.57 g, 52.5 mmol) in H₂O (30.00 mL) at 0°C, cooled to -5°C using ice-bath followed by addition of benzenesulfonyl chloride, (5.30 g, 3.84 mL, 30.00 mmol) in three portions over a period of 1 h. The reacting mixture was then warmed to room temperature and allowed to stir for 4 h. Upon completion of the reaction, 20 % aqueous HCl solution was added with continuous stirring to avoid foaming on the surface until the pH 2.00 was attained. The solid separated out and was allowed to settle down over night and the product isolated via suction filtration. The filtered crude product was washed with pH 2.20 buffer and dried in a vacuum oven at 60°C for 12 h to afford crude solid which was purified by column chromatography on Merck silica gel F (Mesh 200-300) using CHCl₃/CH₃OH, 9:1 solvent system to afford benzenesulfonamides (1a-k) in good to excellent yields (73.20 – 96.60%).

2.2.1.1 1-(Phenylsulfonyl) pyrrolidine-2-carboxylic acid (1a)

Yield 6.11 g (95.7%), mp 75-77°C, R_f = 0.77 (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (CDCl₃) δ: 7.92-7.90 (d, J = 7.60 Hz, 2H, Ar-H), 7.68-7.60 (m, 3H, Ar-H), 4.32-4.30 (dd, J₁ = 3.20 Hz, J₂ = 12.00 Hz, 1H, CH-COOH), 3.56-3.54 (m, 1H, CH_a of CH₂-N), 3.33-3.27 (m, 1H, CH_b of CH₂-N), 2.18-2.15 (m, 1H, CH), 1.97-1.95 (m, 2H, CH₂), 1.83-1.79 (m, 1H, CH). IR (KBr) cm⁻¹: 3064.89 (CH aromatic), 2956.88 (CH aliphatic), 1728.21 (C=O of COOH), 1352.13, 1157.33 (SO₂ two bands), 689.58 (Ar-H). MS: in m/z (rel. %): 211 (10%), 210 (100%), 141 (39%), 70.10 (11%). Anal. calcd. for C₁₁H₁₃NO₄S (255.29): C, 51.75; H, 5.13; N, 5.49. Found: C, 51.72; H, 4.92; N, 5.35.

2.2.1.2 1-(Phenylsulfonyl) piperidine-2-carboxylic acid (1b)

Yield 6.50 g (96.6%), mp 81-82°C, R_f = 0.79 (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (CDCl₃) δ: 10.06-9.95 (s-br, 1H, OH of COOH), 7.81-7.78 (d, J = 8.76 Hz, 2H, Ar-H), 7.55-7.46 (m, 3H, Ar-H), 4.78-4.77 (d, J = 5.00 Hz, 1H, CH-COOH), 3.77-3.74 (d, J = 10.00 Hz, 1H, CH_a of CH₂-N), 3.23-3.16 (dt, J₁ = 2.8 Hz, J₂ = 10.00 Hz, 1H, CH_b of CH₂-N), 2.17-2.13 (m, 1H, CH), 1.71-1.66 (m, 3H, CH₂ & CH), 1.46-1.41 (m, 1H, CH), 1.32-1.23 (m, 1H, CH). MS: in m/z (rel. %): 230.12 (3%), 186.98 (62%), 154.08 (42%), 110.01 (75%), 103.04 (100%), 84.00 (29%),

78.04 (63%), 29.99 (64%). Anal. calcd. for $C_{12}H_{15}NO_4S$ (269.32): C, 53.52; H, 5.61; N, 5.20. Found: C, 53.71; H, 5.59; N, 5.31.

2.2.1.3 2-(Phenylsulfonamido) acetic acid (1c)

Yield 3.94 g (73.2%), mp 160-161°C, $R_f = 0.45$ ($CHCl_3/CH_3OH$, 9:1, at RT). 1H -NMR ($DMSO-d_6$) δ : 12.68 (s-br, 1H, OH of COOH), 8.07-8.04 (t, $J = 6.00$ Hz, 1H, $\underline{NH-CH_2}$), 7.80-7.78 (d, $J = 8.00$ Hz, 2H, Ar-H), 7.65-7.55 (m, 3H, Ar-H), 3.58-3.57 (d, $J = 6.00$ Hz, 2H, $\underline{CH_2-NH}$). IR (KBr) cm^{-1} : 3313.72 (N-H), 3088.21 (C-H aromatic), 2974.23 (CH aliphatic), 1726.30 (C=O of COOH), 1321.31, 1157.29 (SO_2 two bands), 690.51 (Ar-H). MS: in m/z (rel. %): 218.03 ($M^+ + 3$, 3%), 210.07 (28%), 142.01 (26%), 63.96 (100%), 43.01 (37%). Anal. calcd. for $C_8H_9NO_4S$ (215.23): C, 44.65; H, 4.21; N, 6.51. Found: C, 44.45; H, 4.32; N, 6.49.

2.2.1.4 2-(Phenylsulfonamido) propanoic acid (1d)

Yield 4.72 g (82.4%), mp 118-119°C, $R_f = 0.70$ ($CHCl_3/CH_3OH$, 9:1, at RT). 1H -NMR ($DMSO-d_6$) δ : 12.62 (s-br, 1H, OH of COOH), 8.16-8.14 (d, $J = 8.36$ Hz, 1H, $\underline{NH-CH}$), 7.80-7.78 (d, $J = 8.52$ Hz, 2H, Ar-H), 7.64-7.54 (m, 3H, Ar-H), 3.78-3.73 (dt, $J_1 = 7.20$ Hz, $J_2 = 8.36$ Hz, 1H, $\underline{NH-CH-CH_3}$), 1.14-1.12 (d, $J = 7.20$ Hz, 3H, $\underline{CH_3-CH}$). IR (KBr) cm^{-1} : 3327.19, 3267.41 (N-H), 3064.92 (C-H aromatic), 2989.71 (CH aliphatic), 1720.49 (C=O of COOH), 1338.61, 1153.42 (SO_2 two bands), 725.23 (Ar-H). MS: in m/z (rel. %): 157.02 (19%), 141.01 ($PhSO_2^+$, 5%), 93.06 (22%), 44.09 (CO_2^+ , 4%). Anal. calcd. for $C_9H_{11}NO_4S$ (229.26): C, 47.15; H, 4.84; N, 6.11. Found: C, 46.98; H, 4.82; N, 6.06.

2.2.1.5 3-Mercapto-2-(phenylsulfonamido) propanoic acid (1e)

Yield 2.75 g (84.1%), mp 176-177°C, $R_f = 0.35$ ($CHCl_3/CH_3OH$, 9:1, at RT). 1H -NMR ($DMSO-d_6$) δ : 12.95 (s-br, 1H, OH of COOH), 8.35-8.32 (d, $J = 8.40$ Hz, 1H, $\underline{NH-CH}$), 7.77-7.75 (d, $J = 8.64$ Hz, 2H, Ar-H), 7.64-7.53 (m, 3H, Ar-H), 3.93-3.88 (dd, $J_1 = 8.40$ Hz, $J_2 = 20.00$ Hz, 1H, $\underline{NH-CH-CH_a}$), 2.92-2.87 (dd, $J_1 = 5.60$ Hz, $J_2 = 20.00$ Hz, 1H, CH_a of CH_2-S), 2.62-2.56 (dd, $J_1 = 8.22$ Hz, $J_2 = 20.00$ Hz, 1H, CH_b of CH_2-SH). IR (KBr) cm^{-1} : 3294.41 (N-H), 3057.13 (CH aromatic), 2922.21 (CH aliphatic), 1735.89 (C=O of COOH), 1581.60 (C=C), 1328.91, 1147.61 (SO_2 two bands), 688.59 (Ar-H). Anal. calcd. for $C_9H_{11}NO_4S_2$ (261.32): C, 41.37; H, 4.24; N, 5.36. Found: C, 41.34; H, 4.06; N, 5.29.

2.2.1.6 4-(Methylthio)-2-(phenylsulfonamido) butanoic acid (1f)

Yield 2.98 g (82.3%), mp 128-130°C, $R_f = 0.77$ ($CHCl_3/CH_3OH$, 9:1, at RT). 1H -NMR ($DMSO-d_6$) δ : 12.73 (s-br, 1H, OH of COOH), 8.21-8.19 (d, $J = 8.8$ Hz, 1H, $\underline{NH-CH}$), 7.78-7.76 (d, $J = 8.52$ Hz, 2H, Ar-H), 7.63-7.54 (m, 3H, Ar-H), 3.87-3.84 (dt, $J_1 = 4.00$ Hz, $J_2 = 8.80$ Hz, 1H, $\underline{NH-CH-CH_2}$), 2.36-2.25 (m, 2H, CH_2S), 1.91 (s, 3H, CH_3), 1.82-1.73 (m, 2H, $\underline{CH_2-CH_2-S}$). IR (KBr) cm^{-1} : 3253.91 (N-H), 3001.31 (CH aromatic), 2914.39 (CH aliphatic), 1724.41 (C=O of COOH), 1338.62, 1159.22 (SO_2 two bands), 690.51 (Ar-H). MS: in m/z (rel. %): 210.07 (9%), 142.01 ($PhSO_2H^+$, 30%), 141.01 ($PhSO_2^+$, 19%), 77.04 (100%), 43.99 (CO_2^+ , 66%). Anal. calcd. for $C_{11}H_{15}NO_4S_2$ (289.37): C, 45.66; H, 5.22; N, 4.84. Found: C, 45.54; H, 5.19; N, 4.67.

2.2.1.7 3-Methyl-2-(phenylsulfonamido) butanoic acid (1g)

Yield 5.08 g (79.0%), mp 143-144°C, $R_f = 0.76$ ($CHCl_3/CH_3OH$, 9:1, at RT). 1H -NMR ($CDCl_3$) δ : 7.85-7.83 (d, $J = 8.68$ Hz, 2H, Ar-H), 7.58-7.54 (m, 1H, Ar-H), 7.51-7.47 (m, 2H, Ar-H),

5.15-5.13 (d, $J = 12.00$ Hz, 1H, NH-CH), 3.82-3.79 (dd, $J_1 = 4.80$ Hz, $J_2 = 12.00$ Hz, 1H, NH-CH-CH), 2.12-2.07 (m, 1H, CH), 0.97-0.95 (d, $J = 6.80$ Hz, 3H, CH₃-CH), 0.88-0.86 (d, $J = 6.88$ Hz, 3H, CH₃-CH). IR (KBr) cm^{-1} : 3294.41 (N-H), 3089.91 (CH aromatic), 2972.31 (CH aliphatic), 1714.69 (C=O of COOH), 1340.52, 1172.72 (SO₂ two bands), 686.73 (Ar-H). MS: in m/z (rel. %): 212.08 (20%), 142.09 (PhSO₂H⁺, 61%), 141.04 (PhSO₂⁺, 7%), 78.05 (Ph-H, 54%), 77.04 (Ph⁺, 100%), 51.03 (38%), 43.99 (CO₂⁺, 53%). Anal. calcd. for C₁₁H₁₅NO₄S (257.31): C, 51.35; H, 5.88; N, 5.44. Found: C, 49.77; H, 5.70; N, 5.18.

2.2.1.8 3-Hydroxy-2-(phenylsulfonamido) butanoic acid (1h)

Yield 2.88 g (88.9%), 144-146°C, $R_f = 0.55$ (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (DMSO-*d*₆) δ : 12.55 (s-br, 1H, OH of COOH), 7.81-7.79 (d, $J = 8.60$ Hz, 2H, Ar-H), 7.68-7.65 (d, $J = 9.20$ Hz, 1H, NH-CH), 7.59-7.51 (m, 3H, Ar-H), 3.98-3.96 (dq, $J_1 = 3.60$ Hz, $J_2 = 6.40$ Hz, 1H, CH-CH-CH₃), 3.68-3.65 (dd, $J_1 = 3.60$ Hz, $J_2 = 9.20$ Hz, 1H, NH-CH-CH), 2.08 (s, 1H, OH), 1.00-0.99 (d, $J = 6.40$ Hz, 3H, CH₃-CH). IR (KBr) cm^{-1} : 3444.92 (OH free), 3296.33 (N-H), 3016.73 (CH aromatic), 2945.31 (CH aliphatic), 1726.34 (C=O of COOH), 1332.81, 1166.92 (SO₂ two bands), 669.31 (Ar-H). MS: in m/z (rel. %): 259.07 (M⁺, 28%), 195.11 (M⁺ - COOH - OH, 6%), 118.06 (100%), 117.05 (54%), 90.04 (35%). Anal. calcd. for C₁₀H₁₃NO₅S (259.28): C, 46.32; H, 5.05; N, 5.40. Found: C, 46.47; H, 4.99; N, 5.59.

2.2.1.9 5-Amino-5-oxo-2-(phenylsulfonamido) pentanoic acid (1i)

Yield 3.25 g (90.8%), mp 173-174°C, $R_f = 0.34$ (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (DMSO-*d*₆) δ : 12.61 (s-br, 1H, OH of COOH), 8.17-8.15 (d, $J = 8.80$ Hz, 1H, NH-CH), 7.77-7.75 (d, $J = 8.64$ Hz, 2H, Ar-H), 7.61-7.53 (m, 3H, Ar-H), 7.25 (s, 1H, NH_a of CO-NH₂), 6.74 (s, 1H, NH_b of CO-NH₂), 3.74-3.68 (dt, $J_1 = 5.60$ Hz, $J_2 = 8.80$ Hz, 1H, NH-CH-CH₂), 2.08-2.04 (t, $J = 7.60$ Hz, CO-CH₂-CH₂), 1.84-1.82 (m, 1H, CH_a of CH₂-CH₂CO), 1.65-1.63 (m, 1H, CH_b of CH₂-CH₂CO). IR (KBr) cm^{-1} : 3429.43, 3226.92 (N-H), 2978.11 (CH aliphatic), 1739.81 (C=O of COOH), 1683.91 (CO of amide), 1541.12 (C=C), 1321.32, 1170.83 (SO₂ two bands), 603.73 (Ar-H). Anal. calcd. for C₁₁H₁₄N₂O₅S (286.31): C, 46.15; H, 4.93; N, 9.78. Found: C, 46.03; H, 4.95; N, 9.84.

2.2.1.10 3-Phenyl-2-(phenylsulfonamido) propanoic acid (1j)

Yield 3.03 g (79.3%), mp 124-125°C, $R_f = 0.63$ (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (DMSO-*d*₆) δ : 12.71 (s-br, 1H, OH of COOH), 8.30-8.28 (d, $J = 9.00$ Hz, 1H, NH-CH), 7.57-7.53 (m, 3H, Ar-H), 7.45-7.41 (m, 2H, Ar-H), 7.23-7.13 (m, 3H, Ar-H), 7.12-7.11 (m, 2H, Ar-H), 3.88-3.84 (ddd, $J_1 = 5.76$ Hz, $J_2 = 8.96$ Hz, $J_3 = 9.00$ Hz, 1H, NH-CH-CH₂-Ar), 2.96-2.91 (dd, $J_1 = 5.76$ Hz, $J_2 = 20.00$ Hz, 1H, CH_a of CH₂-Ar), 2.73-2.68 (dd, $J_1 = 8.96$ Hz, $J_2 = 20.00$ Hz, 1H, CH_b of CH₂-Ar). IR (KBr) cm^{-1} : 3340.74 (N-H), 3173.11 (OH), 3059.12 (CH aromatic), 2964.42 (CH aliphatic), 1735.92 (C=O of COOH), 1346.32, 1168.91 (SO₂ two bands), 688.62 (Ar-H). MS: in m/z [rel. %]: 294.14 (47%), 214.02 (M⁺ - Ph-CH₂, 10%), 203.08 (12%), 91.05 (Ph-CH₂⁺, 59%), 77.16 (Ph⁺, 100%), 65.04 (SO₂H⁺, 15%). Anal. calcd. for C₁₅H₁₅NO₄S (305.36): C, 59.00; H, 4.95; N, 4.59. Found: C, 58.88; H, 4.83; N, 4.47.

2.2.1.11 2-(Phenylsulfonamido)-3-(4-(phenylsulfonyloxy) phenyl) propanoic acid (1k)

Yield 4.23 g (73.3%), mp 109-110°C, $R_f = 0.61$ (CHCl₃/CH₃OH, 9:1, at RT). ¹H-NMR (CDCl₃) δ : 7.82-7.82 (d, $J = 8.60$ Hz, 2H, Ar-H), 7.74-7.72 (d, $J = 8.52$ Hz, 2H, Ar-H), 7.70-7.68 (m, 1H, Ar-H), 7.56-7.53 (m, 3H, Ar-H), 7.48-7.46 (m, 2H, Ar-H), 7.05-7.03 (d, $J = 8.40$ Hz, 2H, Ar-H), 6.87-6.85 (d, $J = 8.40$ Hz, 2H, Ar-H), 5.10-5.08 (d, $J = 8.80$ Hz, 1H, NH-CH), 4.19-

4.18 (m, 1H, CH), 3.13-3.08 (dd, $J_1 = 5.20$ Hz, $J_2 = 20.00$ Hz, 1H, CH_a of CH₂-Ar), 2.99-2.94 (dd, $J_1 = 6.60$ Hz, $J_2 = 20.00$ Hz, 1H, CH_b of CH₂-Ar). IR (KBr) cm⁻¹: 3225.31 (N-H), 3070.72 (CH aromatic), 2931.82 (CH aliphatic), 1755.20 (C=O of COOH), 1625.31 (C=C), 1363.72, 1161.10 (SO₂ two bands), 686.71 (Ar-H). MS: in m/z (rel. %): 393.12 (40%), 218.03 (18%), 157.02 (77%), 141.00 (PhSO₂⁺, 98%), 134.06 (90%), 94.04 (100%), 78.05 (Ph-H⁺, 28%), 65.04 (SO₂H⁺, 20%). Anal. calcd. for C₂₁H₁₉NO₇S₂ (461.52): C, 54.65; H, 4.15; N, 3.03. Found: C, 54.74; H, 4.25; N, 2.85.

2.2.2 General procedure for N,N-diethylalkanamide of benzenesulfonamide (2a-k)

A three-necked 250 mL flask equipped with magnetic stirring bar was charged with (1a-k) (9.35 mmol) and dichloromethane (DCM) (30.00 mL). The flask was closed and N₂ was bubbled into it continuously. Oxalyl chloride (1.00 mL, 12.16 mmol, 1.30 equiv.) was added via dropping pipette followed by the addition of 1 drop of DMF. The mixture was stirred at room temperature for 2 h and then concentrated to dryness with rotary evaporator (23°C, 40 mmHg). Dichloromethane (DCM) (40.00 mL) was added to the resulting crude acid chloride and the solution was concentrated again. In a separate 250 mL three-necked round bottom flask, equipped with a magnetic stirring bar, a N₂ inlet, a rubber septum, 125-mL pressure equalizing addition funnel and a temperature probe was charged with DCM (20 mL), triethylamine (2.00 mL, 14.03 mmol, 1.50 equiv.) and diethylamine (1.30 mL, 12.16 mmol, 1.30 equiv.) and the mixture was cooled to -15°C. The crude acid chloride was dissolved in DCM (20.00 mL), transferred to the addition funnel and added dropwise to the stirred diethylamine solution at such a rate that the internal temperature was maintained below 10°C.

Upon completion of the addition of the acid chloride solution (ca 30 min), the mixture was stirred at -10 to 0°C for 1 h and at room temperature for 1 h. The mixture was diluted with 2.00M HCl (18.00 mL) and transferred into a 250 mL separatory funnel. The layers were separated, the organic layer was washed with brine (18.00 mL), dried over anhydrous Na₂SO₄, concentrated under reduced pressure, diluted with methanol (18.00 mL) and re-concentrated to give a crude solid. The solid was slurried in methanol (20.00 mL) and water (30.00 mL) was added dropwise with continuous stirring for 10 min. The slurry was stirred at room temperature for 1 h and methanol was removed by rotary evaporator. The resulting residue was transferred into separatory funnel and extracted with DCM. The organic layer was worked up and dried under vacuum/N₂ sweep for 12 h to obtain crude solid which was purified by column chromatography on Merck silica gel F (Mesh 200-300) using CHCl₃/CH₃OH, 9:1 solvent system to afford N,N-diethyl alkanamide substituted benzenesulfonamides (2a-k) in 71.50% - 95.80% yields.

2.2.2.1 N,N-Diethyl-1-(phenylsulfonyl)pyrrolidine-2-carboxamide (2a)

Yield 2.20g (75.9%), mp 84-85°C [Lit. 85-87°C, [22]]. ¹H-NMR (CDCl₃) δ: 7.92-7.90 (d, $J = 7.12$ Hz, 2H, Ar-H), 7.56-7.48 (m, 3H, Ar-H), 4.81-4.78 (dd, $J_1 = 3.60$ Hz, $J_2 = 11.60$ Hz, 1H, CH-CON), 3.58-3.50 (m, 2H, N-CH₂-CH₃), 3.48-3.41 (m, 2H, CH₂-N of pyrrolo), 3.37-3.30 (m, 2H, N-CH₂-CH₃), 2.15-2.07 (m, 2H, CH₂ of pyrrolo), 1.92-1.85 (m, 2H, CH₂ of pyrrolo), 1.29-1.26 (t, $J = 7.08$ Hz, 3H, CH₃-CH₂N), 1.11-1.07 (t, $J = 7.08$ Hz, 3H, CH₃-CH₂N). IR (KBr) cm⁻¹: 2981.93 (CH aromatic), 2926.01 (CH aliphatic), 2860.11 (CH aliphatic), 1649.13 (C=O of amide), 1334.71, 1151.51 (SO₂ two bands), 686.72 (Ar-H). MS: in m/z (rel. %): 210.98 (50%), 169.12 (53%), 140.98 (PhSO₂⁺, 40%), 100.07 (O=C-N (CH₂CH₃)₂⁺, 98%), 77.03 (Ph⁺, 42%), 72.04 (⁺N(CH₂CH₃)₂, 100%), 29.04 (CH₃CH₂⁺, 27%). Anal. calcd. for C₁₅H₂₂N₂O₃S (310.42): C, 58.04; H, 7.14; N, 9.02. Found: C, 57.98; H, 7.25; N, 9.02.

2.2.2.2 *N,N*-Diethyl-1-(phenylsulfonyl) piperidine-2-carboxamide (2b)

Yield 2.90 g (95.8%), mp 128-129°C {Lit. 127-129°C, [22]}. $^1\text{H-NMR}$ (CDCl_3) δ : 7.73-7.71 (d, $J = 8.60$ Hz, 2H, Ar-H), 7.54-7.42 (m, 3H, Ar-H), 4.90-4.88 (dd, $J_1 = 2.00$ Hz, $J_2 = 8.00$ Hz, 1H, CH-CON), 3.79-3.76 (m, 2H, $\text{N-CH}_2\text{-CH}_3$), 3.33-3.28 (m, 2H, $\text{N-CH}_2\text{-CH}_3$), 3.18-3.15 (m, 1H, CH_a of $\text{CH}_2\text{-N}$ piperidine), 3.10-3.07 (m, 1H, CH_b of $\text{CH}_2\text{-N}$ piperidine), 1.78-1.65 (m, 3H, CH & CH_2 of piperidine), 1.61-1.47 (m, 3H, CH & CH_2 of piperidine), 1.29-1.26 (t, $J = 7.16$ Hz, 3H, $\text{CH}_3\text{-CH}_2\text{N}$), 0.98-0.94 (t, $J = 7.12$ Hz, 3H, $\text{CH}_3\text{-CH}_2\text{N}$). Anal. calcd. for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$ (324.45): C, 59.23; H, 7.46; N, 8.63. Found: C, 59.17; H, 7.29; N, 8.55.

2.2.2.3 *N,N*-Diethyl-2-(phenylsulfonamido) acetamide (2c)

Yield 2.23 g (88.2%), mp 201-202°C. $^1\text{H-NMR}$ (CDCl_3) δ : 7.89-7.85 (m, 2H, Ar-H), 7.64-7.48 (m, 3H, Ar-H), 5.92 (s-br, 1H, NH), 3.76-3.75 (d, $J = 5.08$ Hz, 2H, $\text{CH}_2\text{-NH}$), 3.31-3.25 (q, $J = 7.12$ Hz, 2H, $\text{N-CH}_2\text{CH}_3$), 3.18-3.12 (q, $J = 7.16$ Hz, 2H, $\text{N-CH}_2\text{CH}_3$), 1.12-1.09 (t, $J = 7.16$ Hz, 3H, $\text{CH}_3\text{-CH}_2$), 1.03-0.99 (t, $J = 7.12$ Hz, 3H, $\text{CH}_3\text{-CH}_2$). IR (KBr) cm^{-1} : 3294.42 (N-H), 3057.21 (CH aromatic), 2985.94 (CH aliphatic), 1726.33 (C=O of amide), 1625.32 (C=C), 1327.02, 1166.89 (SO_2 two bands), 688.62 (Ar-H). Anal. calcd. for $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ (270.35): C, 53.31; H, 6.71; N, 10.36. Found: C, 53.19; H, 6.84; N, 10.51.

2.2.2.4 *N,N*-Diethyl-3-methyl-2-(phenylsulfonamido) butanamide (2g)

Yield 2.11 g (72.3%), mp 89-90°C. $^1\text{H-NMR}$ (CDCl_3) δ : 7.82-7.79 (d, $J = 8.72$ Hz, 2H, Ar-H), 7.54-7.43 (m, 3H, Ar-H), 5.83-5.81 (d, $J = 9.16$ Hz, 1H, NH-CH), 3.84-3.81 (dd, $J_1 = 4.16$ Hz, $J_2 = 9.16$ Hz, 1H, NH-CH-CH), 3.17-2.99 (m, 4H, $2 \times \text{CH}_2\text{-CH}_3$), 1.83-1.81 (m, 1H, CH), 1.05-1.01 (d, $J = 15.88$ Hz, 3H, $\text{CH}_3\text{-CH}$), 0.93-0.89 (t, $J = 7.20$ Hz, 3H, $\text{CH}_3\text{-CH}_2$), 0.87-0.83 (t, $J = 7.10$ Hz, 3H, $\text{CH}_3\text{-CH}_2$), 0.87-0.83 (t, $J = 7.10$ Hz, 3H, $\text{CH}_3\text{-CH}_2$), 0.87-0.83 (d, $J = 14.20$ Hz, 3H, $\text{CH}_3\text{-CH}$). IR (KBr) cm^{-1} : 3257.81 (N-H), 2966.52 (CH aliphatic), 1639.53 (C=O of amide), 1325.11, 1165.03 (SO_2 two bands), 605.63 (Ar-H). Anal. calcd. for $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$ (312.43): C, 57.67; H, 7.74; N, 8.97. Found: C, 57.44; H, 7.83; N, 9.09.

2.2.2.5 4-[(3-(Diethylamino)-3-oxo-2-(phenylsulfonamido) propyl)] phenyl benzenesulfonate (2k)

Yield 3.46 g (71.5%), mp 72-73°C. $^1\text{H-NMR}$ (CDCl_3) δ : 7.81-7.79 (d, $J = 7.40$ Hz, 2H, Ar-H), 7.75-7.74 (d, $J = 7.40$ Hz, 2H, Ar-H), 7.68-7.64 (m, 1H, Ar-H), 7.54-7.50 (m, 3H, Ar-H), 7.45-7.41 (m, 2H, Ar-H), 7.06-7.03 (d, $J = 8.44$ Hz, 2H, Ar-H), 6.88-6.85 (d, $J = 8.44$ Hz, 2H, Ar-H), 4.23-4.21 (d, $J = 9.20$ Hz, 1H, NH-CH), 4.24-4.21 (dd, $J_1 = 9.20$ Hz, $J_2 = 13.60$ Hz, 1H, NH-CH-CH), 3.17-3.14 (m, 1H, CH_a of $\text{CH}_2\text{-Ar}$), 2.98-2.95 (m, 1H, CH_b of $\text{CH}_2\text{-Ar}$), 2.88-2.80 (m, 4H, $2 \times \text{CH}_2\text{-CH}_3$), 0.86-0.82 (t, $J = 7.10$ Hz, 3H, $\text{CH}_3\text{-CH}_2$), 0.78-0.75 (t, $J = 7.16$ Hz, 3H, $\text{CH}_3\text{-CH}_2$). IR (KBr) cm^{-1} : 3248.11 (N-H), 3072.63 (CH aromatic), 2974.21 (CH aliphatic), 1689.61 (C=O of amide), 1625.31 (C=C), 1371.42, 1161.43 (SO_2 two bands), 686.72 (Ar-H). MS: in m/z (rel. %): 416.03 ($\text{M}^+ - \text{O}=\text{C}-\text{N}(\text{CH}_2\text{CH}_3)_2$, 88%), 359.10 (77%), 269.07 (100%), 218.11 (62%), 140.89 (PhSO_2^+ , 21%), 100.07 ($\text{O}=\text{C}-\text{N}(\text{CH}_2\text{CH}_3)_2^+$, 78%), 72.04 ($^+\text{N}(\text{CH}_2\text{CH}_3)_2$, 47%), 29.04 (CH_3CH_2^+ , 6%). Anal. calcd. for $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_6\text{S}_2$ (516.64): C, 58.12; H, 5.46; N, 5.42. Found: C, 57.97; H, 5.39; N, 5.31.

2.3 Antibacterial Activity Assays

The antimicrobial properties of the sulfonamides were investigated in form of the general sensitivity testing and minimum inhibitory concentration (MIC) with respect to freshly cultured

targeted organisms. The two organisms of interest in this present study are one gram positive (*Staphylococcus aureus* ATCC 6538) and one gram negative (*Escherichia coli* ATCC 25922) organisms which are associated with the gastrointestinal tract damage in man and animal.

2.3.1 Preparation of the inoculum

The standard strains of *S. aureus* and *E. coli* used were obtained from Test Center of Antimicrobial Materials, TIPC, Beijing. No clinically isolated organism was used based on in-availability of such as at the time of this study. The strains were propagated on nutrient agar plates and maintained on the plate at 4°C. The isolates were sub-cultured in nutrient broth at 37°C for 8 h prior to antibacterial testing.

2.3.2 Antibacterial sensitivity testing of the synthesized compounds

Agar well diffusion technique as described by Adeniyi et al. [23] and co-workers was used to determine the antibacterial activity of the synthesized compounds [23]. Sensitivity test agar plates were seeded with 0.10 mL of an overnight culture of each bacterial strain (equivalent to $10^7 - 10^8$ CFU mL⁻¹). The seeded plates were allowed to set and a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.30 mL of each sulfonamide solution in appropriate solvent at a concentration of 1000 µg/mL (0.02 g of sulfonamide dissolved in 20.00 mL distilled water). All the plates were incubated at 37°C for 24 h. The assay was conducted at regular intervals of 24 h until marked decline in the potency of the sulfonamide solution to inhibit the growth of the test organisms was noticed. Zones of clearance round each well means inhibition and the diameter of such zones were measured. The procedure was repeated for the streptomycin (standard). Selectivity index (S.I.) is the ratio of zone of inhibition of compound to that of the streptomycin.

2.3.3 Determination of minimum inhibitory concentration (MIC)

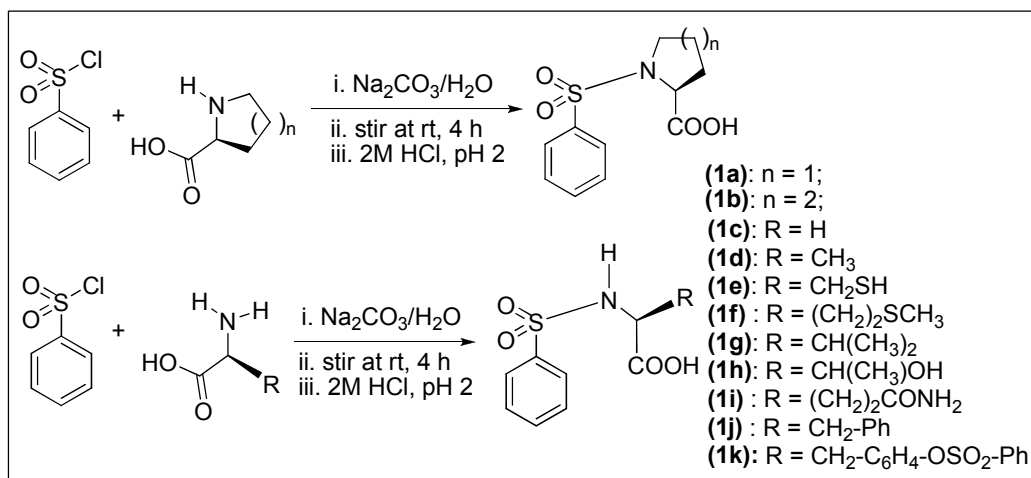
Agar well dilution method as described by Russell and Furr was used to determine the minimum inhibitory concentration (MIC) of the sulfonamides and streptomycin [24]. Different dilutions of the sulfonamides were prepared first, at ≤ 100.00 µg/mL to give final concentrations in the range of 100.00, 50.00, 25.00 and 12.50 µg/mL. The different dilutions of sulfonamide derivatives that could not inhibit the microbial growth at ≤ 100.00 µg/mL were later prepared at ≤ 1000.00 µg/mL to give final concentrations in the range of 1000.00, 500.00, 250.00, 125.00 and 62.50 µg/mL. Two milliliter (2.00 mL) of each dilution was mixed with 18.00 mL of Mueller Hinton agar (MHA, Difco, France) and poured into Petri-dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial strains and incubated overnight. The plates were then examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective sulfonamide. The procedure was repeated for streptomycin (standard).

3. RESULTS AND DISCUSSION

3.1 Chemistry

Benzenesulfonyl chloride and its para-substituted counterparts were earlier used in the protection of amino functional group, identification of amino acid and distinguishing among three classes of amine. However, we have herein successfully used benzenesulfonyl chloride as the cost effective and highly efficient main precursor in order to synthesize our targeted substituted benzene sulfonamide derivatives (1a-k) in the present work. Benzene sulfonyl chloride underwent condensation reaction with secondary amine of two different amino acids to afford *N,N*-disubstituted benzene sulfonamides (1a) and (1b), while its treatment with primary amine functionality of nine other amino acids in alkaline medium generated *N*-substituted benzene sulfonamide (1c-k) according to Scheme 1. It is important to note that amide formation is a fundamental reaction of great interest in organic chemistry [25,26]. The development of efficient methods for the synthesis of amides remains good tools because of their importance in chemistry and biology, with a wide range of industrial and pharmaceutical applications and as valuable intermediates in organic synthesis [27].

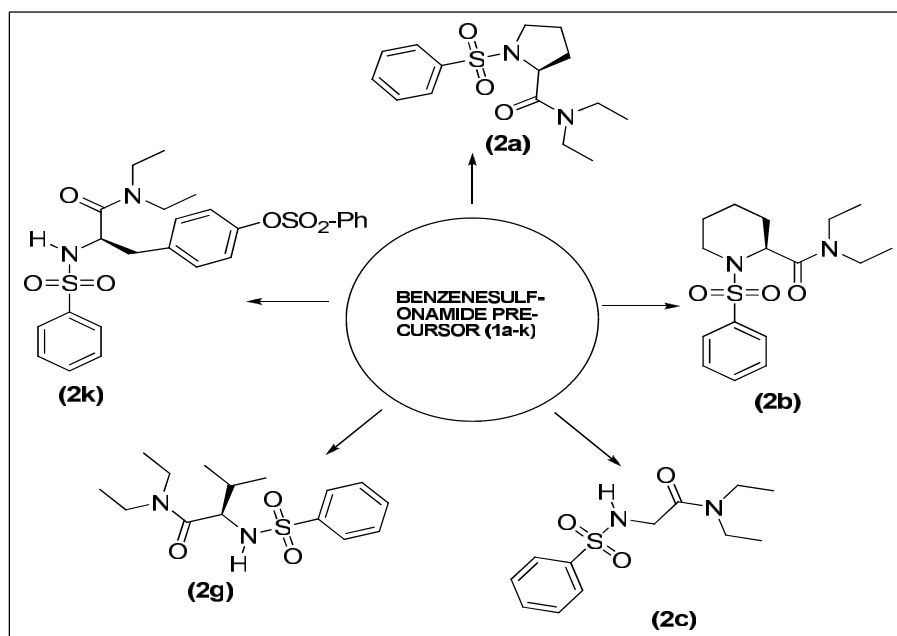
In continuation of our effort in search for therapeutically useful sulfonamides [28], we have here in synthesized benzenesulfonamides and their *N,N*-diethyl amide bearing scaffolds. In the earlier published work, Ajani et al. [28] observed that the work-up process to get the α -toluenesulfonamides in solid form from acidified aqueous medium was very difficult due to high polarity of such sulfonamides unlike the benzenesulfonamides which automatically crystallized out easily after acidification [28]. This arbitrary solubility trend in α -toluenesulfonamide is a strong indication that insertion of a sp^3 hybridized carbon between the phenyl and SO_2 unit (i.e. Ph-CH₂-SO₂Cl) confers different behaviour on the α -toluenesulfonamides, contrary to that of the common sulfonamides such as benzenesulfonamides and *p*-tolylsulfonamide. Although, the technique of synthesis was the same but the solvent used, product yields and the antibacterial activity were never the same. Hence, the CH₂ present in-between SO₂ and Phenyl ring in earlier reported sulfonamides [28] gave them different chemical behaviours and biological trends as compared to the one in the present study.



Scheme 1. Synthesis of benzenesulfonamide derivatives (1a-k)

The carboxylic acid end of the prepared benzenesulfonamide (1a-k) was converted to the corresponding *N,N*-diethylsubstituted alkanamide of benzene sulfonamides (2a-k). Thus, some selected benzene sulfonamides containing free carboxyl side chain were further treated via a one-pot two-step mechanism, to produce some selected new (2c), (2g) and (2k) and known series of *N,N*-diethylated alkanamido benzene sulfonamides (2a), (2b) [22], in good to excellent yield (Scheme 2). This involved, first, reaction of the sulfonamide-carboxylic acid with oxalyl chloride in presence of one drop of DMF catalyst to produce the acid chloride, which was converted to *N,N*-diethyl substituted arylsulfonamide by treating it with diethylamine in the presence of triethylamine base using dichloromethane (DCM) as solvent according to a known procedure [29].

The comparative study of (1a) with (2a) was established according to spectroscopic result in order to validate efficient conversion protocol. For instance, the stretching vibration frequencies at 3064.89 cm^{-1} and 1728.21 cm^{-1} depicted the presence of CH aromatic and C=O acid respectively, in the infrared spectrum of (1a) while SO_2 functionality appeared as two bands at 1352.13 cm^{-1} and 1157.33 cm^{-1} in the same compound. On the contrary, the C=O amide of compound (2a) appeared at lower frequency 1649 cm^{-1} than that of (1a). This experience confirmed the conversion of COOH in (1a) to $\text{CO-N}(\text{CH}_2\text{CH}_3)_2$ in (2a). In a similar manner, the $^1\text{H-NMR}$ spectrum of (1a) in CDCl_3 showed five aromatic protons as two-proton doublet ($J = 7.60\text{ Hz}$) and three-proton multiplet at $\delta\ 7.92\text{-}7.90$ and $7.68\text{-}7.60$ respectively. The CH-COOH of (1a) resonated as doublet of doublet ($J_1 = 3.20\text{ Hz}$, $J_2 = 12.00\text{ Hz}$) at $\delta\ 4.32\text{-}4.30$ while all other six pyrrolidine protons appeared upfield from $\delta\ 3.56\text{-}3.54$ to $1.83\text{-}1.79$. Since, (2a) was structurally related with (1a), similar $^1\text{H-NMR}$ signals were observed in (2a). In addition to these, there were extra two-proton CH_2 multiplets at $\delta\ 3.58\text{-}3.50$ and $3.37\text{-}3.30$ as well as two CH_3 triplets ($J = 7.08\text{ Hz}$) at $1.29\text{-}1.26$ and $1.11\text{-}1.07$ in the $^1\text{H-NMR}$ spectrum of (2a). This $^1\text{H-NMR}$ spectral behaviour further confirmed the effective conversion of $-\text{COOH}$ functionality in (1a) to $-\text{CO-N}(\text{CH}_2\text{CH}_3)_2$ in (2a).



Scheme 2. Conversion of benzenesulfonamides to *N,N*-diethylamides

3.2 Antimicrobial Activity

The antibacterial general sensitivity testing (inhibition zone, mm) of all the series of sixteen synthesized benzene sulfonamides along side with that of streptomycin clinical standard were assayed on test organisms (*Escherichia coli* and *Staphylococcus aureus*) using agar diffusion technique [23] while minimum inhibitory concentration test was carried out using Russell and Fur method (1977) [24]. The choice of *E. coli* as the Gram -ve organism is because it is easily transmissible through food, water, soil, animal and man [30]. *E. coli* is a normal flora of human body which causes a lot of vancomycin-resistant *Enterococci* and methicillin-resistant *Staphylococcus aureus* (MRSA) [31].

Based on our previous report, the choice of streptomycin as clinical standards is due to the fact that at low concentrations, streptomycin only inhibits growth of the bacteria through induction of prokaryotic ribosomes to misread mRNA [20] and it also possesses broad spectrum of antibacterial activity. The biological relevance of the synthesized sulfonamides here in was authenticated by the *in vitro* screening against *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and *Escherichia coli* ATCC 25922 (*E. coli*). The reported selectivity index (S.I.) was duly calculated by comparing zones of inhibition (Z.O.I) of compounds to that of streptomycin (i.e. Z.O.I of compound/ Z.O.I. of streptomycin standard). There were reported cases of *E. coli* and *S. aureus* being susceptible to streptomycin [14,32].

The comparative study of activity of the benzene sulfonamides with that of streptomycin standard was commensurate using selectivity index on both *E. coli* and *S. aureus*. The selectivity index of benzene sulfonamide derivatives along side with that of streptomycin was evaluated on *E. coli* (Fig. 1). The selectivity index of this series of sulfonamide varied from 0.97 to 0.31; hence, streptomycin is more active than all the benzene sulfonamide on *E. coli*. Since increasing intensity of selectivity index connoted improved antibacterial activity; thus, the compound with most probable activity of this class was (1b) (S.I. = 0.97) while the least probable activity was (2c) (S.I. = 0.31). The activities of other benzene sulfonamides were between the two extremists as shown in Fig. 1. They were categorized into: most active (S.I. > 0.80), moderately active (0.60 < S.I. < 0.80) and least active (S.I. < 0.60). Bearing this classification in mind, it was noticeable that the occurrence of the most active scaffolds in a decreasing order of activity was (1b) \approx (1k) > (1d) > (2a) \approx (2g) > (2k) > (1i) \approx (2b) > (1j); that of moderate activity was (1c) \approx (1e) \approx (1f) > (1g) whereas the least activity was in order of (1a) \approx (1h) > (2c).

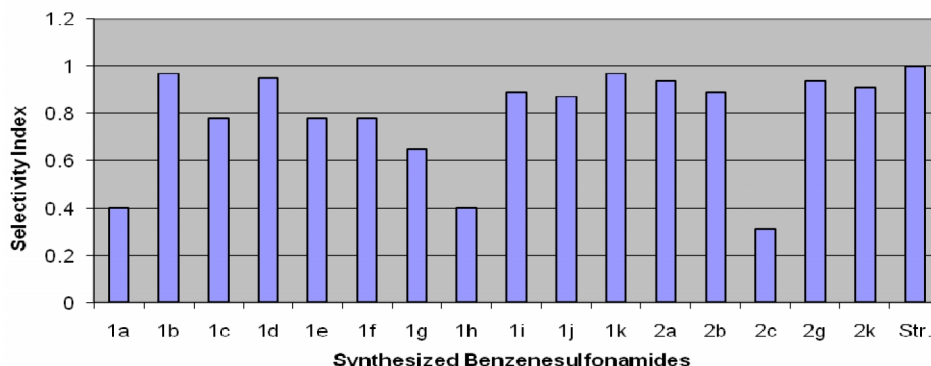


Fig. 1. Antibacterial activity of benzenesulfonamides against *E. coli*

Furthermore, the selectivity index of benzenesulfonamides was also investigated on *S. aureus* and the values varied from 1.60 to 0.28 (Fig. 2). In comparing with the activity of streptomycin, five compounds (1b), (1j), (1k), (2b) and (2k) were more active than streptomycin on *S. aureus*; one compound (1a) had invariably similar activity with the streptomycin standard while all other compounds were less active than streptomycin. Nevertheless, by comparing the trend of activity within the series, the highly active compounds in order of priority of potency were (1k) \approx (2b) \approx (2k) > (1b) \approx (1j) > (1a) > (1f) > (1g) ($0.95 < \text{S.I.} < 1.60$); the moderate activity was found in (2a) \approx (2c) \approx (2g) (S.I. = 0.62) while the remaining five benzenesulfonamides (1e) > (1d) > (1c) \approx (1i) > (1h) ($0.28 < \text{S.I.} < 0.48$) were the series with least activity.

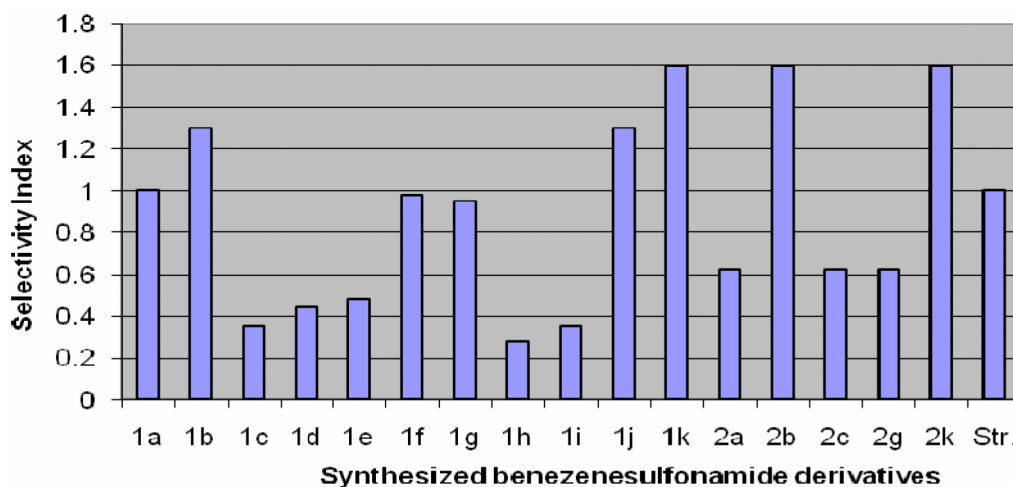


Fig. 2. Antibacterial activity of benzenesulfonamides against *S. aureus*

The minimum inhibitory concentration (MIC) was determined in order to authenticate the actual concentration responsible for the benzenesulfonamide activity observed on both *E. coli* and *S. aureus*. The result was as shown in Table 1. Thus, to start with, the lowest concentration of these sulfonamides that inhibited the growth of *E. coli* varied from 25.00 $\mu\text{g/mL}$ to 1000.00 $\mu\text{g/mL}$. Hence, the compound that had highest potency was (2b) with MIC value of 25.00 $\mu\text{g/mL}$ while the least active ones were (1e) and (1h) with MIC of 1000.00 $\mu\text{g/mL}$. Other sulfonamides exhibited the potency at diverse ranges. They were compounds (1b), (1d), (2a), (2g), (2k) and (1i) with MIC values between 50.00 $\mu\text{g/mL}$ and 100.00 $\mu\text{g/mL}$; (1a), (1f), (1g), (2c) and (1c) with MIC values between 125.00 $\mu\text{g/mL}$ and 500.00 $\mu\text{g/mL}$ and (1e) and (1h) with MIC value of 1000.00 $\mu\text{g/mL}$. This class of sulfonamide followed a peculiar trend in activity on *E. coli*, as it was noticed that all the *N,N*-disubstituted sulfonamides (2a), (2b), (2c), (2g) and (2k) showed better activity than their corresponding non-substituted sulfonamides (1a), (1b), (1c), (1g) and (1k) on *E. coli* (Table 1). This was in line with earlier findings of Dobek et al., (1980) who reported that *N,N*-disubstituted thiosemicarbazone were more active than the non- and mono-substituted analogs [21].

Table 1. MIC test of benzenesulfonamides on targeted organisms ($\mu\text{g/mL}$)

Organisms → Compd. No↓	<i>E. coli</i> ATCC 25922		<i>S. aureus</i> ATCC 6538	
	at 100 $\mu\text{g/mL}$	at 1000 $\mu\text{g/mL}$	at 100 $\mu\text{g/mL}$	at 1000 $\mu\text{g/mL}$
(1a)	>100.00	125.00	>100.00	125.00
(1b)	50.00	< 1000.00	100.00	< 1000.00
(1c)	>100.00	500.00	>100.00	1000.00
(1d)	50.00	< 1000.00	>100.00	500.00
(1e)	>100.00	1000.00	>100.00	250.00
(1f)	>100.00	125.00	62.50	< 1000.00
(1g)	>100.00	250.00	>100.00	250.00
(1h)	>100.00	1000.00	>100.00	500.00
(1i)	100.00	< 1000.00	>100.00	500.00
(1j)	250.00	< 1000.00	50.00	< 1000.00
(1k)	125.00	< 1000.00	62.50	< 1000.00
(2a)	50.00	< 1000.00	>100.00	125.00
(2b)	25.00	< 1000.00	25.00	< 1000.00
(2c)	>100.00	250.00	>100.00	250.00
(2g)	50.00	< 1000.00	>100.00	125.00
(2k)	62.50	< 1000.00	25.00	< 1000.00
Str.	12.50	< 1000.00	>100.00	125.00

>100.00 means that if there was no growth inhibition at 100.00 $\mu\text{g/mL}$, it was repeated at 1000.00 $\mu\text{g/mL}$, <1000.00 $\mu\text{g/mL}$ means that growth inhibition has already been experienced at lower concentration less than or equal to 100.00 $\mu\text{g/mL}$; hence, there is no need to repeat the test at 1000.00 $\mu\text{g/mL}$. – means no activity was observed even at 1000.00 $\mu\text{g/mL}$. Str. means Streptomycin clinical reference.

Considering the MIC test of benzenesulfonamides on the gram positive organism (*S. aureus*), it was observed that compounds (2k), (2b), (1j), (1k), (1f) and (1b) inhibited the microbial growth at varying MIC values $\leq 100.00 \mu\text{g/mL}$ which were more active than streptomycin; whereas, all other compounds were active on *S. aureus* at higher concentrations (between 125.00 $\mu\text{g/mL}$ and 1000.00 $\mu\text{g/mL}$). Specifically speaking, MIC value of the most potent in this series (2b) and (2k) on *S. aureus* was reported to be 25.00 $\mu\text{g/mL}$ which were two folds more active than (1j), with MIC value of 50.00 $\mu\text{g/mL}$ and four times more active than (1b) (100.00 $\mu\text{g/mL}$). The compound (2b) emerged to be the most active sulfonamide which was five times more active than streptomycin. The MIC value of (1j) was reported to be 50.00 $\mu\text{g/mL}$ which established it to be ten times more active than (1d), (1h) and (1i) with MIC value of 500.00 $\mu\text{g/mL}$. The compound with least activity was (1c) (1000.00 $\mu\text{g/mL}$).

Furthermore, from the structure activity relationship (SAR) study, it was observed that the nature of side chains (R) of the sulfonamides (Scheme 1) and the presence of *N,N*-diethylated amido moieties $\{(\text{CH}_3\text{CH}_2)_2\text{N}-\text{C}=\text{O}\}$ (Scheme 2) of the amide contributed immensely toward synergistic or antagonistic effect on the reported *in vitro* antibacterial activity. For instance, the least active compounds (1e) and (1h) had something in common as their side chains $\text{R} = \text{SH}$ and OH respectively, are hydrides of group 6 elements, indicating that the hydride of group 6 elements led to antagonistic effect which resulted in poor activity as seen in the case of MIC screening in *E. coli* (Table 1). In addition, compounds (2a), (2b), (2c), (2g) and (2k) were structurally related with (1a), (1b), (1c), (1g) and (1k), but the only disparity was the presence of *N,N*-diethyl amide chain in the former,

which probably accounted for their better activities than the latter as seen in the case of MIC screening in *S. aureus*. Thus, there was a clear indication that presence of *N,N*-diethyl amide led to a synergistic effect and an upward trend in the activity of (2a), (2b), (2c), (2g) and (2k) against *S. aureus*. This incidence corroborated the earlier finding of Dobek et al. (1980) showing that *N,N*-diethyl amide functionality led to increase in bioactivity of benzenesulfonamides [21].

4. CONCLUSION

Structural derivatives of sulfonamide have resulted in the development of a family of highly successful antibiotics that have saved millions of lives over the years. Hence, we have herein achieved the synthesis of *N,N*-diethylsubstituted amide bearing sulfonamides by amidation of benzenesulfonamide precursors in order to investigate their antibacterial properties. The result of antibacterial screening showed *N,N*-diethyl-1-benzenesulfonyl piperidine-2-carboxamide (2b) to be the most active antibacterial agent on the test organisms used in this study.

ACKNOWLEDGEMENTS

O.O.A. is grateful to the Academy of Science for the Developing World (TWAS) and Chinese Academy of Sciences (CAS) for the sponsorship of this present work under the 2009 TWAS-CAS Post-Graduate Fellowship Award.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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