Research Article

Amidine Sulfonamides and Benzene Sulfonamides: Synthesis and Their Biological Evaluation

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New amidine and benzene sulfonamide derivatives were developed and structures of the new products were confirmed by elemental and spectral analysis (FT-IR, ESI-MS, ¹HNMR, and ¹³CNMR). In vitro, developed compounds were screened for their antibacterial and antifungal activities against medically important bacterial strains, namely, *S. aureus*, *B. subtilis*, and *E. coli*, and fungi, namely, *A. flavus*, *A. parasiticus*, and *A. sp.* The antibacterial and antifungal activities have been determined by measuring MIC values ($\mu\text{g/mL}$) and zone of inhibitions (mm). Among the tested compounds, it was found that compounds **3b**, **9a**, and **9b** have most potent activity against *S. aureus*, *A. flavus*, and *A. parasiticus*, respectively, and were found to be more active than sulfamethoxazole and itraconazole with MIC values $40 \mu\text{g/mL}$. In contrast, all the compounds were totally inactive against the *A. sp.* except **10b** and **15b** to show activity to some extent.

1. Introduction

Sulfonamides are basis of several drug groups, known as sulfa drugs. Any compound that has sulfonamide moiety (SO_2NH_2) in its structure is referred to as sulfonamide. They comprise substantial class of pharmaceutical drugs, containing various kinds of pharmacological agents having antitumor [1], antibacterial [2], anticonvulsant [3, 4], diuretic [5, 6], hypoglycemic [7], and protease inhibitory activity [8–10] or antithyroid activity [11] among others. Sulfonamides are mostly used to treat the bacterial infectious cells because they do not significantly affect the antigenic properties of the infective organism or the development of specific antibodies [12]. Bacteria have liability to acquire resistance against sulfonamides by changing their cell wall permeability, enhancing essential metabolites production, or increasing production of enzyme [13]. In this way sulfonamides become ineffective to inhibit their production. But their ineffectiveness in drug therapy can be abated, due to inductive effect of SO_2 group. Sulfonamides having first pK_a value around 10 are

less soluble in water; therefore, they may readily crystallize in kidney but with advancement in medical science, and new sulfonamides have been synthesized having lower pK_a value (5–6) to avoid crystallization in kidney [14]. The compounds having pyridine and amide functional group exhibit various biological activities like antifungal and antibacterial. So these biological activities encourage us to synthesize the sulfonamides containing such important functional groups [15–18]. The motivation behind this research work was to synthesize some novel sulfonamides (Schemes 1–4) having antimicrobial properties. Different amines were chosen and reacted with sulfonyl chlorides. As a result of substitution, different functional groups were added and resulting compounds exhibited antibacterial and antifungal activities.

2. Experimental

2.1. Chemistry. Chemicals used in present work were of analytical grade obtained from E-Merck (Germany), Sigma Aldrich (USA), and BDH (UK) without further purification

SCHEME 1: Amidine sulfonamides.

SCHEME 2: Benzene sulfonamides (series 1).

SCHEME 3: Benzene sulfonamides (series 2).

to synthesize desired compounds, and high purity water ($0.01 \mu\text{S}/\text{cm}$) was prepared in our own laboratory using Milli-Q purification system (USA). Alpha IR spectrometer (FTIR-ATR) and NMR spectrometer, Bruker, were used to record the IR and ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra, respectively. PG-T80⁺ UV-Vis spectrophotometer (UK) and Flash HT Plus elemental analyzer (Thermo Scientific, UK) were used for λ_{max} , and concentration of hydrogen (H), carbon (C), nitrogen (N), and sulfur (S) of synthesized compounds, respectively, while the melting point was measured by Gallenkamp apparatus. JMS-HX-110 spectrometer with electron spray ionization (ESI) interface was used for mass spectra. The ^1H NMR and ^{13}C NMR spectra of all the synthesized compounds were measured using MeOD and

concentration of all the compounds was 10–20 mg in 0.8–1.0 mL of solvent. Purification and progress of the synthesized compounds were confirmed on precoated TLC silica plate (Merck-Germany).

2.2. Antimicrobial Assay. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Aspergillus flavus* ATCC 9643, *Aspergillus parasiticus* ATCC 15517, and *Acremonium* sp. ATCC 200667 were collected from Mycology Department, University of Punjab, Lahore, Pakistan, and were maintained in tryptic soy agar (TSA) and potato dextrose agar (PDA) medium, respectively, slants at 5°C until use. A series of four 2-fold dilutions (320, 160, 80, and $40 \mu\text{g}/\text{mL}$) were made from stock solution of $640 \mu\text{g}/\text{mL}$ in dimethyl sulphoxide (DMSO). All the dilutions were made sterile in an autoclave at 121°C for 30 min with 15 psi pressure after filtration through $0.22 \mu\text{m}$ membrane filter. The minimal inhibitory concentration (MIC) was reported as absence of no observable growth by the lowest concentration of tested compounds after twofold serial dilution. Five individually numbered test tubes with screw caps were sterilized. Tube 1 was filled with 2 mL of tryptic soy broth culture media including the stock solution of synthesized compounds. 1.0 mL of this solution was introduced into 2 tubes and diluted with 1.0 mL culture media and we repeated the procedure up to tube 5. The tubes were incubated at 25°C for 72 hrs. Ciprofloxacin and sulfamethoxazole (sulfa drug) were used as reference (positive control to check

SCHEME 4

the sensitivity of tested bacterial strains). $1-3 \times 10^8$ cfu/mL of each of Gram negative *E. coli* and Gram positive *S. aureus* and *B. subtilis* was obtained after adjusting the optical density of inoculum at 0.2–0.3 and 0.3–0.4 (620 nm), respectively, while fungal suspension (*A. flavus*, *A. parasiticus*, and *A. sp.*) with cell density of 10^5 cfu/mL was studied in present work and the itraconazole was used as reference antifungal agent. All the compounds and reference solutions were applied (50 μ L) onto a 6 mm sterile filter paper disc separately and the inoculated plates incubated at 37°C for 24 hrs. The zones of inhibition (mm) were measured and we evaluated the antibacterial activities.

2.3. General Procedure for Synthesis of Sulfonamides. A simple method in aqueous media under dynamic pH control is adopted for synthesis of sulfonamides. Filtration after acidification is involved for isolation of products [19–21]. All the amines were weighed accurately and dissolved completely by addition of distilled water by constant stirring using magnetic stirrer. The pH of the reaction contents was strictly monitored and maintained at 8–10 at regular intervals during the experimental reaction using Na_2CO_3 solution (1 M). Then benzene sulfonyl chloride or p-toluene sulfonyl chloride was accurately weighed and added carefully into the above solution. The reaction was carried in round bottom flask equipped with magnetic stirrer. During stirring sulfonyl chloride initially floats on the surface and the completion

of reaction was examined by the change in pH value due to formation of HCl by the consumption of sulfonyl chlorides during the reaction. On completion of the reaction pH was adjusted at 2–3 using HCl solution (2 M). The precipitates formed were filtered through Whatman filter paper number 42, washed several times with distilled water, and recrystallized using methanol and dried using rotary evaporator.

2.4. N-[Imino[(phenylsulfonyl)amino]methyl]-N-methylglycine (3a, $\text{C}_{10}\text{H}_{13}\text{N}_3\text{O}_4\text{S}$). Yield: 397.9 mg (64.7%); m.p.: 172–174°C; TLC: R_f = 0.72 (H_2O -BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu}$ = 3215 ($\text{O}-\text{H}_{\text{carboxylic}}$, stretching), 3084 ($\text{C}-\text{H}_{\text{aromatic}}$, stretching), 1768 ($\text{C}=\text{O}_{\text{carboxylic}}$, stretching), 1707 ($\text{C}=\text{N}_{\text{imine}}$, stretching), 1438 ($\text{O}-\text{H}_{\text{carboxylic}}$, bending), 1033 ($\text{S}=\text{O}_{\text{stretching}}$), 1165 ($-\text{N}-\text{S}=\text{O}_{\text{stretching}}$), 1458 ($\text{C}=\text{C}_{\text{aromatic}}$, stretching), 706 ($\text{O}-\text{S}_{\text{stretching}}$); UV-Vis (methanol, c = 2×10^{-5} mol dm^{-3}): $\lambda_{\text{max}}(\epsilon)$ = 208 (25050) nm ($\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$); ^1H NMR (500 MHz, MeOD) δ = 9.91 (br, s, 1H, NH), 7.98 (d, J = 7.48 Hz, 1H, CH), 7.68 (t, J = 7.59 Hz, 1H, CH), 7.56 (d, J = 7.63 Hz, 1H, CH), 4.71 (d, J = 13.16 Hz, 2H, CH_2), 3.15 (s, 3H, CH_3); ^{13}C NMR (125 MHz, MeOD) δ = 168 (C-16), 155 (C-11), 141 (C-1), 135 (C-4), 129 (C-3), 51 (C-15), 35 (C-14); ESI-MS: m/z = 273.38 [$\text{M} + 2$] $^+$, 271.31 [M] $^+$.

2.5. N-(Imino{[(4 methylphenyl)sulfonyl]amino}methyl)-N-methylglycine (3b, $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_4\text{S}$). Yield: 779.9 mg (82.7%);

m.p.: 185–187°C; TLC: $R_f = 0.64$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3224$ (O-H_{carboxylic}, stretching), 2992 (C-H_{aromatic}, stretching), 1767 (C=O_{carboxylic}, stretching), 1699 (C=N_{imine}, stretching), 1438 (O-H_{carboxylic}, bending), 1028 (S=O_{stretching}), 1199 (-N-S=O_{stretching}), 1498 (C=C-C_{aromatic}, stretching), 701 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 222$ (22500) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 9.96$ (br, s, 1H, NH), 7.71 (s, 1H, CH), 7.38 (d, $J = 8.11$ Hz, 1H, CH), 4.68 (s, 2H, CH₂), 3.15 (s, 3H, CH₃), 2.26 (s, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 168$ (C-17), 156 (C-12), 143 (C-1), 138 (C-4), 129 (C-2), 52 (C-16), 35 (C-15), 22 (C-7); ESI-MS: $m/z = 287.39$ [M + 2]⁺, 285.34 [M]⁺.

2.6. *N*-[[Bis(phenylsulfonyl)amino](imino)methyl]-*N*-methylglycine (**4a**, C₁₆H₁₇N₃O₆S₂). Yield: 931.8 mg (81.3%); m.p.: 210–212°C; TLC: $R_f = 0.67$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3062$ (O-H_{carboxylic}, stretching), 1767 (C=O_{carboxylic}, stretching), 1621 (C=N_{imine}, stretching), 1445 (O-H_{carboxylic}, bending), 1037 (S=O_{stretching}), 1163 (-N-S=O_{stretching}), 1445 (C=C-C_{aromatic}, stretching), 689 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 210$ (17250) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 10.86$ (br, s, 1H, NH), 8.21 (t, $J = 7.61$ Hz, 1H, CH), 8.08 (d, $J = 7.61$ Hz, 1H, CH), 7.93 (s, 1H, CH), 4.75 (s, 2H, CH₂), 3.16 (s, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 168$ (C-25), 156 (C-13), 141 (C-1), 132 (C-4), 131 (C-5), 53 (C-24), 37 (C-23); ESI-MS: $m/z = 413.59$ [M + 2]⁺, 411.48 [M]⁺.

2.7. *N*-[[Bis[(4-methylphenyl)sulfonyl]amino](imino)methyl]-*N*-methylglycine (**4b**, C₁₈H₂₁N₃O₆S₂). Yield: 872.1 mg (82.2%); m.p.: 195–197°C; TLC: $R_f = 0.63$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3265$ (O-H_{carboxylic}, stretching), 1707 (C=O_{carboxylic}, stretching), 1600 (N-H_{amine}, bending), 1660 (C=N_{imine}, stretching), 1399 (O-H_{carboxylic}, bending), 1043 (S=O_{stretching}), 1182 (-N-S=O_{stretching}), 1445 (C=C-C_{aromatic}, stretching), 685 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 222$ (19000) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 10.76$ (br, s, 1H, NH), 7.81 (d, $J = 8.11$ Hz, 1H, CH), 7.78 (d, $J = 8.14$ Hz, 1H, CH), 4.73 (s, 2H, CH₂), 3.15 (s, 3H, CH₃), 2.46 (s, 3H, CH₃); ¹³CNMR (500 MHz, MeOD) $\delta = 168$ (C-27), 156 (C-14), 143 (C-1), 139 (C-4), 132 (C-6), 53 (C-26), 37 (C-24), 21 (C-25); ESI-MS: $m/z = 441.53$ [M + 2]⁺, 439.58 [M]⁺.

2.8. *N*-[Imino[[[4-methylphenyl)sulfonyl](phenylsulfonyl)amino]methyl]-*N*-methylglycine (**6a**, C₁₇H₁₉N₃O₆S₂). Yield: 626.4 mg (70.6%); m.p.: 182–184°C; TLC: $R_f = 0.61$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3277$ (O-H_{carboxylic}, stretching), 1707 (C=O_{carboxylic}, stretching), 1599 (N-H_{amine}, bending), 1659 (C=N_{imine}, stretching), 1399 (O-H_{carboxylic}, bending), 1040 (S=O_{stretching}), 1185 (-N-S=O_{stretching}), 1444 (C=C-C_{aromatic}, stretching), 684 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 218$ (26150) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 10.79$ (br, s, 1H, NH), 8.21 (d, $J = 7.61$ Hz, 1H, CH), 8.10 (s, H, CH),

7.91 (s, H, CH), 7.87 (d, $J = 8.11$ Hz, 1H, CH), 7.73 (s, H, CH), 7.73 (d, $J = 8.01$ Hz, 1H, CH), 4.73 (s, 2H, CH₂), 3.15 (s, 3H, CH₃), 2.44 (s, 3H, CH₃); (125 MHz, MeOD) $\delta = 168$ (C-26), 156 (C-14), 143 (C-1), 141 (C-17), 132 (C-20), 131 (C-2), 129 (C-3), 53 (C-25), 39 (C-24), 23 (C-7); ESI-MS: $m/z = 427.53$ [M + 2]⁺, 425.48 [M]⁺.

2.9. *N*-(Pyridin-2-yl)benzene Sulfonamide (**9a**, C₁₁H₁₀N₂O₂S). Yield: 869.6 mg (83.1%); m.p.: 145–147°C; TLC: $R_f = 0.61$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3367$ (N-H_{amine}, stretching), 1666 (N-H_{amine}, bending), 1024 (S=O_{stretching}), 1177 (-N-S=O_{stretching}), 1445 (C=C-C_{aromatic}, stretching), 687 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 230$ (43800) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 11.49$ (br, s, 1H, NH), 7.91 (d, $J = 5.21$ Hz, 1H, CH), 7.81 (d, $J = 7.61$ Hz, 1H, CH), 7.65 (t, $J = 8.31$ Hz, 1H, CH), 7.61 (t, $J = 7.51$ Hz, 1H, CH), 7.55 (t, $J = 7.61$ Hz, 1H, CH), 7.25 (t, $J = 7.21$ Hz, 1H, CH), 7.15 (d, $J = 8.39$ Hz, 1H, CH); ¹³CNMR (125 MHz, MeOD) $\delta = 148$ (C-2), 145 (C-6), 140 (C-11), 137 (C-4), 132 (C-14), 129 (C-13), 127 (C-12), 115 (C-5), 112 (C-3); ESI-MS: $m/z = 236.31$ [M + 2]⁺, 234.28 [M]⁺.

2.10. 4-Methyl-*N*-(pyridin-2-yl)benzene Sulfonamide (**9b**, C₁₂H₁₂N₂O₂S). Yield: 935.6 mg (82.1%); m.p.: 178–180°C; TLC: $R_f = 0.72$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3328$ (N-H_{amine}, stretching), 1665 (N-H_{amine}, bending), 1019 (S=O_{stretching}), 1161 (-N-S=O_{stretching}), 1455 (C=C-C_{aromatic}, stretching), 680 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 224$ (15380) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 11.43$ (br, s, 1H, NH), 7.97 (d, $J = 8.11$ Hz, 1H, CH), 7.61 (d, $J = 8.31$ Hz, 1H, CH), 7.25 (t, $J = 7.21$ Hz, 1H, CH), 7.20 (s, 1H, CH), 7.11 (t, $J = 8.41$ Hz, 1H, CH), 7.15 (d, $J = 8.39$ Hz, 1H, CH), 2.48 (s, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 148$ (C-2), 145 (C-6), 143 (C-14), 137 (C-4), 129 (C-13), 115 (C-5), 111 (C-3), 21 (C-17); ESI-MS: $m/z = 250.35$ [M + 2]⁺, 248.30 [M]⁺.

2.11. *N*-(Phenylsulfonyl)-*N*-(pyridin-2-yl)benzene Sulfonamide (**10a**, C₁₇H₁₄N₂O₄S₂). Yield: 909.8 mg (71.7%); m.p.: 166–168°C; TLC: $R_f = 0.62$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3348$ (N-H_{amine}, stretching), 1619 (N-H_{amine}, bending), 1030 (S=O_{stretching}), 1186 (-N-S=O_{stretching}), 1445 (C=C-C_{aromatic}, stretching), 688 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 215$ (12830) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 8.13$ (m, 1H, CH), 8.11 (m, 1H, CH), 7.95 (m, 1H, CH), 7.88 (m, 1H, CH), 7.81 (m, 1H, CH), 7.65 (qd, $J = 8.39$ Hz, 1.21 Hz, 1H, CH), 7.48 (qd, $J = 7.29$ Hz, 5.21 Hz, 1H, CH); ¹³CNMR (125 MHz, MeOD) $\delta = 148$ (C-6), 142 (C-13), 138 (C-2), 131 (C-16), 130 (C-14), 117 (C-5), 113 (C-3); ESI-MS: $m/z = 376.45$ [M + 2]⁺, 374.44 [M]⁺.

2.12. 4-Methyl-*N*-(phenylsulfonyl)-*N*-(pyridin-2-yl)benzene Sulfonamide (**10b**, C₁₉H₁₈N₂O₄S₂). Yield: 1140.5 mg (84.3%); m.p.: 164–166°C; TLC: $R_f = 0.68$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3348$ (N-H_{amine}, stretching), 1621 (N-H_{amine}, bending), 1027 (S=O_{stretching}), 1162 (-N-S=O_{stretching}),

1445 (C=C-C_{aromatic}, stretching), 681 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 222$ (13855) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 8.19$ (s, H, CH), 8.02 (d, $J = 8.09$ Hz, 1H, CH), 7.87 (t, $J = 8.41$ Hz, 1H, CH), 7.68 (d, $J = 8.11$ Hz, 1H, CH), 7.61 (d, $J = 8.37$ Hz, 1H, CH), 7.55 (d, $J = 7.27$ Hz, 1H, CH), 2.46 (s, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 148$ (C-6), 138 (C-2), 132 (C-15), 117 (C-5), 113 (C-3), 22 (C-27); ESI-MS: $m/z = 404.55$ [M + 2]⁺, 402.51 [M]⁺.

2.13. 4-Methyl-N-(phenylsulfonyl)-N-(pyridin-2-yl)benzene Sulfonamide (12a, C₁₈H₁₆N₂O₄S₂). Yield: 529.5 mg (65.1%); m.p.: 188–190°C; TLC: $R_f = 0.76$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3280$ (N-H_{amine}, stretching), 1620 (N-H_{amine}, bending), 1021 (S=O_{stretching}), 1158 (-N-S=O_{stretching}), 1445 (C=C-C_{aromatic}, stretching), 682 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 220$ (42550) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 8.15$ (d, $J = 7.69$ Hz, 1H, CH), 8.07 (d, $J = 8.11$ Hz, 1H, CH), 7.98 (d, $J = 7.61$ Hz, 1H, CH), 7.91 (t, $J = 8.37$ Hz, 1H, CH), 7.65 (d, $J = 8.17$ Hz, 1H, CH), 7.51 (d, $J = 7.17$ Hz, 1H, CH), 7.45 (d, $J = 7.17$ Hz, 1H, CH), 2.44 (s, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 147$ (C-6), 138 (C-2), 132 (C-15), 117 (C-5), 112 (C-3), 21 (C-27); ESI-MS: $m/z = 390.53$ [M + 2]⁺, 388.49 [M]⁺.

2.14. N-Methyl-N-(phenylsulfonyl)alanine (15a, C₁₀H₁₃NO₄S). Yield: 786.1 mg (51.7%); m.p.: 132–134°C; TLC: $R_f = 0.72$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3215$ (O-H_{carboxylic}, stretching), 1743 (C=O_{carboxylic}, stretching), 1444 (O-H_{carboxylic}, bending), 1041 (S=O_{stretching}), 1183 (-N-S=O_{stretching}), 1444 (C=C-C_{aromatic}, stretching), 688 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 215$ (13070) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 7.73$ (d, $J = 7.69$ Hz, 1H, CH), 7.53 (d, $J = 7.51$ Hz, 1H, CH), 7.36 (t, $J = 7.61$ Hz, 1H, CH), 4.51 (q, $J = 7.17$ Hz, 1H, CH), 3.75 (br, s, 1H, OH), 2.71 (s, 3H, CH₃), 1.11 (d, $J = 7.17$ Hz, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 178$ (C-14), 138 (C-1), 131 (C-4), 62 (C-12), 29 (C-11), 15 (C-13); ESI-MS: $m/z = 245.38$ [M + 2]⁺, 243.35 [M]⁺.

2.15. N-Methyl-N-[(4-methylphenyl)sulfonyl]alanine (15b, C₁₁H₁₅NO₄S). Yield: 910.9 mg (63.6%); m.p.: 140–142°C; TLC: $R_f = 0.70$ (H₂O-BuOH-Acetic acid 1:4:1); IR (FTIR): $\bar{\nu} = 3268$ (O-H_{carboxylic}, stretching), 1710 (C=O_{carboxylic}, stretching), 1449 (O-H_{carboxylic}, bending), 1054 (S=O_{stretching}), 1146 (-N-S=O_{stretching}), 1422 (C=C-C_{aromatic}, stretching), 677 (O-S_{stretching}); UV-Vis (methanol, $c = 2 \cdot 10^{-5}$ mol dm⁻³): $\lambda_{\max}(\epsilon) = 220$ (17250) nm (mol⁻¹ dm³ cm⁻¹); ¹HNMR (500 MHz, MeOD) $\delta = 7.69$ (d, $J = 8.09$ Hz, 1H, CH), 7.32 (d, $J = 7.91$ Hz, 1H, CH), 4.48 (t, $J = 7.17$ Hz, 1H, CH), 3.75 (br, s, 1H, OH), 2.69 (s, 3H, CH₃), 2.39 (s, 3H, CH₃), 1.08 (d, $J = 7.17$ Hz, 3H, CH₃); ¹³CNMR (125 MHz, MeOD) $\delta = 178$ (C-14), 138 (C-1), 133 (C-4), 129 (C-5), 61 (C-12), 29 (C-11), 15 (C-13); ESI-MS: $m/z = 259.38$ [M + 2]⁺, 257.35 [M]⁺.

3. Results and Discussion

A total of twelve novel sulfonamides were synthesized in aqueous basic media by simple reaction of creatine (amidine derivatives), amino pyridine (benzene sulfonamide series 1), and methyl alanine (benzene sulfonamide series 2) with benzene sulfonyl chloride and para toluene sulphonyl chloride, respectively, with continuous stirring and details of reaction conditions are explained in Experimental section and synthetic pathways of sulfonamides are explained in Schemes 1, 2, 3, and 4. Compounds **3a**, **3b**, **9a**, **9b**, **15a**, and **15b** were synthesized in equimolar concentration of creatine, amino pyridine, and methyl alanine with benzene sulphonyl chloride and para toluene sulphonyl chloride, respectively, while **4a**, **4b**, **9a**, and **9b** were obtained by bimolar concentration of respective sulphonyl chlorides, respectively. Compounds **6a** and **12a** were synthesized by the reaction of **3b** and **9b** with benzene sulphonyl chloride and para toluene sulphonyl chloride in equimolar concentration, respectively. All the compounds except compound **15a** (57.5%) were obtained in good yield. Elemental analysis was performed for the conformation of all the compounds and measurement of absorption maximum ($\lambda_{\max}$) provided the justification. The analytical data of synthesized sulfonamides are presented in Table 1. The synthesized compounds were characterized by FT-IR, and the characteristics band at 1621–1707 cm⁻¹ for imine stretching of amidine sulfonamides, 3280–3367 cm⁻¹ of N-H amide stretching for benzene sulfonamides (series 1), 3250–3268 cm⁻¹ for O-H stretching (benzene sulfonamide series 2), and 1082–1199 cm⁻¹ for (-N-S=O) and 1019–1054 cm⁻¹ (S=O) for all compounds reveals the formation of sulfonamides. [M + 2]⁺ peaks obtained by ESI-MS represented the isolation of sulfonyl group in all synthesized compounds. The structures of all the compounds were also confirmed by ¹HNMR and ¹³CNMR by dissolving in MeOD. ¹HNMR spectra of compounds **3a** and **3b** showed a broad signal at δ 9.91, 9.96 while signal at δ 10.76–10.86 for **4a**, **4b**, and **9a** and 11.43, 11.49 ppm for **9a** and **9b** corresponds to NH group of sulfonamide. A broad singlet at δ 3.75 ppm due to -OH group was also obtained for compounds **15a** and **15b**. The characteristics C-SO-NH signals at δ 155–156 ppm and δ 138–140 ppm for amidine and benzene sulfonamides, respectively, were showed by ¹³CNMR which identified the structures correctly.

Synthesized compounds were also screened for their antibacterial against Gram negative bacteria *E. coli* and Gram positive *S. aureus* and *B. subtilis* by following the guidelines of CLSI [22, 23] using ciprofloxacin and sulfamethoxazole as reference antibacterial agents. Antifungal activity was also evaluated for synthesized compounds against three strains, namely, *A. flavus*, *A. parasiticus*, and *A. sp.* using itraconazole as reference antifungal agent. Among the bacterial strains, compound **3b** has excellent antibacterial activities having MIC 40 µg/mL against *S. aureus* with zone of inhibition comparable with control drug (ciprofloxacin). The pK_a of compound **3b** is 10.8 which has electron withdrawing inductive effect (mild acidic) for which supports its cell permeability against the particular bacterial strain to bind

TABLE 1: Physiochemical and analytical data of sulfonamides.

Compounds	Molecular weight	Elemental analysis (found/cal.) %				^a pK _a
		C	H	N	S	
3a	271.29	44.33/44.27	4.85/4.83	15.42/15.49	11.83/11.82	10.8
3b	285.32	46.35/46.30	5.39/5.31	14.68/14.73	11.29/11.24	10.8
4a	411.45	46.77/46.71	4.15/4.16	10.26/10.21	15.65/15.59	4.7
4b	439.51	49.17/49.19	4.89/4.82	9.58/9.56	14.57/14.59	4.7
6a	425.48	47.96/47.99	4.54/4.50	7.88/7.98	15.12/15.07	4.7
9a	234.28	56.33/56.39	4.28/4.30	11.88/11.96	13.61/13.69	8.5
9b	248.30	58.03/58.05	4.81/4.87	11.22/11.28	12.85/12.91	8.5
10a	374.44	54.50/54.53	3.68/3.77	7.51/7.48	17.15/17.13	5.3
10b	402.49	56.73/56.70	4.52/4.51	6.88/6.96	15.86/15.93	5.3
12a	388.46	55.63/55.65	4.11/4.15	7.16/7.21	16.46/16.51	5.3
15a	243.28	49.43/49.37	5.41/5.39	5.73/5.76	13.16/13.18	5.0
15b	257.31	51.33/51.35	5.81/5.88	5.36/5.44	12.49/12.46	4.8

^apK_a values from ACD, Inc.TABLE 2: Zone^a of inhibition and MIC^b of sulfonamides against pathogenic bacterial strains.

Compounds	Name of bacteria					
	Gram (+) bacterial strains				Gram (–) bacterial strains	
	<i>S. aureus</i> (ATCC 25923)		<i>B. subtilis</i> (ATCC 6633)		<i>E. coli</i> (ATCC 25922)	
	Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of inhibition	MIC
3a	—, —, —	—	—, —, —	—	9, —, —	>160
3b	32, 29, 26	40	8, —, —	>160	—, —, —	—
4a	—, —, —	—	—, —, —	—	—, —, —	—
4b	—, —, —	—	—, —, —	—	28, 25, 22	80
6a	—, —, —	—	—, —, —	—	9, —, —	>160
9a	—, —, —	—	9, —, —	160	9, —, —	>160
9b	—, —, —	—	8, —, —	>160	—, —, —	—
10a	9, —, —	>160	—, —, —	—	14, 10, —	160
10b	27, 24, 22	80	—, —, —	—	—, —, —	—
12a	9, —, —	>160	28, 24, 21	80	—, —, —	—
15a	29, 26, 21	80	—, —, —	—	—, —, —	—
15b	—, —, —	—	27, 24, 22	80	28, 25, 20	80
Ciprofloxacin ^c	36, 34, 31	0.5	36, 33, 31	0.5	35, 34, 31	0.5
Sulfamethoxazole ^d	—, —, —	—	16, 13, —	160	33, 28, 25	40

^aZone of inhibition was measured in mm at concentration of 160, 80, and 40 µg mL^{−1}.^bMIC (minimum inhibitory concentrations) were measured in µg mL^{−1}.^cControl drug; ^dsulfa drug.

the proteins. As far as SAR is concerned, amide functional group further supports its activity. Compounds **4b** and **15b** showed better activity against *E. coli* and compounds **3a**, **4a**, **4b**, and **6a** have no activity against the Gram positive *S. aureus* and *B. subtilis* while **10b**, **12a**, **15a**, and **15b** have good activity against these bacterial strains which is higher (MIC 80 µg/mL) than already existing sulfa drug (sulfamethoxazole; MIC 160 µg/mL). The MIC values and zone of inhibitions are presented in Table 2. The activities of above-mentioned compounds are their high permeability into cell due to their acidic behavior by virtue of their lower pK_a values (4.7–5.3), while the pK_a value of sulfamethoxazole

is 5.7. The electrostatic interaction of methyl group with protein and pyridine further supports their antibacterial activities [24].

Among the fungal strains, compounds **9a** and **9b** have excellent activity against *A. parasiticus* and *A. flavus*, respectively, while **10a** and **15a** showed better activity against *A. parasiticus* and *A. sp.* was insensitive to all strains except compound **15b** (MIC 160 µg/mL). The amide bond formations in the above-mentioned synthesized compounds have excellent activities against the fungal strains as far as the SAR is concerned [25–27]. It is concluded that newly synthesized sulfonamides exhibited greater activity than reference

TABLE 3: Zone^a of inhibition and MIC^b of sulfonamides against pathogenic fungal strains.

Compounds	<i>A. parasiticus</i> (ATCC 15517)		Name of fungi <i>A. flavus</i> (ATCC 9643)		<i>A. sp.</i> (ATCC 200667)	
	Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of inhibition	MIC
3a	—, —, —	—	—, —, —	—	—, —, —	—
3b	—, —, —	—	12, —, —	160	—, —, —	—
4a	9, —, —	>160	—, —, —	—	—, —, —	—
4b	—, —, —	—	—, —, —	—	—, —, —	—
6a	—, —, —	—	—, —, —	—	—, —, —	—
9a	24, 21, 18	40	—, —, —	—	—, —, —	—
9b	22, 19, 13	80	22, 19, 15	40	9, —, —	>160
10a	21, 18, 12	80	—, —, —	—	—, —, —	—
10b	—, —, —	—	24, 20, 18	80	13, —, —	160
12a	—, —, —	—	12, —, —	160	—, —, —	—
15a	24, 21, 18	80	—, —, —	—	—, —, —	—
15b	11, —, —	>160	—, —, —	—	15, 12, —	160
Itraconazole ^c	18, 11, —	160	21, 19, 15	80	12, —, —	>160

^aZone of inhibition was measured in mm at concentration of 160, 80, and 40 $\mu\text{g mL}^{-1}$.

^bMIC (minimum inhibitory concentrations) were measured in $\mu\text{g mL}^{-1}$.

^cControl drug.

itraconazole. The MIC values and zone of inhibitions are reported in Table 3.

4. Conclusion

Amidine and benzene sulfonamides derivatives were synthesized and evaluated biologically. Among the synthesized compounds **3b** was proved potent antibacterial agent with MIC 40 $\mu\text{g/mL}$ and zone of inhibition comparable with ciprofloxacin and more effective than sulfamethoxazole. Synthetic compounds **9a** and **9b** showed better inhibition than itraconazole against *A. parasiticus* and *A. flavus*, respectively, with MIC 40 $\mu\text{g/mL}$.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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